MINIREVIEW

Lipids and cell death in yeast

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Abstract

Understanding lipid-induced malfunction represents a major challenge of today's biomedical research. The connection of lipids to cellular and organ dysfunction, cell death, and disease (often referred to as lipotoxicity) is more complex than the sole lipotoxic effects of excess free fatty acids and requires genetically tractable model systems for mechanistic investigation. We herein summarize recent advances in the field of lipid-induced toxicity that employ the established model system for cell death and aging research of budding yeast *Saccharomyces cerevisiae*. Studies in yeast have shed light on various aspects of lipotoxicity, including free fatty acid toxicity, sphingolipid-modulated cell death as well as the involvement of cardiolipin and lipid peroxidation in the mitochondrial pathways of apoptosis. Regimens used range from exogenously applied lipids, genetic modulation of lipolysis and triacylglyceride synthesis, variations in sphingolipid/ceramide metabolism as well as changes in peroxisome function by either genetic or pharmacological means. In future, the yeast model of programmed cell death will further contribute to the clarification of crucial questions of lipid-associated malfunction.

Introduction

Lipid-associated pathologies, including metabolic syndrome, diabetes, and other cardiovascular diseases, represent a strong burden to both individuals and society. Increasing obesity and advancing demographic aging constitute cardinal risk factors that make lipid-associated diseases a major health problem (Garbarino & Sturley, 2009). It is thus of utmost interest to understand the mechanisms behind such pathologies, on both the organismal and cellular levels. Common to these diseases is the decline of cellular and organ function that is associated with disturbed lipid homeostasis and altered lipid metabolism frequently culminating in cell death.

The connection of lipids to cell death is complex and likely involves distinct mechanisms that we are only beginning to understand. Lipotoxicity is generally referred to as the toxic consequences of lipid overload and frequently connected to free fatty acid (FFA) accumulation in nonadipose tissues (Unger & Orci, 2002; Schaffer, 2003; Brookheart *et al.*, 2009). Storage of FFA into neutral lipids is therefore believed to be an effective defense against lipotoxicity (Listenberger *et al.*, 2003; Garbarino & Sturley, 2009). Excess FFA are suspected to cause cellular dysfunction and to finally culminate in the induction of apoptosis, so-called lipoapoptosis, but may also induce other types of cell death such as necrosis (Navina *et al.*, 2011; Khan *et al.*, 2012). FFA-induced cell death may be responsible for the loss of pancreatic beta-cells during type 2 diabetes and contributes to other lipid-associated pathologies through killing of hepatocytes, cardiomyocytes, and renal parenchymal cells (for review see Brookheart *et al.*, 2009). Nevertheless, the paradigm that lipotoxicity results solely from FFA overload has shifted to a more general definition, as toxic accumulation of various lipid species has been reported (Garbarino & Sturley, 2009). For instance, free cholesterol and oxidized lipoproteins are also known to induce cell death in different cell culture models, and increasing evidence indicates that sphingolipids (e.g. ceramide, a known inducer of apoptosis), phospholipids, and cardiolipin modulate cell death and stress during lipid-associated diseases (Schaffer, 2003; Brookheart *et al.*, 2009; Garbarino & Sturley, 2009). Furthermore, lipid metabolism, including lipolysis and fatty acid oxidation (FAO), may contribute to essential cellular survival mechanisms, meeting energy demands and providing replenishment of metabolites to intermediate metabolism or lipid signaling molecules (Zechner *et al.*, 2012).

In this review, we summarize current knowledge on lipid-associated toxicity in *Saccharomyces cerevisiae*, which represents the yeast species most commonly applied for cell death research. To provide a thematic overview, we briefly introduce the yeast model system to study programmed cell death, including apoptosis and regulated forms of necrosis, and give a short outline of the main players of lipid metabolism and homeostasis in yeast.

Yeast as a model to study programmed cell death

The apoptotic core machinery present in higher eukaryotes is conserved in yeast to a degree that makes it a suitable model organism to approach crucial questions on human apoptosis (Greenwood & Ludovico, 2009; Carmona-Gutierrez *et al.*, 2010). Moreover, yeast undergoes nonapoptotic types of cell death upon certain stimuli, including for instance necrosis during aging (Eisenberg *et al.*, 2009; Eisenberg *et al.*, 2010). Multiple yeast orthologs of essential mammalian apoptotic proteins have been identified, including the caspase Yca1p (Madeo *et al.*, 2002), the serine protease Nma111p, which constitutes the homolog of mammalian HtrA2/Omi (Fahrenkrog *et al.*, 2004), and crucial mitochondrial death effectors such as endonuclease G (yeast Nuc1p) (Büttner *et al.*, 2007) or cytochrome *c* release (Ludovico *et al.*, 2002). While numerous cell death scenarios have been shown to depend on Yca1p, almost as many appear Yca1p independent (Madeo *et al.*, 2009). Conserved proteasomal, mitochondrial, and epigenetically regulated cell death pathways have been reported (Eisenberg *et al.*, 2007; Carmona-Gutierrez *et al.*, 2010). Intriguingly, yeast cells appear to harbor a functional mitochondrial cell death machinery that enables mammalian B-cell lymphoma 2 (BCL-2) family proteins to activate and inhibit cascades of apoptosis (Khoury & Greenwood, 2008; Silva *et al.*, 2011). Comparable to mammalian cells, yeast death induced by heterologous expression of B-cell lymphoma 2-associated protein X (BAX), a proapoptotic member of the BCL-2 protein family, involved insertion into mitochondrial membranes, mitochondrial dysfunction, and cytochrome c release (Priault *et al.*, 2003), and the antiapoptotic proteins BCL-2 and BCL-X_L could prevent these death-related changes (Tao *et al.*, 1997). Only recently, a yeast member of the BCL-2 family has been identified, namely the proapoptotic yeast BH3-only protein Ybh3p (Büttner *et al.*, 2011).

At first glance, the advantage for a unicellular organism to undergo apoptosis is not obvious. However, several studies depicted physiological scenarios, such as the process of chronological and replicative aging (Laun *et al.*, 2001; Fabrizio *et al.*, 2004; Herker *et al.*, 2004), in which death of single cells appears to favor the survival of the whole clonal yeast population, thus providing a teleological explanation for this unicellular suicide (Büttner *et al.*, 2006; Carmona-Gutierrez *et al.*, 2010). Importantly, many of the typical morphological changes indicative of apoptotic death are present in yeast, and the techniques to monitor these changes are established. A combination of precise measurements of survival with the direct assessment of morphological markers of apoptosis vs. necrosis allows both quantification and morphological differentiation of cell death. Survival is usually determined by two main strategies: (1) using fluorescent vital and dead dyes (Teng & Hardwick, 2009; Carmona-Gutierrez *et al.*, 2010; Eisenberg *et al.*, 2010); and (2) plating cells on agar plates to assess the ability to form colonies (clonogenic survival). While DNA degradation during apoptosis can be monitored by the TUNEL assay, the apoptotic exposure of phosphatidylserine to the outer leaflet of the plasma membrane is visualized by annexin V (AnnV) staining. Costaining of AnnV with propidium iodide (PI) is used to discriminate early-apoptotic ($AnnV^+/PI^-$), late-apoptotic and secondary necrotic ($AnnV^+/PI^+$) as well as primary necrotic ($AnnV^-/PI^+$) cells, the latter being also convicted by monitoring the nuclear-cytosolic translocation of yeast HMGB1 protein Nhp6Ap (Eisenberg *et al.*, 2009; Eisenberg *et al.*, 2010). For more details on the use of different markers of cell death in yeast and its potential problems, we refer to the relevant literature (Váchová & Palková, 2005; Carmona-Gutierrez *et al.*, 2010; Eisenberg *et al.*, 2010; Pereira & Saraiva, 2013).

Frequently, impaired growth of yeast cell cultures has been interpreted as increased fractions of cells undergoing cell death; however, such strategies bear potential pitfalls. While a stressed cell can suffer from delayed growth and the inability to divide and form a colony (senescence), it may still be alive with an active metabolism. In addition, the lack of growth after spotting cells on agar plates including a stress substance (drop test) could indicate cell death, but is indistinguishable from profound growth arrest. Therefore, we will emphasize the readouts used by the different studies reviewed herein (which are preferably a combination of distinct methods) instead of simply referring to cell death or toxicity observed.

Lipid metabolism in yeast at a glance

Among the major lipid constituents of a eukaryotic cell, glycerolipids, nonesterified or free fatty acids (FFA), phospholipids, sphingolipids, and sterols represent conserved energy stores and structural membrane components present in yeast. Extensive research using the budding yeast *S. cerevisiae* has led to fundamental insights into lipid

metabolism and lipid signaling, but also into lipid homeostasis in response to cellular stress (Cowart & Obeid, 2007; Kohlwein & Petschnigg, 2008; Hannun & Obeid, 2011; Raychaudhuri *et al.*, 2012).

Under normal physiological conditions, excess amounts of FFA and sterols are sequestered and stored as neutral lipids in lipid storage particles (also called lipid droplets), which contain a hydrophobic core mainly composed of triacylglycerols (TAG) and steryl esters (SE) (Rajakumari *et al.*, 2008; Kohlwein, 2010b). Once mobilized from TAG, fatty acids serve as intermediates for the synthesis of sphingolipids and phospholipids or for energy production (at least in higher eukaryotes) via FAO. In yeast, unlike in mammalian cells, FAO solely resides within peroxisomes (Van Roermund *et al.*, 2003). Notably, while yeast is in principle capable of growing on media containing fatty acids as the sole carbon source, it remains elusive if FFA mobilized from TAG are utilized for energy production in yeast. Under conditions of defective neutral lipid synthesis (TAG and SE), FFA accumulate and are mainly redirected into the pathway of phospholipid generation. Phospholipid synthesis is achieved either via the cytidine diphosphate-diacylglycerol (CDP-DAG) pathway from phosphatidic acid (PA) or via the Kennedy pathway, particularly in the presence of exogenous choline and ethanolamine (Carman & Zeimetz, 1996).

While the same subclasses of phospholipids exist in yeast and mammals, including PA, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, and cardiolipin, the fatty acid composition in mammalian cells is rather complex. In yeast, which in contrast to mammals completely lacks intrinsic polyunsaturation activity, the saturated fatty acids (SFA) palmitic (C_{16}) and stearic (C_{18}) acids as well as their monounsaturated derivates palmitoleic ($C_{16:1}$) and oleic ($C_{18:1}$) acids constitute the vast majority of fatty acids present (Schneiter *et al.*, 1999). Complex sphingolipids as well as ergosterol, the yeast counterpart to mammalian cholesterol, are specifically enriched in lipid rafts, which constitute dynamic microdomains within the plasma membrane regulating the function of associated proteins, signaling pathways, and membrane trafficking (Simons & Gerl, 2010). Beside these structural roles, intermediates of the sphingolipid metabolism such as ceramides, long-chain sphingoid bases (LCB), and their phosphates function as bioactive signaling lipids during numerous cellular processes (Simons & Gerl, 2010; Hannun & Obeid, 2011).

The first step in *de novo* sphingolipid synthesis is mediated by serine palmitoyltransferase (SPT), which is composed of the subunits Lcb1p and Lcb2p, and catalyzes the condensation of serine predominantly with palmitoyl-coenzyme A (CoA) to form 3-ketodihydrosphingosine (Hanada, 2003; Cowart & Obeid, 2007; Breslow *et al.*, 2010). 3-Ketodihydrosphingosine is only transiently generated and further reduced to the LCBs dihydrosphingosine and phytosphingosine (PHS), the central metabolites of sphingolipid metabolism. Through N-acylation of the LCBs, preferably with C_{24}–C_{26} acyl-CoAs, the ceramide species dihydroceramide and phytoceramide are formed (Cowart & Obeid, 2007; Hannun & Obeid, 2011). This conversion requires the ceramide synthases Lag1p (longevity assurance gene 1) or its paralog Lac1p (longevity assurance gene 1 cognate) (Schorling *et al.*, 2001). In addition to the briefly described *de novo* route, several studies support a salvage pathway of ceramide synthesis through hydrolysis of complex sphingolipids by inositol phosphosphingolipid phospholipase C, Isc1p (a homolog of mammalian neutral sphingomyelinases) (Cowart & Obeid, 2007; Kitagaki *et al.*, 2007). Interestingly, the Isc1p pathway may be particularly important for the generation of mitochondrial ceramides and maintenance of mitochondrial function (Kitagaki *et al.*, 2007).

As depicted in a simplified scheme in Fig. 1, the metabolism of FFA, TAG, phospholipids, and sphingolipids is tightly connected. Genetic or pharmacological modification of enzymatic activities or exogenous supply with specific lipid species most probably not only alters one specific metabolite concentration, but interferes with the complete network, thus complicating the determination of causal factor(s) for observed effects. In this review, we focus on TAG, FFA, sphingolipids, and the phospholipid cardiolipin, as these lipid species have been most frequently connected to *S. cerevisiae* cell death.

