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Decoding Calcium Signaling Across the Nucleus

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Calcium (Ca^{2+}) is an important multifaceted second messenger that regulates a wide range of cellular events. A Ca^{2+}-signaling toolkit has been shown to exist in the nucleus and to be capable of generating and modulating nucleoplasmic Ca^{2+} transients. Within the nucleus, Ca^{2+} controls cellular events that are different from those modulated by cytosolic Ca^{2+}. This review focuses on nuclear Ca^{2+} signals and their role in regulating physiological and pathological processes.

The role of calcium (Ca^{2+}) signaling in regulating a variety of cellular processes has been widely described, showing the multifunctional nature of this ion. Even though the mechanisms that allow Ca^{2+} to modulate a wide range of events have not been completely elucidated, it is appreciated that the amplitude, the spatiotemporal profile (e.g., single transient, wave, oscillation), as well as the intracellular compartments where Ca^{2+} signals occur are all involved in orchestrating Ca^{2+} increases that correspond to specific cellular functions mediated by Ca^{2+}. In this review, we will briefly discuss the machinery that regulates intracellular Ca^{2+} signaling and then highlight mechanisms of Ca^{2+} signaling in the nucleus as well as cellular events that are modulated by nucleoplasmic Ca^{2+}.

Intracellular Ca^{2+}-Signaling Toolkit

Although the variety of biological processes regulated by Ca^{2+} can be partially explained by the spatiotemporal organization of Ca^{2+} increases within the cell, a crucial question in this signaling cascade is how cells can generate, regulate, and coordinate such Ca^{2+}-evoked responses. The remarkable plasticity of Ca^{2+} signaling emerges not only from the origin of the intracellular Ca^{2+} signals (i.e., endoplasmic reticulum, mitochondria, or nucleus) but also from the molecular toolkit expressed by different cell types, as well as from the interplay between the cellular compartments. Below, we will briefly review some of the components of the Ca^{2+} toolkit that have been described to date. Although we will not discuss the components at length and we will not present a complete list of the Ca^{2+} signaling molecules, the discussion will provide a sense of the variety of components of the Ca^{2+} signaling network that can be used by different cell types to generate a specific cellular event.

Intracellular Ca^{2+} signals can be initiated by the influx of Ca^{2+} from the extracellular environment or by the mobilization of Ca^{2+} from intracellular stores. The latter depends mainly on the binding of hormones or growth factors to specific receptor recruiting and generating a variety of second messengers that, in turn, will control Ca^{2+} mobilization from different intracellular stores (reviewed in Refs. 10, 87). One of the major signaling cascades triggered by the activation of transmembrane receptors involves phospholipase C (PLC), which generates inositol-1,4,5-trisphosphate ($InsP_3$). $InsP_3$ diffuses throughout the cell to bind to its specific receptor ($InsP_3R$), which is a ligand-gated Ca^{2+} release channel (reviewed in Refs. 19, 30). Three isoforms of $InsP_3R$ have been described, and although these receptors are mainly located in the endoplasmic reticulum, they can also be detected at the plasma membrane (25), portions of the Golgi complex (83), acidic stores (35), and within the nucleus (29, 45, 59). Moreover, the expression pattern of $InsP_3R$ isoforms varies among different cell types (108). Although $InsP_3$ is required to activate $InsP_3R$, the opening of the channel can be modulated by Ca^{2+} itself, ATP, and proteins such as Chromogranin-B (reviewed in Refs. 19, 24, 30). It is also known that the affinity of $InsP_3R$ for $InsP_3$ is highest for $InsP_3R$-II, intermediate in $InsP_3R$-I, and lowest for $InsP_3R$-III (52, 74).

The intracellular increase in Ca^{2+} concentrations itself may activate a second class of receptors: the ryanodine receptors (RyR). Similar to $InsP_3R$, RyR forms channels that are permeable to Ca^{2+}. So far, three isoforms of RyR have been identified (reviewed in Refs. 57, 112). RyR-I is abundantly expressed in skeletal muscle but also is found in cardiac muscle, cerebellum, and other tissues (33, 40, 75). RyR-II is the major isoform found in cardiac muscle (71, 79) but is also expressed in other tissues (33, 55, 72, 96), whereas RyR-III is expressed within hippocampal neuron cerebellum as well as in smooth muscle cells of many organs (33, 40, 46). Ca^{2+} is the major trigger of RyR opening, but some RyR channel subtypes are also activated by cyclic ADP-ribose (cADPR) (70, 100). Moreover, FK506 binding protein,

magnesium, calmodulin, and a number of small molecules such as ATP are able to modulate the activity of RyRs (Ref. 68; reviewed in Refs. 11, 112).

