Acta Neuropathol (2001) 101:174–178
DOI 10.1007/s004010000276

SHORT ORIGINAL COMMUNICATION

**T. Masaki · K. Matsumura · A. Hirata · H. Yamada
A. Hase · T. Shimizu · H. Yorifuji · K. Motoyoshi
K. Kamakura**

Expression of dystroglycan complex in satellite cells of dorsal root ganglia

Received: 15 March 2000 / Revised, accepted: 28 June 2000 / Published online: 21 December 2000

Abstract In Schwann cells, the transmembrane glycoprotein β-dystroglycan composes the dystroglycan complex together with the extracellular glycoprotein α-dystroglycan, which binds laminin-2 (α2/β1/γ1), a major component of the Schwann cell basal lamina. In the Schwann cell cytoplasm, β-dystroglycan is anchored to a dystrophin isoform, Dp116. In this study, we investigated the expression of β-dystroglycan, Dp116 and the laminin-α2 chain in satellite cells of rat dorsal root ganglia (DRGs). Immunohistochemical study showed that immunoreactivities for β-dystroglycan and Dp116 were both localized to the outer rim of neuron-satellite cell and axon-Schwann cell units, indicating that both satellite and Schwann cells expressed these proteins in DRGs. Immunoreactivity for the laminin-α2 chain was detected in a similar location, indicating that the basal lamina surrounding satellite and Schwann cells in DRGs contained laminin-2. Ultrastructurally, immunoreactivity for the cytoplasmic domain of β-dystroglycan as well as that for Dp116 was most intense in the cytoplasm just underlying the outer membrane of satellite cells. The immunoreactivity for laminin was associated with the outer surface of those cells, suggesting that it was localized in the surrounding basal lamina. These results indicate that the dystroglycan complex is expressed in the satellite cell outer membrane and involved in the adhesion with the basal lamina through the interaction with laminin-2.

T. Masaki · A. Hirata · K. Motoyoshi · K. Kamakura (✉)
Third Department of Internal Medicine,
National Defense Medical College,
3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan
Tel.: +81-42-9951617, Fax: +81-42-9965202

K. Matsumura · H. Yamada · A. Hase · T. Shimizu
Department of Neurology and Neuroscience,
Teikyo University School of Medicine,
2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan

H. Yorifuji
Department of Anatomy, National Defense Medical College,
3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan

Keywords Dystroglycan · Dp116 · Laminin · Spinal dorsal root ganglia · Satellite cell

Introduction

Dystroglycan (DG) is encoded by a single gene and cleaved into two proteins α- and β-DG by post-translational processing [2, 7]. α-DG is an extracellular glycoprotein anchored to the cell membrane by a transmembrane glycoprotein β-DG, and the complex comprised of α- and β-DG is called the DG complex [1, 2, 3]. In peripheral nerve, the DG complex is expressed in Schwann cells [11, 26]. Biochemical analysis shows that α-DG derived from bovine peripheral nerve binds laminin-2 [26, 27, 28], a major component of the Schwann cell basal lamina [9, 16, 21]. On the cytoplasmic side, β-DG is anchored to a dystrophin isoform, Dp116 [20]. Recently, we have shown that the immunoreactivity for the cytoplasmic domain of β-DG (β-DG-IR) is localized in the outermost cytoplasm of myelin-forming Schwann cells as well as in that of non-myelin-forming ones [10]. These data suggest that the DG complex is an adhesion apparatus binding the Schwann cell outer membrane with the basal lamina. Indeed, the DG complex has been shown to be involved in the schwannoma cell adhesion to laminin [12].

