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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. Lukáš Čajánek, PhD
Habilitation thesis	Molecular Control of Primary Cilium by Distal Appendages and Associated Proteins
Reviewer	Aniruddha Mitra, PhD
Reviewer's home unit, institution	Institute of Biotechnology of the Czech Academy of Sciences

The habilitation thesis *"Molecular Control of Primary Cilium by Distal Appendages and Associated Proteins"* by Mgr. Lukáš Čajánek presents a substantial body of work on the molecular regulation of primary ciliogenesis, with a particular focus on the CEP164–TTBK2 module as well as intraflagellar transport (IFT), kinesin function, and ciliary signaling. The topic is highly relevant, given the central role of cilia in development, tissue homeostasis, and human disease.

A major strength of the thesis is its combination of long-term focus and mechanistic depth. The candidate has made key contributions to establishing CEP164-mediated recruitment of TTBK2 as a trigger for ciliogenesis and has extended this work through structural, biochemical, and functional analyses. Importantly, the work goes beyond initiation and reveals broader roles of TTBK2 in ciliary maintenance, axoneme stability, IFT recruitment, and signaling.

The second part of the thesis significantly broadens the scope by addressing the regulation of IFT and its coupling to ciliary function. Notably, the identification of the tubulin transport module within the cilium and the characterization of ciliary kinesins provide important mechanistic insights into how cilia grow and function as signaling organelles. The work further connects ciliary biology to signaling pathways, including a nuanced view of WNT signaling, highlighting that while it may not directly control ciliogenesis, key ciliary regulators such as TTBK2 can modulate core signaling components like Dishevelled.

The manuscript is well written, logically organized, and demonstrates a strong command of the field. The author critically addresses current limitations, particularly in linking phosphorylation events to function, which adds to the scientific maturity of the work. I have a few questions aimed at placing these findings into a broader context.

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

1. Many of the proteins discussed as TTBK2 substrates contain extensive intrinsically disordered regions, and the thesis also mentions recent work proposing CEP164–TTBK2 phase separation. Given this, how should we think about the CEP164–TTBK2 module mechanistically? Do you view it primarily as a classical structured recruitment complex, or rather as a dynamic, multivalent assembly where phosphorylation modulates transient interactions and condensate-like behavior?

2. TTBK1 and TTBK2 share very high similarity within the kinase domain, yet TTBK2 is generally indispensable for ciliogenesis whereas TTBK1 only compensates in specific contexts such as neural rosettes. What do you consider the key feature that makes TTBK2 functionally unique? Is the decisive factor primarily CEP164-mediated recruitment to distal appendages, differences in substrate accessibility, or do you think there is meaningful substrate specificity beyond localization alone?

3. A recurring challenge throughout the thesis is moving from phosphosite identification to establishing direct kinase–substrate relationships and clear functional consequences of specific phosphorylation events. What experimental strategy do you consider will become most powerful for bridging this gap? Among the known TTBK2 substrates, which protein or specific phosphorylation event would you prioritize next as the most informative mechanistically, and why?

4. Many mechanistic conclusions about the CEP164–TTBK2 module rely on cultured cell systems such as RPE1 cells. How generalizable do you think these findings are across different cell types, particularly in differentiated or non-dividing cells with long-lived cilia? What do you see as the main limitations of these model systems, and where would you expect the strongest divergence compared to intact tissues or specialized cilia in vivo? In this context, do you envision CEP164–TTBK2 acting primarily during cilia assembly, or also playing a continuous role in ciliary maintenance?

5. Your work highlights that several proteins function in both ciliogenesis and mitosis. Does this challenge the way we typically categorize proteins as “ciliary” or “mitotic”? How should we think about protein function in systems where the same components are reused across different cellular contexts, and does this call for a shift in how we build and interpret models in molecular biology?

Conclusion

The habilitation thesis entitled “*Molecular Control of Primary Cilium by Distal Appendages and Associated Proteins*” by Mgr. Lukáš Čajánek, PhD **fulfils** requirements expected of a habilitation thesis in the field of Molecular Biology and Genetics.

Date: 30.04.2026 Signature: