

Results

Possible homologues of Tom13 and Tom38

The BLAST searches allowed us to find homologues of Tom13 and Tom38 only in closely related organisms. Their amino acid sequence alignments are shown in Fig. S1. In addition to the two Tom38 homologues listed in Fig. S1, *Candida glabrata* CAG59822 and *Debaromyces hansenii* CAG89850 show weak homology to Tom38. Interestingly, residues 48–68 of Tom13 are conserved well among these Tom13 homologues.

Effects of Tom13 depletion on the 450-kD TOM40 complex

Mitochondria were isolated from wild-type cells (Fig. S2, WT) and GAL-TOM13 cells (Fig. S2, Tom13 $\downarrow\downarrow$) 18 h after shift from galactose-containing medium to galactose-free medium and were analyzed by BN-PAGE and immunoblotting with anti-Tom40 antibodies (Fig. S2 A). The amount of the 450-kD TOM40 complex was decreased significantly in Tom13 $\downarrow\downarrow$ mitochondria 18 h after the shift (Fig. S2 A, lanes 3 and 4) as compared with that in wild-type mitochondria (Fig. S2 A, lanes 1 and 2), while the total amount of Tom40 in Tom13 $\downarrow\downarrow$ mitochondria was similar to that in wild-type mitochondria. This result suggests that depletion of Tom13 affects primarily the assembly of Tom40 into the TOM40 complex, but not the stability of Tom40 itself. The amount of the TOM40 complex in Tom13 \downarrow mitochondria, which were prepared 14 h after shift to galactose-free medium, was similar to that in wild-type mitochondria (unpublished data).

Interactions between Tom38 and Sam50 as detected by coimmunoprecipitation

Yeast mitochondria were solubilized with 1% digitonin and subjected to immunoprecipitation with antibodies against Sam50 (in the SAM complex) or Tom22 (in the TOM40 complex). The immunoprecipitates were analyzed for Sam50, Tom38, Tom22, and Tom40 (Fig. S2 B). Tom38 was coprecipitated with anti-Sam50 antibodies, but not with anti-Tom22 antibodies. As a control, Tom40 was coprecipitated with anti-Tom22 antibodies, but not with anti-Sam50 antibodies. This confirms that Tom38 is a subunit of the SAM complex, which contains Sam50 and Mas37.

Materials and methods

Yeast strains and growth conditions

Wild-type *Saccharomyces cerevisiae* strains used in this study are W303-1A (MAT α ade2-1 his3-11,15 ura3-1 leu2-3,112 trp1-1 can1-100) and W303-AB (MAT α/α ade2-1/ade2-1 his3-11,15/his3-11,15 ura3-1/ura3-1 leu2-3,112/leu2-3,112 trp1-1/trp1-1 can1-100/can1-100).

TOM13-HA, TOM13-FLAG, TOM38-FLAG, and SAM50-FLAG, yeast strains expressing the COOH-terminally HA-tagged Tom13 (Tom13-HA), the FLAG-tagged Tom13 (Tom13-FLAG), the FLAG-tagged Tom38 (Tom38-FLAG), and the FLAG-tagged Sam50 (Sam50-FLAG), respectively, instead of corresponding wild-type proteins, were constructed as follows. DNA fragments encoding the triple-HA epitope (for Tom13-HA) or the triple-FLAG epitope (for Tom13-FLAG, Tom38-FLAG, and Sam50-FLAG) were amplified from plasmid pTYE249, pTYE247, pTYE280, and pTYE247 (Yoshihisa et al., 2003) by PCR using primers Tom13-HA-F (5'-GCA GCA TTG AAA GAG ATT TCA AGC CCT GGC ACC CGT GGG AGG GTT GCG TCC AAG TTC CTT GGC GAA TTG GGT ACC CAA TC-3') and Tom13-HA-R (5'-TCT ACC GTA TGT GTG TGT GTA TTT ATT TAT GTA GGT TGC TAA TGC TTT GGT GAT CGT TTA CAG GAA ACA GCT ATG AC-3'), primers Tom13-FLAG-F (5'-GCA GCA TTG AAA GAG ATT TCA AGC CCT GGC ACC CGT GGG AGG GTT GCG TCC AAG TTC CTT GGC GAA TTG GGT ACC CAA TC-3') and Tom13-FLAG-R (5'-TCT ACC GTA TGT GTG TGT GTA TTT ATT TAT GTA GGT TGC TAA TGC TTT GGT GAT CGT TTA CAG GAA ACA GCT ATG AC-3'), primers Tom38-FLAG-F (5'-GGT CCA GTT TGC ACA AGA CAC CCT GAA GAA CTT CGT TCA GGG CGA ATT GGG TAC CGG G-3') and Tom38-FLAG-R (5'-TCA TCT ATA TCG TTT ACA TAC TAC AGC TTG AAA GTG ATT ACA GGA AAC AGC TAT GAC-3'), and primers Sam50-FLAG-F (5'-GCT CAC GAA AAT GAT TTG ATA AGA AAA GGA TTC CAG TTT GGT CTT GGT CTG GCA TTT TTA GGC GAA TTG GGT ACC GGG-3') and Sam50-FLAG-R (5'-AGA ATA TGT TGC TAT ATA ACA TAT GAA AAA TAT TGA ATG GGA AGC TAG GCG ATA GCT TCA CAG GAA ACA GCT ATG AC-3'), respectively. The amplified DNA fragments were integrated into the 3'-end of the corresponding wild-type genes of the haploid strain W303-1A.

GAL-TOM13 and GAL-TOM38, yeast strains expressing Tom13 and Tom38 under the control of the *GAL7* promoter, respectively, were constructed as follows. DNA fragments containing the *GAL7* promoter were amplified from plasmid pCgHIS3-GAL7 (Yamamoto et al., 2002) by PCR using primers Tom13-off-F (5'-CAT CAC TGT AAT ATT AGA AAC

A

Sc.Tom13		MTEVVG-		
Eg.NP_982554	MVRCAIDGYSCWSSRGCTRCAAGWQTSEIWHSLAPGDIITTYVATWPNNWSRGGIRRCHRRSRGGRRQQRLTSAQHGWPVSKSYCEQPNMAEBIILGNIAEEA	-MAEEVFNDIPEEL	100	
K1.CAH02448			13	
Cg.CAG58951			0	
Mg.EAA52191			0	
Gz.XP_386039	MKVNLKTIVFVFDSESQRQAACSSPPAKHKAQAVGGGLQHPTFLAVTQVIIHPPPSYPRFCR	-MSQEYINPSTLNS	60	
Ca.EAK98533			13	
Nc.XP_326594			0	
Dh.CAG89329			12	
Y1.CAG83104			13	
Sp.NP_595347			11	
Um.XP_401673	MQPPSLDDTQEHTNISSGLTDNETLQIDSRTVLAKPRLQPIEKPIASSSLRSAEASPGSTPSEAQKQHP	-MEKNTVTP--KT	70	
Sc.Tom13	-FWESVSDDDESED-	-KDCMEVQNTVS-	LVSFVG-	SCSIN
Eg.NP_982554	HAALLASAGDEATKXSGAIRLPIEDSLDG-	-DFGSRGTAATTDLSTTS-	AGTSQQLVERTGGISFGRLLSVAG	SCSIN
K1.CAH02448	HATILSRGDLSLVVMNSHDLEQDTDDLREENRQVLTNLDATGVSVRSRSPSTTS-	-TTTLSQLSSEKNGVSGFKILSFAG	SCSIN	
Cg.CAG58951	-MIREN-	-SVLSTSTSSS-	DLRELEEVEASDVRGVWRVWLRYLV	SSSIN
Mg.EAA52191	-MASDETSSHLLAEHSVITIRSDSEHYS-	-AGEENTTSPASSGSGPVLYQPPFTVVSLLVR-	SAAIN	
Gz.XP_386039	LTWIKTTTPKISASYPPPLLERTPRLESTMADEHNLHABEGVTMHSDIELYS-	-AGDDLS-SPPSNNSP-VVLYKPVFTWVSLMR-	GTAIN	
Ca.EAK98533	SNSLLIDELESNDLUVLVEQLSPDEVILN--ADIINT-ERLIEESRS	-LEEQPS--YVIDIFGILK	KAAIN	
Nc.XP_326594	-MSAEEISNPLAESGVTISSDSEQYS-	-APESASPQSPSSSSPAVVLQYQPPFTVWSLFR-	SAVIN	
Dh.CAG89329	GIKLODNNDIETEKYVEETSEFLNEALLNNQNNEEINNTTGNQLSSEDVSYA-	-DAINASMFPLQEEKEESVYNINLWSILR-	KSSIN	
Y1.CAG83104	QDIPLEVVEESDEQILTTEEGERQYAPGPRITFS	-MPAIGRFLG	RCCIN	
Sp.NP_595347	LFSQVIHIFKY-	AA	-IN	
Um.XP_401673	LANSIISVLDLPIIIPRHKDRRSSSEQDIDEKEGLAPTELQDAQDSDAFDSDSDEPEQGQQPRPSYPPTGSLTAAFPNPYSSIPLSRRMALISGSIFIN	**	170	
Sc.Tom13	LLLPFLNGMMLGFGELFAHELCWR--	FNWPNH-RNKGYK--VYPESEKRKIAALKEISSP--	GTRGR-	VAS-KFL
Eg.NP_982554	LLLPFLNGLMLGFGELLAHELWKR-	FSWFDKERNRGYR-IYPEVRAAELQERERQ-	RALSR-	AAGPDGFL
K1.CAH02448	LVLVFLINGLMLGFGELVAHELWN--	WNWPNR-KNTGYR-IFPVSRRKFYEDITEHPSNSGKTVND-	TESNDKFL	165
Cg.CAG58951	LVLVFLINGLMLGFGELVAHELWQ-	-YKWFYHGRDSAHK--IFPVSRRKLQAPAQTHEQDQEHIISKAQHGAYRLRERWTSMKSFKL-	125	
Mg.EAA52191	LLLPFLINGMMMGYGELFAHEIAFR-	LGWSGTKVPPMTR--RTRHHSVPGPVBEVID-RPRPLSTSDD--	LTSLE	126
Gz.XP_386039	LLLPFLINGMMLGFGELFAHEAAFR-	LGWGTTKVFPPLSR--RRAHIPGPGIELRENYCTPRPSLDD--	IASLE	212
Ca.EAK98533	LVLVFLINGMMLGFGELFAHEIGFR-	YNWIGARVEPPRR--MVQKKNESASKYL-		129
Nc.XP_326594	LFLPFVNGMMLGFGELFAHEAAFR-	LGWSNTKVFPVSR--RDARPIGPGVVE-RPRRRVDLDDHLDLTSLE-		130
Dh.CAG89329	LFLPFVNGMMLGFGELFAHEIGFR-	YNWVGAKYVEPPRR--IARKNQNQSAFL-		147
Y1.CAG83104	LVLVFLINGMMMGYGELVAHEIGFA-	WGMMSGARVSIMNR--LMRRM-		100
Sp.NP_595347	LGLPFVNGMLGFGELFAHAFIH--	SLGWAP--GHTRIYS--IQRHQ-		71
Um.XP_401673	LGLPFVNGMLGFGFEIFARVVVAPALGITGVGWAGETARAVANWSNWGKTRSSSTQGEKRWSGDLAQQRQPGDFDDWSATNDGVASGPRIEV	*	261	

