university of groningen

University Medical Center Groningen

University of Groningen

Impaired biosynthesis of the non-bilayer lipids phosphatidylethanolamine or cardiolipin does not affect peroxisome biogenesis and proliferation in Saccharomyces cerevisiae

Kawalek, Adam; Jagadeesan, Chandhuru; van der Klei, Ida J.

Published in:
Biochemical and Biophysical Research Communications

DOI:
10.1016/j.bbrc.2016.10.033

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2016

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Kawalek, A., Jagadeesan, C., & van der Klei, I. J. (2016). Impaired biosynthesis of the non-bilayer lipids phosphatidylethanolamine or cardiolipin does not affect peroxisome biogenesis and proliferation in Saccharomyces cerevisiae. *Biochemical and Biophysical Research Communications*, *480*(2), 228-233. https://doi.org/10.1016/j.bbrc.2016.10.033

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 24-04-2025

Biochemical and Biophysical Research Communications 480 (2016) 228–233

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Impaired biosynthesis of the non-bilayer lipids phosphatidylethanolamine or cardiolipin does not affect peroxisome biogenesis and proliferation in *Saccharomyces cerevisiae*

Adam Kawałek [1], Chandhuru Jagadeesan, Ida J. van der Klei*

Molecular Cell Biology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands

ARTICLE INFO

Article history:
Received 3 October 2016
Accepted 13 October 2016
Available online 14 October 2016

Keywords:
Peroxisome
Non-bilayer forming lipids
Cardiolipin
Phosphatidylethanolamine

ABSTRACT

The non-bilayer forming lipids cardiolipin (CL) and phosphatidylethanolamine (PE) modulate membrane curvature, facilitate membrane fusion and affect the stability and function of membrane proteins. Yeast peroxisomal membranes contain significant amounts of CL and PE.

We analysed the effect of CL deficiency and PE depletion on peroxisome biogenesis and proliferation in *Saccharomyces cerevisiae*. Our data indicate that deletion of *CRD1*, which encodes cardiolipin synthase, does not affect peroxisome biogenesis or abundance, both at peroxisome repressing (glucose) or inducing (oleate) growth conditions. Analysis of strains deficient in one of the three PE biosynthesis pathways (*psd1, psd2* or the triple deletion strain *eki1 cki1 dpl1*) revealed that in all three strains peroxisome numbers were reduced upon growth of cells on oleic acid, whereas the *psd1* strain also showed a reduction in peroxisome abundance upon growth on glucose. Because PE is an intermediate of the phosphatidylcholine (PC) biosynthesis pathway, PE depletion affects PC formation. PC however can be synthesized by an alternative pathway when choline is supplemented to the growth medium. Because the addition of choline resulted in suppression of the peroxisome phenotypes in phosphatidylserine decarboxylase mutant strains, we conclude that peroxisome biogenesis and proliferation are not crucially dependent on CL or PE.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Peroxisomes are cell organelles present in almost all eukaryotic cells. Enzymes localized in peroxisomes are involved in a variety of metabolic processes including β-oxidation of fatty acids and hydrogen peroxide detoxification [1,2].

The main phospholipid constituents of *Saccharomyces cerevisiae* peroxisomal membranes are phospatidylcholine (PC) (48%), phosphatidylethanolamine (PE) (23%) and phosphatidylinositol (16%) [3]. In addition, these membranes contain substantial levels (up to 7%) of cardiolipin (CL) [3]. A similar composition was reported for peroxisomal membranes of the yeast *Pichia pastoris* [4].

PC has a cylindrical shape and is a typical lipid of biological membranes. Recent studies indicated that depletion of PC results in disturbed peroxisomal membrane fluidity in *S. cerevisiae* [5]. CL and PE have an inverted cone shape and are non-bilayer forming lipids. Such lipids have been reported to modulate membrane curvature, facilitate membrane fusion [6,7], affect binding of peripheral membrane proteins and formation of membrane protein complexes [8]. In mitochondria CL and PE play crucial roles in various aspects of organelle function and dynamics [7,9–12]. So far, the functions of CL and PE in the peroxisomal membrane are unknown.

In *S. cerevisiae* CL biosynthesis occurs solely in mitochondria and requires among others the *CRD1* gene (Fig. 1A) [13,14]. The biosynthesis of PE is more complex and involves 3 pathways which occur in different cell compartments (Fig. 1B) [15]. Phosphatidylserine (PS) can be decarboxylated by phosphatidylserine decarboxylase 1 (Psd1) at the inner mitochondrial membrane [16,17] or by phosphatidylserine decarboxylase 2 (Psd2) in vacuole/Golgi membranes [18]. Additionally, PE can be formed by

* Corresponding author. Molecular Cell Biology, University of Groningen, P.O. Box 11103, 9300 CC Groningen, The Netherlands.

E-mail addresses: a.kawalek@ibb.waw.pl (A. Kawałek), chandhuruj@gmail.com (C. Jagadeesan), i.j.van.der.klei@rug.nl (I.J. van der Klei).

[1] Present address: Institute of Biochemistry and Biophysics PAS, Department of Microbial Biochemistry, Pawińskiego 5A, 02-106 Warsaw, Poland.

http://dx.doi.org/10.1016/j.bbrc.2016.10.033
0006-291X/© 2016 Elsevier Inc. All rights reserved.

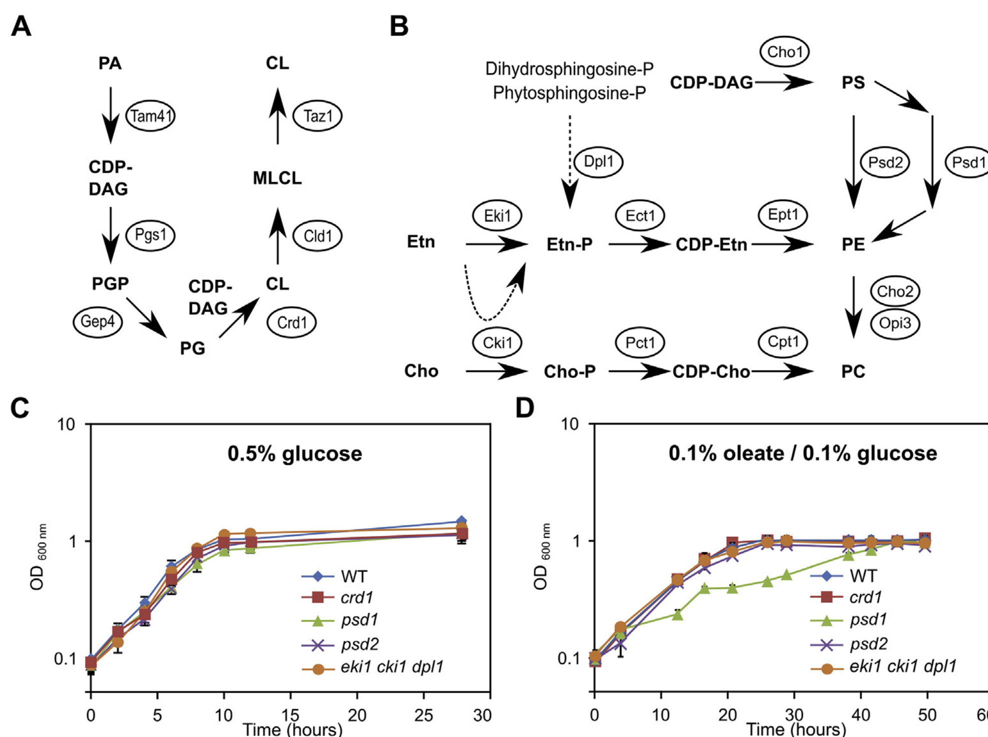

Fig. 1. Impact of impaired cardiolipin and phosphatidylethanolamine synthesis on growth of *S. cerevisiae* cells. (**A**) Schematic overview of cardiolipin synthesis. Phosphatidic acid (PA) is converted to cytidine diphosphate - diacylglycerol (CDP-DAG) and further to phosphatidylglycerolphosphate (PGP). PGP is dephosphorylated to phosphatidylglycerol (PG). PG is condensed with CDP-DAG to form unremodeled CL. Acyl-chains in CL can be remodelled by the deacylase Cld1p and the transacylase Taz1p with monolysocardiolipin (MLCL) as an intermediate. (**B**) Pathways involved in synthesis of phosphatidylethanolamine (PE) and phosphatidylcholine (PC). CDP-DAG is condensed with serine to form phosphatidylserine (PS). PS can be decarboxylated by mitochondrial PS decarboxylase (Psd1) or PS decarboxylase localized to the vacuole/Golgi apparatus (Psd2) to form PE. Alternatively, ethanolamine (Etn) can be incorporated into PE by the Kennedy pathway. An intermediate of this pathway – ethanolamine phosphate (Etn-P) can also derive from degradation of sphingolipids. PE can be methylated to form PC. Alternatively, PC can be formed by incorporation of choline via the Kennedy pathway. Cho – choline, Cho-P – choline phosphate, CDP-Cho - CDP - choline. Growth of WT, *crd1*, *psd1*, *psd2* and *eki1 cki1 dpl1* cells in medium containing 0.5% glucose (**C**) or a mixture of 0.1% oleate and 0.1% glucose (**D**). Data represent mean optical density at 600 nm ± SD (n = 3).

incorporation of ethanolamine through the Kennedy pathway localized to microsomes [19]. Ethanolamine utilized in this route can be taken up from the growth medium or be released within the cell by phospholipases, which is followed by phosphorylation by ethanolamine kinase 1 (Eki1) [20]. Ethanolamine can also be phosphorylated by choline kinase 1 (Cki1) [21]. Additionally, ethanolamine phosphate can derive from degradation of sphingolipids by dihydrosphingosine-1-phosphate lyase (Dpl1) [22].

All 3 pathways were previously shown to contribute to PE abundance in peroxisomal fractions of *S. cerevisiae* [23]. Moreover, PE depletion was reported to result in a slight decrease in peroxisome size and defects in growth on oleic acid, a substrate that is metabolized by peroxisomal β-oxidation [23]. Here we analysed the role of both non-bilayer lipids, CL and PE, on peroxisome biogenesis and proliferation. Our data indicate that CL and PE are not crucial for peroxisome biogenesis or proliferation.

2. Materials and methods

2.1. Strains and growth conditions

The *S. cerevisiae* strains used in this study are listed in Table 1. *S. cerevisiae* cells were grown at 30 °C in mineral medium [24] containing 0.5% glucose or a mixture of 0.1% glucose, 0.1% oleic acid and 0.05% Tween 80. Culture medium was supplemented with leucine (30 μg/ml), histidine (20 μg/ml), uracil (30 μg/ml) and lysine (30 μg/ml).

2.2. Cloning and construction of yeast strains

The plasmids and primers used in this study are listed in Table 2 and Table 3.

Plasmid pTDH3_GFPSKL was constructed by amplification of the GFP_SKL open reading frame with additional *Hin*dIII/*Sal*I sites in a PCR reaction using primers TDH3_GFPSKL_F and TDH3_GFPSKL_R and plasmid pHIPX7_eGFPSKL as a template, followed by *Hin*dIII/*Sal*I digestion and ligation with *Hin*dIII/*Sal*I digested

Table 1
S. cerevisiae strains used in this study.

S. cerevisiae[a]	Description	Reference
Wild-type (WT)	BY4742 MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	EUROSCARF
crd1	BY4742 *CRD1::kanMX4*	EUROSCARF
psd1	BY4742 *PSD1::kanMX6*	This study
psd2	BY4742 *PSD2::kanMX4*	EUROSCARF
eki1 cki1 dpl1	BY4742 *EKI1::kanMX6 CKI1::HPH DPL1::NAT*	This study
psd1 psd2	BY4742 *PSD1::kanMX6 PSD2::NAT*	This study
WT GFP-SKL	WT P_{TDH3}-GFP-SKL	This study
crd1 GFP-SKL	*crd1* P_{TDH3}-GFP-SKL	This study
psd1 GFP-SKL	*psd1* P_{TDH3}-GFP-SKL	This study
psd2 GFP-SKL	*psd2* P_{TDH3}-GFP-SKL	This study
eki1 cki1 dpl1 GFP-SKL	*cki1 eki1 dpl1* P_{TDH3}-GFP-SKL	This study
psd1 psd2 GFP-SKL	*psd1 psd2* P_{TDH3}-GFP-SKL	This study

[a] All *S. cerevisiae* strains listed here require leucine, lysine, histidine and uracil for growth.

Table 2
Primers used in this study.

Primer name	Sequence
ScPSD1_del_F	GCCAGTTAAGAACGCCTTGGCGCAAGGGAGGACGCTCCTCATGGGGAGGACAGCTGAAGCTTCGTACGC
ScPSD1_del_R	CAGGTATGTGGTTCCAAGTGTTTGTCGCTCTTTGAATTTGTCACAAATTCGCATAGGCCACTAGTGGATCTG
ScPSD2_del_F	GTATCAATTGGTAAAGAATCCTCGATTTTCAGGAGCATCCAACGACGAAGCCCACACACCATAGCTTCAA
ScPSD2_del_R	TACTCATCCCGACTTTGACTAACGTTTCAATGCGTTCTGAAGAGTTTTTCACGTTTTCGACACTGGATGG
ScEKI1_del_F	TACGAAAGTAGTAGCAGAAATTAACAGATACAGATCTGCAATTTGGCATACAGCTGAAGCTTCGTACGC
ScEKI1_del_R	TAACTCCCAATGTAATTAAATCGCCCCAAAAGACAGACATTTTTTCTTTACGCATAGGCCACTAGTGGATCTG
ScCKI1_del_F	ACTGATGTCACAGATAGTTTGGGTTCGACTTCGTCGGAATATATTGAGATTCCCACACACCATAGCTTCAA
ScCKI1_del_R	GAACTTGAAAGAGCTGAAATTTTTGCATTCTTCTTCGGTGATTATGCCTAACGTTTTCGACACTGGATGG
ScDPL1_del_F	TACCGAGCAAGTAGGCTAGCTTCTGTAAAGGGATTTTTCCATCTAATACACCCACACACCATAGCTTCAA
ScDPL1_del_R	ACATTGCACTCTCGTTCTTTAAATTATGTATGAGATTTGATTCTATATAGCGTTTTCGACACTGGATGG
TDH3_GFPSKL_F	TGACAAGCTTATGGTGAGCAAGGGCGA
TDH3_GFPSKL_R	TGACGTCGACTTACAGCTTCGACTTGTACAGC

Table 3
Plasmids used in this study.

Plasmid	Description	Reference
pUG6	contains gentamicin resistance cassette (*kanMX6*); amp^R	[39]
pENTR-221-HPH	pENTR-221 containing hygromycin marker; kan^R	[40]
pENTR-221_NAT	pENTR-221 containing nourseothricin marker; kan^R	[40]
pHIPX7_eGFPSKL	contains eGFP-SKL under the control of *H. polymorpha* P_{TEF1} promoter; contains *S. cerevisiae LEU2* gene for complementation of leucine auxotrophy; kan^R	[41]
pTDH3_mcherrysfGFP	contains tandem protein (mcherry + sfGFP) expressed from *S. cerevisiae* P_{TDH3} promoter; contains zeocine resistance cassette; amp^R	Sanjeev Kumar (unpublished)
pTDH3_GFPSKL	contains eGFP-SKL under the control of *S. cerevisiae* P_{TDH3} promoter; contains zeocine resistance cassette; amp^R	this study

pTDH3_mcherrysfGFP. Transformed *E. coli* DH5α were selected on LB agar plates supplemented with 100 μg/ml ampicillin.

Transformation of *S. cerevisiae* cells was performed by the Li-Ac method [25]. Selection of yeast transformants was performed on YPD agar plates supplemented with 200 μg/ml hygromycin B, 100 μg/ml gentamycin, 200 μg/ml zeocine or 100 μg/ml nourseothricin.

S. cerevisiae psd1 deletion strain was obtained by replacement of

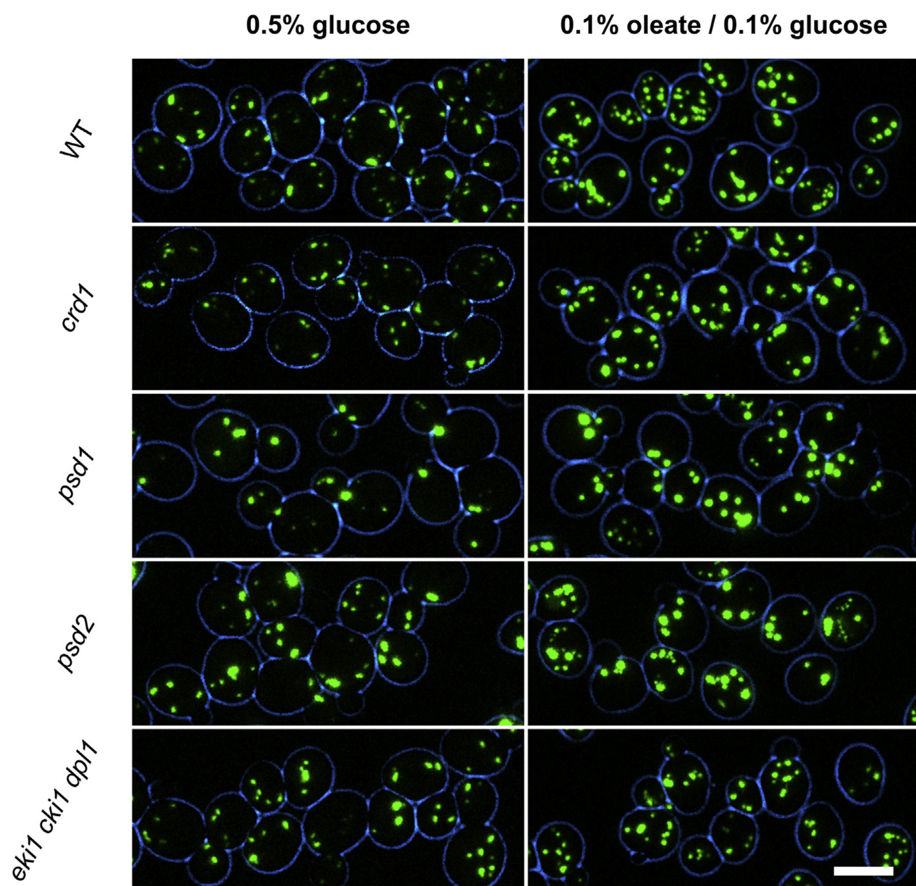

Fig. 2. Intracellular localization of GFP-SKL in *S. cerevisiae* cells impaired in the biosynthesis of CL or PE. Fluorescence microscopy of WT and the indicated mutants producing GFP-SKL, growing exponentially in medium containing 0.5% glucose or a mixture of 0.1% oleate and 0.1% glucose. Scale bar represents 5 μm.

