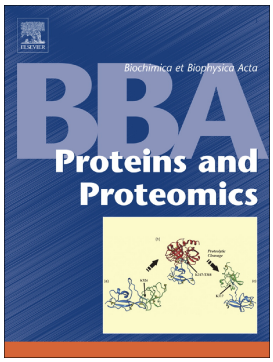
Accepted Manuscript



In vitro studies of disease-linked variants of human tRNA nucleotidyltransferase reveal decreased thermal stability and altered catalytic activity

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PII: S1570-9639(18)30018-9
DOI: doi:10.1016/j.bbapap.2018.02.002
Reference: BBAPAP 40065

To appear in:

Received date: 17 November 2017
Revised date: 9 February 2018
Accepted date: 14 February 2018

Please cite this article as: M. Leibovitch, P.J. Hanic-Joyce, P.B.M. Joyce , In vitro studies of disease-linked variants of human tRNA nucleotidyltransferase reveal decreased thermal stability and altered catalytic activity. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Bbapap(2018), doi:10.1016/j.bbapap.2018.02.002

Title: *In vitro* studies of disease-linked variants of human tRNA nucleotidyltransferase reveal decreased thermal stability and altered catalytic activity

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Abstract

Mutations in the human TRNT1 gene encoding tRNA nucleotidyltransferase (tRNA-NT), an essential enzyme responsible for addition of the CCA (cytidine-cytidine-adenosine) sequence to the 3'-termini of tRNAs, have been linked to disease phenotypes including congenital sideroblastic anemia with B-cell immunodeficiency, periodic fevers and developmental delay (SIFD) or retinitis pigmentosa with erythrocyte microcytosis. The effects of these disease-linked mutations on the structure and function of tRNA-NT have not been explored. Here we use biochemical and biophysical approaches to study how five SIFD-linked amino acid substitutions (T154I, M158V, L166S, R190I and I223T), residing in the N-terminal head and neck domains of the enzyme, affect the structure and activity of human tRNA-NT *in vitro*. Our data suggest that the SIFD phenotype is linked to poor stability of the T154I and L166S variant proteins, and to a combination of reduced stability and altered catalytic efficiency in the M158V, R190I and I223T variants.

Keywords: tRNA nucleotidyltransferase; TRNT1; SIFD; thermal stability; CCA

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