INVENTORY OF SUPPLEMENTAL INFORMATION

Supplemental Information

Tam41 is a CDP-diacylglycerol synthase for cardiolipin biosynthesis in mitochondria

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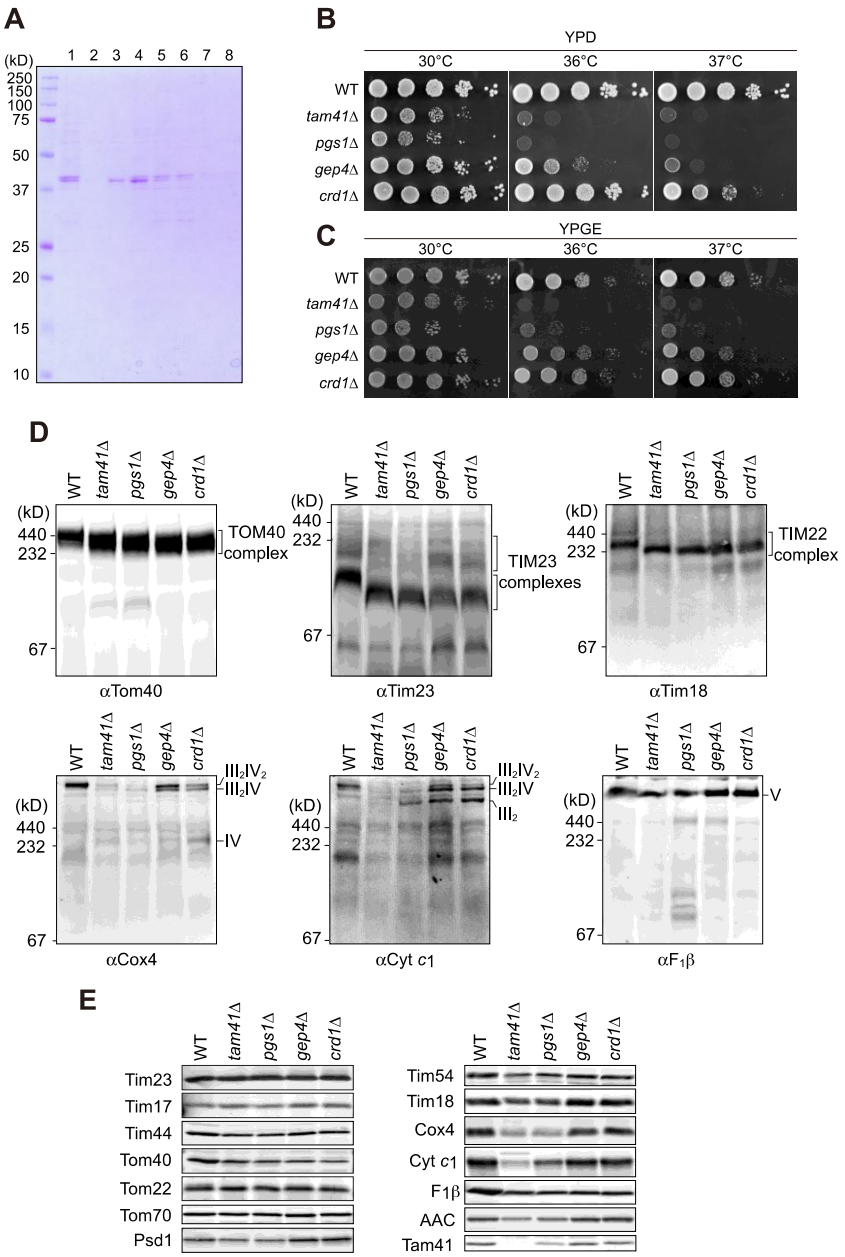
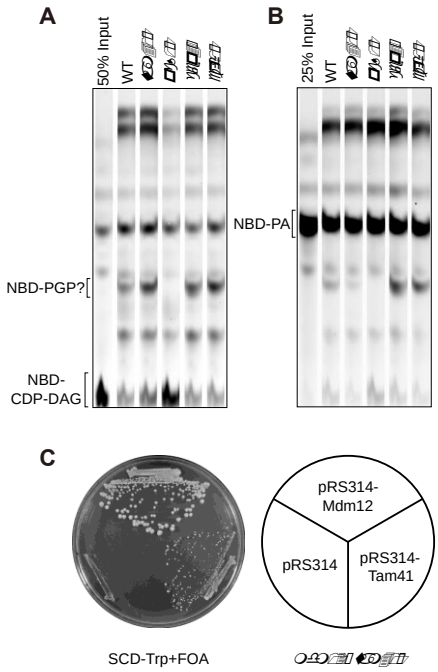


Tamura et al. Figure S1



Tamura et al. Figure S2

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Purification of Tam41 and phenotypes of CL-deficient mutants

(A) Eluted fraction containing Tam41-His6 from Ni-NTA chromatography followed by dialysis (lane 1) was subjected to ion exchange chromatography with SP-sepharose. A flow-through fraction (lane 2) and fractions eluted with buffer (20 mM Tris-HCl pH 7.5, 10 % glycerol, 0.2% Triton X-100, 2 mM β-mercaptoethanocontaining 100 mM NaCl (lanes 3 and 4), 200 mM NaCl (lanes 5 and 6) and 300 mM (lanes 7 and 8) were collected. Proteins in the fractions were analyzed by SDS-PAGE followed by CBB-staining. For the CDP-DAG synthase assay, the first eluted fraction (lane 3) was used (Figure 2). (B, C) Serial dilutions of the indicated cells were spotted onto YPD (B) and YPGE (C) and cultivated for 2 days and 4days, respectively. (D) Digitonin-solubilized mitochondria isolated from the indicated cells cultured in YPGalLac were subjected to BN-PAGE. Protein complexes were analyzed by immunoblotting with the indicated antibodies. (E) Mitochondria isolated from the indicated cells cultured in YPGalLac were analyzed by immunoblotting with the indicated antibodies.

Figure S2. Possible transport of CDP-DAG between the ER and mitochondria

(A, B) *In vitro* CDP-DAG import assay. Liposomes with NBD-CDP-DAG (A) or NBD-PA (B) were incubated with mitochondria isolated from the indicated cells for 50 min at 30°C. Phospholipids were extracted and analyzed by TLC. (C) *mdm12*Δ*tam41*Δ cells with the *URA3-CEN* plasmid harboring the *MDM12* gene were transformed with the *TRP1-CEN* plasmid harboring the *MDM12* (pRS314-Mdm12) or the *TAM41* gene (pRS314-Tam41), or with the empty vector (pRS314). The resulting cells were streaked on to a SCD-Trp+FOA plate and cultivated for 5 days.

Table S1. Yeast strains used in this study

SUPPLEMENTAL EXPEIMENTL PROCEDURES

Blue native PAGE

Blue native PAGE was performed as described previously (Wittig et al., 2006). Crude mitochondrial fractions (500 µg protein) isolated from yeast cells cultivated in YPGalLac (1% yeast extract, 2% polypeptone, 2% galactose, 2% lactic acid pH 5.6) were solubilized in 250 µl of 1% digitonin buffer (1% digitonin, 10 mM Tris-HCl pH 7.5, 50 mM KCl, 0.1 mM EDTA, 10% glycerol). After a clarifying spin (20000 x *g* for 15 min), Coomassie dye was added to the supernatant from 20x concentrated stock solution (4% CBB G-250, 100 mM 6-amminohexanoic acid). 20 µl of the sample was loaded to a 4%-13% blue native gel. The electrophoresis was performed with the voltage limited to 250 V and the current limited to 7 mA for 200 min in a cold room.

In vitro lipid import assay

In vitro assay for phospholipid import into isolated mitochondria was performed as described previously (Tamura et al., 2012). POPC (catalog number 850457), POPE (catalog number 850757) and NBD-PA (catalog number 810174) were purchased from Avanti Polar Lipids. To obtain NBD-CDP-DAG, NBD-PA and purified Tam41 were incubated in the presence of CTP for 1 h at 30°C and the resulting phospholipids were separated on a TLC plate. Bands of the TLC plate containing NBD-CDP-DAG, which are visible due to fluorescence of NBD was scraped off and subjected to lipid extraction. 100 µl of 3 mM lipid mixture (77:20:3% POPC/POPE/NBD-PA or 79.2:20.5:0.3% POPC/POPE/NBD- CDP-DAG) in chloroform was dried under nitrogen gas and vortexed in 400 µl of import buffer (300 mM sucrose, 10 mM Tris-HCl, pH 7.5, 150 mM KCl, 2 mM $MgCl_2$, 1 mM DTT) with 2 mM CTP or 4 mM glycerol-3-phosphate. The lipid solution was kept at room temperature for 1 h and extruded 20 times using an Avanti Mini-Extruder with 0.1 µm polycarbonate membranes to form unilamellar liposomes. Mitochondria (250 µg protein) were suspended in 50 µl of import buffer and incubated with 50 µl of

liposome suspension at 30°C. To stop reactions, 400 µl of ice-cold SEM buffer (250 mM sucrose, 10 mM MOPS-KOH pH 7.2, 1 mM EDTA) was added to the sample. Mitochondria were then precipitated by centrifugation at 12,000 x *g* for 10 min and washed with SEM buffer. Phospholipids were extracted from the mitochondria and analyzed by TLC.

SUPPLEMENTAL REFERENCES

Tamura, Y., Harada, Y., Yamano, K., Watanabe, K., Ishikawa, D., Ohshima, C., Nishikawa, S., Yamamoto, H., and Endo, T. (2006). Identification of Tam41 maintaining integrity of the TIM23 protein translocator complex in mitochondria. J. Cell Biol. *174*, 631-637.

