The long-term interest of our laboratory on the regulation of EC coupling from within the sarcoplasmic reticulum has materialized in a funded NIH research project for the study of ***calsequestrin roles in health and disease***. This is a summary of background and aims of the project. I thank Chulhee Kang (a Co-PI in this project), Carlo Manno, Carolina Figueroa, Claudio Pérez, Clara Franzini-Armstrong, Sheila Riazi, Natalia Kraeva, Dirk Gillespie and Dezso Boda for multiple contributions to this project.

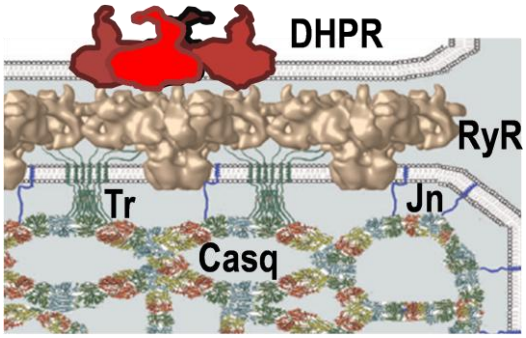

Fig 1. **The couplon**. Includes Ryanodine Receptors, DiHydroPyridine Receptors Triadin, Calsequestrin and Junctin. Modified from (1).

Calsequestrin is the main Ca storage molecule in the SR. Ionic Ca regulates multiple cell functions, including contraction, secretion, synaptic transmission, fertilization, nuclear pore regulation and transcription, which span from ms to years. Electrical signals are "efficient", entailing relatively modest ionic movements. Chemical signaling instead, especially when relying on Ca, requires large gradients with intricate spatial and temporal patterns, the fastest of which involve 1000-fold $[Ca^{2+}]$ changes in 1 ms. High concentrations of cytosolic Ca, $[Ca^{2+}]_{cyto}$, are produced by gating of "Ca release" channels, RyRs or IP_3 receptors (IP_3Rs). To accomplish the large and coordinated release flux, these are spatially clustered. In skeletal muscle the quantity of released Ca can be as high as 200 µmol per liter of cytosol in one twitch[2,3] an amount that is no more than 17% of the total SR content. Considering that the volume of the storage SR compartment (terminal cisternae, TC) is ~2% of the cell volume, the total [Ca] in the TC must be between 35 and 175 mM (detailed in [4]); because resting $[Ca^{2+}]_{SR}$ is ~0.5 mM[5], most Ca must be bound to a buffer molecule. Calsequestrin (we use Casq to designate both gene and protein) is the main buffer of releasable Ca[6]. By measuring directly the buffering power of the SR, we found that 75% of released Ca^{2+} comes from Casq[7]. In heart muscle, where Ca release per beat is less than in a skeletal muscle twitch, Casq (of isoform 2) has a similar buffering role and the contribution to released Ca is roughly estimated at 50%[8].

Casq is part of the couplon. Casq is linked allosterically to a group of (at least) 5 proteins named the *couplon* by Ríos and colleagues[10,11], and depicted for skeletal muscle in fig 1. Note that Casq forms ramified polymers that fill the TC and Tr polymerizes in bundles. RyR and Tr make contact outside the SR. In cardiomyocytes DHPR and RyR are not in contact, and the linkages with Jn and Tr (which in the heart is a shorter product of the single Tr gene[12]) have not been equally demonstrated.

Casq has unique interactions with Ca *in vitro*. Soon after discovery of Casq it was found that its Ca binding capacity increases disproportionately with both [Casq] and $[Ca^{2+}]$[13,14]. Together with other changes in physicochemical properties[15–17], this indicated that high capacity Ca binding requires the protein to polymerize. Largely through work of Kang and colleagues, this property is now understood in molecular terms. Of 309 crystal structures of proteins with Ca bound, deposited in the Cambridge Structural Database, all but 5 have Ca^{2+} coordinated by 6 to 8 ligands, largely consisting of -CO, -COO, -OH and H_2O oxygens[18]; unlike the transition metals in metalloenzymes, Ca has no preference for coordination geometry. This ability to be coordinated by multiple ligands in flexible geometry is a major reason for the privileged position of Ca in all biology. Among other properties, it endows proteins with the capability to fine tune Ca affinity and, in the case of Casq, establish a continuum of sites of decreasing affinity and different but crucial roles.

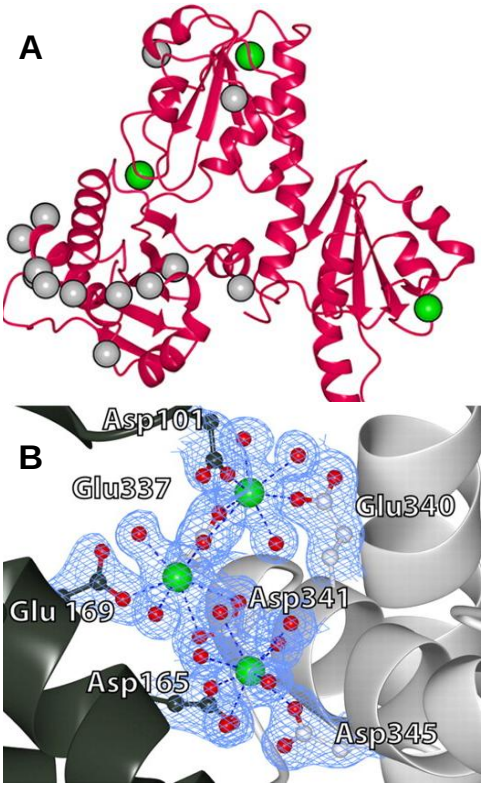

Fig 2. A. Casq monomer, with identified Ca. B. Low affinity sites at dimer interface[9].

Casq is unique for consisting of 3 nearly identical thioredoxin-fold domains (I, II and III)[19] with highly negative net charge (from -62 to -86). Fig 2A shows its structure (human or hCasq1), with identified bound Ca in high (green) and low affinity sites (grey)[9]. Note for future reference a total of 15 sites. Occupancy of the high affinity sites is essential for both secondary and tertiary structures. Most relevant for Ca storage function is the ("cross-linking") role of Ca in establishing 4-nary or polymeric structure. Ca ions, coordinated at the interfaces between monomers, are crucial to polymerization. 2B shows detail in an oligomer crystal, at the "back-to-back" inter-monomer interface near the C terminus. Ca ions (green in 2B) are coordinated by both monomers (black and gray) and water. Similar coordination occurs at the front-to-front interface. The Ca titration of Casq *in vitro* (fig 3) features multiple stages associated with progressive Casq polymerization[20,21]. These observations constitute the basis of Kang's dynamic polymerization model, which postulates that (1) sites on neighboring Casq monomers are better at coordinating Ca than a single peptide chain (which needs to fold—and pay the consequent entropy penalty–if it is to individually bind the ion), (2) Ca binding causes compaction of a hydrophobic core and brings

the charged residues to the surface, which in turn (3) favors electrostatic association of protomers and makes further binding possible.

A key prediction of the model is that the polymerization state of Casq inside TC will change upon large changes in $[Ca^{2+}]_{SR}$. The prediction underpins our Hypothesis 1, tested with Aim 1.

Casq endows the SR with unique properties. P Pape with frog[22,23] and Ríos with mouse muscle[7,24] demonstrated that Casq endows the SR with strongly nonlinear, and functionally advantageous Ca-buffering — maximal at the resting $[Ca^{2+}]_{SR}$ and much lower upon SR depletion. There are indications that the change is associated with depolymerization of the Casq network, and that it tends to occur more easily in a mouse model of MH[25,26]. Meanwhile in cardiac muscle, certain mutations of Casq (isoform 2) associated with CPVT[27] appear to prevent Casq polymerization *in vitro*[28]. A major aim of this proposal is to elucidate the relationships between Casq polymerization and function, and its incidence in disease of cardiac and skeletal muscle.

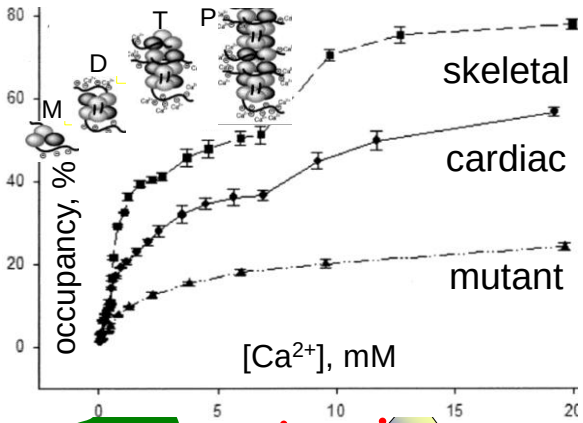

Fig 3. D… T… polymerization o… upon Ca^{2+} b… mutant is Casq… sp.

Casq appears to have additional *gating* roles. Casq, Jn and Tr constitute the deeper reaches of the couplon. One of their roles[23-32] could be control of termination of Ca signals. In both striated muscles Ca transients must terminate rapidly, to permit relaxation. The termination of Ca transients is achieved through (*i*) fast Ca removal from the cytosol and (*ii*) concerted termination of Ca release flux. In turn, termination of flux can be achieved by (*iia*) reducing unitary flux (which occurs when the store depletes and Ca gradient falls) and (*iib*) closing channels. Casq is implicated in all of these. Namely (*i*) it helps SERCA function by buffering $[Ca^{2+}]_{SR}$, (*iia*) it "protects" unitary flux by buffering Ca at the source and (*iib*) it is thought to mediate, by contact, closing of the RyR associated with the decay of $[Ca^{2+}]_{SR}$ that results from Ca release. There is broad agreement on the importance of *i* and *iia*. The significance of *iib*, however, is hotly debated in cardiac muscle[29,30]. We have provided evidence for its operation in skeletal muscle[31], but the issue remains controversial[23]. Aim 2 is the elucidation of the gating roles of Casq.

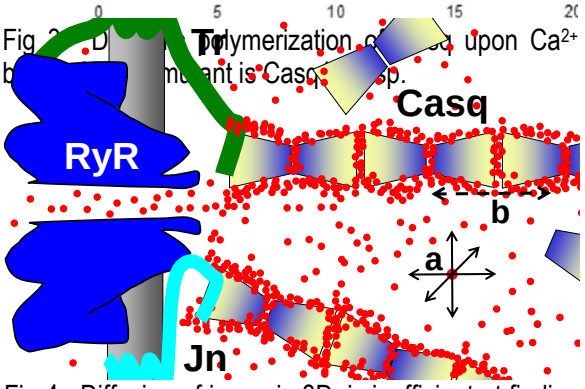

Fig 4. Diffusion of ion **a**, in 3D, is inefficient at finding the channel. Adsorption to "Casq wire" (**b**) increases efficiency by decreasing dimensionality to 1.

Calcium wires and reduction of dimensionality. *In vivo*, Casq forms a dense network, basically a linear ramified polymer, ending with pillars that lead to the junctional membrane, near the mouth of the RyR channels (fig 8). The final attachment to the RyR is made through Tr and Jn (figs 1, 4). The elaborate supramolecular assembly may be necessary for gating the RyR, and is believed to additionally assist the Ca release function by leading Ca ions to within molecular distances from the channel mouth, a role dubbed "Ca wires" [32]. This idea is consistent with *diffusion enhancement by reduction of dimensionality* (DERD), a concept introduced by Max Delbrück[33] to justify the diffusional advantage gained by cellular agonists when they solubilize in the plasma membrane in their travel to receptors (therefore reducing their diffusion to a 2D "walk"). We will test experimentally for the first time whether the presence of polymeric Casq enhances diffusion of Ca by DERD (fig 4) Then, by numerical treatment of DERD models[34–36] we will evaluate how a virtual Casq polymer will enhance diffusion in a liquid. Thus Aims 1C contain the first experimental test of the DERD concept in Ca signaling, as well as the first numeric test of DERD extended to dimension 1. (Reducing dimensionality to 1D should further the enhancement of diffusion gained in 2D. Indeed, analytical treatment shows that, everything else being equal, diffusion times are reduced as 33, 5, 0.3 in going from 3 to 2 to 1D[33]).

Ca-Casq binding *in vitro* does not match the SR *in vivo*. While it is clear that Casq accounts for the majority of signaling Ca[7,37], our measures of its binding properties *in vitro* are strikingly at odds with its contribution to Ca signaling in cells. Fig 5 illustrates the problem. Total Ca in the SR *vs* free $[Ca^{2+}]_{SR}$ is represented in black, (with different *x* scales in A and B)[7]. Binding curves for purified rabbit and recombinant hCasq1, *in vitro*, at concentrations comparable to those *in vivo*, are plotted in color[9,21] (data for rabbit, pink, are the same as in fig 3). Note first the enormous difference between the range of free $[Ca^{2+}]$ measured *in vivo* and that used in the experiments

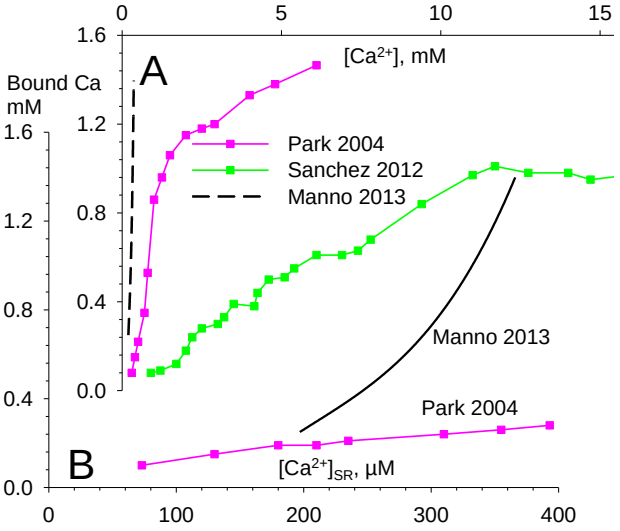

Fig 5. Binding by SR in mice[7] (black) compared with data from human[9] (green) or rabbit[21] Casq1 *in vitro* (pink)

in vitro. The slope of the curves is the buffering power *B* (Eq 3). As shown, *B* is an order of magnitude greater in the SR *in vivo* than in Casq solutions of equivalent concentration *in vitro*. These two sets of measurements represent the best efforts of the two PIs, and, arguably, the state of the art. Their striking discrepancy reveals that the native configuration in the SR environment endows this protein with properties that cannot be readily reconstituted *in vitro*. Our proposal seeks to solve this conundrum through rigorous comparison of buffering properties *in vitro* –under a number of conditions– and in cells. Its significance lies in the fact that this has never been done systematically.

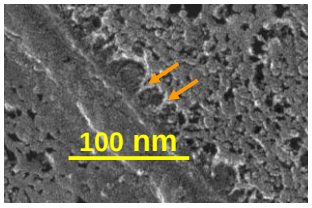

Fig 8. Pillars of Casq1 in TC. S Boncompagni and C Franzini-Armstrong.

Casq in disease. CPVT. Given its implications in control of Ca release it is logical that Casq mutations will result in disease. 15 mutations in Casq2 are linked to a grave ventricular tachycardia (CPVT2)[27,38]. The mutations cause release of RyR2 from gating control by Casq[26] or loss of SR buffering[29], which in turn may be due to reduction in Casq expression or loss of its ability to polymerize, needed for high-capacity Ca binding.

MH and phenotypic variability. The skeletal muscle disease Malignant Hyperthermia, MH, has many features in common with CPVT, as it is defined by catastrophic events of loss of control of Ca release[39]. 40% of MH patients have no identified mutation. The others have mutations in RyR1, but the variability of their phenotype, even for identical mutations, is baffling[39]. Other genetic diseases, including CCD[40], are more frequent and severe than MH and often complicate the MH phenotype. Based on the MH-CPVT similarity, the high homology between Casq1 and 2, and the observation of MH susceptibility in Casq1-null mice[27], mutations of Casq1 have been sought to explain MH and CCD. Our collaborators at the MHRU found a variant in exon 1 of Casq in 16 of 205 MH probands[41]. It was scored "probably damaging" by commercial software, as replacing the highly conserved Met87 by Thr at the dimer interface should hamper dimerization. Our own evaluation of probable damage is presented with fig 23. Thus, alterations of the Casq1 gene can contribute to the variable penetrance and phenotypes of RyR1 mutations, and others might be causative of disease. We will provide the 1st test ever of the impact of a Casq1 variant on cellular phenotype.

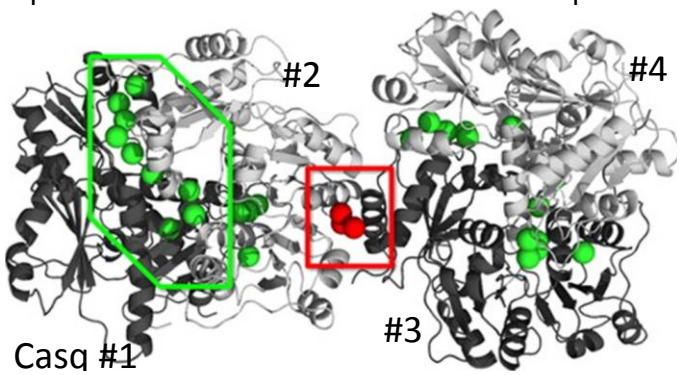

Fig 6. Ca^{2+} identified in front-to-front (green) and back-to-back interfaces of Casq1 tetramer. Modified from[9].

Drug toxicity and posttranslational modification. Kang's group contributed other observations that may explain Casq links to disease. They found that small hydrophobic compounds like doxorubicin, trifluoperazine and cocaine, accumulate in the SR/ER, bind to Casq1 and Casq2 with high affinity, and render them unable to form a proper Ca-linked polymer[42]. This may explain common side effects of these drugs, including muscle cramps and arrhythmias. The state of phosphorylation and glycosylation of Casq2 is modified in cardiac muscle from patients and animals with heart failure[43,44]. Kang demonstrated that the normal glycan profile of Casq1 (with GlycNAc2 and 1-4 mannoses attached at site Asn316) was ideally permissive for dimer formation[45]; by contrast, lack of glycosylation in recombinant Casq1 destabilizes it and its excess (found in newly synthesized Casq in the ER) prevents its correct targeting[46,47]. 2 phosphorylation sites in hCasq, identified by Kang[48], increase its Ca binding capacity.

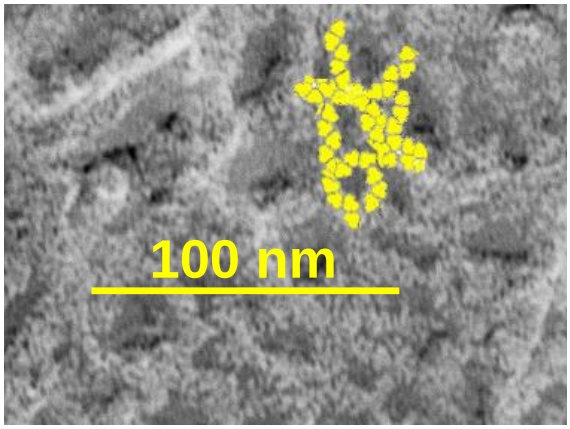

Fig 7. Casq network and schematic polymer.

Two hypotheses emerge from these observations and underpin our Aim 3: CPVT-causing mutations have their effects by altering either Casq's buffering ability or its interactions with Tr and Jn. They do it directly —by local changes that either hamper stability of the monomers and their polymerization[49] or modify affinity for Tr/Jn— or indirectly, by interfering with posttranslational modifications that alter charge (phosphorylation) or steric profile (glycosylation). Kang's lab will synthesize the mutants and determine their Ca binding, polymerization and affinities for couplon proteins, while the Ríos lab will test whether these mutations have functional consequences, by expressing them in adult and developing muscle.

C. Approach.

The studies goal is to *establish what Casq does and understand how it is done*. We will describe the mechanisms whereby Casq supports Ca release from the SR under fast-changing store load, and how they fail in disease. Throughout the project runs the concept that Casq functions as a dynamic polymer, and its Ca-binding properties and interaction with other proteins depend on $[Ca^{2+}]$ via changes in polymerization. Ca binding is central to determining the protein's secondary, tertiary and quaternary structures, via sites of progressively lower affinity and greater capacity. 3 specific hypotheses, derived from this concept, are tested in 3 Aims. Aims 1 and 2 address the two main functions of Casq, one established –Ca store– the other controverted –sensor of $[Ca^{2+}]_{SR}$ and controller of RyR. Aim 1 tests the hypothesis that Casq polymerization, a phenomenon demonstrated *in vitro*, operates reversibly in working muscle. Aim 2 tests whether Casq affects gating of the RyR. Aim 3 examines how these storage and gating functions operate when Casq is altered by disease-linked mutations. Most tests have an *in vitro* component, whereby the underlying physical chemistry is described and quantified, and studies *in vivo*.

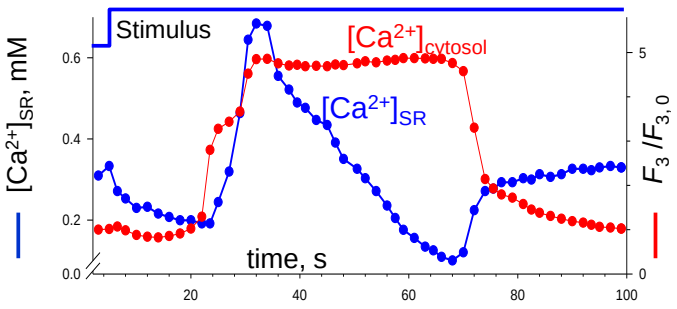

Fig 9. Upon low Mg-induced Ca release, $[Ca^{2+}]_{SR}$ *increases*, suggesting collapse of Casq network.

REFERENCES

1. Boncompagni S, Thomas M, Lopez JR, Allen PD, Yuan Q, Kranias EG, Franzini-Armstrong C, Perez CF. Triadin/Junctin double null mouse reveals a differential role for Triadin and Junctin in anchoring CASQ to the jSR and regulating Ca(2+) homeostasis. *PloS One*. 2012;7:e39962.
2. Pape PC, Jong DS, Chandler WK, Baylor SM. Effect of fura-2 on action potential-stimulated calcium release in cut twitch fibers from frog muscle. *J Gen Physiol*. 1993;102:295–332.
3. Baylor SM, Hollingworth S. Sarcoplasmic reticulum calcium release compared in slow-twitch and fast-twitch fibres of mouse muscle. *J Physiol*. 2003;551:125–138.
4. Royer L, Ríos E. Deconstructing calsequestrin. Complex buffering in the calcium store of skeletal muscle. *J Physiol*. 2009;587:3101–3111.
5. Sztretye M, Yi J, Figueroa L, Zhou J, Royer L, Ríos E. D4cpv-calsequestrin: a sensitive ratiometric biosensor accurately targeted to the calcium store of skeletal muscle. *J Gen Physiol*. 2011;138:211–229.
6. MacLennan DH, Wong PT. Isolation of a calcium-sequestering protein from sarcoplasmic reticulum. *Proc Natl Acad Sci U S A*. 1971;68:1231–1235.
7. Manno C, Sztretye M, Figueroa L, Allen PD, Ríos E. Dynamic measurement of the calcium buffering properties of the sarcoplasmic reticulum in mouse skeletal muscle. *J Physiol*. 2013;591:423–442.
8. Bers DM. Excitation-contraction coupling and cardiac contractile force [Internet]. Dordrecht: Kluwer Academic; 2001 [cited 2013 Sep 13]. Available from: http://www.tandfonline.com/toc/rwhi20/
9. Sanchez EJ, Lewis KM, Danna BR, Kang C. High-capacity Ca2+ binding of human skeletal calsequestrin. *J Biol Chem*. 2012;287:11592–11601.
10. Stern MD, Song LS, Cheng H, Sham JS, Yang HT, Boheler KR, Ríos E. Local control models of cardiac excitation-contraction coupling. A possible role for allosteric interactions between ryanodine receptors. *J Gen Physiol*. 1999;113:469–489.
11. Stern MD, Pizarro G, Ríos E. Local control model of excitation-contraction coupling in skeletal muscle. *J Gen Physiol*. 1997;110:415–440.
12. Marty I, Fauré J, Fourest-Lieuvin A, Vassilopoulos S, Oddoux S, Brocard J. Triadin: what possible function 20 years later? *J Physiol*. 2009;587:3117–3121.

13. Aaron BM, Oikawa K, Reithmeier RA, Sykes BD. Characterization of skeletal muscle calsequestrin by 1H NMR spectroscopy. *J Biol Chem*. 1984;259:11876–11881.

14. Tanaka M, Ozawa T, Maurer A, Cortese JD, Fleischer S. Apparent cooperativity of Ca2+ binding associated with crystallization of Ca2+-binding protein from sarcoplasmic reticulum. *Arch Biochem Biophys*. 1986;251:369–378.

15. He Z, Dunker AK, Wesson CR, Trumble WR. Ca(2+)-induced folding and aggregation of skeletal muscle sarcoplasmic reticulum calsequestrin. The involvement of the trifluoperazine-binding site. *J Biol Chem*. 1993;268:24635–24641.

16. Mitchell RD, Simmerman HK, Jones LR. Ca2+ binding effects on protein conformation and protein interactions of canine cardiac calsequestrin. *J Biol Chem*. 1988;263:1376–1381.

17. Ikemoto N, Nagy B, Bhatnagar GM, Gergely J. Studies on a metal-binding protein of the sarcoplasmic reticulum. *J Biol Chem*. 1974;249:2357–2365.

18. Katz AK, Glusker JP, Beebe SA, Bock CW. Calcium Ion Coordination: A Comparison with That of Beryllium, Magnesium, and Zinc. *J Am Chem Soc*. 1996;118:5752–5763.

19. 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;5:476–483.

20. Park H, Wu S, Dunker AK, Kang C. Polymerization of calsequestrin. Implications for Ca2+ regulation. *J Biol Chem*. 2003;278:16176–16182.

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

22. Pape PC, Fénelon K, Lamboley CRH, Stachura D. Role of calsequestrin evaluated from changes in free and total calcium concentrations in the sarcoplasmic reticulum of frog cut skeletal muscle fibres. *J Physiol*. 2007;581:319–367.

23. Fénelon K, Lamboley CRH, Carrier N, Pape PC. Calcium buffering properties of sarcoplasmic reticulum and calcium-induced Ca(2+) release during the quasi-steady level of release in twitch fibers from frog skeletal muscle. *J Gen Physiol*. 2012;140:403–419.

24. Royer L, Sztretye M, Manno C, Pouvreau S, Zhou J, Knollmann BC, Protasi F, Allen PD, Ríos E. Paradoxical buffering of calcium by calsequestrin demonstrated for the calcium store of skeletal muscle. *J Gen Physiol*. 2010;136:325–338.

25. Launikonis BS, Zhou J, Royer L, Shannon TR, Brum G, Ríos E. Depletion “skraps” and dynamic buffering inside the cellular calcium store. *Proc Natl Acad Sci U S A*. 2006;103:2982–2987.

26. Manno C, Figueroa L, Royer L, Pouvreau S, Lee C-S, Volpe P, Nori A, Zhou J, Meissner G, Hamilton SL, Rios E. Altered Ca2+ concentration, permeability and buffering in the myofiber Ca2+ store of a mouse model of malignant hyperthermia. *J Physiol*. 2013;

27. Faggioni M, Knollmann BC. Calsequestrin 2 and arrhythmias. *Am J Physiol Heart Circ Physiol*. 2012;302:H1250–1260.

28. Qin J, Valle G, Nani A, Nori A, Rizzi N, Priori SG, Volpe P, Fill M. Luminal Ca2+ regulation of single cardiac ryanodine receptors: insights provided by calsequestrin and its mutants. *J Gen Physiol*. 2008;131:325–334.

29. Györke S, Stevens SCW, Terentyev D. Cardiac calsequestrin: quest inside the SR. *J Physiol*. 2009;587:3091–3094.

30. MacLennan DH, Chen SRW. Store overload-induced Ca2+ release as a triggering mechanism for CPVT and MH episodes caused by mutations in RYR and CASQ genes. *J Physiol*. 2009;587:3113–3115.

31. Sztretye M, Yi J, Figueroa L, Zhou J, Royer L, Allen P, Brum G, Ríos E. Measurement of RyR permeability reveals a role of calsequestrin in termination of SR Ca(2+) release in skeletal muscle. *J Gen Physiol*. 2011;138:231–247.

32. MacLennan DH, Reithmeier RA. Ion tamers. *Nat Struct Biol*. 1998;5:409–411.

33. Delbrück M, Adam G. Reduction of dimensionality in biological diffusion processes. In: Structural Chemistry and Molecular Bilogy. Eds. Rich A & Davidson N. San Francisco: Freeman; 1968. p. 198–215.

34. Berg HC, Purcell EM. Physics of chemoreception. *Biophys J*. 1977;20:193–219.

35. Axelrod D, Wang MD. Reduction-of-dimensionality kinetics at reaction-limited cell surface receptors. *Biophys J*. 1994;66:588–600.

36. DeLisi C. The biophysics of ligand-receptor interactions. *Q Rev Biophys*. 1980;13:201–230.

37. Murphy RM, Larkins NT, Mollica JP, Beard NA, Lamb GD. Calsequestrin content and SERCA determine normal and maximal Ca2+ storage levels in sarcoplasmic reticulum of fast- and slow-twitch fibres of rat. *J Physiol*. 2009;587:443–460.

38. Faggioni M, Kryshtal DO, Knollmann BC. Calsequestrin mutations and catecholaminergic polymorphic ventricular tachycardia. *Pediatr Cardiol*. 2012;33:959–967.

39. Maclennan DH, Zvaritch E. Mechanistic models for muscle diseases and disorders originating in the sarcoplasmic reticulum. *Biochim Biophys Acta*. 2011;1813:948–964.

40. Durham W, Wehrens X, Sood S, Hamilton S. Diseases associated with altered ryanodine receptor activity. In: Calcium Signalling and Disease. Springer; 2007. p. 273–321.

41. Kraeva N, Zvaritch E, Frodis W, Sizova O, Kraev A, MacLennan DH, Riazi S. CASQ1 gene is an unlikely candidate for malignant hyperthermia susceptibility in the North American population. *Anesthesiology*. 2013;118:344–349.

42. Kim E, Tam M, Siems WF, Kang C. Effects of drugs with muscle-related side effects and affinity for calsequestrin on the calcium regulatory function of sarcoplasmic reticulum microsomes. *Mol Pharmacol*. 2005;68:1708–1715.

43. Kiarash A, Kelly CE, Phinney BS, Valdivia HH, Abrams J, Cala SE. Defective glycosylation of calsequestrin in heart failure. *Cardiovasc Res*. 2004;63:264–272.

44. Ram ML, Kiarash A, Marsh JD, Cala SE. Phosphorylation and dephosphorylation of calsequestrin on CK2-sensitive sites in heart. *Mol Cell Biochem*. 2004;266:209–217.

45. Sanchez EJ, Lewis KM, Munske GR, Nissen MS, Kang C. Glycosylation of skeletal calsequestrin: implications for its function. *J Biol Chem*. 2012;287:3042–3050.

46. Milstein ML, McFarland TP, Marsh JD, Cala SE. Inefficient glycosylation leads to high steady-state levels of actively degrading cardiac triadin-1. *J Biol Chem*. 2008;283:1929–1935.

47. Jacob S, Sleiman NH, Kern S, Jones LR, Sala-Mercado JA, McFarland TP, Sabbah HH, Cala SE. Altered calsequestrin glycan processing is common to diverse models of canine heart failure. *Mol Cell Biochem*. 2013;377:11–21.

48. Sanchez EJ, Munske GR, Criswell A, Milting H, Dunker AK, Kang C. Phosphorylation of human calsequestrin: implications for calcium regulation. *Mol Cell Biochem*. 2011;353:195–204.

49. Kumar A, Chakravarty H, Bal NC, Balaraju T, Jena N, Misra G, Bal C, Pieroni E, Periasamy M, Sharon A. Identification of calcium binding sites on calsequestrin 1 and their implications for polymerization. *Mol Biosyst*. 2013;9:1949–1957.