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The long and winding road home: How junctin and triadin find their way to the junctional SR

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The function of cardiac myocytes is to contract. To sustain the structures and domains involved in the activation of contraction, cardiac myocytes, like all other cells in the body, must perform basic cellular functions such as protein synthesis and post-translational modification, phospholipid, and steroid synthesis, and protein trafficking. In non-muscle cells, these vital functions are performed by the endoplasmic reticulum (ER). Cardiac myocytes have a specialized form of the ER called the sarcoplasmic reticulum (SR). The SR performs specialized roles in Ca^{2+} signaling, with the junctional SR (jSR) being important in Ca^{2+} release and the longitudinal SR in Ca^{2+} reuptake.

The generally accepted view was that the SR performed all the roles of the ER. However, recent research has shown that the ER also exists in striated muscle [1-3]. While the ER and SR are continuous, they have distinct roles and thus distinct protein compositions. The rough ER (rER) has attached ribosomes that synthesize proteins. The trafficking of proteins from the ER to the jSR is the focus of an intriguing study by Sleiman *et al* published recently in the *Journal of Molecular and Cellular Cardiology*. The study is significant because the mechanisms that control the transit of proteins to the jSR are poorly understood.

Excitation-contraction (EC) coupling is the coordinated process by which an action potential triggers cell contraction. It is initiated in specialized regions (called “dyads”) where the jSR comes into close apposition (20-30 nm) to the sarcolemma of cardiac myocytes. Activation of sarcolemmal voltage-gated $\text{Ca}_v1.2$ channels during the action potential causes a small influx of Ca^{2+} that activates ryanodine receptors (RyR) expressed in the nearby jSR via the mechanism of Ca^{2+} -induced Ca^{2+} release. A central tenet of this model is that dyads must be structurally stable in order to ensure the fidelity and strength of EC coupling, and even small changes in the jSR molecular composition or geometry could induce dramatic changes in EC coupling [4, 5]. Thus, trafficking of jSR proteins could, at least in principle, have an impact on EC coupling.

Accordingly, the structure of both the jSR and T-tubules is important for normal Ca^{2+} signaling. Clusters of $\text{Ca}_v1.2$ channels localize to the T-tubule sarcolemma in the dyads. Addressing the mechanism of this clustering, recent research has shown that the protein bridging integrator 1 (BIN1) anchors microtubules to the membrane and localizes $\text{Ca}_v1.2$ channels to the T-tubules [6]. In addition, BIN1 has been shown to have a role in Ca^{2+} diffusion. The lumen of the T-tubules is continuous with the Ca^{2+} -rich extracellular space, and so a ‘fuzzy space’ of dense protective inner membrane folds in order to limit Ca^{2+} diffusion near the T-tubule-jSR zones [7-10]. The protein bridging integrator 1 (BIN1) has recently been implicated in creating these folds and thus regulating Ca^{2+} flux into the cell [11].

The jSR is a complex structure with several proteins contributing to its architecture and function. Figure 1 shows a cartoon of the proposed protein composition of the jSR. Junctophilin-2 is a protein that is anchored to the jSR in its C-terminus and contacts the T-tubules through lipid-interacting motifs in its N-terminus [12]. These proteins are hypothesized to provide a molecular bridge between the jSR and the T-tubules. Calsequestrin (CSQ) is a Ca^{2+} -binding protein that ensures high levels of Ca^{2+} close to RyRs [13]. Two proteins, junctin (JCT) and triadin (TRD), anchor CSQ to the SR membrane. JCT (26 kDa) [14] and TRD (95 kDa) [15] have a single trans-membrane domain. Because these proteins contain binding sites for the RyR as well as for CSQ, they allow conformational coupling between the Ca^{2+} -binding protein and the Ca^{2+} release channel [16]. Triadin is hypothesized to maintain dyad structure, contribute to healthy EC coupling, and recruit microtubules and molecular motors. In addition, TRD is localized exclusively to the jSR [17]. Both TRD and JCT knockout mice show changes in the jSR architecture, and, with RyRs and CSQ, form the “core complex of proteins required for Ca^{2+} release” [18, 19].

The study by Sleiman *et al* investigates the cellular mechanisms for sorting and trafficking of TRD and JCT to the jSR [20]. Using fluorescent imaging on cultured adult rat ventricular myocytes transfected with canine forms of TRD and JCT, the authors tracked the movement of the proteins TRD and JCT from the rER surrounding the nuclei to the jSR of these cells. They found that TRD and JCT proteins moved radially and away from the nuclei, developing their punctate pattern in the cell body over 48 hours. They introduced CSQ2-DsRed, a form of calsequestrin tagged with the DsRed fluorescent protein, which, when expressed, does not traffic to the jSR, but rather polymerizes ectopically in the rough ER. Co-expression of CSQ2-DsRed and TRD or JCT arrested TRD and JCT trafficking, presumably via binding of CSQ2-DsRed with TRD and JCT.

Furthermore, a version of TRD with the CSQ2 binding site removed ($^{\text{del}}$ TRD) was able to traffic normally when co-expressed with CSQ2-DsRed. This suggests that these proteins follow the same trafficking pathway. The $^{\text{del}}$ TRD protein segregated from the CSQ-DsRed signal over time, and the authors suggest that the trapping of CSQ2-DsRed reveals a novel smooth ER network surrounding and connecting the nuclei. They hypothesize that the interface between the $^{\text{del}}$ TRD and CSQ-DsRed signals also reveals the transition zone between ER and SR. Of interest, the authors found that high levels of CSQ2-DsRed increased the amount of smooth ER and moved the ER-SR transition farther from the

nucleus. Lastly, and perhaps most interestingly, the authors found that disrupting the microtubule network also interrupted TRD and JCT traffic, leading to over-expression of TRD and JCT at juxtannuclear sites with little expression of these proteins in the rest of the cell.

The impact of a paper is not limited to the questions it answers, but by the new questions it raises. The work by Sleiman *et al* is no exception, as it raises a number of interesting questions that warrant further investigation. For example, while the study describes a common pathway for JCT, TRD, and CSQ trafficking and identifies the time course of this pathway, many aspects of this pathway, such as its regulation, rate, and fidelity, are unclear. What is the baseline trafficking and turnover rate of these proteins at specific jSR puncta? Is trafficking up-regulated after injury, and if so, by what means? It is possible that the proteins progress sequentially, that is, the oldest TRD and JCT proteins could populate the edge of the cell, and newest by the nucleus. Is there a mechanism to ensure uniform distribution of these proteins throughout the cell? Furthermore, if this pathway is crucial to jSR composition, a logical, testable claim is that the pathway should be highly reliable. Lastly, does fusion of vesicles containing new TRD and JCT alter jSR geometry and hence local Ca^{2+} control of RyRs during EC coupling? Uncovering these answers could help elucidate how the jSR recovers from the disruption of SR network seen in heart failure and after acute injury.

Another interesting finding is the implication of the microtubule network in jSR protein trafficking. Due to the limited cell division of myocytes, the cardiac microtubule network has been understudied and its purposes are still largely unknown. The images we do have of the microtubule network suggest it to be a vast, dynamic entity [21, 22]. What is the fidelity of the microtubule network, and how does it develop? Based on this study, it seems that the microtubule network must be in place at least in some capacity to allow formation of the jSR. How dynamic is this network, and how does it recover in pathology? As the authors stated, microtubule-mediated defects in junctophilin-2 trafficking has been implicated in Ca^{2+} handling dysfunction and heart failure [21]. It has yet to be seen if TRD and JCT trafficking may also impair Ca^{2+} signaling and have a role in pathology. There are several molecular motors, both kinesins and dyneins, which are expressed in the heart. Which ones are implicated in this trafficking pathway, and do they have other roles in the heart? These molecular motors could hold promise of therapeutic targets if they are specific in their cardiac roles. More work, too, needs to be done expanding our knowledge of this novel, smooth ER network. What are the other differences in protein composition in these ER/SR networks, and what factors determine what parts of the ER network transition to jSR? Instead of arresting CSQ trafficking by adding the DS-Red tag, there may be other physiological markers of the smooth ER that would enable a more systematic, unmanipulated study of the network. In addition, the authors found the smooth ER network expanded upon over expression of CSQ, diminishing the jSR network. Does this have a pathological correlate that could be tested in an animal model? If so, what are the effects of this expansion, and could this expansion be modified pharmacologically for a therapeutic benefit?

The work by Sleiman *et al* adds to a growing body of evidence that suggests that the SR is a dynamic organelle. Vega *et al* [23] found that SR “boutons” expressing functional RyRs can move throughout the cell, approaching or moving away from the sarcolemma of ventricular myocytes. Microtubules and the molecular motors dynein and kinesin 1 (Kif5b) were proposed to be important regulators of SR motility.

Interestingly, new research shows that changes in the dyad structure can likely contribute to Ca^{2+} instability during pathology. Zhang *et al* [24] found that T-tubules and SR physically uncouple in human heart failure. Song *et al* [25] found that remodeled T-tubules move away from the Z-lines in heart failure, leading to loss of local control and Ca^{2+} instability. Recently, Zhang *et al* [21] has shown that microtubule desensitification is implicated in T-tubule remodeling and disruption of dyadic proteins in heart failure. Similar studies have been done in other pathological states besides heart failure. Wagner *et al* [26], for example, used STED nanoscopy to assay remodeling post-myocardial infarction (MI) and found proliferative membrane changes, early EC uncoupling, and SR network fracturing.

These findings suggest that the jSR plays a vital role in normal physiology, and that loss of jSR organization can lead to pathology. The endoplasmic reticulum and SR span myocytes, forming Ca^{2+} homeostatic regulatory domains and the rough ER synthesizes all resident ER/SR proteins, which are then trafficked via diffusion or targeting to their locations [27-30]. However, the mechanisms and pathways of this trafficking is largely unknown. Thus, despite its importance, surprisingly little is known concerning jSR biogenesis and how the jSR may repopulate after injury. The study by Sleiman *et al* represents an important contribution to this field.

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References

1. Nori A, Bortoloso E, Frasson F, Valle G, Volpe P. Vesicle budding from endoplasmic reticulum is involved in calsequestrin routing to sarcoplasmic reticulum of skeletal muscles. *The Biochemical journal*. 2004; 15:505–12. [PubMed: 14728599]
2. Villa A, Podini P, Nori A, Panzeri MC, Martini A, Meldolesi J, et al. The endoplasmic reticulum-sarcoplasmic reticulum connection. II. Postnatal differentiation of the sarcoplasmic reticulum in skeletal muscle fibers. *Experimental cell research*. 1993; 209:140–8. [PubMed: 8223998]
3. Volpe P, Villa A, Podini P, Martini A, Nori A, Panzeri MC, et al. The endoplasmic reticulum-sarcoplasmic reticulum connection: distribution of endoplasmic reticulum markers in the sarcoplasmic reticulum of skeletal muscle fibers. *Proc Natl Acad Sci U S A*. 1992; 89:6142–6. [PubMed: 1631100]
4. Soeller C, Cannell MB. Numerical simulation of local calcium movements during L-type calcium channel gating in the cardiac diad. *Biophys J*. 1997; 73:97–111. [PubMed: 9199775]
5. Gomez AM, Valdivia HH, Cheng H, Lederer MR, Santana LF, Cannell MB, et al. Defective excitation-contraction coupling in experimental cardiac hypertrophy and heart failure. *Science*. 1997; 276:800–6. [PubMed: 9115206]
6. Hong TT, Smyth JW, Gao D, Chu KY, Vogan JM, Fong TS, et al. BIN1 Localizes the L-Type Calcium Channel to Cardiac T-Tubules. *PLoS Biol*. 2010; 8:1–14.

7. Pasek M, Simurda J, Christie G. The functional role of cardiac T-tubules explored in a model of rat ventricular myocytes. *Philos Trans A Math Phys Eng Sci.* 2006; 364:1187–206. [PubMed: 16608703]
8. Lederer WJ, Niggli E, Hadley RW. Sodium-calcium exchange in excitable cells: fuzzy space. *Science.* 1990; 248:283. [PubMed: 2326638]
9. Shepherd N, McDonough HB. Ionic diffusion in transverse tubules of cardiac ventricular myocytes. *Am J Physiol.* 1998; 275
10. Swift F, Stromme TA, Amundsen B, Sejersted OM, Sjaastad I. Slow diffusion of K⁺ in the T tubules of rat cardiomyocytes. *J Appl Physiol.* 2006; 101
11. Hong TT, Yang H, Zhang S, Cho HC, Kalashnikova M, Sun B, et al. Cardiac BIN1 folds T-tubule membrane, controlling ion flux and limiting arrhythmia. *Nature medicine.* 2014; 20:624–32.
12. Garbino A, Wehrens XH. Emerging role of junctophilin-2 as a regulator of calcium handling in the heart. *Acta Pharma Sinica.* 2010; 31:1019–21.
13. Rossi D, Barone V, Giacomello E, Cusimano V, Sorrentino V. The Sarcoplasmic Reticulum: An Organized Patchwork of Specialized Domains. *Traffic.* 2008; 9:1044–9. [PubMed: 18266914]
14. 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. [PubMed: 8530521]
15. Kim KC, Caswell AH, Talvenheimo JA, Brandt NR. Isolation of a terminal cisterna protein which may link the dihydropyridine receptor to the junctional foot protein in skeletal muscle. *Biochemistry.* 1990; 29:9281–9. [PubMed: 2176846]
16. Beard NA, Laver DR, Dulhunty AF. Calsequestrin and the calcium release channel of skeletal and cardiac muscle. *Pro Biophys Mol Biol.* 2004; 85:33–69.
17. Fourest-Lieuvain A, Rendu J, Osseni A, Pernet-Gallay K, Rossi D, Oddoux S, et al. Role of triadin in the organization of reticulum membrane at the muscle triad. *J Cell Sci.* 2012; 125:3443–53. [PubMed: 22505613]
18. Glover L, Quinn S, Ryan M, Pette D, Ohlendieck K. Supramolecular calsequestrin complex. *European journal of biochemistry / FEBS.* 2002; 269:4607–16. [PubMed: 12230573]
19. 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
20. Sleiman NH, McFarland TP, Jones L, Cala SE. Transitions of protein traffic from cardiac ER to junctional SR. *J Mol Cell Cardiol.* 2015
21. Zhang C, Chen B, Guo A, Zhu Y, Miller JD, Gao S, et al. Microtubule-Mediated Defects in Junctophilin-2 Trafficking Contribute to Myocyte T-Tubule Remodeling and Ca²⁺ Handling Dysfunction in Heart Failure. *Circ Res.* 2014
22. Oddoux S, Zaai KJ, Tate V, Kenea A, Nandkeolyar SA, Reid E, et al. Microtubules that form the stationary lattice of muscle fibers are dynamic and nucleated at Golgi elements. *J Cell Biol.* 2013; 203:205–13. [PubMed: 24145165]
23. Vega AL, Santana LF. Dynamic Changes in Sarcoplasmic Reticulum Structure in Ventricular Myocytes. *Journal of Biomedicine and Biotechnology.* 2011; 2011:1–14.
24. Zhang H, Li R, Xu M, Xu S, Lai Y, Wu H, et al. Ultrastructural uncoupling between T-tubules and sarcoplasmic reticulum in human heart failure. *Cardiovascular research.* 2013; 98:269–76. [PubMed: 23405000]
25. Song L, Sobie EA, McCulle SL, Lederer WJ, Balke CW, Cheng H. Orphaned ryanodine receptors in the failing heart. *Proc Natl Acad Sci U S A.* 2006; 103:4305–10. [PubMed: 16537526]
26. Wagner E, Lauterbach MA, Kohl T, Westphal V, Williams GSB, Steinbrecher JH, et al. Stimulated Emission Depletion Live-Cell Super-Resolution Imaging Shows Proliferative Remodeling of T-Tubule Membrane Structures After Myocardial Infarction. *Circ Res.* 2012; 111:402–14. [PubMed: 22723297]
27. Franzini-Armstrong C. Structure of sarcoplasmic reticulum. *Federation proceedings.* 1980; 39:2403–9. [PubMed: 7371874]
28. Bers D, Shannon TR. Calcium movements inside the sarcoplasmic reticulum of cardiac myocytes. *J Mol Cell Cardiol.* 2013; 58:59–66. [PubMed: 23321551]

29. Fleischer S, Inui M. Biochemistry and biophysics of excitation-contraction coupling. *Annu Rev Biophys Biophys Chem.* 1989; 18:333–64. [PubMed: 2660829]
30. Cala SE, Scott BT, Jones L. Intralumenal sarcoplasmic reticulum Ca(2+)-binding proteins. *Semin Cell Biol.* 1990; 1:265–75. [PubMed: 2103513]

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Figure 1.

Cartoon of the jSR. RyR = ryanodine receptor, CSQ = calsequestrin, TRD = triadin, JCT = junction, Ca_v1.2 = voltage-gated calcium channel, BIN1 = bridging integrator 1, JP2 = junctophilin-2.