

RESEARCH ARTICLE

Myonuclear Degeneration in *LMNA* Null MiceMichel Mittelbronn^{1,2}; Teresa Sullivan³; Colin L. Stewart³; Antje Bornemann²¹ Institute of Neuropathology, University Hospital of Zürich, Switzerland.² Institute of Brain Research, University of Tübingen, Tübingen, Germany.³ Cancer and Developmental Biology Laboratory, National Cancer Institute-Frederick Cancer Research and Development Center, Md.**Keywords**

Electron microscopy, myotendinous junction, nucleocytoplasmic integrity.

Corresponding author:

Antje Bornemann, MD, Institute of Brain Research, University Tübingen, Calwerstr. 3, D-72076 Tübingen, Germany (E-mail: antje.bornemann@med.uni-tuebingen.de)

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Abstract

Lamins A/C, the major constituent of the nuclear lamina, confer mechanical stability to nuclei. We examined the myonuclei of *LMNA* null mice at the myotendinous junctions (MTJ), the site of longitudinal force transmission from contractile proteins to extracellular proteins. The right soleus and rectus femoris muscles of five null mutants aged 5–7 weeks and two wild-type animals aged 5 weeks and 6 months were examined by electron microscopy. The myofibers merging into the tendons were assessed for nuclear disintegration and cytoplasmic degeneration. The myofibers of the wild-type rectus femoris and soleus muscles revealed 19–27 nuclei/50 myofibers and 5–8/20, respectively, with no signs of degeneration. The rectus femoris muscle fibers of the null mice contained 75–117 myonuclei/50 myofibers, the soleus muscle, 13–36 nuclei/20 myofibers. Eleven to twenty-one per 50 myonuclei of the rectus femoris myonuclei showed chromatin clumping, nuclear fragmentation, nuclear inclusions and invaginations, and intranuclear filaments. The values were 12–19/50 for the soleus myonuclei. Moreover, 5–12/50 rectus femoris myofibers and 5–14/20, of the soleus myofibers showed cytoplasmic degeneration. None of these changes was found distal to the MTJ. These results favor the notion that myonuclei lacking a functional lamina are susceptible to mechanical stress *in vivo*. These alterations may contribute to the development of early joint contractures, a feature of ADEDMD.

INTRODUCTION

The autosomal dominant form of Emery-Dreifuss muscular dystrophy (ADEDMD) is caused by mutations in the *LMNA* gene. The clinical phenotype is characterized by contractures at the elbows, Achilles' tendons, and spine and progressive muscle wasting in a humero-peroneal distribution (5). In contrast with muscular dystrophies such as dystrophinopathies and dysferlinopathies, the contractures appear early in the disease. The pathogenetic mechanism(s) underlying this process is not clear.

The *LMNA* gene that is mutated in ADEDMD encodes for lamins A and C, the A-type lamins which are generated from the same gene by alternate splicing, and which are major constituents of the nuclear lamina (3, 4). The proteins contain chromatin binding sites and also bind to transcription factors and to RNA processing enzymes. This has led to the hypothesis that lamins A/C have a role in organizing chromatin, regulating transcription, and controlling differentiation (3, 4).

Lamins A/C are also attached to the inner nuclear membrane through interactions with integral membrane proteins. This has led to the additional hypothesis that *LMNA* mutations result in fragile and mechanically unstable nuclei (3, 4). In effect, lamins A/C are essential for the maintenance of normal nuclear architecture: More than 80% of the embryonic fibroblast nuclei from *LMNA* null mice revealed herniation of the nuclear membranes (12). When

mechanical strain was placed on nuclei of embryonic fibroblasts *in vitro*, *LMNA*^{-/-} nuclei showed significantly larger deformations than wild-type cells (7). In a different approach, in which single mouse embryonic fibroblast nuclei were compressed *in vitro*, most ^{-/-} nuclei became damaged, resulting in strands of DNA protruding into the cytoplasm, while the majority of ^{+/+} nuclei remained largely intact (1). Taken together, there is compelling *in vitro* evidence for the A-type lamins having a role in the mechanical stabilization of nuclei.

