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Invited review

Ca^{2+} sparks as a plastic signal for skeletal muscle health, aging, and dystrophy

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Abstract

Ca^{2+} sparks are the elementary units of intracellular Ca^{2+} signaling in striated muscle cells revealed as localized Ca^{2+} release events from sarcoplasmic reticulum (SR) by confocal microscopy. While Ca^{2+} sparks are well defined in cardiac muscle, there has been a general belief that these localized Ca^{2+} release events are rare in intact adult mammalian skeletal muscle. Several laboratories determined that Ca^{2+} sparks in mammalian skeletal muscle could only be observed in large numbers when the sarcolemmal membranes are permeabilized or the SR Ca^{2+} content is artificially manipulated, thus the cellular and molecular mechanisms underlying the regulation of Ca^{2+} sparks in skeletal muscle remain largely unexplored. Recently, we discovered that membrane deformation generated by osmotic stress induced a robust Ca^{2+} spark response confined in close spatial proximity to the sarcolemmal membrane in intact mouse muscle fibers. In addition to Ca^{2+} sparks, prolonged Ca^{2+} transients, termed Ca^{2+} bursts, are also identified in intact skeletal muscle. These induced Ca^{2+} release events are reversible and repeatable, revealing a plastic nature in young muscle fibers. In contrast, induced Ca^{2+} sparks in aged muscle are transient and cannot be re-stimulated. Dystrophic muscle fibers display uncontrolled Ca^{2+} sparks, where osmotic stress-induced Ca^{2+} sparks are not reversible and they are no longer spatially restricted to the sarcolemmal membrane. An understanding of the mechanisms that underlie generation of osmotic stress-induced Ca^{2+} sparks in skeletal muscle, and how these mechanisms are altered in pathology, will contribute to our understanding of the regulation of Ca^{2+} homeostasis in muscle physiology and pathophysiology.

Ca^{2+} sparks: elemental unit of intracellular Ca^{2+} release in striated muscle

Excitation-contraction coupling in skeletal and cardiac muscle requires close association between voltage-gated Ca^{2+} channels of the dihydropyridine receptor (DHPR) class in the sarcolemmal membrane and Ca^{2+} release channels of ryanodine receptor (RyR) in the sarcoplasmic reticulum (SR). In the heart, the entry of extracellular Ca^{2+} via DHPR triggers opening of RyR to amplify Ca^{2+} signaling through the Ca^{2+}-induced Ca^{2+} release (CICR) mechanism[1–3]. Membrane depolarization, rather than external Ca^{2+} entry, triggers SR Ca^{2+} release in skeletal muscle. In skeletal muscle, CICR represents an important amplification mechanism following voltage-induced Ca^{2+} release (VICR), especially under stress conditions[4–6].

The close juxtaposition of the transverse-tubular (TT) invagination of the sarcolemma and the SR terminal cisternae allows relay of the depolarizing signal[7–10]. These TT invaginations run in close spatial proximity to the SR terminal cisternae and establish the triad junction complex in skeletal muscle fibers[11,12]. This membrane structure allows contact between RyR and DHPR to ensure tight control of the Ca^{2+} release machinery to limit Ca^{2+} leak from SR. A majority of RyR channels in the muscle fiber are coupled with DHPR at the triad junctional region. Under normal physiological condition, Ca^{2+} sparks termination and repression in skeletal muscle can be achieved from either deactivation of the DHPR

voltage sensor or intrinsic inactivation of the RyR channel[13].

The elementary units of Ca^{2+} release from SR in striated muscle cells are discreet events known as Ca^{2+} sparks. These events are visualized by laser confocal scanning microscopy as a localized increase of signal from a fluorescent Ca^{2+} indicator dye loaded within a muscle cell. Ca^{2+} sparks were first discovered in cardiac muscle as quantal Ca^{2+} release events that originate from paracrystalline arrays of RyR channels on the SR surface, and therefore represent the elemental units of CICR[1,14,15]. The discovery of Ca^{2+} sparks has revolutionized understanding of the physiology and pathophysiology of Ca^{2+} signaling in cardiac and smooth muscles[16,17]. However, since the discovery of Ca^{2+} sparks in cardiac muscle, investigators have had difficulty in detecting these localized Ca^{2+} release events in intact adult mammalian skeletal muscle (Table 1).

Challenges in observation of Ca^{2+} sparks in skeletal muscle fibers

A major obstacle facing research on Ca^{2+} spark in skeletal muscles is the intrinsic difficulty in measuring spontaneous Ca^{2+} sparks in intact adult mammalian skeletal muscle fibers [18], as most available studies were conducted with amphibian muscle or permeabilized mammalian muscle[19,20]. Ca^{2+} sparks were also soon detected in neonatal mammalian skeletal muscle[21] where they were attributed to the activity of the type 3 RyR[22], which is preferentially expressed in mammalian skeletal muscle during fetal development and present at low expression levels in a minority of adult skeletal muscles[23]. While rare observations of Ca^{2+} sparks have been made in resting intact adult mammalian fibers[18,21], significant numbers of events in mammalian skeletal fibers were only observed in cells where the sarcolemmal integrity was disrupted by various physical or chemical skinning methods[20,24]. Since the sarcolemmal membrane is a major regulator of Ca^{2+} release at the triad junction, disruption of membrane integrity of skeletal muscle will likely alter the intracellular Ca^{2+} release machinery. Thus, most of the biophysical studies with Ca^{2+} sparks in skeletal muscle were carried out under non-physiological conditions. This disadvantage, coupled with the intrinsic difficulties with monitoring Ca^{2+} spark activity in intact mammalian muscle fibers, has limited our understanding of the cellular and molecular mechanisms underlying the regulation of Ca^{2+} spark in skeletal muscle and the adaptive changes of Ca^{2+} spark in muscle aging and disease.

Osmotic stress-induced Ca^{2+} sparks in skeletal muscle

Recently, our laboratory discovered that transient osmotically-induced membrane deformation resulted in a fluttered Ca^{2+} spark response adjacent to the sarcolemmal membrane in intact mouse muscle fibers[25,26] (Figure 1). In a series of serendipitous experiments with mouse skeletal muscle fibers, we discovered that subtle alterations of membrane structure produced drastic elevation of intracellular Ca^{2+} spark activity. When bathed in isotonic solution, isolated intact muscle fibers from non-exercised mice do not reveal any spontaneous Ca^{2+} spark activity, confirming that Ca^{2+} sparks are suppressed under resting conditions. After equilibration in isotonic solution (290 mOsm), fibers were perfused with either a hypotonic (170 mOsm) or hypertonic (450 mOsm) solution. Cell volume was markedly altered by exposure to solutions of varying osmolarity, swelling in a hypotonic environment and shrinking upon exposure to hypertonic conditions. These changes in cell volume result in alterations to the fluorescent Ca^{2+} dye signal observed, which may reflect dilution due to water entry or a decrease in intracellular Ca^{2+} that could influence Ca^{2+} spark induction. Shrinkage of the fiber, resulting from either hypertonic solution or a return to isotonic solution after exposure to hypotonic solution, induces a remarkable elevation of Ca^{2+} spark activity (Figure 1A). Kinetic analysis reveals the presence of two modes of Ca^{2+} spark signaling, the first being short release events similar to those seen in cardiac muscle and permeabilized skeletal muscle. A second group contains events with extended openings of the Ca^{2+} release machinery, known as Ca^{2+} bursts (Figure 1B). These Ca^{2+} burst events uncovered in our intact muscle preparation may have a significant physiological function, as they are not observed in permeabilized skeletal muscle fibers.

Table 1. Differential nature of Ca^{2+} sparks in cardiac and skeletal muscle.

Muscle type	RyR isoforms	Resting state sparks	DHPR/RyR interaction
Cardiac	RyR2	Spontaneous	Coupled activation[14]
Skeletal	RyR1 (major isoform) RyR3 (minor isoform)	Silent	Inhibitory suppression[69]

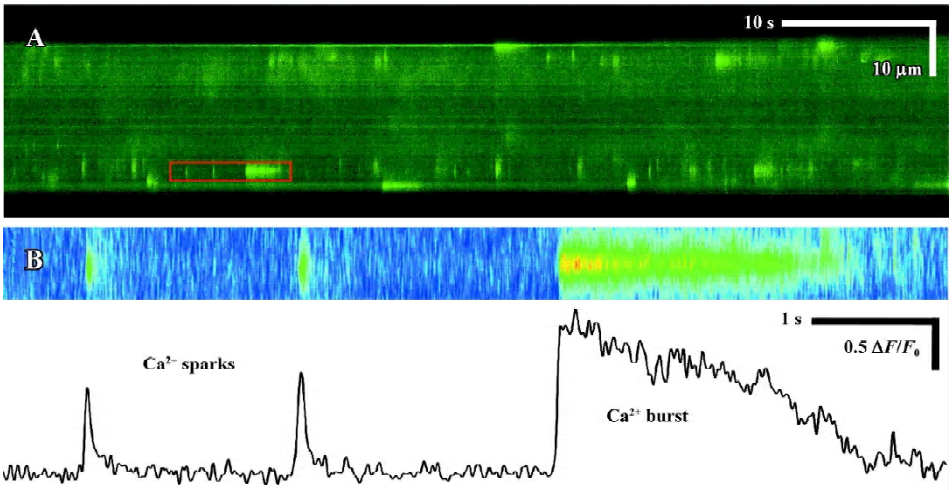

Figure 1. Ca^{2+} sparks and Ca^{2+} bursts appear in the periphery of intact mouse skeletal muscle fibers following osmotic shock. (A) A transverse confocal microscopy line scan image of Fluo-4 fluorescent signal in an intact mouse flexor digitorum brevis muscle fiber. Both short duration Ca^{2+} sparks and longer duration Ca^{2+} burst can be observed from the same spatial location (red box). (B) Pseudocolor magnified image of boxed area in panel A (top) shows relative duration of Ca^{2+} sparks and bursts. Amplitude ($\Delta F/F_0$) tracing for this boxed area (bottom) is shown as well.

The sudden increase in Ca^{2+} spark activity following cell shrinkage is reversible in young, healthy muscle fibers as it returns back to baseline levels in 10 to 15 min after return to normal cell volume. These Ca^{2+} sparks are absent in the presence of high levels of ryanodine, indicating the spark activity is dependent on RyR channel activity. Short term removal of extracellular Ca^{2+} does not have a major effect on osmotic shock-induced Ca^{2+} spark activity, suggesting that osmotic stress-induced sparks are not induced by Ca^{2+} entry from outside the fiber. Our discovery of legitimate manifestations of Ca^{2+} sparks in skeletal muscle enables us to address some fundamental questions in skeletal muscle physiology.

Peripheral confined nature of osmotic stress-induced Ca^{2+} sparks

The numerous Ca^{2+} sparks induced upon shrinkage of the fiber appear exclusively in the periphery of the cell (Figure 2A). The osmotic stress-induced Ca^{2+} spark response is localized near the sarcolemma surfaces despite global swelling of the TT system by osmotic shock. Electron microscopy studies from Chawla *et al* have shown that hypertonic treatment induces subtle changes of triad junction structure in skeletal muscle[27]. We have replicated these experiments and found similar swelling of triad structures throughout the muscle fiber, without significant disruption of sarcolemmal or myofibrillar integrity. It is well known that RyR channels and other associated Ca^{2+} release machinery are found throughout the TT system, and since osmotic shock results in global swelling of TT, the spatial restriction of Ca^{2+} sparks suggests two criteria for the regulation of Ca^{2+} sparks. First, a signal for the initiation of Ca^{2+} sparks must be spatially

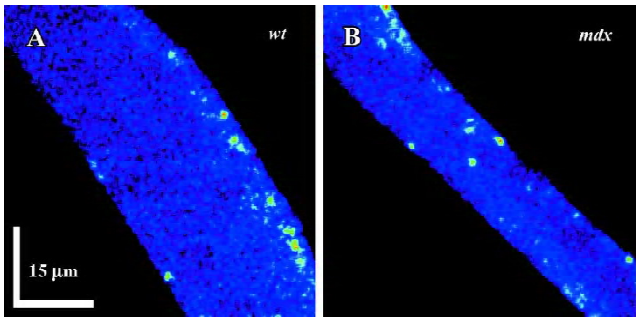

Figure 2. Peripheral nature of Ca^{2+} sparks in wild type muscle is lost in dystrophic muscle. (A) Pseudocolor image of Ca^{2+} sparks in a *wt* mouse skeletal muscle fiber reveals Ca^{2+} sparks are found primarily in the cell periphery close to the sarcolemmal membrane. (B) In dystrophic muscle fibers from *mdx* mice, Ca^{2+} sparks can be found both at the periphery and much closer to the center of the fiber. As the Ca^{2+} spark response continues, more events appear in the fiber center.

confined, eg, either membrane-delimited signaling cascades or localized cytosolic factors should be involved in triggering the SR Ca^{2+} release machinery. Second, a coordinated mechanism must be present to prevent propagation of the Ca^{2+} spark signaling from the periphery toward the central region of a healthy muscle fiber.

Spatial inhomogeneities of SR Ca^{2+} release are not unique to skeletal muscle, as cardiac and smooth muscles also display preferential SR Ca^{2+} release at the periphery of the muscle fiber, at least at the initial stages of cell depolarization[28–32]. Indeed, Ca^{2+} release following membrane deformation is a common phenomena observed in many cell types[33]. While in many of these cases subsarcolemmal Ca^{2+} sparks or waves have been attributed to the limited TT system within these cell types[34], other cell types do display well developed TT

structures[35]. In addition, a previous study also identified osmotic-shock induced Ca^{2+} sparks and Ca^{2+} waves in amphibian skeletal muscle[36].

Role of ryanodine receptor isoforms in Ca^{2+} spark induction

While Ca^{2+} sparks are mediated by opening of RyR, it is not clear how the expression of different RyR isoforms contributes to Ca^{2+} spark formation, particularly within adult mammalian skeletal muscle fibers. Various studies have determined that two RyR isoforms are expressed in mammalian skeletal muscle, RyR1 and RyR3[37–39]. While most adult skeletal muscle primarily expresses RyR1, the RyR3 protein is expressed mainly in neonatal muscle fibers and at a low level in a minority of adult skeletal muscles[40,41]. The function of RyR1 is tightly controlled by DHPR while RyR3 acts as an secondary component that amplifies RyR1-mediated Ca^{2+} release in neonatal skeletal muscle[42]. Overexpression of RyR3 in cultured myotubes[22,43] and non-excitable cells[44] has been shown to produce Ca^{2+} sparks. Homozygous ablation of RyR1 in mice results in post-natal lethality due to defective excitation-contraction coupling[45], while ablation of RyR3 produces viable animals[46–48]. The viability of the *ryr3*(-/-) mouse provides an opportunity to use osmotic shock to determine the contribution of RyR3 to the generation of Ca^{2+} sparks (Table 1).

Osmotic shock-induced Ca^{2+} sparks also occur in *ryr3*(-/-) muscle with a spatial distribution similar to that seen in wild type (*wt*) muscle, suggesting that RyR1 alone is sufficient to produce the dynamic Ca^{2+} spark signal in skeletal muscle fibers. Although RyR3 is not essential for induction of Ca^{2+} sparks in skeletal muscle, kinetic analysis reveals that the absence of RyR3 in the adult muscle fiber leads to significant changes in the elemental properties of Ca^{2+} spark signaling. Our results show that the spatial restriction, initiation rate and amplitude of individual Ca^{2+} release events are altered in the *ryr3*(-/-) muscle, whereas the peripheral localization of active Ca^{2+} release sites and their cross-talk remain unchanged. These changes could reflect the contribution of residual RyR3 function in adult skeletal muscle fibers or adaptations that take place in the developing skeletal muscle to compensate for the loss of RyR3 expression.

Uncontrolled Ca^{2+} sparks as a dystrophic signal in skeletal muscle

In young, healthy muscle fibers, osmotic shock-induced Ca^{2+} spark activity is transient and eventually returns to a silent mode several minutes after the initial shock[25]. This response is plastic in nature as typical muscle fiber can receive up to three osmotic shocks and still maintain a reproducible response. Thus, membrane deformation can induce spontaneous Ca^{2+} spark activity in intact mammalian skeletal muscle in a reversible and repeatable manner.

Duchenne and Becker muscular dystrophy results from mutations within the dystrophin gene. Dystrophin is a protein that links actin in the muscle cytoskeleton to laminin in the extracellular matrix through the dystroglycan complex. It is likely that the dystrophic phenotype is not a direct result of alterations to the myofibrillar structures, rather it is a disruption of sarcolemmal membrane integrity that normally confers control of intracellular Ca^{2+} homeostasis that leads to muscle degeneration[49]. To determine if Ca^{2+} spark signaling was altered in dystrophic skeletal muscle, we used a mouse model that lacks dystrophin, the muscular dystrophic (*mdx*) mouse[50]. One hallmark of the *mdx* muscle is its increased fragility during endurance training and hypotonic shock[51]. Although Ca^{2+} sparks do not appear in *mdx* muscle fibers at a resting state, there is a striking difference in the manifestation of Ca^{2+} sparks following osmotic shock. Similar to *wt* muscle fibers, exposure of the *mdx* muscle to either hypotonic or hypertonic solution also converts a resting and apparently silent muscle fiber into a highly active Ca^{2+} signaling state. In contrast to the transient Ca^{2+} spark activity seen in *wt* fibers, either a hypotonic or hypertonic shock results in sustained Ca^{2+} spark activity that is irreversible in the time period of observation[25] (Table 2).

Differences in the membrane-deformation-induced Ca^{2+} spark response in *mdx* muscle are not limited to its irreversible nature. As with *wt* fibers, Ca^{2+} sparks in *mdx* muscle are usually localized in the peripheral region at the initial stage.

Table 2. Ca^{2+} sparks as an indicator of skeletal muscle integrity.

	Spatial localization	Plasticity of induction	Spark frequency
Young wild type	Peripheral	Highly plastic	Baseline
Young dystrophic	Peripheral → Central	Uncontrolled	Increased
Aged wild type	Peripheral	Static	Decreased

Surprisingly, Ca^{2+} sparks progressively penetrate into the center of the *mdx* muscle fiber at later stages following osmotic shock (Figure 2B). Furthermore, the peak amplitude of Ca^{2+} bursts in *mdx* muscle appears to decline with time after the osmotic shock, possibly due to reduced Ca^{2+} content in the SR. Resting cytosolic Ca^{2+} concentrations are elevated in both *wt* and *mdx* fibers following hypotonic shock, however cytosolic Ca^{2+} levels in *mdx* fibers are consistently increased over *wt*. These results suggest that membrane deformation results in a leaky SR Ca^{2+} release machinery in *mdx* muscle. Although our results do not exclude potential contribution of other factors, it suggests that a leaky intracellular Ca^{2+} release pathway can function as a primary trigger for the dystrophic signal cascade in mammalian skeletal muscle.

Compromised Ca^{2+} spark signaling in aged skeletal muscle

Aging effects on muscle function have been associated with muscle fiber denervation, loss of motor units, and motor unit remodeling. Since functional alterations occur before significant muscle wasting becomes evident, changes in the excitation-contraction coupling machinery and intracellular Ca^{2+} homeostasis may act as causative factors for, or adaptive responses to, muscle aging. Altered function of several triad junction proteins, including DHPR[52,53], calsequestrin[54,55] and SERCA[56,57], have been shown to contribute to disrupted Ca^{2+} homeostasis in aged skeletal muscle. It has been suggested that cumulative uncoupling of the VICR process may be part of the causative and/or adaptive changes during muscle aging[58,59]. However, limitations in resolution of Ca^{2+} sparks in intact muscle have prevented the detailed examination of the mechanisms that underlie changes in Ca^{2+} homeostasis during muscle aging.

Extending our initial discovery of Ca^{2+} sparks in healthy young muscle, we have identified a phenotypic change of Ca^{2+} spark signaling in aged skeletal muscle. Although this Ca^{2+} spark response is located in the periphery of both young and aged muscle fibers, it appears that the plastic nature of Ca^{2+} sparks in young muscle is compromised in aged skeletal muscle where the duration of the Ca^{2+} spark response is diminished and cannot be restimulated by additional rounds of osmotic shock (Table 2). Using biochemical assays, we found that the expression of MG29 was significantly altered in aged skeletal muscle. MG29 is a synaptophysin-family protein that is essential for maintenance of membrane structure in skeletal muscle[60–62]. One can expect that compromised Ca^{2+} spark signaling in aged muscle may be linked to the changes in membrane coupling that result from altered MG29 expression.

Our studies identified a loss of plastic Ca^{2+} spark signaling in young *mg29*(-/-) muscles, in a fashion very similar to that seen in aged skeletal muscle. As with aged *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. In addition, *mg29*(-/-) mice display muscle weakness at age 6 months or younger, which resembles the atrophic phenotype of aged *wt* mice. Relative to young *wt* muscle, aged *wt* muscle appears to contain a diminished Ca^{2+} reservoir responsible for Ca^{2+} spark generation that rapidly depletes, since repeated osmotic shocks do not elicit additional Ca^{2+} spark activity. This may result from the presence of a smaller SR Ca^{2+} pool in aged *wt* and young *mg29*(-/-) skeletal muscle, relative to young *wt* skeletal muscle fibers, or segregation of the intracellular Ca^{2+} release machinery. These and other phenotypic similarities between young *mg29*(-/-) and aged *wt* skeletal muscle suggest the possibility that *mg29*(-/-) mice could mimic some Ca^{2+} related aspects of skeletal muscle aging and may serve as a model for muscle aging under certain conditions.

Conclusions

Since the maintenance of proper Ca^{2+} homeostasis is essential for normal muscle contractile function and survival of muscle fibers, it is not surprising that aberrant Ca^{2+} spark activity in skeletal muscle appears in aged and dystrophic muscle fibers. The function of Ca^{2+} spark activity is well defined in cardiac muscle[63], however there has been relatively little investigation of Ca^{2+} sparks in skeletal muscle. Although the mechanisms underlying the membrane-deformation responses in skeletal muscle may involve changes in multiple cellular factors, our ability to resolve these elemental SR Ca^{2+} release events in intact muscle fibers provides a useful tool to address some of the fundamental questions relating to the nature of SR Ca^{2+} release in skeletal muscle health and disease. The osmotic shock-induced Ca^{2+} sparks in young, healthy skeletal muscle are plastic in nature, a characteristic that is lost during muscular dystrophy and aging. While dystrophic muscle displays uncontrolled Ca^{2+} spark activity, osmotic stress-induced Ca^{2+} sparks in aged skeletal muscle appear to be static.

Our discovery of osmotic stress-induced Ca^{2+} sparks not only opens the way for us to monitor the *in vivo* function of Ca^{2+} sparks, but also raises several important questions: (1) What are the factors limiting the osmotic shock-induced Ca^{2+} sparks to the periphery of muscle fibers? (2) What is the

physiological role of peripherally confined Ca^{2+} sparks in mammalian muscle cells? (3) What are the consequences of loss of spatial confinement or mechanisms regulating either the frequency, magnitude or propagation of Ca^{2+} sparks in skeletal muscle?

While it is unlikely that these spatially confined Ca^{2+} spark signals can directly contribute to myofibril contraction, there are many potential cell biological functions for these peripherally localized Ca^{2+} signals. For example, localized Ca^{2+} sparks may participate in the regulation of cytoskeletal structure or may reflect changes in cytoskeletal organization. The defects observed in dystrophic mouse muscle suggest that this is certainly a possibility. Another potential physiological function of Ca^{2+} sparks could be in volume regulation. Ca^{2+}-dependent K^+ channels located at the sarcolemmal membrane may respond to a local increase of Ca^{2+} sparks and therefore participate in maintenance of cell volume[17,64–66]. Indeed, Ca^{2+}-activated K^+ channels have been shown to be down-regulated in aged smooth muscle cells, leading to impaired arterial tone[67,68]. We have also found in recent experiments that these peripheral Ca^{2+} sparks may be linked to Ca^{2+} entry into the myofiber. These studies and future experiments will lead to a better understanding of the regulatory role of induced Ca^{2+} sparks in the physiology of healthy muscles and the pathophysiology of muscle disease and aging.

References

1 Cheng H, Lederer WJ, Cannell MB. Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. Science 1993; 262: 740–4.

2 Lopez-Lopez JR, Shacklock PS, Balke CW, Wier WG. Local calcium transients triggered by single L-type calcium channel currents in cardiac cells. Science 1995; 268: 1042–5.

3 Wier WG, Balke CW. Ca^{2+} release mechanisms, Ca^{2+} sparks, and local control of excitation-contraction coupling in normal heart muscle. Circ Res 1999; 85: 770–6.

4 Fong PY, Turner PR, Denetclaw WF, Steinhardt RA. Increased activity of calcium leak channels in myotubes of Duchenne human and *mdx* mouse origin. Science 1990; 250: 673–6.

5 Takagi A, Kojima S, Ida M, Araki M. Increased leakage of calcium ion from the sarcoplasmic reticulum of the *mdx* mouse. J Neurol Sci 1992; 110: 160–4.

6 Brotto MA, Nosek TM, Kolbeck RC. Influence of ageing on the fatigability of isolated mouse skeletal muscles from mature and aged mice. Exp Physiol 2002; 87: 77–82.

7 Rios E, Ma JJ, Gonzalez A. The mechanical hypothesis of excitation-contraction (EC) coupling in skeletal muscle. J Muscle Res Cell Motil 1991; 12: 127–35.

8 Rios E, Pizarro G, Stefani E. Charge movement and the nature of signal transduction in skeletal muscle excitation-contraction coupling. Annu Rev Physiol 1992; 54: 109–33.

9 Meissner G. Ryanodine receptor/Ca^{2+} release channels and their regulation by endogenous effectors. Annu Rev Physiol 1994; 56: 485–508.

10 Schneider MF. Control of calcium release in functioning skeletal muscle fibers. Annu Rev Physiol 1994; 56: 463–84.

11 Franzini-Armstrong C, Jorgensen AO. Structure and development of E-C coupling units in skeletal muscle. Annu Rev Physiol 1994; 56: 509–34.

12 Ito K, Komazaki S, Sasamoto K, Yoshida M, Nishi M, Kitamura K, *et al*. Deficiency of triad junction and contraction in mutant skeletal muscle lacking junctophilin type 1. J Cell Biol 2001; 154: 1059–67.

13 Cheng H, Song LS, Shirokova N, Gonzalez A, Lakatta EG, Rios E, *et al*. Amplitude distribution of calcium sparks in confocal images: theory and studies with an automatic detection method. Biophys J 1999; 76: 606–17.

14 Wang SQ, Stern MD, Rios E, Cheng H. The quantal nature of Ca^{2+} sparks and *in situ* operation of the ryanodine receptor array in cardiac cells. Proc Natl Acad Sci USA 2004; 101: 3979–84.

15 Wier WG, ter Keurs HE, Marban E, Gao WD, Balke CW. Ca^{2+} 'sparks' and waves in intact ventricular muscle resolved by confocal imaging. Circ Res 1997; 81: 462–9.

16 Kamishima T, Quayle JM. Ca^{2+}-induced Ca^{2+} release in cardiac and smooth muscle cells. Biochem Soc Trans 2003; 31: 943–6.

17 Nelson MT, Cheng H, Rubart M, Santana LF, Bonev AD, Knot HJ, *et al*. Relaxation of arterial smooth muscle by calcium sparks. Science 1995; 270: 633–7.

18 Conklin MW, Barone V, Sorrentino V, Coronado R. Contribution of ryanodine receptor type 3 to Ca^{2+} sparks in embryonic mouse skeletal muscle. Biophys J 1999; 77: 1394–403.

19 Klein MG, Cheng H, Santana LF, Jiang YH, Lederer WJ, Schneider MF. Two mechanisms of quantized calcium release in skeletal muscle. Nature 1996; 379: 455–8.

20 Zhou J, Brum G, Gonzalez A, Launikonis BS, Stern MD, Rios E. Ca^{2+} sparks and embers of mammalian muscle. Properties of the sources. J Gen Physiol 2003; 122: 95–114.

21 Shirokova N, Garcia J, Rios E. Local calcium release in mammalian skeletal muscle. J Physiol 1998; 512 (Pt 2): 377–84.

22 Ward CW, Schneider MF, Castillo D, Protasi F, Wang Y, Chen SR, *et al*. Expression of ryanodine receptor RyR3 produces Ca^{2+} sparks in dyspedic myotubes. J Physiol 2000; 525 Pt 1: 91–103.

23 Sutko JL, Airey JA, Murakami K, Takeda M, Beck C, Deerinck T, *et al*. Foot protein isoforms are expressed at different times during embryonic chick skeletal muscle development. J Cell Biol 1991; 113: 793–803.

24 Kirsch WG, Uttenweiler D, Fink RH. Spark- and ember-like elementary Ca^{2+} release events in skinned fibres of adult mammalian skeletal muscle. J Physiol 2001; 537: 379–89.

25 Wang X, Weisleder N, Collet C, Zhou J, Chu Y, Hirata Y, *et al*. Uncontrolled calcium sparks act as a dystrophic signal for mammalian skeletal muscle. Nat Cell Biol 2005; 7: 525–30.

26 Ward CW, Lederer WJ. Ghost sparks. Nat Cell Biol 2005; 7: 457–9.

27 Chawla S, Skepper JN, Hockaday AR, Huang CL. Calcium waves

induced by hypertonic solutions in intact frog skeletal muscle fibres. J Physiol 2001; 536: 351–9.
28 Ohi Y, Yamamura H, Nagano N, Ohya S, Muraki K, Watanabe M, *et al*. Local Ca^{2+} transients and distribution of BK channels and ryanodine receptors in smooth muscle cells of guinea-pig vas deferens and urinary bladder. J Physiol 2001; 534: 313–26.
29 Berlin JR. Spatiotemporal changes of Ca^{2+} during electrically evoked contractions in atrial and ventricular cells. Am J Physiol 1995; 269: H1165–70.
30 Huser J, Lipsius SL, Blatter LA. Calcium gradients during excitation-contraction coupling in cat atrial myocytes. J Physiol 1996; 494 (Pt 3): 641–51.
31 Boyden PA, Pu J, Pinto J, Keurs HE. Ca^{2+} transients and Ca^{2+} waves in purkinje cells: role in action potential initiation. Circ Res 2000; 86: 448–55.
32 Cordeiro JM, Spitzer KW, Giles WR, Ershler PE, Cannell MB, Bridge JH. Location of the initiation site of calcium transients and sparks in rabbit heart Purkinje cells. J Physiol 2001; 531: 301–14.
33 Jakab M, Furst J, Gschwentner M, Botta G, Garavaglia ML, Bazzini C, *et al*. Mechanisms sensing and modulating signals arising from cell swelling. Cell Physiol Biochem 2002; 12: 235–58.
34 Haddock PS, Coetzee WA, Cho E, Porter L, Katoh H, Bers DM, *et al*. Subcellular $[Ca^{2+}]_i$ gradients during excitation-contraction coupling in newborn rabbit ventricular myocytes. Circ Res 1999; 85: 415–27.
35 Heinzel FR, Bito V, Volders PG, Antoons G, Mubagwa K, Sipido KR. Spatial and temporal inhomogeneities during Ca^{2+} release from the sarcoplasmic reticulum in pig ventricular myocytes. Circ Res 2002; 91: 1023–30.
36 Martin CA, Petousi N, Chawla S, Hockaday AR, Burgess AJ, Fraser JA, *et al*. The effect of extracellular tonicity on the anatomy of triad complexes in amphibian skeletal muscle. J Muscle Res Cell Motil 2003; 24: 407–15.
37 Takeshima H, Nishimura S, Matsumoto T, Ishida H, Kangawa K, Minamino N, *et al*. Primary structure and expression from complementary DNA of skeletal muscle ryanodine receptor. Nature 1989; 339: 439–45.
38 Giannini G, Clementi E, Ceci R, Marziali G, Sorrentino V. Expression of a ryanodine receptor-Ca^{2+} channel that is regulated by TGF-beta. Science 1992; 257: 91–4.
39 Hakamata Y, Nishimura S, Nakai J, Nakashima Y, Kita T, Imoto K. Involvement of the brain type of ryanodine receptor in T-cell proliferation. FEBS Lett 1994; 352: 206–10.
40 Conti A, Gorza L, Sorrentino V. Differential distribution of ryanodine receptor type 3 (RyR3) gene product in mammalian skeletal muscles. Biochem J 1996; 316 (Pt 1): 19–23.
41 Murayama T, Ogawa Y. Roles of two ryanodine receptor isoforms coexisting in skeletal muscle. Trends Cardiovasc Med 2002; 12: 305–11.
42 Yang D, Pan Z, Takeshima H, Wu C, Nagaraj RY, Ma J, *et al*. RyR3 amplifies RyR1-mediated Ca^{2+}-induced Ca^{2+} release in neonatal mammalian skeletal muscle. J Biol Chem 2001; 276: 40210–4.
43 Fessenden JD, Wang Y, Moore RA, Chen SR, Allen PD, Pessah IN. Divergent functional properties of ryanodine receptor types 1 and 3 expressed in a myogenic cell line. Biophys J 2000; 79: 2509–25.
44 Rossi D, Simeoni I, Micheli M, Bootman M, Lipp P, Allen PD, *et al*. RyR1 and RyR3 isoforms provide distinct intracellular Ca^{2+} signals in HEK 293 cells. J Cell Sci 2002; 115: 2497–504.
45 Takeshima H, Iino M, Takekura H, Nishi M, Kuno J, Minowa O, *et al*. Excitation-contraction uncoupling and muscular degeneration in mice lacking functional skeletal muscle ryanodine-receptor gene. Nature 1994; 369: 556–9.
46 Takeshima H, Ikemoto T, Nishi M, Nishiyama N, Shimuta M, Sugitani Y, *et al*. Generation and characterization of mutant mice lacking ryanodine receptor type 3. J Biol Chem 1996; 271: 19649–52.
47 Futatsugi A, Kato K, Ogura H, Li ST, Nagata E, Kuwajima G, *et al*. Facilitation of NMDAR-independent LTP and spatial learning in mutant mice lacking ryanodine receptor type 3. Neuron 1999; 24: 701–13.
48 Balschun D, Wolfer DP, Bertocchini F, Barone V, Conti A, Zuschratter W, *et al*. Deletion of the ryanodine receptor type 3 (RyR3) impairs forms of synaptic plasticity and spatial learning. EMBO J 1999; 18: 5264–73.
49 Lynch GS, Rafael JA, Chamberlain JS, Faulkner JA. Contraction-induced injury to single permeabilized muscle fibers from *mdx*, transgenic *mdx*, and control mice. Am J Physiol Cell Physiol 2000; 279: C1290–4.
50 Allamand V, Campbell KP. Animal models for muscular dystrophy: valuable tools for the development of therapies. Hum Mol Genet 2000; 9: 2459–67.
51 Leijendekker WJ, Passaquin AC, Metzinger L, Ruegg UT. Regulation of cytosolic calcium in skeletal muscle cells of the *mdx* mouse under conditions of stress. Br J Pharmacol 1996; 118: 611–16.
52 Delbono O, O'Rourke KS, Ettinger WH. Excitation-calcium release uncoupling in aged single human skeletal muscle fibers. J Membr Biol 1995; 148: 211–22.
53 Renganathan M, Messi ML, Delbono O. Dihydropyridine receptor-ryanodine receptor uncoupling in aged skeletal muscle. J Membr Biol 1997; 157: 247–53.
54 Margreth A, Damiani E, Bortoloso E. Sarcoplasmic reticulum in aged skeletal muscle. Acta Physiol Scand 1999; 167: 331–8.
55 Narayanan N, Jones DL, Xu A, Yu JC. Effects of aging on sarcoplasmic reticulum function and contraction duration in skeletal muscles of the rat. Am J Physiol 1996; 271: C1032–40.
56 Chen B, Jones TE, Bigelow DJ. The nucleotide-binding site of the sarcoplasmic reticulum Ca-ATPase is conformationally altered in aged skeletal muscle. Biochemistry 1999; 38: 14887–96.
57 Schoneich C, Viner RI, Ferrington DA, Bigelow DJ. Age-related chemical modification of the skeletal muscle sarcoplasmic reticulum Ca-ATPase of the rat. Mech Ageing Dev 1999; 107: 221–31.
58 Delbono O, Renganathan M, Messi ML. Excitation-Ca^{2+} release-contraction coupling in single aged human skeletal muscle fiber. Muscle Nerve 1997; Suppl 5: S88–92.

59 Payne AM, Delbono O. Neurogenesis of excitation-contraction uncoupling in aging skeletal muscle. Exerc Sport Sci Rev 2004; 32: 36–40.
60 Takeshima H, Shimuta M, Komazaki S, Ohmi K, Nishi M, Iino M, *et al*. Mitsugumin29, a novel synaptophysin family member from the triad junction in skeletal muscle. Biochem J 1998; 331 (Pt 1): 317–22.
61 Nishi M, Komazaki S, Kurebayashi N, Ogawa Y, Noda T, Iino M, *et al*. Abnormal features in skeletal muscle from mice lacking mitsugumin29. J Cell Biol 1999; 147: 1473–80.
62 Pan Z, Yang D, Nagaraj RY, Nosek TA, Nishi M, Takeshima H, *et al*. Dysfunction of store-operated calcium channel in muscle cells lacking mg29. Nat Cell Biol 2002; 4: 379–83.
63 Sjaastad I, Wasserstrom JA, Sejersted OM. Heart failure – a challenge to our current concepts of excitation-contraction coupling. J Physiol 2003; 546: 33–47.
64 Vergara C, Ramirez BU. Age-dependent expression of the apamin-sensitive calcium-activated K^+ channel in fast and slow rat skeletal muscle. Exp Neurol 1997; 146: 282–5.
65 Alioua A, Mahajan A, Nishimaru K, Zarei MM, Stefani E, Toro L. Coupling of c-Src to large conductance voltage- and Ca^{2+}-activated K^+ channels as a new mechanism of agonist-induced vasoconstriction. Proc Natl Acad Sci USA 2002; 99: 14560–5.
66 Toro L, Alioua A, Mahajan A, Nishimaru K, Zarei MM, Stefani E. MaxiK, c-Src and vasoconstriction. J Muscle Res Cell Motil 2004; 25: 616–17.
67 Toro L, Marijic J, Nishimaru K, Tanaka Y, Song M, Stefani E. Aging, ion channel expression, and vascular function. Vascul Pharmacol 2002; 38: 73–80.
68 Marijic J, Li Q, Song M, Nishimaru K, Stefani E, Toro L. Decreased expression of voltage- and Ca^{2+}-activated K^+ channels in coronary smooth muscle during aging. Circ Res 2001; 88: 210–16.
69 Zhou J, Yi J, Royer L, Launikonis BS, Gonzalez A, Garcia J, *et al*. A probable role of dihydropyridine receptors in repression of Ca^{2+} sparks demonstrated in cultured mammalian muscle. Am J Physiol Cell Physiol 2006; 290: C539–53.