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Translational regulation during stage transitions in malaria parasites

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Abstract

The complicated life cycle of the malaria parasite involves a vertebrate host and a mosquito vector, and translational regulation plays a prominent role in orchestrating the developmental events in the two transition stages, gametocytes and sporozoites. Translational regulation is executed in both global and transcript-specific manners. *Plasmodium* uses a conserved mechanism involving phosphorylation of eIF2 α to repress global protein synthesis during the latent period of sporozoite development in the mosquito salivary glands. Transcript-specific translational regulation is achieved by a network of RNA-binding proteins (RBPs), among which the Dhh1 RNA helicase DOZI and Puf family RBPs are by far the best studied in *Plasmodium*. While the DOZI complex defines a new P granule with a role in protecting certain gametocyte mRNAs from degradation, the Puf proteins appear to repress expression of mRNAs in both gametocytes and sporozoites. These examples underscore the significance of translational regulation in *Plasmodium* development.

Keywords

Plasmodium; translation; gametocytes; sporozoites; RNA-binding protein

Introduction

The malaria parasite's life cycle involves a vertebrate host and an invertebrate vector. Infection of both is achieved by a specialized parasite stage: gametocytes developed in the blood of vertebrates start the infections in the mosquitoes, while sporozoites in the mosquito salivary gland initiate infections in vertebrates (Fig. 1). Progression through the parasite life cycle is dictated by a highly regulated molecular program. Advances in genomics and genome-wide analysis technologies have contributed to significant advancements in our understanding of the molecular and cellular basis of gene regulation in these parasites. Multiple studies comparing the transcriptome and proteome data in the *Plasmodium* life

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Conflicts of interest

The authors declare no conflicts of interest.

cycle revealed a discrepancy between the relative abundance of mRNAs and the proteins encoded by them, suggestive of extensive global posttranscriptional regulation in the malaria parasite.^{1–3} Recent polysome profiling on the asexual erythrocytic cycle of *P. falciparum*, which compares the steady-state mRNAs and polysome-associated mRNAs as a proxy for actively translated mRNAs, similarly demonstrated that, for more than 30% of the parasite genes, there is a delay in peak transcript abundance in the polysomal fraction as compared to the steady-state mRNA fraction.⁴ Translational control is more obvious in gametocytes and sporozoites (Fig. 1). It has long been recognized that the mRNAs of two transmission-blocking vaccine candidates, P25 and P28, are abundantly present in mature gametocytes, but that the proteins are mostly expressed after transmission to the mosquito in the subsequent zygote and ookinete stages.^{5, 6} Similarly, several of the upregulated in infectious sporozoites (UIS) transcripts are not translated until transmission back to the vertebrate host.⁷ Genome-wide profiling further confirms the presence of many abundant transcripts with no corresponding protein products in the two transition stages.^{8, 9} Here, we aim to provide an update of the knowledge in translational regulation in the malaria parasites with a focus on the two transmission stages: gametocytes and sporozoites.

Conserved protein synthesis pathway and unique features in malaria parasites

The process of translation is highly conserved in eukaryotes and can be divided into initiation, elongation, termination, and ribosome recycling. As most eukaryotic mRNAs have a 5' m⁷GpppN cap structure at the 5' terminus, the cap-dependent translation pathway is the most commonly used in the translation of their mRNAs. During the initiation phase, a preassembled 43S preinitiation complex (containing 40S ribosome subunit, Met-tRNA_i, and eukaryotic initiation factors (eIFs) 1, 1A, 2, 3, and 5) is recruited to the mRNA with the help of the eIF-4F complex, which consists of the cap-binding protein eIF4E, the scaffold protein eIF4G and the DEAD-box RNA helicase eIF4A. This complex then scans the 5' untranslated region (UTR) of the mRNA. Recognition of the first AUG triplet through base pairing with the anticodon in Met-tRNA triggers the hydrolysis of the eIF2-bound GTP, and thus releases the initiation factors and enables the association of the 60S ribosome subunit to form the translation-competent 80S ribosome. Additionally, a cap-independent pathway is employed by many eukaryotic mRNAs by using an internal ribosome entry site (IRES). An IRES element directly recruits the 40S subunits to the vicinity of a downstream AUG, thus bypassing the requirement of a 5' cap. Elongation involves the binding of an aminoacyl-tRNA to the ribosomal A-site, formation of a peptide bond by transfer of the amino acid or peptide attached to the tRNA in the P-site to the aminoacyl-tRNA in the A-site, and translocation of the mRNA with the newly formed peptidyl-tRNA from the A-site to the P-site. When the termination codon enters the A-site, termination factors bind to the ribosome and promote hydrolysis of the peptidyl-tRNA. Ribosome subunits are then recycled for further rounds of protein synthesis. In most cases, initiation is the rate-limiting step of translation and, naturally, is the most common target of regulation.¹⁰

The malaria parasites are predicted to use the conserved eukaryotic protein translation pathway described above, as the translational machinery, including tRNAs, aminoacyl-

tRNA synthetases, ribosomes, and most of the translation factors, is highly conserved in *Plasmodium*.¹¹ *Plasmodium* carries both eukaryotic and prokaryotic translation machineries to suit the needs of its three compartments where active translation occurs: the cytosol, mitochondrion and apicoplast (a non-photosynthetic plastid relic found in most apicomplexan parasites). The latter two compartments have been found to be the drug targets of several antimalarials.¹¹ For example, apicoplast is the target of several antibiotics (e.g., tetracyclines, clindamycin, and macrolides) that act as translation inhibitors.¹² Compartmentalization also requires targeting of the aminoacyl-tRNA-coupling enzymes to different compartments, and recent evidence of bipolar presence of some aminoacyl-tRNA synthetases in both cytosol and apicoplast presents an opportunity for designing inhibitors targeting this essential process.^{13–15} Another interesting characteristic of protein synthesis in malaria parasites is that the nuclear genomes contain a small number of structurally distinct rRNA units that are transcribed in a stage-specific manner.^{16,17} These rRNAs are referred to as A-type rRNA, which is expressed in the asexual blood stages and liver stages, and O-type and S-type rRNAs, which are associated with the sporogonic development.¹⁸ Since rRNA conformation and dynamics are inextricably linked to the function of the ribosome, controlling the expression of different rRNA genes could allow the parasite to regulate ribosomes and translation efficiency in response to changing environments in different hosts. Whereas the two rRNA units expressed during sporogony of *P. bergheri* appear to be functionally equivalent and possibly confer a gene-dosage effect,¹⁹ the GTPase sites of the *P. falciparum* A- and S-type large subunit (LSU) rRNAs have been found to drastically affect the growth of transformed yeast expressing chimeric LSU molecules.²⁰

While bioinformatic analysis predicts the conservation of the translation machinery in the malaria parasite, some ribosomal proteins have been found to play other roles besides protein synthesis. The ribosomal proteins P0 and P2 are both components of the 60S ribosomal subunit. P0 has been found to also have surface expression on merozoites, and antibodies against P0 inhibit erythrocyte invasion in both *P. falciparum* and *P. yoelii*.²¹ Additionally, PfP2 is exported to the surface of infected erythrocytes for 6–8 h during early schizogony, starting at 26–28 h after merozoite invasion.^{22,23} The surface-exposed PfP2 is present mostly as SDS-resistant homotetramers, and export is concomitant with extensive oligomerization of the protein. Antibodies against PfP2 cause the parasite to arrest development at the first nuclear division, likely through interference with lipid import in infected erythrocytes.²³ These proteins appear to have important, unexpected functions in parasite development in addition to their roles in protein translation.

Mechanisms of translational regulation in *Plasmodium* development

Compared to transcriptional regulation, translational control of existing mRNAs allows the cell to more rapidly respond to external stimuli. After transcription, mature mRNAs are exported from the nucleus to the cytoplasm for translation into proteins. In the cytoplasm, mRNAs are regulated to specify the time, amount, and duration of protein production by factors that affect the localization, stability, and translation efficiency of mRNAs.²⁴ Translational control is especially critical during early development of metazoans, as early embryonic development relies on the stockpiled maternal mRNAs, owing to the absence of transcription. Normal developmental programs thus require that the translation of several of

these accumulated mRNAs must be efficiently blocked during their synthesis and deposition, but later become activated in response to fertilization.²⁵ In malaria parasites, the gametocytes in the vertebrate blood and sporozoites in the salivary glands of mosquitoes are analogous to the egg of a metazoan (Fig. 1). These transition stages must remain quiescent for a very long period of time in the host or vector where they formed. For instance, *P. falciparum* gametocytes can be present in patients for more than a month after the clearance of the asexual blood-stage parasites.²⁶ These transition stages store translationally repressed mRNAs to be used for subsequent development.^{9,27,28} Similarly, sporozoites can remain infectious for over 2 weeks while residing in the mosquito salivary gland.²⁹ Once the gametocytes are taken up by a mosquito or sporozoites are injected into the vertebrate host, they undergo rapid developmental changes with the activation of translationally repressed mRNAs.

