

Ultrastructure and function of nuclear microtubules in *Plasmodium falciparum* gametocytes



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Introduction

Plasmodium falciparum is the most prevalent and fatal species among human malaria parasites. The parasite has a complex lifecycle in its human host, including asexual and sexual stages. The sexual-stage parasites (gametocytes) undergo a dramatic morphological change during their maturation. The shape-changing ability of gametocytes is regulated by their cytoskeletons, with microtubules playing a crucial role. We have identified an unusual microtubule-based structure in the nucleus of early-stage gametocytes that emanates from a microtubule organizing center (MTOC) associated with the nuclear membrane. In this project, we aim to study the nuclear microtubules' dynamics and function in *P. falciparum* gametocytes. We hypothesise that nuclear microtubules play an important role in the shape and organization of the gametocyte's nucleus. We further propose that the MTOC serves as a nucleation point for the cytoplasmic microtubules, thereby initiating the formation of a structure that controls gametocyte morphology during its 12-day development period.

1. Nuclear microtubules assemble in stage II and disassemble in stage IV

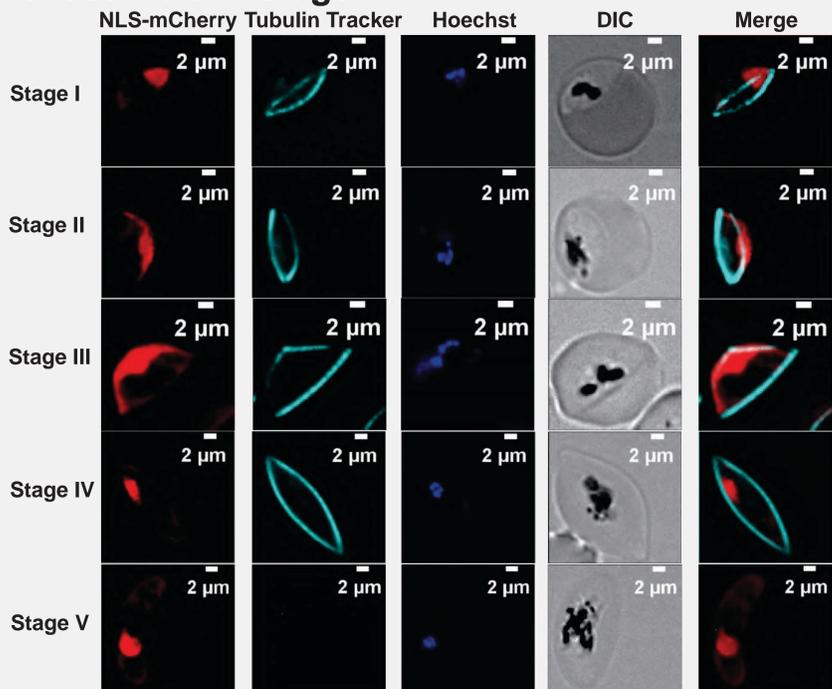


Figure 1. Live-cell fluorescence imaging of stage I to stage V gametocytes in *P. falciparum* NLS-mCherry parasite lines. NLS-mCherry appears to label an elongated nucleus (red) that overlaps with a population of microtubules labelled with Tubulin Tracker (cyan) and Hoechst-labelled DNA (blue).

3. Four distributions of nuclear microtubule bundles are observed

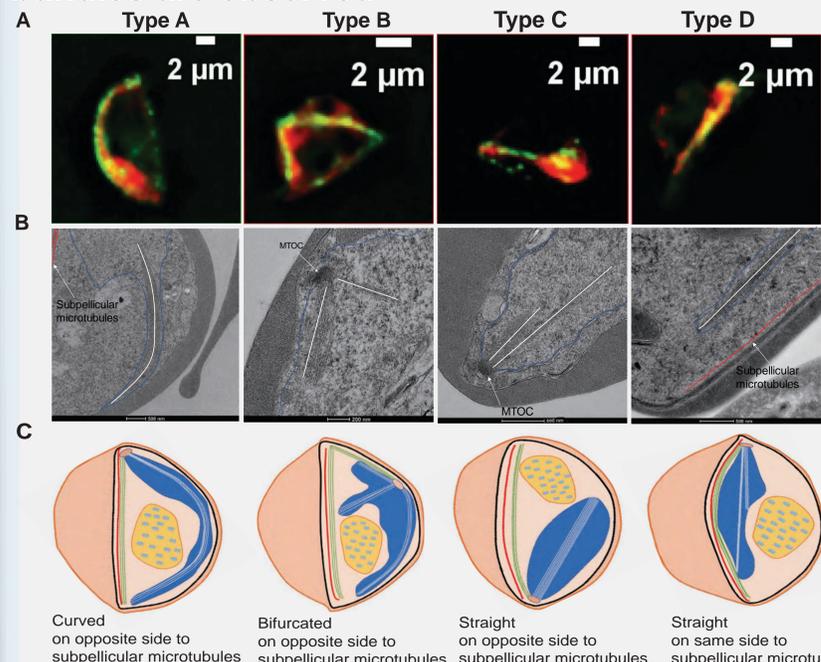
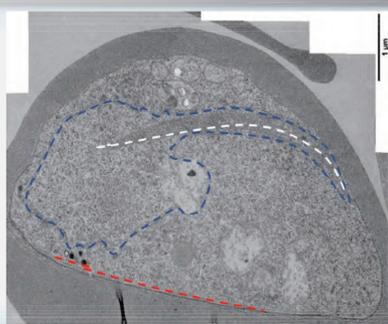


Figure 3. **A.** Live-cell imaging of PfEB1-GFP/NLS-mCherry co-transfectant stage III gametocytes, nucleus (red) and PfEB1-GFP (green). **B.** Transmission electron micrographs of stage III gametocytes. **C.** Diagram illustrating the different organizations of nuclear microtubule bundles.

5. Nuclear microtubule bundles elongate the nucleus

Figure 5. Transmission electron microscopy of stage III gametocyte (PfEB1-GFP).



