

Posudek oponenta na diplomovou práci

oponentský posudek

Jméno posuzovatele:

Tomáš Vomastek

Datum:

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Autor:

Bc. Marharyta Ramanava

Název práce:

Introducing of an experimental approach to study mitotic translation in human cell lines

Cíle práce

In the presented thesis, Bc. Marharyta Ramanava focused on two related projects. The first aim was to determine the interactome of eIEF4E initiation factors in different phases of the cell cycle. To this end, she established hTERT-RPE-1 cell lines that inducibly express eIEF4E initiation factors tagged with either GFP or 3xFLAG epitope. These cell lines were used for immunoprecipitation of eIEF4E factors and subsequent analysis of the immune complexes by Western blotting and mass spectrometry. The aim of the second part of the study was to characterize gene-specific translation at different phases of the cell cycle. To this end, the author isolated various ribosomal fractions and analyzed the amount of selected mRNAs using qRT-PCR.

Struktura (členění) práce, odpovídá požadovanému? ANO

Rozsah práce (počet stran): 92

Je uveden anglický abstrakt a klíčová slova? ANO

Je uveden seznam zkratk? ANO

Literární přehled:

Odpovídá tématu? ANO

Je napsán srozumitelně? ANO

Použil(a) autor(ka) v rešerši relevantní údaje z literárních zdrojů? ANO

Jsou použité literární zdroje dostatečné a jsou v práci správně citovány? ANO

Materiál a metody:

Odpovídají použité metody experimentální kapitole? ANO

Kolik metod bylo použito? cca. 30

Jsou metody srozumitelně popsány? ANO

Experimentální část:

Je vysvětlen cíl experimentů? ANO

Je dokumentace výsledků dostačující? ANO

Postačuje množství experimentů k získání odpovědí na zadané otázky? ANO

Diskuze:

Je opravdu diskuzí, nejde jen o konstatování vlastních výsledků? ANO (i když v omezeném rozsahu, viz komentář níže)

Jsou výsledky porovnávány s literaturou? ANO

Jsou uvedeny nějaké hypotézy či návrhy na další řešení problematiky? ANO

Závěry (Souhrn) :

Jsou výstižné? ANO

Formální úroveň práce (obrazová dokumentace, grafika, text, jazyková úroveň):

The thesis is written in English, and as far as I can tell, the presented thesis contains infrequent grammatical errors, typos and stylistic clumsiness that are typical for this kind of work. Some details escaped the author's attention, e.g. on page 10 the abbreviation of the N-terminal domain should be NTD and not NDT; two tables on page 27 have the same number, "the name "FavorPrep™™ Plasmid Extraction" contains "TM" twice. There is also no explanation as to why some bars in graphs in Fig. 22 are blue/black. Despite these shortcomings, the overall quality of the text is very good, although additional proofreading would be beneficial.

The introduction part is well written and contains all the information needed to understand the problematics of translation initiation and translation efficiency. Personally, I would welcome more figures to illustrate the text, e.g. the figure(s) showing schematically the interacting partners of the eIF4E initiation factors. Also, in my opinion, the introduction and Aims of the thesis would fit better after the literature review. This is not a criticism, just a feeling that it would be easier for the potential reader to understand the text flow.

The Results section describes in detail the results of the experiments. It is evident that the author spent substantial time by adopting new techniques and their extensive troubleshooting. Using these techniques, she was able to carry out initial experiments on the targeted proteomics of eIF4E1 immune complexes and the translation efficiency of selected mRNAs. The amount of data is impressive and the way they were obtained are clear and well described. I would just like to point out that in Fig. 23 the control immunoprecipitation of a cell line not expressing eIF4E is missing. Some of the results are also discussed in this section.

Given that some results have already been discussed in the Results section, the Discussion section mainly summarizes the results. This is understandable, as the work focused primarily on adopting and troubleshooting of new techniques and the characterization of newly developed reagents. However, I think that the data obtained from the targeted proteomics provides some results that could be discussed in more detail. This section thus somewhat diminishes the quality of this overall good thesis.

Splnění cílů práce a celkové hodnocení:

Despite the comments mentioned above, the methodological and professional level of the work is very good. From the results presented, it is clear that the objectives of this study were achieved. The author has used several experimental approaches and many procedures and techniques. This shows the competence of the candidate in conducting the research. Based on the quality of Marharyta Ramanava's diploma thesis, I recommend that this thesis be accepted as the fulfillment of the requirements for the degree of Master of Science.

Otázky a připomínky oponenta:

1. Progression through the cell cycle and the effectiveness of cell synchronization was determined by Western blot using cell cycle markers such as CyclinA2. This is a tedious and hardly quantifiable method. I think that analysis by flow cytometry would be much more informative as it could quantitatively measure the proportion of cells in each phase of the cell cycle. Given the simplicity of flow cytometry, it is quite surprising that it was not used. Could the author comment on this?

2. Although the proteomic experiment was only performed once, it appears that eIEF4E immune complexes from all phases of the cell cycle consistently contained similar set of proteins, including the bait itself, eIEF4G and isoforms of PABPC. Could the author present the model of the eIEF4E interactome based on the published data and discuss the obtained data in this context?

3. I did not find any information on how many micrograms of total proteins was loaded on the SDS-PAGE (Figs. 12, 18, 20, 28). The text only describes the preparation of the lysates without mentioning the determination of the protein concentration. Does this mean that the protein concentration was not measured and it was assumed that an equal amount of proteins was present in all samples?

Návrh hodnocení oponenta

výborně velmi dobře dobře nevyhověl(a)

Podpis oponenta: