Physiological consequences of Elongator complex inactivation in Eukaryotes

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Akademisk avhandling

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Abstract
Mutations found in genes encoding human Elongator complex subunits have been linked to neurodevelopmental disorders such as familial dysautonomia (FD), rolandic epilepsy and amyotrophic lateral sclerosis. In addition, loss-of-function mutations in genes encoding Elongator complex subunits cause defects in neurodevelopment and reduced neuronal function in both mice and nematodes. The Elongator complex is a conserved protein complex comprising six subunits (Elp1p-Elp6p) found in eukaryotes. The primary function of this complex in yeast is formation of the 5-methoxycarbonylmethyl (mcm^5) and 5-carbamoylmethyl (ncm^5) side chains found on wobble uridines (U_{34}) in tRNAs. The aim of this thesis is to investigate the physiological consequences of Elongator complex inactivation in humans and in the yeast *Saccharomyces cerevisiae*.
Inactivation of the Elongator complex causes widespread defects in a multitude of different cellular processes in *S. cerevisiae*. Thus, we investigated metabolic alterations resulting from Elongator complex inactivation. We show that deletion of the *S. cerevisiae ELP3* gene leads to widespread metabolic alterations. Moreover, all global metabolic alterations observed in the *elp3Δ* strain are not restored in the presence of elevated levels of hypomodified tRNAs that normally have the modified nucleoside mcm^5s^2U. Collectively, we show that modified wobble nucleosides in tRNAs are required for metabolic homeostasis.
Elongator mutants display sensitivity to DNA damage agents, but the underlying mechanism explaining this sensitivity remains elusive. We demonstrate that deletion of the *S. cerevisiae ELP3* gene results in post-transcriptional reduction of Ixr1p levels. Further, we show that the reduced Ixr1p levels prevent adequate Rnr1p levels upon treatment with DNA damage agents. These findings suggest that reduced Ixr1p levels could in part explain why Elongator mutants are sensitive to DNA damage agents.
Depletion of Elongator complex subunits results in loss of wobble uridine modifications in plants, nematodes, mice and yeast. Therefore, we investigated whether patients with the neurodegenerative disease familial dysautonomia (FD), who have lower levels of the ELP1 protein, display reduced amounts of modified wobble uridine nucleosides. We show that tRNA isolated from brain tissue and fibroblast cell lines derived from FD patients have 64–71% of the mcm^5s^2U nucleoside levels observed in total tRNA from non-FD brain tissue and non-FD fibroblasts. Overall, these results suggest that the cause for the neurodegenerative nature of FD could be translation impairment caused by reduced levels of modified wobble uridine nucleosides in tRNAs. Thus, our results give new insight on the importance of modified wobble uridine nucleosides for neurodevelopment.

Keywords
Elongator complex, wobble uridine modifications, *IKBKAP*, IKAP, Familial dysautonomia, Untargeted Metabolic profiling.

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