New kid on the block: lipid droplets in the nucleus

Antonio D. Barbosa[1] and Symeon Siniossoglou[1].

1. Cambridge Institute for Medical Research, University of Cambridge, Cambridge CB2 0XY, UK

Correspondence: S. Siniossoglou, Cambridge Institute for Medical Research
University of Cambridge, The Keith Peters Building, Hills Road, Cambridge, CB2 0XY, UK
Tel: 0044 1223 762641
Email: ss560@cam.ac.uk

Running title: Lipid droplets in the nucleus.

Keywords: Lipid droplet, nuclear membrane, nucleus, endoplasmic reticulum, lipid.

Abbreviations: ER, endoplasmic reticulum; INM, inner nuclear membrane; ONM, outer nuclear membrane; nLD, nuclear lipid drople; TG, triacylglycerol.

Abstract
The regulation of lipid homeostasis is essential for normal cell physiology and its disruption can lead to disease. Lipid droplets are ubiquitous organelles dedicated to storing non-polar lipids which are used for metabolic energy production or membrane biogenesis. Lipid droplets normally emerge from, and associate with, the endoplasmic reticulum and interact with other cytoplasmic organelles to deliver the stored lipids. Recently, lipid droplets were found to reside also at the inner side of the nuclear envelope and inside the nucleus in yeast and mammalian cells. This unexpected finding raises fundamental questions about the nature of the inner nuclear membrane, its connection with the endoplasmic reticulum and the pathways of lipid droplet formation. In this viewpoint we will highlight recent developments relating to these questions and discuss possible roles of lipid droplets in nuclear physiology.

Introduction
Cells contain a myriad of lipids which perform key roles as structural components of membranes, energy storage molecules and signal transducers. In order to maintain homeostasis, cells continuously integrate growth and nutritional signals to decide when and where specific lipids must be produced, remodelled or turned over. In eukaryotes, the central site of lipid synthesis is the endoplasmic reticulum (ER), with contribution from other organelles such as mitochondria, the Golgi complex and the vacuole/lysosome. These lipids can be stored or utilized locally, or transported to various organelle membranes via vesicular or non-vesicular pathways.

Unlike most cytoplasmic membrane-bound organelles, the nucleus is often viewed as detached from the busy network of lipid metabolism. Recent studies led to the surprising identification of lipid droplets (LDs) inside the nuclei of both budding yeast and mammalian cells. LDs are ubiquitous organelles that emerge from the ER membrane and normally reside at the cytoplasm. Within their core, LDs store “non-polar” or neutral lipids, mainly triacylglycerol (TG) and steryl esters, and are coated by a phospholipid monolayer and a

complement of proteins involved in LD function. LDs act as a lipid reservoir that provides fatty acids used for energy production during starvation, or other lipid precursors that are used for membrane biogenesis to support organelle or cell growth [1,2].

The functional significance of LDs in the nucleus is currently enigmatic; these LDs display overall similar morphology as their cytoplasmic counterparts, based on neutral lipid stains and various EM techniques- but some evidence exists to show certain differences in their protein and neutral lipid composition [3,4]. In this commentary, we will adopt the term "nuclear lipid droplet (nLD)" although exactly how their precise biochemical and structural properties relate to those of the "canonical" LDs in the cytoplasm remains to be established. We will discuss possible pathways for nLD formation and speculate on their functions in nuclear physiology.

Fat nuclei: LDs in the nucleus

The nuclear envelope forms a barrier that separates the cytoplasm from the nucleus and consists of two closely apposed phospholipid bilayers (Fig.1); the outer nuclear membrane (ONM) is continuous with the ER and participates in the normal ER functions such as protein secretion, while the inner nuclear membrane (INM) faces the nucleoplasm and is involved in multiple nuclear functions such as chromatin anchoring and DNA repair [5]. The two membranes are connected at the highly curved membrane domains of the nuclear pore complexes (NPCs).

