The Ca\textsuperscript{2+} movements that control many cellular functions, including contraction of striated and smooth muscle cells and vesicular secretion from endocrine cells and nerve terminals, are increasingly recognized to involve macromolecular Ca\textsuperscript{2+}-signaling complexes that, in addition to the key Ca\textsuperscript{2+}-transporting proteins, include large numbers of associated proteins that provide a variety of regulatory, structural and Ca\textsuperscript{2+}-sensing/buffering functions. The importance of working out the subtle interactions within these macromolecular complexes is underscored by the genetic diseases that have been associated with mutations of their constituent proteins.

In this issue, Terentyev et al\textsuperscript{1} use a spectrum of molecular and electrophysiological techniques to demonstrate that triadin (TRD) plays an unexpectedly important role in regulating junctions of cardiac sarcoplasmic reticulum (SR; Figure). It has been previously suggested that TRD and junctin are integral membrane proteins of the junctional SR, and serve as the ryanodine receptors (RyRs), found primarily in dyadic junctions of cardiac sarcoplasmic reticulum (SR; Figure).2 The large (\approx 4500 aa) cytoplasmic domain of RyR appears to have multiple binding sites for an ever-growing list of proteins that includes: calmodulin, PKA, FKBP 12.6,3 The major findings of the present communication are that overexpression of TRD leads to 3-fold increase in open probability of RyRs in bilayers, a 60% increase in spontaneous spark frequency with only minor decreases in spark amplitude (\approx 10%) and SR Ca\textsuperscript{2+} content (\approx 30%), as well as a marked alteration in the voltage dependence of Ca\textsuperscript{2+} release. The authors propose the activity of RyRs to be directly modulated by the level of expression of TRD, most likely mediated by amino acid residues 200 to 224 of TRD, directly modulated by the level of expression of TRD, most likely mediated by amino acid residues 200 to 224 of TRD, and the gain factor of the Ca\textsuperscript{2+} channel.

Voltage-Dependence of CICR

In the present context of regulation of I\textsubscript{Ca}-gated Ca\textsuperscript{2+} release (CICR) via interaction of TRD with RyRs, and the resultant significant change in the bell-shaped voltage-dependence of Ca\textsuperscript{2+} release (Figure 2 of Terentyev et al),\textsuperscript{1} it may be appropriate to consider the present understanding of cardiac Ca\textsuperscript{2+} signaling cascade, including the possible steric interactions between RyRs and the cytoplasmic tail of the \(\alpha_{1c}\) subunit of the Ca\textsuperscript{2+} channel.

The dominant Ca\textsuperscript{2+}-signaling pathway that underlies cardiac EC coupling involves activation of the \(\alpha_{1c}\) subunit of Ca\textsuperscript{2+} channel and mandatory influx of Ca\textsuperscript{2+} through the channel leading to release of Ca\textsuperscript{2+} from the RyR,\textsuperscript{2} which in turn inactivate the Ca\textsuperscript{2+} channel helping to terminate the release process.\textsuperscript{5} Deviations from a strict Ca\textsuperscript{2+}-dependent process, however, were recognized early when quantifying the gain of CICR. It was surprising to find that the gain of CICR was voltage-dependent, showing \approx 10X higher gain at negative voltages of \textasciitilde -30 to \textasciitilde -40 mV where \(I_{Ca}\) was minimally activated. Recent studies introducing small segments of the carboxylic tail (ie, LA peptide)\textsuperscript{6} of the Ca\textsuperscript{2+} channel \(\alpha_{1c}\) subunit into atrial myocytes suggest that only the apocalmodulin binding domain of the LA peptide was the critical domain required to enhance the spontaneous spark frequency and the gain factor of the Ca\textsuperscript{2+} channel (dihydropyridine receptors [DHPR]) uncoupled central release sites. The specific interaction of the LA peptide with the RyR at \textasciitilde -30 mV and \textasciitilde -40 mV, but not at \textasciitilde +10 mV, could provide for the voltage-dependence of CICR, as well as the 4X higher spontaneous frequency of peripheral Ca\textsuperscript{2+} sparks (where DHPR and RyRs are coexpressed), as compared with the DHPR-uncoupled central sites of atrial myocytes.\textsuperscript{6} These findings suggest that CICR mechanism maybe regulated also by molecular processes that could involve direct protein–protein interaction.

In this issue, using adenoviral transfection of adult rat myocytes, Terentyev et al\textsuperscript{1} provide compelling evidence that overexpression of TRD leads to altered voltage-dependence of I\textsubscript{Ca}-gated Ca\textsuperscript{2+} release, such that the small \(I_{Ca}\) activated at \textasciitilde -30 mV and \textasciitilde -20 mV produce the same amount of Ca\textsuperscript{2+} release as that at 0 mV to \textasciitilde +60 mV, making the voltage-dependence of Ca\textsuperscript{2+} release less bell-shaped. This more sigmoid voltage-dependence of peak Ca\textsuperscript{2+} release approxi-
mates those recorded in skeletal muscle. Such “squaring off” of the voltage-dependence of Ca\(^{2+}\) release has been previously reported when intracellular Ca\(^{2+}\) pools were increased by incubating the myocytes in 10 mmol/L Ca\(^{2+}\), even when \(I_{\text{Ca}}\) was triggered in solutions with normal Ca\(^{2+}\) concentrations or by introducing large concentrations of low-affinity Ca\(^{2+}\) buffers (eg, citrate) into the SR, even though in the former case the voltage-dependence of the rate of Ca\(^{2+}\) release continued to remain bell-shaped.

In contrast, large increases in Ca\(^{2+}\) content of the SR by overexpression of the endogenous Ca\(^{2+}\) buffering protein, cardiac CSQ via adenoviral approach, or via the transgenic mouse approach failed to change the voltage-dependence of \(I_{\text{Ca}}\)-triggered Ca\(^{2+}\) release significantly, even though producing sharply opposite results on the efficacy of \(I_{\text{Ca}}\) to release SR Ca\(^{2+}\). In myocytes overexpressing CSQ (adenoviral approach), there was a large enhancement of both caffeine- and \(I_{\text{Ca}}\)-triggered Ca\(^{2+}\) transients. In transgenic CSQ overexpressing mice, \(I_{\text{Ca}}\)-triggered Ca\(^{2+}\) release was markedly suppressed, and coordinated activation of Ca\(^{2+}\) sparks failed to occur, leading to smaller and slower Ca\(^{2+}\) transients, even though caffeine triggered stores were 3 to 5 times larger than in wild mice. In this model, interestingly, increasing the Ca\(^{2+}\) sensitivity of RyRs with 0.2 to 0.5 mmol/L of caffeine restored coordinated sarcomeric Ca\(^{2+}\) striping. Irrespective of the differences in the 2 sets of data, the voltage-dependence of \(I_{\text{Ca}}\)-triggered Ca\(^{2+}\) release was not significantly different between the 2 models, suggesting that CSQ serves a dual role in regulating CICR, ie, CSQ enhances Ca\(^{2+}\) release by increasing SR Ca\(^{2+}\) content and, at the same time, CSQ inhibits Ca\(^{2+}\) release by its Ca\(^{2+}\)-dependent binding to the RyR, possibly via its interaction with TRD. Consistent with this idea are in vitro data suggesting that CSQ binds to TRD (Figure), and that both proteins together may represent the sarcoplasmic Ca\(^{2+}\) sensor that regulates the intraluminal Ca\(^{2+}\) sensitivity of the RyR.

When comparing the voltage-dependence of the Ca\(^{2+}\) current of control and TRD overexpressing myocytes it becomes quite clear that even though there is no effect on \(I_{\text{Ca}}\), the ability of \(I_{\text{Ca}}\) to trigger Ca\(^{2+}\) release is strongly enhanced only when \(I_{\text{Ca}}\) is very small, suggesting either an increased sensitivity of RyRs to Ca\(^{2+}\) (easily testable from bilayer single RyRs studies), or that the gating of RyRs is fundamentally altered in TRD overexpressing myocytes. It is suprising to note that the Ca\(^{2+}\) release actually may precede \(I_{\text{Ca}}\), (Figure 2 of Terentyev et al), suggesting that the depolarization signal may directly regulate Ca\(^{2+}\) release process similar to the mechanism of skeletal muscle. It is intriguing to consider whether the level of expression of TRD in part determines the “purity” of the CICR mechanism. If that were the case, does the higher expression of TRD drive the reaction toward a less Ca\(^{2+}\)-dependent phenotype as found in skeletal muscle? In this respect it would be critical to determine the stoichiometry of TRD and RyR in control, and TRD-overexpressing myocytes, as well as in skeletal muscle. A cursory quantification of the gain of \(I_{\text{Ca}}\)-gated Ca\(^{2+}\) release, based on the data of their Figure 2, suggests orders of magnitude increase in the amplification factor at \(-30\) mV, \(-20\) mV and \(+60\) mV, allowing the \(I_{\text{Ca}}\)-induced Ca\(^{2+}\) release to behave more like the depolarization-induced Ca\(^{2+}\) release of skeletal muscle. Quantifying the gain of \(I_{\text{Ca}}\)-gated Ca\(^{2+}\) release corrected for the Ca\(^{2+}\) content of SR as a ratio of the extent of TRD overexpression may provide critical insight in determining how TRD amplifies Ca\(^{2+}\) release.

Adenovirus-Mediated Versus Transgenic TRD Overexpression

It should be noted that chronic overexpression of TRD in cardiac tissue via a transgenic approach resulted in a quite different EC coupling phenotype. Unlike the results from rat myocytes acutely overexpressing TRD presented by Terentyev et al in this current issue, chronic overexpression of TRD in mouse myocytes increased spark amplitude, and SR Ca\(^{2+}\) load, but did not change spark frequency. Inactivation of \(I_{\text{Ca}}\) was slowed, consistent with impaired CICR. There are many possible reasons for the discrepancies between acute and chronic overexpression of TRD. For example, chronic overexpression of TRD causes down-regulation of the RyR and junctin, which may in turn contribute to the observed differences in the TRD transgenic Ca\(^{2+}\) signaling phenotype. On the other hand, keeping adult cardiomyocytes in culture for over 48 hours (necessary for the adenovirus transfection experiments) significantly changes myocyte structure and protein expression (ie, loss of t-tubules, down-regulation of K-channels). Furthermore, in neither model system the exact subcellular location of the overexpressed TRD is known. Depending on the degree of association of the overexpressed TRD molecules with the native RyRs and their stoichiometry, the resultant phenotype could be quite different. For example, rapid production of TRD protein driven by a highly active adenovirus promoter may lead to TRD that is
Arrhythmia and TRD
If indeed TRD is an important regulator of the SR Ca\(^{2+}\) release channel (RyR, Figure), as the data of Terentyev et al suggest, it may not be surprising that TRD overexpression also would increase the proclivity for arrhythmogenesis in a manner similar the abnormalities of expression of other SR associated Ca\(^{2+}\)-signaling proteins. Specifically, recent clinical data\(^{15,16}\) strongly suggest that mutations that render cardiac CSQ or RyR dysfunctional can cause a syndrome of catecholamine-induced polymorphic ventricular tachycardia (CPVT). The finding of catecholamine-induced Ca\(^{2+}\) waves that trigger delayed after-depolarizations in TRD overexpressing myocytes reported here\(^1\) resembles results obtained in myocytes that have decreased levels of functional CSQ\(^7\) or harbor CPVT-linked RyR mutations.\(^{18}\) Together, this raises the exciting possibility that mutations in or increased expression of TRD may represent a novel mechanism that could be responsible for the CPVT syndrome in humans. However, the data from transgenic mice with cardiac-targeted overexpression of TRD appear to suggest otherwise. It has been reported that chronic overexpression of TRD in mouse myocytes was not accompanied by catecholamine-induced arrhythmia, and Ca\(^{2+}\) release was less sensitive to catecholamines.\(^{13}\) Finally, TRD transgenic mice appeared to develop cardiac hypertrophy,\(^{14}\) not a usual feature of the clinical CPVT syndrome.

What can we conclude? The data given by Terentyev et al\(^1\) demonstrate that TRD plays a critical role in the regulation of EC coupling, clearly a major step forward. On the other hand, the large discrepancies of the experimental results between the adenovirus-based increased TRD expression levels reported here and TRD transgenic mice reported previously\(^{13,14}\) suggests that the function of TRD in regulation of CICR and cardiac pathophysiology maybe more complex than what might be predicted from the available data.

References
7. Sham JSK, Cleemann L, Morad M. Epinephrine stimulates Ca release in cardiomyocytes by enhancing Ca loading of sarcoplasmic reticulum. Biophys J. 1992;61:22. (Abstract.)

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