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REVIEW

The elusive role of store depletion in the control of intracellular calcium release

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Abstract The contractile cycle of striated muscles, skeletal and cardiac, is controlled by a cytosolic [Ca^{2+}] transient that requires rapid movements of the ion through channels in the sarcoplasmic reticulum (SR). A functional signature of these channels is their closure after a stereotyped time lapse of Ca^{2+} release. In cardiac muscle there is abundant evidence that termination of release is mediated by depletion of the Ca^{2+} store, even if the linkage mechanism remains unknown. By contrast, in skeletal muscle the mechanisms of release termination are not understood. This article reviews measurements of store depletion, the experimental evidence for dependence of Ca^{2+} release on the [Ca^{2+}] level inside the SR, as well as tests of the molecular nature of putative intra-store Ca^{2+} sensors. Because Ca^{2+} sparks exhibit the basic release termination mechanism, much attention is dedicated to the studies of store depletion caused by sparks and its relationship with termination of sparks. The review notes the striking differences in volume, content and buffering power of the stores in cardiac vs. skeletal muscle, differences that explain why functional depletion is much greater for cardiac than skeletal muscle stores. Because in skeletal muscle store depletion is minimal and reduction in store [Ca^{2+}] does not appear to greatly inhibit Ca^{2+} release, it is concluded that decrease in free SR [Ca^{2+}] does not mediate physiological termination of Ca^{2+} release in this type of muscle. In spite of the apparent absence of store depletion and its putative channel closing effect, termination of Ca^{2+} sparks is faster and more robust in skeletal than cardiac muscle. A gating role of a hypothetical ''proximate store'' constituted by polymers of calsequestrin and associated proteins is invoked in an attempt to preserve a role for store depletion and unify mechanisms in both types of striated muscle.

Keywords Skeletal muscle · Cardiac muscle · Sarcoplasmic reticulum · Calcium sparks · Calsequestrin

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Robust mechanisms terminate intracellular Ca^{2+} release in striated muscles

Skeletal and cardiac muscles share a striated structure, which reflects an organized arrangement of contractile filaments and membrane-bound organelles. The structural similarity reflects an ability for contraction and relaxation that is fast by comparison with that of smooth muscle. The striated muscles also share a basic switching *on* or *off* by Ca^{2+} ions, moving into the cytosol largely from intracellular stores to bind to an allosteric regulatory protein on the contractile filaments. By contrast, the role of Ca^{2+} in smooth muscle is more nuanced, to the point that its release from stores causes relaxation (Wray et al. 2005).

The functional cycles of both types of striated muscle demand full relaxation, which requires robust closure of the Ca^{2+} release channels. Figure 1 shows

the evolution of Ca^{2+} release flux (back-calculated from Ca^{2+} transients), in skeletal and cardiac muscle, during a voltage clamp depolarizing pulse. The records show that all muscles have a provision to stop Ca^{2+} release, even under continuous membrane depolarization, a stop that manifests itself as a sharp peak followed by decay in the evolution of the cytosolic Ca^{2+} transient.

The fast deterministic closure of Ca^{2+} release channels capable of activation by Ca^{2+} (CICR) constitutes a ''paradox of control'', a paradox also manifested in the fact that Ca^{2+} release has a graded dependence on the primary stimulus (which is depolarization in skeletal muscle, and Ca^{2+} influx via I_{Ca} in the heart).

A way out of this paradox was provided by Ca^{2+} sparks (Cheng et al. 1993), which result from synchronous opening of a variable number of clustered channels (González et al. 2000; Wang et al. 2004). The source clusters were identified with ''couplons'', comprising in the cardiac case all the channels in one dyad and in the skeletal case the channels on one side of a junctional segment of t tubule and its associated SR (Stern et al. 1997). If such sources are sufficiently separated they can respond individually in a regenerative manner, mediated by CICR, while the global response remains graded. This ''local control'' theory identifies a crucial component of any explanation, the fact that the sources are multiple and separate, but leaves fundamental questions unanswered.

In cardiac muscle, the limitation of activation during a spark to less than ten channels out of a two-hundred channel couplon remains unexplained (Stern and Cheng 2004). It is accepted that cardiac SR Ca^{2+} overload sharply increases opening of release channels. Largely as an extension of this effect, SR depletion is believed to close channels and together with an ill-defined inactivation process terminate release in the heart.

Skeletal muscle presents two problems; in the amphibian less than 5 channels are involved in a spark if the fiber is intact, and up to 30 if the membrane is disrupted (Ríos et al. 1999; Baylor et al.2002; Chandler et al. 2003; rev. by Baylor 2005). What stops the activation from fully invading couplons of up to 100 channels?

In mammalian skeletal muscle the signal is instead composed of ''embers'' produced by channels opening individually (Csernoch et al. 2004). Sparks occur, but usually under artificial circumstances, which include membrane permeabilization (Kirsch et al. 2001; Zhou et al. 2003), excess of ROS (Isaeva and Shirokova 2003; Isaeva et al. 2005), osmotic disruption or strenuous exercise (Pan et al. 2002). There is, however, a strong spontaneous termination, patent in the whole cell record (Fig. 1b). It has not been possible to reproduce this global response based on the summation of individual embers (Csernoch et al. 2004). By analogy with the cardiac role, here again it is possible that depletion translates the joint activity of many channels to a signal that terminates release.

This article will review existing measurements of depletion in skeletal muscle, as well as the evidence for gating effects of depletion that enable rapid channel closure. Cardiac muscle, where these issues have been assiduously studied, will be reviewed summarily as a term of comparison.

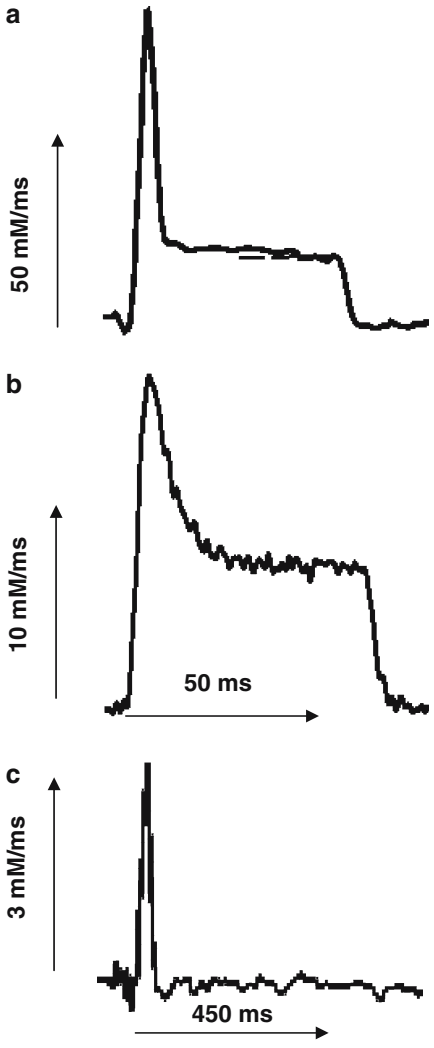

Fig. 1 The time course of Ca^{2+} release flux during sustained membrane depolarization to a highly activating potential. (**a**) frog semitendinosus. (**b**) rat EDL. (**c**) Guinea pig ventricular myocyte. Depolarizing pulse lasted 80 ms (**a**, **b**) or 150 ms (**c**). Panel C is part of Fig. 3 of Sipido and Wier (1991), reproduced with permission. Note in every case the fast decay of flux after and early peak

The activating and inhibitory sites on Ca^{2+} release channels/ryanodine receptors

The ryanodine receptor is modulated by the endogenous ligands Ca^{2+}, Mg^{2+}, H^+, ATP and inorganic phosphate (rev. by Meissner 1994). Of critical importance to EC coupling is the modulation of the ryanodine receptor by Ca^{2+} and Mg^{2+}. The modulation by cytosolic Ca^{2+} constitutes both a paradigm and a confounding factor for the establishment of the putative role of intra-SR Ca^{2+}.

In vertebrate skeletal muscle there are two types of sites on the ryanodine receptor that bind both Ca^{2+} and Mg^{2+}. There is a high affinity Ca^{2+}-activation site (K_D ~ 1 μM for Ca^{2+} and ~ 25 μM for Mg^{2+}; Zhou et al. 2004) and a low affinity site (K_D ~ 1 mM) that keeps the ryanodine receptor shut when either Ca^{2+} or Mg^{2+} are bound (Meissner 1994; Laver et al. 1997). The low affinity inhibitory site will be at least half-saturated at normal resting myoplasmic [Mg^{2+}] (Lamb and Stephenson 1992). Ca^{2+} binding to the high affinity Ca^{2+}-activation site causes the ryanodine receptor to open —the CICR mechanism. Mg^{2+} present in the myoplasm competes with activator Ca^{2+} at this site limiting the rate at which Ca^{2+} can occupy it and elicit activation.

The control by Ca^{2+} and Mg^{2+} sites also varies with taxonomic class. Skinned fibre studies first showed that mammalian skeletal muscle was less susceptible to CICR than amphibian skeletal muscle (Endo 1985). Under more physiological conditions, Shirokova et al. (1996) found markedly different waveforms of voltage-induced Ca^{2+} release in mammalian and amphibian skeletal muscle. In particular the ratio of peak over steady release levels was greater in frog muscle (Fig. 1). Additionally, the ratio of peak over steady release exhibited a clear maximum at intermediate membrane depolarization in the frog, while it was essentially voltage-independent in the rat. The greater peak of Ca^{2+} release in the frog was attributed to CICR. The failure to image Ca^{2+} sparks in voltage-pulsed rat muscle under the same conditions that produced sparks in frog muscle (Shirokova et al. 1998), together with the finding of embers in response to low voltage depolarization in the rat (Csernoch et al. 2004) and mouse (Csernoch et al. 2006) confirmed the disparity. The paucity of CICR in mammalian muscle was also evident in comparisons of release elicited by caffeine and membrane depolarization (Lamb et al. 2001). The need for a lesser peak of Ca^{2+} release in the mammal is attributed to the diffusional advantage derived from the presence of two triad junctions in every sarcomere of mammalian muscle, while muscles of other taxonomic classes only have one (Shirokova et al. 1996, after a suggestion by Elizabeth Stephenson).

In skinned fibres, conditions that produce Ca^{2+} sparks have now been found for mammalian skeletal muscle (Kirsch et al. 2001; Zhou et al. 2003). However, they are consistent with lack of CICR (Zhou et al. 2004). In frog muscle, there is a strong negative correlation between spark frequency and [Mg^{2+}] in the μM to mM range. This is indicative of competition with Ca^{2+} for the CICR activation site on the ryanodine receptor. Similar increases in [Mg^{2+}] affect little the ability of rat skinned fibres to produce sparks. In fact, sparks can be observed in rat muscle in the presence of > 5 mM Mg^{2+} (Zhou et al. 2004). Thus sparks are produced under conditions that saturate with Mg^{2+} both the activating and inhibitory sites of the mammalian ryanodine receptor.

These results suggest that even though ryanodine receptors present in skeletal muscle (of the molecular isoform 1, homologous to the amphibian α isoform) possess the Ca^{2+}/Mg^{2+} activation and inhibitory sites, their physiology in situ includes other Ca^{2+} release modulators. It has been suggested that isoform β of the RyR, abundant in the amphibian, is responsible for the CICR response (Ríos and Zhou 2004; Zhou et al. 2004), while isoform α in the amphibian and the homologous RyR1 in the mammal have their ability to respond to Ca^{2+} strongly inhibited by SR accessory proteins (Murayama and Ogawa 2004) and the DHPR (Lee et al. 2004; Zhou et al. 2006). SR proteins that may inhibit αRyR and RyR1 include FKBP, calsequestrin and triadin.

Structure and biochemistry of the Ca2+ stores provide clues for the role of depletion

In rabbit ventricular myocytes the junctional (j) SR forms flat terminal cisternae that appear in thin sections as linear profiles (Fig. 2a), with an average diameter of 592 nm (Brochet et al. 2005). The interiors of the cisternae are electron dense because of the presence of calsequestrin. The cisternae, described as "pancake-shaped" (Fig. 2b), form junctions with *t* tubules near the Z lines. Each cisterna has 66 RyRs on average, grouped in one or two clusters. Estimates of relative volume are varied. According to Brochet et al. (2005) the jSR and free SR occupy 2.0% and 2.2%, respectively, of the "cytoplasmic" volume (total volume minus mitochondria, nuclei, and SR volumes). jSR of (rat) ventricle is instead 0.3% in measurements of Page et al. (1971), with the lower number due in part to

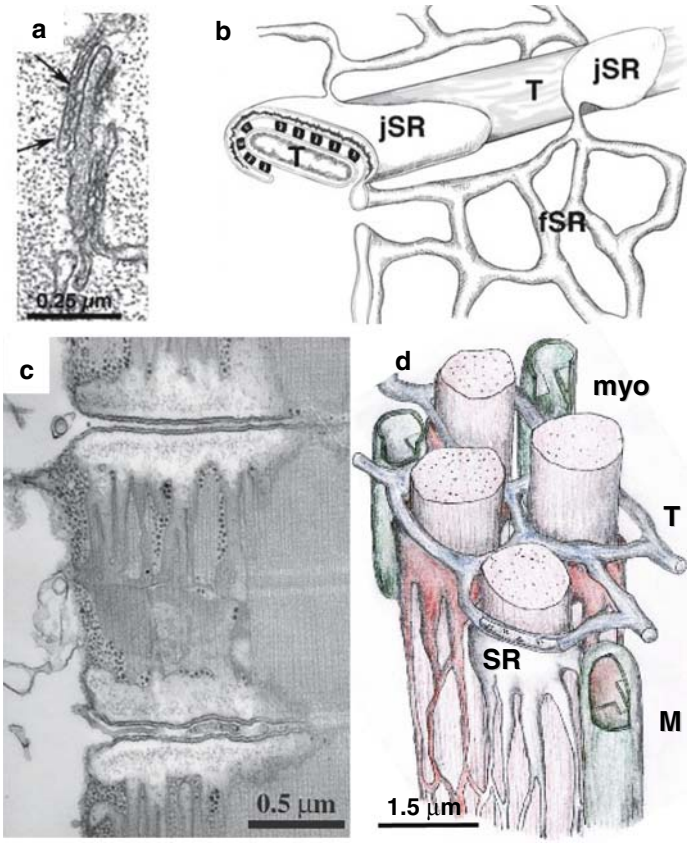

Fig. 2 Comparative structure of terminal cisternae and junctional SR of cardiac and skeletal muscle. (**a** and **b**), rabbit ventricular myocytes. (**c**) electron micrograph of thin section of guppy muscle (representing here skeletal muscle) and (**d**), schematic structure of amphibian and other non-mammalian muscle. Note the striking differences in geometry, volume and arrangement of terminal cisternae. **a** and **b** are from Brochet et al. (2004), and **c** is from Franzini-Armstrong (1999), reproduced with permission

a normalization by cell rather than cytoplasmic volume. These numbers are sharply different than those of skeletal muscle: 4.1% for frog fast twitch muscle (Mobley and Eisenberg 1975) or 1.6% for white muscle of guinea pig (Eisenberg and Kuda 1975).

Thus, there is a large difference between cardiac and skeletal muscle in volume of the Ca^{2+} source organelle (Fig. 2). There is also a large difference in buffering capacity of these compartments. In cardiac muscle, Ca^{2+} buffering power (ratio of bound over free SR-lumenal $[Ca^{2+}]$) is estimated at between ~2 (Shannon et al. 2000) and ~10 (Shannon and Bers 1997). In frog skeletal muscle a number can be derived dividing total calcium content (2–5 mM relative to cytosolic volume, Ríos et al. 2001) by SR volume (12% of cytosolic volume), for a total concentration of 16–42 mM inside the SR. The ratio of this total by free $[Ca^{2+}]_{SR}$, 0.4 mM (Launikonis et al. 2005) or the more conventional figure of 1 mM, yields a buffering power of 22 to 105. An alternative calculation, possible in skeletal muscle, is to multiply the total concentration of calsequestrin, at 0.11 mM relative to SR volume (Volpe and Simon 1991) and its molar binding capacity (80 moles Ca^{2+} per mol; Park et al. 2004) to obtain total calsequestrin binding capacity, 8.8 mM, which should be a lower bound of the SR capacity. The associated buffering power is the ratio of capacity and dissociation constant (1 mM; Volpe and Simon 1991), or 8.8. Therefore in skeletal muscle the Ca^{2+} store volume is several-fold higher and the buffering power is between 3 and 30 times higher than in cardiac muscle, which results in a roughly 100 times greater availability of releasable calcium. Finally, and as pointed out by Brochet et al. (2005) geometric differences make the terminal cisternae of the cardiac SR less accessible for refilling from the longitudinal SR.

Based on these differences the skeletal muscle Ca^{2+} seems much more resistant to depletion. But of course depletion depends also on the rates of Ca^{2+} release.

Physiological depletion of store Ca^{2+}. Cardiac muscle

During a beat, 30 to 70 μmoles of Ca^{2+} are released per litre of cytosol; this release comprises 25 to 63% of content (Bassani and Bers 1995; Shannon et al. 2003; Chen et al. 1996). Interestingly, the peak release flux of up to 3 mM/s (Sipido and Wier 1991) requires only 2–5% of the cellular complement of RyRs to be simultaneously open (Bers 2001; p. 192). This provision of an

overabundant functional reserve of channels also requires the presence of a tight control mechanism, which usually keeps most of the channels closed during release activity.

Recently it was possible to measure the depletion locally associated with Ca^{2+} sparks in cardiac muscle. From the measurement of ''blinks'' of fluo-5N inside the SR, the nadir of reduction of free [Ca^{2+}] was estimated at 54% of resting value, while the reduction in calsequestrin-bound calcium was put at 28% (Brochet et al. 2005).

Thus in the heart Ca^{2+} release causes a large fractional depletion, in spite of the fact that a large functional reserve of channels remains inactive. Therefore, it is possible to envision for cardiac muscle mechanisms that derive a robust signal for termination of release from lumenal SR depletion or other variables that directly depend on lumenal [Ca^{2+}] levels. Such mechanisms have been sought, and apparently found.

The effects of calcium load on activation of release in cardiac muscle

Many observations reveal various activating effects of elevated $[Ca^{2+}]_{SR}$ and inhibitory effects of reduced $[Ca^{2+}]_{SR}$. Activating effects have been described since Fabiato and Fabiato (1972), including the production of Ca^{2+} waves and periodic contractions, and an increase in spark frequency. Diaz et al. (1997) could predict Ca^{2+} wave production assuming that spontaneous release occurs whenever $[Ca^{2+}]_{SR}$ surpasses a fixed threshold. Wave propagation appears to be due to three effects of the increased store [Ca^{2+}]: an enhancement in the sensitivity of channels to activation by cytosolic Ca^{2+}, a reduction of the inhibition that usually follows release and the increase in release flux, which directly favors Ca^{2+}-dependent activation (Bers 2001, p. 232).

The activating effect is very non-linear (Shannon et al. 2000) —the dependence between fractional release and load has a pronounced upward curvature. This non-linearity may be intrinsic to the dependency of activation with lumenal [Ca^{2+}] or it may be indirect, a consequence of positive feedback at the cytosolic side of the channels.

These global effects also have local manifestations, chiefly the increase in frequency of Ca^{2+} sparks with total SR [Ca^{2+}]. A most compelling demonstration of the effect is in the transient nature of the reduction of spark frequency that ensues in the continuous presence of the channel blocker tetracaine, even when the drug is used at concentrations that fully eliminate sparks when first applied (Gyorke et al. 1997).

The activation effect of lumenal Ca^{2+} is also supported by work with channels reconstituted in bilayers. As reviewed by Gyorke et al. (2002), the evidence supports both an indirect ''feed-through'' effect of lumenal Ca^{2+} acting on the cytosolic side, and a direct effect of Ca^{2+} on the lumenal (trans) side of the channels. The joint evidence strongly indicates that the activation is due to lumenal Ca^{2+} acting on the lumenal face of channels or associated proteins.

There is also evidence of a converse effect: reduction of release activity associated with SR depletion. It is useful to summarize the effect of lumenal Ca^{2+} as a change in P_o of the generic release channel and then analyze this effect into its kinetic components. The change in steady P_o must include an increase in the opening transition rate k_{ON} (as suggested by the increase in frequency of local events and the reduction of mean closed time in bilayer-reconstituted channels). Additionally, it could include a reduction in the closing rate $k_{OFF.}$ If this was the case, then the converse would apply, depletion would increase $k_{OFF,}$ literally terminating release. In that case the effect of lumenal Ca^{2+} could provide a mechanism of termination of sparks and spontaneous reduction of Ca^{2+} release (''inactivation'') at the cell-wide level.

Several lines of evidence support release termination by store depletion; most compelling are the effects of extrinsic Ca^{2+} buffers, including maleate, citrate and ADA. They increase the total calcium content in the SR in direct proportion to their affinity for Ca^{2+}, and increase amount of global Ca^{2+} release and amplitude of Ca^{2+} sparks by prolongation of release time (i.e., sparks rise time) exclusive of other mechanisms (Terentyev et al. 2002). The striking correlation among buffer affinity, SR load, duration of cell-wide release and rise time of sparks constitutes the strongest evidence to this date for a determinant role of depletion in termination of Ca^{2+} release. Sobie et al. (2002) anticipated this result with a model that assumed the Ca^{2+} affinity of the cytosolic activator site to be linearly dependent on the SR lumenal [Ca^{2+}]. The lumenal Ca^{2+} dependence of activator affinity plus the rapid spread of gating state in an allosterically connected cluster were sufficient to simulate realistic termination of sparks, provided that the store operated as relatively isolated organelles of limited capacity. While all the assumptions in the model of Sobie et al. (2002) have not been confirmed in detail, it is now clear that the terminal cisternae of cardiac SR do in fact deplete strongly during individual sparks (see previous section) and therefore depletion is taken to be a critical

determinant of spark termination in the heart. This dependence has been rationalized as the consequence of Ca^{2+} binding to an intra-SR "Ca^{2+} sensor", which may not necessarily be on the RyR molecule.

Molecular nature of the lumenal "Ca^{2+} sensor"

The lumenal Ca^{2+} sensor remains unidentified. Because the P_o of purified channels in bilayers was unchanged upon trans [Ca^{2+}] increase (Gyorke et al. 2004), the action of SR-lumenal Ca^{2+} on the release channels may be indirect. Native channels showed instead a marked sensitivity of P_o to lumenal [Ca^{2+}], which suggests that the lumenal Ca^{2+} sensor is part of a protein or complex of proteins that are lost during the purification process.

In striated muscles SR Ca^{2+} is buffered mostly by calsequestrin, a low-affinity Ca^{2+} binding protein whose role in the regulation of SR Ca^{2+} release is under intense investigation. Modification of the cellular levels of calsequestrin produced conflicting results. In transgenic mice the overexpression of calsequestrin resulted in diminished Ca^{2+} transients and contractions, even though the SR Ca^{2+} content was significantly higher (Wang et al. 2000). An opposite result was described by Terentyev et al. (2003). Using adenoviruses to overexpress calsequestrin in cardiomyocytes these authors obtained results similar to the ones observed applying exogenous buffers (Terentyev et al. 2002). The magnitude of both I_{Ca}-induced Ca^{2+} transients and spontaneous Ca^{2+} sparks were increased, which was attributed to a slower termination of Ca^{2+} release from the SR. Also, as observed with the exogenous buffers, the recovery time for repetitive Ca^{2+} sparks was longer, another result consistent with the existence of a threshold $[Ca^{2+}]_{SR}$ above which Ca^{2+} sparks are generated spontaneously.

Due to its role as a calcium buffer in the SR lumen, calsequestrin is an obvious candidate sensor. However, its addition caused no change in the activity of purified RyRs in bilayers (Gyorke et al. 2004).

The junctional SR protein triadin 1 physically links calsequestrin to the lumenal side of the release channel (Guo and Campbell 1995; Zhang et al. 1997). The adenovirus-mediated overexpression of triadin 1 in cardiomyocytes has been shown to enhance cardiac EC coupling (Terentyev et al. 2005). In particular the P_o of native channels reconstituted from myocytes overexpressing triadin 1 was 5-fold higher than that of channels from control cells. Moreover, when these cells overexpressed a triadin mutant lacking the domain responsible for interaction with calsequestrin, the P_o was unchanged. Addition of triadin and/or junctin to the trans side of the bilayer resulted in a significant increase in the open probability of the purified channel (Gyorke et al. 2004). However the channel was still unresponsive to changes in trans Ca^{2+}. A subsequent addition of calsequestrin inhibited the channel activity but restored the ability of the ryanodine receptor to respond to changes in luminal Ca^{2+}. These results suggest that the function requires a supramolecular complex in which calsequestrin act as the sensor, while triadin and/or junctin are responsible for the interaction between calsequestrin and ryanodine receptor.

The impressive series of studies by Gyorke and colleagues took a dramatic turn with the demonstration of de-stabilizing effects of an antisense-induced reduction in calsequestrin content. The intervention resulted in spontaneous Ca^{2+} release and membrane depolarization associated with the early termination of a refractoriness that prevents re-entry, which was in turn traced to an accelerated restoration of $[Ca^{2+}]_{SR}$ after its depletion during a beat. These observations (Terentyev et al. 2003; Viatchenko-Karpinsky et al. 2004) provide a clear pathogenesis mechanism for hereditary arrhythmias associated with mutations of the human *CSQ2* gene (the syndrome named catecholaminergic polymorphic ventricular tachycardia or CPVT; Lahat et al. 2001) and suggest compelling approaches to the treatment of this syndrome.

It is surprising to see that these steady advances in the understanding of Ca^{2+} control in the heart cannot be transferred to skeletal muscle in a straightforward way.

Depletion of store Ca^{2+} in skeletal muscle

In frog skeletal muscle, release by individual action potentials (quantified from the amount of H^+ displaced from EGTA introduced in the cytosol) reached 14% in fiber segments (Pape et al. 1995). This was in good agreement with a figure of 10%, obtained with the first three-dimensional model of calcium movements in skeletal muscle cells (Cannell and Allen 1984).

In toad muscle, prolonged tetanic activity resulted in reduction of contractile force (fatigue) which was accompanied by a reduction in releasable Ca^{2+} store content to approximately 40% of resting level (Kabbara and Allen 1999). The same authors later used fluo-5N to directly evaluate SR content and found that $[Ca^{2+}]_{SR}$ falls by 40% in a 500 ms 100 Hz tetanus (Kabbara and Allen 2001). These measurements are

again in rough agreement with the 3-D model of Cannell and Allen (1984). More recently, using SEER of mag-indo-1 in manually skinned frog fibers Launikonis et al. (2006a) measured a depletion of 10% upon release caused by a single twitch elicited by electrical stimulation. In these skinned fibers the t tubular system reseals and recovers the ability to generate action potentials, therefore the flux and amount of Ca^{2+} release are presumably close to those in a physiological twitch. Finally Rudolf et al. (1996) estimated a 17% reduction by a single twitch in mouse tibialis anterior expressing the biosensor D1ER (Palmer et al. 2004). In summary, estimates of depletion caused by a single twitch range from 10 to 17% in frogs and mammals, but repeated twitches and tetani may accumulate much greater depletion. By comparison, voltage clamp depolarization that activates Ca^{2+} release maximally, causes a flux waveform that reaches a peak (and thereafter decays rapidly) in ~5 ms. Before peak flux, some 250 μM (or 10% of content) may be released. Therefore, whether release is elicited by action potentials or clamp depolarization, the spontaneous process of termination is in full force when depletion has only reached the 10–20% range. Depletion of such small magnitude could hardly have a determinant role in release termination.

The local store depletion left behind by the Ca^{2+} release underlying a spark was first brought into focus by evidence that fluorescence in sparks increases monoexponentially up to a peak, and at this point makes a sharp break to start a rapid decay (Lacampagne et al. 1999). Such time course was interpreted to reflect a roughly constant flux during the rise time of the spark. This inference was supported by modelling in which a constant flux active during the rise time was adequate to stimulate fluorescence measured in sparks (Baylor et al. 2002; Chandler et al. 2003). Also in agreement was the computation of rate of signal mass production, a quantity proportional to release flux under most conditions (Zhuge et al. 2002, 2004; Zhou et al. 2005), in large sparks recorded at high temporal resolution (Fig. 3). In both frog (A, C) and rat muscle (B, D) the signal mass increased at a roughly constant rate (red), consistent in simulations with a current of 75 pA flowing during 4 ms (black). The constancy of the release current suggests that the concentration gradient driving it is also constant, and implies that even the largest spark currents fail to significantly lower the intra-SR $[Ca^{2+}]$.

Launikonis et al. (2006a) used SEER of mag-indo-1 in the SR to evaluate the local depletion resulting from the generation of Ca^{2+} sparks in frog muscle. Ca^{2+} sparks were imaged with cytosolic rhod-2 and their automatically determined locations were used to define in simultaneously obtained images of intra-store $[Ca^{2+}]$ the spatiotemporal regions where the expected depletion should start. Unlike the visible ''blinks'' of cardiac muscle, the images of $[Ca^{2+}]_{SR}$ in skeletal muscle did not show depletion visible by eye. Figure 4a–c show an average of fluorescence ratio (related monotonically with $[Ca^{2+}]_{SR}$) in 6300 spatiotemporal areas centered on sparks. This average depletion image, named ''skrap'', has a magnitude of at most 7%, but is typically 1%. Its most surprising feature can be seen in the temporal profiles of Fig. 4c. The skrap reaches its nadir several tens of milliseconds later than the peak of the

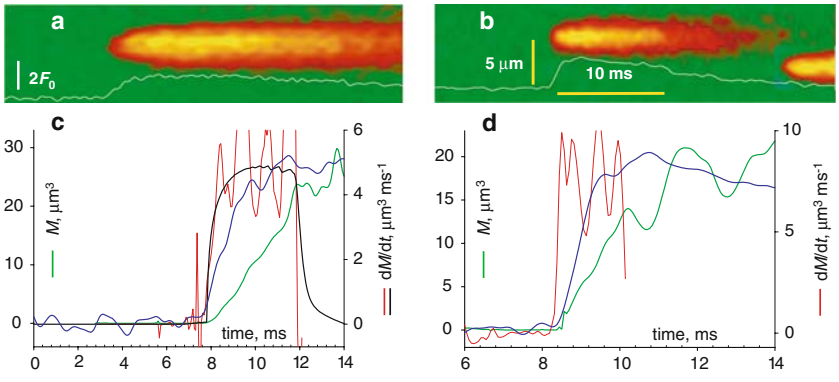

Fig. 3 Time course of release flux in large sparks. (**a**, **b**) fluorescence of fluo-4 in line scans of high temporal resolution of a frog (**a**) or rat (**b**) skeletal muscle fiber. (**c**, **d**) corresponding evolutions of central fluorescence (blue), and signal mass (green), the volume integral of the increase in normalized fluorescence, approximated as $M(t) \approx 1.206\ \Delta F/F_0(t,x = 0) \times \mathrm{FWHM}(t)^3$, where FWHM is full width at half magnitude (Chandler et al. 2003). Note that for both species M increases linearly during the first 3 to 4 ms (as shown by the time derivative, in red). The black trace in **c** is dM/dt in a simulation with a source of 75 pA, open for 4 ms. This simulation approximates the experimentally determined dM/dt, in red. From Zhou et al. (2005)

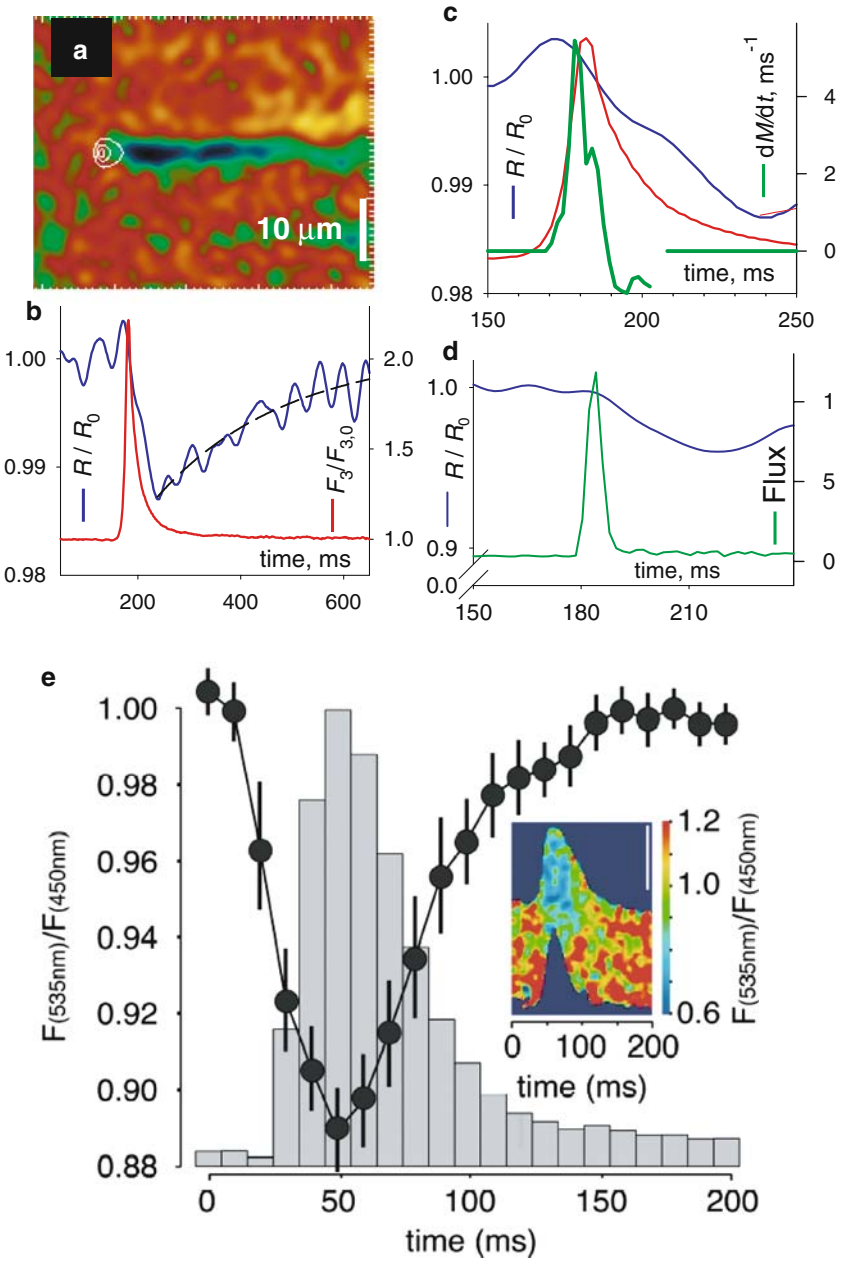

Fig. 4 Various measurements of SR depletion accompanying Ca^{2+} release into the cytosol. (**a**) the average ''skrap'', image of the local deficit in $[Ca^{2+}]$ left inside the store by the release that underlies a spark. The vertical coordinate of linescan images **a** and **e** is space and the horizontal coordinate is time. This image is an average of those corresponding to 6300 individual sparks detected in frog muscle fibers stained with rhod-2 in the cytosol and mag-indo-1 in the SR. It displays the ratio $R(x,t)$ of fluorescence excited by different laser lines in two different ranges of emission of the dye in the SR (a ratio that varies monotonically with $[Ca^{2+}]_{SR}$, as detailed in Launikonis et al. 2006). (**b**) blue: temporal profile of image (**a**). Red, temporal profile of the average of all spark images, obtained from the fluorescence of rhod-2 simultaneously with the images of $[Ca^{2+}]_{SR}$. Both plots are represented normalized to their initial, pre-spark values. The exponential decay of the skrap, after nadir has an initial amplitude of 0.015 and a τ of 251 ms. (**c**) spark and skrap profiles at an expanded temporal scale. Green, the rate of production of spark mass M, which is proportional to the flux of Ca^{2+} release underlying the spark. Note that the depletion reaches nadir ~50 ms after the end of Ca^{2+} release. (**d**) temporal profiles of depletion and Ca^{2+} release flux, measured as in **a**–**c**, in a frog muscle fiber that released Ca^{2+} upon an action potential. Note similar delay of the nadir of depletion. **a**–**d** are from Launikonis et al. (2006), with slight modifications. (**e**) circles, ratio of fluorescence at two emission ranges in an intact mouse tibialis anterior fiber expressing D1ER, averaged over the fiber and 15 twitches repeated at 1 Hz. Bars are an average of fiber shortening. Inset: ratio of linescans during contraction. Bar, 50 μm. Note that the nadir of depletion occurs at the same time as the peak of contractile displacement, or tens of ms later than the peak of the cytosolic Ca^{2+} transient. From Rudolf et al. (2006), reproduced with permission.

spark. While there may be some disagreement in cardiac muscle, the consensus view for skeletal muscle is that the peak of the spark corresponds in time to the end or release, the closing of the involved channels. Therefore the decay of free $[Ca^{2+}]$ in the SR paradoxically continues for tens of milliseconds after the release flux ends. A lag of depletion was also observed accompanying the release elicited by electrical stimulation of mechanically skinned fibers (Fig. 4d; Launikonis et al. 2006a), and the same paradox was found in the evolution of $[Ca^{2+}]_{SR}$ measured with D1ER in mouse muscle (Fig. 4e; Rudolf et al. 2006). As these authors stated, the lag may be real, or it may reflect some kinetic limitation in the response of the dye. Because the lag has been reported using different dyes, under different experimental circumstances and in muscles of different taxonomic classes, we are inclined to believe that it is real. A lag was also found between the peak of sparks in cardiac muscle (which is reached at 10 to 19 ms) and the nadir of ''blinks'' (at ~29 ms; Brochet et al. 2005).

Using SEER on frog muscle, we found that a mild but sustained release-inducing stimulus (a solution with low Ca^{2+} and low Mg^{2+}) often elicited an increase in $[Ca^{2+}]_{SR}$ that was cotemporal with the release into the cytosol (Launikonis et al. 2006a). Seeing this as a violation of the principle of conservation of matter and noting a symmetry with the observed lag in depletion–also a violation of conservation, but in the direction of mass destruction— we proposed that both are consequences of the existence of a third Ca^{2+} compartment, in addition to the free lumenal SR and the cytosol. This compartment, hypothetically envisioned as Ca^{2+} bound to calsequestrin, would be invisible to the monitoring dyes and would contribute most of the Ca^{2+} in a spark. Hence we named it the ''proximate store''.

In terminal cisternae of skeletal muscle calsequestrin forms linear polymers, visible as a fibrillar network (Franzini-Armstrong et al. 1987). Bonds with triadin and junctin (Zhang et al. 1997) place calsequestrin polymers near the lumenal mouth of RyRs. Ca^{2+} favours formation of these polymers and stabilizes them, occupying a layer where it is adsorbed rather than bound (Park et al. 2004). In this layer Ca^{2+} is able to diffuse laterally (i.e. along the polymer) and should be readily deliverable to the open channels (Park et al. 2004). This polymer-specific bound Ca^{2+} could constitute the proximate store. Its presence, as an intermediary between free SR lumen and cytosol, would buffer the spark-associated depletion and postpone it in time; in this view, the delayed decrease in $[Ca^{2+}]_{SR}$ observed after a spark would be caused by refilling of the proximate store. The $[Ca^{2+}]_{SR}$ transient during prolonged cell-wide release is explained likewise. In the well-loaded SR, calsequestrin is polymerized; during Ca^{2+} release $[Ca^{2+}]_{SR}$ decreases near release sites, which should cause widespread depolymerization of calsequestrin with consequent loss of binding sites and release of Ca^{2+} to the SR lumen

While obviously speculative, the idea of a proximate store has other virtues. It justifies the detailed tethering by triadin and junctin of calsequestrin to the lumenal side of ryanodine receptors. Disassembly of the putative store by depolymerization explains the observation (Ikemoto et al. 1991) of a transient increase in $[Ca^{2+}]$ inside SR vesicular fractions prior to release elicited pharmacologically. A dynamic role for calsequestrin beyond that of a simple buffer brings the control of Ca^{2+} release in skeletal muscle close to that in the heart, where as discussed in the previous section calsequestrin appears to have both buffering and gating roles. It should be remarked, however, that no quantitative modelling of a proximate store has been made, as we have not found a way to reasonably and testably limit the many alternative possibilities.

Consequences of store depletion in skeletal muscle

What, in addition to reduction in unitary current, are the functional consequences of store depletion in skeletal muscle? The results of experiments that explore effects of increases in load have been surprisingly inconsistent with those that impose a load reduction. Meissner et al. (1986) showed in vesicle preparations that increasing intravesicular Ca^{2+} shifted the $^{45}Ca^{2+}$ flux activation curve to lower cytosolic $[Ca^{2+}]$. Later Donoso et al. (1995) reported an increased rate of Ca^{2+} efflux in rabbit and frog skeletal muscle vesicles when Ca^{2+} SR was augmented. In 1996 Tripathy and Meissner found that RyRs in bilayers increased their activity when trans $[Ca^{2+}]$ was changed from 0.1 μM to 250 μM. Raising $[Ca^{2+}]$ over 1.5 mM reduced channel activity. The effects were dependent on the holding potential, which indicated that lumenal Ca^{2+} accessed the cytosolic activating and inactivating Ca^{2+} sites.

Pape et al. (1995, 1998) and Pape and carrier (1998) described an increase in release permeability with decreasing SR content, reaching 7-fold at or near the 20% content level and then decreasing beyond this point. By contrast, Schneider and collaborators (1987) found that the steady release under voltage clamp proceeds at a roughly constant permeability, i.e., with flux that decays in direct proportion to content. Reasoning that the divergent results could stem from the different techniques used, Pizarro and Ríos (2004)

combined the direct measurement of $[Ca^{2+}]_{cyto}$ of Schneider et al. (1987) with the evaluation of Ca^{2+} release by EGTA and Phenol Red used by Pape and colleagues, trying also to distinguish the effects on the peak of the waveform of release flux from those on the steady level reached later (Fig. 1A). Their results confirmed qualitatively that permeability increases with progressive depletion; the increase however affected only the steady flux level, it was substantially lower than reported before and was essentially negligible unless SR content was reduced by 30% or more (a feature also described by Pape and Carrier 1998). Therefore, the effect of store depletion in skeletal muscle is one of promotion of cell-wide Ca^{2+} release, in sharp contrast with that found in heart muscle. Pizarro and Ríos (2004) additionally found a rough constancy of the early peak of release flux during a voltage pulse, and a post-peak decay that became slower and less pronounced as SR load decreased. These features imply that the effect only occurs after the establishment of full flux –or channel opening— and therefore indicate a cytosolic locus of Ca^{2+} action. The mechanism probably consists in relief of Ca^{2+}-dependent channel closing (Ca^{2+}-dependent inactivation or CDI) as $[Ca^{2+}]_{cyto}$ near open channels cannot reach the usual high values if the store is depleted.

Launikonis et al. (2006b) studied in frog muscle the effects of changes in $[Ca^{2+}]_{SR}$ on the properties of Ca^{2+} sparks occurring under mild stimulation. Changes in $[Ca^{2+}]_{SR}$ were imposed by manipulation of $[Ca^{2+}]_{cyto}$ and measured with SEER of Mag-Indo-1; to neutralize the effect of cytosolic calcium (reported by Zhou et al. 2005), sparks were always imaged in 100 nM $[Ca^{2+}]_{cyto.}$ The increase in $[Ca^{2+}]_{SR}$ from an average of 250 μM to about 400 μM was accompanied by an increase in spark frequency by about 70%, and the effect reversed upon the inverse change in $[Ca^{2+}]_{SR}$. However, the power of the effect was weak, as attested by a correlation coefficient ρ^2 of less than 0.06. While the average increase in $[Ca^{2+}]_{SR}$ was modest in these experiments, in individual cases it was greater than 3-fold. Not even the largest increases in $[Ca^{2+}]_{SR}$ were able to induce the sharp increases in release activity observed in cardiomyocytes under similar changes.

After the considerations above it seems clear that reduction of free SR Ca^{2+} per se cannot provide a robust mechanism of release termination, global or local, in skeletal muscle. It is not just that the magnitude of depletion caused by physiological activity is limited, but substantial imposed depletion has either release-promoting effects (under voltage stimulation) or causes weak inhibition (in sparks activated by mild promoters of CICR).

These observations, largely carried out in non-mammalian muscle, leave the termination mechanism of Ca^{2+} release totally unsettled. The problem may be less critical in mammalian muscle, where there are no spontaneous Ca^{2+} sparks and the elementary local events of Ca^{2+} release are instead ‘‘embers’’, which reflect individual channel openings controlled by membrane depolarization (Csernoch et al. 2003, 2006). Even in mammals, though, the flux of Ca^{2+} release spontaneously decays after an early peak (Fig. 1B), and it has not been possible to quantitatively reconstitute the cell-wide release waveform as a sum of embers. A ‘‘termination principle’’ is missing in the mammal as well.

Calsequestrin and accessory proteins in skeletal muscle

Gating effects of calsequestrin on skeletal muscle RyRs were first described by Kawasaki and Kasai (1994) as a loss of the activation by Ca^{2+} of release from heavy SR vesicles upon a treatment that induced loss of calsequestrin from the vesicles. A role of triadin in the effect was first postulated by Ohkura et al. (1998) also from observations on heavy SR, while Szegedi et al. (2001) found that only a dephosphorylated form of calsequestrin promoted opening of reconstituted channels. More recently Beard et al. (2002 2005) reproduced for reconstituted skeletal muscle channels most of the effects described for cardiac channels. The evidence suggests both a direct facilitation of channel opening by Ca^{2+} on the lumenal side (demonstrated on purified RyRs) and a basal calsequestrin-mediated inhibition, which depends on the presence of triadin or junctin and is relieved upon increasing lumenal $[Ca^{2+}]$. This relief appears as a $[Ca^{2+}]$—dependent activation that reaches maximum at 1 mM lumenal $[Ca^{2+}]$. The concentration range of these effects is therefore adequate for their physiological relevance. Wei et al. (2006) added to this picture evidence that calsequestrin depolymerizes and detaches from the junctional SR membrane upon prolonged exposure to low (100 μM) lumenal $[Ca^{2+}]$, while calsequestrin monomers remain attached. In this situation the native regulation is lost and channels respond with increase in P_o to reduction of lumenal $[Ca^{2+}]$, a response that could underlie the increase in steady permeability induced by depolarization in (frog) fibers with strongly depleted SR (Pape et al. 1995, 1998; Pizarro and Ríos 2004). In spite of these occasional agreements, cellular observations cannot yet be explained on the basis of the interactions with associated proteins demonstrated in subcellular systems.

Genetic manipulation of the calsequestrin endowment has given additional insights. Paolini et al. (2005) preliminarily showed that a mouse null for the skeletal isoform of calsequestrin (CSQ1), which was viable, had terminal cisternae with greatly altered structural properties. The properties (some of which were shared with cardiac jSR) included flattened cisternae with a junctional membrane that contains multiple –rather than double—rows of RyRs, often forming longitudinally oriented, multi-layered junctions with *t* tubules. Surprisingly, K.O. mice did not show many differences in behaviour, and their EDL muscles –apparently devoid of any calsequestrin—did not exhibit changes in twitch amplitude and had an increased resistance to fatigue (Paolini et al. 2006). These results imply that CSQ1 is important in cellular ontogenesis, but also underscore the shortcomings of the knockout approach to genetic engineering, which allows compensatory changes the most time to develop.

A more conservative approach is transient gene silencing; Wang et al. (2006) used small interference RNA to reduce expression of either or both calsequestrin isoforms in the myogenic C_2C_{12} cell line, which usually expresses both isoforms. Again here the results were surprising: the CSQ1 knockdown myotubes, with less than 10% expression of the skeletal isoform, had normal release of Ca^{2+} in response to various stimuli, while a comparable reduction in CSQ2 did diminish the response. The reduced response in turn was traced to a lower density of SERCA1 and ryanodine receptors, associated with lower Ca^{2+} uptake and release in the CSQ2 knockdown myotubes. The transient gene silencing approach therefore failed to isolate an effect of the lack of calsequestrin, but was consistent, in a way, with the surprisingly modest effects of CSQ1 ablation on contractile performance of these muscle models (Paolini et al. 2006). In view of these results it is again clear that a full reduction of cellular EC coupling to its elementary molecular interactions is far from being achieved.

Conclusions and speculation

The available evidence leads to a number of conclusions:

(i) There are extraordinary differences in fluxes of Ca^{2+} release and reuptake between skeletal and cardiac muscle, as well as in the spatial extent, volume and buffering capacity of the storage organelles that define these fluxes. The differences seem to be in correspondence with very different functional demands for the two systems. Accordingly, one should not expect major similarities in the degree of physiological store depletion, or, by extension, in the functional role of depletion in skeletal and cardiac muscle.

(ii) In agreement with the first conclusion, the loss of Ca^{2+} after one action potential is ~50% in cardiac muscle, while in skeletal muscle it is 15%.

(iii) a robust termination of release is a common feature of all striated muscles. A role for depletion in this termination process seems well-established for cardiac muscle only. Stern and Cheng (2004) concluded that the termination of Ca^{2+} release is the central problem in the field of cardiac muscle EC coupling. From the vantage point of our comparison between striated muscles we must add several qualifications to agree. First, in view of the abundantly demonstrated effects of extrinsic buffers and calsequestrin, the central role of depletion should not be in doubt for cardiac muscle. What remains unclear is how a 50% depletion of the store can bring about channel closure robustly, every time. Thus, from the standpoint of cardiac muscle, the issue is lack of good theories, rather than data.

Moving on to skeletal muscle, the functional role of depletion appears weak, not just because the magnitude of the depletion caused by a single action potential is modest, but also because

(iv) measured effects of increased load are modest (a weak promotion of sparks) and

(v) the effect of major store depletion, an increase in the steady permeability reached during long-lasting depolarizations, is contradictory with a role in termination of Ca^{2+} release.

These largely negative conclusions regarding the role of depletion in skeletal muscle appear incongruous with the consensus that prevails in cardiac muscle. The dissonance seems even greater if one recalls that release termination is sharper, more robust, in frog skeletal muscle, where sparks have a narrowly distributed rise time centered at 4 ms, than in cardiac muscle, where spark rise times are 10–15 ms and open channel times are thought to surpass the time to peak. Thus, Ca^{2+} sparks terminate earlier and more conclusively in the frog, where depletion is negligible, than in heart muscle, where depletion is substantial.

One possible interpretation of this discrepancy is that the termination mechanism operative in the heart is insufficient for skeletal muscle, which therefore must

have developed a much better terminator of its own. Ca^{2+}-dependent inactivation, or CDI, could be such mechanism. Inactivation by the elevated $[Ca^{2+}]_{cyto}$ near open channels could both prevent continued propagation of activity by acting on closed channels and terminate Ca^{2+} release by working on open channels. This mechanism is especially attractive because elevated Ca^{2+} has a much greater inhibitory effect on skeletal than cardiac muscle channels in bilayer studies. CDI predicts among other properties a negative correlation between local $[Ca^{2+}]_{cyto}$ and rise time in Ca^{2+} sparks. Spark amplitude is an imperfect measure of local $[Ca^{2+}]_{cyto}$, due to off-focus errors and limited kinetic response of dyes. A negative correlation between spark amplitude and rise time has not been clearly demonstrated; an absence of correlation is found instead (Lacampagne et al. 1999; Ríos et al. 1999). This result is in itself evidence of autoinactivation, because the absence of effects by local $[Ca^{2+}]_{cyto}$ should result in a positive correlation with rise time ($[Ca^{2+}]_{cyto}$ would rise progressively at increasing channel open times). Together with the evidence from global measurements (reviewed by Pizarro and Ríos 2004), this property of sparks constitute indirect evidence of a release-terminating effect of cytosolic Ca^{2+}.

In addition to a fully different termination strategy valid for skeletal muscle only, it is possible to imagine a shared mechanism that upholds a role of depletion and operates in parallel with CDI. The hypothesis is an extension of the notion that Ca^{2+} ions adsorbed to linear polymers of calsequestrin constitute a proximate store (Launikonis et al. 2006a). We propose that depletion of the proximate store contributes to the robust signal needed for channel closure, and that the effect is mediated by depolymerization of calsequestrin. Given the structural differences, in cardiac muscle calsequestrin depletion would essentially course in parallel with substantial depletion of free SR-lumenal Ca^{2+}, while depletion of the lumen of the larger and better buffered skeletal muscle SR would be smaller and could be delayed. This speculation therefore accounts for both the differences in magnitude and time course of the local reduction in free SR $[Ca^{2+}]$ and the essential similarity of interactions of skeletal and cardiac RyRs with intra-SR proteins and Ca^{2+} in bilayer-reconstituted systems. Better biosensors, alternative genetic manipulations and quantitative modeling will put these speculations to the test within the next few years. In the meantime, the sobering conclusion is that the role of store content in the control of skeletal muscle EC coupling remains unknown.

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References

Bassani RA, Bers DM (1995) Rate of diastolic Ca release from the sarcoplasmic reticulum of intact rabbit and rat ventricular myocytes. Biophys J 68:2015–2022

Baylor SM (2005) Calcium sparks in skeletal muscle fibers. Cell Calcium 37(6):513–530

Baylor SM, Hollingworth S, Chandler WK (2002) Comparison of simulated and measured calcium sparks in intact skeletal muscle fibers of the frog. J Gen Physiol 120:349–368

Beard NA, Casarotto MG, Wei L, Varsanyi M, Laver DR, Dulhunty AF (2005) Regulation of ryanodine receptors by calsequestrin: effect of high luminal Ca^{2+} and phosphorylation. Biophys J 88:3444–3454

Beard NA, Sakowska MM, Dulhunty AF, Laver DR (2002) Calsequestrin is an inhibitor of skeletal muscle ryanodine receptor calcium release channels. Biophys J 82:310–320

Bers DM (2001) Excitation–Contraction Coupling and cardiac contractile force. 2nd edn. Kluwer Academic Publishers

Brochet DX, Yang D, Di Maio A, Lederer WJ, Franzini-Armstrong C, Cheng H (2005) Ca^{2+} blinks: rapid nanoscopic store calcium signaling. Proc Natl Acad Sci USA 102(8):3099–3104

Cannell MB, Allen DG (1984) Model of calcium movements during activation in the sarcomere of frog skeletal muscle. Biophys J 45:913–925

Chandler WK, Hollingworth S, Baylor SM (2003) Simulation of calcium sparks in cut skeletal muscle fibers of the frog. J Gen Physiol 121(4):311–324

Chen W, Steenbergen C, Levy LA, Vance J, London RE, Murphy E (1996) Measurement of free Ca^{2+} in sarcoplasmic reticulum in perfused rabbit heart loaded with 1,2-bis(2-amino-5,6-difluorophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid by 19F NMR. J Biol Chem 271(13):7398–7403

Cheng H, Lederer WJ, Cannell MB (1993) Calcium sparks: elementary events underlying excitation–contraction coupling in heart muscle. Science 262:740–744

Csernoch L, Pouvreau S, Jacquemond V (2006) Voltage-activated calcium release events in mouse skeletal muscle fibers. Biophys J 90:68a

Csernoch L, Zhou J, Stern MD, Brum G, Ríos E (2004) The elementary events of Ca^{2+} release elicited by membrane depolarization in mammalian muscle. J Physiol 557:43–58

Diaz ME, Trafford AW, O′Neill SC, Eisner DA (1997) Measurement of sarcoplasmic reticulum Ca^{2+} content and sarcolemmal Ca^{2+} fluxes in isolated rat ventricular myocytes during spontaneous Ca^{2+} release. J Physiol 501(Pt 1):3–16

Donoso P, Prieto H, Hidalgo C (1995) Luminal calcium regulates calcium release in triads isolated from frog and rabbit skeletal muscle. Biophys J 68(2):507–515

Eisenberg BR, Kuda AM (1975) Stereological analysis of mammalian skeletal muscle. II. White vastus muscle of the adult guinea pig. J Ultrastruct Res 51(2):176–187

Endo M (1985) Calcium release from sarcoplasmic reticulum. Physiol Rev 25:181–230

Fabiato A, Fabiato F (1972) Excitation-contraction coupling of isolated cardiac fibers with disrupted or closed sarcolemmas. Calcium-dependent cyclic and tonic contractions. Circ Res 31(3):293–307

Franzini-Armstrong C (1999) The sarcoplasmic reticulum and the control of muscle contraction. FASEB J. 13(Suppl 2): S266–S270

Franzini-Armstrong C, Kenney LJ, Varriano-Marston E (1987) The structure of calsequestrin in triads of vertebrate skeletal muscle: a deep-etch study. J Cell Biol 105(1):49–56

González A, Kirsch WG, Shirokova N, Pizarro G, Brum G, Pessah IN, Stern MD, Cheng H, Ríos E. (2000) Involvement of multiple intracellular release channels in calcium sparks of skeletal muscle. Proc Natl Acad Sci USA 97:4380–4385

Guo W, Campbell KP (1995) Association of triadin with the ryanodine receptor and calsequestrin in the lumen of the sarcoplasmic reticulum. J Biol Chem 270:9027–9030

Gyorke I, Hester N, Jones LR, Gyorke S (2004) The role of calsequestrin, triadin, and junctin in conferring cardiac ryanodine receptor responsiveness to luminal calcium. Biophys J 86:2121–2128

Gyorke S, Lukyanenko V, Gyorke I. (1997) Dual effects of tetracaine on spontaneous calcium release in rat ventricular myocytes. J Physiol 500(Pt 2):297–309

Gyorke S, Gyorke I, Lukyanenko V, Terentyev D, Viatchenko-Karpinski S, Wiesner TF (2002) Regulation of sarcoplasmic reticulum calcium release by luminal calcium in cardiac muscle. Front Biosci 7:d1454–d163

Ikemoto N, Antoniu B, Kang JJ, Meszaros LG, Ronjat M (1991) Intravesicular calcium transient during calcium release from sarcoplasmic reticulum. Biochemistry 30(21):5230–5237

Isaeva EV, Shkryl VM, Shirokova N (2005) Mitochondrial redox state and Ca^{2+} sparks in permeabilized mammalian skeletal muscle. J Physiol 565:855–872

Isaeva EV, Shirokova N (2003) Metabolic regulation of Ca^{2+} release in permeabilized mammalian skeletal muscle fibres. J Physiol 547:453–462

Kawasaki T, Kasai M (1994) Regulation of calcium channel in sarcoplasmic reticulum by calsequestrin. Biochem Biophys Res Commun 199(3):1120–1127

Kabbara AA, Allen DG (2001) The use of the indicator fluo-5N to measure sarcoplasmic reticulum calcium in single muscle fibres of the cane toad. J Physiol 534(Pt 1):87–97

Kabbara AA, Allen DG (1999) The role of calcium stores in fatigue of isolated single muscle fibres from the cane toad. J Physiol 519(Pt 1):169–176

Kirsch WG, Uttenweiler D, Fink RHA (2001) Spark- and ember-like elementary Ca^{2+} release events in skinned fibres of adult mammalian skeletal muscle. J Physiol 537:379–389

Lacampagne A, Ward CW, Klein MG, Schneider MF (1999) Time course of individual Ca^{2+} sparks in frog skeletal muscle recorded at high time resolution. J Gen Physiol 113:187–198

Lahat H, Pras E, Olender T, Avidan N, Ben-Asher E, Man O, Levy-Nissenbaum E, Khoury A, Lorber A, Goldman B, Lancet D, Eldar M (2001) A missense mutation in a highly conserved region of CASQ2 is associated with autosomal recessive catecholamine-induced polymorphic ventricular tachycardia in Bedouin families from Israel. Am J Hum Genet 69:1378–1384

Lamb GD, Cellini MA, Stephenson DG (2001) Different Ca^{2+} releasing action of caffeine and depolarisation in skeletal muscle fibres of the rat. J Physiol 531:715–728

Lamb GD, Stephenson DG (1992) Importance of Mg^{2+} in excitation-contraction coupling. NIPS 7:270–274

Launikonis BS, Zhou J, Royer L, Shannon TR, Brum G, Ríos E (2005) Confocal imaging of $[Ca^{2+}]$ in cellular organelles by SEER, shifted excitation and emission rationing. J Physiol 567:523–543

Launikonis BS, Zhou J, Royer L, Shannon TR, Brum G, Ríos E (2006a) Depletion ''skraps'' and dynamic buffering inside the cellular Ca^{2+} store. Proc Natl Acad Sci USA 103:2982–2987

Launikonis BS, Zhou J, Santiago D, Brum G, Ríos E (2006b) The changes in Ca^{2+} sparks associated with measured modifications of intra-store Ca^{2+} concentration in skeletal muscle. J Gen Physiol (In press)

Laver DR, Baynes TM, Dulhunty AF (1997) Magnesium inhibition of ryanodine-receptor calcium channels: evidence for two independent mechanisms. J Membr Biol 156(3):213–229

Lee EH, Lopez JR, Li J, Protasi F, Pessah IN, Kim do H, Allen PD (2004) Conformational coupling of DHPR, RyR1 in skeletal myotubes is influenced by long-range allosterism: evidence for a negative regulatory module. Am J Physiol Cell Physiol 286(1):C179–C189

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

Meissner G, Darling E, Eveleth J (1986) Kinetics of rapid Ca^{2+} release by sarcoplasmic reticulum. Effects of Ca^{2+}, $Mg^{2+,}$ and adenine nucleotides. Biochemistry 25(1):236–244

Mobley BA, Eisenberg BR (1975) Sizes of components in frog skeletal muscle measured by methods of stereology. J Gen Physiol 66(1):31–45

Murayama T, Ogawa Y (2004) RyR1 exhibits lower gain of CICR activity than RyR3 in the SR: evidence for selective stabilization of RyR1 channel. Am J Physiol Cell Physiol 287:C36–C45

Ogawa Y, Murayama T, Kurebayashi N (2002) Ryanodine receptor isoforms of non-mammalian skeletal muscle. Front Biosci 7:d1184–d1194

Ohkura M, Furukawa K, Fujimori H, Kuruma A, Kawano S, Hiraoka M, Kuniyasu A, Nakayama H, Ohizumi Y (1998) Dual regulation of the skeletal muscle ryanodine receptor by triadin and calsequestrin. Biochemistry 37(37):12987–1293

Page E, McCallister LP, Power B (1971) Stereological measurements of cardiac ultrastructures implicated in excitation–contraction coupling. Proc Natl Acad Sci USA 68(7):1465–1466

Pan Z, Yang D, Nagaraj RY, Nosek TA, Nishi M, Takeshima H, Cheng H, Ma J (2002) Dysfunction of store-operated calcium channel in muscle cells lacking mg29. Nat Cell Biol 4(5):379–383

Pape PC, Jong DS, Chandler WK (1998) Effects of partial sarcoplasmic reticulum calcium depletion on calcium release in frog cut muscle fibers equilibrated with 20 mM EGTA. J Gen Physiol 112(3):263–295

Pape PC, Carrier N (1998) Effect of sarcoplasmic reticulum (SR) calcium content on SR calcium release elicited by small voltage-clamp depolarizations in frog cut skeletal muscle fibers equilibrated with 20 mM EGTA. J Gen Physiol 112:161–179

Pape PC, Jong D-S, Chandler WK (1995) Calcium release and its voltage dependence in frog cut muscle fibers equilibrated with 20 mM EGTA. J Gen Physiol 106:259–336

Palmer AE, Jin C, Reed JC, Tsien RY (2004) Bcl-2-mediated alterations in endoplasmic reticulum Ca^{2+} analyzed with an improved genetically encoded fluorescent sensor. Proc Natl Acad Sci USA 101(50):17404–17409

Paolini C, Allen PD, Protasi F (2005) Cardiac-like calcium release units in skeletal muscle fibers expressing uniquely the cardiac isoforms of calsequestrin. Biophs J 88: 322a

Paolini C, Quarta M, Nori A, Volpe P, Reggian C, Allen PD, Protasi F (2006) Any role for calsequestrin in EC coupling? Initial studies on a skeletal calsequestrin knock-out mouse. Biophys J meeting supplement:5a

Park H, Park IY, Kim E, Youn B, Fields K, Dunker AK, Kang C (2004) Comparing skeletal and cardiac calsequestrin structures and their calcium binding: a proposed mechanism for coupled calcium binding and protein polymerization. J Biol Chem 279:18026–18033

Pizarro G, Ríos E (2004) How source content determines intracellular Ca^{2+} release kinetics. Simultaneous measurement of [Ca^{2+}] transients and [H^{+}] displacement in skeletal muscle. J Gen Physiol 124(3):239–258

Ríos E, Shirokova N, Kirsch WG, Pizarro G, Stern MD, Cheng H, González A (2001) A preferred amplitude of calcium sparks in skeletal muscle. Biophys J 80(1):169–183

Ríos E, Zhou J (2004) Control of dual isoforms of Ca^{2+} release channels in muscle. Biol Res 37(4):583–591

Ríos E, Stern MD, González A, Pizarro G, Shirokova N (1999) Calcium release flux underlying Ca^{2+} sparks of frog skeletal muscle. J Gen Physiol 114:31–48

Rudolf R, Magalhaes PJ, Pozzan T. (2006) Direct in vivo monitoring of sarcoplasmic reticulum Ca^{2+} and cytosolic cAMP dynamics in mouse skeletal muscle. J Cell Biol 173(2):187–193

Schneider MF, Simon BJ, Szucs G (1987) Depletion of calcium from the sarcoplasmic reticulum during calcium release in frog skeletal muscle. J Physiol 392:167–192

Shannon TR, Bers DM (1997) Assessment of intra-SR free [Ca] and buffering in rat heart. Biophys J 73(3):1524–1531

Shannon TR, Ginsburg KS, Bers DM (2000) Potentiation of fractional sarcoplasmic reticulum calcium release by total and free intra-sarcoplasmic reticulum calcium concentration. Biophys J 78(1):334–343

Shannon TR, Guo T, Bers DM (2003) Ca^{2+} scraps: local depletions of free [Ca^{2+}] in cardiac sarcoplasmic reticulum during contractions leave substantial Ca^{2+} reserve. Circ Res 93:40–45

Shirokova N, Garcia J, Pizarro G, Ríos E (1996) Ca^{2+} release from the sarcoplasmic reticulum compared in amphibian and mammalian skeletal muscle. J Gen Physiol 107(1):1–18

Shirokova N, Garcia J, Ríos E (1998) Local calcium release in mammalian skeletal muscle. J Physiol 512:377–384

Sipido KR, Wier WG (1991) Flux of Ca^{2+} across the sarcoplasmic reticulum of guinea-pig cardiac cells during excitation–contraction coupling. J Physiol 435:605–630

Sobie EA, Dilly KW, Cruz JD, Lederer WJ, Jafri MS (2002) Termination of Ca^{2+} sparks: an investigative mathematical model of calcium-induced calcium release. Biophys J 83:59–78

Stern MD, Cheng H (2004) Putting out the fire: what terminates calcium-induced calcium release in cardiac muscle? Cell Calcium 35:591–601

Stern MD, Pizarro G, Ríos E (1997) Local control model of excitation–contraction coupling in skeletal muscle. J Gen Physiol 110:415–440

Szegedi C, Sarkozi S, Herzog A, Jona I, Varsanyi M (1999) Calsequestrin: more than 'only' a luminal Ca^{2+} buffer inside the sarcoplasmic reticulum. Biochem J 337(Pt 1): 19–22

Terentyev D, Cala SE, Houle TD, Viatchenko-Karpinski S, Gyorke I, Terentyeva R, Williams SC, Gyorke S (2005) Triadin overexpression stimulates excitation–contraction coupling and increases predisposition to cellular arrhytmia in cardiac myocytes. Circ Res 96:651–658

Terentyev D, Viatchenko-Karpinski S, Gyorke I, Volpe P, Williams SC, Gyorke S (2003) Calsequestrin determines the functional size and stability of cardiac intracellular calcium stores: mechanism for hereditary arrhythmia. Proc Natl Acad Scien USA 100:11759–11764

Terentyev D, Viatchenko-Karpinski S, Valdivia HH, Escobar AL, Gyorke S (2002) Luminal Ca^{2+} controls termination and refractory behavior of Ca^{2+}-induced Ca^{2+} release in cardiac myocytes. Circ Res 91(5):414–420

Tripathy A, Meissner G (1996) Sarcoplasmic reticulum lumenal Ca^{2+} has access to cytosolic activation and inactivation sites of skeletal muscle Ca^{2+} release channel. Biophys J 70(6):2600–2615

Viatchenko-Karpinski S, Terentyev D, Gyorke I, Terentyeva R, Volpe P, Priori SG, Napolitano C, Nori A, Williams SC, Gyorke S (2004) Abnormal calcium signaling and sudden cardiac death associated with mutation of calsequestrin. Circ Res 94(4):471–477

Volpe P, Simon BJ (1991) The bulk of Ca^{2+} released to the myoplasm is free in the sarcoplasmic reticulum and does not unbind from calsequestrin. FEBS Lett 278(2):274–278

Wang SQ, Stern MD, Ríos E, Cheng H (2004) The quantal nature of Ca^{2+} sparks and in situ operation of the ryanodine receptor array in cardiac cells. Proc Natl Acad Sci USA 101:3979–3984

Wang W, Cleemann L, Jones LR, Morad M (2000) Modulation of focal and global Ca^{2+} release in calsequestrin-overexpressing mouse cardiomyocytes. J Physiol 524:399–414

Wang Y, Xu L, Duan H, Pasek DA, Eu JP, Meissner G (2006) Knocking down type 2 but not type 1 calsequestrin reduces calcium sequestration and release in C2C12 skeletal muscle myotubes. J Biol Chem (In press) doi/10.1074/jbc.M600090200

Wei L, Varsanyi M, Dulhunty AF, Beard NA (2006) The conformation of calsequestrin determines its ability to regulate skeletal ryanodine receptors. Biophys J (In press) doi:10.1529/biophysj.106.082610

Wray S, Burdyga T, Noble K (2005) Calcium signalling in smooth muscle. Cell Calcium 38:397–407

Zhang L, Kelley J, Schmeisser G, Kobayashi YM, Jones LR (1997) Complex formation between junctin, triadin, calsequestrin, and the ryanodine receptor. Proteins of the cardiac junctional sarcoplasmic reticulum membrane. J Biol Chem 272:23389–23397

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

Zhou J, Launikonis BS, Ríos E, Brum G (2004) Regulation of Ca^{2+} sparks by Ca^{2+} and Mg^{2+} in mammalian and amphibian muscle. An RyR isoform-specific role in excitation–contraction coupling? J Gen Physiol 124:409–428

Zhou J, Brum G, Gonzalez A, Launikonis BS, Stern MD, Rios E. (2005) Concerted vs. sequential. Two activation patterns of vast arrays of intracellular Ca^{2+} channels in muscle. J Gen Physiol 126:301–309

Zhou J, Yi J, Royer L, Launikonis BS, González A, Garcia J, Ríos E (2006) A probable role of dihydropyridine receptors in repression of Ca^{2+} sparks demonstrated in cultured mammalian muscle. Am J Physiol Cell Physiol 290:C539–C553

Zhuge R, Fogarty KE, Tuft RA, Walsh JV Jr (2002) Spontaneous transient outward currents arise from microdomains where BK channels are exposed to a mean Ca^{2+} concentration on the order of 10 μM during a Ca^{2+} spark. J Gen Physiol 120(1):15–27

Zhuge R, Fogarty KE, Tuft RA, Lifshitz LM, Sayar K, Walsh JV Jr (2000) Dynamics of signaling between Ca^{2+} sparks and Ca^{2+}- activated K(+) channels studied with a novel image-based method for direct intracellular measurement of ryanodine receptor Ca^{2+} current. J Gen Physiol 116:845–864