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TRIC Channels and Sarcoplasmic/Endoplasmic Reticulum Calcium Homeostasis

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Abstract

TRIC represents a novel class of trimeric intracellular cation channels. Two TRIC isoforms have been identified in both the human and mouse genomes: TRIC-A - a subtype predominantly expressed in the sarcoplasmic reticulum (SR) of muscle cells, and TRIC-B - a ubiquitous subtype expressed in the endoplasmic reticulum (ER) of all tissues. Genetic ablation of either TRIC-A or TRIC-B leads to compromised K^+ permeation and Ca^{2+} release across the SR/ER membrane, supporting the hypothesis that TRIC channels provide a counter balancing K^+ flux that reduces SR/ER membrane depolarization for maintenance of the electrochemical gradient that drives SR/ER Ca^{2+} release. TRIC-A and TRIC-B appear to have differential functions in Ca^{2+} signaling in excitable and non-excitable cells. *Tric-a*$^{-/-}$ mice display defective Ca^{2+} sparks and spontaneous transient outward currents in arterial smooth muscle and develop hypertension, in addition to skeletal muscle dysfunction. Knockout of TRIC-B results in abnormal IP_3 receptor-mediated Ca^{2+} release in airway epithelial cells, respiratory defects and neonatal lethality. Double-knockout mice lacking both TRIC-A and TRIC-B show embryonic lethality due to cardiac arrest. Such an aggravated lethality indicates that TRIC-A and TRIC-B share complementary physiological functions in Ca^{2+} signaling in embryonic cardiomyocytes. *Tric-a*$^{-/-}$*Tric-b*$^{+/-}$ mice are viable and susceptible to stress-induced heart failure. Recent evidence suggests that TRIC-A directly modulates the function of the cardiac ryanodine receptor (RyR_2) Ca^{2+} release channel, which in turn controls store-overload induced Ca^{2+} release from the SR. Thus, the TRIC channels, in addition to providing a counter-current for SR/ER Ca^{2+} release, may also function as accessory proteins that directly modulate the RyR/IP_3 receptor channel functions.

Keywords

RYR; IP_3R; counter-ion; Ca^{2+} signaling

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INTRODUCTION

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 plasma membrane 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+} channel triggers opening of the ryanodine receptor (RyR) located in the SR membrane through the Ca^{2+} induced Ca^{2+} release (CICR) mechanism[11–13]. 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 (VICR)[14–16]. In this case, CICR represents a secondary mechanism for amplification of VICR in skeletal muscle. While many studies have focused on understanding the mechanisms that contribute to control of CICR and VICR, the detailed ionic-flux events that take place across the SR/ER during Ca^{2+} release are largely un-known. 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 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, active uptake of Ca^{2+} into the ER/SR would lead to accumulation of a positive potential within the SR/ER lumen which would tend to inhibit Ca^{2+}-pumping function. Thus, robust counter-ion movements are essential to balance the SR/ER membrane potential and to maintain efficient Ca^{2+} release and uptake mechanisms[17–22]. While channels selective for monovalent cations have been reported in SR membrane[17, 21, 23–25], searching for the molecular identity of these channels and other accessory proteins that modulate the operation of CICR and VICR has emerged as an important area of muscle physiology and cardiovascular research.

Takeshima and colleagues developed an immuno-proteomic approach to search for novel proteins involved in myogenesis, Ca^{2+} signaling and maintenance of membrane integrity in striated muscle cells[26]. A combination of monoclonal antibody-immunohistochemistry, cDNA library screening and gene knockout techniques was employed to identify a group of novel proteins termed mitsugumins (MG) that play important roles in muscle physiology and cardiovascular diseases. For example, MG29, one protein isolated from this immuno-proteomic library, is a synaptophysin-related membrane protein that is essential for the maturation and development of the triad structure in skeletal muscle[27–29]. MG29 may act as a molecular marker of aging that can shield skeletal muscle against aging-related decreases in Ca^{2+} homeostatic capacity[30–33]. Another gene isolated using the same approach was junctophilin (JP) that physically links the transverse-tubule to the SR membrane[34–38], allowing the formation of triad junctions which provide the structural framework for E-C coupling. Recent studies have also linked JP dysfunction and JP polymorphisms to the development of various cardiovascular diseases[39–43]. More recently, another MG protein, MG53, has been shown to be a muscle-specific member of the TRIM family of proteins that plays an important role in the repair of injuries to the plasma membrane of muscle cells[44–49]. Defects in MG53 function are associated with muscular dystrophy and cardiac

dysfunction[46, 50, 51]. Encouragingly, recombinant MG53 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[44, 52].

In 2007, we discovered TRIC channels located at the SR/ER of multiple cell type[53]. In the human and mouse genomes, two 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 (Fig. S1 of ref 53), but its biological function in regulation of gene transcription has yet to be examined. As a first step towards understanding the physiological function of TRIC, we generated knockout mice carrying deletion of either *Tric-a* or *Tric-b*. While mutant mice lacking TRIC-A survive past their adolescent age, homozygous ablation of *Tric-b* is lethal as the *Tric-b*$^{-/-}$ mice died at the neonatal stage. Moreover, aggravated embryonic lethality is observed with the *Tric-a*$^{-/-}$*Tric-b*$^{-/-}$ mice, demonstrating the essential role of TRIC in development[53]. Our collaborative studies established that both TRIC-A and TRIC-B can function as K^+-permeable channels with distinct conductance and regulatory properties[53, 54]. 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 counter-ion channels that provide the flow of K^+ ions into the SR during the acute phase of Ca^{2+} release[53, 55, 56] (Fig. 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 Fill and colleagues showed that the RyR channel could provide certain amount of counter ion movement associated with Ca^{2+} release from the SR membrane, due to the high permeability of the RyR channel to monovalent cations[57–59]. 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 Ca^{2+} signaling across the SR/ER membrane. In this review article, we summarize key properties of TRIC-A and TRIC-B in controlling intracellular Ca^{2+} homeostasis and signaling, and provide some recent evidence supporting the role for TRIC in modulating the RyR Ca^{2+} release channel and operation of store-overload induced Ca^{2+} release 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[53] (Fig. 1B). In their primary structures, three or four 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, while 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 three 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[60, 61].

