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„Analysis of Mutual Interactions of the Human Multifactor Complex Components in Translation Initiation“

Name of the Student: Mgr. Terezie Prilepskaja

Name of the Supervisor: prof. Leoš Shivaya Valášek, Ph.D.

Evaluation of doctoral thesis

Dear chair, dear committee members,

The doctoral thesis by Mgr. Terezie Prilepskaja concisely describes the complex aspects of human translation initiation and provides a detailed insight into the extensive work she performed to defend her research before a scientific committee.

The first part of the thesis – „Introduction“ – offers an overview of the current knowledge on eukaryotic translation initiation, highlighting the mechanism of start codon selection on the small ribosomal subunit. It focuses primarily on three eukaryotic initiation factors: eIF1, eIF3, and eIF5, and their interplay in orchestrating and regulating this process. The section is well-written, though it contains some typos and missing verbs that could be corrected later. The introduction clearly demonstrates the main focus of her doctoral research, and it is well connected to the aims of the thesis, which are clearly stated in the „Aims of the Study“ section.

The Materials and Methods section is detailed, containing extensive information ranging from specific cell lines and antibodies to a comprehensive list of oligonucleotides. It covers the construction of plasmids, cell cultivations, and multiple assays, all of which were essential for the scientific study highlighted in the doctoral thesis. Since this section includes all the necessary information to understand the experiments she performed, I have no specific comments.

The Results are divided into two sections. The first section includes two high-profile publications in NAR and Cell Reports, in which Terezie is either the first author or a co-author. I had the pleasure of reading these papers prior to the doctoral thesis. Given that these publications underwent thorough peer-review, I focused my critical assessment on the second section, “Additional Unpublished Results.” In this section, Terezie primarily focused on eIF3c, a component of the eIF3 multi-component complex, to investigate its role in start codon selection and its binding interactions with eIF5 and eIF1. The results section showcases the extensive work Terezie conducted to elucidate the mechanistic insights into how eIF3c, along with its depletion or mutation, can regulate the protein levels of other initiation factors, eIF5 and eIF1, and how these events impact

start codon or near-start codon selection. I found this part particularly valuable, as it demonstrates the remarkable amount of effort Terezie invested to produce convincing data, which I believe will contribute to a major publication. My specific comments and questions are provided below.

The final part of the doctoral thesis, comprising the "Discussion" and "Conclusions," appropriately addresses both published and unpublished findings. While I would have appreciated a few more schematic images to better visualize and understand the discussed mechanistic implications, the overall quality remains very high. Particularly noteworthy is Terezie's ability to generate mutated forms of eIF3c, a challenging task, that positions her and her colleagues well for detailed investigations into the functional aspects of start codon selection in humans, especially in relation to eIF5.

Comments/questions:

1. When you refer to the open or closed conformation, are you specifically talking about the conformation of the 40S subunit in terms of initiator tRNA accommodation? Do you mean the movement or closure of the head towards the body of the 40S subunit?

2. When testing the start codon selection with downregulated eIF3c, did the constructs used contain any 5'-UTR before the start codon? Did you consider the implications of the Kozak sequence on start codon selectivity, especially in relation to the length of the 5'-UTRs?

3. Does the upregulation of certain near-cognate codons as start sites have structural implications for why this might occur? Did you attempt to explain it based on structural mechanistic insights?

4. Could the upregulation of eIF1 and eIF5 in eIF3 KD cells be driven by the presence of alternative start sites on the transcripts that encode these initiation factors?

5. It is interesting that some of these initiation factors have long unstructured C- or N-terminal tails. Could these intrinsically disordered tails have roles in translation regulation beyond recognizing their interacting partners? For instance, did you investigate whether these tails contain specific short amino acid repeats that might suggest additional functions?

6. Recent research indicates that visualizing eIF5 as a complete structure on the 40S subunit is challenging. Have you considered an approach to stabilize eIF5 on the subunit to potentially elucidate the mechanistic details of its role in mammalian translation initiation?

In conclusion, I am confident that Mgr. Terezie Prilepskaja`s scientific publications, as well as her doctoral thesis, meet the internationally recognized standards. Therefore, I highly recommend awarding her dissertation with a PhD degree.

In Brno, 01.06.2024

Sincerely yours,

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