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Spingolipids and Mitochondrial Function in Budding Yeast

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Abstract

Background—Spingolipids (SLs) are not only key components of cellular membranes, but also play an important role as signaling molecules in orchestrating both cell growth and apoptosis. In *Saccharomyces cerevisiae*, three complex SLs are present and hydrolysis of either of these species is catalyzed by the inositol phosphosphingolipid phospholipase C (Isc1p). Strikingly, mutants deficient in Isc1p display several hallmarks of mitochondrial dysfunction such as the inability to grow on a non-fermentative carbon source, increased oxidative stress and aberrant mitochondrial morphology.

Scope of Review—In this review, we focus on the pivotal role of Isc1p in regulating mitochondrial function via SL metabolism, and on Sch9p as central signal transducer. Sch9p is one of the main effectors of the target of rapamycin complex 1 (TORC1), which is regarded as a crucial signaling axis for regulation of Isc1p-mediated events. Finally, we describe the retrograde response, a signaling event originating from mitochondria to the nucleus which results in the induction of nuclear target genes. Intriguingly, the retrograde response also interacts with SL homeostasis.

Major Conclusions—All the above suggests a pivotal signaling role for SLs in maintaining correct mitochondrial function in budding yeast.

General Significance—Studies with budding yeast provide insight on SL signaling events that affect mitochondrial function.

Keywords

S. cerevisiae; spingolipids; mitochondrial function; Isc1p; Sch9p; retrograde response

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1. Introduction¹

Sphingolipids (SLs) are lipid species characterized by the presence of a sphingoid base as structural backbone. These sphingoid bases are either sphingosine, dihydrosphingosine (DHS) or phytosphingosine (PHS) [1]. In yeast research, DHS and PHS are termed long chain bases (LCBs). In general, SLs serve as important parts of membranes, but also take part as signaling molecules in the regulation of cell division [2], cell death [3], lifespan [4] and autophagy [5].

SL biosynthetic pathways are highly conserved between mammalian and yeast cells [6-8]. In *Saccharomyces cerevisiae* *de novo* SL biosynthesis (Fig. 1) typically starts by the condensation of serine and palmitoyl Coenzyme A (palmitoyl CoA) to generate 3-ketodihydrosphingosine (3-keto DHS) by the target of Myriocin, namely serine palmitoyltransferase (SPT) [9-12]. As for yeast, 3-keto DHS is reduced to DHS, which then is processed into either dihydroceramide (dhCer) or PHS. Subsequently, both dhCer and PHS are converted into the central yeast SL phytoceramide (phytoCer). PhytoCer serves as precursor in the formation of the three complex SLs by addition of polar headgroups: (i) addition of phospho-inositol to phytoCer by inositolphosphoceramide (IPC) synthase yields IPC; (ii) addition of mannose to IPC by mannose inositolphosphoceramide (MIPC) synthase yields MIPC; (iii) addition of a second phospho-inositol residue to MIPC by inositolphosphotransferase (Ipt1p) leads to the generation of the end product mannose diinositolphosphoceramide (M(IP)₂C) [13-15]. These three complex SLs can be hydrolyzed by the inositol phosphosphingolipid phospholipase C (Isc1p) back to phytoCer [16]. Functionally, Isc1p is essential in the coordination of cellular morphology [17] and cell cycle [18]. Also, Isc1p is involved in tolerance or sensitivity to toxic agents such as Na⁺ and Li⁺ [19], H₂O₂ [20], acetic acid [21], methyl methanesulfate and hydroxyurea [22]. Furthermore, studies with *isc1* mutants have implicated a pivotal role for SLs in the regulation of mitochondrial function and dysfunction [20, 21, 23-27].

The documented role for Isc1p in coordinating mitochondrial function seems to be independent of the retrograde response [28]. The retrograde response is a signaling event originating from the mitochondria that results in the induction of various nuclear target genes by signal transduction proteins [29, 30]. The retrograde response in *S. cerevisiae*, however, also affects SL homeostasis [31, 32] and interacts with additional signaling pathways [29].

Next to the link between SLs and mitochondrial function derived from studies with *isc1* mutants and the retrograde response, a few additional reports have linked SLs to mitochondrial function. For instance, Myriocin-induced cell death, and thus decreased *de*

¹Abbreviations in order of appearance: SLs; sphingolipids; DHS, dihydrosphingosine; PHS, phytosphingosine; LCBs, long chain bases; palmitoyl CoA, palmitoyl coenzyme A; SPT, serine palmitoyltransferase; dhCer, dihydroceramide; IPC, inositolphosphoceramide; MIPC, mannose inositolphosphoceramide; Ipt1, inositolphosphotransferase; M(IP)₂C, mannose diinositolphosphoceramide; Isc1p, inositol phosphosphingolipid phospholipase C; PG, phosphatidylglycerol; CL, cardiolipin; OMM, outer mitochondrial membrane; CLS, chronological lifespan; ETC, electron transport chain; COX, cytochrome c oxidase; HOG pathway, high osmolarity glycerol pathway; CAPP, ceramide-activated protein phosphatase; TORC1, target of rapamycin complex 1; TOR, target of rapamycin; MAPK, mitogen activated protein kinase; ROS, reactive oxygen species; ψ_m , mitochondrial membrane potential; PDRE, Pdr1p/Pdr3p response elements; nSMase, neutral sphingomyelinase; MA-nSMase, mitochondrial-associated nSMase

novo SL biosynthesis, is abrogated in ρ^0 cells [33], which lack mitochondria DNA (mtDNA) and a functional respiratory chain. Likewise, ρ^0 cells are insensitive to Suloctidil and dihydromotuporamine C [33], both compounds that are known to affect SL biosynthesis in mammalian cells [34, 35]. In addition, whereas sub-lethal LCB doses restore viability of yeast mutants defective in SL biosynthesis [36-38] and affect gene expression [39], exogenously added LCBs can kill several fungal species [39-43]. In *S. cerevisiae* however, loss of the mtDNA increases tolerance to LCBs [44], which is dependent on the retrograde response [44]. Such results indicate that SLs indeed are important players in mitochondrial function.

2. Inositol phosphosphingolipid phospholipase C (Isc1p) and mitochondrial function

The inositol phosphosphingolipid phospholipase C (Isc1p) is well documented as the enzyme responsible for the hydrolysis of complex SLs to generate phytoCer [16, 45]. Isc1p activity increases from early exponential to late exponential/post-diauxic growth phase and is regulated by phosphatidylglycerolphosphate synthase (Pgs1p) [23, 25], which is required for the synthesis of phosphatidylglycerol phosphate and subsequent synthesis of phosphatidylglycerol (PG) and cardiolipin (CL) [46]. The mitochondria-associated lipids PG and CL themselves, as well as phosphatidylserine are known activators of Isc1p [45, 47]. Isc1p mainly resides in the ER, but localizes to the outer mitochondrial membrane (OMM) during the late exponential and post-diauxic growth phase [23, 25, 27, 48].

2.1 *isc1* mutants display characteristics of mitochondrial dysfunction

Several studies with *isc1* mutants have pointed to inherent compromised mitochondrial function. One of the main initial observations with *isc1* mutants is their decreased growth rate during late logarithmic and stationary growth phase [23]. Cells lacking Isc1p show decreased chronological lifespan (CLS) [20], a measure of survival of a non-dividing yeast population [49]. This decreased CLS or premature ageing of *isc1* mutants is associated with increased oxidative stress and apoptosis [20, 50]. Indicative for mitochondrial dysfunction, these mutants display defective growth on a nonfermentable carbon source [24-26, 28, 50]. In addition, *isc1* mutants exhibit an increased frequency of petite formation [27], a hallmark of yeast cells with mitochondrial defects [51]. Additional observations from *isc1* mutants indicating aberrant mitochondrial function are the facts that they are characterized by mitochondrial hyperpolarization and mitochondrial fragmentation [26] as well as abnormal mitochondrial morphology [21]. Moreover, *isc1* mutants exhibit increased sensitivity to toxic stimuli, such as hydrogen peroxide (H₂O₂) and ethidium bromide [27], that are reported as increasingly toxic to cells with defective mitochondria [52-54]. A direct link between Isc1p and the mitochondrial respiratory chain is also suggested since *isc1* mutants display lower cytochrome c content [21] and decreased levels of the mitochondrial electron transport chain (ETC) complex IV (cytochrome c oxidase, COX) subunits Cox3p and Cox4p [25]. Hence, decreased COX activity and oxygen consumption rate have also been observed in *isc1* mutants [26]. Interestingly, loss of the mitochondrial genome in *isc1* mutants attenuates the decreased CLS associated with *isc1* mutants, indicating that mitochondrial dysfunction contributes to the shortened CLS in *isc1*

mutants [24]. Summarizing, all these reports indicate that *isc1* mutants indeed are characterized by extensive mitochondrial dysfunction.

