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REVIEW
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Histone Modification Landscapes as a Roadmap for Malaria Parasite Development

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Plasmodium falciparum remains the deadliest parasite species in the world, responsible for 229 million cases of human malaria in 2019. The ability of the *P. falciparum* parasite to progress through multiple life cycle stages and thrive in diverse host and vector species hinges on sophisticated mechanisms of epigenetic regulation of gene expression. Emerging evidence indicates such epigenetic control exists in concentric layers, revolving around core histone post-translational modification (PTM) landscapes. Here, we provide a necessary update of recent epigenome research in malaria parasites, focusing specifically on the ability of dynamic histone PTM landscapes to orchestrate the divergent development and differentiation pathways in *P. falciparum* parasites. In addition to individual histone PTMs, we discuss recent findings that imply functional importance for combinatorial PTMs in *P. falciparum* parasites, representing an operational histone code. Finally, this review highlights the remaining gaps and provides strategies to address these to obtain a more thorough understanding of the histone modification landscapes that are at the center of epigenetic regulation in human malaria parasites.

Keywords: histone post-translational modifications, histone combinations, epigenetic regulation, malaria, *Plasmodium*, gametocyte

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INTRODUCTION

Malaria persists as a global burden to public health and in 2019, was responsible for 409000 deaths. Of the five *Plasmodium* species that infect humans, *P. falciparum* is the most likely to cause severe disease and accounts for the vast majority of deaths from malaria (WHO, 2020). The complexities of the *P. falciparum* life cycle are evident from the array of developmental stages and cycles that are compartmentalized into diverse host cell types within both the human and the mosquito vector (Sherman, 1979). The unique biology of each stage is underpinned by the expression of stage-specific gene sets, regulated at the epigenetic, transcriptional, post-transcriptional, and post-translational levels (Bozdech et al., 2003; Young et al., 2005; Lopez-Barragan et al., 2011; van Biljon et al., 2019). Recent studies involving a diverse set of chromatin-based technologies, including a combination of chromatin immunoprecipitation coupled with next-generation sequencing (ChIP-seq), quantitative mass spectrometry (MS), fluorescence *in situ* hybridization (FISH), and histone mutagenesis and

Abbreviations: Ac, acetylation; EDC, exoerythrocytic developmental cycle; gdv1, gametocyte development protein 1; HAT, histone acetyltransferase; HDAC, histone deacetylase; HDM: histone demethylase; histone PTMs, histone post-translational modifications; HMT, histone methyltransferase; HP1, heterochromatin protein 1; IDC, intraerythrocytic developmental cycle; JMJC, jumonji-C containing demethylase; LSD, lysine-specific demethylase; me, methylation.

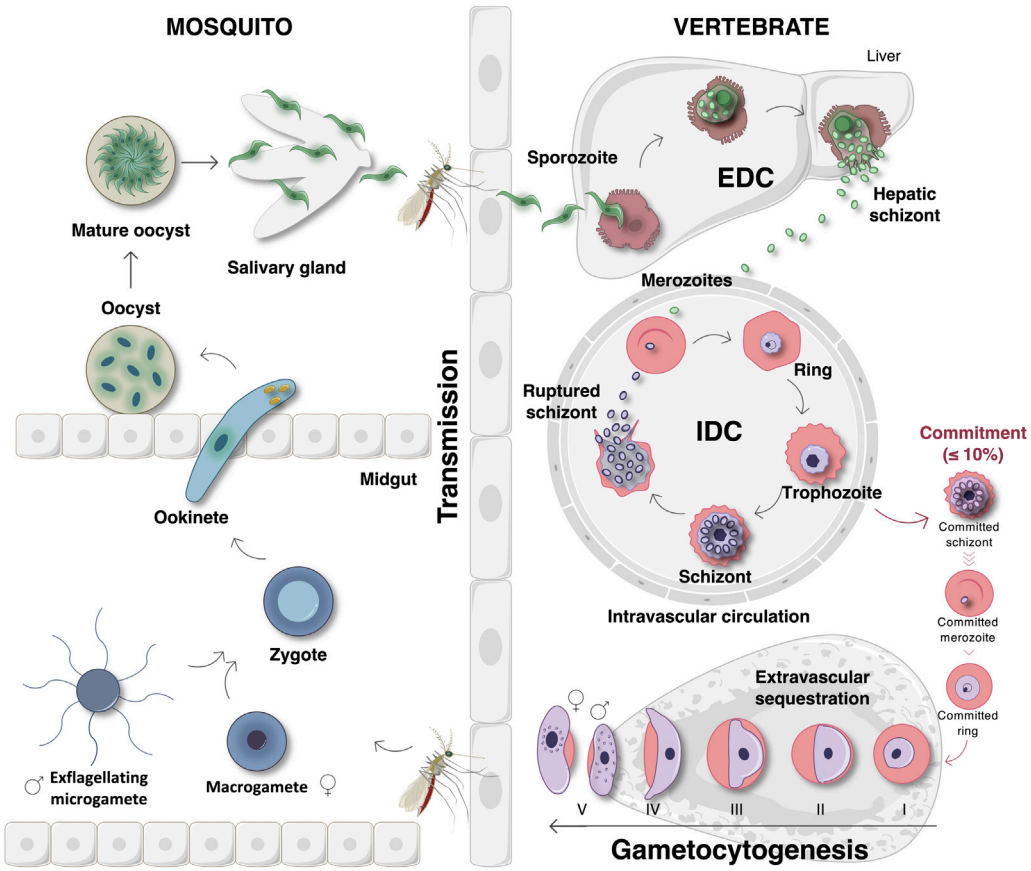

FIGURE 1 | The life cycle of *P. falciparum* parasites. The life cycle begins when an *Anopheles* mosquito vector injects sporozoites into the circulatory system of a human host. The sporozoites then travel to and invade liver cells to initiate the exoerythrocytic developmental cycle (EDC) that results in the formation hepatic schizonts. Once mature, these hepatic schizonts release merozoites into the peripheral circulation. Merozoites invade erythrocytes and initiate the 48-hour intraerythrocytic developmental cycle (IDC) that involves asexual proliferation through ring, trophozoite, and schizont stages. Schizonts then rupture and release new daughter merozoites that repeat this cycle once again. Within each IDC, a small proportion (≤10%) of the parasites will deviate from this fate and instead commit to gametocytogenesis. Sexually committed merozoites invade erythrocytes, forming stage I gametocytes that sequester in the bone marrow where subsequent maturation into gametocyte stages II–IV occurs. Gametocytogenesis yields mature stage V gametocytes that re-enter into the host's circulatory system where they are ideally situated for transmission to the mosquito during feeding. Once taken up during a blood meal, gametogenesis ensues in the mosquito midgut in which female macrogametes are formed and male gametocytes undergo exflagellation to form microgametes. Thereafter, the microgametes fertilize macrogametes, forming diploid zygotes. Zygote development involves maturation into an ookinete and then an oocyst that contains new, maturing sporozoites. Finally, the mature oocysts rupture, releasing sporozoites that travel to the mosquito's salivary glands where they will be transmitted to a new host during feeding. Image was created with BioRender.com.

