Identification and characterization of a novel member of the dystrobrevin gene family

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Abstract A new member of the dystrobrevin gene family was identified using a bioinformatics approach. Sequence analysis indicates that this gene, named DTN-B, is highly homologous to the rabbit A0, the previously described dystrobrevin (DTN), *Torpedo* 87 kDa and to the C-terminus of dystrophin. The coiled-coil domain, shown to be the site of interaction between dystrobrevins and dystrophin, is highly conserved. Immunostaining studies indicate that DTN-B and DTN expression is absent in affected muscle fibers from DMD patients and carriers.

Key words: Dystrobrevin B; Dystrobrevin; Dystrophin associated protein; Monoclonal antibody A0

1. Introduction

Dystrophin, the protein product of the Duchenne muscular dystrophy (DMD) gene, is a membrane associated protein localized to the cytoplasmic surface of the muscle membrane. Although this gene has been shown to be defective in DMD patients, the pathogenetic mechanisms of the progressive muscular wasting are not yet completely understood. A number of membrane associated proteins have been described in mammals to interact with dystrophin. These proteins are known as the dystrophin associated proteins (DAPs) [1]. The DAP complex is composed of three different groups: the dystroglycan (α and β), the sarcoglycan (α, β, γ and δ), and the cytoplasmic (syntrophin and dystrobrevin) subcomplexes; for a review see [2,3].

Biochemical studies have shown that the DAP complex is conserved in various organisms including *Torpedo californica*. When dystrophin is purified from *Torpedo*, it is found in association with two proteins of 58 kDa and 87 kDa [4,5]. The 87 kDa protein shows some homology to the dystrophin COOH-terminal region, which contains a syntrophin binding site [6,7] and a coiled-coil domain [8].

A0 is a 94 kDa protein purified from rabbit skeletal muscle [1]. A monoclonal antibody (MA0) raised by immunization of mice with a purified DAP complex was shown to react with A0 and to recognize a 62 kDa and a 94 kDa band [9]. A partial cDNA corresponding to the rabbit A0 protein was sequenced, and based on the sequence homologies and on the similarity in molecular weight, the authors concluded that A0 is the rabbit homolog of the *Torpedo* 87 kDa [9].

Dystrobrevins (DTN) are dystrophin related proteins recently identified in both humans and mice [10,11]. DTN encodes proteins of various sizes (DTN-1, DTN-2 and DTN-3), which are predominantly expressed in brain, skeletal and cardiac muscle and are highly homologous to dystrophin, particularly in the C-terminus where two heptad leucine repeats are located. These leucines, predicted to form coiled-coils, have been recently demonstrated to interact with dystrophin [12] as previously postulated [13]. Sequence homologies and biochemical studies led the authors to conclude that dystrobrevins represent the homolog of the rabbit A0 and *Torpedo* 87 kDA proteins [10,11].

Using a bioinformatics approach, we have identified a novel member of the cytoplasmic group of dystrophin related proteins. This new gene is homologous but not identical to the previously reported dystrobrevins. We thus named it dystrobrevin B (DTN-B). Sequence analysis reveals that DTN-B is more closely related to the partial rabbit A0 sequence than the previously reported dystrobrevins.

The coiled-coil domain in the C-terminus is highly conserved in DTN-B, implying that this region could also be the interaction site of DTN-B and dystrophin.

2. Materials and methods

2.1. cDNA library screening

Teratocarcinoma/neuron (mature hNT neuron, Stratagene 937233) and skeletal muscle (Clontech) human cDNA libraries were used to characterize the human full-length cDNA. A mouse embryo cDNA library (Clontech) was screened in order to obtain the murine full-length cDNA. Plating hybridization and washing conditions were as previously described [14]

2.2. cDNA sequence analysis

cDNA sequence analysis and nucleotide and protein database searches were performed as previously described [15]. Data on similarity/identity were obtained using the Bestfit program of the GCG software package, Version 8.1. The multiple alignment analyses were generated using the PileUp program of the Wisconsin GCG software package, Version 8.1.

2.3. Expression studies

For Northern blot analysis, commercial Northern blots (Clontech) were used according to the manufacturer's recommendation.

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Abbreviations: DAP, dystrophin associated protein; DTN, dystrobrevin; DMD, Duchenne muscular dystrophy; RACE, rapid amplification of cDNA ends; VR, variable region; UTR, untranslated region

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2.4. RACE

3′-RACE was performed from 1 μg of total RNA from human skeletal muscle. Reverse transcriptase (BRL) was from the semi-random oligo 5′-NNNNNNSS-3′. Nested amplifications were performed with specific forward primers TO2865 (1190–1213 bp) and VR2 (1634–1656 bp) of the DTN-B2 sequence, together with standard RACE primers. PCR conditions are reported below.

