A novel function of lipid droplets in regulating longevity

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Abstract
Growing evidence supports the view that LDs (lipid droplets) are dynamic organelles that can serve both as an intracellular signalling compartment and as an organizing platform orchestrating many vital processes in eukaryotic cells. It has become clear that the LDs-confined deposition and lipolytic degradation of neutral lipids define longevity in multicellular eukaryotic organisms and yeast. We summarize the evidence in support of the essential role that LDs play in longevity regulation and propose several molecular mechanisms by which these dynamic organellar compartments control the aging process in multicellular eukaryotes and yeast.

Introduction
Found in most eukaryotic and some prokaryotic cells, LDs (lipid droplets) have also been referred to as lipid bodies, lipid particles, oil bodies and by many other different names that reflect their cellular and biochemical context in various biological systems [1,2]. LDs have long been viewed as relatively inert organellar compartments [3,4]. They were believed to function only as a site for storing excess energy and stockpiling fatty acids and sterols in the form of neutral lipids, mainly triacylglycerols and steryl esters [1,5,6]. Surrounded by a monolayer of phospholipids and associated proteins, the neutral lipid core of LDs can be hydrolysed in a regulated fashion by lipases [7,8]. The resulting retrieval of stored NEFAs [non-esterified (‘free’) fatty acids], phospholipids and sterols provides energy via fatty acid oxidation during times of nutrient scarcity, maintains the homoeostasis of membrane lipids during cell growth and division and modulates the levels of free sterols inside and outside of the cell [1–8].

Novel functions of LDs
An important recent advance in our understanding of the cell biology of LDs is that they constitute highly dynamic organellar compartments whose protein and lipid composition, de novo formation from the ER (endoplasmic reticulum) template, growth, fragmentation, shrinkage due to lipolysis, movement and association with other organelles is regulated by a complex network of protein machines [4–6,8–19]. It has also become clear that LDs are dynamically integrated into several vital processes and can serve both as an intracellular signalling compartment and as an organizing platform orchestrating these processes. In yeast, the phosphorylation and activation of the LDs-associated lipase Tgl4p by CDK (cyclin-dependent kinase) 1 controls cell-cycle progression by mobilizing triacylglycerols, thereby providing fatty acids and phospholipids for the secretory vesicle-dependent bud formation and ultimately enabling the G1/S phase transition [20]. Furthermore, it appears that LDs in WAT (white adipose tissue) of mice function as a hub in a regulatory network that, by modulating the synthesis and secretion of adipokines and the lipokine C16:1n7-palmitoleate, is central to the multiple defects in metabolic homoeostasis associated with obesity, insulin resistance, Type 2 diabetes, atherosclerosis and inflammatory disorders [12,14,21–25]. Moreover, by recruiting certain proteins from other cellular locations, temporally housing them, managing their availability and modulating their activities, LDs have been proposed to play important roles in the regulation of various cellular processes [4,14,18]. It has been suggested that, by sequestering proteins, LDs function as an organizing platform that (i) removes excess proteins from other organellar compartments, inactivates them and/or stores them for later use; (ii) promotes the refolding of unfolded proteins targeted to LDs by recruiting molecular chaperones from the cytosol; (iii) provides a surface for the ordered degradation of partially unfolded and misfolded LD-bound proteins that otherwise can form toxic aggregates in the cytosol; and (iv) delivers some LD-associated proteins to their target organellar compartments via permanent or transient contact sites [4,14,18,26–28].