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 which collect, classify, and average electron-microscopic (EM) images of purified TRIC-A particles allow us to reconstruct their three-dimensional volumes at a high resolution. TRIC-A reconstruction based on negatively-stained EM images of purified TRIC-A preparations, shows a bullet-shaped homo-trimeric structure[53]. Although homo-trimeric channels are uncommon, P2X receptor and bacterial porin channels are known as trimeric channels[62, 63].

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 Christopher Miller and colleagues[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/or 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 towards 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 due to 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 approximately 10 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 ~4-fold higher than that of the RyR (e.g. 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 (Fig. 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[67], 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] (Fig. 2B). Thus, TRIC-A deficiency impairs RyR-mediated Ca^{2+} release, resulting in SR Ca^{2+} overload. TRIC-A channels thus probably function as counter-ion 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[68–71]. 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 due to overload of the SR Ca^{2+} store and reduced membrane permeability for K^+ ions.

While the pathological changes that took place in the *Tric-a*$^{-/-}$ muscle are consistent with a role for TRIC channels in providing counter-ion 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 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 electrical 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 due to elevated resting tonus of vascular smooth muscle cells (VSMCs)[73] (Fig. 3). There are two Ca^{2+} release mechanisms known to regulate VSMCs activities: one is local Ca^{2+} sparks generated by spontaneous RyR opening that activate cell-surface Ca^{2+}-dependent K^+ channels and lead to hyperpolarization; and the other is the agonist-induced IP_3 receptor (IP_3R) activation that evokes global Ca^{2+} transients, which frequently accompany Ca^{2+} waves and oscillations and induce contraction. Cross-talk between IP_3R-mediated Ca^{2+} release and RyR-mediated Ca^{2+} release in smooth muscle has been reported by other investigators[74, 75], however a role for TRIC channels in facilitating the respective Ca^{2+} release processes has not been examined before. In *Tric-a*$^{-/-}$ VSMCs, RyR-mediated Ca^{2+} sparks are significantly compromised, depressing the hyperpolarization signaling and elevating resting membrane potential. Under such depolarized conditions, voltage-dependent L-type Ca^{2+} channels are highly activated, leading to increased cytoplasmic Ca^{2+} concentration thus elevating resting myogenic tone in *Tric-a*$^{-/-}$ VSMCs[73]. On the other hand, agonist-induced Ca^{2+}-release through IP_3Rs is facilitated due to SR Ca^{2+} overloading in the *Tric-a*$^{-/-}$ VSMCs. In *Tric-a*$^{-/-}$ condition, RyR mediated Ca release is compromised while IP_3Rs function normally, therefore, TRIC-A channels seem to selectively support RyR-mediated Ca^{2+} -release, while IP_3R-mediated Ca^{2+} release might be maintained by TRIC-B channels in *Tric-a*$^{-/-}$ VSMCs. This conclusion is further supported by transgenic mice overexpressing TRIC-A in smooth muscle[76]. In contrast to the phenotype of *Tric-a*$^{-/-}$ mice, the transgenic mice develop persistent hypotension. In VSMSs overexpressing TRIC-A, Ca^{2+} spark generation is highly facilitated and Ca^{2+}-dependent K^+ channels are thus activated. Under such hyperpolarizing conditions, the L-type Ca^{2+} channels are inactivated and resting tonus is decreased in *Tric-a*-overexpressing VSMCs[76].

Role of TRIC-B in IP_3 receptor-mediated Ca^{2+} signaling in non-muscle cells

Tric-b$^{-/-}$ neonatal mice are cyanotic and die shortly after birth due to respiratory failure (Fig. 4). In mutant neonates, the lung alveolus was deflated and surfactant phospholipids

were insufficient in extracellular space[77]. In immunoblotting analysis, the expression of TRIC-B and IP_3R channels was observed in the wild-type lung, but TRIC-A and RyR channels were undetectable. There are mainly two 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 of *Tric-b*$^{-/-}$ 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*$^{-/-}$ lung[77].

Since both the metabolic conversion and surfactant secretion are activated in Ca^{2+}-dependent manners, impaired Ca^{2+} release was proposed in *Tric-b*$^{-/-}$ type II alveolar cells. Indeed, IP_3R-mediated Ca^{2+} release was compromised and intracellular Ca^{2+} stores were overloaded in the mutant type II cells[77] (Fig. 4C). TRIC-B channels therefore seem to facilitate agonist-induced Ca^{2+} release by providing counter-K^+ 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_3R 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. Activation of the Ca^{2+}/calmodulin-dependent enzyme phosphorylase requires both cAMP generated by glucagon and Ca^{2+} release triggered by adrenaline to liberate glucose units from glycogen in hepatocytes. Excess glycogen deposits were detected in the mutant liver of *Tric-b*$^{-/-}$ neonates, suggesting that Ca^{2+}-dependent glucose release is inhibited in the mutant hepatocytes (D.Y. and H.T. unpublished observation). Based on these observations with *Tric-b*$^{-/-}$ neonates, we can reasonably hypothesize that TRIC-B channels exert a major impact on IP3-sensitive Ca^{2+} stores of non-excitable cells.

