PARASITE BIOLOGY

A new malaria gene regulator

Homeodomain protein 1 (HDP1), a DNA-binding protein unrelated to AP2 transcription factors, has been identified as a regulator of gametocyte maturation in *Plasmodium falciparum*.

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Plasmodium parasites have a complex life cycle involving vertebrate and insect hosts, yet only a limited set of transcription factors that regulate parasite adaptation throughout this life cycle have been identified. In addition to the general preinitiation-complex transcription factors, just a single family of 27 apetala 2 (AP2) domain-containing transcription factors has been demonstrated to play important roles in *Plasmodium* development[1]. But now, Campelo Morillo et al.[2] identify another, distinct class of DNA sequence-specific regulators that control parasite development. Homeodomain protein 1 (HDP1, PF3D7_1466200) binds a specific DNA motif and enhances the expression of genes required for the maturation of sexual-stage parasites, called gametocytes, that are essential for malaria transmission.

Human malaria infections begin when the bite of a *Plasmodium*-infected anopheles mosquito introduces sporozoite-stage parasites (Fig. 1). The initial sporozoite stage first invades hepatocytes and, over the course of 6 days, produces tens of thousands of progeny merozoites that then invade

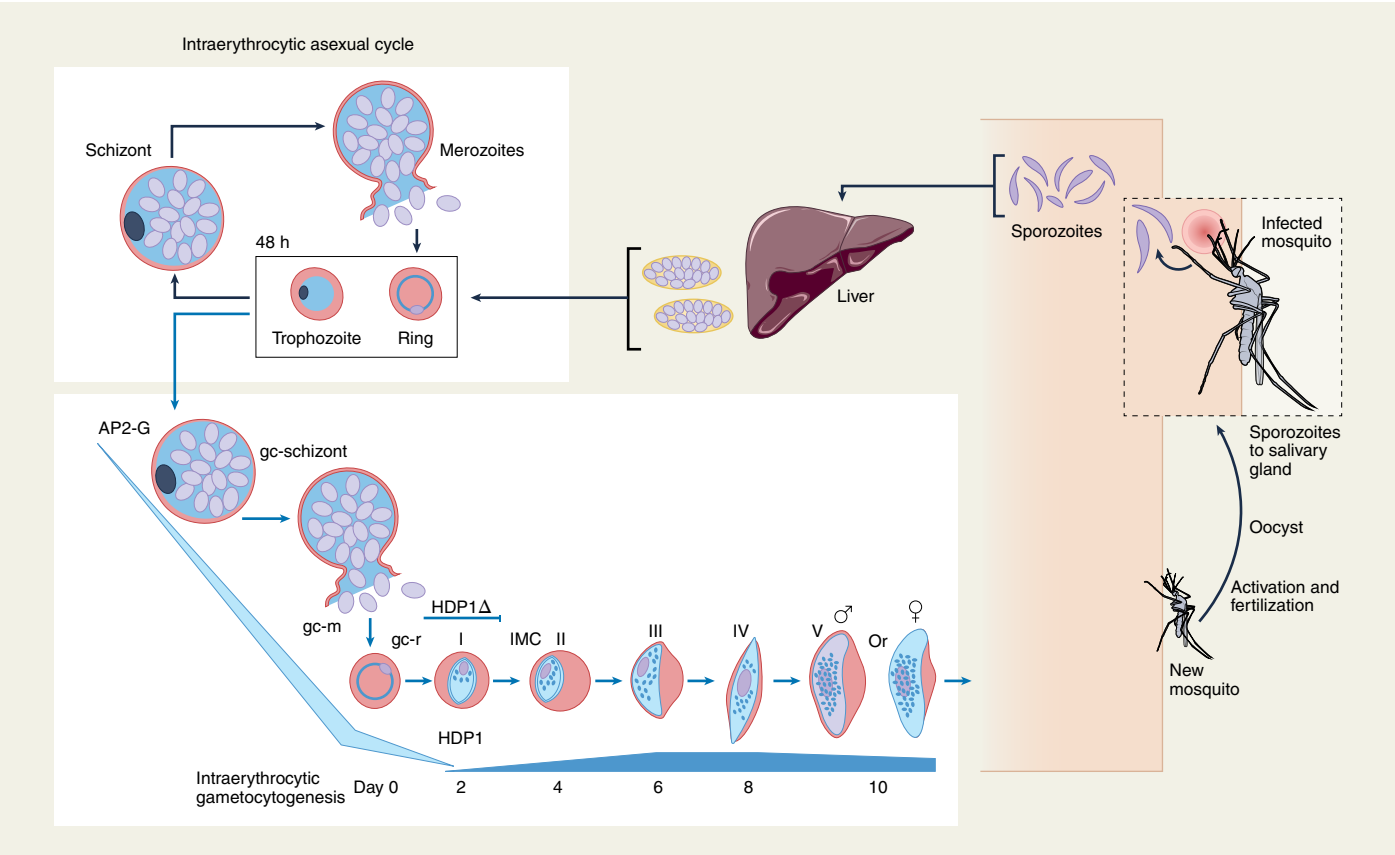

