Spotlight

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The sound of sexual commitment breaks the silencing of malaria parasites

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A fundamental binary decision is made by malaria parasites at every asexual cycle in the blood between further proliferation and differentiation into gametocytes, the mosquito transmissible stages. Recent studies on *Plasmodium* epigenetic regulation, transcriptional control and genetic basis of gametocyte production are merging today to unveil players and propose molecular mechanisms of this key branch point in the malaria parasite life cycle.

Twenty years ago the search for the *Plasmodium falciparum* chloroquine resistance determinants stumbled on the polymorphic *var* gene family. This turned out to encode the elusive PfEMP1 polymorphic antigens, whose turnover on the infected erythrocyte surface was intensely studied to understand parasite immune evasion, cytoadhesion and sequestration. Having then established its molecular basis, we now know that *P. falciparum* antigenic variation is largely controlled by the epigenetic silencing of *var* genes, all physically located in the parasite nucleus periphery, with only one *var* gene productively expressed per asexual cycle [1].

This year independent studies again combined in a breakthrough in our understanding of how blood stage parasites decide to differentiate into gametocytes. In studying heterochromatin silencing, the reversible downregulation of heterochromatin protein 1 (PfHP1), a repressive mark of *var* genes, de-silenced expression of 52 of 60 *var* genes but also spectacularly increased gametocyte production by 25-fold, with half of the parasites in the subsequent cycle entering gametocytogenesis and half developmentally blocked [2]. Independently, erasure of heterochromatin silencing by downregulating the repressive histone deacetylase 2 (PfDha2), besides similarly deregulating *var* expression, also induced gametocytogenesis threefold [3]. Noticeably, in other disruptions of heterochromatin silencing, knocking out the PfSIR2A or SIR2B deacetylase or PfSET2/vs histone methyltransferase genes, the resulting *var* deregulation was uncoupled with gametocyte overproduction.

An epigenetic control of malaria sexual differentiation had been invoked to explain how individual *P. falciparum* schizonts can dictate their progeny to alternatively develop all sexually or asexually, or why parasite isolates/clones exhibit intrinsically different gametocyte conversion rates. The above studies importantly identify the first molecular players. These results also suggest that the loss of gametocyte production that typically accompanies parasite cultivation or animal passage may be due to severe, but possibly breakable, epigenetic locks and not only to gene mutations.

A key piece of the incipient jigsaw is the upregulation of one gene that could not pass unnoticed in the above experiments. One of the two transcripts to be first derepressed after PfHP1 knockdown, also upregulated after PfDha2 depletion, encoded AP2-G, the Apetala2 transcription factor family member recently identified as a conserved master regulator of *P. falciparum* and *Plasmodium berghei* sexual differentiation [4,5]. Null mutations in *ap2-g* uniquely marked human and rodent parasites unable to produce gametocytes, and *ap2-g* disruption or downregulation (*P. falciparum*) prevented gametocytogenesis. In the defective parasites 23 *P. falciparum* and 307 *P. berghei* genes were more than twofold downregulated, 7 of the former encoding early gametocyte-enriched proteins and several among the latter coding for sexual stage proteins.

Importantly, *ap2-g*, alone in the *ap2* family, and together with few euchromatic genes, turned out to be under heritable silencing control as its locus is repressively marked by PfHP1 and the associated histone3 lysine9 trimetylation (H3K9me3). Furthermore, *ap2-g* shares with *var* genes a perinuclear location [6]. Consistently, the H3K9me3 repressive code at the *ap2-g* promoter disappears upon PfHP1 downregulation, leading late asexual stages to activate *ap2-g* transcription before switching on early gametocyte genes after reinvasion. Derepression was instead undetectable in PfDha2-depleted parasites, possibly reflecting the lower magnitude of sexual induction.

These results can be merged to propose how integration of epigenetic and transcriptional controls regulate such a critical decision. They generate key cues and new questions on the molecular mechanism of developmental commitment.

It has been proposed that in natural infections *P. falciparum* has constant gametocyte conversion rates, and that stochastic fluctuations enable parasites to use a bet hedging strategy to face changing environments. Although measuring this parameter is truly challenging, analysis of controlled *P. falciparum* infections used to treat neurosyphilis, free of confounding effects from

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Keywords: malaria parasite transmission; gametocyte differentiation; developmental commitment; parasite antigenic variation; epigenetic silencing; AP2 transcription factors.