The presence of LDs in the nucleus was reported in a number of rodent and human hepatocyte-derived cell types [3,6,7]. Hepatocyte nLDs were later found to associate with two nuclear structures: (a) premyelocytic leukemia (PML) bodies, a membrane-less body that is involved in many nuclear functions; and (b) the type I nucleoplasmic reticulum, a specific type of invagination of the nuclear envelope, where the INM loses its apposition with the ONM and extends deep inside the nuclear interior [4]; in contrast, the type II nucleoplasmic reticulum, which is formed by the invagination of both the ONM and INM, does not appear to associate with nLDs [4,8]. Imaging analyses suggest that nLDs originate from a very low-density lipoproprotein (VLDL) precursor found within the lumen of the type I nucleoplasmic reticulum invaginations [8] (Fig. 1A). Local alterations at the INM that surrounds these luminal LD precursors appear to give rise to nLDs [8]. The molecular mechanisms associated with these changes remain to be established. The abundance of nLDs increases by addition of fatty acid (oleate) and, so far, have not been detected in significant numbers in cell types other than hepatocytes.

Similar to mammals, and with the exception of hepatocytes, wild-type yeast does not seem to naturally generate nLDs under standard growth conditions. However, recent studies have identified LDs in the nucleus of some yeast mutants [9-12]. More specifically, mutants lacking seipin (Sei1), a conserved protein required for LD budding from the ER, or components of the general phospholipid biosynthetic machinery - Ino2, Ino4 and Cds1 - display nLDs. In the latter case, striking electron micrographs document the formation of large LDs that accumulate between the two leaflets of the INM [12]. Membrane bridges connecting the INM to nLDs can be also seen [12]. nLDs have been also detected in wild-type yeast cells cultivated in oleic acid [11,12]. The presence of nLDs in this genetically tractable organism may provide a useful platform to understand the molecular mechanisms underlying the generation and function of nLDs (Fig. 1B).

LD biogenesis at the INM

LD biogenesis requires both enzymes that generate neutral lipids and machinery to efficiently pack them in the core of LDs. Therefore, to investigate the nature of nLDs, we first need to ask: (a) if the acyltransferases catalysing the final step in the synthesis of neutral lipids are present and active at the INM; (b) whether the INM possess the lipid and protein machinery required for LD budding into the nucleus; and (c) whether mature and functional LDs can be accommodated within the nucleus.

(a) Eukaryotes synthesize TG and steryl esters via dedicated integral membrane acyltransferases which normally reside and act at the ER [13]. If the size of their extraluminal domains can be accommodated through the pore channel, ER membrane proteins can reach the INM by diffusion through the nuclear pore membrane; according to this "diffusion-retention" model, binding/assembly to nuclear factors will retain integral membrane proteins to the INM while others will diffuse back out to the ONM/ER [14]. In yeast, a second pathway for the active, karyopherin-mediated, import of INM proteins has been also described [15]. Most neutral lipid acyltransferases in yeast and mammals have sizes that would allow them to access the INM based on the diffusion-retention model. Consistently two acyltransferases, DGAT2 in hepatocytes and Lro1 in yeast, have been shown to localize at the INM [4,16]. Notably, the enzyme that catalyzes the penultimate step in TG synthesis, phosphatidate phosphatase (PAP, also known as lipin) and which lacks a membrane anchor, has been also shown to translocate in the nucleus in several model organisms [17]. Whether lipin and TG acyltransferase activity are coupled at the INM, as one would expect if TG is *de novo* made there, is currently not known.

(b) The question of how budding of nascent LDs at the INM is regulated is more challenging to address. It is generally accepted that LD formation is initiated by accumulation of neutral lipids within the two leaflets of the ER membrane [1,2]. Recent models propose that cytosolic LD emergence, which predominates in most cell types, is influenced by the ER membrane phospholipid composition, by either controlling intrinsic curvature [18] or surface density of phospholipids [19] at sites of LD biogenesis. However, the phospholipid nature of the INM remains largely unknown. Whether phospholipids made at the ONM/ER can diffuse via the pore membrane and freely equilibrate to the INM, or the INM can generate its own distinct lipid composition, for example by import of enzymes such as lipin, remains unknown. It is therefore difficult to predict whether a hypothetical neutral lipid "lens" at the INM would behave in a manner that is similar to the ONM/ER. Notably, fluorescent reporters document an asymmetry in the levels of a key regulator of LD biogenesis, diacylglycerol, between INM and ONM/ER, consistent with the possibility that LD emergence is differentially regulated on the two sides of the nuclear envelope [12]. Moreover, although synthesis and packing of neutral lipids appear highly coordinated events on the ER membrane, it is possible that under certain circumstances, these two processes are spatially segregated. It is interesting to note that nLDs in hepatocytes originate from a luminal ER precursor which is then "internalized" into the nucleus [8]; and, reciprocally, TG generated by the acyltransferase Lro1 at the INM is stored predominantly in LDs bound to the ONM, even when yeast cells are engineered to rely on Lro1 which is constitutively targeted at the INM [16]. This last observation suggests that synthesis of neutral lipids at the INM in yeast is not enough to drive efficient LD formation at

this compartment, at least under conditions where these LDs must be consumed to ensure survival in stationary phase.

