BOC.201100103
Revised version

Role of the nuclear envelope in calcium signalling

Jean-Pierre Mauger[1,2].
[1] Inserm UMR S757; [2] Univ Paris Sud; Orsay, France

Author for correspondance :
Jean-Pierre Mauger
Inserm UMR S757, Bât. 443, Université Paris Sud, 91405 Orsay cedex, France
Tel: 33 1 69 15 75 60
Fax: 33 1 69 15 58 93
email: jean-pierre.mauger@u-psud.fr

Running title: Nuclear envelope and calcium signalling
Key words: endoplasmic reticulum, nuclear envelope, nucleoplasmic reticulum, calcium signalling, cell biology.

Summary

The endoplasmic reticulum is the major Ca^{2+} store inside the cell. Its organisation in specialised sub-domains allows the local delivery of Ca^{2+} to specific cell areas on stimulation. The nuclear envelope, which is continuous with the endoplasmic reticulum, has a double role: it insulates the nucleoplasm from the cytoplasm and it stores Ca^{2+} around the nucleus. Furthermore, all the constituents of the signalling cascade leading to Ca^{2+} mobilisation are found in the nuclear envelope; this allows the nuclear Ca^{2+} to be regulated autonomously. On the other hand, cytosolic Ca^{2+} transients can propagate within the nucleus via the nuclear pore complex. The variations in nuclear Ca^{2+} concentration are important for controlling gene transcription and for progression in the cell cycle. Recent data suggest that invaginations of the nuclear envelope modify the morphology of the nucleus and may affect Ca^{2+} dynamics in the nucleus and regulate transcriptional activity.

Introduction

The endoplasmic reticulum (ER) is a three-dimensional network of membranous tubules and cisternae that spreads throughout the cell from the nucleus to the plasma membrane. The interphase ER can be divided into the nuclear and the peripheral ER and the ER network can have different morphologies in different cell areas, in conjunction with different functions (Baumann and Walz, 2001). It has many different cell functions, including translocation of proteins across the ER membrane, integration of proteins into the membrane, folding and modification of proteins in the lumen, synthesis of phospholipids and steroids (see Baumann and Walz, 2001, Voeltz, et al., 2002). The ER is also recognised as a major Ca^{2+} store inside the cells characterised by the presence of Ca^{2+} pumps that accumulate Ca^{2+}, Ca^{2+}-binding proteins that retain the ion in the lumen of the store and Ca^{2+} channels to rapidly release Ca^{2+} on stimulation (for reviews, see Meldolesi and Pozzan, 1998, Papp, et al., 2003). The mobilisation of Ca^{2+} accumulated in this store plays a major role in agonist-induced Ca^{2+} signalling in stimulated cells and the Ca^{2+} ion is used as an intracellular signal that regulates a variety of cell functions (for reviews, see Berridge, et al., 2003, Rizzuto and Pozzan, 2006). The widespread distribution of the ER throughout the cell allows the delivery of Ca^{2+} in precise areas of the cell and the fine regulation of specific cell functions, like stimulation of ion channels in the plasma membrane, regulation of mitochondrial metabolism and regulation of gene expression in the nucleus (Rizzuto and Pozzan, 2006). Heterogeneity of the ER Ca^{2+} store depends on the non-random distribution of Ca^{2+}-handling proteins and the localisation of the Ca^{2+} release channels which can give rise to local Ca^{2+} signals and microdomains in the cytosol (Papp, et al., 2003). Regulation of the Ca^{2+} level in the nucleus is an example of a complex form of local Ca^{2+} signalling. Increases in Ca^{2+} concentration in the nucleus can have specific effects, different from those observed in the cytoplasm. Ca^{2+} is essential in signalling pathways that regulate gene expression by stimulating the translocation of transcription factors from the cytosol to the nucleus; Ca^{2+} also translocates or activates enzymes that regulate nuclear transcription factor activity and modifies the structure of chromatin (Flavell and Greenberg, 2008, Mellstrom, et al., 2008). The use of selective buffers for cytoplasmic or nucleoplasmic Ca^{2+} allowed the direct demonstration that cell proliferation is dependent on Ca^{2+} signals within the nucleus rather than in the cytoplasm (Rodrigues, et al., 2007). A similar approach in a liver cell line indicated that nucleoplasmic Ca^{2+} is required for mitogen-activated protein kinase-mediated gene transcription (Pusl, et al., 2002). The nucleus is bordered by the nuclear envelope (NE) which has a double function as it insulates the nucleoplasm from the cytoplasm and may be directly involved in Ca^{2+} signalling inside the nucleus. Recent reviews describe in detail various aspects of nuclear calcium signalling, (Alonso and Garcia-Sancho, 2011, Bootman, et al., 2009). In the present review I will focus on the role of the NE in nuclear Ca^{2+} homeostasis and in the mechanisms involved in nuclear Ca^{2+} signalling.

Structure of the nuclear envelope

The use of fluorescent dyes which cannot exchange between discontinuous membranes provided evidence that the ER is a single membrane system (Terasaki and Jaffe, 1991) and the use of luminal GFP-tagged proteins demonstrates that large molecules can rapidly diffuse within the luminal space defined by the ER and the NE membranes (Subramanian and Meyer, 1997) (for review, see Voeltz, et al., 2002).This continuous membrane system is organised in different specialised sub-domains like sER, rER or NE, which are morphologically distinct and have different functions. So, most membrane proteins are shared between the sER and rER, but several proteins involved in translocation or processing of newly synthesised

proteins are enriched in the rER (for reviews, see Levine and Rabouille, 2005, Voeltz, et al., 2002). The NE separates the nuclear and the cytoplasmic compartments of interphase cells. When observed by light microscopy, the nucleus appears delimited by a membrane in continuity with the ER, but electron microscopy reveals a double membrane layer around the nucleus. Therefore, the NE can be further subdivided into three morphologically and biochemically different structures: the outer nuclear membrane (ONM), the inner nuclear membrane (INM) and the nuclear pore complex (NPC) (for reviews, see Baumann and Walz, 2001, Gerace and Burke, 1988). The ONM is continuous with the ER, is decorated with ribosomes and contains a lot of proteins in common with the ER. It also displays a set of unique proteins, some of which may tether the nucleus to the actin cytoskeleton and the centrosome; others interact through the lumen with proteins of the INM. The INM contains many distinct proteins that contact the underlying lamina and chromatin (Batrakou, et al., 2009, Hetzer, 2010, Prunuske and Ullman, 2006). Proteomic analysis of the NE reveals cell type-specific differences in the composition of protein sub-complexes and suggests a functional complexity of the NE (Schirmer and Gerace, 2005). The ONM and the INM are continuous at each NPC that span the NE and regulates transport between the nucleus and the cytoplasm. The NPC is a 120 MDa protein complex which shows eight-fold rotational symmetry with an outer diameter of 100 nm and a central transport channel measuring 40 nm in diameter. It is composed of about 30 distinct protein components, some of high molecular weight and others duplicated many times per NPC (Batrakou, et al., 2009). The NPC mediates communication and selective exchange between the nucleoplasm and the cytoplasm.

The NE can be observed either by electron microscopy or by conventional microscopy after the immunostaining of proteins present in, or associated with, either of the membranes of the nuclear envelope. These studies illustrate the variety of the nuclear shape depending on cell type or cell physiology. Such approaches allow the identification of dynamic tubular channels which appear as deep, narrow invaginations of the nuclear envelope. These invaginations, or infoldings, first described in 1979 (Bourgeois, et al., 1979), have been observed in a lot of cell types (Clubb and Locke, 1998, Collado-Hilly, et al., 2010, Fricker, et al., 1997, Johnson, et al., 2003, Langevin, et al., 2010, Lui, et al., 2003, Wittmann, et al., 2009). The invaginations of the NE have been recently classified into two main classes depending on whether the ONM is involved (Malhas, et al., 2011). Type I invaginations are those where the only the INM invaginates into the nucleoplasm, they can be branched and ramified. The type II invaginations involve both the INM and the ONM. Studies by electron microscopy and recently by three-dimensional structured illumination microscopy have resolved the bilamellar structure of these type II invaginations and revealed the presence of NPC (Schermelleh, et al., 2008). The tubules delimited by these type II invaginations contain cytoplasmic components like actin (Clubb and Locke, 1998, Johnson, et al., 2003), and mitochondria (Clubb and Locke, 1998, Lui, et al., 2003). These invaginations, which have been called the nucleoplasmic reticulum increase the area of exchange between the cytoplasm and the nucleoplasm and make contact deep inside the nucleus with chromatin and sometimes extend to near the nucleolus. Fig. 1 illustrates the presence of invaginations of the NE in polarised MDCK epithelial cells.

It has been suggested that cells that are highly de-differentiated or cancerous have an increased incidence of invaginations (Johnson, et al., 2003). Nuclear infoldings are dynamic structures which can also be formed following various physiological stimuli. Stimulation of synaptic NMDA receptors increases the percentage of infolded nuclei in hippocampal neurons via a process that requires the ERK-MAP kinase pathway and new protein synthesis. In contrast, death signalling pathways triggered by extrasynaptic NMDA receptors cause a rapid

loss of infoldings (Wittmann, et al., 2009). The presence of NE invaginations could also depend on the physiological status of the cell, we have found that they are much more prominent in polarised MDCK epithelial cells than in non-polarised proliferating cells (Collado-Hilly, et al., 2010). The mechanical environment of the cells could also influence the cell nucleus and stretching of fibroblasts induces loss of nuclear invaginations (Langevin, et al., 2010). The formation of invaginations of the nuclear envelope increases the number of NPCs, which may provide more anchoring sites for active genes and enhance gene expression (Akhtar and Gasser, 2007). Furthermore, the infoldings dynamically modify the geometry of the nucleus and form unequally sized nuclear compartments (Wittmann, et al., 2009). These modifications of nuclear geometry and the generation of signalling microdomains may be particularly relevant for diffusion in and out of the nucleus.

Nuclear envelope as a Ca^{2+} store

The lumen of the nuclear envelope is continuous with the lumen of the ER (Subramanian and Meyer, 1997), so it can be assumed that Ca^{2+} accumulated anywhere in the ER can diffuse freely to the nuclear envelope. However, this does not imply that Ca^{2+} is uniformly transported within the ER throughout the cell and we can ask whether the nucleus has its own Ca^{2+} transporting system. An active ATP-dependent Ca^{2+} accumulation has been measured in isolated liver nuclei and this was associated with an increase in the intranuclear free Ca^{2+} concentration (Nicotera, et al., 1989). The use of Ca^{2+}-sensitive fluorescent probes and confocal analysis indicates that Ca^{2+} is accumulated within the NE of isolated liver nuclei (Gerasimenko, et al., 1995), and measurements of the Ca^{2+} concentration in the NE with low-affinity fluorescent Ca^{2+} probes in intact cells gave a resting value of 250-350 µM (Petersen, et al., 1998). The SERCAs are responsible for the active Ca^{2+} transport in the ER and it has been demonstrated that the Ca^{2+} pumps of the NE are identical to that of the ER (Lanini, et al., 1992). The SERCA family includes the products of three genes, named SERCA1, SERCA2 and SERCA3, each giving rise to alternatively spliced mRNA and protein isoforms (for reviews, see Wuytack, et al., 2002). Some studies have reported the presence of SERCA on the NE by using BODIPY-thapsigargin, but the hydrophobic nature of this molecule casts some doubt on the specificity of the labelling (Collado-Hilly, et al., 2010). Immunohistochemical experiments directly demonstrated the presence of the SERCA2 isoform on the NE of epithelial cells (Collado-Hilly, et al., 2010, Lee, et al., 1997a) or muscle cells (Abrenica and Gilchrist, 2000). The Ca^{2+} pump has also been found in the invaginations of the NE (Avedanian, et al., 2011, Collado-Hilly, et al., 2010). Since the ONM is continuous with the ER membrane, the SERCA is more likely present on the ONM, but to our knowledge there is no EM study indicating the presence of SERCA on the INM. In some cell types a sodium-calcium exchanger located in the INM may function to transfer Ca^{2+} between the nucleoplasm and the lumen of the envelope (Xie, et al., 2002). Functional studies indicated that Ca^{2+} concentration in the nucleoplasm decreases after stimulation. This could be due either to direct pumping through the INM or diffusion through the NPC.

The Ca^{2+} pumps can accumulate Ca^{2+} in specific areas of the cell and Ca^{2+}-binding proteins in the lumen of the ER provide a releasable Ca^{2+} pool near the Ca^{2+} release channels (Papp, et al., 2003). The Ca^{2+}-storage protein calreticulin has been localised by immunocytochemistry in the NE and in invaginations of the NE of various tissues, including human oocytes and embryos and epithelial cells (Balakier, et al., 2002, Bedard, et al., 2005, Collado-Hilly, et al., 2010, Echevarria, et al., 2003). Therefore, the presence of the Ca^{2+}-binding protein in the nuclear envelope confirms its role as a Ca^{2+} store which may provide Ca^{2+} to the nucleus.

The localisation of Ca^{2+}-releasing channels is critical for generating spatially complex Ca^{2+} signals and in this case to induce nuclear Ca^{2+} signals. Inositol trisphosphate (IP_3), produced from membrane phosphoinositides on hormonal stimulation, binds to the IP_3 receptor (IP_3R) and releases Ca^{2+} from intracellular stores. The IP_3R family includes the products of three genes, named IP_3R1, IP_3R2, IP_3R3 which display specific tissue distribution and sometimes different sub-cellular localisations (Mikoshiba, 2007, Vermassen, et al., 2004). The presence of IP_3R in the nucleus is suspected from direct measurements of IP_3-induced Ca^{2+} release from liver nuclei and from radiolabelled IP_3 binding to isolated nuclei (Malviya, et al., 1990, Nicotera, et al., 1990). However, there are few reports directly demonstrating the localisation of IP_3R in the nucleus or the NE, possibly because of the low density of receptors found in this organelle. Immunohistochemical experiments performed in situ confirmed the presence of IP_3R on the perinuclear ER and on the nuclear envelope in epithelial cells (Collado-Hilly, et al., 2010, Lee, et al., 1997b, Leite, et al., 2003, Nakanishi, et al., 1996, Siefjediers, et al., 2007, Yule, et al., 1997), cardiomyocytes (Bare, et al., 2005) skeletal muscle cells (Cardenas, et al., 2005, Kusnier, et al., 2006), smooth muscle cells (Vermassen, et al., 2003), and Xenopus oocytes (Kume, et al., 1993). IP_3R has also been detected on the nucleoplasmic reticulum of HeLa cells (Lui, et al., 2003), liver cells (Echevarria, et al., 2003), MDCK cells (Collado-Hilly, et al., 2010), cardiomyocytes (Guatimosim, et al., 2008) and smooth muscle cells (Avedanian, et al., 2011). Some studies suggest that IP_3R2 is more often associated with the nucleus (Bare, et al., 2005, Laflamme, et al., 2002, Leite, et al., 2003), but according to the tissue, any receptor subtype could be localised at the nucleus (for review, see Vermassen, et al., 2004). However, immunohistochemical experiments performed by conventional microscopy failed to determine whether the IP_3Rs are localised on the ONM or the INM. The ONM shares many features with the ER membranes and, indeed, IP_3Rs have been found on the ONM of isolated nuclei by electrophysiological measurements of IP_3-induced currents (Mak and Foskett, 1994, Stehnobittel, et al., 1995a). However, the contamination of the preparation by ER membranes cannot be completely excluded. Recently, the cytoplasmic domain of IP_3R has been directly visualized on isolated nuclei of Sf9 cells by freeze-dry rotary shadowing for electron microscopy, and was located on the cytoplasmic side of the NE (Cardenas, et al., 2010). While there is direct evidence for the localisation of IP_3 on the ONM, its presence on the INM is debated. Functional experiments suggest that IP_3 can induce the release of Ca^{2+} directly through the INM (see below), but to our knowledge there is no immunohistochemical evidence at the EM level for the localisation of IP_3R on the INM. It should be noted that some studies describe the presence of IP_3R on small vesicles inside the nucleus that could be responsible for nuclear Ca^{2+} transients (Huh, et al., 2006, Yoo, et al., 2005). Whether these vesicles are different from invaginations of the NE is not clear. Finally, ryanodine receptors have directly been visualised with BODIPY-Ry on the NE of nuclei isolated from pancreatic cells (Gerasimenko, et al., 2003). They have also been located by immunohistochemical experiments in the NE of cardiac cells (Abrenica and Gilchrist, 2000) and in the NE and the nucleoplasmic reticulum of myoblastic cells (Marius, et al., 2006). These data clearly indicate that the NE of many cell types has all the characteristics of a Ca^{2+} store which can be mobilised on stimulation to deliver a Ca^{2+} signal within or in the close proximity of the nucleus.

Production of IP_3 in the NE

The canonical pathway that leads to the mobilisation of intracellular Ca^{2+} involves the activation of either seven-transmembrane domains receptors or receptor tyrosine kinases, which induces the hydrolysis of phosphatidylinositol 4,5-bisphosphate and the production of IP_3 (Berridge, et al., 2003). The mobility of IP_3 released from the plasma membrane is

sufficient to mobilise Ca^{2+} from the ER anywhere in the cytoplasm, including the nuclear envelope (Allbritton, et al., 1992). However, the various components of the signalling cascade leading to Ca^{2+} mobilisation have also been found in the nucleus. Phosphoinositides (as well as the enzymes responsible for their synthesis, namely phosphatidylinositol and phosphatidylinositol 4-phosphate kinases) have long been known to be present in the nucleus (for review, see Irvine, 2003). The fact that these nuclear inositol lipids resist detergents suggests that they are localised inside the nucleus, possibly on structures called speckles, and virtually not in the nuclear envelope. But it is possible that invaginations of the NE could be enriched in PIP2 such that it appears to be intranuclear (Irvine, 2006). Different phospholipase C isoforms have been identified in the nucleus and may provide in situ production of IP_3 and DAG. PLC-β contains at its C-terminal domain a region responsible for nuclear localisation (Cocco, et al., 2006). PLC-β is present both in whole nuclei and in nuclei treated with detergent, suggesting that it is probably not present on NE (for review, see (Visnjic and Banfic, 2007).

If we assume that the local production of IP_3 in the nucleus mobilises Ca^{2+} locally, it is still unclear how the PLC is stimulated. Several studies have described the translocation to the nucleus of growth factor receptors after stimulation (for review, see Wells and Marti, 2002), but their role in the change in nuclear Ca^{2+} concentration and growth factor-mediated responses have only recently been investigated. For example, experiments performed on a hepatic cell line indicated that the HGF receptor, c-Met, rapidly translocated to the nucleus upon stimulation with HGF and appeared both at the NE and inside the nucleus. The c-Met-induced nuclear Ca^{2+} signal depended on the hydrolysis of PIP2 and formation of IP_3 in the nucleus. This is different from the Ca^{2+} signal induced by vasopressin which depended on the formation of IP_3 in the cytoplasm (Gomes, et al., 2008). Insulin also induced Ca^{2+} oscillations in rat hepatocytes and data suggest that the insulin receptor translocated to the nucleus to initiate an IP_3-dependent nuclear Ca^{2+} signal (Rodrigues, et al., 2008). Various G protein-coupled receptors have also been found in the nucleus in various cell types (for review, see Gobeil, et al., 2006). For example, the metabotropic glutamate receptor, mGlu5R, has been found in the nucleus of neurons, and immunohistochemical experiments using confocal or electron microscopy suggest that it is localised on the membrane of the NE (O'Malley, et al., 2003). Experiments performed on HEK293 cells expressing mutant mGlu5 receptor leading to the loss of G-protein coupling, indicated that the nuclear mGlu5 receptor couples to $G_{q/11}$ and PLC to generate IP_3 and mobilise nuclear Ca^{2+} (Kumar, et al., 2008). In intact cells, extracellular ligands like glutamate or quisqualate reach nuclear receptors via both sodium-dependent transporters and cystine glutamate exchangers (Jong, et al., 2005). Another study in cardiomyocytes indicated that the type 1 and 2 angiotensin receptors (AT1R and AT2R, respectively) are localised on nuclear membranes. Application of angiotensin II to these receptors increased NFκB mRNA expression, and stimulation of AT1R induced a Ca^{2+} signal in isolated nuclei via an IP_3R-mediated mechanism. Angiotensin II can be produced in situ in intact cardiomyocytes and activates this intranuclear pathway (Tadevosyan, et al., 2010).

