

HABILITATION THESIS REVIEWER'S REPORT

Masaryk University

Applicant

Mgr. Jiří Kohoutek, Ph.D.

Habilitation thesis

Role of transcription cyclin-dependent kinases and their cyclins in cellular processes

Reviewer

Prof. RNDr. Ondřej Slabý, Ph.D., Head of Department

Reviewer's home unit, institution

Department of Biology, Faculty of Medicine, Masaryk University

General Assessment

The habilitation thesis provides a well-structured and systematic analysis of cyclin-dependent kinases and their cyclins in transcription and development. It combines an extensive literature review with original research work, supported by broad variety of methodologies ranging from animal models to molecular assays. The writing style is formal and precise, with a clear logical progression from historical context through mechanistic insights to developmental and clinical implications. Figures, appendices, and a detailed list of references enhance the clarity and scholarly complexity of the thesis. Overall, the work demonstrates original research results, methodological skills, and significant scientific contribution.

Scientific Contribution

The habilitation thesis makes an important contribution to the scientific community by advancing the understanding of transcriptional cyclin-dependent kinases - CDKs and their associated cyclins in cellular and developmental processes. Through a combination of genetic models, biochemical assays, and molecular analyses, the work provides mechanistic insights into how CDK9, CDK12, and CDK13 regulate RNA polymerase II activity, DNA damage response pathways, and pre-mRNA processing. These findings expand current knowledge of transcriptional regulation beyond the role of CDKs in the regulation of the cell cycle, highlighting novel directions to embryogenesis, immune tolerance, and genomic stability. The comparison of experimental data with a comprehensive review of the literature ensures that the thesis not only reports original results but also integrates them into the field of molecular biology.

The thesis also contributes to the community by identifying new links for research and potential therapeutic applications. The demonstration of CDK12 role in DNA damage repair and its relevance to synthetic lethality with CHK1 inhibitors underscores translational implications for oncology. Similarly, insights into the developmental functions of CDK13 and its association with congenital abnormalities provide a foundation for future studies in developmental biology and disease modeling. By combining intriguing molecular discoveries with clinically relevant perspectives, the thesis enriches both basic science and applied research, offering a following investigation into transcriptional regulation in cancer therapy.

Methodological Quality

The thesis employs a broad methodological background combining *in vivo* and *in vitro* biochemical and molecular biological approaches. Genetically engineered mouse models provide valuable developmental insights, though their interpretation is sometimes limited by

embryonic lethality and compensatory mechanisms. Cell line-based studies and functional assays yield mechanistic data. Molecular techniques, including immunoprecipitation, mass spectrometry, and ChIP, are appropriate and appropriately described in publications included in habilitation thesis.

Style, Language and Format

The thesis is written in a formal academic style consistent with scientific writing in molecular and cellular biology. The language is precise and specialized, with frequent use of research field-specific terminology. Chapters are structured in a logical and hierarchical format, beginning with historical context, moving through mechanistic details, and converging in developmental and clinical implications. The use of citations follows standard academic conventions, supporting claims with references to peer-reviewed literature. Figures and appendices are integrated to illustrate experimental results and provide supplementary data, enhancing clarity and depth. I do appreciate the applicant's effort to unify the graphical format of the presented schemes throughout the thesis, which appropriately summarizes the given context of the subchapters. The overall formatting of the thesis is professional, with graphically appealing consistency along with presented schemes.

Overall evaluation

In conclusion, the habilitation thesis of Dr. Jiří Kohoutek presents a well-structured and scientifically solid study on transcriptional CDKs and their cyclins, combining extensive literature analysis with original experimental contributions. The work provides novel insights into the roles of CDK9, CDK12, and CDK13 in transcriptional regulation, genomic stability, development, and potential therapeutic applications, thereby significantly contributing to both basic and translational research. Overall, a habilitation work represents a valuable addition to the scientific community and I fully recommend it for successful defense.

Reviewer's questions for the habilitation thesis defence (number of questions up to the reviewer)

- 1) If cyclin T1 and cyclin T2 are able to recognize and associate with various transcription factors, how is their binding specificity mediated?
- 2) Is it possible to envision the clinical use of CDK12 inhibitors, given the very high amino acid similarity between CDK12 and CDK13?
- 3) Can the applicant speculate on the mechanism by which depletion of CDK13 leads to significant developmental defects?

Conclusion

The habilitation thesis entitled "Role of transcription cyclin-dependent kinases and their cyclins in cellular processes" by Jiří Kohoutek **fulfils** requirements expected of a habilitation thesis in the field of Molecular Biology and Genetics.

Date: 23.8.2025

Signature: