Trends in Parasitology

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Forum

Histone lactylation: a new epigenetic axis for host–parasite signalling in malaria?

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Epigenetic modifications play important roles in the biology of malaria parasites. The new epigenetic mark histone lactylation, discovered only recently in humans, is also present in malaria parasites. It may have important functions as a key player in the epigenetic repertoire of *Plasmodium*.

Are histones lactylated in malaria parasites?

Epigenetic phenomena control many aspects of gene expression. Epigenetic changes can be fast, flexible and reversible, making them ideal for responding to changing environments. In the malaria parasite *Plasmodium*, epigenetic marks control virulence pathways including antigenic switching and alternate invasion pathways. Classical histone marks like acetylation and methylation, plus their protein 'writers' and 'readers', have all been identified. Thus, although *Plasmodium* genomes are unusual in many ways, they apparently make conventional use of epigenetics to control gene expression, particularly where rapid responses to varying host conditions can be beneficial. In fact, epigenetics may be particularly prominent in malaria parasites, which encode an unusual paucity of specific transcription factors.

Lactylation is a new epigenetic modification: it was discovered only recently in mammalian cells. In a 2019 *Nature* paper, Zhang *et al.* described the lactylation of histone lysine residues as a novel epigenetic feature in mouse and human cells [1]. A total of 28 lactylated lysines (KLas) were detected across four core histones (Figure 1A). The modification was dependent on lactate levels and could be altered by adding extracellular lactate to cultured cells or by stimulating intracellular glycolysis.

This nascent field has since focussed largely on human biology, but lactyl epigenetic marks could be particularly important in malaria parasites, which are exposed to high and fluctuating lactate levels in their host environment. This is because parasites in the bloodstream respire by glycolysis, producing lactate, and hyperlactataemia is characteristic of severe malarial disease. Therefore, the report from Zhang *et al.* immediately prompted the question of whether lactylated histones might exist in *Plasmodium*. If so, then blood lactate could act as a signal for the status of the infected host that could be directly translated to virulence responses via histone lactylation and modulation of parasite gene expression.

I therefore examined mass spectrometry datasets from laboratory-cultured asexual *Plasmodium falciparum* parasites. (Mass spectrometry was previously conducted on *P. falciparum* histones to detect acetylated residues [2], but lactylated residues were not reported – nor, presumably, were they sought in this 2006 analysis.) A characteristic shift of 72.021 Da was detected on histone-derived peptides: five were detected on three parasite histones (Figure 1B). Therefore, *P. falciparum* does indeed have histone lactylation. These parasites had been cultured normally without added lactate, so only a subset of the most abundant modifications would probably be detected. Interestingly, the same modifications were not found on histones from sexual 'gametocyte' stages. A similar analysis of a recently published dataset from gametocytes [3] revealed only two KLas on a single histone (Figure 1B). Western blots with a pan-KLa antibody that was previously used in human cells [1] further showed that parasite histone lactylation was inducible (Figure 1C) [4].

Lactate and severe malaria

Hyperlactataemia is a cardinal feature of *P. falciparum* malaria and a strong predictor of severe disease. It correlates with parasite load, which likewise predicts severe disease. Its aetiology (recently reviewed in [5]) includes glycolysis by parasites and also by human tissues when normal oxygenation is impeded by parasitized cells sequestered in capillaries. The result is potentially fatal metabolic acidosis and respiratory distress. The clinical threshold for hyperlactataemia, 5 mM, is readily reached in malaria patients. In a study conducted in 2009 [6], I reported that 29 of a cohort of 109 Gambian patients exceeded this level, reaching a maximum of 15.3 mM blood lactate (Figure 2). *In vitro,* lactate at and above the 5 mM clinical threshold clearly induces lactylation of parasite histones (Figure 1C).

Proposed effect of histone lactylation in *Plasmodium*

Since hyperlactataemia correlates with parasite load and also with severe disease, it could simultaneously provide a crude 'quorum sensor' and a measure of host stress. Malaria parasites might have usefully evolved to respond to these signals by altering their growth and virulence.

In the above-referenced Gambian patient study, we found that hyperlactataemia correlated, in the causative parasites, with high expression of virulence genes in the *'var'* family and also of their regulators – histone deacetylase enzymes called sirtuins. Therefore, we originally proposed that the parasites might sense hyperlactatemia and then activate histone deacetylation to modulate epigenetically controlled *var* genes [6]. However, new evidence of histone lactylation now supports a simpler theory: hyperlactataemia could directly

A

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H4  PfH4     SGRGKGGKGLGKGGAKRHRKILRDNIQGITKP... 76  AKRKTVTAMDIVYSLK   91
    HsH4     SGRGKGGKGLGKGGAKRHRKVLRDNIQGITKP... 76  AKRKTVTAMDVVYALK   91
             ********************:***********       **********:**:**

H3  PfH3     ARTKQTARKSTAGKAPRKQLASKAARKS... 55  QKSTDLLIRKLPFQRLVREIAQDYK   79
    PfH3.3   ARTKQTARKSTGGKAPRKQLASKAARKS... 55  QKSTDLLIRKLPFQRLVREIAQEYK   79
    HsH3     ARTKQTARKSTGGKAPRKQLATKAARKS... 55  QKSTELLIRKLPFQRLVREIAQDFK   79
             ***********.*********:*****       ****:******************:::

H2A PfH2A    ----------------------MSA-KGKTGRKKAS... 114  NVLLPKKSQLKAGTANQDY-  132
    PfH2A.Z  EVPGKVIGGKVGGKVGGKVLGLGKGGKGKTGSGKTKK... 138  KALMNKVPLPPTAQKKPKKN  157
    HsH2A    ----------------------SG-RGKQGGKARA... 113  AVLLPKKTESHHKAKGK---  129
    HsH2A.Z  ----------------------AGGKAGKDSGKAKT... 114  HKSLIGKKGQQKTV------- 127
                                   ..   . . *          :  *: *

H2B PfH2B    --------VSKKPAKAKKTGTGPDGKKKRKKSRYDSYGLYIFKVLKQVHPDTGISRKSMN   52
    PfH2BZ   ---SGKGPAQKSQ-AAKKTAGKTLGPRHKRKRRTESFSLYIFKVLKQVHPETGVTKKSMN   56
    HsH2B    PEPAKSAPAPKKGSKKAVTKAQKKDGKKRKRSRKESYSIYVYKVLKQVHPDTGISSKAMG   60
                    . *.      *     . ::::: * :*:.:*::*********:**:: *:*.

             PfH2B   IMNSFLVDTFEKIATEASRLCKYTRRDTLSSREIQTAIRLVLPGELAKHAVSEGTKAVTKFTSK--  116
             PfH2BZ  IMNSFINDIFDRLVTEATRLIRYNKKRTLSSREIQTAVRLLLPGELSKHAVSEGTKAVTKYTTSAA  122
             HsH2B   IMNSFVNDIFERIAGEASRLAHYNKRSTITSREIQTAVRLLLPGELAKHAVSEGTKAVTKYTSSK-  125
                     *****: * *::::. **:** :*.:: *::*******:**:*****:*************:*:.
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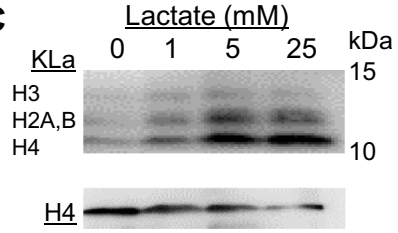
B

P. falciparum histone & residue	Equivalent modification in mammalian cells?
H3.3, K 56	Yes: *Hs*H3, K 56
H2B, K 49	No
H2AZ, K 10, 14	H2AZ is an apicomplexan-specific variant
H2BZ, K 8, 18, 53	H2BZ is an apicomplexan-specific variant *Pf*H2BZ K8 = *Hs*H2B K11

C



Trends in Parasitology

Figure 1. Evidence for histone lactylation in *Plasmodium falciparum* parasites. (A) Alignments of histones from *P. falciparum* and *Homo sapiens*. Lactylated lysine (KLa) sites identified in human cells [1] are highlighted in blue, and those in *P. falciparum* in red (asexual stages) and green (gametocytes). (B) Table of KLa sites identified in *P. falciparum* histones in one or more of six independent mass spectrometry datasets from asexual parasites, or 12 datasets from gametocytes at days 4, 8, and 12 of maturation. All mass spectrometry was on protein extracted from normal *in vitro* cultures without added lactate. (C) Western blot with pan-KLa antibody on *P. falciparum* histones from trophozoite-stage parasites after 16 h of exposure to increasing levels of added lactate. Total histone H4 is shown as a control.

affect the epigenetic control of *Plasmodium* virulence genes by causing histone lactylation within those genes (rather than acting 'indirectly' through sirtuin induction and altered histone acetylation). Lactylated histones in human leukocytes do indeed stimulate gene transcription, similar to the well-characterised effect of acetylated histones [1]. Since the basic tenets of epigenetics are largely conserved even in this early-diverging eukaryote, it is probable that lactylation stimulates transcription in *Plasmodium* as well.