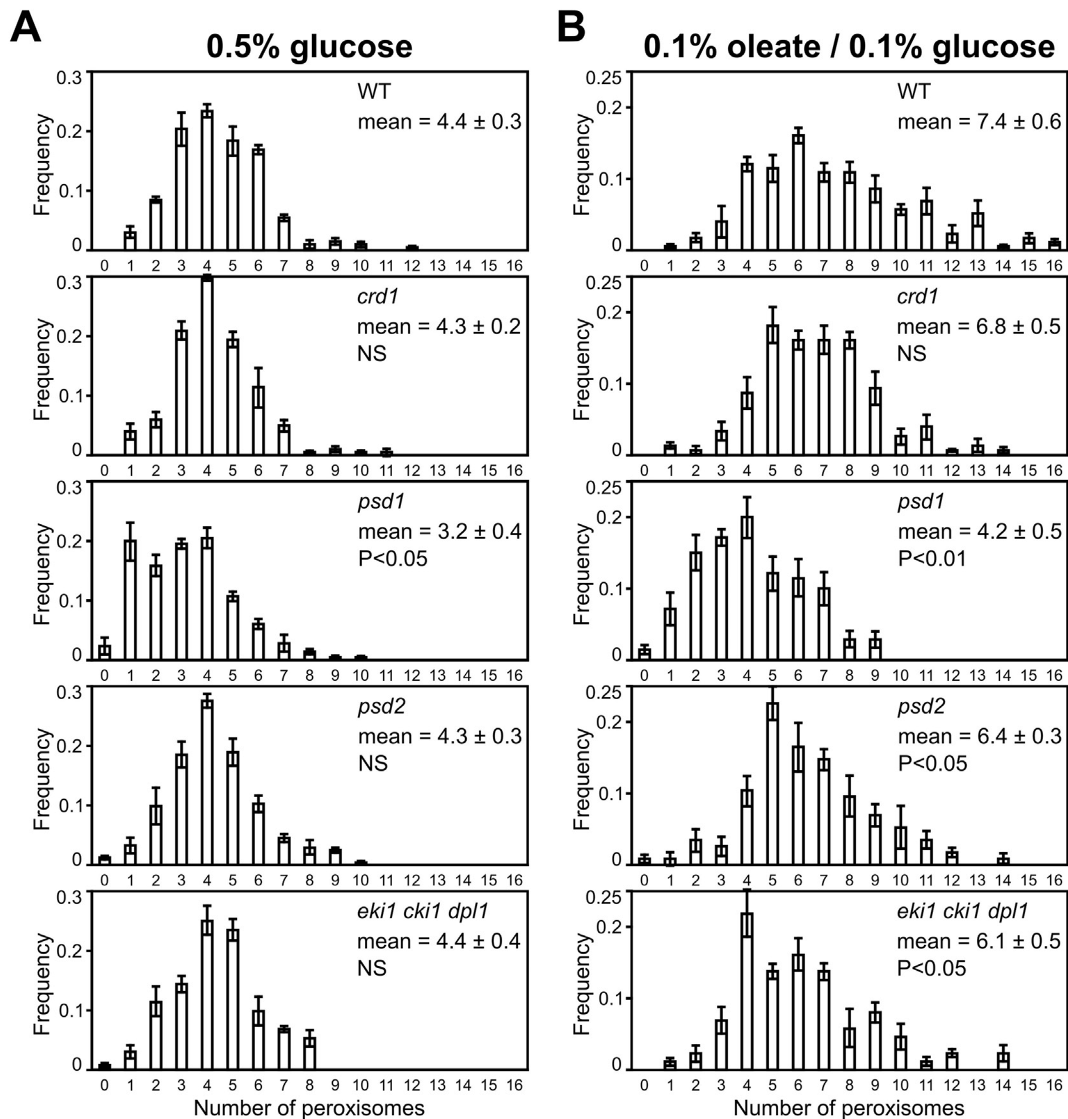

Fig. 3. Quantification of peroxisome numbers. Peroxisomes were visualized by the matrix marker GFP-SKL. Cells were grown for 6 h in medium containing 0.5% glucose (**A**) or for 12 h in medium containing 0.1% oleate and 0.1% glucose (**B**). Frequency distributions and mean number of peroxisomes ±SD of WT and mutant strains grown on the indicated media. Fluorescent spots were quantified in >150 non-dividing cells of at least 2 independent cultures (n > 2). P values were calculated using a Student *t*-test. NS - not significant (P > 0.05).

nucleotides +1 to +1185 from the start codon of the *PSD1* gene (YNL1696) by the gentamycin resistance cassette. To this end, primers ScPSD1_del_F and ScPSD1_del_R were used to amplify the gentamicin resistance cassette using plasmid pUG6 as the template. The obtained PCR product was used for transformation of WT (BY4742) cells.

The *psd1 psd2* double deletion strain was constructed by deletion of *PSD2* (YGR170W) in *psd1* cells. Nucleotides +1 to +3180 from the start codon of *PSD2* were replaced by a nourseothricin resistance cassette. To this end, primers ScPSD2_del_F and ScPSD2_del_R were used to amplify the nourseothricin resistance cassette using plasmid pENTR-221_NAT as the template. The obtained PCR product was used for transformation of *psd1* cells.

The *eki1 cki1 dpl1* triple deletion strain was constructed by replacing nucleotides +1 to +1492 of *EKI1* (YDR147W) gene with a gentamicin resistance cassette, followed by replacing nucleotides +310 to +1677 of *CKI1* (YLR133W) gene with a hygromycin resistance cassette and subsequent replacement of nucleotides +1 to +1770 (whole open reading frame) of the *DPL1* (YDR2943) gene with a nourseothricin resistance cassette. The corresponding deletion cassettes were amplified by PCR using primers ScEKI1_del_F and ScEKI1_del_R and pUG6 plasmid, primers ScCKI1_del_F and ScCKI1_del_R and pENTR-221_HPH plasmid, primers ScDPL1_del_F and ScDPL1_del_F and pENTR-221_NAT plasmid, respectively.

2.3. Fluorescence microscopy and image analysis

Fluorescence microscopy images were captured using a Carl Zeiss confocal microscope LSM510 equipped with photomultiplier tubes (Hamamatsu Photonics). The GFP signal was visualized using a 488 nm argon ion laser (Lasos) and a 500–550 nm bandpass emission filter. Images we acquired using ZEN 2012 software (Carl Zeiss). Before analysis, *S. cerevisiae* cells were fixed for 1 h with 4% formaldehyde in 0.1 M sodium phosphate buffer pH 7.2. The presented fluorescence microscopy images represent maximum intensity projections in the GFP channel. Additionally, bright field images were false coloured in blue to mark cell edges.

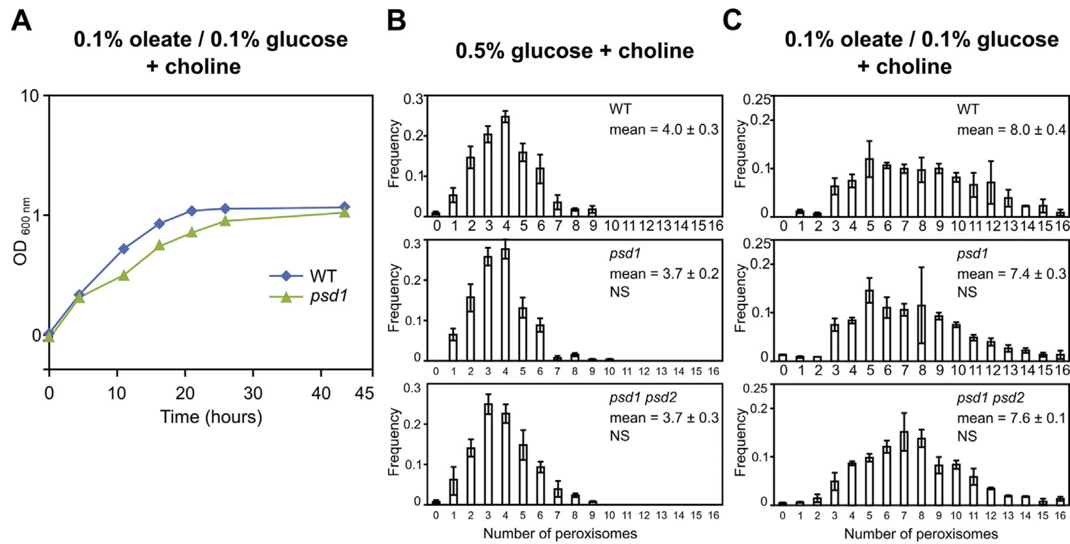

Fig. 4. Impact of choline supplementation on growth and peroxisome numbers. (**A**) Growth of WT and *psd1* cells on the indicated medium supplemented with 2 mM choline. Data represent mean optical densities at 600 nm ± SD (n = 3). Quantification of peroxisome distribution and mean numbers as detailed at Fig. 4. Cells were grown on glucose (**B**) or oleate medium (**C**) supplemented with 2 mM choline.

Peroxisome quantification in *S. cerevisiae* was performed manually by counting GFP spots in individual cells throughout the Z-stacks.

3. Results

3.1. Reduction in PE biosynthesis or CL depletion do not have major effects on peroxisome biogenesis or proliferation in S. cerevisiae

We started our studies with the analysis of the effect of CL deficiency and reduced PE levels. To this purpose we used an *S. cerevisiae* mutant lacking cardiolipin synthase (*crd1*), which results in a complete block in CL biosynthesis [26]. In addition we studied three mutants (*psd1*, *psd2* and *eki1 cki1 dpl1*) (Fig. 1B), in which one of the three PE biosynthetic pathways is blocked, leading to significantly reduced PE levels [23]. Cells were grown on defined mineral medium lacking yeast extract to prevent the presence of lipid precursors (ethanolamine, choline) or lyso-lipids, including lyso-phosphatidylethanolamine [27–29].

As shown in Fig. 1C, all mutant strains grew similar as the wild-type (WT) control on medium containing glucose. However, when cells were grown on medium containing oleate, the *psd1* strain showed an increased doubling time, but the final optical densities (OD_{600}) of all strains were the same (Fig. 1D).

Fluorescence microscopy analysis of WT and mutant cells exponentially growing on glucose or oleic acid containing media showed that the matrix marker protein GFP-SKL was properly imported in peroxisomes in all strains (Fig. 2). Quantification of peroxisome numbers revealed that *crd1* cells displayed similar numbers of peroxisomes as WT control cells both upon growth on glucose or oleate (Fig 3A, B). Interestingly, deletion of *PSD1* resulted in a reduction of peroxisome numbers both on glucose and oleate (Fig 3A, B). Deletion of *PSD2* or *EKI1 CKI1 DPL1* had no effect on peroxisome abundance in glucose-grown cells, whereas the numbers were slightly decreased upon growth on oleate (Fig. 3B).

3.2. Addition of choline to the growth medium suppresses the impact of phosphatidylserine decarboxylase deficiency on peroxisome proliferation

PE may also be methylated to form phosphatidylcholine (PC) (Fig. 1B). Thus impaired PE biosynthesis indirectly affects the formation of PC. To allow PE independent formation of PC, media were supplemented with choline, which can be used in the Kennedy pathway for the formation of PC (Fig. 1B). This largely suppressed the growth defect of *psd1* cells in oleate containing medium (Fig. 4A, compare Fig. 1D). Concomitantly, no significant reduction in peroxisome numbers was observed anymore upon growth of *psd1* cells on glucose or oleic acid (Fig 4B, C). Previously, the simultaneous deletion of *PSD1* and *PSD2* was shown to enhance the depletion of cellular PE relative to that observed in *PSD1* or *PSD2* single deletion strains [23]. Growth of *psd1 psd2* cells in medium containing glucose or oleate supplemented with choline was not altered relative to *psd1* cells (data not shown). Furthermore, at these conditions peroxisome abundance was not reduced (Fig 4B, C).

These data indicate that the effect of impaired PE synthesis on peroxisomes and oleic acid growth is likely to be associated with hampered synthesis of PC.

4. Discussion

CL has been detected in peroxisomal membranes isolated from *S. cerevisiae*, *P. pastoris* and castor bean [3–5,23,30]. However, here we show that a complete block in CL biosynthesis (in *crd1* cells) does not affect peroxisome formation, function (reflected in normal growth on oleate) or proliferation. Our data are consistent with studies in plants which revealed no effect of CL deficiency on peroxisome morphology [31]. Our observations raise the question as to why CL is present in yeast peroxisomal membranes. Possibly, CL deficiency is compensated by other non-bilayer forming lipids, such as PE. In mitochondria CL and PE are playing partially overlapping roles and only a simultaneous block in mitochondrial PE and CL synthesis (by deletion of *PSD1* and *CRD1*) is lethal [7,32]. Alternatively, the reported presence of CL in peroxisomal membrane fractions is due to contamination with other, CL containing membranes. Indeed, CL was not detected in peroxisomes isolated form *Candida tropicalis* [33] or rat liver [34,35]. Mitochondrial contamination could be due to artificial association of both organelles occurring during the peroxisome isolation procedure [36], but both organelles may also form physical contacts *in vivo* [37,38].

In contrast to CL, PE represents a major fraction of the

peroxisomal phospholipids, which is unlikely due to contamination by other organelles [3,4,33]. We show that deletion of *PSD1* resulted in a growth defect on media containing oleic acid, suggesting a partial defect in peroxisome function. However, this growth defect was largely restored upon addition of choline to the medium, indicating that the growth problem was indirectly caused by a defect in PC biosynthesis (Fig. 1).

Similarly, the reduction in peroxisome numbers observed in the *psd1* mutant was abolished by supplementation of the medium with choline. This is in line with recent data that indicate that peroxisome numbers are not changed in *S. cerevisiae psd1* and *psd1 psd2* cells grown on rich YPO (yeast extract, peptone, oleate) medium, which contains trace amounts of ethanolamine and choline [23]. Hence, we speculate that the decrease in peroxisome numbers in *S. cerevisiae* PE biosynthesis mutants in the absence of choline is indirectly caused by impaired PC formation, which is considered as essential in yeast [15].

Overall, our data indicate that unlike in mitochondria, a reduced content of cellular CL or PE does not affect peroxisome biogenesis or proliferation.

Acknowledgements

IvdK is supported by the Marie Curie Initial Training Networks (ITN) program PerFuMe (Grant Agreement Number 316723).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2016.10.033.

References

[1] L. Pieuchot, G. Jedd, Peroxisome assembly and functional diversity in eukaryotic microorganisms, Annu. Rev. Microbiol. 66 (2012) 237–263.
[2] J.J. Smith, J.D. Aitchison, Peroxisomes take shape, Nat. Rev. Mol. Cell Biol. 14 (2013) 803–817.
[3] E. Zinser, C.D. Sperka-Gottlieb, E.V. Fasch, S.D. Kohlwein, F. Paltauf, G. Daum, Phospholipid synthesis and lipid composition of subcellular membranes in the unicellular eukaryote *Saccharomyces cerevisiae*, J. Bacteriol. 173 (1991) 2026–2034.
[4] T. Wriessnegger, G. Gübitz, E. Leitner, E. Ingolic, J. Cregg, B.J. de la Cruz, G. Daum, Lipid composition of peroxisomes from the yeast *Pichia pastoris* grown on different carbon sources, Biochim. Biophys. Acta BBA Mol. Cell Biol. Lipids 1771 (2007) 455–461.
[5] V.V. Flis, A. Fankl, C. Ramprecht, G. Zellnig, E. Leitner, A. Hermetter, G. Daum, Phosphatidylcholine supply to peroxisomes of the yeast *Saccharomyces cerevisiae*, PLoS One 10 (2015) e0135084.
[6] L. Chernomordik, Non-bilayer lipids and biological fusion intermediates, Chem. Phys. Lipids 81 (1996) 203–213.
[7] A.S. Joshi, M.N. Thompson, N. Fei, M. Hüttemann, M.L. Greenberg, Cardiolipin and mitochondrial phosphatidylethanolamine have overlapping functions in mitochondrial fusion in *Saccharomyces cerevisiae*, J. Biol. Chem. 287 (2012) 17589–17597.
[8] E. van den Brink-van der Laan, J. Antoinette Killian, B. de Kruijff, Nonbilayer lipids affect peripheral and integral membrane proteins via changes in the lateral pressure profile, Biochim. Biophys. Acta BBA Biomembr. 1666 (2004) 275–288.
[9] L. Böttinger, S.E. Horvath, T. Kleinschroth, C. Hunte, G. Daum, N. Pfanner, T. Becker, Phosphatidylethanolamine and cardiolipin differentially affect the stability of mitochondrial respiratory chain supercomplexes, J. Mol. Biol. 423 (2012) 677–686.
[10] T. Becker, S.E. Horvath, L. Böttinger, N. Gebert, G. Daum, N. Pfanner, Role of phosphatidylethanolamine in the biogenesis of mitochondrial outer membrane proteins, J. Biol. Chem. 288 (2013) 16451–16459.
[11] C.D. Baker, W.B. Ball, E.N. Pryce, V.M. Gohil, Specific requirements of nonbilayer phospholipids in mitochondrial respiratory chain function and formation, Mol. Biol. Cell 27 (2016) 2161–2171.
[12] M.A. Frohman, Role of mitochondrial lipids in guiding fission and fusion, J. Mol. Med. 93 (2014) 263–269.
[13] M.G. Baile, Y.-W. Lu, S.M. Claypool, The topology and regulation of cardiolipin biosynthesis and remodeling in yeast, Chem. Phys. Lipids 179 (2014) 25–31.
[14] M. Schlame, M.L. Greenberg, Biosynthesis, remodeling and turnover of mitochondrial cardiolipin, Biochim. Biophys. Acta BBA Mol. Cell Biol. Lipids, http://dx.doi.org/10.1016/j.bbalip.2016.08.010.
[15] R. Birner, M. Bürgermeister, R. Schneiter, G. Daum, Roles of phosphatidylethanolamine and of its several biosynthetic pathways in *Saccharomyces cerevisiae*, Mol. Biol. Cell 12 (2001) 997–1007.
[16] C.J. Clancey, S.C. Chang, W. Dowhan, Cloning of a gene (*PSD1*) encoding phosphatidylserine decarboxylase from *Saccharomyces cerevisiae* by complementation of an *Escherichia coli* mutant, J. Biol. Chem. 268 (1993) 24580–24590.
[17] S.E. Horvath, L. Böttinger, F.-N. Vögtle, N. Wiedemann, C. Meisinger, T. Becker, G. Daum, Processing and topology of the yeast mitochondrial phosphatidylserine decarboxylase 1, J. Biol. Chem. 287 (2012) 36744–36755.
[18] P.J. Trotter, D.R. Voelker, Identification of a non-mitochondrial phosphatidylserine decarboxylase activity (*PSD2*) in the yeast *Saccharomyces cerevisiae*, J. Biol. Chem. 270 (1995) 6062–6070.
[19] F. Gibellini, T.K. Smith, The Kennedy pathway—de novo synthesis of phosphatidylethanolamine and phosphatidylcholine, IUBMB Life 62 (2010) 414–428.
[20] K. Kim, K.-H. Kim, M.K. Storey, D.R. Voelker, G.M. Carman, Isolation and characterization of the *Saccharomyces cerevisiae EKI1* gene encoding ethanolamine kinase, J. Biol. Chem. 274 (1999) 14857–14866.
