LABORATORY OF REGULATION
OF GENE EXPRESSION

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Evaluation Report for the Ph.D. award

Name of the Student: **RNDr. Klára Frydrýšková**

Title of Thesis: **Noncanonical human eIF4Es in and out of the RNA granules**

Name of the Supervisor: **RNDr. Martin Pospíšek, PhD.**

Dear chair, dear committee members,

Here I submit to your hands the evaluation report on the dissertation work of RNDr. Klára Frydrýšková for the award of Degree of Doctor of Philosophy (Ph.D.).

RNDr. Frydrýšková's work is based on two already accepted papers, one of which features RNDr. Frydrýšková's as a solo first author (BMC Mol Biol) and the other as a second author (Mol Genet Genomics). One more manuscript (a review article featuring RNDr. Frydrýšková as the first author) is under review in WIREs and is expected to be published soon.

As the title states, her work deals with the less known forms of the eIF4E initiation factor that has been implicated in mediating the interaction between mRNA and a small ribosomal subunit during canonical translation initiation. Altogether three aims are listed and largely fulfilled.

This thesis has classical organization and exemplary graphic layout, it is written in English which is valued, yet the English grammar and vocabulary suffers significantly from numerous imperfections (missing articles and punctuation, typos, wrong word usage, incorrect sentence structures, etc.). Nonetheless, it does provide the reader with a broad overview of the entire problematic, detailed description of all results, and

insightful and critical discussion, followed by a comprehensive and up-to-date list of references.

Below I list several minor issues that I noticed followed by my specific questions:

- Abstract; the sentence beginning with: "Heat stress-induced re-localization..." is missing a verb and makes no sense as such
- also, some critical (for an outside reader) typos like e4F instead of eIF4F (page 14) or eIF3-B instead of eIF4E3-B (page 45) should have been avoided to prevent confusion
- Introduction; the overview of canonical translation initiation is not so well-described, for example
    - it is impossible to say what comes first, whether the formation of the eIF4F complex with mRNA or the 43S PIC; I think both occur simultaneously
    - the description of the AUG selection process is rather naive and imperfect; in fact, references in the entire chapter 2 are rather scarce - just reviews at the beginning are not enough
    - What purpose the description of RAN translation serves for this thesis?
    - What is the real difference between 43S and 48S PICs? I do not think these terms were used correctly.
    - Page 45; "Due to its Trp to Cys substitution, 4E3 is unique in its cap-binding properties as contacts between 4E3 and the cap are more extensive than in any other 4E-family members. The binding affinity of 4E3 is 10 to 40 fold lower than that of 4E1." More extensive contacts, yet lower binding affinity? Please explain.
    - Chapter 3.2. does not say anything about the PB formation, contrary to the promise in its title.
- Results; some chapters are extremely short, like 3.9. (just a title), or 3.8.6. and 3.8.7. (just one sentence) – could have been organized better, under "Others" for example
- Material and Methods; most of this section is exemplary, with the exception of the PCR protocol (less well-arranged); also, the list of chemicals is missing (not sure if it is mandatory, though)
- labeling of figures could be placed underneath each figure – it is a common praxis, I think
- Discussion & my main questions
    - eIF4E3-A can be found in SGs only and was proposed to compensate for attenuated roles of eIF4E1 during translation initiation under certain stress conditions. How would you prove or disprove this suggestion?
    - Page 141; "The truncated isoform of human eIF4E3, eIF4E3_B, lacks an important part of the eIF4G-binding consensus, including the Trp73Ala residue. In this study, we demonstrated that eIF4E3_B neither localized to SGs or PBs nor bound scaffold protein eIF4G (Fig. 16G, Fig. 18G and data shown in the enclosed publication only). This outcome is in agreement with the reported importance of the conserved

motif containing a Trp73Ala residue, which is critical for eIF4E1 interaction with both eIF4G and 4E-BPs and, consequently, for eIF4E1 localization to both SGs and PBs (Marcotrigiano et al. 1999; Joshi et al. 2005; Ferrero et al. 2012)." I do not understand this. First of all, what is Trp73Ala(74?) "residue"? Then, according to the Introduction, eIF4G localizes neither to SGs nor to PBs, and so does not the 4E3-B lacking Trp73Ala. Where does the agreement for "the importance of the conserved motif containing a Trp73Ala residue, which is critical for eIF4E1 interaction with eIF4G..." come from then?

- Your model in Fig. 62 is certainly interesting. Please, suggest a couple of experiments how it could be tested.
- With respect to the occurrence of 4E2 with eIF3 (the description of which on page 111 is, regretfully, rather outdated and not so clear) in the same complex, I would be curious to see if it is specific for 4E2 only and not for other two 4E forms. Perhaps only then one could come up with exciting theories concerning its physiological importance. Let's say, for the purpose of this examination, that it is specific. What experiments would you propose test it?

Taken together, this thesis represents a rather large amount of the quality work of this PhD candidate and clearly demonstrates her experimental, as well as intellectual skills that are required to obtain a Ph.D. degree. I have known Klára since her early days with Dr. Martin Pospisek and remember her skepticism concerning the difficulty of this rather demanding project that was, at the end of the day, successfully overpowered by her genuine drive, hard work and high spirits. That is great, I am really happy for her and gladly recommend acceptance of this PhD. work!

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