Translational regulation in blood stages of the malaria parasite *Plasmodium spp.*: systems-wide studies pave the way

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The malaria parasite *Plasmodium spp.* varies the expression profile of its genes depending on the host it resides in and its developmental stage. Virtually all messenger RNA (mRNA) is expressed in a monocistronic manner, with transcriptional activation regulated at the epigenetic level and by specialized transcription factors. Furthermore, recent systems-wide studies have identified distinct mechanisms of post-transcriptional and translational control at various points of the parasite lifecycle. Taken together, it is evident that 'just-in-time' transcription and translation strategies coexist and coordinate protein expression during *Plasmodium* development, some of which we review here. In particular, we discuss global and specific mechanisms that control protein translation in blood stages of the human malaria parasite *Plasmodium falciparum*, once a cytoplasmic mRNA has been generated, and its crosstalk with mRNA decay and storage. We also focus on the widespread translational delay observed during the 48-hour blood stage lifecycle of *P. falciparum*—for over 30% of transcribed genes, including virulence factors required to invade erythrocytes—and its regulation by *cis*-elements in the mRNA, RNA-processing enzymes and RNA-binding proteins; the first-characterized amongst these are the DNA- and RNA-binding Alba proteins. More generally, we conclude that translational regulation is an emerging research field in malaria parasites and propose that its elucidation will not only shed light on the complex developmental program of this parasite, but may also reveal mechanisms contributing to drug resistance and define new targets for malaria intervention strategies. © 2016 The Authors. *WIREs RNA* published by Wiley Periodicals, Inc.
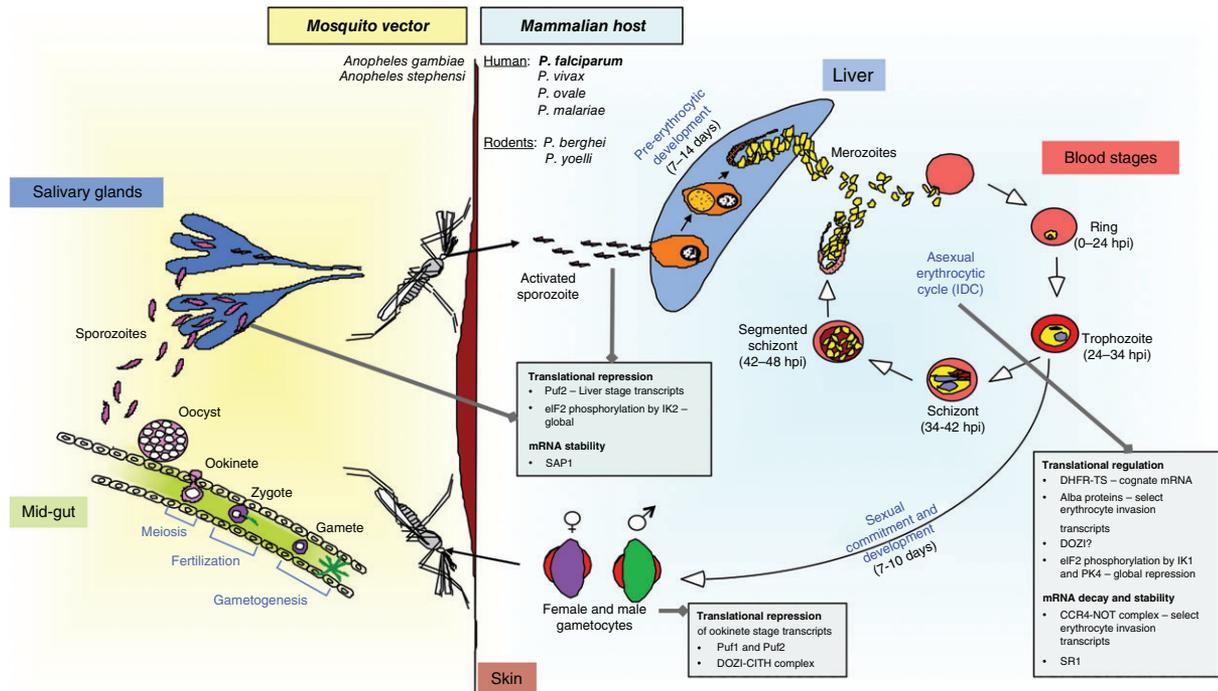
INTRODUCTION

Plasmodium spp., the causative agents of malaria, are unicellular protozoan parasites belonging to the phylum Apicomplexa that use a multi-stage developmental program to transition between their mammalian host and mosquito vector: while replicative stages in the host are strictly haploid and intra-

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cellular, cell division in the vector occurs in an extracellular milieu, within a cyst-like structure. Each developmental stage (Figure 1) has a distinct morphology and physiology that is determined by its gene expression profile, as revealed by stage-specific transcriptomic and proteomic analyses of human and rodent malaria parasites.[1–35] The best studied amongst these is the 48-hour intraerythrocytic developmental cycle (IDC) of the lethal human malaria parasite, *Plasmodium falciparum*, when a single parasite replicates within mature erythrocytes to produce 8–32 daughter cells (i.e., merozoites; Figure 1). Indeed, systems-wide studies have shown that the *P. falciparum* IDC is characterized by a cyclic pattern

FIGURE 1 | Lifecycle of *Plasmodium spp.* parasites in the mammalian host and mosquito vector. The timing of different stages of *P. falciparum* in the human host is indicated. Instances of translational regulation and mRNA decay are also indicated. Note that the monkey malaria parasite *P. knowlesi* has recently been identified to infect humans.

of steady-state messenger RNA (mRNA) expression, with more than 75% of the genes attaining peak mRNA levels at only one time-point of the 48-h cycle.[3,11,19] Moreover, for approximately 30% of genes, a delay is observed between peak mRNA and protein levels;[11,21] in select cases, this corresponds to a delay in ribosome association.[5,6]

Research in the past decade has shown that gene expression in malaria parasites is governed by epigenetic, transcriptional, post-transcriptional, and post-translational regulatory mechanisms.[36–40] Because protein production is the primary outcome of gene expression, mRNA translation, in particular, is coordinated in time and space to fine-tune protein levels and respond to a variety of environmental cues:[41] in *Plasmodium spp.*, this occurs not just during the IDC, but also facilitates developmental stage transitions. The prevalence of post-transcriptional control is also supported by the apparent lack of specialized transcription factors encoded by the parasite genome, relative to genes encoding RNA-binding and -regulatory proteins.[42–44]