2.5. PCR conditions

PCR amplifications were performed as previously described [16] using the following forward primers: 640F (694–719 bp), TO2864 (962–985 bp), TO4094 (1743–1764 bp), VRCF (1855–1877 bp), RH5′ (1975–1994 bp), EX20A (1715–1735 bp), TO3342 (1530–1549 bp) of the DTN-B1 sequence, and reverse primers: VRBR (1764–1743 bp), VRCR (1876–1854 bp), TO8080 (1903–1884 bp), RH3′ (2187–2169 bp), 3′UTRB (1991–1968 bp), 1225R (1315–1290 bp) of the DTN-B1 sequence, VR2 3′UTR (1683–1663 bp) of the DTN-B2 sequence, and A54R (1456–1436 bp) of mDTN-B sequence. PCR conditions were: 30–35 cycles with denaturation at 96°C (30 s), annealing at 55–60°C (1 min) and extension at 68°C (2–8 min).

2.6. FISH, YAC and RH mapping

FISH, YAC and RH mapping were performed as previously described [16]

2.7. Immunoblot analysis of fusion proteins and muscle tissue

GST fusion proteins and homogenate from human muscular tissue were prepared as previously described [16]. Samples were run on 11% SDS-PAGE [17] and transferred to nitrocellulose sheets [18]. Membranes were incubated for 2 h at room temperature with MA0 antibody (0.25 mg/ml diluted 1:500 in PBS with 3% non-fat milk, 0.05% Tween 20 and 0.05% NP40), washed and incubated 1h with peroxidase conjugated anti mouse IgG (diluted 1:10 000 in PBS with 0.5% non-fat milk, 0.05% Tween 20 and 0.05% NP40). Immunoreactive bands were visualized by ECL according to the specifications of the manufacturer (Amersham).

2.8. Immunohistochemistry

Skeletal muscle biopsy specimens were from normal controls, DMD carriers and affected patients. Immunostaining was performed with the streptavidin-biotin peroxidase method (YLEM kit, Italy). Endogenous peroxidase was blocked with hydrogen peroxide 0.3% in absolute methanol for 30 min. After rinsing with PBS, sections were incubated for 30 min with normal serum. The primary antibodies were diluted (MA0 0.25 mg/ml 1:200 and the monoclonal antibody specific for dystrophin C-terminus, YLEM, 1:50) in PBS containing 0.5% BSA. Biotinylated anti-mouse immunoglobulins (YLEM kit) were used as a secondary antibody. After washing in PBS, the sections were incubated in streptavidin conjugated to horseradish peroxidase. As a negative control, the primary antibody was omitted in every set of staining. In all cases, serial sections were stained with routine histochemistry (PAS, Gomori, NADH, etc.).

3. Results

3.1. Identification and characterization of the human and murine DTN-B cDNAs

The sequence corresponding to the *Torpedo* 87 kDa protein (GenBank accession no. L06945) was used as a query to search the dbEST database using the BLAST-N algorithm. Several ESTs were identified. Sequence analysis revealed that some of them corresponded to different isoforms of the recently described DTN [10] and were excluded from the present study. A number of human ESTs revealed to be highly homologous but not identical, suggesting the existence of a different gene which we named dystrobrevin B (DTN-B). These ESTs were further characterized. The IMAGE clone 48908 derived from a human infant brain cDNA library (EST H14817) was entirely sequenced. Analysis of the consensus sequence (2247 bp, GenBank accession number Y12712) revealed the presence of a putative initiation codon at position 184 and a stop codon (TAG) at nucleotide 1885, thus indicating that this clone corresponds to the full-length cDNA sequence. The coding region spans 1701 bp resulting in a predicted protein of 567 amino acids, with a predicted molecular weight of 64 kDa.

Using the sequence corresponding to human DTN-B, two non-overlapping mouse ESTs (AA036262 and AA545996, derived from a mouse embryo and a mouse T cell cDNA library, respectively) were identified and sequenced. Oligonucleotides (TO2864 and AA54R) were used to amplify cDNA from a mouse embryo at embryonic day 13.5. The PCR product was sequenced and assembled with the sequences already available. This effort allowed us to assemble a 2192 bp partial murine cDNA (GenBank accession number Y15742). Sequence analysis revealed a start codon at position 93, which is conserved in the human counterpart, and an open reading frame of 2102 bp, which is still open at the 3′ end. RT-PCR and screening of cDNA libraries failed to identify a mouse cDNA clone containing the stop codon. Sequence comparison of the mouse and human sequences revealed that the two genes are highly homologous (90.6% identity at the nucleotide level, and 94.9% identity and 95.7% similarity at the protein level), indicating that mDTN-B represents the bona fide murine homolog of DTN-B. Sequence analysis shows that the murine counterpart does not contain a 90 bp stretch present in the human sequence (position 1829–1919 of the Y12712 sequence) that includes the stop codon, suggesting that the murine gene encodes a protein larger than what was found in human.

3.2. Characterization of different splice forms for human DTN-B

We undertook an extensive study of all available EST clones together with RT-PCR and 3′ RACE experiments on cDNA from skeletal muscle using the oligonucleotides described in Section 2 and in Fig. 1A.

RT-PCR with oligo 640F and 1225R gave an amplification product larger than expected. Sequence analysis of this PCR product showed the presence of an additional 90 bp at position 1262. This region was designated site variable region A (VRA, GenBank accession number Y15720) and corresponds to the VR3 described in the mouse dystrobrevin [11]. RT-PCR with oligo EX 20A and 3′ UTRB gave three different amplification products of 278, 224 and 188 bp. Sequence analysis of these products revealed the presence of 90 additional bp in the 224 bp product (VRB, GenBank accession number Y15719) at position 1829 and the absence of 54 bp (VRC) in the same position. The PCR product of 188 bp was identical to the sequence from clone 48908, which included VRC, while the fragment of 278 bp contained VRB and VRC. RT-PCR data were confirmed by the existence in the dbEST database of human cDNA clones containing the three different regions. In summary, clone 48908 contained only VRC. Clones 182424 (breast), 593023 (pancreas) and 36952 (infant brain) contain VRA, VRB and VRC and correspond to the larger isoform DTNB-1 of 71 kDa (GenBank accession number Y15722). Clone 1187400 (prostate) contains VRB and does not display VRC. All these results are summarized in Fig. 1.

A human adult skeletal muscle cDNA library was screened with clone 48908. One clone, skm 19.1, was isolated and entirely sequenced. This cDNA clone was revealed to contain the full-length cDNA sequence and to include the VRA region

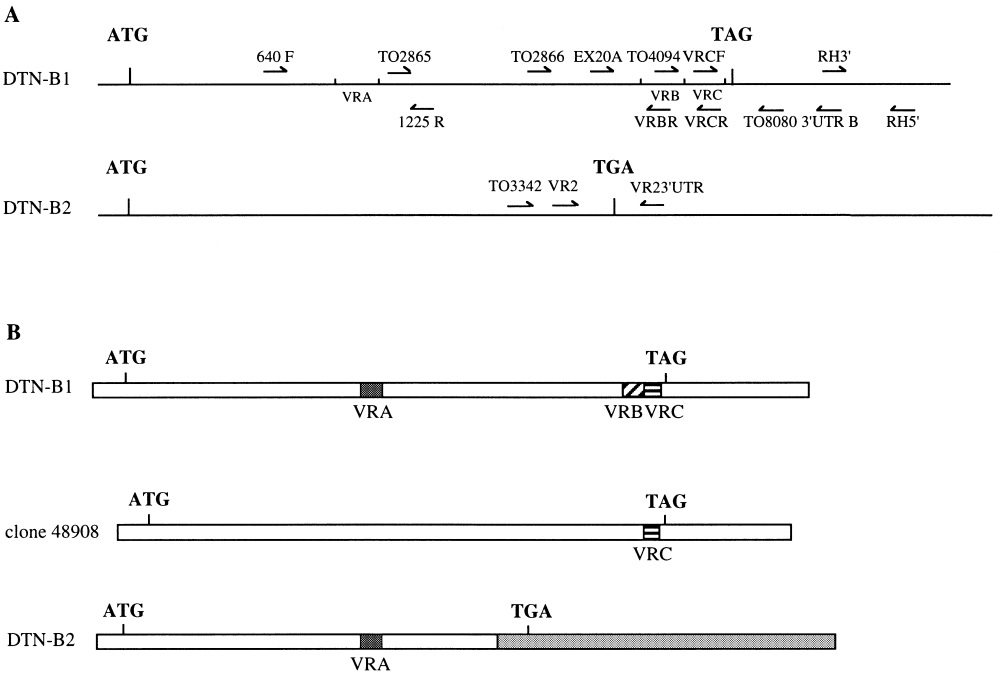

Fig. 1. Schematic representation of the different isoforms identified for DTN-B; all of the oligonucleotides used for RT-PCR and RACE experiments are indicated in A. DTN-B1 (A and B, top) corresponds to the longer isoform characterized and contains all three identified variable regions (VRA, VRB, VRC). DTN-B2 (A, bottom and B) corresponds to the isoform displaying VRA and an alternative 3′ end (gray), the primer VR2 used to perform RACE 3′ experiments is indicated (A, bottom). Primers RH5′ and RH3′ used for the radiation hybrid mapping and screening of the YAC libraries are indicated. DTN-B2 (A and B, bottom) corresponds to the shorter isoform.

with a different C-terminus, resulting in a smaller isoform (DTN-B2) similar to DTN-3 [19]. At amino acid 525 of skm 19.1, the sequence diverges from clone 48908 and after 97 nucleotides a TGA stop codon occurs resulting in a predicted protein product of 559 aa. 3′ RACE experiments on skm cDNA using primers TO2865 and VR2 were performed. Sequence analysis of the derived products confirmed the results obtained by analysis of the skeletal muscle cDNA. Furthermore, an additional EST clone (1268910 from human tonsil cells) recently deposited in the dbEST database was shown to contain the same 3′ end displayed in the skm 19.1 cDNA clone.

In order to determine the genomic structure of the 3′ ends of the DTN-B1 and DTN-B2 genes, we carried out PCR

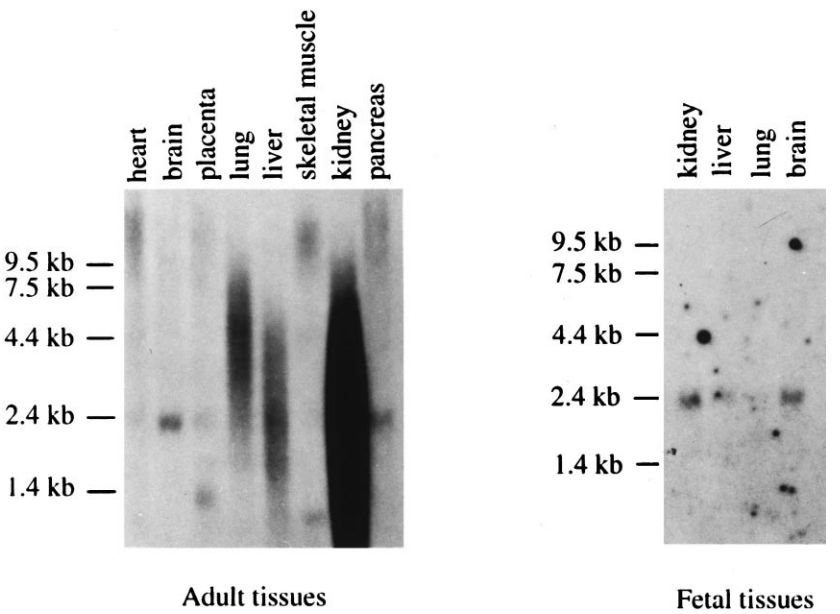

Fig. 2. Northern blot analysis of the DTN-B gene. Poly(A)$^+$ RNA from multiple adult and fetal tissues was hybridized to a probe corresponding to the 3′ UTR of the DTN-B gene. A 1 kbp band was observed in heart and skeletal muscle, as well as a ubiquitously expressed 2.4 kbp band, and a 4.4 kbp band detected only in heart and skeletal muscle (left panel). In fetal tissues only the 2.4 kbp band was observed in all lanes (right panel).

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DTN      1 MIEDSGKRGNTMAERRQLFAEMRAQDLDRIRLSTYRTACKLRFVQKKCNLHLVDIWNVIEALRENALNNLDPNTELNVSR 80
DTN-B    1 MIEESGNKRKTMAEKRQLFIEMRAQNFDVIRLSTYRTACKLRFVQKRCNLHLVDIWNMIEAFRDNGLNTLDHTTEISVSR 80
         81 LEAVLSTIFYQLNKRMPTTHQIHVEQSISLLLNFLLAAFDPEGHGKISVFAVKMALATLCGGKIMDKLRYIFSMISDSSG 160
         81 LETVISSIYYQLNKRLPSTHQISVEQSISLLLNFMIAAYDSEGRGKLTVFSVKAMLATMCGGKMLDKLRYVFSQMSDSNG 160
        161 VMVYGRYDQFLREVLKLPTAVFEGPSFGYTEQSARSCFSQQKKVTLNGFLDTLMSDPPPQCLVWLPLLHRLANVENVFHP 240
        161 LMIFSKFDQFLKEVLKLPTAVFEGPSFGYTEHSVRTCFPQQRKIMLNMFLDTMMADPPPQCLVWLPLMHRLAHVENVFHP 240
        241 VECSYCHSESMMGFRYRCQQCHNYQLCQDCFWRGHAGGSHSNQHQMKEYTSWKSPAKKLTNALSKSLSCASSREPLHPMF 320
        241 VECSYCRCESMMGFRYRCQQCHNYQLCQNCFWRGHAGGPHSNQHQMKEHSSWKSPAKKLSHAISKSLGCVPTREPPHPVF 320
        321 PDQPEKPLNLAHIVDTWPPRPVTSMNDTLFSHSVPSSGSP.....FITR.....SMLESSNR 372
        321 PEQPEKPLDLAHIV...PPRPLTNMNDTMVSHMSSGVPTPTKRLQYSQDIPSHLADEHALIASYVARLQHCARVLDSPSR 397
        373 IDEEHRLIARYAARLAAESSSSQPPQQRSAPDISFTIDANKQQRQLIAELENKNREILQEIQRLRLEHEQASQPTPEKAQ 452
        398 IDEEHRLIARYAARLAAEAGNVTRP....PTDLSFNFDANKQQRQLIAELENKNREILQEIQRLRLEHEQASQPTPEKAQ 473
        453 QNPTLLAELRLLRQRKDELEQRMSALQESRRELMVQLEGLMKLLK.....TQGSGTHRSSRSHSISRPIPMPIRSASA 525
        474 QNPTLLAELRLLRQRKDELEQRMSALQESRRELMVQLEELMKLLKEEEQKQAAQATGSPHTSPTHGGGRPMPMPVRSTSA 553
        526 CSTPTHTPQDSLTGVGGDVQEAFAQSSRRNLRNDLLVAADSITNTMSSLVKELNSEVGSETESNVDSEFARTQFEDLVPS 605
        554 GSTPTHCPQDSLSGVGGDVQEAFAQGTRRNLRNDLLVAADSITNTMSSLVKELHSAEEGAEEEEEKMQNGKDRG..... 627
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Fig. 3. Alignment of the DTN and DTN-B proteins. The homology between the two proteins is very high, as shown by the vertical lines corresponding to identical amino acids. The amino acids defined by the dots are conserved changes. The following domains are recognized in both proteins: an EF-hand motif (solid line); a putative zinc finger ZZ domain (dashed line); a syntrophin binding site (box); the two helices of the coiled-coil domain (italics); and the MA0 epitope (bold).

analysis on genomic DNA using primer pairs Ex20A/VRBR, TO4094/VRCR, and VRCF/TO8080 (Fig. 1A). VRCF/TO8080 failed to amplify genomic DNA. Sequence analysis of the amplification products obtained with the first Ex20A/VRBR and TO4094/VRCR primer pairs revealed the presence of intron/exon boundaries at position 1735 and 1825 of the DTN-B1 sequence, thus demonstrating that the variable regions B and C represent alternatively spliced exons.

Primer pair TO3342/VR23′UTR, located across the alternative 3′ sequence of DTN-B2, amplified an 8 kbp band on genomic DNA. Sequence analysis of the product revealed the presence of a splice site junction at position 1564 of DTN2-B2 cDNA.

3.3. Northern analysis

Commercial Northern blots of human poly(A)$^+$ mRNA from adult (heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas) and fetal tissues (brain, lung, liver, and kidney) were hybridized with a probe corresponding to the 3′ UTR of DTN-B1. Analysis under high stringency revealed in adult tissues the presence of a 1 kbp band in heart and skeletal muscle, a 1.2 kbp band in placenta, a ubiquitously expressed 2.4 kbp band, and a fainter 4.4 kbp band detected only in heart and skeletal muscle (Fig. 2, left panel). The 4.4 kb band specific for muscle was confirmed on different Northern blots after longer exposure (data not shown). In fetal tissues, only the 2.4 kbp band was observed in all lanes (Fig. 2, right panel). The differently sized bands observed might be due to the extensive alternatively spliced forms observed in these proteins. The same expression pattern was obtained using the entire coding region as probe (data not shown).

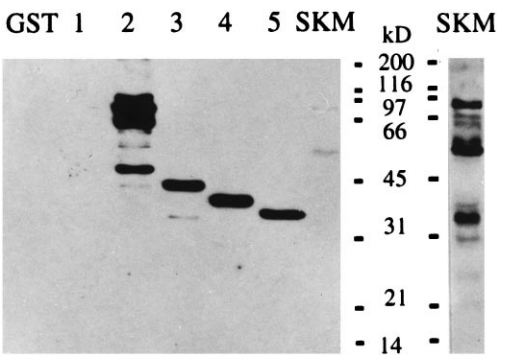

Fig. 4. Deletional analysis of the MA0 epitope and Western blot analysis of human skeletal muscle homogenate. GST corresponds to the vector alone; lane 1: GST-DTN-B (aa 12–346); lane 2: GST-DTN-B (aa 95–597); lane 3: GST-DTN-B (aa 465–574); lane 4: GST-DTN-B (aa 465–537); lane 5: GST-DTN-B (aa 465–505). As shown, the MA0 epitope encompasses the region of aa 465–505. SKM: human skeletal muscle homogenate after 1 min (left panel) and 1 h (right panel) exposure.

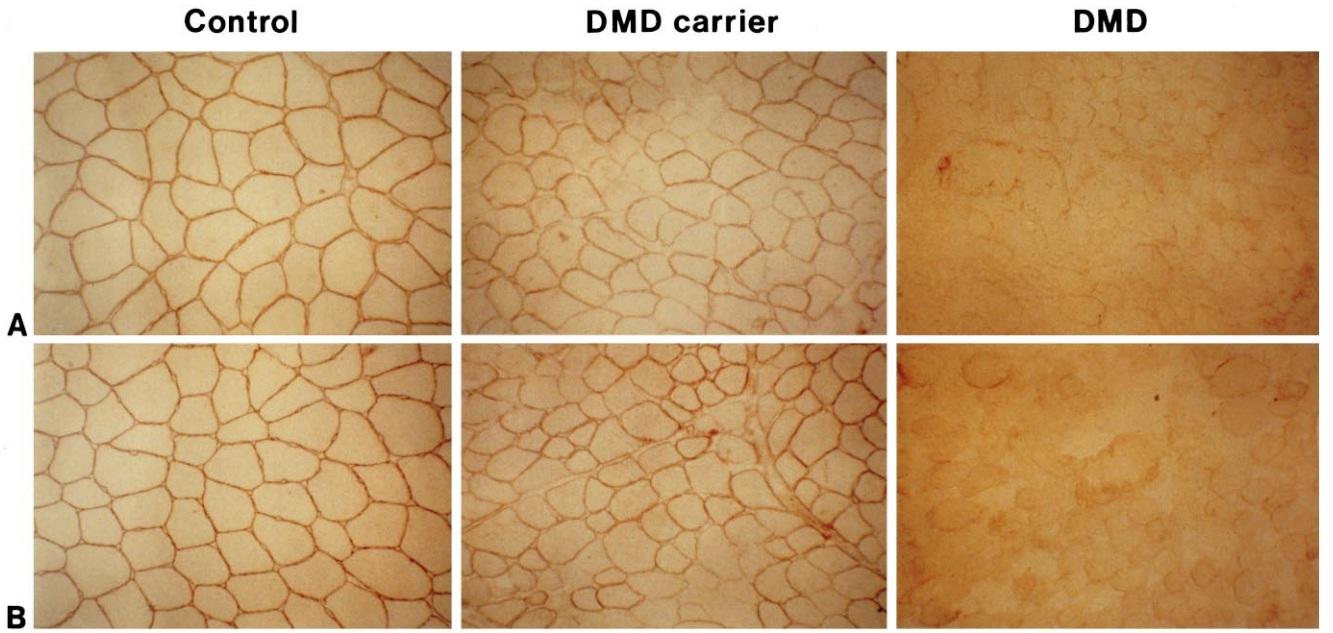

Fig. 5. Immunohistochemical study in muscle biopsies from normal controls, DMD carriers and DMD patients. In row A, the tissue specimens were stained with MA0 monoclonal antibody and visualized with biotynilated anti-mouse IgG. In comparison, in row B an antibody specific for the dystrophin C-terminus was used on the same sections. The same pattern is observed with the two antibodies; in both cases the immunoreactivity is concentrated at the sarcolemma of healthy fiber from normal controls and DMD carriers, while expression is absent in affected muscle fibers from DMD patients and carriers.

3.4. Sequence analysis of the DTN-B predicted protein product

BLAST-X, BLAST-P and BLAST-N analyses against the public databases confirmed the homology of DTN-B with dystrophin, dystrobrevins, *Torpedo* 87 kDa and the partial rabbit A0 sequence. In detail, Bestfit analysis revealed 98% similarity and 96% identity between the partial rabbit A0 sequence and DTN-B1. Homology between the DTN-B1 and DTN human proteins (isoform DTN1, GenBank accession number U46744) is 82.6% similarity and 76.7% identity.

The homology between rabbit A0 and the human DTN is lower than between A0 and DTN-B. These data suggest that DTN-B is more closely related to rabbit A0 than DTN.

Analysis of the DTN-B1 predicted protein product shows an EF-hand motif (aa 113–171) that potentially binds calcium, a putative zinc finger (ZZ) domain (aa 237–286), a syntrophin binding site (aa 399–448) and a coiled-coil motif (aa 425–517). All of these domains are also present in the DTN predicted protein product (Fig. 3). Recently, Sadoulet et al. demonstrated that in DTN the site which binds to dystrophin corresponds to the first five heptad repeats of the coiled-coil domain [12]. As shown in Fig. 3, the region encompassing the coil-coiled domain is very similar between the DTN and the DTN-B proteins, suggesting that this could also be the site of interaction with dystrophin for DTN-B.

3.5. Immunoblot analysis of the DTN-B protein and human skeletal muscle homogenate with the MA0 monoclonal antibody

Immunoblot analysis of glutathione *S*-transferase (GST) fusion proteins corresponding to different regions of the DTN-B1 protein and of fresh homogenate of human muscle was performed with the MA0 monoclonal antibody [9]. As shown in Fig. 4, MA0 reacts with the extracts in lane 2 (aa 95–597), lane 3 (aa 465–574), lane 4 (aa 465–537), and lane 5 (aa 465–505), while it does not react with the extracts in lane 1 (aa 12–346) and with the vector alone (GST). This deletion analysis indicates that the smaller portion of the protein recognized by MA0 is aa 465–505. This region encompasses a portion of the coiled-coil domain and is 100% identical to DTN (Fig. 3). Therefore, MA0 cannot distinguish between DTN and DTN-B. In human skeletal muscle homogenate (SKM), the MA0 antibody recognized two bands of 94 and 62 kDa after short exposure (1 min) (SKM, Fig. 4, left panel). The same Western blot exposed for 1 h (Fig. 4, right panel) revealed differently sized bands, suggesting that this antibody recognizes different isoforms belonging to both the DTN and DTN-B proteins, or to additional dystrophin associated proteins not yet identified. However, no additional sequences were retrieved in the dbEST database using the amino acid sequence recognized by MA0 as a query.

3.6. Expression of dystrobrevins in normal controls, DMD carriers and DMD patients

Immunohistochemical staining of frozen sections from human quadriceps was performed using the MA0 antibody. As shown in Fig. 5, MA0 immunostained muscle fiber sections from normal controls at the sarcolemma (Fig. 5A, control). In sections from DMD patients, no immunolabelling was detected (Fig. 5A, DMD) and in DMD carriers not all fibers were immunostained (Fig. 5A, carrier). The same experiments were performed with dystrophin antibody and a similar labelling pattern was obtained (Fig. 5B). No cytoplasmic staining was observed in any of the biopsies studied.

3.7. FISH, YAC and RH mapping in human, and genetic mapping in the mouse

Clone 48908 was used as a probe for FISH mapping experiments and was mapped to human 2p23. Oligonucleotides (RH3′–RH5′), designed from the DTN-B consensus sequence in order to amplify genomic DNA (Fig. 1A), were used to

screen the YAC libraries available at the YAC Screening Center, San Raffaele Biomedical Science Park, and the radiation hybrid panel (Genebridge 4, Research Genetics). One clone, YAC 783G10, containing the markers D2S171 and WI-6305, and known to map on the 2p23 region, was identified. RH screening confirmed that the gene is linked to the 2p23 region (16.84 cR from WI-3742). Linkage mapping of the mDTN-B was carried out in BSS backcross from the Jackson laboratories [20]. This analysis mapped the mouse DTN-B transcript to the proximal region of mouse chromosome 12 and co-segregates with the marker D12mit37, in a region syntenic to human chromosome 2 (data not shown).

