

# Review: Dystroglycan in the Nervous System

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## Abstract

Dystroglycan is part of a large complex of proteins, the dystrophin-glycoprotein complex, which has been implicated in the pathogenesis of muscular dystrophies for a long time. Besides muscular degeneration many patients manifest symptoms of neurological and cognitive dysfunction. Recent findings suggest that dystroglycan is implicated in brain development, synapse formation and plasticity, nerve-glia interactions and maintenance of the blood-brain barrier.

Most research so far has focused on the functions of dystroglycan in muscle and neuromuscular junctions, while its role in the brain and interneuronal synapses has been neglected.

This review will give an overview of the biochemistry of dystroglycan, its interaction with other proteins as well as its confirmed and hypothetical functions in the nervous system.

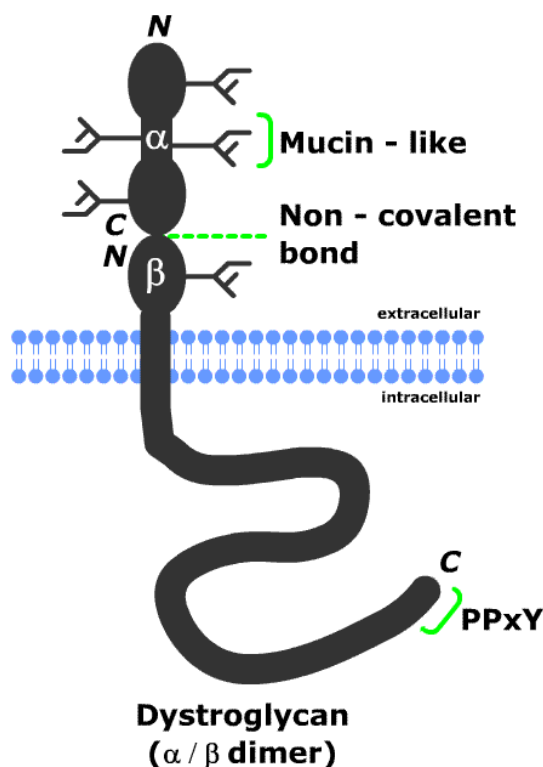
## Introduction

In 1987 a laminin-binding membrane protein was found in brain tissue and was termed cranin (Smalheiser and Schwartz, 1987). Eight years passed until this protein proved to be identical with dystroglycan, which was previously only thought to be associated with muscle membranes (Smalheiser and Kim, 1995).

Dystroglycan gives mechanical stability to muscle fibres by linking the extracellular matrix on the extracellular surface with the actin cytoskeleton on the intracellular side. Furthermore, it is implicated in the formation of neuromuscular junctions. The function of dystroglycan and its associated proteins is impaired in a large group of hereditary diseases, the muscular dystrophies. Muscular dystrophies are among the most prevalent hereditary diseases and affect not only muscle, but do also cause neurological and cognitive symptoms. Recent findings revealed the presence of dystroglycan in many structures of the adult brain and peripheral nervous system which hint that it plays a broader role in neuronal functioning than has been expected.

## Structure and Biochemistry

The dystroglycan gene (*DAG1*) encodes a single propeptide which is cleaved to yield two glycoproteins,  $\alpha$ -dystroglycan and  $\beta$ -dystroglycan (Holt *et al.*, 2000). The roles of this cleavage and the protease or proteases involved are still unknown. Astonishingly, the two resultant proteins immediately associate again by a non-covalent bond to form a tightly bound heterodimer. This complex is anchored in the cell membrane by the membrane-spanning  $\beta$ -dystroglycan and binds to extracellular partners via  $\alpha$ -dystroglycan (Figure 1). This  $\alpha$  -  $\beta$  - dimer is from now on simply referred to as 'dystroglycan', while the complex formed by dystroglycan together with its intracellular binding partners is referred to as 'dystroglycan complex'.



**Figure 1:** The mature dystroglycan heterodimer. Dystroglycan is translated as a single propeptide that contains both  $\alpha$  – and  $\beta$  – subunits. The propeptide is cleaved, but both subunits form non – covalent bonds and reassemble to a tightly bound heterodimer.  $\beta$  – dystroglycan anchors the complex in the cell membrane and binds to intracellular partners, while  $\alpha$  – dystroglycan binds to the extracellular matrix and other binding partners. The heterodimer is subject to extensive glycosylation. Carbohydrates make up the half of the mass of mature dystroglycan. The mucin – like domain of  $\alpha$  – dystroglycan and the PPxY motif of  $\beta$  – dystroglycan are marked. C: C – terminal, N: N – terminal.

## Glycosylation

Glycosylation is quite extensive and about half of the molecular weight of  $\alpha$ -dystroglycan is made up of oligosaccharides. The glycans are anchored on a mucin – like domain in the middle of  $\alpha$ -dystroglycan.

While only a single form of the dystroglycan protein is known there seems to be a wide scope for different forms of glycosylation. Depending on the extent of glycosylation, the molecular mass of dystroglycan can range from around 156 kDa in skeletal muscle to 140 kDa in cardiac muscle and 120 kDa in brain and peripheral nerve (Muntoni *et al.*, 2004a). In the myotubes formed by the C2C12 cell line two different forms of dystroglycan were detected (McDearmon *et al.*, 2001). This suggests that even single cells (or syncytia) are able to produce differently glycosylated forms of dystroglycan, possibly targeted to distinct structures on the cell surface. Most of the extracellular interactions of dystroglycan are dependent on glycosylation, and different glycoforms show distinct binding characteristics to endogenous ligands, lectins and antibodies (Ervasti *et al.*, 1997). However, all of the known glycoforms in healthy organisms do bind to all of the known ligands of dystroglycan. Thus, the differences observed between glycoforms *in vivo* do not seem to change their biological function fundamentally.

## Degradation

In peripheral nerve and other tissues, the cytosolic end of  $\beta$ -dystroglycan can be cleaved by matrix metalloproteinases (MMPs), leading to the formation of a 30 kDa dystroglycan fragment and the disintegration of the dystroglycan complex (Yamada *et al.*, 2001b). The same study did not find this fragment in brain, but it is likely that other cleavage mechanisms are active there.

## Localisation

Dystroglycan is distributed throughout the basement membranes of the whole body and was found in most tissues and organs examined in rodents (Durbeej *et al.*, 1998), primates (Royuela *et al.*, 2003) and teleost fish (Guyon *et al.*, 2003). Homologues have also been identified in *D. melanogaster* (Dekkers *et al.*, 2004) and *C. elegans* (Grisoni *et al.*, 2002; Segalat, 2002).

Zaccaria *et al.* employed an antibody against  $\alpha$  – dystroglycan to map the distribution of dystroglycan in adult mouse brain (Zaccaria *et al.*, 2001a) and found dystroglycan in neurons of the cerebral cortex, hippocampus, olfactory bulb, basal ganglia, thalamus, hypothalamus, brainstem and cerebellum, but not at other areas. Dystroglycan was also found on perivascular astrocytes and blood vessels. On neurons, dystroglycan was

preferentially located to somata and apical dendrites, filling the cytosol. Electron microscopical examination revealed that dystroglycan staining was primarily found on postsynaptic specialisations and endoplasmic reticulum. The antibody against dystroglycan was bound at somata and apical dendrites of pyramidal cells in cortex and CA1 – CA3 of the hippocampus. The subiculum and granule cells of the dentate gyrus were also weakly stained. In the olfactory bulb, staining was observed on a subset of both excitatory and inhibitory dendro - dendritic synapses between mitral and granule cells. In the brainstem the reticular formation, vestibular nucleus, raphe and olivary nuclei were stained. In the cerebellum, Purkinje cells were the only neurons stained strongly.

The results of Zaccaria *et al.* match previous studies that detected dystroglycan on the mRNA – level (Gorecki *et al.*, 1994) with one exception: dystroglycan mRNA has not been detected in the cortex, while dystroglycan protein has. This discrepancy might be due to a lower turnover of dystroglycan in these areas, accompanied by a lower mRNA level.

In the superior cervical ganglion, a part of the sympathetic nervous system, dystroglycan was found on the postsynaptic apparatus of intraganglionic, cholinergic synapses (Zaccaria *et al.*, 1998a). Dystroglycan reactivity was also seen on synapses formed by dissociated neurons of the superior cervical ganglion *in vitro* (Gingras and Ferns, 2001b).

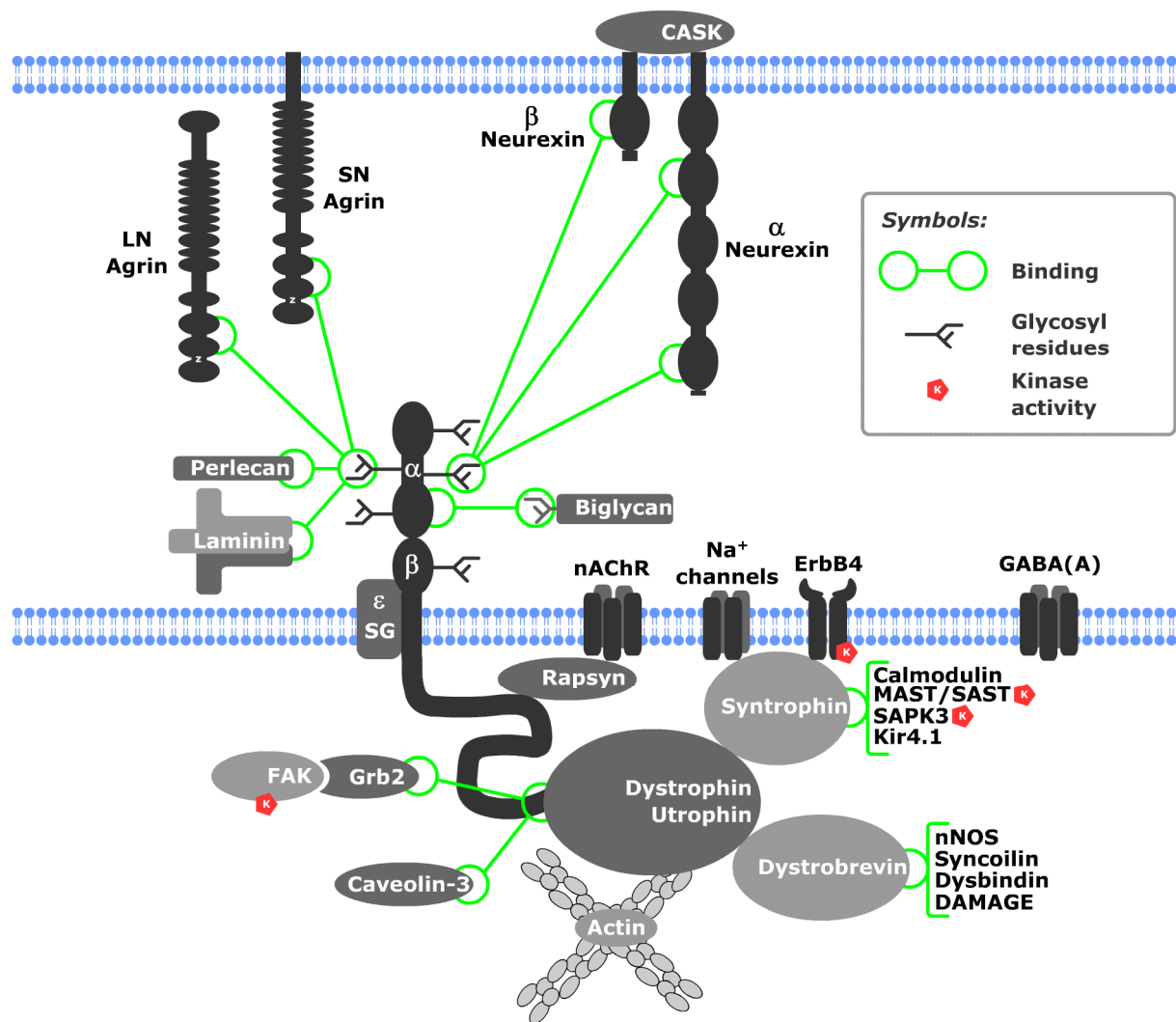
Dystroglycan and associated proteins have been found in the vitreal border and at ribbon synapses of the outer plexiform layer of the retina (Blank *et al.*, 2002). Contrasting the findings on other synapses, dystroglycan is located to the *presynaptic* side of the synapses of the outer plexiform layer (Blank *et al.*, 1997).

### **Binding Partners and Interactions**

Dystroglycan has been found to interact with a multitude of proteins on both the extracellular and the intracellular side (Figure 2), confirming its versatile role in many cellular functions. Dystroglycan has no apparent ability to transduce signals from the extracellular to the intracellular side by itself, so any signals that involve the binding of dystroglycan are dependent on secondary mechanisms, like the clustering of proteins. In the following section all of the binding partners and their confirmed localizations inside the nervous system are reviewed.

**Table 1:** Direct and indirect binding partners of dystroglycan. Indirect binding is diagrammed by hierarchical indention (e.g. dystroglycan binds dystrophin, which binds dystrobrevin, which binds dysbindin). *Abbreviations:* Neur.: expression in neurons, Glia: expression in glia. A + sign marks evidence for presence, a – sign marks evidence for absence.

Binding partner	Main function	Neur.	Glia	References
<b>Extracellular / Transcellular</b>				
Laminin	Extracellular matrix	+	+	(Colognato and Yurchenco, 2000c; Ido <i>et al.</i> , 2004a; Indyk <i>et al.</i> , 2003)
Perlecan	Extracellular matrix	+	+	(Shee <i>et al.</i> , 1998b)
Agrin	Extracellular matrix, transmembrane protein of neurons, messenger	+	+	(Smith and Hilgenberg, 2002d) - Review
Biglycan	Messenger, extracellular matrix		+	(Bowe <i>et al.</i> , 2000c)
Neurexin	Transmembrane protein of neurons, recruits presynaptic apparatus	+		(Sugita <i>et al.</i> , 2001a; Dean <i>et al.</i> , 2003a)
<b>Intracellular</b>				
Dystrophin	Adaptor, binds actin	+	+	(Knuesel <i>et al.</i> , 2001a; Mehes <i>et al.</i> , 2003)
Dystrobrevin	Adaptor	+	+	(Blake <i>et al.</i> , 1998)
nNOS	Nitric oxide synthesis	+	+	
Dysbindin	unknown	+		(Benson <i>et al.</i> , 2001a)
DAMAGE	Putative signalling molecule	+	+	(Albrecht and Froehner, 2004b)
Syncoilin	Desmin binding			(Newey <i>et al.</i> , 2001a)
Desmuslin	Desmin binding			(Mizuno <i>et al.</i> , 2001)
Syntrophin	Adaptor	+	+	(Gorecki <i>et al.</i> , 1997b; Guadagno and Moukhles, 2004a)
Calmodulin	Calcium signalling	+	+	
Sodium channels		+	+	(Gee <i>et al.</i> , 1998a)
Kir4.1 potassium channel	Inward rectifier		+	(Connors <i>et al.</i> , 2004b)
Grb2	Adaptor	+	+	
ErbB4	Neuregulin receptor	+	+	(Garcia <i>et al.</i> , 2000)
SAPK3	Kinase			(Hasegawa <i>et al.</i> , 1999)
MAST, SAST	Putative binding of microtubules, kinases	+	+	(Yano <i>et al.</i> , 2003; Lumeng <i>et al.</i> , 1999a)
TAPP	Phosphatidylinositol 3,4-bisphosphate binding			(Hogan <i>et al.</i> , 2004)
Utrophin	Adaptor (binding profile resembles dystrophin)	+	+	(Knuesel <i>et al.</i> , 2002a; Mehes <i>et al.</i> , 2003)
Actin		+	+	
Ezrin	Connects dystroglycan to actin in glia cells		+	(Spence <i>et al.</i> , 2004a)
Grb2	Adaptor	+	+	
FAK (focal adhesion kinase)	Integrin binding, signalling, neuronal migration, cell cycle, cell survival, putative role in synaptic plasticity	+	+	(Burgaya <i>et al.</i> , 1995a; Parsons, 2003) - Reviews
Caveolin-3	Formation of caveolae	-	+	(Ikezu <i>et al.</i> , 1998b)
Rapsyn	Acetylcholine receptor clustering	+		(Burns <i>et al.</i> , 1997c)
Nicotinic ACh receptors		+		
ERK, MEK2	Kinases	+	+	(Spence <i>et al.</i> , 2004c)
Dynamin 1	Endocytosis	+		(Zhan <i>et al.</i> , 2005a)
<b>Transmembrane</b>				
Sarcoglycan	Unclear, might stabilize dystroglycan	+	+	(Nishiyama <i>et al.</i> , 2004a; Xiao and LeDoux, 2003b)
Sarcospan	Unknown	-	-	(Crosbie <i>et al.</i> , 1997a; Imamura <i>et al.</i> , 2000c)



**Figure 2:** Possible molecular interactions of dystroglycan in neurons and glia. Not all known interactions are depicted, see table 1 for comparison. The domain structures of agrin and laminin are delineated. Laminin, perlecan, agrin, neurexin and biglycan bind to extracellular  $\alpha$  dystroglycan. The binding of biglycan does not depend on the glycosylation of dystroglycan, while binding of other extracellular partners does. The binding of agrin and laminin and probably also perlecan is competitive. The SN variant of agrin is bound to cell membranes, while the LN variant is secreted and has an additional laminin – binding domain. A small ‘z’ marks the z – splice site of agrin. The  $\beta$  - neurexins have one binding site for dystroglycan while  $\alpha$  neurexins have two. Neurexins are connected to intracellular scaffolding complexes via CASK. The intracellular portion of dystroglycan contacts the cytoskeleton, ion channels and other proteins through adaptor proteins. Caveolin -3 and Grb2 compete with Dystrophin for the binding site near the C – terminal of dystroglycan. GABA(A) – receptors colocalize with dystroglycan in some neurons, but there has been no proof of a direct interaction. *Abbreviations:*  $\alpha$ : alpha subunit of dystroglycan,  $\beta$ : beta subunit of dystroglycan,  $\epsilon$  SG: sarcoglycan epsilon, SN / LN agrin: short N-terminal variant / long N-terminal variant of agrin. GABA(A): GABA(A) receptors, nAChR: nicotinic acetylcholine receptors, z: z splice site of agrin. See text for references. Agrin domain structure was adapted from (Smith and Hilgenberg, 2002c), dystroglycan domain structure was adapted from (Winder, 2001), neurexin domain structure was adapted from (Sugita *et al.*, 2001c).

## Extracellular / Transcellular

Five extracellular or transcellular binding partners for dystroglycan could be identified up to now. The three heparan sulphate proteoglycans *laminin*, *agrin* and *perlecan* are abundant components of the extracellular matrix of many tissues. The small proteoglycan *biglycan* is a messenger molecule the roles of which are still not known in its entirety. The transmembrane proteins of the *neurexin* family are found on neurons and have drawn attention as important mediators of presynaptic differentiation. The binding of biglycan is not dependent on glycosylation of dystroglycan (Bowe *et al.*, 2000b), while the binding of the other ligands can be attenuated or totally abolished by a lack of glycosylation.

## Laminin

**Laminins** are large heterotrimeric glycoproteins and are a major component of the extracellular matrix. The three subunits ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) form a large aggregate in the shape of a cross. To date, five  $\alpha$ , three  $\beta$  and three  $\gamma$  subunits have been identified. Laminin 1 ( $\alpha1\beta1\gamma1$ ), laminin 2 (‘merosin’,  $\alpha2\beta1\gamma1$ ), laminin 3 ( $\alpha1\beta2\gamma1$ ), laminin 4 ( $\alpha2\beta2\gamma1$ ) and laminin 10 ( $\alpha5\beta1\gamma1$ ) are known to bind to dystroglycan through their G domains (Cognato and

Yurchenco, 2000b; Ido *et al.*, 2004b). Most laminins bind to integrins on the cell surface and to agrin and other laminin molecules in the extracellular matrix (Colognato and Yurchenco, 2000a).

**Localisation:** The expression profile of laminin in the brain does not always seem to fit its classical roles in other tissues. The laminin  $\gamma 3$  subunit is expressed in many areas of the brain and seems not to be associated with basement membranes (Koch *et al.*, 1999). One study found laminin  $\alpha 2$  subunits on neurites and potentially synaptic structures (Hagg *et al.*, 1997b). In adult hippocampus,  $\alpha 2$  immunoreactivity is associated with dendritic spines (Patton *et al.*, 1998b) and a disruption of long term synaptic plasticity can be observed in cerebellar slice cultures of  $\alpha 2$  deficient mice (Anderson *et al.*, 2005). The  $\gamma 1$  subunit was found on somata and proximal processes (Hagg *et al.*, 1997a). A newer study found that all subunits are expressed in the whole hippocampus, and that  $\alpha 5$ ,  $\beta 1$  and  $\gamma 1$  are expressed in hippocampal neurons, suggesting that laminin 10 ( $\alpha 5\beta 1\gamma 1$ ) is the primary isoform in these neurons (Indyk *et al.*, 2003). Laminins in the hippocampus are degraded via a plasmin-mediated mechanism after excitotoxic injury, which in turn triggers neuronal apoptosis. Dystroglycan binds to laminin 10 (Ido *et al.*, 2004c) and could be involved in these processes.

## Perlecan

**Perlecan** binds a multitude of proteins of the extracellular matrix. It also binds to acetylcholinesterase at the neuromuscular junction.

**Localisation:** Small amounts of perlecan can be found in the intact adult hippocampus. Contrasting the findings on laminin expression, perlecan expression is *elevated* in neurons and glia after excitotoxic injury (Shee *et al.*, 1998a).

## Agrin

**Agrin** is encoded by a single gene, but the occurrence of alternative splicing at three sites and two alternate transcriptional start sites produces a wide array of gene products. Of the splice sites, the z site has the most prominent influence on the function of agrin: agrin<sub>z+</sub> contains inserts at the z splice-site and causes clustering of acetylcholine receptors at the neuromuscular junction. Conversely, agrin<sub>z-</sub> does not contain inserts at this site and does not show clustering activity at the NMJ. The z- isoform is expressed by both neuronal and non-neuronal cells, while the z+ isoform is only expressed by neurons, and dystroglycan binds both of them. Furthermore, agrin isoforms with a long (LN) or a short (SN) N – terminus are produced, dependent on the transcriptional start site. The long isoforms bind laminin and are expressed in various cells and tissues, including muscle and motoneurons. The short isoforms lack a laminin binding site and are not secreted, but exist as transmembrane proteins specifically expressed by neurons. It was also suggested that agrin interacts with neurons through neuronal integrins, but it is left unclear whether this interaction is caused by laminins bound to the agrin or not (Burgess *et al.*, 2002). Agrin and laminin compete for the same binding site on dystroglycan (Gee *et al.*, 1994). For a good review of the known roles of agrin in the nervous system, see (Smith and Hilgenberg, 2002b).

## Biglycan

**Biglycan** was found to be a binding partner of dystroglycan and is upregulated in dystrophic muscle (Bowe *et al.*, 2000a). Besides dystroglycan, it has been found to bind collagen (Pogany *et al.*, 1994), apolipoprotein E (Klezovitch and Scanu, 2001) and others.

Biglycan was found to be upregulated during brain development (Kikuchi *et al.*, 2000a) and at sites of mechanical brain injury for at least up to six months after the lesion (Stichel *et al.*, 1995). It is expressed by glia cells, was shown to be neurotrophic (Junghans *et al.*, 1995) and to stimulate growth of microglial cells in vitro (Kikuchi *et al.*, 2000b). Intracerebral injection of biglycan into the nucleus basalis magnocellularis facilitated learning and activated the cholinergic system (Hasenohrl *et al.*, 1995; Huston *et al.*, 2000).

## Neurexin

**Neurexins** are a family of cell-surface proteins exclusively found on neurons. Three genes encoding neurexins are known, each of the genes has two promoters that can give rise to either a long ( $\alpha$  neurexin) or a short ( $\beta$  neurexin) transcript (Figure 2, top). Due to extensive alternative splicing more than thousand isoforms of neurexin are possible (Missler *et al.*, 1998). They probably serve as signalling receptors and cell adhesion molecules. During synapse formation, the  $\beta$  neurexins on the presynaptic side are bound to neuroligin anchored on the postsynaptic side and initiate the formation of the presynaptic active zone. The  $\alpha$  neurexins do not bind neuroligin but are receptors for messenger molecules termed neurexophilins.

Sugita *et al.* found that dystroglycan is a binding partner of both  $\alpha$  and  $\beta$  neurexins in rat brain (Sugita *et al.*, 2001b).  $\beta$  neurexins have one binding site for dystroglycan, while the larger  $\alpha$  neurexins have two binding sites, giving them the potential ability to cluster dystroglycan. Dystroglycan is the only known membrane-bound ligand for the  $\alpha$ -neurexins known so far, and the expression of dystroglycan and  $\alpha$ -neurexins in postnatal brain development in rats shows some correlation. The function and ultrastructural localisation of this significant interaction are still unknown.

## Intracellular

The most important intracellular ligands of dystroglycan are dystrophin and utrophin, which act as adaptors between dystroglycan and other binding partners, including the actin cytoskeleton. Newer findings have added rapsyn, actin, ezrin, Grb2/FAK and caveolin-3 to the group of binding partners.

### Dystrophin

The **dystrophin** gene is the largest gene described in the human genome. The gene contains 7 promoters that give rise to several tissue specific isoforms with a great variability in molecular weight. The first three promoters produce proteins with a molecular weight of 427 kDa and are associated with cortex/hippocampus, cerebellar Purkinje cells and muscle, respectively. The other promoters produce proteins with a lower molecular weight: 260 kDa (primarily found in the retina), 140 kDa (brain, retina, kidney), 116 kDa (Schwann cells) and 71 kDa (most tissues including brain). The full-length isoforms (427 kDa) bind to actin with the N – terminal end; the shorter isoforms are unable to bind to actin. All isoforms seem to bind dystroglycan and the other members of the dystrophin-glycoprotein complex (DGC).

*Localisation:* In brain, dystrophin was found at postsynaptic GABA(A) receptors of the cortex, hippocampus and cerebellum (Knuesel *et al.*, 2001b). It is colocalized with dystroglycan in most if not all instances (Levi *et al.*, 2002a).

### Utrophin

**Utrophin** is structurally and functionally closely related to dystrophin and can partially substitute for dystrophin when dystrophin function is disturbed (i.e. through mutations).

The binding of utrophin and dystrophin is abolished when dystroglycan is phosphorylated on a tyrosine residue (James *et al.*, 2000; Ilsley *et al.*, 2001). This phosphorylation is triggered by cell adhesion, but the kinase involved is still unknown.

*Localisation:* It is found in large sensory and motor brain stem neurons (Knuesel *et al.*, 2002b). Different to dystrophin, utrophin is located extrasynaptically and seems to give support to neuronal membranes (Knuesel *et al.*, 2001c).

Dystrophin/utrophin bind other intracellular members of the DGC: **dystrobrevin** (which in turn binds neuronal nitric oxide synthase / nNOS, dysbindin (Benson *et al.*, 2001b), syncoilin (Newey *et al.*, 2001b) and DAMAGE (Albrecht and Froehner, 2004a)), and **syntrophin** (binds Calmodulin, Na<sup>+</sup> channels (Gee *et al.*, 1998b), the potassium channel Kir4.1 (Connors *et al.*, 2004a), Grb2, ErbB4, MAST/SAST (Yano *et al.*, 2003; Lumeng *et al.*, 1999b) and might also be responsible for the correct localisation of aquaporin-4 (Neely *et al.*, 2001)).

*Localisation:* Dystrobrevin has been found to be expressed in the developing and adult brain (Lien *et al.*, 2004; Enigk and Maimone, 1999) and retina (Blank *et al.*, 2002). Isoforms of syntrophin are found at all sites where dystrophin and utrophin are located, with the exception of Purkinje neurons (Gorecki *et al.*, 1997a).

### Actin

**Actin** was found to be able to directly bind to dystroglycan (Chen *et al.*, 2003), through a mechanism not dependent on dystrophin or utrophin. Overexpression of dystroglycan in cultured cells caused aberrant bundling of actin filaments.

### Ezrin

**Ezrin** binds to the juxtamembrane region of dystroglycan and the two proteins are colocalized in microvilli (Spence *et al.*, 2004b). Ezrin interacts with actin, and disturbance of the dystroglycan-ezrin interaction reduced microvilli-like surface protrusions in cell culture.

*Localisation:* Ezrin was found in glia cells, but not in neurons.

### Grb2 and FAK

Growth factor receptor 2 (**Grb2**) is an adaptor protein involved in signal transduction. It binds dystroglycan with high affinity and competes with dystrophin for the binding site on dystroglycan (Russo *et al.*, 2000). Via Grb2, dystroglycan can interact with the C – terminal domain of focal adhesion kinase (**FAK**) (Cavaldesi *et al.*, 1999b). As the name implies, FAK is most commonly, but not exclusively, associated with focal adhesions, and the N – terminal domain of FAK binds to intracellular portions of integrins. Thus, FAK might act as a direct functional connection between dystroglycan and the integrin system. The N – terminal domain of FAK shows sequence similarities with ezrin and other related proteins, but does not seem to be able to directly bind to dystroglycan (Cavaldesi *et al.*, 1999a).

Autophosphorylation of FAK is important for its function, and the major isoform found in brain neurons (FAK<sup>6,7</sup>) is marked by its stronger tendency towards autophosphorylation. Activation of FAK recruits kinases of the Src – family, which influence many important intracellular signalling cascades (Girault *et al.*, 1999a).

Up to now, the Dystroglycan/Grb2/FAK – interaction has not been investigated *in vivo*.

*Localisation:* In the nervous system, FAK is ubiquitously expressed but tightly regulated during development. Highest levels in the brain are reached during late embryonic stages. In adult rats, FAK is especially concentrated in the cortex and hippocampus, particularly at neural cell bodies and large dendrites. Neurons in primary culture exhibit reactivity in growth cones and perikarya at early stages and a diffuse distribution at later stages. Cultured astrocytes show FAK reactivity associated with tight junctions (Burgaya *et al.*, 1995b). For a review, see (Girault *et al.*, 1999b).

### **Caveolin-3**

**Caveolin-3** can bind to the C – terminus of beta dystroglycan and competes with dystrophin for this binding site (Sotgia *et al.*, 2000). Consistently, overexpression of caveolin-3 in skeletal muscle leads to a disruption of the dystroglycan – dystrophin interaction which is accompanied by muscular dystrophy (Galbiati *et al.*, 2000).

