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Epigenetic memory takes center stage in the survival strategy of malaria parasites

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Malaria parasites run through a complex life cycle in the vertebrate host and mosquito vector. This not only requires tightly controlled mechanisms to govern stage-specific gene expression but also necessitates effective strategies for survival under changing environmental conditions. In recent years, the combination of different -omics approaches and targeted functional studies highlighted that *Plasmodium falciparum* blood stage parasites use heterochromatin-based gene silencing as a unifying strategy for clonally variant expression of hundreds of genes. In this article, we describe the epigenetic control mechanisms that mediate alternative expression states of genes involved in antigenic variation, nutrient uptake and sexual conversion and discuss the relevance of this strategy for the survival and transmission of malaria parasites.

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Introduction

The life cycle of malaria parasites (*Plasmodium* spp.) is characterised by a succession of distinct cell differentiation and division phases allowing the parasites to progress through the diverse environments encountered during their difficult journey. With the exception of blood stage parasites, the life cycle proceeds in a linear progressive manner where fertilisation and meiosis in the mosquito midgut as well as the intermittent mitotic proliferation steps (sporogony, intra-hepatic schizogony, male gametogenesis) produce functionally specialised daughter cells (ookinetes, sporozoites, merozoites, microgametes) that are distinct from their progenitors and determined to initiate the next phase of the life cycle (midgut epithelium traversal, hepatocyte invasion, erythrocyte invasion, fertilisation). In contrast, during the blood stage of infection the parasite population undergoes vegetative growth through repeated rounds of intra-erythrocytic schizogony, and parasites have a choice to enter either one of two alternative developmental pathways — mitotic proliferation or gametocyte differentiation. Importantly, while continuous asexual proliferation secures the supply of sexual precursor cells that are essential for malaria transmission, this strategy lies in conflict with the host's adaptive immune system and other environmental pressures that jeopardise parasite survival during this phase of the infection.

It has long been recognised that *Plasmodium* parasites employ antigenic variation in order to escape immune recognition and to establish persistent infection [1]. In an effort to understand the regulatory mechanisms underlying antigenic variation of *var*/PfEMP1 in *P. falciparum*, epigenetics became an important field of investigation in malaria research. The term 'epigenetics' describes heritable changes in gene activity that are established through reversible chromatin modifications and occur independent of changes in the DNA sequence [2]. As a consequence, genetically identical cells adopt clonally variant phenotypes and propagate them through subsequent cell divisions (epigenetic or cellular memory). We now know that *P. falciparum* blood stage parasites make extensive use of epigenetic control not only to regulate antigenic variation but also to drive clonally variant expression of many other proteins involved in red blood cell (RBC) invasion, solute transport or sexual differentiation. In this review, we will discuss recent advances in our understanding of epigenetic control mechanisms in *P. falciparum* and how epigenetic gene regulation influences parasite survival and transmission.

The BIG picture — how –omics approaches shaped our understanding of the epigenetic landscape in *P. falciparum*

Epigenetic changes in gene expression are commonly orchestrated by alteration of chromatin structure via reversible post-translational modifications (PTMs) of histones. Histone PTMs recruit effectors that can in turn oligomerize and condense nucleosomal arrays (e.g. heterochromatin protein 1 (HP1)), re-organise local chromatin structure (e.g.

SWI/SNF-family remodellers) or place/remove other influential histone modifications (e.g. methyltransferases (HKMT), demethylases, acetyltransferases or deacetylases (HDAC)) [3]. The first proteomics studies of *P. falciparum* histone extracts mapped more than 40 PTMs, with acetylation being the most abundant modification [4,5]. Except for H3K27me3 and H3K36me3, most H3 and H4 modifications present in other eukaryotes have been identified in *P. falciparum*, and *Plasmodium*-specific modifications were mainly detected on the heavily acetylated tails of the H2A.Z and H2B.Z histone variants. More recent studies identified additional histone phosphorylation and acetylation modifications [6,7], and with the improved sensitivity of state-of-the-art mass spectrometry the identification of further PTMs is awaited.

Genome-wide distribution of histone PTMs as defined by chromatin immunoprecipitation followed by array hybridization (ChIP-on-chip) or next generation sequencing (ChIP-seq) proved to be highly informative to understand epigenetic regulation in *P. falciparum* [8]. These studies revealed that most of the *Plasmodium* genome is in a transcriptionally competent euchromatic state [9,10•]. The AT-rich intergenic regions are demarcated by a unique nucleosome subtype containing H2A.Z/H2B.Z [11•,12•]. Interestingly, these regions are dynamically marked across the cell cycle by various histone modifications (e.g. H3K4me3, H3K9ac, H4K8ac) that correlate with transcriptional activity of the neighbouring gene, but do not appear to dictate whether a gene is expressed or not [10•,13].

In contrast to these euchromatic features, the subtelomeric regions of all chromosomes and some chromosome-internal islands are characterised by H3K9me3 and PfHP1, evolutionary conserved marks of heterochromatin and gene silencing [9,14,15]. These domains incorporate over 400 genes (approx. 8% of the coding genome) that primarily encode members of proteins families implicated in functions at the host-parasite interface. An elegant study investigating transcriptional profiles of isogenic clones revealed that basically all heterochromatic gene classes are subject to clonally variant gene expression (CVGE) and that CVGE was hardly detected for genes outside heterochromatic domains [16••], suggesting that H3K9me3/PfHP1-dependent gene regulation is the major pathway for ''true'' epigenetic inheritance in *P. falciparum*. DNA methylation, which has a major importance in heterochromatin formation and epigenetic regulation in other eukaryotes, was believed to be absent in *Plasmodium* parasites. A recent study challenges this view by detecting predominantly CHH-type DNA methylation at most parts of the genome using sequencing of bisulphite-treated genomic DNA [17•]. However, given the asymmetric nature of this modification and broad distribution in both euchromatic and heterochromatic regions it appears unlikely that CHH-type DNA methylation plays a major role in heritable silencing in *P. falciparum*.

Playing hide-and-seek — antigenic variation

PfEMP1 is the major antigen and virulence factor on the surface of RBCs and is encoded by approximately 60 *var* gene paralogs [18]. PfEMP1 interacts with various receptors on endothelial cells or other RBCs and thus mediates sequestration of infected RBCs (iRBCs) in the microvasculature, which contributes substantially to severe disease outcome [19]. Antigenic variation of *var*/PfEMP1 is a complex process that builds on two main pillars. First, only one *var* gene is transcribed in individual parasites whereas all other members are silenced (mutual exclusion or singular gene choice). Mitotic inheritance of these transcriptional states prevents premature presentation of the full antigenic repertoire by the expanding population. Second, infrequent switches in mutually exclusive *var* activation give rise to subpopulations that can proliferate in the presence of adaptive immune responses targeting previous PfEMP1 variants.

Silenced *var* loci are associated with H3K9me3/PfHP1 throughout the cell cycle [14,20,21]. In contrast, nucleosomes at the active *var* promoter are characterised by H3K9ac and H3K4me2/3 [20] and the variant histones H2A.Z/H2B.Z [12•] (Figure 1). Histone-modifying enzymes known to participate in *var* regulation include the sirtuin-like HDACs PfSIR2A (required for silencing of the upsA/upsC/upsE *var* genes and some other subtelomeric gene families including *rif* [22–25] and PfSIR2B (required mainly for silencing of upsB *var* genes) [24]. A putative ortholog of the H3K9me-specific HKMT SU(VAR)3-9 (PfSET3/PfHKMT1) has been localised to the nuclear periphery in blood stages but functional characterisation of this factor is still missing [15,26]. Intriguingly, two recent studies identified a rather unexpected role for the H3K36me-specific HKMT PfSET2 in *var* gene regulation [27•,28]. While *var* gene coding regions are associated with H3K36me3 irrespective of their transcriptional state, H3K36me3 is reduced/absent at the transcriptional start site and intron of the active *var* gene [27•,28] (Figure 1). In PfSET2 knockout parasites, most *var* genes are de-repressed and display reduced H3K36me3 occupancy along the entire transcription unit [27•]. However, this is only observed during the active phase of *var* transcription in ring stage parasites. During schizogony, where *var* transcription is repressed, H3K36me3 occupancy is restored to wild type levels in a PfSET2-independent manner [27•]. It therefore remains unclear if PfSET2/H3K36me3 participates in epigenetic memory or specifically mediates transcriptional repression in ring stages.

While the above mechanisms explain how most *var* genes are silenced it remains largely elusive how the transition between the silenced and active states is achieved. Several lines of evidence suggest that *var* gene activation occurs explicitly in a perinuclear *var* expression site (VES) and that *var* gene switching involves re-positioning