Ca^{2+} ions are important second messengers in many cellular signal transduction pathways. Compromised Ca^{2+} homeostasis and signaling have been linked to many human diseases, including muscle dysfunction and heart failure.1–5 Two principal sources provide Ca^{2+} to the cell: channels in the plasma membrane (PM) that allow external Ca^{2+} to enter the cell and internal stores sequestered in the endoplasmic reticulum (ER) or sarcoplasmic reticulum (SR) that release Ca^{2+}. Junctional membrane complexes between PM and ER/SR are present in all excitable cells, providing effective mechanisms for cross-talk between Ca^{2+} channels/transporters in the PM and Ca^{2+} release channels in intracellular membranes.6–10 A central focus in cardiovascular research is to understand the basic mechanisms that underlie the control of Ca^{2+} signaling.
in the heart and to search for ways to correct the defective Ca^{2+} signaling process associated with arrhythmogenesis and heart failure.

In the heart, entry of extracellular Ca^{2+} via the L-type Ca^{2+}-permeable channel triggers opening of the ryanodine receptor (RyR) located in the SR membrane through the Ca^{2+}-induced Ca^{2+} release (CICR) mechanism. In skeletal muscle, it is membrane depolarization, rather than external Ca^{2+}, that triggers Ca^{2+} release as the close proximity between the transverse-tubular invagination of PM, and the terminal cisternae of SR permits direct relay of the depolarizing signal via voltage-induced Ca^{2+} release. In this case, CICR represents a secondary mechanism for amplification of voltage-induced Ca^{2+} release in skeletal muscle. Although many studies have focused on understanding the mechanisms that contribute to control of CICR and voltage-induced Ca^{2+} release, the detailed ionic-flux events that take place across the SR/ER during Ca^{2+} release are largely unknown. The ER/SR Ca^{2+} load is maintained by the Ca^{2+} uptake and release processes, both of which are electrogenic events. The efflux of Ca^{2+} through the RyR channels will lead to the generation of a negative potential inside the SR/ER lumen, and this would be expected to limit the release of Ca^{2+} from the SR/ER. Likewise, the active uptake of Ca^{2+} into the ER/SR would lead to the accumulation of a positive potential within the SR/ER lumen that would tend to inhibit Ca^{2+}-pumping function. Thus, robust counterion movements are essential to balance the SR/ER membrane potential and to maintain efficient Ca^{2+} release and uptake mechanisms. However, channels selective for monovalent cations have been reported in SR membrane, searching for the molecular identity of these channels and other accessory proteins that modulate the operation of CICR and voltage-induced Ca^{2+} release has emerged as an important area of muscle physiology and cardiovascular research.

Yamazaki et al. developed an immunoproteomic approach to search for novel proteins involved in myogenesis, Ca^{2+} signaling, and maintenance of membrane integrity in striated muscle cells. A combination of monoclonal antibody-immunohistochemistry, cDNA library screening, and gene knockout techniques was used to identify a group of novel proteins termed mitsugumin that play important roles in muscle physiology and cardiovascular diseases. For example, mitsugumin 29, one protein isolated from this immunoproteomic library, is a synaptophysin-related membrane protein that is essential for the maturation and development of the triad structure in skeletal muscle. Mitsugumin 29 may act as a molecular marker of aging that can shield skeletal muscle against aging-related decreases in Ca^{2+} homeostatic capacity. Another gene isolated using the same approach was junctophilin that physically links the transverse-tubule to the SR membrane, allowing the formation of triad junctions that provide the structural framework for E-C coupling. Recent studies have also linked junctophilin dysfunction and junctophilin polymorphisms to the development of various cardiovascular diseases. More recently, another mitsugumin protein, mitsugumin 53, has been shown to be a muscle-specific member of the tripartite motif family of proteins that plays an important role in the repair of injuries to the PM of muscle cells. Defects in mitsugumin 53 function are associated with muscular dystrophy and cardiac dysfunction. Encouragingly, recombinant mitsugumin 53 protein can be used to modulate membrane repair, which would have important implication for the treatment of muscular dystrophy and other human diseases associated with membrane repair defects.

In 2007, we discovered trimeric intracellular cation channels (TRIC) located at the SR/ER of multiple cell type. In the human and the mouse genomes, 2 isoforms of TRIC were identified: TRIC-A, a subtype predominantly expressed in SR of muscle cells, and TRIC-B, a ubiquitous subtype expressed in ER of all tissues. TRIC-A is also present in the nuclear membrane (Figure S153), but its biological function in regulation of gene transcription has yet to be examined. As a first step toward understanding the physiological function of TRIC, we generated knockout mice carrying deletion of either Tric-a or Tric-b. Although mutant mice lacking TRIC-A survive past their adolescent age, homozygous ablation of Tric-b is lethal because the Tric-b−/− mice died at the neonatal stage. Moreover, aggravated embryonic lethality is observed with the Tric-a−/− and Tric-b−/− mice, demonstrating the essential role of TRIC in development. Our collaborative studies established that both TRIC-A and TRIC-B can function as K+‐permeable channels with distinct conductance and regulatory properties. We found that genetic ablation of TRIC-A or TRIC-B lead to compromised K+‐permeability and Ca^{2+} release across the SR/ER membrane, supporting the hypothesis that TRIC could function as counterion channels that provide the flow of K+ ions into the SR during the acute phase of Ca^{2+} release (Figure 1A). In addition, TRIC may also participate in modulating ER/SR membrane potential between Ca^{2+} release events. In other words, TRIC likely helps maintain overall Ca^{2+} homeostasis, and its function may become particularly important during periods of repetitive release events.

Studies from Guo et al., Gillespie et al., and Gillespie and Fill showed that the RyR channel could provide certain amount of counter ion movement associated with Ca^{2+} release from the SR membrane because of the high permeability of the RyR channel to monovalent cations. Clearly, further studies are required to define the contribution of the intrinsic...
K⁺-permeability of the RyR channel and its relationship to the TRIC channel and the overall Ca²⁺ signaling across the SR/ER membrane. In this review article, we summarize key properties of TRIC-A and TRIC-B in controlling intracellular Ca²⁺ homeostasis and signaling and provide some recent evidence supporting the role for TRIC in modulating the RyR Ca²⁺ release channel and operation of store-overload–induced Ca²⁺ release (SOICR) from the SR membrane. These findings highlight the important role of TRIC in cardiac physiology and disease.

### Biochemical and Biophysical Properties of TRIC Channels

TRIC subtypes are composed of ≈300 amino acid residues. TRIC-A and TRIC-B share patches of sequence identities and similar hydropathy profiles that suggest the existence of multiple transmembrane segments (Figure 1B). In their primary structures, 3 or 4 ER/SR membrane-spanning segments are proposed by protein-structural analysis using computer algorithms. In limited proteolysis of muscle SR vesicles, the amino-terminal region of TRIC-A was resistant to protease digestion, whereas the carboxyl-terminal tail was sensitive to digestion. The amino- and carboxyl-terminal regions are, therefore, assigned to the SR/ER luminal and cytoplasmic sides, respectively. Additional observations in epitope-tagged TRIC-A proteins expressed in cultured cells support the existence of 3 transmembrane segments in the primary structure. Moreover, the hydrophobic loop connecting putative transmembrane segments, TM1 and TM2, is likely associated with membranous environments at the cytoplasmic side and may constitute the channel pore region. The proposed transmembrane topology of TRIC subtypes thus bears an overall resemblance to that of glutamate receptor channels.

Solubilized TRIC-A protein can be purified using an affinity resin conjugated with a specific monoclonal antibody. Application of chemical cross-linkers to the SR vesicles containing endogenous TRIC-A and purified recombinant TRIC-A protein produced dimeric and trimeric products of TRIC-A. Combined computer algorithms that collect, classify, and average electron-microscopic (EM) images of purified TRIC-A particles allow us to reconstruct their 3-dimensional volumes at a high resolution. TRIC-A reconstruction based on negatively stained EM images of purified TRIC-A preparations shows a bullet-shaped homotrimeric structure. Although homotrimeric channels are uncommon, P2X receptor and bacterial porin channels are known as trimeric channels.

As mentioned above, TRIC-A and TRIC-B were identified as SR/ER-resident membrane proteins bearing several
biochemical and structural features consistent with ion channels. To test this possibility, recombinant TRIC-A and TRIC-B proteins were purified from cDNA-transfected yeast cells and reconstituted into artificial lipid bilayer membranes. The reconstitution experiments demonstrated that both TRIC-A and TRIC-B form voltage-dependent cation channels, permeable to monovalent cations K\(^+\) and Na\(^+\) and impermeable to anions and divalent cations.\(^{54-64}\) Both TRIC subtypes are much more active at positive (cytosolic side positive relative to the SR luminal side)-holding potentials than at negative potentials. The channel characteristics observed in the purified TRIC preparations bear close resemblance to the SR K\(^+\) channel previously identified by Miller et al.\(^{17,24,65}\) indicating that TRIC-A and TRIC-B are subtypes of the SR K\(^+\) channel. Although detailed electrophysiological features remain to be defined, our current results on TRIC subtypes suggest that they are ideally suited to carry counter currents in response to loss of positive charge within the lumen of the SR/ER during Ca\(^{2+}\) release and to compensate for charge movements during SR/ER Ca\(^{2+}\) uptake.

**Role of TRIC-A in SR Ca\(^{2+}\) Homeostasis in Skeletal and Smooth Muscles**

As a first step toward understanding the physiological function of TRIC subtypes, we produced knockout mice carrying deletion of either the Tric-a or Tric-b gene. Perhaps not totally unexpected because of its ubiquitous expression pattern, homozygous ablation of TRIC-B is lethal; Tric-b\(^{-/-}\) mice died at the neonatal stage. Interestingly, mutant mice lacking the muscle-specific subtype of TRIC-A, Tric-a\(^{-/-}\), survive past their adolescent age. Thus, Tric-a\(^{-/-}\) can provide a useful animal model for studying the role of TRIC in muscle function in adults.

Skeletal muscle contains TRIC-A and TRIC-B isoforms as predominant and minor components, respectively. TRIC-A is \(\approx10\)-fold more than TRIC-B in skeletal muscle.\(^{54}\) Using microsomal membrane vesicles derived from rabbit skeletal muscle, we showed that TRIC-A is abundantly expressed in skeletal muscle, which is \(\approx4\)-fold higher than that of the RyR (eg, TRIC-A/RyR=5, in rabbit skeletal muscle).\(^{66}\) Ultrastructural analysis using electron microscopy showed that Tric-a\(^{-/-}\) skeletal muscle frequently develops vacuolated SR elements with the formation of Ca\(^{2+}\) deposits that are rarely observed in wild-type muscle (Figure 2A). The RyR activator caffeine could still be able to release Ca\(^{2+}\) from the overloaded SR in Tric-a\(^{-/-}\) muscle, whereas elemental Ca\(^{2+}\) release events, for example, osmotic stress-induced Ca\(^{2+}\) sparks,\(^{66}\) were significantly reduced. Moreover, isolated Tric-a\(^{-/-}\) muscle often displayed alternans behavior reflected by the transient and alternate increases of contractile force during fatigue stimulations\(^{66}\) (Figure 2B). Thus, TRIC-A deficiency impairs RyR-mediated Ca\(^{2+}\) release, resulting in SR Ca\(^{2+}\) overload. TRIC-A channels thus probably function as counterion channels to support physiological Ca\(^{2+}\) release across the SR in skeletal muscle.

Alternans also occurs in cardiac muscle, but the exact mechanism underlying cardiac alternans is unknown. Altered coupling between RyR-mediated intracellular Ca\(^{2+}\) release and various Ca\(^{2+}\) influxes across the sarcolemmal membrane, including L-type Ca\(^{2+}\) channel and Na\(^+\)/Ca\(^{2+}\) exchanger, may contribute to the alternan behavior in cardiac muscle.\(^{66-71}\) Furthermore, SR Ca\(^{2+}\) content is thought to be an important determinant of Ca\(^{2+}\) alternans.\(^{72}\) Thus, we speculate that the mechanical alternans observed in the Tric-a\(^{-/-}\) skeletal muscle may represent instability of the SR Ca\(^{2+}\) release machinery because of overload of the SR Ca\(^{2+}\) store and reduced membrane permeability for K\(^+\) ions.

Although the pathological changes that took place in the Tric-a\(^{-/-}\) muscle are consistent with a role for TRIC channels in providing counterion movements associated with Ca\(^{2+}\) release, it is also possible that TRIC may participate in limiting the electronegative influence of Ca\(^{2+}\) release from the SR, especially under conditions of repetitive stimulations where a succession of fast Ca\(^{2+}\) release events would lead to the accumulation of negative potential inside the SR lumen. Without the TRIC-mediated K\(^+\) movement, the SR will be more electronegative than normal, which would electrically favor Ca\(^{2+}\) uptake. At the same time, the reduced electrochemical driving force for Ca\(^{2+}\) would work to reduce Ca\(^{2+}\) leak that would promote Ca\(^{2+}\) overload inside the SR. Smaller driving force means smaller Ca\(^{2+}\) currents through the RyR channel, which would make CICR less likely.\(^{57-59}\) This model would explain why TRIC ablation resulted in SR Ca\(^{2+}\) overload and abnormal CICR. A direct test of this model would require quantitative assessment of the changes in electric potential across the ER/SR membrane as a function of the Ca\(^{2+}\) release flux under controlled stimulation conditions.

Tric-a\(^{-/-}\) mice also develop hypertension even at young-adult stages because of elevated resting tonus of vascular smooth
muscle cells (VSMCs)\(^7\) (Figure 3). There are 2 \(\text{Ca}^{2+}\) release mechanisms known to regulate VSMCs activities: one is local \(\text{Ca}^{2+}\) sparks generated by spontaneous RyR opening that activate cell-surface \(\text{Ca}^{2+}\)-dependent K\(^+\) channels and lead to hyperpolarization, and the other is the agonist-induced IP\(_3\) receptor (IP\(_3\),R) activation that evokes global \(\text{Ca}^{2+}\) transients, which frequently accompany \(\text{Ca}^{2+}\) waves and oscillations and induce contraction. Cross-talk between IP\(_3\)-R-mediated \(\text{Ca}^{2+}\) release and RyR-mediated \(\text{Ca}^{2+}\) release in smooth muscle has been reported by other investigators\(^7\)\(^3\)\(^5\); however, a role for TRIC channels in facilitating the respective \(\text{Ca}^{2+}\) release processes has not been examined before. In \(\text{Tric-a}^{-/-}\) VSMCs, RyR-mediated \(\text{Ca}^{2+}\) sparks are significantly compromised, depressing the hyperpolarization signaling and elevating resting membrane potential. Under such depolarized conditions, voltage-dependent L-type \(\text{Ca}^{2+}\) channels are highly activated, leading to increased cytoplasmic \(\text{Ca}^{2+}\) concentration and elevating resting myogenic tone in \(\text{Tric-a}^{-/-}\) VSMCs.\(^7\) However, agonist-induced \(\text{Ca}^{2+}\)-release through IP\(_3\),Rs is facilitated because of SR \(\text{Ca}^{2+}\) overloading in the \(\text{Tric-a}^{-/-}\) VSMCs. In \(\text{Tric-a}^{-/-}\) condition, RyR-mediated \(\text{Ca}^{2+}\) release is compromised, whereas IP\(_3\),Rs function normally; therefore, TRIC-A channels seem to support RyR-mediated \(\text{Ca}^{2+}\)-release selectively, whereas IP\(_3\),R-mediated \(\text{Ca}^{2+}\) release might be maintained by TRIC-B channels in \(\text{Tric-a}^{-/-}\) VSMCs. This conclusion is further supported by transgenic mice overexpressing TRIC-A in smooth muscle.\(^7\)\(^6\) In contrast to the phenotype of \(\text{Tric-a}^{-/-}\) mice, the transgenic mice develop persistent hypotension. In VSMCs overexpressing TRIC-A, \(\text{Ca}^{2+}\) spark generation is highly facilitated and \(\text{Ca}^{2+}\)-dependent \(\text{K}^{+}\) channels are thus activated. Under such hyperpolarizing conditions, the L-type \(\text{Ca}^{2+}\) channels are inactivated and resting tonus is decreased in \(\text{Tric-a}^{-/-}\) VSMCs.\(^7\)\(^6\)

**Role of TRIC-B in IP\(_3\)R-Mediated \(\text{Ca}^{2+}\) Signaling in Nonmuscle Cells**

\(\text{Tric-b}^{-/-}\) neonatal mice are cyanotic and die shortly after birth because of respiratory failure (Figure 4). In mutant neonates, the lung alveolus was deflated and surfactant phospholipids were insufficient in extracellular space.\(^7\)\(^7\) In immunoblotting analysis, the expression of TRIC-B and IP\(_3\),R channels was observed in the wild-type lung, but TRIC-A and RyR channels were undetectable. There are mainly 2 cell types in alveolar epithelium: one is the squamous type I cells that surround the alveolar spaces and the other is the cylindrical type II cells that contribute to surfactant phospholipid secretion. In the alveolus...

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**Figure 3.** \(\text{Tric-a}^{-/-}\) mice show hypertension, whereas transgenic \(\text{Tric-a}\) mice show hypotension phenotypes. A, Systolic blood pressure (SBP) was monitored by telemetric recording. \(\text{Tric-a}\) knockout and \(\text{Tric-a}\) transgenic mice exhibited daytime hypertension and hypotension, respectively. B, The membrane potential of isolated vascular smooth muscle cells (VSMCs) was controlled by the whole-cell patch-clamp technique to monitor spontaneous transient outward currents (STOCs). \(\text{Tric-a}\) knockout VSMCs exhibit insufficient STOCs because of impaired \(\text{Ca}^{2+}\) sparks, whereas \(\text{Tric-a}\) transgenic (TG#20) VSMCs show facilitated STOCs because of an enhanced \(\text{Ca}^{2+}\) spark generation. C, Fura-2 \(\text{Ca}^{2+}\) imaging demonstrated elevated resting \(\text{Ca}^{2+}\) levels and store \(\text{Ca}^{2+}\) overloading in \(\text{Tric-a}^{-/-}\) VSMCs. WT indicates wild type.