transcriptomics (microarrays and RNA-sequencing), have revealed that histone post-translational modifications (PTMs) are foundational in generating these stage-specific gene expression fingerprints and are thus proposed to be central to *P. falciparum* parasite life cycle regulation (Comeaux and Duraisingh, 2007; Issar et al., 2009; Trelle et al., 2009; Crowley et al., 2011; Kaur et al., 2016; Saraf et al., 2016; Coetzee et al., 2017; Gomez-Diaz et al., 2017; Gupta and Bozdech, 2017; Gupta et al., 2017; Ngwa et al., 2017; Herrera-Solorio et al., 2019; Witmer et al., 2020; Connacher et al., 2021; Kumar et al., 2021; Ngwa et al., 2021). Here, we review the current knowledge regarding epigenetic regulation in *P. falciparum* parasites with a specific focus on the recent developments in our understanding of how histone PTM landscapes orchestrate life cycle progression.

P. FALCIPARUM PARASITE LIFE CYCLE

Malaria infections are initiated after sporozoite infection of liver cells. After exoerythrocytic development (EDC), merozoites are released into the peripheral circulation to invade erythrocytes, initiating a new asexual intraerythrocytic developmental cycle (IDC) (**Figure 1**) (Kappe et al., 2004). Here, ring, trophozoite and schizont development leads to population expansion and the release of new daughter merozoites to repeat the cycle (Dvorak et al., 1975). Parasite survival and transmission are ensured by the divergence of a small proportion (≤10%) of the parasites that commit to sexual differentiation. In *P. falciparum*, this process is uniquely associated with stage differentiation with stage I gametocytes sequestering in the bone marrow to maturate into stages II-IV (**Figure 1**) (Sinden et al., 1978; Carter and Graves, 1988; Aguilar et al., 2014; Joice et al., 2014).

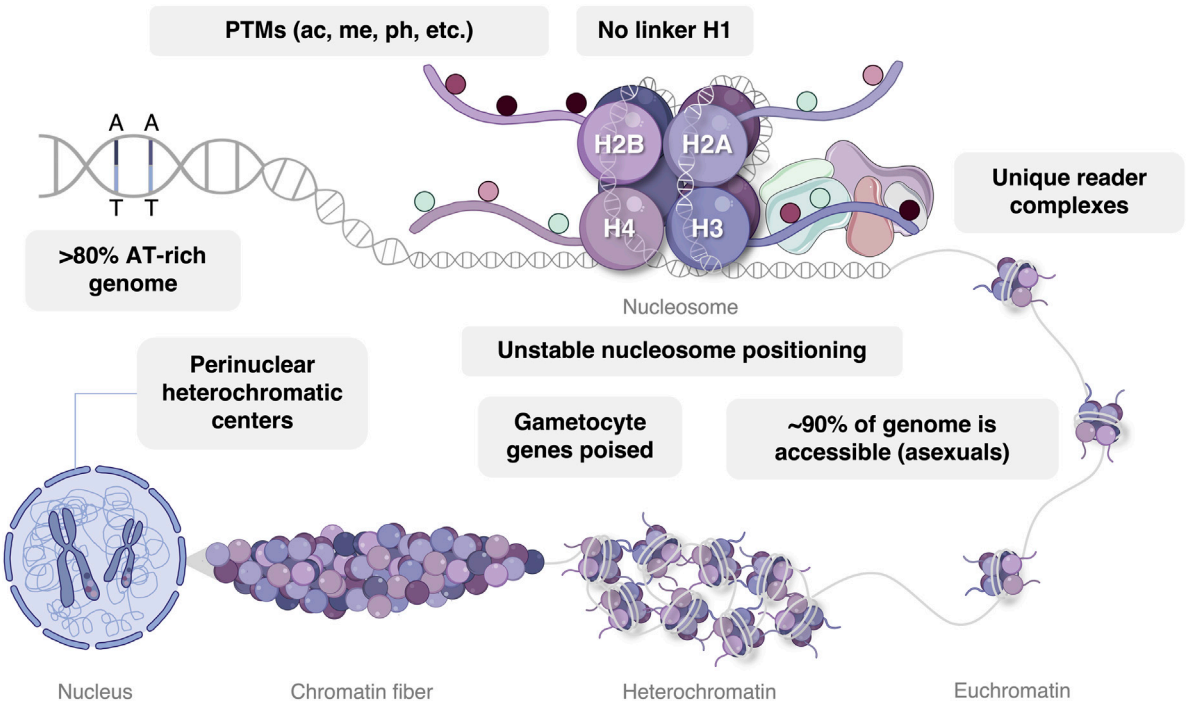

FIGURE 2 | Epigenetic gene regulation in *P. falciparum* parasites. DNA with an AT bias is wrapped around a nucleosome with conventional eukaryotic histone proteins, including H2A, H2B, H3, and H4. The majority of histone post-translational modifications (PTMs) are read by unique epigenetic reader complexes consisting of somewhat divergent writer and eraser enzymes. In asexual parasites, the majority of chromatin exists in an euchromatic conformational state, while gametocyte chromatin exhibits an expansion of H3K9me3-mediated heterochromatin and is stage-specifically poised for transcriptional activation. Perinuclear heterochromatic centers reside in the nucleus associated with *var* gene regulation. Image was created with BioRender.com.

This process of gametocytogenesis yields mature stage V male and female gametocytes that re-enter the host's circulatory system for transmission to female *Anopheles* mosquitoes during feeding (Smalley et al., 1981; Aguilar et al., 2014). Since stage V gametocytes are the only transmissible forms of the malaria parasite, they have become the focus of the discovery and development of transmission-blocking compounds to aid in malaria elimination strategies (Delves, 2012; Birkholtz et al., 2016). Following gametogenesis of micro- (male) and macro- (female) gametocytes in the mosquito midgut, haploid gametes are fertilized to produce a diploid zygote that transforms into a tetraploid motile ookinete (**Figure 1**). The maturation of ookinetes into oocysts results in the formation of sporozoites, which can be transmitted to a new host during feeding (Sinden et al., 1978; Billker et al., 1998).

