Dissecting the molecular mechanisms that control cilia beating – around and inside the N-DRC "black box"

Motile cilia and longer but similar in structure, flagella are eukaryotic cell protrusions. A complex structural composition of these organelles enables their bending and motility. The movement of these several micrometer-long "nanomachines" plays an important role in the proper functioning of multiple organisms. In humans, motile cilia are assembled by the epithelial cells lining respiratory tracks, brain ventricles, in females – Fallopian tube and in males – flagella are assembled by sperm cells. Moreover, the beating of motile cilia of a specific type, so-called nodal cilia, that are assembled during the early embryonic development, cause that the main visceral organs of our body such as the heart, liver, or pancreas are positioned asymmetrically. Lack or defects of cilia and flagella or their improper functioning due to the mutations in genes encoding ciliary proteins, cause among others, ciliary primary dyskinesia (PCD). This genetic syndrome manifests by recurring infections of the respiratory tracts, infertility, reversed position of the internal organs, and rarely hydrocephalus. Although the diagnostics of PCD was recently significantly improved, in numerous cases, the causative mutation remains unknown. More detailed knowledge regarding cilia structure and motility regulation will enable a better understanding of the PCD causes and identification of the additional genetic markers that could help to diagnose this rare genetic disorder.

It estimates that the assembly and functioning of these several-several dozen micrometer long organelles involve several hundred proteins, but in the case of nearly half of them, it remains unknown how they affect cilia and flagella formation and functioning. Cilia and flagella although differ in their length, have a similar structure. The skeleton of these organelles is composed of microtubules; two microtubules are positioned in the central part of the cilium while nine pairs of microtubules (known as outer doublets) are located uniformly at the cilium circumference. The outer doublet microtubules are docking sites for dyneins, motor proteins enabling the shift of the adjacent outer doublets causing in effect, a cilium bend. To transform cilium bending into cilium beating, the activity of the dynein arms has to be coordinated. Dynein arms activity regulation and coordination are controlled by other ciliary structures, docked to either central microtubules or outer doublets. Radial spokes and nexin links (N-DRC) are outer doublet complexes.

The nexin link is composed of eleven proteins and performs two important functions. First, N-DRC connects two neighboring outer doublets and restricts their shift caused by dyneins' activity. Second, N-DRC seems to be a specific "intermediary" between dyneins and radial spokes and coordinates the activity of ciliary structures located nearby. Importantly, three proteins, the subunits of the N-DRC were related to PCD. At the moment it is unclear how N-DRC interacts with and coordinates other ciliary complexes and what kind of processes take place inside the N-DRC complex; more precisely, how the posttranslational modifications of N-DRC proteins and binding of the different signaling factors such as calcium ions, calmodulin, or ATP affects the activity of the N-DRC complex.

Our recent studies showed that N-DRC can interact/bind to dyneins and radial spokes not only directly, but also indirectly via "connectors" – small protein complexes. Therefore, our goal is to identify proteins that connect N-DRC with other ciliary structures, and to elucidate the role of these connectors and changes inside the N-DRC complex in the cilia beating regulation.

The proposed studies will be conducted using a cilia *Tetrahymena thermophila*. This model organism assembles cilia similar to cilia present in the human body. Proteins connecting N-DRC with other ciliary structures will be identified using BioID and immunoprecipitation approaches followed by mass spectrometry using cells expressing N-DRC proteins as fusions with either mutated biotin ligase of 3HA tag. Protein localization will be verified using genetic, biochemical, and microscopies methods. Next, we will analyze a phenotype of *Tetrahymena* cells with eliminated individual N-DRC proteins. We will also study the role of the domains present in the N-DRC proteins and the significance of posttranslational modification of selected N-DRC proteins. Moreover, we will attempt to identify protein kinase(s) responsible for the phosphorylation of N-DRC proteins.

Obtained data will not only significantly improve our understanding of the mechanisms that regulate cilia beating, but also may improve the PCD diagnostics, a better understanding of the genetic background of PCD, and subsequently in the future may contribute to developing gene therapies for individuals affected with this genetic disorder.