[Frontiers In Bioscience, Landmark, 23, 1166-1194, January 1, 2018]

Lipid metabolism and transport define longevity of the yeast *Saccharomyces cerevisiae*

Darya Mitrofanova[1], Pamela Dakik[1], Melissa McAuley[1], Younes Medkour[1], Karamat Mohammad[1], Vladimir I. Titorenko[1]

[1]Department of Biology, Concordia University, Montreal, Quebec H4B 1R6, Canada

TABLE OF CONTENTS

1. ABSTRACT

Emergent evidence indicates that certain aspects of lipid synthesis, degradation and interorganellar transport play essential roles in modulating the pace of cellular aging in the budding yeast *Saccharomyces cerevisiae*. The molecular mechanisms underlying the vital roles of lipid metabolism and transport in defining yeast longevity have begun to emerge. The scope of this review is to critically analyze recent progress in understanding such mechanisms.

2. INTRODUCTION

Lipids are water-insoluble amphiphilic biomolecules; they are structurally diverse and generated by an intricate network of integrated metabolic pathways (1-6). Lipids are known to play key roles in the organization and function of biological membranes, energy homeostasis, signal transduction, vesicular trafficking, organelle biogenesis, and regulated cell death (5-14). The initial indications that lipids may also modulate the rate of cellular and organismal aging came from observations that longevity-extending mutations in the IGF-1 (insulin/insulin-like growth factor 1) and TORC1 (target of rapamycin complex 1) signaling pathways elicit an increase in the concentration of storage lipids in the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster* and laboratory mice (reviewed in reference 15). In this review, we outline the important conceptual advance in our understanding of how lipid metabolism and transport control the pace of cellular aging in the yeast *Saccharomyces cerevisiae*. We critically evaluate several mechanisms underlying the essential roles of lipids in defining yeast longevity. We outline the most important unanswered questions and suggest directions for future research.

3. SOME LIPIDS ARE CRITICAL FOR LONGEVITY OF THE YEAST *SACCHAROMYCES CEREVISIAE*

3.1. Sphingolipids define yeast replicative and chronological lifespans

In *S. cerevisiae*, the *de novo* synthesis of sphingolipids begins in the endoplasmic reticulum (ER) where the serine palmitoyltransferase (SPT) protein complex catalyzes a condensation of serine with palmitoyl-CoA to form 3-ketodihydrosphingosine (Figure 1) (16-18). The activity of SPT in the ER can inhibited by the amino fatty acid antibiotic myriocin derived from certain thermophilic fungi (19, 20). 3-ketodihydrosphingosine is transformed into dihydrosphingosine (DHS), which is then undergoes conversion into phytosphingosine (PHS) in the ER; DHS and PHS are sphingoid backbone bases of all sphingolipids (Figure 1) (17, 21, 22). An acyl-CoA ester of hexacosanoic fatty acid having twenty six carbon atoms is then used as a fatty acid donor for the synthesis of dihydroceramide (dhCer) or phytoceramide (phytoCer) from DHS or PHS (respectively) in a reaction that is catalyzed by ceramide synthase (CerS) and confined to the ER (Figure 1) (22-27). The activity

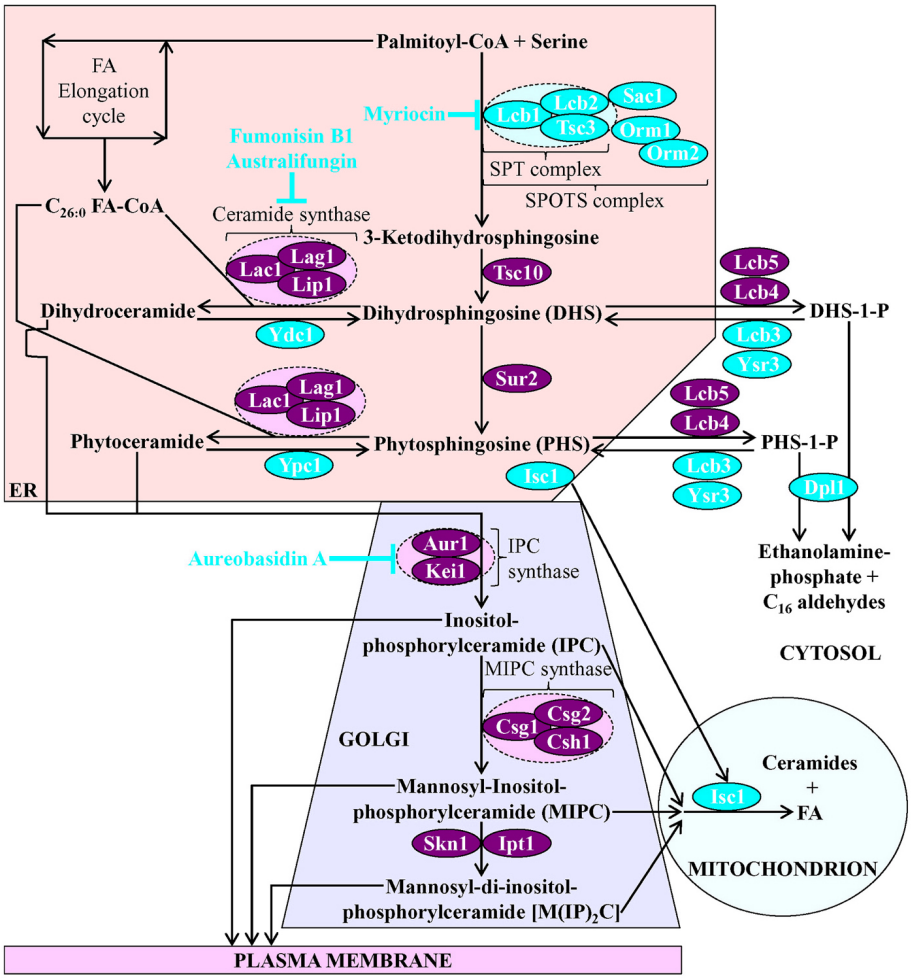

Figure 1. Sphingolipid metabolism in the yeast *Saccharomyces cerevisiae*. 3-ketodihydrosphingosine, dihydrosphingosine and phytosphingosine (DHS and PHS, respectively; two sphingoid backbone bases of all sphingolipids), acyl-CoA esters of very long-chain fatty acids (including the acyl-CoA ester of hexacosanoic fatty acid ($C_{26:0}$ FA-CoA)), as well as dihydroceramide and phytoceramide are all generated in the endoplasmic reticulum (ER). After being transported from the ER to the Golgi apparatus, phytoceramide undergoes conversion into complex sphingolipids, including inositol-phosphorylceramide (IPC), mannosyl-inositol-phosphorylceramide (MIPC) and mannosyl-di-inositol-phosphorylceramide ($M(IP)_2C$). Following IPC, MIPC and $M(IP)_2C$ synthesis in the Golgi, these complex sphingolipids can be either sorted to the plasma membrane or used to regenerate ceramides in a reaction catalyzed by Isc1 in mitochondria. After DHS and PHS are synthesized in the ER, they can undergo phosphorylation in the cytosol. Such phosphorylation yields DHS-1-phosphate and PHS-1-phosphate (respectively), which can be further catabolized into ethanolamine-phosphate and aliphatic aldehydes having sixteen carbon atoms (C_{16} aldehydes). Enzymes that catalyze anabolic or catabolic reactions of sphingolipid metabolism are displayed in green or red color, respectively. See text for more details. Abbreviations: Aur1, aureobasidin A resistance 1; Csg1 and Csg2, calcium-sensitive growth 1 and 2 (respectively); Csh1, CSG1/SUR1 homolog1; Dpl1, dihydrosphingosine phosphate lyase 1; ER, endoplasmic reticulum; FA, fatty acid; Ipt1, inositolphosphotransferase 1; IPC, inositol-phosphorylceramide; Isc1, inositol phosphosphingolipid phospholipase C 1; 3-KDHS, 3-ketodihydrosphingosine; Kei1, Kex2-cleavable protein essential for inositol-phosphorylceramide synthesis 1; Lac1, longevity-assurance gene cognate 1; Lag1, longevity assurance gene 1; Lip1, Lag1/Lac1 interacting protein 1; Lcb1, Lcb2, Lcb3, Lcb 4 and Lcb5, long-chain base proteins 1, 2, 3, 4 and 5 (respectively); MIPC, mannosyl-inositol-phosphorylceramide; $M(IP)_2C$, mannosyl-di-inositol-phosphorylceramide; Npr1, nitrogen permease reactivator 1; Orm1 and Orm2, orosomucoid 1 and 2 (respectively); PM, plasma membrane; Sac1, suppressor of actin 1; SPOTS, serine palmitoyltransferase, Orm1/2 and Sac1; Sit4, suppressor of initiation of transcription 4; Slm1/2, synthetic lethal with Mss4 protein 1 or 2; SPT, serine palmitoyltransferase; Sur2, suppressor 2 of Rvs161 and rvs167 mutations; Tsc3 and Tsc10, temperature-sensitive suppressors of Csg2 mutants 3 and 10; Ydc1, yeast dihydroceramidase 1; Ypc1, yeast phytoceramidase 1; Ysr3, yeast sphingolipid resistance 3.

of the CerS protein complex in the ER can be inhibited by mycotoxins fumonisin B1 and australifungin (28, 29). dhCer or phytoCer are transported from the ER to the Golgi apparatus by the coat protein complex II vesicle-mediated flow as well as by the Nvj2-facilitated transfer via the ER-Golgi membrane contact sites (Figure 1) (30-32). In the Golgi apparatus, a stepwise attachment of different polar groups converts dhCer and phytoCer into such complex sphingolipids as inositol-phosphorylceramide (IPC), mannosyl-inositol-phosphorylceramide (MIPC) and mannosyl-di-inositol-phosphorylceramide ($M(IP)_2C$) (Figure 1) (33, 34). The Aur1/Kei1-dependent synthesis of IPC in the Golgi apparatus can be inhibited by aureobasidin A, an antifungal cyclic depsipeptide antibiotic (33, 35, 36). A vesicular flow delivers these complex sphingolipids

from the Golgi apparatus to the plasma membrane (PM) (Figure 1) (1, 37). After being synthesized in the Golgi apparatus, these complex sphingolipids can also be used to replenish the cellular pool of ceramides. Such replenishment occurs in mitochondria and is catalyzed by Isc1, an inositol phosphosphingolipid phospholipase C which is translocated from the ER to mitochondria during the post-diauxic growth phase (Figure 1) (38-40). Following DHS and PHS synthesis in the ER, these sphingoid backbone bases of sphingolipids can be used not only for ceramide synthesis in the ER but also for phosphorylation in the cytosol (Figure 1) (37, 41). The products of such phosphorylation, DHS-1-phosphate and PHS-1-phosphate (respectively), can be then converted into such non-sphingolipid molecules as ethanolamine-phosphate and aliphatic aldehydes having sixteen carbon atoms (42).

There are two different ways to study aging of the budding yeast *S. cerevisiae*. The first way is to monitor the replicative mode of yeast aging, which is measured as the maximum number of daughter cells that a mother cell can produce before becoming senescent (43-45). It seems that yeast replicative aging mirrors aging of some dividing, mitotically active mammalian and human cells (such as lymphocytes) (43, 44, 46-49) as well as aging of non-dividing cells in certain post-mitotic tissues of laboratory roundworms and humans (49-51). The second way is to examine the chronological mode of yeast aging, which is monitored as the length of time during which a cell remains viable by preserving a reproductive (clonogenic) ability after cell growth and division have been arrested (47, 52-54). Chronological aging in yeast is believed to model aging of non-dividing, post-mitotic mammalian and human cells (such as neurons) (47, 52, 53, 55-59).

Growing evidence supports the notion that certain molecular species of long-chain sphingoid bases of sphingolipids, ceramides and/or more complex sphingolipids (which are formed from ceramides through the covalent attachment of certain polar head groups) may play essential roles in defining the rates of replicative or chronological aging in the yeast *S. cerevisiae*. Specifically, it has been demonstrated that some genetic or pharmacological interventions altering the concentrations of certain sphingolipid classes extend yeast replicative or chronological lifespan (RLS or CLS, respectively).

For example, a single-gene-deletion mutation eliminating the Lag1 subunit of CerS (Figure 1) extends yeast RLS (23) but not CLS (60). Of note, a single-gene-deletion mutation eliminating a different subunit of CerS, Lac1 (Figure 1), does not alter yeast RLS or CLS (61). Two alternative explanations for the essential mechanistic role of Lag1 in yeast RLS have been proposed, namely that 1) lack of Lag1 (but not lack of Lac1) elicits a change in the concentrations of sphingosine and/or ceramide species that are critical for longevity of replicatively aging yeast; or 2) Lag1 determines yeast RLS not because of its distinct effects on sphingosine and/or ceramide concentrations but because of its known physical and functional interactions with many proteins that do not interact with Lac1 and are not related to sphingolipid metabolism (62). The unique role of Lag1 in regulating longevity of replicatively aging yeast is underscored by the observations that both the lack of this subunit of CerS (23) and its moderate overexpression prolong yeast RLS (61), whereas its massive overexpression has an opposite effect on the RLS of *S. cerevisiae* (61). Thus, it is conceivable that the relative level of the Lag1 subunit of CerS exhibits a non-linear dose-response effect on the concentrations of some distinct sphingosine and/or ceramide species with the essential roles in yeast RLS but not in CLS. The identities of such critical species of sphingosine (DHS and/or PHS) and/or ceramide (dhCer and/or phytoCer) remain to be established, perhaps by measuring the concentrations of different sphingolipid metabolism intermediates in yeast mutant strains that exhibit a wide-range expression levels of Lag1 and have quite different RLS.

Single-gene-deletion mutations eliminating Ipt1 and/or Skn1, two inositolphosphotransferases involved in the synthesis of $M(IP)_2C$ from MIPC (63, 64) (Figure 1), have been shown to extend yeast CLS (60, 65). Each of these mutations causes an accumulation of excessive amounts of MIPC and impairs the synthesis of $M(IP)_2C$, the most abundant and complex sphingolipid in *S. cerevisiae* (63, 64). These findings suggest that $M(IP)_2C$ may be an essential negative regulator of yeast CLS and/or MIPC may play an essential stimulatory role in regulating yeast CLS.

A single-gene-deletion mutation eliminating Isc1, an inositol phosphosphingolipid phospholipase C which hydrolyzes complex sphingolipids to produce ceramides (38-40) (Figure 1), has been shown to shorten yeast CLS (66). The most prominent effects of the *isc1Δ* mutation on cellular sphingolipids include a decline in the concentrations of different molecular species of DHS and a rise in the concentrations of dhCer and phytoCer having twenty six carbon atoms (67). The CLS-shortening effect of the *isc1Δ* mutation was likely due to its demonstrated abilities to lower mitochondrial respiration, diminish catalase A activity, stimulate cellular iron accumulation, intensify oxidative damage to cellular macromolecules, decrease cell resistance to oxidative stress, enhance programmed apoptotic cell death, and/or activate Hog1 (a mitogen activated protein kinase (MAPK) of the high osmotic glycerol (HOG) pathway) (66-68). Importantly, the abilities of the *isc1Δ* mutation to shorten yeast CLS, lessen mitochondrial respiration, weaken catalase

A activity and decline cell resistance to oxidative stress can be partially suppressed by a single-gene-deletion mutation that eliminates any of the following four proteins: 1) Sit4, a catalytic subunit of type 2A ceramide-activated protein phosphatase and a downstream effector in the TORC1-Sit4 branch of the nutrient and stress signaling TORC1 pathway (67); 2) Hog1 (68); 3) Tor1, a nutrient-sensing protein kinase component of TORC1, which regulates cell growth, metabolism, stress response and longevity in response to nutrient availability and cellular stresses (69); or 4) Sch9, a nutrient-sensing protein kinase and a downstream effector in the TORC1-Sch9 branch of the TORC1 pathway (69). It needs to be emphasized that both the TORC1-Sit4 and TORC1-Sch9 branches are modulated not only in response to nutrient availability but also in response to concentrations of certain sphingolipid species; some of these sphingolipid species are synthesized *de novo* while others are produced by the Isc1-driven hydrolysis of complex sphingolipids (17, 18, 37, 62, 70-73). Furthermore, the TORC1-Sit4 branch regulates some reactions of the *de novo* sphingosine and ceramide biosynthesis (74), whereas the TORC1-Sch9 branch controls the production of sphingosines, ceramides and complex sphingolipids both biosynthetically and hydrolytically (75). Moreover, the Sch9 protein component of the TORC1-Sch9 branch is required for the translocation of Isc1 from the ER to mitochondria during the post-diauxic growth phase (75). These findings support the notion that the TORC1-Sit4 and TORC1-Sch9 branches, as well as Hog1 and Isc1, are linked nodes of a signaling network that integrates nutrient and sphingolipid signaling to regulate longevity of chronologically aging yeast (37, 62, 69-75). A more detailed description of this signaling network is provided below in this section and schematically depicted in Figure 2.

The identities of molecular species of mitochondrial membrane sphingolipids that may modulate information flow through the TORC1-Sit4/TORC1-Sch9/Hog1/Isc1 signaling nodes remain to be established. These sphingolipid species may include DHS and PHS, two sphingoid backbone bases of sphingolipids whose concentrations in yeast are gradually increased with chronological age (76). Such age-related rise of DHS and PHS in mitochondrial membranes of chronologically aging yeast may shorten CLS by slowing mitochondrial fusion, eliciting mitochondrial fragmentation, lowering mitochondrial respiration and electrochemical membrane potential, compromising ATP synthesis in mitochondria, and lessening the number of mitochondrial DNA copies (76).

Another convincing evidence for the essential roles of sphingolipids in yeast chronological aging has been provided by the demonstration that yeast CLS can be extended by pharmacological and genetic interventions that weaken (but do not completely stop) metabolite flow through the pathway of *de novo* sphingolipid synthesis. Such weakening of metabolite flow through sphingolipid synthesis pathway was achieved via a partial inhibition of the initial, SPT-driven step of the pathway using 1) relatively low concentrations of myriocin, an inhibitor of SPT enzymatic activity (77) (Figure 1); or 2) the tetracycline-repressible promoter cassette to lower transcription of genes encoding the Lcb1 or Lcb2 subunits of the SPT protein complex (77) (Figure 1). By eliciting a partial inhibition of SPT, both these CLS-extending interventions have been shown to decrease the concentrations of DHS, PHS, DHS-1-phosphate, PHS-1-phosphate and IPC (77) (Figure 1). Such decline in the abundance of sphingosine species and/or IPC is likely to be responsible for the observed abilities of both these interventions to lower protein kinase activities of Pkh1 (Pkb-activating kinase homolog protein 1) and Pkh2, two sphingolipid-activated protein kinases that phosphorylate a specific residue in the activation loop of the nutrient-sensing protein kinase Sch9 (77). Pkh1 and Pkh2 are likely stimulated by PHS (78). Although the Pkh1/2-Sch9 branch of a network that integrates nutrient and sphingolipid signaling is the primary target of both these interventions, myriocin treatment also elicits the following pro-longevity changes in other pathways possibly integrated into this network: 1) it weakens the pro-aging PKA (protein kinase A) pathway; 2) it attenuates the pro-aging TORC1 pathway; 3) it activates the anti-aging Snf1 (sucrose non-fermenting) pathway; and 4) it stimulates the anti-aging ATG (autophagy) pathway (79). These effects of myriocin treatment on different nodes and hubs comprising the nutrient and sphingolipid signaling network are believed to be responsible for the global changes in transcription of numerous nuclear genes seen in myriocin-treated yeast (79). It is presently unclear which of the above effects of myriocin treatment on signaling and transcription are due to the decline in the abundance of sphingosine species and IPC that occurs in yeast exposed to myriocin (62). Some of these effects could be due to the abilities of certain sphingolipid species to act in minor quantities as signaling molecules that bind to specific protein components integrated into the nutrient and sphingolipid signaling network, whereas others could be caused by the abilities of bulk quantities of sphingolipids to influence general physical properties of cellular membranes and/or create functionally distinct membrane domains (62).

In sum, emergent evidence indicates that the metabolic pathway for the biosynthetic and hydrolytic production of sphingosines, ceramides and complex sphingolipids is integrated into an intricate network with certain nutrient- and sphingolipid-sensing signaling pathways. This network defines yeast CLS and is schematically depicted in Figure 2. The nutrient and sphingolipid signaling network includes three hubs,

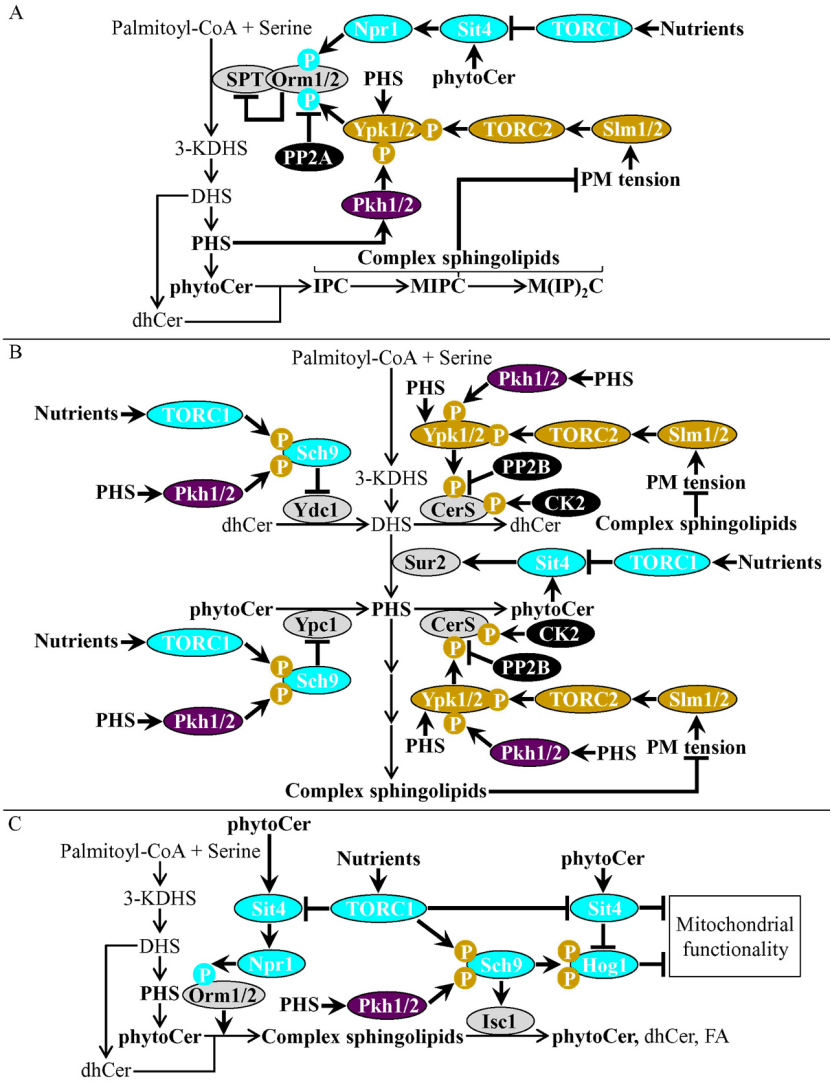

Figure 2. Sphingolipid metabolism, nutrient-sensing signaling and sphingolipid-controlled signaling pathways are integrated into a network. This network includes three hubs. Each hub is a chemical reaction (or several chemical reactions) of sphingolipid metabolism linked to several nodes or branches of nutrient-sensing and sphingolipid-controlled signaling pathways. (A) The SPT hub of this network links the initial reaction of sphingolipid metabolism to the TORC1-Sit4-Npr1, TORC2-Ypk1/2 and Pkh1/2-Ypk1/2 branches of nutrient- and sphingolipid-dependent signaling. (B) The CerS/Ydc1/Ypc1/Sur2 hub of this network links reactions of sphingosine and ceramide metabolism to the TORC2-Ypk1/2, Pkh1/2-Ypk1/2, TORC1-Sch9, Pkh1/2-Sch9 and TORC1-Sit4-Npr1 branches of nutrient- and sphingolipid-dependent signaling. (C) Enzymes involved in the synthesis and hydrolysis of complex sphingolipids constitute a network hub linked to the TORC1-Sit4-Npr1, TORC1-Sch9 and Pkh1/2-Sch9 branches of nutrient- and sphingolipid-dependent signaling. Inhibiting or activating phosphorylations are displayed in red or blue color, respectively. See text for more details. Abbreviations: CerS, ceramide synthase; CK2, casein kinase 2; dhCer, dihydroceramide; DHS, dihydrosphingosine; FA, fatty acid; HOG, high osmotic glycerol; IPC, inositol-phosphorylceramide; Isc1, inositol phosphosphingolipid phospholipase C 1; 3-KDHS, 3-ketodihydrosphingosine; MIPC, mannosyl-inositol-phosphorylceramide; $M(IP)_2C$, mannosyl-di-inositol-phosphorylceramide; Npr1, nitrogen permease reactivator 1; Orm1 and Orm2, orosomucoid 1 and 2 (respectively); PHS, phytosphingosine; phytoCer, phytoceramide; Pkh, Pkb-activating kinase homolog; PM, plasma membrane; PP2A, protein phosphatase type 2A; PP2B, protein phosphatase type 2B; Pkh, Pkb-activating kinase homolog; Sit4, suppressor of initiation of transcription 4; Slm1/2, synthetic lethal with Mss4 protein 1 or 2; SPT, serine palmitoyltransferase; Sur2, suppressor 2 of Rvs161 and rvs167 mutations; TOR, target of rapamycin; TORC1, TOR complex 1; TORC2, TOR complex 2; Ydc1, yeast dihydroceramidase 1; Ypc1, yeast phytoceramidase 1; Ypk1/2, yeast protein kinase 1 or 2

each representing a chemical reaction (or several chemical reactions) in the sphingolipid metabolism pathway which is linked to several nodes or branches of nutrient- and sphingolipid-sensing signaling pathways.

At the SPT hub of this network, the SPT protein complex catalyzing the initial reaction of sphingolipid metabolism is inhibited by non-phosphorylated forms of the Orm1 and Orm2 proteins (37, 73, 80-84) (Figure 2A). This Orm1/2-dependent inhibition of SPT can be relieved via phosphorylation of Orm1 and Orm2 by the protein kinase Npr1 of the TORC1-Sit4-Npr1 branch of the nutrient-sensing TORC1 signaling pathway; the catalytic subunit of type 2A protein phosphatase

Sit4, another component of this branch, can be inhibited by TORC1 and activated by phytoCer (37, 67, 73, 83, 84) (Figure 2A). Orm1 and Orm2 can also be phosphorylated (and SPT inhibition can be relieved) via partially overlapping TORC2-Ypk1/2 and Pkh1/2-Ypk1/2 signaling branches; a common component of these branches, the protein kinase Ypk1/2, phosphorylates Orm1 and Orm2 at sites that differ from sites phosphorylated by the protein kinase Npr1 of the TORC1-Sit4-Npr1 branch (37, 73, 82-84) (Figure 2A). Both the TORC2-Ypk1/2 and Pkh1/2-Ypk1/2 branches are modulated by sphingolipids. The TORC2-Ypk/2 branch of the TORC2 signaling pathway is activated if a decline in the abundance of complex sphingolipids increases tension of the PM; this allows the phosphatidylinositol-4,5-bisphosphate binding proteins Slm1 and Slm2 to move from the MCC (membrane compartment containing Can1)/eisosome domain of the PM to the MCT (membrane compartment containing TORC2) area of the PM, where they activate Ypk1/2 phosphorylation by the PM-associated TORC2 complex (37, 73, 82, 85, 86) (Figure 2A). The Pkh1/2-Ypk1/2 branch is activated by PHS, a sphingoid base of sphingolipids which stimulates both protein components of this signaling branch (37, 78) (Figure 2A). The TORC1-Sit4-Npr1-, TORC2-Ypk1/2- and Pkh1/2-Ypk1/2-dependent phosphorylations of Orm1 and Orm2 can be offset (and SPT inhibition can be restored) by the complex between Cdc55 and Pph21/Pph22, a regulatory and catalytic subunits (respectively) of yeast protein phosphatase 2A (PP2A) (37, 73, 87) (Figure 2A). Altogether, these findings indicate that the SPT hub of the nutrient and sphingolipid signaling network is modulated by three feedback loops. One of these feedback loops acts in a negative manner (i.e. SPT is indirectly inhibited by complex sphingolipids), whereas two others are positive feedback loops (i.e. SPT is indirectly activated by PHS and phytoCer) (Figure 2A). It is conceivable that these three feedback loops orchestrate a delicate tuning of SPT activity in response to the availability of nutrients and the extent of cellular stress (which is exhibited in part as changes in sphingolipid concentrations), thus defining longevity of chronologically aging yeast.

Another hub of the nutrient and sphingolipid signaling network includes the CerS protein complex, ceramidases Ydc1 and Ypc1, and the sphinganine C4-hydroxylase Sur2 (Figure 2B). These proteins and protein complexes are involved in the synthesis of dhCer and phytoCer from DHS and PHS, hydrolysis of dhCer and phytoCer to DHS and PHS, and conversion of DHS into PHS (respectively) (Figure 1). At the CerS/Ydc1/Ypc1/Sur2 hub, CerS is activated in response to phosphorylation at different sites by 1) Ypk1/2, a common component of partially overlapping TORC2-Ypk1/2 and Pkh1/2-Ypk1/2 signaling branches (88, 89); and 2) casein kinase 2 (CK2), a Ser/Thr protein kinase with many cellular functions (90, 91) (Figure 2B). The TORC2-Ypk1/2 branch is attenuated by complex sphingolipids, whereas both components of the Pkh1/2-Ypk1/2 branch are activated by PHS (37, 78, 73, 82, 85, 86) (Figure 2B). The TORC2-Ypk1/2 branch is also weakened by calcineurin, a Ca^{2+}/calmodulin-regulated type 2B protein phosphatase (PP2B) which stimulates the dephosphorylation of CerS sites phosphorylated by Ypk1/2 (88, 89) (Figure 2B). Ceramidases Ydc1 and Ypc1 are components of the CerS/Ydc1/Ypc1/Sur2 hub whose abundance can be decreased if the nutrient-sensing protein kinase Sch9 represses transcription of the YDC1 and YPC1 genes (75) (Figure 2B). Sch9 is a common component of the partially overlapping TORC1-Sch9 and Pkh1/2-Sch9 signaling branches; it can be activated if concomitantly phosphorylated at different sites by nutrient-sensing TORC1 and PHS-dependent Pkh1/2 (77, 79, 82, 92-94) (Figure 2B). The Sur2 component of the CerS/Ydc1/Ypc1/Sur2 hub can be activated by Sit4, a catalytic subunit of PP2A which can be inhibited by nutrient-sensing TORC1 and stimulated by phytoCer (37, 67, 73, 74, 83, 84) (Figure 2B). Thus, the CerS/Ydc1/Ypc1/Sur2 hub of the nutrient and sphingolipid signaling network is under control of the following four feedback loops: 1) a positive feedback loop in which CerS is indirectly activated by PHS; 2) a negative feedback loop in which CerS is indirectly inhibited by complex sphingolipids; 3) a negative feedback loop in which Ydc1 and Ypc1 are indirectly inhibited by PHS; and 4) a positive feedback loop in which Sur2 is indirectly activated by phytoCer (Figure 2B). It is tempting to speculate that these four feedback loops allow to coordinate the synthesis and breakdown of DHS, PHS, dhCer and phytoCer in response to the intracellular nutrient and stress status, thereby being essential for regulating longevity of chronologically aging yeast.

The third hub of the nutrient and sphingolipid signaling network includes enzymes involved in the synthesis and hydrolysis of complex sphingolipids (Figure 2C). The synthesis of complex sphingolipids in the Golgi apparatus is activated by phosphorylated forms of the Orm1 and Orm2 proteins; a mechanism of such activation remains unknown (84) (Figure 2C). Orm1 and Orm2 are phosphorylated by the protein kinase Npr1 of the TORC1-Sit4-Npr1 signaling branch; the Sit4 component of this branch can be suppressed by nutrient-sensing TORC1 and can be stimulated by phytoCer (37, 74, 84) (Figure 2C). The hydrolysis of complex sphingolipids in mitochondria is catalyzed by Isc1, whose translocation from the ER to mitochondria during the post-diauxic growth phase requires the nutrient-sensing protein kinase Sch9 (75). The Sch9-driven stimulation of complex sphingolipids hydrolysis is under positive control of the TORC1-Sch9 and Pkh1/2-Sch9 signaling branches, as nutrient-sensing TORC1 and PHS-dependent Pkh1/2 can activate

Sch9 by phosphorylating different sites of this protein (77, 79, 82, 92-94) (Figure 2C). Besides their essential roles in regulating the synthesis and hydrolysis of complex sphingolipids (as well as other reactions of sphingolipid metabolism; see Figures 2A and 2B), the TORC1-Sit4 and TORC1-Sch9 branches are nodes of a signaling subnetwork that modulates mitochondrial functionality in response to nutrient status and phytoCer concentration (Figure 2C). This signaling subnetwork integrates the TORC1-Sit4 and TORC1-Sch9 branches with Hog1 (a MAPK which is phosphorylated by Sch9) and Isc1, thereby coordinating sphingolipid metabolism and mitochondrial function and regulating longevity of chronologically aging yeast (37, 62, 69-75) (Figure 2C). Together, these findings indicate that the third hub of the nutrient and sphingolipid signaling network is controlled by a positive feedback loop which indirectly activates complex sphingolipid synthesis by phytoCer, as well as by a positive feedback loop which indirectly stimulates complex sphingolipid hydrolysis by PHS (Figure 2C). By coordinating complex sphingolipid metabolism and mitochondrial functionality in response to the intracellular nutrient and stress status, these feedback loops are likely to play essential role in regulating longevity of chronologically aging yeast.

3.2. Triacylglycerol metabolism is a longevity assurance process

Triacylglycerols (TAGs) are uncharged (and therefore called ″neutral″) lipids synthesized in the ER and then deposited in lipid droplets (LDs) (72, 95, 96). The hydrolytic degradation of TAGs stored in LDs can provide free fatty acids (FFAs) and diacylglycerols (DAGs) for the production of energy, synthesis of phospholipid and sphingolipid constituents of cellular membranes, and generation of some signaling lipids (7, 72, 95-99).

The metabolic pathways of TAG synthesis and degradation in yeast cells are well known (95, 96, 98, 99); they are schematically depicted in Figure 3. The *de novo* synthesis of TAGs begins in the ER where two glycerol-3-phosphate/dihydroxyacetone phosphate (Gro-3-P/DHAP) acyltransferases, Sct1 and Gpt2, catalyze the formation of lysophosphatidic acid (LPA) or acyl-DHAP from fatty acyl-CoA esters (FA-CoAs) and Gro-3-P or DHAP, respectively (100, 101) (Figure 3). FA-CoAs, which serve as co-substrates in these Sct1- and Gpt2-driven reactions, are synthesized *de novo* from acetyl-CoA by the cytosolic acetyl-CoA carboxylase Acc1 and FA synthase complex Fas1/Fas2 (102-107) (Figure 3). LPA can also be formed from acyl-DHAP in an Ayr1-driven reduction reaction (108) (Figure 3). The LPA acyl-transferases Slc1, Slc4, Loa1 and Ale1 catalyze the conversion of LPA to PA in an acyl CoA-dependent reaction (109-113) (Figure 3). PA can then be used as a substrate in two different reactions, each yielding a distinct precursor molecule for a biosynthetic pathway that contributes to TAG formation *de novo*. One of these reactions is catalyzed by the cytidine diphosphate (CDP)-DAG synthase Cds1; this reaction converts PA to CDP-DAG, which is then used for the synthesis of the phospholipids phosphatidylserine (PS), phosphatidylcholine (PC) and phosphatidylinositol in the ER and also of the phospholipid phosphatidylethanolamine (PE) in mitochondria (95-99, 114) (Figure 3). The other reaction is catalyzed by the PA phosphatases Pah1, App1, Dpp1 and Lpp1; this reaction converts PA to DAG (115-117) (Figure 1). DAG is then acylated to TAG in the following two reactions: 1) an FA-CoA-dependent reaction catalyzed by Dga1, Are1 and Are2 (118, 119); and 2) a phospholipid (mainly PE and PC)-dependent reaction catalyzed by Lro1 (120) (Figure 3). After being *de novo* synthesized in the ER, TAGs are deposited in LDs. To provide FFAs and DAGs needed to support growth and division of rapidly proliferating yeast, these TAGs can undergo hydrolysis which is catalyzed by the TAG lipases Tgl1, Tgl3, Tgl4, Tgl5 and Ayr1; all these TAG lipases reside in LDs (121-125) (Figure 3). DAGs can also be hydrolyzed, likely by Tgl3, to yield monoacylglycerols (MAGs) (124) (Figure 3). The lipolytic degradation of MAGs in LDs is catalyzed by the MAG lipase Yju3 (126) (Figure 3). FFAs generated in LDs as the products of TAG, DAG and MAG hydrolysis can be reactivated to FA-CoAs by the long chain acyl-CoA synthetases Faa1, Faa4 and Fat1, which form a complex in LDs (72, 127-129) (Figure 3). After being formed in LDs, these FA-CoAs can undergo beta-oxidation in peroxisomes, which associate with LDs in rapidly proliferating yeast cells (72, 130-137) (Figure 3). Peroxisomal oxidation of these FA-CoA species produces acetyl-CoA, which can then be used for 1) energy production in mitochondria; and/or 2) FA-CoAs formation and its subsequent utilization for the *de novo* synthesis of TAGs and phospholipids in the ER (72, 98, 99, 131, 133) (Figure 3). In addition, the FA-CoA and DAG species formed in LDs can be used for the synthesis of membrane and signaling lipids in yeast cells that undergo rapid growth and division (7, 72, 95, 97, 98, 138-141) (Figure 3).

The intensities of lipid fluxes via metabolic pathways for TAG synthesis and degradation in yeast cells are modulated by different mechanisms, controlled in space and time, and altered in response to certain changes in environmental, nutritional and developmental conditions. Indeed, many reactions of TAG synthesis and degradation are catalyzed by partially redundant enzymes that have different substrate specificities (Figure 3); this may allow to modulate the cellular concentrations of TAGs, DAGs, phospholipids and FFAs under different internal and external conditions (95, 97-99). Also, some enzymes involved in TAG synthesis (i.e. Gpt2, Ayr1, Slc1, Loa1 and Dga1), TAG hydrolysis (i.e. Tgl1 and Yju3) and FFA reactivation (i.e. Faa1, Faa4 and Fat1) in yeast

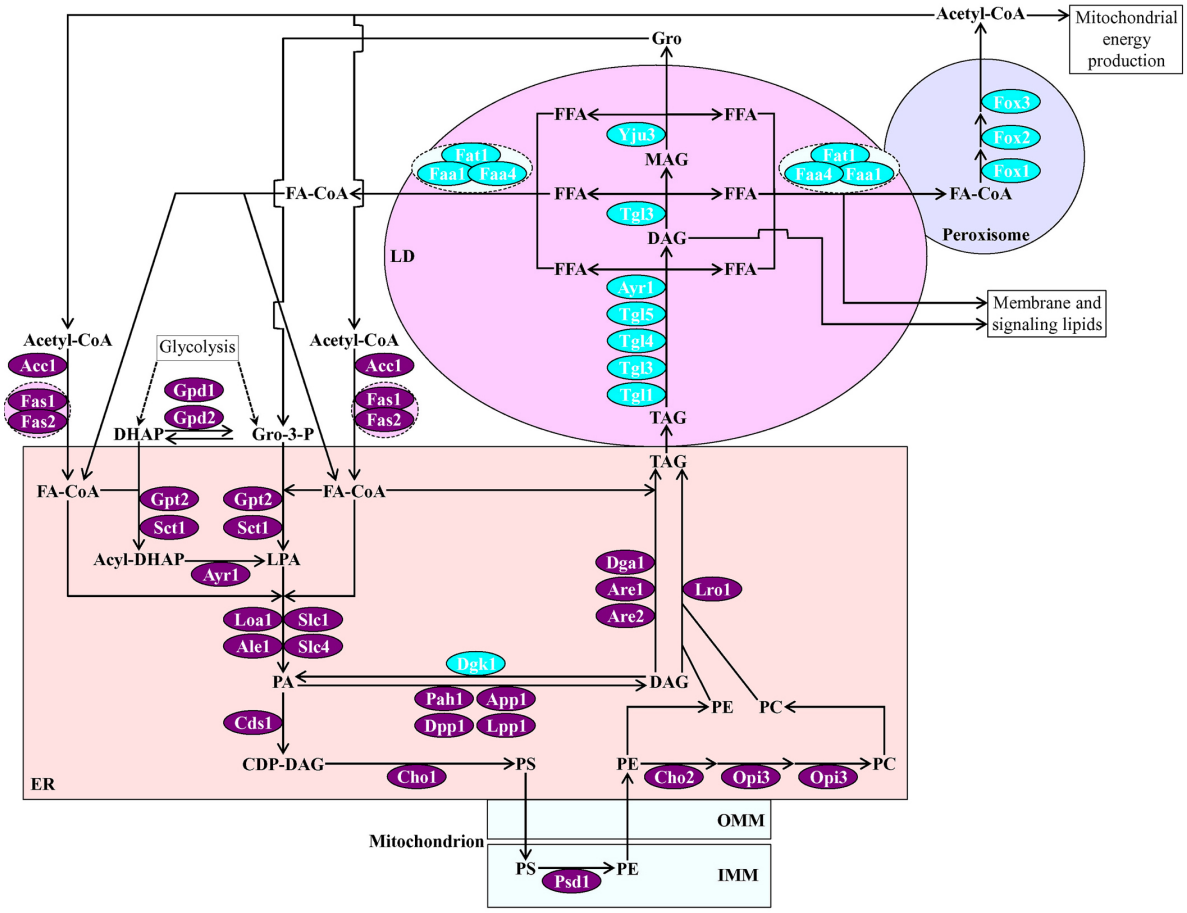

Figure 3. Pathways for the synthesis of phospholipids in the endoplasmic reticulum (ER) and mitochondria partially overlap with the anabolic branch of TAG (triacylglycerol) metabolism in the ER. The catabolic branch of TAG metabolism is confined to lipid droplets (LDs) and peroxisomes. Enzymes that catalyze anabolic or catabolic reactions of TAG metabolism are displayed in green or red color, respectively. See text for more details. Abbreviations: Acc1, acetyl-CoA carboxylase 1; Ale1, acyltransferase for lysophosphatidylethanolamine 1; App1, actin patch protein 1; Are1/2, acyl-coenzyme A: cholesterol acyl transferase-related enzymes 1 and 2; Ayr1, acyl-dihydroxyacetone-phosphate reductase 1; CDP, cytidine diphosphate; Cds1, CDP-diacylglycerol synthase 1; Cho1/2, choline requiring 1 and 2; DAG, diacylglycerol; Dga1, diacylglycerol acyltransferase 1; Dgk1, diacylglycerol kinase 1; Dpp1, diacylglycerol pyrophosphate phosphatase 1; DHAP, dihydroxyacetone phosphate; Faa1/4, fatty acid activation 1 and 2; FA-CoA, fatty acyl-CoA ester; Fas1/2, fatty acid synthetases 1 and 2; Fat1, fatty acid transporter 1; FFA, free fatty acid; Fox1/2/3, fatty acid oxidation 1, 2 and 3; Gpd1/2, glycerol-3-phosphate dehydrogenases 1 and 2; Gpt2, glycerol-3-phosphate acyltransferase; Gro, glycerol; Gro-3-P, glycerol-3-phosphate; OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; Loa1, lysophosphatidic acid: oleoyl-CoA acyltransferase 1; Lpp1, lipid phosphate phosphatase 1; LPA, lysophosphatidic acid; Lro1, lecithin cholesterol acyl transferase related open reading frame 1; MAG, monoacylglycerol; Opi3, overproducer of inositol 3; PA, phosphatidic acid; Pah1, phosphatidic acid phosphohydrolase 1; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; Psd1, phosphatidylserine decarboxylase 1; Sct1, suppressor of choline-transport mutants 1; Slc1/4, sphingolipid compensation 1 and 4; Tgl1/3/4/5, triglyceride lipases 1, 3, 4 and 5.

cells exhibit dual localization to the ER and LDs; this may also be used as a mechanism for adapting the anabolic and catabolic branches of TAG metabolism to certain changes in intracellular and extracellular conditions (72, 95, 98, 99). In addition, the ER and LDs form physical contacts and share lipid intermediates of TAG metabolism with many other cellular structures, including mitochondria, peroxisomes, vacuoles, the nucleus, the Golgi apparatus and the PM; this allows to maintain lipid homeostasis of the entire cell under different environmental, nutritional and developmental conditions (134-137, 140-149). Furthermore, TAG lipolysis supplies (while TAG synthesis removes) certain TAG metabolism intermediates that in yeast cells play essential roles in cell cycle progression and cytokinesis; these pathways of TAG metabolism 1) are controlled by the cyclin-dependent kinases Pho85-Pho80 and/or Cdc28 at two different cell-cycle checkpoints, and 2) modulate the intracellular concentrations of lipid species that control cell-cycle progression activities of the phosphatase $PP2A^{Cdc55}$ and morphogenesis checkpoint kinase Swe1 (138-140, 150-153). Moreover, TAG synthesis in yeast cells is under the tight control by such key nutrient-sensing protein kinases as Tor1, PKA and Snf1; this allows to sustain cellular homeostasis of lipids under different conditions of nutrient availability (72, 95, 97, 153-157).

Given that TAG metabolism in *S. cerevisiae* is spatially and temporally integrated into many vital cellular processes confined to different cellular compartments and controlled by some key signaling pathways in response to specific changes in intracellular and extracellular conditions, it is not surprising that TAG synthesis and degradation have been shown to define longevity of chronologically aging yeast. It seems that there are two different ways of delaying yeast chronological aging by differently altering the age-related dynamics of changes in intracellular TAG concentration. These two ways are described below.

One of these ways of aging delay has been discovered by studies of yeast cultured in a nutrient-rich liquid medium initially containing 2% glucose (158, 159). Under these so-called non-caloric restriction (non-CR) conditions yeast cells are not limited in the supply of calories (47, 160, 161). In non-CR yeast, the intracellular concentration of TAGs is substantially increased during logarithmic (L), diauxic (D) and post-diauxic (PD) phases (161). After entering stationary (ST) phase, yeast cells cultured under non-CR conditions gradually consume TAGs accumulated in LDs during the preceding L, D and PD phases of growth (161). It has been found that in non-CR yeast 1) single-gene-deletion mutations eliminating the TAG lipases Tgl3 and/or Tgl4 increase TAG concentration and extend CLS; 2) a simultaneous lack of DAG acyltransferases Dga1 and Lro1 in the *dga1Δlro1Δ* mutant strain decreases TAG concentration and shortens CLS; and 3) the overexpression of the DAG acyltransferase Dga1 rises TAG concentration and prolongs CLS (158). It was therefore concluded that an increase in the abundance of TAGs seen in *tgl3Δ*, *tgl4Δ*, *tgl3Δtgl4Δ* and Dga1 overexpressing cells under non-CR conditions is responsible for the extension of their CLS (158, 159). A "radical sink" mechanism may underlie the ability of increased concentration of TAGs to serve as a longevity assurance factor in chronologically aging non-CR yeast (Figure 4) (159). In this mechanism, an age-related accumulation of reactive oxygen species (ROS) in non-CR yeast elicits oxidative damage to different kinds of biological macromolecules, especially to unsaturated FFA known to be highly susceptible to such damage (159). Genetic manipulations that increase an incorporation of unsaturated FFA into TAGs (i.e. the *tgl3Δ* and/or *tgl4Δ* mutations or Dga1 overexpression) may intensify the flow of these susceptible to oxidative damage unsaturated FFA into LDs, where TAGs are stored. This may decrease the abundance of unsaturated FFA in cellular membranes, thereby lowering the extent of an age-related oxidative damage to membrane lipids and proteins (and perhaps to water-soluble macromolecules, such as proteins, DNA and RNA) and extending the CLS of non-CR yeast (Figure 4) (159). Moreover, because genetic manipulations that increase the incorporation of unsaturated FFA into TAGs may sequester the major target molecules of an age-related oxidative damage inside the hydrophobic core of LDs, this is expected to limit the distribution of oxidative damage to water-soluble molecules outside LDs and thus to prolong the CLS of non-CR yeast as well (Figure 4) (159). The "radical sink" mechanism, which has been proposed to explain how the accumulation of bulk quantities of TAGs by non-CR cells of some yeast mutants may extend CLS, provides a framework for future studies aimed at testing its validity. It remains to be established if TAGs stored in LDs of these long-lived mutant cells amass oxidatively damaged unsaturated FFA. Another challenge is to assess if membrane-associated and/or water-soluble macromolecules in these mutant cells exhibit a lowered extent of oxidative damage. In the future it would be also interesting to investigate if pharmacological interventions that can extend yeast CLS under non-CR conditions may (akin to the aging-delaying *tgl3Δ*, *tgl4Δ* and *tgl3Δtgl4Δ* mutations or Dga1 overexpression) elicit an accumulation of excessive TAG quantities.

The other way of delaying yeast chronological aging by altering the age-related dynamics of TAGs has been discovered by studies of yeast placed on a CR diet; this diet was imposed by culturing *S. cerevisiae* in a nutrient-rich liquid medium initially containing 0.2.% or 0.5.% glucose (161, 162). CR is a dietary intervention that delays aging not only in yeast (47, 58, 161) but also in multicellular eukaryotes across phyla (163-167). In yeast cultured under CR conditions, the intracellular concentration of TAGs is increased during L and D phases to reach a significantly lower steady-state level than that in yeast cultured under non-CR conditions (161). CR yeast cells completely consume TAGs during the subsequent PD phase, unlike non-CR yeast cells that maintain a relatively high concentration of TAGs through the entire CLS (161). Yeast cells cultured under non-CR conditions (but not yeast cells cultured under CR conditions) amass ethanol (131, 161). This product of glucose fermentation has been shown to decrease yeast CLS (168), however a mechanism by which ethanol shortens longevity of chronologically aging yeast remains unknown. Ethanol accumulated by yeast cells cultured under non-CR conditions has been shown to suppress the synthesis of Fox1, Fox2 and Fox3, all of which are the core enzymes of peroxisomal beta-oxidation of FFAs (169, 170). Because of the resulting low efficiency of FFA oxidation in peroxisomes of prematurely aging non-CR yeast cells, they accumulate FFAs (161). Moreover, it has been shown that 1) a close physical association of peroxisomes with LDs promotes the lipolytic degradation of TAGs within LDs, thus providing bulk quantities of FFAs for beta-oxidation in yeast peroxisomes (130, 131, 135, 136); and 2) lack of peroxisomal Fox1, Fox2 or Fox3 in the *fox1Δ*, *fox2Δ* or *fox3Δ* mutant strain elicits an accumulation

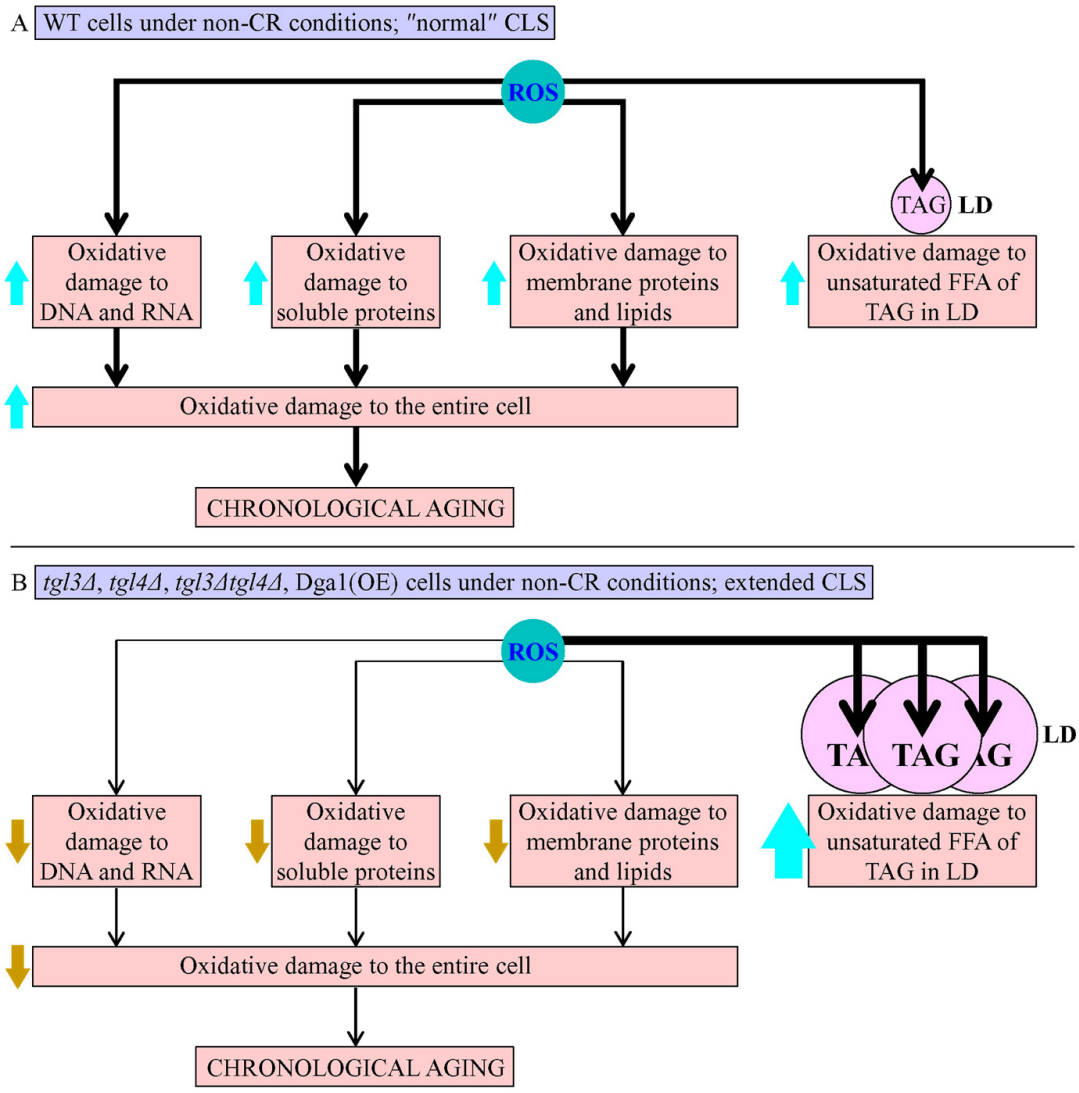

Figure 4. A "radical sink" mechanism may explain how the accumulation of triacylglycerols (TAG) by non-CR cells of some yeast mutants may extend yeast chronological lifespan (CLS). (A) An age-related accumulation of reactive oxygen species (ROS) in non-CR cells of wild-type (WT) strain elicits an oxidative damage to water-soluble molecules (i.e. DNA, RNA, proteins and metabolites) as well as to membrane proteins and lipids, and also to unsaturated free fatty acids (FFA) that are incorporated into TAG stored in lipid droplets (LD). The substantial oxidative damage to water-soluble and membrane-associated molecules elicits a massive oxidative damage to the entire cell, thereby accelerating yeast chronological aging. (B) The *tgl3Δ*, *tgl4Δ* and *tgl3Δtgl4Δ* mutations as well as Dga1 overexpression (OE) increase the incorporation of unsaturated FFA into TAG, thereby intensifying the flow of these unsaturated FFA (which are very susceptible to oxidative damage) into LD. This may decrease the abundance of unsaturated FFA in cellular membranes, thus lowering the extent of oxidative damage to membrane proteins and lipids as well as to water-soluble DNA, RNA, proteins and metabolites. This, in turn, lowers the extent of oxidative damage to the entire cell and decelerates yeast chronological aging. The thickness of black arrows is proportional to the extent of oxidative damage to various molecules, degree of oxidative damage to the entire cell or efficiency with which chronological aging is accelerated. Arrows next to the boxes showing names of affected processes denote those of them that are intensified (red arrows) or weakened (blue arrows). See text for more details.

of electron-dense arrays of FFAs (which are called "gnarls"), as well as a deposition of bulk quantities of TAGs, within yeast LDs (130, 135, 136). Based on all these findings, a mechanism has been proposed for how a CR diet may extend yeast CLS by altering the spatiotemporal dynamics of TAG synthesis in the ER, TAG lipolysis in LDs and beta-oxidation of TAG-derived FFAs in peroxisomes (10, 131, 161, 171-173). This mechanism is schematically depicted in Figure 5.

In this mechanism, yeast cells grown under non-CR conditions amass ethanol. The accumulated ethanol weakens peroxisomal oxidation of FA-CoAs because it represses the synthesis of Fox1, Fox2 and Fox3 (10, 131, 161, 171, 172) (Figure 5). The ensuing build-up of FA-CoAs in peroxisomes creates a negative feedback loop which attenuates the transport of FA-CoAs from associated LDs, where these FA-CoAs are formed from TAG-derived FFAs. This elicits an accumulation of

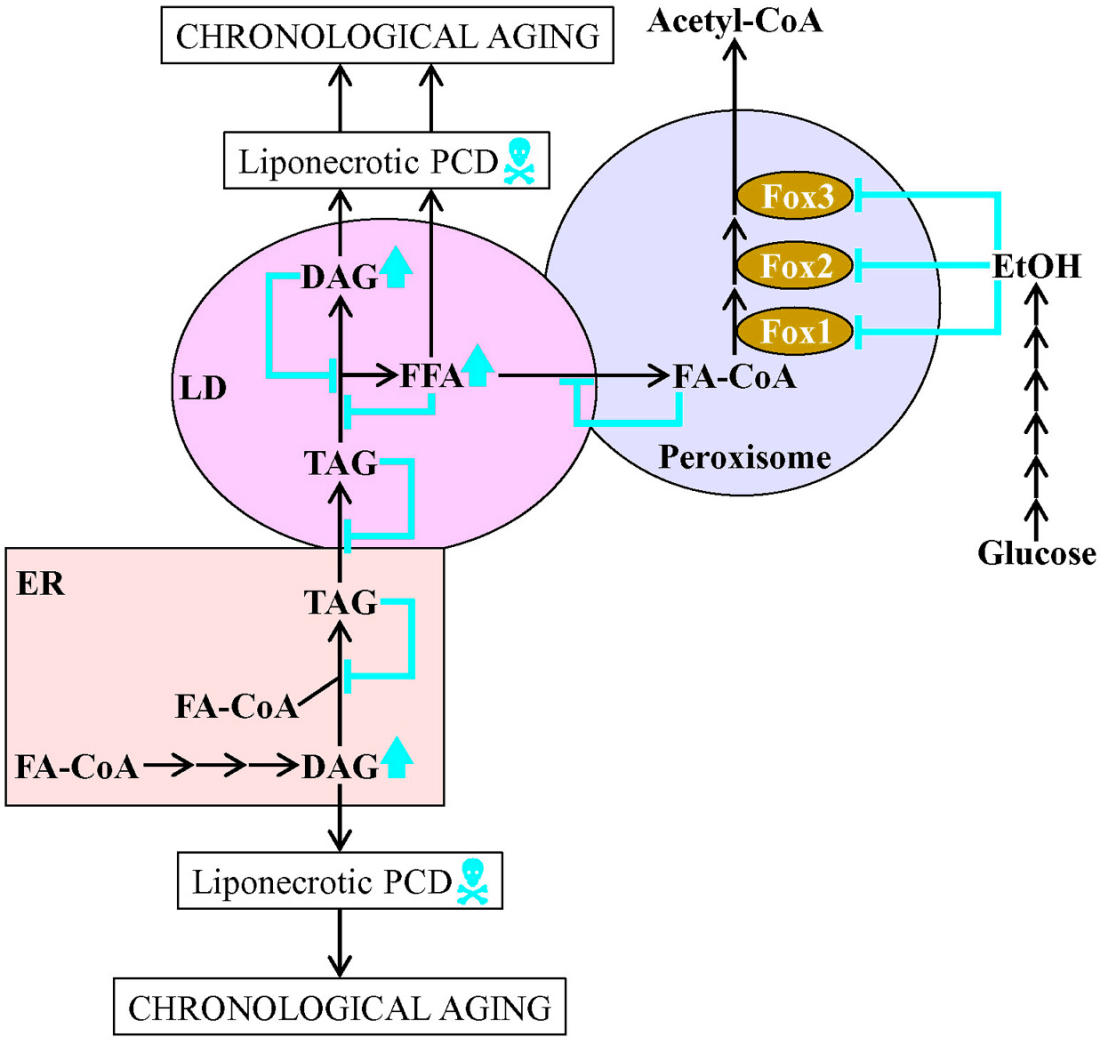

Figure 5. A mechanism through which a build-up of ethanol by chronologically aging yeast cells grown under non-CR conditions may shorten their longevity by altering the spatiotemporal dynamics of triacylglycerol (TAG) synthesis in the endoplasmic reticulum (ER), TAG lipolysis in lipid droplets (LD) and beta-oxidation of TAG-derived free fatty acids (FFA) in peroxisomes. Yeast cells under non-CR conditions accumulate ethanol, which then represses the synthesis of Fox1, Fox2 and Fox3. This elicits a build-up of fatty acyl-CoA esters (FA-CoA) in peroxisomes, thereby initiating several negative feedback loops that weaken TAG lipolysis in LD, TAG transport from the ER to LD and TAG synthesis from diacylglycerol (DAG) in the ER. The resulting build-up of FFA and DAG in the ER and LD shortens yeast CLS because these two lipids trigger an age-related form of liponecrotic programmed cell death (PCD). Red arrows next to the names of lipid classes denote those of them whose concentrations are increased in non-CR yeast. Inhibition bars displayed in red color signify negative feedback loops. See text for more details.

arrays of FFAs (gnarls) within LDs of non-CR yeast, thus initiating several negative feedback loops that weaken TAG lipolysis in LDs, TAG transport from the ER to LDs and TAG synthesis from DAGs in the ER (10, 131, 161, 171, 172) (Figure 5). The resulting build-up of FFAs and DAGs in the ER and LDs shortens the CLS of non-CR yeast because these two lipid classes are known to elicit an age-related form of liponecrotic programmed cell death (PCD) (10, 173) (Figure 5). Because yeast cells grown under CR conditions do not accumulate ethanol (161), they are not susceptible to liponecrotic PCD and thus live longer than non-CR yeast (10, 131, 161, 171-173). In the above mechanism, age-related liponecrotic PCD shortens longevity of non-CR yeast. Because proteins that execute this mode of PCD in chronologically aging yeast have been identified (10, 173), it would be interesting to investigate if single-gene-deletion mutations eliminating these proteins can extend longevity of yeast cultured under non-CR conditions.

3.3. Some mitochondrial membrane phospholipids define yeast chronological lifespan

A high-throughput chemical genetic screen for low molecular weight chemical compounds capable

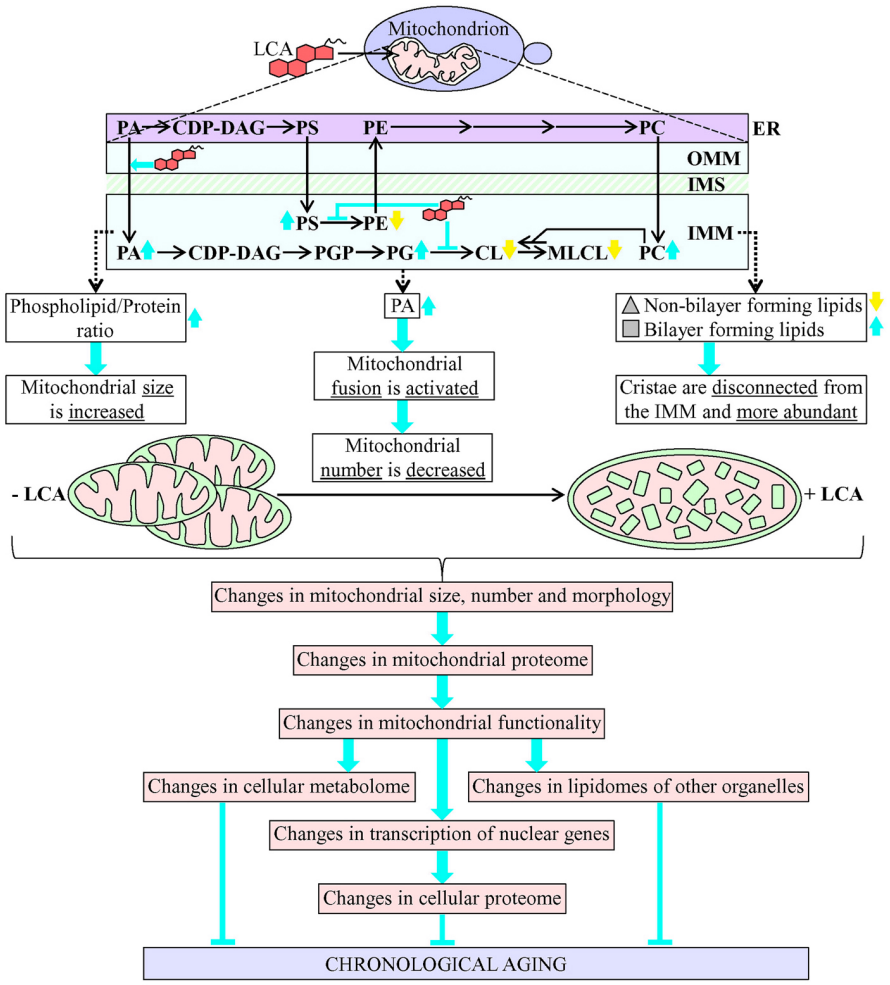

Figure 6. Exogenously added lithocholic bile acid (LCA) enters the yeast cell, accumulates in a double membrane delimiting mitochondria, and elicits major changes in the abundance and composition of mitochondrial membrane phospholipids. These changes in mitochondrial membrane phospholipids initiate a cascade of downstream events that gradually develop an anti-aging cellular pattern, thus extending longevity of chronologically aging yeast. See text for more details. Abbreviations: CDP-DAG, cytidine diphosphate-diacylglycerol; Cl, cardiolipin; ER, endoplasmic reticulum; OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; IMS, intermembrane space; MLCL, monolysocardiolipin; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; PGP, phosphatidylglycerol-phosphate

of prolonging yeast CLS has identified lithocholic bile acid (LCA) as one of such geroprotectors (162). Yeast cells do not produce LCA or other bile acids, all of which are synthesized and released into an ecosystem by animals and humans (174-177). If LCA is added exogenously to yeast cultured in a liquid medium, this highly hydrophobic bile acid enters the yeast cell, is delivered to mitochondria, accumulates mainly in the inner mitochondrial membrane (IMM) and also associates with the outer mitochondrial membrane (OMM) (178). A body of evidence supports the notion that LCA slows down yeast chronological aging because it instigates specific changes in the concentrations of mitochondrial membrane phospholipids (178-183). This evidence has recently been thoroughly discussed (184-188). We therefore briefly summarize below the data confirming that certain LCA-dependent changes in mitochondrial membrane phospholipids play essential roles in the ability of LCA to extend yeast CLS. These data are integrated into a model presented in Figure 6.

After being sorted to a double membrane delimiting mitochondria, LCA elicits three major changes in the abundance and composition of mitochondrial membrane phospholipids. These major changes are depicted in Figure 6 and outlined beneath.

First change: LCA significantly increases the phospholipid/protein ratio of mitochondrial membranes; this rise in the abundance of all membrane phospholipid classes in mitochondria causes a substantial enlargement of these organelles (178) (Figure 6).

Second change: LCA considerably augments the relative concentration of PA, likely by activating its transfer from the ER to the OMM via mitochondria-

ER contact sites and the ensuing movement of PA from the OMM via the intermembrane space (IMS) to the IMM (178, 183). PA is a so-called "fusogenic" lipid class known to stimulate a fusion of two or more small mitochondria into a single mitochondrion (182). This LCA-driven increase in the relative concentration of PA causes a substantial decline in the number of mitochondria (178) (Figure 6).

Third change: LCA differently affects the relative concentrations of different phospholipid classes as follows: a) it causes a rise in PS, phosphatidylglycerol (PG) and PC; and b) it causes a decline in PE, cardiolipin (CL) and monolysocardiolipin (MLCL) (178, 183) (Figure 6). These effects of LCA are believed to be instigated by an LCA-dependent attenuation of Psd1 and Crd1, which catalyze the conversion of PS into PE and of PG into CL (respectively) (178, 183) (Figure 6). These LCA-driven changes in different phospholipids not only decrease the relative concentrations of the non-bilayer forming classes of phospholipids but also increase the relative concentrations of the bilayer forming classes of phospholipids (178, 183) (Figure 6). The non-bilayer forming classes of phospholipids are known to enhance membrane curving for the IMM, whereas the bilayer forming classes of phospholipids have the opposite effect on IMM curving (178, 189-192). Because LCA elicits these divergent effects on the non-bilayer forming and bilayer forming classes of phospholipids, many cristae in mitochondria of yeast treated with LCA are disconnected from the IMM and amass within mitochondrial matrix as flat bilayers (178, 184, 186, 188) (Figure 6). Moreover, because LCA increases the phospholipid/protein ratio of mitochondrial membranes (see above), this bile acid also rises the abundance of such disconnected cristae inside mitochondria (178, 184, 186, 188) (Figure 6).

Taken together, these data indicate that the LCA-driven changes in mitochondrial membrane phospholipids play a causal role in enlarging mitochondria, lessening mitochondrial number, and increasing the abundance of mitochondrial cristae that are disconnected from the IMM and accumulate within mitochondrial matrix as flat bilayers (178, 184, 186, 188) (Figure 6).

The above changes in the abundance and morphology of mitochondria in LCA-treated yeast lead to significant changes in the concentrations of many mitochondrial proteins; these mitochondrial proteins have been implicated in such longevity-defining processes as the tricarboxylic acid cycle, glyoxylate cycle, electron transport chain, amino acid synthesis, heme synthesis and attachment, iron-sulfur clusters synthesis and assembly, NADPH synthesis, ROS detoxification, protein import and folding, stress response and protection, mitochondrial division, mitochondrial DNA replication and maintenance, and synthesis and translation of mitochondrial RNA (181, 183) (Figure 6). The LCA-driven changes in mitochondrial proteome of LCA-treated yeast alter the age-related chronology of several longevity-defining mitochondrial processes, including mitochondrial respiration, membrane potential preservation, ROS homeostasis maintenance and ATP synthesis (178, 183) (Figure 6). These LCA-dependent alterations in mitochondrial functionality allow mitochondria to operate as signaling platforms that a) orchestrate a longevity-extending transcriptional program for many nuclear genes that are controlled by a discrete set of ten transcriptional factors, thus altering the entire cellular proteome; b) promote changes in the lipidomes of cellular organelles other than mitochondria; and c) elicit changes in the concentrations of certain water-soluble metabolites located outside of mitochondria (178, 181, 183, 188) (Figure 6).

In sum, the LCA-driven changes in mitochondrial membrane phospholipids trigger a cascade of downstream events that gradually lead to the development of a cellular pattern extending yeast CLS. In the future it would be interesting to investigate mechanisms through which LCA-dependent changes in mitochondrial functionality prompt changes in membrane lipidomes and water-soluble metabolomes outside of mitochondria. Another challenge is to assess the timetable of events that, in response to LCA-driven changes in mitochondrial membrane phospholipids, lead to the development of a pro-longevity pattern of the entire yeast cell.

4. SUMMARY AND PERSPECTIVE

Recent studies have provided an important conceptual advance in our understanding of the mechanisms that underlie the vital roles of sphingolipids, TAGs and mitochondrial membrane phospholipids in controlling the pace of cellular aging in the yeast *S. cerevisiae*. The essential mechanistic role of lipid metabolism and transport in defining longevity of this unicellular eukaryote further supports the notion that some aspects of the maintenance of lipid homeostasis are essential for healthy aging in evolutionarily distant organisms. These eukaryotic organisms include not only laboratory strains of budding yeast, roundworms (15, 193-213), fruit flies (214-223) and mammals (208, 224-241), but also humans (208, 232-235, 241-246). The major challenge now is to get a greater insight into the mechanisms through which lipid metabolism and transport define lifespan and healthspan in multicellular model organisms and humans.

5. ACKNOWLEDGMENTS

We are grateful to other members of the Titorenko laboratory for discussions. This study was supported by grants from the Natural Sciences and

Engineering Research Council (NSERC) of Canada (RGPIN 2014-04482) and Concordia University Chair Fund (CC0113). V.I.T. is a Concordia University Research Chair in Genomics, Cell Biology and Aging.

6. REFERENCES

1. van Meer, G., Voelker, D. R., Feigenson, G. W.: Membrane lipids: where they are and how they behave. *Nat Rev Mol Cell Biol*, 9, 112-124 (2008) DOI: 10.1038/nrm2330

2. Bou Khalil, M., Hou, W., Zhou, H., Elisma, F., Swayne, L. A., Blanchard, A. P., Yao, Z., Bennett, S. A., Figeys, D.: Lipidomics era: accomplishments and challenges. *Mass Spectrom Rev*, 29, 877-929 (2010) DOI: 10.1002/mas.20294

3. Shevchenko, A., Simons, K.: Lipidomics: coming to grips with lipid diversity. *Nat Rev Mol Cell Biol*, 11, 593-598 (2010) DOI: 10.1038/nrm2934

4. Brügger, B.: Lipidomics: analysis of the lipid composition of cells and subcellular organelles by electrospray ionization mass spectrometry. *Annu Rev Biochem*, 83, 79-98 (2014) DOI:10.1146/annurev-biochem-060713-035324

5. Simons, K.: The Biology of Lipids: Trafficking, Regulation, and Function. Cold Spring Harbor Laboratory Press, ISBN 978-1936113-39-2, 334 pages (2011)

6. Holthuis, J. C., Menon, A. K.: Lipid landscapes and pipelines in membrane homeostasis. *Nature*, 510, 48-57 (2014) DOI: 10.1038/nature13474

7. Zechner, R., Zimmermann, R., Eichmann, T. O., Kohlwein, S. D., Haemmerle, G., Lass, A., Madeo, F.: FAT SIGNALS - lipases and lipolysis in lipid metabolism and signaling. *Cell Metab*, 15, 279-291 (2012) DOI: 10.1016/j.cmet.2011.12.018

8. Bogdanov, M., Dowhan, W., Vitrac, H.: Lipids and topological rules governing membrane protein assembly. *Biochim Biophys Acta*, 1843, 1475-1488 (2014) DOI: 10.1016/j.bbamcr.2013.12.007

9. Eisenberg, T., Büttner, S.: Lipids and cell death in yeast. *FEMS Yeast Res*, 14, 179-197 (2014) DOI: 10.1111/1567-1364.12105

10. Richard, V. R., Beach, A., Piano, A., Leonov, A., Feldman, R., Burstein, M. T., Kyryakov, P., Gomez-Perez, A., Arlia-Ciommo, A., Baptista, S., Campbell, C., Goncharov, D., Pannu, S., Patrinos, D., Sadri, B., Svistkova, V., Victor, A., Titorenko, V. I.: Mechanism of liponecrosis, a distinct mode of programmed cell death. *Cell Cycle*, 13, 3707-3726 (2014) DOI: 10.4161/15384101.2014.965003

11. Volmer, R., Ron, D.: Lipid-dependent regulation of the unfolded protein response. *Curr Opin Cell Biol*, 33, 67-73 (2015) DOI: 10.1016/j.ceb.2014.12.002

12. Arlia-Ciommo, A., Svistkova, V., Mohtashami, S., Titorenko, V.I.: A novel approach to the discovery of anti-tumor pharmaceuticals: searching for activators of liponecrosis. *Oncotarget*, 7, 5204-5225 (2016) DOI: 10.18632/oncotarget.6440

13. Jackson, C. L., Walch, L., Verbavatz, J. M.: Lipids and their trafficking: An integral part of cellular organization. *Dev Cell*, 39, 139-153 (2016) DOI: 10.1016/j.devcel.2016.09.030

14. Mårtensson, C. U., Doan, K. N., Becker, T.: Effects of lipids on mitochondrial functions. *Biochim Biophys Acta*, 1862, 102-113 (2017) DOI: 10.1016/j.bbalip.2016.06.015

15. Hansen, M., Flatt, T., Aguilaniu, H.: Reproduction, fat metabolism, and life span: what is the connection? *Cell Metab*, 17, 10-19 (2013) DOI: 10.1016/j.cmet.2012.12.003

16. Hanada, K.: Serine palmitoyltransferase, a key enzyme of sphingolipid metabolism. *Biochim Biophys Acta*, 1632, 16-30 (2003) DOI: 10.1016/S1388-1981(03)00059-3

17. Dickson, R. C.: Thematic review series: sphingolipids. New insights into sphingolipid metabolism and function in budding yeast. *J Lipid Res*, 49, 909-921 (2008) DOI: 10.1194/jlr.R800003-JLR200

18. Dickson, R. C.: Roles for sphingolipids in *Saccharomyces cerevisiae. Adv Exp Med Biol*, 688, 217-231 (2010) DOI: 10.1007/978-1-4419-6741-1_15

19. Fujita, T., Inoue, K., Yamamoto, S., Ikumoto, T., Sasaki, S., Toyama, R., Chiba, K., Hoshino, Y., Okumoto, T.: Fungal metabolites. Part

11. A potent immunosuppressive activity found in *Isaria sinclairii* metabolite. *J Antibiot (Tokyo)*, 47, 208-215 (1994)
DOI: 10.7164/antibiotics.47.208

20. Miyake, Y., Kozutsumi, Y., Nakamura, S., Fujita, T., Kawasaki, T.: Serine palmitoyltransferase is the primary target of a sphingosine-like immunosuppressant, ISP-1/myriocin. *Biochem Biophys Res Commun*, 211, 396-403 (1995)
DOI: 10.1006/bbrc.1995.1827

21. Beeler, T., Bacikova, D., Gable, K., Hopkins, L., Johnson, C., Slife, H., Dunn, T.: The *Saccharomyces cerevisiae TSC10/YBR265w* gene encoding 3-ketosphinganine reductase is identified in a screen for temperature-sensitive suppressors of the Ca2+-sensitive csg2Delta mutant. *J Biol Chem*, 273, 30688-30694 (1998)
DOI: 10.1074/jbc.273.46.30688

22. Grilley, M. M., Stock, S. D., Dickson, R. C., Lester, R. L., Takemoto, J. Y.: Syringomycin action gene *SYR2* is essential for sphingolipid 4-hydroxylation in *Saccharomyces cerevisiae. J Biol Chem*, 273, 11062-11068 (1998)
DOI: 10.1074/jbc.273.18.11062

23. D'mello, N. P., Childress, A. M., Franklin, D. S., Kale, S. P., Pinswasdi, C., Jazwinski, S. M.: Cloning and characterization of *LAG1*, a longevity-assurance gene in yeast. *J Biol Chem*, 269, 15451-15459 (1994)

24. Haak, D., Gable, K., Beeler, T., Dunn, T.: Hydroxylation of *Saccharomyces cerevisiae* ceramides requires Sur2p and Scs7p. *J Biol Chem*, 272, 29704-29710 (1997)
DOI: 10.1074/jbc.272.47.29704

25. Guillas, I., Kirchman, P. A., Chuard, R., Pfefferli, M., Jiang, J. C., Jazwinski, S. M., Conzelmann, A.: C26-CoA-dependent ceramide synthesis of *Saccharomyces cerevisiae* is operated by Lag1p and Lac1p. *EMBO J*, 20, 2655-2665 (2001)
DOI: 10.1093/emboj/20.11.2655

26. Schorling, S., Vallée, B., Barz, W. P., Riezman, H., Oesterhelt, D.: Lag1p and Lac1p are essential for the Acyl-CoA-dependent ceramide synthase reaction in *Saccharomyces cerevisiae. Mol Biol Cell*, 12, 3417-3427 (2001)
DOI: 10.1091/mbc.12.11.3417

27. Vallée, B., Riezman, H.: Lip1p: a novel subunit of acyl-CoA ceramide synthase. *EMBO J*, 24, 730-741 (2005)
DOI: 10.1038/sj.emboj.7600562

28. Merrill, A. H. Jr., Sullards, M. C., Wang, E., Voss, K. A., Riley, R. T.: Sphingolipid metabolism: roles in signal transduction and disruption by fumonisins. *Environ Health Perspect*, 109 Suppl 2, 283-289 (2001)
DOI: 10.1289/ehp.01109s2283

29. Mandala, S. M., Thornton, R. A., Frommer, B. R., Curotto, J. E., Rozdilsky, W., Kurtz, M. B., Giacobbe, R. A., Bills, G. F., Cabello, M. A., Martín, I, Peláez, F., Harris, G. H.: The discovery of australifungin, a novel inhibitor of sphinganine N-acyltransferase from *Sporormiella australis*. Producing organism, fermentation, isolation, and biological activity. *J Antibiot (Tokyo)*, 48, 349-356 (1995)
DOI: 10.7164/antibiotics.48.349

30. Funato, K., Riezman, H.: Vesicular and nonvesicular transport of ceramide from ER to the Golgi apparatus in yeast. *J Cell Biol*, 155, 949-959 (2001)
DOI: 10.1083/jcb.200105033

31. Kajiwara, K., Ikeda, A., Aguilera-Romero, A., Castillon, G. A., Kagiwada, S., Hanada, K., Riezman, H., Muñiz, M., Funato, K.: Osh proteins regulate COPII-mediated vesicular transport of ceramide from the endoplasmic reticulum in budding yeast. *J Cell Sci*, 127, 376-387 (2014)
DOI: 10.1242/jcs.132001

32. Liu, L. K., Choudhary, V., Toulmay, A., Prinz, W. A.: An inducible ER-Golgi tether facilitates ceramide transport to alleviate lipotoxicity. *J Cell Biol*, 216, 131-147 (2017)
DOI: 10.1083/jcb.201606059

33. Nagiec, M. M., Nagiec, E. E., Baltisberger, J. A., Wells, G. B., Lester, R. L., Dickson, R. C.: Sphingolipid synthesis as a target for antifungal drugs. Complementation of the inositol phosphorylceramide synthase defect in a mutant strain of *Saccharomyces cerevisiae* by the *AUR1* gene. *J Biol Chem*, 272, 9809-9817 (1997)
DOI: 10.1074/jbc.272.15.9809

34. Sato, K., Noda, Y., Yoda, K.: Kei1: a novel subunit of inositolphosphorylceramide synthase, essential for its enzyme activity

and Golgi localization. *Mol Biol Cell*, 20, 4444-4457 (2009)
DOI: 10.1091/mbc.E09-03-0235

35. Heidler, S. A., Radding, J. A.: The *AUR1* gene in *Saccharomyces cerevisiae* encodes dominant resistance to the antifungal agent aureobasidin A (LY295337). *Antimicrob Agents Chemother*, 39, 2765-2769 (1995)
DOI: 10.1128/AAC.39.12.2765

36. Hashida-Okado, T., Ogawa, A., Endo, M., Yasumoto, R., Takesako, K., Kato, I.: *AUR1*, a novel gene conferring aureobasidin resistance on *Saccharomyces cerevisiae*: a study of defective morphologies in Aur1p-depleted cells. *Mol Gen Genet*, 251, 236-244 (1996)

37. Teixeira, V., Costa, V.: Unraveling the role of the Target of Rapamycin signaling in sphingolipid metabolism. *Prog Lipid Res*, 61, 109-133 (2016)
DOI: 10.1016/j.plipres.2015.11.001

38. Sawai, H., Okamoto, Y., Luberto, C., Mao, C., Bielawska, A., Domae, N., Hannun, Y. A.: Identification of *ISC1* (*YER019w*) as inositol phosphosphingolipid phospholipase C in *Saccharomyces cerevisiae*. *J Biol Chem*, 275, 39793-39798 (2000)
DOI: 10.1074/jbc.M007721200

39. Vaena de Avalos, S., Okamoto, Y., Hannun, Y. A.: Activation and localization of inositol phosphosphingolipid phospholipase C, Isc1p, to the mitochondria during growth of *Saccharomyces cerevisiae*. *J Biol Chem*, 279, 11537-11545 (2004)
DOI: 10.1074/jbc.M309586200

40. Kitagaki, H., Cowart, L. A., Matmati, N., Montefusco, D., Gandy, J., de Avalos, S. V., Novgorodov, S. A., Zheng, J., Obeid, L. M., Hannun, Y. A.: *ISC1*-dependent metabolic adaptation reveals an indispensable role for mitochondria in induction of nuclear genes during the diauxic shift in *Saccharomyces cerevisiae*. *J Biol Chem*, 284, 10818-10830 (2009)
DOI: 10.1074/jbc.M805029200

41. Nagiec, M. M., Skrzypek, M., Nagiec, E. E., Lester, R. L., Dickson, R. C.: The *LCB4* (*YOR171c*) and *LCB5* (*YLR260w*) genes of *Saccharomyces* encode sphingoid long chain base kinases. *J Biol Chem*, 273, 19437-19442 (1998)
DOI: 10.1074/jbc.273.31.19437

42. Saba, J. D., Nara, F., Bielawska, A., Garrett, S., Hannun, Y. A.: The *BST1* gene of *Saccharomyces cerevisiae* is the sphingosine-1-phosphate lyase. *J Biol Chem*, 272, 26087-26090 (1997)
DOI: 10.1074/jbc.272.42.26087

43. Bitterman, K. J., Medvedik, O., Sinclair, D. A.: Longevity regulation in *Saccharomyces cerevisiae*: linking metabolism, genome stability, and heterochromatin. *Microbiol Mol Biol Rev*, 67, 376-99 (2003)
DOI: 10.1128/MMBR.67.3.376-399.2003

44. Steinkraus, K. A., Kaeberlein, M., Kennedy, B. K.: Replicative aging in yeast: the means to the end. *Annu Rev Cell Dev Biol*, 24, 29-54 (2008)
DOI: 10.1146/annurev.cellbio.23.090506.123509

45. Steffen, K. K., Kennedy, B. K., Kaeberlein, M.: Measuring replicative life span in the budding yeast. *J Vis Exp*, 28, 1209 (2009)
DOI: 10.3791/1209

46. Kaeberlein, M.: Lessons on longevity from budding yeast. *Nature*, 464, 513-519 (2010)
DOI: 10.1038/nature08981

47. Longo, V. D., Shadel, G. S., Kaeberlein, M., Kennedy, B.: Replicative and chronological aging in *Saccharomyces cerevisiae*. *Cell Metab*, 16, 18-31 (2012)
DOI: 10.1016/j.cmet.2012.06.002

48. Denoth Lippuner, A., Julou, T., Barral, Y.: Budding yeast as a model organism to study the effects of age. *FEMS Microbiol Rev*, 38, 300-325 (2014)
DOI: 10.1111/1574-6976.12060

49. McCormick, M. A., Delaney, J. R., Tsuchiya, M. *et al.*: A comprehensive analysis of replicative lifespan in 4,698 single-gene deletion strains uncovers conserved mechanisms of aging. *Cell Metab*, 22, 895-906 (2015)
DOI: 10.1016/j.cmet.2015.09.008

50. Ghavidel, A., Baxi, K., Ignatchenko, V., Prusinkiewicz, M., Arnason, T. G., Kislinger, T., Carvalho, C. E., Harkness, T. A.: A genome scale screen for mutants with delayed exit from mitosis: Ire1-independent induction of autophagy integrates ER homeostasis into mitotic lifespan. *PLoS Genet*, 11:e1005429 (2015)
DOI: 10.1371/journal.pgen.1005429

51. Janssens GE, Veenhoff LM. Evidence for the hallmarks of human aging in replicatively aging yeast. *Microb Cell*, 3, 263-274 (2016) DOI: 10.15698/mic2016.07.510

52. Fabrizio, P., Longo, V. D.: The chronological life span of *Saccharomyces cerevisiae*. *Methods Mol Biol*, 371, 89-95 (2007) DOI: 10.1007/978-1-59745-361-5_8

53. Longo, V. D., Fabrizio, P.: Chronological aging in *Saccharomyces cerevisiae*. *Subcell Biochem*, 57:101-21 (2012) DOI: 10.1007/978-94-007-2561-4_5

54. Piper, P. W.: Maximising the yeast chronological lifespan. *Subcell Biochem*, 57, 145-59 (2012) DOI: 10.1007/978-94-007-2561-4_7

55. Longo, V. D., Kennedy, B. K.: Sirtuins in aging and age-related disease. *Cell*, 126, 257-268 (2006) DOI: 10.1016/j.cell.2006.07.002

56. Burtner, C. R., Murakami, C. J., Kennedy, B. K., Kaeberlein, M.: A molecular mechanism of chronological aging in yeast. *Cell Cycle*, 8:1256-1270 (2009) DOI: 10.4161/cc.8.8.8287

57. Burtner, C. R., Murakami, C. J., Olsen, B., Kennedy, B. K., Kaeberlein, M.: A genomic analysis of chronological longevity factors in budding yeast. *Cell Cycle*, 10, 1385-1396 (2011) DOI: 10.4161/cc.10.9.15464

58. Arlia-Ciommo, A., Leonov, A., Piano, A., Svistkova, V., Titorenko, V. I.: Cell-autonomous mechanisms of chronological aging in the yeast *Saccharomyces cerevisiae*. *Microb Cell*, 1, 163-178 (2014) DOI: 10.15698/mic2014.06.152

59. Arlia-Ciommo, A., Piano, A., Leonov, A., Svistkova, V., Titorenko, V. I.: Quasi-programmed aging of budding yeast: a trade-off between programmed processes of cell proliferation, differentiation, stress response, survival and death defines yeast lifespan. *Cell Cycle*, 13, 3336-3349 (2014) DOI: 10.4161/15384101.2014.965063

60. Powers, R. W. 3rd, Kaeberlein, M., Caldwell, S. D., Kennedy, B. K., Fields, S.: Extension of chronological life span in yeast by decreased TOR pathway signaling. *Genes Dev*, 20, 174-184 (2006) DOI: 10.1101/gad.1381406

61. Jiang, J. C., Kirchman, P. A., Allen, M., Jazwinski, S. M.: Suppressor analysis points to the subtle role of the *LAG1* ceramide synthase gene in determining yeast longevity. *Exp Gerontol*, 39, 999-1009 (2004) DOI: 10.1016/j.exger.2004.03.026

62. Huang, X., Withers, B. R., Dickson, R. C.: Sphingolipids and lifespan regulation. *Biochim Biophys Acta*, 1841, 657-664 (2014) DOI: 10.1016/j.bbalip.2013.08.006

63. Dickson, R. C., Nagiec, E. E., Wells, G. B., Nagiec, M. M., Lester, R. L.: Synthesis of mannose-(inositol-P)$_2$-ceramide, the major sphingolipid in Saccharomyces cerevisiae, requires the *IPT1* (*YDR072c*) gene. *J Biol Chem*, 272, 29620-29625 (1997) DOI: 10.1074/jbc.272.47.29620

64. Thevissen, K., Idkowiak-Baldys, J., Im, Y. J., Takemoto, J., François, I. E., Ferket, K. K., Aerts, A. M., Meert, E. M., Winderickx, J., Roosen, J., Cammue, B. P.: *SKN1*, a novel plant defensin-sensitivity gene in *Saccharomyces cerevisiae*, is implicated in sphingolipid biosynthesis. *FEBS Lett*, 579, 1973-1977 (2005) DOI: 10.1016/j.febslet.2005.02.043

65. Aerts, A. M., François, I. E., Bammens, L., Cammue, B. P., Smets, B., Winderickx, J., Accardo, S., De Vos, D. E., Thevissen, K.: Level of M(IP)$_2$C sphingolipid affects plant defensin sensitivity, oxidative stress resistance and chronological life-span in yeast. *FEBS Lett*, 580, 1903-1907 (2006) DOI: 10.1016/j.febslet.2006.02.061

66. Almeida, T., Marques, M., Mojzita, D., Amorim, M. A., Silva, R. D., Almeida, B., Rodrigues, P., Ludovico, P., Hohmann, S., Moradas-Ferreira, P., Côrte-Real, M., Costa, V.: Isc1p plays a key role in hydrogen peroxide resistance and chronological lifespan through modulation of iron levels and apoptosis. *Mol Biol Cell*, 19, 865-876 (2008) DOI: 10.1091/mbc.E07-06-0604

67. Barbosa, A. D., Osório, H., Sims, K. J., Almeida, T., Alves, M., Bielawski, J., Amorim, M. A., Moradas-Ferreira, P., Hannun, Y. A., Costa, V.: Role for Sit4p-dependent mitochondrial dysfunction in mediating the shortened chronological lifespan and oxidative stress sensitivity of Isc1p-deficient cells. *Mol Microbiol*, 81, 515-527 (2011) DOI: 10.1111/j.1365-2958.2011.07714.x

68. Barbosa, A. D., Graça, J., Mendes, V., Chaves, S. R., Amorim, M. A., Mendes, M. V., Moradas-Ferreira, P., Côrte-Real, M., Costa, V.: Activation of the Hog1p kinase in Isc1p-deficient yeast cells is associated with mitochondrial dysfunction, oxidative stress sensitivity and premature aging. *Mech Ageing Dev*, 133, 317-330 (2012)
DOI: 10.1016/j.mad.2012.03.007

69. Teixeira, V., Medeiros, T. C., Vilaça, R., Moradas-Ferreira, P., Costa, V.: Reduced TORC1 signaling abolishes mitochondrial dysfunctions and shortened chronological lifespan of Isc1p-deficient cells. *Microb Cell*, 1, 21-36 (2014)
DOI: 10.15698/mic2014.01.121

70. Spincemaille, P., Cammue, B. P., Thevissen, K.: Sphingolipids and mitochondrial function, lessons learned from yeast. *Microb Cell*, 1, 210-224 (2014)
DOI: 10.15698/mic2014.07.156

71. Spincemaille, P., Matmati, N., Hannun, Y. A., Cammue, B. P., Thevissen, K.: Sphingolipids and mitochondrial function in budding yeast. *Biochim Biophys Acta*, 1840, 3131-3137 (2014)
DOI: 10.1016/j.bbagen.2014.06.015

72. Jazwinski, S. M.: Mitochondria to nucleus signaling and the role of ceramide in its integration into the suite of cell quality control processes during aging. *Ageing Res Rev*, 23, 67-74 (2015)
DOI: 10.1016/j.arr.2014.12.007

73. Eltschinger, S., Loewith, R.: TOR complexes and the maintenance of cellular homeostasis. *Trends Cell Biol*, 26, 148-159 (2016)
DOI: 10.1016/j.tcb.2015.10.003

74. Woodacre, A., Lone, M. A., Jablonowski, D., Schneiter, R., Giorgini, F., Schaffrath, R.: A novel Sit4 phosphatase complex is involved in the response to ceramide stress in yeast. *Oxid Med Cell Longev*, 2013, 129645 (2013)
DOI: 10.1155/2013/129645

75. Swinnen, E., Wilms, T., Idkowiak-Baldys, J., Smets, B., De Snijder, P., Accardo, S., Ghillebert, R., Thevissen, K., Cammue, B., De Vos, D., Bielawski, J., Hannun, Y. A., Winderickx, J.: The protein kinase Sch9 is a key regulator of sphingolipid metabolism in *Saccharomyces cerevisiae*. *Mol Biol Cell*, 25, 196-211 (2014)
DOI: 10.1091/mbc.E13-06-0340

