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Yeast TOM complex humanization and its effect on growth under stress

MHryc

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Mitochondrial protein import

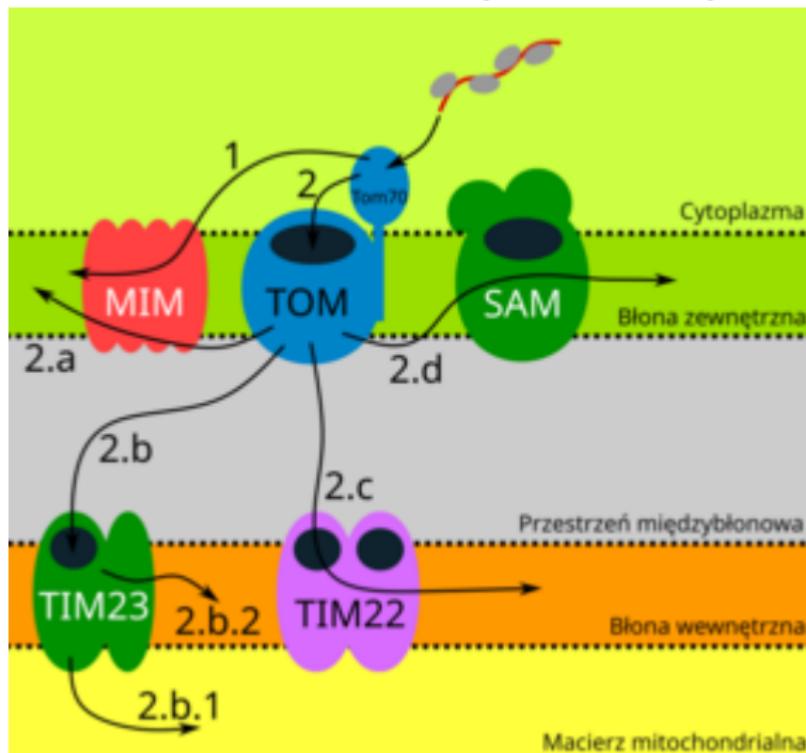


Figure 1: Diagram of mitochondrial protein import paths.

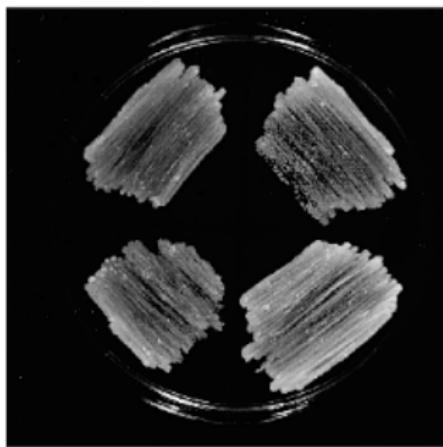
Tom70 and Tom71 subunits

$\Delta 71$

WT

$\Delta 71$

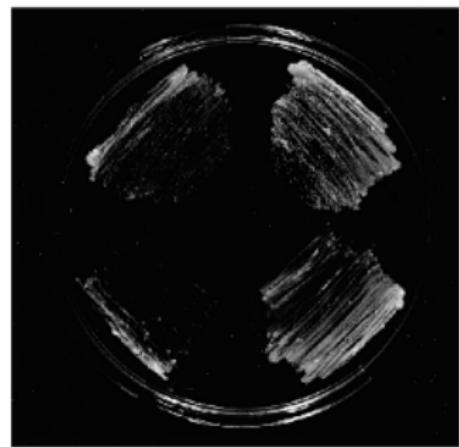
WT



$\Delta 70/71$

$\Delta 70$

30°C



$\Delta 70/71$

$\Delta 70$

37°C

Figure 2: Effects of receptor gene(s) deletion under different growth temperature.

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Engineering strains

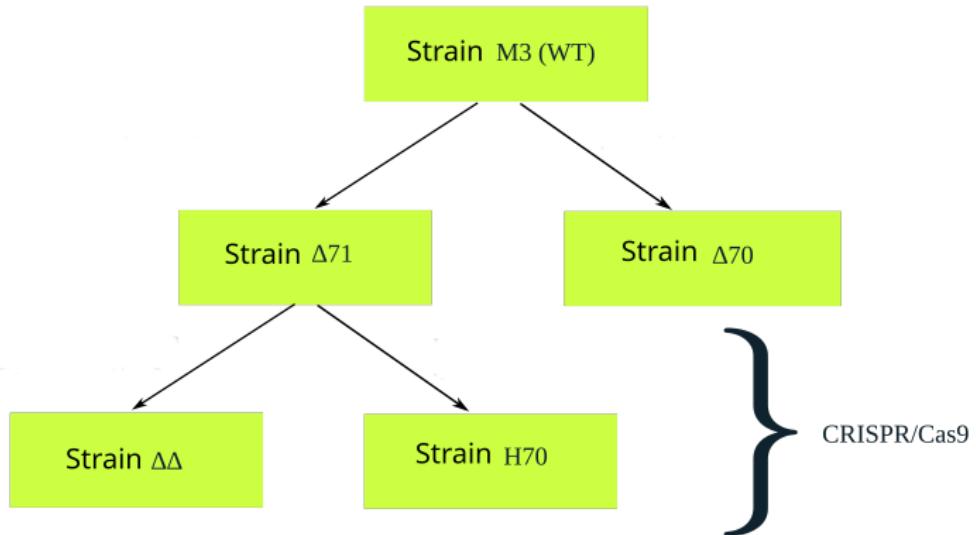


Figure 3: Mutants

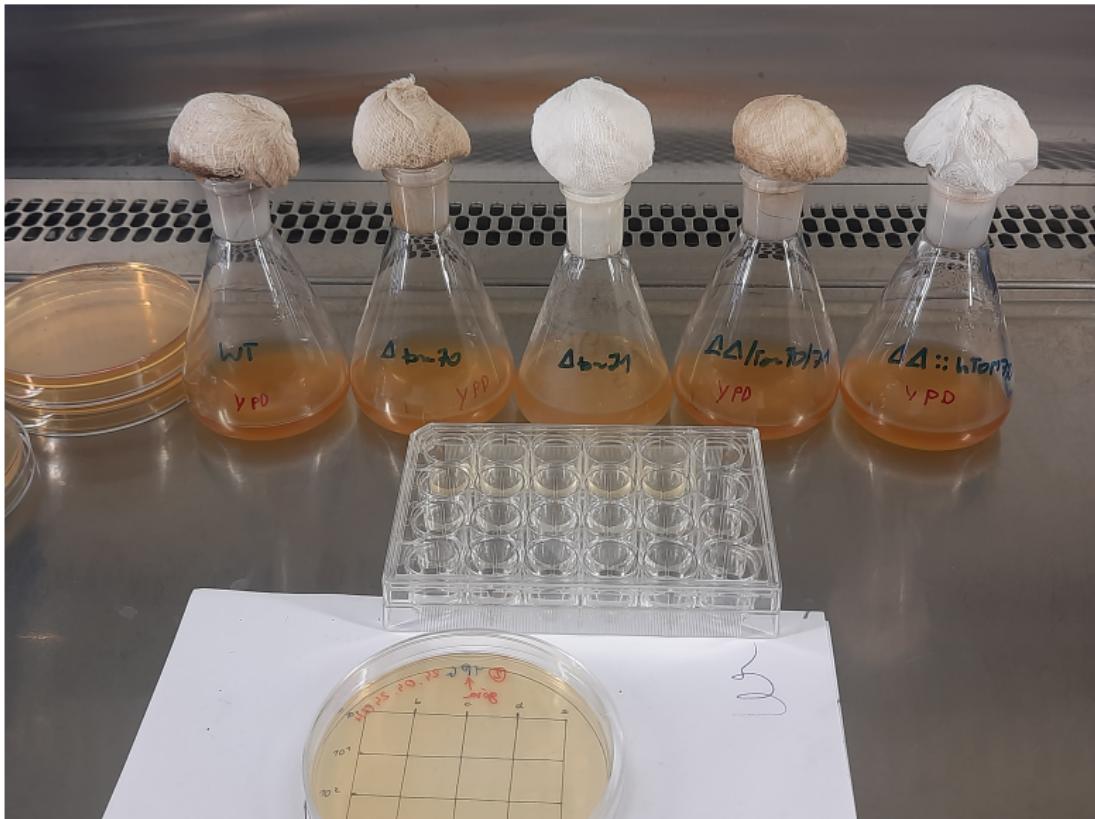
Strain genotyping

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Figure 4: Diagram of primer pairs used for genotyping.

Solid media



Liquid media



Statistical analysis

To compare the growth of **H70** and **DeltaDelta** strains in liquid media, **t-test** implementation from SciPy Python's library was used.

