MECHANISMS OF DISEASE

Molecular mechanisms of muscular dystrophies: old and new players

Kay E Davies and Kristen J Nowak‡*

Abstract | The study of the muscle cell in the muscular dystrophies (MDs) has shown that mutant proteins result in perturbations of many cellular components. MDs have been associated with mutations in structural proteins, signalling molecules and enzymes as well as mutations that result in aberrant processing of mRNA or alterations in post-translational modifications of proteins. These findings have not only revealed important insights for cell biologists, but have also provided unexpected and exciting new approaches for therapy.

Sarcolemma
The muscle-cell membrane.

*Department of Physiology, Anatomy and Genetics, MRC Functional Genetics Unit, South Parks Road, Oxford OX1 3QX, UK.
‡Centre for Medical Research, University of Western Australia, Western Australian Institute for Medical Research, B Block, QEII Medical Centre, Nedlands 6009, Western Australia.
e-mails: kay.davies@human-anatomy.oxford.ac.uk; knowak@cyllene.uwa.edu.au
doi:10.1038/nrm2024
Published online 13 September 2006

The term muscular dystrophy (MD) encompasses over 30 different inherited diseases, all involving progressive weakness and degeneration of skeletal muscle (BOX 1). MDs can be devastating diseases with weakened skeletal muscles leading to loss of ambulation, difficulties in breathing and eating and, in most cases, premature death. Key features of dystrophic muscle include central nuclei, small regenerating fibres and accumulation of connective tissue and fatty tissue (see FIG. 1 for cross-sections of normal and dystrophic muscle). MDs do not usually involve the central nervous system or peripheral nerves (although some do), despite the close anatomical relationship between skeletal muscle and nerves, but they can affect the heart and other organs. Traditionally the MDs have been grouped according to their clinical and pathological manifestations (see Supplementary information S1 (box) for the more common types of MD).

Genetically, MDs can be inherited in a dominant or recessive manner or, in many cases, caused by *de novo* mutations, which are therefore sporadic. As the genetic causes have become apparent, MDs have been named to reflect the defective gene, for example, 'laminopathies', 'titinopathies', 'dystrophinopathies' and 'dysferlinopathies', with the same defective gene possibly causing more than one disease phenotype; these gene-describing titles sometimes group clinically distinct diseases together. For example, mutations in the gene that encodes the giant sarcomeric protein titin can cause both tibial MD (TMD) and the more severe limb girdle MD (LGMD2J), depending on whether the patient is heterozygous for the mutation (TMD) or homozygous for the mutation (LGMD2J)[1].

Moreover, some mutated genes not only cause more than one clinically distinct MD, but can also cause diseases that do not primarily affect skeletal muscle. Mutations in the *LMNA* gene, which encodes the nuclear proteins lamin A and lamin C can cause MDs[2,3] as well as other diseases such as the premature ageing disease Hutchinson–Gilford progeria syndrome[4–9]. To further complicate the situation, more than one gene can produce the same MD phenotype; for example, Emery–Dreifuss MD (EDMD) can be caused by autosomal dominant mutations in the *LMNA* gene[3] or X-linked mutations in the gene encoding another nuclear protein, emerin[10].

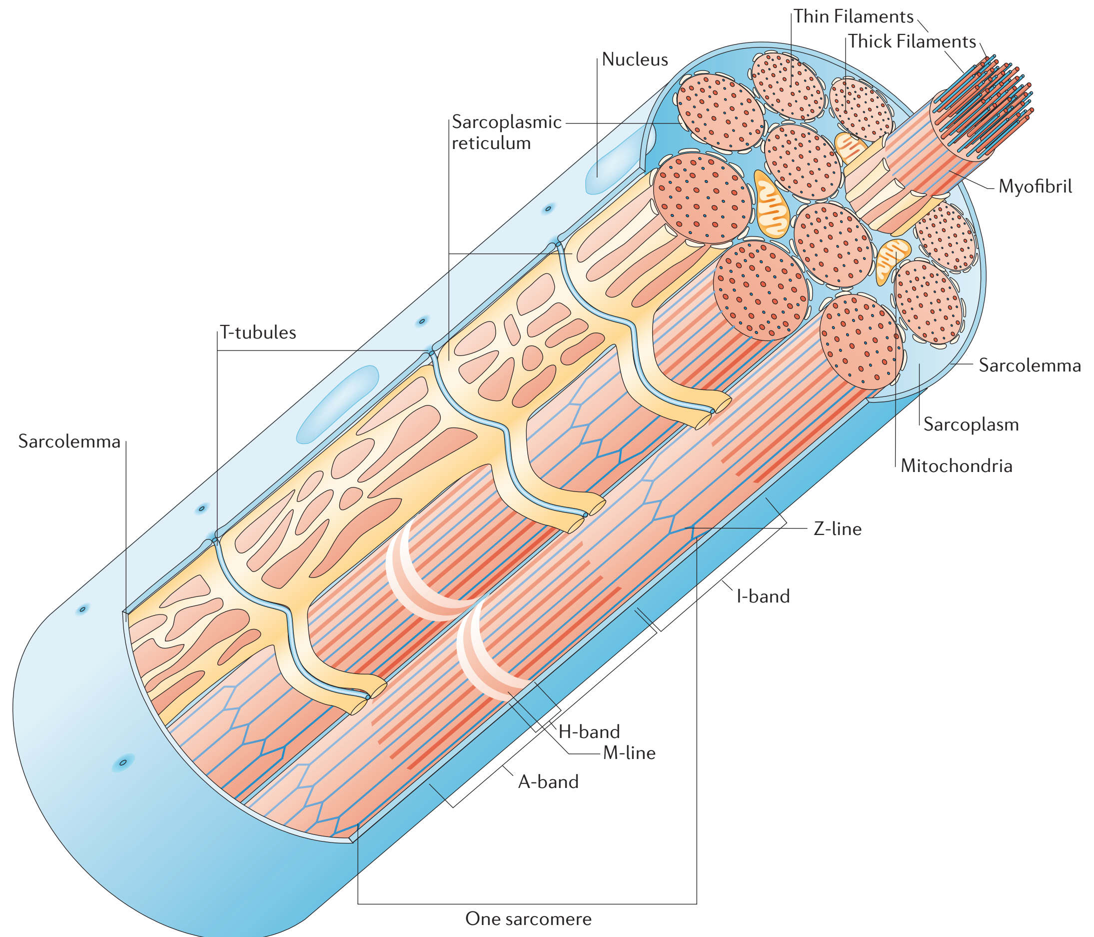
Dystrophin was the first mutant protein shown to cause MD. Mutations of the dystrophin gene, the largest gene in the human genome, cause the most common MD, Duchenne MD (DMD), as well as the milder phenotype of Becker MD (BMD)[11]. Differing mutations within the dystrophin gene determine whether a patient shows a DMD or BMD phenotype. DMD results from an absence of dystrophin or expression of a non-functional protein, whereas BMD has been associated with reduction of wild-type dystrophin or expression of a partially functional protein.

Although DMD was first described in the 1830s, it was not until 1975 that electron microscopy and biochemical analyses indicated that patients with DMD had a defect in the plasma membrane (sarcolemma) of muscle fibres[12]. The gene was located to Xp21 using linkage analysis with restriction fragment length polymorphisms[13]. The causative gene was identified in 1987 and encodes the dystrophin protein[11] (so-called because its deficiency causes dystrophy), which is localized at the sarcoplasmic surface of the sarcolemma. This was one of the first genes to be identified by positional cloning and its discovery led to the identification of other genes involved in MDs.

In this review, we discuss the vast array of skeletal-muscle proteins that are currently associated with MD (FIGS 2,3). Historically, defects in structural proteins have been predominantly associated with MD. However, we

© 2006 Nature Publishing Group

Box 1 | **The structure of a skeletal-muscle fibre**



Skeletal muscle is the muscle attached to the skeleton. Hundreds or thousands of muscle fibres bundle together to make up an individual skeletal muscle. Muscle fibres (myofibres) are long, cylindrical structures that are bound by a plasma membrane (the sarcolemma) and an overlying basal lamina and when grouped into bundles (fascicles) they make up muscle. The sarcolemma forms a physical barrier against the external environment and also mediates signals between the exterior and the muscle cell.

The sarcoplasm is the specialized cytoplasm of the striated-muscle fibre that contains the usual subcellular elements along with the Golgi apparatus, abundant myofibrils, a modified endoplasmic reticulum known as the sarcoplasmic reticulum (SR), glycogen granules, myoglobin and mitochondria. Transverse (T)-tubules invaginate the sarcolemma (see the figure), which allows action-potential signals to penetrate the cell and activate the SR. As shown in the figure, the SR forms a network around the myofibrils, storing and providing the Ca^{2+} that is required for muscle contraction.

Myofibrils are contractile units that consist of an ordered arrangement of longitudinal myofilaments. Myofilaments can be either thick filaments (comprised of myosin) or thin filaments (comprised of actin). Together they produce movement by contraction through the sliding filament model. The characteristic 'striations' of skeletal and cardiac muscle are readily observable by light microscopy as alternating light and dark bands on longitudinal sections (see the figure). The light band, (known as the I-band) is made up of thin filaments and is isotropic in polarized light, whereas the dark band (known as the A-band) is made up of thick filaments and is anisotropic. The M-line (for 'mittelscheibe' or middle disc) bisects the A-band and the Z-line ('zwischenscheibe,' or between disc; also known as the Z-disk or Z-band) defines the lateral boundary of each sarcomeric unit. Contraction of the sarcomere occurs when the Z-lines move closer together, making the myofibrils contract, and therefore the whole muscle cell and then the entire muscle contracts.

Protein assemblies known as costameres, which are located on the peripheral myofibrils of the myofibre, line up with the Z-disk. Costameres physically link the sarcomeres, which produce force through contraction, with the sarcolemma and, are proposed to transmit this force across the sarcolemma to the extracellular matrix and on to neighbouring muscle cells. So, during both contraction and relaxation, sarcomere length remains consistent between muscle cells within skeletal muscle.

Myofibre
An individual skeletal-muscle cell that consists of several nuclei formed by the fusion of myoblasts. Also known as a myocyte.

Sarcoplasm
The cytoplasm of a striated-muscle fibre.

Sarcoplasmic reticulum
Modified endoplasmic reticulum of muscle cells that is adapted to contain large stores of Ca^{2+} that can be released readily to initiate contraction on receiving signals relayed by the T-tubules.

Transverse (T)-tubule
A deep invagination of the plasma membrane of striated-muscle cells that extends perpendicularly from the surface. Muscle contraction occurs when depolarization of the T-tubule membrane triggers the release of Ca^{2+} from the sarcoplasmic reticulum.

Sarcomere
The fundamental unit of muscle contraction, which extends from one Z-line to another.

