

Evolutionary conservation within the apical complex: Insight from newly discovered conoid-associated proteins in *Toxoplasma gondii*

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One of the defining features of the group Apicomplexa is the assemblage of structural and secretory elements forming the apical complex, which play pivotal roles in host cell invasion and proliferation. The complexity of its architecture, however, differs among apicomplexan lineages. An elaborate apical complex is found in the Coccidia and consists of the apical polar ring that serves as an organizing centre for an array of subpellicular microtubules, and the mobile conoid that sits within the apical polar ring. The conoid consists of tubulin fibres and associated proteins tightly organized into a hollow barrel that protrudes during invasion. Despite extensive characterization of the apical complex through ultrastructural studies, our knowledge of its molecular composition, function and evolutionary origins is limited. To address this issue, we selected several protein markers that localize in close proximity to the conoid in *Toxoplasma* and fused them with a promiscuous biotin ligase to use as baits for proximity biotinylation assay. Furthermore, we applied a novel spatial proteomics technology termed LOPIT (Localisation of Organelle Proteins by Isotopic Tagging) to simultaneously map the localization of several thousand proteins on a cell-wide scale. Our proteomic data provided new candidate proteins associated with the *Toxoplasma* apex, and several of them were localized to different parts of the conoid by 3D-SIM super-resolution microscopy. Thirteen of these proteins have clear orthologs in *Plasmodium*, despite the apparent absence of the conoid in this widely studied parasite. Most of these proteins seem to be specifically expressed in the invasive stages within the mosquito vector and their localization in *Plasmodium* is currently being investigated. Although the majority of conoid-associated proteins have very restricted taxonomic distribution within Apicomplexa, few proteins are also found in other eukaryotic taxa suggesting that this highly specialized cytoskeletal assembly partially evolved from pre-existing cellular structures.