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Revealing the Molecular Determinants of Gender in Malaria Parasites

Malaria parasites have a complex life cycle with asexual multiplication in a vertebrate host and obligate sexual reproduction in the mosquito; however, commitment to sexual development begins in the vertebrate with differentiation of female and male gametocytes. In this issue of *Cell*, Khan et al. (2005) used elegant approaches to purify male and female gametocytes and elucidated their respective proteome, providing the basis for understanding sexual development in this pathogen.

In malaria, the mosquito is much more than a simple vehicle by which the *Plasmodium* parasites travel between mammalian hosts. A series of developmental processes central to the life cycle of this unicellular parasite occur in the mosquito, beginning with sexual reproduction. Sex plays a key role in *Plasmodium* biology, especially in generating diverse parasite populations that rapidly adapt and survive in the face of inhibitory forces such as immunity and antimalarial drugs. Fertilization of male and female gametes occurs in the midgut soon after the mosquito ingests a blood meal from an infected host. However, parasites do not enter the mosquito as gametes; rather they are taken up as gametocytes, forms derived in the vertebrate blood stream following a process of early sexual differentiation. The molecular events that trigger sexual differentiation, the development of gametocytes, and fertilization are all essential steps in the transmission of this parasite to the mosquito and subsequently to its next vertebrate host. Consequently, these events have long been thought to be potential targets of new drugs or vaccines designed to interrupt the parasite life cycle.

Elucidation of the proteomes of male and female gametocytes from *Plasmodium berghei*, a rodent model of human malaria, by Khan et al. (2005) provides a major step forward in understanding sexual development and in opening new avenues to develop strategies to block parasite transmission. This is important for the two major *Plasmodium* species that infect humans, *P. falciparum* and *P. vivax*. While both species are responsible for substantial morbidity in human populations, *P. falciparum* can be lethal and is responsible for several million deaths each year throughout the developing world (Snow et al., 2005).

The availability of the genome sequences of different *Plasmodium* species, including *P. falciparum*, and the model rodent malaria species *P. yoelii* and *P. berghei*, have been landmark steps in accelerating our progress in understanding these parasites and the basis of pathogenesis in their respective vertebrate host (Carlton et al., 2002; Gardner et al., 2002; Hall et al., 2005). This information has been essential for analyzing the transcriptome and proteome of these *Plasmodium* species through their complex developmental life cycle, and these studies have provided a global view of protein expression at different stages in the parasite life cycle (Florens et al., 2002; Hall et al., 2005; Lasonder et al., 2002). This has included gametocyte stages of both *P. falciparum* and *P. berghei*; however, these stages have been analyzed as mixtures of male and female forms and have also been contaminated with other stages of the asexual life cycle. The paper by Khan et al. presented in this issue provides an elegant solution to this problem in their development of a novel approach to separate and purify male and female gametocytes in quantities sufficient to allow detailed proteomic analysis. The male and female gametocyte proteomes provide a revealing snapshot of the different *P. berghei* proteins, the vast majority of which have close relatives in the human malaria parasite *P. falciparum*, that are required for the functional role of these two cell types. This work provides an excellent foundation to decipher the role of sex-specific proteins in the function of gametocytes as well as the development of gametes.

When comparing the proteome of male versus female gametocytes, the first and most striking observation is that they are so different. Perhaps this is not so surprising considering the divergent roles they must perform. The functions of these cells are reflected in their proteome with the terminally differentiated male gametocyte biased toward expression of proteins associated with genome replication and axoneme formation, consistent with the requirements to produce eight motile gametes per gametocyte. The female gametocyte is prepared for continued development and requires a broad array of functions. Proteins involved in protein expression and mitochondrial function were more prevalent in this cell type. Surprisingly, apicoplast proteins were not gender specific, and the fact that this organelle is maternally transferred suggests that it has an important function in both male and female gametocytes. The apicoplast organelle is essential in the asexual stages of *Plasmodium* and contains functions involved in fatty-acid and isoprenoid biosynthesis (McFadden and Roos, 1999).

Perhaps the most interesting feature of male and female gametocyte proteomes is the sex-specific expression of a number of kinases and phosphatases, suggesting that there are different modes of signal transduction. Male and female gametocytes are in a state of cell cycle arrest, and activation to form gametes results from the signals and cues present within the mosquito gut. The calcium-dependent protein kinase (CDPK4) has been shown to be important in activation of gamete formation, probably by control of a signal transduction cascade (Billker et al., 2004). Khan et al. suggest that certain kinases and phosphatases are sex specific and play important roles in signal transduction in the male and female. To provide evidence for this, they constructed parasites lacking the genes

encoding either male-specific mitogen-activated protein kinase 2 (MAP2) or female-specific NIMA-associated kinase (NEK4). These results showed that MAP2 is required for differentiation of male gametocytes after genome replication while NEK4 is required post-fertilization. The role of MAP2 in development of male gametes has recently been confirmed in an independent study (Rangarajan et al., 2005). The identification of sex-specific proteins in male and female gametocytes combined with the ability to construct *P. berghei* parasites that lack expression of specific proteins has provided a powerful approach to dissect the role of these proteins in sexual development of *Plasmodium*.

Another interesting feature of this work has been the suggestion that presynthesized proteins are stored within the female and male gametocyte for use in subsequent stages of development. Following uptake of gametocytes into the mosquito gut, the process of gamete differentiation and zygote formation after fertilization is a very rapid process. Storage of proteins for these processes would be absolutely essential providing, for example, fast provision of axonemal components for rapid assembly of the motor complex and flagella for male gamete motility. Additionally, it has been shown previously that repressed mRNA species are stored in the female gametocyte, allowing rapid expression for development of both this cell type and the fertilized zygote (Hall et al., 2005). It is not clear why the female gametocyte stores different protein and mRNA species, although it may possibly be related to the timing for which these particular functions are required.

Although the work by Khan et al. (2005) has provided the identity of the proteins involved in gametocyte and gamete function in *Plasmodium*, there is still a long way to go before we fully understand the main events in this process. For example, although we have some knowledge with respect to the cues that trigger gamete production, the specific proteins involved are as yet unknown. Nevertheless, the work described in this paper heralds an exciting time as we now have the identity of many male and female gametocyte-specific proteins and the genetic tools to construct loss-of-function mutants and perform other genetic manipulations. This knowledge has the potential to lead to identification of novel drug targets and ultimately methods to interrupt transmission of *Plasmodium* to the mosquito vector.

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