4. Discussion

Dystrobrevins are part of the cytoplasmic subcomplex of the DAPs. Using a bioinformatics approach we have identified a novel member of the dystrobrevin gene family, which we named DTN-B. The previously reported DTN has been postulated to be the human homolog of A0 [10]. A0 is a 94 kDa protein isolated in rabbit using a biochemical approach. This protein is recognized by a monoclonal antibody, MA0, obtained by immunizing the mouse with the DAP complex [9]

Sequence analysis revealed that DTN-B is more closely related to the rabbit A0 sequence than DTN. This data may suggest that DTN-B corresponds to the human homolog of rabbit A0, although further characterization of the human DTN-B protein, together with a complete characterization of the rabbit A0 full-length cDNA, is necessary to be conclusive.

DTN and DTN-B share the same domains: an EF-hand motif, a putative zinc finger ZZ, a coiled-coil domain, and a syntrophin binding site. In addition to these motifs, the DTN protein presents a tyrosine kinase substrate domain which is also present in *Torpedo* 87 kDa where it is believed to play a role in the clustering of the nAChR [8]. Dystrophin and DTN have been recently shown to interact in vitro through the first heptad repeat in the coil-coiled domain [12]. In the same region, the homology between DTN and DTN-B is very high (Fig. 3) (21/25 amino acids are identical), suggesting that DTN-B could also interact with dystrophin through the same binding site.

Western blot analysis was carried out with the MA0 antibody on skeletal muscle. The results partially overlap with what was described in mouse using Ab 308 and Ab 433, two antibodies produced using synthetic peptides corresponding to residues 308–328 (Ab 308) and 433–451 (Ab 433) of the murine DTN protein [11]. Alignment of murine DTN and mDTN-B protein sequences shows that 6/21 aa of Ab 308 peptide and 19/19 aa of Ab 433 peptide are identical. Our immunoblot analysis indicates that MA0 recognizes a ladder of bands in skeletal muscle. In particular, a 94 kDa band was observed. A similarly sized band was also detected in the same mouse tissue using Ab 433 [11]. This band, which had not been observed with different dystrobrevin antibodies, including Ab 308, was believed not to correspond to a dystrobrevin isoform. Our data indicate that the 94 kDa band can be detected with an antibody (MA0) which, like Ab 433, recognizes both DTN and DTN-B. Moreover, the fact that Ab 308, which is specific for DTN, does not recognize this larger band indicates that the 94 kDa band corresponds either to a larger DTN-B isoform not yet identified or to a new dystrobrevin protein. Furthermore, our deletion analysis showed that MA0 has a different epitope when compared to Ab 433, suggesting that the 94 kDa band recognized by both antibodies is very unlikely to correspond to an aspecific product, but in our opinion corresponds to a dystrobrevin related protein. This dystrobrevin related protein could explain the larger murine isoform and the 4.4 kbp band observed in human muscles on Northern blot, for which we were unable to identify a corresponding cDNA.

In normal individuals, dystrophin and associated proteins have been shown to be located at the muscle membrane [21]. Several groups have also shown that in DMD patients, both dystrophin and DAP immunoreactivity of muscle sarcolemma was either absent or reduced [22]. In muscle of DMD carriers, both healthy and affected fibers (mosaicism) are present, and this situation is reflected by the dystrophin and DAP immunostaining [23].

Immunostaining analysis on muscle fibers from controls, DMD carriers and patients shows that MA0 recognized proteins have a sarcolemma localization and that the sarcolemmal immunostaining is absent in dystrophic muscle fibers. Since MA0 recognizes both the DTN and the DTN-B proteins, the absence of detectable signal in DMD muscle fibers suggests that DTN-B is a dystrophin associated protein, although these results should be reproduced by using a DTN-B specific antibody to be conclusive.

The DTN-B gene was mapped to human 2p23 and to the mouse chromosome 12 syntenic region, further supporting that they are true orthologs and not just homologous genes. No obvious candidate diseases that map to these regions could be recognized. This of course does not exclude the possible involvement of DTN-B in the pathogenesis of genetic disorders. Functional studies will elucidate the possible role of DTN-B in the physiology of muscle and in muscular dystrophies.

5. Note added in proof

During the review process of this paper, Peters et al. reported the isolation of the same transcript named β-dystrobrevin in human (Peters et al. (1997) J. Biol. Chem. 272, 31561–31569), while Blake et al. reported the isolation of the β-dystrobrevin mouse homolog (Blake et al. (1998) Proc. Natl. Acad. Sci. USA 95, 241–246).

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