Caveolins are thought to act as scaffolding proteins in the formation of *caveolae*, 50 – 100 nm large invaginations found on the membranes of most cell types.

*Localisation:* In muscle, caveolin-3 is preferentially localized to neuromuscular junctions, probably interacting with dystroglycan (Carlson *et al.*, 2003). In brain, caveolins 1, 2 and 3 are expressed. Caveolin 3 was predominantly found on the endfeet of astrocytic processes, but not on neurons (Ikezu *et al.*, 1998a). Dystroglycan is also present at astrocytic endfeet (Guadagno and Moukhles, 2004b).

### **Rapsyn**

The cytoplasmic juxtamembrane region of dystroglycan binds rapsyn, a protein which is essential for the clustering and stabilisation of acetylcholine receptors at the neuromuscular junction, as well as the linkage of the utrophin complex to these clusters (Cartaud *et al.*, 1998). Rapsyn was also found to cluster GABAA receptors *in vitro* (Yang *et al.*, 1997).

*Localisation:* Besides muscular endplates, rapsyn was found to be expressed in brain and cultured neurons of the parasympathetic ciliary ganglion (Burns *et al.*, 1997b).

### **ERK, MEK2**

The kinases MEK2 (mitogen-activated protein kinase kinase 2) and ERK (extracellular signal-regulated kinase) bind to dystroglycan and are colocalized with dystroglycan in cultured fibroblasts (Spence *et al.*, 2004d). MEK2 and ERK are part of an important signalling cascade, the receptor tyrosine kinase/Ras/Raf/MAP kinase pathway. These kinases are involved in a vast number of cellular processes, including synaptic plasticity.

### **Dynamin 1**

Dynamin is a GTPase that is involved in endocytosis in all cell types including neurons, where it is involved in the recycling of presynaptic vesicles and receptor internalization. Zhan *et al.* report that dynamin 1 has an affinity for dystroglycan (Zhan *et al.*, 2005b). They also showed that dynamin immunoreactivity is colocalized with dystroglycan reactivity in the presynaptic portions of synapses in the outer plexiform layer of the retina. Furthermore, they found that fibroblastic cells with a disruption of the dystroglycan showed increased endocytosis of transferrin compared to control.

### **Transmembrane**

The sarcoglycans and sarcospan are transmembrane proteins associated with the dystroglycan complex.

### **Sarcoglycan**

Sarcoglycans are important for the stability of dystroglycan. Six sarcoglycans ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$ ,  $\zeta$ ) are known, but only  $\epsilon$ -sarcoglycan is expressed in brain.

*Localisation:*  $\epsilon$ -sarcoglycan is accumulated on neurons in the olfactory bulb, cortex, hippocampus, cerebellar cortex and substantia nigra and is colocalised with  $\alpha 2$ -laminin (Nishiyama *et al.*, 2004b; Xiao and LeDoux, 2003a). It is also expressed in astrocytes. The neuronal expression pattern overlaps with that of other components of the DGC.  $\beta$ ,  $\delta$ , and  $\epsilon$ -sarcoglycans were found on the myelin sheath of peripheral nerve (Imamura *et al.*, 2000b).



## Sarcospan

Sarcospan is a transmembrane protein that is associated with the DGC via Sarcoglycan. It seems to be absent from neurons and nervous tissue (Crosbie *et al.*, 1997b; Imamura *et al.*, 2000a).

## Human Diseases and Animal Models

Total gene-knockout of dystroglycan is lethal in early embryonic stages in rodents. There are no known diseases that involve a mutation in the dystroglycan gene and no mutations have been described so far. However, there are several common hereditary diseases that are caused by aberrant glycosylation of dystroglycan, which leads to a loss of its main functions.

Five distinct human diseases that affect the glycosylation of dystroglycan have been identified up to now: Fukuyama type congenital muscular dystrophy (Kobayashi *et al.*, 1998a), MDC1C (Brockington *et al.*, 2001a), MDC1D (Longman *et al.*, 2003b), muscle-eye-brain disease (Yoshida *et al.*, 2001a) and Walker-Warburg syndrome (Beltran-Valero *et al.*, 2002b; Kim *et al.*, 2004b). All of them are caused by a mutation in a glycosyltransferase.

**Table 2:** Genetic diseases that are known to be caused by a hypoglycosylation of dystroglycan.

Disease	Gene	Function	References
Fukuyama type congenital muscular dystrophy	Fukutin	Putative glycosyltransferase	(Kobayashi <i>et al.</i> , 1998b)
MDC1C	Fukutin-related protein	Putative glycosyltransferase	(Brockington <i>et al.</i> , 2001b)
MDC1D	LARGE	Putative glycosyltransferase	(Longman <i>et al.</i> , 2003a)
Muscle-eye-brain disease	POMGnT1	O-mannosyl glycan synthesis	(Yoshida <i>et al.</i> , 2001b)
Walker-Warburg syndrome	POMT1, POMT2	Putative O-mannosyltransferases	(Beltran-Valero <i>et al.</i> , 2002a; Kim <i>et al.</i> , 2004a; van Reeuwijk <i>et al.</i> , 2005)

Defects in the glycosylation of dystroglycan have grave consequences on brain development, and these effects might well obscure other defects in the nervous system. All forms of hypoglycosylation of dystroglycan in the brain lead to failures of neuronal migration, anomalies in the retina, disruption of cortical layering and lissencephaly, to varying degrees (Muntoni *et al.*, 2004b). Targeted deletion of dystroglycan in brain leads to similar symptoms (Moore *et al.*, 2002b). The cause of these disorders lies mainly in the disruption of interactions between neurons and glia cells during early brain development. Accordingly, masking of dystroglycan by antibodies inhibited granule neuron migration in cerebellar slice cultures (Qu and Smith, 2004). In addition to the defects in brain development, some forms of myodystrophy cause neurofibrillary degeneration in the cortex (Oka *et al.*, 1999).

Interestingly, deficiency of FAK, a kinase that binds to the dystroglycan complex, can give rise to comparable abnormalities in brain development (Beggs *et al.*, 2003). For a review of the roles of FAK in neuronal migration, see (Nikolic, 2004).

The myd<sup>LARGE</sup> mouse is an established animal model for human myodystrophies (Grewal and Hewitt, 2002). These mice have a mutation in the gene encoding the glycosyltransferase LARGE, which parallels the mutation in the human disease MDC1D. Besides muscle pathology, myd<sup>LARGE</sup> mice exhibit eye and brain defects resembling those seen in human myodystrophies (Holzfeind *et al.*, 2002).

The aberrant glycosylation seen in human myodystrophies and in myd<sup>LARGE</sup> mice abolishes the interaction of dystroglycan with its main extracellular ligands: laminin, agrin and neurexin (Michele *et al.*, 2002). It can be presumed that binding to perlecan is affected as well. It is likely that the loss of binding to these molecules is the main mechanism of the pathology of these diseases, although erroneous cellular targeting of incorrectly glycosylated dystroglycan might also play a role. A mutation in the dystrophin gene causes Duchenne muscular dystrophy, a disease with symptoms comparable to those caused by hypoglycosylation of dystroglycan.

## Infectious Diseases

Alpha dystroglycan is the cellular receptor for lymphocytic choriomeningitis virus, lassa fever virus and mycobacterium leprae (Cao *et al.*, 1998; Rambukkana *et al.*, 1998). *M. leprae*, the causative agent of leprosy,

targets dystroglycan/ $\alpha$ 2-laminin – complexes on Schwann cells and leads to demyelination and secondary nerve degeneration.

