Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

Calcium Signaling in Cardiomyocyte Function

Guillaume Gilbert, Kateryna Demydenko, Eef Dries, Rosa Doñate Puertas, Xin Jin, Karin Sipido, and H. Llewelyn Roderick

Laboratory of Experimental Cardiology, Department of Cardiovascular Sciences, KU Leuven, BE3000 Leuven, Belgium

Correspondence: llewelyn.roderick@kuleuven.be

Rhythmic increases in intracellular Ca^{2+} concentration underlie the contractile function of the heart. These heart muscle-wide changes in intracellular Ca^{2+} are induced and coordinated by electrical depolarization of the cardiomyocyte sarcolemma by the action potential. Originating at the sinoatrial node, conduction of this electrical signal throughout the heart ensures synchronization of individual myocytes into an effective cardiac pump. Ca^{2+} signaling pathways also regulate gene expression and cardiomyocyte growth during development and in pathology. These fundamental roles of Ca^{2+} in the heart are illustrated by the prevalence of altered Ca^{2+} homeostasis in cardiovascular diseases. Indeed, heart failure (an inability of the heart to support hemodynamic needs), rhythmic disturbances, and inappropriate cardiac growth all share an involvement of altered Ca^{2+} handling. The prevalence of these pathologies, contributing to a third of all deaths in the developed world as well as to substantial morbidity makes understanding the mechanisms of Ca^{2+} handling and dysregulation in cardiomyocytes of great importance.

THE PHYSIOLOGY OF CALCIUM SIGNALING IN CARDIOMYOCYTES

The Heart and Circulation

The pump function of the heart is mediated by the synchronous contraction of its constituent muscle cells, the cardiomyocytes. Cardiomyocyte contraction is triggered by an electrical stimulus, the action potential (AP), which induces transient depolarization of the cardiomyocyte plasma membrane (the sarcolemma). The AP is generated by specialized cells known as pacemaker cells located at the sinoatrial node (SAN), which set the frequency of contraction of the heart. This electrical signal propagates through specialized conduction pathways and via myocyte-to-myocyte transfer through gap junctions, across the atria until it reaches the atrioventricular node (Rohr 2004). After a short delay to allow atrial systole, the impulse propagates through the ventricles via the bundle of His and Purkinje fibers to induce ventricular systole (Stephenson et al. 2012). Opening and closing of valves further defines the filling and ejection of blood from the chambers. Eventually, blood is propelled from the left ventricle into the aorta to the systemic circulation and from the right ventricle into the pulmonary artery to the lungs. The AP is a transient phenomenon and repolarization of the cardiomyocytes is associated with relaxation, again in a sequential manner for each chamber of the heart. This relaxation phase (diastole) is important for the filling of the chambers, and is an essential part

Editors: Geert Bultynck, Martin D. Bootman, Michael J. Berridge, and Grace E. Stutzmann
Additional Perspectives on Calcium Signaling available at www.cshperspectives.org

 doi: 10.1101/cshperspect.a035428
Cite this article as *Cold Spring Harb Perspect Biol* 2020;12:a035428

of the cardiac cycle (Fukuta and Little 2008). This highly coordinated contraction and relaxation of the four chambers of the heart is necessary for optimal support of the circulation and ensures a supply of nutrients and signals throughout the body.

ECC and Ca^{2+}: Generation of the Ca^{2+} Transient and Induction of Contraction

The mechanism by which the AP is transduced into the contractile response is termed "excitation–contraction coupling" (ECC) (Fozzard 1977; Bers 2002; Fearnley et al. 2011; Eisner 2014). Changes in intracellular Ca^{2+} concentration are central to this mechanism and couple (within a few msec) membrane depolarization with the engagement of the actomyosin cytoskeleton (via the energy of ATP hydrolysis) and thus sarcomere contraction (Fig. 1). During diastole (when the heart is relaxed), the cells are in their most extended state; their membrane potential (i.e., difference between inside and out) lies between −70 mV and −90 mV for atrial and ventricular cardiomyocytes and −60 mV for nodal cells. This negative resting membrane potential is close to the equilibrium potential for K^+ channels, which are the dominant conductance (through Kir2.x channels) at rest (Miake et al. 2003; Zobel et al. 2003; Cordeiro et al. 2015). The negative equilibrium potential results from the large K^+ gradient across the sarcolemma. This ionic gradient is generated and maintained by the Na^+K^+ATPase pumping three Na^+ ions out of the cell in exchange for two K^+ ions (Glitsch 1979; Gadsby 1984; Shattock et al. 2015). This pump also maintains the Na^+ gradient across the cell membrane, compensating for the Na^+ influx during the upstroke of the AP. Arrival of the AP results in an increase in membrane potential (depolarization) to the activation threshold of sodium channels (Na_V; pore-forming subunit $Na_V1.5$), −40 mV, and induces a rapid influx of Na^+ (Fig. 1(1)). This increase in intracellular Na^+ further depolarizes the cell, to an extent that the threshold for activation of voltage-gated L-type Ca^{2+} channels (LTCCs) is crossed (comprising its pore-forming subunit, $Ca_V1.2$). The activation of these channels and ensuing Ca^{2+} influx underlies the plateau phase of the AP (Fig. 1(2)). Although the magnitude of this Ca^{2+} influx is not by itself sufficient to induce effective contraction, it represents a key triggering step in ECC. Specifically, Ca^{2+} entering the cell is amplified by Ca^{2+} release from the sarcoplasmic reticulum (SR) Ca^{2+} store through a mechanism called Ca^{2+}-induced Ca^{2+} release (CICR) (Fabiato and Fabiato 1977; Fabiato 1983; Roderick et al. 2003). Through this mechanism, the Ca^{2+} entry signal arising via LTCC stimulates the opening of ryanodine receptor Ca^{2+} channels (RyR; type 2 isoform) located on the SR (Cannell et al. 1987; Beuckelmann and Wier 1988; Stern 1992; Wang et al. 2001). The RyR channel is a multimeric protein complex that is comprised of an RyR tetramer as well as a number of accessory proteins that contribute to modulating its activity under basal conditions as well as in response to physiological agonists and altering cell conditions. One of the first such proteins to be described is the archetypal Ca^{2+} sensor CaM, which acts to inhibit the channel activity (Xu and Meissner 2004). An interaction with the immunophilin FKBP12.6 is also well established. Although this interaction was at one time considered dynamic (Wehrens et al. 2003), evidence now supports a model in which the interaction is highly stable and serves to maintain the closed state of RyRs (Guo et al. 2010). Other interacting proteins include protein phosphatases and kinases that may act to couple kinase signaling cascades with RyR function (Zalk et al. 2007). In the SR, Ca^{2+} is primarily stored bound to the low-affinity Ca^{2+}-binding protein calsequestrin (Cala et al. 1990). Notably, calsequestrin associates with the RyR through the proteins junctin and triadin (Györke et al. 2004). This efficient coupling between plasmalemma Ca^{2+} influx and Ca^{2+} release from the SR is ensured through a close apposition (10–15 nm) of the associated channels (LTCC and RyR2) and their host membranes in a confined space called the dyadic cleft (Fig. 1(3); Hayashi et al. 2009; Scriven et al. 2010; Pinali et al. 2013). This architecture ensures that RyRs are exposed to high levels of Ca^{2+} entering the cardiomyocyte during the AP, which are necessary for their opening and subsequent Ca^{2+} release.

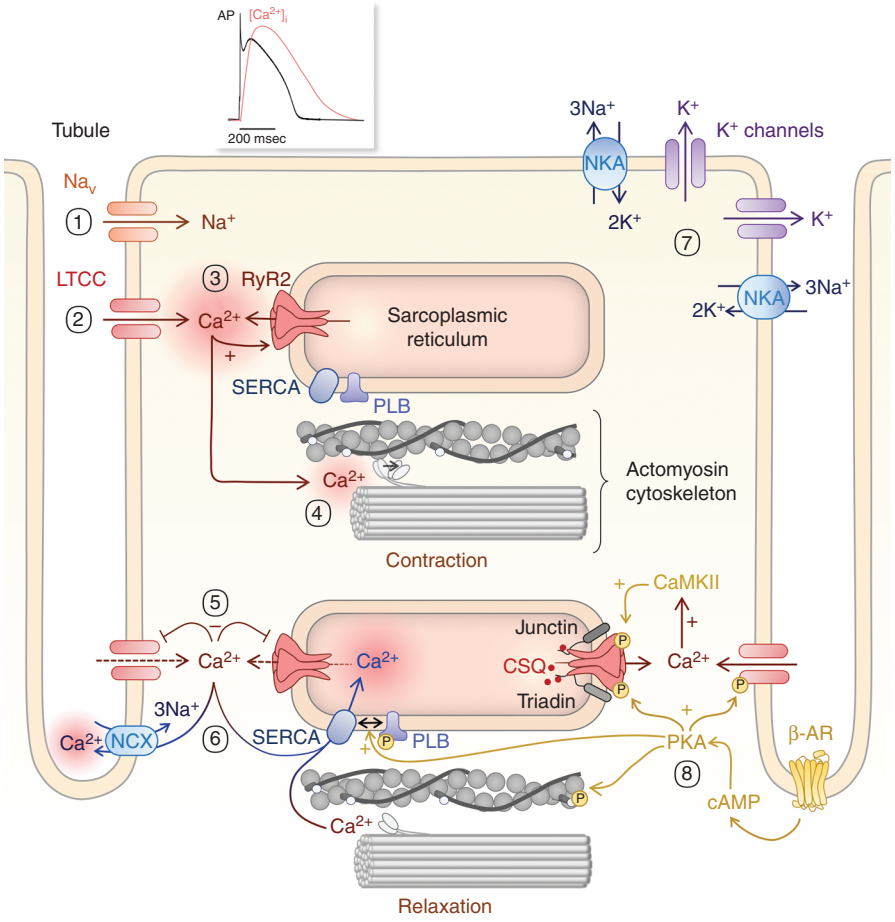

Figure 1. Excitation–contraction coupling (ECC). (1) An action potential depolarizes the cardiomyocyte and induces Na^+ influx through voltage-gated Na^+ channels (Na_v). (2) This further depolarizes the cell membrane and induces Ca^{2+} influx through voltage-gated L-type Ca^{2+} channels (LTCCs). (3) This Ca^{2+} entry stimulates Ca^{2+} release via dyadic RyR2 on the SR (4), which in turn triggers cell contraction through activating myofilament crossbridges. (5) LTCC inactivate and RyR close. (6) Cytosolic Ca^{2+} is then moved out of the cell by the Na^+/Ca^{2+} exchanger (NCX) and pumped back into the SR by SERCA2a, thereby decreasing cytosolic Ca^{2+} concentration and bringing about relaxation. (7) A family of K^+ channels participate in cell repolarization with K^+ efflux as a last step for returning membrane potential to its resting value before a new cycle starts. (8) Tuning ECC to meet cardiovascular demands involves β-adrenergic pathways that induce the activation of CaMKII and of cAMP/PKA, which phosphorylates voltage-gated LTCCs and RyRs to enhance their activity and phospholamban (PLB) to remove its inhibition of SERCA activity.

However, these couplons do not provide a sufficient Ca^{2+} influx to overcome the high-buffering capacity of the cardiomyocyte cytosol to induce RyR-mediated Ca^{2+} release throughout the volume of large ventricular cardiomyocytes (150 µm long × 20 µm deep on average) and induce contraction (Hüser et al. 1996; Smyrnias et al. 2010). To overcome this problem, and convey the AP to the entire cell volume, ventricular cardiomyocytes are endowed with a highly elaborate network of membrane tubules extending from invaginations of the sarcolemma (Smyrnias et al. 2010; Scardigli et al. 2018a). This network comprises transverse tubules (T-tubules [TTs]), which are distributed along the Z-lines of the sarcomere and axial

tubules that are arranged along the long axis of the cardiomyocyte (together, the transverse and axial tubule system [TATS]). Further, TATS brings the sarcolemma and SR and its associated LTCCs and RyRs, respectively, into close proximity, forming couplons to generate a homogenous cell-wide Ca^{2+} transient (Scriven et al. 2010; Pinali et al. 2013). At this location, RyRs are organized in clusters, thereby optimizing release at each site (Walker et al. 2014; Shen et al. 2019). Although clusters of more than 100 RyRs have been reported, recent superresolution imaging experiments describe average cluster sizes ranging between 9 and 23 RyRs (Jayasinghe et al. 2018b; Shen et al. 2019). The localization of dyads to the Z-line of the sarcomere ensures exposure of contractile machinery to maximal Ca^{2+} elevation and flux (Fig. 1(4)). In atrial cardiomyocytes or ventricular cardiomyocytes from fetal and neonatal animals (Mackenzie et al. 2004; Zima and Blatter 2004; Bootman et al. 2006), TATS are however either absent or at a very low density (Brandenburg et al. 2016). In these cells, Ca^{2+} entry across the sarcolemma and its coupling with subsarcolemmal RyRs is sufficient to support their relatively lower contraction. Through the action of a number of players, such as kinases (e.g., calmodulin kinase II [CaMKII] and protein kinase A [PKA]), phosphatases, and reactive/nitric oxygen species, Ca^{2+} release in the dyad is regulated by many signal transduction cascades (for review, see Zalk et al. 2007).

Cardiomyocyte Relaxation and Ca^{2+} Clearance

Depending on species, size of animal, and level of activity, the heart beats between 1 and 12 times per second. Thus, subsequent to systole and to allow the next round of contraction to occur, efficient mechanisms are required to bring about relaxation (diastole), and allow refilling of the chambers with blood. An initial step in this relaxation phase is the termination of mechanisms responsible for raising cytosolic Ca^{2+}. RyR2 release channels behave stochastically and their opening terminates rapidly through a combination of attrition, store depletion, regulation of their gating by luminal Ca^{2+}, and inactivation at the cytosolic side (Cannell and Kong 2017). LTCC also rapidly inactivates by a Ca^{2+}- and voltage-dependent mechanism (Cens et al. 2006; Tadross et al. 2008). Repolarization of the cell membrane potential is the result of an outward current of K^+ via different voltage-activated K^+ channels (I_{Kr}, I_{Ks}) and, finally, opening of K^+ channels that maintain the resting membrane potential (I_{K1}-Kir2.1, 2.2, and 2.3) (Fig. 1(6); Cordeiro et al. 2015). Overall, these channel activities determine the time course and phases of the AP (Carmeliet 1999; Chiamvimonvat et al. 2017). It is also important to note that it is not possible to trigger a new AP during the plateau phase of the ventricular cardiomyocyte AP. This refractory period protects the heart from prematurely unwanted contractions (Burton and Cobbe 2001).

Active mechanisms substantially contribute to return intracellular cytosolic Ca^{2+} concentration to its resting levels and to bring about relaxation. These mechanisms also serve to replenish intracellular Ca^{2+} stores to prepare the cell for its next contraction. The primary contributor to cytosolic Ca^{2+} clearance as well as SR refilling is the sarco/endoplasmic reticulum Ca^{2+} ATPases (SERCA) pump. The SERCA 2a isoform is primarily expressed in the heart where it is localized to the SR membrane proximal to RyR2 and the contractile machinery (Dally et al. 2010). Subsequent to its Ca^{2+}-dependent activation ($K_{1/2}$ 0.4 μM) (Lytton et al. 1992), via ATP hydrolysis, SERCA actively pumps Ca^{2+} from the cytosol into the SR (Fig. 1(5)). Ca^{2+} is also extruded out of the cell by a Na^+/Ca^{2+} exchanger (NCX) in its forward mode to exchange one Ca^{2+} out of the cell and three Na^+ into the cell (Fig. 1(5); Shattock et al. 2015). Although expressed in cardiomyocytes, the plasma membrane Ca^{2+} ATPase does not make a major contribution to Ca^{2+} extrusion during ECC (Hammes et al. 1998; Bers 2002). Although SERCA dominates in cytosolic Ca^{2+} clearance, its contribution varies according to species ranging between 70% and 90% of all Ca^{2+} removed from the cytosol (Bassani et al. 1994; Piacentino et al. 2003). The remainder is predominantly via NCX. In steady state, extrusion via NCX equals influx via LTCC and Ca^{2+} release and reuptake into the

SR are in equilibrium at a set level of Ca^{2+} in the store (Eisner 2014). Dysregulation of any of these transport mechanisms will lead to a new equilibrium, with a new filling level of the store (e.g., an increase in intracellular Na^+ will raise the Ca^{2+} store filling by reducing NCX-mediated Ca^{2+} extrusion) (Eisner 2014).

Tuning ECC to Modulate Cardiac Function

Heart function is dynamically regulated to meet the changing needs of the organism during alterations in the body activity, by the autonomic nervous system (Lymperopoulos et al. 2013; Eisner 2014; Wang et al. 2018). For example, during the fight-or-flight response, cardiomyocytes show inotropic (increased force of contraction) and lusitropic (increased relaxation) responses (Lymperopoulos et al. 2013). An increase in heart rate is also observed, although this is mediated by an effect on the spontaneous generation of AP at the SAN. This fight-or-flight response is primarily mediated by the adrenergic system and its hormone mediators noradrenaline and adrenaline. In altering cardiomyocyte function, the latter acts through β-adrenergic receptors (primarily β1) expressed on the cardiomyocyte plasma membrane. β-Adrenergic receptors are 7-transmembrane G-protein-coupled receptors (GPCRs) that couple via the stimulatory G-protein (Gs) to activate adenylate cyclases and increase levels of the intracellular messenger cyclic AMP (cAMP) (Wang et al. 2018). Increased cAMP activates protein kinase A (PKA), which then phosphorylates multiple targets involved in ECC to augment cardiomyocyte contraction, and in turn cardiac function. Indeed, PKA phosphorylation targets each of the key functional components of ECC. Specifically, PKA phosphorylation of LTCC results in increased Ca^{2+} current, and phosphorylation of RyR leads to greater sensitivity to Ca^{2+} (Valdivia et al. 1995; van der Heyden et al. 2005). PKA also phosphorylates myofilament proteins (troponin C, myosin-binding protein C [MyBPC] and troponin I), most notably troponin I, decreasing the affinity of the myofilaments for Ca^{2+} and thereby enhancing crossbridge cycling (Fig. 1 (8); Metzger and Westfall 2004). In bringing about the effects of PKA, and modulation of Ca^{2+} handling, phospholamban (PLB) is an important target. PLB is a small 5 kDa protein that interacts with SERCA, inhibiting its activity. On phosphorylation by PKA, PLB dissociates from SERCA resulting in its disinhibition (Fig. 1; for review, see MacLennan and Kranias 2003). As well as bringing about the cardiomyocyte lusitropic response via more rapid clearance of cytosolic Ca^{2+}, enhancing of SERCA activity results in an elevated SR Ca^{2+} load. The latter in turn increases Ca^{2+} availability in the SR for release, also sensitizing the open probability of RyRs. Many of the proteins targeted by PKA, most notably RyR and PLB, are also phosphorylated during β-adrenergic stimulation by CaMKII with similar consequences for cardiomyocyte function (Ullrich et al. 2012; Grimm et al. 2015; Uchinoumi et al. 2016; Potenza et al. 2018). Recently, a number of new micropeptide regulators of SERCA such as DWORF have been described (Nelson et al. 2016). Although these proteins are not modulated by PKA, their interaction with SERCA appears to act in competition with PLB. A direct regulation of SERCA by a long noncoding RNA has also been reported (Vervliet et al. 2018; Zhang et al. 2018). β-Adrenergic stimulation also shortens the AP duration by enhancing K^+ channel activity (Hegyi et al. 2018). Together, these combined actions of β-adrenergic stimulation facilitates the increased heart rate and cardiac output required under times of stress.

To balance the sympathetic system in the heart, the parasympathetic system with its principal mediator acetylcholine (ACh) lower the heart rate. ACh acts on the M2 muscarinic GPCRs within the heart. On activation of M2 ACh receptors $G_{\beta\gamma}$ subunits are liberated, and through activation of GIRK channels (also known as IK_{ACh}) result in a more negative resting membrane potential and slower heart rate (for review, see Saw et al. 2018).

Multiple circulating hormones act on cardiomyocyte activity to modify contractility, and in so doing, couple other organ systems and cell types with cardiomyocyte function (Drawnel et al. 2013; Mayourian et al. 2018; Smyrnias et al. 2018). Those modulations act through

altered Ca^{2+} signals as well as through changes in myofilament response to Ca^{2+} (Kobayashi and Solaro 2005). Notable cardioactive hormones include angiotensin II, which is produced in the kidney, endothelin-1, which is released locally and systemically by the endothelium, and by ATP. These hormones/mediators are also produced by cardiomyocytes themselves, thus eliciting an autocrine action (Dostal and Baker 1999; Drawnel et al. 2013). Of these hormones, many engage GPCRs, but in contrast to the β-adrenergic receptors, act via $G_{\alpha q}$ to promote phospholipase C–dependent hydrolysis of phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-trisphosphate ($InsP_3$) and diacylglycerol (DAG). DAG acts through protein kinase C (PKC) and $InsP_3$ acts on $InsP_3$ receptors ($InsP_3R$) to modify ECC (Kockskämper et al. 2008b; Drawnel et al. 2013; Smyrnias et al. 2018). In particular, PKC phosphorylates myofilament proteins to alter contractility, and on the Na^+/H^+ exchanger to modify intracellular pH and Na^+ (Russell and Molenaar 2000). In so doing, myofilament sensitivity to Ca^{2+} is altered and Ca^{2+} balance further modulated through NCX consequent to changes in Na^+ (Despa and Bers 2013; Garciarena et al. 2013). The role of $InsP_3$ in regulation of ECC is discussed below.

Membrane Tubules and Microdomain Signaling in Cardiomyocytes

Cell-wide increases in Ca^{2+} and efficient contraction rely on TATS in adult ventricular cardiomyocytes (Heinzel et al. 2002, 2011; Smyrnias et al. 2010; Crocini et al. 2014, 2016). Owing to its essential role in ECC, the complexity of TATS has gained great interest over recent years, with detailed knowledge arising from the advances in superresolution and electron microscopical imaging technologies that are able to visualize TATS in all their detail (Ferrantini et al. 2013; Soeller and Baddeley 2013; Crocini et al. 2014; Crossman et al. 2017). The density of TATS varies substantially through development, across the chambers of the heart, between species and during pathology (Song et al. 2006; Jayasinghe et al. 2012; Frisk et al. 2016; Manfra et al. 2017; Jones et al. 2018). During early postnatal development, the organization of TATS in rat cardiomyocytes is completed by 10–12 days postbirth (Ziman et al. 2010), although at this stage, very few LTCCs lie in close proximity to RyRs (Franzini-Armstrong et al. 2005). With development, the colocalization of LTCC with RyR increases (Sedarat et al. 2000), constructing the mature couplon in the dyad to enable efficient CICR. The structure and organization of TATS shows species-dependent differences, with mice having narrower tubules (~0.17 μm) (Kong et al. 2017) compared with humans, pigs, and rats (~0.25 μm) (Soeller and Cannell 1999) or rabbits (~0.45 μm) (Savio-Galimberti et al. 2008; Kong et al. 2017; Rog-Zielinska et al. 2018). TATS are also more densely distributed in small rodents compared with large mammals including human, with TT flanking each sarcomere (Fig. 2; Manfra et al. 2017). The density of TATS in ventricular cardiomyocytes seems to associate with a high heart rate and the need for a very fast contraction and relaxation (e.g., in mice with a heart rate of 600 bpm). In atrial cardiomyocytes, TATS have been generally considered to be absent or rudimentary in nature. Recent data, however, shows that the nature of TATS in atrial cardiomyocytes is more diverse, with observed differences in right versus left atria, and between species. This diversity is possibly associated with different atrial workloads and cardiomyocyte width (Frisk et al. 2014; Brandenburg et al. 2016, 2018; Gadeberg et al. 2016; Denham et al. 2018). High-resolution microscopy studies in small and large mammals showed distinct populations of atrial cardiomyocytes that possess different configurations of TATS (i.e., organized, disorganized, and empty cells) (Frisk et al. 2014; Glukhov et al. 2015; Yue et al. 2017).

Structural differences in TATS have an impact on cardiomyocyte physiology and function. With a high TATS density, the majority of RyRs reside within couplons (Fig. 2A; Louch et al. 2006; Scriven et al. 2010; Wong et al. 2013). In this situation, for example in small rodents including mice and rats, AP-depolarization and RyR-mediated Ca^{2+} release show high-temporal fidelity. However, a low TATS density (e.g., humans and pigs) results in a significant number of

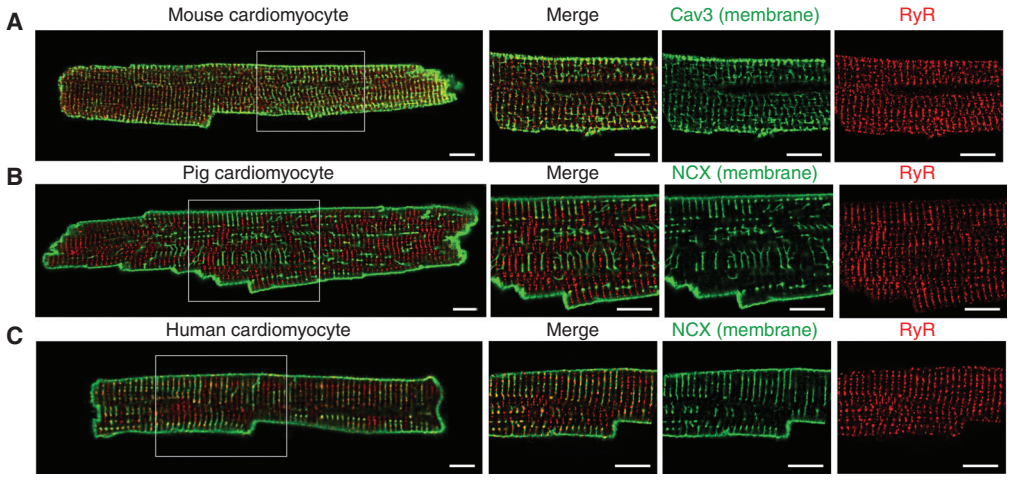

Figure 2. Difference in the TATS in isolated cardiomyocytes from mouse (*A*), pig (*B*), and human (*C*). The TATS is stained in green (Caveolin-3 [Cav3] or NCX) and RyRs are stained in red. Scale bar, 10 µm. Images were acquired with a Nikon A1R confocal microscope using a 60× oil immersion objective. A4× zoom of the white square is shown (unpubl.).

RyR that lie outside of couplons/dyad cleft (i.e., noncoupled) and are therefore likely not exposed to the same Ca^{2+} concentration directly following the AP (Fig. 2B,C; Heinzel et al. 2002; Louch et al. 2004; Dries et al. 2013). As a consequence, this fraction of RyRs is activated with a delay, resulting in inhomogeneous Ca^{2+} release during a cell transient/contraction (Heinzel et al. 2002). This role of TATS in Ca^{2+} transient generation is further illustrated in cells in which they are normally absent or sparse, such as atrial cells (Bootman et al. 2011; Brandenburg et al. 2016). On electrical stimulation, these cells show an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) that initially occurs at the cell periphery and, which then propagates to the center of the cell via a combination of CICR and Ca^{2+} diffusion (Mackenzie et al. 2004; Sheehan et al. 2006). Conversely, disruption of TATS in ventricular cells by osmotic shock or through maintenance in culture for 6 days leads to inhomogeneous Ca^{2+} release during transients (Lipp et al. 1996; Brette et al. 2005; Smyrnias et al. 2010). The efficiency of CICR is similarly low in ventricular cardiomyocytes isolated from neonatal hearts, which show little evidence of TATS (Haddock et al. 1999). The mechanisms underlying the expression and maintenance of TATS is not yet fully resolved. Titin cap protein (telethonin), junctophilin 2, and the Bin/Amphiphysin/Rvs domain protein amphiphysin II (AmpII or BIN-1) have all been shown, however, to modulate the biogenesis and maintenance of TATS (Chen et al. 2013; Ibrahim et al. 2013; Reynolds et al. 2013; Hong et al. 2014; Fu and Hong 2016).

NON-CANONICAL Ca^{2+} SIGNAL GENERATION IN THE HEART

In addition to the Ca^{2+} signaling mechanisms underlying ECC (described above), other Ca^{2+} transport pathways are expressed in the heart contributing to a more complex picture of the role of Ca^{2+} in cardiomyocyte function. These additional Ca^{2+} signaling pathways interact with the canonical ECC signaling Ca^{2+} mechanism to selectively and simultaneously control ECC as well as other cellular processes (Fig. 3).

$InsP_3$ and $InsP_3$ Receptors

Inositol 1,4,5-trisphosphate receptor ($InsP_3R$) intracellular Ca^{2+} release channels are expressed in cardiomyocytes (Lipp et al. 2000; Kockskämper et al. 2008b; Garcia et al. 2017). Although all

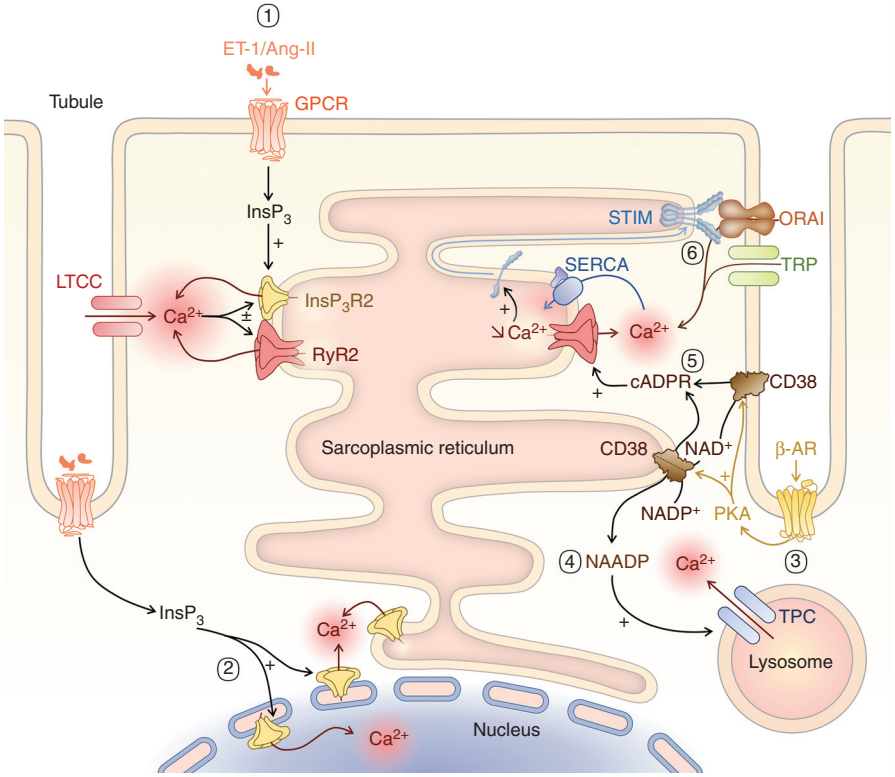

Figure 3. Noncanonical Ca^{2+} channels. (1) G-protein-coupled receptors activated by endothelin-1 (ET-1) or angiotensin-II (Ang-II), produce $InsP_3$ that activates Ca^{2+} release via $InsP_3$ receptors (IP_3RII), which cross talks with RyR2 to modify Ca^{2+}-induced Ca^{2+} release (CICR). (2) Ca^{2+} release from $InsP_3R2$ present at the perinuclear SR and the nuclear envelope stimulates nuclear Ca^{2+} signaling pathways. (3) β-Adrenergic receptor (β-AR) activation leads to the activation of protein kinase A (PKA), which in turn enhances the activity of the CD38 enzyme. (4) CD38 produces nicotinic acid adenine dinucleotide phosphate (NAADP) that promotes Ca^{2+} leak via endolysosomal two-pore channels (TPCs). (5) Activation of CD38 also generates cyclic ADP-ribose (cADPR), which acts on the RyR2 to enhance its activity. (6) To refill the SR as well as affect cytosolic Ca^{2+} levels, the STIM/ORAI/TRP system may be engaged.

three isoforms of $InsP_3Rs$ have been identified in the heart, the type 2 $InsP_3R$ is predominant (Perez et al. 1997; Lipp et al. 2000). $InsP_3R$ expression is required in cardiac development and contributes to heart function during the embryonic and neonatal stages (Rosemblit et al. 1999; Sasse et al. 2007; Kockskämper et al. 2008b). $InsP_3R$ expression level declines during postnatal heart maturation, as the number of RyRs increases. Ca^{2+} release via $InsP_3R$ is initiated by $InsP_3$ generated downstream to the activation of $G_{\alpha q}$-coupled GPCR signaling cascades, for example, by endothelin-1 and angiotensin II (Fig. 3; Berridge 1993). As for all $InsP_3R$ isoforms, $InsP_3R2$ activity shows a bell-shaped dependency for Ca^{2+}, with high activity occurring at Ca^{2+} levels present at diastole (Foskett et al. 2007). Perhaps making this isoform most appropriate for its function in the heart, it shows the highest sensitivity to $InsP_3$ (Fig. 3(1); Mak and Foskett 2015; Vervloessem et al. 2015).

$InsP_3R$ expression varies substantially across the chambers of the heart, with ~10-fold higher expression in the atria than ventricle (Lipp et al. 2000). Greater expression levels are also observed in cardiomyocytes of the SAN (Kapoor et al. 2015) and the Purkinje conduction fibers (Gorza et al. 1993; Hirose et al. 2008) in which

ECC is not the primary function. In the adult ventricle, $InsP_3R$ is reported to be expressed at a substantially lower level than RyRs (10- to 50-fold) (Moschella and Marks 1993) and owing to this lower expression, as well their lower conductance compared with RyRs, have not been considered to play a substantial role in regulation of ECC in this heart region. Despite this low expression, roles for $InsP_3R$ in regulation of ECC, hypertrophic gene expression, Purkinje fiber function, and SAN activity are reported (Domeier et al. 2008; Hirose et al. 2008; Higazi et al. 2009; Nakayama et al. 2010; Kapoor et al. 2015). The ability of $InsP_3Rs$ to influence cardiomyocyte function despite low expression is made possible through their localization to functionally relevant cellular subcompartments and local signaling events (Fig. 3(2); Wu et al. 2006; Higazi et al. 2009; Ljubojevic et al. 2014; Hohendanner et al. 2015a; Wullschleger et al. 2017). In atrial and ventricular cardiomyocytes, $InsP_3Rs$ are colocalized with RyRs in the subsarcolemmal space or at dyads, respectively (Lipp et al. 2000; Harzheim et al. 2009). At these locations, Ca^{2+} released via $InsP_3Rs$ modulates ECC by bringing proximal RyRs closer to the threshold for activation (Harzheim et al. 2009; Horn et al. 2013; Hohendanner et al. 2015b; Wullschleger et al. 2017) leading to sensitization of Ca^{2+} release during the transient. Depending on the model studied, this channel cross talk induces SR Ca^{2+} leak and elicits inotropic and/or pro-arrhythmic effects (Mackenzie et al. 2002; Zima and Blatter 2004; Proven et al. 2006; Bootman et al. 2007; Domeier et al. 2008; Kockskämper et al. 2008a; Namekata et al. 2008; Signore et al. 2013; Blanch and Egger 2018). Although positive inotropic effects of $InsP_3Rs$ are observed in atrial cardiomyocytes, the impact of $InsP_3$ signaling on contractility in ventricular cardiomyocytes is less obvious with effects on cellular automaticity more often reported (Harzheim et al. 2009; Blanch and Egger 2018).

Two-Pore Channels and Nicotinic Acid Adenine Dinucleotide Phosphate

Nicotinic acid adenine dinucleotide phosphate (NAADP) is an intracellular signaling messenger that stimulates Ca^{2+} release via transient receptor potential (TRP) channels and two-pore channels (TPCs) from the endo/lysosome (Calcraft et al. 2009; Pitt et al. 2010; Grimm et al. 2012). NAADP is a potent second messenger, which acts at nanomolar levels to trigger micromolar release of Ca^{2+} from TPCs. Several lines of evidence support a role for NAADP signaling in cardiomyocyte ECC. Specifically, NAADP triggers Ca^{2+} release from microsomes prepared from SR; it activates single RyR2 incorporated into bilayer lipid membranes and direct application of NAADP increases the amplitude of Ca^{2+} transient and frequency/amplitude of Ca^{2+} sparks in intact guinea pig cardiomyocytes (Bak et al. 2001; Mojzisova et al. 2001; Macgregor et al. 2007a; Collins et al. 2011). Although a role for NAADP as a physiologically relevant messenger in the heart was for a long time not clear, the identification of its production downstream of β-adrenergic stimulation makes it an important regulator of cardiomyocyte function (Macgregor et al. 2007a; Lewis et al. 2012). NAADP is produced following β-adrenergic stimulation through activation of the enzyme CD38, an ADP-ribosyl cyclase, and is thereby proposed to contribute to both the inotropic and arrhythmogenic effects of this signaling pathway (Macgregor et al. 2007a; Lewis et al. 2012; Nebel et al. 2013; Warszta et al. 2014; Lin et al. 2017). To elicit its inotropic effect, NAADP is proposed to enhance Ca^{2+} loading of the SR (Macgregor et al. 2007a; Collins et al. 2011). A requirement for CaMKII in mediating this effect of NAADP has been shown (Capel et al. 2015). Reminiscent of the cross talk between $InsP_3Rs$ and RyRs, Ca^{2+} release from the NAADP-sensitive stores sensitizes RyR2 at lysosomal–endoplasmic reticulum nanojunctions leading to spontaneous diastolic Ca^{2+} release events (Fig. 2(4); Macgregor et al. 2007a; Nebel et al. 2013; Warszta et al. 2014).

RyRs and cADPR

Cyclic ADP-ribose (cADPR) is a second messenger that is also produced by ADP-ribosyl cyclase (CD38) in mammalian cardiomyocytes after sympathetic or angiotensin II stimulation (Ga-

lione et al. 1998; Higashida et al. 1999, 2000; Xie et al. 2005). cADPR modulates the peak amplitude of Ca^{2+} transients and hence cardiomyocyte contractility (Rakovic et al. 1996; Cui et al. 1999; Macgregor et al. 2007a). Mechanisms involved in mediating this action of cADPR include increased RyR activity and greater SR Ca^{2+} load (Rakovic et al. 1999; Lukyanenko et al. 2001; Macgregor et al. 2007b; Zhang et al. 2009). Consistent with this hypothesis, a higher frequency of Ca^{2+} sparks is observed in response to increased cADPR. However, the relatively slow kinetics for the development of the full effect of cADPR on Ca^{2+} release, and evidence for a cADPR-responsive component independent of RyR2 is suggestive of a more complex mechanism (Cui et al. 1999; Prakash et al. 2000). Apart from the role in Ca^{2+} homeostasis, the ADPR-cyclase and hence cADPR are implicated in the development of angiotensin II–induced hypertrophic responses (Gul et al. 2008, 2009) and in cellular toxicity following ischemia/reperfusion injury (Fig. 3(5); Xie et al. 2005).

Ca^{2+} Influx and STIM/Orai

A functional store-operated Ca^{2+} entry (SOCE) pathway is described in cardiomyocytes (Voelkers et al. 2010; Bootman and Rietdorf 2017). As in other cellular systems, in cardiomyocytes this pathway is activated in response to Ca^{2+} depletion of the SR, for example by treatment with SERCA inhibitors (cyclopiazonic acid or thapsigargin) and/or GPCR agonists (for review, see Hunton et al. 2002, 2004; Uehara et al. 2002; Kojima et al. 2012; Luo et al. 2012; Avila-Medina et al. 2018). The molecular components of SOCE, including the ER/SR Ca^{2+} sensors STIM1 and STIM2 and the plasma membrane channel ORAI are also all expressed in cardiomyocytes, further supporting the presence of this pathway in this cell type (Correll et al. 2015; Zhao et al. 2015). Although the role of SOCE in cardiomyocytes is not fully resolved, it has been shown to participate in regulation of gene transcription and Ca^{2+} homeostasis (Ohba et al. 2007; Voelkers et al. 2010; Hulot et al. 2011). SOCE activity is developmentally regulated, being readily detectible in fetal and neonatal cardiomyocytes, but is thereafter down-regulated, with low-to-nondetectable activity in healthy adult cardiomyocytes (Uehara et al. 2002). As such, it does not significantly contribute to ECC in healthy adult cardiomyocytes (Fig. 3(6)).

