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Nuclear Lipids and Cell Fate

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ABSTRACT

Lipids are known not only as components of cell membranes but also as part of the nuclear fraction. In the nucleus lipids are present with a specific composition and metabolism and their behaviour, in different physiological and pathological conditions, is completely independent from that of the cell membrane. The nuclear lipid fraction is constituted by glycerolphospholipids, sphingolipid and cholesterol which have an active metabolism thanks to the presence of lipid enzymes responsible for their synthesis and catabolism. Nuclear lipid metabolites are in equilibrium when the cells are resting whereas, when the cells are submitted to various stimuli, they interact in different ways in relation to cell type and localization: nuclear membrane, nuclear matrix and chromatin, playing different structural and/or functional roles. The focus of this mini-review is to highlight the implications of nuclear lipid metabolism on the cell fate regulation when a proliferative or apoptotic stimulus is applied.

Keywords: apoptosis, cell cycle, cholesterol, differentiation, phosphatidylinositol, phosphatidylcholine, proliferation, sphingomyelin
Abbreviations: CE, ceramide; **CHO**, cholesterol; **DAG**, diacylglycerol;dsRNA, double strand RNA; **GST**, glutathione-*S*-transferase; **IP3**, inositol 1,4,5 triphosphate, **NGF**, Nerve Growth Factor; **N-SMase**, neutral sphingomyelinase; **PC**, phosphatidylcholine; **PC-PLD**, phosphatidylcholine dependent phospholipase D; **PH**, pleckstrin homology; **PI**, phosphatidylinositol; **PI-PLC**, phosphatidylinositol-specific phospholipase C; **PK3**, phosphoinositide-3-kinase; **PLs**, phospholipids; **PS**, phosphatidylserine; **PtdIns**, phosphoinositides; **PTEN**, Phosphatase and tensin homolog deleted on chromosome ten; **R-SM-synthase**, reverse sphingomyelin-synthase; **SM**, sphingomyelin; **SM-synthase**, sphingomyelin-synthase

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INTRODUCTION

For many years the lipids were considered as essential structural elements of cell membranes or as a source of energy. Numerous studies have shown that lipids are present at the nuclear level where they play different roles in relation to their localization: nuclear membrane, nuclear matrix and chromatin. In the nuclear membrane, constituted by an outer and inner leaflet separated by the perinuclear space, the communication between cytoplasm and nucleoplasm is controlled by nuclear pores which perforate the double membrane and regulate the bidirectional traffic of ions, proteins, nucleotides and RNA (Tomassoni *et al*. 1999). It has been demonstrated by using specific fluorescent probes, diphenylhexatriene and its cationic derivative trimethylammonium-diphenylexatriene, that the lipid composition is responsible for the nuclear membrane permeability and fluidity (Albi *et al*. 1997). Conversely, the lipid composition of the nuclear matrix is fundamental for maintaining its rigidity, making this structure an anchor for the organization of nuclear chromatin and the regulation of many important intra-nuclear biochemical events (Albi and Viola Magni 2003a). Instead the presence of the lipids in chromatin has been the object of discussion for many years. The first observations were made by Chayen *et al*. (1957, 1959) on plant cells by using 3,4-benzpyrene and the acid-hematein reaction on tissue fixed in calcium-formaldehyde-Reinecke solution. Gahan (1965) confirmed these results with a histochemical study on chromosomes of animal cells. The data have been supported by biochemical analysis made on nucleohistones isolated from calf thymus which have demonstrated a high amount of the sphingomyeline (SM, Chayen and Gahan 1958; Chayen *et al*. 1959). Although these results have been strongly questioned (Gurr *et al*. 1963), following other biochemical evidences have shown the presence of neutral lipids and phospholipids (PLs) in chromatin isolated from a large variety of tissues (Jackson *et al*. 1968; Shepherd *et al*. 1970; Viola Magni *et al*. 1985) including many tumour cells (Awad and Spector 1976; Manzoli *et al*. 1977). However, the biochemical evidences were not convincing and the presence of lipids in the chromatin was generally criticized. Tata *et al*. (1972) and Garrod and Hancock (1985) thought that the presence of lipids in isolated chromatin is due to contamination from the membranes during the extraction procedure and as a consequence their presence must be considered an index of ina-

