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*Invited Review*

# The role of dystroglycan, a novel receptor of laminin and agrin, in cell differentiation

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**Summary.** Dystroglycan was originally identified as the extracellular and transmembrane constituents of a large oligomeric complex of sarcolemmal proteins associated with dystrophin, the protein product of the Duchenne muscular dystrophy (DMD) gene. During the last few years, dystroglycan has been demonstrated to be a novel receptor of not only laminin but also agrin, two major proteins of the extracellular matrix having distinct biological effects. The fact that the drastic reduction of dystroglycan in the sarcolemma, caused by the absence of dystrophin, leads to muscle cell death in DMD patients and *mdx* mice indicates that, as a laminin receptor, dystroglycan contributes to sarcolemmal stabilization during contraction and stretch of striated muscle cells. Dystroglycan is also expressed in the neuromuscular junction and non-muscle tissues such as kidney, brain and peripheral nerve, and, as a receptor of laminin/agrin, has been implicated in such diverse and specific developmental processes as epithelial morphogenesis, synaptogenesis and myelinogenesis. These findings point to the fundamental role of dystroglycan in the cellular differentiation process shared by many different cell types. In this paper, we review the recent publications on the biological functions of dystroglycan and discuss its roles in cell differentiation.

**Key words:** Dystroglycan, Laminin, Agrin, Dystrophin, Extracellular matrix

## Introduction

In skeletal muscle, dystrophin, a large membrane-associated cytoskeletal protein encoded by the Duchenne muscular dystrophy (DMD) gene, exists in a large oligomeric complex (the dystrophin-glycoprotein complex or DGC) tightly associated with several sarcolemmal proteins, which are collectively called the dystrophin-associated proteins (DAPs) (for overviews on the DGC and the DAPs see Tinsley et al., 1994; Campbell, 1995; Ozawa et al., 1995; Worton, 1995). In DMD patients and *mdx* mice, the absence of dystrophin leads to a drastic reduction of all the DAPs in the sarcolemma. Extensive studies over the last several years have indicated that the DAPs are classified into three groups: (1) the syntrophin complex; (2) the sarcoglycan complex; and (3) the dystroglycan complex (Fig. 1). The syntrophin complex is comprised of the members of the syntrophin family, α-, ß1- and ß2-syntrophin, which are cytoplasmic proteins complexed with the carboxyl-terminal domain of dystrophin or its homologues. The sarcoglycan complex is comprised of three transmembrane glycoproteins, α-, ß- and γ-sarcoglycan. Although the physiological functions of the syntrophin and sarcoglycan complexes remain obscure at present, recent studies have demonstrated that primary deficiency of α-, ß- and γ-sarcoglycan causes three forms of autosomal recessive muscular dystrophies, LGMD2D, LGMD2E and LGMD2C, respectively, suggesting that the sarcoglycan complex plays a crucial role in the stabilization of the sarcolemma.

In contrast to the syntrophin and sarcoglycan complexes, the dystroglycan complex has been characterized extensively during the last few years and this has led to the amazing discovery that it is a receptor of the extracellular matrix (ECM) proteins laminin and agrin in various tissues. As a receptor of laminin/agrin, the dystroglycan complex is presumed to play roles in diverse and specific physiological processes such as epithelial morphogenesis, synaptogenesis and myelinogenesis, as well as stabilization of the sarcolemma. In this paper, we review the recent publications on the biological functions of the dystroglycan complex and discuss its roles in cell differentiation in various tissues.

## The primary structure of dystroglycan

Dystroglycan was originally identified as the 156 kDa and 43 kDa constituents of the DGC in skeletal muscle (Ervasti et al., 1990). Interestingly, cDNA cloning disclosed that both the 156 kDa and 43 kDa

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proteins are encoded by a single 5.8 kb mRNA and that post-translational processing of a 97 kDa precursor protein resulted in two mature proteins (Ibraghimov-Beskrovnzaya et al., 1992). The 156 kDa and 43 kDa proteins were found to correspond to the amino- and carboxyl-terminal halves of the dystroglycan precursor protein and were named α- and ß-dystroglycan, respectively (Ibraghimov-Beskrovnaya et al., 1992). Dystroglycan has no significant homology with any known proteins. There is a signal sequence in the amino-terminus and a single transmembrane domain of 24 amino acids near the carboxyl-terminus. This suggests that α- and ß-dystroglycan are extracellular and transmembrane proteins, respectively (Ibraghimov-Beskrovanaya et al., 1992). Tryptic peptide mapping of purified α- and ß-dystroglycan and amino-terminal amino acid sequencing of purified ß-dystroglycan have identified serine 654 of the dystroglycan precursor as the single cleavage site (Deyst et al., 1995; Smalheiser and Kim, 1995). The complex comprised of α- and ß-dystroglycan is called the dystroglycan complex. However, the precise nature of the interaction between these two proteins has not yet been characterized. The dystroglycan gene, containing two exons, is localized on chromosome 3p21 and 9 in human and mouse, respectively (Ibraghimov-Beskrovnaya et al., 1993; Górecki et al., 1994). Thus far, no disease-causing mutations have been identified in the dystroglycan gene.

## Glycosylation of α-dystroglycan

The molecular mass of α-dystroglycan ranges between 120 kDa to 190 kDa among different tissues, whereas the core protein has a molecular mass of only about 70 kDa, suggesting that it may be a target of extensive post-translational modification, such as glycosylation. This is consistent with the facts that: (1) α-dystroglycan contains three potential N-glycosylation sites and numerous potential O-glycosylation sites (Ibraghimov-Beskrovnaya et al., 1992); and (2) α-dystroglycan is far more negatively charged than predicted from the primary structure (Gee et al., 1993; Smalheiser and Kim, 1995). Indeed, the results of lectin staining and digestion with deglycosylation enzymes or O-sialoglycoprotease indicate that α-dystroglycan is a mucin-type glycoprotein, which is defined as a glycoprotein heavily glycosylated in the O-sites (Ervasti and Campbell, 1991; Smalheiser and Kim, 1995; Yamada et al., 1996a). The most prominent feature of mucin domains of glycoproteins is the high content of proline residues in the vicinity of serine and threonine O-glycosylation sites. Such a characteristic sequence can be found in the middle portion of α-dystroglycan, suggesting that this may be a mucin domain. This region is expected to take the form of a rigid rod, since complex secondary and tertiary structures are hindered by heavy glycosylation (Fig. 1). Consistent with this prediction, analysis by electron microscopy demonstrated that the isolated cardiac muscle α-dystroglycan consisted of two globular domains connected by a rod-like segment (Brancaccio et al., 1995). At present, there is no direct evidence which indicates that α-dystroglycan contains glycosaminoglycan chains as originally proposed.

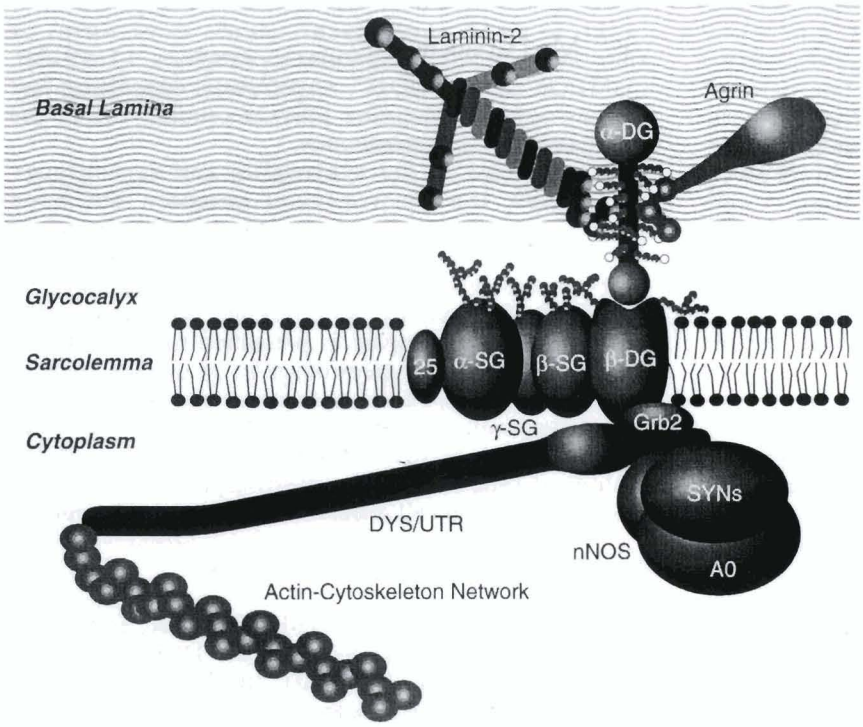


**Fig. 1.** The molecular organization of the DGC in skeletal muscle. α-DG: α-dystroglycan; ß-DG: ß-dystroglycan; α-SG: α-sarcoglycan; ß-SG: ß-sarcoglycan; γ-SG: γ-sarcoglycan; 25: the 25 kDa DAP; SYN: syntrophin; nNOS: neuronal nitric oxide synthase; DYS: dystrophin; UTR: utrophin.

## The dystroglycan complex and signal transduction

The intracellular signal transduction pathways activated by adhesion of cells to other cells or the ECM play crucial roles in cell differentiation, migration and proliferation. The prototypical cell adhesion molecules are the cell surface receptors for the ECM glycoproteins. As a receptor of the ECM glycoproteins laminin and agrin (discussed below), the dystroglycan complex fulfills this criterion, raising a possibility that it may be involved in the signaling pathways. This is supported by the fact that the intracellular carboxyl-terminal tail of ß-dystroglycan contains a phosphotyrosine consensus sequence and several proline-rich regions which could associate with SH2 and SH3 domains of signaling proteins (Ibraghimov-Beskrovnaya et al., 1992). Recently, the carboxyl-terminal tail of ß-dystroglycan was found to bind to Grb2, an adaptor protein in the signaling pathways, via the two SH3 domains, suggesting that ß-dystroglycan may indeed play a role in signal trasduction (Yang et al., 1995). Since ß-dystroglycan binds to dystrophin through the last 15 amino acids of the carboxyl-terminus (Jung et al., 1995), the interaction with GrB2 may also modulate the interaction of ß-dystroglycan with the submembranous dystrophin-cytoskeleton and affect the molecular organization of the dystroglycan complex in the cell membrane.

## Biological functions of the dystroglycan complex

In contrast to dystrophin, whose expression is restricted to muscle and nervous tissues, dystroglycan is broadly expressed in a variety of tissues. Northern blot analysis demonstrated the expression of a single 5.8 kb dystroglycan mRNA in not only skeletal and cardiac muscles but also non-muscle tissues such as brain, lung, liver, pancreas, placenta and kidney (Ibraghimov-Beskrovnaya et al., 1992, 1993). Immunoblot analysis has demonstrated, on the other hand, that the molecular mass of α-dystroglycan differs between muscle and non-muscle tissues. In striated muscle, the molecular mass of α-dystroglycan is 156 kDa or over, while it is 120 kDa in lung, kidney, brain and peripheral nerve (Ibraghimov-Beskrovnaya et al., 1992; Evarsti and Campbell, 1993; Gee et al., 1993; Matsumura et al., 1993; Yamada et al., 1994; Smalheiser and Kim, 1995). This difference is explained by different levels of glycosylation of α-dystroglycan (Ervasti and Campbell, 1993; Ibraghimov-Beskovnaya et al., 1993). Thus far, the cellular distribution, molecular organization and biological functions of the dystroglycan complex have been investigated in detail in striated muscle, kidney, brain and peripheral nerve.

### *1. Striated muscle (Fig. 1)*

In skeletal muscle, the dystroglycan complex was localized in the sarcolemma by immunohistochemistry and immunoelectron microscopy (Ervasti et al., 1990; Ervasti and Campbell, 1991; Ibraghimov-Beskrovnaya et al., 1992; Cullen et al., 1994; Wakayama et al., 1995). In cardiac muscle, it is localized in the transverse tubules in addition to the sarcolemma (Klietsch et al., 1993). The molecular organization of the skeletal muscle dystroglycan complex has been investigated extensively. The DGC was isolated from the detergent extracts of the skeletal muscle membrane fractions using wheat germ agglutinin (WGA) and DEAE chromatography followed by sucrose density gradient centrifugation (Ervasti et al., 1990; Ervasti and Campbell, 1991). Both α- and ß-dystroglycan were identified as major constituents of the isolated DGC, together with dystrophin and the components of the sarcoglycan and syntrophin complexes (Ervasti et al., 1990; Ervasti and Campbell, 1991). The dystroglycan complex was dissociated from dystrophin and the sarcoglycan and syntrophin complexes by *n*-octyl-ß-D-glucoside (Yoshida et al., 1994). Alpha-dystroglycan, but not ß-dystroglycan, was extracted from the membranes or dissociated from the DGC at pH 12 (Ervasti and Campbell, 1991). In addition, a probe which labels the hydrophobic segments of proteins reacted with ß-dystroglycan, but not with α-dystroglycan (Ervasti and Campbell, 1991). Together with the primary structure and the localization in the sarcolemma, these findings indicate that α-dystroglycan is an extracellular peripheral membrane protein, while ß-dystroglycan is an integral membrane protein of the sarcolemma.

The most amazing discovery relevant to the physiological functions of α-dystroglycan is that it binds laminin, a major component of the ECM basal lamina, with high affinity (Ibraghimov-Beskrovnaya et al., 1992; Ervasti and Campbell, 1993). Laminin is a heterotrimer made up of three chains of classes α, ß and γ , and exists in numerous trimeric isoforms in different tissues (for review see Burgeson et al., 1994; Sanes, 1994). Laminin α chains have globular domain (G) repeats in the carboxyl-terminus, and laminin-1, comprising the α1, ß1, and γ1 chains, binds to α-dystroglycan via the last two G repeats in the α1 chain (Gee et al., 1993). Later α-dystroglycan was also shown to bind laminin-2, comprising the α2, ß1 and γ1 chains, which is a striated muscle isoform of laminin (Sunada et al., 1994). The binding of laminin-1 and 2 to α-dystroglycan is $Ca^{2+}$-dependent. Heparin inhibits the binding of laminin-1 more effectively than laminin-2 (Pall et al., 1996). On the cytoplasmic side of the sarcolemma, the carboxyl-terminal tail of ß-dystroglycan is anchored to the cysteine-rich/carboxyl-terminal domains of dystrophin (Suzuki et al., 1994; Jung et al., 1995). The carboxyl-terminal domain of dystrophin interacts with α1-syntrophin which, in turn, binds to neuronal nitric oxide synthase, a signaling enzyme (Brenman et al., 1996). On the other hand, the amino-terminal domain of dystrophin interacts with F-actin (Winder et al., 1995). These findings indicate that the DGC spans the sarcolemma to link the ECM basal lamina with the subsarcolemmal

actin-cytoskeleton and a signaling pathway (Fig. 1).

In DMD patients and *mdx* mice, absence of dystrophin causes a severe reduction in all of the DAPs, including the dystroglycan complex, and eventually leads to muscle cell death. In the patients with LGMD2C, LGMD2D or LGMD2E, the loss of the whole sarcoglycan complex leads to muscle cell death. Furthermore, deficiency of the laminin α2 chain in the basal lamina leads to muscle cell death in congenital muscular dystrophy (CMD) patients and *dy* mice. Thus, the disruption of the linkage anywhere between the subsarcolemmal cytoskeleton and the ECM via the DGC leads to muscle cell death (Fig. 2) (for overviews see Campbell, 1995; Ozawa et al., 1995; Worton, 1995). All these findings indicate that the DGC contributes to the sarcolemmal stability during contraction and stretch of mature muscle cells. At present, the role of the dystroglycan complex in muscle cell differentiation remains unknown.

*2. Neuromuscular junction (Fig. 1)*

Immunohistochemical analysis demonstrated that all the components of the DAPs were highly enriched in the neuromuscular junction (Matsumura et al., 1992). In addition, utrophin, an autosomal homologue of dystrophin, is selectively localized at the neuromuscular junction, and is associated with the DAPs, that is, the dystroglycan, sarcoglycan and syntrophin complexes (Matsumura et al., 1992). Recently α-dystroglycan was shown to bind agrin, a major component of the basal lamina of the neuromuscular junction, with high affinity (Bowe et al., 1994; Campanelli et al., 1994; Gee et al., 1994; Sugiyama et al., 1994). Agrin has the G repeats homologous to those of laminin α chains in the carboxyl-terminus and mediates the clustering of acetylcholine receptors (AChRs) in the postsynaptic membrane of the neuromuscular junction (for overviews see Fallon and Hall, 1994; Carbonetto and Lindenbaum,

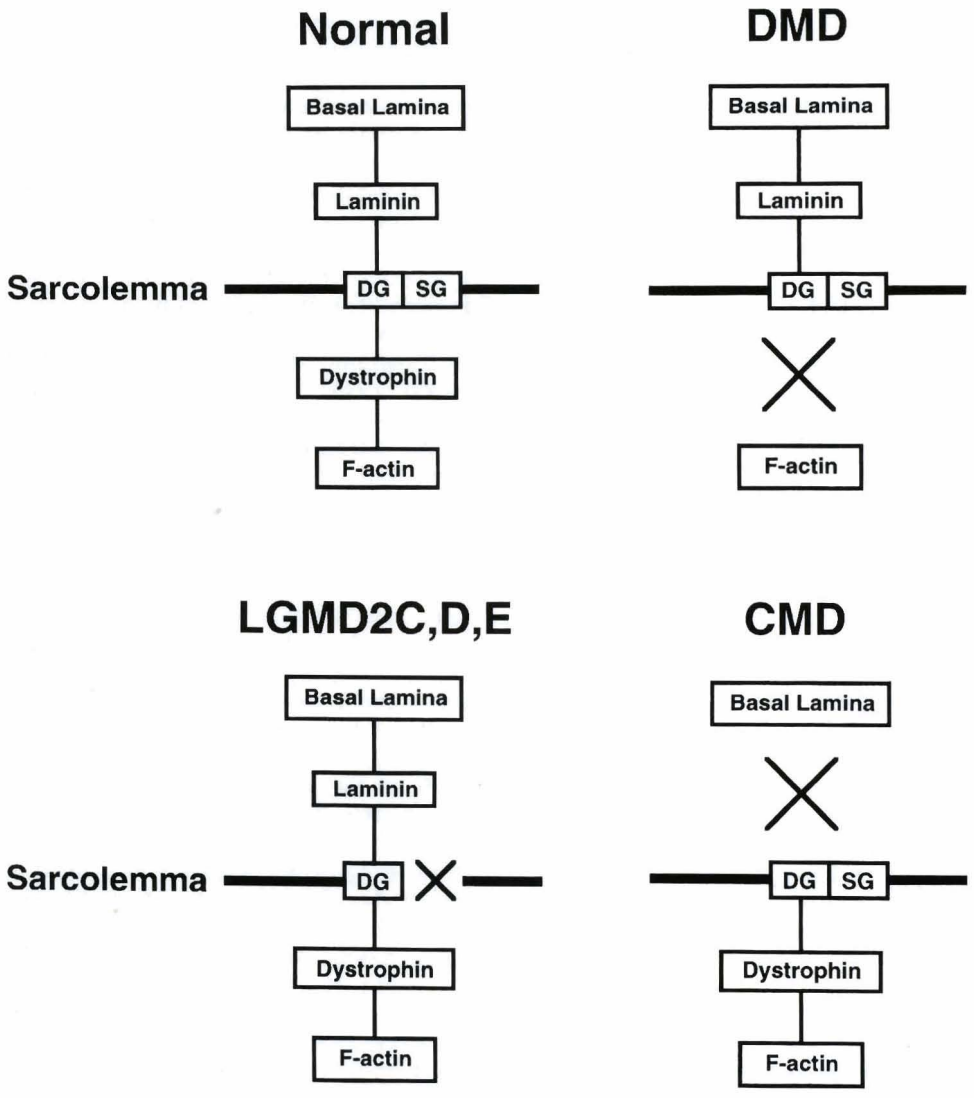


**Fig. 2.** Disruptions of the link between the basal lamina and the cytoskeleton via the DGC in muscular dystrophies. DG: the dystroglycan complex; SG: the sarcoglycan complex.

1995). These findings indicate that, in the neuromuscular junction, agrin is anchored to the submembranous utrophin-cytoskeleton via the identical sets of membrane proteins as laminin-2. In the Torpedo postsynaptic membrane, on the other hand, dystrophin, utrophin or the components of the sarcoglycan and syntrophin complexes are not detected in the isolated dystroglycan complex (Bowe et al., 1994).

Similar to laminin-1 and 2, the binding of agrin to α-dystroglycan is $Ca^{2+}$-dependent and inhibited by heparin. Agrin has several isoforms generated by alternative mRNA splicing at two sites in the carboxyl-terminus (named A and B in chick and Y and Z in rat). Agrin isoforms are expressed among many different tissues, but the neuronal isoform, which has inserts of four and eight amino acids at the A (Y) and B (Z) sites respectively, has the highest activity of AChR clustering. The neuronal isoform is secreted by nerve terminals and accumulates in the junctional basal lamina. Since the AChR clustering activity of agrin is $Ca^{2+}$-dependent and inhibited by heparin, it was proposed that the dystroglycan complex might be the functional agrin receptor itself (Bowe et al., 1994; Gee et al., 1994; Campanelli et al., 1994). This was supported by the finding that α-dystroglycan was deficient in the mutant muscle cells which are defective in proteoglycan synthesis and response to agrin. The 43 kDa AChR associated protein rapsyn was suggested to function as a linker between the AChRs and the dystroglycan complex (Apel et al., 1995).

This view is still disputed for several reasons (for more extensive reviews see Fallon and Hall, 1994; Carbonetto and Lindenbaum, 1995). Firstly, the isoform of agrin, which lacks amino acid inserts at the A (Y) and B (Z) sites and thus is inactive for AChR clustering, binds to α-dystroglycan with higher affinity than the active isoform (Sugiyama et al., 1994). Secondly, studies looking at the effects of monoclonal antibody IIH6 against α-dystroglycan, which inhibits the binding of laminin/agrin to α-dystroglycan, on the agrin-induced clustering of AChRs gave somehow confusing results. In three studies, the clustering was affected, to varying extents, by IIH6 (Campanelli et al., 1994; Gee et al., 1994; Cohen et al., 1995), whereas it was not affected in one study (Sugiyama et al., 1994). Thirdly, the domains of agrin necessary to induce AChR clustering and the heparin-binding site of agrin were found to be overlapping, but not identical to the α-dystroglycan-binding site (Gesemann et al., 1996; Hopf and Hoch, 1996). These findings suggest the existence and involvement of another and hitherto-unidentified functional agrin receptor for AChR clustering.

It should be noted, on the other hand, that the 120 kDa brain and peripheral nerve α-dystroglycan are both positive for $VVAB_4$, a lectin which binds Gal/NAc-terminated saccharides (Smalheiser and Kim, 1995; Yamada et al., 1996a). Interestingly, $VVAB_4$-positive glycoconjugates, selectively localized at the neuromuscular junction, were reported to play a role in the agrin-induced clustering of AChRs (Martin and Sanes, 1995). Although skeletal muscle α-dystroglycan with a molecular mass of 156 kDa is distributed throughout the sarcolemma and was reported negative for $VVAB_4$ (Martin and Sanes, 1995), a possibility exists that the fraction of α-dystroglycan localized and enriched in the neuromuscular junction may be glycosylated differently from the extrajunctional form and be positive for $VVAB_4$. These findings suggest that α-dystroglycan may be identical to the $VVAB_4$-positive glycoconjugate involved in the agrin-induced clustering of AChRs in the neuromuscular junction. In any case, more studies are needed to clarify the precise role of the dystroglycan complex in AChR clustering.

### 3. Kidney

It has been known that the α6ß1 integrin in the cell surface binds to the E8 fragment of laminin-1 and antibody against the α6 subunit of integrin interfers with kidney tubule development *in vitro*. On the other hand, it has also been known that antibodies to the E3 fragment of laminin-1, which contains the α-dystroglycan-binding site, inhibit kidney tubule development, suggesting the existence of a cell surface laminin-1-receptor distinct from the α6ß1 integrin. Recently, dystroglycan was localized to the basal side of epithelial cells in developing kidney, and dystroglycan mRNA, together with that of laminin α1 chain, was shown to increase during kidney epithelial differentiation (Durbeej et al., 1995). Furthermore, monoclonal antibody IIH6 against α-dystroglycan, which inhibits the binding of laminin/agrin to α-dystroglycan, perturbed epithelial development in kidney organ culture (Durbeej et al., 1995). These findings indicate that, in concert with the α6ß1 integrin, the dystroglycan complex acts as a receptor of laminin-1 during epithelial morphogenesis in kidney.

### 4. Brain

In brain, biochemical studies had led to the identification of a laminin-binding protein with a molecular mass of 120 kDa, called cranin or LBP120 (Smalheiser and Schwartz, 1987; Gee et al., 1993). Surprisingly, amino acid sequencing of the purified cranin/LBP120 demonstrated that it was identical to α-dystroglycan (Gee et al., 1993; Smalheiser and Kim, 1995). Similar to striated muscle, brain α-dystroglycan is complexed with ß-dystroglycan. However, the other components of the DGC are apparently not detected in the isolated brain dystroglycan complex (Gee et al., 1993; Smalheiser and Kim, 1995), indicating differences in the composition of the proteins associated with the dystroglycan complex from skeletal muscle. The cellular distribution of the dystroglycan complex in brain is not fully established. *In situ* hybridization has demonstrated the expression of dystroglycan mRNA in the hippocampal formation (Anmon's horn and the dentate

gyrus), cerebellum, olfactory bulb, thalamus and the choroid plexus (Górecki et al., 1994). However, the localization of α-dystroglycan has been confirmed only in the regions of synaptic contact upon the Purkinje cell dendrites by immunohistochemical analysis (Smalheiser and Kim, 1995).

Isolated brain α-dystroglycan binds laminin in a $Ca^{2+}$-dependent manner *in vitro* (Ibraghimov-Beskrovnaya et al., 1992; Ervasti and Campbell, 1993; Gee et al., 1993; Smalheiser and Kim, 1995). However, basal lamina does not exist surrounding neuronal cells in mature brain and the native ligands for the neuronal α-dystroglycan remain elusive. Neurexins, a large family of receptors found on nerve terminals, were proposed as candidates, based on the fact that they have the G repeats homologous to those of laminin α chains and agrin in the carboxyl-terminus (Carbonetto and Lindenbaum, 1995). Sugar moieties of brain α-dystroglycan have been characterized by lectin and antibody staining (Smalheiser and Kim, 1995). One interesting feature is that brain α-dystroglycan is HNK-1 positive, which indicates that it has 3-sulphated glucuronic acid. Since the HNK-1 carbohydrate epitope, contained in a number of glycoproteins of nervous system, has been implicated in cell adhesion, to laminin in particular (Schachner and Martini, 1995), it would be interesting to see if the HNK-1 carbohydrate epitope is involved in the binding of brain α-dystroglycan with the ligands.