Satellite cells of dorsal root ganglia (DRGs) are homologous to Schwann cells [23], because both of them arise from neural crest, differentiate to peripheral glia and share many common proteins, such as S-100 protein, glial fibrillary acidic protein (GFAP), tubulin, microtubule-associated protein 1 (MAP-1), Schwann cell myelin protein, neurotrophins and their receptors, GDNF, fibroblast growth factor-2, transforming growth factor (TGF)-β, EGF receptor, NaG and Krox-20 [4, 5, 6, 8, 13, 14, 17, 22, 30, 31, 32]. Satellite cells almost completely envelope each of the neuronal perikarya, and they have the basal lamina on their outer membrane surface. We considered therefore that, similar to Schwann cells, DRG satellite cells might express the DG complex as a laminin receptor. In this respect, it is interesting to note that Rambukkana et

al. [18] recently reported that DG is a Schwann cell receptor for *Mycobacterium leprae*, which causes significant damage to peripheral nerve. Thus, clarifying the precise localization of the DG complex in the peripheral nervous system may provide important clues for understanding the molecular pathogenesis of leprosy. In this study, we demonstrate that DRG satellite cells express β-DG and Dp116 in the cytoplasm just underlying the outer membrane. We also indicate that the basal lamina surrounding DRG satellite cells contains laminin-2. These results indicate that the DG complex is involved in the adhesion of the satellite cell outer membrane with the basal lamina through the interaction with laminin-2.

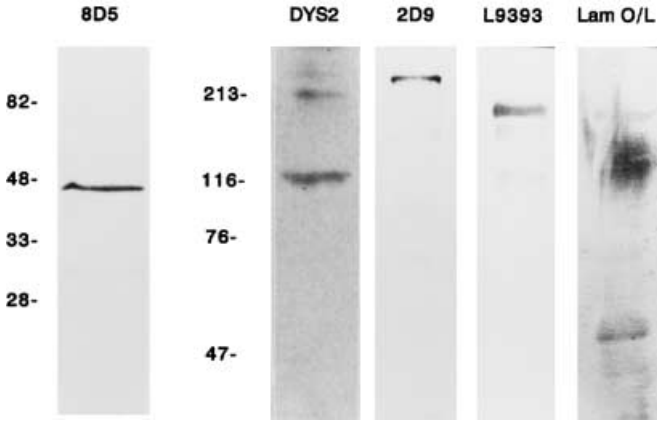


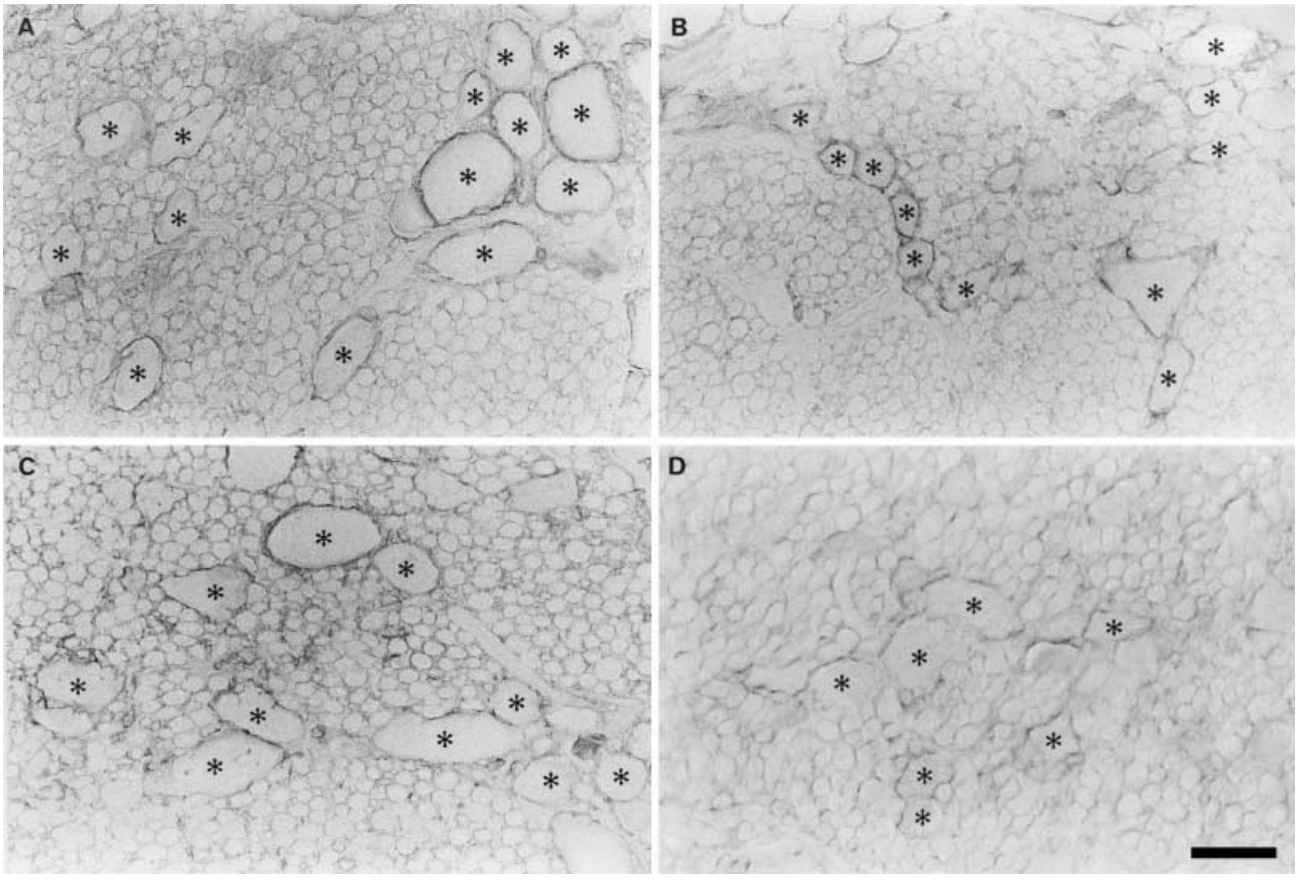
Fig. 1 Monoclonal antibody 8D5 recognizes a 43-kDa band corresponding to β-DG. Monoclonal antibody DYS2 mainly recognizes a 116-kDa band corresponding to Dp116. Monoclonal antibody 2D9 recognizes a 320-kDa band corresponding to laminin α2 chain. Polyclonal antibody L9393 recognizes a 200-kDa band corresponding to laminin β1 and/or γ1 chains. Lam O/L analysis reveals a broad band around 120 kDa corresponding to α-DG. Molecular weight standards (Da $\times 10^{-3}$) are shown on the left (*DG* dystroglycan, *Lam O/L* Laminin blot overlay)

Materials and methods

Tissue preparation

Fifteen male adult rats (Wistar, 12–13 weeks old) were used in this study. The experimental procedures followed the welfare rules and regulations for treatment of animals of National Defense Medical College. The animals were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight) and were transcardially perfused with 50 ml phosphate-buffered saline (PBS, 10 mM sodium phosphate buffer, pH 7.4, containing 0.15 M NaCl) followed by 400 ml of 100 mM sodium phosphate buffer containing 4% paraformaldehyde. After perfusion, L5 DRGs were dissected out, and were immersed in the 4% paraformaldehyde at 4°C overnight. Samples were then infiltrated with 30% sucrose in

Fig. 2 Light photomicrographs showing the IRs for β-DG (**A**), Dp116 (**B**), laminin (**C**) and laminin α2 chain (**D**) in rat DRGs. The IRs for these four proteins show a similar localization pattern; they are selectively localized on the outer rim of each axon-Schwann cell unit and neuron-satellite cell unit (*asterisks*) (*IR* immunoreactivity, *DRG* dorsal root ganglion). *Bar* 30 μm



PBS overnight, mounted in OCT compound (Miles, Elkhart, Ind.), and quickly frozen in isopentane cooled in liquid nitrogen. The frozen samples were stored at –80°C. DRGs were also dissected out immediately after sacrifice without transcardial perfusion, mounted in the OCT compound and quickly frozen for immunohistochemistry, or homogenized for immunoblotting, as described previously [10].

Immunohistochemical, immunoelectron microscopic, immunoblot and laminin blot overlay analyses

The frozen samples were cut into 10-μm-thick sections on a cryostat. The frozen sections were placed on gelatin-coated slides. These were immunostained according to the methods of Vectastain Elite ABC kit (Vector Laboratories, Burlingame, Calif.). In brief, after three washes in PBS, sections were treated with PBS containing 5% bovine serum albumin and 0.05% Triton X-100 (referred to as buffer A in this study) for 1 h. Sections were then incubated for 2 h with one of the following antibodies: 8D5 (1:30), DYS2 (1:200), 2D9 (1:5) or L9393 (1:2,000) in buffer A. After washing three times in buffer A, the sections were incubated for 1 h with biotin-conjugated anti-mouse immunoglobulin (1:200, PharMingen, Calif.) or biotin-conjugated anti-rabbit IgG (1:200, Vector Laboratories) in buffer A. After three further washes in PBS, the sections were incubated for 30 min with the Vectastain Elite ABC reagent, washed again three times in PBS, and incubated for 3 min in DAB solution (50 mM TRIS buffer pH 7.4, containing 0.05% 3,3'-diaminobenzidine tetrahydrochloride) supplemented with 0.01% H_2O_2. Immunoelectron microscopy, immunoblot and laminin blot overlay (Lam O/L) analyses were performed as described previously [10, 26]. For immunoblotting, 10% or 7.5% SDS-PAGE was performed; 10 μl of homogenized samples were applied in each lane.

Miscellaneous

Mouse monoclonal antibodies 8D5 against the C terminus of β-DG, DYS2 against the C terminus of dystrophin and 2D9 against the proximal portion of the globular domain of laminin-α2 chain have been described previously [26, 27, 28]. The β-DG is a type I transmembrane protein with the N and C termini located extra- and intracellularly, respectively; thus, the 8D5 epitope is located in the cytoplasmic domain of β-DG. L9393, a rabbit polyclonal antibody against laminin, was purchased from Sigma (St. Louis, Mo.).

Results and discussion

To determine the expression of the components of the DG complex and laminin, we performed the immunoblot and Lam O/L analyses of rat DRGs (Fig. 1).

Monoclonal antibody 8D5 recognized a 43-kDa band corresponding to β-DG. Monoclonal antibody DYS2 mainly recognized a 116-kDa band corresponding to Dp116. Lam O/L analysis revealed a broad band around 120 kDa corresponding to α-DG. These results closely resembled those reported previously in bovine peripheral nerve [20, 26, 27, 28], and indicate that the DG complex, comprised of α-DG, β-DG and Dp116, is expressed in rat DRGs. On the other hand, monoclonal antibody 2D9 recognized a 320-kDa band corresponding to laminin α2 chain. Polyclonal antibody L9393 recognized a 200-kDa band corresponding to laminin β1 and/or γ1 chains. These results suggest that the laminin-2 heterotrimer (α2/β1/γ1) is expressed in rat DRGs.

Immunohistochemical analysis of rat DRGs was performed using antibodies, 8D5, DYS2, 2D9 and L9393. The IRs for β-DG, Dp116, laminin α2 chain and laminin were selectively localized on the outer rim of each DRG neuron-satellite cell unit and axon-Schwann cell unit (Fig. 2).

Immunoelectron microscopic analysis was performed using antibodies 8D5, DYS2 and L9393. 2D9 did not work in the immunoelectron microscopic analysis. β-DG-IR was most intense in the cytoplasm just underly-

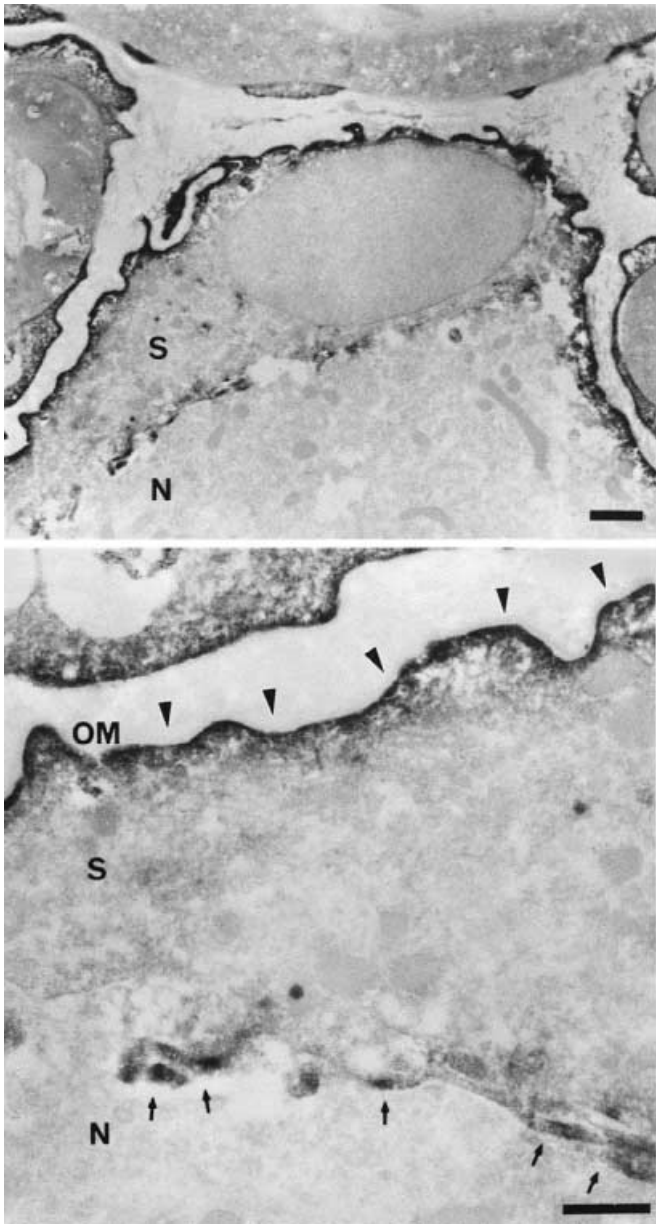


Fig. 3 Immunoelectron photomicrographs showing the β-DG-IR in rat DRGs. *Upper panel* β-DG-IR seems to be associated with the outer membrane of satellite cells. *Lower panel* The photomicrograph with higher magnification shows that β-DG-IR is most intense in the cytoplasm just underlying the satellite cell outer membrane (*arrowheads*). The cytoplasm just underlying the satellite cell inner membrane apposing the neuronal perikaryon lacks or shows only faint β-DG-IR (*arrows*) (*S* satellite cell, *N* neuronal perikaryon, *OM* satellite cell outer membrane). *Bar upper panel* 1 μm, *lower panel* 500 nm

