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An integrated mechanism of cardiomyocyte nuclear Ca^{2+} signaling

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Abstract

In cardiomyocytes, Ca^{2+} plays a central role in governing both contraction and signaling events that regulate gene expression. Current evidence indicates that discrimination between these two critical functions is achieved by segregating Ca^{2+} within subcellular microdomains: transcription is regulated by Ca^{2+} release within nuclear microdomains, and excitation–contraction coupling is regulated by cytosolic Ca^{2+} . Accordingly, a variety of agonists that control cardiomyocyte gene expression, such as endothelin-1, angiotensin-II or insulin-like growth factor-1, share the feature of triggering nuclear Ca^{2+} signals. However, signaling pathways coupling surface receptor activation to nuclear Ca^{2+} release, and the phenotypic responses to such signals, differ between agonists. According to earlier hypotheses, the selective control of nuclear Ca^{2+} signals by activation of plasma membrane receptors relies on the strategic localization of inositol trisphosphate receptors at the nuclear envelope. There, they mediate Ca^{2+} release from perinuclear Ca^{2+} stores upon binding of inositol trisphosphate generated in the cytosol, which diffuses into the nucleus. More recently, identification of such receptors at nuclear membranes or perinuclear

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Disclosures

None.

sarcolemmal invaginations has uncovered novel mechanisms whereby agonists control nuclear Ca^{2+} release. In this review, we discuss mechanisms for the selective control of nuclear Ca^{2+} signals with special focus on emerging models of agonist receptor activation.

Keywords

Nuclear Ca^{2+} ; Cardiomyocyte; Insulin-like growth factor-1; Endothelin-1; Angiotensin II; Sarcolemmal receptor

1. Introduction

Calcium homeostasis is regulated by the combined action of a variety of channels, transporters, and binding proteins which allow cells to increase or decrease intracellular Ca^{2+} concentration on demand [1]. Ca^{2+} -releasing events, or Ca^{2+} transients, occur when Ca^{2+} channels embedded within either the plasma membrane or in select internal membranes open, allowing Ca^{2+} to move down its electrochemical gradient from either external sources or intracellular Ca^{2+} stores, flooding the cytosolic compartment. Cytosolic Ca^{2+} increases a remarkable 50-fold this way with each heart beat (0.1 μM in diastole to $\approx 5 \mu\text{M}$ in systole). Then, Ca^{2+} is rapidly removed from the cytosol by Na^+ - Ca^{2+} exchangers and ATP-dependent transporters that pump Ca^{2+} out of the cell or back into intracellular stores [2]. This Ca^{2+} cycle defines the Ca^{2+} transient, whereas repeated Ca^{2+} cycles comprise a Ca^{2+} oscillation [3,4].

Ca^{2+} oscillations can be tuned in frequency, amplitude, and duration, providing a biological signal with limitless possible combinations for encoding information [5]. Cardiac contraction provides an excellent example of the importance of Ca^{2+} oscillations, and the need to maintain them under fine control [6]. Under normal conditions, the human heart beats once every second, therefore, each cardiomyocyte undergoes a full, coordinated Ca^{2+} cycle nearly 60 times per minute [2]. Many biological inputs ultimately exert control over heart rate by impacting various components governing Ca^{2+} oscillation.

Although Ca^{2+} oscillations are central to driving cardiomyocyte contraction, non-contractile Ca^{2+} -dependent signaling has emerged as an important regulatory mechanism of both transcriptional control and structural remodeling in the heart. In a wonderfully intricate manner, Ca^{2+} manages to regulate these processes independent of the whole-cell Ca^{2+} oscillations that drive contraction. Ca^{2+} -mediated changes in gene expression often occur in response to agonist binding to receptors at the plasma membrane, or sarcolemma [7,8]. This mechanism allows cells to reprogram their gene expression profiles to meet ever-changing cardiac demand. Ca^{2+} -mediated signaling can also influence transcriptional control of cardiomyocyte development [9], differentiation [10], survival [11], hypertrophic growth [12,13], metabolism [14] and cell death [11]. At present, we are only beginning to understand how a cardiomyocyte decodes a Ca^{2+} signal to alter gene expression without interfering with, or being controlled by, the essential and ongoing process of contraction [15]. A growing body of evidence indicates that such discrimination is accomplished by triggering local Ca^{2+} release in segregated subcellular compartments (cytosol versus nucleus) or specific sub-regions of these compartments, generating microdomains of

localized Ca^{2+} -signaling events. In this review, we focus on mechanisms currently proposed to explain such selective control of nuclear Ca^{2+} signals.

2. Cytosolic source of nuclear Ca^{2+} signals

Although it is currently controversial whether the initiation of nuclear Ca^{2+} signals derives from cytosolic Ca^{2+} entry into the nucleus, or generated by the nuclear release of Ca^{2+} , there is evidence that both mechanisms exist in cardiomyocytes (as summarized in Fig. 1). Indeed, several studies in cardiomyocytes and other cell types suggest that elevations nuclear Ca^{2+} are the direct consequence of changes in cytosolic Ca^{2+} [16–18]. On the other hand, it has also been shown in different cardiac muscle cells that changes in nuclear Ca^{2+} can be regulated independent of cytosolic Ca^{2+} and derive from Ca^{2+} released inside, or in close proximity to, the nucleus [18–20].