Similar to other eukaryotes, the life of a *Plasmodium spp.* mRNA begins in the nucleus, when the transcriptional machinery is recruited to the promoter of a gene to synthesize pre-mRNA (or nascent mRNA) that corresponds to the 5′-untranslated region (UTR), coding sequence (CDS), introns, and 3′UTR of the gene (Figure 2). Regulatory events include the synthesis of cryptic RNAs from cognate promoters of select *P. falciparum* genes, which may be cotranscriptionally degraded by exoribonucleases;[45] antisense transcription from a downstream promoter[27,46] (Figure 2); transcription from intronic promoters for select *P. falciparum* virulence gene families;[47,48] and stage-dependent regulation of specific genes by the 27-member specialized transcription factor family ApiAP2 (Apicomplexan Aptela-2).[49] Subsequent processing of the pre-mRNA via splicing (for intron-containing genes), accompanied by the addition of the m⁷GpppN cap structure to the 5′ end and the poly(A) tail to the 3′ end, results in a mature mRNA that is competent for cytoplasmic export (Figure 2). In the cytoplasm, the mature mRNA faces three fates: (1) decoding of its message by the 80S ribosome and tRNAs charged with amino acids to synthesize specific polypeptides, i.e., translation, (2) degradation by exo- or endo-ribonucleases, i.e., decay, and (3) sequestration within specific ribonucleoprotein (RNP) complexes to inhibit translation and/or decay, i.e., repression (Figure 2). The equilibrium amongst these processes is a key determinant of
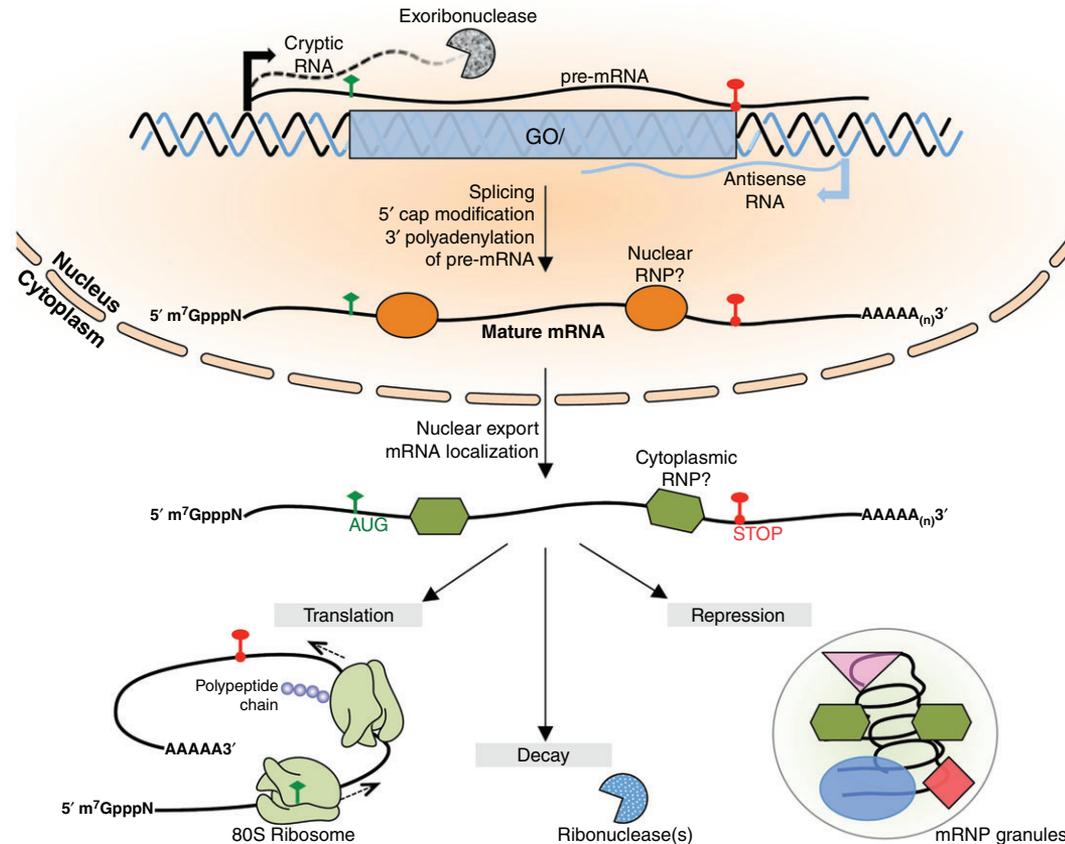
FIGURE 2 | Stages of mRNA maturation and cytoplasmic outcomes. Once a pre-mRNA is transcribed in the nucleus from a given *GOI* (gene of interest), it is co- and post-transcriptionally processed by splicing, 5′ cap modification, and 3′ polyadenylation to yield a mature mRNA. For select *GOIs* in *Plasmodium falciparum*, so-called cryptic RNAs and antisense RNAs have been described in asexual blood stages, which may regulate transcription and/or translation. The mature mRNA is then bound by nuclear ribonucleoprotein (RNP) complexes and transported to the cytoplasm where it faces three outcomes: (1) Translation, mediated by the 80S ribosome to synthesize a polypeptide chain; (2) Decay, mediated by ribonucleases; and (3), Repression, which may occur in the context of mRNA-RNP (mRNP) granules composed of different classes of proteins such as RNA helicases, RNA-binding proteins, and translation-associated factors.

the proteomic signature at any given stage of parasite growth, although the steady-state protein levels also depend on protein degradation rates.

Here, we summarize the current knowledge of translational regulation in malaria parasites, both at the global and mRNA-specific levels, with an emphasis on mechanisms that are prevalent during the *P. falciparum* IDC. We discuss how insights from transcriptome-wide studies of ribosome occupancy and mRNA decay, the identification of *cis*-acting elements in the UTRs of select mRNAs, and the characterization of the mRNA interactomes of *trans*-acting RNA-binding proteins (RBPs), have provided a complex, but as yet incomplete, picture of translational control in this unicellular pathogen. Because translational regulation during *Plasmodium spp.* stage transitions has recently been reviewed elsewhere,[36] we will discuss these findings in brief, where relevant.

CORE TRANSLATION APPARATUS AND ITS REGULATION IN *PLASMODIUM*

The core translation machinery is highly conserved in *Plasmodium spp.* with regard to its repertoire of ribosome components, translation factors, tRNA molecules, and aminoacyl-tRNA synthetases (reviewed in Jackson et al.).[50] However, certain unique features have been noted as the malaria parasite progresses from mammalian development (consisting of liver stages, the IDC, and sexual stages, i.e., gametocytes; Figure 1) to mosquito stages (consisting of gamete, zygote, ookinete, oocyst, and sporozoite stages; Figure 1).

Translation Machinery

In contrast to most eukaryotes, which have ribosomal DNA (rDNA) clusters comprising hundreds of

tandemly repeated units, *Plasmodium* genomes feature 4–8 single-copy rDNA units on different chromosomes: these differ in sequence as well as expression profile during lifecycle progression, and are broadly classified into A-type (liver and blood asexual stage-specific), O-type (ookinete-specific), and S-type (sporozoite-specific) rDNA.[51–59] Moreover, the different rRNAs are functionally divergent. For example, heterologous complementation studies in yeast showed that *P. falciparum* S-Type rRNA were not functionally equivalent to A-Type rRNA.[58] This hinted at the presence of regulatory elements within the different rRNAs that could contribute to translational control, either globally or for a subset of mRNAs. Additionally, in the rodent malaria parasite *P. berghei*, S-type rDNA loci appear to be dispensable for lifecycle completion and only affect parasite fitness,[60] whereas in a second rodent parasite *P. yoelii*, an S-type rRNA gene was associated with oocyst development defect (ODD) by quantitative trait locus analysis.[61] Not only was this phenotype verified by targeted genetic knockout of the rDNA locus, ODD was also observed upon episomal complementation of the mutant strain,[61] emphasizing the importance of tight regulation of rRNA dose and timing of expression. The concept of specialized ribosomes with stage-specific functions is enticing, but due to alterations in rDNA sequence and locus numbers across different *Plasmodium* species, a generalized interpretation requires further research.

Recently, high-resolution cryo-electron microscopy (cryo-EM) reconstructions of the *P. falciparum* blood stage, cytoplasmic 80S ribosome either bound to the antimalarial drug emetine[62] or bound to tRNAs[63] were published. While the core structure was well conserved, some *P. falciparum*-specific differences were identified including large rRNA expansion segments (ESs) at the periphery of the 18s rRNA; these ESs may be targeted by the translational regulation machinery *in vivo* and present as potential targets for drug intervention.[62,63] Additionally, both studies noted the striking absence of PfRACK1 (receptor for activated C kinase protein)[64] from the conserved binding site near the mRNA exit channel; RACK1 has been shown to associate with eukaryotic ribosomes in stoichiometric quantities and is thought to be involved in translational regulation, especially internal ribosomal entry site (IRES)-mediated, cap-independent translation initiation.[65] Absence of RACK1 from the *Plasmodium* blood stage ribosome could either be due to loss during purification or could point to important mechanistic differences in *P. falciparum* translational regulation; of note, PfRACK1 copurified with blood stage polysomes.[5]

Moreover, RACK1-ribosome association may be regulated in a stage- and rRNA-dependent manner.

Another notable aspect is the moonlighting, extra-ribosomal function of *Plasmodium* 60S-ribosomal stalk proteins P0 and P2. P0 has been found on the merozoite and gametocyte surface and has been implicated in the invasion process[66] while P2 is found on the infected erythrocyte surface during early schizogony and is thought to be involved in regulation of nuclear division.[67] Extra-ribosomal functions of eukaryotic ribosomal proteins, such as regulation of ribosome biogenesis and stress sensing, have been previously reported,[68] and it appears that *Plasmodium spp.* ribosomal proteins may also have evolved alternate functions. Overall, the heterogeneity in *Plasmodium* rRNAs, ribosome structure and the function of ribosomal proteins need to be better understood, especially to shed light on translational regulation and parasite development.

Global Regulation of Translation

Translation in eukaryotes can be divided into three phases, initiation, elongation, and termination, all of which are subject to regulation (Figure 3). In *Plasmodium spp.*, mechanistic conservation has been experimentally demonstrated for some of the phases. For example, in model eukaryotes, during cap-dependent translation initiation, the 5' m[7]GpppN cap structure of an mRNA is bound by eIF4E (eukaryotic translation Initiation Factor 4E), which is part of the heterotrimeric translation initiation complex eIF4F consisting of the helicase eIF4A, the scaffold protein eIF4G, and eIF4E[69] (Figure 3). Subsequently, interaction of eIF4F with poly(A)-binding protein (PABP), decorating the 3' poly(A)-tail, results in the formation of a closed-loop mRNA-RNP (mRNP) structure that both promotes efficient translation and deters mRNA decay (Figure 2). The interactions of *P. falciparum* eIF4E,[70,71] eIF4F,[72,73] and PABP[72,73] have been demonstrated *in vitro*. Next, eukaryotic translation begins when the 80S ribosome and the methionine-loaded initiator tRNA are assembled on the start codon with the help of eIF2[69] (Figure 3); *Plasmodium* eIF2 has been annotated, but not functionally characterized. However, phosphorylation of the alpha subunit of eIF2, eIF2α, under conditions of stress, one of the most common modes of global mRNA translational repression in eukaryotes, has been demonstrated in *Plasmodium* (Figure 3).[40] The three eIF2α serine/threonine kinases encoded by the parasite genome, eIK1, eIK2, and PK4, are differentially expressed during its lifecycle and exhibit specialized function for stress response and stage-specific
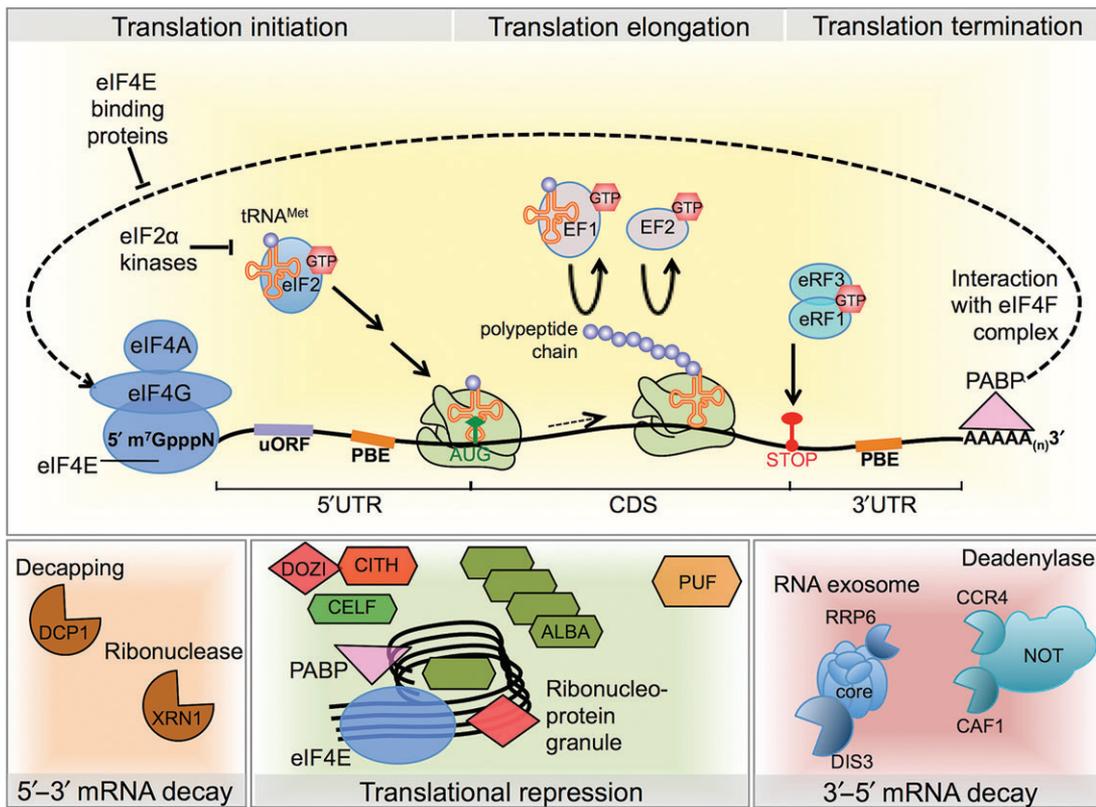
FIGURE 3 | Molecular basis of translational regulation in malaria parasites. Translational regulation in the cytoplasm can occur during the initiation, elongation, and termination phases. This can be co-regulated by factors that mediate decay and repression. eIF, eukaryotic Initiation Factor; tRNA^{met}, tRNA charged with methionine; eEF, eukaryotic Elongation Factor; eRF, eukaryotic Release Factor; eIF4F complex, eIF4E + eIF4G + eIF4A; PABP, Poly(A)-Binding Protein; UTR, Untranslated Region; CDS, Coding Sequence; uORF, upstream Open Reading Frame; PBE, Puf-Binding Element.

repression of translation in sporozoites and schizonts[74–78] (Table 1). Additionally, the translation elongation complex, which contains four subunits EF1α, β, γ, and δ, was purified from *P. falciparum* blood stages under native conditions[79] (Figure 3), although its functionality remains uncharacterized. Given that new modes of regulation of elongation are being discovered in model organisms,[80] and that translational accuracy and efficiency of the AT-rich mRNAs of *P. falciparum* may be determined by ribosome stalling at low complexity regions such as asparagine-encoding AAT tracts,[81] translation elongation in malaria parasites needs to be further elucidated. This is especially important because the GTP-dependent elongation factor PfeEF2 was recently described as a target of the small molecule inhibitor DDD107498[82] (discussed below), although PfeEF2 function in translation elongation has not been demonstrated *in vitro* or *in vivo*. Finally, while eukaryotic peptide chain release factors (eRFs; Figure 3) that facilitate translation termination have been annotated in *Plasmodium spp.* (http://plasmodb.org), their function is not yet characterized.

Translation in the Apicoplast and Mitochondria

There is evidence that, in addition to bulk translation in the cytoplasm, both *Plasmodium* organelles of endosymbiotic origin, the apicoplast and the mitochondrion, are translationally active.[83–85] The apicoplast, which is a nonphotosynthetic remnant of the plastid, is a peculiarity of several organisms belonging to the phylum Apicomplexa and is required for fatty acid and isoprenoid biosynthesis, and parasite survival.[86] The 35 kb apicoplast genome encodes its own set of rRNAs, tRNAs, some ribosomal proteins and the bacterial translation elongation factor EF-Tu,[87] while the highly reduced 6 kb genome of the mitochondrion encodes only three proteins and fragmented rRNAs.[88,89] To achieve functional translation in each organelle, many factors including ribosomal proteins, translation factors, and tRNA synthetases, have to be encoded by the nuclear genome, with some factors shared between the cytoplasm and these organelles.[50,90–92] Furthermore, nuclear-encoded, divergent *P. falciparum* homologues of the bacterial initiation factors IF1, 2, and

TABLE 1 | Molecular Regulators of Translation, Decay, and Repression That Have Been Characterized in *Plasmodium spp.*

Biological Process	Protein/Protein Complex	Species	Lifecycle Stage	Expressed in Asexual Stages?	Remarks	References
Translation	eIF2 α Kinases: IK1, IK2, PK4	*P. falciparum* *P. berghei*	IDC, Sporozoite, Gametocytes	Yes	IK1 is primarily transcribed in asexual blood stages, IK2 in sporozoites and PK4 during the IDC and in gametocytes; IK1 and IK2 are not essential to parasite growth (all stages of development) whereas the *PK4* gene cannot be deleted in blood stages	73–77
	PfDZ50	*P. falciparum*	IDC	Yes	DDX6/Dhh1 RNA helicase and homolog of DOZI; RNA-binding and RNA helicase activities of PfDZ50 have been demonstrated *in vitro* as has its ability to bind to PfeIF4E and repress translation	112
	PfAlba1	*P. falciparum*	IDC	Yes	PfAlba1 is essential to blood stage development; The *in vivo* RNA interactome of PfAlba1 consists of >105 mRNAs, including mRNAs encoding erythrocyte invasion proteins such as Rap1, AMA1, RhopH3 and CDPK1	120,122
mRNA decay	PfCaf1	*P. falciparum*	IDC	Yes	Deadenylase subunit of the CCR4-NOT complex; Of the 1031 mRNAs that are misregulated upon PfCaf1 depletion, mRNAs encoding erythrocyte egress and invasion proteins are over-represented	125
	RNA exosome (RRP6, DIS3)	*P. falciparum*	IDC	Yes	PfRRP6 and PfDIS3 coimmunoprecipitate with the exosome and localize to the nucleus and cytoplasm, respectively, of ring stage parasites	45
Translational repression	PbDOZI-CITH complex	*P. berghei*	Female gametocytes	Yes	PbDOZI and PbCITH localize to the cytoplasm in P-granule-like structures, with the PbDOZI-CITH complex composed of 11 proteins; Targets include ~730 mRNAs, several of which are repressed in sexual stages and translated only in the zygote and ookinete	130–132
	Puf2	*P. falciparum* *P. berghei* *P. yoelii*	Gametocytes, Sporozoites	Yes	PfPuf2 binds to the Puf-binding element in the 5′ and 3′UTRs of two mRNAs, Pfs25 and Pfs28, in gametocytes, and translationally represses them; PbPuf2 and PyPuf2 translationally regulate the UIS mRNAs in sporozoites	138–142
	Bruno/CELF	*P. falciparum* *P. berghei*	IDC, Female gametocytes	Yes	PfCELF1 localizes to punctate structures in the cytoplasm; ~1100 mRNA targets of PfCELF1 have been identified using *in vitro* RNA-binding assays; PbCELF2 associates with the DOZI-CITH complex	132,144
	Alba1, 2 and 3	*P. berghei* *P. falciparum*	Female gametocytes, IDC	Yes	PbAlba1, 2 and 3 associate with the DOZI-CITH complex; PfAlba1, 2 and 3 bind to RNA *in vitro*; The cytoplasmic localization of PfAlba1 and 2 in trophozoites and schizonts is punctate and reminiscent of P-granules	120,132

3 were recently characterized, and specific isoforms localized to either the apicoplast or mitochondrion.[93] Organellar translation is of particular interest, in view of being the predicted target of inhibitors of prokaryotic translation, which are also active against malaria parasites[94–96] (discussed below).

LESSONS FROM SYSTEMS-WIDE STUDIES OF *P. FALCIPARUM* ASEXUAL BLOOD STAGES

The first instance of translational regulation during the *P. falciparum* IDC was observed in 2002 for dihydrofolate reductase–thymidylate synthase (DHFR-TS), a bifunctional enzyme that is essential for parasite growth.[97] Binding of PfDHFR-TS to its cognate mRNA repressed translation,[98] which could be alleviated by antifolate treatment; importantly, such a treatment did not affect steady-state PfDHFR-TS mRNA levels.[99] These findings supplemented previous studies in *P. berghei*, where researchers had reported translational repression in gametocyte stages (discussed below). Nonetheless, the widespread prevalence of translational control in blood stages became evident only in the postgenomic area.

Transcriptomics and Proteomics

The draft genome of *P. falciparum* was first published in 2002[43] and was closely followed by the transcriptome and proteome of several lifecycle stages.[3,9,19] The transcriptome of the 48-h IDC was measured independently by two groups in 2003: Le Roch et al. used 25 base-long oligonucleotide arrays to analyze the transcriptomes of rings [8–16 hours post erythrocyte invasion (hpi)], trophozoites (22–30 hpi), schizonts (36–42 hpi), and merozoites,[19] while Bozdech et al. performed a higher time-resolution study, where they used 70 base-long oligonucleotide arrays to measure the IDC transcriptome at 1-h intervals.[3] Together, it was revealed that at least 3240 of the then-annotated approximately 5400 *P. falciparum* genes (the latest annotation being ~5770 genes) were transcribed during this stage and exhibited a cyclic pattern of expression, with >75% of the mRNAs reaching peak steady-state levels at only one time-point; a study in 2011 extended this analysis to 4670 IDC mRNAs.[11] This resulted in the assignment of a 'Fourier phase' to several genes, which corresponded to peak timing of mRNA expression. Notably, transcripts that encoded proteins belonging to the same biological process such as DNA replication, protein translation (peak mRNA at 8–12 hpi), proteasomal degradation, and so on, were co-expressed, leading to a model of 'just-in-time' transcription, i.e., induction of gene expression occurred only at one specific point of the lifecycle, exactly when protein function was required. Moreover, when the proteome of select lifecycle stages including a few blood stage time-points was measured by Multidimensional Protein Identification Technology (MudPIT)[9] and mass spectrometry (MS),[25] and of the IDC at 2-h intervals by two-dimensional DIfferential Gel Electrophoresis (2D-DIGE),[11] a periodic pattern of protein expression was observed for the IDC. However, this pattern was more discontinuous than the transcriptional cascade, with two distinct breaks at 6–14 and 26–34 hpi, indicative of replenishment/restocking of proteins at these time-points.[11]