Sphingolipid-modulated cell death

Constituting not only structural elements in cellular membranes but also essential signaling molecules, sphingolipids including ceramides are involved in a variety of cellular processes. Variations in hydroxylation, unsaturation, chain length, and head groups lead to a diversity of sphingolipids in mammals and account for their wide range of functions. In yeast, the basic sphingolipid metabolism is conserved, but the complexity within is largely reduced. Only three complex sphingolipids are synthesized, which are inositol phosphoceramide, mannosyl-inositol phosphoceramide, and mannosyl-(inositol phosphate)$_2$ ceramide (Dickson & Lester, 2002), rendering yeast an ideal model system to study the complex relationship between sphingolipids, cell death subroutines, and aging (Fig. 2).

A general downregulation of sphingolipid synthesis by genetic or pharmacological blockage of the first step of sphingolipid biosynthesis (via depletion of the SPT subunits Lcb1p and Lcb2p or via administration of the SPT inhibitor myriocin, respectively), has been shown to cause growth retardation, but to extend the yeast

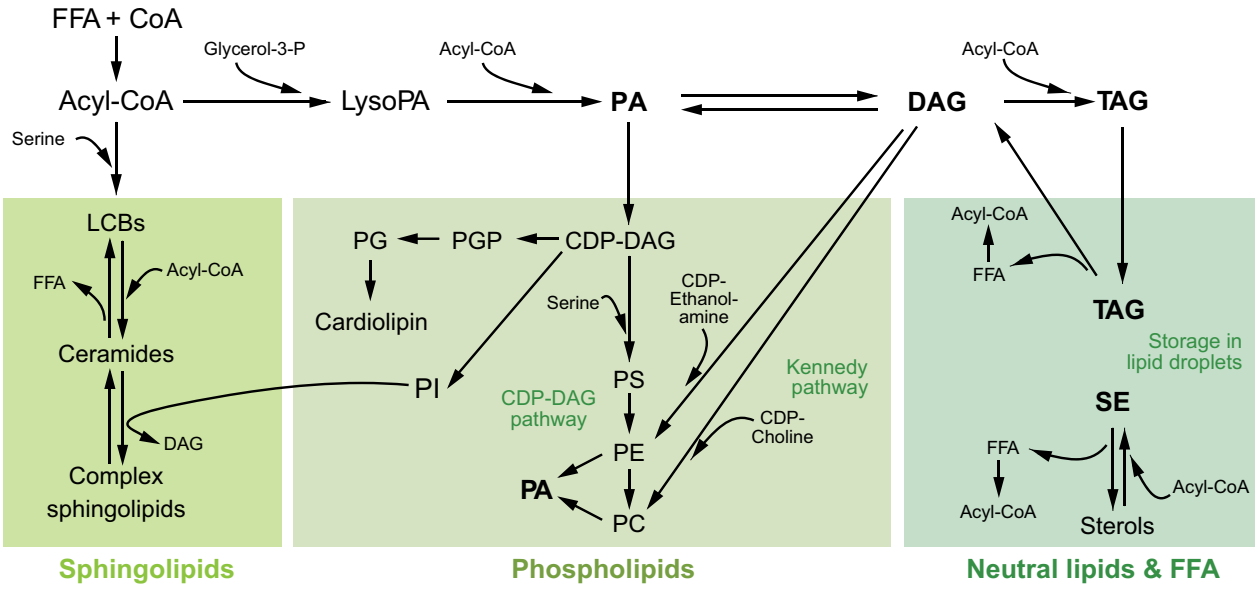

Fig. 1. Simplified overview of selected aspects of yeast lipid metabolism. Pathways and metabolites of the basic lipid homeostasis that are relevant to cell death have been depicted, including the synthesis of sphingolipids, phospholipids, and neutral lipids. The central metabolite phosphatidic acid (PA), which originates from glycerol-3-phosphate and free fatty acids (FFA), activated by condensation with coenzyme A (CoA), is used to synthesize diacylglycerol (DAG) and subsequently triacylglycerol (TAG), neutral lipids that can be stored in lipid droplets together with sterol esters (SE) generated from sterols (e.g. ergosterol). PA also serves as precursor for the generation of phospholipids via the cytidine diphosphate-diacylglycerol (CDP-DAG) pathway, leading to the formation of phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), or cardiolipin. Cardiolipin is synthesized via phosphatidylglycerophosphate (PGP), which is dephosphorylated to generate phosphatidylglycerol (PG). Alternatively, the Kennedy pathway can supply PE and PC from DAG. The first step of sphingolipid synthesis requires acyl-CoA (mostly palmitoyl-CoA), leading to the long-chain sphingoid bases (LCB), which are used to build ceramides. In the following, PI is utilized to generate complex sphingolipids from ceramides. Note that for reasons of simplicity, cofactors such as ATP and NADH have been omitted.

chronological life span (CLS). The authors suggest that this life span prolongation by the decrease in sphingolipid levels is partly due to reduced Sch9p protein kinase activity (Huang *et al.*, 2012). Additionally, reduction in SPT activity conferred resistance to hydrogen peroxide and heat shock as indicated by drop tests (Huang *et al.*, 2012). In contrast, a selective reduction in complex sphingolipids accompanied by an increase in ceramides has been shown to trigger apoptotic death as indicated by the loss of clonogenic survival and the occurrence of DNA fragmentation and chromatin condensation (Kajiwara *et al.*, 2012). Apoptotic death was accompanied by enhanced endoplasmic reticulum (ER) stress and an increase in cytosolic Ca^{2+} concentration causal for this mode of death (Kajiwara *et al.*, 2012). The authors achieved a depletion of complex sphingolipids by the administration of aureobasidin A (AbA), an antifungal drug that has been shown to inhibit the *AUR1*-encoded inositol phosphorylceramide synthase (IPC synthase) activity, thus blocking the first step in the generation of complex sphingolipids from ceramides (Nagiec *et al.*, 1997). Previously, AbA has been reported to cause yeast growth arrest via both a reduction in complex sphingolipids and a simultaneous increase in ceramides (Cerantola *et al.*, 2009). In a mutant deficient in this IPC synthase activity, accumulation of ceramides upon exogenous PHS supplementation has been shown to cause cell death (Nagiec *et al.*, 1997). Consistently, overexpression of Aur1p could protect yeast cells from growth arrest induced by heat stress and high osmolarity, probably via reduction in ceramide levels (Yang *et al.*, 2006). These findings imply the existence of a yeast cell death subroutine activated by ceramide overload.

A membrane-permeable ceramide (C_2-ceramide, N-acetyl-D-sphingosine) has been shown to cause cell death in rapidly proliferating yeast cells, while C_2-dihydroceramide had no effect (Carmona-Gutierrez, 2011; Galluzzi *et al.*, 2012). Loss of clonogenic survival was accompanied by massive generation of reactive oxygen species (ROS) and mitochondrial fragmentation. Flow cytometric quantification of phosphatidylserine externalization, loss of membrane integrity, and DNA fragmentation demonstrated that cellular demise occurred with both apoptotic and necrotic features (Carmona-Gutierrez *et al.*, 2011). C_2-ceramide-induced cell killing occurred in a way strictly depending on mitochondrial function, as abrogation of

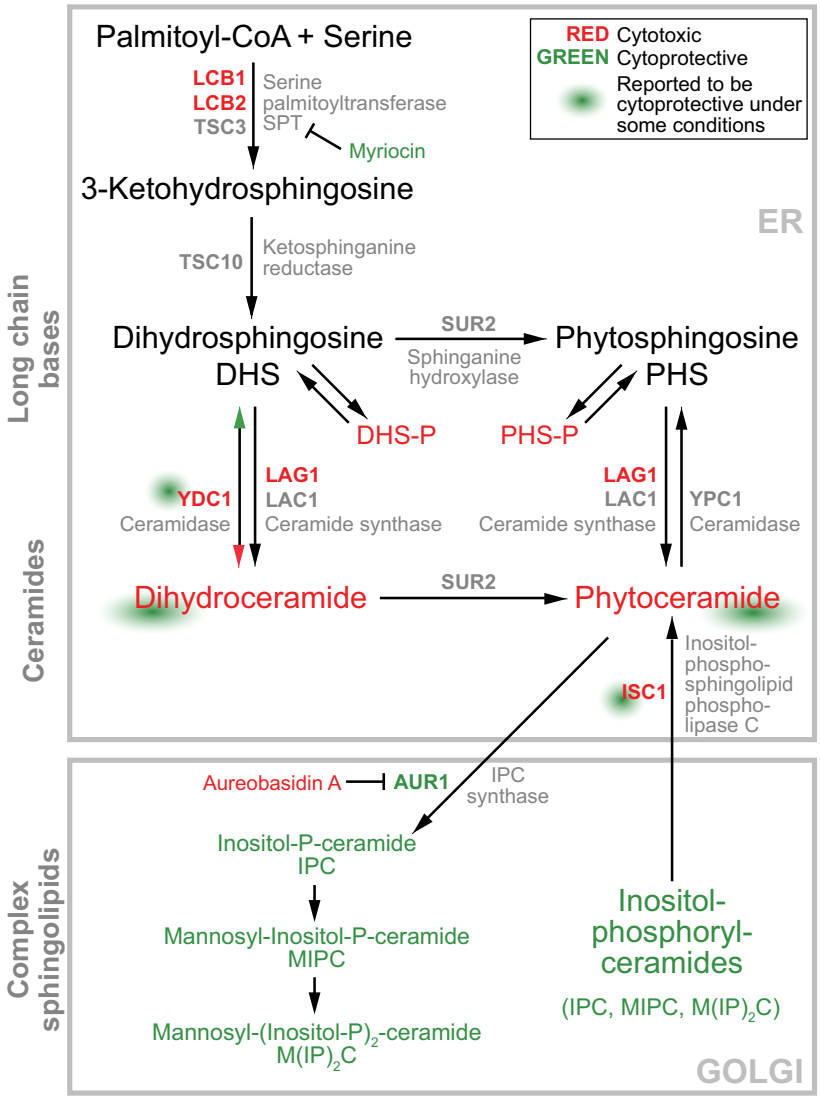

Fig. 2. Schematic illustration of yeast sphingolipid metabolism and its connection to cell death. Palmitoyl-CoA serves as a precursor to generate the long-chain sphingoid bases dihydrosphingosine (DHS) and phytosphingosine (PHS) and the corresponding phosphates (DHS-P and PHS-P). DHS and PHS can be amide-linked to fatty acids to build ceramides, which in turn are converted into complex sphingolipids (inositol phosphorylceramides) via the addition of phosphoinositol. Enzymes and metabolites that have been connected to cell death are depicted in red, if they have been shown to promote cellular demise, or in green, if they have been demonstrated to harbor cytoprotective functions. The green shadow indicates controversial results with respect to cytotoxicity vs. cytoprotection.

the mitochondrial DNA (but not the deletion of the yeast caspase *YCA1*) conferred cytoprotection, pointing to a pivotal role of mitochondria in ceramide-mediated cell death. In this line, treatment of isolated yeast mitochondria with C_{16}-ceramide has been shown to result in the formation of protein-permeable channels responsible for mitochondrial outer membrane permeabilization (MOMP), suggesting a putative mechanism for the release of apoptogenic factors in the course of mitochondria-mediated apoptosis (Siskind *et al.*, 2008). Auxiliary proteins seem not to be essential for this pore-forming activity of ceramides, as C_2-ceramide and C_{16}-ceramide (but not C_2-dihydroceramide or C_{16}-dihydroceramide) are capable of pore formation in artificial membranes completely lacking proteins (Siskind & Colombini, 2000). However, the ceramide channels in isolated mitochondria could be blocked/disassembled by the subsequent addition of antiapoptotic members of the BCL-2 family (Siskind *et al.*, 2008), while simultaneous treatment with activated human BAX and C_{16}-ceramide in concentrations where they only exerted minor effects on their own has been shown to result in MOMP in a synergistic fashion

(Ganesan *et al.*, 2010). Consistently, trehalose, a disaccharide that acts as an inhibitor of ceramide channels (Ganesan *et al.*, 2010), blocked MOMP triggered by activated BAX, and mitochondria isolated from BCL-X_L-expressing yeast cells were resistant to C_{16}-ceramide pore formation (Siskind *et al.*, 2008). Applying yeast cells heterologously expressing human BAX to screen mammalian cDNA libraries for suppressors of BAX-induced growth arrest led to the identification of the murine sphingomyelin synthase 1 (mSMS1) as an inhibitor of the toxic consequences of BAX expression (Yang *et al.*, 2006). In addition, expression of mSMS1 was able to prevent growth inhibition induced by various other stimuli, in particular by challenge with exogenous C_2-ceramide and PHS, the precursor of phytoceramide. In mammalian cells, mSMS1 catalyzes the transfer of phosphocholine from phosphatidylcholine to ceramide, thereby generating sphingomyelin and diacylglycerol (DAG) and decreasing the level of ceramide (Huitema *et al.*, 2004). Thus, the authors suggest that the cytoprotective effect of mSMS1 expression in yeast is attributed to a reduction in ceramide levels, which might, analogous to mammalian cells, increase upon toxic insults. While these data implicate that excess ceramide triggers the mitochondrial pathway of apoptosis governed by mammalian BCL-2 family members, it remains to be shown whether cell death linked to the recently identified yeast BH3-only protein Ybh3p involves ceramides.

