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Abnormal Ion Homeostasis and Cell Damage in Muscular Dystrophy

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1. Introduction

Disruption of cytoskeletal organization caused by genetic defects in the components of the dystrophin-glycoprotein complex (DGC) results in muscular dystrophy and/or cardiomyopathy in human patients and animal models. Accumulating evidence obtained from studies by using skeletal muscle fibers, cultured myotubes, and cardiac muscle preparations from dystrophic animals suggest that defects in DGC components cause altered membrane properties in the sarcolemma of myocytes. For example, disruption of the DGC can cause increased susceptibility to mechanical stress or increased permeability to ions such as Ca^{2+} and Na^{+}, leading to a chronic increase in the concentrations of intracellular Ca^{2+} ($[Ca^{2+}]_i$) and Na^{+} ($[Na^{+}]_i$). Abnormal ion homeostasis, especially under conditions of mechanical stress, is thought to be a key molecular event in the pathology of muscular dysgenesis. In this chapter, we will review the stretch-induced cell damage pathways that result in abnormal Ca^{2+} and Na^{+} concentrations. In particular, we will focus on stretch-activated channels, transient receptor potential cation channels, and Na^{+}-dependent ion transporters, which have been reported to be of critical pathological significance. We will also discuss the therapeutic potential of these ion handling membrane proteins for the treatment of muscular dystrophy.

2. Sarcolemmal weakness and abnormal ion homeostasis in muscular dystrophy

Table 1 shows the genes and their products, which are involved in muscular dystrophy. Disruption of some genes (highlighted in pink) lead to heart failure, most often caused by dilated cardiomyopathy, as well as muscular dystrophy. In Duchenne muscular dystrophy (DMD), in which the protein dystrophin is defective, expression of dystrophin-associated proteins is also greatly reduced (Ervasti and Campbell, 1991). In addition, other types of muscular dystrophy are caused by mutations in genes encoding the components of the DGC (Campbell, 1995; Duclos et al., 1998; Nigro et al., 1997). The DGC is a multi-subunit complex (Campbell, 1995; Campbell & Kahl, 1989; Tinsley et al., 1994) that spans the sarcolemma to structurally link extracellular matrix proteins such as laminin to the actin cytoskeleton (Ervasti & Campbell, 1993), providing mechanical strength to the muscle cell membranes. Therefore, disruption of the DGC could significantly destroy membrane integrity or stability during contraction/relaxation, and cause cell damage. Importantly, defects in different

genes cause similar end-stage symptoms, i.e., muscle dysgenesis. Understanding the pathway that leads to cell damage is important for the development of common therapeutic strategies, not only for muscular dystrophy, but also for inherited and non-inherited cases of dilated cardiomyopathy. Two animal models are commonly used for the study of muscular dystrophy: the dystrophin-deficient mouse (*mdx*), which is representative of human DMD, and the delta-sarcoglycan-deficient hamster (BIO14.6), which is a model for human delta-sarcoglycanopathy.

inheritance	disease	gene	gene product	locus	OMIM number
X-linked	Duchenne Muscular Dystrophy	*DMD*	dystrophin	Xp21.2	300377
	Becker Muscular Dystrophy	*BMD*	dystrophin	Xp21.2	300376
	Emery-Dreifus Muscular Dystrophy	*EMD,FHL1*	emerin, FHL1	Xq28, Xq27.2	310300
Autosomal Recessive	Fukuyana-type	*FCMD*	fukutin	9q31-q33	607440
	Merosin-deficient	*LAMA2*	laminin α-2 chain	6q22-q23	156225
	Integrin-deficient	*ITGA7*	integrin α-7	12q13	600536
	Ullrich	*COL6A1,2,3*	collagenVI	2q37, 21q22.3	254090
	Muscle-eye-brain（MEB)	*POMGNT1*	POMGNT1	1p34-p33	606822
	Walker-Warburg syndrome(WWS)	*POMT1,2*	POMT1,2	9q34.1, 14q24.3	236670
	Limb-girdle				
	Calpainopathy	*CAPN3*	calpain-3	15q15.1-q21.1	114240
	Dysferlinopathy, Miyoshi distal	*DYSF*	dysferlin	2p13	603009
	Gamma-sarcoglycanopathy	*SGCG*	γ-sarcoglycan	13q12	253700
	Alpha-sarcoglycanopathy	*SGCA*	α-sarcoglycan	17q21	600119
	Beta-sarcoglycanopathy	*SGCB*	β-sarcoglycan	4q12	600900
	Delta-sarcoglycanopathy	*SGCD*	δ-sarcoglycan	5q33	601411
	Telethoninopathy	*TCAP*	telethonin	17q12	604488
	LGMD2H	*TRIM32*	E3 ubiquitin-protein ligase	9q31-q34.1	254110
	LGMD2I	*FKRP*	fukutin-related protein	19q13.32	606596
	LGMD2J	*TTN*	titin	2q31	608807
	LGMD2K	*POMT1*	POMT1	9q34.1	609308
	LGMD2L	*ANO5*	Ca^{2+} activated Cl^- channel	11p12-p13	611307
	LGMD2M	*FKTN*	fukutin	9q31	607440
	LGMD2N	*POMT2*	POMT2	14q24.3	607439
	LGMD2O	*POMGNT1*	POMGNT1	1p34-p33	606822
Autosomal Dominant	Limb-girdle				
	Myotilinopathy(LGMD1A)	*MYOT*	myotilin	5q31	604103
	LGMD1B	*LMNA*	lamin A/C	1q21.2	150330
	Caveolinopathy(LGMD1C)	*CAV3*	caveolin-3	3p25	601253
	LGMD1D	*unknown*	unknown	6q23	603511
	Facioscapulohumeral (FSHD)	*DUX4*	DUX4	4q35	158900
	Myotonic dystrophy	*DMPK*	myotonica protein kinase	19q13.2-q13.3	605377
	Myoclonus dystonia	*SGCE*	ε-sarcoglycan	7q21.3	604149

OMIM number: National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/)

Common gene and gene product of cardiomyopathy

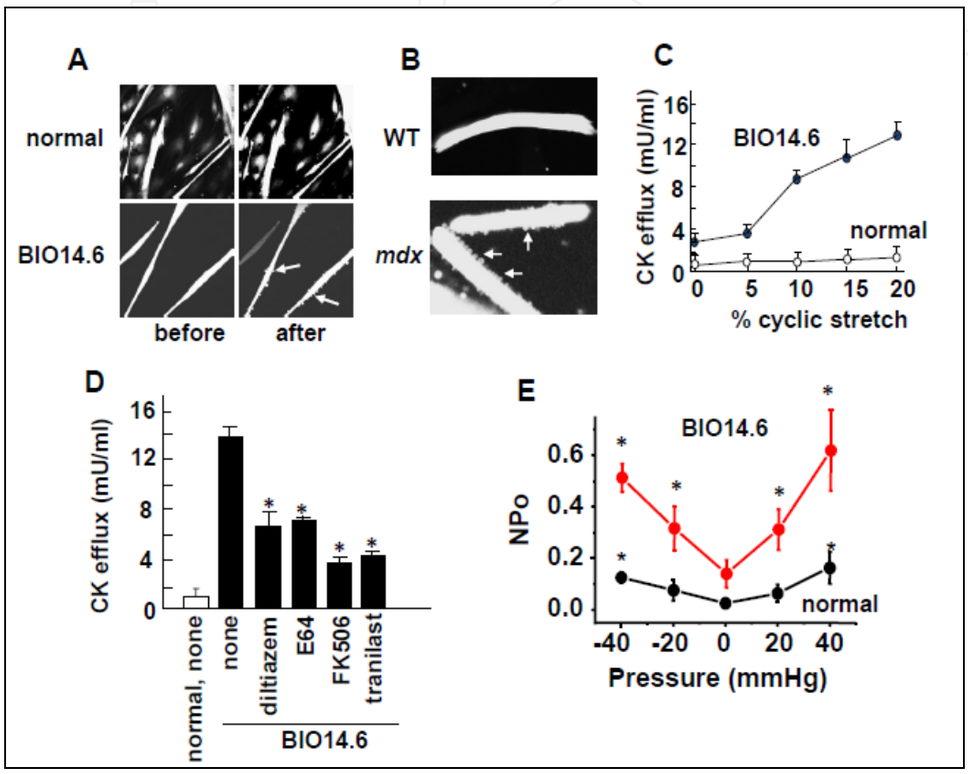
POMGNT;protein O-mannose beta 1,2-N-acetylglucosaminyltransferase

POMT ; protein O-mannosyltransferase

Table 1. Genes and their products responsible for muscular dystrophy

In an initial investigation, we found that sarcolemma from BIO14.6 hamster cardiomyopathic hearts has a fragile nature and is highly susceptible to mechanical stress, as evidenced by the ease of extraction of sarcolemma and T-tubules by relatively weak mechanical homogenization in low strength homogenization buffer (Tawada-Iwata et al., 1993). As a result, the amount of dystrophin extracted from BIO14.6 hamsters was 5 times more than that extracted from control hamsters, despite similar or lower dystrophin expression levels in BIO14.6 hamsters (Iwata et al., 1993a). The physical association between dystrophin and dystroglycan in BIO14.6 hearts was very weak (Iwata et al., 1993b). Mechanical membrane weakness was also observed in the skeletal muscle myotubes from BIO14.6 hamsters. Under hypo-osmotic stress (70% osmolarity), extensive cell bleb formation was seen in cultured BIO14.6 myotubes; however, this was not observed in control myotubes (Fig.1 A). Similarly, bleb formation was also seen in *mdx* mouse fibers, but not in control fibers (Fig.1 B). Upon

cyclic stretching of up to 20% elongation for 1 h, creatine phosphokinase (CK) efflux (a marker of cell damage) was elevated with increasing strength of stretch in BIO14.6 myotubes, but not in controls (Fig.1 C). Such cell damage in *mdx* myotubes has also been reported by other groups (Menke & Jockusch, 1991; Petrof et al., 1993). These data suggest that skeletal and cardiac muscle is highly sensitive to mechanical stretch in animal models of DGC deficiency.



(A) Hypo-osmotic stress-induced cell damage in cultured dystrophic myotubes. Normal and BIO14.6 myotubes were preloaded with 5 mM calcein-AM and exposed to hypo-osmotic medium (70% osmolarity) for 17 min. Extensive bleb formation was observed in BIO14.6 myotubes (arrow). (B) Hypo-osmotic stress-induced cell damage in the single skeletal muscle fibers from WT and *mdx* muscle. Note the extensive bleb formation in *mdx* fibers. (C) Cyclic stretch of up to 20% for 1 h induced CK efflux from BIO14.6 myotubes, indicating the high susceptibility of dystrophic myotubes to mechanical stress. (D) Effect of various agents on stretch-induced CK efflux. The TRPV2 inhibitor tranilast effectively blocked CK efflux. The Ca^{2+} channel blocker diltiazem, the Ca^{2+}-dependent protease calpain inhibitor E64, and the Ca^{2+}-dependent phosphatase calcineurin inhibitor FK506 also blocked CK efflux, suggesting that these Ca^{2+} handling and Ca^{2+} effector proteins are involved in stretch-induced membrane damage. (E) A stretch-induced Ca^{2+}-permeable channel is activated in BIO14.6 myotubes. Positive or negative pressures were applied to the pipette using cell-attached patches that were held at -60 mV. $BaCl_2$ (110 mM) was used as the charge carrier. Note the higher open probability (NP_o) in BIO14.6 myotubes.

Fig. 1. Mechanical membrane weakness in the DGC-defective myotubes

It has been reported that myocyte degeneration may be caused by increased membrane permeability to Ca^{2+}, which is probably linked with membrane weakness. Many studies have reported chronic elevations in $[Ca^{2+}]_i$ underneath the sarcolemma, or within other intracellular compartments, in the skeletal muscle fibers or myotubes from DMD patients and *mdx* mice

(Brown, 1997; Mallouk et al., 2000; Robert et al., 2001). Elevated $[Ca^{2+}]_i$ has been causally linked to a greater rate of protein degradation, catalyzed by the Ca^{2+}-dependent protease calpain (Alderton & Steinhardt, 2000a; MacLennan et al., 1991; Spencer et al., 1995; Turner et al., 1988). Concurrently, myocyte contractile activity would cause physical damage to the sarcolemma, leading to leakage of cytosolic enzymes such as CK. The $[Ca^{2+}]_i$ in the muscle tissue is regulated by numerous ion channels, Ca^{2+} pumps, and transporters in the sarcolemma and sarcoplasmic reticulum (SR). Of these, the sarcolemmal Ca^{2+}-permeable channels (Ca^{2+}-specific leak channels) or the mechanosensitive non-selective cation channels, which contribute to abnormal Ca^{2+} handling in dystrophic myocytes, have been the focus of attention.

In 1990, the Steinhardt group (Fong et al., 1990) reported the existence of Ca^{2+} leak channels with a higher open probability in myotubes from *mdx* mice or DMD patients. These channels were activated by the L-type Ca^{2+} channel blocker nifedipine, but inhibited by the other dihydropyridine compounds AN406 and AN1043 (Alderton et al., 2000a). Treatment with the calpain inhibitor leupeptin decreased opening probabilities of the leak channels and prevented elevation in resting $[Ca^{2+}]_i$ in *mdx* myotubes (Turner et al., 1993). The leak channels were activated by store depletion in control myotubes (Hopf et al., 1996). From these data, Steinhardt et al. proposed a mechanism of myocyte degeneration caused by leak channel activation; contraction-induced sarcolemmal tears in dystrophin-deficient myotubes lead to localized Ca^{2+} entry, which initiates the repair of the defect. Activation of proteolysis is thought to be essential for the activation of leak channels, which accelerate Ca^{2+} entry and further increase Ca^{2+}–dependent proteolysis, the final common pathway of cell damage (Alderton & Steinhardt, 2000b). However, molecular identification and characterization of Ca^{2+} leak channels has not been carried out.

Mechanosensitive stretch-activated channels (SACs) have been described to contribute to increased Ca^{2+} permeability in dystrophic myotubes. The open probability of these channels increases when negative pressure is applied to a patch pipette. This is observed in both control and *mdx* muscle, but to a greater extent in *mdx* myotubes and fibers (Franco-Obregon & Lansman, 1994). SACs are non-selective cation channels permeable to Na^+, K^+, Ca^{2+}, and Ba^{2+}, and have a conductance of 13 pS when a patch pipette is filled with 110 mM Ca^{2+} (Franco & Lansman, 1990). In addition, a stretch-inactivated channel has been reported to exist only in *mdx* muscle cells (Franco et al., 1990), which was later reported to have identical conductance properties to SACs (Franco-Obregon & Lansman, 2002). These channels are blocked by Gd^{3+}, streptomycin (Hamill & McBride, 1996), and the spider venom toxin GsMtx4 (Suchyna et al., 2000). Abnormalities in SACs have been detected in the recordings from the muscle biopsy samples of DMD patients (Vandebrouck et al., 2001; Vandebrouck et al., 2002a). These results suggest that SACs are important for pathological Ca^{2+} entry into the dystrophin-deficient muscle, at early stages of the pathogenesis.

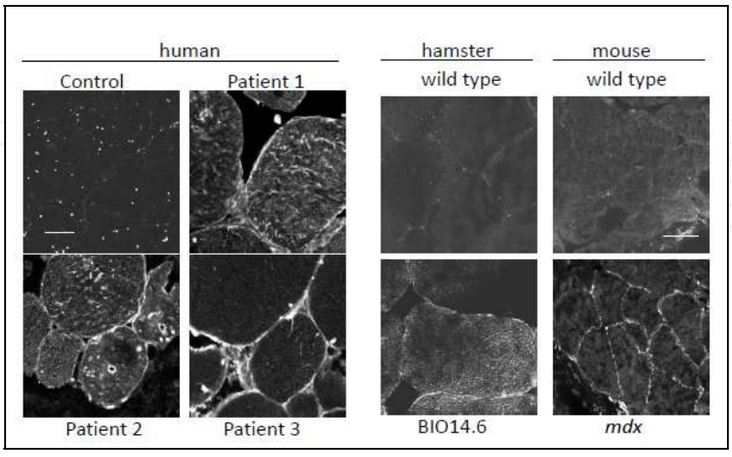
Research has shown that store-operated Ca^{2+} channels (SOCs) may also be involved in the pathogenesis of muscular dystrophy. SOCs have voltage-independent properties and a unitary conductance between 7 and 8 pS (with 110 mM Ca^{2+} in a patch pipette). Their open probability increases when luminal Ca^{2+} in the SR is depleted by the Ca^{2+} pump inhibitor thapsigargin. SOC activity was reported to be about twice as high in *mdx* compared to wild-type mice, and contributes to increased $[Ca^{2+}]_i$ in DMD (Vandebrouck et al., 2002b). Recently, Ca^{2+}-independent phospholipase A2 was found to be localized in the sarcolemma of *mdx* muscle, and its enzymatic product, lysophosphatidylcholine, was found to trigger Ca^{2+} entry through SOCs (Boittin et al., 2006). SACs and SOCs share several biophysical and

pharmacological properties in adult muscle fibers; they have the same unitary conductance, and a similar sensitivity to Gd^{3+}, SKF-96365, 2-aminoethoxydiphenyl borate (2-APB), GsMTx4 toxin, and IGF-1 stimulation (Ducret et al., 2006). These observations suggest that SACs and SOCs may share common constituents, although molecular identification of these components is still required.

3. Therapeutic targets for muscular dystrophy

3.1 TRPV channels

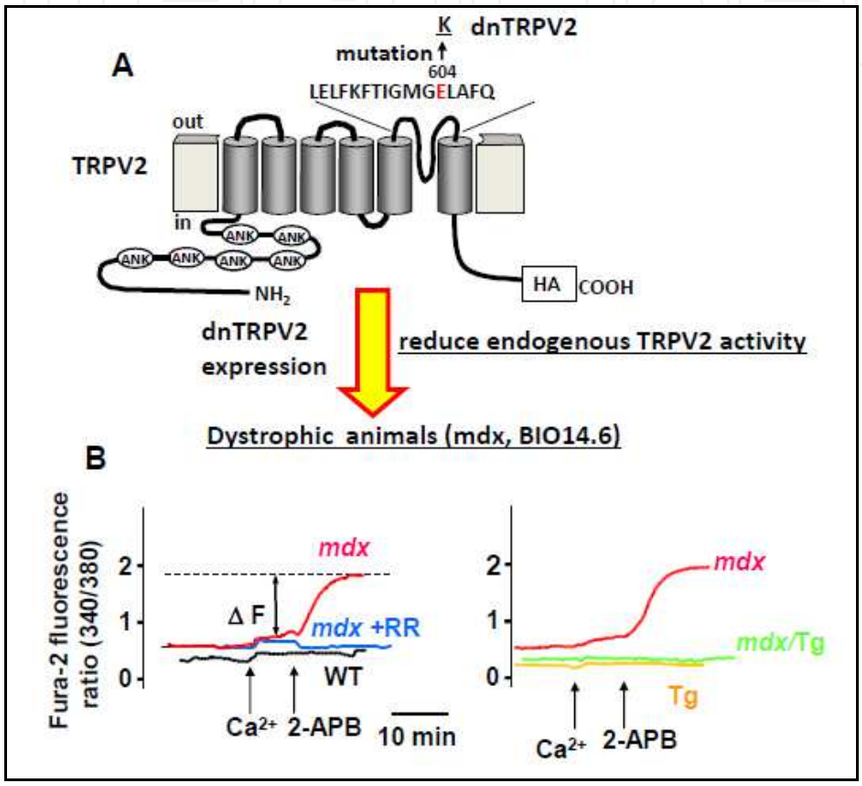
Since a large body of evidence indicates the pathological significance of increased $[Ca^{2+}]_i$ in muscular dystrophy, much effort has been made to identify the genes responsible for abnormal Ca^{2+} handling in this disease. Our group has shown that stretch-sensitive cation-selective channels, similar to those recorded in *mdx* skeletal muscle, are active in cultured myotubes prepared from BIO14.6 hamsters (Nakamura et al., 2001). Positive or negative pressure increases the open probability of this channel in BIO14.6 myotubes (Fig.1 E). To identify the Ca^{2+} entry pathway responsible for myocyte degeneration, we searched for mammalian homologs of the *Drosophila* stretch sensor *NompA,* which were expressed in the striated muscle. We were successful in identifying a candidate that belongs to the transient receptor potential (TRP) channel family, which is similar to *NompA*. Many members of this family are Ca^{2+}-permeable cation channels sensitive to physical stimuli such as osmotic stress or heat (Montell et al., 2002). The candidate channel was previously reported as growth factor responsive channel (GRC) (Kanzaki et al., 1999), and later renamed TRP vanilloid type 2 (TRPV2) channel. We showed that TRPV2 is activated by mechanical stimuli and plays a critical role in the pathogenesis of muscular dystrophy and cardiomyopathy (Iwata et al., 2003; Muraki et al., 2003). TRPV2 is normally localized in the intracellular membrane compartment, but translocates to the plasma membrane in response to stretch or growth factor stimulation. Importantly, TRPV2 was observed to accumulate in the sarcolemma of the skeletal muscle from human patients with muscular dystrophy, BIO14.6 hamsters, and *mdx* mice (Fig.2) (Iwata et al., 2003), thus contributing to a sustained increase in $[Ca^{2+}]_i$ in diseased myocytes.



TRPV2 was immunolocalized in the frozen sections of the skeletal muscle from dystrophic patients and a non-dystrophic control, or from wild-type and dystrophic animals (Iwata et al., 2003). Note extensive sarcolemmal localization of TRPV2 in dystrophic patients and animal models. Bars, 50 μm.

Fig. 2. Immunohistochemical localization of TRPV2

In order to determine whether TRPV2 contributes to Ca^{2+}-induced muscle damage, we used a dominant-negative mutant strategy. We produced loss-of-function TRPV2 mutants in the pore region of the protein, which have a dominant-negative effect on the channel function by forming a non-functional oligomer, thereby abrogating the activity of endogenous TRPV2 (Fig.3 A). This dominant-negative TRPV2 mutant was incorporated into *mdx* mice by using a transgenic strategy or into BIO14.6 hamsters by adenoviral transfer. We found that these approaches significantly reduced resting $[Ca^{2+}]_i$ as well as the increase in $[Ca^{2+}]_i$ induced by high Ca^{2+} and the TRPV2 agonist 2-APB observed in dystrophic muscles (Fig.3 B).



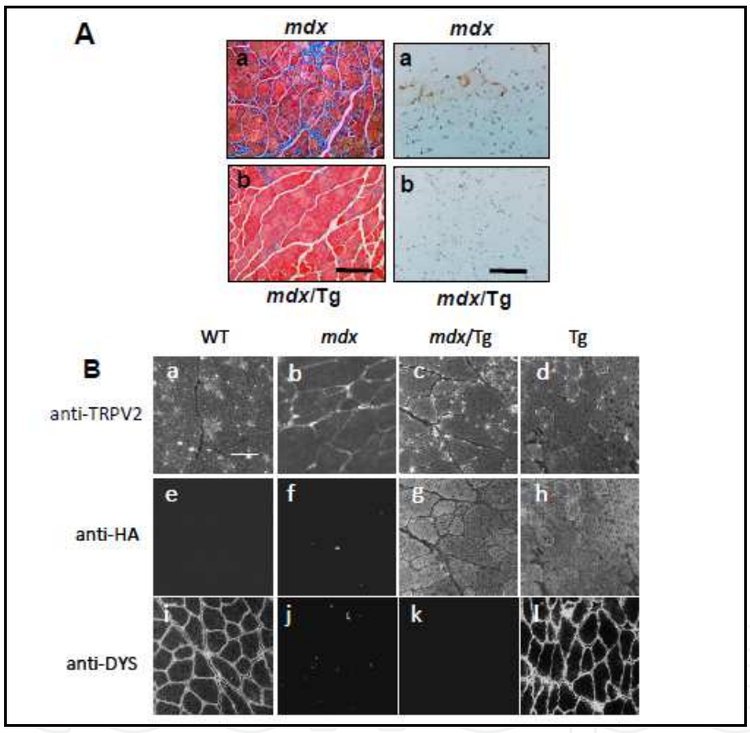
(A) Transgenic (Tg) mice overexpressing the TRPV2 mutant with a mutation in the putative pore region (Glu604) were produced, and crossed with *mdx* mice to introduce dnTRPV2 and inhibit endogenous TRPV2 activity. ANK, ankyrin repeat domain. HA, epitope tag. (B) Agonist (2-APB)-induced $[Ca^{2+}]_i$ increase in the isolated flexor digitorum brevis fibers of *mdx* mice, which is inhibited by ruthenium red (RR) (left). Note that no large $[Ca^{2+}]_i$ increase was detected in the fibers from wild-type mice. Such $[Ca^{2+}]_i$ increases in the *mdx* fibers were markedly reduced by expression of dnTRPV2 (*mdx*/Tg) (right) (Iwata et al., 2009).

