FORUM: MALARIA

Molecular secrets of a parasite

Research shows how the malaria parasite *Plasmodium falciparum* manipulates the expression of its *var* genes to avoid recognition by the host immune system. Four experts comment on the implications of these results for our understanding of gene regulation in general and the development of antimalaria vaccines. SEE LETTER P.223

THE PAPER IN BRIEF

- *Plasmodium falciparum* is devious. It uses 60 different *var* genes to express slightly different versions of one protein, PfEMP1, on the surface of the host's infected erythrocytes (red blood cells).
- Moreover, the parasite expresses one *var* gene at a time, making it much harder for the immune system to recognize infected erythrocytes than if there were just one *var* gene.
- On page 223 of this issue, Jiang *et al.*[1] show that the gene *pfSETvs* silences the expression of the remaining 59 *var* genes at any one time*.
- The protein product of *pfSETvs* modifies *var* genes through a H3K36me3 mark — that is, by adding three methyl (me) groups to lysine amino-acid residue (K) 36 of the histone (H) 3 protein associated with these genes.
- When the authors deleted *pfSETvs*, almost all 60 of the *var* genes were expressed simultaneously in a single parasite, and the proteins they encode made their way to the surface of infected erythrocytes (Fig. 1).

Unusual use of a mark

SWAMINATHAN VENKATESH & JERRY L. WORKMAN

Switching identity to evade immune detection is a common trick. What is surprising is Jiang and colleagues' finding that *P. falciparum* uses the H3K36me3 mark in an uncommon way[2] to silence its identity-determining *var* genes.

In multicellular organisms, gene-silencing mechanisms work by reorganizing chromatin (complexes of DNA and associated proteins) into a tight, inflexible structure, to diminish access to the DNA. In plants and animals, methylation of specific lysine residues on histones (H3K9, H3K27 and H4K20) is crucial for engaging proteins that form this repressive structure[3].

But not all methylation marks silence gene expression. H3K4 and H3K36 residues, for example, are methylated during gene transcription, maintaining the transcriptional competence of the chromatin template. Specifically, H3K36me2 and H3K36me3 are selectively enriched in coding DNA regions, functioning to preserve chromatin structure and so prevent initiation of transcription at inappropriate regions[4]. It was unexpected, therefore, when Jiang *et al.* found that *P. falciparum* uses H3K36me3 to coat not just the coding regions but also the promoter sequence of most *var* genes, thereby blocking their transcription.

Interestingly, experimental manipulations in yeast that mis-target the methyltransferase protein Set2, and so H3K36me3, to gene promoters repress transcription[5]. This raises two questions. Do *Plasmodium* parasites use a similar histone methyltransferase protein to add H3K36me3 to the *var* genes? And if so, what leads to its unusual localization to *var*-gene promoters in *Plasmodium*?

Jiang and co-authors' answers to these questions reveal previously unknown facets of parasite biology. It turns out that PfSETvs — the histone methyltransferase that functions in *P. falciparum* — shows sequence similarity to a fly protein involved in activating transcription. Whereas there is considerable uncertainty about whether the fly protein targets the H3K4 or H3K36 residues, the authors convincingly show that PfSETvs occupies the silent *var* genes and adds the H3K36me3 mark only at early stages of parasite infection. Intriguingly, in *P. falciparum*, PfSETvs is responsible for the addition of H3K36me3 to promoters and coding regions of only *var* genes and members of other variant-gene families that carry this mark. However, the methyltransferase responsible for adding H3K36me3 to other genes is unidentified.

What is the advantage of using H3K36me3 for gene silencing, instead of other marks that have evolutionarily conserved silencing activity? The answer might lie in the easy reversibility of the methylated and unmethylated states to allow *var*-gene switching. But first it is necessary to know how a single, specific *var* gene is turned on while all the others are silenced. Jiang and co-workers' analysis suggests that a long non-coding RNA (lncRNA) generated from the transcription of an active *var* gene in the opposite (antisense) direction removes PfSETvs from this gene's promoter, allowing initiation of its transcription (Fig. 1). Identifying parasite proteins that interact with H3K36me3 might clarify the exact mechanism of H3K36me3-mediated silencing. It might also clarify how the parasite differentiates H3K36me3-enriched active coding regions from the H3K36me3-enriched silent *var*-gene promotors to target the silencing complexes.

Swaminathan Venkatesh *and* **Jerry L. Workman** *are at the Stowers Institute for Medical Research, Kansas City, Missouri 64110, USA.*
e-mail: jlw@stowers.org

Repertoire unveiled

MATS WAHLGREN & MARIA TERESA BEJARANO

The subject of Jiang and colleagues' work, PfEMP1, is a key target of immunity. *P. falciparum* expresses this adhesive protein on the surface of infected human erythrocytes to sequester itself within blood vessels and, thus, avoid destruction in the spleen. Therefore, specific antibodies that protect humans against severe malaria target PfEMP1 to overcome the obstruction to the blood flow caused by the parasite-infected erythrocytes[6].

To evade immunity, *P. falciparum* varies the proteins it expresses on the surface of the infected host erythrocytes. Even in persistent malaria infections, the protein variants that

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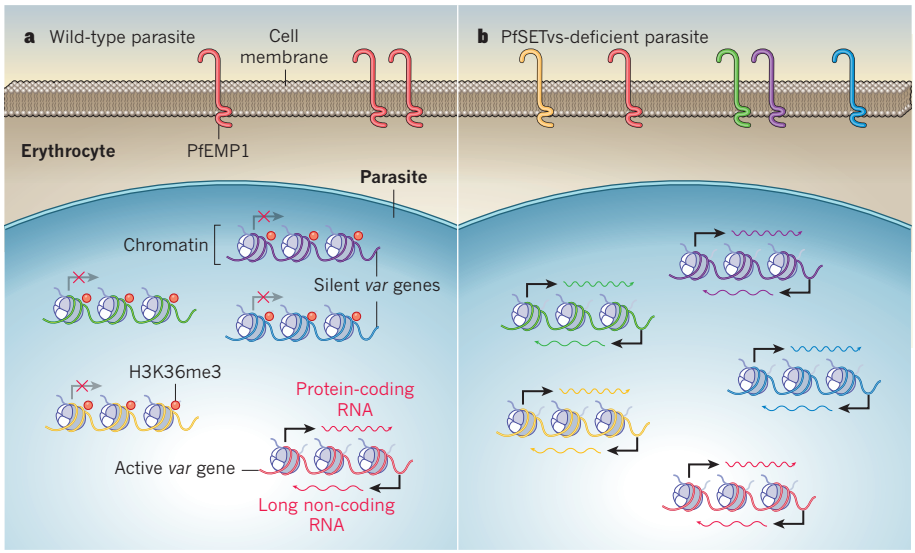

Figure 1 | Regulation of *var*-gene silencing. **a**, In wild-type *Plasmodium falciparum*, the protein PfSETvs (not shown) adds the H3K36me3 mark to chromatin containing all but one *var* genes, thereby silencing their expression. Consequently, a single version of identity-determining PfEMP1 — the protein product of *var* genes — is expressed on the surface of an infected erythrocyte. Long non-coding RNA is expressed in the antisense direction only in the active *var* gene. **b**, Jiang *et al.*[1] find that PfSETvs loss results in the simultaneous expression of all *var* genes, and so the infected erythrocyte displays several varieties of PfEMP1.

appear later are distinct from those of the parental parasite in terms of their antigenic determinants — the triggers for an immune response. This antigenic variation reflects a fundamental element of parasitism[7]. PfEMP1 belongs to one of several families of variant proteins that are expressed on the surface of erythrocytes infected with *P. falciparum*[8–10].

This is a powerful parasitic defence strategy, and immunity develops slowly in patients with malaria. That is because antibodies to a single PfEMP1 variant block sequestration only of the parasites expressing that variant, and their cross-reactivity with other PfEMP1 variants is limited. Consequently, anti-PfEMP1 antibodies of different specificities are needed to protect against the glut of the protein's variants that develop in an infected individual, especially in the most vulnerable — children and pregnant women.

Could *P. falciparum* lacking *PfSETvs* — which Jiang *et al.* find expresses the whole repertoire of PfEMP1-encoding *var* genes — be used for vaccination? A vaccine based on this mutant could allow the generation of a full repertoire of antibodies to protect against malaria, including the severe forms of the infection.

Human vaccines against bacteria and viruses are often based on killed, live attenuated or inactivated microorganisms. For *P. falciparum*, a unicellular organism, advances in the development of live whole-cell vaccines against malaria have mainly come from studies of pre-erythrocytic stages of the parasite's life cycle[11], although vaccination with its blood stages has also been tried[12]. Moreover, *Babesia bovis*, a parasite related to *P. falciparum* that infects cattle, is used in a live vaccine in several countries and protects the animals against severe forms of the disease. So a vaccine based on the whole, blood-stage, *PfSETvs*-deficient parasite could potentially be developed and, to improve its efficiency, be combined with a vaccine based on a parasite form that is maturing in its mosquito vector[11].

Parasites expressing the complete repertoire of variant genes do not appear spontaneously in nature nor during *in vitro* growth. PfSETvs-mediated silencing therefore seems robust. Still, PfSETvs might not be the only protein involved in regulating variant-gene families. Indeed, *P. falciparum* often loses the capacity to activate and express genes encoding PfEMP1 *in vitro*, generating parasites that would not be expected to survive in a human host. Although the present study implicates antisense lncRNA in activating *var* genes, to target the variant genes of *P. falciparum* with drugs and vaccines, the mechanisms that initiate and regulate their activation must be explored further. ■

Mats Wahlgren *and* **Maria Teresa Bejarano**
are at the Center for Infectious Disease Research, Department of Microbiology, Cell and Tumorbiology, and at the Center for Infectious Medicine, Department of Medicine, Karolinska Institutet, SE 17177 Stockholm, Sweden.
e-mail: mats.wahlgren@ki.se

1. Jiang, L. *et al. Nature* **499,** 223–227 (2013).
2. Wagner, E. J. & Carpenter, P. B. *Nature Rev. Mol. Cell Biol.* **13,** 115–126 (2012).
3. Shilatifard, A. *Annu. Rev. Biochem.* **75,** 243–269 (2006).
4. Venkatesh, S. *et al. Nature* **489,** 452–455 (2012).
5. Strahl, B. D. *et al. Mol. Cell. Biol.* **22,** 1298–1306 (2002).
6. Miller, L. H., Ackerman, H. C., Su, X. & Wellems, T. E. *Nature Med.* **19,** 156–167 (2013).
7. Chen, Q. *et al. Nature* **394,** 392–395 (1998).
8. Baruch, D. I. *et al. Cell* **82,** 77–87 (1995).
9. Fernandez, V., Hommel, M., Chen, Q., Hagblom, P. & Wahlgren, M. *J. Exp. Med.* **190,** 1393–1404 (1999).
10. Niang, M., Yan Yam, X. & Preiser, P. R. *PLoS Pathog.* **5,** e1000307 (2009).
11. Epstein, J. E. *et al. Science* **334,** 475–480 (2011).
12. Pombo, D. J. *et al. Lancet* **360,** 610–617 (2002).

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GEOPHYSICS

A third way to rift continents

Rifting of continents is usually explained by one of two mechanisms based on effects that originate far from the zone of rifting. Laboratory experiments show that this geodynamic process can also be caused by local effects.

W. ROGER BUCK

Vast continental regions have experienced volcanism precisely where 1,000-kilometre-scale crustal blocks were pulling apart. For example, such rifts began to cut across much of Africa about 140 million years ago, and distributed, low-flux volcanism continues in that region today (Fig. 1). Such broadly distributed, synchronous activity is hard to fit into standard theories of rifting and volcanism. Writing in the *Journal of Geophysical Research*, Fourel *et al.*[1] suggest an explanation for this activity based on laboratory experiments with fluids whose densities depend on temperature and composition.

Radiation of heat to space cools the strong outer layer of the Earth, called the lithosphere, which overlies the hot, convecting interior. Minerals contract as they cool, and this can make the cold lithosphere denser than the