Interestingly, six out of seven lactylated residues found in *Plasmodium* thus far (Figure 1) were in variant histones – H3.3, H2AZ, and H2BZ – not in standard core histones H2A, H2B, H3, and H4. If histone lactylation is particularly abundant in variant histones, this would be significant because they are known to be associated with active genes, particularly active virulence genes [7,8]. For example, both H2AZ/H2BZ and H3.3 are found in the promoters of active but not inactive *var* genes, and H2BZ-containing nucleosomes tend to be acetylated [7,8]; Figure 1 now suggests that they are also lactylated.

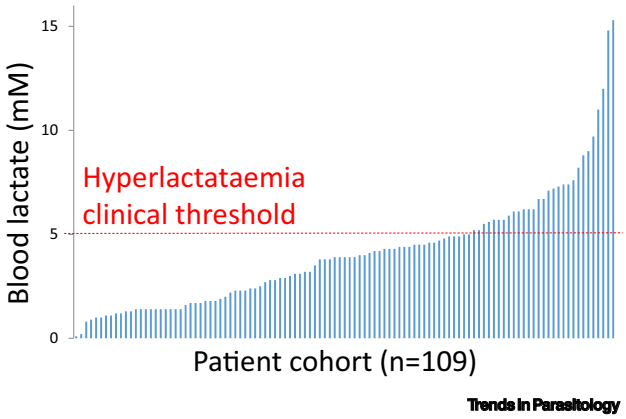


Figure 2. Blood lactate in malaria patients frequently exceeds a 5 mM threshold. Blood lactate levels (rank-ordered) measured in 109 Gambian malaria patients. Data replotted from [6].

Biological implications of histone lactylation in *Plasmodium*

In some circumstances it might be beneficial for parasites to upregulate virulence genes in stressed hosts experiencing hyperlactataemia. For example:

(i) Elevated expression of *var* genes can help parasites to cytoadhere to epithelial cells, sequestering them from the blood flow to avoid splenic clearance and thus replicate more efficiently. A recent study in Malian patients suggested that parasites in high-parasitaemia, symptomatic infections tend to cytoadhere earlier and more efficiently than parasites in lower-level, asymptomatic infections [9].

(ii) Increased conversion to sexual gametocytes (gametocytogenesis) is beneficial in promoting mosquito-borne transmission from stressed hosts. Although conversion takes almost 2 weeks in *P. falciparum*, in most other species it takes only 1–2 days and could therefore promote transmission quite acutely. Gametocytogenesis is a virulence phenotype that was linked to lactate in a recent publication: when parasites were cultured in clinically achievable levels of added lactate, they converted at a higher rate [10]. This conversion is well established to require a suite of gametocyte-specific, epigenetically controlled genes, including the *Plasmodium*-specific transcription factor AP2-G, which is a clear candidate for regulation via histone lactylation.

(iii) It could be beneficial for parasites in hyperlactataemic hosts to upregulate stress-resistance genes, since such hosts are prone to severe disease with associated inflammation and fever exerting high levels of oxidative stress and thermal stress on the causative parasites. Indeed, we have generated preliminary evidence (L.O. Anagu, PhD thesis, University of Keele, 2020) suggesting that moderate lactate exposure can improve stress-resistance in cultured parasites. Genes that might be induced here are unknown but antioxidant and chaperone pathways are likely candidates. Again, such pathways could, *in vivo*, improve parasite survival in a stressed hyperparasitaemic host. Overall, lactyl epigenetic marks clearly have potential to affect parasite virulence in multiple ways.

What is the molecular mechanism of histone lactylation?

Epigenetic marks are usually dynamic, added and removed by opposing enzymes, for example, histone acetyltransferases and deacetylases (HATs/HDACs). Histone lactylation enzyme(s) have yet to be fully identified in mammalian cells but there is evidence for the HAT p300 [1]. This lacks a direct homologue in *Plasmodium*, where other GNAT-family HATs may perform its functions [11]. *Plasmodium* has a relatively small HAT/HDAC repertoire [11,12], only some of which have been experimentally confirmed. There is one MYST-family and up to ten putative GNAT-family acetyltransferases, but only two have been fully characterised as HATs and most are probably not histone-directed. There are five HDACs, most of them confirmed as genuine HDACs, including the sirtuins mentioned above [6]. Table 1 highlights the enzymes experimentally reported as essential or inessential in erythrocytic *Plasmodium* parasites (data from [11,12] and *PlasmoDB.org*). Some HATs/HDACs could plausibly 'moonlight' upon lactyl as well as acetyl modifications, although it is theoretically possible that *Plasmodium* has unique lactylation enzymes as well.

Could histone lactylation be important more broadly in apicomplexan parasites?

