

Dysferlin and the plasma membrane repair in muscular dystrophy

Dimple Bansal and Kevin P. Campbell

Howard Hughes Medical Institute, Department of Physiology and Biophysics and Department of Neurology, University of Iowa, Roy J. & Lucille A. Carver College of Medicine, Iowa City, IA 52242, USA

Muscular dystrophy covers a group of genetically determined disorders that cause progressive weakness and wasting of the skeletal muscles. Dysferlin was identified as a gene mutated in limb-girdle muscular dystrophy (type 2B) and Miyoshi myopathy. The discovery of dysferlin revealed a new family of proteins, known as the ferlin family, which includes four different genes. Recent work suggests the function of dysferlin in membrane repair and demonstrates that defective membrane repair is a novel mechanism of muscle degeneration. These findings reveal the importance of a basic cellular function in skeletal muscle and a new class of muscular dystrophy where the defect lies in the maintenance, not the structure, of the plasma membrane. Here, we discuss the current knowledge of dysferlin function in the repair of the plasma membrane of the skeletal muscle cells.

Muscular dystrophy includes a diverse group of inherited myogenic disorders characterized by progressive muscle weakness and wasting. Although skeletal muscle is the tissue primarily affected in muscular dystrophies, structural and functional abnormalities can also be detected in other tissues such as cardiac muscle, smooth muscle and brain [1–3]. A great amount of variation can be observed in the distribution and severity of the affected muscles in different types of muscular dystrophies. Several criteria are used to classify muscular dystrophies; these include: age of onset, rate of disease progression, mode of inheritance, muscle-group involvement and, most commonly, the genetic cause or the gene affected [4]. Since the discovery of dystrophin [5], a large number of genes either associated with or linked to various types of muscular dystrophies have been identified, thus providing a genetic basis for the classification of muscular dystrophies. Table 1 shows a list of various types of muscular dystrophies, their mode of inheritance and the responsible gene. Interestingly, 12 of these muscular dystrophies are related to the dystrophin–glycoprotein complex (DGC) – extracellular matrix proteins or glycosylation enzymes required for the processing of dystroglycan. The DGC is a multisubunit complex of proteins, present at the muscle sarcolemma, that connects the extracellular matrix to the intracellular actin cytoskeleton. A mutation in one protein component of the DGC disrupts the stability of the whole complex, which in turn makes the muscle cell membrane

susceptible to contraction-induced injuries [6]. Skeletal muscle cells are very susceptible to plasma membrane injuries because they generate force by contraction [7,8]. A direct or indirect association of a large number of muscular dystrophies with the DGC reflects the need for maintaining the structural integrity of the plasma membrane in skeletal muscle cells. The cells have therefore evolved mechanisms to maintain plasma membrane integrity. Plasma membrane repair is a basic cellular mechanism the primary function of which is to mend any physical injuries to the plasma membrane [7,9,10]. Recent data show that a defect in plasma membrane repair could cause muscle degeneration, which can lead to muscular dystrophy. Dysferlin was identified as a gene that is mutated in limb-girdle muscular dystrophy (LGMD) type 2B and Miyoshi myopathy [11,12] and was recently shown to have a role in the process of membrane repair in skeletal muscle cells [13]. These studies demonstrated that dysferlin-null mice maintain functional DGCs [13,14] and a stable plasma membrane structure; however, they slowly develop progressive muscular dystrophy [15] caused by the defect in the repair of muscle cell plasma membrane [13]. Therefore, in contrast to DGC-linked muscular dystrophies, dysferlin-linked muscular dystrophies introduce a new class of the disease where the repair, not the structure, of the plasma membrane is disrupted. This is a novel mechanism of muscle degeneration. This review discusses the current understanding of dysferlin function in the repair of the plasma membrane of muscle cells.

LGMD

LGMDs are a group of muscular dystrophies characterized by predominant weakness and wasting of muscles of the pelvic and shoulder girdle. There is a wide range of clinical heterogeneities in this group of muscular dystrophies, which can be attributed to the involvement of a large number of different genes. There are two types of LGMDs – autosomal dominant (LGMD type 1) and autosomal recessive (LGMD type 2). Sixteen genetically different LGMDs have been identified, of which 12 LGMDs have been defined by the primary gene involved. Work is in progress to identify the genes responsible for the remaining four LGMDs; these have been mapped to a defined region of specific chromosomes by linkage analysis.

LGMD2B and Miyoshi myopathy

LGMD2B and Miyoshi myopathy are two autosomal recessive muscle diseases. They are clinically distinct

Corresponding author: Kevin P. Campbell (kevin-campbell@uiowa.edu).

Table 1. Various muscular dystrophies and the genes linked to them^a

Disease	Mode of inheritance	Gene locus	Gene product
Duchenne and/or Becker muscular dystrophy	XR	Xp21	Dystrophin
Limb-girdle muscular dystrophy (LGMD)			
LGMD1A	AD	5q22	Myotilin
LGMD1B	AD	1q11–q21	Lamin A and/or C
LGMD1C	AD	3p25	Caveolin-3
LGMD1D	AD	6q23	Not identified
LGMD1E	AD	7q	Not identified
LGMD1F	AD	2q	Not identified
LGMD2A	AR	15q15	Calpain-3
LGMD2B	AR	2p13	Dysferlin
LGMD2C	AR	13q12	γ -Sarcoglycan
LGMD2D	AR	17q12	α -Sarcoglycan
LGMD2E	AR	4q12	β -Sarcoglycan
LGMD2F	AR	5q23	δ -Sarcoglycan
LGMD2G	AR	17q11	TCAP
LGMD2H	AR	9q31	TRIM32
LGMD2I	AR	19q13	FKRP
LGMD2J	AR	2q31	Titin
Distal muscular dystrophy (DMD)			
Miyoshi myopathy	AR	2p13	Dysferlin
Tibial muscular dystrophy	AD	2q31	Titin
Congenital muscular dystrophy (CMD)			
MDC1A	AR	6q22	Laminin α 2
MDC1B	AR	1q42	Not identified
MDC1C	AR	19q13	FKRP
MDC1D	AR	22q12	LARGE
Fukuyama CMD	AR	9q31–q33	Fukutin
α 7 integrin congenital myopathy	AR	12q13	α 7 Integrin
Rigid spine CMD	AR	1p36	Selenoprotein N1
Muscle–eye–brain disease	AR	1p32	POMGnT1 glycosyltransferase
Walker–Warburg syndrome	AR	9q34	POMT1
Other types of muscular dystrophy			
Emery–Dreifuss muscular dystrophy	XR	Xq28	Emerin
Emery–Dreifuss muscular dystrophy	AD and AR	1q11–q23	LaminA/C
Bethlem myopathy/Ullrich syndrome	AD	21q22	Collagen VI α 1
	AD	21q22	Collagen VI α 2
	AD	2q37	Collagen VI α 3
Epidermolysis bullosa and muscular dystrophy	AR	8q24-qter	Plectin
Oculopharyngeal muscular dystrophy	AD	14q11.2–q13	Poly-A-binding protein 2
Facioscapulohumeral muscular dystrophy	AD	4q35	Not identified
Myotonic dystrophy	AD	19q13	Myotonin protein kinase
Myotonic dystrophy type 2	AD	3q21.3	ZNF9

^aAbbreviations: AD, autosomal dominant; AR, autosomal recessive; FKRP, fukutin-related protein; MDC, muscular dystrophy congenital type; TCAP, telethonin; XR, X-linked recessive.

because they differ in both muscle groups that initially show the onset of the disease and predominant weakness and wasting in the respective patients. LGMD2B is a predominantly proximal muscular dystrophy with an onset in the late teens. The patients show normal mobility in childhood with a slowly progressive muscle weakness and wasting [16]. The anterior muscles of distal legs and distal arms are relatively normal in these patients, even at the later stages of the disease. By contrast, Miyoshi myopathy is a predominantly distal muscular dystrophy with early involvement of the posterior compartments of the lower limb. Although previously described mainly in Japan, Miyoshi myopathy has been increasingly recognized in Western countries over the past few years. The disease onset is generally in the late teens with an initial involvement of the muscles of distal lower limbs; a common early symptom is the inability of the patients to stand on tiptoes. Although these two diseases affect different muscle groups, they share some similarities, suggesting a possible common mechanism of muscle pathology. For example in both diseases, muscles of the limb and girdle

are primarily affected, the symptoms usually appear in the late teens, the diseases progress slowly, and high levels of creatine kinase, a skeletal-muscle-specific enzyme, are detected in the serum of the patients [16].

Genetic-linkage mapping revealed that dysferlin is the gene that is mutated in both LGMD2B and Miyoshi myopathy patients [11,12]. In these patients, several mutations causing from partial to complete loss of dysferlin have been identified [17,18]. However, no correlation between the type or location of the mutation and the clinical phenotype has been established. Therefore, LGMD2B and Miyoshi myopathy cannot be distinguished on the basis of mutation analysis [18]. Furthermore, the same mutation in dysferlin was responsible for both LGMD2B and Miyoshi myopathy phenotypes in one family, suggesting that there are other genetic factors that can affect the clinical symptoms and severity of these diseases in the patients [18,19]. Although search for these modifier genes is in progress, no gene has been identified that can explain the variations in the severity of the dysferlin-linked muscular dystrophy.

Box 1. C2 protein domains

The C2 domains are independently folded protein domains of ~120–140 amino acid residues that were initially defined as the second-constant sequence (hence called C2) in protein kinase C [38,39]. They are found in several proteins, involved in signal transduction, vesicle trafficking and membrane fusion, and are known to interact with phospholipids and proteins [27,40–43]. They are implicated mainly in two different pathways in the cell: (i) generation of the second-messenger lipids, involved in transduction pathways, and (ii) membrane trafficking and fusion. The cytoplasmic phospholipases A2 belong to the first category and members of the second category are proteins involved in the docking of the synaptic vesicles to the plasma membrane and/or their fusion, such as synaptotagmins, rabphilin 3A, munc 13, DOC2 proteins and RIM. C2 domains are most studied and best characterized in synaptotagmins. Synaptotagmins are a very

well-studied family of proteins that play a role in vesicle trafficking and membrane fusion and regulate important cellular events including synaptic transmission and plasma membrane repair. Synaptotagmins contain two C2 domains and one N-terminal transmembrane domain, whereas dysferlin contains six C2 domains and a C-terminal transmembrane domain. In the membrane fusion process, C2 domains can interact with negatively charged phospholipids and proteins; this is a consequence of the modification of their surface electrostatic potential caused by calcium binding. The binding of calcium to the C2 domains primarily involves aspartyl side-chains that act as bidentate ligands for these ions [40,41]. The C2 domains of both dysferlin and otoferlin possess these aspartyl residues, suggesting that their interactions with other proteins or phospholipids are calcium dependent.

Dysferlin and other members of the ferlin protein family

Dysferlin is a mammalian gene that shows homology to fer-1 gene of *C. elegans* [11]. Fer-1 is a spermatogenesis factor that is specifically expressed in primary spermatocytes of *C. elegans*. In spermatids, mutations in fer-1 cause infertility by impairing the fusion of large vesicles (termed membranous organelles) with the plasma membrane [20,21]. The fusion of these vesicles with the plasma membrane adds extra membrane to the plasma membrane at the fusion site; this is required for the extension of the pseudopodia that cause crawling of the spermatids. Thus, mutations in fer-1 lead to immotile spermatids and consequently to sterility in *C. elegans* [20,21]. Because of structural and sequence similarities between dysferlin and fer-1, it was suggested that dysferlin might also be a vesicle-associated membrane protein involved in the docking and fusion of vesicles in the skeletal muscle cells. After the identification of dysferlin, several novel genes showing protein structure and sequence homology to dysferlin were identified [22,23]. Therefore, a new family of mammalian proteins was discovered and called ferlin. Until now, four genes have been identified that belong to the ferlin family (Table 2). All members of the ferlin family have a conserved protein structure and share two common characteristics: the presence of (i) several C2 domains (Box 1) and (ii) a single transmembrane domain at their C terminus (Figure 1). Of the four members of the ferlin family, only dysferlin and otoferlin have been linked to human disease.

In humans, dysferlin is located on chromosome 2p13 and encompasses 55 exons spanning over 150 kb of genomic DNA [24]. The dysferlin gene encodes a 230-kDa protein with widespread expression in tissues such as skeletal muscle, cardiac muscle, kidney, placenta, lung and brain. Although dysferlin is most abundantly expressed in skeletal and cardiac muscles, there is no evidence for cardiac muscle dysfunction in dysferlin-deficient patients.

Immunohistochemical studies showed that, in skeletal muscle, dysferlin is located at the plasma membrane, as well as in cytoplasmic vesicles, [13] and is absent in human patients with LGMD2B and Miyoshi myopathy [14]. In the skeletal muscle cells of human fetus, dysferlin was detected in 5–6-week-old embryos, when limb development starts to show regional differentiation [25].

Dysferlin contains six C2 domains and a single transmembrane domain located at its C terminus. Dysferlin is a type II transmembrane protein with a membrane topology suggesting that it anchors to the plasma membrane by its C-terminal transmembrane domain, whereas the N-terminal part of the protein resides in the cytoplasm of the muscle fiber (Figure 2).

The protein structure of dysferlin is similar to that of synaptotagmins. In vertebrates, There are 13 different synaptotagmins with diverse functions [26] such as synaptic-vesicle fusion [27–29] and plasma membrane repair [30]. During the exocytosis of synaptic vesicle, synaptotagmin I and II work as calcium sensors [31]. The C2 domains of synaptotagmins are homologous to those of protein kinase C [32]. The C2 domain of protein kinase C binds to those parts of the membrane containing anionic lipids, such as phosphatidylserine, in the presence of micromolar concentrations of calcium. The calcium ions act as a 'bridge' between negatively charged lipids and negatively charged amino acid residues on the surface of the C2 domain. The C2 domains of synaptotagmins also bind to the synaptic SNARE proteins synaptobrevin, SNAP-25 and syntaxin [29]. These proteins are important components of the synaptic-vesicle fusion machinery and their interaction with synaptotagmins mediates the fusion of synaptic vesicles with the plasma membrane during the exocytosis of these vesicles. The similarities between dysferlin and synaptotagmins suggest a possible function of dysferlin in calcium-dependent vesicle fusion with the plasma membrane.

Table 2. List of protein members of the ferlin family

Gene name	Gene symbol	Gene locus	Open reading frame (amino acids)	Protein mass (kDa)	Linked human disease
Dysferlin	DYSF	2p13	2080	230	Limb-girdle type 2B and Miyoshi myopathy
Otoferlin	OTOF	2p23	1230	140	Autosomal recessive nonsyndromic deafness (DFNB9) ^a
Myoferlin	MYOF	10q24	2061	230	Not known
Fer1L4	FER1L4	20q11	1952	215	Not known

^aDFNB9 = A specific type of autosomal recessive deafness

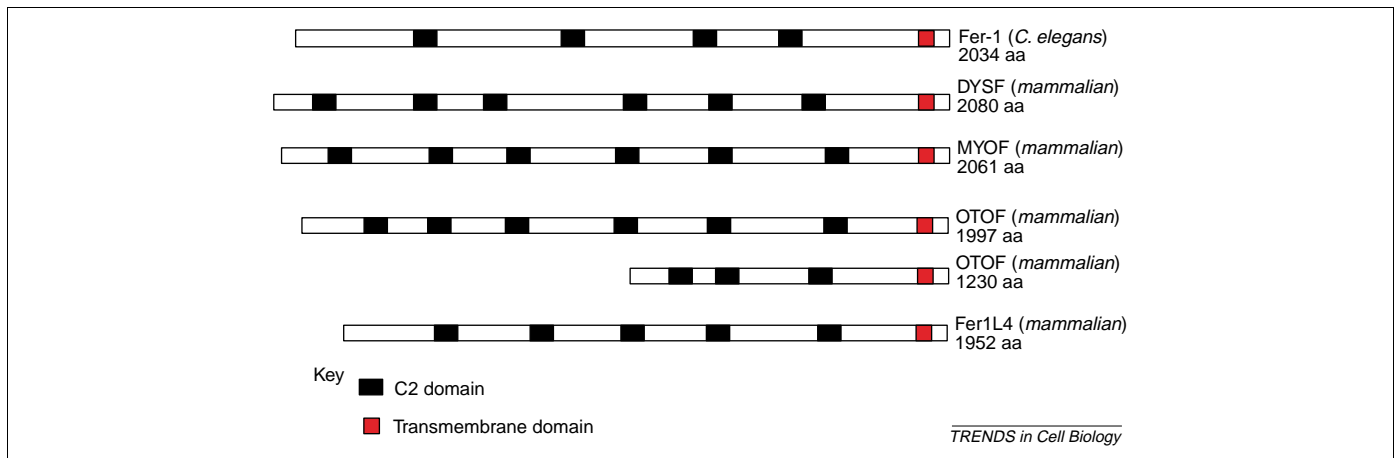


Figure 1. Conserved protein structure of the ferlin family. All ferlin proteins have variable number of C2 domains throughout their length and a transmembrane domain at their C terminus. Sequence analysis of dysferlin and myoferlin and the longer isoform of otoferlin suggest the presence of six C2 domains, whereas data on the small isoform of otoferlin suggest only three C2 domains and those on Fer1L4 suggest five C2 domains. Although the *C. elegans* fer-1 protein is very similar to dysferlin in size, it has only four C2 domains.

Interestingly, mutations in otoferlin, the second-identified member of ferlin family, are responsible for DFNB9, a specific type of autosomal recessive deafness in humans [23]. Sequence and northern blot analysis on the RNA isolated from human tissues suggests that otoferlin is smaller than dysferlin, and shows 64% similarity and 31% identity to dysferlin at the level of amino acid sequence. Otoferlin encodes a 1230-amino-acid protein with a molecular mass of 140 kDa. Otoferlin has three predicted C2 domains and a transmembrane domain at its C terminus [23]. Dysferlin shows homology to otoferlin mainly at the C-terminal region, and the C2 domains of otoferlin correspond to the last three C2 domains of dysferlin. In addition to the 140-kDa isoform, a longer isoform of otoferlin was detected in human and mouse tissues [33]. This isoform encodes a protein composed of

1997 amino acid residues and with a molecular mass of ~227 kDa. Similar to dysferlin, the longer isoform possesses six C2 domains and a single transmembrane domain. The transcripts for the long isoform of otoferlin (six C2 domains) could be detected in both human and mouse tissues; however, transcripts for shorter isoforms (three C2 domains) could only be detected in human tissues [33]. At present, the basis of this difference is unknown, but the protein expression profiles of these isoforms, as well as identifying their function, will be useful to understand this difference. Primarily, mouse otoferlin shows expression in the cochlea, vestibule and brain, although a basal level of expression could also be detected in several other tissues including lung, kidney, skeletal muscle and heart [23].

The other two members of the ferlin family are myoferlin [22,34] and Fer1L4 [35]. The sequence of myoferlin is highly homologous to that of dysferlin, whereas Fer1L4 is most homologous to otoferlin. Myoferlin is also thought to be a type II transmembrane protein, containing six C2 domains. Immunohistochemical analysis suggested that, similar to dysferlin, myoferlin is present on the plasma membrane but, in contrast to dysferlin, it is also present in the nucleus of the muscle fibers. Myoferlin is upregulated at the plasma membrane of muscle fibers in the dystrophin-deficient mdx mouse [36,37] and is hypothesized to play a role in muscle regeneration and repair [34]. Fer1L4 is the most recently identified member of ferlin family. The open reading frame of Fer1L4 predicts a 1952-amino-acid protein with five C2 domains [35]. Although no association between any human disease and the genes encoding myoferlin and Fer1L4 has been identified, efforts are in progress to investigate a possible link.

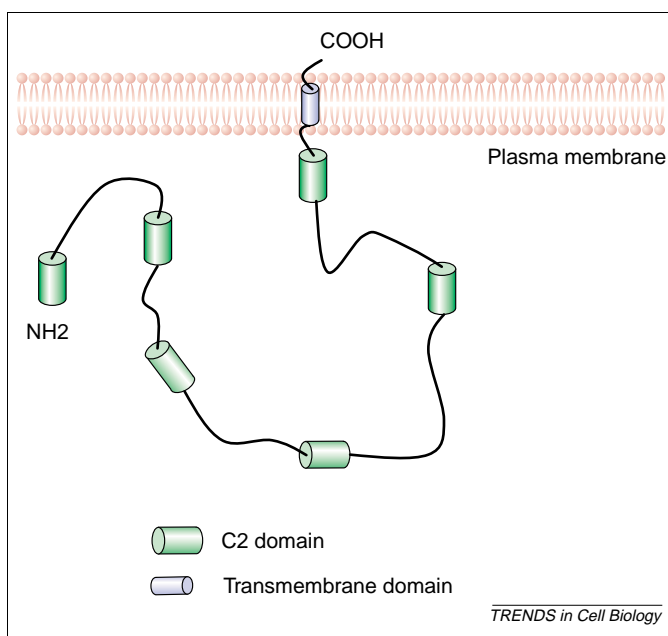


Figure 2. Predicted protein structure and topology of dysferlin. The predicted topology of dysferlin suggests that this protein anchors to the plasma membrane by its C-terminal transmembrane domain and the N terminus of the protein resides in the cytoplasm.

Dysferlin and membrane fusion

As discussed earlier, on the basis of homology between dysferlin and the fer-1 gene of *C. elegans*, it is thought that dysferlin plays a role in vesicle trafficking and membrane fusion in muscle cells. The first C2 domain of dysferlin binds to phospholipids in a calcium-dependent manner [44]. A missense mutation in this domain has been

identified in some patients; in both LGMD2B and Miyoshi myopathy patients, the mutation can give rise to a range of phenotypic severity. Interestingly, *in vitro* studies showed that introduction of this mutation in the first C2 domain of dysferlin results in altered binding properties of this domain to phospholipids over a range of calcium concentrations [44]. These studies suggest that the normal calcium-dependent binding of the C2 domains of dysferlin to membrane phospholipids is crucial for the function of dysferlin and further support the idea that, in skeletal muscle cells, dysferlin plays a role in vesicle trafficking and membrane fusion.

Dysferlin and the DGC

The DGC is an essential component of the muscle sarcolemma and is required for the structural stability of the plasma membrane [37,45]. Mutations in several components of the DGC result in different types of muscular dystrophy [37,45]. The loss of one of the DGC components destabilizes the whole complex at the sarcolemma and causes loss of the transmembrane link between the extracellular matrix and intracellular cytoskeleton [6,46]. Extensive studies performed on the mouse models showed that, in DGC-linked muscular dystrophies, the sarcolemma is structurally unstable and very susceptible to damage [6,46]. Although dysferlin is not an integral component of the DGC, its distribution is altered in DGC-linked muscular dystrophies [47]. In several different types of the muscular dystrophies, dysferlin expression is reduced on the plasma membrane and increased in the cytoplasmic vesicles [47]. Although it is not clear what causes this alteration in the dysferlin distribution in these muscular dystrophies, the change in the location suggests that the function of dysferlin has been affected. Therefore, it is possible that dysferlin has a direct or indirect functional association with DGC. Recent data, however, demonstrated that dysferlin-null mice show normal expression of various DGC components, and the DGC is stable and functional in dysferlin-null muscle [13]. The presence of a stable and functional DGC suggests a structurally stable sarcolemma in dysferlin-null muscle cells. Further evidence supporting this came from studies involving exercise-induced damage to the plasma membrane of muscle cells. Physical exercise, such as running, causes stress to the skeletal muscle, and under such conditions, muscle cells with structurally unstable sarcolemma are more susceptible to membrane damage than their normal counterparts [48,49]. It has been suggested that eccentric contraction, which involves the development of tension while the muscle is being lengthened, and is caused during downhill running, is most damaging to the muscle membrane [50]. A recent study showed that, although in the mdx mice downhill running on the treadmill caused severe membrane damage, dysferlin-null mice, similar to wild-type mice, exhibited no significant increase in membrane damage. These results suggest that the muscle cells of dysferlin-null mice have a structurally stable sarcolemma [13]. Furthermore, they indicate that, in dysferlin-deficient muscle, the pathological mechanism of muscle degeneration is different from the DGC-linked muscular dystrophies.

Defective plasma membrane repair in dysferlin-null muscle cells

The plasma membrane of a cell provides a physical barrier between extracellular and intracellular environments, and the maintenance of this barrier is crucial for cell survival. Plasma membrane repair is a basic cellular process required to reseal membrane disruptions [10,51,52]. Recent studies show that, in dysferlin-null mice, the muscle cells are defective in repairing the membrane disruptions and suggest that, in dysferlin-deficient muscular dystrophies, impaired membrane repair, caused by dysferlin loss, is responsible for muscle degeneration [13]. Several studies have previously suggested the presence of membrane irregularities in the skeletal muscle cells of patients carrying mutations in dysferlin [53]. The plasma membrane irregularities, such as disrupted plasma membrane, thickening of basal lamina and accumulations of small vesicles under membrane disruptions, were observed by electron microscopical analysis of skeletal muscle biopsies from patients with dysferlin mutations [53]. Membrane irregularities, such as vesicle accumulations under the disrupted membrane, were also reported in the skeletal muscle cells of dysferlin-null mice [13]. Taken together, these findings suggest that dysferlin-deficient muscle cells show disruptions in the plasma membrane, which could arise from defective repair of the plasma membrane.

To repair a disrupted membrane, additional membrane must be added to the surface at or near the membrane disruption site [52,54]. Studies have shown that this additional membrane comes from an intracellular source and is targeted to the disruption site in the form of vesicles [52,54–57]. These vesicles fuse with each other and with the plasma membrane in the presence of high levels of calcium entering the cell at sites of membrane disruption [56]. Recent data demonstrate that, in the muscle fibers that underwent membrane damage, dysferlin is enriched at the membrane disruption sites [13]. A membrane disruption causes the transient loss and reduction of the sarcolemma markers at the disruption site and the enrichment of markers for proteins involved in the resealing of the disruption [30]. Therefore, the enrichment of dysferlin on the membrane disruption sites suggests that, because of the fusion of the dysferlin vesicles at these sites, dysferlin molecules accumulate. Furthermore, accumulation of vesicles under the membrane disruption sites have been identified in the muscle cells of both patients with mutations in dysferlin and dysferlin-null mice. This also suggests that dysferlin deficiency renders these vesicles unable to repair membrane disruption. Interestingly, vesicle accumulation under the plasma membrane has also been reported in spermatocytes of *C. elegans* with mutated *fer-1* [20,21]. These data also suggest that it is not the targeting but the fusion of the vesicles that has been compromised in the absence of functional dysferlin, indicating a role for this protein in membrane fusion.

A direct assessment of the membrane repair ability of the dysferlin-deficient muscle cells was performed using a membrane repair assay. The authors showed that a disruption on the surface of wild-type skeletal muscle

cells is efficiently repaired in a calcium-dependent manner. However dysferlin-null muscle cells could not repair a similar disruption to the plasma membrane [13]. Another recent study reconfirms this finding and identifies annexins A1 and A2 as the proteins interacting with dysferlin and suggests that annexins play a role in dysferlin-mediated membrane repair [58].

Membrane-repair model

In the light of the recent findings, a model describing dysferlin-mediated membrane repair in the skeletal muscle cells is presented in Figure 3. The model states that a membrane disruption causes influx of extracellular calcium into the muscle fiber and creates a transient zone of high calcium around the injury site. Dysferlin-carrying vesicles are targeted to the disruption sites, where they fuse with each other and the plasma membrane in the presence of localized high levels of calcium ions. Dysferlin is thought to play a role at the fusion step of this repair process, facilitating vesicle docking and fusion with the plasma membrane by interacting with other dysferlin molecules, annexins or some other unknown protein-binding partner(s) at the plasma membrane. The fusion of vesicles causes addition of membrane to the plasma membrane, thereby patching and resealing the disrupted membrane. Therefore, in the LGMD2B and Miyoshi myopathy patients, because of a lack of dysferlin, the

disrupted membrane is not able to reseal, and thus, a membrane-disrupted muscle fiber is unable to survive.

Concluding remarks

The degeneration of skeletal muscle is the most common pathological feature of the muscular dystrophies, and several different mechanisms for the muscle degeneration have been proposed in different types of muscular dystrophies. There are a large number of muscular dystrophies that are thought to show a primary defect in the structural and functional maintenance of plasma membrane of the skeletal muscle cells. Physical stress can cause muscle degeneration by damaging the plasma membrane, when either the structural integrity of the membrane is compromised or the maintenance of the membrane is defective. The DGC-linked muscular dystrophies, which comprise a large group of extensively studied muscular dystrophies, share a common pathological mechanism of muscle degeneration; here, the structural integrity of the sarcolemma is compromised because the loss of the DGC sensitizes the muscle to contraction-induced injuries [4,6,37,46]. A recent study showed that muscular dystrophies characterized by dysferlin deficiency are defective in repairing injuries to the plasma membrane. This finding introduced a new class of muscular dystrophies in which the defect lies in the maintenance, and not in the structure, of the sarcolemma,

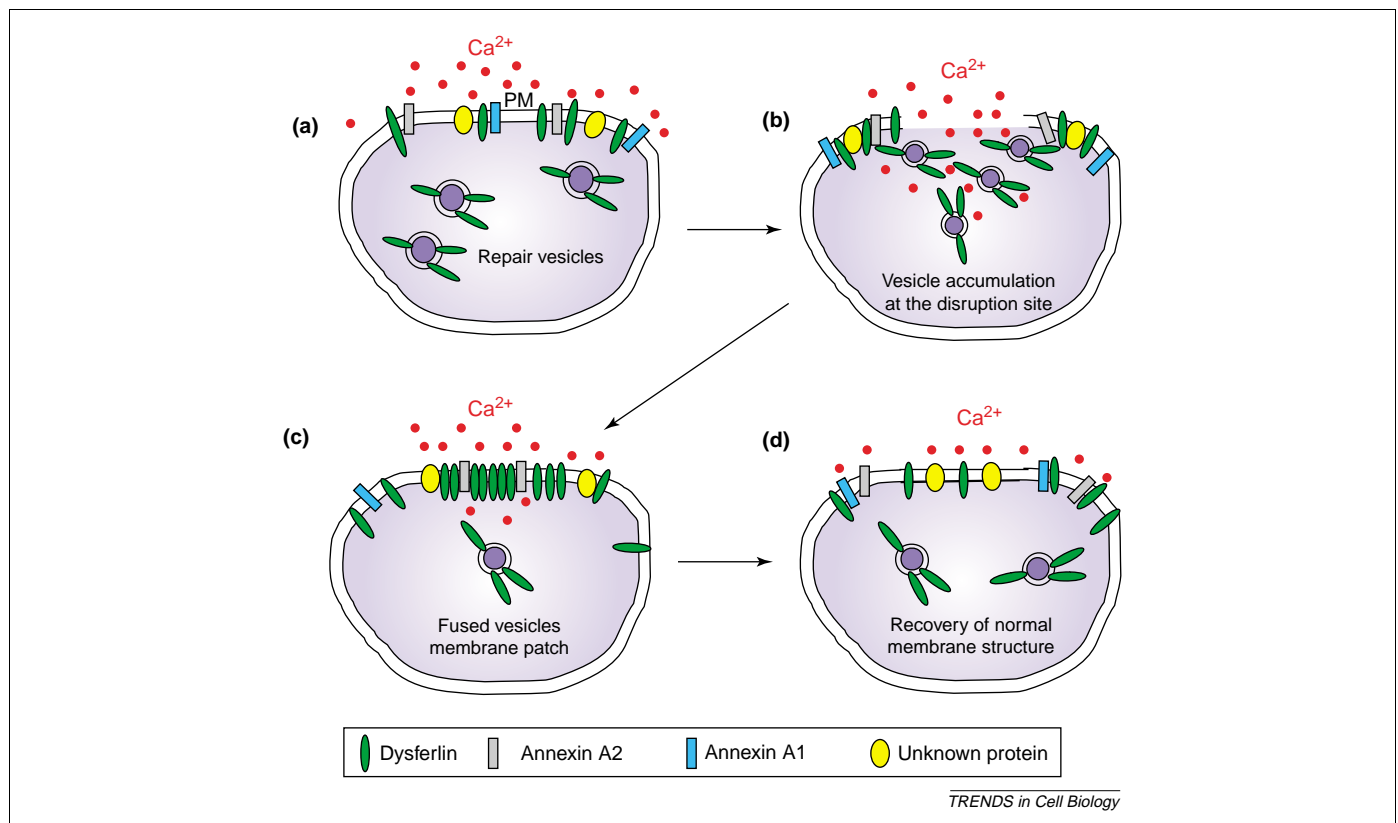


Figure 3. Dysferlin-mediated membrane repair model. In normal muscle fiber, dysferlin is localized at the plasma membrane (where it interacts with annexin A1 and A2) and cytoplasmic vesicles (a). A disrupted membrane causes diffusion of calcium in the muscle fiber and creates a zone of high calcium around the disruption site and dissociates dysferlin from annexin A1. Dysferlin-carrying repair vesicles are targeted to the disruption site, where they accumulate and fuse with one another and the plasma membrane in the presence of localized high levels of calcium. Dysferlin, present on the vesicles, facilitates vesicle docking and fusion with the plasma membrane by interacting with the annexin A2, other dysferlin molecules and with some other unknown protein-binding partner(s) at the plasma membrane (b,c). Fusion of the repair vesicles with plasma membrane puts a membrane patch across the membrane disruption and thereby reseals the disrupted plasma membrane (d).

leading to muscle degeneration. Plasma membrane repair is a basic cellular function required to overcome physical injuries to the plasma membrane. Skeletal muscle is a highly specialized tissue whose primary function is to generate physical force; therefore, it is more susceptible to plasma membrane damage and requires more efficient membrane repair machinery. The importance of membrane repair in skeletal muscle cells is further highlighted by the fact that dysferlin, although ubiquitously expressed, shows the most prominent expression in the skeletal muscle.

Current data demonstrate a role for dysferlin in the repair of muscle-fiber plasma membrane [13]; however, future work is required to further dissect this role at a molecular level. Because of its similarities to synaptotagmins, it is very probable that dysferlin acts as a calcium sensor and facilitates membrane fusion in the presence of high levels of calcium when a cell experiences an injury to the membrane. Moreover, the presence of multiple C2 domains can facilitate the recruitment of membrane fusion machinery by interacting with other proteins. A recent study demonstrated an interaction between dysferlin and annexins A1 and A2 and suggested a role for these proteins in vesicle fusion and aggregation in dysferlin-mediated membrane repair [58]. The interaction of dysferlin with the phospholipid- and calcium-binding annexins is in agreement with its role in membrane fusion. Although no link between annexins and muscular dystrophy has been identified, calpain-3 and caveolin-3, the other two proteins that have been associated with dysferlin [58], cause specific types of muscular dystrophy [4]. Although future work is required to understand the function of each of these proteins in the membrane repair pathway, our current knowledge suggests that there might be several other types of muscular dystrophies, which will show a defect in this pathway.

The recent advancements in understanding the pathological mechanism of muscle degeneration in dysferlin-deficient muscle cells have introduced a novel pathway to be explored in understanding the maintenance of muscle sarcolemma. Further work is required to identify other components of this membrane repair machinery. This might help to explain the variation observed in the phenotype of dysferlin-deficient patients, and such components will serve as candidate genes for the muscle diseases of unknown etiology. These findings also open new areas for therapeutic approaches, where enhancing the membrane repair efficiency of muscle fibers could be useful in reducing the disease severity of those muscular dystrophies where the primary defect lies in increased susceptibility to membrane damage.

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