2.2 *isc1* mutants show aberrant mitochondrial SL composition

The aforementioned phenotypes in *isc1* mutants can be correlated to aberrancies in mitochondrial SL composition. Intriguingly, wild type yeast cell mitochondria are enriched in α -hydroxylated phytoCer and depleted in sphingoid bases as compared to whole cells [27]. Except for α -OH-C₁₄-phytoCer and C₂₆-phytoCer levels, *isc1* mutants display decreased levels of all SLs, with the most prominent decreases in the levels of α -OH-C₂₄-phytoCer and α -OH-C₂₆-phytoCer species. Also, during CLS *isc1* mutants display an abnormal SL composition: decreased levels of DHS and α -OH-phytoCer and increased levels C₂₆-dhCer and C₂₆-phytoCer [50]. In addition, exogenously supplied C₁₂-phytoCer restores the ability of *isc1* mutants to grow on a non-fermentable carbon source [25]. This suggests that Isc1p-mediated generation of phytoCer in mitochondria is important for mitochondrial function [27].

2.3 Mitochondrial dysfunction in *isc1* mutants is caused by a misregulation of gene expression

The mitochondrial dysfunction-related phenotypes prevalent in *isc1* mutants are, however, caused by a misregulation of gene expression, and not by an intrinsic mitochondrial defect [28]. For instance, deficient growth on a non-fermentable carbon source of *isc1* mutants is not characterized by a loss of mtDNA, nor do isolated mitochondria from *isc1* mutants exhibit differences in the rate of oxygen consumption [28], indicating that *isc1* mutants have a functional respiratory chain. However, *isc1* mutants display little oxygen consumption rate in the post-diauxic shift phase [26], suggesting that additional mechanisms repress mitochondrial function in intact cells during the post-diauxic shift phase. Nonetheless, during the diauxic shift *isc1* mutants are unable to up-regulate genes that are predominantly involved in the utilization of non-fermentable carbon sources, and to down-regulate genes involved in nutrient uptake and amino acid metabolism, independently of the retrograde response [28]. These results suggest an indispensable role of Isc1p in metabolic adaptation and a crucial signaling role for SLs in orchestrating mitochondrial function in intact cells.

2.4 Mechanistic insights into the mitochondria-related function of Isc1p

Mechanistic insights in the underlying signaling pathways that mediate Isc1p-related changes in mitochondrial function were provided by studies showing that loss of either Sit4p, Hog1p, Tor1p or Sch9p attenuates mitochondrial dysfunction-related phenotypes in *isc1* mutants [24, 26, 48, 50]. In the next paragraphs we will provide more details on the interplay between Isc1p and Sit4p/CAPP, the high osmolarity glycerol pathway (HOG pathway) and the TORC1/Sch9p pathway (as summarized in Fig. 2).

Sit4p/CAPP—Sit4p is the catalytic subunit of the ceramide-activated protein phosphatase (CAPP), which is activated by Cer [55]. Sit4p is inactivated by the target of rapamycin complex 1 (TORC1) [56], which is part of the highly conserved target of rapamycin (TOR) nutrient signaling pathway [57]. A regulatory role for Sit4p in SL biosynthesis was recently

suggested as *sit4* mutants display an aberrant SL composition [43]. More specifically, Sit4p was suggested to regulate Sur2p or Cer synthase activity [43]. Sur2p converts DHS to PHS [58]. Cer synthases such as Lag1p and Lac1p generate dhCer from DHS and phytoCer from PHS [6].

Barbosa and coworkers initially linked Sit4p to SLs and mitochondrial function based on the observation that cells lacking Isc1p display increased Sit4p activity [50]. Thus, Sit4p dependent Sur2p or Cer synthase activity [43] could point to a compensatory mechanism for the lack of phytoCer production in *isc1* mutants. This compensatory mechanism could, for instance, be responsible for the increased generation of α OH-C₁₄-phytoCer and C₂₆-phytoCer levels in *isc1* mutant mitochondria [27]. Also, loss of Sit4p attenuates the shortened CLS and H₂O₂ sensitivity in *isc1* mutants. In addition, *isc1 sit4* mutants have a restored mitochondrial function during the diauxic shift and are able to grow on a non-fermentable carbon source [50]. Hence, Sit4p is a downstream target of SLs in regulating mitochondrial function.

