Brazilian Journal of Medical and Biological Research (2009) 42: 17-20
ISSN 0100-879X Review

Nuclear calcium signaling: a cell within a cell

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Calcium (Ca^{2+}) is a versatile second messenger that regulates a wide range of cellular functions. Although it is not established how a single second messenger coordinates diverse effects within a cell, there is increasing evidence that the spatial patterns of Ca^{2+} signals may determine their specificity. Ca^{2+} signaling patterns can vary in different regions of the cell and Ca^{2+} signals in nuclear and cytoplasmic compartments have been reported to occur independently. No general paradigm has been established yet to explain whether, how, or when Ca^{2+} signals are initiated within the nucleus or their function. Here we highlight that receptor tyrosine kinases rapidly translocate to the nucleus. Ca^{2+} signals that are induced by growth factors result from phosphatidylinositol 4,5-bisphosphate hydrolysis and inositol 1,4,5-trisphosphate formation within the nucleus rather than within the cytoplasm. This novel signaling mechanism may be responsible for growth factor effects on cell proliferation.

Key words: Calcium; Proliferation; Receptor tyrosine kinases; Nucleus

Presented at the IV Miguel R. Covian Symposium, Ribeirão Preto, SP, Brazil, May 23-25, 2008.

Research supported by NIH (#DK57751, #DK34989, and #DK45710), and by CNPq, FAPEMIG, and Howard Hughes Medical Institute.

Received July 3, 2008. Accepted September 29, 2008

Introduction

Intracellular Ca^{2+} can regulate cellular processes as distinct as cell death and proliferation (1). To achieve this versatility, there is increasing evidence that the spatial patterns of Ca^{2+} signals may determine their specificity (2). Ca^{2+} signals in nuclear and cytoplasmic compartments occur independently in several different cell types (3). However, the mechanisms and pathways that promote localized increases of free Ca^{2+} levels in the nucleus have not been entirely defined.

Recently, ligand-dependent translocation of receptor tyrosine kinases (RTKs) to the nucleus has been reported (4-7). RTKs can activate phospholipase C (PLC) that hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2), generating two intracellular products: inositol 1,4,5-trisphosphate ($InsP_3$), a universal Ca^{2+}-mobilizing second messenger, and diacylglycerol, an activator of protein kinase C (PKC) (8,9). It has also been reported that the interior of the nucleus has all the Ca^{2+} signaling machinery necessary to produce nuclear Ca^{2+} signaling (10-15). The translocation of RTK to the nucleus indicates a new mechanism by which RTK increases Ca^{2+} in the nucleus and a new paradigm to explain the mechanism and pathways that promote nuclear Ca^{2+} signaling. This review highlights the recent advances in this area.

The nucleus contains the machinery needed to locally increase Ca^{2+}

PLC hydrolyzes PIP_2 to generate $InsP_3$ (16), and $InsP_3$ then binds to the $InsP_3$ receptor ($InsP_3R$) to release Ca^{2+}

from internal stores. It is well established that components necessary for $InsP_3$-mediated Ca^{2+} signaling are present in the plasma membrane and the endoplasmic reticulum, and there is evidence that these components are also present in the nuclear envelope as well. These components include PIP kinase (PIPK) (17,18), which synthesizes PIP_2, plus PLC (19) and the $InsP_3R$ (20-22). $InsP_3R$ is found on both the cytoplasmic and the intranuclear side of the nuclear membrane (11,23), and the nuclear envelope contains sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pumps for Ca^{2+} reuptake as well (24). The nucleus, therefore, is equipped to produce $InsP_3$ and to release and take up free Ca^{2+}, independent of cytosolic $InsP_3$ or Ca^{2+}. Although Ca^{2+} can spread passively from the cytosol into the nucleus under certain circumstances (25-27), intranuclear $InsP_3$ can increase Ca^{2+} directly within the nucleus as well, both in isolated nuclei (12,20,28) and in nuclei within intact cells (23,29,30). Moreover, RTKs may selectively activate nuclear isoforms of PLC (18,31). However, until recently it was not known whether such receptors use this mechanism to increase Ca^{2+} in the nucleus. Two additional details about nuclear Ca^{2+} signaling have recently been established. First, the relative distribution of $InsP_3R$ isoforms in the nucleus and cytosol can differ among cell types (21). Because each $InsP_3R$ isoform has distinct sensitivities to $InsP_3$ (32) and to Ca^{2+} (33,34), this differential distribution provides a mechanism by which the nucleus may be more sensitive than the cytosol to $InsP_3$-mediated Ca^{2+} release in certain cell types (21). Second, $InsP_3$-gated Ca^{2+} stores are found not only within the nuclear envelope, but also along a nucleoplasmic reticulum (23). PIPK and PIP_2 are present in the interior of the nucleus (14), and insulin and hepatocyte growth factor (HGF) can induce $InsP_3$ production in nuclei (6,7,35). These findings suggest that Ca^{2+} signaling machinery is present not only along the nuclear envelope but within the interior of the nucleus as well, which may provide an additional level of spatial control of nuclear Ca^{2+} signaling. In fact, Ca^{2+} signals induced by HGF and insulin begin in the nucleus (6,7); nuclear Ca^{2+} signals are initiated in both SKHep-1 cells and primary hepatocytes when PIP_2 is hydrolyzed to form $InsP_3$ (6,7). Moreover, both the HGF receptor (c-met) and insulin receptor translocate to the nucleus (Figure 1). Translocation of the HGF receptor to the nucleus depends upon the adaptor protein Gab1, that contains a nuclear localization sequence and importin-β1, and the formation of Ca^{2+} signals depends upon this translocation (6). Transport of proteins through the nuclear pore complex typically involves importins α/β and exportins. Specifically, importin-β binds to the classical lysine-rich nuclear localization signal in the cargo, and importin-β interacts with the importin-β/cargo complex to guide it through the nuclear pore (6). Together, these data indicate that RTKs can activate the calcium signaling machinery within the nucleus.

