MicroReview

The molecular machinery of translational control in malaria parasites

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Summary

Translational control regulates the levels of protein synthesized from its transcript and is key for the rapid adjustment of gene expression in response to environmental stimuli. The regulation of translation is of special importance for malaria parasites, which pass through a complex life cycle that includes various replication phases in the different organs of the human and mosquito hosts and a sexual reproduction phase in the mosquito midgut. In particular, the quiescent transmission stages rely on translational control to rapidly adapt to the new environment, once they switch over from the human to the mosquito and vice versa. Three control mechanisms are currently proposed in *Plasmodium*, (1) global regulation that acts on the translation initiation complex; (2) mRNA-specific regulation, involving *cis* control elements, mRNA-binding proteins and translational repressors; and (3) induced mRNA decay by the Ccr4-Not and the RNA exosome complex. The main molecules controlling translation are highly conserved in malaria parasites and an increasing number of studies shed light on the interwoven pathways leading to the up or downregulation of protein synthesis in the diverse plasmodial stages. We here highlight recent findings on translational control during life cycle progression of *Plasmodium* and discuss the molecules involved in regulating protein synthesis.

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Introduction

Gene expression in a cell is up or downregulated in response to environmental triggers, such as changes in temperature, pH or nutrient availability. In general, the regulation of gene expression can occur on three levels, the transcriptional, the post-transcriptional and the translational level. In contrast to transcriptional control, the regulation of protein synthesis by post-transcriptional and translational mechanisms enables the cell to rapidly adjust to the environmental changes and protein synthesis can be activated or stopped within minutes after perception of the signal (reviewed in López-Maury et al., 2008).

Rapid responses to environmental changes are particularly important for the protozoan malaria parasite *Plasmodium*. Currently, five human pathogenic malaria parasites are known. These parasites pass through a life cycle that switches between a human and a mosquito host and includes various phases of stage conversions (Fig. 1). Fast successive stage conversions occur during the intrahepatocytic replication phase in the human liver, which takes place over a period of 6–16 days, and the following recurrent intraerythrocytic replication cycles that each last between 1 and 3 days; both replication phases are dependent on the *Plasmodium* species. Another, slower progressing phase of replication occurs in the oocysts, which are attached to the midgut epithelium of the mosquito. The oocyst-specific replication phase requires between 7 and 30 days and in addition to the species dependency is influenced by the outside temperature (reviewed in Antinori et al., 2012).

Other processes of stage conversion are related to sexual reproduction. The sexual phase begins with the formation of sexual precursor cells, the gametocytes, in the human red blood cells in response to host factors (Fig. 1) (e.g. Usui et al., 2019). Following a period of maturation, which lasts between 2 and 10 days and again is species-dependent, mature gametocytes are able to transform into fertile gametes within less than 10 min, once taken up by a blood-feeding mosquito.
Sexual reproduction is initiated in the mosquito midgut, triggered by environmental factors (Fig. 1). The resulting zygote then develops to a motile ookinete during a period of 1 day. The exit of the ookinete from the mosquito midgut has to occur before the peritrophic membrane that protects the midgut epithelium from invaders has fully matured. Hence, the time the ookinete needs to develop is fixed and thus independent from species (reviewed in Dyer and Day, 2000; Talman et al., 2004; Kooij and Matuschewski, 2007; Pradel, 2007; Bennink et al., 2016; Josling et al., 2018). In summary, the asexual replication phases are closely timed and expected to be predominantly mediated by internal triggers, while the sexual commitment and reproduction phase is highly dependent on external signals (Fig. 1).

The plasmodial genome comprises roughly 4,700 genes coding for core proteins as well as a varying number of subtelomeric multigene families, distributed across 14 chromosomes (Otto et al., 2014). Each life cycle stage requires approximately two-thirds of genes for optimal growth, which is proposed to be a consequence of functional optimization caused by genomic reductions during the evolution of *Plasmodium* (Bozdech et al., 2003; Bushell et al., 2017). During intraerythrocytic replication alone, roughly 80% of genes is active; however, for more than 30% of these, a delay in peak transcript and

Fig. 1. Life cycle of *P. falciparum* in the human host and mosquito vector. The various asexual replication phases and the sexual reproduction phase are indicated (blue text box). Stages during life cycle progression particularly dependent on extracellular signals are indicated (grey text box). ECM, extracellular matrix; PL, phospholipid; XA, xanthurenic acid.
protein levels was observed (Bozdech et al., 2003; Bunnik et al., 2013). The mRNA abundance highly fluctuates throughout the development of *Plasmodium*, and varying degrees of transcription and transcript stability have been observed during the plasmodial life cycle (Painter et al., 2017; 2018). The half-life of an mRNA varies from 1 to 138 min, dependent on the metabolic activity and life-span of the respective parasite stage (Shock et al., 2007; Caro et al., 2014). These numbers illustrate that only a small portion of the plasmodial genome may actually be truly specific to a particular developmental stage, while a strong translational control is responsible for the regulation of protein synthesis during life cycle progression of *Plasmodium*. Notably, stages that strongly respond to external triggers and require ‘waiting for the right moment’ will have to be highly dependent on translational regulation to be able to adjust rapidly to environmental changes. Translational control mechanisms would thus particularly affect the transmission stages, i.e. the quiescent gametocytes and salivary gland sporozoites (Fig. 1).

Among the types of translational regulation that are currently known in *Plasmodium*, global regulation targets most mRNAs of the cell at the same time, while mRNA-specific regulation affects only a small number of mRNAs and involves among other cis control elements and mRNA-binding proteins. The third known mechanism of translational regulation is the enzymatically catalyzed mRNA decay by different ribonuclease complexes. The majority of work to date was performed using the human malaria parasite *P. falciparum*, the main causative agent of 445,000 deaths by malaria per year (World Health Organisation, 2018). This review focuses on the diverse mechanisms, the different life cycle stages of malaria parasites employ to regulate translation of cytoplasmic mRNAs. The review further highlights the plasmodial proteins that are part of the translational control machinery.

**Global regulation of translation initiation**

Global regulation of protein synthesis involves mechanisms by which the translation of most mRNAs in a cell is regulated and typically targets initiation, which is the rate-limiting step of translation. The block of translation initiation mainly occurs by the modification of translation initiation factors (termed eukaryotic initiation factors (eIFs)) and is generally achieved by changes in the phosphorylation status of the eIFs or the regulators that interact with them (reviewed in Gebauer and Hentze, 2004; Szostak and Gebauer, 2013). The core translation machinery is highly conserved in malaria parasites, including the eIFs (Table 1). While the knowledge on global regulation of translation in *Plasmodium* is rudimentary, some evidence of such regulation has been provided.

**Regulation by phosphorylation of eIF2α**

A key mechanism of global regulation is the phosphorylation of eIF2α, which together with eIF2β and eIF2γ forms the eIF2 complex. eIF2 is able to bind GTP and subsequently interacts with the methionine-loaded initiator tRNA, thereby constituting the ternary complex. The ternary complex then assembles with the small ribosomal subunit and the eIFs 1, 1A, 3 and 5 to form the 43S pre-initiation complex (PIC) (Fig. 2). During docking of the 43S complex to the start codon AUG, eIF2-bound GTP is hydrolyzed and the subsequent exchange of GDP to GTP is catalyzed by the guanine nucleotide exchange factor eIF2B (composed of subunits α to ε). This GTP exchange can be blocked by phosphorylation of eIF2α at Ser51, resulting in sequestration of eIF2B at GDP-bound eIF2 (reviewed in Gebauer and Hentze, 2004). The arrest of translation initiation by eIF2α phosphorylation particularly occurs under cell stress, and phosphorylated eIF2α then accumulates in stress granules (see below). A number of kinases are able to phosphorylate eIF2α, dependent on the type of stress signal perceived. In humans, phosphorylation is catalyzed by GCN2 (general control non-derepressible-2) during amino acid starvation, while PKR (protein kinase activated by double-stranded RNA) is activated during viral infection and PERK (protein kinase R-like endoplasmic reticulum kinase) and HRI (heme-regulated inhibitor) under endoplasmic reticulum and erythrocytic oxidative stress respectively (reviewed in Donnelly et al., 2013).

In *Plasmodium*, three eIF2α kinases were identified (Ward et al., 2004; Fennell et al., 2009), termed eukaryotic eIF2α kinases eIK1 and eIK2 (also known as UIS1) and protein kinase PK4. These are able to phosphorylate eIF2α at Ser51. While transcript for all of the kinases is detected in the asexual and sexual blood stages, peak expression of eIK2 is found in the salivary gland sporozoites, as was shown in rodent and human malaria parasites (Le Roch et al., 2003; Tarun et al., 2008; Zhang et al., 2010; reviewed in Zhang et al., 2013). Here, eIK2 mediates translational arrest by phosphorylation of eIF2α. Salivary gland sporozoites deficient of eIK2 lack stress granules as shown via poly(A)-tail labeling of mRNA accumulations. The sporozoites further exhibit deregulated transcript expression and in consequence lose their infectivity (Zhang et al., 2010). The salubrinal-sensitive eIF2α-specific phosphatase UIS2 reverts eIF2α phosphorylation, once the sporozoites entered the mammalian host. It has been postulated that the stress granule-associated RNA-binding protein PuF2 (Pumilio and fem-3 binding factor homology 2) represses synthesis of the phosphatase in the salivary gland sporozoites (Zhang et al., 2016). Accordingly, PuF2-knock-out (KO) sporozoites exhibit decreased *uis2* mRNA levels, while general protein levels are increased. These data indicate that lack
Table 1. Molecules of the translational control machinery of *P. falciparum* strain 3D7.

| Protein | PF3D7 gene ID | Role in translational control | References |
|---------|---------------|-------------------------------|------------|
| **Global regulation** | | | |
| Eukaryotic translation initiation factor 2-alpha kinase eIF1/GCN2 | PF3D7_1444500 | Linked to amino acid starvation in the asexual blood stages of *P. falciparum* | Fennell et al. (2009) |
| Eukaryotic translation initiation factor 2-alpha kinase eIF2/US1 | PF3D7_0107600 | Phosphorylates eIF2α in quiescent salivary gland sporozoites in *P. berghei* | Zhang et al. (2010) |
| Eukaryotic translation initiation factor 2-alpha kinase/PK4/PERK | PF3D7_0628200 | Crucial for asexual blood stage replication in *P. falciparum* and *P. berghei* | Zhang et al. (2012) |
| Eukaryotic translation initiation factor eIF2α | PF3D7_0728000 | Subunit of eIF2, part of the ternary complex/43S complex | – |
| Eukaryotic translation initiation factor eIF2β | PF3D7_1010600 | Subunit of eIF2, part of the ternary complex/43S complex | – |
| | PF3D7_1410600 | Subunit of eIF2, part of the ternary complex/43S complex | – |
| Translation initiation factor eIF-2Bα | PF3D7_0828500 | Subunit of guanine nucleotide exchange factor eIF-2B | – |
| Translation initiation factor eIF-2Bβ | PF3D7_1250600 | Subunit of guanine nucleotide exchange factor eIF-2B | – |
| Translation initiation factor eIF-2Bγ | PF3D7_1013900 | Subunit of guanine nucleotide exchange factor eIF-2B | – |
| Translation initiation factor eIF-2Bδ | PF3D7_1326400 | Subunit of guanine nucleotide exchange factor eIF-2B | – |
| | PF3D7_1468700 | Subunit of eIF4F, RNA helicase | – |
| Eukaryotic translation initiation factor 4A | PF3D7_0351500 | Subunit of eIF4F, cap-binding protein | – |
| Eukaryotic translation initiation factor 4E | PF3D7_0111800 | Putative eIF4E homologue | – |
| | PF3D7_1312900 | Subunit of eIF4F, scaffold | – |
| Polyadenylate-binding protein PABP1 | PF3D7_1224300 | Binds poly(A) tail and eIF4G, mediates mRNA pseudo-circularization in cytosol, a homologue is present on the surface of *P. yoelii* sporozoites | Minns et al. (2018) |
| Polyadenylate-binding protein PABP2 | PF3D7_0923900 | Binds poly(A) tail and eIF4G, mediates mRNA pseudo-circularization, in nucleus | Minns et al. (2018) |
| Polyadenylate-binding protein PABP3 | PF3D7_0629400 | Binds poly(A) tail and eIF4G, mediates mRNA pseudo-circularization | – |
| Polyadenylate-binding protein-interacting protein Paip1 | PF3D7_1107300 | Mediates binding between PABP and eIF4A in other eukaryotes | – |
| **RNA-binding proteins** | | | |
| DNA/RNA-binding protein Alba 1 | PF3D7_0814200 | Binds transcripts, involved in maintaining mRNA homeostasis and translational repression | Chêne et al. (2012); Vembar et al. (2015) |
| DNA/RNA-binding protein Alba 2 | PF3D7_1346300 | Putative DNA/RNA-binding protein | Chêne et al. (2012); Goyal et al. (2012) |
| DNA/RNA-binding protein Alba 3 | PF3D7_1006200 | Putative DNA/RNA-binding protein, inhibits transcription in vitro by binding to DNA | Muñoz et al. (2017) |
| DNA/RNA-binding protein Alba 4 | PF3D7_1347500 | Involved in the stabilization/destabilization of transcripts in *P. yoelii* | – |
| ATP-dependent RNA helicase DDH1/DDX6/DOZI/DDZ50 | PF3D7_0320800 | Decapping activator, binds eIF4E, translational repressor in *P. berghei* | Mair et al. (2006); (2010); Tarique et al. (2013) |
| Trailer hitch homolog CITH | PF3D7_1474900 | Homologue of Lsm14a, translational repressor in *P. berghei* | Mair et al. (2010) |
| mRNA-binding protein Puf1 | PF3D7_0518700 | RNA binding activity demonstrated, involved in gametocyte maturation | Cui et al. (2002); Shrestha et al. (2016) |
| mRNA-binding protein Puf2 | PF3D7_0417100 | Represses *uis2* and other transcripts in salivary gland sporozoites in *P. berghei*, involved in gametocyte development, translational regulation of *p25* and *p28* transcripts in gametocytes | Miao et al. (2010); (2013); Gomes-Santos et al. (2011); Silva et al. (2016); Sai et al. (2018) |
| mRNA-binding protein Puf3 | PF3D7_0621300 | Participates in ribosomal biogenesis | Liang et al. (2018) |
| CUGBP Elav-like family member CELF1 | PF3D7_1359400 | Homologue of Bruno, binds RNA, might be involved in pre-mRNA processing | Wongswamset et al. (2014) |
| CUGBP Elav-like family member CELF2 | PF3D7_1409800 | Homologue of Bruno, part of the DOZI/CITH-complex in *P. berghei* | Mair et al. (2010) |
| RNA-binding protein Musashi sporozoite and liver stage asparagine-rich protein SAP1/SLARP | PF3D7_0916700 | Part of the DOZI/CITH-complex in *P. berghei* | Mair et al. (2010); Aly et al. (2008; 2011); Silvie et al. (2008) |

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| Protein | PF3D7 gene ID | Role in translational control | References |
|---------|----------------|-------------------------------|------------|
| 7-Helix-1 | PF3D7_0525400 | Component of stress granules, interacts with DOZI/CITH/Puf2, translational regulation of p25 and p28 transcripts in gametocytes | Bennink et al. (2018) |
| CNOT complex/Deadenylation | | | |
| CCR4 domain-containing protein Ccr4-1 | PF3D7_0519500 | Deadenylase of CNOT; crucial for male gametocytogenesis and gametogenesis in *P. yoelii* | Hart et al. (2019) |
| CCR4 domain-containing protein Ccr4-2 | PF3D7_1363500 | Deadenylase of CNOT | – |
| CCR4 domain-containing protein Ccr4-3 | PF3D7_0107200 | Deadenylase of CNOT | – |
| CCR4-NOT transcription complex subunit Not1 | PF3D7_1103800 | CNOT subunit | – |
| CCR4-NOT transcription complex subunit Not2 | PF3D7_1128600 | CNOT subunit | – |
| CCR4-NOT transcription complex subunit Not4 | PF3D7_1235300 | CNOT subunit | – |
| CCR4-NOT transcription complex subunit Not5 | PF3D7_1006100 | CNOT subunit | – |
| Protein CAF40 | PF3D7_0507600 | CNOT subunit | – |
| NOT family protein, putative | PF3D7_1417200 | CNOT subunit | – |
| CCR4-associated factor CAF1 | PF3D7_1434000 | CNOT subunit | – |
| CCR4-associated factor CAF1/Pop2 | PF3D7_0811300 | Deadenylase of CNOT; crucial for asexual blood stage replication, gametocytogenesis and gametogenesis in *P. berghei*, *P. yoelii* and *P. falciparum* | Balu et al. (2010); (2011); Hart et al. (2019) |
| poly(A)-specific ribonuclease PARN | PF3D7_1443500 | Involved in nonsense-mediated mRNA decay in other eukaryotes | – |
| Decapping/5' -3' degradation | | | |
| mRNA-decaping enzyme DCP1 | PF3D7_1032100 | Subunit of the decapping complex | – |
| mRNA-decaping enzyme DCP2 | PF3D7_1309890 | Subunit of the decapping complex | – |
| U6 snRNA-associated Sm-like protein LSm1 | PF3D7_1124400 | Subunit of the Lsm1-7-Pat1 ring, mediates binding of DCP1-DCP2 to poly(A) tail in other eukaryotes | – |
| U6 snRNA-associated Sm-like protein LSm2 | PF3D7_0520300 | Subunit of the Lsm1-7-Pat1 ring, mediates binding of DCP1-DCP2 to poly(A) tail in other eukaryotes | – |
| U6 snRNA-associated Sm-like protein LSm3 | PF3D7_0819900 | Subunit of the Lsm1-7-Pat1 ring, mediates binding of DCP1-DCP2 to poly(A) tail in other eukaryotes | – |
| U6 snRNA-associated Sm-like protein LSm4 | PF3D7_1107000 | Subunit of the Lsm1-7-Pat1 ring, mediates binding of DCP1-DCP2 to poly(A) tail in other eukaryotes | – |
| U6 snRNA-associated Sm-like protein LSm5 | PF3D7_1443300 | Subunit of the Lsm1-7-Pat1 ring, mediates binding of DCP1-DCP2 to poly(A) tail in other eukaryotes | – |
| U6 snRNA-associated Sm-like protein LSm6 | PF3D7_1325000 | Subunit of the Lsm1-7-Pat1 ring, mediates binding of DCP1-DCP2 to poly(A) tail in other eukaryotes | – |
| U6 snRNA-associated Sm-like protein LSm7 | PF3D7_1209200 | Subunit of the Lsm1-7-Pat1 ring, mediates binding of DCP1-DCP2 to poly(A) tail in other eukaryotes | – |
| Exoribonuclease Xrn1 homologue | PF3D7_0909400 | mRNA degradation in other eukaryotes | – |
| Exoribonuclease Xrn1 homologue | PF3D7_1106300 | Putative Xrn1 homologue | – |
| The RNA exosome | | | |
| Exosome complex component RRP4 | PF3D7_0410400 | KH domain protein, exosome subunit | – |
| Exosome complex component RRP40 | PF3D7_1307000 | KH domain protein, exosome subunit | – |
| Exosome complex component RRP41 | PF3D7_1427800 | PH domain protein, exosome subunit, exoribonuclease activity demonstrated | Jiang et al. (2018) |
| Exosome complex component RRP42 | PF3D7_1340100 | PH domain protein, exosome subunit | – |
| Exosome complex component RRP45 | PF3D7_1364500 | PH domain protein, exosome subunit | – |
| Exosome complex component MTR3 | PF3D7_0209200 | PH domain protein, exosome subunit | – |
| Exosome complex exonuclease RRP6 | PF3D7_1449700 | 3'-5' exoribonuclease, activity demonstrated | Zhang et al. (2018); Droll et al. (2018); Jiang et al. (2018) |
| Exosome complex exonuclease RRP44/ DIS3 | PF3D7_1359300 | 3'-3' exoribonuclease, activity demonstrated | – |
| Exosome complex component CSL4 | PF3D7_0720000 | Exosome subunit | – |
| Helicase SKI2W | PF3D7_0909900 | Exosome-associated helicase | – |

Data were compiled from (Shock et al., 2007; Cui et al., 2015; Vembar et al., 2016; Jiang et al., 2018) as well as the databases www.plasmDB.org and mpmp.huji.ac.il.
Fig. 2. The molecular machinery of translational regulation. The molecular interactions during translation initiation and select mechanisms of translational control, i.e. (1) stalled translation initiation, (2) translational repression, and (3) 3′-to-5′ and 5′-to-3′ mRNA decay, are depicted. Circled numbers represent translation initiation factors; the red cross represents the initiator tRNA. 3′-UTR-BS, 3′-untranslated region binding sequence; IRP, IRE-binding protein; IRE, iron-responsive element; IncRNA, long non-coding RNA; uORF, upstream open reading frame; 43S PIC, 43 S preinitiation complex; RBP, RNA-binding protein.
of Pu2 leads to premature activity of the phosphatase in salivary gland sporozoites, subsequently resulting in the premature transformation into liver stages, while still inside of the mosquito (see below; Gomes-Santos et al., 2011; Müller et al., 2011). While eIF2 acts in salivary gland sporozoites, PK4 phosphorylates eIF2α in the sexual and sexual blood stages. So far, the gene encoding PK4 could not be knocked-out in the asexual blood stages of P. berghai, thus functional data are yet missing (Zhang et al., 2012). Interestingly, treatment of malaria with the frontline drug artemisinin and its derivatives leads to increased eIF2α phosphorylation, mediated by PK4. The resulting repression of translation can cause the parasites to enter latency and accounts for treatment failure. In accord, inhibition of PK4 blocks parasites from entering latency, ensuring treatment efficacy (Zhang et al., 2017). The third plasmodial eIF2α kinase, eIK1, a homolog of GCN2, is dispensable for blood stage growth. Parasites lacking eIK1, however, are unable to phosphorylate eIF2α under amino acid-limiting conditions (Fennell et al., 2009).

**eIF4E-binding proteins in translational control**

Global regulation of translation can also occur by changing the availability of the cap-binding protein eIF4E, which together with the scaffold protein eIF4G and the RNA helicase eIF4A forms the cap-binding complex eIF4F (Fig. 2). The interaction between eIF4E and eIF4G is crucial for recruitment of the 43S complex to the 5′-cap of the mRNA and the binding between eIF4E and eIF4G requires a small domain in eIF4G that is shared by the 4E-binding protein (4E-BP). When hypophosphorylated, 4E-BP binds to eIF4E and, in consequence, blocks binding to eIF4G, hence the 43S complex cannot be recruited to the cap structure (reviewed in Gebauer and Hentze, 2004). 4E-BP hyperphosphorylation, however, mediates the release of 4E-BP from eIF4E, making the factor accessible to eIF4G. 4E-BP hyperphosphorylation is mediated by the mTOR (mechanistic Target of Rapamycin) kinase (reviewed in Gingras et al., 1999).

While not much is known yet about the translational control in *Plasmodium* that involves sequestration of eIF4E, homologs of the three eIF4F components are expressed (Table 1) (Shaw et al., 2007; Tuteja, 2009; Tuteja and Pradhan, 2009). In *P. falciparum*, a DHH1/DDX6-like RNA helicase, DOZI (termed DZ50 in this study), has been identified as a potential eIF4A protein. DOZI was shown to interact with eIF4E, resulting in impaired translation (Tarique et al., 2013). It is worth mentioning in this context that DOZI has been assigned additional functions in other life cycle stages, e.g. as a stress granule-resident translational repressor in gametocytes and as a potential mediator of decapping during mRNA decay (see below). Notably, malaria parasites have lost most of the mTORC components through genomic reduction, including the mTOR kinase (Serfontein et al., 2010; van Dam et al., 2011; McLean and Jacobs-Lorena, 2017), thus the kinase responsible for the phosphorylation of potential eIF4E-BP has yet to be identified.

**The roles of PABP in translational control**

The factors eIF4G and eIF4A that are linked to the 5′-cap via eIF4E can interact with the poly(A)-binding protein (PAPB), mediated by the PABP-interacting protein Paip1 (Fig. 2). These interactions facilitate mRNA pseudo-circularization, which promotes transcript stabilization (reviewed in Derry et al., 2006). Most *Plasmodium* genomes encode three PABPs (Table 1; Aurrecoechea et al., 2009; Tuteja and Pradhan, 2009). In eukaryotes, PABPs have multiple roles in controlling translation, which range from protecting the 5′-cap by inhibiting decapping to promoting 3′-deadenylation by interacting with the Ccr4-Not (CNOT) complex (Khanna and Kiledjian, 2004; Webster et al., 2018; see below). Interestingly, the PABP-mediated mRNA closed-loop provides the physical contact between mRNA-binding proteins (RBPs), which typically target the 3′-UTR (untranslated region), and the 5′-UTR of the transcript in order to suppress translation, as it was shown for BRUNO in *Drosophila* (Castagnetti et al., 2000). BRUNO/CELF homologues were described in *Plasmodium* (Table 1; see below). Notably, the PABPs of the rodent malaria parasite *P. yoelii* are not only present in the parasite nucleus and cytoplasm, but are reported to also be deposited on the sporozoite surface by a yet unknown mechanism, presumptively to bind exogenous RNA (Minns et al., 2018).

**mRNA-specific regulation of translation**

While global control mechanisms regulate translation of most mRNAs in a cell at the same time, mRNA-specific control mechanisms regulate the translation of specific mRNA species or of a defined group of mRNAs. Measures of transcript-specific, post-transcriptional gene regulation include the usage of antisense transcripts or RNA interference (RNAi). It can be further driven by specific regulatory RBPs complexes and by the recognition of cis translational control elements within the UTRs of mRNAs. These cis control elements include e.g. upstream AUG start codons and upstream open reading frames (uAUGs and uORFs respectively), internal ribosome entry sequences (IRES) and secondary structures.

