Upstream Open Reading Frames (uORFs) in the Apicomplexan Parasites *Plasmodium falciparum* and *Toxoplasma gondii*: small yet powerful regulators of translation

Chhaminder Kaur[1], and Swati Patankar [1,*]

[1]Department of Biosciences & Bioengineering, Indian Institute of Technology Bombay, Powai, Mumbai 400076, India

* **Author for correspondence:** Swati Patankar, E-mail: patankar@iitb.ac.in

ABSTRACT

During their complex life cycles, the Apicomplexan parasites, *Plasmodium falciparum* and *Toxoplasma gondii* employ several genetic switches to regulate their gene expression. One such switch is mediated at the level of translation through upstream Open Reading Frames (uORFs). As uORFs are found in the upstream regions of a majority of genes in both the parasites, it is essential that their roles in translational regulation be appreciated to a greater extent. This review provides a comprehensive summary of studies that show uORF-mediated gene regulation in these parasites and highlights examples of clinically and physiologically relevant proteins that exhibit uORF-mediated regulation. In addition to these examples, several studies that use bioinformatics, transcriptomics, proteomics, and ribosome profiling also indicate the possibility of widespread translational regulation by uORFs. Further analysis of genome-wide datasets will reveal novel genes involved in key biological pathways such as cell-cycle progression, stress-response, and pathogenicity. The cumulative evidence from studies presented in this review suggests that uORFs will play crucial roles in regulating gene expression during clinical disease caused by these important human pathogens.

Key words: *P. falciparum*, *T. gondii*, upstream ORFs, translational regulation

INTRODUCTION

Eukaryotic translation initiation is a tightly regulated, multi-step process that involves scanning of messenger RNA (mRNA) by the preinitiation complex (Kozak, 1980). This complex, comprising of the small ribosomal subunit and numerous initiation factors, scans the mRNA for an authentic start codon (AUG) present in the coding sequence (CDS) (Kozak, 1991). The selection of the start codon is governed by the sequence surrounding the AUG codon i.e. the Kozak sequence, availability of initiation factors, molecules that provide energy, and methionyl-tRNAs (reviewed in Hinnebusch, 2011).

Other than these factors, the presence of start codon(s) that lie upstream of the start codon of the main CDS confers another layer of regulation. This is due to the scanning model of translation initiation where the ribosomes recognize the 5′ cap and move along the mRNA towards the 3′ end. During this process, the ribosomes encounter upstream start codons (uAUGs) before the main CDS and therefore, these uAUGs are capable of engaging the ribosome (Kozak, 2002). Similar to uAUGs, upstream open reading frames (uORFs), defined as an upstream start codon followed by an in-frame stop codon, also engage the scanning ribosome with varying capacities, which in turn alters the level of the protein encoded by the main CDS (reviewed in Morris and Geballe, 2000). The presence of these alternative initiation sites constitutes a "hurdle" for the ribosome and usually results in repression of translation of the main CDS. This repression can be relieved by the cellular translation machinery with a multitude of strategies, as and when required (Wang and Rothnagel, 2004; Iacono *et al.*, 2005). Hence, uAUGs and uORFs act as regulatory elements in the 5' leader sequences of eukaryotic mRNAs. Interestingly, as translation regulation allows the organism to respond more rapidly than transcriptional regulation, uORFs (rather than uAUGs) are used by cells to handle a wide range of environmental changes, affecting the survivability of the cell.

The earliest known evidence for uORF involvement in translational control was shown for

Saccharomyces cerevisiae General Control Non-depressible 4 (GCN4), a transcription factor that controls amino acid biosynthesis under conditions of starvation (reviewed in Hinnebusch, 1988). After these early reports, translation regulation by uORFs during stress conditions was shown in numerous organisms including *Homo sapiens*, *Mus musculus*, *Drosophila melanogaster*, *Neurospora crassa*, *Danio rerio*, *Arabidopsis thaliana*, *Zea mays*, and higher plants (Iacono *et al.*, 2005; Barbosa *et al.*, 2013; Chew *et al.*, 2013; von Arnim *et al.*, 2014; Lei *et al.*, 2015; Young and Wek, 2016; Zhang *et al.*, 2018, 2019; Silva *et al.*, 2019; Wu *et al.*, 2019; Chen and Tarn, 2019).

Indicators of uORF-mediated gene regulation

Regulation of translation is one mode of post-transcriptional gene regulation (PTGR). The presence of PTGR in cells can be inferred by several key cellular features. One such feature is a lack of correlation between peak mRNA levels and protein abundance for a given gene and/or temporal delay between the transcription and the translation of the gene (de Sousa Abreu *et al.*, 2009; Maier *et al.*, 2009; Liu *et al.*, 2016). These can be explained by multiple factors such as stability of mRNA, secondary structure of the transcript, post-translational modifications and *cis*-regulatory elements including uORFs (Araujo *et al.*, 2012; Carpenter *et al.*, 2014). Therefore, while studying regulation of a gene by uORFs, a delay between the transcription and translation of the gene is often used as a preliminary indicator of translational control (Figure 1).

Other indirect indicators that point towards uORF-mediated gene regulation are the proteins/factors that the translation machinery uses to overcome the challenge posed by the uORF. To cope with uORF-mediated translation repression, and allow protein synthesis to occur from the CDS, a cell adopts unconventional mechanisms of translation: reinitiation, leaky scanning, ribosome shunting, and the use of internal ribosome entry sites (IRES) (Reviewed in

Morris and Geballe, 2000; Skabkin *et al.*, 2013; Silva *et al.*, 2019). In brief, reinitiation is a phenomenon where the ribosome, rather than dissociating after termination at a uORF, successfully re-initiates translation at the start codon of the main CDS. Leaky scanning occurs when the ribosome scans past and skips the start codon of the uORF, consequently initiating translation at the start codon of the main CDS (reviewed in Silva *et al.*, 2019). Even though other unconventional translation mechanisms, such as ribosome shunting and IRES have been observed in eukaryotes, they are more prevalent in viruses (Yang and Wang, 2019) and hence, the discussion here will be limited to reinitiation and leaky scanning. Factors that promote reinitiation and leaky scanning are indicators of a dampening of global translation and up-regulation of certain genes. For some genes that are controlled by reinitiation/leaky scanning, there is an involvement of uORFs in regulating gene expression, particularly during a cellular stress response. Therefore, an indirect indicator of uORF-mediated translational control can be the up-regulation and/or modification of factors that regulate either reinitiation by employing reinitiation factors, or leaky scanning by phosphorylating the eukaryotic initiation factor 2 alpha (eIF2α) (Figure 1).

The phosphorylation status of eIF2α was demonstrated for the first time in the case of the GCN4 transcript, in which the translation of the CDS is regulated by uORFs. The choice of translating the CDS rather than the uORFs is driven by phosphorylation of eIF2α (reviewed in Hinnebusch, 2005). Similarly, during the integrated stress response (ISR), phosphorylated SceIF2α promotes translation of genes required for handling the stressor *via* reinitiation (Dever *et al.*, 1992; Lu *et al.*, 2004). Similar mechanisms that involve relieving uORF-mediated repression by phosphorylated eIF2α have been discovered for numerous genes (Vattem and Wek, 2004; Dang Do *et al.*, 2009; Zhao *et al.*, 2010; Palam *et al.*, 2011; Baird *et al.*, 2014; Zach *et al.*, 2014; Aktas *et al.*, 2015; Cnop *et al.*, 2017; Guan *et al.*, 2017; Asano, 2021). The phosphorylation of eIF2α is carried out by members of the eIF2α kinase family (Pakos-

Zebrucka *et al.*, 2016; Wek, 2018; Costa-Mattioli and Walter, 2020) and leads to global inhibition of protein synthesis and up-regulation of genes involved in mediating the adaptive response. These studies indicate that the phosphorylation status of eIF2α is a global indicator for translational regulation of large numbers of genes, some of which could be controlled by uORFs.