B

Figure S1. Tom13 and Tom38 from various organisms. Amino acid sequence alignments of Tom13 homologues (A) and of Tom38 homologues (B). The alignments were made by ClustalW with classification of amino acid types: red (A, V, F, P, M, I, L, and W), blue (D and E), pink (R and K), and green (S, T, Y, H, C, N, G, and Q) (<http://www.ebi.ac.uk/clustalw/#>). Asterisk, identical residues; colon, conserved residues within the same amino-acid types (see the above color classification); dot, semi-conserved residues. Sc, *Saccharomyces cerevisiae*; Kl, *Kluyveromyces lactis*; Eg, *Eremothecium gossypii*; Cg, *Candida glabrata*; Mg, *Magnaporthe grisea*; Gz, *Gibberella zeae*; Ca, *Candida albicans*; Nc, *Neurospora crassa*; Dh, *Debaryomyces hansenii*; Yl, *Yarrowia lipolytica*; Sp, *Schizosaccharomyces pombe*; Um, *Ustilago maydis*.

ATC ACC CCC CTT CTT ACG AAA CTG CCA CAA GAC AGA AAT AAG CTT GGG TCT TCT GGA GC-3') and Tom13-off-R (5'-GTC TTT GTC TTC TGA TTC GTC ATC TGA CAC GCT CTC CCA GAA TCC CAC AAC CTC TGT CAT TTT TGA GGG AAT ATT CAA CT-3') and primers Tom38-off-F (5'-TGG TGT GAA GGC CTC TTG TAT GTC GTC TCA TAT AGG GCT AAA ACG AAG CCA TCG TGC ACA AAG CTT GGG TCT TCT GGA GC-3') and Tom38-off-R (5'-TAA CGG AAA TGT GTC AAA TAT CCG TTT TAC TGG CAT GGG AAC ACT GAA TGA

ACT TAC CAT TTT TGA GGG AAT ATT CAA CT-3'), respectively. The amplified DNA fragments were integrated into the chromosome upstream of the corresponding gene of the wild-type haploid strain W303-1A.

TOM13/ Δ *tom13* and *TOM38*/ Δ *tom38*, yeast diploid strains carrying a complete null mutation in the chromosomal copy of the *TOM13* gene and the *TOM38* gene, respectively, were constructed as follows. DNA fragments containing the *Candida glabrata* *HIS3* marker were amplified from plasmid pCgHIS3 (Kitada et al., 1995) by PCR using primers Tom13-delta-F (5'-CAT CAC TGT AAT ATT AGA AAC ATC ACC CCC CTT CTT ACG AAA CTG CCA CAA GAC AGA AAT GTT GTA AAA CGA CGG CCA GT-3') and Tom13-delta-R (5'-TAA TCT ACC GTA TGT GTG TGT GTA TTT ATT TAT GTA GGT TGC TAA TGC TTT GGT GAT CGT CAC AGG AAA CAG CTA TGA CC-3') and primers Tom38-delta-F (5'-GTA AGT TCA TTC AGT GTT CCC ATG CCA GTA AAA CGG ATA TGT TGT AAA ACG ACG GCC AGT-3') and Tom38-delta-R (5'-GGG TGT CTT GTG CAA ACT GGA CCA ATT CTT TAC ATT CGT TCA CAG GAA ACA GCT ATG ACC-3'), respectively. The amplified DNA fragments, flanked by 60 base pairs of the sequences upstream and downstream of the *TOM13* or *TOM38* gene, were integrated into the chromosome of the wild-type diploid strain W303-AB.

Yeast strains were grown in YPD (1% yeast extract, 2% polypeptone, and 2% glucose), lactate (+0.1% glucose) medium, and lactate (+0.1% galactose) medium (Daum et al., 1982; with minor modifications).

References

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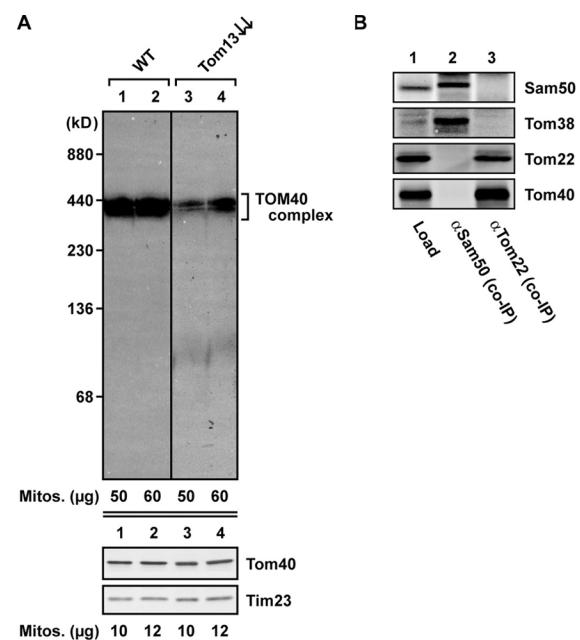


Figure S2. Roles of Tom13 in the TOM40 complex assembly in vivo and Tom38-Sam50 interactions detected by coimmunoprecipitation. (A) Effects of Tom13 depletion on the 450-kD TOM40 complex. Mitochondria isolated from yeast strains W303-1A (WT) and GAL-TOM13 (Tom13 $\downarrow\downarrow$) after cultivation in lactate medium (+0.1% glucose) for 18 h at 23°C. Mitochondria were solubilized as in Fig. 4 A and subjected to BN-PAGE analyses followed by immunoblotting with anti-Tom40 antibodies (top). The position of the 450-kD TOM40 complex is indicated. 50 µg (lanes 1 and 3) and 60 µg (lanes 2 and 4) of mitochondrial proteins (Mitos.) were loaded. The amounts of Tom40 and Tim23 in 10 and 12 µg of mitochondrial proteins (Mitos.) were analyzed by SDS-PAGE and immunoblotting with anti-Tom40 and anti-Tim23 antibodies (bottom). (B) Mitochondria were solubilized as in Fig. 4 A and subjected to immunoprecipitation with anti-Sam50 (α Sam50) or anti-Tom22 (α Tom22) antibodies. Immunoprecipitates (lanes 2 and 3) as well as solubilized proteins before immunoprecipitation (lane 1) were analyzed by SDS-PAGE and immunoblotting with anti-Sam50, anti-Tom38, anti-Tom22, and anti-Tom40 antibodies. The amounts of proteins in lanes 2 and 3 are 20-fold excess over that in lane 1.