

Control of muscle ryanodine receptor calcium release channels by proteins in the sarcoplasmic reticulum lumen

Nicole A. Beard, Lan Wei,* and Angela F. Dulhunty

John Curtin School of Medical Research, Australian National University, ACT 2601, Australia

*Current address: Department of Physiology and Pharmacology, University of Rochester, Rochester, NY, USA

Summary

1. Many biological processes which are governed by intracellular calcium signals rely on intracellular stores that provide a reliable, controlled release of calcium into the cytoplasm. Calcium release through the ryanodine receptor, the main ion channel in the sarcoplasmic reticulum (the calcium store in muscle) is the key determinant of muscle force.

2. Calsequestrin, the main calcium buffer in the sarcoplasmic reticulum, provides a pool of calcium for release through the ryanodine receptor and acts as a luminal calcium sensor for the channel *via* its interactions with triadin and junctin. Until recently, how calsequestrin communicated the store Ca^{2+} load to the RyR remained elusive.

3. Calsequestrin 1 (skeletal calsequestrin) has been shown to both inhibit and activate the skeletal ryanodine receptor 1, dependent on whether it's bound to the ryanodine receptor 1 directly, or indirectly, *via* anchoring proteins

4. The phosphorylation status of calsequestrin 1 is deemed important; it influences calsequestrin's Ca^{2+} binding capacity, the way in which calsequestrin 1 regulates the ryanodine receptor 1 and how it interacts with the key anchoring protein junctin.

5. In skeletal muscle junctin plays a more critical role than triadin in the mechanism which control of Ca^{2+} release from the SR.

6. The close relationship between altered expression and dysfunction of calsequestrin in several skeletal and cardiac disorders highlights the critical role that calsequestrin plays in maintaining Ca^{2+} homeostasis and regulation of muscle contraction.

Introduction

Cellular signaling in most eukaryotic organisms depends on Ca^{2+} release from intracellular Ca^{2+} stores. The efficacy of this release depends on both the Ca^{2+} binding capacity of the store proteins and the activity of Ca^{2+} release channels in the store membrane. The Ca^{2+} store in striated muscle, the sarcoplasmic reticulum (SR) is pivotal to the vital function of movement, respiration and heart beat.

Excitation contraction (EC) coupling is the mechanism linking depolarisation of the surface membrane with Ca^{2+} release from the SR and initiates skeletal and cardiac contraction. EC coupling depends on activation of

the SR Ca^{2+} release channel (the ryanodine receptor (RyR)) by an interaction with the dihydropyridine receptor (DHPR), the major Ca^{2+} channel in the surface membrane. In skeletal muscle this interaction is mechanical, whereby the II-III loop of the DHPR is thought to physically couple to part of the cytoplasmic portion of the RyR, causing channel activation. In cardiac muscle, Ca^{2+} ions enter the cytoplasm through the DHPR channel initiating Ca^{2+} -activated Ca^{2+} release through the RyR. Both the RyR and DHPR are essential for skeletal and cardiac muscle function, as failure in their expression leads to death at or before birth.

Both the skeletal (RyR1) and cardiac (RyR2) isoforms form the hub of giant macromolecular complexes with transmembrane, luminal and cytoplasmic proteins. The activity of the channels is set by the integrated effects of associated proteins/ligands, covalent modification by redox reactions and phosphorylation. Luminal interactions, such as that with the major SR Ca^{2+} binding protein – calsequestrin (CSQ) – are essential for communicating the [Ca^{2+}] in the SR store to the RyR.¹⁻³

Calsequestrin

CSQ is a small acidic Ca^{2+} binding glycoprotein which resides within the terminal cisternae of the SR,^{4,5} and which has a moderate affinity and high capacity for Ca^{2+} .⁶ CSQ binds to the transmembrane proteins triadin and junctin (Figure 1A), forming a protein complex in the lumen of the SR capable of associating with, and communicating store Ca^{2+} load to, the RyR.⁷⁻⁹ It is thought that in the absence of Ca^{2+} , CSQ is a random coil monomer and folds into thioredoxin-like domains (found in several Ca^{2+} binding proteins). The protein then dimerise and polymerise as [Ca^{2+}] increases to ~1 mM (Figure 1A). It is this polymer which ensures that free Ca^{2+} within the SR is buffered to 1 mM¹⁰ even though the total [Ca^{2+}] varies greatly during Ca^{2+} release cycles¹¹ and falls to ~0.5 mM after a single heart beat (cardiac) or to as low as 100 μM (skeletal) after experimentally-induced Ca^{2+} release.^{12,13} Polymerisation allows CSQ to bind Ca^{2+} with high capacity, and to transmit the information about the [Ca^{2+}] to triadin and junctin so that the multi-protein complex in the lumen of the SR forms a luminal Ca^{2+} transduction machine (Figure 1A). It appears that *in vivo*, control of the RyR *via* CSQ in skeletal muscle is mostly mediated by junctin and triadin (see below).

Two CSQ isoforms are found in muscle, encoded by different genes. Skeletal CSQ1 is the only isoform

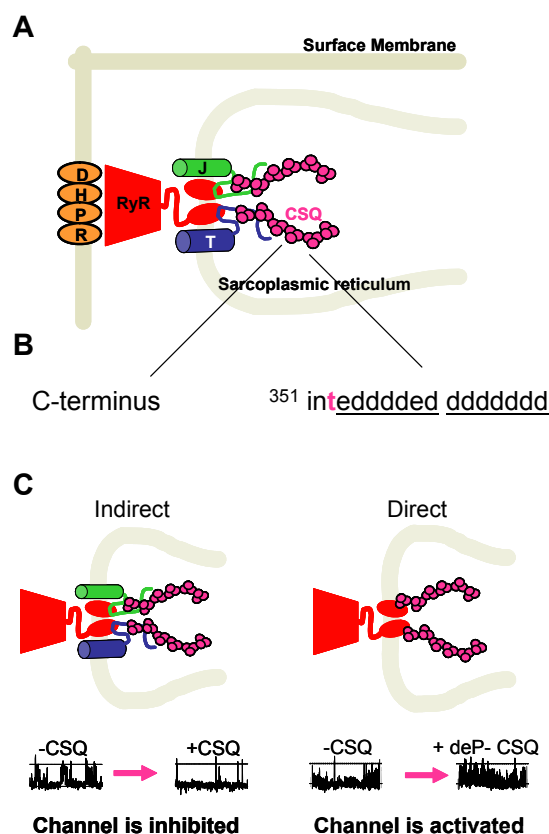


Figure 1. Association of CSQ with triadin, junctin and the RyR in skeletal muscle. **A:** Schematic structure of the skeletal triad junction. DHPR tetrads in the t-tubule membrane oppose every other RyR embedded in the SR membrane. The RyR1 is associated with CSQ1 via two anchoring proteins, triadin (T) and junctin (J). **B:** Rabbit skeletal CSQ1 sequence C-terminal region, with phosphorylatable threonine residue in purple, and the acidic rich region responsible for CSQ1 associating with triadin and junctin (and the proposed Ca²⁺ binding motif) underlined. **C:** CSQ1 regulates the skeletal RyR1 via two distinct mechanisms, one which involves inhibition of RyR1 activity by binding to triadin and junctin (left), and the other by activating the RyR1 in a phosphorylation-dependent manner by binding directly to the RyR1 itself (right). Beneath are shown single channel recordings (3 s) illustrating the effect of each mechanism when CSQ1 binds to the native RyR1 complex (left) or to purified RyR1s (right). Whilst the panel on the left hand side shows native RyR1 inhibition after addition of phosphorylated CSQ1, very similar inhibition of channel activity is observed after the addition of dephosphorylated CSQ1 (data not shown). In contrast, only dephosphorylated CSQ1 (deP-CSQ) activated the purified RyR1 (Figure 1C, right panel).

expressed in fast-twitch skeletal muscle, and is the major isoform expressed in slow-twitch skeletal muscle, but is absent in the heart.¹⁴⁻¹⁶ In contrast, CSQ2, (cardiac CSQ) is the sole isoform expressed in cardiac muscle, is a minor transcript in slow-twitch skeletal muscle and is absent in

fast-twitch muscle. Whilst the CSQ sequence is highly conserved across isoforms and species, (reviewed in Beard, Laver & Dulhunty, 2004¹⁷), the CSQ isoforms differ mainly in their C-terminal region, with CSQ2 having an extended C-terminus (residues 367-391) containing 71% acidic residues.

The C-terminus of CSQ contains one (CSQ1) or two (CSQ2) glycosylation sites as well as one (CSQ1) or three (CSQ2) phosphorylation sites for casein kinase II (CKII)^{18,19} (Figure 1B). This region, which is rich in acidic residues and contains the single binding site for triadin, is believed to form part of the binding site for junctin and is also purported to form a Ca²⁺ binding sink upon CSQ1 polymerisation.²⁰⁻²²

Triadin and junctin

Junctin and triadin are integral transmembrane proteins found in the SR of cardiac and skeletal muscle. Both triadin and junctin have a single membrane spanning domain, a short cytoplasmic N-terminus and a longer, highly charged C-terminal tail in the SR lumen.^{7,23} Junctin is expressed in many tissues and is a splice variant of aspartyl β-hydroxylase, missing the N-terminal catalytic subunit of this enzyme. Junctin (~27 kDa) has been characterized mainly in cardiac muscle and has been found to associate with triadin, the RyR and CSQ.⁹ Unlike the binding site on CSQ1 for triadin (see paragraph above), the association between junctin and CSQ1 or CSQ2 does not involve one discrete site, but is likely to contain several binding motifs.²⁰

CSQ influences RyR activity

CSQ is normally considered to interact with the RyR only via triadin and junctin, forming a luminal protein complex capable of controlling release through the RyR, termed the luminal Ca²⁺ transduction machine. In artificial lipid bilayer experiments, CSQ1 has been shown to inhibit channel activity of native skeletal RyRs under physiological or resting Ca²⁺ conditions.² Under native conditions, the RyR1 is embedded into the lipid bilayer with the full complement of associated proteins, which includes triadin, junctin and CSQ1. An early report indicated that CSQ1 can also bind to the RyR1 directly,²⁴ a finding which we have repeated in our laboratory. When the RyR was purified (and triadin, junctin and CSQ removed), addition of exogenous CSQ1 activated the RyR1 (under certain conditions, see section entitled the role of CSQ phosphorylation in the RyR response to luminal Ca²⁺). These data led to the hypothesis that there are two distinct mechanisms for CSQ1 association with, and regulation of, the RyR1 (Figure 1C): an indirect association (via triadin and junctin) whereby CSQ1 inhibits RyR1 activity (Figure 1C, left panel); and a direct interaction between CSQ1 and the RyR1, resulting in channel activation (Figure 1C, right panel). The contribution of a direct interaction between CSQ1 and the RyR1 to CSQ1-mediated regulation of SR Ca²⁺ release is unknown. The fact that CSQ1 significantly inhibits RyR1 channel activity when all proteins are present (*i.e.* native

RyR1),^{1,10} indicates either that the direct activating skeletal CSQ1-RyR1 interaction does not occur, or that if CSQ1 does bind to the RyR1 directly, channel activation is masked by the inhibition that occurs when CSQ1 binds to triadin and junctin.

Cardiac CSQ has been reported not to alter purified RyR2 activity in the heart, but to activate²⁵ or inhibit³ RyR2 activity when anchoring proteins triadin and junctin are associated with the RyR2 at low (20 μ M) luminal Ca^{2+} .³ Association of CSQ2 with native RyR2s confers responsiveness to luminal Ca^{2+} , particularly over the physiologically relevant ranges of 100 μ M-1 mM Ca^{2+} .²⁶ So CSQ2 is a major luminal Ca^{2+} sensor in the heart. The role of CSQ2 on stabilizing RyR2 release has been defined further and it is reported that CSQ2 can inhibit RyR2 specifically through its interaction with triadin.²⁷ No systematic study on the action(s) of CSQ2 on native and purified RyR2 under physiological [Ca^{2+}] has been published thus far.

The role of CSQ1 phosphorylation on the RyR1 response to luminal Ca^{2+}

CSQ phosphorylation was originally thought to be essential for retention of CSQ1 in the SR, as CSQ1 lacks the conventional luminal targeting sequence (KDEL).²⁸ However, mutation of the phosphorylation site did not alter targeting of CSQ1 to the SR lumen,²⁸ thus there must be alternative reasons for CSQ1 phosphorylation *in vivo*. Whilst these have not been definitively identified, there are several lines of evidence to support the hypothesis that CSQ1 phosphorylation may alter the response of the RyR1 to CSQ1 and thus regulate Ca^{2+} release. CSQ1 can be isolated from skeletal muscle tissue in both predominantly non-phosphorylated^{18,29} or predominantly phosphorylated forms,^{1,30} with the phosphorylation level estimated to be 1 mmol P_i /mol CSQ.³⁰ ATP entry pathways into the SR have been described³¹ and CKII (for which CSQ contains a consensus sequence and for which CSQ1 and CSQ2 are *in vitro* phosphorylation targets) has been localized to the SR.³² Therefore, it seems likely that a CSQ1 phosphorylation cycle may have regulatory impact *in vivo*. The sequence required for CKII catalysis is S/TXXD/E. The only threonine with this CKII consensus sequence in rabbit skeletal CSQ is located at the beginning of the C-terminus (³⁵³TEDD; Figure 1B) which can be CKII-phosphorylated *in vitro*.¹⁸

CSQ1 activation of purified skeletal RyRs (see above) is dependant on the phosphorylation status of CSQ1. Dephosphorylated CSQ1 induced an almost 5-fold increase in skeletal RyR open probability (Szegedi *et al.*, 1999²⁴ and Figure 1C, right panel), whilst the phosphorylated form of CSQ1 was unable to regulate the purified channel, despite their similar high affinity binding to the RyR1.³³ Curiously, it was found that 1% of dephosphorylated CSQ1 was sufficient to activate the RyR1,²⁴ which is depicted in Figure 1C, right panel). In contrast, native skeletal RyRs under resting luminal [Ca^{2+}] (1 mM) failed to show a difference between phosphorylated and dephosphorylated

CSQ1, since both inhibit RyR1s in a similar manner (Beard *et al.*;¹ Figure 1C, left panel).

Despite not having any influence on RyR1 channel gating, the phosphorylation status of CSQ1 does influence the Ca^{2+} binding capacity of CSQ1, which is reduced by more than 1/3rd when skeletal CSQ is dephosphorylated.³⁴ This reduction in Ca^{2+} binding capacity may reflect a reduced ability of dephosphorylated CSQ1 to polymerise.²² The major effects of CSQ1 phosphorylation/dephosphorylation on the conformation of CSQ1 and Ca^{2+} binding capacity did not affect the way in which CSQ1 influences RyR1s (at [Ca^{2+}] of 100 μ M-1 mM,³⁴ which occur *in vivo*).¹² Apparently, CSQ1 can still function as a physiological regulator of the skeletal RyR and maintain store load, despite having a reduced capacity for binding Ca^{2+} .

CSQ1 dephosphorylation alters the CSQ1/junctin interaction and enhances SR Ca^{2+} depletion

CSQ1 phosphorylation does not alter the way in which it interacts with the anchoring protein triadin, but surprisingly does alter the interaction with junctin and the functional action of skeletal CSQ on RyR1. Junctin has been shown to bind very strongly to CSQ,^{21,34} and this process is highly Ca^{2+} -dependent.²¹ Junctin's strongest association with phosphorylated CSQ1 is in the absence of Ca^{2+} , although a significant proportion of junctin associates with phosphorylated CSQ1 even at 1 mM Ca^{2+} .^{21,34} In marked contrast, at low luminal Ca^{2+} , dephosphorylation of CSQ1 significantly reduces (at 100 μ M Ca^{2+}) or completely abolishes (100 nM Ca^{2+}) its association with junctin, although binding at resting [Ca^{2+}] is unaffected. The reduced ability of dephosphorylated CSQ1 to bind to junctin at low luminal Ca^{2+} is associated with a change in the way CSQ regulates the RyR in skeletal muscle.

Dephosphorylated CSQ1 actually activates the native skeletal RyR in this situation (low Ca^{2+}), which is in stark contrast to the strong inhibition imposed at 1 mM Ca^{2+} .³⁴ Indeed, at low luminal Ca^{2+} , dephosphorylated CSQ1 regulates the RyR1 in a manner strikingly similar to the CSQ2-imposed activation of RyR2 in the heart (at 1 mM luminal Ca^{2+} ²⁶) or the phosphorylation-dependant activation of the purified skeletal RyR (when triadin and junctin are absent) (with 1 mM luminal Ca^{2+} ²⁴). Although the mechanisms are undefined, it appears that when the Ca^{2+} stores are low, dephosphorylation of CSQ1 would enhance Ca^{2+} depletion of the SR. It is tempting to speculate that the mechanism for this activation is somehow linked to dephosphorylated CSQ1's reduced binding to junctin. This provides insight into the previously obscure roles of triadin and junctin and suggests that the function of the two anchoring proteins may differ.

Junctin mediates the inhibitory action of CSQ1 on the skeletal RyR

It appears that skeletal junctin plays a pivotal role in inhibiting RyR1 when Ca^{2+} stores are depleted and in maintaining a normal intracellular Ca^{2+} cycling in a

phosphorylation- and Ca²⁺-dependent manner. We were interested in investigating the role of junctin in modulating SR Ca²⁺ release (*via* the RyR1), particularly as there have been no reports on the effects of junctin on the RyR1 directly, or its role in facilitating CSQ1 regulation of the RyR1.

A review of current literature does reveal, however, the potential role of junctin in modulating the SR/Ca²⁺ release. Junctin overexpression in mouse hearts elicits hearts with impaired muscle relaxation and contractility, with decreases in expression of the cardiac forms of RyR and triadin, and with a reduced SR Ca²⁺ store.^{23,35-37} Down-regulation of junctin in cardiac myocytes increases the fractional shortening and maximal rates of muscle contraction/relaxation.³⁸⁻⁴⁰ Knockout of junctin in a mouse model causes enhanced Ca²⁺ spark amplitude and frequency, a reduction in spark duration⁴¹ and an apparent overload of the SR Ca²⁺ store (but no alterations in the expression levels of other Ca²⁺ handling proteins). Thus it appears that junctin influences the size of the SR Ca²⁺ store and Ca²⁺ release in the heart.

The first study to look at the direct effects of junctin on the cardiac RyR channel elegantly showed that junctin and triadin added together cannot confer RyR2 responsiveness to luminal Ca²⁺, but that the two proteins can communicate the activatory message of CSQ2 to the RyR2.³ Triadin appears to be important for stabilizing the cardiac CSQ-RyR interaction.²⁷ The only experiments so far to dissect the individual roles of triadin and junctin in regulating the RyR1 has been done in our laboratory. These experiments consistently revealed that junctin alone is essential for maintaining the skeletal luminal transduction machine (Figure 2 and unpublished data) and that the minimal construct of the luminal transduction machine is RyR1/junctin/CSQ1. In lipid bilayers, purified rabbit skeletal triadin, junctin and CSQ were re-associated with the purified RyR1 sequentially or in combination, and their effects on channel activity recorded at 1 mM luminal Ca²⁺. Our initial results (Figure 2) indicate that junctin alone (but not triadin alone), mediates skeletal RyR inhibition by CSQ1. In the presence of junctin alone (using protein concentrations previously reported to modulate RyR function in cardiac RyR in lipid bilayers³), CSQ1 induced an around 60% RyR1 inhibition, reminiscent of CSQ1s effect on the native RyR1.² Triadin alone could not potentiate any effect of CSQ1 on the purified RyR1, despite extended incubation times or different concentrations of proteins added to the channel (N=5). We find that junctin plays a role in mediation of the normal inhibitory action of skeletal CSQ on the skeletal RyR in a luminal Ca²⁺- and phosphorylation-dependent manner. Thus, the characteristic decline in RyR1 activity induced by phosphorylated or dephosphorylated CSQ1 in the reassembled RyR1/triadin/junctin/CSQ1 complex in the presence of physiological Ca²⁺ concentrations is mostly mediated by junctin. This conclusion supports our previous proposal that (a) junctin plays a more critical role than triadin in native skeletal RyR modulation and (b) that an enhanced interaction between junctin and phosphorylated

CSQ1 (compared with dephosphorylated CSQ) in the presence of low luminal Ca²⁺ is likely to be important in RyR1 regulation by CSQ1 and maintenance of SR Ca²⁺ under these conditions.

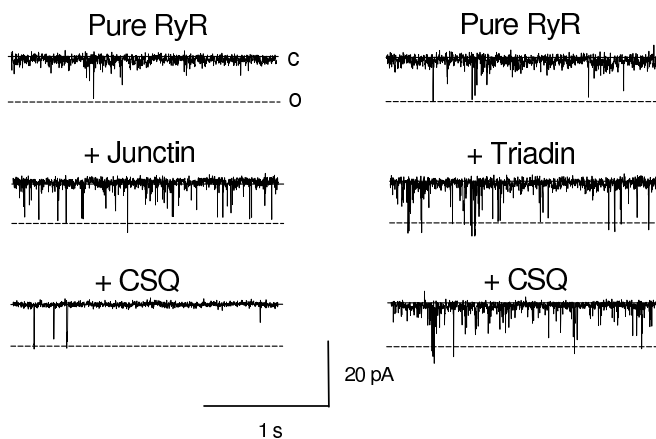


Figure 2. Junctin, but not triadin, potentiates CSQ1 inhibition of the skeletal RyR. Recordings (3s) of purified skeletal RyR activity, at -40 mV, where channel opening is downward from zero current (c) to maximum open conductance (o). Addition of 5 $\mu\text{g/ml}$ junctin (2nd row left) or 5 $\mu\text{g/ml}$ triadin (2nd row right) each activated the RyR1 channel. Addition of 16 $\mu\text{g/ml}$ CSQ1 to junctin-associated channels caused channel inhibition (3rd row left). The addition of 16 $\mu\text{g/ml}$ CSQ1 to triadin-associated channels did not result in any channel regulation (3rd row right).

The role of CSQ in skeletal and cardiac function and disease

Store-dependent Ca²⁺ release processes require a well maintained intracellular Ca²⁺ store and are essential to the function of most eukaryotic cell types. The role of CSQ in regulating the RyR and governing the Ca²⁺ store in both skeletal and cardiac muscle appears pivotal to store-dependent Ca²⁺ release in these tissues, particularly in skeletal muscle. This role is in part dependant on Ca²⁺- and phosphorylation-dependent structural changes in CSQ, as well as on its associations with Ca²⁺ and with the anchoring protein triadin and more importantly in skeletal muscle, with junctin. The crucial role of CSQ in store Ca²⁺ regulation processes is highlighted by the influence of altered CSQ expression, CSQ point mutation and CSQ-targeted drug-induced toxicity on several disease states. Skeletal muscle fibres from patients with Duchenne muscular dystrophy^{42,43} and in a mouse model of streptozotocin-induced diabetes show altered expression of CSQ,⁴⁴ which leads to muscle necrosis and altered levels of SR Ca²⁺. Seven point mutations on cardiac CSQ2 have thusfar been identified, which lead to sudden cardiac death as a result of ventricular fibrillation in the form of catecholaminergic polymorphic ventricular tachycardia following exposure to catecholamines, stress or moderate exercise (reviewed in Gyorko & Terentyev, 2008⁴⁵). Long

term use of common chemotherapeutic agents (anthracyclines) can lead to cardiotoxicity and death.⁴⁶ The cause of death is believed to be due to mishandling of Ca²⁺ by the cardiac SR. Chronic exposure to anthracyclines causes both changes in expression of Ca²⁺ handling proteins, (including CSQ2) and effects due to binding to CSQ2. The drugs may bind to other SR proteins, but these have not yet been identified. The close relationship between CSQ2 and these diseases demonstrates its critical role in Ca²⁺ homeostasis and regulation of muscle contraction and further emphasizes the physiological and clinical importance of investigating the interactions between the members of the luminal transduction machine and the way in which they control SR Ca²⁺ handling.

Conclusions

CSQ, the main Ca²⁺ buffering protein in the lumen of the SR, is an important regulator of the RyR in skeletal muscle. CSQ1 can either activate or inhibit the RyR1, dependent on whether it binds directly to the RyR or associates with the channel *via* anchoring proteins triadin and junctin. CSQ1 inhibition of RyR1, thought to be mediated through triadin and junctin, is believed the physiological control mechanism of Ca²⁺ release in skeletal muscle. CSQ is capable of undergoing phosphorylation *in vivo*. This mechanism appears to control the Ca²⁺ binding capacity of CSQ1, and the way in which it binds to junctin and regulates the native RyR1 under low Ca²⁺ conditions. These data highlighted the role that junctin may play in controlling RyR1 activity and we postulate that junctin inhibits RyR1 when Ca²⁺ stores are depleted and maintains a normal intracellular Ca²⁺ cycling in a phosphorylation- and Ca²⁺-dependent manner. Further investigation of junctin has revealed that junctin, and not triadin, is likely to play a more critical role in native skeletal RyR modulation, and that the minimal protein composition to transmit CSQ1 inhibitory message to the RyR1 is RyR1/junctin/CSQ1.

References

1. Beard NA, Casarotto MG, Wei L, Varsányi M, Laver DR, Dulhunty AF. Regulation of ryanodine receptors by calsequestrin: effect of high luminal Ca²⁺ and phosphorylation. *Biophys. J.* 2005; **88**: 3444-54.
2. Beard NA, Sakowska MM, Dulhunty AF, Laver DR. Calsequestrin is an inhibitor of skeletal muscle ryanodine receptor calcium release channels. *Biophys. J.* 2002; **82**: 310-20.
3. Gyorke I, Hester N, Jones LR, Gyorke S. The role of calsequestrin, triadin, and junctin in conferring cardiac ryanodine receptor responsiveness to luminal calcium. *Biophys. J.* 2004; **86**: 2121-8.
4. Franzini-Armstrong C, Kenney LJ, Varriano-Marston E. The structure of calsequestrin in triads of vertebrate skeletal muscle: a deep-etch study. *J. Cell. Biol.* 1987; **105**: 49-56.
5. Wagenknecht T, Hsieh CE, Rath BK, Fleischer S, Marko M. Electron tomography of frozen-hydrated isolated triad junctions. *Biophys. J.* 2002; **83**: 2491-501.
6. MacLennan DH, Wong PT. Isolation of a calcium-sequestering protein from sarcoplasmic reticulum. *Proc. Natl. Acad. Sci. U S A.* 1971; **68**: 1231-5.
7. Jones LR, Zhang L, Sanborn K, Jorgensen AO, Kelley J. Purification, primary structure, and immunological characterization of the 26-kDa calsequestrin binding protein (junctin) from cardiac junctional sarcoplasmic reticulum. *J. Biol. Chem.* 1995; **270**: 30787-96.
8. Mitchell RD, Simmerman HK, Jones LR. Ca²⁺ binding effects on protein conformation and protein interactions of canine cardiac calsequestrin. *J. Biol. Chem.* 1988; **263**: 1376-81.
9. Zhang L, Kelley J, Schmeisser G, Kobayashi YM, Jones LR. Complex formation between junctin, triadin, calsequestrin, and the ryanodine receptor. Proteins of the cardiac junctional sarcoplasmic reticulum membrane. *J. Biol. Chem.* 1997; **272**: 23389-97.
10. Wei L, Varsanyi M, Dulhunty AF, Beard NA. The conformation of calsequestrin determines its ability to regulate skeletal ryanodine receptors. *Biophys. J.* 2006; **91**: 1288-301.
11. Fryer MW, Stephenson DG. Total and sarcoplasmic reticulum calcium contents of skinned fibres from rat skeletal muscle. *J. Physiol. (Lond)* 1996; **493**: 357-70.
12. Launikonis BS, Zhou J, Royer L, Shannon TR, Brum G, Rios E. Depletion "skraps" and dynamic buffering inside the cellular calcium store. *Proc. Natl. Acad. Sci. U S A.* 2006; **103**: 2982-87.
13. Shannon TR, Guo T, Bers DM. Ca²⁺ scraps: local depletions of free [Ca²⁺] in cardiac sarcoplasmic reticulum during contractions leave substantial Ca²⁺ reserve. *Circ. Res.* 2003; **93**: 40-5.
14. Arai M, Tomaru K, Takizawa T *et al.* Sarcoplasmic reticulum genes are selectively down-regulated in cardiomyopathy produced by doxorubicin in rabbits. *J. Mol. Cell. Cardiol.* 1998; **30**: 243-54.
15. Biral D, Volpe P, Damiani E, Margreth A. Coexistence of two calsequestrin isoforms in rabbit slow-twitch skeletal muscle fibers. *FEBS Lett.* 1992; **299**: 175-8.
16. Damiani E, Margreth A. Characterization study of the ryanodine receptor and of calsequestrin isoforms of mammalian skeletal muscles in relation to fibre types. *J. Muscle. Res. Cell. Motil.* 1994; **15**: 86-101.
17. Beard NA, Laver DR, Dulhunty AF. Calsequestrin and the calcium release channel of skeletal and cardiac muscle. *Prog. Biophys. Mol. Biol.* 2004; **155**: 33-69.
18. Cala SE, Jones LR. Phosphorylation of cardiac and skeletal muscle calsequestrin isoforms by casein kinase II. Demonstration of a cluster of unique rapidly phosphorylated sites in cardiac calsequestrin. *J. Biol. Chem.* 1991; **266**: 391-8.
19. Cala SE, Miles K. Phosphorylation of the cardiac isoform of calsequestrin in cultured rat myotubes and rat skeletal muscle. *Biochim. Biophys. Acta*

