1492
doi:10.1017/S1431927622006031
Microsc. Microanal. 28 (Suppl 1), 2022

Visualizing the Intracellular Niche of Human-Infecting Microsporidia Using Serial Block Face Scanning Electron Microscopy

Noelle V. Antao[1], Cherry Lam[1], Alina Davydov[1], Joseph Sall[1], Feng-Xia Liang[1], Damian Ekiert[1,2*] and Gira Bhabha[1*]

[1] Department of Cell Biology, NYU Grossman School of Medicine, New York, NY, USA.
[2] Department of Microbiology, NYU Grossman School of Medicine, New York, NY, USA.
Corresponding authors: gira.bhabha@gmail.com, damian.ekiert@ekiertlab.org

Microsporidia are single-celled, eukaryotic parasites that can infect most animals including humans, where they most commonly cause enteric infections [1][2]. As obligate intracellular parasites, microsporidia have evolved highly reduced genomes and must co-opt metabolites from the host cell for successful reproduction and development [3,4]. The high degree of host dependence suggests that microsporidia likely establish close associations with various host organelles to ensure the rapid import of metabolites. However, little is known about the intracellular niche of human-infecting microsporidia, and how these pathogens develop in the host. This is, in part, because tools commonly used to study other pathogens, such as genetic manipulation, are not available for microsporidia. We used correlative light and serial block-face scanning electron microscopy (SBF-SEM) to analyze the intracellular niche of *E. intestinalis* at different stages of parasite development. We chose to use en face embedding of tissue culture monolayers, because at early time points, infection rates are very low (~10-15%). Therefore, we first used light microscopy to identify and record the shape and position of infected cells relative to the surrounding cells as well as its location on the dish (grid number/ position). The infected monolayer was next fixed, stained and embedded in Durcupan [5,6]. The light microscopy images were then used to correlate and identify the infected cell of interest for imaging by SBF-SEM. Analysis of our SBF-SEM datasets, allowed us to generate complete 3D reconstructions of the intracellular niche of *E. intestinalis* at 24 h and 48 h post infection (Figure 1A). Similar to recently published data [7], we found that early in infection, parasites are tightly associated with host cell mitochondria and ER (Figure 1A). We also observed that the host mitochondria were less tubular and more fragmented in infected cells, a finding that was corroborated by light microscopy (Figure 1B). SBF-SEM analysis of the different intracellular developmental stages of *E. intestinalis* provided a wealth of new information on how organelles in the parasite develop. In particular we could examine the development of an invasion organelle that is unique to microsporidia called the polar tube (PT) [8][9] (Figure 2). These findings have given us insights into the intracellular niche of a human-infecting microsporidia and how it is manipulated during parasite development. Additionally, we have gained insights into the development of the PT, an organelle important for invasion of the host.

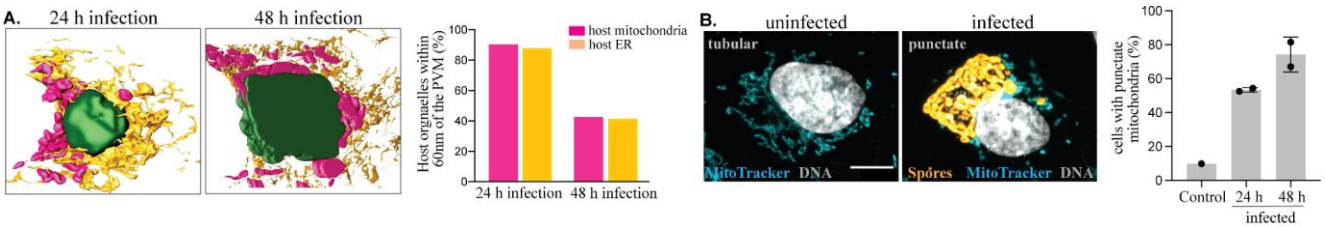

Figure 1. Examining the intracellular niche of *E. intestinalis* A. 3D reconstruction of the host cell niche at 24 h and 48 h post infection. Host mitochondria (pink), Host ER (yellow) and parasitophorous vacuole containing developing parasites (green) B. Micrographs of control (tubular mitochondria) and infected cells (punctate mitochondria). Cyan - MitoTraker, Grey - DNA and orange - FISH probe for E. intestinalis. Scale bar 10 µm.

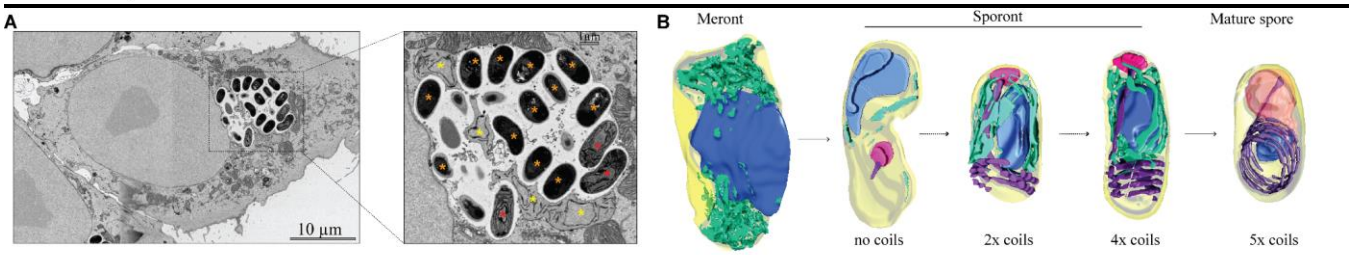

Figure 2. Characterising the intracellular developmental stages of *E. intestinalis* A. Single slice from a SBF-SEM dataset of Vero cells (African green monkey epithelial cells) infected with *E. intestinalis* for 48 h. Inset shows parasitophorous vacuole containing developing parasites (2.5X magnified). Meronts (yellow asterix), Sporonts (red asterix) and mature spores (orange asterix). B. 3D reconstruction of intracellular developmental stages of *E. intestinalis* - meronts, sporonts and mature spores. Parasite organelles - nucleus (blue), ER like membrane (green), anterior organelle (pink), polar tube (purple) and spore outline (yellow). PT coil number indicated. Scale bars 1µm and 10 µm.

References:

[1] J Vávra and J Lukeš, Adv Parasitol. **82** (2013) p. 253.
[2] LM Weiss, Oxford Medicine Online (2011). doi:10.1093/med/9780198570028.003.0056
[3] MD Katinka et al., Nature **414** (2001), p. 450.
[4] N Corradi et al., Nat Commun. **1** (2010), p. 77.
[5] TJ Deerinck et al. in "NCMIR methods for 3D EM: a new protocol for preparation of biological specimens for serial block face scanning electron microscopy" (2010).
[6] AM Seligman, HL Wasserkrug and JS Hanker, J Cell Biol. **30** (1966), p. 424.
[7] J Flores et al., J Cell Sci. **134** (2021). doi:10.1242/jcs.253757
[8] A Magaud, A Achbarou and I Desportes-Livage, J Eukaryot Microbiol. **44** (1997), p. 81S.
[9] Y Xu and LM Weiss. Int J Parasitol. **35** (2005), p. 941.

https://doi.org/10.1017/S1431927622006031 Published online by Cambridge University Press