Identification of the putative "Ciliary Localization Signal" in the axonemal beta-tubulin of *Tetrahymena thermophila*

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Ciliogenesis relies on intraflagellar transport (IFT) to move tubulin heterodimers, axonemal molecular motors and other associated proteins, from the site of synthesis in the cell body to the site of function in the cilium. Beside the molecular mechanisms of IFT are poorly understood, it has been found that the two core IFT proteins, IFT74 and IFT81, have a role in the transport of tubulin along the cilium (Bhogaraju et al, Science, 2013). However, the putative signals involved in the incorporation of tubulin heterodimers into the axoneme have not been characterized.

In the ciliated protozoa *Tetrahymena thermophila,* beta-tubulin is represented by two identical isotypes named BTU1 and BTU2, which are the most conserved and are known as ciliary tubulins, and six more divergent beta-tubulins, named beta-like (BLTs), and numbered from 1 to 6. We previously demonstrated (Pucciarelli et al., PlosOne, 2012) that two BLTs, the BLT1 and the BLT4, were not detectable in somatic cilia and basal bodies and they were preferentially incorporated into the microtubule arrays of the mitotic and meiotic apparatus of the nuclei. In contrast the BTU2 was detected mainly in cilia. These results suggest that cilia and nuclei must recognize specific "key signals" to ensure the accurate targeting to and retention of proteins within these compartments. Here we propose a putative Ciliary Localization Signal (CLS) identified on the sequence of *T. thermophila* BTU2, which is responsible for the axonemal localization of this tubulin isotype. We analyzed the subcellular localization of a chimeric construct formed by the N-terminus of the BLT1 combined with the intermediate domain and the C-terminus of the BTU2, and we also over-expressed mutants of this chimera and of the BTU2 wild type, in *T. thermophila* cells. These analysis revealed that the ^{39}DS^{40} residues (located upstream to the ^{49}VYYNEATGGRYV^{60} motif reported as the dynein binding site in *Drosophila*) together with the axonemal ^{427}EGEF^{431} motif of the C-terminal domain may have a role in the localization of this isotype into the somatic cilium. By mapping the ^{39}DS^{40} residues on three dimensional model of *Tetrahymena* BTU2, we found that they are located in the beta-tubulin region that faces the lumen of the microtubule. Our results support the hypothesis of an active transport of tubulin heterodimers during axoneme formation. This study gives a contribution to the understanding of the molecular mechanisms of IFT-mediated ciliogenesis.