Description for the general public

1. Research project objectives / Research hypothesis

Besides microtubular skeleton composed of 9 doublets of peripheral microtubules and two central microtubules, motile cilia form multi-protein complexes such as outer and inner dynein arms, nexin-dynein regulatory complex and radial spokes. Radial spokes (RS) assemble as triplets (RS1, RS2 and RS3). As was shown using tomography and 3D reconstruction, each radial spoke of the triplet has slightly different architecture, especially spoke RS3. Radial spokes transmit mechanochemical signals from the pair of central microtubules to the dynein arms and are indispensable for the generation of cilia beating.

The comparison of the proteins isolated from the axonemes of the wild type and *Chlamydomonas* mutant cells, lacking entire radial spokes ("spokeless"), let to the identification of 23 proteins (RSP1-23) that build the radial spokes. However, in *Chlamydomonas* the RS3 spoke is reduced to the knob-like structure that is present in "spokeless" mutants. Thus identified 23 RSP proteins are subunits of RS1 and RS2 and the protein composition of spoke RS3 remains unknown. It is noteworthy that opposite to *Chlamydomonas*, in human and *Tetrahymena*, the RS3 is a full-size structure.

The differences in the radial spokes architecture may reflect the differences in the protein composition of the individual radial spokes and result in the radial spokes functional diversity. This hypothesis is supported by our data indicating that two out of three paralogs of RSP3 and RSP4/6 are RS3 spoke-specific. Our goal is to identify RS3 protein subunits and to reveal the role of RS3 in cilia beating regulation. We will also analyze the role of the RSP proteins that have domains suggesting involvement in the signal transduction in cilia beating regulation. Moreover, we will conduct bioinformatics analyses in the search for human orthologs of the identified RS3 proteins and next verify if they indeed localize in motile cilia.

2. Research project methodology

Ciliate *Tetrahymena thermophila* is well-known model to analyze ciliary proteins and their role in the regulation of cilia beating. The RS3 spoke-specific proteins will be identified by proximity labeling assay using mutated version of the BirA* ligase tagged to the verified RS3-specific proteins. The biotinylated proteins will be identified using mass spectrometry. Identified putative RS3 proteins will be expressed as HA-tagged fusion proteins under the control of their native promoters either in wild-type cells or in *Tetrahymena* mutants lacking either entire radial spoke RS3 or its fragment. Interactions between proteins will be assayed using biochemical and microscopic methods.

Tetrahymena mutants with deletion of the selected genes or expression of HA-tagged proteins under the control of their native promoters will be obtained using standard methods. We will also perform the phenotypic analyses of all mutants of RS3-specific proteins, including analysis of cells trajectories and cilia beating pattern.

3. Expected impact of the research project on the development of science

Our analysis will extend the general knowledge concerning the protein composition of the ciliary structures and the molecular mechanisms that regulate cilia beating (the protein composition of the RS3 spoke and its role in the transmission of signals that regulate cilia beating).

Motile cilia play an important role in the human body and lack or disfunction of motile cilia leads to a multi-symptom human disorder called primary ciliary dyskinesia (PCD), resulting in a male and female infertility, infections of the respiratory tracks, improper left-right asymmetry of the organs in the human body and rarely in hydrocephalus. Often the diagnosis of PCD is difficult. Identification of new ciliary proteins may in the future, help to develop new genetic markers of PCD (diagnostics) and gene therapy.