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Research Article

RESEARCH ARTICLE

# Get1p and Get2p are required for maintenance of mitochondrial morphology and normal cardiolipin levels

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**One sentence summary:** Cardiolipin synthase genetically interacts with genes required for maintenance of mitochondrial morphology, suggesting functional redundancy.
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## ABSTRACT

Cardiolipin (CL) is the signature phospholipid of mitochondrial membranes. CL deficiency leads to defects in mitochondrial function. Using a targeted synthetic lethality screen to identify defects that exacerbate CL deficiency, we determined that deletion of mitochondrial morphology genes in cells lacking CL leads to severe growth defects. We show that ER membrane proteins Get1p and Get2p are required for maintaining normal levels of CL. We propose that these proteins regulate the level of CL by maintaining wild type-like tubular mitochondrial morphology. The genetic interactions observed in this study identify novel physiological modifiers that are required for maintenance of CL levels and mitochondrial morphology.

**Keywords:** cardiolipin; mitochondria morphology; Barth syndrome

Cardiolipin (CL) is a unique anionic phospholipid that is enriched in mitochondrial membranes. It has two phosphatidyl moieties linked by glycerol, giving it a dimeric structure (Lecocq and Ballou 1964). CL is synthesized in the inner mitochondrial membrane by CL synthase, encoded by *CRD1* in yeast (Jiang, Rizavi and Greenberg 1997; Chang *et al.* 1998; Tuller *et al.* 1998). *De novo* synthesis is followed by a two-step remodeling process in which CL is deacylated by Cld1p to monolysocardiolipin (MLCL), which is reacylated by the *TAZ1* encoded transacylase tafazzin (Gu *et al.* 2004; Beranek *et al.* 2009). In humans, a mutated tafazzin gene is responsible for the severe genetic disorder known as Barth syndrome (Bione *et al.* 1996). In both yeast and human cells, loss of function of tafazzin results in a reduction in total CL levels, accumulation of MLCL and aberrant CL fatty acyl species (Vreken *et al.* 2000; Gu *et al.* 2004; Valianpour *et al.* 2005). CL is important for many mitochondrial functions, including mitochondrial protein import (Jiang *et al.* 2000; Gebert *et al.* 2009), Fe-S biogenesis (Patil *et al.* 2013), apoptosis (Gonzalvez *et al.* 2008; Schafer *et al.* 2009), aging (Zhou *et al.* 2009) and assembly of protein complexes in the mitochondrial inner membrane including ATP synthase and respiratory chain complexes (Jiang *et al.* 2000; Koshkin and Greenberg 2000; Zhang, Mileykovskaya and Dowhan 2002, 2005; Pfeiffer *et al.* 2003; Claypool *et al.* 2008; Wenz *et al.* 2009). For more detailed discussion of the functions of CL, we refer the reader to reviews (Gohil and Greenberg 2009; Joshi *et al.* 2009; Osman, Voelker and Langer 2011; Mileykovskaya and Dowhan 2014; Ren, Phoon and Schlame 2014).

In addition to the above-mentioned functions, CL deficiency also affects mitochondrial morphology. We have shown that cells lacking mitochondrial phospholipids CL and phosphatidylethanolamine (PE) have fragmented mitochondria due to a defect in mitochondrial fusion (Joshi *et al.* 2012).

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**Table 1.** *CRD1* genetically interacts with mitochondrial morphology genes. The yeast strains used in this study are isogenic to BY4741 and BY4742. The *crd1*Δ*::URA3 MATα* was crossed with mitochondrial morphology mutants obtained from *MATa* yeast deletion collection. The heterozygous diploids were sporulated and tetrad dissected. The synthetic interaction between *CRD1* and mitochondrial morphology mutants was determined by examining the growth of the double mutant compared to isogenic parental strains and wild type. Unviable double mutants indicated synthetic lethal interaction, viable double mutants with growth defect indicated a synthetic sick interaction and with no growth defect indicate no genetic interaction. Key: (+)—no genetic interaction; (–)—synthetic lethal; (+/–)—synthetic sick.

| | | *crd1*Δ | |
|---|---|---|---|
| Functional groups | | 30°C | 37°C |
| GET complex | *get1*Δ | + | +/– |
| | *get2*Δ | + | + |
| | *get3*Δ | + | +/– |
| UPS complex | *ups1*Δ | – | – |
| | *ups2*Δ | – | – |
| | *ups3*Δ | + | + |
| ERMES complex | *gem1*Δ | +/– | +/– |
| Mitochondrial dynamics | *dnm1*Δ | +/– | – |
| | *fis1*Δ | + | + |
| | *mdv1*Δ | + | + |
| | *mdm36*Δ | + | +/– |
| | *ugo1*Δ | – | – |
| | *fzo1*Δ | – | – |
| | *mgm1*Δ | + | + |
| Mitochondrial inheritance | *mdm31*Δ | + | + |
| | *mdm32*Δ | + | + |
| F-box protein association | *mfb1*Δ | + | + |
| Imported protein cleavage | *cym1*Δ | + | +/– |
| Fatty acid synthesis | *htd2*Δ | + | +/– |
| Similarity to hemolysins | *mam3*Δ | + | +/– |

Mitochondria from cells with mutated tafazzin exhibit abnormal mitochondrial shape further supporting the role of CL in mitochondrial morphology (Xu *et al.* 2005; Acehan *et al.* 2007, 2011). Interestingly, several mutants that exhibit defective mitochondrial morphology have altered levels of CL, PE, or both (Osman *et al.* 2009; Tamura *et al.* 2009; Kuroda *et al.* 2011). To better understand the functional relationship between mitochondrial morphology and CL, we performed a targeted synthetic lethality screen to identify mutations in mitochondrial morphology genes that exhibit genetic interaction with the *crd1*Δ mutant. The mitochondrial morphology mutants screened are listed in Table 1. Synthetic lethal interactions between two genes suggest functional redundancy. Thus, we hypothesized that genetic interactions may identify mitochondrial morphology proteins required to regulate levels of CL. Using the genetic screen, we identified both lethal interactions (in which double mutants were not viable) and sick interactions (in which double mutants exhibited defective growth compared to isogenic parent strains). The genetic screen showed that the GET complex genes genetically interacted with *CRD1*. We also showed the novel function of ER membrane proteins Get1p and Get2p in maintaining normal levels of CL and mitochondrial morphology.

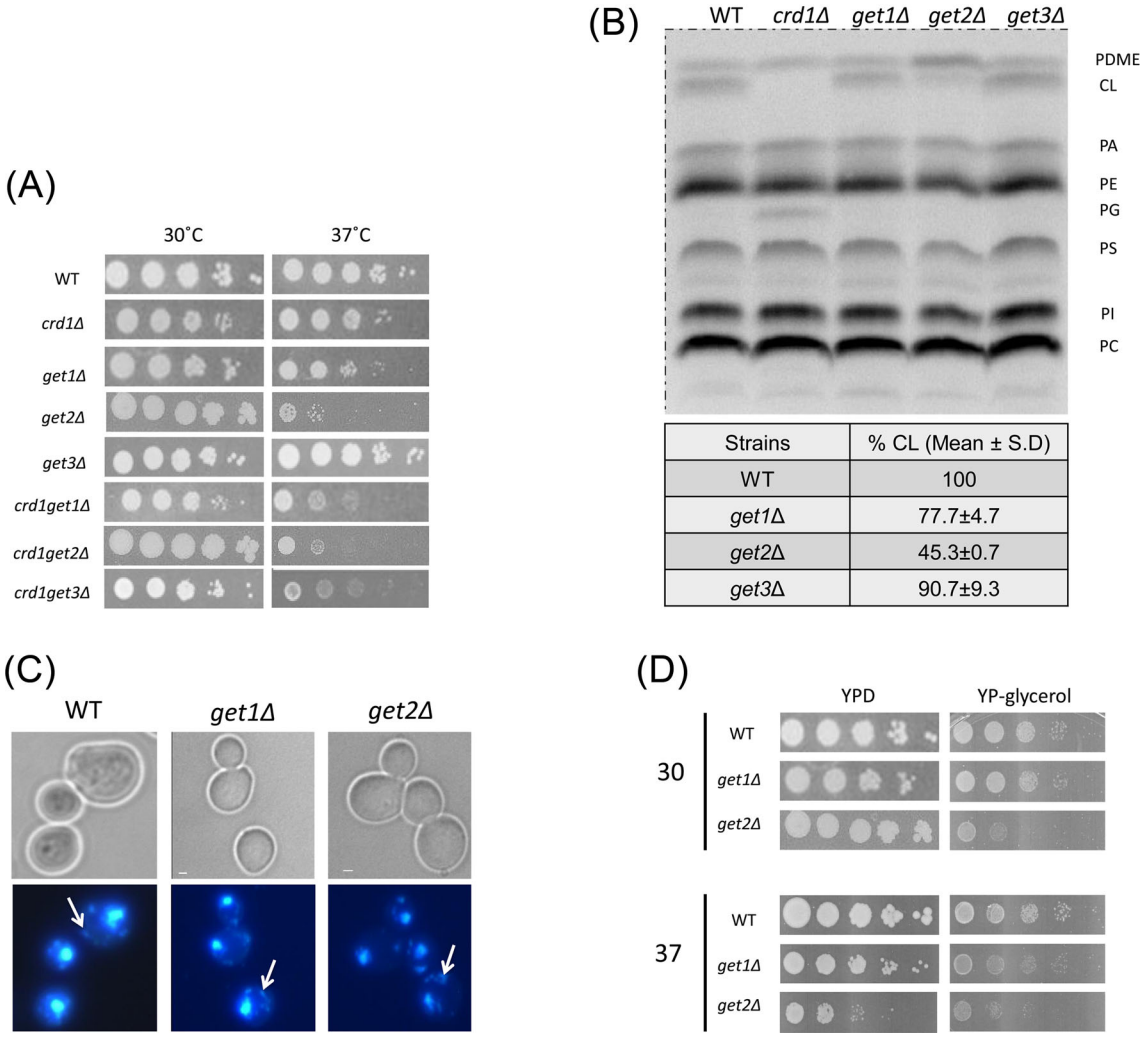
GET proteins (Get1p and Get2p) are localized to ER membrane and Get3p is a cytosolic protein. The GET complex is involved in targeting newly synthesized tail-anchored (TA) proteins to the ER membrane. This process involves formation of the Get3p-TA complex in the cytosol, which is then recruited by the Get1/2p receptor proteins present in the ER membrane to insert the TA proteins in the lipid bilayer (Schuldiner *et al.* 2008; Mariappan *et al.* 2011; Stefer *et al.* 2011; Wang *et al.* 2011). In the absence of the GET complex, TA proteins are mistargeted to other organelles such as mitochondria and peroxisomes. In our screen, we found that *CRD1* exhibits a synthetic sick interaction with *GET1* and *GET3* at elevated temperature, as both *crd1*Δ*get1*Δ and *crd1*Δ*get3*Δ have growth defects compared to the parent strains (Fig. 1A). Surprisingly, deletion of *crd1*Δ did not exacerbate the growth defect of *get2*Δ (data not shown). These findings suggest that, at elevated temperature, CL is essential for the growth of *get1*Δ and *get3*Δ but not *get2*Δ, or that the function of Get1p or Get3p, but not Get2p, is essential for growth of *crd1*Δ. It is interesting to speculate that CL may be involved in targeting proteins to the mitochondria or required to clear mistargeted proteins from mitochondrial membranes. Alternatively, it is possible that the GET proteins may be involved in regulating cellular levels of CL.