2.1. Ca^{2+} diffusion through the nuclear pore complex

It was initially held that primary access for Ca^{2+} to the nuclear compartment occurred through passive diffusion of cytosolic Ca^{2+} through nuclear pores connecting the nucleus and the cytoplasm. This is a reasonable hypothesis given that the nuclear pore complex, a multiprotein structure integral to the nuclear envelope (NE), has an approximate diameter of 8nm although this was estimated in isolated *Xenopus* oocyte nuclei [21]. Although Ca^{2+} has an ionic radius of only $0.99^{\circ}\text{\AA}$, its hydrophobicity in solution gives it an effective diameter of $12^{\circ}\text{\AA}$ per hydrated ion (or 1.2nm). This would allow unlimited traffic of Ca^{2+} ions between the cytoplasm and nucleus as postulated by pioneer studies in amphibian and insect cells [22,23].

The concept of passive diffusion of Ca^{2+} into the nucleus from a cytoplasmic source is supported in cardiomyocytes by several lines of evidence, such as the observations of synchronous elevations in nuclear and cytoplasmic Ca^{2+} [16]. Indeed, the cytosolic Ca^{2+} wave propagated during cardiomyocyte contraction can invade the nucleoplasm via diffusion [16], favored by the lower Ca^{2+} buffer capacity of the nucleus [24]. In this model, the NE functions as a barrier and the nuclear pores provide the entryway for regulated diffusion of high cytosolic Ca^{2+} , and this has also been observed in mouse neuroblastoma cells [25].

Like any gate, the nuclear pore is subject to regulation. Diffusion through nuclear pores is complex and regulated by several mechanisms, including passive diffusion of small molecules (up to 10kDa), Ca^{2+} -regulated transport of intermediate molecules (10–70kDa) and also involves active transport for larger molecules [26]. Ca^{2+} itself can influence diffusion through nuclear pores, and Ca^{2+} store depletion decreases diffusion of intermediate but not small size molecules or ions [26–28]. Consistent with this, atomic force microscopy demonstrates that the nuclear pore complex is a dynamic structure capable of responding to changes in intracellular Ca^{2+} [29]. Indeed, some hormones that increase cytosolic Ca^{2+} levels also increase permeability of the nuclear pore complex [30]. Thus, regulation of nuclear pore function is complex, rendering it capable of either increasing or decreasing the traffic of Ca^{2+} and other middle size molecules into and out of the nucleus. The origin of a

Ca^{2+} signal and its proximity to the nucleus can also be important factors influencing regulated diffusion of cytosolic Ca^{2+} into the nucleus [16,31,32].

2.2. Whole-cell Ca^{2+} oscillations

Propagation of cytosolic Ca^{2+} oscillations into the nucleus has also been reported in different cell types. In adrenal chromaffin and pituitary cells, both high and low frequency Ca^{2+} oscillations invade the nucleus, and interestingly high frequency oscillations are more able to do so than those of low frequency [33]. It is not clear whether in cardiomyocytes cytosolic Ca^{2+} diffusion into the nucleus during cardiac contraction is a function of the frequency and amplitude of cytosolic Ca^{2+} fluctuations, however, it has been proposed that during cardiomyocyte hypertrophy the nuclear machinery can decode distinct Ca^{2+} oscillatory patterns and respond specifically [34]. Distinct patterns of Ca^{2+} oscillations can alter the threshold of activation for Ca^{2+} as a second messenger by preferentially activating or inactivating distinct Ca^{2+} -dependent compartments, resulting in directed, downstream changes in gene expression or cell function [35–38]. This may be of particular importance during inotropic and chronotropic responses where oscillatory patterns of Ca^{2+} handling are quickly shaped in response to hormonal stimulation.

3. Perinuclear sources of nuclear Ca^{2+} signals

A second hypothesis for the generation of nuclear Ca^{2+} signals has been proposed based on the ability of the nuclear envelope itself to function as a perinuclear Ca^{2+} store, capable of releasing Ca^{2+} into the nuclear matrix in response to stimulation [39]. This concept is supported by the identification of specific perinuclear structures that generate, modulate, and shape nuclear Ca^{2+} signals.

3.1. Perinuclear endoplasmic reticulum

Rough ER is distinguished by the abundance of attached protein-synthesizing ribosomes and is the type of ER most closely associated with the NE. Recent studies have explored the importance of a perinuclear subset of rough ER in the regulation of nucleoplasmic Ca^{2+} . Over-expression of calsequestrin-2 targeted to the perinuclear rough ER promotes amplified RyR2-mediated nuclear Ca^{2+} transients in both adult and neonatal cardiomyocytes [40], suggesting that perinuclear rough ER acts as a source, in addition to the NE, for nucleoplasmic Ca^{2+} transients. For instance, in atrial cardiomyocytes, cytosolic Ca^{2+} sparks must be close to the nucleus (within $5\mu\text{m}$) to contribute to a nuclear Ca^{2+} signal [41], demonstrating the importance of localized events in the perinuclear endoplasmic reticulum for nuclear Ca^{2+} signaling.

3.2. The nuclear envelope

Two membranes comprise the NE: the outer nuclear membrane (ONM), which is a structure continuous with the endoplasmic reticulum (ER), and the inner nuclear membrane (INM), which is in direct contact with the nuclear matrix. The perinuclear space (PS) is the space between the ONM and INM [42]. The NE of cardiomyocytes harbors Ca^{2+} , which can generate Ca^{2+} signals independent of cytosolic Ca^{2+} [26,39,43]. Fluorescence recovery after photobleaching of Fluo-5N in intact adult cardiomyocytes demonstrates that the lumen of

the sarcoplasmic reticulum (SR) and the PS are interconnected, enabling rapid diffusion of intraluminal Ca^{2+} ions and limiting the generation of spatial gradients during Ca^{2+} release events [20]. In other words, Ca^{2+} inside the NE is connected to cytoplasmic Ca^{2+} stores and fully available for rapid signaling, only requiring nuclear Ca^{2+} channels to trigger release.

