Identification of IP_3 Pathway Components in *Plasmodium falciparum* and *P. chabaudi* Using Gene Co-expression Networks

Lyang Higa Cano[1], Celia Regina da Silva Garcia[2], Ronaldo Fumio Hashimoto[1]

1. Instituto de Matemática, Estatística e Ciência da Computação, Departamento de Ciências da Computação, Universidade de São Paulo, São Paulo, Brasil.
2. Department of Clinical and Toxicological Analysis, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil.

Plasmodium falciparum, the main causative agent of malaria, remains a global health challenge. Several *Plasmodium* species exhibit circadian rhythmicity patterns during their intraerythrocytic cycle (IEC) [1], which appears to increase parasite fitness and immune evasion [2-5]. This rhythmicity is disrupted under in vitro conditions [6], indicating that synchronization depends on signals from the host. Among the proposed host cues, melatonin, a hormone produced in a circadian manner, has been widely studied as a key regulator of parasite rhythms [7, 8]. Experimental data suggest that melatonin acts through an IP_3 signaling cascade [8], triggering intracellular calcium release and gene expression changes [8]. Although this pathway is supported by pharmacological and transcriptomic evidence [8], most of its molecular components in *Plasmodium* remain unknown [8]. In this work, we present a computational strategy to identify and investigate candidate components of the IP_3 signaling pathway. Our approach uses Gene Co-expression Networks (GCNs) [9] and a Guilt-by-Association (GBA) heuristic [10]. To reduce the false positives commonly found with GBA [11], we employ a topological strategy for threshold selection [12]. We then combined these network results with domain architecture comparisons and structural analysis. We first validated our GCN model by confirming its ability to find well-known functional modules, including components of the tubulin complex [13] and key enzymes of the glycolysis pathway [14]. We then applied the validated model to transcriptomic datasets [15, 16] from parasites either synchronized or unsynchronized with the host circadian cycle. The results suggest biologically plausible predictions, highlighting SR25 (a putative GPCR), a candidate for $G_{\alpha q}$-like protein, and MDR1 as a possible IP_3 receptor. Interestingly, the inferred pathway appears to be more closely connected to K^+-induced signaling than to direct melatonin effects [17]. To complement these results, we implemented a comparative domain analysis framework. Although no new domains were detected in the well-characterized PLC protein from *P. falciparum* [18, 19], structural comparison with the human ortholog demonstrated that the pipeline can recover biologically consistent features, supporting its application to less-characterized candidates. We also developed a method that combines our GCN model and the inferred IP_3 module to search for genes potentially related to this pathway among the 1408 genes in *P. falciparum* annotated with unknown function [20]. This is an important challenge, as about one third of the genes in the *P. falciparum* genome still lack functional annotation.

Keywords: *Plasmodium, melatonin, Ca^{2+}, IP_3 Receptor, Gene Co-expression Networks.*

References

[1] Célia R. S. Garcia, Regina P. Markus, and Luciana Madeira. “Tertian and Quartan Fevers: Temporal Regulation in Malarial Infection”. en. In: Journal of Biological Rhythms 16.5 (Oct. 2001), pp. 436–443.

[2] Philip G. McQueen and F. Ellis McKenzie. “Host Control of Malaria Infections: Constraints on Immune and Erythropoeitic Response Kinetics”. en. In: PLoS Computational Biology 4.8 (Aug. 2008). Ed. by Rob J. De Boer, e1000149.

[3] Igor M. Rouzine and F. Ellis McKenzie. “Link between immune response and parasite synchronization in malaria”. en. In: Proceedings of the National Academy of Sciences 100.6 (Mar. 2003), pp. 3473–3478.

[4] Aidan J. O’Donnell et al. “Fitness costs of disrupting circadian rhythms in malaria parasites”. en. In: Proceedings of the Royal Society B: Biological Sciences 278.1717 (Aug. 2011), pp. 2429–2436.

[5] Sarah E. Reece, Kimberley F. Prior, and Nicole Mideo. “The Life and Times of Parasites: Rhythms in Strategies for Within-host Survival and Betweenhost Transmission”. en. In: Journal of Biological Rhythms 32.6 (Dec. 2017), pp. 516–533.

[6] William Trager and James B. Jensen. “Human Malaria Parasites in Continuous Culture”. en. In: Science 193.4254 (Aug. 1976), pp. 673–675.

[7] Carlos T. Hotta et al. “Calcium-dependent modulation by melatonin of the circadian rhythm in malarial parasites.” en. In: Nature Cell Biology 2.7 (July 2000), pp. 466–468.

[8] Bárbara K. M. Dias, Abhinab Mohanty, and Célia R. S. Garcia. “Melatonin as a Circadian Marker for Plasmodium Rhythms”. en. In: International Journal of Molecular Sciences 25.14 (July 2024), p. 7815.

[9] Sipko Van Dam et al. “Gene co-expression analysis for functional classification and gene–disease predictions”. en. In: Briefings in Bioinformatics (Jan. 2017).

[10] Oliver, S. (2000). Guilt-by-association goes global. Nature, 403(6770), 601-603.

[11] Jesse Gillis and Paul Pavlidis. “ “Guilt by Association” Is the Exception Rather Than the Rule in Gene Networks”. en. In: PLoS Computational Biology 8.3 (Mar. 2012).

[12] Bhavesh R Borate et al. “Comparison of threshold selection methods for microarray gene co-expression matrices”. en. In: BMC Research Notes 2.1 (2009), p. 240.

[13] Malabika Chakrabarti, Nishant Joshi, and Geeta Kumari. “Interaction of Plasmodium falciparum apicortin with alpha- and beta-tubulin is critical for parasite growth and survival”. In: Scientific Reports 11 (2021).

[14] David D. Van Niekerk et al. “A detailed kinetic model of glycolysis in Plasmodium falciparum-infected red blood cells for antimalarial drug target identification”. en. In: Journal of Biological Chemistry 299.9 (Sept. 2023), p. 105111.

[15] Amit K. Subudhi et al. “Malaria parasites regulate intra-erythrocytic development duration via serpentine receptor 10 to coordinate with host rhythms”. en. In: Nature Communications 11.1 (June 2020), p. 2763.

[16] Filipa Rijo-Ferreira et al. “The malaria parasite has an intrinsic clock”. en. In: Science 368.6492 (May 2020), pp. 746–753.

[17] Miriam S. Moraes et al. “Plasmodium falciparum GPCR-like receptor SR25 mediates extracellular K+ sensing coupled to Ca2+ signaling and stress survival”. en. In: Scientific Reports 7.1 (Aug. 2017), p. 9545.

[18] Andreas Raabe et al. “Genetic and transcriptional analysis of phosphoinositidespecific phospholipase C in Plasmodium”. en. In: Experimental Parasitology 129.1 (Sept. 2011), pp. 75–80.

[19] Paul-Christian Burda et al. “Global analysis of putative phospholipases in Plasmodium falciparum reveals an essential role of the phosphoinositide-specific phospholipase C in parasite maturation”. en. In: mBio (July 2023).

[20] Cristina Aurrecoechea et al. “PlasmoDB: a functional genomic database for malaria parasites”. In: Nucleic Acids Research 37.suppl_1 (Jan. 2009), pp. D539–D543.