2. PfEB1 is located along the full length of nuclear microtubules and is concentrated at the opposite end to Pfcenrin

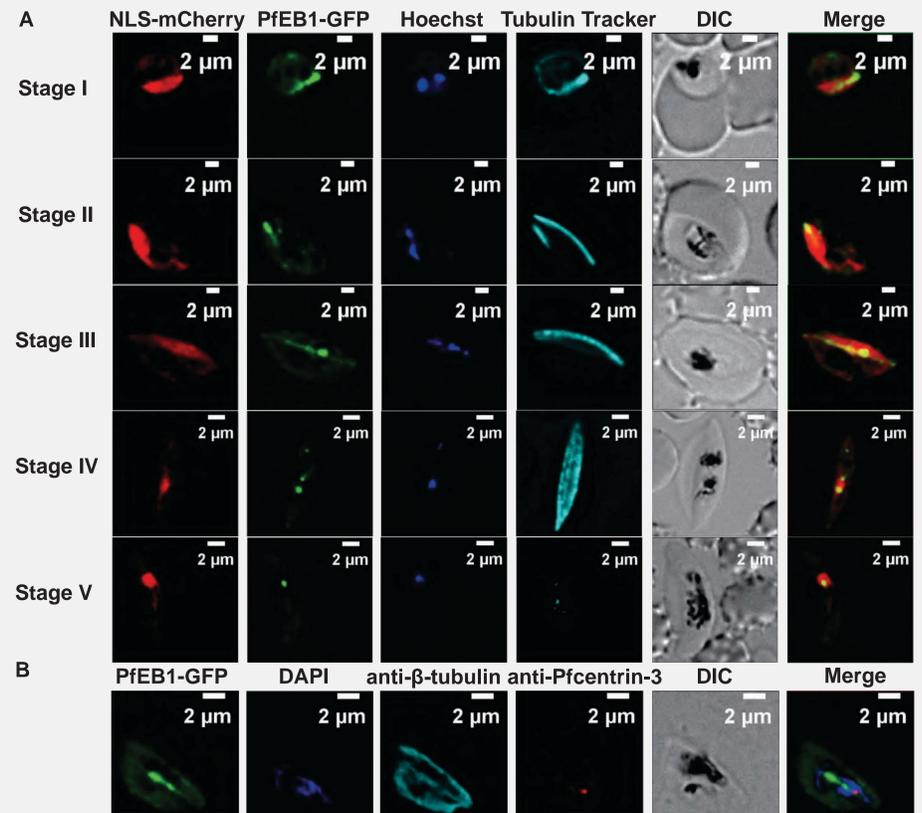


Figure 2. **A.** Live-cell fluorescence imaging of stage I to stage V gametocytes in the PfEB1-GFP/NLS-mCherry co-transfectant parasite lines. mCherry (red) delineates the nucleus and PfEB1-GFP (green) appears to be in the same compartment. Tubulin Tracker (cyan) and Hoechst (blue) labelled microtubules and DNA. **B.** Immunofluorescence microscopy showing anti-Pfcenrin-3 (red) and anti-β-tubulin (cyan) labeling PfEB1-GFP in a stage III gametocyte.

4. Nuclear microtubules associated with peripheral microtubules arise from the MTOC

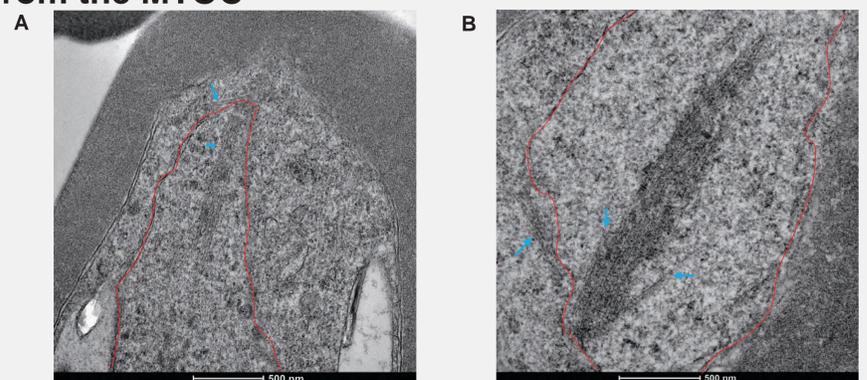


Figure 4. Transmission electron micrographs of stage III gametocytes (**A.** 3D7C; **B.** PfEB1-GFP) show the nuclear microtubule bundles impinging on the MTOC. Cytoplasmic microtubules appear to arise from the cytoplasmic face of the MTOC.

6. Transmission Electron Tomography

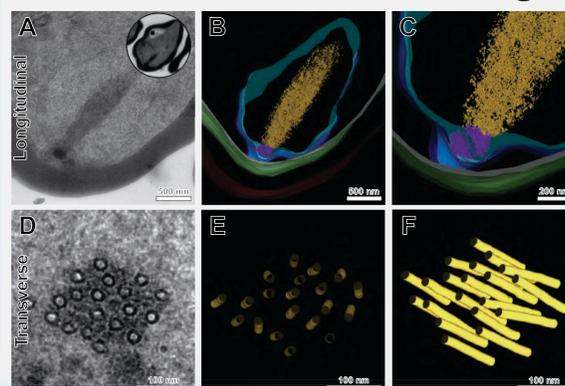


Figure 6. **A.** Longitudinal profile of a bundle of nuclear microtubules emanating from a MTOC embedded within the inner nuclear membrane and occupying the perinuclear space. **B** and **C** model view corresponding to **A.** **D.** Transverse profile of nuclear microtubules arranged in a rhomboid bundle. **E** and **F** model views corresponding to **D.** Colour legend: Red, erythrocyte plasma membrane; Green, parasitophorous vacuole; White, parasite plasma membrane; Blue, outer nuclear membrane; Cyan, inner nuclear membrane; Purple, MTOC; Gold, nuclear microtubules.

Summary

1. Nuclear microtubule bundles form in stage II and disassemble in stage IV gametocytes in *P. falciparum* gametocytes;
2. Nuclear microtubule bundles are associated with elongation of the nucleus and can be classified into four organizational types;
3. Nuclear microtubules emanate from an MTOC (indicated by Pfcenrin-3 labeling), which the plus-end binding protein, PfEB1, is concentrated at the other end;
4. The MTOC is also the site of initiation of cytoplasmic microtubules.