Fig. 1 | HDP1 is required for gametocyte maturation after stage I. *P. falciparum* sporozoites released during an infected mosquito bite initiate a human infection. Sporozoites replicate in hepatocytes for ~6 days, producing 10,000–30,000 new progeny, called merozoites, that invade red blood cells. If the AP2-G transcription factor is expressed by the developing intraerythrocytic parasite, all of the merozoites produced are committed to gametocyte development (gc-m). AP2-G continues to be expressed for the first 48 hours as the parasite develops from a gametocyte-committed ring (gc-r) into a stage I gametocyte (light-blue bar), which begins to express HDP1 (dark-blue bar). HDP1 traffics to the nucleus, binds a specific DNA motif and enhances expression of the downstream genes, including those required for IMC formation. The IMC is required for gametocytes to transition from stage I to stage II, and in the absence of HDP1 (HDPΔ), IMC formation is inhibited and further development is blocked. Republished with permission of Annual Reviews, Inc. from ref. [5]. Permission conveyed through Copyright Clearance Center, Inc.

erythrocytes. As the intra-erythrocytic parasite replicates asexually, a subpopulation of parasites express a specific AP2 transcription factor, AP2-G, which commits the newly produced merozoites to sexual differentiation[3,4]. In the most virulent human malaria species, *P. falciparum*, AP2-G (PF3D7_1222600) is only expressed during the first 2 days of gametocytogenesis, yet sexual maturation continues for 10 days as the parasite differentiates through five distinct morphologic stages[3]. No additional transcription factors or genetic regulators had been associated with *P. falciparum* gametocyte maturation[5] before this report by Campelo Morillo et al. demonstrated the critical role of HDP1.

HDP1 expression increases during early gametocyte development as AP2-G expression declines, and parasites lacking *hdp1* initiate normal levels of gametocyte commitment but fail to mature to stage II gametocytes, which is consistent with HDP1 acting downstream of sexual commitment (Fig. 1). This phenotype was confirmed by tagging the 3′ end of the gene with an autocatalytic glmS ribozyme, which allowed inducible RNA degradation of *hdp1* RNA. Careful analysis of HDP1's 3,078 amino acid (aa) sequence identified nuclear localization signals and a C-terminal helix–turn–helix structural motif, suggesting a role in DNA binding. C-terminal-reporter-tagged HDP1 indeed localized to the nucleus and was resistant to solubilization with ≤600 nM salt, which is consistent with chromatin association. Furthermore, recombinant HDP1 encoding the predicted DNA-binding domain (aa 3011–3071) recognized a dimer of the DNA motif, GTGCAC, suggesting sequence-specific binding, which was also supported by chromatin immunoprecipitation followed by DNA sequencing (ChIP–seq) analysis. Overall, 85% of the HDP1–GFP ChIP–seq binding summits were within the upstream region of genes, with the greatest concentration of binding just 5′ of transcription start sites. Moreover, one of the two sequence motifs enriched within 100 bp of 78.1% of the HDP1–GFP ChIP–seq binding summits included the motif recognized by the recombinant protein, again strongly supporting the ability of HDP1 to bind to specific DNA motifs. Additionally, 59% of the 156 significantly downregulated genes in the *hdp1*-knockout parasites had upstream HDP1–GFP ChIP–seq binding summits, including *hdp1* itself and 11 of the 13 downregulated genes involved in inner membrane complex (IMC) formation[6]. The IMC forms just under the parasite plasma membrane and is essential for the stage I to stage II gametocyte transition. The downregulation of these 11 genes is likely to be responsible for blocking the transition from stage I to II gametocytes in *hdp1*-knockout parasites[6].

Together, the data are consistent with HDP1 functioning as a homeodomain-like protein with a major role in *P. falciparum* sexual maturation. Interestingly, homologues of the core 60-aa helix–turn–helix domain were found in other *Plasmodium* and *Coccidia* species, such as *Toxoplasma gondii*, but not other Apicomplexa, suggesting that these are divergent members of the large family of homeodomain proteins that regulate development in many species[7,8].

While the present study has begun to elucidate the role of *hdp1* in parasite development, future studies using targeted mutations in HDP1 and its DNA binding sites, as well as studies to determine HDP1 binding partners, will be important to further characterize the function of this unique homeodomain protein. Of particular interest is the role of the N-terminal 3,000 aa of HDP1. The ability of an N-terminal reporter tag to block gene function and have the same phenotype as the HDP1 knockout merits further investigation. Protein–protein interactions along with the chromatin landscape, which is known to be dynamic in *P. falciparum*[9], have been shown to affect the DNA-binding affinity and specificity of homeodomain proteins[8] and could play an important role in HDP1 activity. Once bound to DNA, homeodomain proteins could enhance access to the transcription start site or could help to direct further chromatin modification to regulate expression[8]. It is also intriguing to speculate on whether HDP1 has additional functions later in gametocyte maturation or in the mosquito or liver stages. Later gametocyte stages cannot be tested using the glmS ribozyme system, which degrades only RNA and not already translated protein. Instead, a ligand-mediated protein degradation system that allows HDP1 expression through stage II and induces degradation only at later time points is needed. Finally, the important developmental role of HDP1 suggests that additional genes with isolated, predicted DNA-binding-like domains should be carefully examined to uncover other gene regulators involved in the development of this important human pathogen. ❐

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References

1. Balaji, S., Babu, M. M., Iyer, L. M. & Aravind, L. *Nucleic Acids Res.* **33**, 3994–4006 (2005).
2. Campelo Morillo, R. A. et al. *Nat. Microbiol.* https://doi.org/10.1038/s41564-021-01045-0 (2022).
3. Kafsack, B. F. C. et al. *Nature* **507**, 248–252 (2014).
4. Sinha, A. et al. *Nature* **507**, 253–257 (2014).
5. Josling, G. A., Williamson, K. C. & Llinás, M. *Annu. Rev. Microbiol.* **72**, 501–519 (2018).
6. Parkyn Schneider, M. et al. *PLoS Pathog.* **13**, e1006659 (2017).
7. Bürglin, T. R. & Affolter, M. *Chromosoma* **125**, 497–521 (2016).
8. Bobola, N. & Merabet, S. *Curr. Opin. Genet. Dev.* **43**, 1–8 (2017).
9. Hollin, T. & Le Roch, K. G. *Front. Cell Infect. Microbiol.* **10**, 618454 (2020).

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Competing interests

The author declares no competing interests.