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Solid medium phenotyping

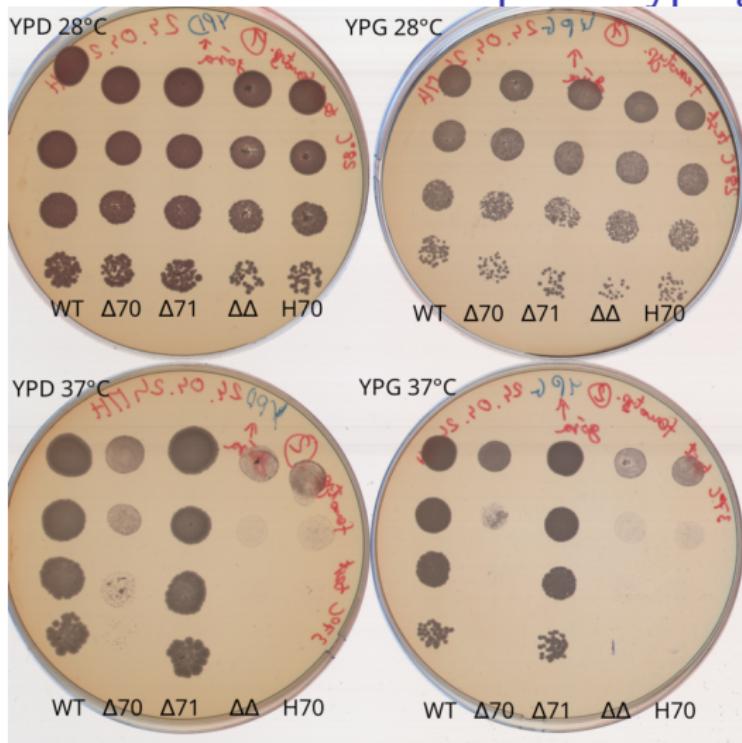
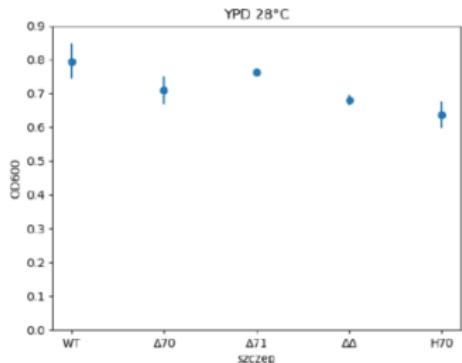
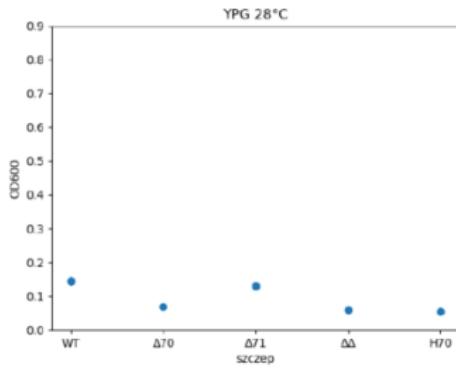


Figure 5: Scan after 24h of growth under different temperature-medium growth conditions

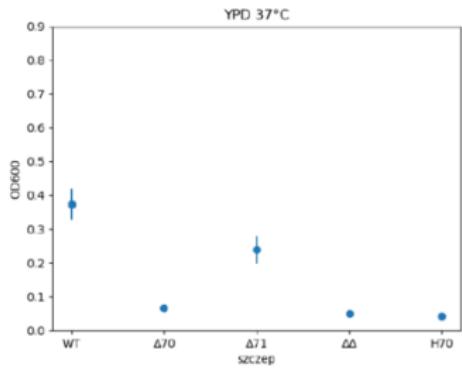
Liquid medium phenotyping (1)



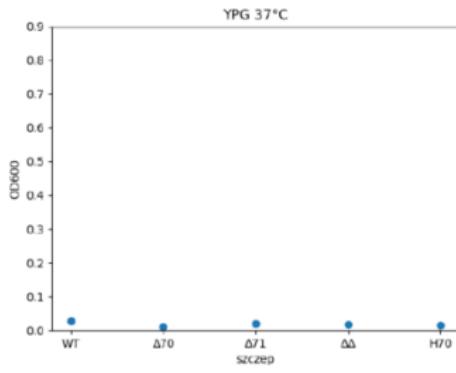
(a) YPD 28°C



(b) YPG 28°C

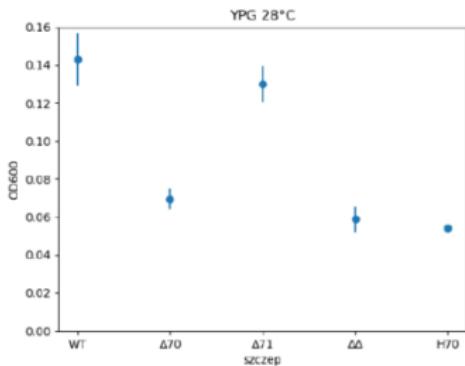


(c) YPD 37°C

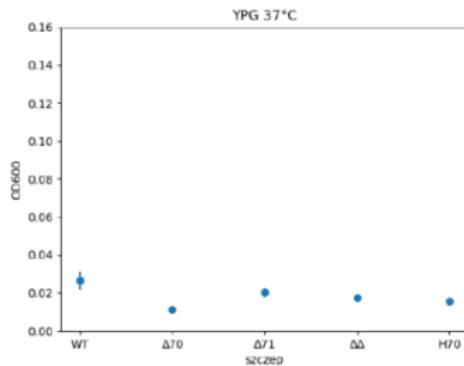


(d) YPG 37°C

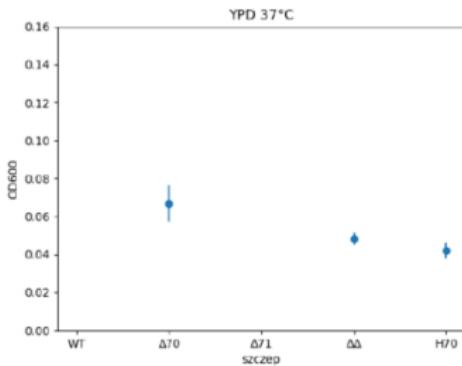
Liquid medium phenotyping (2)



(a) YPG 28°C



(b) YPG 37°C



(c) YPD 37°C

Results of the analysis

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YPD 28°C	1.824	0.118
YPG 28°C	1.104	0.312
YPD 37°C	2.076	0.083
YPG 37°C	1.414	0.207
*YPD 37°C	3.814	0.012

Figure 6: * calculated after removing one extreme sample.

Conclusions

- ① Humanization with human Tom70 analog did not mitigate the growth defect
- ② Strain M3 (used as wild type in this study) exhibits Tom70 preference, while according to the literature other strains exhibit Tom71 preference
- ③ Double knockout mutation (deletion of Tom70 and Tom71 genes) is not lethal in M3 yeast strain

Future direction

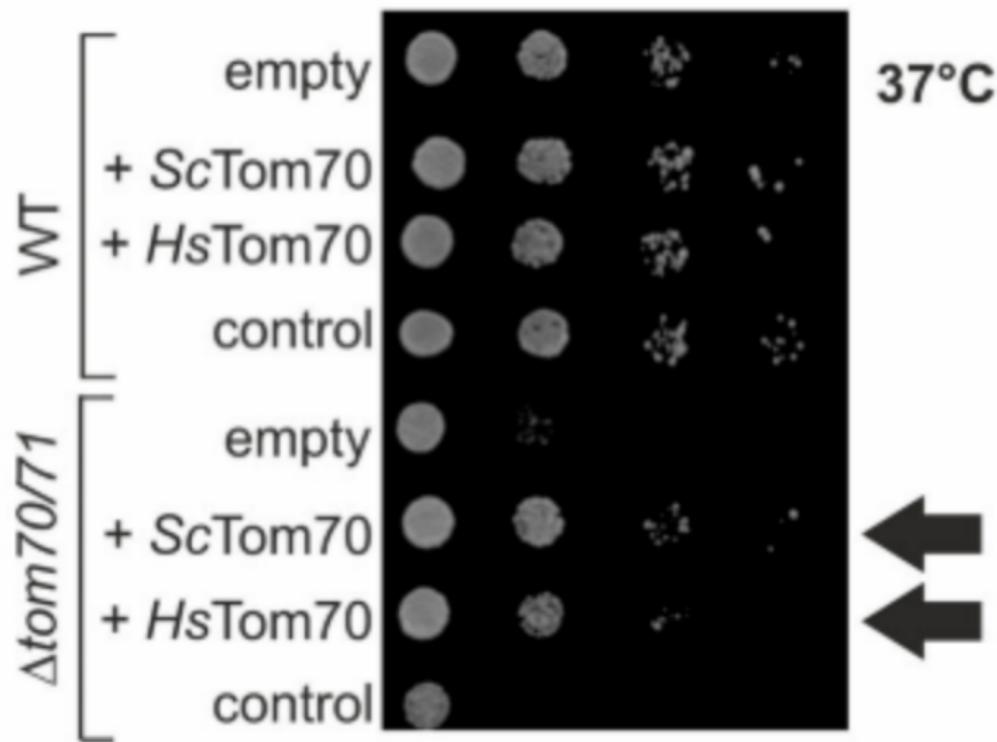


Figure 7: Results of a spot assay of yeast strains with humanized TOM complex components.

Sources

- J. Schlossmann, et al. "Tom71, a novel homologue of the mitochondrial preprotein receptor Tom70." *Journal of Biological Chemistry*, doi: 10.1074/jbc.271.30.17890.
- S. Lenhard, et al. "The Orf9b protein of SARS-CoV-2 modulates mitochondrial protein biogenesis." *Journal of Cell Biology*, doi: 10.1083/jcb.202303002.