Thereafter, when the transcriptomic profiles of seven different *P. falciparum* stages—including four IDC time-points[19]—were compared to their protein profiles, it became evident that there was a delay of 11–18 h between peak mRNA and protein levels for approximately 30% of the 2584 analyzed genes.[21] This hinted at widespread translational regulation, especially during the transition from merozoite to ring stages, and from ring to trophozoite stages,[21] supporting a model of 'just-in-time' translation for several mRNAs. A subsequent study revisited this question, but for a reduced set of genes and at 2-h intervals of the IDC.[11] Using microarrays for transcriptome analysis, and 2D-DIGE followed by MS for proteomic analysis, the steady-state expression patterns of 125 proteins (with 2.9 isoforms per protein on average) were compared to their mRNA profiles. Again, a delay of 11–15 h was observed between peak mRNA and protein levels for a majority of the full-length protein isoforms. However, using a mathematical model, the authors attributed this delay to rates of protein translation (k_{transl}) and degradation (k_{deg}) alone, and ruled out checkpoints between transcription and translation, lending support to the 'just-in-time' transcription model. Nevertheless, given the relatively small number of proteins analyzed in this study and the assumptions of k_{transl} and k_{deg} made by the researchers, it remains unclear if the model can fully explain the widespread translational delay observed during this stage. In general, the genome-wide picture of translation regulation that has emerged from these studies is incomplete and needs to be further elucidated by evaluating the blood stage proteome at high time-resolution, using quantitative proteomic analysis techniques such as SILAC (Stable Isotope Labeling by Amino acids in Cell culture) and iTRAQ (Isobaric Tagging reagents for Relative and Absolute Quantification), both of which have been adapted to *P. falciparum in vitro* culture.[25,100]

Ribosome Occupancy Studies

To globally identify instances of translational regulation, genome-wide approaches that correlate ribosomal occupancy of mRNAs to their steady-state levels have been developed,[101] with the premise that ribosome–mRNA association is an indicator of active protein production. One such study by Bunnik et al. utilized sucrose gradients to isolate polysome-mRNA complexes from three time-points of the *P. falciparum* IDC—0, 18, and 36 hpi—and analyzed protein and RNA composition by MudPIT and directional RNA-seq, respectively.[5] Upon comparing polysome profiles to the trancriptome at each time-point, the authors found that for 1749 mRNAs, peak steady-state levels and polysome association peaks were coincident, while for 738 mRNAs, there was a partial delay, with polysome occupancy sustained into the next time-point, even in the absence of detectable steady-state mRNA levels. Finally, for 1280 mRNAs, there was a marked delay in polysome occupancy (of up to 18 h) indicating that these mRNAs were maintained (or possibly stored) in a translationally inactive state until the time when protein synthesis was required, supporting the model of 'just-in-time' translation. Such a mechanism was especially evident for proteins that are required during or immediately after erythrocyte invasion, and are involved in remodeling the erythrocyte as the parasite establishes its niche.

A second study by Caro et al. utilized ribosome profiling to evaluate the diverse pool of mRNA footprints (28–30 nt long) generated by actively translating 80S ribosomes in five IDC stages—11, 21, 31, and 45 hpi and merozoites (considered as the 2 h time-point); the ribosome footprints were identified by directional RNA-seq.[6] When the ribosome footprint density of 3605 genes was compared to mRNA abundance at each time-point, a strong correlation was apparent for 3110 genes, all of which had a single mRNA peak; this indicated that there was no apparent delay between mRNA production and ribosome association. This was in contrast to the observations by Bunnik et al. and could be partly explained by: (1) the different time-points analyzed in the two studies, and (2) the fact that Bunnik et al. analyzed polysomes (i.e., two or more ribosomes per mRNA), whereas Caro et al. assessed both monosomes and polysomes. Next, the authors calculated the translational efficiency (TE) of each mRNA as: ribosome footprint rpkM/mRNA rpkM; where rpkM is the reads per kilobase of exon model per million sequencing reads mapped; and identified 177 mRNAs with high TE and 124 mRNAs with low TE at each time-point of the IDC tested. These corresponded, respectively, to translationally up- or downregulated mRNAs, although the authors did not rule out translational regulation of mRNAs that did not exhibit extreme TE patterns. Notably, 73 of the 177 mRNAs with elevated TE encode proteins involved in merozoite egress and/or erythrocyte invasion, key parasite virulence factors.

Furthermore, the two studies identified potential *cis*-regulators of translation. Bunnik et al. mapped polysome-associated sequencing reads to the 5′UTR and 3′UTR of select mRNAs as well as to intronic and intergenic sequences: e.g., 409 mRNAs showed twofold higher coverage in the 5′UTR as compared to the CDS. The authors correlated this higher coverage to the presence of upstream Open Reading Frames (uORFs) in the 5′UTR (Figure 3) that could 'trap' the 80S ribosome and inhibit translation of the coding sequence, as has been observed in yeast and mammals;[102] however, they did not further validate this hypothesis. In contrast, Caro et al., who also mapped ribosomal footprints to the 5′UTR of a subset of genes, found that ribosome occupancy in the 5′UTR did not correlate with the presence of predicted uORFs. The exceptions were *var2csa*, which encodes the surface virulence antigen PfEMP1-CSA (Erythrocyte Membrane Protein 1-Chondroitin Sulphate A), and *PF3D7_0531000*, which encodes a conserved *Plasmodium spp.* protein of unknown function. Indeed, it has been demonstrated that the 360-base uORF in the 5′UTR sequence of *var2csa* represses translation of the CDS and that this effect can be transiently reversed *in vitro*.[103–105] An independent bioinformatic study found 22860 uORFs in the −350 to −1 position of 5211 *P. falciparum* genes, with an over-representation in select virulence genes such as *var*.[106] Moreover, the uORFs from the 5′UTR of three genes were able to repress translation of a downstream reporter gene.[106] Overall, it is possible that uORF translation may not only inhibit translation initiation of the CDS, but may also result in protein products such as functional small peptides.[107] Additionally, the presence of ribosomes in the 5′UTR may be dependent on IRESs and other sequences,[108] which remain undescribed in *P. falciparum*, but, if they exist, might have effects on translation elongation and termination. It is also important to note that in the ribosome profiling study by Caro et al.,[6] some of the footprints might have originated from RNP complexes that migrate at the same size as the 80S ribosome in sucrose gradients[109] and should be cautiously interpreted. Overall, systems-wide studies support translational control in blood stages of *P. falciparum*, although the extent of translational delay and its contribution to steady-state protein levels still remains unclear.

mRNA Decay during the IDC

Studies in yeast and mammals have shown that the half-life ($t_{1/2}$) of an mRNA is closely related to its biological role and can be altered in response to different environmental stimuli and during development; this in turn affects translational capacity.[110] Therefore, an in-depth analysis of mRNA decay in *P. falciparum* may shed light on instances of translational regulation. To this end, DeRisi and colleagues measured the $t_{1/2}$ of >4700 *P. falciparum* mRNAs at four time-points—10, 20, 30, and 44 hpi—using Actinomycin-D treatment (to block transcription) and 70-nt microarrays.[111] While mRNA $t_{1/2}$ varied from as little as 1 min to >138 min and was not correlated to abundance or CDS length, the mRNAs of proteins that participate in the same biological pathway exhibited similar patterns of decay, as has been observed in yeast.[112,113] Moreover, for 2744 mRNAs, $t_{1/2}$ increased during the IDC with a mean of 9.5 min at 10 hpi to a mean of 65.4 min at 44 hpi. The lengthening of $t_{1/2}$ during a single developmental cycle appears to be unique to *Plasmodium spp*. One explanation for this may be the phasic expression of mRNA decay components, with most profiles showing peak mRNA abundance at 10 and 20 hpi (http://plasmodb.org), although it is unclear if the corresponding proteins exhibit a similar profile. Finally, the correlation between ribosomal occupancy of an mRNA, rate of translation, and its decay rate remains to be measured, as does the contribution of other regulatory factors, including mRNA sequestration, to maintain this developmentally regulated pattern of decay.