NUCLEOSOME AND CHROMATIN STRUCTURE IN *P. FALCIPARUM*

The *P. falciparum* nucleosome consists of 147 bp of DNA associated with an octamer of four histone proteins, typically the H2A, H2B, H3, and H4 core histones, each of which display particular functional and stage-specific characteristics (**Figure 2**) (Miao et al., 2006; Trelle et al., 2009). Within certain nucleosomes, these core histones may be exchanged for their variants, namely, H2A.Z, H2B.Z, H3.3, and H3Cen, which may also be modified by an array of chemical groups. These variant histones are typically incorporated to demarcate or influence the properties of specific chromatin domains. For example, distinct intergenic regions of the *P. falciparum* genome are demarcated by double-variant nucleosomes containing H2A.Z and H2B.Z while H3.3 is incorporated to index euchromatic coding regions and subtelomeric repeat regions (Bartfai et al., 2010; Hoeijmakers et al., 2013; Petter et al., 2013; Fraschka et al., 2016). Despite sequence divergence, *P. falciparum* histones have not adapted increased binding affinity for the extremely A + T-rich (~80%) genome (Gardner et al., 2002; Silberhorn et al., 2016). Combined with the apicomplexan-specific absence of the H1 linker histone (Gardner et al., 2002; Sullivan et al., 2006) and short nucleosome repeat length (155 bp) (Silberhorn et al., 2016), this evolution results in a general lack of stable nucleosome patterns in asexual *P. falciparum* parasites (Hoeijmakers et al., 2012). Except for a few internal gene clusters that are brought into proximity with subtelomeric heterochromatin *via* chromatin looping, typical eukaryotic topologically associating domains are therefore largely absent in *P. falciparum* parasites (Lemieux et al., 2013; Bunnik et al., 2014; Bunnik et al., 2018).

Three-dimensional chromatin structure influences the accessibility of DNA to chromatin-binding proteins and

transcription factors, thereby enhancing or repressing gene expression (Zheng and Xie, 2019). As such, the rearrangement of genome spatial organization occurs throughout the parasite's life cycle and corresponds with changes in transcriptional activity (Ay et al., 2014; Bunnik et al., 2014). A relatively simple and dichotomous nuclear organization characterizes the asexual parasite with the majority of the genome (~90%) in a euchromatic state and with the remainder of the DNA present in perinuclear heterochromatic centers (Hoeijmakers et al., 2012; Brancucci et al., 2014; Coleman et al., 2014). Trophozoites exhibit the most euchromatic and accessible genome organization, in line with the increased level of transcriptional activity required in preparation of schizogony (Ay et al., 2014). Contrasting with this restricted distribution of heterochromatin in the asexual parasite stages, repressive factors become more prolific in the gametocyte stages (Brancucci et al., 2014; Coetzee et al., 2017; Bunnik et al., 2018; Fraschka et al., 2018), reflecting the specific transcriptional environment that underlies sexual differentiation and development (Poran et al., 2017; Walzer et al., 2018; van Biljon et al., 2019).

Chromatin status is largely influenced by post-translational modification of the N-terminal tails of histone proteins that make up the core of the nucleosome (Goll and Bestor, 2002). Quantitative mass spectrometry and antibody-based techniques such as Western blotting has been used to identify histone PTMs in *P. falciparum* parasites with acetylated, methylated, phosphorylated, ubiquitinated, and SUMOylated histones quantitatively detected throughout the life cycle (Miao et al., 2006; Salcedo-Amaya et al., 2009; Trelle et al., 2009; Treeck et al., 2011; Dastidar et al., 2013; Gupta et al., 2013; Ukaegbu et al., 2014; Cobbold et al., 2016; Kaur et al., 2016; Saraf et al., 2016; Coetzee et al., 2017; Gupta et al., 2017; Bui et al., 2019; Green et al., 2020). Acetylation and methylation marks indeed form the major component of histone PTMs in *P. falciparum* and have been accurately detected and quantified over multiple life cycle stages. This provides confidence as to their biological relevance, and indeed, a large number of these marks have been extensively functionally validated. The same is not true for the less prevalent marks, particularly for crotonylation and formylation. Quantitative detection of less abundant (or perhaps time-point specific) histone PTMs, including crotonylation, require antibody-based enrichment of histone PTMs prior to mass spectrometric analysis where, for example, H2AK3crK5cr, H2BK18cr, and H4K78cr was identified in *P. falciparum* asexual parasites (Wang and Zhang, 2020). However, besides detection, no information on the importance of crotonylation to parasite development is currently available and it remains to be seen if this histone PTM is functionally distinct from acetylation in the parasite. Remarkably, lysine crotonylation catalyzed by p300 increased transcriptional activation more potently than lysine acetylation in a cell-free system (Sabari et al., 2015) but such functional validation of a biological role for this histone PTM in *Plasmodium* is lacking.

In addition to individual PTMs, histones of mammalian cells for example are also readily modified with distinct patterns of co-existing PTMs at multiple sites, e.g., methylation of lysine 4 with lysine 9 acetylation of histone H3 (Muller and Muir, 2015). While the functional relevance of a relatively small number of single histone PTMs is well documented, evidence indicating that histone PTMs act in concert with one another to regulate transcriptional programs suggests that combinatorial histone PTMs landscapes contribute an additional layer of regulation in eukaryotes (Berger, 2002; Zhao and Garcia, 2015). The compendium of co-existing histone PTMs on specifically histone H3 and H3.3 in *P. falciparum* parasites was recently updated and revealed that PTM combinations are prevalent in asexual parasites and gametocytes and are considerably diverse and stage-stratified, as determined by quantitative mass spectrometric analysis of whole histone N-terminal tails (von Grüning et al., 2022). Histone PTM crosstalk influences the addition, removal, and binding of effector proteins between histone PTMs in combination (Hunter, 2007). While the enzymes responsible for depositing and removing these individual and combinatorial PTMs are somewhat divergent from other organisms based on homology (Miao et al., 2006; Cui et al., 2008a; Miao et al., 2010a), *P. falciparum* parasites employ a typical eukaryotic histone PTMs repertoire (Miao et al., 2006; Chung et al., 2009; Saraf et al., 2016; Coetzee et al., 2017), including functional acetylation, methylation and phosphorylation (**Figure 3**) to expand the regulatory capacity of nucleosomes using a limited set of effector proteins (summarized in **Table 1**).

HISTONE MODIFYING ENZYMES IN *P. FALCIPARUM*

The methylation of histones in *P. falciparum* is mediated by ten histone methyltransferases (HMTs), all of which belong to the evolutionarily conserved SET [Su (var)3-9, Enhancer of Zeste and Trithorax] domain-containing protein family (Cui et al., 2008a). The site specificities of all, except two (SET5 and SET9) of these HMTs have been determined through recombinant protein expression, pull-down proteomics, mutagenesis, and histone lysine methyltransferase (Cui et al., 2008a; Volz et al., 2012; Jiang et al., 2013; Chen et al., 2016; Ngwa et al., 2021) (**Table 1**). Less is known regarding the three Jumonji-C (JmjC) domain-containing histone demethylases (HDMs) and the one lysine-specific demethylase 1 (LSD1) that demethylate histones in *P. falciparum,* however, JMJC1 and JMJ3 have predicted and known H3K36-specificity (Cui et al., 2008a; Matthews et al., 2020). Although the precise mechanisms by which histone methyltransferases and demethylases modify histones remain relatively unclear, several studies have demonstrated their importance for asexual and sexual stage development through genetic disruption and chemical interrogation (Jiang et al., 2013; Ukaegbu et al., 2014; Zhang et al., 2018; Coetzee et al., 2020; Matthews et al., 2020; Connacher et al., 2021; Reader et al., 2021) (**Table 1**).

