Tamura et al., http://www.jcb.org/cgi/content/full/jcb.200603087/DC1

Supplemental results

Tetrad analysis shows that Tam41 is not an essential yeast protein

Disruption of the *TAM41* gene was performed as follows. A DNA fragment containing *Candida glabrata HIS3* gene was amplified by PCR using pCgHIS3 (Kitada et al., 1995) as a template with primers 5'-GATTGAGCTAATAATTTGAAT-TAATAGGAGCTGCTTTTTACTTTGATATATCCT-GAAGTTGTTGTAAAACGACGGCCAGT-3' and 5'-TTGATTTACTAATACGGCTGAGCAAAAGTCTCT-GAGTATTCTTTAATGTGAACCCTTTTGCACAGGAAA-CAGCTATGACC-3'. The amplified DNA fragment, flanked by 40 base pairs of the sequences upstream and downstream of the *TAM41* gene, was introduced into the WT diploid strain W303-AB, and His^+ transformants were selected. Disruption of the *TAM41* gene on one of the two chromosomes was confirmed by PCR, and the resulting strain was named *TAM41/tam41*Δ. When we subjected *TAM41/tam41*Δ to tetrad analysis, all of the four spores grew normally on YPD at 23°C (Fig. S1 A), and the haploid *tam41* disruption mutant (*tam41*Δ) was obtained.

Temperature-sensitive growth of the *tam41*Δ strain

Growth of the strain (*tam41*Δ) with chromosomal *TAM41* deletion was tested on both fermentable (SCD) and nonfermentable (SCLac) media at various temperatures. *tam41*Δ cells showed slow growth at elevated temperature (37°C) as compared with that at 23°C, and the temperature-sensitive growth was more prominent on SCLac than on SCD (Fig. S1 B).