Fig. 3. Production of a dominant-negative (dn) TRPV2 mutant

Transgenic or adenoviral expression of dnTRPV2 resulted in a 40%–70% reduction of impaired muscle function, as determined by an increased number of central nuclei and improvements in fiber size variability, fibrosis, apoptosis, elevated serum creatine kinase levels, and reduced muscle performance in dystrophic animals (Fig.4 A) (Iwata et al., 2009). Furthermore, *mdx* muscles were largely protected from eccentric work-induced force drop by the same transgenic strategy (Zanou et al., 2009). These results suggest that the entry of Ca^{2+} through TRPV2 channels precedes and is involved in membrane damage. Interestingly,

expression of dnTRPV2 also promoted the removal of endogenous TRPV2 from the sarcolemma (Fig.4 B), suggesting that Ca^{2+} entering the cell via TRPV2 is required for the sarcolemmal retention of TRPV2 expression in dystrophic muscles. The pathological importance of TRPV2 was also verified using a pharmacological approach. A non-selective cation channel blocker tranilast was shown to be an effective inhibitor of TRPV2 (Iwata et al., 2005). Oral administration of tranilast reduced various symptoms of muscular dystrophy, such as elevated serum CK levels, progressive muscle degeneration, and increased infiltration of immune cells (Fig.5 A) (Iwata et al., 2005). Therefore, specific inhibitors of TRPV2 could be potentially useful and effective treatments for various muscle degenerative diseases, including hereditary diseases.

Although the relationship between muscular dystrophy and other types of TRPV channel has not yet been investigated, TRPV4 was recently identified as a responsive gene in inherited neurodegenerative disease, which indirectly causes muscle atrophy (Deng et al., 2010; Landoure et al., 2010).



(A) Masson's trichrome staining (left) or TUNEL labeling (right) of the gastrocnemius muscle sections from *mdx* (a) or *mdx*/Tg (b) mice. Note the beneficial effect of dnTRPV2 (*mdx*/Tg). Scale bar, 100 μm. (B) Immunohistochemical analysis of TRPV2 (a-d) and dystrophin (i-l) in frozen cross sections of skeletal muscle. Dominant negative TRPV2 was immunolocalized with rat anti-HA (e-h). Note the removal of TRPV2 from the sarcolemma promoted by dnTRPV2 (*mdx*/Tg). Scale bar, 50 μm.

Fig. 4. Dominant-negative TRPV2 prevents muscle degeneration in *mdx* mice

3.2 TRPC channels

TRPC channels represent another important candidate for a therapeutic target in the treatment of muscular dystrophy. TRPC1, 4, and 6 are expressed in the sarcolemma of the

skeletal muscle (Vandebrouck et al., 2002b). Knockdown of TRPC1 and TRPC4, but not TRPC6, was reported to reduce abnormal Ca^{2+} influx in dystrophic fibers (Vandebrouck et al., 2002b). TRPC1 has been shown to form the stretch-activated channel (Maroto et al., 2005), although contrary data also exists (Gottlieb et al., 2008). TRPC1, together with its binding partner caveolin-3, accumulates at higher levels in the sarcolemma of the dystrophin-deficient muscle and contributes to abnormal Ca^{2+} influx, which is activated by reactive oxygen species and Src kinase (Allen & Whitehead, 2011; Gervasio et al., 2008). Furthermore, mice lacking the scaffolding protein Homer-1, which interacts with TRPC1, exhibit myopathy associated with increased spontaneous cation influx (Stiber et al., 2008b). A recent study (Millay et al., 2009) also showed that overexpression of TRPC3 resulted in a phenotype of muscular dystrophy nearly identical to that observed in dystrophic animal models with abnormal DGC. Transgene-mediated inhibition of TRPC channels dramatically reduced the dystrophic phenotype in this animal model. These results suggest that Ca^{2+} entry through TRPC channels is sufficient to induce muscular dystrophy *in vivo*, and that TRPC channels are also promising therapeutic targets for muscular dysgenesis.

3.3 STIM1 and Orai1

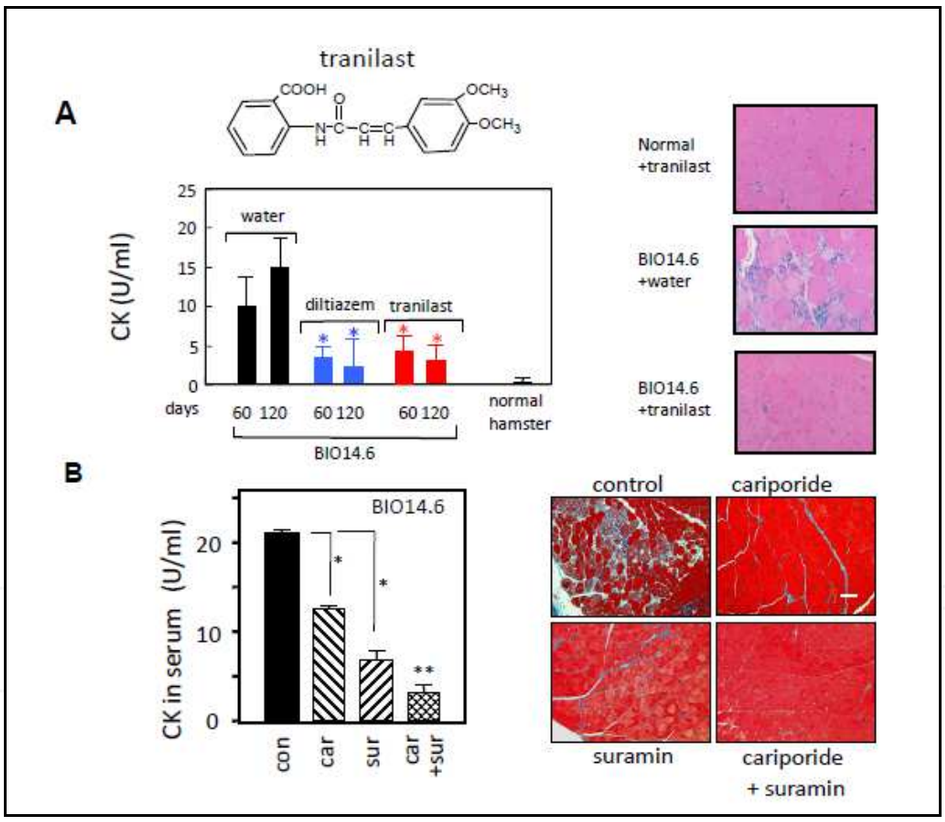
Recently, the molecules involved in store-operated Ca^{2+} entry (SOCE) were identified. Stromal interaction molecules (STIM) were identified as the endoplasmic reticulum (ER) Ca^{2+} sensor, and found to interact with sarcolemmal Orai1 channels after Ca^{2+} store depletion to trigger SOCE. STIM1 and Orai1 are highly expressed in skeletal muscle: STIM1 is pre-localized at junctions of the SR with the T-tubule system, which contains pre-localized Orai1 (Stiber et al., 2008a). STIM1/Orai1 couples with TRPC channels (Dirksen, 2009) and the resulting STIM1/Orai1/TRPC1 ternary complexes have been shown to assemble during store depletion, thereby contributing to SOCE (Yuan et al., 2007; Zeng et al., 2008). Recently, the amount of STIM1/Orai1 proteins was reported to be upregulated in *mdx* muscle fibers, and the thresholds for the activation and deactivation of SOCE shifted to higher SR Ca^{2+} concentrations. This contributes to increased Ca^{2+} influx during long stimulation periods in *mdx* muscles (Edwards et al., 2010). In contrast, knockdown or inhibition of STIM1/Orai1 function was reported to cause myopathy resulting from impaired muscle development, which is different to muscular dystrophy caused by DGC defects. For example, mice lacking either STIM1 or Orai1 display skeletal muscle myopathy (Stiber et al., 2008a), and severe combined immunodeficiency patients characterized by loss-of-function mutations in STIM1/Orai1 signaling display similar skeletal muscle myopathy (Feske et al., 2006). Knockdown of STIM1 or expression of the Orai1 dominant negative E106Q caused a marked decrease in SOCE in skeletal muscle myotubes (Lyfenko & Dirksen, 2008). These findings suggest that STIM1/Orai1 proteins are involved in the fine-tuning of Ca^{2+} regulation, and physiological levels are required for normal skeletal muscle function.

3.4 Na^+-dependent ion transporters

In addition to $[Ca^{2+}]_i$, $[Na^+]_i$ is also reported to be elevated in skeletal muscle of *mdx* mice (Dunn et al., 1993). The increase in $[Na^+]_i$ is accompanied by a compensatory increase in membrane-bound Na^+/K^+ ATPase contents (Dunn et al., 1995). A possible cause of increased $[Na^+]_i$ in the *mdx* skeletal muscle may be enhanced activity of stretch-activated channels, since it was inhibited by Gd^{3+} and streptomycin, which are known to be broad inhibitors of this channel type (Yeung et al., 2003a; Yeung et al., 2003b). It has also been

shown that increases in $[Na^+]_i$ under the sarcolemma in *mdx* mice may be due to alterations in localization and gating properties of Nav1.4; the skeletal muscle isoform of the voltage-gated sodium channel may be correlated with increased cell death because $[Na^+]_i$ overload is reversed by tetrodotoxin, a specific Nav1.4 blocker (Hirn et al., 2008).

Recently, we have shown that the sarcolemmal Na^+/H^+ exchanger (NHE), which is known to be stimulated in response to various stimuli, including growth factors and osmotic stress, is significantly activated in dystrophic myocytes (from BIO14.6 hamsters). This is evidenced by an alkaline shift in the intracellular pH (pH_i) dependent on NHE activity, enhanced $^{22}Na^+$ influx, and elevated $[Na^+]_i$ (Iwata et al., 2007). In dystrophic myotubes, NHE was found to be a major Na^+ influx pathway, since the specific NHE inhibitor cariporide markedly (65%) inhibited it. Interestingly, NHE inhibition also significantly reduced the increase in intracellular Ca^{2+} and stretch-induced CK release in dystrophic myotubes, and ameliorated myopathic damage *in vivo* (Fig.5 B) (Iwata et al., 2007), indicating that the inhibition of NHE protects muscle cells against injury. Elevation in $[Na^+]_i$ may contribute to abnormal Ca^{2+} homeostasis by influencing the activity of the Na^+/Ca^{2+} exchanger.



(A) Beneficial effect of the Ca^{2+}-handling drugs diltiazem and tranilast. Chemicals were administered orally to 30-day-old BIO14.6 hamsters for 60 or 120 days. The TRPV2 inhibitor tranilast effectively reduces CK release (left) and prevents muscle degeneration (H&E staining, right) (Iwata et al., 2005). (B) Beneficial effect of the P2 receptor antagonist suramin (sur) and the NHE inhibitor cariporide (car). In particular, note an excellent amelioration of muscle degeneration by combined administration of the two chemicals (Masson's trichrome staining of the quadriceps muscle sections, right) (Iwata et al., 2007). Scale bar, 100 μm.

Fig. 5. Effects of pharmacological agents on muscle degeneration in BIO14.6 hamsters

Furthermore, ATP was released more easily from the dystrophic myotubes in response to mechanical stretch. Thus, it is likely that P2 receptor stimulation with ATP activates the NHE, thereby leading to $[Ca^{2+}]_i$ overload (Iwata et al., 2007). These molecules also represent good targets for muscular dystrophy therapy. In fact, combined treatment with the P2 receptor antagonist suramin and the NHE1-specific inhibitor cariporide resulted in efficient amelioration of muscular dystrophy in BIO14.6 hamsters (Fig.5 B).

3.5 SR proteins

Recently, the occurrence of increased Ca^{2+} sparks, an indication of abnormalities in the SR Ca^{2+} release channel ryanodine receptor (RyR1), was reported in dystrophic fibers (Bellinger et al., 2009; Wang et al., 2005). RyR1 from *mdx* mice were shown to be excessively cystein-nitrosylated, which was coupled with depletion of calstabin-1 (calcium channel-stabilizing binding protein-1, also known as FKBP12) from RyR1. As a consequence, this led to increased spontaneous RyR1 openings (Ca^{2+} sparks), and reduced specific muscle force. Prevention of calstabin-1 depletion from RyR1 inhibited SR Ca^{2+} leak, reduced muscle damage, improved muscle function, and increased exercise performance in *mdx* mice (Bellinger et al., 2009).

One strategy to reduce the effects associated with chronic Ca^{2+} leak from activated channels and membrane rupture is to increase the rate of Ca^{2+} reuptake into the SR, by overexpression of the SR Ca^{2+} pump SERCA1. Indeed, transgenic overexpression of SERCA1 dramatically rescued the dystrophic phenotype of delta-sarcoglycan-null mice (Goonasekera et al., 2011). Furthermore, Ca^{2+} removal by adenovirus-mediated overexpression of SERCA1a reduced susceptibility to contraction-induced damage in *mdx* mice (Morine et al., 2010). These results suggest that Ca^{2+} is a common risk factor in the transmission of most genetic defects to downstream necrosis pathways in muscular dystrophy. Thus, control of $[Ca^{2+}]_i$ would provide a universal therapeutic strategy that reduces the dystrophic phenotype.

3.6 Other candidates

Dystrophic muscles are always exposed to oxidative and immune stress, as well as mechanical stress. Although not discussed in detail in this chapter, there are many important stress-dependent factors leading to muscle injury in muscular dystrophy (Lawler, 2011; Tidball & Wehling-Henricks, 2007). For example, NADPH oxidase in the muscles was recently reported to be an important source of oxidative stress, which stimulates stretch-activated Ca^{2+} entry in dystrophic muscles (Whitehead et al., 2010). Futhermore, nitric oxide (NO) synthase in macrophages may be another risk factor that promotes membrane injury in dystrophic muscles (Villalta et al., 2009). Therapeutic interventions that regulate stress pathways may be useful in protecting against dystrophic phenotypes.

4. Conclusion

As described above, disruption of the DGC results in increased mechanical stress and abnormal ion homeostasis. Sustained increases in $[Ca^{2+}]_i$ are the key pathological event leading to muscle degeneration (Fig.6). Many key players contribute to abnormal Ca^{2+} handling. Among these molecules, we consider TRPV2 to have high therapeutic potential for the treatment of muscular dystrophy, because most TRPV2 localizes to the intracellular

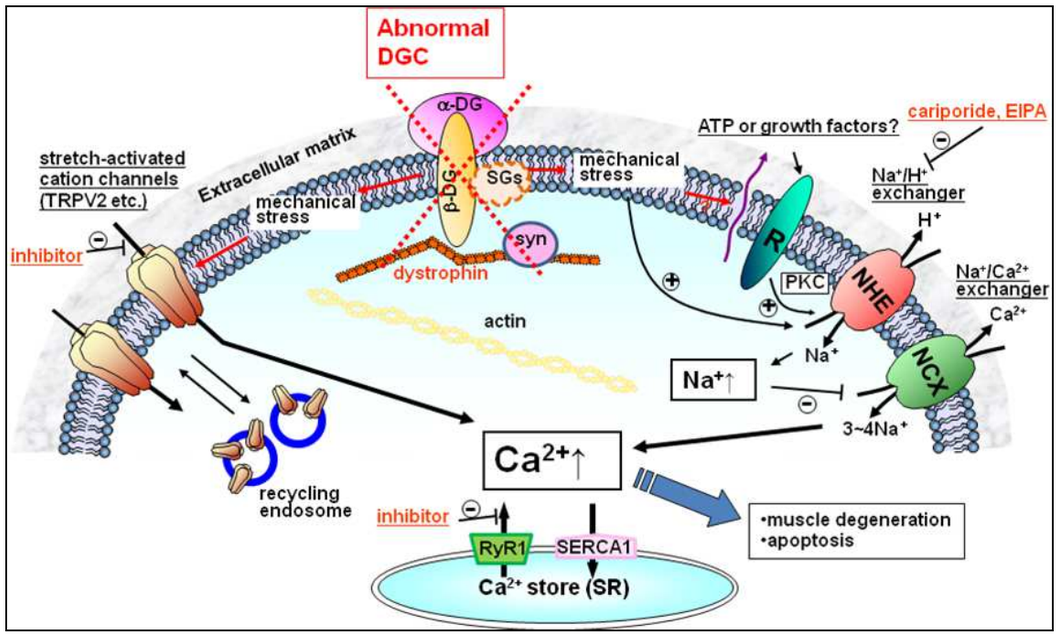


Fig. 6. Schematic drawing showing a possible pathway leading to abnormal Ca^{2+} handling and subsequent muscle degeneration in DGC-defective dystrophic muscles, according to our recent studies (Iwata et al., 2003; 2009; 2007; 2005) Increased mechanical stress caused by DGC defects induces sarcolemmal translocation and TRPV2 activation, leading to sustained $[Ca^{2+}]_i$ increase. On the other hand, mechanical stress also induces release of bioactive substances such as ATP and growth factors, which, in turn, activates NHE, increases $[Na^+]_i$, and results in further increase in $[Ca^{2+}]_i$ via inhibition of the NCX forward mode. Ca^{2+} handling proteins in the SR may also contribute to cytosolic Ca^{2+} overload. Therapeutic intervention at the level of Ca^{2+}-handling proteins would be useful for reducing the dystrophic phenotype.

membranes in the healthy skeletal muscles, while it translocates to the surface membrane upon muscle degeneration. Hence, specific inhibitors against TRPV2 are expected to act only on degenerative muscles. Given the lack of a definitive strategy to cure muscular dystrophy, identifying new therapeutic targets appears to be extremely important. We hope that this chapter might help to provide an opportunity to promote such studies.

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6. References

Alderton, J. M. & Steinhardt, R. A. 2000a. Calcium influx through calcium leak channels is responsible for the elevated levels of calcium-dependent proteolysis in dystrophic myotubes. *Journal of Biological Chemistry*, 275(13): 9452-9460.

Alderton, J. M. & Steinhardt, R. A. 2000b. How calcium influx through calcium leak channels is responsible for the elevated levels of calcium-dependent proteolysis in dystrophic myotubes. *Trends in Cardiovascular Medicine*, 10(6): 268-272.

Allen, D. G. & Whitehead, N. P. 2011. Duchenne muscular dystrophy--what causes the increased membrane permeability in skeletal muscle? *International Journal of Biochemistry and Cell Biology*, 43(3): 290-294.

Bellinger, A. M.; Reiken, S.; Carlson, C.; Mongillo, M.; Liu, X.; Rothman, L.; Matecki, S.; Lacampagne, A. & Marks, A. R. 2009. Hypernitrosylated ryanodine receptor calcium release channels are leaky in dystrophic muscle. *Nature Medicine*, 15(3): 325-330.

Boittin, F. X.; Petermann, O.; Hirn, C.; Mittaud, P.; Dorchies, O. M.; Roulet, E. & Ruegg, U. T. 2006. Ca^{2+}-independent phospholipase A2 enhances store-operated Ca^{2+} entry in dystrophic skeletal muscle fibers. *Journal of Cell Science*, 119(Pt 18): 3733-3742.

Brown, R. H., Jr. 1997. Dystrophin-associated proteins and the muscular dystrophies. *Annual Review of Medicine*, 48: 457-466.

Campbell, K. P. 1995. Three muscular dystrophies: loss of cytoskeleton-extracellular matrix linkage. *Cell*, 80(5): 675-679.

Campbell, K. P. & Kahl, S. D. 1989. Association of dystrophin and an integral membrane glycoprotein. *Nature*, 338(6212): 259-262.

Deng, H. X.; Klein, C. J.; Yan, J.; Shi, Y.; Wu, Y.; Fecto, F.; Yau, H. J.; Yang, Y.; Zhai, H.; Siddique, N.; Hedley-Whyte, E. T.; Delong, R.; Martina, M.; Dyck, P. J. & Siddique, T. 2010. Scapuloperoneal spinal muscular atrophy and CMT2C are allelic disorders caused by alterations in TRPV4. *Nature Genetics*, 42(2): 165-169.

Dirksen, R. T. 2009. Checking your SOCCs and feet: the molecular mechanisms of Ca^{2+} entry in skeletal muscle. *J Physiol*, 587(Pt 13): 3139-3147.

Duclos, F.; Straub, V.; Moore, S. A.; Venzke, D. P.; Hrstka, R. F.; Crosbie, R. H.; Durbeej, M.; Lebakken, C. S.; Ettinger, A. J.; van der Meulen, J.; Holt, K. H.; Lim, L. E.; Sanes, J. R.; Davidson, B. L.; Faulkner, J. A.; Williamson, R. & Campbell, K. P. 1998. Progressive muscular dystrophy in alpha-sarcoglycan-deficient mice. *Journal of Cell Biology*, 142(6): 1461-1471.

Ducret, T.; Vandebrouck, C.; Cao, M. L.; Lebacq, J. & Gailly, P. 2006. Functional role of store-operated and stretch-activated channels in murine adult skeletal muscle fibres. *J Physiol*, 575(Pt 3): 913-924.

Dunn, J. F.; Bannister, N.; Kemp, G. J. & Publicover, S. J. 1993. Sodium is elevated in mdx muscles: ionic interactions in dystrophic cells. *Journal of the Neurological Sciences*, 114(1): 76-80.

Dunn, J. F.; Burton, K. A. & Dauncey, M. J. 1995. Ouabain sensitive Na^+/K^+-ATPase content is elevated in mdx mice: implications for the regulation of ions in dystrophic muscle. *Journal of the Neurological Sciences*, 133(1-2): 11-15.

Edwards, J. N.; Friedrich, O.; Cully, T. R.; von Wegner, F.; Murphy, R. M. & Launikonis, B. S. 2010. Upregulation of store-operated Ca^{2+} entry in dystrophic mdx mouse muscle. *Am J Physiol Cell Physiol*, 299(1): C42-50.

Ervasti, J. M. & Campbell, K. P. 1993. A role for the dystrophin-glycoprotein complex as a transmembrane linker between laminin and actin. *Journal of Cell Biology*, 122(4): 809-823.

Feske, S.; Gwack, Y.; Prakriya, M.; Srikanth, S.; Puppel, S. H.; Tanasa, B.; Hogan, P. G.; Lewis, R. S.; Daly, M. & Rao, A. 2006. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature*, 441(7090): 179-185.

Fong, P. Y.; Turner, P. R.; Denetclaw, W. F. & Steinhardt, R. A. 1990. Increased activity of calcium leak channels in myotubes of Duchenne human and mdx mouse origin. *Science*, 250(4981): 673-676.

Franco-Obregon, A., Jr. & Lansman, J. B. 1994. Mechanosensitive ion channels in skeletal muscle from normal and dystrophic mice. *J Physiol*, 481 (Pt 2): 299-309.

Franco-Obregon, A. & Lansman, J. B. 2002. Changes in mechanosensitive channel gating following mechanical stimulation in skeletal muscle myotubes from the mdx mouse. *J Physiol*, 539(Pt 2): 391-407.

Franco, A., Jr. & Lansman, J. B. 1990. Calcium entry through stretch-inactivated ion channels in mdx myotubes. *Nature*, 344(6267): 670-673.

Gervasio, O. L.; Whitehead, N. P.; Yeung, E. W.; Phillips, W. D. & Allen, D. G. 2008. TRPC1 binds to caveolin-3 and is regulated by Src kinase - role in Duchenne muscular dystrophy. *Journal of Cell Science*, 121(Pt 13): 2246-2255.

Goonasekera, S. A.; Lam, C. K.; Millay, D. P.; Sargent, M. A.; Hajjar, R. J.; Kranias, E. G. & Molkentin, J. D. 2011. Mitigation of muscular dystrophy in mice by SERCA overexpression in skeletal muscle. *Journal of Clinical Investigation*, 121(3): 1044-1052.

Gottlieb, P.; Folgering, J.; Maroto, R.; Raso, A.; Wood, T. G.; Kurosky, A.; Bowman, C.; Bichet, D.; Patel, A.; Sachs, F.; Martinac, B.; Hamill, O. P. & Honore, E. 2008. Revisiting TRPC1 and TRPC6 mechanosensitivity. *Pflugers Archiv. European Journal of Physiology*, 455(6): 1097-1103.

Hamill, O. P. & McBride, D. W., Jr. 1996. The pharmacology of mechanogated membrane ion channels. *Pharmacological Reviews*, 48(2): 231-252.

Hirn, C.; Shapovalov, G.; Petermann, O.; Roulet, E. & Ruegg, U. T. 2008. Nav1.4 deregulation in dystrophic skeletal muscle leads to Na^+ overload and enhanced cell death. *Journal of General Physiology*, 132(2): 199-208.

Hopf, F. W.; Turner, P. R.; Denetclaw, W. F., Jr.; Reddy, P. & Steinhardt, R. A. 1996. A critical evaluation of resting intracellular free calcium regulation in dystrophic mdx muscle. *American Journal of Physiology*, 271(4 Pt 1): C1325-1339.

Iwata, Y.; Katanosaka, Y.; Arai, Y.; Komamura, K.; Miyatake, K. & Shigekawa, M. 2003. A novel mechanism of myocyte degeneration involving the Ca^{2+}-permeable growth factor-regulated channel. *Journal of Cell Biology*, 161(5): 957-967.

Iwata, Y.; Katanosaka, Y.; Arai, Y.; Shigekawa, M. & Wakabayashi, S. 2009. Dominant-negative inhibition of Ca^{2+} influx via TRPV2 ameliorates muscular dystrophy in animal models. *Human Molecular Genetics*, 18(5): 824-834.

Iwata, Y.; Katanosaka, Y.; Hisamitsu, T. & Wakabayashi, S. 2007. Enhanced Na^+/H^+ Exchange Activity Contributes to the Pathogenesis of Muscular Dystrophy via Involvement of P2 Receptors. *American Journal of Pathology*, 171(5):1576-1587.

Iwata, Y.; Katanosaka, Y.; Shijun, Z.; Kobayashi, Y.; Hanada, H.; Shigekawa, M. & Wakabayashi, S. 2005. Protective effects of Ca^{2+} handling drugs against abnormal Ca^{2+} homeostasis and cell damage in myopathic skeletal muscle cells. *Biochemical Pharmacology*, 70(5): 740-751.

Iwata, Y.; Nakamura, H.; Fujiwara, K. & Shigekawa, M. 1993a. Altered membrane-dystrophin association in the cardiomyopathic hamster heart muscle. *Biochemical and Biophysical Research Communications*, 190(2): 589-595.

Iwata, Y.; Nakamura, H.; Mizuno, Y.; Yoshida, M.; Ozawa, E. & Shigekawa, M. 1993b. Defective association of dystrophin with sarcolemmal glycoproteins in the cardiomyopathic hamster heart. *FEBS Letters*, 329(1-2): 227-231.

Kanzaki, M.; Zhang, Y. Q.; Mashima, H.; Li, L.; Shibata, H. & Kojima, I. 1999. Translocation of a calcium-permeable cation channel induced by insulin-like growth factor-I. *Nat Cell Biol*, 1(3): 165-170.

Landoure, G.; Zdebik, A. A.; Martinez, T. L.; Burnett, B. G.; Stanescu, H. C.; Inada, H.; Shi, Y.; Taye, A. A.; Kong, L.; Munns, C. H.; Choo, S. S.; Phelps, C. B.; Paudel, R.; Houlden, H.; Ludlow, C. L.; Caterina, M. J.; Gaudet, R.; Kleta, R.; Fischbeck, K. H. & Sumner, C. J. 2010. Mutations in TRPV4 cause Charcot-Marie-Tooth disease type 2C. *Nature Genetics*, 42(2): 170-174.

Lawler, J. M. 2011. Exacerbation of pathology by oxidative stress in respiratory and locomotor muscles with Duchenne muscular dystrophy. *J Physiol*, 589(Pt 9): 2161-2170.

Lyfenko, A. D. & Dirksen, R. T. 2008. Differential dependence of store-operated and excitation-coupled Ca^{2+} entry in skeletal muscle on STIM1 and Orai1. *J Physiol*, 586(Pt 20): 4815-4824.

MacLennan, P. A.; McArdle, A. & Edwards, R. H. 1991. Effects of calcium on protein turnover of incubated muscles from mdx mice. *American Journal of Physiology*, 260(4 Pt 1): E594-598.

Mallouk, N.; Jacquemond, V. & Allard, B. 2000. Elevated subsarcolemmal Ca^{2+} in mdx mouse skeletal muscle fibers detected with Ca^{2+}-activated K^+ channels. *Proceedings of the National Academy of Sciences of the United States of America*, 97(9): 4950-4955.

Maroto, R.; Raso, A.; Wood, T. G.; Kurosky, A.; Martinac, B. & Hamill, O. P. 2005. TRPC1 forms the stretch-activated cation channel in vertebrate cells. *Nat Cell Biol*, 7(2): 179-185.

Menke, A. & Jockusch, H. 1991. Decreased osmotic stability of dystrophin-less muscle cells from the *mdx* mouse. *Nature*, 349(6304): 69-71.

Millay, D. P.; Goonasekera, S. A.; Sargent, M. A.; Maillet, M.; Aronow, B. J. & Molkentin, J. D. 2009. Calcium influx is sufficient to induce muscular dystrophy through a TRPC-dependent mechanism. *Proceedings of the National Academy of Sciences of the United States of America*, 106(45): 19023-19028.

Montell, C.; Birnbaumer, L. & Flockerzi, V. 2002. The TRP channels, a remarkably functional family. *Cell*, 108(5): 595-598.

Morine, K. J.; Sleeper, M. M.; Barton, E. R. & Sweeney, H. L. 2010. Overexpression of SERCA1a in the *mdx* diaphragm reduces susceptibility to contraction-induced damage. *Human Gene Therapy*, 21(12): 1735-1739.

Muraki, K.; Iwata, Y.; Katanosaka, Y.; Ito, T.; Ohya, S.; Shigekawa, M. & Imaizumi, Y. 2003. TRPV2 is a component of osmotically sensitive cation channels in murine aortic myocytes. *Circulation Research*, 93(9): 829-838.

Nakamura, T. Y.; Iwata, Y.; Sampaolesi, M.; Hanada, H.; Saito, N.; Artman, M.; Coetzee, W. A. & Shigekawa, M. 2001. Stretch-activated cation channels in skeletal muscle

myotubes from sarcoglycan-deficient hamsters. *Am J Physiol Cell Physiol*, 281(2): C690-699.

Nigro, V.; Okazaki, Y.; Belsito, A.; Piluso, G.; Matsuda, Y.; Politano, L.; Nigro, G.; Ventura, C.; Abbondanza, C.; Molinari, A. M.; Acampora, D.; Nishimura, M.; Hayashizaki, Y. & Puca, G. A. 1997. Identification of the Syrian hamster cardiomyopathy gene. *Human Molecular Genetics*, 6(4): 601-607.

Petrof, B. J.; Shrager, J. B.; Stedman, H. H.; Kelly, A. M. & Sweeney, H. L. 1993. Dystrophin protects the sarcolemma from stresses developed during muscle contraction. *Proceedings of the National Academy of Sciences of the United States of America*, 90(8): 3710-3714.

Robert, V.; Massimino, M. L.; Tosello, V.; Marsault, R.; Cantini, M.; Sorrentino, V. & Pozzan, T. 2001. Alteration in calcium handling at the subcellular level in *mdx* myotubes. *Journal of Biological Chemistry*, 276(7): 4647-4651.

Spencer, M. J.; Croall, D. E. & Tidball, J. G. 1995. Calpains are activated in necrotic fibers from *mdx* dystrophic mice. *Journal of Biological Chemistry*, 270(18): 10909-10914.

Stiber, J.; Hawkins, A.; Zhang, Z. S.; Wang, S.; Burch, J.; Graham, V.; Ward, C. C.; Seth, M.; Finch, E.; Malouf, N.; Williams, R. S.; Eu, J. P. & Rosenberg, P. 2008a. STIM1 signalling controls store-operated calcium entry required for development and contractile function in skeletal muscle. *Nat Cell Biol*, 10(6): 688-697.

Stiber, J. A.; Zhang, Z. S.; Burch, J.; Eu, J. P.; Zhang, S.; Truskey, G. A.; Seth, M.; Yamaguchi, N.; Meissner, G.; Shah, R.; Worley, P. F.; Williams, R. S. & Rosenberg, P. B. 2008b. Mice lacking Homer 1 exhibit a skeletal myopathy characterized by abnormal transient receptor potential channel activity. *Molecular and Cellular Biology*, 28(8): 2637-2647.

Suchyna, T. M.; Johnson, J. H.; Hamer, K.; Leykam, J. F.; Gage, D. A.; Clemo, H. F.; Baumgarten, C. M. & Sachs, F. 2000. Identification of a peptide toxin from Grammostola spatulata spider venom that blocks cation-selective stretch-activated channels. *Journal of General Physiology*, 115(5): 583-598.

Tawada-Iwata, Y.; Imagawa, T.; Yoshida, A.; Takahashi, M.; Nakamura, H. & Shigekawa, M. 1993. Increased mechanical extraction of T-tubule/junctional SR from cardiomyopathic hamster heart. *American Journal of Physiology*, 264(5 Pt 2): H1447-1453.

Tidball, J. G. & Wehling-Henricks, M. 2007. The role of free radicals in the pathophysiology of muscular dystrophy. *Journal of Applied Physiology*, 102(4): 1677-1686.

Tinsley, J. M.; Blake, D. J.; Zuellig, R. A. & Davies, K. E. 1994. Increasing complexity of the dystrophin-associated protein complex. *Proceedings of the National Academy of Sciences of the United States of America*, 91(18): 8307-8313.

Turner, P. R.; Schultz, R.; Ganguly, B. & Steinhardt, R. A. 1993. Proteolysis results in altered leak channel kinetics and elevated free calcium in *mdx* muscle. *Journal of Membrane Biology*, 133(3): 243-251.

Turner, P. R.; Westwood, T.; Regen, C. M. & Steinhardt, R. A. 1988. Increased protein degradation results from elevated free calcium levels found in muscle from *mdx* mice. *Nature*, 335(6192): 735-738.

Vandebrouck, C.; Duport, G.; Cognard, C. & Raymond, G. 2001. Cationic channels in normal and dystrophic human myotubes. *Neuromuscular Disorders*, 11(1): 72-79.

Vandebrouck, C.; Duport, G.; Raymond, G. & Cognard, C. 2002a. Hypotonic medium increases calcium permeant channels activity in human normal and dystrophic myotubes. *Neuroscience Letters*, 323(3): 239-243.

Vandebrouck, C.; Martin, D.; Colson-Van Schoor, M.; Debaix, H. & Gailly, P. 2002b. Involvement of TRPC in the abnormal calcium influx observed in dystrophic (*mdx*) mouse skeletal muscle fibers. *Journal of Cell Biology*, 158(6): 1089-1096.

Villalta, S. A.; Nguyen, H. X.; Deng, B.; Gotoh, T. & Tidball, J. G. 2009. Shifts in macrophage phenotypes and macrophage competition for arginine metabolism affect the severity of muscle pathology in muscular dystrophy. *Human Molecular Genetics*, 18(3): 482-496.

Wang, X.; Weisleder, N.; Collet, C.; Zhou, J.; Chu, Y.; Hirata, Y.; Zhao, X.; Pan, Z.; Brotto, M.; Cheng, H. & Ma, J. 2005. Uncontrolled calcium sparks act as a dystrophic signal for mammalian skeletal muscle. *Nat Cell Biol*, 7(5): 525-530.

Whitehead, N. P.; Yeung, E. W.; Froehner, S. C. & Allen, D. G. 2010. Skeletal muscle NADPH oxidase is increased and triggers stretch-induced damage in the *mdx* mouse. *PLoS One*, 5(12): e15354.

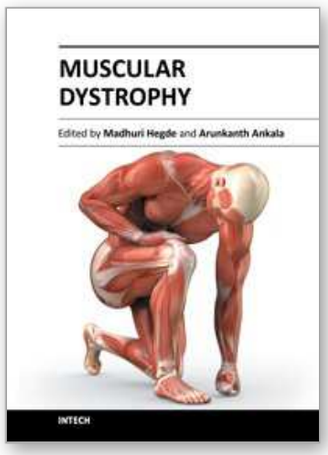
Yeung, E. W.; Ballard, H. J.; Bourreau, J. P. & Allen, D. G. 2003a. Intracellular sodium in mammalian muscle fibers after eccentric contractions. *Journal of Applied Physiology*, 94(6): 2475-2482.

Yeung, E. W.; Head, S. I. & Allen, D. G. 2003b. Gadolinium reduces short-term stretch-induced muscle damage in isolated *mdx* mouse muscle fibres. *J Physiol*, 552(Pt 2): 449-458.

Yuan, J. P.; Zeng, W.; Huang, G. N.; Worley, P. F. & Muallem, S. 2007. STIM1 heteromultimerizes TRPC channels to determine their function as store-operated channels. *Nat Cell Biol*, 9(6): 636-645.

Zanou, N.; Iwata, Y.; Schakman, O.; Lebacq, J.; Wakabayashi, S. & Gailly, P. 2009. Essential role of TRPV2 ion channel in the sensitivity of dystrophic muscle to eccentric contractions. *FEBS Letters*, 583(22): 3600-3604.

Zeng, W.; Yuan, J. P.; Kim, M. S.; Choi, Y. J.; Huang, G. N.; Worley, P. F. & Muallem, S. 2008. STIM1 gates TRPC channels, but not Orai1, by electrostatic interaction. *Molecular Cell*, 32(3): 439-448.



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With more than 30 different types and subtypes known and many more yet to be classified and characterized, muscular dystrophy is a highly heterogeneous group of inherited neuromuscular disorders. This book provides a comprehensive overview of the various types of muscular dystrophies, genes associated with each subtype, disease diagnosis, management as well as available treatment options. Though each different type and subtype of muscular dystrophy is associated with a different causative gene, the majority of them have overlapping clinical presentations, making molecular diagnosis inevitable for both disease diagnosis as well as patient management. This book discusses the currently available diagnostic approaches that have revolutionized clinical research. Pathophysiology of the different muscular dystrophies, multifaceted functions of the involved genes as well as efforts towards diagnosis and effective patient management, are also discussed. Adding value to the book are the included reports on ongoing studies that show a promise for future therapeutic strategies.

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