AbA administration was demonstrated to induce apoptotic cell death that was inhibited by deletion of the yeast metacaspase *YCA1* as well as by deletion of its downstream effector *RSM23*, which codes for the yeast homolog of the mammalian mitochondrial mediator of apoptosis (Berger *et al.*, 2000; Madeo *et al.*, 2002). In addition, the absence of several proteins implicated in mitochondrial cell death such as the two cytochrome *c* isoforms Cyc1p and Cyc7p and the adenine nucleotide transporters Aac1p and Aac3p conferred resistance to AbA (Berger *et al.*, 2000). Interestingly, death induced by block of the IPC synthase Aur1p (e.g. via AbA administration) may not solely be explained by a mere accumulation of ceramides. Genetic or pharmacological downregulation of SPT activity, leading to an overall decline in ceramide species, did not prevent, but exacerbate AbA-induced cell death (Kajiwara *et al.*, 2012). In this line, inhibition of the assembly of glycosylphosphatidylinositol, which has been demonstrated to cause reduced levels of complex sphingolipids (Kajiwara *et al.*, 2008), aggravated AbA-mediated apoptosis (Kajiwara *et al.*, 2012). These findings led the authors to conclude that not a change in ceramide levels, but rather a reduction in cytoprotective complex sphingolipids is critically involved in yeast cell death upon treatment with AbA, leading to the activation of the mitochondrial pathway of apoptosis (Kajiwara *et al.*, 2012).

Two yeast ceramidases have been characterized so far, the phytoceramidase Ypc1p and the dihydroceramidase Ydc1p. As both enzymes function in the hydrolysis of ceramides to generate free LCB, namely PHS and dihydrosphingosine, their absence normally results in increased levels of ceramides and complex sphingolipids (Mao *et al.*, 2000a, b). Still, reverse activities upon inhibition of the Lag1p/Lac1p synthesis pathway have been reported (Schorling *et al.*, 2001; Cerantola *et al.*, 2009). The absence of Ydc1p has been shown to result in increased sensitivity to heat stress (Mao *et al.*, 2000b), arguing again for a prodeath role of excess ceramides and/or complex sphingolipids. However, the overexpression of this ceramidase and concomitantly decreased levels of phytoceramides and dihydroceramides provoked premature aging and apoptosis preceded by mitochondrial fragmentation and dysfunction in another study (Aerts *et al.*, 2008). Cellular demise during aging could be prevented by external administration of ceramide, leading the authors to suggest that apoptosis triggered by overexpression of Ydc1p is most likely due to decreased ceramide levels rather than due to increased LCB (Aerts *et al.*, 2008). As mentioned above, Ypc1p and Ydc1p may also harbor reverse ceramidase (meaning ceramide synthase) activity, catalyzing the generation of phytoceramide from FFA instead of using Acyl-CoA, which is used by the regular ceramide synthases Lag1p and Lac1p to generate phytoceramide (Mao *et al.*, 2000a, b; Schorling *et al.*, 2001). In this line, overexpression of Ydc1p or Ypc1p was reported to partially correct for loss of ceramide synthesis in *lag1*Δ*lac1*Δ cells (Schorling *et al.*, 2001). Thus, discrepancies with respect to the cytocidal or cytoprotective effect of increased or decreased ceramide levels (e.g. after modulation of ceramidase activity) might be due to complex changes in specific ceramide species and potentially other lipids.

The absence of the ceramide-generating enzymes Isc1p or Lag1p has been shown to confer resistance to acetic acid, thus linking acetic acid-induced apoptosis to sphingolipid metabolism (Rego *et al.*, 2012). The deletion of these enzymes prevented acetic acid-induced ROS production, mitochondrial fragmentation and degradation, as well as cytochrome *c* release and subsequent apoptosis. Performing lipidomic analysis, the authors could show that upon treatment with acetic acid, the levels of most phytoceramide and dihydroceramide species decreased, while α-hydroxy-C_{20}-phytoceramide levels increased dramatically in a way strictly depending on the presence of both Isc1p and Lag1p. External administration of C_2-phytoceramide triggered cell death *per se* and additionally aggravated acetic acid-induced death in a way that was not influenced by deletion of *ISC1* or *LAG1* (Rego *et al.*, 2012). In consistence with these results, suggesting a

prodeath function for Isc1p and Lag1p, deletion of *LAG1* extended replicative life span (RLS), and its expression sharply declined with replicative age possibly as a prosurvival response of aging cells to cope with progressing stress (D'mello *et al.*, 1994; Jiang *et al.*, 2004).

In contrast to observed resistance of the *ISC1* null mutant to acetic acid, deletion of *ISC1* has been reported to result in sensitivity to oxidative stress as well as a shortened CLS (Kitagaki *et al.*, 2007; Almeida *et al.*, 2008). Isc1p represents the only yeast member of the family of sphingomyelinases (SMases) and translocates from ER to mitochondria upon induction of respiration (de Avalos *et al.*, 2004), where it integrates into the outer mitochondria membrane (Kitagaki *et al.*, 2007). This seems to be essential for mitochondrial function, as the deletion of *ISC1* and the concomitant loss of more than 90% mitochondrially located ceramide (α-hydroxy-C_{26}-phytoceramide) triggered the generation of respiratory-deficient cells when exposed to elevated temperature (Kitagaki *et al.*, 2007). Death of *ISC1*-disrupted cells during aging and upon treatment with hydrogen peroxide was accompanied by typical markers of apoptosis and prevented by deletion of *YCA1*. Interestingly, while *ISC1* deficiency caused a general decrease in most ceramide species and an increase in α-hydroxy-C_{20}-phytoceramide upon treatment with acetic acid (Rego *et al.*, 2012), specific species, namely C_{26}-dihydroceramide and C_{26}-phytoceramide, were upregulated during chronological aging (Barbosa, 2011). Only recently, premature aging, sensitivity to oxidative stress as well as mitochondrial defects of *ISC1*-disrupted cells could be circumvented by additional deletion of *SIT4*, the gene that codes for the catalytic subunit of the ceramide-activated protein phosphatase type 2A (Barbosa, 2011). The authors suggest that the upregulation of the C_{26}-ceramide species causes an activation of Sit4p that is essentially involved in the lethal consequences of *ISC1* deletion. It remains to be clarified whether death in *ISC1*-disruptant cells is selectively caused by (1) loss of general ceramides leading to dysfunctional mitochondria during aging; (2) an accumulation of complex sphingolipids; (3) an increase in specifically C_{26}-ceramides that might act via the ceramide-activated phosphatase Sit4p or (4) a combination of these changes that may vary between distinct cellular conditions. In a different approach, the phosphates of LCB have been suggested to be the toxic sphingolipid species causing yeast cell death (Zhang *et al.*, 2001).

In sum, the results concerning the toxicity of an overload or decline in LCB, LCB phosphates, ceramides, or complex sphingolipids are quite complex if not controversial (Fig. 2), which most probably is due to an intricate network of enzyme activities and metabolites, where small and distinct modifications have far-reaching results that differ depending on the growth phase, culture condition, and applied stresses.

Fatty acid-induced cell death

Disturbances in cellular lipid homeostasis can have multifarious reasons depending on the respective metabolites and enzymes involved, but often culminate in lipotoxic cell death. Processes such as the esterification of DAG to TAG or of sterol to SE, generating neutral lipids, are thought to be cytoprotective, in part due to their buffering of excess FFA. Subsequently, these neutral lipids are retained in lipid droplets, which act as a storage facility for lipids in an inert form. As DAG and PA serve as precursors for the synthesis of both TAG and phospholipids, these biosynthesis pathways are tightly connected. Interestingly, neutral storage lipids appear nonessential for yeast cell growth, as cells lacking all four acyltransferases necessary for TAG and SE synthesis, namely Are1p, Are2p, Dga1p, and Lro1p, readily grow under standard synthetic medium conditions (Sandager *et al.*, 2002). As this quadruple mutant completely lacks neutral lipids, it represents an ideal system to study lipotoxicity induced by FFA accumulation and is therefore frequently used as a model for human pathological conditions associated with lipid overload (Garbarino & Sturley, 2005; Garbarino *et al.*, 2009; Petschnigg *et al.*, 2009; Kohlwein, 2010a; Rockenfeller *et al.*, 2010). Treatment of yeast cells lacking all four acyltransferases with the SFA palmitate ($C_{16:0}$) and stearate ($C_{18:0}$) did not affect cell growth, while unsaturated fatty acids (UFA) such as palmitoleate ($C_{16:1}$), oleate ($C_{18:1}$), or linoleate ($C_{18:2}$) severely impaired growth (Garbarino *et al.*, 2009). In this respect, shorter chain length as well as a higher degree of unsaturation correlated with higher toxicity. Furthermore, UFA impaired cell survival and caused accumulation of ROS and activation of the unfolded protein response. Performing annexin V and propidium iodide (AnnV/PI) costaining, the authors suggest that these cells undergo apoptotic cell death (Garbarino *et al.*, 2009), while another study reported necrotic cell death under similar conditions (Rockenfeller *et al.*, 2010). Petschnigg *et al.* (2009) could show that the genetic blockage of TAG synthesis in the quadruple deletion mutant caused sensitivity (as quantified by growth and clonogenic cell survival) to exogenous oleic acid and to a lesser extend to palmitoleic acid, while no toxicity was detectable for the SFA palmitic and stearic acid. This toxicity induced by unsaturated FFA seems to be due to a lack of TAG synthesis rather that SE synthesis, as reintroduction of Dga1p or Lro1p prevented the growth arrest, while Are1p or Are2p, which are mainly responsible for the generation of SE, failed to reinstate growth (Petschnigg *et al.*, 2009). Short-term exposure of

the quadruple deletion mutant to oleic acid caused massive accumulation of membranes and severely altered cellular lipid profiles, leading to accumulation of phospholipids and a phospholipid fatty acid profile that shifted toward unsaturation and toward C_{18} rather than C_{16}. Interestingly, administration of palmitic acid ($C_{16:0}$) could prevent oleic acid ($C_{18:1}$)-induced toxicity. As this protection came along with a lipid profile that resembled those of wild-type cells (in particular with respect to the ratio of SFA to UFA), the authors suggest that oleic acid-mediated cell death is caused by alterations in acyl chain distribution in membrane phospholipids (Petschnigg *et al.*, 2009).

Treatment of yeast cells with different nutritive oils including pumpkin seed, olive, rapeseed, walnut, linseed, and salmon oils had no marked effect on wild-type yeast cells. However, upon concomitant addition of TAG lipase to externally hydrolyze TAG and as such to mimic the microenvironment of the mammalian intestine, the oils displayed differential grades of cytotoxicity (determined using clonogenic survival), with linseed oil being the most toxic one (Rockenfeller *et al.*, 2010). Interestingly, linseed oil contains the highest amount of polyunsaturated fatty acids (PUFA) of the tested cooking oils. Dying cells exhibited massive accumulation of ROS and loss of plasma membrane integrity indicating necrotic death, while externalization of phosphatidylserine indicative of apoptosis was absent. Using above-described yeast cells devoid of TAG and SE synthesis instead of wild-type cells, the extent of cell death upon treatment with different FFA was again demonstrated to correlate with the degree of unsaturation, which is in line with above-mentioned findings after treatment with nutritive oils as well as with previous results (Garbarino *et al.*, 2009). While palmitic and stearic acid had no effect, oleic ($C_{18:1}$), linoleic ($C_{18:2}$), and linolenic ($C_{18:3}$) acid caused the generation of ROS and subsequent cell death. Linoleic acid could be detected in mitochondrial membranes, indicating that externally supplied FFA can be incorporated into intracellular membranes (Rockenfeller *et al.*, 2010). In contrast to a previous study (Garbarino *et al.*, 2009), this death was shown to be of necrotic nature as indicated by flow cytometric quantification of plasma membrane rupture, nuclear-cytosolic translocation of the yeast HMGB1 homolog Nhp6Ap, and an absence of apoptotic phosphatidylserine externalization (Rockenfeller *et al.*, 2010). Remarkably, the necrotic death of the quadruple mutant could be inhibited by abrogation of the mitochondrial DNA, rendering the cells respiratory deficient.

