M U N I

COMMENTARY TO HABILITATION THESIS[1]

Molecular Control of Primary Cilium by Distal Appendages and Associated Proteins

Lukáš Čajánek

Let's imagine a tiny (~several micrometers long), hair-like structure on the surface of nearly every vertebrate cell, carefully orchestrating vital biological processes. These are cilia, evolutionarily conserved organelles with crucial roles in development and tissue homeostasis. Although once overlooked, cilia have gradually captured attention owing to their critical involvement in a wide range of disorders. Given this, a thorough understanding of how cilia are regulated is not just a scientific pursuit – it is key to unlocking new therapies for cilia-related diseases and uncovering the hidden mechanisms driving cilia biology and its associated pathologies. This manuscript discusses the regulatory mechanisms and functional consequences of primary cilia biology, along with my contributions to advancing this understanding, with a particular focus on events governed by proteins localized to the distal end of the centriole or its immediate vicinity.

In the first part, I focus on the role of CEP164, a component of the distal appendages of the mother centriole, and its effector, Tau Tubulin Kinase 2 (TTBK2). Together, they form one of the key modules regulating ciliogenesis in vertebrates. The primary contribution of my work to this theme lies in the identification of CEP164-mediated recruitment of TTBK2 to the mother centriole as a trigger for ciliogenesis in human cells. Subsequent follow-up work provided a structural basis for the CEP164–TTBK2 interaction, thereby identifying the underlying mechanism by which CEP164 mutations cause ciliopathies. Significant attention has been devoted to resolving the mechanisms underlying TTBK2 activity in cilia. This led to the identification of several novel substrates of the kinase and numerous phosphorylation sites. Importantly, in selected cases, we were also able to provide functional annotation of the identified phosphosites. Notably, our work linked the inhibitory phosphorylation of a tubulin-depolymerizing kinesin to the regulation of primary cilia length, thus providing a mechanistic foundation for the role of the CEP164–TTBK2 module beyond the initiation of ciliogenesis. In addition, our work capitalizing on the use of human pluripotent stem cells (hPSCs) as a model system established that cilia are not required for self-renewal, in contrast to intact centrosomes. However, primary cilia become critical for fine-tuning the proliferation of hPSC-derived neural progenitors, where ciliogenesis is also controlled by TTBK1, unlike in most other model systems.

---

[1] The commentary must correspond to standard expectations in the field and must include a brief characteristic of the investigated matter, objectives of the work, employed methodologies, obtained results and, in case of co-authored works, a passage characterising the applicant's contribution in terms of both quality and content.

The second part of this manuscript is devoted to the regulation of intraflagellar transport (IFT) in cilia and the role of cilia in cell signaling. Regarding the former, the key contribution of my work lies in the identification of the module responsible for the binding and transport of tubulin within the cilium. In additional work, we identified novel ciliary kinesin required for proper cilium assembly and functionality, including the ability of the cilium to act as a signaling organelle. Continuing along the line of ciliary signaling, our work demonstrated that, while the WNT signaling pathway does not appear to play a major role in the regulation of ciliogenesis, TTBK2, a key regulator of ciliogenesis, is able to modulate the activity of Dishevelled (DVL), a key component of the WNT pathway.

The papers listed below represent examples I selected to highlight my contributions to the main theme of my thesis. They comprise 11 original research articles, one methodological paper, and three reviews. My contribution to each manuscript, as well as the journal metrics at the time of publication, is detailed in the tables below. The asterisk (*) indicates publications where I served as the corresponding author. The manuscripts are attached as correspondingly numbered appendices and are highlighted in **blue** in the main text of the manuscript for clarity. In situations when the full-text is not freely available from the publishers' websites, original manuscripts deposited to PubMed Central are provided as an open-access alternative.

[1][2] **Čajánek L***, Smite S, Ivashchenko O, Huranova M. Cilia at the crossroad: convergence of regulatory mechanisms to govern cilia dynamics during cell signaling and the cell cycle. *Cell & Bioscience*. 2025 Jun 7;15(1):81. doi: 10.1186/s13578-025-01403-z.(JCR 2024, IF = 6.2, Q1 – Biochemistry & Molecular Biology)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
NA-review article	0	45	50

[2] Lacigová A, **Čajánek L*.** Phosphorylation at the Helm: Kinase-Mediated Regulation of Primary Cilia Assembly and Disassembly. *Cytoskeleton.* 2025 Mar 10. doi:10.1002/cm.22012. (JCR 2024, IF = 1.6, Q4 – Cell Biology)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
NA-review article	100	40	70

[3] Benk Vysloužil D, Bernatík O, Lánská E, Renzová T, Binó L, Lacigová A, Drahošová T, Lánský Z, **Čajánek L*.** Tau-tubulin kinase 2 restrains microtubule-depolymerizer KIF2A to support primary cilia growth**.** *Cell Communication and Signaling.* 2025 Feb 10;23(1):73. doi: 10.1186/s12964-025-02072-8.(JCR 2024, IF = 8.9, Q1 – Cell Biology)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)

[2] Bibliographic record of a published scientific result, which is part of the habilitation thesis.