## Synaptic function

There exists a large quantity of research on the role of dystroglycan in the function of neuromuscular synapses, opposed to a relatively small number of studies that deal with dystroglycan at interneuronal synapses. It can be assumed that there is at least some functionality of dystroglycan common to both types of synapses. It is therefore warranted to examine the current theories about dystroglycan in the neuromuscular junction and to subsequently check for possible parallels in interneuronal synapses.

## Function at the NMJ

At the neuromuscular junction, dystroglycan in the postsynaptic membrane acts as a binding partner for molecules of the extracellular matrix and is involved in the formation of postsynaptic clusters of acetylcholine receptors in response to agrin and laminin. There exists a complex interplay between dystroglycan and the muscle specific kinase MuSK. MuSK does not bind agrin directly, but it is an important part of a signal cascade involved in the clustering of acetylcholine receptors induced by agrin. *In vitro* agrin alone is sufficient to trigger acetylcholine receptor clustering, the formation of a synapse-specific extracellular matrix and the recruitment of the subsynaptic apparatus (reviewed in (Patton, 2003)).

Although MuSK alone is sufficient to trigger clustering *in vitro*, muscular synaptogenesis is disturbed in chimeric mice lacking dystroglycan (Cote *et al.*, 1999). Conversely, overexpression of dystroglycan in cultured myotubes interferes with agrin-induced acetylcholine receptor clustering (Heathcote *et al.*, 2000), as does overexpression of its  $\beta$ -subunit (Kahl and Campanelli, 2003). The inhibitory activity is dependent on an intact juxtamembrane region, but not on those sites that bind extracellular and most intracellular ligands (like dystrophin/utrophin and probably rapsyn). These contradictive findings have not been integrated into one satisfying theory so far.

Laminin is also able to induce clustering of acetylcholine receptor *in vitro* through a mechanism different to that of agrin (Lee *et al.*, 2002). This action of laminin is dependent on dystroglycan (Jacobson *et al.*, 2001). Expression of the laminin  $\beta$ 2 – chain is crucial for the development of motoric endplates. Furthermore, the number of presynaptic active zones and junctional folds in  $\beta$ 2 knockout mice is reduced and the exclusion of Schwann cells from the synaptic cleft is hampered. Generally, the active exclusion of glia cells by certain laminin isoforms might be crucial for synaptic stability at neuromuscular junctions (Patton *et al.*, 1998a).

Mice with a disrupted glycosylation of dystroglycan (*myd*<sup>LARGE</sup>) show disorganized muscular endplates, simplified presynaptic endings and a decreased binding of bungarotoxin to muscular acetylcholine receptors (Ruth Herbst and Reginald Bittner, unpublished observation).

Dystroglycan seems to stabilize acetylcholine receptor clusters by forming a supportive intracellular scaffold, while MuSK is needed for the initiation of clustering. The role of dystroglycan seems to be the general binding of extracellular matrix molecules, rather than binding to agrin specifically.

It is important to be aware that neither MuSK nor a functional equivalent has been found at interneuronal synapses up to now. Moreover, molecules of the extracellular matrix are largely absent from the narrow interneuronal synaptic clefts.

Rapsyn is essential for clustering of acetylcholine receptors at the neuromuscular junction triggered by activation of MuSK, and it associates with dystroglycan. Rapsyn – knockout mice do not form acetylcholine receptor clusters at neuromuscular junctions and die within hours after birth. Isoforms of rapsyn are found in the nervous system (Burns *et al.*, 1997a), but acetylcholine clustering was still observed in the superior cervical ganglion of rapsyn – knockout mice (Feng *et al.*, 1998b). It was found that the neurons of the superior cervical ganglion express a short isoform of rapsyn that does not support clustering of acetylcholine receptors and that clustering in these interneuronal synapses is not dependent on rapsyn. *In vivo*, clustering of neuronal acetylcholine receptors can be induced by full-length rapsyn, but these clusters do not reach the cell surface. For a more detailed review of the role of rapsyn and other ligands of dystroglycan in the neuromuscular junction see (Banks *et al.*, 2003).

Dystroglycan is essential for the clustering of acetylcholinesterase at the synaptic cleft (Peng *et al.*, 1999; Arikawa-Hirasawa *et al.*, 2002). It has been proposed that dystroglycan binds to perlecan, which in turn binds to acetylcholinesterase, building a ‘bridge’ between dystroglycan and the enzyme. Newer studies complicate that model, showing that MuSK is also involved in the formation of this complex (Cartaud *et al.*, 2004).

## Function at Interneuronal Synapses

So far, the presence of dystroglycan has been confirmed for postsynaptic terminals of cholinergic and GABAergic interneuronal synapses. Ionotropic receptors for acetylcholine and GABA share some characteristics and are distantly related to each.

The reported localization of dystroglycan in central synapses (Zaccaria *et al.*, 2001a), retina and autonomic ganglia can be accounted for by the presence of cholinergic / GABAergic terminals. Gene knockout of one of the major binding partners of dystroglycan, dystrophin, affects the expression of acetylcholine receptors, GABA receptors and rapsyn in the brain, thus strengthening this connection (Wallis *et al.*, 2004). However, a closer investigation would be necessary to exclude the presence of dystroglycan at other types of synapses.

## Dystroglycan and Cholinergic Synapses of the Superior Cervical Ganglion

The group of Zaccaria *et al.* was one of the first to investigate the function of dystroglycan at interneuronal synapses, namely the cholinergic intraganglionic synapses of the superior cervical ganglion (SCG). The SCG is part of the sympathetic nervous system and is ideally suited for the study of the dystroglycan complex and synapse formation for several reasons. Most important are its parallels to the neuromuscular system: nicotinic acetylcholine receptors of the SCG are similar in sequence and biophysical properties to those at the NMJ, autonomic preganglionic neurons are related to motoneurons and cultured neurons of the SCG form functional synapses with myotubes *in vitro* (Kobayashi *et al.*, 1984; Nurse and O'Laigue, 1975). Most of the ligands of dystroglycan have been found to be present in the SCG, including agrin, dystrophin (Gingras and Ferns, 2001a), neurexin (Mochida, 1995), nitric oxide synthase (Dun *et al.*, 1993) and rapsyn (Feng *et al.*, 1998a).

In the SCG, dystroglycan is localized to postsynaptic specializations together with acetylcholine receptors, dystrophin and spectrin.

Transection of postganglionic axons (axotomy) leads to the detachment of presynaptic boutons and the disassembly of the synapses supplying the postganglionic neurons, which is followed by a reassembly when the severed axons regenerate. Zaccaria *et al.* observed a rapid decline of postsynaptic dystroglycan, dystrophin and acetylcholine receptors after axotomy, followed by a decrease of intraganglionic synapses. When the severed axons regenerated, the number of synapses and reactivity for dystroglycan and dystrophin increased, accompanied by a slower increase of acetylcholine receptors (Zaccaria *et al.*, 1998b). On the mRNA level, acetylcholine receptor mRNA and dystrophin mRNA were decreased by postganglionic nerve crush, while dystroglycan mRNA were increased (Zaccaria *et al.*, 2001b).

The number of acetylcholine receptors and dystroglycan reactivity was found to be reduced in *mdx* mice that express a mutant dystrophin that cannot bind actin. Furthermore, the time-course of the decrease of intraganglionic synapses after axotomy was slowed in these mice, and the decrease of acetylcholine receptors, dystroglycan and dystrophin in relation to synapse number was totally abolished (Zaccaria *et al.*, 2000). This suggests that the decrease seen in control wildtype mice is mediated by dystrophin and actin.

The work of Zaccaria *et al.* highlights the role of dystroglycan in synaptic plasticity after neuronal injury and might also hint on an involvement of the dystroglycan complex in other forms of plasticity, i.e. those involved in learning and memory. It also poses an explanation for the autonomic imbalance observed in some cases of muscular dystrophy (Lanza *et al.*, 2001; Yotsukura *et al.*, 1995).