**Figure 4.** \(\text{Tric-b}\) knockout shows respiratory lethality phenotype. A, \(\text{Tric-b}^{-/-}\) neonates were cyanotic and died shortly after birth because of respiratory failure. B, The lungs from \(\text{Tric-b}^{-/-}\) neonates were deflated because the synthesis and secretion of surfactant phospholipids were impaired in alveolar type II epithelial cells. C, Fura-2 \(\text{Ca}^{2+}\) imaging showed impaired ATP-evoked transients and facilitated ionomycin-induced responses in cultured alveolar type II cells from \(\text{Tric-b}^{-/-}\) neonates, indicating IP\(_3\), receptor (IP\(_3\),R)-mediated \(\text{Ca}^{2+}\) release is disturbed, despite the endoplasmic reticulum stores are overloaded with \(\text{Ca}^{2+}\). Reprinted from Yamazaki et al\(^7\)\(^7\) with permission of the publisher. Copyright © 2009, Company of Biologists.
of Tric-b<sup>−/−</sup> neonates, excess glycogen deposits and insufficient phospholipid lamellar bodies could be observed indicating ultrastructural defects in the type II cells. Type II cells preserve glycogen during the late embryonic stage, and stored glycogen is converted into surfactant lipids just before delivery. It is likely that the metabolic conversion of glycogen into phospholipids is disrupted in the mutant type II cells, leading to defective surfactant secretion into the alveolar space in the Tric-b<sup>−/−</sup> lung.77

Because both the metabolic conversion and the surfactant secretion are activated in Ca<sup>2+</sup>-dependent manners, impaired Ca<sup>2+</sup> release was proposed in Tric-b<sup>−/−</sup> type II alveolar cells. Indeed, IP<sub>R</sub>-mediated Ca<sup>2+</sup> release was compromised, and intracellular Ca<sup>2+</sup> stores were overloaded in the mutant type II cells (Figure 4C). TRIC-B channels, therefore, seem to facilitate agonist-induced Ca<sup>2+</sup> release by providing counter-K<sup>+</sup> ions to the ER in type II alveolar cells and enable them to exert the specialized cellular functions required for neonatal breathing.

As in alveolar epithelial cells, hepatocytes contain TRIC-B and IP<sub>R</sub> channels but not TRIC-A and RyR channels. Hepatocytes also preserve glycogen during the late embryonic development and supply glucose to peripheral tissues after birth. The activation of the Ca<sup>2+</sup>/calmodulin-dependent enzyme phosphorylase requires both cAMP generated by glucagon and Ca<sup>2+</sup> release triggered by adrenaline to liberate glucose units from glycogen in hepatocytes. Excess glycogen deposits were detected in the mutant liver of Tric-b<sup>−/−</sup> neonates, suggesting that Ca<sup>2+</sup>-dependent glucose release is inhibited in the mutant hepatocytes (D. Yamazaki and H. Takeshima, unpublished observation, 2013). On the basis of these observations with Tric-b<sup>−/−</sup> neonates, we can reasonably hypothesize that TRIC-B channels exert a major effect on IP<sub>3</sub>-sensitive Ca<sup>2+</sup> stores of nonexcitable cells.

**Contribution of TRIC-A and TRIC-B to Ca<sup>2+</sup> Signaling in Cardiac Development and Contraction**

Through cross-breeding of the Tric-a<sup>+/−</sup> and Tric-b<sup>+/−</sup> mice, we found an aggravated embryonic lethality in the TRIC-A and TRIC-B double knockout (DKO) mice (eg, the DKO embryos die between E9 and E11, perhaps as a result of abnormal heart function; Figure 5A). This aggravated lethality suggests that TRIC-A and TRIC-B subtypes share a complementary function in the heart. In the looped cardiac tube from E8.5 to E9.5 DKO embryos, irregular cytoplasmic vacuoles were formed. EM observations revealed extensively swollen SR/ER structures in DKO myocytes. Such structures were not present in animals that carried a single functional copy of the Tric-b gene. As in high Ca<sup>2+</sup>-containing organelles, fixative solutions containing oxalate form electron-dense calcium-oxalate deposits, were detectable in EM analysis. Such deposits were frequently detected in the SR/ER in DKO myocytes but not in Tric-a<sup>+/−</sup> and Tric-b<sup>+/−</sup> double heterozygotes myocytes. Fluorometric Ca<sup>2+</sup> imaging of cardiac myocytes from the DKO embryos shows that the amplitudes of spontaneous Ca<sup>2+</sup> oscillations were depressed at E8.5 (Figure 5B). However, remarkably larger caffeine-evoked Ca<sup>2+</sup> transients were observed in E8.5 DKO myocytes. The elevated caffeine-evoked Ca<sup>2+</sup> transients, together with the insoluble deposits in EM observations, indicate severe SR/ER Ca<sup>2+</sup> overloading in DKO myocytes.

