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Unveiling the intricacies of intracellular Ca^{2+} regulation in the heart

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ABSTRACT Recent studies have provided valuable insight into the key mechanisms contributing to the spatiotemporal regulation of intracellular Ca^{2+} release and Ca^{2+} signaling in the heart. In this research highlight, we focus on the latest findings published in *Biophysical Journal* examining the structural organization of Ca^{2+} handling proteins and assessing the functional aspects of intracellular Ca^{2+} regulation in health and the detrimental consequences of Ca^{2+} dysregulation in disease. These important studies pave the way for future mechanistic investigations and multiscale understanding of Ca^{2+} signaling in the heart.

The human heart consists of billions of myocytes that work cooperatively to facilitate contraction and relaxation to pump blood around the body. For cardiac contraction to occur, electrical activity originating from the sinoatrial node must be converted to mechanical force through a process called excitation-contraction (EC) coupling. This process relies on the precise spatiotemporal regulation of intracellular Ca^{2+} release, which is governed by the intricate interplay of various factors including cell ultrastructure alongside the expression and localization of numerous ion channels, Ca^{2+} handling proteins, protein kinases, and second messengers. In addition to its role in EC coupling, Ca^{2+} also acts as a signaling molecule responsible for regulating gene expression through excitation-transcription (ET) coupling. This process occurs similarly in perhaps (partially) overlapping microdomains yet over a longer timescale. In this research highlight we summarize the latest findings published in the *Biophysical Journal* on the regulation of intracellular Ca^{2+} in the heart, which involves multiple spatial and temporal scales (Fig. 1). We discuss both the structural and molecular aspects involved, delve into the proarrhythmic consequences of Ca^{2+} instabilities in disease and examine the role and significance of Ca^{2+} signaling in gene transcription.

On a beat-to-beat basis, Ca^{2+} enters the cell via L-type Ca^{2+} channels (LTCCs) and triggers the release of a larger amount of Ca^{2+} from the sarcoplasmic reticulum (SR) intracellular Ca^{2+} store. This Ca^{2+} binds to myofilaments to generate contraction and is then removed from the cytosol to facilitate relaxation primarily by reuptake into the SR by sarco/endoplasmic Ca^{2+} ATPase (SERCA) and extrusion via Na^{+}-Ca^{2+} exchanger (NCX). Release of Ca^{2+} from the SR occurs via tetrameric ryanodine receptors (RyRs) that are arranged in clusters along the z-lines and form dyads with closely apposed LTCCs along t-tubules. Due to the spatial arrangement of RyRs in clusters, Ca^{2+} released from one RyR can trigger the release of Ca^{2+} from other nearby RyRs should the amplitude of release be above threshold and in close enough proximity. As such, RyR properties including cluster size, density, and spacing are suggested to be key determinants of Ca^{2+} release and have been shown to vary within both atrial and ventricular myocytes in health and disease (1–6). While it is not possible to study individual RyRs within clusters experimentally, a recent modeling study has provided insight into key determinants of RyR opening in response to changes in local Ca^{2+} concentration. Using a statistical model of a single RyR tetramer, Greene et al. (7) found that a minimum of three out of four subunits must be in the open state in order for a RyR to activate in response to increased local Ca^{2+}. The subunit cooperativity required for effective Ca^{2+} sensing is also important at the cluster level, with occurrence and properties of Ca^{2+} sparks determined by closed and open state subunit interactions (7).

Just as RyR properties influence Ca^{2+} release from the SR, the organization and regulation of SERCA dictates the rate of Ca^{2+} reuptake. SERCA is inhibited by the protein phospholamban (PLB) with its inhibition released primarily following PLB phosphorylation but also to a lesser extent by Ca^{2+} binding. Through molecular dynamics simulations and cellular FRET studies, Raguimova et al. (8)

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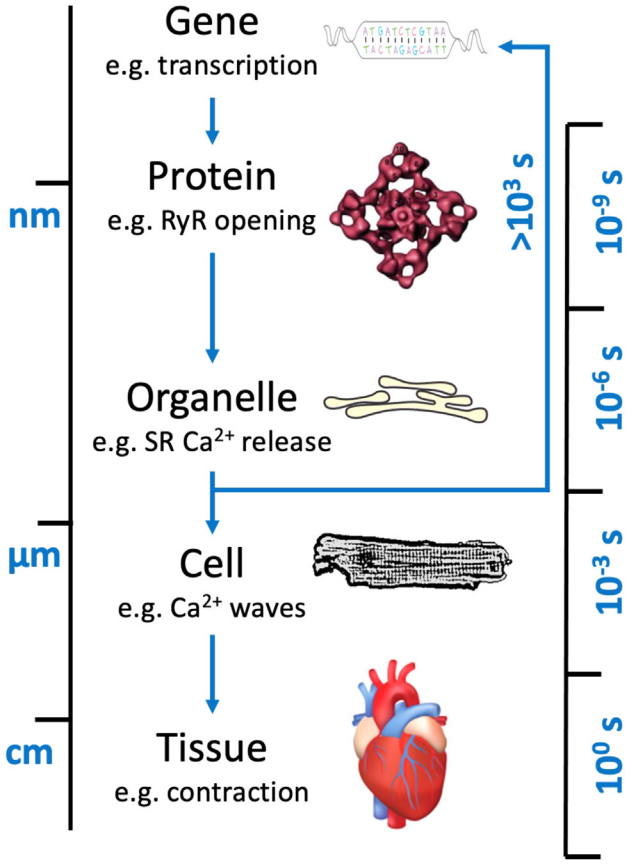

FIGURE 1 Spatial and temporal scales involved in intracellular Ca^{2+} regulation in the heart.

demonstrated that binding of a single Ca^{2+} ion causes structural changes in the SERCA-PLB complex that relieves PLB inhibition of SERCA independent of PLB dissociation. They suggest that the controlling modules and catalytic elements of the SERCA-PLB complex string together after Ca^{2+} binding in a structure that favors ATP utilization and thus promotes SERCA activity (8). Interestingly, SERCA activity is increased where it exists in dimers (9). Bovo et al. (9) saw increased dimerization when SERCA expression was increased, which in turn was associated with greater turnover rate and catalytic efficacy that promoted faster Ca^{2+} reuptake and loading.

These studies highlight the importance of protein structure in the regulation of intracellular Ca^{2+}. Recent research has also demonstrated how changes in modulation and organization of these proteins are associated with dysfunctional Ca^{2+} handling. Due to NCX being electrogenic, Ca^{2+} and voltage are closely coupled in cardiac myocytes. As such, Ca^{2+} released from the SR between beats that is extruded by NCX can result in arrhythmogenic inward currents that cause membrane depolarizations. This is often observed in heart failure where changes in Ca^{2+} handling protein localization (e.g., LTCC, RyR, NCX) associated with t-tubule loss promote proarrhythmic Ca^{2+} waves (10–12). Greene and Shiferaw (13) also found increased propensity for Ca^{2+} wave propagation with a reduction in the Ca^{2+} binding protein calmodulin. As calmodulin interacts with and modulates RyR, lower concentrations are associated with increased Ca^{2+} sparks and RyR open probability (P_o) (13). Interestingly, Meng et al. (14) recently reported changes in RyR P_o that are mediated by lysosomes. They found that enhanced lysosomal Ca^{2+} transport was associated with elevated RyR P_o and increased spontaneous Ca^{2+} release from the SR (14). While aberrant Ca^{2+} release in individual myocytes does not necessarily result in an arrhythmia in the intact heart due to dispersion of the depolarization to the surrounding tissue through cellular coupling, Greene et al. (15) showed that Ca^{2+} waves can synchronize across cells leading to action potential abnormalities. This was observed at a critical pacing rate at which spatially discordant beat-to-beat alternations of action potential duration that promote the emergence of reentrant arrhythmias also occur (15,16). Given that this phenomenon is attributed to the coupling of Ca^{2+} and voltage via NCX and the dependence of wave propagation on SR load and LTCC recovery from inactivation (15), it demonstrates dangerous arrhythmogenic consequences resulting from the dysregulation of key spatiotemporal Ca^{2+} regulatory proteins.

The regulation of intracellular Ca^{2+} is not limited to its crucial involvement in EC coupling but, due to the actions of Ca^{2+} as a signaling molecule, extends to modulating gene transcription via ET coupling. A recent study by Moise and Weinberg has shed light into the physiological importance of this process (17). The authors developed a Ca^{2+} feedback model of the sinoatrial node that demonstrated Ca^{2+} dependence of gene transcription, whereby a target intracellular Ca^{2+} concentration, which is typically an output of electrical activity, governs ion channel mRNA levels. Moise and Weinberg showed that, in quiescent sinoatrial node cells, where intracellular Ca^{2+} concentration is below target, ion channel expression is upregulated in a manner that initiates automaticity and subsequently brings Ca^{2+} levels to target ranges (17). In addition to its role modulating ion channel expression, Ca^{2+} is also involved in hypertrophic signaling via inositol 1,4,5-trisphosphate receptors (IP_3Rs). IP_3Rs are found on the SR and release Ca^{2+} downstream of G-protein-coupled receptor activation (18,19). Ca^{2+} signals generated by sequential IP_3R Ca^{2+} release spikes have recently been modeled in different cell types by Friedhoff et al. (19). In a cardiac cell model, Hunt et al. (18) showed Ca^{2+} released via IP_3Rs elevates the fraction of the cycle at which intracellular Ca^{2+} is raised during the Ca^{2+} transient (duty cycle). This correlated to the level of downstream transcription factor activation in the nucleus, indicating a possible mechanism for IP_3R-mediated hypertrophic signaling (18). Further investigation is required to fully elucidate how cardiac cells distinguish between EC and ET coupling Ca^{2+} fluxes that ultimately

determine physiological and pathophysiological processes. While actions are likely dependent on the different spatiotemporal Ca^{2+} flux profiles, given free cytosolic Ca^{2+} diffusion rates of $\sim$400 $\mu m^2/s$ have been calculated in *Xenopus laevis* oocytes (double that previously assumed in living cells) (20), it is clear that all signal transduction is occurring at ultrarapid rates.

In summary, this research highlight presents emerging findings on spatiotemporal regulation of intracellular Ca^{2+} in the heart. It is hoped that this brief synopsis will be of interest to researchers in this continually evolving field, fostering further advancements in understanding the complex multispatial and multitemporal mechanisms underlying Ca^{2+} (dys)regulation.

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