The ONM of embryonic chicken cardiomyocytes harbors the sarco/endoplasmic reticulum Ca^{2+} -ATPase 2 (SERCA2), which actively transports Ca^{2+} from the cytosol surrounding the nucleus into the PS [44]. Although Ca^{2+} -ATPases have not been identified in the INM, there is a $\text{Na}^+/\text{Ca}^{2+}$ exchanger complex in the IMM, suggesting a mechanism for maintaining Ca^{2+} homeostasis in the nucleoplasm through $\text{Na}^+/\text{Ca}^{2+}$ exchange and allowing restoration of resting Ca^{2+} levels by transfer of Ca^{2+} to the NE or PS [45,46]. Thus, in addition to the intraluminal equilibrium of Ca^{2+} stores between the PS and the SR, the NE participates in active uptake of Ca^{2+} from both inside and outside the nucleus, thereby exhibiting Ca^{2+} storage capabilities similar to the ER. Buffering of cytoplasmic Ca^{2+} by binding proteins and organelles in the cytoplasm can also directly and indirectly influence nuclear Ca^{2+} levels.

3.3. The nucleoplasmic reticulum

In 2003, a model, derived from SKHep1 epithelial cells, of the dynamic interconnections between ER and NE membranes was extended to structures inside the nuclear matrix, comprising a nucleoplasmic reticulum (NR). The NR is a continuous reticular network that invaginates the INM of the NE toward the nuclear matrix [47]. Subsequently, these nuclear structures were reported in neonatal and adult cardiomyocytes [48], as well as in many other mammalian and plant cells [49]. Interestingly, this discrete Ca^{2+} rich structure can regulate Ca^{2+} signals in specific subregions of the nucleus, as Ca^{2+} releasing channels are localized to specific regions of the NR, forming nucleoplasmic Ca^{2+} -release hotspots.

Invaginations of both the ONM and INM into the nuclear matrix have been described in many cell types [49]. The lumen of these structures, or “NE tunnels” as they have been called, contains both ER and mitochondrial markers [50,51], indicating that they bring cytoplasmic elements and organelles in close physical proximity to the nuclear matrix. As a result, they comprise intranuclear signaling microdomains that can segregate, generate, or amplify Ca^{2+} release from the NE, possibly in response to T-tubule invaginations.

3.4. Perinuclear mitochondria

Mitochondria can also shape the dynamics of nuclear Ca^{2+} [50,52] by acting as a perinuclear buffer, thereby influencing the magnitude and duration of nuclear Ca^{2+} signals.

Mitochondria are often found in close proximity to the ER, particularly in the perinuclear region, and can rapidly uptake Ca^{2+} released from InsP_3 -receptors, buffering the impact of InsP_3 -mediated cytoplasmic Ca^{2+} release [53]. During E–C coupling this same process of mitochondrial Ca^{2+} uptake through the VDAC/mitochondrial Ca^{2+} uniporter (MCU) complex can buffer the amplitude of cytosolic Ca^{2+} transients, theoretically limiting the passive diffusion of cytosolic Ca^{2+} into the nucleus [54].

All the above-mentioned sources of Ca^{2+} can contribute or shape nuclear Ca^{2+} , but despite the multiple sources of nuclear Ca^{2+} signals, whether the origin is nuclear or perinuclear,

remains in dispute. A recent report may help reconcile these two models. In situ calibration of Ca^{2+} concentrations in the nucleus and cytosol of murine adult cardiomyocytes reveals that nuclear Ca^{2+} transients evoked by electrical stimulation consist of at least two separate components, a passive component of Ca^{2+} diffusion from the cytosol to the nucleus through nuclear pores and an active component of Ca^{2+} release via InsP_3 receptors [18]. Thus, nuclear Ca^{2+} transients in adult cardiomyocytes arising from electrical stimulation are both dependent and independent of cytosolic Ca^{2+} . Together, these studies unveil the complex regulation of cardiomyocyte nuclear Ca^{2+} signals through combined actions of highly structured nuclear and perinuclear sub-compartments.

4. Nucleus-initiated nuclear Ca^{2+} release

Despite advances in our understanding of the nuclear Ca^{2+} store, the question remains whether these specialized structures can be activated *independent* of cytosolic Ca^{2+} release. For instance, does targeted stimulation by a hormone act directly on the nuclear Ca^{2+} release machinery to trigger Ca^{2+} release?

4.1. Nucleus-restricted molecular tools

To address this question, targeted molecular tools were engineered such that their mode of action is restricted to a specific subcellular compartment. The high-capacity, low-affinity Ca^{2+} buffer protein, parvalbumin (PV) was targeted to either the nucleus or the cytosol by addition of a peptide signal that directs the protein to a specific subcellular location [55]. These fusion proteins were used in HepG2 cells to reveal that nuclear Ca^{2+} buffering suppresses ATP induction of nuclear Ca^{2+} signals without affecting the rise in cytosolic Ca^{2+} . By contrast, cytosol-targeted PV accomplished the opposite, blocking cytosolic Ca^{2+} transients but sparing nuclear transients. In the same study, EGF-mediated activation of the transcription factor Elk-1 was abolished specifically by buffering nuclear Ca^{2+} , indicating that, in this context, nucleus-restricted Ca^{2+} increases drive the regulation of gene expression [56].

When this technology was applied to cardiomyocytes, global E–C coupling-related Ca^{2+} transients were largely decreased by buffering cytosolic Ca^{2+} , as expected. However, buffering nuclear Ca^{2+} had the surprising effect of decreasing the amplitude of Ca^{2+} oscillations while increasing both their frequency and action potential duration [48]. Together, these findings suggest that a component of nuclear Ca^{2+} release helps shape global Ca^{2+} transients during the cardiac contraction cycle, likely via a nucleus-delimited E–C coupling process.

4.2. Nuclear Ca^{2+} contribution to E–C coupling

The concept that nuclear Ca^{2+} is an important component of E–C coupling was first suggested by structural studies demonstrating the existence of perinuclear T-tubules and nuclear Ca^{2+} microdomains along with RyR2 [48] and L-type Ca^{2+} release channels [57,58]. Amplification of cytosolic Ca^{2+} signals by this nuclear E–C coupling mechanism may occur through passive diffusion of Ca^{2+} from the nucleus to the cytosol through nuclear pores, and

is favored by the nuclear-to-cytosolic Ca^{2+} gradient and relatively lower Ca^{2+} buffering capacity of the nucleus [18].

4.3. Nuclear Ca^{2+} signals independent of E–C coupling

Although nuclear Ca^{2+} signals coordinated with cardiac E–C coupling exhibit features both dependent and independent of changes in cytosolic Ca^{2+} , activation of nuclear Ca^{2+} in response to certain stimuli can occur entirely independent of cytosolic Ca^{2+} . For example, this has been demonstrated for insulin-like growth factor-1 (IGF-1) receptor signaling [59]. Treating neonatal cardiomyocytes with IGF-1 initiates an InsP_3 -dependent nuclear Ca^{2+} transient that precedes the cytosolic transient [59]. Blocking the nuclear Ca^{2+} transient with targeted PV abrogated both the nuclear and cytosolic responses, whereas cytosol-localized PV blocked only the cytosolic response, indicating that the signal originates in the nucleus [58]. This cytosol-independent nuclear Ca^{2+} response is required for activation of the transcription factor MEF-2C, a mediator of IGF-1-induced cardiomyocyte hypertrophy [58]. Using a similar approach, a nucleus-restricted InsP_3 buffer inhibited the hypertrophic response of neonatal cardiomyocytes to either IGF-1 or ET-1 [60]. A different study showed that phenylephrine-stimulated cardiomyocytes showed an increased frequency of nuclear Ca^{2+} sparks leading to nuclear Ca^{2+} transients [61], which is also in favor of the notion that both InsP_3 - and Ca^{2+} -dependent signaling occur within the nucleus in response to hypertrophic agonists to regulate cardiac transcription, and this signaling is not dependent on cytosolic Ca^{2+} release.

Thus, current evidence suggests that, depending on whether a nuclear Ca^{2+} transient arises during an E–C coupling cycle or in response to activation of a receptor on the plasma membrane, the elevation in nuclear Ca^{2+} can be either partially dependent or fully independent of cytosolic Ca^{2+} . Considering that cardiac contraction is a continuous, episodic process that itself can be shaped by extracellular stimuli, the details of the interplay between nuclear and cytoplasmic Ca^{2+} signaling are likely to be complex. With the exception of extreme situations, such as cardioplegic arrest during surgery, receptor activation in cardiomyocytes must always occur simultaneously with E–C coupling. Therefore, Ca^{2+} signals arising from nuclear stores are constantly influenced by regulatory signals that diffuse from the cytosol. How nuclear Ca^{2+} stores detect, decode, and respond appropriately to all the possible inputs delivered at a given time remains an important and unanswered question.

5. Ca^{2+} channels mediating nuclear Ca^{2+} transients

Cardiomyocytes harbor three classes of ion channels that mediate the rapid mobilization of Ca^{2+} ions from intracellular stores: inositol 1,4,5-trisphosphate receptors (InsP_3Rs), ryanodine receptors (RyRs) [10] and NAADP-sensitive endolysosomal Ca^{2+} channels [62]. While two pore channels (TPCs) have been proposed as the NAADP-sensitive Ca^{2+} channels at endolysosomal stores [63], the best-characterized channels that release Ca^{2+} from intracellular stores are InsP_3Rs and RyRs. Both of the latter families form high-conductance, low-selectivity cation channels by association of four high-molecular mass protein monomers in combination with a large number of accessory proteins [64–68]. The InsP_3R family consists of at least three distinct gene products, encoding monomers of

molecular mass around 300 kDa, with three conserved and characterized domains (i.e. binding, regulatory/coupling, and channel pore). All three of these proteins bind their endogenous agonist InsP_3 and share approximately 70% amino acid identity with one another [64,65]. There is general agreement that the type-2 InsP_3R is the predominant InsP_3R isoform in cardiomyocytes [69,70], although its expression levels are nearly 100-fold lower than that of RyRs, the primary release channels during E-C coupling [71]. Thus, InsP_3Rs are not the primary source of Ca^{2+} release. That said, InsP_3R localization in cardiomyocytes is concentrated in the NE where it is found at the ONM, INM, and at the nucleoplasmic reticulum [48,72–74]. Accordingly, the introduction of InsP_3 into the nucleus has been shown to evoke Ca^{2+} release from the NE [75–77]. Release of Ca^{2+} from the NE can be inhibited by microinjection of heparin (an InsP_3R antagonist) into the nucleus, [76] or by an antibody against the InsP_3R [77,78]. InsP_3 triggers rapid nuclear Ca^{2+} signals in permeabilized atrial cardiomyocytes that can be blocked by InsP_3 inhibition, but not by inhibition of RyR [74]. In isolated cardiomyocyte nuclei, InsP_3 triggers increases in nucleoplasmic Ca^{2+} and decreases in the PS [74]. InsP_3 increases the frequency of perinuclear Ca^{2+} sparks in permeabilized neonatal cardiomyocytes [61]. InsP_3 also induces prominent monophasic nuclear Ca^{2+} transients in immortalized HL-1 cells, where InsP_3R type-1 is localized to the perinuclear region [79]. Thus, it is not the relative abundance of InsP_3Rs , but rather their strategic localization to nuclear membranes, that renders these Ca^{2+} channels relevant for gating nuclear Ca^{2+} signals in cardiomyocytes.

Release of Ca^{2+} via the RyR has been implicated in the regulation of nuclear Ca^{2+} signals, as suggested in the proposed mechanism 4 from Fig. 1, but this has been restricted to neonatal cardiomyocytes, whereas evidence for nuclear localization of RyRs in adult cardiomyocytes has not been clearly established. Hypothetically, this could be a difference lost with differentiation, due to the importance of E-C coupling in promoting an adult cardiomyocyte phenotype. Similarly in a different myocyte cell, photo-release of caged Ca^{2+} directly within the nucleus of C2C12 myoblasts triggers localized Ca^{2+} -induced Ca^{2+} release (CICR) in the nucleus which could be suppressed by the RyR inhibitor dantrolene [80]. RyR1 was found to localize to intranuclear extensions of the NE in C2C12 myoblasts. RyR2 was also found to localize to the perinuclear region of embryonic chick cardiomyocytes [44], and the nucleoplasmic reticulum of neonatal rat cardiomyocytes [48], providing structural evidence that nuclear RyRs control locally-restricted nuclear CICR in neonatal cells. Together, these studies unveil selective regulation of nuclear Ca^{2+} signals by specific InsP_3R and RyR isoforms localized to nuclear membranes, where they mediate Ca^{2+} release from perinuclear Ca^{2+} stores by the combined action of the second messengers InsP_3 and Ca^{2+} . These mechanisms could play important roles in the establishment of the adult cardiac phenotype, and also may be involved in pathological conditions where neonatal gene programs are activated.

6. Diffusion of cytosolic second messengers into the nucleus

Activation of nuclear Ca^{2+} channels can occur via diffusion into the nucleus of second messengers initially generated in the cytoplasm or via second messengers generated on-site. Several second messengers traverse the nuclear pore to promote nuclear Ca^{2+} release [76,81].

6.1. Diffusion of Ca^{2+}

Ca^{2+} itself is an excellent candidate for regulating the initiation and maintenance of nuclear Ca^{2+} signals. Analogous to signaling events in the cytosol, an increase in nucleoplasmic Ca^{2+} facilitates Ca^{2+} release from InsP_3Rs [82,83]. Indeed, various isoforms of InsP_3R are either activated or inhibited by high Ca^{2+} concentrations [64,65,68,84,85]. It is possible to envision a mechanism of nuclear Ca^{2+} regulation of CICR similar to that of cytoplasmic CICR, where an increase in nuclear Ca^{2+} serves as a positive modulator, initiating, amplifying and ultimately terminating the signal at NE Ca^{2+} -release hotspots [18,24,86]. This positive feedback between nuclear Ca^{2+} and nuclear Ca^{2+} release channels would then operate as an amplifying mechanism to direct nucleoplasmic Ca^{2+} -mediated signaling.

6.2. Diffusion of InsP_3

Evidence exists for diffusion of InsP_3 from the cytosol to the nucleus, as illustrated in Fig. 1. FRET-based InsP_3 biosensors have revealed that either stimulation with ET-1 or delivery of InsP_3 directly into the cytosol induces diffusion of InsP_3 into the nucleus, with delayed kinetics relative to cytosolic concentration [87]. Diffusion of InsP_3 into the nucleus initiates a CaM/CaMK II signaling pathway ultimately controlling HDAC5 phosphorylation and nuclear export [73]. These findings confirm the ability of cytosolic InsP_3 to diffuse into the nucleus and are consistent with the documented timing of nuclear Ca^{2+} transients in response to extracellular ET-1. This is illustrated in mechanism 2 from Fig. 1. However, diffusion of InsP_3 from the cytosol is not sufficient to explain the rate of nuclear Ca^{2+} responses which exceed that of InsP_3 diffusion. InsP_3 generated at the plasma membrane has a limited capacity for traversing large distances in the cell (20–50 μm) [88,89]. The cytosolic diffusion rate of InsP_3 is 15 $\mu\text{m}/\text{s}$ [89] and is compatible with the kinetics of the nuclear Ca^{2+} signal in response to ET-1. As with Ca^{2+} , it is unclear how nuclear InsP_3Rs discriminate between InsP_3 generated in the cytoplasm versus InsP_3 generated in the nucleus. Rates of InsP_3 degradation are likely an important factor. InsP_3 is rapidly hydrolyzed to inactive forms, such as InsP_2 [90]. Therefore, cytoplasm-localized InsP_3Rs encounter the bulk of InsP_3 generated in the cytoplasm, whereas, the concentration of cytoplasmic InsP_3 at the nucleus is relatively small. This argument hints at the ability to generate InsP_3 inside the nucleus, near its site of action, as will be discussed below.

7. Generation of second messenger inside the nucleus

Increasing evidence indicates that nuclear Ca^{2+} signals can be initiated by second messengers generated in and/or around the nucleus. The nucleus of many cell types is equipped with a toolkit for inositol lipid metabolism [91], as nuclear membranes harbor enzymatic systems for the production and degradation of InsP_3 : phospholipases [92,93], PIP_2 [94] and kinases [91,95]. However, and importantly, these have mainly been identified in cell types different than cardiomyocytes. Evidence for a nuclear phosphoinositide cycle in cardiomyocytes has indeed been more elusive. In particular, nuclear $\text{PLC}\epsilon$ has been detected in neonatal cardiomyocytes, and its knock down in the nucleus inhibited the hypertrophy of neonatal cardiomyocytes induced by ET-1, isoproterenol or norepinephrine [96]. $\text{PLC}\beta 3$ and $\text{PLC}\gamma$ have been observed in the nucleus in confocal studies in cardiomyocytes leading to nuclear Ca^{2+} signals, but further subcellular fractionation experiments are necessary to

confirm such observations, specifically as the localization of these enzymes in perinuclear microdomains could account for the effects on nuclear Ca^{2+} [58], as proposed in mechanism 1 from Fig. 2. One study did suggest that $\text{PLC}\beta 1$ was localized to the nucleus in adult cardiomyocytes [97], although others have questioned the antibody used in this study (Santa Cruz, G12 $\text{PLC}\beta$ antibody). Conversely, Zhang et al. [98] suggested that the substrate PIP_2 is not at the nuclear membrane in neonatal cardiomyocytes, rather that $\text{PLC}\epsilon$ is at the nucleus. However, this localization was not related to nuclear InsP_3 generation, but instead to the production of DAG from PI4P for controlling PKD phosphorylation and nuclear import [96]. Disruption of nuclear localization of $\text{PLC}\epsilon$ did not affect nuclear Ca^{2+} transients induced by ET-1, while $\text{PLC}\beta$ knock down did affect it [98], in agreement with the notion that perinuclear $\text{PLC}\beta$ is necessary for nuclear Ca^{2+} regulation [58]. Other studies, however, have shown that $\text{PLC}\beta$ isoforms also adopt nuclear localization. Nuclear $\text{PLC}\beta 1$ have been reported in adult cardiomyocytes [97] whereas nuclear $\text{PLC}\beta 3$ and $\text{PLC}\gamma$ have been shown in neonatal cardiomyocytes [58]. Moreover, stimulation of neonatal cardiomyocytes with IGF-1 induces a fast and transient increase in intracellular InsP_3 levels [59]. This effect is reversed by preincubating cells with the $\text{G}\alpha\text{i}$ blocker pertussis toxin [59] or by over-expressing the $\text{G}\beta\gamma$ scavenger peptide βARKct [58], indicating that a G protein-sensitive PLC isoform, likely $\text{PLC}\beta$, is involved in this response. Subcellular analysis of PLC activation by means of a fluorescent reporter demonstrates that PIP_2 hydrolysis occurs exclusively in the perinuclear region upon IGF-1 stimulation, without affecting the peripheral PIP_2 pool [58], although the dynamic range for the fluorescent PIP_2 reporter in the nuclear region seems to be lower than the one observed in the plasma membrane [98], which may reflect local differences in phosphoinositide composition and availability. Thus, different nuclear PLC isoforms may contribute differently on nuclear Ca^{2+} regulation or nuclear lipid signaling in cardiomyocytes.

A central unanswered question is how nuclear PLC isoforms are activated by an extracellular ligand. In cancer cells, ERK-mediated kinases activate nuclear $\text{PLC}\beta$ [99]. However, the kinetics of this MAPK cascade are slower (15–30min) than the fast nuclear response to IGF-1 of cardiomyocytes (<15s). This suggests that IGF-1 increases nuclear PLC activity via a different mechanism. Moreover, evidence indicates that not all agonists use the same signaling mechanisms to provoke a nuclear Ca^{2+} transient [100].

8. Working models of receptor-initiated nuclear Ca^{2+} release

As mentioned above, different extracellular ligands trigger a range of signaling mechanisms to achieve specific regulation of nuclear Ca^{2+} release [100], as shown in Fig. 2. For example, a number of agonists induce nuclear Ca^{2+} release in cardiomyocytes. Endothelin-1 (ET-1) is perhaps the best studied and therefore much of our understanding of nuclear Ca^{2+} signaling has been built upon mechanisms triggered by ET-1 [19,73,101]. However, other agonists, such as IGF-1 [59], insulin [102], angiotensin II (Ang II) [103], ATP [53], extracellular Ca^{2+} [104], and testosterone [105], also mobilize cardiomyocyte nuclear Ca^{2+} , although a full understanding of underlying mechanisms remains elusive.

8.1. Activation of sarcolemmal receptors

ET-1 receptor A (ETA) signals via the classic model for ligand activation of cell membrane receptors [100]. Binding of ET-1 to its cognate receptor in the cardiomyocyte plasma membrane triggers a dose-dependent increase in nuclear Ca^{2+} via Gq-mediated activation of PLC, which triggers hydrolysis of sarcolemmal PIP_2 to generate InsP_3 [19,73]. InsP_3 generated in the cytosol diffuses into the nucleus and activates perinuclear Ca^{2+} release through type-2 InsP_3 receptors, as shown in pathway 3 from Fig. 2. In this model, the selective regulation of nuclear Ca^{2+} is achieved by the strategic localization of InsP_3 Rs in nuclear membranes. Two physically segregated signaling hubs can be identified: one at the plasma membrane, formed by the ET-1/ET-1 receptor complex–Gq–PLC β – PIP_2 generating InsP_3 , which then diffuses through nuclear pores to the second hub, comprising the nuclear InsP_3 R and the perinuclear Ca^{2+} store. This chain of events is consistent with the kinetics of ET-1-elicited nuclear Ca^{2+} transients, i.e. ranging between 30 and 60 s. It remains an intriguing question why the second messenger, InsP_3 , is produced in excess far from the target site for its effect. One possibility is that by filtering the InsP_3 signal through cytosolic elements that facilitate or limit InsP_3 diffusion into the nucleus, the signal can be tuned to facilitate an appropriate response. Importantly, ET-1 receptor localization is not restricted to the sarcolemma but can also be detected in intracellular membranes, especially in the vicinity of the NE.

8.2. Perinuclear receptors

This working model is designed to explain the rapid effects of IGF-1 on nuclear Ca^{2+} release. A key component of this model is that a significant fraction of plasma membrane IGF-1R in cardiomyocytes is localized to perinuclear plasma membrane invaginations, or perinuclear T-tubules. This brings the surface receptor complex into close proximity of NE Ca^{2+} stores and provides direct access for extracellular ligands to the juxtannuclear receptor, thereby facilitating a localized nuclear response [58]. In this near-nuclear receptor model [100], plasma membrane IGF-1 receptors are directly apposed to the NE where they stimulate local production of InsP_3 mediated by Gai-dependent activation of perinuclear PLC. InsP_3 then enters the nucleus to activate InsP_3 Rs and release Ca^{2+} into the nucleoplasm [59]. This perinuclear Ca^{2+} signaling microdomain provides spatial insulation of nuclear Ca^{2+} signals from large cytosolic Ca^{2+} fluctuations [57].

This model is characterized by a single signaling hub, as all components share close proximity to the nucleus, thus avoiding the rate-limiting process of long-distance InsP_3 diffusion and degradation. This likely contributes to the fact that cardiomyocytes display the fastest nuclear Ca^{2+} response to IGF-1 of any cell type described to date, with a nuclear peak of only 2–6 s. This model is also potentially more efficient, because the total amount of InsP_3 necessary for triggering a nuclear Ca^{2+} response is significantly smaller than that required in the classic model. In summary, this model connects surface receptor activation directly with the nuclear Ca^{2+} store through a specialized and highly structured perinuclear Ca^{2+} microdomain, which is independent and physically separated from the cytosolic Ca^{2+} machinery.

8.3. Nuclear membrane receptors

A third working model situates a fraction of classical plasma membrane receptors (Ang II, ET-1 receptors or α 1-adrenergic receptors) and their downstream signaling elements intracellularly, at nuclear membranes [106]. Activation of GPCRs located in nuclear membranes sets in motion a signaling cascade involving G protein–PLC–PIP₂ hydrolysis to produce InsP₃ directly in the nucleoplasm promoting a very rapid InsP₃ receptor release of nuclear Ca²⁺ from NE stores [107]. Similar to the near-nucleus receptor model, a single signaling hub can be identified in this model, as all the signaling elements are recruited to act together in close proximity in the nucleus. However, an important difference between the two models is the way in which ligands gain access to their cognate receptors. Indeed, it is a matter of debate whether the nuclear-delimited receptors are activated by the same ligands as their plasma membrane counterparts or by alternative ligands made available through intracrine mechanisms [106]. Mechanisms of ligand action may be receptor-specific, as will be discussed next.

8.3.1. Angiotensin II receptors—Functional Ang II receptors (AT1 and AT2) have been detected in the cardiomyocyte nuclear membrane [106]. Other components of the renin–angiotensin system, such as angiotensinogen, angiotensin converting enzyme (ACE) and renin are also expressed in adult cardiomyocytes and found to act intracellularly [108]. Thus, cardiomyocytes possess the potential for an entirely cell-autonomous renin–angiotensin axis. In addition, cardiomyocytes can take up and activate circulating prorenin [109]. Functional ACE has been detected in the nuclei of neonatal cardiomyocytes, suggesting that this can be a site of Ang II production [110]. Activation of AT1 receptors in nuclei isolated from cardiomyocytes triggers increases in nucleoplasmic Ca²⁺ via release from InsP₃ receptors [107]. This intracellular Ang II-mediated Ca²⁺ transient is required for downstream activation of transcription. Together, these facts provide evidence for an intracrine pathway of intracellular Ang II production which can activate Ca²⁺-dependent transcriptional events involved in the expression of specific cardiac genes through the modulation of InsP₃ levels and nuclear Ca²⁺ release.

8.3.2. ET-1 receptors—In contrast to type A ET receptors (ETA) that localize to the sarcolemma (see Section 8.1), ETB receptors localize to nuclear membranes of adult cardiomyocytes [111]. This localization is not the consequence of surface receptor internalization, as demonstrated by the absence of N-glycosylated ETB receptor in nuclear protein fractions [112]. Moreover, endocytosed ETB receptor is destined for lysosomal degradation and does not traffic to the NE, suggesting that a significant pool of ETB receptor is targeted directly to nuclear membranes following biosynthesis [112]. Photo-release of caged ET-1 induces InsP₃-dependent nucleoplasmic Ca²⁺ increases that can be selectively blocked by intracellular delivery of an ETB antagonist, but not by an extracellular exposure to the same drug [112]. It is well established that ET-1 can be produced, stored, and secreted by cardiomyocytes under basal or stimulated conditions [113], suggesting that nuclear ETB can be activated by intracellular ET-1, comprising an intracrine system of nuclear Ca²⁺ release control.

8.3.3. α 1-Adrenergic receptors—Evidence is also available for the nuclear localization and signaling of α 1-adrenergic receptors in cardiomyocytes. α 1-Adrenergic receptors localize to the nucleus of adult mouse cardiomyocytes and colocalize with nuclear G α q and PLC β 1 [97]. Endogenous nuclear α 1-adrenergic receptor signaling complex can be activated locally by extracellular catecholamines since cardiomyocytes uptake catecholamines through a norepinephrine-uptake mechanism. Activation of nuclear-delimited α 1-adrenergic receptors leads to receptor oligomerization, ERK phosphorylation [114], nuclear PKC δ activation and phosphorylation of cardiac TnI, which in turn increase myocyte contractility [115]. Although nuclear α 1-adrenergic receptor signaling has been extensively demonstrated in cardiomyocytes, the exact involvement of nuclear receptor signaling on nuclear Ca²⁺ homeostasis remains unknown and future studies are needed to clarify the link between these 2 pathways.

9. Conclusion

Nuclear Ca²⁺ signals provide a strategic mechanism which allows cardiomyocytes to segregate the control of Ca²⁺-dependent transcription from cytosolic Ca²⁺ transients mediating E–C coupling [15]. Although different models have been proposed to explain the selective control of nuclear Ca²⁺ release in response to hormone stimulation [100], current evidence suggests that cardiomyocytes harbor a variety of mechanisms to activate and regulate nuclear Ca²⁺ release (Fig. 1). Furthermore, organization and signaling through the same receptor type may be different when localized to the sarcolemma versus the nuclear environment (Fig. 2).

There are many fascinating and unresolved questions. First, it is intriguing that cardiomyocytes are equipped with multiple mechanisms for controlling a common Ca²⁺-dependent transcriptional response. As discussed earlier, it may be that the diversity of mechanisms, all culminating in nuclear Ca²⁺ release, provides a physiological filter that encodes information regarding both the cellular environment and the nature of the specific agonist. Shaping the magnitude and temporal dynamics of nuclear Ca²⁺ release could impact the constellation of genes affected and the duration of the transcriptional or translational response. This is certainly plausible considering that agonists that use different mechanisms to control nuclear Ca²⁺ release also differ in their phenotypic effects. For example, Ang II and ET-1 generally mediate the expression of maladaptive genes and pathological cardiac hypertrophy, whereas IGF-1 mediates adaptive physiological cardiac hypertrophy [116]. In addition, many of these extracellular ligands activate other pathways different than Ca²⁺ signals, and their compartmentation in different subcellular locations may be a key for achieving selective responses.

Second, when a single agonist promotes nuclear Ca²⁺ release via multiple pathways, how is this decoded by the nucleus? As an example, ET-1 can initiate a signal at a sarcolemmal receptor and/or at a nuclear-membrane receptor. Decoding these signals by the nuclear machinery may lead to divergent phenotypic responses, or be integrated to give rise to a specified phenotype. Such a response could be further tuned to the initiating signal depending upon the balance of flux between the mechanisms. Third, it is not known whether there is cross-talk among the various mechanisms controlling nuclear Ca²⁺ or how the

resulting nuclear Ca^{2+} signals are coordinated with other important pathways activated by the same ligand or receptor.

Finally, current knowledge regarding mechanisms activating nuclear Ca^{2+} signals and their downstream consequences has been limited to experimental models, primarily in vitro. Applying this knowledge in the context of human heart disease may provide new therapeutic tools that harness mechanisms through which cardiomyocytes decode and respond to agonist-activated nuclear Ca^{2+} signals.

Thus, the conceptual framework of nuclear Ca^{2+} signals is in rapid flux, with exciting new insights emerging. Further elucidation of underlying mechanisms is necessary to fully define the complex roles of Ca^{2+} in the governance of contractile function and intracellular signaling in the heart.

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Abbreviations

Ang II	angiotensin II
E-C	excitation-contraction
ER	endoplasmic reticulum
ET-1	endothelin-1
IGF-1	insulin-like growth factor-1
INM	inner nuclear membrane
InsP₃	inositol 1,4,5-trisphosphate
InsP₃R	InsP ₃ receptor
NE	nuclear envelope
ONM	outer nuclear membrane
PI4P	phosphatidylinositol-4-phosphate
PIP₂	phosphatidylinositol bisphosphate
PLC	phospholipase C
PS	perinuclear space
RyR	ryanodine receptor
SR	sarcoplasmic reticulum

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Fig. 1.

Nuclear and cytosolic pathways controlling nuclear Ca^{2+} homeostasis in cardiomyocytes. Different pathways can lead to nuclear Ca^{2+} signals. Pathway 1: InsP_3 is generated by PLC either in the plasma membrane or in peri/intra-nuclear compartments. Pathway 2: InsP_3 can diffuse in and out of the nucleus. Pathway 3: InsP_3 from cytosolic or nuclear origin triggers Ca^{2+} release inside and/or outside the nucleus. Pathway 4: Ca^{2+} triggers Ca^{2+} release from RyRs in the cytoplasm or in the nucleus of cardiomyocytes (nuclear RyR is present only in neonatal cardiac myocytes). Pathway 5: Ca^{2+} diffusion from the cytosol can also generate nuclear Ca^{2+} transients and vice versa.

Fig. 2.

An integrated view of receptor-initiated nuclear Ca^{2+} signals in cardiomyocyte. Different agonists can activate receptors located in the sarcolemma (1 and 3) or intracellular receptors (2). Pathway 1: Perinuclear receptors located in deep T-tubules can lead to PLC-dependent InsP_3 generation in peri/nuclear compartments. Pathway 2: Intracellular receptors can be activated by lipophilic ligands (steroid hormones) or endocytosed ligands, leading to PLC-dependent InsP_3 generation in peri/nuclear compartments. Pathway 3: Receptors located in peripheral sarcolemma lead to generation of InsP_3 and its diffusion into the nucleus triggers nuclear Ca^{2+} signals.