

REVIEW

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PATHWAYS AND MECHANISMS OF TRANSMEMBRANE CALCIUM IONS EXCHANGE IN THE CELL NUCLEUS

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A variety of nuclear calcium functions includes regulations of gene transcription and permeability of nuclear pore complexes, activation of numerous enzymes involved in the apoptosis, and therefore cell fate determination. Thus, the pathways of Ca^{2+} entry into the nucleus, the interplay between nuclear and cytosolic calcium signals, calcium accumulation in the nuclear depot, extrusion and subsequent destinies are of particular interest. In this review, we systematized literature data and our results about aspects of calcium transport in the cell. We especially concentrated on calcium transport systems and general mechanisms of calcium exchange in the central organelle – the nucleus. We also described the general structure of the cell nucleus, nuclear envelope transporters, and the role of Ca^{2+} in the nucleus and disturbances of nuclear calcium signaling.

Key words: nucleus, nucleoplasm, nuclear envelope, nuclear transporters, calcium signaling.

Calcium ions (Ca^{2+}) play an important role in regulation of many biochemical, biophysical and physiological processes, acting as cytoplasmic signal transducers that transmit information from the plasma membrane to intracellular structures [1-7]. Changes in the Ca^{2+} concentration affect virtually all aspects of cellular life from birth to death. Ca^{2+} is an essential cellular messenger. Ca^{2+} signaling pathways coordinate a wide range of cellular processes from fertilization, proliferation, secretion of neurotransmitters and other substances, regulation of activity of different enzymes, muscle contraction, neurotransmission, cell growth to apoptosis or necrosis [8-13]. The mechanisms of Ca^{2+} exchange in the cytosol, on the one hand, and in the endoplasmic reticulum and mitochondria as calcium depots [14-17], on the other hand, have been well studied. However, there is great interest in the understanding the pathways and effects of changes in the intranuclear Ca^{2+} concentration, since it has been found that fluctuation in the level of Ca^{2+} in the nucleoplasm is likely to control the activation of nuclear transcription factors, breakdown of the nuclear

membrane during cell division, and apoptosis [18]. Furthermore, Ca^{2+} transport systems and channels involved in the Ca^{2+} redistribution within a cell were found to exist in nuclear envelope [19]. The European conference on Ca^{2+} signaling in the cell nucleus, held in Baia Paraellios, Calabria, Italy in 1997, was a springboard for summarization of existing numerous, sometimes polar theories and experimental data related to transmembrane Ca^{2+} exchange and Ca^{2+} -signaling in the cell nucleus [20].

Published data on the calcium exchange in the nucleus are contradictory and limited, though it is known that the nuclear envelope contains its own system of signal transduction and generation of secondary messengers, and the nucleus lumen may serve as a Ca^{2+} depot. Moreover, there is a reason to consider the Ca^{2+} exchange processes in the nucleus, cytosol, and endoplasmic reticulum as interconnected and integrated processes. All this has generated considerable interest in the nucleus as an equally important player in the processes of intracellular Ca^{2+} exchange.

I. General concepts of the Ca^{2+} level, localization, and distribution in the cell

As it was noted earlier, Ca^{2+} is an important signal messenger; changes in its concentration induced by various factors regulate many cellular functions. Minor changes in cytoplasmic Ca^{2+} affect enzyme activities, initiate signaling cascades, cause muscle contraction, nerve impulse transmission, or secretion. Hence, there is a strict control of cytoplasmic Ca^{2+} , whose concentration in the resting state of the cell does not exceed 50-300 nM. The main Ca^{2+} store is the lumen of the endoplasmic reticulum (ER), where the Ca^{2+} concentration is substantially higher – 100-500 μM . Ca^{2+} release from the ER occurs through two types of channels: ryanodine and IP_3 -sensitive [21]. Other vital subcellular structures that can accumulate Ca^{2+} are mitochondria. At rest, the concentration of Ca^{2+} in mitochondria is not high – about 200 nM [22]. However, due to the close association of mitochondria with the sites of Ca^{2+} release into the cytosol, the mitochondria serve as a buffer system and can accumulate Ca^{2+} to a level of 10-100 μM , whereas the concentration of cytoplasmic Ca^{2+} can increase to 2-3 μM [23]. The highest cellular Ca^{2+} concentration was detected in plant vacuoles, where this ion is essential for the balance of organic anions. In some cases, its level in these compartments can reach several mM [24].

The idea that nuclear Ca^{2+} and cytosolic Ca^{2+} are regulated independently emerged soon after the development of new technologies (confocal microscopy), which enable to monitor Ca^{2+} changes in several compartments simultaneously. Electron probe microanalysis [25] allowed comparing the Ca^{2+} concentrations in different cell compartments of one sample of myocytes. Thus, the Ca^{2+} concentration in peripheral cytosol was 570 μM (this value increased to 840 μM upon mechanical stretch); in the central cytosol – 400 μM (increased to 840 μM). It is interesting to note that the Ca^{2+} concentration in the nucleus was 180 μM (increased to 300 μM), in the nuclear envelope – 933 μM (increased to 1530 μM). Such results allowed suggesting that the nuclear envelope performs the function of a barrier for Ca^{2+} . Furthermore, high Ca^{2+} concentration in the lumen of nuclear envelope indicates its high Ca^{2+} capacity and the ability to store Ca^{2+} to increase the Ca^{2+} level in the nucleoplasm. The difference in the increases in Ca^{2+} concentrations in nucleus and cytoplasm can be explained either by the buffering

property of the cytoplasm or by the mechanism of independent regulation of the Ca^{2+} permeability by the nuclear membrane [26]. According to other data, the Ca^{2+} concentration in the lumen of the nucleus is close to that in the lumen of the endoplasmic reticulum and in the resting state is found to be 100-300 μM . The Ca^{2+} concentration in the nucleoplasm is approximately 100 nM and corresponds to the cytoplasmic level [27].

Using aequorin as a Ca^{2+} sensor in a cell resting state it was shown that there was no difference between the Ca^{2+} concentration in the cytoplasm and the nucleus. However, under the influence of IP_3 , the Ca^{2+} accumulation in the nucleus and the formation of the Ca^{2+} gradient between the nucleus and the cytoplasm were observed [18, 28]. It might occur due to the Ca^{2+} release from the nuclear envelope through the IP_3 channels localized in the inner nuclear membrane. Some signaling events were shown to occur without an increase in the Ca^{2+} concentration in the cytosol, with an increase in the Ca^{2+} level in the nucleus solely, in particular, in oocyte during fertilization [18, 29]. Also, an increase in the cytoplasmic Ca^{2+} concentration is not necessarily accompanied by an increase in free nuclear Ca^{2+} , particularly when Ca^{2+} signaling occurs in the cytosol [26]. An increase in the cytoplasmic Ca^{2+} concentration under the influence of histamine, ATP, and ionomycin did not correlate with an increase in the concentration of nuclear Ca^{2+} . This indicates that in the nucleus there is an independent mechanism for Ca^{2+} regulation [26]. Thus, Ca^{2+} was shown to be a specific regulator of nuclear events, acting via Ca^{2+} binding proteins, such as calmodulin, calreticulin and calpain.

II. Functional role of Ca^{2+} in the nucleus

Changes in the Ca^{2+} concentration in the nucleus regulate basic nuclear functions, such as gene expression, and activate nuclear transcription factors. In addition, Ca^{2+} was shown to be involved in the development of apoptosis and a breakdown of the nuclear membrane [18].

Ca^{2+} is the central ion in many signal transduction cascades. Signaling cascades regulate gene expression through the stimulation of transcription factor translocation from the cytosol into the nucleus, or through translocation or activation of enzymes that regulate the transcription factor activity or the chromatin structure. An increase in the Ca^{2+} concentration in the nucleus may be not an indispensable factor for such events. However, it is necessary for the

initiation of gene transcription. For example, Ca^{2+} activates transcription through nuclear factor of activated T-cells (NFAT). This factor is typically located in the cytosol; however, in response to an increase in the cytoplasmic Ca^{2+} concentration, it translocates to the nucleus and is dephosphorylated by calcineurin. NFAT translocation to the nucleus occurs in the form of the calcineurin-NFAT complex, so, an increased nucleoplasmic Ca^{2+} concentration provides this association and, hence, maintains the transcriptional activity of NFAT.

The CREB factor (cAMP response element-binding protein) is an example of a protein that is regulated by both nuclear and cytoplasmic Ca^{2+} signals. Studies on neurons showed that depolarization leads to CREB phosphorylation by calmodulin-dependent kinase 4, which forms the signal that is necessary but not sufficient for CREB-dependent transcription. It was revealed that the level of the Ca^{2+} -calmodulin complex in the nucleus, which depends on the “history” of activation of NMDA receptors on the presynaptic neuronal membrane, is crucial for the activation of CREB and CRE site of gene promoters that promotes neuronal survival and a long-term neuroprotective effect [30]. In the nucleus, CREB binds additional proteins, and forms a transcriptionally active complex – this event requires an increase in the concentration of nuclear Ca^{2+} . The introduction of Ca^{2+} -buffer into the nucleus terminates CREB-dependent transcription. Moreover, an increase in the Ca^{2+} level in the nucleus was found to be essential for the activation of CBP (CREB-binding protein), which acts as a CREB co-activator [31].

Another interesting mechanism for the neuroprotective effect of the nuclear Ca^{2+} -calmodulin complex, in addition to CREB activation, is the modulation of the nuclear export of transcriptional co-repressors (SMRT, HDAC) and, as a result, activation of corresponding gene transcription. For example, it is known that the gene of neuroprotective myocyte enhancer factor-2 (MEF2) transcription factor is generally repressed by histone deacetylase 2 (HDAC2), and one of the ways of its activation is Ca^{2+} -calmodulin kinase-dependent HDAC2 export from the nucleus [32, 33]. Nuclear Ca^{2+} -calmodulin signaling is also noteworthy for its role in synaptic plasticity and the processes of long-term memory formation [34]. The genes regulated by nuclear Ca^{2+} level and involved in memory consolidation include VEGFD, Arc, Dnmt3a2, Homer1, Npas4, and nr4a1 [35].

The expression of two other genes, which encode synaptic cell adhesion molecules and regulate synaptic transmission and neuronal plasticity, also depends on Ca^{2+} transients in the nucleus. The products of these genes are leucine-rich repeat transmembrane proteins (Lrrtm1 and Lrrtm2), whose peak activity is observed on the day of birth. It should be noted that the promoters of the Lrrtm genes contain the CRE site, and hence, their activation via nuclear Ca^{2+} -calmodulin signaling pathway described above is evident [36].

The activity of the downstream antagonist regulatory element modulator (DREAM) is also known to be regulated by nuclear Ca^{2+} . This protein contains Ca^{2+} -binding EF motifs and is directly regulated by an increase in the nuclear Ca^{2+} concentration. At the low concentration of Ca^{2+} , DREAM represses the gene transcription by binding to DNA, and with the increased concentration of Ca^{2+} DREAM dissociates [37, 38].

In the experiments on acinar cells of the pancreas, it was found that an increase in the Ca^{2+} level in the cytoplasm and nucleoplasm under the influence of some agonists, e.g., cholecystokinin, caused an increase in the expression of immediate early genes (IEG): c-fos, c-jun, and c-myc. Changes in the expression level were observed at the concentration of extracellular Ca^{2+} 1 mM and were most pronounced at the concentration of 10 mM [39].

Ca^{2+} signals formed in the cytoplasm and the nucleoplasm can synergistically control critical cellular events. However, independent nuclear Ca^{2+} signals can regulate specific nuclear processes. For example, Ca^{2+} is involved in the regulation of nuclear pore permeability. Reduction of Ca^{2+} in the nuclear membrane leads to the protein influx through the nuclear pore. It is believed that there is a Ca^{2+} -sensor in the nuclear membrane, that is pore membrane protein gp210 (nucleoporin 210 kDa), which anchors the nuclear pore proteins. A significant amount of protein is localized in the nuclear pore lumen, where it can respond to changes in the Ca^{2+} concentration [40–42]. A decrease in the Ca^{2+} concentration under the influence of chelators prevents the energy-dependent import of histones and the energy-dependent transport of dextrin molecules [41, 43].

The localization of IP_3 receptors on the nuclear membrane enables the formation of the nuclear-specific Ca^{2+} signals (even though they are initiated in the cytosol) that might be essential to regulating the gene transcription. Similar channels were discovered

on cardiomyocytes, and such mechanism, along with periodic fluctuations in the cytosolic Ca^{2+} level, may be employed in the induction of cardiac hypertrophy [44].

An equally important function of nuclear Ca^{2+} , along with cytosolic Ca^{2+} , is regulation of apoptosis. This role of nuclear Ca^{2+} seems obvious, given the localization in the nucleus of the Ca^{2+} -dependent endonucleases and proteases involved in chromatin fragmentation and protein digestion during apoptosis [45]. It was shown that an increase in the Ca^{2+} level in the nucleoplasm correlates with the development of apoptosis as a result of the activation of these enzymes, whereas Ca^{2+} release from the nucleus is accompanied by elevated activity of anti-apoptotic Bcl-2 proteins [20].

Thus, changes in the nuclear Ca^{2+} concentration trigger activation of transcription factors (in particular their phosphorylation/dephosphorylation), their dissociation or binding to the corresponding DNA site, changes in the localization of the transcriptional repressors. However, the functional role of Ca^{2+} in the nucleus is not confined to the effect on gene transcription that generates increasing interest in the processes of ion exchange in the nucleus.

III. The functional organization of the cell nucleus

For a long time, the structural complexity of the nucleus was underestimated, and the nucleus was seen only as a repository of genetic information packed in chromatin fibrils that are freely distributed in the amorphous nucleoplasm. Since the nucleus does not contain a system of internal membranes (as in the cytoplasm), its compartmentalization was not considered at the time [46]. However, the development of electron microscopy and immunohistochemistry enabled to visualize distinct nuclear compartments [47, 48]. Thus, the discovery of different subnuclear compartments at the beginning of the 20th century can be considered the renaissance in research of nucleus hyperfine structure. Thus, in 1903, the neuroscientist Santiago Ramon Cajal first described the small argentophilic bodies associated with the nucleolus, which he named nucleolar accessory bodies, or coiled bodies [47]. In 1999, Joseph Gall renamed these structures Cajal bodies in honor of the discoverer [47, 49]. In 1910 Cajal gave a detailed description of another nuclear domain – interchromatin compartment – called nuclear speckles [48]. The existence of nucleolus within the nucleus

was reported in 1835. The monumental monograph published by Montgomery (1898) with remarkable 346 drawings of nuclei and nucleoli from different cell types is convincing evidence of existence of a large dense structure within the nucleus [48, 50]. However, details of the nucleolus structure and functions were revealed much later with the development of electron microscopy techniques.

According to modern views, a part of the nucleus, which is characterized by a subset of macromolecules and specialized functions, is considered a nuclear compartment [51, 47]. The nuclear compartments include nucleolus, chromosome territories, interchromatin domain, transcriptional sites and nuclear bodies (Cajal body, PML) [46, 47, 51-53] (Fig. 1).

The nucleolus, the most noticeable nuclear structure, is assembled around the rRNA genes and formed by ribonucleoproteins. However, the function of the nucleolus is not just restricted to the synthesis of rRNA and biogenesis of the ribosomes [48]. According to C. H. Waddington, the researcher of the nucleolus, the nucleolus should not be considered a relatively simple organelle with a single function, comparable to a particular part of an automobile. It is rather a whole production line than a single machine tool. Thus, the nucleolus is the site for rRNA processing with the involvement of small nucleolar RNAs and is also the site of formation of signal recognition particle RNA [48, 54]. The nucleolus is characterized by much higher protein/RNA ratio than the mature ribosome. It also contains proteins involved in DNA replication and repair, as well as cell cycle regulators [55]. For instance, nucleostemin, a protein which shuttles between the nucleolus and nucleoplasm, is found inside the nucleolus. While transporting to the nucleoplasm, it binds and inactivates p53 [56]. Besides, there is a small p53 pool within the nucleolus, which interacts with molecular partners other than those with which it usually forms the complex in the nucleoplasm [48].

IV. Ion exchange systems in the nuclear membrane

Recent reported data have indicated that the nuclear envelope is involved in a wide variety of cellular processes ranged from the transport of macromolecules through nuclear pore complexes to signal transduction, cell cycle regulation, and regulation of embryonic and postembryonic development [35, 57, 58]. Despite the obvious role of the nuclear en-

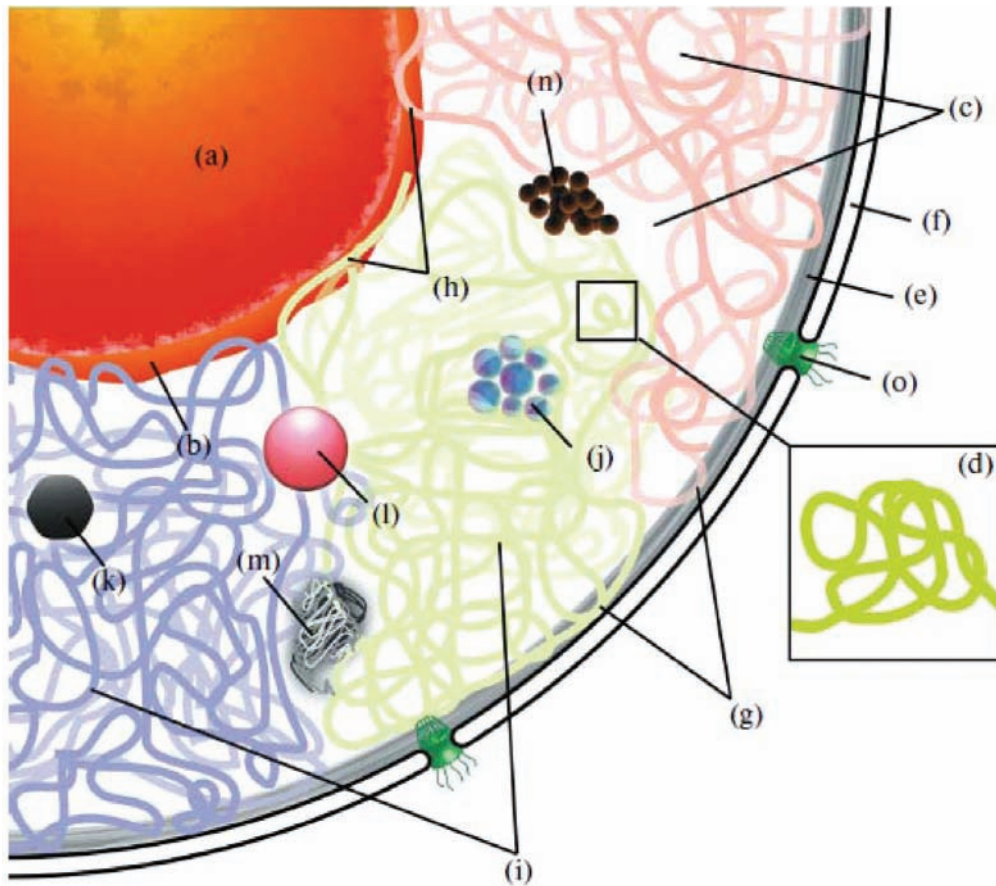


Fig. 1. Structure of the cell nucleus with the indication of all known nuclear compartments [51]: (a) – nucleolus, (b) – perinuclear space, (c) – interchromatin domain, (d) – topologically associated domain, (e) – lamina, (f) – nuclear envelope, (g) – lamina associated domains, (h) – nucleolus associated domains, (i) – chromosome territories, (j) – Polycomb body, (k) – insulator body, (l) – promyelocytic leukemia oncoprotein body (PML body), (m) – Cajal body, (n) – nuclear speckles, (o) – nuclear pore complex

velope in the cell functioning, some aspects, including the electrophysiological properties of the double membrane, remain poorly understood. The reasons for the limited knowledge about this essential nuclear structure are the following. First, researchers for a long time focused on the study of nuclear envelope proteins involved in the transport of macromolecules and proteins associated with chromatin [57]. Second, gaps in the understanding of "nuclear electrophysiology" are associated with difficulties in purifying of the nuclear envelope from the endoplasmic reticulum membranes. In intact cells and isolated nuclei, the inner nuclear membrane is inaccessible for patch clamp pipette that complicates electrophysiological studies. In addition, methods for separating the inner nuclear membrane from the outer one have not been developed yet [57].

The features of the nuclear-cytoplasmic Ca^{2+} transport may account for the structural peculiarities of the nuclear envelope, which has an inner and outer membranes with the perinuclear space between them. The nuclear envelope is usually 30-50 nm wide in somatic cells. The inner and outer nuclear membranes differ in structure and function. The outer membrane is continuous with the endoplasmic reticulum membrane, so the perinuclear space is connected with the lumen of the endoplasmic reticulum [48, 57]. In the link sites of inner and outer membranes, the nuclear envelope has many nuclear pore complexes that are supramolecular structures responsible for preserving the integrity of the double nuclear membrane and for the transport of molecules between the cytoplasm and the nucleoplasm [19, 59, 60].

The most well-studied nuclear transport structures are nuclear pore complexes (NPC) that cross the double nuclear membrane. The molecular mass of the NPC is about 10^8 Da; the diameter is about 120 nm. A three-dimensional analysis of the NPC structure revealed that it has octagonal rotational symmetry around large central channel (diameter of 40-70 nm) surrounded by eight small peripheral channels (diameter of 10 nm (in the open state)) [61, 62]. Interestingly, this multi-protein complex is involved in the nuclear-cytoplasmic transport of not only macromolecules (RNA, proteins) but also ions [37]. However, the question remains: whether this transport is regulated, or it occurs via passive ion diffusion? By recently, researchers who sought to establish whether the NPC provides for constitutive ion conductivity, or it remains, for some time, in a closed state, obtained discrepant results depending on the electrophysiological approach [57, 63, 64]. It is believed that the NPCs transport ions steadily (at least through peripheral channels if the central channel is partially blocked [57]), but their conductivity can vary depending on the effect of such factors as the concentrations of Ca^{2+} and ATP [65]. The NPC is likely to serve as a mechanism for a delay in equilibration between the cytoplasmic and the nucleoplasmic Ca^{2+} concentration.

Early electron microscopic studies revealed that the NPC is formed by about 100 proteins [57]. Later, the proteomic analysis of yeast and mammalian NPCs, however, disproved the existence of such a large number of proteins, and demonstrated that the NPC contains approximately 30 different core proteins that have numerous copies [66]. It is interesting to note that the NPCs from all eukaryotes have common structure indicating the conservatism of the nuclear pore components organisation. Electron microscopy analysis established that the NPC has an octagonal symmetry and spoke wheel-shaped architecture. The core structure, embedded in the nuclear envelope, is formed by the nucleoporin proteins (Nup) and bounded by cytoplasmic and nuclear rings with attached filaments whose ends are attached to the terminal ring [20, 37]. Nup proteins play a crucial role in the formation of the central channel through which active molecule transport occurs. The permeability limit of this transport machinery was determined by studying the transport of fluorescently labeled dextrans of different sizes and gold particles. It was reported that molecules larger than 40 kD and 5 nm could not permeate the

NPC [48, 67]. So, the molecules of water, small metabolites, and metal ions can freely diffuse through a nuclear pore. The transport of macromolecules (>40 kDa) through the NPC is highly selective, since only specific molecules that contain nuclear transport signals, namely nuclear export signal (NES) or nuclear localization signal (NLS) can pass across this barrier. It was shown that a cargo up to 39 nm (tRNA, some proteins, ribosomal subunits, viral particles) can be translocated through the pores either by directly binding to the NPC components or by forming a complex with soluble transport proteins, karyopherins [48, 68]. The transport of particles with a diameter of approximately 50 nm (mRNA in complex with proteins) is also possible, but it requires a rearrangement of the particle to reduce its diameter [69].

The function of NPC is not only bidirectional transport of molecules. The NPC, namely Nup84, was shown to be involved in anchoring of some telomeres and their length regulation. Deletion of Nup84 led to disruption of DNA repair by non-homologous end joining (NHEJ) [70].

It is important that the nuclear pores also mediate the Ca^{2+} transport between the nucleoplasm and the cytosol. This transport is likely to be regulated and depends on the Ca^{2+} concentration in the lumen (details will be discussed below). In response, the Ca^{2+} level in lumen affects the transport of molecules through nuclear pores [37, 71]. However, there is no consensus yet on the peculiarities of Ca^{2+} regulation of the nuclear pores conductivity for different molecules.

Taking into account the features of the NPC functioning, it has been suggested that ion transporters must contain the nuclear localization signal (NLS) sequence to be translocated from their synthesis sites to the nucleus and to be incorporated into nuclear membranes. Presently, the entire spectrum of sequences attributed to NLS has not been determined; however, at least two types of nuclear localization sequences are known: pentapeptide KKRRK and NLS formed by two clusters of basic amino acid residues separated by 10 residues [72]. Thus, the presence or absence of certain transport system in the nuclear envelope can be established by its amino acid sequence.

The existence of various ion exchange systems in the nuclear envelope of animal and plant cells was revealed using electrophysiological techniques (in particular, the patch-clamp). Although the localiza-

tion of all nuclear ion channels and pumps has not been determined yet, it was found that some of them function in the outer nuclear membrane, others in the inner nuclear membrane, and certain transporters are located in both membranes [57, 67, 73].

The only now cloned nuclear transporter is chloride channel-27 (NCC27/CLIC1), which exists in the internal nuclear membrane of plant and some animal cells [66]. NCC27/CLIC1 was determined to contain the nuclear localization signal NLS, formed from two sequences of basic amino acids separated by a fragment of 10 amino acids (which ensures the transport of this protein from the place of its synthesis in the cytosol to the inner nuclear membrane). Given the two-component structure of the chloride channel NLS, Marjori Matzke and colleagues performed a screening of ion transport proteins in *Arabidopsis* (using the INTERPRO database) for the presence of two-component NLS in their composition to identify potential ion channels and pumps localized in the inner membrane of the plant cell nucleus [57]. Thus, potential-dependent Cl^- , K^+ -channels, cAMP-dependent ion channels and Ca^{2+} -ATPase were detected in the nuclear envelope. However, the sequence mentioned above was not found in the composition of, e.g., V-type H^+ -ATPase [66].

The presence of a two-component NLS sequence does not guarantee that the protein will be exclusively nuclear. Moreover, the INTERPRO database allows identifying only two-component NLS in proteins but not other types of NLS [74]. Thus, the range of existing nuclear transporters is likely to be wider and the use of other methods is required for a thorough study of this issue.

An interesting example of the ion channels functioning in nuclear membrane is the involvement of some nuclear membrane ion channels of legume roots in the generation of Ca^{2+} -spikes, which occur as a result of the interaction of rhizobial symbionts with the roots of these plants. For example, DMII, CASTOR, and POLLUX are the nuclear membrane cation channels which have higher selectivity to K^+ over Na^+ and Ca^{2+} . Such ionic conductivity of these transporters led to hyperpolarization of the inner nuclear membrane and the Ca^{2+} release from the lumen into the nucleoplasm. An increase in the Ca^{2+} concentration in the nucleoplasm might induce the expression of endosymbiotic genes [75, 76].

Proteome analysis, which enables to distinguish nuclear proteins from microsomal membrane

proteins, demonstrated that Ca^{2+} channels, Zn^{2+} transporters, Na^+/H^+ exchangers are also localized in the nucleus [57].

It was found, using antiserum to the N- and C-terminal epitopes of ATP-regulated P2X7 channels, that these transporters exist in the outer nuclear membrane of all hippocampal neurons (whether they are excitatory or inhibitory). Immunoassay electron microscopy data revealed that P2X7 crosses nuclear membranes, since antiserum labels were detected on the cytoplasmic and nucleoplasmic sides of the nuclear envelope [77]. Binding of ATP to the cytoplasmic site of the receptor induced the activation of a non-selective Ca^{2+} channel in the nuclear membrane, and, thus, changes in the Ca^{2+} concentration in the nucleoplasm [77, 78].

Interestingly, these purinergic receptors are concentrated in the presynaptic membranes exclusively of excitatory neurons. However, their content in nuclear membranes is high in both excitatory and inhibitory neurons [77]. Probably, different changes in the nucleoplasm Ca^{2+} concentration in excitatory and inhibitory neurons under the influence of activated P2X7 affect in different degree the transcriptional activity of genes involved in neuronal plasticity or affect different neuronal plasticity-related genes in these neurons.

Apart from the potential-dependent K^+ channels [79], ATP-sensitive K^+ channels (K_{ATP}) were also shown to operate in the nuclear membrane (at least in some cells). For example, in the β -cells of mice pancreatic islet, along with plasma membrane K_{ATP} , the nuclear K_{ATP} was detected by fluorescent dye glibenclamide-BODIPY-FL. The plasma membrane K_{ATP} in pancreatic cells is known to be involved in the regulation of insulin secretion from β -cells: the increase in ATP concentration in these cells, as a result of glucose uptake from blood, leads to the closure of ATP-sensitive K^+ -channels in the plasma membrane and the Ca^{2+} influx through potential-dependent Ca^{2+} channels in response to the plasma membrane depolarization. A further increase in the cytosolic Ca^{2+} concentration is a prerequisite event for the fusion of insulin-containing secretory vesicles and the insulin secretion from pancreatic β cells [80]. The nuclear K_{ATP} channels closure, in response to an increase in ATP level, leads to increased nucleoplasmic Ca^{2+} concentration. The latter, in turn, induces the CREB phosphorylation and expression of the c-Myc transcription factor, which is responsible for cell growth and proliferation [81].

From all of those above, it can be concluded that the nuclear membranes contain the following ion exchange systems:

| Inner membrane | Outer membrane |
|---|--|
| NPC | NPC |
| Voltage-gated K ⁺ channels (VGKCs) | Voltage-gated K ⁺ channels (VGKCs) |
| K _{ATP} ? | K _{ATP} ? |
| P2X7 | P2X7 NCC27 CLIC1 |
| | Cyclic nucleotide-gated channels (CNGs) |
| Ca ²⁺ channels: - IP3R - RyR - IP4R | Ca ²⁺ channels: - IP3R - RyR - NADPR |
| Ca ²⁺ -ATPase? | Na ⁺ /H ⁺ exchanger? |
| Na ⁺ /H ⁺ exchanger? | Na ⁺ /Ca ²⁺ exchanger (NCX) |
| | Na ⁺ ,K ⁺ -ATPase |

A general scheme for the localization of known nuclear transporters in a nuclear envelope is presented in Fig. 2 [57]. Some of the nuclear envelope transporters (40% of the total amount) are present in the membranes of other organelles. Thus, in addition to specific functions, a nuclear membrane, to some extent, “recapitulates” the functions of some cellular organelles [57].

V. ATP-hydrolases and nuclear membrane

Nuclear membrane has been long known to have ATPase activity. ATP analog S-dinitrophenyl-6-mercaptapurine riboside triphosphate was found to inhibit nuclear ATPase activity. The inhibition was observed only on the isolated membrane in the presence of DNase but not on the whole nuclei and heparin vesicles that indicates that ATPases are localized on the inner side of the nuclear envelope. It was believed that their biological role is the involvement in RNA transport processes, since the ATPase activity increases in the RNA preparations, and the mRNA-protein complexes are transported from the nucleus more efficiently in the presence of ATP [82].

Some studies have demonstrated a functioning Na⁺,K⁺-ATPase in the nuclear envelope (for example, in hepatocyte nuclei [83], in HEK293 cell nuclei [84]), where this pump provides a driving force for Ca²⁺ transport into the nuclear lumen via Na⁺/

Ca²⁺ exchanger (NCX) (similar to the “cooperation” of Na⁺,K⁺-ATPase and NCX in the plasma membrane). Thus, the presence of Na⁺,K⁺-ATPase in isolated HEK293 cell nuclei was confirmed by immunoblotting (using antibodies to the α-subunit of the protein), enzyme assay (determination of the ouabain-sensitive ATPase activity), and immunocytochemistry. In the latter case, to confirm the presence of Na⁺,K⁺-ATPase in the nuclear envelope (and not only in nuclear preparations with contaminants of the endoplasmic reticulum and fragments of plasma membranes) the authors incubated isolated nuclei with anti-Na⁺,K⁺-ATPase α-subunits on the background of staining with antibodies against the NPC protein. Overlap of the signals indicated the functioning of both proteins in the nuclear envelope [84].

A question concerning the localization of Na⁺,K⁺-ATPase in the nuclear envelope (inner, outer or both membranes) drew much attention. To answer this question, a method for isolation the nuclear outer and inner membranes [85, 86], followed by proteins separation of both membranes by immunoblotting (using antibodies to the Na⁺,K⁺-ATPase β-subunit) was used. The results obtained by Galva revealed that this enzyme is present exclusively in the inner nuclear membrane [84].

There are data demonstrating the localization of V-type H⁺-ATPases (V-ATPase) in the outer and inner nuclear membranes. V-ATPase was suggested to generate H⁺-gradients, which mediate the proton-coupled transport of substrate [87]. Moreover, the corroboration that V-ATPase functionally operates in the nuclear envelope will make researchers revise the accepted ideas about the regulation of nuclear pH: probably, the transport of protons through the nuclear pore complexes is restricted, and V-ATPase plays a crucial role in maintaining the nuclear pH.

Apart from Na⁺,K⁺-ATPases and H⁺-ATPases, nuclear envelope also contains Ca²⁺-ATPases. The significance of Ca²⁺-ATPase as a system of active Ca²⁺ transport into the nuclear lumen was evidenced by the findings that inhibition of Ca²⁺-ATPase impede the histones import through the nuclear pore, which conductivity depends on the Ca²⁺ level in the nuclear store [71].

VI. Ca²⁺ transport systems in the nucleus

Various research sources demonstrated the existence of both transport systems in the nuclear envelope – Ca²⁺ release system from the lumen into cytosol or nucleoplasm via Ca²⁺ channels and Ca²⁺ flux into the lumen (Ca²⁺-ATPase, NCX) [57, 67, 88].

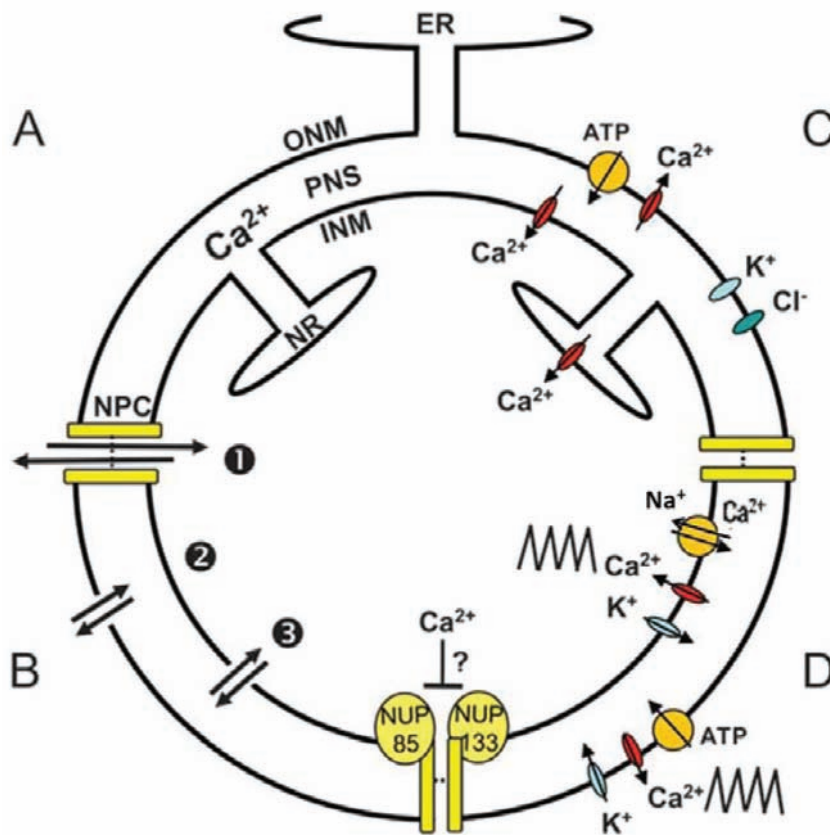


Fig. 2. Ion exchange systems in the nuclear envelope [57]. (A) – structure of the nuclear envelope: ONM – outer nuclear membrane, INM – inner nuclear membrane, PNS – perinuclear space, ER – endoplasmic reticulum, NR – nucleoplasmic reticulum, NPC – nuclear pore complex. (B) – three routes of ion transport across the nuclear envelope: 1 – across the NPC, 2 – across the ONM, 3 – across the INM. (C-D) – ion transport systems in the nuclear envelope: active transport systems depicted as the yellow spheres

Ca^{2+} release occurs via ligand-controlled Ca^{2+} channels of both nuclear membranes - outer (release of Ca^{2+} from the lumen into cytosol) and inner (release of Ca^{2+} from the lumen into nucleoplasm) [20]. Both membranes contain ryanodine (RyRs) and IP_3 receptors. NAADPRs, as well as an enzyme for the synthesis of cADP-ribose and nicotinic acid adenine dinucleotide phosphate (NAADP) are localized in the inner membrane, while other Ca^{2+} channels, which operate in plasma membrane, endoplasmic reticulum or lysosomes (e.g., TRP), were not found in the nuclear envelope [37, 38].

IP_3 -regulated Ca^{2+} channels. According to the published data, the leading role in the Ca^{2+} release from the nucleus is played by IP_3 -regulated Ca^{2+} channels located in both nuclear membranes. Although the proportion of the IP_3 channels on the inner membrane is only 1%, their functioning provides an essential part of the Ca^{2+} release into the nucleo-

plasm [37]. Evidence in support of the existence of functionally active IP_3 receptors in an isolated nucleus was found before the European Conference in Calabria, in 1990 [89].

Although IP_3 -regulated Ca^{2+} channels have been found on the membranes of Golgi apparatus, lysosomes, endosomes (what would be expected since these compartments are formed during biogenesis of endoplasmic reticulum membranes), the significance of these channels as a system for Ca^{2+} transport from the store into the cytosol is most evident for the endoplasmic reticulum. A typical IP_3 receptor has tertiary structure and consists of three parts: N-terminal ligand-binding domain (on the cytosolic or nucleoplasmic side), a regulatory domain in the central part of the molecule (binding sites for Ca, ATP, chromogranin B, protein kinase phosphorylation) and C-terminal domain, which is Ca^{2+} channel [76, 90]. It is important to note that even

a high level of IP_3 is not enough to trigger channel activation: the combined effect of IP_3 and Ca^{2+} on the receptor is necessary. The dependence of the IP_3 -induced release of Ca^{2+} into the cytosol on $[Ca^{2+}]$ has a bell-shaped form with the optimum cytosolic Ca^{2+} concentration of 600 nM, that is likely to be a protective mechanism and prevent an excessive increase in the cytosolic Ca^{2+} level [91].

Ryanodine receptors (RyRs). Unlike in skeletal muscle cells, RyRs in the membranes of the sarcoplasmic reticulum and nucleus in smooth muscle cells, do not directly contact the L-type voltage-gated Ca^{2+} channels (VGCCs) in plasma membranes. Therefore, for their activation in smooth muscle cells, just the changes in the VGCCs conformation are insufficient, Ca ions that enter through the VGCCs or IP_3 Rs into the cytosol and bind to the cytosolic site of RyRs are required. The affinity of the ion channels for Ca^{2+} increases when they bind endogenous ligand (cyclic ADP-ribose). However, the affinity for Ca^{2+} can also change under the influence of other factors (Ca^{2+} , Mg^{2+} , H^+ , ATP, calmodulin, protein kinase C and A, methylxanthines (e.g., caffeine)) [90, 92].

In smooth muscle cells, the release of Ca^{2+} from the RyRs cluster increases the local Ca^{2+} concentration. This event is called a calcium spark. Calcium sparks can modulate the plasma membrane excitability causing hyperpolarization by activation of Ca^{2+} -activated K^+ channels of small and large conductance (SK channels and BK channels, respectively) or depolarization by activation of Ca^{2+} -activated Cl channels [90].

NAADPR. The nicotinic acid adenine dinucleotide phosphate (NAADP) receptors are found mainly in the endolysosomal compartments, where the high proton concentration, for the NAADP synthesis from $NADP^+$ in the presence of ADP-ribosyl cyclase (CD38), is maintained [93]. The NAADP-activated Ca^{2+} release from these compartments occurs through NAADPR, called two-pore channels (TPC2). The relatively small amount of Ca^{2+} released from endolysosomes through these channels can induce global Ca^{2+} waves due to stimulation of Ca -induced Ca^{2+} release from the stores via RyRs [94, 95]. Moreover, in some cases, NAADP itself may act as a RyRs activator.

Research on isolated nuclei of pancreatic acinar cells demonstrated that active NAADPRs also exist in the nuclear envelope [96]. These transporters are inhibited by ruthenium red and ryanodine (inhibitors

of RyRs) but not caffeine [67]. Remarkably, these receptors are specific exclusively to NAADP and cannot be activated by NAADP analogs, β -NADP and NAAD [97]. However, the functional role of the NAADP receptors in the nucleus still requires further study. Thus, taking into consideration the location of calcium channels in the nuclear envelope, Ca^{2+} releases from the lumen in both directions: into the cytosol (via IP_3 -mediated Ca^{2+} channels and RyRs) and into the nucleoplasm (via activation of the IP_3 -mediated Ca^{2+} channels, RyRs and NAADPRs). However, what route is preferred at the molecular level – is a question for further investigation.

