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A “rough” journey to the sarcoplasmic reticulum – implications of altered calsequestrin trafficking for cardiac arrhythmia

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First identified almost 40 years ago as a low-affinity high capacity Ca buffering protein located in the sarcoplasmic reticulum (SR) of muscle,[1] the story of calsequestrin continues to be full of surprises. Far from being only a “simple” Ca store that provides Ca necessary for muscle contraction, studies have implicated a role of calsequestrin in a variety of diseases ranging from ventricular arrhythmias[2] to autoimmune eye disorders.[3] The work by McFarland and colleagues on calsequestrin trafficking in adult cardiac muscle reported in the current issue sheds light on several puzzling aspects of calsequestrin biology.

To determine calsequestrin trafficking in cardiac muscle, the authors used adenovirus mediated gene transfer and expressed calsequestrin fused with a fluorescent tag, DsRed, in adult rat ventricular myocytes. DsRed is fluorescent only in its tetrameric form. Monomeric DsRed is non-fluorescent, but can be detected by anti-DsRed antibodies. Using these reagents, McFarland and coworkers determined that calsequestrin is first made in the perinuclear rough endoplasmic reticulum (ER) as a monomer. The calsequestrin monomer then traffics directly into its target cellular compartment, the junctional sarcoplasmic reticulum. Cellular trafficking occurs only as calsequestrin monomer, since tetrameric calsequestrin is retained completely in the perinuclear cisternae, the rough ER compartment. Polymerization of native cardiac calsequestrin occurs first in the junctional SR, which may explain why calsequestrin is retained completely in this cellular compartment. Native calsequestrin apparently never reaches the free SR, suggesting that the free SR in cardiac muscle is analogous to ER-Golgi intermediate compartment of non-muscle cells. The intricate subcellular processing and trafficking of calsequestrin in cardiac muscle reported here by McFarland and coworkers may help explain several puzzling features of calsequestrin biology discussed next.

Calsequestrin polymerization and binding to triadin/junctin determine its localization in the junctional SR compartment

Why is a small protein without membrane spanning domain such as calsequestrin retained in the junctional SR? It should easily diffuse into the free SR that is contiguous with the junctional SR. Yet biochemical, electron microscopy and confocal studies clearly show that calsequestrin is only found in the terminal cisternae of the junctional SR in close contact with the cardiac ryanodine receptor.[2] Calsequestrin polymerizes into strands via end to end interaction in the presence of millimolar Ca.[4] The authors showed previously that calsequestrin polymerization

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is responsible for its retention in the ER compartment of non-muscle cells, the first cellular compartment with ionic conditions favorable of calsequestrin polymerization.[5] Here they report that calsequestrin appears in the junctional SR of adult cardiac myocytes immediately after its emergence in the rough ER of the perinuclear envelope, suggesting that junctional SR of cardiac muscle may be analogous to the ER of non-muscle cells. Based on their work in non-muscle cells, the authors argue that junctional SR is the first subcellular compartment with ionic conditions favorable for calsequestrin polymerization, and suggest that calsequestrin trafficking directly from the rough ER to the junctional SR is responsible for its exclusive subcellular localization in cardiac muscle. While this is a compelling argument, it cannot be the complete story. For example: Why does the tetrameric calsequestrin-DsRed eventually traffic to free SR, whereas the monomer form does not (Figure 5H)? This suggests that factors other than sequence of trafficking and polymerization contribute to calsequestrin's location in the cell. One possibility is that heteropolymerization of CSQ-DsRed monomers with native rat calsequestrin favors CSQ-DsRed monomers over tetramers. More likely, the explanation lies in the calsequestrin binding proteins triadin and junctin. Biochemical studies suggest that triadin and junctin anchor calsequestrin to the RyR2 protein complex.[6] But calsequestrin is a much more abundant protein, and binding of calsequestrin monomers to triadin and junctin would not be sufficient to retain all calsequestrin in the terminal cisternae. However, based on the work by the Cala group, it is intriguing to speculate that triadin and junctin help anchor a mesh of linear calsequestrin polymers to the mouth of the RyR2 release channels. This idea is directly supported by the colocalization of triadin, junctin, RyR2 and calsequestrin in immunolabeling studies,[7] and by the presence of polymeric calsequestrin juxtaposed to the SR luminal surface of the RyR2 "feet" on electron micrographs.[8] The critical role of triadin and junctin for calsequestrin trafficking has been confirmed by the experiments in triadin knock-out mice, which also exhibit an almost complete loss of junctin. Deletion of triadin and junctin protein causes (1) an overall decrease in calsequestrin protein (but not mRNA) and (2), a significant accumulation of calsequestrin in the free SR.[9] Collectively; these data indicate that two factors are critical for calsequestrin trafficking in cardiac muscle: Polymerization and binding to triadin/junctin.

Altered calsequestrin trafficking and localization may explain why calsequestrin mutations cause catecholaminergic polymorphic ventricular tachycardia

Work over the last decade has elucidated the role of calsequestrin in cardiac muscle. While calsequestrin is dispensable for bulk Ca release and contractile function, it is critically important to prevent spontaneous openings of RyR2 channels that trigger premature beats and ventricular tachycardia under conditions of adrenergic stress.[10] Experimental evidence suggest several possible mechanisms: calsequestrin inhibits RyR2 channels either via direct protein-protein interaction,[11] via binding to triadin/junctin[12] or via local buffering of Ca close to the RyR2 luminal Ca regulatory site.[13] Regardless of the exact molecular mechanism, it is clear that calsequestrin has to be within the nanodomain of the Ca release channel to exert its regulation of RyR2-mediated Ca release. Hence, calsequestrin localization within the cell determines its function in biology and provides a likely explanation why calsequestrin mutations cause catecholaminergic polymorphic ventricular tachycardia. Early work using adenoviral-mediated gene transfer demonstrated a dominant-negative effect of the disease-causing calsequestrin mutations D307H[14] and R33Q[15] on SR Ca release function. However, the same mutations have a recessive inheritance pattern in humans, suggesting that the underlying disease mechanism is likely different. Knock-in mice homozygous for the two mutations reproduced the human arrhythmia phenotype, whereas heterozygous mice were not affected. Surprisingly, mutant calsequestrin was either undetectable (D307H)[16] or drastically reduced to very low levels (R33Q).[17] The work by the Cala group reported here provides a

compelling explanation for this surprising result. Since the D307H mutations causes both a 50% reduction in calsequestrin binding to triadin and junctin and conformational changes that may interfere with normal polymerization,[18] one can predict that although mutant protein is made, it is not retained in the junctional SR. Recent work supports this hypothesis. The trafficking defect can be overcome by overexpressing mutant D307H using the much stronger alphaMHC promoter in the calsequestrin null background.[19] Now mutant calsequestrin D307H protein is detectable in the heart, and is apparently targeted in sufficient amounts to the junctional SR to rescue the phenotype of calsequestrin null mice. Similarly, the R33Q mutation also interferes with calsequestrin polymerization[20] and binding to the RyR2 protein complex.[15]

Altered calsequestrin trafficking may contribute to arrhythmia risk in heart failure

On the other end of the spectrum, mass spectrometry analysis of calsequestrin suggests that more calsequestrin is retained in the rough ER of the perinuclear cisternae during disease states such as cardiac hypertrophy or heart failure.[21] While highly speculative, several consequences can be predicted based on increased calsequestrin retention in the rough ER. Studies have consistently demonstrated that the total amount of calsequestrin in cardiac muscle is not altered in heart failure.[2] However, if more calsequestrin is located in the perinuclear rough ER, then less will be in the junctional SR, where calsequestrin importantly regulates RyR2 mediated Ca release. Global calsequestrin reductions of as little as 25% increase SR Ca leak and cause susceptibility to ventricular arrhythmias.[22] Thus, one might speculate that altered calsequestrin trafficking could also contribute to the SR Ca leak and increased risk for ventricular arrhythmias typically found in heart failure.

Clearly, more work on the cell biology of calsequestrin in cardiac muscle needs to be done. Nevertheless, the provocative work by McFarland et al reported in the current issue provides a unifying hypothesis for how altered calsequestrin trafficking may contribute to increased arrhythmia risk associated with inherited and acquired forms of heart disease in humans.

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