HIGH RESOLUTION STRUCTURAL STUDIES OF THE CILIARY TRANSITION ZONE IN THE MODEL ORGANISM CHLAMYDOMONAS REINHARDTII: A MORPHO-FUNCTIONAL APPROACH

*Gentile M, Paccagnini E, Pratelli A, Mencarelli C and Lupetti P

Department of Life Science, University of Siena

Cilia and flagella are highly conserved organelles during evolution and are present on the surface of many eukaryotic cells where they play an active role on cell motility, fluid propulsion on the surface of the ciliated epithelia, and in sensing mechanical and chemical stimuli from the extracellular matrix. More recently an indirect involvement of ciliary sensing and turnover has also been involved in the regulation of cell cycle. Defects in the assembly or maintenance of functional components in cilia and flagella along with their structural alterations lead to the onset of a growing class of human diseases defined as ciliopathies. Although eukaryotic cilia and flagella have been the subject of numerous studies, there is still much to understand about their functional morphology.

Several evidences indicate that the protein complexes, synthesized in the cytoplasmic compartment, are involved in growth and functioning of cilia/flagella after their selective admission into the flagellar compartment via the ciliary pore, also known as the Transition Zone (TZ), located at the base of the flagella.

In order to study these processes one can take consistent advantage from the use of reliable model organisms such as Chlamydomonas reinhardtii: a biflagellate green alga whose flagella tuned out having strong ultrastructural, genomic and proteomic homologies with the cilia present in several districts of the human body.

The main focus of our research group has been the ciliary TZ ultrastructure and its morphological changes along the flagellar regeneration in both wild type Chlamydomonas cells and from a mutant with a deletion of the gene that synthesizes the protein CEP290 (known as nephocystin-6 in human cilia). Because of some evidence indicative for a dynamic nature of ciliary pore along flagellar regeneration, the electron microscopy observations have been conducted on cell regenerating their flagella which were fixed at incremental times after flagellar amputation by cell exposure to ph shock.

3D models of TZ were obtained by double-tilt axis electron tomography of about 250 nm thick sections from flat embedded samples. Such strategy was selected because 3D analysis allows to detect the different structural and functional components in crowded cell districts like the TZ, displaying the spatial distribution of their electron dense components and, though indirectly, also the interactions occurring amongst TZ's structural components. Post imaging processing by appropriate combined sets of open source packages and subsequent rendering of the obtained 3D density maps were adopted to analyze comparatively the TZ 3D structure in wt and CEP290 mutant. In order to obtain a further ultrastructural and morphological detail, we also averaged 3D density maps from both regenerating flagella and from full length ones.

The comparative analysis of 3D models from TZs of wt sample, revealed the occurrence of TZ structural changes along flagellar regeneration. Interestingly no consistent variation was observed along flagellar regeneration of CEP290 mutants.

Recent immuno-flurescence and immuno-electron microscopy studies by other research groups indicated that CEP290 is located at the level of the transition zone but the role plaid but such protein within the ciliary pore, is still poorly understood though there is evidence indicative for the involvement of this protein in ciliary assembly and functioning. To establish the exact localization of CEP290 protein we developed a reliable and reproducible ad-hoc pre-embedding immuno- electron microscopy method. By achieving this goal, we could establish CEP290 localization along flagellar regeneration in the model organism Chlamydomonas reinhardtii, adding new and partly different evidence compared to previously published papers.. We are now testing the new method to localize other TZ constructive modules with the aim of producing new data also on the eventual interactions occurring amongst TZ components. We are also planning to extend our studies to other model organisms and mammalian cells

Keywords: cilia, transition zone, electron microscopy

AN IMMUNOELECTRONMICROSCOPY ANALYSIS OF THE INTERACTIONS BETWEEN IFT PROTEINS AND THE FLAGELLAR TIP COMPONENTS

*Pratelli A, Gentile M, Paccagnini E, Lupetti P and Mencarelli C

Department of Life Science, University of Siena

Cilia and flagella are dynamics organelles that undergo cycles of assembly/disassembly in a manner that is temporally coordinated with the cell cycle.

These organelles lack the machinery required for protein synthesis and thus rely on protein precursors that are synthetised in the cell body. A bidirectional transport process - known as the IntraFlagellar Transport (IFT)- moves precursors up to the ciliary tip (anterograde transport), where ciliary assembly takes place, and turnover products back to the cell body (retrograde transport). IFT plays a crucial function to ensure the maintenance of the flagellar homeostasis.

Molecular actors of IFT are two specific molecular motors (kinesin II as the anterograde motor, and dynein 1b as the retrograde one) and the IFT particles, macromolecular complexes that are moved by motors along the axoneme, between doublet surface and the flagellar membrane, and act as platforms for cargo transport.

At the distal end of the organelle, the anterograde transport phase is converted into the retrograde phase. The distal ciliary district, where turnaround takes place, is thus crucial for ciliary functioning. However, despite its key role, very limited structural and molecular information is currently available on this compartment, and nothing is known on the interactions that occur between the distal end and the IFT components.

In order to contribute to the knowledge of IFT turnaround, we have undertaken a detailed electronmicroscopy and immunoelectronmicroscopy analysis of the flagellar tip district in Chlamydomonas reinhardtii. Our studies revealed that IFT protein specifically and differentially interact with structural components of the axonemal tip

Keywords: cilia, flagellar tip, immunoelectron microscopy

* *Speaker*