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# Cytoskeletal organization during cell division in apicomplexan parasites

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Apicomplexan parasites employ a complex cytoskeletal system for cell division that is largely unexplored. Recent data suggest an unconventional division machinery that divides the nucleus and separates the apicoplast [1], a single non-photosynthetic plastid that is essential for parasite survival. By using *Toxoplasma gondii* as a most suitable laboratory model for apicomplexan parasites it has been shown that the parasite's unconventional division apparatus is based on microtubules and their organizing center (the centriole-centrosome complex) and perhaps other cytoskeletal fibers that associate with the apicoplast and the pellicle (the parasite's outer membrane system) to generate the force required for coordinated separation of the genome and the apicoplast.

By employing our newly developed methods that allow preservation and three-dimensional imaging of cytoskeletal structures in *T. gondii* we were able to obtain new information on the complex and unusual motility system of apicomplexan parasites [2,3,4]. To understand the cytoskeletal organization and the functional correlation with cell divison in apicomplexan parasites we have employed the following two methods using high resolution field emission scanning electron microscopy (HRFESEM). The first method utilized membrane-lysed cytoskeleton-stabilized cells which allows direct imaging of cytoskeletal structures underneath the plasma membrane. The second method is based on removing Epon from thick-sectioned material with Epon solvent and viewing this sectioned material with HRFESEM. In addition to microtubules (20-nm diameter) we were able to visualize a complex meshwork of interconnected fibers that include intermediate-sized filaments (10-nm diameter), and actin-like filaments (7-nm diameter).

Material used for low voltage field emission scanning electron microscopy (LVFESEM) was prepared by extracting the cells attached to coverslips with 0.15% Triton X-100 in cytoskeletal stabilization buffer (PHEM) consisting of 60 mM Pipes, 25 mM HEPES, 10mM ethylene glycol bis (beta-aminoethyl ether)-N,N,N', N-tetra acetic acid, 2 mM $MgCl_2$, pH 6.9. Samples were fixed in 2% glutaraldehyde in 100 mM HEPES, pH 7.4 containing 0.2% tannic acid. After rinses with 100 mM Hepes, cells were postfixed in 0.1% $OsO_4$ for 10 minutes, 1% uranyl acetate for 10 min, dehydrated through an ethanol series, and critical-point dried. After Argon ion sputter coating with a thin layer of Pt (about 1-nm), the cells were viewed with the Hitachi S-900 FESEM at the Integrated Microscopy Resource in Madison, WI, operating at 1.5 kV. For our second method, human fibroblasts and *Toxoplasma* parasites were cultured on coverslips, fixed with 1% glutaraldehyde in 0.1M HEPES buffer (pH 7.2) plus 0.05% saponin and 0.2% tannic acid. After washing in buffer, they were

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postfixed in 0.1% osmium tetroxide and 1% uranyl acetate, and embedded in Epon. 200nm thick sections were cut orthogonally to the cell substrate. Ribbons were collected on cover glass strips fitting into the Hitachi S-900 stage. The Epon was extracted from the sections with the Polysciences Epoxy Resin Removal Kit. The extracted sections were critical point dried, and Argon ion sputter coated with a thin layer of Pt. Micrographs were obtained with the Hitachi S-900 FESEM in Madison, WI, operating at 1.5kV.

By using these methods we could visualize the replication of daughter cells on the complex scaffolding of cytoskeletal fibers and flattened membrane vesicles, the inner membrane complex. We expect to reveal mechanisms that play a role in generating the force between cytoskeletal structures and the parasite's membrane system that are required for coordinated cell division in apicomplexan parasites.

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