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## "CYCLIC GMP-PKG SIGNALING AND THE REGULATION OF $CA^{2+}$ DYNAMICS IN THE INTRAERYTHROCYTIC LIFE CYCLE PROGRESSION OF *PLASMODIUM FALCIPARUM*"

By

Rute Isabel Honorio
PhD Biomedical Sciences,
Molecular Biology, Genetics, and Cancer Program
Bachelor's in Pharmaceutical Sciences, University of Sao Paulo, 2021

Thesis Advisors:
Dr. Andew P Thomas
Distinguished Professor and Chair of the
Department of Pharmacology, Physiology & Neuroscience,

Celia Regina da Silva Garcia
Full Professor – University of Sao Paulo – Brazil
Department of Clinical and Toxicological Analyses – School of Pharmaceutical Sciences

Friday, May 15th, 2026
1:00 PM
MSB, H609b

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## Abstract

The crosstalk among signaling pathways that act through calcium ion ($Ca^{2+}$) concentration in *Plasmodium falciparum* is not fully understood, and this is further complicated by the parasite's exposure to multiple environmental conditions throughout its life cycle, transitioning between the mosquito vector and the human host. An important player that increases cytosolic $Ca^{2+}$ concentration is the cyclic GMP (cGMP)-activated Protein Kinase G (PKG) pathway. This pathway is crucial for the final step of the intraerythrocytic stage of the parasite in the human host, as its activation triggers the process of egress of the daughter parasites, the merozoites, from the red blood cells (RBC); however, how PKG orchestrates the increase in cytosolic $Ca^{2+}$ concentration essential for egress remains unclear. The aim of this study is to understand this crucial signaling pathway, focusing on finding the organelles and downstream signaling that increase cytosolic $Ca^{2+}$ concentration. First, we focused on characterizing the response to increased activity of the cGMP-PKG signaling pathway in parasites within RBCs, from mid trophozoites to late schizont stages, the growth and replicating stages, respectively. To increase activity in this pathway, we used zaprinast, an inhibitor of the phosphodiesterase enzyme that degrades cGMP, thereby increasing PKG activation. There is a stage-dependent difference in the zaprinast response, ranging from no cytosolic $Ca^{2+}$ increase in early/mid trophozoites, followed by a small transient peak in replicating parasites, to a two-phase response characterized by a transient peak followed by a second, persistent $Ca^{2+}$ response in schizonts. To identify the $Ca^{2+}$ sources for these two phases, the $Ca^{2+}$ pump inhibitor cyclopiazonic acid (CPA) was used to deplete the endoplasmic reticulum (ER) $Ca^{2+}$ content, and chloroquine was used to disrupt digestive vacuole (DV) homeostasis, both of which are important $Ca^{2+}$ storage organelles for the parasite. We identified downstream of cGMP the ER as the source of $Ca^{2+}$ for the first transient response, and the DV as the source of $Ca^{2+}$ for the second, persistent response. A further understanding of the role of cGMP-PKG signaling during egress came from our identification of a cell-autonomous increase in cytosolic $Ca^{2+}$ concentration preceding egress. This $Ca^{2+}$ increase initiates 30 to 25 minutes before egress and takes the form of $Ca^{2+}$ oscillations with progressively increasing amplitude leading up to egress. During egress there is a further increase in cytosolic $Ca^{2+}$ of the free merozoites, and $Ca^{2+}$ continues to oscillate at a higher amplitude. To further investigate the $Ca^{2+}$ oscillation mechanisms, trophozoite-stage parasites isolated from RBCs were used as an experimental model. In this preparation it was possible to identify that the oscillations depend on extracellular $Ca^{2+}$ and on PKG. Our results indicate that activation of the cGMP-PKG signaling pathway increases cytosolic $Ca^{2+}$ concentration from three sources: the ER, the DV, and extracellular $Ca^{2+}$.

In a separate study, we screened 10 compounds from an antimalarial candidate library and identified a low half-maximal inhibitory concentration ($IC_{50}$) against *Plasmodium falciparum*, indicating promising compounds to further investigate the mechanism of action.