NUCLEAR LIPIDS IN THE NERVOUS SYSTEM: WHAT THEY DO IN HEALTH AND DISEASE

Abbreviated title:

Neural nuclear lipids in health and disease

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Abstract

In the last twenty years it has been widely demonstrated that cell nucleus contains neutral and polar lipids localized in nuclear membranes, nucleoli, nuclear matrix and chromatin. Nuclear lipids may show specific organization forming nuclear lipid microdomains and have both structural and functional roles. Depending on their localization, nuclear lipids play different roles such as the regulation of nuclear membrane and nuclear matrix fluidity but they also can act as platforms for vitamin and hormone function, for active chromatin anchoring, and for the regulation of gene expression, DNA duplication and transcription. Crosstalk among different kinds of lipid signalling pathways influence the physiopathology of numerous cell types. In neural cells the nuclear lipids are involved in cell proliferation, differentiation, inflammation, migration and apoptosis. Abnormal metabolism of nuclear lipids might be closely associated with tumorigenesis and neurodegenerative diseases such as Alzheimer disease and Parkinson disease among others.

KEYWORDS:

Nucleus, lipid, sphingolipids, polyphosphoinositides, cholesterol, nuclear lipid microdomains,

neurodegenerative diseases

Introduction

Cell nucleus contain glycerophospholipids, sphingolipids, and cholesterol [1-3]. Major nuclear glycerophospholipids include polyphosphoinositides (PPIn) including phosphatidylinositol phosphate (PIP), phosphatidylinositol 4,5- bisphosphate (PI4,5P2), and phosphatidylinositol 1,4,5-trisphosphate (PI1,4,5P3), phosphatidylcholine (PC), phosphatidylserine (PS), and phosphatidylethanolamine (PE). Nucleus also contains sphingolipids, including sphingomyelin (SM), ceramide, ceramide-1-phosphate (C1P), sphingosine, sphingosine-1-phosphate (S1P) and gangliosides as well as cholesterol and its hydroxyl and oxygenated derivatives. Many enzymes involved in lipid metabolism and many lipid receptors are known to be localized in the nucleus or are translocated to the nucleus in response to a variety of stimuli [for reviews see [1-8]].

The signaling pathway of PPIn has been extensively studied. Increasing evidence suggest that the nucleus constitutes a functionally distinct compartment of inositol lipid metabolism and that the regulation of the nuclear PI pool is independent from the plasma membrane counterpart [5,8]. The pathways for the interconversion of phosphoinositides in the nucleus are illustrated in Fig. 1. PPIn are phosphorylated by specific kinases (PIPKs). PIPK type I phosphorylates PI4P to PI4,5P2; PIPK type II phosphorylates PI5P to PI5P4,5P2; PI3K (C2α, C2β, and C2γ isoforms) phosphorylates PI to PI3P. PI3K indirectly regulates the PPIn-dependent protein kinase-1 (PDK1) and interacts with the AKT/PKB pathway. The nuclear activities of PI3K and AKT are antagonized by a variety of phosphatases that can translocate to the nucleus such as Src homology 2 domain containing inositol-5′-phosphatase 2 (SHIP2) known to be one of lipid phosphatases converting PI3,4,5P3 to PI3,4P2. PPIn are degraded by specific PI-dependent phospholipase C (PI-PLC). Different PI-PLCs isoforms have been identified in the nucleus, namely PI-PLC-β1, γ1, δ1 and ζ. [5,6]. PLC-mediated cleavage of PI4,5P2 generates the two second messengers diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP3). DAG stimulates PKC isoforms and IP3 modulates nuclear calcium level thereby regulating gene transcription, DNA replication, and nuclear envelope (NE) breakdown [7]. IP3 can also be converted via successive kinases to inositol tetrakisphosphate (IP4), inositol pentakisphosphate (IP5), and inositol hexakisphosphate (IP6) which have distinct regulatory functions in protein deacetylation (IP4) and RNA export (IP6).

Plasmalogen and PC are metabolized by PC-dependent PLC that produce DAG and phosphocholine. In addition, the nucleus contains phospholipase D (PLD), DAG-kinases and DAG-lipases, monoacylgycerol-lipase, cyclooxygenases, lipoxygenases, and neuroaminidases [2,4,9-11].

There are differences between nuclear and non-nuclear compartments in terms of agonist responses and properties of enzymes involved in phospholipid metabolism found in these

subcellular fractions. For example, during retinoic-acid induced differentiation of neuroblastoma cells, there is stimulation of phospholipase A2 (PLA2), PLC, PLD, and generation of lipid mediators in the nucleus but not in the plasma membrane or the cytosol. Determination of kinetic parameters indicates that retinoic acid treatment does not affect the Km value, but Vmax values for PE and plasmalogen-PLA2 activities are increased in the nuclear fraction of neuroblastoma LA-N-1 cells [2].

Sphingolipids are metabolized by sphingomyelinase (SMase), sphingomyelin-synthase (SM-synthase), reverse sphingomyelin-synthase (RSM-synthase), ceramidase, ceramide kinase (CerK) and sphingosine kinases (SK) in multiple nuclear compartments, including chromatin, NE, and nuclear matrix [1,3,11,12] (Fig. 2). Similar to SMase, reverse SM synthase catalyzes the degradation of SM but with the difference that it catalyzes the transfer of the phosphocholine group from SM to DAG, forming ceramide and PC. Nuclear SM, SMase, SM-synthase and RSM-synthase are localized in specific nuclear lipid microdomains (NLM) that act as platform for vitamin and drug action, and for chromatin anchoring thereby regulating gene expression [13-15]. Interestingly, reverse SM synthase is also found in chromatin [16].

The activity of all these enzymes leads to the generation of nuclear lipid mediators such as DAG, ceramide, sphingosine, S1P, arachidonic acid, eicosanoids, platelet activating factor and various inositol phosphates. Nuclear DAG serves as a chemoattractant for some isoforms of PKC that migrate to the nucleus in response to a variety of agonists. DAG-kinases and DAG-lipases terminate DAG-dependent signals and control many DAG-dependent nuclear events [2,4,6,10,9] . It has been suggested that DAG derived from PI is shuttled directly to a DAG kinase (DAGK) present in the nucleus, while DAG derived from PC is not accessible to DAGK. Thus, nuclei may contain at least two distinct pools of DAG that are generated by the action of two distinct phospholipases [2,3]. Interestingly, in an in vitro model of ischemia in rat hippocampal cells and in in vivo experiments, during transient ischemia-reperfusion of the forebrain and following kainate-induced seizures of hippocampal neurons, DAGKζ migrates outside the nucleus in vivo and never relocates to the nucleus. Since these conditions result later in cell death [17-20], it has been hypothesized that nuclear export of DAGKζ could somehow facilitate neuronal apoptosis.

Nuclear membranes contain a variety of receptors (including those for endocannabinoids, platelet activating factor (PAF), prostaglandin E2 (PGE2), thromboxane, inositol 1,4,5-trisphosphate (IP3), retinoic acid, vitamin D and peroxisome proliferator receptors) [2] that can initiate nuclear signal transduction pathways and also cross-talk with other subcellular organelles and with the plasma membrane. A variety of stimuli modulates the activity of the nuclear enzymes or promotes their translocation to the nucleus. The stimulation of signaling pathways involving lipid

second messengers leads to changes in permeability to ions, in nuclear calcium levels, in transcription, and in protein and mRNA trafficking. These changes regulate cellular processes such as differentiation, proliferation, and cell death.

Differences in the lipid composition of cellular membranes depend on the physiopathological state, and also on the *in vivo* and *in vitro* growth environments but in some instances the differences might be an artifact of the *in vitro* culture conditions [21]. Fatty acid composition and the function of lipids may be different in cells that use aerobic glycolysis and those that respire to obtain energy. This might have consequences in conditions such as ischemia and hypoxia and in cancer since some tumour cells can switch between glycolytic and oxidative metabolism in a reversible fashion [22]. It is known that, in contrast to most untransformed tissues, which use dietary lipids, cancer cells frequently re-activate de novo lipogenesis. In some tumours, increased content of saturated fatty acids in PC correlates with higher tumour grade and lower survival, while a shift in polyunsaturated PI is correlated with invasiveness [23]. Recently, the components of membranes of subcellular organelles, especially those of mitochondria, have been intensely studied. The lipidomes of highly purified mitochondria isolated from normal brain, from brain tumours (astrocytoma and ependymoblastoma) grown *in vivo*, from astrocytoma cultured cells and from non-tumorigenic astrocytes have been compared. Major differences have been found between normal tissue and tumour tissue and between *in vivo* and *in vitro* growth environments in the content or in the fatty acid composition of ethanolamine glycerophospholipids, phosphatidylglycerol and cardiolipin [21]. The *in vitro* experimental conditions produced lipid and electron chain transport abnormalities in cultured non-tumorigenic astrocytes that were similar to those associated with tumorigenicity. For example, hydroxylated fatty acid molecular species were found in cardiolipin from tumour cells grown *in vitro*, but were not found in cardiolipin from the solid tumours grown *in vivo*, suggesting the possibility that hydroxylated fatty acids are produced as an artifact of the *in vitro* environment [21]. The lipid composition of nuclear membranes and nuclear lipid microdomains (NLM) obtained from cultured hepatocytes and from hepatoma cells has recently been compared [24]. NLMs of cancer cells exhibit reduced saturated very-long-chain fatty acid (24:0) SM and increased long-chain fatty acid (16:0) SM, as well as increased SM containing unsaturated fatty acids. The content of signaling proteins in NLMs is also modified in hepatoma cells [24]. Unfortunately, no similarly detailed studies concerning the composition of the nuclear membrane of neural cells are available at the moment.

We will overview the data concerning the physiopathological role of nuclear lipids, in particular the role of nuclear phosphoinositides, sphingolipid, cholesterol and its metabolites in the nervous system.

Role of the nuclear phosphatidylinositide pathway in the brain

Phospholipases C

PI-PLC β1 is the isoenzyme best characterized and is involved in the regulation of differentiation and proliferation in cultured myoblasts, adipocytes and erythroid leukemia cells [5]. The interactome of nuclear PI-PLC β1 in erythroid leukemia cells comprises proteins involved in cellular metabolic processes, gene expression, transport, developmental processes, translation, RNA splicing and processing, response to oxidative stress, and regulation of apoptosis [5]. It is known that PI-PLCs are highly expressed in the nucleus of cultured rat glioma cells [25]. Montaña et al. [26] have studied the distribution of the PI-PLCβ1 isoform in the adult rat nervous system *in vivo*, and the phenotype of cells expressing PI-PLCβ1, and also its subcellular localization in cortical neurons by immunofluorescence staining and confocal laser scanning. PI-PLCβ1-positive cells are colocalized with GABAergic neurons and glial processes of the spinal cord white matter. In cortical neurons, PI-PLCβ1 is found at speckles, a nuclear compartment that lacks membrane structures. Garcia del Caño et al. [6,9] have shown the localization of PLC-β1 and DAGL-α, which is much more abundant than DAGL-β in brain, in the neuronal nuclear compartment and also the PLC/DAG-dependent production of the endocannabinoid 2-arachidonylglycerol.

To study the functions of a protein is useful to identify the proteins that are able to interact with it as well as to study the consequences of its overexpression and knockdown. An interesting interactor of PI-PLCβ1 is α-synuclein. This protein, highly expressed in brain, is also the major component of neurodegenerative plaques and it is mutated in some forms of familial Parkinson disease (PD). *In vitro* experiments show that α-synuclein binds strongly to PI-PLCβ1 and promotes the release of Ca^{2+} from the endoplasmic reticulum [27]. It has been shown that the expression of α-synuclein increases the cellular level of PI-PLCβ1 by protecting it against degradation from enzymes such as calpain. Interestingly, oxidative stress that is often correlated with neurodegeneration, down-regulates PLCβ1 thereby promoting α-synuclein aggregation [28]. Nuclear α-synuclein inhibits histone acetylation and promotes death in SH-SY5Y neuroblastoma cells while cytoplasmatic α-synuclein is neuroprotective [29]; α-synuclein binds to DNA in a conformation-specific manner and causes a conformational transition that could play a significant role in neuronal cell dysfunction [30]. It is worth noting that α-synuclein has been found in the nuclei of some neurons and oligodendroglia from brains of patients with multiple system atrophy [31,32]. Increased levels of nuclear α-synuclein are associated with

Parkinson-linked mutations of α-synuclein [29,33]. Experiments with transgenic flies expressing nuclear or cytoplasmatic α-synuclein have demonstrated that nuclear α-synuclein is associated with loss of dopaminergic neurons [29].

It is worth noting that the PLC β1-knockout mice develop epileptic seizures from the fourth week of life, and show a selective loss of somatostatin-containing interneurons in the hippocampus [34], excessive adult hippocampal neurogenesis, and an abnormal migration of adult-born granule neurons [35]. These knockout mice also exhibit symptoms similar to human schizophrenia such as locomotor hyperactivity, impaired prepulse inhibition of the startle response, lack of barbering and nesting behaviors, socially subordinate status, impaired learning, and lack of type II theta rhythm which has been implicated in working memory [36]. In schizophrenia-affected patients, deletions of PLCβ1 gene in orbito-frontal cortex samples [37], as well as down-regulation of PLCβ1 transcript in the dorsolateral prefrontal cortex have been found [38], and a PLCβ1 deletion-associated early-onset epileptic encephalopathy has been reported [39]. Altogether these observations suggest that PLCβ1 has a role in the normal and pathological development of cortical and hippocampal circuitry.

PI-PLCγ1 is also found in the nucleus. NGF treatment leads to the activation of the shorter isoform of phosphatidylinositol kinase enhancer (PIKE-S), by triggering the nuclear translocation of PI-PLCγ1, which acts as a physiological guanine nucleotide exchange factor for PIKE-S through its SH3 domain. The ability of PI-PLCγ1 of activating PIKE does not depend on its phospholipase activity but results in PI3K/AKT activation in the nucleus and NGF-induced neuroprotection [40].

In rat hippocampal neurons, a massive increase in the intracellular Ca^{2+} elicited by ionomycin, thapsigargin or glutamate induces PI-PLCδ1 translocation from cytoplasm to nucleus as well as nuclear shrinkage. The nuclear translocation of PI-PLCδ1 is mediated by a Ca^{2+} -dependent direct interaction with importin b1. Furthermore, overexpression of GFP-PLCδ1 facilitates ionomycin-induced nuclear shrinkage in embryonic fibroblasts derived from PI-PLCδ1 knockout mice [41]. Therefore, nuclear translocation and the PI-PLC activity of PI-PLCδ1 may regulate the nuclear scaffold during Ca^{2+}-induced cell death, such as in ischemia and excitotoxicity.

Phosphatidylinositol 4,5-bisphosphate and phosphatidylinositol 3,4,5-trisphosphate effectors

PI4,5P2 is found in NE and intra-nuclear structures. It binds and regulates nuclear PI4,5P2 effectors some of which have recently been identified. These effectors are involved in a variety of processes, including transcription, mRNA processing, mRNA export, chromatin remodeling, stress responses, DNA repair, and mitosis [42]. A PI4,5P2 effector is Speckle Targeted PIPKIα Regulated-Poly(A)

Polymerase (Star-PAP). Start-PAP is a poly(A) polymerase critical for the 3′ cleavage, polyadenylation and expression of select mRNAs. PIPKIα, PI4,5P2 and Star-PAP function together in a complex to control the mRNA processing and expression of specific mRNAs, such as the ones codifying the proapoptotic protein BIK and heme-oxygenase-1 [42]. BIK is a proapoptotic protein while heme-oxygenase-1 catalizes heme degradation generating biliverdin, Fe^{2+} and CO. This enzyme has a neuroprotective role since it protects against oxidative injury, regulates apoptosis, and modulates inflammation [43]. PI4,5P2 directly activates Star-PAP and also regulates other components of the complex, such as casein kinase I PKCδ required to activate Star-PAP following oxidative stress and DNA damage respectively [42].

Another interesting nuclear PI4,5P2 effector is steroidogenic factor 1 (SF-1). The transcriptional activity of SF-1 is regulated by a direct association with PI4,5P2. SF-1 binds PI4,5P2 with the head group exposed to the surface and this allows its phosphorylation by a polyphosphate multikinase. The association of SF-1 with PI3,4,5P3 stabilizes its binding to coactivators leading to enhancement of the transcriptional activity of SF-1[44].

It is worth noting that steroids are not only synthesized in adrenal glands and gonads, but they are also synthesized within the brain and rapidly modulate neuronal excitability. For example, neurosteroids are endogenous regulators of seizure susceptibility, anxiety, and stress [45].

Phosphatidylinositol 3- kinase effectors. AKT and its role in neuronal survival.

AKT also known as protein kinase B (PKB) is the canonical downstream signaling effector of PI3K and it is also an oncogene with critical roles in cell growth. Three AKT isoforms encoded by different genes have been identified in mammalian cells [46]. AKT3 is predominantly localized within the nucleus and at the NE while AKT1 and AKT2 mainly localize at the plasma membrane and cytosol, and may translocate to the nucleus after growth factor stimulation. The nuclear PI3K/AKT pathway is involved in mRNA processing and exportation, DNA replication and repair, ribosome biogenesis, cell survival and tumourigenesis [47].

Nuclear PI3K/AKT signaling is implied in neuronal survival. Nuclear PI3K and its upstream regulator PIKE are necessary and sufficient to mediate the anti-apoptotic effect in PC12 cells stimulated with nerve growth factor (NGF) and in growth factor- stimulated HeLa and HEK-293 cells by preventing DNA fragmentation [48]. Recently some nuclear targets of AKT involved in survival have been identified and they include nucleophosmin /B23, Acinus and ribosomal protein S3 (RPS3) [48-52] (Fig.3). The nucleolar phosphoprotein B23 dynamically shuttles between the nucleus and cytoplasm as well as from the nucleolus to the nucleoplasm in the S-phase of the cell cycle.

Addition of PI3,4,5P3 to cell nuclei mimicks the antiapoptotic effect but requires nuclear AKT [48]. The neuroprotective effect of PI3,4,5P3 appears to be due to the interaction between AKT and the nuclear PI3,4,5P3 receptor, B23 [49], which prevents the caspase-3 dependent degradation of B23 and promotes survival [50]. The nuclear protein Acinus, after caspase-3 mediated proteolysis, is required for apoptotic chromatin condensation. AKT-phosphorylated Acinus forms a complex with the antiapoptotic protein zyxin, and this association prevents the caspase-3 mediated cleavage of Acinus and subsequent chromatin condensation. Another AKT substrate for neuronal survival is ribosomal protein S3 (RPS3), a conserved protein of the ribosomal 40S subunit, which is required for ribosome biogenesis. RPS3 shuttles between the cytoplasm and the nucleus, and acts in both the compartments with extra-ribosomal functions including apoptosis [52]. In PC12 cells, AKT-mediated phosphorylation of RPS3 increases nuclear accumulation of RPS3 and decreases its pro-apoptotic effect. Overexpression of RPS3 in the PC12 cells or in the primary hippocampal neurons induces neuronal apoptosis by cooperating with the transcription factor E2F1 and causing up-regulation of pro-apoptotic proteins, Bim and death protein 5/harakiri. AKT-dependent phosphorylation of RPS3 inhibits pro-apoptotic protein induction and leads to nuclear accumulation of RPS3, thereby promoting neuronal survival. Thus, these findings imply that upon NGF stimulation not only AKT translocates into the nucleus, but also triggers its target proteins, that have a proapoptotic function in the cytoplasm, to move into the nucleus preventing neuronal death. (Fig. 3). It is worth noting that only some aspects of this mechanism have been studied in primary neurons. Being PC12 a pheochromocytoma-derived cell line, it is important to clarify whether this prosurvival signaling pathway is similar in neurons *in vitro* and also *in vivo*.

Nuclear phosphatases

The activity of the kinases such as PI3K and AKT are antagonized by a variety of phosphatases such as phosphatase and tensin homolog deleted on chromosome TEN (PTEN) and SHIP2 that can translocate to the nucleus.

In astrocytes, the phosphatase SHIP2 is found the nucleus, at nuclear speckles, and in the cytoplasm [53,54]. Phosphorylated SHIP2 on S132 can be found in the nucleus and nuclear speckles. Nuclear SHIP2 interacts with the nuclear lamina proteins Lamin A/C and the PP2A regulatory subunit PR130B [53]. In unstimulated astrocytoma cells, SHIP2 has a perinuclear and cytoplasmic localization. In serum-stimulated cells, SHIP2 can be localized at the plasma membrane and at focal contacts in polarized cells, suggesting that it could play a role in adhesion and migration. Similarly to plasma membrane AKT, nuclear AKT may rely on the nuclear production of PI3,4P2 from PI3,4,5P3 by SHIP2 to maintain or achieve full activation. In the glioblastoma cell line 1321 N1, that does not express PTEN, lowering SHIP2 expression has an impact on the levels of PI3,4,5P3, cell

morphology and cell proliferation. SHIP2 stimulates cell proliferation by decreasing the expression of key regulatory proteins of the cell cycle such as p27 [54].

PTEN nuclear translocation is an essential step in excitotoxic *(in vitro)* and ischemic *(in vivo)* neuronal injuries. NMDA induces PTEN nuclear translocation and cell death. While nuclear overexpression of a dominant negative mutant PTEN reduces NMDA receptor-mediated excitotoxicity, specific blockade of PTEN nuclear translocation with the Tat-K13 peptide prevents excitotoxicity in cultured neurons and protects against ischemic brain damage *in vivo* [55]. However, the mechanism involved in survival is unknown. Whether nuclear PTEN in neurons is acting by dephosphorylating PI3,4,5P3 or by directly interacting with p53 and thereby altering transcription activity, or if it is independent of the phosphatase activity, remains to be tested.

Role of neural nuclear sphingolipids

Evidence from *in vitro* and *in vivo* studies have highlighted the role of ceramide, ceramide 1-phosphate, sphingosine, sphingosine-1-phosphate and gangliosides in neural cell proliferation, apoptosis and differentiation.

It is known that nuclear SM metabolism is independent on the non-nuclear one. Extranuclear and nuclear enzymes involved in sphingolipid metabolism exhibit different properties. In HN9 embryonic hippocampal cells nuclear SMase and SM-synthase regulate the ceramide level directly in the nucleus and differ from those present in the homogenate in optimum pH, Km and Vmax [56]. In neural cell cultures NGF induces generation of low levels of ceramide at plasma membrane and results in differentiation and neuritic outgrowth [57] whereas serum deprivation results in ceramide increase and apoptosis [56].

We and others have demonstrated that the nuclear levels of distinct sphingolipid subspecies change under different cellular physiological states. For example, changes in ceramide content have been described *in vitro* after serum deprivation in embryonic hippocampal cells in culture [56] or during Fas-induced apoptosis in Jurkat cells [58] and *in vivo* during liver regeneration [59]. GM1 increases during axogenesis in cultured neuroblastoma cells and in cultured cortical neurons [12]. Chromatin-associated SM synthase and SMase control dynamic oscillations in SM concentrations during the cell cycle both *in vivo* [60,61] and *in vitro* [62] when SM plays a role in RNA maturation [3,63,16].

Ceramide as mediator of apoptosis and neurodegeneration

Different apoptotic stimuli in different types of cells result in an increase of nuclear ceramide. We have demonstrated that serum deprivation induces apoptosis in HN9 embryonic hippocampal cells. Serum deprivation induces, after 1 hour, a rise of intracellular ceramide level, followed by

translocation of Bax, dysregulation of calcium, cytocrome c release and caspase 3 activation [64,65]. The ceramide that increases in the early phase of apoptosis is localized in the nucleus. The extranuclear SMase activity increases after 8-15 hours of serum deprivation, suggesting a role in later phases of apoptosis [56]. In Jurkat T cells, Fas ligand simultaneously stimulates neutral SMase and inhibits SM synthase activities in a caspase-3-dependent manner, which results in the time- and dose-dependent accumulation of nuclear ceramide [58]. Alterations in protein traffic may contribute to the apoptotic effect ceramide. Ceramide regulates nuclear protein import in smooth muscle cells by inducing p38 mitogen-activated protein kinase activation and the subsequent relocalization of two nuclear transport proteins, importin A and cellular apoptosis susceptibility gene [66]. The inhibition of nuclear import by exogenously supplemented ceramide results in diminished expression of proliferation protein markers, including cyclin A and proliferating cell nuclear antigen, and in reduced proliferative capacity [66].

Alzheimer disease (AD) is a neurodegenerative disorder of the central nervous system and the most common form of dementia. The pathogenic hallmarks of AD include extracellular amyloid-containing plaques, intracellular neurofibrillary tangles consisting of hyperphosphorylated tau protein and death of cholinergic neurons of the basal forebrain. Amyloid plaques are mainly formed by aggregated amyloid β peptide (Aβ) generated by the hydrolysis of amyloid precursor protein. Many studies in cultured cells have demonstrated that Aβ is mainly generated in lipid rafts, that Aβ affects sphingolipid metabolism and that sphingolipids regulate the production of Aβ [67]. Aβ induces activation of nSMase increasing ceramide levels, which result in cell death, while ceramide increases the stability and S1P directly activates the beta-secretase, one of the enzymes responsible for the production of Aβ [68]. Increasing SM and inhibition of glucosylceramide synthetase (the first step in ganglioside formation) leads to lower Aβ levels [68].

Ceramide localized in NLM is involved in 1,25-dihydroxy vitamin D_3 induced differentiation in embryonic hippocampal cells [69]. A portion of the nuclear 1,25-dihydroxy vitamin D_3 receptor (VDR) is found in NLM. The integrity of these microdomains is necessary for the effect of 1,25-dihydroxy vitamin D_3 on this process (Fig.4). Treatment with exogenous SMase or serum deprivation, which increases SMase activity in these cells, changes the lipid composition of the NLM, impairs the localization of the receptor in the NLM and prevents neuronal differentiation [13]. Conversely, treatment of serum-deprived cells with a high concentration of 1,25-dihydroxy vitamin D_3 increases the content of VDR as well as that of SM in NLM and allows differentiation [13].

The apoptotic role of ceramide has also been observed *in vivo* in nuclei of liver cells. The ligature of portal branches induces apoptosis as shown by TUNEL assay and analysis of DNA

degradation [59]. In ligated liver lobes, nuclear SMase activity and ceramide levels increase after 30 min and 1 hour respectively while no similar variations are found in the plasma membrane [59].

Drastic alterations in the sphingolipid content (mainly increase in ceramide levels) and in the gene expression of several enzymes that control sphingolipid metabolism (serine palmitoyl transferase, SMases) have been observed in the brain of human AD patients [for recent reviews see [67,68]].

Sphingosine, S1P, and fingolimod

It is known that cAMP rapidly decreases nuclear concentrations of ceramide and stimulates the nuclear localization of SK1 while concomitantly increases sphingosine and S1P levels [11]. A nuclear S1P target has recently been discovered, linking nuclear S1P to epigenetic regulation of gene expression. S1P binds to the histone deacetylases1 (HDAC1) and HDAC2 and inhibits their enzymatic activity [70]. SK2 is associated with HDAC1 and HDAC2 in repressor complexes and is selectively enriched in the promoters of the genes encoding the cyclin-dependent kinase inhibitor p21 or the transcriptional regulator c-fos, where it increases local histone H3 acetylation and transcription [70]. S1P acts not only as a second messenger, but also as a ligand of five G-protein coupled receptors (S1PR) found to be involved in a wide range of physiopathological processes in different tissues, including the nervous system. Both S1P synthesis and S1PR expression are required for embryonic neurogenesis whereas in the adult nervous system, S1P/S1PR axis regulates neurotransmission, promotes survival and affects differentiation [71,72]. All S1PR are found in the nucleus of neural tissue; the nuclear subtype 3 is overexpressed in malignant compared to normal tissue [73]. The role of nuclear S1PR is unknown.

An analogue of sphingosine, FTY720 (fingolimod) is able to enter the nucleus, where it is phosphorylated by SK2 and the nuclear FTY720-phosphate, as S1P, binds and inhibits class I HDACs, enhancing specific histone acetylations and gene expression [74,70]. FTY720-phosphate binds S1P1 to downregulate activated microglial production of pro-inflammatory cytokines as tumour necrosis factor-α, interleukin-1β, and interleukin-6. FTY720 also upregulates microglial production of brain-derived neurotrophic factor and glial cell-derived neurotrophic factor [75].

FTY720 decreases production of Aβ in cultured cortical neurons [76]. Interestingly, this decrease is independent of known downstream signaling pathways of S1PRs, but whether this effect is due to the action of FTY720 on protein acetylation has not been investigated.

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system characterized by infiltration of immune cells and progressive damage to myelin and axons together with reactive astrogliosis and activated microglia. The pathology is generally believed to reflect autoimmune attack upon myelin autoantigens. In lymph nodes, fingolimod induces the internalization of type 1 S1PR (S1P1) in T cells. As a result, lymphocytes no longer respond to the gradient of S1P and remain sequestered in the lymph node. However, fingolimod has beneficial effects in the CNS independent of its effects on immune cell trafficking [77]. Choi et al., [78] using animals in which S1P1 was deleted in specific cell types, have found that S1P1 of astrocytes is necessary for the protective action of fingolimod in experimental allergic encephalitis, a model of MS. When FTY720 is given to mice, it is phosphorylated and accumulates in the brain, enhances histone acetylation and expression of genes associated with memory and learning, and rescues memory deficits [74,70]. Hait et al. [74] have tested the effects of FTY720 on memory in SK2-/- mice. These mice have reduced both S1P levels and histone acetylation, and display deficits in spatial memory and impaired contextual fear extinction that are rescued by FTY720, suggesting that this drug may be useful to facilitate extinction of aversive memories [74]. In addition, FTY720 exerts an antidepressive action in mice subjected to chronic unpredictable stress, a model of reactive depression. Fingolimod treatment also enhances histone acetylation and adult neurogenesis in the hippocampus of these mice [79] supporting the idea that this drug could have a broader application in neurodegenerative diseases.

Administration of FTY720 in a rat model of AD obtained by bilateral hippocampus injection of Aβ decreases death in the hippocampus and increases memory compared with control rats [80]. Similarly, in rats injected with Aβ in the cortex, the administration of the combination of FTY720 and memantine reduces hippocampal death [81]. Unexpectedly, treatment of APP transgenic mice harboring mutations found in familial AD with FTY720 for 6 days results in a decrease in Aβ40, and an increase in Aβ42 levels in brains [76]. These results highlight the necessity to further study the mechanism of the sphingolipid analogue and Aβ42 metabolism in order to avoid adverse effects in humans.

Ceramide-1-phosphate

Rovina et al. [82] identified nuclear export and import signals in the primary sequence of CerK. This finding suggests that nuclear ceramide can be further metabolized into C1P, which may exhibit unique nuclear functions that need to be explored. C1P binds to the Ca^{2+} -binding regions in the C2 domain of cPLA2α and promotes its translocation to the perinuclear region of cells [83].

Arachidonic acid, produced by the action of PLA2, can be converted by lipoxygenases to 5-oxo-eicosatetraenoic acid. This metabolite binds and activates the transcription factor PPARδ1, promoting neuroblastoma survival [84]. Although many studies correlate S1P function to increased survival, differentiation and proliferation in neuronal and glial cells, the relevance of C1P/Ceramide axis remains to be clarified. During retinoic acid-induced differentiation of SH-SY5Y neuroblastoma cells there is a down regulation of CerK expression [85]. We have demonstrated that CerK is involved in the anti-proliferative action of vitamin D_3 and its analogues in human neuroblastoma cells [86]. The inhibition of CerK, the enzyme responsible for C1P synthesis, by specific gene silencing or pharmacological inhibition, drastically reduces cell proliferation. The treatment with Vitamin D_3 or with its structural analogue ZK191784 induces a significant decrease in CerK expression and C1P content, and an increase of ceramide. Notably, the treatment of SH-SY5Y cells with vitamin D_3, the antagonist of vitamin D_3 receptor ZK159222, the inhibitor of histone deacetylases trichostatin A, or COUP-TFI-siRNA prevented the decrease of CerK expression elicited by vitamin D_3 supporting the involvement of VDR/COUP-TFI/histone deacetylase complex in CerK regulation. Altogether, these findings provide the first evidence that CerK/C1P axis acts as molecular effector of the anti-proliferative action of vitamin D_3 and its analogues, thereby representing a new possible target for anti-cancer therapy of human neuroblastoma. Whether nuclear CerK is involved in this antiproliferative effect has not been explored.

Gangliosides

Gangliosides are important components of neuronal cell membranes that play a critical role in neuronal and brain development. They are functionally involved in neurotransmission and are thought to support the formation and stabilization of functional synapses and neural circuits required as the structural basis of memory and learning [87]. Besides the nuclear membrane localization, GD3 has also been found in chromatin [88] after Aβ-induced cell death of cultured cerebrocortical neurons. In rat cortical neurons, Aβ increases GD3 accumulation concomitant with a decrease of SM, the source of ceramide, and induction of the 2,8-sialyltransferase, the enzyme that forms GD3 from GM3. GD3 synthase knockdown by RNA interference prevents Aβ- induced entry into S phase and apoptosis, supporting a role for GD3 in cell cycle activation and cell death [88]. Moreover, after Fas-induced apoptosis in HUT-78 T-lymphoma cells, GD3 translocation from the cytosol into the nucleus correlates with histone H1 phosphorylation, suggesting that GD3 may have an epigenetic role in the transcriptional regulation of specific genes [89]. It is worth noting that

after CD95/Fas triggering, raft-like microdomains could be detected in mitochondrial membranes. It has been hypothesized that some "small" mitochondria, possibly derived from their fission process, can reach the NE and strictly interact with it contributing to molecular trafficking of molecules such as GD3 [90].

The multiple roles of GM1 have been recently reviewed [12]. GM1 is located in membrane rafts, where it associates with specific proteins that have glycolipid-binding domains. GM1 interacts with proteins that modulate mechanisms such as ion transport, neuronal differentiation, G protein-coupled receptors, and neurotrophin receptors. GM1 occurs at high concentrations in the nuclear membrane of differentiating neuroblastoma and cultured embryonic neurons obtained from cortex and from the superior cervical ganglion [12]. GM1 is associated with the inner NE in neurons, astrocytes and neuroblastoma cells, and plays a prominent role in nuclear Ca2+ homeostasis. This ganglioside is tightly associated with a Na^+/Ca^{2+} exchanger (NCX) which mediates the countertransport of three Na^+ ions for one Ca^{2+} ion against a Ca^{2+}gradient. Ca^{2+} uptake experiments with isolated nuclei and imaging using Ca^{2+} sensors have demonstrated that GM1 association with NCX potentiates Na^+ and Ca^{2+} exchange between the nucleoplasm and the NE lumen, thus protecting the nucleus from degradative enzymes activated by prolonged Ca^{2+} elevation. Moreover, studies using cultured neurons obtained from knockout mice engineered to lack GM2/GD2 synthase, which results in deficient synthesis of GM2, GD2, and GM1, have demonstrated key regulatory roles for nuclear membrane–associated GM1 in Ca^{2+} homeostasis [12]. Cultured neurons from these mice are highly vulnerable to Ca^{2+} -induced apoptosis but they are rescued to some extent by GM1 and more effectively by LIGA-20, a membrane-permeant derivative of GM1 that traverses the plasma membrane more effectively than GM1 and inserts into the NE.

Huntington disease (HD) is a neurodegenerative disorder caused by the expansion of a polyglutamine stretch in the protein huntingtin (Htt), and it is characterized by extensive neurodegeneration of striatum and cortex. Synthesis of the ganglioside GM1 is reduced in fibroblasts from HD patients and in cell models of HD [91], and decreased GM1 levels contribute to increase HD cell susceptibility to apoptosis. Administration of GM1 restores ganglioside levels in HD cells and promotes activation of AKT and phosphorylation of mutant Htt, leading to decreased mutant Htt toxicity and increased survival of HD cells.

Studies in rat have indicated that the synthesis of distinct ganglioside subspecies in the nucleus is differentially regulated during development. GM1, GM3, and c-series gangliosides are abundant in mature rat brain but there are relatively more GM3 and GD3 during embryonic development [1].

The GM2/GD2 synthase knockout mice develop late-onset neurological disease and are susceptible to kainate-induced seizures accompanied by increased apoptosis in CA3 hippocampal neurons. Consistent with Ca^{2+} dysregulation, kainate-induced seizures are also attenuated by LIGA-20 administration to the mutant mice. This neuroprotective effect correlates with the ability of LIGA-20 to cross the blood-brain barrier, enter brain cells, insert into the NE, and potentiate the NCX [12]. Since many studies have reported neurotrophic and neuro-protective effects of applied gangliosides, clinical trials have been designed to test their efficacy as therapeutic agents [for a review see [12]]. GM1 therapy shows partial restoration of depleted striatal dopamine and nigrostriatal neuron recovery in animal models of Parkinson disease (PD), and also reduces motor symptoms and slows symptom progression over a 2-year period in patients [12]. Another clinical study has reported that ganglioside combined with methylprednisolone in early acute spinal injury promotes the recovery of nerve function of patients and improves the prognosis compared to treatment with only methylprednisolone [92].

Moreover, intraventricular infusion of ganglioside GM1 induces phosphorylation of mutant Htt at specific serine amino acid residues that attenuate Htt toxicity, and restores normal motor function in already symptomatic HD mice [93]. The *in vitro* experiments suggest a role of nuclear GM1 in the protective effect, since mutant HD dysregulates calcium and interferes with numerous transcription factors. However, it is important to note that the alteration of gangliosides found in the post-mortem brain samples of three HD patients (grade 3) [94,95] is different from that found in the R6/1 and YAC128 mouse models and in the HD cultured fibroblasts [91,94]. In the latter, GM1 is reduced whereas an increase in GD3 and GM1 is observed in the caudate nucleus and in the cerebellum of HD patients, respectively [94,95]. Quantitative analyses of a larger number of human samples with varying grades of pathology are required to evaluate the impact of altered sphingolipid metabolism on HD. In addition, it would also be convenient to develop new animal models of the disease, since the current models do not mimic all the neurodegenerative changes observed in human HD.

Nuclear endocannabinoids and cannabinoid receptors

Endocannabinoids (N- arachidonoylethanolamine/anandamide and 2-arachidonylglycerol) and their receptors are involved in the regulation of energy balance, addiction, synaptic plasticity, neuroprotection, pain, anxiety, and depression [96-99]. Mammalian tissues contain at least two types of cannabinoid receptors, CB1 and CB2, both coupled to G proteins. CB1 is expressed mainly

by neurons of the central and peripheral nervous system. CB2 is primarily associated with immune function and is expressed on immune cells, including microglial cells within the nervous system [100]. CB2 expression has been found in gliomas, [101] and in human peripheral nerves after injury, particularly in painful neuromas [102]. It is worth noting that the effects of the cannabinoids are not always mediated by cannabinoid receptors, since they may also derive from the metabolism of the arachidonic acid present in their structure, and the generation of many bioactive lipids. CB1 maintain homeostasis in the central nervous by inhibiting excessive neuronal excitation and activity. Endocannabinoids that are synthesized and released following neurotransmitter binding to postsynaptic receptors function as retrograde synaptic messengers acting on presynaptic CB1 receptors to inhibit the release of neurotransmitters. Cannabinoid receptor immunoreactivity and binding activity are located on plasma and nuclear membranes whereas only low affinity binding is observed in the chromatin fraction [103]. Recently, CB1 has also been found in mitochondria, where it is overexpressed after traumatic brain injure and modulates apoptosis [104,105]. Endocannabinoids protect primary neurons, astrocytes and oligodendrocytes from apoptosis through mechanisms that include the control of glutamate homeostasis, calcium influx, the toxicity of reactive oxygen species, glial activation and the induction of autophagy [106]. Accumulating evidence suggests that endocannabinoids and their receptors also modulate progenitor cell proliferation and differentiation [107]. The modulatory effect on brain immune responses together with their effect on neurogenesis supports the concept that selected cannabinoids have potential as therapeutic agents for management of neurodegenerative disorders, such as AD, MS, HD, amyotrophic lateral sclerosis (ALS), and PD. In neuronal cells of adult brain, the activation of metabotropic receptors coupled to PLC-β1 is linked to endocannabinoid signaling through the production of DAG, which could be systematically metabolized by DAGL to produce an increase of 2-arachidonylglycerol, the most abundant endocannabinoid in the brain [98]. Interestingly, it has been recently reported that endocannabinoids might be synthesized also in the nucleus. Garcia del Caño et al. [6,9] hypothesized that nuclear endocannabinoids might regulate transcription acting as one of the physiological agonists at the nuclear receptor peroxisome proliferator activated receptor gamma (PPARgamma) or as a precursor of several types of prostaglandins. It will be interesting to know whether the nuclear pathway is involved in neuroprotection or in proliferation and differentiation of progenitor/stem cells. These processes are extremely important to be considered when developing new therapies for neurodegenerative disorders [106,107].

Cholesterol and cholesterol-derived lipids

Brain is the richest source of cholesterol in the body, accounting approximately 23% of total body cholesterol. Most brain cholesterol is present in myelin, neural membranes, and small amounts of cholesterol are associated with the nucleus [63,108-110]. Cholesterol affects the physicochemical properties of neural membranes, and activities of membrane-bound enzymes, receptors, and ion channels and it is the precursor of neurosteroids. In the nucleus, cholesterol metabolites modulate transcription. Cholesterol is not uniformly distributed within membranes. The plasma membrane contains nano/micro-domains that are enriched in cholesterol and sphingomyelin, the so-called 'lipid rafts'. These rafts represent highly dynamic structures that recruit downstream signaling molecules upon activation by external or internal signals. Cholesterol is involved in synaptogenesis, turnover, maintenance and stabilization of synapses and is a limiting factor for outgrowth of neurites.. Evidence deriving from *in vitro* and *in vivo* experiments in animal models and from observations of patients support the importance of alterations in cholesterol metabolism in the pathogenesis of neurodegenerative diseases such as AD, HD, PD, and even to the cognitive deficits typical of aging [67,111-113,68]. Astrocytes bearing the mutated Htt produce and secrete less cholesterol bound to apoE lipoproteins *in vitro*. HD neural stem-derived neurons display neurite outgrowth defects compared with control neurons. Addition of cholesterol, or conditioned media from wild type astrocytes, but not from HD astrocytes, revert the neuritic defect, suggesting that reduced glial cholesterol is detrimental for HD neurons [112]. Many *in vitro* studies have reported that cholesterol derivatives such as hydroxyl cholesterols and ketocholesterols have toxic effects on neurons, oligodendrocytes and microglia [67,111-113,68]. Moreover, oxysterols modify specific sites of the Aβ peptide thus enhancing Aβ aggregation and its neurotoxicity [111]. The increased ratio of 27-hydroxycholesterol to 24-hydroxycholesterol in AD brains is consistent with AD pathogenesis [111]. The expression of several genes involved in the cholesterol biosynthesis are reduced in striatum and cortex of HD mice and in post mortem cortical tissue collected from HD patients (see below) [112].

Oxysterols and neurogenesis

Oxysterols are not only intermediates in the cholesterol elimination pathway but also constitute important signaling molecules. Oxysterols including 24- hydroxycholesterol act as endogenous ligands for the nuclear receptor Liver X receptor (LXR). Recently it has been demonstrated that LXR is involved in neural development by regulating cell division, ventral midbrain neurogenesis, and dopaminergic neuronal differentiation [114]. In mice deletion of both LXRα and LXRβ slows

ventral midbrain neurogenesis, causing a decrease in dopaminergic neurons at birth. Immnunohistochemical experiments have shown that this results from an impairment in the cell cycle progression and from the reduced expression of several genes involved in the genesis and differentiation of dopaminergic neurons. To gain insight into the mechanism, *in vitro* experiments have been performed. Overexpression or activation of LXRs by classic oxysterol ligands increases the number of ventromedial embryonic primary cultures and dopaminergic differentiation of mouse embryonic stem cells [114]. Deletion of LXRs has the opposite effect. It is possible that some oxysterols may enhance adult neurogenesis and promote maturation of newly generated neurons. Recently other ligands responsible for midbrain neurogenesis have been identified [115]. While cholic acid increases survival and neurogenesis of specific red nucleus neurons, 24,25-epoxycholesterol promotes dopaminergic neurogenesis. The latter also promotes dopaminergic differentiation of embryonic stem cells, suggesting that LXR ligands may thus contribute to the development of cell replacement and regenerative therapies for PD, and likely to other neurodegenerative diseases such as amyotrophic lateral sclerosis.

The alterations found in the adult LXR double-knockout mice indicate that the function of LXR is not limited to the developing ventral midbrain. These mice progressively accumulate lipids in astrocytes and ventricular/periventricular cells, show abnormal blood-brain barrier, increased reactive microglia, astrogliosis, and degeneration of adult spinal cord motor neurons and midbrain dopaminergic neurons [116]. These results indicate that LXRs play an important role also in the adult brain, by regulating the maintenance of both midbrain dopaminergic and motor neurons.

Oxysterols and neurodegeneration

LXR are also involved in the regulation of inflammatory responses by reducing the nuclear factor-kappaB transcriptional activity and the expression of inflammatory mediators as well as cytokines and chemokines in the central nervous system. Accordingly, the natural and synthetic agonists of LXR can attenuate the imbalance of cholesterol metabolism and the overactivation of microglia and astrocytes in inflammation, and are widely used in a variety of neurodegenerative disease animal models [117].

The expression of genes involved in the biosynthesis of cholesterol and the amount of cholesterol and of 24-hydroxycholesterol are reduced in murine models of HD. In HD patients, the decrease of plasma 24-hydroxycholesterol correlates with disease progression evaluated as motor and neuropsychiatric dysfunction and brain atrophy [112]. Total plasma cholesterol is significantly reduced only in advanced stages. In cellular and animal models of HD the translocation of sterol

regulatory element-binding protein (SREBP) to the nucleus is reduced, resulting in decreased cholesterol synthesis. Some *in vitro* experiments have highlighted some aspects of the mechanism underlying the cholesterol changes. Wild type Htt is able to bind to some nuclear receptors involved in lipid metabolism (LXR, PPARgamma and VDR). Overexpression of Htt activates LXR while a lack of Htt results in a reduction of LXR-mediated transcription. It is possible that mutant Htt is less able to up-regulate LXR and LXR-targeted genes such as those involved in the synthesis of cholesterol and ApoE, involved in the transport of cholesterol from astrocytes to neurons. In addition, in oligodendrocytes mutant Htt inhibits the regulatory effect of peroxisome-proliferator-activated receptor gamma co-activator 1 alpha (PGC1a) on cholesterol metabolism and on the synthesis of myelin basic protein [112].

Future directions

Glycerophospholipids, sphingolipids, and cholesterol are not only integral components of the nuclear membranes and intranuclear domains but also act as messengers that regulate many cell processes. Evidence derived from different cell types indicate that distinct species have unique nuclear functions and act via temporally and spatially specific mechanisms. Despite the significant progress in this field, the role of nuclear glycerophospholipids, sphingolipids, endocannabinoids, and cholesterol and their derivatives in neurons, glia and microglia needs further investigation. Many nuclear effectors of the PPIn pathway involved in erythroid differentiation have been studied, but the ones involved in neuronal differentiation need identification. In the few cases in which the effectors are known (α-synuclein, for example) further effort is necessary to individuate the specific nuclear role. The mechanism involved in the signal transduction elicited by nuclear S1P and endocannabinoid receptors requires to be uncovered . It will be interesting to ascertain whether the nuclear envelope possesses S1P transporters and to discover whether the inside-out signaling of S1P between the interior and exterior of the cell is extended to the nucleus. It is necessary to identify the different lipid species and to quantify the changes occurring in physiological and pathological conditions in distinct subcellular localizations in order to allow a more complete understanding of how nuclear lipid metabolism coordinates global changes in cell function leading to activation, proliferation, differentiation or cell death. These studies need to be extended to NLM to reveal their role in the communication between the nucleus and the rest of the cell. Lipidomic profiling has revealed changes in the fatty acid composition in different phospholipids in cancer tissue compared with normal tissue and during cancer progression [23], and also studies on the lipid profile in subcellular compartments such as mitochondria have been performed and nuclear membrane

[118,21,24] but no information on nuclear lipidome in neural cells is yet available. The specific composition of lipids may help to discriminate low- and high-grade tumours as well as malignant cells from benign ones. Moreover, combined with transcriptome/ proteome analyses, lipidomic data could also unravel new potential lipid-related nuclear targets for the development of drugs to be administered alone or in combination with currently used chemotherapy. Although nuclear lipid alterations have scarcely been so far investigated directly in the context of neurodegenerative diseases, they might have an important role. It is, however, our suggestion that this area of research would be worthy of future investigations.

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Legends

Fig.1. Phosphoinositide metabolism in the nucleus.

Pathways for interconversion of phosphoinositides. Black arrows indicate kinase reactions while discontinous arrows indicate phosphatases. Some of the nuclear localization of the enzymes are also specified. The bulk of PI is found in membranes where they are substrates for kinases (PIPK), phosphatases and phospholipases. PIPKs share significant sequence homology but differ in the substrate specificities, subcellular localisations and functions. Type I (PIPKI) are PIPKs that phosphorylate PI4P to PI4,5P2 and are called PI4P 5-kinases because they phosphorylate at the D-5 hydroxyl group. Type II PIPKs (PIPKII) phosphorylate PI5P at the D-4 site and are called PI5P 4-kinases. PIPKIα and PIPKIγ are found in different subcellular localizations, including the nucleus, whereas PIPKIβ localizes at the perinuclear region. PI4,5P2 generated by PIPKIα at sites of concentrated pre-mRNA processing factors known as nuclear speckles regulates the activity of the nuclear poly(A) polymerase Star-PAP. PIPKIIα and PIPKIIβ are also found at speckles. Class I and class II PI3Ks have been reported to be localized in the nucleus while Class III (called PIKFyve in humans) is involved in vesicular trafficking and autophagy, and in EGF receptor nuclear translocation [119]. Class II PI3Ks, which comprise the PI3K-C2α, -C2 β, and -C2 γ isoforms, preferentially phosphorylate PI to yield PI3P, however, they can also yield PI3,4P2. Class I PI3Ks

phosphorylate both PI4P and PI4,5P2 to yield *in vivo* PI3,4P2 and PI3,4,5P3, respectively. PI3,4,5P3 is a crucial activator of phosphoinositide-dependent kinase 1 (PDK1) and thus the serine/threonine protein kinase AKT. The activity of the kinases is antagonized by a variety of phosphatases that can translocate to the nucleus (PTEN, SHIP1 and 2).

Fig. 2. Sphingolipid metabolism overview.

SMase, SK, CDase, SMSase have been found in the nucleus.

CDase: Ceramidase. CerS: Ceramide Synthase; CerK, Ceramide kinase; GCS, glucosylceramidesynthase; GCase, glucosyl ceramidase; SK, sphingosine kinase; SMase, sphingomyelinase; SMS, sphingomyelin synthase; S1P, sphingosine-1-phosphate; SPL, S1P lyase; SPPase, Sphingosine-1-phosphase phosphatase.

Fig. 3. Nuclear AKT signaling in neuronal survival.

Upon NGF stimulation, AKT is recruited to the plasma membrane, where it binds PI3,4,5P3and is fully activated following phosphorylation in two residues (●). AKT-mediated phosphorylation of ribosomal protein 3 (RPS3) increases nuclear accumulation of RPS3 and blocks its apoptotic effect. AKT is translocated to the nucleus, and phosphorylates zyxin and interacts with the isoform p48 of the ErbB3 and with B23, leading to inhibition of chromatin condensation and DNA fragmentation. Nuclear AKT also increases survival by phosphorylating and inhibiting the FOXO family members of transcription factors (not shown). NGF stimulates the translocation of PLCγ to the nucleus. There, it binds and activates PIKE, which in turn activates nuclear PI3K, that might contribute to activate nuclear AKT. The mechanism of activation of the nuclear AKT by NGF is still unclear: it is not known whether it is identical to that of cytoplasmatic AKT [46]. Dp5/Hrk: death protein 5/hara-kiri. ↑: activation ;┬ inhibition. Dashed arrows indicate translocation.

Fig. 4. Role of NLM on differentiation in HN9 hippocampal cells.

Vitamin D_3 induces differentiation in HN9 cells. In the inner nuclear membrane, vitamin D_3 bound to its receptor (VDR) is found in lipid microdomains (NLM) which are rich in cholesterol, PC and SM. Decrease of SM in NLM (by treatment with exogenous SMase or by serum deprivation) leads to a loss of VDR and impairs differentiation. Serum-deprived cells treated with a higher concentration of vitamin D3 (↑VD3) express NGF, show neurite elongation and exhibit increased SM and VDR content in NLM. NLM might act as a platform for vitamin D_3 effect on transcription.

Figure 1

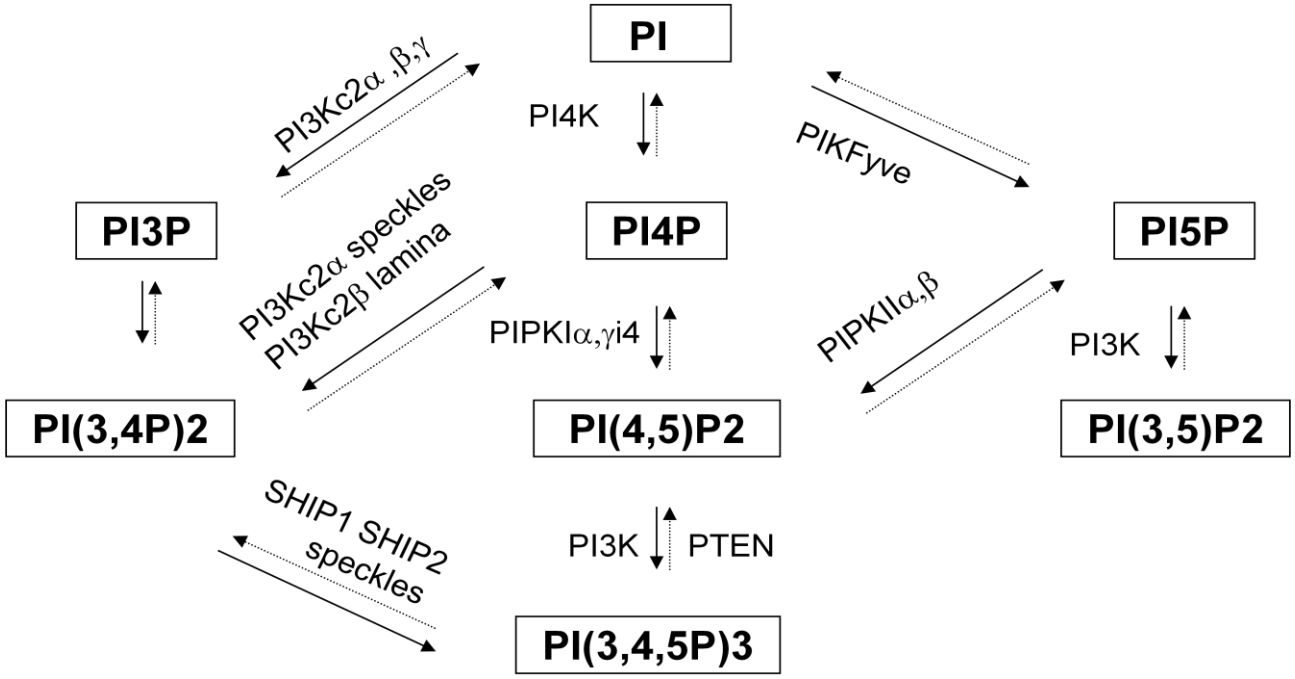

PI
PI3Kc2α ,β,γ
PI4K
PIKFyve
PI3P
PI4P
PI5P
PI3Kc2α speckles
PI3Kc2β lamina
PIPKIα,γi4
PIPKIIα,β
PI3K
PI(3,4P)2
PI(4,5)P2
PI(3,5)P2
SHIP1 SHIP2
speckles
PI3K
PTEN
PI(3,4,5P)3