Ca^{2+} is accumulated in the nucleus depot via the Ca^{2+} -ATPase and Na^+/Ca^{2+} -exchanger – Ca^{2+} extrusion systems from the cytosol and nucleoplasm. Another mechanism for Ca^{2+} transport to the nuclear lumen, proposed in 1993, is IP_4 (inositol-1,3,4,5-tetrakisphosphate)-mediated Ca^{2+} uptake, which remains puzzling [98, 99]. It was assumed that the concentration of free Ca^{2+} in the cytosol/nucleoplasm is the main factor in the “choice” between ATP- and IP_4 -mediated Ca^{2+} transport into the depot: the IP_4 -mediated mechanism is triggered at the Ca^{2+} concentration $> 1 \mu M$ [98]. It is interesting that the “nuclear” functions of IP_4 and other inositol polyphosphates include not only the regulation of Ca^{2+} signaling but also gene transcription, chromatin remodeling, DNA repair, RNA export, and mRNA and tRNA editing [100, 101].

A summary scheme of the pathways that regulate the Ca^{2+} concentration in the nucleus is presented in Fig. 3 [37]. Inositol-1,4,5-triphosphate (IP_3) as a ligand of IP_3 -regulated Ca^{2+} channels can be formed from PI-4,5-P2, the component of plasma or nuclear membranes, since the nuclear envelope has its own pool of PI-4,5-P2 and phospholipase C β . Another mechanism of Ca^{2+} mobilization involves the translocation of ligand-stimulated phospholipase C from the plasma membrane to the nuclear membrane. In addition, Ca^{2+} can diffuse in and out the nucleus through the NPC. The supposed mechanism (although it has not been shown in all studies) is also presented in this scheme. This pathway implies that the hormone-receptor complex is translocated into the nucleus and activates nuclear phospholipase C and synthesis of IP_3 . In the nucleus IP_3 serves as a substrate for the synthesis of other inositol polyphosphates, which modulate gene expression.

Ca^{2+} -ATPase. It is believed that Ca^{2+} accumulation in the perinuclear store occurs as a result of

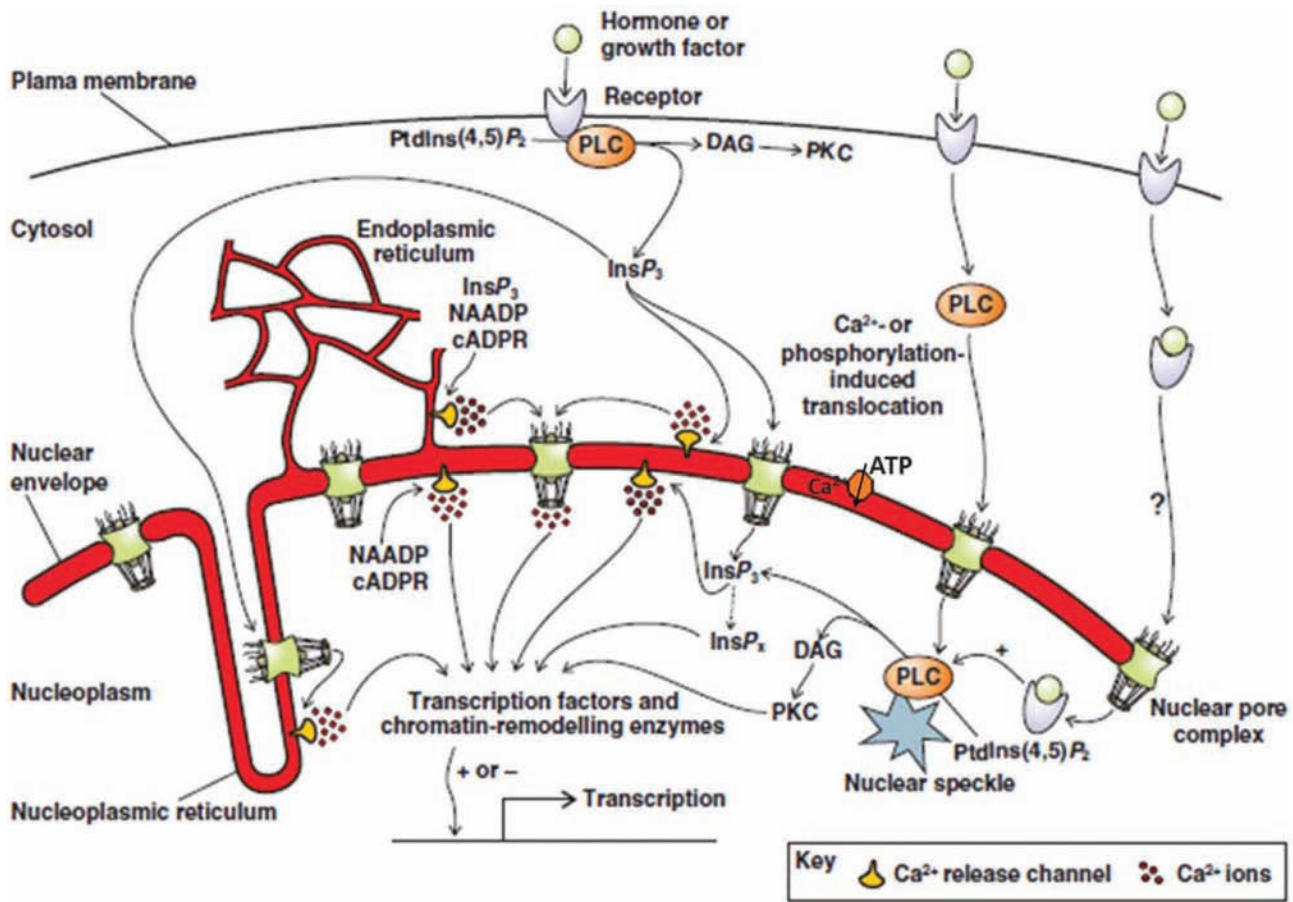


Fig. 3. Ca^{2+} signaling pathways and transport in the nucleus [37]

the activation of nuclear Ca^{2+} -ATPases [85, 102]. The Ca^{2+} -ATPase activity and ATP-dependent Ca^{2+} accumulation were for the first time shown in isolated hepatocyte nuclei using a Ca^{2+} -sensitive probe Indo-1. Addition of ATP to the incubation medium resulted in an increase in the Ca^{2+} concentration in hepatocyte nuclei from 100 to 540–800 nM. Removal of ATP from the medium led to a passive release of accumulated Ca^{2+} along its concentration gradient [103]. The subnuclear localization of Ca^{2+} -ATPase has not been completely established. Most researchers believe that the enzyme is located only on the outer nuclear membrane [104, 85]. Ca^{2+} -ATPase has been recently shown to exist in the nuclear envelope of osteoblasts [105], neurons [106], oocytes [107] and lymphocyte [108].

Structures of nuclear Ca^{2+} -ATPase and sarco-plasmic reticulum Ca^{2+} -ATPase are similar that was confirmed by comparative Ca^{2+} -ATPase proteolysis and its reaction with antibodies. However, the nuclear Ca^{2+} -ATPase activity is partially inhibited by thapsigargin and is insensitive to 2,5-di-tert-bu-

tyl-1,4-benzohydroquinone (DBHQ), whereas sarco-plasmic reticulum Ca^{2+} -ATPase is inhibited by both inhibitors [109].

NCX. Unlike Ca^{2+} -ATPase, $\text{Na}^+/\text{Ca}^{2+}$ -exchanger (NCX) is a system of secondary active transport, which uses the Na^+ gradient (generated by Na^+/K^+ -ATPase) for Ca^{2+} transport against the concentration gradient [110]. Contrary to Ca^{2+} -ATPase, NCX has low affinity to Ca^{2+} and high capacity, and is able to work in reverse mode under certain conditions (e.g., in heart, NCX provides Ca^{2+} influx into the myocyte and extrusion of Na^+ after membrane depolarization during systole as a result of voltage-dependent Na^+ channels opening) [111, 112].

Generally, $\text{Na}^+/\text{Ca}^{2+}$ exchangers are associated with the functioning of excitable cells, however NCX expression either in the inner nuclear membrane solely or in both the plasma membrane and the nuclear envelope was found in many types of unexcitable cells, [37]. It is known that the nucleus is characterized by the presence of splice-variant of NCX1 isoform. It should be noted that nuclear NCX

is characterized by atypical interaction with the gangliosides GM1, although the protein configuration is the same as that of the plasma membrane NCX [113]. Gangliosides, as components of the membrane environment, are involved in the regulation of $\text{Na}^+/\text{Ca}^{2+}$ exchanger activity, similarly to the function of inositol-4,5 bisphosphate (PIP2) in the plasma membrane NCX.

The localization of Na^+/K^+ -ATPase and Na^+/H^+ exchanger in the inner nuclear membrane gives a reason to suggest that these systems are involved in the generation of primary transport gradient for the NCX operation in the nucleus. Thus, again on the example of the functioning of these transport systems, we can observe recapitulation of the plasma membrane features by the nuclear envelope.

VII. Relationship between cytoplasmic and intranuclear Ca^{2+}

The question of the mutual influence of cytoplasmic and nuclear Ca^{2+} levels, namely: whether an increase in Ca^{2+} concentration in the nucleoplasm is the only response to Ca^{2+} waves in the cytosol and the amplitudes of the Ca^{2+} signals in both compartments differ just slightly, or the increase in Ca^{2+} level in the nucleus occurs independently of cytosol event and plays a physiological role, is still open. In this aspect, it is important to note that Ca^{2+} diffuses in the cytosol at a lower rate than in the nucleus [37]. Therefore, Ca^{2+} influx through the voltage-dependent plasma membrane channels induces Ca^{2+} signals in the cytosol and increases only slightly (after a short delay) Ca^{2+} level in the nucleus. In contrast, Ca^{2+} released from the organelles near the nucleus is more likely to cause Ca^{2+} transients there than at a distance in the cytosol [20, 114]. Confocal microscopy analysis of hepatocyte nuclei revealed that changes in the Ca^{2+} concentration in the cytosol induced rapid changes in the Ca^{2+} level in the nucleoplasm (measured using dextrans labeled with fluorescent calcium indicators: calcium-Green 1 and Fura 2) [116]. These results suggest that changes in cytoplasmic Ca^{2+} affect the Ca^{2+} level in the nucleus.

It is believed that the nucleus has an independent calcium signaling system, which can generate its own transients [67, 38]. Therefore, this organelle does not simply imitate the processes of changing the Ca^{2+} concentration in the cytosol but can initiate calcium signals. However, the relationship between cytoplasmic and nuclear calcium is obvious and can be observed in some cases: for example, the activa-

tion of gene transcription is initiated by an increase in the concentration of cytosolic free Ca^{2+} . Moreover, an increase in the cytosolic Ca^{2+} level triggers the NFAT activation: its dephosphorylation by Ca^{2+} -calmodulin-dependent phosphatase calcineurin results in exposure of the nuclear localization signal and translocation of NFAT as a complex with calcineurin to the nucleus. An increased Ca^{2+} level in the nucleus is necessary to maintain NFAT transcriptional activity since only under this condition calcineurin supports the active NFAT dephosphorylated state [37].

Interestingly, proteins that can be translocated into the nucleus and trigger the further stages of Ca^{2+} signaling in the nucleus can serve as a link between cytoplasmic and nuclear calcium. One such protein is calmodulin, a universal Ca^{2+} -binding protein that functions in all eukaryotic cells. It is known that calmodulin is constitutively present in the nucleus, however additional calmodulin enters nucleus from the cytosol after cell stimulation (it was demonstrated on neurons). Since calmodulin molecule does not contain nuclear localization signal, it was assumed that it engages other carrier proteins containing NLS and translocates into the nucleus after an increase in cytosolic Ca^{2+} level [37]. In this case, nuclear Ca^{2+} transients are likely to be a response to a global increase in the Ca^{2+} level in the cytoplasm, while calmodulin in a complex with Ca^{2+} activates the nuclear Ca^{2+} -calmodulin-dependent kinase involved in the transcription regulation through the mechanisms described in part III [30, 35, 38].

Several mechanisms are suggested for Ca^{2+} signals in the nucleus (Fig. 4) [38]. One of them postulates the existence of a connection between secondary messengers generated in the cytosol and Ca^{2+} -signaling in the nucleus. Thus, IP_3 , a product of membrane phospholipid phosphatidylinositol-4,5-bisphosphate PIP2 in the cytosol, diffuses to the nucleus, where it binds to type II IP_3 receptors in the nucleoplasmic reticulum and induces the Ca^{2+} release into the nucleoplasm. However, the question arises: why IP_3 , which diffuses freely in the cytosol, targets precisely at the IP_3 receptors in the nucleus, and not the IP_3 receptors of endoplasmic reticulum, where the level of IP_3 receptors is higher? The most likely reason is the different ligand-binding affinities of three types of IP_3 receptors, namely: $\text{IP}_3\text{R2} > \text{IP}_3\text{R1} > \text{IP}_3\text{R3}$ [116]. Therefore, if the cell nuclei express $\text{IP}_3\text{R2}$ in a sufficient concentration, Ca^{2+} -signaling in response to IP_3 will begin in this compartment.

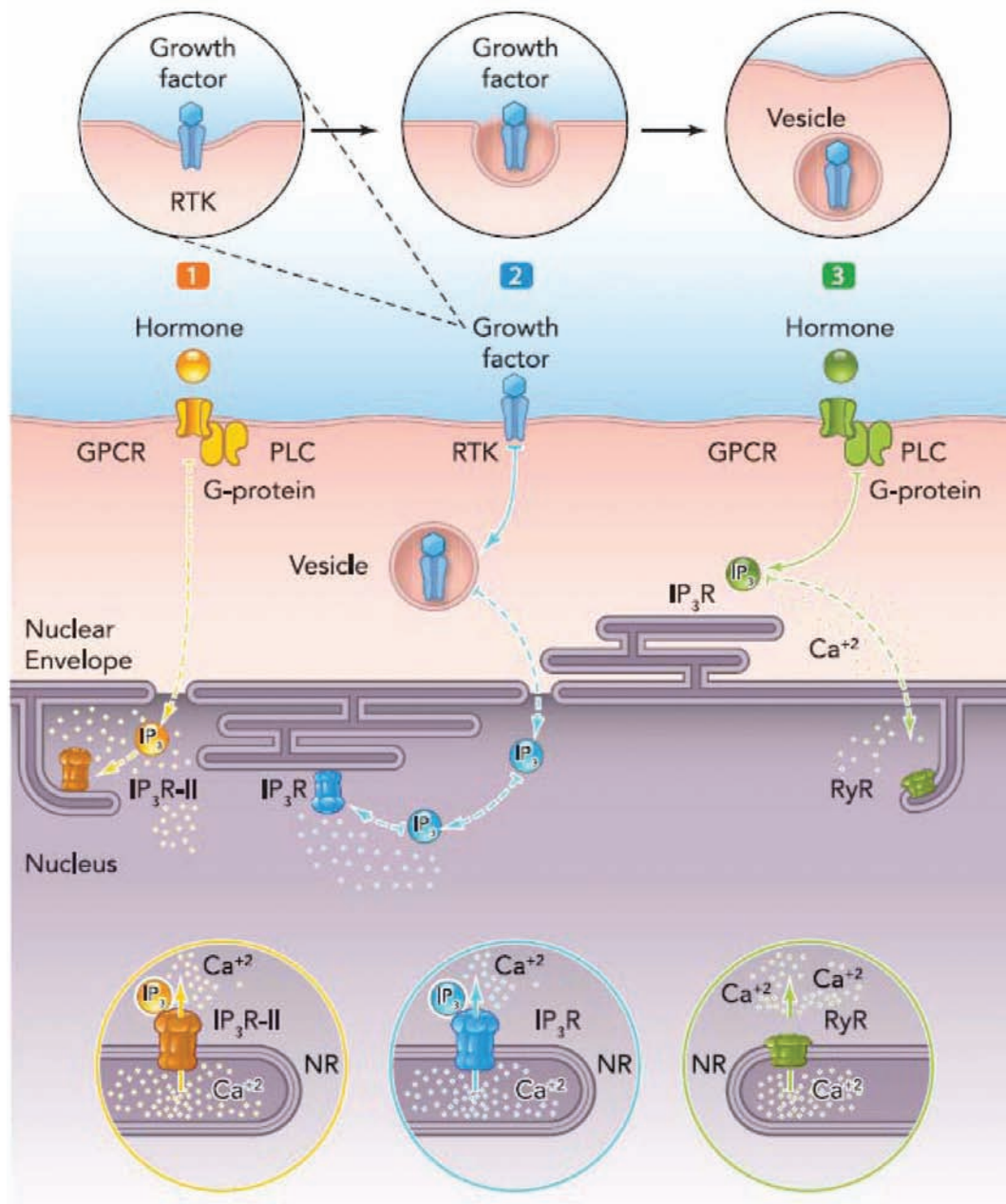


Fig. 4. Mechanisms for the Ca^{2+} concentrations increases in the nucleus [38]. 1 – the binding of an agonist to its receptor on the plasma membrane induces IP_3 generation by membrane phospholipase C (PLC). IP_3 diffuses in the cytosol and activates IP_3 receptors located in the nucleoplasmic reticulum; 2 – translocation of the growth factor-receptor tyrosine kinase (RTK) complex to the nucleus activates generation of the nuclear IP_3 ; 3 – the binding of an agonist to its receptor on the plasma membrane induces IP_3 production with the involvement of membrane phospholipase C (PLC). IP_3 diffuses in the cytosol and activates IP_3 receptors located on the endoplasmic reticulum membrane. Ca^{2+} can diffuse from endoplasmic reticulum to the nucleus and activates IP_3 receptors located in the nucleoplasmic reticulum