In addition to the well known mechanisms above, a growing body of evidence shows that members of a third and a fourth class of intracellular Ca^{2+} channels can be involved in controlling Ca^{2+} release from organelles within the cell. One family of these receptors, named two pore channels (TPCs), acts as Ca^{2+} channels and is responsible for the mobilization of Ca^{2+} from acidic organelles in response to stimulation by nicotinic acid adenine dinucleotide phosphate (NAADP) (15, 34). There are three isoforms of TPCs in mammals, with TPC1 and TPC3 expressed in endosomes and TPC2 present primarily in lysosomes (15, 34, 92). It has been shown that TPC-induced Ca^{2+} release may trigger further Ca^{2+} release through the activation of juxtaposed $InsP_3R$ or RyR, suggesting a broader role of TPCs in the regulation of cellular activities (Refs. 16, 34; reviewed in Ref. 102).

Another channel shown to be involved in intracellular Ca^{2+} signaling is the TRP2 that belongs to the transient receptor potential (TRP) superfamily of ion channels (Refs. 53, 54; reviewed in Refs. 5, 104). Besides its location at the plasma membrane and the primary cilium, TRP2 is also found in high concentrations in the endoplasmic reticulum membrane functioning as an intracellular Ca^{2+}-release channel that augments $InsP_3$-induced intracellular Ca^{2+} release (54).

Within a cell, Ca^{2+} signaling may be seen as a shifting balance that controls cell physiology. When this signaling pathway is activated but tightly controlled, Ca^{2+} regulates a broad spectrum of biological processes, including secretion, motility, gene transcription, proliferation, differentiation, contraction, cell death, and others (10). Therefore, it is important that the cells express a toolkit not only to allow Ca^{2+} release from intracellular stores but also to reestablish the intracellular free Ca^{2+} to baseline levels. To accomplish this, cytosolic Ca^{2+} can be *1*) extruded into the extracellular environment by the action of the plasma membrane Ca^{2+}-ATPase (PMCA) or the sodium Ca^{2+} exchanger (NCX), or *2*) pumped back into the lumen of the Ca^{2+} stores, such as endoplasmic reticulum and mitochondria, by the action of proteins like the sarco-/endoplasmic Ca^{2+}-ATPase (SERCA) and mitochondrial Ca^{2+} uptake 1 (MCU1) (Ref. 82; reviewed in Refs. 12, 84).

Although understanding the components of the intracellular Ca^{2+} toolkit constitutes a powerful strategy to familiarize the reader with the diversity and peculiarities of the molecular repertoire involved in intracellular Ca^{2+} signaling, the reader should be reminded that these components act in a coordinated fashion to regulate specific cellular functions triggered by increases in Ca^{2+}. Also, despite the broad expression of the components of the Ca^{2+} toolkit, Ca^{2+} signaling may occur in distinct microdomains of the cell (9). Evidence showing components of the Ca^{2+} toolkit in the nucleus identifies this cellular compartment as a pivotal microdomain for independent and localized Ca^{2+} signaling. In the following sections, we will describe the mechanisms underlying nuclear Ca^{2+} signaling as well as its relevance for physiological and pathological processes.

Nucleoplasmic Ca^{2+} Signaling

There are several reports in the literature suggesting that nuclear Ca^{2+} signals can result from passive diffusion of Ca^{2+} from the cytosol to the nucleoplasm (14, 48, 61, 98). This view evolved from observations made by numerous groups showing not only the existence of nuclear-cytosolic Ca^{2+} gradients (94, 107) but also the presence of several components of the Ca^{2+} toolkit within the nucleus capable of autonomously controlling nuclear Ca^{2+} homeostasis (reviewed in Refs. 37, 87, 91).

For instance, $InsP_3$-sensitive pools (77), as well as a high-affinity, $InsP_3$ binding site, were demonstrated to exist in isolated liver nuclei, with $InsP_3$ being able to release $^{45}Ca^{2+}$ directly from isolated nuclei (63). Also using isolated rat liver nuclei, it was shown that ATP increases the free Ca^{2+} concentration in the nucleoplasm (76). However, it was not initially clear which intranuclear structures contained Ca^{2+} nor into which intranuclear compartments Ca^{2+} was released by $InsP_3$. Indeed, the first report demonstrating that $InsP_3$ is able to cause the release of Ca^{2+} from the nuclear envelope into the nucleoplasm came a few years later. It was then shown that the nuclear envelope actively takes up Ca^{2+} via a Ca^{2+}-activated ATPase and that $InsP_3$ releases Ca^{2+} from this envelope store into the nucleoplasm (39). A subsequent study, in which $InsP_3$ was injected in the nucleus of *Xenopus laevis* oocytes, also demonstrated that $InsP_3$ causes an increase in nuclear Ca^{2+}, even when cytosolic $InsP_3Rs$ were blocked (48). Moreover, photorelease of caged $InsP_3$ in the nucleus of starfish oocytes was shown to trigger nucleoplasmic Ca^{2+} increase (94). Like $InsP_3$, cADPR also could raise Ca^{2+} in isolated hepatocyte nuclei (1, 39) and induce Ca^{2+} oscillations in the nucleus of starfish oocytes (94). These findings illustrate some of the initial evidence that supported the hypothesis that the nucleus could autonomously trigger nucleoplasmic Ca^{2+} transients. This hypothesis was strengthened by additional data showing that the inner and/or the outer membranes of the nuclear envelope express several components of the Ca^{2+} toolkit. In particular, it was shown that the nuclear envelope

accumulates Ca^{2+} via a Ca^{2+}-ATPase pump (SERCA) (56, 76) located in its outer leaflets (38, 49) and a Na^+/Ca^{2+}-exchanger (109, 110) located in its inner membrane (109), and releases Ca^{2+} via channels that are sensitive to $InsP_3$ (39, 76), cADPR (1, 39), and NAADP (36).

There are reports that the $InsP_3R$ (17, 49, 101) and the RyR (93) can be present on both leaflets of the nuclear envelope, whereas ADP-ribosyl (CD38), an enzyme required for generation of cADPR, is found to be only associated with the inner membrane of the nuclear envelope (1). Although it is widely accepted that the nucleus also holds the apparatus required to produce nuclear $InsP_3$, including PIP_2 as well as PLC (Ref. 65; reviewed in Ref. 27), and that PLC can be found associated with the nuclear membrane (28, 64), it is still not completely clear whether PIP_2 is localized in the nucleus. It has been proposed that nuclear PIP_2 could be confined within a lipid bilayer forming intranuclear membrane invaginations (51). Alternatively, there are reports that PIP_2 is located in the nucleoplasm rather than at the nuclear envelope (13, 78, 106). Nevertheless, there are several reports showing that nuclear PIP_2 hydrolysis involves the translocation of activated tyrosine kinase receptors from the plasma membrane to the nucleus (2, 20, 44, 89). For instance, IGF-1 (66), integrins (20), hepatocyte growth factor (44), and insulin (89) represent some agonists known to preferentially cause nuclear PIP_2 breakdown, producing $InsP_3$ and consequently Ca^{2+} signals in the nucleus. Moreover, it has been demonstrated that, upon growth factor stimulation, activation of nuclear PLC can occur through an additional pathway that involves relocation of MAP kinase to the nucleus (93). Even though the identification of several components of the Ca^{2+} toolkit within the nucleus represented solid evidence that could explain how Ca^{2+} is released from the nuclear envelope directly into the nucleoplasm, recognition and characterization of a structure named the nucleoplasmic reticulum (NR) (29) increased appreciation for the role of nuclear Ca^{2+} signaling, as it provided direct evidence of a compartment that stores Ca^{2+} deep in the nucleus and releases it within the nucleoplasm in a process that could occur completely independent of the cytosolic Ca^{2+} increases. The NR was shown to be continuous with the endoplasmic reticulum/nuclear envelope (29), as observed by lamin-A staining (FIGURE 1). Additional morphological characterization showed that the NR can be divided into two types (reviewed in Ref. 62). The type I NR is characterized by invagination of the inner nuclear membrane, whereas NR type II is formed by invagination of both the outer and inner nuclear membrane; and, although structurally different, both NR subtypes can coexist within a single nucleus (31, 32, 62). Indeed, similar intranuclear extensions of the endoplasmic reticulum had been described previously and were thought to be widespread among mammalian cell types (32) and to be dynamic structures that become altered during cell proliferation or disease states (105). The NR has also been identified in plant cells, and it has been suggested that it regulates nuclear Ca^{2+} signaling in these cell types as well (22, 81). By showing that the NR gives rise to localized Ca^{2+} gradients in the nucleoplasm, a potential mechanism was revealed by which Ca^{2+}-dependent events can be regulated differentially in the nucleus, just as they are in the cytosol.

The NR was shown to express functional $InsP_3R$ (29) and RyR (67), giving rise to local Ca^{2+} signals in the nuclear interior, triggered by either $InsP_3$ (29) or Ca^{2+}, respectively (67). To date, it is not known whether the TPCs or TRPC2 are also expressed along the NR. It is also not known whether and how the various intracellular Ca^{2+} channels in the NR interact to coordinate nuclear Ca^{2+} signals and

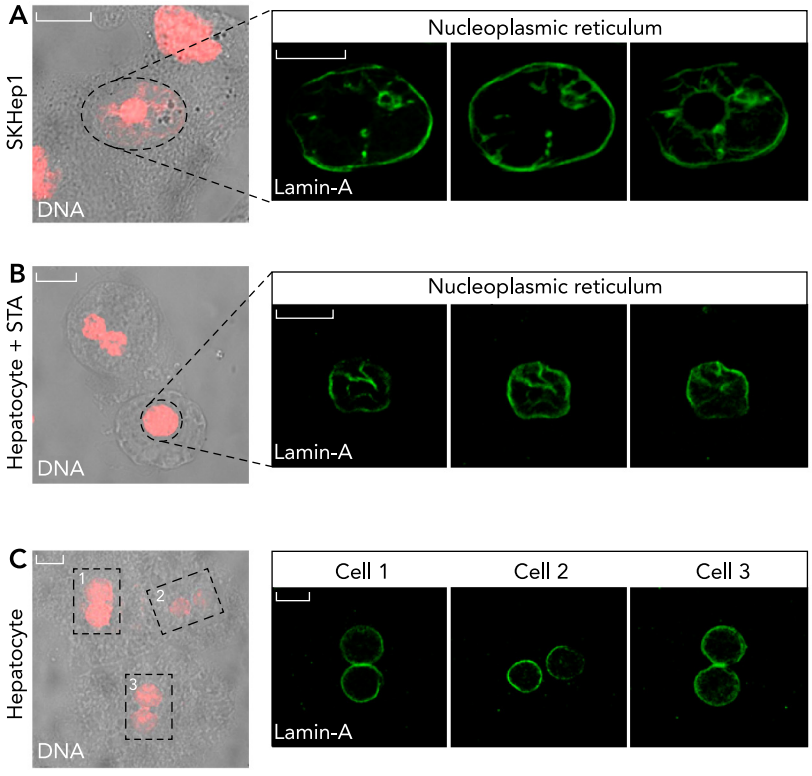

Figure 1. **The nucleus contains the nucleoplasmic reticulum structure**
The nuclear envelope was labeled with an antibody against lamin-A (green) in SkHep1 (*A*), an adenocarcinoma liver cell line, and freshly isolated primary hepatocytes treated (*B*) or not treated (*C*) with staurosporine (STA) for 4 h, respectively. DNA was stained with propidium iodide. Note the presence of the nucleoplasmic reticulum (NR) in a cell line as well as in a primary culture. In the two *top* panels, the images represent three focal planes, depicting the three dimensionality of the NR. In the cell lineage used here, the NR seems to be constitutively present (*A*). In the primary culture of hepatocytes, the NR, not present under control condition, can be induced by drugs, such as STA (compare *B* with *C*), corroborating the findings that the NR is a dynamic and inducible structure. Bar in *A* = 5 μm; bars in *B* and *C* = 10 μm.

cellular functions. Although more studies are needed to clarify these points, there are suggestions that the NR expresses $InsP_3$-kinase isoform B (IP3KB) (73), which can inactivate $InsP_3$ (26, 111) and SERCA (21), indicating that nuclear Ca^{2+} signals can be locally initiated and terminated within the nucleus.

Although the data described above demonstrate the potential independent behavior of the nucleus relative to the cytosol, they cannot rule out that, in response to some stimuli, the nuclear Ca^{2+} signaling machinery needs to be activated by intermediate effectors located in the cytosol. For instance, it was shown in a liver cell line that extracellular ATP preferentially activates nuclear Ca^{2+} release via $InsP_3$ that diffuses from the cytosol to the nucleus to activate nuclear $InsP_3R$ (59). Indeed, at present, different mechanisms for nuclear Ca^{2+} increases have been considered. One mechanism relies on the expression pattern of $InsP_3R$ isoforms in the cell (FIGURE 2A). It involves cytosolic to nucleoplasmic $InsP_3$ diffusion to preferentially bind to the type II $InsP_3R$ concentrated in the nucleus. The main question regarding this model is how $InsP_3$, as it diffuses across the cytosol, does not trigger Ca^{2+} release from the $InsP_3R$ located in the endoplasmic reticulum. The answer may rely on the differing affinity of the various $InsP_3R$ isoforms to $InsP_3$ as described above: $InsP_3R$-II > $InsP_3R$-I > $InsP_3R$-III (52, 74). Therefore, if type II $InsP_3R$ expression is concentrated in the nucleus, Ca^{2+} signals are more likely to start there (59). A second mechanism consists of nuclear $InsP_3$ formation (FIGURE 2B). In support of this, it has been demonstrated that growth factors and their respective receptors translocate from the plasma membrane to the nucleus to stimulates the hydrolysis of nuclear PIP_2 with the formation of $InsP_3$ within the nucleus and consequently nuclear Ca^{2+} signals (2, 23, 44, 86, 89). Another possible mechanism is the stimulation of nuclear RyR (FIGURE 2C). This might occur when Ca^{2+} released from nuclear stores stimulates further Ca^{2+} release from RyR present along the NR. Although more experimental evidence is needed to support the importance of this pathway, the presence of RyR in the NR and its functionality was already described in C2C12 cells and in cardiomyocytes (45, 67). Finally, but no less important, is the model that predicts the juxtaposition of the components of the Ca^{2+} toolkit with the nuclear envelope. Recently, it was shown using cardiomyocytes that the invaginations of the plasma membrane, known as T-tubules, concentrate the machinery to generate $InsP_3$ in direct apposition to the components of the nuclear Ca^{2+} toolkit. Thus, after stimulation from an extracellular ligand, $InsP_3$ is generated in the vicinity of the nucleus diffusing into it and triggering independent nuclear Ca^{2+} signals (50).

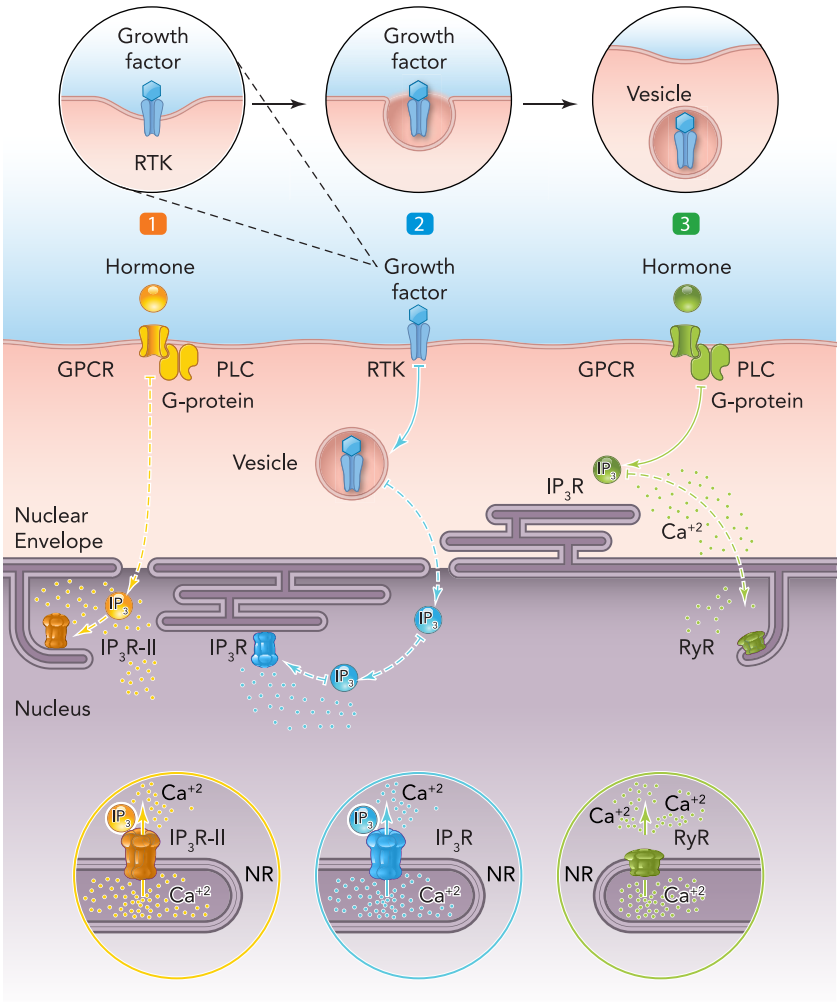

Figure 2. **Working hypotheses to trigger nuclear Ca^{2+}**
Different pathways to generate nuclear Ca^{2+} signals have been proposed. In *1* (*left*), the binding of an agonist to its transmembrane G-protein-coupled receptor (GPCR) produces $InsP_3$ (IP_3) in the cytosol. IP_3 diffuses to the nucleus and binds to the high-affinity, type II $InsP_3R$ (IP_3R-II), located along the nucleoplasmic reticulum (NR), to release Ca^{2+} in the nucleoplasm. In *2* (*middle*), the binding of a growth factor to a receptor tyrosine kinase (TKR) may result in the translocation of the receptor to the nucleus to generate nuclear IP_3. IP_3 binds to the IP_3R to release Ca^{2+} in the nucleoplasm. The topology of internalized TRK is not known, and the cartoon shows TKR as being inside the vesicle merely for simplicity. In *3* (*right*), another putative pathway is initiated following the binding of a hormone to GPCR at the plasma membrane. The IP_3 generated within the cytosol can release Ca^{2+} from the endo-/sarcoplasmic reticulum, and Ca^{2+} may reach the nucleus to trigger Ca^{2+}-induced Ca^{2+} release by activating the ryanodine receptor (RyR) in the NR.

These studies have clearly shown that the nucleus can be completely autonomous in terms of Ca^{2+} regulation. The presence of the Ca^{2+} signaling machinery in the nuclear interior has several implications, in physiological and pathological states, some of which will be described below.

Cellular Functions Mediated by Nuclear Ca^{2+}

Ca^{2+} signaling regulates a variety of cellular functions, and, more recently, it has been shown that Ca^{2+} increases in the nucleoplasm regulate events

that are distinct from the ones mediated by cytosolic Ca^{2+} (2, 6, 29, 43, 87, 89, 91). For instance, nuclear Ca^{2+} promotes translocation of protein kinase C (PKC) to the nuclear envelope, whereas cytosolic Ca^{2+} causes PKC translocation to the plasma membrane (29). Also, nuclear but not cytosolic Ca^{2+} generated by insulin receptor activation regulates hepatocyte proliferation after partial hepatectomy (2). This finding was in agreement with previous data that showed that buffering nuclear Ca^{2+} regulates the cell cycle by arresting cells in an early phase of mitosis (90). In fact, it was found that nuclear Ca^{2+} signals regulate the rate of cell proliferation both in vitro and in xenographic tumors (3, 89). One possible mechanism by which nuclear Ca^{2+} may control growth is by activating the promoter region of genes involved in cell proliferation (4). In addition to its direct effect on tumor growth, impaired nuclear Ca^{2+} signaling can sensitize adenocarcinoma cells to radiotherapy, in part by downregulating metalloproteinase and growth factor receptor expression and activation induced by X-ray irradiation (3).

Nuclear Ca^{2+} is also involved in cardiomyocyte hypertrophy. For example, buffering Ca^{2+} in the nucleus causes nuclear enlargement in neonatal cardiomyocytes, an early sign of cardiac hypertrophy (45). Nuclear Ca^{2+} activates the calcineurin/NFAT signaling cascade, culminating in increased ANP expression (45), a well known cardiomyocyte hypertrophic marker (88). However, cardiac hypertrophy is an adaptive response that can be induced in pathological and physiological conditions. Indeed, nuclear Ca^{2+} is indispensable for induction of hypertrophy in both health and disease. Regardless of the precise cellular compartment in which $InsP_3$ is produced, it must reach the nucleus interior to trigger nuclear Ca^{2+}-mediating hypertrophy (6).

Also dependent on the spatial properties of Ca^{2+} signals are transcriptional responses, since they are strongly influenced by the intracellular localization of Ca^{2+} transients. For example, neuronal gene expression is differentially controlled by nuclear and cytoplasmic Ca^{2+} signals: signaling pathways activated by cytoplasmic Ca^{2+} target the serum-response element (SRE), whereas increases in nuclear Ca^{2+} are critical for cyclic-AMP-response element (CRE)-dependent calcium-activated transcription (8, 47). Also, the transcriptional factor cyclic-AMP response element-binding protein (CREB) can function as a nuclear Ca^{2+}-responsive transcription factor (97) that is involved in mediating Ca^{2+}-dependent transcription of a number of genes, including c-fos (41). Nuclear Ca^{2+} buffering inhibits CREB-mediated gene transcription (47). In addition, nuclear Ca^{2+} controls, through nuclear calcium-/calmodulin (CaM)-dependent protein kinases, especially CaM Kinase IV, the activity of the coactivator CREB-binding protein (CBP) (18). CBP interacts with CREB and with many transcription factors (42), a mechanism whereby nuclear Ca^{2+} can modulate the expression of different genes. Nuclear Ca^{2+} is similarly required for epidermal growth factor (EGF)-mediated transcriptional activation of Elk-1 (85). An additional target directly regulated by nuclear Ca^{2+} signaling is the downstream regulatory element antagonist modulator (DREAM) (69). All DREAM family members bind specifically to DNA and regulate transcription negatively. An increase in the concentration of nuclear Ca^{2+} causes DREAM to dissociate from its DNA binding sites and thereby allows the transcription of its target genes (58). On the other hand, nuclear Ca^{2+} also can negatively regulate the activity of transcription factors. For example, chelation of nuclear Ca^{2+} raises the activity of the transcription enhancer factor (TEAD) (103).

It is widely known that Ca^{2+} transients are crucial for synaptic activity and neuro-adaptations (reviewed in Ref. 7) including survival (114), memory consolidation (60), and pain (99). For example, histone deacetylases (HDACs) have a pivotal role linking external stimuli with gene expression. Inhibition of nuclear Ca^{2+} signaling interferes with the subcellular distribution of members of class IIa HDACs in hippocampal neurons, representing a novel transcriptional pathway regulated by nuclear Ca^{2+} (95). Also, synaptic activity is required for neuronal survival. Part of this neuroprotection is afforded by the resulting increase in Ca^{2+} in the cell nucleus due to activation of CREB (80) and inhibition of DREAM family members (58). Together, these changes increase the transcriptional activity of neurons, as already discussed in this review, and result in the triggering of a neuroprotective program referred to as Activity Inhibitor of Death (AID) (114). The core of this program consists of the robust induction of nuclear Ca^{2+}-related genes involved in survival and differentiation processes, including ATF3, GADD45β, GADD45γ, IFI202b, NPAS4, NR4A1, SERPINb2, Inhibin β (114), and BTG2 (113). Another important role of nuclear Ca^{2+} signaling in the nervous system is its relationship to pain. Inducing Ca^{2+} signals in the nucleoplasm increases the expression of genes that modulate neuronal excitation and morphology. In this case, neurons became more susceptible to painful stimuli, contributing to the amplification of nociceptive sensitivity (99).

Together, these data show that a large amount of information regarding nuclear Ca^{2+} signaling and cell function has been acquired in recent years. These findings not only contribute to our understanding of basic aspects of several physiological processes but also permit nuclear Ca^{2+} to be considered as a potential therapeutic target to treat diseases.

Future Perspectives for Nuclear Ca^{2+} Signaling

The characterization of the NR as a nuclear Ca^{2+} compartment, together with evidence demonstrating the presence of components of the components of the Ca^{2+} toolkit in the nuclear interior, add one more level of complexity to the already multifaceted field of Ca^{2+} signaling. A remaining intriguing question would be to understand whether and how the different intracellular Ca^{2+} channels present in the nucleus interact to decode Ca^{2+} signals to produce a specific cellular response. Moreover, the existence of micro-domains within the nucleus might also be important to tightly regulate nuclear Ca^{2+} signals and should be considered and investigated. ■

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