Costamere
An assembly of sub-sarcolemmal proteins that physically connects the Z-disk of the sarcomere of peripheral myofibrils to the sarcolemma and the basement membrane.

now understand that the underlying mechanisms not only involve loss of structural proteins but also defective enzymes, disruption of sarcolemma-repair mechanisms and the loss of signalling molecules. Judging from the location of mutant proteins, which can be found at the edge of the muscle cell at the sarcolemma, in the extracellular matrix (ECM), at the nuclear membrane or within the muscle cell in the sarcoplasm or the sarcomeres, location does not seem to dictate whether a mutant protein can cause MD. Proteins that are involved in

© 2006 Nature Publishing Group

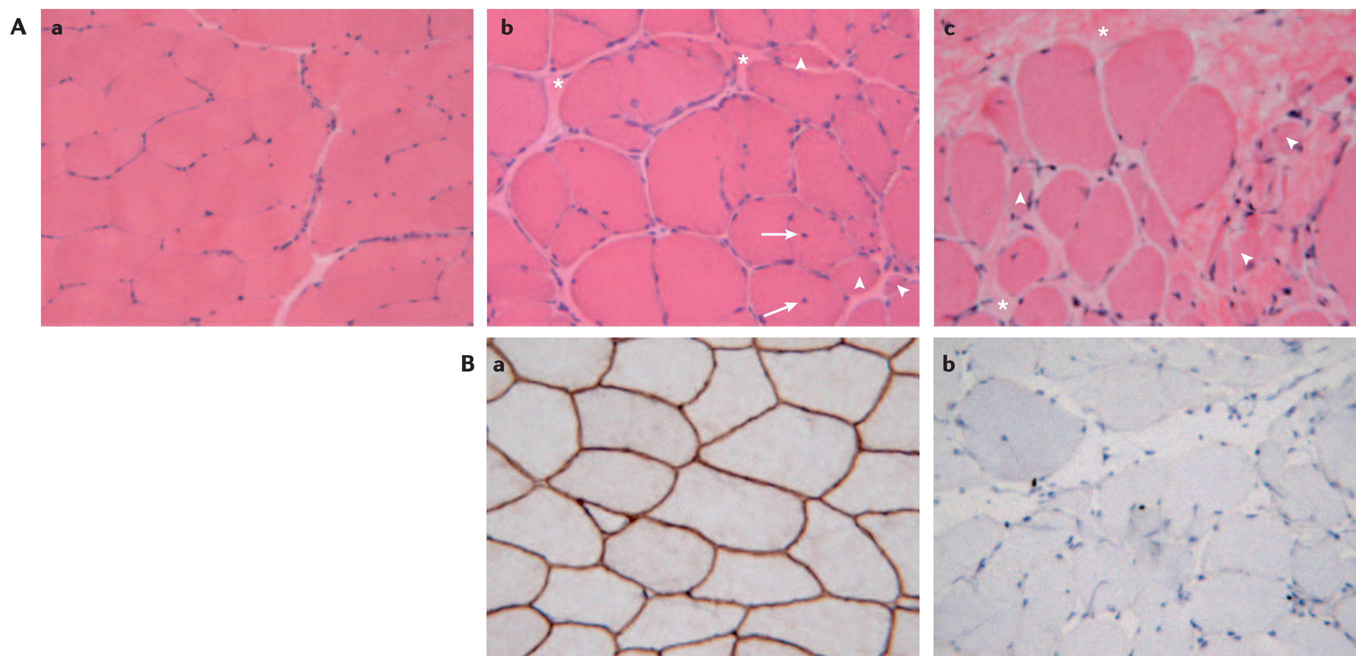


Figure 1 | **Microscopic images of cross-sections of human skeletal muscle. A** | Haematoxylin and eosin staining of normal muscle (**a**) and dystrophic muscle (**b** and **c**). In normal muscle, myofibres are approximately equal in diameter with nuclei (stained blue) located around the periphery. The characteristic features of dystrophic muscle are reflective of successive rounds of degeneration and regeneration. These include central nuclei (indicated by arrows), a variation in fibre size with smaller myofibres present (indicated by arrowheads) and a build-up of connective tissue between muscle fibres (indicated by asterisks). The muscle in (**c**) shows more dystrophic features than that in (**b**). **B** | Immunostaining of normal muscle (**a**) and dystrophic muscle (**b**) using an antibody against the dystrophin protein. Dystrophin is present in the normal muscle section (brown staining) and is localized at the sarcolemma (**a**). Dystrophin is absent in the muscle section from a patient with muscular dystrophy with a dystrophin mutation (**b**). Images courtesy of V. Fabian and P. Morling, Royal Perth Hospital, Western Australia.

post-translational modifications can also cause MD, as can expansions or deletions of nucleotide-repeat sequences that are not necessarily in protein-coding regions of the genome (BOX 2). For further information about the more common MDs, associated mutant proteins and their known binding partners, see Supplementary information S1 (box) and Supplementary information S2 (table). As we begin to decipher the intricate web of protein interactions within the skeletal-muscle fibre, new strategies for the development of effective treatments for these disorders are becoming apparent (BOX 3).

Dystrophin helps link the ECM and cytoskeleton

Despite being the first mutant protein shown to cause MD, the exact function of dystrophin has not yet been elucidated. Dystrophin anchors the sarcolemma to the actin cytoskeleton in the sarcoplasm and therefore has an important structural role during muscle contraction and muscle stretch. Dystrophin is expressed at the sarcolemma and is enriched at the costameres and sites of cell–cell contact, namely the myotendinous junction and the neuromuscular junction (NMJ). Dystrophin is thought to be an elastic and flexible protein owing to triple helical repeats located in its rod domain[14] and therefore dystrophin probably protects the muscle cell from the stresses caused by the force created during muscle contraction. Muscle fibres that lack dystrophin are less stiff than normal fibres[15].

The protective role of dystrophin. The N terminus of dystrophin shares high homology with spectrins and α-actinins and therefore dystrophin belongs to the spectrin superfamily[11]. The N terminus and spectrin repeats of dystrophin bind to the cytoskeleton through filamentous (F)-actin[16,17]. The ~1,200 amino acids that are found between these two regions of dystrophin provide an extended lateral connection between dystrophin and F-actin, with 1 dystrophin molecule binding to the equivalent of 24 actin monomers, which are present as polymerized actin[18] (FIG. 2). The actin that binds to dystrophin (γ-actin) is a different type of actin from that found in the thin filaments of sarcomeres (α-actin)[19]. Accordingly, in the absence of dystrophin, costameric actin (γ-actin) disappears, whereas the actin in the contractile apparatus (α-actin) is retained[19].

Dystrophin also binds to dystroglycan through the cysteine-rich domain and to α-dystrobrevin through the C-terminal domain (see FIG. 2); dystroglycan and α-dystrobrevin are involved in various MDs (see below). The C terminus of dystrophin binds to the dystrophin-associated protein complex (DAPC), a group of proteins that can be purified as a macromolecular structure from muscle-fibre membranes that have been solubilized by detergents[20,21]. The DAPC consists of cytoplasmic, transmembrane and extracellular proteins, and therefore provides a strong mechanical link and mediates interactions between the intracellular cytoskeleton and the ECM[22] (FIGS 2,3).

Myotendinous junction
The junction of muscle fibres and tendons.

Neuromuscular junction
The junction of a motor neuron with a muscle fibre.

Spectrin superfamily
Closely related protein members that bind to actin and contain differing numbers of tandem homologous repeats comprised of a three-α-helix motif.

© 2006 Nature Publishing Group

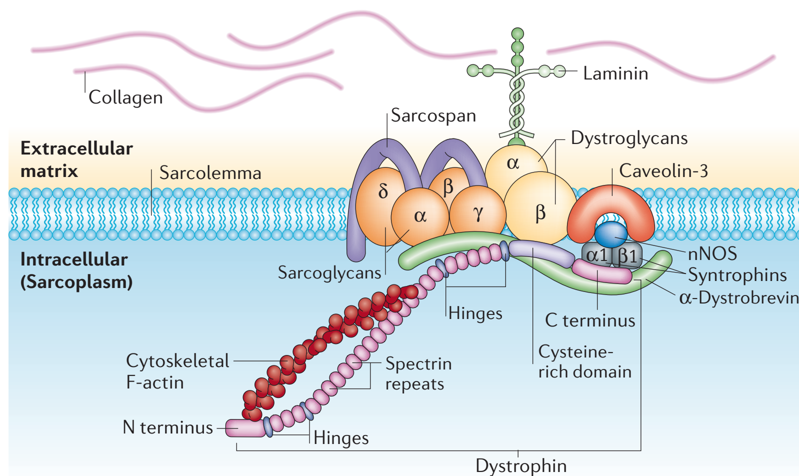


Figure 2 | **Dystrophin binds to the DAPC at the sarcolemma.** Dystrophin, which is localized at the sarcolemma, has a long central rod domain made up of spectrin repeats, which are interspersed with hinge regions. The C terminus is preceded by a cysteine-rich domain. Dystrophin binds to the dystrophin-associated protein complex (DAPC) through its C terminus. The DAPC is comprised of sarcoplasmic proteins (α-dystrobrevin, syntrophins and neuronal nitric oxide synthase (nNOS)), transmembrane proteins (β-dystroglycan, the sarcoglycans, caveolin-3 and sarcospan) and extracellular proteins (α-dystroglycan and laminin). Many members of the DAPC are also associated with muscular dystrophy, either owing to mutations in the genes that encode them (for example, α-, β-, γ- and δ-sarcoglycan, laminin or caveolin-3), or through mutant binding partners (for example, nNOS, syntrophin, α-dystrobrevin, α-dystroglycan, β-dystroglycan or sarcospan). The N terminus of dystrophin binds to the cytoskeleton through filamentous (F)-actin. Therefore, the DAPC provides a strong mechanical link between the intracellular cytoskeleton and the extracellular matrix. Loss of sarcolemmal integrity is thought to occur when a mutant protein of the DAPC is expressed, resulting in muscle fibres that are more susceptible to damage.

It is thought that mutant protein members of the DAPC cause a loss of sarcolemmal integrity and therefore render muscle fibres more vulnerable to damage[23]. In the absence of dystrophin, the DAPC becomes destabilized, leading to diminished levels of the other DAPC proteins[20,21]. Therefore, the protective role of dystrophin and the other members of the DAPC; that is, anchoring the sarcolemma to the internal actin cytoskeleton and to the ECM, is lost. The increasingly fragile sarcolemma is subjected to mechanical injury, which results in progressive muscle-fibre damage and sarcolemmal leakage. Regeneration gradually fails as the pool of endogenous satellite cells ceases to compensate for the damaged muscle fibres. The extent of necrosis and membrane weakness is exacerbated by physical exercise, but is improved by muscle immobilization.

Satellite cell
A myogenic stem cell that is located under the basement membrane of muscle fibres.

Syntrophin
A family that consists of widely expressed adaptor proteins, which are highly concentrated at the postsynaptic membrane of the NMJ (similar to dystrophin).

Signalling: α-dystrobrevin and syntrophin

The dystrobrevins are sarcoplasmic proteins that are highly concentrated at the sarcolemma, the NMJ and the myotendinous junction, and are thought to have a role in signalling, although this is not fully understood. α-Dystrobrevin-null mice have a mild skeletal and cardiac-muscle disease phenotype[24]. Although the DAPC is retained, neuronal nitric oxide synthase (nNOS) is displaced from the sarcolemma into the sarcoplasm in the absence of α-dystrobrevin[24], probably through the loss of the interaction between α-dystrobrevin and syntrophin[25]. nNOS interacts with α1-syntrophin and β1-syntrophin[26] (FIGS 2,3) and it is thought that the disruption of signalling facilitated by nNOS leads to disease, which emphasizes the role of α-dystrobrevin in signalling. Overexpression of nNOS in dystrophin-deficient mice greatly improves the integrity of the sarcolemma of muscle fibres and therefore lessens the degree of dystrophy, perhaps owing to the anti-inflammatory properties of nNOS[27].

Syntrophin links to the ECM through dystrophin (two syntrophin proteins bind to one dystrophin protein) and is thought to associate with kinases, ion channels and several signalling proteins (for example, GRB2 (REF. 28)). Therefore, by localizing signalling molecules, syntrophin creates signal-transduction complexes at the DAPC. Knockout mice for either syntrophin[29] or nNOS[30] do not have an MD phenotype, although they have abnormal NMJs. However, α-syntrophin and nNOS[31] are not found at the sarcolemma in the absence of dystrophin, which indicates that the ability of syntrophin to associate with the sarcolemmal DAPC depends solely on its interaction with dystrophin. By contrast, α-syntrophin localizes to the NMJ in the absence of dystrophin, raising the possibility that the protein responsible for its localization at this site is utrophin (a functional autosomal paralogue of dystrophin that is expressed at the NMJ[32]). The lack of utrophin in the abnormal NMJs of α-syntrophin-knockout mice further supports this notion[29].

Syntrophin and α-dystrobrevin are required for normal muscle function, despite not obviously having a mechanical role in conjunction with dystrophin, which supports the suggestion that these proteins might have roles in signalling. In the absence of dystrophin, both α-dystrobrevin and α-syntrophin are almost completely lost from the sarcolemma. However, these proteins can be restored at the sarcolemma in mice that express truncated forms of dystrophin transgenes that lack the α-dystrobrevin- and syntrophin-binding sites[33]. In these mice, the structure and function of the muscle is normal[33], indicating that both syntrophin and α-dystrobrevin can function maximally despite losing their link to dystrophin. These findings therefore imply that syntrophin and α-dystrobrevin might have a role(s) other than structural (for example, in signalling) and that syntrophin and α-dystrobrevin associate with the DAPC in other ways apart from through dystrophin. *In vitro* studies have shown that α-dystrobrevin might associate with the sarcoglycans[34].

Binding partners of α-dystrobrevin. The other binding partners of α-dystrobrevin include the intermediate-filament proteins synemin[35], dysbindin[36] and syncoilin[37]. Syncoilin is localized to the NMJ and costameres and binds to desmin[38] (FIG. 3). As desmin interacts directly with proteins at the Z-line of the sarcomere, syncoilin and desmin link α-dystrobrevin at the sarcolemma to the site of contraction. Although the amounts of α-dystrobrevin can be affected in some MDs, mutations in α-dystrobrevin itself have so far only been shown to cause congenital heart disease[39]. Synemin is present at the sarcomeric Z-line where it binds to desmin, vimentin[40] and α-actinin[41]. Synemin also binds

© 2006 Nature Publishing Group

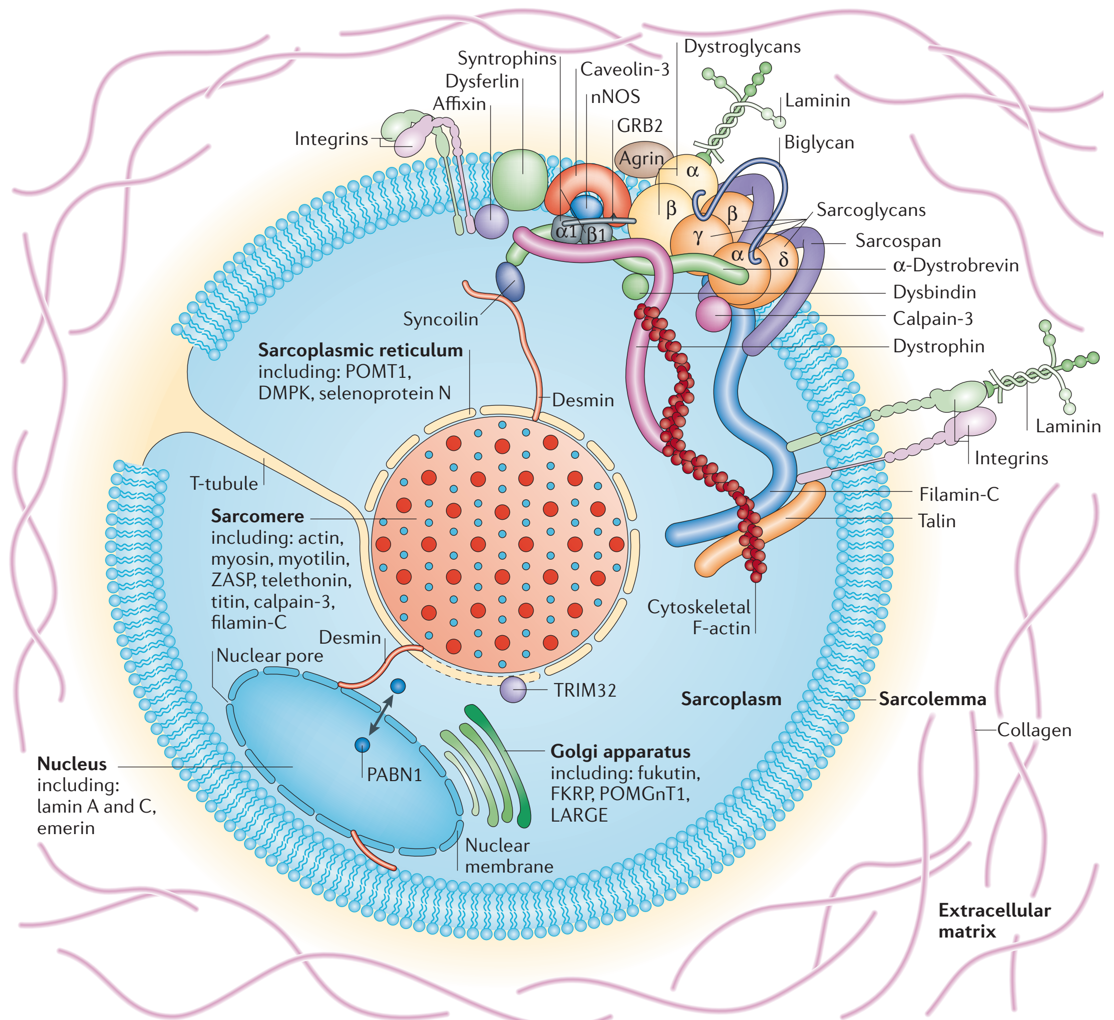


Figure 3 | **A cross-section of a myofibre showing the approximate position of important component proteins.** For simplicity, only one myofibril and one T-tubule are indicated within the sarcoplasm, and the T-tubule has not been drawn the whole way through the myofibre. The extracellular matrix (ECM) surrounds the sarcolemma and consists of collagen and laminin. Laminin binds to integrin and α-dystroglycan at the sarcolemma. α-Dystroglycan is part of the dystrophin-associated protein complex (DAPC), a group of proteins (including the sarcoglycans and α-dystrobrevin) that binds to dystrophin. Dystrophin links the ECM with the sarcomere where contraction occurs — at the sarcolemma, dystrophin binds to α-dystrobrevin, which in turn binds to syncoilin. Syncoilin interacts with desmin, a protein that localizes to the Z-disk of the sarcomere. This link can also occur through laminin binding to integrin, which in turn binds to filamin-C; this protein binds to myotilin at the sarcomere (not shown). Mutations in proteins that are associated with the nucleus (lamin A and lamin C, PABPN1 and emerin), the sarcoplasmic reticulum (POMT1, DMPK and selenoprotein N) as well as the Golgi apparatus (fukutin, FKRP, POMGnT1 and LARGE) can cause muscular dystrophy when mutated. DMPK, dystrophia myotonica protein kinase; FKRP, fukutin-related protein; nNOS, neuronal nitric oxide synthase; PABPN1, poly(A)-binding protein, nuclear-1; POMGnT1, protein *O*-linked mannose β1,2-*N*-acetylglucosaminyltransferase; POMT1, protein *O*-mannosyltransferase-1; TRIM32, tripartite motif-32; ZASP, Z-band alternatively spliced PDZ-motif protein.

Laminins
A family of glycoproteins that makes up the main non-collagenous component of the basement membrane. One type of laminin, laminin-2, is found in the brain and muscle fibres. Laminin-2 is composed of an α2, β1 and γ1 chain. In muscle, one end of laminin-2 binds to α-dystroglycan and the other end binds to the ECM.

Sarcoglycan complex
A sub-complex within the dystrophin-associated protein complex composed of four glycosylated transmembrane proteins each with a short intracellular domain, a single transmembrane region and a large extracellular domain.

to both dystrophin and utrophin, highlighting a role for synemin in connecting the intermediate-filament proteins (desmin and vimentin), and the sarcomeric proteins (α-actinin and desmin) to the costameres at the sarcolemma[42].

The dystroglycans: important DAPC members

Loss of dystroglycan results in embryonic lethality in the mouse[43], perhaps explaining why no dystroglycan mutations have been identified in humans so far. However, if dystroglycan is selectively disrupted in mature muscle fibres it leads to an instability and degeneration of the fibres[44]. Dystroglycan is post-translationally cleaved into two subunits, α-dystroglycan and β-dystroglycan, which remain non-covalently linked[45]. α-Dystroglycan is attached to the extracellular cell surface through the transmembrane β-dystroglycan subunit, whereas β-dystroglycan binds directly to α-dystrobrevin (FIGS 2,3). The presence of glycan carbohydrate moieties on α-dystroglycan is necessary for binding to ECM proteins such as laminin, agrin and perlecan[46]. The dystroglycan complex is associated with the sarcoglycan complex (FIGS 2,3).

© 2006 Nature Publishing Group

Box 2 | Repeat instability: expansions and deletions

Expansions or deletions of nucleotide repeats, not necessarily contained within the coding region of a gene, can cause muscular dystrophies (MDs). These repeats are variable in number in the normal human genome and disease has been associated with a decreased or increased number of repeats. The dystrophia myotonica protein kinase (*DMPK*)[118] and zinc finger protein-9 (*ZNF9*)[119] genes cause myotonic dystrophy (DM) type-1 and type-2 respectively. DM1 is the most common inherited MD that affects adults. These gene expansions are transcribed into RNA but do not modify the protein-coding region of any other gene. Instead, it has been shown that they modify the splicing of other genes such as the chloride channel (in the case of DM1) and the insulin receptor (in the case of DM2)[120], which helps to explain why MD affects other organs in addition to skeletal muscle.

An expansion of alanines within the coding region of the poly(A)-binding protein, nuclear-1 gene (*PABPN1*) produces MD[121]. The ubiquitously expressed PABPN1 is involved in mRNA synthesis and trafficking and regulates the length of mRNA poly(A) tails[122]. The normal protein can shuttle between the nucleus and the sarcoplasm[123]; however, PABPN1 expansions cause filamentous intranuclear aggregates that concentrate into discrete 'speckles' in the nucleus[124].

Deletions of repeated 3.3-kb units located on chromosome 4 (4q35.2) also cause MD[125]. As no transcribed genes have been identified within these repeats, one suggestion is that the deleted repeats derepress neighbouring genes by a position effect and lead to their upregulation (reviewed in REF. 126). Transgenic mice that overexpress one of these genes, *FRG1*, develop an MD similar to facioscapulohumeral MD in humans[127], indicating that dysregulation of *FRG1* might be the cause of this MD. FRG1 is a nuclear protein that is thought to splice pre-mRNA and aberrant splicing of genes was confirmed in this study in the affected mice[127].

The expression of mutant dystrophin proteins that lack the ability to bind to β-dystroglycan produces a marked loss of all sarcoglycan proteins, as well as α-dystroglycan[47], indicating that the sarcoglycan complex is assembled and anchored in the DAPC through β-dystroglycan.

The sarcoglycan complex. In skeletal and cardiac muscle the predominant sarcoglycan complex consists of β-sarcoglycan and δ-sarcoglycan together as a core, bound by α-sarcoglycan and γ-sarcoglycan[48] (FIGS 2,3). Despite being transmembrane components of the DAPC, none of the sarcoglycans bind to dystrophin directly *in vivo.* The absence of dystrophin usually initiates disruption and destabilization of the sarcoglycan complex, making the sarcolemma leaky. Mutations in the genes that encode α-, β-, γ- and δ-sarcoglycan cause limb girdle MDs (reviewed in REF. 49; see Supplementary information S2 (table)). In most instances the protein encoded by the mutated sarcoglycan gene is absent in patients with MD, with a reduction or complete loss of the other sarcoglycans. Although the exact function of the sarcoglycan complex is not known, it seems to have mechanical, non-mechanical and signalling roles[50].

At the surface of the muscle cell, each member of the sarcoglycan complex forms a functional unit by associating closely with sarcospan, which has four transmembrane regions[51] (FIGS 2,3). Extracellularly, the sarcoglycan complex binds to a small proteoglycan, biglycan, through α-sarcoglycan and γ-sarcoglycan[52] and through biglycan the sarcoglycan complex then binds to α-dystroglycan (FIG. 3). Intracellularly, the sarcoglycan complex interacts with the cytoskeletal protein filamin-C through the sarcoplasmic tails of γ-sarcoglycan and δ-sarcoglycan[53] (FIG. 3). Filamin-C also binds to the integrin complex and closely links to the sarcomere through the Z-line[53] (FIG. 3). In dystrophin-deficient mice, filamin-C is upregulated and recruited away from the sarcomere to the sarcolemma[53].

Caveolin-3 and dysferlin. The β-dystroglycan subunit also binds to caveolin-3 in the DAPC, as well as the adaptor protein GRB2 (FIG. 3)[54]. Caveolin-3 is the muscle-specific isoform of the caveolin family[55] and is the main structural component of caveolae at the sarcolemma. Caveolin-3 is also located in T-tubules during muscle development and therefore it is not surprising that caveolin-3-null mice have T-tubule defects[56]. Other putative functions of caveolin-3 include inhibition of nNOS enzymatic activity[57], interactions with several signalling molecules[58] and involvement in the regulation of energy metabolism[59].

Overexpression of caveolin-3 results in an MD phenotype in mice, leading to a marked reduction in the amounts of dystrophin and other DAPC members[60]. These findings might result from the excess caveolin-3 displacing dystrophin as both proteins bind to the same site on β-dystroglycan[61]. As β-dystroglycan anchors dystrophin to the sarcolemma, this linkage is destroyed or reduced and therefore results in a dystrophic phenotype. By contrast, dominant expression of mutant caveolin-3 proteins in patients with MD often causes a loss or reduction of the total amounts of caveolin-3, but preservation of dystrophin and the DAPC[62]. However, sometimes α-dystroglycan and nNOS are selectively absent[63] or dysferlin is mislocalized[64]. Dominant expression of a mutant caveolin-3 protein can result in targeting of the mutant caveolin-3 as well as the wild-type caveolin-3 to the proteasomal-degradation pathway, with retention at the Golgi complex[65], which explains the absence of both these proteins in the sarcolemma of patients.

Caveolin-3 binds to dysferlin[64], which is a ubiquitously expressed member of the ferlin protein family. The novel, integrin-linked kinase-binding protein affixin was recently identified as a binding partner for dysferlin[66] (FIG. 3). As affixin also interacts with α-actinin and F-actin[67], it links integrin, dysferlin and caveolin-3 to the cytoskeleton. However, dysferlin mutations are not thought to cause MD solely through a disruption of the link between integrins and the cytoskeleton. Instead, there is strong evidence that MD probably arises owing

Caveolae
Small invaginations of the plasma membrane that are associated with the control of signal-transduction events.

Ferlin protein family
Family of proteins with a C-terminal transmembrane domain and four to seven C2 domains. These proteins mediate fusion and vesicle trafficking in a Ca^{2+}-dependent manner.

© 2006 Nature Publishing Group

Box 3 | Potential therapies for MDs

Currently there is no cure for any of the muscular dystrophies (MDs), with only palliative and symptomatic treatment available for patients. The identification of the causative gene for Duchenne MD and Becker MD nearly 20 years ago was accompanied by a surge of optimism that a therapy or cure would soon follow. Unfortunately, translating the knowledge of the causative gene and its mutation into genetic therapy for MDs has proven to be a difficult task. There have been studies of possible treatments that function at the molecular level (chimeroplasts, short fragment homologous recombination and antisense oligonucleotide exon skipping), the cellular level (delivery of myoblasts, stem cells, viruses and plasmids) and the pharmacological level (myostatin blockade, upregulation of an alternative gene, proteasome-degradation prevention and stop-codon read-through using aminoglycoside antibiotics) (reviewed in REFS 128,129).

Two promising therapeutic approaches are the delivery of a normal replacement for the defective gene using adeno-associated virus (AAV) vectors[130] and the use of antisense oligonucleotides to induce exon skipping of the mutation-containing exon or to extend a deletion. This produces an in-frame transcript that is translated into a functional, albeit smaller, protein[131]. One of the main hurdles facing therapeutic approaches is effective delivery of the therapeutic product to skeletal muscle, which makes up 30–40% of the total human body mass. Fortunately, recent success with systemic delivery has shown significant promise. For example, weekly intravenous injections of a morpholino oligonucleotide into the dystrophin-null mouse model, *mdx*, produced body-wide expression of dystrophin[132]. Also, a single injection of an AAV8 virus into hamsters produced sustained expression of the previously missing δ-sarcoglycan protein in both skeletal muscle and heart for longer than 12 months[133]. A novel combination of both viral delivery and antisense technology was accomplished in *mdx* mice, in which a single tail-vein injection of an AAV vector expressing an antisense U1 small nuclear RNA led to successful body-wide expression of a dystrophin isoform for at least 12 weeks[134].

to defective muscle-membrane repair and maintenance processes[68]. Although, to survive, all cells must have a fast and proficient way to reseal their membranes after injury, highly active tissues such as skeletal muscle have a more crucial need for this repair process. A membrane-resealing action that requires Ca^{2+} is disrupted in mouse myofibres that lack dysferlin[68].

Glycosylation of α-dystroglycan

Although the primary sequence of α-dystroglycan is predicted to have a molecular mass of 72 kDa, the molecular mass in mammalian skeletal muscle is 156 kDa, which reflects post-translational modifications (predominantly *O*-linked glycosylation)[45,69]. No human disease has been shown to be caused by dystroglycan mutations so far, but many proteins that glycosylate α-dystroglycan, or are thought to do so, have been identified as causing MD when mutated[70]. Aberrant glycosylation of α-dystroglycan (but not β-dystroglycan) is the primary cause of a range of MDs that are characterized by muscle weakness and sometimes by brain and ocular abnormalities[71–77]. Common to all these disorders is a uniform or variable reduction of glycosylation, which produces an α-dystroglycan protein that is less glycosylated[70]. This decrease in glycosylation affects the binding of α-dystroglycan to agrin, laminin and neurexin.

Proteins of the Golgi apparatus and the sarcoplasmic reticulum mediate post-translational modifications, such as the attachment of carbohydrate molecules to form glycoproteins. Mutations in four proteins of the Golgi apparatus (fukutin, fukutin-related protein (FKRP), LARGE and protein *O*-linked mannose β1,2-*N*-acetylglucosaminyltransferase (POMGnT1)) and two proteins of the sarcoplasmic reticulum (protein *O*-mannosyltransferase-1 (POMT1) and selenoprotein N) have been shown to cause an MD phenotype. Although the exact biochemical function of each of these proteins remains unknown, all except selenoprotein N are glycoproteins that potentially modify and process α-dystroglycan post-translationally.

Myoblast
An undifferentiated, mononucleated cell that is a precursor of a muscle cell.

Proteins of the Golgi apparatus. The *LARGE* gene was initially identified as having deletions in meningioma tumours, and is ubiquitously expressed, although the LARGE protein is most predominantly found in skeletal muscle, heart and brain and this protein is predicted to have two putative glycosyltransferase domains[78]. POMGnT1 is constitutively expressed and is involved in the synthesis of *O*-mannose glycan[79], a reaction that is rarely found in mammals, although *O*-mannose glycan is a laminin-binding ligand of α-dystroglycan[80]. MD arises from loss-of-function mutations in the *POMGnT1* gene and patients have reduced amounts of α-dystroglycan and laminin-α2 (REF. 79).

Fukutin, which is thought to have a role in neuronal migration, has a hydrophobic Golgi-signal-anchor sequence that coincides with its transmembrane region and so is found in secretory granules and Golgi bodies[81,82]. Similar to patients with *POMGnT1* mutations, patients with a mutant fukutin gene have reduced amounts of α-dystroglycan and laminin-α2 and therefore have a disrupted basal lamina[83]. FKRP is highly enriched in skeletal muscle and heart[75]. Although the enzymatic activity of FKRP has not yet been proven, an alteration of dystroglycan processing was seen after overexpression of FKRP *in vitro*[81]. Patients with *FKRP* mutations have a variable reduction of laminin-α2 and α-dystroglycan levels[84]; the severity of α-dystroglycan depletion directly correlates with the severity of the MD phenotype[85].

O-mannosylation: from yeasts to humans. Protein *O*-mannosyltransferases are conserved throughout evolution from yeasts to humans and have been shown to be crucial in yeasts owing to their important roles in the maintenance of cell integrity and cell-wall rigidity. Although rare in humans, *O*-mannosylation occurs in skeletal muscle, brain and nerve. In mammals, POMT1 and POMT2 are both required for *O*-mannosyltransferase activity, which produces a functional α-dystroglycan[86]. Mutant POMT1 proteins cause a range of congenital

© 2006 Nature Publishing Group

MDs with variable severity, some of which can be associated with mental retardation[87]. Patients with *POMT1* mutations lack glycosylated α-dystroglycan[74].

The glycosylation of α-dystroglycan is essential for its interaction with intracellular and extracellular binding partners. An increasing number of potential glycoproteins have been identified, which when mutated lead to disruption of α-dystroglycan glycosylation and subsequent MD, indicating similar pathways of disease pathogenesis.

ECM proteins associated with MD

The ECM enhances the elastic properties of myofibres, adds strength to the sarcolemma and is involved in tissue differentiation, repair and regeneration. The ECM is composed of a meshwork of insoluble proteins with carbohydrate chains, such as collagen, proteoglycans, laminins and fibronectins. Among the ECM proteins, collagen VI and laminin have been identified as causing MD when mutated. Collagen VI is a ubiquitous cell-adhesion protein that does not bind directly to the DAPC. It forms a microfibrillar network in the ECM in close association with the basement membrane where it interacts with many other proteins. Laminin-α2 forms the link between α-dystroglycan at the sarcolemma and the basal lamina[45] (FIGS 2,3) and has a role in organizing a structured basement membrane as well as linking the basement membrane from neighbouring cells through receptors on the cell surface. Laminin-α2 also binds to the heterodimeric receptor α7β1 integrin at the sarcolemma[88], which in turn binds to filamin-C[89] (see below and FIG. 3).

Interaction between α7β1 integrin and laminin-2 seems to be essential for the maintenance of mature skeletal muscle[90]. Integrin binds to laminin in the ECM through an extracellular domain, whereas intracellularly it interacts with cytoskeletal actin through molecules such as talin[91] (FIG. 3). Upregulation of α7β1 integrin has been observed in muscle biopsies from patients with DMD[92]. Despite the mechanism being unknown, amelioration of the severe phenotype of mice lacking both dystrophin and utrophin was achieved when these mice were crossed with transgenic mice that overexpress α7BX2 integrin[93]. Double α7 integrin- and γ-sarcoglycan-knockout mice have profound, rapid muscle degeneration, which leads to death before 1 month of age[94], which indicates a weakened cellular attachment to the ECM.

Proteins of the sarcolemma and ECM have traditionally been those most readily associated with MDs, especially as many were first identified owing to their involvement in these diseases. As the pathogenesis of MD involves weakened muscle-cell membranes, it is perhaps not difficult to understand how mutant sarcolemmal and ECM proteins would cause this defect. However, more recently, mutations in genes that encode proteins of other cell compartments, such as the sarcomere (which has been conventionally associated with muscle diseases other than dystrophies, such as the myopathies[95]), have been shown to produce MDs. These findings have broadened our knowledge of disease mechanisms in skeletal muscle.

Basement membrane
Amorphous extracellular matrix material, composed of proteins such as collagen, laminin and fibronectin, that surrounds individual muscle fibres. It is comprised of two layers, the basal lamina and the reticular lamina.

Myofibrillogenesis
The transition from premyofibril to myofibril, which is a long, highly organized bundle of contractile proteins or filaments in the sarcoplasm of the myofibre.

Sarcomere-associated proteins and MD

Myotilin, telethonin, titin, the Z-band alternatively spliced PDZ-motif protein (ZASP; also known as cypher), calpain-3 and tripartite motif-32 (TRIM32) are all sarcomere-associated proteins that are involved in MDs. Calpain-3 and TRIM32 are also found in the sarcoplasm.

Anchoring titin. Telethonin is implicated in signal transmission in myofibrillogenesis and it anchors titin to the Z-line[96]. Telethonin interacts with the growth factor myostatin, which is a negative regulator of myoblast proliferation and therefore probably promotes muscle regeneration and growth[97]. MD patients with an absence of telethonin have preservation of Z-lines and other sarcomeric features as well as retention of DAPC members at the sarcolemma[98]. This finding indicates that MD arises from disruption of the signalling roles, rather than the structural roles, of telethonin.

A Ser residue near the C terminus of telethonin (Ser157) is phosphorylated by the kinase activity of titin in early differentiating myocytes[99]. Titin is the largest protein known, having a molecular weight in humans of 4,200 kDa. Titin forms a continuous filament system with each titin molecule spanning half a sarcomere from the Z-disk to the M-line. It is known as a 'molecular spring' because its I-band region has elastic properties and as a 'ruler' because it functions as a principal sarcomeric template and stabilizer during myofibrillogenesis.

Recent findings indicate that, owing to its pseudo-symmetric structure, telethonin mediates the antiparallel assembly of two titin molecules, perhaps explaining how sarcomeric filaments are crosslinked and stabilized[100]. The most N-terminal 30 kDa of titin proteins from adjacent sarcomeres are anchored at the Z-line; the proteins continue for approximately 1 μm until the C-terminal 250 kDa regions of opposing titins localize at the M-line, the middle of the sarcomere where actin and myosin filaments overlap.

Proteins that affect sarcomere formation. The C terminus of titin binds to calpain-3. The calpains are intracellular, Ca^{2+}-activated, non-lysosomal cysteine proteases that function in several signalling pathways. Calpain-3 is the sole skeletal-muscle-specific isoform[101] and cleaves the C-terminal portion of filamin-C, moderating the interaction between filamin-C and the sarcoglycans[102] (FIG. 3). Filamin-C binds to the sarcoglycan proteins and to integrin[89] at the sarcolemma, and also to myotilin[103] and FATZ1 (filamin, actin and telethonin-binding protein at the Z-disk) at the sarcomere[89]. Patients with titin mutations can have a secondary calpain-3 deficiency[104]. Calpain-3 is implicated in processes that involve remodelling of the cytoskeleton during myoblast fusion and repair. Mutated calpain-3 was the first enzyme, rather than a structural protein, to be associated with MD[101]. An increase in apoptosis is seen in muscle that is deficient in calpain-3 (REF. 105) with studies in mice indicating that calpain-3 is required for sarcomere formation and the maintenance of sarcomeric structural integrity[106], perhaps owing to its binding with titin.

© 2006 Nature Publishing Group

TRIM32 is an E3-ubiquitin ligase that is involved in the ubiquitin–proteasome pathway of protein degradation[107]. TRIM32 binds to the head and neck region of myosin and ubiquitylates actin, a finding which led to the suggestion that TRIM32 is involved in the maintenance and degradation of myofibrils during muscle remodelling[107]. It is hypothesized that disruption of the ubiquitin-degradation pathway leads to a disruption of the internal equilibrium of myofibre proteins.

Myotilin (which is a myofibrillar protein with titin-like immunoglobulin domains) is thought to have a crucial role during sarcomere assembly by stabilizing and anchoring thin filaments at the Z-line — patients with myotilin mutations show Z-line streaming and myofibre degeneration[108]. Myotilin is expressed mainly in striated muscle; its N-terminal half binds to α-actinin[109] and its C terminus binds to filamin-C[103], with myotilin, α-actinin and filamin-C all binding to actin. α-Actinin is the main Z-line protein, which explains the Z-line streaming seen in patients with myotilin mutations. ZASP is also located at the Z-line and binds to α-actinin[110]. It is predominantly expressed in skeletal and cardiac muscle and, like nNOS and syntrophin, it contains PDZ motifs[110].

Mutations in these proteins that are at the 'frontline' of muscle contraction, that is, at the sarcomere, cause MD. Similar to proteins of the DAPC, it is now recognized that sarcomeric proteins not only have important structural roles but also have signalling roles, such as those involved in muscle-cell proliferation, fusion, maintenance, regeneration and repair. It is perhaps defects in these signalling functions that are more likely to produce MD.

How do nuclear proteins cause MD?

Each skeletal-muscle fibre is multinucleated as these cells originate from the fusion of many myoblasts. Lamin A, lamin C, emerin and nesprin all contribute to the nuclear membrane and nuclear envelope, which link the cytoskeleton to the inside of the nucleus, maintain the physical architecture of the nucleus and function as a scaffold for other nuclear proteins that are involved in transcription, DNA replication and chromatin organization. Emerin and lamin A and C interact with each other directly, are localized at the inner nuclear membrane in almost all tissues and cause MD when mutated[3,10].

Emerin is involved in differentiation. Although the exact function of emerin has not yet been elucidated, proposed functions include nuclear assembly, stabilization of the nuclear envelope and the regulation of gene expression[111]. Both emerin and lamin C must bind to lamin A for correct localization to the nuclear envelope[112]. Most mutations in the emerin gene result in a deficiency of emerin protein[113], which makes nuclei more fragile because of destabilization of the peripheral nuclear lamina[114].

Although emerin is ubiquitously expressed, the complete loss of emerin seems to affect skeletal and cardiac muscle only. Why emerin mutations selectively affect certain organs is so far unclear; however, it is possible that the nuclear envelope of muscle cells is more susceptible to the absence of emerin than other cell types. Alternatively, there might be as-yet-unknown interactions between emerin and other muscle-specific proteins that are disrupted only in muscle, or there might be a functionally similar protein(s) in non-striated-muscle cells that can compensate for the absence of emerin. As muscle nuclei endure external physical forces during contraction, mechanical destruction owing to a weakened lamina is likely to be a pathological cause of these types of MD.

Emerin-null mice do not have any overt phenotype despite abnormal amounts of the retinoblastoma protein (Rb1) and MyoD[115]. Both Rb1 and MyoD regulate muscle differentiation and are crucial for muscle development. As a result, the emerin-null mice show a delay in the rate of both muscle regeneration and myotube formation[115]. These findings indicate that loss of emerin produces inefficient responses to differentiation signals when cells are required to exit from the cell cycle and begin to terminally differentiate[115].

Lamins are associated with many diseases. Lamins provide the nuclear envelope with mechanical stability by forming a meshwork arrangement, the nuclear lamina, under the inner nuclear membrane. As discussed above, *LMNA* (which encodes lamin A and lamin C) is a perfect example of how the same mutant gene can be involved in a spectrum of clinical phenotypes with a range of age of onsets, severities and rates of progression. So far, a genotype–phenotype correlation between mutant lamin A and lamin C and the tissue-specific diseases they cause has not been elucidated. It is unknown why only certain muscles of the body are affected in some diseases when the causative mutant protein is expressed ubiquitously. A possible explanation could be that certain mutations disrupt interactions with binding partners that are found only in some tissues (and therefore only these tissues are affected) or, conversely, that selective tissues have compensatory proteins that protect that particular tissue from disease. The exact reasons for the differing disease phenotypes remain to be unravelled.

An in-depth microarray study using muscle mRNA from patients with a range of neuromuscular disorders has shown that, similar to emerin, mutations in *LMNA* lead to disruption of the interaction between the nuclear envelope and Rb1 and MyoD[116]. In support of this finding, a decrease in the amounts of MyoD and therefore decreased differentiation potential was found in myoblasts that were deficient for lamin A and lamin C[117]. The similar expression profiles revealed by these two studies indicate that mutations in two different genes might cause MD through a common pathway.

Conclusions

Several proteins of the skeletal-muscle fibre can produce one or more types of MD when mutated. A dystrophic phenotype can occur owing to expression of a mutant protein at the sarcolemma, the sarcomere, the nuclear membrane or the sarcoplasm. More than one gene can cause the same clinical disease and, furthermore, the

Z-line streaming
A common pathological feature in which the dense Z-line material extends into one or both of the adjacent I-bands and, in advanced cases, into the whole sarcomere.

PDZ motif
Protein–protein interaction domain that is thought to have a role in directing intracellular proteins to several protein complexes.

© 2006 Nature Publishing Group

same gene can produce a spectrum of MD phenotypes, as well as diseases that do not necessarily affect the musculature.

The field has moved forward from a belief that MDs are caused predominantly by absent or defective structural proteins to a realization that signalling molecules, enzymes and proteins involved in post-translational modifications, such as glycosylation, also cause MDs. The study of MD is leading to a better understanding of skeletal-muscle function through the analysis of muscle dysfunction. However, the causative mechanisms behind some MDs remain to be elucidated. Although no curative therapy is currently available for any of the MDs, immense progress is being made using several approaches (BOX 3). The more we improve therapeutic delivery systems and the more we understand about the protein network of the skeletal-muscle fibre, the better armed we shall be in the crusade against MD.

1. Hackman, P. *et al.* Tibial muscular dystrophy is a titinopathy caused by mutations in *TTN*, the gene encoding the giant skeletal-muscle protein titin. *Am. J. Hum. Genet.* **71**, 492–500 (2002).
2. Muchir, A. *et al.* Identification of mutations in the gene encoding lamins A/C in autosomal dominant limb girdle muscular dystrophy with atrioventricular conduction disturbances (LGMD1B). *Hum. Mol. Genet.* **9**, 1453–1459 (2000).
3. Bonne, G. *et al.* Mutations in the gene encoding lamin A/C cause autosomal dominant Emery–Dreifuss muscular dystrophy. *Nature Genet.* **21**, 285–288 (1999).
4. Novelli, G. *et al.* Mandibuloacral dysplasia is caused by a mutation in *LMNA*-encoding lamin A/C. *Am. J. Hum. Genet.* **71**, 426–431 (2002).
5. Chen, L. *et al. LMNA* mutations in atypical Werner's syndrome. *Lancet* **362**, 440–445 (2003).
6. Fatkin, D. *et al.* Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease. *N. Engl. J. Med.* **341**, 1715–1724 (1999).
7. De Sandre-Giovannoli, A. *et al.* Homozygous defects in *LMNA*, encoding lamin A/C nuclear-envelope proteins, cause autosomal recessive axonal neuropathy in human (Charcot–Marie–Tooth disorder type 2) and mouse. *Am. J. Hum. Genet.* **70**, 726–736 (2002).
8. Eriksson, M. *et al.* Recurrent *de novo* point mutations in lamin A cause Hutchinson–Gilford progeria syndrome. *Nature* **423**, 293–298 (2003).
9. De Sandre-Giovannoli, A. *et al.* Lamin A truncation in Hutchinson–Gilford progeria. *Science* **300**, 2055 (2003).
10. Bione, S. *et al.* Identification of a novel X-linked gene responsible for Emery–Dreifuss muscular dystrophy. *Nature Genet.* **8**, 323–327 (1994).
11. Koenig, M., Monaco, A. P. & Kunkel, L. M. The complete sequence of dystrophin predicts a rod-shaped cytoskeletal protein. *Cell* **53**, 219–226 (1988).
One of the groundbreaking papers in the field, which describes the protein product of the dystrophin gene, the first gene associated with MD.
12. Mokri, B. & Engel, A. G. Duchenne dystrophy: electron microscopic findings pointing to a basic or early abnormality in the plasma membrane of the muscle fiber. *Neurology* **25**, 1111–1120 (1975).
13. Davies, K. E. *et al.* Linkage analysis of two cloned DNA sequences flanking the Duchenne muscular dystrophy locus on the short arm of the human X chromosome. *Nucleic Acids Res.* **11**, 2303–2312 (1983).
14. Grum, V. L., Li, D., MacDonald, R. I. & Mondragon, A. Structures of two repeats of spectrin suggest models of flexibility. *Cell* **98**, 523–535 (1999).
15. Pasternak, C., Wong, S. & Elson, E. L. Mechanical function of dystrophin in muscle cells. *J. Cell Biol.* **128**, 355–361 (1995).
16. Hemmings, L., Kuhlman, P. A. & Critchley, D. R. Analysis of the actin-binding domain of α-actinin by mutagenesis and demonstration that dystrophin contains a functionally homologous domain. *J. Cell Biol.* **116**, 1369–1380 (1992).
17. Rybakova, I. N., Amann, K. J. & Ervasti, J. M. A new model for the interaction of dystrophin with F-actin. *J. Cell Biol.* **135**, 661–672 (1996).
18. Rybakova, I. N. & Ervasti, J. M. Dystrophin–glycoprotein complex is monomeric and stabilizes actin filaments *in vitro* through a lateral association. *J. Biol. Chem.* **272**, 28771–28778 (1997).
19. Rybakova, I. N., Patel, J. R. & Ervasti, J. M. The dystrophin complex forms a mechanically strong link between the sarcolemma and costameric actin. *J. Cell Biol.* **150**, 1209–1214 (2000).
20. Ervasti, J. M., Ohlendieck, K., Kahl, S. D., Gaver, M. G. & Campbell, K. P. Deficiency of a glycoprotein component of the dystrophin complex in dystrophic muscle. *Nature* **345**, 315–319 (1990).
Describes four members of the DAPC and illustrates that their expression is diminished in DMD patients, a crucial finding in the understanding of the pathogenesis of dystrophic muscle.
21. Yoshida, M. & Ozawa, E. Glycoprotein complex anchoring dystrophin to sarcolemma. *J. Biochem. (Tokyo)* **108**, 748–752 (1990).
22. Rando, T. A. The dystrophin–glycoprotein complex, cellular signaling, and the regulation of cell survival in the muscular dystrophies. *Muscle Nerve* **24**, 1575–1594 (2001).
23. Petrof, B. J., Shrager, J. B., Stedman, H. H., Kelly, A. M. & Sweeney, H. L. Dystrophin protects the sarcolemma from stresses developed during muscle contraction. *Proc. Natl Acad. Sci. USA* **90**, 3710–3714 (1993).
Shows that dystrophin-deficient muscle is more susceptible to injury after damage by mechanical stress caused by contraction, supporting the hypothesis that dystrophin reinforces the sarcolemma.
24. Grady, R. M. *et al.* Role for α-dystrobrevin in the pathogenesis of dystrophin-dependent muscular dystrophies. *Nature Cell Biol.* **1**, 215–220 (1999).
25. Newey, S. E., Benson, M. A., Ponting, C. P., Davies, K. E. & Blake, D. J. Alternative splicing of dystrobrevin regulates the stoichiometry of syntrophin binding to the dystrophin protein complex. *Curr. Biol.* **10**, 1295–1298 (2000).
26. Brenman, J. E. *et al.* Interaction of nitric oxide synthase with the postsynaptic density protein PSD-95 and α1-syntrophin mediated by PDZ domains. *Cell* **84**, 757–767 (1996).
27. Wehling, M., Spencer, M. J. & Tidball, J. G. A nitric oxide synthase transgene ameliorates muscular dystrophy in *mdx* mice. *J. Cell Biol.* **155**, 123–131 (2001).
28. Oak, S. A., Russo, K., Petrucci, T. C. & Jarrett, H. W. Mouse α1-syntrophin binding to Grb2: further evidence of a role for syntrophin in cell signaling. *Biochemistry* **40**, 11270–11278 (2001).
29. Adams, M. E. *et al.* Absence of α-syntrophin leads to structurally aberrant neuromuscular synapses deficient in utrophin. *J. Cell Biol.* **150**, 1385–1398 (2000).
30. Crosbie, R. H. *et al. Mdx* muscle pathology is independent of nNOS perturbation. *Hum. Mol. Genet.* **7**, 823–829 (1998).
31. Adams, M. E., Mueller, H. A. & Froehner, S. C. *In vivo* requirement of the α-syntrophin PDZ domain for the sarcolemmal localization of nNOS and aquaporin-4. *J. Cell Biol.* **155**, 113–122 (2001).
32. Love, D. R. *et al.* An autosomal transcript in skeletal muscle with homology to dystrophin. *Nature* **339**, 55–58 (1989).
The first report about utrophin, an autosomal homologue of dystrophin. This protein has been actively investigated for treatment of MD as its upregulation has been shown to ameliorate dystrophic features.
33. Crawford, G. E. *et al.* Assembly of the dystrophin-associated protein complex does not require the dystrophin COOH-terminal domain. *J. Cell Biol.* **150**, 1399–1410 (2000).
34. Yoshida, M. *et al.* Biochemical evidence for association of dystrobrevin with the sarcoglycan–sarcospan complex as a basis for understanding sarcoglycanopathy. *Hum. Mol. Genet.* **9**, 1033–1040 (2000).
35. Mizuno, Y. *et al.* Desmuslin, an intermediate filament protein that interacts with α-dystrobrevin and desmin. *Proc. Natl Acad. Sci. USA* **98**, 6156–6161 (2001).
36. Benson, M. A., Newey, S. E., Martin-Rendon, E., Hawkes, R. & Blake, D. J. Dysbindin, a novel coiled-coil-containing protein that interacts with the dystrobrevins in muscle and brain. *J. Biol. Chem.* **276**, 24232–24241 (2001).
37. Newey, S. E. *et al.* Syncoilin, a novel member of the intermediate filament superfamily that interacts with α-dystrobrevin in skeletal muscle. *J. Biol. Chem.* **276**, 6645–6655 (2001).
38. Poon, E., Howman, E. V., Newey, S. E. & Davies, K. E. Association of syncoilin and desmin: linking intermediate filament proteins to the dystrophin-associated protein complex. *J. Biol. Chem.* **277**, 3433–3439 (2002).
39. Ichida, F. *et al.* Novel gene mutations in patients with left ventricular noncompaction or Barth syndrome. *Circulation* **103**, 1256–1263 (2001).
40. Granger, B. L. & Lazarides, E. Synemin: a new high molecular weight protein associated with desmin and vimentin filaments in muscle. *Cell* **22**, 727–738 (1980).
41. Bellin, R. M. *et al.* Molecular characteristics and interactions of the intermediate filament protein synemin. Interactions with α-actinin may anchor synemin-containing heterofilaments. *J. Biol. Chem.* **274**, 29493–29499 (1999).
42. Bhosle, R. C., Michele, D. E., Campbell, K. P., Li, Z. & Robson, R. M. Interactions of intermediate filament protein synemin with dystrophin and utrophin. *Biochem. Biophys. Res. Commun.* **346**, 768–777 (2006).
43. Williamson, R. A. *et al.* Dystroglycan is essential for early embryonic development: disruption of Reichert's membrane in *Dag1*-null mice. *Hum. Mol. Genet.* **6**, 831–841 (1997).
44. Cohn, R. D. *et al.* Disruption of *Dag1* in differentiated skeletal muscle reveals a role for dystroglycan in muscle regeneration. *Cell* **110**, 639–648 (2002).
45. Ibraghimov-Beskrovnaya, O. *et al.* Primary structure of dystrophin-associated glycoproteins linking dystrophin to the extracellular matrix. *Nature* **355**, 696–702 (1992).
Describes the dystroglycans and finds that the larger α-dystroglycan is reduced in dystrophin-deficient mice, thereby disrupting the link between dystrophin, the sarcolemma and the ECM.
46. Michele, D. E. & Campbell, K. P. Dystrophin–glycoprotein complex: post-translational processing and dystroglycan function. *J. Biol. Chem.* **278**, 15457–15460 (2003).
47. Chamberlain, J. S. *et al.* Interactions between dystrophin and the sarcolemma membrane. *Soc. Gen. Physiol. Ser.* **52**, 19–29 (1997).
48. Ozawa, E., Mizuno, Y., Hagiwara, Y., Sasaoka, T. & Yoshida, M. Molecular and cell biology of the sarcoglycan complex. *Muscle Nerve* **32**, 563–576 (2005).
49. Laval, S. H. & Bushby, K. M. Limb–girdle muscular dystrophies — from genetics to molecular pathology. *Neuropathol. Appl. Neurobiol.* **30**, 91–105 (2004).
50. Barton, E. R. Impact of sarcoglycan complex on mechanical signal transduction in murine skeletal muscle. *Am. J. Physiol. Cell Physiol.* 290, C411–C419 (2006).
51. Hayashi, K. *et al.* Sarcospan: ultrastructural localization and its relation to the sarcoglycan subcomplex. *Micron* **37**, 591–596 (2006).
52. Rafii, M. S. *et al.* Biglycan binds to α- and γ-sarcoglycan and regulates their expression during development. *J. Cell. Physiol.* 1 Aug 2006 (doi:10.1002/jcp.20740).

© 2006 Nature Publishing Group

53. Thompson, T. G. *et al.* Filamin 2 (FLN2): a muscle-specific sarcoglycan interacting protein. *J. Cell Biol.* **148**, 115–126 (2000).
54. Yang, B. *et al.* SH3 domain-mediated interaction of dystroglycan and Grb2. *J. Biol. Chem.* **270**, 11711–11714 (1995).
55. Way, M. & Parton, R. G. M-caveolin, a muscle-specific caveolin-related protein. *FEBS Lett.* **378**, 108–112 (1996).
56. Galbiati, F. *et al.* Caveolin-3 null mice show a loss of caveolae, changes in the microdomain distribution of the dystrophin–glycoprotein complex, and T-tubule abnormalities. *J. Biol. Chem.* **276**, 21425–21433 (2001).
57. Venema, V. J., Ju, H., Zou, R. & Venema, R. C. Interaction of neuronal nitric-oxide synthase with caveolin-3 in skeletal muscle. Identification of a novel caveolin scaffolding/inhibitory domain. *J. Biol. Chem.* **272**, 28187–28190 (1997).
58. Song, K. S. *et al.* Expression of caveolin-3 in skeletal, cardiac, and smooth muscle cells. Caveolin-3 is a component of the sarcolemma and co-fractionates with dystrophin and dystrophin-associated glycoproteins. *J. Biol. Chem.* **271**, 15160–15165 (1996).
59. Scherer, P. E. & Lisanti, M. P. Association of phosphofructokinase-M with caveolin-3 in differentiated skeletal myotubes. Dynamic regulation by extracellular glucose and intracellular metabolites. *J. Biol. Chem.* **272**, 20698–20705 (1997).
60. Galbiati, F. *et al.* Transgenic overexpression of caveolin-3 in skeletal muscle fibers induces a Duchenne-like muscular dystrophy phenotype. *Proc. Natl Acad. Sci. USA* **97**, 9689–9694 (2000).
61. Sotgia, F. *et al.* Caveolin-3 directly interacts with the C-terminal tail of β-dystroglycan. Identification of a central WW-like domain within caveolin family members. *J. Biol. Chem.* **275**, 38048–38058 (2000).
62. Minetti, C. *et al.* Mutations in the caveolin-3 gene cause autosomal dominant limb–girdle muscular dystrophy. *Nature Genet.* **18**, 365–368 (1998).
63. Herrmann, R. *et al.* Dissociation of the dystroglycan complex in caveolin-3-deficient limb girdle muscular dystrophy. *Hum. Mol. Genet.* **9**, 2335–2340 (2000).
64. Matsuda, C. *et al.* The sarcolemmal proteins dysferlin and caveolin-3 interact in skeletal muscle. *Hum. Mol. Genet.* **10**, 1761–1766 (2001).
65. Galbiati, F., Volonte, D., Minetti, C., Bregman, D. B. & Lisanti, M. P. Limb-girdle muscular dystrophy (LGMD-1C) mutants of caveolin-3 undergo ubiquitination and proteasomal degradation. Treatment with proteasomal inhibitors blocks the dominant negative effect of LGMD-1C mutants and rescues wild-type caveolin-3. *J. Biol. Chem.* **275**, 37702–37711 (2000).
66. Matsuda, C. *et al.* Dysferlin interacts with affixin (β-parvin) at the sarcolemma. *J. Neuropathol. Exp. Neurol.* **64**, 334–340 (2005).
67. Yamaji, S. *et al.* Affixin interacts with α-actinin and mediates integrin signaling for reorganization of F-actin induced by initial cell–substrate interaction. *J. Cell Biol.* **165**, 539–551 (2004).
68. Lennon, N. J. *et al.* Dysferlin interacts with annexins A1 and A2 and mediates sarcolemmal wound-healing. *J. Biol. Chem.* **278**, 50466–50473 (2003).
69. Holt, K. H., Crosbie, R. H., Venzke, D. P. & Campbell, K. P. Biosynthesis of dystroglycan: processing of a precursor propeptide. *FEBS Lett.* **468**, 79–83 (2000).
70. Barresi, R. & Campbell, K. P. Dystroglycan: from biosynthesis to pathogenesis of human disease. *J. Cell Sci.* **119**, 199–207 (2006).
71. Kobayashi, K. *et al.* An ancient retrotransposal insertion causes Fukuyama-type congenital muscular dystrophy. *Nature* **394**, 388–392 (1998).
72. Beltran-Valero de Bernabe, D. *et al.* Mutations in the *FKRP* gene can cause muscle–eye–brain disease and Walker–Warburg syndrome. *J. Med. Genet.* **41**, e61 (2004).
73. Taniguchi, K. *et al.* Worldwide distribution and broader clinical spectrum of muscle–eye–brain disease. *Hum. Mol. Genet.* **12**, 527–534 (2003).
74. Beltran-Valero de Bernabe, D. *et al.* Mutations in the *O*-mannosyltransferase gene *POMT1* give rise to the severe neuronal migration disorder Walker–Warburg syndrome. *Am. J. Hum. Genet.* **71**, 1033–1043 (2002).
75. Brockington, M. *et al.* Mutations in the fukutin-related protein gene (*FKRP*) cause a form of congenital muscular dystrophy with secondary laminin α2 deficiency and abnormal glycosylation of α-dystroglycan. *Am. J. Hum. Genet.* **69**, 1198–1209 (2001).
76. Longman, C. *et al.* Mutations in the human *LARGE* gene cause MDC1D, a novel form of congenital muscular dystrophy with severe mental retardation and abnormal glycosylation of α-dystroglycan. *Hum. Mol. Genet.* **12**, 2853–2861 (2003).
77. Brockington, M. *et al.* Mutations in the fukutin-related protein gene (*FKRP*) identify limb girdle muscular dystrophy 2I as a milder allelic variant of congenital muscular dystrophy MDC1C. *Hum. Mol. Genet.* **10**, 2851–2859 (2001).
78. Kanagawa, M. *et al.* Molecular recognition by LARGE is essential for expression of functional dystroglycan. *Cell* **117**, 953–964 (2004).
79. Yoshida, A. *et al.* Muscular dystrophy and neuronal migration disorder caused by mutations in a glycosyltransferase, POMGnT1. *Dev. Cell* **1**, 717–724 (2001).
80. Endo, T. *O*-Mannosyl glycans in mammals. *Biochim. Biophys. Acta* **1473**, 237–246 (1999).
81. Esapa, C. T. *et al.* Functional requirements for fukutin-related protein in the Golgi apparatus. *Hum. Mol. Genet.* **11**, 3319–3331 (2002).
82. Saito, Y., Mizuguchi, M., Oka, A. & Takashima, S. Fukutin protein is expressed in neurons of the normal developing human brain but is reduced in Fukuyama-type congenital muscular dystrophy brain. *Ann. Neurol.* **47**, 756–764 (2000).
83. Ishii, H., Hayashi, Y. K., Nonaka, I. & Arahata, K. Electron microscopic examination of basal lamina in Fukuyama congenital muscular dystrophy. *Neuromuscul. Disord.* **7**, 191–197 (1997).
84. Topaloglu, H. *et al.* *FKRP* gene mutations cause congenital muscular dystrophy, mental retardation, and cerebellar cysts. *Neurology* **60**, 988–992 (2003).
85. Brown, S. C. *et al.* Abnormalities in α-dystroglycan expression in MDC1C and LGMD2I muscular dystrophies. *Am. J. Pathol.* **164**, 727–737 (2004).
86. Manya, H. *et al.* Demonstration of mammalian protein *O*-mannosyltransferase activity: coexpression of POMT1 and POMT2 required for enzymatic activity. *Proc. Natl Acad. Sci. USA* **101**, 500–505 (2004).
87. van Reeuwijk, J. *et al.* The expanding phenotype of *POMT1* mutations: from Walker–Warburg syndrome to congenital muscular dystrophy, microcephaly, and mental retardation. *Hum. Mutat.* **27**, 453–459 (2006).
88. von der Mark, H. *et al.* Skeletal myoblasts utilize a novel β1-series integrin and not α6β1 for binding to the E8 and T8 fragments of laminin. *J. Biol. Chem.* **266**, 23593–23601 (1991).
89. Gontier, Y. *et al.* The Z-disc proteins myotilin and FATZ-1 interact with each other and are connected to the sarcolemma via muscle-specific filamins. *J. Cell Sci.* **118**, 3739–3749 (2005).
90. Mayer, U. *et al.* Absence of integrin α7 causes a novel form of muscular dystrophy. *Nature Genet.* **17**, 318–323 (1997).
91. Horwitz, A., Duggan, K., Buck, C., Beckerle, M. C. & Burridge, K. Interaction of plasma membrane fibronectin receptor with talin — a transmembrane linkage. *Nature* **320**, 531–533 (1986).
92. Hodges, B. L. *et al.* Altered expression of the α7β1 integrin in human and murine muscular dystrophies. *J. Cell Sci.* **110**, 2873–2881 (1997).
93. Burkin, D. J., Wallace, G. Q., Nicol, K. J., Kaufman, D. J. & Kaufman, S. J. Enhanced expression of the α7β1 integrin reduces muscular dystrophy and restores viability in dystrophic mice. *J. Cell Biol.* **152**, 1207–1218 (2001).
94. Allikian, M. J., Hack, A. A., Mewborn, S., Mayer, U. & McNally, E. M. Genetic compensation for sarcoglycan loss by integrin α7β1 in muscle. *J. Cell Sci.* **117**, 3821–3830 (2004).
95. Laing, N. G. & Nowak, K. J. When contractile proteins go bad: the sarcomere and skeletal muscle disease. *Bioessays* **27**, 809–822 (2005).
96. Gregorio, C. C. *et al.* The NH2 terminus of titin spans the Z-disc: its interaction with a novel 19-kD ligand (T-cap) is required for sarcomeric integrity. *J. Cell Biol.* **143**, 1013–1027 (1998).
97. Nicholas, G. *et al.* Titin-cap associates with, and regulates secretion of, Myostatin. *J. Cell Physiol.* **193**, 120–131 (2002).
98. Vainzof, M. *et al.* Telethonin protein expression in neuromuscular disorders. *Biochim. Biophys. Acta* **1588**, 33–40 (2002).
99. Mayans, O. *et al.* Structural basis for activation of the titin kinase domain during myofibrillogenesis. *Nature* **395**, 863–869 (1998).
100. Zou, P. *et al.* Palindromic assembly of the giant muscle protein titin in the sarcomeric Z-disk. *Nature* **439**, 229–233 (2006).
Describes how two titin proteins form an antiparallel sandwich with one telethonin molecule, providing insight into how sarcomeres are anchored and crosslinked and perhaps explaining the mechanical stability of the Z-line.
101. Richard, I. *et al.* Mutations in the proteolytic enzyme calpain 3 cause limb–girdle muscular dystrophy type 2A. *Cell* **81**, 27–40 (1995).
The first description that mutations within an enzyme, calpain-3, rather than a predominantly structural protein, cause MD.
102. Guyon, J. R. *et al.* Calpain 3 cleaves filamin C and regulates its ability to interact with γ- and δ-sarcoglycans. *Muscle Nerve* **28**, 472–483 (2003).
103. van der Ven, P. F. *et al.* Indications for a novel muscular dystrophy pathway. γ-filamin, the muscle-specific filamin isoform, interacts with myotilin. *J. Cell Biol.* **151**, 235–248 (2000).
104. Haravuori, H. *et al.* Secondary calpain3 deficiency in 2q-linked muscular dystrophy: titin is the candidate gene. *Neurology* **56**, 869–877 (2001).
105. Baghdiguian, S. *et al.* Calpain 3 deficiency is associated with myonuclear apoptosis and profound perturbation of the IκBα/NF-κB pathway in limb–girdle muscular dystrophy type 2A. *Nature Med.* **5**, 503–511 (1999).
106. Kramerova, I., Kudryashova, E., Tidball, J. G. & Spencer, M. J. Null mutation of calpain 3 (p94) in mice causes abnormal sarcomere formation *in vivo* and *in vitro*. *Hum. Mol. Genet.* **13**, 1373–1388 (2004).
107. Kudryashova, E., Kudryashov, D., Kramerova, I. & Spencer, M. J. Trim32 is a ubiquitin ligase mutated in limb girdle muscular dystrophy type 2H that binds to skeletal muscle myosin and ubiquitinates actin. *J. Mol. Biol.* **354**, 413–424 (2005).
108. Salmikangas, P. *et al.* Myotilin, the limb–girdle muscular dystrophy 1A (LGMD1A) protein, cross-links actin filaments and controls sarcomere assembly. *Hum. Mol. Genet.* **12**, 189–203 (2003).
109. Salmikangas, P. *et al.* Myotilin, a novel sarcomeric protein with two Ig-like domains, is encoded by a candidate gene for limb–girdle muscular dystrophy. *Hum. Mol. Genet.* **8**, 1329–1336 (1999).
110. Faulkner, G. *et al.* ZASP: a new Z-band alternatively spliced PDZ-motif protein. *J. Cell Biol.* **146**, 465–475 (1999).
111. Ozawa, R. *et al.* Emerin-lacking mice show minimal motor and cardiac dysfunctions with nuclear-associated vacuoles. *Am. J. Pathol.* **168**, 907–917 (2006).
112. Vaughan, A. *et al.* Both emerin and lamin C depend on lamin A for localization at the nuclear envelope. *J. Cell Sci.* **114**, 2577–2590 (2001).
113. Yates, J. R. *et al.* Genotype–phenotype analysis in X-linked Emery–Dreifuss muscular dystrophy and identification of a missense mutation associated with a milder phenotype. *Neuromuscul. Disord.* **9**, 159–165 (1999).
114. Fidzianska, A. & Hausmanowa-Petrusewicz, I. Architectural abnormalities in muscle nuclei. Ultrastructural differences between X-linked and autosomal dominant forms of EDMD. *J. Neurol. Sci.* **210**, 47–51 (2003).
115. Melcon, G. *et al.* Loss of emerin at the nuclear envelope disrupts the Rb1/E2F and MyoD pathways during muscle regeneration. *Hum. Mol. Genet.* **15**, 637–651 (2006).
116. Bakay, M. *et al.* Nuclear envelope dystrophies show a transcriptional fingerprint suggesting disruption of Rb–MyoD pathways in muscle regeneration. *Brain* **129**, 996–1013 (2006).
Shows that mutations in two genes, one encoding the nuclear-envelope proteins lamin A and lamin C and one encoding emerin, lead to similar MDs that involve the Rb1 and MyoD transcriptional regulatory pathway. These MDs are similar to another MD, FSHD, which is also thought to involve perturbations of the nuclear envelope.
117. Frock, R. L. *et al.* Lamin A/C and emerin are critical for skeletal muscle satellite cell differentiation. *Genes Dev.* **20**, 486–500 (2006).
118. Mahadevan, M. *et al.* Myotonic dystrophy mutation: an unstable CTG repeat in the 3′ untranslated region of the gene. *Science* **255**, 1253–1255 (1992).
119. Liquori, C. L. *et al.* Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of *ZNF9*. *Science* **293**, 864–867 (2001).

© 2006 Nature Publishing Group

120. Day, J. W. & Ranum, L. P. RNA pathogenesis of the myotonic dystrophies. *Neuromuscul. Disord.* **15**, 5–16 (2005).
121. Brais, B. *et al.* Short GCG expansions in the *PABP2* gene cause oculopharyngeal muscular dystrophy. *Nature Genet.* **18**, 164–167 (1998).
122. Wahle, E. A novel poly(A)-binding protein acts as a specificity factor in the second phase of messenger RNA polyadenylation. *Cell* **66**, 759–768 (1991).
123. Calado, A., Kutay, U., Kuhn, U., Wahle, E. & Carmo-Fonseca, M. Deciphering the cellular pathway for transport of poly(A)-binding protein II. *RNA* **6**, 245–256 (2000).
124. Davies, J. E., Berger, Z. & Rubinsztein, D. C. Oculopharyngeal muscular dystrophy: potential therapies for an aggregate-associated disorder. *Int. J. Biochem. Cell Biol.* **38**, 1457–1462 (2006).
125. Hewitt, J. E. *et al.* Analysis of the tandem repeat locus D4Z4 associated with facioscapulohumeral muscular dystrophy. *Hum. Mol. Genet.* **3**, 1287–1295 (1994).
126. van der Maarel, S. M. & Frants, R. R. The D4Z4 repeat-mediated pathogenesis of facioscapulohumeral muscular dystrophy. *Am. J. Hum. Genet.* **76**, 375–386 (2005).
127. Gabellini, D. *et al.* Facioscapulohumeral muscular dystrophy in mice overexpressing *FRG1*. *Nature* **439**, 973–977 (2006).
The first indication that, in mice, the transgenic overexpression of a gene, *FRG1*, which neighbours a repeat-element region, can lead to an MD that is phenotypically similar to human FSHD. These findings reveal a possible mechanism behind the human disease.
128. Chakkalakal, J. V., Thompson, J., Parks, R. J. & Jasmin, B. J. Molecular, cellular, and pharmacological therapies for Duchenne/Becker muscular dystrophies. *FASEB J.* **19**, 880–891 (2005).
129. Nowak, K. J. & Davies, K. E. Duchenne muscular dystrophy and dystrophin: pathogenesis and opportunities for treatment. *EMBO Rep.* **5**, 872–876 (2004).
130. Blankinship, M. J., Gregorevic, P. & Chamberlain, J. S. Gene therapy strategies for Duchenne muscular dystrophy utilizing recombinant adeno-associated virus vectors. *Mol. Ther.* **13**, 241–249 (2006).
131. Wilton, S. D. & Fletcher, S. Modification of pre-mRNA processing: application to dystrophin expression. *Curr. Opin. Mol. Ther.* **8**, 130–135 (2006).
132. Alter, J. *et al.* Systemic delivery of morpholino oligonucleotide restores dystrophin expression bodywide and improves dystrophic pathology. *Nature Med.* **12**, 175–177 (2006).
Outlines the delivery of morpholino oligonucleotides through an intravenous injection in a dystrophic mouse model, resulting in body-wide expression of dystrophin and improvement in muscle function.
133. Zhu, T. *et al.* Sustained whole-body functional rescue in congestive heart failure and muscular dystrophy hamsters by systemic gene transfer. *Circulation* **112**, 2650–2659 (2005).
134. Denti, M. A. *et al.* Body-wide gene therapy of Duchenne muscular dystrophy in the *mdx* mouse model. *Proc. Natl Acad. Sci. USA* **103**, 3758–3763 (2006).
An exciting description of body-wide delivery of a small nuclear RNA through transduction of an AAV in a dystrophic mouse model, which led to persistent exon skipping of dystrophin and subsequent recovery of functional and biochemical parameters.

Acknowledgements
K.E.D. is supported by the Medical Research Council, UK, Muscular Dystrophy Association, USA, the Muscular Dystrophy Campaign, UK, and the Association Française Contre les Myopathies. K.J.N. is funded by the Australian National Health and Medical Research Council.

Competing interests statement
The authors declare no competing financial interests.

DATABASES
The following terms in this article are linked online to:
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
dystrophin | *DMPK* | *FKRP* | *FRG1* | *LARGE* | *LMNA* | *PABPN1* | *POMGnT1* | *POMT1* | *ZNF9*
UniProtKB: http://ca.expasy.org/sprot
affixin | calpain-3 | caveolin-3 | desmin | dysbindin | dysferlin | α-dystrobrevin | emerin | filamin-C | FKRP | fukutin | GRB2 | myotilin | POMGnT1 | POMT1 | POMT2 | α-sarcoglycan | β–sarcoglycan | δ–sarcoglycan | γ–sarcoglycan | synemin | α1-syntrophin | β1-syntrophin | telethonin | titin | TRIM32 | utrophin | vimentin | ZASP
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
BMD | DM1 | DM2 | DMD | EDMD | Hutchinson–Gilford progeria syndrome | LGMD2J | TMD

FURTHER INFORMATION
Kay Davies' homepage:
http://www.mrcfgu.ox.ac.uk/kedavies.html

SUPPLEMENTARY INFORMATION
See online article: S1 (box) | S2 (table)
Access to this links box is available online.

© 2006 Nature Publishing Group