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Master Thesis Evaluation

Filip Nemčko

Title: „Ribosomal protein Rpl22 regulates the splicing of its own transcripts“

Filip Nemčko has conducted his Master thesis work in the group of doc. RNDr. Petr Folk and was supervised by Mgr. Kateřina Abrhámová, Ph.D. He investigated the functional relationship between paralogs of ribosomal proteins (RPs) in the yeast *Saccharomyces cerevisiae*. RP genes are highly expressed in *S. cerevisiae* and are among the few genes that contain introns. Their introns differ from those of non-ribosomal proteins and were suggested to control the expression of their host genes as well as their paralogous partner. Indeed, out of seven tested pairs of RP paralogs, Mr. Nemčko identified one pair, Rpl22a and Rpl22b that exhibited a complex auto- and cross-regulation circuit. In a heroic attempt to determine the underlying mechanism of this regulatory circuit, Filip constructed and tested a series of whole-gene and intron-deletion mutants of *RPL22A* and *RPL22B* and made several interesting observations. First, both paralogs exhibit a reciprocal positive cross-regulation and a negative auto-regulation and this regulation depended on the presence of both introns. Second, the observed differences in *RPL22B* mRNA level in the different mutant backgrounds could be recapitulated with a splicing reporter that carried only the *RPL22B* intron. This suggested that *RPL22B* mRNA levels are regulated at the level of pre-mRNA splicing, and that the intron modulates splicing efficiencies. A splicing reporter carrying the *RPL22A* intron did not recapitulate the endogenous situation. Third, using the yeast-three-hybrid system, Mr. Nemčko discovered that both paralogs, Rpl22a and Rpl22b, bind to a specific region within the *RPL22B* intron, while mutant proteins without functional RNA binding domain failed to bind. Fourth, the cross-regulation of Rpl22 is likely evolutionary conserved since the Rpl22 ortholog from the yeast *Kluyveromyces lactis* was able to bind to the same intronic region of *RPL22B* and was able to regulate *RPL22* mRNA levels in *S. cerevisiae* and *K. lactis.* And finally, Filip Nemčko used sequence comparisons and RNA folding of several yeast *Rpl22* introns to predict the Rpl22-binding site and its

folding into a putative stem-loop structure.

Overall, Filip Nemčko presents a well-written thesis, which comprises several interesting findings. The introduction is very interesting to read and contains precise numbers from the actual literature and is complemented with beautiful comprehensive figures. Filip nicely introduces the specific features of Rpl22 and the dissimilarities between both paralogs including the very interesting difference in codon bias. All experiments appear well planned and are presented in a logical order. Experimental approaches and tools are appropriate for the investigated questions and all experiments seem carefully performed and provided reproducible and quantitative data, which were subjected to thorough statistical analyses. Hypotheses, research aims and questions are clearly formulated and the results are well described in a logical and comprehensive way. All presented conclusions are supported by the data; however, the story seems somehow incomplete and would have benefited from a functional study either with smaller intron deletions or with structure probing experiments to further confirm his observations and conclusions.

Mr. Nemčko certainly lost precious time during his unsuccessful attempt to validate published data. It is very appreciated that he shares his critical view on previous observations and discusses them based on his own data. Moreover, Mr. Nemčko acknowledges gaps in his thesis work and provides missing data, for example supporting experiments that have been performed by other lab members. The relevance and importance of his findings are discussed with recent literature without overstating them. It would have been nice if Filip provided a model of how Rpl22 binding to the introns might modulate splicing efficiencies in two different directions. Does Rpl22 binding stabilize the stem-loop structure and this way brings functional splicing elements in close proximity? Does it block splicing relevant sequences?

The thesis work of Filip Nemčko is part of a publication, where he is co-first author. Only four minor flaws dim the overall very good impression of the thesis: 1) There are many errors in the use of articles and a few imprecise formulations. 2) The Outlook is missing with suggestions for future research directions or experiments. 3) The exposure of the primer extension experiment is too low. 4) Size markers are missing on the gels.

In summary, Filip Nemčko has presented a very interesting, comprehensive and successful Master thesis. Without hesitation, I grade it with

„very good“ (velmi dobře)

Sincerely

Jun. Prof. Michaela Müller-McNicoll

I have the following questions for Filip:

1.) Is it possible to use Cyclohexamide (CHX) to inhibit NMD in the different yeast mutants and re-test the *RPL22A* reporter or endogenous pre-mRNAs for splicing regulation? What would you expect?

2) Did you use a reporter with the entire gene or swapped introns to test for splicing regulation? What would you expect?

3) The yeast-3-hybrid system is quite artificial. Could you use RIP to confirm Rpl22 binding to the intron?

4) Why is the complete intron is not bound by Rpl22a or Rpl22b?

5) How do you make sure that the structure of the I2 region is actually occurring in the cells? Please provide structure predictions of the separate I2 region within the reporter sequences!

6) Did you test for transcription termination of the intron construct by Northern blot?

7) Did you try to generate a mutant lacking only the I2 region? If so, why did it fail?

8) What other functional analysis could you do to test the intronic Rpl22 binding site I2? Which experiment would you do to confirm its structure and which to confirm Rpl22 binding?

9) The intron structure might be conserved without sequence conservation! How can you test this?

10) Why does Rpl22 from *S. Pombe* not bind to intron I2? What is the difference?