Expression and characterization of a *Plasmodium falciparum* copper-binding protein (Sco1)

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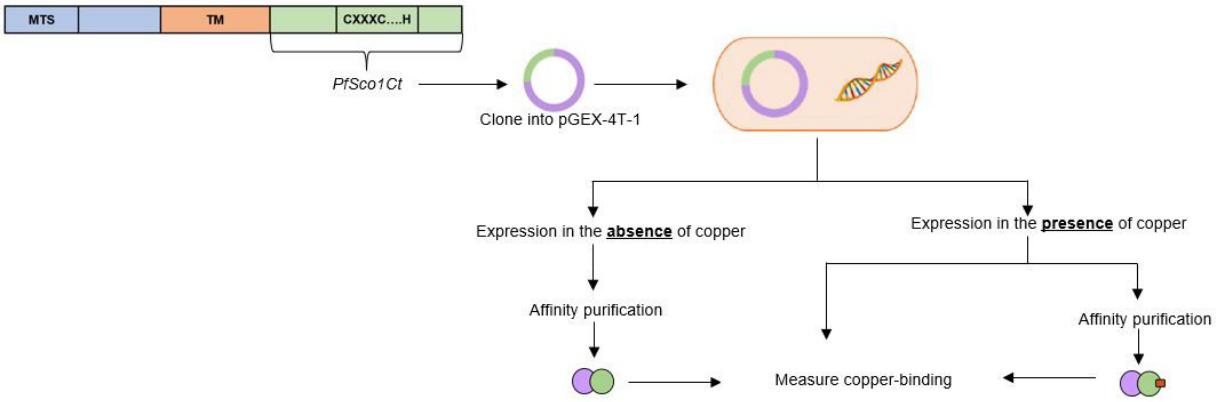

INTRODUCTION: Malaria is an infectious disease caused by the protozoan of the genus *Plasmodium.* Five species infect humans; *P. knowlesi*, *P. ovale*, *P. malariae*, *P. vivax*, and *P. falciparum* (Antinori *et al*., 2012). *In vitro* studies have shown *P. falciparum* ring-to-trophozoite development is inhibited by the copper chelator neocuproine (Asahi *et al*., 2014). Excess copper is toxic to the parasite. Copper is an essential metal in eukaryotic metabolism and is required by enzymes such as cytochrome *c* oxidase for activity (Camponeschi and Banci, 2018). Cytochrome *c* oxidase has two copper sites (Cu_A and Cu_B) in the Cox2 and Cox1 domains respectively. The Cu_A site is metallated by Sco1. Sco1 hasbeen implicated in the regulation of cellular copper uptake and efflux. A Sco1 homolog with the characteristic CX3C copper-binding motif and a conserved copper-binding histidine has been identified in the *P. falciparum* genome.

METHODOLOGY: A 3D structure of *Pf*Sco1 (PF3D7_0708900) was modeled on the human Sco1 structure. The carboxy-terminal domain of the putative *P. falciparum* Sco1 (*PfSco1Ct*) was amplified from *P. falciparum* genomic DNA, cloned, and expressed as a glutathione-s-transferase fusion protein (rGST-*Pf*Sco1Ct). Copper-binding was examined by three methods in both an *in vitro* and *in vivo* setting using the copper-catalyzed oxidation of ascorbate, the BCA copper-release assay, and by examining the growth of the *E. coli* host bacteria expressing rGST-*Pf*Sco1Ct in copper enriched media.

RESULTS: The homology modeling showed that *Pf*Sco1 has the copper-binding CX3C copper-binding motif and the conserved copper-binding histidine as the human Sco1. The affinity-purified rGST-*Pf*Sco1Ct bound copper and inhibited the copper-catalyzed oxidation of ascorbate. The BCA copper-release assay showed that rGST-*Pf*Sco1Ct binds copper and preferentially binds cuprous copper (Cu^{+}) rather than cupric copper (Cu^{2+}). *E. coli* host bacterial growth was reduced in the presence of 8 mM $CuCl_2$, while the bacteria expressing rGST-*Pf*Sco1Ct continued to grow. This result suggests that rGST-*Pf*Sco1Ct bound copper in the *in vivo E. coli* environment. Affinity-purified rGST-*Pf*Sco1Ct expressed in low, 0.5 mM $CuCl_2$ bound copper as shown by the BCA release assay and did not inhibit the copper-catalyzed oxidation of ascorbate.

DISCUSSION AND CONCLUSION: *P. falciparum* parasites express Sco1. The rGST-*Pf*Sco1Ct binds Cu^{+} *in vitro* and *in vivo*. Understanding *P. falciparum* copper homeostasis and characterizing the proteins of the Plasmodial “cuprome” has interesting potential for novel antimalarial drug development

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