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329 WGS-based Identification of Recurrent Mutations That Confer Adaptation to Translation Termination Defects in Yeast.

Yury Barbitoff, Andrew Matveenko, Anton Matiiv, Svetlana Moskalenko, Dmitrii Polev, Alexandra Beliavskaia, Alexander Predeus and Galina Zhouravleva

St. Petersburg State University,St. Petersburg,Russia; Bioinformatics Institute,St. Patersburg,Russia,Russia; Vavilov Institute of General Genetics,Russia; University of Liverpool,Liverpool,United Kingdom,Bioinformatics Institute

Baker's yeast Saccharomyces cerevisiae is a well-known eukaryotic model organism that is widely used to study mechanisms of fundamental cellular processes. One example of such processes is the termination of translation, a crucial stage in protein synthesis. Termination of translation in yeast, like in most eukaryotes, is controlled by two main release factors, eRF1 and eRF3, encoded by the essential SUP45 and SUP35 genes, respectively. Previously we showed that even though these genes are essential yeast cells can maintain viability upon nonsense mutations in them (sup35-n and sup45-n). Interestingly, viability of the cells harboring these mutant alleles is increased after growth in the absence of wild-type allele, suggesting that additional mutations may arise during the first stage of selection. In this study we set off to identify such mutations and characterize their role in conferring cellular adaptation to translation termination defects.

We first constructed a chromosome-level de novo reference genome assembly of one yeast strain from the Peterhof Genetic Collection, 1A-D1628, using data from Oxford Nanopore Technologies (ONT) MinION sequencer. The assembly was further polished using both raw ONT data and paired-end Illumina reads. The resulting reference assembly contained 23 contigs, including all but one yeast chromosomes assembled in a single contig. We then used the obtained assembly as the reference to search for genetic variants present in 100 yeast clonal cultures obtained by substitution of the wild-type allele of either SUP45 or SUP35 gene for the respective nonsense mutant copy. We identified 559 mutations arising after plasmid shuffling procedure, 428 of which were uniquely present in strains resulting from substitution to the mutant allele. 100 of such mutations occured 3 or more times in strains that harbored the mutant allele of SUP35 or SUP45. The role of these mutations in survival of yeast cells lacking functional termination factors is currently under examination. Dissection of the adaptive mutations that help cells survive upon severe translational defects would provide new insights into the mechanisms of translational regulation and may suggest new strategies for disease therapy.

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