Published in final edited form as:

Ageing Res Rev. 2008 July ; 7(3): 177–188. doi:10.1016/j.arr.2007.12.003.

Altered Ca^{2+} sparks in aging skeletal and cardiac muscle

Noah Weisleder and Jianjie Ma

Department of Physiology and Biophysics, Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854

Abstract

Ca^{2+} sparks are the fundamental units that comprise Ca^{2+} -induced Ca^{2+} release (CICR) in striated muscle cells. In cardiac muscle, spontaneous Ca^{2+} sparks underlie the rhythmic CICR activity during heart contraction. In skeletal muscle, Ca^{2+} sparks remain quiescent during the resting state and are activated in a plastic fashion to accommodate various levels of stress. With aging, the plastic Ca^{2+} spark signal becomes static in skeletal muscle, whereas loss of CICR control leads to leaky Ca^{2+} spark activity in aged cardiomyocytes. Ca^{2+} spark responses reflect the integrated function of the intracellular Ca^{2+} regulatory machinery centered around the triad or dyad junctional complexes of striated muscles, which harbor the principal molecular players of excitation-contraction coupling. This review highlights the contribution of age-related modification of the Ca^{2+} release machinery and the effect of membrane structure and membrane cross-talk on the altered Ca^{2+} spark signaling during aging of striated muscles.

Introduction

Aging effects on cardiac and skeletal muscle function are associated with the loss of contractility and loss of muscle mass. Since muscle dysfunction can be observed prior to significant muscle atrophy, alteration of intracellular Ca^{2+} signaling may contribute to, or serve as an adaptive response to, muscle aging phenotypes. Although many factors may contribute to the progressive sarcopenia and weakness of contraction in aging muscle (i.e., muscle fiber switch, changes in metabolic capacity, intracellular redox state and handling, denervation, cellular repair machinery, etc.), maintenance of the cellular homeostatic capacity for Ca^{2+} appears to be the central player, as Ca^{2+} is vital to the many physiological events up and downstream of contractile function.

During aging, the skeletal and cardiac muscle must adapt to physiological and pathophysiological stresses, many of which come in pulsatile signals such as strenuous exercise, fatigue, trauma, and hormonal stimulation that often involve elevated Ca^{2+} cycling over and above the normal physiological settings. Ca^{2+} sparks are the building blocks of intracellular Ca^{2+} signaling, and are inherently pulsatile in nature. The spatial and temporal aspects of Ca^{2+} sparks reflect the integrated function of Ca^{2+} release, uptake and recycling events occurring under normal and pathophysiological conditions with development and aging. This review will discuss recent advances made in characterizing the changes in Ca^{2+} spark

© 2008 Elsevier Ireland Ltd. All rights reserved.

Address correspondence to, Jianjie Ma, 732-235-4494, maj2@umdnj.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

signaling during aging, and the molecular components that contribute to the altered pulsatility of Ca^{2+} sparks in skeletal and cardiac muscle.

Differential properties of Ca^{2+} sparks in cardiac and skeletal muscle

Excitation-contraction (E-C) coupling in striated muscle requires that membrane depolarization results in release from Ca^{2+} from the sarcoplasmic reticulum (SR). This is facilitated by junctional membrane complexes (dyad or triad) that provide a structural context where the dihydropyridine receptor (DHPR), a voltage-gated Ca^{2+} channel, in the sarcolemmal membrane can be held in close physical proximity to the ryanodine receptor (RyR) Ca^{2+} in the SR. While the molecular machinery is similar in striated muscle, both cardiac and skeletal muscle display distinct mechanisms to control SR Ca^{2+} release. In cardiac muscle, these proteins interact at the dyad junction where DHPR facilitates extracellular Ca^{2+} entry that induces RyR activation through an amplification process of Ca-induced Ca^{2+} release (CICR) to allow for the rhythmic contraction of the heart (Bers, 2001). Due to the voluntary nature of skeletal muscle contraction the release of Ca^{2+} from the SR in skeletal muscle fibers is more tightly controlled. Thus, membrane depolarization takes the place of external Ca^{2+} entry as the signal to induce intracellular Ca^{2+} release. The triad junction in skeletal muscle fibers is comprised of a transverse (t)-tubule invagination of the sarcolemma and a pair of terminal cisternae of SR. This configuration holds the DHPR and RyR in close juxtaposition to permit direct relay of the membrane depolarization signal in a process known as voltage-induced Ca^{2+} release (VICR) (Rios et al., 1991; Rios et al., 1992; Meissner, 1994; Schneider, 1994). In skeletal muscle, VICR can be followed by CICR, especially under stress conditions (Fig. 1).

The global increase in intracellular Ca^{2+} that triggers contraction in striated muscle is the summation of smaller, discreet events known as Ca^{2+} sparks (Csernoch, 2007). These localized quantal Ca^{2+} release events can be visualized using confocal microscopy imaging of Ca^{2+} indicator dyes. The initial discovery of Ca^{2+} sparks was made in cardiac muscle, and further study these events originate from RyR channel arrays in the SR membrane (Cheng et al., 1993; Wier et al., 1997; Wang et al., 2004a). These and other discoveries of the function of Ca^{2+} sparks in the physiology and pathophysiology of cardiac and smooth muscles has revolutionized our understanding of Ca^{2+} signaling in these muscle types (Nelson et al., 1995; Kamishima and Quayle, 2003).

Ca^{2+} sparks in cardiac muscle appear spontaneously throughout the cardiomyocyte. This spontaneous activity has functional consequences for muscle contractility as the summation of these events leads to the rhythmic CICR activity required for synchronized contraction of the heart. Elegant studies by Cheng and colleagues demonstrated a tight coupling between Ca^{2+} influx through DHPR and intracellular Ca^{2+} release through RyR channels (Wang et al., 2001; Cheng and Wang, 2002). Thus, Ca^{2+} influx with discrete spatial restrictions, known as Ca^{2+} sparklets, serve as a trigger for the onset of Ca^{2+} sparks and also for the global CICR response in cardiac muscle.

Compared with cardiac muscle, the molecular machinery controlling Ca^{2+} signaling and homeostasis in skeletal muscle has many different properties. First, the genes encoding RyR Ca^{2+} release channel and DHPR are different isoforms from those in cardiomyocytes (McPherson and Campbell, 1993). Second, the ultrastructural arrangement of the junctional complex between sarcolemma and SR are different. Cardiomyocytes have less developed t-tubule system that primarily form dyad junctions in the periphery of the cell, while skeletal muscle has a more ordered t-tubule system that forms a triad junction structure running throughout the muscle fiber. The t-tubule system in skeletal muscle is thought to serve an inhibitory role on SR Ca^{2+} release (Lee et al., 2004; Zhou et al., 2006), which further contributes

to the differences in Ca^{2+} sparks between skeletal and cardiac muscle. Third, cardiac muscle contraction is dependent on transmission of excitation from one myocyte to another through a syncytial arrangement involving gap junction connections, whereas skeletal muscle contraction is under the control of innervation of single motor units through neuromuscular junctions. Fourth, Ca^{2+} movement across the sarcolemma of skeletal muscle is limited by the slow kinetics of the L-type Ca^{2+} current in skeletal muscle (Sanchez and Stefani, 1978). In cardiac muscle, voltage-induced activation of the L-type Ca^{2+} current produces the initial Ca^{2+} influx signal that allows amplification of intracellular Ca^{2+} release through CICR. As a result, sufficient Ca^{2+} extrusion mechanisms must be present in the cardiac muscle to balance the internal Ca^{2+} concentration by removing excess Ca^{2+} from the myocyte. To provide this capacity to cardiomyocytes, Na^{2+} - Ca^{2+} exchanger (NCX) is abundant in cardiac muscle while it is less abundant in skeletal muscle (Eisner and Sipido, 2004).

