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REVIEWS

Spotlight

Commitment Isn't for Everyone

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The majority of malaria parasites during human infection are asexual and are unable to be transmitted to mosquitoes. Only sexually differentiated parasites (gametocytes) can be successfully transmitted to complete the lifecycle. In a recent study by Bancells *et al*. (*Nat. Microbiol.* 2019;4:144–154), a new route of sexual conversion is identified that does not require a prior round of replication.

During blood stage infection by the malaria parasite *Plasmodium falciparum*, the majority of parasites undergo cyclic asexual replication every 48 hours, resulting in an exponential increase in parasite load and classic disease symptoms such as fever, anaemia and metabolic acidosis. Asexual replication encompasses development of an early ring-stage parasite into the maturing trophozoite that undergoes schizogony to form newly infectious merozoites, which upon release from the red blood cell will reinvade and initiate a new round of replication. However, in each cycle a small proportion of cells will commit to sexual development requiring 10-12 days to reach full maturity. The sexual form of the *Plasmodium* parasite is the gametocyte, which is absolutely required for onward transmission to the mosquito host to complete the full lifecycle and for the spread of the disease [1].

For nearly four decades, the prevailing model for gametocyte formation has been that sexually committed parasites undergo schizogony to form merozoites that will all go on to sexual differentiation following egress and red blood cell invasion [2]. Along with this view, it is thought that all sexually committed merozoites from the same schizont are predestined to a given sexual fate, ultimately resulting in either all female or all male gametocytes [3,4]. Recent studies have identified a transcription factor, AP2-G, as the master regulator of gametocytogenesis [5,6]. AP2-G is expressed in committed schizonts and is thought to drive transcription of genes required for sexual differentiation, as parasites lacking AP2-G are unable to produce gametocytes [5,6] and there is a linear correlation between the level of *pfap2-g* transcript and the gametocyte conversion rate [5].

Studying sexual commitment has been challenging for three major reasons: First, only a minor subpopulation of parasites undergoes sexual conversion. Second, sexually committed parasites cannot readily be purified, thereby hindering their characterization. Third, there is a widespread variability in the number of gametocytes produced by any given *Plasmodium* strain during *in vitro* culture, not to mention the inter-laboratory variability. To circumvent these challenges, Bancells *et al*. [7] take advantage of an established regulatable system in which PfAP2-G is fused to an FKBP destabilization domain (PfAP2-G-DD) [5], which in the absence of a small molecule ligand called Shld-1 is marked for degradation by the proteasome. This results in a system in which exogenous Shld-1 can be used to selectively stabilize PfAP2-G to induce timed, regulatable sexual conversion and to interrogate the timing of sexual commitment.

Using an elegant combination of the PfAP2-G-DD system, microscopy, and single-cell RNA-seq (scRNA-seq), Bancells *et al*. challenge the prevailing dogma regarding sexual commitment. Their findings convincingly demonstrate that *Plasmodium* parasites can commit to sexual development by two different paths (Figure 1). As has been previously demonstrated, a minority of parasites develop into sexually committed schizonts that are PfAP2-G positive and will solely produce gametocytes upon re-invasion of red blood cells [7]. This is called next cycle conversion (NCC). Surprisingly, Bancells *et al*. show that stabilization of PfAP2-G in early ring-stage parasites enables a second route to sexual conversion that bypasses the committed schizont phase. This means that a single schizont can potentially produce a mixture of asexual and sexual progeny, which is demonstrated by using plaque assays [7]. Mixed asexual and sexual parasite plaques result from a sub-population of schizonts that do not express PfAP2-G. Rather, PfAP2-G is expressed upon red blood cell invasion and this results in gametocyte conversion in the same cycle (same-cycle conversion, SCC). Despite significant differences between *P. falciparum* and rodent malaria parasites, SCC has also been recently demonstrated in the rodent *Plasmodium berghei* parasite by conditionally over-expressing PbAP2-G [8]. The existence of SCC is further supported by scRNA-seq experiments demonstrating that PfAP2-G stabilisation in rings results in the production of a subpopulation of cells in the same cycle that are transcriptionally almost identical to early gametocytes [7]. Bancells *et al*. also use the PfAP2-G-DD system to dissect the timing of commitment, and identify the early ring stage as the essential period during which *pfap2-g* levels peak. Although the PfAP2-G protein is still present in the nucleus for the first stage of gametocyte development, it appears not to be required after the ring stage [7].

These exciting findings bring up many new directions and questions for future investigation. While the authors show conclusively that malaria parasites can convert to sexual development via two

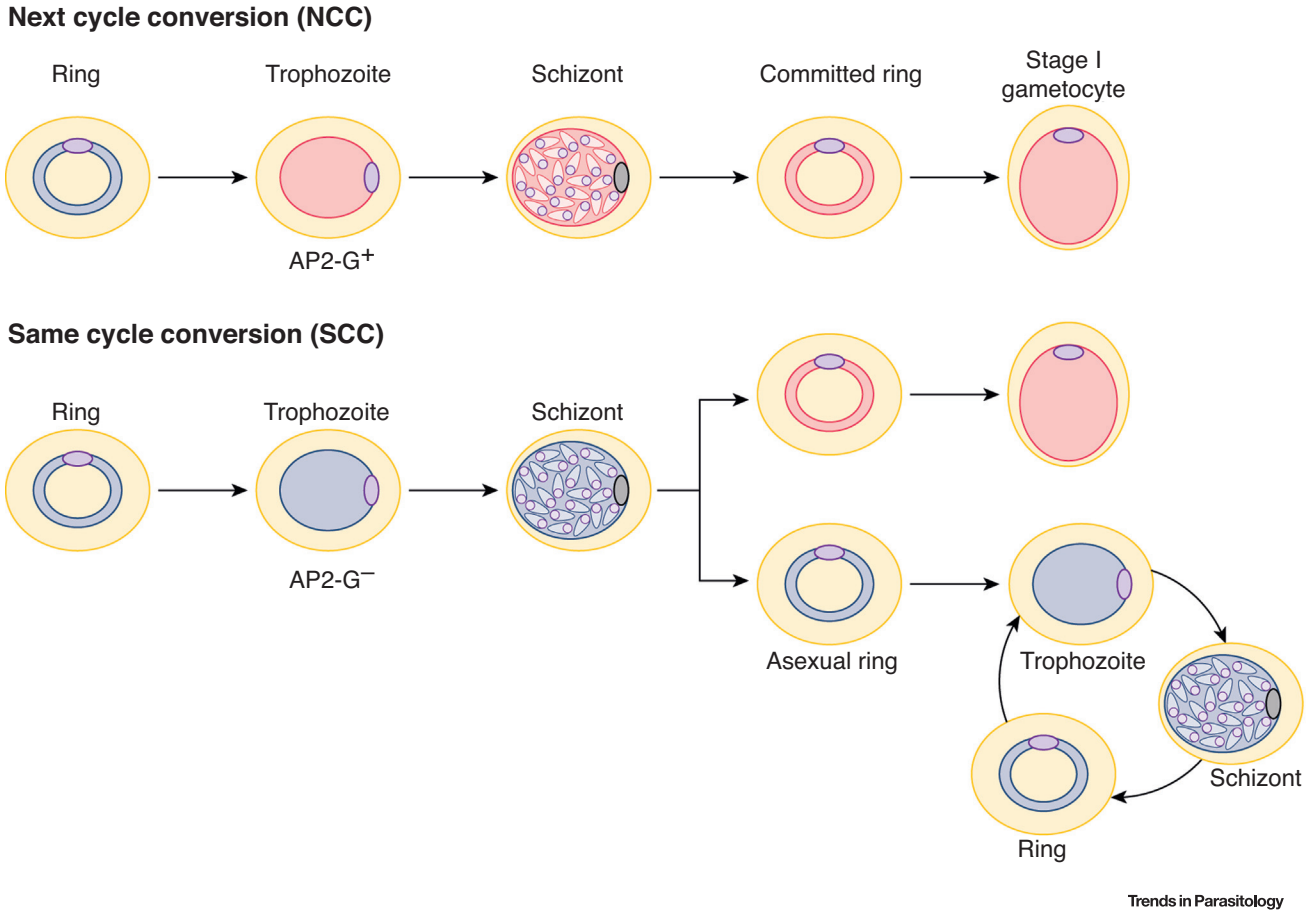

Figure 1. Model Showing the Two Routes to Sexual Conversion in *P. falciparum*. PfAP2-G-positive cells are shown in red. In the case of next cycle conversion (NCC), PfAP2-G expression begins in trophozoites and results in every merozoite in a single schizont also expressing PfAP2-G. Following re-invasion, these parasites develop into committed rings and then into stage I gametocytes. Alternatively, in same cycle conversion (SCC) PfAP2-G is first expressed in early rings. These parasites develop directly into stage I gametocytes without undergoing replication, egress, and re-invasion. This means that a single PfAP2-G-negative schizont can have both asexual and sexual progeny in the following cycle.

different routes *in vitro*, the extent to which both occur during natural infections remains unknown. Further, the difference between gametocytes produced via NCC and SCC remains to be fully examined. Understanding the functional differences between SCC and NCC gametocytes may reveal why malaria parasites are able to convert using two routes. Alternatively, the two routes could simply be redundant and allow malaria parasites to convert either rapidly or more gradually depending on various environmental triggers. The scRNA-seq shows that NCC and SCC early gametocytes are very similar at the transcript level, though intriguingly a small number of transcripts such as *pfg14.748* were more highly expressed in NCC cells [7]. These differences are surprising and warrant further investigation. The authors also demonstrate that the percent gamete egress is similar for NCC and SCC-derived gametocytes, although they do not directly look at the male:female sex ratio.

While several regulators of commitment and PfAP2-G have now been identified [9–11], their roles in SCC and NCC remain to be explored. Notably, the *pfap2-g* locus is epigenetically regulated and so must become euchromatic for PfAP2-G to be expressed and commitment to occur [9–11]. In the case of SCC, it is unknown whether the locus has already been de-repressed in the previous cycle or if these epigenetic changes occur in rings. Additionally, the identification of the ring stage as the essential period of development for PfAP2-G expression points to the existence of a yet-to-be-identified ring-stage transcription factor that

activates transcription of *pfap2-g*. Similarly, identification of the environmental factors that trigger SCC and NCC respectively and how these connect to transcriptional and epigenetic regulators will be crucial to better understanding commitment.

While many questions remain, the results of the study by Bancells *et al*. [7] require that we rethink how *Plasmodium* parasites commit to sexual differentiation. In light of the critical role that the gametocyte plays in malaria transmission, efforts to target any aspect of gametocyte commitment, conversion, or maturation will remain high priorities in antimalarial elimination strategies.

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https://doi.org/10.1016/j.pt.2019.03.012

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Forum

Increasing Complexity Threatens the Elimination of Extra-Amazonian Malaria in Brazil

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Extra-Amazonian malaria has been reported to be endemic in Brazil since the end of the 19th century. Currently, only a few cases are reported annually. However, recent findings of unexpected *Plasmodium* infections with uncertain transmission cycles in the Extra-Amazonian region could pose a threat to the malaria elimination agenda in Brazil.

Malaria in the Extra-Amazonian Region

Extra-Amazonian malaria has been reported to be endemic in Brazil since the end of the 19th century [1]. In the region, autochthonous cases of malaria have two profiles (introduced and indigenous) that occur in very distinct areas. The cycles consist primarily of the transmission of *Plasmodium* by *Anopheles* mosquitoes from the *Kerteszia* and *Nyssorhynchus* subgenus [2]. The Brazilian Extra-Amazonian region comprises 17 states and the Federal District (DF) and is home to approximately 86% of the Brazilian population. From 2007 to 2017, 806 autochthonous cases of malaria were confirmed in the Extra-Amazonian region (Figure 1). Moreover, recent reports are indicating an upsurge in the incidence of Extra-Amazonian malaria with a substantial increase in the number of cases, totaling 103 confirmed cases from January to July 2018, an increase of 35% in relation to the same period in 2017 [3].

There are two main autochthonous malaria transmission cycles in the Extra-Amazonian region:

(i) Introduced malaria cases, secondary from imported cases (e.g., malaria acquired in the Amazon region) occurring in plains, lowlands, and plateau areas where malaria was formerly endemic. Introduced malaria cases are caused by the introduced *Plasmodium* and are usually transmitted by *Anopheles* species from subgenus *Nyssorhynchus* (*Anopheles darlingi* as the primary vector). Although there were tremendous sanitary improvements and landscape changes in the Extra-Amazonian region since malaria was first considered nonendemic, the majority of Brazil is still highly receptive for malaria transmission [1,2].

(ii) Autochthonous bromeliad-malaria transmission cycle occurring in the Atlantic Forest biome. Bromeliad-malaria consists of the transmission of *Plasmodium vivax* and *Plasmodium malariae* to humans primarily by *Anopheles* (*Kerteszia*) *cruzii*, and to a lesser extent by other *Kerteszia* species, namely *Anopheles bellator* and *Anopheles homunculus*. These mosquito species almost exclusively lay their eggs in the water accumulated in phytotelmata bromeliads, which are found abundantly in the Atlantic Forest biome. Bromeliad-malaria also includes simian malaria transmission (*Plasmodium brasilianum* and