Consistent with the genetic interaction data, we found that GET complex mutants *get1*Δ and *get2*Δ, but not *get3*Δ, exhibited a decrease in CL compared to WT (Fig. 1B), suggesting a possible role of these ER membrane proteins in regulating the levels of CL. While loss of mitochondrial DNA leads to a decrease in CL (Chen *et al.* 2010), this could not explain the reduced CL levels in *get1*Δ and *get2*Δ, as DAPI staining indicated the presence of mitochondrial DNA in these mutants (Fig. 1C). Even though the *get1*Δ and *get2*Δ mutants were not deficient in mtDNA, *get2*Δ exhibited a severe growth defect whereas *get1*Δ exhibited only a mild growth defect on non-fermentable media (Fig. 1D), suggesting that reduced mitochondrial function may be due to a decrease in CL. Defects in mitochondrial morphology as well as reduced CL levels were restored by expression of *GET2* from a 2$\mu$ plasmid in *get2*Δ (Figs 1E and 2F). In contrast, overexpression of *CRD1* in *get2*Δ neither restored CL levels nor improved mitochondrial morphology (Fig. 1E and F), suggesting that import of either precursors of CL or enzymes required for CL biosynthesis in the mitochondria might be affected in the *get2*Δ mutants. We also found that cellular levels of other phospholipids were unchanged (data not shown). In addition to its role in protein targeting, Get1p and Get2p may facilitate lipid flow from the ER to the mitochondria possibly by creating ER-mitochondria encounter structure (ERMES)-like contact sites. To test if Get2p is required to form an additional ERMES-like contact to facilitate ER-mitochondria lipid, we overexpressed *GET2* in ERMES mutants. *GET2* overexpression did not rescue the growth defect of the ERMES mutants (Fig. 1G), indicating that Get2p might not facilitate ER-mitochondria lipid flow, as does ERMES. Thus, the mechanism by which Get1p and Get2p regulate CL levels remains unknown. We propose that the decrease in CL observed in *get1*Δ and *get2*Δ is not due to a defect in ER-mitochondria lipid exchange and could be a consequence of defects in mitochondrial function.

We also observed genetic interaction between *CRD1* and the UPS complex genes (Table 1). Of the three UPS proteins, Ups1p and Ups2p are required for maintenance of normal mitochondrial morphology and to regulate levels of mitochondrial phospholipids such as CL and mitochondrial PE. Ups1p is required for shuttling of phosphatidic acid, a precursor for CL biosynthesis, between outer and inner mitochondrial membranes (Connerth *et al.* 2012). Consequently, the *ups1*Δ mutant exhibits decreased levels of CL, while deletion of Ups2p in *ups1*Δ restores CL levels (Osman *et al.* 2009). Ups1p also enhances conversion of PE to PC whereas Ups2p suppresses it. Therefore, *ups1*Δ exhibits increased mitochondrial PE levels and deletion of Ups2

results in decreased PE. Thus Ups1p and Ups2p antagonistically regulate CL and mitochondrial PE levels (Tamura *et al.* 2009). As expected *ups2*Δ*crd1*Δ (Table 1) is not viable, as depletion of both CL and mitochondrial PE is lethal in yeast cells (Gohil, Thompson and Greenberg 2005). However, we found that *CRD1* is synthetically lethal with *UPS1* as *crd1*Δ*ups1*Δ is not viable (Fig. 2 and Table 1). Both Ups1p and CL are required for efficient mitochondrial protein import and for normal mitochondrial morphology, which are both impaired when both are deleted.

*CRD1* exhibits synthetic sick interaction with *GEM1* (Table 1). Gem1p, an integral component of the ERMES complex, regulates the number and size of this complex (Kornmann, Osman and Walter 2011). The complex consists of Mdm10p, Mmm1p, Mdm12p and Mdm34p. In addition to its role in mitochondrial protein import, maintenance of mitochondrial morphology and mitochondrial DNA, the ERMES complex is believed to facilitate phospholipid exchange by tethering ER and mitochondrial membranes (Kornmann *et al.* 2009). In this study, we show that deletion of *GEM1* in the *crd1*Δ mutant results in growth defects at 30°C and 37°C (Table 1). Both ERMES mutants and *gem1*Δ exhibit decreased levels of CL. Our results are consistent with previous findings (Kornmann *et al.* 2009; Kornmann, Osman and Walter 2011). Thus, the synthetic genetic interactions support the role




| Strains | % CL (Mean ± S.D) |
|---|---|
| WT | 100 |
| *get1*Δ | 77.7±4.7 |
| *get2*Δ | 45.3±0.7 |
| *get3*Δ | 90.7±9.3 |

**Figure 1.** Get1p and Get2p are required for maintenance of mitochondrial morphology and CL levels. **(A)** *CRD1* is synthetically sick with *GET1* and *GET3*. Yeast cells were pre-cultured overnight in YPD at 30°C, serially diluted and spotted on YPD plates. The plates were incubated at 30°C and 37°C for 3–4 days. **(B)** Quantitation of CL in *get1*Δ, *get2*Δ and *get3*Δ mutants. Yeast cells were grown in YPD with $^{32}P_i$ at 30°C until early stationary phase. Total phospholipids were extracted and analyzed by 1D- TLC as described (Vaden *et al.* 2005). The developed chromatograms were analyzed using phosphorimaging and phospholipids were quantified using Image Quant software. The TLC image is representative of two independent experiments. Values are mean ± SD ($n = 2$). **(C)** The *get1*Δ and *get2*Δ mutants do not exhibit loss of mitochondrial DNA. Fluorescence microscopy was performed using an Olympus BX41 epifluorescence microscope. Images were acquired using an Olympus Q-Color3 digitally charge-coupled device camera operated by QCapture2 software. All pictures were taken at 1000 X. To stain mitochondrial DNA, yeast cells were cultured to the mid-log phase, fixed in 70% ethanol at room temperature for 30 min, washed two times with distilled water, and stained with 1 $\mu$g/ml DAPI (Sigma) for 5 min. **(D)** GET complex mutants exhibit decreased mitochondrial function. Yeast cells were pre-culture in liquid YPD for 1 day, washed twice with water, serially diluted and spotted on YPD and YP-glycerol plates. The plates were incubated at 30°C and 37°C for 2–3 days. **(E)** Over expression of *GET2* restores CL levels in *get2*Δ. Yeast cells were grown until early stationary phase, total phospholipids were extracted and analyzed as in Fig. 1B. Values are mean ± SD ($n = 2$). **(F)** Over expression of *CRD1* cannot restore mitochondrial morphology defect in *get2*Δ. Mitochondria were visualized by transforming the cells with pYX122 expressing GFP fused to the mitochondrial presequence, pre Su9 (provided by Dr Benedikt Westermann). These cells were grown in synthetic media at 30°C until log phase for observation under fluorescence microscopy. **(G)** *GET2* overexpression does not rescue growth defect of ERMES mutants. Yeast cells were pre-cultured overnight at 30°C, serially diluted and spotted on leucine deficient synthetic media. The plates were incubated at 30°C for 3–4 days.

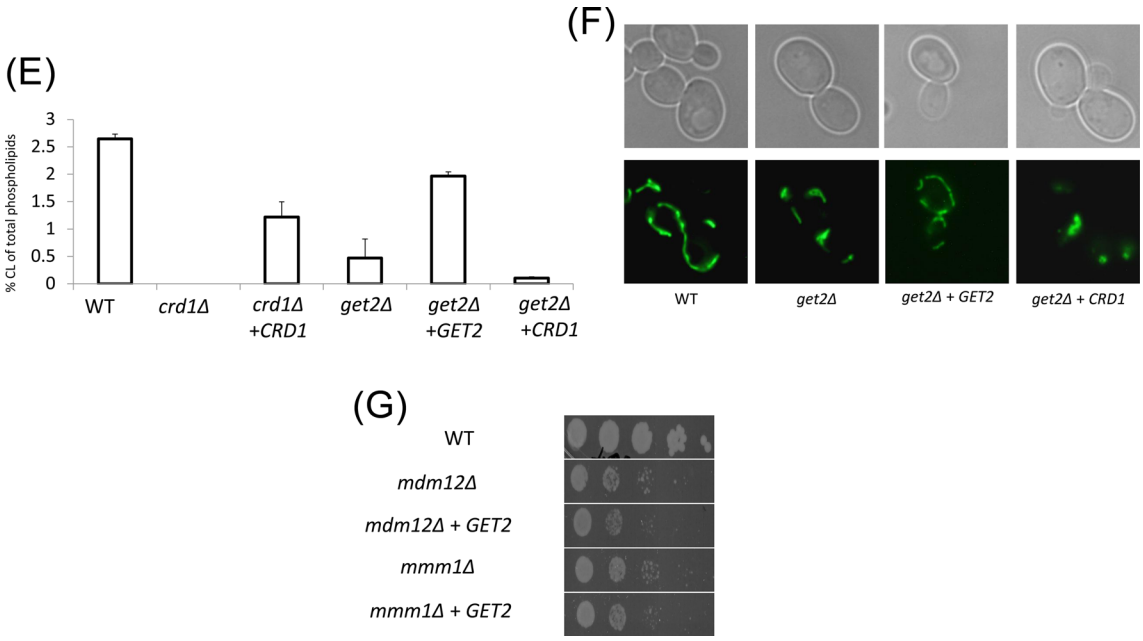



**Figure 1.** (*Continued*).

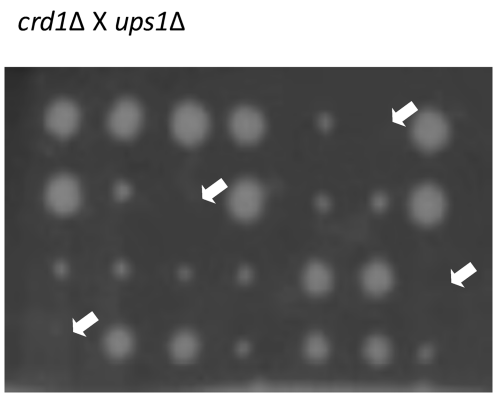



**Figure 2.** *CRD1* genetically interacts with *UPS1*. *CRD1* is synthetically lethal with *UPS1*. Heterozygous diploid strain with deletion of *CRD1* and *UPS1* was sporulated and tetrad dissected. Arrows indicate unviable double mutant haploid spore on YPD plate incubated at 30°C for 3–4 days.

of ERMES and Gem1p in tethering ER-mitochondrial membranes required for phospholipid transport and normal mitochondrial function.

*CRD1* genetically interacted with genes involved in mitochondrial fusion and fission. Mitochondria are dynamic organelles that constantly undergo fusion and fission. In yeast, Fzo1p, Ugo1p and Mgm1p are required for fusion, while Dnm1p, Fis1p and Mdv1p are required for fission (Hoppins, Lackner and Nunnari 2007). We have shown that mitochondrial PE and CL have overlapping roles in mitochondrial fusion (Joshi *et al.* 2012). Loss of both CL and mitochondrial PE results in degradation of Mgm1p leading to defective fusion. In this study, we show that *CRD1* is synthetically lethal with mitochondrial fusion genes *UGO1* and *FZO1* whereas deletion of *CRD1* does not exacerbate the growth defect of *mgm1*Δ (Table 1). On the other hand, deletion of *DNM1* in *crd1*Δ led to severe growth defects, while *crd1*Δ*mdv1*Δ and *crd1*Δ*fis1*Δ grew normally. This suggests that CL is functionally redundant with Dnm1p but not Mdv1p and Fis1p. The double mutant *crd1*Δ*mdm36*Δ also exhibited growth defects at elevated temperature, suggesting a synthetic sick interaction. Mdm36p is required for normal mitochondrial morphology and inheritance and with Num1p forms a mitochondria-ER-cortex-anchor tether that anchors mitochondria to the cortex. Mdm36p is also involved in formation of a Dnm1p and Num1p complex at the cell cortex that promotes mitochondrial division (Lackner *et al.* 2013). It is interesting to observe that both *crd1*Δ*dnm1*Δ and *crd1*Δ*mdm36*Δ exhibit growth defects, as both Mdm36p and Dnm1p are required for anchoring of mitochondria to cortex. Dnm1p and Mdm36p may regulate CL levels. Alternatively, CL may play a role in anchoring mitochondria to cortex.

*CRD1* does not exhibit genetic interaction with mitochondrial inheritance genes *MDM31* and *MDM32*, as both *crd1mdm31*Δ and *crd1mdm32*Δ grew normally (Table 1). *CRD1* also did not exhibit genetic interaction with *MFB1*, which is required to maintain tubular mitochondrial morphology. However, *CRD1* genetically interacted with *CYM1*, *HTD2* and *MAM3* (Table 1). Cym1p is a mitochondrial metalloprotease (Kambacheld *et al.* 2005), Htd2p is a mitochondrial dehydratase involved in fatty acid synthesis (Kastaniotis *et al.* 2004) and Mam3p is similar to hemolysins (Entian *et al.* 1999). However, all three proteins are required for normal mitochondrial morphology. Depletion of CL in *cym1*Δ, *htd1*Δ and *mam3*Δ might cause severe defect in mitochondrial function in these mutants. It is also possible that *cym1*Δ, *htd1*Δ and *mam3*Δ might regulate CL levels by altering mitochondrial morphology. During the course of this study, independent reports identified three novel mutants *mcp1*Δ, *mcp2*Δ and *fmp30*Δ that also exhibit both abnormal mitochondrial morphology and altered CL levels (Kuroda *et al.* 2011; Tan *et al.* 2013).

Thus, using a targeted genetic screen, we show that *CRD1* genetically interacts with several genes required for maintenance of mitochondrial morphology, suggesting functional redundancy. In particular, we found that *crd1*Δ exhibits synthetic sick interactions with GET complex mutants. We also demonstrate that ER membrane proteins Get1p and Get2p are required

for maintenance of CL levels and mitochondrial morphology. How these ER membrane proteins regulate mitochondrial morphology and mitochondrial phospholipid levels remains to be investigated. Elucidating the mechanism of regulation of CL levels by the GET complex may uncover a novel pathway for mitochondrial phospholipid homeostasis. In addition, further characterization of the genetic interactions between *crd1*Δ and mitochondrial morphology mutants summarized in Table 1 may identify novel cellular functions of CL.

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***Conflict of interest.*** None declared.

## REFERENCES

Acehan D, Vaz F, Houtkooper RH *et al.* Cardiac and skeletal muscle defects in a mouse model of human barth syndrome. *J Biol Chem* 2011;**286**:899–908.

Acehan D, Xu Y, Stokes DL *et al.* Comparison of lymphoblast mitochondria from normal subjects and patients with Barth syndrome using electron microscopic tomography. *Lab Invest* 2007;**87**:40–8.

Beranek A, Rechberger G, Knauer H *et al.* Identification of a cardiolipin-specific phospholipase encoded by the gene CLD1 (YGR110W) in yeast. *J Biol Chem* 2009;**284**:11572–8.

Bione S, D'Adamo P, Maestrini E *et al.* A novel X-linked gene, G4.5. is responsible for Barth syndrome. *Nat Genet* 1996;**12**: 385–9.

Chang SC, Heacock PN, Mileykovskaya E *et al.* Isolation and characterization of the gene (CLS1) encoding cardiolipin synthase in *Saccharomyces cerevisiae*. *J Biol Chem* 1998;**273**:14933–41.

Chen S, Liu D, Finley RL, Jr *et al.* Loss of mitochondrial DNA in the yeast cardiolipin synthase crd1 mutant leads to upregulation of the protein kinase Swe1p that regulates the G2/M transition. *J Biol Chem* 2010;**285**:10397–407.

Claypool SM, Oktay Y, Boontheung P *et al.* Cardiolipin defines the interactome of the major ADP/ATP carrier protein of the mitochondrial inner membrane. *J Cell Biol* 2008;**182**:937–50.

Connerth M, Tatsuta T, Haag M *et al.* Intramitochondrial transport of phosphatidic acid in yeast by a lipid transfer protein. *Science* 2012;**338**:815–8.

Entian KD, Schuster T, Hegemann JH *et al.* Functional analysis of 150 deletion mutants in *Saccharomyces cerevisiae* by a systematic approach. *Mol Gen Genet* 1999;**262**:683–702.

Gebert N, Joshi AS, Kutik S *et al.* Mitochondrial cardiolipin involved in outer-membrane protein biogenesis: implications for Barth syndrome. *Curr Biol* 2009;**19**:2133–9.

Gohil VM, Greenberg ML. Mitochondrial membrane biogenesis: phospholipids and proteins go hand in hand. *J Cell Biol* 2009;**184**:469–72.

Gohil VM, Thompson MN, Greenberg ML. Synthetic lethal interaction of the mitochondrial phosphatidylethanolamine and cardiolipin biosynthetic pathways in *Saccharomyces cerevisiae*. *J Biol Chem* 2005;**280**:35410–6.

Gonzalvez F, Schug ZT, Houtkooper RH *et al.* Cardiolipin provides an essential activating platform for caspase-8 on mitochondria. *J Cell Biol* 2008;**183**:681–96.

Gu Z, Valianpour F, Chen S *et al.* Aberrant cardiolipin metabolism in the yeast taz1 mutant: a model for Barth syndrome. *Mol Microbiol* 2004;**51**:149–58.

Hoppins S, Lackner L, Nunnari J. The machines that divide and fuse mitochondria. *Annu Rev Biochem* 2007;**76**:751–80.

Jiang F, Rizavi HS, Greenberg ML. Cardiolipin is not essential for the growth of *Saccharomyces cerevisiae* on fermentable or nonfermentable carbon sources. *Mol Microbiol* 1997;**26**:481–91.

Jiang F, Ryan MT, Schlame M *et al.* Absence of cardiolipin in the crd1 null mutant results in decreased mitochondrial membrane potential and reduced mitochondrial function. *J Biol Chem* 2000;**275**:22387–94.

Joshi AS, Thompson MN, Fei N *et al.* Cardiolipin and Mitochondrial Phosphatidylethanolamine Have Overlapping Functions in Mitochondrial Fusion in *Saccharomyces cerevisiae*. *J Biol Chem* 2012;**287**:17589–97.

Joshi AS, Zhou J, Gohil VM *et al.* Cellular functions of cardiolipin in yeast. *Biochim Biophys Acta* 2009;**1793**:212–8.

Kambacheld M, Augustin S, Tatsuta T *et al.* Role of the novel metallopeptidase Mop112 and saccharolysin for the complete degradation of proteins residing in different subcompartments of mitochondria. *J Biol Chem* 2005;**280**:20132–9.

Kastaniotis AJ, Autio KJ, Sormunen RT *et al.* Htd2p/Yhr067p is a yeast 3-hydroxyacyl-ACP dehydratase essential for mitochondrial function and morphology. *Mol Microbiol* 2004;**53**:1407–21.

Kornmann B, Currie E, Collins SR *et al.* An ER-mitochondria tethering complex revealed by a synthetic biology screen. *Science* 2009;**325**:477–81.

Kornmann B, Osman C, Walter P. The conserved GTPase Gem1 regulates endoplasmic reticulum-mitochondria connections. *P Natl Acad Sci USA* 2011;**108**:14151–6.

Koshkin V, Greenberg ML. Oxidative phosphorylation in cardiolipin-lacking yeast mitochondria. *Biochem J* 2000;**347**:687–91.

Kuroda T, Tani M, Moriguchi A *et al.* FMP30 is required for the maintenance of a normal cardiolipin level and mitochondrial morphology in the absence of mitochondrial phosphatidylethanolamine synthesis. *Mol Microbiol* 2011; **80**:248–65.

Lackner LL, Ping H, Graef M *et al.* Endoplasmic reticulum-associated mitochondria-cortex tether functions in the distribution and inheritance of mitochondria. *P Natl Acad Sci USA* 2013;**110**:E458–67.

Lecocq J, Ballou CE. On the Structure of Cardiolipin. *Biochemistry* 1964;**3**:976–80.

Mariappan M, Mateja A, Dobosz M *et al.* The mechanism of membrane-associated steps in tail-anchored protein insertion. *Nature* 2011;**477**:61–6.

Mileykovskaya E, Dowhan W. Cardiolipin-dependent formation of mitochondrial respiratory supercomplexes. *Chem Phys Lipids* 2014;**179**:42–8.

Osman C, Haag M, Potting C *et al.* The genetic interactome of prohibitins: coordinated control of cardiolipin and phosphatidylethanolamine by conserved regulators in mitochondria. *J Cell Biol* 2009;**184**:583–96.

Osman C, Voelker DR, Langer T. Making heads or tails of phospholipids in mitochondria. *J Cell Biol* 2011;**192**:7–16.

Patil VA, Fox JL, Gohil VM *et al.* Loss of cardiolipin leads to perturbation of mitochondrial and cellular iron homeostasis. *J Biol Chem* 2013;**288**:1696–705.

Pfeiffer K, Gohil V, Stuart RA *et al.* Cardiolipin stabilizes respiratory chain supercomplexes. *J Biol Chem* 2003;**278**: 52873–80.

Ren M, Phoon CK, Schlame M. Metabolism and function of mitochondrial cardiolipin. *Prog Lipid Res* 2014; **55**:1–16.

Schafer B, Quispe J, Choudhary V *et al*. Mitochondrial outer membrane proteins assist Bid in Bax-mediated lipidic pore formation. *Mol Biol Cell* 2009;**20**:2276–85.

Schuldiner M, Metz J, Schmid V *et al*. The GET complex mediates insertion of tail-anchored proteins into the ER membrane. *Cell* 2008;**134**:634–45.

Stefer S, Reitz S, Wang F *et al*. Structural basis for tail-anchored membrane protein biogenesis by the Get3-receptor complex. *Science* 2011;**333**:758–62.

Tamura Y, Endo T, Iijima M *et al*. Ups1p and Ups2p antagonistically regulate cardiolipin metabolism in mitochondria. *J Cell Biol* 2009;**185**:1029–45.

Tan T, Ozbalci C, Brugger B *et al*. Mcp1 and Mcp2, two novel proteins involved in mitochondrial lipid homeostasis. *J Cell Sci* 2013;**126**:3563–74.

Tuller G, Hrastnik C, Achleitner G *et al*. YDL142c encodes cardiolipin synthase (Cls1p) and is non-essential for aerobic growth of *Saccharomyces cerevisiae*. *FEBS Lett* 1998; **421**:15–18.

Vaden DL, Gohil VM, Gu Z *et al*. Separation of yeast phospholipids using one-dimensional thin-layer chromatography. *Anal Biochem* 2005;**338**:162–4.

Valianpour F, Mitsakos V, Schlemmer D *et al*. Monolysocardiolipins accumulate in Barth syndrome but do not lead to enhanced apoptosis. *J Lipid Res* 2005;**46**:1182–95.

Vreken P, Valianpour F, Nijtmans LG *et al*. Defective remodeling of cardiolipin and phosphatidylglycerol in Barth syndrome. *Biochem Bioph Res Co* 2000;**279**:378–82.

Wang F, Whynot A, Tung M *et al*. The Mechanism of Tail-Anchored Protein Insertion into the ER Membrane. *Mol Cell* 2011;**43**:738–50.

Wenz T, Hielscher R, Hellwig P *et al*. Role of phospholipids in respiratory cytochrome bc(1) complex catalysis and supercomplex formation. *Biochim Biophys Acta* 2009;**1787**:609–16.

Xu Y, Sutachan JJ, Plesken H *et al*. Characterization of lymphoblast mitochondria from patients with Barth syndrome. *Lab Invest* 2005;**85**:823–30.

Zhang M, Mileykovskaya E, Dowhan W. Gluing the respiratory chain together. Cardiolipin is required for supercomplex formation in the inner mitochondrial membrane. *J Biol Chem* 2002;**277**:43553–6.

Zhang M, Mileykovskaya E, Dowhan W. Cardiolipin is essential for organization of complexes III and IV into a supercomplex in intact yeast mitochondria. *J Biol Chem* 2005;**280**:29403–8.

Zhou J, Zhong Q, Li G *et al*. Loss of cardiolipin leads to longevity defects that are alleviated by alterations in stress response signaling. *J Biol Chem* 2009;**284**:18106–14.