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Differential expression of dystrophin, utrophin and dystrophin-associated proteins in peripheral nerve

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The dystrophin–glycoprotein complex is a novel laminin receptor in skeletal muscle. Dystrophin-associated proteins are comprised of an extracellular glycoprotein of 156 kDa (156DAG), transmembrane glycoproteins of 50 kDa (50DAG), 43 kDa (43DAG) and 35 kDa (35DAG), and a cytoskeletal protein of 59 kDa (59DAP). The laminin-binding 156DAG and 43DAG are encoded by a single gene and are now called α- and β-dystroglycan, respectively. In neuromuscular junctions, utrophin, an autosomal homologue of dystrophin, is associated with sarcolemmal proteins identical or immunologically homologous to the dystrophin-associated proteins. Here we demonstrate the co-localization of Dp116 (a 116 kDa protein product of the DMD gene), full-size utrophin, α- and β-dystroglycan, 59DAP and 35DAG in a thin rim surrounding the outermost layer of myelin sheath of peripheral nerve fibers. The α-dystroglycan in peripheral nerve had molecular weight of 120 kDa instead of 156 kDa, suggesting different levels of glycosylation between skeletal muscle and peripheral nerve. In sharp contrast to skeletal muscle, however, full-size dystrophin and 50DAG were undetectable in peripheral nerve. Our results demonstrate the varied expression of the components of the dystrophin/utrophin–glycoprotein complex between skeletal muscle and peripheral nerve suggesting the complex may exist in varied compositions and have varied functions in these two tissues.

Dystrophin; Dp116; Utrophin; Dystrophin-associated protein; Dystroglycan; Peripheral nerve

1. INTRODUCTION

Dystrophin is a large cytoskeletal protein encoded by the Duchenne muscular dystrophy (DMD) gene [1,2]. In skeletal muscle, dystrophin exists in a large oligomeric complex tightly associated with an extracellular glycoprotein of 156 kDa (156DAG), transmembrane glycoproteins of 35 kDa (35DAG), 43 kDa (43DAG) and 50 kDa (50DAG), and a cytoskeletal protein of 59 kDa (59DAP) [3–7]. The 156DAG and 43DAG are encoded by a single gene and these proteins are now called α- and β-dystroglycan, respectively [8,9]. The α-dystroglycan (156DAG) binds laminin, a major protein component of the extracellular matrix [8]. It is heavily glycosylated, two-thirds of its molecular mass being accounted for by sugar residues [8]. The glycoprotein-binding site exists in the cysteine-rich/C-terminal domains of dystrophin [10,11]. Dystrophin also interacts with F-actin through the actin-binding site(s) in the N-terminal domain [12–14]. These findings indicate that the dystrophin–glycoprotein complex spans the sarcolemma to link the subsarcolemmal actin–cytoskeleton with the extracellular matrix [7,8].

Utrophin (previously called dystrophin-related protein or DRP), an autosomal homologue of dystrophin, is localized exclusively to the neuromuscular junction in adult skeletal muscle [15,16]. Utrophin has cysteine-rich C-terminal domains which are highly homologous to those of dystrophin [15]. In the neuromuscular junction, utrophin exists in a protein complex homologous to the dystrophin–glycoprotein complex, suggesting that the utrophin–glycoprotein complex may have similar function(s) to the dystrophin–glycoprotein complex in this specialized region of sarcolemma [17].

Recently distal transcripts of the DMD gene have been identified and their protein products have been shown to be expressed differently from the full-size 400 kDa dystrophin [18–23]. A protein of 71 kDa called Dp71 (also called apo-dystrophin-1) is expressed in non-muscle tissues, such as brain, liver and gut [18–22]. A protein of 116 kDa called Dp116 has been reported to be expressed in Schwann cells of peripheral nerve [23]. While the physiological functions of these proteins remain unknown, the fact that both Dp71 and Dp116 share the cysteine-rich/C-terminal domains of dystrophin which are involved in the interaction with the glycoprotein complex, suggested a possibility that Dp71 and Dp116 could exist in a complex similar to the dystrophin–glycoprotein complex of skeletal muscle [18–23]. The elucidation of the physiological functions of these newly identified DMD gene products and their potential protein complexes could provide insight into the molecular mechanism of nervous system dysfunc-

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tion frequently observed in DMD patients. In the present study, we investigated the status of expression of dystrophin, utrophin and the dystrophin-associated proteins in peripheral nerve.

2. MATERIALS AND METHODS

2.1. *Antibodies*

The following monoclonal antibodies against distinct domains of dystrophin were used in this study: A1C, against amino acid residues 215–264 [24,25]; $VIA4_2$, against the cysteine-rich/C-terminal domains [6,7,11,26,27]; and DYS2 against the last 17 amino acids (Novocastra Laboratories). Two antibodies against the C-terminus of utrophin were used: affinity-purified rabbit antibody against the last 12 amino acids [16,17], and a monoclonal antibody, DRP1, against the last 11 amino acids (Novocastra Laboratories). Sheep antibodies against 156DAG, 59DAP, 50DAG, 43DAG and 35DAG were affinity-purified as described [26–28]. IIH6, a monoclonal antibody against 156DAG, and $IVD3_1$, a monoclonal antibody against 50DAG, were characterized previously [4,6–8,27,28].

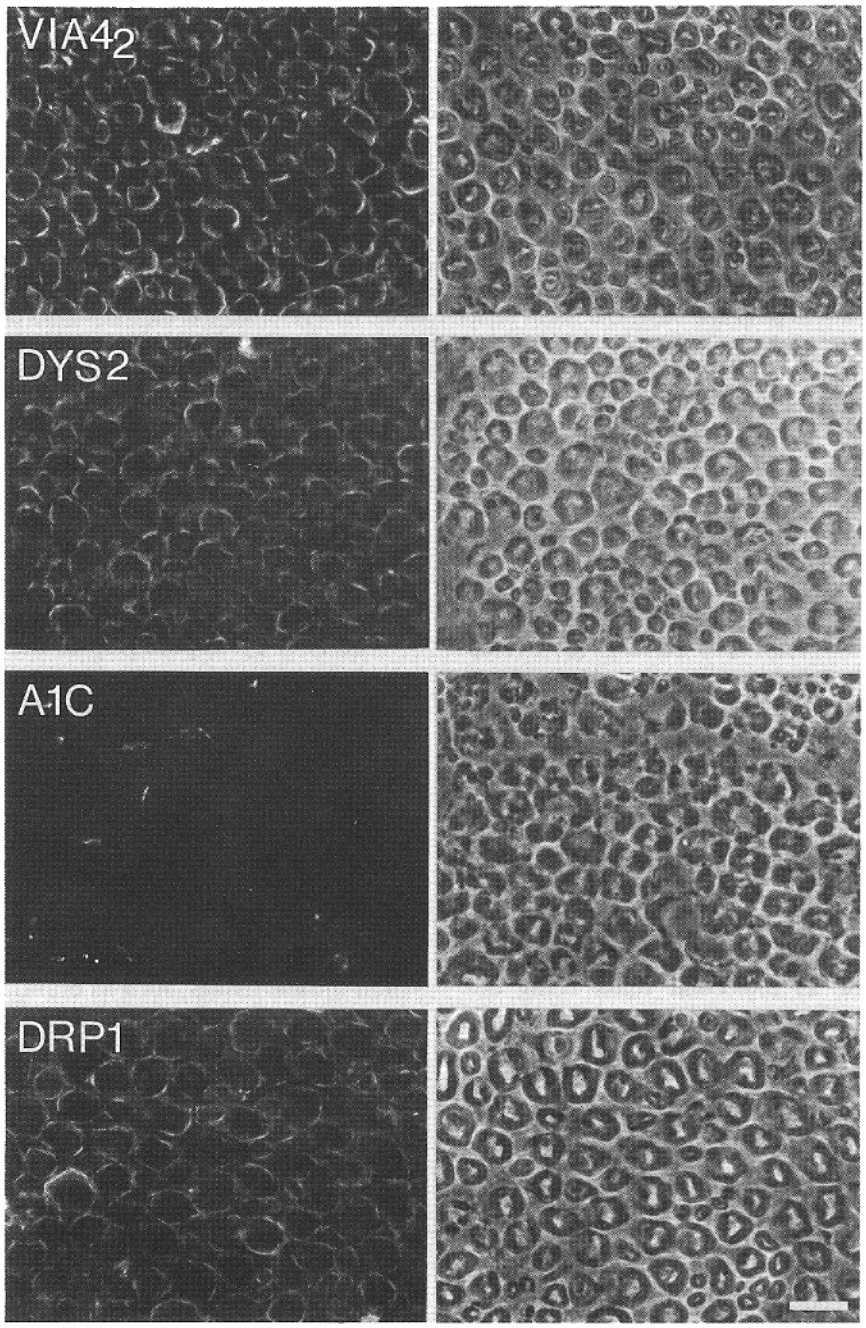

Fig. 1. Immunohistochemical analysis of the DMD gene products and utrophin in rabbit sciatic nerve. 7-μm thick transverse cryosections from rabbit sciatic nerve were immunostained with monoclonal antibodies against distinct domains of dystrophin ($VIA4_2$, DYS2 and A1C) and utrophin (DRP1). Phase-contrast images of the same visual field are shown on the right. $VIA4_2$, DYS2 and DRP1 stained a thin rim surrounding the outermost layer of myelin sheath of nerve fibers. A1C gave no signal. Bar = 20 μm.

2.2. *Immunohistochemistry*

After sacrificing a rabbit, skeletal muscle and sciatic nerve were excised immediately, snap-frozen in isopentane cooled in liquid nitrogen and stored at −80°C until use. Indirect immunofluorescence microscopy of 7 μm-thick cryosections from rabbit skeletal muscle and sciatic nerve was performed as described previously [26–28].

2.3. *3–12% SDS-PAGE and immunoblot analysis*

Cryosections from rabbit skeletal muscle and sciatic nerve were extracted in 50 vols. of SDS-buffer as described previously [17,28]. 3–12% SDS-PAGE and immunoblotting were performed as described [3,4]. 25 and 125 μl of SDS-extracts were applied for Coomassie blue staining of the gel and immunoblotting, respectively.

3. RESULTS AND DISCUSSION

Immunohistochemistry demonstrated intense staining of rabbit sciatic nerve by antibodies against the C-terminal domains of dystrophin, $VIA4_2$ and DYS2 (Fig. 1). Staining was positive in a thin rim surrounding the outermost layer of myelin sheath of nerve fibers, as revealed by phase contrast images of the same visual field (Fig. 1). Antibody against the N-terminal domain of dystrophin, A1C, gave no signal (Fig. 1). Antibody against the C-terminus of utrophin, DRP1, also stained a thin rim surrounding the outermost layer of myelin sheath of nerve fibers (Fig. 1). The same staining pattern was obtained with antibodies against 156DAG, 59DAP, 43DAG and 35DAG (Fig. 2). In sharp contrast, antibody against 50DAG did not stain peripheral nerve (Fig. 2).

On immunoblot analysis of SDS extracts of rabbit sciatic nerve, antibodies against the C-terminal domains of dystrophin, $VIA4_2$ and DYS2, stained a band with a molecular weight of 110–120 kDa, but full-size 400 kDa dystrophin was not detected (Fig. 3). This 110–120 kDa band was not stained by antibody against the N-terminal domain of dystrophin, A1C (Fig. 3). These findings, together with the immunohistochemical data described above, indicated that this 110–120 kDa band was Dp116, a recently-identified protein product of the 3′ region of the DMD gene [23]. Affinity-purified antibody against the C-terminus of utrophin, on the other hand, stained full-size 400 kDa utrophin in peripheral nerve (Fig. 3). Multiple bands with lower molecular weight were also stained weakly by this antibody (Fig. 3). These bands could be proteolytic fragments of utrophin or alternative protein products encoded by the 3′ region of the utrophin gene [19].

Antibody against 156DAG stained a band of 120 kDa in sciatic nerve, while it stained a band of 156 kDa in skeletal muscle (Fig. 4). Antibody against 43DAG stained a band of 43 kDa in both skeletal muscle and sciatic nerve (Fig. 4). However, antibody against 50DAG did not stain a band in sciatic nerve while it stained a band of 50 kDa in skeletal muscle (Fig. 4). Antibodies against 59DAP and 35DAG were not strong enough to produce conclusive results in this experiment (not shown).

Here we demonstrated the co-localization of Dp116, utrophin, dystroglycan, 59DAP and 35DAG in a thin rim surrounding the outermost layer of myelin sheath of peripheral nerve fibers. We also demonstrated that the α-dystroglycan (156DAG) had molecular weight of 120 kDa in peripheral nerve. Recently it has been shown that the α-dystroglycan has molecular weight of 120 kDa in brain [8,29], and that a laminin-binding brain protein of 120 kDa called LBP120 has an amino acid sequence identical to α-dystroglycan [30], raising the possibility that the α-dystroglycan might be glycosylated to different levels between skeletal muscle and brain. Our results suggest that the α-dystroglycan in peripheral nerve might have a similar glycosylation pattern as the brain form. In any case, the demonstration of laminin-binding dystroglycan in a restricted region of peripheral nerve could stimulate research to elucidate its function in the development and maintenance of the structural organization of peripheral nerve.

In sharp contrast to skeletal muscle, full-size dystrophin and 50DAG were undetectable in peripheral nerve by both immunohistochemical and immunoblot analyses. Although the failure to detect 50DAG in peripheral

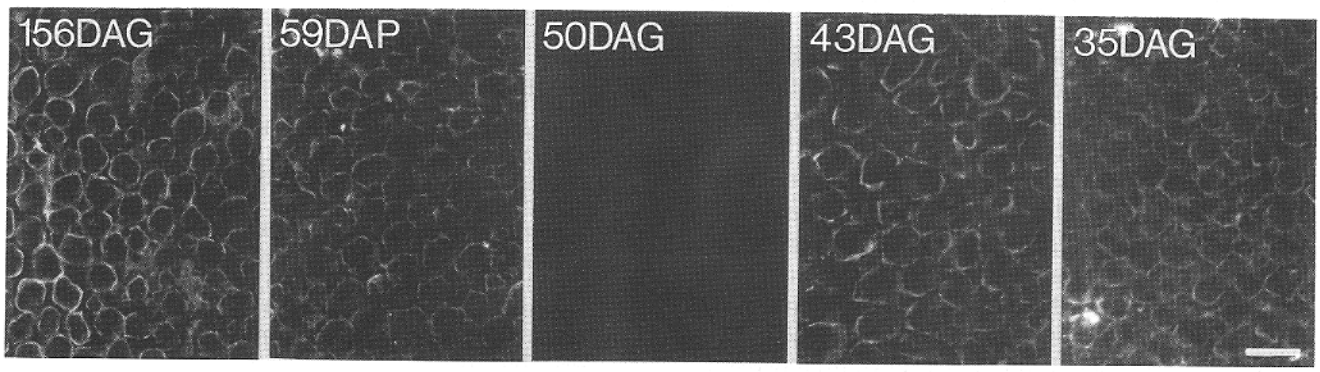

Fig. 2. Immunohistochemical analysis of the dystrophin-associated proteins in rabbit sciatic nerve. 7-μm thick transverse cryosections from rabbit sciatic nerve were immunostained with a monoclonal antibody against 50DAG, $IVD3_1$, and affinity-purified antibodies against 156DAG, 59DAP, 43DAG and 35DAG. Antibodies against 156DAG, 59DAP, 43DAG and 35DAG stained a thin rim surrounding the outermost layer of myelin sheath of nerve fibers. Antibody against 50DAG gave no signal. Bar = 20 μm.

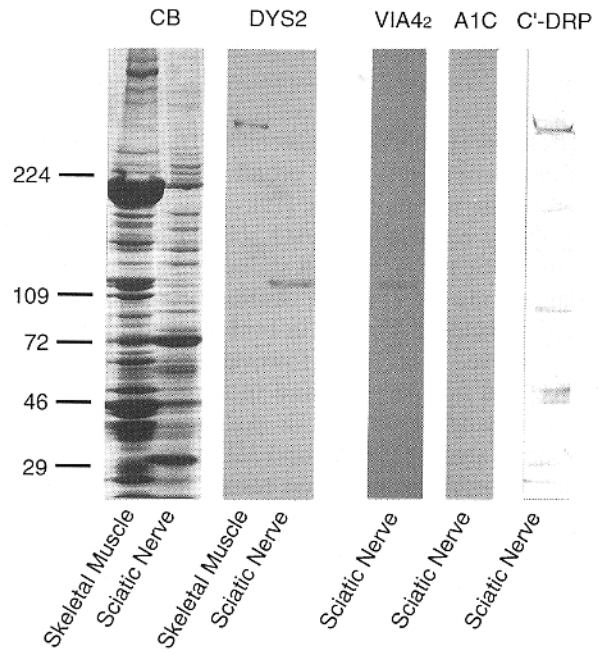

Fig. 3. Immunoblot analysis of the DMD gene products and utrophin in rabbit sciatic nerve. SDS extracts of rabbit skeletal muscle and sciatic nerve were separated by 3–12% SDS-PAGE and immunoblotted with monoclonal antibodies against distinct domains of dystrophin (DYS2, VIA4$_2$ and A1C) and affinity-purified antibody against the C-terminus of utrophin (C′-DRP). CB shows the gel stained with Coomassie blue. DYS2 detected full-size 400 kDa dystrophin in skeletal muscle and a band of 110–120 kDa in sciatic nerve. VIA4$_2$ also detected a band of 110–120 kDa in sciatic nerve, while A1C did not stain this 110–120 kDa band. C′-DRP detected full-size 400 kDa utrophin in sciatic nerve, together with less intensely stained bands of lower molecular weight. Molecular weight standards (Da $\times 10^{-3}$) are shown on the left.

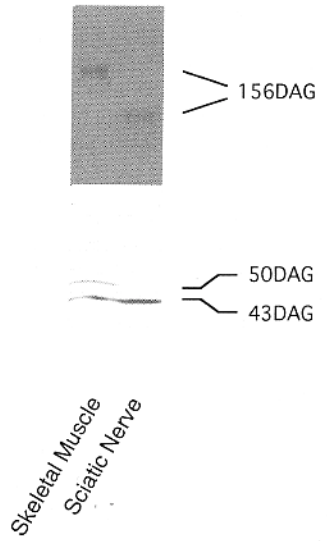

Fig. 4. Immunoblot analysis of the dystrophin-associated proteins in rabbit sciatic nerve. SDS-exrtracts of rabbit skeletal muscle and sciatic nerve were separated by 3–12% SDS-PAGE and immunoblotted with IIH6, a monoclonal antibody against 156DAG (upper panel), and a cocktail of affinity-purified antibodies against 50DAG and 43DAG (lower panel). IIH6 stained a band of 120 kDa in sciatic nerve, while it stained a band of 156 kDa in skeletal muscle. Antibody against 43DAG stained a band of 43 kDa in both skeletal muscle and sciatic nerve. Antibody against 50DAG did not stain a band in sciatic nerve, while it stained a band of 50 kDa in skeletal muscle. Antibodies against 59DAP and 35DAG were not strong enough to produce conclusive results in this experiment.

nerve in this study could be explained by different levels of glycosylation of this protein between skeletal muscle and peripheral nerve, our findings raise an intriguing possiblity that the putative complex, which could involve Dp116, utrophin and the dystrophin-associated proteins, may lack 50DAG in peripheral nerve. The clarification of this putative complex and identification of its components await further biochemical investigation.

Recently we have demonstrated the specific deficiency of 50DAG in sarcolemma of patients with severe childhood autosomal recessive muscular dystrophy with DMD-like phenotype (SCARMD) [28], and the deficiency in all the dystrophin-associated proteins, including 50DAG, in sarcolemma of DMD patients [27]. These findings suggested that the deficiency of the 50DAG may be the common denominator leading to muscle cell necrosis in these two diseases. Interestingly, SCARMD patients never present with the nervous system dysfunction which is found in a substantial percentage of DMD patients. Taking our results into consideration, one hypothesis to explain this phenotypic difference could be that 50DAG is not expressed in nervous system and thus, its specific deficiency causes only muscular dysfunction in SCARMD. It would be of utmost importance to clarify whether 50DAG is expressed in brain.

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REFERENCES

[1] Hoffman, E.P., Brown, R.H. and Kunkel, L.M. (1987) Cell 51, 919–928.
[2] Koenig, M., Monaco, A.P. and Kunkel, L.M. (1988) Cell 53, 219–228.
[3] Campbell, K.P. and Kahl, S.D. (1989) Nature 338, 259–262.
[4] Ervasti, J.M., Ohlendieck, K., Kahl, S.D., Gaver, M.G. and Campbell, K.P. (1990) Nature, 345, 315–319.
[5] Yoshida, M. and Ozawa, E. (1990) J. Biochem. 108, 748–752.
[6] Ervasti, J.M., Kahl, S.D. and Campbell, K.P. (1991) J. Biol. Cem. 266, 9161–9165.
[7] Ervasti, J.M. and Campbell, K.P. (1991) Cell 66, 1121–1131.
[8] Ibraghimov-Beskrovnaya, O., Ervasti, J.M., Leveille, C.J., Slaughter, C.A., Sernett, S.W. and Campbell, K.P. (1992) Nature 355, 696–702.

[9] Ibraghimov-Beskrovnaya, O., Milatovich, A., Ozcelik, T., Yang, B., Francke, U. and Campbell, K.P. (1993) Hum. Mol. Genet. (in press).
[10] Suzuki, A., Yoshida, M., Yamamoto, H. and Ozawa, E. (1992) FEBS Lett. 308, 154–160.
[11] Matsumura, K., Tome, F.M.S., Ionasescu, V.V., Ervasti, J.M., Anderson, R.D., Romero, N.B., Simon, D., Kaplan J.C., Fardeau, M. and Campbell, K.P. (1993) J. Clin. Invest. 92, 866–871.
[12] Levine, B.A., Moir, A.J.D., Patchell, V.B. and Perry, S.V. (1992) FEBS Lett. 298, 44–48.
[13] Way, M., Pope, B., Cross, R.A., Kendrick-Jones, J. and Weeds, A.G. (1992) FEBS Lett. 301, 243–245.
[14] Hemmings, L., Kuhlmann, P.A. and Critchley, D.R. (1992) J. Cell Biol. 116, 1369–1380.
[15] Tinsley, J.M., Blake, D.J., Roche, A., Fairbrother, U., Riss, J., Byth, B.C., Knight, A.E., Kendrick-Jones, J., Suther, G.K., Love, D.R., Edwards, Y.H. and Davies, K.E. (1992) Nature 360, 591–593.
[16] Ohlendieck, K., Ervasti, J.M., Matsumura, K., Kahl, S.D., Leveille, C.J. and Campbell, K.P. (1991) Neuron 7, 499–508.
[17] Matsumura, K., Ervasti, J.M., Ohlendieck, K., Kahl, S.D. and Campbell, K.P. (1992) Nature 360, 588–591.
[18] Bar, S., Barnea, E., Levy, Z., Neuman, S., Yaffe, D. and Nudel, U. (1990) Biochem. J. 272, 557–560.
[19] Blake, D.J., Love, D.R., Tinsley, J., Morris, G.E., Turley, H., Gatter, K., Dickson, G., Edwards, Y.H. and Davies, K.E. (1992) Hum. Mol. Genet. 1, 103–109.
[20] Lederfein, D., Levy, Z., Augier, N., Mornet, D., Morris, G., Fuchs, O., Yaffe, D. and Nudel, U. (1992) Proc. Natl. Acad. Sci. USA 89, 5346–5350.
[21] Hugnot, J.P., Gilgenkrantz, H., Vincent, N., Chafey, P., Morris, G.E., Monaco, A.P., Brewald-Netter, Y., Koulakoff, A., Kaplan, J.C., Kahn, A. and Chelly, J. (1992) Proc. Natl. Acad. Sci. USA 89, 7506–7510.
[22] Tinsley, J.M., Blake, D.J. and Davies, K.E. (1993) Hum. Mol. Genet. 2, 521–524.
[23] Byers, T.J., Lidov, H.G.W. and Kunkel, L.M. (1993) Nature Genet. 4, 77–81.
[24] Shimizu, T., Matsumura, K., Hashimoto, K., Mannen, T., Ishiguro, T., Eguchi, C., Nonaka, I., Yoshida, M. and Ozawa, E. (1988) Proc. Jpn. Acad. B 64, 205–208.
[25] Shimizu, T., Matsumura, K., Sunada, Y. and Mannen, T. (1989) Biomed. Res. 10, 405–409.
[26] Ohlendieck, K. and Campbell, K.P. (1991) J. Cell Biol. 115, 1685–1694.
[27] Ohlendieck, K., Matsumura, K., Ionasescu, V.V., Towbin, J.A., Bosch, E.P., Weinstein, S.L., Sernett, S.W. and Campbell, K.P. (1993) Neurology 43, 795–800.
[28] Matsumura, K., Tome, F.M.S., Collin, H., Azibi, K., Chaouch, M., Kaplan, J.C., Fardeau, M. and Campbell, K.P. (1992) Nature 359, 320–322.
[29] Ervasti, J.M. and Campbell, K.P. (1993) J. Cell Biol. 122, 809–823.
[30] Gee, S.H., Blacher, R.W., Douville, P.J., Provost, P.R., Yurchenco, P.D. and Carbonetto, S. (1993) J. Biol. Chem. 268, 14972–14980.