Histone acetylation is regulated by four histone acetyltransferases (HATs) including proteins from the MYST and GNAT families (Cui et al., 2008a). The acetyltransferase activities of GCN5 and MYST are specific to the lysine residues of H3 and H4, respectively (Cui et al., 2007b; Miao et al., 2010a),

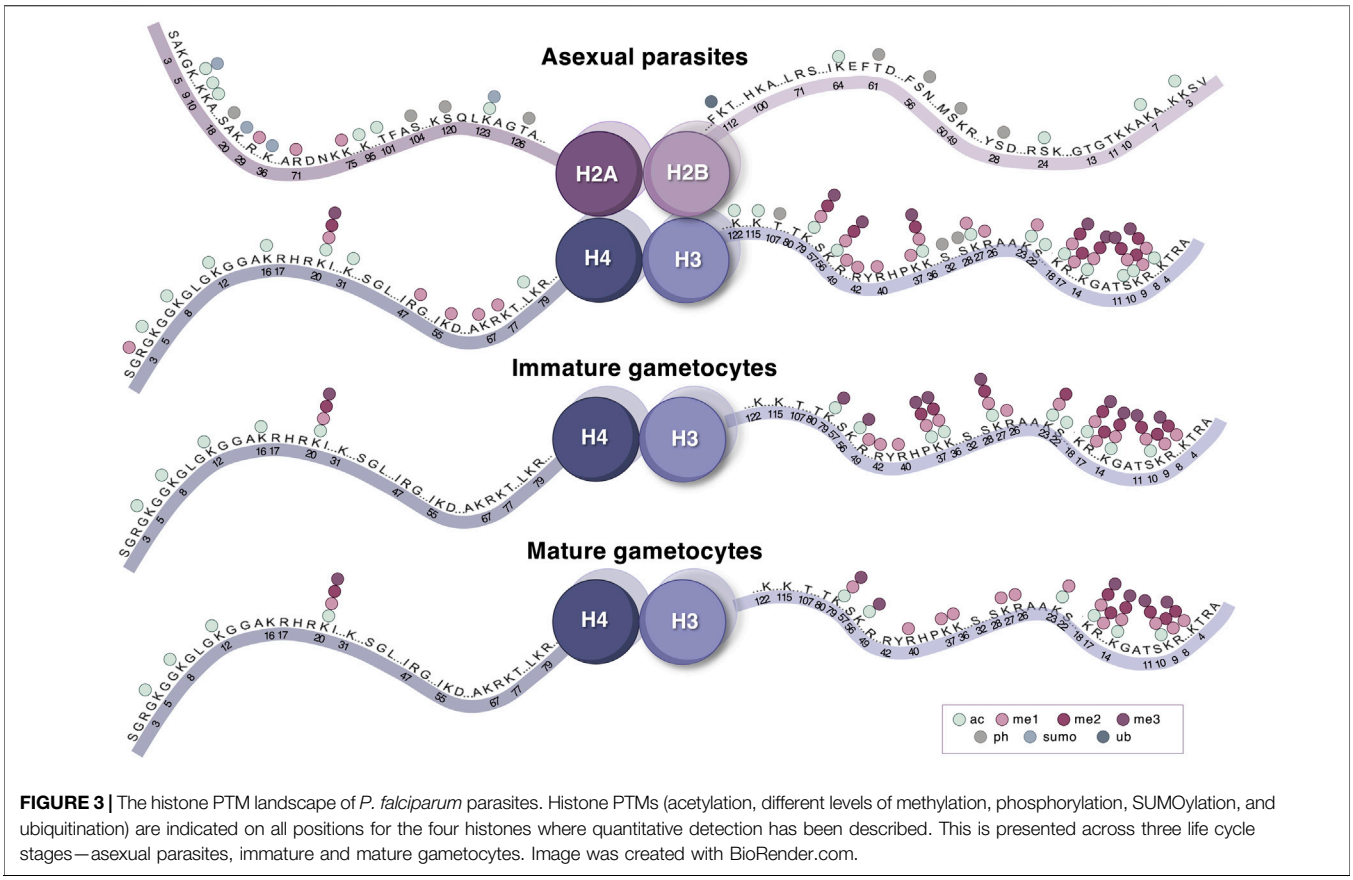

FIGURE 3 | The histone PTM landscape of *P. falciparum* parasites. Histone PTMs (acetylation, different levels of methylation, phosphorylation, SUMOylation, and ubiquitination) are indicated on all positions for the four histones where quantitative detection has been described. This is presented across three life cycle stages—asexual parasites, immature and mature gametocytes. Image was created with BioRender.com.

with the functional importance of these enzymes evident from the transcriptional deregulation arising from their genetic and chemical disruption (Cui et al., 2007b; Cui et al., 2008b; Chaal et al., 2010; Bhowmick et al., 2020; Miao et al., 2021) (**Table 1**). Acetyltransferase activities are antagonized by a repertoire of three histone deacetylases (HDACs) and two sirtuin histone deacetylase proteins in *P. falciparum* (Kanyal et al., 2018). Interestingly, the *P. falciparum* genome encodes for an essential YEATS-domain protein (PF3D7_0807000). Its orthologues in mammalians and yeast are readers of crotonylation. It would therefore be interesting to see if similar activity is present in Plasmodia and if so, crotonylation could be important to biological processes in the parasite despite it being a rarely identified PTM (**Table 1**).

The chromatin reorganization induced by these enzymes results either from a direct physical change in nucleosome structure or the recruitment of epigenetic "reader complexes" that mediate subsequent chromatin remodeling (Latchman, 2010; Abel and Le Roch, 2019) (**Table 1**). To date, five putative reader complexes have been identified in *P. falciparum* parasites, however, the vast majority remain to be functionally validated (Hoeijmakers et al., 2019). These *P. falciparum* readers largely exist in complexes with other reader proteins and/or transcription factors that would presumably require sequential binding occurrences for recruitment (Hoeijmakers et al., 2019). Such cooperation between epigenetic regulatory proteins is exemplified in the recruitment of the GCN5/ADA2 reader complex by H3K4me3 *via* interaction with PHD1 (Hoeijmakers et al., 2019). Additionally, evidence suggests that as in other organisms, epigenetic complexes in *P falciparum* may be recruited to or "flavored" to specific histone PTMs or combinations of PTMs, as determined by peptide pull-down coupled with mass spectrometry. For example, GCN5/ADA2 core reader complex has at least two distinct "flavors" with a unique composition of reader proteins, such as the presence or absence of PHD1 or PHD2 (Hoeijmakers et al., 2019; Miao et al., 2021). Additionally, the intricate nature of multiprotein reader complexes supports the existence of unique sets of functional histone PTMs combinations in *P. falciparum* parasites whereby different reader proteins bind to PTMs using reader domains. Whether these modifications directly influence chromatin structure via the recruitment of such readers or by virtue of their biochemical properties, HMs are undoubtedly at the core of epigenetic regulation.

HISTONE PTM LANDSCAPES IN *P. FALCIPARUM*

Although up to 230 histone PTMs have been identified in various qualitative and quantitative proteomic based approaches *P. falciparum* parasites (Salcedo-Amaya et al.,

TABLE 1 | Catalogue of certain classes of chromatin-associated proteins in *P. falciparum*.

Protein	Gene ID	Essentiality[a]	PTM	Note[b]	Ref
Writers					
Histone lysine methyltransferases (HKMTs)					
PfSET1	PF3D7_0629700	Dispensable (Pf)	H3K4	PbSET1 methylate H4R3, H3K4me3 enriched at intergenic regions	Cui et al. (2008a)
PfSET2 (SETvs)	PF3D7_1322100	Dispensable (Pf)	H3K36	*var* gene silencing	Jiang et al. (2013); Ukaegbu et al. (2014)
PfSET3 (G9a)	PF3D7_0827800	Essential (Pb, Pf)	H3K9		Lopez-Rubio et al. (2009); Volz et al. (2010)
PfSET4	PF3D7_0910000	Dispensable (Pb, Pf)	H3K4	H3K4me3 enriched at intergenic regions	Cui et al. (2008a); Volz et al. (2010)
PfSET5	PF3D7_1214200	Dispensable (Pf)	H3K5/K8/K12		Cui et al. (2008a); Volz et al. (2010)
PfSET6	PF3D7_1355300	Essential (Pf)	H3K4		Cui et al. (2008a); Volz et al. (2010)
PfSET7	PF3D7_1115200	Dispensable (Pb, Pf)	H3K4, H3K9, H3K27		Chen et al. (2016)
PfSET8	PF3D7_0403900	Dispensable (Pb, Pf)	H4K20	H4K20me1/me2/me3; PbSET8 methylate H4R23, H3K59	Cui et al. (2008a); Volz et al. (2010)
PfSET9	PF3D7_0508100	Essential (Pb, Pf)	H4K20		Cui et al. (2008a)
PfSET10	PF3D7_1221000	Dispensable (Pf)	H3K4	H3K4me1, H3K4me2; H3K4me3 enriched at intergenic regions	Volz et al. (2012); Ngwa et al. (2021)
Histone acetyltransferases (HATs)					
PfGCN5	PF3D7_0823300	Defective (Pf)	H3K9, H3K14	Also have a bromodomain, active *var* gene	Fan et al. (2004b)
PfHAT1	PF3D7_0416400				Cui et al. (2008a)
PfMYST	PF3D7_1118600	Essential (Pb, Pf)	H4K5/K8/K12/K16		Miao et al. (2010a)
Protein arginine methyltransferase (PRMT)					
PfPRMT1	PF3D7_1426200	Dispensable (Pb, Pf)	H3R3	Putative class I, H4R3me	Fan et al. (2009)
PfPRMT4/CARM1	PF3D7_0811500	Essential (Pb, Pf)		Putative class I	
PfPRMT5	PF3D7_1361000	Dispensable (Pf)		Putative class II	
Erasers					
Histone demethylases (HDMs)					
PfJmjC1	PF3D7_0809900	Dispensable (Pf)	H3K9, H3K36		Cui et al. (2008b); Volz et al. (2010)
PfJmjC2	PF3D7_0602800	Dispensable (Pb, Pf)			Cui et al. (2008b); Volz et al. (2010)
PfJmj3	PF3D7_1122200	Dispensable (Pf)	H3K36		Cui et al. (2008b); Matthews et al. (2020)
PfLSD1	PF3D7_1211600	Dispensable (Pb, Pf)			Cui et al. (2008b); Volz et al. (2010)
Histone deacetylases (HDACs)					
PfSIR2A	PF3D7_1328800	Dispensable (Pb, Pf)		Transcriptional regulatory protein involved in telomere maintenance and regulation of *var* gene expression	Merrick and Duraisingh. (2010); Mancio-Silva et al. (2013)
PfSIR2B	PF3D7_1451400	Dispensable (Pb, Pf)		Transcriptional regulatory protein involved in telomere maintenance and regulation of *var* gene expression	Petter et al. (2011)
PfHDAC1	PF3D7_0925700	Essential (Pb, Pf)		Class I histone deacetylase 1	Chaal et al. (2010)
PfHDAC2	PF3D7_1008000	Essential (Pb, Pf)		Class II histone deacetylase 2	Coleman et al. (2014)
PfHDAC3/PfHDA2	PF3D7_1472200	Essential (Pf)		Class II histone deacetylase, putative, *var* gene silencing and sexual differentiation	Chaal et al. (2010)
Readers					
Bromodomains: Acetylated histone binding					
PfSET1	PF3D7_0629700	Dispensable (Pf)	H3K4	Also has PHD-finger domain	Cui et al. (2008b)
PfGCN5	PF3D7_0823300	Defective (Pf) Dispensable (Pb)	H3K9/K14ac		Hoeijmakers et al. (2019); Miao et al. (2021)

(Continued on following page)

TABLE 1 | (*Continued*) Catalogue of certain classes of chromatin-associated proteins in *P. falciparum*.

Protein	Gene ID	Essentiality[a]	PTM	Note[b]	Ref
			Writers		
Histone lysine methyltransferases (HKMTs)					
				Also has HAT domain, forms part of SAGA-like complex that can bind H3K4me3 *via* a PHD-domain	
PfBDP1	PF3D7_1033700	Essential (Pb, Pf)	H2B.ZK3/K8/K13/K14/K18ac H3K18/K27ac, H4K5/K8/K12ac	Erythrocyte invasion	Josling and Llinas. (2015); Hoeijmakers et al. (2019)
PfBDP2	PF3D7_1212900	Essential (Pb, Pf)	H2B.ZK3/K8/K13/K14/K18ac H4K5/K8/K12ac		Josling and Llinas. (2015); Hoeijmakers et al. (2019)
PfBDP3	PF3D7_0110500	Dispensable (Pf)			Fleck et al. (2021)
PfBDP4	PF3D7_1475600	Defective (Pf)			Fleck et al. (2021)
PfBDP5/TAF1	PF3D7_1234100	Essential (Pf)			Fleck et al. (2021)
PfBDP6/TAF2	PF3D7_0724700	Essential (Pf)			Fleck et al. (2021)
PHD-finger/ZF-CW domain: Methylated and acetylated histone binding					
PfSET1	PF3D7_0629700	Dispensable (Pf)	H3K4		Cui et al. (2008a)
PfSET2	PF3D7_1322100	Dispensable (Pf)			Jiang et al. (2013); Ukaegbu et al. (2014)
PfSET10	PF3D7_1221000	Dispensable (Pf)			Ngwa et al. (2021)
PfSUMO ligase	PF3D7_1360700	Essential (Pb, Pf)			Reiter et al. (2016)
PfLSD1	PF3D7_1211600	Dispensable (Pb, Pf)			Kaur et al. (2016)
PHD finger containing protein	PF3D7_0310200	Dispensable (Pf)			Cowell et al. (2018)
PfPHD1	PF3D7_1008100	Defective (Pf) Dispensable (Pf)	H3K9ac, H3K4me2/me3	Forms part of SAGA-like complex	Miao et al. (2010a); Hoeijmakers et al. (2019)
EELM2 domain containing	PF3D7_1141800	Dispensable (Pf)	H2B.ZK13/K14/K18ac		Hoeijmakers et al. (2019)
PfPHD2	PF3D7_1433400	Essential (Pb, Pf)			Miao et al. (2010a); Hoeijmakers et al. (2019)
PfZFP	PF3D7_0420000	Dispensable (Pf)			Ngwa et al. (2021)
Chromodomain-like: Methylated histone binding					
PfHP1	PF3D7_1220900	Essential (Pb, Pf)	H3K9me3	Sexual differentiation, regulation of *var* family	Flueck et al. (2009); Perez-Toledo et al. (2009)
PfMYST	PF3D7_1118600	Essential (Pb, Pf)		Also has HAT and ZnF_C2H2 domains, occupy *var* gene promoter, intergenic regions	Miao et al. (2010b)
Chromo-domain protein	PF3D7_1140700	Dispensable (Pf)			Hoeijmakers et al. (2019)
PfCHD1	PF3D7_1023900	Dispensable (Pf)	H3K9me3	chromodomain-helicase-DNA-binding protein 1 homolog, putative	Watzlowik et al. (2021)

