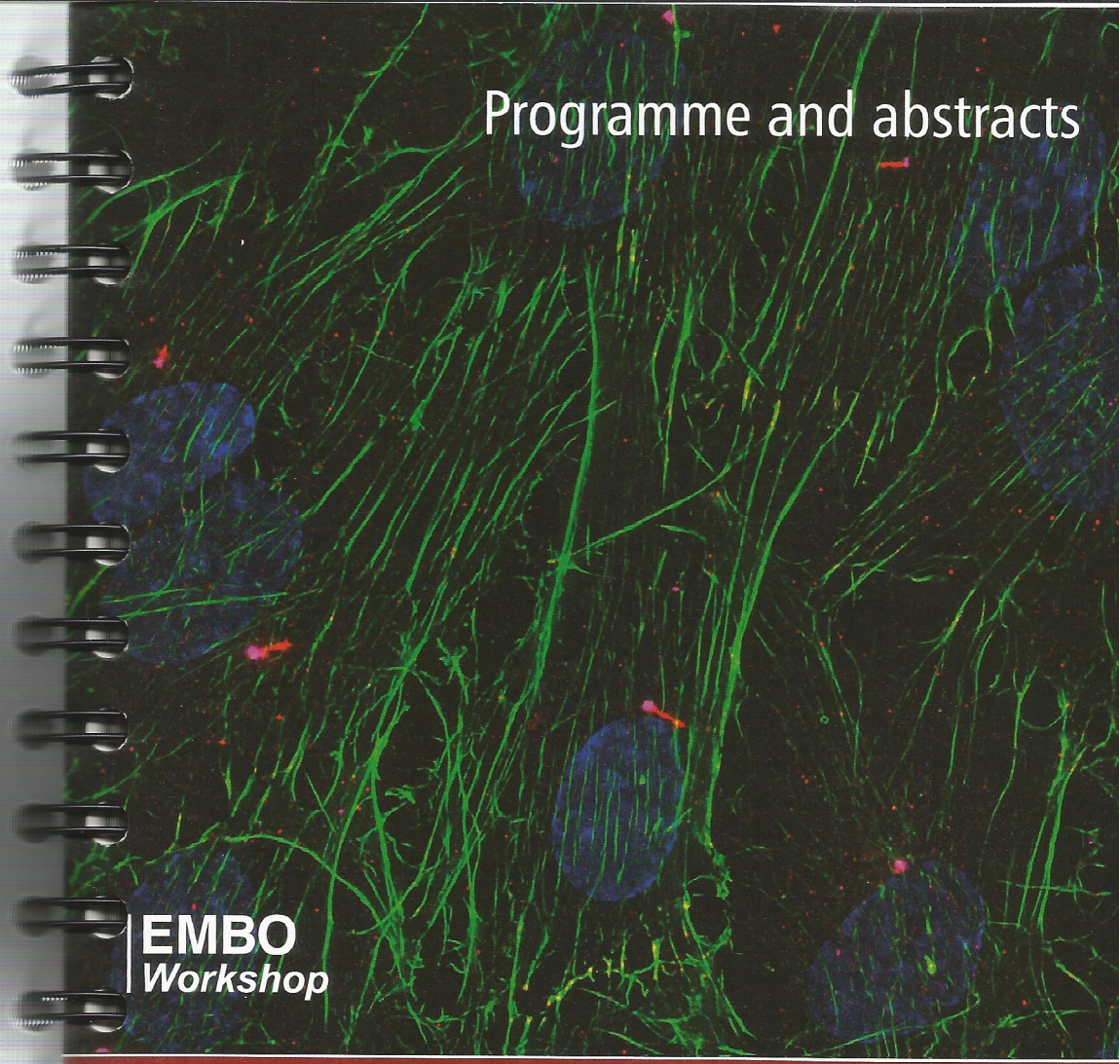

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Poster presentation
Structural biology of cilia and basal bodies

T7-P-04

Tetrahymena CILIA CAP IS BUILT IN A MULTI-STEP PROCESS: A STUDY BY ATOMIC FORCE MICROSCOPY

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Objectives: In motile cilia, a basal body organizes the axoneme composed of nine microtubule doublets surrounding a central pair of singlet microtubules. The distal ends of axonemal microtubules are attached to the membrane by microtubule-capping structures. Since little is known about the early steps of cilium assembly, it was our aim to get insight into the first assembly stages and structure of motile cilia. Methods: Atomic force microscopy (AFM) in tapping mode, with resolution at nanometer range and with minimum sample manipulation, was used to study the first steps of cilia assembly in Tetrahymena. Results: We show that cilia assembly requires transient assembly of structures, composed of three components that are placed asymmetrically on an early elongating axoneme wall. In small uncapped axonemes the microtubule central pair was never observed. We show that the cilia cap assembly is a multi-step process in which structures of different sizes and shapes are put together in close proximity before the axoneme appears capped. The ciliary cap seems to be fully formed by the time cilia reach ~1.5 μm in length as by then it is indistinguishable from the caps of fully mature cilia of ~7 μm length. Conclusions: We show that AFM is a powerful technique to study cilia assembly, providing images of different ciliary sub-structures such as the basal body, transition zone, and ciliary caps that correlate to those previously obtained by EM. Based in our interpretations of AFM images we propose a speculative model for Tetrahymena cilia assembly. Funding: PEst-OE/QUI/UI0612/2013