Dystroglycan was found to be colocalized with agrin and other synaptic markers in synapses of the SCG *in vitro* as well as *in vivo*, together with acetylcholine receptors and PSD95 (Gingras and Ferns, 2001c). Dystroglycan / agrin staining was found in the form of small puncta on somata and proximal dendrites, which parallels the ultrastructural distribution in central neurons. SCG neurons expressed the membrane-bound isoform of agrin with the z insert (z+), while the secreted form was detected at a low level and could have also been produced by glial cells. Cultures of SCG neurons from agrin – deficient embryonic mice showed a reduction of synapse number and aberrant matching of pre- and postsynaptic terminals compared to control cultures (Gingras *et al.*, 2002). Synapse formation could be partially rescued by adding soluble z+ agrin to the culture medium, while z- agrin did not have an effect. Adding agrin antibodies to control cultures mimicked the effect of agrin deficiency. *In vivo*, agrin-deficiency impaired the matching of pre- and postsynaptic terminals, but the total number of synaptic terminals was not affected. Functional studies *in vivo* also revealed that paired pulse depression and post-tetanic potentiation were enhanced, which might hint to a defect of the presynaptic machinery. It has still to be confirmed whether the interaction between presynaptic agrin and postsynaptic dystroglycan is involved in the actions of agrin in the SCG or not. The different effects of z+ and z- agrin, both of which bind dystroglycan, weakens this hypothesis to some degree.

## Agrin in the CNS

Disturbed agrin expression hampers synaptic differentiation in hippocampal neurons (Bose *et al.*, 2000) and selectively attenuates the clustering of GABA receptors {Ferreira 1999 86 /id}. Agrin is also synthesized by retinal cells, is active in the clustering of acetylcholine receptors and colocalizes with synapses and gephyrin, a molecule involved in the clustering of certain GABA subunits (Koulen *et al.*, 1999; Mann and Kroger, 1996).

However, dystroglycan was clustered at GABAergic synapses in cultures of nerve cells with a total knockout of agrin (Levi *et al.*, 2002b). This suggests that a loss of clustering of dystroglycan is not the reason for the effects observed *in vivo*, and that the interaction with other molecules (like neurexin) is responsible for the clustering of dystroglycan.

Agrin also participates in the formation of neuroeffector junctions of the autonomic nervous system, for instance, it is located to parasympathetic varicosities of the rat urinary bladder (Gingras *et al.*, 2005). Application of agrin to rat adrenal slices led to a rapid switch in the phenotype of developing synapses between the splanchnic nerve and the chromaffin cells, with the synapses shifting from electrical coupling to cholinergic transmission (Martin *et al.*, 2005).

It can be concluded that agrin is involved in the formation of central synapses and that it is localized to those types of synapses that also contain dystroglycan, but its exact function and its relation to dystroglycan remain unknown. The questions surrounding agrin in the central nervous system are further reviewed in (Smith and Hilgenberg, 2002a).

## The Neurexin Connection

The  $\alpha$  – and  $\beta$  – neurexins are key factors for the formation and function of presynaptic active zones. Clustering of presynaptic  $\beta$  – neurexin by postsynaptic neuroligin is sufficient to trigger the formation of the whole presynaptic apparatus (Dean *et al.*, 2003b), and  $\alpha$  – neurexin is implicated in the function of presynaptic calcium channels and vesicular exocytosis (Missler *et al.*, 2003a). The only known binding partners for  $\beta$  – neurexins are neuroligin and dystroglycan. So far, the induction of presynaptic specialisations has only been demonstrated for neuroligin but not dystroglycan.

Most research on the neuroligin / neurexin system is focused on its role in presynaptic differentiation in glutamatergic synapses, but more recent publications show that these molecules are also involved in the formation of GABAergic synapses (Levinson *et al.*, 2005). It was also shown that the synaptogenic cue conferred by the binding of neuroligin to  $\beta$  - neurexin is not unidirectional: Not only does postsynaptic neuroligin induce presynaptic differentiation via neurexin binding; presynaptic neurexin can also induce postsynaptic differentiation via neuroligin binding in both glutamatergic and GABAergic synapses (Graf *et al.*, 2004). In the same experiments Graf *et al.* also demonstrated that dystroglycan was not clustered by neurexin like neuroligin was.

Conversely to  $\beta$  – neurexins, the  $\alpha$  – neurexins do not bind to neuroligin, which leaves dystroglycan as their only known postsynaptic binding partner. It seems that  $\alpha$  – neurexins are of greater importance for GABAergic than for glutamatergic synapses. Total knockout of  $\alpha$  – neurexins leads to a drastic reduction of GABAergic synapses in the brainstem and neocortex, while the number of glutamatergic synapses is unaffected (Missler *et al.*, 2003b). This could hint on a functional similarity between neuroligin and dystroglycan in inhibitory synapses. Neuroligin and dystroglycan could be colocalized on postsynaptic terminals, giving rise to functional redundancy.

The hypothesis of a presynaptic differentiation being triggered by dystroglycan via presynaptic neurexin is questioned by the work of Levi *et al.* (Levi *et al.*, 2002c), who examined the time course of GABAergic synapse formation *in vitro*. They found that dystroglycan was clustered at synaptic loci *after* synaptic vesicles and the GABA receptors appeared at the nascent synapse. It is therefore unlikely that dystroglycan is essential for the major steps of presynaptic differentiation.

It should be added that newer studies indicate that the action of neurexins on synaptic function is not confined to presynaptic terminals and describe postsynaptic effects (Kattenstroth *et al.*, 2004).

The presence of neurexin at neurons from the superior cervical ganglion has been demonstrated *in vitro* (Mochida, 1995), indicating that neurexin could also be of importance for cholinergic synapses.

## Dystroglycan at Central GABAergic Synapses

Dystroglycan, dystrophin and syntrophin are clustered together with GABA(A) receptors in a subset of GABAergic synapses in the brain (Knuesel *et al.*, 1999b; Levi *et al.*, 2002d; Brunig *et al.*, 2002b), and the number of GABA receptors at these synapses is reduced by about 50% in *mdx* mice that have a mutation in the dystrophin gene (Knuesel *et al.*, 1999a).

The decreased number of GABA receptors leads to alterations in synaptic plasticity. Short term depression, short term potentiation and the initial phase of long term potentiation (LTP) are facilitated in the CA1 region of hippocampal slices from *mdx* mice, and these effects are likely to be conferred by reduced GABAergic activity (Vaillend and Billard, 2002).

A study that employed mice with a targeted deletion of dystroglycan in brain contrasts these findings. It found LTP upon high – frequency stimulation to be blunted at CA3 – CA1 synapses in hippocampal slices, while other parameters of neurotransmission were unchanged (Moore *et al.*, 2002a). The study used a Cre/Lox system that affects a large proportion of glia and neurons in the brain (Zhuo *et al.*, 2001), but it might be possible that the suppression of dystroglycan in the GABAergic cells was not sufficient. Furthermore, the knockout of dystroglycan led to a gross alteration of brain architecture, which makes it hard to compare functional findings between knockout and control group.

The importance of dystroglycan in the formation of GABAergic synapses is questioned by *in vitro* cultures of neurons with a targeted deletion of dystroglycan. Lévi *et al.* showed that synapses of cultured neurons formed in

the absence of dystroglycan (Levi *et al.*, 2002e). Furthermore, they showed that the clustering of dystroglycan at synapses could still be observed in the absence of agrin, gephyrin and dystrophin.

Brunig *et al.* found that GABA receptors and gephyrin are sometimes mistargeted to glutamatergic synapses under certain culture conditions, but the dystroglycan associated complex was exclusively found opposed to GABAergic terminals (Brunig *et al.*, 2002a). This means that a) clustering of GABA receptors is independent of the dystrophin associated complex, b) clustering of GABA receptors can be triggered both by glutamatergic and GABAergic terminals, c) dystroglycan can only be clustered by GABAergic, but not glutamatergic terminals, d) clustering of dystroglycan and GABA receptors is mediated by parallel mechanisms that are not interdependent. The mechanisms behind the clustering of GABA receptors remain unknown.

Again, the findings suggest that the role of dystroglycan lies in the modulation and plasticity of synaptic function rather than in the direct initiation of synapse formation.

## **GABA, Glutamate and Susceptibility for Seizures**

Through its function at inhibitory GABAergic synapses, it might be hypothesized that malfunction of dystroglycan and its associated proteins leads to phenomena of neuronal overexcitation, e.g. seizures and excitotoxic injury. The results so far are contradictory: Yoshihara *et al.* described *reduced* seizure susceptibility in *mdx* mice deficient in dystrophin (Yoshihara *et al.*, 2003). They found reductions of the kainic acid receptor density in the brain and attributed the increased resistance towards seizures to this observation. They also found that the resistance to seizures induced by GABA – antagonists was not changed. Dystroglycan is not found at glutamatergic synapses, so the reduction of kainate receptors is likely to be caused by secondary mechanisms, i.e. in response to decreased inhibitory activity of GABAergic synapses or changes in brain development.

The dystroglycan associated complex is also involved in the reaction to chemically induced seizures, as expression of dystrophin in neurons is altered in response to seizures, along with agrin and GABA(A) receptors (Knuesel *et al.*, 2001d; O'Connor *et al.*, 1995).

Knuesel *et al.* found that knockout of utrophin, another protein associated with dystroglycan and located extrasynaptically, increased the vulnerability towards excitotoxicity and seizures induced by kainate (Knuesel *et al.*, 2002c). They also observed that, upon excitotoxic injury, utrophin is expressed in neurons of the dentate gyrus that do not normally express this protein. They deduce that expression of utrophin is necessary for the structural stability and survival of neurons after injury, which points out to the fact that the influence of the dystroglycan complex on neuronal functioning is not solely confined to synaptic transmission.

## **Myoclonus Dystonia**

Myoclonus dystonia is a hereditary movement disorder with symptoms of dystonia together with jerky movement and tremor, resembling myoclonus. Different genetic causes have been found for the disease, one being a mutation in the  $\epsilon$  – sarcoglycan gene (Borges *et al.*, 2000; Hjermind *et al.*, 2003; Marechal *et al.*, 2003; Doheny *et al.*, 2002a). The transmembrane sarcoglycan complex is associated with dystroglycan in muscle and other tissues, and it has been hypothesized that sarcoglycans protect dystroglycan from degradation by matrix metalloproteinases (Yamada *et al.*, 2001a).  $\epsilon$  – sarcoglycan is the only member of the sarcoglycan family that was found to be expressed in central neurons.

Myoclonus dystonia is associated with symptoms of anxiety, depression, obsessive – compulsive disorder and agoraphobia, epilepsy and cognitive deficits (Saunders-Pullman *et al.*, 2002b; Doheny *et al.*, 2002b; Foncke *et al.*, 2003). The movement symptoms are responsive to ethanol and benzodiazepines, and comorbidity with alcohol addiction is reported (Saunders-Pullman *et al.*, 2002a). In view of these findings, it seems very likely that the mutation of  $\epsilon$  – sarcoglycan affects the stability of the dystroglycan complex at central GABAergic synapses, thereby leading to a reduction in synapse number and inhibitory transmission.

## **The Link between Dysbindin and Schizophrenia**

Dysbindin is a binding partner of the dystroglycan complex and its mutation has recently been associated with schizophrenia. However, a later study found that, contrary to expectations, dysbindin expression is decreased in the *presynaptic* glutamatergic terminals in a subgroup of patients suffering from schizophrenia (Talbot *et al.*, 2004). The dystroglycan complex is absent from these nerve terminals, which suggests that dysbindin also has a function independent of the DGC and that the DGC is not involved in the symptoms observed.

## **Myelination and Glia**

Targeted deletion of dystroglycan in Schwann cells leads to impaired myelination of axons and to a selective perturbation of the clustering of  $\text{Na}^+$  – channels at the nodes of Ranvier (Saito *et al.*, 2003).

The ion channels on a myelinated axon are distributed in correspondence with the periodic segments of myelination (nodal, paranodal, juxtaranodal, internodal). This distribution is upheld by the coordinated interaction between membrane proteins on the respective structures of the myelinating Schwann cell and their

binding partners on the membrane of the axon (reviewed in (Scherer and Arroyo, 2002)). The nodal segments of the axon are not tightly wrapped by the myelin sheath, but the Schwann cells extend microvilli that contact the surface of the nodal axon. These microvilli bear dystroglycan, which has proofed to be essential for the clustering of Na<sup>+</sup> - channels in the nodal region. The deletion of dystroglycan in the study of Saito *et al.* resulted in malformation of the microvilli and a dispersal of the Na<sup>+</sup> - channels along the axon, while the other ion channels were still confined to their axonal segments. This suggests that dystroglycan of the Schwann cell microvilli might interact with an unknown binding partner on the axonal surface that is able to bind to the Na<sup>+</sup> - channels. It is intriguing to speculate that agrin, neurexin or a neurexin - related molecule might be that binding partner. Neurexin was detected on myelinated nerves innervating the *Torpedo* electric organ (Russell and Carlson, 1997). The proteins caspr and caspr2 are located to the axonal membrane and confer axonal specialisation at the paranodal and juxtaparanodal region, respectively. Both are structurally related to neurexins (in fact, caspr was termed 'neurexin IV' when it was first discovered, but proofed to be part of a distinct class of proteins afterwards).

However, it is also possible that dystroglycan is solely responsible for the stabilisation of the microvilli, and that other proteins are involved in the interaction with the axonal surface.

Dystroglycan also has important functions in astrocytes and for the functioning of the blood – brain barrier. In astrocytes, dystroglycan seems to be responsible for the aggregation of the potassium channel Kir4.1 and the aquaporin AQP4 in response to laminin (Guadagno and Moukhles, 2004c). *Mdx* mice with mutations of dystrophin also exhibit failures in the aggregation of AQP4 (Vajda *et al.*, 2002), together with malformation and increased permeability of the blood – brain barrier (Nico *et al.*, 2003).

## Outlooks

In spite of its importance in various diseases the role of the dystroglycan complex in the nervous system remains enigmatic. This can be accounted for by its versatility: on one hand, defects involving the dystroglycan complex have a multitude of consequences which overshadow one another. On the other hand, dystroglycan seemingly transcends classical concepts of molecular biology by acting as both a mechanical building block and as a molecule in signal transduction. Lastly, the multitude of interactions with other cellular systems complicates the matter even more. It is likely that the molecular complex around dystroglycan serves a multitude of subtle synaptic functions instead of a single, clear – cut one.

Dystroglycan and its associated molecules will certainly proof to be a worthwhile object of investigation in the next years and decades. Dystroglycan has so far only been characterized at central GABAergic and sympathetic cholinergic synapses, but not at other synapses and locations (e.g. central cholinergic synapses). Besides GABAergic and cholinergic synapses, other types of synapses might carry dystroglycan as well. Glycinergic synapses would be possible candidates, as they are phylogenetically related to nicotinic and muscarinic receptors. For the general investigation of the roles of dystroglycan in synaptic functioning, the superior cervical ganglion might proof as a useful model system, both *in vivo* and *in vitro*. Besides synaptic function, another short – term goal could be the investigation of myelination in patients with muscular dystrophies.

Long – term goals include the complete understanding of dystroglycan in brain development and synaptic plasticity. To achieve such an understanding, the mechanisms of this system must be investigated on different temporal and spatial scales and have to be integrated into a single theory.

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