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Factors modulating tRNA biogenesis and function in *Saccharomyces cerevisiae*

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Abstract

Transfer RNA (tRNA) genes are transcribed by RNA polymerase III as precursors that undergo multiple processing steps to form mature tRNAs. These steps include processing of the 5' leader and 3' trailer sequences, addition of a 3' CCA tail, removal of introns, and formation of modified nucleosides. The mature tRNAs carry amino acids to the ribosome where proteins are synthesized. The aim of this thesis is to identify and characterize factors that influence tRNA biogenesis and function in *Saccharomyces cerevisiae*.

Nonsense suppressor tRNAs are encoded by mutated tRNA genes and able to read stop codons. The *SUP4* gene encodes such a suppressor tRNA that base-pairs with UAA stop codons. By screening for mutations that impair the nonsense suppression of the *SUP4*-encoded tRNA, we identified a loss-of-function mutation in the *YPK9* gene. Inactivation of Ypk9p causes a reduction in the readthrough of UAA stop codon. We found that phenotypes of *ypk9Δ* cells including decreased UAA readthrough and sensitivity to Mn^{2+} are counteracted by increasing the cellular levels of putrescine, one type of polyamine. Importantly, cells lacking Ypk9p show reduced levels of putrescine. Our results suggest that the *YPK9* gene product influences the cellular levels of putrescine, which plays a role in maintaining the fidelity of translation termination.

The Elongator complex, consisting of Elp1p-Elp6p six proteins, catalyzes the formation of U_{34} modifications in the anticodon region of 11 tRNA species. Elongator mutants display pleiotropic phenotypes that are caused by decreased tRNA functionality. We found that the genetic background, largely due to a polymorphism at the *SSD1* locus, influences the pleiotropic phenotypes of Elongator mutants.

In a genetic screen for factors that are essential for the survival of cells encoding a destabilized tRNA^{Ser}_{CGA}, several gene products were identified. We demonstrate that mutations in these genes result in reduced levels of the destabilized tRNA^{Ser}_{CGA}, suggesting a role for these gene products in tRNA^{Ser}_{CGA} biosynthesis.

Keywords

Translation, tRNA biogenesis, suppressor tRNA, Elongator complex, wobble uridine modifications, *SUP4*, *YPK9*, *SSD1*, serine tRNA, synthetic lethality, yeast

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