The above discussion focusses on the principal human malaria parasite, *P. falciparum*. However, other *Plasmodium* species that are able to achieve high parasitaemias do cause hyperlactataemia, including the zoonotic macaque parasite *P. knowlesi* and the rodent model species *P. berghei* and *P. yoelii.* These were recently reported to raise blood lactate in mice to ~12 mM and 18 mM respectively [13], mimicking levels in severe human malaria. As in *P. falciparum* infections, this correlates with respiratory distress in human patients and with equivalent phenotypes in mice. Could all *Plasmodium* species therefore have evolved to sense blood lactate and modulate virulence phenotypes accordingly – and if so, which phenotypes would be modulated? The rodent species do not show classical cytoadherence and do not have *var* genes, but *P. knowlesi* has a gene family called *sicavar*, analogous

Table 1. HAT and HDAC enzymes identified in *P. falciparum*

Function	Family	Gene	Gene name	Characterised?	Targets?	Essential?
HAT	MYST	PF3D7_1118600	*Pf*MYST	Y	Histones	Y
	GNAT	PF3D7_0823300	*Pf*GCN5	Y	Histones	Y
		PF3D7_0416400	*Pf*HAT1	–	Histones?	Y
		PF3D7_1227800	*Pf*ELP3	–	Histones?	?
		PF3D7_1323300		–	Histones?	N
		PF3D7_0805400		–		N
		PF3D7_1003300	*Pf*ARD1	–	Non-histone	Y
		PF3D7_0109500		–	Non-histone	?
		PF3D7_0629000	*Pf*GNA1	Y	Non-histone	Y
		PF3D7_1020700		–		N
		PF3D7_1437000		–		Y
HDAC	Class I	PF3D7_0925700	*Pf*HDAC1	Y	Histones	Y
	Class II	PF3D7_1472200	*Pf*HDA1	–		Y
		PF3D7_1008000	*Pf*HDA2	Y	Histones	Y
	Class III	PF3D7_1328800	*Pf*Sir2a	Y	Histones	N
		PF3D7_1451400	*Pf*Sir2b	–	Histones?	N

to but technically different from the *var* gene family. If *sicavar* genes were also affected by histone lactylation, this would imply an evolutionarily conserved pathway. All *Plasmodium* species could also share the epigenetic control of gametogenesis and stress-resistance.

Beyond the *Plasmodium* genus, related apicomplexan parasites (*Toxoplasma*, *Babesia*, *Cryptosporidium*, etc.) also have public-health importance. Only a few of these are blood-dwelling; they may reside in niches where lactate levels vary, and they may use epigenetics to control their biology, but none of them cause hyperlactataemia like *Plasmodium*. An initial protein lactylome was recently published for *Toxoplasma gondii*, identifying a wide variety of lactylated proteins, including histones [14]. However, whether or how these are functional remains to be elucidated. In *Toxoplasma*, the key stress-induced phenotypic switch from tachyzoite to bradyzoite is, for example, controlled by a transcription factor rather than an epigenetic switch (albeit the control of that transcription factor is not yet fully understood). Thus far, it appears that the biology proposed in this article might have evolved uniquely in *Plasmodium* due to the unique features of mammalian malaria. Box 1 lists some of the many questions that await further research on this topic.

Box 1. Open questions

- What is the full catalogue of histone lactyl modifications in *Plasmodium*? Are parasite-specific histone variants, particularly those associated with active genes, preferentially or uniquely lactylated? Are modified histone sites conserved across *Plasmodium* species?
- What are the temporal dynamics of histone lactylation after exposure to exogenous or endogenously generated lactate?
- Which enzymes control histone lactylation in *Plasmodium*? Are 'moonlighting' HAT and HDAC enzymes entirely responsible?
- Where does histone lactylation occur throughout *Plasmodium* genomes? By combining chromatin immunoprecipitation (ChIP-seq) and RNA sequencing (RNA-seq), the gene types most affected by histone lactylation could be identified along with resultant changes in expression, thus pinpointing the likely biological roles for the epigenetic mark. Would the affected genes be similar in different *Plasmodium* species?
- Does histone lactylation control virulence phenotypes, such as cytoadherence, gametocytogenesis and stress-resistance – and if so, which target genes are responsible?
- In human malaria patients, is there a correlation between in-host hyperlactataemia, parasite virulence phenotype(s) and expression of lactyl-modified genes?

Concluding remarks

Histone lactylation is an entirely new epigenetic pathway in the protozoan parasite *Plasmodium*. It suggests a novel mechanism of host–parasite interaction in malaria: a disease in which the host frequently develops the potentially fatal complications of hyperlactataemia and respiratory distress. If malaria parasites have indeed evolved to sense and respond to this epigenetically then the implications for virulence and malarial disease are compelling.

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Declaration of interests

The author declares no competing interests.

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