Figure 2

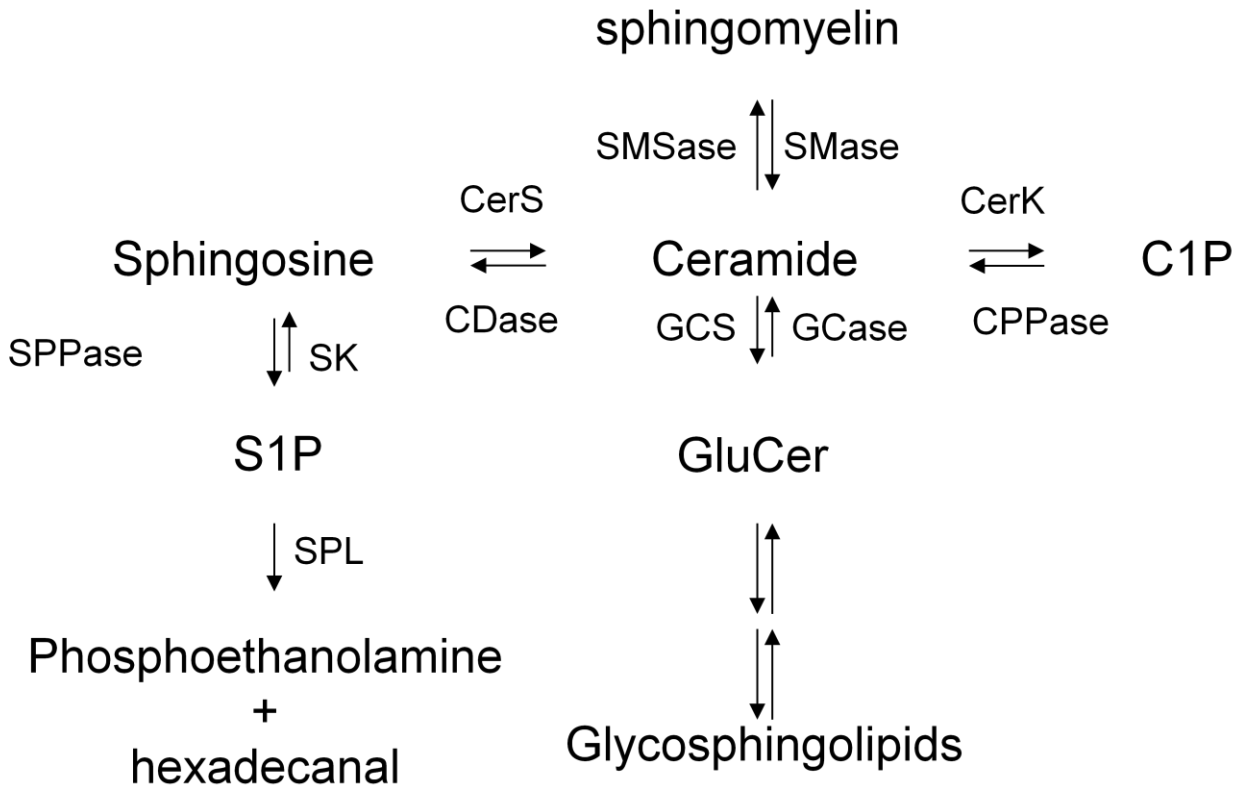
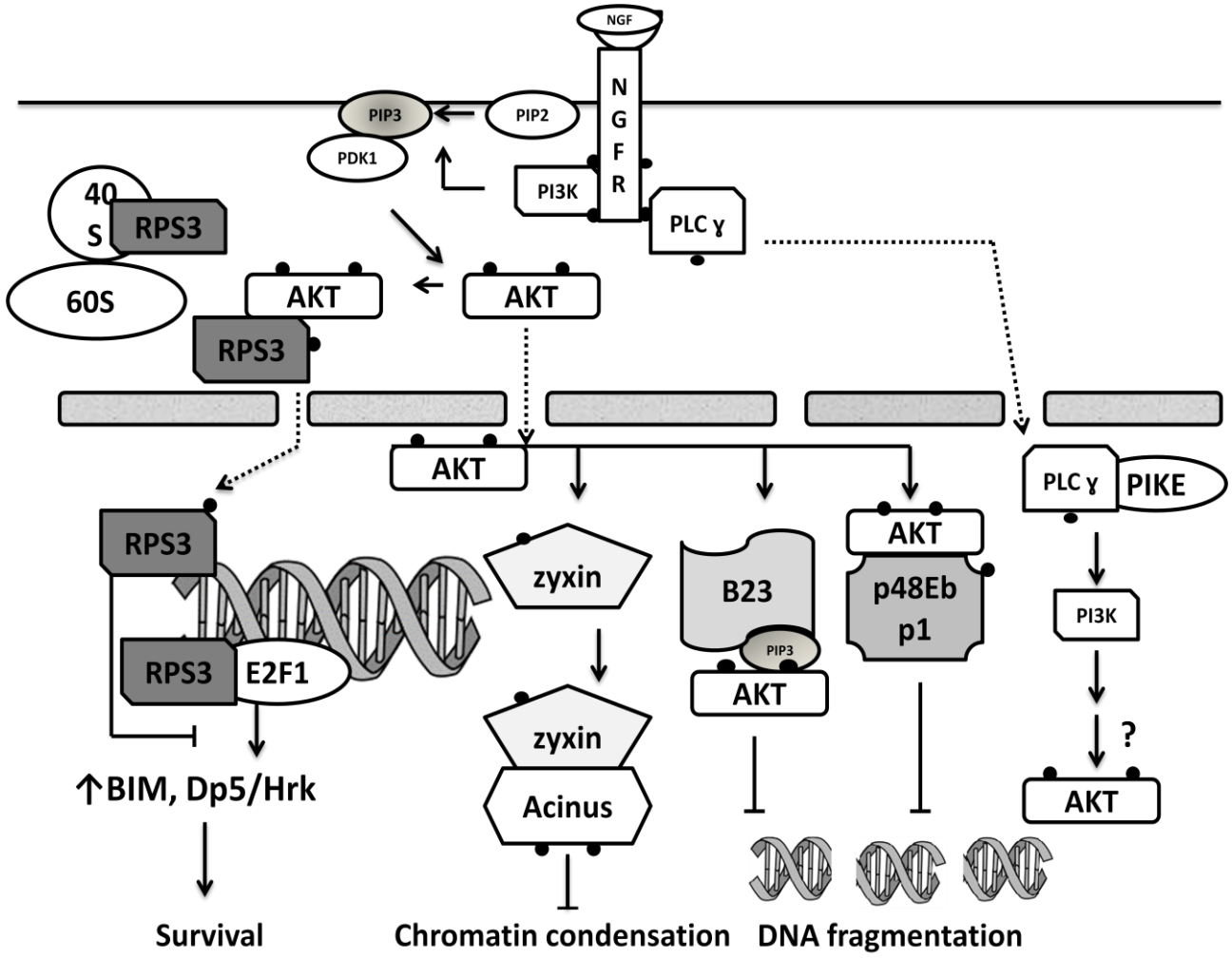

sphingomyelin
SMSase
SMase
CerS
CerK
Sphingosine
Ceramide
C1P
CDase
GCS
GCase
CPPase
SPPase
SK
S1P
GluCer
SPL
Phosphoethanolamine
+
hexadecanal
Glycosphingolipids

Figure 3


NGF
NGFR
PIP3
PIP2
PDK1
PI3K
PLC γ
40S
RPS3
60S
AKT
AKT
RPS3
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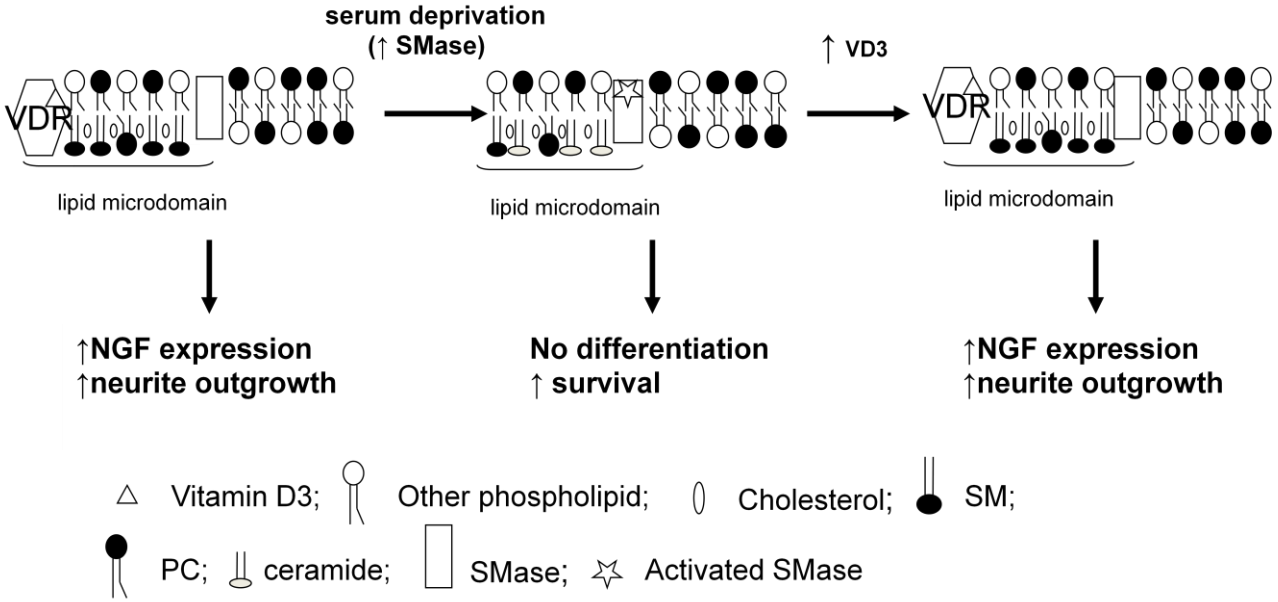
PLC γ
PIKE
RPS3
RPS3
E2F1
zyxin
B23
PIP3
AKT
AKT
p48Eb p1
PI3K
?
AKT
zyxin
Acinus
↑BIM, Dp5/Hrk
Survival
Chromatin condensation
DNA fragmentation

Figure 4


serum deprivation
(↑ SMase)
↑ VD3
VDR
VDR
lipid microdomain
lipid microdomain
lipid microdomain
↑NGF expression
↑neurite outgrowth
No differentiation
↑ survival
↑NGF expression
↑neurite outgrowth
Vitamin D3;
Other phospholipid;
Cholesterol;
SM;
PC;
ceramide;
SMase;
Activated SMase