Abstract

The cilium/flagellum is a complex organelle protruding from the cell body and functioning in motility, sensing, and signalling. It is composed of hundreds of protein constituents, the majority of which comprise the flagellar cytoskeleton – the microtubule-based axoneme.

Because the flagellum lacks ribosomes, its protein constituents have to be imported from the cell body and delivered to proper locations. Moreover, these proteins have to retain their function over a considerable length of time, despite the mechanical stress caused by flagellar beating and due to environmental exposure. This raises the question whether and where protein turnover occurs.

Previously, it was established that *Chlamydomonas reinhardtii* flagella are dynamic structures (Marshall & Rosenbaum, 2001). In contrast, in the *Trypanosoma brucei* flagellum axonemal proteins are remarkably stable (Vincensini *et al.*, 2018). However, the questions of axonemal assembly and stability were so far investigated only for a small number of proteins and during relatively short periods. Moreover, in these experiments expression of studied proteins was controlled by non-native regulatory elements.

To elucidate the site of incorporation of proteins from all major axonemal complexes and to find out if and where the protein turnover occurs, *T. brucei* as a model organism was selected. *T. brucei* is capable of homology recombination, which enables rapid protein tagging in their endogenous loci. Examined proteins were fused to two tags – a fluorescent protein to monitor total protein in the cell and the HaloTag enabling labelling of the protein molecules present in the cell at a particular time and monitor their behaviour thereafter.

These experiments confirmed that the addition of constituents of all major axonemal complexes to a growing axoneme occurs exclusively at its distal end. Surprisingly, the turnover of these proteins in mature flagella of *T. brucei* was observed. This turnover happens around cytokinesis and exclusively affects a short region at the distal end of the flagellum. Finally, the data indicate differences in relative cytoplasmic pools of the studied axonemal proteins. In conclusion, this work provides a systematic analysis of the axonemal growth and turnover, contribution to the knowledge of these aspects of basic flagellar biology.

Keywords: cilium, flagellum, axoneme, turnover, protein incorporation**,** *Trypanosoma brucei*, HaloTag