**Results**

During our practical we screened the *S.cerevisae* samples T5, T6, T7 and T8 for interesting mutations that could potentially be responsible for reverting the heat-sensitive phenotype of *∆TOM1* *S.cerevisae* strains. The sample T5 contains only one haploid strain named YDK1364 and was used as a reference (Figure XXX). All strains found in T6 to T8 arose from the strain YDK1364 found in T5, but as mutations emerged in strains S1364-1 to S1364-8, these strains became more resistant to high-temperature stress (i.e. 37°C). Therefore, we excluded all mutations that co-occurred in T5 and any of the samples of interest T6 to T8. Since each of the samples is composed of two pooled haploid *S.cerevisae* strains (Figure XXX), we excluded all homozygous mutations from further analysis, since it is highly unlikely that two strains have the same mutation suppressing the *∆TOM1* heat sensitivity phenotype.

Ein Bild, das Text enthält.

Automatisch generierte Beschreibung

**Figure XXX:** Growth characteristics of *S.cerevisae* strains cultured in Yeast Extract–Peptone–Dextrose medium (YPD) at different temperatures. Strain names are indicated on the left of the growth assay photographs. *S.cerevisae* cells were plated onto agar plates and were cultured for up to 7 days at increasing temperature starting from 16°C to 37°C. (A) Wildtype yeast grows well at all tested temperatures. (B) Depicted is the growth behavior of the *∆Tom1* strain YDK1364, which is highly sensitive to heat. (C) Growth of mutated *∆Tom1* yeast strains derived from YDK1364 that exhibit lower heat susceptibility due to accumulation of mutations that counteract the *∆Tom1* phenotype. Depicted are representative growth assays of the different yeast strains.

In this first screening approach, we included only mutations with a Phred-scaled quality score of over 200. Furthermore, we focussed on mutations that interact genetically or physically with *TOM1* or genes that encode for ribosomal proteins of the large (RPL) or small (RPS) subunit, because they have observed to accumulate and form detergent-insolubleble. Using our pipeline described in the Variant calling and annotation of the Methods section, we identified nine mutations that may explain why samples T6, T7, and T8 are less susceptible to high temperatures compared to our reference T5.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample** | **Gene Name** | **Mutation Type** | **Chromosome** | **Interactors** |
| T6 | *YGR160W* | Inframe insertion | VII | Unknown |
| T6 | *PMT1* | Frameshift | IV | *HAS1* |
| T7 | *KRE6* | Stop gained | XVI | *TOM1*, *RPL1B*, *RPL34B* |
| T7 | *KRE9* | Missense | X | *MRPL17*, *MRPL25*, *MRPL38*, *RPL10*, *RPL11B*, *RPL15A*, *RPL1B*, *RPL24A*, *RPL2A*, *RPL3* |
| T7 | *ISC1* | Missense | V | *RPL40B* |
| T7 | *FLO9* | Inframe insertion | I | *IMG2*, *YAR1* |
| T7 | *VTC4* | Missense | X | TOM1(Physical) |
| T8 | *KRE6* | Missense | XVI | *TOM1*, *RPL1B*, *RPL34B* |
| T8 | *ROT1* | Missense | XIII | *RPL4B*, *RPS25A* |

**Table YY:** Table depicting all heterozygous mutations found in the sample T6 to T8, which might be potential suppressors of the *∆TOM1* phenotype. Annotation of the mutation type was done by SnpEff. With the expection of VTC4, only genetic interactions are shown. All intergenic and synonymous mutations were excluded from further analysis.

**Discussion:**

In sample T6, we found two potential high-quality mutation candidates, namely an inframe insertion in YGR160W and frameshift mutation in *PMT1*. On closer inspection, we found that *YGR160W* was flagged as a dubious gene, which is unlikely to encode a functional protein, thus in spite of the quality we discarded it from further research. In contrast, *PMT1* codes for an O-mannosyltransferase that is involved in ER quality control among other things.

REF: <https://www.ncbi.nlm.nih.gov/pubmed/8367478>

<https://www.ncbi.nlm.nih.gov/pubmed/21147851>

As a result of the enormous global genetic interaction network that has been created by M. Constanzo and colleagues, *PMT1* has been shown to genetically interact with *HAS1*, which codes for an ATP-dependent RNA helicase that is involved in the biogenesis of the 40S and 60S ribosome subunits.

REF: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5661885/>

<https://www.ncbi.nlm.nih.gov/pubmed/23788678>

In sample T7, we found an already described *∆TOM1* suppressor gene named *KRE6* as well as some putative candidates that might be worth investigating in more depth. In T7, we identified a nonsense mutation in the known extragenic suppressor *KRE6* of *∆Tom1*. The premature stop codon is inserted at nucleotide position 1431 of 2161, which strongly suggests that translation of the *KRE6* transcript results in the formation of a truncated protein. *KRE6* codes for a type II membrane protein involved in the synthesis of β-(1,6)-glucan, which is an essential constituent of the fungal cell wall.

REF: <https://www.ncbi.nlm.nih.gov/pubmed/8321211>

<https://www.ncbi.nlm.nih.gov/pubmed/21193403>

In spite of the fact that the function of the KRE6 protein is not directly involved stress responses, Sasaki and colleagues found that mutation in the *KRE6* gene acts as a weak suppressor of heat sensitivity mediated by *TOM1* deletion.

REF: <https://www.ncbi.nlm.nih.gov/pubmed/10660055>

Although the authors were not able to decipher the underlying mechanism responsible for restoring heat tolerance in *∆TOM1* *S.cerevisae* with mutated *KRE6*, but they concluded that the mutation may activate unknown suppressor genes of *∆TOM1*.

According to the Biological General Repository for Interaction Datasets (BioGRID, https://thebiogrid.org/), *KRE6* has been shown to exhibit genetic interaction with *TOM1* itself as well as multiple genes coding for mitochondrial and cytoplasmic ribosomal proteins of the large subunit (Table YY). Moreover, we found a missense mutation in gene *KRE9*, which is also involved to be involved in the synthesis of β-(1,6)-glucan like KRE6 protein. The deletion of *KRE9*  has long been known to have a deleterious effect on the growth of *S.cerevisae* by altering the composition of its cell wall and thus causes defects to its integrity.

REF: <https://www.ncbi.nlm.nih.gov/pubmed/8413233>

Similar to *KRE6*, *KRE9* also interacts with several gene coding for the small and large subunit of ribosomes (Table YY). It is also conceivable that *KRE9* mutation alone or in combination with the mutation in *Kre6* has an impact on the transcription of ribosomal genes and thus may passively counteract the stress response associated with the accumulation of protein related to *∆Tom1*. Another gene related to ribosome biogenesis was also found to be mutated in sample T7, namely *ISC1*, which has been reported to interact genetically *RBL40B*.

REF: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3198156/>

While the protein ISC1 is not directly linked to the regulation of ribosome biosynthesis, RPL40B is involved in the maturation of the 60S ribosomal subunit.

REF: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3488107/>

Interestingly, the null mutation of *ISC1* has been associated with heat sensitivity, which implies that the mutation observed in sample T7 does not yield a non-functional protein. Therefore, we concluded that the mutation in *ISC1* is most likely not responsible for counteracting the heat-sensitive phenotype resulting form the deletion of *TOM1*.

Last but not least, we also identified a mutation in a direct physical interactor of the TOM1 protein in sample T7 named *VTC4*, which is a component of the vacuolar transporter chaperone (VTC) complex.

REF: <https://www.ncbi.nlm.nih.gov/pubmed/12584253>

Unfortunately, the nature of the interaction between VTC4 and TOM1 is not described and thus it is not possible to evaluate whether a mutation in this gene has an impact on the *∆TOM1* phenotype. A possible interesting scenario could be that TOM1 is an inhibitor of VTC4.

In T8, we observed a missense mutation in *KRE6*. In addition, we discovered another missense mutation in a gene named *ROT1*, which codes for a chaperone involved in protein folding.

REF: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2488298/>

Similar to the other gene candidates, *ROT1* also interacts genetically with ribosomal genes, namely *RPL4B* and *RPS25A*.

Interestingly, most of the mutations we found in samples T6, T7, and T8 were indirectly linked to ribosomal genes. These findings are of particular interest since deletion of *TOM1 S.cerevisae* has been associated with greatly increased levels of ribosomal proteins. Under normal conditions, the E3 ubiquitin ligase TOM1rapidly removes excess of ribosomal proteins via proteasomal degradation.

REF <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5026473/>

In conclusion, we found one known as well as seven potential new suppressors of *∆TOM1*. The known suppressor *KRE6*, has been found to be mutated in T7 and T8. Due to the heterozygosity of the mutation, we conclude that *KRE6* may explain the partially restored capability of *S.cerevisae* to grow at 37°C in one of the two strains found in each sample. However, it is important to note that while the mutation in T7 introduces a additional stop codon, the spontaneous mutation in T8 only affected one amino acid and consequently affects the function of the protein to a lesser extent (Table YY).

REF: <https://www.ncbi.nlm.nih.gov/pubmed/10660055>

Since most of the mutated genes found in our samples were associated with biogenesis or regulation of ribosomes, it is would be interesting to investigate whether these mutations suppress the accumulation of ribosomal proteins in the absence of *TOM1*. Take together our data provide the basis for further investigations aimed at clarifying whether accumulation of ribosomal proteins may be causative of the heat sensitivity of yeast lacking *TOM1*.