A more definitive role for uORFs in translational regulation is provided by the presence of ribosomal footprints on the 5' leader of the transcripts undergoing PTGR (Schneider-Poetsch *et al.*, 2010; Garreau de Loubresse *et al.*, 2014). This provides a snapshot of the dynamics of translation on each transcript by determining the positions of the ribosomes engaged in elongating an ORF (Brar *et al.*, 2012; Ingolia *et al.*, 2014). Such studies in yeast and humans revealed that uORFs are the major contributors of ribosome occupancy in the 5' leaders of transcripts (Calvo *et al.*, 2009; Brar *et al.*, 2012; Ingolia *et al.*, 2014; Johnstone *et al.*, 2016), suggesting that the presence of ribosome footprints in the 5' leader of the transcript is a distinctive feature that indicates PTGR *via* uORFs. Ribosome footprints along the entire length of certain genes show that when the upstream regions are loaded with ribosomes, the CDS has lower ribosome occupancy (Ingolia *et al.*, 2014). These data reinforce the notion that the presence of uORFs stalls the ribosome before it can reach the main CDS, resulting in repression of CDS translation. The conclusive proof of a uORF regulating the translation of a particular gene is provided when mutation of the start codon of the uORF results in a loss of repression/regulation of the gene (Harigai *et al.*, 1996; Reynolds *et al.*, 1996; Ruan *et al.*, 1996; Schlüter *et al.*, 2000; Sarrazin *et al.*, 2000; Diba *et al.*, 2001; Kwon *et al.*, 2001; Jousse *et al.*, 2001; Warnakulasuriyarachchi *et al.*, 2003; Zhang and Dietrich, 2005; Lee *et al.*, 2007; Song *et al.*, 2007; Calvo *et al.*, 2009; Devlin *et al.*, 2010; Spevak *et al.*, 2010; Qiao *et al.*, 2011; Armata *et al.*, 2013; Tennen *et al.*, 2013; Bancells and Deitsch, 2013; Wu *et al.*, 2014; Capell *et al.*, 2014; Kumar *et al.*, 2015; Guerrero-González *et al.*, 2016). The direct and indirect

evidences that lead to the involvement of uORFs in mediating gene expression regulation have been shown in Figure 1.

Translational regulation mediated via uORFs in apicomplexan parasites, Plasmodium falciparum and Toxoplasma gondii

Apicomplexans belong to a large phylum of parasitic alveolates and due to their complex life cycles involving multiple hosts including humans, some members of the phylum cause widespread occurrence of diseases. For example, malaria and toxoplasmosis are caused by *P. falciparum* and *T. gondii* respectively (Jacobs, 1963; Sabin and Olitsky, 1937). These parasites exhibit many developmental stages in different hosts and so must regulate expression of their genes in a highly coordinated fashion for survival and transmission in order to complete their life cycles. Gene expression is regulated at multiple levels, including transcription and translation (White *et al.*, 2014; Vembar *et al.*, 2014, 2015, 2016; Holmes *et al.*, 2017; Bennink and Pradel, 2019; Hollin and Le Roch, 2020; Sharma *et al.*, 2020).

There is evidence for uORFs playing substantive roles in translational control in apicomplexan parasites; this evidence includes high frequencies and a widespread distribution of uORFs among large numbers of genes (Bunnik *et al.*, 2013; Caro *et al.*, 2014; Kumar *et al.*, 2015; Srinivas *et al.*, 2016; Hassan *et al.*, 2017; Holmes *et al.*, 2019; Markus *et al.*, 2021). Additionally, ribosome profiling studies in *P. falciparum* and *T. gondii* parasites reveal footprints in the 5' leader sequences of transcripts (Lacsina *et al.*, 2011; Bunnik *et al.*, 2013; Caro *et al.*, 2014; Hassan *et al.*, 2017; Holmes *et al.*, 2019). Recent discoveries of clinically important genes, such as *var2csa* in *P. falciparum* (Chan *et al.*, 2017) and *BFD1* in *T. gondii* (Waldman *et al.*, 2020) that are regulated translationally by uORFs further reinforce the impact of these small, yet important features in translational regulation of gene expression. In the next sections, the major findings of these and other reports will be summarized and the need to

further understand the phenomenon of uORF-mediated PTGR in apicomplexan parasites will be highlighted in detail.

UPSTREAM ORFS IN PLASMODIUM FALCIPARUM

A long uORF regulates translation of the var2csa gene

The first example of uORF-mediated translational regulation was shown for a gene implicated in pregnancy-associated malaria (PAM), also termed malaria in pregnancy (MiP): *var2csa* (Lavstsen *et al.*, 2003; Amulic *et al.*, 2009; Bancells and Deitsch, 2013). This gene is a variant of the *var* gene family in *P. falciparum* that consists of ~60 *var* genes encoding Erythrocyte Membrane Protein 1 (PfEMP1). These proteins help the parasite evade clearance by the spleen of the host by binding to the endothelial lining of blood vessels (Kraemer and Smith, 2006). The *var* gene family has also been implicated in cerebral malaria, one of the major symptoms of severe malaria caused by *P. falciparum* that results due to sequestration of infected RBCs to capillaries in the brain (reviewed in van der Heyde *et al.*, 2006). This sequestration is due to binding of PfEMP1 proteins to receptors such as CD36, thrombospondin, and intercellular adhesion molecule 1 (ICAM1) found on the surface of different cell types (Baruch *et al.*, 1996; Smith *et al.*, 2000, 2013; Rowe *et al.*, 2009).

The transcription profile of members of this gene family is unusual, with only one of the *var* genes expressed at a given time (Scherf *et al.*, 1998) and a switch of gene expression between different *var* genes occurring at the rate of 0.03-2% (Gatton *et al.*, 2003). The mechanisms that regulate the switch are complex and regulation occurs at various levels including sub-nuclear organisation, epigenetic regulation, *cis*-acting DNA elements, transcriptional regulation as well as translational repression (reviewed in Deitsch and Dzikowski, 2017). Due to the importance of the *var* gene family in multiple clinical manifestations of severe malaria caused by *P. falciparum*, understanding the expression of the

members of this gene family has been of intense interest.

One variant of this gene family that has attracted clinical attention is *var2csa*. Parasites expressing *var2csa* adhere to chondroitin sulphate A (CSA) found in the placenta of pregnant women and block the supply of oxygen and nutrition to the foetus, thereby resulting in pregnancy-associated malaria (PAM) (Salanti *et al.*, 2003, 2004). While mRNA from the *var2csa* gene can be detected in asexual stages, the translation of the VAR2CSA protein is observed only in parasites that are selected for adherence to CSA in lab cultures (Mok *et al.*, 2008; Chan *et al.*, 2017). This lack of correlation between transcription and translation is a clear indicator of PTGR (Figure 2). Interestingly, this PTGR of *var2csa* is regulated by a 360 nucleotide-long uORF in the 5' leader of the transcript (Dzikowski *et al.*, 2007; Amulic *et al.*, 2009; Bancells and Deitsch, 2013).

As expected, the 360 nucleotide-long uORF causes translational repression of the *var2csa* gene, hence, it was of interest to understand how this repression is relieved to express the VAR2CSA protein when required. In cultured parasites, detailed molecular analysis showed that this switch depends on reinitiation of the *var2csa* gene, after the uORF is translated (Bancells and Deitsch, 2013). In parasites derived from placental samples, this reinitiation was shown to occur due the presence of *Plasmodium* Translation Enhancing Factor (PTEF). PTEF is highly up-regulated in parasites sequestered in the placenta and allows efficient reinitiation of translation at the *var2csa* CDS when over-expressed in cultured parasites (Chan *et al.*, 2017).

The molecular factors that lead to the expression of PTEF in a CSA-rich environment are still unknown and further studies need to be undertaken to understand the structure and the interacting partners of PTEF in the asexual stages to provide further clarity regarding its role. These detailed studies are particularly important, as recent reports have shown that the serum of non-pregnant individuals (men and children) contain antibodies recognizing the VAR2CSA protein (Reviewed in Gnidehou and Yanow, 2021). While the authors discuss technical issues

such as cross-reactivity to other proteins that cannot be ruled out, they also mention that deregulation of the uORF-mediated repression of the *var2csa* gene might play a role in these clinical findings.

High prevalence of uORFs in the P. falciparum genome leads to repression of translation

Reports establishing translational regulation of the *var2csa* gene led to an interest in understanding whether this phenomenon was observed in other genes as well. Interestingly, subsequent studies showed that regulation by uORFs could be more prevalent in *P. falciparum* than previously anticipated. For example, the *P. falciparum* transcriptome displays widespread occurrence of uORFs (Caro *et al.*, 2014), with 99% of the transcripts containing at least one uORF in their 5' leader (Kumar *et al.*, 2015). This number is extremely high when compared to human transcripts where only 49% of the transcripts contain at least one uORF in their leader (Calvo *et al.*, 2009). With a prediction of an average of 11 uORFs per CDS (Kaur *et al.*, 2020), *P. falciparum* exhibits the highest ever recorded number of uORFs in a transcript. These high frequencies of uORFs are a reflection of the distinctive genome of *P. falciparum* whose composition is skewed heavily towards adenine (A) and thymine (T) nucleotides (Gardner *et al.*, 2002). This leads to a high probability of finding AT-rich start and stop codons in the 5′ leader sequence, thereby giving rise to uAUGs and uORFs. The presence of multiple uAUGs and uORFs causes translational repression of the main CDS, posing a significant challenge to the parasite's cytoplasmic translation machinery (Kumar *et al.*, 2015).

   Each uORF has a very different ability to engage the scanning ribosome and experimental approaches have been used to study the features that contribute to the repressive capacity of uORFs. Such features are the Kozak sequence, codon composition, length of the uORF, and the distance between the uORF and the CDS (Kaur *et al.*, 2020). Further, bioinformatics was used to predict translatability of an ORF (therefore, the repressiveness of the uORF) by

calculating the probability of translation initiation and elongation of ORFs in the *P. falciparum* genome (Srinivas *et al.*, 2016). The model utilizes positional features comprising of the Kozak sequence and compositional features comprising of the codon topography of the ORF to predict translation initiation and elongation probabilities of the ORF respectively.

The notion of translation repression by certain uORFs is reinforced by the presence of ribosome footprints on the 5' leaders of transcripts expressed in the intra-erythrocytic asexual stages of *P. falciparum*, supporting the hypothesis that these huge numbers of uORFs are able to engage ribosomes. Ribosome profiling studies found rampant occurrence of ribosome density associated with 5′ leaders of transcripts compared to the 3' UTRs (Bunnik *et al.*, 2013; Caro *et al.*, 2014). The data suggest active translation of uORFs present in the 5' leader sequence, with some instances where transcripts showed a higher abundance of ribosome footprints on the 5' leader than on the CDS, leading to low translational efficiency of the CDS (Caro *et al.*, 2014). As the repressive capability of different uORFs has now been assessed by experimental/bioinformatics analysis and ribosome profiling, a holistic analysis of all these genome-wide datasets would shed light on specific uORFs regulating expression of classes of genes. Based on the evidence that uORF-mediated regulation is a strategy employed to handle stress responses, such classes of genes might be physiologically relevant in host-pathogen interactions and establishing the pathogenicity of this parasite.

Another indicator of translational control of gene expression, a delay between the peak of transcript abundance and translation of those transcripts, has also been observed in *P. falciparum*. Early reports of a cascade of gene expression in synchrony with the asexual life cycle stages (Bozdech *et al.*, 2003; Foth *et al.*, 2011) suggested that transcription occurs only when the protein is required. Subsequent studies showed an absence of correlation between the peaks of transcripts and their encoded protein products for ~30% of the genes (Le Roch *et al.*, 2004; Bunnik *et al.*, 2013), indicative of PTGR for these genes. Interestingly, the *var* gene

family that is under multiple forms of regulation, has ~5 times more uAUGs and uORFs than other genes (Kumar *et al.*, 2015).

Translation repression of the downstream gene in the presence of uORFs can be alleviated by unconventional translation mechanisms: reinitiation and/or other mechanisms including leaky scanning. The role of reinitiation in translation of VAR2CSA during PAM has been discussed in the previous section. However, the use of non-canonical translation mechanisms to circumvent translation repression caused by the uORFs is not limited to this gene. Indeed, it was demonstrated that reinitiation occurs in the case of the hsp70 gene in the presence of a native uORF and synthetic uORFs suggesting that there is widespread occurrence of reinitiation in the asexual stages of *P. falciparum* (Kaur *et al.*, 2020).

More and more evidence points towards uORFs playing roles in translational regulation during the asexual stages of the intra-erythrocytic developmental cycle (IDC). As there may be parasite-specific proteins, such as PTEF, that are required for handling huge numbers of uORFs, a better understanding of PTGR by uORFs may lead to new targets of development of therapeutic interventions for the asexual stages of *P. falciparum* that are the major cause of the clinical symptoms of malaria.

Upstream ORFs in stress conditions

The role of uORFs in the stress response in yeast and mammals is well studied (Hinnebusch, 2005; Silva *et al.*, 2019; Houston *et al.*, 2020). However, this area of research requires more focus in *P. falciparum*, more so because of the widespread occurrence of uORFs. During its complicated life cycle, *P. falciparum* faces a variety of external conditions that are hostile to the parasite. As is the case with other parasites, *P. falciparum* has also evolved to use complex strategies to adapt to the changing environment (Camus *et al.*, 1995). While the shift of host from mosquito to human is one of the major challenges faced by the parasite due to drastic

differences in the two hosts' biology, understanding how the parasite responds to various stress conditions that it faces in the human erythrocytes holds importance from the clinical perspective of malaria treatment.

During the IDC, *P. falciparum* experiences a periodic rise in temperature every 48 hours due to the host inflammatory response (Brown, 1912). The temperature during these febrile episodes can elevate to 40-41°C (Kwiatkowski, 1989). The adaptive response to the cyclical heat stress experienced by intra-erythrocytic parasites has been studied at the level of the transcriptome (Oakley *et al.*, 2007; Rawat *et al.*, 2019). However, as translational responses afford a rapid adaptation mechanism, it would be informative to study whether uORFs play a role in heat stress by checking the phosphorylation status of PfeIF2α and differential ribosome occupancy during this stress condition.

Another stress faced by *P. falciparum* during its intra-erythrocytic cycle is the lack of essential amino acids, especially isoleucine. This stress arises from the fact that inside the red blood cell, the parasite salvages amino acids by degrading haemoglobin (Francis *et al.*, 1997). However, of the twenty amino acids, isoleucine is completely absent in the α and β chains of haemoglobin (Sherman, 1977). Therefore, the parasite depends on an exogenous supply of isoleucine through the plasma of the host (Liu *et al.*, 2006). Since isoleucine is an essential amino acid, the human host also depends on external sources of isoleucine to survive (Soeters *et al.*, 2004) and in situations of malnourishment, isoleucine pools in the human host can drop significantly (Baertl *et al.*, 1974).

Lack of an exogenous supply of isoleucine can lead to a delayed-growth phenotype, where the parasites enter a hibernatory state as a response (Babbitt *et al.*, 2012). This response has been linked to phosphorylation of PfeIF2α *via* PfeIK1, an orthologue of GCN2 that is responsible for phosphorylation of eIF2α under nutrient starvation conditions in yeast (Hinnebusch, 2005; Fennell *et al.*, 2009; Babbitt *et al.*, 2012). A possible role of uORFs in

translation of the genes required for adaptive response to this nutritional stress faced by *P. falciparum* can be illustrated by identifying genes having differential ribosome occupancy in parasites that are deprived of isoleucine. Further, ribosome profiling of PfeIK1 knock-out parasites would also reveal classes of genes that are under regulation by uORFs.

There is preliminary evidence to support the notion that translational regulation mediated by uORFs occurs during isoleucine starvation stress. The Maf1 protein is a part of the Target of Rapamycin Complex 1 (TORC1) pathway that responds to stress caused by nutrient deprivation in *S. cerevisiae* and mammals (Loewith and Hall, 2011). Maf1 represses transcription of highly abundant tRNAs and ribosomal RNAs through its function as a regulator of RNA polymerase III (Upadhya *et al.*, 2002; Boguta, 2013; Moir and Willis, 2015). In nutrient-rich conditions, Maf1 remains inactive due to phosphorylation (Pluta *et al.*, 2001; Shor *et al.*, 2010), while under starvation conditions, it is de-phosphorylated and the activated protein binds and inhibits RNA polymerase III (Vannini *et al.*, 2010). Although the majority of proteins involved in the TORC1 pathway have been lost in the *Plasmodium* genus during genome reduction, an orthologue of Maf1 has been identified in *P. falciparum* that is essential for its recovery from the hibernatory state induced due to isoleucine starvation (Serfontein *et al.*, 2010; McLean and Jacobs-Lorena, 2017). Ribosome profiling data show that Maf1 CDS is poorly translated despite being transcribed in all stages of IDC (Caro *et al.*, 2014). However, a significant presence of ribosome footprints on the 5' leader sequence of the transcript points towards a possible uORF-mediated regulation that needs to be further investigated (Figure 2).

Another physiologically important stressor is treatment with anti-malarial drugs as they constitute a source of oxidative damage to the parasite. For example, chloroquine and artemisinin induce free radical production (Pandey *et al.*, 2001; Haynes and Krishna, 2004; Zhang *et al.*, 2010). Interestingly, parasites treated with dihydroartemisinin (DHA), a derivative of artemisinin show enhanced phosphorylation of eIF2α, a key regulator of stress

adaptation (Zhang *et al.*, 2017), suggesting a possible role of PTGR in overcoming the drug-induced stress. Increased cases of resistance to anti-malarial drugs suggest that parasites have evolved to enhance their adaptive response to drug-induced stress, thus decreasing drug susceptibility (Rocamora *et al.*, 2018). This has been shown in the case of artemisinin, where increased levels of phosphorylated PfeIF2α induces latency in parasites, thereby causing then to re-emerge later when the drug pressure has subsided (Zhang *et al.*, 2017). These studies could be extended by identifying genes that have repressive uORFs due to enhanced ribosome occupancy.

Clearly, there are gaps in our understanding of the adaptation responses mounted by *P. falciparum* during these stress conditions. Filling in these gaps by studying the role of uORFs in stress responses would be necessary to gain deeper insights into parasite biology, especially in conditions of clinical relevance.

UPSTREAM ORFS IN TOXOPLASMA GONDII

Ribosome profiling in T. gondii points towards widespread translational regulation by uORFs

Similar to *P. falciparum*, the transcripts of *T. gondii* also have widespread occurrence of uORFs. At least one uORF has been predicted in 90% of transcripts with annotated 5' leader sequences (Markus *et al.*, 2021). This number is 1.8 times higher than the reported number in human transcripts where only 49% of the transcripts contain at least one uORF in their leader sequence (Calvo *et al.*, 2009). Evidence of translation occurring in the 5' leaders of transcripts in *T. gondii* has been provided in two recent ribosome profiling studies that demonstrate a high prevalence of ribosome footprints on the long 5' leaders of transcripts (Hassan *et al.*, 2017; Holmes *et al.*, 2019). Ribosomal occupancy on uORFs is indicative of the fact that ribosomes are engaged in translating uORFs rather than the CDS, thereby exerting translational control

over the expression of the gene.

In an attempt to study translational control of genes that provide an adaptive advantage to the stress posed by the extracellular environment, comparative ribosome profiling of extracellular and intracellular tachyzoites was performed. This study identified more than a thousand genes that vary at the level of ribosome occupancy in intracellular and extracellular parasites, implying there is a widespread usage of translational regulation to cope with the stress imposed by the extracellular environment on *T. gondii*. Additionally, this study confirmed changes in gene expression between the two sets of parasites occurring at the translation level, involving regulation by uORFs and mRNA secondary structure (Hassan *et al.*, 2017). The widespread occurrence of uORFs warrants further examination of the role played by uORFs in mediating stress response.

Translation regulation of the arginine transporter TgApiAT1

T. gondii depends on nutrients derived from its host (Coppens, 2014; Zuzarte-Luís and Mota, 2018) and a nutrient for which the parasite is auxotrophic is the amino acid arginine (Fox *et al.*, 2004). Hence, there is a dedicated plasma membrane transporter (TgApiAT1) for the uptake of arginine (Rajendran *et al.*, 2017). Depletion of this amino acid results in the formation of bradyzoites, the latent forms of this parasite (Fox *et al.*, 2004; Butcher *et al.*, 2011). In order to maintain the virulent tachyzoite stages and cause infection, parasites need to sense the availability of arginine and respond accordingly to maintain the intracellular levels of arginine by regulating the expression of the transporter TgApiAT1.

The arginine-dependent expression of TgApiAT1 is mediated *via* an upstream ORF present in the 5' leader sequence of the transcript (Rajendran *et al.*, 2019) (Figure 2). The uORF codes for a conserved peptide that is hypothesized to function in a similar manner to the arginine attenuator peptide found in *S. cerevisiae* (ScAAP) (Rajendran *et al.*, 2019). The

ScAAP stalls the ribosome and prevents it from reaching the downstream CDS in arginine-rich conditions. Conversely, in the conditions of arginine scarcity, ribosomes are able to reach and translate the downstream CDS (Wu *et al.*, 2012; Wei *et al.*, 2012). A similar switch is used by *T. gondii* for modulating the TgApiAT1-dependent uptake of arginine in varying arginine conditions (Rajendran *et al.*, 2019). Given the extensive occurrence of uORFs in *T. gondii*, we believe that this might be among the first of many studies that unravel the existence of uORF-mediated translational regulation.

Upstream ORFs play a crucial role in development of latent cysts in T. gondii

Inside the intermediate mammalian host, *T. gondii* parasites divide asexually to form tachyzoites, which develop into tissue cyst bradyzoites under certain conditions (reviewed in Cerutti *et al.*, 2020). Bradyzoites are the latent stages of *T. gondii* that persist and cause reinfection when the immune system of the host lapses (Dubey, 1998; Montoya and Liesenfeld, 2004). While the host immune response can lead to stress that initiates bradyzoite formation *in vivo* (Bohne *et al.*, 1993; Lüder *et al.*, 1999), conversion of tachyzoites to bradyzoites *in vitro* can be induced under various stress conditions, such as pH change, heat shock, nutritional stress, stress to the endoplasmic reticulum (ER), mitochondrial inhibition, presence of nitric oxide, signalling through secondary messengers such as cAMP, and other *in vivo* factors (Bohne *et al.*, 1993; Soete *et al.*, 1993; Weiss *et al.*, 1995, 1998; Dubey, 1998; Kirkman *et al.*, 2001; Fox *et al.*, 2004; Narasimhan *et al.*, 2008). Stage conversion that can be triggered by a multitude of external stressors is highly reminiscent of an integrated stress response (ISR) that is controlled by uORFs in other eukaryotes (Reviewed in Young and Wek, 2016).

Another indicator of translational regulation, possibly through uORFs, is phosphorylation of eIF2α which has also been reported for bradyzoite conversion. TgIF2α is phosphorylated during alkaline stress when the developmental shift from tachyzoite to bradyzoite occurs

(Sullivan *et al.*, 2004; Narasimhan *et al.*, 2008). Disruption of this phosphorylation by either deleting TgIF2KB (Augusto *et al.*, 2020) or inhibiting TgIF2KA (Augusto *et al.*, 2018), both kinases responsible for phosphorylating TgIF2α, leads to significant loss of stage conversion.

The molecular factor responsible for the stage conversion was unidentified until the recent discovery of a master regulator, the Bradyzoite Formation Deficient 1 (BFD1) protein that encodes a transcription factor, which triggers the conversion of tachyzoites to the latent tissue cyst form (Waldman *et al.*, 2020). Stress-dependent expression of BFD1 appears to be regulated at the translational level because although the transcript is detected both in tachyzoites and in bradyzoites (a marginal 1.5- to 3.6-fold upregulation in bradyzoites) the protein is expressed only in bradyzoites (Waldman *et al.*, 2020) (Figure 2).

As bradyzoites can be formed in culture by a variety of stressors and their stage conversion coincides with the phosphorylation of TgIF2α, it would not be far-fetched to infer that uORFs play a role in the process. Most satisfyingly, evidence for the involvement of uORFs in translational regulation was provided by the observation that parasites expressing BFD1 without its 5' leader can differentiate into bradyzoites even in the absence of any stress. This strongly alludes to the presence of regulatory *cis*-acting elements in the 5' leader that act as a switch to turn on gene expression under stress conditions. The translational switch of the gene has been hypothesized to be under the control of four uORFs present in its 2.7 kb-long 5' leader sequence (Waldman *et al.*, 2020).

Understanding the control of BFD1 gene expression will have crucial implications from a clinical perspective, as it would allow development of drugs that inhibit the conversion of tachyzoites to persistent bradyzoites. Even though the number of parasites that transition from tachyzoites to bradyzoites can be suppressed by the small molecules tanshinone IIA and hydroxyzine (Murata *et al.*, 2017), there are no known drugs that eradicate them. It would be possible to develop such drugs if light could be shed on the molecular mechanism that controls

the switch to bradyzoite formation.

CONCLUDING REMARKS

Given the sheer number of uORFs and wide prevalence of ribosomal footprints on the 5′ leader sequences in the Apicomplexan parasites, *P. falciparum* and *T. gondii*, their role in mediating translational regulation is certainly under-recognized. Efforts to understand translational regulation in these parasites is gradually gaining momentum (reviewed in Rao *et al.*, 2017) and in this review we highlight selected examples of genes that are regulated by uORFs giving rise to clinically relevant patho-physiology in the life cycles of these parasites. Due to the requirement of novel translation factors that promote non-canonical strategies of handling the "hurdles" created by uORFs, such as reinitiation and leaky scanning, further research in this area may lead to the identification of parasite-specific, essential proteins that might serve as drug targets for therapeutics. We conclude by predicting that, with transcriptome, proteome, ribosome profiling and bioinformatics analyses giving genome-wide pointers towards genes and pathways that might be subjected to uORF-mediated PTGR, the role of uORFs in regulating translation will surely be an area of intense research in the future.

FINANCIAL SUPPORT

CONFLICTS OF INTEREST

The authors declare there are no conflicts of interest.

ETHICAL STANDARDS

Not applicable

REFERENCES

Aktas, B. H., Bordelois, P., Peker, S., Merajver, S. and Halperin, J. A. (2015). Depletion of eIF2·GTP·Met-tRNAi translation initiation complex up-regulates BRCA1 expression in vitro and in vivo. *Oncotarget* **6**, 6902–6914. doi: 10.18632/oncotarget.3125.

Amulic, B., Salanti, A., Lavstsen, T., Nielsen, M. A. and Deitsch, K. W. (2009). An Upstream Open Reading Frame Controls Translation of *var2csa*, a Gene Implicated in Placental Malaria. *PLoS Pathogens* **5**, e1000256. doi: 10.1371/journal.ppat.1000256.

Araujo, P. R., Yoon, K., Ko, D., Smith, A. D., Qiao, M., Suresh, U., Burns, S. C. and Penalva, L. O. F. (2012). Before It Gets Started: Regulating Translation at the 5′ UTR. *Comparative and Functional Genomics* **2012**, 1–8. doi: 10.1155/2012/475731.

Armata, I. A., Balaj, L., Kuster, J. K., Zhang, X., Tsai, S., Armatas, A. A., Multhaupt-Buell, T. J., Soberman, R., Breakefield, X. O., Ichinose, H. and Sharma, N. (2013). Dopa-responsive dystonia: functional analysis of single nucleotide substitutions within the 5' untranslated GCH1 region. *PloS One* **8**, e76975. doi: 10.1371/journal.pone.0076975.

Asano, K. (2021). Origin of translational control by eIF2α phosphorylation: insights from genome-wide translational profiling studies in fission yeast. *Current Genetics*. doi: 10.1007/s00294-020-01149-w.

Augusto, L., Martynowicz, J., Staschke, K. A., Wek, R. C. and Sullivan, W. J. (2018). Effects of PERK eIF2α Kinase Inhibitor against *Toxoplasma gondii*. *Antimicrobial Agents and Chemotherapy* **62**,. doi: 10.1128/AAC.01442-18.

Augusto, L., Martynowicz, J., Amin, P. H., Carlson, K. R., Wek, R. C. and Sullivan, W. J. (2020). *TgIF2K-B is an eIF2α kinase in Toxoplasma gondii that responds to oxidative stress and optimizes pathogenicity*, preprint. Microbiology doi: 10.1101/2020.09.24.312744.

Babbitt, S. E., Altenhofen, L., Cobbold, S. A., Istvan, E. S., Fennell, C., Doerig, C., Llinas, M. and Goldberg, D. E. (2012). *Plasmodium falciparum* responds to amino acid starvation by entering into a hibernatory state. *Proceedings of the National Academy of Sciences* **109**, E3278–E3287. doi: 10.1073/pnas.1209823109.

Baertl, J. M., Placko, R. P. and Graham, G. G. (1974). Serum proteins and plasma free amino acids in severe malnutrition. *The American Journal of Clinical Nutrition* **27**, 733–742. doi: 10.1093/ajcn/27.7.733.

Baird, T. D., Palam, L. R., Fusakio, M. E., Willy, J. A., Davis, C. M., McClintick, J. N., Anthony, T. G. and Wek, R. C. (2014). Selective mRNA translation during eIF2 phosphorylation induces expression of IBTKα. *Molecular Biology of the Cell* **25**, 1686–1697. doi: 10.1091/mbc.E14-02-0704.

Bancells, C. and Deitsch, K. W. (2013). A molecular switch in the efficiency of translation reinitiation controls expression of *var2csa* , a gene implicated in pregnancy-associated malaria: *var2csa* is expressed by translation reinitiation. *Molecular Microbiology* **90**, 472–488. doi: 10.1111/mmi.12379.

Barbosa, C., Peixeiro, I. and Romão, L. (2013). Gene expression regulation by upstream open reading frames and human disease. *PLoS genetics* **9**, e1003529. doi: 10.1371/journal.pgen.1003529.

Baruch, D. I., Gormely, J. A., Ma, C., Howard, R. J. and Pasloske, B. L. (1996). *Plasmodium falciparum* erythrocyte membrane protein 1 is a parasitized erythrocyte receptor for adherence to CD36, thrombospondin, and intercellular adhesion molecule 1. *Proceedings of the National Academy of Sciences of the United States of America* **93**, 3497–3502. doi: 10.1073/pnas.93.8.3497.

Bennink, S. and Pradel, G. (2019). The molecular machinery of translational control in malaria parasites. *Molecular Microbiology* **112**, 1658–1673. doi: 10.1111/mmi.14388.

Boguta, M. (2013). Maf1, a general negative regulator of RNA polymerase III in yeast. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms* **1829**, 376–384. doi: 10.1016/j.bbagrm.2012.11.004.

Bohne, W., Heesemann, J. and Gross, U. (1993). Induction of bradyzoite-specific *Toxoplasma gondii* antigens in gamma interferon-treated mouse macrophages. *Infection and Immunity* **61**, 1141–1145. doi: 10.1128/IAI.61.3.1141-1145.1993.

Bozdech, Z., Llinás, M., Pulliam, B. L., Wong, E. D., Zhu, J. and DeRisi, J. L. (2003). The Transcriptome of the Intraerythrocytic Developmental Cycle of *Plasmodium falciparum*. *PLoS Biology* **1**, e5. doi: 10.1371/journal.pbio.0000005.

Brar, G. A., Yassour, M., Friedman, N., Regev, A., Ingolia, N. T. and Weissman, J. S. (2012). High-resolution view of the yeast meiotic program revealed by ribosome profiling. *Science (New York, N.Y.)* **335**, 552–557. doi: 10.1126/science.1215110.

Brown, W. H. (1912). MALARIAL PIGMENT (HEMATIN) AS A FACTOR IN THE PRODUCTION OF THE MALARIAL PAROXYSM. *The Journal of Experimental Medicine* **15**, 579–597. doi: 10.1084/jem.15.6.579.

Bunnik, E. M., Chung, D.-W. D., Hamilton, M., Ponts, N., Saraf, A., Prudhomme, J., Florens, L. and Le Roch, K. G. (2013). Polysome profiling reveals translational control of gene expression in the human malaria parasite *Plasmodium falciparum*. *Genome Biology* **14**, R128. doi: 10.1186/gb-2013-14-11-r128.

Butcher, B. A., Fox, B. A., Rommereim, L. M., Kim, S. G., Maurer, K. J., Yarovinsky, F., Herbert, D. R., Bzik, D. J. and Denkers, E. Y. (2011). *Toxoplasma gondii* rhoptry kinase ROP16 activates STAT3 and STAT6 resulting in cytokine inhibition and arginase-1-dependent growth control. *PLoS pathogens* **7**, e1002236. doi: 10.1371/journal.ppat.1002236.

Calvo, S. E., Pagliarini, D. J. and Mootha, V. K. (2009). Upstream open reading frames cause widespread reduction of protein expression and are polymorphic among humans. *Proceedings of the National Academy of Sciences* **106**, 7507–7512. doi: 10.1073/pnas.0810916106.

Camus, D., Zalis, M. G., Vannier-Santos, M. A. and Banic, D. M. (1995). The art of parasite survival. *Brazilian Journal of Medical and Biological Research = Revista Brasileira De Pesquisas Medicas E Biologicas* **28**, 399–413.

Capell, A., Fellerer, K. and Haass, C. (2014). Progranulin transcripts with short and long 5' untranslated regions (UTRs) are differentially expressed *via* posttranscriptional and translational repression. *The Journal of Biological Chemistry* **289**, 25879–25889. doi: 10.1074/jbc.M114.560128.

Caro, F., Ahyong, V., Betegon, M. and DeRisi, J. L. (2014). Genome-wide regulatory dynamics of translation in the *Plasmodium falciparum* asexual blood stages. *eLife* **3**,. doi: 10.7554/eLife.04106.

Carpenter, S., Ricci, E. P., Mercier, B. C., Moore, M. J. and Fitzgerald, K. A. (2014). Post-transcriptional regulation of gene expression in innate immunity. *Nature Reviews Immunology* **14**, 361–376. doi: 10.1038/nri3682.

Cerutti, A., Blanchard, N. and Besteiro, S. (2020). The Bradyzoite: A Key Developmental Stage for the Persistence and Pathogenesis of Toxoplasmosis. *Pathogens (Basel, Switzerland)* **9**,. doi: 10.3390/pathogens9030234.

Chan, S., Frasch, A., Mandava, C. S., Ch'ng, J.-H., Quintana, M. del P., Vesterlund, M., Ghorbal, M., Joannin, N., Franzén, O., Lopez-Rubio, J.-J., Barbieri, S., Lanzavecchia, A., Sanyal, S. and Wahlgren, M. (2017). Regulation of PfEMP1–VAR2CSA translation by a Plasmodium translation-enhancing factor. *Nature Microbiology* **2**, 17068. doi: 10.1038/nmicrobiol.2017.68.

Chen, H.-H. and Tarn, W.-Y. (2019). uORF-mediated translational control: recently elucidated mechanisms and implications in cancer. *RNA biology* **16**, 1327–1338. doi: 10.1080/15476286.2019.1632634.

Chew, G.-L., Pauli, A., Rinn, J. L., Regev, A., Schier, A. F. and Valen, E. (2013). Ribosome profiling reveals resemblance between long non-coding RNAs and 5' leaders of coding RNAs. *Development (Cambridge, England)* **140**, 2828–2834. doi: 10.1242/dev.098343.

Cnop, M., Toivonen, S., Igoillo-Esteve, M. and Salpea, P. (2017). Endoplasmic reticulum stress and eIF2α phosphorylation: The Achilles heel of pancreatic β cells. *Molecular Metabolism* **6**, 1024–1039. doi: 10.1016/j.molmet.2017.06.001.

Coppens, I. (2014). Exploitation of auxotrophies and metabolic defects in *Toxoplasma* as therapeutic approaches. *International Journal for Parasitology* **44**, 109–120. doi: 10.1016/j.ijpara.2013.09.003.

