Abstract

The tip of the eukaryotic flagellum is one of the most important regions of the flagellum in many eukaryotic cells. Several important functions have been associated with the flagellar tip. However, its protein composition remains largely unknown. The main aim of this thesis was to identify flagellar tip proteins and characterize them. Using the parasitic model organism *Trypanosoma brucei* and the TrypTag.org project, a unique resource localizing every protein encoded in the trypanosome's genome, we were able to identify, for the first time, the complete catalog of flagellar tip proteins in a eukaryotic organism. In *T. brucei* the full complement comprises 78 proteins localizing exclusively to the flagellar tip or being highly enriched there. To characterize these proteins, we established new reagents and approaches. First, we developed antibody markers labeling the tip of an assembling trypanosome flagellum via recognizing the flagella connector. This enabled us to study the tip-specific processes. Second, we developed a rapid and cloning-free approach for tagging and inducible overexpression of trypanosome proteins. We demonstrated that the approach is well suited for overexpression of large proteins, such as some of the flagellum tip proteins. This enables the study of overexpression phenotypes and generates material for protein purification for subsequent biochemical characterization of the proteins of interest. Third, we optimized the ultra-expansion microscopy, a rapid and versatile super-resolution approach, for its use in kinetoplastid parasites *T. brucei* and *Leishmania major*. This approach was used to characterize the microtubule-based cytoskeleton of the organisms and was highly instrumental in localizing the flagellum tip proteins in respect to the axoneme and in characterizing depletion phenotypes. The final set of flagellar tip proteins consisted of 78 proteins. Based on the evolutionary analysis, a majority of them are evolutionarily conserved. Only a minority was determined as a structural part of flagellum, while others were a part of either the soluble matrix or membrane-associated structures. Functionally, these proteins can be divided into several cohorts, based on whether their depletion affected axonemal construction, or length regulation. In conclusion, we identified the full complement of the flagellar tip proteins in *Trypanosoma brucei* and characterized their

functions in respect to the axoneme. Furthermore, the established approaches are applicable for characterization of other proteins in *T. brucei*.

This thesis was written in a short format as a commentary because it consists of 3 published papers, one first-author and two co-author, and one first-author manuscript in preparation. A part of the PhD project was supported by the GAUK project (103120) awarded by Charles University.