Data from *in vivo* studies are not as straightforward as *in vitro*. Muscle biopsies from patients with genetically confirmed ADEDMD showed ultrastructural myonuclear alterations. Two out of three muscle biopsies showed clumping of heterochromatin and detachment of chromatin from the nuclear membrane (11). Reichart *et al* found nuclear membrane invagination in 10% and peripheral chromatin condensation in 20% of the myonuclei studied (9). Myonuclei of three biopsies from genetically confirmed ADEDMD cases revealed changes in chromatin distribution as well as pseudoinclusions. The percentage of affected myonuclei was not specified (6). Sabatelli *et al* studied the biopsies from three patients with genetically confirmed ADEDMD and found reduced peripheral heterochromatin, condensed clumps detached from the inner nuclear membrane, and sharp borders between residual heterochromatic areas and interchromatin in 10% of the myofibers (10).

Table 1. Numbers of altered myonuclei and myofibers.

Age	Mutation	Muscle	Myonuclei/fibers merging into the tendon	Myonuclei disintegrating*	Myofibers degenerating†
5 weeks	<i>LMNA</i> ^{-/-}	Rectus femoris‡	75/50	17/50	12/50
		Soleus§	28/20	18/50	10/20
7 weeks	<i>LMNA</i> ^{-/-}	Rectus femoris	91/50	15/50	10/50
		Soleus	13/20	13/50	7/20
7 weeks	<i>LMNA</i> ^{-/-}	Rectus femoris	117/50	21/50	11/50
		Soleus	36/20	12/50	14/20
7 weeks	<i>LMNA</i> ^{-/-}	Rectus femoris	82/50	21/50	13/50
		Soleus	31/20	19/50	5/20
7 weeks	<i>LMNA</i> ^{-/-}	Rectus femoris	79/50	12/50	5/50
		Soleus	22/20	13/50	10/20
5 weeks	<i>LMNA</i> ^{+/+}	Rectus femoris	27/50	0/50	0¶
		Soleus	8/20	0/50	0¶
6 months	<i>LMNA</i> ^{+/+}	Rectus femoris	19/50	0/50	0¶
		Soleus	5/20	0/50	0¶

*Number of disintegrating myonuclei per 50 nuclei counted. †Number of degenerating myofibers per 50 (rectus femoris muscle) and 20 (soleus muscle) fibers counted, respectively. ‡Of a total of 50 fibers per muscle. §Of a total of 20 fibers per muscle. ¶Number of degenerating myofibers across the whole section.

The *in vivo* mechanism by which *LMNA* mutations contribute to these myonuclear alterations is at present unknown. It is in particular open to discussion if and in which way the observed ultrastructural nuclear changes are related to mechanical stress. In the present study, we examined the myonuclear and cytoplasmic ultrastructure at the MTJs of *LMNA*^{-/-} null mice as the MTJs are the site of longitudinal force transmission from contractile proteins to extracellular proteins. The MTJ is particularly vulnerable to damage (13). Susceptibility of myonuclei to mechanical stress *in vivo* is expected to be revealed at this site.

MATERIAL AND METHODS

Animals

The derivation of *LMNA*^{-/-} mice has been previously described (12). Five knockout animals ranging in age from 5–7 weeks were used as well as two wild-type animals aged 5 weeks and 6 months (Table 1). The animals were killed by CO₂ asphyxiation and skinned. They were then immersion-fixed in 2% glutaraldehyde and 4% freshly prepared paraformaldehyde (PFA) (the 5-week-old null mutant and its wild-type litter mate) or 0.5% glutaraldehyde and 3% PFA (the 7-week-old null mice and the 6-month-old wild-type animal) in 0.1M cacodylate buffered at pH 7.2.

Electron microscopy

The right soleus and rectus femoris muscles were excised from the fixed animals and prepared for electron microscopy. The myonuclei of 50 myofibers merging into the tendon of the rectus femoris and 20 myofibers of the soleus muscle merging into the Achilles tendon were counted at the ultrastructural level in order to assess the ratio of myonuclei per myofiber at these sites. Furthermore, myonuclei undergoing disintegration were assessed in a total of 50 myonuclei of every muscle studied. Finally, the number of myofibers showing

signs of degeneration was assessed from 50 (rectus femoris muscle) or 20 fibers (soleus muscle) merging into the respective tendons.

RESULTS

Myonuclear degeneration

The number of myonuclei in myofibers merging into the tendon was elevated in knockout mice when compared with those in the wild-type animals (Figure 1; Table 1). Many of the nuclei were internalized. The numbers of myonuclei of the rectus femoris muscles ranged from 75/50 to 117/50, whereas they ranged from 13/20 to 36/20 in soleus muscles. The wild-type mice showed 27/50 and 19/50 myonuclei in the rectus femoris muscle and 8/20 and 5/20 myonuclei in the soleus muscle.

Many myonuclei of the *LMNA* null rectus femoris and soleus muscles showed signs of degeneration (Figure 2; Table 1). These consisted of chromatin clumping (Figure 2B–F), nuclear disintegration (Figure 2D–F), intranuclear inclusions or invaginations (Figure 2D,G–I), and/or intranuclear filaments measuring 10–12 nm in diameter (Figure 2J). Chromatin clumping occasionally occurred in conjunction with the perinuclear arrangement of felt-like filaments (Figure 2B) or perinuclear concentric tubules (Figure 2C).

Myofiber degeneration

A number of myofibers facing the tendon showed one of two types of pathological changes: “Splitting” (Figure 3A,B) or sub-sarcolemmal absence of myofibrils (Figure 4). Both patterns were considered to be signs of degeneration. The number of myofibers showing degeneration is indicated in Table 1. “Splitting” was accompanied by delicate connective tissue strands extending between the myofiber fragments (Figure 3A,B). This created

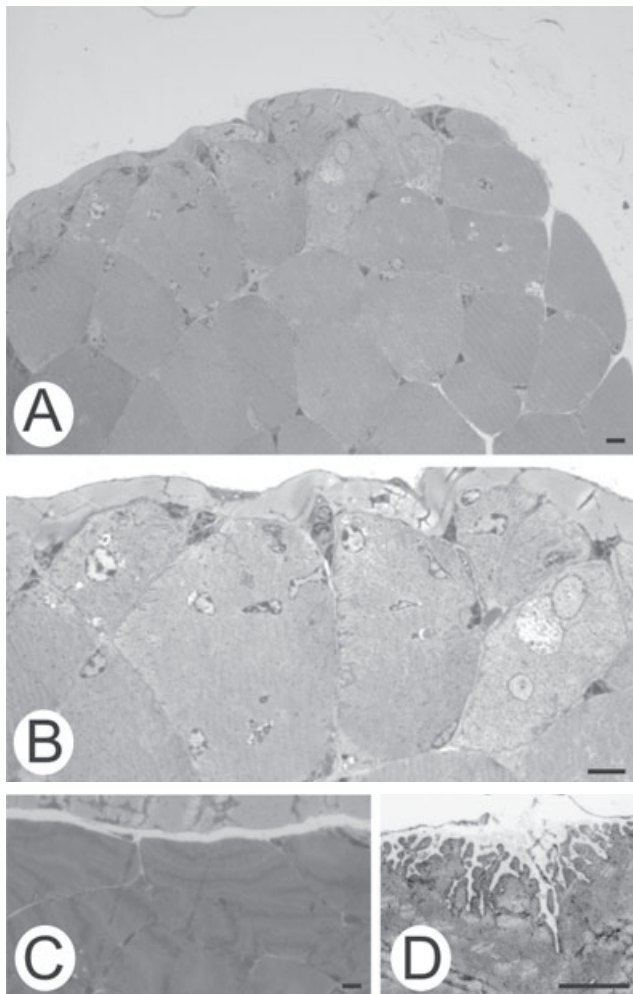


Figure 1. Myofibers of the rectus femoris muscle facing the tendon. **A, B.** Null mouse. **A.** The number of myonuclei per myofiber is increased in muscle adjacent to tendon. **B.** Higher magnification. **C, D.** Wild-type mouse. **C.** This was not seen in the wild-type mouse. **D.** The electron micrograph shows the junctional folds of a myofiber merging with the tendon. Bars, 10 μ m.

cytoplasmic surfaces that were smooth (Figure 3B). This pattern differs from myofibers of normal mice that form junctional foldings at the tendon interface (Figure 3C).

The second pattern of myofiber degeneration consisted of a loss of subsarcolemmal myofibrils from the surface directed toward the tendon (Figure 4). Myonuclei were frequently found in this myofibril-free space (Figure 4A,B). These myonuclei often showed one of the degenerative features shown in Figure 2 (Figure 4B). The myofibril-free space also contained sarcotubular profiles, honeycomb structures and polysomes (Figure 4C). None of these patterns was found distal to the MTJ.

DISCUSSION

We have demonstrated that *LMNA* null mice have both myonuclear disintegration and cytoplasmic degeneration at the MTJ. The MTJ

is the site where force is transmitted from the myofibers to the tendon. Moreover, MTJs are affected in the Emery–Dreifuss phenotype of muscular dystrophy, resulting in joint contractures early in the course of the disease (5).

The null mice showed increased myonuclear numbers in the myofibers facing the tendons when compared with the wild-type mice (Figure 1; Table 1). Elevated myonuclear numbers are not a constant finding at the MTJ. In a detailed *in vivo* study of mouse extensor digitorum longus muscle, Bruusgaard *et al* found that five out of 19 MTJ showed a nucleic density that was 80%–100% higher when compared with the length of the myofibers (2). The numbers of myonuclei found in myofibers facing the tendon ranged from 75 to 117 per 50 myofibers of the rectus femoris, and from 13 to 36 per 20 myofibers of the soleus muscle (Table 1). The values for the wild-type mice were 27/50, 19/50, 8/20 and 5/20, respectively. This finding suggests that the number of myonuclei at the MTJ is increased in *LMNA*^{-/-} mice when compared with normal animals. The reason for this is not clear. Myonuclei of healthy mice are immobile within the myofiber over periods of up to 4 days (2). It is suggested that myonuclei of *LMNA*^{-/-} mice do in fact move, at least in the myotendinous region.

Many of the myonuclei of the MTJ showed signs of disintegration (Figure 2). Several of the features demonstrated here are similar to those shown in human biopsies from patients with genetically confirmed ADEDMD. Clumping of heterochromatin (Figure 2B–F) (9–11), detachment of chromatin from the nuclear membrane (Figure 2F) (11), pseudoinclusions (Figure 2D,G,H) (6) are features that are shared by human biopsies and *LMNA*^{-/-} mice. Nuclear fragmentation, intranuclear filaments and intranuclear tubular structures illustrated in Figure 2I and J have so far not been reported in human biopsies of ADEDMD. Another feature hitherto unreported in human biopsies was perinuclear felt-like filaments and concentric tubules as shown in Figure 2B and C. It is not clear whether these surrounding structures are cytoplasmic in origin, or whether they are derived from nuclear envelope structures.

We recently observed myofiber “splitting” as shown in Figure 3 in 0.5%–7% of myofibers in biopsy and autopsy tissue from three patients with ADEDMD (8). The percentages found in the human tissue were below most of the percentages detected in the *LMNA*^{-/-} mice (Table 1). The paucity of affected fibers in human material might be explained by the distance of these sites from the MTJ of the respective muscles as diagnostic muscle biopsies are usually taken from the belly of the muscle rather than from the ends. The human condition of ADEDMD being heterozygous may also add to the milder phenotype.

The MTJ are specialized junctions for force transmission from contractile, cytoskeletal proteins to extracellular, structural proteins (13). Cytoplasmic “splitting” accompanies loss of the junctional folding (Figure 3B,C). Membrane folding has been identified as a mechanically important specialization of the MTJ because it reduces contractile stress generated by the myofiber by increasing its distribution over a larger junctional area. Loss of the junctional folding is associated with an increase in MTJ stress during loading (13). Hence, the accompanying cytoplasmic “splitting” and the blunting of the foldings may increase the mechanical stress at the MTJ, thereby aggravating the course of the disease.

In conclusion, we demonstrate myonuclear disintegration at the MTJ in *LMNA*^{-/-} mice. This may be well be interpreted as an

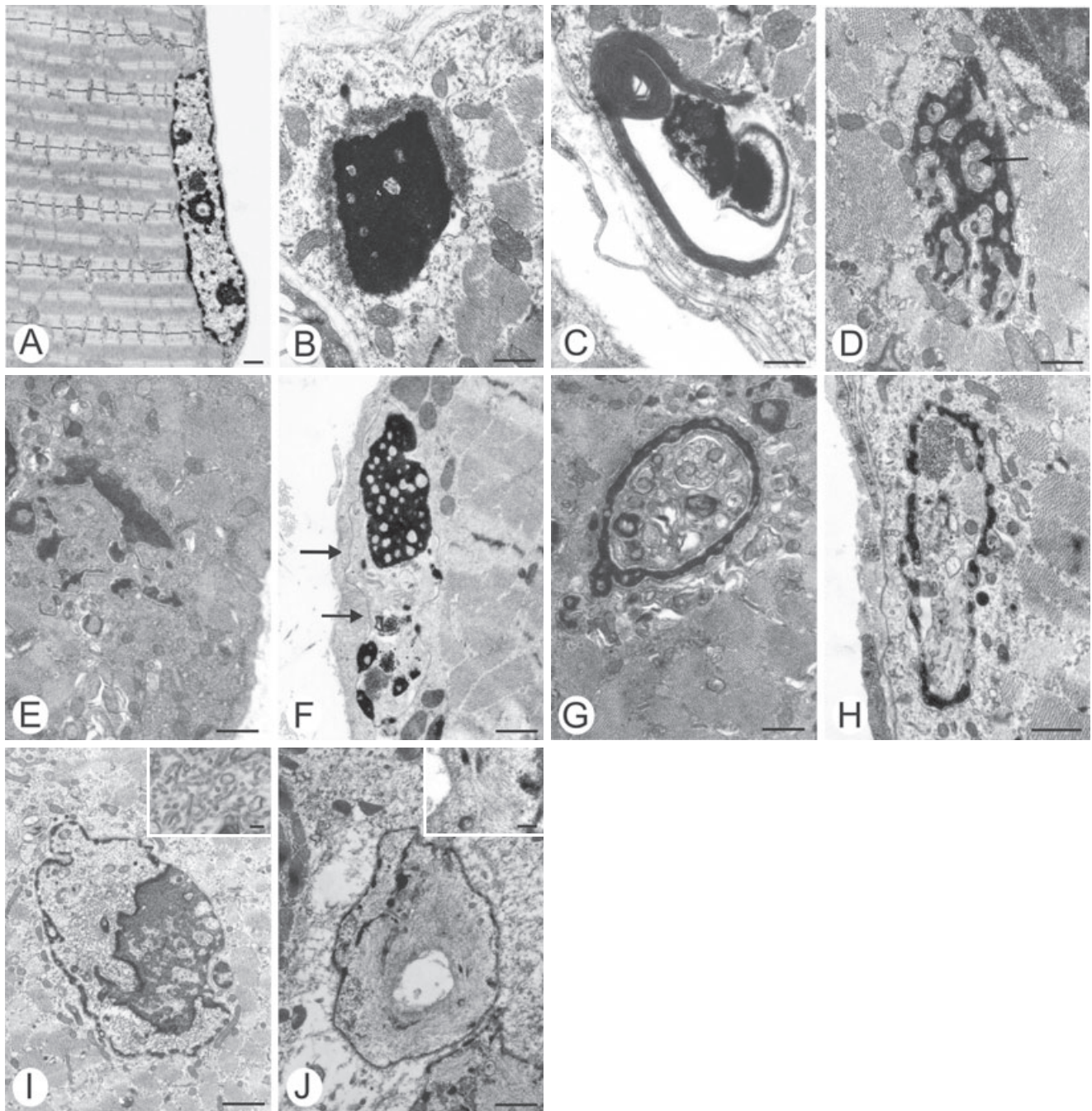


Figure 2. **A.** Normal myonucleus of the wild-type mouse. **B–J.** The spectrum of myonuclear changes in null mutants. **B.** Condensed chromatin, surrounded by a felt-like rim. **C.** Condensed chromatin, surrounded by concentric lamellae. **D.** Fragmented nucleus containing

tubular profiles (arrow). **E.** Condensed fragmented chromatin. **F.** Condensed fragmented chromatin with inclusions. Note nuclear membrane (arrows). **G, H.** Pseudoinclusions. **I.** Intracellular tubular profiles. **J.** Intracellular filaments. Bars, 1 μ m.

in vivo example of the increased nuclear vulnerability to mechanical stress in the absence of a functionally intact nuclear lamina. The accompanying cytoplasmic degeneration is most likely a result of the nuclear instability. The pathomechanism linking nuclear disintegration and cytoplasmic degeneration remains to be established.

Loss of, or mutations within, the *LMNA* gene resulted in ADEDMD, which led to mechanical weakening of the nuclei; this pathology may be compounded by alterations to signaling pathways, together with effects on chromatin organization/transcription of specific genes.

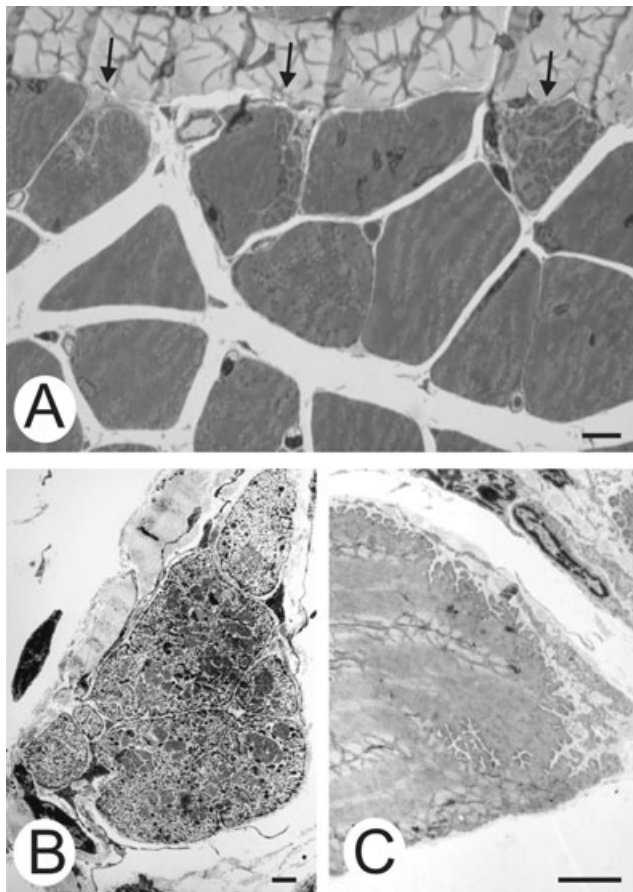


Figure 3. Cytoplasmic pathological changes. **A.** Three myofibers show “splitting” (arrows). Bar, 10 μ m. **B.** The cytoplasmic surfaces abutting the extracellular matrix of a split myofiber are smooth. **C.** In contrast, this myofiber of the wild-type mouse shows indentations wherever its surface contacts extracellular matrix. Bars, 5 μ m.

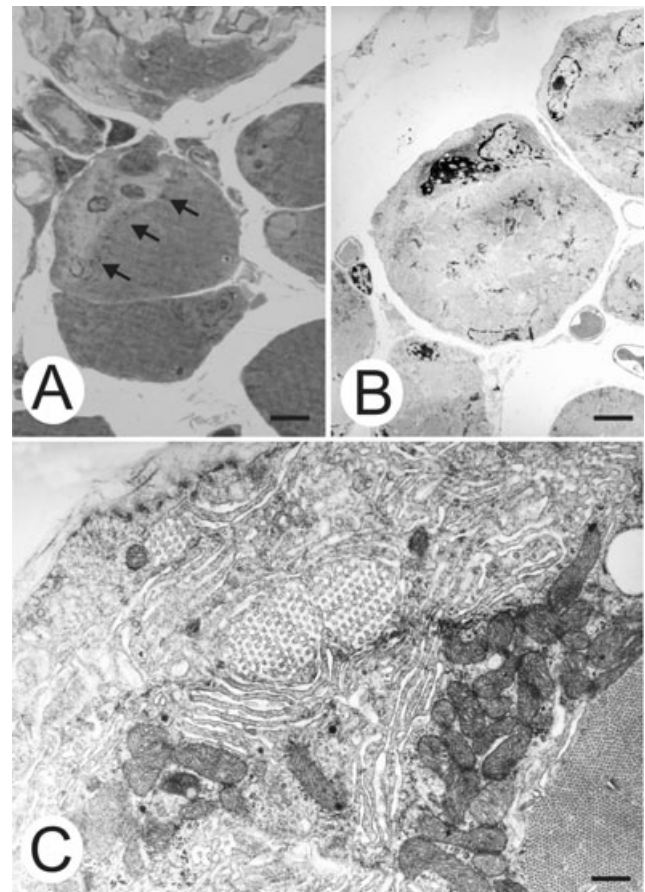


Figure 4. Subsarcolemmal myofibrillar loss. **A.** Myofibrils of the central myofiber do not reach the cell border. Arrows denote the border of the filamented cytoplasm. **B.** Electron micrograph of a parallel non-serial section of the myofiber shown in **A.** One myonucleus in the myofibril-free subsarcolemmal region shows clumped chromatin. Bars, 50 μ m. **C.** Higher magnification of a subsarcolemmal area containing honeycomb structures, sarcotubular profiles, and polysomes. Bar, 5 μ m.

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