Contribution of TRIC-A and TRIC-B to Ca^{2+} signaling in cardiac development and contraction

Through cross-breeding of the *Tric-a*$^{-/-}$ and *Tric-b*$^{+/-}$ mice, we found an aggravated embryonic lethality in the TRIC-A and TRIC-B double knockout (DKO) mice, e.g. the DKO embryos die between E9 and E11, perhaps as a result of abnormal heart function (Fig. 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 E8.5-9.5 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^{2+}-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*$^{+/-}$ and *Tric-b*$^{+/-}$ DHE (double heterozygotes) myocytes. Fluorometric Ca^{2+} imaging of cardiac myocytes from the DKO embryos shows that the amplitudes of spontaneous Ca^{2+} oscillations were depressed at E8.5 (Fig. 5B). However, remarkably larger caffeine-evoked Ca^{2+} transients were observed in E8.5 DKO myocytes. The elevated caffeine-evoked Ca^{2+} transients, together with the insoluble deposits in EM observations, indicate severe SR/ER Ca^{2+}-overloading in DKO myocytes.

In embryonic cardiomyocytes bearing immature intracellular stores, Ca^{2+} signaling or spontaneous Ca^{2+} oscillations are predominantly composed of Ca^{2+} influx, but significant contribution of RyR_2-mediated CICR is also detectable[78]. RyR_2 is the major cardiac Ca^{2+} release channel that regulates intracellular Ca^{2+} homeostasis. Lately, results from studies of the inducible, cardiac-specific RyR2 knockdown mice 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_2-knockout mice show cardiac arrest at similar embryonic stages, and share similar characteristic phenotypes of swollen and Ca^{2+}-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 appear 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*$^{-/-}$*Tric-b*$^{+/-}$ mice allows us to examine the physiological roles of TRIC subtypes in adult cardiac function. Compared with wild-type mice, the *Tric-a*$^{-/-}$ *Tric-b*$^{+/-}$ mice show bradycardia and arrhythmic heart beats (Fig. 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 non-stressful conditions, frequent AV block was observed with the *Tric-a*$^{-/-}$ *Tric-b*$^{+/-}$ mice. Moreover, physiological stress applied to these mice, such as treatment with isoproterenol, caused high incidence of sudden death when compared to wild-type mice (unpublished observations, Yamazaki et al). It would be interesting to know whether this phenotype is linked to altered Ca^{2+} signaling in the cardiomyocytes.

TRIC control of store-overload induced Ca^{2+} release (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[11]. 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 store-overload-induced Ca^{2+} release (SOICR) [82, 83, 4].

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, 81]. 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 after depolarizations (DADs) and triggered activities[3, 71, 84]. These SOICR-evoked DADs and triggered activities are a major cause of ventricular tachyarrhythmias and sudden death in patients with catecholaminergic polymorphic ventricular tachycardia (CPVT) 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 appears to be no spontaneous Ca^{2+} waves (SOICR) in cardiomyocytes isolated from the TRIC-A and TRIC-B double knockout 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 employed stable, inducible HEK293 cells expressing the RyR_2. In this cellular model, elevation of extracellular Ca^{2+} ($[Ca]_o$) 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]. Co-expression of TRIC-A and RyR_2 in HEK293 cells led to the apparent suppression of SOICR (Fig. 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 (Fig. 6B) This reduced ER Ca^{2+} store is unlikely to be due to Ca^{2+} leakage from the TRIC channel, because over-expression 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 appears to be specific to TRIC-A, as co-expression 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

Since 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*-knockout[73] and the hypotension phenotype of *Tric-a*-transgenic[76] demonstrate that the expression level of TRIC-A channels in vascular smooth muscle cells (VSMCs) sets the resting blood pressure at the whole animal level. Gene association analysis in a Japanese population identified several single nucleotide polymorphisms (SNPs) around the *Tric-a* gene that increase hypertension risk and diminish the efficacy of antihypertensive drugs[73]. These risk SNPs are likely to be associated with a relatively low expression of the *Tric-a* gene in VSMCs. Therefore, *Tric-a* SNPs 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, since activators of TRIC-A channels are thought to directly stimulate hyperpolarization signaling 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 defected type I collagen; structural mutations and altered posttranslational 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 Arabic 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*$^{-/-}$ neonatal mice; for example, insufficient bone density as revealed in quantitative tomography scanning, and impaired mineralization as demonstrated in histological analysis (D.Y. and H.T. unpublished observation). Therefore, *Tric-b*$^{-/-}$ 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 further examine *Tric*-mutant mice to define new phenotypes related to health and disease, and also to investigate corresponding pathologies in human diseases.

Conclusion and Discussion

The discovery of TRIC channels has potential importance for our understanding of Ca^{2+} 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^{2+} 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 (Fig. 1A). For example, high H^+ permeability is detected in the SR/ER membrane and is, in part, responsible for the counter-transport of H^+ and Ca^{2+} mediated by Ca^{2+} pumps[91]. Along with the SR K^+ channel, several other K^+ and Cl^--selective currents were detected in intracellular organelles, whereas their molecular identities remain to be solved. Recent studies from Fill and colleagues suggested that RyR and IP_3R channels can provide certain extent of counter-current movement due to the non-selective nature of the Ca^{2+} release channels. In their recent publication, Guo et al[57] used pharmacological inhibitors of the SR K^+ channel and concluded that TRIC-mediated counter ion movement does not contribute to the overall SR Ca^{2+} release property in cardiac muscle. Note that their experiments were conducted using the replacement of K^+ ions with Cs^+ ions that resulted in only ~70% inhibition of the cation current through the SR K^+ channel, which may not be sufficient to cause detectable impact on the SR Ca^{2+} release property.

Aside from a role for TRIC channels in modulating the acute phase of Ca^{2+} release from the SR/ER store, TRIC-mediated movement of K^+ ions could also help in limiting the electronegative influence of Ca^{2+} release on the overall Ca^{2+} homeostasis inside the SR/ER. Under conditions of repetitive stimulations with fast Ca^{2+} release in succession, TRIC may in effect function as a biologic “voltage clamp” for the ER/SR membrane that helps normalize SR/ER potential and thus sustain normal Ca^{2+} uptake and release. Direct evaluation of the role of both TRIC channel subtypes in cardiac Ca^{2+} 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 in order to define the physiological function of TRIC subtypes in adult muscle and heart cells. Such studies would help define the role for TRIC channels in controlling the maturation of SR and the integrity of intracellular Ca^{2+} 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^{2+} 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^{2+} 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_3R channel to modulate the overall SR and ER Ca^{2+}

homeostasis. While many studies have shown that cross-talk between IP_3R and RyR can modulate Ca^{2+} signaling in muscle and heart cells in response to physiological and pathological stresses[74, 75, 92–96], understanding the potential role of TRIC-A and TRIC-B in mediating IP_3R/RyR cross-talk for regulation of Ca^{2+} signaling will certainly require further studies.

While many studies have suggested that altered function of store-overload induced Ca^{2+} release from the SR in cardiomyocytes may contribute to the development of cardiac arrhythmias, searching for accessory proteins that modulate the RyR_2 channel function and SR Ca^{2+} homeostasis should yield important clues to the function of SOICR in physiological and pathophysiological settings. Since TRIC-A can potentially modulate RyR_2-mediated SOICR, one can envision that potential therapeutic interventions can be introduced to target the TRIC/RyR interaction for restoring defective Ca^{2+} 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 or TRIC-B to the development of arrhythmia and other stress-induced heart diseases, and whether or not these are correlated with the altered intracellular Ca^{2+} signaling and homeostasis in the cardiovascular system.

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Nonstandard Abbreviations and Acronyms

TRIC	trimeric intracellular cation channels
ER	endoplasmic reticulum
SR	sarcoplasmic reticulum
RyR	ryanodine receptor
IP_3R	IP_3 receptor
CICR	Ca^{2+} induced Ca^{2+} release
VICR	voltage induced Ca^{2+} release
VSMC	vascular smooth muscle cell
SOICR	store overload induced Ca^{2+} release

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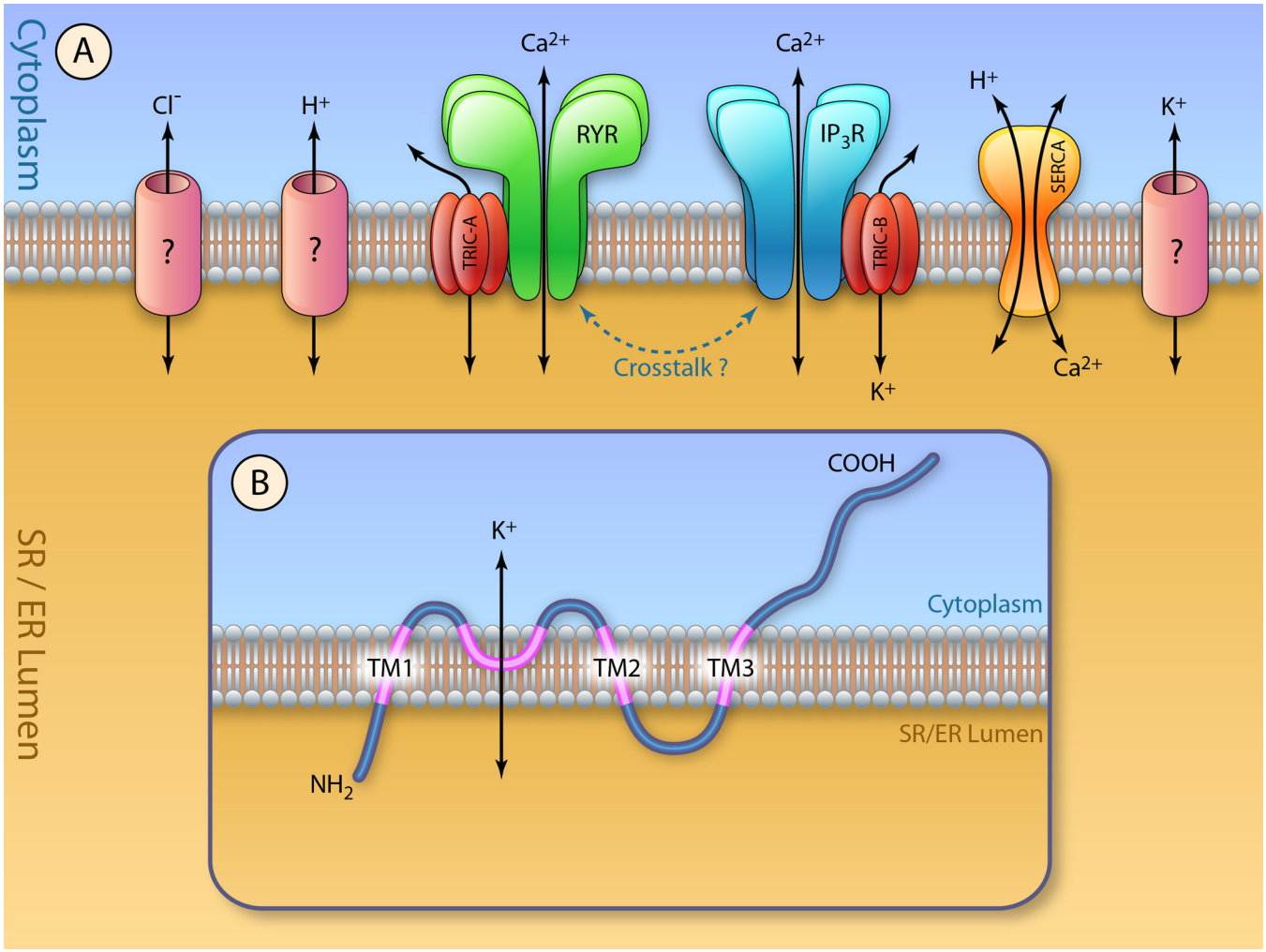

