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Nuclear Calcium in Cardiac Myocytes

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Abstract

Calcium (Ca^{2+}) is a universal second messenger involved in the regulation of various cellular processes including electrical signaling, contraction, secretion, memory, gene transcription and cell death. In heart, Ca^{2+} governs cardiomyocytes contraction, is central in electrophysiological properties and controls major signaling pathway implicated in gene transcription. How cardiomyocytes decode Ca^{2+} signal to regulate gene expression without interfering with, or being controlled by “contractile” Ca^{2+} that floods the entire cytosol during each heart beat is still elusive. In this review we summarize recent findings on nuclear Ca^{2+} regulation and its downstream signaling in cardiomyocytes. We will address difficulties in reliable quantification of nuclear Ca^{2+} fluxes and discuss its role in the development and progression of cardiac hypertrophy and heart failure. We also point out key open questions to stimulate future work.

1. Introduction

Calcium (Ca^{2+}) is a universal second messenger underlying key cellular processes varying from gene transcription to cell death.¹ In cardiac muscle, Ca^{2+} is best known for its role in beat-to-beat contractile activation. During each heartbeat, a transient rise in the cytoplasmic free Ca^{2+} concentration ($[\text{Ca}^{2+}]_{\text{cyto}}$) is followed by Ca^{2+} removal, completing the Ca^{2+} cycle that governs contraction and relaxation. In addition to this fundamental role in mediating cardiac myocyte contraction, in recent years a broader role for Ca^{2+} in cellular signaling has emerged.² It is intriguing how a cardiomyocyte decodes Ca^{2+} signal to regulate gene expression without interfering with, or being controlled by “contractile” Ca^{2+} , given the prevailing conditions in which $[\text{Ca}^{2+}]_{\text{cyto}}$ increases up to 20-fold (100 nM in diastole to 1–2 μM in systole) and Ca^{2+} floods the entire cytosol during each contractile cycle. It became evident that Ca^{2+} -dependent signaling regulation works via specific Ca^{2+} -binding proteins, but how molecular components involved in these processes may distinguish contractile vs. signaling Ca^{2+} still remains unknown and even controversial. On one hand, Ca^{2+} oscillations can vary in frequency, baseline, amplitude and duration, providing a biological signal with unlimited combinations for encoding information. On the other hand, a growing body of

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evidence suggests that such discrimination is attained by triggering spatially segregated Ca^{2+} release, generating subcellular microdomains for minutely regulated local Ca^{2+} -signaling events.³

In this review we will discuss nuclear Ca^{2+} signaling in cardiomyocytes, with focus on current understanding of its regulation and role in gene transcription. We will also address difficulties in reliably measuring nuclear Ca^{2+} concentration, $[\text{Ca}^{2+}]_{\text{nuc}}$, and tackle its involvement in development and progression of cardiac diseases. In addition, we will identify some unanswered questions to encourage further work.

2. Role of Nuclear Calcium in Cardiac Myocytes

Maintaining cardiac output, which is generally proportional to the tissues' need for oxygen, is one of the most intricate functions of circulatory system. Acute cardiac adaptation to increased oxygen demand is ensured by specific neurohormonal mediators (such as endothelin-1, angiotensin II, epinephrine and norepinephrine) which can very rapidly increase myocardial contractility and heart rate.⁴⁻⁶ On the other hand, long-term responses require complex cellular mechanisms allowing cardiomyocytes to reprogram their gene expression profile to meet changing cardiac demand. The mechanism of long-term cardiac reprogramming initiation and maintenance, as well as how it turns into maladaptive remodeling, is still not fully understood. However, over the past two decades, activation of Ca^{2+} -dependent transcription factors in a process termed excitation-transcription coupling (ETC), has emerged as a connecting link integrating extracellular signaling information and subsequent cardiomyocyte reprogramming. Pioneering work identified activation of key transcription factors via Ca^{2+} -dependent signaling pathways in adult cardiomyocytes, including nuclear factor of activated T cells (NFAT) responding to calcineurin (CaN) activation and myocyte enhancer factor 2 (MEF2) responding to CaMKII activation and histone deacetylase (HDAC) phosphorylation.^{7, 8} Since the known Ca^{2+} -dependent targets for these transcription factors (CaN-NFAT and CaMKII-HDAC) exist both in the cytosol and nucleus and can translocate, their transcriptional activation effects may be influenced by both $[\text{Ca}^{2+}]_{\text{cyto}}$ and $[\text{Ca}^{2+}]_{\text{nuc}}$. There is also evidence of locally regulated (peri)nuclear Ca^{2+} -signaling events and strategic organization of molecular components involved in ETC on the nuclear envelope and in the perinuclear regions.⁹⁻¹¹ Local increase in $[\text{Ca}^{2+}]_{\text{nuc}}$, derived from Ca^{2+} released inside, or in the close proximity to the nucleus, in contrast to global increase in $[\text{Ca}^{2+}]_{\text{cyto}}$, is currently believed to have a central role in the regulation of gene expression in cardiomyocytes.¹² Another transcription factor, cyclic-AMP-response element-binding protein (CREB), and its co-activator CREB-binding protein (CBP) are known to decode Ca^{2+} signals across the nucleus. Their activation requires high-amplitude changes in $[\text{Ca}^{2+}]_{\text{nuc}}$ ¹³ and they enhance expression of genes important in anti-oxidative and anti-apoptotic processes.¹⁴ However, we are not aware of direct evidence for $[\text{Ca}^{2+}]_{\text{nuc}}$ activation of CREB in adult cardiomyocytes. Therefore, better understanding of the regulation as well as reliable quantification of $[\text{Ca}^{2+}]_{\text{nuc}}$ in single cardiomyocytes is essential for understanding ongoing physiological and pathophysiological processes in the heart.

3. Nuclear Calcium Regulation

Although the nucleus is an autonomous subcellular compartment, well-defined by the nuclear envelope (NE), numerous nuclear pores complexes (NPCs) penetrate the NE and allow bidirectional passive diffusion of ions (including Ca^{2+}) making the nucleoplasm only partly insulated from the surrounding cytoplasm. Thus, each cytoplasmic $[\text{Ca}^{2+}]$ transient (CaT) also elicits a nucleoplasmic CaT.¹⁵ Similarly, any stimuli that cause an elevation of $[\text{Ca}^{2+}]_{\text{cyto}}$ (i.e. mechanical stretch, increased heart rate or non-excitatory stimulation) will also lead to an increase in $[\text{Ca}^{2+}]_{\text{nuc}}$.

On the other hand, accumulating evidence suggests that nucleoplasmic CaTs follow distinct kinetics and may – in part – be regulated independently from cytoplasmic CaTs.^{15, 16} A central role in the independent regulation of $[\text{Ca}^{2+}]_{\text{nuc}}$, is attributed to the NE which, beside its contribution to nuclear structure, also acts as a functional Ca^{2+} store to regulate $[\text{Ca}^{2+}]_{\text{nuc}}$.¹⁷ Even though recent studies provided compelling evidence that both mechanisms of $[\text{Ca}^{2+}]_{\text{nuc}}$ regulation co-exist in cardiomyocytes, their relative contribution under different conditions, as well as functional consequences of the specific message they transmit, remain elusive.

3.1 Passive diffusion

It was initially showed that Ca^{2+} ions can enter the nucleus via passive diffusion of cytoplasmic Ca^{2+} through NPCs transversing the NE. The NE consists of the inner and outer nuclear membrane with the space between them acting as a functional Ca^{2+} store, akin to, and with its lumen connected to, the sarcoplasmic reticulum (SR).¹⁸ The nuclear membranes fuse at many sites to form pores that harbor the NPCs. These multiprotein complexes are the major gateway for ions (including Ca^{2+}) and small molecules to diffuse along their concentration gradients between the cytosol and nucleoplasm, given the approximate pore diameter of 8 nm.¹⁹ Although the conductance of NPCs may change in response to factors such as Ca^{2+} and ATP, therefore influencing the kinetics of Ca^{2+} traffic,²⁰ the pore itself does not entirely close, allowing punctate passages for Ca^{2+} between the cytoplasm and the nucleus.

In addition, NE of numerous cell types (including cardiomyocytes) is interrupted by infoldings that reach deep into the nucleoplasm and may even traverse the nucleus completely (for review see ²¹). The NE invaginations are lined by both, the inner and the outer nuclear membranes and filled with cytosol, SR and even mitochondria.²² Recently demonstrated presence of NPCs on the NE invaginations ensures effective nucleo-cytoplasmic ion diffusion and cargo transport in regions that would otherwise be remote from the nuclear periphery, by decreasing the diffusion delay and by increasing membrane surface area.²³

Whole cell Ca^{2+} oscillations—The Ca^{2+} cycle in cardiomyocytes that governs contraction and relaxation on a beat-to-beat basis consists of a transient rise and decay of $[\text{Ca}^{2+}]_{\text{cyto}}$. During the cardiac action potential, myocyte membrane depolarization leads to opening of the voltage-dependent L-type Ca^{2+} channels, inducing an inward Ca^{2+} current. The increase in local intracellular $[\text{Ca}^{2+}]$ triggers the release of Ca^{2+} stored in the SR

through Ca^{2+} release channels – ryanodine receptors (RyR) – in a positive feedback fashion. The transient increase in cytoplasmic free $[\text{Ca}^{2+}]$ allows Ca^{2+} binding to the myofilament protein troponin C. The conformational change of troponin regulatory complex leads to initiation of cross-bridge formation between actin and myosin, causing myocyte contraction. For relaxation to occur, free cytoplasmic $[\text{Ca}^{2+}]$ has to decline and allow Ca^{2+} to dissociate from troponin. Intracellular Ca^{2+} is mostly taken up by an ATP-dependent Ca^{2+} pump, the sarco-endoplasmic reticulum calcium-ATPase (SERCA), into the SR. To a quantitatively smaller extent, cytoplasmic Ca^{2+} is removed from the cell by the electrogenic sodium-calcium exchanger. About 1% of the activating Ca^{2+} is taken up by mitochondria or extruded via the sarcolemmal Ca^{2+} -ATPase.²⁴ The dynamic regulation of Ca^{2+} transport mechanisms is essential at varying heart rates.²⁵ To achieve this, several cellular kinases and phosphatases regulate proteins involved in excitation-contraction coupling, providing their suitable activation and inhibition under different physiological conditions. In healthy hearts, Ca^{2+} -handling proteins are regulated by α - and β -adrenergic receptor (α -AR and β -AR) stimulation upon the activation of sympathetic nerves, coupled to G_q and G_s protein-coupled signaling cascades, respectively. β -AR stimulation activates PKA which causes increased Ca^{2+} entry via L-type Ca^{2+} channels and enhances SR Ca^{2+} uptake (by phospholamban phosphorylation and dis-inhibition of SERCA). α -AR stimulation can also promote Ca^{2+} release from the SR and NE via inositol-1,4,5-trisphosphate receptors (IP₃R) located on the SR and NE. However, in ventricular myocytes the IP₃R-mediated Ca^{2+} release has greater impact on $[\text{Ca}^{2+}]_{\text{nuc}}$ vs. the rise and fall of $[\text{Ca}^{2+}]_{\text{cyto}}$ that drives contraction and relaxation. On the other hand, local IP₃R-mediated Ca^{2+} release can influence signaling (see below).

It is well established that due to the passive diffusion of Ca^{2+} ions through NPCs, each cytoplasmic CaT also elicits a nucleoplasmic CaT.¹⁵ It has been proposed that NE can function as ‘diffusion filter’ allowing only a limited fraction of cytoplasmic Ca^{2+} to enter the nucleoplasm, as well as introducing a kinetic delay in the equilibration of $[\text{Ca}^{2+}]_{\text{nuc}}$ and $[\text{Ca}^{2+}]_{\text{cyto}}$.¹²

Based on NE invaginations and NPC, SERCA and RyR distribution around nucleus,²³ the rise in $[\text{Ca}^{2+}]_{\text{nuc}}$ is driven mainly by the RyR-dependent rise in $[\text{Ca}^{2+}]_{\text{cyto}}$ and Ca^{2+} diffusion via NPCs (including invaginations) which slows rise times and peak $[\text{Ca}^{2+}]_{\text{nuc}}$ (Figure 1). As SERCA is mainly expressed on the outer NE facing the cytoplasm (where there is also much more SERCA on SR), most Ca^{2+} probably has to diffuse out of the nucleus to be pumped back into the NE and SR, accounting for the much slower $[\text{Ca}^{2+}]_{\text{nuc}}$ decline. In line with this hypothesis, quantitative analysis of nucleoplasmic vs. cytoplasmic CaTs revealed significant nucleoplasmic-to-cytoplasmic $[\text{Ca}^{2+}]$ gradients during the cardiac cycle. Higher diastolic $[\text{Ca}^{2+}]$ and lower systolic $[\text{Ca}^{2+}]$ in the nucleus was explained by significantly slower CaTs kinetics in the nucleoplasm than in the cytoplasm.¹⁶ Still, the amplitude of the nuclear CaT was found to be proportional to the amplitude of the cytoplasmic CaT, providing evidence for the passive nature of nuclear CaT. One of the most important consequences of markedly slower kinetics of nucleoplasmic CaTs is the non-proportional increase in diastolic $[\text{Ca}^{2+}]_{\text{nuc}}$ vs. $[\text{Ca}^{2+}]_{\text{cyto}}$ when diastole is shortened at higher heart rates. An increase in pacing frequency is, therefore, enough to cause profound differences in the level of $[\text{Ca}^{2+}]_{\text{nuc}}$ vs. $[\text{Ca}^{2+}]_{\text{cyto}}$.¹⁶