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dequate chromatin preparation. But, the clear demonstration that lipids are really present at the chromatin level and that they do not derive from the nuclear membrane, , was given from Albi *et al*. (1994) by using the radioiodination of the fatty acids tecnique. The rat hepatocyte nuclei were isolated and radioiodinated by the lactoperoxidase method under saturating and nonsaturating conditions and the radioactivity associated with the PLs present in the nuclei and in the extracted chromatin was monitored. In both conditions the lipid extract from the chromatin do not appear labelled excluding the probability of the membrane contamination. If the radioiodination was made directly on the chromatin, the labelling was present in the lipid extract. The quantitative analysis has demonstrated that chromatin-associated lipids represent 10% of the amount present in the entire nucleus and differ in composition from those present in the whole nuclei (**Table 1**). Also the turnover is different from the microsomal and nuclear membrane lipids (Albi and Viola Magni 2004). The independence of chromatin lipids from those localised in the various cell sites resulted in them being described as a new "minor component of chromatin" (Albi and Viola Magni 2004). These observations justified previous results that showed modifications of the chromatin lipid fraction during cell proliferation (Viola Magni *et al*. 1985) and maturation (Albi *et al*. 1991) were not evident in microsomal and nuclear membrane lipids. The localization of PLs in the chromatin has been confirmed by a cytochemical study (Fraschini *et al*. 1992). During the last ten years many studies highlighted that an active lipid metabolism is present in the nucleus, independent of that from other cellular membranes, that generates intranuclear second messengers (D'Santos *et al*. 2000; Cocco *et al*. 2001; Albi and Viola Magni 2004).

Table 1 Comparison of the amounts of the different lipids in hepatocyte nuclei and purified chromatin (based on Albi *et al*. 1994).

	Nuclei	Chromatin
Phospholipid	123.9 ± 10.1	12.5 ± 3.6
Phosphatidylethanolamine	25.6 ± 2.3	2.5 ± 0.8
Phosphatidylserine	3.2 ± 0.5	0.7 ± 0.3
Phosphatidylinositol	14.5 ± 2.4	2.8 ± 0.7
Phosphatidylcholine	68.5 ± 13.2	9.5 ± 0.5
Sphingomyelin	6.5 ± 0.5	2.4 ± 1.0

Values are given in nmol/mg DNA and represent the mean ± D.S of six experiments.

INTRANUCLEAR GLYCEROPHOSPHOLIPIDS

The most important glycerophospholipids in the nucleus are phosphatidylinositol (PI) and phosphatidylcholine (PC) since they represent the source of important second messengers (Divecha and Irvine 1995).

Phosphatidylinositol

Viola Magni *et al*. (1985) have demonstrated that PI present at the chromatin level is 10% of the total chromatin PLs. In rat hepatocytes, this lipid increases during cell differentiation (Albi *et al*. 1991; Rubbini *et al*. 1997) whereas its catabolism is stimulated during cell proliferation or apoptosis (Albi 2003a) PI is the substrate of phos-phatidylinositol-dependent phospholipase C (PI-PLC). Its hydrolysis produces two fundamental second messengers: inosi-tol 1,4,5 trisphosphate (1,4,5 P3) and diacylglycerol (DAG). Several distinct PI-PLC isoforms have been purified from a variety of mammalian tissues and a total of 16 amino acid sequences (14 mammalian enzymes and 2 *Drosophila* enzymes) have been found from the nucleotide sequences of their corresponding cDNAs (Rhee 1989). PI-PLC β1 isoform is specifically located in the nucleus (Divecha *et al*. 1993). Two variants of the β1 isoform "a" and "b" were identified in quiescent fibroblasts (Cocco *et al*. 1999) and in rat hepatocytes (Neri *et al*. 1997). These were discovered in different sites: the first, variant a, is localized in the cytoplasm while variant b is present in the nucleus, similar to variant γ1 and δ1 (Liu *et al*. 1996). The use of specific lipid-binding domains known as pleckstrin homology (PH) of the PI-PLC δ1 fused with glutathione-*S*-transferase (GST) to form a probe PI-PLC δ1PH-GST has revealed a nuclear labelling that corresponds to 17-21% of the total cellular labelling (Lemmon and Ferguson 2000). Immunocytochemical analysis showed that isoform β1 is associated exclusively to the chromatin, colocalising with DNA replication sites, whereas isoform γ1 is present in the nuclear membrane (Albi and Viola Magni 2004). These isoforms present different chemico-physical characteristics and play different roles in cell cycle progression; isoform β1 should trigger DNA replication whereas isoform γ1 should be involved in the G2/M cell phase transition (Albi and Viola Magni 2004).

Formerly, Banfic *et al*. (1993) hypothesized that nuclear inositol has a regulatory function and is a crucial element of DNA replication and of the genetic expression during cell proliferation as a response to partial hepatectomy. This pioneer hypothesis was supported by experimental data that pointed out the involvement of the phosphoinositides (PtdIns), as the derivates of PI phosphorylated on three of five potential phosphorylation sites by phosphoinositide kinase (PK), in the maintenance of the chromatin structure (Osborne *et al*. 2001). Specific antibodies have been used to reveal the presence of PtdIns (4-5)P_2 in the nucleoplasm but also in the domains of the nucleus that contain components for the transcription process, including RNA polymerase II and the splicing factor SC-35 (Osborne *et al*. 2001). PtdIns are important second messengers that interact with proteins and play a role in cell proliferation and apoptosis (Tolker and Cantley 1997). The presence in the nucleus of either PtdIns or enzymes responsible for their metabolism, PI-PLC and phosphoinositide-3-kinase (PK3), has suggested the existence of the intranuclear PI cycle (Cocco *et al*. 1995) that can constitute a signalling system (Divecha and Irvine 1995).

Phosphatidylcholine

PC is the most abundant glycerophospholipid in the nucleus (Divecha and Irvine 1995) where it is synthesised from phosphatidylserine (PS) by a "choline base exchange complex" (Albi and Viola Magni 1994) or from SM by reverse sphingomyelin-synthase (Albi and Viola Magni 2006) and it is catabolised by phosphatidylcholine-dependent phospholipase C (PC-PLC; Albi and Viola Magni 2004) and by phosphatidylcholine-dependent phospholipase D (PC-PLD; Baldassarre *et al*. 1997).

The base exchange enzyme complex has been found in hepatocyte and liver nuclei and in nuclear membranes. Its activity is Ca^{2+}-dependent: in fact it is higher when the nuclei are pre-incubated with CaCl (Albi and Viola Magni 1997). The activity is similar in the liver nuclei and nuclear membranes and corresponds to that reported for the brain light microsomes. Instead the hepatocyte nuclei show, after incubation for 90 min, an activity higher than that present in the liver nuclei and nuclear membrane. The difference in speed between the reaction in microsomes and in nuclei may be explained by the different composition in unsaturated fatty acids of PC. In fact, the composition of PC present in the microsomes shows an enrichment in tetraene and exaene fractions which increase the speed of reaction, whereas hepatocyte nuclei contain many unsaturated fatty acids of the monoenic fraction which have an opposite effect. The role of PC has been investigated by increasing its content with a "choline base exchange reaction" in isolated nuclear membranes. In fact, after this reaction, the fluorescence anisotropy of the bilayer surface increases whereas that the hydrophobic core decreases indicating an increase of the fluidity in the nuclear membrane (Tomassoni *et al*. 1999). The presence of this enzyme complex can explain the increase in PC observed during hepatocyte maturation (Albi *et al*. 1991). In contrast, nuclear PC catabolism is due

to two phospholipases, C and D. The first is localized either in the nuclear membrane or in the chromatin with different chemical-physics characteristics (Albi and Viola Magni 2004), whereas the second has two isoforms: PC-PLD1 which is localized at the nuclear membrane level and PC-PLD2 which is present inside the nucleus (Baldassarre *et al*. 1997).

SPHINGOMYELIN AND CHOLESTEROL

In the nucleus SM is present with different concentration and metabolism in relation to its localization (Neicheva and Peeva 1995; Martelli *et al*. 2002; Ledeen and Wu 2004). Recently an intranuclear SM cycle has been described (Albi and Viola Magni 2006, **Fig. 1**). In fact, this lipid is synthesised from PC by sphingomyelin-synthase (SM-synthase), is catabolised by neutral-sphingomyelinase (N-SMase) and it is used as source of phosphocholine to synthesise PC by RSM-synthase (Albi and Viola Magni 2006). Moreover, inside the nucleus, SM is associated with cholesterol (CHO) through van der Waals's forces and this interaction changes in relation to SM fatty acid composition and SM metabolism (Albi and Viola Magni 2006).

In the nuclear membrane, isolated by the method of Kay and Johnston (1975), the interaction SM-CHO has been demonstrated by SMase treatment which induces a degradation of SM with production of ceramide (CE) and phosphocholine and a reduction of the CHO with an increase of CHO ester (Albi and Viola Magni 2006). The modification of in CHO content modifies the nuclear membrane fluidity and consequently the transportation of mRNA, regulating the cellular functions (Tomassoni *et al*. 1999). Recently it has been shown that in the nucleus SM and CHO are associated to form nuclear lipid rafts (Cascianelli 2006). In fact, the authors demonstrate that extracting the microdomains from purified nuclei by using Triton X-100 detergent at a low temperature followed by a sucrose density gradient, the lipid rafts present a morphology similar to that previously reported for microvillar membrane (Braccia 2003) Biochemical analyses showed that the nuclear microdomains are characterized by the lipid fraction constituted by CHO, SM and PC in a 1:1:1 ratio. The presence in the nuclear microdomains of lamin B as a specific protein of the nuclear membrane and STAT3 transcription factor suggested that the nuclear lipid rafts could represent a platform for the process of transcription in the nuclear membrane (Cascianelli 2006).

Moreover the activity of N-SMase and SM-synthase, previously described in the nuclear membrane (Albi and Viola Magni 2004), is associated to the nuclear lipid rafts (Cascianelli 2006), suggesting a metabolism of SM in loco which could modify the structure and function of these microdomains.

The nuclear N-SMase activity has been discovered also in association with the nuclear matrix of rat hepatocytes (Tamiya-Koizumi *et al*. 1989) together to the SM-synthase activity (Albi *et al*. 2003b) indicating that also the rigidity of nuclear matrix can be easily modified influencing its role.

In the chromatin, the SM is present in a higher percentage with respect to other nuclear lipids (Albi 1994) and it is characterized from high levels of saturated fatty acids not found in the nuclear membrane SM which permit a strong interaction with CHO (Albi and Viola Magni 2006). The authors demonstrated that in the chromatin two pools of CHO are present: one soluble, the "SM-free CHO" and another that can be extracted only after digestion with SMase or proteinase K, the "SM-linked CHO". The regulation of the SM-free CHO pool depends on the SMase activity which in turn depends on SM-synthase (Albi 2006). In fact these enzymes are also present at the chromatin level with chemical and physical characteristics different with respect to the same enzymes present in the cytoplasm and nuclear membrane (Albi and Viola Magni 2003a; **Table 2**).

The role of nuclear SM was supposed by Novello *et al*. (1975) who demonstrated that SM *in vitro* stimulates the activity of DNA polymerase. Manzoli *et al*. (1972) showed that SM takes part in the organization of DNA, changing its structure. The SM-DNA interaction is due to the content of the trimethylammonium group, positively charged, which is able to bind cationic groups and to form the double-stranded DNA. When the SM concentration increases, the spatial competition between the molecules occurs with a consequent quick denaturation of DNA (Manzoli *et al*. 1972). The function of SM in maintaining double strand stabilization has been supported by a study performed on rat hepatocyte nuclei treated with RNase and DNase (Micheli *et al*. 1998). The results showed the existence in the nucleus of an "Intranuclear Complex" formed by proteins, a very small amount of DNA lipids and an RNAase-resistant RNA which is a double strand RNA (dsRNA). Lipid analysis highlighted that SM and PC and CHO are the only lipids associated to dsRNA (Rossi *et al*. 2006) and the treatment with SMase opens this RNA, making it sensitive to RNase (Micheli *et al*. 1998).