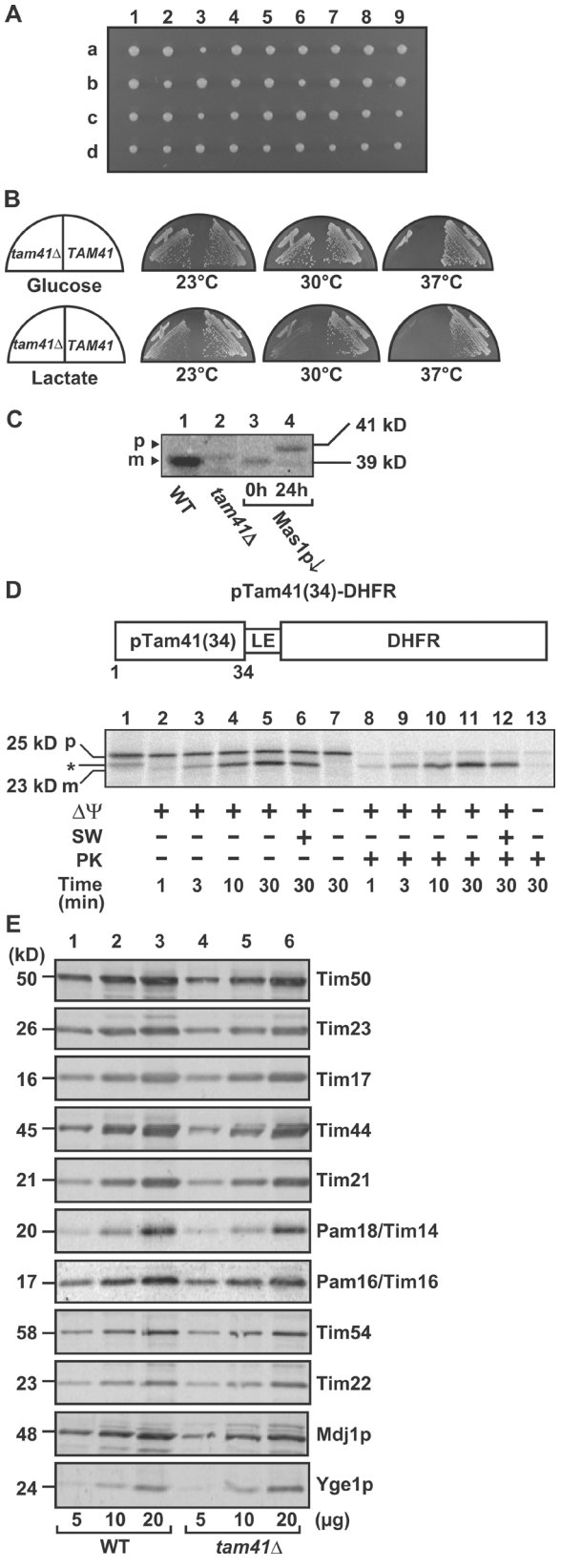

Figure S1. **Additional characterization of Tam41.** (A) One of the two chromosomal *TAM41* genes in a yeast diploid strain, W303, was disrupted, the diploid cells were sporulated, and nine different asci were dissected. The four spores recovered from each ascus were allowed to germinate and to grow on YPD for 45 h at 23°C. (B) The yeast *tam41* disruption mutant strain carrying pRS316-Tam41 (*TAM41*) or pRS316 (*tam41*Δ) was grown on SCD(-Ura) (Glucose) and SCLac(-Ura) (Lactate) at 23, 30, and 37°C. (C) Yeast G10 strain, in which the *MAS1* gene for MPP is under the control of *GAL10* promoter (Mas1p⊠; Geli et al., 1990), was cultivated in YPGal medium at 30°C (lane 3) or YPD medium at 30°C for 24 h to deplete MPP (lane 4), and total cell extracts were prepared. Total cell extracts were also prepared for W303-1A (WT) and *tam41*Δ strains cultivated in YPD medium at 23°C (lanes 1 and 2). Proteins in the extracts were analyzed by SDS-PAGE and immunoblotting with anti-Tam41 antibodies. p, precursor form; m, mature form. (D) The radiolabeled fusion protein pTam41(34)-DHFR (as schematically shown in the top panel) were incubated with isolated yeast mitochondria (D273-10B) at 30°C for indicated times with or without ΔΨ. The mitochondria were then subjected to osmotic swelling (SW) and further treated with or without 100 μg/ml PK for 30 min on ice. The mitochondria or mitoplasts were reisolated by centrifugation, and proteins were analyzed by SDS-PAGE and radioimaging. Asterisk indicates the form possibly starting from the second Met (residue 22) of the fusion protein. (E) Mitochondria were isolated from WT and *tam41*Δ cells grown at 23°C in YPD medium. Indicated amounts of mitochondrial proteins were separated by SDS-PAGE and analyzed by immunoblotting with antibodies against the indicated proteins.

In vivo conversion of the 41-kD precursor form of Tam41 to the 39-kD mature form

To confirm that the 41-kD precursor form of Tam41 is converted to the 39-kD mature from in vivo, we analyzed the apparent molecular mass of endogenous Tam41 by immunoblotting with anti-Tam41 antibodies before and after depletion of Mas1p, a subunit of matrix processing peptidase (MPP; Fig. S1 C). Endogenous Tam41 migrated as a 39-kD protein (Fig. S1 C, lane 1), whereas it exhibited a 41-kD form after inhibiting the presequence cleavage by depletion of Mas1p (Fig. S1 C, lane 4).

The N-terminal 34 residues of the Tam41 precursor functions as a mitochondrial targeting signal

The N-terminal possible presequence of the Tam41 precursor was tested for its ability to direct a nonmitochondrial protein to mitochondria in vitro. For this purpose, a fusion protein (pTam41[34]-DHFR) consisting of residues 1–34 of the Tam41 precursor and mouse DHFR was synthesized with reticulocyte lysate and was incubated with isolated yeast mitochondria. pTam41(34)-DHFR ("p") was converted to the smaller form ("m"), which was resistant to proteinase K treatment, in a $\Delta\Psi$-dependent manner (Fig. S1 D), indicating that residues 1–34 of the Tam41 precursor can function as a mitochondrial targeting signal.

The gene for pTam41(34)-DHFR was constructed as follows. A DNA fragment for the N-terminal 34 residues of Tam41 was amplified from pRS316-Tam41 by PCR using primers 5'-CCGGATCCATAATTTGAATTAATAGGAGCTGCTTT-3' and 5'-GGCTCGAGCCCCTCCTTTATTTGAGTACTCAGAAG-3'. The amplified DNA was digested with BamHI and XhoI and introduced into pUC119/DHFR (Yamano et al., 2005) to give pUC119/pTam41(34)-DHFR. The BamHI–PstI fragment containing the gene for pTam41(34)-DHFR was subcloned into pGEM-4z.

The steady-state levels of translocator proteins and MMC proteins did not change by the Tam41 depletion

The steady-state levels of translocator proteins and MMC proteins were examined for WT and *tam41*Δ mitochondria by immunoblotting with antibodies against known components of the mitochondrial translocators and MMC proteins (Fig. S1 E).

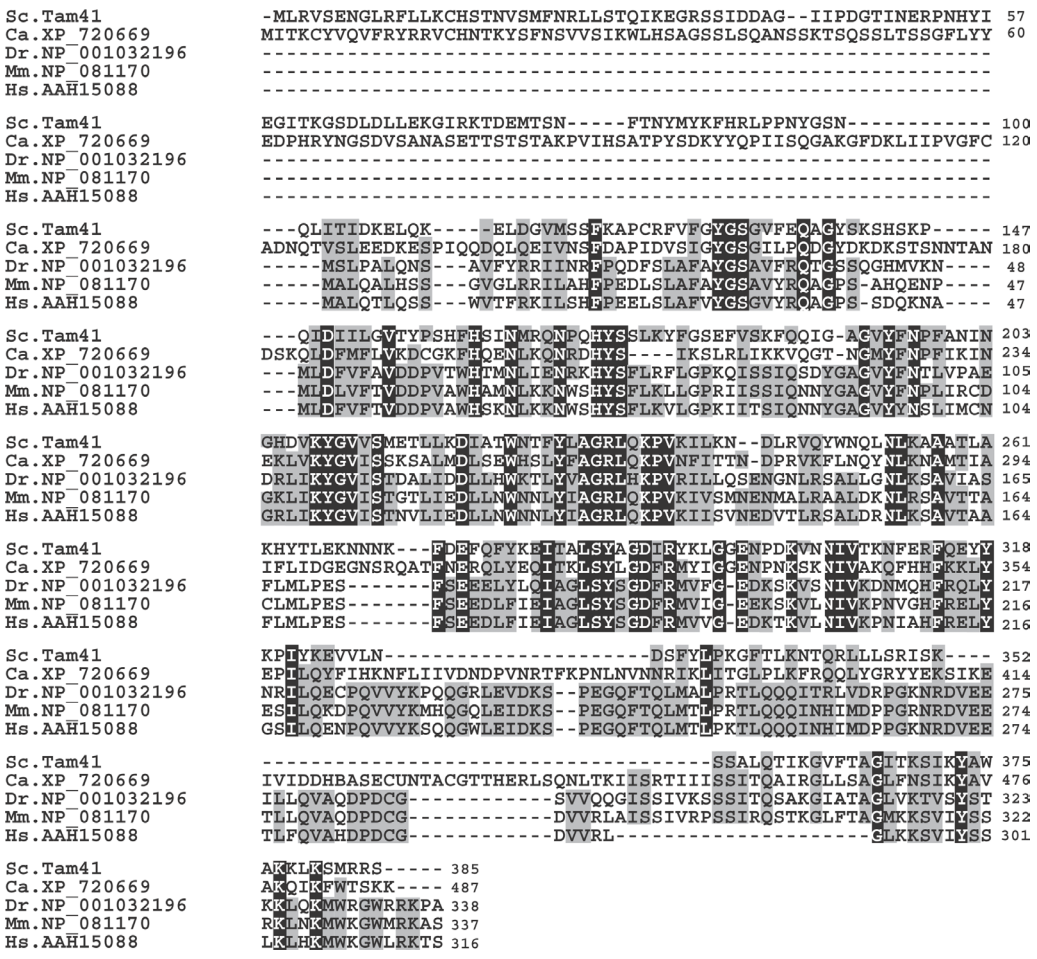

Figure S2. **Tam41 from various organisms.** Amino acid sequence alignments of Tam41 homologues. Identical residues are shown in black and similar residues in gray. Sc, *Saccharomyces cerevisiae*; Ca, *Candida albicans*; Dr, *Danio rerio*; Mm, *Mus musculus*; Hs, *Homo sapiens*.

Table S1. **Yeast strains**

Strain	Genotype	Source
D273-10B	*MATα*	
W303	*MAT***a***/MATα ade2/ ade2 his3/ his3 ura3/ ura3 leu2/ leu2 trp1/ trp1 can1/ can1*	R. Rothstein (Columbia University, New York, NY)
W303-1A	*MAT***a** *ade2 his3 ura3 leu2 trp1 can1*	R. Rothstein
G10	*MAT***a** *ade2 his4 ura3 leu2 trp1 mas1::URA3 GAL-MAS1*	Geli et al., 1990
YTY4100	*MAT***a***/MATα ade2/ ade2 his3/ his3 ura3/ ura3 leu2/ leu2 trp1/ trp1 can1/ can1 TAM41/ tam41::CgHIS3*	This study
YTY4101	*MAT***a***/MATα ade2/ ade2 his3/ his3 ura3/ ura3 leu2/ leu2 trp1/ trp1 can1/ can1 TAM41/ tam41::CgHIS3* [pRS316 (*URA3*)]	This study
YTY4102	*MAT***a***/MATα ade2/ ade2 his3/ his3 ura3/ ura3 leu2/ leu2 trp1/ trp1 can1/ can1 TAM41/ tam41::CgHIS3* [pRS316-Tam41 (*URA3*)]	This study
YTY4110	*MAT***a** *ade2 his3 ura3 leu2 trp1 can1 TIM23-FLAG::CgHIS3*	This study
YTY4111	*MAT***a** *ade2 his3 ura3 leu2 trp1 can1 TIM23-FLAG::CgHIS3 tam41::CgTRP1*	This study
YTY4112	*MAT***a** *ade2 his3 ura3 leu2 trp1 can1 TIM17-FLAG::CgHIS3*	This study
YTY4113	*MAT***a** *ade2 his3 ura3 leu2 trp1 can1 TIM17-FLAG::CgHIS3 tam41::CgTRP1*	This study
YTY4114	*MAT***a** *ade2 his3 ura3 leu2 trp1 can1 TIM50-FLAG::CgHIS3*	This study
YTY4115	*MAT***a** *ade2 his3 ura3 leu2 trp1 can1 TIM50-FLAG::CgHIS3 tam41::CgTRP1*	This study
YTY4116	*MAT***a** *ade2 his3 ura3 leu2 trp1 can1 TIM21-FLAG::CgHIS3*	This study
YTY4117	*MAT***a** *ade2 his3 ura3 leu2 trp1 can1 TIM21-FLAG::CgHIS3 tam41::CgTRP1*	This study
YTY4118	*MAT***a** *ade2 his3 ura3 leu2 trp1 can1 PAM16-FLAG::CgHIS3*	This study
YTY4119	*MAT***a** *ade2 his3 ura3 leu2 trp1 can1 PAM16-FLAG::CgHIS3 tam41::CgTRP1*	This study
YTY4120	*MAT***a** *ade2 his3 ura3 leu2 trp1 can1 PAM18 -FLAG::CgHIS3*	This study

The results show that the levels of Tim50, Tim23, Tim17, Tim44, Tim21, Pam18, Pam16, Tim54, Tim22, Mdj1p, and Yge1p are similar between wild-type and *tam41*Δ mitochondria.

Possible homologues of Tam41

The BLAST searches allowed us to find homologues of Tam41 in various organisms. Their amino acid sequence alignments are shown in Fig. S2. Although only Tam41 proteins from *Saccharomyces cerevisiae* and *Candida albicans* have N-terminal extensions, which probably function as mitochondrial targeting presequences, the other Tam41 homologues are also predicted to be localized to mitochondria (MITOP; http://ihg.gsf.de/ihg/mitoprot.html).

References

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