76. Yi, J. K., Xu, R., Jeong, E., Mileva, I., Truman, J. P., Lin, C. L., Wang, K., Snider, J., Wen, S., Obeid, L. M., Hannun, Y. A., Mao, C.: Aging-related elevation of sphingoid bases shortens yeast chronological life span by compromising mitochondrial function. *Oncotarget*, 7, 21124-21144 (2016)
DOI: 10.18632/oncotarget.8195

77. Huang, X., Liu, J., Dickson, R. C.: Down-regulating sphingolipid synthesis increases yeast lifespan. *PLoS Genet*, 8:e1002493 (2012)
DOI: 10.1371/journal.pgen.1002493

78. Liu, K., Zhang, X., Lester, R. L., Dickson, R. C.: The sphingoid long chain base phytosphingosine activates AGC-type protein kinases in *Saccharomyces cerevisiae* including Ypk1, Ypk2, and Sch9. *J Biol Chem*, 280, 22679-22687 (2005)
DOI: 10.1074/jbc.M502972200

79. Liu, J., Huang, X., Withers, B. R., Blalock, E., Liu, K., Dickson, R. C.: Reducing sphingolipid synthesis orchestrates global changes to extend yeast lifespan. *Aging Cell*, 12, 833-841 (2013)
DOI: 10.1111/acel.12107

80. Han, S., Lone, M. A., Schneiter, R., Chang, A.: Orm1 and Orm2 are conserved endoplasmic reticulum membrane proteins regulating lipid homeostasis and protein quality control. *Proc Natl Acad Sci USA*, 107, 5851-5856 (2010)
DOI: 10.1073/pnas.0911617107

81. Breslow, D. K., Collins, S. R., Bodenmiller, B., Aebersold, R., Simons, K., Shevchenko, A., Ejsing, C. S., Weissman, J. S.: Orm family proteins mediate sphingolipid homeostasis. *Nature*, 463, 1048-1053 (2010)
DOI: 10.1038/nature08787

82. Roelants, F. M., Breslow, D. K., Muir, A., Weissman, J. S., Thorner, J.: Protein kinase Ypk1 phosphorylates regulatory proteins Orm1 and Orm2 to control sphingolipid homeostasis in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA*, 108, 19222-19227 (2011)
DOI: 10.1073/pnas.1116948108

83. Liu, M., Huang, C., Polu, S. R., Schneiter, R., Chang, A.: Regulation of sphingolipid synthesis through Orm1 and Orm2 in yeast. *J Cell Sci*, 125, 2428-2435 (2012)
DOI: 10.1242/jcs.100578

84. Shimobayashi, M., Oppliger, W., Moes, S., Jenö, P., Hall, M. N.: TORC1-regulated protein kinase Npr1 phosphorylates Orm to stimulate complex sphingolipid synthesis. *Mol Biol Cell*, 24, 870-881 (2013) DOI: 10.1091/mbc.E12-10-0753

85. Berchtold, D., Piccolis, M., Chiaruttini, N., Riezman, I., Riezman, H., Roux, A., Walther, T. C., Loewith, R.: Plasma membrane stress induces relocalization of Slm proteins and activation of TORC2 to promote sphingolipid synthesis. *Nat Cell Biol*, 14, 542-547 (2012) DOI: 10.1038/ncb2480

86. Niles, B. J., Mogri, H., Hill, A., Vlahakis, A., Powers, T.: Plasma membrane recruitment and activation of the AGC kinase Ypk1 is mediated by target of rapamycin complex 2 (TORC2) and its effector proteins Slm1 and Slm2. *Proc Natl Acad Sci USA*, 109:1536-1541 (2012) DOI: 10.1073/pnas.1117563109

87. Sun, Y., Miao, Y., Yamane, Y., Zhang, C., Shokat, K. M., Takematsu, H., Kozutsumi, Y., Drubin, D. G.: Orm protein phosphoregulation mediates transient sphingolipid biosynthesis response to heat stress via the Pkh-Ypk and Cdc55-PP2A pathways. *Mol Biol Cell*, 23:2388-2398 (2012) DOI: 10.1091/mbc.E12-03-0209

88. Aronova, S., Wedaman, K., Aronov, P. A., Fontes, K., Ramos, K., Hammock, B. D., Powers, T.: Regulation of ceramide biosynthesis by TOR complex 2. *Cell Metab*, 7:148-158 (2008) DOI: 10.1016/j.cmet.2007.11.015

89. Muir, A., Ramachandran, S., Roelants, F. M., Timmons, G., Thorner, J.: TORC2-dependent protein kinase Ypk1 phosphorylates ceramide synthase to stimulate synthesis of complex sphingolipids. *Elife*, 3:10.7.554/eLife.03779 (2014)

90. Kobayashi, S. D., Nagiec, M. M.: Ceramide/long-chain base phosphate rheostat in *Saccharomyces cerevisiae*: regulation of ceramide synthesis by Elo3p and Cka2p. *Eukaryot Cell*, 2, 284-294 (2003) DOI: 10.1128/EC.2.2.284-294.2003

91. Fresques, T., Niles, B., Aronova, S., Mogri, H., Rakhshandehroo, T., Powers, T.: Regulation of ceramide synthase by casein kinase 2-dependent phosphorylation in *Saccharomyces cerevisiae*. *J Biol Chem*, 290, 1395-1403 (2015) DOI: 10.1074/jbc.M114.621086

92. Urban, J., Soulard, A., Huber, A., Lippman, S., Mukhopadhyay, D., Deloche, O., Wanke, V., Anrather, D., Ammerer, G., Riezman, H., Broach, J. R., De Virgilio, C., Hall, M. N., Loewith, R.: Sch9 is a major target of TORC1 in *Saccharomyces cerevisiae*. *Mol Cell*, 26, 663-674 (2007) DOI: 10.1016/j.molcel.2007.04.020

93. Voordeckers, K., Kimpe, M., Haesendonckx, S., Louwet, W., Versele, M., Thevelein, J. M.: Yeast 3-phosphoinositide-dependent protein kinase-1 (PDK1) orthologs Pkh1-3 differentially regulate phosphorylation of protein kinase A (PKA) and the protein kinase B (PKB)/S6K ortholog Sch9. *J Biol Chem*, 286, 22017-22027 (2011) DOI: 10.1074/jbc.M110.200071

94. Swinnen, E., Ghillebert, R., Wilms, T., Winderickx, J.: Molecular mechanisms linking the evolutionary conserved TORC1-Sch9 nutrient signalling branch to lifespan regulation in *Saccharomyces cerevisiae*. *FEMS Yeast Res*, 14, 17-32 (2014) DOI: 10.1111/1567-1364.12097

95. Kohlwein, S. D.: Triacylglycerol homeostasis: insights from yeast. *J Biol Chem*, 285, 15663-15667 (2010) DOI: 10.1074/jbc.R110.118356

96. Koch, B., Schmidt, C., Daum, G.: Storage lipids of yeasts: a survey of nonpolar lipid metabolism in *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Yarrowia lipolytica*. *FEMS Microbiol Rev*, 38, 892-915 (2014) DOI: 10.1111/1574-6976.12069

97. Kohlwein, S. D.: Obese and anorexic yeasts: experimental models to understand the metabolic syndrome and lipotoxicity. *Biochim Biophys Acta*, 1801, 222-229 (2010) DOI: 10.1016/j.bbalip.2009.12.016

98. Henry, S. A., Kohlwein, S. D., Carman, G. M.: Metabolism and regulation of glycerolipids in the yeast *Saccharomyces cerevisiae*. *Genetics*, 190, 317-349 (2012) DOI: 10.1534/genetics.111.130286

99. Klug, L., Daum, G.: Yeast lipid metabolism at a glance. *FEMS Yeast Res*, 14, 369-388 (2014) DOI: 10.1111/1567-1364.12141

100. Athenstaedt, K., Daum, G.: Biosynthesis of phosphatidic acid in lipid particles and endoplasmic reticulum of *Saccharomyces cerevisiae*. *J Bacteriol*, 179, 7611-7616 (1997)
DOI: 10.1128/jb.179.24.7611-7616.1997

101. Zheng, Z., Zou, J.: The initial step of the glycerolipid pathway: identification of glycerol 3-phosphate/dihydroxyacetone phosphate dual substrate acyltransferases in *Saccharomyces cerevisiae*. *J Biol Chem*, 276, 41710-41716 (2001)
DOI: 10.1074/jbc.M104749200

102. Stoops, J. K., Wakil, S. J.: The isolation of the two subunits of yeast fatty acid synthetase. *Biochem Biophys Res Commun*, 84, 225-231 (1978)
DOI: 10.1016/0006-291X(78)90286-3

103. Wieland, F., Renner, L., Verfürth, C., Lynen, F.: Studies on the multi-enzyme complex of yeast fatty-acid synthetase. Reversible dissociation and isolation of two polypeptide chains. *Eur J Biochem*, 94, 189-197 (1979)
DOI: 10.1111/j.1432-1033.1979.tb12885.x

104. Mohamed, A. H., Chirala, S. S., Mody, N. H., Huang, W. Y., Wakil, S. J.: Primary structure of the multifunctional alpha subunit protein of yeast fatty acid synthase derived from *FAS2* gene sequence. *J Biol Chem*, 263, 12315-12325 (1988)

105. Hasslacher, M., Ivessa, A. S., Paltauf, F., Kohlwein, S. D.: Acetyl-CoA carboxylase from yeast is an essential enzyme and is regulated by factors that control phospholipid metabolism. *J Biol Chem*, 268, 10946-10952 (1993)

106. Fichtlscherer, F., Wellein, C., Mittag, M., Schweizer, E.: A novel function of yeast fatty acid synthase. Subunit alpha is capable of self-pantetheinylation. *Eur J Biochem*, 267, 2666-2671 (2000)
DOI: 10.1046/j.1432-1327.2000.01282.x

107. Leibundgut, M., Maier, T., Jenni, S., Ban, N.: The multienzyme architecture of eukaryotic fatty acid synthases. *Curr Opin Struct Biol*, 18, 714-725 (2008)
DOI: 10.1016/j.sbi.2008.09.008

108. Athenstaedt, K., Daum, G.: 1-Acyldihydroxyacetone-phosphate reductase (Ayr1p) of the yeast *Saccharomyces cerevisiae* encoded by the open reading frame *YIL124w* is a major component of lipid particles. *J Biol Chem*, 275, 235-240 (2000)
DOI: 10.1074/jbc.275.1.235

109. Benghezal, M., Roubaty, C., Veepuri, V., Knudsen, J., Conzelmann, A.: *SLC1* and *SLC4* encode partially redundant acyl-coenzyme A 1-acylglycerol-3-phosphate O-acyltransferases of budding yeast. *J Biol Chem*, 282, 30845-30855 (2007)
DOI: 10.1074/jbc.M702719200

110. Chen, Q., Kazachkov, M., Zheng, Z., Zou, J.: The yeast acylglycerol acyltransferase LCA1 is a key component of Lands cycle for phosphatidylcholine turnover. *FEBS Lett*, 581, 5511-5516 (2007)
DOI: 10.1016/j.febslet.2007.10.061

111. Jain, S., Stanford, N., Bhagwat, N., Seiler, B., Costanzo, M., Boone, C., Oelkers, P.: Identification of a novel lysophospholipid acyltransferase in *Saccharomyces cerevisiae*. *J Biol Chem*, 282, 30562-30569 (2007)
DOI: 10.1074/jbc.M706326200

112. Riekhof, W. R., Wu, J., Jone, J. L., Voelker, D. R.: Identification and characterization of the major lysophosphatidylethanolamine acyltransferase in *Saccharomyces cerevisiae*. *J Biol Chem*, 282, 28344-28352 (2007)
DOI: 10.1074/jbc.M705256200

113. Ayciriex, S., Le Guédard, M., Camougrand, N., Velours, G., Schoene, M., Leone, S., Wattelet-Boyer, V., Dupuy, J. W., Shevchenko, A., Schmitter, J. M., Lessire, R., Bessoule, J. J., Testet, E.: *YPR139c*/*LOA1* encodes a novel lysophosphatidic acid acyltransferase associated with lipid droplets and involved in TAG homeostasis. *Mol Biol Cell*, 23, 233-246 (2012)
DOI: 10.1091/mbc.E11-07-0650

114. Shen, H., Heacock, P. N., Clancey, C. J., Dowhan, W.: The *CDS1* gene encoding CDP-diacylglycerol synthase in *Saccharomyces cerevisiae* is essential for cell growth. *J Biol Chem*, 271, 789-795 (1996)
DOI: 10.1074/jbc.271.2.789

115. Han, G. S., Wu, W. I., Carman, G. M.: The *Saccharomyces cerevisiae* Lipin homolog is a Mg^{2+}-dependent phosphatidate phosphatase enzyme. *J Biol Chem*, 281, 9210-9218 (2006)
DOI: 10.1074/jbc.M600425200

116. Carman, G. M., Han, G. S.: Phosphatidic acid phosphatase, a key enzyme in the regulation of lipid synthesis. *J Biol Chem*, 284, 2593-1597 (2009)
DOI: 10.1074/jbc.R800059200

117. Chae, M., Han, G. S., Carman, G. M.: The *Saccharomyces cerevisiae* actin patch protein App1p is a phosphatidate phosphatase enzyme. *J Biol Chem*, 287, 40186-40196 (2012)
DOI: 10.1074/jbc.M112.421776

118. Oelkers, P., Cromley, D., Padamsee, M., Billheimer, J. T., Sturley, S. L.: The *DGA1* gene determines a second triglyceride synthetic pathway in yeast. *J Biol Chem*, 277, 8877-8881 (2002)
DOI: 10.1074/jbc.M111646200

119. Sorger, D., Daum, G.: Synthesis of triacylglycerols by the acyl-coenzyme A: diacyl-glycerol acyltransferase Dga1p in lipid particles of the yeast *Saccharomyces cerevisiae*. *J Bacteriol*, 184, 519-524 (2002)
DOI: 10.1128/JB.184.2.519-524.2002

120. Oelkers, P., Tinkelenberg, A., Erdeniz, N., Cromley, D., Billheimer, J. T., Sturley, S. L.: A lecithin cholesterol acyltransferase-like gene mediates diacylglycerol esterification in yeast. *J Biol Chem*, 275, 15609-15612 (2000).
DOI: 10.1074/jbc.C000144200

121. Athenstaedt, K., Daum, G.: YMR313c/ TGL3 encodes a novel triacylglycerol lipase located in lipid particles of Saccharomyces cerevisiae. J Biol Chem, 278, 23317-23323 (2003)
DOI: 10.1074/jbc.M302577200

122. Athenstaedt, K., Daum, G.: Tgl4p and Tgl5p, two triacylglycerol lipases of the yeast *Saccharomyces cerevisiae* are localized to lipid particles. *J Biol Chem*, 280, 37301-37309 (2005)
DOI: 10.1074/jbc.M507261200

123. Jandrositz, A., Petschnigg, J., Zimmermann, R., Natter, K., Scholze, H., Hermetter, A., Kohlwein, S. D., Leber, R.: The lipid droplet enzyme Tgl1p hydrolyzes both steryl esters and triglycerides in the yeast, *Saccharomyces cerevisiae*. *Biochim Biophys Acta*, 1735, 50-58 (2005)
DOI: 10.1016/j.bbalip.2005.04.005

124. Kurat, C. F., Natter, K., Petschnigg, J., Wolinski, H., Scheuringer, K., Scholz, H., Zimmermann, R., Leber, R., Zechner, R., Kohlwein, S. D.: Obese yeast: triglyceride lipolysis is functionally conserved from mammals to yeast. *J Biol Chem*, 281, 491-500 (2006)
DOI: 10.1074/jbc.M508414200

125. Ploier, B., Scharwey, M., Koch, B., Schmidt, C., Schatte, J., Rechberger, G., Kollroser, M., Hermetter, A., Daum, G.: Screening for hydrolytic enzymes reveals Ayr1p as a novel triacylglycerol lipase in *Saccharomyces cerevisiae*. *J Biol Chem*, 288, 36061-36072 (2013)
DOI: 10.1074/jbc.M113.509927

126. Heier, C, Taschler, U., Rengachari, S., Oberer, M., Wolinski, H., Natter, K., Kohlwein, S. D., Leber, R., Zimmermann, R.: Identification of Yju3p as functional orthologue of mammalian monoglyceride lipase in the yeast *Saccharomyces cerevisiae*. *Biochim Biophys Acta*, 1801, 1063-1071 (2010)
DOI: 10.1016/j.bbalip.2010.06.001

127. Huh, W. K., Falvo, J. V., Gerke, L. C., Carroll, A. S., Howson, R. W., Weissman, J. S., O'Shea, E. K.: Global analysis of protein localization in budding yeast. *Nature*, 425, 686-691 (2003)
DOI: 10.1038/nature02026

128. Natter, K., Leitner, P., Faschinger, A., Wolinski, H., McCraith, S., Fields, S., Kohlwein, S. D.: The spatial organization of lipid synthesis in the yeast *Saccharomyces cerevisiae* derived from large scale green fluorescent protein tagging and high resolution microscopy. *Mol Cell Proteomics*, 4, 662-672 (2005)
DOI: 10.1074/mcp.M400123-MCP200

129. Black, P. N., DiRusso, C. C.: Yeast acyl-CoA synthetases at the crossroads of fatty acid metabolism and regulation. *Biochim Biophys Acta*, 1771, 286-298 (2007)
DOI: 10.1016/j.bbalip.2006.05.003

130. Binns, D., Januszewski, T., Chen, Y., Hill, J., Markin, V. S., Zhao, Y., Gilpin, C., Chapman, K. D., Anderson, R. G., Goodman, J. M.: An intimate collaboration between peroxisomes and lipid bodies. *J Cell Biol*, 173, 719-731 (2006)
DOI: 10.1083/jcb.200511125

131. Goldberg, A. A., Bourque, S. D., Kyryakov, P., Boukh-Viner, T., Gregg, C., Beach, A., Burstein, M. T., Machkalyan, G., Richard,

V., Rampersad, S., Titorenko, V. I.: A novel function of lipid droplets in regulating longevity. *Biochem Soc Trans*, 37, 1050-1055 (2009)
DOI: 10.1042/BST0371050

132. Titorenko, V. I., Terlecky, S. R.: Peroxisome metabolism and cellular aging. *Traffic*, 12:252-259 (2011)
DOI: 10.1111/j.1600-0854.2010.01144.x

133. Beach, A., Burstein, M. T., Richard, V. R., Leonov, A., Levy, S., Titorenko, V. I.: Integration of peroxisomes into an endomembrane system that governs cellular aging. *Front Physiol*, 3, 283 (2012)
DOI: 10.3389/fphys.2012.00283

134. Leonov, A., Titorenko, V. I.: A network of interorganellar communications underlies cellular aging. *IUBMB Life*, 65, 665-674 (2013)
DOI: 10.1002/iub.1183

135. Gao, Q., Goodman, J. M.: The lipid droplet - a well-connected organelle. *Front Cell Dev Biol*, 3, 49 (2015)
DOI: 10.3389/fcell.2015.00049

136. Hashemi, H. F., Goodman, J. M.: The life cycle of lipid droplets. *Curr Opin Cell Biol*, 33, 119-124 (2015)
DOI: 10.1016/j.ceb.2015.02.002

137. Dakik, P., Titorenko, V. I.: Communications between mitochondria, the nucleus, vacuoles, peroxisomes, the endoplasmic reticulum, the plasma membrane, lipid droplets and the cytosol during yeast chronological aging. *Front Genet*, 7, 177 (2016)
DOI: 10.3389/fgene.2016.00177

138. Kurat, C. F., Wolinski, H., Petschnigg, J., Kaluarachchi, S., Andrews, B., Natter, K., Kohlwein, S. D.: Cdk1/Cdc28-dependent activation of the major triacylglycerol lipase Tgl4 in yeast links lipolysis to cell-cycle progression. Mol Cell, 33, 53-63 (2009)
DOI: 10.1016/j.molcel.2008.12.019

139. Chauhan, N., Visram, M., Cristobal-Sarramian, A., Sarkleti, F., Kohlwein, S. D.: Morphogenesis checkpoint kinase Swe1 is the executor of lipolysis-dependent cell-cycle progression. *Proc Natl Acad Sci USA*, 112, E1077-E1085 (2015)
DOI: 10.1073/pnas.1423175112

140. Yang, P. L., Hsu, T. H., Wang, C. W., Chen, R. H.: Lipid droplets maintain lipid homeostasis during anaphase for efficient cell separation in budding yeast. *Mol Biol Cell*, 27, 2368-2380 (2016)
DOI: 10.1091/mbc.E16-02-0106

141. Barbosa, A. D., Siniossoglou, S.: Function of lipid droplet-organelle interactions in lipid homeostasis. *Biochim Biophys Acta*, 1864, 1459-1468 (2017)
DOI: 10.1016/j.bbamcr.2017.04.001

142. van Zutphen, T., Todde, V., de Boer, R., Kreim, M., Hofbauer, H. F., Wolinski, H., Veenhuis, M., van der Klei, I. J., Kohlwein, S. D.: Lipid droplet autophagy in the yeast *Saccharomyces cerevisiae*. *Mol Biol Cell*, 25, 290-301 (2014)
DOI: 10.1091/mbc.E13-08-0448

143. Barbosa, A. D., Savage, D. B., Siniossoglou, S.: Lipid droplet-organelle interactions: emerging roles in lipid metabolism. *Curr Opin Cell Biol*, 35, 91-97 (2015)
DOI: 10.1016/j.ceb.2015.04.017

144. Shpilka, T., Welter, E., Borovsky, N., Amar, N., Mari, M., Reggiori, F., Elazar, Z.: Lipid droplets and their component triglycerides and steryl esters regulate autophagosome biogenesis. *EMBO J*, 34, 2117-2131 (2015)
DOI: 10.15252/embj.201490315

145. Vevea, J. D., Garcia, E. J., Chan, R. B., Zhou, B., Schultz, M., Di Paolo, G., McCaffery, J. M., Pon, L. A.: Role for lipid droplet biogenesis and microlipophagy in adaptation to lipid imbalance in yeast. *Dev Cell*, 35, 584-599 (2015)
DOI: 10.1016/j.devcel.2015.11.010

146. Eisenberg-Bord, M., Shai, N., Schuldiner, M., Bohnert, M.: A tether is a tether is a tether: tethering at membrane contact sites. *Dev Cell*, 39, 395-409 (2016)
DOI: 10.1016/j.devcel.2016.10.022

147. Wong, L. H., Levine, T. P.: Lipid transfer proteins do their thing anchored at membrane contact sites… but what is their thing? *Biochem Soc Trans*, 44, 517-527 (2016)
DOI: 10.1042/BST20150275

148. Dimmer, K. S., Rapaport, D.: Mitochondrial contact sites as platforms for phospholipid

exchange. *Biochim Biophys Acta*, 1862, 69-80 (2017)
DOI: 10.1016/j.bbalip.2016.07.010

149. Schuldiner, M., Bohnert, M.: A different kind of love - lipid droplet contact sites. *Biochim Biophys Acta*, pii: S1388-1981(17)30109-9 (2017)

150. Santos-Rosa, H., Leung, J., Grimsey, N., Peak-Chew, S., Siniossoglou, S.: The yeast lipin Smp2 couples phospholipid biosynthesis to nuclear membrane growth. *EMBO J*, 24, 1931-1941 (2005)
DOI: 10.1038/sj.emboj.7600672

151. Choi, H. S., Su, W. M., Morgan, J. M., Han, G. S., Xu, Z., Karanasios, E., Siniossoglou, S., Carman, G. M.: Phosphorylation of phosphatidate phosphatase regulates its membrane association and physiological functions in *Saccharomyces cerevisiae*: identification of SER(602), THR(723), and SER(744) as the sites phosphorylated by *CDC28* (*CDK1*)-encoded cyclin-dependent kinase. *J Biol Chem*, 286, 1486-1498 (2011)
DOI: 10.1074/jbc.M110.155598

152. Choi, H. S., Su, W. M., Han, G. S., Plote, D., Xu, Z., Carman, G. M.: Pho85p-Pho80p phosphorylation of yeast Pah1p phosphatidate phosphatase regulates its activity, location, abundance, and function in lipid metabolism. *J Biol Chem*, 287, 11290-11301 (2012)
DOI: 10.1074/jbc.M112.346023

153. Pascual, F., Carman, G. M.: Phosphatidate phosphatase, a key regulator of lipid homeostasis. *Biochim Biophys Acta*, 1831, 514-522 (2013)
DOI: 10.1016/j.bbalip.2012.08.006

154. Shirra, M. K., Patton-Vogt, J., Ulrich, A., Liuta-Tehlivets, O., Kohlwein, S. D., Henry, S. A., Arndt, K. M.: Inhibition of acetyl coenzyme A carboxylase activity restores expression of the *INO1* gene in a *snf1* mutant strain of *Saccharomyces cerevisiae*. *Mol Cell Biol*, 21, 5710-5722 (2001)
DOI: 10.1128/MCB.21.17.5710-5722.2001

155. Su, W. M., Han, G. S., Casciano, J., Carman, G. M.: Protein kinase A-mediated phosphorylation of Pah1p phosphatidate phosphatase functions in conjunction with the Pho85p-Pho80p and Cdc28p-cyclin B kinases to regulate lipid synthesis in yeast. *J Biol Chem*, 287, 33364-33376 (2012)
DOI: 10.1074/jbc.M112.402339

156. Xu, Z., Su, W. M., Carman, G. M.: Fluorescence spectroscopy measures yeast PAH1-encoded phosphatidate phosphatase interaction with liposome membranes. *J Lipid Res*, 53, 522-528 (2012)
DOI: 10.1194/jlr.M022798

157. Madeira, J. B., Masuda, C. A., Maya-Monteiro, C. M., Matos, G. S., Montero-Lomelí, M., Bozaquel-Morais, B. L.: TORC1 inhibition induces lipid droplet replenishment in yeast. *Mol Cell Biol*, 35, 737-746 (2015)
DOI: 10.1128/MCB.01314-14

158. Handee, W., Li, X., Hall, K. W., Deng, X., Li, P., Benning, C., Williams, B. L., Kuo, M. H.: An energy-independent pro-longevity function of triacylglycerol in yeast. *PloS Genet*, 12, e1005878 (2016)
DOI: 10.1371/journal.pgen.1005878

159. Li, X., Handee, W., Kuo, M. H.: The slim, the fat, and the obese: guess who lives the longest? *Curr Genet*, 63, 43-49 (2017)
DOI: 10.1007/s00294-016-0617-z

160. Sinclair, D. A.: Toward a unified theory of caloric restriction and longevity regulation. *Mech Ageing Dev*, 126, 987-1002 (2005)
DOI: 10.1016/j.mad.2005.03.019

161. Goldberg, A. A., Bourque, S. D., Kyryakov, P., Gregg, C., Boukh-Viner, T., Beach, A., Burstein, M. T., Machkalyan, G., Richard, V., Rampersad, S., Cyr, D., Milijevic, S., Titorenko, V. I.: Effect of calorie restriction on the metabolic history of chronologically aging yeast. *Exp Gerontol*, 44, 555-571 (2009)
DOI: 10.1016/j.exger.2009.06.001

162. Goldberg, A. A., Richard, V. R., Kyryakov, P., Bourque, S. D., Beach, A., Burstein, M. T., Glebov, A., Koupaki, O., Boukh-Viner, T., Gregg, C., Juneau, M., English, A. M., Thomas, D. Y., Titorenko, V. I.: Chemical genetic screen identifies lithocholic acid as an anti-aging compound that extends yeast chronological life span in a TOR-independent manner, by modulating housekeeping longevity assurance processes. *Aging (Albany NY)*, 2, 393-414 (2010)
DOI: 10.18632/aging.100168

163. Colman, R. J., Anderson, R. M., Johnson, S. C., Kastman, E. K., Kosmatka, K. J., Beasley, T. M., Allison, D. B., Cruzen, C., Simmons, H. A., Kemnitz, J. W., Weindruch, R.: Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science*, 325, 201-204 (2009)
DOI: 10.1126/science.1173635

164. Fontana, L., Partridge, L., Longo, V. D.: Extending healthy life span - from yeast to humans. *Science*, 328, 321-326 (2010)
DOI: 10.1126/science.1172539

165. Colman, R. J., Beasley, T. M., Kemnitz, J. W., Johnson, S. C., Weindruch, R., Anderson, R. M.: Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. *Nat Commun*, 5, 3557 (2014)
DOI: 10.1038/ncomms4557

166. de Cabo, R., Carmona-Gutierrez, D., Bernier, M., Hall, M. N., Madeo, F.: The search for antiaging interventions: from elixirs to fasting regimens. *Cell*, 157, 1515-1526 (2014)
DOI: 10.1016/j.cell.2014.05.031

167. Lee, C., Longo, V.: Dietary restriction with and without caloric restriction for healthy aging. *F1000Res*, 5, F1000 Faculty Rev-117 (2016)

168. Fabrizio, P., Gattazzo, C., Battistella, L., Wei, M., Cheng, C., McGrew, K., Longo, V. D.: Sir2 blocks extreme life-span extension. *Cell*, 123, 655-667 (2005)
DOI: 10.1016/j.cell.2005.08.042

169. Hiltunen, J. K., Mursula, A. M., Rottensteiner, H., Wierenga, R. K., Kastaniotis, A. J., Gurvitz, A.: The biochemistry of peroxisomal beta-oxidation in the yeast *Saccharomyces cerevisiae*. *FEMS Microbiol Rev*, 27, 35-64 (2003)
DOI: 10.1016/S0168-6445(03)00017-2

170. van der Klei, I. J., Yurimoto, H., Sakai, Y., Veenhuis, M.: The significance of peroxisomes in methanol metabolism in methylotrophic yeast. *Biochim Biophys Acta*, 1763, 1453-1462 (2006)
DOI: 10.1016/j.bbamcr.2006.07.016

171. Beach, A., Titorenko, V. I.: In search of housekeeping pathways that regulate longevity. *Cell Cycle*, 10, 3042-3044 (2011)
DOI: 10.4161/cc.10.18.16947

172. Beach, A., Titorenko, V. I.: Essential roles of peroxisomally produced and metabolized biomolecules in regulating yeast longevity. Subcell Biochem, 69, 153-167 (2013)
DOI: 10.1007/978-94-007-6889-5_9

173. Sheibani, S., Richard, V. R., Beach, A., Leonov, A., Feldman, R., Mattie, S., Khelghatybana, L., Piano, A., Greenwood, M., Vali, H., Titorenko, V. I.: Macromitophagy, neutral lipids synthesis, and peroxisomal fatty acid oxidation protect yeast from “liponecrosis”, a previously unknown form of programmed cell death. *Cell Cycle*, 13, 138-147 (2014)
DOI: 10.4161/cc.26885

174. Lefebvre, P., Cariou, B., Lien, F., Kuipers, F., Staels, B.: Role of bile acids and bile acid receptors in metabolic regulation. *Physiol Rev*, 89, 147-191 (2009)
DOI: 10.1152/physrev.00010.2008

175. Goldberg, A. A., Kyryakov, P., Bourque, S. D., Titorenko, V. I.: Xenohormetic, hormetic and cytostatic selective forces driving longevity at the ecosystemic level. *Aging (Albany NY)*, 2, 461-470 (2010)
DOI: 10.18632/aging.100186

176. Gomez-Perez, A., Kyryakov, P., Burstein, M. T., Asbah, N., Noohi, F., Iouk, T., Titorenko, V. I.: Empirical validation of a hypothesis of the hormetic selective forces driving the evolution of longevity regulation mechanisms. *Front Genet*, 7, 216 (2016)
DOI: 10.3389/fgene.2016.00216

177. Kyryakov, P., Gomez-Perez, A., Glebov, A., Asbah, N., Bruno, L., Meunier, C., Iouk, T., Titorenko, V. I.: Empirical verification of evolutionary theories of aging. *Aging (Albany NY)*, 8, 2568-2589 (2016)
DOI: 10.18632/aging.101090

178. Beach, A., Richard, V. R., Leonov, A., Burstein, M. T., Bourque, S. D., Koupaki, O., Juneau, M., Feldman, R., Iouk, T., Titorenko, V. I.: Mitochondrial membrane lipidome defines yeast longevity. *Aging (Albany NY)*, 5, 551-574 (2013)
DOI: 10.18632/aging.100578

179. Burstein, M. T., Kyryakov, P., Beach, A., Richard, V. R., Koupaki, O., Gomez-Perez, A., Leonov, A., Levy, S., Noohi, F., Titorenko, V. I.: Lithocholic acid extends longevity of chronologically aging yeast only if added at

certain critical periods of their lifespan. *Cell Cycle*, 11, 3443-3462 (2012)
DOI: 10.4161/cc.21754

180. Richard, V. R., Leonov, A., Beach, A., Burstein, M. T., Koupaki, O., Gomez-Perez, A., Levy, S., Pluska, L., Mattie, S., Rafesh, R., Iouk, T., Sheibani, S., Greenwood, M., Vali, H., Titorenko, V. I.: Macromitophagy is a longevity assurance process that in chronologically aging yeast limited in calorie supply sustains functional mitochondria and maintains cellular lipid homeostasis. *Aging (Albany NY)*, 5, 234-269 (2013)
DOI: 10.18632/aging.100547

181. Beach, A., Richard, V. R., Bourque, S., Boukh-Viner, T., Kyryakov, P., Gomez-Perez, A., Arlia-Ciommo, A., Feldman, R., Leonov, A., Piano, A., Svistkova, V., Titorenko, V. I.: Lithocholic bile acid accumulated in yeast mitochondria orchestrates a development of an anti-aging cellular pattern by causing age-related changes in cellular proteome. *Cell Cycle*, 14, 1643-1656 (2015)
DOI: 10.1080/15384101.2015.1026493

182. Vögtle, F. N., Keller, M., Taskin, A. A., Horvath, S. E., Guan, X. L., Prinz, C., Opalińska, M., Zorzin, C., van der Laan, M., Wenk, M. R., Schubert, R., Wiedemann, N., Holzer, M., Meisinger, C.: The fusogenic lipid phosphatidic acid promotes the biogenesis of mitochondrial outer membrane protein Ugo1. *J Cell Biol*, 210, 951-960 (2015)
DOI: 10.1083/jcb.201506085

183. Leonov, A., Arlia-Ciommo, A., Bourque, S. D., Koupaki, O., Kyryakov, P., Dakik, P., McAuley, M., Medkour, Y., Mohammad, K., Di Maulo, T., Titorenko, V. I.: Specific changes in mitochondrial lipidome alter mitochondrial proteome and increase the geroprotective efficiency of lithocholic acid in chronologically aging yeast. *Oncotarget*, 8, 30672-30691 (2017)
DOI: 10.18632/oncotarget.16766

184. Arlia-Ciommo, A., Piano, A., Svistkova, V., Mohtashami, S., Titorenko, V. I.: Mechanisms underlying the anti-aging and anti-tumor effects of lithocholic bile acid. *Int J Mol Sci*, 15, 16522-16543 (2014)
DOI: 10.3390/ijms150916522

185. Beach, A., Leonov, A., Arlia-Ciommo, A., Svistkova, V., Lutchman, V., Titorenko, V. I.: Mechanisms by which different functional states of mitochondria define yeast longevity. *Int J Mol Sci*, 16, 5528-5554 (2015)
DOI: 10.3390/ijms16035528

186. Burstein, M. T., Titorenko, V. I.: A mitochondrially targeted compound delays aging in yeast through a mechanism linking mitochondrial membrane lipid metabolism to mitochondrial redox biology. *Redox Biol*, 2, 305-307 (2014)
DOI: 10.1016/j.redox.2014.01.011

187. Medkour, Y., Titorenko, V. I.: Mitochondria operate as signaling platforms in yeast aging. *Aging (Albany NY)*, 8, 212-213 (2016)
DOI: 10.18632/aging.100914

188. Medkour, Y., Dakik, P., McAuley, M., Mohammad, K., Mitrofanova, D., Titorenko, V. I.: Mechanisms underlying the essential role of mitochondrial membrane lipids in yeast chronological aging. *Oxid Med Cell Longev*, 2017, 2916985 (2017)
DOI: 10.1155/2017/2916985

189. McMahon, H. T., Boucrot, E.: Membrane curvature at a glance. *J Cell Sci*, 128, 1065-1070 (2015)
DOI: 10.1242/jcs.114454

190. Jarsch, I. K., Daste, F., Gallop, J. L.: Membrane curvature in cell biology: An integration of molecular mechanisms. *J Cell Biol*, 214, 375-387 (2016)
DOI: 10.1083/jcb.201604003

191. Mårtensson, C. U., Doan, K. N., Becker, T.: Effects of lipids on mitochondrial functions. *Biochim Biophys Acta*, 1862, 102-113 (2017)
DOI: 10.1016/j.bbalip.2016.06.015

192. Tatsuta, T., Langer, T.: Intramitochondrial phospholipid trafficking. *Biochim Biophys Acta*, 1862, 81-89 (2017)
DOI: 10.1016/j.bbalip.2016.08.006

193. Gerisch, B., Rottiers, V., Li, D., Motola, D. L., Cummins, C. L., Lehrach, H., Mangelsdorf, D. J., Antebi, A.: A bile acid-like steroid modulates *Caenorhabditis elegans* lifespan through nuclear receptor signaling. *Proc Natl Acad Sci USA*, 104, 5014-5019 (2007)
DOI: 10.1073/pnas.0700847104

194. Wang, M. C., O'Rourke, E. J., Ruvkun, G.: Fat metabolism links germline stem cells

and longevity in *C. elegans*. *Science*, 322, 957-960 (2008)
DOI: 10.1126/science.1162011

195. Narbonne, P., Roy, R.: *Caenorhabditis elegans* dauers need LKB1/AMPK to ration lipid reserves and ensure long-term survival. *Nature*, 457, 210-214 (2009)
DOI: 10.1038/nature07536

196. Soukas, A. A., Kane, E. A., Carr, C. E., Melo, J. A., Ruvkun, G.: Rictor/TORC2 regulates fat metabolism, feeding, growth, and life span in *Caenorhabditis elegans*. *Genes Dev*, 23, 496-511 (2009)
DOI: 10.1101/gad.1775409

197. Shmookler Reis, R. J., Xu, L., Lee, H., Chae, M., Thaden, J. J., Bharill, P., Tazearslan, C., Siegel, E., Alla, R., Zimniak, P., Ayyadevara, S.: Modulation of lipid biosynthesis contributes to stress resistance and longevity of *C. elegans* mutants. *Aging (Albany NY)*, 3, 125-147 (2011)
DOI: 10.18632/aging.100275

198. Ackerman, D., Gems, D.: The mystery of *C. elegans* aging: an emerging role for fat. Distant parallels between *C. elegans* aging and metabolic syndrome? *Bioessays*, 34, 466-471 (2012)
DOI: 10.1002/bies.201100189

199. Hou, N. S., Taubert, S.: Function and regulation of lipid biology in *Caenorhabditis elegans* aging. *Front Physiol*, 3, 143 (2012)
DOI: 10.3389/fphys.2012.00143

200. Antebi, A.: Regulation of longevity by the reproductive system. *Exp Gerontol*, 48, 596-602 (2013)
DOI: 10.1016/j.exger.2012.09.009

201. Haynes, C. M., Fiorese, C. J., Lin, Y. F.: Evaluating and responding to mitochondrial dysfunction: the mitochondrial unfolded-protein response and beyond. *Trends Cell Biol*, 23, 311-318 (2013)
DOI: 10.1016/j.tcb.2013.02.002

202. Kniazeva, M., Han, M.: Fat chance for longevity. *Genes Dev*, 27, 351-354 (2013)
DOI: 10.1101/gad.214189.113

203. Magner, D. B., Wollam, J., Shen, Y., Hoppe, C., Li, D., Latza, C., Rottiers, V., Hutter, H., Antebi, A.: The NHR-8 nuclear receptor regulates cholesterol and bile acid homeostasis in *C. elegans*. *Cell Metab*, 18, 212-224 (2013)
DOI: 10.1016/j.cmet.2013.07.007

204. O'Rourke, E. J., Kuballa, P., Xavier, R., Ruvkun, G.: ω-6 Polyunsaturated fatty acids extend life span through the activation of autophagy. *Genes Dev*, 27, 429-440 (2013)
DOI: 10.1101/gad.205294.112

205. Mahanti, P., Bose, N., Bethke, A., Judkins, J. C., Wollam, J., Dumas, K. J., Zimmerman, A.M., Campbell, S. L., Hu, P. J., Antebi, A., Schroeder, F. C.: Comparative metabolomics reveals endogenous ligands of DAF-12, a nuclear hormone receptor, regulating *C. elegans* development and lifespan. *Cell Metab*, 19, 73-83 (2014)
DOI: 10.1016/j.cmet.2013.11.024

206. Folick, A., Oakley, H. D., Yu, Y., Armstrong, E. H., Kumari, M., Sanor, L., Moore, D. D., Ortlund, E. A., Zechner, R., Wang, M. C.: Aging. Lysosomal signaling molecules regulate longevity in *Caenorhabditis elegans*. *Science*, 347, 83-86 (2015)
DOI: 10.1126/science.1258857

207. Niso-Santano, M., Malik, S. A., Pietrocola, F., Bravo-San Pedro, J. M., Mariño, G., Cianfanelli, V., Ben-Younès, A., Troncoso, R., Markaki, M., Sica, V., Izzo, V., Chaba, K., Bauvy, C., Dupont, N., Kepp, O., Rockenfeller, P., Wolinski, H., Madeo, F., Lavandero, S., Codogno, P., Harper, F., Pierron, G., Tavernarakis, N., Cecconi, F., Maiuri, M. C., Galluzzi, L., Kroemer, G.: Unsaturated fatty acids induce non-canonical autophagy. *EMBO J*, 34, 1025-1041 (2015)
DOI: 10.15252/embj.201489363

208. Schroeder, E. A., Brunet, A.: Lipid profiles and signals for long life. *Trends Endocrinol Metab*, 26, 589-592 (2015)
DOI: 10.1016/j.tem.2015.08.007

209. Schulz, A. M., Haynes, C. M.: UPR^{mt}-mediated cytoprotection and organismal aging. *Biochim Biophys Acta*, 1847, 1448-1456 (2015)
DOI: 10.1016/j.bbabio.2015.03.008

210. Aguilaniu, H., Fabrizio, P., Witting, M.: The role of dafachronic acid signaling in development and longevity in *Caenorhabditis elegans*: Digging deeper using cutting-edge analytical chemistry. *Front Endocrinol (Lausanne)*, 7, 12 (2016)
DOI: 10.3389/fendo.2016.00012

211. Kim, H. E., Grant, A. R., Simic, M. S., Kohnz, R. A., Nomura, D. K., Durieux, J., Riera, C. E., Sanchez, M., Kapernick, E., Wolff, S., Dillin, A.: Lipid biosynthesis coordinates a

mitochondrial-to-cytosolic stress response. *Cell*, 166, 1539-1552 (2016)
DOI: 10.1016/j.cell.2016.08.027

212. Lemieux, G. A., Ashrafi, K.: Investigating connections between metabolism, longevity, and behavior in *Caenorhabditis elegans*. *Trends Endocrinol Metab*, 27, 586-596 (2016)
DOI: 10.1016/j.tem.2016.05.004

213. Watts, J. L.: Using *Caenorhabditis elegans* to uncover conserved functions of omega-3 and omega-6 fatty acids. *J Clin Med*, 5, E19 (2016)
DOI: 10.3390/jcm5020019

214. Grönke, S., Mildner, A., Fellert, S., Tennagels, N., Petry, S., Müller, G., Jäckle, H., Kühnlein, R. P.: Brummer lipase is an evolutionary conserved fat storage regulator in *Drosophila*. *Cell Metab*, 1, 323-330 (2005)
DOI: 10.1016/j.cmet.2005.04.003

215. Teleman, A. A., Chen, Y., Cohen, S. M.: 4E-BP functions as a metabolic brake used under stress conditions but not during normal growth. *Genes Dev*, 19, 1844-1848 (2005)
DOI: 10.1101/gad.341505

216. Skorupa, D. A., Dervisefendic, A., Zwiener, J., Pletcher, S. D.: Dietary composition specifies consumption, obesity, and lifespan in *Drosophila melanogaster*. *Aging Cell*, 7, 478-490 (2008)
DOI: 10.1111/j.1474-9726.2008.00400.x

217. Bjedov, I., Toivonen, J. M., Kerr, F., Slack, C., Jacobson, J., Foley, A., Partridge, L.: Mechanisms of life span extension by rapamycin in the fruit fly *Drosophila melanogaster*. *Cell Metab*, 11, 35-46 (2010)
DOI: 10.1016/j.cmet.2009.11.010

218. Alic, N., Hoddinott, M. P., Vinti, G., Partridge, L.: Lifespan extension by increased expression of the *Drosophila* homologue of the IGFBP7 tumour suppressor. *Aging Cell*, 10, 137-147 (2011)
DOI: 10.1111/j.1474-9726.2010.00653.x

219. Partridge, L., Alic, N., Bjedov, I., Piper, M. D.: Ageing in *Drosophila*: the role of the insulin/Igf and TOR signalling network. *Exp Gerontol*, 46, 376-381 (2011)
DOI: 10.1016/j.exger.2010.09.003

220. Katewa, S. D., Demontis, F., Kolipinski, M., Hubbard, A., Gill, M. S., Perrimon, N., Melov, S., Kapahi, P.: Intramyocellular fatty-acid metabolism plays a critical role in mediating responses to dietary restriction in *Drosophila melanogaster*. *Cell Metabolism*, 16, 97-103 (2012)
DOI: 10.1016/j.cmet.2012.06.005

221. Karpac, J., Biteau, B., Jasper, H.: Misregulation of an adaptive metabolic response contributes to the age-related disruption of lipid homeostasis in *Drosophila*. *Cell Rep*, 4, 1250-1261 (2013)
DOI: 10.1016/j.celrep.2013.08.004

222. Emran, S., Yang, M., He, X., Zandveld, J., Piper, M. D.: Target of rapamycin signalling mediates the lifespan-extending effects of dietary restriction by essential amino acid alteration. *Aging (Albany NY)*, 6, 390-398 (2014)
DOI: 10.18632/aging.100665

223. Minois, N., Rockenfeller, P., Smith, T. K., Carmona-Gutierrez, D.: Spermidine feeding decreases age-related locomotor activity loss and induces changes in lipid composition. *PLoS One*, 9, e102435 (2014)
DOI: 10.1371/journal.pone.0102435

224. Blüher, M., Kahn, B. B., Kahn, C. R.: Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science*, 299, 572-574 (2003)
DOI: 10.1126/science.1078223

225. Chiu, C. H., Lin, W. D., Huang, S. Y., Lee, Y. H.: Effect of a C/EBP gene replacement on mitochondrial biogenesis in fat cells. *Genes Dev*, 18, 1970-1975 (2004)
DOI: 10.1101/gad.1213104

226. Picard, F., Kurtev, M., Chung, N., Topark-Ngarm, A., Senawong, T., Machado De Oliveira, R., Leid, M., McBurney, M. W., Guarente, L.: Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. *Nature*, 429, 771-776 (2004)
DOI: 10.1038/nature02583

227. Bordone, L., Guarente, L.: Calorie restriction, SIRT1 and metabolism: understanding longevity. *Nat Rev Mol Cell Biol*, 6, 298-305 (2005)
DOI: 10.1038/nrm1616

228. Argmann, C., Dobrin, R., Heikkinen, S., Auburtin, A., Pouilly, L., Cock, T. A., Kutnikova, H., Zhu, J., Schadt, E. E., Auwerx, J.: Ppargamma2 is a key driver

of longevity in the mouse. *PLoS Genet*, 5, e1000752 (2009)
DOI: 10.1371/journal.pgen.1000752

229. Enns, L. C., Morton, J. F., Treuting, P. R., Emond, M. J., Wolf, N. S., Dai, D. F., McKnight, G. S., Rabinovitch, P. S., Ladiges, W. C.: Disruption of protein kinase A in mice enhances healthy aging. *PLoS One*, 4, e5963 (2009)
DOI: 10.1371/journal.pone.0005963

230. Ranieri, S. C., Fusco, S., Panieri, E., Labate, V., Mele, M., Tesori, V., Ferrara, A. M., Maulucci, G., De Spirito, M., Martorana, G. E., Galeotti, T., Pan, G.: Mammalian life-span determinant p66shcA mediates obesity-induced insulin resistance. *Proc Natl Acad Sci USA*, 107, 13420-13425 (2010)
DOI: 10.1073/pnas.1008647107

231. Streeper, R. S., Grueter, C. A., Salomonis, N., Cases, S., Levin, M. C., Koliwad, S. K., Zhou, P., Hirschey, M. D., Verdin, E., Farese, R. V. Jr.: Deficiency of the lipid synthesis enzyme, DGAT1, extends longevity in mice. *Aging (Albany NY)*, 4, 13-27 (2012)
DOI: 10.18632/aging.100424

232. Gonzalez-Covarrubias, V.: Lipidomics in longevity and healthy aging. *Biogerontology*, 14, 663-672 (2013)
DOI: 10.1007/s10522-013-9450-7

233. Gonzalez-Covarrubias, V., Beekman, M., Uh, H. W., Dane, A., Troost, J., Paliukhovich, I., van der Kloet, F. M., Houwing-Duistermaat, J., Vreeken, R. J., Hankemeier, T., Slagboom, E. P.: Lipidomics of familial longevity. *Aging Cell*, 12, 426-434 (2013)
DOI: 10.1111/acel.12064

234. Jové, M., Naudí, A., Aledo, J. C., Cabré, R., Ayala, V., Portero-Otin, M., Barja, G., Pamplona, R.: Plasma long-chain free fatty acids predict mammalian longevity. *Sci Rep*, 3, 3346 (2013)
DOI: 10.1038/srep03346

235. Naudí, A., Jové, M., Ayala, V., Portero-Otín, M., Barja, G., Pamplona, R.: Membrane lipid unsaturation as physiological adaptation to animal longevity. *Front Physiol*, 4, 372 (2013)
DOI: 10.3389/fphys.2013.00372

236. Canaan, A., DeFuria, J., Perelman, E., Schultz, V., Seay, M., Tuck, D., Flavell, R. A., Snyder, M. P., Obin, M. S., Weissman, S. M.: Extended lifespan and reduced adiposity in mice lacking the *FAT10* gene. *Proc Natl Acad Sci USA*, 111, 5313-5318 (2014)
DOI: 10.1073/pnas.1323426111

237. Jové, M., Naudí, A., Ramírez-Núñez, O., Portero-Otín, M., Selman, C., Withers, D. J., Pamplona, R.: Caloric restriction reveals a metabolomic and lipidomic signature in liver of male mice. *Aging Cell*, 13, 828-837 (2014)
DOI: 10.1111/acel.12241

238. Martin-Montalvo, A., Sun, Y., Diaz-Ruiz, A., Ali, A., Gutierrez, V., Palacios, H. H., Curtis, J., Siendones, E., Ariza, J., Abulwerdi, G. A., Sun, X., Wang, A. X., Pearson, K. J., Fishbein, K. W., Spencer, R. G., Wang, M., Han, X., Scheibye-Knudsen, M., Baur, J. A., Shertzer, H. G., Navas, P., Villalba, J. M., Zou, S., Bernier, M., de Cabo, R.: Cytochrome b_5 reductase and the control of lipid metabolism and healthspan. *NPJ Aging Mech Dis*, 2, 16006 (2016)
DOI: 10.1038/npjamd.2016.6

239. Green, C. L., Mitchell, S. E., Derous, D., Wang, Y., Chen, L., Han, J. J., Promislow, D. E. L., Lusseau, D., Douglas, A., Speakman, J. R.: The effects of graded levels of calorie restriction: IX. Global metabolomic screen reveals modulation of carnitines, sphingolipids and bile acids in the liver of C57BL/6 mice. *Aging Cell*, 16, 529-540 (2017)
DOI: 10.1111/acel.12570

240. Miller, K. N., Burhans, M. S., Clark, J. P., Howell, P. R., Polewski, M. A., DeMuth, T. M., Eliceiri, K. W., Lindstrom, M. J., Ntambi, J. M., Anderson, R. M.: Aging and caloric restriction impact adipose tissue, adiponectin, and circulating lipids. *Aging Cell*, 16, 497-507 (2017)
DOI: 10.1111/acel.12575

241. Bozek, K., Khrameeva, E.E., Reznick, J., Omerbašić, D., Bennett, N. C., Lewin, G.R., Azpurua, J., Gorbunova, V., Seluanov, A., Regnard, P., Wanert, F., Marchal, J., Pifferi, F., Aujard, F., Liu, Z., Shi, P., Pääbo, S., Schroeder, F., Willmitzer, L., Giavalisco, P., Khaitovich, P.: Lipidome determinants of maximal lifespan in mammals. *Sci Rep*, 7, 5 (2017)
DOI: 10.1038/s41598-017-00037-7

242. Mapstone, M., Cheema, A. K., Fiandaca, M. S., Zhong, X., Mhyre, T. R., MacArthur, L. H., Hall, W. J., Fisher, S. G., Peterson, D.

R., Haley, J. M., Nazar, M. D., Rich, S. A., Berlau, D. J., Peltz, C. B., Tan, M. T., Kawas, C. H., Federoff, H. J.: Plasma phospholipids identify antecedent memory impairment in older adults. *Nat Med*, 20, 415-418 (2014) DOI: 10.1038/nm.3466

243. Cheng, S., Larson, M. G., McCabe, E. L., Murabito, J. M., Rhee, E. P., Ho, J. E., Jacques, P. F., Ghorbani, A., Magnusson, M., Souza, A. L., Deik, A. A., Pierce, K. A., Bullock, K., O'Donnell, C. J., Melander, O., Clish, C. B., Vasan, R. S., Gerszten, R. E., Wang, T. J.: Distinct metabolomic signatures are associated with longevity in humans. *Nat Commun*, 6, 6791 (2015) DOI: 10.1038/ncomms7791

244. Mielke, M. M., Bandaru, V. V., Han, D., An, Y., Resnick, S. M., Ferrucci, L. Haughey, N. J.: Factors affecting longitudinal trajectories of plasma sphingomyelins: the Baltimore Longitudinal Study of Aging. *Aging Cell*, 14, 112-121 (2015) DOI: 10.1111/acel.12275

245. Fabbri, E., Yang, A., Simonsick, E. M., Chia, C. W., Zoli, M., Haughey, N. J., Mielke, M. M., Ferrucci, L., Coen, P. M.: Circulating ceramides are inversely associated with cardiorespiratory fitness in participants aged 54-96 years from the Baltimore Longitudinal Study of Aging. *Aging Cell*, 15, 825-831 (2016) DOI: 10.1111/acel.12491

246. Jové, M., Naudí, A., Gambini, J., Borras, C., Cabré, R., Portero-Otín, M., Viña, J., Pamplona, R.: A stress-resistant lipidomic signature confers extreme longevity to humans. *J Gerontol A Biol Sci Med Sci*, 72, 30-37 (2017) DOI: 10.1093/gerona/glw048

Abbreviations: ATG, autophagy; Aur1, aureobasidin A resistance 1; CerS, ceramide synthase; CK2, casein kinase 2; CLS, chronological lifespan; Csg1 and Csg2, calcium-sensitive growth 1 and 2 (respectively); Csh1, CSG1/SUR1 homolog1; DHS, dihydrosphingosine; Dpl1, dihydrosphingosine phosphate lyase 1; ER, endoplasmic reticulum; FA, fatty acid; IGF-1: insulin/insulin-like growth factor 1; HOG, high osmotic glycerol; IPC, inositol-phosphorylceramide; Ipt1, inositolphosphotransferase 1; Isc1, inositol phosphosphingolipid phospholipase C 1; Kei1, Kex2-cleavable protein essential for inositol-phosphorylceramide synthesis 1; Lac1, longevity-assurance gene cognate 1; Lag1, longevity assurance gene 1; Lip1, Lag1/Lac1 interacting protein 1; Lcb1, Lcb2, Lcb3, Lcb 4 and Lcb5, long-chain base proteins 1, 2, 3, 4 and 5 (respectively); MAPK, mitogen activated protein kinase; MCC, membrane comspartment containing Can1; MCT, membrane compartment containing TORC2; MIPC, mannosyl-inositol-phosphorylceramide; $M(IP)_2C$, mannosyl-di-inositol-phosphorylceramide; Orm1 and Orm2, orosomucoid 1 and 2 (respectively); PHS, phytosphingosine; PKA, protein kinase A; Pkh, Pkb-activating kinase homolog; PP2A, protein phosphatase type 2A; PP2B, protein phosphatase type 2B; RLS, replicative lifespan; Sac1, suppressor of actin 1; Snf, sucrose non-fermenting; SPT, serine palmitoyltransferase; SPOTS, serine palmitoyltransferase, Orm1/2 and Sac1; Sur2, suppressor 2 of Rvs161 and rvs167 mutations; TOR, target of rapamycin; TORC1, TOR complex 1; Tsc3 and Tsc10, temperature-sensitive suppressors of Csg2 mutants 3 and 10; Ydc1, yeast dihydroceramidase 1; Ypc1, yeast phytoceramidase 1; Ysr3, yeast sphingolipid resistance 3; Ypk1/2, yeast protein kinase 1 or 2

Key Words: Aging, Longevity, Mechanisms, Lipid metabolism, Review

Send correspondence to: Vladimir I. Titorenko, Department of Biology, Concordia University, 7141 Sherbrooke Street, West, Science Pavilion, Office SP-501-13, Montreal, Quebec, Canada H4B 1R6, Tel: 514-848-2424, Fax: 514-848-2881, E-mail: vladimir.titorenko@concordia.ca