In this model, the degree of the kinetic delay might be subject to modulation, therefore regulating the portion of cytoplasmic Ca^{2+} diffusing to the nucleus upon specific stimuli. First of all, a greater expression of NPCs would allow a more rapid equilibration of $[\text{Ca}^{2+}]_i$ and $[\text{Ca}^{2+}]_{\text{nuc}}$ with a tendency to minimize nucleoplasmic-to-cytoplasmic $[\text{Ca}^{2+}]$ gradients. Increased permeability of NPCs would have similar effect on Ca^{2+} traffic between the nucleus and the cytoplasm. Additionally, changes in Ca^{2+} buffering capacity of the nucleus could favor or diminish nucleoplasmic CaTs propagation. It is also tempting to speculate that – similar to T-tubular sarcolemmal invaginations that are critical for coordinated Ca^{2+} cycling throughout the myocyte – NE invaginations may be critical for fine control of nucleoplasmic Ca^{2+} regulation. Fewer nuclear invaginations, as we see in heart failure, may also magnify such cytosol-nuclear $[\text{Ca}^{2+}]$ gradients.²³ However, further experimental work is necessary to confirm or dismiss these possibilities in cardiomyocytes.

Local Ca^{2+} oscillations - SR Ca^{2+} leak—In addition to changes of $[\text{Ca}^{2+}]_{\text{nuc}}$ as a result of whole cell CaTs, brief, spontaneous, local Ca^{2+} release events from the SR – so-called Ca^{2+} sparks (RyR release) and Ca^{2+} puffs (IP_3R release) – may also give rise to nuclear Ca^{2+} signal. Bootman et al. showed that if the spontaneous Ca^{2+} release occurs within the 5 μM distance from the nucleus, it may briefly increase $[\text{Ca}^{2+}]_{\text{nuc}}$.²⁶ The advantage of controlling specific cellular processes by elementary Ca^{2+} signals is their rapid local action at relatively low energy cost to the cells, in contrast to global Ca^{2+} changes. Since the elementary Ca^{2+} signals have only a limited spatial spread (usually 2–4 μm), and the $[\text{Ca}^{2+}]$ declines sharply with distance from the site of origin, regulation of cellular activities relies on close localization of the Ca^{2+} channels and their targets. Existence of structural elements that correspond to these requirements were recently described in cardiomyocytes.²⁷ Subcellular organizational units formed by an association between the SR and the T-tubule segments of plasma membrane (dyads) were found to be frequently located in the space between the nearest myofibril and the NE. The linear distance between RyRs from the nearest dyads and the NE is as low as ≈ 200 nm or occasionally less. Ca^{2+} released from these dyads would reach the NE in less than 1 ms, in that way creating an effective perinuclear microdomain that can influence $[\text{Ca}^{2+}]_{\text{nuc}}$.

Although we have convincing structural and functional evidence showing that $[\text{Ca}^{2+}]_{\text{nuc}}$ may be regulated by local cytoplasmic Ca^{2+} release events in the close proximity to the nucleus, how this affects downstream signaling and cellular processes under different physiological and pathophysiological conditions, requires future studies.

3.2 Receptor Stimulated regulation of Nuclear Calcium

In addition to $[\text{Ca}^{2+}]_{\text{nuc}}$ regulation via passive diffusion of Ca^{2+} ions from the cytoplasm, several lines of evidence suggest that there is an additional, entirely independent source of Ca^{2+} in the nucleus. First, the nucleus is a cellular compartment with its own perinuclear Ca^{2+} store (NE) that can actively store and release Ca^{2+} .²³ Second, the NE expresses Ca^{2+} -regulating proteins, including IP_3R Ca^{2+} release channels (facing the nucleoplasm and the cytoplasm) and Ca^{2+} -buffering proteins.^{23, 28, 29} Third, specific stimuli may – via IP_3R -mediated Ca^{2+} release from the NE – increase $[\text{Ca}^{2+}]_{\text{nuc}}$ independently from $[\text{Ca}^{2+}]_{\text{cyto}}$.^{10, 15}

Key component of the nuclear Ca^{2+} -regulatory machinery independent of $[\text{Ca}^{2+}]_{\text{cyto}}$ is the G-protein coupled receptor (GPCR)-dependent activation of phospholipase C, followed by the generation of IP_3 and IP_3R stimulation (Figure 1). The high affinity type 2 IP_3R (IP_3R_2) is the predominant subtype in cardiac myocytes, which in ventricular myocytes is concentrated in the NE.³⁰ One of the first experimental evidence of this concept in cardiomyocytes came from the work of Zima et al., showing that treatment of isolated nuclei with IP_3 leads to NE Ca^{2+} depletion via IP_3Rs , that is paralleled by an increase of $[\text{Ca}^{2+}]_{\text{nuc}}$.³¹ Further studies revealed that a number of agonists such as ET-1,¹⁰ angiotensin II (ATII)³² or insulin-like growth factor 1 (IGF-1)³³ may use various signaling mechanisms to stimulate specific GPCRs upstream of IP_3 generation. The structural organization of this signaling may also involve either receptors and IP_3 production at T-tubule membranes that come very close to the nucleus,³³ or even GPCR-complexes right on the NE (Figure 2).³² Clearly, local IP_3R -mediated Ca^{2+} release can modify $[\text{Ca}^{2+}]_{\text{nuc}}$ that is driven by global CaT. Mechanisms of receptor-specific ligand action will be discussed next.

ET-1 receptors—Several years ago, Wu and colleagues were the first to show that ET-1, which activates GPCRs to produce IP_3 , elicits local nuclear envelope Ca^{2+} release via IP_3R .¹⁰ They proposed the mechanism in which receptor signaling complex including the GPCR, phospholipase C and IP_3 precursor phosphatidylinositol 4,5-bisphosphate is located on the plasma membrane and IP_3R is located on the NE accessing local nuclear Ca^{2+} store. The communication between these two signaling hubs is achieved via IP_3 diffusion. Fluorescence resonance energy transfer-based IP_3 biosensors confirmed that ET-1 triggers IP_3 production at the plasma membrane that precedes the rise of IP_3 level in the nucleus and that most of the IP_3R in adult ventricular myocytes is largely localized to the NE.³⁴ These findings confirm the ability of cytoplasmic IP_3 to diffuse to the nucleus and this sequence of events correspond well to the kinetics of ET-1-induced rise in $[\text{Ca}^{2+}]_{\text{nuc}}$. That is consistent with the longer range of IP_3 signaling ($\approx 50 \mu\text{m}$; greater than the radius of a myocyte),³⁵ although IP_3 is also hydrolyzed to its inactive forms such as IP_2 .³⁶ An obvious question is why does cell produce excess of IP_3 far from its target? Whether it is to fine tune the nuclear Ca^{2+} response in terms of its kinetics or amplitude, or for the interplay with cytoplasmic IP_3R , complete understanding of regulatory mechanism behind this seemingly inefficient cellular strategy needs further clarification.

At the same time, a fraction of GPCRs stimulated by ET-1 appear to be localized at the nuclear membrane, where intracrine ET-1 signaling evokes IP_3 -dependent increases in $[\text{Ca}^{2+}]_{\text{nuc}}$.³⁷ In this regulatory model, ET-1 receptors are targeted directly to the NE following biosynthesis³⁸ and activated selectively by endogenous ET-1, which is produced, stored and secreted by cardiomyocytes.³⁹ Similar to the previous mechanism, ET-1 stimulation resulted in an IP_3R -mediated increase in $[\text{Ca}^{2+}]_{\text{nuc}}$.

Although we know that two different ET-1 receptor subtypes are responsible for these two different regulatory mechanisms, with type A ET-1 receptor being located at sarcolemma and responding to exogenous ET-1⁴⁰ and type B ET-1 receptor found at the NE and being activated by intracellular ET-1,³⁷ functional importance and activation pattern for each mechanism is still not fully understood.

Angiotensin II receptors—Two different subtypes of functional ATII receptors, ATII receptor 1 and ATII receptor 2, have been detected in cardiomyocytes.⁴¹ Similarly to ET-1 receptors, ATII receptors can be located at the T-tubular network of the plasma membrane and on the NE. Again, the fraction of the receptor found on the NE is a result of intracellular synthesis and trafficking, rather than endocytosis. Exposure of isolated nuclei to ATII transiently raised $[Ca^{2+}]_{nuc}$, whereas the use of subtype specific agonists revealed that ATII receptor 1 is specifically responsible for nuclear $[Ca^{2+}]$ response.³² When a specific inhibitor of IP₃R was applied, ATII-mediated increase in $[Ca^{2+}]_{nuc}$ was completely blocked, consistent with the ATII effects being IP₃-dependent. In isolated cardiomyocytes, application of ATII increased diastolic $[Ca^{2+}]$ in both the cytoplasm and the nucleus. The increase in $[Ca^{2+}]_{nuc}$ was much larger than the increase in diastolic $[Ca^{2+}]_{cyto}$ and it was prevented by cardiomyocytes pretreatment with IP₃R blocker.²³

Although blockers of the renin-angiotensin system are widely used in the treatment of hypertension and heart failure, the exact molecular mechanism of how activation of different ATII receptor subtypes at different subcellular localization is orchestrated still remains elusive.

α 1-Adrenergic Receptors—Presence and signaling function of another GPCR, α -AR, on the NE has been demonstrated in cardiomyocytes.^{42, 43} When two different subtypes of functional α -ARs (α 1A and α 1B) were expressed as GFP fusion proteins in α 1AB-KO cardiomyocytes, both could be observed at nuclear and perinuclear locations.⁴⁴ Although evidence for colocalization of both α 1A and α 1B subtypes with G-protein and PLC in the nuclear membrane is available, the involvement of α 1-AR in the regulation of $[Ca^{2+}]_{nuc}$ remains unknown.

β -Adrenergic Receptors—In a series of experiments, Boivin et al. presented strong evidence for the presence and functional role of β -ARs on the NE.⁴⁵ The best investigated downstream effector of β -ARs is the enzyme adenylyl cyclase, which catalyzes the conversion of ATP to cAMP, and is also identified in the NE.⁴⁶ Rise in cAMP levels activates cAMP-dependent protein kinases (PKA). In turn, PKA increases the phosphorylation state of target proteins involved in the regulation of Ca^{2+} cycling (L-type Ca^{2+} channels, phospholamban, RyR). Some of these phosphorylation events increase the Ca^{2+} content of the cardiomyocytes, in this way not only elevating force but also hastening relaxation.⁴⁷ As NE contains components for regulation of the Ca^{2+} equilibrium, we cannot exclude partial role of nuclear β -ARs in the regulation of $[Ca^{2+}]_{nuc}$.

IGF-1 receptors—In a recent study, Ibarra et al. proposed an exciting extension to the previously described models of receptor-mediated $[Ca^{2+}]_{nuc}$ regulation.³³ They provided evidence that an entire signaling complex, in this case IGF-1 receptor, G-protein, phospholipase C, and IP₃ production, is situated in the close proximity to the nucleus due to deep plasma membrane infoldings towards the nuclear envelope. IP₃ production close to the IP₃R at the NE greatly reduce the amount of IP₃ required for increase in $[Ca^{2+}]_{nuc}$ and provides spatial insulation of nuclear Ca^{2+} signals from large cytoplasmic Ca^{2+} oscillations.

There is now sufficient evidence for the existence of NE-proximal pathways (Figure 2). However, it remains to be clarified what the relative importance of different structural pathways is vs. the traditional surface membrane IP_3 production and if these pathways have functionally discrete physiological functions.

4. Nuclear Ca^{2+} in hypertrophy and heart failure

Heart failure (HF) is characterized by impaired systolic and diastolic function with abnormalities of Ca^{2+} handling in cardiomyocytes underlying contractile dysfunction in failing hearts.^{48, 49} Current research is focused on better understanding the early triggers for alterations of intracellular Ca^{2+} homeostasis, as well as whether Ca^{2+} -dependent signaling pathways (e.g. via CaMKII and calcineurin) may initiate and/or facilitate the progression of cardiac remodeling (such as hypertrophy) to severe HF. Although substantial progress has been made towards understanding the role of altered *cytoplasmic* Ca^{2+} homeostasis in hypertrophy and HF,⁵⁰ not as much data are available on *nucleoplasmic* Ca^{2+} homeostasis. It may, however, be a critical component to cardiac remodeling by influencing protein expression via nuclear Ca^{2+} -dependent gene transcription.¹⁰

As previously mentioned, NE contains invaginations that reach deep into the nucleoplasm and may facilitate intranuclear regulation of ions in regions that would otherwise be remote from the nuclear periphery.²¹ Our most recent work showed that there is a progressive decrease in NE invagination density in hypertrophied and failing hearts, associated with alterations of $[Ca^{2+}]_{nuc}$ in electrically stimulated cardiomyocytes. Changes in nuclear CaTs occurred at an earlier disease stage than did cytoplasmic CaT changes, with an onset so early that they may well be involved in the development and progression of hypertrophy and HF.²³ As expected, due to the slower kinetics of nucleoplasmic CaTs, the frequency-dependent higher increase in diastolic $[Ca^{2+}]_{nuc}$ vs. diastolic $[Ca^{2+}]_{cyto}$ was even more pronounced in cardiomyocytes from hypertrophied and failing hearts. Higher pacing frequencies may, therefore, be causally involved in the remodeling processes leading to HF, in particular when considering the often elevated heart rates of patients with HF.

Significant changes in specific proteins of the NPC in cardiomyocytes from failing human hearts were recently reported.⁵¹ These changes, related to ventricular function, could be accompanied by alterations in the nucleo-cytoplasmic transport and regulation of gene expression. In addition, mutations in genes encoding proteins of the inner and outer nuclear membrane as well as nucleoskeleton cause early onset of cardiomyopathy with altered nuclear positioning, shape, and chromatin organization.^{52, 53} Indeed, such changes could also interfere with the nucleoplasmic Ca^{2+} handling and nuclear Ca^{2+} -mediated gene expression.

Increased Ca^{2+} leak via RyRs is another well-established feature of HF.⁵⁴ Even more, RyR-mediated SR Ca^{2+} leak itself promotes myocardial remodeling, including eccentric hypertrophy, dilatation and contractile dysfunction in RyR2^{R4496C/+} mice under pressure overload.⁵⁵ It is tempting to speculate that RyR leak in perinuclear dyads may have a critical role in promoting hypertrophy as it may directly increase $[Ca^{2+}]_{nuc}$.

GPCRs (α -adrenergic, ET-1 and ATII receptors) that induce IP₃ production are also more activated during the development and progression of HF.^{56, 57} There are also higher levels of IP₃R2 expression in HF, as well as downstream activation of nuclear CaMKII-dependent nuclear signaling.^{58, 59} Elevated [Ca²⁺]_{nuc} may enhance the IP₃ sensitivity of IP₃ receptors⁶⁰ and could well synergize with other factors which elevate [Ca²⁺]_{nuc} in HF, as we have shown for AII and high pacing frequency.²³ Indeed, increased IP₃R2 expression was proposed to be essential during hypertrophy and HF.⁶¹ In line with this notion, we recently observed increased perinuclear IP₃R2 expression, and higher diastolic [Ca²⁺]_{nuc} vs. [Ca²⁺]_{cyto} in HF cardiomyocytes treated with ATII.²³

Complex regulation of [Ca²⁺]_{nuc} in cardiomyocytes seems to be an important determinant of cardiac remodeling and may contribute to the development and progression of hypertrophy and HF. Normalization of nucleoplasmic Ca²⁺ levels and its regulation may, therefore, be a novel therapeutic approach for preventing adverse cardiac remodeling.

5. Measuring nuclear [Ca²⁺]

As [Ca²⁺]_{nuc} in cardiomyocytes regulates transcription and its alterations are implicated in remodelling processes leading to HF, quantification of subcellularly resolved Ca²⁺ signals is essential for understanding physiological and pathological processes in the heart.

Advancement of confocal laser microscopy together with the development of chemical fluorescent Ca²⁺ indicators provides the basis for visualization of whole cell and subcellular [Ca²⁺] fluxes. In principle, upon excitation, these indicators emit light at particular wavelengths and the emitted fluorescence intensity or emission spectrum is changed in a Ca²⁺ bound state.⁶²

The dissociation constant (K_d) as a measure of Ca²⁺ binding affinity is crucial for the selection of the appropriate Ca²⁺ indicator for the cellular compartment of interest. Low affinity, high K_d dyes (e.g. Fluo-5N and Mag-fluo-4) are used for the visualization of [Ca²⁺] changes in the SR or NE, whereas, high affinity, low K_d indicators (e.g. Fluo-3 and Fluo-4) are preferred for measuring changes in cytosolic and nucleoplasmic free [Ca²⁺]. The use of fluorescent Ca²⁺ indicators for simultaneous quantification of cytoplasmic and nucleoplasmic Ca²⁺ fluctuations gets complicated somewhat as properties of fluorescent Ca²⁺-indicators in intracellular compartments may differ, due to the specific physicochemical properties of the environment. *In situ* calibration of the most frequently used Ca²⁺ indicator, Fluo-4, revealed significant differences in the apparent K_d between cytoplasm and nucleoplasm of adult cardiomyocytes,¹⁶ confirming that without proper independent determination of the indicator properties in the nucleoplasm vs. the cytoplasm, any quantitative analysis of [Ca²⁺]_{nuc} versus [Ca²⁺]_{cyto} is not reliable.

Targeted molecular tools, engineered in such way that their mode of action is restricted to a specific subcellular compartment is a promising new strategy to avoid artefacts of commonly used chemical Ca²⁺ indicators.⁶³ Protein-based Ca²⁺ probes such as cameleons,⁶⁴ pericams⁶⁵ or aequorins⁶⁶ have been successfully targeted to the nucleus. Differences in behaviour between [Ca²⁺]_{nuc} and [Ca²⁺]_{cyto} have been reported in neurons using cameleon,⁶⁷ and ratiometric pericam has been used to monitor [Ca²⁺]_{nuc} in beating

neonatal cardiomyocytes.⁶⁸ However, improvement in dynamic range, in combination with ultra-fast kinetics needed to reliably monitor Ca^{2+} changes in cardiomyocytes is a continuing challenge.

6. Conclusion

At present, due to limited tools for measuring and selectively blocking/buffering nuclear Ca^{2+} , we have only begun to understand how a cardiomyocyte decodes a complex array of nuclear Ca^{2+} signals to reprogram gene expression profile and meet constantly varying cardiac demand. It is striking that a simple ion such as Ca^{2+} can regulate so many different cellular processes and it seems that current research has opened more questions than the answers it has provided. Thus, understanding the regulation of nuclear Ca^{2+} in cardiomyocytes will undoubtedly remain an exciting research focus and a topic of debate for some time.

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References

1. Bers DM. Calcium cycling and signaling in cardiac myocytes. *Annu Rev Physiol.* 2008; 70:23–49. [PubMed: 17988210]
2. Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: Dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol.* 2003; 4:517–529. [PubMed: 12838335]
3. Ibarra C, Vicencio JM, Varas-Godoy M, Jaimovich E, Rothermel BA, Uhlen P, Hill JA, Lavandero S. An integrated mechanism of cardiomyocyte nuclear Ca signaling. *J Mol Cell Cardiol.* 2014; 75C: 40–48. [PubMed: 24997440]
4. Moravec CS, Reynolds EE, Stewart RW, Bond M. Endothelin is a positive inotropic agent in human and rat heart in vitro. *Biochem Biophys Res Commun.* 1989; 159:14–18. [PubMed: 2647079]
5. Salas MA, Vila-Petroff MG, Palomeque J, Aiello EA, Mattiazzi A. Positive inotropic and negative lusitropic effect of angiotensin II: intracellular mechanisms and second messengers. *J Mol Cell Cardiol.* 2001; 33:1957–1971. [PubMed: 11708841]
6. Kambayashi M, Miura T, Oh BH, Rockman HA, Murata K, Ross J Jr. Enhancement of the force-frequency effect on myocardial contractility by adrenergic stimulation in conscious dogs. *Circulation.* 1992; 86:572–580. [PubMed: 1353420]
7. Molkentin JD, Lu JR, Antos CL, Markham B, Richardson J, Robbins J, Grant SR, Olson EN. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell.* 1998; 93:215–228. [PubMed: 9568714]
8. Passier R, Zeng H, Frey N, Naya FJ, Nicol RL, McKinsey TA, Overbeek P, Richardson JA, Grant SR, Olson EN. CaM kinase signaling induces cardiac hypertrophy and activates the MEF2 transcription factor in vivo. *J Clin Invest.* 2000; 105:1395–1406. [PubMed: 10811847]
9. Rinne A, Kapur N, Molkentin JD, Pogwizd SM, Bers DM, Banach K, Blatter LA. Isoform- and tissue-specific regulation of the Ca^{2+} -sensitive transcription factor NFAT in cardiac myocytes and heart failure. *Am J Physiol Heart Circ Physiol.* 2010; 298:H2001–H2009. [PubMed: 20304816]
10. Wu X, Zhang T, Bossuyt J, Li X, McKinsey TA, Dedman JR, Olson EN, Chen J, Brown JH, Bers DM. Local InsP_3 -dependent perinuclear Ca^{2+} signaling in cardiac myocyte excitation-transcription coupling. *J Clin Invest.* 2006; 116:675–682. [PubMed: 16511602]
11. Mishra S, Gray CBB, Miyamoto S, Bers DM, Brown JH. Location Matters: Clarifying the Concept of Nuclear and Cytosolic CaMKII Subtypes. *Circ Res.* 2011; 109:1354–1362. [PubMed: 21998325]

12. Bootman MD, Fearnley C, Smyrniak I, MacDonald F, Roderick HL. An update on nuclear calcium signalling. *J Cell Sci.* 2009; 122:2337–2350. [PubMed: 19571113]
13. Chawla S, Hardingham GE, Quinn DR, Bading H. CBP: A signal-regulated transcriptional coactivator controlled by nuclear calcium and CaM kinase IV. *Science.* 1998; 281:1505–1509. [PubMed: 9727976]
14. Watson PA, Reusch JEB, McCune SA, Leinwand LA, Luckey SW, Konhilas JP, Brown DA, Chicco AJ, Sparagna GC, Long CS, Moore RL. Restoration of CREB function is linked to completion and stabilization of adaptive cardiac hypertrophy in response to exercise. *American Journal of Physiology - Heart and Circulatory Physiology.* 2007; 293:H246–H259. [PubMed: 17337597]
15. Kockskaemper J, Seidlmayer L, Walther S, Hellenkamp K, Maier LS, Pieske B. Endothelin-1 enhances nuclear Ca^{2+} transients in atrial myocytes through $\text{Ins}(1,4,5)\text{P}_3$ -dependent Ca^{2+} release from perinuclear Ca^{2+} stores. *J Cell Sci.* 2008; 121:186–195. [PubMed: 18089647]
16. Ljubojevic S, Walther S, Asgarzoei M, Sedej S, Pieske B, Kockskaemper J. In Situ Calibration of Nucleoplasmic versus Cytoplasmic Ca^{2+} Concentration in Adult Cardiomyocytes. *Biophys J.* 2011; 100:2356–2366. [PubMed: 21575569]
17. Mauger JP. Role of the nuclear envelope in calcium signalling. *Biol Cell.* 2012; 104:70–83. [PubMed: 22188206]
18. Wu X, Bers DM. Sarcoplasmic reticulum and nuclear envelope are one highly interconnected Ca^{2+} store throughout cardiac myocyte. *Circ Res.* 2006; 99:283–291. [PubMed: 16794184]
19. Keminer O, Peters R. Permeability of single nuclear pores. *Biophys J.* 1999; 77:217–228. [PubMed: 10388751]
20. Hohendanner F, McCulloch AD, Blatter LA, Michailova AP. Calcium and IP_3 dynamics in cardiac myocytes: experimental and computational perspectives and approaches. *Front Pharmacol.* 2014; 5:35. [PubMed: 24639654]
21. Malhas A, Goulbourne C, Vaux DJ. The nucleoplasmic reticulum: form and function. *Trends Cell Biol.* 2011; 21:362–373. [PubMed: 21514163]
22. Lui PPY, Kong SK, Kwok TT, Lee CY. The nucleus of HeLa cell contains tubular structures for Ca^{2+} signalling. *Biochem Biophys Res Commun.* 1998; 247:88–93. [PubMed: 9636660]
23. Ljubojevic S, Radulovic S, Leitinger G, Sedej S, Sacherer M, Holzer M, Winkler C, Pritz E, Mittler T, Schmidt A, Sereinig M, Wakula P, Zissimopoulos S, Bisping E, Post H, Marsche G, Bossuyt J, Bers DM, Kockskaemper J, Pieske B. Early remodeling of perinuclear Ca^{2+} stores and nucleoplasmic Ca^{2+} signaling during the development of hypertrophy and heart failure. *Circulation.* 2014; 130:244–255. [PubMed: 24928680]
24. Bers DM. Cardiac excitation-contraction coupling. *Nature.* 2002; 415:198–205. [PubMed: 11805843]
25. Koivumaki JT, Korhonen T, Takalo J, Weckstrom M, Tavi P. Regulation of excitation-contraction coupling in mouse cardiac myocytes: integrative analysis with mathematical modelling. *BMC Physiology.* 2009; 9:16. [PubMed: 19715618]
26. Lipp P, Thomas D, Berridge MJ, Bootman MD. Nuclear calcium signalling by individual cytoplasmic calcium puffs. *EMBO J.* 1997; 16:7166–7173. [PubMed: 9384593]
27. Escobar M, Cardenas C, Colavita K, Petrenko NB, Franzini-Armstrong C. Structural evidence for perinuclear calcium microdomains in cardiac myocytes. *J Mol Cell Cardiol.* 2011; 50:451–459. [PubMed: 21147122]
28. Gerasimenko O, Gerasimenko J. New aspects of nuclear calcium signalling. *J Cell Sci.* 2004; 117:3087–3094. [PubMed: 15226390]
29. Echevarria W, Leite MF, Guerra MT, Zipfel WR, Nathanson MH. Regulation of calcium signals in the nucleus by a nucleoplasmic reticulum. *Nat Cell Biol.* 2003; 5:440–446. [PubMed: 12717445]
30. Perez PJ, Ramos-Franco J, Fill M, Mignery GA. Identification and functional reconstitution of the type 2 inositol 1,4,5-trisphosphate receptor from ventricular cardiac myocytes. *J Biol Chem.* 1997; 272:23961–23969. [PubMed: 9295347]
31. Zima AV, Bare DJ, Mignery GA, Blatter LA. IP_3 -dependent nuclear Ca^{2+} signalling in the mammalian heart. *J Physiol.* 2007; 584:601–611. [PubMed: 17761776]

32. Tadevosyan A, Maguy A, Villeneuve LR, Babin J, Bonnefoy A, Allen BG, Nattel S. Nuclear-delimited angiotensin receptor-mediated signaling regulates cardiomyocyte gene expression. *J Biol Chem.* 2010; 285:22338–22349. [PubMed: 20463030]
33. Ibarra C, Vicencio JM, Estrada M, Lin Y, Rocco P, Rebellato P, Munoz JP, Garcia-Prieto J, Quest AF, Chiong M, Davidson SM, Bulatovic I, Grinnemo KH, Larsson O, Szabadkai G, Uhlen P, Jaimovich E, Lavandero S. Local control of nuclear calcium signaling in cardiac myocytes by perinuclear microdomains of sarcolemmal insulin-like growth factor 1 receptors. *Circ Res.* 2013; 112:236–245. [PubMed: 23118311]
34. Remus TP, Zima AV, Bossuyt J, Bare DJ, Martin JL, Blatter LA, Bers DM, Mignery GA. Biosensors to measure inositol 1,4,5-trisphosphate concentration in living cells with spatiotemporal resolution. *J Biol Chem.* 2006; 281:608–616. [PubMed: 16249182]
35. Allbritton NL, Meyer T, Stryer L. Range of messenger action of calcium ion and inositol 1,4,5-trisphosphate. *Science.* 1992; 258:1812–1815. [PubMed: 1465619]
36. Sanchez X, Carrasco MA, Vergara J, Hidalgo C. Inositol 1,4,5-triphosphate phosphatase activity in membranes isolated from amphibian skeletal muscle [corrected. *FEBS Lett.* 1991; 279:58–60. [PubMed: 1995344]
37. Boivin B, Chevalier D, Villeneuve LR, Rousseau E, Allen BG. Functional endothelin receptors are present on nuclei in cardiac ventricular myocytes. *J Biol Chem.* 2003; 278:29153–29163. [PubMed: 12756260]
38. Merlen C, Farhat N, Luo X, Chatenet D, Tadevosyan A, Villeneuve LR, Gillis MA, Nattel S, Thorin E, Fournier A, Allen BG. Intracrine endothelin signaling evokes IP₃-dependent increases in nucleoplasmic Ca²⁺ in adult cardiac myocytes. *J Mol Cell Cardiol.* 2013; 62:189–202. [PubMed: 23756157]
39. Thomas PB, Liu EC, Webb ML, Mukherjee R, Hebbar L, Spinale FG. Exogenous effects and endogenous production of endothelin in cardiac myocytes: potential significance in heart failure. *Am J Physiol.* 1996; 271:H2629–H2637. [PubMed: 8997325]
40. Bers DM. Membrane receptor neighborhoods: snuggling up to the nucleus. *Circ Res.* 2013; 112:224–226. [PubMed: 23329788]
41. Tadevosyan A, Vaniotis G, Allen BG, Hebert TE, Nattel S. G protein-coupled receptor signalling in the cardiac nuclear membrane: evidence and possible roles in physiological and pathophysiological function. *J Physiol.* 2012; 590:1313–1330. [PubMed: 22183719]
42. Wright CD, Wu SC, Dahl EF, Sazama AJ, O'Connell TD. Nuclear localization drives alpha1-adrenergic receptor oligomerization and signaling in cardiac myocytes. *Cell Signal.* 2012; 24:794–802. [PubMed: 22120526]
43. Wu SC, Dahl EF, Wright CD, Cypher AL, Healy CL, O'Connell TD. Nuclear localization of alpha1A-adrenergic receptors is required for signaling in cardiac myocytes: an "inside-out" alpha1-AR signaling pathway. *J Am Heart Assoc.* 2014; 3:e000145. [PubMed: 24772522]
44. Wright CD, Chen Q, Baye NL, Huang Y, Healy CL, Kasinathan S, O'Connell TD. Nuclear alpha1-adrenergic receptors signal activated ERK localization to caveolae in adult cardiac myocytes. *Circ Res.* 2008; 103:992–1000. [PubMed: 18802028]
45. Boivin B, Lavoie C, Vaniotis G, Baragli A, Villeneuve LR, Ethier N, Trieu P, Allen BG, Hebert TE. Functional beta-adrenergic receptor signalling on nuclear membranes in adult rat and mouse ventricular cardiomyocytes. *Cardiovasc Res.* 2006; 71:69–78. [PubMed: 16631628]
46. Vaniotis G, Allen BG, Hebert TE. Nuclear GPCRs in cardiomyocytes: an insider's view of beta-adrenergic receptor signaling. *Am J Physiol Heart Circ Physiol.* 2011; 301:H1754–H1764. [PubMed: 21890692]
47. Neumann J. The cardiac beta-adrenoceptors: Where and why? *Cardiovasc Res.* 2006; 71:6–7. [PubMed: 16716280]
48. Lehnart SE, Maier LS, Hasenfuss G. Abnormalities of calcium metabolism and myocardial contractility depression in the failing heart. *Heart Fail Rev.* 2009; 14:213–224. [PubMed: 19434491]
49. Piacentino V, Weber CR, Chen XW, Weisser-Thomas J, Margulies KB, Bers DM, Houser SR. Cellular basis of abnormal calcium transients of failing human ventricular myocytes. *Circ Res.* 2003; 92:651–658. [PubMed: 12600875]

50. Hasenfuss G, Pieske B. Calcium cycling in congestive heart failure. *J Mol Cell Cardiol.* 2002; 34:951–969. [PubMed: 12234765]
51. Tarazon E, Rivera M, Rosello-Lleti E, Molina-Navarro MM, Sanchez-Lazaro IJ, Espana F, Montero JA, Lago F, Gonzalez-Juanatey JR, Portoles M. Heart failure induces significant changes in nuclear pore complex of human cardiomyocytes. *PLoS One.* 2012; 7:e48957. [PubMed: 23152829]
52. Banerjee I, Zhang J, Moore-Morris T, Pfeiffer E, Buchholz KS, Liu A, Ouyang K, Stroud MJ, Gerace L, Evans SM, McCulloch A, Chen J. Targeted ablation of nesprin 1 and nesprin 2 from murine myocardium results in cardiomyopathy, altered nuclear morphology and inhibition of the biomechanical gene response. *PLoS Genet.* 2014; 10:e1004114. [PubMed: 24586179]
53. Worman HJ, Ostlund C, Wang Y. Diseases of the nuclear envelope. *Cold Spring Harb Perspect Biol.* 2010; 2:a000760. [PubMed: 20182615]
54. Fischer TH, Herting J, Tirilomis T, Renner A, Neef S, Toischer K, Ellenberger D, Forster A, Schmitto JD, Gummert J, Schondube FA, Hasenfuss G, Maier LS, Sossalla S. Ca^{2+} /calmodulin-dependent protein kinase II and protein kinase A differentially regulate sarcoplasmic reticulum Ca^{2+} leak in human cardiac pathology. *Circulation.* 2013; 128:970–981. [PubMed: 23877259]
55. Sedej S, Schmidt A, Denegri M, Walther S, Matovina M, Arnstein G, Gutsch EM, Windhager I, Ljubojevic S, Negri S, Heinzel FR, Bisping E, Vos MA, Napolitano C, Priori SG, Kocksamper J, Pieske B. Subclinical abnormalities in sarcoplasmic reticulum Ca^{2+} release promote eccentric myocardial remodeling and pump failure death in response to pressure overload. *J Am Coll Cardiol.* 2014; 63:1569–1579. [PubMed: 24315909]
56. Pieske B, Beyer mann B, Breu V, Löffler BM, Schlotthauer K, Maier LS, Schmidt-Schweda S, Just H, Hasenfuss G. Functional effects of endothelin and regulation of endothelin receptors in isolated human nonfailing and failing myocardium. *Circulation.* 1999; 99:1802–1809. [PubMed: 10199875]
57. van de Wal RM, Plokker HW, Lok DJ, Boomsma F, van der Horst FA, van Veldhuisen DJ, van Gilst WH, Voors AA. Determinants of increased angiotensin II levels in severe chronic heart failure patients despite ACE inhibition. *Int J Cardiol.* 2006; 106:367–372. [PubMed: 16337046]
58. Ai X, Curran JW, Shannon TR, Bers DM, Pogwizd SM. Ca^{2+} /calmodulin-dependent protein kinase modulates cardiac ryanodine receptor phosphorylation and sarcoplasmic reticulum Ca^{2+} leak in heart failure. *Circ Res.* 2005; 97:1314–1322. [PubMed: 16269653]
59. Bossuyt J, Helmstadter K, Wu X, Clements-Jewery H, Haworth RS, Avkiran M, Martin JL, Pogwizd SM, Bers DM. Ca^{2+} /calmodulin-dependent protein kinase II δ and protein kinase D overexpression reinforce the histone deacetylase 5 redistribution in heart failure. *Circ Res.* 2008; 102:695–702. [PubMed: 18218981]
60. Taylor CW, Tovey SC. IP_3 receptors: toward understanding their activation. *Cold Spring Harb Perspect Biol.* 2010; 2:a004010. [PubMed: 20980441]
61. Harzheim D, Movassagh M, Foo RS, Ritter O, Tashfeen A, Conway SJ, Bootman MD, Roderick HL. Increased InsP_3 Rs in the junctional sarcoplasmic reticulum augment Ca^{2+} transients and arrhythmias associated with cardiac hypertrophy. *Proc Natl Acad Sci U S A.* 2009; 106:11406–11411. [PubMed: 19549843]
62. Takahashi A, Camacho P, Lechleiter JD, Herman B. Measurement of intracellular calcium. *Physiol Rev.* 1999; 79:1089–1125. [PubMed: 10508230]
63. Alonso MT, Garcia-Sancho J. Nuclear Ca^{2+} signalling. *Cell Calcium.* 2011; 49:280–289. [PubMed: 21146212]
64. Miyawaki A, Llopis J, Heim R, McCaffery JM, Adams JA, Ikura M, Tsien RY. Fluorescent indicators for Ca^{2+} based on green fluorescent proteins and calmodulin. *Nature.* 1997; 388:882–887. [PubMed: 9278050]
65. Nagai T, Sawano A, Park ES, Miyawaki A. Circularly permuted green fluorescent proteins engineered to sense Ca^{2+} *Proc Natl Acad Sci U S A.* 2001; 98:3197–3202. [PubMed: 11248055]
66. Brini M, Murgia M, Pasti L, Picard D, Pozzan T, Rizzuto R. Nuclear Ca^{2+} concentration measured with specifically targeted recombinant aequorin. *EMBO J.* 1993; 12:4813–4819. [PubMed: 8223490]

67. Ikeda M, Sugiyama T, Wallace CS, Gompf HS, Yoshioka T, Miyawaki A, Allen CN. Circadian dynamics of cytosolic and nuclear Ca^{2+} in single suprachiasmatic nucleus neurons. *Neuron*. 2003; 38:253–263. [PubMed: 12718859]
68. Robert V, Gurlini P, Tosello V, Nagai T, Miyawaki A, Di Lisa F, Pozzan T. Beat-to-beat oscillations of mitochondrial $[\text{Ca}^{2+}]$ in cardiac cells. *EMBO J*. 2001; 20:4998–5007. [PubMed: 11532963]

Figure 1. Nucleoplasmic $[Ca^{2+}]$ regulation

$[Ca^{2+}]_{nuc}$ is the sum of Ca^{2+} that enters the nucleoplasm by diffusion from the cytoplasm through nuclear pores (red arrows) and Ca^{2+} that is released from the NE in a specific and regulated manner upon GPCRs stimulation (blue arrows). ECM, extracellular matrix; ET-1, endothelin-1; ATII, angiotensin II; NE, norepinephrine; GPCR, G protein-coupled receptor; PLC, phospholipase C; IP_3 , inositol-1,4,5-trisphosphate; IP_3R , IP_3 receptors; RyR, ryanodine receptor; SERCA, SR- Ca^{2+} -ATPase; DAG, diacylglycerol; TRPC, transient receptor potential cation channel; LCC, L-type calcium channel.

Figure 2. Three models of membrane receptor signaling to IP_3 -dependent Ca^{2+} signals

(A) In the traditional model, receptors (as for ET-1 in this example) activate IP_3 production at the cell periphery. The IP_3 has to diffuse a long way to the nuclear IP_3R to cause local $[\text{Ca}^{2+}]_{\text{nuc}}$ elevation by release from the Ca^{2+} store in the nuclear envelope. (B) The model indicating the insulin-like growth factor 1 receptor (IGF-1R) complex in plasma membrane invaginations, reducing the diffusional distance for IP_3 to reach the nucleus. (C) A third model where functional G-protein coupled receptors can exist near or on the nuclear envelope.