Costa-Mattioli, M. and Walter, P. (2020). The integrated stress response: From mechanism to disease. *Science (New York, N.Y.)* **368**,. doi: 10.1126/science.aat5314.

Dang Do, A. N., Kimball, S. R., Cavener, D. R. and Jefferson, L. S. (2009). eIF2alpha kinases GCN2 and PERK modulate transcription and translation of distinct sets of mRNAs in mouse liver. *Physiological Genomics* **38**, 328–341. doi: 10.1152/physiolgenomics.90396.2008.

de Sousa Abreu, R., Penalva, L. O., Marcotte, E. M. and Vogel, C. (2009). Global signatures of protein and mRNA expression levels. *Molecular BioSystems* 10.1039.b908315d. doi: 10.1039/b908315d.

Deitsch, K. W. and Dzikowski, R. (2017). Variant Gene Expression and Antigenic Variation by Malaria Parasites. *Annual Review of Microbiology* **71**, 625–641. doi: 10.1146/annurev-micro-090816-093841.

Dever, T. E., Feng, L., Wek, R. C., Cigan, A. M., Donahue, T. F. and Hinnebusch, A. G. (1992). Phosphorylation of initiation factor 2 alpha by protein kinase GCN2 mediates gene-specific translational control of GCN4 in yeast. *Cell* **68**, 585–596. doi: 10.1016/0092-8674(92)90193-g.

Devlin, A. H., Thompson, P., Robson, T. and McKeown, S. R. (2010). Cytochrome P450 1B1 mRNA untranslated regions interact to inhibit protein translation. *Molecular Carcinogenesis* **49**, 190–199. doi: 10.1002/mc.20589.

Diba, F., Watson, C. S. and Gametchu, B. (2001). 5'UTR sequences of the glucocorticoid receptor 1A transcript encode a peptide associated with translational regulation of the glucocorticoid receptor. *Journal of Cellular Biochemistry* **81**, 149–161.

Dubey, J. P. (1998). Advances in the life cycle of *Toxoplasma gondii*. *International Journal for Parasitology* **28**, 1019–1024. doi: 10.1016/S0020-7519(98)00023-X.

Dzikowski, R., Li, F., Amulic, B., Eisberg, A., Frank, M., Patel, S., Wellems, T. E. and Deitsch, K. W. (2007). Mechanisms underlying mutually exclusive expression of virulence genes by malaria parasites. *EMBO reports* **8**, 959–965. doi: 10.1038/sj.embor.7401063.

Fennell, C., Babbitt, S., Russo, I., Wilkes, J., Ranford-Cartwright, L., Goldberg, D. E. and Doerig, C. (2009). PfeIK1, a eukaryotic initiation factor 2α kinase of the human malaria parasite *Plasmodium falciparum*, regulates stress-response to amino-acid starvation. *Malaria Journal* **8**, 99. doi: 10.1186/1475-2875-8-99.

Foth, B. J., Zhang, N., Chaal, B. K., Sze, S. K., Preiser, P. R. and Bozdech, Z. (2011). Quantitative time-course profiling of parasite and host cell proteins in the human malaria parasite *Plasmodium falciparum*. *Molecular & cellular proteomics: MCP* **10**, M110.006411. doi: 10.1074/mcp.M110.006411.

Fox, B. A., Gigley, J. P. and Bzik, D. J. (2004). *Toxoplasma gondii* lacks the enzymes required for de novo arginine biosynthesis and arginine starvation triggers cyst formation. *International Journal for Parasitology* **34**, 323–331. doi: 10.1016/j.ijpara.2003.12.001.

Francis, S. E., Sullivan, D. J. and Goldberg, D. E. (1997). Hemoglobin metabolism in the malaria parasite *Plasmodium falciparum*. *Annual Review of Microbiology* **51**, 97–123. doi: 10.1146/annurev.micro.51.1.97.

Gardner, M. J., Hall, N., Fung, E., White, O., Berriman, M., Hyman, R. W., Carlton, J. M., Pain, A., Nelson, K. E., Bowman, S., Paulsen, I. T., James, K., Eisen, J. A., Rutherford, K., Salzberg, S. L., Craig, A., Kyes, S., Chan, M.-S., Nene, V., Shallom, S. J., Suh, B., Peterson, J., Angiuoli, S., Pertea, M., Allen, J., Selengut, J., Haft, D., Mather, M. W., Vaidya, A. B., Martin, D. M. A., Fairlamb, A. H., Fraunholz, M. J., Roos, D. S., Ralph, S. A., McFadden, G. I., Cummings, L. M., Subramanian, G. M., Mungall, C., Venter, J. C., Carucci, D. J., Hoffman, S. L., Newbold, C., Davis, R. W., Fraser, C. M. and Barrell, B. (2002). Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* **419**, 498–511. doi: 10.1038/nature01097.

Garreau de Loubresse, N., Prokhorova, I., Holtkamp, W., Rodnina, M. V., Yusupova, G. and Yusupov, M. (2014). Structural basis for the inhibition of the eukaryotic ribosome. *Nature* **513**, 517–522. doi: 10.1038/nature13737.

Gatton, M. L., Peters, J. M., Fowler, E. V. and Cheng, Q. (2003). Switching rates of *Plasmodium falciparum var* genes: faster than we thought? *Trends in Parasitology* **19**, 202–208. doi: 10.1016/s1471-4922(03)00067-9.

Gnidehou, S. and Yanow, S. K. (2021). VAR2CSA Antibodies in Non-Pregnant Populations. *Trends in Parasitology* **37**, 65–76. doi: 10.1016/j.pt.2020.09.012.

Guan, B.-J., van Hoef, V., Jobava, R., Elroy-Stein, O., Valasek, L. S., Cargnello, M., Gao, X.-H., Krokowski, D., Merrick, W. C., Kimball, S. R., Komar, A. A., Koromilas, A. E., Wynshaw-Boris, A., Topisirovic, I., Larsson, O. and Hatzoglou, M. (2017). A Unique ISR Program Determines Cellular Responses to Chronic Stress. *Molecular Cell* **68**, 885-900.e6. doi: 10.1016/j.molcel.2017.11.007.

Guerrero-González, M. de la L., Ortega-Amaro, M. A., Juárez-Montiel, M. and Jiménez-Bremont, J. F. (2016). Arabidopsis Polyamine oxidase-2 uORF is required for downstream translational regulation. *Plant physiology and biochemistry: PPB* **108**, 381–390. doi: 10.1016/j.plaphy.2016.08.006.

Harigai, M., Miyashita, T., Hanada, M. and Reed, J. C. (1996). A *cis*-acting element in the BCL-2 gene controls expression through translational mechanisms. *Oncogene* **12**, 1369–1374.

Hassan, M. A., Vasquez, J. J., Guo-Liang, C., Meissner, M. and Nicolai Siegel, T. (2017). Comparative ribosome profiling uncovers a dominant role for translational control in *Toxoplasma gondii*. *BMC genomics* **18**, 961. doi: 10.1186/s12864-017-4362-6.

Haynes, R. K. and Krishna, S. (2004). Artemisinins: activities and actions. *Microbes and Infection* **6**, 1339–1346. doi: 10.1016/j.micinf.2004.09.002.

Hinnebusch, A. G. (1988). Mechanisms of gene regulation in the general control of amino acid biosynthesis in *Saccharomyces cerevisiae*. *Microbiological Reviews* **52**, 248–273.