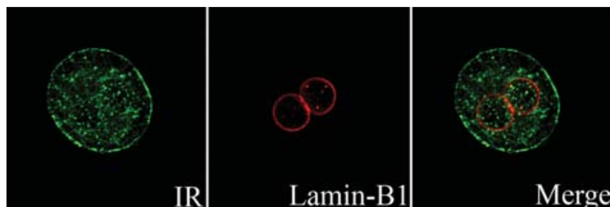

Figure 1. The insulin receptor translocates to the nucleus. Confocal immunofluorescence images of the insulin receptor (IR) after 5-min stimulation with insulin (10 nM). Isolated rat hepatocytes were double-labeled with a polyclonal antibody against insulin receptor B (BD Biosciences, USA) and a monoclonal antibody against the nuclear membrane marker Lamin-B1 (Abcam, USA) and then incubated with secondary antibodies conjugated to Alexa 488 and 555 (Invitrogen, USA), respectively. Images were collected with a Zeiss LSM 510 confocal microscope using a 63X, 1.4-NA objective lens with excitation at 488 nm and observation at 505-550 nm to detect Alexa 488 (green), and excitation at 543 nm and observation at 560-610 nm to detect Alexa 555 (red).

Increases in Ca^{2+} within the nucleus have specific cellular effects

Nuclear Ca^{2+} signaling directly regulates cellular functions such as activation of kinases within the nucleus (23,36), protein transport across the nuclear envelope (11,37), and transcription of certain genes (38-40). For example, nuclear Ca^{2+} activates calmodulin kinase IV (36) and induces translocation of intranuclear but not cytosolic PKC (23). Gene transcription mediated by either the cAMP response element (CRE), CRE binding protein (CREB), or CREB binding protein (CBP) specifically depends upon increases in nuclear Ca^{2+}, whereas gene transcription mediated by the serum response element instead is mediated by increases in cytoplasmic Ca^{2+} (38,39). Transcriptional activation of Elk-1 by epidermal growth factor also depends upon nuclear rather than cytosolic Ca^{2+} (40). Moreover, Ca^{2+} can bind to and directly regulate certain nuclear transcription factors (41), and can affect DNA structure as well (42). Nuclear Ca^{2+} can negatively regulate the activity of transcription factors as well (43). This was demonstrated by examining the relative effects of nuclear and cytosolic Ca^{2+} on the activity of the transcription enhancer factor TEF/TEAD. Chelation of nuclear but not cytosolic Ca^{2+} increased TEAD activity to twice that of controls, providing evidence that nuclear Ca^{2+} negatively regulates the activity of this transcription factor. Collec-

tively, these findings show that nuclear Ca^{2+} regulates the expression of certain genes. Exogenous expression of the Ca^{2+} buffering protein parvalbumin has shown that intracellular Ca^{2+} regulates cell growth (44), but lack of effective experimental tools has made it difficult to demonstrate whether the effect of Ca^{2+} on cell growth is due to nuclear or cytosolic Ca^{2+} signals. Initial functional studies of nuclear Ca^{2+} on gene transcription relied on microinjection of Ca^{2+} chelators into either the nucleus or cytosol of individual cells (39), but it is impractical to use this labor-intensive approach to conduct biochemical, cell population, or *in vivo* studies. However, a newer approach has been developed in which cells are infected with adenovirus to deliver Ca^{2+} chelators such as parvalbumin that are targeted to be expressed in either the nucleus or cytosol (45). Nuclear Ca^{2+} stimulates cell proliferation rather than apoptosis and specifically permits cells to advance through early prophase (45). Furthermore, nuclear Ca^{2+} regulates cell proliferation in multiple cell lines and *in vivo* (45).

Conclusions and future directions

The current evidence suggests that nucleoplasmic Ca^{2+} regulates cell cycle progression. RTKs move to the nucleus to generate $InsP_3$ and therefore Ca^{2+} signals within the nucleus, and this nuclear Ca^{2+} signaling is important for cell proliferation. Further work is needed to identify the mechanism by which RTKs move to the nucleus and how nucleoplasmic Ca^{2+} control the pathways involved in cell cycle progression.

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