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Annex No. 10 to the MU Directive on Habilitation Procedures and Professor Appointment Procedures

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	MUDr. Petr Džubák, Ph.D.
Reviewer's home unit, institution	Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc

General Assessment:

The habilitation manuscript comprises 43 pages of text and 13 appended publications on 168 pages, all of which have been published in international peer-reviewed journals.

The author is the first or corresponding author on seven of these papers.

The introduction, with a slightly poetic touch, situates the work within the historical context of the discovery of cell-cycle regulators and CDK/cyclin complexes and classifies CDKs into three groups (cell-cycle regulators, transcription-regulating CDKs, and atypical CDKs). It then focuses on kinases controlling RNAPII transcription and clearly formulates the guiding questions posed at the beginning and throughout the research: how the switch between active and inactive P-TEFb is executed; whether P-TEFb activity is constitutive or phase-restricted across the cell and transcription cycles; and how its association with diverse transcription factors is established and regulated. Already here, as well as in the discussions accompanying later chapters and in the concluding synthesis, the author's knowledge of the field is evident, supported by an extensive and up-to-date reference list (135 citations).

Scientific contribution:

In the submitted habilitation thesis, the author advances the understanding of:

(a) The function of P-TEFb.

The thesis frames RNAPII elongation as the key transcriptional checkpoint and shows that CDK9/P-TEFb mediates pause release via CTD Ser2 phosphorylation and modulation of NELF/DSIF. The author mechanistically links the active "small" and inactive "large" P-TEFb complexes to upstream signals that mobilize P-TEFb from the 7SK/HEXIM1 reservoir. This framework, as originally reported (Appendices 1–4), supports subsequent analyses in differentiation, development, and stress responses.

(b) Role of CDK12 and CDK13 in the regulation of transcription (Appendices 5-8).

Contrary to prior assumptions, cyclin K binds CDK12/13—not CDK9, forming distinct complexes. The author demonstrates their contribution to RNAPII Ser2 phosphorylation and selective control of DNA-damage response genes.

(c) Impact of transcription kinases and their associated cyclins in developmental processes (Appendices 9-13).

The thesis maps essential in vivo roles of these factors: cyclin T2 (and cyclin K) in early embryogenesis; Loss of CDK13 causes CHDFIDD-like craniofacial, intellectual, and cardiac defects; and oocyte CDK12 as a prerequisite for female fertility.

Style, Language, and Formatting

The thesis is written in clear, appropriate academic English, with a precise use of molecular biology terminology. Its structure is coherent (Commentary, Introduction, three mechanistic chapters, Summary/Future Directions, Appendices), moving from historical context to mechanism and developmental relevance. The bibliography is comprehensive and up-to-date, featuring strong primary sources in high-quality journals. In-text citations generally support claims and avoid over-reliance on reviews or self-citations. Schematics are informative and visually consistent. Formatting is professional, just with minor inconsistencies.

Overall evaluation

The thesis integrates original mechanistic work on P-TEFb with a redefinition of cyclin K partnerships to CDK12/13 and their selective control of DDR gene transcription, and it documents developmental consequences in vivo. It clarifies how transcriptional kinases regulate RNAPII elongation and genome maintenance, linking these processes to embryogenesis and fertility. Overall, this is a coherent and well-supported habilitation thesis that meets the standards for habilitation, and I recommend it for a successful defence.

Reviewer's questions for the habilitation thesis defence

What key challenges do you foresee in developing irreversible covalent CDK12 inhibitors while maintaining sufficient selectivity over CDK13?

Which rational combination strategies (targets and agents) could maximize the therapeutic window for pharmacological CDK12 inhibition, and which biomarkers would most effectively stratify patients?

Which mechanisms could, in your view, explain the absence of statistically significant changes in RNAPII CTD Ser2 and Ser5 phosphorylation in the brains of conditional Cdk12 knockout mice?

Conclusion

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

Date: 29 August 2025 Signature: