Chapter 14
Disturbed Ca^{2+} Homeostasis in Muscle-Wasting Disorders

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Abstract Ca^{2+} is essential for proper structure and function of skeletal muscle. It not only activates contraction and force development but also participates in multiple signaling pathways. Low levels of Ca^{2+} restrain muscle regeneration by limiting the fusion of satellite cells. Ironically, sustained elevations of Ca^{2+} also result in muscle degeneration as this ion promotes high rates of protein breakdown. Moreover, transforming growth factors (TGFs) which are well known for controlling muscle growth also regulate Ca^{2+} channels. Thus, therapies focused on changing levels of Ca^{2+} and TGFs are promising for treating muscle-wasting disorders. Three principal systems govern the homeostasis of Ca^{2+}, namely, excitation-contraction (EC) coupling, excitation-coupled Ca^{2+} entry (ECCE), and store-operated Ca^{2+} entry (SOCE). Accordingly, alterations in these systems can lead to weakness and atrophy in many hereditary diseases, such as Brody disease, central core disease (CCD), tubular aggregate myopathy (TAM), myotonic dystrophy type 1 (MD1), oculopharyngeal muscular dystrophy (OPMD), and Duchenne muscular dystrophy (DMD). Here, the interrelationship between all these molecules and processes is reviewed.

Keywords EC coupling · Ca^{2+} channel · Myogenesis · Intracellular Ca^{2+} · Atrophy

14.1 Introduction

Numerous biological processes depend on the levels of intracellular Ca^{2+}. The neuromuscular transmission (NMT) is an emblematic example. It begins with the arrival of an action potential (AP) to the nerve terminal, with the ensued release and accumulation of acetylcholine (ACh) into the synaptic cleft. Subsequently, precise coordination of the gating of many types of ion channels (and transporters) results in a transitory increase in the levels of free myoplasmic Ca^{2+} ($[Ca^{2+}]_i$). More specifically, the influx of Na^+ through skeletal muscle ACh receptors depolarizes the

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membrane and thereby activates voltage-gated Na^+ channels, an AP is fired, and a process known as excitation-contraction (EC) coupling begins. During EC coupling, the voltage sensors of a voltage-gated Ca^{2+} channel ($Ca_V1.1$) activate the opening of ryanodine receptors (RyR1s, located in the sarcoplasmic reticulum or SR), which allows a massive release of Ca^{2+} to the cytosol. The resulting rise of $[Ca^{2+}]_i$ activates, in turn, not only the contractile machinery but also the SR Ca^{2+} ATPase (SERCA) that pumps Ca^{2+} back into the SR (reviewed recently in [1]).

Many human diseases course with skeletal muscle weakness, which (not surprisingly) can be explained by alterations in either NMT or EC coupling. Nevertheless, such modifications can also elicit a chronic loss of muscle mass. For example, by inhibiting the activity of the Ca^{2+}-calmodulin-dependent protein kinase (CamK). This kinase is important to not only stimulate the differentiation of precursor cells (myoblasts) [2] but also to induce transactivation of genes involved in hypertrophy. Apparently, CamK stimulates hypertrophy by inactivating a protein named glycogen synthase kinase 3 beta (GSK3β) [3], whose function is to limit the synthesis of proteins. Thus, by downregulating CamK, low levels of Ca^{2+} are well suited to generate atrophy. Paradoxically, a sustained rise of $[Ca^{2+}]_i$ also results in muscle wasting. This is because the amount of muscle mass depends on a balance between protein synthesis and degradation, and the elevated levels of Ca^{2+} can activate proteases and thereby promote the breakdown of proteins (Fig. 14.1) [4]. Accordingly, both agonists of the CamK signaling pathway and inhibitors of Ca^{2+}-dependent proteases represent intriguing candidates for treating the pathological loss of skeletal muscle (reviewed in [4, 5]). Herein, the interrelationship between all these physiological and pathological processes is reviewed. An emphasis is put on the role of Ca^{2+} as a critical node that manages the transition, from a healthy muscular structure to weakness and atrophy.

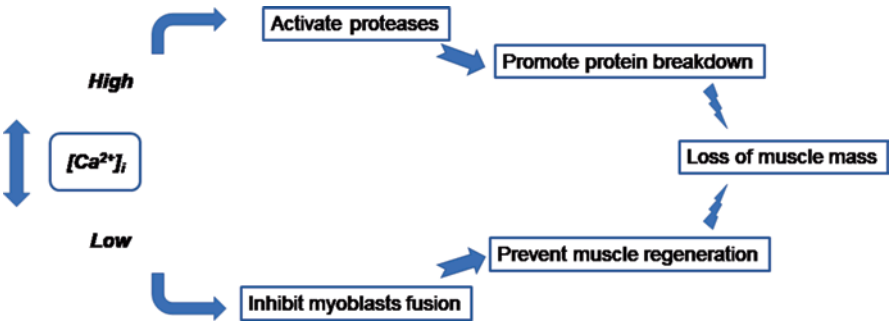

Fig. 14.1 The scheme depicts how pathological alterations of $[Ca^{2+}]_i$ can lead to atrophy. Changes in the levels of Ca^{2+}, in the up-and-down direction, activate two different signaling pathways that converge in promoting a significant loss of muscle mass. **High**: Sustained elevations of Ca^{2+} can activate a Ca^{2+}-dependent protease (calpain) and thereby result in the breakdown of proteins and atrophy. **Low**: On the other hand, a decrease in resting Ca^{2+} levels leads to an impaired formation of myotubes, preventing the proper regeneration of muscle and thus promoting the development of atrophy. See the text for further details

14.2 Dynamic Changes in Myoplasmic Ca^{2+}

The following three major physiological processes contribute to regulating the homeostasis of Ca^{2+}. They reflect the expression and activity of both Ca^{2+} channels and the SERCA pump.

14.2.1 Excitation-Contraction (EC) Coupling

EC coupling is the process by which an AP induces contraction and force development. A transitory increase in $[Ca^{2+}]_i$ (Ca^{2+} transient) is responsible for activating the contractile machinery, whose relaxation occurs as the Ca^{2+} levels return to normal values, thanks to the activity of SERCA. The source of Ca^{2+} for EC coupling is the SR, and it has been firmly established that extracellular Ca^{2+} is irrelevant for this process. For example, in the absence of extracellular Ca^{2+}, the skeletal muscle fiber contracts vigorously, for several minutes [6]. Additionally, the maximum levels of both $[Ca^{2+}]_i$ and contractile force can be elicited at membrane potentials where the influx of Ca^{2+} is practically null [7, 8]. Moreover, in 1973 Schneider and Chandler published what is known as the hypothesis of the physical link for EC coupling. It states that mobile particles embedded in the sarcolemma (voltage sensors) sense APs and mechanically activate the release of Ca^{2+} from the SR [9]. The molecular identity of voltage sensors was subsequently defined. They form part of a voltage-gated Ca^{2+} channel, also known as the dihydropyridine receptor (DHPR), or $Ca_V1.1$ [10, 11]. The junctional gap between transverse tubes of the sarcolemma (T-tubes) and terminal cisterns of the SR contains electron dense structures, termed "feet." They reflect the presence of the SR Ca^{2+} release channel, also known as RyR1 [12]. Indeed, mice knockout for the RyR1 gene lack feet [13]. Thus, $Ca_V1.1$ and RyR1 are both essential for EC coupling. Accordingly, they are also critical for survival [14–16].

14.2.2 Excitation-Coupled Ca^{2+} Entry (ECCE)

The Ca^{2+}-conducting activity of $Ca_V1.1$ is irrelevant for EC coupling [17]. This fact indirectly reinforces the concept that the SR is the only source of Ca^{2+} for this process (see Sect. 14.2.1). Nevertheless, it has been proposed that the entry of Ca^{2+} through $Ca_V1.1$ might participate in replenishing the SR during sustained depolarizations. A process known as excitation-coupled Ca^{2+} entry (ECCE, [18]) provides indirect support for this speculation. ECCE is a slow increase in the entry of Ca^{2+} in response to either sustained or repetitive depolarization (for review see [19]). A large amount of data suggests that in both, developing myotubes and adult muscle fibers, an entry of Ca^{2+} via $Ca_V1.1$ represents the underlying mechanism for ECCE [20–22].

The following direct evidence supports the notion that ECCE effectively contributes to SR Ca^{2+} loading. Robin and Allard (2015) reported that the SR Ca^{2+} loading is potentiated in response to an increase in the magnitude of Ca^{2+} current associated to ECCE. Moreover, they also found that Mn^{2+} is not only able to permeate during ECCE but also produces quenching of the fluo-5 N trapped in the SR [22]. Although these findings could be interpreted to suggest that ECCE is physiologically relevant, neither the development nor performance of skeletal muscle is altered in response to the elimination of Ca^{2+} influx via $Ca_V1.1$ [23]. Thus, the possibility that *a reduced* magnitude of ECCE be of pathophysiological relevance is practically null. Nevertheless, future work may lead to the exciting discovery that, conversely, *an increase* in ECCE leads to pathological symptoms.