[a]*Essentiality/dispensable nature as indicated by Phenoplasm: http://phenoplasm.org/(Sanderson and Rayner, 2017) or https://www.sanger.ac.uk/group/plasmogem/(Gomes et al., 2015), with a focus on* P. falciparum *data.*

Pf = P. falciparum; *Pb = the rodent malaria parasite* P. berghei.

2009; Trelle et al., 2009; Saraf et al., 2016), only 46 of these have been quantitatively validated (Coetzee et al., 2017) (**Figure 3**). The *P. falciparum* histone PTM repertoire expands beyond the typical eukaryotic histone modifications (e.g., H3K4me3, H3K9me3, and H3K36me3) and contains several less common modifications, including H4K20ac, H3.3K79me3, and H3K23me1 (Coetzee et al., 2017). Similar to the hierarchical cascades of gene expression that are associated with each asexual and sexual stage (Bozdech et al., 2003; van Biljon et al., 2019), strikingly unique and stage-specific histone PTM landscapes characterize each stage of the *P. falciparum* parasite life cycle (**Figure 3**) (Coetzee et al., 2017).

Asexual Blood Stage PTM Landscape

The greatest proportion of histone PTMs in the asexual parasite stages consists of acetylation and mono-methylation, both of which are associated with transcriptionally permissive chromatin (Saraf et al., 2016; Coetzee et al., 2017). Throughout the IDC, the mostly euchromatic nature of the genome is marked by nucleosomes containing acetylated histones H3 (K9, K14, and K56) and H4 (K5, K8, K12, and K16) in addition to H3K4me3 and H4K20me1, most of which are positively correlated with gene expression in *P. falciparum* (Gupta et al., 2013). The large transcriptional disruption (~60% of the genome) and effects on growth that arise from inhibiting histone acetylation and methylation in *P. falciparum* parasites, highlight the crucial

nature of these histone PTMs for parasite regulation (Cui et al., 2007a; Cui et al., 2008b; Matthews et al., 2020; Connacher et al., 2021). Regarding combinatorial histone PTMs in the asexual parasite stages, co-existing H3K4me3, H3K9ac, and H3K14ac on histone H3 are achieved through the individual actions of PfGCN5 and PfSET7 (Fan et al., 2004a; Fan et al., 2004b; Salcedo-Amaya et al., 2009; Cui and Miao, 2010; Chen et al., 2016).

Throughout the asexual life cycle, dynamic patterns of H3K18ac and H3K27ac mark the transcriptional start sites of active genes with the AP2-I transcription factor and bromodomain protein 1 (BDP1) reader protein exhibiting similar temporal enrichment at these sites as determined by ChIP-seq and RNA-seq (Tang et al., 2020). Ring and schizonts stages have increased abundance of H4K20me1 and H4K8ac, respectively while acetylation of H3K9 and H3K14 become highly abundant in trophozoites (Gupta et al., 2013; Coetzee et al., 2017). H3K37me1, a novel histone PTMs in *P. falciparum* which has been associated with licensing of replication origins in yeast (Santos-Rosa et al., 2021; von Grüning et al., 2022), is prominent in trophozoite stages. Similarly, on histone H3.3, K37me3 steadily increases in abundance from asexual parasites and immature gametocytes to mature gametocytes (von Grüning et al., 2022). Interestingly, the methylation of H3R17 and H3R40 is more prevalent compared to H3K4me3, suggesting that these histone PTMs may be additional to the canonical acetylation modifications (e.g., H3K9a and H3K14ac) that are involved in transcriptional activation in trophozoites (von Grüning et al., 2022). In addition to this, the connectivity between histone PTMs is exemplified by H3K9acK14ac that is present, and together with the binding of effector proteins, likely contributes to the euchromatic nature of the trophozoite genome (von Grüning et al., 2022). H3K9ac also co-exists with H3K36me3 and exhibits the most notable crosstalk in the trophozoite stages, highlighting the exclusive nature of these particular combinations for asexual parasites, evidenced by quantitative middle-down mass spectrometry (von Grüning et al., 2022). Overall, PTMs on variant histones have also been identified, yet not reproducibly quantified (Saraf et al., 2016; Coetzee et al., 2017). Nonetheless, the C-terminal SQ motif of histone H2A has been shown to be phosphorylated in response to DNA damage (Goyal et al., 2021) in addition to the qualitative detection of serine phosphorylation and lysine acetylation (Coetzee et al., 2017). Similarly, lysine acetylation and methylation, and serine phosphorylation, have also been detected on H3.3, and variant histones H2A.Z, H2B.Z, and centromeric H3 (Saraf et al., 2016; Coetzee et al., 2017; von Grüning et al., 2022). SUMOylation, a PTM that is linked to antagonizing transcription and chromatin remodeling, has been predicted on a combined ten lysine sites on the N-termini of histone H2A and H2A.Z, with histone H4 being a putative target for SUMOylation as well (Issar et al., 2008; Ryu and Hochstrasser, 2021). This suggests that SUMOylation might well be an integral part of the histone PTM language of the parasite. Together with the abundance of individual activating modifications, these combinations provide a critical roadmap that underpins the "just-in-time" transcription of the asexual stages. Interestingly, another ApiAP2 transcription factor family member that is thought to act downstream of AP2-G, AP2-G2, occupancy is highly correlated with repressive H3K36me3, H4K20me3 and H3K27me3 in asexual parasites as determined by pairwise Pearson correlation comparing read counts (Singh et al., 2020). Although AP2-G2 is not essential for asexual proliferation, it is, however, essential for gametocyte maturation where AP2-G2 functions by repressing genes necessary for asexual replication (Singh et al., 2020). Together, this indicates that although present at lower abundance, repressive PTMs are an equally important facet of transcriptional regulation during asexual proliferation. Interestingly, negative crosstalk has been observed between the novel arginine PTMs, H3R40me1, and H3R17me1 in the trophozoite stages (von Grüning et al., 2022). It remains to be seen if H3R40me1 is important to microgametogenesis, similar to its involvement in sporulation in yeast, a process similar to male gametogenesis (Govin et al., 2010) and if H3R17me1 serves independently from canonical euchromatic PTMs in trophozoites to de-represses silenced chromatin as in mammalian cells (Miller and Grant, 2013). In model organisms, H3K56me1&2 have been implicated as docking sites for DNA replication and repair enzymes (Lee et al., 2012; Yu et al., 2012; Janssen et al., 2019), inviting the question as to whether the peak abundance of these histone PTMs in the ring-stage parasites may be indicative of the parasite's preparation for genome replication in the proceeding stages.