While spontaneous Ca^{2+} sparks underlie the rhythmic contraction of cardiac muscle, these spontaneous events must be suppressed at the resting state of skeletal muscle, since contraction of skeletal muscle occurs on a voluntary basis. Moreover, maintenance of quiescent Ca^{2+} release machinery while at rest is essential for development of skeletal muscle and to avoid aberrant contraction. Thus it is not surprising that investigators have rarely detected spontaneous Ca^{2+} sparks in intact adult mammalian skeletal muscle (Shirokova et al., 1998; Conklin et al., 1999). Early studies reported numerous spontaneous Ca^{2+} sparks in amphibian skeletal muscle (Klein et al., 1996) and in neonatal mammalian skeletal muscle (Shirokova et al., 1998). Such activity in these muscle preparations were attributed to the action of the type 3 RyR (Ward et al., 2000), an RyR isoform highly expressed in skeletal muscle during fetal development but only found in small quantities in adult muscle (Sutko et al., 1991). Until recently, significant numbers of Ca^{2+} sparks were only observed in adult mammalian skeletal fibers following permeabilization of their sarcolemmal membrane with various physical or chemical methods (Kirsch et al., 2001; Zhou et al., 2003). Because of these intrinsic difficulties with monitoring CICR activity in intact mammalian skeletal muscle fibers, the cellular and molecular mechanisms underlying the regulation of CICR in skeletal muscle and the adaptive changes of CICR in muscle aging remain largely unknown. It has been suggested that cumulative uncoupling of the VICR process may be part of the causative and/or adaptive changes during muscle aging (Delbono et al., 1997; Payne and Delbono, 2004). However, limitations in resolution of elemental Ca^{2+} release units in intact muscle have prevented the detailed examination of the mechanisms that underlie changes in Ca^{2+} homeostasis during muscle aging.

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

Recently, our group discovered that transient osmotically induced membrane deformation results in a fluttered Ca^{2+} spark response adjacent to the sarcolemmal membrane in intact mammalian muscle fibers (Wang et al., 2005; Ward and Lederer, 2005) (Fig. 2A). These stress-induced Ca^{2+} sparks are reversible and repeatable in young and healthy muscle fibers, and therefore are of plastic nature in these muscles. Our studies also suggest a physiological function for Ca^{2+} sparks in skeletal muscle, linking enhanced Ca^{2+} spark activity to muscle exercise (Wang et al., 2005). These findings suggest that Ca^{2+} sparks are rarely, if ever, completely quiescent in skeletal muscle *in vivo*. Although the mechanisms underlying the membrane-deformation responses in skeletal muscle may involve changes in multiple cellular factors, our ability to resolve these elemental Ca^{2+} release events from SR 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.

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. 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 (Fig. 2C) (Weisleder et al., 2006). We reasoned that stress-induced Ca^{2+} sparks are a representation of the normal physiological response of healthy skeletal muscle, whose dysfunction may signify altered Ca^{2+} handling in aging muscle.

Understanding the cellular mechanism underlying the compromised Ca^{2+} spark signaling in aged muscle should provide valuable insights into potential therapeutic targets for improvement of muscle function during aging. We determined that stress-induced Ca^{2+} sparks do not result from the movement of Cl^- ions across the cell membrane, as replacement of Cl^- with glutamate in the extracellular solution produces a similar response (Weisleder et al., 2007b). Removal of extracellular Ca^{2+} does not impact Ca^{2+} spark characteristics in the short term, suggesting that intracellular Ca^{2+} release events contribute to the Ca^{2+} spark response following osmotic stress. These intracellular Ca^{2+} release events originate from RyR1, the dominant RyR isoforms present in adult skeletal muscle, as they can be observed in muscle fibers isolated from *ryr3*($-/-$) mice (Weisleder et al., 2007a). Moreover, individual muscle fibers under voltage-clamp control also display peripherally confined Ca^{2+} sparks in response to osmotic stress, suggesting that changes in membrane potential are not necessary to induce Ca^{2+} sparks in skeletal muscle (unpublished observations). One of the unique features of stress-induced Ca^{2+} sparks in healthy young skeletal muscle is the confinement to the peripheral region of the muscle fiber. Since RyR channels and other associated Ca^{2+} release machinery are found throughout the t-tubule system, and since osmotic shock results in global swelling of t-tubule membrane (Chawla et al., 2001), the spatial restriction of Ca^{2+} sparks suggests that either membrane delimited signals or spatially restricted cytosolic factors are involved in the initiation and/or restriction of these Ca^{2+} sparks. In addition, a coordinated control mechanism must also be involved in preventing stress-induced Ca^{2+} sparks from propagating toward the central of the muscle fiber, as otherwise uncontrolled Ca^{2+} sparks can turn into a dystrophic signal for skeletal muscle (Wang et al., 2005).

Unlike aged skeletal muscle where there is an abrogated stress-induced Ca^{2+} spark response, aged cardiac muscle appears to display increase in the frequency of spontaneous Ca^{2+} spark activity at basal condition, whereas the amplitude of global systolic Ca^{2+} transients are reduced compared to a young cardiomyocytes (Lim et al., 2000; Isenberg et al., 2003; Howlett et al., 2006). These and other studies have shown that the diastolic cytosolic Ca^{2+} level (Howlett et al., 2006) and the releasable Ca^{2+} pool (Dibb et al., 2004) from the SR are not very different between young and senescent cardiomyocytes. Therefore, a leaky Ca^{2+} pathway across the SR membrane due to modification of the RyR2 channel, or its associated components, likely contributes to the elevated Ca^{2+} spark activity in senescent cardiomyocytes. In addition, increased extrusion of Ca^{2+} from the cytosol is also probably present in aged cardiomyocytes to account for the lack of an elevation in the cytosol resting Ca^{2+} (Janczewski et al., 2002). Recent studies have indicated that the efficiency of SR Ca^{2+} release may further suffer from the reduced coupling between Ca^{2+} sparklets and Ca^{2+} sparks in aged cardiomyocytes (Wang et al., 2004b). Thus, aging can compromise the efficiency of Ca^{2+} spark signaling in cardiomyocytes, altering the pulsatile nature of Ca^{2+} in cardiomyocytes and impacting cardiac function (Lindner et al., 2002).

Several clues to the molecular machinery that may be altered in aged cardiac muscle are provided by studies of pathologic states that also display SR with a leaky Ca^{2+} phenotype. Mutations in various proteins associated with cardiomyocyte Ca^{2+} handling can cause a predisposition to sudden death cardiac events. One such protein is RyR2, where specific mutations result in a leaky RyR Ca^{2+} release channel that can result in arrhythmogenesis under stress conditions (Jiang et al., 2002; George et al., 2003; Lehnart et al., 2004; Kannankeril et al., 2006; Liu et al., 2006). Another series of mutations in calsequestrin (CSQ2) have been

shown to induce leaky Ca^{2+} release from the SR that is mediated at least in part by induction of increased RyR2 expression (Terentyev et al., 2003; di Barletta et al., 2006; Terentyev et al., 2006; Dirksen et al., 2007; Song et al., 2007). The role of CSQ in control of SR Ca^{2+} release is emphasized by findings that overexpression of CSQ results in reduced Ca^{2+} spark frequency and co-ordination, mirroring the effect of CSQ mutations on SR Ca^{2+} release (Wang et al., 2000). Shin, et al. demonstrated that in skeletal muscle CSQ is also involved in retrograde regulation of RyR function and store-operated Ca^{2+} entry (SOCE) (Shin et al., 2003). Additional evidence is available that CSQ function is altered during muscle aging (Narayanan et al., 1996; Margreth et al., 1999). Thus, changes in the SR luminal Ca^{2+} buffering capacity, or modification of the RyR release machinery from the luminal side, certainly plays an important role in the retrograde regulation of Ca^{2+} signaling in striated muscle (Ma and Pan, 2003; Brochet et al., 2005). Exactly how alteration of the retrograde signaling process influences Ca^{2+} spark activity during striated muscle aging is not clear at present, which should constitute an interesting avenue for future studies.

Alteration of intracellular membrane ultrastructure during muscle aging

While it is clear that various proteins, including DHPR (Delbono et al., 1995; Renganathan et al., 1997) and SERCA (Chen et al., 1999; Schoneich et al., 1999), display altered function in aged skeletal muscle and thus are likely to contribute to the altered Ca^{2+} homeostasis in aging, there are additional factors that contribute to Ca^{2+} homeostasis in young and aged muscle. Our recent study found that one major phenotype of aged skeletal muscle is the segregation of a portion of the SR Ca^{2+} store away from the normal VICR machinery (Weisleder et al., 2006). Repeated depolarization of intact muscles in the absence of extracellular Ca^{2+} resulted in decreasing specific force generation. When caffeine was applied to liberate the remaining RyR-containing Ca^{2+} store, young muscles generated minimal additional force, indicating that little Ca^{2+} remained in the SR. In contrast, a large force was generated by aged skeletal muscles, suggesting that a portion of the pool of total Ca^{2+} within the SR of aged fibers cannot be mobilized by VICR. This apparent segregation of Ca^{2+} release would require functional discontinuity of the Ca^{2+} release control machinery and the SR Ca^{2+} store itself. The cause of such changes was not immediately evident until electron microscopy examination of the aged muscle ultrastructure indicated significant disruption of intracellular membrane systems at the skeletal muscle triad junction.

Several defects in intracellular membrane structures have been observed in aged skeletal muscle. The most striking defect is the increased prevalence of tubular aggregates within the fast twitch muscles of aged mice (Agbulut et al., 2000). These tubular aggregates arise from the SR and are known to contain SERCA1, sarcalumenin, CSQ and RyR1 (Chevessier et al., 2004). Such structures are found in human muscle, particularly following burn injury or in patients with tubular aggregate myopathy (Agbulut et al., 2000). Since these structures contain RyR1 and SERCA1, and they are physically uncoupled from the DHPR on the t-tubules at the triad junction, they were an intriguing candidate for the source of this uncoupled Ca^{2+} pool. However, further studies confirmed that these aggregates were only observed in fast twitch fibers. The uncoupled Ca^{2+} pool appeared to be much greater in muscles that are primarily slow twitch type (Weisleder et al., 2006), arguing against that tubular aggregates correlate with the segregated Ca^{2+} pool in aged skeletal muscle. While most male mice from inbred strains display these tubular aggregates (Agbulut et al., 2000), the increase in quantity of these structures with age may have phenotypic effects outside of E-C coupling. Such severe disruptions of cellular structure could result in some degree of contractile dysfunction or even the death of muscle fibers.

A subtle defect in aged skeletal muscle ultrastructure observed during our studies is SR fragmentation and t-tubule swelling (Weisleder et al., 2006). In contrast with the tubular

aggregates, the extent of these defects correspond with the size of the uncoupled Ca^{2+} pool in a given muscle type. Additionally, electron microscopy studies of aged human muscle revealed similar disruptions of the triad junctions, but not an increased presence of large tubular aggregates (Boncompagni et al., 2006). Thus, it is likely that one factor contributing to the compromised Ca^{2+} spark signaling in aged skeletal muscle may originate from the disruption of the connection between the t-tubule and SR membrane surfaces.

MG29 as a marker of age-related dysfunction in skeletal muscle

Identification of molecular markers of muscle aging, and their contribution to aging-related muscle dysfunction, has recently emerged as a focus in E-C coupling studies and geriatric medical research in general. It has been suggested that cumulative uncoupling of the VICR process may be part of the causative and/or adaptive changes during muscle aging (Payne and Delbono, 2004). If disruption of the triad junction membrane structure contributes to muscle dysfunction during aging, it would be advantageous to understand the molecular basis for this change. Previously, we discussed some of the proteins that display altered expression or function during muscle aging. These proteins generally have a direct role in handling intracellular Ca^{2+} ions and only an indirect role in establishing and maintaining the structural integrity of the triad junction. Therefore, other candidate molecules may contribute more directly to aging-related changes in triad architecture. We have recently established that mitsugumin 29 (MG29) is a candidate for one of these molecular components (Fig. 2B).

MG29 is a synaptophysin-family member protein localized in the triad junction of skeletal muscle (Takeshima et al., 1998; Komazaki et al., 1999; Nishi et al., 1999; Komazaki et al., 2001), where the t-tubule invagination of sarcolemmal membrane touches the terminal cisternae of SR. We know that MG29 is essential for the maturation and development of the triad junction, as swollen t-tubules and fragmented SR networks were observed in muscle fibers isolated from the *mg29*^{-/-} mice (Nishi et al., 1999; Komazaki et al., 2001). Interestingly, some of these ultrastructural defects were also observed in aged wild type muscle, raising the possibility that defective membrane integrity or membrane cross-talk may be partially responsible for defects associated with aging (Boncompagni et al., 2006; Weisleder et al., 2006).

Using biochemical assays, we found that the expression of MG29 is significantly reduced in aged skeletal muscle (Weisleder et al., 2006) (Fig. 2B). A series of studies on the role of MG29 in muscle Ca^{2+} signaling (Pan et al., 2002; Brotto et al., 2004; Pan et al., 2004) have provided intriguing observations that allowed us to formulate the hypothesis that MG29 could act as a sentinel, shielding muscle from aging induced alterations in Ca^{2+} homeostasis. There were two important observations in these initial studies that suggest this is the case. First, the *mg29*^{-/-} mice display contractile alterations and muscle atrophy at ages of 6 month or younger (Nagaraj et al., 2000), which resemble the atrophic phenotype of wild type mice at ages 27 months or older. Second, SOCE in *mg29*^{-/-} muscle is significantly down-regulated (Pan et al., 2002), which is similar to the dysfunctional properties of SOCE identified in various cell types from aged animals (Papazafiri and Kletsas, 2003; Vanterpool et al., 2005), including skeletal muscle (unpublished observations).

To further test if young *mg29*^{-/-} muscle displays structural and Ca^{2+} handling defects that are similar to aged wild type muscle, we conducted osmotic stress induced Ca^{2+} spark experiments and electron microscopy examinations. Indeed, 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 wild type skeletal muscle (Weisleder et al., 2006) (Fig. 2C). Furthermore, we also identified a segregated Ca^{2+} pool in muscle from young *mg29*^{-/-} mice that was very similar to the size of the pool in aged wild type skeletal muscle (Weisleder et al., 2006). These

alterations to cellular Ca^{2+} handling are complemented by the similar defects in triad junction membrane ultrastructure in the young *mg29(-/-)* muscle and the aged wild type muscle. Fragmentation of SR and swelling of t-tubules appear in *mg29(-/-)* muscle in a similar percentage of fibers as in the aged wild type muscle. In contrast, the young *mg29(-/-)* muscle does not display significant numbers of tubular aggregates, further suggesting that these large aggregates do not harbor a major portion of the segregated intracellular Ca^{2+} pool.

Establishing whether reduced MG29 expression in aged skeletal muscle acts as a causal factor in the progression of skeletal muscle aging should be the goal of future investigations. Assuming MG29 does act as a sentinel against aging effect on skeletal muscle, an important investigation would be to examine how decreased MG29 modulates cellular Ca^{2+} handling. MG29 can function in triad junction organization and can also directly modulate RyR function (Pan et al., 2004). One approach to examine the role of MG29 in aging skeletal muscle would be to rescue the expression of MG29 in aged mouse muscle through transgenic and molecular approaches and assay if compromised Ca^{2+} spark signaling, the segregated Ca^{2+} store and alterations to triad junction structure are restored to a level similar to the young wild type fibers. If the primary function of MG29 is to directly modulate RyR-mediated SR Ca^{2+} release, one would predict that transient rescue of MG29 expression in the aged muscle fibers should restore the plastic nature of Ca sparks in the mutant muscle without significantly improving the disrupted triad junction ultrastructure in aged muscle fibers. On the other hand, if altered triad junction integrity in the chronic absence of MG29 is responsible for compromised Ca^{2+} spark signaling in aged wild type muscle, or young *mg29(-/-)* muscle, one would expect that transient rescue of MG29 expression would not be sufficient to remodel the triad membrane network, and would therefore fail to rescue the plastic nature of Ca^{2+} spark signaling. By establishing the molecular function of MG29 in protection from aging in skeletal muscle, one can envision targeting that aspect of MG29 activity to be an effective therapeutic approach in rescuing some aspects of age-related skeletal muscle dysfunction.

Disruption of membrane cross-talk following aging

While ultrastructural studies suggest that a physical uncoupling of the t-tubule and SR membranes takes place at the triad junction in aged skeletal muscle fibers, the strongest evidence for disruption of E-C coupling in aged skeletal muscle comes from functional studies. This “E-C uncoupling” mechanism was originally proposed by Delbono and colleagues (Delbono et al., 1995), based on their groundbreaking studies of Ca^{2+} handling in aged skeletal muscle. These findings have been reviewed elsewhere (Payne and Delbono, 2004), but essentially their results indicate that denervation of muscle fibers leads to uncoupling of the interaction between DHPR and RyR. These modifications can be reversed by muscle-specific expression of IGF-1 (Renganathan et al., 1998; Gonzalez et al., 2003; Moreno et al., 2006), and perhaps NGF (Heath et al., 1998). Additional growth regulatory factors can contribute to the rejuvenation of aged skeletal muscle. For example, restoration of Notch signaling has been proposed to be an attractive intervention for improving the regenerative capacity of aged skeletal (Conboy et al., 2003). While growth factors have well defined anti-aging capacity, clinical application of such an approach is limited by the currently available technology as systemic application of growth factors is known to produce detrimental effects.

While it is clear that there is physical and functional uncoupling of the triad junction in aged skeletal muscle, the molecular players remain to be defined. Our finding that MG29 may contribute to this process is a first step towards a more comprehensive understanding of the factors that maintain E-C coupling integrity in aged skeletal muscle. We have also identified junctophilin as another candidate molecule that can directly influence coupling of the t-tubule membrane with the SR membrane (Fig. 3). The junctophilins (JPs) are unique SR resident membrane proteins that can span the junctional gap distance between the t-tubule and SR

membranes in striated muscle tissues, or the gap between plasma membrane and endoplasmic reticulum in neuron cells (Takeshima et al., 2000; Ito et al., 2001; Komazaki et al., 2002). Four JP subtypes have been identified: JP1 is predominantly present in skeletal muscle, JP2 is found in cardiac and skeletal muscle, and JP3 and JP4 are mainly present in neural tissues (Nishi et al., 2000; Takeshima et al., 2000; Nishi et al., 2003). JPs have a conserved domain structure with multiple membrane occupation and recognition nexus (MORN) motifs on the amino-terminus that are thought to facilitate interaction with the sarcolemma and a single transmembrane domain on the carboxyl-terminus that inserts into the SR membrane (Fig. 3A). These terminal domains are linked by a helical region that spans the junctional gap, which likely provides a structural framework to modulate the distance between the t-tubule and SR membranes to allow elastic coupling between DHPR and RyR in striated muscles. Mathematical modeling of E-C coupling indicated that the gap length between t-tubule and SR membranes is an essential determinant of the triggering efficiency of RyR-mediated SR Ca^{2+} release by DHPR (Stern et al., 1999; Sobie et al., 2002; Cannell et al., 2006). As several studies suggest that JPs are required to maintain proper alignment of t-tubule structure and SR membranes, it is not surprising that JPs are essential to facilitate the cross-talk between proteins associated with these membrane systems. JP1 knockout mice exhibited deficiency in skeletal muscle triad junctions and neonate lethality (Ito et al., 2001; Komazaki et al., 2002). Genetic ablation of JP2 in mice caused widening of the gap size of the junctional membrane complex, and deficient Ca^{2+} transients in cardiomyocytes, resulting in embryonic lethality (Takeshima et al., 2000).

Mutations in JP2 have been linked to hypertrophic cardiomyopathy and Ca^{2+} handling dysfunction (Landstrom et al., 2007; Matsushita et al., 2007). Altered expression of JP2 is observed in models of cardiomyopathy (Minamisawa et al., 2004), and as an early event in the transition towards heart failure (Xu et al., 2007). Since muscle specific JPs are essential for the survival of mice, it has proven difficult to examine the function of JP in adult muscle Ca^{2+} handling or during the aging process. Indeed, this mirrors a prevalent technical challenge in aging studies. The function of essential genes, the absence of which often leads to embryonic lethality, cannot be studied in aging animals using conventional knockout approaches. Use of powerful small hairpin RNA interference (shRNA) technology has allowed us to knockdown JP genes in cultured muscle cells and live animals (Hirata et al., 2006), therefore providing a useful tool to examine the role of membrane coupling in the stress-induced Ca^{2+} spark signaling in skeletal muscle physiology and pathophysiology. Such studies indicate that silencing of JP expression in adult skeletal muscle leads to uncontrolled CICR activity, accompanied by the disruption of the triad junction, in skeletal muscle fibers (Fig. 3C). One can expect that similar changes in Ca^{2+} spark signaling in aged muscle may be linked to the changes in membrane coupling that result from altered protein expression. We have recently observed that JP expression decreases in aging skeletal muscle (Fig. 3B). This finding suggests that decreased JP expression in aging skeletal muscle may contribute to some aspects of the progressive nature of altered Ca^{2+} spark signaling and contractile dysfunction in aging skeletal muscle fibers, and perhaps to aging of the cardiomyocytes as well.

Conclusion

Ca^{2+} sparks are pulsatile signals involved in muscle function that represent the integrated capacity of muscle cells to function under normal physiological and stressed conditions. Loss of the plastic nature of the Ca^{2+} sparks may contribute to, or be an adaptive response to, the altered condition of the musculoskeletal and cardiovascular systems during aging. As an adaptive response to cellular stress, Ca^{2+} sparks provide an index of the capacity of muscle to maintain proper Ca^{2+} homeostasis and signaling. In addition to direct modification of the Ca^{2+} release machinery, changes in membrane structure or membrane cross-talk as the result of aging have significant influence on the Ca^{2+} spark activity. Identification of proteins

involved in maintenance of membrane integrity at junctional complexes in muscle should provide important insights into the function of Ca^{2+} signaling in muscle physiology and aging, as well as providing unique therapeutic targets for restoration of age-related muscle dysfunction.

References

- Agbulut O, Destombes J, Thiesson D, Butler-Browne G. Age-related appearance of tubular aggregates in the skeletal muscle of almost all male inbred mice. *Histochem Cell Biol* 2000;114:477–481. [PubMed: 11201609]
- Bers, DM. *Excitation-Contraction Coupling and Cardiac Contractile Force*. New York: Springer; 2001. p. 452
- Boncompagni S, d'Amelio L, Fulle S, Fano G, Protasi F. Progressive disorganization of the excitation-contraction coupling apparatus in aging human skeletal muscle as revealed by electron microscopy: a possible role in the decline of muscle performance. *J Gerontol A Biol Sci Med Sci* 2006;61:995–1008. [PubMed: 17077192]
- Brochet DX, Yang D, Di Maio A, Lederer WJ, Franzini-Armstrong C, Cheng H. Ca^{2+} blinks: rapid nanoscopic store calcium signaling. *Proc Natl Acad Sci U S A* 2005;102:3099–3104. [PubMed: 15710901]
- Brotto MA, Nagaraj RY, Brotto LS, Takeshima H, Ma JJ, Nosek TM. Defective maintenance of intracellular Ca^{2+} homeostasis is linked to increased muscle fatigability in the MG29 null mice. *Cell Res* 2004;14:373–378. [PubMed: 15538969]
- Cannell MB, Crossman DJ, Soeller C. Effect of changes in action potential spike configuration, junctional sarcoplasmic reticulum micro-architecture and altered t-tubule structure in human heart failure. *J Muscle Res Cell Motil* 2006;27:297–306. [PubMed: 16897575]
- Chawla S, Skepper JN, Hockaday AR, Huang CL. Calcium waves induced by hypertonic solutions in intact frog skeletal muscle fibres. *J Physiol* 2001;536:351–359. [PubMed: 11600671]
- 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;38:14887–14896. [PubMed: 10555971]
- Cheng H, Lederer WJ, Cannell MB. Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. *Science* 1993;262:740–744. [PubMed: 8235594]
- Cheng H, Wang SQ. Calcium signaling between sarcolemmal calcium channels and ryanodine receptors in heart cells. *Front Biosci* 2002;7:d1867–d1878. [PubMed: 12161336]
- Chevessier F, Marty I, Paturneau-Jouas M, Hantai D, Verdiere-Sahuque M. Tubular aggregates are from whole sarcoplasmic reticulum origin: alterations in calcium binding protein expression in mouse skeletal muscle during aging. *Neuromuscul Disord* 2004;14:208–216. [PubMed: 15036331]
- Conboy IM, Conboy MJ, Smythe GM, Rando TA. Notch-mediated restoration of regenerative potential to aged muscle. *Science* 2003;302:1575–1577. [PubMed: 14645852]
- 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;77:1394–1403. [PubMed: 10465751]
- Csernoch L. Sparks and embers of skeletal muscle: the exciting events of contractile activation. *Pflügers Arch* 2007;454:869–878. [PubMed: 17342530]
- Delbono O, O'Rourke KS, Ettinger WH. Excitation-calcium release uncoupling in aged single human skeletal muscle fibers. *J Membr Biol* 1995;148:211–222. [PubMed: 8747553]
- Delbono O, Renganathan M, Messi ML. Excitation- Ca^{2+} release-contraction coupling in single aged human skeletal muscle fiber. *Muscle Nerve Suppl* 1997;5:S88–S92. [PubMed: 9331393]
- di Barletta MR, Viatchenko-Karpinski S, Nori A, Memmi M, Terentyev D, Turcato F, Valle G, Rizzi N, Napolitano C, Gyorko S, Volpe P, Priori SG. Clinical phenotype and functional characterization of *CASQ2* mutations associated with catecholaminergic polymorphic ventricular tachycardia. *Circulation* 2006;114:1012–1019. [PubMed: 16908766]
- Dibb KM, Rueckschloss U, Eisner DA, Isenberg G, Trafford AW. Mechanisms underlying enhanced cardiac excitation contraction coupling observed in the senescent sheep myocardium. *J Mol Cell Cardiol* 2004;37:1171–1181. [PubMed: 15572047]

- Dirksen WP, Lacombe VA, Chi M, Kalyanasundaram A, Viatchenko-Karpinski S, Terentyev D, Zhou Z, Vedamoorthy S, Li N, Chiamvimonvat N, Carnes CA, Franzini-Armstrong C, Gyorke S, Periasamy M. A mutation in calsequestrin, CASQ2D307H, impairs Sarcoplasmic Reticulum Ca²⁺ handling and causes complex ventricular arrhythmias in mice. *Cardiovasc Res* 2007;75:69–78. [PubMed: 17449018]
- Eisner DA, Sipido KR. Sodium calcium exchange in the heart: necessity or luxury? *Circ Res* 2004;95:549–551. [PubMed: 15375019]
- George CH, Higgs GV, Lai FA. Ryanodine receptor mutations associated with stress-induced ventricular tachycardia mediate increased calcium release in stimulated cardiomyocytes. *Circ Res* 2003;93:531–540. [PubMed: 12919952]
- Gonzalez E, Messi ML, Zheng Z, Delbono O. Insulin-like growth factor-1 prevents age-related decrease in specific force and intracellular Ca²⁺ in single intact muscle fibres from transgenic mice. *J Physiol* 2003;552:833–844. [PubMed: 12937290]
- Heath BM, Xia J, Dong E, An RH, Brooks A, Liang C, Federoff HJ, Kass RS. Overexpression of nerve growth factor in the heart alters ion channel activity and beta-adrenergic signalling in an adult transgenic mouse. *J Physiol* 1998;512(Pt 3):779–791. [PubMed: 9769421]
- Hirata Y, Brotto M, Weisleder N, Chu Y, Lin P, Zhao X, Thornton A, Komazaki S, Takeshima H, Ma J, Pan Z. Uncoupling store-operated Ca²⁺ entry and altered Ca²⁺ release from sarcoplasmic reticulum through silencing of junctophilin genes. *Biophys J* 2006;90:4418–4427. [PubMed: 16565048]
- Howlett SE, Grandy SA, Ferrier GR. Calcium spark properties in ventricular myocytes are altered in aged mice. *Am J Physiol Heart Circ Physiol* 2006;290:H1566–H1574. [PubMed: 16327019]
- Isenberg G, Borschke B, Rueckschloss U. Ca²⁺ transients of cardiomyocytes from senescent mice peak late and decay slowly. *Cell Calcium* 2003;34:271–280. [PubMed: 12887974]
- Ito K, Komazaki S, Sasamoto K, Yoshida M, Nishi M, Kitamura K, Takeshima H. Deficiency of triad junction and contraction in mutant skeletal muscle lacking junctophilin type 1. *J Cell Biol* 2001;154:1059–1067. [PubMed: 11535622]
- Janczewski AM, Spurgeon HA, Lakatta EG. Action potential prolongation in cardiac myocytes of old rats is an adaptation to sustain youthful intracellular Ca²⁺ regulation. *J Mol Cell Cardiol* 2002;34:641–648. [PubMed: 12054851]
- Jiang D, Xiao B, Zhang L, Chen SR. Enhanced basal activity of a cardiac Ca²⁺ release channel (ryanodine receptor) mutant associated with ventricular tachycardia and sudden death. *Circ Res* 2002;91:218–225. [PubMed: 12169647]
- Kamishima T, Quayle JM. Ca²⁺-induced Ca²⁺ release in cardiac and smooth muscle cells. *Biochem Soc Trans* 2003;31:943–946. [PubMed: 14505454]
- Kannankeril PJ, Mitchell BM, Goonasekera SA, Chelu MG, Zhang W, Sood S, Kearney DL, Danila CI, De Biasi M, Wehrens XH, Pautler RG, Roden DM, Taffet GE, Dirksen RT, Anderson ME, Hamilton SL. Mice with the R176Q cardiac ryanodine receptor mutation exhibit catecholamine-induced ventricular tachycardia and cardiomyopathy. *Proc Natl Acad Sci U S A* 2006;103:12179–12184. [PubMed: 16873551]
- Kirsch WG, Uttenweiler D, Fink RH. Spark- and ember-like elementary Ca²⁺ release events in skinned fibres of adult mammalian skeletal muscle. *J Physiol* 2001;537:379–389. [PubMed: 11731572]
- Klein MG, Cheng H, Santana LF, Jiang YH, Lederer WJ, Schneider MF. Two mechanisms of quantized calcium release in skeletal muscle. *Nature* 1996;379:455–458. [PubMed: 8559251]
- Komazaki S, Ito K, Takeshima H, Nakamura H. Deficiency of triad formation in developing skeletal muscle cells lacking junctophilin type 1. *FEBS Lett* 2002;524:225–229. [PubMed: 12135771]
- Komazaki S, Nishi M, Kangawa K, Takeshima H. Immunolocalization of mitsugumin29 in developing skeletal muscle and effects of the protein expressed in amphibian embryonic cells. *Dev Dyn* 1999;215:87–95. [PubMed: 10373013]
- Komazaki S, Nishi M, Takeshima H, Nakamura H. Abnormal formation of sarcoplasmic reticulum networks and triads during early development of skeletal muscle cells in mitsugumin29-deficient mice. *Dev Growth Differ* 2001;43:717–723. [PubMed: 11737152]
- Landstrom AP, Weisleder N, Batalden KB, Bos JM, Tester DJ, Ommen SR, Wehrens XH, Claycomb WC, Ko JK, Hwang M, Pan Z, Ma J, Ackerman MJ. Mutations in JPH2-encoded junctophilin-2

- associated with hypertrophic cardiomyopathy in humans. *J Mol Cell Cardiol* 2007;42:1026–1035. [PubMed: 17509612]
- Lee EH, Lopez JR, Li J, Protasi F, Pessah IN, Kim do H, Allen PD. Conformational coupling of DHPR and RyR1 in skeletal myotubes is influenced by long-range allostereism: evidence for a negative regulatory module. *Am J Physiol Cell Physiol* 2004;286:C179–C189. [PubMed: 13679303]
- Lehnart SE, Wehrens XH, Laitinen PJ, Reiken SR, Deng SX, Cheng Z, Landry DW, Kontula K, Swan H, Marks AR. Sudden death in familial polymorphic ventricular tachycardia associated with calcium release channel (ryanodine receptor) leak. *Circulation* 2004;109:3208–3214. [PubMed: 15197150]
- Lim CC, Apstein CS, Colucci WS, Liao R. Impaired cell shortening and relengthening with increased pacing frequency are intrinsic to the senescent mouse cardiomyocyte. *J Mol Cell Cardiol* 2000;32:2075–2082. [PubMed: 11040110]
- Lindner M, Bohle T, Beuckelmann DJ. Ca²⁺-handling in heart failure--a review focusing on Ca²⁺ sparks. *Basic Res Cardiol* 2002;97:179–182. [PubMed: 12479239]
- Liu N, Colombi B, Memmi M, Zissimopoulos S, Rizzi N, Negri S, Imbriani M, Napolitano C, Lai FA, Priori SG. Arrhythmogenesis in catecholaminergic polymorphic ventricular tachycardia: insights from a RyR2 R4496C knock-in mouse model. *Circ Res* 2006;99:292–298. [PubMed: 16825580]
- Ma J, Pan Z. Retrograde activation of store-operated calcium channel. *Cell Calcium* 2003;33:375–384. [PubMed: 12765683]
- Margreth A, Damiani E, Bortoloso E. Sarcoplasmic reticulum in aged skeletal muscle. *Acta Physiol Scand* 1999;167:331–338. [PubMed: 10632636]
- Matsushita Y, Furukawa T, Kasanuki H, Nishibatake M, Kurihara Y, Ikeda A, Kamatani N, Takeshima H, Matsuoka R. Mutation of junctophilin type 2 associated with hypertrophic cardiomyopathy. *J Hum Genet* 2007;52:543–548. [PubMed: 17476457]
- McPherson PS, Campbell KP. The ryanodine receptor/Ca²⁺ release channel. *J Biol Chem* 1993;268:13765–13768. [PubMed: 8390976]
- Meissner G. Ryanodine receptor/Ca²⁺ release channels and their regulation by endogenous effectors. *Annu Rev Physiol* 1994;56:485–508. [PubMed: 7516645]
- Minamisawa S, Oshikawa J, Takeshima H, Hoshijima M, Wang Y, Chien KR, Ishikawa Y, Matsuoka R. Junctophilin type 2 is associated with caveolin-3 and is down-regulated in the hypertrophic and dilated cardiomyopathies. *Biochem Biophys Res Commun* 2004;325:852–856. [PubMed: 15541368]
- Moreno RJ, Messi ML, Zheng Z, Wang ZM, Ye P, D'Ercole JA, Delbono O. Role of sustained overexpression of central nervous system IGF-I in the age-dependent decline of mouse excitation-contraction coupling. *J Membr Biol* 2006;212:147–161. [PubMed: 17334835]
- Nagaraj RY, Nosek CM, Brotto MA, Nishi M, Takeshima H, Nosek TM, Ma J. Increased susceptibility to fatigue of slow- and fast-twitch muscles from mice lacking the MG29 gene. *Physiol Genomics* 2000;4:43–49. [PubMed: 11074012]
- 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–C1040. [PubMed: 8897807]
- Nelson MT, Cheng H, Rubart M, Santana LF, Bonev AD, Knot HJ, Lederer WJ. Relaxation of arterial smooth muscle by calcium sparks. *Science* 1995;270:633–637. [PubMed: 7570021]
- Nishi M, Komazaki S, Kurebayashi N, Ogawa Y, Noda T, Iino M, Takeshima H. Abnormal features in skeletal muscle from mice lacking mitsugumin29. *J Cell Biol* 1999;147:1473–1480. [PubMed: 10613905]
- Nishi M, Mizushima A, Nakagawara K, Takeshima H. Characterization of human junctophilin subtype genes. *Biochem Biophys Res Commun* 2000;273:920–927. [PubMed: 10891348]
- Nishi M, Sakagami H, Komazaki S, Kondo H, Takeshima H. Coexpression of junctophilin type 3 and type 4 in brain. *Brain Res Mol Brain Res* 2003;118:102–110. [PubMed: 14559359]
- Pan Z, Hirata Y, Nagaraj RY, Zhao J, Nishi M, Hayek SM, Bhat MB, Takeshima H, Ma J. Co-expression of MG29 and ryanodine receptor leads to apoptotic cell death: effect mediated by intracellular Ca²⁺ release. *J Biol Chem* 2004;279:19387–19390. [PubMed: 15039443]
- Pan Z, Yang D, Nagaraj RY, Nosek TA, Nishi M, Takeshima H, Cheng H, Ma J. Dysfunction of store-operated calcium channel in muscle cells lacking mg29. *Nat Cell Biol* 2002;4:379–383. [PubMed: 11988740]

- Papazafiri P, Kletsas D. Developmental and age-related alterations of calcium homeostasis in human fibroblasts. *Exp Gerontol* 2003;38:307–311. [PubMed: 12581795]
- Payne AM, Delbono O. Neurogenesis of excitation-contraction uncoupling in aging skeletal muscle. *Exerc Sport Sci Rev* 2004;32:36–40. [PubMed: 14748548]
- Renganathan M, Messi ML, Delbono O. Dihydropyridine receptor-ryanodine receptor uncoupling in aged skeletal muscle. *J Membr Biol* 1997;157:247–253. [PubMed: 9178612]
- Renganathan M, Messi ML, Delbono O. Overexpression of IGF-1 exclusively in skeletal muscle prevents age-related decline in the number of dihydropyridine receptors. *J Biol Chem* 1998;273:28845–28851. [PubMed: 9786885]
- Rios E, Ma JJ, Gonzalez A. The mechanical hypothesis of excitation-contraction (EC) coupling in skeletal muscle. *J Muscle Res Cell Motil* 1991;12:127–135. [PubMed: 1648106]
- Rios E, Pizarro G, Stefani E. Charge movement and the nature of signal transduction in skeletal muscle excitation-contraction coupling. *Annu Rev Physiol* 1992;54:109–133. [PubMed: 1562172]
- Sanchez JA, Stefani E. Inward calcium current in twitch muscle fibres of the frog. *J Physiol* 1978;283:197–209. [PubMed: 309941]
- Schneider MF. Control of calcium release in functioning skeletal muscle fibers. *Annu Rev Physiol* 1994;56:463–484. [PubMed: 8010748]
- 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;107:221–231. [PubMed: 10360678]
- Shin DW, Pan Z, Kim EK, Lee JM, Bhat MB, Parness J, Kim do H, Ma J. A retrograde signal from calsequestrin for the regulation of store-operated Ca²⁺ entry in skeletal muscle. *J Biol Chem* 2003;278:3286–3292. [PubMed: 12419813]
- Shirokova N, Garcia J, Rios E. Local calcium release in mammalian skeletal muscle. *J Physiol* 1998;512 (Pt 2):377–384. [PubMed: 9763628]
- Sobie EA, Dilly KW, dos Santos Cruz J, Lederer WJ, Jafri MS. Termination of cardiac Ca(2+) sparks: an investigative mathematical model of calcium-induced calcium release. *Biophys J* 2002;83:59–78. [PubMed: 12080100]
- Song L, Alcalai R, Arad M, Wolf CM, Toka O, Conner DA, Berul CI, Eldar M, Seidman CE, Seidman JG. Calsequestrin 2 (CASQ2) mutations increase expression of calreticulin and ryanodine receptors, causing catecholaminergic polymorphic ventricular tachycardia. *J Clin Invest* 2007;117:1814–1823. [PubMed: 17607358]
- Stern MD, Song LS, Cheng H, Sham JS, Yang HT, Boheler KR, Rios E. Local control models of cardiac excitation-contraction coupling. A possible role for allosteric interactions between ryanodine receptors. *J Gen Physiol* 1999;113:469–489. [PubMed: 10051521]
- Sutko JL, Airey JA, Murakami K, Takeda M, Beck C, Deerinck T, Ellisman MH. Foot protein isoforms are expressed at different times during embryonic chick skeletal muscle development. *J Cell Biol* 1991;113:793–803. [PubMed: 2026650]
- Takeshima H, Komazaki S, Nishi M, Iino M, Kangawa K. Junctophilins: a novel family of junctional membrane complex proteins. *Mol Cell* 2000;6:11–22. [PubMed: 10949023]
- Takeshima H, Shimuta M, Komazaki S, Ohmi K, Nishi M, Iino M, Miyata A, Kangawa K. Mitsugumin29, a novel synaptophysin family member from the triad junction in skeletal muscle. *Biochem J* 1998;331 (Pt 1):317–322. [PubMed: 9512495]
- Terentyev D, Nori A, Santoro M, Viatchenko-Karpinski S, Kubalova Z, Gyorke I, Terentyeva R, Vedamoorthy S, Blom NA, Valle G, Napolitano C, Williams SC, Volpe P, Priori SG, Gyorke S. Abnormal interactions of calsequestrin with the ryanodine receptor calcium release channel complex linked to exercise-induced sudden cardiac death. *Circ Res* 2006;98:1151–1158. [PubMed: 16601229]
- Terentyev D, Viatchenko-Karpinski S, Gyorke I, Volpe P, Williams SC, Gyorke S. Calsequestrin determines the functional size and stability of cardiac intracellular calcium stores: Mechanism for hereditary arrhythmia. *Proc Natl Acad Sci U S A* 2003;100:11759–11764. [PubMed: 13130076]
- Vanterpool CK, Pearce WJ, Buchholz JN. Advancing age alters rapid and spontaneous refilling of caffeine-sensitive calcium stores in sympathetic superior cervical ganglion cells. *J Appl Physiol* 2005;99:963–971. [PubMed: 15845773]

- Wang SQ, Song LS, Lakatta EG, Cheng H. Ca²⁺ signalling between single L-type Ca²⁺ channels and ryanodine receptors in heart cells. *Nature* 2001;410:592–596. [PubMed: 11279498]
- Wang SQ, Stern MD, Rios E, Cheng H. The quantal nature of Ca²⁺ sparks and in situ operation of the ryanodine receptor array in cardiac cells. *Proc Natl Acad Sci U S A* 2004a;101:3979–3984. [PubMed: 15004280]
- Wang SQ, Wei C, Zhao G, Brochet DX, Shen J, Song LS, Wang W, Yang D, Cheng H. Imaging microdomain Ca²⁺ in muscle cells. *Circ Res* 2004b;94:1011–1022. [PubMed: 15117829]
- Wang W, Cleemann L, Jones LR, Morad M. Modulation of focal and global Ca²⁺ release in calsequestrin-overexpressing mouse cardiomyocytes. *J Physiol* 2000;524(Pt 2):399–414. [PubMed: 10766921]
- Wang X, Weisleder N, Collet C, Zhou J, Chu Y, Hirata Y, Zhao X, Pan Z, Brotto M, Cheng H, Ma J. Uncontrolled calcium sparks act as a dystrophic signal for mammalian skeletal muscle. *Nat Cell Biol* 2005;7:525–530. [PubMed: 15834406]
- Ward CW, Lederer WJ. Ghost sparks. *Nat Cell Biol* 2005;7:457–459. [PubMed: 15867931]
- Ward CW, Schneider MF, Castillo D, Protasi F, Wang Y, Chen SR, Allen PD. Expression of ryanodine receptor RyR3 produces Ca²⁺ sparks in dyspedic myotubes. *J Physiol* 2000;525(Pt 1):91–103. [PubMed: 10811728]
- Weisleder N, Brotto M, Komazaki S, Pan Z, Zhao X, Nosek T, Parness J, Takeshima H, Ma J. Muscle aging is associated with compromised Ca²⁺ spark signaling and segregated intracellular Ca²⁺ release. *J Cell Biol* 2006;174:639–645. [PubMed: 16943181]
- Weisleder N, Ferrante C, Hirata Y, Collet C, Chu Y, Cheng H, Takeshima H, Ma J. Systematic ablation of RyR3 alters Ca²⁺ spark signaling in adult skeletal muscle. *Cell Calcium* 2007a;42:548–555. [PubMed: 17412417]
- Weisleder N, Ferrante C, Hirata Y, Collet C, Csernoch L, Ma J. Inhibition of osmotic stress-induced Ca sparks in skeletal muscle by bromoenol lactone, an inhibitor of calcium-independent phospholipase A2 (iPLA2). *Biophys J*. 2007b 2007 Biophysical Society Annual Meeting.
- Wier WG, ter Keurs HE, Marban E, Gao WD, Balke CW. Ca²⁺ 'sparks' and waves in intact ventricular muscle resolved by confocal imaging. *Circ Res* 1997;81:462–469. [PubMed: 9314826]
- Xu M, Zhou P, Xu SM, Liu Y, Feng X, Bai SH, Bai Y, Hao XM, Han Q, Zhang Y, Wang SQ. Intermolecular failure of L-type Ca²⁺ channel and ryanodine receptor signaling in hypertrophy. *PLoS Biol* 2007;5:e21. [PubMed: 17214508]
- Zhou J, Brum G, Gonzalez A, Launikonis BS, Stern MD, Rios E. Ca²⁺ sparks and embers of mammalian muscle. Properties of the sources. *J Gen Physiol* 2003;122:95–114. [PubMed: 12835473]
- Zhou J, Yi J, Royer L, Launikonis BS, Gonzalez A, Garcia J, Rios E. A probable role of dihydropyridine receptors in repression of Ca²⁺ sparks demonstrated in cultured mammalian muscle. *Am J Physiol Cell Physiol* 2006;290:C539–C553. [PubMed: 16148029]

Figure 1. Integrative control of Ca^{2+} spark signaling in striated muscle physiology and aging
 Ca^{2+} sparks, as a pulsatile signal for intracellular Ca^{2+} release, represent the integrated function of Ca^{2+} signaling in striated muscles. DHPR acts as a voltage sensor that controls opening of RyR to allow for VICR in skeletal muscle. In cardiac muscle, DHPR provides the fast-activating L-type Ca current, allowing extracellular Ca^{2+} entry to trigger CICR. CSQ is the major Ca^{2+} buffering protein in the SR lumen, which has an additional role in modulating RyR function through retrograde signaling. MG29 is a synaptophysin family protein present in skeletal muscle that contributes to maintenance of the triad junction membrane integrity, whose expression is decreased in aged skeletal muscle. JP is an SR resident membrane protein that facilitates formation of triad junction in skeletal muscle or dyad junction in cardiac muscle by providing the physical link with the sarcolemmal membrane. Mutations in JP2, or altered expression of JP2, has been linked with altered Ca^{2+} signaling in skeletal and cardiac muscle in disease and aging. Unlike the spontaneous Ca^{2+} sparks in cardiac muscle, Ca^{2+} sparks are quiescent in skeletal muscle. Stresses applied to skeletal muscle leads to a plastic response involving Ca^{2+} spark activation. This stress response is lost in aged skeletal muscle, whereas uncontrolled Ca^{2+} sparks act as a dystrophic signal in mammalian skeletal muscle. In contrast to the compromised Ca^{2+} spark signal in aged skeletal muscle, increased Ca^{2+} spark activity was observed in senescent cardiomyocytes, likely reflecting the loss of pulsatility during aging.

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

(A) Osmotic-stress applied to an intact skeletal muscle isolated from the young mice (3 month) induced robust Ca^{2+} spark activity revealed by transverse line-scan confocal imaging of fluo-4 Ca^{2+} indicator fluorescence at the periphery of the muscle fiber. The area boxed in red is magnified in the bottom section with a trace recording of the fluorescence intensity ($\Delta F/F_0$). In addition to the fast Ca^{2+} sparks, a group of long-lasting Ca^{2+} burst activities was also observed in skeletal muscle. For details, see Weisleder et al., 2007. (B) Western blot showed that the expression of MG29 is reduced in aged skeletal muscle (27 month) compared with the young skeletal muscle (2 month). (C) Osmotic-stress induced Ca sparks is reversible and repeatable in young skeletal muscle (black), and is transient in aged skeletal muscle (red). The

transient nature of stress-induced Ca sparks is also observed in young skeletal muscle derived from the *mg29*($-/-$) mice (*blue*). See Weisleder et al., 2006 for details.

Figure 3. Junctophilin (JP) and Ca signaling in striated muscle

(A) JP contains the characteristic MORN motifs at the amino-terminus, with a single TM segment at the carboxyl-terminus that inserts into the SR membrane. Between the MORN and TM domains is a α -helical rich motif. JP1 is predominantly expressed in skeletal muscle, and JP2 is present in both cardiac and skeletal muscle. (B) The expression of JP2 is decreased in aged skeletal muscle (27 month) compared with the adolescent skeletal muscle (2 and 6 months). Similar observations were also reported for hypertrophic and dilated cardiomyopathies in Minamisawa et al., 2004. (C) Silencing of JP in adult skeletal muscle leads to compromised CICR and disrupted triad junction formation. Wild type skeletal muscle responds to caffeine stimulation (arrow) with a fast onset of intracellular Ca^{2+} release that

terminates rapidly. Muscle fibers treated with siRNA against JP (*red*) displayed elevated resting cytosolic Ca^{2+} ; the amplitude of caffeine-induced Ca release is reduced and termination of caffeine-induced Ca^{2+} release is significantly slower than scrambled siRNA controls (*black*). Panel modified from Hirata et al., 2006.