Amino acid substitutions in translation termination factor eRF3 of yeast Saccharomyces cerevisiae that lead to decreased GTPase activity

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The final step in protein synthesis, translation termination, occurs when one of three termination codons (UAA, UGA, or UAG) in mRNA reaches the ribosomal A-site. In eukaryotes, translation termination requires two factors: eRF1 (eukaryotic release factor) and eRF3. The eRF1 protein belongs to class 1 release factors, responsible for the recognition of the stop codon and peptidyl-tRNA hydrolysis, and eRF3 – to class 2 release factors, functioning to stimulate the work of class 1 factors due to its GTPase activity. In the yeast *S. cerevisiae* release factors are encoded by the *SUP45* and *SUP35* genes. Factor eRF3 of *S. cerevisiae* (Sup35p) consists of three domains. C-domain is essential for translation termination and possesses GTP- and eRF1-binding sites. N-domain is responsible for Sup35p aggregation and [*PSI*$^+$] prion formation. M-domain is involved in the maintenance of [*PSI*$^+$]. Both *sup35* mutations and [*PSI*$^+$] cause reduction of the translation termination fidelity and lead to the nonsense suppression. It was shown that *sup35* mutations affecting the N-domain have an influence on [*PSI*$^+$] appearance and maintenance. In this work, we have studied three *sup35-m* (missense) mutations located inside the C-domain of Sup35p. We reconstituted *in vitro* eukaryotic translation termination using purified ribosomal subunits, termination factors eRF1 and C-domain of eRF3 (wild type or mutant variants). It has been shown that amino acid substitutions in translation termination factor eRF3 of yeast *S. cerevisiae* lead to decreased GTPase activity. It is possible that these mutations disrupt the GTPase activity of eRF3, resulting in suppression and reduction of the translation termination fidelity. This work was supported by RSF grant 18-14-00050 “Genetic and epigenetic regulation of translation termination”, RFBR grant 19-04-00173 and the State Research Program 0112-2016-0015. Part of experimental work was done in the resource centre of SPBU “Centre for Molecular and Cell Technologies”.