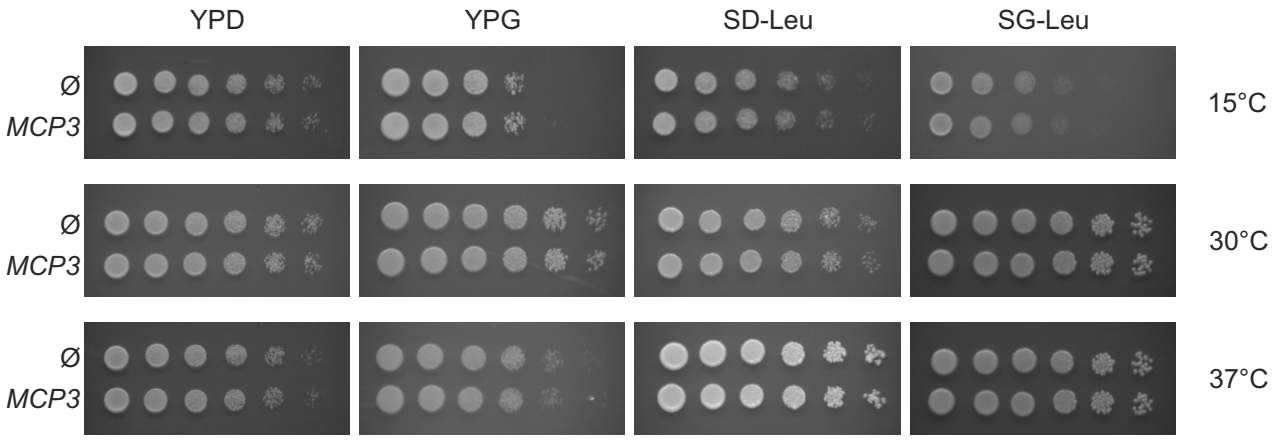
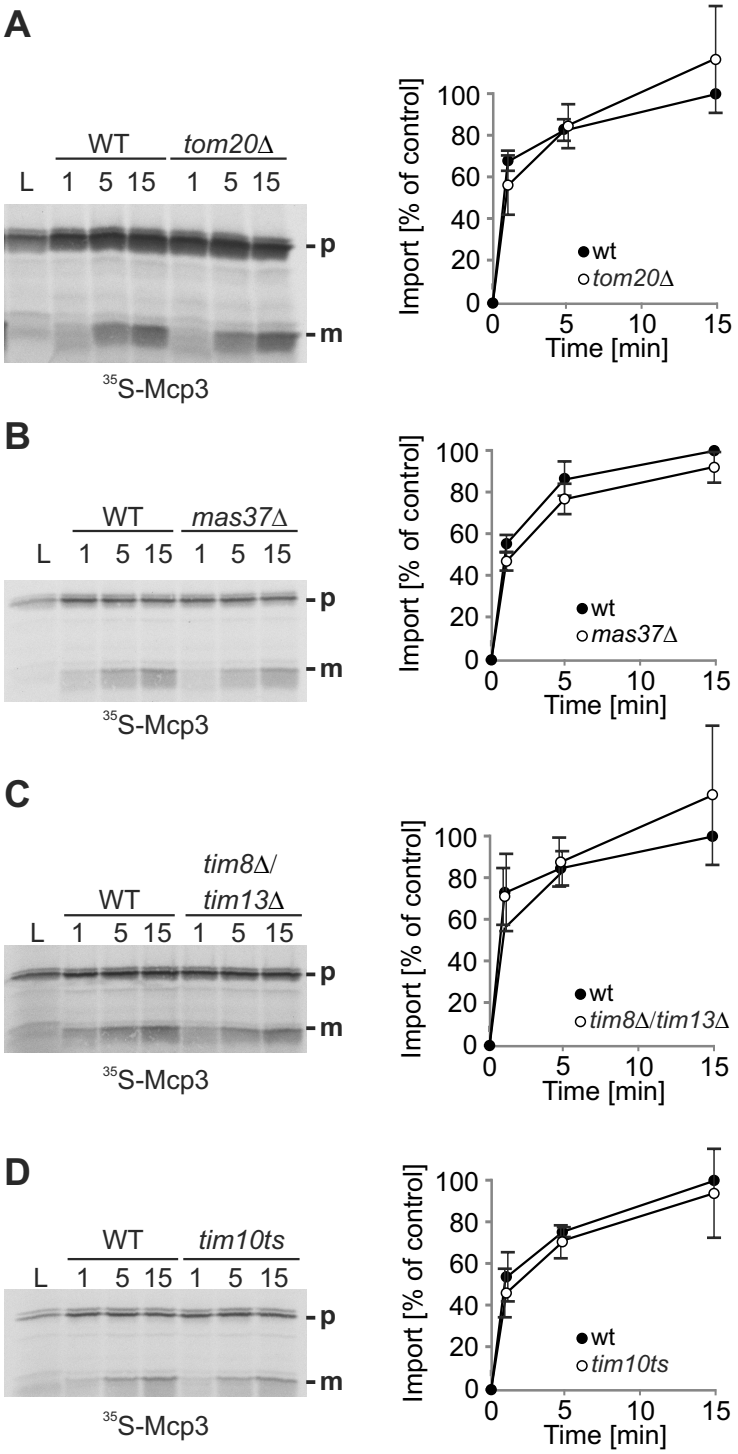
**Sinzel et al. Appendix**

Table of Contents




**Appendix Figure S1. Over-expression of Mcp3 has no influence on the growth of yeast cells.** Wild-type cells were transformed with the empty plasmid pYX142 (Ø) or pYX142 encoding Mcp3 (*MCP3*). Cells were grown to an $OD_{600}$ of 1.0 in minimal medium lacking leucine and spotted in a 1:5 dilution series on YPD, YPG, SD-Leu or SG-Leu plates directly and in a 1:5 dilution series. Plates were incubated at the indicated temperatures.




**Appendix Figure S2. Import of Mcp3 is independent of receptor Tom20, TOB subunit Mas37/Sam37 and the IMS import chaperones Tim8/10/13.**

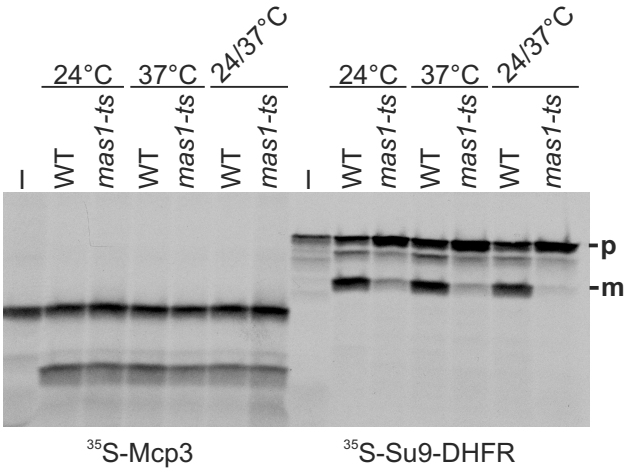
**(A)** Mitochondria from wild-type and *tom20*Δ cells were isolated and incubated with radiolabelled Mcp3 for the indicated time periods (1, 5, 15 min). After import mitochondria were reisolated and analysed by SDS-PAGE and autoradiography. Bands corresponding to the mature (m) form were quantified. Import after 15 min into wild-type mitochondria was set to 100%. The mean with standard deviations of three independent experiments (n=3) is depicted in the graph.

**(B)** Mitochondria from wild-type and mas37Δ cells were isolated. Import and analysis was performed as in (A).

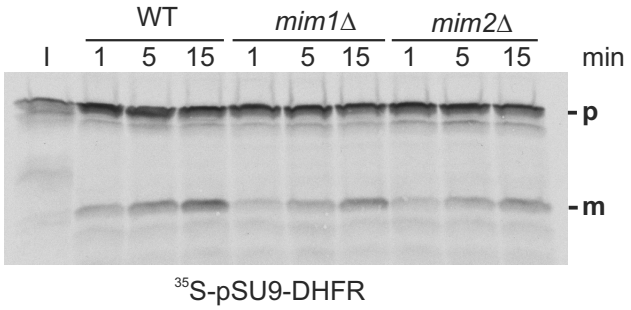
**(C)** Mitochondria from wild-type and *tim8*Δ/*tim13*Δ cells were isolated. Import and analysis was performed as in (A).

**(D)** Mitochondria from wild-type and the temperature sensitive mutant *tim10-1* (*tim10ts*) were isolated after growth at 24°C. Prior to import mitochondria were incubated for 15 min at the non-permissive temperature. Import and analysis were performed as in (A).

l, 20% of radiolabelled precursor protein used in each import reaction; p, precursor; m, mature form.




**Appendix Figure S3. MPP is not responsible for processing of Mcp3.** Mitochondria from wild-type (WT) and *mas1-ts* cells were isolated after growth at the permissive temperature 24°C (24°C, 24/37°C) or the non-permissive temperature 37°C (37°C). Next mitochondria were incubated with radiolabelled Mcp3 ($^{35}$S-Mcp3, left part) without preincubation at the non-permissive temperature (24°C, 37°C) or with in vitro shift for 15 min to 37°C prior to the import reaction (24/37°C). Radiolabelled Su9-DHFR as bona fide substrate of MPP was applied in the same way as control for impaired MPP function in mitochondria of the mutant strain (right part). After import mitochondria were reisolated and analysed by SDS-PAGE and autoradiography. I, 20% of radiolabelled precursor protein used in each import reaction; p, precursor; m, mature form.




**Appendix Figure S4. Mitochondria lacking Mim1 or Mim2 display an import defect for matrix destined proteins.** Mitochondria from wild-type (WT), *mim1*Δ and *mim2*Δ cells were isolated and incubated with radiolabelled $^{35}$S-pSu9-DHFR for the indicated time periods (1, 5, 15 min). After import mitochondria were reisolated and analysed by SDS-PAGE and autoradiography. Bands corresponding to the mature (m) and precursor (p) form are indicated.

Appendix Table 1: *S. cerevisiae* and *E. coli* strains used in this study

| name | genotype | reference |
|---|---|---|
| *S. cerevisiae* | | |
| W303a | MAT a; *ade2-1; can1-100; his3-11; leu2-3,112; trp1*Δ*2; ura3-52* | (Thomas and Rothstein, 1989) |
| W303α | MAT α; *ade2-1; can1-100; his3-11; leu2-3,112; trp1*Δ*2; ura3-52* | (Thomas and Rothstein, 1989) |
| YPH499 | MAT a; *ade2-101; his3*Δ*200; leu2*Δ*1; ura3-52; trp1*Δ*63; lys2-801* | (Sikorski and Hieter, 1989) |
| BY4741 | Mat a; *his3*Δ1; *leu2*Δ0; *met15*Δ0; *ura3*Δ0 | Euroscarf (http://web.uni-frankfurt.de/fb15/mikro/euroscarf/ |
| YMS018 | W303a; *fun14*Δ::Kan | this study |
| YMS019 | W303a; *fun14*Δ::His3 | this study |
| YKD291 | W303a; *mdm10*Δ::His3 | (Tan *et al.*, 2013) |
| YKD461 | W303a; *mmm1*Δ::Kan | (Tan *et al.*, 2013) |
| YKD227 | W303a; *mmm2*Δ::Kan | (Tan *et al.*, 2013) |
| YKD301 | W303a; *mdm12*Δ::His3 | (Tan *et al.*, 2013) |
| YKD303 | W303a; *mim2*Δ::His3 | (Dimmer *et al.*, 2012) |
| YKD145 | W303a; *mim1*Δ::Kan | (Dimmer *et al.*, 2012) |
| YKD132 | W303α; *tom20*Δ::His3 | (Muller et al., 2011) |
| YDR251 | YPH499; *mas37*Δ::His3 | (Habib et al., 2005) |
| 2535 | YPH499; *tom40*Δ::Kan; pFL39-*TOM40* | (Wenz *et al.*, 2014) |
| 3007; tom40-2522 | YPH499; *tom40*Δ::Kan; pFL39-*TOM40*-25 | (Wenz *et al.*, 2014) |
| pTIM23t | YPH499; *tim23*Δ::Kan; pRS315-p*TIM23*t | (Gevorkyan-Airapetov et al., 2009) |
| pTIM23-Y70A,L71A-t | YPH499; *tim23*Δ::Kan; pRS315-p*TIM23*-Y70AL71A-t | (Gevorkyan-Airapetov et al., 2009) |
| YMS063 | BY4741; *fun14*Δ::Kan | Euroscarf |
| YMS087 | BY4741; *atp23*Δ::Kan | Euroscarf |
| YMS102 | BY4741; *pcp1*Δ::Kan | Euroscarf |
| YMS103 | BY4741; *prd1*Δ::Kan | Euroscarf |
| YMS106 | BY4741; *yta12*Δ::Kan | Euroscarf |
| YMS107 | BY4741; *pim1*Δ::Kan | Euroscarf |
| YMS108 | BY4741; *yta10*Δ::Kan | Euroscarf |
| YMS109 | BY4741; *imp1*Δ::Kan | Euroscarf |
| YMS110 | BY4741; *imp2*Δ::Kan | Euroscarf |
| YMS111 | BY4741; *oct1*Δ::Kan | Euroscarf |
| YMS116 | BY4741; *yme1*Δ::Kan | Euroscarf |
| JSY7452 | MAT α *ade2-1; can1-100; his3-11;15; leu2-3; trp1-1; ura3-1* | (Kondo-Okamoto *et al.*, 2008) |
| JSY8283 | JSY7452; *tom70*Δ::Trp1; *tom71*Δ::His3 | (Kondo-Okamoto *et al.*, 2008) |
| GA74-1A | MAT a *ade8*; *his3*; *leu2*; *trp1*; *ura3* | (Koehler et al., 1998) |
| CK14 | GA74D; *tim10*Δ::His3; *tim10-1*:Trp1 | (Koehler et al., 1998) |
| MB2 | MAT a/α, ADE2/*ade2-101*$^{ochre}$; *his3*/*his3-*Δ*200*; *leu2*/*leu2-*Δ*1*; *lys2-801*$^{amber}$/*lys2-801*$^{amber}$; *trp1-289*/TRP1; *ura3-52*/*ura3-52* | (Maarse et al., 1992) |
| TU008/ YDR2628 | MB2; *tim8*Δ::Ura3; *tim13*Δ::His3 | (Paschen et al., 2000) |
| MYM104 | MAT α; Ura3-52; trp1-1; leu2-3; leu2-112; his3-11; his3-15 | (Witte et al., 1988) |
| MYM105 | MYM104; mas1-ts | (Witte et al., 1988) |
| YTW224 | YPH499; *TOM22-10His* | (Meisinger et al., 2001) |
| YIA30 | W303a; *yme*Δ*1*::Kan | (Arnold et al., 2006) |
| YKD870 | W303a; *yme1*Δ::Kan; *mim2*Δ::His3 | this study |
| *E. coli* | | |
| MS094 | W3110; pVG18-MPPHis | (Geli, 1993) |
| BL21(DE3) | | Thermofisher Scientific, Darmstadt, Germany |
| MH1 | | NEB biolabs, Frankfurt, Germany |