Mitochondrial Calcium in Cardiomyocytes

Cardiomyocyte Ca^{2+} dynamics are also influenced by mitochondria. They constitute about one-third of the volume of cardiomyocytes being arranged as bricks spanning the sarcomere (Bossen et al. 1978). Via the tricarboxylic acid (TCA) cycle and oxidative phosphorylation, mitochondria are responsible for ~90% of ATP generated in cardiomyocytes. Although mitochondria take up Ca^{2+} from the cytosol during ECC and show a high capacity for Ca^{2+} accumulation (Wei et al. 2012, 2015), their influence on Ca^{2+} dynamics is more subtle, especially under physiological conditions (Drago et al. 2012; Williams et al. 2013; Boyman et al. 2014). Ca^{2+} uptake into mitochondria is, however, an important regulator of mitochondrial metabolism. It augments the activity of pyruvate dehydrogenase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase, which are key enzymes of the TCA cycle as well as the activity of cytochrome *c* oxidase and F_0-F_1-ATPase, which are components of the electron transport chain (ETC) (Denton et al. 1988; Balaban 2009). By augmenting mitochondrial ATP generation, Ca^{2+} thus coordinates cellular metabolism, and the dynamic changes in the energy requirements of the cardiac myocyte, including for Ca^{2+} clearance mechanisms and actomyosin-mediated contraction that are enhanced during the fight-or-flight response (Balaban 2009; Kwong et al. 2015). During pathology such as following ischemia, excessive mitochondrial Ca^{2+} uptake is toxic, resulting in increased reactive oxygen species generation and permeability transition pore opening associated with cell death mechanisms (Santulli et al. 2015).

Cardiomyocyte mitochondrial Ca^{2+} has been reported to either mirror changes in cytosolic Ca^{2+} showing short-lived transients (Robert et al. 2001; Kettlewell et al. 2009) or to

show steady-state Ca^{2+} changes that increase according to the frequency of cytosolic Ca^{2+} transients (Paillard et al. 2017). Reasons for these different models are not clear but are in part proposed to be methodological (Dedkova and Blatter 2013). The mitochondrial Ca^{2+} uptake mechanism has a low affinity, in the tens of μM, precluding its activation by bulk cytosolic Ca^{2+} elevations generated during ECC and in response to hormonal stimulation that do not reach this level (Collins et al. 2002; Kirichok et al. 2004; Kettlewell et al. 2009; Dedkova and Blatter 2013). Mitochondrial Ca^{2+} uptake sites are, however, localized to within tens of nm of Ca^{2+} release channels on the SR/ER, exposing them to a microdomain of Ca^{2+} in the tens of μM, which is sufficiently high to activate the low-affinity Ca^{2+} uniporter (Hajnóczky et al. 1995; Csordás et al. 1999, 2010). These ER/SR mitochondrial uptake sites are known as "hotspots" and structurally as mitochondrial-associated membranes (MAMs) (Rizzuto et al. 2004). Supporting colocalization of sites for mitochondrial Ca^{2+} uptake and SR Ca^{2+} release in cardiomyocytes, cardiomyocyte mitochondria show a gradient in Ca^{2+} from the Ca^{2+} release sites at the junctional SR to the center of the sarcomere (Lu et al. 2013).

Mitochondrial Ca^{2+} uptake primarily occurs via the voltage-dependent anion channel (VDAC) of the outer mitochondrial membrane (OMM) (Shimizu et al. 2015) and a low affinity mitochondrial Ca^{2+} uniporter (MCU) of the inner mitochondrial membrane (IMM) (Kirichok et al. 2004). Other mechanisms of mitochondrial Ca^{2+} uptake have been described, including a rapid mode of mitochondrial uptake (RaM) (Buntinas et al. 2001) via mitochondrial RyR1 (Beutner et al. 2005) and connexin 43 (Gadicherla et al. 2017). The molecular identification of the MCU in the last decade (Baughman et al. 2011; De Stefani et al. 2011) has led to new insights into its role in mitochondrial Ca^{2+} sequestration in the heart (Pan et al. 2013; Kwong et al. 2015; Wu et al. 2015). A common finding of these studies is that MCU function is not required for baseline function but is necessary for adaptive responses of the heart to stress or work. This is illustrated by results of loss-of-function studies, which showed that MCU was required for heart rate acceleration at the level of pacemaker cells and inotropy of ventricular cardiomyocytes (Kwong et al. 2015; Rasmussen et al. 2015; Wu et al. 2015). The greatest contribution of these effects of loss of MCU function was to limit increases in ATP generation during periods of greater stress/workload and not on Ca^{2+} buffering (Drago et al. 2012). As a consequence of insufficient ATP, the increase in SERCA function required to bring about chronotropic and inotropic responses is prevented (Kwong et al. 2015; Rasmussen et al. 2015; Wu et al. 2015). MCU is associated with a number of regulatory proteins, including MICU1 and MICU2 and EMRE (Perocchi et al. 2010; Vais et al. 2016). Of particular importance in the context of the cardiomyocyte is MICU1, which sets the threshold of activation and properties of Ca^{2+} uptake via MCU (Csordás et al. 2013). Notably, whereas MCU and MICU1 interact in a stoichiometric manner in liver, a reduced proportion of MCU is associated with MICU1 in cardiomyocytes (Paillard et al. 2017). Through this decreased interaction with MICU1, cardiomyocyte mitochondrial Ca^{2+} uptake shows a higher affinity and lower cooperativity than in nonmuscle tissues such as liver (Paillard et al. 2017). Whereas MCU is positioned close to RyR to facilitate mitochondrial Ca^{2+} uptake (De La Fuente et al. 2016), Ca^{2+} extrusion mechanisms, including the $Na^+/Ca^{2+}/Li^+$ exchanger NCXL (Palty et al. 2010; Luongo et al. 2017), are localized to regions of the mitochondria distinct from the Ca^{2+} uptake route (De La Fuente et al. 2018). Furthermore, an electrogenic $Ca^{2+}/2H^+$ exchanger (potentially the leucine zipper EF-hand-containing transmembrane protein 1 [LETM1]) has also been proposed to extrude Ca^{2+} out of the mitochondria (Jiang et al. 2009; Haumann et al. 2018).

REMODELING OF ECC IN DISEASE

Given its fundamental role in regulation of cardiac function, it is not surprising that disturbances in the organization and regulation of Ca^{2+} signals result in cardiac dysfunction (Hasenfuss and Pieske 2002; Wehrens et al. 2005;

Luo and Anderson 2013; Marks 2013). Indeed, altered Ca^{2+} handling is observed during hypertrophic remodeling and heart failure, as well as during diseases associated with rhythmic disturbances, including atrial fibrillation (Berridge et al. 2003; Fearnley et al. 2011; Mozaffarian et al. 2015; Denham et al. 2018). Perturbations in Ca^{2+} handling are also apparent in hearts remodeled because of mutations in myofilament proteins (hypertrophic as well as dilated cardiomyopathies). Further, mutations in Ca^{2+}-handling proteins, including RyRs (e.g., during catecholaminergic polymorphic ventricular tachycardia [CPVT]) and PLB are described to have a direct effect on cardiomyocyte function (Wehrens et al. 2005; Liu et al. 2015).

Cardiac remodeling (i.e., changes in structure and function), including altered Ca^{2+} handling, is seen in diseases with different etiologies, and is often the first step in progression to frank heart failure (Marks 2013; Schirone et al. 2017). Despite variability in the disease phenotype, some general features and patterns are common. They have been characterized in detail in animal models (e.g., during pressure overload). In the early stages of pathological remodeling, the change of ECC is adaptive, with augmented ECC observed (Ohkusa et al. 1997; Lymperopoulos et al. 2013). This is characterized by enhanced Ca^{2+} transient amplitude, greater contraction, and more effective relaxation (SR Ca^{2+} sequestration). As remodeling progresses to failure, ECC capacity decreases, eventually reaching a point that effective contraction is no longer possible (Gomez et al. 1997; Marks 2013; Høydal et al. 2018; Munro et al. 2018). Under these conditions, intracellular Ca^{2+} release becomes less homogenous, the kinetics of Ca^{2+} release and reuptake are slower, Ca^{2+} transient amplitude is lower, and ultimately these maladaptations combine to decrease contractile function (Fig. 4; Gomez et al. 1997; Mozaffarian et al. 2015; Høydal et al. 2018). This decline in ECC is brought about through a combined alteration in membrane architecture and Ca^{2+}-handling machinery. The Ca^{2+} influx through LTCC is generally unchanged, but because of changes in cytoarchitecture, it may have a different microdistribution (Sanchez-Alonso et al. 2016). A particular feature of the remodeling of membrane architecture is alteration in TATS (Song et al. 2006; Heinzel et al. 2008; Lenaerts et al. 2009; Lyon et al. 2009; Crossman et al. 2015, 2017; Crocini et al. 2016; Seidel et al. 2017; Dries et al. 2018; Høydal et al. 2018). During the early stages of pathological remodeling, the TATS becomes disorganized and membrane/SR distances increased (Xu et al. 2007; Wu et al. 2012; Jones et al. 2018). As disease progresses, together with cardiomyocyte hypertrophy, a further disorganization (seen in rodent models) and decrease in density (recorded in large mammals) of TATS is observed, resulting in an increase in the fraction of noncoupled RyRs (Song et al. 2006; Sachse et al. 2012; Seidel et al. 2017; Dries et al. 2018; Munro et al. 2018). Consequently, a lower proportion of RyRs is directly activated by Ca^{2+} influx during the initial phase of the AP. The noncoupled RyRs are activated, albeit with a delay, by Ca^{2+} diffusion from the coupled RyRs (Dries et al. 2018). Owing to decreased synchrony in RyR activation, the Ca^{2+} flux from the SR is lower, consequently reducing Ca^{2+} transient amplitude and contraction. The rate of Ca^{2+} clearance is also substantially reduced in disease (Fig. 4). This is manifest as a slower rate of decline of the Ca^{2+} transient/contraction and an increase in cytosolic Ca^{2+} during relaxation (Kubalova et al. 2005; Hohendanner et al. 2013). Poor cardiomyocyte relaxation contributes to the overall decrease in diastolic function and consequently poor filling of the ventricle. A reduction in SERCA expression and/or activity is primarily responsible for this reduced Ca^{2+} clearance (Røe et al. 2018). Notably, in vivo re-expression of SERCA reverses many of the features of disease-associated remodeling of Ca^{2+} handling raising its potential as a therapy (Lyon et al. 2011). Disappointingly, clinical trials have not shown a similar benefit, which is possibly related to methodological hurdles to reach the necessary increase in SERCA expression within failing cardiomyocytes. Further advances in this area may yet provide benefit (Greenberg et al. 2016).

Further adding to the perturbation of Ca^{2+} handling in disease is a dysregulation of SR Ca^{2+}

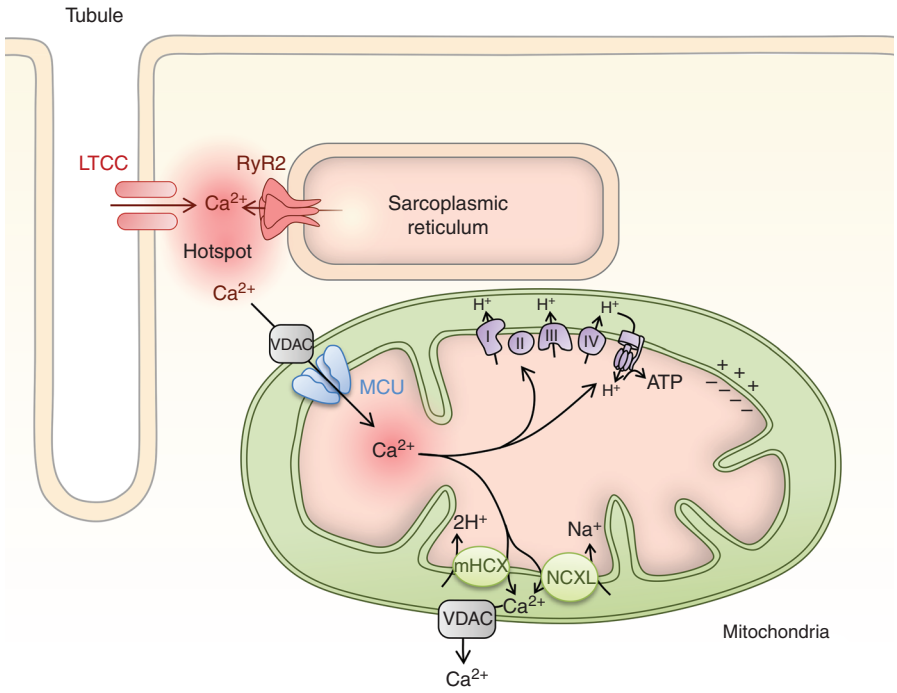

Figure 4. Mitochondrial Ca^{2+}. Mitochondria are densely packed in the cardiomyocyte being aligned along the myofilaments as bricks. Their Ca^{2+} uptake sites are closely localized to the junctional sarcoplasmic reticulum (SR) and a microdomain of elevated Ca^{2+} generated by SR Ca^{2+} release via RyRs. Ca^{2+} enters the mitochondria through the voltage-gated anion channel (VDAC) of the outer mitochondrial membrane and the mitochondrial Ca^{2+} uniporter (MCU) of the inner mitochondrial membrane. Ca^{2+} acts at multiple sites in the electron transport chain and tricarboxylic acid (TCA) cycle to modulate mitochondrial metabolism and ATP production. Ca^{2+} is extruded from the mitochondria through a $Na^+/Li^+/Ca^{2+}$ exchanger (NCXL) and/or a Ca^{2+}/H^+ exchanger (mHCX).

release. At the nanoscale, RyR clusters are reorganized in heart failure, which is associated with an increase in their activity (Kolstad et al. 2018). Atrial fibrillation is also associated with changes in RyR cluster size, distribution, and activity (Macquaide et al. 2015). Increased RyR channel activity in diastole, observed as increased spark frequency, is very often seen, including in late-stage human heart failure (Fischer et al. 2014; Dries et al. 2018). Although RyR expression is not substantially altered in disease, posttranslational modifications including phosphorylation by PKA and CaMKII, oxidation, as well as remodeling of RyR clusters, contribute to the increase in spontaneous Ca^{2+} release events (Fischer et al. 2014; Ho et al. 2014; Grimm et al. 2015; Fu et al. 2016; Uchinoumi et al. 2016; Walweel et al. 2017; Dries et al. 2018). The greater frequency of these spontaneous Ca^{2+} release events can lead to a leak of Ca^{2+} from the SR as well as an increase in the propensity of Ca^{2+} waves (Cheng et al. 1996; Venetucci et al. 2008; Curran et al. 2010; Dries et al. 2018). These Ca^{2+} waves stimulate NCX activity, which because of its electrogenic nature, leads to the generation of delayed after depolarizations (DADs), triggering APs that may propagate to neighboring cardiomyocytes and induce arrhythmia (Venetucci et al. 2008). A similar proarrhythmic phenotype arises because of CPVT-associated mutations in RyR2 (Priori and Chen 2011). These mutated forms of RyRs show enhanced sensitivity to luminal and/or cytosolic Ca^{2+} and show increased spontaneous

openings. Although the heart can accommodate for these more active RyRs under normal conditions, during periods of increased catecholamine stimulation and associated increased SR store loading, large spontaneous Ca^{2+} release events induce NCX activity and AP generation. This can trigger arrhythmogenic events, ventricular tachycardia, and eventually sudden cardiac death. The identification of small molecules such as K201 and EL0 that stabilize the closed state of the RyR may reverse the pathological SR Ca^{2+} leak observed in heart failure as well as in CPVT (Li et al. 2017).

A re-expression of a gene program expressed in the fetus/neonate (the fetal gene program) characterized by atrial and brain natriuretic factors (NPPA and NPPB) is often described during hypertrophy. Notably this gene program includes a number of proteins involved in Ca^{2+} handling. Indeed, increases in expression of $InsP_3R$ (Harzheim et al. 2010), proteins involved in SOCE (Correll et al. 2015), and of T-type Ca^{2+} channels are observed during cardiac pathology (Izumi et al. 2003). Moreover, the increase in the expression of these proteins contributes to pathological alterations of cardiomyocyte physiology (Izumi et al. 2003; Harzheim et al. 2010; Correll et al. 2015).

Increased $InsP_3R$ expression, as well as the mechanisms responsible for $InsP_3$ generation, are observed in hypertrophy and heart failure, as well as in atrial fibrillation (Yamda et al. 2001; Harzheim et al. 2010; Drawnel et al. 2012; Signore et al. 2013). As a consequence, Ca^{2+} release independent of ECC is enhanced (Harzheim et al. 2009; Nakayama et al. 2010; Hohendanner et al. 2015b). Although $InsP_3$-mediated Ca^{2+} release remains of a low magnitude, through promoting Ca^{2+} release via neighboring Ca^{2+}-sensitive RyRs, it leverages increased Ca^{2+} spark events that may lead to arrhythmogenic cell-wide Ca^{2+} activity (Hohendanner et al. 2015b). The summation of increased RyR sensitivity and greater $InsP_3$-mediated Ca^{2+} release may create a perfect storm in which the cardiomyocyte is sensitized to spontaneous release that, in turn, via activation of NCX, induces the generation of APs and tissue-wide arrhythmia.

SOCE is also increased in hypertrophied cardiomyocytes (Hulot et al. 2011; Luo et al. 2012). Remarkably, the overwhelming majority of studies showed that stress-induced overexpression of SOCE-associated proteins and, hence, the enhanced activity of SOCE in cardiomyocytes contribute to, and sustain, hypertrophic remodeling (Hunton et al. 2002, 2004; Ohba et al. 2007; Voelkers et al. 2010; Hulot et al. 2011). Attenuation of SOCE components suppresses this induction of hypertrophy in neonatal cardiac myocytes (Voelkers et al. 2010; Luo et al. 2012). Increased SOCE through transgenic STIM1 overexpression is also sufficient to induce hypertrophic remodeling of the heart, promoting enhanced CnA/NFAT and CaMKII signaling. Moreover, in advance of disease development, STIM1 overexpression induces spontaneous Ca^{2+} transients, increased LTCC current and Ca^{2+} sparks—effects that may underlie the sudden death observed in these mice (Correll et al. 2015). Together these combined roles of SOCE in cardiomyocytes would suggest that SOCE inhibition may be of therapeutic benefit (Kojima et al. 2012).

Ca^{2+} Triggering of Hypertrophic Remodeling

Alterations in Ca^{2+} handling are a cue for cardiac remodeling during disease. The connection between Ca^{2+} and cardiomyocyte growth—hypertrophy associated with disease—was first uncovered by examining the responses of neonatal cardiomyocytes to altered frequency of electrical stimulation (McDonough et al. 1994; Tavi et al. 2004). In vivo, tachypacing or catecholamine infusion is also sufficient to elicit a hypertrophic response (Kubalova et al. 2005). The absence of hypertrophy in mice deficient for PLB that show a constitutive inotropic state is not sufficient to trigger hypertrophy, and suggests that enhanced Ca^{2+} cycling per se is not, however, sufficient for the induction of hypertrophic gene expression (Kiriazis and Kranias 2000). Indeed, increases in nuclear Ca^{2+} are now considered to play an important role in activation/modulation of cardiomyocyte gene expression (Fig. 5; Wu et al. 2006; Guatimosim et al. 2008; Higazi et al.

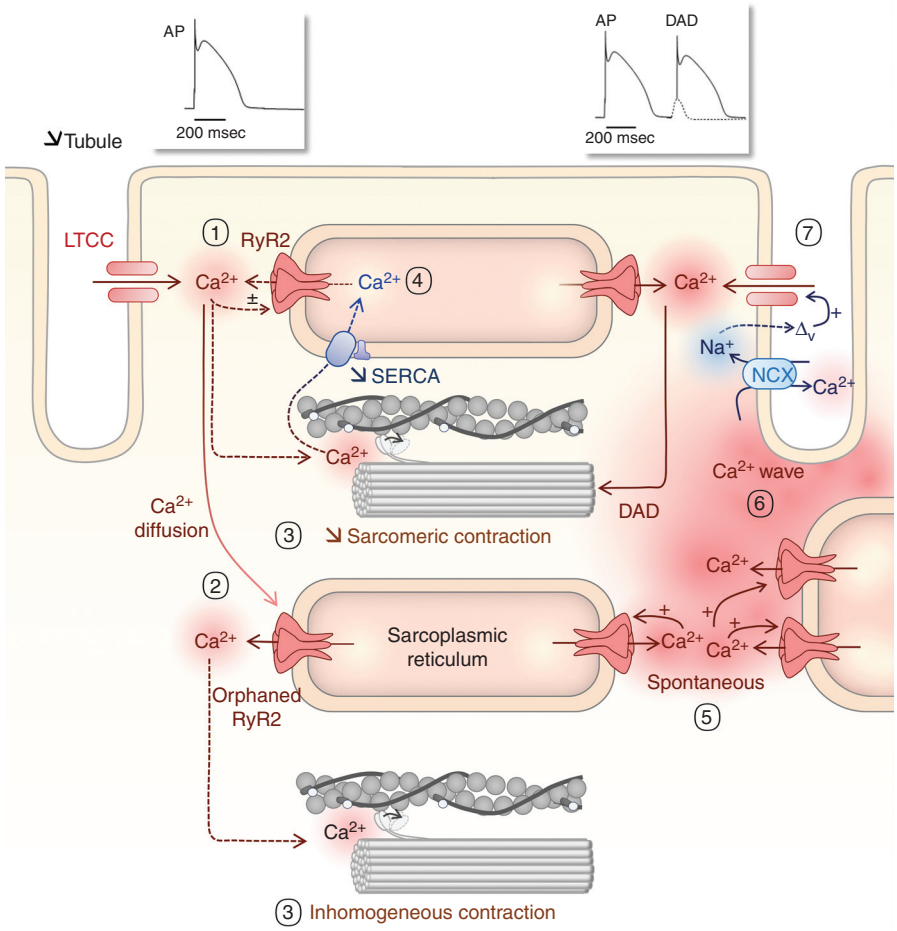

Figure 5. Remodeling of excitation–contraction coupling (ECC) in disease. (1) The coupling between Ca^{2+} influx and Ca^{2+} release is compromised because of a loss of tight connections between the transverse and axial tubule system (TATS) and the sarcoplasmic reticulum (SR) with an atrophy and remodeling of the tubular network. (2) RyRs thus become uncoupled and are then activated with a delay by Ca^{2+} diffusion across the cell from coupled RyRs. (3) Consequently, activation of myofilaments is less homogeneous and contraction impaired. (4) SERCA expression is reduced leading to an elevation in diastolic Ca^{2+} and reduced SR Ca^{2+}, which in turn leads to a decrease in Ca^{2+} release via RyRs during each ECC cycle, thereby reducing contraction amplitude. (5) RyRs display spontaneous releases of Ca^{2+}, (6) generating Ca^{2+} waves. (7) This cytosolic Ca^{2+} overload leads to NCX activation, triggering after-depolarizations and potentially a spontaneous AP.

2009; Arantes et al. 2012). Moreover, in the absence of these nuclear Ca^{2+} changes, altered frequency of cytosolic Ca^{2+} oscillations may not be sufficient to increase hypertrophic gene expression (Higazi et al. 2009). In generating nuclear-specific Ca^{2+} changes, $InsP_3Rs$ have been proposed to play a role (Wu et al. 2006; Guatimosim et al. 2008; Higazi et al. 2009; Arantes et al. 2012). Since $InsP_3Rs$ are activated downstream of $G_{\alpha q}$-coupled receptors by circulating hormones or phospholipase C_ε (Zhang et al. 2013), this mechanism can be engaged independent of the Ca^{2+} changes associated with ECC (Fig. 5).

The role of Ca^{2+} in hypertrophic gene expression is further substantiated by gain- and loss-of-function studies involving downstream sensors and mediators of Ca^{2+}. Through a num-

ber of studies, key roles for a pathway involving calmodulin (CaM) (Obata et al. 2005), the archetypal Ca^{2+} sensor, calcineurin (CnA) a calcium-dependent phosphatase and its downstream transcription factor—the nuclear factor of activated T-cells (NFAT) in hypertrophic gene expression has been identified (Fig. 5(5) and 5(6); Molkentin et al. 1998; Sussman et al. 1998; Bourajjaj et al. 2008). Nuclear Ca^{2+} changes have been invoked in regulation of this pathway through maintaining CnA in the nucleus and prolonging its dephosphorylation of NFAT (Higazi et al. 2009; Olivares-Florez et al. 2018). Indeed, nuclear translocation of CaM is important in the activity of nuclear CnA (Zhu and McKeon 1999; Oda et al. 2018). The involvement of this CnA/NFAT pathway in hypertrophy induced by a pathological tropomyosin mutation suggests a more general role for this pathway in cardiac pathology (Sussman et al. 1998). Ca^{2+} also elicits its hypertrophic effects via CaMKII. This kinase phosphorylates the type II histone deacetylase (HDAC) causing its translocation out of the nucleus and loss of inhibition of MEF2-dependent gene expression (Fig. 5(7) and 5(8); Zhang et al. 2002; Wu et al. 2006; Backs et al. 2008).

Ca^{2+} Handling and Hypertrophic Remodeling Are Interdependent

Notably, while ECC and the gene expression underlying hypertrophic remodeling are selectively controlled by Ca^{2+}, these cell mechanisms are intimately linked. Indeed, at the same time that Ca^{2+} stimulates the induction of hypertrophy, the expression of Ca^{2+}-handling proteins involved in ECC is altered during disease. Thus, the Ca^{2+} signals that stimulate hypertrophy are modified. As a consequence, the hypertrophic response is augmented and sustained. Clear examples of this feedback/feedforward system are illustrated by the roles of $InsP_3Rs$ and SERCA2a. As indicated above, SERCA activity/expression is often decreased during heart failure contributing to the associated diminished contractility. Re-expression of SERCA leads, however, to a reversal of the hypertrophic phenotype: SR Ca^{2+} leak, β-adrenergic receptor distribution and ECC return to a healthy state (Lyon et al. 2011). $InsP_3R$ expression is increased and the nuclear membrane invaginations where it is localized decreased during disease (Ljubojevic et al. 2014). Nuclear $InsP_3$ signaling is also a cue for the activation of CnA/NFAT and CaMKII/HDAC pathways and the induction of the hypertrophic response (Wu et al. 2006; Higazi et al. 2009; Plačkić et al. 2016). Notably, $InsP_3$ signaling suppresses the expression of microRNA miR-133 (Figs. 5(2), 5(3), and 6; Drawnel et al. 2012), which is not only an inhibitor of hypertrophy-related genes but also of $InsP_3R$ expression (Li et al. 2018). Thus, enhanced $InsP_3$ signaling that may initiate hypertrophy, is self-augmenting and sustaining. Further, $InsP_3R$ expression has also been reported to be under the control of NFAT (Sankar et al. 2014; Olivares-Florez et al. 2018), which is again sensitive to $InsP_3$ signaling independent of Ca^{2+} changes associated with ECC (Higazi et al. 2009). CnA/NFAT signaling also shows feedback regulation. In particular, NFAT stimulates induction of RCAN1.4 (regulator of CnA) also known as DCSR1 and calcipressin), which then directly inhibits the activity of CnA (Oh et al. 2010). These links between modifications in expression of certain Ca^{2+}-handling proteins, in particular those that show disease-dependent changes, are particularly interesting targets for therapeutic intervention.

CONCLUSIONS AND PERSPECTIVES

Cardiomyocytes rely on Ca^{2+} signaling for their core task as contractile units in the heart, and Ca^{2+} dysregulation is one of the main contributors to failure of the heart as pump. Yet, Ca^{2+} signaling in cardiomyocytes is equally pivotal as a mechanism for regulating cardiomyocyte growth and physiological remodeling. Further, Ca^{2+}-handling mechanisms are remodeled in disease, generating a unique link with contraction and arrhythmias in heart disease (Denham et al. 2018). Such feedback loops involving these dual roles of Ca^{2+} in the cardiomyocyte may represent ideal targets for therapy (Hulot et al. 2012; Gabisonia and Recchia 2018). To gain greater insight into these mechanisms and to develop strategies for their exploitation for

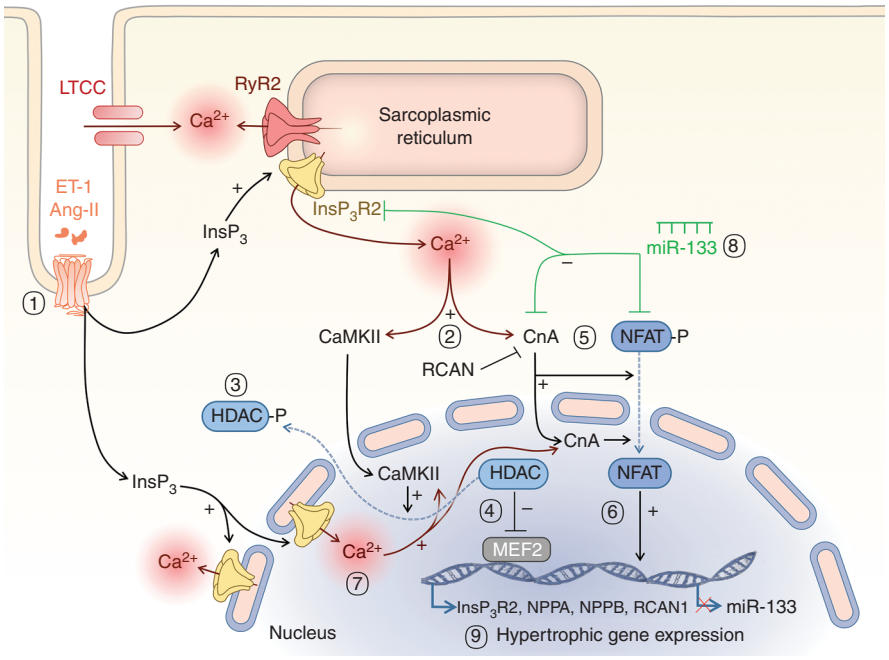

Figure 6. Excitation–transcription coupling. (1) $InsP_3R$-mediated cytosolic Ca^{2+} increase induces activation of different signaling pathways: (2) Ca^{2+} activates cytosolic and nuclear CaMKII that phosphorylates the inhibitory transcription factor histone deacetylase (HDAC) and (3) induces its export out of the nucleus. (4) MEF2 is activated and this triggers hypertrophic genes expression. (5) Ca^{2+} increases, including via $InsP_3Rs$, activates calcineurin (CnA), leading to dephosphorylation of nuclear factor of activated T-cells (NFAT) and its translocation to the nucleus. (6) NFAT then activates the transcription of hypertrophic genes including fetal genes ($InsP_3R$, NPPA, NPPB, RCAN1) and can also repress miR-133. (7) Nuclear Ca^{2+} signals arising from $InsP_3R$ can contribute to activation of nuclear CnA and trigger pathways for hypertrophy (9). (7) miR-133 suppresses expression of NFAT, CnA, and $InsP_3R$, which will inhibit the hypertrophic responses previously described (8).

therapeutic ends, novel approaches and methodologies are required. Further insights into cardiomyocyte physiology and mechanisms underlying ECC are expected to emerge with the development of new technologies including live cell imaging, superresolution microscopy, and in particular automated image analysis and artificial intelligence for high throughput and discovery (Sacconi et al. 2006; Jayasinghe et al. 2018a). Using multiomics approaches and single-cell analysis, new molecular targets and their interactions that determine the physiological status of the cardiomyocyte will be discovered (Barwari et al. 2016; Perrino et al. 2017; See et al. 2017; Thienpont et al. 2017; Gilsbach et al. 2018), for example, microRNAs, long noncoding RNAs, and epigenetic modifications that govern the expression of Ca^{2+}-handling proteins. Notably, some of these microRNAs are found in the circulation in plasma (sometimes in exosomes) and act on the cardiomyocyte compartment as well as being biomarkers of disease progression (Hoefer et al. 2015; Devaux et al. 2017). As our understanding and methodology for physiological analysis of intact tissue develops, analysis of the interactions of the multiple cell types in the heart (e.g., fibroblasts with cardiac myocytes) and their heterogeneity will be made possible (Perbellini et al. 2018; Scardigli et al. 2018b). Through these means, a true picture of how Ca^{2+} signaling at the cellular level is regulated to control cardiac function will emerge.

COMPETING INTEREST STATEMENT

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Work in the authors' laboratories is supported by grants from the Research Foundation Flanders (FWO). Specifically, Odysseus grant (90663) and Project Grant (G08861N) to H.L.R., Project Grant (G091815N) to K.S., and Post-doctoral Fellowships to G.G. (12W6218N) and E.D. (12Q1117N).

REFERENCES

Arantes LA, Aguiar CJ, Amaya MJ, Figueiró NC, Andrade LM, Rocha-Resende C, Resende RR, Franchini KG, Guatimosim S, Leite MF. 2012. Nuclear inositol 1,4,5-trisphosphate is a necessary and conserved signal for the induction of both pathological and physiological cardiomyocyte hypertrophy. *J Mol Cell Cardiol* **53:** 475–486. doi:10.1016/j.yjmcc.2012.06.017

Avila-Medina J, Mayoral-Gonzalez I, Dominguez-Rodriguez A, Gallardo-Castillo I, Ribas J, Ordoñez A, Rosado JA, Smani T. 2018. The complex role of store operated calcium entry pathways and related proteins in the function of cardiac, skeletal and vascular smooth muscle cells. *Front Physiol* **9:** 257. doi:10.3389/fphys.2018.00257

Backs J, Backs T, Bezprozvannaya S, McKinsey TA, Olson EN. 2008. Histone deacetylase 5 acquires calcium/calmodulin-dependent kinase II responsiveness by oligomerization with histone deacetylase 4. *Mol Cell Biol* **28:** 3437–3445. doi:10.1128/MCB.01611-07

Bak J, Billington RA, Timar G, Dutton AC, Genazzani AA. 2001. NAADP receptors are present and functional in the heart. *Curr Biol* **11:** 987–990. doi:10.1016/S0960-9822(01)00269-X

Balaban RS. 2009. The role of Ca^{2+} signaling in the coordination of mitochondrial ATP production with cardiac work. *Biochem Biophys Acta* **1787:** 1334–1341. doi:10.1016/j.bbabio.2009.05.011

Barwari T, Joshi A, Mayr M. 2016. MicroRNAs in cardiovascular disease. *J Am Coll Cardiol* **68:** 2577–2584. doi:10.1016/j.jacc.2016.09.945

Bassani JW, Bassani RA, Bers DM. 1994. Relaxation in rabbit and rat cardiac cells: Species-dependent differences in cellular mechanisms. *J Physiol* **476:** 279–293. doi:10.1113/jphysiol.1994.sp020130

Baughman JM, Perocchi F, Girgis HS, Plovanich M, Belcher-Timme CA, Sancak Y, Bao XR, Strittmatter L, Goldberger O, Bogorad RL, et al. 2011. Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. *Nature* **476:** 341–345. doi:10.1038/nature10234

Berridge MJ. 1993. Inositol trisphosphate and calcium signalling. *Nature* **361:** 315–325. doi:10.1038/361315a0

Berridge MJ, Bootman MD, Roderick HL. 2003. Calcium signalling: Dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol* **4:** 517–529. doi:10.1038/nrm1155

Bers DM. 2002. Cardiac excitation–contraction coupling. *Nature* **415:** 198–205. doi:10.1038/415198a

Beuckelmann DJ, Wier WG. 1988. Mechanism of release of calcium from sarcoplasmic reticulum of guinea-pig cardiac cells. *J Physiol* **405:** 233–255. doi:10.1113/jphysiol.1988.sp017331

Beutner G, Sharma VK, Lin L, Ryu SY, Dirksen RT, Sheu SS. 2005. Type 1 ryanodine receptor in cardiac mitochondria: Transducer of excitation-metabolism coupling. *Biochem Biophys Acta* **1717:** 1–10. doi:10.1016/j.bbamem.2005.09.016

Blanch ISJ, Egger M. 2018. Obstruction of ventricular Ca^{2+}-dependent arrhythmogenicity by inositol 1,4,5-trisphosphate-triggered sarcoplasmic reticulum Ca^{2+} release. *J Physiol* **596:** 4323–4340. doi:10.1113/JP276319

Bootman MD, Rietdorf K. 2017. Tissue specificity: Store-operated Ca^{2+} entry in cardiac myocytes. *Adv Exp Med Biol* **993:** 363–387. doi:10.1007/978-3-319-57732-6_19

Bootman MD, Higazi DR, Coombes S, Roderick HL. 2006. Calcium signalling during excitation-contraction coupling in mammalian atrial myocytes. *J Cell Sci* **119:** 3915–3925. doi:10.1242/jcs.03223

Bootman MD, Harzheim D, Smyrnias I, Conway SJ, Roderick HL. 2007. Temporal changes in atrial EC-coupling during prolonged stimulation with endothelin-1. *Cell Calcium* **42:** 489–501. doi:10.1016/j.ceca.2007.05.004

Bootman MD, Smyrnias I, Thul R, Coombes S, Roderick HL. 2011. Atrial cardiomyocyte calcium signalling. *Biochem Biophys Acta* **1813:** 922–934. doi:10.1016/j.bbamcr.2011.01.030

Bossen EH, Sommer JR, Waugh RA. 1978. Comparative stereology of the mouse and finch left ventricle. *Tissue Cell* **10:** 773–784. doi:10.1016/0040-8166(78)90062-9

Bourajjaj M, Armand AS, da Costa Martins PA, Weijts B, van der Nagel R, Heeneman S, Wehrens XH, De Windt LJ. 2008. NFATc2 is a necessary mediator of calcineurin-dependent cardiac hypertrophy and heart failure. *J Biol Chem* **283:** 22295–22303. doi:10.1074/jbc.M801296200

Boyman L, Chikando AC, Williams GS, Khairallah RJ, Kettlewell S, Ward CW, Smith GL, Kao JP, Lederer WJ. 2014. Calcium movement in cardiac mitochondria. *Biophys J* **107:** 1289–1301. doi:10.1016/j.bpj.2014.07.045

Brandenburg S, Kohl T, Williams GS, Gusev K, Wagner E, Rog-Zielinska EA, Hebisch E, Dura M, Didié M, Gotthardt M, et al. 2016. Axial tubule junctions control rapid calcium signaling in atria. *J Clin Invest* **126:** 3999–4015. doi:10.1172/JCI88241

Brandenburg S, Pawlowitz J, Fakuade FE, Kownatzki-Danger D, Kohl T, Mitronova GY, Scardigli M, Neef J, Schmidt C, Wiedmann F, et al. 2018. Axial tubule junctions activate atrial Ca^{2+} release across species. *Front Physiol* **9:** 1227. doi:10.3389/fphys.2018.01227

Brette F, Despa S, Bers DM, Orchard CH. 2005. Spatiotemporal characteristics of SR Ca^{2+} uptake and release in detubulated rat ventricular myocytes. *J Mol Cell Cardiol* **39:** 804–812. doi:10.1016/j.yjmcc.2005.08.005

Buntinas L, Gunter KK, Sparagna GC, Gunter TE. 2001. The rapid mode of calcium uptake into heart mitochondria (RaM): Comparison to RaM in liver mitochondria. *Biochem Biophys Acta* **1504:** 248–261. doi:10.1016/S0005-2728(00)00254-1

Burton FL, Cobbe SM. 2001. Dispersion of ventricular repolarization and refractory period. *Cardiovasc Res* **50:** 10–23. doi:10.1016/S0008-6363(01)00197-3

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

Cala SE, Scott BT, Jones LR. 1990. Intralumenal sarcoplasmic reticulum Ca^{2+}-binding proteins. *Semin Cell Biol* **1:** 265–275.

