Muscle aging is associated with compromised Ca^{2+} spark signaling and segregated intracellular Ca^{2+} release

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Reduced homeostatic capacity for intracellular Ca^{2+} ($[Ca^{2+}]_i$) movement may underlie the progression of sarcopenia and contractile dysfunction during muscle aging. We report two alterations to Ca^{2+} homeostasis in skeletal muscle that are associated with aging. Ca^{2+} sparks, which are the elemental units of Ca^{2+} release from sarcoplasmic reticulum, are silent under resting conditions in young muscle, yet activate in a dynamic manner upon deformation of membrane structures. The dynamic nature of Ca^{2+} sparks appears to be lost in aged skeletal muscle. Using repetitive voltage stimulation on isolated muscle preparations, we identify a segregated $[Ca^{2+}]_i$ reserve that uncouples from the normal excitation–contraction process in aged skeletal muscle. Similar phenotypes are observed in adolescent muscle null for a synaptophysin-family protein named mitsugumin-29 (MG29) that is involved in maintenance of muscle membrane ultrastructure and Ca^{2+} signaling. This finding, coupled with decreased expression of MG29 in aged skeletal muscle, suggests that MG29 expression is important in maintaining skeletal muscle Ca^{2+} homeostasis during aging.

Introduction

Effective muscle contractile performance is contingent upon the maintenance of Ca^{2+} homeostasis and signaling, which requires that intracellular Ca^{2+} ($[Ca^{2+}]_i$) be readily available for release. In skeletal muscle, excitation–contraction (E–C) coupling is primarily mediated by conformational coupling between voltage sensors of the sarcolemmal membrane and ryanodine receptor (RyR) Ca^{2+} release channel of the sarcoplasmic reticulum (SR; Ma et al., 1988; Rios et al., 1992; Franzini-Armstrong and Jorgensen, 1994). A secondary process, called Ca^{2+}-induced Ca^{2+} release (CICR), amplifies $[Ca^{2+}]_i$ release in skeletal muscle, particularly under stress conditions in muscle fatigue and dystrophy (Fong et al., 1990; Takagi et al., 1992; Brotto et al., 2002).

Aging effects on muscle function have been associated with muscle fiber denervation, loss of motor units, and motor unit remodeling. Because functional alterations occur before significant muscle wasting becomes evident, changes in the E–C coupling machinery and $[Ca^{2+}]_i$ homeostasis may act as causative factors for, or adaptive responses to, muscle aging (Larsson and Edstrom, 1986; Faulkner et al., 1995; Delbono, 2002). We show that stress-induced Ca^{2+} sparks, which are the elemental events of CICR in striated muscles (Cheng et al., 1993; Klein et al., 1996), are severely compromised in aged skeletal muscle. In addition, we find that muscle aging is associated with the development of a segregated SR Ca^{2+} pool that uncouples from the normal E–C coupling machinery. We present evidence to suggest that mitsugumin-29 (MG29) may act as a sentinel against the effects of age on skeletal muscle Ca^{2+} homeostasis.

Results and discussion

To delineate the contribution of Ca^{2+} sparks to the aging phenotype in skeletal muscle, intact flexor digitorum brevis (FDB) fibers isolated from young (2–4 mo) and aged (26–27 mo) mice

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Abbreviations used in this paper: ANOVA, analysis of variance; CICR, Ca^{2+}-induced Ca^{2+} release; E–C, excitation–contraction; EDL, extensor digitorum longus; $[Ca^{2+}]_o$, extracellular Ca^{2+}; FDB, flexor digitorum brevis; $[Ca^{2+}]_i$, intracellular Ca^{2+}; RyR, ryanodine receptor; SR, sarcoplasmic reticulum; TT, transverse tubule; VICR, voltage-induced Ca^{2+} release; wt, wild-type.

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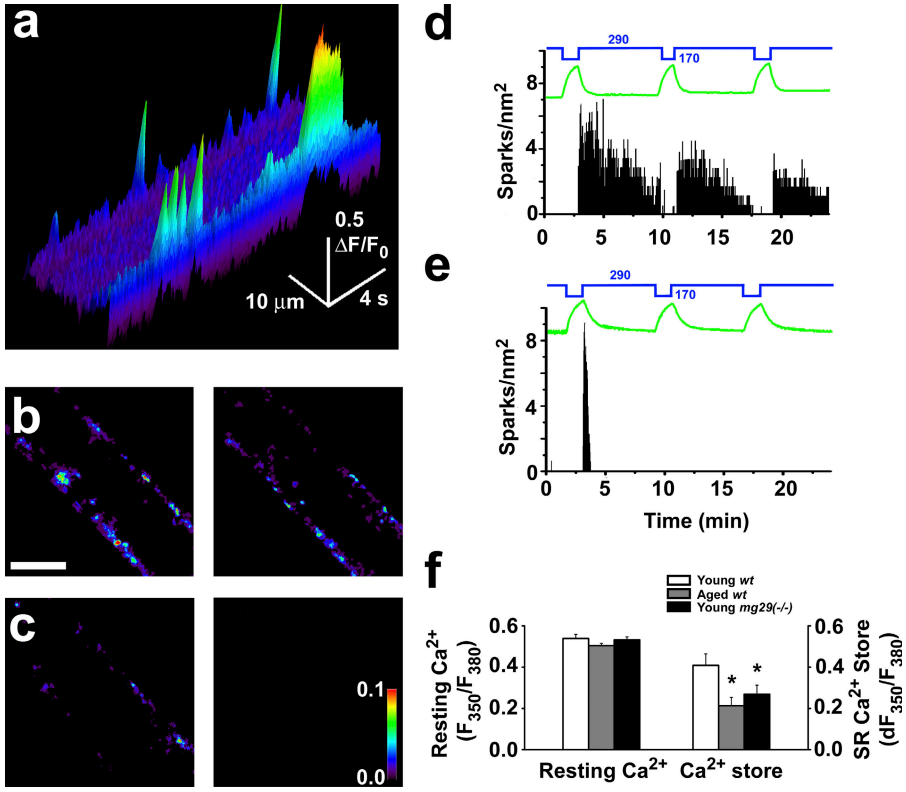

Figure 1. **Loss of plastic Ca^{2+} spark signaling in aged skeletal muscle.** Intact FDB muscle fibers were treated with hypotonic shock to generate a Ca^{2+} spark response. (a) Cross section line scan image of young FDB muscle fiber after osmotic shock. Distinct Ca^{2+} spark and burst events are seen in the periphery of the muscle fiber. (b) Sparks over the span of 2 min were binned using a custom-designed IDL data processing software routine (left). Plasticity of Ca^{2+} sparks in young fibers is shown with the persistent response seen after a second osmotic shock on the same cell (right). Bar, 15 μm. (c) Aged skeletal muscle fibers show an initial response after osmotic shock (left); however, this response is blunted and cannot be observed in subsequent osmotic shocks (right). Pseudocolor represents the average number of Ca^{2+} release events at each point within the fiber, with a scale of 0.0–0.1 in sparks/s. (d) A diary plot of spark activity versus changes in fiber volume (green line) illustrates young fibers can produce three or more responses to osmotic shock ($n = 16$). Blue numbers and lines indicate the osmolarity of perfusion solutions. (e) Aged fibers only display a rapidly terminating response after the first shock, which cannot be restimulated ($n = 14$). (f) Determination of $[Ca^{2+}]_i$- (left) and caffeine/ryanodine-sensitive SR Ca^{2+} store (right) in FDB fibers as measured by changes in fura-2 fluorescence. Error bars are the mean ± SEM. * indicates $P < 0.05$ by analysis of variance (ANOVA).

were treated with osmotic shock to induce Ca^{2+} sparks. Exposure of the muscle fiber to a hypotonic solution leads to swelling of the fiber. Upon return to isotonic solution, the recovery of cell volume to normal is accompanied by a robust, peripherally localized Ca^{2+} spark response (Fig. 1 a; Wang et al., 2005). Young muscle fibers display a dynamic Ca^{2+} spark response to repeated stress cycles, with each round of osmotic shock generating Ca^{2+} sparks that continue for several minutes (Fig. 1, b and d). This Ca^{2+} spark response is located in the periphery of both young and aged muscle fibers. The dynamic nature of this Ca^{2+} spark response is significantly reduced in aged muscle (Fig. 1 c). Relative to young muscle (Fig. 1 d), aged muscle appears to contain a diminished capacity for the generation of dynamic Ca^{2+} sparks with repeated osmotic stresses (Fig. 1 e).

The diminished Ca^{2+} spark response in aged muscle could result from changes in resting $[Ca^{2+}]_i$ levels or altered Ca^{2+} storage inside the SR. We measured $[Ca^{2+}]_i$ levels and SR Ca^{2+} storage in FDB muscle fibers obtained from young and aged mice. As shown in Fig. 1 f, the resting $[Ca^{2+}]_i$ levels appear to be similar between young and aged skeletal muscle fibers, whereas the caffeine/ryanodine-mobilized SR Ca^{2+} pool is significantly less in aged skeletal muscle. Thus, the reduced caffeine/ryanodine-mobilized SR Ca^{2+} store may represent one potential factor for the compromised Ca^{2+} spark signaling associated with muscle aging.

