Copper uptake in the intracellular parasite *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular parasite capable of infecting virtually all warm-blooded animals and up to one-third of the human population. During acute infection, rapidly replicating tachyzoites of *T. gondii* invades host cells and replicates within a parasitophorous vacuole, eventually lysing the host cell to propagate infection, whereas environmental stress triggers differentiation into slow-growing bradyzoites that persist within tissue cyst and resist immune clearance and current therapies. Like other eukaryotes, *T. gondii* depends on essential trace metals such as copper, a critical cofactor for mitochondrial cytochrome c oxidase (complex IV). However, the mechanisms by which *T. gondii* acquires and regulates copper remain poorly understood. *T. gondii* encodes three copper transporters in the genome, two of which (Ctr1 and Ctr2) are expressed. Here, we identify and functionally characterize two major copper transporters in Toxoplasma gondii, Ctr1 and Ctr2. We generated knockout strains for each gene and found that both genes were involved in copper homeostasis, but with distinct phenotypes. Loss of Ctr1 caused severe defects in intracellular replication, mitochondrial respiration, and complex IV activity. Remarkably, supplementation with exogenous copper rescued the Ctr1-deficient phenotypes, suggesting that another transporter with lower affinity can compensate for copper uptake under certain conditions. On the other hand, we found out that Ctr2 is dispensable during tachyzoite growth but is copper responsive and required to maintain bradyzoite viability both in vitro and in vivo. These findings provide the first comprehensive characterization of copper transport and homeostasis in *T. gondii* and demonstrate that multiple Ctr transporters function cooperatively to ensure sufficient copper acquisition to sustain essential mitochondrial and metabolic functions during infection.