JCI The Journal of Clinical Investigation

Chain-reaction Ca^{2+} signaling in the heart

Sandor Györke, … , Dmitry Terentyev, W. Jonathan Lederer

J Clin Invest. 2007;117(7):1758-1762. https://doi.org/10.1172/JCI32496.

Commentary

Mutations in Ca^{2+}-handling proteins in the heart have been linked to exercise-induced sudden cardiac death. The best characterized of these have been mutations in the cardiac Ca^{2+} release channel known as the ryanodine receptor type 2 (RyR2). RyR2 mutations cause "leaky" channels, resulting in diastolic Ca^{2+} leak from the sarcoplasmic reticulum (SR) that can trigger fatal cardiac arrhythmias during stress. In this issue of the *JCI*, Song et al. show that mutations in the SR Ca^{2+}-binding protein calsequestrin 2 (CASQ2) in mice result not only in reduced CASQ2 expression but also in a surprising, compensatory elevation in expression of both the Ca^{2+}-binding protein calreticulin and RyR2, culminating in premature Ca^{2+} release from cardiac myocytes and stress-induced arrhythmia (see the related article beginning on page 1814). In the context of these findings and other recent reports studying CASQ2 mutations, we discuss how CASQ2 influences the properties of Ca^{2+}-dependent regulation of RyR2 and how this contributes to cardiac arrhythmogenesis.

DK070947 and DK30534. The authors express their appreciation to Richard Ajioka for assistance with Figure 2.

Address correspondence to: Jerry Kaplan, Department of Pathology, University of Utah School of Medicine, 50 North Medical Drive, Salt Lake City, Utah 84106, USA. Phone: (801) 581-7427; Fax: (801) 581-4517; E-mail: jerry.kaplan@path.utah.edu.

1. Nemeth, E., and Ganz, T. 2006. Regulation of iron metabolism by hepcidin. *Annu. Rev. Nutr.* **26**:323–342.
2. Nemeth, E., et al. 2004. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science.* **306**:2090–2093.
3. Babitt, J.L., et al. 2007. Modulation of bone morphogenetic protein signaling in vivo regulates systemic iron balance. *J. Clin. Invest.* **117**:1933–1939. doi:10.1172/JCI31342.
4. Wrighting, D.M., and Andrews, N.C. 2006. Interleukin-6 induces hepcidin expression through STAT3. *Blood.* **108**:3204–3209.
5. Pietrangelo, A., et al. 2007. STAT3 is required for IL-6-gp130-dependent activation of hepcidin in vivo. *Gastroenterology.* **132**:294–300.
6. Verga Falzacappa, M.V., et al. 2007. STAT3 mediates hepatic hepcidin expression and its inflammatory stimulation. *Blood.* **109**:353–358.
7. Massague, J., Seoane, J., and Wotton, D. 2005. Smad transcription factors. *Genes Dev.* **19**:2783–2810.
8. Wang, R.H., et al. 2005. A role of SMAD4 in iron metabolism through the positive regulation of hepcidin expression. *Cell Metab.* **2**:399–409.
9. Babitt, J.L., et al. 2006. Bone morphogenetic protein signaling by hemojuvelin regulates hepcidin expression. *Nat. Genet.* **38**:531–539.
10. Papanikolaou, G., et al. 2004. Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. *Nat. Genet.* **36**:77–82.
11. Lin, L., Goldberg, Y.P., and Ganz, T. 2005. Competitive regulation of hepcidin mRNA by soluble and cell-associated hemojuvelin. *Blood.* **106**:2884–2889.
12. Zhang, A.S., et al. 2007. Evidence that inhibition of hemojuvelin shedding in response to iron is mediated through neogenin. *J. Biol. Chem.* **282**:12547–12556.
13. Fukuda, S., et al. 2007. Potentiation of astrogliogenesis by STAT3-mediated activation of BMP-Smad signaling in neural stem cells. *Mol. Cell. Biol.* doi:10.1128/MCB.02435-06.
14. Truksa, J., Peng, H., Lee, P., and Beutler, E. 2006. Bone morphogenetic proteins 2, 4, and 9 stimulate murine hepcidin 1 expression independently of Hfe, transferrin receptor 2 (Tfr2), and IL-6. *Proc. Natl. Acad. Sci. U. S. A.* **103**:10289–10293.
15. Goswami, T., and Andrews, N.C. 2006. Hereditary hemochromatosis protein, HFE, interaction with transferrin receptor 2 suggests a molecular mechanism for mammalian iron sensing. *J. Biol. Chem.* **281**:28494–28498.

Chain-reaction Ca^{2+} signaling in the heart

Sandor Györke,[1] Brian M. Hagen,[2] Dmitry Terentyev,[1] and W. Jonathan Lederer[2]

[1]Department of Physiology and Cell Biology and OSU Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University, Columbus, Ohio, USA.
[2]Institute of Molecular Cardiology, Medical Biotechnology Center, University of Maryland Biotechnology Institute, Baltimore, Maryland, USA.

Mutations in Ca^{2+}-handling proteins in the heart have been linked to exercise-induced sudden cardiac death. The best characterized of these have been mutations in the cardiac Ca^{2+} release channel known as the ryanodine receptor type 2 (RyR2). RyR2 mutations cause "leaky" channels, resulting in diastolic Ca^{2+} leak from the sarcoplasmic reticulum (SR) that can trigger fatal cardiac arrhythmias during stress. In this issue of the *JCI*, Song et al. show that mutations in the SR Ca^{2+}-binding protein calsequestrin 2 (CASQ2) in mice result not only in reduced CASQ2 expression but also in a surprising, compensatory elevation in expression of both the Ca^{2+}-binding protein calreticulin and RyR2, culminating in premature Ca^{2+} release from cardiac myocytes and stress-induced arrhythmia (see the related article beginning on page 1814). In the context of these findings and other recent reports studying CASQ2 mutations, we discuss how CASQ2 influences the properties of Ca^{2+}-dependent regulation of RyR2 and how this contributes to cardiac arrhythmogenesis.

Intracellular Ca^{2+} handling: how the beat goes on

In the normal heart, the cycling of intracellular Ca^{2+} in cardiomyocytes is critical to the heart's mechanical contraction and relaxation. On a beat-to-beat basis, Ca^{2+} entry through voltage-gated Ca^{2+} channels in the sarcolemma (including the transverse tubules) locally activates cardiac ryanodine receptor type 2 (RyR2) Ca^{2+}-release channels found in large clusters in the junctional sarcoplasmic reticulum (jSR) across a very narrow (15 nm) junctional gap (Figure 1A). The release of Ca^{2+} from functional clusters of RyR2s depends on this Ca^{2+} influx and works by the mechanism of Ca^{2+}-induced Ca^{2+} release (CICR). The release of Ca^{2+} from the RyR2 clusters is visualized as Ca^{2+} sparks. The Ca^{2+} sparks amplify the initial Ca^{2+} influx trigger signal and combine to produce an elevation of cell-wide myoplasmic $[Ca^{2+}]$ called the *Ca^{2+} transient*. This increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) leads to activation of the contractile proteins and hence to generation of the heartbeat (1). Ca^{2+} release to the cytosol is accompanied by a reciprocal decline in the $[Ca^{2+}]$ within the SR ($[Ca^{2+}]_{SR}$) for both the $[Ca^{2+}]_i$ transient (2) and for Ca^{2+} sparks (3). This reduction in $[Ca^{2+}]_{SR}$ contributes to deactivation or closure of RyR2s, resulting in Ca^{2+} release termination and induction of a refractory state that prevents Ca^{2+} release during the diastole (4–6). Relaxation occurs following Ca^{2+} reuptake into the SR through the phospholamban-regulated (PLN-regulated) sarcoplasmic or endoplasmic reticulum Ca^{2+} ATPase 2 (SERCA2a). Ordered Ca^{2+} cycling is essential to normal rhythmic activity of the heart, and disturbances in Ca^{2+} handling have previously been shown to underlie diverse Ca^{2+}-dependent cardiac arrhythmias (7–9). In this issue of the *JCI*, catecholamine-induced polymorphic ventricular tachycardia (CPVT) caused by mutations in calsequestrin 2 (CASQ2) is the subject of the study by Song et al. (10); Ca^{2+} signaling plays a central role in the dysfunction.

It is appreciated that RyR2 activity underlies SR Ca^{2+} release and generation of the cytosolic Ca^{2+} transient that is required for muscle contraction. However, just how RyR2 is activated, how sensitive it is to $[Ca^{2+}]$ in both cytosolic and lumenal compartments, and how RyR2-mediated Ca^{2+} release from the SR is regulated depends in

Nonstandard abbreviations used: $[Ca^{2+}]_i$, cytosolic Ca^{2+} concentration; $[Ca^{2+}]_{SR}$, SR Ca^{2+} concentration; CASQ2, calsequestrin 2; CICR, Ca^{2+}-induced Ca^{2+} release; CPVT, catecholamine-induced polymorphic ventricular tachycardia; jSR, junctional SR; PLN, phospholamban; P_o, open probability; RyR2, ryanodine receptor type 2; SERCA2a, sarcoplasmic or endoplasmic reticulum Ca^{2+} ATPase 2; SR, sarcoplasmic reticulum.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* **117**:1758–1762 (2007). doi:10.1172/JCI32496.

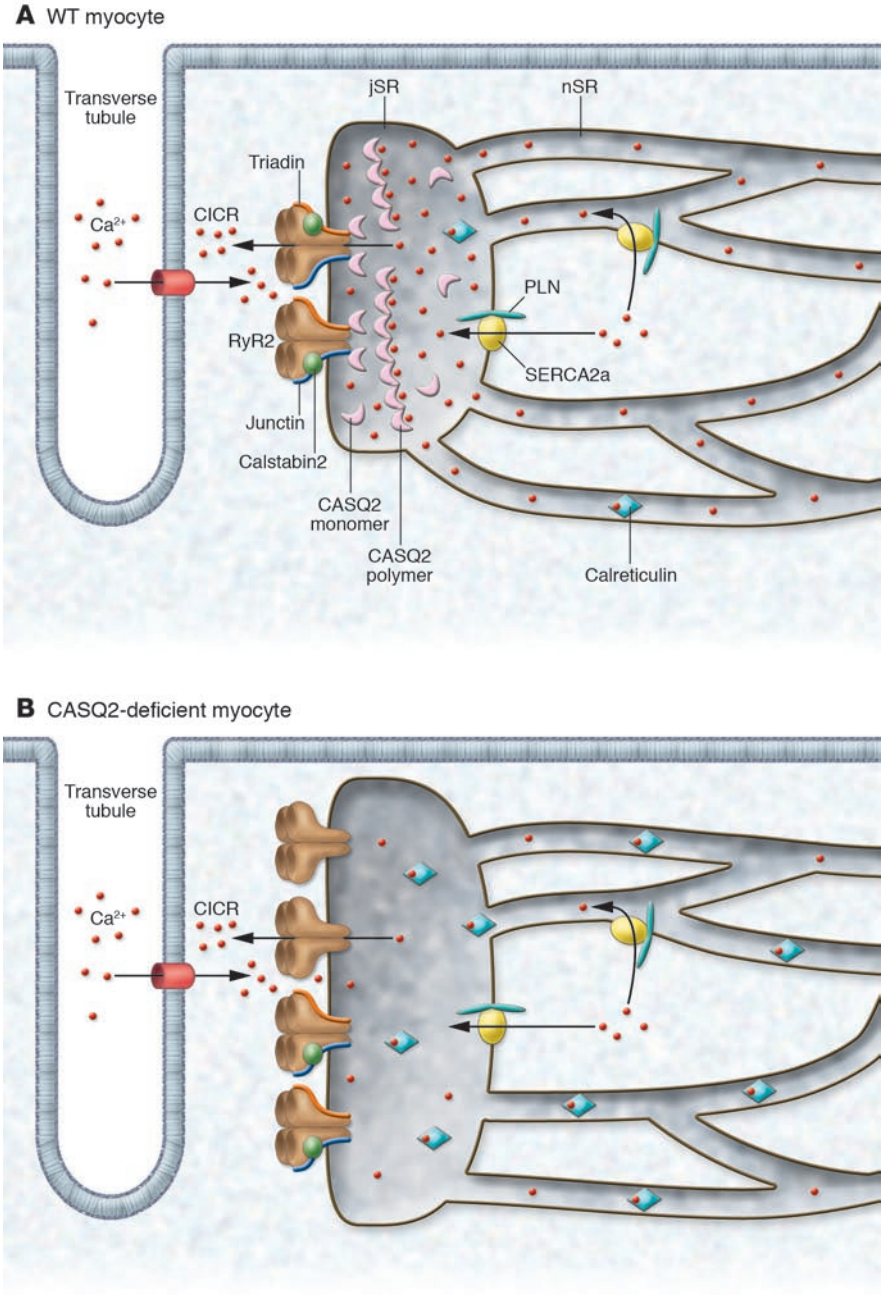

Figure 1
Intracellular Ca^{2+} handling in cardiomyocytes. (**A**) Calcium transients begin with the initial influx of Ca^{2+} via L-type Ca^{2+} channels followed by Ca^{2+} release from the SR via RyR2s, which culminates in contraction. During relaxation, Ca^{2+} reuptake occurs via the PLN-regulated Ca^{2+} pump SERCA2a. The major Ca^{2+} buffering protein in the SR is CASQ2. High $[Ca^{2+}]_{SR}$ converts monomeric CASQ2 (bound to the RyR2-triadin-junctin complex) to the polymeric CASQ2 form that buffers Ca^{2+} and remains close to the complex in the jSR. Calstabin2 and monomeric CASQ2 bind to the complex and stabilize RyR2 activity. (**B**) Altered Ca^{2+} handling in CASQ2-deficient myocytes. As Song et al. report (10), in CASQ2-deficient mouse myocytes, RyR2 expression is significantly upregulated and calreticulin abundance is slightly increased. There is a decrease in Ca^{2+} in the SR. Despite altered Ca^{2+} handling in these animals under resting conditions, these compensatory changes in protein expression appear to help maintain relatively normal heart function. However, under catecholamine- or exercise-induced stress, RyR2 instability increases, leading to an increased risk of cardiac arrhythmia. nSR, nonjunctional SR.

complex ways on five interacting proteins: the SR transmembrane proteins, RyR2, junctin, and triadin; CASQ2, located within the SR lumen; and FKBP12.6 (also known as calstabin2), which is tightly bound to the large cytosolic regulatory domain of RyR2 (11–13) (Figure 1A).

CASQ2

CASQ2 is the major Ca^{2+}-binding and -buffering protein that resides entirely within the SR and binds the Ca^{2+} that is released during Ca^{2+} sparks and during the $[Ca^{2+}]_i$ transient (5, 11, 14). It normally exists in monomeric and polymeric forms, with the polymers dynamically self assembling when $[Ca^{2+}]_{SR}$ is in submillimolar range (15, 16). Full CASQ2 polymerization is thought to occur at high mM $[Ca^{2+}]_{SR}$. Polymeric CASQ2 has a high Ca^{2+}-binding capacity (16) and is located close (~5 nm) to the clustered RyR2s (17) that are organized in a paracrystalline array in the jSR membrane. Monomeric CASQ2 forms a quaternary complex with RyR2 and the intrinsic membrane proteins triadin and junctin, and this conformation decreases the likelihood or probability that the RyR2 channel will be triggered to open by the low diastolic $[Ca^{2+}]_i$. The low open probability (P_o) of RyR2 channels under physiological conditions prevents the RyR2s from opening when they are not triggered by Ca^{2+} influx across the sarcolemmal and transverse tubule membranes, and thus provides a margin of safety. The interactions among CASQ2 and other members of this complex are weakened by elevated $[Ca^{2+}]_{SR}$. Consequently, at elevated $[Ca^{2+}]_{SR}$, the P_o of RyR2s increases and results in increased Ca^{2+} release from the SR in cardiomyocytes. Thus, CASQ2 appears to play at least two different roles in cardiac myocytes: as a Ca^{2+} storage reservoir in the SR and as an active modulator of the Ca^{2+} release process. As a Ca^{2+} storage molecule, CASQ2 is thought to supply the bulk of Ca^{2+} required for contractile activation. As a modulator of Ca^{2+} release, CASQ2 controls RyR2 P_o (via protein-protein interactions involving triadin and junctin) in a manner that depends on the amount of Ca^{2+} within the SR lumen. Given the importance of these SR proteins for Ca^{2+} handling, it is not surprising that genetic alterations of these proteins lead to cardiac disease.

CPVT

Disturbances in the regulation of intracellular Ca^{2+} in the heart were linked explicitly to electrical abnormalities and

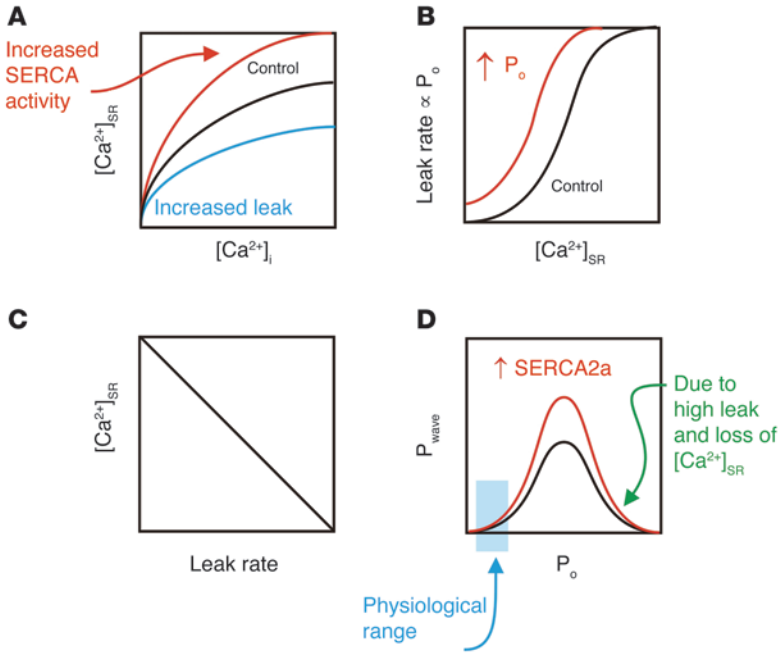

Figure 2

Ca^{2+}-dependent arrhythmogenesis. (**A**) Relationship between $[Ca^{2+}]_{SR}$ and diastolic $[Ca^{2+}]_i$. As $[Ca^{2+}]_i$ increases, so does $[Ca^{2+}]_{SR}$. (**B**) As $[Ca^{2+}]_{SR}$ increases, so does SR Ca^{2+} leak. Any additional features that increase RyR2 openings (P_o) will also increase Ca^{2+} leak. (**C**) As leak increases, there is an increasing loss of Ca^{2+} in the SR. (**D**) Probability of generating a cellular arrhythmia (i.e., a wave) (P_{wave}). $[Ca^{2+}]_{SR}$ is the primary factor in Ca^{2+} overload arrhythmogenesis because it affects P_o. However, as the leak increases, there is loss of Ca^{2+} from within the SR. Thus, increasing P_o has a biphasic effect on P_{wave}. The relationship is biphasic because at low P_o, $[Ca^{2+}]_{SR}$ remains sufficiently high to produce substantial Ca^{2+} efflux and sustain the propagation of Ca^{2+} waves. When P_o is very high, the Ca^{2+} leak outpaces SERCA2a; there is a net loss of $[Ca^{2+}]_{SR}$, and P_{wave} decreases. Increased SERCA2a activity (red curve) shifts the curve (48). The physiological range occurs at very low RyR2 P_o (about 10^{-4} s^{-1}).

arrhythmogenesis over 30 years ago. These arrhythmias developed during Ca^{2+} overload, a state of the cardiac myocyte in which intracellular Ca^{2+} levels are elevated (7–9, 18). During Ca^{2+} overload, increased Ca^{2+} instability within the myocyte was observed, and during diastole, the rate of appearance of elementary Ca^{2+} release events, Ca^{2+} sparks, changed from rare to frequent. Additionally, Ca^{2+} sparks could trigger a chain reaction in the form of propagating waves of CICR within the myocytes (1, 19, 20) whereas under control conditions, Ca^{2+} waves are not seen at all. Increases in lumenal $[Ca^{2+}]$ sensitized RyR2s to activation by cytosolic Ca^{2+}, contributing to generation of Ca^{2+} waves above a certain $[Ca^{2+}]_{SR}$ threshold (21, 22). Such cellular arrhythmogenesis during Ca^{2+} overload underlies changes in both automaticity and electrical conduction that contribute to arrhythmogenesis (23–25).

CPVT is a type of Ca^{2+}-dependent triggered arrhythmia that was initially identified as resulting from mutations in *RyR2* (26) and more recently from mutations in *CASQ2* (27). CPVT occurs in the absence of structural heart disease and is characterized by episodes of syncope, seizures, or sudden death, usually elicited during physical activity or stress. Seven autosomal recessive mutations in *CASQ2* are linked to CPVT (28–31) in addition to the more than 60 arrhythmogenic mutations in *RyR2* (32, 33). The fact that mutations in the SR Ca^{2+} release channel (RyR2) and in the SR Ca^{2+}-binding protein CASQ2 both result in the same phenotype (exercise-induced sudden cardiac death or CPVT) suggests a common mechanism linked to aberrant regulation of SR Ca^{2+} release. The study by Song et al. reported in this issue of the *JCI* (10) examines the consequences of mutating (or deleting) *CASQ2* in mice and how the changes in expression of the CASQ2 protein and other proteins involved in Ca^{2+} signaling lead to cardiac arrhythmias, specifically CPVT. These mouse models, as well as a *CASQ2* knockout mouse described by Knollmann et al. in a recent issue of the *JCI* (34), emulate the exercise-induced CPVT that has been linked to mutations in the *CASQ2* gene in humans (30, 31).

Song et al. (10) constructed two lines of mutant mice: first, homozygous $CASQ^{307/307}$ mice, which possessed a D307H missense mutation in their *CASQ2* gene, a mutation that has previously been identified in a number of Bedouin families in Israel with recessive CPVT (31). The second animal line, homozygous $CASQ^{\Delta E9/\Delta E9}$ mice, possessed a truncation mutation causing loss of *CASQ2* exon 9. Complete absence of *CASQ2* (homozygous 62delA and 532+1 G/A) was previously shown to be associated with CPVT in patients with otherwise functionally and structurally normal hearts (30). The finding that these mutations did not cause structural heart disease in humans was puzzling given the presumed importance of CASQ2 to Ca^{2+} handling and the expectation that absence of this protein would be incompatible with life. Both the study by Song et al. (10) and the recent work with *CASQ2* knockout mice (34) help to resolve this enigma. The results show that there are a multitude of compensatory mechanisms that develop when *CASQ2* is mutated or knocked out. Song et al. (10) demonstrate that CASQ2-deficient mice show a compensatory (albeit small) increase in expression of the lumenal Ca^{2+}-binding protein calreticulin. Additionally, RyR2 expression was dramatically (6-fold) enhanced in a possible attempt by the cells to compensate for the reduced SR Ca^{2+} content (Figure 1B). The logic behind such compensatory changes may be that, along with increased SR volume demonstrated by Knollmann et al. (34), these changes should help the SR to maintain its Ca^{2+} storage function. However, these compensatory mechanisms clearly proved inadequate to fully restore normal Ca^{2+} handling. Indeed the SR Ca^{2+} content remained significantly reduced in CASQ2-deficient myocytes. Moreover, under conditions of stress, the mice developed malignant arrhythmias characteristic of CPVT. Given the similar manifestations of arrhythmia caused by *CASQ2* mutations and arrhythmia associated with Ca^{2+} overload, one may expect that these two disease states have the same underlying mechanism. However, in reality the situation is more complex, as detailed below.

Leak and the overload paradox

A critical factor in this story is determination of Ca^{2+} concentration within the SR itself (6), which is assessed by measuring the average Ca^{2+} efflux or "leak," the Ca^{2+} reaccumulation by SERCA2a, and levels of the Ca^{2+}-buffer CASQ2. One paradox that has emerged from the current work of Song et al. (10) and another recent study (34) is that CASQ2-deficient myocytes have low $[Ca^{2+}]_{SR}$ yet possess an increased probability of Ca^{2+} release — a phenomenon known as the *overload paradox*. Although the paradox is not fully resolved in these studies, key questions are raised and specific future experiments suggested. The overload paradox is found in other arrhythmic diseases including heart failure (35–37) and CPVT due to RyR2 mutations (38). A common feature in these arrhythmic diseases, including those associated with CASQ2 mutations, is increased SR Ca^{2+} leak. This term refers to the loss of Ca^{2+} from the SR by any means. Ca^{2+} sparks represent a clear and visible loss of Ca^{2+} from the SR; indeed, whenever any RyR2 opens, there is a loss of SR Ca^{2+}. In addition, whenever either RyR2s or inositol-1,4,5-trisphosphate receptors open, Ca^{2+} leaks out of all of the Ca^{2+} storage organelles (SR, ER, and nuclear envelope) because they are interconnected (39). Mutations in both RyR2 and CASQ2 lead to CPVT and result in a leaky SR. The question is thus raised, How does SR leak tie in with arrhythmogenesis? This will be discussed here in the context of the role played by CASQ2 under normal and disease conditions.

Under control conditions, SR Ca^{2+} leak is low and is thought to occur almost exclusively through RyR2 Ca^{2+} release channels. Clustered RyR2s (20–300 in number) in the jSR produce Ca^{2+} sparks when activated. In contrast, single or very small clusters of RyR2s ("rogue" RyR2s) may produce SR Ca^{2+} release that may not be readily visible as Ca^{2+} sparks (40). The low SR Ca^{2+} leak in ventricular myocytes is due to the low P_o of the RyR2 Ca^{2+} release channels under diastolic conditions and the low sensitivity of RyR2s to $[Ca^{2+}]_i$. Importantly, however, RyR2 sensitivity to $[Ca^{2+}]_i$ can be modulated by many factors, including $[Ca^{2+}]_{SR}$ (41, 42), phosphorylation of critical proteins such as RyR2 itself, and possibly important proteins such as CASQ2, junctin, triadin, and calstabin2 (11, 13, 43).

Although Ca^{2+} sparks do not normally trigger other Ca^{2+} sparks, during Ca^{2+} overload, a Ca^{2+} spark chain reaction can occur, and this reaction appears as propagated waves of elevated $[Ca^{2+}]_i$ (19). Such reactions may also arise when RyR2 sensitivity to $[Ca^{2+}]_i$ is increased (e.g., by increased $[Ca^{2+}]_{SR}$, mutations in RyR2 (38, 44), or mutations of CASQ2). As the RyR2 P_o increases, so does the SR Ca^{2+} leak rate. Under steady state conditions, the increased leak tends to deplete the SR of Ca^{2+} whether the leak is due to Ca^{2+} sparks or to rogue RyR2 openings. While the probability of arrhythmogenic Ca^{2+} waves increases with RyR2 P_o, SR Ca^{2+} content tends to decrease (Figure 2). Thus, there is a biphasic relationship between the probability of an arrhythmogenic wave and P_o. The common thread in cellular arrhythmogenesis is a disturbance in Ca^{2+}-signaling stability, and this instability underlies multicellular conductance abnormalities (24, 38). This work raises the question, How do CASQ2 mutations lead to arrhythmogenesis?

Molecular mechanism of CPVT

Catecholamines activate protein kinase A (PKA), which phosphorylates RyR2 and also PLN (Figure 2B). When phosphorylated, PLN no longer inhibits SERCA2a and $[Ca^{2+}]_{SR}$ increases. This phosphorylation-dependent modulation of $[Ca^{2+}]_{SR}$ is an important physiological modulation of cardiac Ca^{2+} signaling by catecholamines and is not arrhythmogenic. However, when CASQ2 is mutated, the relationship between $[Ca^{2+}]_{SR}$ and RyR2 behavior may be different, and this difference underlies CPVT. As discussed above, CASQ2 affects RyR2 sensitivity to $[Ca^{2+}]_i$ by binding to the homologous SR transmembrane proteins triadin and junctin (11, 12). At low $[Ca^{2+}]_{SR}$, CASQ2 is tightly bound to triadin and junctin (Figure 1), and in this four-protein complex conformation, RyR2 is inhibited. As $[Ca^{2+}]_{SR}$ increases, the CASQ2-RyR2 complex is weakened and RyR2 becomes more sensitive to $[Ca^{2+}]_i$. In the absence of normal CASQ2, which is capable of inhibiting RyR2 in a Ca^{2+}-dependent manner, the RyR2 complex will be more sensitive to $[Ca^{2+}]_i$, increased Ca^{2+} leak from the SR would be expected to be seen, and there will be a propensity for Ca^{2+} waves and arrhythmogenesis at lower $[Ca^{2+}]_{SR}$ (28). The compensatory increase in the Ca^{2+}-binding protein calreticulin is small, and calreticulin does not seem to interact with the RyR2-triadin-junctin complex. The effects of the known mutations in increasing the sensitivity of RyR2 to $[Ca^{2+}]_i$ combine with the actions of PKA on RyR2 and on PLN to produce the CICR chain reaction and the CPVT phenotype (28, 45). The resolution of the overload paradox in CPVT may simply be that in CPVT, the Ca^{2+} overload phenomenon occurs at much lower $[Ca^{2+}]_{SR}$.

The compensatory increase in RyR2 abundance may further exacerbate the problem if the RyR2 proteins are added to the already large jSR clusters (at this point, however, we do not know the fate of the overexpressed RyR2 proteins). More RyR2 proteins at the jSR may increase the overall likelihood that one is activated to produce the Ca^{2+} chain reaction. Additionally, calstabin2 expression, which remained unchanged compared with wild type, may become insufficient for stabilizing RyR2 activity due to a significantly reduced calstabin2/RyR2 ratio.

Complexities in CASQ2 investigations and future studies

Using an animal model to investigate an important disease provides significant insight into molecular pathophysiology but also highlights the tools we use and the complexity of the diseases we study. CPVT is recapitulated in mouse models with the CASQ2 D307H mutation as shown in the Song et al. study (10). There are, however, many questions raised by this investigation that motivate future work. Previous studies did show expression of the mutant CASQ2 protein, but they demonstrated that the D307H mutation impairs monomeric CASQ2 binding to triadin or/and disrupts formation of polymeric CASQ2 (45–47). This loss of CASQ2 function in animals with the D307H mutation may accelerate CASQ2 protein degradation, and the disparities in CASQ2 abundance may depend on differences in the genetic background of the mice used in the various studies. This raises the question of how Ca^{2+} signaling remains quasinormal in the D307H mice when there is virtually no CASQ2 to buffer the SR Ca^{2+}. As Song et al. suggest (10), the overexpression of calreticulin (which is normally only present in cardiomyocytes at low levels) may substitute for CASQ2 loss. Finally, does calreticulin interact with the RyR2-junctin-triadin complex to modulate signaling? The cellular investigation of these questions remains to be carried out. A possibly even more perplexing question is raised by the elevation of RyR2 expression: How does this feature of the CPVT phenotype contribute to the disease? Finally, it is interesting to note that the two functional knockouts in the Song et al. study exhibit a different set of compensatory mechanisms compared with the mutant CASQ2 mouse described by Knollmann et al. (34). Whereas the principal changes reported in the Song et al. study are increased RyR2 and calreticulin levels, Knollmann et al. (34) observed expansion of SR volume and down-

regulation of triadin and junctin. In future studies, it will be interesting to determine how these different adaptive changes support the same disease phenotype. In summary then, either mutations in the RyR2 channel that render it leaky due to decreased binding of calstabin2 as previously shown (44) or those in CASQ2 that impair inhibition of the RyR2 channel from the luminal SR side can result in a diastolic SR Ca^{2+} leak that triggers fatal cardiac arrhythmias.

Acknowledgments

We thank the National Heart, Lung, and Blood Institute for continued support of S. Györke and W.J. Lederer and the National Institute of Arthritis and Musculoskeletal and Skin Diseases and the Muscle Training Program, University of Maryland School of Medicine for support to B.M. Hagen.

Address correspondence to: W.J. Lederer, Institute of Molecular Cardiology, Medical Biotechnology Center, University of Maryland Biotechnology Institute, 725 West Lombard Street, Baltimore, Maryland 21201, USA. Phone: (410) 706-8181; Fax: (410) 510-1545; E-mail: lederer@umbi.umd.edu.

1. Cheng, H., Lederer, W.J., and Cannell, M.B. 1993. Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. *Science.* **262**:740–744.
2. Shannon, T.R., Guo, T., and Bers, D.M. 2003. Ca2+ scraps: local depletions of free [Ca2+] in cardiac sarcoplasmic reticulum during contractions leave substantial Ca2+ reserve. *Circ. Res.* **93**:40–45.
3. Brochet, D.X., et al. 2005. Ca^{2+} blinks: rapid nanoscopic store calcium signaling. *Proc. Natl. Acad. Sci. U. S. A.* **102**:3099–3104.
4. Sobie, E.A., Dilly, K.W., dos Santos Cruz, J., Lederer, W.J., and Jafri, M.S. 2002. Termination of cardiac Ca^{2+} sparks: an investigative mathematical model of calcium-induced calcium release. *Biophys. J.* **83**:59–78.
5. Terentyev, D., et al. 2003. Calsequestrin determines the functional size and stability of cardiac intracellular calcium stores: mechanism for hereditary arrhythmia. *Proc. Natl. Acad. Sci. U. S. A.* **100**:11759–11764.
6. Terentyev, D., Viatchenko-Karpinski, S., Valdivia, H.H., Escobar, A.L., and Gyorke, S. 2002. Luminal Ca^{2+} controls termination and refractory behavior of Ca^{2+}-induced Ca^{2+} release in cardiac myocytes. *Circ. Res.* **91**:414–420.
7. Kass, R.S., Lederer, W.J., Tsien, R.W., and Weingart, R. 1978. Role of calcium ions in transient inward currents and aftercontractions induced by strophanthidin in cardiac Purkinje fibres. *J. Physiol. (Lond.).* **281**:187–208.
8. Aronson, R.S., Gelles, J.M., and Hoffman, B.F. 1973. Effect of ouabain on the current underlying spontaneous diastolic depolarization in cardiac Purkinje fibers. *Nature New Biol.* **245**:118–120.
9. Ferrier, G.R., Saunders, J.H., and Mendez, C. 1973. A cellular mechanism for the generation of ventricular arrhythmias by acetylstrophanthidin. *Circ. Res.* **32**:600–609.
10. Song, L., et al. 2007. Calsequestrin 2 (CASQ2) mutations increase expression of calreticulin and ryanodine receptors, causing catecholaminergic polymorphic ventricular tachycardia. *J. Clin. Invest.* **117**:1814–1823. doi:10.1172/JCI31080.
11. Gyorke, I., Hester, N., Jones, L.R., and Gyorke, S. 2004. The role of calsequestrin, triadin, and junctin in conferring cardiac ryanodine receptor responsiveness to luminal calcium. *Biophys. J.* **86**:2121–2128.
12. Zhang, L., Kelley, J., Schmeisser, G., Kobayashi, Y.M., and Jones, L.R. 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.
13. Wehrens, X.H., Lehnart, S.E., and Marks, A.R. 2005. Intracellular calcium release and cardiac disease. *Annu. Rev. Physiol.* **67**:69–98.
14. Cala, S.E., and Jones, L.R. 1983. Rapid purification of calsequestrin from cardiac and skeletal muscle sarcoplasmic reticulum vesicles by Ca2+-dependent elution from phenyl-sepharose. *J. Biol. Chem.* **258**:11932–11936.
15. Wang, S., et al. 1998. Crystal structure of calsequestrin from rabbit skeletal muscle sarcoplasmic reticulum. *Nat. Struct. Biol.* **5**:476–483.
16. Park, H., et al. 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.
17. Franzini-Armstrong, C., and Protasi, F. 1997. Ryanodine receptors of striated muscles: a complex channel capable of multiple interactions. *Physiol. Rev.* **77**:699–729.
18. Lederer, W.J., and Tsien, R.W. 1976. Transient inward current underlying arrhythmogenic effects of cardiotonic steroids in Purkinje fibres. *J. Physiol. (Lond.).* **263**:73–100.
19. Cheng, H., Lederer, M.R., Lederer, W.J., and Cannell, M.B. 1996. Calcium sparks and [Ca2+]i waves in cardiac myocytes. *Am. J. Physiol.* **270**:C148–C159.
20. Berlin, J.R., Cannell, M.B., and Lederer, W.J. 1989. Cellular origins of the transient inward current in cardiac myocytes. Role of fluctuations and waves of elevated intracellular calcium. *Circ. Res.* **65**:115–126.
21. Diaz, M.E., Trafford, A.W., O'Neill, S.C., and Eisner, D.A. 1997. Measurement of sarcoplasmic reticulum Ca2+ content and sarcolemmal Ca2+ fluxes in isolated rat ventricular myocytes during spontaneous Ca2+ release. *J. Physiol. (Lond.).* **501**:3–16.
22. Kubalova, Z., et al. 2004. Modulation of cytosolic and intra-sarcoplasmic reticulum calcium waves by calsequestrin in rat cardiac myocytes. *J. Physiol.* **561**:515–524.
23. Mohler, P.J., et al. 2003. Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. *Nature.* **421**:634–639.
24. Weiss, J.N., et al. 2005. The dynamics of cardiac fibrillation. *Circulation.* **112**:1232–1240.
25. Shiferaw, Y., Watanabe, M.A., Garfinkel, A., Weiss, J.N., and Karma, A. 2003. Model of intracellular calcium cycling in ventricular myocytes. *Biophys. J.* **85**:3666–3686.
26. Priori, S.G., et al. 2002. Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. *Circulation.* **106**:69–74.
27. Eldar, M., Pras, E., and Lahat, H. 2003. A missense mutation in the CASQ2 gene is associated with autosomal-recessive catecholamine-induced polymorphic ventricular tachycardia. *Trends Cardiovasc. Med.* **13**:148–151.
28. Terentyev, D., et al. 2006. Abnormal interactions of calsequestrin with the ryanodine receptor calcium release channel complex linked to exercise-induced sudden cardiac death. *Circ. Res.* **98**:1151–1158.
29. di Barletta, M.R., et al. 2006. Clinical phenotype and functional characterization of CASQ2 mutations associated with catecholaminergic polymorphic ventricular tachycardia. *Circulation.* **114**:1012–1019.
30. Postma, A.V., et al. 2002. Absence of calsequestrin 2 causes severe forms of catecholaminergic polymorphic ventricular tachycardia. *Circ. Res.* **91**:e21–e26.
31. Lahat, H., Pras, E., and Eldar, M. 2004. A missense mutation in CASQ2 is associated with autosomal recessive catecholamine-induced polymorphic ventricular tachycardia in Bedouin families from Israel. *Ann. Med.* **36**(Suppl. 1):87–91.
32. Priori, S.G., and Napolitano, C. 2005. Cardiac and skeletal muscle disorders caused by mutations in the intracellular Ca^{2+} release channels. *J. Clin. Invest.* **115**:2033–2038. doi:10.1172/JCI25664.
33. George, C.H., Jundi, H., Thomas, N.L., Fry, D.L., and Lai, F.A. 2007. Ryanodine receptors and ventricular arrhythmias: emerging trends in mutations, mechanisms and therapies. *J. Mol. Cell. Cardiol.* **42**:34–50.
34. Knollmann, B.C., et al. 2006. *Casq2* deletion causes sarcoplasmic reticulum volume increase, premature Ca^{2+} release, and catecholaminergic polymorphic ventricular tachycardia. *J. Clin. Invest.* **116**:2510–2520. doi:10.1172/JCI29128.
35. Song, L.-S., et al. 2006. Orphaned ryanodine receptors in the failing heart. *Proc. Natl. Acad. Sci. U. S. A.* **103**:4305–4310.
36. Kubalova, Z., et al. 2005. Abnormal intrastore calcium signaling in chronic heart failure. *Proc. Natl. Acad. Sci. U. S. A.* **102**:14104–14109.
37. Wehrens, X.H., et al. 2006. Ryanodine receptor/calcium release channel PKA phosphorylation: a critical mediator of heart failure progression. *Proc. Natl. Acad. Sci. U. S. A.* **103**:511–518.
38. Lehnart, S.E., et al. 2006. Stabilization of cardiac ryanodine receptor prevents intracellular calcium leak and arrhythmias. *Proc. Natl. Acad. Sci. U. S. A.* **103**:7906–7910.
39. Wu, X., and Bers, D.M. 2006. Sarcoplasmic reticulum and nuclear envelope are one highly interconnected Ca2+ store throughout cardiac myocyte. *Circ. Res.* **99**:283–291.
40. Sobie, E.A., et al. 2006. The Ca(2+) leak paradox and "rogue ryanodine receptors": SR Ca(2+) efflux theory and practice. *Prog. Biophys. Mol. Biol.* **90**:172–185.
41. Gyorke, I., and Gyorke, S. 1998. Regulation of the cardiac ryanodine receptor channel by luminal Ca2+ involves luminal Ca2+ sensing sites. *Biophys. J.* **75**:2801–2810.
42. Gyorke, S., et al. 2002. Regulation of sarcoplasmic reticulum calcium release by luminal calcium in cardiac muscle. *Front. Biosci.* **7**:d1454–d1463.
43. Terentyev, D., Viatchenko-Karpinski, S., Gyorke, I., Terentyeva, R., and Gyorke, S. 2003. Protein phosphatases decrease sarcoplasmic reticulum calcium content by stimulating calcium release in cardiac myocytes. *J. Physiol.* **552**:109–118.
44. Wehrens, X.H., et al. 2003. FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death. *Cell.* **113**:829–840.
45. Viatchenko-Karpinski, S., et al. 2004. Abnormal calcium signaling and sudden cardiac death associated with mutation of calsequestrin. *Circ. Res.* **94**:471–477.
46. Houle, T.D., Ram, M.L., and Cala, S.E. 2004. Calsequestrin mutant D307H exhibits depressed binding to its protein targets and a depressed response to calcium. *Cardiovasc. Res.* **64**:227–233.
47. Dirksen, W.P., et al. 2007. A mutation in calsequestrin, CASQ2D307H, impairs sarcoplasmic reticulum Ca2+ handling and causes complex ventricular arrhythmias in mice. *Cardiovasc. Res.* **75**:69–78.
48. Venetucci, L.A., Trafford, A.W., and Eisner, D.A. 2007. Increasing ryanodine receptor open probability alone does not produce arrhythmogenic calcium waves: threshold sarcoplasmic reticulum calcium content is required. *Circ. Res.* **100**:105–111.