The HOG pathway—The HOG pathway is involved in the response to hyperosmotic stress, in which the Hog1p mitogen activated protein kinase (MAPK) cascade plays a central role [59-62]. MAPK cascades are conserved signaling pathways that are crucial in e.g. the regulation of gene expression [63]. The HOG pathway in yeast is initiated in response to unfavorable osmotic conditions by activation of MAPK kinase Pbs2p through the Sln1p or Sho1p branch that activate the redundant MAPKKK Ssk2p and Ssk22p or Ste11p, respectively. Activation of Pbs2p results in phosphorylation of Hog1p, which in turn translocates to the nucleus and initiates Hog1p-mediated phosphorylation of transcriptional regulators that drive transcription and cell cycle. Finally, the action of Hog1p restores the osmotic balance, its activity decreases and is exported back to the cytoplasm. For a detailed review the reader is referred to [62].

Hog1p is known to affect mitochondrial function as well as dysfunction [24, 64-68]. Transient activation of Hog1p is essential for cellular responses during osmotic stress [59-62] and results in the induction of transcription of genes involved in mitochondrial function [65-67]. In addition, Hog1p controls correct functioning of the respiratory metabolism in *Candida albicans* [68]. However, prolonged Hog1p activation results in cell death [69], inhibition of mitochondrial respiration and a concomitant increase in levels of reactive oxygen species (ROS) [64]. In addition, growth on a nonfermentable carbon source is impaired during osmotic stress [64]. These observed phenotypes resulting from prolonged Hog1p activation are also characteristic of *isc1* mutants [20, 21, 23-27]. This implies that activation of Hog1p controls correct mitochondrial functioning, but can also result in its dysfunction upon prolonged activation. Hence, Hog1p activation should be highly controlled in order to regulate mitochondrial function and cell survival.

The HOG pathway has also been shown to involve interaction with SLs [24, 26, 70]. For instance, inhibition of *de novo* SL biosynthesis increases Hog1p phosphorylation [70] while *isc1* mutants display Hog1p hyperphosphorylation [24, 26]. In addition, exogenous addition of C₂-Cer increases phosphorylated Hog1p levels [24, 26]. Similarly as reported for *isc1 sit4* mutants [50], loss of Hog1p in *isc1* mutants increases H₂O₂ tolerance,

attenuates the decreased CLS, allows growth on a non-fermentable carbon source and restores mitochondrial function [24]. Thus, SL signaling affects Hog1p phosphorylation and is a negative upstream regulator of mitochondrial function.

The TORC1/Sch9p pathway—The TORC1 pathway is part of the highly conserved TOR nutrient signaling pathway and is important for metabolic adaptation during the diauxic shift [57, 71]. TORC1 activity directly reflects both nutrient availability and absence of stress factors in the growth medium. Two branches execute the majority of TORC1-mediated effects (Fig. 2). The Tap42p branch is inhibited upon phosphorylation by TORC1 and regulates Sit4p activity. The Sch9p branch is activated upon TORC1-mediated phosphorylation [57]. Alternatively, Sch9p activity is modulated by SLs via the action of Pkh1 and SLs themselves [72]. Pkh1p and its paralog Pkh2p (Pkh1/2p) are functionally redundant protein kinases homologous to mammalian 3-phosphoinositide-dependent protein kinase PDK1 [73, 74] and are important kinases in the regulation of cell processes such as aging, endocytosis and stress resistance [15]. The TORC1/Sch9p signaling axis also regulates CLS in yeast [4, 75]. For a recent review on TORC1/Sch9p the reader is referred to [57].

The TORC1/Sch9p signaling axis plays a pivotal role in Isc1p localization, SL biosynthesis and the regulation of mitochondrial function [26, 48, 76]. For instance, Sch9p represses the expression of genes involved in the ETC [76], but also affects the expression of SL biosynthetic genes in a TORC1 dependent manner [48]. Concomitantly *sch9* mutants display altered abundance of several LCBs, Cer and complex SL [48]. Strikingly, an important role for Sch9p in Isc1p localization was demonstrated as loss of Sch9p attenuates the localization of Isc1p to the mitochondria during the post-diauxic growth phase [48]. Additionally, Teixeira and coworkers showed that the TORC1/Sch9p pathway is activated in *isc1* mutants [26]. Moreover, loss of either Tor1p or Sch9p abolishes the aberrant mitochondrial dysfunction-related phenotypes associated with *isc1* mutants such as decreased oxidative stress tolerance, decreased CLS, inability to grow on a non-fermentable carbon source, mitochondrial hyperpolarization, mitochondrial fragmentation and increased apoptotic cell death and ROS production during chronological aging [26, 48]. Hence, while Sch9p affects SL biosynthesis, the kinase also regulates the correct translocation of Isc1p to the OMM during the post-diauxic shift, and vice versa SL species generated by Isc1p target the TORC1/Sch9p axis as crucial signal transducer in regulating mitochondrial function.

Teixeira and coworkers also further integrated a putative connection between the TORC1/Sch9p pathway and previous mechanistic studies performed on *isc1* mutants such as the connection with the HOG pathway and Sit4p/CAPP and reported extensive interactions (Fig. 2) [26]. For instance, *isc1 sit4* and *isc1 tor1* mutants show increased Hog1p phosphorylation as compared to *isc1* mutant, indicating that Sit4p and TORC1 block Hog1p phosphorylation in a *isc1* mutant [26]. Whether the Sit4p-mediated block on Hog1p phosphorylation is TORC1-dependent has not yet been addressed. Their results also indicate that Sch9p causes Hog1p phosphorylation in *isc1* mutants, and that Sch9p phosphorylates Hog1p in response to exogenously added C₂-Cer [26]. Therefore, the TORC1/Sch9p signaling axis appears to act as a crucial signal transducer between on one hand SLs and on

the other hand Hog1p in regulating mitochondrial function in *isc1* mutants. An overview of the thus far identified interactions is given in Fig. 2

Additional indications concerning a central signal transducing role for Sch9p in regulating mitochondrial function originates from *isc1*-like phenotypical observations in a yeast model for Niemann-Pick type C1 disease based on *ncr1* mutants [77, 78]. Niemann Pick type C1 is a lipid storage disorder characterized by neurodegeneration [79] and is caused by mutations in NPC1 in 95 % of all cases [80-82]. Yeast *ncr1* mutants lack the orthologue of NPC1 [83]. These *ncr1* mutants exhibit increased sensitivity to oxidative stress and decreased CLS, characterized by increased levels of oxidative stress markers. Additionally, these mutant cells are unable to grow on a non-fermentative carbon source, show decreased mitochondrial membrane potential (ψ_m) and mitochondrial fragmentation [77]. In line with mammalian Niemann Pick type C1 cells [84], accumulation of LCBs is observed in the yeast *ncr1* mutants caused by an increased turnover of complex SLs. In addition, *ncr1* mutants show increased Sch9p phosphorylation due to the action of the Pkh1/2p pathway. Similarly as observed for *isc1* mutants loss of either Sch9p or Pkh1p restores the aberrant phenotypes of *ncr1* mutants. In contrast, in *isc1* mutants Sch9p phosphorylation is independent of Pkh1p [26]. Furthermore, *ncr1 sch9* cells exhibit restored SL levels [77]. Such results indicate that the role of Sch9 as central signal transducer in the regulation of mitochondrial function is not restricted to the *isc1* mutant genetic background.

3. The retrograde response interacts with sphingolipid homeostasis

The retrograde response is typically referred to as a signaling event, originating from the mitochondria, to the nucleus, resulting in the induction of various nuclear target genes by signal transduction pathways [29, 30]. In *S. cerevisiae*, the retrograde response is used as a sensor for mitochondrial dysfunction and results in metabolic adaptations, which are largely dependent on three signal transduction genes namely *RTG1*, *RTG2* and *RTG3* [29, 30]. Interestingly, the retrograde response is linked to SL homeostasis and multidrug resistance [31, 32, 85, 86]. In addition, the retrograde response interacts with additional pathways such as the TORC1/Sch9p signaling axis as exemplified by decreased TORC1-dependent Sch9p phosphorylation after dropping ψ_m [87].

Crucial in orchestrating the link between the retrograde response and SL biosynthesis is Pdr3p. Like Pdr1p, Pdr3p is a Zn₂Cys₆-cluster-containing transcription factor [88, 89], which typically recognizes Pdr1p/Pdr3p response elements (PDREs). Therefore, PDREs are found in the promoters of all known genes regulated by these transcription factors [85, 90]. In addition, both Pdr1p and Pdr3p appear important players in multidrug resistance as gain of function mutations in *PDR1* and *PDR3* increase expression of their target genes encoding multidrug efflux pumps [85]. Furthermore, *PDR3* expression increases upon loss of the mitochondrial genome during the retrograde response and is regulated by both Pdr3p itself [86] and Sit4p [91].

