DOI 10.18699/SBB-2022-041

Amplification of the release factor genes as a mechanism of adaptation in yeast

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Key words: release factors, yeast, eRF1, eRF3, gene amplification, copy number variation, chromosome instability, WGS

Motivation and Aim: Termination of translation in eukaryotes is conferred by two release factors, eRF1 and eRF3. In baker's yeast these factors are encoded by essential genes *SUP45* and *SUP35*. Despite being essential, several viable nonsense mutations in these genes have been discovered [1, 2]. The survival of such mutants involves feedback control of stop codon readthrough, however, the exact molecular basis remains unclear.
Methods and Algorithms: To find out the mechanisms of yeast adaptation to nonsense mutations in the release factor genes, we performed whole genome sequencing of 100 yeast strains carrying various mutant alleles of the *SUP35* and *SUP45* genes (*sup35-n/sup45-n*) on centromeric plasmids using Illumina HiSeq 2500. Aditionally, 13 strains carrying *sup35-n/sup45-n* mutant alleles on the chromosomes were sequenced and analyzed. The sequencing data were aligned to the reference genome of the U-1A-D1628 strain, obtained previously [3], after which the search for genetic variations and copy number analysis were performed. The number of copies of the release factor genes was additionally confirmed with qPCR.
Results: SNV analysis of WGS data did not show any frequent changes in the yeast genome, which could potentially be adaptive to translation termination malfunction. At the same time, we found a significant increase in the number of copies of plasmids carrying *sup35-n/sup45-n* mutant alleles compared to wild-type alleles, which was confirmed using qPCR. In four sequenced strains carrying the *sup35-n/sup45-n* alleles as a single chromosomal copy, we found a duplication of the entire chromosome II (containing the *SUP45* gene) or a region on the chromosome IV, bearing the *SUP35* gene [4]. Interestingly, the borders of the duplicated region on chromosome IV are exactly the same as in previously reported duplication in the *sup35-222* strain, containing a downregulating single-nucleotide substitution in the *SUP35* promoter [5]. In addition, three of the 13 strains were found to have aneuploidies on chromosomes XI and XIII, which do not contain alleles of the release factor genes.
Conclusion: We thus conclude that increasing gene dosage of the *SUP45* and *SUP35* genes is a primary mechanism for increasing the survival of cells with impaired termination of translation. The observed genomic instability which did not alter the release factor genes copy number indicates, that other mechanisms might also be involved, which, however, are less wide-spread.

Acknowledgements: The study is supported by the RSF grant 18-14-00050, by the State research program 0112-2016-0015, as well as by the resource centres “Development of Molecular and Cell Technologies” and “Biobank” of SPbSU.

References

1. Moskalenko S.E., Chabelskaya S.V., Inge-Vechtomov S.G., Philippe M., Zhouravleva G.A. Viable nonsense mutants for the essential gene *SUP45* of *Saccharomyces cerevisiae*. *BMC Mol Biol*. 2003;4:2.
2. Chabelskaya S., Kiktev D., Inge-Vechtomov S., Philippe M., Zhouravleva G. Nonsense mutations in the essential gene *SUP35* of *Saccharomyces cerevisiae* are non-lethal. *Mol Genet Genomics*. 2004;272(3):297-307.
3. Barbitoff Y.A., Matveenko A.G., Matiiv A.B., Maksiutenko E.M., Moskalenko S.E., Drozdova P.B., ..., Zhouravleva G.A. Chromosome-level genome assembly and structural variant analysis of two laboratory yeast strains from the Peterhof Genetic Collection lineage. *G3 (Bethesda)*. 2021;11(4):jkab029.
4. Maksiutenko E.M., Barbitoff Y.A., Matveenko A.G., Moskalenko S.E., Zhouravleva G.A. Gene amplification as a mechanism of yeast adaptation to nonsense mutations in release factor genes. *Genes (Basel)*. 2021;12(12):2019.
5. Matveenko A.G., Drozdova P.B., Moskalenko S.E., Tarasov O.V., Zhouravleva G.A. Whole genome sequencing data and analyses of the underlying *SUP35* transcriptional regulation for a *Saccharomyces cerevisiae* nonsense suppressor mutant. *Data Brief*. 2019;23:103694.