3D Electron Imaging Reveals Structural Development of Malaria Parasites

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The *Plasmodium falciparum* gametocyte has five distinct stages of development, where it transforms from a round stage I gametocyte into a typically falciform shaped stage V gametocyte. This process, known as gametocytogenesis, is a crucial step for the parasite's transformation from human host to *Anopheles* mosquito vector for sexual reproduction. A bilayer cisternal compartment membrane structure known as inner membrane complex (IMC) has been found beneath the parasite plasma membrane of the late stage gametocytes [1]. The IMC has a few distinct cellular functions, among which its role in stability and shape of the gametocyte is of great interest. Furthermore, the expansion of the IMC is accompanied by an increase in microtubule number and length [2]. Microtubules start to disassemble in stage V, whereas the IMC still determines the cylindrical shape of the gametocyte. In this work, we introduce 3D Scanning Electron Microscopy Block Face (BF-SEM) imaging of gametocytes and use it, in a combination with high-resolution electron tomography (ET), to elucidate the genesis and expansion of the molecular structures that drive gametocyte elongation.

We constructed a high-resolution 3D atlas of gametocytes to define their ultrastructure at different stages of development. The genesis and organization of IMC, along with the assembly of the underpinning microtubules were demonstrated as a distinct mechanism (Fig. 1). Membrane material is initially deposited in a thin semi-circular string of IMC plates at the periphery of the roughly spherical stage I gametocyte. The nascent IMC forms individual segments acting to seed microtubule formation. Then the IMC expands laterally and the parasites become highly elongated and encased in a microtubule network. The microtubules are depolymerized in stage V, but small membrane-bound stubs remain at the parasite periphery. In this work, we also characterized two IMC proteins, PhIL1 and PIP1, by gene knockdown strategies. BF-SEM imaging showed that the IMC of knockdown parasites is under-developed and they exhibit a swollen digestive vacuole, indicating an important role for PhIL1 and PIP1 in the membrane trafficking events that drive IMC genesis. High resolution ET also revealed unusual intranuclear microtubules. Although similar structures have been observed in other apicomplexa, their function has not yet been investigated. In our study, the novel microtubule structure was validated by multiple imaging techniques and various sample preparation procedures (Fig.2). These imaging data showed unusual elongated nuclei stretched by concentrated microtubule bundle in stage II-III gametocytes. This suggests a structural role for these microtubules separate from the sub-pellicular microtubules. The function of intranuclear microtubule bundles are being investigated by chemical modulation and gene knockdown.

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References:

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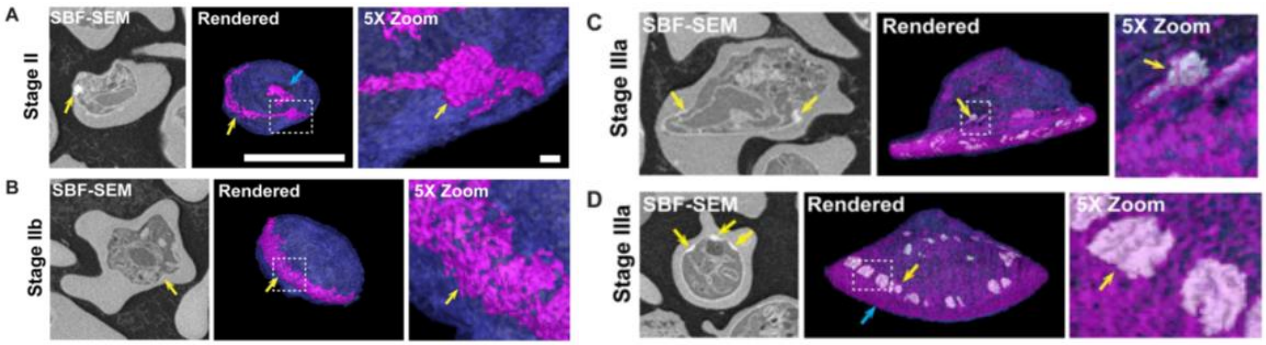

Fig 1. IMC development in early stage (II-III) gametocytes. Rendered models (right) and individual BF-SEM sections (left) are shown. The rendered 3D models reveal (A) a narrow semi-circular strip of thickened membrane at the periphery of stage II gametocytes (yellow arrow), transitioning (B) to a ribbon of disc-like plates in stage IIb (yellow arrow). (C) In stage III of development, these plates have expanded. Regions of thickening (yellow arrow, rendered in white) Determinants of P. falciparum gametocyte elongation. Scale bars: 5 µm. 5x Zoom images are shown on the right. Scale bars: 1 µm.

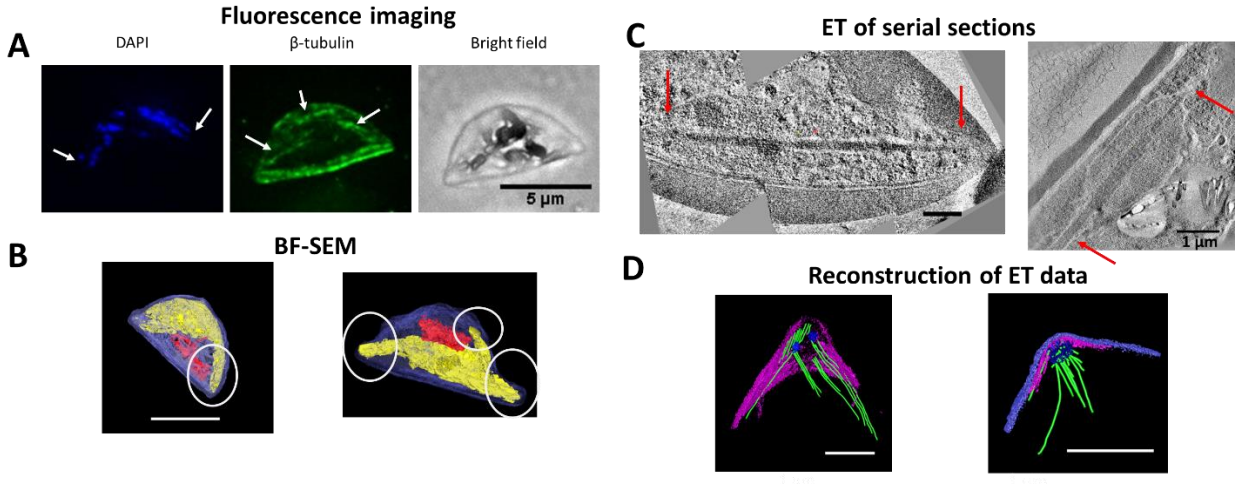

Fig 2. Elongated nucleus stretched by novel intranuclear microtubule structures in stage II and III gametocytes, which were found in three imaging methods, i.e. fluorescence imaging (A), BF-SEM (B) and ET (C). 3D models (D) were reconstructed from ET data. Microtubule is in green, IMC is in magenta and parasite membrane is in blue.

Elongated nuclei and tubular structures are indicted by arrows and circles.

Sample preparation:

Fluorescence imaging: paraformaldehyde fixation.

BF-SEM: glutaraldehyde fixation followed by heavy metal staining.

ET left: high pressure freezing and freeze substitution.

ET right: glutaraldehyde fixation and heavy metal staining.

Scale bar in BF-SEM model is 5 µm.