Calcraft PJ, Ruas M, Pan Z, Cheng X, Arredouani A, Hao X, Tang J, Rietdorf K, Teboul L, Chuang KT, et al. 2009. NAADP mobilizes calcium from acidic organelles through two-pore channels. *Nature* **459:** 596–600. doi:10.1038/nature08030

Cannell MB, Kong CHT. 2017. Quenching the spark: Termination of CICR in the submicroscopic space of the dyad. *J Gen Physiol* **149:** 837–845. doi:10.1085/jgp.201711807

Cannell MB, Berlin JR, Lederer WJ. 1987. Effect of membrane potential changes on the calcium transient in single rat cardiac muscle cells. *Science* **238:** 1419–1423. doi:10.1126/science.2446391

Capel RA, Bolton EL, Lin WK, Aston D, Wang Y, Liu W, Wang X, Burton RA, Bloor-Young D, Shade KT, et al. 2015. Two-pore channels (TPC2s) and nicotinic acid adenine dinucleotide phosphate (NAADP) at lysosomal-sarcoplasmic reticular junctions contribute to acute and chronic β-adrenoceptor signaling in the heart. *J Biol Chem* **290:** 30087–30098. doi:10.1074/jbc.M115.684076

Carmeliet E. 1999. Cardiac ionic currents and acute ischemia: From channels to arrhythmias. *Physiol Rev* **79:** 917–1017. doi:10.1152/physrev.1999.79.3.917

Cens T, Rousset M, Leyris JP, Fesquet P, Charnet P. 2006. Voltage- and calcium-dependent inactivation in high voltage-gated Ca^{2+} channels. *Prog Biophys Mol Biol* **90:** 104–117. doi:10.1016/j.pbiomolbio.2005.05.013

Chen B, Guo A, Zhang C, Chen R, Zhu Y, Hong J, Kutschke W, Zimmerman K, Weiss RM, Zingman L, et al. 2013. Critical roles of junctophilin-2 in T-tubule and excitation–contraction coupling maturation during postnatal development. *Cardiovasc Res* **100:** 54–62. doi:10.1093/cvr/cvt180

Cheng H, Lederer MR, Lederer WJ, Cannell MB. 1996. Calcium sparks and $[Ca^{2+}]$i waves in cardiac myocytes. *Am J Physiol* **270:** C148–C159. doi:10.1152/ajpcell.1996.270.1.C148

Chiamvimonvat N, Chen-Izu Y, Clancy CE, Deschenes I, Dobrev D, Heijman J, Izu L, Qu Z, Ripplinger CM, Vandenberg JI, et al. 2017. Potassium currents in the heart: Functional roles in repolarization, arrhythmia and therapeutics. *J Physiol* **595:** 2229–2252. doi:10.1113/JP272883

Collins TJ, Berridge MJ, Lipp P, Bootman MD. 2002. Mitochondria are morphologically and functionally heterogeneous within cells. *EMBO J* **21:** 1616–1627. doi:10.1093/emboj/21.7.1616

Collins TP, Bayliss R, Churchill GC, Galione A, Terrar DA. 2011. NAADP influences excitation–contraction coupling by releasing calcium from lysosomes in atrial myocytes. *Cell Calcium* **50:** 449–458. doi:10.1016/j.ceca.2011.07.007

Cordeiro JM, Zeina T, Goodrow R, Kaplan AD, Thomas LM, Nesterenko VV, Treat JA, Hawel L III, Byus C, Bett GC, et al. 2015. Regional variation of the inwardly rectifying potassium current in the canine heart and the contributions to differences in action potential repolarization. *J Mol Cell Cardiol* **84:** 52–60. doi:10.1016/j.yjmcc.2015.04.010

Correll RN, Goonasekera SA, van Berlo JH, Burr AR, Accornero F, Zhang H, Makarewich CA, York AJ, Sargent MA, Chen X, et al. 2015. STIM1 elevation in the heart results in aberrant Ca^{2+} handling and cardiomyopathy. *J Mol Cell Cardiol* **87:** 38–47. doi:10.1016/j.yjmcc.2015.07.032

Crocini C, Coppini R, Ferrantini C, Yan P, Loew LM, Tesi C, Cerbai E, Poggesi C, Pavone FS, Sacconi L. 2014. Defects in T-tubular electrical activity underlie local alterations of calcium release in heart failure. *Proc Natl Acad Sci* **111:** 15196–15201. doi:10.1073/pnas.1411557111

Crocini C, Ferrantini C, Scardigli M, Coppini R, Mazzoni L, Lazzeri E, Pioner JM, Scellini B, Guo A, Song LS, et al. 2016. Novel insights on the relationship between T-tubular defects and contractile dysfunction in a mouse model of hypertrophic cardiomyopathy. *J Mol Cell Cardiol* **91:** 42–51. doi:10.1016/j.yjmcc.2015.12.013

Crossman DJ, Young AA, Ruygrok PN, Nason GP, Baddelely D, Soeller C, Cannell MB. 2015. T-tubule disease: Relationship between T-tubule organization and regional contractile performance in human dilated cardiomyopathy. *J Mol Cell Cardiol* **84:** 170–178. doi:10.1016/j.yjmcc.2015.04.022

Crossman DJ, Shen X, Jüllig M, Munro M, Hou Y, Middleditch M, Shrestha D, Li A, Lal S, Dos Remedios CG, et al. 2017. Increased collagen within the transverse tubules in human heart failure. *Cardiovasc Res* **113:** 879–891. doi:10.1093/cvr/cvx055

Csordás G, Thomas AP, Hajnóczky G. 1999. Quasi-synaptic calcium signal transmission between endoplasmic reticulum and mitochondria. *EMBO J* **18:** 96–108. doi:10.1093/emboj/18.1.96

Csordás G, Várnai P, Golenár T, Roy S, Purkins G, Schneider TG, Balla T, Hajnóczky G. 2010. Imaging interorganelle contacts and local calcium dynamics at the ER-mitochondrial interface. *Mol Cell* **39:** 121–132. doi:10.1016/j.molcel.2010.06.029

Csordás G, Golenár T, Seifert EL, Kamer KJ, Sancak Y, Perocchi F, Moffat C, Weaver D, de la Fuente Perez S, Bogorad R, et al. 2013. MICU1 controls both the threshold and cooperative activation of the mitochondrial Ca^{2+} uniporter. *Cell Metab* **17:** 976–987. doi:10.1016/j.cmet.2013.04.020

Cui Y, Galione A, Terrar DA. 1999. Effects of photoreleased cADP-ribose on calcium transients and calcium sparks in myocytes isolated from guinea-pig and rat ventricle. *Biochem J* **342:** 269–273. doi:10.1042/bj3420269

Curran J, Brown KH, Santiago DJ, Pogwizd S, Bers DM, Shannon TR. 2010. Spontaneous Ca waves in ventricular myocytes from failing hearts depend on Ca^{2+}-calmodulin-dependent protein kinase II. *J Mol Cell Cardiol* **49:** 25–32. doi:10.1016/j.yjmcc.2010.03.013

Dally S, Corvazier E, Bredoux R, Bobe R, Enouf J. 2010. Multiple and diverse coexpression, location, and regulation of additional SERCA2 and SERCA3 isoforms in nonfailing and failing human heart. *J Mol Cell Cardiol* **48:** 633–644. doi:10.1016/j.yjmcc.2009.11.012

Dedkova EN, Blatter LA. 2013. Calcium signaling in cardiac mitochondria. *J Mol Cell Cardiol* **58:** 125–133. doi:10.1016/j.yjmcc.2012.12.021

De La Fuente S, Fernandez-Sanz C, Vail C, Agra EJ, Holmstrom K, Sun J, Mishra J, Williams D, Finkel T, Murphy E, et al. 2016. Strategic positioning and biased activity of the

mitochondrial calcium uniporter in cardiac muscle. *J Biol Chem* **291:** 23343–23362. doi:10.1074/jbc.M116.755496

De La Fuente S, Lambert JP, Nichtova Z, Fernandez Sanz C, Elrod JW, Sheu SS, Csordás G. 2018. Spatial separation of mitochondrial calcium uptake and extrusion for energy-efficient mitochondrial calcium signaling in the heart. *Cell Rep* **24:** 3099–3107.e4. doi:10.1016/j.celrep.2018.08.040

Denham NC, Pearman CM, Caldwell JL, Madders GWP, Eisner DA, Trafford AW, Dibb KM. 2018. Calcium in the pathophysiology of atrial fibrillation and heart failure. *Front Physiol* **9:** 1380. doi:10.3389/fphys.2018.01380

Denton RM, McCormack JG, Midgley PJ, Rutter GA, Thomas AP. 1988. The role of Ca^{2+} in the hormonal control of intramitochondrial metabolism in heart, liver, and adipose tissue. *Adv Second Messenger Phosphoprotein Res* **21:** 157–164.

Despa S, Bers DM. 2013. Na^+ transport in the normal and failing heart—Remember the balance. *J Mol Cell Cardiol* **61:** 2–10. doi:10.1016/j.yjmcc.2013.04.011

De Stefani D, Raffaello A, Teardo E, Szabò I, Rizzuto R. 2011. A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. *Nature* **476:** 336–340. doi:10.1038/nature10230

Devaux Y, Creemers EE, Boon RA, Werfel S, Thum T, Engelhardt S, Dimmeler S, Squire I. 2017. Circular RNAs in heart failure. *Eur J Heart Fail* **19:** 701–709. doi:10.1002/ejhf.801

Domeier TL, Zima AV, Maxwell JT, Huke S, Mignery GA, Blatter LA. 2008. IP_3 receptor-dependent Ca^{2+} release modulates excitation–contraction coupling in rabbit ventricular myocytes. *Am J Physiol Heart Circulatory Physiol* **294:** H596–H604. doi:10.1152/ajpheart.01155.2007

Dostal DE, Baker KM. 1999. The cardiac renin-angiotensin system: Conceptual, or a regulator of cardiac function? *Circ Res* **85:** 643–650. doi:10.1161/01.RES.85.7.643

Drago I, De Stefani D, Rizzuto R, Pozzan T. 2012. Mitochondrial Ca^{2+} uptake contributes to buffering cytoplasmic Ca^{2+} peaks in cardiomyocytes. *Proc Natl Acad Sci* **109:** 12986–12991. doi:10.1073/pnas.1210718109

Drawnel FM, Wachten D, Molkentin JD, Maillet M, Aronsen JM, Swift F, Sjaastad I, Liu N, Catalucci D, Mikoshiba K, et al. 2012. Mutual antagonism between IP_3RII and miRNA-133a regulates calcium signals and cardiac hypertrophy. *J Cell Biol* **199:** 783–798. doi:10.1083/jcb.201111095

Drawnel FM, Archer CR, Roderick HL. 2013. The role of the paracrine/autocrine mediator endothelin-1 in regulation of cardiac contractility and growth. *Br J Pharmacol* **168:** 296–317. doi:10.1111/j.1476-5381.2012.02195.x

Dries E, Bito V, Lenaerts I, Antoons G, Sipido KR, Macquaide N. 2013. Selective modulation of coupled ryanodine receptors during microdomain activation of calcium/calmodulin-dependent kinase II in the dyadic cleft. *Circ Res* **113:** 1242–1252. doi:10.1161/circresaha.113.301896

Dries E, Santiago DJ, Gilbert G, Lenaerts I, Vandenberk B, Nagaraju CK, Johnson DM, Holemans P, Roderick HL, Macquaide N, et al. 2018. Hyperactive ryanodine receptors in human heart failure and ischaemic cardiomyopathy reside outside of couplons. *Cardiovasc Res* **114:** 1512–1524. doi:10.1093/cvr/cvy088

Eisner D. 2014. Calcium in the heart: From physiology to disease. *Exp Physiol* **99:** 1273–1282. doi:10.1113/expphysiol.2013.077305

Fabiato A. 1983. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. *Am J Physiol* **245:** C1–C14. doi:10.1152/ajpcell.1983.245.1.C1

Fabiato A, Fabiato F. 1977. Calcium release from the sarcoplasmic reticulum. *Circ Res* **40:** 119–129. doi:10.1161/01.RES.40.2.119

Fearnley CJ, Roderick HL, Bootman MD. 2011. Calcium signaling in cardiac myocytes. *Cold Spring Harb Perspect Biol* **3:** a004242. doi:10.1101/cshperspect.a004242

Ferrantini C, Crocini C, Coppini R, Vanzi F, Tesi C, Cerbai E, Poggesi C, Pavone FS, Sacconi L. 2013. The transverse-axial tubular system of cardiomyocytes. *Cell Mol Life Sci* **70:** 4695–4710. doi:10.1007/s00018-013-1410-5

Fischer TH, Eiringhaus J, Dybkova N, Förster A, Herting J, Kleinwächter A, Ljubojevic S, Schmitto JD, Streckfuss-Bömeke K, Renner A, et al. 2014. Ca^{2+}/calmodulin-dependent protein kinase II equally induces sarcoplasmic reticulum Ca^{2+} leak in human ischaemic and dilated cardiomyopathy. *Eur J Heart Failure* **16:** 1292–1300. doi:10.1002/ejhf.163

Foskett JK, White C, Cheung KH, Mak DO. 2007. Inositol trisphosphate receptor Ca^{2+} release channels. *Physiol Rev* **87:** 593–658. doi:10.1152/physrev.00035.2006

Fozzard HA. 1977. Heart: Excitation-contraction coupling. *Ann Rev Physiol* **39:** 201–220. doi:10.1146/annurev.ph.39.030177.001221

Franzini-Armstrong C, Protasi F, Tijskens P. 2005. The assembly of calcium release units in cardiac muscle. *Ann NY Acad Sci* **1047:** 76–85. doi:10.1196/annals.1341.007

Frisk M, Koivumäki JT, Norseng PA, Maleckar MM, Sejersted OM, Louch WE. 2014. Variable t-tubule organization and Ca^{2+} homeostasis across the atria. *Am J Physiol Heart Circ Physiol* **307:** H609–H620. doi:10.1152/ajpheart.00295.2014

Frisk M, Ruud M, Espe EK, Aronsen JM, Røe AT, Zhang L, Norseng PA, Sejersted OM, Christensen GA, Sjaastad I, et al. 2016. Elevated ventricular wall stress disrupts cardiomyocyte t-tubule structure and calcium homeostasis. *Cardiovasc Res* **112:** 443–451. doi:10.1093/cvr/cvw111

Fu Y, Hong T. 2016. BIN1 regulates dynamic t-tubule membrane. *Biochim Biophys Acta* **1863:** 1839–1847. doi:10.1016/j.bbamcr.2015.11.004

Fu Y, Shaw SA, Naami R, Vuong CL, Basheer WA, Guo X, Hong T. 2016. Isoproterenol promotes rapid ryanodine receptor movement to bridging integrator 1 (BIN1)-organized dyads. *Circulation* **133:** 388–397. doi:10.1161/circulationaha.115.018535

Fukuta H, Little WC. 2008. The cardiac cycle and the physiologic basis of left ventricular contraction, ejection, relaxation, and filling. *Heart Fail Clin* **4:** 1–11. doi:10.1016/j.hfc.2007.10.004

Gabisonia K, Recchia FA. 2018. Gene therapy for heart failure: New perspectives. *Curr Heart Fail Rep* **15:** 340–349. doi:10.1007/s11897-018-0410-z

Gadeberg HC, Bond RC, Kong CH, Chanoit GP, Ascione R, Cannell MB, James AF. 2016. Heterogeneity of T-tubules in pig hearts. *PLoS ONE* **11:** e0156862. doi:10.1371/journal.pone.0156862

Gadicherla AK, Wang N, Bulic M, Agullo-Pascual E, Lissoni A, De Smet M, Delmar M, Bultynck G, Krysko DV, Camara A, et al. 2017. Mitochondrial Cx43 hemichannels contribute to mitochondrial calcium entry and cell death in the heart. *Basic Res Cardiol* **112:** 27. doi:10.1007/s00395-017-0618-1

Gadsby DC. 1984. The Na/K pump of cardiac cells. *Annu Rev Biophys Bioeng* **13:** 373–398. doi:10.1146/annurev.bb.13.060184.002105

Galione A, Cui Y, Empson R, Iino S, Wilson H, Terrar D. 1998. Cyclic ADP-ribose and the regulation of calcium-induced calcium release in eggs and cardiac myocytes. *Cell Biochem Biophys* **28:** 19–30. doi:10.1007/BF02738307

Garcia MI, Karlstaedt A, Chen JJ, Amione-Guerra J, Youker KA, Taegtmeyer H, Boehning D. 2017. Functionally redundant control of cardiac hypertrophic signaling by inositol 1,4,5-trisphosphate receptors. *J Mol Cell Cardiol* **112:** 95–103. doi:10.1016/j.yjmcc.2017.09.006

Garciarena CD, Youm JB, Swietach P, Vaughan-Jones RD. 2013. H^+-activated Na^+ influx in the ventricular myocyte couples Ca^{2+}-signalling to intracellular pH. *J Mol Cell Cardiol* **61:** 51–59. doi:10.1016/j.yjmcc.2013.04.008

Gilsbach R, Schwaderer M, Preissl S, Grüning BA, Kranzhöfer D, Schneider P, Nührenberg TG, Mulero-Navarro S, Weichenhan D, Braun C, et al. 2018. Distinct epigenetic programs regulate cardiac myocyte development and disease in the human heart in vivo. *Nat Commun* **9:** 391. doi:10.1038/s41467-017-02762-z

Glitsch HG. 1979. Characteristics of active Na transport in intact cardiac cells. *Am J Physiol* **236:** H189–H199. doi:10.1152/ajpheart.1979.236.2.H189

Glukhov AV, Balycheva M, Sanchez-Alonso JL, Ilkan Z, Alvarez-Laviada A, Bhogal N, Diakonov I, Schobesberger S, Sikkel MB, Bhargava A, et al. 2015. Direct evidence for microdomain-specific localization and remodeling of functional L-type calcium channels in rat and human atrial myocytes. *Circulation* **132:** 2372–2384. doi:10.1161/circulationaha.115.018131

Gomez AM, Valdivia HH, Cheng H, Lederer MR, Santana LF, Cannell MB, McCune SA, Altschuld RA, Lederer WJ. 1997. Defective excitation-contraction coupling in experimental cardiac hypertrophy and heart failure. *Science* **276:** 800–806. doi:10.1126/science.276.5313.800

Gorza L, Schiaffino S, Volpe P. 1993. Inositol 1,4,5-trisphosphate receptor in heart: Evidence for its concentration in Purkinje myocytes of the conduction system. *J Cell Biol* **121:** 345–353. doi:10.1083/jcb.121.2.345

Greenberg B, Butler J, Felker GM, Ponikowski P, Voors AA, Desai AS, Barnard D, Bouchard A, Jaski B, Lyon AR, et al. 2016. Calcium upregulation by percutaneous administration of gene therapy in patients with cardiac disease (CUPID 2): A randomised, multinational, double-blind, placebo-controlled, phase 2b trial. *Lancet* **387:** 1178–1186. doi:10.1016/S0140-6736(16)00082-9

Grimm C, Hassan S, Wahl-Schott C, Biel M. 2012. Role of TRPML and two-pore channels in endolysosomal cation homeostasis. *J Pharmacol Exp Ther* **342:** 236–244. doi:10.1124/jpet.112.192880

Grimm M, Ling H, Willeford A, Pereira L, Gray CB, Erickson JR, Sarma S, Respress JL, Wehrens XH, Bers DM, et al. 2015. CaMKIIδ mediates β-adrenergic effects on RyR2 phosphorylation and SR Ca^{2+} leak and the pathophysiological response to chronic β-adrenergic stimulation. *J Mol Cell Cardiol* **85:** 282–291. doi:10.1016/j.yjmcc.2015.06.007

Guatimosim S, Amaya MJ, Guerra MT, Aguiar CJ, Goes AM, Gómez-Viquez NL, Rodrigues MA, Gomes DA, Martins-Cruz J, Lederer WJ, et al. 2008. Nuclear Ca^{2+} regulates cardiomyocyte function. *Cell Calcium* **44:** 230–242. doi:10.1016/j.ceca.2007.11.016

Gul R, Kim SY, Park KH, Kim BJ, Kim SJ, Im MJ, Kim UH. 2008. A novel signaling pathway of ADP-ribosyl cyclase activation by angiotensin II in adult rat cardiomyocytes. *Am J Physiol Heart Circ Physiol* **295:** H77–H88. doi:10.1152/ajpheart.01355.2007

Gul R, Park JH, Kim SY, Jang KY, Chae JK, Ko JK, Kim UH. 2009. Inhibition of ADP-ribosyl cyclase attenuates angiotensin II-induced cardiac hypertrophy. *Cardiovasc Res* **81:** 582–591. doi:10.1093/cvr/cvn232

Guo T, Cornea RL, Huke S, Camors E, Yang Y, Picht E, Fruen BR, Bers DM. 2010. Kinetics of FKBP12.6 binding to ryanodine receptors in permeabilized cardiac myocytes and effects on Ca sparks. *Circ Res* **106:** 1743–1752. doi:10.1161/circresaha.110.219816

Györke I, Hester N, Jones LR, Györke S. 2004. The role of calsequestrin, triadin, and junctin in conferring cardiac ryanodine receptor responsiveness to luminal calcium. *Biophys J* **86:** 2121–2128. doi:10.1016/S0006-3495(04)74271-X

Haddock PS, Coetzee WA, Cho E, Porter L, Katoh H, Bers DM, Jafri MS, Artman M. 1999. Subcellular $[Ca^{2+}]_i$ gradients during excitation-contraction coupling in newborn rabbit ventricular myocytes. *Circ Res* **85:** 415–427. doi:10.1161/01.RES.85.5.415

Hajnóczky G, Robb-Gaspers LD, Seitz MB, Thomas AP. 1995. Decoding of cytosolic calcium oscillations in the mitochondria. *Cell* **82:** 415–424. doi:10.1016/0092-8674(95)90430-1

Hammes A, Oberdorf-Maass S, Rother T, Nething K, Gollnick F, Linz KW, Meyer R, Hu K, Han H, Gaudron P, et al. 1998. Overexpression of the sarcolemmal calcium pump in the myocardium of transgenic rats. *Circ Res* **83:** 877–888. doi:10.1161/01.RES.83.9.877

Harzheim D, Movassagh M, Foo RS, Ritter O, Tashfeen A, Conway SJ, Bootman MD, Roderick HL. 2009. Increased $InsP_3Rs$ in the junctional sarcoplasmic reticulum augment Ca^{2+} transients and arrhythmias associated with cardiac hypertrophy. *Proc Natl Acad Sci* **106:** 11406–11411. doi:10.1073/pnas.0905485106

Harzheim D, Talasila A, Movassagh M, Foo RS, Figg N, Bootman MD, Roderick HL. 2010. Elevated $InsP_3R$ expression underlies enhanced calcium fluxes and spontaneous extra-systolic calcium release events in hypertrophic cardiac myocytes. *Channels (Austin)* **4:** 67–71. doi:10.4161/chan.4.1.10531

Hasenfuss G, Pieske B. 2002. Calcium cycling in congestive heart failure. *J Mol Cell Cardiol* **34:** 951–969. doi:10.1006/jmcc.2002.2037

Haumann J, Camara AKS, Gadicherla AK, Navarro CD, Boelens AD, Blomeyer CA, Dash RK, Boswell MR, Kwok WM, Stowe DF. 2018. Slow Ca^{2+} efflux by Ca^{2+}/H^+ exchange in cardiac mitochondria is modulated by Ca^{2+} re-uptake via MCU, extra-mitochondrial pH, and

H^+ pumping by FOF1-ATPase. *Front Physiol* **9:** 1914. doi:10.3389/fphys.2018.01914

Hayashi T, Martone ME, Yu Z, Thor A, Doi M, Holst MJ, Ellisman MH, Hoshijima M. 2009. Three-dimensional electron microscopy reveals new details of membrane systems for Ca^{2+} signaling in the heart. *J Cell Sci* **122:** 1005–1013. doi:10.1242/jcs.028175

Hegyi B, Bossuyt J, Ginsburg KS, Mendoza LM, Talken L, Ferrier WT, Pogwizd SM, Izu LT, Chen-Izu Y, Bers DM. 2018. Altered repolarization reserve in failing rabbit ventricular myocytes: Calcium and β-adrenergic effects on delayed- and inward-rectifier potassium currents. *Circ Arrhythm Electrophysiol* **11:** e005852. doi:10.1161/CIRCEP.117.005852

Heinzel FR, Bito V, Volders PG, Antoons G, Mubagwa K, Sipido KR. 2002. Spatial and temporal inhomogeneities during Ca^{2+} release from the sarcoplasmic reticulum in pig ventricular myocytes. *Circ Res* **91:** 1023–1030. doi:10.1161/01.RES.0000045940.67060.DD

Heinzel FR, Bito V, Biesmans L, Wu M, Detre E, von Wegner F, Claus P, Dymarkowski S, Maes F, Bogaert J, et al. 2008. Remodeling of T-tubules and reduced synchrony of Ca^{2+} release in myocytes from chronically ischemic myocardium. *Circ Res* **102:** 338–346. doi:10.1161/circresaha.107.160085

Heinzel FR, MacQuaide N, Biesmans L, Sipido K. 2011. Dyssynchrony of Ca^{2+} release from the sarcoplasmic reticulum as subcellular mechanism of cardiac contractile dysfunction. *J Mol Cell Cardiol* **50:** 390–400. doi:10.1016/j.yjmcc.2010.11.008

Higashida H, Egorova A, Higashida C, Zhong ZG, Yokoyama S, Noda M, Zhang JS. 1999. Sympathetic potentiation of cyclic ADP-ribose formation in rat cardiac myocytes. *J Biol Chem* **274:** 33348–33354. doi:10.1074/jbc.274.47.33348

Higashida H, Zhang J, Hashii M, Shintaku M, Higashida C, Takeda Y. 2000. Angiotensin II stimulates cyclic ADP-ribose formation in neonatal rat cardiac myocytes. *Biochem J* **352:** 197–202. doi:10.1042/bj3520197

Higazi DR, Fearnley CJ, Drawnel FM, Talasila A, Corps EM, Ritter O, McDonald F, Mikoshiba K, Bootman MD, Roderick HL. 2009. Endothelin-1-stimulated InsP3-induced Ca^{2+} release is a nexus for hypertrophic signaling in cardiac myocytes. *Mol Cell* **33:** 472–482. doi:10.1016/j.molcel.2009.02.005

Hirose M, Stuyvers B, Dun W, ter Keurs H, Boyden PA. 2008. Wide long lasting perinuclear Ca^{2+} release events generated by an interaction between ryanodine and IP3 receptors in canine Purkinje cells. *J Mol Cell Cardiol* **45:** 176–184. doi:10.1016/j.yjmcc.2008.05.008

Ho HT, Liu B, Snyder JS, Lou Q, Brundage EA, Velez-Cortes F, Wang H, Ziolo MT, Anderson ME, Sen CK, et al. 2014. Ryanodine receptor phosphorylation by oxidized CaMKII contributes to the cardiotoxic effects of cardiac glycosides. *Cardiovasc Res* **101:** 165–174. doi:10.1093/cvr/cvt233

Hoefer IE, Steffens S, Ala-Korpela M, Bäck M, Badimon L, Bochaton-Piallat ML, Boulanger CM, Caligiuri G, Dimmeler S, Egido J, et al. 2015. Novel methodologies for biomarker discovery in atherosclerosis. *Eur Heart J* **36:** 2635–2642. doi:10.1093/eurheartj/ehv236

Hohendanner F, Ljubojević S, MacQuaide N, Sacherer M, Sedej S, Biesmans L, Wakula P, Platzer D, Sokolow S, Herchuelz A, et al. 2013. Intracellular dyssynchrony of diastolic cytosolic $[Ca^{2+}]$ decay in ventricular cardiomyocytes in cardiac remodeling and human heart failure. *Circ Res* **113:** 527–538. doi:10.1161/circresaha.113.300895

Hohendanner F, Maxwell JT, Blatter LA. 2015a. Cytosolic and nuclear calcium signaling in atrial myocytes: IP_3-mediated calcium release and the role of mitochondria. *Channels* **9:** 129–138. doi:10.1080/19336950.2015.1040966

Hohendanner F, Walther S, Maxwell JT, Kettlewell S, Awad S, Smith GL, Lonchyna VA, Blatter LA. 2015b. Inositol-1,4,5-trisphosphate induced Ca^{2+} release and excitation-contraction coupling in atrial myocytes from normal and failing hearts. *J Physiol* **593:** 1459–1477. doi:10.1113/jphysiol.2014.283226

Hong T, Yang H, Zhang SS, Cho HC, Kalashnikova M, Sun B, Zhang H, Bhargava A, Grabe M, Olgin J, et al. 2014. Cardiac BIN1 folds T-tubule membrane, controlling ion flux and limiting arrhythmia. *Nat Med* **20:** 624–632. doi:10.1038/nm.3543

Horn T, Ullrich ND, Egger M. 2013. "Eventless" $InsP_3$-dependent SR-Ca^{2+} release affecting atrial Ca^{2+} sparks. *J Physiol* **591:** 2103–2111. doi:10.1113/jphysiol.2012.247288

Høydal MA, Kirkeby-Garstad I, Karevold A, Wiseth R, Haaverstad R, Wahba A, Stølen TL, Contu R, Condorelli G, Ellingsen O, et al. 2018. Human cardiomyocyte calcium handling and transverse tubules in mid-stage of post-myocardial-infarction heart failure. *ESC Heart Fail* **5:** 332–342. doi:10.1002/ehf2.12271

Hulot JS, Fauconnier J, Ramanujam D, Chaanine A, Aubart F, Sassi Y, Merkle S, Cazorla O, Ouillé A, Dupuis M, et al. 2011. Critical role for stromal interaction molecule 1 in cardiac hypertrophy. *Circulation* **124:** 796–805. doi:10.1161/circulationaha.111.031229

Hulot JS, Senyei G, Hajjar RJ. 2012. Sarcoplasmic reticulum and calcium cycling targeting by gene therapy. *Gene Ther* **19:** 596–599. doi:10.1038/gt.2012.34

Hunton DL, Lucchesi PA, Pang Y, Cheng X, Dell'Italia LJ, Marchase RB. 2002. Capacitative calcium entry contributes to nuclear factor of activated T-cells nuclear translocation and hypertrophy in cardiomyocytes. *J Biol Chem* **277:** 14266–14273. doi:10.1074/jbc.M107167200

Hunton DL, Zou L, Pang Y, Marchase RB. 2004. Adult rat cardiomyocytes exhibit capacitative calcium entry. *Am J Heart Physiol Circ Physiol* **286:** H1124–H1132. doi:10.1152/ajpheart.00162.2003

Hüser J, Lipsius SL, Blatter LA. 1996. Calcium gradients during excitation-contraction coupling in cat atrial myocytes. *J Physiol* **494:** 641–651. doi:10.1113/jphysiol.1996.sp021521

Ibrahim M, Siedlecka U, Buyandelger B, Harada M, Rao C, Moshkov A, Bhargava A, Schneider M, Yacoub MH, Gorelik J, et al. 2013. A critical role for Telethonin in regulating t-tubule structure and function in the mammalian heart. *Hum Mol Genet* **22:** 372–383. doi:10.1093/hmg/dds434

Izumi T, Kihara Y, Sarai N, Yoneda T, Iwanaga Y, Inagaki K, Onozawa Y, Takenaka H, Kita T, Noma A. 2003. Reinduction of T-type calcium channels by endothelin-1 in

failing hearts in vivo and in adult rat ventricular myocytes in vitro. *Circulation* **108:** 2530–2535. doi:10.1161/01.CIR.0000096484.03318.AB

Jayasinghe I, Crossman D, Soeller C, Cannell M. 2012. Comparison of the organization of T-tubules, sarcoplasmic reticulum and ryanodine receptors in rat and human ventricular myocardium. *Clin Exp Pharmacol Physiol* **39:** 469–476. doi:10.1111/j.1440-1681.2011.05578.x

Jayasinghe I, Clowsley AH, de Langen O, Sali SS, Crossman DJ, Soeller C. 2018a. Shining new light on the structural determinants of cardiac couplon function: Insights from ten years of nanoscale microscopy. *Front Physiol* **9:** 1472. doi:10.3389/fphys.2018.01472

Jayasinghe I, Clowsley AH, Lin R, Lutz T, Harrison C, Green E, Baddeley D, Di Michele L, Soeller C. 2018b. True molecular scale visualization of variable clustering properties of ryanodine receptors. *Cell Rep* **22:** 557–567. doi:10.1016/j.celrep.2017.12.045

Jiang D, Zhao L, Clapham DE. 2009. Genome-wide RNAi screen identifies Letm1 as a mitochondrial Ca^{2+}/H^+ antiporter. *Science* **326:** 144–147. doi:10.1126/science.1175145

Jones PP, MacQuaide N, Louch WE. 2018. Dyadic plasticity in cardiomyocytes. *Front Physiol* **9:** 1773. doi:10.3389/fphys.2018.01773

Kapoor N, Tran A, Kang J, Zhang R, Philipson KD, Goldhaber JI. 2015. Regulation of calcium clock-mediated pacemaking by inositol-1,4,5-trisphosphate receptors in mouse sinoatrial nodal cells. *J Physiol* **593:** 2649–2663. doi:10.1113/JP270082

Kettlewell S, Cabrero P, Nicklin SA, Dow JA, Davies S, Smith GL. 2009. Changes of intra-mitochondrial Ca^{2+} in adult ventricular cardiomyocytes examined using a novel fluorescent Ca^{2+} indicator targeted to mitochondria. *J Mol Cell Cardiol* **46:** 891–901. doi:10.1016/j.yjmcc.2009.02.016

Kiriazis H, Kranias EG. 2000. Genetically engineered models with alterations in cardiac membrane calcium-handling proteins. *Annu Rev Physiol* **62:** 321–351. doi:10.1146/annurev.physiol.62.1.321

Kirichok Y, Krapivinsky G, Clapham DE. 2004. The mitochondrial calcium uniporter is a highly selective ion channel. *Nature* **427:** 360–364. doi:10.1038/nature02246

Kobayashi T, Solaro RJ. 2005. Calcium, thin filaments, and the integrative biology of cardiac contractility. *Annu Rev Physiol* **67:** 39–67. doi:10.1146/annurev.physiol.67.040403.114025

Kockskämper J, Seidlmayer L, Walther S, Hellenkamp K, Maier LS, Pieske B. 2008a. Endothelin-1 enhances nuclear Ca^{2+} transients in atrial myocytes through Ins(1,4,5)P3-dependent Ca^{2+} release from perinuclear Ca^{2+} stores. *J Cell Sci* **121:** 186–195. doi:10.1242/jcs.021386

Kockskämper J, Zima AV, Roderick HL, Pieske B, Blatter LA, Bootman MD. 2008b. Emerging roles of inositol 1,4,5-trisphosphate signaling in cardiac myocytes. *J Mol Cell Cardiol* **45:** 128–147. doi:10.1016/j.yjmcc.2008.05.014

Kojima A, Kitagawa H, Omatsu-Kanbe M, Matsuura H, Nosaka S. 2012. Presence of store-operated Ca^{2+} entry in C57BL/6J mouse ventricular myocytes and its suppression by sevoflurane. *Br J Anaesth* **109:** 352–360. doi:10.1093/bja/aes212

Kolstad TR, van den Brink J, MacQuaide N, Lunde PK, Frisk M, Aronsen JM, Norden ES, Cataliotti A, Sjaastad I, Sejersted OM, et al. 2018. Ryanodine receptor dispersion disrupts Ca^{2+} release in failing cardiac myocytes. *eLife* **7:** e39427. doi:10.7554/eLife.39427

Kong CHT, Rog-Zielinska EA, Orchard CH, Kohl P, Cannell MB. 2017. Sub-microscopic analysis of t-tubule geometry in living cardiac ventricular myocytes using a shape-based analysis method. *J Mol Cell Cardiol* **108:** 1–7. doi:10.1016/j.yjmcc.2017.05.003

Kubalova Z, Terentyev D, Viatchenko-Karpinski S, Nishijima Y, Gyorke I, Terentyeva R, da Cunha DN, Sridhar A, Feldman DS, Hamlin RL, et al. 2005. Abnormal intrastore calcium signaling in chronic heart failure. *Proc Natl Acad Sci* **102:** 14104–14109. doi:10.1073/pnas.0504298102

Kwong JQ, Lu X, Correll RN, Schwanekamp JA, Vagnozzi RJ, Sargent MA, York AJ, Zhang J, Bers DM, Molkentin JD. 2015. The mitochondrial calcium uniporter selectively matches metabolic output to acute contractile stress in the heart. *Cell Rep* **12:** 15–22. doi:10.1016/j.celrep.2015.06.002

Lenaerts I, Bito V, Heinzel FR, Driesen RB, Holemans P, D'Hooge J, Heidbüchel H, Sipido KR, Willems R. 2009. Ultrastructural and functional remodeling of the coupling between Ca^{2+} influx and sarcoplasmic reticulum Ca^{2+} release in right atrial myocytes from experimental persistent atrial fibrillation. *Circ Res* **105:** 876–885. doi:10.1161/circresaha.109.206276

Lewis AM, Aley PK, Roomi A, Thomas JM, Masgrau R, Garnham C, Shipman K, Paramore C, Bloor-Young D, Sanders LE, et al. 2012. β-Adrenergic receptor signaling increases NAADP and cADPR levels in the heart. *Biochem Biophys Res Commun* **427:** 326–329. doi:10.1016/j.bbrc.2012.09.054

Li N, Wang Q, Sibrian-Vazquez M, Klipp RC, Reynolds JO, Word TA, Scott L, Salama G, Strongin RM, Abramson JJ, et al. 2017. Treatment of catecholaminergic polymorphic ventricular tachycardia in mice using novel RyR2-modifying drugs. *Int J Cardiol* **227:** 668–673. doi:10.1016/j.ijcard.2016.10.078

Li N, Zhou H, Tang Q. 2018. miR-133: A suppressor of cardiac remodeling? *Front Pharmacol* **9:** 903. doi:10.3389/fphar.2018.00903

Lin WK, Bolton EL, Cortopassi WA, Wang Y, O'Brien F, Maciejewska M, Jacobson MP, Garnham C, Ruas M, Parrington J, et al. 2017. Synthesis of the Ca^{2+}-mobilizing messengers NAADP and cADPR by intracellular CD38 enzyme in the mouse heart: Role in β-adrenoceptor signaling. *J Biol Chem* **292:** 13243–13257. doi:10.1074/jbc.M117.789347

Lipp P, Hüser J, Pott L, Niggli E. 1996. Spatially non-uniform Ca^{2+} signals induced by the reduction of transverse tubules in citrate-loaded guinea-pig ventricular myocytes in culture. *J Physiol* **497:** 589–597. doi:10.1113/jphysiol.1996.sp021792

Lipp P, Laine M, Tovey SC, Burrell KM, Berridge MJ, Li W, Bootman MD. 2000. Functional InsP3 receptors that may modulate excitation–contraction coupling in the heart. *Curr Biol* **10:** 939–942. doi:10.1016/S0960-9822(00)00624-2

Liu GS, Morales A, Vafiadaki E, Lam CK, Cai WF, Haghighi K, Adly G, Hershberger RE, Kranias EG. 2015. A novel

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

human R25C-phospholamban mutation is associated with super-inhibition of calcium cycling and ventricular arrhythmia. *Cardiovasc Res* **107:** 164–174. doi:10.1093/cvr/cvv127

Ljubojevic S, Radulovic S, Leitinger G, Sedej S, Sacherer M, Holzer M, Winkler C, Pritz E, Mittler T, Schmidt A, et al. 2014. Early remodeling of perinuclear Ca^{2+} stores and nucleoplasmic Ca^{2+} signaling during the development of hypertrophy and heart failure. *Circulation* **130:** 244–255. doi:10.1161/circulationaha.114.008927

Louch WE, Bito V, Heinzel FR, Macianskiene R, Vanhaecke J, Flameng W, Mubagwa K, Sipido KR. 2004. Reduced synchrony of Ca^{2+} release with loss of T-tubules—A comparison to Ca^{2+} release in human failing cardiomyocytes. *Cardiovasc Res* **62:** 63–73. doi:10.1016/j.cardiores.2003.12.031

Louch WE, Mørk HK, Sexton J, Strømme TA, Laake P, Sjaastad I, Sejersted OM. 2006. T-tubule disorganization and reduced synchrony of Ca^{2+} release in murine cardiomyocytes following myocardial infarction. *J Physiol* **574:** 519–533. doi:10.1113/jphysiol.2006.107227

Lu X, Ginsburg KS, Kettlewell S, Bossuyt J, Smith GL, Bers DM. 2013. Measuring local gradients of intramitochondrial $[Ca^{2+}]$ in cardiac myocytes during sarcoplasmic reticulum Ca^{2+} release. *Circ Res* **112:** 424–431. doi:10.1161/circresaha.111.300501

Lukyanenko V, Györke I, Wiesner TF, Györke S. 2001. Potentiation of Ca^{2+} release by cADP-ribose in the heart is mediated by enhanced SR Ca^{2+} uptake into the sarcoplasmic reticulum. *Circ Res* **89:** 614–622. doi:10.1161/hh1901.098066

Luo M, Anderson ME. 2013. Mechanisms of altered Ca^{2+} handling in heart failure. *Circ Res* **113:** 690–708. doi:10.1161/circresaha.113.301651

Luo X, Hojayev B, Jiang N, Wang ZV, Tandan S, Rakalin A, Rothermel BA, Gillette TG, Hill JA. 2012. STIM1-dependent store-operated Ca^{2+} entry is required for pathological cardiac hypertrophy. *J Mol Cell Cardiol* **52:** 136–147. doi:10.1016/j.yjmcc.2011.11.003

Luongo TS, Lambert JP, Gross P, Nwokedi M, Lombardi AA, Shanmughapriya S, Carpenter AC, Kolmetzky D, Gao E, van Berlo JH, et al. 2017. The mitochondrial Na^{+}/Ca^{2+} exchanger is essential for Ca^{2+} homeostasis and viability. *Nature* **545:** 93–97. doi:10.1038/nature22082

Lymperopoulos A, Rengo G, Koch WJ. 2013. Adrenergic nervous system in heart failure: Pathophysiology and therapy. *Circ Res* **113:** 739–753. doi:10.1161/circresaha.113.300308

Lyon AR, MacLeod KT, Zhang Y, Garcia E, Kanda GK, Lab MJ, Korchev YE, Harding SE, Gorelik J. 2009. Loss of T-tubules and other changes to surface topography in ventricular myocytes from failing human and rat heart. *Proc Natl Acad Sci* **106:** 6854–6859. doi:10.1073/pnas.0809777106

Lyon AR, Bannister ML, Collins T, Pearce E, Sepehripour AH, Dubb SS, Garcia E, O'Gara P, Liang L, Kohlbrenner E, et al. 2011. SERCA2a gene transfer decreases sarcoplasmic reticulum calcium leak and reduces ventricular arrhythmias in a model of chronic heart failure. *Circ Arrhythm Electrophysiol* **4:** 362–372. doi:10.1161/CIRCEP.110.961615

Lytton J, Westlin M, Burk SE, Shull GE, MacLennan DH. 1992. Functional comparisons between isoforms of the sarcoplasmic or endoplasmic reticulum family of calcium pumps. *J Biol Chem* **267:** 14483–14489.

Macgregor A, Yamasaki M, Rakovic S, Sanders L, Parkesh R, Churchill GC, Galione A, Terrar DA. 2007a. NAADP controls cross-talk between distinct Ca^{2+} stores in the heart. *J Biol Chem* **282:** 15302–15311. doi:10.1074/jbc.M611167200

Macgregor AT, Rakovic S, Galione A, Terrar DA. 2007b. Dual effects of cyclic ADP-ribose on sarcoplasmic reticulum Ca^{2+} release and storage in cardiac myocytes isolated from guinea-pig and rat ventricle. *Cell Calcium* **41:** 537–546. doi:10.1016/j.ceca.2006.10.005

Mackenzie L, Bootman MD, Laine M, Berridge MJ, Thuring J, Holmes A, Li WH, Lipp P. 2002. The role of inositol 1,4,5-trisphosphate receptors in Ca^{2+} signalling and the generation of arrhythmias in rat atrial myocytes. *J Physiol* **541:** 395–409. doi:10.1113/jphysiol.2001.013411

Mackenzie L, Roderick HL, Berridge MJ, Conway SJ, Bootman MD. 2004. The spatial pattern of atrial cardiomyocyte calcium signalling modulates contraction. *J Cell Sci* **117:** 6327–6337. doi:10.1242/jcs.01559

MacLennan DH, Kranias EG. 2003. Phospholamban: A crucial regulator of cardiac contractility. *Nat Rev Mol Cell Biol* **4:** 566–577. doi:10.1038/nrm1151

Macquaide N, Tuan HT, Hotta J, Sempels W, Lenaerts I, Holemans P, Hofkens J, Jafri MS, Willems R, Sipido KR. 2015. Ryanodine receptor cluster fragmentation and redistribution in persistent atrial fibrillation enhance calcium release. *Cardiovasc Res* **108:** 387–398. doi:10.1093/cvr/cvv231

Mak DO, Foskett JK. 2015. Inositol 1,4,5-trisphosphate receptors in the endoplasmic reticulum: A single-channel point of view. *Cell Calcium* **58:** 67–78. doi:10.1016/j.ceca.2014.12.008

Manfra O, Frisk M, Louch WE. 2017. Regulation of cardiomyocyte T-tubular structure: Opportunities for therapy. *Curr Heart Fail Rep* **14:** 167–178. doi:10.1007/s11897-017-0329-9

Marks AR. 2013. Calcium cycling proteins and heart failure: Mechanisms and therapeutics. *J Clin Invest* **123:** 46–52. doi:10.1172/JCI62834

Mayourian J, Ceholski DK, Gonzalez DM, Cashman TJ, Sahoo S, Hajjar RJ, Costa KD. 2018. Physiologic, pathologic, and therapeutic paracrine modulation of cardiac excitation-contraction coupling. *Circ Res* **122:** 167–183. doi:10.1161/circresaha.117.311589

McDonough PM, Stella SL, Glembotski CC. 1994. Involvement of cytoplasmic calcium and protein kinases in the regulation of atrial natriuretic factor secretion by contraction rate and endothelin. *J Biol Chem* **269:** 9466–9472.

Metzger JM, Westfall MV. 2004. Covalent and noncovalent modification of thin filament action: The essential role of troponin in cardiac muscle regulation. *Circ Res* **94:** 146–158. doi:10.1161/01.RES.0000110083.17024.60

Miake J, Marbán E, Nuss HB. 2003. Functional role of inward rectifier current in heart probed by Kir2.1 overexpression and dominant-negative suppression. *J Clin Invest* **111:** 1529–1536. doi:10.1172/JCI200317959

Mojžišová A, Križanová O, Žáčiková L, Komínková V, Ondriaš K. 2001. Effect of nicotinic acid adenine dinucleo-

tide phosphate on ryanodine calcium release channel in heart. *Pflugers Arch* **441:** 674–677. doi:10.1007/s004240000465

Molkentin JD, Lu JR, Antos CL, Markham B, Richardson J, Robbins J, Grant SR, Olson EN. 1998. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell* **93:** 215–228. doi:10.1016/S0092-8674(00)81573-1

Moschella MC, Marks AR. 1993. Inositol 1,4,5-trisphosphate receptor expression in cardiac myocytes. *J Cell Biol* **120:** 1137–1146. doi:10.1083/jcb.120.5.1137

Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, Despres JP, Fullerton HJ, Howard VJ, et al. 2015. Heart disease and stroke statistics—2015 update: A report from the American Heart Association. *Circulation* **131:** e29–e322. doi:10.1161/CIR.0000000000000152

Munro ML, Shen X, Ward M, Ruygrok PN, Crossman DJ, Soeller C. 2018. Highly variable contractile performance correlates with myocyte content in trabeculae from failing human hearts. *Sci Rep* **8:** 2957. doi:10.1038/s41598-018-21199-y

Nakayama H, Bodi I, Maillet M, DeSantiago J, Domeier TL, Mikoshiba K, Lorenz JN, Blatter LA, Bers DM, Molkentin JD. 2010. The IP_3 receptor regulates cardiac hypertrophy in response to select stimuli. *Circ Res* **107:** 659–666. doi:10.1161/circresaha.110.220038

Namekata I, Fujiki S, Kawakami Y, Moriwaki R, Takeda K, Kawanishi T, Takahara A, Shigenobu K, Tanaka H. 2008. Intracellular mechanisms and receptor types for endothelin-1-induced positive and negative inotropy in mouse ventricular myocardium. *Naunyn Schmiedebergs Arch Pharmacol* **376:** 385–395. doi:10.1007/s00210-007-0228-9

Nebel M, Schwoerer AP, Warszta D, Siebrands CC, Limbrock AC, Swarbrick JM, Fliegert R, Weber K, Bruhn S, Hohenegger M, et al. 2013. Nicotinic acid adenine dinucleotide phosphate (NAADP)-mediated calcium signaling and arrhythmias in the heart evoked by β-adrenergic stimulation. *J Biol Chem* **288:** 16017–16030. doi:10.1074/jbc.M112.441246

Nelson BR, Makarewich CA, Anderson DM, Winders BR, Troupes CD, Wu F, Reese AL, McAnally JR, Chen X, Kavalali ET, et al. 2016. A peptide encoded by a transcript annotated as long noncoding RNA enhances SERCA activity in muscle. *Science* **351:** 271–275. doi:10.1126/science.aad4076

Obata K, Nagata K, Iwase M, Odashima M, Nagasaka T, Izawa H, Murohara T, Yamada Y, Yokota M. 2005. Overexpression of calmodulin induces cardiac hypertrophy by a calcineurin-dependent pathway. *Biochem Biophys Res Commun* **338:** 1299–1305. doi:10.1016/j.bbrc.2005.10.083

Oda T, Yamamoto T, Kato T, Uchinoumi H, Fukui G, Hamada Y, Nanno T, Ishiguchi H, Nakamura Y, Okamoto Y, et al. 2018. Nuclear translocation of calmodulin in pathological cardiac hypertrophy originates from ryanodine receptor bound calmodulin. *J Mol Cell Cardiol* **125:** 87–97. doi:10.1016/j.yjmcc.2018.10.011

Oh M, Dey A, Gerard RD, Hill JA, Rothermel BA. 2010. The CCAAT/enhancer binding protein β (C/EBPβ) cooperates with NFAT to control expression of the calcineurin regulatory protein *RCAN1–4*. *J Biol Chem* **285:** 16623–16631. doi:10.1074/jbc.M109.098236

Ohba T, Watanabe H, Murakami M, Takahashi Y, Iino K, Kuromitsu S, Mori Y, Ono K, Iijima T, Ito H. 2007. Up-regulation of TRPC1 in the development of cardiac hypertrophy. *J Mol Cell Cardiol* **42:** 498–507. doi:10.1016/j.yjmcc.2006.10.020

Ohkusa T, Hisamatsu Y, Yano M, Kobayashi S, Tatsuno H, Saiki Y, Kohno M, Matsuzaki M. 1997. Altered cardiac mechanism and sarcoplasmic reticulum function in pressure overload-induced cardiac hypertrophy in rats. *J Mol Cell Cardiol* **29:** 45–54. doi:10.1006/jmcc.1996.0250

Olivares-Florez S, Czolbe M, Riediger F, Seidlmayer L, Williams T, Nordbeck P, Strasen J, Glocker C, Jansch M, Eder-Negrin P, et al. 2018. Nuclear calcineurin is a sensor for detecting Ca^{2+} release from the nuclear envelope via IP3R. *J Mol Med* **96:** 1239–1249. doi:10.1007/s00109-018-1701-2

Paillard M, Csordas G, Szanda G, Golenár T, Debattisti V, Bartok A, Wang N, Moffat C, Seifert EL, Spat A, et al. 2017. Tissue-specific mitochondrial decoding of cytoplasmic Ca^{2+} signals is controlled by the stoichiometry of MICU1/2 and MCU. *Cell Rep* **18:** 2291–2300. doi:10.1016/j.celrep.2017.02.032

Palty R, Silverman WF, Hershfinkel M, Caporale T, Sensi SL, Parnis J, Nolte C, Fishman D, Shoshan-Barmatz V, Herrmann S, et al. 2010. NCLX is an essential component of mitochondrial Na^+/Ca^{2+} exchange. *Proc Natl Acad Sci* **107:** 436–441. doi:10.1073/pnas.0908099107

Pan X, Liu J, Nguyen T, Liu C, Sun J, Teng Y, Fergusson MM, Rovira II, Allen M, Springer DA, et al. 2013. The physiological role of mitochondrial calcium revealed by mice lacking the mitochondrial calcium uniporter. *Nat Cell Biol* **15:** 1464–1472. doi:10.1038/ncb2868

Perbellini F, Watson SA, Bardi I, Terracciano CM. 2018. Heterocellularity and cellular cross-talk in the cardiovascular system. *Front Cardiovasc Med* **5:** 143. doi:10.3389/fcvm.2018.00143

Perez PJ, Ramos-Franco J, Fill M, Mignery GA. 1997. Identification and functional reconstitution of the type 2 inositol 1,4,5-trisphosphate receptor from ventricular cardiac myocytes. *J Biol Chem* **272:** 23961–23969. doi:10.1074/jbc.272.38.23961

Perocchi F, Gohil VM, Girgis HS, Bao XR, McCombs JE, Palmer AE, Mootha VK. 2010. MICU1 encodes a mitochondrial EF hand protein required for Ca^{2+} uptake. *Nature* **467:** 291–296. doi:10.1038/nature09358

Perrino C, Barabási AL, Condorelli G, Davidson SM, De Windt L, Dimmeler S, Engel FB, Hausenloy DJ, Hill JA, Van Laake LW, et al. 2017. Epigenomic and transcriptomic approaches in the post-genomic era: Path to novel targets for diagnosis and therapy of the ischaemic heart? Position paper of the European Society of Cardiology Working Group on Cellular Biology of the Heart. *Cardiovasc Res* **113:** 725–736. doi:10.1093/cvr/cvx070

Piacentino V III, Weber CR, Chen X, Weisser-Thomas J, Margulies KB, Bers DM, Houser SR. 2003. Cellular basis of abnormal calcium transients of failing human ventricular myocytes. *Circ Res* **92:** 651–658. doi:10.1161/01.RES.0000062469.83985.9B

Pinali C, Bennett H, Davenport JB, Trafford AW, Kitmitto A. 2013. Three-dimensional reconstruction of cardiac sarco-

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

plasmic reticulum reveals a continuous network linking transverse-tubules: This organization is perturbed in heart failure. *Circ Res* **113:** 1219–1230. doi:10.1161/circresaha.113.301348

Pitt SJ, Funnell TM, Sitsapesan M, Venturi E, Rietdorf K, Ruas M, Ganesan A, Gosain R, Churchill GC, Zhu MX, et al. 2010. TPC2 is a novel NAADP-sensitive Ca^{2+} release channel, operating as a dual sensor of luminal pH and Ca^{2+}. *J Biol Chem* **285:** 35039–35046. doi:10.1074/jbc.M110.156927

Plačkić J, Preissl S, Nikonova Y, Pluteanu F, Hein L, Kockskamper J. 2016. Enhanced nucleoplasmic Ca^{2+} signaling in ventricular myocytes from young hypertensive rats. *J Mol Cell Cardiol* **101:** 58–68. doi:10.1016/j.yjmcc.2016.11.001

Potenza DM, Janicek R, Fernandez-Tenorio M, Camors E, Ramos-Mondragon R, Valdivia HH, Niggli E. 2018. Phosphorylation of the ryanodine receptor 2 at serine 2030 is required for a complete beta-adrenergic response. *J Gen Physiol* **151:** 131–145. doi:10.1085/jgp.201812155

Prakash YS, Kannan MS, Walseth TF, Sieck GC. 2000. cADP ribose and $[Ca^{2+}]_i$ regulation in rat cardiac myocytes. *Am J Physiol Heart Circ Physiol* **279:** H1482–H1489. doi:10.1152/ajpheart.2000.279.4.H1482

Priori SG, Chen SR. 2011. Inherited dysfunction of sarcoplasmic reticulum Ca^{2+} handling and arrhythmogenesis. *Circ Res* **108:** 871–883. doi:10.1161/circresaha.110.226845

Proven A, Roderick HL, Conway SJ, Berridge MJ, Horton JK, Capper SJ, Bootman MD. 2006. Inositol 1,4,5-trisphosphate supports the arrhythmogenic action of endothelin-1 on ventricular cardiac myocytes. *J Cell Sci* **119:** 3363–3375. doi:10.1242/jcs.03073

Rakovic S, Galione A, Ashamu GA, Potter BV, Terrar DA. 1996. A specific cyclic ADP-ribose antagonist inhibits cardiac excitation-contraction coupling. *Curr Biol* **6:** 989–996. doi:10.1016/S0960-9822(02)00643-7

Rakovic S, Cui Y, Iino S, Galione A, Ashamu GA, Potter BV, Terrar DA. 1999. An antagonist of cADP-ribose inhibits arrhythmogenic oscillations of intracellular Ca^{2+} in heart cells. *J Biol Chem* **274:** 17820–17827. doi:10.1074/jbc.274.25.17820

Rasmussen TP, Wu Y, Joiner ML, Koval OM, Wilson NR, Luczak ED, Wang Q, Chen B, Gao Z, Zhu Z, et al. 2015. Inhibition of MCU forces extramitochondrial adaptations governing physiological and pathological stress responses in heart. *Proc Natl Acad Sci* **112:** 9129–9134. doi:10.1073/pnas.1504705112

Reynolds JO, Chiang DY, Wang W, Beavers DL, Dixit SS, Skapura DG, Landstrom AP, Song LS, Ackerman MJ, Wehrens XH. 2013. Junctophilin-2 is necessary for T-tubule maturation during mouse heart development. *Cardiovasc Res* **100:** 44–53. doi:10.1093/cvr/cvt133

Rizzuto R, Duchen MR, Pozzan T. 2004. Flirting in little space: The ER/mitochondria Ca^{2+} liaison. *Sci STKE* **2004:** re1. doi:10.1126/stke.2152004re1

Robert V, Gurlini P, Tosello V, Nagai T, Miyawaki A, Di Lisa F, Pozzan T. 2001. Beat-to-beat oscillations of mitochondrial $[Ca^{2+}]$ in cardiac cells. *EMBO J* **20:** 4998–5007. doi:10.1093/emboj/20.17.4998

Roderick HL, Berridge MJ, Bootman MD. 2003. Calcium-induced calcium release. *Curr Biol* **13:** R425. doi:10.1016/S0960-9822(03)00358-0

Røe AT, Ruud M, Espe EK, Manfra O, Longobardi S, Aronsen JM, Norden ES, Husebye T, Kolstad TRS, Cataliotti A, et al. 2018. Regional diastolic dysfunction in post-infarction heart failure: Role of local mechanical load and SERCA expression. *Cardiovasc Res* **115:** 752–764. doi:10.1093/cvr/cvy257

Rog-Zielinska EA, Kong CHT, Zgierski-Johnston CM, Verkade P, Mantell J, Cannell MB, Kohl P. 2018. Species differences in the morphology of transverse tubule openings in cardiomyocytes. *Europace* **20:** iii120–iii124. doi:10.1093/europace/euy245

Rohr S. 2004. Role of gap junctions in the propagation of the cardiac action potential. *Cardiovasc Res* **62:** 309–322. doi:10.1016/j.cardiores.2003.11.035

Rosemblit N, Moschella MC, Ondriašova E, Gutstein DE, Ondriaš K, Marks AR. 1999. Intracellular calcium release channel expression during embryogenesis. *Dev Biol* **206:** 163–177. doi:10.1006/dbio.1998.9120

Russell FD, Molenaar P. 2000. The human heart endothelin system: ET-1 synthesis, storage, release and effect. *Trends Pharmacol Sci* **21:** 353–359. doi:10.1016/S0165-6147(00)01524-8

Sacconi L, Tolic-Nørrelykke IM, D'Amico M, Vanzi F, Olivotto M, Antolini R, Pavone FS. 2006. Cell imaging and manipulation by nonlinear optical microscopy. *Cell Biochem Biophys* **45:** 289–302. doi:10.1385/CBB:45:3:289

Sachse FB, Torres NS, Savio-Galimberti E, Aiba T, Kass DA, Tomaselli GF, Bridge JH. 2012. Subcellular structures and function of myocytes impaired during heart failure are restored by cardiac resynchronization therapy. *Circ Res* **110:** 588–597. doi:10.1161/circresaha.111.257428

Sanchez-Alonso JL, Bhargava A, O'Hara T, Glukhov AV, Schobesberger S, Bhogal N, Sikkel MB, Mansfield C, Korchev YE, Lyon AR, et al. 2016. Microdomain-specific modulation of L-type calcium channels leads to triggered ventricular arrhythmia in heart failure. *Circ Res* **119:** 944–955. doi:10.1161/circresaha.116.308698

Sankar N, deTombe PP, Mignery GA. 2014. Calcineurin-NFATc regulates type 2 inositol 1,4,5-trisphosphate receptor ($InsP_3R2$) expression during cardiac remodeling. *J Biol Chem* **289:** 6188–6198. doi:10.1074/jbc.M113.495242

Santulli G, Xie W, Reiken SR, Marks AR. 2015. Mitochondrial calcium overload is a key determinant in heart failure. *Proc Natl Acad Sci* **112:** 11389–11394. doi:10.1073/pnas.1513047112

Sasse P, Zhang J, Cleemann L, Morad M, Hescheler J, Fleischmann BK. 2007. Intracellular Ca^{2+} oscillations, a potential pacemaking mechanism in early embryonic heart cells. *J Gen Physiol* **130:** 133–144. doi:10.1085/jgp.200609575

Savio-Galimberti E, Frank J, Inoue M, Goldhaber JI, Cannell MB, Bridge JH, Sachse FB. 2008. Novel features of the rabbit transverse tubular system revealed by quantitative analysis of three-dimensional reconstructions from confocal images. *Biophys J* **95:** 2053–2062. doi:10.1529/biophysj.108.130617

Saw EL, Kakinuma Y, Fronius M, Katare R. 2018. The non-neuronal cholinergic system in the heart: A comprehen-

sive review. *J Mol Cell Cardiol* **125:** 129–139. doi:10.1016/j.yjmcc.2018.10.013

Scardigli M, Ferrantini C, Crocini C, Pavone FS, Sacconi L. 2018a. Interplay between sub-cellular alterations of calcium release and T-tubular defects in cardiac diseases. *Front Physiol* **9:** 1474. doi:10.3389/fphys.2018.01474

Scardigli M, Müllenbroich C, Margoni E, Cannazzaro S, Crocini C, Ferrantini C, Coppini R, Yan P, Loew LM, Campione M, et al. 2018b. Real-time optical manipulation of cardiac conduction in intact hearts. *J Physiol* **596:** 3841–3858. doi:10.1113/JP276283

Schirone L, Forte M, Palmerio S, Yee D, Nocella C, Angelini F, Pagano F, Schiavon S, Bordin A, Carrizzo A, et al. 2017. A review of the molecular mechanisms underlying the development and progression of cardiac remodeling. *Oxid Med Cell Longev* **2017:** 3920195. doi:10.1155/2017/3920195

Scriven DR, Asghari P, Schulson MN, Moore ED. 2010. Analysis of Cav1.2 and ryanodine receptor clusters in rat ventricular myocytes. *Biophys J* **99:** 3923–3929. doi:10.1016/j.bpj.2010.11.008

Sedarat F, Xu L, Moore ED, Tibbits GF. 2000. Colocalization of dihydropyridine and ryanodine receptors in neonate rabbit heart using confocal microscopy. *Am J Physiol Heart Circ Physiol* **279:** H202–H209. doi:10.1152/ajpheart.2000.279.1.H202

See K, Tan WLW, Lim EH, Tiang Z, Lee LT, Li PYQ, Luu TDA, Ackers-Johnson M, Foo RS. 2017. Single cardiomyocyte nuclear transcriptomes reveal a lincRNA-regulated de-differentiation and cell cycle stress-response in vivo. *Nat Commun* **8:** 225. doi:10.1038/s41467-017-00319-8

Seidel T, Navankasattusas S, Ahmad A, Diakos NA, Xu WD, Tristani-Firouzi M, Bonios MJ, Taleb I, Li DY, Selzman CH, et al. 2017. Sheet-like remodeling of the transverse tubular system in human heart failure impairs excitation-contraction coupling and functional recovery by mechanical unloading. *Circulation* **135:** 1632–1645. doi:10.1161/circulationaha.116.024470

Shattock MJ, Ottolia M, Bers DM, Blaustein MP, Boguslavskyi A, Bossuyt J, Bridge JH, Chen-Izu Y, Clancy CE, Edwards A, et al. 2015. Na^+/Ca^{2+} exchange and Na^+/K^+-ATPase in the heart. *J Physiol* **593:** 1361–1382. doi:10.1113/jphysiol.2014.282319

Sheehan KA, Zima AV, Blatter LA. 2006. Regional differences in spontaneous Ca^{2+} spark activity and regulation in cat atrial myocytes. *J Physiol* **572:** 799–809. doi:10.1113/jphysiol.2005.103267

Shen X, van den Brink J, Hou Y, Colli D, Le C, Kolstad TR, MacQuaide N, Carlson CR, Kekenes-Huskey PM, Edwards AG, et al. 2019. 3D dSTORM imaging reveals novel detail of ryanodine receptor localization in rat cardiac myocytes. *J Physiol* **597:** 399–418. doi:10.1113/JP277360

Shimizu H, Schredelseker J, Huang J, Lu K, Naghdi S, Lu F, Franklin S, Fiji HD, Wang K, Zhu H, et al. 2015. Mitochondrial Ca^{2+} uptake by the voltage-dependent anion channel 2 regulates cardiac rhythmicity. *eLife* **4**. doi:10.7554/eLife.04801

Signore S, Sorrentino A, Ferreira-Martins J, Kannappan R, Shafaie M, Del Ben F, Isobe K, Arranto C, Wybieralska E, Webster A, et al. 2013. Inositol 1,4,5-trisphosphate receptors and human left ventricular myocytes. *Circulation* **128:** 1286–1297. doi:10.1161/circulationaha.113.002764

Smyrnias I, Mair W, Harzheim D, Walker SA, Roderick HL, Bootman MD. 2010. Comparison of the T-tubule system in adult rat ventricular and atrial myocytes, and its role in excitation–contraction coupling and inotropic stimulation. *Cell Calcium* **47:** 210–223. doi:10.1016/j.ceca.2009.10.001

Smyrnias I, Goodwin N, Wachten D, Skogestad J, Aronsen JM, Robinson EL, Demydenko K, Segonds-Pichon A, Oxley D, Sadayappan S, et al. 2018. Contractile responses to endothelin-1 are regulated by PKC phosphorylation of cardiac myosin binding protein-C in rat ventricular myocytes. *J Mol Cell Cardiol* **117:** 1–18. doi:10.1016/j.yjmcc.2018.02.012

Soeller C, Baddeley D. 2013. Super-resolution imaging of EC coupling protein distribution in the heart. *J Mol Cell Cardiol* **58:** 32–40. doi:10.1016/j.yjmcc.2012.11.004

Soeller C, Cannell MB. 1999. Examination of the transverse tubular system in living cardiac rat myocytes by 2-photon microscopy and digital image-processing techniques. *Circ Res* **84:** 266–275. doi:10.1161/01.RES.84.3.266

Song LS, Sobie EA, McCulle S, Lederer WJ, Balke CW, Cheng H. 2006. Orphaned ryanodine receptors in the failing heart. *Proc Natl Acad Sci* **103:** 4305–4310. doi:10.1073/pnas.0509324103

Stephenson RS, Boyett MR, Hart G, Nikolaidou T, Cai X, Corno AF, Alphonso N, Jeffery N, Jarvis JC. 2012. Contrast enhanced micro-computed tomography resolves the 3-dimensional morphology of the cardiac conduction system in mammalian hearts. *PLoS ONE* **7:** e35299. doi:10.1371/journal.pone.0035299

Stern MD. 1992. Theory of excitation-contraction coupling in cardiac muscle. *Biophys J* **63:** 497–517. doi:10.1016/S0006-3495(92)81615-6

Sussman MA, Lim HW, Gude N, Taigen T, Olson EN, Robbins J, Colbert MC, Gualberto A, Wieczorek DF, Molkentin JD. 1998. Prevention of cardiac hypertrophy in mice by calcineurin inhibition. *Science* **281:** 1690–1693. doi:10.1126/science.281.5383.1690

Tadross MR, Dick IE, Yue DT. 2008. Mechanism of local and global Ca^{2+} sensing by calmodulin in complex with a Ca^{2+} channel. *Cell* **133:** 1228–1240. doi:10.1016/j.cell.2008.05.025

Tavi P, Pikkarainen S, Ronkainen J, Niemela P, Ilves M, Weckström M, Vuolteenaho O, Bruton J, Westerblad H, Ruskoaho H. 2004. Pacing-induced calcineurin activation controls cardiac Ca^{2+} signalling and gene expression. *J Physiol* **554:** 309–320. doi:10.1113/jphysiol.2003.053579

Thienpont B, Aronsen JM, Robinson EL, Okkenhaug H, Loche E, Ferrini A, Brien P, Alkass K, Tomasso A, Agrawal A, et al. 2017. The H3K9 dimethyltransferases EHMT1/2 protect against pathological cardiac hypertrophy. *J Clin Invest* **127:** 335–348. doi:10.1172/JCI88353

Uchinoumi H, Yang Y, Oda T, Li N, Alsina KM, Puglisi JL, Chen-Izu Y, Cornea RL, Wehrens XHT, Bers DM. 2016. CaMKII-dependent phosphorylation of RyR2 promotes targetable pathological RyR2 conformational shift. *J Mol Cell Cardiol* **98:** 62–72. doi:10.1016/j.yjmcc.2016.06.007

Uehara A, Yasukochi M, Imanaga I, Nishi M, Takeshima H. 2002. Store-operated Ca^{2+} entry uncoupled with ryanodine receptor and junctional membrane complex in heart

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

muscle cells. *Cell Calcium* **31:** 89–96. doi:10.1054/ceca.2001.0257

Ullrich ND, Valdivia HH, Niggli E. 2012. PKA phosphorylation of cardiac ryanodine receptor modulates SR luminal Ca^{2+} sensitivity. *J Mol Cell Cardiol* **53:** 33–42. doi:10.1016/j.yjmcc.2012.03.015

Vais H, Mallilankaraman K, Mak DD, Hoff H, Payne R, Tanis JE, Foskett JK. 2016. EMRE is a matrix Ca^{2+} sensor that governs gatekeeping of the mitochondrial Ca^{2+} uniporter. *Cell Rep* **14:** 403–410. doi:10.1016/j.celrep.2015.12.054

Valdivia HH, Kaplan JH, Ellis-Davies GC, Lederer WJ. 1995. Rapid adaptation of cardiac ryanodine receptors: Modulation by Mg^{2+} and phosphorylation. *Science* **267:** 1997–2000. doi:10.1126/science.7701323

van der Heyden MA, Wijnhoven TJ, Opthof T. 2005. Molecular aspects of adrenergic modulation of cardiac L-type Ca^{2+} channels. *Cardiovasc Res* **65:** 28–39. doi:10.1016/j.cardiores.2004.09.028

Venetucci LA, Trafford AW, O'Neill SC, Eisner DA. 2008. The sarcoplasmic reticulum and arrhythmogenic calcium release. *Cardiovasc Res* **77:** 285–292. doi:10.1093/cvr/cvm009

Vervliet T, Robinson EL, Roderick HL. 2018. Lnc'ing Ca^{2+}, SERCA and cardiac disease. *Cell Calcium* **72:** 132–134. doi:10.1016/j.ceca.2018.05.005

Vervloessem T, Yule DI, Bultynck G, Parys JB. 2015. The type 2 inositol 1,4,5-trisphosphate receptor, emerging functions for an intriguing Ca^{2+}-release channel. *Biochem Biophys Acta* **1853:** 1992–2005. doi:10.1016/j.bbamcr.2014.12.006

Voelkers M, Salz M, Herzog N, Frank D, Dolatabadi N, Frey N, Gude N, Friedrich O, Koch WJ, Katus HA, et al. 2010. Orai1 and Stim1 regulate normal and hypertrophic growth in cardiomyocytes. *J Mol Cell Cardiol* **48:** 1329–1334. doi:10.1016/j.yjmcc.2010.01.020

Walker MA, Williams GSB, Kohl T, Lehnart SE, Jafri MS, Greenstein JL, Lederer WJ, Winslow RL. 2014. Superresolution modeling of calcium release in the heart. *Biophys J* **107:** 3018–3029. doi:10.1016/j.bpj.2014.11.003

Walweel K, Molenaar P, Imtiaz MS, Denniss A, Dos Remedios C, van Helden DF, Dulhunty AF, Laver DR, Beard NA. 2017. Ryanodine receptor modification and regulation by intracellular Ca^{2+} and Mg^{2+} in healthy and failing human hearts. *J Mol Cell Cardiol* **104:** 53–62. doi:10.1016/j.yjmcc.2017.01.016

Wang SQ, Song LS, Lakatta EG, Cheng H. 2001. Ca^{2+} signalling between single L-type Ca^{2+} channels and ryanodine receptors in heart cells. *Nature* **410:** 592–596. doi:10.1038/35069083

Wang J, Gareri C, Rockman HA. 2018. G-protein-coupled receptors in heart disease. *Circ Res* **123:** 716–735. doi:10.1161/circresaha.118.311403

Warszta D, Nebel M, Fliegert R, Guse AH. 2014. NAD derived second messengers: Role in spontaneous diastolic Ca^{2+} transients in murine cardiac myocytes. *DNA Repair (Amst)* **23:** 69–78. doi:10.1016/j.dnarep.2014.05.007

Wehrens XH, Lehnart SE, Huang F, Vest JA, Reiken SR, Mohler PJ, Sun J, Guatimosim S, Song LS, Rosemblit N, et al. 2003. FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death. *Cell* **113:** 829–840. doi:10.1016/S0092-8674(03)00434-3

Wehrens XH, Lehnart SE, Marks AR. 2005. Intracellular calcium release and cardiac disease. *Annu Rev Physiol* **67:** 69–98. doi:10.1146/annurev.physiol.67.040403.114521

Wei AC, Liu T, Winslow RL, O'Rourke B. 2012. Dynamics of matrix-free Ca^{2+} in cardiac mitochondria: Two components of Ca^{2+} uptake and role of phosphate buffering. *J Gen Physiol* **139:** 465–478. doi:10.1085/jgp.201210784

Wei AC, Liu T, O'Rourke B. 2015. Dual effect of phosphate transport on mitochondrial Ca^{2+} dynamics. *J Biol Chem* **290:** 16088–16098. doi:10.1074/jbc.M114.628446

Williams GS, Boyman L, Chikando AC, Khairallah RJ, Lederer WJ. 2013. Mitochondrial calcium uptake. *Proc Natl Acad Sci* **110:** 10479–10486. doi:10.1073/pnas.1300410110

Wong J, Baddeley D, Bushong EA, Yu Z, Ellisman MH, Hoshijima M, Soeller C. 2013. Nanoscale distribution of ryanodine receptors and caveolin-3 in mouse ventricular myocytes: Dilation of t-tubules near junctions. *Biophys J* **104:** L22–L24. doi:10.1016/j.bpj.2013.02.059

Wu X, Zhang T, Bossuyt J, Li X, McKinsey TA, Dedman JR, Olson EN, Chen J, Brown JH, Bers DM. 2006. Local InsP3-dependent perinuclear Ca^{2+} signaling in cardiac myocyte excitation-transcription coupling. *J Clin Invest* **116:** 675–682. doi:10.1172/JCI27374

Wu HD, Xu M, Li RC, Guo L, Lai YS, Xu SM, Li SF, Lü QL, Li LL, Zhang HB, et al. 2012. Ultrastructural remodelling of Ca^{2+} signalling apparatus in failing heart cells. *Cardiovasc Res* **95:** 430–438. doi:10.1093/cvr/cvs195

Wu Y, Rasmussen TP, Koval OM, Joiner ML, Hall DD, Chen B, Luczak ED, Wang Q, Rokita AG, Wehrens XH, et al. 2015. The mitochondrial uniporter controls fight or flight heart rate increases. *Nat Commun* **6:** 6081. doi:10.1038/ncomms7081

Wullschleger M, Blanch J, Egger M. 2017. Functional local crosstalk of inositol 1,4,5-trisphosphate receptor- and ryanodine receptor-dependent Ca^{2+} release in atrial cardiomyocytes. *Cardiovasc Res* **113:** 542–552. doi:10.1093/cvr/cvx020

Xie GH, Rah SY, Kim SJ, Nam TS, Ha KC, Chae SW, Im MJ, Kim UH. 2005. ADP-ribosyl cyclase couples to cyclic AMP signaling in the cardiomyocytes. *Biochem Biophys Res Commun* **330:** 1290–1298. doi:10.1016/j.bbrc.2005.03.114

Xu L, Meissner G. 2004. Mechanism of calmodulin inhibition of cardiac sarcoplasmic reticulum Ca^{2+} release channel (ryanodine receptor). *Biophys J* **86:** 797–804. doi:10.1016/S0006-3495(04)74155-7

Xu M, Zhou P, Xu SM, Liu Y, Feng X, Bai SH, Bai Y, Hao XM, Han Q, Zhang Y, et al. 2007. Intermolecular failure of L-type Ca^{2+} channel and ryanodine receptor signaling in hypertrophy. *PLoS Biol* **5:** e21. doi:10.1371/journal.pbio.0050021

Yamda J, Ohkusa T, Nao T, Ueyama T, Yano M, Kobayashi S, Hamano K, Esato K, Matsuzaki M. 2001. Up-regulation of inositol 1,4,5 trisphosphate receptor expression in atrial tissue in patients with chronic atrial fibrillation. *J Am Coll Cardiol* **37:** 1111–1119. doi:10.1016/S0735-1097(01)01144-5

Yue X, Zhang R, Kim B, Ma A, Philipson KD, Goldhaber JI. 2017. Heterogeneity of transverse-axial tubule system in

mouse atria: Remodeling in atrial-specific Na^+–Ca^{2+} exchanger knockout mice. *J Mol Cell Cardiol* **108:** 50–60. doi:10.1016/j.yjmcc.2017.05.008

Zalk R, Lehnart SE, Marks AR. 2007. Modulation of the ryanodine receptor and intracellular calcium. *Annu Rev Biochem* **76:** 367–385. doi:10.1146/annurev.biochem.76.053105.094237

Zhang CL, McKinsey TA, Chang S, Antos CL, Hill JA, Olson EN. 2002. Class II histone deacetylases act as signal-responsive repressors of cardiac hypertrophy. *Cell* **110:** 479–488. doi:10.1016/S0092-8674(02)00861-9

Zhang X, Tallini YN, Chen Z, Gan L, Wei B, Doran R, Miao L, Xin HB, Kotlikoff MI, Ji G. 2009. Dissociation of FKBP12.6 from ryanodine receptor type 2 is regulated by cyclic ADP-ribose but not β-adrenergic stimulation in mouse cardiomyocytes. *Cardiovasc Res* **84:** 253–262. doi:10.1093/cvr/cvp212

Zhang L, Malik S, Pang J, Wang H, Park KM, Yule DI, Blaxall BC, Smrcka AV. 2013. Phospholipase Cε hydrolyzes perinuclear phosphatidylinositol 4-phosphate to regulate cardiac hypertrophy. *Cell* **153:** 216–227. doi:10.1016/j.cell.2013.02.047

Zhang Y, Jiao L, Sun L, Li Y, Gao Y, Xu C, Shao Y, Li M, Li C, Lu Y, et al. 2018. LncRNA *ZFAS1* as a SERCA2a inhibitor to cause intracellular Ca^{2+} overload and contractile dysfunction in a mouse model of myocardial infarction. *Circ Res* **122:** 1354–1368. doi:10.1161/circresaha.117.312117

Zhao G, Li T, Brochet DX, Rosenberg PB, Lederer WJ. 2015. STIM1 enhances SR Ca^{2+} content through binding phospholamban in rat ventricular myocytes. *Proc Natl Acad Sci* **112:** E4792–E4801. doi:10.1073/pnas.1423295112

Zhu J, McKeon F. 1999. NF-AT activation requires suppression of Crm1-dependent export by calcineurin. *Nature* **398:** 256–260. doi:10.1038/18473

Zima AV, Blatter LA. 2004. Inositol-1,4,5-trisphosphate-dependent Ca^{2+} signalling in cat atrial excitation-contraction coupling and arrhythmias. *J Physiol* **555:** 607–615. doi:10.1113/jphysiol.2003.058529

Ziman AP, Gómez-Viquez NL, Bloch RJ, Lederer WJ. 2010. Excitation–contraction coupling changes during postnatal cardiac development. *J Mol Cell Cardiol* **48:** 379–386. doi:10.1016/j.yjmcc.2009.09.016

Zobel C, Cho HC, Nguyen TT, Pekhletski R, Diaz RJ, Wilson GJ, Backx PH. 2003. Molecular dissection of the inward rectifier potassium current (I_{K1}) in rabbit cardiomyocytes: Evidence for heteromeric co-assembly of $K_{ir2.1}$ and $K_{ir2.2}$. *J Physiol* **550:** 365–372. doi:10.1113/jphysiol.2002.036400