

# When sparks get old

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Sparks are transient local elevations of Ca ion concentration observed in different types of muscle cells. Such local Ca<sup>2+</sup> signals can be provoked in skeletal muscle cells by altering the osmotic pressure of the extracellular solution. In this issue, Weisleder et al. (see p. 639) demonstrate that the Ca<sup>2+</sup> response to osmotic stress is substantially altered in aged muscle. The study presents evidence for a link between this finding and a reduced expression of mitsugumin 29 (MG29), a small membrane protein of the sarcoplasmic reticulum (SR).

## Ca signaling in skeletal muscle

Skeletal muscles are the motors that carry us through life. They consist of fibrous giant cells that are specifically designed for efficient nerve-controlled shortening. To switch the contractile machinery on and off, these cells raise and lower their cytoplasmic Ca<sup>2+</sup> concentration (for review see Melzer et al., 1995). To allow the concentration increase to occur with sufficient speed, skeletal muscle fibers are equipped with a unique mechanism to activate Ca<sup>2+</sup> release from their Ca<sup>2+</sup> storage compartment, the SR. The mechanism involves tight coupling of the probability that Ca<sup>2+</sup> release channels (ryanodine receptors [RyRs]) in the SR membrane are open to cell membrane voltage. The muscle fiber membrane is rapidly depolarized by action potentials that propagate from the postsynaptic region of the nerve–muscle endplate throughout the cell surface and into the cell by means of transversal tubular infoldings (T system). The signal transmission from the T system to the SR is performed by an assembly of proteins in the contact region between the two membrane systems. A modified L-type Ca<sup>2+</sup> channel protein (dihydropyridine receptor [DHPR]) acts as the voltage sensor and transmits a conformational command signal to the RyR (Dirksen, 2002). Besides activating the contractile filaments, the released Ca<sup>2+</sup> exerts a positive feedback on the opening of the release channels in a process termed Ca-induced Ca release (CICR).

## Ca sparks in skeletal muscle

Time-resolved confocal microscopy using fluorescent probes revealed localized Ca<sup>2+</sup> release events termed Ca<sup>2+</sup> sparks

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Abbreviations used in this paper: CICR, Ca-induced Ca release; DHPR, dihydropyridine receptor; MG29, mitsugumin 29; RyR, ryanodine receptor; SR, sarcoplasmic reticulum.

(Klein et al., 1996) that result from clustered openings of RyRs, probably under the participation of CICR. In frog muscle, their frequency was found to increase with fiber membrane depolarization, supporting the hypothesis of a voltage-dependent recruitment of Ca<sup>2+</sup> release quanta (Klein et al., 1997). However, to the research community's disappointment, such quantal events were not detectable in skeletal muscle fibers of mammals, shedding doubts on a general physiological role of spark-based quantal Ca<sup>2+</sup> release in skeletal muscle. Only muscle fibers whose outer membranes were permeabilized by saponin (which leaves the SR membrane functional) showed sparklike events (Kirsch et al., 2001).

Sparks are also more readily seen in developing muscle cells but not in regions of these cells that respond with Ca<sup>2+</sup> release to membrane depolarization (Shirokova et al., 1999). It has been proposed that the DHPR–RyR alignment prevents sparks from occurring (Zhou et al., 2006). The search for conditions to elicit Ca<sup>2+</sup> sparks in intact mammalian muscle finally led to success when Wang et al. (2005) demonstrated localized Ca<sup>2+</sup> fluctuations in isolated mouse muscle fibers challenged with external solutions of different osmolarities. An increase of osmolarity to ~150%, which was synchronous with an increase of the external Ca<sup>2+</sup> concentration (by raising CaCl<sub>2</sub> from 2.5 to 50 mM), elicited shrinkage and localized Ca<sup>2+</sup> transients in the periphery of the fibers. Such signals could also be observed on return to a normosmotic bath solution after a temporary decrease in osmolarity to 60%, which caused cell swelling.

In normal mouse muscle fibers, the spark activity during recovery from swelling ceased within several minutes. In contrast, fibers from the mdx mouse, an animal model of human Duchenne muscular dystrophy resulting from lack of the cytoskeletal protein dystrophin, showed persistent, apparently irreversible spark activity. A defective Ca<sup>2+</sup> response has long been suggested to be responsible for the death of muscle fibers in this devastating muscle disease (Tutdibi et al., 1999). The results of Wang et al. (2005) provided further direct evidence for this notion.

## Sparking in old age

In the present issue of this journal, a new study from the Ma laboratory (Weisleder et al., 2006) reports experiments on muscle fibers from mice close to the end of their natural life span (~2 yr). These fibers exhibit just the opposite effect to that seen in dystrophic muscle (i.e., a drastic shortening of the period of spark activity after brief hyposmotic stimulation). Weakening of skeletal muscle is one of the burdens accompanying the aging

process, and part of the loss of muscle force in old age has been attributed to alterations in the Ca<sup>2+</sup> release machinery. For instance, it has been shown that the DHPR/RyR ratio decreases with age (Renganathan et al., 1997). The now discovered drastic changes in osmotic stress-provoked local Ca<sup>2+</sup> response is a new facet of altered Ca<sup>2+</sup> regulation in aged muscle.

Interestingly, Weisleder et al. (2006) observed a very similar phenomenon in young mice deficient in the protein MG29. This protein normally sits in the membrane area of the SR that faces the T system (i.e., in a region critical for Ca<sup>2+</sup> handling). It resembles the synaptophysins, which are found in the membrane of synaptic vesicles, and is one of several small proteins that are suspected to modulate the Ca<sup>2+</sup> release mechanism. MG29 has been reported to affect store-operated Ca<sup>2+</sup> entry and the susceptibility of muscle to fatigue (Pan et al., 2002). Because the level of MG29 was found to be reduced by ~50% in skeletal muscle of old mice, Weisleder et al. (2006) propose that this protein plays a role in the weaker Ca<sup>2+</sup> response to osmotic stimulation of aged muscle fibers. They suggest that the substantially altered spark activity might result from a reduced sensitivity of the RyRs to CICR. Another contributor to the rapid decline of spark frequency could be the reduced load of Ca<sup>2+</sup> in the SR that was found for muscle fibers of both aged and MG29-deficient mice. Evidence from reconstituted RyRs and from single cell experiments indicates that the concentration of Ca<sup>2+</sup> on the luminal side of the SR affects the opening probability of Ca<sup>2+</sup> release channels (Herrmann-Frank and Lehmann-Horn, 1996; Launikonis et al., 2006). Calsequestrin, the major luminal Ca<sup>2+</sup>-binding protein of the SR, has been suggested to serve as a Ca<sup>2+</sup> sensor to modulate RyR under the participation of triadin and junctin, two further SR proteins of the T system–SR junction (Beard et al., 2005).

Other interesting parallels between old mice and MG29-null mice reported by Weisleder et al. (2006) include muscle atrophy and characteristic changes in ultrastructure, indicating a disruption of the alignment of the SR and T system and fragmentation of SR regions. The structural changes and the altered force response to depolarization and caffeine led to the suggestion that the T system loses control of sections of the SR, possibly as a result of the loss of MG29 as an essential component in maintaining SR–T system alignment. In view of the similarity of MG29 with synaptophysin, a membrane protein of still unknown function concentrated in the clear and dense core synaptic vesicles, the study of muscle might ultimately be useful for the understanding of regulated exocytosis in neurons and neurosecretory cells.

There is no doubt that the age-related dysfunction of muscle is a complex process, and it remains to be established whether MG29 is indeed a key element in this process, as the new results seem to indicate. MG29's possible involvement in the generation of sparks is intriguing and may open a new avenue to understanding the mechanism that links osmotic changes to Ca<sup>2+</sup> signaling in muscle. Are the sparks observed under these conditions indeed comparable with the stereotyped signals found in other muscle preparations? They may simply be artifacts caused by a mechanical perturbation of the membranes leading to local leaks of Ca<sup>2+</sup> into the cytoplasm. Alternatively, are

they part of a signal transduction chain involving specific mechanosensitive elements, perhaps even designed to modulate the Ca<sup>2+</sup> signal during the mechanical stress of muscle contraction? If so, is MG29 part of the mechanism? There is much work ahead. Sparks may get old, but not old fashioned.

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