

## Reality check for malaria proteomics

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### Abstract

New studies highlight the wide diversity of post-translational protein modifications in the intra-erythrocytic stages of the malaria parasite, raising new avenues for inquiry.

Now is an exciting time to be in malaria research. The science is moving at an ever faster pace, and the malaria research community has been challenged by Bill and Melinda Gates to re-engage with the ambitious concept of global eradication of malaria. Fundamental to any new efforts to attack the parasite (*Plasmodium*) or its mosquito vectors (*Anopheles* species) is the need to understand the regulation and molecular organization of parasite development throughout its complex life cycle (Figure 1). A new study by Foth *et al.* [1] published in *Genome Biology* adds a significant new dimension to this understanding by using methods that detect and delineate a diversity of post-translational modifications to proteins in the asexual stages of the parasite infecting the red blood cells of its human host, the stage that causes the debilitating clinical symptoms of malaria.

#### 'Just-in-time' regulation and its exceptions

The sequencing of the genome of *Plasmodium falciparum* in 2002 made possible high-throughput global analysis of the transcriptome [2-5]. Interpreted in the light of the limited previous work on the expression of individual proteins, these transcriptome analyses suggested that a significant fraction of the genome is regulated in a 'just-in-time' manner; that is, immediate translation (implicitly of bioactive proteins) of newly synthesized transcripts [3]. The first proteomic studies emerged soon after, looking at large datasets from individual or multiple parasite life stages [6-12].

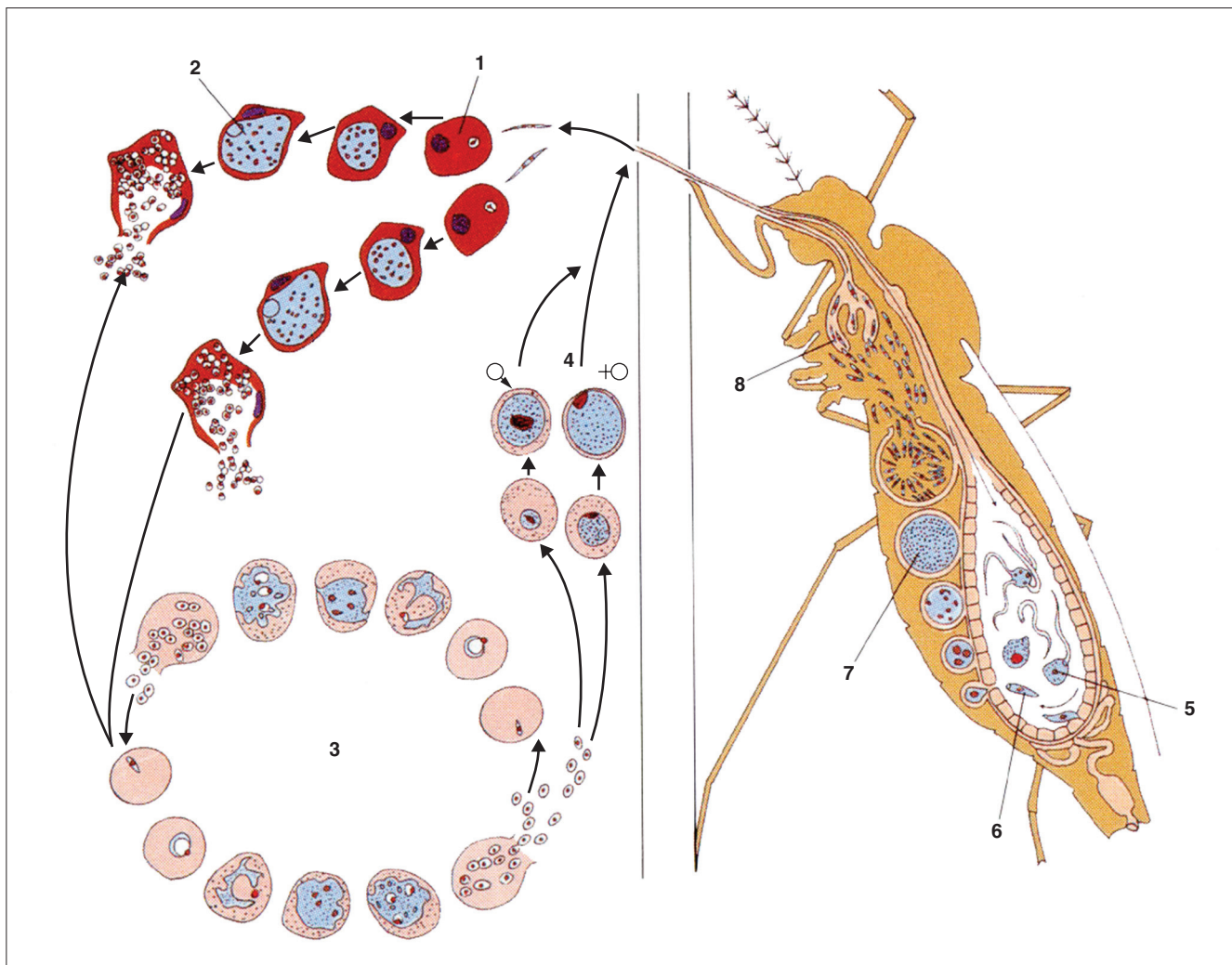
While proteomic studies confirmed the expression of many proteins as consistent with the 'just-in-time' hypothesis, they also found that a previously described disjunction of transcription and translation [13] was not the rarity suspected,

