

## Heavy metal detoxification in *Toxoplasma gondii*

Copper homeostasis must be tightly regulated in all cell types, as although this micronutrient is required as a cofactor for crucial enzymes, excess copper causes toxicity and cell death. Mammalian cells utilise copper in nutritional immunity to combat invading intracellular pathogens, with successful pathogens employing their own detoxifying measures both to counteract host-driven mechanisms and ensure sufficient uptake to maintain replication. However, such detoxification and transporting mechanisms are poorly understood in the obligate intracellular parasite *Toxoplasma gondii*, causative agent of the zoonotic disease Toxoplasmosis.

We hypothesize that a putative copper transporting P-type ATPase (CuTP) functions as a copper exporter, protecting *Toxoplasma* from copper stress. We genetically deleted CuTP and found the parasites were less able to replicate under standard growth conditions. Although *Toxoplasma* requires copper in the mitochondria, we saw no defects in mitochondrial morphology or copper-dependent mitochondrial processes. However, the growth phenotype could be rescued and growth restored when copper was removed by chelation. Supporting our hypothesis,  $\Delta cutp$  mutants displayed hypersensitivity to copper but not to other transition metals, demonstrating the potential selectivity of this transporter.

We are currently investigating not only the role of CuTP in *Toxoplasma*, but the mechanism behind copper toxicity within the pathogen, with current research exploring links between copper accumulation and reactive oxygen species accumulation and mis-metalation as possible parasite death mechanisms. We suggest that although we see slight ROS accumulation under high copper conditions, copper-induced oxidative stress may not be the key contributor to *Toxoplasma* death, with future research validating this and investigating possible cuproptosis in *Toxoplasma*.