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## Behind the stoNE wall: A fervent activity for nuclear lipids

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### ABSTRACT

The four main types of biomolecules are nucleic acids, proteins, carbohydrates and lipids. The knowledge about their respective interactions is as important as the individual understanding of each of them. However, while, for example, the interaction of proteins with the other three groups is extensively studied, that of nucleic acids and lipids is, in comparison, very poorly explored. An iconic paradigm of physical (and likely functional) proximity between DNA and lipids is the case of the genomic DNA in eukaryotes: enclosed within the nucleus by two concentric lipid bilayers, the wealth of implications of this interaction, for example in genome stability, remains underassessed. Nuclear lipid-related phenotypes have been observed for 50 years, yet in most cases kept as mere anecdotal descriptions. In this review, we will bring together the evidence connecting lipids with both the nuclear envelope and the nucleoplasm, and will make critical analyses of these descriptions. Our exploration establishes a scenario in which lipids irrefutably play a role in nuclear homeostasis.

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## Abbreviations

<b>Acc1</b>	Acetyl-CoA Carboxylase 1	<b>INM</b>	Inner Nuclear Membrane
<b>ACSL3</b>	Long-chain-fatty-acid-CoA ligase 3	<b>KASH domain</b>	Klarsicht-Anc-1-Syne-1 Homology domain
<b>AGPAT2</b>	1-Acyl-sn-Glycerol-3-Phosphate AcylTransferase 2	<b>LAP1</b>	Lamina-Associated Polypeptide 1
<b>AH</b>	Amphipatic Helix	<b>LBR</b>	Delta(14)-sterol reductase Lamin B Receptor
<b>Apo B/C-III/E</b>	Apolipoprotein B-100/C-III/E	<b>LCB</b>	Long-Chain Base
<b>ATGL</b>	Patatin-like phospholipase domain-containing protein 2	<b>LEM domain</b>	LAP2-Emerin-MAN1 domain
<b>ATM</b>	serine/threonine-protein kinase Ataxia Telangiectasia-Mutated	<b>LINC</b>	Linker of Nucleoskeleton and Cytoskeleton
<b>ATR</b>	serine/threonine-protein kinase Ataxia Telangiectasia and Rad3-related protein	<b>MMS</b>	Methyl Methanesulfonate
<b>BAR domain</b>	Bin-Amphiphysin-Rvs domain	<b>MTP</b>	Microsomal Triglyceride transfer Protein
<b>CCT<math>\alpha</math></b>	CTP:phosphocholine CytidylylTransferase alpha	<b>MVB</b>	MultiVesicular Body
<b>CDP-Choline</b>	Cytidine DiPhosphate-choline	<b>NCS</b>	Nucleolar Channel System
<b>CDP-DAG</b>	Cytidine DiPhosphate choline-DiAcylGlycerol	<b>NE</b>	Nuclear Envelope
<b>Cds1</b>	Cytidine diphosphate-Diacylglycerol Synthase 1	<b>NET</b>	Envelope transmembrane protein
<b>CE</b>	Cholesterol Esters	<b>NHEJ</b>	Non-Homologous End Joining
<b>CEPT1</b>	Choline/Ethanolamine PhosphoTransferase	<b>nLD</b>	nuclear Lipid Droplet
<b>CHMP7</b>	CHarged Multivesicular body Protein 7	<b>NPC</b>	Nuclear Pore Complex
<b>clD</b>	cytoplasmic Lipid Droplet	<b>NR</b>	Nucleoplasmic Reticulum
<b>cnep1</b>	CTD nuclear envelope phosphatase 1	<b>NUMEN (ENDOD1)</b>	NUclear Membrane Endo/exoNuclease (Endonuclease domain-containing 1 protein)
<b>CPT</b>	Camptothecin	<b>OA</b>	Oleic Acid
<b>DAB</b>	3,3'-DiAminoBenzidine	<b>Osh1</b>	Oxysterol-binding protein 1
<b>DAG</b>	DiAcylGlycerol	<b>ONM</b>	Outer Nuclear Membrane
<b>DDR</b>	DNA Damage Response	<b>PA</b>	Phosphatidic Acid
<b>DGAT 1/2</b>	Diacylglycerol O-AcylTransferase 1/2	<b>PAP1</b>	Phosphatidic Acid Phosphatase 1
<b>DHCR24</b>	Delta(24)-sterol reductase	<b>PC</b>	PhosphatidylCholine
<b>DHCR7</b>	7-DeHydroCholesterol Reductase	<b>PDAT</b>	Phospholipid-Diacylglycerol AcylTransferase
<b>DSB</b>	Double-Stranded deoxyribonucleic acid Breaks	<b>PFOA</b>	PerFluoroOctanoic Acid
<b>EBP</b>	3-beta-hydroxysteroid-Delta(8),Delta(7)-isomerase	<b>PG</b>	PhosphatidylGlycerol
<b>EPHB2</b>	EPHrin type-B receptor 2	<b>PI</b>	PhosphatidylInositol
<b>ER</b>	Endoplasmic Reticulum	<b>PIP</b>	PhosphoInositol Phosphate
<b>ESCRT-III</b>	Endosomal Sorting Complexes Required for Transport-III	<b>PIP<sub>2</sub></b>	Phosphatidyl-Inositol-biPhosphate
<b>FRAP</b>	Fluorescence Recovery After Photobleaching	<b>PIP<sub>3</sub></b>	Phosphatidyl-Inositol-1,4,5-triPhosphate
<b>GFP</b>	Green Fluorescent Protein	<b>PLC-<math>\delta</math></b>	PhosphoLipase C delta
<b>GPAT 3/4</b>	Glycerol-3-Phosphate AcylTransferase 3/4	<b>PML</b>	ProMyelocytic Leukemia protein
<b>HDAC 1/2</b>	Histone DeAcetylase 1/2	<b>PS</b>	PhosphatidylSerine
<b>HMG-CoA</b>	3-Hydroxy-3-MethylGlutaryl-coenzyme A reductase	<b>rDNA</b>	ribosomal DNA
<b>HU</b>	Hydroxyurea	<b>RNAi</b>	RNA interference
<b>IP<sub>3</sub></b>	Inositol-1,4,5-triPhosphate	<b>RPA</b>	Replication Protein A
<b>IP<sub>3</sub>R</b>	Inositol-1,4,5-triPhosphate Receptor	<b>S1P</b>	Sphingosine-1-Phosphate
<b>IDR</b>	Intrinsically Disordered Region	<b>ssDNA</b>	single-stranded DeoxyriboNucleic Acid
<b>IMS</b>	Inter-Membrane Space	<b>SUN domain</b>	Sad1-UNC-84 domain
<b>ING</b>	INHibitor of Growth	<b>TAG</b>	TriAcylGlycerol
		<b>TERT</b>	TElomerase Reverse Transcriptase
		<b>TMX4</b>	thioredoxin-related transMembrane protein 4
		<b>TopoII<math>\beta</math></b>	DNA Topoisomerase 2-beta
		<b>VLDL</b>	Very-Low-Density-Lipoprotein

## 1. Introduction

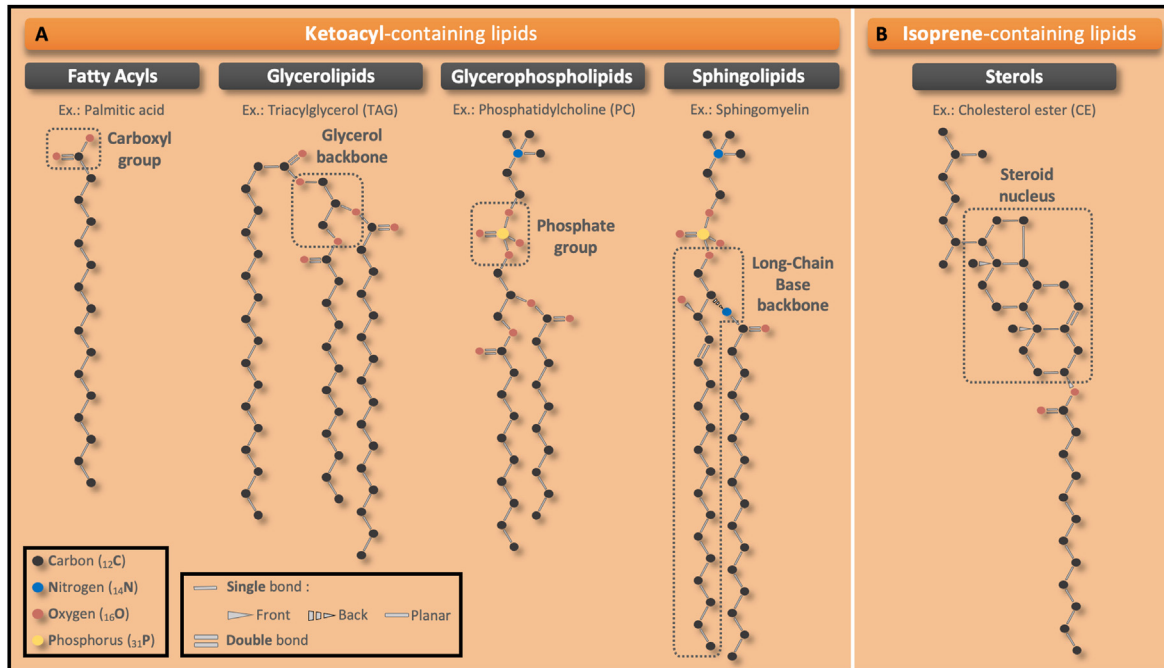
The nucleus, the primary repository of genetic material in eukaryotic cells, is delimited by the nuclear envelope (NE) in a discontinuous manner. This envelope consists of two bilayers interrupted by macromolecular proteinaceous rings named the nuclear pore complexes (NPC) [1]. The NPC dynamically interact with DNA/RNA molecules, and proteins. Historically, the in-depth study of nuclear membranes as an individual cellular entity dates back to the first characterization of their morphology through early electron microscopy in the 1950s [2], and their biochemical composition by thin-layer chromatography analysis in the 1970s [3,4]. More recently, in the 2010s, biophysical studies provided the fundamental insights into intricate structural details and nuclear membranes' thermodynamic behaviour [5].

From a functional perspective, efforts have mainly unravelled the function of the NE serving as a physical barrier: it isolates the genome, warranting its own metabolism, and acts as a diffusion barrier, tightly controlling the ins and outs of nucleo-cytoplasmic macromolecular exchange. To go beyond and understand all possible scales of NE's functions, an expanding set of descriptive and causative studies in the field of human pathophysiology is gathering (reviewed in Ref. [6]). The emerging picture features an active NE's role in regulating genome-associated processes. Yet, this field is developed mostly from a protein-centred perspective. The significance of lipids in NE's active functions, in particular in genome homeostasis, has not gained comparable attention yet. In this review, we will give stage to the contribution of lipids to NE function and to nuclear homeostasis.

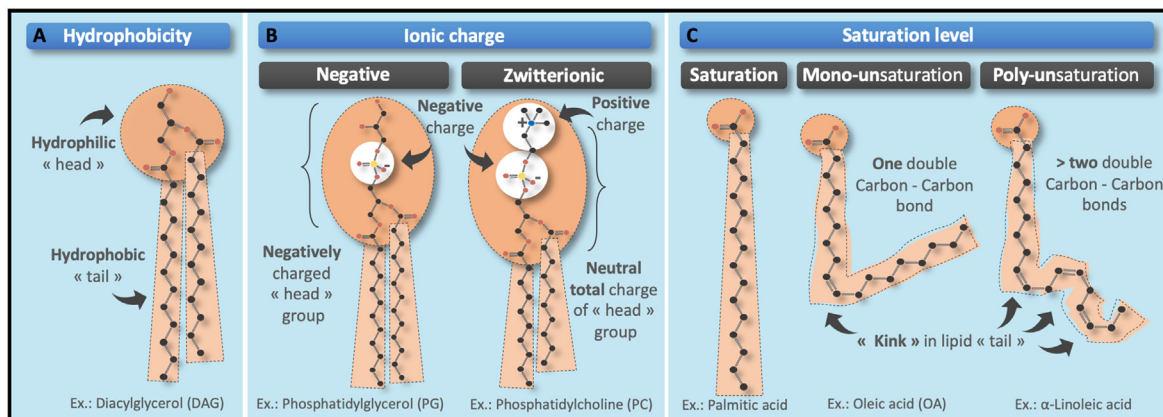
### 1.1. Basic concepts

**Lipid classification.** Among the four types of biomolecules - nucleic acids, proteins, lipids, and carbohydrates - lipids stand out as the most heterogeneous group, owing to their exceptional structural and functional diversity. According to the LIPID MAPS classification system [7,8], all lipid species are biosynthesized either from ketoacyl groups by the carbanion-mediated mechanism of chain extension or from isoprene groups *via* the alternatively mediated carbocation pathway. Therefore, the first level of lipid classification delineates two fundamental groups: 1) the ketoacyl-containing lipids, which include six categories - fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, and polyketides, and 2) the isoprene-containing lipids, which encompass the categories of sterols and phenol lipids. Given the focus of this review, we would like to highlight the distinctive structural features defining five of the eight aforementioned categories, especially the presence of 1) a hydrocarbon chain with a terminal carboxyl group for fatty acyls, the category that includes the class of **Fatty Acids** (FA); a glycerol group for 2) **Glycerolipids** and 3) **Glycerophospholipids** (the latter also containing a phosphate group); 4) a sphingoid base backbone (also known as Long-Chain nitrogenous Bases (LCB)) for **Sphingolipids**; 5) a four-ring-core structure for **Sterols** (Fig. 1).

**Lipid-related physico-chemical properties important for biological membranes.** Membranes' structure and thermodynamic behaviour in a cellular context are predominantly determined by the physical and biochemical properties of their composing lipids [9], mostly by:



**Fig. 1. Lipid classification.** Two-dimensional diagrams of skeletal formulas depicting (A) ketoacyl-containing, and (B) isoprene-containing lipid categories. All lipids are classified using the LIPID MAPS system (see Section 1.1). **From left to right:** each lipid category (fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterols) is illustrated by one example of lipid class with its characteristic functional groups, depicted by dashed rectangles: palmitic acid (hexadecanoic acid,  $C_{16}H_{32}O_2$ ) with carboxyl group (COOH); triacylglycerol (1-dodecanoyl-2-hexadecanoyl-3-octadecanoyl-*sn*-glycerol, TG 12:0/16:0/18:0,  $C_{49}H_{94}O_6$ ) with glycerol backbone ( $C_3H_5O_3$ ); phosphatidylcholine (1,2-didecanoyl-*sn*-glycero-3-phosphocholine, PC 10:0/10:0,  $C_{28}H_{56}NO_8P$ ) with phosphate group ( $PO_4$ ); sphingomyelin (N-(hexadecanoyl)-sphing-4-enine-1-phosphocholine, SM d18:1/16:0,  $C_{39}H_{79}N_2O_6P$ ) with long-chain base backbone ( $C_{18}H_{37}NO_2$ ); cholesterol ester (cholest-5-en-3 $\beta$ -yl dodecanoate, CE 12:0,  $C_{39}H_{68}O_2$ ) with steroid nucleus ( $C_{19}$ ). Note that all skeletal formulas are represented in a linear manner without illustration of a « kink » (*trans* conformation) for the sphingosine molecule. Atoms are shown as colored circles: black for carbon ( $_{12}C$ ), blue for nitrogen ( $_{14}N$ ), red for oxygen ( $_{16}O$ ), and yellow for phosphorus ( $_{31}P$ ). Chemical bonds are depicted by gray lines: one line for a single bond, two lines for a double bond. Note that the two-dimensional stereochemistry of skeletal formulas is indicated by the Natta projection method: solid line for a planar chemical bond, solid wedge for a front bond, and dashed wedge for a back bond.



**Fig. 2. Physico-chemical lipid properties** (see Section 1.1). **(A) Hydrophobicity** is illustrated using an example of a diacylglycerol molecule (1-octadecanoyl-2-hexadecanoyl-sn-glycerol, DG (18:0/16:0/0:0),  $C_{37}H_{72}O_5$ ). The hydrophilic « head », containing functional groups (carboxyl group and glycerol backbone), is shown by a dark orange circle with a dotted outline. The hydrophobic « tails », composed of a hydrocarbon chain (i.e. acyl chain), are depicted by light orange rectangles with dashed outlines. **(B) Ionic charge.** From left to right: phosphatidylglycerol molecule exemplifies a negatively charged lipid species, in the « head » of which a **negative** ionic charge is conferred by a phosphate group ( $PO_4^-$ ). A phosphatidylcholine molecule illustrates an example of a **zwitterionic** lipid species, defined by the presence of a neutral total « head » charge due to an equal number of opposite charges (one negative from a phosphate group ( $PO_4^-$ ) and one positive from quaternary amine group of choline ( $C_5H_{14}NO^+$ )). **(C) Saturation level.** From left to right: **saturation**, which is defined by an absence of double carbon-carbon bonds in a hydrocarbon chain (lipid « tail »), is illustrated with a molecule of palmitic acid (hexadecanoic acid,  $C_{16}H_{32}O_2$ ). **Mono-unsaturation** (one carbon-carbon double bond), and **poly-unsaturation** (two or more), are exemplified by oleic acid (9Z-octadecenoic acid (d5),  $C_{18}H_{34}O_2$ ); and  $\alpha$ -linoleic acid (9Z,12Z,15Z-octadecatrienoic acid,  $C_{18}H_{30}O_2$ ), respectively. Double carbon-carbon bonds induce « kinks » in a « tail » structure, that are indicated with dark gray arrows.

- 1. Hydrophobicity:** physical property defined as the “relative tendency to prefer non-aqueous to an aqueous environment” [10]. For example, all membrane glycerophospholipids are composed of a hydrophobic “tail” (hydrocarbon chain) and a hydrophilic “head” (functional glycerol and phosphate groups linked to yet another molecular species such as choline, ethanolamine ...). The molecules simultaneously exhibiting hydrophobic and hydrophilic natures (Fig. 2A) are classified as **amphipathic**.
- 2. Ionic charge:** chemical property designated as “an electric charge of an ion, created by the gain (negative charge) or loss (positive charge) of one or more electrons from one or a group of atoms” [11]. The biomolecules exhibiting an equal number of opposite electric charges are defined as **zwitterionic**, while some glycerophospholipids appear negatively charged due to the non-counteracted presence of a phosphate group (Fig. 2B).
- 3. Saturation state:** physico-chemical feature specific for the hydrophobic lipid “tail”. The term “saturated” means that, in a given hydrocarbon chain, each carbon atom is bound to the maximal number of possible hydrogens. On the contrary, the hydrocarbon chain with at least one carbon-carbon double bond is termed as “unsaturated”. In function of the number of double bonds, lipid “tails” can be classified as mono-unsaturated or poly-unsaturated, characterized by one or two and more double bonds, respectively (Fig. 2C).

## 2. The nuclear envelope as a vital cellular entity (and its physiological functions)

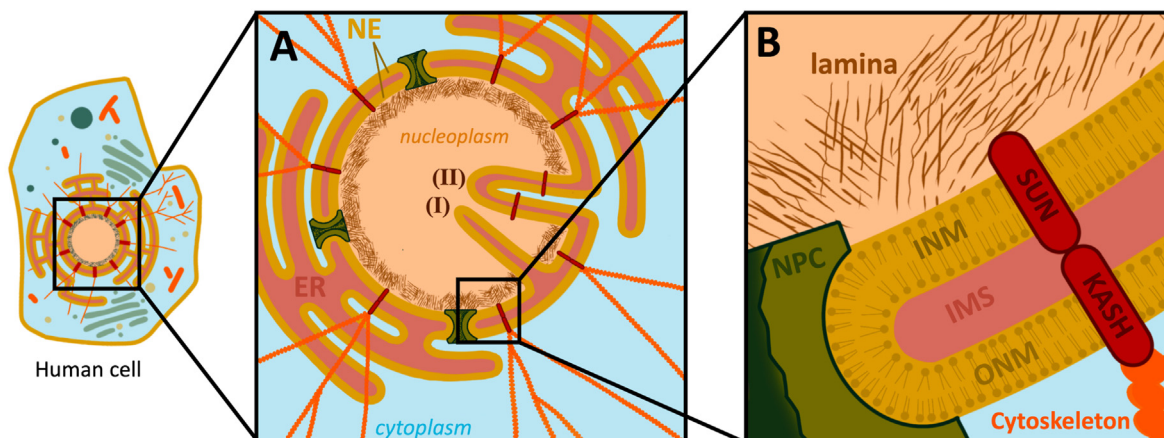
### 2.1. NE structural organization

The endogenous compartmentalization of eukaryotic cells, which establishes most organelles [12], particularly well illustrates the importance of intracellular membranes. Membranes were initially defined by the « fluid and mosaic model », which stated « a two-dimensional oriented solution of integral proteins [...] in the viscous phospholipid bilayer » [13]. Recently revisited [14], the updated model also considers: 1) the membrane asymmetry, 2) the dynamic association of membrane components into multi-

molecular complexes, 3) the existence of defined domains enriched in specific lipids, and 4) *cis*- and *trans*-membrane linkages (where, “*cis*-linkages” refer to the intra-membrane protein-lipid interactions, while “*trans*-linkages” involve the membrane’s connections to intracellular elements (for example, the cytoskeleton), and the extracellular environment (extracellular matrix)). It also accounts for rotational and lateral mobilities of its components, compressibility, molecules’ residence time and activities, the whole depicting a denser and more mosaic model than the original one. Among the cellular membranes fitting into this description there is the Nuclear Envelope (NE), which encloses the nuclear genome, structurally and functionally defining the nucleus. An early electron microscopy study of thin-layer sections from mammalian cells, performed by Watson in 1955 [2], presented its first morphological definition, including 1) the Outer Nuclear Membrane (ONM), which is continuous with the membranes of the Endoplasmic Reticulum (ER); 2) the Inner Nuclear Membrane (INM), facing a nucleoplasm and separated from the ONM by the Inter-Membrane Space (IMS), i.e. the perinuclear space, which is structurally continuous with the ER lumen; and 3) the Nuclear Pore Complexes (NPC), ring-shaped structures at the sites of INM/ONM fusion (Fig. 3). Cell imaging technologies, including three-dimensional reconstructions, have provided detailed ultrastructural insights into these morphological features. For example, the physiological width of the NE was found to be about 50 nm in *Homo sapiens* cell lines [15], and 18 nm in *Saccharomyces cerevisiae* [16]. Also, the architecture of the NPC was defined as composed of 1) the core, at the central transport channel and exhibiting rotational symmetry along the perpendicular and parallel axis of the NE; 2) the asymmetrical extensions, namely cytoplasmic filaments and nuclear baskets (reviewed in Ref. [17]).

Functionally, the highly selective bi-directional nucleo-cytoplasmic exchange of macromolecules (such as RNA and proteins) through the NPC restricts their free diffusion (reviewed in Ref. [18]). Other than this transport, the nucleo-cytoplasmic dialogue implies the transmission of internal and external mechanical forces through the NE. In most multicellular eukaryotic species, including plants, this process of mechanotransduction from the extracellular matrix, as well as the organization of nuclear position, is coordinated by the Linker of Nucleoskeleton and Cytoskeleton (LINC)





**Fig. 3. General features of the human nuclear envelope.** (A) Zoom into the nucleus, defined by two membranes, the nuclear envelope (NE): the inner one is in contact with the nucleoplasm; the outer one is in contact with the cytoplasm and in continuity with the endoplasmic reticulum (ER). These membranes can undergo remodelling, for instance, the invagination of the inner nuclear membrane (I) or both (II), a structure termed nucleoplasmic reticulum (see [section 4.1](#)). (B) Important protein complexes are found at the NE: in green, the Nuclear Pore Complex (NPC), allowing nucleo-cytoplasmic transport and scaffolding the physical connection between the inner nuclear membrane (INM) and the outer nuclear membrane (ONM), otherwise separated by the inter-membrane space (IMS). In red, the LINC complex, part of the NE transmembrane proteome, is depicted given its importance in NE structure. LINC is formed by SUN domain- and KASH domain-containing proteins, both transmembrane proteins bridged with each other in the IMS. KASH proteins bind the cytoskeleton in the cytoplasm (in orange), while SUN proteins bind the nucleoskeleton (the lamina, in brown) in the nucleoplasm.

complex ([Fig. 3](#)) [19–21]. LINC is composed by SUN domain- and KASH domain-containing proteins, both of which are part of the Nuclear Envelope Transmembrane (NET) proteome at the INM and at the ONM, respectively. The LINC complex connects the three types of cytoskeletal proteins, represented by microtubules, intermediate filaments and actin filaments, to the nucleoskeleton, mainly composed of the nuclear lamina, intimately underlying the INM and containing type V intermediate filament proteins, the lamins ([Fig. 3](#)). Of note, lamin-like proteins fulfil this same function in plants [22] and beyond [23]. Further, even eukaryotes lacking the lamina, as budding yeast, exploit LINC-like complexes to warrant nuclear homeostasis [24]. LINC complex dysregulation participates to nuclear softening [25] and leads to dramatic (unscheduled) NE remodelling that matches high migration rates and metastatic behaviour [26,27]. Also, the LINC complex interacts with and tethers chromatin, which fulfils roles in structuring genome territories, modulating gene expression, and preventing DNA damage [28,29].

Across various phylogenetic kingdoms, core nuclear functions have been kept through evolution, from single cell eukaryotes to metazoans, such as the proteins involved in lipid metabolism [12]. However, other features are far from conserved, as for example the simplistic picture of the NE as a uniform spherical structure. First, a diversity of nuclear shapes, clearly departing from sphericity, have been reported (see next [section](#)). Second, the same way the ONM branches into the cytoplasm in what we know as the Endoplasmic Reticulum (ER), recurrent observations report the INM branching into the nucleoplasm, known as the Nucleoplasmic Reticulum (NR)-type I, a dynamic intranuclear network that penetrates the nucleus in vertical and/or horizontal orientations, forming tubular, branching and interconnecting extensions (see [section 4.1](#)). Analogously, invaginations of both nuclear membranes, known as NR-type II, also exist (see [section 4.1](#)) ([Fig. 3](#)).

## 2.2. Lipid determinants of NE composition

The early characterization of the NE's lipid composition started with biochemical analyses from mammalian cell extracts, including rat liver [4], calf thymus [3], and bovine liver [30]. These investigations revealed that nuclear membranes contain

approximately 0.33 mg of lipids per 1 mg of proteins, indicating that lipid fraction is equal, on average, to 30 % of total mass. Detailed thin-layer chromatography analyses unveiled: 1) the prevalence of glycerophospholipid species, that comprise  $\approx 62\%$  of total lipid fraction and are composed of phosphatidylcholine (PC) ( $\approx 53\text{--}55\%$ ), phosphatidylethanolamine ( $\approx 23\text{--}25\%$ ), phosphatidylinositol (PI) (8 %) and phosphatidylserine (PS) (6 %); as well as 2) a lesser contribution of cholesterol and sphingomyelin. Recently, Dazzoni et al. [5] revisited these assessments using liquid-state Nuclear Magnetic Resonance, a technique enabling metabolomic analysis through the detection of proton ( $^1\text{H}$ ), carbon ( $^{13}\text{C}$ ), and phosphorus ( $^{31}\text{P}$ ) nuclei distribution in biological samples. The authors detected the enrichment of PC, PI and cholesterol within the nuclear membranes of the human HEK293-T cell line. Overall, these results point at glycerophospholipids and cholesterol species as the main lipid components of NE.

The condensation reaction of serine and palmitoyl-CoA by serine palmitoyltransferase, a conserved enzyme localized in the NE and the ER, yields products collectively referred to as Long-Chain Bases (LCB) (see [section 1.1](#) § [Fig. 1](#)). LCB can be further esterified with a FA, generating sphingolipids. Although sphingolipids abundance at the NE is very low, the local concentration of their derivative, ceramide, in the vicinity of NPC suggests a compartmentalized role for sphingolipids at the NE [31]. This may be important because sphingolipids are generally long molecules, a feature presumably important at bending sites where ONM and INM meet (discussed in [section 3.2](#)). Further, the sphingolipid precursors, the LCB, are not only enriched at the NE, but also essential to restrict excessive NE fluidity [32].

A loosely packed unsaturated glycerophospholipid (see [section 1.1](#) § [Fig. 2C](#)) profile appears to feature the NE membranes (reviewed in Ref. [33]). Compared to the rigid plasma membrane, enriched in cholesterol, glycerophospholipids bearing tightly packed saturated FA, and a high density of sphingolipids in its outer leaflet [34], the nuclear membranes thus appear more fluid and flexible. These features likely confer plasticity that is important to preserve nuclear integrity (and likely genome stability) under compression, such as during migration, when cells experience a passage through constrained spaces, or in the face of sudden alterations in external/internal pressure. At the same time, however,

the NE needs to remain sufficiently impermeable to ensure that nucleo-cytoplasmic exchange of macromolecules occurs only through the nuclear pores, where traffic is exquisitely controlled, and not through unscheduled NE ripening, which arises if the envelope is too fluid [32,35]. On the one hand, the observation that the NE is spherical to elliptical in many eukaryotic cells may stem from a balance between intra-nuclear and extranuclear forces. On the other hand, depart from sphericity may be ascribed to this comparatively more fluid nature, which renders it a highly modifiable structure, and this throughout the cell cycle [36], in different cell types [37] or in different pathological scenarios [38]. Interesting examples of NE that depart from sphericity include human granulocytes, which are multilobed [39], an anatomy important to establish chromosome territories, themselves essential to shape the transcriptional patterns that define their identity [40]. Also, the process of spermatogenesis leads to various NE shapes. In metazoans, spermatogenesis is accompanied by a compact NE and chromatin [41], and a decrease in NPC, likely reflecting that nucleo-cytoplasmic transport is mostly dispensable in these cells [42]. Reinforcing the fundamental contribution of nuclear membranes' lipid profiles, NE shape diversity has been well described in different species [43,44] and, when comparing the present species of the eukaryotic domain across evolution, the most conserved NE proteins are involved in lipid metabolism [11], suggesting that the lipids in membranes are the limiting factor for changes in NE morphology and function.

The individualized lipidomic characterization of the ONM and the INM is hampered by the difficulty in separating one from each other for analytical purposes. As briefly stated earlier, the direct physical contact of the INM with the nuclear content and the barrier imposed by the nuclear pores presume a unique lipid signature for the INM. An asymmetry in lipid distribution across the NE, separating the INM from ONM, can arise from the physical constraints imposed by membrane lipids themselves. The highly curved regions, such as the INM-ONM fusion sites, can selectively restrict the transfer of certain lipids according to their shape [45], which include lipid species promoting negative curvature (PA, DAG, PE, cholesterol), positive one (PI, lysoglycerophospholipids) and zero curvature (PC) (Fig. 4A). Alternatively, a targeted enrichment of specific lipids at the INM could establish the asymmetry. In this line, a subset of recently published studies demonstrated an enrichment of DAG at the INM of yeast cells [46,47]. In mammalian cells, the need of supporting a fluid INM was reinforced by the finding that the FA composing those DAG molecules were mostly of a poly-unsaturated nature [48]. Functionally, the presence of poly-unsaturated DAG at the INM has implications in sculpting the INM proteome. For example, SUN2, the INM-resident component of the LINC complex, which transmits forces between the nucleo- and the cytoskeleton and maintains a stable distance between the two NE membranes, possesses a lipid-binding amphipathic helix (AH) in its nucleoplasmic domain. By intercalation of the AH within the membrane packing defects (Fig. 4B) created by DAG molecules, SUN2 was stabilized and resistant to proteasomal degradation [48]. Thus, a decrease in INM packing defects concurs with a modification of LINC stoichiometry, thus NE architecture.

As a balancing mechanism, the rate-limiting enzyme in PC synthesis, CTP:phosphocholine cytidyltransferase (Pct1 in *S. cerevisiae*, Pcyt1 in *Drosophila melanogaster*, CCT $\alpha$  in mammals) localizes to the nucleus and is regulated at the INM [49]. Low levels of PC at the INM, or an increase in DAG, as stated in the case of SUN2, cause lipid packing defects that elicit CCT $\alpha$  adsorption to the membrane via a deployed AH, in turn activating it. The soluble product of CCT $\alpha$ , CDP-Choline, is used along with DAG to yield PC. In this sense, launching this reaction would simultaneously increase the amount of PC while consuming DAG. Although the enzyme of

the pathway responsible for this latter reaction, CEPT1, has been described at the NE [50], whether the reaction occurs at the INM has not been elucidated. PC synthesis, either at the INM directly, or originating from the ONM, would help restore correct membrane packing (Fig. 4B), subsequently entailing CCT $\alpha$  drop-off the membrane, elegantly halting its own biosynthetic activity [49].

### 2.3. Physiological roles of the nuclear envelope

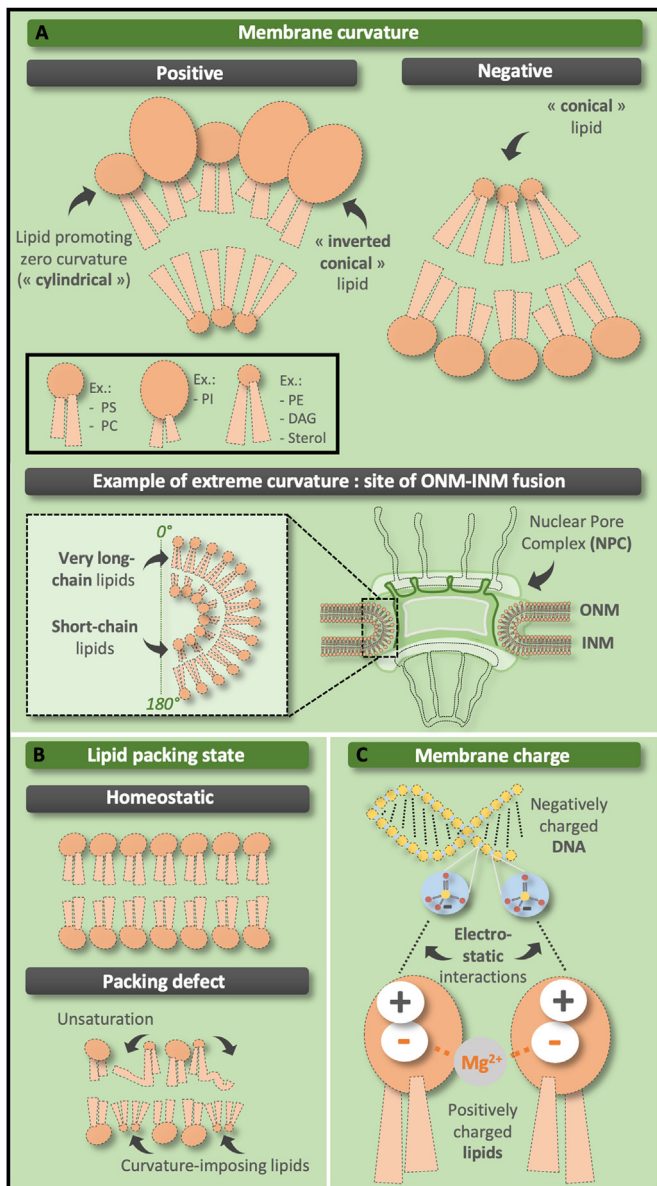
The NE fulfils numerous functions. For the sake of focus, we will briefly state those less concerned with the topic of this review, while we will deliberately expand on those in full link with it.

#### 2.3.1. A barrier for nucleo-cytoplasmic transport

The NE compartmentalizes the cell by confining the genetic material, therefore opposing to the free flow of biomolecules. This is a hallmark of eukaryotic cells and, for communication and transport to be possible, the NE barrier needs to be overcome. There is an increasing body of evidence indicating that the two nuclear membranes can be remodelled to give rise to vesicles that will permit the transfer of material from the nucleus to the cytoplasm, a process termed NE budding. This mechanism would be used by healthy cells to transport of RNA granules, aggregated proteins, for the disposal of INM proteins and of mis-assembled nuclear pore complexes (reviewed in Ref. [51]). However, the classical route, and the best studied, implies the stable piercing of the NE by sophisticated, macromolecular complexes termed the nuclear pores that act as gates to transport macromolecules in a carefully controlled manner. Transport through the pores has been extensively addressed in the past (reviewed in Ref. [17]). Non-canonical aspects of transport and how this process is deregulated in pathology is presented in a clever manner in Ref. [52]. Also, regulatory aspects that have drawn less attention, such as the importance of calcium in regulating nucleo-cytoplasmic transport, have been addressed in Ref. [53]. To stay on focus, these aspects will not be further discussed here.

#### 2.3.2. The NE in calcium signalling

Intracellular calcium acts as a second messenger in multiple signalling pathways in the cell. Calcium is stored inside the ER and transient opening of ER channels (and timely closing) in response to the adequate cues endows such cascades. These processes are understood with great detail concerning calcium release into the cytoplasm. It took longer to realize, and it is therefore less well understood, that the IMS being a continuum with the ER lumen, there also exists a calcium pool that could be released from the NE into the nucleoplasm. Not only there is the calcium, but the full set of molecules participating to the cascade are found at the NE. These include the initial actor phospholipase C delta; (PLC- $\delta$ ), present in the INM [54], and which cleaves phosphatidyl-inositol-4,5-bisphosphate (PIP<sub>2</sub>) into DAG and inositol-1,4,5-triphosphate (IP<sub>3</sub>). IP<sub>3</sub> is the key unlocking IP<sub>3</sub>-receptors (IP<sub>3</sub>R), also found at the INM [55], which subsequently permits calcium flow from the IMS into the nucleoplasm [56]. Release from other type of receptors, namely ryanodine receptors, has also been reported from the NE [57–60]. It is important to specify that, although one could think that, calcium being an ion, it is sufficiently small to freely access the nucleus via the nuclear pores, this is rarely observed, and studies have drawn the clear notion that the permeability of nuclear pores to calcium is regulated [61,62], with nuclear calcium eventually being very slowly back to the cytoplasm (discussed in Ref. [63]). The direct release of calcium from the NE into the nucleoplasm has been documented in multiple studies [64–66]. Importantly, this controls key cellular functions such as progression through early prophase, thus proliferation [67], establishment of specific transcriptional



**Fig. 4. Lipid-dependent physico-chemical properties of membranes. (A) Membrane curvature.** Based on the relative size between a « head » and a « tail », all membrane lipids can be divided into three groups (at the top, from left to right): lipids promoting zero membrane curvature (i.e. « cylindrical »), like phosphatidylserine and phosphatidylcholine; supporting positive curvature (i.e. « inverted conical »), such as phosphatidylinositol; and negative curvature (i.e. « conical »), for example, phosphatidylethanolamine, diacylglycerol, and sterol. The prevalence of one of these lipid groups changes membrane shape, thereby dictating membrane curvature. Specifically, lipids with an « inverted conical » shape, featuring a « head » larger than the « tail », induce positive membrane curvature (top, left), whereas « conical » lipids, that are characterized by a smaller « head », conversely promote negative membrane curvature (top, right). At the sites of Nuclear Pore Complexes (NPC) insertion, which are defined as the fusion sites of Outer and Inner Nuclear Membranes (ONM and INM, respectively), the establishment of membrane curvature could be supported by combining different lengths of lipid acyl chains (bottom). In this case, the extremely high curvature is stabilized by the prevalence of very long-chain lipids in the outer membrane leaflet (occupying the major part of hydrophobic space), and the enrichment of short-chain lipids in the inner leaflet. (B) **Membrane lipid packing state.** In contrast to the homeostatic packing state (top), a packing defect of a biological membrane (bottom) is marked by an elevated presence of curvature-triggering lipid species (see Fig. 4A) and hydrocarbon chain unsaturations (see Fig. 2C). These factors disrupt the geometric arrangement of membrane lipids, as highlighted by dark gray arrows. (C) **Membrane charge.** In the context of biological membranes, bivalent cations such as Mg<sup>2+</sup> (gray circle) bind to the negatively charged functional groups (white circles) of zwitterionic lipid species (bottom). This results in a positive total charge of lipid « heads », allowing

signatures [68], and pathological cues such as hypertrophy in cardiomyocytes [69]. Also, nuclear calcium activates calcineurin, which in turn dephosphorylates topoisomerase II beta (TopoIIβ). TopoIIβ cuts (and later seals) the two strands of DNA to release topological constraints at different chromosomal scales. On a local scale, calcium-elicited TopoIIβ cuts target selected promoters of the Early Response Genes, a small set of genes whose products orchestrate a vast network in response to neural activity. This ultimately mediates lasting changes in neuronal morphology and synapse organization [70]. On a genome-wide scale, TopoIIβ warrants chromosome territories establishment and prevents excessive chromosome entanglement during interphase [71,72]. Thus, NE-commanded calcium release into the nucleus shapes nuclear processes including cell cycle progression, transcriptional and epigenetic landscapes, and even DNA integrity.

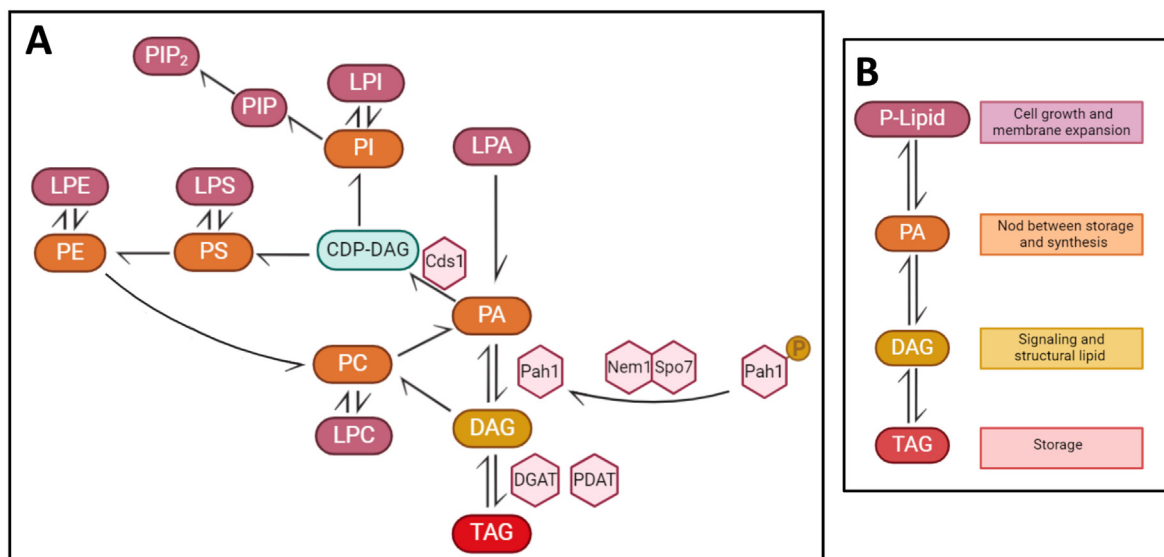
### 2.3.3. NE as a site for lipid synthesis

The ER is the most active organelle in lipid synthesis, modification, and sorting (discussed in Ref. [73]). As a specialized ER subdomain, the NE is likely to be active in lipid synthesis, some of which have been evoked above when describing specific NE lipid compositions. In this section, we present an overview of NE lipid-synthesizing activities that may sustain lipid production that feeds other subcellular compartments.

**2.3.3.1. Phosphatidic acid metabolism.** The discovery of the NE as a metabolically active region hosting phospholipid-synthesizing enzymes starts with the yeast protein Pah1, a central enzyme in glycerophospholipid metabolism, evolutionary conserved from fungi to metazoans. Pah1 is the member of the protein family of Mg<sup>2+</sup>-dependent Phosphatidic Acid Phosphatases (PAP1) including lipin (*H. sapiens*), lipin-1 (*Caenorhabditis elegans*), and Ned1 (*Schizosaccharomyces pombe*). In response to elevated Phosphatidic Acid (PA) levels, Pah1 is dephosphorylated (activated) by the NE-resident Nem1-Spo 7 phosphatase complex (CNEP-1/NEPR-1 in *C. elegans*), thus dephosphorylating PA to produce diacylglycerol (DAG), the precursor of triacylglycerol (TAG), the FA storage currency [74] (Fig. 5). Phenotypically, absence of Pah1, which phenocopies absence of Nem1 and Spo7, is characterized by severe metabolic changes in yeast: increased levels of PA, PI, PE, and ergosterol esters, decreased levels of DAG, TAG, and PC [75]. This metabolic status, reflecting the activation of glycerophospholipid synthesis over TAG production, is accompanied by the presence of irregular nuclei with an expanded NE [74]. This abnormal expansion is documented in multiple organisms [76–80]. Glycerophospholipid synthesis from PA goes well beyond the NE and the ER, as PA is imported into the mitochondria to feed the synthesis of the mitochondria-specific glycerophospholipid cardiolipin [81,82]. Reciprocally, genetic inhibition of PA channelling into glycerophospholipid synthesis by deficient Cds1 orients lipid metabolism toward energy storage and, in this case, the accumulation of PA and downstream TAG leads to the emergence of lipid droplets (LD, the TAG storage organelle) both from the ONM towards the cytoplasm (cytoplasmic lipid droplets, cLD), and from the INM towards the nucleoplasm (nuclear lipid droplets, nLD) [46] (Fig. 5). In U2OS human cells, a dysregulated accumulation of PA at the INM results in enhanced Lipin-1b isoform tethering to the NE and nLD formation [83]. Overall, the NE-localized PAP1 enzymes orchestrate the metabolism of PA at this location, catalyzing *de novo* TAG synthesis and simultaneously providing the negative regulatory control for

them to subsequently interact with the negatively charged phosphate backbone (blue circles) of DNA molecules (top), thus establishing electrostatic interactions between lipids and DNA.





**Fig. 5. Glycerolipid pathway in *S. cerevisiae*.** (A) Pathway illustrating the connections linking phosphatidic acid (PA) and its conversion into glycerophospholipids or its channelling towards storage. Lipids are indicated in rounded boxes, while particularly relevant enzymes are shown as hexagonal ones. For nomenclature, see the text. (B) Functional categories attributed to the mentioned lipids depending on which aspect of lipid metabolism they play on.

glycerophospholipid synthesis.

**2.3.3.2. Phosphatidylcholine metabolism.** In yeast and mammalian cells, the glycerophospholipid-synthesizing pathways share numerous evolutionally conserved features, especially the Kennedy pathway-mediated generation of PC, one of the major glycerophospholipid species in these organisms. The rate-limiting step of PC biosynthesis is catalyzed by CTP:phosphocholine cytidyltransferase (CCT). The sole CCT enzyme encoded in yeast, Pct1, as well as its mammalian orthologue, CCT $\alpha$  (encoded by *PCYT1A*), both act as sensors detecting low PC levels at the NE (discussed above) and mediate subsequent PC replenishment [49]. In actively growing yeast cultures, GFP-tagged Pct1 displays a predominant INM localization, while it is released into the nucleoplasm either when cells reach stationary (non-proliferating) phase, or upon artificial increase of PC levels following supplementation with choline [49]. In a subset of human cell lines (U2OS, HeLa and Caco-2), the translocation of the nucleoplasm-resident CCT $\alpha$  to the INM is detected in response to overloading cells with the unsaturated FA oleic acid [84], likely because this increases the unsaturation of INM glycerophospholipid tails, thus of packing defects (Fig. 4B), attracting CCT $\alpha$  AH for binding. Also, free OA as well as its channelling towards DAG formation elicit CCT $\alpha$  activation [85]. Lastly, in a very recent study, the presence of the anionic glycerophospholipid PS was reported to spark the association of CCT $\alpha$  with the inner nuclear membrane, thus PC synthesis [86]. The channelling of the generated CCT products to other cellular destinations can rely on the fact that its product, CDP-Choline, is soluble, thus can reach other membranes within the cell by diffusion. Additionally, the localization of CCT $\alpha$  at the INM (at least, in mouse embryo-derived NIH/3T3 fibroblasts, Chinese Hamster Ovary (CHO) cells, and in hepatocytes-derived human cells lines) can prime the growth of intra-nuclear glycerophospholipid-containing structures such as the nucleoplasmic reticulum type I [87,88], and the generation of nLD [83,85,89,90], discussed in detail in section 4.

**2.3.3.3. Triacylglycerol metabolism.** The critical steps of *de novo* TAG synthesis can be achieved either by the enzymatic activity of diacylglycerol-*O*-acyltransferases (DGAT 1/2 families), which use

fatty acyl-CoA to convert DAG to TAG; or by phospholipid-diacylglycerol acyltransferases (PDAT), which use one acyl group from a glycerophospholipid as a substrate to generate DAG-derived TAG, thus yielding a lysoglycerophospholipid (Fig. 5A). Hence, the previously described Pah1/Lipin activity mediating DAG production at the NE is not sufficient to consider nuclear membranes as active sites for TAG anabolism. In this sense, DGAT activities are mostly reported to occur throughout the ER, and indeed TAG-containing LD are born from this location. In the same line, it was established that, in rich medium-growing yeast cells, Lro1 (belongs to PDAT family) also localizes all over the ER. However, once cells reach the stationary (non-proliferating) phase, Lro1 localizes to a given subdomain of the INM, where it actively creates TAG at the expense of the INM phospholipid acyl chains [47]. This implies a smart coupling between the need of creating, in this nutrient-poor context, TAG reservoirs, at the same time than restricting membrane proliferation. This is reminiscent to, but different from, the role played by lipin at the DAG/PA crossroad (section 2.3.3.1). PDATs with a topology similar to that of Lro1 are present in fungi, green algae, plants, the flagellates Euglenozoa and the fungal-like Oomycetes [47,91,92]. However, the green algal *Chlamydomonas reinhardtii* PDAT and the *Arabidopsis thaliana* PDAT1 were proposed to use chloroplast's instead of NE's membrane glycerophospholipids to synthesize TAG [91,93], suggesting that different cell types may use different acyl chain sources during stress. Importantly, the NE contribution to TAG synthesis is not exclusive to these organisms. In this sense, in U2OS and hepatocytes-derived human cell lines, a subset of TAG metabolism-related enzymes, in particular DGAT1/DGAT2, GPAT3/GPAT4, AGPAT2 and ACSL3, has been detected at the nucleoplasmic reticulum membranes and nLD surface [89,94].

**2.3.3.4. Sterol metabolism.** Even if the sterol-synthesizing pathways are diversified depending on the phylogenetic group, thereby leading to the production of different sterol species [95], the enrichment at the NE of related enzymes has been revealed as an evolutionarily conserved process [96]. Firstly, there are the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductases, which catalyze the rate-limiting step for mevalonate production

(the first and indispensable sterol precursor). Interestingly, at increased expression levels, Hmg1 (in *S. cerevisiae*) and HMGRs (in *H. sapiens*) induce NE deformation, observed in the form of stacked pairs of membranes localizing around the nucleus and assigned as nuclear-associated 'karmellae' [97,98]. The molecular mechanism underlying this overproliferation remains non-understood. In line, NE-derived membrane stacks invading the nucleoplasm upon overexpression of a nucleolar protein were decorated with HMGR [99], indicative of the NE localization of this protein. In *S. cerevisiae*, Hmg1 and Hmg2 nucleate as a platform in the junction established between the nucleus and the lysosome-like yeast organelle, the vacuole. There, the congregation of these enzymes boosts sterol production [100], from where sterol species may travel through ER membranes and continue their processing down the pathway. Well-known transporters dispatch them to other cellular membranes, like the Golgi, the endosomes or the plasma membrane [101]. Moreover, a direct on-site exchange may occur to replenish the vacuole sterol contents, as the transporter protein Osh1 resides at this contact site between the NE and the vacuole [102]. Secondly, the human sterol isomerase EBP, which converts  $\Delta 8$ -sterols to their corresponding  $\Delta 7$ -isomers, was detected by confocal and electron microscopy at the NE in human monocytic leukemia-derived THP-1 cell line [103]. Also, fluorescently tagged constructs of DHCR7 and DHCR24 (7- and 24-dehydrocholesterol reductases, respectively), which catalyze the two last steps of the cholesterol biosynthetic pathway, were noted at the NE in cell culture and in transgenic mouse models [104]. An important mention goes for the protein Lamin B Receptor (LBR). The C-terminal domain of LBR possesses C14-sterol reductase activity [105] and is broadly distributed among eukaryotes, and presumably present in the ER of the last eukaryotic common ancestor [22]. However, its N-terminal TUDOR domain, which binds lamin B and the heterochromatin in the nucleoplasmic space [106,107], is restricted to Metazoa [22]. LBR's sterol biosynthetic capacity at the NE has been elegantly demonstrated [108], and its deficiency has been linked to Greenberg skeletal dysplasia or Pelger-Huët anomaly [109]. Recent biochemical studies in HeLa cells revealed that proper INM localization of LBR depends on its physical interaction with another INM transmembrane protein, TMEM147 [110]. siRNA-mediated TMEM147 silencing globally decreases cholesterol esters (CE) levels, induces coordinated transcriptional downregulation of LBR and, intriguingly, of the aforementioned DHCR7. Overall, these new experimental data position TMEM147 as a novel regulator of sterol homeostasis at the NE [110].

