ÚOCHB AV ČR
IOCB PRAGUE
Ústav organické chemie a biochemie
Akademie věd České republiky, v. v. i.
Institute of Organic Chemistry and Biochemistry
of the Czech Academy of Sciences

Dr. Hana Cahová
Junior research group leader
Institute of Organic Chemistry and Biochemistry
of the Czech Academy of Sciences
Flemingovo namesti 542/2
CZ - 16610 Prague 6
Email: cahova@uochb.cas.cz

Prof. Dana Holá
Chair of the Doctoral board
Faculty of Science, Charles University

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Evaluation Report for the Ph.D. award
"Interplay between the tRNA anticodon stem and small ribosomal proteins forming the decoding site during stop codon readthrough"
of MSc. Zuzana Čapková (Pavlíková).

Dear Chair and Committee Members,

I hereby submit the evaluation report of Zuzana Čapková's doctoral thesis for the award of the Degree of Doctor of Philosophy (Ph.D.).
Zuzana Čapková's Ph.D. thesis is based on three accepted or published manuscripts. In two manuscripts, published in Nature and accepted in Nature Structural and Molecular Biology, Zuzana is the first author. In the third manuscript, published in Nucleic Acids Research, she is the second author. All three works are of excellent quality and demonstrate the high standard of the laboratory where the work was performed.
As the title suggests, the entire work is dedicated to the exploration of stop codon readthrough in interplay with ribosomal proteins and the tRNA anticodon stem. In the first manuscript, where Zuzana is the second author, she contributed to the identification of a group of four yeast tRNAs ($tRNA^{Cys}$, $tRNA^{Trp}$, $tRNA^{Gln}$, and $tRNA^{Tyr}$) that induce readthrough at the stop codon. In this manuscript, the authors also reported the development of a new method, YARIS (Yeast Applied Readthrough Inducing System).
The second work, on which this thesis is based, was a breakthrough in the field and was published in the prestigious journal Nature. In this work, Zuzana contributed to the discovery of a new trypanosomatid named *Blastocrithidia nonstop* and to understanding the mechanism by which this organism overcomes the problem of widespread UGA stop codons in the coding regions of its nuclear genome. She found that shortening the anticodon stem of tRNATrp from the canonical 5 base pairs to 4 base pairs is essential for efficient readthrough of the stop codon in this organism. In general, she

observed that the anticodon stem, particularly some base pairing in this region, plays a very important role in proper decoding.
The author's most recent work was recently accepted in Nature Structural and Molecular Biology. Here, she shows that the N-terminus of the eS30 ribosomal protein extends into the decoding pocket and interacts with the anticodon stem of $tRNA^{Gln}$ and other tRNAs. Specifically, Arg10 is responsible for this contact, as its mutation to Ala led to the disruption of the readthrough capability of $tRNA^{Gln}$. The authors also studied other ribosomal proteins and their interactions in the codon-anticodon minihelix region.
In general, Zuzana presents an excellent piece of scientific work for her PhD and has set the bar high for future PhD students. The PhD thesis is written in very good English, and I did not find any significant mistakes. The structure is clear, logical, and typical for this field.
The only criticism I have is that the author did not properly introduce the topic to experts from fields other than RNA translation. For example, on the first page of the "Current State of Knowledge" section, the author describes various mutations in the ribosome and ribosomal proteins without including any figures. Unfortunately, I do not have this structure memorized to visualize it easily. This issue occurs a few times throughout the entire work. Additionally, sometimes the text goes into extreme detail and loses its flow, requiring many sentences to be read several times to understand what the author intended to convey.
This criticism is not meant to diminish the value of the scientific work. Rather, it should serve as advice for the future preparation of any scientific work, including grant proposals, lectures, or articles.

I would like to ask the author to answer the following questions:

1. On page 12, the author claims that mRNA containing premature termination codons (PTCs) is also a target for degradation by nonsense-mediated decay. Unfortunately, this issue is covered only by this sentence. Can you please explain the molecular mechanisms of this process in more detail?

2. On page 18: Is it known why the essential protein eS30 is synthesized as a fusion protein with the ubiquitin-like protein FUBI in the majority of eukaryotes? What is the role of FUBI?

3. The understanding of stop codon readthrough seems to be critical for future therapeutic use in human medicine, as mentioned by the author. Concerning this topic, I would like to ask two questions:
   - Does any human disease exist that is connected with mutations in the anticodon stem of any tRNA?
   - Could the author suggest one example of a therapeutic approach to an inherited human genetic disease connected with a gene carrying a PTC?

In conclusion, I would like to stress that Zuzana Čapková (Pavlíková) has demonstrated that she is capable of excellent independent scientific work. I gladly recommend that her work be accepted for PhD defense. I wish her all the best in her future career and look forward to her future scientific contributions.

Hana Cahová, Ph.D.

Flemingovo nám. 2
166 10 Praha 6
Czech Republic
+420 220 183 333
uochb@uochb.cas.cz
www.uochb.cz
IČ: 61388963
DIČ: CZ61388963