Immunohistochemical analysis revealed that α- and ß-dystroglycan were expressed in the outer plexiform layer of retina, where both dystrophin and utrophin, but not laminin, were present (Montanaro et al., 1995). *In situ* hybridization identified two neuronal populations, photoreceptors and retinal ganglion cells, that expressed dystroglycan mRNA (Montanaro et al., 1995).

### *5. Peripheral nerve*

In peripheral nerve, utrophin, α- and ß-dystroglycan, but not dystrophin and α-sarcoglycan, are expressed, indicating differences in the composition of the proteins associated with the dystroglycan complex from skeletal muscle (Matsumura et al., 1993). Indeed, the components of the sarcoglycan complex were not detected in the peripheral nerve dystroglycan complex isolated from the detergent extracts of the peripheral nerve membrane fractions using WGA and laminin-affinity chromatography (Yamada et al., 1996a). Alpha- and ß-dystroglycan are localized to the Schwann cell membrane (Yamada et al., 1994, 1996a). It is noteworthy that, in contrast to the other well known myelin proteins, their expression is restricted to the Schwann cell outer membrane that abuts on the endoneurial basal lamina, but not the compact myelin or the inner membrane (Yamada et al., 1996a). On the other hand, utrophin and Dp116, a 116 kDa protein product of the DMD gene (Byers et al., 1993), are localized in the Schwann cell cytoplasm (Yamada et al., 1996a). Since both utrophin and Dp116 are cytoskeletal proteins sharing the ß-dystroglycan-binding site of dystrophin, these proteins are the candidates that anchor the dystroglycan complex in the Schwann cell outer membrane to the underlying cytoskeleton.

In peripheral nerve, laminin-2 is expressed in the endoneurial basal lamina (Leivo and Engvall, 1988; Sanes et al., 1990), and is a ligand of the Schwann cell dystroglycan complex (Yamada et al., 1994, 1996a). Glycosylation of α-dystroglycan (sialylation in particular) was implicated in the interaction with laminin 2 (Yamada et al., 1996a). Similar to brain α-dystroglycan, Schwann cell α-dystroglycan is HNK-1 positive, raising the possibility that the HNK-1 carbohydrate epitope may also be involved in the interaction with the ECM ligands (Yamada et al., 1996a). In addition, laminin-2, an isoform of agrin, which lacks inserts at both A (Y) and B (Z) sites and thus is inactive for AChR clustering, is also expressed in the endoneurial basal lamina of peripheral nerve, and is a ligand of the Schwann cell dystroglycan complex (Ruegg et al., 1992; Yamada et al., 1996b). The interaction of laminin-2 and agrin with Schwann cell α-dystroglycan is $Ca^{2+}$-dependent (Yamada et al., 1994, 1996b). These findings indicate that the dystroglycan complex is a dual receptor for laminin-2 and agrin in the Schwann cell outer membrane.

The results of *in vitro* experiments had shown that the deposition of laminin in the basal lamina was essential for ensheathment and myelination by Schwann cells (Eldridge et al., 1989; Anton et al., 1994; Obremski and Bunge, 1995). Furthermore, peripheral myelination is known to be greatly disturbed in CMD patients and *dy* mice deficient in laminin α2 chain (Arahata et al., 1993; Sunada et al., 1994; Xu et al., 1994; Tomé et al., 1994; Helbling-Leclerc et al., 1995; Shorer et al., 1995). These findings, all together, point to the role for the interaction of the Schwann cell dystroglycan complex with laminin-2 in peripheral myelinogenesis. So far, the activity of agrin has been discussed only in terms of AChR clustering, and the biological functions of the agrin isoform lacking amino acid inserts at both A (Y) and B (Z) sites remain to be elucidated. It would be of interest to see if, in addition to laminin-2, the agrin isoform, inactive for AChR clustering, is also involved in peripheral myelinogenesis.

## How does the dystroglycan complex mediate diverse cell differentiation processes? (Fig. 3)

At present, the mechanism by which the dystroglycan complex mediates such diverse and specific biological processes like sarcolemmal stabilization, epithelial morphogenesis, synaptogenesis and myelinogenesis is unclear. We may envisage several hypothetical scenarios. Firstly, the interaction of different ECM ligands with the dystroglycan complex may transmit downstream intracellularly distinct signals for each of these processes (Fig. 3a). Differential glycosylation of α-dystroglycan among different tissues

may also be involved in this mechanism. As discussed above, however, this scenario is not necessarily supported by the recent findings on the agrin-induced AChR clustering in the neuromuscular junction.

The other three scenarios propose the existence and involvement of more specific and functional cell surface receptors for each of the cell differentiation processes described above. In the second scenario, we may envisage that the interaction of different ECM ligands with the dystroglycan complex may transmit intracellular signals, which, in turn, induce the more specific and functional cell surface receptors, such as the members of the integrin family for instance, to interact with these ligands (Fig. 3b). Thirdly, the dystroglycan complex may function as a helper protein or co-receptor; the initial and high-affinity binding of the ECM ligands to the dystroglycan complex may enable the more specific and functional cell surface receptors to interact with these ligands (Fig. 3c). Without the initial interaction with the dystroglycan complex, it may require very high concentrations of these ligands to interact with the functional cell surface receptors. Fourthly, the dystroglycan complex may function purely as a structural protein in the maturational stages of development in a broad range of cell types; the binding of the ECM ligands to the cell surface dystroglycan complex may trigger the reorganization of the submembranous dystrophin/utrophin-cytoskeleton, potentially via Grb2 and other signaling/adaptor proteins (Fig. 3d). This process could be critical for the maturation of the cytoskeletal network that supports the cell membrane. Clearly, many more future studies

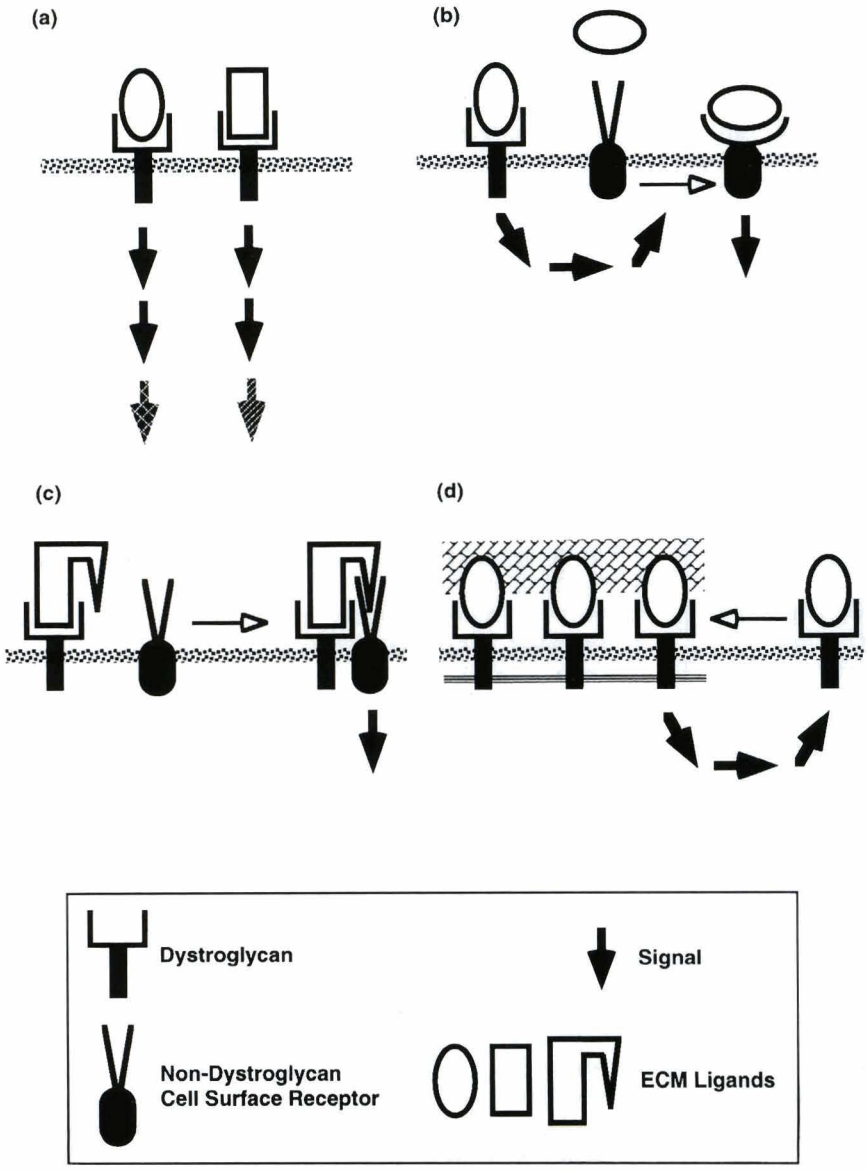


**Fig. 3.** Schematic model of the biological functions of the dystroglycan complex in cell differentiation.

are needed before we will know if any of these hypotheses, alone or in various combinations, hold true.

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