While long-chain SFA have been shown to be nontoxic to wild-type cells, even when administered in high doses (Black & DiRusso, 2007; Petschnigg *et al.*, 2009), treatment with the medium-chain saturated FFA decanoic acid ($C_{10:0}$) caused rapid cell death (Stratford & Anslow, 1996). The synthetic branched-chain carbonic acid valproic acid (C_8), an inhibitor of class I histone deacetylases therapeutically used to treat neurological/neuropsychiatric disorders (such as mental illness or epilepsy), has been demonstrated to trigger apoptotic death of yeast cells (Mitsui *et al.*, 2005; Sun *et al.*, 2007). Valproic acid-induced cell killing depended on the presence of Yca1p when administered in low doses, while the mode of death switched to Yca1p-independent death with autophagic features when applied in higher concentration (Mitsui *et al.*, 2005). In addition, apoptotic death induced by low doses of valproic acid was preceded by the accumulation of neutral lipids as indicated by increased amount of lipid droplets. Cell death required the presence of the NAD^+-dependent histone deacetylase Sir2p (but not the histone deacetylases Rpd3p and Hda1p), indicating that valproic acid exerts toxic functions beyond the simple inhibition of class I histone deacetylases such as Rpd3p (Sun *et al.*, 2007).

Whether FFA are utilized to synthesize phospholipids or TAG is mainly governed by the activity of the phosphatidate phosphatase Pah1p (Han *et al.*, 2006), the yeast homolog of human lipin-1 (Péterfy *et al.*, 2001). Pah1p catalyzes the dephosphorylation of PA to generate DAG that gets converted to TAG. Deletion of *PAH1* resulted in reduced levels of DAG and TAG and elevated PA content, which caused heat sensitivity, respiratory deficiency, and transcriptional upregulation of the enzymatic machinery necessary for phospholipid synthesis (Santos-Rosa *et al.*, 2005; Han *et al.*, 2006). These changes came along with depletion of lipid droplets and enhanced apoptotic death in stationary phase (Fakas *et al.*, 2011). The absence of this enzyme provoked an imbalanced lipid profile (in particular an increased palmitoleic acid-to-oleic acid ratio) that rendered cells vulnerable to external administration of SFA and UFA, in particular to palmitoleic acid (Fakas *et al.*, 2011), while wild-type cells were unaffected, but counteracted with enhanced PA phosphatase activity and concomitant increase in TAG content. Especially in stationary phase, the lack of *PAH1* led to drastically enhanced masses of phospholipids and FFA, while the TAG content was largely reduced. The defect in lipid droplet formation observed in the *PAH1* deletion mutant, but not its sensitivity toward palmitoleic acid could be reversed by simultaneous deletion of *DGK1*, the gene coding for the DAG kinase that catalyzes the opposed reaction, thus generating PA (Fakas *et al.*, 2011).

Nma111p (alias Ynm3p), the yeast homolog of the serine protease HtrA2/Omi, has been shown to specifically interact with Faa1p, the major long-chain acyl-CoA synthetase involved in uptake and metabolism of FFA (Tong *et al.*, 2006). The deletion of *NMA111* was demonstrated to cause

enhanced FFA uptake along with intracellular accumulation of TAG and FFA. Under the conditions used, the phenotypes observed for *NMA111* null mutants were accompanied by generation of ROS and apoptotic DNA fragmentation (Tong *et al.*, 2006).

In sum, cellular FFA overflow (derived from exogenous sources upon impairment of TAG synthesis or by changes in FFA uptake and activation) induces programmed cell death in yeast with predominantly necrotic markers. FFA toxicity appears to depend on the degree of unsaturation and may comprise different pathways involving mitochondria and phospholipid synthesis.

Lipolysis, FAO, peroxisomes, and aging

Energy metabolism, nutrient signaling, and aging are intimately connected. While caloric restriction (CR) extends life span and contributes to health span throughout phyla, nutrient overload promotes aging and favors the development of age-associated diseases (Baur, 2006; Schlotterer *et al.*, 2009; Fontana *et al.*, 2010; Weinberger *et al.*, 2010). Much knowledge has been acquired concerning the role of nutrient-sensing pathways in regulating longevity under different alimentary conditions from yeast to humans (Fontana *et al.*, 2010). However, we are just beginning to uncover the full relevance of lipids and lipid metabolism to aging.

As mentioned above, yeast FAO solely resides within peroxisomes (Van Roermund *et al.*, 2003). Central during the oxidation process, the Fox1-3p enzymes catalyze the cycling steps during FAO, providing acetyl-CoA for biosynthetic or energy demands. Interesting hypotheses have been framed that integrate peroxisomes to a complex network with the ER and lipid droplets that governs cellular aging (Goldberg *et al.*, 2009; Beach & Titorenko, 2012). In addition to maintaining lipid and hydrogen peroxide homeostasis, peroxisomes may harbor important signaling functions during development, differentiation, and aging (Goldberg *et al.*, 2009; Beach & Titorenko, 2012; Manivannan *et al.*, 2012). Central to these hypotheses is the FAO activity of peroxisomes that may (1) contribute to the decline of otherwise toxic FFA and/or (2) provide anaplerotic replenishment of tricarboxylic acid cycle intermediates as part of an age-relevant survival process known as retrograde signaling. While the causal interrelation of FAO to these processes has not been demonstrated directly, several studies provide first evidence that peroxisomal FAO activity is crucial to healthy aging.

With progressing age, a general decline in peroxisomal function is apparent in mammals (Manivannan *et al.*, 2012). Importantly, an age-related decrease in peroxisomal and mitochondrial FAO activity has been demonstrated in rats and mice (Périchon & Bourre, 1996; Tucker & Turcotte, 2002; Houtkooper, 2011) influencing FFA and DAG homeostasis. In yeast, FFA and DAG are known triggers of necrotic or apoptotic cell death (Zhang *et al.*, 2001; Low *et al.*, 2005; Petschnigg *et al.*, 2009; Rockenfeller *et al.*, 2010), which both are associated with cellular demise during aging (Herker *et al.*, 2004; Eisenberg *et al.*, 2009). In line with this, deletion of the gene coding for peroxiredoxin Pmp20p or the peroxisome transport protein Pex6p culminated in necrotic cell death in the yeasts *Hansenula polymorpha* or *S. cerevisiae*, respectively (Bener Aksam *et al.*, 2008; Jungwirth *et al.*, 2008).

Interestingly, energy storages in the form of TAG-containing lipid droplets are built up during logarithmic growth and show the highest density upon entry into stationary phase (Kurat *et al.*, 2006). Thus, during chronological aging, yeast cells are well equipped with TAG that could in principle serve as substrates for lipolysis and peroxisomal FAO required for antiaging functions of peroxisomes. Consistent with the idea that fatty acids satisfy metabolic demands during aging, stationary-phase quiescent cells displayed increased transcripts of FAO genes compared with nonquiescent cells, which are mainly composed of stressed and apoptotic cells (Allen *et al.*, 2006).

It remains to be demonstrated whether yeast efficiently activates TAG storages for lipolysis and subsequent FAO during chronological or replicative aging. First indications that lipolysis and FAO are at least active under CR conditions come from studies using *PEX5* deletion mutant cells exhibiting increased levels of TAG, DAG, and FFA and fail to respond to the life-prolonging effects of CR (Burstein *et al.*, 2012). Pex5p is required for peroxisomal biogenesis through import of peroxisomal proteins, indicating that the increase in TAG and DAG may be a result of impaired peroxisomal function. Addressing the potential lipolytic and FAO activity as well as the consequences of mutations in the lipases Tgl3,4,5p (Athenstaedt & Daum, 2003, 2005), which lead to TAG accumulation with an 'obese' phenotype, or in the FAO enzymes Fox1-3p (Hiltunen *et al.*, 2003) during aging and upon known life-extending regimens should help to dissect the causal roles of lipolysis and FAO in mediating antiaging mechanisms.

Using a chemical genetic screen, Goldberg *et al.* (2010) further identified the bile acid lithocholic acid (LCA), a cholesterol derivate, as a life-extending lipid that reveals its maximum antiaging capacity under CR conditions. Although to a lower extent, other bile acids exerted antiaging effects under CR as well, including deoxycholic and chenodeoxycholic acid. Supplementation of LCA to chronological aging of yeast cells reduced the amount of FFA and DAG, while TAG levels increased. LCA concomitantly lowered the occurrence of necrotic cell death as indicated by reduced numbers of Ann V^-/PI^+ cells (Goldberg *et al.*,

2010). Interestingly, a bile acid-like steroid modulated *C. elegans* life span (Gerisch *et al.*, 2007). The fact that aging of phylogenetically distant organisms such as yeast and worms can be modulated by bile acids suggests that conserved cellular pathways exist that respond to this class of lipids.

In recent years, cytoprotective autophagy appears to be crucial for healthy aging throughout phyla (Madeo *et al.*, 2010). Intriguingly, autophagy may be interrelated to functional lipolysis, albeit the precise mechanism of how lipophagy contributes to antiaging needs further clarification (Singh *et al.*, 2009; Zechner & Madeo, 2009; Singh & Cuervo, 2012). In chronological aging of yeast cells subjected to CR and concomitantly treated with LCA, mitophagy was required for lipid homeostasis (Richard *et al.*, 2013). Deletion of the mitophagy essential gene *ATG32* strongly impaired the response of chronologically aging yeast cells to LCA treatment under CR. While the lack of *ATG32* provoked alterations in mitochondrial membrane lipids, it also substantially affected lipids of the plasma membrane and the ER (Richard *et al.*, 2013).

In sum, lipids and lipid metabolism contribute to aging by various mechanisms, including FFA- and DAG-induced age-associated programmed cell death as well as metabolic consequences of FAO on survival-ensuring processes such as autophagy or retrograde signaling. The genetic tractability and ease of use make yeast a prime candidate for the elucidation of conserved cellular mechanisms that underlie some of the known, but also yet-uncovered effects of lipids and lipid metabolism on aging and age-associated disease.

Cardiolipin and mitochondrial lipid oxidation

Cardiolipin, (diphosphatidylglycerol) is a unique anionic phospholipid of the inner mitochondrial membrane carrying four acyl chains. In yeast, the first step of cardiolipin *de novo* synthesis in mitochondria is catalyzed by the phosphatidylglycerophosphate synthase (Pgs1p), using CDP-DAG and glycerol-3-phosphate to form phosphatidylglycerophosphate, which then is dephosphorylated to phosphatidylglycerol. The cardiolipin synthase Crd1p catalyzes the irreversible conversion into cardiolipin, which in turn is subjected to deacylation and reacylation reactions, commonly referred to as cardiolipin remodeling (reviewed in Joshi *et al.*, 2009). The specific composition of fatty acids within cardiolipin is essential for its proper function, as abnormal cardiolipin remodeling underlies the genetic cardiomyopathic disorder, Barth syndrome (Vreken *et al.*, 2000; Schlame *et al.*, 2002). Most cardiolipin species found across phyla contain one or two different types of acyl chains, thus providing structural uniformity and symmetry. Loss of the respective transacylase activity (encoded by the evolutionary conserved tafazzin gene) causes the accumulation of varying, non-uniform cardiolipin species typical for Barth syndrome (Schlame *et al.*, 2005). In yeast, tafazzin activity is encoded by *TAZ1*, and yeast cells devoid of *TAZ1* have been successfully applied to model Barth syndrome, recapitulating pathological features such as alterations in cardiolipin metabolism and respiratory supercomplexes, mitochondrial dysfunction, and oxidative stress (Brandner *et al.*, 2005; Chen *et al.*, 2008; Claypool *et al.*, 2008; Whited *et al.*, 2013). Cardiolipin has been shown to interact with a large number of mitochondrial proteins, including the ADP-ATP carrier, the phosphate carrier, various respiratory chain proteins, and cytochrome *c*, and to be involved in essential cellular processes such as mitochondrial biogenesis, mitochondrial protein import, ceramide synthesis, aging, and apoptosis (reviewed in Joshi *et al.*, 2009). Deletion of the gene encoding the yeast cardiolipin synthase Crd1p results in complete absence of cardiolipin and a heat-sensitive phenotype. In wild-type mitochondria, cardiolipin accounts for about 12% of total phospholipids and can be replaced by its precursor phosphatidylglycerol in mitochondria of cells deleted in *CRD1* upon growth on nonfermentable carbon sources (Gonzalvez *et al.*, 2005b). Mitochondrial transmembrane potential was mildly reduced upon deletion of *CRD1*, and the change in mitochondrial network organization observed in late exponential cells as well as upon entry into stationary phase was absent (Gonzalvez *et al.*, 2005b). Lack of Crd1p has been suggested to provoke mitochondrial dysfunction, as indicated by decreased mitochondrial transmembrane potential, defects in protein import, and instability of mitochondrial DNA (Jiang *et al.*, 2000). In addition, yeast cells devoid of Crd1p have been shown to exhibit reduced activities of several mitochondrial iron–sulfur cluster enzymes, indicating that mitochondrial iron homeostasis requires Crd1p (Patil *et al.*, 2013).

With respect to the mitochondrial pathway of apoptosis governed by the BCL-2 protein family, cardiolipin has been shown to be necessary for proper targeting of truncated BH3 interacting domain (tBID), a proapoptotic member of the BCL-2 protein family, to liposomes and isolated mitochondria and for cytochrome *c* release (Lutter *et al.*, 2000; Kim *et al.*, 2004). Insertion of BAX into liposomes was demonstrated to require cardiolipin (Lucken-Ardjomande *et al.*, 2008). In this line, tBID and BAX have been shown to cooperate with cardiolipin to form supramolecular openings in artificial membranes, a process that could be inhibited by BCL-X_L (Kuwana *et al.*, 2002). While the proapoptotic function of tBID and/or BAX has been shown to require cardiolipin by several groups, others demonstrated a decline in mitochondrial

cardiolipin in mammalian cells upon induction of apoptosis (Matsko *et al.*, 2001; McMillin & Dowhan, 2002; Ott *et al.*, 2002). Another study provided a potential explanation for these at first sight contrary findings. Applying liposomes, isolated mitochondria, and liver cells, it was demonstrated that not cardiolipin *per se*, but rather its metabolic product monolysocardiolipin is instrumental for membrane binding and relocation of tBID to mitochondria (Esposti *et al.*, 2003). Upon apoptosis induction in liver cells, the authors observed a downregulation of cardiolipin and a concomitant accumulation of monolysocardiolipin, indicating a transition of cardiolipin into monolysocardiolipin during apoptosis, which enforced membrane binding of tBID and facilitated the release of apoptogenic factors (Esposti *et al.*, 2003).

In mitochondria isolated from wild-type yeast cells, but not from *CRD1* deficient cells, incubation with tBID caused an inhibition of respiration and ATP synthesis, indicating that tBID alters mitochondrial function in a way strictly depending on cardiolipin (Gonzalvez *et al.*, 2005a, b). However, other groups found that cytochrome *c* release and cell killing mediated by heterologously expressed BAX did not depend on the presence of cardiolipin (Iverson *et al.*, 2004; Gonzalvez *et al.*, 2005b; Polčic *et al.*, 2005). Instead, cardiolipin has been found to be required for tight binding of cytochrome *c* to the inner mitochondrial membrane (Iverson *et al.*, 2004). As in the absence of cardiolipin, its precursor phosphatidylglycerol was suggested to substitute for its function (Chang *et al.*, 1998), it seemed possible that BAX might cooperate with phosphatidylglycerol instead of cardiolipin to permeabilize mitochondria. However, in strains lacking both cardiolipin and phosphatidylglycerol (due to deletion of *PGS1*), the heterologous expression of BAX still provoked mitochondrial permeabilization (Polčic *et al.*, 2005).

Accumulating evidence indicates a role for cardiolipin oxidation in early mitochondrial apoptosis (Shidoji *et al.*, 1999; Kagan *et al.*, 2005; Korytowski *et al.*, 2011). Cytochrome *c* has been shown to be bound to the inner mitochondrial membrane via an interaction with cardiolipin, which in turn has to be disrupted in order for apoptotic cytochrome *c* release to occur (Ott *et al.*, 2002). This interaction and as such mitochondrial attachment of cytochrome *c* have been shown to drastically decrease with the degree of cardiolipin oxidation (Shidoji *et al.*, 1999; Nomura *et al.*, 2000). In addition, cytochrome *c* has been demonstrated to possess an intrinsic cardiolipin-specific peroxidase activity, leading to peroxidized cardiolipin and a decreased retention of cytochrome *c* (Kagan *et al.*, 2005). In this line, oxidation of cardiolipin increased binding of tBID to mitochondria (Korytowski *et al.*, 2011). Thus, it seems feasible that the effects of cardiolipin in BAX- or tBID-mediated mitochondrial permeabilization vary depending on the degree of cardiolipin oxidation and/or peroxidation. Highlighting the emerging role of lipid oxidation in the mitochondrial pathway of apoptosis, the expression of BAX triggered oxidation of mitochondrial lipids in yeast that was causal for its lethal activity (Priault *et al.*, 2003).

Synthetic antitumor alkylphospholipid-induced yeast cell death

Cellular membranes become increasingly recognized as targets for the treatment of different pathologies. The membrane-bound O-acyltransferase Gup1p has been shown to be involved in lipid metabolism, in particular in the maintenance of lipid raft integrity and glycosylphosphatidylinositol anchor remodeling. Deletion of *GUP1* resulted in enhanced necrotic death during chronological aging and upon treatment with acetic acid (Tulha *et al.*, 2012).

Synthetic antitumor alkylphospholipids, mostly analogues of (lyso)phosphatidylcholine, represent a class of chemotherapeutic compounds that integrate into cell membranes, where interference with lipid metabolism and signal transduction pathways essentially contribute to their cytotoxic action. A number of proteins associated with the plasma membrane have been suggested to be targeted by alkylphospholipids depending on the specific cell type used (for review see Van Blitterswijk & Verheij, 2008). Edelfosine, the prototype alkylphospholipid, selectively triggers apoptosis in cancer cells and is thought to act mainly via two targets, (1) the ER where it interferes with phosphatidylcholine biosynthesis and triggers ER stress (Boggs *et al.*, 1995; Nieto-Miguel *et al.*, 2007; Gajate *et al.*, 2012); and (2) the plasma membrane, where edelfosine selectively accumulates in and causes modification of lipid rafts, leading to disturbances of membrane trafficking and signaling, thereby compromising cellular survival (Van der Luit *et al.*, 2002; Wright *et al.*, 2004; Zaremberg *et al.*, 2005; Mollinedo *et al.*, 2011). The preferred uptake of edelfosine by metabolically active, proliferating cells such as cancer cells represents a main aspect of its antitumor activity (Mollinedo *et al.*, 1997; Gajate & Mollinedo, 2002). In addition, several alkylphospholipids display promising antifungal and antiprotozoal, in particular antileishmanial, activity (Lux *et al.*, 2000). Thus, understanding the mechanism underlying alkylphospholipids-induced cellular demise in cancer cells as well as in infectious protozoans and fungi is crucial, and the use of the yeast model system to further elucidate the mechanism of the cytocidal action of alkylphospholipids shows promise.

Unbiased genetic screens in yeast demonstrated that edelfosine caused prominent alterations in the biophysical

structure of lipid rafts and indicates a role for sphingolipid and sterol metabolism in edelfosine cytotoxicity (Zaremberg *et al.*, 2005). Further studies indicate that edelfosine directly acts on lipid raft integrity, triggering internalization of sterols and displacement of the plasma membrane located proton pump Pma1p from lipid rafts. The endocytosis and subsequent vacuolar degradation of Pma1p, the major regulator of intracellular pH, caused intracellular acidification and subsequent cell death (Cuesta-Marbán *et al.*, 2013; Czyz *et al.*, 2013). As in mammalian cells, treatment with α-tocopherol could inhibit edelfosine-induced yeast cell death (Zhang *et al.*, 2007; Bitew *et al.*, 2010). Yeast cells challenged with edelfosine exhibited alterations in mitochondrial transmembrane potential, accumulation of ROS, and apoptotic DNA fragmentation. Cotreatment with α-tocopherol as well as inhibition of the respiratory chain via rotenone could prevent the generation of ROS and subsequent apoptotic cell death (Zhang *et al.*, 2007). The protective effect of α-tocopherol with respect to edelfosine treatment in mammalian cells has been attributed to the inhibition of PUFA peroxidation (Wagner *et al.*, 1996). As yeast cells lack PUFA (Schneiter *et al.*, 1999; Ejsing *et al.*, 2009), the cytoprotectivity of α-tocopherol must involve additional biological activities. In this respect, α -tocopherol has been shown to prevent sterol replacement and Pma1p internalization, two early events after the addition of edelfosine, suggesting that α-tocopherol exerts its protective effects against edelfosine (at least in part) at the plasma membrane (Bitew *et al.*, 2010). The internalization and subsequent vacuolar degradation of Pma1p seem to be characteristics for alkylphospholipid-mediated cytotoxicity in yeast, as not only edelfosine, but also miltefosine and perifosine altered lipid raft composition and caused Pma1p replacement. The insertion of edelfosine (and that of miltefosine) into the plasma membrane was mediated by the flippase subunit Lem3p (Hanson *et al.*, 2003). Notably, this study led to the recent finding that the human homolog of yeast Lem3p (TMEM30a) is essentially involved in the uptake of edelfosine and other bioactive choline phospholipids in human cells (Chen *et al.*, 2011). Thus, yeast cells provide a valuable tool to decipher the molecular mechanisms underlying the cytocidal activities of antitumor antibiotics.

Outlook

Understanding lipid-induced malfunction clearly represents a major challenge of today's biomedical research. In sum, the connection of lipids to cellular and organ dysfunction, cell death, and disease is more complex than the sole lipotoxic effects of excess FFA accumulation and requires genetically tractable model systems for mechanistic investigation. As one of such models, yeast has already been used to address critical questions of lipid-induced cell death, of which important studies are summarized in Table 1. Given its genetic tractability and the fact that regulation of nutrient and lipid metabolism by cellular signaling pathways is conserved (Kohlwein, 2010a; Raychaudhuri *et al.*, 2012), yeast appears as a perfect tool to further address central questions:

(1) What are the toxic lipid species that finally induce programmed cell death?

(2) Which cell death routines are activated during lipotoxicity?

(3) Can intracellular nutrient signaling delay or prevent lipid-induced cell death?

(4) Are changes in lipid metabolism and associated lipidomic as well as metabolomic alterations causally connected to lipid-associated cell death?

(5) What are the major sites/subcellular locations of lipotoxicity?

(6) What is the role of autophagy/lipophagy during lipotoxic events?

Several yeast studies revealed that sphingolipid homeostasis is required for cellular function and stress resistance, resembling knowledge obtained throughout phyla. In particular, altered ceramide levels are among the major lipidomic changes that occur upon induction of cell death or aging in yeast. However, it remains unclear whether and which ceramide species are the actual toxic triggers. Alternatively, (un)expected changes in metabolic precursors or downstream effectors (including more complex sphingolipids) may also explain observed phenotypes. Functional genetic experiments are required to solve these problems and may shed light on some of the discrepancies reported by studies reviewed herein.

Similarly, neutral lipid storages have been recognized as cytoprotective resorts that prevent toxic FFA accumulation. The mechanisms of FFA-induced cell death may involve multiple subroutines, including lipid peroxidation and the overflow to the phospholipid pathway affecting cellular membranes and physiology. During aging of yeast, changes in FFA levels may not only affect cellular survival through induction of age-relevant cell death, but also reflect altered FAO metabolism as has been observed in aging mammals (Houtkooper, 2011). It is, thus, tempting to speculate that a sustained flux through FAO is required to maintain cellular survival processes during both yeast and mammalian aging.

As yeast harbors a basically conserved although less complex machinery of metabolism and signaling, it will continue to serve as a valuable model for the investigation of the molecular basis underlying human disease, including that of lipid-associated malfunction.

Table 1. Lipid- or lipid metabolism-related regimens affect cell death and survival in yeast. The table summarizes the most important genetic or pharmacological regimens associated with lipids and lipid metabolism that lead to cell death or cell protection in the yeast *Saccharomyces cerevisiae*. If monitored by referenced studies, alterations in lipid profiles are depicted. The cell death and stress markers that led to respective phenotype conclusions are also included