Both Pdr1p and Pdr3p are reported to affect SL homeostasis, e.g. by their observed regulation of *IPT1* expression [31]. In line with the reported activation of Pdr3p during the retrograde response [86], loss of the mitochondrial genome causes a Pdr3p-dependent

increase of *IPT1* expression [31]. Concomitantly, ρ^0 cells display an aberrant SL composition as compared to WT cells, which is restored to normal-state levels of most complex SLs upon loss of Pdr3p [31]. Next to *IPT1*, expression of additional genes encoding enzymes involved in SL biosynthesis respond to increased activity of Pdr1p/Pdr3p such as (i) *LAC1*, (ii) *SUR2* and (iii) *LCB2* [32], a component of SPT [37]. These studies indicate that compromised mitochondrial function initiates signaling events that result in the Pdr3p-dependent induction of SL biosynthesis.

In addition, despite the fact that the retrograde response is not activated in *isc1* mutants [28], several observations during the retrograde response are also observed in *isc1* mutants and are linked to SL signaling (Fig. 3). For instance, while Rtg2p or Rtg3p upregulate expression of *SWE1* [92], encoding the Swe1p protein kinase involved in G2/M transition [93], phytoCer generated by Isc1p regulates Swe1p levels [18, 94]. Additionally, the protein product of *RSB1*, whose expression of the encoding gene increases in a Pdr3p-dependent manner upon loss of the mitochondrial genome [44, 95, 96], removes LCBs from the cell [97] and is also increased in *isc1* mutants [20]. Furthermore, while expression of Pdr5p is dependent on Pdr3p during the retrograde response [95], *isc1* mutants exhibit increased expression of the gene encoding Pdr5p and its paralog Pdr15p [20, 98]. These findings suggest that SL signaling activates pathways in *isc1* mutants that resemble responses to retrograde signaling as summarized in Fig. 3.

4. Conclusion

Thus far the most intriguing insights into a direct connection between SLs and mitochondrial function in *S. cerevisiae* mainly originate from studies that focused on Isc1p and the retrograde response. An overview of the thus far identified interactions is given in Fig. 2 and 3.

Several reports indicate that the translocation of Isc1p to the OMM is crucial in the regulation of mitochondrial function during the post-diauxic growth phase [20, 21, 23-27, 48]. Loss of Isc1p is detrimental for cellular mitochondrial function [20, 21, 23-26], resulting from a misregulation of gene expression [28]. Sch9p is responsible for the correct translocation of Isc1p from the ER to the OMM [48], however, Sch9p itself is a downstream signaling target of Isc1p, and affects SL biosynthesis [26, 48].

Nevertheless, the TORC1/Sch9p signaling axis acts as the central switch to pass upstream signaling events to downstream effectors, where Sch9p and Sit4p combine Isc1p-mediated phytoCer signaling. Thus far, Hog1p has been identified as their downstream target more specifically with Sch9p phosphorylating Hog1p, and Sit4p blocking Hog1p phosphorylation [26]. Intriguingly, while loss of Sit4p suppresses mitochondrial dysfunction-related phenotypes in *isc1* mutants [50], *isc1 sit4* mutants exhibit increased phosphorylated Hog1p levels as compared to *isc1* mutants [26]. Given the reports that indicate that transient Hog1p activation promotes mitochondrial function [65-68], and prolonged Hog1p activation is detrimental [64, 69], these results also imply that Hog1p phosphorylation is a delicate event in order to preserve mitochondrial function in yeast. The role of the TORC1/Sch9p axis is not restricted to the *isc1* mutant genetic background, as Sch9p was also

shown to be crucial in regulating mitochondrial function in a yeast model for Niemann Pick type C1 [77, 78]. Hence, Sch9p acts as a critical switch in the regulation of mitochondrial function in yeast; whether Hog1p is the sole downstream target in the regulation of mitochondrial function in response to SL remains to be elucidated.

The role of the retrograde response is limited in Isc1p-mediated effects on mitochondrial function [28]. Still, the retrograde response is linked to the induction of SL biosynthetic genes [31, 32]. As such, during the retrograde response, the central transcription factor Pdr3p induces expression of SL biosynthetic genes by binding to the PDRE in the promoters of its target genes [29]. Among these targets are several genes involved in SL biosynthesis such as *IPT1*, *LCB2*, *SUR2* and *LAC1* [31, 32]. In addition, Sit4p affects Pdr3p expression and SL biosynthesis [91]. Whether this effect is mediated via a Pdr3p mechanism is yet to be shown. In addition, loss of ψ_m decreases TORC1-mediated Sch9p phosphorylation [87]. Despite the fact that the retrograde response is not activated in *isc1* mutants [28], several cellular responses during the retrograde response are also observed in *isc1* mutants [18, 20, 44, 92, 94-96, 98]. This indicates that loss of mitochondrial quality, which triggers the retrograde response, interacts with SL homeostasis, and SL signaling activates pathways in *isc1* mutants that resemble responses to retrograde signaling.

