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).
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 (Khouri & 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-XL 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 esterified sterols (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 & Zeiment, 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 polynsaturation activity, the saturated fatty acids (SFA) palmitic (C16) and stearic (C18) acids as well as their monounsaturated derivatives palmitoleic (C16:1) and oleic (C18:1) acids constitute the vast majority of fatty acids present (Schneider 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 C24–C26 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...
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 (IPCSynthase) 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 subroutines 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
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 C16-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 C2-ceramide and C16-ceramide (but not C2-dihydroceramide or C16-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 C16-ceramide in concentrations where they only exerted minor effects on their own has been shown to result in MOMP in a synergistic fashion.

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.
(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-XL-expressing yeast cells were resistant to C16-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 C2-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 Bfh3–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 lag1Δlac1Δ 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 (Regó 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-C20-phytoceramide levels increased dramatically in a way strictly depending on the presence of both Isc1p and Lag1p. External administration of C20-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 (Regó et al., 2012). In consistence with these results, suggesting a
prodeath function for Isc1p and Lag1p, deletion of \( \text{LAG1} \) 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 \textit{et al.}, 1994; Jiang \textit{et al.}, 2004).

In contrast to observed resistance of the \( \text{ISC1} \) null mutant to acetic acid, deletion of \( \text{ISC1} \) has been reported to result in sensitivity to oxidative stress as well as a shortened CLS (Kitagaki \textit{et al.}, 2007; Almeida \textit{et al.}, 2008). Isc1p represents the only yeast member of the family of sphingomyelinases (SMases) and translates from ER to mitochondria upon induction of respiration (de Avalos \textit{et al.}, 2004), where it integrates into the outer mitochondrial membrane (Kitagaki \textit{et al.}, 2007). This seems to be essential for mitochondrial function, as the deletion of \( \text{ISC1} \) and the concomitant loss of more than 90% mitochondrial locally located ceramide (\( \alpha \)-hydroxy-C\(_{26}\)-phytosphingosine) triggered the generation of respiratory-deficient cells when exposed to elevated temperature (Kitagaki \textit{et al.}, 2007). Death of \( \text{ISC1} \)-disrupted cells during aging and upon treatment with hydrogen peroxide was accompanied by typical markers of apoptosis and prevented by deletion of \( \text{YCA1} \). Interestingly, while \( \text{ISC1} \) deficiency caused a general decrease in most ceramide species and an increase in \( \alpha \)-hydroxy-C\(_{26}\)-phytosphingosine upon treatment with acetic acid (Rego \textit{et al.}, 2012), specific species, namely C\(_{26}\)-dihydroceramide and C\(_{26}\)-phytosphingosine, were upregulated during chronological aging (Barbosa, 2011). Only recently, premature aging, sensitivity to oxidative stress as well as mitochondrial defects of \( \text{ISC1} \)-disrupted cells could be circumvented by additional deletion of \( \text{SIT4} \), 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 Sip4p that is essentially involved in the lethal consequences of \( \text{ISC1} \) deletion. It remains to be clarified whether death in \( \text{ISC1} \)-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 protein phosphatase Sip4p 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 \textit{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 \textit{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 & Sturler, 2005; Garbarino \textit{et al.}, 2009; Petschnigg \textit{et al.}, 2009; Kohlwein, 2010a; Rockenfeller \textit{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 \textit{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 \textit{et al.}, 2009), while another study reported necrotic cell death under similar conditions (Rockenfeller \textit{et al.}, 2010). Petschnigg \textit{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 than 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 reestablish growth (Petschnigg \textit{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 C18 rather than C16. Interestingly, administration of palmitic acid (C16:0) could prevent oleic acid (C18:1)-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 (C18:1), linoleic (C18:2), and linolenic (C18:3) 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 Nhp6A1p, 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 (C10:0) caused rapid cell death (Stratford & Anslow, 1996). The synthetic branched-chain carbonic acid valproic acid (C8), 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 opposite 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 healthspan 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 (Athens-Taedt & 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).
Cardiolipin and mitochondrial lipid oxidation

Cardiolipin, (diphasphatidylglycerol) 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 phosphatidylglyceroxophosphate 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 (Gonzalez 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 (Gonzalez 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 (Lunken-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-XI (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 wt-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 (Iversen et al., 2004; Gonzalvez et al., 2005b; Policic et al., 2005). Instead, cardiolipin has been found to be required for tight binding of cytochrome c to the inner mitochondrial membrane (Iversen 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 PGSI), the heterologous expression of BAX still provoked mitochondrial permeabilization (Policic 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 a-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 a-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 a-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 a-tocopherol must involve additional biological activities. In this respect, a -tocopherol has been shown to prevent sterol replacement and Pma1p internalization, two early events after the addition of edelfosine, suggesting that a-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 alklyphospholipid-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 LCBl, general sphingolipids | Growth impairment; increased CLS; heat shock and H₂O₂ 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 Ca²⁺ 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. | H₂O₂, 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/ mitochondrial dependent | Carmona-Gutierrez et al. (2011) and Galluzzi et al. (2012) |
| **YDC1 overexpression** | n.d. | Reduced CLS preventable by exogenous C₂₆-dihydroceramide; apoptotic death | Clonogenicity; TUNEL, AnnV/PI, MF | Aerts et al. (2008) |
| **ISC1 or LAG1 deletion** | General ceramide decreased, but increased α-hydroxy-C₂₀-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 C₂₆-phytoceramide; ceramide increased during CLS | Reduced CLS, sensitivity to H₂O₂, 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 Edelfosine** | Lipidomic alterations in PC metabolism; reduced sterols and sphingolipids in lipid rafts | Cell death, Pma1p degradation-dependent acidification | Clonogenicity, drop tests | Zaremba et al. (2005) and Cyż et al. (2013) |
| **Edelfosine** | n.d. | α-tocopherol inhibitable apoptotic cell death | Growth, ROS, TUNEL | Zhang et al. (2007) |
| **GU1 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$_2$O$_2$ 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.

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