14.2.3 Store-Operated Ca^{2+} Entry (SOCE)

SOCE is the process in which a decrease in the load of SR Ca^{2+} induces a protein of the SR to oligomerize and directly activate a Ca^{2+} channel of the sarcolemma: STIM is the SR protein, whereas Orai is the Ca^{2+} channel. Three isoforms of Orai have been identified in human, namely, Orai1, Orai2, and Orai3. They conform the well-known calcium release-activated Ca^{2+} channels (CRAC) [24]. STIM, on the other hand, consists of two isoforms, which have been detected in vertebrates (STIM1 and STIM2). The principal isoforms that underlie SOCE in skeletal muscle are STIM1 and Orai1 [25]. The C-terminal portion of STIM1 is cytosolic and presents domains critical for binding to—and activating—Orai1. On the other hand, the NH2-terminal segment of STIM1 is located in the lumen of the SR. It contains two regions that are critical for sensing the levels of luminal Ca^{2+}. More specifically, the following domains, EF-hand and sterile alpha-motif (SAM), are thought to constitute the sensor of Ca^{2+} (EF-SAM). Under normal levels of SR Ca^{2+} loading, the binding of Ca^{2+} to EF-SAM keeps STIM1 in its monomeric form. However, the EF-SAM conforms dimers and oligomers in response to depletion and thus promotes both binding of STIM1 to Orai1 and the subsequent entry of Ca^{2+} [21, 24, 26, 27].

It has been proposed that SOCE participates in refilling the SR of Ca^{2+}, but this idea is controversial. Evidently, an SR depletion is required for activating SOCE, but this condition is difficult to reach, not only physiologically but also experimentally [28]. The following evidence supports the view that SOCE, in effect, contributes to refilling the SR of Ca^{2+}. Mice knockout for myostatin (Sect. 14.3.3) develop a severe reduction in expression levels of STIM1 and Orai1, which correlates with an inhibition of SOCE and a faster SR depletion (induced by repetitive release of Ca^{2+}) [29]. Indeed, this tendency to readily exhaust the SR might explain why those mice deficient in myostatin also exhibit a significant muscle weakness (low specific force), in the face of an excessive muscle mass [30].

14.3 Myogenesis

14.3.1 Myogenesis Is Critical for Muscle Growth and Force Development

This is a brief explanation of how precursor cells contribute to the genesis and regeneration of skeletal muscle. The reader is encouraged to consult more extensive reviews on this topic [31–33]. During the embryonic development, precursor cells (termed myoblasts) fuse and form multinucleated cells, known as myotubes. The myoblasts withdraw from the cell cycle, adopt a spindle shape, and align with each other—forming a braid—and the fusion occurs. Subsequently, the myotubes are transformed into muscle fibers, through a maturation process that involves (among other things) the formation of T-tubes. The fusion of myoblasts is also known as "terminal differentiation" because it implies that DNA from the fused myoblasts will no longer replicate, and thereby the cell proliferation is arrested. In the adults, myotubes continually form. The corresponding precursor cells are known as satellite cells (SCs). Although not fully differentiated, proliferating myoblasts and SCs are committed to the myogenic lineage (i.e., they already express transcription factors of the MyoD family). Depending on specific conditions, precursor cells can be either mitotically quiescent or induced to proliferate. For example, injury stimulates SCs to proliferate, and the resulting colony provides for generating both a stock of quiescent cells and a significant number of fusion-competent myoblasts. The latter eventually will either form a new fiber or fuse into injured fibers contributing to healing [31–33].

In vitro, the fusion of myoblasts is often quantified as the "fusion index": that is, the number of nuclei per myotube, divided by the total number of nuclei per field of observation. The fusion index is crucial for in vivo conditions because the myofiber size and thereby the contractile strength depend on the number of nuclei in the fiber. Accordingly, it is well known that the number of nuclei in the myofiber declines during atrophy. Conversely, the restoration of muscle mass requires myonuclear accretion [34]. Remarkably, SCs also contribute to a robust neuromuscular junction (NMJ) [35, 36]. Indeed, the deterioration of NMJs, in aging, is more closely related to deficiencies in SCs and myogenesis rather than to denervation [36].

