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INVITED REVIEW

SOCE in the cardiomyocyte: the secret is in the chambers

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Abstract

Store-operated Ca^{2+} entry (SOCE) is an ancient and ubiquitous Ca^{2+} signaling pathway that is present in virtually every cell type. Over the last two decades, many studies have implicated this non-voltage dependent Ca^{2+} entry pathway in cardiac physiology. The relevance of the SOCE pathway in cardiomyocytes is often questioned given the well-established role for excitation contraction coupling. In this review, we consider the evidence that STIM1 and SOCE contribute to Ca^{2+} dynamics in cardiomyocytes. We discuss the relevance of this pathway to cardiac growth in response to developmental and pathologic cues. We also address whether STIM1 contributes to Ca^{2+} store refilling that likely impacts cardiac pacemaking and arrhythmogenesis in cardiomyocytes.

Keywords Stromal interaction molecule 1 (STIM1) · Store-operated calcium entry · Store-operated calcium channels · Cardiac muscle

In cardiomyocytes, each action potential triggers a rapid rise in cytosolic Ca^{2+} creating a Ca^{2+} transient that is necessary to initiate and sustain sarcomeric contraction. Opening of Na channels depolarizes the T-tubular membrane which activates L-type Ca^{2+} channels (LTCCs) [22]. This relatively minor Ca^{2+} influx from the T-tubule membrane leads to a much greater release of Ca^{2+} from the sarcoplasmic reticulum (SR) through the action of ryanodine receptors type-2 (RyR2). Once that Ca^{2+} transient reaches its peak and Ca^{2+} levels are sufficiently high, Ca^{2+} binds to troponin leaving actin open to bind with myosin that culminates in sarcomeric contraction. To relax the cardiomyocytes, cytosolic Ca^{2+} levels decline as Ca^{2+} is re-sequestered into the SR or is extruded across the plasma membrane. These are the major cyclical events underlying excitation contraction coupling (ECC) and highlight the significance of the amplitude of the Ca^{2+} transient as a determinant of contractility as discussed in many recent reviews [19, 100]. In cardiomyocytes, a reduction in the peak combined with an increase in the width of the Ca^{2+} transient is a hallmark of the changes that occur in the failing cardiomyocyte and reflects a reduction in SR Ca^{2+} stores. Insufficient RYR2-Ca^{2+} stores fail to activate the contractile complex and thus reduce the force of contraction representing failure at the cardiomyocyte level. When depletion of Ca^{2+} stores from cardiomyocytes becomes more widespread, organ dysfunction manifests as a reduction in systolic function of the left ventricle or leads to the generation of arrhythmias. Because of the changes to Ca^{2+} transients that occur in heart failure, tremendous effort is underway to identify therapeutic strategies focused on Ca^{2+} signaling [68].

Additional determinants of the Ca^{2+} transient include Ca^{2+} extrusion across the sarcolemma by the Na/Ca^{2+} exchanger (NCX) and plasma membrane ATPase (PMCA), Ca^{2+} buffering capacity in the SR by calsequestrin, and Ca^{2+} flux into and out of other organelles. Thus, there is a growing view that the Ca^{2+} transients are the culmination of the integrative actions of many cellular processes and likely involve new and previously not considered components. A greater understanding of the contribution of these processes to the normal Ca^{2+} transients is likely to alter how we think about calcium signaling in the mammalian cardiomyocyte. Moreover, these factors may disrupt or distort the Ca^{2+} transient and thereby influence disease

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states. Diversion of Ca^{2+} from the cytosol into non-SR organelles including the mitochondria and perhaps nuclei may, in theory, lower the amplitude of the Ca^{2+} transient and activate injurious signaling pathways or alter gene expression. In contrast, Ca^{2+} release from endosomes or lysosomes via Two Pore channels (TPC) could facilitate greater Ca^{2+} release from RyR2 channels via Ca^{2+} induced Ca^{2+} release during diastole and regulate autophagy in cardiomyocytes [13, 26, 29]. Ca^{2+} uptake into organelles may shift the balance of cytosolic and SR Ca^{2+} levels, prevent Ca^{2+}-dependent inactivation of certain channels (e.g., cav1.2), and make less Ca^{2+} available to the contractile apparatus. Whether these additional pathways represent primary or indirect mechanism for controlling Ca^{2+} stores remains to be determined, but these pathways are emerging as important regulators of diastolic Ca^{2+} and Ca^{2+} oscillations during pathologic conditions [94].

Non-voltage dependent Ca^{2+} entry, where Ca^{2+} flux occurs independent of an action potential, has been implicated in cardiomyocyte signaling during stress conditions including neurohormonal activation, mechanical stretch, and RyR2 store depletion. Examples of these currents include transient receptor potential channels (TRPC) and store operated Ca^{2+} entry (SOCE) channels [16, 67, 92]. For the most part, these Ca^{2+} signaling pathways have been studied in the context of disease states including heart failure or arrhythmias while the precise function of these pathways under normal physiologic functions remained poorly defined. This review focuses on one of these mechanisms: the sensing of RyR2 store depletion by the S/ER Ca^{2+} sensor stromal interaction molecule 1 (STIM1) and activation of non-voltage dependent Ca^{2+} entry through members of the family of Orai channels. It is now widely accepted that SOCE operates to one extent or another in different cardiomyocytes (e.g., atrial, SAN, and ventricular). Many questions remain about STIM1 signaling in cardiomyocytes and the differences in the STIM1-SOCE signaling in cardiomyocytes compared with non-excitable cells. Nevertheless, reconciling SOCE's role in cardiac pathophysiology has important implications as it might offer novel therapeutic targets for cardiac arrhythmias and the treatment of heart failure.

SOCE is a well-established signaling pathway in non-excitable cells first introduced by Putney and colleagues in 1986 [79]. When the stores are full, no Ca^{2+} influx was detected. Because Ca^{2+} stores are finite and Ca^{2+} entry is triggered upon SR depletion, this Ca^{2+} entry pathway was originally thought to occur in a manner analogous to a capacitor, hence the name capacitive calcium entry, where communication between the cell exterior and the ER/SR stores was thought to be direct. Decades of investigation has now establish that extracellular Ca^{2+} entry in response to the internal Ca^{2+} store depletion provides a key mechanism to maintain cellular Ca^{2+} homeostasis and thereby fulfills the requirements of the Cell Boundary Theorem in all cell types (non-excitable or not) [80]. Leak from internal stores (IP3R or RYR2) or impaired buffering of Ca^{2+} may alter Ca^{2+} transients directly, but only changes in the rate of SOCE are able to modify resting Ca^{2+} levels. Indeed SOCE is activated by store depletion (rather than cell stimulation with agonists of the G-protein coupled and tyrosine kinase receptors pathway) in order to sustain low amplitude cytosolic Ca^{2+} signaling, refill internal stores, and maintain resting cytosolic Ca^{2+} levels [47, 79]. It is fascinating to note that many of the key features and biophysical characteristics of SOC current were determined decades prior to the identification of the key components of the SOCE complex in 2005. In fact, recent work has established how these key players interact structurally and provide the opportunity to unravel the molecular details for Ca^{2+} selectivity, inward rectification, and small unitary conductance that characterize SOC currents [78].

Stromal interaction molecule (STIM) proteins are a family of Ca^{2+} sensors located in the S/ER membrane that are activated by S/ER Ca^{2+} store depletion [56, 117]. STIM proteins share a common domain structure that includes the EF-SAM domain in the N-terminus (a single pass transmembrane domain) and cytosolic domains including coiled coil regions and a polybasic regions. The EF-SAM domain for both STIM1 and STIM2 resides in the lumen of the ER and exhibits high affinity of Ca^{2+}, 200uM and 500 uM for STIM1 and STIM2 respectively [56, 117] (Fig. 1A). The EF-SAM domains bind Ca^{2+} under resting conditions with replete stores and thereby folds into a compact globular structure. Upon store depletion, STIM1 is decalcified and the EF-SAM domains become less structured or unfolded. It seems that loss of Ca^{2+} in the EF-SAM domain signals to the cytosolic portions of STIM1 and promotes multimerization and migration of STIM1 into punta. Store depletion releases an inhibitory clamp between coiled coiled 1 (CC1) and the CRAC activation domain (CAD) that is comprised of the other coiled coil domains (CC2 and CC3 domains) (Fig. 2) [21, 59, 66, 122]. STIM1 migrates from the conventional ER into ER tubules that align underneath the plasma membrane. Orai channels are the pore that contain four membrane-spanning units that share homology with tetraspanin, claudins, and transmembrane AMPA regulators, proteins that make up the tight junctions and are often implicated in adhesive signaling in cells [111] (Fig. 1B). The Orai channel's cytosolic domains at their N- and C-terminus are important for interaction with STIM1 and confer store operation to the Ca^{2+} channels. STIM1 can make contact with Orai channels in order to activate SOCE (Fig. 2). How these events occur in excitable cells such as striated muscle has been subject of considerable debate. In resting myocytes, STIM1 puncta exist as large macromolecular complexes that are required for rapid activation of SOCE. In fact, STIM1 couples Orai1 and activates SOCE with each action potential in SAN cardiomyocytes and skeletal muscle fibers [98, 116]. In addition, a second pool of STIM1 is recruited for SOCE during

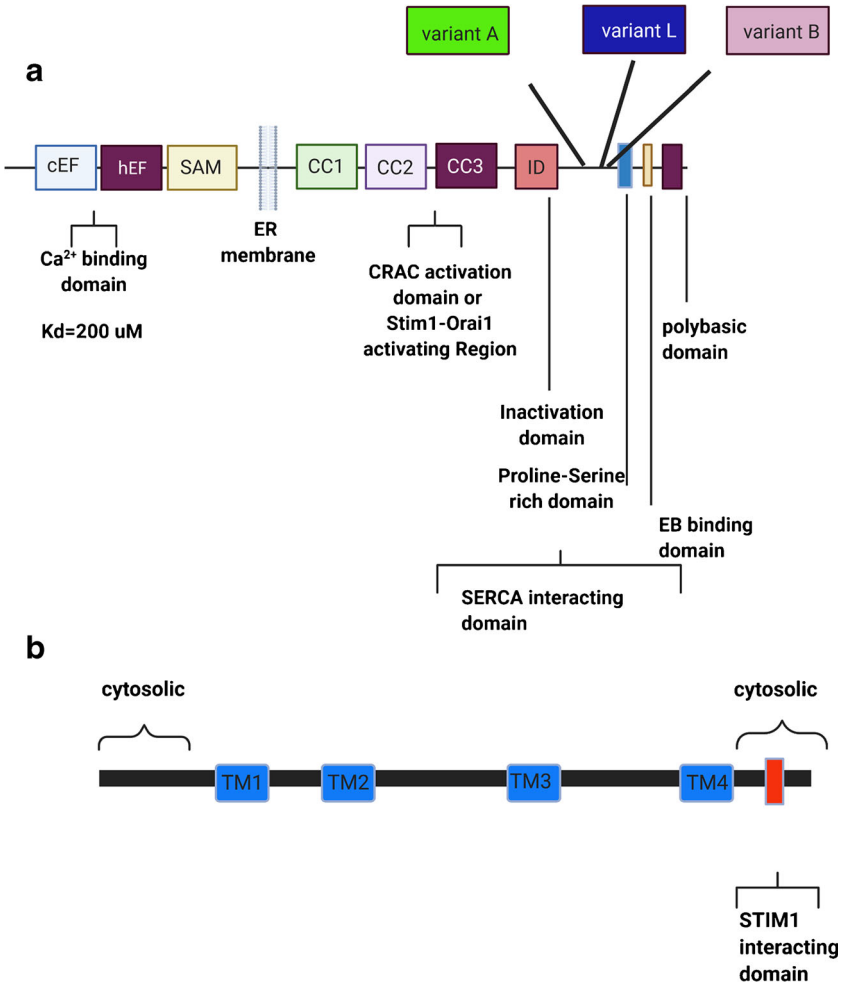

Fig. 1 Schematic of STIM1 and Orai1 identifying functional domains. (A) STIM1 is divided into luminal domain of the ER and the cytosol. To the left of the ER membrane is the calcium binding regions in STIM1. Sterile alpha motif (SAM) domains are important for STIM1 dimer formation and interact with the EF hand domains. Also depicted are STIM1 interaction domains for Orai1 (SOAR), SERCA interacting domain, and the EB1 domain. Three alternative spliced variants are depicted as A, B, and L. (B) Orai1 contains four transmembrane domains. STIM1 interacts and activates Orai1 by interacting with the C-terminal cytosolic domain

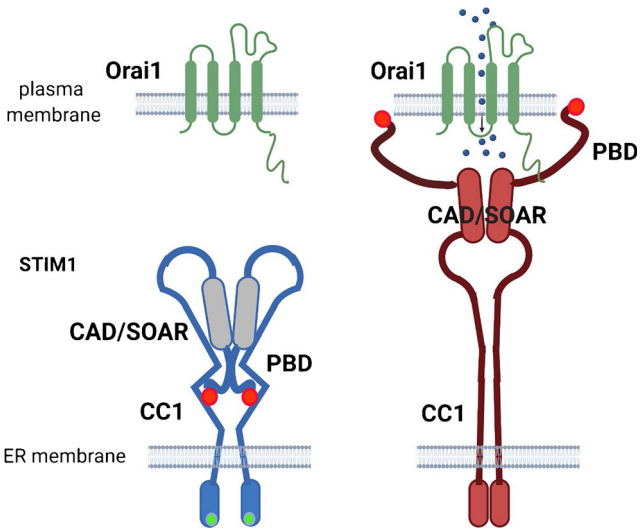

Fig. 2 Cartoon of STIM1 and Orai1 molecules in the resting and store depleted states to demonstrate domain interactions. In the resting state, STIM1 domains including CAD/SOAR and PBD domains are buried in the CC1-CC2 domains and Orai1 channels are closed. Upon depletion of ER-Ca^{2+} stores, PBD domain interacts with plasma membrane lipids and CAD/SOAR makes contact with Orai1 C-terminal domain. Orai1 channels open to create SOCE

exercise [52]. As proposed by the Protasi and Dirksen groups, STIM1-Orai1 remodel in skeletal muscle in response to fatigue during intense exercise [10]. Interestingly, T-tubules containing Orai1 were shown to undergo remodeling where the Orai1 containing TT assemble near STIM1 to augment SOCE. Given the complexity of the SR structures in striated muscle, it will be important to identify factors that maintain constitutive STIM1-Orai1 coupling and how these differ from the augmented SOCE associated with fatigue.

Interest in the role SOCE plays in the immune response peaked with the identification of STIM1 and Orai1 mutations in families suffering from severe combined immunodeficiency [24, 81, 105, 117] [30, 48]. As additional families with STIM1 or Orai1 mutations were identified, the clinical phenotype expanded to include muscle hypotonia, muscle atrophy, and weakness [23, 77]. No cardiac phenotype was described for patients harboring the loss of function mutations for STIM1 or Orai1. Notably, these patients are critically ill and require bone marrow transplantation but often succumb to overwhelming infections and sepsis, so a detailed cardiac phenotyping is often not possible [77]. However, none of the surviving patients exhibits cardiac dysfunction. Animal

models designed to address the role of STIM1 and SOCE in cardiomyocytes are therefore the principle method to define the function of SOCE in cardiac physiology. A growing consensus highlights the role of STIM1 Ca^{2+} signaling in cardiac growth and contractility as well as its participation in cardiac pacemaking and arrhythmogenesis[82, 83].

Far more patients carrying the gain of function (GOF) mutations for both STIM1 and Orai1 genes have been described. These patients present with an unusual clinical syndrome that involves tubular aggregate myopathy, hyposplenism, and platelet dysfunction as well as hypocalcemia [7–9, 20, 32, 60, 63, 65, 69, 71]. These dominant mutations occur in the hot spots for the STIM1 gene that involve the EF hands located in the S/ER lumen or in the coiled coil CC1-2 domain, both of which are required for SOCE. These patients exhibit muscle weakness with variable penetrance ranging from a severe disability to only mild exercise intolerance in adulthood. Skeletal muscle biopsies from these patients reveal specific myopathic features including aggregation of membranous material seen with Gomori staining. Immunofluorescence studies show that Orai1, calsequestrin, and SERCA pumps colocalize with STIM1 in these aggregates indicating that the aggregates contain S/ER membranes. Ultrastructural studies with transmission electron microscopy reveal large arrays of SR membrane that occupy portions of the muscle fiber and limit muscle contraction. Functional studies for SOCE in cultured myoblasts reveal augmented SOCE which is consistent with formation of preformed STIM1-Orai1 punctae, although no SOC currents have been characterized. As for the cardiac phenotype in these patients, several patients with EF-SAM GOF mutations have required implantation of permanent pacemakers for heart rhythm abnormalities, findings consistent with a role of STIM1 in cardiac pacemaking [31, 107]. While these studies bring to light the role of SOCE in cardiac and skeletal muscle physiology and offer insight to its role is disease, a greater understanding of the genetic variability of STIM1 and Orai1 will be important to understand the role SOCE plays in human cardiac physiology.

The role of STIM1 and SOCE in the heart

SOCE was first characterized in the murine embryonic cardiomyocytes (e9.0) where depletion of internal stores with CPA or thapsigargin activated Zn-inhibited Ca^{2+} entry. Intermittent SOCE activation following depletion of RYR2 Ca^{2+} stores provided Ca^{2+} needed for spontaneous Ca^{2+} oscillation [103]. Additional studies by the Marchase group demonstrated SOCE in neonatal rat cardiomyocytes and proposed that this Ca^{2+} entry pathway contributed to the activation of the calcineurin/NFAT signaling pathway that is so important in neonatal heart growth [36]. Subsequently, SOCE currents and fluorescence measurements were made in adult rat cardiomyocytes both of which resembled Orai1 currents [37]. Once STIM1 and Orai1 were identified as components of the SOCE machinery, several groups sought to determine if SOCE is operant in cells obtained from cardiac muscle of humans, mice, rates, zebrafish, and drosophila [35, 58, 99] (Table 2). Such analysis was complicated because cardiac tissue is composed of many cells including cardiomyocytes, cardiac fibroblasts, and endocardial cells, all of which express STIM and Orai proteins. Isolation of primary cells or the use of immunofluorescence of cardiac sections was required to demonstrate STIM1 and Orai1 proteins in cardiomyocytes. What has become clear from these studies is that STIM1 and Orai expression varies considerably in the heart depending on the cardiac chamber, developmental state, and even species. These differences in developmental expression were first addressed in a careful study of SOCE in neonatal and adult rat myocytes performed in the Hill lab [58]. These studies show that SOCE is robust in neonatal cardiomyocytes, occurring in 100% of cardiomyocytes. In contrast, SOCE occurs in only 10% of adult cardiomyocytes. These findings are consistent with data from our lab in which we used a LacZ reporter for the endogenous STIM1 promoter to characterize STIM1 expression during cardiac development (Fig. 3) [115, 116]. This pattern of expression for STIM1 in the heart finds that STIM1 is present early in sinus tissue that become the SAN and coronary sinus. Embryonic and neonatal expression of STIM1-LacZ can be seen at low levels in ventricular cardiomyocytes (Fig. 3A, B, and C). The pattern of STIM1-LacZ then becomes restricted in the adult heart, as seen at 3 weeks, where it is seen in the SAN, coronary sinus CMs, and subset of CMs in the ventricle and interventricular septum (Figure D, F). Why is STIM1 and SOCE so enriched in neonatal but not adult cardiomyocytes? Over the immediate days and weeks after birth, a series of events are undertaken to establish a functional Ca^{2+} store by increasing RyR2 expression, expanding the SR Ca^{2+} buffering capacity (calreticulin is replaced by calsequestrin), and increasing the Ca^{2+} SR pump activity. During fetal and neonatal cardiomyocyte growth, SR Ca^{2+} stores combine with Ca^{2+} entry to create rhythmic oscillations in Ca^{2+} that are needed for cardiac differentiation and proliferation via Ca^{2+}-dependent gene expression (e.g., calcineurin and CamK) [91]. As the T-tubule membranes invade from the sarcolemma and connect with terminal cisternae to coordinate ECC, SOCE is minimized as RyR2-cav1.2 coupling provides the Ca^{2+} transients for adult cardiomyocytes (Fig. 4).

Integration of SOCE into Ca^{2+} signaling of cardiomyocytes

Why would SOCE vary so much in cardiomyocytes? One consideration is that Ca^{2+} dynamics vary considerably among

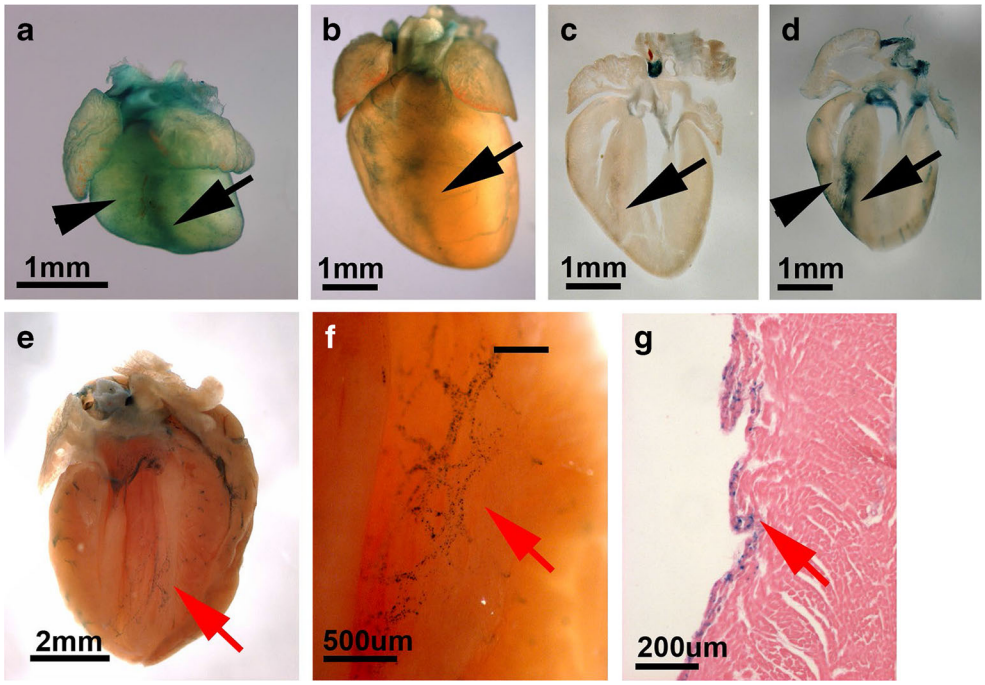

Fig. 3 STIM1 expression during cardiac development. Mice carrying a fusion construct for STIM1-LacZ were used to detect cardiac expression for STIM1. X-gal staining shows STIM1-LacZ expression in the ventricles of $STIM1^{+/gt}$ mice or $STIM1^{gt/gt}$ mice. (A) At E15.5 in whole mount $STIM1^{+/gt}$ hearts, Stim1-LacZ is widely expressed at low levels in the ventricles. (B) LacZ is present on the epicardial surface (black arrows) and developing coronaries of the heart from neonatal (P2) mice in whole mount $STIM1^{+/gt}$ hearts. (C) In 200-μm thick vibrotome sections of P2 $STIM1^{+/gt}$ hearts shows LacZ in the interventricular septum (black arrows) and (D) P2 $STIM1^{gt/gt}$ hearts STIM1-LacZ is observed in the septum (black arrows) and epicardium (black arrow heads). (E, F) Whole mount micrographs of $STIM1^{+/gt}$ hearts from adult mice with display ventricular cavity. (G) Wax sectioned $STIM1^{+/gt}$ hearts reveal STIM1-LacZ in a subpopulation of cells in the left ventricular wall and septum (red arrows)

mammalian cardiomyocytes of different species. Larger mammals including feline, human, and rabbit cardiomyocytes maintain very low resting Ca^{2+} levels that reflects the relative contributions of the SR Ca^{2+} stores and NCX1 where the SR Ca^{2+} is 2–3-fold greater than NCX1 activity [5, 44, 76]. In contrast, the SR Ca^{2+}-NCX1 relationship is different for rodent cardiomyocytes where a greater resting Ca^{2+} levels reflect SR Ca^{2+} that is tenfold greater than contributions by the NCX1 activity [5]. The impact of the variation in SOCE by species becomes clear when cardiomyocytes are stimulated at high frequency that generate repetitive Ca^{2+} transients. High-frequency stimulation of larger mammalian cardiomyocytes

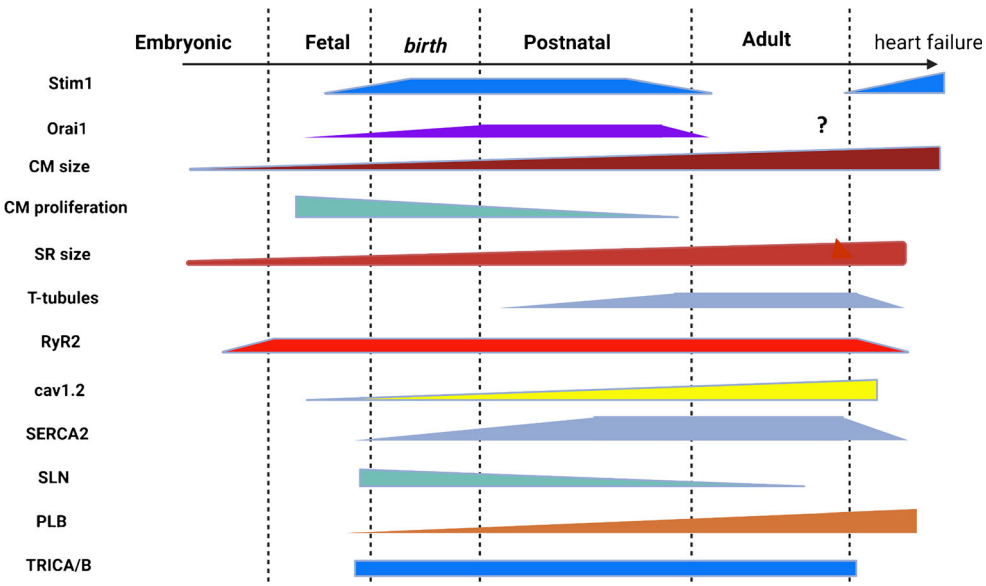

Fig. 4 Time line for STIM1 and Orai1 expression during cardiac development and heart failure. Cardiomyocyte (CM) size and proliferation expand during the neonatal period. Developmental expression pattern for key Ca^{2+} handling proteins (based on published data) culminates in the formation of the mature cardiac SR. STIM1 and Orai1 are present during neonatal phase and then exhibit a limited expression pattern. As STIM1 and Orai1 expression decays, T-tubule and cav1.2 expression becomes the dominant Ca^{2+} entry pathway for CM contraction. STIM1 and Orai1 are re expressed in cardiomyocytes during heart failure

activates refilling mechanisms and reduces Ca^{2+} extrusion resulting in greater SR Ca^{2+} load. In rodents, complex regulation of SR Ca^{2+}-NCX1 relationship and cytosolic Na levels can influence Ca^{2+} dynamics because even at sedentary conditions Ca^{2+} transients occur at high frequency. High cytosolic Na levels in rodent cardiomyocytes will limit NCX1 extrusion, lead to a reduction in fractional Ca^{2+} release, and result in a negative force-frequency. Because the SR Ca^{2+} stores are maintained in maximal state in rodent cardiomyocytes, it is possible that STIM1 and Orai1 are minimized and SOCE sidelined. When SOCE was assessed in cardiomyocytes with leaky RYR2 channels, in a mouse model of catecholaminergic ventricular tachycardia (CPVT), Orai1 channels and Ca^{2+} entry typical of localized SOCE contributed to arrhythmogenic Ca^{2+} waves. Here, leaky RYR2 stores lead to partial depletion of Ca^{2+} stores activated SOCE, which would raise resting Ca^{2+} levels. Additional factors within the the SR may influence Ca^{2+} store release and SOCE activation [104]. Recently, trimeric intracellular cation channels (TRIC) were identified as intracellular K channels located in the SR membrane of muscle that can counterbalance SR Ca^{2+} release and maintain SR membrane polarity during Ca^{2+} release. A role for TRIC-A in coordinating RyR2-Ca^{2+} oscillations involves SOCE as a mechanism. Here, SOCE was robust in myocytes of TRIC-$A^{-/-}$ mice implicating SOCE in the establishment of SR Ca^{2+} load [121]. Interestingly, TRICA/$B^{-/-}$ mice die from embryonic heart failure that involves diminished RYR2 Ca^{2+} stores [120]. The muscle phenotype of the TRIC-$A^{-/-}$ mice resembles the Ca^{2+} defects described for mSTIM1$^{-/-}$ muscle fibers. Ca^{2+} transients are not sustained when STIM1 KO fibers are stimulated at high frequency and result in poor muscle performance, fatigue, and reduced contractility [52]. It is clear from the emerging evidence from several groups that SOCE operates in a complex electrophysiological landscape and a great deal of work will be required to determine how SOCE contribute to the Ca^{2+} signaling of cardiomyocytes.

Properties of SOC currents

Because adult cardiomyocytes express such low levels of STIM1 and Orai1, it is important to establish whether the Ca^{2+} entry activated by store depletion shares features typical for a SOC current. Orai channels have very well-defined features; therefore, validating the SOCE assays with whole cell current measurements has been important to distinguish the Ca^{2+} entry from another SOC like channel. In addition to store dependence, Orai channels display specific features including (1) a very positive reversal potential characteristic of a highly selective Ca^{2+} current, (2) Ca^{2+}-dependent inactivation, (3) monovalence in divalent free conditions, and (4) specific pharmacology (see below) [6]. Efforts to record the currents from cardiomyocytes require inactivation of Na, K, and cav1.2 channels using specific solutions, pharmacologic inhibitors, and current clamp configurations. To date, traditional Orai currents that meet all of these criteria have only been resolved in SA nodal cells [116] and RV cardiomyocytes [85] but have not been in ventricular cardiomyocytes [116], coronary sinus CM [115], or rat pressure overloaded ventricular CM [35]. Cardiomyocytes do exhibit a store depletion–activated current but the classical features of Orai currents such as inward rectification, reversal potential that indicate calcium selectivity, are not characteristics of this current [119]. Rather the thapsigargin induces a non-selective current might represent another STIM1 gated channels such as transient receptor potential channel (TRPC, TRPV, or TRPM) or a STIM1 target protein such as a transporter or exchanger [45]. It will be important to identify the precise molecular entity that underlies the store operated Ca^{2+} entry in adult cardiomyocytes. Any future therapeutic strategy designed around SOCE in cardiomyocytes would require a higher level of confidence that Orai channels are the pore for SOCE.

STIM alternative splicing and cardiac expression

The importance of alternative splice variants for STIM1 has emerged recently as a way to regulate SOCE at the tissue level (Table 1). At least four spliced variants are known to exist for STIM1 (STIM1-S, STIM1-L, and STIM1-A) and there are two alternative translational start sites. STIM1-L results from a slicing event between exon 12 and 13 that affects STIM1 domain structure by adding a 106-amino acid peptide containing an actin binding domain into the C-terminal end of STIM1 [18, 62, 88]. Spliced variants differ in their capacity for STIM1-oligomerization and Orai1-trapping in specialized regions of the plasma membrane and gating kinetics of SOCE as reported in striated muscle (Fig. 1A). STIM1-S and STIM1-L variants have been described in neonatal cardiomyocytes whereas adult myocytes only express STIM1-S. STIM1-L-Orai1 interaction has only been identified in adult cardiomyocytes subjected to pressure overload; the significance of this complex is unknown in neonatal cardiomyocytes [58]. STIM1-L can facilitate rapid SOCE in association with G-protein coupled receptor activated Ca^{2+} entry [88]. Alternatively STIM1-L may activate TRPC channels; a class of non-selective cation channels found to be regulated by STIM1 as SOC channels [34]. A third splice variant (STIM1-A) was recently identified for STIM1: an insertion of novel exon 11 that creates a new domain in the cytosolic portion of STIM1. This variant is highly expressed in the testes, astrocytes, and the heart where it accounts for more than 60% of total STIM1 compared with only 10% in skeletal muscle. Far less is known about STIM1-B [43]. There are several interesting differences between STIM1-A and

Table 1 Alternative splice variants in STIM1, tissue distribution, and putative function

Isoform	Tissue distribution	Putative effect on SOCE
STIM1-A	Heart, testes	Dominant negative effect on SOCE
STIM1-B	Unknown	Unknown
STIM1-S	Ubiquitous	Activates Orai channels
STIM1-L	Striated muscle	Reduces SOCE latency, activates TRPC channels

STIM1-S including its localization to adherens junctions, the cellular domain structure critical for cardiomyocyte mechanotransduction. Functionally this spliced variant was found to limit SOC current in a dominant negative manner where it can compete with STIM1-S and STIM-L. STIM1-A includes a 30 amino acid peptide inserted after the CAD domain that might alter STIM1 CAD domain interaction with Orai channels. Interestingly, predictive algorithms for alternative splicing identify as many as 20 alternative transcripts for STIM1. STIM2 is a second member of the Ca^{2+} sensor family and may regulate basal Ca^{2+} levels [56]. STIM2 is also subject to alternative splicing and at least three spliced variants have been described [62]. STIM2.2 is the dominant variant expressed and activates Orai1 channels. STIM2.1 results from an insertion of eight amino acids into the CAD domain of STIM2. The STIM2.1 variants can disrupt the STIM2-Orai1 interaction while retaining STIM oligomerization [96]. As a result, the STIM2.2 can inhibit Orai1 Ca^{2+} currents, reduce SOCE, and prevent calcineurin-dependent NFAT translocation into the nucleus [62]. STIM2.3 contains an extended C' terminus beyond STIM2.2 but not much else is known about this isoform [62]. The presence of different STIM species with distinct actions on SOCE adds to the complexity of SOCE regulation and raises the possibility of an array of STIM proteins that might contribute to SOCE in different cell types *a*nd other functions assigned to STIM1 that might not involve SOCE. Given the controversies surrounding SOCE in cardiac muscle, it may be important to better define the pattern of expression and function of all spliced variants of STIM in cardiac cells.

Animal models for SOCE in the heart

Global knockout models for STIM1 and Orai1 are lethal during the first month of life because of profound muscle weakness and limited muscle growth [52, 98]. STIM1/Orai1 KO mice exhibit several phenotypes including defects in immune responsiveness, skeletal muscle hypotonia, and bone and enamelization defects [48]. In order to target STIM1 deletion in cardiomyocytes, mice carrying flox alleles for the STIM1 gene are crossed with transgenic mice carrying either constitutive Cre recombinase or the Tamoxifen inducible Cre, both under the control of the myosin heavy chain promoter [116]. These models delete STIM1 early in cardiac development or in adults following Tamoxifen administration and phenotypes include SAN dysfunction, dilated cardiomyopathy, and both atrial and ventricular arrhythmias (Table 2). Mice from these models, including our own (Fig. 5), exhibit sudden cardiac death that occurs later in adult life, around 35 to 50 weeks of life [17, 35, 102, 106]. Mice exhibit progressive decline in heart rates and LV function starting around 20 weeks of life. Collins and colleagues in a series of studies provided a thorough molecular characterization of the cardiomyopathy and showed that these mice exhibit unchecked ER stress resulting in marked impairment glucose metabolism and mitochondrial remodeling [14–16]. In addition, STIM1 cardiac specific knockout mice exhibit adverse remodeling of the myofibrillar apparatus that contribute to reduced systolic function [73]. Electrical instability including ventricular arrhythmias has also been described in STIM1 KO mice [12]. Collectively these studies implicate STIM1 in cardiac pacemaking, interatrial conduction, and arrhythmogenesis and show how STIM1 expression in specific subtypes of cardiomyocytes at specific developmental periods can influence cardiac electrophysiology. Additional evidence for a functional role of STIM1 and SOCE in cardiomyocytes can be gleamed from gain of function studies in humans and mice. Some of the patients with STIM1 mutations (gain of function) exhibit a cardiac phenotype that ranges from conduction disease requiring a pacemaker to left ventricular hypertrophy. While the details of the how these mutations cause cardiac disease are unknown, transgenic mice overexpressing STIM1 specifically in the heart or in cardiomyocytes [17, 119] exhibit augmented Ca^{2+} stores that trigger spontaneous Ca^{2+} sparks and therefore serve as arrhythmic substrates.

Cellular localization of STIM1 and Orai1

Excitation contraction coupling in the cardiomyocytes occurs at the triad, the intracellular location where the RyR2 channels located in the terminal cisternae of the SR abuts cav1.2 channels located in the t-tubule membranes; these local membrane domains create the large, synchronized Ca^{2+} transients required for myocyte contraction. Understanding how STIM1 and Orai1 integrate into the architecture of these membrane domains will provide mechanistic insight into the fundamental

Table 2 Cardiac phenotype for animal models with altered STIM1 and Orai1 function

Species	Design	Phenotype	Reference
Rat	Gene silencing STIM1 and Orai1	Blunted cardiac growth	[106]
Rat and mouse	Adenoassociated virus delivery of shRNA for STIM1	Cardiac atrophy SOC currents	[4, 35]
Mouse	Tamoxifen inducible cardiac restricted gene knockout	Cardiac atrophy Impaired response to cardiac hypertrophy	[73]
Mouse	Cardiac restricted gene knockout	Sinoatrial node dysfunction PLB-STIM1 regulation of SR Ca2+ release	[116, 119]
Mouse	STIM1-S transgenic mouse line	Cardiac failure Mitochondrial abnormalities	[17]
Feline	Gene silencing	Reduced SR Ca^{2+} stores and widened action potential duration	[102]
Rodent	Gene silencing and overexpression	Blunted hypertrophic response to neurohormonal agonists.	[58]
Mouse	Cardiac restricted STIM1 deletion	Late inset LV dysfunction ER stress	[14, 16]
Drosophila	Gene deletion	Dilated sarciomyocytes Myofibril disorganization	

properties of SOCE in cardiomyocytes (Fig. 6). However, it has been very difficult to localize Orai channels in cardiac membranes or measure its Ca^{2+} current given its small unitary conductance. STIM1 is present in the longitudinal SR at the Z-lines, a sub-compartment of the S/ER. STIM1 in this locale is believed to sense the need to refill the local S/ER stores and trigger Orai1-dependent Ca^{2+} entry. STIM1 is also detected in the outer membranes of the nucleus and in the nuclear invaginations called nucleoplasmic reticulum [51] as well as the adherens junction [11]. Establishing the function of STIM1 and Orai1 at these different localizations is needed to better understand how SOCE contributes to cardiomyocyte signaling. Data from our lab demonstrates STIM1 in the SR at the I-band near the Z-line of SAN cells and Orai1 is detected in the sarcolemma membranes [116]. Store depletion or rhythmic oscillations of Ca^{2+} do not induce redistribution of STIM1 to the sarcolemma membrane. We continue to explore these different domains of STIM1 and to determine if they are functionally related and involve Orai1-Ca^{2+} entry or contribute to cell processes in different ways.

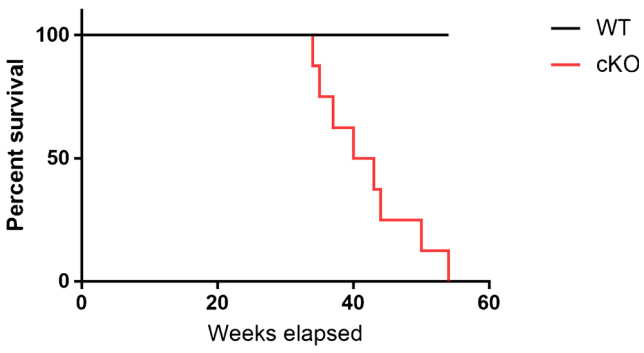

Fig. 5 Kaplan-Meyer curve of survival for WT (n=8) and cardiac specific STIM1 knockout (n=18) mice. Cohorts of mice from both genotypes were followed for 1 year. Fifty percent mortality was observed at 40 weeks for the cSTIM1 KO mice

STIM1 signaling independent of Orai1

STIM1 is a multifunctional signaling protein in the SR membrane that can interact with many channels, transporters, and signaling molecules [45, 114]. STIM1 binding partners include EB1, STING, POST, nuclear transporters (importin and exportin), TRPC, PMCA, phospholambdan, and SERCAs [28, 40, 97, 113, 119] (Fig. 6). How STIM1 regulates the function of these targets remains unclear but is believed to be independent of Orai channels. For example, STIM1 has previously been shown to interact with and attenuate the voltage gated Ca^{2+} channel cav1.2 [109] (Fig. 6A). Although this interaction was mapped out in neurons and smooth muscle cells, it has been difficult to demonstrate the relevance of this interaction in STIM1 KO mice [41, 72, 110]. In fact, L-type voltage currents appear to be unchanged in cardiomyocytes as well skeletal and smooth muscle from STIM1 KO mice [102]. It is interesting to note that the cav1.2 blocker amlodipine was recently shown to activate STIM1 proteins and induce SOCE in smooth muscle cells via a mechanism that is independent of cav1.2. Here amlodipine was able to activate STIM1 directly and induce the shift in phenotype for vascular smooth muscle cells from the contractile to synthetic. Given the widespread use of these agents, it will be important understand the full clinical implications of this interaction [39]. Similar Orai1 agonists have been identified and can also influence vascular smooth muscle function [2].

STIM and SERCA pumps

Proteomic studies have found that both STIM1 and STIM2 proteins interact with SERCA pumps in skeletal muscle as

