Malaria Parasite Epigenetics: When Virulence and Romance Collide

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Blood-stage malaria parasites evade the immune system by switching the protein exposed at the surface of the infected erythrocyte. A small proportion of these parasites commits to sexual development to mediate mosquito transmission. Two studies in this issue (Brancucci et al., 2014; Coleman et al., 2014) shed light on shared epigenetic machinery underlying both of these events.

Malaria, which kills up to a million people each year, is caused by the protozoan parasite *Plasmodium*. The malaria parasite life cycle has multiple stages, but only the blood stage causes pathology. After invasion of human erythrocytes, merozoites develop from rings into trophozoites and schizonts, which must sequester in the capillary endothelium to prevent clearance by the spleen. Cytoadherence is mediated by the binding of the parasite ligand PfEMP1, exposed on the surface of the infected erythrocyte, to human endothelial cell receptors such as CD36, ICAM1, and chondroitin sulfate A. PfEMP1 is encoded by the ~60-member *var* gene family, which undergoes clonal antigenic variation. Only a single *var* gene is expressed in a parasite clone, but spontaneous switching can occur whereby an alternative *var* gene is expressed, which may result in a different cytoadherence phenotype. There is evidence that this process contributes significantly to immune evasion and that repeated exposure of individuals to multiple PfEMP1 isoforms gradually builds up their immunity to severe disease. Mutually exclusive expression of PfEMP1 is known to be governed by an epigenetic control mechanism involving heterochromatin-based gene silencing (Guizetti and Scherf, 2013).

The existence of the sexual malaria parasite forms (male and female gametocytes that fertilize soon after being taken up in a mosquito blood meal) has been known for more than a century. However, how gametocytes develop from asexual blood-stage parasites has been a complete mystery until very recently. Teams working on the most lethal form of malaria parasite *P. falciparum* (Kafsack et al., 2014) and the mouse malaria parasite *P. berghei* (Sinha et al., 2014) both used independent forward and reverse genetic approaches to identify the transcription factor AP2-G as the master regulator of commitment to sexual development. This protein activates early sexual-stage genes, thereby committing the parasite to this alternative developmental program. In the absence of a functional copy of this gene, no gametocytes whatsoever could develop in either species. Interestingly, two previous studies had identified epigenetic marks characteristic of heterochromatic silencing at *the ap2-g* locus (Flueck et al., 2009; Lopez-Rubio et al., 2009). The notion of expression of this transcriptional activator being epigenetically controlled was attractive because it is known that only a subpopulation of asexual blood-stage parasites switch to sexual development. The two studies in the current issue of *Cell Host & Microbe* provide direct evidence that the epigenetic machinery underlying the mutually exclusive expression of *var* genes is also responsible for regulating the switch to sexual development in malaria parasites.

Brancucci et al. (2014) show, using transcript profiling, that conditional knockdown of the *Plasmodium falciparum* heterochromatin protein 1 (PfHP1), a major player in gene silencing, results in derepression of a large number of parasite genes. The list includes the clonally variant family of *var* genes encoding PfEMP1 and results in a breakdown of the normal mutually exclusive expression pattern, implying that multiple PfEMP1 variants are expressed on the surface of a single infected erythrocyte (Figure 1). Another gene they found to be derepressed encodes the transcription factor *PfAP2-G*, which induces the switch to sexual differentiation. In asexual blood-stage parasites prior to knockdown, PfHP1 localized to the nuclear periphery, the site expected for heterochromatin, but disappeared several hours after induction of protein degradation. Remarkably, around 50% of parasites in the culture had switched to sexual development in the cycle following degradation of PfHP1 as assessed by a marker of early gametocytes, thereby demonstrating epigenetic control of the switch to sexual commitment. Interestingly, the parasites not converting to gametocytes showed a complete developmental arrest in the cycle following PfHP1 depletion. This pre-S phase arrest could be rescued and growth resumed as soon as normal PfHP1 protein levels were restored by adding back the stabilizing ligand Shield1, demonstrating that PfHP1 is essential for asexual proliferation.

Coleman et al. (2014) describe the essential role of another component of the parasite's epigenetic machinery, histone deacetylase 2 (PfHda2). Histone deacetylases promote silencing by removing acetyl groups (active chromatin marks) from histones. Several histone deacetylases have been described in *P. falciparum* and implicated in subtelomeric virulence gene silencing (Tonkin et al., 2009). PfHda2 colocalizes with PfHP1 to discrete foci at the nuclear periphery known to consist of chromosome end clusters containing heterochromatic virulence gene loci. Using an inducible knockdown line, the authors show that PfHda2, like PfHP1, contributes to the maintenance of heterochromatin at both virulence gene loci and the *api2-g* locus,

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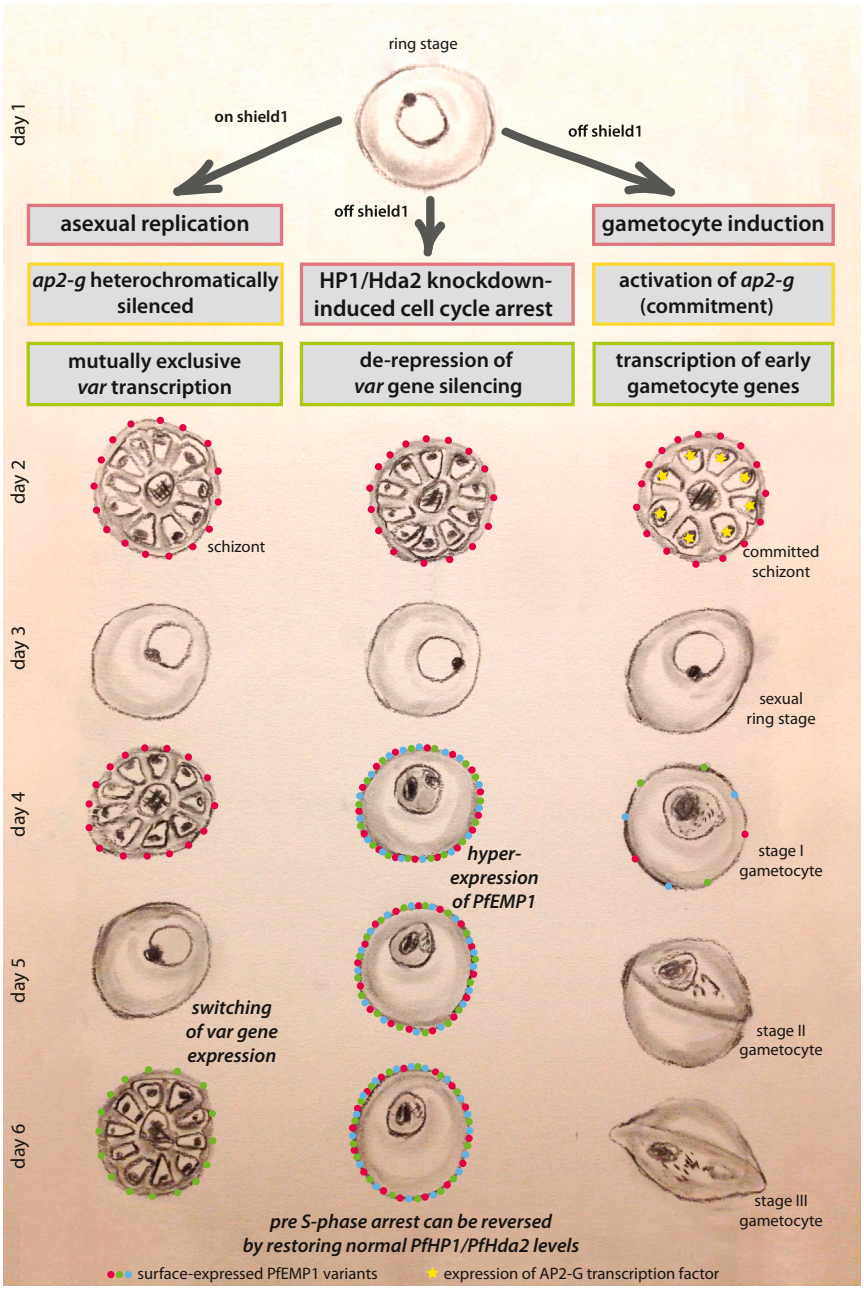

Figure 1. The Fate of a *P. falciparum* Ring-Stage Parasite upon Inducible PfHP1 or PfHda2 Knockdown

The left column shows the asexual replicative cycle before induction of protein degradation (on Shield1) characterized by a heterochromatically silenced *ap2-g* locus and mutually exclusive *var* gene transcription, resulting in the expression of a single PfEMP1 variant on the surface of the infected erythrocyte. Occasional switches in *var* gene expression result in the presentation of a new PfEMP1 variant. Depletion of PfHP1 or PfHda2 by the removal of the stabilizing ligand Shield1 interferes with the establishment and maintenance of heterochromatin and results in the activation of heterochromatically silenced genes. PfHP1- or PfHda2-depleted parasites will either go into a pre-S phase cell-cycle arrest (middle column) or commit to gametocyte formation (right column). The cell-cycle arrest phenotype accompanies a breakdown of virulence gene silencing in the subsequent ring stage and the resulting expression of multiple PfEMP1 variants on the surface of the infected erythrocyte. Induction of gametocytogenesis, be it natural or induced by either protein knockdown, involves derepression of the otherwise silenced *ap2-g* locus. The newly expressed AP2-G transcription factor will then activate early gametocyte genes.

as its absence leads to a derepression of *var* genes and other virulence gene families as well as a 3-fold increase in the rate of sexual commitment through derepression of PfAP2-G (Figure 1). Furthermore, a 50% replication phenotype was observed. Mathematical modeling exploring the implications of a shared epigenetic machinery that regulates both antigenic variation and sexual conversion suggested that low switching rates (~1% per generation) lead to a prolonged period of infection (due to extended immune evasion) and consequent lengthening of the duration of infectivity to mosquitos. The authors suggest that this link could also explain the concept of reproductive restraint in terms of an adaptation to maintain chronic infection.

Although both PfHP1 and PfHda2 depletion clearly interfere with heterochromatin formation, the two knockdown phenotypes differ markedly in their severity, with the PfHP1 phenotype being stronger in every respect. To explain the differences between the two phenotypes, we need to bear in mind that the two proteins serve quite different functions. PfHda2 is a histone modifier removing acetyl groups from histone 3 acetylated at lysine 9 (H3K9ac, a euchromatic mark), while PfHP1 is a histone reader, recognizing the heterochromatic histone 3 lysine 9 trimethyl (H3K9me3) mark placed by a histone methyltransferase. Depletion of PfHda2 creates an imbalance resulting in a relative hyperacetylation of H3K9 residues in nucleosomes along heterochromatic gene loci. Indeed, an increase in H3K9ac and concomitant decrease in H3K9me3 was observed in heterochromatic loci upon Hda2 depletion. Presumably, the residual Hda2 protein in the knockdown parasites still contributes to deacetylation of H3K9ac. That would explain why the replication defect remains stable over many cycles and why a knockout was not achieved.

PfHP1 has more far-reaching roles than Hda2 in the establishment and maintenance of heterochromatin. HP1 not only binds to and thereby protects H3K9me3 marks from demethylase activity, but also recruits methyltransferases to neighboring nucleosomes to promote the spread of heterochromatin. HP1 also facilitates chromosome compaction by directly bridging neighboring nucleosomes (Canzio et al., 2011). In the

absence of HP1, the level of compaction typical for heterochromatin cannot be achieved, and the H3K9me3 mark will be lost rapidly.

Heterochromatin has been linked to a G1/S phase transition checkpoint in other organisms. It is conceivable that a similar checkpoint is in place in the blood-stage malaria parasite, as it is crucial to closely monitor the integrity of its heterochromatic domains to prevent revelation of its entire antigenic repertoire simultaneously. The 50% replication defect in the Hda2 knockdown probably reflects the proportion of the parasite population not fit for S phase entry due to insufficient heterochromatin integrity. In the HP1 knockdown, which exhibits a complete arrest in the cycle following HP1 depletion, none of the asexual parasites fulfil the checkpoint criteria, exemplified by the complete breakdown of *var* gene silencing and the resulting hyperexpression of PfEMP1 on the surface of the host cell.

Similar to the 50% replication defect in the Hda2 knockdown, half of the HP1 knockdown population commits to gametocytogenesis in the cycle of HP1 depletion. This probably reflects the proportion of the population expressing AP2-G to levels sufficient to activate early gametocyte genes.

These two papers report the induction of gametocytogenesis by gene knockdown. Until now, the only way of inducing gametocytogenesis in vitro has been applying environmental stress. The question remains: what induces gametocytogenesis in vivo? Is there an external signal? Is it merely stochastic? Also, it remains to be explored experimentally whether there is a direct link between the frequency of *var* gene switching and the rate of gametocyte conversion.

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REFERENCES

Brancucci, N.M.B., Bertschi, N.L., Zhu, L., Niederwieser, I., Chin, W.H., Wampfler, R., Freymond, C., Rottmann, M., Felger, I., Bozdech, Z., and Voss, T.S. (2014). Cell Host Microbe *16*, this issue, 165–176.

Canzio, D., Chang, E.Y., Shankar, S., Kuchenbecker, K.M., Simon, M.D., Madhani, H.D., Narlikar, G.J., and Al-Sady, B. (2011). Mol. Cell *41*, 67–81.

Coleman, B.I., Skillman, K.M., Jiang, R.H.Y., Childs, L.M., Altenhofen, L.M., Ganter, M., Leung, Y., Goldowitz, I., Kafsack, B.F.C., Marti, M., et al. (2014). Cell Host Microbe *16*, this issue, 177–186.

Flueck, C., Bartfai, R., Volz, J., Niederwieser, I., Salcedo-Amaya, A.M., Alako, B.T., Ehlgen, F., Ralph, S.A., Cowman, A.F., Bozdech, Z., et al. (2009). PLoS Pathog. *5*, e1000569.

Guizetti, J., and Scherf, A. (2013). Cell. Microbiol. *15*, 718–726.

Kafsack, B.F., Rovira-Graells, N., Clark, T.G., Bancells, C., Crowley, V.M., Campino, S.G., Williams, A.E., Drought, L.G., Kwiatkowski, D.P., Baker, D.A., et al. (2014). Nature *507*, 248–252.

Lopez-Rubio, J.J., Mancio-Silva, L., and Scherf, A. (2009). Cell Host Microbe *5*, 179–190.

Sinha, A., Hughes, K.R., Modrzynska, K.K., Otto, T.D., Pfander, C., Dickens, N.J., Religa, A.A., Bushell, E., Graham, A.L., Cameron, R., et al. (2014). Nature *507*, 253–257.

Tonkin, C.J., Carret, C.K., Duraisingh, M.T., Voss, T.S., Ralph, S.A., Hommel, M., Duffy, M.F., Silva, L.M., Scherf, A., Ivens, A., et al. (2009). PLoS Biol. *7*, e84.

How a Virus Blocks a Cellular Emergency Access Lane to the Nucleus, STAT!

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Early in viral infection, the STAT1 transcription factor is rapidly transported into the nucleus using a nonconventional import mechanism to establish an antiviral state. In this issue, Xu et al. (2014) show how the Ebola virus VP24 protein precisely blocks specialized STAT1 import while leaving other cellular import processes intact.

As anyone stuck in traffic watching an ambulance race past can appreciate, it is important to make sure that emergency responders have special access to roadways to ensure their rapid response. A similar emergency response is mounted in cells that sense viral infection via the interferon (IFN) signaling cytokines, allowing the host to rapidly establish an antiviral state by upregulating the expression of hundreds of genes, known collectively as interferon-stimulated genes (ISGs). Key to this signaling cascade is the phosphorylation and nuclear import of STAT1 and STAT2 (signal transducer and activator of transcription) proteins, which are powerful transcription activators that switch on ISG expression. As part of the rapid immune response, STAT1 nuclear import is mediated in a noncanonical way, akin to an ‘‘emergency access