Abstract

The eukaryotic flagellum is a membrane-bound protruding organelle with a cytoskeleton of microtubules. Flagella are found in unicellular as well as multicellular organisms, performing a variety of functions. Motile flagella enable cell locomotion, like in protists or spermatozoa, but can also create flows of fluids or mucus, like in respiratory airways. Flagella also act as cellular “antennas”, as their surface can probe the environment with sensorial receptors. The flagellar ultrastructure is often regarded as widely conserved among eukaryotes, however significant differences have been reported for the structure of the distal flagellar tip between organisms. The tip is where the flagellum grows and where intra-flagellar transport must unload and load cargo, making it a hub of flagellar-specific processes that are still relatively under-explored.

In humans, genetic mutations that impair proper flagellar function cause primary ciliary dyskinesia, a collective term for numerous pathologies which are still not fully characterized. To elucidate the ultrastructure of the human flagellar tip, we performed cryo-electron tomography on intact spermatozoa, plunge-frozen in their native environment. The results revealed drastic differences compared to commonly studied model organisms. Additionally, a novel extensive structure (named TAILS) was discovered decorating the lumen of sperm tip microtubules. These results together highlight the power of cryo-electron tomography in displaying complex cellular structures in their native environment, as well as the importance of studying the human system directly.

Lastly, a multi-pronged approach was designed to biochemically identify and characterize TAILS, based on a reverse structural biology perspective. This included obtaining high-resolution structures of TAILS produced with different cryo-electron microscopy techniques, the first ever flagellar tip proteome and an evolutionary overview of TAILS conservation.