

Influence of Wobble Uridine Modifications on Eukaryotic Translation

Hasan Tükenmez

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Fakultetsopponent: Dr. Sebastian Leidel,
Max Planck Institute for Molecular Biomedicine, Münster, Germany



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Author

Hasan Tükenmez

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Abstract

Elongator is a conserved six subunit protein (Elp1p-Elp6p) complex that is required for the formation of ncm^5 and mcm^5 side chains at wobble uridines in transfer RNAs (tRNAs). Moreover, loss-of-function mutations in any gene encoding an Elongator subunit results in translational defects and a multitude of phenotypic effects. This thesis is based on investigations of effects of wobble uridine modifications on translation.

In *Saccharomyces cerevisiae*, $\text{ncm}^5\text{U}_{34^-}$, $\text{mcm}^5\text{U}_{34^-}$ and $\text{mcm}^5\text{s}^2\text{U}_{34^-}$ -modified wobble nucleosides in tRNAs are important for proper codon-anticodon interactions. My colleagues and I (hereafter we) showed that mcm^5 and s^2 groups at wobble uridine in tRNAs are vital for maintaining the reading frame during translation, as absence of these modifications increases the frequency of +1 frameshifting. We also showed that +1 frameshifting events at lysine AAA codons in Elongator mutants are due to slow entry of the hypomodified $\text{tRNA}_{\text{A}^{\text{Lys}}_{\text{s}^2\text{UUU}}}$ to the ribosomal A-site.

Ixr1p is a protein that plays a key role in increasing production of deoxynucleotides (dNTPs) in responses to DNA damage, via induction of Ribonucleotide reductase 1 (Rnr1p), in *S. cerevisiae*. We showed that expression of Ixr1p is reduced in *elp3Δ* mutants due to a post-transcriptional defect, which results in lower levels of Rnr1p in responses to DNA damage. Collectively, these results suggest that high sensitivity of Elongator mutants to DNA damaging agents might be partially due to reductions in Ixr1p expression and hence Rnr1p levels.

Elongator mutant phenotypes are linked to several cellular processes. To probe the mechanisms involved we investigated the metabolic perturbations associated with absence of a functional *ELP3* gene in *S. cerevisiae*. We found that its absence results in widespread metabolic perturbations under both optimal (30°C) and semi-permissive (34°C) growth conditions. We also found that changes in levels of certain metabolites (but not others) were ameliorated by elevated levels of hypomodified tRNAs, suggesting that amelioration of perturbations of these metabolites might be sufficient for suppression of the Elongator mutant phenotypes.

A mutation in the *IKBKAP* (*hELP1*) gene results in lower levels of the full-length hELP1 protein, which causes a neurodegenerative disease in humans called familial dysautonomia (FD). We showed that the levels of $\text{mcm}^5\text{s}^2\text{U}$ -modified wobble nucleoside in tRNAs are lower in both brain tissues and fibroblast cell lines derived from FD patients than in corresponding materials derived from healthy individuals. This suggests that FD may result from inefficient translation due to partial loss of $\text{mcm}^5\text{s}^2\text{U}$ -modified nucleosides in tRNAs.

Keywords

tRNA modification, frameshifting, Elongator complex, DNA damage response, metabolomics, familial dysautonomia

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