*Regulation via cis translational control elements*

In general, uAUGs and uORFs act as negative regulators by reducing the translation rate of the main downstream
ORF (dORF). The uAUGs and uORFs in the 5’ UTR of mRNAs are first encountered by the scanning ribosome, leading to a reduced probability to reach the main AUG and reduced concentrations of translation factors (reviewed in Hood et al., 2009). In rare cases, the uORF-encoded peptide itself confers negative translational regulation of the main dORF (Fang et al., 2000; Law et al., 2001). A mechanism that allows translation of the main dORF despite one or more uORFs is translation initiation via internal ribosome entry sequences (IRESs), which mediate cap-independent translation initiation (Fig. 2). IRESs are sequences within the 5’ UTR of a transcript that fold into a structure resembling the 5’-cap structure and are recognized by the ribosome to initiate translation. They are mainly found in viral RNAs, enabling fast protein synthesis utilizing the host translation machinery. The mechanisms for ribosome recruitment to the IRESs are quite diverse, as some structures can recruit both ribosomal subunits without using any eIFs, while others use a subset of eIFs or even proteins that are usually not associated with translation to initiate translation (reviewed in Plank and Kieft, 2012).

A secondary structure affecting translation can be found in the mRNA of the human iron storage protein ferritin, which is involved in controlling the amount of intracellular iron. The ferritin transcript exhibits a stem loop motif in its 5’ UTR, referred to as an iron-responsive element (IRE). In iron-deficient cells, the two iron-regulatory proteins (IRPs) IRP1 and IRP2 bind to the IRE, thereby sterically blocking the recruitment of the 43S complex to the mRNA, inhibiting translation of ferritin and eventually decreasing the amount of iron sequestered within the storage protein (Goossen et al., 1990; Muckenthaler et al., 1999).

In Plasmodium, regulation of translation via 5’ UTRs was suggested to be a widespread mechanism to control protein expression (Bunnik et al., 2013). While no IRESs have yet been described in malaria parasites, an IRP-like protein that shows 47% sequence identity with human IRP1 has been identified in P. falciparum (PlIRPα) which is capable of binding to a mammalian consensus IRE in an iron-regulated manner. Furthermore, database searches also identified putative plasmodial IRESs, to which recombinant and endogenous PlIRPα binds in in vitro assays (Loyevsky et al., 2001; 2003). Another study investigating the expression of a typical house-keeping gene encoding the phosphoglutamase 2 revealed that partial deletions of the 5’ UTR affect the efficiency of translation, confirming that 5’ UTR sequences or structures contribute to the regulation of protein synthesis in P. falciparum (Hasenkamp et al., 2013).

The expression of the major virulence factor of P. falciparum, erythrocyte membrane protein 1 (PIEMP1), is under strict control of both transcriptional and translational regulation mechanisms. PIEMP1 is encoded by the multicycopy var gene family, comprising 60 genes coding for different PIEMP1 variants. Significantly, only one of the 60 var genes is active at a given time, resulting in mutually exclusive expression of the protein. This concept, referred to as antigenic variation, is achieved through the escape of one of the 60 loci from epigenetic silencing and regulation of polymerase II-dependent transcription initiation (Kyes et al., 2007; reviewed in Guizetti and Scherf, 2013). Furthermore, each var gene is associated with one of four conserved 5’ upstream (ups) regions (upsA, upsB, upsC and upsE). Recently, a 5’ UTR sequence within the upsC region has been identified, that is essential for singular var gene choice and additionally inhibits translation initiation of the corresponding transcript, indicating that a single regulatory element might be capable of controlling var gene expression at both transcriptional and translational levels (Brancucci et al., 2012; 2014). These data indicate that translational control may significantly contribute to the regulation of PIEMP1 synthesis, as it has also been demonstrated for the PIEMP1 variant VAR2CSA. VAR2CSA is implicated in pregnancy-associated malaria mediating the parasite-placenta adherence (Salanti et al., 2003; 2004). It is specifically synthesized in infected pregnant individuals, while in non-pregnant individuals, the mRNA but no protein is detectable (Lavstsen et al., 2005; Tuikue Ndam et al., 2005; Duffy et al., 2006). It was shown that in non-pregnant individuals, VAR2CSA is translationally repressed by a uORF that negatively influences the translation rate of the main dORF (Mok et al., 2008; Amulic et al., 2009). Translational repression is released through the P. falciparum translation enhancing factor (PTEF) that binds to ribosomes and stimulates efficient release of repression and subsequent translation of VAR2CSA (Chan et al., 2017). In fact, there is a high probability for uAUGs and uORFs in the Plasmodium genome, due to its high AT-content. However, var 5’ UTRs appear to be significantly enriched in uAUGs and uORFs compared to the 5’ UTRs of other genes (Brancucci et al., 2014; Kumar et al., 2015).

Translational repression by RNA-binding proteins

Translational repression allows the cell to rapidly react to external stimuli. It enables the temporary storage of transcripts and their subsequent reintroduction into the translation machinery after the release of repression. The repressed transcripts are contained in messenger ribonucleoprotein particles (mRNPs) including translational repressor proteins, which condense to cytosolic granules. While such granules of a cell are highly diverse in composition and function, currently two main types are described, i.e. stress granules, which store the mRNA to later reintroduce them to protein synthesis, and granules...
with function in degradation (e.g. termed P-bodies). External stimuli that demand a change in the protein repertoire of the cell can lead to a release of repression of the transcripts and active translation or final decay (reviewed in Anderson and Kedersha, 2007; 2009).

*Plasmodium* parasites employ transcript-repression by RBPs especially in the transmission stages in order to proactively prepare for the change of hosts and the parasite exhibits a large number of putative RBPs that might be involved in this process (Reddy et al., 2015). Transcripts coding for proteins needed after transmission are already transcribed and stored in mRNPs in earlier life cycle stages, while translation preferentially takes place after transmission when repression is released. In *Plasmodium* parasites, this phenomenon was first described for the *P. berghei* surface antigens P25 and P28. While transcripts were abundantly detected in the gametocyte stages, proteins were synthesized in zygotes and ookinetes after transmission to the mosquito (Hall et al., 2005). Further studies described DOZI (development of zygote inhibited; transmission to the mosquito (Vembar et al., 2015)). Another member of the plasmodial ALBA family, ALBA4, constitutes mRNPs in the rodent malaria parasite *P. yoelii* throughout its life cycle. KO studies revealed a role for the protein in regulating the development of the parasite’s transmission stages. Moreover, P25 and P28 expressions were further shown to be regulated in *P. falciparum* by a member of the Puf family. Puf proteins are defined by a conserved RNA-binding domain and known to be important eukaryotic regulators of translation by repressing protein synthesis or promoting degradation of transcripts (reviewed in Miller and Olivas, 2011). They bind to conserved Puf-binding elements (PBEs) in their target sequences, which are usually located in the 3′ UTR. In the genome of *P. falciparum*, three members of the Puf family, Puf1-3, are encoded (Cui et al., 2011; Fan et al., 2004; Liang et al., 2018). While Puf1 is involved in gametocyte maturation (Shrestha et al., 2016), Puf3 participates in ribosomal biogenesis (Liang et al., 2018). Puf2-KO promotes sexual stage differentiation in *P. falciparum* and further leads to a shift in the male-to-female ratio of gametocytes (Miao et al., 2010). Moreover, translationally repressed transcripts, including p25 and p28, and their protein products P25 and P28 were significantly upregulated in Puf2-KO gametocytes, indicating that the deletion of Puf2 leads to the loss of translational regulation of these transcripts and increased translation (Miao et al., 2013). PBEs have been identified in the p28 3′ UTR and the p25 5′ UTR, demonstrating that PI2Puf2 is a versatile regulator of translation and acts through both 3′ and 5′ UTRs. Significantly, in rodent malaria species, no effect on the male-to-female ratio in gametocytes was observed in Puf2-KO parasites (Gomes-Santos et al., 2011; Müller et al., 2011; Lindner et al., 2013). Studies conducted in *P. berghei* and *P. yoelii* demonstrated that Puf2-KO parasites are affected during mosquito-to-human transmission, where Puf2 represses transcripts of salivary gland
sporozoites that are important for parasite development in hepatocytes (see above; Lindner et al., 2013; Silva et al., 2016). Another component of sporozoite-specific stress granules that regulates expression of genes important for the intrahepatic phase is SAP1 (also known as SLARP; sporozoite and liver asparagine-rich protein 1), and lack of SAP1 in P. yoelii causes transcript degradation as well as loss of infectivity (Aly et al., 2008, 2011; Silvie et al., 2008). This makes SAP1 an interesting target for genetically attenuated parasites, which could be used for vaccination without establishing an infection (van Schaijk et al., 2014).

In addition to the above RBPs, we recently identified a novel key player in translational regulation during the sexual phase of P. falciparum, termed 7-Helix-1. The protein constitutes stress granules in female gametocytes and here interacts with known translational repressors such as DOZI, CITH and Pul2. The 7-Helix-1 complex further binds p25 and p28 transcripts and 7-Helix-1-KO leads to reduced P25 synthesis in sexual stage parasites (Bennink et al., 2018). It is thus postulated that 7-Helix-1 might function in the re-initiation of translation of repressed transcripts, once gametogenesis is initiated.

**Antisense transcripts in translational control**

Another measure of post-transcriptional negative gene regulation is the usage of antisense transcripts or of RNAi via microRNA (miRNA). RNAi relies on the interaction of short RNA sequences with the target mRNA and involves proteins such as Dicer and Argonaute. Argonaute proteins are the main constituents of the so-called RNA-induced silencing complex (RISC), that finally degrades the target mRNA and thereby prevents protein synthesis. In contrast to this, antisense transcripts are long RNA sequences that are complementary to the target mRNA, and base pairing between the two strands alone impairs translation, without the need for participating enzymes (Fig. 2).

No Dicer or Argonaute-coding genes nor the corresponding protein products have been identified in Plasmodium parasites, as was shown using various genetic approaches and comparative genomics, suggesting that no functional RNAi-based gene regulation mechanism is active in the malaria parasite (Baum et al., 2009; reviewed in Mueller et al., 2014). However, a widespread distribution of antisense transcripts has been detected in the asexual and sexual stages of Plasmodium parasites (Patankar et al., 2001; Kyes et al., 2002; Gunasekera et al., 2004; Militello et al., 2005; Lu et al., 2007). The synthesis of these transcripts is catalyzed by RNA polymerase II, as it is sensitive to the potent RNA polymerase II inhibitor α-amanitin (Militello et al., 2005). Notably, production of antisense transcripts was reported for at least 24% of protein-coding genes (Siegel et al., 2014) and a recent study confirmed the regulatory function of antisense transcripts in translational control (Filarsky et al., 2018). The authors demonstrated that expression of the gametocyte development protein 1 (GDV1) is controlled via a gdv1 antisense RNA and that elimination of antisense RNA leads to GDV1 synthesis, which promotes sexual commitment. The underlying mechanism likely involves interference of gdv1 antisense RNA with transcription, stability or translation of the corresponding mRNA (Filarsky et al., 2018).

**mRNA decay**

A non-reversible stop of translation is the induced degradation of transcript. mRNA decay can occur both in 5′ to 3′ and in 3′ to 5′ direction, and usually involves poly(A)-tail shortening (deadenylation), as the rate-limiting, initial step in the decay pathway. Deadenylation is then followed by either mRNA 3′-to-5′ degradation or mRNA decapping and 5′-to-3′ degradation. In rare cases, transcripts can also be destroyed through endoribonucleolytic decay, thereby generating two RNA fragments with one unprotected end each, which are then further degraded by exonucleases (reviewed in Garneau et al., 2007). Two major complexes are involved in mRNA decay; the CNOT and the RNA exosome complex (Fig. 2).

**The CNOT complex**

CNOT is a highly conserved multi-subunit complex that contributes to regulating gene expression from mRNA synthesis in the nucleus to its degradation in the cytoplasm. CNOT comprises the two deadenylases Ccr4 (carbon catabolite repressor 4) and CAF1 (Ccr4-associated factor 1) and the evolutionally conserved scaffold protein Not1 as well as a number of species-specific core proteins. These include e.g. Not5 that mediates binding to the mRNA and Not4, an E3 ligase that acts in quality control by co-translationally tagging aberrant proteins for proteasomal degradation (reviewed in Miller and Reese, 2012; Collart et al., 2013; Inada and Makino, 2014; Collart, 2016). In the nucleus, CNOT is involved in the control of gene expression, e.g. via interaction with transcription factors or the RNA polymerase II, while in the cytoplasm, it particularly acts as a deadenylase complex. CNOT is present in polysomes, where mRNAs are translated, and in cytoplasmic granules, where mRNAs are repressed and degraded.

CNOT contributes to the rate-limiting step of poly(A)-tail shortening via the deadenylation activities of Ccr4 and CAF1. While the latter removes adenosine monophosphates from free poly(A) tails, the former trims the PABP-bound poly(A) tail during removal of PABP (Webster et al., 2019).
The RNA exosome complex

Following its deadenylation, an mRNA can also be degraded in the 3’ to 5’ direction by the RNA exosome complex. The exosome is a multi-protein complex found in archaeal and eukaryotic cells that is capable of degrading various types of RNA molecules (reviewed in Kilchert et al., 2016). The core of the exosome contains a six-membered ring structure formed by RNase PH proteins accompanied by three S1-KH cap proteins. Two proteins with 3’-5’ exoribonuclease (as well as endoribonuclease) activity are attached to the nine-subunit core, termed Rrp44/Dis3 and Rrp6. In eukaryotic cells, the exosome complex is present in the cytoplasm and nucleus, here particularly in the nucleolus. In the nucleus, the exosome mainly degrades cryptic transcripts and RNAs that are incorrectly processed; in the cytoplasm, however, its prime function lies in transcript degradation. Because the RNA needs to be unwound prior to its degradation by the exosome, helicases are needed, such as Mtr4 and superkiller Ski2. Exosome-specificity factors recruit the helicases to their substrates, which are often proteins binding AU-rich elements (AREs) at the 3’-UTR of the mRNA. Significantly, in the Plasmodium genome, the prediction and identification of AREs are nearly impossible due to the exceptionally high AT-content of the genome.

The majority of the exosome core subunits as well as Rrp44/Dis3, Rrp6 and Ski2 are conserved in P. falciparum (Table 1) (Shock et al., 2007; Jiang et al., 2018). First functional data on the plasmodial exosome mainly describe its function in regulating gene expression. Homologs of exosome subunits are found in the parasite cytoplasm and nucleus and for Rrp6, Rrp41 and Rrp44/Dis3 catalytic activities for single-stranded RNA were demonstrated (Jiang et al., 2018). Asexual blood stages lacking Rrp44/Dis3 exhibited upregulated expression of coding and regulatory RNA as well as cryptic transcripts linked to virulence gene families (Droll et al., 2018). Furthermore, asexual blood stages which are deficient of the noncanonical Rrp44/Dis3 homolog PfRNase II are impaired in the expression of virulence-related var genes (Zhang et al., 2014). This 3’-5’ RNase is not part of the exosome, but associated with chromatin, where it promotes the expression of approximately 200 genes. Because these reports relate to an involvement of the plasmodial exosome in post-transcriptional regulation, a direct link between the plasmodial exosome and translational control has yet to be provided.

Conclusions

To date, the key pathways of translational control in malaria parasites remain underinvestigated and only a handful of conserved regulators have been functionally studied. Evidence collected during the last few years indicate that select components of the translational control machinery exert more than one function or have roles...
outside of regulating protein synthesis, as it has been shown for DOZI and PABP. New arrays of techniques such as ribosome profiling, large-scale protein–protein interaction screenings, functional protein knock-down approaches and quantitative high-throughput proteomics and transcriptomics will allow to tackle decoding the translational control machinery from different angles. Results gained are likely to improve our understanding on how the malaria parasite is able to adapt so quickly to its new environment. Significantly, the molecular machinery of translational control in Plasmodium represents an attractive new target for antimalarial interventions and the increasing numbers of small-molecule inhibitors targeting mRNA translation and upstream pathways in humans provide a starting point for the generation of new types of chemotherapeutics.

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Conflicts of interest
The authors have no conflict of interest to declare.

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