The second mechanism provides for an increase in the nuclear Ca^{2+} level as a consequence of independent from PM and cytosol IP_3 generation in the nuclear membrane. The growth factor-receptor complex is translocated from the plasma membrane into the nucleus, where it stimulates hydrolysis of PIP_2 to IP_3 on the nuclear membrane. IP_3 , in turn, stimulates via IP_3R_2 the Ca^{2+} release into the nucleoplasm. Another mechanism suggests that IP_3 , generated as a result of activation of plasma membrane receptors, causes the release of Ca^{2+} from the IP_3 receptors on the endoplasmic reticulum membrane. Endoplasmic reticulum calcium stimulates RyR in the nucleoplasmic reticulum and further increase in the Ca^{2+} concentration in the nucleoplasm (CICR- Ca^{2+} -induced Ca^{2+} release) [116, 38].

Furthermore, there is a mode suggesting that the nuclear IP_3 receptors are stimulated by inositol triphosphate synthesized in membrane invaginations T-tubules located close to the nuclear envelope. This mechanism as a version of the first one may occur in skeletal muscles and cardiomyocytes, which are characterized by the presence of T-tubules [117]. Described mechanisms indicate that the nucleus can be either an independent generator of Ca^{2+} signaling or can act co-operatively with the events initiated in the cytosol.

VIII. The nuclear transmembrane Ca^{2+} exchange disorders and “nuclear” pathologies

To date, examples of “nuclear” pathologies (related to calcium signaling) have been established mainly for neurons in the brain and are often associated with the stimulation of extrasynaptic NMDA receptors (e-NMDARs) [55, 58]. The signal from synaptic NMDA receptors is known to cause Ca^{2+} transients in the cytosol and nucleus (through the activation of VGCCs in PM and IP_3 channels in ER) and ultimately leads to the gene expression that provides neuroprotection and contributes to the neuron survival. In this aspect, e-NMDARs act as antagonists: their activation leads to an elevation in the Ca^{2+} concentration to a level at which some destructive processes resulting in excitotoxic neuronal death are triggered: the free radical generation, the mitochondrial inner membrane potential disruption and the activation of hydrolases [55]. These events are associated with CREB dephosphorylation and degradation, HDAC II accumulation in the nucleus and translocation of the proapoptotic factor FOXO3a

to the nucleus [35]. However, the question still remains how the nucleus distinguishes between Ca^{2+} transients caused by the stimulation of synaptic and extrasynaptic NMDA receptors.

Some neurodegenerative disorders, in particular, Alzheimer's and Huntington's diseases, as well as hypoxic conditions and strokes, are associated with an increased level of e-NMDARs activation and abnormalities in Ca^{2+} -signaling in neuronal nuclei [118, 119]. Interestingly, increased activation of e-NMDARs upon Alzheimer's disease can be a consequence of the β -amyloid-induced excessive glutamate release from glial cells. The activation of e-NMDARs, in turn, leads to the β -amyloid formation, neuronal death, and synapse loss, and such vicious circle results in impaired synaptic plasticity and brain cognitive dysfunction [58, 63]. This kind of nuclear Ca^{2+} signaling dysfunctions was called “nuclear calciopathy” [35].

In addition, changes in nuclear Ca^{2+} concentration cause cardiomyocyte hypertrophy: both physiological (regulated by insulin-like growth factor IGF-1) and pathological (induced by endothelin 1 (ET-1)) [64]. Autocrine and paracrine factors, that act during stress and induce cardiac muscle hypertrophy, activate the phosphatidylinositol cascade, which results in an increase in the intracellular Ca^{2+} concentration [65]. Arantes L.A.M. et al. demonstrated that the increase in nuclear, not cytosolic, IP_3 level is a key stage in the development of cardiomyocyte hypertrophy and inducer of the activation of nuclear Ca^{2+} -calmodulin-dependent kinase II, which, in turn, induces histone deacetylase export from the nucleus (through phosphorylation) and gene expression involved in increasing the cardiomyocyte size. To understand the role of nuclear IP_3 and nuclear Ca^{2+} in these processes, a vector containing the IP_3R ligand-binding domain bound to a nuclear localization signal, and serving as a buffer for nuclear IP_3 was used. A decrease in the level of free nuclear IP_3 inhibited hypertrophy processes [64].

The phenomenon of “nuclear calciopathy” can certainly occur under other pathological conditions. However, it should be established in which cases the disorder of transmembrane calcium metabolism and calcium signaling in the nucleus is one of the prerequisites of pathology and has a therapeutic effect.

Thus, the nucleus is an essential component of the Ca^{2+} accumulation system in the cell. The specificity of the nucleus as a central player in the realization of genetic information defines the range of particular functions of nuclear calcium. The

functioning of its own systems involved in the Ca^{2+} transport (IP_3 -, IP_4 -receptors, ryanodine receptors, NAADP receptors, Ca^{2+} -ATPase, $\text{Na}^+/\text{Ca}^{2+}$ -exchanger) indicates that the nuclear envelope can autonomously generate calcium signals. However, detailed mechanisms for normalizing the Ca^{2+} level in the nucleoplasm remain to be clarified. In addition, the mechanism for increasing nuclear Ca^{2+} is still an open question.

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ШЛЯХИ ТА МЕХАНІЗМИ ТРАНСМЕМБРАННОГО ОБМІНУ ІОНІВ Ca^{2+} В КЛІТИННОМУ ЯДРІ

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Функції Ca^{2+} в клітинному ядрі різноманітні (регуляція транскрипції генів, проникності ядерних пор, активація ензимів, залучених в апоптоз) та визначають долю будь-якого типу клітини. Тому шляхи надходження Ca^{2+} до ядра, питання взаємозв'язку цитозольних та ядерних кальцієвих сигналів, накопичення та виходу Ca^{2+} з ядерних депо та їхня подальша роль являють неабиякий інтерес. В огляді узагальнено дані літератури і результати власних досліджень щодо особливостей транспорту Ca^{2+} в клітині, зокрема зосереджено увагу на транспортних системах та загальних механізмах обміну цього вторинного месенджера в центральній органелі клітини – ядрі. Також розглянуто загальну структуру клітинного ядра, транспортери ядерної оболонки, функції Ca^{2+} в ядрі та порушення кальцієвого сигналіngu в ядрі.

Ключові слова: ядро, нуклеоплазма, ядерна оболонка, ядерні транспортери, кальцієвий сигналінг.

ПУТИ И МЕХАНИЗМЫ ТРАНСМЕМБРАННОГО ОБМЕНА ИОНОВ Ca^{2+} В КЛЕТОЧНОМ ЯДРЕ

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Функции Ca^{2+} в клеточном ядре разнообразны (регуляция транскрипции генов, проницаемости ядерных пор, активация энзимов, вовлеченных в апоптоз) и определяют судьбу любого типа клетки. Поэтому пути поступления Ca^{2+} в ядро, вопросы взаимосвязи цитозольных и ядерных кальциевых сигналов, накопления и выхода Ca^{2+} из ядерных депо и их дальнейшая роль представляют большой интерес. В обзоре обобщены данные литературы и результаты собственных исследований об особенностях транспорта Ca^{2+} в клетке, в частности сосредоточено внимание на транспортных системах и общих механизмах обмена этого вторичного мессенджера в центральной органелле клетки – ядре. Также рассмотрены общая структура клеточного ядра, транспортеры ядерной оболочки, функции Ca^{2+} в ядре и нарушения кальциевого сигналинга в ядре.

Ключевые слова: ядро, нуклеоплазма, ядерная оболочка, ядерные транспортеры, кальциевый сигналинг.

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