Tamura, Y., Endo, T., Iijima, M., and Sesaki, H. (2009). Ups1p and Ups2p antagonistically regulate cardiolipin metabolism in mitochondria. J. Cell Biol. *185*, 1029-1045.

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Winston, F., Dollard, C., and Ricupero-Hovasse, S.L. (1995). Construction of a set of convenient *Saccharomyces cerevisiae* strains that are isogenic to S288C. Yeast (Chichester, England) *11*, 53-55.

Wittig, I., Braun, H.P., and Schagger, H. (2006). Blue native PAGE. Nat Protoc *1*, 418-428.

Table S1. Yeast strains used in this study

Name	Genotype
FY833 (WT)	*MAT* a *his3 leu2 lys2 trp1 ura3*
W303-1A (WT)	*MAT* a *ade2 his3 leu2 trp1 ura3 can1*
Cds1FLAG	*MAT* a *his3 leu2 lys2 trp1 ura3 CDS1-FLAG* ::*kanMX6*
Cds1FLAG↓	*MAT pCDS1* ::*kanR-tet07-TATA CDS1-FLAG* ::*HIS3 URA3* ::*CMV-tTA his3 leu2 met15*
*tam41*Δ	*MAT* a *ade2 his3 leu2 trp1 ura3 can1 tam41*Δ ::*CgHIS3*
*pgs1*Δ	*MAT* a *ade2 his3 leu2 trp1 ura3 can1 pgs1*Δ ::*hphMX*
*gep4*Δ	*MAT* a *ade2 his3 leu2 trp1 ura3 can1 gep4*Δ ::*URA3*
*crd1*Δ	*MAT* a *ade2 his3 leu2 trp1 ura3 can1 crd1*Δ ::*CgHIS3*
*psd1*Δ	*MAT* a *ade2 his3 leu2 trp1 ura3 can1 psd1*Δ ::*CgHIS3*
*itr1*Δ	*MATa ade2 his3 ura3 leu2 trp1 can1 itr1* ::*CgHIS3*
*tam41*Δ*itr1*Δ	*MATa ade2 his3 ura3 leu2 trp1 can1 tam41*Δ ::*CgHIS3 itr1* ::*CgTRP1*
*psd1*Δ*itr1*Δ	*MAT* a *ade2 his3 leu2 trp1 ura3 can1 psd1*Δ ::*CgHIS3 itr1*Δ ::*CgHIS3*
*crd1*Δ*itr1*Δ	*MAT* a *ade2 his3 leu2 trp1 ura3 can1 crd1*Δ ::*CgHIS3 itr1*Δ ::*CgHIS3*
*gep4*Δ	*MAT* a *his3 leu2 met15 ura3 gep4*Δ ::*kanMX4*
*gep4*Δ*itr1*Δ	*MAT* a *his3 leu2 met15 ura3 gep4*Δ ::*kanMX4 itr1*Δ ::*CgHIS3*
*ups1*Δ	*MAT* a *his3 leu2 lys2 trp1 ura3 ups1*Δ ::*kanMX4*
*ups1*Δ*itr1*Δ	*MAT* a *his3 leu2 lys2 trp1 ura3 ups1*Δ ::*kanMX4 itr1*Δ ::*CgHIS3*
*mdm12*Δ*tam41*Δ	*MAT* a *his3 leu2 lys2 trp1 ura3 mdm12*Δ ::*kanMX4 tam41*Δ ::*HIS3*
*tam41*Δ*pgs1*Δ	*MATa ade2 his3 ura3 leu2 trp1 can1 tam41*Δ*::CgHIS3 pgs1*Δ ::*hphMX*
Itr1HA	*MATa ade2 his3 ura3 leu2 trp1 can1 ITR1-3HA* ::*CgHIS3*
*Itr1HAtam41*Δ	*MATa ade2 his3 ura3 leu2 trp1 can1 ITR1-3HA* ::*CgHIS3 tam41*Δ*::CgTRP1*
Itr2HA	*MATa ade2 his3 ura3 leu2 trp1 can1 ITR2-3HA* ::*CgHIS3*
*Itr2HAtam41*Δ	*MATa ade2 his3 ura3 leu2 trp1 can1 ITR2-3HA* ::*CgHIS3 tam41*Δ*::CgTRP1*

Source	Parental strain
Winston et al., 1995 R. Rothstein (Columbia University, NY, NY)	
This study	FY833
Open Biosystems	R1158
Tamura et al., 2006	W303-1A
This study	W303-1A
This study	W303-1A
This study	W303-1A
This study	W303-1A
This study	W303-1A
This study	W303-1A
This study	W303-1A
This study	W303-1A
This study	W303-1A
Open Biosystems	BY4741
This study	BY4741
Tamura et al., 2009	FY833
This study	FY833
This study	FY833
This study	FY833
This study	W303-1A
This study	W303-1A
This study	W303-1A
This study	W303-1A