LDs regulate longevity in multicellular eukaryotic organisms
One of the recently discovered new and unexpected functions of LDs is the essential role they play in longevity regulation [21,29–35]. It appears that various interventions that inhibit the deposition of neutral lipids in LDs or activate their lipolytic degradation in fat storage tissues of the nematode Caenorhabditis elegans, the fruitfly Drosophila melanogaster or laboratory mice also extend the lifespans of these organisms. In fact, at present unknown humoral signals produced
and secreted by somatic cells in the gonad of C. elegans promote the nuclear localization of the forkhead transcription factor DAF-16 (decay-accelerating factor-16) in intestinal cells, the principal site of neutral lipid storage in this organism [29]. DAF-16 then induces the expression of a gene encoding a specific lipase, thereby promoting the lipolysis of neutral lipids stored in LDs of intestinal cells and extending longevity by a yet-to-be-established mechanism [29]. Furthermore, the elimination of LD-associated lipases Brummer or ATGL (adipose triacylglycerol lipase) in the fat body of flies or WAT of mice respectively shortens lifespan [30,31]. Moreover, by greatly diminishing the levels of stored neutral lipids in WAT, both the elimination of WAT-specific insulin receptor and the replacement of the C/EBPα (CCAAT/enhancer-binding protein α) protein with its parologue C/EBPβ extend longevity in mice [32–34]. Importantly, a low-calorie dietary regimen known as CR (calorie restriction) extends lifespan in a wide spectrum of organisms and delays the onset of age-related diseases in rodent models [21]. The life-extending effect of CR in worms and flies is mediated by the so-called sirtuins, a family of NAD+-dependent protein deacetylases and ADP ribosylases [21,33]. In mice, CR diet increases the abundance of the sirtuin SIRT1, which has been shown to repress transcription of genes that are required for the LD-confined accumulation of neutral lipids in WAT [21,35]. It has therefore been proposed that SIRT1 delays aging of CR mice by shifting the balance between the opposing processes of LD formation and degradation in WAT towards lipolytic degradation of neutral lipids in this fat storage tissue [21,35].

Altogether, these findings imply that the LD-confined deposition and lipolytic degradation of neutral lipids in fat storage tissues define longevity of multicellular eukaryotic organisms. A key challenge for the future will be to establish the molecular mechanisms by which a delicate balance between the opposing processes of neutral lipids accumulation and their lipolytic degradation regulates longevity in these organisms. Recent studies suggested three such mechanisms (Figure 1). First, the size of LDs in WAT of mice modulates the ability of the ER in this tissue to synthesize and send for secretion various antihyperglycaemic and pro-hyperglycaemic protein hormones that control metabolic homeostasis in other tissues [21,22,36,37]. In the intestine of worms, LDs that accumulate in this fat storage tissue contribute sterols for the biosynthesis in the ER and the subsequent secretion of the lipophilic hormones dafachronic acid and pregnenolone, both of which delay cellular aging in other tissues [37]. Thus it is conceivable that the dynamics of LD-confined deposition and lipolytic degradation of neutral lipids in fat storage tissues modulates their ability to synthesize in the ER and then secrete humoral signals that control longevity-related processes in other tissues (Figure 1A). Secondly, the lipolysis of LD-deposited neutral lipids results in the release of the lipokine C16:1n7-palmitoleate, an unsaturated fatty acid, from WAT of mice [24]. This recently discovered lipid hormone regulates systemic carbohydrate and lipid homeostasis in muscle and liver by promoting muscle insulin-sensitivity and attenuating hepatic steatosis [24]. Hence, the LD-derived C16:1n7-palmitoleate produced in and then secreted by WAT of mice could function as a signalling molecule that plays an important role in longevity regulation (Figure 1B). Thirdly, in response to enhanced lipolysis of LD-deposited neutral lipids, WAT of mice and the fat body of flies activate certain regulatory networks that stimulate mitochondrial biogenesis and fatty acid β-oxidation in mitochondria [25,34,38]. This, in turn, decreases the levels of saturated fatty acids secreted by these fat storage tissues, thereby reducing the risk of lipotoxicity in other tissues and preventing muscle insulin resistance and hepatic steatosis [23–25,39]. Importantly, such a stimulation of mitochondrial biogenesis and fatty acid β-oxidation in WAT of mice expressing the β-parologue of C/EBP increases their lifespan [34]. Therefore it is likely that this third mechanism, which is confined to fat storage tissues, plays an important role in delaying cellular aging in peripheral tissues and increasing longevity of the entire organism (Figure 1C).

**LDs play an essential role in longevity regulation in yeast**

Recent findings suggest that LDs play an essential role in longevity regulation not only in multicellular eukaryotic organisms but also in yeast [9,40–45]. The budding yeast *Saccharomyces cerevisiae*, a genetically and biochemically manipulatable unicellular eukaryote with annotated genome, is an advantageous model for understanding the molecular mechanisms of cellular aging in multicellular eukaryotes [40]. There are two different ways to monitor yeast aging. Yeast replicative aging is defined by the maximum number of daughter cells that a mother cell can produce before senescence and mimics aging of mitotically active mammalian cells [41]. By contrast, chronological aging is measured by the length of time a yeast cell remains viable in a non-dividing state and mimics aging of postmitotic mammalian cells [42]. Both the replicative and chronological aging of yeast can be slowed down by CR [40,43], a low-calorie diet that extends lifespan in various multicellular eukaryotic organisms and delays the onset or reduces the incidence of many age-related diseases in rodent models [21]. A CR diet can be imposed in yeast by reducing the glucose concentration from 2% to 0.5% in growth medium [40]. Chronologically aging non-CR yeast grown on 2% glucose (but not CR yeast grown on 0.5% glucose) accumulate ethanol, a product of glucose fermentation [40,43]. Importantly, ethanol modulates longevity in chronologically aging yeast by a yet-to-be-characterized mechanism [43]. Of note, ethanol in yeast cells suppresses the synthesis of certain proteins localized to peroxisomes [44]. Thus it is tempting to speculate that the accumulation of ethanol in non-CR yeast represses the synthesis of Fox1p, Fox2p and Fox3p, all of which are the core enzymes of fatty acid β-oxidation in peroxisomes [45]. We therefore propose that, because of the resulting low efficiency of fatty acid oxidation in peroxisomes of prematurely aging non-CR yeast, they accumulate NEFAs.
Figure 1 | Possible mechanisms by which the deposition and lipolytic degradation of neutral lipids in LDs of fat storage tissues define longevity of multicellular eukaryotic organisms

(A) The size of LDs in WAT of mice modulates the ability of the ER in this tissue to synthesize and send for secretion various pro- and anti-aging adipokines that control longevity-related processes in other tissues. Overloading LDs in WAT with neutral lipids results in the recruitment of macrophages to this fat storage tissue. Macrophages that infiltrate WAT secrete some of the pro-aging adipokines, whereas adipocytes of WAT secrete others. In the intestine of worms, LDs that accumulate in this fat storage tissue contribute sterols for the biosynthesis in the ER and the subsequent secretion of the lipophilic hormones dafachronic acid and pregnenolone, both of which delay cellular aging in other tissues. (B) The lipolysis of LD-deposited neutral lipids results in the release of the lipokine C16:1n7-palmitoleate, an unsaturated fatty acid, from WAT of mice. This LD-derived lipid hormone regulates longevity-related processes in muscle and liver. (C) In response to enhanced lipolysis of LD-deposited neutral lipids, adipocytes in WAT of mice increase the level of the G-protein α stimulatory subunit (Gαs), whereas cells in the fat body of flies activate transcription of numerous genes positively regulated by the HNF4 (hepatocyte nuclear factor 4). This, in turn, stimulates mitochondrial biogenesis and β-oxidation of NEFAs in mitochondria, thereby decreasing the levels of saturated NEFAs secreted by these fat storage tissues. Saturated NEFAs are lipotoxic in peripheral tissues and cause muscle insulin resistance and hepatic steatosis. Thus the stimulation of mitochondrial biogenesis and NEFA β-oxidation in fat storage tissues displaying enhanced lipolysis of neutral lipids plays an important role in delaying cellular aging in peripheral tissues. Abbreviation: Ac-CoA, acetyl-CoA.
Figure 2 | A possible mechanism linking longevity and lipid dynamics in the ER, LDs and peroxisomes of yeast placed on a calorie-rich diet

LDs in yeast cells function as a hub in a regulatory network that modulates neutral lipids synthesis in the ER and fatty acid oxidation in peroxisomes. The LD-peroxisome association leads to peroxisome invasion into the lipid core of LDs, thereby generating the so-called pexopodum. Ethanol accumulated in yeast placed on a calorie-rich diet represses the synthesis of Fox1p, Fox2p and Fox3p, thereby suppressing peroxisomal oxidation of NEFAs (‘FFA’) that originate from triacylglycerols (TAG) synthesized in the ER and deposited within LDs. The resulting build-up of arrays of NEFAs (gnarls) within LDs of these yeasts initiates several negative feedback loops regulating the metabolism of TAG. Due to the action of these negative feedback loops, chronologically aging yeast placed on a calorie-rich diet not only amass TAG in LDs but also accumulate DAG and NEFAs in the ER. The resulting remodelling of lipid dynamics in these yeasts shortens their lifespan by causing their premature death due to the following: (i) necrosis triggered by the inability of their peroxisomes to oxidize NEFAs; (ii) lipoapoptosis initiated in response to the accumulation of DAG and NEFAs; and (iii) a DAG-induced reorganization of the PKC-dependent signal transduction network affecting multiple stress response- and longevity-related processes. Abbreviation: FFA-CoA, CoA esters of fatty acids.

It should be emphasized that fatty acid β-oxidation in yeast peroxisomes is facilitated by their physical association with LDs. The extensive physical contact existing between peroxisomes and LDs stimulates the lipolysis of neutral lipids within LDs, thereby initiating the conversion of newly formed NEFAs into their CoA esters that then get imported and oxidized by peroxisomes [9]. The LD-peroxisome association can lead to peroxisome invasion into the lipid core of LDs, thereby generating the so-called pexopodum that exist as individual peroxisomes or as their extensions penetrating the core of LDs [9]. Importantly, pexopodia of yeast mutants that were unable to oxidize NEFAs due to the absence of Fox1p, Fox2p or Fox3p caused the accumulation of the so-called gnarls within the core of LDs [9]. Gnarls represent electron-dense arrays of NEFAs accumulated in excessive amounts within LDs of fox1Δ, fox2Δ and fox3Δ mutants [9]. In addition, lack of Fox1p, Fox2p or Fox3p resulted in the build-up of triacylglycerols within the core of LDs [9]. By extending these observations to lipid dynamics in LDs and peroxisomes of chronologically aging non-CR yeast, one could expect that the accumulation of ethanol in non-CR yeast and the resulting repression of the synthesis of Fox1p, Fox2p and Fox3p cause the build-up of arrays of NEFAs (gnarls) and triacylglycerols within the core of their LDs.

Taken together, these findings suggest a mechanism linking yeast longevity and lipid dynamics in the ER, LDs and peroxisomes (Figure 2). In this mechanism, LDs in yeast cells function as a hub in a regulatory network that modulates neutral lipids synthesis in the ER and fatty acid oxidation in peroxisomes. We hypothesize that ethanol accumulated in yeast placed on a calorie-rich diet represses the synthesis of Fox1p, Fox2p and Fox3p, thereby suppressing peroxisomal oxidation of NEFAs that originate from triacylglycerols synthesized in the ER and deposited within LDs. In our
hypothesis, the resulting build-up of arrays of NEFAs (gnarls) within LDs of non-CR yeast initiates several negative feedback loops regulating the metabolism of triacylglycerols. Owing to the action of these negative feedback loops, chronologically aging non-CR yeast not only amass triacylglycerols in LDs but also accumulate DAG (diacylglycerol) and NEFAs in the ER (Figure 2). Of note, it has been recently revealed that (i) loss of peroxisome function triggers necrosis [46,47]; (ii) both NEFAs and DAG induce lipoapoptosis (a caspase-and mitochondria-independent form of programmed cell death) [48]; and (iii) the accumulation of DAG triggers a PKC (protein kinase C)-dependent signal transduction network affecting multiple stress response- and longevity-related processes [49,50]. We therefore hypothesize that the proposed remodelling of lipid dynamics in chronologically aging non-CR yeast shortens their lifespan by causing their premature death due to (i) necrosis triggered by the inability of their peroxisomes to oxidize NEFAs; (ii) lipoapoptosis initiated in response to the accumulation of DAG and NEFAs; and (iii) the DAG-induced reorganization of the PKC-dependent signal transduction network affecting multiple longevity-related cellular targets (Figure 2).

Recently, we assessed the effect of CR on the dynamics of age-related changes in the proteomes and lipidomes of ER, LDs and peroxisomes in chronologically aging yeast. We also examined how mutations that extend yeast longevity by impairing lipid metabolism in these organelles influence their morphologies, abundance, association with each other and protein and lipid compositions during chronological aging. Our findings support the proposed model (Figure 2) for a mechanism in which LDs function as a hub in a regulatory network that defines the chronological lifespan of yeast by modulating both neutral lipids synthesis in the ER and fatty acid oxidation in peroxisomes. By orchestrating several negative feedback loops that co-ordinate lipid dynamics in a diet- and genotype-dependent fashion, this network controls age-related necrotic cell death and multiple stress response-related processes. Implementing our understanding of the mechanism linking longevity and age-related dynamics of LDs, we developed a lifespan assay that was used for a high-throughput screening of extensive libraries of small molecules. We identified five groups of novel anti-aging drugs that significantly delay yeast aging by remodelling lipid metabolism in the ER, LDs and peroxisomes and by activating a distinct set of stress response-related processes in mitochondria.

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