LABORATORY OF

BIOLOGY OF CYTOSKELETON

Microtubules, tubulin isotypes, γ-tubulin complexes, regulation of microtubule nucleation, signal transduction

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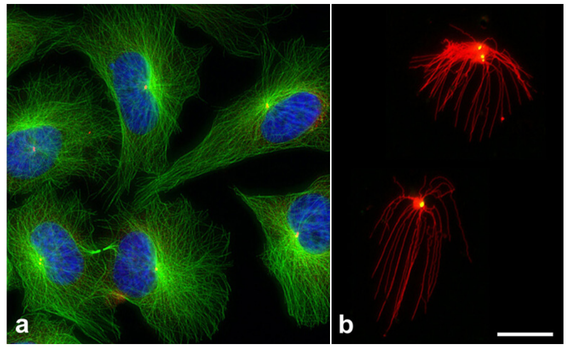
Microtubules are intracellular dynamic polymers made up of polymorphic αβ-tubulin heterodimers and a large number of microtubule-associated proteins. Microtubules are required for vital processes in eukaryotic cells, including cell division, maintenance of cell shape, intracellular transport, and signal transduction. The organization of microtubules in cells is controlled by microtubule organizing centres (MTOCs) as centrosomes. The key components of MTOCs are γ-tubulin complexes essential for microtubule nucleation. The regulatory mechanisms of γ-tubulin complex activation are just about to begin to be understood.

The long-term research programme of the laboratory includes study of the structure-function relationships of microtubule proteins and their interactions with other cytoskeletal elements in cells under normal and pathological conditions. In recent years, the research efforts have concentrated on elucidation of the molecular mechanisms governing microtubule nucleation and dynamics and the role of γ-tubulin in these processes. It has been shown that γ-tubulin is post-translationally modified and forms complexes with protein tyrosine kinases and phosphatases. An important role in the regulation of microtubule nucleation is played by the GIT/βPIX/PAK signalling complex. We have shown that the properties of γ-tubulin change during differentiation events and that γ-tubulin-2 could have a pro-survival function in neurons. The presence of γ-tubulin complex proteins was demonstrated in membrane-associated complexes and in the nuclei, where they modulate DNA damage G2/M checkpoint activation through tumour suppressor protein C53. We have also shown that C53 is important regulator of microtubule organization in cells under ER stress, and that microtubule dynamics in vitro and in cells could be modulated by nanosecond-pulsed electric fields.

Our current work focuses on deciphering the regulatory mechanisms of microtubule nucleation in activated mast cells and the role of signal transduction molecules in this event. We also study dysregulation of microtubule organization in brain cancer cells and the function of neuronal γ-tubulin-2 isotype. Finally, we define the new roles of actin-associated profilin in the regulation of centrosomal microtubule nucleation. To address these questions, we use techniques of molecular biology, biochemistry, and immunology, as well as a variety of microscopic techniques, including superresolution microscopy, live-cell imaging and quantification of microtubule nucleation and dynamics.



Microtubule nucleation from centrosomes in cells and in vitro.
(a) Human osteosarcoma cells U2OS stained for microtubules with antibody to β-tubulin (green) and for centrosomes with antibody to γ-tubulin (red). DNA in blue. (b) Centrosomes isolated from U2OS cells by sucrose gradient centrifugation were incubated with 1.2 mg/ml tubulin in the presence of 1 mM GTP for 20 min at 37°C. After fixation, centrosomes were centrifuged through a glycerol cushion onto a coverslip and immunostained for pericentrin to mark centrosomes (green) and for β-tubulin to mark microtubules (red). Scale bar for (a) and (b), 20 μm.

Selected publications:

1. Sulimenko V., Dráberová E., Dráber P.*: γ-Tubulin in microtubule nucleation and beyond. Front. Cell Dev. Biol. 10: e880761, 2022.
2. Klebanovych A, Vinopal S., Dráberová E., Sládková V., Sulimenko T., Sulimenko V., Vosecká V., Macůrek L., Legido A., Dráber P.*: C53 interacting with UFM1-protein ligase 1 regulates microtubule nucleation in response to ER stress. Cells 11: e555, 2022.
3. Shapoval O., Sulimenko V., Klebanovych A., Rabyk M., Shapoval P., Kaman O., Rydvalová E., Filipová M., Dráberová E., Dráber P.*, Horák D.*: Multimodal fluorescently labeled polymer-coated GdF3 nanoparticles inhibit degranulation in mast cells. Nanoscale 13: 19023-19037, 2021.
4. Nejedlá M., Klebanovych A., Sulimenko V., Sulimenko T., Dráberová E., Dráber P.*, Karlsson R.*: The actin regulator profilin 1 is functionally associated with the mammalian centrosome. Life Science Alliance 4: e202000655, 2021.
5. Chafai D.E*., Vostárek F., Dráberová E., Havelka D., Arnaud-Cormos D., Leveque P., Janáček J., Kubínová L., Cifra M., Dráber P*: Microtubule cytoskeleton remodelling by nanosecond pulsed electric fields. Adv. Biosystems 4: e2000070, 2020.