Dysregulation of calcium homeostasis in muscular dystrophies

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Muscular dystrophies are a group of diseases characterised by the primary wasting of skeletal muscle, which compromises patient mobility and in the most severe cases originate a complete paralysis and premature death. Existing evidence implicates calcium dysregulation as an underlying crucial event in the pathophysiology of several muscular dystrophies, such as dystrophinopathies, calpainopathies or myotonic dystrophy among others. Duchenne muscular dystrophy is the most frequent myopathy in childhood, and calpainopathy or LGMD2A is the most common form of limb-girdle muscular dystrophy, whereas myotonic dystrophy is the most frequent inherited muscle disease worldwide. In this review, we summarise recent advances in our understanding of calcium ion cycling through the sarcolemma, the sarcoplasmic reticulum and mitochondria, and its involvement in the pathogenesis of these dystrophies. We also discuss some of the clinical implications of recent findings regarding Ca^{2+} handling as well as novel approaches to treat muscular dystrophies targeting Ca^{2+} regulatory proteins.

Introduction

Ca^{2+} ions are involved in the regulation of a variety of cellular processes such as muscle contraction, secretion, proliferation or cell death (Ref. 1). Therefore, there is a need of precise temporal and spatial control of Ca^{2+} fluxes and Ca^{2+} concentration within the cell. Indeed, Ca^{2+} concentration is tightly controlled in all eukaryotic cells by complex interactions among voltage sensors, Ca^{2+} transporters, Ca^{2+} channels, Ca^{2+} exchangers, Ca^{2+}-binding proteins, ion pumps and ion exchangers (Refs. 2, 3, 4). This fine regulation of Ca^{2+} homeostasis allows this ion to act as one of the most important second messengers in signal transduction.

In skeletal muscle fibres, the status of the excitation–contraction–relaxation cycle is determined by the cytosolic Ca^{2+} levels: high

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Ca^{2+} levels are crucial for triggering muscle contraction, while low levels are critical for initiating muscle relaxation. Hence, a fast Ca^{2+}-cycling mechanism and an efficient storage process are absolutely essential in muscle fibres. During normal contractions, local cytosolic Ca^{2+} concentration is greatly increased reaching up to 20 μM (Refs. 5, 6). In addition to these Ca^{2+} transients associated with muscle contraction, smaller but prolonged increases in intracellular Ca^{2+} levels associated with long-duration fatigue have been described (Ref. 7). Additionally, small but persistent increases in basal intracellular Ca^{2+} concentration likely occur during certain muscle diseases and in ageing (Ref. 8). Since changes in cytosolic Ca^{2+} levels are particularly frequent and diverse in the skeletal muscle, a tight control in Ca^{2+} homeostasis is most significant in this system. In this regard, small changes in the Ca^{2+} handling apparatus in the skeletal muscle might result in major pathophysiological consequences. Indeed, abnormal expression patterns of ion-regulatory proteins have been reported repeatedly in muscular dystrophy (Refs. 9, 10, 11, 12, 13).

Existing evidence implicates Ca^{2+} dysregulation as a common underlying event in the pathophysiology of muscular dystrophies, a group of diseases characterised by a primary degeneration of skeletal muscle tissue that results in progressive muscle weakness. This review examines Ca^{2+} handling in muscular dystrophies, with a special focus on dystrophinopathies, calpainopathies and myotonic muscular dystrophies. We also summarise recent advances in our understanding of Ca^{2+} ion cycling through the sarcolemma, the sarcoplasmic reticulum (SR) and mitochondria, and its involvement in the pathogenesis of these dystrophies. Finally, we discuss some of the clinical implications of these findings, and in particular, novel pharmacological therapeutic approaches for muscular dystrophies that target Ca^{2+} regulatory proteins.

Calcium handling in the skeletal muscle

Structure and physiology of the skeletal muscle

The skeletal muscle is under the control of the somatic nervous system through innervation of muscle fibres by motoneurons. Muscle fibres are long, cylindrical multinucleated cells that originate from the fusion of myoblasts during embryonic development and behave as a single unit. Each fibre contains several bundles of myofibrils, with inter-digitating myosin (thick) and actin (thin) filaments arranged longitudinally in repeating units known as sarcomeres. This particular arrangement of the cytoskeletal elements accounts for the banding pattern, characteristic of skeletal fibres. Actin filaments are bound to the Z line, which forms the borders of the sarcomere. At this location, deep invaginations of the sarcolemma (T-tubules) permit the conduction of the electrical impulses to the cell interior (Fig. 1).

A single motoneuron innervating one or more muscle fibres with similar functional properties constitutes a motor unit. A muscle is formed by many motor units, each one bringing its own specific contribution (Ref. 14). Motor units differ in the size of motoneurons and also in the type of innervated muscle fibres. They are divided into three categories: (a) slow; (b) fast fatigable; and (c) fast fatigue-resistant motor units (Ref. 15).

(a) *Slow motor units:* Constituted by type I, slow twitch muscle fibres, are essential for sustained muscular contraction. Type I fibres contract very slowly and generate small force, but they are very resistant to fatigue. They contain high mitochondria density and they generate ATP by the aerobic metabolism.
(b) *Fast fatigable motor units:* Constituted by type IIB fibres, or type IIX in humans, are used for tasks that require large brief bursts of energy such as sprinting. Type IIB/IIX or fast glycolytic fibres have a fast contraction velocity but they fatigue easily. They contain few mitochondria and they generate ATP mainly by the anaerobic system.
(c) *Fast fatigue-resistant motor units:* Constituted by type IIA, fast twitch A fibres or fast oxidative fibres, which contain many mitochondria and, thus, can generate ATP by aerobic metabolism. They have a fast contraction velocity and they are resistant to fatigue, although not as much as type I fibres (Ref. 16).

Some of the fibre-type differences in twitch kinetics are also likely due to differences in Ca^{2+} handling. Indeed, the amount of Ca^{2+} released in type II fibres is up to fourfold larger than in type I fibres, there is a faster decline of the calcium transient in type II fibres (Ref. 14) and the proportion of Ca^{2+} that binds troponin to activate contraction is substantially smaller (Ref. 5). On the other hand, type I fibres show increased affinity for SR Ca^{2+} uptake, higher

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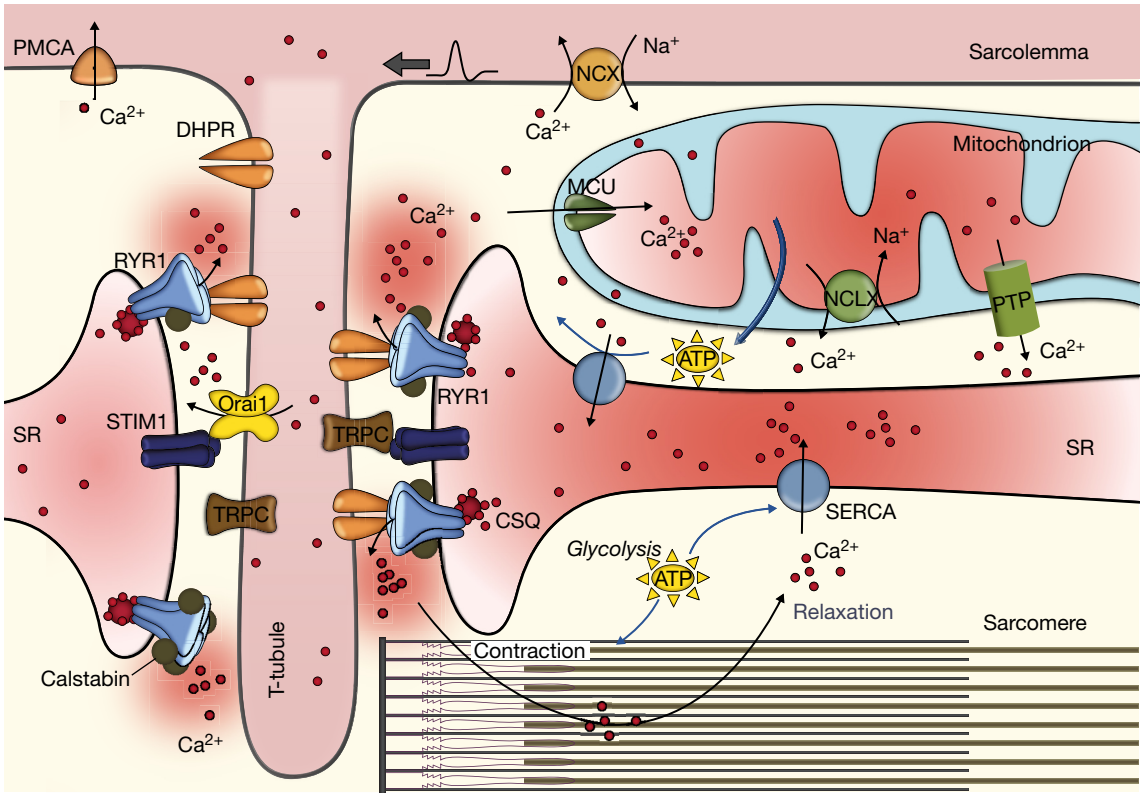

Representation of calcium signalling dynamics in the muscle fibre

Figure 1. Representation of calcium signalling dynamics in the muscle fibre. Signalling begins when sarcolemmal depolarisation reaches the T-tubules. In these structures, DHPR respond with a conformational change that activates closely apposed RyR1, resulting in Ca^{2+} release from the sarcoplasmic reticulum (SR). Ca^{2+} diffuses out to activate contraction by binding with Troponin C. Relaxation occurs as Ca^{2+} is returned into the SR by SERCA ATPase or is pumped out of the cell by NCX or PMCA. A portion of this Ca^{2+} reaches the mitochondria and driven by the negative membrane potential, it enters through MCU and LETM1 importers, where it stimulates the metabolism to provide the ATP required for maintaining contraction. Ca^{2+} can be released from mitochondria through NCLX exchanger and the mitochondrial PTP. Low SR Ca^{2+} levels are detected by STIM1 and activate SOCE, an extracellular Ca^{2+} influx through Orai1 or TRPC channels to refill intracellular Ca^{2+} stores.

relative levels of SR Ca^{2+} loading and increased SR Ca^{2+} leakage compared with type II fibres (Refs. 17, 18). Muscle fibres display a high plasticity, being able to adapt to changing demands by modifying size or fibre-type composition (Ref. 19). Specific types of muscle fibres are preferentially affected in different forms of muscular dystrophies. In Duchenne muscular dystrophy, for example, type IIB fibres are progressively replaced by type I and IIA fibres (Ref. 20). Recently, we have also found in collaboration with Dr Spencer's group that slow fibres are preferentially involved in Limb-girdle muscular dystrophy-type 2A (LGMD2A) dystrophic patients (Ref. 21). Interestingly, some very specific muscle types present in extra ocular muscles show great resistance to damage in Duchenne muscular dystrophy, partially due to an enhanced Ca^{2+} buffering (Ref. 22).

The Ca^{2+} cycle during excitation–contraction (E–C)

Skeletal muscle contraction is initiated by depolarisation of the sarcolemma in response to

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acetylcholine release from motoneurons. The sarcolemmal depolarisation propagates down the T-tubular system and activates dihydropyridine receptors (DHPRs) (Refs. 5, 23). The resulting conformational change of DHPRs activates closely apposed ryanodine receptors (RyRs), which are Ca^{2+} release channels localised in the SR membranes (Ref. 24). As these release channels open, Ca^{2+} moves from the SR into the cytosol, driven by a steep Ca^{2+} concentration gradient. Afterwards, Ca^{2+} diffuses throughout the sarcomere and binds to various cytosolic constituents or Ca^{2+} buffers. Binding of Ca^{2+} to troponin triggers the contractile response of the myofibrils: Ca^{2+}-bound troponin undergoes a conformational change that leads to the movement of tropomyosin, which unmasks myosin-binding sites on actin filaments and allows myosin and actin ATP-dependent cross-bridge cycling and shortening of the muscle. Lastly, relaxation is initiated by Ca^{2+} diffusion from the myofilaments to the cytosol and Ca^{2+} transfer back to the SR by the sarco/endoplasmic reticulum Ca^{2+} pump SERCA (Ref. 25). The sarcolemmal Na^{+}/Ca^{2+}-exchanger (NCX) and the plasmalemmal Ca^{2+}-ATPase (PMCA) are other mechanisms involved in relaxation, since they remove cytosolic Ca^{2+} to the extracellular space (Ref. 26). Figure 1 illustrates the major intracellular movements of Ca^{2+} taking place during E–C coupling in skeletal muscle fibres.

Cytosolic Ca^{2+} buffers

Cytosolic Ca^{2+} buffering is the rapid binding of cytosolic incoming Ca^{2+} ions to different cellular binding sites, and constitutes a critical process implicated in the precise regulation of Ca^{2+} signalling and homeostasis. Ca^{2+} buffers are cytosolic Ca^{2+}-binding proteins that modulate intracellular Ca^{2+} transients by controlling diffusion of free Ca^{2+} ions within the cytosol. This process occurs at the sub-second scale, much faster than Ca^{2+} sequestration into intracellular stores. Concentration of Ca^{2+} buffers and other parameters, such as mobility and Ca^{2+}-binding affinity, are important for determining their specific role in modulation of Ca^{2+} signalling (Ref. 27).

In skeletal muscle fibres, the most significant Ca^{2+} buffers in order of binding occurrence in time are, first, troponin C is a component of thin filaments that allows interaction of actin and myosin and initiates contractile activation when its regulatory sites are bound to Ca^{2+}. Different types of muscle fibres have specific versions of troponin C with different Ca^{2+}-binding capacities. Second, ATP acts as a cytosolic mobile buffer for Ca^{2+} and Mg^{2+} (Ref. 5). Then parvalbumin plays an important role in regulating the speed of relaxation in mice (Ref. 28), although it is expressed at very low concentrations in human skeletal muscle (Ref. 29). Afterwards, Ca^{2+} binds to calmodulin, contributing to the regulation of contractile function via myosin light-chain kinase. It is also conducive to the activation of Ca^{2+}-dependent signalling pathways involved in muscle gene regulation. Lastly, Ca^{2+} binds to SERCA pumps for Ca^{2+} sequestration into the SR.

Contribution of sarcolemma to Ca^{2+} homeostasis

In skeletal muscle, Ca^{2+} entry through the sarcolemma has been shown to play an important role in store repletion (Ref. 30), limiting fatigue under conditions of extensive exercise (Ref. 31), activation of NFAT transcription factor signalling (Refs. 32, 33) and muscle differentiation (Ref. 34). The sarcolemma has a very low permeability to Ca^{2+} and a very particular organisation with the majority of the sarcolemma internalised as the tubular system, which allows for tightly regulated Ca^{2+} trans-sarcolemmal fluxes. Aside from voltage-gated channels, Ca^{2+} may enter the muscle fibre via SOCE or store-operated Ca^{2+} entry (Ref. 30); ECCE, or excitation-coupled Ca^{2+} entry (Ref. 35); SAC or stretch-activated cation channels (Ref. 36); SIC or stretch-inhibitable cation channels (Ref. 37); and leak channels (Ref. 38). Previous studies have shown that dysregulation of Ca^{2+} entry may lead to severe muscle pathologies. In particular, several genetic mouse models of muscular dystrophy point towards an enhanced Ca^{2+} influx through SOCE or SAC that leads to Ca^{2+}-dependent apoptosis and muscle degeneration (Refs. 39, 40). On the other hand, Ca^{2+} extrusion from cells may also play an important role in maintaining Ca^{2+} homeostasis. In muscle fibres, Ca^{2+} is pumped out of the cell by the Na^{+}/Ca^{2+} exchangers NCX1-3 and the Ca^{2+}-ATPase PMCA isoforms 1, 3 and 4, localised at the sarcolemma (Ref. 14).

Ca^{2+}-handling proteins in the sarcoplasmic reticulum

The SR, the major reservoir of intracellular Ca^{2+} in the muscle fibre, is a highly specialised form of

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endoplasmic reticulum. It is organised as an extensive tubular network surrounding myofibrils with periodical dilated end sacs or terminal cisternae. A central T-tubule flanked on both sides by two terminal cisternae from the SR constitutes a triad, a highly specialised anatomical structure that is the basis of E–C coupling (Ref. 41). In the SR, the balance between Ca^{2+} storage, release and reuptake is achieved through the coordinated action of three major types of Ca^{2+}-handling proteins: luminal Ca^{2+}-binding proteins, SR Ca^{2+} release channels and Ca^{2+}-ATPase pumps for Ca^{2+} reuptake (Ref. 42). Ca^{2+}-handling proteins are heterogeneously distributed within the SR, which results in functionally distinct subdomains where Ca^{2+} release and reuptake occur in the terminal cisternae and the longitudinal SR, respectively.

(a) *Luminal Ca^{2+}-binding proteins:* Within the SR lumen, a large proportion of Ca^{2+} is bound to Ca^{2+}-binding proteins that perform a dual function as Ca^{2+} reservoir components and as endogenous regulators of Ca^{2+} fluxes. Luminal Ca^{2+}-binding proteins help to reduce the concentration of free Ca^{2+} within the SR, which assists in the reuptake of Ca^{2+} to the SR. They determine normal and maximal Ca^{2+} levels in the SR, in combination with SERCA pump (Ref. 43). Intraluminal Ca^{2+} levels have been reported to range between high micromolar and low milimolar concentrations, thus, constituting a steep electrochemical gradient aimed at the cytosol. The most significant Ca^{2+}-binding proteins in the SR are: calsequestrin (CSQ), which is a moderate-affinity, high-capacity Ca^{2+}-binding proteins located in the terminal cisternae region; histidine-rich Ca^{2+}-binding protein (HRC), which has been found to bind SERCA and triadin, and therefore, might be involved in the regulation of Ca^{2+} release by RyRs and sequestration (Refs. 44, 45); sarcalumenin, a Ca^{2+}-binding/shuttle protein localised in the longitudinal SR; and calreticulin. In particular, CSQ is involved in the SR Ca^{2+}-loading capacity (Ref. 43), while also modulating activity of RyR channels (Ref. 46). In the skeletal muscle, CSQ1 is the main isoform, although CSQ2 is also expressed in type I fibres. In fact, CSQ1 has been found to both inhibit and activate RyR1 depending on its binding manner to RyR1 (Ref. 47). Recent work supports an essential role of CSQ1 in the mechanism of RyR1 channel closure and termination of SR Ca^{2+} release in mouse skeletal muscle (Ref. 48).

(b) *SR Ca^{2+} release channels:* Activation of these channels results in Ca^{2+} release from SR, which contributes to elevation of cytosolic Ca^{2+} levels, whereas Ca^{2+} sequestration to SR assists termination of Ca^{2+} signals in the cytosol. RyRs, the largest known ion channels, are responsible for Ca^{2+} release from the sarco/endoplasmic reticulum and they also play a central role in the regulation of cytosolic and SR luminal Ca^{2+} levels. At least three isoforms are expressed in mammals, RyR1-3, being RyR1 the predominant isoform expressed in the skeletal muscle. RyR2 and RyR3 are expressed in cardiac and immature skeletal muscle, respectively (Refs. 49, 50, 51, 52). RyR1 is essential for the E–C coupling in the skeletal muscle and its gating is strictly controlled by DHPR activation. In fact, Ca^{2+} release through RyR1 is the key determinant of muscle force since skeletal muscle contraction depends on Ca^{2+} released from the SR (Ref. 7). Cytosolic Ca^{2+} activates RyR1 in the low micromolar, but acts as an inhibitor in the low milimolar range. In addition, luminal Ca^{2+} levels are also able to modulate RyR1 activity, since previous reports show that high luminal Ca^{2+} levels enhance RyR1 responsiveness to cytosolic agonist (Ref. 49).

Ca^{2+} release through RyR1 is also modulated by post-translational modifications and a vast variety of proteins and small molecules, both in the SR lumen and cytosol. In addition to Ca^{2+} and DHPR channels, other positive regulators of RyR1 activity in the cytosol are ATP, CaMKII and PKA. Calstabin-1 stabilises the closed stage of the channel and calmodulin has a biphasic effect on RyR1, functioning as an activator at the low cytosolic Ca^{2+} levels and as and inhibitor at the high cytosolic Ca^{2+} levels. On the luminal side, RyR1 interacts with the CSQ–junctin–triadin complex, which plays a dual role as luminal Ca^{2+} sensor and RyR activity modulator.

RyRs can be phosphorylated by several kinases, including CaMKII and PKA. A recent study has implicated PKA phosphorylation of RyR1 in skeletal muscle enhancement of contractile force (Ref. 53). However, PKA hyper-phosphorylation of RyR1 might lead to

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SR Ca^{2+} leak under prolonged pathological stress (Refs. 54, 55). Finally, RyR1 activity can also be modulated by oxidative stress through modifications of cysteine thiol residues, such as S-nitrosylation and S-glutathionylation. Although the effect of RyR1 oxidative modifications may vary depending on which residue is modified, exposure of skeletal muscle to nitric oxide has been reported to increase RyR1 activity (Refs. 49, 51). Interestingly, RyRs have been found to release Ca^{2+} spontaneously under conditions of luminal Ca^{2+} overload in a process known as SOICR or Store Overload-Induced Ca^{2+} Release (Refs. 56, 57). This mechanism has been recently linked to cardiac and muscular disorders, such as malignant hyperthermia or catecholaminergic polymorphic ventricular tachycardia (Ref. 58).

The other major SR Ca^{2+} release channels are the inositol 1,4,5-triphosphate receptors (IP3Rs). These Ca^{2+} channels are gated by the combined binding of IP3 and Ca^{2+}, which initiates a slow wave of internal store Ca^{2+} release that often occurs at specific subcellular locations (Ref. 59). Whereas in myoblasts IP3Rs have a major role regulating Ca^{2+} homeostasis during skeletal muscle development, the roles that IP3Rs may play in adult skeletal muscle are controversial. A recent study has not found any evidence of IP3R affecting global Ca^{2+} levels in adult mouse skeletal muscle (Ref. 60). Conversely, a previous work supports a role of IP3Rs mainly at the neuromuscular junction (Ref. 61), and recent evidence indicates that crosstalk between IP3R1 and RyR1 underlies activation of stress-induced Ca^{2+} sparks in adult mice skeletal muscle (Ref. 62).

(c) *Ca^{2+}-ATPase pumps:* Ca^{2+} reuptake into the SR after contraction relies on the Ca^{2+} ATPase pump SERCA, which actively transports Ca^{2+} from the cytosol to the SR against a large concentration gradient at the expense of ATP hydrolysis. Three genes localised on different chromosomes encode for SERCA1, 2 and 3 proteins, although isoform diversity is further increased by alternative splicing. Skeletal fast muscle fibres express SERCA1a exclusively, while SERCA2a is expressed in slow fibres and cardiac muscle. During development or regeneration, muscle fibres express the neonatal isoform SERCA1b (Refs. 23, 25). SERCA pumps are highly affected by changes in cell energetics and ATP supply, since they are, together with myosin ATPases, major ATP consumers (Ref. 63). SERCA pumps are also a substantial pathway for SR Ca^{2+} leakage and this leakage is increased by high cytosolic ADP levels (Ref. 64). Low levels of free intraluminal Ca^{2+} are, thus, necessary so that Ca^{2+} leakage does not metabolically compromise muscle function (Ref. 43).

In addition to ATP, SERCA activity is modulated by several cytosolic and luminal proteins, and also by post-translational modifications. The endogenous inhibitory proteins phospholamban and sarcolipin modulate SERCA activity in slow and fast-twitch fibres, respectively (Ref. 65). Phosphorylation by PKA or CaMKII, results in SERCA activation by dissociation of phospholamban and sarcolipin from the Ca^{2+} pump. N-glycosylation and other post-translational modifications such as glutathiolation have been found to affect SERCA activity in different ways: SERCA activity can be reversibly increased by oxidative modifications under normal or mild oxidative stress levels, but high oxidant levels present in some pathological conditions may further lead to irreversible SERCA inactivation and degradation (Refs. 66, 67). This particularly susceptibility of SERCA to oxidative and nitrative modifications has led to the hypothesis of SERCA functioning as a sensor of cellular stress (Ref. 68). Moreover, these data suggest a central role of SERCA in energy production and consumption through regulation of Ca^{2+} homeostasis.

Ca^{2+} in mitochondria

Mitochondria are located in close proximity to the SR in the skeletal muscle, and they account for about 15% of the cytosolic volume in oxidative fibres (Refs. 69, 70). There is a significant interplay between the mitochondria and the SR, through the mitochondria-associated SR membrane (MAM), which is a structural element essential for cell physiology and Ca^{2+} homeostasis (Refs. 71, 72). Besides playing a central role in muscle bioenergetics, mitochondria are also able to store Ca^{2+} transiently and therefore, they contribute significantly to Ca^{2+} homeostasis in the muscle fibre. In fact, their ability to rapidly uptake Ca^{2+}

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for later release in response to different stimuli makes them very good cytosolic Ca^{2+} buffers (Ref. 73). Ca^{2+} fluxes between mitochondria and SR are believed to help shape mitochondrial metabolism and ATP synthesis to the physiological demands of skeletal fibres (Ref. 74). At the same time, mitochondria have been found to modulate intracellular Ca^{2+} transients in skeletal muscle during E–C coupling (Ref. 75). The outer mitochondrial membrane is permeable to Ca^{2+}, unlike the inner mitochondrial membrane. Influx into the mitochondrial matrix is driven by the negative membrane potential and occurs through two types of importers: the low-affinity mitochondrial Ca^{2+} uniporter (MCU) (Ref. 76) and a high-affinity mitochondrial Ca^{2+}/H^{+} exchanger, LETM1. In skeletal muscle fibres, Ca^{2+} release from mitochondria can occur via a Na^{+}/Ca^{2+} exchange protein (NCLX) or via Ca^{2+}-induced–Ca^{2+}-release pathways (Ref. 76). Also, at high matrix Ca^{2+} levels mitochondria release Ca^{2+} through the permeability transition pore (PTP), a mechanism that can lead to cell death and might have an important role in muscular dystrophies (Refs. 77, 78). Interestingly, in humans, mutations in the mitochondrial calcium uptake 1, a regulator of MCU, causes dysfunctional Ca^{2+} uptake and results in clinical and pathological features that overlap with those of mitochondrial myopathies, congenital core myopathies and muscular dystrophies (Ref. 79).

Calcium dysregulation in muscular dystrophies

In this section, we will discuss current knowledge regarding Ca^{2+} homeostasis in dystrophinopathies, calpainopathies and myotonic muscular dystrophies. The main Ca^{2+}-related pathways altered in these pathologies are summarised in Table 1.

Dystrophinopathies

Duchenne muscular dystrophy (DMD) is an inherited X-linked neuromuscular disease that affects 1 in 3500 male births and it is characterised by severe and progressive skeletal muscle degeneration, weakness and premature death caused by respiratory failure and cardiac dysfunction (Ref. 80). DMD results from deficiency in the structural protein dystrophin (Ref. 81), a 427 kDa protein located beneath the sarcolemma whose major role is to protect and maintain muscle fibre integrity by linking intracellular cytoskeletal actin to the extracellular matrix. This is achieved through its association to a group of plasma membrane glycoproteins known as the dystrophin–glycoprotein complex (DGC) (Ref. 82). Dystrophin is essential for sarcolemmal stability, and thus dystrophin-deficient muscle fibres are prone to endure recurrent membrane damage (Ref. 83). This sarcolemma fragility, however, does not fully explain the onset and progression of DMD and, up to date, the exact cause of muscle fibre death still remains unsolved. The pathological events most widely accepted to occur in DMD muscle fibres as a result of dystrophin deficiency include membrane fragility and increased basal intracellular Ca^{2+} levels, which leads to calpain activation, protein degradation, mitochondrial PTP opening and, ultimately, fibre death by necrosis (Refs. 9, 11, 13, 84, 85, 86). Studies performed more than 30 years ago in muscle biopsies (Ref. 85) and later on, in human foetuses and premature infants (Ref. 87), showed increased Ca^{2+} accumulation in prenecrotic DMD fibres, which suggests that Ca^{2+} dysregulation is an early event in the pathophysiology of this disorder. Afterwards, many other studies have demonstrated increased intracellular Ca^{2+} levels in dystrophic muscles from DMD mouse models (*mdx* mice) and Duchenne patients compared to normal muscles (Refs. 13, 84, 88, 89). Increased intracellular Ca^{2+} levels observed in dystrophin-deficient fibres is a complex process that involves, at least, trans-sarcolemmal Ca^{2+} fluxes, SR Ca^{2+} leakage and abnormal SR Ca^{2+} levels.

High cytosolic Ca^{2+} levels may result from enhanced Ca^{2+} influx through the dystrophin-deficient sarcolemma (Refs. 90, 91). This abnormal influx seems to occur mostly through TRPC channels, a type of mechanosensitive voltage-independent Ca^{2+} channels with increased expression in dystrophic muscle fibres (Ref. 40). Indeed, several groups have found that blockage of TRPC channels in dystrophic mice reduces the abnormal extracellular Ca^{2+} influx. Furthermore, overexpression of TRPC3 channels in normal mouse skeletal muscle enhances Ca^{2+} influx and results in a phenotype that closely resembles DGC-lacking dystrophic mice (Ref. 86). TRPC channels can function as both, stretch-activated and store-operated channels, and therefore they are also involved in SOCE response, an influx of extracellular Ca^{2+} triggered by SR depletion of Ca^{2+} that is enhanced in dystrophic fibres (Ref. 39). An increased SOCE associated with TRPC1 channels has been reported in dystrophic

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Table 1. Calcium-related mechanisms affected in muscular dystrophies

Disease	Protein affected	Subcellular localisation	Proposed mechanism	System	References
Duchenne (DMD)	Dystrophin	Sarcolemma-cytoskeleton	Absence causes membrane fragility and transarcolemmal Ca^{2+} entry	Mouse mdx and human DMD myotubes	90, 91
	TRPC channels	Sarcolemma	Increased expression causes high Ca^{2+} entry (SOCE)	mdx fibres, transgenic mice, mouse myotubes	39, 40, 86, 107
	NCX	Sarcolemma	Misfunction: reversal of function causes high Ca^{2+} entry	human myotubes – rat spinal cord cocultures	93
	Orai1	Sarcolemma	Increased expression causes increased SOCE	mdx muscles	96
	RyR1	SR triad, transmembrane	Misfunction causes SR Ca^{2+} leakage to the cytosol	mdx muscles	101
	IP3R	SR transmembrane	Increased levels, increased activity causes high cytosolic Ca^{2+}. Also involved in mitochondrial defects	mdx and DMD myotubes *C. elegans*, zebrafish	105, 106, 107, 110, 111
	SERCA1	SR network, transmembrane	Reduced activity (controversial)	mdx muscles	117, 118, 165
	CSQ1, CSQ-like proteins	SR lumen	Reduced expression results in low SR Ca^{2+} levels	mdx muscle	119, 120
	sarcalumenin	SR lumen	Reduced expression results in low SR Ca^{2+} levels	mdx fibres	121
	–	Mitochondria	Calcium overload induces apoptosis Dramatic fragmentation early in the pathology	mdx myotubes *C. elegans*, zebrafish	109, 110, 111

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Table 1. Calcium-related mechanisms affected in muscular dystrophies *(continued)*

Disease	Protein affected	Subcellular localisation	Proposed mechanism	System	References
LGMD2A	Calpain 3	Triad, cytoskeleton	Absence results in dysregulation of SR Ca^{2+} release	Capn3 knockout mouse	133
	RyR1	SR triad, transmembrane	Reduced expression causes reduced SR Ca^{2+} release	Capn3 knockout mouse and LGMD2A muscles	21, 133, 134
	AldoA	Triad, cytosol	Reduced localisation at the triad could cause misregulation of RyR1 activity	Capn3 knockout mouse	133
	CaMKII	Triad, cytosol	Reduced expression and activity affect SR Ca^{2+} uptake and release	Capn3 knockout mouse and LGMD2A muscles	21
	–	Mitochondria	Abnormal distribution and structure, oxidative stress, ATP deficit cause Ca^{2+} misregulation	Capn3 knockout mouse and LGMD2A muscles	141, 142
Myotonic Dystrophy (DM)	DHPR	Sarcolemma, triad	Aberrant mRNA splicing enhances gating properties. Reduced expression in DM1 muscles	DM muscles and mouse model	148, 150
	RyR1	SR triad, transmembrane	Reduced protein expression in DM1 and DM2. Aberrant mRNA splicing causes reduced activity	DM1 and DM2 muscles DM1 myotubes	149, 150, 153
	SERCA1	SR network, transmembrane	Aberrant mRNA splicing may cause increased $[Ca^{2+}]i$	DM1 myotubes	149
	SERCA2	SR network, transmembrane	Reduced protein levels in DM2	DM2 muscles	153
	CSQ2	SR lumen (slow fibres)	Reduced protein expression in DM1 and DM2 muscles causes reduced SR Ca^{2+} concentration	DM1 and DM2 muscles	153
	JPH1	Triad, cytosol	Reduced protein in DM1 and DM2 muscles may cause reduced E–C coupling	DM1 and DM2 muscles	153

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fibres, and remarkably, it can be restored to normal levels by inducing expression of minidystrophin, a smaller and partially functional dystrophin variant (Ref. 92). Finally, enhanced Ca^{2+} influx in dystrophic fibres has also been reported to occur by reversal NCX sarcolemmal channels (Ref. 93) and also by the Orai1/Stim SOCE pathway. In addition to TRPC channels, other proteins may also be responsible for increased SOCE in DMD, such as stromal interaction molecule 1 (STIM1), which acts as an SR Ca^{2+} sensor that can trigger SOCE through both TRPCs and Orai1 channels (Ref. 94). In this regard, it has been recently reported increased Orai1 (Refs. 95, 96) and STIM1 (Ref. 95) expression in dystrophic muscles from *mdx* mice. This indicates that enhanced activation of SOCE pathways may contribute to the disrupted Ca^{2+} homeostasis in DMD pathology. Several other proteins have been involved in this enhanced SOCE activity in dystrophin-deficient muscle myotubes, such as phospholipase C, protein kinase C (Ref. 97), phospholipase A2 (iPLA2) and its derived metabolites (Ref. 98). In this regard, increased iPLA2 activity has been known for more than 10 years in DMD muscles (Ref. 99). Therefore, specific inhibitors of these pathways may be of interest to reduce Ca^{2+} influx and subsequent degeneration of dystrophic muscle (Ref. 100).

Another source of elevated cytosolic Ca^{2+} levels may involve SR Ca^{2+} leakage through RyRs or IP3Rs. Regarding RyRs, several studies have pointed to abnormally high S-nitrosylation of RyR1 cysteine residues in *mdx* mice causing calstabin-1 depletion from the RyR complex, that lead to unstable and leaking RyR1 channels at resting conditions (Refs. 101, 102, 103). These abnormal S-nitrosylation levels seem to be due to inducible nitric oxide synthase (iNOS), which was also found increased in dystrophic *mdx* muscles.

A recent study has found evidence of significant Ca^{2+} leakage through IP3Rs as well as through RyRs in dystrophic *mdx* myotubes contributing to the increased intracellular Ca^{2+} levels (Ref. 104), which suggests that IP3R malfunction may also be involved in DMD Ca^{2+} mishandling. Indeed, previous studies have shown increased IP3R and IP3 levels, and enhanced IP3R-dependent Ca^{2+} transients in myotubes from DMD patients and *mdx* mice (Refs. 105, 106, 107). Moreover, induced expression of minidystrophin in dystrophin-deficient myotubes reverts the IP3R-dependent increase in Ca^{2+} release to normal levels (Ref. 108), which suggests an IP3R involvement in DMD impaired Ca^{2+} homeostasis. Finally, there seems to be a connection between IP3R and mitochondria that may play a relevant role in Ca^{2+} dysregulation. Previous work in *mdx* dystrophic myotubes has shown mitochondrial Ca^{2+} overload due to increased SR Ca^{2+} release (Ref. 109). Interestingly, overexpression of Bcl-2 antiapoptotic protein in *mdx* myotubes prevents this mitochondrial Ca^{2+} overload by inhibiting IP3Rs (Ref. 110). More recently, abnormal mitochondrial fragmentation has been observed in *Caenorhabditis elegans* and zebrafish models of DMD, where involvement of cytochrome c in muscle fibre death was demonstrated, acting in part through an interaction with IP3R (Ref. 111).

In DMD and in many other neuromuscular disorders such as sarcoglinopathies, desminopathies, LGMD2B muscular dystrophy and Pompe disease, the cardiac and respiratory muscles are affected and, consequently, there is an increased risk of respiratory and cardiac failure in these patients that leads to early mortality (Ref. 84). Abnormal Ca^{2+} homeostasis and mitochondrial dysfunction have also been reported in these muscles. In particular, previous works in mdx cardiac myocytes have shown that stretch-activate channels contribute to abnormal Ca^{2+} influx (Ref. 112), and excessive cytosolic Ca^{2+} signals induced by mechanical stress result in mitochondrial dysfunction (Ref. 113). More recently, higher mitochondrial Ca^{2+} levels have been reported in cardiac dystrophic myocytes and activation of DHPR seems to contribute to abnormal mitochondrial Ca^{2+} handling (Ref. 114). Evidence for early mitochondrial dysfunction in dystrophic cardiomyopathy is further supported by alterations in mitochondrial citric acid cycle-related parameters observed in ex vivo mdx hearts preceding overt cardiomyopathy (Ref. 115).

Another controversial issue is whether SERCA expression or function is reduced, unchanged or increased in dystrophin-deficient fibres, with recent examples in the literature supporting all different possibilities (Refs. 96, 116, 117, 118). Regardless the need for further studies to establish a clear understanding on this subject, the majority of the studies agree with a reduced SERCA activity in *mdx* muscles. In this line, nitrative stress could be causing a reduced SERCA activity in dystrophic fibres, since SERCA is highly sensitive to reactive nitrogen

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species (Ref. 68) and high iNOS levels have been reported in *mdx* fibres (Ref. 101).

Ca^{2+} binding proteins and decreased SR Ca^{2+} buffering have also been involved in the pathology of dystrophinopathies. In particular, in the SR lumen, reduction of CSQ1, CSQ-like proteins and sarcalumenin has been observed in *mdx* dystrophic muscles (Ref. 119, 120, 121). A significant reduction of the SR Ca^{2+} buffering would have a direct negative impact on the Ca^{2+} reuptake capacity of SERCA, which in turn, would result in the increased cytosolic Ca^{2+} levels. Remarkably, less affected muscles in the *mdx* muscular dystrophy mouse model, such as the extraocular and intrinsic laryngeal muscles, exhibit higher expression levels of several Ca^{2+}-handling proteins such as CSQ, calmodulin and/or SERCA (Refs. 122, 123), which suggests that these proteins are playing a protective role in DMD pathology.

Calpainopathies

LGMDs are a large group of hereditary muscular dystrophies characterised by progressive proximal weakness and a predominant involvement of the scapula, pelvic girdle and trunk muscles without affecting cardiac or facial muscles (Ref. 124). Among the recessive forms of LGMDs, calpainopathy or LGMD2A is the most frequent muscular dystrophy with a prevalence of 82–100% in genetically isolated populations such as the Basque Country (Refs. 125, 126). LGMD2A is caused by mutations in the gene encoding Calpain 3 (CAPN3), a non-lysosomal cysteine protease necessary for normal muscle function and regeneration (Refs. 127, 128). The exact pathogenic mechanisms that underlie muscular dystrophic features caused by mutations in CAPN3 are still unclear, and in view of the lack of effective therapies available for LGMD2A patients, understanding these pathological mechanisms is needed in order to advance in the design of potential treatments.

In skeletal muscle CAPN3 is present in different subcellular locations including myofibrils, membrane and cytoplasm. Most of the CAPN3 is bound within the contractile filament network, where it remains in its inactive state through its association with titin, a giant protein that connects the Z line to the M line in the sarcomere (Refs. 129, 130). Absence of CAPN3 from mouse and human muscles results in sarcomere disorganisation (Refs. 127, 131), which suggests that CAPN3 is involved in sarcomere repair and maintenance and may also be acting as a sensor of its integrity and functionality (Ref. 129). Remarkably, sarcomere alignment is preserved in mice expressing an inactive form of CAPN3 (*Capn3cs/cs* mice), indicating that the structural role of CAPN3 and not the enzymatic one is involved in sarcomeric remodelling (Ref. 132).

In the membranes, CAPN3 is localised in the triad protein complex and plays an essential role in preserving its structure and function. CAPN3 has been found to interact with RyR1 (Ref. 132), the main SR Ca^{2+} release channel, and regulate Ca^{2+} release from SR (Ref. 133). CAPN3 has also been found to interact with the Ca^{2+}-handling protein CSQ by immunoprecipitation analysis (Ref. 132). This interaction is proposed to occur by means of connecting proteins such as triadin, since CSQ and CAPN3 are localised in different cellular compartments. In CAPN3 knockout mice (C3KO, *Capn3-/-*), reduced expression of RyR1 is concomitant with the reduced SR Ca^{2+} release (Refs. 133, 134). Also, it has been previously reported slower Ca^{2+} reuptake into SR after SR Ca^{2+} depletion in CAPN3 mice, although the basis of these finding needs further analysis (Ref. 134). However, loss of CAPN3 proteolytic activity in *Capn3cs/cs* mice does not affect Ca^{2+} homeostasis (Ref. 132), which indicates that the structural role of CAPN3 is a key for maintenance of Ca^{2+} homeostasis. In this line, we have recently shown in a collaboration study that muscles from LGMD2A patients with deficient CAPN3 also expressed lower RyR1 protein levels. Moreover, we found a correlation between CAPN3 and RyR1 expression levels that supports a structural function of CAPN3 in the preservation of the triad protein complex (Ref. 21).

Another CAPN3-binding partner in the triad complex is aldolase A (AldoA) (Ref. 133), a glycolytic enzyme that may modulate Ca^{2+} handling in the muscle fibre through multiple mechanisms, including direct regulation of RyR activity by intermediates and products of glycolysis, and modulation of SERCA activity through local changes of glycolytically derived ATP (Ref. 135). In the skeletal muscle, AldoA directly interacts with RyR1 and *in vitro* it has been shown to modulate RyR1 activity (Refs. 133, 136). Interestingly, CAPN3 knockout mice show decreased levels of AldoA in the

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sarcomere due to impaired recruitment of AldoA to the triads (Ref. 133). Remarkably, mutations in the gene encoding AldoA result in severe skeletal muscle damage in humans (Refs. 137, 138).

Ca^{2+} calmodulin kinase II (CaMKII) is another triad component that is modulated by CAPN3. In this regard, our group has found that CaMKII signalling is compromised in CAPN3 knockout mice and, thus, we propose that impaired Ca^{2+}-mediated signalling and weakened muscle adaptation are pathogenic mechanisms likely operating in CAPN3-deficient muscles (Ref. 21). CaMKII is a multifunctional protein activated by muscle contraction-induced Ca^{2+} increase that regulates several Ca^{2+}-handling proteins (Ref. 7). In slow twitch skeletal fibres, CaMKII may increase SERCA2 activity through direct phosphorylation or by phosphorylation of its inhibitory protein phospholamban (Refs. 67, 139). On the other hand, CaMKII is also able to modulate Ca^{2+} release through phosphorylation of related proteins, such as triadin, which inhibits RyR1 activity when unphosphorylated (Ref. 140). Thus, decreased CaMKII signalling levels, comparable to the ones observed in CAPN3 deficient muscles, may result in reduction of SR Ca^{2+} release during contractions as well as reduced Ca^{2+} reuptake by SERCA. However, this hypothesis needs verification through experimental analysis.

Finally, there seems to be a connection between CAPN3 and mitochondrial function, since LGMD2A patients and CAPN3 knockout mice show abnormal distribution and structure of mitochondria in skeletal fibres (Ref. 141). Moreover, CAPN3-deficient muscles show ATP production deficits and increase oxidative stress levels (Ref. 142). Accordingly, muscles with higher percentage of slow twitch fibres, which have higher oxidative metabolism, are the most affected in CAPN3 knockout mice (Refs. 21, 142). These mitochondrial abnormalities are likely associated to a severe Ca^{2+} dysregulation in CAPN3-deficient muscle fibres, although future experiments are still needed in order to elucidate the pathogenic mechanisms involved in the mitochondrial dysfunction observed in LGMD2A.

Myotonic dystrophy

Myotonic dystrophy type 1 (DM1), the most frequent adult form of muscular dystrophy (Ref. 143), is an inherited autosomal dominant disease caused by an unstable expansion of the CTG triplet repeat located in the 3′ untranslated region of *DMPK* gene that codes for myotonic dystrophy protein kinase. This gene is located in the long arm of chromosome 19 and it is predominantly expressed in skeletal muscle (Ref. 144). DM2 form is a rare disease associated with a CCTG tetranucleotide repeat expansion, located in the first intron of the zinc finger protein 9 (ZNF9) gene in chromosome 3. Thus, both DM1 and DM2 originate from long non-coding repeat expansions, which cause a similar chronic, slowly progressing, multisystemic disease with a dominant inheritance pattern. Expanded mRNA molecules in the cell nucleus affect expression and/or re-allocation of RNA-binding proteins and lead to alteration in RNA processing (Refs. 145, 146, 147). The multi-systemic nature of both diseases is thought to be caused by aberrant splicing affecting a number of proteins involved in multiple cellular processes. It has recently been shown that myotonic dystrophy is associated with deregulated alternative splicing of the gene CACNA1S that encodes for DHPR alpha 1S subunit (DHPRα1s), a voltage sensor that plays a central role in E–C coupling (Ref. 148). In DM1 and DM2, skipping of CACNA1S exon 29 is enhanced, which increases DHPRα1s channel conductance and voltage sensitivity. The long-term consequences are very likely harmful to muscle, especially when DHPR missplicing is combined with deregulated alternative splicing of other genes related to Ca^{2+}-handling or E–C coupling such as the ones encoding chloride channel type 1, SERCA and RyR1 (Refs. 149, 150). This would likely lead to a chronic Ca^{2+} overload, a common scenario occurring in other forms of muscular dystrophies. In fact, several studies have reported that Ca^{2+}-signalling pathways are consistently affected in DM muscles, DM myotubes and mouse models of this disease (Refs. 151, 152, 153). These findings suggest that the combined effect of misregulated splicing of several genes involved in Ca^{2+} regulation and E–C coupling contributes to muscle degeneration in myotonic dystrophy. In particular, a recent study performed on human DM samples demonstrates abnormal expression of mRNA and proteins involved in muscle Ca^{2+} release, reuptake, storage and signalling, such as junctophilin-1 (JPH1), SERCA2, RyR1, CSQ2 and DHPR (Ref. 153). In DM muscles they

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found reduced protein levels of JPH1, but not mRNA, which is consistent with a general dysregulation of Ca^{2+} homeostasis since JPH1 undergoes Ca^{2+}-dependent proteolysis when intracellular Ca^{2+} levels are elevated for a sustained period (Refs. 154, 155). Interestingly, in most cases mRNA levels of Ca^{2+}-handling molecules were found increased while the protein levels were decreased, which suggests that the translational block may be one of the underlying mechanisms of DM.

Therapeutic targeting of mitochondria and calcium homeostasis in muscular dystrophies

Over the years, several pharmacological and molecular therapies that target Ca^{2+} homeostasis and/or mitochondrial function have been proposed as potential treatments for muscular dystrophies (summarised in Table 2). Different studies have demonstrated beneficial effects of RyR stabilisers on different animal models of muscular dystrophy and heart failure (Refs. 50, 101, 156). In particular, treatment of mouse models of Duchenne and LGMD2E muscular dystrophy with the RyR stabiliser S107 results in reduced RyR1 nitrosylation, reversion of calstabin-1 depletion and an overall improvement of muscle function and exercise performance (Refs. 101, 156). Interestingly, a recent study has demonstrated that S107, as well as the RyR inhibitor dantrolene, synergise with antisense-mediated exon-skipping therapies and increases muscle function in mdx mice and human DMD myotubes (Ref. 157). Also, several Ca^{2+} channel blockers such as nifedipine, diltiazem and verapamil have shown to improve muscle structure and function in dystrophic mice (Refs. 158, 159), but they have failed to prove significant beneficial effects on DMD patients in clinical trials (Ref. 160). Likewise, pharmacological or molecular blockage of stretch-activated channels (TRPC, TRPV2) has also shown a beneficial effect on several dystrophic models (Refs. 89, 161).

Angiotensin II-receptor blockers, β-blockers and angiotensin-converting-enzyme inhibitors have shown beneficial effects for the treatment of cardiomyopathies in clinical trials and animal models (Refs. 162, 163). Interestingly, some pharmacological agents from these groups have also been reported to increase SERCA2 expression and/or function, and therefore they are potential enhancers of cardiac Ca^{2+} homeostasis (Ref. 164). In this line, different strategies comprising overexpression of SERCA1 or SERCA2 in skeletal muscle of dystrophic mice, such as treatment with BGP-15, or AAV-mediated gene transfer have consistently shown a significant improvement in the dystrophic phenotype (Refs. 117, 118, 165), which further supports that reduced SERCA activity is a contributing factor in the pathology of muscular dystrophies.

Finally, several therapeutic approaches targeting mitochondrial dysfunction have been shown to improve muscle function in different dystrophic mouse models. These include treatment with cyclosporine A and cyclophilin D inhibitors (Debio 025) for impaired mitochondrial PTP opening, treatment with pargyline for excessive accumulation of reactive oxygen species or overexpression of peroxisome proliferator-activated receptor γ coactivator 1-gene α (PGC1 α) for mitochondrial dysfunction in postnecrotic dystrophic muscles (Ref. 166). In this line, an antioxidant that improves mitochondrial respiratory function, idebenone, has shown protection of cardiac and skeletal muscle in mdx mice and trends of efficacy in a Phase II study with DMD patients (Ref. 167).

Conclusions and future address

The full extent to which intracellular Ca^{2+} signalling influences the physiopathology of primary and secondary muscle diseases is only beginning to be understood. In the forthcoming years, future technological advances, including new powerful Ca^{2+} indicators are needed in order to accelerate and target-specific Ca^{2+}-signalling pathways within cells. Some promising genetically encoded Ca^{2+} indicators from invertebrates or algae are already under development and will likely provide us with new clues to understanding the role of Ca^{2+} in degenerative processes. Since these indicators are both, incorporated at the genome level and translated as fluorescent proteins that modify their emission intensity as a function of Ca^{2+} levels, they can be used for long-term experiments over days to months and they can also be introduced into in vivo animal models to test Ca^{2+} activity over a lifetime (Refs. 168, 169). Furthermore, other imaging technologies such as fluorescence resonance energy transfer-based (FRET) analysis can demonstrate Ca^{2+}-dependent responses at the single protein level (Ref. 170). In the near future, these

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Table 2. Treatments targeting calcium homeostasis and mitochondrial function in muscular dystrophies

Method	Effect on Ca^{2+} homeostasis / mitochondrial function	System	Clinical / pharmacological use	Comments	References
Pharmacological					
K201 / JTV519	RyR stabiliser / regulator Inhibits SERCA pumps	Mouse model of heart failure	Candidate cardioprotective reagent. Prevents Ca^{2+} leakage from SR	Antagonist of multiple ion channels Improves cardiac and muscle function	50
S107 (K201 analogue)	RyR stabiliser / regulator	mdx and $Sgcb^{-/-}$ mice	Prevents Ca^{2+} leakage from SR	Ameliorates muscular dystrophic phenotype Enhances exon skipping	101, 156, 157
Dantrolene	RyR antagonist	mdx mouse and DMD myotubes	Muscle relaxant. Treatment for malignant hyperthermia	Enhances exon skipping	157
Nifedipine	DHPR calcium blocker	mdx mice. DMD clinical trial	Antihypertensive, antianginal	Improves dystrophic muscle function. No significant effect on DMD clinical trials	158, 160
Diltiazem Verapamil	Non-DHPR calcium blocker. Inhibitor SERCA2 function	mdx mice. DMD clinical trial	Vasodilator Antianginal, antiarrythmic	Protect dystrophic fibres from degeneration. No significant effects on DMD clinical trials	159, 160, 164
BGP-15	Increases SERCA activity	mdx, dko mice	Insulin sensitiser Pharmacological inducer of HSP70	Ameliorating effect on dystrophic mice	118
Enalapril	Increases SERCA2 expression and function	mdx mice. DMD clinical trial	Angiotensin-converting enzyme inhibitor	Improves muscle strength and reduces fibrosis. Likely preserves cardiac function in DMD patients	162, 164
Carvedilol	Increases SERCA2 expression and function	DMD clinical trials	β-blocker	Likely preserves cardiac function in DMD patients	163, 164

(continued on next page)

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Table 2. Treatments targeting calcium homeostasis and mitochondrial function in muscular dystrophies *(continued)*

Method	Effect on Ca^{2+} homeostasis / mitochondrial function	System	Clinical / pharmacological use	Comments	References
Losartan	Increases SERCA2 expression and function	mdx mice	Angiotensin II receptor blocker. Blocks TGF-β signalling	Preserves cardiac function Improves respiratory function	162, 164
Resveratrol	Increases SERCA2 expression	mdx mice	Antioxidant SIRT activator	Heart diseases Ameliorateing effect on dystrophic mice	162, 164
Streptomycin	Blocker of stretch-activated channels (TRPCs)	mdx mice	Amynolycoside Does not affect ribosomal readthrough	Protects against muscle damage	89
Cyclosporin A Debio 025	Mictochondrial PTP inhibitors. Reduce SERCA2 expression	mdx, $Sgcb^{-/-}$, $Col6a1^{(-/-)}$ mice	Immunosuppressants	Improve muscle function in dystrophic mouse models	164, 166
Idebenone	Improves mitochondrial function	mdx, DMD patients	Antioxidant	Ameliorating effect on dystrophic mice. Trends of efficacy in DMD Phase II clinical study	162, 167
Pargyline	Monoamine oxidase B inhibitor	$Col6a1^{(-/-)}$ and mdx mice	Decrease ROS accumulation generated by MAOB	Ameliorating effect on dystrophic mice	166
Molecular					
AAV-mediated transfer of SERCA	Increases SERCA1/2 expression and function	mdx, $Sgcb^{-/-}$		Ameliorating effect on dystrophic mice	117, 165
Adenoviral-dominant negative TRPV2	Reduces TRPV2 signalling	δ-sarcoglycan deficient hamster		Ameliorating effect on dystrophic mice	161
PGC1α gene transfer	Restores mitochondrial function	mdx mice		Ameliorating effect on dystrophic mice	166

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developments will contribute to the design of new experiments with potential applications in basic science and translational research studies (Ref. 171).

The target of these research lines in the field of muscular dystrophies should be focused in the first place on disorders where Ca^{2+} homeostasis seems to play a central role, such as in malignant hyperthermia, central core disease or arrythmogenic cardiomyopathies (Ref. 50). Nevertheless, as awareness of Ca^{2+}-signalling dysregulation in other muscular diseases progresses, such as LGMD2A, attention should be addressed to specific elements of intracellular Ca^{2+}-signalling cascades in these entities. From a therapeutical point of view, some progress in these areas is already taking place: the DHPR blocker nifedipine and the SR leaky-channel blockers dantrolene and S-107, for instance, have been successfully tested in animal models of muscular dystrophy (Refs. 101, 156, 158). It is very likely that whatever achievements may come from these on-going research projects, they will be useful for several other muscular disorders sharing similar underlying Ca^{2+} pathological mechanisms.

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Conflicts of interest

None.

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Further reading, resources and contacts

Disease pages in OMIM:

Duchenne muscular dystrophy, http://www.omim.org/entry/310200

Becker muscular dystrophy, http://www.omim.org/entry/300376

LGMD2A muscular dystrophy, http://www.omim.org/entry/253600

Myotonic dystrophy 1, http://www.omim.org/entry/160900

Myotonic dystrophy 2, http://www.omim.org/entry/602668

ClinicalTrialsGov offers a complete list of worldwide clinical trials on muscular dystrophies and other medical conditions: http://clinicaltrials.gov/

The Muscular Dystrophy Association provides information on muscular dystrophies as well as helpful resources for patients: http://mda.org/

Treat Neuromuscular Disorders (TREAT-NMD) provides ample information about muscular dystrophies and new therapeutic developments: http://www.treat-nmd.eu/

Features associated with this article

Figure

Figure 1. Representation of calcium signalling dynamics in the muscle fibre.

Tables

Table 1. Calcium-related mechanisms affected in muscular dystrophies.

Table 2. Treatments targeting calcium homeostasis and mitochondrial function in muscular dystrophies.

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