MOLECULAR REGULATORS OF TRANSLATION

In silico analyses of the *Plasmodium* genome have identified conserved RNA metabolism pathways,[38,44] and homologues of the major classes of RNA-binding and -processing proteins;[44] however, experimental validation of these proteins, and their contribution to translational control in *Plasmodium spp*. is still lagging behind. In the following sections, we delineate the few *cis*- and *trans*-acting molecular players that have been characterized thus far.

Translational Regulation: Timing and Ribosome Association

Besides global repression of translation initiation via eIF2α phosphorylation, in higher eukaryotes, translation initiation can also be regulated by eIF4E-binding proteins, which inhibit eIF4E–eIF4G interaction, in turn inhibiting binding of PABP to the eIF4F complex and preventing the cap-dependent loading of the ribosome onto an mRNA (Figure 3).[114] One potential eIF4E-binding protein PfDZ50, a DDX6/DHH1-like RNA helicase, and the homolog of DOZI (Development of Zygote Inhibited; see below), was recently described in *P. falciparum* blood stages[115] (Table 1). Immunofluorescence studies showed that PfDZ50 localized to the cytoplasm during the IDC, though without an obvious pattern. Next, *in vitro* assays demonstrated that recombinant PfDZ50 binds to both DNA and RNA, hydrolyzes ATP, unwinds RNA, and represses translation in a reticulocyte lysate system. Finally, recombinant PfDZ50 interacted with PfeIF4E *in vitro* and this interaction relieved PfDZ50-mediated translational repression, most likely by titrating away PfDZ50 from reticulocyte eIF4E. Nonetheless, the impact of PfDZ50-PfeIF4E interaction *in vivo*, and PfDZ50's mRNA targets are not known. Also, whether signaling pathways such as MAPK (mitogen-activated protein kinase) can regulate translation initiation[116] in *Plasmodium spp*. is an open question.

Alternatively, secondary structure elements or *cis*-acting sequences in the 5′UTR can induce and regulate cap-independent initiation of translation,[108] all of which remain unexplored in *Plasmodium spp*. For example, the 5′UTR of select *var* genes contains a *cis*-element that putatively inhibits the translation of the CDS;[117] whether this element mediates cap-independent translation of a uORF or prevents ribosomal loading at the AUG of the CDS is unclear. Another mode of regulating translation at 5′UTRs was recently reported in *P. falciparum* that infected erythrocytes expressing the sickle-cell variant of hemoglobin, HbS.[118] Lamonte et al. found that the human miRNAs miR-451 and let-7i were overexpressed in HbS-erythrocytes, and that these miRNAs translocated into the parasite cell and were trans-spliced to the 5′ end of the mRNAs encoding essential *P. falciparum* genes. This resulted in translational repression by preventing ribosomal loading and consequently, defects in parasite growth: nevertheless, how widespread this phenomenon of host resistance is, remains to be seen. It is also unclear whether such a regulatory mechanism has any fitness benefits or evolutionary significance for *Plasmodium spp*. given the lack of success in detecting small (<25 nt) parasite-derived RNAs[119] and the absence of a functional RNAi pathway.[120]

Trans-acting regulators of translation include RBPs of the Alba (Acetylation lowers binding affinity) family[121–124] (Figure 3; Table 1); Alba proteins

were initially characterized in archaea as DNA-binding proteins,[121,125] but a divergence of their function in other protozoan parasites such as *Toxoplasma gondii*,[126] *Trypanasoma cruzi*,[127] and *Leishmania infantum*[128] toward RNA regulation has been described as has their role in stress adaptation in plants.[129] In *P. falciparum*, it was recently shown that PfAlba1 binds to a subset of IDC mRNAs including those encoding erythrocyte invasion proteins such as RAP1, AMA1, RhopH3, and CDPK1, and may regulate their association with the ribosome in a stage-dependent manner, thus determining the timing of protein expression.[124] In particular, this transcriptome-wide analysis evidenced the presence of translational RNA regulons in *P. falciparum*, which are most likely composed of mRNAs encoding proteins of the same biological process, e.g., erythrocyte invasion. Given that *Plasmodium spp.* encode up to six Alba family proteins (PfAlba1-6),[44] that the PfAlbas appear to have different cellular localization and DNA/RNA specificity patterns,[122,123] and that PfAlba1-4 coprecipitate with polysomes of blood stages,[5] the roles of these proteins in regulating translation needs to be evaluated in greater detail. Furthermore, the diversification of *Plasmodium* Alba1 and Alba2 by the addition of an RGG domain and of Alba4 by the addition of a membrane-tethering ENTH/VHS module,[122] indicates that these proteins may perform distinct functions. Lastly, the evolutionary proximity of the Albas to RNA-binding components of RNase P/RNase MRP RNP complexes[121] also has implications for the crosstalk between translation regulation and mRNA decay. It is interesting to conjecture that the Alba proteins, which are of archaeal origin, may be master regulators of *Plasmodium* translation.[130]

mRNA Decay and Stabilization

mRNA decay can take place in a 5′–3′ or 3′–5′ manner, and typically involves decapping, removal of the poly(A)-tail, i.e., deadenylation, and degradation by ribonucleases; this is different from nonsense-mediated mRNA decay which is mediated by Upf1 and involved endonucleolytic cleavage.[131] In *Plasmodium spp.*, mRNA decapping enzymes such as DCP1 and DCP2, and members of the CCR4/NOT complex, implicated in transcription initiation and elongation and mRNA deadenylation, are conserved[38,44,111] (Figure 3). Preliminary studies in *P. falciparum* showed that disruption of the deadenylase subunit Caf1 of the CCR4/NOT complex altered gene expression during the IDC, in particular, mistimed expression of proteins involved in erythrocyte invasion and

egress, resulting in a proliferation defect[132] (Table 1). Upon deadenylation, a transcript is vulnerable to 3′–5′ degradation by the RNA exosome, a complex of nine noncatalytic core proteins and associated exoribonucleases (exoRNases) which is involved in RNA processing, quality control and decay, both in the nucleus and cytoplasm.[133,134] Putative homologues of some of the core proteins, as well as the exoRNases DIS3/RRP44 (exoRNaseII-related family) and RRP6 (RNase D family) are found in the *Plasmodium* genome,[38,44,45,111] but the exact composition of the complex still remains undefined (Figure 3). Using coimmunoprecipitation assays, Zhang et al. demonstrated that PfDis3 and PfRRP6 associated with the exosome core, and using immunofluorescence assays, localized PfDIS3 and PfRRP6 to the cytoplasm and the nucleus, respectively, of ring stage parasites[45] (Table 1). Furthermore, they showed that PfRNaseII, a noncanonical exoribonuclease, post-transcriptionally regulates ~200 genes—including a subset of *var* genes and noncoding RNAs (ncRNAs)—in an RNA exosome-independent fashion, and identified, for the first time, the presence of cryptic RNAs in the malaria parasite. Nonetheless, the regulation of mRNA decay and its correlation to translation is not well understood in *Plasmodium spp.*

