The dystrophin-associated protein complex

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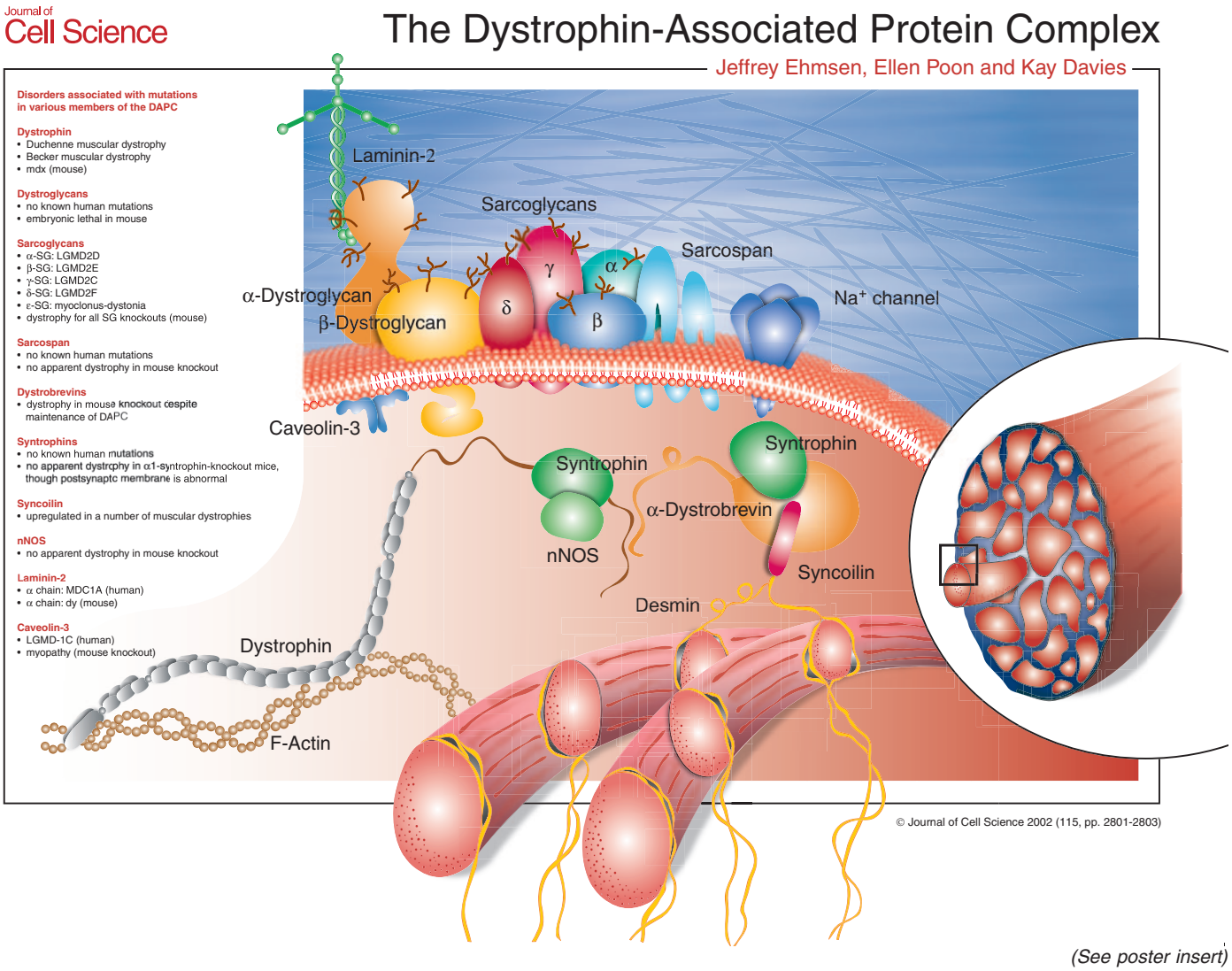
The lethal muscle-wasting disorder, Duchenne muscular dystrophy, is caused by mutations or deletions in the dystrophin gene. In skeletal and cardiac muscle, dystrophin associates with various proteins to form the dystrophin-associated protein complex (DAPC). The DAPC is thought to play a structural role in linking the actin cytoskeleton to the extracellular matrix, stabilizing the sarcolemma during repeated cycles of contraction and relaxation, and transmitting force generated in the muscle sarcomeres to the extracellular matrix (Petrof et al., 1993). There is also evidence that the DAPC is involved in cell signalling via its interactions with calmodulin, Grb2 and nNOS (Rando, 2001). Various members of the DAPC, such as the sarcoglycans, have already been implicated in a number of muscle diseases, illustrating the vital role this complex plays in the maintenance of muscle integrity. This brief review offers a glimpse of the major known proteins that constitute the DAPC and the defects caused by their absence.

Dystrophin

The muscle isoform of dystrophin is a 427 kDa protein consisting of an N-terminal actin-binding domain, a central rod-like domain comprising 24 spectrin-like triple helical coiled coils, and a cysteine-rich C-terminus that allows assembly of the DAPC. Dystrophin stretches laterally along F-actin filaments, binding primarily via three sites within the N-terminal region (Norwood et al., 2000) and electrostatically through a cluster of basic repeats (11-17) within the rod domain (Amann et al., 1998). Although remarkable evolutionary conservation exists, with even the *C. elegans* homologue possessing the same number of spectrin repeats, much of the rod domain appears dispensable and a dystrophin molecule comprising at least eight integral repeats remains relatively



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functional (Harper et al., 2002). Dystrophin deficiency results in loss of the associated protein complex and severe muscular dystrophy, underscoring the central role that dystrophin plays in assembling and maintaining the link between cytoskeletal actin and the extracellular matrix.

Dystroglycans

The widely expressed α and β dystroglycans make up the core of the DAPC, establishing the transmembrane link between laminin-2 and dystrophin. Both proteins are produced from a single post-translationally modified polypeptide, and are heavily glycosylated prior to being sorted to their respective extracellular and transmembrane locations. These glycosylation patterns are developmentally regulated and largely correlate with the diversity of binding partners in different tissues (Winder, 2001). Total deletion of dystroglycan in mouse is embryonic lethal owing to disruption of formation of the extra-embryonic basement membrane, and the void of naturally occurring mutations or other dystroglycan-associated diseases suggests that its function is indispensable for survival (Williamson et al., 1997). Disrupting the dystroglycan-dystrophin link causes a Duchenne-like phenotype, whereas disruption of the laminin-dystroglycan link causes congenital muscular dystrophy. Defects in post-translational modification of dystroglyan may also be pathogenic, as a nonsense mutation in the glycosyltransferase, Large, is the primary defect in the myodystrophy mouse (Grewal et al., 2001).

Sarcoglycans

Five transmembrane proteins, all expressed primarily in skeletal muscle, constitute the sarcoglycan family: α (50 kDa, also called adhalin), β (43 kDa), γ (35 kDa), δ (35 kDa) and ε (50 kDa). The β, γ and δ sarcoglycans co-purify, with β and δ forming an especially tight link, whereas α sarcoglycan may be spatially separated. Dystrophin and γ sarcoglycan can interact directly, and δ sarcoglycan appears to be coordinated to the dystroglycan complex (Chan et al., 1998). Mutations abolishing the expression of any one of the sarcoglycans cause loss of the others from the sarcolemma; the four recessive limb girdle muscular dystrophies 2D, 2E, 2C and 2F are caused by absence of the α, β, γ or δ sarcoglycans, respectively (Bushby, 1999).

Sarcospan

Sarcospan is a 25 kDa membrane protein with four transmembrane domains and intracellular N- and C-termini, a unique feature for transmembrane proteins of the DAPC (Crosbie et al., 1997). Expression is seen predominantly in skeletal and cardiac muscle, but shorter isoforms exist in other tissues. No human disease is currently known to be associated with sarcospan deficiency, and sarcospan-null mice maintain expression of all sarcoglycans at the sarcolemma and do not develop muscular dystrophy (Lebakken et al., 2000).

α-Dystrobrevins

Alternative splicing produces five α-dystrobrevin isoforms, differing as C-terminal truncations. Three of these are found in muscle, but only α-dystrobrevin-2 is abundantly expressed at the sarcolemma. This isoform contains two tandem α-helical syntrophin-binding sites, which may be alternatively spliced to modulate the stoichiometry of syntrophin association with the DAPC (Newey et al., 2000). As dystrobrevin contains several tyrosine kinase consensus sites, protein-protein interactions may also be regulated by tyrosine phosphorylation. Dystrobrevin associates with dystrophin via coiled-coil interactions, but an independent link with the sarcoglycan/sarcospan complex might also exist as dystrobrevin and syntrophin can bind to the DAPC in the absence of the C-terminal region of dystrophin (Crawford et al., 2000). Intriguingly, dystrobrevin-knockout mice present with a DMD-like phenotype while retaining the DAPC and sarcolemmal integrity. Loss of dystrobrevin is expected to disrupt the syncoilin-mediated link between the DAPC and desmin intermediate filaments (see below), perhaps contributing to dystrophy. Alternatively, the coordinate reduction of sarcolemmal nNOS that is observed in these knockouts suggests that signalling defects may be at fault (Grady et al., 1999).

Syntrophins

All three of the 58 kDa syntrophin isoforms are found at the neuromuscular junction in skeletal muscle, but only the α1 and β1 isoforms are present along the sarcolemma. Each contains two pleckstrin homology (PH) domains, which are modules of ~100 amino acids found in many signalling proteins. Within each first syntrophin PH domain is a PDZ domain capable of facilitating homo- and hetero-dimerization with other PDZ-containing proteins. Indeed, through these types of interactions, the syntrophins may function as modular adaptors in recruiting signalling proteins to the sarcolemma and DAPC: binding interactions exist with skeletal muscle sodium channels, nNOS, serine/threonine kinases, MAST205 and stress-activated protein kinase-3 (Rando, 2001). The highly conserved C-terminal 57 amino acids [constituting the syntrophin-unique (SU) domain] probably contain binding sites for dystrophin family members. No human disease has been correlated with syntrophin mutations. Mice lacking α1 syntrophin (the predominant muscle isoform) display no overt phenotype, but nNOS is absent from the sarcolemma and the postsynaptic membrane is grossly abnormal (Kameya et al., 1999; Adams et al., 2000).

Syncoilin

Syncoilin was first identified via its interaction with α-dystrobrevin in muscle (Newey et al., 2001). Sequence analysis revealed the presence of a unique N-terminus domain and a coiled-coil domain that is typical of those found in intermediate filament proteins. Syncoilin is highly expressed in skeletal, cardiac and smooth muscle at the sarcolemma, Z-lines and neuromuscular junction. Through its interaction with desmin, syncoilin is thought to provide a link between the DAPC at the sarcolemma and the intermediate filament protein network (Poon et al., 2002). Its upregulation in a range of muscular dystrophies may be a compensatory mechanism against muscle damage.

nNOS

The production of nitric oxide (NO) by nNOS is important for increasing local blood flow to match the increased metabolic load of contracting muscles,

such as during exercise. The presence of nNOS at the sarcolemma is mediated through PDZ domain interactions with syntrophin, and it is lost in a number of muscular dystrophies including DMD. Indeed, patients with DMD show abnormal blood vessel constriction presumably due to lack of nNOS at the sarcolemma, and it is the only other protein known to correlate so closely with the fiber-type-specific onset of dystrophy. This observation is in accordance with the supposed role of the DAPC in signalling and the contribution of ischemic stress to muscle degeneration. However, abolishing nNOS expression alone in mice does not cause overt dystrophy (Crosbie et al., 1998; Chao et al., 1998).

Laminin-2

Laminin-2 is composed of α2, β1 and γ1 chains and binds to α-dystroglycan and the α7β1 integrin complex. Laminins are thought to form the structural part of the basement membranes along with collagen IV, nidogen and perlecan. Mutations of the laminin α2 gene cause severe congenital muscular dystrophy but do not appear to cause damage to the sarcolemma (Patton, 2000).

Caveolin-3

Caveolae are vesicular invaginations of the plasma membrane and are found in most cell types. The primary scaffolding protein of skeletal muscle caveolae is caveolin-3, and although traditionally considered distinct from the DAPC, a number of studies imply an important relationship. Caveolin-3 interacts directly with the C-terminus of β-dystroglycan, co-fractionates with dystrophin and α-sarcoglycan, and shows elevated expression in Duchenne muscular dystrophy. Transgenic mice overexpressing caveolin-3 present with DMD-like pathology, suggesting that competitive downregulation of dystrophin at the sarcolemma may occur. Mutations in caveolin-3 are associated with autosomal dominant limb-girdle muscular dystrophy (LGMD-1C), hyperCKemia and rippling muscle disease (Galbiati et al., 2001).

Sodium channels

Voltage-gated sodium channels bind to syntrophin PDZ domains, an interaction that may influence ion conduction properties or association with other channels (Gee et al., 1998). However, even in the absence of syntrophin, normal sodium channel distribution is seen along the sarcolemma, suggesting that this interaction is not essential for channel localization (Adams et al., 2001; Ribaux et al., 2001).

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