DIOSCURI
CENTRE FOR RNA-PROTEIN INTERACTIONS
IN HUMAN HEALTH AND DISEASE

Warsaw, 14th September 2021

prof. dr hab. Gracjan Patryk Michlewski

To: Rada Dyscypliny Nauki Biologiczne Uniwersytetu Jagiellońskiego

Review of mgr Anna Salerno-Kochan PhD thesis

entitled: „Structural characterization of Mei-P26 protein – a central regulator of RNA biosynthesis during cell fate decision"

Regulation of gene expression is essential for faithful and timely production of proteins at all stages of cellular and organismal development. This regulation is achieved at the level of transcription and post-transcriptionally. Transcription factors binding to DNA are responsible for transcriptional regulation, whereas RNA-binding proteins (RBPs) are key players in post-transcriptional regulation. RBPs bind to nascent transcripts and control all steps of RNA metabolism, from 5' and 3' modifications, splicing, export to cytoplasm, translation, turnover, and many others. Some RBPs are crucial factors that inhibit or promote cellular differentiation. Importantly, over the last decade thousands of new RBPs have been identified and are still awaiting detailed structural and functional characterisation.

The focus of this PhD thesis was a protein called Mei-P26, expressed in the germline of *Drosophila melanogaster*. The protein is homologous to TRIpartite Motif NHL (TRIM-NHL) and few other proteins important for early stages of cellular differentiation. They consist of RING, B-Box (BB), Coiled-Coil (CC) and NHL domains. The RING domain is responsible for E3 ubiquitin ligase activity, BB for protein interactions, CC for homo and heterodimerisation, whereas NHL domain of these proteins have been shown to bind RNA as well as proteins.

The main aim of this PhD was to produce, purify and characterise DmMei-P26 (which I will be calling Mei-P26). Mgr Anna Salerno-Kochan used several experimental approaches to achieve these goals. First, she tried to used bacteria and yeast for production Mei-P26 and its truncated versions. These approaches have not yield positive results. Then she employed baculovirus expression system and was successful in obtaining large quantity of pure and stable NHL domain of Mei-P26 (Mei-P26 NHL). She used this protein to establish preferential binding to U-rich ssRNAs in low micromolar Kd. Moreover, she managed to obtain high-resolution crystal structures of this domain, which she compared with other known NHL structures. Finally, in collaboration with Prof. Bujnicki, mgr Anna Salerno-Kochan mapped RNA contacts in the domain that allowed her to design mutants with impaired RNA-binding activity.

In my view the biggest achievement and discoveries of this PhD thesis are:

1. Establishing condition for production and purification of Mei-P26 NHL domain.
2. Atomic structure of the Mei-P26 NHL domain.
3. Lack of interaction between Mei-P26 mutants (page 99) to SEQ3 oligonucleotide UUUUACA, opposed to low micromolar binding of similar mutants to U9 oligonucleotide (UUUUUUUUU).

The results from point 3 indicate that Mei-P26 NHL has both specific and unspecific RNA-binding activities. This might be important in the context of understanding its function as well as exploring structure-function relationship of similar RBPs. The organisation and the presentation of the PhD thesis is excellent. The thesis consists of table of contents, acronyms, abbreviations, abstract (in English and Polish), introduction, aims of the studies, scope of the research, materials and methods, results, discussion, conclusion, future

perspectives, list of figures, list of tables and references. Most figures are well presented, however the font on few of them is too small (for example Fig. 10). The introduction feels too much like a textbook for year 1 undergraduate students. It would be much better to better introduce structural and functional roles of NHL-containing proteins. The aims are very clear and concise. The section describing materials and methods is succinct, presenting all necessary details. I very much appreciate the results section, which shows negative results. Too often we only see the final, polished version of the study. By reading about the journey towards the positive results we learn much more about the limitations and challenges working with similar proteins. Also, we see the perseverance of the PhD candidate that allowed her, at the end, to obtain great quality results. Finally, the discussion section is adequate but could be improved with more references and comparisons with other RBPs.

Below, I am presenting more details comments regarding selected parts of the PhD thesis.

1. In the abstract we do not see that Mei-P26 is associated with TRIM proteins. We only learn this in passing on page 27.
2. In paragraph 2.2.2. on page 25 we can read that CLIP-seq and RIP-seq are the methods that 'allowed the *de novo* discovery of multiple non-canonical RBPs'. This is in fact not true. These methods are protein-cantered and rely on the immunoprecipitation of known RBPs. The methods that allowed discoveries of new RBPs are mostly associated with mass spectrometry (for example RNP capture).
3. Mei-P26's homolog TRIM-NHL belongs to a large family of proteins that perform multitude of functions. Some of them are also binding to RNA. I would like to see more introduction and discussion about this family of proteins.

4. On page 35 we can read that 22nt RNA duplex forms miRISC. This is not true. Only one strand from the duplex is retained with Ago and forms mRISC, capable of binding to target mRNAs.
5. On page 73 there is a spelling mistake – crated – should be created.
6. To claim that Mei-P26 NHL 'has a strong preference toward poly-uridine tracts' one would have to analyse other oligonucleotides, including poly C, poly A, poly G and other RNAs enriched in these nucleotides. Thus far, most tested RNAs are U-rich and only contain few substitutions.
7. I am not sure what is the added value of list of figures with figure legends.

In summary, PhD thesis of mgr. Anna Salerno-Kochan is extremely interesting and important addition to the field. I am not surprised that the results are in the final stages of the review process in the prestigious Nucleic Acids Research Journal. I am thus asking for the PhD thesis to be awarded distinction. Despite the recent advances of machine learning structure prediction such as AlphaFold, determining protein structure-function relationship remains high on the priority list towards understanding how cells and organisms work and respond to environment. The results obtained by mgr. Anna Salerno-Kochan are valuable addition to the field of RBPs and RNA-protein interactions.

The PhD thesis fulfils all criteria from the 'Ustawa o stopniach naukowych I tytule naukowym oraz o stopniach i tytule w zakresie sztuki (Dz.U. z 2016 r. Poz. 882)'.

With best regards,

prof. dr hab. Gracjan Patryk Michlewski