Besides mRNA decay components, other *trans*-acting factors may regulate mRNA stability. These include splicing regulators such as *P. falciparum* SR1 (Serine/Arginine-rich protein 1), which shows both nuclear and cytoplasmic localization.[135] Indeed, it was recently shown that PfSR1 binds to 64 transcripts via two purine-rich RNA motifs, similar to its human counterpart SRSF1, and that PfSR1 overexpression resulted in the early detection of gametocyte-specific intron-less transcripts.[136] However, the molecular basis of PfSR1-mediated post-transcriptional regulation is not known. Also, it remains to be seen whether the other splicing factors encoded by the parasite genome perform similar post-transcriptional functions, as has been observed for several mammalian SR proteins.[137]

Translational Repression

The presence of translational repression in malaria parasites was first evidenced by the analysis of a *P. berghei* surface antigen P28 (previously called Pbs21). While *p28* mRNA was abundantly transcribed in mature gametocytes in the host, the protein was only expressed after transmission to mosquitoes, in zygotes and ookinetes.[138,139] Subsequent studies identified a similar regulatory pattern for another *P. berghei* mRNA that encodes a mosquito-stage

surface antigen P25 and 7 other mRNAs.[14] Thereafter, Waters, Mair, and colleagues described PbDOZI, and its interacting partner CITH (CAR-I/Trailer Hitch Homolog) as central regulators of translational repression in *P. berghei* female gametocytes[140–142] (Figure 3; Table 1). PbDOZI/CITH-containing mRNP complexes appeared as punctate P granule-like structures in the cytoplasm and bound to 731 transcripts (488 for PbDOZI and 551 for PbCITH, with an overlap of 154) including *p25* and *p28*; 211 of these were downregulated in gametocytes upon PbDOZI/CITH depletion.[140–142] The authors also determined the composition of PbDOZI-CITH-containing mRNA-protein (mRNP) complexes using tandem affinity purification and identified RBPs such as PbBRUNO/CELF and PbAlbas, and translation factors such as PbeIF4E and PbPABP, as components of the complex; notably, the complex did not contain RNases.[142] Taken together, these studies validated the presence of a so-called 'maternal mRNA repressome' in *P. berghei*, which is coordinated by PbDOZI-CITH at the level of mRNA stability and translational repression and is essential for developmental stage transitions. The question that remains is whether a similar complex exists in *P. falciparum* gametocytes.

In addition to regulation by the PbDOZI-CITH complex, the mRNAs of P25 and P28 are regulated by *cis*-acting elements in the 5′UTR and 3′UTR sequences. A 47-base *cis*-acting regulatory element in the 3′UTR of translationally repressed mRNAs of *P. berghei* gametocytes was first identified by *in silico* analysis.[14] Thereafter, using a GFP reporter system, the impact of the 47-base element on TE was measured in *P. berghei* gametocytes, as was the impact of the 5′ and 3′UTRs of *p25*, *p28* and *pb000245.02.0*, another translationally repressed gametocyte mRNA.[143] This led to the identification of a U-rich *cis*-regulatory motif in the 5′UTR of *p25* that was homologous to the 47-base element, and was necessary and sufficient to confer translational repression. Nonetheless, it remains unclear if the binding of PbDOZI, PbCITH, or other members of the complex to *p25*, *p28*, and so on, depends on these *cis*-elements.

Adding further complexity to translational regulation in sexual stages is the observation that *pfs25* and *pfs28*, *P. falciparum* homologs of *p25* and *p28*, are regulated by the Puf (Pumilio and fem-3 binding factor homolog) family of RBPs[144–147] (Figure 3; Table 1); Puf proteins regulate diverse processes in eukaryotes by binding to the 3′UTRs of their mRNA targets and repressing translation and/or promoting degradation.[148] In *P. falciparum*, PfPuf1 and PfPuf2

were shown to be differentially expressed in gametocytes, and to bind *in vitro* to the RNA sequence of the Nanos-responsive element from the *hunchback* mRNA of *Drosophila melanogaster*.[144,145] Subsequent analysis demonstrated an essential role for PfPuf2 in repressing gametocytogenesis, in particular male differentiation,[146] and translational repression of *pfs25* and *pfs28* mRNAs via binding to Puf-binding elements (PBEs) in the 5′ and 3′UTRs of its target mRNAs[147] (Figure 2). Simultaneously, several groups assessed the contribution of *P. berghei* and *P. yoelli* Puf proteins to translational repression and parasite development.[149–153] A key conclusion from these studies was that Puf2 binds to and inhibits the translation of UIS (Upregulated in Infectious Sporozoites) mRNAs in sporozoite stages, with the proteins being translated only after liver cell invasion. Notably, the UIS mRNAs are also post-transcriptionally regulated at the level of stability by sporozoite asparagine-rich protein 1 (SAP1).[154]

Although systems-wide studies of *P. falciparum* have hinted at pervasive, temporal translational repression during the IDC, other than PfAlba1, molecular regulators of translation are poorly described; indeed, the impact of the DOZI-CITH complex and Puf proteins during this stage in *P. falciparum* is a black box. A putative component of the *P. falciparum* DOZI-CITH complex, Bruno/CELF, was recently characterized[155] (Figure 3 and Table 1). Wongsombat et al. identified two Bruno/CELF homologs in the *P. falciparum* genome, *CELF1* and *CELF2*, and showed that GFP-tagged PfCELF1 localized to both the nucleus and cytoplasm of IDC stages. Next, using an *in vitro* UV crosslinking-based assay, they demonstrated that, PfCELF1, but not PfCELF2, bound to 12-mer RNA sequences. Moreover, using *in vitro* RNA immunoprecipitation-microarray analysis, they found that PfCELF1 bound to 1376 features from 1040 mRNAs, including the *CELF1* mRNA, while PfCELF2 bound to 26 features from 22 mRNAs. Bioinformatic analysis of PfCELF1's mRNA targets led to the identification of a UG-rich motif that the authors predict is the binding site of this protein. One of the proposed functions of PfCELF1 was the regulation of splicing, although it was not validated. Moreover, the contribution of the 180-odd *Plasmodium* RBPs[44] to the translational delay observed in blood stages, which could be partially achieved by the storage of mRNAs in structures similar to the PbDOZI-CITH-containing maternal granules, is not explored. Again, the PfAlba proteins and PfCELF1 are front-runners for such a storage mechanism given their punctate localization in the cytoplasm of

trophozoite and schizont stages,[122,155] which is reminiscent of P-granules (Table 1). Overall, the mechanism of translational repression in malaria parasites is complex, but as yet in its nascent stages of clarification. Future work could focus on specific sets of mRNAs that are subject to translational control at different stages of the parasite lifecycle as well as RBPs that exhibit features similar to P-granule components.[44]