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Appendix Table 2. Primers used in this study

| Primers for gene-targeting | | |
|---|---|---|
| name | sequence | remarks |
| PFa-For-Fun14K | 5' ACG CTA GAG GGG CAA GAA GGA AGA ACT TAA AAT AAT AGG TGT AAA *CGT ACG CTG* | amplification of KanMX4 or HISMX6 cassette |
| PFa-Rev-Fun14K | 5' AAC GAA AGA ATA TAA CCC TCG TTT ATA TCT GGT CAT TTG TCT TGC *ATC GAT GAA* | amplification of KanMX4 or HISMX6 cassette |
| Primers for cloning | | |
| name | sequence | remarks |
| FpYX-Fun14 | 5' GGG GAA TTC ATG ACT TTG GCT TTT AAT ATG CA | amplification of *MCP3* ORF 5' contains *Eco*RI restriction site |
| RpYX-Fun14 | 5' GGG AAG CTT TCA TTT GTT AGC ATT TAA ACT TGC | amplification of MCP3 ORF 3' contains *Hin*dIII restriction site |
| FUN14intHArev | 5' GGG GGA TCC *TGC GTA GTC AGG CAC ATC ATA CGG ATA* CCC TAA AGA ATC ATT GAA TAT CA | amplification of *MCP3* presequence 3' contains *BamH*I restriction site and encodes the HA tag |
| Fun14intHAfwd | 5' GGG GGA TCC GCA GCT GTC AAA CAA CAG G | amplification of *MCP3* mature part 5' (starting 4 codons downstream of predicted Imp1 cleavage site) contains *BamH*I restriction site |
| D70G_Fun14fwd | 5' GAT ATT CAA TGG TTC TTT AGG G | site directed mutagenesis for D70G amino acid exchange (sense) |
| D70G_Fun14rev | 5' CTA TAA GTT ACC AAG AAA TCC C | site directed mutagenesis for D70G amino acid exchange (antisense) |
| F14PromFwd | 5' GGG GAG CTC GTG GCT TAA AGA CGA TAA TGC | amplification of *MCP3* promoter 5' contains *Sac*I restriction site |
| F14PromRev | 5' GGG GAA TTC TTT ACA CCT ATT ATT TTA AGT TCT T | amplification of *MCP3* promoter 3' contains *Eco*RI restriction site |
| F14TermFwd | 5' GGG AAG CTT GCAAGA CAA ATG ACC AGA TAT A | amplification of *MCP3* terminator 5' contains *Hin*dIII restriction site |
| F14TermRev | 5' GGG GTC GAC AGC GTT GAA AAA GGT AGA AAT TA | amplification of *MCP3* terminator 3' contains *Sal*I restriction site |
| delta-TMD1-Arev | 5' GGG GGA TCC CTG CTT GTG ACT ACT TAT TTT G | amplification of aa 1-105 coding sequence of MCP3, contains *BamH*I restriction site |
| delta-TMD1-Bfwd | 5' GGG GGA TCC TAT GTC GGT ATT ACA AGC ATG | amplification of aa 129-198 coding sequence of MCP3, contains *BamH*I restriction site |
| delta-TMD2-rev | 5' GGG AAG CTT TTA ATC AAT AAG CAG TTT CTT CAA GT | amplification of aa 1-171 coding sequence of MCP3, contains *Hin*dIII restriction site |
| RpYX-Fun14HA | 5' GGG AAG CTT TTT GTT AGC ATT TAA ACT TGC TAA | amplification of MCP3 ORF 3' contains *Hin*dIII restriction site, stop codon omitted for fusion with HA-tag |