Dystroglycan

Dystroglycan was first purified from rabbit skeletal muscle as a membrane glycoprotein component of the dystrophin–glycoprotein complex (DGC).[1] In muscle, it binds to both laminin-2 in the extracellular matrix and to dystrophin in the cytoskeleton.[2] Mutation of genes encoding a number of proteins associated with dystroglycan in the DGC leads to distinct forms of muscular dystrophy.[3] Dystroglycan is also expressed in a wide variety of developing and adult non-muscle tissues where it mediates cellular interactions with the extracellular matrix.

Synonyms

α-dystroglycan: SL 156,[4] 156-dystrophin-associated glycoprotein (DAG),[5] cranin,[6] laminin binding protein (LBP)-120.[7] β-dystroglycan: 43-DAG,[5] A3a.[8]

Protein properties

Dystroglycan is composed of two subunits.[9] The α subunit is an extracellular peripheral membrane glycoprotein which ranges in size from around 120 kDa to over 190 kDa depending on the tissue source and method of analysis.[1,6,7,10] The β subunit is an integral membrane glycoprotein that, in contrast to the α subunit, displays a conserved molecular weight of around 43 kDa in a wide range of species and tissues.[1,8] Both of these subunits are the post-translationally derived products of a single mRNA transcript encoded by a single dystroglycan gene.[9] The considerable heterogeneity in the apparent molecular weight of α-dystroglycan is most probably the result of differential carbohydrate modification.[2,6] Indeed, α-dystroglycan is extensively glycosylated and tends to migrate as a broad band in SDS-polyacrylamide gels. The nature of these modifications is only partially understood at present. There are both *N*- and *O*-linked carbohydrate moieties present on α-dystroglycan. The *N*-linked sugars are of the high mannose variety. The *O*-linked sugars are less well characterized, but are thought to be of a mucin-type with an unusual *O*-mannosyl glycosidic linkage.[11]

Dystroglycan is expressed in a broad array of adult and developing tissues and cell types.[9,31] In general, dystroglycan is localized to some, but not all, cellular domains that are in close apposition to laminin-containing extracellular matrix. Examples include the sarcolemma, the neuromuscular junction, the Schwann cell outer membrane surrounding peripheral nerve, epithelial basement membranes, smooth muscle, and the cellular constituents of Reichert's membrane in the developing rodent embryo (Fig. 1). Dystroglycan is expressed early in mouse development with abundant expression in the egg cylinder stage embryo, and in the maternal decidual tissue.

At least several binding partners for both α- and β-dystroglycan are known. Complementing dystroglycan's juxtaposed localization to basement membranes *in vivo* is the ability of α-dystroglycan to bind laminin-1[9] and laminin-2[12] *in vitro* with high affinity. As α-dystroglycan binding to these laminins appears to be mediated through the G domains at the carboxyl terminus of the laminin α chain,[7] it is likely that α-dystroglycan binds to other laminin heterotrimeric isoforms. Dystroglycan also binds to agrin,[10] an extracellular matrix proteoglycan critically involved in neuromuscular junction formation, through a conserved G domain.[13] It is not yet clear whether α-dystroglycan exhibits specific binding to a single extracellular ligand in cases where more than one of its potential ligands are co-expressed within the extracellular matrix. The apparently homogeneous primary structure of dystroglycan indicates that ligand selectivity would have to be regulated by post-translational mechanisms, such as carbohydrate modification. Some data indicates that heparan sulphate proteoglycans might mediate ligand binding specificity.[14] Alternatively, in at least one tissue, peripheral nerve, dystroglycan may be able to bind both agrin and laminin-2 through overlapping binding sites.[15]

β-dystroglycan also binds to several related intracellular ligands. In skeletal muscle, at the sarcolemma, β-dystroglycan binds to dystrophin which, in turn, binds to F-actin.[2] This interaction is mediated between the carboxyl-terminal 15 amino acids of β-dystroglycan and a larger cysteine-rich domain in dystrophin.[16,17] This same interacting region is also present in dystrophin isoforms

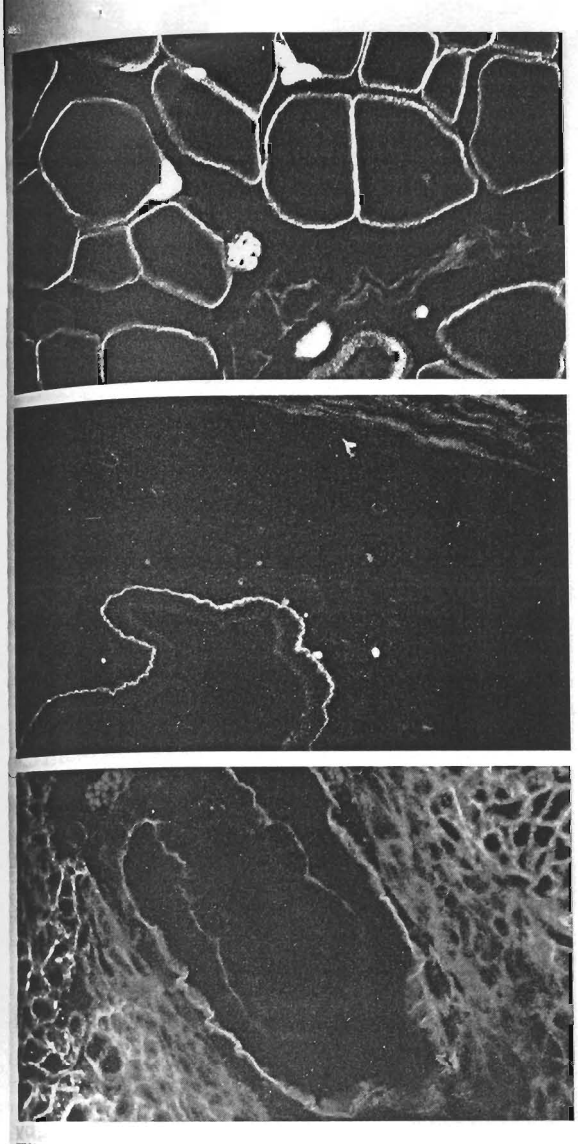

Figure 1. Localization of dystroglycan protein in various tissues. Top: section of mouse skeletal muscle showing dystroglycan localization in the sarcolemma, neuromuscular junction, peripheral nerve bundle, and the wall of a major blood vessel. Middle: section of mouse uterus showing dystroglycan localization to the lumenal epithelial basement membrane, and smooth muscle in the myometrium. Bottom: Parasaggital section through an embryonic day 6.5 mouse embryo *in utero* showing embryonic dystroglycan localization to Reichert's membrane, and a basement membrane between the visceral endoderm, and ectodermal layers, and maternal localization to the decidual cells surrounding the embryo.

derived from alternative promoters within in the dystrophin gene, which are expressed in non-muscle tissues, and is also conserved in utrophin, a broadly expressed dystrophin homologue. In fact, utrophin may replace dystrophin as β-dystroglycan's binding partner at the neuromuscular junction, indicating that distinct dystroglycan complexes can exist within the same cell type[18]. Interestingly, the proline-rich carboxyl terminus of β-dystroglycan also interacts with the cell signalling molecule grb-2 via its SH3 domain.[19] However, a cell signalling function for dystroglycan has yet to be characterized.

The sarcoglycans are a group of four intergal membrane proteins that are known to interact with dystroglycan in muscle. The details of their association with dystroglycan are unclear at present. The sarcoglycans do form a tight complex with dystroglycan that can only be dissociated by relatively harsh biochemical treatments.[20] One possible role for the sarcoglycans may be to stabilize the association of α-dystroglycan with the sarcolemma. Although α- and β-dystroglycan can be isolated together *in vitro* as a non-covalently associated unit,[20] this connection may be tenuous *in vivo* and could require the support of other proteins like the sarcoglycans.[21] Figure 2 summarizes the current state of understanding of dystroglycan and its binding partners in skeletal muscle, where this complex is best understood, but similar complexes are likely to exist in other tissues. Defining dystroglycan's molecular partners in non-muscle tissues is a clear target for future research.

Dystroglycan function has been inferred from several lines of evidence. First, based on both the abundance of dystroglycan in muscle and the consequences of disrupting the dystroglycan-mediated linkage between the extracellular matrix and the cytoskeleton, as occurs in several forms of muscular dystrophy, it was proposed that dystroglycan served as a mechanical linkage protecting the sarcolemma from contraction-induced shear forces.[5] Accordingly, dystroglycan behaves as a cell adhesion molecule in Schwannoma cell cultures.[22] Antibody inhibition studies have revealed important roles for dystroglycan in various developmental contexts. These show that dystroglycan function is necessary for acetylcholine receptor clustering[23] and epithelial morphogenesis.[24] However, the mechanistic details for dystroglycan's involvement in these processes have remained elusive. The phenotype of the dystroglycan-null mutant mouse offers a plausible hypothesis for these developmental effects of inhibiting dystroglycan function. The dystroglycan-null mutant mice die early in embryonic development as a result of structural and functional defects in Reichert's membrane, a laminin-rich extraembryonic basement membrane.[25] The details of the phenotype indicate that dystroglycan is required for the development of Reichert's membrane, possibly by mediating the assembly of laminin networks present in that structure. Therefore, dystroglycan may also be mediating the assembly of the extracellular matrix in developing neuromuscular synapses, epithelia and other tissues in which it is expressed. Perhaps dystroglycan acts in the assembly of extracellular matrices

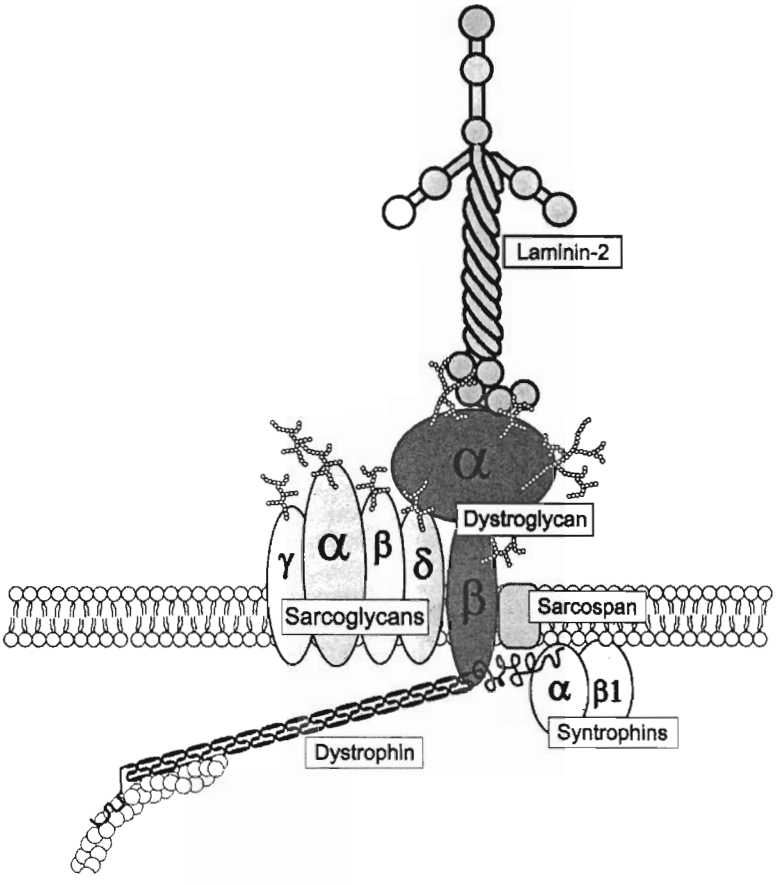

Figure 2. Model of the DGC. Dystroglycan is a central component of the DGC as it is known to link laminin-2 in the extracellular matrix, and dystrophin in the cytoskeleton. Mutations in genes encoding proteins associated with dystroglycan–dystrophin, each of the sarcoglycans, and the laminin $\alpha 2$ chain lead to distinct forms of muscular dystrophy. The sarcoglycans form a subcomplex within the DGC. The relationship of sarcospan, a newly identified component of the DGC, is not yet full characterized.

during embryonic development or tissue remodeling and persists in mature tissues as a stable linkage between the cell and extracellular matrix.

Purification

α- and β- dystroglycan were first purified from skeletal muscle as a part of a glycoprotein complex which anchors dystrophin to the muscle cell membrane. This complex, the DGC, is purified from digitonin-extracted rabbit skeletal muscle microsomes by affinity chromatography on succinylated wheat germ agglutinin–sepharose followed by DEAE chromatography and a final step on a sucrose gradient.[1] A similar scheme has been used to purify dystroglycan from bovine peripheral nerve.[26] Dystroglycan was purified as an agrin receptor from the post-synaptic membranes of the electric organ from *Torpedo californica* utilizing an immunoaffinity approach.[10] α- Dystroglycan can be purified by pretreatment of skeletal muscle microsomes with high pH[14] or by laminin or lectin affinity chromatography from brain homogenates.[6,7]

Activities

α-Dystroglycan binds to laminin and agrin isoforms with affinities in the low nanomolar range as judged by blot overlay assays.[2,12] This binding is calcium-dependent, salt-sensitive, and heparin-inhibitable. β-Dystroglycan binds tightly and specifically to dystrophin in a variety of *in vitro* assays.[16,17]

Antibodies

Monoclonal antibodies specific for either α- or β-dystroglycan have been raised by several laboratories. Some of these are commercially available. Applications

and cross-reactivities for these reagents vary. One monoclonal antibody, IIH6, developed in our laboratory, blocks the association between α-dystroglycan and laminin or agrin *in vitro*.[2,12] This blocking antibody also specifically inhibits neuromuscular junction formation and kidney epithelial morphogenesis *in vivo*[23,24]. Polyclonal antibodies have been raised against α-dystroglycan, β-dystroglycan, or both subunits by several laboratories. In general, the polyclonal antibodies against dystroglycan tend to be widely species cross-reactive. Currently, no polyclonal antibodies against dystroglycan are commercially available.

Genes

Only a single dystroglycan gene is known to exist in each species in which it has been identified. Both the genomic structure and coding potential of this gene are highly conserved among mammalian species.[9,27] The primary amino acid sequence encoded by the dystroglycan gene is rather well conserved between mammals and the electric ray *Torpedo*. Clones exhibiting similarity to dystroglycan have recently been discovered in invertebrate species (*Drosophila*, *C. elegans*) (S. Baumgartner; personal communication). In humans, this gene is located at 3p21.[27] In mice, it is located in a syntenic region at the distal tip of chromosome 9[28](EMBL/GenBank U48854). In both of these species, dystroglycan is organized into two exons. A single 5.8 kb mRNA species has been characterized in each of the tissues and developmental stages that have been analysed.[9,27] Full length or partial cDNA clones exist for human (GenBank L19711), rabbit (GenBank X64393), and mouse (EMBL Z34532) dystroglycan.

Mutant phenotype/disease states

Despite the fact that mutations in genes encoding a number of dystroglycan's binding partners within the DGC lead to distinct forms of muscular dystrophy in humans and amimals,[3] there is as yet no linkage between mutations in the dystroglycan gene and muscular dystrophy. Nevertheless, a common feature of muscular dystrophies involving other DGC components is a reduction or destabilization of dystroglycan in association with the sarcolemma.[1,21] A likely explanation for the lack of a null mutant form of dystroglycan linked to muscular dystrophy is provided by the phenotype of the dystroglycannull mutant mouse that dies during early embryogenesis.[25] Therefore, certain DGC components may support the muscle-specific functions of dystroglycan, but not its non-muscle roles.

Structure

Very little is known about the structure of dystroglycan at present. Electron microscopic analysis of purified preparations of α-dystroglycan suggests that it is an elongated, dumb-bell-shaped molecule.[29] Ultrastructural analysis *in situ* also argues for an extended conformation of α-dystroglycan.[30]

Web sites

www-camlab.physlog.uiowa.edu

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