0	65	35	80

[4] Binó L, **Čajánek L*.** Tau tubulin kinase 1 and 2 regulate ciliogenesis and human pluripotent stem cells-derived neural rosettes. *Scientific Reports.* 2023 Aug 9;13(1):12884. doi: 10.1038/s41598-023-39887-9. (JCR 2023, IF = 3.8, Q1 – Multidisciplinary Sciences)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
0	100	70	90

[5] Rosa E Silva I, Binó L, Johnson CM, Rutherford TJ, Neuhaus D, Andreeva A, **Čajánek L**, van Breugel M. Molecular mechanisms underlying the role of the centriolar CEP164-TTBK2 complex in ciliopathies. *Structure.* 2022 Jan 6;30(1):114-128.e9. doi: 10.1016/j.str.2021.08.007.(JCR 2022, IF = 5.7, Q1 – Biochemistry & Molecular Biology)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
0	15	10	15

[6] Bino L, Mikulenkova E, Stepanek L, Bernatik O, Vyslouzil D, Pejskova P, Gorilak P, Huranova M, Varga V, **Čajánek L***. A protocol for generation and live-cell imaging analysis of primary cilia reporter cell lines. *STAR PROTOCOLS*. 2022;3(1). doi:10.1016/j.xpro.2022.101199 Q4.(JCR 2022, IF = 1.3, Q4 – Biochemistry Research Methods)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
0	55	35	65

[7] Bernatik O, Paclikova P, Kotrbova A, Bryja V, **Cajanek L***. Primary Cilia Formation Does Not Rely on WNT/β-Catenin Signaling. *Front Cell Dev Biol.* 2021 Feb 26;9:623753. doi: 10.3389/fcell.2021.623753.(JCR 2021. IF=6.081, Q1 – Developmental Biology)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
0	60	40	75

[8] Pejskova P, Reilly ML, Bino L, Bernatik O, Dolanska L, Ganji RS, Zdrahal Z, Benmerah A, **Cajanek L*.** KIF14 controls ciliogenesis via regulation of Aurora A and is important for Hedgehog signaling**.** *Journal of Cell Biology***.** 2020 Jun 1;219(6):e201904107. doi: 10.1083/jcb.201904107.(JCR 2020. IF=10.539, Q1- Cell Biology)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
0	70	55	90

[9] Bernatik O, Pejskova P, Vyslouzil D, Hanakova K, Zdrahal Z, **Cajanek L*.** Phosphorylation of multiple proteins involved in ciliogenesis by Tau Tubulin kinase 2. *Molecular Biology of the Cell***.** May 1;31(10):1032-1046. doi: 10.1091/mbc.E19-06-0334. (JCR 2020. IF= 4.138, Q3 – Cell Biology)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
0	60	40	80

[10] Hanáková K, Bernatík O, Kravec M, Micka M, Kumar J, Harnoš J, Ovesná P, Paclíková P, Rádsetoulal M, Potěšil D, Tripsianes K, **Čajánek L**, Zdráhal Z, Bryja V**.** Comparative

phosphorylation map of Dishevelled 3 links phospho-signatures to biological outputs. *Cell Communication and Signaling***.** 2019 Dec 23;17(1):170. doi: 10.1186/s12964-019-0470-z.(JCR 2019. IF=4.344, Q2 – Cell Biology)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10	0	10	10

[11] Renzova T, Bohaciakova D, Esner M, Pospisilova V, Barta T, Hampl A, **Čajánek L*.** Inactivation of PLK4-STIL Module Prevents Self-Renewal and Triggers p53-Dependent Differentiation in Human Pluripotent Stem Cells. *Stem Cell Reports*. 2018 Oct 9;11(4):959-972. doi: 10.1016/j.stemcr.2018.08.008. (JCR 2018, IF= 5.499, Q1 – Cell Biology)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
5	70	55	90

[12] Bohaciakova D, Renzova T, Fedorova V, Barak M, Kunova Bosakova M, Hampl A, **Čajánek L*.** An Efficient Method for Generation of Knockout Human Embryonic Stem Cells Using CRISPR/Cas9 System . *Stem cells and development*. 2017 Nov 1;26(21):1521-1527. doi: 10.1089/scd.2017.0058. JCR 2017. IF= 3.315, Q2 – Hematology)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
5	60	50	80

[13] Bryja V, Červenka I, **Čajánek L.** The connections of Wnt pathway components with cell cycle and centrosome: side effects or a hidden logic. *Crit Rev Biochem Mol Biol*. 2017 Dec;52(6):614-637. doi: 10.1080/10409238.2017.1350135. (JCR 2017. IF=5.279, Q1 - Biochemistry & Molecular Biology)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
NA-review article	0	40	40

[14] Čajánek L, Nigg EA**.** Cep164 triggers ciliogenesis by recruiting Tau tubulin kinase 2 to the mother centriole. *Proc Natl Acad Sci U S A*. 2014 Jul 15;111(28):E2841-50. doi: 10.1073/pnas.1401777111. (JCR 2014. IF=9.674, Q1 - Multidisciplinary Sciences)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
95	0	75	90

[15] Bhogaraju S, **Cajanek L**, Fort C, Blisnick T, Weber K, Taschner M, Mizuno N, Lamla S, Bastin P, Nigg EA, Lorentzen E**.** Molecular basis of tubulin transport within the cilium by IFT74 and IFT81. *Science*. 2013 Aug 30;341(6149):1009-12. doi: 10.1126/science.1240985. JCR 2013. IF= 31.477, Q1 - Multidisciplinary Sciences)

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	0	10	10