14.3.2 Role of Ca^{2+} in Skeletal Muscle Development

Myogenesis involves a dramatic change in phenotype which in turn depends on a coordinated activation of skeletal muscle-specific genes [37–39]. Apart from the expression of myogenic factors (e.g., MyoD, Myf5, Myf6, and myogenin), this process requires Ca^{2+}. More precisely, a Ca^{2+}-dependent signaling pathway that involves calmodulin and the family of transcription factors known as NFAT leads to the

fusion of myoblasts (for review see [5, 39, 40]). The recent discovery of a feedback mechanism by which SOCE and NFATc3 control the fusion of myoblasts highlights the relevance of this Ca^{2+}-dependent pathway [41].

Because myogenesis requires Ca^{2+}, a reduced entry of this ion tends to inhibit the proper regeneration of muscle. Ironically, however, sustained elevations of $[Ca^{2+}]_i$ also contribute to the degeneration of skeletal muscle (Fig. 14.1). This is because Ca^{2+}-dependent proteases lead to protein degradation (i.e., calpains, which contain Ca^{2+}-binding domains) [4]. Indeed, an increase in intracellular Ca^{2+} is frequently observed in both congenital myopathies and muscular dystrophies (see Sect. 14.4). Additionally, high rates of protein breakdown have been reported in many muscle-wasting diseases [42].

During myogenesis, the expression of several proteins involved in the homeostasis of Ca^{2+} is induced. An intricate relationship exists because Ca^{2+}, in turn, regulates the expression of at least two of these proteins (i.e., SERCA and $Ca_V1.1$) [43–45]. Therefore, dissecting the role of a specific protein in myogenesis is complicated. Nevertheless, the use of knockout animals has provided irrefutable proofs pointing to a leading role for $Ca_V1.1$ and RyR1. For example, it has been reported that dyspedic and dysgenic mice (i.e., RyR1 and $Ca_V1.1$ knockout) die both at birth. More interestingly, these two strains of mice also develop malformations, consisting in delayed development of skeletal muscle [14–16, 46]. Thus, RyR1 and $Ca_V1.1$ are both of paramount relevance for not only EC coupling (Sect. 14.2.1) but also myogenesis. On the other hand, a recent work elegantly showed that the Ca^{2+}-conducting activity of $Ca_V1.1$ is irrelevant for skeletal muscle development and function [23]. Thus, most likely this protein exerts its regulatory actions via mechanical control of RyR1 (as opposed to regulating the entry of Ca^{2+}, see Sect. 14.2).

In mice, the voltage-gated Ca^{2+} channel isoform $Ca_V3.2$ is expressed during embryonic development and then gradually disappears, after birth [47, 48]. In 2000, Biglenga et al. proposed that the entry of Ca^{2+} through this channel stimulates myogenesis [49]. More recently, this idea was tested and discarded because the fusion of myoblasts was unaltered by nickel (a $Ca_V3.2$ blocker) [50]. In addition to $Ca_V3.2$, both Orai1 (see Sect. 14.2.3) and a transient receptor potential channel (TRCP1) have also been proposed as necessary for myogenesis [51, 52].

14.3.3 Transforming Growth Factors Regulate Both Myogenesis and Ca^{2+} Channels

Several extracellular signaling factors participate in controlling distinct phases of myogenesis. For example, the hepatocyte growth factor (HGF) and fibroblast growth factor (FGF) are both considered of critical relevance for SCs activation [53]. Myostatin (growth differentiation factor 8, GDF-8) is a member of the transforming growth factor-β (TGF-β) superfamily, and it has also proven essential to regulate myogenesis [54, 55]. The TGF-β superfamily includes many other types of

growth factors, which, similarly to myostatin, also inhibits the development of skeletal muscle. Specifically, in less than 24 h, the bone morphogenetic protein type 2 (BMP-2) and transforming growth factor β1 (TGF-β1) decrease both the expression of MyoD and myogenin. The effect on these transcription factors precedes a drastic inhibition of myotube formation (Fig. 14.2) [56], which saturates at nanomolar concentrations [57].

Because myogenesis requires Ca^{2+} (Sect. 14.3.2), it is possible that BMP-2 and TGF-ß1 arrest this process by interfering with the activity of Ca^{2+} channels. In support of this view, both growth factors also inhibit the functional expression of Ca_V3 channels (in semi-differentiated myotubes, see Fig. 14.2). Moreover, TGF-β1, but not BMP-2, also downregulates the activity of $Ca_V1.1$ [56]. Although these data suggest that $Ca_V1.1$ and Ca_V3 channels participate in myogenesis, a role for only $Ca_V1.1$ has been firmly established (see Sect. 14.3.2).

14.4 Role of Ca^{2+} in Diseases That Course with Skeletal Muscle Atrophy

The calcium ions are of paramount relevance in the context of muscle atrophy (Sect. 14.3.2). Thus, not surprisingly, the list of diseases in which alterations in the homeostasis of Ca^{2+} and skeletal muscle atrophy concur is vast. This section discusses examples where dysregulation of Ca^{2+} channels and SERCA has been observed. It also explains how such dysregulation contributes to understanding the corresponding loss of muscle mass. It is highly recommended to consult the following excellent reviews on these topics [58, 59].

14.4.1 Congenital Myopathies

14.4.1.1 Brody Disease

Brody disease is a congenital myopathy characterized by muscle cramping that usually manifests after exercise (especially in the cold) and is accompanied by impairment of muscle relaxation. Muscles from the legs, arms, and eyelids are principally affected, and they slowly return to relaxation if maintained at rest (reviewed in [60]). This disease is linked to mutations in the gene encoding the skeletal muscle SERCA (i.e., SERCA1) [61]. A related myopathy has also been observed but in the absence of SERCA mutations (termed Brody syndrome). Thus, in more general terms, these disorders are just referred to as "Brody myopathy." It has been reported that patients with advanced phases of this myopathy also show skeletal muscle weakness and atrophy (of both type I and type II fibers) [60, 62, 63].

A reduced SERCA activity is observed in muscle samples of Brody myopathy patients, and this alteration explains an increase in time needed for myoplasmic Ca^{2+}

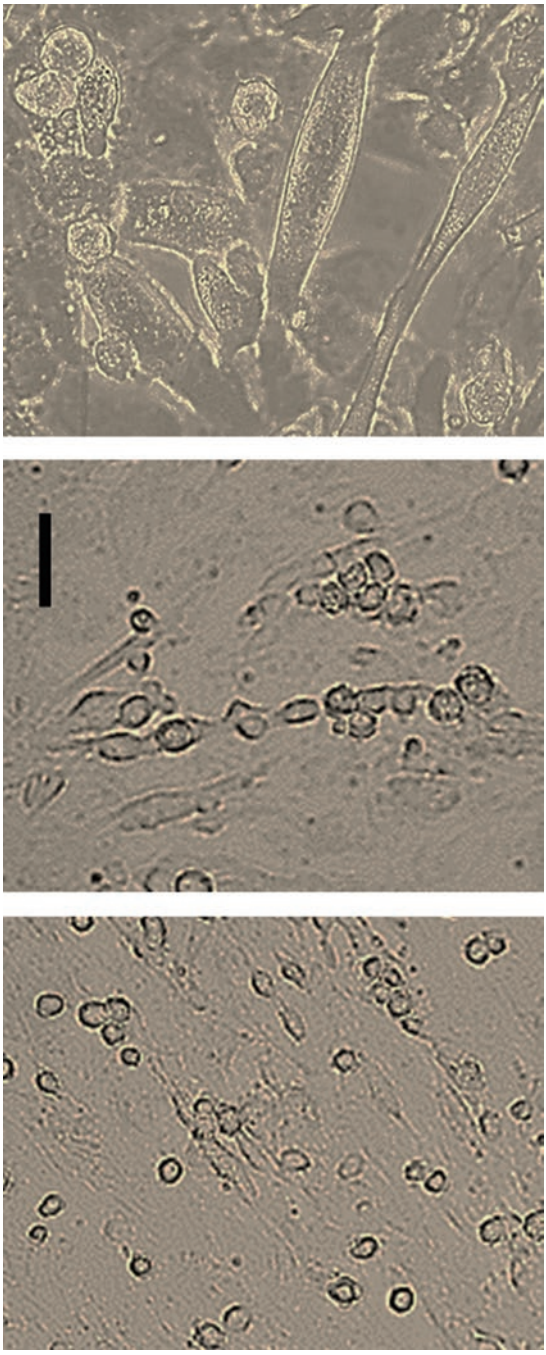

Fig. 14.2 TGF-β1 and BMP-2 inhibit myotube formation. Light-field images of myoblasts that were obtained from newborn mice and then kept 6 days under control differentiation conditions (upper panel) and the presence of either BMP-2 (5 nM, middle panel) or TGF-β1 (40 pM, lower panel). The scale bar represents 50 μm

extrusion after repetitive stimulation. Although this mechanism underlies the damaged muscle relaxation, stiffness, and cramping [64, 65], the primary functional defect responsible for the loss of SERCA activity remains unknown [60]. Likewise, the molecular basis underlying loss of muscle mass has yet to be elucidated. Because an increase in time needed for myoplasmic Ca^{2+} extrusion is ostensibly involved in this myopathy, it seems reasonable to speculate that an elevated level of $[Ca^{2+}]_i$ recruits Ca^{2+}-dependent proteases and thereby induces protein degradation (Fig. 14.1; see also Sect. 14.3.2). Dantrolene and verapamil, two inhibitors of EC coupling, are promising therapeutic agents for Brody myopathy. They limit the amount of Ca^{2+} released, and thereby the low Ca^{2+} pumping capacity readily restores the normal resting $[Ca^{2+}]_i$ levels, preventing Ca^{2+} overload ([65], discussed in [60]). Thus, in the near future, it will be interesting to investigate if these compounds also prevent the development of atrophy.

14.4.1.2 Central Core Disease

The following congenital myopathies have been related to mutations in the gene encoding RyR1: central core disease (CCD), multiminicore disease (MmD), core myopathies with rods, centronuclear myopathy (CNM), and congenital fiber-type disproportion (CFTD). They conform the also known as "RyR1-related congenital myopathies" (RyR1-RCM) [66, 67]. CCD was the first one being linked to RyR1, and thus the corresponding mutations have been more thoroughly investigated.

CCD is of early onset and courses with proximal weakness, wasting, and skeletal deformities. These symptoms can range from very mild to extremely severe. The diagnosis is based on the identification of areas located within the center of the myofiber, depleted of mitochondria and with poor oxidative enzymatic activity (for recent reviews, see [68, 69]).

Several CCD RyR1 mutant proteins exhibit an overactive or "leaky" behavior that depletes the SR of Ca^{2+} and thereby decreases the magnitude of the Ca^{2+} transient [43, 45, 70]. Another set of mutations, located nearby the pore leaning segment of RyR1 (i.e., exon 102, within the C-terminus region), results in mutant proteins with poor Ca^{2+} permeability. Thus, rather than being leaky, these "pore mutations" result in a functional uncoupling of SR Ca^{2+} release from the electrical stimulus (termed "EC uncoupling") [71–73]. A third mechanism indicates that certain CCD mutations induce a reduced expression level of RyR1 and thus also promote a lower magnitude of Ca^{2+} transients [74–77]. These three primary defects (i.e., leaky, Ca^{2+}-impermeable, and decreased expression) are not mutually exclusive. For example, it has been reported that the Y4864H mutation results in mutant RyR1 proteins that exhibit both, low expression level and altered functional properties (leaky behavior). Remarkably, this mutation also elicits a reduced magnitude of Ca^{2+} transients, and this defect is attributed to a modified gating of the channel (as opposed to a reduced number of Ca^{2+} release units) [77].

Although mutations located in many regions of the RyR1 result in leaky behavior, evidence exists suggesting that this alteration ultimately depends on a structural modification of the protein portion facing the lumen of the SR. In particular, it has been reported that the leak depends on a reduced threshold for store overload-induced Ca^{2+} release (SOICR) [78].

As reviewed above (Sect. 14.3.2), mice knockout for RyR1 exhibit several malformations, including a delayed development of skeletal muscle. Conceivably, these alterations could simply arise from the physical absence of RyR1. Nevertheless, the following evidence indicates that they are due to the inevitable loss of SR Ca^{2+} release. A point RyR1 mutation that renders Ca^{2+} impermeable channels (equivalent to I4897T in humans) also inhibits the fusion of C2C12 myoblasts [45]. Moreover, mice knock-in for the same mutation also exhibit a delayed development, which includes a reduced and amorphous skeletal muscle, and very small myotubes [72]. Thus, a reduced level of SR Ca^{2+} release is sufficient for disrupting myogenesis and thereby also contributes to explaining the atrophy seen in the corresponding CCD patients (Fig. 14.1).

On the contrary, in patients expressing leaky CCD mutations, the atrophy is likely due to a sustained increase in the levels of $[Ca^{2+}]_i$ [43, 45, 70]. More specifically, Ca^{2+}-dependent proteolysis [4] may result in increased rate of protein degradation [42] and thereby promote the corresponding loss of muscle mass (Fig. 14.1).

In a mouse model of CCD, the I4897T mutation (see above) was found to induce the development of endoplasmic reticulum stress, unfolded protein response, mitochondrial reactive oxygen species (ROS) production, muscle weakness, and atrophy. Currently, it is unclear how this Ca^{2+}-impermeable mutant protein results in all these alterations. Nevertheless, it is important to note that they were reverted by treatment with the chemical chaperone 4-phenylbutyrate (4-PBA) [79]. Similarly to 4-PBA, agonists of the G_s subgroup of G-protein-coupled receptors have also been reported to be of therapeutic potential in CCD [45, 80]. These findings are encouraging since no effective treatment exists for CCD.