Potential implications for the described connection between SLs and mitochondrial function in yeast are diverse and can serve to understand SL-related human pathologies or specific mammalian/human systems. For instance, Isc1p is homologous to mammalian neutral sphingomyelinases (nSMases) involved in the hydrolysis of the mammalian SL sphingomyelin to Cer [16]. In mammalian cells, four nSMases have been cloned and purified, with nSMase2 being the best studied isoform [99]. Only recently, the fourth nSMase was identified in mice, termed MA-nSMase (mitochondrial-associated nSMase) and was shown to localize to both ER and mitochondria, [100]. In addition the presence of a putative human MA-nSMase encoding gene has been reported [99]. As Isc1p in yeast is implicated in generating SLs that initiate signaling events that regulate mitochondrial function, the question arises whether human MA-nSMase serves the same purpose. With regard to the study of human SL-related pathologies using yeast as a model, yeast studies have revealed new insights into the neurodegenerative condition Niemann Pick type C1. Cells that lack NPC1 accumulate lipids such as cholesterol and Sph [84, 101] and exhibit markers of oxidative stress and mitochondrial dysfunction [102-106]. However, the specific mechanisms that lead to neurodegeneration during Niemann Pick type C1 are not fully elucidated. Interestingly, *ncr1* mutants not only exhibit hallmarks of oxidative stress and mitochondrial dysfunction, they also accumulate LCBs [77]. In addition, these LCBs are proposed to be crucial in regulating mitochondrial function in *ncr1* mutants [77]. Hence this suggests that yeast may reveal important insights into cellular events regarding SL signaling and mitochondrial function in higher eukaryotes.

In conclusion, all the above findings clearly support the importance of a feedback/feed-forward loop between SLs and mitochondrial function.

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Highlights

- Sphingolipids (SLs) are components of cellular membranes and signaling molecules
- Isc1p-mediated phytoCer generation is crucial for mitochondrial function
- In response to SLs Sch9p transduces signals to regulate mitochondrial function
- The retrograde response affects SL homeostasis
- SLs are important signaling molecules in the regulation of mitochondrial function

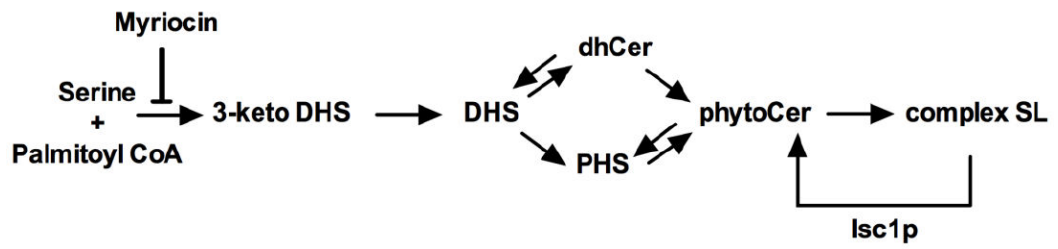


Fig. 1.
De novo SL biosynthetic pathway in *S. cerevisiae*. Myriocin inhibits SPT.

