THE 67TH INTERNATIONAL

OPEN READINGS

CONFERENCE FOR STUDENTS OF PHYSICS AND NATURAL SCIENCES

BOOK OF ABSTRACTS | 2024

Vilnius University

VILNIUS UNIVERSITY PRESS

Editors:

Martynas Keršys
Rimantas Naina
Vincentas Adomaitis
Emilijus Maskvytis

Cover and Interior Design:

Goda Grybauskaitė

Vilnius University Press
9 Saulėtekio Av., III Building, LT-10222 Vilnius
info@leidykla.vu.lt, www.leidykla.vu.lt/en/
www.knygynas.vu.lt, www.journals.vu.lt

Bibliographic information is available
on the Lithuanian Integral Library Information System (LIBIS) portal www.ibiblioteka.lt
ISBN 978-609-07-1051-7 (PDF)

The ASCH domain-containing protein from Thermus thermophilus acts as a tRNA deacetylase

Greta Gakaite[1], R Statkevičiūtė[1], R Meškys[1]

[1]Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Science Centre, Vilnius University, Vilnius, Lithuania
greta.gakaite@chgf.stud.vu.l

Proteins containing the ASC-1 homology (ASCH) domain are present in all domains of life, including several prokaryotic viruses. It is suggested that these 103–120 amino acid long domains could have functions in transcription co-activation, RNA metabolism, and translation regulation in prokaryotes. However, despite their high abundance in nature, most proteins of the ASCH superfamily are considered hypothetical due to a lack of experimental data. One of the well-characterized proteins containing the ASCH domain is an amidohydrolase YqfB from Escherichia coli. The primary substrate of YqfB is the modified nucleoside N4-acetylcytidine (ac4C), which is highly abundant in both eukaryotic and prokaryotic RNA molecules. Recently, we identified a novel RNA-binding ASCH domain-containing protein TthASCH from the thermophilic bacterium Thermus thermophilus, which was shown to be active towards ac4C in vitro. This study aimed to investigate the catalytic mechanism of enzymatic ac4C hydrolysis and to confirm whether TthASCH can act as an eraser of the ac4C modification of tRNA. To reveal the amino acids that may be crucial for its catalytic activity of TthASCH, we purified 13 mutants of TthASCH and determined their amidohydrolase activity towards ac4C by using thin-layer chromatography. The results suggested a catalytic dyad (Lys-Glu) and Arg acting as an oxyanion hole, hence, TthASCH forms an active center different compared to YqfB. Furthermore, we present experimental evidence that the synthesis of TthASCH in E. coli leads to decreased ac4C levels in tRNA molecules. This study expands the knowledge of the possible functional diversity of proteins belonging to the ASCH superfamily, as no tRNA ac4C erasers have been reported to date.