14.4.1.3 Tubular Aggregate Myopathy

Tubular aggregate myopathy (TAM) is a condition characterized by the presence of "tubular aggregates," cramps, weakness, and myalgia. Such aggregates contain proteins of the SR and thereby are thought to represent structural alterations of this organelle. A genetic cause of the disease was recently found. Specifically, in 2013 Böhm and collaborators discovered a form of TAM that is inherited with an autosomal dominant pattern and is associated with mutations in the gene encoding STIM1 [81]. This finding was confirmed more recently [82–84]. Most of the naturally occurring mutations in STIM1 are punctual substitutions, and they are positioned within the NH2-terminal sequence, just where the EF-hand is located (Sect. 14.2.3). Accordingly, these mutations result in mutant proteins that exhibit an altered

capability to bind luminal Ca^{2+} and thereby also present constitutive oligomerization [81, 83, 85]. The principal role of STIM1 is to activate the entry of Ca^{2+} via Orai1 channels (during SOCE, Sect. 14.2.3). Thus, prominent levels of SOCE may represent an important functional defect of this myopathy. Indeed, TAM has also been linked to mutations in Orai1, and the corresponding mutant proteins allow an exacerbated influx of Ca^{2+} [86–88].

A TAM STIM1 mutation that consists of an extension of amino acids (I484RfsX21) was reported recently. Remarkably, it resides in the cytosolic part of the protein (C-terminal portion) and, in contrast to mutations of the lumen, it inhibits the entry of Ca^{2+} [84]. In addition, TAM has been linked to three different mutations in the gene encoding calsequestrin (CASQ1, which is responsible for Ca^{2+} storage in the SR). Interestingly, while all CASQ1 mutant proteins show a reduced ability to store Ca^{2+}, only two appear to stimulate SOCE [89]. These findings suggest that TAM, and the corresponding atrophy, can both arise from other pathophysiological mechanisms, in addition to elevated levels of SOCE.

14.4.2 Muscular Dystrophies

14.4.2.1 Myotonic Dystrophy Type 1 (MD1)

This disease is caused by the expansion of a CTG repeat in the gene encoding a protein kinase termed MDPK. Increased excitability, delayed relaxation, atrophy, and weakness represent the most common symptoms. The CTG-repeat expansion results in both lower MDPK protein levels and trapping of the corresponding mRNA into nuclear foci. Interestingly, muscle degeneration has been related to increased rates of myofibrillar protein breakdown [42], which in turn could be explained by an exacerbated activity of Ca^{2+}-dependent proteases [4]. Indeed, elevated levels of $[Ca^{2+}]_i$ have been observed in myotubes derived from both MD1 patients and DMPK knockout mice [90–92]. Nevertheless, it is important to note that a deficiency in DMPK has functional effects in neither cardiac nor skeletal muscle. Thus, the MD1 symptoms likely arise from toxic effects of the trapped transcripts, rather than to decreased levels of the protein [93]. Transcripts of at least both, transcription factors and alternative splicing factors can be trapped, which explains why in this myopathy the expression of multiple genes is altered. Remarkably, the trapping of mRNAs modifies not only the function but also the structure of the nuclei [94].

MD1 has also been associated with misregulated alternative splicing; for example, MD1 patients show repressed alternative splicing of exon 29 in $Ca_V1.1$. Of note, the degree of exon skipping correlates with the severity of muscle weakness, suggesting that the corresponding functional alteration in $Ca_V1.1$ contributes to exacerbating symptoms [95]. Additionally, the alternative splicing of both RyR1 and SERCA (1 and 2) is misregulated. Thus, aberrant splicing of the corresponding transcripts most likely also contribute (by affecting Ca^{2+}-dependent pathways) [92, 96].

14.4.2.2 OPMD

Oculopharyngeal muscular dystrophy, or OPMD, is a late-onset autosomal dominant congenital myopathy. The first symptoms begin between the fifth and sixth decades of life. They consist of progressive drooping of eyelids (ptosis), swallowing difficulty (dysphagia), muscle atrophy, and proximal upper and lower weakness. OPMD is linked to mutations in the gene encoding poly(A)-binding protein nuclear 1 (PABPN1). The OPMD mutations consist of an expansion of a tract that contains 10 alanines (to 12–17). The pathological hallmark is that the nuclei of skeletal muscle fibers develop aggregates or inclusions (termed intranuclear inclusions, INI), which contain a misfolded PABPN1 and sequester poly(A) RNA [97, 98]. This disease is also frequently accompanied by other severe symptoms, such as weakness and atrophy of the tongue, dysphonia, limitation of upward gaze, and facial muscle weakness [99].

Although the precise underlying mechanism is not yet clear, it has been proposed that the INIs generate toxic effects, likely by interfering with the cellular traffic of poly(A) RNA, and thus affecting gene expression [97, 98]. The expression of at least 202 genes is misregulated, as shown by microarray assays performed in muscle fibers from a mouse model of OPMD [100]. A recent study reported that an OPMD mutant protein (PABPN1-17A) promotes structural alterations of the nucleus, which contributes to explaining the wide range of genes whose expression is misregulated [101].

Interestingly, PABPN1 stimulates the fusion of myoblasts, and this property is missing in the PABPN1-17A mutant protein [101]. Thus, an altered capacity to regenerate muscle may explain the corresponding muscle atrophy and weakness in OPMD. In C2C12 myotubes, PABPN1-17A also elicits many alterations in the homeostasis of Ca^{2+} [101]. For example, it promotes a ~50% reduction of the magnitude of Ca^{2+} transients. This effect can be explained by parallel changes in the expression of RyR1 and SR Ca^{2+} content. In fibers from adult mice, however, this mutant protein is unable to modify the magnitude of Ca^{2+} transients [101]. This finding indirectly supports the notion that atrophy, due to inability to stimulate myogenesis (Fig. 14.1), likely represents the most significant pathophysiological consequence of PABPN1 mutant proteins [101–104].

14.4.2.3 Duchenne Muscular Dystrophy

The absence of dystrophin, a cytosolic protein that is critical for proper structure of the muscle, results in a genetic disorder known as Duchenne muscular dystrophy (DMD). This disease is characterized by shorter lifespan, cardiac involvement, and skeletal muscle degeneration and weakness. An increased structural fragility of muscle fibers and altered homeostasis of Ca^{2+} represent two relevant pathophysiological mechanisms. Indeed, an increased entry of Ca^{2+} (which promotes protein degradation and higher levels of ROS) has been proposed to explain the

corresponding atrophy [42, 105]. Accordingly, myotubes of mdx mice (a commonly used model of DMD) exhibit a higher activity of Ca^{2+} channels at resting membrane potentials, compared with controls. This hyperactivity is due to the presence of a mechano-transducing Ca^{2+} channel, which likely contributes to the high influx of Ca^{2+} [106, 107]. Although the identity of the corresponding stretch-activated Ca^{2+} channel(s) (SACs) has yet to be firmly established, members of the transient receptor potential channel (TRPC) family may be involved. TRPCs participate in muscle differentiation, and thus changes in their function/expression might also contribute to generating the corresponding loss of muscle mass. For a recent and comprehensive review, see [108].

An exacerbated SOCE has also been linked to DMD. For example, muscle fibers from mdx mice show not only increased levels of SOCE but also higher expression level of both Orai1 and STIM1 [109, 110]. Accordingly, it has been reported that the severity of this disease can be reduced by expressing a dominant negative Orai1, in two mouse models of DMD [111].

Like in many human myopathies, no effective treatment exists for DMD (other than palliatives focused on easing the symptoms). Thus, the search for a more effective treatment continues. With regard to "fixing" alterations in the homeostasis of Ca^{2+}, pharmacological approaches have been investigated. More precisely, the efforts have focused on using blockers of Ca^{2+} channels, as well as on regulating the activity and expression of SERCA (reviewed in [112, 113]). Knocking down the expression and activity of myostatin (see Sect. 14.3.2) also represents a promising therapy. This intervention is particularly beneficial to counteract muscle weakness and wasting, in not only DMD [114, 115] but also many other disorders [116].

14.5 Conclusions

In skeletal muscle fibers, much work has evolved in acquiring a deep knowledge of the mechanisms that control the homeostasis of Ca^{2+}, under both physiological and pathological conditions. Meanwhile, significant efforts have firmly established a pivotal role for Ca^{2+} in determining the amount of muscle mass. Accordingly, it is now generally accepted that this ion controls not only muscle mechanical properties but also the corresponding development, regeneration, atrophy, and hypertrophy. Therefore, treating wasting disorders with therapies based on a precise tune-up of the activity/expression of Ca^{2+} channels and transporters could eventually become a daily clinical practice.

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