but might represent a 'master strategy' by which quiescent stages of the parasite life cycle are pre-programmed for rapid developmental transitions - for example, when the cell-cycle-arrested gametocytes are transferred from the human bloodstream into the stomach of the mosquito vector. Here, induction of gametogenesis (see Figure 1) by mosquito-derived xanthurenic acid, and a fall in temperature of the bloodmeal, activates calcium- and protein-kinase-mediated pathways that control gamete formation [14]. Transcripts for as many as 370 proteins expressed in the gamete or in the zygote (for example, the candidate vaccine targets P25 and P28), were found to be stabilized by a DDX6-class RNA helicase, DOZI [15]. These mRNAs are translated within minutes following ingestion of infected blood into the mosquito's stomach.

There is a second (and reciprocal) life-stage transition when another cell-cycle-arrested form (the sporozoite) leaves the mosquito salivary gland and enters the liver of the human host to initiate infection (see Figure 1) but, interestingly, here there is less compelling evidence for translational control [16]. It is somewhat surprising, therefore, that a growing body of evidence, exemplified by the study of Foth *et al.* [1], indicates that translational control can regulate differentiation of the rapidly replicating asexual stage of the parasite during its pathogenic development inside red blood cells.

#### Post-translational regulation in *P. falciparum*

Exciting though high-throughput global transcriptome and proteome comparisons are, they do not grapple with the fact that development of eukaryotic organisms is significantly regulated by post-translational modifications of protein structure and function, for example, protease cleavage [17],

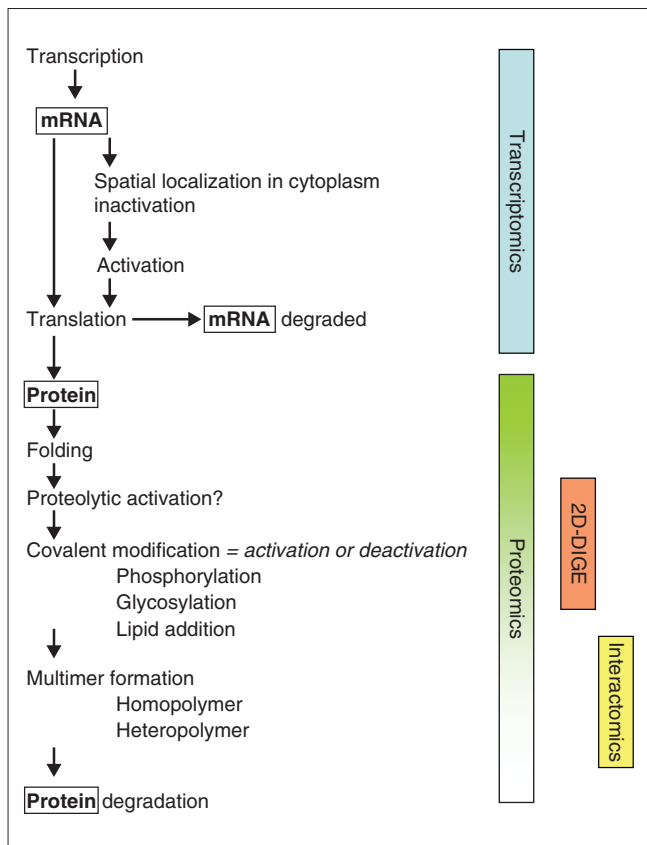


**Figure 1**  
 A generic life cycle of *Plasmodium* species. Sporozoites delivered from the salivary glands of a biting mosquito (8) enter the human bloodstream and are carried to the liver, where they infect hepatocytes (1) and produce liver-stage schizonts. These burst open to release merozoites, which enter red blood cells and undergo multiple rounds of replication as the erythrocytic schizont (3). The stages shown at (3) are those analyzed by Foth *et al.* [1]. A minority of merozoites at each cycle form the sexual stage gametocytes (4), which persist in the blood until ingested by another mosquito. Within minutes, gametes differentiate in the mosquito gut and fertilization follows (5). The zygote then develops into an ookinete (6), which penetrates the gut wall to form another 'schizogonic' stage, the oocyst (7). Daughter sporozoites are released from the oocysts and invade the salivary glands (8). Gametocytes (4) are terminally arrested cells while within the bloodstream. The expression of many proteins required for gamete function just minutes after the parasite is ingested by the mosquito is under translational control. Sporozoites (8), which are similarly responsible for transmission between hosts, have not yet exhibited similar regulation of gene expression: note that their development in the new host is less urgent. Figure modified with permission from [20].

phosphorylation, glycosylation, covalent addition of lipid groups and formation of molecular complexes (Figure 2). Foth *et al.* [1] now make the first substantive effort to understand how changes in both protein structure and protein amount modulate *Plasmodium* development in its asexual blood stages. There have been previous attempts to produce quantitative data on protein expression levels, but the elegant and logistically demanding methodology of that work, using radiolabeling methods [18], lacked the higher-throughput potential of the methods deployed by Foth *et al.* These authors [1] used experimentally standardized two-dimensional

difference gel electrophoresis (2D-DIGE) with fluorescent labeling to compare protein expression in four samples (each of a 6-hour 'bandwidth') taken from cultures from infected red blood cells 34, 38, 42 and 46 hours post-invasion.

Analysis of some 9,000 spots in the gels showed that the abundance of 278 proteins changed more than 1.4-fold between samples, the most extreme being the translation initiation factor eIF5a, which exhibited a 15-fold change. Detailed analysis including identification by mass spectrometry (MS) was achieved for 54 proteins, a small but



**Figure 2**  
Application of ‘omics’ technologies to understanding the regulation of expression of functional proteins. The area in which 2D-DIGE approaches (as applied by Foth *et al.* [1]) are particularly valuable is indicated.

significant return for the massive investment made when compared with previous less discriminatory approaches using multidimensional protein identification technology (MudPIT) or one-dimensional gel/liquid chromatography/MS technologies [6-11], methods that have identified many hundreds of proteins at individual life stages.

What the new data lack in quantity is, however, more than compensated for by the new information on protein abundance and isoform changes. Foth *et al.* [1] detected multiple isoforms for 50% of all the proteins identified. Different isoforms of equivalent mobility ( $M_r$ ) were considered to be due to changes in phosphorylation. An increase in mobility between two isoforms was interpreted to be due to post-translational protein cleavage (or proteasomal degradation). One protein, enolase, was described in no less than seven different isoforms, of which two appeared to be truncated.

By comparing the proteomic data from these samples with previous transcriptomic data from comparable samples [5], Foth *et al.* [1] found that expression of some proteins or isoforms - for example, the chaperone protein HSP40 and

four actin isoforms - were concordant with the ‘just-in-time’ synthesis model. Interestingly, peak protein abundance of another actin isoform was delayed following transcription, indicating regulated post-translational modification. The expression of yet other proteins, for example HSP60, was negatively correlated with their mRNA levels.

**Looking to the future**

Where does this leave us? Reductionists can argue strongly that this paper [1] reinforces the concept that it is essential to treat each molecule and pathway separately and investigate each and every one in depth, whereas ‘synthesizers’ can emphasize that such global approaches have the potential, perhaps not fully realized in this work, to understand ‘master regulatory mechanisms’, which require consideration before examining individual pathways, each of which will be, by definition, unique. It will be interesting to see whether the application of systems approaches to data of this type will permit resolution of these questions at the global level.

Above all, Foth *et al.* [1] provide a healthy reality check as to the complexity of the molecular mechanisms regulating the development of this important parasite, which should caution the researcher against making assumptions as to the time and place of protein activity from transcriptome, or indeed proteome, analyses. Even the phenotypic analysis of genetic mutations may not provide unequivocal solutions to these questions [19]. For those enjoying the ‘thrill of the academic chase’ there is clearly ample room for more exciting research. For those seeking to control this global scourge, an understanding of the fundamental yet multifaceted mechanisms regulating parasite development may bring ways of interrupting the parasite’s life cycle, or perhaps of generating new attenuated strains for therapy or transmission blockade.

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