Within eukaryotic mRNAs, most of the known *cis* translational control elements are localized in the UTRs, including 5' UTR length, secondary structure, upstream AUGs (uAUGs), upstream open reading frames (uORFs) and IRESs, all of which affect translational efficiency. Polysomal profiling of *P. falciparum* during asexual erythrocytic development identified more than 400 mRNAs with increased polysomal coverage of the 5' UTR and a subset of mRNAs with greater coverage in the 3' UTR, suggesting that UTRs may also play important roles in translational regulation of temporally expressed genes.⁴ Moreover, many *P. falciparum* genes possess long 5' UTRs,^{30,31} and the length of the 5' UTR may affect translation efficiency, as shown for the 5' UTR of the *phosphoglucosmutase* gene.³¹ Whether these 5' UTRs contain functional IRES elements remains to be experimentally determined. In addition, long 5' UTRs often harbor uAUGs and uORFs, which have important roles in translational control. A good example of a uORF controlling translation efficiency in the malaria parasites is the *var2csa* gene, which encodes the PfEMP1 protein responsible for parasite sequestration to placenta. Each PfEMP1 is encoded by one of the ~ 60 *var* genes in the parasite genome that display mutually exclusive expression patterns in individual parasites. Whereas *var* gene expression is primarily regulated at transcription, the finding of apparent discrepancy between abundant *var2csa* mRNA and sparsely translated VAR2CSA protein suggests that the expression of this gene is also controlled at the translational level.³² In the 5' UTR of the *var2csa* mRNA, a 360 bp uORF mediates translational repression of the *var2csa* mRNA, a process that requires active translation of the uORF.^{33,34} Translational control of the downstream VAR2CSA does not appear to involve the “leaky scanning” or IRES mechanism but uses translation re-initiation. The uORF sequence, the immediate upstream sequence of the uORF, and the inter-cistronic region (both sequence and length) all affect the efficiency of translation re-initiation at the downstream VAR2CSA ORF.³⁴ Translational regulation by the 5' UTRs is further extended to other *var* genes, which are particularly enriched in uAUGs.³⁵ Although these uAUGs have not been confirmed to serve as translation initiation sites (TISs), 5' UTR sequences containing these uAUGs conferred translation repression of the downstream ORF.³⁵ During the ribosome scanning of the 5' UTR, selection and preference of a particular AUG as the TIS depends on the sequence context of the TIS. *Plasmodium* appears to prefer similar sequence context to the Kozak sequence found in higher eukaryotes, and purines at -3 and

+4 positions are essential for efficient translation, as exemplified from the analysis of the *Pfhsp86* gene.³⁶

Translation can be regulated globally or in an mRNA-specific manner. Global control enables the cell to respond to conditions such as stress and nutrient starvation to co-regulate many or most of expressed transcripts. One prominent example of global translation regulation is through phosphorylation of the α subunit of eIF2 during cellular stress conditions. Phosphorylation of eIF2 α at S51 (S59 in PfeIF2 α) converts it to an inhibitor of its guanine nucleotide exchange factor eIF2B. The resulting reduction of eIF2–GTP levels downregulates global translation.³⁷ This pathway is important for development and stage transition in the malaria parasite. The parasite genome encodes three eIF2 α kinases (IK1, IK2, and PK4) that are differentially expressed in development. IK1's function is analogous to GCN2 and regulates stress response to amino acid starvation during asexual development,³⁸ whereas PK4 is needed for global repression of protein synthesis in late schizonts.³⁹ Interestingly, IK2 is predominantly expressed in salivary gland sporozoites, and it controls the latency of sporozoites by inhibiting global protein translation (described in more detail below)⁴⁰ In comparison, mRNA-specific control is mostly achieved through a network of RNA-binding proteins (RBPs) that bind to *cis*-elements in the specific transcripts, which are located most often in the 3' UTRs. These mRNA–protein complexes accumulate in the cytoplasm of both gametocytes and sporozoites as cytoplasmic granules, where the mRNAs are either protected from degradation and translationally repressed or are targeted for degradation.^{28,29} Although mRNA-specific translation control was documented more than two decades ago,^{5,6} the molecular mechanisms are only now beginning to be deciphered.

Translational regulation during vertebrate-to-mosquito transition

The Dhh1 RNA helicase DOZI complex in *Plasmodium* gametocytes

By comparing the transcriptomes and proteomes of *P. berghei* gametocytes and ookinetes, a dozen genes potentially regulated at the translational level were identified, among which ten have orthologues in *P. falciparum*, including P25 and P28.⁹ *In silico* analysis identified a 47-base U-rich motif in the putative 3' UTRs of many of the *P. berghei* translationally repressed genes expressed in gametocytes. Further study of P25, P28, and two other genes, *PBANKA_131860* and *PBANKA_146130* (PF3D7_1248400 and PF3D7_1454900 in *P. falciparum*), in *P. berghei* determined that such motifs in the 3' UTR of P28 and 5' UTR of P25 confer translation repression of the mRNAs, regardless of their positions relative to the coding regions.⁴¹ Interestingly, this 47-base motif is not present in *P. falciparum*, and the *trans*-element(s) that mediates translation repression through the U-rich motifs remains elusive. Knockout (KO) of *DOZI* (development of zygote inhibited), a homologue of the Dhh1 RNA helicase in *P. berghei*, causes a near complete loss of P25 and P28 mRNAs and significant reduction of many other mRNA species in unactivated gametocytes, suggesting a role for DOZI in controlling mRNA stability in the female gametocyte.²⁷ Further characterization of the DOZI complex in *P. berghei* gametocytes identified 16 major factors (Table 1), which define a *Plasmodium* P granule as an ancient mRNA ribonucleoprotein (mRNP).²⁸ Consistent with its role in protecting certain gametocyte mRNAs from

degradation, this mRNP integrates the components commonly found in mammalian stress granules: eIF4E, poly(A)-binding protein (PABP), and translational regulators including homologue of *Drosophila* BRUNO (HoBo), and homologue of Musashi (HoMu), but RNA degradation factors often present in P bodies were not detected. One major component of the DOZI complex, CITH, is a homologue of the worm CAR-I and fly Trailer Hitch, which contains a LSM14 domain and a FDF motif. These structures are known to compete with the enhancer of mRNA decapping 3 (EDC3) for binding to DDX6 helicases in other species. Similar to knocking out DOZI, disruption of CITH resulted in degradation of a large number of putative TR mRNAs, thus providing further confirmation of its role in conferring mRNA stability. Interestingly, although disruptions of DOZI and CITH both cause failures of processes from zygote to ookinete transformation and downregulation of many TR transcripts, there are still certain differences. While *DOZI* KO gametes fertilize normally with appropriate fusion of the male and female nuclei, the parasites fail to complete meiotic replication. In contrast, *CITH* KO gametes also fertilize normally and go through meiotic DNA replication to establish tetraploidy, but they fail to develop into the motile, banana-shaped ookinetes. This phenotype aligns well with a comparison of the up- or downregulated transcripts upon disruptions of DOZI and CITH, which revealed that there are only partially overlapping sets of affected transcripts. Among these are a number of meiosis-associated transcripts that were exclusively depleted in the *DOZI* KO line.²⁸ Biochemical characterization of the recombinant PfDOZI demonstrated that it has RNA-dependent ATPase, RNA-binding and RNA-unwinding activities, and that it interacts with PfeIF4E and inhibits translation *in vitro*.⁴² Studies of *Toxoplasma gondii* homologue of DOZI (TgHoDI) demonstrated its involvement in cytoplasmic mRNA granules in tachyzoites and functional complementation of the *dhh1* mutants in yeast, indicating functional conservation.⁴³

The role of Puf proteins in translational repression in *Plasmodium*

The Puf family RBPs, named after the Pumilio in *Drosophila* and fem-3 binding factor in *Caenorhabditis elegans*, are ubiquitously present throughout all eukaryotic branches.⁴⁴ Puf proteins are defined by a conserved RNA-binding domain, referred to as the Pumilio homology domain (PUM-HD), which contains eight α -helical repeats of ~ 38 amino acids and folds into an arc shape. Pufs use the concave surface to bind the 8–9 nucleotide (nt) Puf-binding element (PBE) in target mRNAs, which contain the core sequence UGU. Bioinformatic searches for canonical PBEs in Puf-associated mRNAs in yeast, *Drosophila*, *C. elegans*, and humans have identified that the overwhelming majority of putative PBEs are located in the 3' UTRs, lending support to a paradigm of Puf regulation through PBEs in the 3' UTRs. The recent identification of a functional PBE in the 5' UTR of a *P. falciparum* transcript suggests a revision to extend Puf regulation through 5' UTRs.⁴⁵ Puf proteins interact with and recruit additional factors and typically lead to translation repression, deadenylation, and/or mRNA decay, or, in some cases, translation activation. These combined interactions can ultimately initiate and/or accelerate the turnover of target mRNAs.

Two Puf family proteins (named Puf1 and Puf2) are expressed in malaria gametocytes and sporozoites stages.^{46,47} Functional studies of Puf1 did not reveal noticeable phenotypic

changes in the development of the rodent parasite *P. berghei*.^{48,49} Puf2, however, appears to play important roles in both gametocyte and sporozoite stages, and functions of Puf2 conform to the presumptive ancestral roles of the Puf families in promoting proliferation (asexual replication) and suppressing differentiation (sexual development). *Puf2* KO in both *P. falciparum* and *P. berghei* promotes differentiation of the sexual stages and a switch to the production of more male gametocytes.^{48,50} Analysis of gene expression by microarrays showed that *PfPuf2* KO results in a great disturbance of transcript abundance in gametocytes. It is noteworthy that almost all homologues of the putative translationally repressed transcripts identified earlier in *P. berghei*, including *P25* and *P28*, were upregulated in the *PfPuf2* KO line, indicating that Puf2 disruption causes the loss of translation regulation to a number of transcripts in gametocytes.⁴⁵ Moreover, *P25* and *P28* were expressed at much higher levels in the *PfPuf2* KO line, indicating greater access of these mRNAs to the translational machinery. Interestingly, the 118 upregulated transcripts in stage V gametocytes in the *PfPuf2* KO line only had 11 and 8 common transcripts overlapping with the 370 and 183 transcripts with more than twofold downregulation in the *PbDOZI* and *PbCITH* KO lines, respectively,²⁸ among which are seven translationally repressed transcripts. This indicates that the DOZI complex and PfPuf2 control substantially different sets of mRNA populations to accomplish similar effects. While PfPuf2 mediates translation repression using a conserved mechanism requiring binding to the PBEs, it is remarkable that the recombinant PfPuf2 could even bestow translation repression in an evolutionarily distant reconstituted translation system.⁴⁵ Also noteworthy is the identification of a functional PBE present in the 5' UTR of *pfs25*, albeit the extent of Puf regulation through 5' UTR needs further evaluation in *Plasmodium*.

Translational regulation during mosquito-to-vertebrate transition

Similarly, *Plasmodium* uses both general transcriptional regulators and mRNA-binding proteins to regulate protein translation in sporozoites as they await transmission from the mosquito vector to the vertebrate host. Studies by several laboratories have identified a group of UIS mRNAs in salivary gland sporozoites, which are much less abundant in the less infectious sporozoites derived from mosquito midguts.^{51,52} Further investigations of this group of transcripts revealed that many are partially or totally translationally repressed until after parasites are transmitted and productively infect a host hepatocyte (e.g., UIS3, UIS4).^{7,8,53,54} Moreover, several other UIS genes encode proteins that regulate translational regulation in sporozoites, such as UIS1 (IK2) and Puf2. Taken together, the sporozoite's investment in producing and maintaining translational regulation consumes a significant portion of the parasite's activities before transmission.

Much of the work in understanding translational regulation in sporozoites has come from several recent studies on the Puf2 protein in the rodent-infectious species *P. yoelii* and *P. berghei*.^{29,48,49} Genetic disruption of Puf2 led to the progressive loss of sporozoite infectivity while it remained in the mosquito salivary gland, and ultimately led to the premature dedifferentiation of the sporozoites into a liver trophozoite-like form. Owing to the shorter time required for sporozoites to develop and invade the mosquito salivary glands with *P. yoelii* compared to *P. berghei* (14 days versus 18 days), it was observed that the *pypuf2*⁻ parasites instead resembled wild-type parasites on day 14.²⁹ Microarray and RNA

sequencing analyses of the *Puf2* KO sporozoites revealed that the transcript abundance of several hundred transcripts was substantially increased and decreased, even in fully infectious day-14 *pypuf2*[−] parasites. This suggests a role for Puf2 earlier in sporozoite development, which when disrupted causes global deregulation of key regulatory processes and ultimately leads to the loss of infectivity during prolonged salivary gland residence times. Interestingly, both increases and decreases in the transcript abundances of specific mRNAs were observed in these parasites, indicating a potentially multifaceted role for Puf2 in protecting some transcripts while targeting others for degradation. While there is likely therapeutic potential in manipulating Puf2's functions, the important work of demonstrating the role of Puf2 in human infectious parasites remains. In addition, many other RBPs are present in sporozoites that may play similar or complementary roles to provide specificity for mRNA binding or to act as effector proteins to silence, protect, or degrade the selected targets.

In addition to the role of mRNA-binding proteins to translationally regulate specific mRNAs, the eIK2 serine/threonine kinase (previously identified as UIS1) was found to be a more global regulator of sporozoite translation through its phosphorylation of the α subunit of eIF2. ⁴⁰ Phosphorylated eIF2 α was observed in cytoplasmic foci in salivary gland sporozoites, although these foci did not colocalize with the discrete Puf2 foci also present in these parasites. *PbeIK2* KO leads to the partial loss of some sporozoite functions (e.g., gliding motility, infectivity) and the loss of eIF2 α phosphorylation. Release from any translational repression caused by the subsequent dephosphorylation of eIF2 α is likely to occur after sporozoite transmission to the new vertebrate host. However, the phosphatase(s) responsible for this critical event has yet to be identified.

Future perspectives

It is apparent that *Plasmodium* uses sophisticated ways to regulate protein expression during its life cycle. Translational control, both global and mRNA specific, regulates many of the developmental steps, especially during stage transitions. While phosphorylation of eIF2 α is a conserved mechanism that the parasites employ to shut down global protein synthesis during the quiescent stages (gametocytes, sporozoites and perhaps in the dormant liver forms—hypnozoites), the parasites have a large array of RBPs, which potentially regulate the translation of specific mRNAs. In addition, the presence of alternative splicing sites in *P. falciparum*, with many occurring in the UTRs, may also have a significant impact on protein translation.^{55,56} Further functional studies on the translational regulators and the large repertoire of mRNAs they control will provide more in-depth understanding of translational regulation in *Plasmodium* and shed light on how they control the developmental process.

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Figure 1.

Illustration of the life cycle of the malaria parasite. In the mammalian host, a slender sporozoite initiates infection of a hepatocyte and stretches it fourfold as the parasite develops into a liver-stage schizont, which releases thousands of merozoites to start the asexual erythrocytic cycle. A small proportion of the parasites exit the asexual developmental cycle and differentiate into male and female gametocytes, which circulate in the blood of the vertebrate host. Upon ingestion by a female mosquito vector, the gametocytes form male and female gametes, which mate to produce a zygote. The sporogonic development of the parasite in oocysts on the mosquito midgut eventually produces thousands of sporozoites, which enter the salivary glands and there wait to be injected into the next mammalian host. The quiescent transition stages, gametocytes and sporozoites, store a large number of translationally repressed mRNAs, which are used in subsequent developmental stages. These mRNAs are regulated by many RBPs such as Puf2, DOZI, and CITH.

Table 1

Components of an evolutionarily conserved DOZI complex identified in *P. berghei* expressed at the gametocyte stage.

Protein name	Gene ID in <i>P. berghei</i>	Gene ID in <i>P. falciparum</i>
Conserved <i>Plasmodium</i> protein, unknown function	PBANKA_111520	PF3D7_0515600
Conserved <i>Plasmodium</i> protein, unknown function	PBANKA_121470	PF3D7_0323800
Phosphoglycerate mutase	PBANKA_040840	PF3D7_0310300
Poly(A)-binding protein PABP	PBANKA_143920	PF3D7_1224300
Lsm14 domain protein CITH	PBANKA_130130	PF3D7_1474900
DEAD-box RNA helicase DOZI	PBANKA_121770	PF3D7_0320800
Conserved <i>Plasmodium</i> protein, unknown function	PBANKA_070470	PF3D7_0825900
Homologue of BRUNO HoBo	PBANKA_103270	PF3D7_1409800
Conserved <i>Plasmodium</i> protein, unknown function	PBANKA_143520	PF3D7_1220000
Homologue of RNA-binding protein Musashi HoMu	PBANKA_081770	PF3D7_0916700
eIF4E	PBANKA_041280	PF3D7_0315100
Enolase	PBANKA_121430	PF3D7_1015900
DNA/RNA-binding protein Alba 1 (ALBA1)	PBANKA_142330	PF3D7_0814200
DNA/RNA-binding protein Alba 2 (ALBA2)	PBANKA_135920	PF3D7_1346300
DNA/RNA-binding protein Alba 3 (ALBA3)	PBANKA_120440	PF3D7_1006200
DNA/RNA-binding protein Alba 4 (ALBA4)	PBANKA_136030	PF3D7_1347500