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Malarial Parasites Accumulate Labile Zinc Pools

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The malarial parasite, *Plasmodium falciparum*, is an intracellular pathogen and partially dependent on nutrient uptake for survival. In this issue of *Chemistry & Biology*, Marvin et al. demonstrate that zinc is essential for parasite growth and that the parasite maintains substantial labile cytosolic and mitochondrial zinc pools.

Microbial pathogens require transition metals for structural, catalytic, and signaling functions. While these roles have been extensively studied in pathogenic bacteria and fungi, fewer studies have examined the contribution of transition metals to human protozoan pathogen biology. Given the essential roles these metals play, a thorough and quantitative understanding of the mechanisms governing the dynamics of uptake, subcellular distribution, and utilization should provide basic insights that may be exploited for therapeutic purposes.

P. falciparum is an important example of a human protozoan parasite. This pathogen is responsible for the form of malaria associated with the highest rates of morbidity and mortality. To date, studies of transition metal biology in *P. falciparum* have focused predominantly on iron and, to a lesser extent, copper metabolism (Rasoloson et al., 2004). Several factors have contributed to this emphasis. *P. falciparum* completes a part of its complex life cycle inside human red blood cells (RBCs). During this period, the parasite imports a substantial amount of hemoglobin (Hb) into a specialized digestive vacuole where Hb is proteolytically degraded into peptides/amino acids and the iron-containing heme cofactor, which is predominantly detoxified by polymerization into hemozoin (Francis et al., 1997). Despite the large amount of iron that could be derived from heme, the parasite is exquisitely sensitive to iron chelators (Mabeza et al., 1999). Similarly, copper chelation is inhibitory to parasite growth (Rasoloson et al., 2004), suggesting these metals play critical biological roles.

Notably, key insights into iron metabolism in *P. falciparum* have been gained through the use of complementary spectroscopic approaches. These modalities have been invaluable in quantitatively mapping the subcellular distribution of various iron-containing species at high spatial resolution in parasite-infected RBCs (Bonifacio et al., 2008; Egan et al., 2002; Kang et al., 2011). In this issue of *Chemistry and Biology*, Marvin et al. (2012) build on the theme of using complementary spectroscopic techniques to gain key insights into metal biology in *P. falciparum*. The authors use X-ray fluorescence microprobe (XFM) microscopy, confocal microscopy with zinc-specific fluorescent sensors, and inductively-coupled plasma-mass spectrometry (ICP-MS) to quantitatively analyze the accumulation of zinc and several other metals in *P. falciparum*-infected RBCs. They demonstrate that infected RBCs accumulate intracellular zinc at levels up to 4-fold above those in uninfected RBCs as determined by XFM. Zinc accumulation occurs in a stage-dependent manner, with levels being minimal in early-stage (rings), significantly increased in mid-stage (trophozoites) and maximal in late-stage (schizonts) parasites. Additionally, zinc appears to accumulate in both the parasite's cytosol and mitochondrion, reaching local concentrations up to ~20-fold that in uninfected RBCs. A substantial fraction of the zinc pool is labile as determined by quantitative fluorescence imaging with the Zinbo5 sensor. Furthermore, the parasite is susceptible to zinc restriction by the potent chelating agent, TPEN, and zinc supplementation reverses this effect. Parasites treated with TPEN fail to accumulate a labile zinc pool, and their mitochondrial membrane potential is dissipated. Altogether, these data indicate that *P. falciparum* accumulates a labile zinc pool and that zinc limitation by a fairly specific and potent chelator induces parasite death in a process that involves mitochondrial dysfunction.

While the authors have focused on zinc fluxes and characterize these in the most detail, Marvin et al. (2012) provide additional insight into *P. falciparum*-induced changes in the RBC "metallome". Using ICP-MS, they demonstrate intracellular increases in copper (2-fold), magnesium (~2-fold), and calcium (6-fold) above uninfected RBC levels. Interestingly, a significant increase in intracellular iron levels in parasitized RBCs (~14–15 mM) was not detected. This reinforces the hypothesis that the labile iron pool upon which the parasite is dependent is quite small relative to what is potentially available.

The Marvin et al. (2012) paper raises several key biological questions. What are the mechanism(s) governing zinc uptake and subcellular distribution in the parasite? As the zinc concentration in infected RBCs exceeds that in normal RBCs, additional zinc must be acquired from an extracellular source. The authors propose a two-step process whereby zinc is first imported into the RBC cytosol by resident transporter(s), and subsequently into the parasite via parasite-synthesized transporter(s) (Figure 1). They suggest the *P. falciparum* gene PF07_0065 (PF3D7_0715900) as a candidate for zinc import based on a temporally consistent transcriptional profile and its 29% identity to the mouse Znt2 transporter. However, no genetic, biochemical, or cell biology data testing this hypothesis is currently available. To speculate, bioinformatics analyses suggest that PF07_0065 is a six transmembrane domain protein lacking discernible N-terminal

signal peptide, apicoplast, or mitochondrial targeting sequences. As zinc does not appreciably accumulate in either the nucleus or the digestive vacuole, PF07_0065 might be predicted to localize to the parasite cell membrane assuming it plays a role in zinc trafficking. In this scenario, trafficking mechanisms to explain how zinc in the RBC cytosol enters the parasite's cytosol and mitochondrion are still lacking. This speculative model is subject to the caveat that protein trafficking targeting signals cannot be predicted with complete certainty, and there are alternative mechanisms for protein subcellular localization in *P. falciparum* and other cell types. For example, the Znt2 transporter in mouse mammary epithelial cells localizes to the inner mitochondrial membrane despite the absence of a classical mitochondrial targeting sequence (Seo et al., 2011). If this were also true in *P. falciparum*, this could explain the partial accumulation of zinc within the mitochondrion. However, without invoking targeting of PF07_0065 to multiple compartments, it becomes difficult to reconcile the observed subcellular trafficking and distribution of zinc with a single parasite-derived transport mechanism. Further genetic and biochemical studies will be needed to more fully address this question.

Another future challenge lies in defining the essential zinc-dependent process(es) that are most reliant on the accumulated labile zinc pool. Establishing a specific role for the mitochondrial zinc pool and the circumstances under which it communicates with the cytosolic pool will also be crucial. An important consideration worth keeping in mind is that TPEN is a potent, cell-permeable chelator capable of perturbing both the labile and to some extent the more tightly protein-bound structural/catalytic zinc pools. Consequently, direct disruption of essential protein function may contribute to TPEN-induced toxicity. Therefore, whether perturbation of the labile zinc pool per se versus direct depletion of zinc from essential proteins underlies TPEN-induced toxicity is presently unresolved. It will be informative to determine how selectively sequestering the labile zinc pool impacts parasite viability. Furthermore, monitoring parasite growth kinetics over the course of the RBC developmental cycle during these studies will provide a high-resolution picture of the cell cycle phase most sensitive to perturbation of the labile zinc pools.

Overall, the study by Marvin et al. (2012) describes the intriguing phenomenon of an accumulated labile zinc pool in *P. falciparum* and vulnerability of the parasite to zinc restriction. The authors propose that this susceptibility may potentially be exploited in drug development efforts. Given the widespread resistance to current antimalarial drugs, this may indeed represent a new opportunity, especially upon gaining a more complete understanding of the molecular mechanism(s) underlying zinc fluxes in the parasite.

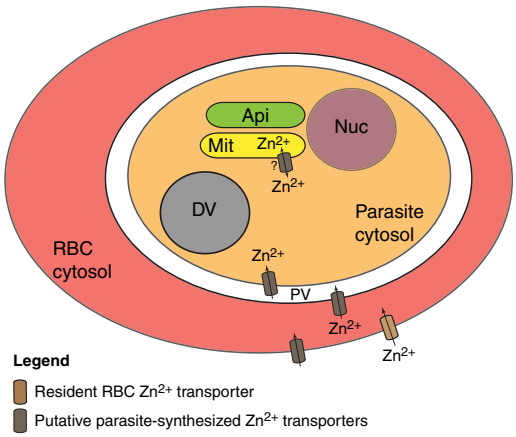

Figure 1. Putative Transport Routes for Zinc Uptake from the External Medium and Accumulation in the *P. falciparum* Cytosol and Mitochondrion Are Illustrated

Zinc transporters natively present on the RBC surface perhaps together with unknown parasite-derived transporters exported to the RBC membrane may mediate initial uptake of zinc into the RBC cytosol. Parasite-synthesized transporters could then facilitate zinc transport across the parasitophorous vacuole (PV) and into the parasite cytosol. Whether these are general divalent metal or zinc-specific transport mechanisms will be important to establish. The cytosolic and mitochondrial labile zinc pools may then dynamically communicate with each other. Marvin et al. (2012) propose that the PF07_0065 could be a zinc-specific transporter, but if and where it functions along the zinc transport route remain to be established. Api, apicoplast; Mit, mitochondrion; Nuc, nucleus; PV, parasitophorous vacuole, which is a virtual space between the inner parasite plasma membrane and the outer parasitophorous vacuolar membrane derived from the RBC membrane during parasite invasion.

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