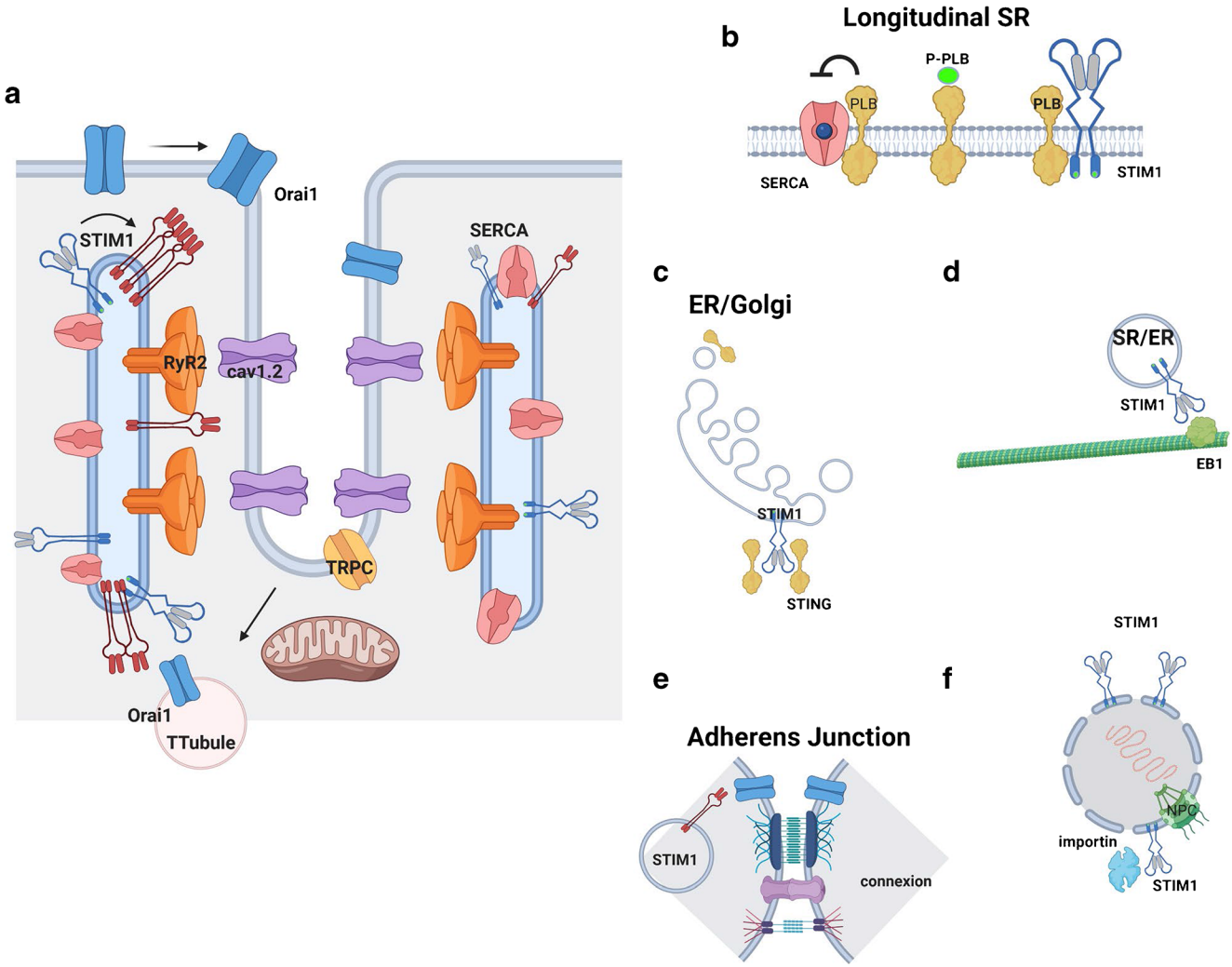

Fig. 6 STIM1 localizes in the cardiac SR/ER membrane to different regions to interact with different cellular targets. (A) Cardiomyocyte (CM) membrane structures include the tubule membrane, a radial projection of the sarcolemma membrane, and the sarcoplasmic reticular (SR) membranes, the large Ca^{2+} storage organelle. Key molecules involved in Ca^{2+} handling include the ryanodine receptor (RYR2) and SR Ca^{2+} pump (SERCA2a). Components of SOCE are depicted in specific membranes and include Orai1 channels and STIM1. (B) Alternative STIM1 target proteins included phospholambdan (PLB) and the SERCA2a in the longitudinal SR membrane. (C) STIM1 interacts with stimulator of interferon genes (STING) in the ER membrane. Dissociation of STIM1 from STING permits STING to traffic to the golgi to activate IFN1 signaling. (D) STIM1 in the ER membrane interacts with the tip attachment protein (TAC) by an interaction with Microtubule Associated Protein (also known as EB1). (E) STIM1 and Orai1 channels localize to the adherens junctions of CMs. Depicted are gap junctions (purple), desmosomes (blue), and tight junctions (red). (F) STIM1 localizes to the nuclear membrane where it interacts with importin, a protein involved in cytosolic-nuclear translocation

well as non-excitable cells (Fig. 6A and B) [25, 45, 50]. In non-excitable cells, STIM1 knockdown cells refill ER stores through microdomains involving SERCA pumps, mitochondria, and Orai1 channels [1]. Our recent work has shed light onto the mechanism by which STIM1 regulates SERCA function [90, 119]. We first described a developmentally controlled relationship between STIM1 and sarcolipin (SLN), an endogenous inhibitor of SERCA. In embryonic muscle, STIM1 exhibits low levels of expression while SLN expression is robust in an effort to keep SR Ca^{2+} stores low. However, STIM1 is robustly expressed while SLN expression is low and is only detected in atrial cells and slow twitch muscle fibers. These results suggest that the primary role for STIM1 in cardiomyocytes is to establish a functional SR Ca^{2+} store in neonatal mice which may not be required in adult cells. To better understand the function of STIM1, we utilized a screen of a phage display library to identify novel STIM1 interacting partners. Phospholamban (PLN), a micropeptide that regulates the SERCA pump, was identified to interact directly with STIM1 using this cell free assay (Fig. 6B) [83, 119]. PLN in its monomeric, dephosphorylated form can bind SERCA pumps and inhibit the ATPase activity and thereby decrease Ca^{2+} store refilling. Adrenergic stimulation of the cardiac membranes leads to inhibition of PLN interaction with

SERCA and the associated decrease in store filling. To place the STIM1-PLN interaction into physiologic context, gain and loss of function studies were used to test the idea that Ca^{2+} load in the cardiomyocyte is determined by a relationship involving STIM1-PLN and SERCA2a. STIM1 was shown to sequester PLN from SERCA2a and thereby enhance SR Ca^{2+} loading in the cardiomyocyte under homeostatic conditions. This mechanism may explain observations from several groups in which overexpression of STIM1 in cardiomyocytes leads to greater spark frequency in cardiomyocytes. Our data also provides context for the low levels of STIM1 expression in resting adult cardiomyocytes. Here, STIM1 assembles in large macromolecular complexes at the Z-line where it can influence Ca^{2+} pumping and promote store refilling via greater SERCA2 efficiency. STIM1 can also interact with SERCA pumps and influence S/ER Ca^{2+} loading [40, 57, 87]. In many cell types, STIM1 may influence SERCA activity to shape the dynamics of Ca^{2+} signaling. In fact, a common thread in many studies that evaluate models of STIM1 overexpression in cardiomyocytes is that increased levels of STIM1 lead to greater Ca^{2+} store release which results in cell damage and apoptosis [17, 102]. Importantly, deletion of STIM1 from the cardiomyocytes was not associated with a change in SR Ca^{2+} content, whereas SAN cells from cardiac restricted STIM1 KO mice displayed a reduction in SR Ca^{2+} stores. It is likely that deletion of STIM1 from the cardiomyocyte may lead to a compensatory effect for other components of the Ca^{2+} transient.

STIM1 and stimulator of interferon genes

STIM1 activates SOCE in immune cells to upregulate cytokine production via calcineurin-NFAT signaling and loss of SOCE results in immunodeficiency. STIM1 also seems to coordinate a second component of the immune response involving innate immunity and the type I interferon (IFN1) response [97]. STIM1 interacts with stimulator of interferon genes (STING), an adaptor protein located in the ER that is central to IFN1 response. Cells lacking STIM1 exhibit a dramatic upregulation of IFN1 resulting from constitutive movement of STING to the golgi apparatus from the ER (Fig. 6C). SOCE does not appear to influence the protein-protein interaction and is independent of STIM1-Orai1 interaction. This interaction likely accounts for the autoimmune phenomenon described in patients lacking STIM1 as well as participation in the response to viral infections [97]. STING has been implicated in cardiac hypertrophy and might be activated by release of mitochondria or nuclear dsDNA fragments released in the sick cardiomyocytes. There is evidence that STING is important in the remodeling of the heart during sepsis where systemic inflammation results in cardiac dysfunction [53]. It will be important to understand if STIM1 restrains STING in the cardiomyocyte and limits the effects of STING on mitophagy. Certainly, the significance of cardiac inflammation has being recognized in many disease states including cardiac hypertrophy, myocarditis, and infarction. The relevance of the putative pathway may have critical importance in the COVID-19 pandemic where IFN1 may influence the cardiovascular outcomes during infection which certainly involves the cardiac manifestations of the infection [55]. Additional work will be need to understand the significance of this STIM1, STING, and IFN1 in cardiomyocyte remodeling.

STIM1 and microtubules

The migration of STIM1 in the ER to the plasma membrane has been recognized for sometime as a key step in SOCE: STIM1 resides in the perinuclear ER, is attached to microtubules, and then is delivered to specialized regions of plasma membrane to activate SOCE. STIM1 requires a direct protein-protein interaction via end-binding protein 1 (EB1 proteins) to attach to microtubules (Fig. 6D). STIM1–EB1 interact when ER Ca^{2+} stores are full and STIM1 in ER tubules aggregate at the tip of the microtubules that appear as STIM1-comets. Ca^{2+} store depletion then stops STIM1-comets, dissociate STIM1-EB1, and create STIM1 punctae near Orai1 channels in the cell membrane. Importantly STIM1, via interaction with EB1, remodels the SR into tubules rather then sheets, a process that is independent of SOCE. We also note that EB1 has been shown to guide gap junctions (connexins) from the interior of the cardiomyocyte to the adherns junctions, where STIM1 is also present (Fig. 6E). Gap junctions promote electrical coupling between cardiomyocytes and therefore contributes to excitability. Whether a similar role for EB1-STIM1 exists in cardiomyocytes is not known but STIM1 has been shown to be targeted the adherens junction to activate local SOCE. More generally, microtubules maintain cardiomyocyte structural integrity and fidelity of the myofibril assembly. STIM1 attached to microtubules can would link the SR Ca^{2+} stores to the microtubules. Whether the SR-microtubule connection is important structural support of the myocyte is not known. But, $STIM1^{-/-}$ cardiomyocytes from several different models (mice and flies) exhibit myofibril derangement supporting the hypothesis that deletion of STIM1 might disrupt the microtubular lattice and destabilize myofibril integrity [73, 75].

The role of STIM1 and SOCE in cardiac hypertrophy

Increased wall stress on cardiomyocytes requires an adaptive response to normalize the stress or the heart fails. Cardiomyocytes respond to pressure overload, a cause of wall stress, by activating the fetal gene program that leads to

cardiac hypertrophy. Over the last decade, a number of studies tested the idea that STIM1 and Orai are activated during pressure overload as part of the fetal gene program and thereby introduce a deleterious source of Ca^{2+} entry [35, 73, 102, 106, 123]. A common theme present in all of these studies is that STIM1 in cardiomyocytes influences signal transduction pathways associated with cardiac growth. In fact, it is widely accepted that hypertrophic agonists such as pressure overload, catecholamine excess, and TGF-β signaling activate STIM1 Ca^{2+} signaling. Eliminating STIM1 by different techniques (genetic deletion or haploinsufficiency, viral delivery of silencing contracts, or pharmacological inhibition) alters the activation state of pathways governed by calcineurin, a Ca^{2+}-calmodulin regulated serine-threonine phosphatase, and the calmodulin-dependent kinase CamK [35, 58]. Here transcriptional regulation of nuclear factor activated T-cells (NFAT) family can change the expression profiles for genes associated with cardiac growth and hypertrophy. In our own work, 8-week-old male WT and cSTIM1 KO mice were subjected to pressure overload by transaortic constriction. Our studies show that cardiac hypertrophy, assessed as cardiac mass/tibial length, occurred at the same rate for mice of both genotypes indicating hearts underwent hypertrophy to the same degree (Fig. 7A). However, comparison of the effects of pressure overload after 8 weeks on LV systolic function revealed a significant deterioration in the fractional shortening for the cSTIM1 KO compared to WT littermates (Fig. 7B). We note that cSTIM1 KO subjected to TAC had much reduced heart rates, owing to SAN dysfunction, compared to the WT mice after 8 weeks of pressure overload. It is possible that cardiac stress and decompensated LV function results from altered conduction, SAN dysfunction, and arrhythmias.

Work from the Hulot lab has demonstrated that reduction of STIM1 using a cardiotropic adeno-associated virus influenced the response to pressure overload by reducing calcineurin and CamK activity [35]. In a follow-up study, they examined the long-term influence of STIM1 on cardiac function of juvenile and adult mice and found that long-term silencing of STIM1 led to cardiac atrophy as evidenced by the reduced cardiomyocyte size and cardiac function. An interesting observation from this study is that cardiac dilation and impaired left ventricular (LV) function accompanied the reduced myocyte size [4]. Mechanistically, these studies implicate STIM1 in cell growth pathways through links to AKT signaling and show that STIM1 can regulate mTORC2 complex or by influencing the unfolded protein response linked to ER stress [14]. Studies from Mancarella's group used an inducible Cre-lox system to delete STIM1 from the mouse. Here tamoxifen administered to adult mice activated Cre recombinase to delete STIM1 gene from cardiomyocytes. Again, STIM1 deletion for a duration of 40 days led to a dramatic reduction in LV function but not a clear difference in cardiac hypertrophy. These studies therefore suggest that reduction of STIM1 decouples the effects of cardiac hypertrophic growth and LV decompensation [73]. It is well established that LV dysfunction is accompanied by elevated wall stress and increased intracardiac pressures that signal cardiac hypertrophy both in parallel and in series. That each of these models of STIM1 deletion results in LV impairment in the absence of an increase heart weight suggests that the LV dysfunction is related to differences in heart rate variability, ventricular dyschonrony, or capillary rarefraction. These findings are in keeping with limited expression of STIM1 in cardiomyocytes of adult mice.

The contributions of Orai1 channels to the hypertrophic response have been evaluated in two recent studies using different genetic and pharmacologic approaches. Expression of a dominant negative Orai1 transgene in the adult heart using an inducible genetic system demonstrates that blocking Orai1 channels preserves LV function and limits cardiac hypertrophy following pressure overload. These authors also employed novel Orai1 inhibitor JPIII to test whether pharmacologic blockade of Orai1 would be cardioprotective in preventing heart failure from pressure overload [3]. These careful studies offer strong support to the notion that Orai1 blockers might be effective in heart failure. In contrast, the

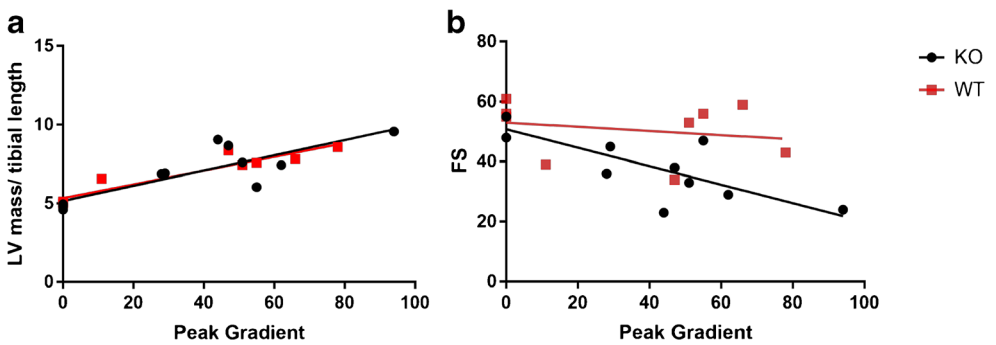

Fig. 7 Cardiac response to pressure overload in the cSTIM1 KO mice is abnormal. (A) Mice (MHC-Cre, *n*=5, STIM1f/f *n*=8, and cSTIM1 KO, *n*=8) were anesthetized and subjected to transverse aortic constriction (TAC) or a sham operation (*n*=3 for each genotype). After 8 weeks, echocardiograms and pressure gradients were determined for these mice. Data are plotted to demonstrate the relationship of change in LV hypertrophy based on extent of pressure overload (A). Similarly, changes in LV systolic function were related to pressure overload (B)

Londono group presented disparate results using a slightly different approach to blunt Orai1 current in the cardiomyocytes. Using an inducible cardiac restricted Cre-lox P system to delete Orai1 from the adult heart, the authors show that angiotensin-induced cardiac stress was much worse in the cardiac specific Orai1 knockout mice. While baseline hemodynamic parameters were normal for the cardiac Orai1 KO, these mice exhibited greater deterioration in cardiac function following weeks of angiotensin infusion as a form of cardiac stress [89]. It is not clear why these different approaches to blunt Orai1 currents in cardiomyocytes were met with opposite results. Further work is needed to know how these pathophysiologic conditions influence properties of the Orai1 currents including Ca^{2+}-dependent inactivation.

The role of SOCE in cardiac pacemaking in SAN cells

Unique among cardiomyocytes, SAN cardiomyocytes (SANC) utilize Ca^{2+} to control the heart rate as opposed to less generating force. Here, rhythmic and spontaneous Ca^{2+} release defines the Ca^{2+} clock as described by the Lakatta lab [49]. The Ca^{2+} clock works in parallel with the membrane clock where oscillations in membrane potential can trigger diastolic depolarization via HCN4 channels activation [54, 64]. Because the expression of STIM1 and Orai1 was so robust in the SAN cells, we considered that SOCE might be an important component of both the membrane and Ca^{2+} clocks during cardiac pacemaking (Fig. 3). Notably, Orai1 currents were detected in SAN cells and displayed many of the features typically described for Orai1 channels including inward rectification, Ca^{2+} selectivity (markedly positive reversal potential), and inhibition by Orai1 blockers. We found that STIM1 and Orai1 punctae are pre-formed in SAN cardiomyocytes. Store depletion did not rearrange STIM1 as is described for many non-excitable cells. Deletion of STIM1 from SAN cardiomyocytes resulted in lower resting Ca^{2+} levels and reduced SR Ca^{2+} stores. The cardiac specific STIM1 KO mice exhibit spontaneous shift from superior and inferior poles within the pacemakers and exaggerated slowing of the heart rate after sustained stimulation. These studies are evidence that STIM1 and SOCE are required for basal pacemaking (Fig. 8). We also found substantial changes to key components of the Ca^{2+} clock in cSTIM1 KO SAN cardiomyocytes. For example, currents attributed to NCX1 and cav1.2 were altered in response to the loss of SOCE and are proposed as compensatory changes to currents in the absence of SOCE. We hypothesize that Orai1-mediated Ca^{2+} entry acts a counter current for the potassium currents during phase 3 of the action potential. Phase 3 is notable for repolarization of the AP for the SAN and coincides with the emptying of SR stores (Fig. 8). Crosstalk between SOCE and these other currents provide the basis of the idea that STIM1 integrates the membrane and Ca^{2+} clock systems for cardiac pacemaking. Further work is needed to understand whether STIM1's role in pacemaking is similar to its role in electrically stimulated muscle fibers where it prevents fatigue. Is replenishment of SR Ca^{2+} stores the main function of SOCE or are there other cell functions that are store operated in these SANC? How does the loss of STIM1 and SOCE influence the threshold for atrial arrhythmias, what are the signaling pathways activated by SOCE that contribute to Ca^{2+} clock and link to the membrane clock.

The role of STIM1 and SOCE in cardiac arrhythmias

Action potentials emerge from the SAN and propagate through the atria to activate the AV node during regular cardiac rhythm (sinus rhythm). Existence of three intra-atrial conduction pathways (superior, septal, and coronary sinus) in the heart has been identified for decades by cardiac electrophysiologists, yet few details about these pathways are known and the characteristics of these conduction cells remain poorly described. Recently we showed that STIM1 expression is conserved in the cardiomyocytes that ensheath the coronary sinus [115]. The CS-CM have SOCE and Isoc currents can be recorded from these cells. We hypothesized that STIM1 CS-CM might have a role in interatrial impulse conduction. In fact, we show that the deletion of STIM1 from these cells leads to slowed conduction, particularly following cholinergic stimulation. When these mice are subjected to rapid atrial pacing, atrial fibrillation was readily induced in the STIM1 KO cells. It is likely that slowed conduction through this pathway acts as a substrate for reentry and a mechanism for the arrhythmia. Taken alongside recent description of STIM1 and SOCE as a mechanism for the ventricular arrhythmias, it is likely that a primary function of STIM1 in these cardiomyocytes is to link dynamic Ca^{2+} signaling with membrane potential changes occurring with action potentials [12, 119].

Pharmacology of SOC channels

Tremendous interest exists in the identification of Orai blockers for the treatment of many inflammatory conditions as well as cardiac diseases [38]. Screens for lead compounds have used interesting assays including high throughput Ca^{2+} influx, NFAT translocation assays, and even protein-protein interaction assays. There are four broad categories of SOC inhibitors including heavy metals (e.g., lanthanides), antibodies, aptamers, and pyrazole derivative. For cardiomyocytes, studies have utilized lanthanides and pyrazole compounds as blockers of SOCE [35]. Lanthanides are the earliest Orai

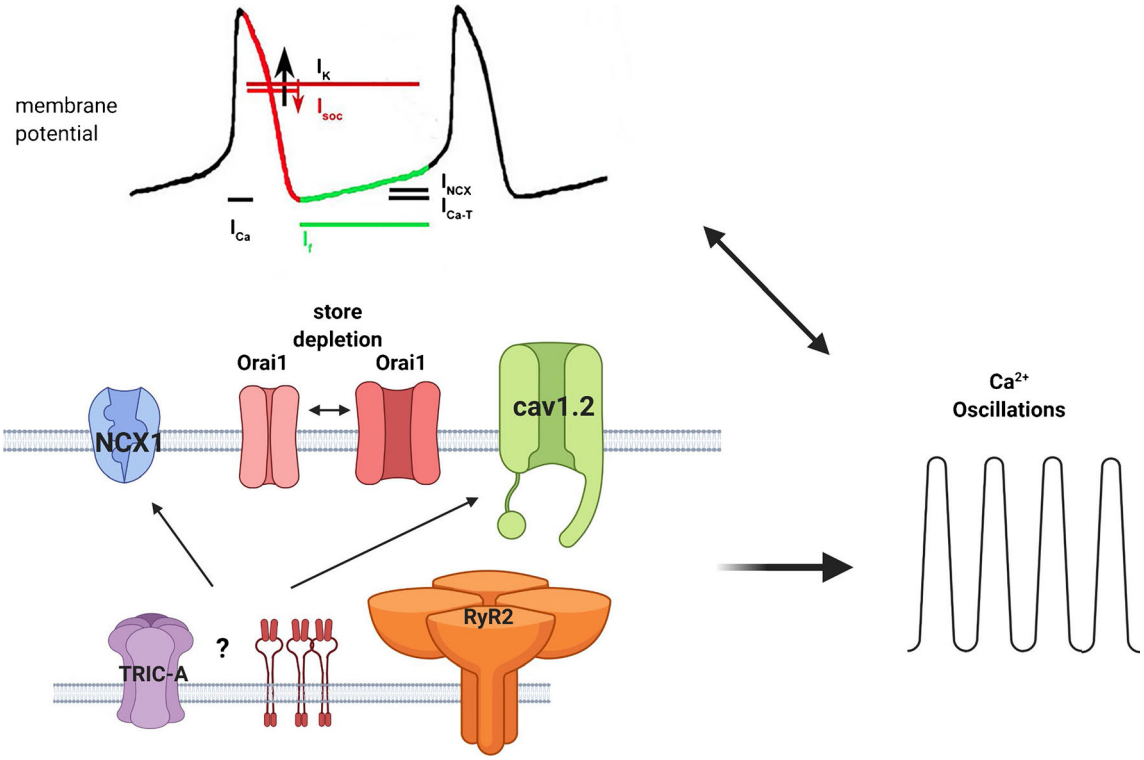

Fig. 8 STIM1 and SOCE regulate sinus node function. A model of action potentials from the SAN. Relevant currents that coordinate diastolic depolarization (green) and repolarization (red). Speculative model for the role of STIM1 in the SAN to regulate Orai1 directly and cav1.2 and NCX1 indirectly. Because SOC current is an inward Ca^{2+} current activated by depletion of SR Ca^{2+} stores, it serves to link the membrane and Ca^{2+} clocks to coordinate diastolic depolarization. STIM1 may integrate RyR2 Ca^{2+} release, TRIC-A signaling to replete Ca^{2+} stores for repetitive Ca^{2+} oscillations

inhibitors but are not very specific as they can block TRPC channels and L-type Ca^{2+} channels [42, 101]. Monclonal antibodies directed against Orai channels might be an attractive therapeutic strategy but Bis(triflourmethyl)pyrozoles were identified by their ability to block NFAT nuclear translocation following T-cell activation [70, 124]. BTP-2 can block SOC channels experimentally and is offered as evidence for Orai1 channel involvement in action potential changes and SOCE [84, 99, 102]. Because of several challenges with BTP-2, caution must be applied to interpreting the experiments as proof of Orai channel involvement. First, BTP-2 must be applied extracellularly in order to block SOC currents, indicating that the drug does not alter intracellular events associated with SOCE activation including STIM1 oligomerization or STIM1-Orai1 interaction [38, 93]. Secondly, specificity of BTP-2 for SOCE is not good as BTP-2 can activate TRPM4 channels [33, 95]. Here, TRPM4 channels, which confer Ca^{2+} activated non-selective currents, can depolarize the cell membrane and thereby limit Ca^{2+} entry. Given that TRPM4 channels are robustly expressed in cardiomyocytes and underlie human cardiac phenotypes ranging from conduction abnormalities to cardiac hypertrophy [33, 46], it would be important to further understand the mechanism of action for BTP-2 in cardiac electrophysiology [112]. Another pharmacologic agent that blocks SOCE with high potency in cardiomyocytes is 2-aminoethyldiphenyl borate (2-ABP) [74, 108]. A curious aspect of 2-ABP is the ability to activate Orai3 channels, independent of STIM1, or store depletion. Orai3 channels are found only in mammals and have been implicated in cardiac hypertrophy based on their expression profile by several investigative groups [86, 118]. Agents that block Orai currents have been identified and are currently in clinical studies for the treatment of acute pancreatitis and covid19 pneumonia. Whether these agents can influence the trajectory of cardiac failure and arrhythmias is not known.

Conclusion

For decades, cardiac Ca^{2+} signaling was viewed from a relatively simplistic perspective that revolved around the release and resequestration of Ca^{2+} by the cav1.2-RyR2-SERCA2a network in order to maintain excitation contraction coupling. However, correction of impaired Ca^{2+} handling in the sick and failing cardiomyocytes has been met with only modest therapeutic success [27, 61]. Given the paucity of new therapies for heart failure, the emergence of SOCE in the cardiomyocyte and the upregulation during cardiac stress is an exciting development in the field and requires more research to refine our understanding of how this pathway influences cardiac

physiology. In particular, STIM1 and/or SOCE might represent a novel targets in clinical situations including arrhythmogenesis, cardiac hypertrophy and failure, and sinoatrial node function.

Abbreviations Ca^{2+}, Calcium; *SAN,* Sinoatrial node; *CPA,* Cyclopiazonic acid; $[Ca^{2+}]_i$, Cytoplasmic calcium concentration; *GoF,* Gain of function; *VCM,* Ventricular cardiomyocytes; *LoF,* Loss of function; *ROCCs,* Receptor-operated calcium channels; *S/ER,* Sarco(endo)plasmic reticulum; *SCID,* Severe-combined immunodeficiency; *STIM1,* Stromal interaction molecule 1; *SOCCs,* Store-operated calcium channels; *SOCE,* Store-operated calcium entry; *TG,* Thapsigargin; *VSM,* Vascular smooth muscle; *VOCCs,* Voltage-operated calcium channels

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Declarations

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