In embryonic cardiomyocytes bearing immature intracellular stores, Ca<sup>2+</sup> signaling or spontaneous Ca<sup>2+</sup> oscillations are predominantly composed of Ca<sup>2+</sup> influx, but significant contribution of RyR<sub>2</sub>-mediated CICR is also detectable.78 RyR<sub>2</sub> is the major cardiac Ca<sup>2+</sup> release channel that regulates intracellular Ca<sup>2+</sup> homeostasis. Recently, results from studies of the inducible, cardiac-specific RyR2 knockout mouse demonstrate that RyR2 loss-of-function can lead to fatal arrhythmias, which exemplifies the important contribution of RyR2 to cardiac arrhythmia and sudden death in humans.13,79 TRIC-DKO and RyR<sub>2</sub>-knockout mice show cardiac arrest at similar embryonic stages and share similar characteristic phenotypes of swollen and Ca<sup>2+</sup>-overloaded SR/ER in embryonic cardiac myocytes. Further biochemical studies showed that myocytes...
derived from the DKO embryos retained normal expression of major SR Ca\(^{2+}\) store–related proteins. Thus, CICR mediated by RyR\(_2\) in Tric-DKO cardiomyocytes seem to be impaired. Insufficient RyR\(_2\) function could lead to SR Ca\(^{2+}\) overload and further disrupt cellular homeostasis in TRIC-DKO cardiomyocytes.

The viable nature of the Tric-a\(^{-/-}\) and Tric-b\(^{-/-}\) mice allows us to examine the physiological roles of TRIC subtypes in adult cardiac function. When compared with wild-type mice, the Tric-a\(^{-/-}\) and Tric-b\(^{-/-}\) mice show bradycardia and arrhythmic heart beats (Figure 5C), which is partly linked to the activated baroreflex response under hypertensive condition and may also reflect an intrinsic defect in cardiac muscle function. Even under nonstressful conditions, frequent AV block was observed with the Tric-a\(^{-/-}\) and Tric-b\(^{-/-}\) mice. Moreover, physiological stress applied to these mice, such as treatment with isoproterenol, caused high incidence of sudden death when compared with wild-type mice (D. Yamazaki et al, unpublished observations). It would be interesting to know whether this phenotype is linked to altered Ca\(^{2+}\) signaling in the cardiomyocytes.

TRIC Control of SOICR

Ca\(^{2+}\) release from the SR in cardiomyocytes is normally controlled by voltage-dependent Ca\(^{2+}\) influx via the L-type Ca\(^{2+}\) channel through the CICR mechanism.\(^{31}\) In addition to CICR, it has long been recognized that SR Ca\(^{2+}\) release can occur spontaneously under conditions of SR Ca\(^{2+}\) overload.\(^{70,72,80,81}\) Considering its dependence on SR Ca\(^{2+}\) load and independence on membrane depolarization, this spontaneous SR Ca\(^{2+}\) release has been referred to as SOICR.\(^{82,83}\)

A number of conditions, including increased extracellular Ca\(^{2+}\) concentrations, high-frequency stimulations, excessive \(\beta\)-adrenergic activation, or digitalis intoxication, can lead to Ca\(^{2+}\) overloading of the SR and subsequently SOICR in cardiac cells.\(^{68,83}\) It is also well recognized that SOICR in the form of Ca\(^{2+}\) waves can enhance the activity of the Na\(^+\)/Ca\(^{2+}\) exchanger, leading to delayed afterdepolarizations and triggered activities.\(^{3,71,84}\) These SOICR-evoked delayed afterdepolarizations and triggered activities are a major cause of ventricular tachyarrhythmias and sudden death in patients with catecholaminergic polymorphic ventricular tachycardia and heart failure.\(^{3,71}\) Thus, understanding the molecular basis and regulation of SOICR is critical for the understanding and treatment of Ca\(^{2+}\)-triggered cardiac arrhythmias and other diseases associated with Ca\(^{2+}\) dysregulation.

It is of interest to note that there seems to be no spontaneous Ca\(^{2+}\) waves (SOICR) in cardiomyocytes isolated from the TRIC-A and TRIC-B DKO mice, despite the heavily overloaded SR. It is unknown why the Ca\(^{2+}\)-overloaded SR in DKO cardiomyocytes does not lead to SOICR. It is possible that TRIC may be required to control the response of RyR\(_2\) channels to SR luminal Ca\(^{2+}\), and that TRIC-deficiency somehow renders RyR\(_2\) channels less sensitive to luminal Ca\(^{2+}\) activation. Reduced luminal Ca\(^{2+}\) sensitivity of RyR\(_2\) may provide an explanation for the lack of SOICR and the built-up of SR Ca\(^{2+}\) load in the DKO cells. This possible regulation of RyR\(_2\) by TRIC in cardiomyocytes has yet to be characterized.

To gain some initial insights into the relationship between TRIC-A and RyR\(_2\), we used stable, inducible HEK293 cells expressing the RyR\(_2\). In this cellular model, the elevation of extracellular Ca\(^{2+}\) \([Ca\text{\textsubscript{o}}]\) can lead to increased Ca\(^{2+}\) store inside the ER, which triggers the opening of the RyR2 channel, leading to SOICR in the form of Ca\(^{2+}\) oscillations.\(^{82,83,85}\) Coexpression of TRIC-A and RyR\(_2\) in HEK293 cells led to the apparent suppression of SOICR (Figure 6A). A direct measurement of ER luminal Ca\(^{2+}\) dynamics using the ER luminal Ca\(^{2+}\) indicator, D1ER,\(^{83,86,87}\) revealed a markedly reduced ER Ca\(^{2+}\) store (Figure 6B). This reduced ER Ca\(^{2+}\) store is unlikely to be because of Ca\(^{2+}\) leakage from the TRIC channel because overexpression of TRIC-A or TRIC-B channel alone in HEK293 cells does not reduce ER Ca\(^{2+}\) content. The SOICR inhibitory effect of TRIC-A seems to be specific to TRIC-A because coexpression of RyR\(_2\) with TRIC-B did not affect SOICR in HEK293 cells. These observations support a specific link between TRIC-A and RyR\(_2\). It remains to be determined how overexpression of TRIC-A in RyR\(_2\)-expressing cells reduces ER Ca\(^{2+}\) content. TRIC-A may involve in maintaining a normal level of ER Ca\(^{2+}\) content by promoting counter ion movement, thus preventing store Ca\(^{2+}\) overload that triggers SOICR. Alternatively, TRIC-A may directly interact with RyR\(_2\), regulate its response to store/luminal Ca\(^{2+}\), thus controlling the occurrence of SOICR.
Genetic Variations of TRIC and Their Roles in Human Diseases

Because TRIC subtypes are involved in various biological functions as described above, it is possible that TRIC channels may have important pathophysiological roles in human diseases. The hypertension phenotype of Tric-a knockout73 and the hypotension phenotype of Tric-a transgenic69 demonstrate that the expression level of TRIC-A channels in VSMCs sets the resting blood pressure at the whole animal level. Gene association analysis in a Japanese population identified several single nucleotide polymorphisms around the Tric-a gene that increase hypertension risk and diminish the efficacy of antihypertensive drugs.73 These risk single nucleotide polymorphisms are likely to be associated with a relatively low expression of the Tric-a gene in VSMCs. Therefore, Tric-a single nucleotide polymorphisms can provide biomarkers for the diagnosis and personalized treatment of essential hypertension. Moreover, the TRIC-A protein is a potential pharmaceutical target for malignant hypertension that is resistant to common depressors because activators of TRIC-A channels are thought to stimulate hyperpolarization signaling directly and to reduce resting tonus in VSMCs.

Osteogenesis imperfecta (OI) is a monogenic hereditary disease characterized by low bone mass with abnormal bone microarchitecture, leading to increased bone fragility and deformity. OI has divergent phenotypic manifestations, and the heterogeneity of clinical symptoms suggests several OI-responsible genes in the human genome.88 Most of the OI cases (≥90%) result from defective type I collagen; structural mutations and altered post-translational modifications lead to its functional impairments. However, OI-causing mutations are also linked to collagen-unrelated genes, and a homozygous deletion mutation in the Tric-b (also referred to as TMEM38B) locus was recently identified in Saudi Arabian and Bedouin Israeli OI pedigrees.89,90 This mutation encodes a truncated form of TRIC-B lacking the third transmembrane segment in our topology model and may severely impair the channel activity in various cell types. However, the pathological mechanism underlying this form of OI remains to be investigated. We recently detected OI-like abnormalities in Tric-b<sup>−/−</sup> neonatal mice (eg, insufficient bone density as revealed in quantitative tomography scanning and impaired mineralization as demonstrated in histological analysis; D. Yamazaki and H. Takeshima, unpublished observation). Therefore, Tric-b<sup>−/−</sup> mice may provide a useful animal model for studying OI associated with the Tric-b mutation. In addition to hypertension and OI, altered expression and genetic mutations of TRIC genes may be associated with other human disorders. We need to examine Tric-mutant mice further to define new phenotypes related to health and disease and also to investigate corresponding pathologies in human diseases.

Discussion and Conclusions

The discovery of TRIC channels has potential importance for our understanding of Ca<sup>2+</sup> signaling and homeostasis in the heart and other tissues. One question that requires further investigation is the extent to which TRIC contributes to ion flux across the ER/SR membrane during the Ca<sup>2+</sup> release and uptake processes. In addition to TRIC, additional molecules are also likely to be involved in balancing the ion fluxes across the SR/ER membrane (Figure 1A). For example, high H<sup>+</sup> permeability is detected in the SR/ER membrane and is, in part, responsible for the countertransport of H<sup>+</sup> and Ca<sup>2+</sup> mediated by Ca<sup>2+</sup> pumps.91 Along with the SR K<sup>+</sup> channel, several other K<sup>+</sup> and Cl<sup>−</sup>-selective currents were detected in intracellular organelles, whereas their molecular identities remain to be solved. Recent studies from Fill et al suggested that RyR and IP<sub>R</sub> channels can provide certain extent of countercurrent movement because of the nonselective nature of the Ca<sup>2+</sup> release channels. In their recent publication, Guo et al<sup>97</sup> used pharmacological inhibitors of the SR K<sup>+</sup> channel and concluded that TRIC-mediated counter ion movement does not contribute to the overall SR Ca<sup>2+</sup> release property in cardiac muscle. Note that their experiments were conducted using the replacement of K<sup>+</sup> ions with Cs<sup>+</sup> ions that resulted in only 70% inhibition of the cation current through the SR K<sup>+</sup> channel, which may not be sufficient to cause detectable effect on the SR Ca<sup>2+</sup> release property.

Aside from a role for TRIC channels in modulating the acute phase of Ca<sup>2+</sup> release from the SR/ER store, TRIC-mediated movement of K<sup>+</sup> ions could also help in limiting the electrochemical gradient of Ca<sup>2+</sup> release on the overall Ca<sup>2+</sup> homeostasis inside the SR/ER. Under conditions of repetitive stimulations with fast Ca<sup>2+</sup> release in succession, TRIC may in effect function as a biological voltage clamp for the ER/SR membrane that helps normalize SR/ER potential and thus sustain normal Ca<sup>2+</sup> uptake and release. Direct evaluation of the role of both TRIC channel subtypes in cardiac Ca<sup>2+</sup> signaling will require the use of specific and potent pharmacological inhibitors that can produce complete inhibition of the TRIC channels. Alternatively, tissue-specific manipulations of TRIC-A or TRIC-B expression in transgenic mice are needed to examine the physiological function of these channels in cardiovascular physiology or disease. For overcoming the lethality of the germline ablation of Tric-b and Tric-a genes, inducible or targeted siRNA silencing of both Tric-a and Tric-b may be required to define the physiological function of TRIC subtypes in adult muscle and heart cells. Such studies would help define the role of TRIC channels in controlling the maturation of SR and the integrity of intracellular Ca<sup>2+</sup> release associated with developmental function of the heart.

It is clear that TRIC-A and TRIC-B have differential functions in regulating SR and ER Ca<sup>2+</sup> homeostasis. TRIC-B is ubiquitously expressed in all tissues and, considering the lethal phenotype produced by TRIC-B ablation, one can envision that TRIC-B may play an essential role in maintaining normal ER cellular function in a wide variety of cell types. In contrast, TRIC-A expression is predominantly targeted to tissues containing excitable cell types, such as the brain and muscles. Thus, TRIC-A may function to meet particular kinetic demands of Ca<sup>2+</sup> release within those excitable cells. A reduction in either TRIC protein would likely lead to instability of ER/SR function and thus could have wide reaching effects in cellular physiology. One possibility is that TRIC-A may interact with the RyR channel, and TRIC-B may associate with the IP<sub>R</sub> channel to modulate the overall SR and ER Ca<sup>2+</sup> homeostasis. Although
many studies have shown that cross-talk between IP₃R and RyR can modulate Ca²⁺ signaling in muscle and heart cells in response to physiological and pathological stresses,[] understanding the potential role of TRIC-A and TRIC-B in mediating IP₃R/RyR cross-talk for regulation of Ca²⁺ signaling will certainly require further studies.

Although many studies have suggested that altered function of SOICR from the SR in cardiomyocytes may contribute to the development of cardiac arrhythmias, searching for accessory proteins that modulate the RyR, channel function and SR Ca²⁺ homeostasis should yield important clues to the function of SOICR in physiological and pathophysiological settings. Because TRIC-A can potentially modulate RyR₂-mediated SOICR, one can envision that potential therapeutic interventions can be introduced to target the TRIC/RyR interaction for restoring defective Ca²⁺ signaling in cardiovascular and potentially other human diseases.

Several lines of evidence have linked mutations in TRIC-B to bone and pulmonary diseases and mutations in TRIC-A to hypertension and muscular diseases. Expansion of these research efforts should provide new insights into the physiological function of TRIC channels in human health and disease. For example, one area of cardiovascular research may focus on establishing the link of genetic mutations in TRIC-A to certain other human diseases.

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Disclosures

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