(c) The organization of the INM with the perinuclear chromatin and lamina anchoring may pose additional restrictions to nLDs once these are formed; lamins form a filamentous meshwork that coats the surface of the INM in animal cells and provide mechanical stability to the nucleus [20]. How nLDs would emerge, and become accessible, from the rigid lamina scaffold is not known. Of note, INM subdomains that concentrate nLDs display local lamin deficiency, supporting the hypothesis of a confined remodelling event to allow nLD budding, although the molecular details of this process remain unknown [4,8].

In summary, the enzymatic machinery that makes neutral lipids is likely to be present at the INM. The pathways governing the packing of neutral lipids into LDs and their emergence on the nuclear side will be determined by the specific phospholipid composition and protein requirements of the INM. These represent fertile areas for future research in the field.

Exploring the biology of LDs in the nucleus

The main function of LDs is the transient storage of fatty acids - and other lipid precursors - to prevent lipotoxicity and provide a source of lipids that can be promptly mobilized for energy production or membrane synthesis. The presence of LDs in the cytoplasm enables their interaction with the other cytoplasmic organelles that catabolize the mobilized fatty acids, namely mitochondria/peroxisomes through beta oxidation and the vacuole/lysosome through lipophagy [21]. The fact that LDs in the nucleus do not have direct access to these organelles raises the possibility that that the primary role of nLDs is not linked to energy storage. Although there is currently little information on the physiological relevance of nLDs, recent studies have provided intriguing clues.

In yeast, under conditions of nLD formation, Opi1 – a transcriptional regulator that controls the expression of several genes encoding phospholipid biosynthetic enzymes– is sequestered on the surface of nLDs [12]. It was proposed that this recruitment could reduce its transcriptional repressor activity and consequently de-repress phospholipid synthesis [12]. The distribution and activity of other nuclear proteins may be also influenced by their recruitment on nLDs, particularly those containing amphipathic helices which are well known to target the phospholipid LD monolayer [22]. CCTα is a soluble enzyme that catalyses the rate-limiting step for the synthesis of phosphatidylcholine (PC). In response to the need for more PC, CCTα is activated by recruitment at the INM via its amphipathic helix to generate the penultimate intermediate in PC synthesis [23-25]. Recently it was reported that in hepatic cell lines and primary mouse hepatocytes, CCTα associates with nLDs [4,8]. The association of CCTα with nLDs correlated with PC synthesis leading to the model that, in liver, nLDs are the major site of CCTα activation and PC synthesis [8]. The reversible association of Opi1 and CCTα with the surface of nLDs could provide the means to regulate their intranuclear functions, in yeast and mammalian cells, respectively. Indeed, examples of the surface of LDs acting as platforms for protein regulation have been previously described, such as the case of histone storage in the Drosophila embryo [26] or the presence of damaged ER proteins on LDs that are cleared by lipophagy in yeast [27]. However, important questions are also raised: the reason behind the discrepancy on the site of CCTα activation – INM vs nLDs – remains

unclear; and whether physiological signals control the absorption of Opi1, or other transcriptional regulators, on nLDs, will need further investigation.

LDs are depots for lipid building blocks used for membrane synthesis. Indeed, TG-mobilized fatty acids and diacylglycerol are important for phospholipid synthesis and bud growth during growth resumption in yeast [28,29]. Therefore, nLDs could store locally, and provide when necessary, fatty acids and other lipid precursors for nuclear membrane growth. The machinery for this appears to be present in the yeast nucleus: lipases required for the mobilization of neutral lipids, are found on nLDs [12]; and the diacylglycerol kinase Dgk1, which converts TG-derived diacylglycerol to membrane phospholipids is also present at the INM [12]. However, whether these enzymes associate with, and are active at, the INM under physiological conditions of nuclear growth is unclear. It should be noted that in recent years several nuclear envelope remodelling events that contribute to both normal nuclear homeostasis and disease, have been described. These include nuclear envelope rupture and repair; nuclear pore complex biogenesis; various nucleophagic pathways; and viral nuclear egress pathways [30]. It is likely that extensive nuclear envelope shape changes in these cases are coordinated with local modification of phospholipid composition. In such a scenario, nLDs could provide a local INM pool of lipids for these remodelling events.

Outlook

The surprising discovery that LDs can populate nuclei raises fundamental questions on both the nuclear and LD fields. How widespread are LDs inside the nuclei of different cell types? Could lesser accumulation of neutral lipids, which are not readily detectable by optical microscopy, be more common than expected at the INM? On the other hand, the observation that LDs can arise inside the nucleus from the perinuclear ER lumen, as in hepatocytes, raises the question of directionality and movement of LDs between compartments. The fact that LD-stored lipid intermediates are normally destined for use in cytoplasmic membranes and organelles raises the question of the relevance of their intranuclear location. In addition to possible roles in nuclear protein regulation and nuclear membrane biogenesis or remodelling, nLDs may also participate in nuclear lipid signalling [31] and nuclear receptor function which is regulated by lipids [32]. Addressing these questions is not straightforward as it will require the specific ablation of nLDs without affecting their cytoplasmic, and more abundant, counterparts. Ultimately, many of the questions relating to nLD formation and function are inextricably linked to the nature and specific properties of INM. Similar to the recent advances in the identification and characterization of INM proteome, developing tools to study the INM lipidome may prove critical in order to elucidate the biogenesis and function of nLDs.

Acknowledgments

Previous work from our group referred to in this Viewpoint article was supported by the Wellcome Trust (108042) and the MRC (G0701446).

Author contributions

ADB and SS jointly wrote the article.

Conflict of interest

The authors declare no conflict of interest.

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Figure legends

Figure 1. Schematic depicting proposed models on the formation and possible roles of nuclear lipid droplets. (A) In hepatocytes, an endoplasmic reticulum luminal precursor gives rise to nuclear lipid droplets by remodelling of an inner nuclear membrane extension; (B) in budding yeast, mutants in phospholipid metabolism, or wild-type cells in the presence of oleate, develop nuclear lipid droplets at the inner nuclear membrane. Out of the two yeast triacylglycerol acyltransferases, Lro1 is present at the inner nuclear membrane, but the triacylglycerol produced is predominantly packed in lipid droplets facing the outer nuclear membrane; currently it has not been shown whether the second acyltransferase, Dga1, is active at the inner nuclear membrane. In both yeast and mammalian systems, nuclear lipid droplets have been shown to recruit nuclear proteins, possibly influencing the distribution of their active pools. The role of the inner nuclear membrane phospholipid composition (in red) or lamina scaffold assembly, in nuclear lipid droplet budding remains unknown. For more details, please see the text.

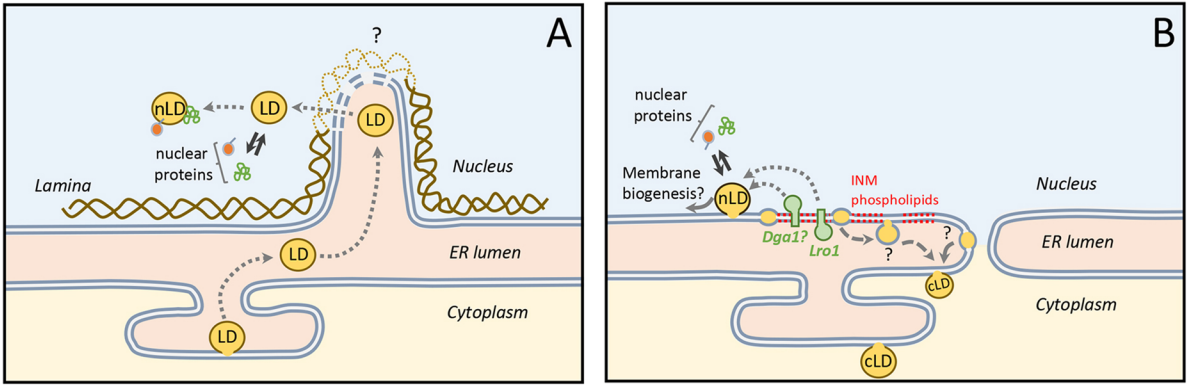
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Figure 1