Posudek na bakalářskou práci

☐ školitelský posudek ☒ oponentský posudek	**Jméno posuzovatele:** Jan Mašek
	Datum: 23/05/2024

Autor:
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Název práce:
Mammalian proteins carrying zinc finger arrays
Savčí proteiny se sadou zinkových prstů

☒ Práce je literární rešerší ve smyslu zveřejněných požadavků (pravidel).
☐ Práce obsahuje navíc i vlastní výsledky.

Cíle práce (předmět rešerše, pracovní hypotéza…)

The proposed thesis goal is to provide: „an overview of the discovery, structure, and function of zinc finger domains (arrays) and then review and discuss selected naturally occurring mammalian zinc finger proteins and their properties, showcasing diverse uses zinc finger arrays have been adapted for throughout evolution.“ as well as discuss „The history and future of zinc fingers in artificial proteins created for gene therapy and research...“.

Struktura (členění) práce:

The thesis starts with an introduction to the Zn finger discovery, followed by a classification of the discovered Zn finger domains based on their structure. Given the high number of genes containing the Zn finger domains, the author further focused on selected Zn finger arrays, describing in more detail their biological roles in mammals. The last part thesis brings the overview of the past usage of engineered zinc fingers, both *Fok*I-conjugated and its alternatives.

Jsou použité literární zdroje dostatečné a jsou v práci správně citovány? Použil(a) autor(ka) v rešerši relevantní údaje z literárních zdrojů?

The thesis builds on a plentiful and well-referenced literature body. It would benefit from referencing every review used with „reviewed in“ which would lay bare the two paragraphs on pages 5 and 6 built solely on 2 review articles, which unnecessarily undermines the otherwise thorough work with literature, especially at the beginning and in the second half of the thesis.

Pokud práce obsahuje (nadstandardně) i vlastní výsledky, jsou tyto výsledky adekvátním způsobem získány, zhodnoceny a diskutovány?

n.a.

Formální úroveň práce (obrazová dokumentace, grafika, text, jazyková úroveň):

The text is accompanied by well-selected illustrations and one table summarizing the number of protein-coding genes containing the C2H2 motif in selected species. In places, a few more illustrations would help the reader understand the text (the effects of the specific examples of Znf arrays (CTCFs, PRDM9) on the 3D structure of the chromatin).
The thesis division into sections is logical, and the text flow keeps a predictable, easy-to-follow structure, containing only a few slip-ups (calling the thesis a „paper“, a typo in „spacial organization“ page 1- Introduction, and missing abbreviation to RING protein).

Splnění cílů práce a celkové hodnocení:

The thesis is well-written and easy to read, with aims largely met. I appreciated the thorough walkthrough of the initial Zn finger discovery via studies of TFIIIA that moved naturally to the description of the structural and mechanical properties of the C2H2 Zn fingers and related molecules.

The following transition to the specific examples of Zn finger arrays was necessary and the selected examples are picked well, covering the KRAB-Zn finger proteins (KZFPs) coevolution with transposable elements, PRDM9 unique role in chromosomal recombination and speciation, and the CTCF role in the regulation of chromatin topology. Needless to say, the chosen protein families are still very large (each being enough complex to serve as a basis for individual theses if not books), and despite the author's valiant efforts, the overview remains superficial and often stops before getting to the truly interesting parts of the most recent experimental evidence.

The last part of the thesis can serve as an epitaph of one chapter in molecular biology when Zn finger arrays as tools were swiftly replaced by CRISPR/Cas technology. In this part, I missed a more focused explanation of the caveats of Zn finger usage that could build on the thorough introduction of the Zn finger structure and functional strengths and limitations.

All in all, I enjoyed reading the thesis, and I am looking forward to learning more about the details I missed from the discussion.

Otázky a připomínky oponenta:

Page 1 – in the introduction, the author states that „zinc finger domains can bind to any non-palindromic sequence in a linear way in the form of a monomer (Klug, 2010)“. How big part of the genome is thus not accessible for the Zn finger proteins, and what are the reasons/consequences?

Page 11 - Related to the KZFP role in early development you mention that „Upon the depletion of the scaffold protein KAP1 or the H3K9 methyltransferase SETDB1 in human or mice ESCs, several TEs become expressed“. – What is the effect of this change in expression on the development? Could you in a similar way comment also the outcomes of the results experimental manipulation of ZFP932 and Gm15446 binding to ERV sequences in ESCs? (page 12)

Page 13 – Comment, happy to discuss - The paragraph concerning the so-called “domestication” of TEs by KZPF as the right model to replace the „Arms Race hypothesis“, builds the arguments based on Imbeault et al., 2017, and a review from Ecco et al., 2017 from the same research group. For a balanced assessment of the phenomenon would be beneficial to consider reports from other researchers who cover the topic of TE domestication from different angles and interpret the findings as a mixed model where both „Arms Race“ and „Domestication“ co-exist as two mechanisms with different functionalities (Yang et al., 2017).

Page 13 – Concerning the PRDM9 function of „PRDM9 as a major component of the meiotic recombination hotspot-forming machinery“ – Is there a mouse knockout of the gene and if so, what happens to the mammalian recombination hotspots in its absence?

Page 20 – The author points out that usage of the „ZFN-mediated knockout of the CCR5 receptor in $CD4_+$ T cells was shown to potentially grant heritable immunity to human immunodeficiency virus (HIV) and even entered clinical trials (Perez et al., 2008; Tebas et al., 2014)“ – Its 10 years later, was the trial successful?

Návrh hodnocení školitele nebo oponenta (bude zveřejněn)

☐ výborně ☒ velmi dobře ☐ dobře ☐ nevyhověl(a)

Podpis školitele/oponenta: