Regulation of mitochondrial RNA-maturation by an RNA-binding domain abundant in Apicomplexa or RAP protein in *Toxoplasma gondii*

Shikha Shikha, Mariana Silva, Stephanie Waypa, Yu Jing Chang, Lilach Sheiner

School of Infection & Immunity, University of Glasgow

Mitochondrial gene expression is essential for nearly all eukaryotes. Apicomplexan parasites, including *Plasmodium* and *Toxoplasma*, possess one of the most reduced mitochondrial genomes known, approximately 5.9 kb in size. This genome encodes only three protein-coding genes, lacks tRNA genes entirely, and contains numerous ribosomal RNA (rRNA) pieces that assemble into functional mitochondrial ribosomes. Despite the essential role of mitochondrial gene expression in parasite viability, the mechanisms that generate mature mitochondrial transcripts from this minimal genome remain largely unexplored. This atypical RNA landscape is marked by the expansion of a family of organelle localized RNA-binding proteins termed RNA-binding domain abundant in Apicomplexa (RAP), which are hypothesized to mediate mitochondrial RNA maturation.

Here, we investigate the function of a RAP protein that is mitochondrially-localised but not a structural component of the *Toxoplasma* mitochondrial ribosome. Conditional knockdown of this protein is lethal, as parasites fail to form plaques upon depletion. Loss of the RAP protein results in complete ablation of complex IV activity, whilst complexes II and V remain unaffected, consistent with a specific defect in mitochondrial translation. Accordingly, depletion of this RAP protein disrupts the assembly of both the small and large mitoribosomal subunits. Northern blot analyses further reveal the accumulation of longer rRNA species, indicating impaired rRNA processing. Complementation of the knockdown line with ectopic expression of the wild-type RAP protein fully rescues mitochondrial translation defects. In contrast, complementation with a RAP-domain deletion or with point mutations of two conserved residues within the RAP domain fails to restore function, demonstrating that the RAP domain is essential for the protein's RNA-maturation activity.

Collectively, these results establish RAP proteins as critical regulators of mitochondrial RNA-maturation in apicomplexan parasites and identify them as key factors underlying the unique and highly complex mitochondrial RNA-maturation landscape of this phylum.