The most notable increase in stage-specific histone PTM abundance is present during the schizont stages (Coetzee et al., 2017), specifically for H3K4ac and H4K16ac, both of which have roles in transcriptional activation and is therefore unsurprising given the increased metabolic and transcriptional activity in schizonts (Bozdech et al., 2003; van Biljon et al., 2019). H3K4ac is abundant in active promoter regions with the levels of this PTM regulated by H3K4 methylation in higher eukaryotes (Guillemette et al., 2011). In line with this, H3K4me2&3 are also present in high abundance in schizonts (Coetzee et al., 2017), with functional data indicating H3K4 methylation also positively influences transcription via the recruitment of the GCN5/ADA2 and the associated BDP1/2 reader complexes (Fan et al., 2004a; Hoeijmakers et al., 2019). Although the roles of H4K16ac are less clear, studies in other organisms have documented this PTM as a high-affinity docking site for chromatin-remodeling complexes that promote transcription (Ruthenburg et al., 2011), with the abundance of H4K16ac in asexual parasites, therefore, presenting a possible core mechanism that promotes euchromatin formation. H4K20me3 also exhibits a schizont stage-specific peak in abundance (Coetzee et al., 2017) and was recently identified by machine learning algorithms to be highly indicative of active gene expression in *P. falciparum* parasites (Read et al., 2019).

Histone PTMs That Regulate Clonally Variant Gene Expression and Phenotypic Plasticity

The involvement of epigenetic regulatory mechanisms in variant expression of several gene families have been extensively studied

in *P. falciparum*. Epigenetic regulation of processes such as immune evasion (e.g., *var*, *rifin*, and *stevor*), erythrocyte remodeling (e.g., *PfMC-2TM*) and lipid metabolism (e.g., acyl-CoA binding proteins) (Rovira-Graells et al., 2012; Gomez-Diaz et al., 2017) ensures heritability and rapid adaptation in response to environmental changes. For example, single *var* gene expression is ensured by H2A.Z/H2B.Z double-variant histones, H3.3 and H3K9ac occupancy (Petter et al., 2013), and heritable transmission is ensured by a poised state associated with H3K4me2 (Volz et al., 2012). Additionally, H3.3 is also present in the promoter region and has thus been proposed to be involved in regulating the single active or poised *var* gene (Fraschka et al., 2016). The remainder of the *var* gene family in perinuclear repressive centers is actively silenced with H3K9me3/HP1 and H3K36me3 (Chookajorn et al., 2007; Flueck et al., 2009; Lopez-Rubio et al., 2009; Salcedo-Amaya et al., 2009; Trelle et al., 2009; Jiang et al., 2013; Ay et al., 2014; Ukaegbu et al., 2014; Bunnik et al., 2019). H3K9me3/HP1 have similar strong mechanistic links with the regulation of the *rif*, *stevor*, *pfmc-2tm*, and lysophospholipase families as well as genes that direct the transition from asexual proliferation to sexual differentiation in *P. falciparum* parasites (Rovira-Graells et al., 2012; Brancucci et al., 2014; Gomez-Diaz et al., 2017).

Histone PTMs That Orchestrate the Asexual to Sexual Stage Transition

Several extrinsic drivers of the switch from proliferation to sexual differentiation in *P. falciparum* have been investigated including increased parasitaemia, antimalarial drugs, and lysophosphatidylcholine (lysoPC) depletion (Trager and Gill, 1992; Buckling et al., 1999; Williams, 1999; Dyer and Day, 2000; Brancucci et al., 2017). Currently, the specific mechanism linking these factors with the switch to sexual differentiation is unclear. Nevertheless, several essential genetic factors have reproducibly been shown to be upregulated upon this cue and drive sexual differentiation in *Plasmodium* parasites (Kafsack et al., 2014; Sinha et al., 2014). The commitment process is largely driven by AP2-G, a member of the apicomplexan-specific Apetala2 (ApiAP2) DNA binding domain-containing protein family (Balaji et al., 2005; Kafsack et al., 2014). During the IDC, the *ap2-g* locus remains silenced within H3K9me3/HP1-mediated heterochromatin (Brancucci et al., 2014; Filarsky et al., 2018) and the expression of gametocyte development protein 1 gene (*gdv1*) is inhibited by its own antisense RNA. The gametocyte essential factor, AP2-G5, further prevents sexual commitment by binding *ap2-g* upstream and exonic regions that are responsible for maintaining local heterochromatin which suppresses *ap2-g* gene expression (Shang et al., 2021). Upon the cue for commitment, GDV1 is upregulated and evicts H3K9me3/HP1, resulting in the de-repression of the *ap2-g* locus (Poran et al., 2017; Filarsky et al., 2018). Sequence-specific binding of AP2-G to promoters results the transcriptional activation of hundreds of genes that are predominantly involved in commitment and early-stage gametocyte development, including those encoding invasion proteins that are co-regulated by a second AP2 TF, AP2-I (Kafsack et al., 2014; Poran et al., 2017; Josling et al., 2020). Following sexual commitment, AP2-G5 downregulates AP2-G as well as the early gametocyte genes activated by AP2-G, resulting in a cascade of gene on/off switching that is required for gametocyte maturation (Shang et al., 2021). Aside from the abovementioned H3K9 modifications, the histone PTM landscape dynamics of commitment remain unknown. It is possible that the interaction between H3K9me3 and PfHP1 is influenced by PTMs that co-exist with H3K9me3, most notably, H3S10ph. Although not proven yet, there may be a negative crosstalk relationship between H3K9me3 and H3S10ph in *Plasmodium*, similar to how H3S10ph evicts HP1 from H3K9me3 and prevents the spread of H3K9me2/me3 across the genome in mammalian cells (Fischle et al., 2005; Hirota et al., 2005; Chen et al., 2018). Interestingly, H3.3 carries less PTMs in combination than H3 in trophozoite stages but increase to up to seven PTMs co-existing on the H3.3 histone tail in immature gametocytes and could be linked to the drastic shift in transcriptional program in immature gametocytes (von Grüning et al., 2022).

In model organisms, differentiation consists of multiple stages, each with characteristic epigenome signatures (Bhanu et al., 2016). Elucidating whether the histone PTMs that characterize differentiation initiation in other eukaryotes, such as H3K4 H3K9, H3K20 methylation (Bhanu et al., 2016) and global histone acetylation (Meshorer et al., 2006; Tan et al., 2013), is also associated with sexual commitment in *P. falciparum* remains an interesting question. The impact of the metabolic shift between asexual and gametocytes on the chromatin milieu also warrants clarification, with the indication that loss of function of acetyl-CoA synthetase leads to chromatin hypoacetylation and that the restriction of *S*-adenosyl-L-methionine availability can cause heterochromatin formation (Prata et al., 2021; Summers et al., 2021).

Histone PTM Landscapes That Direct Gametocytogenesis

The histone PTM landscapes associated with gametocyte development stand in strong contrast with those involved in asexual stage-specific transcriptional regulation (Coetzee et al., 2017) (**Figure 3**). Gametocytogenesis is characterized by fewer typical euchromatic PTMs and an increased abundance of repressive di- and tri-methylation on histone H3 (Coetzee et al., 2017). The presence of these PTMs is particularly notable in the early (I-III) stages of gametocyte development and include H3K9me3, H3K27me2&3, and H3K36me2 (Coetzee et al., 2017), the latter of which has been linked to global repression in asexual stages (Karmodiya et al., 2015). Other less well-characterized PTMs including H3K37me1, H3R17me1 and H3R17me2 are also prominent in the early-stage gametocytes (von Grüning et al., 2022).

During gametocytogenesis, H3K9me3/HP1 patterns undergo substantial changes, leading to large heterochromatin reorganization events (Bunnik et al., 2018; Fraschka et al., 2018). Together with a subtelomeric stretch of chromosome 10, genes encoding merozoite invasion proteins become

strongly associated with the distinct perinuclear H3K9me3/HP1-enriched heterochromatin clusters (Brancucci et al., 2014; Bunnik et al., 2018). Additionally, heterochromatin expansion across the subtelomeric region of chromosome 14 leads to the formation of a topologically associated superdomain (>800 kb) with a strong boundary that is postulated to mediate the expression of cell growth and division inhibitors during gametocyte development (Bunnik et al., 2018), similar to what has been observed in other organisms (Dixon et al., 2012). During gametocytogenesis, certain genomic regions show reduced H3K9me3/HP1 occupancy, including those containing sexual development genes (e.g., *ap2-g*, *pfg14-744*, and *pfg14-748*) (Bunnik et al., 2018; Fraschka et al., 2018).

Transcriptional repression of asexual-stage associated genes that become obsolete in the immature gametocyte stages has been associated with several PTMs including H3K36me2&3 (Connacher et al., 2021). Despite the strong link between H3K36me3 and AP2-G2 occupancy in the asexual stages, fewer H3K36me2&3 enriched genes are associated with AP2-G2 in the early-stage gametocyte, indicating that, similar to the divergent transcriptional programs, regulation by histone PTM landscapes is highly specific to gametocytogenesis. The histone PTM landscape of immature gametocytes is also characterized by an increased abundance of repressive H3K27me2&3, H4K20me3 and H3K27ac, the latter two of which co-occur with AP2-G2 in asexual parasites (Singh et al., 2020). While it is tempting to speculate that these histone PTMs are similarly involved in recruiting transcription factors and chromatin modifying enzymes in *P. falciparum* as in other eukaryotes (Carrozza et al., 2005; Keogh et al., 2005; Maltby et al., 2012; Ren et al., 2021), the functional and mechanistic links between these epigenetic factors remain interesting points for further study.

Arginine methylation combinations may represent a key feature of epigenetic regulation for gametocyte development, exemplified by the exclusive combinations of H3R42me1 with H3K37me1 and H3R40me1 (von Grüning et al., 2022). The abundance of combinatorial H3K14me2 with H3K9me1 or H3K18 methylation offers the first suggestion that co-existing PTMs may also modulate heterochromatin establishment in early-stage gametocytes (von Grüning et al., 2022). PTM crosstalk in the late-stage gametocyte reflects a unique relationship between PTMs compared to other stages of the parasite's life cycle. Late-stage (stages IV/V) gametocytes are transcriptionally poised for subsequent gamete formation, as reflected by equal distribution of hetero- and euchromatin marks and the use of translational repression mechanisms in female gametocytes (Lasonder et al., 2016; Coetzee et al., 2017). The combination of H3K27me1K36me1 (Coetzee et al., 2017; von Grüning et al., 2022), may further contribute towards generating poised mRNAs, congruent with the dependence of H3K27me1 on H3K36me1 to induce transcription in embryonic stem cells (Jung et al., 2013). Ultimately, the histone PTM landscape of the late-stage gametocytes presents a suitable environment in which certain genes remain transcriptionally poised in preparation for rapid fertilization in the mosquito.

CONCLUSION AND FUTURE DIRECTIONS

Given the central nature of histone PTMs for *P. falciparum* reviewed here, further understanding the mechanistic relevance of individual and combinatorial PTMs will help identification of the most suited epigenetic targets for drug-based intervention. Although the stage-specific functional relevance of certain individual histone PTMs has been elucidated, the remaining PTMs and combinations with stage-specific patterns should be investigated. Specifically, the influence of these PTMs on asexual and sexual development and the writers, erasers and readers of the histone PTM landscapes and thus insights into the direct or indirect consequences on transcription. In the future, we foresee the use of the combinatorial histone code as a "barcode" that could define parasite life cycle stages or ongoing biological processes. This barcode is dynamic and associated with remarkable changes to allow shifts in chromatin status in different developmental stages of the parasite. In-depth critical analyses of the available data clearly implicate this changing histone PTM landscape as higher-order mechanisms of regulation of gene expression, providing a clear blueprint associated with required phenotypic changes in the parasite to adapt to host responses (antigenic variation), allow massive population expansion in the host (asexual proliferation) and exploiting host environments to allow differentiation and species continuation (sexual development). However, as histone PTMs are not isolated but co-exist to fine-tune binding of effector proteins, the PTM landscape and combinatorial code may indeed guide a highly specific transcriptional program. Lastly, the cause and/or effect relationship between nutrient metabolism and available substrates to modify the histones requires deconvolution. Taken together, the findings discussed in this necessary update demonstrate that although many factors, including nucleosome positioning, chromatin structure and transcription factors participate in transcriptional regulation in *P. falciparum* parasites, the histone PTM landscapes represent the road maps that are central to the specific biology of each stage. The demonstration of histone PTMs as key determinants of both asexual proliferation and sexual differentiation of *P. falciparum* parasites highlights the individual factors as novel targets for disrupting malaria transmission and underscore the importance of a more thorough understanding of these critical regulators.

AUTHOR CONTRIBUTIONS

LB conceived the work and JC, HvG, and LB wrote and edited the paper.

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