[21] K.-H. Kim, D.R. Voelker, M.T. Flocco, G.M. Carman, Expression, purification, and characterization of choline kinase, product of the *CKI* gene from *Saccharomyces cerevisiae*, J. Biol. Chem. 273 (1998) 6844–6852.
[22] J.D. Saba, F. Nara, A. Bielawska, S. Garrett, Y.A. Hannun, The *BST1* gene of *Saccharomyces cerevisiae* is the sphingosine-1-phosphate lyase, J. Biol. Chem. 272 (1997) 26087–26090.
[23] S. Rosenberger, M. Connerth, G. Zellnig, G. Daum, Phosphatidylethanolamine synthesized by three different pathways is supplied to peroxisomes of the yeast *Saccharomyces cerevisiae*, Biochim. Biophys. Acta BBA Mol. Cell Biol. Lipids 1791 (2009) 379–387.
[24] L.P.V. Dijken, R. Otto, W. Harder, Growth of *Hansenula polymorpha* in a methanol-limited chemostat, Arch. Microbiol. 111 (1976) 137–144.
[25] D. Gietz, A. St Jean, R.A. Woods, R.H. Schiestl, Improved method for high efficiency transformation of intact yeast cells, Nucleic Acids Res. 20 (1992) 1425.
[26] S.-C. Chang, P.N. Heacock, E. Mileykovskaya, D.R. Voelker, W. Dowhan, Isolation and characterization of the gene (*CLS1*) encoding cardiolipin synthase in *Saccharomyces cerevisiae*, J. Biol. Chem. 273 (1998) 14933–14941.
[27] W.R. Riekhof, J. Wu, J.L. Jones, D.R. Voelker, Identification and characterization of the major lysophosphatidylethanolamine acyltransferase in *Saccharomyces cerevisiae*, J. Biol. Chem. 282 (2007) 28344–28352.
[28] W.R. Riekhof, J. Wu, M.A. Gijón, S. Zarini, R.C. Murphy, D.R. Voelker, Lysophosphatidylcholine metabolism in *Saccharomyces cerevisiae*: the role of P-type ATPases in transport and a broad specificity acyltransferase in acylation, J. Biol. Chem. 282 (2007) 36853–36861.
[29] W.R. Riekhof, D.R. Voelker, Uptake and utilization of lysophosphatidylethanolamine by *Saccharomyces cerevisiae*, J. Biol. Chem. 281 (2006) 36588–36596.
[30] G. Daum, J.E. Vance, Import of lipids into mitochondria, Prog. Lipid Res. 36 (1997) 103–130.
[31] R. Pan, A.D. Jones, J. Hu, Cardiolipin-mediated mitochondrial dynamics and stress response in *Arabidopsis*, Plant Cell 26 (2014) 391–409.
[32] V.M. Gohil, M.N. Thompson, M.L. Greenberg, Synthetic lethal interaction of the mitochondrial phosphatidylethanolamine and cardiolipin biosynthetic pathways in *Saccharomyces cerevisiae*, J. Biol. Chem. 280 (2005) 35410–35416.
[33] W.M. Nuttley, A.G. Bodnar, D. Mangroo, R.A. Rachubinski, Isolation and characterization of membranes from oleic acid-induced peroxisomes of *Candida tropicalis*, J. Cell Sci. 95 (1990) 463–470.
[34] Y. Fujiki, S. Fowler, H. Shio, A.L. Hubbard, P.B. Lazarow, Polypeptide and phospholipid composition of the membrane of rat liver peroxisomes: comparison with endoplasmic reticulum and mitochondrial membranes, J. Cell Biol. 93 (1982) 103–110.
[35] D. Hardeman, C. Versantvoort, J.M. van den Brink, H. van den Bosch, Studies on peroxisomal membranes, Biochim. Biophys. Acta BBA Biomembr. 1027 (1990) 149–154.
[36] S. Reumann, R. Singhal, Isolation of leaf peroxisomes from *Arabidopsis* for organelle proteome analyses, Methods Mol. Biol. Clifton N. J. 1072 (2014) 541–552.
[37] Y. Cohen, Y.A. Klug, L. Dimitrov, Z. Erez, S.G. Chuartzman, D. Elinger, I. Yofe, K. Soliman, J. Gärtner, S. Thoms, R. Schekman, Y. Elbaz-Alon, E. Zalckvar, M. Schuldiner, Peroxisomes are juxtaposed to strategic sites on mitochondria, Mol. Biosyst. 10 (2014) 1742–1748.
[38] M. Mattiazzi Ušaj, M. Brložnik, P. Kaferle, M. Žitnik, H. Wolinski, F. Leitner, S.D. Kohlwein, B. Zupan, U. Petrovič, Genome-wide localization study of yeast Pex11 identifies peroxisome–mitochondria interactions through the ERMES complex, J. Mol. Biol. 427 (2015) 2072–2087.
[39] U. Guldener, S. Heck, T. Fielder, J. Beinhauer, J.H. Hegemann, A new efficient gene disruption cassette for repeated use in budding yeast, Nucleic Acids Res. 24 (1996) 2519–2524.
[40] R. Saraya, A.M. Krikken, J.A.K.W. Kiel, R.J.S. Baerends, M. Veenhuis, I.J. van der Klei, Novel genetic tools for *Hansenula polymorpha*, FEMS Yeast Res. 12 (2012) 271–278.
[41] R.J. Baerends, K.N. Faber, A.M. Kram, J.A. Kiel, I.J. van der Klei, M. Veenhuis, A stretch of positively charged amino acids at the N terminus of *Hansenula polymorpha* Pex3p is involved in incorporation of the protein into the peroxisomal membrane, J. Biol. Chem. 275 (2000) 9986–9995.