Additional factors that may contribute to this defective Ca^{2+} spark signaling include changes in membrane ultrastructure or altered expression of Ca^{2+} regulatory proteins in skeletal muscle. We conducted a survey of triad junction proteins and found that the expression level of MG29 (Takeshima et al., 1998), a synaptophysin-related membrane protein, is significantly down-regulated during muscle aging (Fig. 2, a and b). To determine the extent that decreased MG29 levels contribute to age-related alterations in muscle Ca^{2+} homeostasis, muscle fibers obtained from young (3–5 mo) *mg29(−/−)* mice (Nishi et al., 1999) were stressed by osmotic shock. As with aged *wild-type* (*wt*) muscle, there is an initial Ca^{2+} spark response to the first osmotic shock and subsequent osmotic shocks produce little to no Ca^{2+} spark response in young *mg29(−/−)* muscle fibers (Fig. 2 c). Using fura-2 Ca^{2+} measurements, we found that the resting $[Ca^{2+}]_i$ level and SR Ca^{2+} storage are similar between aged *wt* and young *mg29(−/−)* muscle fibers (Fig. 1 f). These results point to a role for MG29 in maintaining normal Ca^{2+} homeostasis that is lost with its diminished expression during aging.

Our previous studies have shown that the *mg29(−/−)* mice display contractile alterations and muscle atrophy at ages of 6 mo or younger (Nishi et al., 1999; Nagaraj et al., 2000) that resemble the atrophic phenotype of aged *wt* mice. Electron microscopy studies reveal similar ultrastructural alterations to triad junction membrane structures of aged *wt* and young *mg29(−/−)* skeletal muscle. Although organized alignment of SR and transverse-tubule (TT) membranes is present in young *wt* muscle, fragmented SR is frequently observed in slow (soleus) and fast (extensor digitorum longus [EDL]) twitch muscles from both young *mg29(−/−)* and aged *wt* skeletal muscles (Fig. 3, b–d). Aged *wt* soleus muscle also displays swelling of the TT that is very similar to that seen in *mg29(−/−)* muscle (Nishi et al., 1999). The development of these defects appears to be progressive during aging, as EDL fibers display

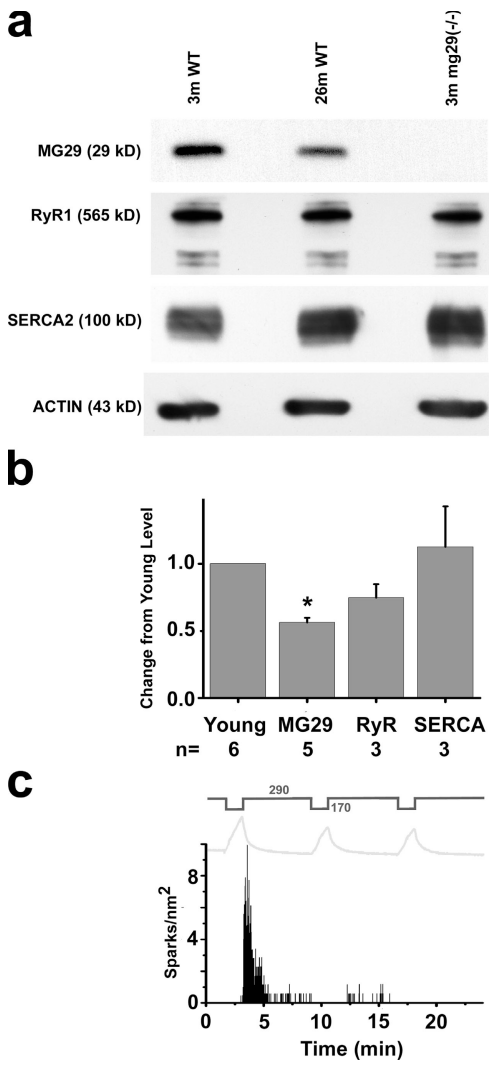

Figure 2. **Reduced expression of MG29 in aged skeletal muscle and compromised Ca^{2+} spark signaling in *mg29(−/−)* muscle.** (a) Western blots were performed for MG29, RyR, and SERCA2 on FDB muscle extracts from young and aged *wt* mice, and from young *mg29(−/−)* mice. (b) Densitometry measurements of altered protein expression with muscle aging. Values are the mean change in protein level versus young *wt* (set at 100%) ± the SEM. *n* represents paired groups of young and aged mice. * represents $P < 0.001$. Levels of RyR1 appear to decrease in aged skeletal muscle, although these differences do not reach a level of statistical significance ($P = 0.064$). Variation in the level of RyR in aged muscle may result from the presence of tubular aggregates (Fig. 3). (c) Osmotic shock–induced Ca^{2+} sparks in *mg29(−/−)* muscle is plotted against changes in fiber volume (gray line) ($n = 14$).

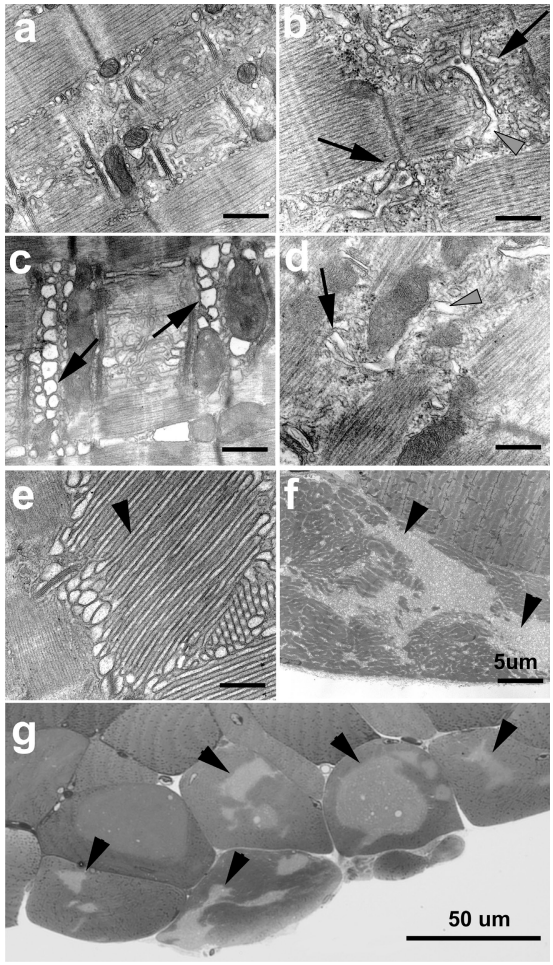

Figure 3. **Disruption of triad junction membrane structures in aged wild-type and young *mg29(−/−)* mice.** EDL from young *wt* (a) and hindlimb muscle from young *mg29(−/−)* animals (b), as well as aged *wt* EDL (c) and soleus (d), were examined by transmission electron microscopy. Many cells from aged EDL display large aggregates of SR (e) that are preferentially found in necrotic cells (f). Light microscopy of a toluidine blue–stained transverse section reveals the necrotic nature of aged muscle fibers containing large SR aggregates (g). Arrows indicate fragmented SR network; black arrowheads indicate tubular aggregates; gray arrowheads point to swollen TT. The presence of intact mitochondria minimizes the risk of fixation artifact in these micrographs. Bars, 0.5 μm, unless otherwise noted.

a continuum of damage ranging from minor SR fragmentation (Fig. 3 c) to formation of large aggregations of SR (Fig. 3 e and Table S1, available at http://www.jcb.org/cgi/content/full/jcb.200604166/DC1; Agbulut et al., 2000; Chevessier et al., 2004). We suspect that aggregation of SR may result from the accumulation of subtle defects in Ca^{2+} signaling and membrane recycling, leading to progressive sarcopenia (Fig. 3, f and g).

The similar structural alterations seen in aged *wt* and young *mg29(−/−)* muscle suggests that further defects in Ca^{2+} homeostasis beyond their Ca^{2+} spark response should be present. Previous studies by Kurebayashi et al. (2003) revealed that repetitive Ca^{2+} release using KCl stimulation on intact *mg29(−/−)* muscle in the absence of extracellular Ca^{2+} ($[Ca^{2+}]_o$) did not lead to complete depletion of the SR Ca^{2+} pool. We now present evidence to show the presence of a segregated SR Ca^{2+} pool that uncouples from the normal voltage-induced Ca^{2+} release (VICR) machinery in young *mg29(−/−)* muscle and aged *wt* muscle (Fig. 4). When isolated, intact muscle fibers are exposed to 0 $[Ca^{2+}]_o$ for 90 min, intermittent nonfatigue voltage stimulation leads to passive depletion of the SR Ca^{2+} pool, followed by minimal caffeine-induced Ca^{2+} release in young *wt* muscle, whereas this response is larger in aged *wt* and young *mg29(−/−)* muscle (Fig. 4 a). In the absence of $[Ca^{2+}]_o$, fatiguing stimulation using repetitive VICR leads to rapid depletion of the voltage-sensitive pool of $[Ca^{2+}]_i$ releasable, revealing striking differences among the three muscle preparations in their subsequent response to

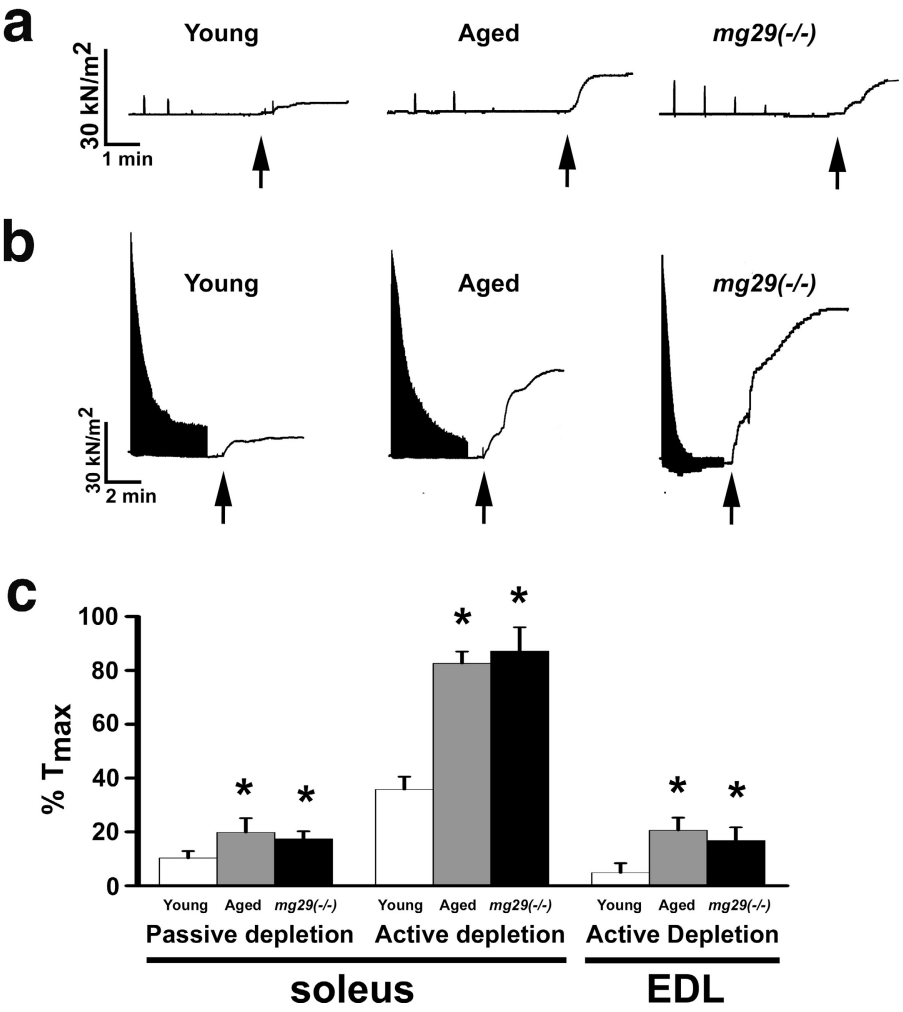

Figure 4. **Segregation of $[Ca^{2+}]_i$ release in aged wild-type and young *mg29(−/−)* muscle.** (a) Force tracings of soleus muscle bundles after passive depletion of $[Ca^{2+}]_i$ stores by single tetanic voltage stimulation contractions once per minute during incubation in 0 $[Ca^{2+}]_o$ for ~90 min. Traces are cropped to only include the final six minutes of the experiment. 30 mM caffeine was added at points indicated by the arrows to induce contraction after the force generated by voltage stimulation could no longer be detected. (b) Tetanic voltage stimulation, for a 5-min duration, was applied to isolated soleus muscles in a solution containing 0 $[Ca^{2+}]_o$. Arrows indicate the application of 30 mM caffeine. Although young *wt* muscles show a minimal response to caffeine, young *mg29(−/−)* and aged *wt* muscles display a significantly enhanced response to caffeine, indicating the presence of a Ca^{2+} pool that cannot be mobilized by extended voltage stimulation. (c) Data from multiple experiments are averaged (mean ± SEM; n = 6–11). Data is provided for both soleus and EDL skeletal muscles. * indicates significant differences, with $P < 0.05$ as measured by ANOVA.

caffeine treatment. As shown in Fig. 4 b, after this fatiguing stimulation, caffeine induces significantly larger Ca^{2+} release in young *mg29(−/−)* and aged *wt* muscles compared with the young wt muscle. Data from multiple experiments are summarized in Fig. 4 c. These findings suggest the presence of a VICR-uncoupled, RyR-sensitive Ca^{2+} pool in aged *wt* and young *mg29(−/−)* muscles; however, the relative sizes of these pools in soleus and EDL muscle fibers are difficult to evaluate because of increased sensitivity of the contractile apparatus of the soleus muscle to caffeine and Ca^{2+} (Singh and Dryden, 1989; Lamb et al., 2001; Jin et al., 2003; Brotto et al., 2006).

The segregated SR Ca^{2+} pool in aged *wt* and young *mg29(−/−)* muscle is not likely to result from major changes in the VICR machinery itself, as the initial VICR responses in young and aged *wt* and young *mg29(−/−)* muscle are generally similar (Fig. 4, a and b). The maximal specific tetanic force produced by aged *wt* EDL muscle (250 ± 15 kN/m^2) is lower than that produced by young *wt* muscle (300 ± 18 kN/m^2), which is consistent with previous studies (Faulkner et al., 1995; Gonzalez et al., 2000). The maximal specific tetanic force in young *mg29(−/−)* EDL muscles is 262 ± 17 kN/m^2, which is similar to that measured in aged *wt* muscle. To test the possibility that changes in contractile machinery during muscle aging might contribute to our measurement of Ca^{2+} release, the force versus pCa relationship was examined in all three types of muscle preparations. As shown in Table S2 (available at http://www.jcb.org/cgi/content/full/jcb.200604166/DC1), the isometric contractile properties of Triton X-100–skinned muscle fibers are similar in young and aged *wt*, as well as young *mg29(−/−)*, mice. Therefore, under these conditions, the force output is an authentic measurement of Ca^{2+} release from the SR, and a segregated SR Ca^{2+} pool must exist in aged *wt* and young *mg29(−/−)* muscle to account for the elevated, caffeine-induced Ca^{2+} release.

We have established that aged muscle fibers have a disrupted Ca^{2+} spark response and a segregated Ca^{2+} store that cannot be mobilized by VICR, which are associated with ultrastructural disruption of triad junctions and the SR network (Fig. 5). One possible explanation for the development of a segregated SR Ca^{2+} pool is that subtle disruption of SR and TT alignment at the triad junction could result in uncoupling of RyR1 and DHPR, which could also lead to the compromised Ca^{2+} spark signaling observed in aged skeletal muscle. An inhibitory role for DHPR on RyR1 function has been proposed by other investigators (Suda and Penner, 1994; Lee et al., 2004; Zhou et al., 2006). If the Ca^{2+} spark response associated with membrane deformation and the segregation of $[Ca^{2+}]_i$ release was solely caused by disruption of the inhibitory effects of DHPR on RyR1 function, one would expect to see an elevated Ca^{2+} spark response

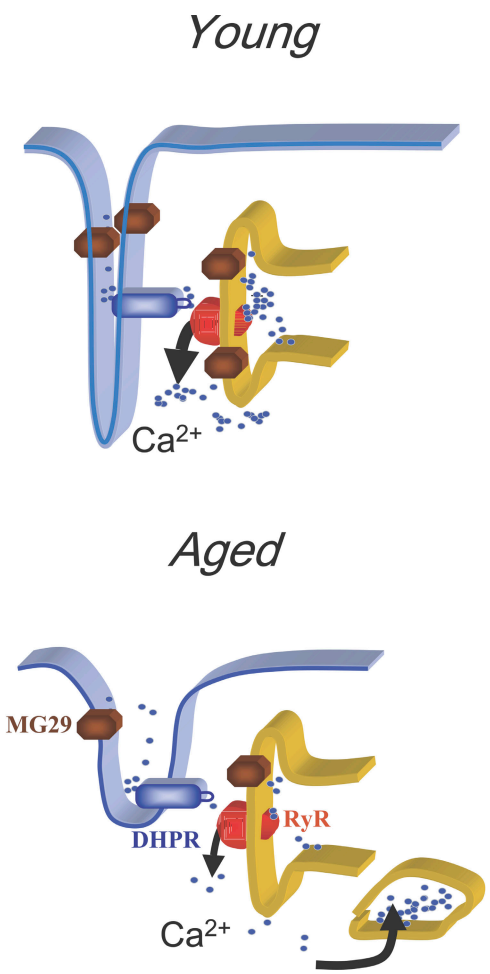

Figure 5. **Schematic diagram to illustrate the segregated Ca^{2+} release process in aged skeletal muscle.** In young, healthy skeletal muscle, plastic activation of Ca^{2+} sparks represents a physiologic response to stress. This process is reduced in aged muscle because of the following two factors: first, that reduced expression of MG29 may lead to an increased threshold for Ca^{2+}-induced activation of RyR; and second, that fragmentation of SR enables generation of a segregated SR Ca^{2+} pool that uncouples from the normal VICR process.

in aged skeletal muscle, as it has been established that DHPR expression and the ratio of DHPR to RyR1 is decreased in aged skeletal muscle (Renganathan et al., 1997). Therefore, the reduced Ca^{2+} spark response observed in aged skeletal muscle suggests that changes in other cellular factors, such as MG29 expression, may play a role in regulation of Ca^{2+} signaling in skeletal muscle at different developmental stages.

MG29 contains a high degree of homology with synaptophysin, a protein thought to be involved with membrane fusion during exocytosis (Alder et al., 1992). Decreased MG29 expression may lead to improper lipid membrane formation or fusion, altering the dynamic process of membrane recycling and SR network formation. In aging *wt* or young *mg29(−/−)* mice, the lack of this synaptophysin family member would suppress the efficient maintenance of triad junction structure, while also generating a fragmented SR network. Recent results from our laboratory have begun to shed light on the physiological function of MG29. We have demonstrated that MG29 increases sensitivity of the RyR channel to CICR when expressed in a heterologous cell system and when reconstituted with RyR in lipid bilayer single-channel studies (Pan et al., 2004). The lack of MG29 in aged and *mg29(−/−)* muscle should decrease the sensitivity of RyR to CICR, in agreement with our current results.

Segregation of Ca^{2+} pools in aged muscle may have a physiological role in maintaining muscle integrity in the face of decreasing homeostatic capabilities. The resulting dampened Ca^{2+} mobilization in aged muscle may be a compensatory mechanism that protects aged fibers from Ca^{2+}-induced injury. It is also possible that the presence of a segregated Ca^{2+} reserve isolated from VICR responses contributes to cellular stress and decreased homeostatic capacity. Although the mechanism of active shuttling of Ca^{2+} from the VICR-responsive to the VICR-nonresponsive pool is not known, selective regulation of this Ca^{2+} shuttling to modulate the VICR-responsive pool would allow for the enhancement of aging skeletal muscle performance and/or protect skeletal muscle during aging. The *mg29(−/−)* mouse represents a model system in which these mechanisms can be examined and these hypotheses can be tested.

Materials and methods

Ca^{2+} imaging and spark analysis

Studies were conducted with *wt* C57Bl6/J male mice (Aged Rodent Colony, maintained by the National Institute on Aging), which were either 2–4 or 26–27 mo old. Male *mg29(−/−)* mice (2–5 mo old), and *wt* animals of the same genetic background (129Sv/J backcrossed to C57Bl6/J) were maintained in local facilities and handled in a manner approved by local regulations. FDB fibers were isolated by enzymatic disassociation in 0.2% type I collagenase (Sigma-Aldrich) for 55 min at 37°C and loaded with 10 μM Fluo-4–AM for 60 min at room temperature. Mean FDB fiber size was 1 mm × 20 μM. Measurements of Ca^{2+} release were performed on a confocal microscope (Radiance 2100; Bio-Rad Laboratories) equipped with an argon laser (488 nm) and a 60×, 1.3 NA, oil immersion objective. For Ca^{2+} spark measurements, fibers were perfused with a 170-mosM hypotonic solution containing (in mM) 70 NaCl, 5 KCl, 10 Hepes, 2.5 $CaCl_2$, 2 $MgCl_2$, pH 7.2, for 60–180 s to induce swelling before perfusion was switched back to the initial Tyrode solution (in mM) 140 NaCl, 5 KCl, 10 Hepes, 2.5 $CaCl_2$, 2 $MgCl_2$, pH 7.2, with an osmolarity of 290 mosM as measured by a Micro Osmometer 3300 (Advanced Instruments). Image analysis was performed using custom routines on IDL software (Research Systems, Inc.; Cheng et al., 1999; Wang et al., 2005).

For determination of resting cytosolic Ca^{2+} levels and total SR Ca^{2+} store, individual FDB fibers were loaded with 10 μM fura-2 AM for 45 min at room temperature in Tyrode solution. 20 μM N-benzyl-p-toluene sulphonamide, a myosin II inhibitor, was applied for 15 min to prevent motion artifact from muscle contraction (Cheung et al., 2002; Pinniger et al., 2005). Fibers were also embedded into silicone grease to maintain their position in the culture dish (Jacquemond, 1997). The ratio of fura-2 fluorescence at excitation wavelength of 350 and 380 nm was measured on a PTI spectrofluorometer (Photon Technology International) to assess the resting $[Ca^{2+}]_i$ level. The SR Ca^{2+} store was measured by addition of 20 mM caffeine plus 5 μM ryanodine in the presence of 0 $[Ca^{2+}]_o$.

Electron and light microscopy

Electron microscopy studies were performed following our previously published protocols (Ito et al., 2001). In brief, skeletal muscles were fixed in 3% paraformaldehyde, 2.5% glutaraldehyde, and 0.1 M cacodylate buffer, pH 7.4, and later postfixed in 1% OsO4 and 0.1 M cacodylate buffer, pH 7.4. Microthin sections were double stained with uranyl acetate and lead citrate. These sections were examined under a transmission electron microscope (JEM-1010; JEOL).

Intact muscle preparation

Intact EDL and soleus muscles were dissected from mice and maintained in modified Ringer's solution containing the following (in mM): 142 NaCl, 4.0 KCl, 2.5 $CaCl_2$, 2.0 $MgCl_2$, 10 glucose, and 10 Hepes, pH 7.4 ± 0.1, continuously bubbled with 100% O_2. EDL muscles had a mean length of

12 mm and a mean mass of 80 mg, whereas soleus muscles had a mean length of 10 mm and a mean mass of 10 mg. Muscles were mounted vertically on a glass-stimulating apparatus (Radnoti) with platinum electrodes and attached to a movable isometric force transducer and to a stationary anchor, which allowed muscles to be stretched until both maximal forces for a given frequency and the frequency producing T_{max} were obtained.

Force measurements during passive depletion and fatigue

After T_{max} was determined, the intact muscles were allowed to equilibrate for 20 min in the Ringer's solution. During equilibration, muscle strips were stimulated with ~100–120 Hz (EDL) or ~60–80 Hz (SOL), 330 mA, 500 ms electrical pulse–trains administered with a periodicity of 1 min to generate T_{max}. After equilibration, the muscles from the passive-depletion group were washed five times in Ringer's solution with the same composition as described in the previous section, except that no $CaCl_2$ was added while 0.1 mM EGTA was added, to create a nominal 0 $[Ca^{2+}]_o$ solution. Muscles were stimulated with one T_{max} every minute in 0 $[Ca^{2+}]_o$ solution until force declined to nondetectable levels; the muscles were then exposed to 30 mM caffeine. Force produced in response to caffeine application was recorded until a stable plateau was obtained. After the passive depletion protocol, muscles were subjected to extensive washes in normal Ringer's solution containing 2.5 Ca^{2+} and then electrically stimulated until force returned to initial equilibration values. In 80% of our preparations, this was achieved. After forces were stable and comparable to the initial levels before the onset of the passive depletion, muscles were returned to the 0 $[Ca^{2+}]_o$ solution for 5 min and subsequently subjected to a 1–5 min fatiguing protocol consisting of the same stimulatory pattern administered at a 1-s periodicity (i.e., 50% duty cycle). In between fatigue runs, muscles were washed in 2.5 Ca^{2+} solution and force was allowed to recover to prefatigue levels before the onset of the next fatigue run. At the end of each fatiguing protocol, muscles were treated with 30 mM caffeine and maximal response to caffeine was recorded. Caffeine was mixed in a small volume of the Ringer's solution and added to the chambers to produce a final concentration of 30 mM in the bathing chamber. Whenever possible, paired experiments were performed with young wild-type animals and aged *wt* or young *mg29(−/−)* animals. Experiments were also conducted with fibers only exposed to passive depletion or fatigue in 0 $[Ca^{2+}]_o$ to confirm that effects of each treatment can be observed independently. The integrity of the fiber contractile apparatus and Ca^{2+}-handling machinery was tested at the conclusion of the protocol by exposure to 100 mM KCl. Only fibers with at least 85% of T_{max} were included for statistical analysis. All force data were normalized to the last tetanic contraction at the end of the equilibration period and just before the start of the fatiguing protocol (this T_{max} = 100%). Absolute force, normalized per cross sectional area (i.e., in kN/m^2) was determined at the end of the equilibration period by the following relationship: Force (in Kg) = (*g* of force) × (muscle length in cm) × 1.06/muscle weight (*g*), where 1.06 represents the density of the muscle strips. Triton X-100–skinned muscle fiber experiments followed the protocols as previously described (Brotto and Nosek, 1996; Brotto et al., 2004).

Statistical analysis

All statistical analysis in this study was conducted using ANOVA, and data is presented as the mean ± SEM.

Online supplemental material

Table S1 describes the parallel disruption of triad junctions in aged *wt* and young *mg29(−/−)* skeletal muscle. Table S2 is an assessment of muscle contractile function in young *wt*, aged *wt*, and young *mg29(−/−)* muscle. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200604166/DC1.

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