Role of nuclear envelope in nuclear Ca^{2+} signalling

The nucleus is separated from the surrounding cytoplasm by the NE, but the two compartments can communicate through the NPC. It is admitted that molecules larger than 70 kDa do not redistribute passively between nucleus and cytoplasm and so need a targeting sequence to be transported through the NPC by facilitated diffusion or active transport. Conversely, metal ions, small metabolites and molecules up to 10 kDa in mass freely exchange between the cytoplasm and the nucleus by passive diffusion through the NPC

(Torok, 2007). The permeability of the pore to proteins of intermediate size may be regulated. Among the factors that modulate the permeability of the nuclear pore, the Ca^{2+} ion is of special interest as both the cytosolic Ca^{2+} concentration and the amount of Ca^{2+} within the NE may be involved. Greber and Gerace (Greber and Gerace, 1995) first demonstrated in mammalian cells that depletion of Ca^{2+} from the NE inhibits both the signal mediated transport of proteins into the nucleus and the passive diffusion of 10 kD fluorescent dextran through the NPC. Similarly, experiments performed in intact nuclei or nuclear membrane from *Xenopus laevi* oocytes demonstrated that depletion of the nuclear Ca^{2+} store regulates movements of 10 kD molecules through the NE (Stehnobittel, et al., 1995b). However the measurement of the diffusion of GFP through the NE of intact cell by real time imaging indicated that it was not affected by depletion of perinuclear Ca^{2+} store (Wei, et al., 2003). A recent study in liver cells, using localised two-photon activation of photoactivatable GFP, demonstrates that hormones that increase cytosolic Ca^{2+} concentration increased the permeability of the nuclear membrane (O'Brien, et al., 2007). These studies and others illustrate the discrepancies on the role of Ca^{2+} on the permeability of the NPC, they also indicate that both luminal and cytoplasmic Ca^{2+} may have an important role in regulating trafficking of macromolecules between the cytoplasm and the nucleoplasm (for review and discussion see Bootman, et al., 2009, Sarma and Yang, 2011). This may provide a mechanism to mediate the entry of transcription factors or other regulatory molecules and to regulate gene transcription in target cells. It is admitted that Ca^{2+} can diffuse freely through the NPC however, whether it can be regulated by Ca^{2+} within the NE is also controversial (Gerasimenko and Gerasimenko, 2004). The regulatory role of Ca^{2+} stored in the lumen of the NE is associated with a switch in the conformation of the nuclear pore complex which may control the diffusion of intermediate sized molecules. Atomic force microscopy (AFM) showed the displacement of a central granule to the cytoplasmic face of the NE under Ca^{2+} depleting conditions (Moore-Nichols, et al., 2002, PerezTerzic, et al., 1996, Stoffler, et al., 2006).

The Ca^{2+} ion could also act directly in the nucleoplasm and regulate gene transcription. It is a small molecule which is assumed to diffuse freely through the NPC, so it can be expected that the Ca^{2+} concentration in the nucleus rapidly equilibrates with the cytosolic Ca^{2+} concentration. Indeed, studies on intact RBL cells (Allbritton, et al., 1994), or neurons (Eder and Bading, 2007, Omalley, 1994), and isolated liver nuclei (Gerasimenko, et al., 1995), demonstrate that the Ca^{2+} signal from the cytosol to the nucleus is not substantially delayed by the NE. Therefore, Ca^{2+} waves that occur in the cytosol of stimulated cells could propagate to the nucleoplasm. However, a major question concerns the Ca^{2+} transients observed in the nucleoplasm and whether they could be generated autonomously and distinguished from cytosolic Ca^{2+} variations. Several groups using fluorescent Ca^{2+} imaging in living cells reported that the Ca^{2+} concentration can be different in the cytosol and in the nucleus. However, it has been recognised that the fluorescent Ca^{2+} indicators can behave differently in the nucleus as they are much brighter in the nucleus than in the cytosol. Both Ca^{2+} binding to the probe and dynamic range of the indicator may be affected. This results in technical artefacts that led to misinterpretation of the data and controversy on the regulation of nuclear Ca^{2+} concentration (for discussion see Bootman, et al., 2009)).

Several experimental approaches have been used to study nuclear Ca^{2+} signals, such as kinetic analysis of Ca^{2+} transients in the cytoplasm and nucleoplasm, and the direct manipulation of the cytoplasmic and nucleoplasmic Ca^{2+} contents in intact cells. Several studies indicate that the Ca^{2+} signal can originate in the nucleus (Fig. 2). Stimulation of HepG2 liver cells with submaximal concentrations of ATP revealed that the Ca^{2+} signal started earlier and was

greater in the nucleus than in the cytosol. This was confirmed by the photorelease of IP_3 inside the cells, which showed that the nuclear IP_3R channels were more sensitive to IP_3 than the cytoplasmic IP_3R, and this was correlated with a higher sensitivity of the type 2 IP_3R present on the nuclear envelope (Leite, et al., 2003). The selective measurements of changes of Ca^{2+} in the cytosol or in the nucleus by targeted aequorin in GH3 pituitary cells indicated that the IP_3-induced nuclear response is not inhibited by the addition of cytosolic heparin, which does not permeate the nuclear pore. Furthermore, the nuclear Ca^{2+} response to IP_3 showed a higher sensitivity and again this was correlated with the presence of IP_3R-2 in the nucleus (Chamero, et al., 2008). In functional experiments on isolated liver nuclei, IP_3 or cyclic ADP ribose evoked intranuclear Ca^{2+} elevations (Gerasimenko, et al., 1995). Similarly, in isolated acinar pancreatic nuclei, NAADP, cyclic ADP ribose or IP_3 reduced the Ca^{2+} concentration inside the nuclear envelope, and this was associated with a transient Ca^{2+} rise in the nucleoplasm (Gerasimenko, et al., 2003).

Cardiomyocytes are of special interest because Ca^{2+}-dependent signalling pathways are regulated under conditions of continual Ca^{2+} transients that mediate cardiac contraction during each heart beat (Molkentin, 2006). Stimulation of ventricular myocytes with endothelin 1, which produces intracellular IP_3, induced local Ca^{2+} release from the NE via IP_3R, and again these data are correlated with the presence of type 2 IP_3R on the nuclear envelope. This local Ca^{2+} release triggers histone deacetylase 5 phosphorylation and nuclear export, which do not occur during the global Ca^{2+} transients at each heart beat (Wu, et al., 2006). The involvement of IP_3-induced Ca^{2+} release in the nuclear Ca^{2+} transients was confirmed by the direct addition of IP_3 to permeabilised cardiomyocytes, which increases nuclear Ca^{2+} (Zima, et al., 2007) Direct labelling of Ca^{2+} in the lumen of the nuclear envelope indicates that it is mobilised to increase the Ca^{2+} concentration in the nucleoplasm. Experiments performed on isolated nuclei also demonstrate that $InsP_3$ induces the release of Ca^{2+} from the nuclear envelope and allows the genesis of Ca^{2+} transients in the nucleoplasm (Zima, et al., 2007). Similar results have been obtained in electrically stimulated atrial myocytes, where addition of endothelin 1 caused an increase of nuclear Ca^{2+}, an effect mediated by the production of IP_3 and the activation of IP_3R (Kockskamper, et al., 2008). The elevation of nuclear Ca^{2+} concentration induced by endothelin 1 in cardiomyocytes is involved in the pathway leading to cardiac hypertrophy (Higazi, et al., 2009).

It is now acknowledged that Ca^{2+} release from the NE participates in the genesis and regulation of Ca^{2+} transients in the nucleoplasm. However, a major controversy concerns the orientation of the Ca^{2+} flux from the NE. Ca^{2+} can be either directly released to the nucleoplasm through the INM or released to the cytoplasm and secondly enter the nucleus through the NPC. One approach to determine whether Ca^{2+} is released directly to the nucleoplasm is to apply IP_3 or IP_3 antagonists to the cytoplasm or to the nucleoplasm and to consider the effects on the cytoplasmic or nucleoplasmic Ca^{2+} transients. These approaches have given conflicting results, possibly due to the cell type used in the experiments or to the geometry of the nucleus (see below). In an experiment performed in rat basophilic leukaemia cells stimulated by IP_3-generating agonist, the injection of heparin-dextran, an inhibitor of the IP_3R which is excluded from the nucleus, suppressed Ca^{2+} transients in the cytosol and in the nucleus, suggesting that nuclear Ca^{2+} increases followed cytosolic Ca^{2+} increases (Allbritton, et al., 1994). But other experiments performed on intact cells led to a different conclusion. So, the injection of heparin in the nucleus of HeLa cells abolished Ca^{2+} increase induced by histamine, while an increase in cytosolic Ca^{2+} was observed (Lui, et al., 1998a). Injection of IP_3 in the nucleus of Xenopus oocytes induced Ca^{2+} increases in the nucleus even if heparin was present in the cytosol (Hennager, et al., 1995). Moreover, the observation of Ca^{2+}

transients in isolated nuclei suggests that Ca^{2+} was directly delivered from the lumen of the NE to the nucleoplasm, because it is assumed that Ca^{2+} released to the incubation medium will immediately diffuse and be diluted far from the nucleus (Gerasimenko, et al., 2003, Gerasimenko, et al., 1995, Zima, et al., 2007). Furthermore, patch-clamp recording from the inner nuclear membrane of Purkinje neurons revealed the presence of $InsP_3R$-activated channels (Marchenko, et al., 2005). Today, there is some consensus that Ca^{2+} can be delivered directly from the lumen of the NE to the nucleoplasm through the INM, more likely through IP_3R or in some cases through RyR. It should be noted that small vesicles have been described inside the nucleoplasm that could accumulate Ca^{2+} and release it in the presence of IP_3 (Yoo, et al., 2005). These putative Ca^{2+} stores in the nucleoplasm can also be involved in some cell types in the genesis of nuclear Ca^{2+} signals. It is not clear whether autonomous nuclear Ca^{2+} signals play a role in physiological responses in intact cells. The whole machinery needed to produce IP_3 locally within the nucleoplasm is present in the nucleus. This could induce Ca^{2+} release through the INM or nucleoplasmic vesicles if we assume that stimuli (hormone or agonist) can access the nucleus as a complex with a membrane receptor or by endocytosis or by local intracellular production.

The presence of IP_3R on the ONM is better documented (see above), but its involvement in the regulation of nuclear Ca^{2+} concentration is difficult to differentiate from the role of IP_3R on the ER in close proximity to the nuclear membrane. Careful analysis of the subcellular origin of nuclear Ca^{2+} signals in Fluo-3-loaded HeLa cells indicates that all signals that increase nuclear Ca^{2+} concentration are of cytoplasmic origin. Ca^{2+} puffs that originate within a 2–3 mm perinuclear zone propagate anisotropically across the entire nucleus. Furthermore, the low buffering capacity of the nucleus allow the substancial prolongation of the Ca^{2+} signal in the nucleus as compared to the small cytosolic signal (Lipp, et al., 1997). In this case, it can be assumed that Ca^{2+} released through the IP_3R toward the cytosol will diffuse to the nucleoplasm through neighbouring NPC and, the absence of Ca^{2+} uptake mechanism in the nucleus allows the Ca^{2+} to rapidly diffuse over the entire nucleoplasm (Lipp, et al., 1997). Several studies agree with this model. So, the direct injection of IP_3 in the nucleus of hamster oocyte in close proximity to the INM did not induce Ca^{2+} release, but when IP_3 was uniformly applied to the cytoplasm it initiated a Ca^{2+} rise in the perinuclear cytoplasm and then in the nucleoplasm. It was concluded that the rise in Ca^{2+} concentration in the nucleoplasm was a consequence of Ca^{2+} release in the perinuclear cytoplasm and diffusion through the NPC. Accordingly, clusters of ER are localised in the perinuclear cytoplasm and serve as a trigger zone for IP_3-induced Ca^{2+} release and propagation to the nucleoplasm (Shirakawa and Miyazaki, 1996). In neonatal myocytes, phenylephrine- or IP_3-evoked nuclear Ca^{2+} waves engulf the entire nucleus without spreading into the bulk cytosol (Luo, et al., 2008). The IP_3R located at the ONM can be activated by IP_3 produced at the plasma membrane in the presence of agonists. This pathway can be facilitated by the structure of the cell as, for example, in the cardiac myocytes, the IP_3R present on the ONM is in close proximity to the T tubules (a likely source of IP_3) and dyads containing RyR2 which can establish local perinuclear Ca^{2+} microdomains and activate IP_3R (Escobar, et al., 2011). On another hand, it is interesting to notice that in some cell types, NPC are also found in an ER subdomain known as annulate lamellae. It was recently found that IP_3R activity is decreased within annulate lamellae and the relief of this inhibition is correlated with the dismantling of the NPC from this ER subdomain (Boulware and Marchant, 2008). It would be interesting to study whether NPC could also regulate Ca^{2+} fluxes and IP_3R activity within the NE.

Influence of the geometry of the nucleus

The geometry of the nucleus may participate in the genesis and pattern of the nuclear Ca^{2+} signal. We have seen above that invaginations of the double membrane of the NE, form a Ca^{2+} store deep inside the nucleus in close proximity to the chromatin. They increase the nuclear surface and the number of NPC and may then facilitate the exchanges between the cytoplasm and nucleus. Wittmann et al (Wittmann, et al., 2009) used a mathematical modelling approach that revealed that the division of the nucleus into unequally sized compartments by infoldings can function as microdomains which generate distinct Ca^{2+} signals. It is assumed that nuclear infoldings decrease the diffusion distances, Ca^{2+} reaches central sites faster and is cleared more quickly. This means that "nuclear inertia" is reduced by membrane infoldings (Queisser, et al., 2011). Both modelling and experimental approaches in hippocampal neurons show that nuclear Ca^{2+} transients evoked by cytosolic Ca^{2+} transients are larger in small nuclear compartments than in large compartments of the same nucleus. Moreover, frequency information in the Ca^{2+} signal is preserved and resolved better in small compartments of infolded nuclei. The authors demonstrated that the presence of nuclear infoldings correlates with transcription-relevant events since synaptic activity-induced phosphorylation of histone H3 on serine 10 was more robust in neurons with infolded nuclei than in neurons with near spherical nuclei (Wittmann, et al., 2009). This work highlights the role of the geometry of neuronal nuclei in Ca^{2+} signals and the activation of transcription-regulating events. The formation of nuclear infoldings in synaptically activated neurons constitutes a structural plasticity that may allow an adaptation to metabolic states related to the regulation of gene expression.

In the preceding example, the invaginations of the NE passively regulate the Ca^{2+} signal generated in the cytosol, they can also be directly involved in the genesis of Ca^{2+} transients in the nucleus. In HeLa cells, the NE invaginations define an extension of the cytosol inside the nucleus where Ca^{2+} can be released or transported after ionomycin stimulation (Lui, et al., 1998b). This demonstrates that the nucleoplasmic reticulum is a Ca^{2+} store. Recently, the IP_3-3-kinase-B has been found, together with IP_3R, in nuclear invaginations of a lung carcinoma cell line, and may modulate the IP_3 concentration locally within the nucleus (Nalaskowski, et al., 2011).

The role of the nucleoplasmic reticulum in Ca^{2+} signalling was characterised in experiments performed in a liver cell line by Echevarria et al (Echevarria, et al., 2003), who demonstrated that a branching intranuclear network which is continuous with the ER and the NE contains calreticulin, IP_3R and Ca^{2+} storing activity. Stimulation of the cells with HGF preferentially induced nuclear Ca^{2+} signals. Furthermore, local photorelease of a small amount of IP_3 resulted in a small increase in Ca^{2+} which began near this nucleoplasmic reticulum. These authors also show that release of Ca^{2+} in the nucleus causes nuclear PKC to translocate to the nuclear envelope, demonstrating a specific effect of localized nuclear Ca^{2+} signals.
We emphasized above the question concerning the direction of the Ca^{2+} release from the nuclear envelope, either directly to the nucleoplasm or to the cytoplasm and diffusion through the NPC. The presence of NE invaginations can facilitate the diffusion of Ca^{2+} from the cytoplasm to the nucleoplasm. The membrane bordering the lumen of the tubule is identical to the ONM and therefore delineates a confined cytosolic space in the deeper part of the nucleus. Brasen et al (Brasen, et al., 2010) calculated that the wrinkled surfaces of cells provide a mechanism for generating microdomains where the Ca^{2+} concentration may be much higher than in the bulk cytosol, and long narrow membrane wrinkles will be most effective in

generating such a high Ca^{2+} microdomain. The ONM lining the tubules more likely contains IP_3R which can release the Ca^{2+} ions toward the cytosol within the narrow wrinkles formed by the NE invagination. This should give rise locally to high concentrations of free Ca^{2+}, whose diffusion into the bulk cytosol is restricted. The Ca^{2+} retained in this confined space of the cytoplasm may diffuse into the nucleoplasm more easily, through the nuclear pores identified in invaginations by electron microscopy (Fig. 2).

Differences between the NE and the peripheral ER

The NE is often presented as a sub-domain of the ER and the peripheral ER is described as an extensive network that branches out of the NE. One can ask what are the respective roles of the NE and the peripheral ER in Ca^{2+} signalling? The protein composition of the ONM is mainly similar to that of the peripheral ER independently of differences which may characterise specialised sub-domains of the ER. The main differences in the protein composition of the peripheral ER and the NE concern the INM which has its own protein components and is underlined by lamina rich in intermediate filaments. Particularly, the NE is riddled with NPC that regulate transport of molecules between the cytoplasm and the nucleoplasm. We have seen above that the Ca^{2+} handling proteins are equally distributed in the peripheral ER and in the NE, this allows the accumulation of Ca^{2+} in a single luminal space and the Ca^{2+} release in the cytoplasm or the nucleoplasm. One important difference between the two structures concerns their spatial organisation. The peripheral ER consists of a dynamic network of membrane sheets and interconnected tubules and the NE forms membrane sheets around the chromatin and nuclear lamins. This has direct involvements in the mechanisms of cell signalling.

The peripheral ER is a network that expands within the cytosol and covers every part of the cell. This ER network is stabilised partly through interactions with cellular organelles such as mitochondria, Golgi, endosomes or plasma membrane. These close juxtapositions of ER membranes with other cell membranes are important for interorganelle exchanges of lipids. These interactions also play an important role in Ca^{2+} signalling. Protein complexes including IP_3 receptor have been extensively studied these last few years and allow the mitochondrial-ER attachment and the transfer of Ca^{2+} to the mitochondria (de Brito and Scorrano, 2010). In the same way, interactions of ER with the plasma membrane have been described in epithelial cells where the IP_3R is concentrated near the apical domain of the plasma membrane (Vermassen, et al., 2004). The ER proteins STIM sense a decrease in the ER Ca^{2+} concentration and activate the ORAI Ca^{2+} channel of the plasma membrane across the junction between the two membranes (Shen, et al., 2011). These local interactions of the peripheral ER associated with the fact that it is constantly rearranged and moves along microtubules, allow the comparison to an octopus which expands its tentacles within the cytosol and regulates locally the Ca^{2+} fluxes.

The NE, although being in continuity with the peripheral ER has an additional role as it separates two cell compartments, the cytoplasm and the nucleus, and consequently, it has a static role. It regulates the transfer of molecules, including Ca^{2+}, between the cytoplasm and the nucleus and it is a Ca^{2+} store that allows the delivery of Ca^{2+} to the nucleoplasm. The dynamics of the NE is limited to the formation of infoldings that increases the surface of exchange between the cytoplasm and the nucleoplams. The invaginations of the NE modulate the transfer of the Ca^{2+} signal from the cytoplasm to the nucleus and allow the release of Ca^{2+} deeply near the chromatin.

Concluding remarks

Over recent years, it has become more and more evident that nuclear Ca^{2+} signals can specifically influence gene transcription and cell-cycle progression. At the molecular level, all the components constituting the Ca^{2+} signalling cascade (from the hormone receptor to the Ca^{2+} release channels) have been found in the nucleus. The nuclear envelope plays a major role by integrating all the molecules that constitute a Ca^{2+} store and allows the local release of Ca^{2+} when stimulated by Ca^{2+}-mobilising agents like IP_3, also produced locally. All this machinery suggests that Ca^{2+} signalling in the nucleus can be independent of cytosolic variations even if the mechanisms involved are not yet clearly established. On the other hand, the global cytosolic Ca^{2+} signals can be easily transmitted to the nucleoplasm through the NPCs, which are freely permeable to small molecules like calcium ions. The exchanges between the cytoplasm and the nucleoplasm depend on the area of exchange and the number of NPCs and these two features are increased in some cell types and under some physiological conditions by the formation of invaginations of the NE. These structures, recently called nucleoplasmic reticulum, have long been observed, but very interesting recent data suggest that they could play important roles in nuclear functions. The nucleoplasmic reticulum allows the delivery of Ca^{2+} locally deep inside the nucleus. It also forms separate compartments of varying sizes within the nucleoplasm and could therefore determine whether the nucleus functions as an integrator or a detector of oscillating Ca^{2+} signals (Queisser, et al., 2011). Until now we have described the generation of Ca^{2+} microdomains by considering their different subcellular location and how each organelle has its own distinct Ca^{2+}-handling properties. A future challenge will be to characterise the signalling pathways and/or the physiological conditions that regulate the formation of the nucleoplasmic reticulum and modify the geometry of the nucleus. These regulatory mechanisms more likely participate in the pattern of the Ca^{2+} signal and possibly in other nuclear processes. The morphology regulation of the Ca^{2+} signal described in the nucleus could be extended to the Ca^{2+} dynamics in the entire cells (Queisser, et al., 2011). In this way, the manipulation of the cell shape and microenvironnement by the micropatterning techniques could be of considerable help (Thery, 2010). Modification of the morphology of the organelles like the nucleus can modulate Ca^{2+} signals and this opens up new ways to describe the generation and propagation of Ca^{2+} signals at the nuclear level and, possibly, at the level of the entire cell.

Abbreviations used: EM, electron microscopy; ER, endoplasmic reticulum; GFP, green fluorescent protein; INM, inner nuclear membrane; IP_3, inositol-1,4,5-trisphosphate; IP_3R, inositol-1,4,5-trisphosphate receptor; MDCK, Madin Darby canine kidney; NE, nuclear envelope; NPC, nuclear pore complex; ONM, outer nuclear membrane; PIP_2, phosphatidylinositol-4,5-bisphosphate; PLC, phospholipase C; RyR, ryanodine receptor, SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase.

Acknowledgements
This study was supported by Inserm. The author is indebted to Mauricette Collado-Hilly (Inserm UMR S757, Univ Paris Sud, Orsay, France) and Danielle Jaillard (CNRS UMR 8195; Univ Paris Sud; CCME, Orsay, France) for providing epifluorescence and electron microscopy images.

References

Abrenica, B. and Gilchrist, J. S. C. (2000) Nucleoplasmic Ca2+ loading is regulated by mobilization of perinuclear Ca2+. Cell Calcium. **28**, 127-136.

Akhtar, A. and Gasser, S. M. (2007) The nuclear envelope and transcriptional control. Nat Rev Genet. **8**, 507-17.

Allbritton, N. L., Meyer, T. and Stryer, L. (1992) Range of Messenger Action of Calcium Ion and Inositol 1,4,5- Trisphosphate. Science. **258**, 1812-1815.

*Allbritton, N. L., Oancea, E., Kuhn, M. A. and Meyer, T. (1994) Source of nuclear calcium signals. Proc Natl Acad Sci USA. **91**, 12458-12462.

Alonso, M. T. and Garcia-Sancho, J. (2011) Nuclear Ca(2+) signalling. Cell Calcium. **49**, 280-9.

Avedanian, L., Jacques, D. and Bkaily, G. (2011) Presence of tubular and reticular structures in the nucleus of human vascular smooth muscle cells. J Mol Cell Cardiol. **50**, 175-86.

Balakier, H., Dziak, E., Sojecki, A., Librach, C., Michalak, M. and Opas, M. (2002) Calcium-binding proteins and calcium-release channels in human maturing oocytes, pronuclear zygotes and early preimplantation embryos. Hum Reprod. **17**, 2938-47.

Bare, D. J., Kettlun, C. S., Liang, M., Bers, D. M. and Mignery, G. A. (2005) Cardiac type 2 inositol 1,4,5-trisphosphate receptor: interaction and modulation by calcium/calmodulin-dependent protein kinase II. J Biol Chem. **280**, 15912-20.

*Batrakou, D. G., Kerr, A. R. and Schirmer, E. C. (2009) Comparative proteomic analyses of the nuclear envelope and pore complex suggests a wide range of heretofore unexpected functions. J Proteomics. **72**, 56-70.

*Baumann, O. and Walz, B. 2001. Endoplasmic reticulum of animal cells and its organization into structural and functional domains. *In* International Review of Cytology A Survey of Cell Biology, Vol 205. K. W. Jeon, editor. Academic Press Inc, 525 B Street/ Suite 1900/San Diego/CA 92101-4495/USA. 149-214.

Bedard, K., Szabo, E., Michalak, M. and Opas, M. (2005) Cellular functions of endoplasmic reticulum chaperones calreticulin, calnexin, and ERp57. Int Rev Cytol. **245**, 91-121.

*Berridge, M. J., Bootman, M. D. and Roderick, H. L. (2003) Calcium signalling: dynamics, homeostasis and remodelling. Nat Rev Mol Cell Biol. **4**, 517-29.

*Bootman, M. D., Fearnley, C., Smyrnias, I., Macdonald, F. and Roderick, H. L. (2009) An update on nuclear calcium signalling. J Cell Sci. **122**, 2337-50.

Boulware, M. J. and Marchant, J. S. (2008) Nuclear pore disassembly from endoplasmic reticulum membranes promotes Ca2+ signalling competency. J Physiol. **586**, 2873-88.

Bourgeois, C. A., Hemon, D. and Bouteille, M. (1979) Structural relationship between the nucleolus and the nuclear envelope. J Ultrastruct Res. **68**, 328-40.

Brasen, J. C., Olsen, L. F. and Hallett, M. B. (2010) Cell surface topology creates high Ca2+ signalling microdomains. Cell Calcium. **47**, 339-49.

*Cardenas, C., Escobar, M., Garcia, A., Osorio-Reich, M., Hartel, S., Foskett, J. K. and Franzini-Armstrong, C. (2010) Visualization of inositol 1,4,5-trisphosphate receptors on the nuclear envelope outer membrane by freeze-drying and rotary shadowing for electron microscopy. J Struct Biol. **171**, 372-81.

Cardenas, C., Liberona, J. L., Molgo, J., Colasante, C., Mignery, G. A. and Jaimovich, E. (2005) Nuclear inositol 1,4,5-trisphosphate receptors regulate local Ca2+ transients and modulate cAMP response element binding protein phosphorylation. J Cell Sci. **118**, 3131-40.

Chamero, P., Manjarres, I. M., Garcia-Verdugo, J. M., Villalobos, C., Alonso, M. T. and Garcia-Sancho, J. (2008) Nuclear calcium signaling by inositol trisphosphate in GH(3) pituitary cells. Cell Calcium. **43**, 205-14.

Clubb, B. H. and Locke, M. (1998) 3T3 cells have nuclear invaginations containing F-actin. Tissue Cell. **30**, 684-91.

*Cocco, L., Faenza, I., Fiume, R., Maria Billi, A., Gilmour, R. S. and Manzoli, F. A. (2006) Phosphoinositide-specific phospholipase C (PI-PLC) beta1 and nuclear lipid-dependent signaling. Biochim Biophys Acta. **1761**, 509-21.

Collado-Hilly, M., Shirvani, H., Jaillard, D. and Mauger, J. P. (2010) Differential redistribution of Ca2+-handling proteins during polarisation of MDCK cells: Effects on Ca2+ signalling. Cell Calcium. **48**, 215-24.

de Brito, O. M. and Scorrano, L. (2010) An intimate liaison: spatial organization of the endoplasmic reticulum-mitochondria relationship. Embo J. **29**, 2715-23.

*Echevarria, W., Leite, M. F., Guerra, M. T., Zipfel, W. R. and Nathanson, M. H. (2003) Regulation of calcium signals in the nucleus by a nucleoplasmic reticulum. Nat Cell Biol. **5**, 440-6.

Eder, A. and Bading, H. (2007) Calcium signals can freely cross the nuclear envelope in hippocampal neurons: somatic calcium increases generate nuclear calcium transients. BMC Neurosci. **8**, 57.

*Escobar, M., Cardenas, C., Colavita, K., Petrenko, N. B. and Franzini-Armstrong, C. (2011) Structural evidence for perinuclear calcium microdomains in cardiac myocytes. J Mol Cell Cardiol. **50**, 451-9.

Flavell, S. W. and Greenberg, M. E. (2008) Signaling Mechanisms Linking Neuronal Activity to Gene Expression and Plasticity of the Nervous System. Annu Rev Neurosci. **31**, 563-590.

*Fricker, M., Hollinshead, M., White, N. and Vaux, D. (1997) Interphase nuclei of many mammalian cell types contain deep, dynamic, tubular membrane-bound invaginations of the nuclear envelope. J Cell Biol. **136**, 531-44.

Gerace, L. and Burke, B. (1988) Functional organization of the nuclear envelope. Annu Rev Cell Biol. **4**, 335-74.

Gerasimenko, J. V., Maruyama, Y., Yano, K., Dolman, N. J., Tepikin, A. V., Petersen, O. H. and Gerasimenko, O. V. (2003) NAADP mobilizes Ca2+ from a thapsigargin-sensitive store in the nuclear envelope by activating ryanodine receptors. J Cell Biol. **163**, 271-82.

Gerasimenko, O. and Gerasimenko, J. (2004) New aspects of nuclear calcium signalling. J Cell Sci. **117**, 3087-94.

*Gerasimenko, O. V., Gerasimenko, J. V., Tepikin, A. V. and Petersen, O. H. (1995) ATP-dependent accumulation and inositol trisphosphate- or cyclic ADP-ribose-mediated release of Ca2+ from the nuclear envelope. Cell. **80**, 439-444.

Gobeil, F., Fortier, A., Zhu, T., Bossolasco, M., Leduc, M., Grandbois, M., Heveker, N., Bkaily, G., Chemtob, S. and Barbaz, D. (2006) G-protein-coupled receptors signalling at the cell nucleus: an emerging paradigm. Can J Physiol Pharmacol. **84**, 287-97.

*Gomes, D. A., Rodrigues, M. A., Leite, M. F., Gomez, M. V., Varnai, P., Balla, T., Bennett, A. M. and Nathanson, M. H. (2008) c-Met Must Translocate to the Nucleus to Initiate Calcium Signals. J Biol Chem. **283**, 4344-51.

Greber, U. F. and Gerace, L. (1995) Depletion of calcium from the lumen of endoplasmic reticulum reversibly inhibits passive diffusion and signal-mediated transport into the nucleus. J Cell Biol. **128**, 5-14.

Guatimosim, S., Amaya, M. J., Guerra, M. T., Aguiar, C. J., Goes, A. M., Gomez-Viquez, N. L., Rodrigues, M. A., Gomes, D. A., Martins-Cruz, J., Lederer, W. J. and Leite, M. F. (2008) Nuclear Ca(2+) regulates cardiomyocyte function. Cell Calcium. **44**, 230-42.

Hennager, D. J., Welsh, M. J. and Delisle, S. (1995) Changes in either cytosolic or nucleoplasmic inositol 1,4,5-trisphosphate levels can control nuclear Ca2+ concentration. J Biol Chem. **270**, 4959-4962.

Hetzer, M. W. (2010) The nuclear envelope. Cold Spring Harb Perspect Biol. **2**, a000539.

Higazi, D. R., Fearnley, C. J., Drawnel, F. M., Talasila, A., Corps, E. M., Ritter, O., McDonald, F., Mikoshiba, K., Bootman, M. D. and Roderick, H. L. (2009) Endothelin-1-stimulated InsP3-induced Ca2+ release is a nexus for hypertrophic signaling in cardiac myocytes. Mol Cell. **33**, 472-82.

Huh, Y. H., Huh, S. K., Chu, S. Y., Kweon, H. S. and Yoo, S. H. (2006) Presence of a putative vesicular inositol 1,4,5-trisphosphate-sensitive nucleoplasmic ca(2+) store. Biochemistry. **45**, 1362-73.

*Irvine, R. F. (2003) Nuclear lipid signalling. Nat Rev Mol Cell Biol. **4**, 349-61.

Irvine, R. F. (2006) Nuclear inositide signalling -- expansion, structures and clarification. Biochim Biophys Acta. **1761**, 505-8.

Johnson, N., Krebs, M., Boudreau, R., Giorgi, G., LeGros, M. and Larabell, C. (2003) Actin-filled nuclear invaginations indicate degree of cell de-differentiation. Differentiation. **71**, 414-24.

*Jong, Y. J., Kumar, V., Kingston, A. E., Romano, C. and O'Malley, K. L. (2005) Functional metabotropic glutamate receptors on nuclei from brain and primary cultured striatal neurons. Role of transporters in delivering ligand. J Biol Chem. **280**, 30469-80.

Kockskamper, J., Seidlmayer, L., Walther, S., Hellenkamp, K., Maier, L. S. and Pieske, B. (2008) Endothelin-1 enhances nuclear Ca2+ transients in atrial myocytes through Ins(1,4,5)P3-dependent Ca2+ release from perinuclear Ca2+ stores. J Cell Sci. **121**, 186-95.

*Kumar, V., Jong, Y. J. and O'Malley, K. L. (2008) Activated nuclear metabotropic glutamate receptor mGlu5 couples to nuclear Gq/11 proteins to generate inositol 1,4,5-trisphosphate-mediated nuclear Ca2+ release. J Biol Chem. **283**, 14072-83.

Kume, S., Muto, A., Aruga, J., Nakagawa, T., Michikawa, T., Furuichi, T., Nakade, S., Okano, H. and Mikoshiba, K. (1993) The Xenopus IP(3) Receptor - Structure, Function, and Localization in Oocytes and Eggs. Cell. **73**, 555-570.

Kusnier, C., Cardenas, C., Hidalgo, J. and Jaimovich, E. (2006) Single-channel recording of inositol trisphosphate receptor in the isolated nucleus of a muscle cell line. Biol Res. **39**, 541-553.

Laflamme, K., Domingue, O., Guillemette, B. I. and Guillemette, G. (2002) Immunohistochemical localization of type 2 inositol 1,4,5-trisphosphate receptor to the nucleus of different mammalian cells. J Cell Biochem. **85**, 219-28.

Langevin, H. M., Storch, K. N., Snapp, R. R., Bouffard, N. A., Badger, G. J., Howe, A. K. and Taatjes, D. J. (2010) Tissue stretch induces nuclear remodeling in connective tissue fibroblasts. Histochem Cell Biol. **133**, 405-15.

Lanini, L., Bachs, O. and Carafoli, E. (1992) The Calcium Pump of the Liver Nuclear Membrane Is Identical to That of Endoplasmic Reticulum. J Biol Chem. **267**, 11548-11552.

Lee, M. G., Xu, X., Zeng, W. Z., Diaz, J., Kuo, T. H., Wuytack, F., Racymaekers, L. and Muallem, S. (1997a) Polarized expression of Ca2+ pumps in pancreatic and salivary gland cells - Role in initiation and propagation of [Ca2+](i) waves. J Biol Chem. **272**, 15771-15776.

Lee, M. G., Xu, X., Zeng, W. Z., Diaz, J., Wojcikiewicz, R. J. H., Kuo, T. H., Wuytack, F., Racymaekers, L. and Muallem, S. (1997b) Polarized expression of Ca2+ channels in pancreatic and salivary gland cells - Correlation with initiation and propagation of [Ca2+](i) waves. J Biol Chem. **272**, 15765-15770.

*Leite, M. F., Thrower, E. C., Echevarria, W., Koulen, P., Hirata, K., Bennett, A. M., Ehrlich, B. E. and Nathanson, M. H. (2003) Nuclear and cytosolic calcium are regulated independently. Proc Natl Acad Sci U S A. **100**, 2975-80.

Levine, T. and Rabouille, C. (2005) Endoplasmic reticulum: one continuous network compartmentalized by extrinsic cues. Curr Opin Cell Biol. **17**, 362-8.

*Lipp, P., Thomas, D., Berridge, M. J. and Bootman, M. D. (1997) Nuclear calcium signalling by individual cytoplasmic calcium puffs. EMBO J. **16**, 7166-7173.

Lui, P. P., Chan, F. L., Suen, Y. K., Kwok, T. T. and Kong, S. K. (2003) The nucleus of HeLa cells contains tubular structures for Ca(2+) signaling with the involvement of mitochondria. Biochem Biophys Res Commun. **308**, 826-33.

Lui, P. P. Y., Kong, S. K., Fung, K. P. and Lee, C. Y. (1998a) The rise of nuclear and cytosolic Ca2+ can be uncoupled in HeLa cells. Pflugers Arch. **436**, 371-376.

*Lui, P. P. Y., Kong, S. K., Kwok, T. T. and Lee, C. Y. (1998b) The nucleus of HeLa cell contains tubular structures for Ca2+ signalling. Biochem Biophys Res Commun. **247**, 88-93.

Luo, D., Yang, D., Lan, X., Li, K., Li, X., Chen, J., Zhang, Y., Xiao, R. P., Han, Q. and Cheng, H. (2008) Nuclear Ca(2+) sparks and waves mediated by inositol 1,4,5-trisphosphate receptors in neonatal rat cardiomyocytes. Cell Calcium. **43**, 165-74.

*Mak, D. O. D. and Foskett, J. K. (1994) Single-channel inositol 1,4,5-trisphosphate receptor currents revealed by patch clamp of isolated Xenopus oocyte nuclei. J Biol Chem. **269**, 29375-29378.

*Malhas, A., Goulbourne, C. and Vaux, D. J. (2011) The nucleoplasmic reticulum: form and function. Trends Cell Biol. **21**, 362-73.

Malviya, A. N., Rogue, P. and Vincendon, G. (1990) Stereospecific Inositol 1,4,5-<P-32>Trisphosphate Binding to Isolated Rat Liver Nuclei - Evidence for Inositol Trisphosphate Receptor-Mediated Calcium Release from the Nucleus. Proc Natl Acad Sci USA. **87**, 9270-9274.

Marchenko, S. M., Yarotskyy, V. V., Kovalenko, T. N., Kostyuk, P. G. and Thomas, R. C. (2005) Spontaneously active and InsP3-activated ion channels in cell nuclei from rat cerebellar Purkinje and granule neurones. J Physiol. **565**, 897-910.

Marius, P., Guerra, M. T., Nathanson, M. H., Ehrlich, B. E. and Leite, M. F. (2006) Calcium release from ryanodine receptors in the nucleoplasmic reticulum. Cell Calcium. **39**, 65-73.

Meldolesi, J. and Pozzan, T. (1998) The endoplasmic reticulum Ca2+ store: a view from the lumen. Trends in Biochem Scie. **23**, 10-14.

*Mellstrom, B., Savignac, M., Gomez-Villafuertes, R. and Naranjo, J. R. (2008) Ca2+-operated transcriptional networks: molecular mechanisms and in vivo models. Physiol Rev. **88**, 421-49.

Mikoshiba, K. (2007) IP(3) receptor/Ca(2+) channel: from discovery to new signaling concepts. J Neurochem. **102**, 1426-46.

Molkentin, J. D. (2006) Dichotomy of Ca2+ in the heart: contraction versus intracellular signaling. J Clin Invest. **116**, 623-6.

Moore-Nichols, D., Arnott, A. and Dunn, R. C. (2002) Regulation of Nuclear Pore Complex Conformation by IP(3) Receptor Activation. Biophys J. **83**, 1421-8.

Nakanishi, S., Fujii, A., Nakade, S. and Mikoshiba, K. (1996) Immunohistochemical localization of inositol 1,4,5-trisphosphate receptors in non-neural tissues, with special reference to epithelia, the reproductive system, and muscular tissues. Cell Tissue Res. **285**, 235-251.

Nalaskowski, M. M., Fliegert, R., Ernst, O., Brehm, M. A., Fanick, W., Windhorst, S., Lin, H., Giehler, S., Hein, J., Lin, Y. N. and Mayr, G. W. (2011) Human Inositol 1,4,5-

Trisphosphate 3-Kinase Isoform B (IP3KB) Is a Nucleocytoplasmic Shuttling Protein Specifically Enriched at Cortical Actin Filaments and at Invaginations of the Nuclear Envelope. J Biol Chem. **286**, 4500-10.

*Nicotera, P., Mcconkey, D. J., Jones, D. P. and Orrenius, S. (1989) ATP Stimulates Ca-2+ Uptake and Increases the Free Ca-2+ Concentration in Isolated Rat Liver Nuclei. Proc Natl Acad Sci USA. **86**, 453-457.

Nicotera, P., Orrenius, S., Nilsson, T. and Berggren, P. O. (1990) An Inositol 1,4,5-Trisphosphate-Sensitive Ca-2+ Pool in Liver Nuclei. Proc Natl Acad Sci USA. **87**, 6858-6862.

O'Brien, E. M., Gomes, D. A., Sehgal, S. and Nathanson, M. H. (2007) Hormonal regulation of nuclear permeability. J Biol Chem. **282**, 4210-7.

O'Malley, D. M. (1994) Calcium permeability of the neuronal nuclear envelope: Evaluation using confocal volumes and intracellular perfusion. Journal of Neuroscience. **14**, 5741-5758.

O'Malley, K. L., Jong, Y. J., Gonchar, Y., Burkhalter, A. and Romano, C. (2003) Activation of metabotropic glutamate receptor mGlu5 on nuclear membranes mediates intranuclear Ca2+ changes in heterologous cell types and neurons. J Biol Chem. **278**, 28210-9.

*Papp, S., Dziak, E., Michalak, M. and Opas, M. (2003) Is all of the endoplasmic reticulum created equal? The effects of the heterogeneous distribution of endoplasmic reticulum Ca2+-handling proteins. J Cell Biol. **160**, 475-9.

PerezTerzic, C., Pyle, J., Jaconi, M., StehnoBittel, L. and Clapham, D. E. (1996) Conformational states of the nuclear pore complex induced by depletion of nuclear Ca2+ stores. Science. **273**, 1875-1877.

Petersen, O. H., Gerasimenko, O. V., Gerasimenko, J. V., Mogami, H. and Tepikin, A. V. (1998) The calcium store in the nuclear envelope. Cell Calcium. **23**, 87-90.

Prunuske, A. J. and Ullman, K. S. (2006) The nuclear envelope: form and reformation. Curr Opin Cell Biol. **18**, 108-16.

Pusl, T., Wu, J. J., Zimmerman, T. L., Zhang, L., Ehrlich, B. E., Berchtold, M. W., Hoek, J. B., Karpen, S. J., Nathanson, M. H. and Bennett, A. M. (2002) Epidermal growth factor-mediated activation of the ETS domain transcription factor Elk-1 requires nuclear calcium. J Biol Chem. **277**, 27517-27.

Queisser, g., Wieger, S. and Bading, H. (2011) Structural dynamics of the cell nucleus: Basis for morphology modulation of nuclear calcium signaling and gene transcription. Nucleus. **2**, 1-7.

*Rizzuto, R. and Pozzan, T. (2006) Microdomains of intracellular Ca2+: molecular determinants and functional consequences. Physiol Rev. **86**, 369-408.

Rodrigues, M. A., Gomes, D. A., Andrade, V. A., Leite, M. F. and Nathanson, M. H. (2008) Insulin induces calcium signals in the nucleus of rat hepatocytes. Hepatology. **48**, 1621-31.

*Rodrigues, M. A., Gomes, D. A., Leite, M. F., Grant, W., Zhang, L., Lam, W., Cheng, Y. C., Bennett, A. M. and Nathanson, M. H. (2007) Nucleoplasmic calcium is required for cell proliferation. J Biol Chem. **282**, 17061-8.

Sarma, A. and Yang, W. (2011) Calcium regulation of nucleocytoplasmic transport. Protein Cell. **2**, 291-302.

Schermelleh, L., Carlton, P. M., Haase, S., Shao, L., Winoto, L., Kner, P., Burke, B., Cardoso, M. C., Agard, D. A., Gustafsson, M. G., Leonhardt, H. and Sedat, J. W. (2008) Subdiffraction multicolor imaging of the nuclear periphery with 3D structured illumination microscopy. Science. **320**, 1332-6.

*Schirmer, E. C. and Gerace, L. (2005) The nuclear membrane proteome: extending the envelope. Trends Biochem Sci. **30**, 551-8.

Shen, W. W., Frieden, M. and Demaurex, N. (2011) Remodelling of the endoplasmic reticulum during store-operated calcium entry. Biol Cell. **103**, 365-80.

*Shirakawa, H. and Miyazaki, S. (1996) Spatiotemporal analysis of calcium dynamics in the nucleus of hamster oocytes. J Physiol. **494**, 29-40.

Siefjediers, A., Hardt, M., Prinz, G. and Diener, M. (2007) Characterization of inositol 1,4,5-trisphosphate (IP3) receptor subtypes at rat colonic epithelium. Cell Calcium. **41**, 303-15.

Stehnobittel, L., Luckhoff, A. and Clapham, D. E. (1995a) Calcium release from the nucleus by InsP(3) receptor channels. Neuron. **14**, 163-167.

Stehnobittel, L., Perezterzic, C. and Clapham, D. E. (1995b) Diffusion across the nuclear envelope inhibited by depletion of the nuclear Ca2+ store. Science. **270**, 1835-1838.

Stoffler, D., Schwarz-Herion, K., Aebi, U. and Fahrenkrog, B. (2006) Getting across the nuclear pore complex: new insights into nucleocytoplasmic transport. Can J Physiol Pharmacol. **84**, 499-507.

*Subramanian, K. and Meyer, T. (1997) Calcium-induced restructuring of nuclear envelope and endoplasmic reticulum calcium stores. Cell. **89**, 963-971.

Tadevosyan, A., Maguy, A., Villeneuve, L. R., Babin, J., Bonnefoy, A., Allen, B. G. and Nattel, S. (2010) Nuclear-delimited angiotensin receptor-mediated signaling regulates cardiomyocyte gene expression. J Biol Chem. **285**, 22338-49.

*Terasaki, M. and Jaffe, L. A. (1991) Organization of the sea urchin egg endoplasmic reticulum and its reorganization at fertilization. Journal of Cell Biology. **114**, 929-940.

Thery, M. (2010) Micropatterning as a tool to decipher cell morphogenesis and functions. J Cell Sci. **123**, 4201-4213.

*Torok, K. (2007) The regulation of nuclear membrane permeability by Ca2+ signaling: a tightly regulated pore or a floodgate? Sci STKE. **2007**, pe24.

*Vermassen, E., Parys, J. B. and Mauger, J. P. (2004) Subcellular distribution of the inositol 1,4,5-trisphosphate receptors: functional relevance and molecular determinants. Biol Cell. **96**, 3-17.

Vermassen, E., Van Acker, K., Annaert, W. G., Himpens, B., Callewaert, G., Missiaen, L., De Smedt, H. and Parys, J. B. (2003) Microtubule-dependent redistribution of the type-1 inositol 1,4,5- trisphosphate receptor in A7r5 smooth muscle cells. J Cell Sci. **116**, 1269-77.

Visnjic, D. and Banfic, H. (2007) Nuclear phospholipid signaling: phosphatidylinositol-specific phospholipase C and phosphoinositide 3-kinase. Pflugers Arch. **455**, 19-30.

*Voeltz, G. K., Rolls, M. M. and Rapoport, T. A. (2002) Structural organization of the endoplasmic reticulum. EMBO Rep. **3**, 944-50.

Wei, X., Henke, V. G., Strubing, C., Brown, E. B. and Clapham, D. E. (2003) Real-time imaging of nuclear permeation by EGFP in single intact cells. Biophys J. **84**, 1317-27.

*Wells, A. and Marti, U. (2002) Signalling shortcuts: cell-surface receptors in the nucleus? Nat Rev Mol Cell Biol. **3**, 697-702.

