Microbiology

How a malaria parasite becomes a male

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Organisms use various strategies for sex determination. The non-genetic mechanism in the malaria-causing parasite *Plasmodium falciparum*, involving a male-specific factor, has now been revealed. **See p.528**

The protozoan parasite *Plasmodium falciparum* is responsible for the vast majority of human malaria cases and deaths. It has a complex life cycle that involves only two developmental decision points, which occur in parasites living in red blood cells. The first of these is whether to continue with a cycle of asexual replication or to differentiate into sexual forms, called gametocytes, that enable human-to-mosquito transmission. The second is whether gametocytes develop as male or female forms. For all other stages of the life cycle, which is split between human and mosquito hosts, the parasite's only option is to proceed to the next stage. On page 528, Gomes *et al.*[1] shed light on a long-standing mystery about how male sex is determined.

Transformation of asexual parasites into gametocytes, termed sexual conversion, starts with expression of the GDV1 protein, which displaces repressive protein–DNA complexes (heterochromatin) from the *pfap2-g* gene. This leads to the expression of *pfap2-g*, which encodes a transcription-factor protein (PfAP2-G) that initiates the gametocyte development programme[2,3].

The mechanism of sex determination in malaria parasites was unknown until now. However, there were some clues. Populations of genetically identical parasites that are haploid (having a single copy of each gene) can produce both male and female gametocytes. This argues against mechanisms of sex determination that involve alternative versions of genes (alleles), or a role for sex chromosomes[4–6]. The observation indicates that sex is instead determined by non-genetic mechanisms.

A previous study[7] using the malaria parasite *Plasmodium berghei*, which can infect mice, identified the gene *md1* as a leading candidate for involvement in male sex determination. Gomes and colleagues follow up on this discovery, and demonstrate that the Md1 protein encoded by this gene is responsible for male sex determination in *P. falciparum*. The authors characterized the role of Md1 using a variety of methods, including harnessing genetically engineered parasites and undertaking analysis of global gene expression (transcriptomics) at the single-cell level. The use of single-cell transcriptomics to measure gene expression in individual parasites was crucial for identifying and separately analysing male, female and sexual-precursor cells (in which sex has not yet been determined) from mixed populations of parasites.

The authors show that several types of RNA are made from the *md1* genomic region (the *md1* locus). The expression of a full-length 'sense' version of messenger RNA for this gene leads to the production of Md1, and to male sex determination (Fig. 1). The expression of an RNA that does not produce protein and is transcribed from the opposite strand of DNA at the *md1* locus – an antisense long non-coding RNA – is specifically associated with development into a female. Moreover, an RNA corresponding to a shorter version of the sense RNA is also expressed, at least in females, revealing a complex transcriptional architecture. Md1 does not seem to be a transcription factor, given that the authors found it to be localized in cytoplasmic spots (foci) and report that it is associated with proteins that are involved in the regulation of mRNA stability or translation. Therefore, rather than regulating transcription, Md1 probably operates after transcription has occurred.

Gomes *et al.* identified a region of Md1 (the amino-terminal domain) that is responsible and sufficient for the determination of male sex, whereas another region of the protein (the carboxy-terminal domain) is needed later on for the correct development of male gametocytes. Previous work identified factors necessary for the maturation of either male or female gametocytes[4]. What makes Md1 unique is that it determines the male sex, rather than being

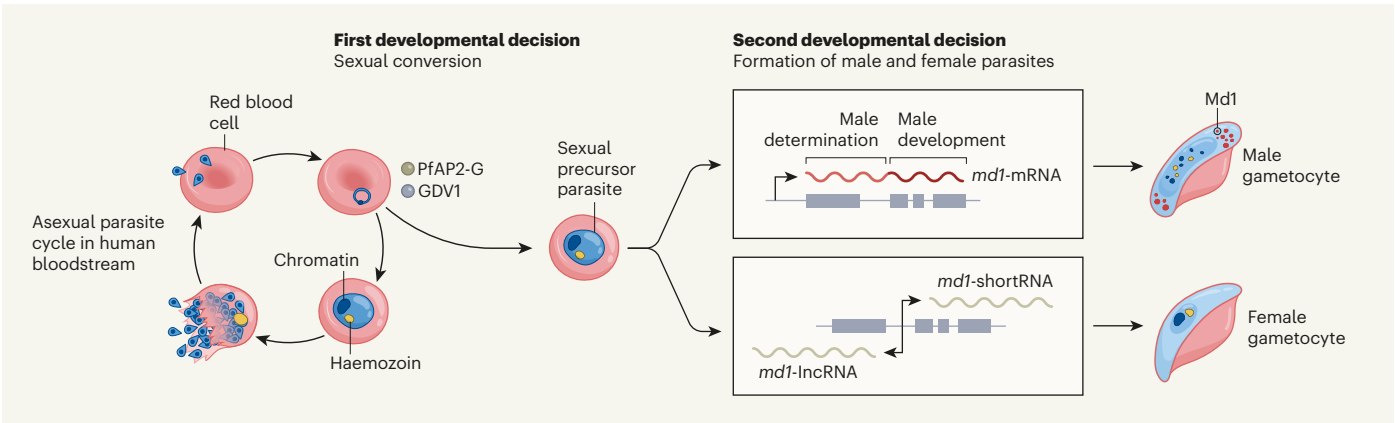

Figure 1 | Crucial decision points in the life cycle of the malaria parasite *Plasmodium falciparum*. Parasites (various forms are shown in blue) contain the pigment haemozoin and the DNA–protein complex chromatin. The parasite life cycle has two key developmental switches. The first requires the proteins GDV1 and PfAP2-G and occurs when the parasite exits the asexual replication cycle and commits to sexual development. The second is when sexual-precursor parasites on the sexual-development pathway form either male or female gametocytes. Gomes *et al.*[1] reveal how male sex is determined. Parasites that express full-length 'sense' transcripts of the *md1* gene (*md1*-mRNA), which encodes the cytoplasmic protein Md1, form male gametocytes. This mRNA for Md1 encodes a region that is necessary and sufficient for male sex determination and a region that is needed for male gametocyte development. Females develop from parasites that predominantly express antisense (*md1*-lncRNA) and shorter sense (*md1*-shortRNA) RNAs from the *md1* genomic region. Whether *md1*-shortRNA is female-specific or also produced in males could not be unambiguously established.

necessary only for maturation after sex has been determined. It is tempting to speculate that, by default, gametocytes would develop into females, and that only those gametocytes for which a transcriptional switch at the *md1* locus occurs, resulting in Md1 production, become males. Testing this hypothesis will require a high-resolution temporal analysis of the RNA species at the *md1* locus in sexual precursors in which sex is not yet specified.

Previous research using a method called a plaque assay, in which the progeny cells produced from individual asexual parasites can be visualized, indicates that sex determination occurs at the same time as, or soon after, commitment to sexual conversion[5,6]. By contrast, Gomes *et al.* show that Md1 expression and transcriptional differentiation between male and female gametocytes start at stage II or III of gametocyte development, which is about four days after sexual conversion, suggesting that sex determination occurs instead at this later stage. The results of the plaque assays (from more than 20 years ago), which used antibodies to distinguish between male and female gametocytes, should be revisited by performing the assays with newer technology and tools such as male–female reporter lines, in which different fluorescent proteins are expressed depending on whether parasites are male or female. If the plaque-assay conclusions are confirmed, it will be necessary to reconcile the apparent discrepancy with Gomes and colleagues' results, perhaps invoking molecular events upstream of the transcriptional switch at *md1*. Such events might pre-commit a parasite to become either male or female several days later.

Life-cycle progression in *P. falciparum* is generally governed by the expression of a cascade of transcriptional regulators of the ApiAP2 family[8]. However, for sexual conversion and sex determination, the parasite uses a complex transcriptional switch at the *gdv1* or *md1* genomic regions, respectively. The *gdv1* locus also encodes an abundantly expressed antisense long non-coding RNA, which has been proposed to act as a repressor of GDV1 expression[3]. By contrast, Gomes and colleagues ruled out a repressor role for the *md1* antisense long non-coding RNA, although its precise mechanism of action is unknown. Of note, antisense long non-coding RNAs are also involved in the regulation of another gene family (the *var* genes) with a fundamental role in parasite biology[9] — mediating parasite virulence and evasion of immune responses. Therefore, complex transcriptional switches involving different RNA species encoded in the same locus emerge as a common theme for fundamental processes in malaria-parasite biology that require robust regulation.

The identification of Md1 as the male sex determinant settles a major outstanding question in malaria-parasite biology and raises several questions. Future research should determine the mechanism underlying the transcriptional switch at the *md1* locus that results in expression of the full-length mRNA and Md1 production, and identify the regulator(s) of *md1* expression. This switch probably involves an element of randomness (stochastic processes) because, in a population of genetically identical parasites under the same environmental conditions, some will develop as males and some as females. However, the switch might nevertheless be modulated by environmental cues to dynamically adjust the sex ratio to the optimal level for different conditions[4,10].

Of note, the proportion of parasites that convert into sexual forms (the sexual-conversion rate) is also affected by the environment. Some cues that enhance baseline sexual-conversion rates have been identified, including depletion of specific lipids and exposure to certain drugs[11,12]; however, the mechanism by which these cues result in *gdv1* and *pfap2-g* expression is unresolved. Future research should address the mechanism by which the *gdv1* and *md1* switches respond to the environment.

Another crucial aspect of sex determination now ripe for exploration is the identification of Md1's molecular targets. Gomes and colleagues' work suggests that Md1 contributes to regulating the stability or translation of some mRNAs. Identifying the specific mRNAs that are targeted and the downstream events in which they participate will be needed to gain a full mechanistic understanding of malaria sex determination.

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Condensed-matter physics

Twin techniques narrow search for elusive particles

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A versatile nanowire system has enabled the hunt for particles that could be useful for quantum computers. The platform can be probed with two techniques simultaneously — minimizing the possibility of false-positive signals. **See p.442**

Majorana particles are curious things. They are unusual excitations comprising a pair of half electrons — and they are their own antiparticles. This means that they annihilate each other when brought together, but remain stable when separated. What makes them really intriguing is that they retain a memory of how they move — a property that could be used to store quantum information in solid-state systems. The only problem is that they are tremendously difficult to find, and there have been instances of false-positive detections. On page 442, Valentini *et al.*[1] report a system in which measurements can be made simultaneously using two techniques, reducing the probability of false positives and revealing a simple explanation for a misleading signature.

Although elementary Majorana particles were proposed 85 years ago by Italian physicist Ettore Majorana[2], they are yet to be detected in high-energy physics experiments. However, analogous 'particles' can be engineered in solid-state systems that comprise semiconducting materials combined with some that exhibit superconductivity (zero electrical resistance). This is because the electrons in superconductors have energies that are equivalent to those of their antiparticles, which are called holes in this context. This symmetry enables the formation of states, known as Majorana bound states, at the ends of wires made from these hybrid materials[3].