

# The trypanosome flagellum

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Movie available online

## Introduction

African Trypanosomes are flagellated protozoan parasites that cause sleeping sickness in humans and Nagana in cattle. During its life cycle, *Trypanosoma brucei* alternates between an insect vector (tsetse fly) and a mammalian host.

Within each of these, the parasite proliferates and undergoes separate periods of differentiation in preparation for each new host/vector environment. The differentiated cell types of the trypanosome life cycle are defined morphologically by the position of the single flagellum, nucleus and kinetoplast (the single mass of mitochondrial DNA). The flagellum is key to these morphological events and hence much attention has focused recently on understanding its role in trypanosome morphogenesis and pathogenicity. However, the tractable cell biology, reverse genetics and advanced genome project mean that the trypanosome is also emerging as an ideal model organism for the studies of eukaryotic flagella and cilia in general.

## Flagellum functions: morphogenesis to pathogenicity

The positioning of the mitochondrial genome in the kinetoplast is a direct consequence of the position and segregation of the flagellum basal bodies (Robinson and Gull, 1991). In addition, the construction of a set of internal cytoskeletal microtubules and filaments are influenced by, and at times defined by, the ontogeny of the external flagellum. Given that these cytoskeletal structures also define three distinct plasma membrane regions (the flagellum membrane, the cell body membrane and the flagellar pocket membrane), the flagellum exhibits a pivotal role in morphogenesis of the trypanosome with many functions beyond those of motility (Gull, 1999).

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The flagellum plays key roles in the morphogenesis, motility and pathogenicity of trypanosomes. Its basal body complex defines both the site of the flagellar pocket, the unique region for surface membrane traffic, and the position of the kinetoplast, the single mass of mitochondrial DNA. The external left-handed helical track of the attached flagellum along the cell body also reflects the internal axis and polarity of cytoskeletal elements involved in organelle positioning and cytokinesis. Duplication or remodelling of these cellular features occurs in a strict temporal order within the cell cycle. Clarifying these events has revealed generic aspects of morphogenesis of eukaryotic flagella and cilia as well as highlighting the requirement for a three-dimensional understanding of complex cell processes.

Elongation of the immature basal body (yellow), which was formed during the last cell cycle, and its movement to a position posterior to the mature basal body is the first morphological marker of entry into a new cell division cycle.

The immature basal body 'matures' (now shown to red), and is capable of initiating a new flagellum. Two new immature basal bodies (yellow) are formed close to the now two mature basal bodies.

The new flagellum is extended out of the flagellum pocket and positioned alongside the old flagellum. Ultimately, a new flagellum pocket forms and basal bodies move apart, thereby segregating the kinetoplast DNA connected to the posterior end of the basal body. The distal tip of the new flagellum remains attached by the connector structure to the side of the old flagellum, hence influencing the helical internal cytoskeleton. Cleavage proceeds after mitosis from the anterior end of the cell, ultimately segregating all duplicated organelles and producing two daughter trypanosomes.

Electron micrograph of the flagellum and associated internal cytoskeletal structures (Sherwin and Gull, 1989a).

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(See poster insert)

The trypanosome flagellum is now recognized as a major contributor to pathogenicity of these parasites. In this context, it contributes motility functions important in moving both the trypanosome and surface molecules, sensory recognition of the host/vector environment and, finally, recognition and attachment necessary for immobilizing the parasite at vector surfaces at certain critical life-cycle stages (Borst and Fairlamb, 1998; Gull, 1999).

The intrinsic feature of the trypanosome flagellum is that the existing flagellum is maintained and a new flagellum is produced during each cell cycle. Given that many of the trypanosome organelles exist as single copies, the basic issue facing the cell is a problem of coordinated duplication and segregation of structures and organelles, some in a conservative and some in a semi-conservative manner (Gull, 1999).

### Flagellum structure

The single flagellum exits the flagellar pocket at the posterior end of the trypanosome and is attached to the cell body along its length. In ultrastructural terms, the flagellum exhibits a canonical '9+2' microtubule axoneme, as well as a lattice-like structure termed the paraflagellar rod (PFR). The flagellum is attached to the cell body by a unique filament and positioned alongside this in the subpellicular array is a set of four specialised microtubules; together these two elements constitute the flagellum attachment zone (FAZ). This FAZ filament and specialised microtubules originate close to the basal bodies at the proximal end of the flagellum in the flagellar pocket zone (Sherwin and Gull, 1989a). Duplication and positioning of the elements within this zone are central to the morphological modulations observed during cell differentiation and cell proliferation (Gull, 1999).

### The flagellum and cell morphogenesis

Modulations in cell form that characterise the life-cycle stages range from what might appear to be simple changes in cell body length (the slender to stumpy bloodstream forms), to major cell-shape- and organelle-positioning

events such as those of the epimastigote form. However simple these cell shape modulations may seem, they must take place within the confines of the existing subpellicular microtubule cytoskeleton, which remains intact during both cell differentiation and cell proliferation (Gull, 1999).

Essentially three processes encapsulate the morphological events involved in cell proliferation and cell differentiation in *T. brucei* – duplication, positioning and segregation. The earliest morphological events in cell proliferation occur with duplication of the basal bodies, formation of the new flagellum and other connected cytoskeletal elements. The precise duplication and positioning of these cytoskeletal elements are crucial to ensure correct segregation to the two daughter cells (Gull, 1999). These early events occur in a strict temporal order and are coordinated with the periodic kinetoplast S-phase to ensure that the duplicated mitochondrial genome is connected to the duplicated basal bodies, securing its inheritance and segregation to the two new daughter cells (Robinson and Gull, 1991).

Once duplication and positioning of the new flagellum and connected elements are achieved, the process of building a new subpellicular array of microtubules begins. This occurs by the insertion of new microtubules between old microtubules, such that the complex is segregated semi-conservatively to each daughter trypanosome (Sherwin and Gull, 1989b). Details of these events vary slightly between the bloodstream and vector forms of the parasite. The details below, poster and movie (see <http://jcs.biologists.org/supplemental>) describe the events of the procyclic (tsetse midgut) form of the parasite.

### Spatial patterning and the flagellum

The precise 2D positioning of the cytoskeletal elements and their temporal order of duplication and positioning throughout the cell proliferation cycle in *T. brucei* is well described. However, it is clear that an appreciation of the 3D spatial positionings are critical for understanding the mechanisms of

inheritance of pattern and form in *T. brucei*. This is exemplified by recent experiments, which, with the discovery of the flagella connector in the procyclic form, have highlighted a templating mechanism involving the trypanosome flagellum. The extending new flagellum is always positioned posterior to the old flagellum and its distal tip is connected to the side of the old flagellum by a distinct structure, the flagella connector. The flagella connector 'tracks' along the old flagellum as the new flagellum extends, replicating the helical path of the old flagellum, until the new flagellum reaches full length. Axis and polarity information embedded in existing cell structure is, therefore, used in a cytotoxic process to influence the morphogenesis of the new cell (Moreira-Leite et al., 2001).

Spatial organisation is an important aspect to our understanding of many cellular processes that are of interest to cell biologists (e.g. mitotic spindle assembly/disassembly, cell division, centrosome duplication and cell polarity). The complexity of cytoskeletal construction and remodelling within the confines of the intact cytoskeleton of *T. brucei* is an example of the difficulties faced in attempting to understand and explain spatial organisation. Construction of the 3D spatial models in this poster and the accompanying movie resulted from a collaboration between laboratory scientists and computer graphic artists. The requirements of providing a distinct spatial blue-print to the graphic artists proved to be an excellent test of the laboratory scientist's knowledge. It led to the formulation of a number of new research programmes and highlighted the lack of real 3D detail in the descriptions of many cell biological systems. The use of new computer technology to enable 3D spatial organisation of different types of cell biological data to be documented, visualized and analysed at high resolution has increasing relevance. However, our experience is that when developed in an interactive manner, it not only facilitates visualisation of complicated events, but also highlights data limitations and enables formulation of new questions.

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