Table 2 Comparison of Km and optimal pH values of the sphingomyelinases localised in different subcellular and subnuclear sites (based on Albi and Viola Magni 2006).

	homogenate	microsomes	nuclear membrane	chromatin	nuclear matrix
Km	1,2 x 10^{-4}	6,2 x 10^{-5}	3,9 x 10^{-4}	2,4 x10^{-5}	n.d.
pH	about 7.2	about 7.2	7.6	8.2	n.d.

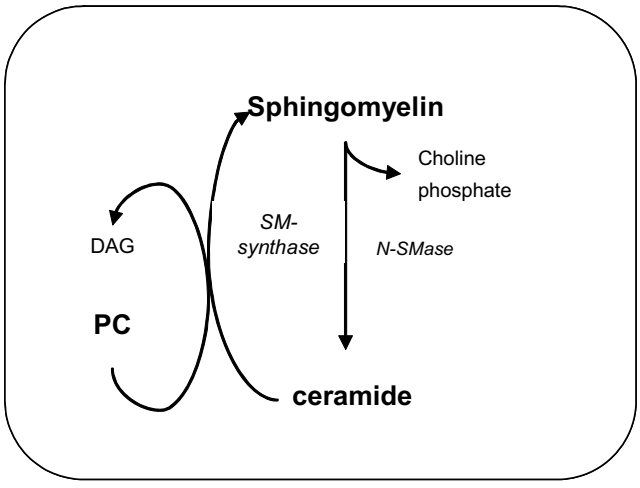

Fig. 1 Nuclear Sphingomyelin cycle. In the nucleus the SM can be hydrolysed by sphingomyelinase (*N-SMase*) to produce ceramide and choline phosphate. The SM catabolized is re-synthesised by sphingomyelin-synthase (*SM-synthase*) by using phosphocholine of phosphatidylcholine (PC) and ceramide freeing diacylglycerol (DAG).

NUCLEAR LIPID METABOLISMS IN RELATION TO CELL FATE

The nuclear lipids constitute a resource of bioactive molecules which are involved in the transduction of various signals and in the regulation of fundamental cellular mechanisms such as proliferation and/or apoptosis (Alessenko and Burlakova 2002; Albi and Viola Magni 2004). In fact in the nucleus either PI-PLC (Cocco *et al*. 2001) and PC-PLC or SMase (Albi and Viola Magni 2004) are involved in cell proliferation thanks to the production of diacylglycerol (DAG) and CE. It is known that a typical response to the factors which stimulate PC and PI hydrolysis is a biphasic increase in DAG with an initial transient peak followed by a more slowly developing, but prolonged, accumulation (Exton 1990). The first peak is due to PI hydrolysis and is associated with Ca^{++} increase, whereas the second peak is due to PC hydrolysis without variations of Ca^{++}. DAG induces PKC activation and therefore a more prolonged increase of DAG means a prolonged activation of PKC. Several substrates are indicated for PKC, especially proteins implicated

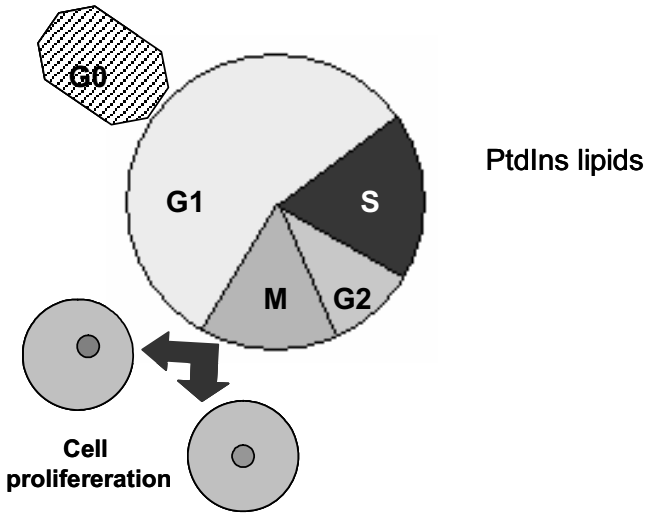

Fig. 2 Role of nuclear PtdIns lipids on cell proliferation. The metabolism of PtdIns is activated during the S-phase of the cell cycle stimulating cell proliferation.

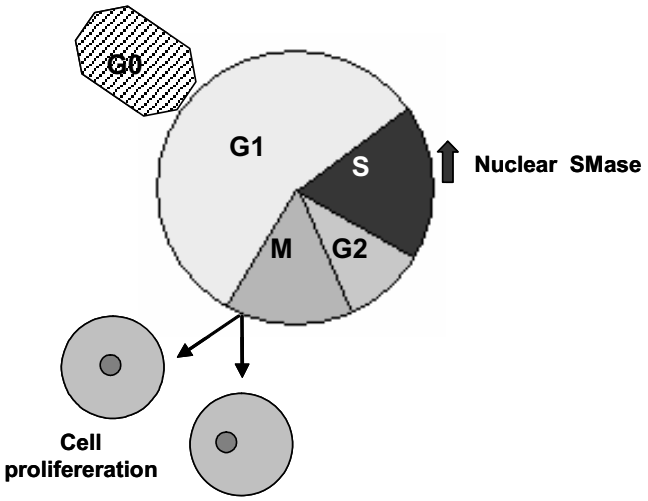

Fig. 3 Role of nuclear sphingomyelinase on cell proliferation. In heaptocytes induced to proliferate after partial hepatectomy, the activation of nuclear SMase reduces SM concentration during the S-phase of the cell cycle facilitating the molecular events that precede DNA synthesis.

in maintaining chromatin structure and in the replication or repair of DNA (Buchner *et al*. 1995). During rat liver regeneration, a very good model to study the cell proliferation, the activity of chromatin PI-PLC increases at 12 hours after operation during G1/S phase transition of the cell cycle (Albi and Viola Magni 2004). On the other hand, the PtdIns extracted from the nuclei of synchronized cells (HeLa) decrease about 50% at 2 and 4 hours after their release during the G1/S phase of the cell cycle and return to the original levels by 9 hours (York and Majerus 1994; **Fig. 2**). Instead, after hepatectomy, chromatin PC-PLC activity increases at the beginning of hepatocyte S-phase suggesting that it could have a role in initiating DNA synthesis through the prolonged activation of the nuclear form of protein kinase C (Albi and Viola Magni 2004).

In the same experimental model, chromatin SM content equivalent to 1.05 μmol/mg proteins decreases when the S-phase of the cell cycle starts with a value of 0.42 μmol/mg proteins thanks to the SMase activation that occurs during rat liver regeneration (Alessenko and Chatterjee 1995; **Fig. 3**). It has been supposed that these low levels may favour the opening of the DNA double helix favouring its duplication, while increased levels of chromatin SM may hinder DNA duplication (Novello *et al*. 1975). The increased activity of SMase may also explain the decrease of the chromatin "SM-linked CHO" fraction and the increase of the free-CHO fraction 18 hrs after hepatectomy, corresponding to the hepatocyte S-phase initiation (Albi and Viola Magni 1996). This increase is very important considering that CHO stimulates cyclin-dependent kinase activity, which is essential for the progression of the S-phase of the cell cycle. Inhibition of CHO synthesis arrests the cell in the S-phase, with a reduction of cdk2 and cdk4 expression, which is followed by an increase in protein p21 synthesis (Reszka *et al*. 2001). At 24 hrs after hepatecomy corresponding to the end of the S-phase, when the CHO values decrease, the SM-synthase increases its activity thus restoring the amount of SM and the SM-CHO bound fraction (Albi and Viola Magni 2006). The involvement of chromatin SMase during cell proliferation has also been demonstrated in a study on rat hepatocytes treated with ciprofibrate, a non-genotoxic hepatocarcinogen, which stimulates the proliferation through a specific nuclear receptor inducing DNA synthesis and peroxisome activity (Albi and Viola Magni 2006).

Many evidences have recently demonstrated that nuclear lipid metabolism is involved not only in cell proliferation but also in blocking the cell cycle and/or apoptosis. The nuclei isolated from PC12 cells treated with NGF are protected from apoptosis induced by various stimuli (Martelli *et al*. 2005). In these cells PtdIns (3,4,5) P3, but not PtdIns (3,4) P2, PtdIns (4,5) P2 or PtdIns (3) P mimic the anti-apoptotic effect of NGF. The involvement of nuclear PtdIns (3,4,5) P3 in the protecting role of NGF has been demonstrated by an experiment in which nuclei isolated from PC12 cells were pre-incubated with phosphatase and tensin homolog deleted on chromosome ten (PTEN) that block the cell cycle, stimulating the dephosphorylation of PtdIns (3,4,5) P3 (Martelli *et al*. 2005). In the liver stimulated to proliferate by ciprofibrate treatment, the withdrawal of the drug induces hepatocytes to apoptose accompanied by an inhibition of chromatin SMase activity and stimulation of SM-synthase with a consequent increase of the amount of SM that could favour chromatin clamps, characteristics of this process (Albi and Viola Magni 2006). In liver lobules after ligature of the portal vein, apoptosis is preceded by an increase of nuclear N-SMase and ceramidase activity, followed by an increase of ceramide and sphingosine concentration (Tsugane *et al*. 1999). This result is not in contrast

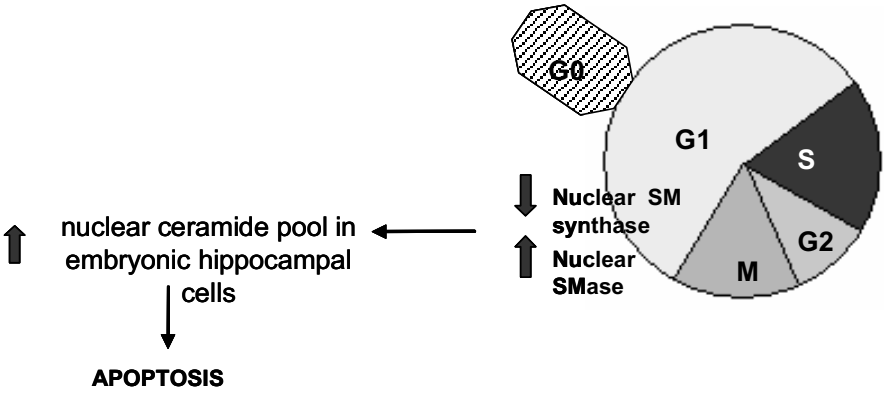

Fig. 4 Role of nuclear sphingomyelin metabolism on apoptotic process. In embryonic hippocampal cells, serum deprivation induces apoptosis increasing sphingomyelinase (SMase) and inhibiting sphingomyelin-synthase (SM-synthase) activity with consequent enrichment of ceramide pool during G1-phase of the cell cycle.

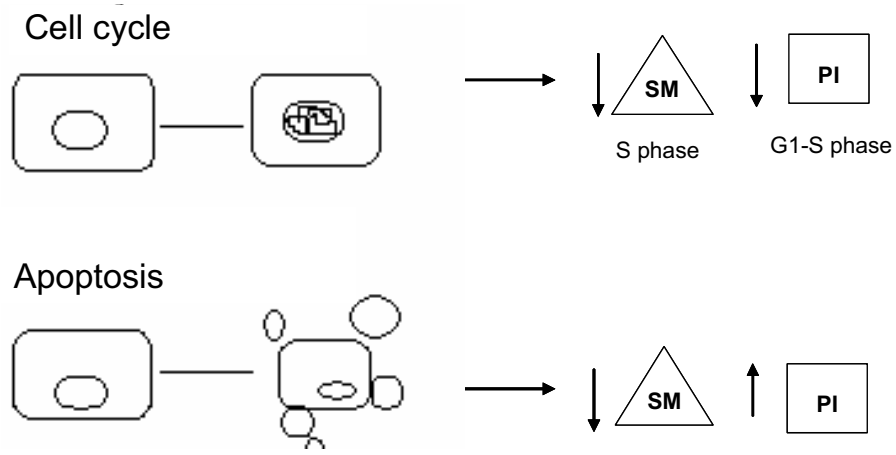

Fig. 5 Variation of the content of sphingomyelin and phosphatidylinositol during cell proliferation and apoptosis. During cell proliferation a reduction of phosphatidylinositol (PI) with consequent increase of diacylglycerol pool occurs in the G1/S phase of the cell cycle transition and then, during the S-phase, sphingomyelin (SM) reduces with a consequent increase of ceramide. In the apoptotic process SM amount decreases very early whereas the PI content increases. ↑ increase; ↓ decrease.

with those reported above since N-SMase in whole nuclei does not have the same behaviour as the chromatin enzyme. A stimulation of the N-SMase activity present in the whole nucleus has been described in the human erythro-myeloblastic cells TF-1-33 induced to apoptosis by ionizing radiation (Jaffrézou *et al*. 2001). In embryonic hippocampal cells a nuclear N-SMase activation together with SM synthase inhibition and a consequent early increase of the nuclear ceramide pool are involved in the induction to apoptosis by serum deprivation (Albi and Viola Magni 2006; **Fig. 4**).

CONCLUSIONS

Studies collected here highlight clearly that the metabolism of nuclear lipid is regulated independently from that of the cytoplasm and is modulated in response to growth factor signalling, cell cycle progression and/or apoptosis (Dygas and Baraska 2001; Albi and Viola Magni 2004; Martelli *et al*. 2004; Ledeen and Wu 2006). The identification of nuclear PI, PC and SM cycles with different mechanisms of regulation in the various subnuclear compartments has permitted to establish novel functions of the lipids: from nuclear membrane fluidity regulation and the formation in this structure of specific domains for transcription process, to nuclear matrix rigidity regulation influencing its role such as DNA replication, relaxation of the superhelical strain in DNA, processing of hnRNA and snRNP, and RNA transport (Fernandes *et al*. 1995), to chromatin organization and transcription process control. All evidences taken together strongly suggest that a cooperation between glicerolipids and sphingolipid metabolism exists in maintaining the cells in an equilibrium state. The modifications of both metabolisms are involved in the cell proliferation or apoptotic process induced by different stimuli (**Fig. 5**). During cell proliferation, in the nuclear membrane the activation of SMase is responsible for its fluidity increase facilitating the mRNA transportation form the nucleus to the cytoplasm (Tomassoni *et al*. 1999) whereas the nuclear matrix stiffens thanks to the inhibition of the same enzyme and consequent SM content increase permitting a correct DNA duplication and making a correct rearrangement of the chromosome more difficult (Albi *et al*. 2003b). As reported above, at the same time an early chromatin activation first of PI-PLC and then PC-PLC permits the maintaining of the high concentration of DAG, and a later activation of SMase determines an enrichment of the CE pool. During apoptosis the early activation of SMase present in the whole nucleus induces a high concentration of CE (Albi and Viola Magni 2006) and PI metabolism is responsible for the enrichment of the DAG pool (Martelli *et al*. 2005). It has been demonstrated that DAG and ceramide are involved in cell function (Hannun and Bell 1993; Divecha *et al*. 1997; Hannun and Obeid 2002; Luberto *et al*. 2002; Pettus *et al*. 2002). It can be suggest that CE and DAG act as mediators of transduction signalling which influences the activity of the intranuclear kinases to direct the cells to proliferation or apoptosis. Therefore further studies on nuclear lipid metabolism could be useful to understand the mechanism of various pathological processes and to develop new therapeutic strategies.

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