ELSEVIER

Bioelectrochemistry 58 (2002) 13–21

Bioelectrochemistry

www.elsevier.com/locate/bioelechem

Functional role of phospholipids in the nuclear events

A.V. Alessenko*, E.B. Burlakova

Institute of Biochemical Physics of the Russian Academy of Sciences, Kosygin str., 4, 117977 Moscow, Russia

Received 17 May 2001; received in revised form 22 May 2002; accepted 30 May 2002

Abstract

This review presents the structural and functional role of phospholipids in chromatin and nuclear matrix as well as the difference in composition and turnover compared to those present in the nuclear membrane. Nuclei have a very active lipid metabolism which seems to play an important role in the transduction of the signals to the genome in response to agonists acting at the plasma membrane level. The evidence on the presence of phospholipid-calcium-dependent protein kinase C (PKC) in nuclei and enzymes of phospholipids turnover is given. Protein kinase C interacts with nuclear phosphoinositol and sphingomyelin cycles products. This fact evidences about possibility that signal transduction events could also occur at the nuclear level during induction of cell proliferation, differentiation and apoptosis.

Keywords: Nuclei; Chromatin; Nuclear matrix; Lipid metabolism; Phospholipids signal transduction; Apoptosis

1. Introduction

The presence of phospholipids in chromatin and nuclear matrix has been demonstrated by histochemical and biochemical techniques in plant and animal cells. They seem to be associated with nucleoli, non-histone proteins of chromatin and nuclear matrix. To assign specific function to these molecules in the nucleus, the effect of several phospholipids on chromatin structure and function has been investigated in cell-free system by exposure of isolated nuclei to phospholipid vesicles. The results indicated that anionic phospholipids could decondense chromatin, affect nucleosome structure and stimulate either synthesis of RNA and DNA.

Although growth factors acting on plasma membrane receptors can induce cell division, the following questions remain still unanswered: what messages transfer the signal from the cell surface to the nucleus, and how those messages exert their effect. Evidence on the occurrence of phospholipid-calcium-dependent protein kinase (kinase C) in the nucleus seems to represent a direct link between the presence and possible functions of nuclear lipids, namely polyphosphoinositides and sphingomyelin, degradation products of which could regulate the activity of this enzyme controlling the phosphorylation of nuclear proteins.

In our review, we present data on the localization and metabolism of phospholipids in nuclear membrane, chromatin and nuclear matrix, evidence of participation of nuclear phospholipids in structure organization of chromatin and nucleic acids synthesis and description of phosphoinositol and sphingomyelin signal transduction cascades in the nuclei.

2. Localization and metabolism of phospholipids in nuclear membrane, chromatin and nuclear matrix

The precise topology of nuclear lipid metabolism and the relationship between nuclear lipids and crucial events are studied very intensively now. Nuclei have a very active lipid metabolism, which seems to play an important role in the transduction of signals to the genome in response to agonists acting at the plasma membrane level [1–5]. The main reports concerned with the demonstration of phospholipids in the nucleus stem from fractionation studies, but presence of phospholipids in define nuclear domains in whole cells and in tissues have been demonstrated by ultrastructural cytochemistry [6–8].

Maraldi et al. [8] also investigated the localization of the phospholipid substrates for the phospholipase by cytochemistry with colloidal gold-labelled enzyme and their data indicated a possible intrachromatin and nucleolus localization.

* Corresponding author. Fax: +7-95-137-41-01.
E-mail address: ales@sky.chph.ras.ru (A.V. Alessenko).

1567-5394/02/$ - see front matter
PII: S1567-5394(02)00135-4

The nucleus is surrounded by a double membrane, which has certain features of a classic lipid bilayer. Many types of lipid metabolism enzymes are located in nuclear membrane [2,9–13], chromatin [9] and nuclear matrix [11–14]. For example, two kinases which phosphorylate respectively phosphatidylinositol (PtdIns) into PtdIns(4)*P* and PtdIns(4,5)P_2, which had previously been thought to be confined mostly to the plasma membrane or to the endoplasmic reticulum, were found in the nuclear membrane of rat liver nuclei [3,14]. It has been shown that the polyphosphoinositide synthesis occurs at the nuclear level [11,12] and takes place not only on the nuclear membrane, but also even within the nucleus. This evidence is supported also by the fact that the specific phosphoinositide kinases are bound to the nuclear matrix fraction [11–13,15].

The presence of phospholipids in chromatin has been demonstrated by histochemical and biochemical techniques in plant and animal cells [16–22]. It was shown that the differences in composition and turnover are comparable with those present in the nuclear membrane [20,22]. It has been shown that after lactoperoxidase radioiodination of isolated hepatocyte nuclei, chromatin phospholipids were unlabelled, whereas all radioactivity was recovered in the nuclear membranes [22]. This evidences that chromatin phospholipids did not appear due to contamination from the nuclear membrane phospholipids. The chromatin phospholipids represent about 10% of those in the entire nuclei and differ in composition [21] and turnover [22] from microsomal and nuclear phospholipids.

Recently, some enzymes of lipid metabolism were found in chromatin: the base lipid exchange protein complex, phospholipase C and A2 [23] and neutral sphingomyelinase (N-SMase) [9,20]. Sphingomyelinases-type C (EC.3.1,4.12) are phospholipases that catalyze the hydrolysis of sphingomyelin to ceramide and phosphocholine [24]. We found that the specific activity of the neutral SMase in the nuclei was one-third of that in the whole cell homogenate [25]. Most of the nuclear N-SMase was solubilized with 1% Triton X-100. The apparent molecular weight of N-SMase in rat liver was of the order of 92 kDa [25].

The presence of SMase in chromatin has a particular relevance since sphingomyelin is one of phospholipids represented in chromatin [19,20]. Sphingomyelin has been found in a non-histone chromosomal fraction [27]. Sphingomyelin is classically considered as a phospholipid typically localized in plasma membrane. However, there is evidence about its location also in the nuclei. This pool of sphingomyelin was inaccessible to a sphingomyelinase added to isolated nuclei implying a location other than in the outer nuclear membrane [28].

The existence of the synthesis of sphingomyelin in chromatin and in nuclear membrane isolated from liver nuclei was shown [9]. The evaluation of the enzyme was performed using [(3) H] phosphatidylcholine as a donor of radioactive phosphorylcholine and by identifying the product isolated by thin layer chromatography [22]. In both fractions, the enzyme phosphatidylcholine:ceramide phosphocholine transferase or sphingomyelin synthase was present, although with higher activity in nuclear membrane. The enzyme present in the chromatin differs in pH optimum and $K_{(m)}$, showing a higher affinity for the substrates than that of nuclear membrane. The results presented show that sphingomyelin synthase is present not only in the cytoplasma at the level of the Golgi apparatus, but also in the nuclei, at the level of either the nuclear membrane or the chromatin [9]. During hepatic regeneration, the chromatin sphingomyelin is synthesized and accumulated in relation to DNA synthesis [20,21,29,30].

Phospholipids have been also found in nuclear matrix, which is considered as a site of synthesis of DNA and RNA [31–35]. Treatment of isolated nuclear matrix with a bacterial phospholipase C results in the release of nucleic acids [34,36]. These data strongly evidence on the amphiphilic/amphipathic nature of phospholipids in some bonding forces within chromatin.

The phospholipid spectra of the nucleus, chromatin and nuclear matrix isolated from the liver cells of intact rats are very different [27]. The phospholipids of nuclear matrix are reached by sphingomyelin, which is also presented in lipoproteins. This assumption is supported by the data of Manzoli et al. [27]. They had shown that the acidic proteins from lymphocyte nuclei are enriched in sphingomyelin. Sphingomyelin is the main component of phospholipids (80%) in nuclear matrix of regenerating liver [33,35].

3. Participation of nuclear phospholipids in structure organization of chromatin and nucleic acids synthesis

The autonomous behavior of chromatin and phospholipids of nuclear matrix is emphasized by sharp changes in their spectra during RNA and DNA synthesis in liver cells after partial hepatectomy [20,21,25,33]. An increase in sphingomyelin concentration is correlated with the level of DNA synthesis in liver cells in regenerating liver. It is known that sphingomyelin in vitro stimulates DNA-polymerase activity [17]. Changes in sphingomyelin content have also been observed in the nuclei of different tumor cells [37].

Based on evidence about high content of sphingomyelin in the nuclear matrix at the moment of RNA and DNA synthesis, we have considered the possibility that this lipid can be incorporated into the replicative and transcriptional complexes associated with the nuclear matrix [33,34,36]. This has been confirmed by digestion of sphingomyelin in the nuclear matrix with sphingomyelinase. As a result of this process, 75% of the newly synthesized DNA and 90% of the newly synthesized RNA have been released from the nuclear matrix [33,36]. The treatment of nuclear matrix with phospholipases (phospholipase C without sphingomyelinase activity, phospholipase A_2 and phospholipase D) has not led to the release of such a high yield of newly

synthesized nucleic acids into the supernatant. Significantly, a release of DNA and RNA from the protein structures in the nuclear matrix in vivo can be brought about by the endogenous sphingomyelinase, whose activity has been detected in the nucleus [20,25,26]. The fact that 45% of sphingomyelin is removed with RNAse digestion strongly indicates that this link may have a functional significance, e.g. in the regulation of RNA synthesis or in nucleoprotein processing [38–40].

Inhibition of DNA polymerase in the nuclear matrix and release of newly synthesized DNA from the nuclear matrix when treated with phospholipases [35,36] indicate a structure-functional role of phospholipids in the replicative complex. These results lead to assumption that sphingomyelin is the main phospholipid involved in the formation of replicative complex in the nuclear matrix. Most of these data have consisted of the documentation of the presence of phospholipids, without much attention to possible relevance, and usually with the implicit inference that they are just as likely to be contaminants from nuclear membrane.

However, Manzoli [31,35,38,41–46], Alessenko [5,33,36,47–51], Magni [10,20,39,40] and their colleagues have investigated the possible role of phospholipids in structure and functional activity of nuclei. The effect of several phospholipids on chromatin structure and function has been investigated in cell-free systems by exposure of isolated nuclei to phospholipid vesicles [38,41,42,52,53]. Cocco et al. [52,53] have investigated the structural changes in chromatin that occur if phosphatidylserine or phosphatidylcholine are added as multilamellar vesicles. The results obtained indicate that anionic phospholipids can decondense chromatin [53], could affect nucleosome structure [52] and stimulate either synthesis of total RNA or that of specific mRNA families [38,42]. Phosphatidylserine had a profound effect on transcription in vitro by removing histones [53]. In addition, for a more simplified system, a stimulatory effect of inositol lipids on the isolated DNA polymerase α activity has been reported [54].

4. Phospholipids signal transduction cascades in the nuclei

Existence of a phospholipid signal transduction cascade based on the polyphosphoinositide and sphingomyelin breakdown has been indicated by the discovery in the nucleus of phospholipase C [13] and sphingomyelinase [25] and protein kinase C (PKC) [55–58]. Recent evidence on the occurrence of PKC in nuclei seems to prove a direct link between the presence and possible functions of nuclear lipids. Phosphatidylinositols, sphingolipids, phosphatidylserine and diacylglycerols, which could regulate the activity of this enzyme, can control the phosphorylation of nuclear proteins [54,59–62].

Specific PKC that associates with liver nuclei has been partially purified. Immunoblotting studies evidence that it is different isotype from the major cytosolic liver PKC [63]. There may also be an independent translocation of PKC to the nucleus [56,64].

Therefore, variation in the nuclear phospholipid content and relative composition may affect the functional activity of this key enzyme. The nuclear domains, the interchromatin fibers and granules, and the nucleolus, are sites in which transcription products are processed and they belong to the nuclear matrix compartment, with which PKC and some enzymes of inositol lipid cycle are also associated [12,13,55].

Metabolism of polyphosphoinositides was changed by cellular differentiation, proliferation and apoptosis. Level of both PtdIns(4)*P* and PtdIns(4,5)P_2 was changed in Friend cells induced to differentiate by treatment with DMSO [65]. Stimulation of Swiss 3T3 cells with IGF-I causes a rapid and transient decrease in nuclear PtdIns(4) and PtdIns(4,5)P_2 and concurrent increase in nuclear diacylglycerols [64]. That this is really happening in the nuclei is based on the results that in the same cells bombesin causes a large identical response in the plasma membrane, but not in nuclei [3,64]. The interpretation of this data by authors of this study is that a signal reaches the nucleus as a result of stimulation of the IGF-I receptor and this signal stimulates a nuclear phosphoinositidase C that hydrolyses PtdIns(4,5)P_2 to generate diacylglycerol and Ins (1,4,5)P_3. Both diacylglycerol and Ins (1,4,5)P_3 are involved in activation of PKC.

Hormones prolactin and erythropoietin added to purified nuclei have activated PKC inside nuclei [66,67]. However, at least in Swiss 3T3 cells in response to IGF-I, the nuclear response required intact cells. It means that nuclei have own PKC and PKC, which is translocated to the nucleus as a result of some stimulation. Nuclear lipids control both these enzymes.

As it was mentioned earlier, sphingomyelin metabolism exists in nuclei and sphingomyelin has the unique ability to accumulate in chromatin and nuclear matrix [5,9,10,20,29,30,34]. Currently, the attention is focused on the sphingomyelin metabolic cycle in nuclei. The products of this metabolism (sphingosine and ceramide) can also exhibit the properties of second messengers [24,68]. The plausible involvement of sphingolipids in relaying cell signals was first revealed in the experiments of Hannun and Bell [69] who examined the inhibitory activity of sphingosine toward PKC in vitro. This sphingomyelin metabolite has subsequently been shown to inhibit many PKC-depended processes in vitro [70,71]. On the other hand, in some studies, sphingosine has been shown to posses numerous properties, some of which manifest themselves independently from protein kinase C [72]. Sphingosine has been shown to enhance phosphorylation of the EGF receptors [73] and to activate purified casein kinase II [74] and other sphingosine-dependent kinases [75].

The phosphorylation reactions in nuclei have a biological significance for the transduction of the mitogenic signals

from cell membrane to nucleus in response to growth factors and other proliferative stimuli. Evidence on the presence of PKC and casein kinase II in nuclei of actively growing cells and enzymes suggested the intriguing possibility that signal transduction events, controlled by sphingomyelin cycle, could also occur at the nuclear level.

We have shown that the activity of sphingomyelinase and level of sphingomyelin, ceramide and sphingosine in nuclei is changed during nucleic acid synthesis in regenerating liver [26,29,30,76]. Albi et al. [10,20] found changes in the activity of the enzyme and level of sphingomyelin in chromatin during DNA synthesis in liver after partial resection as well.

Our data demonstrate that the level of sphingosine (4–5 ng/mg of protein) in nuclei is very low compared with sphingomyelin (2 –4 μg/mg of protein). However, we found a pronounced accumulation of sphingosine in nuclei during activation of transcription, 4–6 h after partial hepatectomy [76] and replication, 20–24 h after operation [76]. Activation of sphingomyelinase located in the cell nuclei also occurs when transcription and replication are enhanced. Thus, activation of sphingomyelin cycle in nucleus of regenerating liver is observed during DNA and RNA synthesis. The changes in the level of the sphingomyelin cycle metabolites (ceramides and sphingosine) during nucleic acid synthesis are of a universal character, suggesting that the mechanism of their action is common for transcription and replication.

At present, sphingosine is known to activate casein kinase II [74], the substrates of which are RNA polymerases I and II, DNA topoisomerases I and II, non-histone chromatin proteins and a number of other proteins involved in the regulation of the cell cycle and proliferation [77]. Sphingosine involves the accumulation of phosphatidic acid, which possesses mitogenic properties and activates DNA synthesis in Swiss 3T3 cells under the action of sphingosine in concentration up to 10 μM [72]. Direct experiments have shown an intimate connection between sphingosine synthesis and cell growth. This has been shown with mutant CHO cells, which are deficient in sphingomyelin synthesis due to thermolability of their serine palmitoyltransferase [78].

It was shown that sphingosine influences on DNA synthesis [72] and activity of key enzymes taking part in replication and transcription. In our experiments, it was shown that sphingosine enhances the activity of RNA polymerases isolated from rat liver [76] and retards DNA methylation [51].

Sphingosine is likely to influence RNA and DNA synthesis by its ability to control phosphorylation of enzyme participating in nucleic acid synthesis and to interact with DNA and to alter its structure. The latest properties of sphingosine have been demonstrated by Manzoli et al. [44] with the method of heat denaturation.

We have also shown the existence of the interaction of sphingosine with DNA in vitro, using fluorescent and spin probes [76,79]. We found that sphingosine can prevent interaction of DNA with AO or even displace AO from its complex with DNA [76]. It means that sphingosine interacts with DNA and influences its structure. Similarly to polyamines, sphingosine has a primary amino group, which forms an ionic bound with the oxygen atom of the phosphate group in DNA molecules. These changes in the structure of DNA via its interaction with sphingosine lead to alteration of the DNA substrate specificity. Sphingosine inhibits DNA-primase in vitro and in vivo [80]. Sphingosine was found to complete with H1 histone for the binding to DNA [81]. Other lipids, such as phosphatidic acid and phosphatidylglycerol, firmly bound precisely with histones [81,82]. Sphingosine and its derivatives, e.g. sphingosylphosphoholine, sharply increased the DNA-binding activity of AP-1 transcriptional factor [83]. The increase in the binding activity of AP-1 preceded the accumulation of c-*fos* mRNA.

Thus, presented data contribute a great deal to the significance of the nuclear sphingomyelin cycle and its particular metabolites in the regulation of cell proliferation. We should mention that the mechanism proposed includes one product (sphingosine) with the ability to activate signal transduction controlled by casein kinase and inhibit pathways controlled by protein kinase C. Thus, sphingosine may emerge as a multifunctional regulator that is able to modulate the activity of many targets such as protein kinases, phosphatidic acid phosphohydrolase [84], diacylglycerol kinase [85] and phospholipase D [86–87] not only in cytoplasma but also in nuclei.

Cell surface receptors for TNF-alpha form one of the components of a specific signal transduction pathway. Phorbol ester-induced activation of protein kinase C decreases the binding activity of TNF-alpha receptors. The involvement of protein kinase C in regulation of TNF receptor activity points to the possible role of sphingosine in cell responses to TNF-alpha. In our experiments, it was shown that TNF-alpha activates sphingomyelinase and accumulates sphingosine in nuclei isolated from liver after injection of TNF to mice [47,49]. We found that activation of sphingomyelin cycle induced by TNF-alpha is an early response, more pronounced in the nuclei as compared with whole cells [49]. It is suggested that sphingomyelin metabolites are involved in transduction of TNF-elicited signals from the cell surface to the nucleus.

Metabolism of sphingomyelin is tightly connected with cholesterol metabolism [88]. Degradation of sphingomyelin by exogenous sphingomyelinase induces a decrease of cholesterol due to an increase of esterification biosynthesis. A relation between sphingomyelin and cholesterol in nuclear membranes following sphingomyelinase activation and during cell proliferation was shown [10]. The content of nuclear sphingomyelin and cholesterol was analyzed before and after sphingomyelinase activation and during hepatic regeneration. The activities of nuclear membrane SM-synthase and SMase were also determined. The results con-

firmed that activation of sphingomyelinase in nuclei of regenerating liver and exogenous sphingomyelinase causes a strong decrease in cholesterol [10]. Changes in the content of sphingomyelin are accompanied by similar behavior of cholesterol. Changes in cholesterol content modify the nuclear membranes fluidity and, as a consequence, mRNA transport [40]. The neutral sphingomyelinase present in the nuclei may, across this mechanism, regulate the cell function.

Phospholipids of nuclei may play crucial role in apoptosis. Apoptosis, programmed cell death, has been shown to play a key role in normal development, differentiation, glandular atrophy following hormonal withdrawal and maturation of the immune system [88]. Interference with apoptosis appears to be one of the mechanisms leading to unrestrained growth and development of cancer. Its hallmark biochemical feature is endonuclease activation, giving rise to internucleosomal DNA fragmentation. There are also characteristic morphological changes, including chromatin condensation, nuclear fragmentation, shrinkage, the formation of dense chromatin masses and apoptotic bodies [88]. Apoptosis is an active process, which is governed by a signal transduction pathway. Up-regulation of c-*myc*, c-*fos* and *p*53 genes is associated with transmission of the apoptotic signal.

Utilization of the sphingomyelin pathway for induction of the apoptotic response has already been demonstrated in a large variety of mammalian cells [89]. Sphingomyelin breakdown with an accumulation of ceramide and/or sphingosine in nuclei may be involved in induction of the apoptosis in vivo and in vitro [89–92].

Prior to the onset of apoptosis induced by portal vein branch ligation of lobes in rat liver, the activity of neutral sphingomyelinase increased in the nuclei of hepatocytes in ligated lobes in parallel with its reaction product, ceramide. This was followed by elevation of ceramidase activity in nuclei in association with an increase of sphingosine. These changes were not observed in nuclei of the nonligated lobes, or in the plasma membranes from either ligated or nonligated lobes [92].

We have shown that cycloheximide (CHI) in sublethal doses (0.3 mg/100 g of body weight) caused apoptosis in liver cells in vivo, inducing c-*myc*, c-*fos* and *p*53 genes, activation of sphingomyelinase and accumulation of free sphingosine in liver cells and nuclei [91]. It was shown that the enhancement of the sphingomyelinase activity precedes the maximum accumulation of mRNA of c-*myc* and c-*fos* genes. Sphingosine in nuclei accumulated concomitantly with an increase of c-*myc* and c-*fos* gene expression and exceeded the control values almost threefold. On the other hand, the level of free sphingosine in nuclei isolated from liver rats injected with CHI at doses (0.01 and 0.05 mg/100g of body weight) that did not increase gene expression did not show any elevation of sphingosine.

The protein products of c-*myc*, c-*fos* and *p*53 genes contribute significantly in transduction of the apoptotic signal. The activity of these proteins depends on PKC. On the other hand, sphingosine functions as one of the inhibitors of PKC, which strongly induce apoptosis in certain cell types. Recently, it was shown that sphingosine decreases bcl-2 expression at both RNA and protein levels [93]. Overexpression of bcl-2 is connected with inhibition of apoptosis in response to a wide spectrum of agents, like chemotherapeutic agents, TNF-alpha and ionizing radiation. Finally, nuclear sphingosine might be important in mediating apoptosis as an endogenous modulator of nuclear PKC and inhibitor of bcl-2 expression.

The last decade has been characterized by rapid development of research investigation of the molecular mechanisms whereby hormones, peptides, growth factors and cytokines regulate cell metabolism, differentiation and proliferation. One general signaling mechanism involves the rapid Ca^{2+} redistribution throughout the cell [94].

The mechanism of regulation of nuclear calcium signaling is vehemently debated currently [95–102]. Nuclear calcium signals control a variety of nuclear functions, including gene transcription [102], DNA synthesis [94], DNA repair, chromatin organization and cleavage of nuclear DNA by nucleases during programmed cell death or apoptosis [98–100]. There are indications that nuclear and cytosolic calcium signals are differentially regulated and are independent of each other [96]. For examples, nuclear calcium concentrations are higher than cytosolic ones in a number of cell systems or lower in smooth muscle and neuronal cells.

The nucleus contains an endoplasmic reticulum-type calcium pump ATPase, which attests to the ATP-mediated nuclear calcium uptake [96]. The nucleus is endowed with functional inositol triphosphate receptors and the necessary machinery for InsP [(3)] production [97]. The nucleus also contains inositol 1,3,4,5-tetraphosphate receptors, which mediates nuclear calcium entry in the presence of InsP [(4)]. InsP [(4)] receptor was located to the inner nuclear membrane. Mechanistic model developed proposed calcium movement in and out of the nucleus. Ca [Image]-ATPase and InsP [(4)] R are located to the outer nuclear membrane and thus intervene in the action of ATP and InsP [(4)], respectively, in eliciting calcium transport to the nuclear envelope. Calcium can be further released to the inner nuclear space by the activation of Imps [(3)] R (located to the inner membrane) by Imps [(3)]. Calcium can diffuse out from the nucleoplasm by the nuclear pores. There are specific Ca^{2+} release channels present in the inner nuclear membrane that can be activated by inositol triphosphate or cADP ribose [99,101].

In conclusion, InsP [(3)] is capable of controlling calcium concentration within the nucleus, which could be important for the control of specific types of gene expression.

Recently, the sphingosine-induced mobilization of Ca^{2+} from intracellular stores was shown [103]. It was found that sphingosine-gated Ca^{2+}-permeable channel is a novel chan-

nel distinct from other characterized intracellular Ca^{2+} channels such as the ryanodyne receptor and the inositol 1,4,5-triphosphate receptors [104,105]. This suggests that a new type of Ca^{2+} channel should exist which is controlled by sphingosine.

It was shown that sphingosine and its derivators influence at Ca^{2+} mobilization in the nuclei. The dose–response relationship for the peak response of Ca^{2+} concentration in nuclei to sphingosine and psychosine was analyzed [106]. Nucleoplasmic Ca^{2+} concentration increased at 15 mM agonist and reached the maximum response at 50 mM lipid concentration. The sphingosine was the most effective lipid assayed. The highest concentrations (75 mM) evoked an increase in Ca^{2+} concentration from 50 to 350 nM. The sphingolipid concentrations used in these experiments were similar to those described as effective in several cell types [103]. It is possible that sphingolipids mobilize Ca^{2+} through a modulatory action on the IP3 pathway [10], or that they could act in a completely independent manner, as recently suggested for sphingosine in fibroblasts [24,107].

5. Conclusion

It has been widely reported that lipids exist as factors bound to chromatin and nuclear matrix components in a number of cell types. The effect of several phospholipids on DNA and chromatin structure and function has been investigated in cell-free systems by exposure of isolated DNA, chromatin or nuclei to phospholipids vesicles. In addition, stimulatory effects of inositol lipids and sphingolipids on isolated DNA polymerase α and RNA polymerases activities have been reported.

Evidence on the occurrence of phospholipid-calcium-dependent protein kinase C (PKC) in the nucleus seems to present a direct link between the presence and possible functions of nuclear lipids, phosphatidylinositol, sphingosine, phosphatidylserine and diacylglycerol, which could regulate the activity of this enzyme controlling the phosphorylation of nuclear proteins. In conclusion, Imps [(3)] and sphingosine are capable of controlling calcium concentration within the nucleus, which could be important for the control of specific types of gene expression during cell growth, differentiation and apoptosis.

The discovery of inositide and sphingomyelin signaling systems in nuclei shows what messages transfer the signals from the cell surface to the nucleus, and how those messages transmit their effect.

References

[1] R.R. Baker, H.Y. Chang, The incorporation of radioactive fatty acids into the phospholipids of nerve-cell-body membranes in vivo. Evidence for highly labelled neuronal nuclei, Biochem. J. 188 (1980) 153–161.
[2] R.R. Baker, H.Y. Chang, Phosphatidylinositol synthase activities in neuronal nuclei and microsomal fractions isolated from immature rabbit cerebral cortex, Biochim. Biophys. Acta 1042 (1990) 55–61.
[3] R.F. Irvine, N. Divecha, Phospholipids in the nucleus-metabolism and possible functions, Cell Biol. 3 (1992) 225–235.
[4] L. Cocco, A.M. Martelli, R.S. Gilmour, A. Ognibene, F.A. Manzoli, R.F. Irvine, Rapid changes in phospholipid metabolism in the nuclei of Swiss 3T3 cells induced by treatment of the cells with insulin-like growth factor I, Biochem. Biophys. Res. Commun. 154 (1988) 1266–1272.
[5] G.N. Filippova, O.V. Borovkova, A.V. Alessenko, Comparison of lipid metabolism in rat liver cells and nuclei during cycloheximide-induced superinduction of nuclear oncogenes, Biokhimia (Moscow) 56 (1991) 892–902.
[6] P.B. Gahan, Histochemical evidence for the presence of lipids on the chromosomes of animal cells, Exp. Cell Res. 39 (1965) 136–144.
[7] A. Fraschini, E. Albi, P.B. Gahan, M.P. Viola Magni, TEM cytochemical study of localization of phospholipids in interphase chromatin in rat hepatocytes, Histochemistry 97 (1992) 225–235.
[8] N.M. Maraldi, N. Zini, S. Squarzoni, R. Del Coco, P. Sabatelli, F.A. Manzoli, Internuclear localization of phospholipids by ultrastructural cytochemistry, J. Histochem. Cytochem. 40 (1992) 1383–1392.
[9] E. Albi, M.V. Magni, Sphingomyelin synthase in rat liver nuclear membrane and chromatin, FEBS Lett. 460 (1999) 369–372.
[10] E. Albi, I. Peloso, M.V. Magni, Nuclear membrane sphingomyelin-cholesterol changes in rat liver after hepatectomy, Biochem. Biophys. Res. Commun. 262 (1999) 692–695.
[11] L. Cocco, R.S. Gilmour, A. Ognibene, A.J. Letcher, F.A. Manzoli, R.F. Irvine, Synthesis of polyphosphoinositides in nuclei of Friend cells. Evidence for polyphosphoinositide metabolism inside the nucleus which changes with cell differentiation, Biochem. J. 248 (1987) 765–770.
[12] N. Zini, A.M. Martelli, L. Cocco, F.A. Manzoli, N.M. Maraldi, Phosphoinositidase C isoforms are specifically localized in the nuclear matrix and cytoskeleton of Swiss 3T3 cells, Exp. Cell Res. 208 (1993) 257–269.
[13] B. Payrastre, M. Nievers, J. Boonstra, M. Breton, A.J. Verkleij, P.M.P. van Bergen, E.N. Henegouwen, A differential localization of phosphoinositide kinase, diacylglycerol kinase, and phospholipase C in the nuclear matrix, J. Biol. Chem. 287 (1992) 5078–5084.
[14] K. Tamiya-Koizumi, H. Umekawa, S. Yoshida, H. Ishihara, K. Kojima, A novel phospholipase A_2 associated with nuclear matrix: stimulation of the activity and modulation of the Ca^{2+} dependency by polyphosphoinositides, Biochim. Biophys. Acta 1002 (1989) 182–188.
[15] S. Capitani, V. Bertagnolo, M. Mazzoni, P. Santi, M. Previati, A. Antonucci, F.A. Manzoli, Lipid phosphorylation in isolated rat liver nuclei. Synthesis of polyphosphoinositides at subnuclear levels, FEBS Lett. 245 (1989) 194–198.
[16] F.A. Manzoli, N.M. Maraldi, L. Cocco, S. Capitani, A. Facchini, Chromatin phospholipids in normal and chronic lymphocyte leukemia lymphocytes, Cancer Res. 37 (1977) 843–849.
[17] F.A. Manzoli, S. Capitani, G. Mazzotti, O. Barnabei, N.M. Maraldi, Role of chromatin phospholipids on template availability and ultrastructure of isolated nuclei, Adv. Enzyme Regul. 20 (1982) 247–262.
[18] T.P. Kulagina, S.A. Shuruta, I.K. Kolomiitseva, V.I. Popov, Difference in metabolic pools of cholesterol and free fatty acids of rat thymocyte chromatin and nuclear membranes, Mol. Biol. (Moscow) 28 (1994) 714–719.
[19] E. Albi, M. Mersel, C. Leray, M.L. Tomassoni, M.P. Viola Magni, Rat liver chromatin phospholipids, Lipids 29 (1994) 715–719.
[20] E. Albi, M.P. Viola Magni, P.B. Gahan, Chromatin phospholipids changes during rat liver development, Cell Biochem. Funct. 9 (1991) 119–123.
[21] M.P. Viola Magni, P.B. Gahan, E. Albi, P.F. Gentillucci, Synthesis of chromatin phospholipids, Basic Appl. Histochem. 31 (1987) 355–364.

[22] M.P. Viola Magni, P.B. Gahan, E. Albi, R. Iapoce, P.F. Gentilucci, Phospholipids in chromatin: incorporation of [^{32}P2-O] in different subcellular fraction of hepatocytes, Cell Biochem. Funct. 4 (1986) 283–288.
[23] E. Albi, M. Viola Magni, Phosphatidylcholine-dependent phospholipase C in rat liver chromatin, Biochem. Biophys. Res. Commun. 265 (1999) 640–643.
[24] A.V. Alessenko, Functions of sphingosine in cell proliferation and death, Biochemistry (Mosc.) 63 (1998) 62–68.
[25] A.V. Alessenko, N.A. Babenko, M.Yu. Pushkareva, V.N. Nikitin, A comparative evaluation of the level of sphingomyelinase activity in liver cell nuclei and in the brain, Biull. Eksp. Biol. Med. 108 (1989) 287–289.
[26] A.V. Alessenko, S. Chatterjee, Neutral sphingomyelinase: localization in rat liver nuclei and involvement in regeneration/proliferation, Mol. Cell Biochem. 143 (1995) 169–174.
[27] F.A. Manzoli, L.A. Cocco, A. Facchini, A.M. Carali, N.M. Maraldi, C.E. Grossi, Phospholipids bound to acidic nuclear proteins in human B and T lymphocytes, Mol. Cell Biochem. 12 (1976) 67–71.
[28] D. Allan, R.J. Raval, Sphingomyelinase-resistant pool of sphingomyelin in the nuclear membrane, Biochim. Biophys. Acta 897 (1987) 355–363.
[29] A.V. Alessenko, E.A. Pantaz, Differences in the composition of nuclear and chromatin phospholipids in proliferating rat liver cells after partial hepatectomy, Biokhimiya (Moscow) 48 (1983) 263–268.
[30] M.Yu. Pushkareva, O.V. Borovkova, A.V. Alessenko, Study of sphingomyelinase activity and sphingomyelin and ceramide level comparison with other lipid fractions of the cell nucleus in regenerating rat liver, Biokhimiya (Moscow) 56 (1991) 903–912.
[31] L. Cocco, A.M. Martelli, A.M. Billi, A. Cataldi, S. Miscia, M.R. Mottola, L. Manzoli, Phospholipids as components of the nuclear matrix: their possible biological significance, Basic Appl. Histochem. 31 (1987) 413–419.
[32] L. Cocco, N.M. Maraldi, F.A. Manzoli, Phospholipid interaction in rat liver nuclear matrix, Biochim. Biophys. Res. Commun. 96 (1980) 890–898.
[33] A.V. Alessenko, V.A. Krasil'nikov, P.Ya. Boikov, Participation of sphingomyelin in the formation of a DNA bond with the nuclear matrix in the replication process, Dokl. Akad. Nauk SSSR 264 (1983) 231–234.
[34] A.V. Alessenko, V.A. Krasil'nikov, P.Ya. Boikov, Phospholipids as structural elements of the nuclear matrix, Dokl. Akad. Nauk SSSR 263 (1982) 730–733.
[35] S. Miscia, A. Cataldi, A. Ognibene, A.M. Martelli, L. Manzoli, A.M. Billi, C. De Marchis, L. Cocco, Evidence for a reduction of the replicative activity of matrix-bound DNA polymerase alpha following treatment with phospholipase C, Cell Biol. Int. Rep. 12 (1988) 347–354.
[36] A.V. Alessenko, Role of lipids in functional activity of cell nuclei, in: J.R. Harris, I.B. Zbarsky (Eds.), Nuclear Structure and Function, Plenum, 1990, pp. 399–404.
[37] M. Splanger, M.L. Coatzee, S.L. Katyal, H.P. Morris, P. Ove, Some biochemical characteristics of rat liver and Morris hepatoma nuclei and nuclear membranes, Cancer Res. 35 (1975) 3131–3145.
[38] S. Capitani, L. Cocco, N.M. Maraldi, S. Papa, F.A. Manzoli, Effects of phospholipids on transcription and ribonucleoprotein processing in isolated nuclei, Adv. Enzyme Regul. 25 (1986) 425–438.
[39] M. Micheli, E. Albi, C. Leray, M.V. Magni, Nuclear sphingomyelin protects RNA from RNase action, FEBS Lett. 431 (1998) 443–447.
[40] E. Albi, M. Micheli, M.P. Viola Magni, Phospholipids and nuclear RNA, Cell Biol. Int. 20 (1996) 407–412.
[41] F.A. Manzoli, N.M. Maraldi, S. Capitani, Effect of phospholipids on the control of nuclear DNA template restriction, in: J.J. Kabara (Ed.), The Pharmacological Effect of Lipids II, AOCS Publisher, Champaign, IL, 1985, pp. 133–156.
[42] L. Cocco, R.S. Gilmour, N.M. Maraldi, S. Papa, F.A. Manzoli, Increase of globin RNA synthesis induced by phosphatidylserine liposomes in isolated erythroleukemic cell nuclei. Morphological and functional features, Biol. Cell 54 (1985) 49–56.
[43] N. Zini, N.M. Maraldi, A.M. Martelli, A. Antonucci, P. Santi, G. Mazzikti, R. Rizzoli, F.A. Manzoli, Phospholipase C digestion induces the removal of nuclear RNA: a cytochemical quantitative study, Histochem. J. 21 (1989) 491–500.
[44] F.A. Manzoli, J.H. Machmore, B. Bonora, S. Capitani, S. Bartoli, Lipid–DNA interactions II. Phospholipids, cholesterol, glycerophosphocholine, sphingosine and fatty acids, Biochim. Biophys. Acta 34 (1974) 1–15.
[45] F.A. Manzoli, S. Capitani, L. Cocco, N.M. Maraldi, G. Mazzotti, O. Barnabei, Lipid metabolism signal transduction in the cell nucleus, Adv. Enzyme Regul. 27 (1988) 83–91.
[46] N.M. Maraldi, S. Marmiroli, L. Cocco, S. Capitani, O. Barnabei, F.A. Manzoli, Nuclear lipid-dependent signal transduction in human osteosarcoma cells, Adv. Enzyme Regul. 37 (1997) 351–375.
[47] G.P. Zhizhina, V.G. Korobko, A.V. Alessenko, Correlation of DNA degradation, induced by tumor necrosis factor-alpha with accumulation of sphingosine in the nucleus and peroxides in DNA, Biokhimiya (Moscow) 59 (1994) 1756–1765.
[48] A.V. Alessenko, P.Ya. Boikov, L.B. Drobot, S.A. Rusakov, G.N. Filippova, Changes in the level of sphingosine in rat liver nuclei and cells during oncogene superexpression induced by cycloheximide, Biokhimiya (Moscow) 59 (1994) 1076–1087.
[49] S.A. Rusakov, G.N. Filippova, M.Yu. Pushkareva, Korobko, A.V. Alessenko, The effect of tumor necrosis factor on the free sphingosine level and sphingomyelinase in murine liver cells and nuclei, Biokhimiya (Moscow) 58 (1993) 724–732.
[50] A.V. Alessenko, E.B. Burlakova, E.A. Pantaz, Effect of sphingomyelin on RNA-polymerase activity in the cell nucleus of normal and regenerating rat liver, Biokhimiya (Moscow) 49 (1984) 621–628.
[51] E.B. Romanenko, A.V. Alessenko, B.F. Vanyushin, Effect of sphingomyelin and antioxidants on the in vitro and in vivo DNA methylation, Biochem. Mol. Biol. Int. 35 (1995) 87–94.
[52] L. Cocco, A.M. Martelli, A.M. Billi, A. Matteucci, M. Vitale, L.M. Neri, F.A. Manzoli, Changes in nucleosome structure and histone H3 accessibility. Iodoacetamidofluorescein labelling after treatment with phosphatidylserine vesicles, Exp. Cell Res. 166 (1986) 465–474.
[53] L. Cocco, S. Papa, N.M. Maraldi, P. Santi, A.M. Martelli, R. Rizzoli, F.A. Manzoli, Chromatin structural transition following histone H1 displacement by phosphatidylserine vesicles and low pH treatment. A multiparametric analysis involving flow cytometry, electron microscopy and nuclease digestion, J. Histochem. Cytochem. 36 (1988) 65–71.
[54] V.L. Sylvia, C.O. Joe, J.O. Norman, G.M. Curtin, D.L. Busbee, Phosphatidylinositol-dependent activation of DNA polymerase alpha, Biochem. Biophys. Res. Commun. 135 (1986) 880–885.
[55] S. Capitani, P.R. Girard, G.J. Mazzei, J.F. Kuo, R. Berezney, F.A. Manzoli, Immunochemical characterization of protein kinase C in rat liver nuclei and subnuclear fractions, Biochem. Biophys. Res. Commun. 142 (1987) 367–375.
[56] T.P. Thomas, H.S. Talwar, W.B. Anderson, Phorbol ester-mediated association of protein kinase C to the nuclear fractions in NIH 3T3 cells, Cancer Res. 48 (1988) 1910–1919.
[57] A.N. Malviya, C. Block, Nuclear protein kinase C and signal transduction, Receptor 3 (1993) 275.
[58] A.V. Alessenko, W.A. Khan, W.C. Wetsel, Y.A. Hannun, Selective changes in protein kinase C isoenzymes in rat liver nuclei during liver regeneration, Biochem. Biophys. Res. Commun. 182 (1992) 1333–1339.
[59] P. Hornbeck, K.-P. Hung, W.E. Paul, Lamin B is rapidly phosphorylated in lymphocytes after activation of protein kinase C, Proc. Natl. Acad. Sci. U. S. A. 85 (1988) 2279–2283.
[60] N. Sahvoun, M. Wolf, J. Berterman, T. Hsieh, M. Sander, H. LeVine, K.-J. Chang, P. Cuatracasas, Protein kinase C phosphorylates topoisomerase II: topoisomerase activation and its possible role in phorbol

ester-induced differentiation in HL-60 cells, Proc. Natl. Sci. U. S. A. 83 (1986) 1603–1604.
[61] A.M. Martelli, R.S. Gilmour, E. Falcieri, F.A. Manzoli, L. Cocci, Mitogen stimulated phosphorylation of nuclear proteins in Swiss 3T3 cells. Evidence for a protein kinase C requirement, Exp. Cell Res. 185 (1989) 191–202.
[62] A.P. Fields, G. Tylor, A.S. Kraft, W.S. May, Role of nuclear protein kinase C in the mitogenic response to platelet-derived growth factor, J. Cell Sci. 96 (1990) 107–114.
[63] P. Rogue, G. Labourdette, A. Masmandi, Y. Yoshida, F.L. Huang, K.P. Huaqng, J. Zwiller, G. Malviya, Rat liver nuclei protein kinase C is the isotype II, J. Biol. Chem. 256 (1990) 4161–4165.
[64] N. Divecha, H. Banfie, R.F. Irvine, The polyphosphoinositide cycle exists in the nuclei of Swiss 3T3 cells under the control of a receptor (for IGF-I) in the plasma membrane and stimulation of the cycle increases nuclear diacylglycerol and apparently induces translocation of protein kinase C to the nucleus, EMBO J. 10 (1991) 3207–3214.
[65] O. Trubiani, A.M. Martelli, L. Manzoli, E. Santavenere, L. Cocco, Nuclear lipids in Friend cells. Shifted profile of diacylglycerol during erythroid differentiation induced by DMSO, Cell Biol. Int. Rep. 14 (1990) 559–566.
[66] A.R. Buckley, P.D. Crowe, D.H. Russel, Rapid activation of protein kinase C in isolated rat liver nuclei by prolactin, a known hepatic mitogen, Proc. Natl. Acad. Sci. U. S. A. 85 (1988) 8649–8653.
[67] M. Mason-Garcia, C.L. Weill, B.S. Beckman, Rapid activation by erythropoietin of protein kinase C in nuclei of erythroid progenitor cells, Biochem. Biophys. Res. Commun. 168 (1990) 490–497.
[68] Y.A. Hannun, C. Luberto, Ceramide in the eukariotic stress response, Trends Cell Biol. 10 (2000) 73–80.
[69] Y.A. Hannun, R.M. Bell, Lysosphingolipids inhibit PKC-implications for the sphingolipidoses, Science 235 (1987) 670–674.
[70] Y.A. Hannun, C.R. Loomis , A.H. Merrill Jr., R.M. Bell, Sphingosine inhibition of protein kinase C activity and of phorbol dibutyrate binding in vitro and in human platelets, J. Biol. Chem. 261 (1986) 12604–12609.
[71] A.H. Merrill Jr., A.M. Sereni, V.L. Stevens, Y.A. Hannun, R.M. Bell, J.M. Kinkade Jr., Inhibition of phorbol-ester-dependent differentiation of human promyelocytic (HL-60) cells by sphinganine and other long-chain bases, J. Biol. Chem. 261 (1986) 12610–12615.
[72] H. Zhang, N.E. Buckley, K. Gibson, S. Spiegel, Sphingosine stimulates cellular proliferation via a protein kinase C-independent pathway, J. Biol. Chem. 265 (1990) 76–81.
[73] M. Faucher, N. Girones, Y.A. Hannun, R.M. Bell, R.J. Davis, Regulation of the epidermal growth factor receptor phosphorylation state by sphingosine in A 431 human epidermoid carcinoma cells, J. Biol. Chem. 263 (1988) 5319–5327.
[74] O.B. Mc Donalds, Y.A. Hannun, C.H. Reynolds, N. Sahyoun, Activation of casein kinase II by sphingosine, J. Biol. Chem. 266 (1991) 21773–21776.
[75] M. Pushkareva, W. Khan, A. Alessenko, N. Sahyoun, Y.A. Hannun, Sphingosine activation of protein kinase in Jurkat T cells. In vitro phosphorylation of endogenous protein substrates and specificity of action, J. Biol. Chem. 267 (1992) 15246–15251.
[76] A.V. Alessenko, Functional role of the sphingomyelin cycle in cell nuclei during activation of cell proliferation and oncogenes expression, in: G. Cevc, F. Paltant (Eds.), Phospholipids: Characterization, Metabolism and Novel Biological Applications, AOCS Press, Champaign, IL, 1995, pp. 299–310.
[77] P.A. Gruppuso, J.M. Boylan, Developmental changes in the activity and cellular localization of hepatic casein kinase II in the rat, J. Cell Biochem. 58 (1995) 65–72.
[78] Y. Aridor-Piterman, Y. Lavie, M. Liscovitch, Bimodal distribution of phosphatidic acid phosphohydrolase in NG 108-15 cells. Modulation of the amphiphilic lipids oleic acid and sphingosine, Eur. J. Biochem. 204 (1992) 561–568.
[79] K. Hanada, M. Nishijima, M. Kisio, A. Hasegawa, S. Fujita, T. Ogawa, Y. Akamatsu, Sphingolipids are essential for the growth of Chinese hamster ovary cells. Restoration of the growth of a mutant defective in sphingoid base biosynthesis by exogenous sphingolipids, J. Biol. Chem. 267 (1992) 23527–23533.
[80] V.A. Krasil'nikov, E.M. Mill, A.V. Alessenko, V.M. Biniukov, Comparison of DNA interaction with sphingomyelin, spermine and magnesium ions using a spin label method, Biofizika (Mosk.) 34 (1989) 953–957.
[81] K. Tamiya-Koizumi, T. Murate, M. Suzuki, C.M. Simbulan, M. Nakagawa, M. Takemura, K. Furuta, S. Izuta, S. Yoshida, Inhibition of DNA primase by sphingosine and its analogues parallels with their growth suppression of cultured human leukemic cells, Biochem. Mol. Biol. Int. 41 (1997) 1179–1189.
[82] A. Koiv, J. Paavo, P.K. Kinnunen, Evidence for ternary complex formation by histone H1, DNA, and liposomes, Biochemistry 34 (1995) 8018–8027.
[83] A. Koiv, P.K. Kinnunen, Binding of DNA to liposomes contaning different derivatives of sphingosine, Chem. Phys. Lipids 72 (1994) 77–78.
[84] A. Berger, D. Rozenthal, S. Spiegel, Sphingosylphosphocholine, a signaling molecule which accumulates In Niemann-Pick disease type A, stimulates DNA-binding activity of the transcription activator protein AP-1, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 5885–5889.
[85] T.J. Mullmann, M.I. Siegel, R.W. Egan, M.M. Billah, Sphingosine inhibits phosphatidate phosphohydrolase in human neutrophils by PKC-independent mechanism, J. Biol. Chem. 266 (1991) 2013–2016.
[86] I. Sakane, K. Yamada, H. Kanoh, Different effects of sphingosine, R59022 and anionic amphiphiles on two diacylglycerol kinase isoenzymes purified from porcine thymus cytosol, FEBS Lett. 255 (1989) 409–413.
[87] Y. Lavie, M. Liscovitch, Activation of phospholipase D by sphingoid bases in NG108-15 neural-derived cells, J. Biol. Chem. 265 (1990) 3868–3872.
[88] S. Chatterjee, Neutral sphingomyelinase action stimulates signal transduction of tumor necrosis factor—in the synthesis of cholesteryl esters in human fibroblasts, J. Biol. Chem. 269 (1994) 879–882.
[89] M.J. Arends, A.H. Wyllie, Apoptosis: mechanisms and roles in pathology, Int. Rev. Exp. Pathol. 32 (1991) 223–254.
[90] Y.A. Hannun, L.M. Obeid, Mechanisms of ceramide-mediated apoptosis, Adv. Exp. Med. Biol. 407 (1997) 145–149.
[91] A.V. Alessenko, A.V. Khrenov, Role of sphingosine in induced apoptosis, Lipids 34 (1999) S75–S76.
[92] A.V. Alessenko, P.Ya. Boikov, G.N. Filippova, A.V. Khrenov, A.S. Loginov, E.D. Makarieva, Mechanisms of cycloheximide-induced apoptosis in liver cells, FEBS Lett. 416 (1997) 113–116.
[93] K. Tsugane, K. Tamiya-Koizumi, M. Nagino, Y. Nimura, S. Yoshida, A possible role of nuclear ceramide and sphingosine in hepatocyte apoptosis in rat liver, J. Hepatol. 31 (1999) 8–17.
[94] C. Sakakura, E.A. Sweeney, T. Shirahama, S. Hakomori, Y. Igarashi, Suppression of bcl-2 gene expression by sphingosine in the apoptosis of human leukemic HL-60 cells during phorbol ester-induced terminal differentiation, FEBS Lett. 379 (1996) 177–180.
[95] B.A. White, C. Bancroft, in: W.Y. Cheung (Ed.), Calcium and Cell Functions, Academic Press, New York, 1987, pp. 109–132.
[96] O.H. Peterson, O.V. Gerasimenko, J.V. Gerasimenko, A.V. Tepikin, The calcium store in the nuclear envelope, Cell Calcium 23 (1998) 87–96.
[97] O.V. Gerasimenko, J.V. Gerasimenko, A.V. Tepikin, O.H. Peterson, Calcium transport pathways in the nucleus, Pflugers Arch. 432 (1996) 1–6.
[98] J.-P. Humbert, N. Matter, J.-C. Artault, P. Kuppler, A.N. Malviya, Discrete distribution of inositol phosphate receptors to inner and outer nuclear membranes, J. Biol. Chem. 271 (1996) 478–485.
[99] M. Yamagushi, K. Oishi, Effect of nuclear Ca^{2+} uptake inhibitors on Ca^{2+}-activated DNA fragmentation in rat liver nuclei, Mol. Cell Biochem. 148 (1995) 33–37.
[100] P. Nicotera, B. Zhivotovsky, S. Orrenius, Nuclear calcium transport and the role of calcium in apoptosis, Cell Calcium 16 (1994) 279–288.

[101] P. Nicotera, A.D. Rossi, Nuclear Ca^{2+}: physiological regulation and role in apoptosis, Mol. Cell. Biochem. 135 (1994) 89–98.
[102] N.L. Allbritton, E. Oancea, M.A. Kuhn, T. Meyer, Source of nuclear calcium signals, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 12458–12462.
[103] J.S. Gilchrist, M.P. Czubryt, G.N. Pierce, Calcium and calcium-binding proteins in the nucleus, Mol. Cell Biochem. 135 (1994) 79–88.
[104] R.A. Sabbadini, R. Betto, A. Teresi, G. Fachechi-Cassano, G. Salviati, The effects of sphingosine on sarcoplasmic reticulum membrane calcium release, J. Biol. Chem. 267 (1992) 15475–15484.
[105] S. Kim, V. Lakhani, D.J. Costa, A.I. Sharara, J.G. Fitz, L.W. Huang, K.G. Peters, L.A. Kindman, Sphingolipid-gated Ca^{2+} release from intracellular stores of endothelial cells is mediated by a novel Ca^{2+}-permeable channel, J. Biol. Chem. 270 (1995) 5266–5269.
[106] R.E. Catalan, B.G. Miguel, M.C. Calcerrada, S. Ruiz, A.M. Marti Bnez, Sphingolipids increase calcium concentration in isolated rat liver nuclei, Biochem. Biophys. Res. Commun. 238 (1997) 347–350.
[107] R. Liu, M.C. Farach-Carson, N.J. Karin, Effects of sphingosine derivatives on MC3T3-E-1 pre-osteoblasts:psychosine elicits release of calcium from intracellular stores, Biochem. Biophys. Res. Commun. 214 (1995) 676–684.