TRANSLATION INHIBITORS AS DRUGS

Translation is an essential, conserved process: therefore, inhibitor design inherently faces the challenge of selectivity for the parasite over host enzymes, which is not easily predicted. Nevertheless, antimalarial activities of antibiotics like chloramphenicol and tetracyclines that inhibit prokaryotic translation were described more than 60 years ago.[156–159] These antibiotics were thereafter shown to target the translation machinery of the apicoplast; as described above, this organelle was obtained by secondary endosymbiosis and, like the mitochondrion, possesses a prokaryotic-type 70S ribosome, albeit with a divergent composition. Indeed, *in silico* analysis revealed that the *Plasmodium spp.* apicoplast and mitochondrial ribosomes have a reduced number of ribosomal proteins with differences in the rRNA sequence and structure.[95] Furthermore, the effect of disrupting apicoplast translation on parasite proliferation is consistently observed only in the second cycle, after cell division and reinvasion, and has been termed the 'delayed death phenotype' (i.e., the effect of the antibiotic against apicoplast translation and/or transcription begins in the parental generation, and accumulates in the progeny, leading to the irreversible loss of apicoplast function and parasite death). This presents a problem with regards to the therapeutic potential of these antibiotics and is a much debated intervention strategy.[160] However, apicoplast-targeted aminoacyl-tRNA synthetases (aaRSs), enzymes that couple cognate tRNAs with amino acids, have attracted attention as therapeutic targets and specific inhibitors against apicoplastic isoleucyl-tRNA synthetase[161] and lysyl-tRNA synthetase[162] have been described.

In recent years, the cytoplasmic translation apparatus has also emerged as a potential target for antimalarials. Firstly, small molecule inhibitors of cytoplasmic aaRSs have been characterized. One such molecule, Cladosporin, was identified in a cell-based high throughput screen of natural compounds against both liver and blood stage proliferation of *P. falciparum*, and was demonstrated to block *de novo* protein synthesis by directly targeting cytoplasmic lysyl tRNA syntethase (KRS).[163] *In silico* docking studies revealed differences in the ATP-binding pocket between the parasite and human enzymes as the cause of Cladosporin's 100-fold higher cytotoxicity against the parasite as compared to human cells.[163] Another drug, borrelidin, was shown to be active against threonyl-tRNA synthetase from bacteria, *Plasmodium spp.*, yeast and humans; in *Plasmodium spp.*, threonyl-tRNA synthetase is shared between the cytoplasm and apicoplast.[92,164] In a separate study, analogues of borrelidin were identified that show higher selectivity and lower cytotoxicity against human cells[165] compared to the original molecule.[166] A further screen of a library of borrelidin analogs identified two compounds with improved features that were able to clear parasites from *P. yoelii* infected mice.[167] Secondly, small molecule inhibitors of ribosome-associated factors have been described. In 2015, Baragan et al. identified PfeEF2 as the target of the antimalarial compound, DDD107498.[82] DDD107498, which is not toxic to mammalian cells and is now in the advanced nonclinical development stage as a therapeutic, inhibited *de novo* protein synthesis and displayed antimalarial activity against multiple lifecycle stages of *P. falciparum*. Therefore, it is tempting to speculate that the inhibition of protein synthesis indeed presents as an effective intervention strategy for attaining multi-stage activity against malaria parasites.

CONCLUSIONS AND FUTURE PERSPECTIVES

The discovery that steady-state mRNA levels do not strictly correlate to steady-state protein levels is not unprecedented and different models have been proposed to describe how eukaryotic mRNA-protein equilibrium is maintained.[168,169] In malaria parasites, the steady-state transcriptome and proteome of different developmental stages, each of which has distinct morphological and physiological properties, appears to be maintained by 'just-in-time' transcriptional and translational regulatory mechanisms. This results in phenotypes such as the transcriptional cascade of the 48-h *P. falciparum* IDC, translational delay to fine-tune protein expression, and translational repression of maternal mRNAs in sexual stages and UIS mRNAs in sporozoite stages. All of these phenomena are likely regulated by *Plasmodium* DNA-binding proteins and/or RBPs and their cofactors. In recent years, one such class of proteins,

the Albas, has emerged as an important regulator of translation in blood stages and potentially, in sexual stages. Therefore, in-depth analysis of *Plasmodium* RBPs will be key to gain further insights into translational control mechanisms in this parasite. We anticipate that the utilization of CRISPR/Cas9-based genome editing techniques,[170] and in the future, CRISPR-based mRNA degradation techniques,[171] will expedite this process. Alternately, *cis*-regulatory sequences can be bioinformatically predicted in the 5′ and 3′UTRs of co-regulated sets of mRNAs and tested for their contribution to translational regulation; of note, Caro et al. described the 5′ and 3′UTRs of 3569 *P. falciparum* IDC mRNAs (average length of 607–1140 nt and 518–622 nt, respectively).[6] Additionally, given that more than 60% of the annotated *Plasmodium* proteins are of unknown function, several of these may contribute to mRNA-binding and translational regulation as illustrated in a recent study on African trypanosomes.[172]

In general, the field of translational regulation has burgeoned in recent years due to the application of techniques such as ribosome profiling,[109] transcriptome-wide detection of mRNA 'epigenetic' modifications,[173,174] and quantitative high-throughput proteomics,[175,176] to name a few. In particular, RNA modifications have emerged as important biological regulators of tRNA, rRNA, mRNA, and ncRNA function. For example, base modifications in human tRNAs have a direct impact on translation rates and disease,[177] while in bacteria, changes in tRNA modifications regulate the TE of stress response proteins.[178] Furthermore, N6-methyl-adenosine (m6A) modifications in mRNA and long ncRNAs regulate RNA tertiary structure and/or recruitment of m6A-binding proteins.[179] Therefore, future studies could focus on whether *Plasmodium* RNAs are subject to post-transcriptional regulation at the level of base modifications. Indeed, such a mechanism may contribute to various parasite phenotypes that are yet to be explored at the molecular level and may even explain the observation that clinical isolates of *P. falciparum* which are resistant to the drug artemisinin exhibit an extended ring phase transcriptomic profile.[180]

To conclude, we would like to emphasize that there are many outstanding questions in the field of *Plasmodium* translational regulation that need to be resolved. These include: Do blood stages simply use translational regulation as a mechanism to fine-tune gene expression (hence its widespread occurrence), whereas the transition stages between the host and vector—which do not have the same precise timing as asexual blood stages—use translational control to rapidly respond to environmental changes by repressing specific sets of mRNAs? Indeed, does mRNA-specific translational regulation occur at all during the IDC, and if yes, how can these specific events be identified? Moreover, are multivariant gene families such as *var* and erythrocyte invasion components subject to both epigenetic and translational control in order to better adapt to host responses? Do changes in tRNA modifications play a role in parasite development and drug resistance by altering blood stage translation outcomes, as has been observed in human disease states[177]? Given that phosphoproteomic studies have identified post-translational modifications (PTMs; such as phosphorylation) of *Plasmodium* eIF, eEF, and eRF proteins (http://plasmodb.org), do these PTMs modulate their translational regulatory function at different stages of the parasite lifecycle? Is ribosomal profiling combined with proteomics the way forward to identify true instances of translational control? Investigating the answers to these questions will reveal the where, when, what, who, how, and how much, of translational control occurs in malaria parasites and may lead to the establishment of new paradigms in RNA biology. Finally, it is noteworthy to mention that all of the systems-wide studies described here were performed using laboratory-adapted *P. falciparum* strains such as 3D7, HB3, DD2, and W2. While they have highlighted important physiological phenomena, their extrapolation to growth of the parasite within humans is not always straightforward. Therefore, in the future, transcriptomic studies of field isolates should be coupled with proteomic studies to reveal true instances of translational regulation in malaria parasites and their contribution to disease severity.

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