Treatment/regimen	Lipid alterations	Phenotype	Death and Stress Markers/Pathway	References
LCB1 LCB2 knockdown *OR* SPT inhibitor myriocin	Reduction in LCBs, general sphingolipids	Growth impairment; increased CLS; heat shock and H_2O_2 resistance	OD; clonogenicity: drop tests	Huang *et al.* (2012)
IPC synthase inhibitor Aureobasidin A	Reduction in complex sphingolipids; increased ceramide	Apoptosis and growth impairment	Clonogenicity, TUNEL, ROS/Yca1p and Ca^{2+} dependent	Cerantola *et al.* (2009) and Kajiwara *et al.* (2012)
Exogenous PHS to IPC synthase mutant cells	Ceramide-3 accumulation	Cell death or senescence	OD, clonogenicity	Nagiec *et al.* (1997)
AUR1 overexpression; Heterologous expression of mSMS1	n.d.	H_2O_2, heat shock, and Bax resistance; resistance to C2-ceramide or PHS	Clonogenicity, drop tests	Yang *et al.* (2006)
Exogenous C2-ceramide	n.d.	Apoptotic and necrotic cell death	TUNEL, AnnV/PI, clonogenicity/ mitochondrion dependent	Carmona-Gutierrez *et al.* (2011) and Galluzzi *et al.* (2012)
YDC1 overexpression	n.d.	Reduced CLS preventable by exogenous C_6-dihydroceramide; apoptotic death	Clonogenicity; TUNEL, AnnV/PI, MF	Aerts *et al.* (2008)
ISC1 or *LAG1* deletion	General ceramide decreased, but increased α-hydroxy-C_{20}-phytoceramide	Resistance to acetic acid-induced cell death/apoptosis	Clonogenicity, ROS, MF, cytochrome c release	Rego *et al.* (2012)
ISC1 deletion	Mitochondrial ceramide decreased, but increased C_{26}-phytoceramide; ceramide increased during CLS	Reduced CLS, sensitivity to H_2O_2, apoptotic cell death	Clonogenicity, ROS, TUNEL/Yca1p and PP2A(Sit4p) dependent	Kitagaki *et al.* (2007) and Almeida *et al.* (2008) and Barbosa (2011)
LAG1 deletion; *LAG1* overexpression	n.d.	Increased RLS; bimodal effect on RLS	RLS analysis	D'mello *et al.* (1994) and Jiang *et al.* (2004)
Edelfosine	Lipidomic alterations in PC metabolism; reduced sterols and sphingolipids in lipid rafts	Cell death, Pma1p degradation-dependent acidification	Clonogenicity, drop tests	Zaremberg *et al.* (2005) and Czyz *et al.* (2013)
Edelfosine	n.d.	α-tocopherol inhibitable apoptotic cell death	Growth, ROS, TUNEL	Zhang *et al.* (2007)
GUP1 deletion	n.d.	Reduced CLS with enhanced necrotic death	Clonogenicity, ROS, AnnV/PI, DAPI staining	Tulha *et al.* (2012)
UFAs applied to TAG/SE devoid cells (quadruple mutant*)	Increased FFA and phospholipids	Apoptotic cell death and activation of UPR	Clonogenicity, AnnV/PI, ROS	Garbarino *et al.* (2009)
UFAs applied to TAG/SE devoid cells (quadruple mutant*)	Accumulation of membranes and unsaturated phospholipids	Necrotic cell death, Dga1p-/Lro1p -complementable growth impairment	Growth, clonogenicity, AnnV/PI, ROS/ mitochondrion dependent	Petschnigg *et al.* (2009) and Rockenfeller *et al.* (2010)
PAH1 deletion	Reduced levels of TAG, DAG; increased PA and phospholipid levels	Apoptotic cell death in stationary phase, FFA sensitivity	Growth, AnnV/PI	Fakas *et al.* (2011)
SFA (Decanoic acid, valproic acid)	Accumulation of neutral lipids	Apoptotic cell death	Growth, clonogenicity, ROS, AnnV/PI, TUNEL/ Yca1p and Sir2p dependent	Stratford & Anslow (1996), Mitsui *et al.* (2005) and Sun *et al.* (2007)

Table 1. Continued

Treatment/regimen	Lipid alterations	Phenotype	Death and Stress Markers/Pathway	References
PEX5 deletion	Increased TAG, DAG and FFA levels	Reduced CLS	Clonogenicity	Goldberg *et al.* (2010)
PEX6 deletion	n.d.	Sensitivity to H_2O_2 and acetic acid; increased necrosis during aging; reduced CLS	Clonogenicity, ROS, AnnV/PI	Meijer *et al.* (2007)

AnnV/PI, annexin V and propidium iodide costaining; CLS, chronological life span; IPC, inositol phosphorylceramide; mSMS1, murine sphingomyelin synthase; MF, mitochondrial fragmentation; OD, optical density; PA, phosphatidic acid; PC, phosphatidylcholine; PHS, phytosphingosine; PP2A, protein phosphatase 2A; RLS, replicative life span; ROS, reactive oxygen species; SFA, saturated fatty acid; SPT, serine palmitoyltransferase; TUNEL, TdT-mediated dUTP nick end labeling; UFA, unsaturated fatty acid
*Please refer to text for explanation.

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Authors' contribution

T.E. and S.B. contributed equally to this work.

References

Aerts AM, Zabrocki P, François IEJA *et al.* (2008) Ydc1p ceramidase triggers organelle fragmentation, apoptosis and accelerated ageing in yeast. *Cell Mol Life Sci* **65**: 1933–1942.

Allen C, Büttner S, Aragon AD *et al.* (2006) Isolation of quiescent and nonquiescent cells from yeast stationary-phase cultures. *J Cell Biol* **174**: 89–100.

Almeida T, Marques M, Mojzita D *et al.* (2008) 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.

Athenstaedt K & Daum G (2003) YMR313c/TGL3 encodes a novel triacylglycerol lipase located in lipid particles of *Saccharomyces cerevisiae*. *J Biol Chem* **278**: 23317–23323.

Athenstaedt K & Daum G (2005) Tgl4p and Tgl5p, two triacylglycerol lipases of the yeast *Saccharomyces cerevisiae* are localized to lipid particles. *J Biol Chem* **280**: 37301–37309.

Barbosa AD, Osório H, Sims KJ, Almeida T, Alves M, Bielawski J, Amorim MA, Moradas-Ferreira P, Hannun YA & Costa V (2011) 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.

Baur JA, Pearson KJ, Price NL *et al.* (2006) Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* **444**: 337–342.

Beach A & Titorenko VI (2012) Integration of peroxisomes into an endomembrane system that governs cellular aging. *Front Physiol* **3**: 283.

Bener Aksam E, Jungwirth H, Kohlwein SD, Ring J, Madeo F, Veenhuis M & van der Klei IJ (2008) Absence of the peroxiredoxin Pmp20 causes peroxisomal protein leakage and necrotic cell death. *Free Radic Biol Med* **45**: 1115–1124.

Berger T, Brigl M, Herrmann JM, Vielhauer V, Luckow B, Schlondorff D & Kretzler M (2000) The apoptosis mediator mDAP-3 is a novel member of a conserved family of mitochondrial proteins. *J Cell Sci* **113**: 3603–3612.

Bitew T, Sveen CE, Heyne B & Zaremberg V (2010) Vitamin E prevents lipid raft modifications induced by an anti-cancer lysophospholipid and abolishes a Yap1-mediated stress response in yeast. *J Biol Chem* **285**: 25731–25742.

Black PN & DiRusso CC (2007) Yeast acyl-CoA synthetases at the crossroads of fatty acid metabolism and regulation. *Biochim Biophys Acta* **1771**: 286–298.

Boggs KP, Rock CO & Jackowski S (1995) Lysophosphatidylcholine and 1-O-octadecyl-2-O-methyl-rac-glycero-3-phosphocholine inhibit the CDP-choline pathway of phosphatidylcholine synthesis at the CTP: phosphocholine cytidylyltransferase step. *J Biol Chem* **270**: 7757–7764.

Brandner K, Mick DU, Frazier AE, Taylor RD, Meisinger C & Rehling P (2005) Taz1, an outer mitochondrial membrane protein, affects stability and assembly of inner membrane protein complexes: implications for Barth syndrome. *Mol Biol Cell* **16**: 5202–5214.

Breslow DK, Collins SR, Bodenmiller B, Aebersold R, Simons K, Shevchenko A, Ejsing CS & Weissman JS (2010) Orm family proteins mediate sphingolipid homeostasis. *Nature* **463**: 1048–1053.

Brookheart RT, Michel CI & Schaffer JE (2009) As a matter of fat. *Cell Metab* **10**: 9–12.

Burstein MT, Kyryakov P, Beach A, Richard VR, Koupaki O, Gomez-Perez A, Leonov A, Levy S, Noohi F & Titorenko VI (2012) Lithocholic acid extends longevity of chronologically aging yeast only if added at certain critical periods of their lifespan. *Cell Cycle* **11**: 3443–3462.

Büttner S, Ruli D, Vögtle F-N *et al.* (2011) A yeast BH3-only protein mediates the mitochondrial pathway of apoptosis. *EMBO J* **30**: 2779–2792.

Büttner S, Eisenberg T, Herker E, Carmona-Gutierrez D, Kroemer G & Madeo F (2006) Why yeast cells can undergo apoptosis: death in times of peace, love, and war. *J Cell Biol* **175**: 521–525.

Büttner S, Carmona-Gutierrez D, Eisenberg T, Ruli D & Madeo F (2007) Conspiracy of yeast killers: the fifth international meeting on yeast apoptosis in Prague, Czech Republic, 3–7 September, 2006. *FEMS Yeast Res* **7**: 351–354.

Carman GM & Zeimetz GM (1996) Regulation of phospholipid biosynthesis in the yeast *Saccharomyces cerevisiae*. *J Biol Chem* **271**: 13293–13296.

Carmona-Gutierrez D, Reisenbichler A, Heimbucher P *et al.* (2011) Ceramide triggers metacaspase-independent mitochondrial cell death in yeast. *Cell Cycle* **10**: 3973–3978.

Carmona-Gutierrez D, Eisenberg T, Büttner S, Meisinger C, Kroemer G & Madeo F (2010) Apoptosis in yeast: triggers, pathways, subroutines. *Cell Death Differ* **17**: 763–773.

Cerantola V, Guillas I, Roubaty C, Vionnet C, Uldry D, Knudsen J & Conzelmann A (2009) Aureobasidin A arrests growth of yeast cells through both ceramide intoxication and deprivation of essential inositolphosphorylceramides. *Mol Microbiol* **71**: 1523–1537.

Chang S-C, Heacock PN, Clancey CJ & Dowhan W (1998) The PEL1 gene (Renamed PGS1) encodes the phosphatidylglycero-phosphate synthase of *Saccharomyces cerevisiae*. *J Biol Chem* **273**: 9829–9836.

Chen S, Tarsio M, Kane PM & Greenberg ML (2008) Cardiolipin mediates cross-talk between mitochondria and the vacuole. *Mol Biol Cell* **19**: 5047–5058.

Chen R, Brady E & McIntyre TM (2011) Human TMEM30a promotes uptake of antitumor and bioactive choline phospholipids into mammalian cells. *J Immunol* **186**: 3215–3225.

Claypool SM, Oktay Y, Boontheung P, Loo JA & Koehler CM (2008) Cardiolipin defines the interactome of the major ADP/ATP carrier protein of the mitochondrial inner membrane. *J Cell Biol* **182**: 937–950.

Cowart LA & Obeid LM (2007) Yeast sphingolipids: recent developments in understanding biosynthesis, regulation, and function. *Biochim Biophys Acta* **1771**: 421–431.

Cuesta-Marbán Á, Botet J, Czyz O *et al.* (2013) Drug uptake, lipid rafts, and vesicle trafficking modulate resistance to an anticancer lysophosphatidylcholine analogue in yeast. *J Biol Chem* **288**: 8405–8418.

Czyz O, Bitew T, Cuesta-Marbán A, McMaster CR, Mollinedo F & Zaremberg V (2013) Alteration of plasma membrane organization by an anticancer lysophosphatidylcholine analogue induces intracellular acidification and internalization of plasma membrane transporters in yeast. *J Biol Chem* **288**: 8419–8432.

de Avalos SV, Okamoto Y & Hannun YA (2004) Activation and localization of inositol phosphosphingolipid phospholipase C, Isc1p, to the mitochondria during growth of *Saccharomyces cerevisiae*. *J Biol Chem* **279**: 11537–11545.

Dickson RC & Lester RL (2002) Sphingolipid functions in *Saccharomyces cerevisiae*. *Biochim Biophys Acta* **1583**: 13–25.

D'mello NP, Childress AM, Franklin DS, Kale SP, Pinswasdi C & Jazwinski SM (1994) Cloning and characterization of LAG1, a longevity-assurance gene in yeast. *J Biol Chem* **269**: 15451–15459.

Eisenberg T, Knauer H, Schauer A *et al.* (2009) Induction of autophagy by spermidine promotes longevity. *Nat Cell Biol* **11**: 1305–1314.

Eisenberg T, Büttner S, Kroemer G & Madeo F (2007) The mitochondrial pathway in yeast apoptosis. *Apoptosis* **12**: 1011–1023.

Eisenberg T, Carmona-Gutierrez D, Büttner S, Tavernarakis N & Madeo F (2010) Necrosis in yeast. *Apoptosis* **15**: 257–268.

Ejsing CS, Sampaio JL, Surendranath V, Duchoslav E, Ekroos K, Klemm RW, Simons K & Shevchenko A (2009) Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. *Proc Natl Acad Sci* **106**: 2136–2141.

Esposti MD, Cristea IM, Gaskell SJ, Nakao Y & Dive C (2003) Proapoptotic Bid binds to monolysocardiolipin, a new molecular connection between mitochondrial membranes and cell death. *Cell Death Differ* **10**: 1300–1309.

Fabrizio P, Battistella L, Vardavas R, Gattazzo C, Liou L-L, Diaspro A, Dossen JW, Gralla EB & Longo VD (2004) Superoxide is a mediator of an altruistic aging program in *Saccharomyces cerevisiae*. *J Cell Biol* **166**: 1055–1067.

Fahrenkrog B, Sauder U & Aebi U (2004) The *S. cerevisiae* HtrA-like protein Nma111p is a nuclear serine protease that mediates yeast apoptosis. *J Cell Sci* **117**: 115–126.

Fakas S, Qiu Y, Dixon JL, Han G-S, Ruggles KV, Garbarino J, Sturley SL & Carman GM (2011) Phosphatidate phosphatase activity plays key role in protection against fatty acid-induced toxicity in yeast. *J Biol Chem* **286**: 29074–29085.