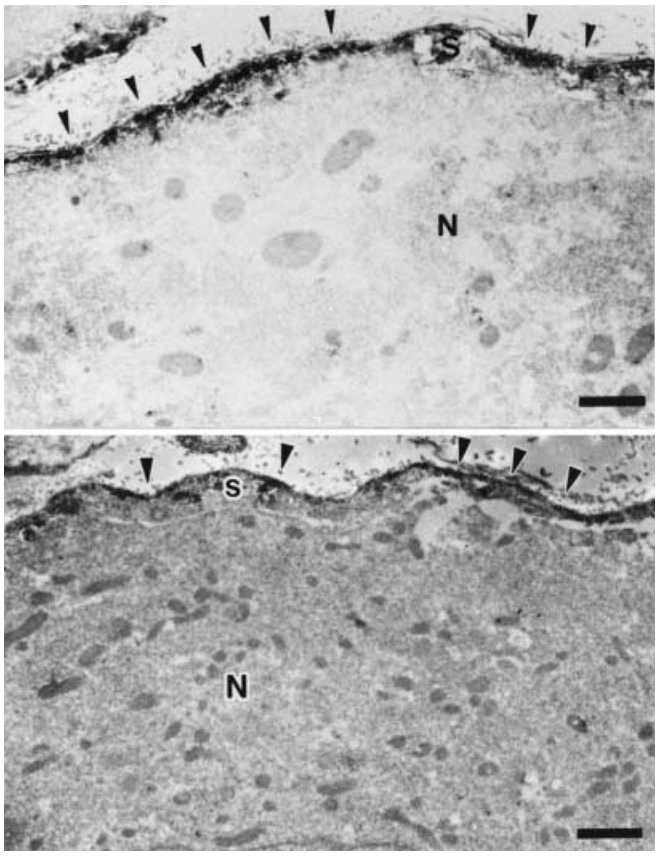


Fig. 4 Immunoelectron photomicrographs showing the IRs for Dp116 and laminin in rat DRGs. *Upper panel* The satellite cell cytoplasm just underlying the outer membrane shows intense Dp116-IR (*arrowheads*). *Lower panel* Laminin-IR is associated with the outer surface of the satellite cell (*arrowheads*) (*S* satellite cell, *N* neuronal perikaryon). *Bars* 1 μm

ing the outer membrane of satellite cells (Fig. 3). In contrast, β-DG-IR was lacking or only faintly detected in the cytoplasm underlying the inner membrane apposing the neuronal perikaryon (Fig. 3).

Dp116-IR was also most intense in the cytoplasm just underlying the outer membrane of satellite cells. Although the basal lamina was less intensely labeled, it seemed to be due to diffusion of reaction product from the adjacent satellite cell cytoplasm (Fig. 4, upper panel). Laminin-IR was associated with the outer surface of satellite cells, suggesting that it was localized in the surrounding basal lamina. (Fig. 4, lower panel).

Here, we have shown, for the first time, that the components of the DG complex, β-DG and Dp116, are expressed in the outer membrane of DRG satellite cells and that laminin-2 is expressed in the basal lamina surrounding these cells. These results indicate that, in DRGs, the DG complex functions as an adhesion apparatus binding the outer membrane of satellite cells with the basal lamina through the interaction with laminin-2. We presume that the DG complex-laminin-2 interaction plays a role in the maintenance or modulation of the morphology and function of the DRG neuron-satellite cell units by anchoring the satellite cell outer membrane to the basal lamina. In this respect, it is noteworthy that Pannese et al. [15] have recently reported that DRG satellite cells permit dynamic perikaryal projections, whereas the basal lamina alone does not. Together with the reports that the DG complex may convey signals from the basal lamina [7, 29], it would be intriguing to envision that the DG complex-laminin-2 interaction may support the dynamic neuron-satellite cell interaction by providing structural anchorage to the basal lamina. On the other hand, in DRGs, satellite cells are known to interact with neurons or with each other through paracrine actions of neurotrophic factors/their receptors, such as NGF/p75, NGF/trk A, BDNF/trk B and TGF-α/EGF receptor [17, 19, 24, 25]. Whereas it is possible that the DG complex, present on the satellite cell outer membrane, may be associated with those cell-cell interactions, further studies will be needed to test the hypothesis.

Acknowledgements Monoclonal antibodies 8D5 and 2D9 were kindly provided by Drs. Louise V.B. Anderson (Newcastle General Hospital) and Hisae Hori (Tokyo Medical and Dental University), respectively. This work was supported by Research Grants (10B-3 and 11B-1) for Nervous and Mental Disorders and Research on Brain Science from Health Science Research Grants from the Ministry of Health and Welfare and Research Grants (09470156, 09770460, 09877121, 10044319, 11470151 and 11670644) from the Ministry of Education, Science, Sports and Culture.

References

1. Deyst KA, Bowe MA, Leszyk JD, Fallon JR (1995) The α-dystroglycan-β-dystroglycan complex. J Biol Chem 270: 25956–25959
2. Ervasti JM, Campbell KP (1991) Membrane organization of the dystrophin-glycoprotein complex. Cell 66: 1121–1131
3. Ervasti JM, Campbell KP (1993) A role for the dystrophin-glycoprotein complex as a transmembrane linker between laminin and agrin. J Cell Biol 122: 809–823
4. Felts PA, Black JA, Dib-Hajj SD, Waxman SG (1997) NaG: a sodium channel-like mRNA shared by Schwann cells and other neural crest derivatives. Glia 21: 269–276
5. Grothe C, Meisinger C, Hertenstein A, Kurz H, Wewetzer K (1997) Expression of fibroblast growth factor-2 and fibroblast growth factor receptor 1 messenger RNAs in spinal ganglia and sciatic nerve: regulation after peripheral nerve lesion. Neuroscience 76:123–135
6. Hammarberg H, Piehl F, Cullheim S, Fjell J, Hokfelt T, Fried K (1996) GDNF mRNA in Schwann cells and DRG satellite cells after chronic sciatic nerve injury. Neuroreport 7: 857–860
7. Ibraghimov-Beskrovnaya O, Ervasti JM, Leveille CJ, Slaughter CA, Sernett SW, Campbell KP (1992) Primary structure of dystrophin-associated glycoproteins linking dystrophin to the extracellular matrix. Nature 355: 696–702
8. Jessen KR, Thorpe R, Mirsky R (1984) Molecular identity, distribution and heterogeneity of glial fibrillary acidic protein: an immunoblotting and immunohistochemical study of Schwann cells, satellite cells, enteric glia and astrocytes. J Neurocyt 13: 187–200
9. Leivo I, Engvall E (1988) Merosin, a specific for basement membranes of Schwann cells, striated muscle, and trophoblast, is expressed late in nerve and muscle development. Proc Natl Acad Sci USA 85: 1544–1548
10. Masaki T, Matsumura K, Saito F, Sunada Y, Shimizu T, Yorifuji H, Motoyoshi K, Kamakura K (2000) Expression of β-dystroglycan and laminin-2 in peripheral nerve under axonal degeneration and regeneration. Acta Neuropathol 99: 289–295

11. Matsumura K, Yamada H, Shimizu T, Campbell KP (1993) Differential expression of dystrophin, utrophin and dystrophin-associated proteins in peripheral nerve. FEBS Lett 334: 281–285
12. Matsumura K, Chiba A, Yamada H, Fukuta-Ohi H, Fujita S, Endo T, Kobata A, Anderson LVB, Kanazawa I, Campbell KP, Shimizu T (1997) A role of dystroglycan in schwannoma cell adhesion to laminin. J Biol Chem 272: 13904–13910
13. Murphy P, Topilko P, Schneider-Maunoury S, Seitanidou T, Evercooren AB-V, Charnay P (1996) The regulation of Krox-20 expression reveals important steps in the control of peripheral glial cell development. Development 122: 2847–2857
14. Naves FJ, Huerta JJ, Garcia-Suares O, Urdangaray N, Esteban I, Del Valle ME, Vega JA (1996) Distribution of immunoreactivity for cytoskeletal (microtubule, microtubule-associated, and neurofilament) proteins in adult human dorsal root ganglia. Anat Rec 244:246–256
15. Pannese E, Procacci P, Berti E, Ledda M (1999) The perikaryal surface of spinal ganglion neurons: differences between domains in contact with satellite cells and in contact with the extracellular matrix. Anat Embryol (Berl) 199: 199–206
16. Patton BL, Miner JH, Chiu AY, Sanes JR (1997) Distribution and function of laminins in the neuromuscular system of developing, adult, and mutant mice. J Cell Biol 139: 1507–1521
17. Pruginin-Bluger M, Shelton DL, Kalcheim C (1997) A paracrine effect for neuron-derived BDNF in development of dorsal root ganglia: stimulation of Schwann cell myelin protein expression by glial cells. Mech Dev 61: 99–111
18. Rambukkana A, Yamada H, Zanazzi G, Mathua T, Salzer JL, Yurchenco PD, Campbell KP, Fischetti VA (1998) Role of α-dystroglycan as a Schwann cell receptor for *Mycobacterium leprae*. Science 282: 2076–2079
19. Ramer MS, Thompson SWN, McMahon SB (1999) Causes and consequences of sympathetic basket formation in dorsal root ganglia. Pain [Suppl] 6: S111-S120
20. Saito F, Masaki T, Kamakura K, Anderson LVB, Fujita S, Fukuta Ohi H, Sunada Y, Shimizu T, Matsumura K (1999) Characterization of the transmembrane molecular architecture of the dystroglycan complex in Schwann cells. J Biol Chem 274: 8240–8246
21. Sanes JR, Engvall E, Butkowski R, Hunter DD (1990) Molecular heterogeneity of basal lamina: isoforms of laminin and collagen IV at the neuromuscular junction and elsewhere. J Cell Biol 111: 1685–1699
22. Stewart HJS, Rougon G, Dong Z, Dean C, Jessen K, Mirsky R (1995) TGF-β upregulate NCAM and L1 expression in cultured Schwann cells suppress cyclic AMP-induced expression of O4 and galactocerebroside, and are widely expressed in cells of the Schwann cell lineage in vivo. Glia 15: 419–436
23. Thomas PK, Scaravilli F, Belai A (1994) Pathologic alterations in cel bodies of peripheral neurons in neuropathy. In: Dyck PT, Thomas PK, Griffin JW, Low PA, Poduslo JF (eds) Peripheral neuropathy, 3rd edn. Saunders, Philadelphia, pp 476–513
24. Wetmore C, Olson L (1995) Neuronal and nonneuronal expression of neurotrophins and their receptors in sensory and sympthetic ganglia suggest new intercellular trophic interactions. J Comp Neurol 353:143–159
25. Xian CJ, Zhou X-F (1999) Neuronal-glial differential expression of TGF-α and its receptor in the dorsal root ganglia in response to sciatic nerve lesion. Exp Neurol 157: 317–326
26. Yamada H, Shimizu T, Tanaka T, Campbell KP, Matsumura K (1994) Dystroglycan is a binding protein of laminin and merosin in peripheral nerve. FEBS Lett 352: 49–53
27. Yamada H, Chiba A, Endo T, Kobata A, Anderson LVB, Hori H, Fukuta-Ohi H, Kanazawa I, Campbell KP, Shimizu T, Matsumura K (1996) Characterization of dystroglycan-laminin interaction in peripheral nerve. J Neurochem 66: 1518–1524
28. Yamada H, Denzer AJ, Hori H, Tanaka T, Anderson LVB, Fujita S, Fukuta-Ohi H, Shimizu T, Ruegg MA, Matsumura K (1996) Dystroglycan is a dual receptor for agrin and laminin-2 in Schwann cell membrane. J Biol Chem 271: 23418–23423
29. Yang B, Jung D, Motto D, Meyer J, Koretzky G, Campbell KP (1995) SH3 domain-mediated interaction of dystroglycan and Grb2. J Biol Chem 270: 11711–11714
30. Yang Q, Hou X, Hamberger A, Wang S, Dahlstrom A, Haglid KG (1995) S-100β immunoreactivity in neurons of the rat peripheral sensory ganglia. Neuroreport 6: 2005–2009
31. Zhou X-F, Rush RA, McLachlan EM (1996) Differential expression of the p75 nerve growth factor receptor in glia and neurons of the rat dorsal root ganglia after peripheral nerve transection. J Neurosci 16: 2901–2911
32. Zhou X-F, Deng Y-S, Chie E, Xue Q, Zhong J-H, McLachlan EM, Rush RA, Xian CJ (1999) Satellite-cell-derived nerve growth factor and neurotrophin-3 are involved in noradrenergic sprouting in the dorsal root ganglia following peripheral nerve injury in the rat. Eur J Neurosci 11: 1711–1722