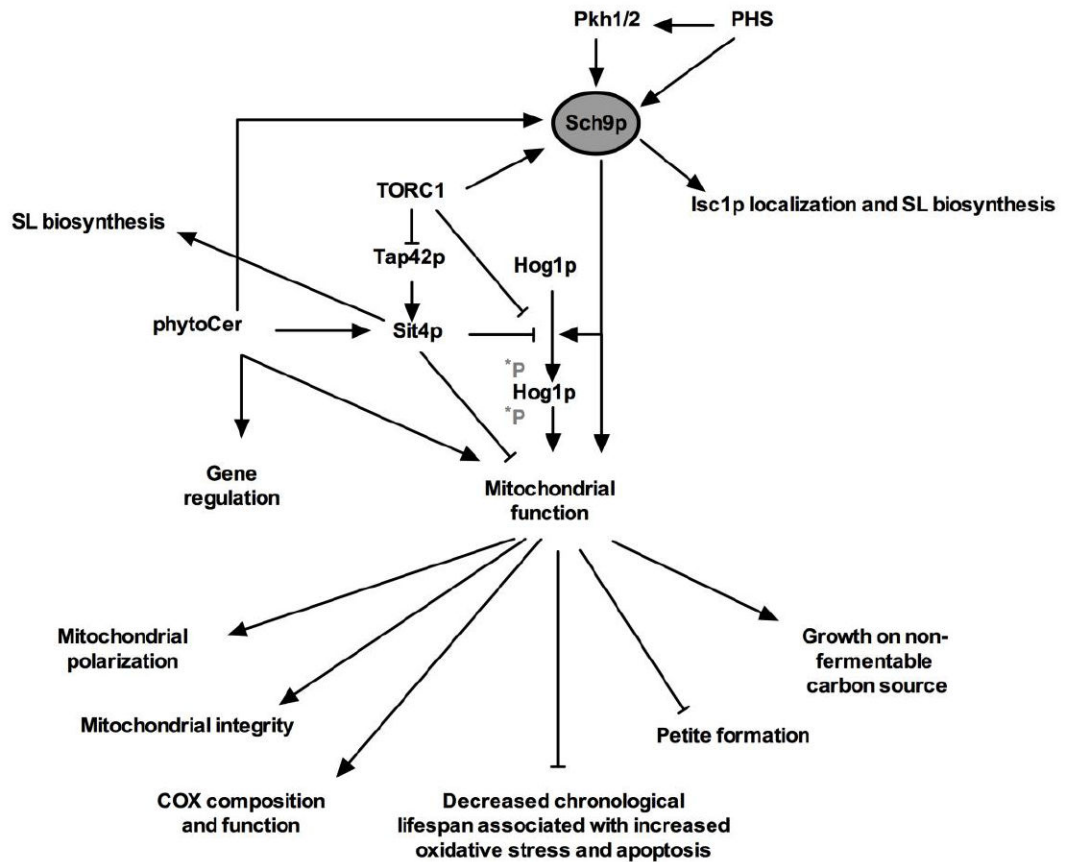


Fig. 2. Interplay between SLs and mitochondrial function. Sch9p governs correct localization of Isc1p to the OMM during the post diauxic shift and affects SL biosynthesis. Sit4p affects SL biosynthesis, represses Hog1p phosphorylation and mitochondrial function. Sch9p and Sit4p combine Isc1p-mediated phytoCer signaling. Adapted from [26].

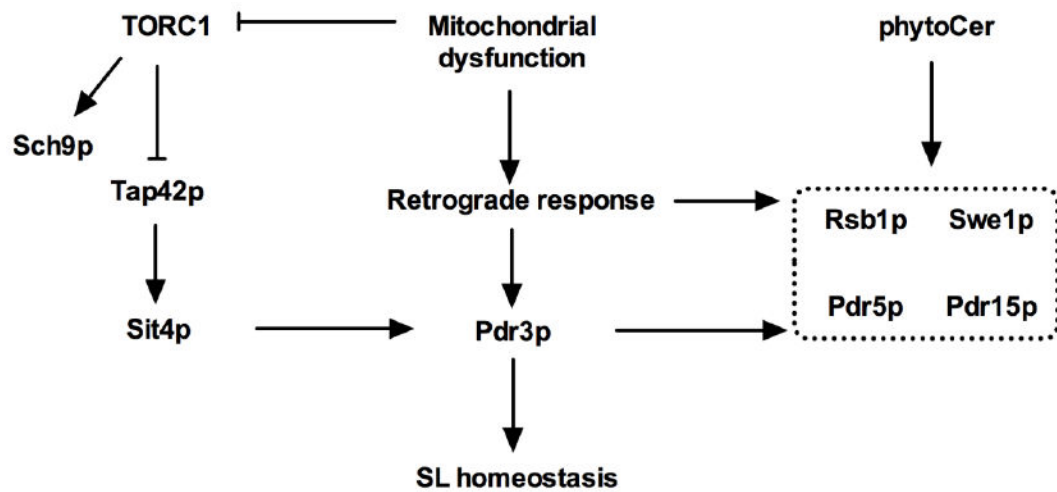


Fig. 3. Interconnections between SLs and the retrograde response in *S. cerevisiae*. Mitochondrial dysfunction affects TORC1-dependent Sch9p phosphorylation and Pdr3p regulates SL homeostasis. Sit4p affects expression of the gene encoding Pdr3p. SL signaling activates pathways in *isc1* mutants that resemble responses to retrograde signaling, and thus affect several common proteins as delineated by the dotted black line.