1471-4922/

 http://dx.doi.org/10.1016/j.pt.2014.09.002

immunity, generally shows waves of gametocytaemia following those of asexual parasitemia. If this suggests a constant conversion rate per parasite generation, mathematical models nevertheless best fitted such data allowing such rate per generation to vary. Furthermore, the possibility to manipulate gametocyte conversion rates *in vitro* also suggests a need to accommodate a responsiveness module introducing flexibility over epigenetic locks in a model of commitment.

The observation that restoring PfHP1 activity fully reverses gametocyte overproduction only if the protein re-accumulates in asexual parasites between 28 and 36 hours postinfection (hpi), whereas rescue is partial or absent if this occurs respectively between 34 and 42 or 40 and 48 hpi, defines a window where commitment can be modulated [2]. Mechanistically, the bistable developmental decision could critically depend on an activity threshold of the *ap2-g* promoter. Developmental branch points in prokaryotic and eukaryotic systems suggest that modification in activity or stability of constitutive (such as PfHP1) or just-in-time upregulated (such as PfDha2) proteins may act on this threshold. Importantly, the presence of eight PfAP2-G recognition sequences within 3.6 kb of the *ap2-g* upstream region further suggests that a positive autoregulatory loop could non-linearly amplify inducing signals and self-sustain sexual commitment. The host cell could also modulate commitment, similar to how neural and muscle cells favor *Toxoplasma gondii* bradyzoite differentiation [7]. The sevenfold to tenfold higher gametocyte production in reticulocyte-rich blood and the emerging role of human bone marrow as a privileged *P. falciparum* gametocyte maturation site raise questions that are now molecularly addressable.

Unlike the *Candida albicans* white-opaque or the *T. gondii* tachyzoite-bradyzoite interconversions, gametocytogenesis is a no-return option for the malaria parasite, formally equivalent to the *Trypanosoma brucei* slender-to-stumpy unidirectional differentiation or *Bacillus subtilis* sporulation. *P. falciparum* asexual schizonts were shown to be either AP2-G-negative or to contain merozoites all expressing nuclearly localized PfAP2-G [4]. This is strong evidence that malaria parasites, like the related *Haemoproteus* or *Leukocytozoon* parasites [8], produce sexually committed schizonts, a point now addressable in the possibly divergent *P. berghei*. However, is it possible that AP2-G is necessary but not sufficient to execute the gametocytogenesis program? Expression of developmental regulators can be detected in non-fully differentiated cells, questioning their predictivity as markers of irreversible commitment [9]. Although PfAP2-G^{+} schizonts and *ap2-g* mRNA positively correlate with subsequent gametocyte production, AP2-G is not yet described in second generation parasites within 24 hpi. Until a deterministic link is established at the single cell level between AP2G and the appearance of early gametocyte markers, it is conceivable that additional checkpoints may be required to irreversibly execute the AP2-G transcriptional expression program. Single cell markers from these and follow-up studies will refine commitment hypotheses, and those able to unambiguously identify sexually committed ring parasites, the only free-circulating stages besides mature gametocytes, would be invaluable in malaria control and field studies.

The perinuclear localization and the inclusion of *ap2-g* under the silencing umbrella governing *var* genes raised the intriguing hypothesis that *ap2-g* and *var* genes compete for the same monoallelic exclusion mechanism ensuring that only one *var* is productively transcribed per asexual parasite [4]. In this way the parasite could 'switch to invisibility', intimately linking the decision to sexually differentiate with a lower antigenic profile during the long gametocytogenesis. Although gametocytes express, but do not surface expose, members of other polymorphic gene families, studies on erythrocyte remodeling by early gametocytes are consistent with this scenario. Evolutionarily, this mechanism would protect transmission stages from the immunity mounting against asexual parasites, through an alternative strategy to that used by *T. brucei* to reduce immune recognition of its transmissible forms [10].

A fascinating mosaic depicting a molecular model of how malaria parasites master a key life style decision is being assembled today from tiles revealing key players of *Plasmodium* epigenetic, transcriptional and developmental controls. Importantly, such breakthrough contributions bear the promise of novel tools and a sharper aim to target parasite transmission.

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