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Review

Sphingolipids and mitochondrial function in budding yeast

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ABSTRACT

Background: Sphingolipids (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 course, 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 a 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 the 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 of 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.

1. Introduction

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 not only 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 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 a precursor in the formation of the three complex SLs by the 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 (Fig. 1) [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], H_2O_2 [20], acetic acid [21], methyl methanosulfate and hydroxyurea

Abbreviations: 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; $\Delta\psi_m$, mitochondrial membrane potential; PDRE, Pdr1p/Pdr3p response elements; nSMase, neutral sphingomyelinase; MA-nSMase, mitochondrial-associated nSMase

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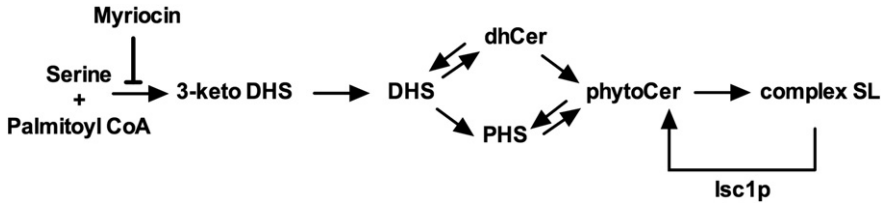

Fig. 1. Pathway in *S. cerevisiae*. Myriocin inhibits SPT.

[22]. Furthermore, studies with *Δisc1* 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 *Δisc1* 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 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, the 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. Δisc1 mutants display characteristics of mitochondrial dysfunction

Several studies with *Δisc1* mutants have pointed to inherent compromised mitochondrial function. One of the main initial observations with *Δisc1* 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 aging of *Δisc1* mutants is associated with increased oxidative stress and apoptosis [20,50]. Indicative for mitochondrial dysfunction, these mutants display defective growth on a non-fermentable carbon source [24–26,28,50]. In addition, *Δisc1* mutants exhibit an increased frequency of petite formation [27], a hallmark of yeast cells with mitochondrial defects [51]. Additional observations from *Δisc1* 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, *Δisc1* mutants exhibit increased sensitivity to toxic stimuli, such as hydrogen peroxide (H_2O_2) and ethidium bromide [27], which are reported to be increasingly toxic to cells with defective mitochondria [52–54]. A direct link between Isc1p and the mitochondrial respiratory chain is also suggested since *Δisc1* 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 *Δisc1* mutants [26]. Interestingly, the loss of the mitochondrial genome in *Δisc1* mutants attenuates the decreased CLS associated with *Δisc1* mutants, indicating that mitochondrial dysfunction contributes to the shortened CLS in *Δisc1* mutants [24]. To summarize, all these reports indicate that *Δisc1* mutants indeed are characterized by extensive mitochondrial dysfunction.

2.2. Δisc1 mutants show aberrant mitochondrial SL composition

The aforementioned phenotypes in *Δisc1* 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-C_{14}-phytoCer and C_{26}-phytoCer levels, *Δisc1* mutants display decreased levels of all SLs, with the most prominent decreases in the levels of α-OH-C_{24}-phytoCer and α-OH-C_{26}-phytoCer species. Also, during CLS *Δisc1* mutants display an abnormal SL composition: decreased levels of DHS and α-OH-phytoCer and increased levels of C_{26}-dhCer and C_{26}-phytoCer [50]. In addition, exogenously supplied C_{12}-phytoCer restores the ability of *Δisc1* mutants to grow on a non-fermentable carbon source [25]. This suggests that Isc1p-mediated phytoCer generation in mitochondria is important for mitochondrial function [27].

2.3. Mitochondrial dysfunction in Δisc1 mutants is caused by a misregulation of gene expression

The mitochondrial dysfunction-related phenotypes prevalent in *Δisc1* 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 *Δisc1* mutants is not characterized by a loss of mtDNA, nor do isolated mitochondria from *Δisc1* mutants exhibit differences in the rate of oxygen consumption [28], indicating that *Δisc1* mutants have a functional respiratory chain. However, *Δisc1* 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 *Δisc1* 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.