Storing fat inside the nucleus

A specific pathway for nuclear lipid droplet formation

The nucleus could be called “the brain” of our cells. And therefore it is not surprising that the nucleus is regarded as the most important and most complex element in our cells. In eukaryotic cells, the nucleus serves as a depositary for the genome, providing a spatial and temporal separation of transcription and translation (*1*). This segregation function is fulfilled by the nuclear envelope (NE). The NE not only serves as a compartment boundary to protect the genome but also as a specific interface at which processes between the nucleus and the cytoplasm are coordinated (*2*). The NE is remarkably dynamic. It expands during growth, disassembles and reforms during mitosis, deforms in response to mechanical stress, and changes during differentiation, aging and diseases (*2*, *3*). Therefore, the complex nature of the nucleus is ultimately related to the complexity of the NE.

During the last decades, scientists have studied various chromatin-centered processes inside the nucleus and the proteins of the NE. But what do we know about the NE beyond its resident proteins? What do we actually know about the NE lipids? Although lipids are fundamental building blocks of all cells, the nature of lipids and a lack of techniques comparable to protein analysis explains our gap of knowledge about the NE (*4*). In addition, the complex organization of the NE is often forgotten. The NE is actually composed of two membranes: an outer nuclear membrane (ONM) and an inner nuclear membrane (INM), which are fused together at sites where the nuclear pore complexes are embedded (*5*, *6*). It is believed that the protein composition of the endoplasmic reticulum (ER), the ONM and the INM differs (*7*). However, less is known about the lipid composition. Seen from the cytoplasm, the INM is a remote territory of the ER, both in distance and difficulty to access. To get there, any lipid or protein must pass through the nuclear pores. The ER and the ONM are highly active in converting nutrients into building blocks for lipids and membranes. In contrast, the INM passively receives its lipids from the ER via the nuclear pore route. In this view, the INM was thought to be metabolically silent and to resemble the ER and the ONM in its lipid composition (*7*, *8*). For my PhD thesis research, together with my

supervisor Prof. Alwin Koehler, I chose to challenge this view by developing tools that would allow us to "see" lipids inside the nucleus.

Since the INM and ONM are only 10–50 nm apart (*9*), biochemical fractionation does not separate them into pure fractions that would be suitable for lipidomics. Therefore, it is also challenging to monitor whether and how the INM responds to changes in cellular lipid metabolism. To overcome these limitations, I established *in vivo* lipid sensors to examine specific precursor lipid species - phosphatidic acid (PA) and diacylglycerol (DAG) (Fig. 1A) at the INM using fluorescence microscopy.

Having established robust lipid sensors for the INM, I examined whether and how they respond to changes in lipid environment. To induce cellular lipid storage, I blocked the phospholipid synthesis pathway by cultivating a temperature-sensitive mutant of Cds1 (*cds1-ts*) at the restrictive temperature. Cds1 converts PA into cytidine diphosphate diacylglycerol (CDP-DAG) to promote new phospholipid synthesis (Fig. 1A). Therefore, the inhibition of Cds1 is expected to increase PA levels and channel PA into the branch of neutral lipid triacylglycerol (TAG) storage (*11*). Surprisingly, we not only observed an increase in PA levels at the INM but also formation of lipid droplets (LDs) inside the nucleus (Fig. 1B), which are organelles for neutral lipid storage that are thought to emerge only from the ER. Moreover, wild-type cells also possess a natural capacity to generate nuclear LDs (nLDs) when grown in fatty acid-rich media. Our data therefore suggest a specific pathway for nLD generation in yeast (*10*).

Taking advantage of electron tomography, we demonstrated that nLDs are connected to the INM (Fig. 1C-H) by Seipin-dependent bridges (*10*). Seipin is an LD biogenesis factor and mutations in the human seipin lead to a severe form of congenital lipodystrophy, a condition characterized by a drastic loss of body fat (*12*). Moreover, I showed that Pah1, the yeast ortholog of human lipin, is found at the INM and deletion of Pah1 affects lipid composition of the INM (*10*). It is known that mutations in lipin cause lipodystrophy in mice. These findings of Seipin and Pah1 at the INM raise the question about their role at the INM in the pathogenesis of human metabolic diseases.

How do these fat-storing organelles affect processes in the nucleus? In contrast to cytoplasmic LDs, nuclear LDs possess a unique location in close

proximity to chromatin. We demonstrated that nLDs have a functional significance: they affect transcriptional regulation of lipid synthesis genes by sequestering Opi1 (*10*) which is a master regulator of glycerophospholipid synthesis (*11*). Therefore, in the future it can be studied to which extent nLDs can impact global gene regulation and whether nLDs can function as scaffolds for organizing the 3D architecture of the genome through dynamic interactions with transcription factors and genes.

The unexpected finding that the INM is able to generate nLDs led us to investigate how cells decide when and where to make LDs across the NE. I discovered that an increased load of unsaturated fatty acids induces their sequestration in cytoplasmic LDs, whereas nLD production at the INM is suppressed. Using lipid saturation biosensors, transcriptomic and lipidomic analysis I could show that the underlying mechanism of decision making at the NE involves partitioning of PA between the INM and the ONM. These results establish lipid saturation as a regulator of lipid storage metabolism at the NE contributing to the topological decision where LDs are made (*13*).

My findings have launched a new field of nuclear biology. The INM is not merely a remote province of the ER, spatially isolated and dependent on lipid supplies. Instead, we have identified the INM as a territory with its own lipid metabolism and striking metabolic adaptability that our work has now moved into focus. Continuing to decipher the lipid component of the NE in the future will allow us to explain key functional features of the NE, including the durability-plasticity dichotomy, demands on the membrane for nuclear pore insertion and the regulation of chromatin at the nuclear periphery and to investigate how perturbed nuclear lipid metabolism is linked to human diseases.

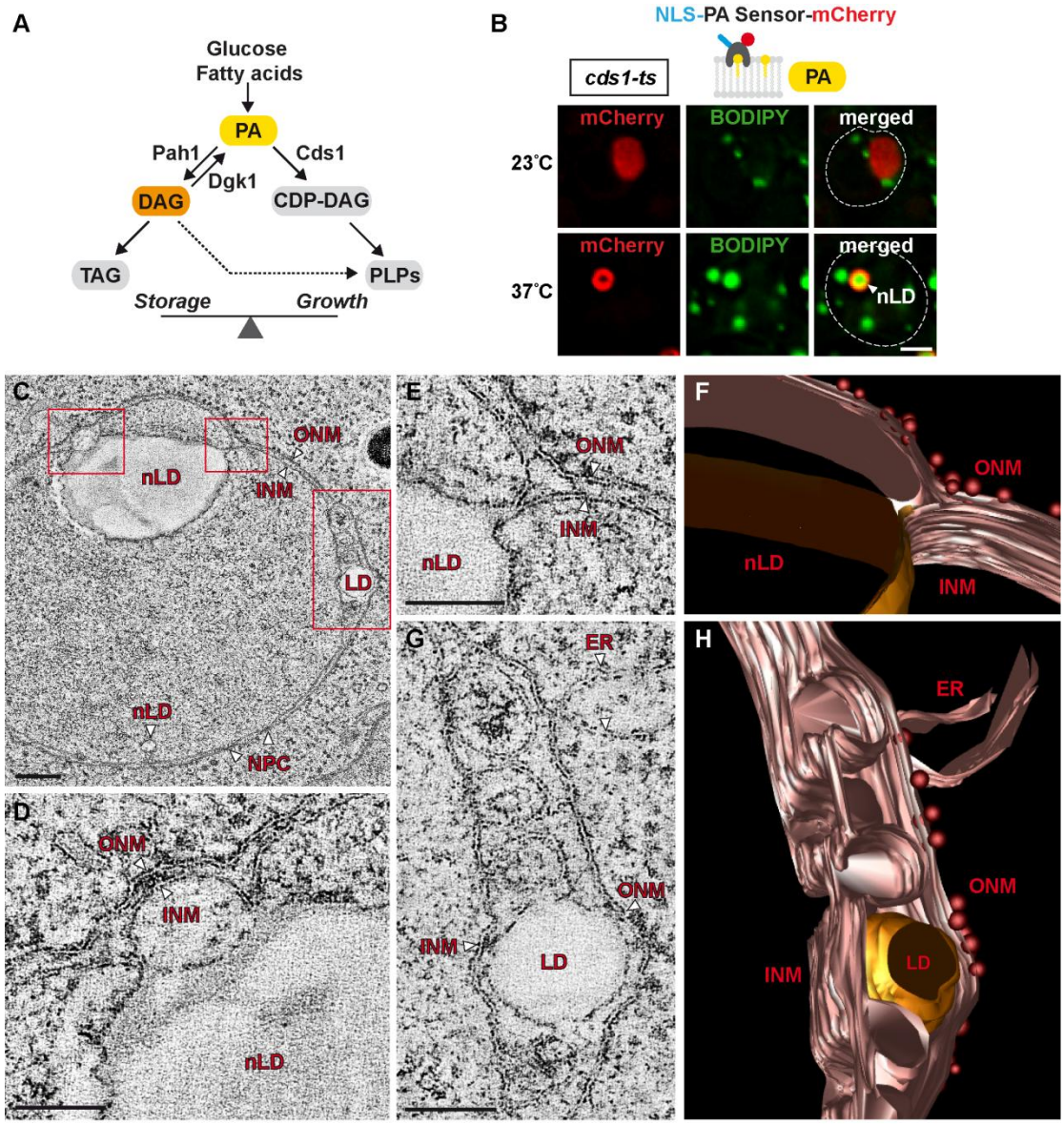

Fig. 1. Nuclear lipid droplets are generated directly from the INM. (**A**) Simplified cartoon of yeast lipid biosynthesis depicting the two major branches leading to synthesis of phospholipids (PLPs) (Growth) or triacylglycerol (TAG) (Storage). Phosphatidic acid (PA) is a central precursor. The Kennedy pathway (dashed line) channels diacylglycerol (DAG) into PLP production. Cytidine diphosphate diacylglycerol, CDP-DAG. (**B**) Live imaging of *cds1-ts* cells expressing the NLS-PA-mCherry sensor grown at the indicated temperatures for 4 hours. BODIPY stains lipid droplets. Nuclear lipid droplet, nLD. Scale bar: 2 μm. (**C-H**) Transmission electron microscopy (TEM) and 3D reconstruction of the nuclear envelope in nuclear lipid droplet (nLD) producing cells. (**D**), (**E**) correspond to the boxed areas in the upper part of (**C**) and show INM-nLD membrane bridges. (**G**) shows a magnification of INM evaginations seen on the right side of (**C**). (**F**) and (**H**) are 3D reconstructions of (**E**) and (**G**), respectively. The ONM is studded with ribosomes (red spheres). Scale bar: 200 nm (**C**) or 100 nm for images (**D**), (**E**), (**G**).

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