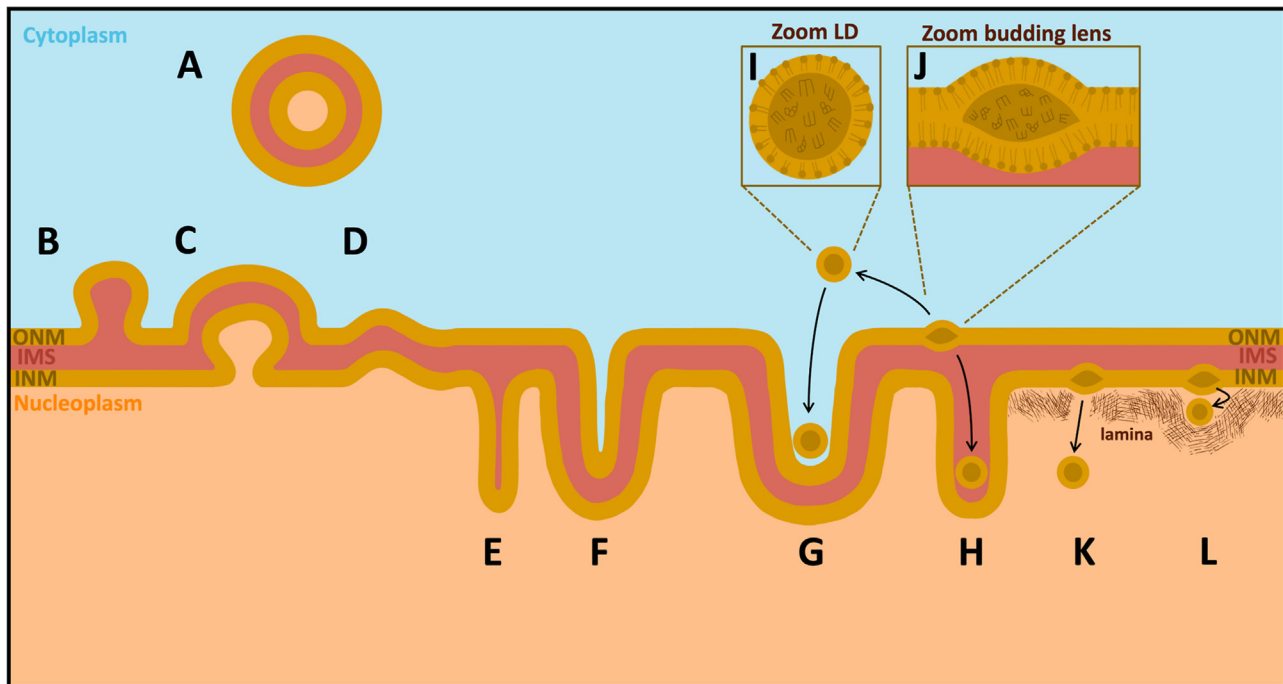
### 2.3.4. The NE as a site for chromatin anchoring and genome stability

Other than acting as gates during nucleo-cytoplasmic transport, NPC have been recurrently implicated in genome stability maintenance processes. The initial observation that mutations in several nucleoporins render cells sensitive to different DNA-damaging agents and entail DNA damage accumulation [111] pointed at the possibility that the pore participates in the response to genome alterations. In line with this, difficult-to-repair DNA double strand breaks (DSB) were seen to relocate to nuclear pores in *S. cerevisiae*, a vision that was enlarged with the observation that critically short telomeres also migrate to, and experienced recombination at, this location [112–117]. The evidence increased with the characterization of replication intermediates under difficulty also locating at this site, such as triplet-nucleotide repeats [118], ribosomal DNA [119] and artificially inserted replication barriers in *S. pombe* [120]. The migration was also observed for other structures that put at risk genome homeostasis, such as DNA opening by the hybridization of a complementary RNA (known as R-loops) [121], and the need for dis-anchoring actively transcribed genes from the pore to concert

this process with their simultaneous replication [122]. On a functional note, this localization assists telomere lengthening via recombination [123], resumption of replication when forks suffer from a block [120,124], promotion of the repair of breaks either via Non-Homologous End Joining (NHEJ) [111,125] or using the sister chromatid [126,127], alleviation of the R-loop load [128] or repair of one-ended DSB by break-induced replication [129]. The outcome of these events can be either beneficial or deleterious for the cell, depending on the cell cycle timeframe when the relocation happens and, likely, the cell type [121,122,128]. The regulation of these choices greatly relies on post-translational modifications of DNA repair-involved factors or the nucleoporins themselves, including phosphorylation, ubiquitination or sumoylation [122,130–132]. It is important to note that most of this knowledge stems from studies in yeast, and that the picture is not always conserved in metazoans, or is less explored, yielding important conceptual gaps or even contradictory descriptions. For example, in cultured human cells, defects in correct cleavage of pre-mRNA molecules were seen to trigger the relocalization of the expressing *loci* to the nuclear pores, which led to downstream defects in replication, and these defects were rescued if relocalization to the pore was actively prevented [133], suggesting a detrimental influence of the pore. The nuclear pore also stands in metazoans as an important hub for organizing chromatin and transcriptional profiles. Several nucleoporins have been reported to induce chromatin decondensation [134] and interact with super-enhancers, *cis*-regulatory elements that control gene activity despite being located away from the gene promoter [135,136].

Beyond the nuclear pores, it is intuitive that the remaining majority of the NE, which is in constant contact with chromatin and anchors it, has a word to say in genome organization and homeostasis. In basal conditions, telomeres, for example, are constitutively localized to the NE in *S. cerevisiae* [137,138], and this association is, despite a myriad of differences, a leitmotiv in eukaryotic species ranging from yeast and flies, to plants and also mammals, and has implications for the preservation of genome architecture (recently reviewed in Ref. [139]). Also, the nucleolus, where ribosomal DNA (rDNA) is concentrated, is tightly tethered to the NE in *S. cerevisiae*, and perturbations in this anchoring has dramatic consequences in rDNA homeostasis (recently reviewed in Ref. [44]). Multiple basal features of genome behaviour are likely maintained thanks to the association with the NE. A beautiful example concerns the inactive X chromosome of mammalian females, known as the Barr body, which clusters against the NE in almost 80 % of interphase cells [140,141]. This association with the nuclear periphery is generally understood to confer a gene-silencing environment, purportedly due to the association with the nuclear lamina [142–144], in contact to which the chromatin displays heterochromatic features [145–147]. Beyond the lamina, the proteins of the LINC complex have been implicated in genome homeostasis. SUN1 interacts with the telomere-protective complex shelterin [148], and this connection exerts an inhibitory activity on unscheduled telomere recombination [149]. Failure to warrant SUN1 interaction with the shelterin complex initiates a damage cascade that culminates with the phosphorylation of lamin A, its removal (erosion), and an aberrant clustering of telomeres [150]. This process may end up in NE rupture and micronuclei formation (Fig. 6A) [151].

The INM (other than the pores) has also been implicated in warranting genome homeostasis. Persistent DSB appear to target the nuclear periphery in a process requiring the canonical DNA break repair protein Rad51, a specific histone variant, H2A.Z, and SUMOylation. This work raised the consideration that persistent DNA break tethering may not take place exclusively at nuclear pores, as described above, but also involve the INM in a broader



**Fig. 6. Remodelling events at the nuclear envelope.** (A) Micronucleus, nucleus-derived body defined by the same two concentric membranes that form the nuclear envelope (NE), and enclosing part of the genome (not depicted). (B) ONM swelling towards the cytoplasm. (C) Bleb of the NE, characterized by both nuclear membranes protruding towards the cytoplasm, thus leading to a herniation of the nucleoplasm. (D) Fold, whose morphology is reminiscent of a wave. (E) Nucleoplasmic reticulum-type I, implies the invagination within the nucleoplasm of the INM only. (F) Nucleoplasmic reticulum-type II, the two nuclear membranes invaginate, which preserves the inter-membrane space (IMS) and the nuclear pores (not depicted). (G) A cytoplasmic lipid droplet (cLD), formed upon budding towards the cytoplasm of lipids concentrated between the two leaflets of the ONM. Subsequently, this cLD can slide within a nucleoplasmic reticulum-type II. In given sections of a nucleus, this structure may be inadvertently classified as being nuclear. (H) A LD nucleated within any of the nuclear membranes' leaflets buds into inter-membrane space and can be trapped into a nucleoplasmic reticulum-type I. Eventual breaching of the nucleoplasmic reticulum-type I tip releases this luminal LD into the nucleoplasm (not depicted). (I) Zoom into the LD, delimited by a single layer of phospholipids and filled with neutral lipids, such as triacylglycerols and/or esterified sterols. (J) Zoom into the neutral lipid lens as it nucleates within the membrane, prior to budding and LD birth. This process can take place at the endoplasmic reticulum membrane, at the ONM and at the INM. (K) If nucleation of lipids occurs within the two leaflets of the INM then the LD buds towards the nucleoplasm, this gives rise to a nuclear lipid droplet (nLD). Its penetration in the nucleus distorts or even may require the disassembly of the lamina. (L) The intrusion of the nLD from the INM may push the lamina without disrupting it. In this case, the lamina coats the nLD as it intrudes deeper in the nucleus.

manner [152]. In this work, Mps3, the *S. cerevisiae* homolog of SUN proteins, had a predominant role in break localization to the INM. This was later refined as a strategy preponderant during the S and G<sub>2</sub> phases of the cell cycle [126], likely with the participation of the telomerase complex [153]. Beyond studies in budding yeast, the same phenomenon was described for breaks in the heterochromatin in association with INM proteins in *Drosophila* [154], heterochromatic DNA DSB and uncapped telomeres in human cells [155], and for difficult-to-repair DSB in *S. pombe* [156] and in *C. elegans* [157]. Thus, DNA ends displaying ssDNA at the tips, eroded telomeres (i.e., which have lost repeats below a critical threshold) or breaks embedded within heterochromatin, show a preference for, and likely benefit from, targeting the INM.

Other than the basal localization, or the active relocation, of genes and chromosome territories to the NE in view of their homeostasis, little is known about the active presence of DNA repair factors that constitutively reside at the NE. Only very recently was the first NET nuclease NUMEN reported to assist DNA repair. NUMEN possesses both endonuclease and 3' → 5' exonuclease activities. It promotes the repair of heterochromatic DNA DSB by NHEJ and can process uncapped telomeres [155]. Importantly for this review, proximity-ligation experiments aiming at identifying the interactors of NUMEN revealed, other than the NHEJ machinery, the LINC complex, the lamin-associated factors of the INM transmembrane proteome and SMPD4, a sphingomyelinase [155]. The only additional protein reported to date to be a *bona-fide* NE-resident protein is Msc1, described as important to repair DNA

DSB in *S. cerevisiae* [158]. It is therefore very likely that a dedicated DNA repair machinery is to be discovered at the INM in the coming years. This likelihood is reinforced when inspired by previous studies with prokaryotic organisms. In them, where no NE exists, chromosomal DNA has been recurrently reported to exploit the plasma membrane to coordinate DNA transactions. Such is the case of DnaA, the protein initiating chromosomal replication in *Escherichia coli*, which can be purified as a soluble protein and can efficiently initiate replication of mini-chromosomes in membrane-free DNA synthesis reactions. However, its conversion from a replicatively inactive into an active form necessitates its association with acidic glycerophospholipids in a lipid bilayer [159], yielding the notion that the initiation of chromosomal DNA replication in this bacterium is a membrane-associated event. Acidic glycerophospholipids of the membrane have been since implicated in finetuning the important control of replication happening once, and only once, per cell cycle, and how, *in vivo*, DnaA forms helical structures along the longitudinal axis of the cell (reviewed in Ref. [160]). Anionic glycerophospholipids also dictate the function of the DNA recombination protein RecA in *E. coli* and *Bacillus subtilis*, where it forms an array of parallel, long bundles under the membrane before and after DNA damage induction [161–165]. In this sense, *E. coli* RecA binds specifically to the anionic glycerophospholipids cardiolipin and phosphatidylglycerol (PG), leading to the inhibition of its binding to single-stranded DNA [166]. Also, in *Mycobacteria*, two pools of RecA have been described, one binding the membrane, the other in a soluble form. Membrane-bound RecA

interacts with the negatively charged glycerophospholipids cardiolipin, PI, PA, and this impedes its DNA-dependent ATPase activity without affecting its ability to bind ATP [167]. These examples illustrate the notion, likely more developed from studies using prokaryotic systems, that proteins implicated in DNA metabolism can be, if not inserted in membranes, at least functionally interacting with them.

Additionally, the association of chromatin with the NE could be also envisioned through the DNA molecule itself, in a direct manner. As a negatively charged polyelectrolyte, DNA can strongly interact with cationic lipids, forming spherical lipoplexes [168], although this association is unlikely to exist *in vivo* given the profile of biologically relevant lipids. Thus, *in vivo*, only zwitterionic and anionic lipids may be worth considering. In the presence of divalent cations such as  $Mg^{2+}$  or  $Ca^{2+}$ , DNA can indeed strongly bind to zwitterionic lipids (Fig. 4C), which elicits DNA condensation [169]. Divalent cations also work as linkers between the negative charge of DNA and the head of anionic glycerophospholipids.

A constant between the NE and genome stability maintenance pathways is the ignorance on which mechanism, or feature, specifies the association of distinct DNA intermediates with particular NPC or NE segments, or of the different molecular outcomes (evoked in Ref. [170]). We propose that these are dictated by the lipid composition of the NE. Indeed, alterations in NE homeostasis are noted to impact genome homeostasis. For example, the use of the non-metabolizable lysoglycerophosphatidylcholine analogue edelfosine, known to accumulate at the NE, induces severe NE deformation [171]. As will be discussed (see section 3.3), the accumulation of such analogue may frustrate cellular attempts for reacylating glycerophospholipids, creating an imbalance that translates in membrane stress (defined as a non-homeostatic composition that perturbs the membrane's behaviour). Consequently, it was reported that edelfosine disrupts telomere clustering but not anchoring and, with it, the association of the sirtuin Sir4 to telomeres decreases. This drives the altered expression of Sir-dependent genes, located at sub-telomeric regions and, consistent with the known shuttling of Sir4 between telomeres and the nucleolus, transcriptional changes also in the latter. Also, we already mentioned that the LINC INM protein SUN2 presents an AH whose intercalation within the membrane packing defects, created by DAG molecules, stabilized it [48]. Importantly, it was found that SUN2 degradation, whenever required, is also fundamental, as preventing it led to an aberrant nuclear architecture, vulnerability to DNA damage and increased mitotic lagging chromosomes [172]. The authors discussed that the local downregulation of LINC complexes by focal SUN2 degradation may be part of the DNA repair process or, alternatively, prevent mechanical-induced damage. We add the consideration that local DAG levels may be critical to channel DNA damage to different pathways. These possibilities require future testing.

Using proteomics to identify novel NET proteins from cultured mesenchymal stem cells, and from correspondingly differentiated adipocytes and myocytes, authors identified the protein sphingomyelinase, SMPD4 [173], already mentioned above as a proximity partner for a NET DNA nuclease [155]. They validated SMPD4 presence at the NE by immunofluorescence, including in a fourth cell type, U2OS cells. SMPD4 was concentrated in the vicinity of NPC. Sphingomyelinase hydrolyzes sphingomyelin and releases ceramide. Biallelic loss-of-function variants in *SMPD4* cause a rare and severe neurodevelopmental disorder with progressive congenital microcephaly and early death. In the rare cases where prolonged survival is documented, all individuals surviving beyond infancy develop insulin-dependent diabetes (besides severe neurodevelopmental disorder and microcephaly), making diabetes one

of the most frequent age-dependent non-cerebral abnormalities [174]. At the cellular level, using human neural stem cells, mitosis entry was found delayed due to difficulties in NE breakdown, as well as there were problems during reassembly, in particular associated to decreased post-mitotic NPC insertion. At the organ level, in embryonic mouse brain, knockdown of *SMPD4* impaired cortical progenitor proliferation and provoked premature differentiation by altering the equilibrium between neurogenic and proliferative progenitor cell divisions [174]. It was proposed that *SMPD4* activity provides sphingomyelin derivatives assisting NE bending and subsequent NPC insertion. However, beyond a sole contribution to NE structuration, the downstream products of *SMPD4* are described to impact genome homeostasis. As such, ceramide can be hydrolyzed to give rise to sphingosine, whose phosphorylation by sphingosine kinase 2 (ShpK2) creates Sphingosine-1-Phosphate (S1P). ShpK2 is present in the nucleus in a complex with the histone deacetylases HDAC1 and HDAC2. This way, generation of S1P by ShpK2 allows its binding to HDACs to inhibit deacetylation of histones, implementing an epigenetic signalling mechanism modifying gene expression [175]. Also, S1P binds and stabilizes telomerase at the nuclear periphery. Telomerase is the enzyme responsible for the maintenance of a normal telomere length (the end of chromosomes otherwise shrinks at the end of each replication cycle, and a critically low telomere length forces cells entry into senescence). Telomerase activity is also key in cancer cells, where it is often increased, to sustain their ability to proliferate indefinitely. The telomerase catalytic subunit, telomerase reverse transcriptase (TERT), is stabilized by phosphorylation. S1P was found to bind TERT residue 684 and mimic its phosphorylation, an event known to stabilize the enzyme. Disrupting the interaction between S1P and TERT either by S1P depletion or mutation of the TERT-interacting site impaired telomere maintenance, promoted senescence in cultured cells and decreased the growth of lung cancer cell xenografts in mice [176]. Likely related to these impacts on the genome by SP1, *Mfsd2b* absence was identified as a top hit in a very recent work aiming at identifying genetic determinants for micronuclei formation (Fig. 6A) in mouse erythrocytes using blood from mutant mice [177]. Indeed, *Mfsd2b* is essential to export S1P out of the cells and, in its absence, intracellular SP1 accumulates [178]. One can envision that excess S1P may lead to dramatic histone deacetylation profiles and/or aberrant TERT stabilization that elicits micronuclei formation pathways.

A last potential (and intriguing) impact of membranes on dictating genome fate is illustrated by a recent work in which authors had the motivation to understand how increased cell size influences cell cycle progression. By artificially holding cells synchronized in the  $G_1$  phase of the cell cycle, they monitored a fraction of cells unable to resume cycling upon release. These cells recurrently were enlarged, displaying increased cellular and nuclear volumes. Whenever some of these enlarged cells managed to resume cycling, they would invariably acquire genome lesions by doing so and would definitely exit the cell cycle when reaching  $G_1$  again [179]. While the authors did not resolve the cause, they reported that these cells were prone to DNA damage, at least in part due to defects in DNA damage signalling and repair. We hypothesize that more cellular volume implying, by definition, more membranes, an increase in the NE surface (for a similar DNA quantity) may interfere with chromosome territories establishment and organization of DNA repair at the nuclear periphery. Supportive of this theory, when we explored the proteomic data the authors provide from these cells, and while they concentrated their attention on DNA repair-related hits, we note the upregulation, among their major hits, of many actors of the cholesterol pathway.



### 3. NE homeostasis

Excluding the massive reorganization (and, in some cases, also dissolution) suffered by the NE during mitosis [180], the nucleus in interphase, under basal conditions, is not particularly prone to global deformation, except in special cases such as in migratory cells, whose extravasation through the endothelium exposes them to high pressures. The strong compression of chromatin in this set-up has been reported to impose a difficulty to ongoing replication forks during DNA synthesis, a stress that can degenerate into DNA damage [181]. Stronger pressure on the nucleus can culminate in NE rupture, meaning the tearing of the two nuclear membranes.

Another important contributor to NE remodelling is its physical attachment/detachment from other organelles. This process underscores the intricate interplay between the nucleus and the rest of the cell and raises the potential impact of such organelles on genome stability. For example, in budding yeast, the nucleus is anchored to the vacuole (the lysosome-like organelle), and this association is dynamic and responsive to external cues [182]. Using the vacuole as a template, the nucleus massively deforms and wraps around it under certain conditions halting cell cycle progression in G<sub>2</sub> phase [183–185]. Reciprocally, detachment from the vacuole allowed it to experience drastic invaginations ([186]; reviewed in Ref. [44]). On another note, overexpression of the cortical ER-resident protein Ist2 created an artificial Velcro-like interaction between the nucleus and the plasma membrane that led to a remarkable nuclear compression [187]. Beyond these “macroscopic”, full NE transitions, we will focus, in the next sections, on “microscopic” (individual proteins, lipids, genome transactions) events that affect NE homeostasis.

#### 3.1. Proteins in NE homeostasis

The number of proteins involved in lipid metabolism present in the nucleus [96] makes of them the first category of nuclear proteins to be conserved during evolution [11]. Defects in the LINC complex, which tethers both NE membranes, or in the NPC both weaken the NE and lead to breakage [188,189]. However, the most severe loss of homeostasis elicited at the NE stems from the absence of NETs [190], involved in multiple processes such as chromatin integrity, lipid processing, signalling, differentiation and structure maintenance [191].

Among key NETs, a study searching novel NET proteins from cultured mesenchymal stem cells, adipocytes and myocytes highlighted Tmx4, a thioredoxin-related protein [173], for which NE localization was validated by microscopy methods. At that time, back in 2019, the authors suspected Tmx4 to exert a potential role in regulating the luminal aspects of NE specific structures, such as the LINC complex and associated torsin A, both of which may be regulated by disulfide oxidation/reduction, based on previous works [192,193]. Since then, human TMX4 was shown last year to indeed exert its activity onto the LINC complex. In response to cues eliciting ER stress, much is known about the pathways engaged throughout the cytoplasmic domains of this organelle. However, the impact on the NE (the perinuclear ER) is poorly understood, with only some studies in yeast exploring the morphological impact of oxidative and genotoxic stresses [194,195]. In the TMX4 study, authors used cyclopiazonic acid, a reversible inhibitor of the sarco/ER calcium pump, which simultaneously mimics ER stresses triggered by perturbation of glycosylation and calcium homeostasis. They observed a selective swelling exclusively of the ONM towards the cytoplasm (Fig. 6B). The ONM excess was removed by the autophagy machinery to restore a normal NE morphology once the stress was over. Importantly, to implement such asymmetric swelling, the LINC complex needed to be disassembled. TMX4 was

found to dismantle the intermolecular disulfide bond that connects the two LINC members, SUN and KASH, by attacking (i.e., forming mixed disulfides bonds with) KASH. TMX4 absence prevented NE swelling in response to ER stress. While this may seem without consequence, TMX4 removal was very badly tolerated by the cells [196]. This is likely because not all nucleo-cytoplasmic transport occurs through the nuclear pores, but specific substrates do so *via* NE blebbing (Fig. 6C), such as the transport of RNA granules, aggregated proteins, inner nuclear membrane proteins, and mis-assembled NPC (already mentioned in section 2.3.1, reviewed in Ref. [51]). Moreover, NE blebbing is a recurrent response to multiple stresses, likely of an alleviating nature [197]. In all these processes, TMX4-mediated LINC complex disengagement may become essential.

The absence of specific NETs that tether chromatin at the INM can lead, first, to altered contacts between the NE and the heterochromatin, which generates chromatin disorganization and damage [198,199]; second, to dramatic NE rupture [35,200]. Upon NE rupture, 1) the DNA becomes exposed to the cytoplasm, thus to the unscheduled access by cellular nucleases; 2) the nuclear contents leak into the cytoplasm; 3) this abrogates accurate import/export dynamics; 4) this is an instigator of inflammatory responses [201] and 5) the rupture also frees the contents of the IMS which, being a subdomain of the ER lumen, will release carefully stored calcium [202]. Mild calcium release can contribute to indirectly harm the DNA because it activates topoisomerase II, which induces unwanted DNA breaks [70]. Strong calcium release is a trigger of apoptosis [203]. These NE transitions can act as oncogenesis instigators [201]. Of note, cancer cells defective in DNA break repair were recently reported to instruct, in a programmed manner, NE rupture through active lamin dismantling [204]. This could release elevated intra-nuclear pressure, although its functional implication, if any, was not explored.

The repair of NE ruptures is fulfilled by the same proteins involved in the rebuilding of the NE at mitosis exit. Membrane sealing is performed by the Endosomal Sorting Complexes Required for Transport (ESCRT-III) [205–207], which is transiently recruited by Charged Multivesicular body Protein 7 (CHMP7). CHMP7 is actively excluded from the nucleus by sustained export [208], therefore managing to bind its activator at the INM, Heh1/Lem2 (in *S. cerevisiae/S. pombe*) [205], only in case of NE rupture. This warrants a precise spatio-temporal recruitment of the sealing machinery only in case of membrane damage. Inability to exclude CHMP7 from the nucleus provokes unrestrained presence and activity of ESCRT-III, which translates into dramatic INM distortions, downstream increase in DNA torsional stress, concomitant DNA unwinding exposing single-stranded DNA, thus a susceptibility for accumulating DNA damage [209]. These mechanisms appear to be conserved from different yeasts to humans [210].

#### 3.2. Lipids in NE homeostasis

In comparison with the knowledge regarding the contribution of proteins (or their absence) to NE fragility, rupture and downstream repair, very little is known about the potential involvement of lipids. The best assessed aspect is likely the role of lipids in sealing broken NE membranes, which mostly occurs in open mitosis [33], yet is not extensively studied in the context of NE rupture. PA can accumulate at focal points of the NE to recruit Chm7, the *S. cerevisiae* homolog of the repair activator factor CHMP7 (see above). Although, in this study, Chm7 was implicated in membrane remodelling to allow accurate NPC insertion and not in NE sealing, it is conceivable that PA could play a role to help Chm7/CHMP7 dock at NE rupture sites [211]. On the same line, PA was reported in *S. cerevisiae* as important to promote severe NE

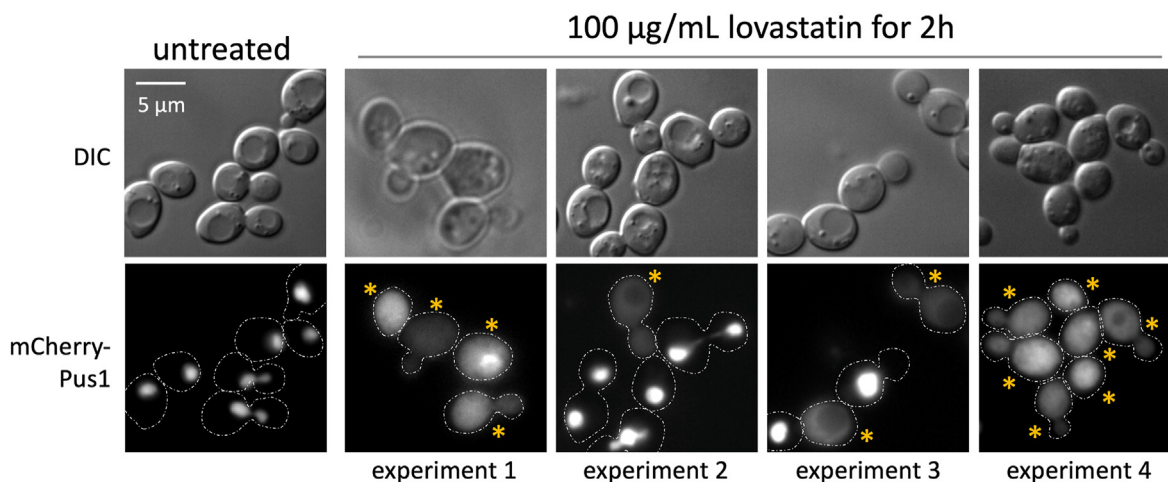
invaginations at a focal point [186], suggesting its concentration imparts negative curvature potential to the NE. Also, the overexpression of the  $\Delta 9$  FA desaturase Ole1 in *Schizosaccharomyces japonicus* rescued defects in NE sealing [212], hinting at the importance of the profile of FA unsaturation in NE integrity. With respect to the intact NE, few studies characterize how lipid disequilibrium affects the NE. NE rupture was described upon lipid-based transfection [213], implying that the interference with a given lipid composition influences the NE stability. In the yeast *S. cerevisiae*, raising the saturation degree of the NE glycerophospholipids led to rigidification, adoption of nuclear cubic shapes and, ultimately, NE rupture [214].

A recurrent line of evidence points at long lipid molecules as key for NE integrity. LCB are present at the NE and are essential to tolerate aneuploidy [32]. Absence of very long chain FA in a hypomorphic mutant of the acetyl-CoA-carboxylase (Acc1) enzyme led to aberrant invaginations of the INM into the perinuclear (IMS) space in *S. cerevisiae* [215]. Reciprocally, *S. pombe* cells suffering from genetically induced NE rupture were alleviated if complemented with overexpression of Elo2, the elongase responsible for generating very long chain FA [35]. While LCB serve to build sphingolipids, and very long chain FA are not sphingolipids, *S. cerevisiae* strains that survive without sphingolipids produce novel  $C_{26}$  FA-containing glycerophospholipids likely as a compensatory mechanism, for they structurally mimic sphingolipids [216]. Thus, long chain lipids are important to preserve NE integrity, purportedly because they support the implementation of high curvature at specific regions, for example where the ONM and the INM fuse to allow NPC insertion (Fig. 4A). Keeping on with the same concept, global lipidome analysis using quantitative mass spectrometry of engineered aneuploid yeast strains, featured by NE deformation, revealed that LCB and their derived product ceramides are elevated compared to euploid controls, and that such elevation happens at the NE. These yeast cells appeared hypersensitive to myriocin, a drug that halts LCB production by specifically inhibiting serine palmitoyl transferase, suggesting that this increase in LCB was important for the NE to accommodate the ploidy alteration. The same increase in LCB and ceramides was found in mouse embryonic fibroblasts trisomic for chromosomes 1, 13, 16, and 19, and in Human Skin Fibroblasts trisomic for chromosomes 21, 18, and 13. Importantly, artificially raising LCB

improves cell fitness and restores NE shape [32,217].

In a recent effort to better understand drivers of NE homeostasis, authors isolated NE lipids from human kidney cells, finding a complex mixture of glycerophospholipids, cholesterol, and an elevated amount of unsaturated FA, the whole featuring a consistent fluidity in comparison to other cellular membranes. This was accompanied by an elasticity two orders of magnitude higher than that of the plasma membrane [5]. The contribution of cholesterol is always complex to decipher, and even counterintuitive, as its effect depends on the surrounding lipid composition of the membrane: it renders saturated membranes more rigid, but unsaturated membranes more flexible, giving rise to a contradictory situation in which the membranes simultaneously become thicker and softer [218]. This may explain the recent pre-print in which the cholesterol-synthesizing activity of the INM LBR led to an accumulation of cholesterol in the NE that was defined as necessary and sufficient for nuclear deformability (*did the NE become softer?*) but also NE rupture (*did it behave as rigid?*) in cells confined *in vitro*. The authors also report that NE rupture associated to an increase in NE cholesterol levels was a feature in invasive cells migrating out of tumour organoids and tumours *in vivo*, thus highlighting an important molecular and cellular feature of metastasis [219]. The increase in sterols facilitating NE deformation may be conserved, as it was also reported in *S. cerevisiae* [186]. However, we note that the opposite, that is, the absence of sterols, may equally impose a challenge to NE homeostasis, as we recurrently observe that 2 h-long treatments with the HMG-CoA-reductase inhibitor lovastatin systematically leads to leakage of nuclear contents into the cytoplasm (Fig. 7), a readout for NE rupture [35].

Last, contribution to NE lipid homeostasis can also stem from other cellular organelles. The Golgi apparatus, through its role in protein and lipid modification and sorting, can also indirectly influence NE homeostasis. Its functional uncoupling from the ER - by disrupting proteins in charge of lipid transport - affects the trafficking of lipids to the nucleus, which are critical for maintaining nuclear structure and integrity. Indeed, when DAG was depleted from the Golgi - to probe if DAG transport (i.e. through vesicular transport) could affect the NE -, it was shown that the rim curvatures of the NE, where the nuclear pore complexes are recruited, manifested a significant loss in membrane curvature [220]. This results in aberrant NE morphology and impaired nuclear function.



**Fig. 7. Lovastatin treatment triggers nuclear contents' leakage in *S. cerevisiae* cells.** *S. cerevisiae* cells expressing mCherry-Pus1 (as a marker of the nucleoplasm) growing exponentially in rich medium were treated (or not) for 2 h with 100  $\mu\text{g}/\text{mL}$  lovastatin and observed using the Differential Interference Contrast (DIC, top row) and fluorescent signals by using the adequate wavelength (mCherry-Pus1, bottom row). Imaging was achieved using a Zeiss Axioimager Z2 microscope and Zeiss software. Cells' contours have been imposed on the bottom row pictures to help identify if the nuclei appear intact, or if leakage of nucleoplasmic contents happens into the cytoplasm (marked by yellow asterisks). Four independent experiments featuring cells exposed to the treatment are shown to account for reproducibility.

### 3.3. The genome in NE homeostasis: insights from “bifunctional” proteins

At the INM there are proteins with LEM domains [221] involved in contacting the chromatin (and the lamina, when applicable), anchoring it to the INM [222,223]. These proteins are involved in the reassembly of the NE after breakdown in metazoans and their absence induces NE invaginations in *C. elegans* [224] as well as in human cells [225]. Overexpression of the chromatin-tethering protein Esc1 induces the overgrowth of the NE in the yeast *S. cerevisiae* [226], so as does the overexpression of the chromatin-binding INM protein LBR in cultured human cells [227]. These examples suggest that the need to attach some genomic regions to the NE, as implied by chromosome territories establishment, conditions the homeostasis of the NE. In this section, we will review the case of some chromatin-contacting INM proteins and explore their impact on NE integrity.

The first example, concerning the SUN domain-containing protein **Mps3** from *S. cerevisiae*, has already been smartly visited for its duality in genome organization and NE homeostasis elsewhere [228]. We will therefore only briefly sketch these features. SUN-domain proteins oligomerize in the form of trimers that traverse the INM [229–231]. In the case of Mps3, N-termini hang in the nucleoplasm contacting and anchoring telomeres, as well as helping concentrate proteins important in their silencing [232]. This Mps3's domain can also tether DSB-containing chromatin to the nuclear periphery, as presented before (see [section 2.3.4](#)). In contrast, Mps3's C-terminal half, hosted in the IMS, harbors the SUN domain, normally involved in contacting a KASH partner inserted within the ONM to establish a LINC complex (or LINC-like, as it is worth noting that the KASH partner in *S. cerevisiae* is either undetermined, or different in different situations [23]). Mutations in conserved residues of its SUN domain, like *mps3-Y502H* and *mps3-F592S*, display synthetic sickness or lethality when combined with the deletion of factors related to lipid metabolism [233]. Failure to degrade Mps3 as mitosis approaches leads to its accumulation in the INM, which entails aberrant nuclear envelope expansion [233]. Mps3 was therefore proposed to play a role in rigidifying the membrane, as its overexpression at cold temperatures was lethal and, reciprocally, multiple *mps3* point mutations showed sensitivity to membrane fluidizing agents [233]. It is therefore evident that Mps3 exerts a dual role in genome organization and in lipid homeostasis, and a full understanding of how it coordinates both activities may be instrumental in learning how the genome and the metabolism of lipids talk to each other. In their analysis, Sosa Ponce et al. [228] propose several exploratory entry points to tackle this issue. Among them, they invoke the physical interaction between the non-catalytic subunit of the telomerase, Est1, and the lysophosphatidic acid-generating enzyme Lro1 (see [section 2.3.3](#)) [234]. As already explained, Lro1 localizes to a segment of the INM specifically when cells stop proliferating, where it consumes membrane glycerophospholipids to create fat reservoirs in the shape of TAG [47]. The concomitant raise in lysophosphatidic acid in the membrane leads to telomere (and telomere silencing) reorganization [171], a reaction during which Mps3 could act as a switch.

Another illustrative example of this duality is the NET **Lamin B Receptor** (LBR), which gathers as multimers in the INM and interacts in the nucleoplasm with the nuclear lamina and the heterochromatin through its N-terminal domain, while its C-terminal domain, located in the perinuclear space, fulfils cholesterol synthesis functions (already introduced in [section 2.3.3](#)). No functional connection between the N- and C-termini of LBR has been described. In fact, mutations abolishing LBR sterol reductase activity do not impact its N-terminal domain [108] and, reciprocally, a

point mutant incapable of contacting chromatin still maintain its sterol-related enzymatic activity [107]. It would be important to assess if a physical coupling exists that would allow the transduction of information from chromatin to cholesterol synthesis and *vice versa*, as already evoked [235].

The complex **Apq12-Brl1-Brr6** resides at the NE and is important for NPC biogenesis [236–240]. Likely due to the role of the nuclear pore in monitoring accurate RNA export from the nucleus, Apq12 is important for mRNA transport [241] and transcribed chromatin docking [236]. Brr6 interacts with chromatin and helps maintain normal chromatin architecture and transcriptional regulation of given *loci* at the NE [242]. Also, *apq12* mutants are delayed in anaphase, re-replicate their DNA and display new budding prior to completion of cytokinesis, suggestive of defects in controlling mitotic progression [243]. Simultaneously, defects in the Apq12-Brl1-Brr6 complex are accompanied by severe loss of NE lipid homeostasis. This way, cells expressing an *apq12-ah* mutant version, in which the protein's amphipathic helix is disrupted, show NE integrity defects, and the overexpression of wild-type Apq12 (but not mutant *apq12-ah*) triggers a striking over-proliferation of the ONM and promotes accumulation of PA at the NE [236,244]. *brr6-1* cells accumulate at, or near the NE, elevated levels of the two main types of neutral lipids, sterol esters and TAG, and sterols tend to accumulate at this location when provided exogenously [238]. Last, mutations affecting Brr6, Apq12, or Brl1 lead to defects in lipid metabolism, increase the sensitivity to lipid synthesis inhibitors, display strong genetic interactions with mutations of this same pathway, and lead to hyper-fluid membranes [245]. In a unifying proposal, a recent work uncovers the human homolog of this complex, CLCC1: in the absence of CLCC1, lipid droplets (LD, organelles important for neutral lipid storage) formed in the IMS fail to fuse with the ONM and cannot be released into the cytoplasm. This fusing ability also applies for fusing the INM and the ONM during nuclear pore complex assembly. Thus, both incomplete nuclear pore insertion and LD trapping contribute to NE deformation and loss of ability to interact with chromatin and warrant NPC-associated functions [246]. Thus, the Apq12-Brl1-Brr6 complex/CLCC1 perfectly illustrates the connection between NE lipid homeostasis and genome integrity.

Last, the **Bqt4-Lem2** tandem ensures correct chromosome docking at the NE and warrants genome integrity in *S. pombe*. Bqt4 is an enigmatic protein that lies in the nucleoplasm and is anchored to the INM *via* its C-terminus [247,248]. Through its N-terminus, Bqt4 interacts, in a competitive manner, with different partners, all of which have roles in docking chromatin to the NE [249]. These include Sad1, Rap1 and Lem2. Since the number of Bqt4 proteins per cell greatly exceeds that of each of its interactors, it is likely that different pools of Bqt4 simultaneously exist in a cell fulfilling different activities [249]. The N-terminal domain of Bqt4 also possesses a DNA-binding ability with no sequence specificity, purported to facilitate random chromosome recruitment to the NE so that, in turn, each relevant sequence becomes stabilized by its appropriate tether. This way, Sad1 (the SUN component of the LINC complex) is important for telomere bouquet formation during meiosis, while Rap1 stabilizes telomeres against the INM in vegetative cells [250]. As such, in the absence of Bqt4, telomeres appear disorganized. On its side, Lem2 displays two transmembrane domains, and both its N- and C-termini fall into the nucleoplasm, where they tether chromatin and assist in its heterochromatinization, respectively [200,251,252]. In its absence, cells display genome instability phenotypes, including loss of pericentromeric heterochromatin and of a model mini-chromosome [35].

Surprisingly, while it is intuitive why Lem2 and Bqt4 are important for genome homeostasis, this did not appear to be their only relevant function, nor even the vital one. The simultaneous



absence of Bqt4 and Lem2 is lethal because the NE suffers dramatic ruptures and nuclear contents leak into the cytoplasm [200]. This prompted the consideration of whether, other than chromosome tethering, Bqt4 and Lem2 have a role in NE structure homeostasis. Lem2 indeed acts as a rheostat ensuring the availability of the precise amount of membrane needed to conform the NE for, in its absence, shortage of membrane in other cellular compartments makes the NE shrink, while excess of available membrane makes it expand [253]. This function may relate to its ability to establish, in concert with the Lunapark homolog Lnp1, the boundaries where the NE stops being so and it transitions into the ER network [254]. Also, Lem2 fulfils a role in nuclear membrane maintenance in concert with the membrane repair system ESCRT-III [205,208,212,255–257].

All these descriptions invoked that Lem2 may display connections with lipid metabolism. A proteomic screen of Lem2 interactors revealed Cho2 (involved in the methylation steps of PC synthesis), Ole1 (the master FA desaturase) and Erg11 (involved in sterol synthesis) [258]. Moreover, Lem2 absence leads to a general decrease in t20:0/24:0 phytoceramide species [35]. With respect to Bqt4, preventing its timely degradation with the use of proteasomal inhibitors revealed severe NE distortions due to uncontrolled membrane proliferation [247], confirming the notion that Bqt4 had an influence on membrane homeostasis. Very recently, an Intrinsically Disordered Region (IDR) spanning amino acids 364–413 of Bqt4 has been identified as responsible for this membrane proliferative ability. This IDR possesses the potential for attracting PA *via* electrostatic interactions [259]. As already explained, PA is both a key substrate for glycerophospholipid synthesis and a powerful membrane modelling brick given its ability to impart negative curvature. Last, Lem2 and Bqt4 were found to ensure the correct distribution of the ceramide metabolism protein Tc14, whose accumulation at the NE renders it fragile [260].

Thus, Lem2 and Bqt4 play a dual role in genome and in lipid homeostasis. The question that remains is whether these two functions crosstalk to each other. This is likely since, first, the NE overproliferation associated to Bqt4 excess causes genome instability [259]. Second, overexpression of Elo2, the elongase responsible for the generation of very long FA, suppresses all the phenotypes associated to Lem2 absence, including loss of pericentromeric heterochromatin and of the model mini-chromosome [35]. Thus, the two functions appear intimately intertwined and changes in one impact the other. The mechanistic understanding of this connection would grant a source of new knowledge.

#### 4. Membrane lipids that invade the nucleoplasm

Other than the NE defining the boundaries of what is the nucleus, the NE membranes and its constituent lipids can gain access to the nuclear interior. Deciding when the NE folding towards the interior “invades the nucleoplasm” is an arbitrary decision. In this review, we will not consider folds, defined as local but relatively consecutive depressions of the NE, as they are likely a normal feature of the nucleus in view of tolerating mechanical pressure (Fig. 6D).

##### 4.1. The nucleoplasmic reticulum (NR)

In diverse organisms, cell types and conditions, the NE emits membrane invaginations that dive deep into the nucleoplasm, referred to as the Nucleoplasmic Reticulum (NR). These invaginations can implicate only the INM, in which case they are termed NR-type I; or both the ONM and the INM, termed NR-type II. While NR-type II ingresses the nucleus by keeping the connection between both membranes, warranted by the LINC complex, and

displays NPC, NR-type I by definition occurs within NPC-devoid segments and forces ONM-INM separation (Fig. 6E and F). An important notion recurrently emerging when describing these structures is their ability to bring the NE close to anywhere within the vastness of the nucleus, which may be relevant given that some nuclear regions lay remotely from the periphery. This is beautifully exemplified by NE-released calcium transients into the nucleoplasm. Since calcium signalling works thanks to a very accurate spatial segregation (reviewed in Ref. [261]), it is difficult to imagine that chromosome regions kept away from the NE could, for example, benefit from calcium-controlled TopoII $\beta$  activation [70]. The physical proximity of a NR invagination to the very vicinity of a specific *locus* could render this possible. As such, NR has been shown to play an active role in intra-nuclear calcium delivery [57,262], (Table 1), in particular in response to synaptic activity [263]. Importantly, these were detected not only in neurons, but also in HeLa cells stimulated with the calcium ionophore ionomycin, which elicited distortions of the NE membrane into the nucleus in the frame of minutes, revealing the NR as a specialized, dynamic nuclear compartment regulated by calcium levels [264].

More generally, the potential reasons for NR formation, and the mechanisms behind these membrane invaginations are subjected to lack of consensus, likely because they cannot be simplified to one or two. Hypotheses are multiple: is the driver the cytoskeleton, the nucleoskeleton, the nuclear membrane-associated proteins, the lipids themselves, the lipid enzymes, the chromatin? Could the NR be created by the detachment of the nucleus from other organelles, or maybe stem from the extracellular matrix? Is it elicited in physiological contexts, or mostly relates to pathology? These concepts have in the past been discussed in comprehensive reviews [306–308]. In this section, we have gathered multiple works reporting NR events (though likely not exhaustively). Table 1 has been arbitrarily divided in four sections with the goal of illustrating this diversity, and to allow the reader elaborate his/her own impression. These include interventions that were shown to elicit NR formation, the description of natural features associated to this structure, a compendium of works linking it to calcium signalling and the NR's recurrent association with nucleoli. Complementarily, we discuss below aspects that may help understand the physiological contributions of the NR in the light of new, recent findings.

Although serial-sections electron microscopy first identified NR-type II tubes with type I ones branching as extensions [309], the most frequently reported event is that of NR-type II formation. Cytoskeletal forces could create pressure against the NE that would drive lipid invagination. For instance, actin has already been observed pushing against the NE and even inside the invaginations, as well as intermediate filaments [310,311] and microtubules [310], (Table 1). Yet, these descriptions mostly concerned either breakdown events, or were associated with NE rupture [312].

More recently, NR-type II was observed in response to DSB created *via* different strategies: 1) the topoisomerase-inhibitor etoposide [313,314]; 2) the clastogen zeocin [186]; 3)  $\gamma$ -irradiation [315]; 4) the DNA-damaging bacterial genotoxin Cytolethal Distending Toxins (CDT) from *Haemophilus ducreyi* [316] and from *Helicobacter hepaticus* [317]; 5) artificially expressed DNA restriction enzymes [314,318]. In the latter works, the formation of these NR-type II invaginations required factors implicated in nucleocytoplasmic force transmission, dynamic microtubules and/or kinesin motors [314,318] suggesting that these actors are likely a means to implement the phenomenon, but not the original trigger. Indeed, defects in KASH, the ONM-located segment of the LINC complex, led to mild invaginations of the two nuclear membranes that did not qualify as NR-type II invaginations, but as NE folds (Fig. 6D) [319]. Since KASH absence abolishes the contact of the NE with the cytoskeleton, this observation reinforces the notion that



**Table 1**  
Works reporting on nucleoplasmic reticulum.

Cell type/Tissue	Condition associated to NR presence	Comment	Reference
<b>Interventions that elicit NR formation</b>			
<i>Drosophila</i> salivary glands	<b>expression of condensin II</b> tethering the INM to chromatin		[265]
Chinese Hamster Ovary cells, mouse embryo-derived NIH/3T3 fibroblasts	<b>CCT<math>\alpha</math> overexpression</b> (with and without oleate supplementation)	CCT $\alpha$ and lamins found needed to make the NR grow	[87,266]
human fibroblasts (GM05757)	treatment with <b>etoposide</b> , which inhibits topoisomerase II after it has cleaved the two DNA strands to remove topological stress, thus preventing religation, thus leading to the accumulation of DNA DSB		[267]
mouse embryo-derived NIH/3T3 fibroblasts	Murine CytoMegalovirus (MCMV) <b>infection</b>	the structure likely represents a virus-induced specialized membrane domain at which the viral particles are preferentially enveloped	[268]
human skin fibroblasts	loading with human immunodeficiency virus type 1 <b>protease</b> (HIV-1 PR)	peptides derived from the intermediate filament protein vimentin proposed as responsible for NR emergence	[269]
<i>Saccharomyces cerevisiae</i>	treatment with the genotoxin <b>zeocin</b> , which creates both single and DSB	likely unrelated to zeocin's ability to damage DNA, likely related to a collateral alteration of lipid metabolism	[186]
mouse embryonic fibroblasts	<b>prelamin A accumulation</b> at the nuclear envelope		[88]
mouse embryonic fibroblasts, rabbit kidney cells	treatment with <b>saquinavir</b> , an anti-retroviral protease inhibitor	protease inhibitors against Human Immunodeficiency Virus are designed to inhibit the HIV aspartyl protease, yet some also inhibit ZMPSTE24, the protease processing Lamin A, thus mimicking laminopathies	[270,271]
artificially assembled nuclei from cell-free <i>Xenopus</i> extracts	LAP2 proteins mediate <b>nuclear envelope-chromatin contacts</b> . LAP2-protein fragments were assessed for their ability to support nuclear envelope reformation, and were found to lead to deep NR structures	suggested to dictate ability to launch DNA replication	[272]
monkey kidney fibroblast-like COS cells	Lamin B Receptor ( <b>LBR</b> ) <b>overexpression</b>		[273]
<b>Description of natural features observed in NR</b>			
spleen lymphocytes of mouse embryo	14.3 % of small lymphocytes display a single and deep NR-type I		[274]
polytene nuclei of salivary gland in <i>Drosophila melanogaster</i>	naturally displaying deep NR		[275]
rat Sertoli cells	NR observed to be filled with <b>microtubules, F-actin, and intermediate filaments</b>		[276]
multiple cancer cell lines	intermediate filament proteins <b>vimentin</b> and <b>keratin</b> fully run along deep NR invaginations		[277]
adrenal gland and human cortex-derived SW-13 cells	presence <i>versus</i> absence of <b>vimentin</b>	cytoplasmic vimentin suggested to trigger NR formation	[278]
multiple mammalian cell types	NR-type II morphology and abundance found to be <b>cell-type dependent</b> :		[279]
	- NRL cells (few vertically oriented tubules)		
	- HeLa cells (numerous non-vertically oriented tubules)		
	- Primary mouse peritoneal exudate cells (often single and vertical)		
	- 3T3, CHO, G8 cells (complex branching network)		
monkey kidney fibroblast-like COS cells	<b>emerin</b> systematically present in NR		[280]
mouse embryo-derived NIH/3T3 fibroblasts	classification of NR-type II invaginations as horizontal to the main plane of the nucleus as being <b>F-actin</b> -positive, and vertical, which are F-actin-negative and perpendicular to the first type		[281]
Chinese Hamster Ovary CHO-K1 cell line	<b>lamin A</b> detected at NR that was more immobile than that associated to the standard nuclear envelope		[282]
living epidermal onion cells and tobacco NT1 cells in culture	extreme manifestation of NR	fully traversed by <b>F-actin</b>	[283] and many references concerning other plants therein
microspore tetrads of <i>Podocarpus macrophyllus</i>	deep and very developed NR-type II and type I	proposed to serve for the optimal export of ribonucleoproteins and other substances	[284]
mouse intestinal smooth muscle cells, nuclei from the endothelium of small blood vessels of rat	NR-type II	suggests that NR correlates with <b>muscle contraction</b>	[285,286]
nuclei isolated from rat heart, pigeon breast, rat femoral muscle cells	NR-type II and frequent, wave-like type I	the NE infoldings from muscle cells from different species persist for several days despite isolation and inactivity	[287]
ascites hepatoma AH602 cells	NR-type I	proposed to be related to <b>malignancy</b>	[288]
collection of 138 invasive breast cancers	out of 273 invasive breast cancers analyzed, 138 displayed NR deformations	this feature was statistically correlated with <b>metastatic</b> behaviors	[289]
<i>Xenopus</i> embryonic cells and multiple cell lines, including	<b>actin</b> found filling NR invaginations	proposed to correlate with the degree of <b>de-differentiation</b>	[290]

Table 1 (continued)

Cell type/Tissue	Condition associated to NR presence	Comment	Reference
human breast cancer MDA-MB-231 cell line			
C57BL/6 mouse subcutaneous connective tissue-derived fibroblasts	alpha smooth muscle <b>actin</b> ( $\alpha$ -SMA) found within the NR invaginations	suggests NR possible involvement in cellular and nuclear mechanotransduction as well as nuclear transport	[291]
human cervical cancer epithelial HeLa cells	detection of <b>DAG</b> at NR		[292]
<b>Calcium Signaling</b>			
lymphoblastoid cell lines	ITPR1 mutations causative of Gillespie syndrome, which concurs with bilateral iris hypoplasia, congenital hypotonia, non-progressive ataxia, and progressive cerebellar atrophy	<i>ITPR1</i> encodes an inositol 1,4,5-triphosphate-responsive calcium channel found in endoplasmic and nucleoplasmic reticula	[293]
pituitary GH (3) cells	deep NR-type II harboring cytoplasmic organelles within and displaying the $Ca^{2+}$ -release system		[294]
intact human vascular smooth muscle cells (hVSMCs), isolated hVSMCs nuclei	characterization using $IP_3$ and ryanodine suggest that the NR may participate in nuclear $Ca^{2+}$ signaling	the presence of nuclear pores suggests that these structures can play a role in nucleo-cytoplasmic transport	[60]
SKHep1 epithelial cells, Chinese Hamster Ovary cells, rat primary hepatocytes	presence of inositol 1,4,5-triphosphate receptor 2 ( $IP_3R2$ ) and of Protein Kinase C at NR. They are activated by $IP_3$ and DAG, respectively, the two products of $PIP_2$ hydrolysis, upstream of $Ca^{2+}$ release	reinforces existence of $Ca^{2+}$ signals generated from the NR	[262]
lung carcinoma-derived H1299 cells	inositol 1,4,5-triphosphate-kinase isoform B ( $IP_3KB$ ) is enriched at NR	inositol 1,4,5-triphosphate, created by $IP_3KB$ , may be involved in modulating nuclear $Ca^{2+}$ signals generated from the NR	[295]
rat adrenal medulla-derived PC12 cells	inositol 1,4,5-triphosphate receptor 1 ( $IP_3R1$ )	$IP_3R1$ is the receptor for inositol 1,4,5-triphosphate, which opens to release $Ca^{2+}$ from the NR	[296]
mouse ovarian granulosa cells	inositol 1,4,5-triphosphate receptors ( $IP_3R$ ), ryanodine receptors (RyR) and $Ca^{2+}$ -ATPase (SERCA)	the NR harbors the three types of proteins allowing $Ca^{2+}$ mobilization	[297]
epidermal cells of the ovary of the flower <i>Lilium regale</i>	branched and deep NR network detected	suggested to be a $Ca^{2+}$ depot	[298]
striated muscle cells C2C12	ryanodine receptors (RyR) detected at NR	receptors at the NR proposed to be important for cardiomyocyte function	[57]
rat neonatal cardiomyocytes	inositol 1,4,5-triphosphate receptors ( $IP_3R$ ), and ryanodine receptors (RyR) present at the NR	receptors for at the NR proposed to be important for cardiomyocyte function	[58]
primary <i>Aplysia</i> giant neurons	NR can form but is not always present in primary neurons	NR formation is not needed for $Ca^{2+}$ release into the nucleoplasm	[299]
<b>Export from nuclei, nucleoli and translation efficiency</b>			
human mouth epidermoid carcinoma, human oviduct tumor, cells isolated from rabbit kidneys	NR-type II contacting nucleoli		[300]
haemopoietic cells of the yolk sac in mice	NR-type II observed in the contact with nucleoli plus derived branching type I		[301]
<i>Noctiluca scintillans</i> (unicellular alga)	nucleoli-associated double-membrane vesicles fusing with the NE	suggested to deliver material from nucleoli out from the nucleus	[302]
primary human mammary epithelial cells and human breast tumor cell lines	ectopic expression of <i>mrtl</i> ( <i>myc</i> -related translation/localization regulatory factor), a protein synthesized from the first open reading frame contained within the human <i>c-myc</i> , which results in loss of Myc from the nucleus	<i>Mrtl</i> is a putative modulator of Myc translation and localization	[303]
mouse C2C12 skeletal muscle cells	myoblast differentiation into myotubes	likely involved in supporting efficient sarcomeric protein mRNA exit from the nucleus	[304]
monkey kidney COS-1 cells	expression of the nucleolar chaperone Nopp140		[99]
<i>Saccharomyces cerevisiae</i>	overexpression of the nuclear pore complex component Nup53		[305]

this network contributes to implementing NR-type II formation. But, why would skeletal forces drive NR-type II emergence in response to DNA breaks? As explained in [section 2.3.4](#), the NE has been described to function as a platform where broken DNA molecules relocate to be assisted during DNA repair [153]. Here, authors propose that, in an analogous manner, invading membranes provide an intra-nuclear **platform for accurate repair** of molecules whose nature or subnuclear compartmentalization prevent them from peripheral relocation. It becomes thus an intriguing question what dictates when the DNA goes meet the NE, when the NE goes meet the DNA. Also, while microtubules push membranes thus implementing invagination, it is however hard to conceive that this can occur without an accompanying, coordinated lipid synthesis

that would remodel membrane composition and render the structure more malleable, in whose absence the NE would tear apart. In this sense, the active synthesis of phospholipids along the whole NR tube, but mostly at the tip of the progressing NR, has been detected using deuterated choline [320], and key enzymes of the phospholipid-synthesis machinery were found at these locations [266,321]. Moreover, these enzymes may not only contribute lipids: CCT $\alpha$  caused deformation of liposomes into tubules *in vitro*, suggesting an additional role in instructing the appropriate high curvature needed at the NR nascent tip [266].

In murine tissues, NR-type II was found only in two cell types naturally displaying polyploidy. The authors also explored cultured human cells and found the formation of NR in three of them, all also

polyploid. This correlation raises the possibility that the chromatin itself can also be the reason why membranes undergo remodelling. Via anchoring, the chromatin exerts a hold over NE membranes and, depending on the **chromatin status**, the forces are not the same [322]. This purports that condensing chromatin would pull the membrane. In support, in *Drosophila* salivary glands, DNA condensation mediated by condensin-II concurs with NR-type II invagination within the nucleus [265]. Furthermore, some studies described that the detachment of chromatin from the NE suppresses NE deformation [323]. Reciprocally, the overexpression of a chromatin-tethering protein induces the overgrowth of the NE, this time towards the cytoplasm, in yeast [226], again suggesting that membranes are actively responding to changes in chromatin anchoring and compensate by synthesizing more attachment surface. This brings us back to [section 3.3](#), where we proposed that the dual role of certain proteins at the INM, mostly NETs, can coordinate INM lipid biology with probing the DNA status.

Another possible contributor to NR formation is, of course, the **lipids composing the NE**. While it is unlikely that this single feature can launch NR formation, it for sure sets the grounds for this to happen with more or less propensity. The recent isolation of human kidney cell NE lipids, found enriched in PC, PI and cholesterol, permitted the measurement of the mixture elasticity by subjecting the reassembled membrane to a magnetic field, which appeared to be two orders of magnitude higher than that of the plasma membrane, thus providing a basis for the NE propensity to fold and manifest membrane fluctuations [5]. This may explain the natural and impressively deep clefts seen in cardiomyocytes nuclei, despite the fact that their presence does not correlate with their contraction status [287], likely attesting of a basal composition able to stand the forces to which they are exposed.

There have been repetitive reports indicating NR-type II dives till contacting nucleoli [264,266,279,281,304,324] and [Table 1](#). The already proposed idea has been that this optimizes, for it shortens, the **export of immature ribosomes**, whose partial assembly first takes place in nucleoli. The same idea could apply to any messenger RNA after its transcription, for a NR tube would allow it to gain immediate access to the cytoplasm, as proposed [325]. In this line, Unr, a cytoplasmic RNA-binding protein with roles in the regulation of mRNA stability and translation, was found to induce the formation of NR tubes in trophoblast giant cells of the developing placenta that were filled with high levels of poly(A) RNA and translation factors, therefore constituting active **sites of translation** [313]. Reciprocally, the tubes can be used in the opposite sense to mediate, as seen in cancer cells, a fast **delivery of extracellular vesicles** and its messenger contents deep into the nucleus [326] using as a docking adaptor the complex formed by VAP-A/ORP3/Rab7 proteins [327]. On a more speculative view, NR could assist in compartmentalization of the genetic material or, literally, in splitting chromosome territories. Given that NR is cell type-specific, this remains an exciting possibility, as invaginations may create specialized compartments within the nucleus and set specific inter-chromosomal interactions, which could have implications for gene regulation or replication timing. This notion has been proposed for onion epidermal cells, where the nucleus is massively crossed by NR in all directions [283].

But what then triggers the formation of NR-type I? While lamin addition permits the progressive growth of NR-type II, along with phospholipids [328], **lamin removal** is necessary to permit NR-type I birth [94]. This is also the case to permit other INM remodelling events, such as nuclear LD emergence [94] or programmed NE rupture in response to DNA damage [204]. To this end, promyelocytic leukemia protein (PML) implements lamin removal. In this set-up, it is conceivable that conditions leading to local lamin depletion may elicit this phenomenon, provided this is sufficient.

The treatment of Schwann cells with tellurium, which inhibits squalene epoxidase, thus hinting at **cholesterol synthesis**, simultaneously provokes a compensatory (and reversible) overexpression of HMG-CoA reductase and synthase, and a marked development of NR-type I [329]. NR-type I has also been reported in response to treatment with the genotoxin methyl metanesulfonate (MMS), specifically in cells of non-cancerous origin, although this ability was unlikely related to its DNA-damaging potential [330]. In a different system, MMS was reported to elicit a sharp and fast downregulation of the sterol metabolism [331]. One plausible link connecting all these elements in cells bearing lamina would be that the restriction of the sterol pathway prevents lamins from being farnesylated, farnesylation being key for anchoring lamins to the NE. It can be speculated that, in the absence of farnesylation, lamin stabilization would not occur, mimicking its removal, thus creating conditions optimal for NR-type I emergence.

But is the NR-type I then just a by-product? Or are there, at least in some instances, any functional aspects that could be associated to this specific type of NR? It was shown that the increased presence of this type of NR correlated with an improved ability to react to DNA lesions exposing single-stranded DNA (ssDNA) tracts [332]. ssDNA accumulates either directly, or as an intermediate of other types of DNA damage, and very powerfully stimulates the alert system of the cell, the DNA Damage Response (DDR). In fact, ssDNA is rapidly coated by the Replication Protein A (RPA) and, together, represent an alarm substrate capable of attracting signalling proteins that will spread the word around the cell (and beyond). The master protein responding to this call is the kinase Ataxia Telangiectasia Rad3-related (ATR), which was curiously shown in the past to 1) have additional capacities such as sensing membrane stretching [333,334] and 2) the degree of poly-unsaturation of membrane glycerophospholipids [335]. In the event of NR-type I presence in the nucleus, ATR adjusts the intensity and kinetics of the DDR (phosphorylation of downstream effectors to respond to DNA damage) to the load of intra-nuclear lipids. This way, a higher amount of intra-nuclear lipids matches a more intense DDR for an equal DNA insult, thus a more efficient engagement of DNA repair mechanisms [332]. Although it was not assessed whether this could be achieved also by instructing the formation of NR-type II, increasing the expansion of NE membranes in an unrelated system (*S. cerevisiae*) in the presence of a genotoxic insult also led to a more robust response to DNA damage [332]. More needs to be done to dissect the drivers, the possible functions and the relevance (if any) of the NR-type I.

#### 4.2. The nucleolar channel system (NCS)

For more than half a century, the Nucleolar Channel System (NCS) is known as an ultrastructural hallmark of the post-ovulation human endometrium [336–339]. Composed by membrane cisternae within the nuclei of endometrial epithelial cells, it recurrently associates with nucleoli (reviewed in Ref. [340]). It appears during a 3-to-4 days window that robustly coincides with the midluteal phase of the menstrual cycle, and is under the apparent control of progesterone, when the endometrium is receptive for fertilized egg implantation [337,341]. This, along with the fact that oral contraceptives and intrauterine devices interfere with the timing or even the appearance of this structure [342–346], makes the NCS be ascribed to fertility. This is reinforced by sporadic characterizations of primary infertility in which the NCS failed to be identified [347–349]. However, a retrospective and prospective study considering both fertile women and women with unexplained infertility failed to find an association between NCS and fertility [350], reinstalling the mystery of the relevance of this organelle.

In an effort to characterize this structure better, cells from endometrial origin were subjected to treatment with the reproductive hormones oestrogen and progesterone, which was sufficient to induce NR-type II formation, although not a *bona-fide* NCS. The process did not require cell division, highlighting that passage through mitosis is not needed to elicit the formation of these membranes. Also, this was reversible, as the membranes disappeared upon removal of the hormonal stimulus [320]. This study suggests that NR-type II is the primary event underlying NCS formation. While the fact that the NCS derives from NR-type II events is expected in view of the presence of well-established ONM and ER proteins in the membranes of this structure [104], the ultrastructural characterization of eventual tubular connections of the NCS with exclusively the INM had set the doubt in the past [351], making some authors write that the NCS derives from NR-type I events [352]. Irrespective of the origin, the final structure of the NCS implies that it is subjected to an extensive and radical remodelling.

#### 4.3. Nuclear lipid droplets (nLD)

##### 4.3.1. nLD types and formation

Nuclear lipid droplets (nLD) are organelles defined by analogy with the cLD, however found in the nucleoplasm of cells. They are also defined as having a core of neutral lipids delimited by a glycerophospholipid monolayer and capable of harboring an evolving proteome on their surface. They were initially described in ultrastructural studies of mouse hepatic parenchymal cells in response to dietary exposure to the food additive bentonite [353], the liver fibrosis/hepatocellular carcinoma-inducing thioacetamide [354], the carcinogenic azo dye DAB [355] and the chemical agent polybrominated biphenyl [356]. Moreover, the excessive accumulation of intra-nuclear lipid inclusions was assigned as the distinctive feature of the pre-lethality in *T* locus-mutated mouse embryos [357,358]. It is to note that, in some of these early studies, the presence of cLD enclosed within a NR-type II were also considered as nuclear, likely overestimating the actual presence of *bona-fide* nLD (Fig. 6G). It is only recently that, using biopsies from diseased human livers, a very careful assessment was done to evaluate the prevalence of one *versus* the other type and try to correlate them with pathological parameters [359].

After a 30-year gap of research in nLD biology, Layerenza et al. [360] were the first to demonstrate the natural presence of nLD in isolated nuclei from rat liver and in cultured rat hepatocytes and human hepatoma HepG2 cells by confocal microscopy. To date, there are at least three described mechanisms of nLD formation in mammalian cells. The first was established by Ohsaki et al. [89] and Soltysik et al. [94] and occurs in cultured human and mouse cells of hepatocyte origin, which are characterized by their physiological capacity to produce Very Low-Density Lipoproteins (VLDL). VLDL particles, consisting of 90 % lipids (predominantly TAG with a lesser contribution of CE and FA) and 10 % apolipoproteins (ApoB, ApoE, and ApoCIII) [361], are first synthesized and assembled within the ER lumen relying on the activity of Microsomal Triglyceride transfer Protein (MTP). Subsequently, these organelles are released into the circulatory system to safely transport hydrophobic lipids from the site of their formation (liver) to adipose, muscle, and cardiac tissues. Under ER stress in the Huh7 human cell line, VLDL precursors (ApoB-free luminal LD) accumulate in the IMS, the INM evolves into NR-type I, inside which these immature lipoproteins slide in an MTP-dependent translocation, then are released into the nucleoplasm following a rupture of the NR-type I tip (Fig. 6H). RNAi-mediated knockdown of SUN proteins leads to a significant increase of nLD in OA-treated cells. Promyelocytic leukemia (PML) isoform II ablation significantly decreases the rate of nLD formation

in Huh7 cells upstream of SUN action, overall revealing a role for PML-II in locally removing SUN proteins to authorize OA-induced nLD birth in the event of ER stress [94]. Once released, these nLD undergo a progressive enrichment of lipid-synthesizing enzymes at the surface, especially CCT $\alpha$  and DGAT2, respectively catalyzing *de novo* PC (which allows nLD surface's growth) and TAG synthesis (which allows nLD contents' increase) [89].

However, a second, alternative mode of nLD emergence has been observed in hepatocytes of mice [362]. In animals lacking the Lamin-Associated Polypeptide 1 (LAP1), huge nLD formed spontaneously at a striking frequency. Like SUN proteins, whose absence is a nLD-promoting factor in the cultured hepatocyte system, LAP1 is a monotopic integral INM transmembrane protein. In further analogy, nLD formed in the absence of LAP1 also emerged from NR-type I ingressions events and were coated by CCT $\alpha$ . However, they did not necessitate oleate supplementation for being predominant, and even were oleate load-irresponsive. They were surrounded by lamins and did not relate to PML or MTP activity. It appears then that neutral lipids that were destined elsewhere end up intruding the nucleus if the INM somehow loses its homeostatic parameters, as exemplified by loss of SUN or LAP1 INM-structuring proteins.

A third type of nLD formation in mammalian cells is featured by cultured U2OS human cells. In it, the INM is not seen to intrude the nucleus in the shape of NR-type I and the neutral lipids filling the future nLD first congregate within the two leaflets of the INM bilayer, eventually budding into the nucleoplasm (Fig. 6K), in a process extremely reminiscent to the birth of canonical LD towards the cytoplasm [83,85]. This occurs in the absence of MTP, in agreement with nLD contents not arising from VLDL metabolism, and does not necessitate PML-II participation, highlighting again that this very specificity likely responds to the involvement of a NR-type I. Of note, this process is also alike to nLD formation in the unicellular eukaryote *S. cerevisiae*, as seen upon genetic manipulation (see below) [46].

In invertebrates, nLD are reported to occur naturally in the intestinal and gonad tissues of *C. elegans* wild-type animals [363]. The homeostasis of these tissues strongly relies on a tight regulation of lipid metabolism, in particular during the self-fertile reproductive period in hermaphrodite nematodes (spanning from the L4 (larval) to the D4 (adult) developmental stages). nLD emergence was age- and cell type-dependent. In the intestinal nuclei of young animals (L4-D1 stages), nLD form between the NE and its associated peripheral heterochromatin and, in emerging, disrupt the lamina layer (Fig. 6K). They expand into the nucleoplasm till reaching an impressive size (4  $\mu$ m diameter). Since these nLD deplete the lamina, they prime the NE for subsequent rupture, and, as such, were recurrently found at sites displaying NE rupture. In comparison with the processes described above, this nLD birth mode is partly reminiscent to that reported for U2OS and *S. cerevisiae* cells (Fig. 6K).

In opposition, a remarkably distinct class of nLD is observed in older intestinal nuclei (D2-D4 stages): the INM intrudes into the nucleoplasm carrying the actual LD already inside (thus implying it was formed in the IMS), yet in this case being able to keep the lamin coat (not depicted, it would look like a hybrid of Fig. 6H and L). These nLD possess a non-negligible size, push the heterochromatin that is associated with the NE regions from where they emerge, disrupting its organization and, with it, potentially chromosome territories. Together, NE rupture and genome reorganizations were purported to underlie intestinal nuclear damage [363]. Recently, Palikaras et al. [364] provided another characterization of age-dependent deposition of nLD in *C. elegans* intestinal cells. The authors employed an elegant technique, Third Harmonic Generation imaging, which allows to visualize lipid content *in vivo* in a label-free and non-invasive manner. nLD significantly accumulated in



the NE of older nuclei (D10), compared to D1 stage nuclei, and this was remarkably diminished in *eat-2(ad465)* and *daf-2(e1370)* *C. elegans* strains, both genetic models for prolonged longevity. Altogether, these findings could be understood in terms of a link between nLD biology and aging. At the molecular level, when considering the striking analogy between nLD from old intestinal nuclei and those emerged upon LAP1 absence in mice hepatocytes, it could be purported that processes (like aging) **debilitating the quality of the INM proteome** (like SUN, or LAP1, or many others: see [section 3](#)) may weaken its resistance to emit NR-type I and with it, the **unscheduled deposition of neutral lipids within the nucleoplasmic space**.

#### 4.3.2. nLD elicitors, genetic determinants, and composition

Oleate treatment remains the most commonly employed model for the artificial induction of nLD in yeast [46], in rat/human cells of hepatocyte origin [83,90,94,365]; and in non-hepatocyte human cells [84,85,366]. In this line, in a mouse model, a dose-dependent accumulation of nLD is detected in hepatocyte's nuclei in response to perfluorooctanoic acid (PFOA), a synthetic organic chemical structurally resembling FA [367]. Recurrently correlated with increased levels of cholesterol and TAG [368,369], it remains badly understood if PFOA supplementation triggers these effects as a structural mimic of FA or indirectly, by perturbing metabolic pathways. In both cases, the notion is that nLD may emerge in **reaction to an excess of lipids**. This is particularly well illustrated in some cancer contexts. EPHB2 (Ephrin type-B Receptor 2) protein expression was highlighted in a panel of human-derived prostate cancer cell lines for being inversely associated with *in vivo* cell aggressiveness. In agreement, EPHB2 silencing increased the proliferation of prostate cancer cells. Simultaneously, this led to the *de novo* accumulation of both cytoplasmic and nuclear LD, which was linked to an increased expression of lipogenic factors such as DGAT1, DGAT2 and PLIN2, and a decrease in lipolytic activities such as ATGL [370]. Thus, EPHB2 helps tame lipogenic activity and this restricts nLD accumulation. Another example is provided by Fatty Acid Binding Protein 7, which was recently reported to translocate FA to the nucleoplasm, where they feed the growth of nLD, and this exerts a boosting activity for glioma [366]. Examples also exist from physiological, non-disease contexts. Abundant nLD were observed in pachytene nuclei of meiotic germ cells from *C. elegans*, but specifically those from which oocytes will derive, and not those from which sperm will be formed. In them, nLD reach a giant size, and were found to have no negative impact. As these cells display highly condensed chromosomes that do not form a peripheral heterochromatin, authors proposed this may be why these giant nLD do not confer any deleterious effects [363]. Considering the nourishing function of oocytes in supporting initial cleavages after fertilization and egg development, these “innocuous” nLD may relate to the storage of a vast lipid excess with developmental purposes.

Genetic modifications are also a recurrent means for artificially inducing nLD formation, and bear a strong potential to uncover mechanistic insights. The characterization of genetically engineered *S. cerevisiae* strains displaying frequent nLD allow us to learn more on the actual drivers, and likely of the potential function, of nLD. Conditional inhibition of *CDS1*, coding for the key enzyme converting PA to CDP-DAG, led to a significant increase in nLD featuring PA and DAG at their surface. A quantitatively similar nLD increase was observed upon deficiency of the *INO4* gene, coding for one of the transcriptional activators of multiple glycerophospholipid-synthesizing genes, which phenocopies the *CDS1* inhibition, as it also results in downregulation of glycerophospholipid synthesis and a concomitant raise in lipid storage (TAG-producing) pathway [46]. Overall, in this model, the primary nLD determinant were the **elevated levels of PA at the INM**, which

is channelled toward lipin/Pah1-mediated TAG production [46]. In support, alterations in the DAG/PA equilibrium have been by now recurrently reported to elicit nLD formation [89,371–373], including those naturally present in the germ cells of *C. elegans*, which became of a massive size when this same axis was mutated [363]. In the same line, the overexpression of a LD-binding protein harbouring a putative PA-binding domain, LdrA, in the multinucleated filamentous fungus *Aspergillus oryzae* triggered the formation of nLD. Mutant versions of the protein manipulating the hydrophobicity of its AH led to almost half of the nuclei displaying nLD [374]. Further, altering DAG metabolism by supplementing *S. cerevisiae* cells with the lysophosphatidic acid analogue edelfosine also led to the formation of nLD [171].

A second important feature dictating nLD birth in *S. cerevisiae* was the INM glycerophospholipid saturation status (see [section 1.1](#) & [Fig. 2C](#)). nLD formation was strongly suppressed in response to high unsaturation at the INM, as manifested after treatment of wild-type cells with linoleic or stearic acid (FA with a higher number of double bonds, compared to OA), or overexpression of the Ole1-Mga2 transcriptional pathway, which increases the ratio of unsaturated-to-saturated FA [375]. Other events altering the lipid homeostasis of the INM also drive nLD formation, as in U2OS cells in the absence of the PC synthesis enzyme (CEPT1), as PC deficiency at the INM favoured nLD emergence [376]. Together, the emerging picture is that a **NE lipid disequilibrium**, or an overload with species with lipotoxic potential, either because of their type, or of their quantity, may elicit nLD formation as a **quenching mechanism** that would alleviate the risk of losing NE homeostasis (a notion further developed in Ref. [377]).

A last but important leitmotiv in the literature regarding nLD induction has been the recurrent use of harmful molecules, as illustrated by the early-days exotic products referred to above. Even nowadays, it has been reported the case for acute exposure to waterborne cadmium in the rare minnows *Gobiocypris rarus*, representing a family of non-mammalian vertebrates [378]. In *S. cerevisiae* cells, nLD are specifically generated in response to DNA replication impairments, meaning the slowing or arresting of replication forks [331]. This was elicited pharmacologically through treatment with genotoxic drugs (methyl methanesulfonate (MMS), hydroxyurea (HU) or camptothecin (CPT)) [331]. This induction was specific to this type of challenge, as the use of other genotoxins that create DNA damage, yet without restraining replication fork progression, did not elicit nLD accumulation. To provide further support to this notion genetically, authors showed that absence of genes coding enzymes that assist replication forks upon DNA damage led to nLD formation and that, reciprocally, overexpression of one of these factors, the exonuclease Exo1, suppressed the genotoxin-induced nLD accumulation [331]. Together, it is possible that the myriad of toxins tested in the past and shown to trigger nLD formation do so, if not out of a perturbation in the NE lipid composition homeostasis, then by creating DNA replicative stress.

But is there anything specific to replicative stress, occurring at DNA, that can elicit nLD birth from the NE? Are these nLD somehow different, for example in their composition, to claim they are distinct from all the lipid-related examples described earlier? Replicative difficulty leads to the arrest or slowing down in the progression of the replication fork, which is signalled by the component Mrc1. Also, problems to duplicate the template leave single-stranded gaps in the vicinity of the fork that are immediately covered by RPA. Both Mrc1 and, as already mentioned, ssDNA-coating RPA, are potent activators of Mec1 (in budding yeast)/ATR (in humans). This initiates the activation of the cascade in response to DNA damage and is important to set in action the cellular strategies necessary to transiently arrest cell cycle progression, engage repair, tolerance mechanisms, and adapt transcriptional programs.

As evoked in the previous section, ATR was reported to be reactive to NE stretching [334] and to the degree of poly-unsaturation of membrane phospholipids [335], as well as to coordinate the intensity and the kinetics of the DDR to the load of intra-nuclear lipids [332]. Thus, first of all, nLD in response to replicative stress could be a commanded event under the control of the DDR, in which case they might fulfil a role. Given the match between intra-nuclear lipids and DDR intensity [332], this could be a means of maintaining this signalling as long as the challenge is still present.

Purification analyses of hepatocytes-derived nLD showed they are composed of the classical neutral lipids (TAG and CE), albeit in very different proportions to those seen in cLD, with CE greatly exceeding TAG. Moreover, they are also (atypically) rich in unesterified cholesterol, and more protein-rich than their cytoplasmic counterparts [360]. The striking enrichment in sterol moieties measured in these nLD matches well the report that nLD in *S. cerevisiae* also emerge spontaneously in cells with a high load of free sterols in membranes [331]. Further, using inhibitors of the cholesterol synthesis pathway in HepG2 cells leads to the detection of the abortive intermediate molecules specifically in nLD [379]. Interestingly, a sharp shut-down of the whole sterol synthesis transcriptional pathway was seen in response to one replicative stressor at the timepoint when nLD generation reached a plateau [331]. Together, it is tempting to imagine that a specific class of nLD rich in sterols is first instructed and later restricted in response to replicative stress, likely as an integral part of the response to challenges during DNA duplication.

## 5. Intra-nuclear lipids

### 5.1. Chromatin-bound lipids

Phospholipids from isolated hepatocyte nuclei were radio-labelled, and the radioactivity associated with chromatin, nuclear membranes and nucleoplasm was monitored. 97 % of the label was recovered in the nuclear membranes, less than 1 % (0.08–0.6 %) was found in chromatin, and the remaining 2 % was present in the nucleoplasm. The most remarkable on qualitative terms was that 35 % of chromatin-associated lipids was sphingomyelin [380]. Thus, up to 3 % of nuclear phospholipids were found in non-membranous nuclear compartments. In the following, we dive into the details of this 3 %.

The ability of lipids to directly bind DNA is documented *in vitro*, as for the unsaturated OA, which recognizes A-T base pairs and can bind tightly at one molecule per 2–3 base pairs [381]; and in prokaryotes, as the lipidomic characterization of lipids bound to DNA in *Pseudomonas aurantiaca*, with PC and PI found to bind loosely, while the neutral species TAG bound very tightly [382]. Lipids can also bind the nucleosome, the complex of histones with DNA. Recent studies *in vitro* showed the dehydrating potential of cholesterol against the nucleosome, which led to chromatin compaction [383]. Also, cholesterol in complex with single-stranded DNA can instruct its bending [384]. Moreover, nucleosomes have the potential to interact with steroid hormones, bile acids and FA (reviewed in Ref. [385]), implying that lipids could affect chromatin states by modulation of the nucleosome physics. *In vivo*, chromatin lipids have been documented to increase during DNA duplication, as shown during both liver regeneration and cell maturation. They localize in the proximity of newly synthesized RNA, and likely associate with it, as digestion of chromatin with RNase, but not with DNase, causes a loss of phospholipids. The composition of the chromatin phospholipid fraction shows an enrichment in sphingomyelin and PS. While sphingomyelin, the most represented species in chromatin, localizes to newly synthesized RNA and likely protects it from unscheduled degradation, PS

has been reported to stimulate both RNA and DNA polymerase activities (reviewed in Ref. [386]). Further, chromatin-bound PS was proposed to serve as the catalyst for NE reformation after mitosis [387].

### 5.2. Lipids within condensates

The phospholipids most frequently reported in the nucleus are glycerophosphoinositides (PIP) [388]. PIP bear inositol as the polar head, which can become phosphorylated at positions 3, 4 and 5 in all possible combinations, thus giving rise to seven possible variants. While the synthesis of PIP at the INM is documented [389,390], an important concern is whether their synthesis can also occur within the nucleoplasm. To this end, as PIP emerge from the enzyme synthesizing them, they must immediately engage in interactions with surrounding proteins and nucleic acids [391], given their poor solubility. For example, the apolar tails can be bound by Steroidogenic Factor 1 [392,393]. Alternatively, the apolar tails can arrange conforming micelles (discussed in Ref. [394]).

The selective extraction of nuclei from mouse NIH/3T3-fibroblasts and rat liver cells allowed the preparations of peripheral (NE-associated) and internal (nucleoplasmic) components. The NE-associated fraction contained PI(4) kinase, while the internal fraction was positive for PI(4,5)P kinase [389]. This differential nuclear localization of the PIP kinases, which are known to act sequentially, strongly suggested a compartmentalization, thus a functionality for PIP metabolism in the nucleus. PI(4)P kinase was confirmed to be intra-nuclear much later, and in company of PI(3)P kinase. These nuclear kinases displayed comparable activities toward their substrates – PI(4)P and PI(3)P – than their cytoplasmic counterparts. Immunofluorescence revealed their association with distinct subnuclear domains, in particular with nuclear speckles, known to contain pre-mRNA processing factors. A pool of PIP<sub>2</sub>, the product of these kinases, was also detected at these structures. Inhibition of mRNA transcription led to dynamic changes in the localization of both the kinases and their PIP<sub>2</sub> products [395]. More recently, different pools of PI(4)P were reported both in close contact with the NE, but also scattered throughout the nucleoplasm and associated with nucleoli and the nuclear speckles [396]. Last year, a PI(4)P pool associated in the cytoplasm with the surface of the Trans Golgi network was reported to titrate, away from the nucleus, a master kinase in the response to DSB, ATM [397]. This suggests that the nuclear pool of PI(4)P may be completely occluded by its nuclear protein interactors. In this sense, the identification of nuclear PI(4)P-associated proteome points at replication, transcription, as well as mRNA and rRNA processing [396]. PIP are therefore irrefutably present inside the nucleus and their levels evolve in response to multiple stimuli, therefore suggesting their potential role in regulating specific nuclear functions [394].

To exert their functions, PIP can act as cofactors for proteins, for example because PIP polar heads locally provide negative charges at the regions where they are embedded, thus creating domains for interaction with positively charged proteins. The nuclear targets of PIP are still incompletely defined. Targeted and global proteomic studies have established lists of potential PIP-interacting proteins, which has pointed at transcription, chromatin remodelling, mRNA maturation, DNA topology and nucleolar biology [398–400]. For example, ING2, one member of the conserved inhibition of growth (ING) family, was described as a *bona-fide* nuclear receptor for PI(5)P, which therefore implicates it in the modulation of histone acetyltransferase and deacetylase complexes [401], and regulates the ability of ING2 to activate p53 and p53-dependent apoptotic pathways [402]. At present, chromatin regulation is established to be under PIP influence: PIP are also reported to interact with a

variety of histones and histone combinations (H1, H2AH2B, H3H4, octamers). *In vitro*, histones caused a dose-dependent aggregation of artificial PC-containing vesicles when harbouring negatively charged glycerophospholipids, in particular PI(4)P [403]. Also, EBP1, a ubiquitous and conserved protein located in both the cytoplasm and nucleolus, and associated with cell proliferation and survival, was found to bind PIP through a C-terminal binding motif, and this domain responsible for its localization to the nucleolus. The point mutation K372N within this domain, found in endometrial tumours, is sufficient to alter EBP1 nucleolar targeting. Mechanistically, EBP1 clustered at the nucleolus with the catalytic subunit of the phosphoinositide 3-kinase (PI(3)K) and its product PIP<sub>3</sub> [404].

PIP can also act as scaffolding platforms. These include splicing speckles, nucleoli, and DNA repair foci. In all cases, PI(4,5)P<sub>2</sub> underlies their organization [392,405], and its absence has a negative impact with functional consequences, as defects in splicing [406], RNA polymerase I transcription [407], and sensing of DNA damage [392], respectively. In particular, the involvement of PI(4,5)P<sub>2</sub> in the organization of membrane-less nuclear compartments, also called condensates, takes us to define sub-nuclear domains with a functional identity, self-structured through the nucleation of their component molecules, yet not delimited by a shielding membrane. However, till recently, there was no evidence in the literature for phospholipids, PIP or other, as *bona-fide* organizers of condensates, as research on this field has classically focused on the interactions established by proteins and the seeding or adjuvant contribution of RNA [408]. It was appreciated just last year that, when mimicking the composition of different *in vivo* condensates (including nuclear ones as those formed by the DNA break-associated factor 53BP1), unbiased metabolomic analyses of the separated condensates yielded phospholipids as major hits, as well as lysoglycerophospholipids, unsaturated FA and acylcarnitines [409]. Phospholipids were able to partition within the condensates in the absence of any transporting protein or transfer mechanism, although it was not ruled out that, *in vivo*, FA binding protein-mediated transfer from nearby membranes or local *in situ de novo* synthesis could provide them. These phospholipids contributed to the phase-separation phenomenon of these condensates. The hydrophobicity of the acyl tails was essential, with 7 carbon-chains being a minimum, yet above 36 carbon-chains being too long, the optimal carbon-chain length being 17–18. The polar head (or the lack of charge) did not appear to be very important, except for PIP<sub>2</sub> and PIP<sub>3</sub>, for which condensates were no longer spherical, and tended to branch, likely through magnesium-dependent rearrangements. On a mechanistic side, *in vitro*, phospholipids tended to increase the number of formed condensates, while the median condensate size remained unchanged [409]. Thus, there is now ground to build on the ways through which lipids could act as organizers of functional platforms in the shape of condensates.

### 5.3. Condensates that remodel membranes

Other than floating within the nucleoplasm, having their own metabolism, or modulating chromatin, condensates have the potential to contact the INM and establish functional interactions with it. These interactions will be, by definition, bidirectional. First, the membrane can remodel the condensate, including its morphology and functionality (elegantly reviewed in Ref. [410]). For example, membranes have been observed to split condensates by sectioning them [411], and condensates could feed the membrane by providing phospholipids, simultaneously altering their own composition while anatomically remodelling the membrane.

Biomolecular condensates typically form spherical droplets in solution, but when the droplets come in contact with a membrane, they tend to spread on the interface through adhesion. Depending

on the interactions established with the membrane surface, the droplet adhesion would possess a contact angle comprised between 180° and 0°, a phenomenon termed partial wetting. The increasing realization is that partial wetting by protein condensates can drive membrane remodelling in multiple biological processes. These include classical endocytic events at the plasma membrane [412,413], generation of autophagosomes [414], and formation of protein storage vacuoles in plants [415]. In fact, the proteins within the condensates, which IDR, lack a stable three-dimensional structure. How do they potently drive membrane bending by crowding together within a condensate? Steric repulsion among IDR will drive the membrane to buckle outward while, when multivalent interactions happen between domains inside the condensate, different forces emerge that shape the membrane inward [416,417]. This strikingly contrasts with the classical view, in which specialized proteins exist that have the ability to shape membranes, including proteins that harbour amphipathic helices or BAR domains. But what advantage would this type of membrane remodelling offer compared to classical protein-driven membrane remodelling? Since lower energy barriers are required to assemble and disassemble condensates, perhaps this endows the system with greater potential to evolve into a variety of shapes that may serve a biological purpose. These include budding, vesiculation, reticulation and variable tubule shapes like pearls, undulations and cylinders [418,419]. In the specific context of the INM, condensates' partial wetting could be at the basis of NR-type I emergence.

Beyond membrane feeding, and membrane shape remodelling, condensates adhered to membranes have been reported to seal membrane ruptures, as in damaged lysosomes, preventing lysosomal contents from leaking into the cytoplasm [420]. This may be equally helpful in repairing transient ruptures at any of the NE membranes. Also, condensates clustering on one side of a membrane have the virtue of synchronizing protein condensates on the other side of the membrane, a phenomenon termed transmembrane coupling [421]. But how can protein condensates on different sides of the membrane recognize each other and become coupled? FRAP studies demonstrated that lipids from the underlying membrane could diffuse faster in the regions without protein, while lipids' diffusion was minimal in regions of the membrane with coupled proteins on both sides. Furthermore, the stronger the protein-protein interactions within the condensate, the higher the reorganization of the underlying lipids. The lipids reorganized this way can, in turn, become coupled, or "registered", a phenomenon occurring by various mechanisms that include line tension, membrane fluctuation, and spontaneous curvature [422–425]. Transmembrane coupling represents a fully unexpected mechanism for transferring information across biological membranes that is independent of transmembrane proteins and that, through the organization of the underlying lipids, bypasses the need of direct contact between proteins to transmit information through a membrane. This encloses an incredible potential that could help understand, for example, how chromatin-binding proteins clustering near or at the NE manage to impact lipid-synthesis activities occurring in the IMS (as discussed in section 3).

### 5.4. RNA – lipid interactions

The potential interaction between lipids and RNA is a neglected research area. The first observation dates back to 40 years ago [426] and few interactions have been described since, mostly *in vitro*. Different features of the lipid membrane, mainly implicating charged residues, are involved in the binding to RNA. Indeed, zwitterionic glycerophospholipids, in which the head group bears a positive and a negative charge simultaneously, can become virtually cationic if calcium or magnesium ions stabilize the negative



charge (Fig. 4C). In this case, the negatively charged RNA molecules can establish electrostatic interactions with the membrane [427,428]. Specific hairpin RNA sequences can bind the membranes of synthetic liposomes and increase their permeability [429]. Moreover, the choline moiety of PC was found to be bound by RNA [430,431]. Interestingly, in a recent study, not only was RNA found to bind lipids in a sequence-dependent manner, but lipids could impact RNA's enzymatic (ribozyme) activity [432]. Remarkably, the interaction between lipids and RNA can locally increase the concentration of RNA, in turn restricting the RNA's molecular interactions elsewhere [433]. At present, even if these events have been assessed *in vitro*, they foster new hypotheses about potential *in vivo* mechanisms. *In vivo*, precedent exists, out of the nucleus, for the ability of RNA and lipids to engage in a biologically relevant interaction: vesicles are released into the extracellular space in the form of exosomes. This occurs after the fusion of the multivesicular bodies (MVBs) in which they were included fuse with the plasma membrane. Exosomes dispatch throughout the organism a myriad of molecules, which include miRNAs, and serve as a long-range communication strategy both in physiological and pathological situations, such as done by immune and cancer cells, respectively. miRNAs contained within exosomes are delivered to the raft-like region of the membrane of MVBs by RNA-binding proteins that transport them. An interesting recent work shows that RNA loading into exosomes can occur through the direct interaction between the miRNAs and the raft-like region of the MVB's membrane. Authors identified, upon bioinformatic analyses, four *bona-fide* frequent raft-binding motifs present within these sequences [434]. Thus, lipids' interaction with diverse types of RNA could impact their biology in the nucleus, especially considering that RNA export is finely regulated at the nuclear periphery. Although this control has historically been attributed to the nuclear pores, it is formally possible that the membrane contributes to the process.

## 6. Concluding remarks and perspectives

In this review, we have considered the genuine lipid nature of the NE, and its inextricable impact on all NE functions. This analysis particularly strongly points at the intricate connection between NE lipid homeostasis and genome stability. While at its infancy, developing this concept may bring fresh understanding to long-standing questions. For example, aneuploid cells with identical genotype do not display the same behaviors, a phenomenon termed phenotypic variability, or "non-genetic" individuality. A small fraction of this variability can be explained to gene copy number imbalances and by stochastic DNA damage leading to differential activation of the DNA damage response. Yet, detailed analyses reveal that these events alone are unlikely to justify the loss of robustness of the system [435]. Lipid metabolism lying beyond genome-encoded functions, and in view of the drastic crosstalk between genome instability and lipid homeostasis, we suggest that NE morphology and lipid biochemistry may underlie phenotypic variability. Nuclear deformation is relevant in migration [436], associated to disease [437], and used by pathologists to stage the grade of human tumours [438]. A deeper understanding that considers the lipid dimension of the NE will be the key in unlocking research in these and other fields in the next years.

## CRediT authorship contribution statement

**Kseniya Samardak:** Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Data curation, Conceptualization. **Janélie Bâcle:** Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Data curation, Conceptualization. **María Moriel-Carretero:** Writing –

review & editing, Writing – original draft, Visualization, Supervision, Investigation, Funding acquisition, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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