Establishing a pipeline for structural characterisation of a mitochondrial RNA-processing complex in apicomplexan parasite Toxoplasma gondii

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Apicomplexa are a phylum of protozoan, obligate parasites. Most are pathogenic, and infect a wide range of hosts causing diseases in both humans and animals. A particularly well studied apicomplexan is *Toxoplasma gondii* which causes toxoplasmosis, a prevalent infectious disease that poses a health risk to immunocompromised individuals and unborn or newborn babies.

Critical mitochondrial complexes in *T. gondii* and related parasites, are very different in composition and structure from the commonly studied ophistokonts (e.g. yeast and mammalian cells), representing both a prominent site of biological divergence and a promising target for antiparasitic drug development. The mitochondrial ribosome (mitoribosome) is a particularly important complex mediating the translation of the three essential proteins necessary for the electron transport chain. The structure consists of 124 total proteins, including 55 clade-specific proteins and ~53 rRNA fragments, highlighting both the highest degree of rRNA fragmentation in any mitoribosome studied thus far and the importance of numerous clade specific proteins including a family of proteins known as RNA binding domain abundant in Apicomplexa - or RAP proteins.

There are 23 predicted RAP proteins in *T. gondii* with four RAP proteins found in the mitoribosome. RAP family members were shown and/or postulated to play a role in RNA stability and processing in both apicomplexans and humans. However, little is known about their mechanism of action. Our preliminary studies provide support to the role of a RAP protein in ribosomal RNA stability and/or processing in *T. gondii*, providing an opportunity for mechanistic studies. My work aims to tackle this through the identification and characterization this mechanism via tools such as proteomics and SPA cryo-EM.

Initial progress involves three complementary avenues of work: 1) Utilizing CRISPR-Cas9 to introduce a Twin-Strep tag to the C-terminus of the RAP protein 2) Utilising CRISPR-Cas9 to introduce TurboID for the purposes of proximity labelling and 3) Optimisation of mitochondrial isolation for future affinity purification/immunoprecipitation directly from mitochondria to enrich for our protein of interest.

This work should allow us to better understand the function of RNA processing complexes on ribosomal RNA in *Toxoplasma gondii*.