FUNCTIONAL GENOMICS IN *Plasmodium*

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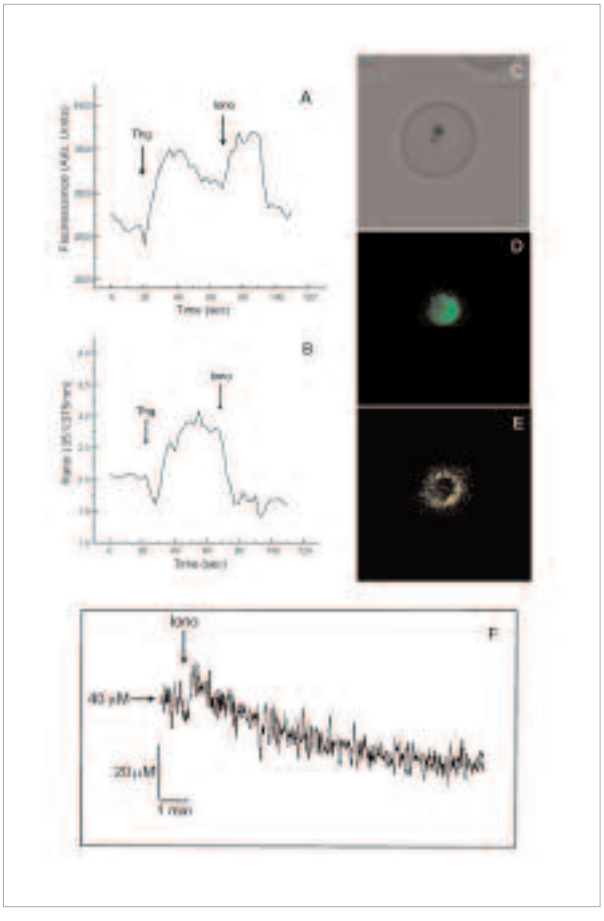

Simultaneous imaging of the [Ca^{2+}] in the PV and cytosol

The significance of this proposal is to investigate the adaptation of the parasite, where *Plasmodium* is capable of sensing the environment and using molecules derived from the host to signal the cell cycle. Using a bioinformatics approach we identified in the genome, four candidates with serpentine receptors in *Plasmodium falciparum*. Briefly, this proposal is based on the importance of the upstream signaling paths (serpentine receptors) and downstream paths (kinases, phosphatases, etc.) in *Plasmodium* for the modulation of transduction signal coupled to survival and replication mechanisms of the parasite. An important aspect of the project emerged with results of the microarray showing that *Pfemp* (*Var* genes) has its genes activated when parasites *P. falciparum* were subjected to treatment with melatonin. Investigation with strains of parasites coming from patient samples (obtained form the strain banks of the Department of Parasitology, ICB, Dr. Gerard Wunderlich, São Paulo and Rondônia) and comparison with culture samples treated or not with hormone could provide vital information for the understanding of the mechanisms of the regulation of the expression of the *Var* genes. One hypothesis is that the hormone could mimic a physiological situation and act in the regulation which is absent from the culture of *Plasmodium falciparum*. To unravel the paths of signal transduction from serpentine receptors in the membrane and target-proteins in the cytosol for the development of new drugs to combat malaria. To study the function of the genes, we plan to use bioinformatics, proteomics and systems Biology such as, for example, the use of synthetic genes with codon optimization to resolve the question of the difficulty of heterologous expression in *Plasmodium*.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

This thematic project commenced in March 2008, following on from an earlier thematic project, the objective of which was to elucidate the molecular and cellular bases of the transduction of the signal of the intra-erythrocyte cycle of *Plasmodium*.

Studies in Cell and Molecular Physiology in *Plasmodium* point to the use of a mechanism for transduction of signal which includes Ca^{2+}-ATPases and proteins regulated by calcium. The sequencing of the Genome of *Plasmodium* in 2002 revealed that 60 per cent of the genes of the parasite do not possess homology with other organisms. In this thematic project, we are investigating what mechanisms the malaria parasite would use to develop within the host cell, an inhospitable environment, using messenger seconds, such as calcium and AMPc to modulate its life cycle.

Our research shows that in the course of evolution, the parasite subverted the endocrine system of the host and sequestered calcium machinery creating an ionic microenvironment so as to adapt itself in the interior of the erythrocyte.

The passage between the intracellular stages, as well as the initiation of the cell division are synchronic processes. Interestingly the synchronicity of the intraerythrocyte forms observed in the cycle of *Plasmodium* is lost in culture. Our discovery that derivatives of tryptophan synchronize malaria parasite is the first evidence that the synchronicity depends on signaling molecules from the host. The hypothesis is based on the capacity of the parasite to perceive signal in the extracellular environment. The search for transduction paths of the hormone signal led us to find the participation of the protein kinase A (PKA).

In this project, we identified a receptor for kinase C *Pfrack* (Madeira et AL, 2003) in *Plasmodium*. This gene (*Pfrack*-GFP) when transfected in mammal cells, blocks the signaling of calcium when stimulated with the agonist ATP (photo attached).

The next question to be answered was: How could the parasite detect an extracellular signal? After a long search, we identified through bioinformatics 4 serpentine receptors in the membrane of *Plasmodium*. This information is fundamental for the understanding of how the parasite identifies and transduces extracellular signals.

Research into the mechanism of signal transduction by the malaria parasite represents a potential goal, as it could lead to the development of new chemotherapies.

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