1310 CD
DOI: 10.1017/S1431927607074648
Microsc Microanal 13(Suppl 2), 2007

Electron Microscopic Tomography of the Microtubule Cytoskeleton and Flagellar Pocket in African Trypanosomes.

M. Shaw*, C. Gadelha*, S. Lacomble*, M.K. Morphew**, E.T O'Toole**, S. Vaughan*, J. R. McIntosh** and K. Gull*.

*Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, OX1 3RE, UK.

**Laboratory for 3-D Electron Microscopy of Cells, Department of Molecular, Cellular, and Developmental Biology, University of Colorado at Boulder, USA.

Trypanosoma brucei causes devastating disease in animals and man (Sleeping Sickness) in sub-Saharan Africa. The surface of the bloodstream form of the parasite is covered by a dense coat composed of a glycosylphosphatidylinositol-anchored protein, the variant surface glycoprotein (VSG). The individual parasite's VSG coat and its switching allows evasion of the host's immune response. The portal whereby all secretion and uptake of proteins occurs is a unique site defined by the exit point of the flagellum from the cell – the flagellar pocket. This is the only area of plasma membrane devoid of microtubules. Secretion of VSGs to the cell surface, removal of surface-associated antibodies and uptake of essential growth factors (e.g., transferrin) occur through the flagellar pocket. This site is therefore the key to the basic cell biology that underlies this parasite's success.

The flagellar pocket is defined by two particular boundaries: the site of docking and interaction of the basal body with the membrane defines the base of the pocket whilst the interaction of a specific flagellar pocket collar with an annulus of differentiated membrane intimately surrounding the emerging flagellum defines a flagellar pocket neck (Figs 1 and 2). Between these two annuli the flagellar pocket membrane is highly dynamic and differentiated from that membrane surrounding the cell body or the flagellum.

We have used a number of electron microscopic approaches (negative staining of whole cytoskeletons, thin sectioning and electron tomography) to define the cytoarchitecture of the pocket and the mechanism of its duplication during the division of the parasite. Our tomographic studies have focussed on the cytoskeletal structures surrounding the flagellar pocket, basal body duplication, the associated cytoskeleton, flagellar attachment zone filament (FAZ filament etc) and new flagellum growth. We have shown how a new flagellar pocket is formed in procyclic (tsetse form) parasites with a major reorientation whereby the basal bodies of the new flagellum move rotationally, so orchestrating division of the flagellar pocket and creation of the new flagellar pocket for the daughter cell.

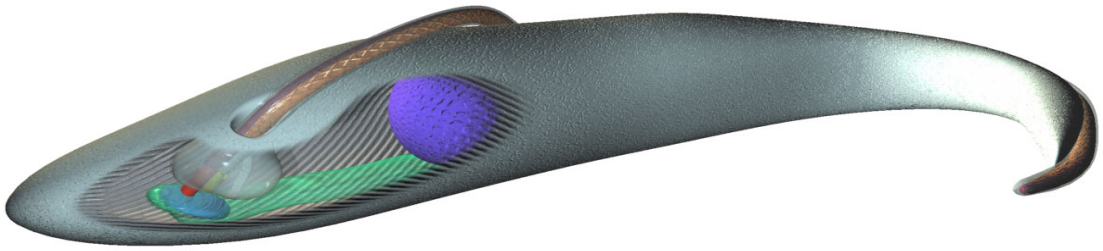

Fig 1. A cutaway cartoon of the African trypanosome illustrating the flagellum and flagellar pocket inside the parasite.

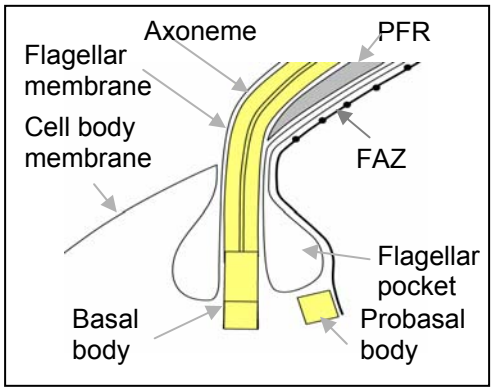

Fig 2. A cartoon of the major structural components of the flagellar pocket.