- 1992; **1118**: 277-87.
20. Kobayashi YM, Alseikhan BA, Jones LR. Localization and characterization of the calsequestrin-binding domain of triadin I. Evidence for a charged beta-strand in mediating the protein-protein interaction. *J. Biol. Chem.* 2000; **275**:17639-46.
 21. Shin DW, Ma J, Kim DH. The asp-rich region at the carboxyl-terminus of calsequestrin binds to Ca²⁺ and interacts with triadin. *FEBS Lett.* 2000; **486**: 178-82.
 22. Wang S, Trumble WR, Liao H, Wesson CR, Dunker AK, Kang CH. Crystal structure of calsequestrin from rabbit skeletal muscle sarcoplasmic reticulum. *Nat. Struct. Biol.* 1998; **5**: 476-83.
 23. Knudson CM, Stang KK, Moomaw CR, Slaughter CA, Campbell KP. Primary structure and topological analysis of a skeletal muscle- specific junctional sarcoplasmic reticulum glycoprotein (triadin). *J. Biol. Chem.* 1993; **268**: 12646-54.
 24. Szegedi C, Sarkozi S, Herzog A, Jona I, Varsanyi M. Calsequestrin: more than 'only' a luminal Ca²⁺ buffer inside the sarcoplasmic reticulum. *Biochem. J.* 1999; **337**: 19-22.
 25. Wei L, Abdellatif YA, Liu D *et al.* Muscle-specific GSTM2-2 on the luminal side of the sarcoplasmic reticulum modifies RyR ion channel activity. *Int. J. Biochem. Cell Biol.* 2008; **40**: 1616-28.
 26. Qin J, Valle G, Nani A *et al.* Luminal Ca²⁺ regulation of single cardiac ryanodine receptors: insights provided by calsequestrin and its mutants. *J. Gen. Physiol.* 2008; **131**: 325-34.
 27. Terentyev D, Viatchenko-Karpinski S, Vedamoorthyrao S *et al.* Protein protein interactions between triadin and calsequestrin are involved in modulation of sarcoplasmic reticulum calcium release in cardiac myocytes. *J. Physiol.* 2007; **583**: 71-80.
 28. Nori A, Gola E, Tosato S, Cantini M, Volpe P. Targeting of calsequestrin to sarcoplasmic reticulum after deletions of its acidic carboxy terminus. *Am. J. Physiol.* 1999; **277**: C974-C81.
 29. O'Brian JJ, Ram ML, Kiarash A, Cala SE. Mass spectrometry of cardiac calsequestrin characterizes microheterogeneity unique to heart and indicative of complex intracellular transit. *J. Biol. Chem.* 2002; **277**: 37154-60.
 30. Varsanyi M, Heilmeyer LM, Jr. Autocatalytic phosphorylation of calsequestrin. *FEBS Lett* 1980; **122**: 227-30.
 31. Shoshan-Barmatz V, Hadad N, Feng W *et al.* VDAC/porin is present in sarcoplasmic reticulum from skeletal muscle. *FEBS Lett.* 1996; **386**: 205-10.
 32. Shoshan-Barmatz V, Orr I, Weil S, Meyer H, Varsanyi M, Heilmeyer LM. The identification of the phosphorylated 150/160-kDa proteins of sarcoplasmic reticulum, their kinase and their association with the ryanodine receptor. *Biochim. Biophys. Acta* 1996; **1283**:89-100.
 33. Herzog A, Szegedi C, Jona I, Herberg FW, Varsanyi M. Surface plasmon resonance studies prove the interaction of skeletal muscle sarcoplasmic reticular Ca²⁺ release channel/ryanodine receptor with calsequestrin. *FEBS Lett.* 2000; **472**:73-7.
 34. Beard NA, Wei L, Cheung SN, Kimura T, Varsanyi M, Dulhunty AF. Phosphorylation of skeletal muscle calsequestrin enhances its Ca²⁺ binding capacity and promotes its association with junctin. *Cell Calc.* 2008; <http://dx.doi.org/10.1016/j.ceca.2008.01.005>
 35. Hong CS, Cho MC, Kwak YG *et al.* Cardiac remodeling and atrial fibrillation in transgenic mice overexpressing junctin. *Faseb. J.* 2002; **16**: 1310-2.
 36. Kirchhefer U, Baba HA, Hanske G *et al.* Age-dependent biochemical and contractile properties in atrium of transgenic mice overexpressing junctin. *Am. J. Physiol.* 2004; **287**: H2216-25.
 37. Kirchhefer U, Hanske G, Jones LR *et al.* Overexpression of junctin causes adaptive changes in cardiac myocyte Ca²⁺ signaling. *Cell Calc.* 2006; **39**: 131-42.
 38. Franzini-Armstrong C, Protasi F, Tijssens P. The assembly of calcium release units in cardiac muscle. *Ann. NY Acad. Sci.* 2005; **1047**: 76-85.
 39. Tijssens P, Jones LR, Franzini-Armstrong C. Junctin and calsequestrin overexpression in cardiac muscle: the role of junctin and the synthetic and delivery pathways for the two proteins. *J. Mol. Cell. Cardiol.* 2003; **35**: 961-74.
 40. Zhang L, Franzini-Armstrong C, Ramesh V, Jones LR. Structural alterations in cardiac calcium release units resulting from overexpression of junctin. *J. Mol. Cell Cardiol.* 2001; **33**: 233-47.
 41. Yuan Q, Fan GC, Dong M *et al.* Sarcoplasmic reticulum calcium overloading in junctin deficiency enhances cardiac contractility but increases ventricular automaticity. *Circ.* 2007; **115**: 300-9.
 42. Culligan K, Banville N, Dowling P, Ohlendieck K. Drastic reduction of calsequestrin-like proteins and impaired calcium binding in dystrophic mdx muscle. *J. Appl. Physiol.* 2002; **92**: 435-45.
 43. Lohan J, Ohlendieck K. Drastic reduction in the luminal Ca²⁺-binding proteins calsequestrin and sarcalumenin in dystrophin-deficient cardiac muscle. *Biochim. Biophys. Acta* 2004; **1689**: 252-8.
 44. Howarth FC, Glover L, Culligan K, Qureshi MA, Ohlendieck K. Calsequestrin expression and calcium binding is increased in streptozotocin-induced diabetic rat skeletal muscle though not in cardiac muscle. *Pflügers Arch.* 2002; **444**: 52-8.
 45. Györke S, Terentyev D. Modulation of ryanodine receptor by luminal calcium and accessory proteins in health and cardiac disease. *Cardiovasc. Res.* 2008; **77**: 245-55.
 46. Charlier HA, Jr., Olson RD, Thornock CM *et al.* Investigations of calsequestrin as a target for anthracyclines: comparison of functional effects of daunorubicin, daunorubicinol, and trifluoperazine. *Mol. Pharmacol.* 2005; **67**: 1505-12.

Received 22 June 2008, in revised form 31 July 2008.

Accepted 13 August 2008.
© N.A. Beard 2008.

Author for correspondence:

Dr N.A. Beard,
John Curtin School of Medical Research,
Australian National University,
PO Box 334,
Canberra, ACT 2601,
Australia

Tel: +61 2 6125 8391
Fax: +61 2 6125 0415
E-mail: nicole.beard@anu.edu.au