ÚOCHB AV ČR
IOCB PRAGUE
Ústav organické chemie a biochemie
Akademie věd České republiky, v. v. i.
Institute of Organic Chemistry and Biochemistry
of the Czech Academy of Sciences

Evaluation of the doctoral thesis submitted by Anna Marešová: „ Linking transcriptional regulation of lipid metabolism with catastrophic mitosis in fission yeast.“

General Remarks:

The submitted thesis describes a substantial amount of work that contributed to the project that was focused on the key players involved in the transcriptional regulation of lipid metabolism genes concerning mitotic fidelity in the fission yeast. In particularly, Anna looked closely in two transcriptional regulators of lipid metabolism genes - Cbf11 and Mga2. She has revealed that they work closely together, which might suggest that their roles are dependent of physical interaction of these proteins. Unfortunately, this has not been confirmed. She also revealed that the Cbf11 roles, that additionally include cell cycle progression regulation, are closely coupled to its canonical DNA-binding capacity. Perhaps the most important finding is the observation that lipid metabolism significantly impacts chromatin structure and gene expression regulation, highlighting how fatty acid synthesis contributes to mitotic fidelity.

Anna’s work has been published in two first author papers and she also contributed by her expertise to two additional papers that are related to her PhD projects. Additionally, she has contributed to two excellent unrelated papers.

Major comments:

The high standard of the published work is self-standing, and adding a description of an unsuccessful attempt to prove the physical interaction between Cbf11 and Mga2 does not add any extra value to the thesis. It is not surprising that the co-immunoprecipitation experiments did not capture the interaction when the partner proteins were not expressed. The data also suggest that Cbf11 was proteolyzed into several shortened variants. I would expect that the experimental design would take the DNA-binding capacity of both proteins into account.

Minor comments:

The use of bullet point lists within the summary of the published papers is somewhat unfortunate. A concise description in the form of two paragraphs would be more appropriate for this purpose.

I was surprised to find that while the description of the student's contribution to the published work extensively covered the actual experiments performed, there was no mention of their contribution to the experimental design. Perhaps this oversight can be addressed during the defence.

Flemingovo nám. 2
166 10 Praha 6
Czech Republic
+420 220 183 333
uochb@uochb.cas.cz
www.uochb.cz
IČ: 61388963
DIČ: CZ61388963

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General questions:

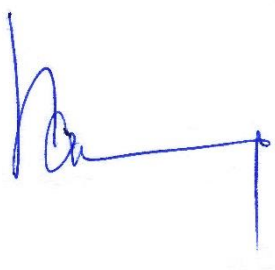
In the introduction, you mention that Mga2 cleavage was prevented by proteasome inhibition, indicating that the proteasome is required to activate Mga2. However, I consider proteasomal cleavage as an ultimate degradation mechanism in the cell. Could you clarify this? Isn't this an outcome of a non-specific inhibition of a different protease?

You mention that "Most DNA-binding proteins achieve their DNA-binding specificity through contacts with functional groups of bases (base readout) and 'reading' of structural properties of DNA (shape readout)." Can you explain the molecular mechanism by which relatively short DNA motifs are recognized by transcription factors with unprecedented specificity, especially considering the limited number of DNA building blocks?

You have briefly described the domain architecture of the studied proteins. Can you show the domain organization, show the intrinsically disordered regions and discuss their putative roles? The Alphafold-derived structures suggest the presence of well-known folded domains. Can you discuss their roles in the context of other nuclear proteins? Additionally, is there any evidence regarding the oligomeric state of the studied transcription factors?

Have you attempted to predict the interaction between Cbf11 and Mga2 using the recently published Alphafold3? This would give you an opportunity to explore the extent of involvement/interference of DNA.

After thoroughly assessing the thesis, I conclude that the candidate has clearly demonstrated her creative abilities. The thesis unreservedly meets the requirements for a dissertation in the field, and I recommend that it be accepted for defence.



Prague 9.6.2024

Václav Veverka, Ph.D.

Flemingovo nám. 2
166 10 Praha 6
Czech Republic
+420 220 183 333
uochb@uochb.cas.cz
www.uochb.cz
IČ: 61388963
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