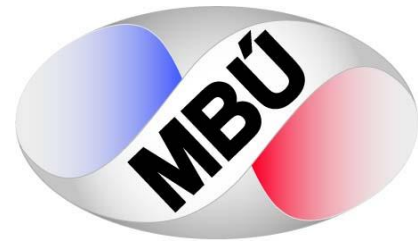


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Reviewer's report of the Thesis **The role of pre-mRNA splicing in human hereditary diseases** by Anna Malinová.

The Thesis focuses on the splicing machinery, namely of the U5 small ribonucleoprotein particle (U5 snRNP), its biogenesis, and mutations involved in hereditary diseases of which the center of attention is retinitis pigmentosa (RP).

Preceding the Thesis are Acknowledgements where I was impressed by Real Madrid contributing to the advancement of science. However, considering the topic of the Thesis, I was surprised that the Irish rock band U2 was not mentioned, and also acknowledgements to the Moravian band U5 from Havírov were most notably missing.

The Thesis consists of 123 pages that are divided into four main Chapters (Literature Review, Materials and Methods, Results, and Discussion) plus References. In general, the Thesis is well written with occasional errors (e. g. confusing "data" with "dates"). However, the overall structure of the Thesis is well organized and it reads well.

The first Chapter contains Literature Review. This Introduction concisely describes splicing, splicing factors, biogenesis of snRNAPs, the roles of splicing in diseases with a special focus on retinitis pigmentosa that is caused by mutations in several splicing factors. This Chapter provides sufficient background information for the subsequent Chapters.

The next Chapter describes the materials and methods used in the study. The information is sufficiently detailed.

The following Chapter contains Results and it is based mainly on two publications in peer-reviewed journals with IF ~ 7-9. AM is the first author of one publication and a coauthor of the second publication.

AM showed that depletion of PRPF8, one of the core U5 snRNA components, caused a lack of association of other U5-specific proteins with this snRNP, and accumulation of incomplete U5 in Cajal bodies. AM then focused on selected

mutations in PRPF8 that are known to be present in patients with RP, and demonstrated that these mutations do not allow proper assembly of snRNPs, and, consequently, negatively affect splicing. Finally, she identified novel interacting partners of PRPF8 and demonstrated their role in the U5 snRNP biogenesis.

**Questions:**

- 1/ In Fig. 22, HSP70, a chaperone, is shown in two dots – do these dots represent two isoforms of the same protein (perhaps originated by alternative splicing) or a non-modified and modified (e. g. phosphorylated) forms of the protein?*
- 2/ The mutations were studied in tissue cultures. The next logical step would be to create an animal (mouse) model with these mutations, and carry out transcriptomic studies of the retina as well as other tissues. Are such animal models available or planned?*
- 3/ The symptoms of RP may develop over many years. In addition to the primary mutations in e. g. PRPF8, could other mutations in the retina caused by UV appear subsequently and contribute to the manifestation of the disease?*
- 4/ Could overexpression of Hsp90 assist in folding of PRPF8 so that the phenotype would be less severe?*
- 5/ The prevalence of RP is approximately 1 in 4000. Are there differences between populations (e. g. is it more frequent in Caucasians than in other races?)? What is the prevalence in the Czech Republic?*

In summary, the Thesis contains a large amount of meticulously documented work that brings new insights into our understanding of U5 snRNP biogenesis and into the molecular basis of RP.

I recommend this Thesis to be classified as passed, and I wish the author all the best in her further professional career.

Prague, 24 July 2017

Libor Krásný