



**Regulation of Sexual Development of Plasmodium
by Translational Repression**

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Science **313**, 667 (2006);
DOI: 10.1126/science.1125129

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Whereas men sought advice from their often broad-reaching networks, women frequently depended on close relationships with male collaborators to initiate the patenting process. Formal institutional sponsorship was also particularly important for women. Many women commented that their TTO provided industry contacts, advice, and encouragement to develop the commercial aspects of their research.

Our interviews also exposed differences between older and younger women scientists. Most senior female faculty we met perceived themselves as being excluded from industry relationships and therefore failed to develop an understanding of how commercial science works. Few made the transition to patenting. Some of the younger (but tenured) female life scientists had begun to incorporate patenting into their research strategy. Nonetheless, many still felt at a disadvantage to their male colleagues because of their limited experience at the academic-industry boundary. It is only among junior faculty that we found parity in attitudes, which were shaped by doctoral and postdoctoral experiences. Regardless of gender, those that experienced patenting during training were undaunted by the challenges of combining academic and commercial science.

Because our data spans 35 years, we can determine whether such generational distinctions are evident in the larger sample. For three Ph.D. cohorts (those earning degrees from 1967 to 1975, 1976 to 1985, and 1986 to 1995),

we examined gender-specific nonparametric cumulative hazard plots. For each cohort, we also calculated the male-to-female ratio of the cumulative hazards. For example, at the 10th year after scientists earned their Ph.D., the cumulative hazard of patenting for male scientists was 4.4 times as high as women in the 1967–1975 cohort, 2.1 times as high in the 1976–1985 cohort, and 1.8 times as high in the 1986–1995 cohort (fig. S1). Thus, consistent with our interview findings, the archival data indicate that the gender gap in patenting rates has been declining.

Our analyses suggest that patenting has become common in the academic life sciences, particularly for highly productive and networked faculty. Among the most senior faculty, a large gender gap persists, reinforced by women's limited commercial networks and traditional views of academic careers. Younger cohorts widely embrace patenting, although a gender gap remains. Increasingly, however, young female faculty are similar to their male colleagues: They view patents as accomplishments and as a legitimate means to disseminate research. If this trend continues, we may observe further declines in the magnitude of the gender gap in commercializing academic science.

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16. F.M. acknowledges financial support from the Cambridge-MIT Institute. T.E.S. acknowledges financial support from the Center for Entrepreneurial Leadership at the Ewing Marion Kauffman Foundation, Kansas City, MO. This material is partly based on work supported by the NSF under grant No. EEC-0345195 (T.E.S.).

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11 January 2006; accepted 13 June 2006
10.1126/science.1124832

Regulation of Sexual Development of *Plasmodium* by Translational Repression

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Translational repression of messenger RNAs (mRNAs) plays an important role in sexual differentiation and gametogenesis in multicellular eukaryotes. Translational repression and mRNA turnover were shown to influence stage-specific gene expression in the protozoan *Plasmodium*. The DDX6-class RNA helicase, DOZI (development of zygote inhibited), is found in a complex with mRNA species in cytoplasmic bodies of female, blood-stage gametocytes. These translationally repressed complexes are normally stored for translation after fertilization. Genetic disruption of *pbdzi* inhibits the formation of the ribonucleoprotein complexes, and instead, at least 370 transcripts are diverted to a degradation pathway.

Translational repression (TR) of mRNAs in higher eukaryotes controls temporal expression of specific protein cascades or directs the location of translation within a cell, and is important after gamete fertilization (zygote formation) in the early embryo when de novo transcription of mRNA is restricted (1–5). The hallmark of repression is the assembly of certain mRNAs together with proteins into qui-

escent messenger ribonucleoprotein particles (mRNPs), where these transcripts are stored for translation at a later time. The DDX6 family of DEAD-box RNA helicases is tightly linked both to storage of mRNAs encoding proteins associated with progression through meiosis into translationally silent mRNPs and with the transport of mRNA to degradation centers in the cell (P-bodies). These helicases are found in orga-

nisms as diverse as yeast (e.g., Dhh1p) and humans (e.g., RCK/p54).

Earlier studies in *Saccharomyces cerevisiae* suggested that Dhh1p was localized to cytoplasmic P-bodies that contain both mRNA and enzymes central to the RNA degradation pathway (e.g., the decapping enzyme), implying that P-bodies harbor transcripts destined for degradation (6–8). More recently, it was proposed that mRNAs also exit P-bodies and re-engage polysomes for translation in a Dhh1p-dependent mechanism (9). With the exception of human RCK/p54, homologs of DDX6 helicases in metazoans have been found exclusively localized to mRNPs involved in TR (2–4).

TR has been described in *Plasmodium* (10–16) in the female gametocyte, the stable, blood-stream precursor cell of the female gamete, where two abundant transcripts are present but not translated. These mRNAs, *p25* and *p28*, en-

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code proteins essential for zygote development and mosquito midgut invasion (17) and are kept in a translationally quiescent state. Translation is only initiated after these sexual forms have been activated during ingestion by a female mosquito, thus triggering gamete formation and subsequent fertilization. Later work showed that in gametocytes, TR most likely affected multiple transcripts and could be an important mechanism of gene regulation at this developmental time point involving mechanisms established in metazoans (16).

In the gametocyte sex-specific proteomes (18), we identified an RNA helicase (DOZI) that is highly up-regulated in female gametocytes and showed high sequence homology to the DDX6 family of RNA helicases. Sequence alignments showed that DOZI formed a specific clade with in all annotated *Plasmodium falciparum* RNA helicases, their *P. berghei* and apicomplexan homologs that cluster specifically with several DDX6 helicases known to be involved in mRNP formation (figs. S1 to S3). DOZI appeared to be the only DDX6-family homolog present in *Plasmodium* and contained the domains involved in RNA-binding and RNA-unwinding activity (19).

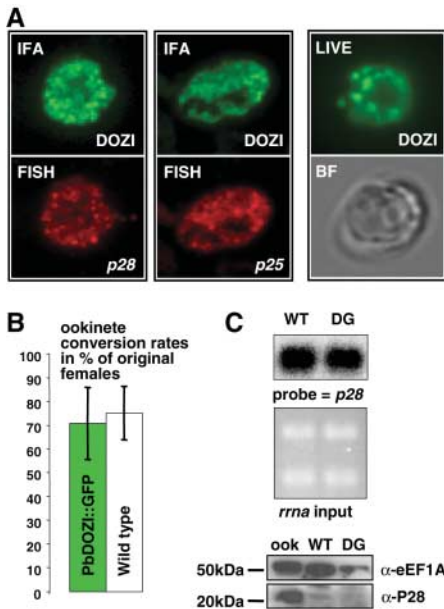


Fig. 1. PbDOZI::GFP, a modified *P. berghei* line expressing GFP-tagged DOZI, showed punctate localization in the cytoplasm of female gametocytes. Translational repression and zygote development were normal. (A) IFA and FISH analysis of female gametocytes showed a similar, punctate localization in the cytoplasm of DOZI::GFP and *p25* and *p28*. Live imaging also showing punctate localization. BF, bright field. (B) PbDOZI::GFP gametocytes showed wild-type development of zygotes into ookinetes. Data show the mean \pm SD. (C) Northern analyses of *p28* mRNA and Western analysis of P28 protein showed storage and translational repression of the transcript as in wild-type parasites. WT, wild type; DG, PbDOZI::GFP; ook, ookinetes.

For cellular localization of DOZI, we generated a modified *P. berghei* line expressing a C-terminal green fluorescent protein (GFP) fusion of endogenous DOZI (DOZI::GFP) (Fig. 1, A to C; fig. S4). A punctate GFP-fluorescence pattern that appeared to be restricted to the cytoplasm of female gametocytes was observed in live and fixed cells after immunofluorescence assay (IFA) analysis with antibodies to GFP (anti-GFP) (Fig. 1A). These transgenic parasites showed wild-type fertilization rates and zygote/ookinete production (Fig. 1B). Steady-state levels and translational repression of *p28* mRNA were the same as those in wild-type parasites (Fig. 1C). Fluorescence in situ hybridization (FISH) analysis of the localization of *p28* and *p25* transcripts, combined with IFA for DOZI, showed that both transcripts were highly abundant in female gametocytes (Fig. 1A) with a punctate localization pattern comparable to that of the helicase. This suggested that the repressed transcripts and DOZI are distributed similarly in the cytoplasm and prompted an analysis of transcripts associated with DOZI.

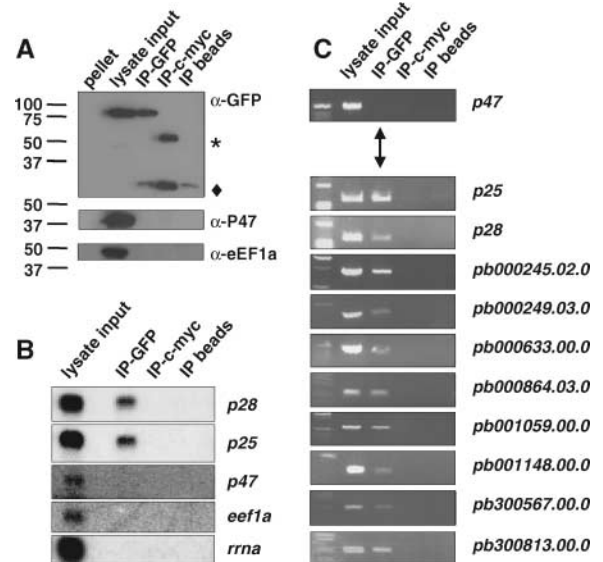
Immunoprecipitations of the DOZI::GFP fusion protein from gametocyte lysates were made with monoclonal anti-GFP (GFPmAb). Eluates were analyzed for DOZI protein and transcript content. Western analysis of total gametocyte lysate input, as well as specific and control precipitates, identified DOZI::GFP only in the specific immunoprecipitates, whereas other control proteins (P47 and eEF1A) were only found in the input material (Fig. 2A). Northern analysis showed the presence of a substantial amount of *p25* and *p28* transcripts and not control RNA species in the GFPmAb eluate (Fig. 2B), suggesting that these mRNAs and DOZI occurred together in an mRNP. Additionally, reverse transcription-polymerase chain reaction (RT-PCR) analysis of the eluates demonstrated that many other transcripts that had been predicted to be transla-

tionally repressed (16) coelute with DOZI::GFP (Fig. 2C). Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis indicated that these same mRNA species were significantly enriched in the DOZI::GFP coeluate (fig. S5) and that they showed the punctate localization pattern in the cytoplasm (fig. S6).

P. berghei parasites were generated that lacked *pbdozi* (fig. S7). The *pbdozi*⁻ null mutants showed normal development of the asexual blood stages and normal production of gametocytes and gametes, but there was a total lack of development of fertilized female gametes (zygotes) into mature ookinetes (Fig. 3A). Normal development into ookinetes requires meiotic DNA replication in the zygote 2 to 3 hours after fertilization of female gametes (18). In *pbdozi*⁻ null mutants, all zygotes aborted development before meiosis. DOZI is also produced in males (18), although in much lower abundance than in females. We crossed the male and female gametes of *pbdozi*⁻ with gametes of mutant parasite lines that are defective in male (20, 21) or in female gamete production (22) (Fig. 3A). Such cross-fertilization assays revealed that male *pbdozi*⁻ gametes were able to fertilize wild-type DOZI-expressing female gametes, resulting in the development of ookinetes. By contrast, development of zygotes from *pbdozi*⁻ females fertilized by wild-type males was incomplete and similar to development of zygotes from the *pbdozi*⁻ line. These crosses demonstrate that the block in zygote/ookinete development is essentially due to a lack of DOZI of female gametocyte origin.

The phenotype of the *pbdozi*⁻ parasites might be expected from the predicted function of DOZI in the storage of translationally repressed mRNAs of P28 and P25 in the female gametocyte, and the later, essential, developmentally regulated use of these and other transcripts in postfertilization (zygote and ookinete devel-

Fig. 2. Immunoprecipitation (IP) experiments and localization of the transcripts *p25* and *p28* to complexes containing the DOZI::GFP fusion protein. (A) Western blot analyses of gametocyte lysates from IPs, with anti-GFP, anti-c-myc, or beads only, showed the presence of DOZI::GFP only in the specific (i.e., IP-GFP) fraction. No contamination with the control proteins P47 or eEF1A was observed; both were only present in the input lysate. Asterisk, anti-c-myc; diamond, protein G. (B) Northern blot analysis of RNA recovered from the IPs as shown in (A) identified *p25* and *p28* transcripts in the IP-GFP fraction. The transcripts of the controls *p47*, *eef1a*, and *rna* were not detected. (C) RT-PCR analyses of IP-eluates showed coelution with DOZI::GFP of *p25* and *p28* transcripts, and mRNAs predicted to be translationally repressed, but not of the control RNA *p47*.



opment) events. Northern analysis of mRNA showed not only a nearly complete loss of transcripts of *p25* and *p28* (Fig. 3B), explaining the absence of P28 and P25 (Fig. 3C), but also down-regulation of an additional three transcripts—*warp*, which encodes an ookinete-specific protein, as well as *pb000245.02.0* and *pb000633.00.0* (Fig. 3B), which earlier studies had predicted to be translationally repressed (16)—indicating a wider role of DOZI in mRNA maintenance.

The full extent of the effect of DOZI depletion on steady-state mRNAs of blood-stage, unactivated gametocytes was analyzed with an oligonucleotide microarray that consisted of 5283 *P. berghei* gene models (16, 23). We observed 370 transcripts to be significantly reduced by more than a factor of 2 in *pbdozi*⁻ as compared to wild-type gametocytes (table S1), including seven of nine genes previously shown to be translationally repressed (16). This subset also included groups of genes that ensure successful development of the parasite in the mosquito, e.g., genes linked to ookinete motility and invasion (table S2). Unexpectedly, transcripts of 92 genes were observed that concomitantly increased in abundance, which might reflect transcriptome responses to altered biological processes in the mutant or indicate a more complex role of DOZI in the regulation of mRNA abundance. These observations were confirmed by RT-qPCR analyses of selected transcripts (table S3, fig. S8).

Together, these data indicate that DOZI has a central role in the silencing and maintenance of steady-state levels of a population of gametocyte-specific transcripts. Furthermore, the loss of DOZI apparently severely affected the capacity of the parasite to store and stabilize a discrete subset of mRNAs in the female gametocyte, resulting

in a failure to synthesize specific proteins and to complete normal zygote development.

Translational repression in *Plasmodium* may function to specifically regulate gene expression during meiosis in the zygote. Posttranscriptional regulatory mechanisms of gene expression in *Plasmodium*, such as translational repression and mRNA homeostasis, might play a central role in development, because the annotation of *Plasmodium* genomes indicates a relative scarcity of transcription factors (24). Indeed, the timing of the appearance of proteins from transcripts that undergo TR in gametocytes can be quite different. For example, P25 and P28 are first detected about 2 hours after female gamete activation and fertilization, whereas WARP may only be detected in developing ookinetes 8 hours after fertilization (25).

Until recently, DDX6 RNA helicases were implicated in mRNA storage in translationally silent mRNP complexes and in the transport of mRNA to P-bodies that serve as centers for mRNA degradation. More recently it has been shown that mRNA may be cycled to and from P-bodies in vegetative *S. cerevisiae* in a DDX6 helicase-dependent manner, at least in unicellular eukaryotes. DDX6 helicases are also expressed in germline cells and control the fate of mRNA species that are required for further development of the cell once it has been fertilized. Our data support a role for DOZI in the storage and silencing of mRNA species in *Plasmodium* gametocytes required after gamete fertilization (fig. S9). However, gametocytes in the blood circulation have a short half-life: The overwhelming majority fail to be transmitted to the mosquito and rapidly decay. Decaying gametocytes contain repressed transcripts that if translated, could produce proteins targeted

by transmission-blocking antibodies (10). Consequently, in decaying blood-borne gametocytes, DOZI may be involved in rapid destruction of the stored mRNAs. By contrast, in the activated female gamete in the mosquito, TR is relieved, allowing the coordinated production of proteins essential for the further development of the parasite and establishment of the infection in the mosquito vector.

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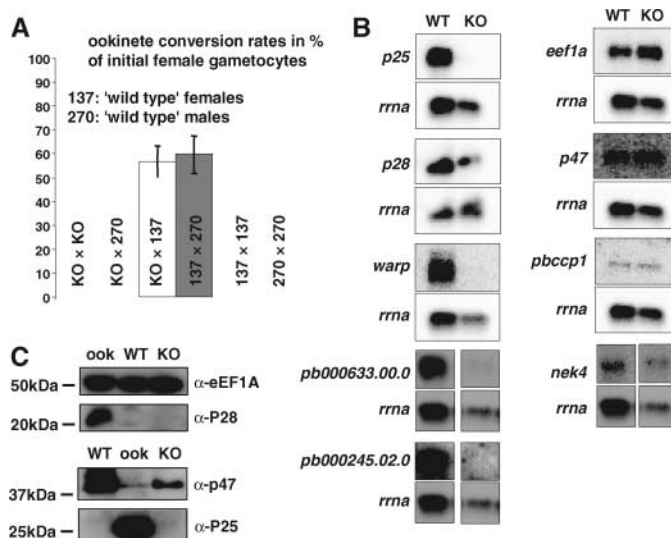
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26. This research was supported by BIOMALPAR Network of Excellence Grant (G.R.M.), The Functional Genomics Initiative of the Wellcome Trust (G.R.M., J.A.M.B.), The Netherlands Organisation for Scientific Research (NWO) Genomics Initiative (S.M.K.), a NSF Graduate Research Fellowship (L.S.G.), and The Johns Hopkins Malaria Research Institute. We thank M. van Dijk for access to pbs47⁻ parasite mutants used in this study, and J. Ramesar for technical assistance. We thank the Johns Hopkins MRI Array Core Facility for providing the microarray equipment. This work has been supported by the NIH—National Institute of Allergy and Infectious Diseases 1R01AI061576-01A1, a Johns Hopkins School of Public Health Faculty Innovation Grant, and the Johns Hopkins Malaria Research Institute. The accession numbers to the array data submitted to the GEO-NCBI (Gene Expression Omnibus—National Center for Biotechnology Information) database (www.ncbi.nlm.nih.gov/projects/geo/) are GSM111868, GSM111871, GSM111872, GSM111873, GSM111874, GSM111875.

Supporting Online Material

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19 January 2006; accepted 7 June 2006
10.1126/science.1125129

Fig. 3. The zygote development of *Pbdozi*⁻ null mutants was completely inhibited because of failure to store transcripts in gametocytes. (A) Analysis of zygote development in standard *in vitro* assays showing the lack of development of *pbdozi*⁻ null zygotes into ookinetes. *Pbdozi*⁻ null female gametes (KO) cross-fertilized with “wild type” males (270) did not develop into ookinetes, whereas “wild type” females (137) cross-fertilized with *pbdozi*⁻ null males (KO) showed normal ookinete development. Data show the mean ± SD.



(B) Northern blot analyses of gametocyte transcripts showed that *p25* and *p28* steady-state levels were substantially reduced in *pbdozi*⁻ null parasites. In addition, we showed transcripts that were also affected (left) or unaffected (right) by the lack of DOZI. (C) Western blot analyses of gametocyte lysates showing the absence of P28 and P25 in both wild-type (WT) and *pbdozi*⁻ null (KO) gametocytes. P28 and P25 were present only in ookinetes (ook). eEF1A and P47 were included as controls.