*Wittmann, M., Queisser, G., Eder, A., Wiegert, J. S., Bengtson, C. P., Hellwig, A., Wittum, G. and Bading, H. (2009) Synaptic activity induces dramatic changes in the geometry of the cell nucleus: interplay between nuclear structure, histone H3 phosphorylation, and nuclear calcium signaling. J Neurosci. **29**, 14687-700.

*Wu, X., Zhang, T., Bossuyt, J., Li, X., McKinsey, T. A., Dedman, J. R., Olson, E. N., Chen, J., Brown, J. H. and Bers, D. M. (2006) Local InsP3-dependent perinuclear Ca2+ signaling in cardiac myocyte excitation-transcription coupling. J Clin Invest. **116**, 675-82.

Wuytack, F., Raeymaekers, L. and Missiaen, L. (2002) Molecular physiology of the SERCA and SPCA pumps. Cell Calcium. **32**, 279-305.

Xie, X., Wu, G., Lu, Z. H. and Ledeen, R. W. (2002) Potentiation of a sodium-calcium exchanger in the nuclear envelope by nuclear GM1 ganglioside. J Neurochem. **81**, 1185-95.

Yoo, S. H., Nam, S. W., Huh, S. K., Park, S. Y. and Huh, Y. H. (2005) Presence of a nucleoplasmic complex composed of the inositol 1,4,5-trisphosphate receptor/ca(2+) channel, chromogranin B, and phospholipids. Biochemistry. **44**, 9246-54.

Yule, D. I., Ernst, S. A., Ohnishi, H. and Wojcikiewicz, R. J. H. (1997) Evidence that zymogen granules are not a physiologically relevant calcium pool - Defining the distribution of inositol 1,4,5-trisphosphate receptors in pancreatic acinar cells. J Biol Chem. **272**, 9093-9098.

*Zima, A. V., Bare, D. J., Mignery, G. A. and Blatter, L. A. (2007) IP3-dependent nuclear Ca2+ signalling in the mammalian heart. J Physiol. **584**, 601-11.

Figure legends

Figure 1

Polarised MDCK cells develop invaginations in the nuclear envelope.

MDCK cells were plates at 10^4 cells/ cm^2 and cultured for 12 to 15 days. (Left): Epifluorescence image of polarised MDCK cells stained with antibodies against emerin, a protein associated with the inner nuclear membrane. The antibodies reveal the presence of invaginations of the nuclear envelope. (Middle): Electron micrograph of polarised MDCK cells shows the narrow invaginations of the nuclear envelope which penetrate deeply the nucleus. (Right): Observations at high magnification of the inset in the middle panel revealed that both the inner and the outer membrane of the nuclear envelope form the invaginations. The presence of a nuclear pore (NP) in invaginations is indicated by the arrow.

Figure 2

Summary of the different mechanisms that regulate nuclear Ca^{2+} concentration.

Ca^{2+} is accumulated from the cytoplasm into the NE by the Ca^{2+} pump (SERCA) localised in the outer nuclear membrane (ONM). Ca^{2+} can be released by IP_3 either through the inner nuclear membrane (INM) directly to the nucleoplasm or through the ONM to the cytoplasm and secondly through the nuclear pore complex (NPC) to the nucleoplasm. The presence of invaginations of the nuclear envelope generate cytosolic microdomains where the Ca^{2+} concentration may be much higher than in the bulk cytosol, this can favour the transfer of Ca^{2+} from the cytoplasm to the nucleoplasm. The local production of IP_3 in the nucleus is indicated.

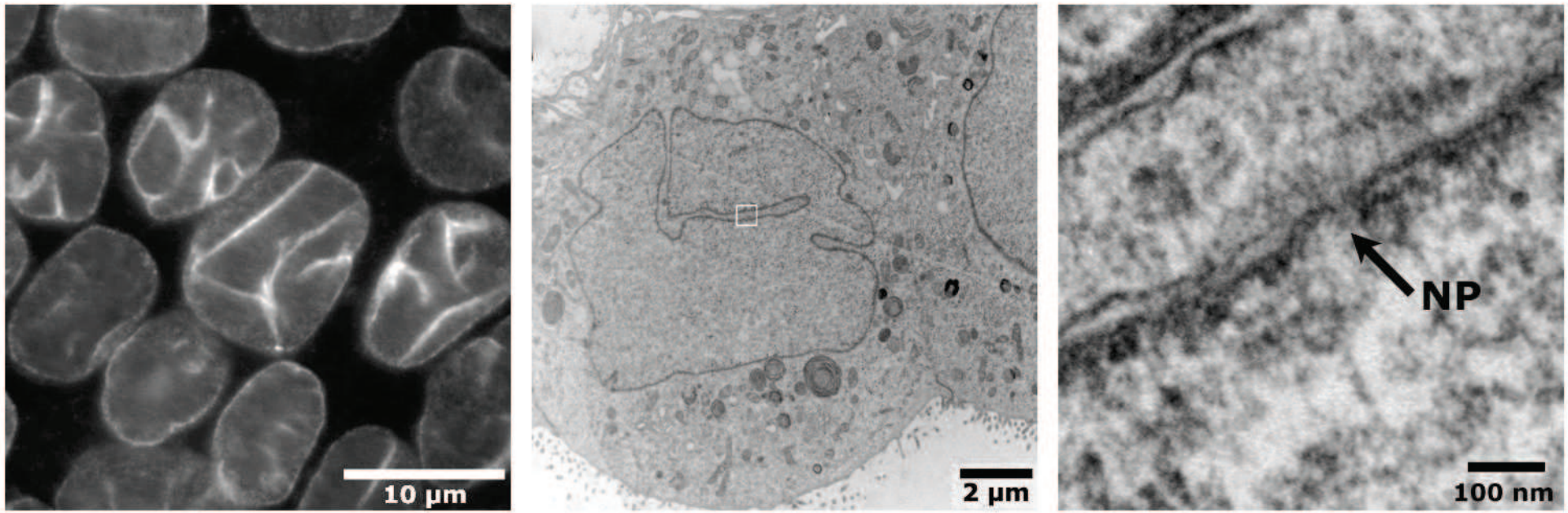

Fig. 1

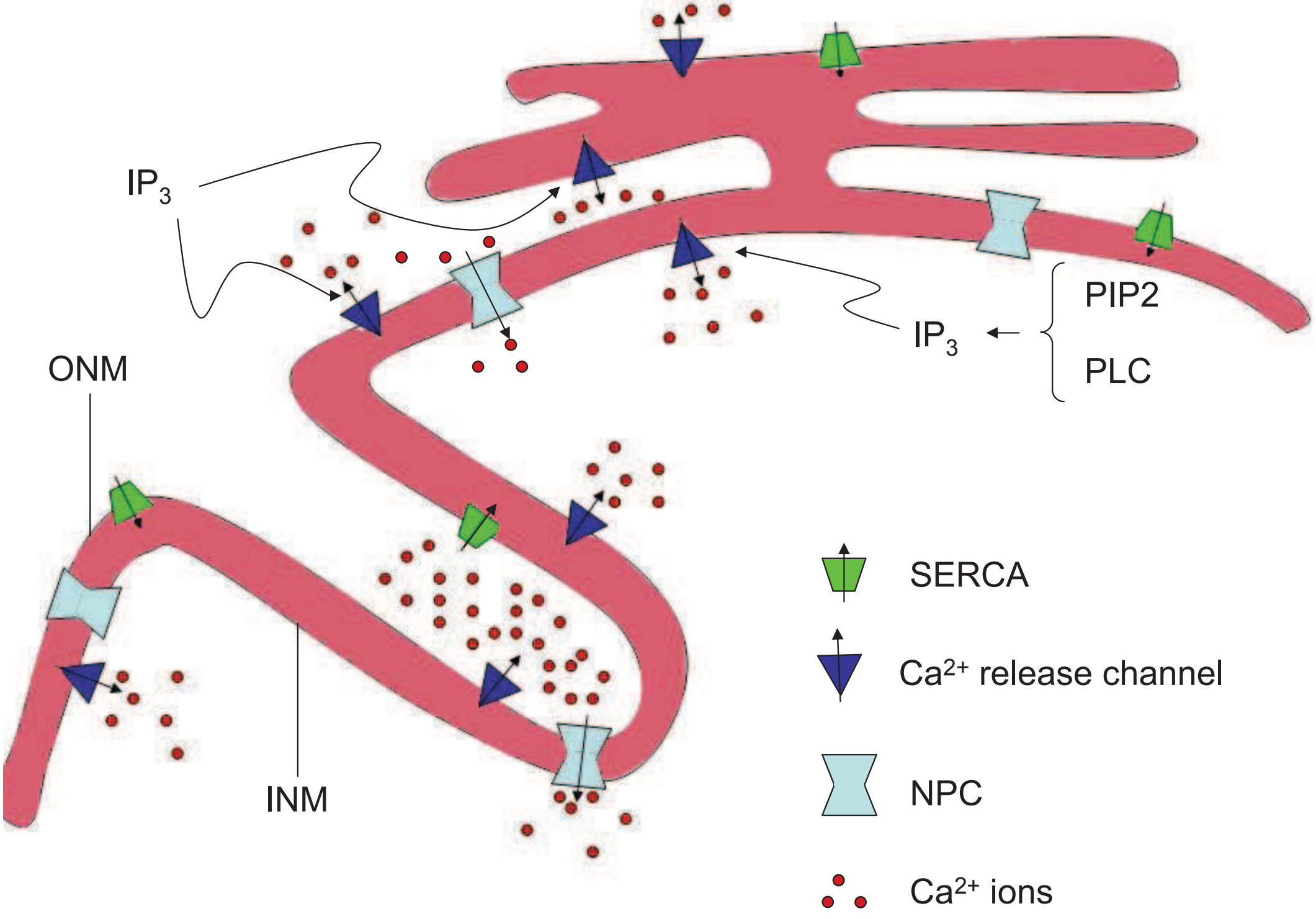
IP3
IP3
PIP2
PLC
ONM
INM
SERCA
Ca2+ release channel
NPC
Ca2+ ions

Fig. 2