Brno, 11.9. 2024

Review of PhD thesis:"Functional significance of DHX38 in pre-mRNA splicing and its role in the mechanisms of retinitis pigmentosa"

PhD candidate: Msc. Mina Rajkovic

Pre-mRNA splicing is in eukaryotic cells and particularly mammals the driving force of protein diversity and has irreplaceable role in many aspects of development and life. Pre-mRNA splicing is catalyzed by one of the largest, if not the largest protein-RNA complexes in humans. It is not surprising that mutations in this machinery have been linked to several human disease. Patients with retinitis pigmentosa (RP), a genetic progressive degeneration of eyes, are found with mutations in many splicing factors. Among those, the helicase DHX38/PRP16 is the main topic of this PhD thesis. Mina's task was to investigate its role in slicing and evaluate its involvement in RP .
The presented PhD thesis contains all the required parts. I very much liked the theoretical introduction and background to the experimental part. Mina has elegantly preesented the necessary basic information in a historical time line. It is not trivial to write an introductory overview to a splicing topic due to the extensive existing literature and literally hundreds of relevant publications. In the experimental part, Mina used several approaches to obtain results that indicate DHX38 association with several key splicing factors, mainly of the U2 snRNP. By using reporter systems, she then showed that the interaction with U2snRNP components facilitates branch site selection and facilitates splicing of suboptimal 5' splice sites. These studies are part of the published work with Mina Rajkovic as the first author.

I have some questions and comments listed below:

1. On page 59/60 you show and state that DHX38 shows nuclear as well as cytoplasmic localisation. Do you have any hypothesis for DHX38 role in the cytoplasm? Has this observation been made by another group?

2. In the next chapter, you show the efforts to uncover DHX38 interactors by using protein based IPs. Is increasing salt concentrations really good aproach to identify interacting proteins and why do you things so? Can you discuss alterantive aproaches how to search for interacting proteins and discuss pros and cons?

3. Page 62. FLAG-DHX38 IP did not reveal coprecipitating snRNAs. What would be the suitable positive control to show that this experiment could lead to also positive answer?

4. Do you think that HEK293 cells are suitable cell line model to search for RP-related mechanisms?

In summary, the PhD thesis fulfils all the criteria to qualify for the PhD title and thus I agree with and am looking forward to the thesis defense.

Stepanka Vanacova

Professor of Molecular Biology and Genetics
CEITEC, Masaryk University
Brno, Czech Republic