Figure 1. Model for TRIC function in Ca^{2+} signaling

(A) TRIC-A and TRIC-B are two isoforms of the trimeric intracellular cation channels. Both TRIC-A and TRIC-B channels can conduct monovalent cations to provide the flow of counter currents associated with release of Ca^{2+} ions from intracellular stores. TRIC-A modulates RyR-mediated Ca^{2+} release from the SR, and TRIC-B facilitates IP_3R-mediated Ca^{2+} release from the ER. Whether or not there is a cross-talk between TRIC-A and TRIC-B mediated intracellular Ca^{2+} signaling remains to be explored. Molecular identify of other channels located on the ER/SR membranes are not known. **(B)** Topology model of TRIC channels on the SR/ER membrane. (Illustration Credit: Ben Smith).

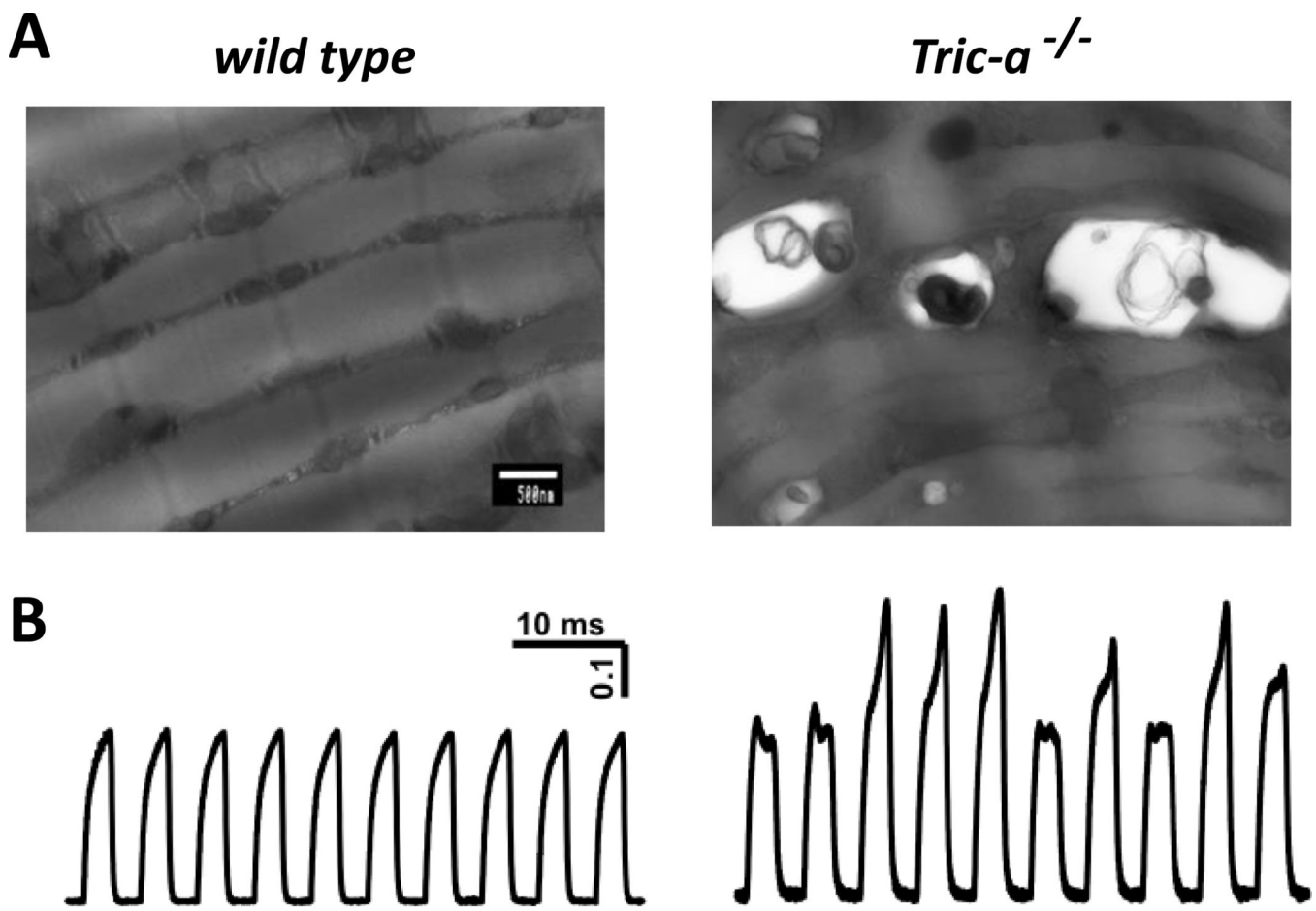

Figure 2. Ablation of TRIC-A leads to SR Ca^{2+} overload and muscle dysfunction

(**A**) Transmission EM image reveals Ca^{2+}-overloaded and swollen SR in *Tric-a*$^{-/-}$ skeletal muscle. Arrows designate electron dense Ca^{2+} deposits in the large-sized vacuoles. (**B**) Skeletal muscle derived from the *Tric-a*$^{-/-}$ mice showed alternans behavior following fatigue stimulation. Isolated muscle bundle from wild type mice showed constant contractile force after a fatiguing stimulation (*left*), whereas muscle bundle derived from the *Tric-a*$^{-/-}$ mice displayed irregular patterns of contractile force following similar fatigue stimulation (*right*). Such fatigue-induced irregular contractile force may reflect instability of SR Ca^{2+} handling in the *Tric-a*$^{-/-}$ muscle. Modified from Zhao et al[66], Ca2+ overload and sarcoplasmic reticulum instability in tric-a null skeletal muscle. *J Biol Chem*. 2010;285:37370–37376.

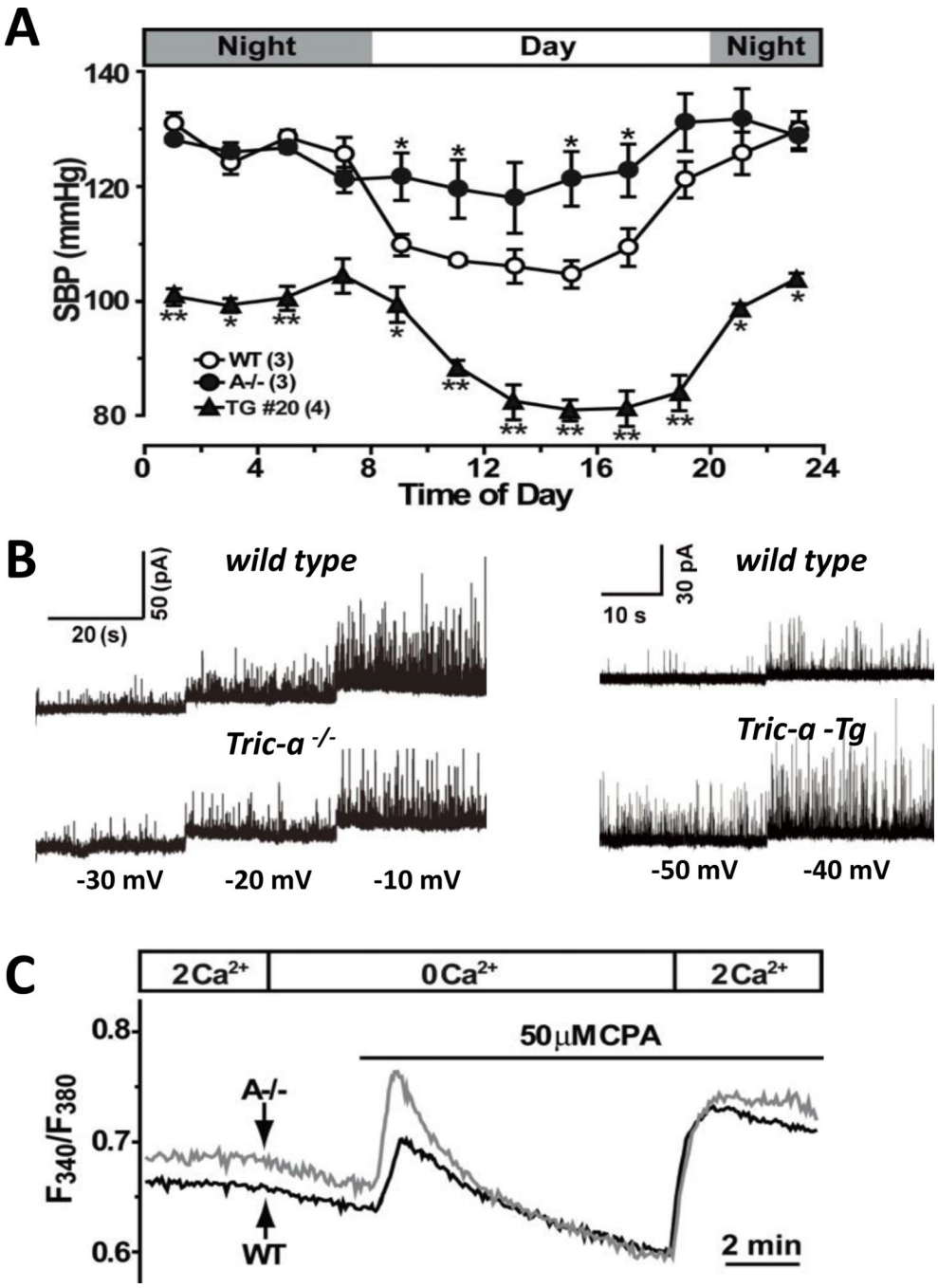

Figure 3. *Tric-a*$^{-/-}$ mice show hypertension while transgenic *Tric-a* mice show hypotension phenotypes

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

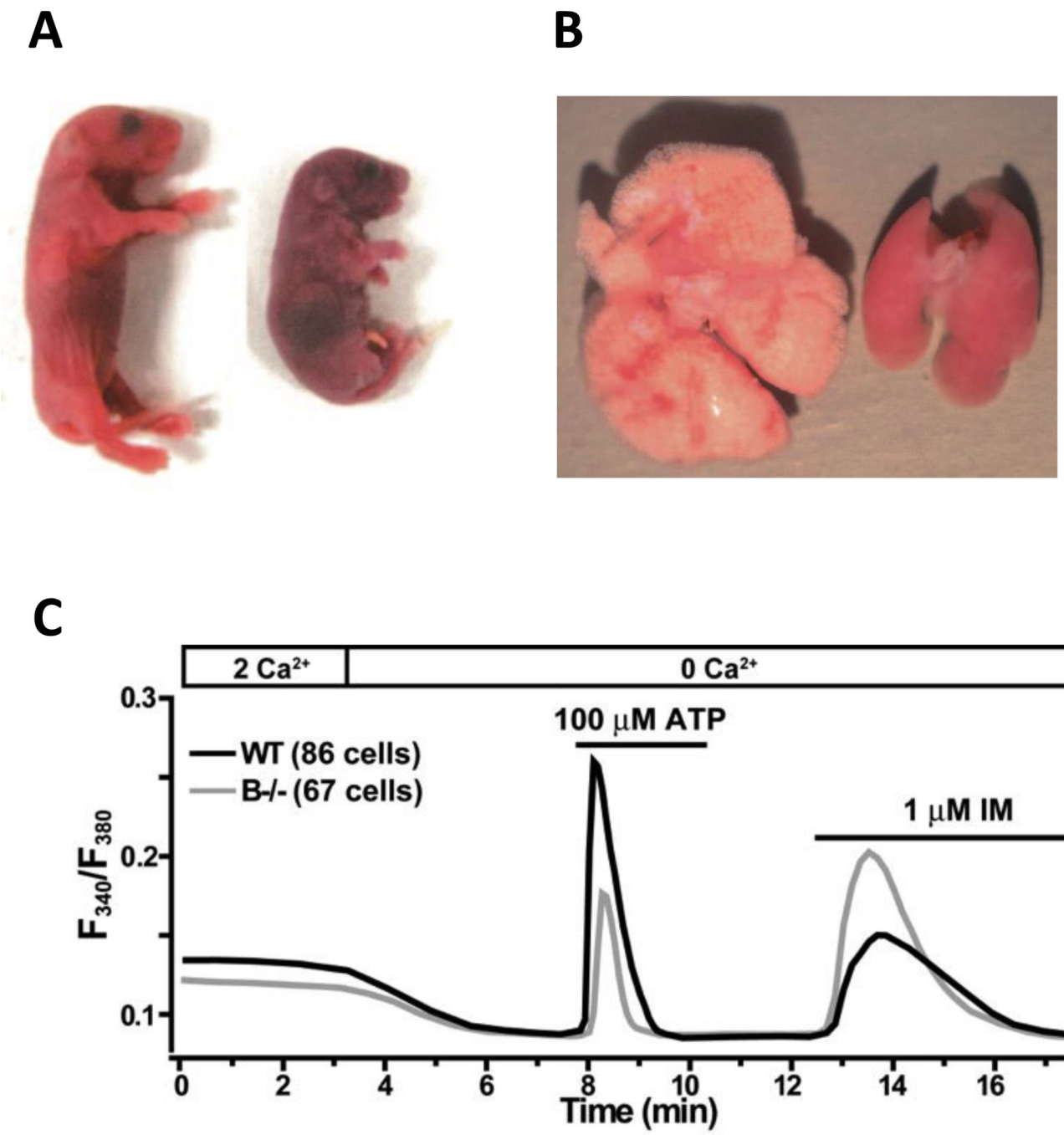

Figure 4. *Tric-b* knockout shows respiratory lethality phenotype

(A) Tric-b−/− neonates were cyanotic and died shortly after birth due to respiratory failure. (B) The lungs from Tric-b−/− neonates were deflated, because the synthesis and secretion of surfactant phospholipids were impaired in alveolar type II epithelial cells. (C) Fura-2 Ca2+ imaging showed impaired ATP-evoked transients and facilitated ionomycin-induced responses in cultured alveolar type II cells from Tric-b−/− neonates, indicating IP3R-mediated Ca2+ release is disturbed despite the ER stores are overloaded with Ca2+. Modified from Yamazaki et al76, Facilitated hyperpolarization signaling in vascular smooth muscle-overexpressing tric-a channels. J Biol Chem. 2013;288:15581–15589

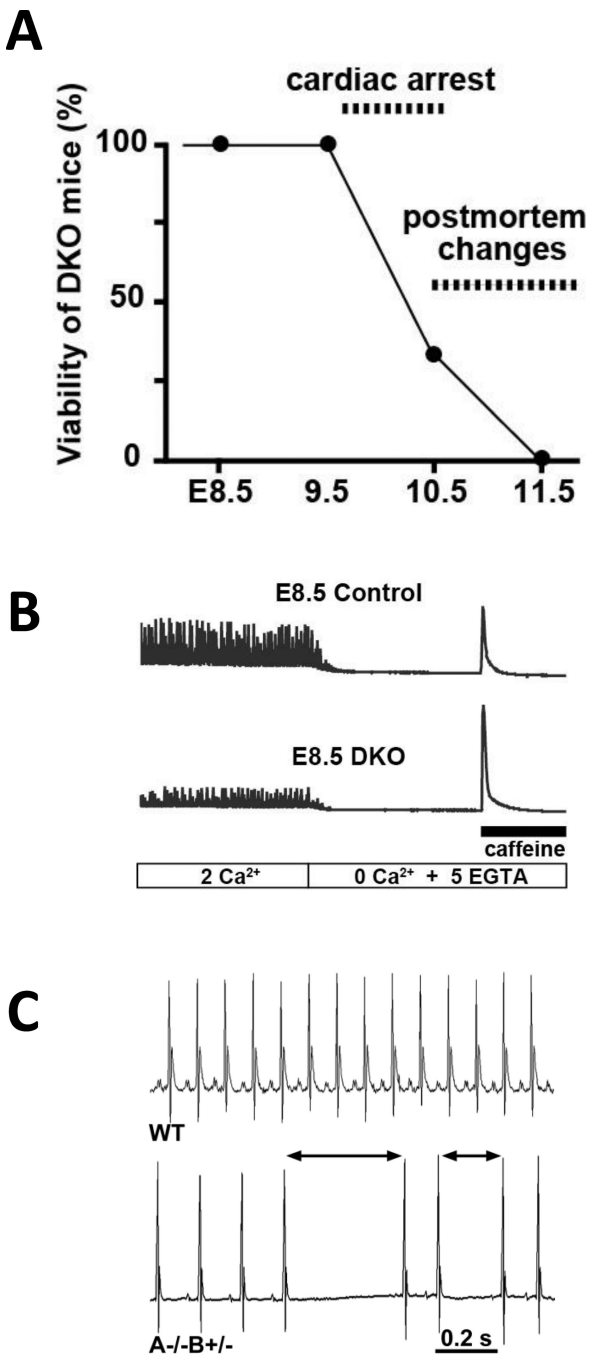

Figure 5. Deletion of both *Tric-a* and *Tric-b* leads to embryonic lethality

(**A**) TRIC-DKO embryos exhibited weak heartbeats at E9.5, stopped beating at ~E10.5, and postmortem autolysis and discoloration at ~E11.5. (**B**) Defective Ca^{2+} signaling in TRIC-DKO cardiomyocytes (*bottom*) compared normal Ca^{2+} signaling in DHE (double heterozygotes, control, *top*) cardiomyocytes (modified from Yazawa et al[53]). (**C**) Telemetry ECG recording of mice under non-treated conditions. *Tric-a*−/−*Tric-b*+/− mice developed bradycardia with occasional arrhythmic events.

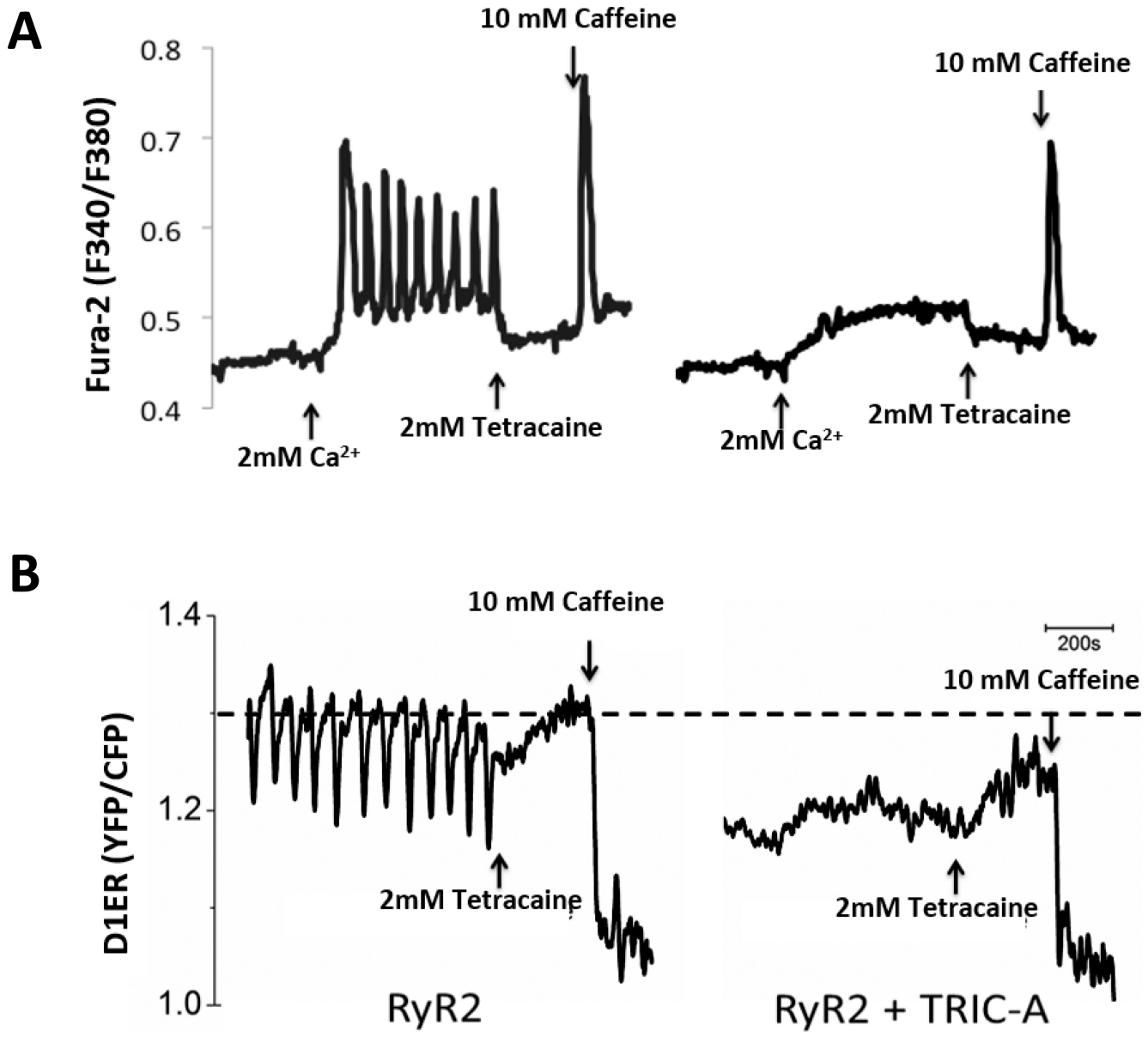

Figure 6. SOICR measurement in HEK293 cells expressing RyR_2 and TRIC-A

(**A**) Fluorometric Ca^{2+} imaging to determine cytosolic Ca^{2+} concentration. HEK293 cells expressing RyR_2 alone show spontaneous Ca^{2+} oscillations caused by SOICR, while co-expression of RyR_2 with TRIC-A suppressed SOICR. (**B**) FRET-based Ca^{2+} sensing protein D1ER was used to determine ER luminal Ca^{2+} concentration. FRET recordings from representative cells expressing RyR_2, or RyR_2 plus TRIC-A, were measured with 2 mM $[Ca]_o$. 2 mM tetracaine inhibited ER Ca^{2+} oscillations and 10 mM caffeine caused depletion of ER Ca^{2+}.