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Mini review

Functional diversity of dystroglycan

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ABSTRACT

During the last 15 years, following its identification and first detailed molecular characterization, the dystroglycan (DG) complex has taken centre stage in biology and biomedicine. Functions in different cells and tissues have been identified for this complex, ranging from its typical role in skeletal muscle as a sarcolemmal stabilizer, highlighted by the recently identified "secondary dystroglycanopathies", to a variety of very diverse functions including embryogenesis, cancer progression, virus particle entry and cell signalling. Such functional promiscuity can be in part explained when considering the multiple domain organization of the two DG subunits, the extracellular α-DG and the transmembrane β-DG, that has been largely scrutinized, but only in part unraveled, exploiting a variety of recombinant and transgenic approaches. Herein, while rapidly recapitulating some of the functions that nowadays can be assigned safely to each DG domain, we also try to envisage a sort of worry list featuring and dwelling on some of the most compelling "mysteries" that should be solved to finally understand DG's functional diversity.

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1. Introduction

1.1. Identification and cloning: DG is a consolidated sarcolemmal marker in vertebrates

Following its pioneering discovery in 1987 as a novel laminin-binding protein (then named cranin) in the plasma membrane of different cell types (Smalheiser and Schwartz, 1987), dystroglycan (DG), along with its corresponding gene (*dag1*), has been first cloned and characterized in Kevin Campbell's laboratory in Iowa City at the beginning of the 90s (Ibraghimov-Beskrovnaya et al., 1992). The DG complex is composed of two subunits, namely α and β. α-DG is a highly glycosylated peripheral membrane protein that establishes a network of interactions with other extracellular proteins such as laminins, agrin, perlecan, neurexin and biglycan; its C-terminal domain interacts noncovalently with the N-terminal extracellular domain of the β-subunit. β-DG crosses the membrane, and its cytosolic domain is anchored to actin through the interaction with dystrophin or utrophin, a dystrophin homologue expressed in non-muscular tissues. The same domain binds also other proteins, such as rapsyn, caveolin-3 and Grb2 (Barresi and Campbell, 2006).

DG belongs to the dystrophin–glycoprotein complex (DGC), together with sarcoglycans, dystrobrevins, syntrophins and sarcospan (for a recent extensive review see Ervasti and Sonnemann, 2008). Such a crowded glycocomplex, which includes other peripheral members or associated proteins, such as nitric oxide synthase and caveolin-3, binds the WW domain of dystrophin (Huang et al., 2000) via a "terminal site" (the last 15 aa) of the β-DG cytodomain, thus contributing to the formation of countless "molecular pillars" that, from the basement membrane surrounding the cell, cross the plasma membrane and eventually protrude through the cell cytoskeleton (Ervasti and Campbell, 1993).

DG has a wide tissue distribution and is expressed in muscle, in the central and peripheral nervous system, in epithelia and endothelia (Durbeej and Campbell, 1999). Typically, in mammals the *dag1* gene encodes for a polypeptide precursor of 895 amino acids, which undergoes a post-translational proteolytic cleavage resulting in two subunits, α and β, that interact noncovalently (Ibraghimov-Beskrovnaya et al., 1992). Indeed, the exact significance of the post-translational process of the DG precursor has not been clarified yet and it will be extensively discussed within the next paragraphs.

1.2. Specialized functions within basement membranes

The primary structure of the DG complex is highly conserved in all the mammals analyzed and maintains a high degree of homology in lower vertebrate species, suggesting that the domain organization and the function of the complex have been conserved during evolution. Nonetheless, although in humans and other mammals DG has been shown to play a major role in sarcolemmal stability, in lower species such as *D. rerio* (zebrafish) abolishing the expression of DG does not impair the embryonic development (as observed in mice, see below), and the adult animals display a severe form of dystrophy only later (Parsons et al., 2002).

The DG gene, or some surrogate forms defined as DG-like, has been found also in invertebrates. Curiously, in *C. elegans* the deletion of the major DG-like gene (*dgn-1*) does not cause a muscle phenotype (Johnson et al., 2006). In *Drosophila*, an entire orthologue DGC has been identified, and shown to establish similar connections with laminins and the dystrophin-linked cytoskeleton (Greener and Roberts, 2000).

Targeted disruption of the DG gene results in an abrupt stop (as early as E6.5) of the mouse embryonic development, confirming the crucial role played by DG for the establishment of the first extra-embryonic basement membrane (Williamson et al., 1997). Recently, it was shown that mice with epiblast deletion of DG as early as E7,5 (maintaining expression of DG at Reichert's membrane) develop defects that resemble the Walker–Warburg syndrome (see next section), but it also appears that DG has no major role in developing the embryo properly (Satz et al., 2008).

Although no muscular diseases have been primarily linked to mutations of the DG gene, alterations of its maturation process and/or of its membrane localization have been observed in many neuromuscular disorders (Michele and Campbell, 2003). The generation of mice with conditional knockout of DG in skeletal muscle and brain contributed to the comprehension of its role in the muscular and central nervous system. While chimaeric mice develop severe muscular dystrophy (Cotè et al., 1999), the skeletal muscle specific ablation of DG results in a mild form of muscular dystrophy, revealing a role of DG in the muscle regeneration promoted by satellite cells (Cohn et al., 2002). In brain, the selected deletion of DG leads to important structural defects (Moore et al., 2002) and the conditional DG knockout in peripheral nerves demonstrates its crucial role for myelination and for the architecture of Ranvier's node (Saito et al., 2003).

At the neuromuscular junction (NMJ), where its localization depends on its interaction with ankyrin (Ayalon et al., 2008), DG binds agrin with high affinity (Gesemann et al., 1996), and is involved in the stabilization of post-synaptic acetylcholine receptor clusters (Jacobson et al., 2001). Recently, a specific role for DG was demonstrated also at the "ribbon synapse" of the retina, where it binds pikachurin, a novel extracellular matrix protein (Sato et al., 2008).

1.3. Our approach: the recombinant domain dissection of DG

Both the DG subunits are organized into subdomains, which are likely to represent autonomously folding units. α-DG is constituted by two domains (N- and C-terminal) separated by an elongated mucin-like region rich of prolines, serines and threonines and highly O-glycosylated (Brancaccio et al., 1995); β-DG is composed of an N-terminal extracellular domain, a transmembrane region and a cytoplasmatic, proline rich C-terminal domain (Fig. 1.1).

For the biochemical characterization of DG we applied an analytical method based on the dissection of DG into domains, each expressed as a recombinant protein. This successful approach enabled us to achieve several results (see also paragraph 3); amongst them, a first structural characterization of the β-DG extracellular domain by NMR that showed the absence of any well defined three-dimensional structure (Bozzi et al., 2003). Indeed, the β-DG extracellular domain should be considered as an ensemble of conformers in a thermodynamic equilibrium, capable of binding their biological partner, the C-terminal domain of α-DG. With such characteristics, the extracellular domain of β-DG belongs to the group of the "natively unfolded proteins" (Bozzi et al., 2003). A recent computational model, though, suggests the presence of at least some secondary structure in part of the β-DG ectodomain (Akhavan et al., 2008). No structural data are available on the cytoplasmatic domain of β-DG, but its high content in proline residues points to a rather disordered conformation.

As far as α-DG is concerned, the crystallographic structure of its N-terminal domain was solved, showing the presence of two subdomains, the first being an Ig-like domain and the second similar to ribosomal protein S6, connected by a flexible loop (Bozic et al., 2004).

1.4. Six dystroglycan "mysteries" …at least

Although the amount of information available on DG is nowadays considerable, at a closer analytical look many points remain obscure and need to be addressed further. In the following paragraphs (depicted in Fig. 1, each in a schematic of the corresponding number), we first highlight what has been already clarified, and then dwell on those details that need to be solved to ultimately unravel the DG's structure and function (see also Fig. 1).