Fontana L, Partridge L & Longo VD (2010) Extending healthy life span—from yeast to humans. *Science* **328**: 321–326.

Gajate C & Mollinedo F (2002) Biological activities, mechanisms of action and biomedical prospect of the antitumor ether phospholipid ET-18-OCH(3) (edelfosine), a proapoptotic agent in tumor cells. *Curr Drug Metab* **3**: 491–525.

Gajate C, Matos-da-Silva M, Dakir el-H, Fonteriz RI, Alvarez J & Mollinedo F (2012) Antitumor alkyl-lysophospholipid analog edelfosine induces apoptosis in pancreatic cancer by targeting endoplasmic reticulum. *Oncogene* **31**: 2627–2639.

Galluzzi L, Vitale I, Senovilla L *et al.* (2012) Independent transcriptional reprogramming and apoptosis induction by cisplatin. *Cell Cycle* **11**: 3472–3480.

Ganesan V, Perera MN, Colombini D, Datskovskiy D, Chadha K & Colombini M (2010) Ceramide and activated Bax act synergistically to permeabilize the mitochondrial outer membrane. *Apoptosis* **15**: 553–562.

Garbarino J, Padamsee M, Wilcox L, Oelkers PM, D'Ambrosio D, Ruggles KV, Ramsey N, Jabado O, Turkish A & Sturley SL (2009) Sterol and diacylglycerol acyltransferase deficiency

triggers fatty acid-mediated cell death. *J Biol Chem* **284**: 30994–31005.

Garbarino J & Sturley SL (2005) Homoeostatic systems for sterols and other lipids. *Biochem Soc Trans* **33**: 1182.

Garbarino J & Sturley SL (2009) Saturated with fat: new perspectives on lipotoxicity. *Curr Opin Clin Nutr Metab Care* **12**: 110–116.

Gerisch B, Rottiers V, Li D, Motola DL, Cummins CL, Lehrach H, Mangelsdorf DJ & Antebi A (2007) A bile acid-like steroid modulates *Caenorhabditis elegans* lifespan through nuclear receptor signaling. *Proc Natl Acad Sci* **104**: 5014–5019.

Goldberg AA, Bourque SD, Kyryakov P *et al.* (2009) A novel function of lipid droplets in regulating longevity. *Biochem Soc Trans* **37**: 1050–1055.

Goldberg AA, Richard VR, Kyryakov P *et al.* (2010) 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* **2**: 393–414.

Gonzalvez F, Pariselli F, Dupaigne P *et al.* (2005a) tBid interaction with cardiolipin primarily orchestrates mitochondrial dysfunctions and subsequently activates Bax and Bak. *Cell Death Differ* **12**: 614–626.

Gonzalvez F, Bessoule J-J, Rocchiccioli F, Manon S & Petit PX (2005b) Role of cardiolipin on tBid and tBid/Bax synergistic effects on yeast mitochondria. *Cell Death Differ* **12**: 659–667.

Greenwood MT & Ludovico P (2009) Expressing and functional analysis of mammalian apoptotic regulators in yeast. *Cell Death Differ* **17**: 737–745.

Han G-S, Wu W-I & Carman GM (2006) The *Saccharomyces cerevisiae* Lipin homolog is a Mg2+-dependent phosphatidate phosphatase enzyme. *J Biol Chem* **281**: 9210–9218.

Hanada K (2003) Serine palmitoyltransferase, a key enzyme of sphingolipid metabolism. *Biochim Biophys Acta* **1632**: 16–30.

Hannun YA & Obeid LM (2011) Many *Ceramides*. *J Biol Chem* **286**: 27855–27862.

Hanson PK, Malone L, Birchmore JL & Nichols JW (2003) Lem3p is essential for the uptake and potency of alkylphosphocholine drugs, edelfosine and miltefosine. *J Biol Chem* **278**: 36041–36050.

Herker E, Jungwirth H, Lehmann KA, Maldener C, Fröhlich K-U, Wissing S, Büttner S, Fehr M, Sigrist S & Madeo F (2004) Chronological aging leads to apoptosis in yeast. *J Cell Biol* **164**: 501–507.

Hiltunen JK, Mursula AM, Rottensteiner H, Wierenga RK, Kastaniotis AJ & Gurvitz A (2003) The biochemistry of peroxisomal β-oxidation in the yeast *Saccharomyces cerevisiae*. *FEMS Microbiol Rev* **27**: 35–64.

Houtkooper RH, Argmann C, Houten SM, Cantó C, Jeninga EH, Andreux PA, Thomas C, Doenlen R, Schoonjans K & Auwerx J (2011) The metabolic footprint of aging in mice. *Sci Rep* **1**: 134.

Huang X, Liu J & Dickson RC (2012) Down-regulating sphingolipid synthesis increases yeast lifespan. *PLoS Genet* **8**: e1002493.

Huitema K, van den Dikkenberg J, Brouwers JFHM & Holthuis JCM (2004) Identification of a family of animal sphingomyelin synthases. *EMBO J* **23**: 33–44.

Iverson SL, Enoksson M, Gogvadze V, Ott M & Orrenius S (2004) Cardiolipin is not required for Bax-mediated cytochrome c release from yeast mitochondria. *J Biol Chem* **279**: 1100–1107.

Jiang F, Ryan MT, Schlame M, Zhao M, Gu Z, Klingenberg M, Pfanner N & Greenberg ML (2000) Absence of cardiolipin in the crd1 null mutant results in decreased mitochondrial membrane potential and reduced mitochondrial function. *J Biol Chem* **275**: 22387–22394.

Jiang JC, Kirchman PA, Allen M & Jazwinski SM (2004) Suppressor analysis points to the subtle role of the LAG1 ceramide synthase gene in determining yeast longevity. *Exp Gerontol* **39**: 999–1009.

Joshi AS, Zhou J, Gohil VM, Chen S & Greenberg ML (2009) Cellular functions of cardiolipin in yeast. *Biochim Biophys Acta* **1793**: 212–218.

Jungwirth H, Ring J, Mayer T, Schauer A, Büttner S, Eisenberg T, Carmona-Gutierrez D, Kuchler K & Madeo F (2008) Loss of peroxisome function triggers necrosis. *FEBS Lett* **582**: 2882–2886.

Kagan VE, Tyurin VA, Jiang J *et al.* (2005) Cytochrome c acts as a cardiolipin oxygenase required for release of proapoptotic factors. *Nat Chem Biol* **1**: 223–232.

Kajiwara K, Watanabe R, Pichler H, Ihara K, Murakami S, Riezman H & Funato K (2008) Yeast ARV1 is required for efficient delivery of an early GPI intermediate to the first mannosyltransferase during GPI assembly and controls lipid flow from the endoplasmic reticulum. *Mol Biol Cell* **19**: 2069–2082.

Kajiwara K, Muneoka T, Watanabe Y, Karashima T, Kitagaki H & Funato K (2012) Perturbation of sphingolipid metabolism induces endoplasmic reticulum stress-mediated mitochondrial apoptosis in budding yeast. *Mol Microbiol* **86**: 1246–1261.

Khan MJ, Alam MR, Waldeck-Weiermair M *et al.* (2012) Inhibition of autophagy rescues palmitic acid-induced necroptosis of endothelial cells. *J Biol Chem* **287**: 21110–21120.

Khoury CM & Greenwood MT (2008) The pleiotropic effects of heterologous Bax expression in yeast. *Biochim Biophys Acta* **1783**: 1449–1465.

Kim T-H, Zhao Y, Ding W-X, Shin JN, He X, Seo Y-W, Chen J, Rabinowich H, Amoscato AA & Yin X-M (2004) Bid-cardiolipin interaction at mitochondrial contact site contributes to mitochondrial cristae reorganization and cytochrome c release. *Mol Biol Cell* **15**: 3061–3072.

Kitagaki H, Cowart LA, Matmati N, Vaena de Avalos S, Novgorodov SA, Zeidan YH, Bielawski J, Obeid LM & Hannun YA (2007) Isc1 regulates sphingolipid metabolism in yeast mitochondria. *Biochim Biophys Acta* **1768**: 2849–2861.

Kohlwein SD (2010a) Obese and anorexic yeasts: experimental models to understand the metabolic syndrome and lipotoxicity. *Biochim Biophys Acta* **1801**: 222–229.

Kohlwein SD (2010b) Triacylglycerol homeostasis: insights from yeast. *J Biol Chem* **285**: 15663–15667.

Kohlwein SD & Petschnigg J (2008) Lipid-induced cell dysfunction and cell death: lessons from yeast. *Curr Hypertens Rep* **9**: 455–461.

Korytowski W, Basova LV, Pilat A, Kernstock RM & Girotti AW (2011) Permeabilization of the mitochondrial outer membrane by Bax/truncated Bid (tBid) proteins as sensitized by cardiolipin hydroperoxide translocation mechanistic implications for the intrinsic pathway of oxidative apoptosis. *J Biol Chem* **286**: 26334–26343.

Kurat CF, Natter K, Petschnigg J, Wolinski H, Scheuringer K, Scholz H, Zimmermann R, Leber R, Zechner R & Kohlwein SD (2006) Obese yeast: triglyceride lipolysis is functionally conserved from mammals to yeast. *J Biol Chem* **281**: 491–500.

Kuwana T, Mackey MR, Perkins G, Ellisman MH, Latterich M, Schneiter R, Green DR & Newmeyer DD (2002) Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. *Cell* **111**: 331–342.

Laun P, Pichova A, Madeo F, Fuchs J, Ellinger A, Kohlwein S, Dawes I, Fröhlich KU & Breitenbach M (2001) Aged mother cells of *Saccharomyces cerevisiae* show markers of oxidative stress and apoptosis. *Mol Microbiol* **39**: 1166–1173.

Listenberger LL, Han X, Lewis SE, Cases S, Farese RV, Ory DS & Schaffer JE (2003) Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proc Natl Acad Sci* **100**: 3077–3082.

Low CP, Liew LP, Pervaiz S & Yang H (2005) Apoptosis and lipoapoptosis in the fission yeast *Schizosaccharomyces pombe*. *FEMS Yeast Res* **5**: 1199–1206.

Lucken-Ardjomande S, Montessuit S & Martinou J-C (2008) Contributions to Bax insertion and oligomerization of lipids of the mitochondrial outer membrane. *Cell Death Differ* **15**: 929–937.

Ludovico P, Rodrigues F, Almeida A, Silva MT, Barrientos A & Côrte-Real M (2002) Cytochrome c release and mitochondria involvement in programmed cell death induced by acetic acid in *Saccharomyces cerevisiae*. *Mol Biol Cell* **13**: 2598–2606.

Lutter M, Fang M, Luo X, Nishijima M, Xie X & Wang X (2000) Cardiolipin provides specificity for targeting of tBid to mitochondria. *Nat Cell Biol* **2**: 754–761.

Lux H, Heise N, Klenner T, Hart D & Opperdoes FR (2000) Ether–lipid (alkyl-phospholipid) metabolism and the mechanism of action of ether–lipid analogues in *Leishmania*. *Mol Biochem Parasitol* **111**: 1–14.

Madeo F, Herker E, Maldener C *et al.* (2002) A caspase-related protease regulates apoptosis in yeast. *Mol Cell* **9**: 911–917.

Madeo F, Carmona-Gutierrez D, Ring J, Büttner S, Eisenberg T & Kroemer G (2009) Caspase-dependent and caspase-independent cell death pathways in yeast. *Biochem Biophys Res Commun* **382**: 227–231.

Madeo F, Tavernarakis N & Kroemer G (2010) Can autophagy promote longevity? *Nat Cell Biol* **12**: 842–846.

Manivannan S, Scheckhuber CQ & van der Klei IJ (2012) The impact of peroxisomes on cellular aging and death. *Front Oncol* **2**: 50.

Mao C, Xu R, Bielawska A & Obeid LM (2000a) Cloning of an alkaline ceramidase from *Saccharomyces cerevisiae* an enzyme with reverse (CoA-independent) ceramide synthase activity. *J Biol Chem* **275**: 6876–6884.

Mao C, Xu R, Bielawska A, Szulc ZM & Obeid LM (2000b) Cloning and characterization of a *Saccharomyces cerevisiae* alkaline ceramidase with specificity for dihydroceramide. *J Biol Chem* **275**: 31369–31378.

Matsko CM, Hunter OC, Rabinowich H, Lotze MT & Amoscato AA (2001) Mitochondrial lipid alterations during Fas- and radiation-induced apoptosis. *Biochem Biophys Res Commun* **287**: 1112–1120.

McMillin JB & Dowhan W (2002) Cardiolipin and apoptosis. *Biochim Biophys Acta* **1585**: 97–107.

Meijer WH, van der Klei IJ, Veenhuis M & Kiel JA (2007) ATG genes involved in non-selective autophagy are conserved from yeast to man, but the selective Cvt and pexophagy pathways also require organism-specific genes. *Autophagy* **3**: 106–116.

Mitsui K, Nakagawa D, Nakamura M, Okamoto T & Tsurugi K (2005) Valproic acid induces apoptosis dependent of Yca1p at concentrations that mildly affect the proliferation of yeast. *FEBS Lett* **579**: 723–727.

Mollinedo F, Fernández M, Hornillos V *et al.* (2011) Involvement of lipid rafts in the localization and dysfunction effect of the antitumor ether phospholipid edelfosine in mitochondria. *Cell Death Dis* **2**: e158.

Mollinedo F, Fernández-Luna JL, Gajate C, Martín-Martín B, Benito A, Martínez-Dalmau R & Modolell M (1997) Selective induction of apoptosis in cancer cells by the ether lipid ET-18-OCH3 (Edelfosine): molecular structure requirements, cellular uptake, and protection by Bcl-2 and Bcl-X(L). *Cancer Res* **57**: 1320–1328.

Nagiec MM, Nagiec EE, Baltisberger JA, Wells GB, Lester RL & Dickson RC (1997) Sphingolipid synthesis as a target for antifungal drugs. *J Biol Chem* **272**: 9809–9817.

Navina S, Acharya C, DeLany JP *et al.* (2011) Lipotoxicity causes multisystem organ failure and exacerbates acute pancreatitis in obesity. *Sci Transl Med* **3**: 107ra110.

Nieto-Miguel T, Fonteriz RI, Vay L, Gajate C, López-Hernández S & Mollinedo F (2007) Endoplasmic reticulum stress in the proapoptotic action of edelfosine in solid tumor cells. *Cancer Res* **67**: 10368–10378.

Nomura K, Imai H, Koumura T, Kobayashi T & Nakagawa Y (2000) Mitochondrial phospholipid hydroperoxide glutathione peroxidase inhibits the release of cytochrome c from mitochondria by suppressing the peroxidation of cardiolipin in hypoglycaemia-induced apoptosis. *Biochem J* **351**: 183.

Ott M, Robertson JD, Gogvadze V, Zhivotovsky B & Orrenius S (2002) Cytochrome c release from mitochondria proceeds by a two-step process. *Proc Natl Acad Sci* **99**: 1259–1263.

Patil VA, Fox JL, Gohil VM, Winge DR & Greenberg ML (2013) Loss of cardiolipin leads to perturbation of mitochondrial and cellular iron homeostasis. *J Biol Chem* **288**: 1696–1705.

Pereira C & Saraiva L (2013) Interference of aging media on the assessment of yeast chronological life span by propidium iodide staining. *Folia Microbiol (Praha)* **58**: 81–84.

Périchon R & Bourre JM (1996) Aging-related decrease in liver peroxisomal fatty acid oxidation in control and clofibrate-treated mice. A biochemical study and mechanistic approach. *Mech Ageing Dev* **87**: 115–126.

Péterfy M, Phan J, Xu P & Reue K (2001) Lipodystrophy in the fld mouse results from mutation of a new gene encoding a nuclear protein, lipin. *Nat Genet* **27**: 121–124.

Petschnigg J, Wolinski H, Kolb D, Zellnig G, Kurat CF, Natter K & Kohlwein SD (2009) Good fat, essential cellular requirements for triacylglycerol synthesis to maintain membrane homeostasis in yeast. *J Biol Chem* **284**: 30981–30993.

Polčic P, Su X, Fowlkes J, Blachly-Dyson E, Dowhan W & Forte M (2005) Cardiolipin and phosphatidylglycerol are not required for the *in vivo* action of Bcl-2 family proteins. *Cell Death Differ* **12**: 310–312.

Priault M, Camougrand N, Kinnally KW, Vallette FM & Manon S (2003) Yeast as a tool to study Bax/mitochondrial interactions in cell death. *FEMS Yeast Res* **4**: 15–27.

Rajakumari S, Grillitsch K & Daum G (2008) Synthesis and turnover of non-polar lipids in yeast. *Prog Lipid Res* **47**: 157–171.

Raychaudhuri S, Young BP, Espenshade PJ & Loewen CJ (2012) Regulation of lipid metabolism: a tale of two yeasts. *Curr Opin Cell Biol* **24**: 502–508.

Rego A, Costa M, Chaves SR, Matmati N, Pereira H, Sousa MJ, Moradas-Ferreira P, Hannun YA, Costa V & Côrte-Real M (2012) Modulation of mitochondrial outer membrane permeabilization and apoptosis by ceramide metabolism. *PLoS One* **7**: e48571.

Richard VR, Leonov A, Beach A, Burstein MT, Koupaki O, Gomez-Perez A, Levy S, Pluska L, Mattie S & Rafesh R (2013) Macromitophagy is a longevity assurance process that in chronologically aging yeast limited in calorie supply sustains functional mitochondria and maintains cellular lipid homeostasis. *Aging* **5**: 234–269.

Rockenfeller P, Ring J, Muschett V *et al.* (2010) Fatty acids trigger mitochondrion-dependent necrosis. *Cell Cycle* **9**: 2836–2842.

Sandager L, Gustavsson MH, Ståhl U, Dahlqvist A, Wiberg E, Banas A, Lenman M, Ronne H & Stymne S (2002) Storage lipid synthesis is non-essential in yeast. *J Biol Chem* **277**: 6478–6482.

Santos-Rosa H, Leung J, Grimsey N, Peak-Chew S & Siniossoglou S (2005) The yeast lipin Smp2 couples phospholipid biosynthesis to nuclear membrane growth. *EMBO J* **24**: 1931–1941.

Schaffer JE (2003) Lipotoxicity: when tissues overeat. *Curr Opin Lipidol* **14**: 281–287.

Schlame M, Towbin JA, Heerdt PM, Jehle R, DiMauro S & Blanck TJJ (2002) Deficiency of tetralinoleoyl-cardiolipin in Barth syndrome. *Ann Neurol* **51**: 634–637.

Schlame M, Ren M, Xu Y, Greenberg ML & Haller I (2005) Molecular symmetry in mitochondrial cardiolipins. *Chem Phys Lipids* **138**: 38–49.

Schlotterer A, Kukudov G, Bozorgmehr F *et al.* (2009) *C. elegans* as model for the study of high glucose– mediated life span reduction. *Diabetes* **58**: 2450–2456.

Schneiter R, Brügger B, Sandhoff R *et al.* (1999) Electrospray ionization tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals acyl chain-based sorting/remodeling of distinct molecular species en route to the plasma membrane. *J Cell Biol* **146**: 741–754.

Schorling S, Vallée B, Barz WP, Riezman H & Oesterhelt D (2001) Lag1p and Lac1p are essential for the Acyl-CoA–dependent ceramide synthase reaction in *Saccharomyces cerevisae*. *Mol Biol Cell* **12**: 3417–3427.

Shidoji Y, Hayashi K, Komura S, Ohishi N & Yagi K (1999) Loss of molecular interaction between cytochrome c and cardiolipin due to lipid peroxidation. *Biochem Biophys Res Commun* **264**: 343–347.

Silva RD, Manon S, Goncalves J, Saraiva L & Corte-Real M (2011) The importance of humanized yeast to better understand the role of bcl-2 family in apoptosis: finding of novel therapeutic opportunities. *Curr Pharm Des* **17**: 246–255.

Simons K & Gerl MJ (2010) Revitalizing membrane rafts: new tools and insights. *Nat Rev Mol Cell Biol* **11**: 688–699.

Singh R & Cuervo AM (2012) Lipophagy: connecting autophagy and lipid metabolism. *Int J Cell Biol* **2012**: 282041.

Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, Tanaka K, Cuervo AM & Czaja MJ (2009) Autophagy regulates lipid metabolism. *Nature* **458**: 1131–1135.

Siskind LJ, Feinstein L, Yu T *et al.* (2008) Anti-apoptotic Bcl-2 family proteins disassemble ceramide channels. *J Biol Chem* **283**: 6622–6630.

Siskind LJ & Colombini M (2000) The lipids C2- and C16-ceramide form large stable channels. *J Biol Chem* **275**: 38640–38644.

Stratford M & Anslow PA (1996) Comparison of the inhibitory action on *Saccharomyces cerevisiae* of weak-acid preservatives, uncouplers, and medium-chain fatty acids. *FEMS Microbiol Lett* **142**: 53–58.

Sun Q, Bi L, Su X, Tsurugi K & Mitsui K (2007) Valproate induces apoptosis by inducing accumulation of neutral lipids which was prevented by disruption of the SIR2 gene *in Saccharomyces cerevisiae*. *FEBS Lett* **581**: 3991–3995.

Tao W, Kurschner C & Morgan JI (1997) Modulation of cell death in yeast by the Bcl-2 family of proteins. *J Biol Chem* **272**: 15547–15552.

Teng X & Hardwick JM (2009) Reliable method for detection of programmed cell death in yeast. *Methods Mol Biol* **559**: 335–342.

Tong F, Black PN, Bivins L, Quackenbush S, Ctrnacta V & DiRusso CC (2006) Direct interaction of *Saccharomyces cerevisiae* Faa1p with the Omi/HtrA protease orthologue Ynm3p alters lipid homeostasis. *Mol Genet Genomics* **275**: 330–343.

Tucker MZ & Turcotte LP (2002) Impaired fatty acid oxidation in muscle of aging rats perfused under basal conditions. *Am J Physiol Endocrinol Metab* **282**: E1102–E1109.

Tulha J, Faria-Oliveira F, Lucas C & Ferreira C (2012) Programmed cell death in *Saccharomyces cerevisiae* is hampered by the deletion of GUP1 gene. *BMC Microbiol* **12**: 80.

Unger RH & Orci L (2002) Lipoapoptosis: its mechanism and its diseases. *Biochim Biophys Acta* **1585**: 202–212.

Váchová L & Palková Z (2005) Physiological regulation of yeast cell death in multicellular colonies is triggered by ammonia. *J Cell Biol* **169**: 711–717.

Van Blitterswijk WJ & Verheij M (2008) Anticancer alkylphospholipids: mechanisms of action, cellular sensitivity and resistance, and clinical prospects. *Curr Pharm Des* **14**: 2061–2074.

Van der Luit AH, Budde M, Ruurs P, Verheij M & van Blitterswijk WJ (2002) Alkyl-lysophospholipid accumulates in lipid rafts and induces apoptosis via raft-dependent endocytosis and inhibition of phosphatidylcholine synthesis. *J Biol Chem* **277**: 39541–39547.

Van Roermund CWT, Waterham HR, Ijlst L & Wanders RJA (2003) Fatty acid metabolism in *Saccharomyces cerevisiae*. *Cell Mol Life Sci* **60**: 1838–1851.

Vreken P, Valianpour F, Nijtmans LG, Grivell LA, Plecko B, Wanders RJA & Barth PG (2000) Defective remodeling of cardiolipin and phosphatidylglycerol in barth syndrome. *Biochem Biophys Res Commun* **279**: 378–382.

Wagner BA, Buettner GR & Burns CP (1996) Vitamin E slows the rate of free radical-mediated lipid peroxidation in cells. *Arch Biochem Biophys* **334**: 261–267.

Weinberger M, Mesquita A, Caroll T, Marks L, Yang H, Zhang Z, Ludovico P & Burhans WC (2010) Growth signaling promotes chronological aging in budding yeast by inducing superoxide anions that inhibit quiescence. *Aging (Albany NY)* **2**: 709–726.

Whited K, Baile MG, Currier P & Claypool SM (2013) Seven functional classes of Barth syndrome mutation. *Hum Mol Genet* **22**: 483–492.

Wright MM, Howe AG & Zaremberg V (2004) Cell membranes and apoptosis: role of cardiolipin, phosphatidylcholine, and anticancer lipid analogues. *Biochem Cell Biol* **82**: 18–26.

Yang Z, Khoury C, Jean-Baptiste G & Greenwood MT (2006) Identification of mouse sphingomyelin synthase 1 as a suppressor of Bax-mediated cell death in yeast. *FEMS Yeast Res* **6**: 751–762.

Zaremberg V, Gajate C, Cacharro LM, Mollinedo F & McMaster CR (2005) Cytotoxicity of an anti-cancer lysophospholipid through selective modification of lipid raft composition. *J Biol Chem* **280**: 38047–38058.

Zechner R & Madeo F (2009) Cell biology: another way to get rid of fat. *Nature* **458**: 1118–1119.

Zechner R, Zimmermann R, Eichmann TO, Kohlwein SD, Haemmerle G, Lass A & Madeo F (2012) FAT SIGNALS – lipases and lipolysis in lipid metabolism and signaling. *Cell Metab* **15**: 279–291.

Zhang X, Skrzypek MS, Lester RL & Dickson RC (2001) Elevation of endogenous sphingolipid long-chain base phosphates kills *Saccharomyces cerevisiae* cells. *Curr Genet* **40**: 221–233.

Zhang H, Gajate C, Yu L, Fang Y & Mollinedo F (2007) Mitochondrial-derived ROS in edelfosine-induced apoptosis in yeasts and tumor cells. *Acta Pharmacol Sin* **28**: 888–894.