Hinnebusch, A. G. (2005). Translational regulation of GCN4 and the general amino acid control of yeast. *Annual Review of Microbiology* **59**, 407–450. doi: 10.1146/annurev.micro.59.031805.133833.

Hinnebusch, A. G. (2011). Molecular mechanism of scanning and start codon selection in eukaryotes. *Microbiology and molecular biology reviews: MMBR* **75**, 434–467, first page of table of contents. doi: 10.1128/MMBR.00008-11.

Hollin, T. and Le Roch, K. G. (2020). From Genes to Transcripts, a Tightly Regulated Journey in *Plasmodium. Frontiers in Cellular and Infection Microbiology* **10**, 618454. doi: 10.3389/fcimb.2020.618454.

Holmes, M. J., Augusto, L. da S., Zhang, M., Wek, R. C. and Sullivan, W. J. (2017). Translational Control in the Latency of Apicomplexan Parasites. *Trends in Parasitology* **33**, 947–960. doi: 10.1016/j.pt.2017.08.006.

Holmes, M. J., Shah, P., Wek, R. C. and Sullivan, W. J. (2019). Simultaneous Ribosome Profiling of Human Host Cells Infected with *Toxoplasma gondii*. *mSphere* **4**,. doi: 10.1128/mSphere.00292-19.

Houston, R., Sekine, S. and Sekine, Y. (2020). The coupling of translational control and stress responses. *Journal of Biochemistry* **168**, 93–102. doi: 10.1093/jb/mvaa061.

Iacono, M., Mignone, F. and Pesole, G. (2005). uAUG and uORFs in human and rodent 5'untranslated mRNAs. *Gene* **349**, 97–105. doi: 10.1016/j.gene.2004.11.041.

Ingolia, N. T., Brar, G. A., Stern-Ginossar, N., Harris, M. S., Talhouarne, G. J. S., Jackson, S. E., Wills, M. R. and Weissman, J. S. (2014). Ribosome profiling reveals pervasive translation outside of annotated protein-coding genes. *Cell Reports* **8**, 1365–1379. doi: 10.1016/j.celrep.2014.07.045.

Johnstone, T. G., Bazzini, A. A. and Giraldez, A. J. (2016). Upstream ORFs are prevalent translational repressors in vertebrates. *The EMBO journal* **35**, 706–723. doi: 10.15252/embj.201592759.

Jousse, C., Bruhat, A., Carraro, V., Urano, F., Ferrara, M., Ron, D. and Fafournoux, P. (2001). Inhibition of CHOP translation by a peptide encoded by an open reading frame localized in the chop 5'UTR. *Nucleic Acids Research* **29**, 4341–4351. doi: 10.1093/nar/29.21.4341.

Kaur, C., Kumar, M. and Patankar, S. (2020). Messenger RNAs with large numbers of upstream open reading frames are translated *via* leaky scanning and reinitiation in the asexual stages of *Plasmodium falciparum*. *Parasitology* **147**, 1100–1113. doi: 10.1017/S0031182020000840.

Kirkman, L. A., Weiss, L. M. and Kim, K. (2001). Cyclic nucleotide signaling in *Toxoplasma gondii* bradyzoite differentiation. *Infection and Immunity* **69**, 148–153. doi: 10.1128/IAI.69.1.148-153.2001.

Kozak, M. (1980). Evaluation of the "scanning model" for initiation of protein synthesis in eucaryotes. *Cell* **22**, 7–8. doi: 10.1016/0092-8674(80)90148-8.

Kozak, M. (1991). Structural features in eukaryotic mRNAs that modulate the initiation of translation. *The Journal of Biological Chemistry* **266**, 19867–19870.

Kozak, M. (2002). Pushing the limits of the scanning mechanism for initiation of translation. *Gene* **299**, 1–34. doi: 10.1016/s0378-1119(02)01056-9.

Kraemer, S. M. and Smith, J. D. (2006). A family affair: *var* genes, PfEMP1 binding, and malaria disease. *Current Opinion in Microbiology* **9**, 374–380. doi: 10.1016/j.mib.2006.06.006.

Kumar, M., Srinivas, V. and Patankar, S. (2015). Upstream AUGs and upstream ORFs can regulate the downstream ORF in *Plasmodium falciparum*. *Malaria Journal* **14**,. doi: 10.1186/s12936-015-1040-5.

Kwiatkowski, D. (1989). Febrile temperatures can synchronize the growth of *Plasmodium falciparum* in vitro. *The Journal of Experimental Medicine* **169**, 357–361. doi: 10.1084/jem.169.1.357.

Kwon, H. S., Lee, D. K., Lee, J. J., Edenberg, H. J., Ahn, Y. H. and Hur, M. W. (2001). Posttranscriptional regulation of human ADH5/FDH and Myf6 gene expression by

upstream AUG codons. *Archives of Biochemistry and Biophysics* **386**, 163–171. doi: 10.1006/abbi.2000.2205.

Lacsina, J. R., LaMonte, G., Nicchitta, C. V. and Chi, J.-T. (2011). Polysome profiling of the malaria parasite *Plasmodium falciparum*. *Molecular and Biochemical Parasitology* **179**, 42–46. doi: 10.1016/j.molbiopara.2011.05.003.

Lavstsen, T., Salanti, A., Jensen, A. T., Arnot, D. E. and Theander, T. G. (2003). Sub-grouping of *Plasmodium falciparum* 3D7 *var* genes based on sequence analysis of coding and non-coding regions. *Malaria Journal* **2**, 27. doi: 10.1186/1475-2875-2-27.

Le Roch, K. G., Johnson, J. R., Florens, L., Zhou, Y., Santrosyan, A., Grainger, M., Yan, S. F., Williamson, K. C., Holder, A. A., Carucci, D. J., Yates, J. R. and Winzeler, E. A. (2004). Global analysis of transcript and protein levels across the *Plasmodium falciparum* life cycle. *Genome Research* **14**, 2308–2318. doi: 10.1101/gr.2523904.

Lee, B., Udagawa, T., Singh, C. R. and Asano, K. (2007). Yeast phenotypic assays on translational control. *Methods in Enzymology* **429**, 105–137. doi: 10.1016/S0076-6879(07)29006-8.

Lei, L., Shi, J., Chen, J., Zhang, M., Sun, S., Xie, S., Li, X., Zeng, B., Peng, L., Hauck, A., Zhao, H., Song, W., Fan, Z. and Lai, J. (2015). Ribosome profiling reveals dynamic translational landscape in maize seedlings under drought stress. *The Plant Journal: For Cell and Molecular Biology* **84**, 1206–1218. doi: 10.1111/tpj.13073.

Liu, J., Istvan, E. S., Gluzman, I. Y., Gross, J. and Goldberg, D. E. (2006). *Plasmodium falciparum* ensures its amino acid supply with multiple acquisition pathways and redundant proteolytic enzyme systems. *Proceedings of the National Academy of Sciences* **103**, 8840–8845. doi: 10.1073/pnas.0601876103.

Liu, Y., Beyer, A. and Aebersold, R. (2016). On the Dependency of Cellular Protein Levels on mRNA Abundance. *Cell* **165**, 535–550. doi: 10.1016/j.cell.2016.03.014.

Loewith, R. and Hall, M. N. (2011). Target of rapamycin (TOR) in nutrient signaling and growth control. *Genetics* **189**, 1177–1201. doi: 10.1534/genetics.111.133363.

Lu, P. D., Harding, H. P. and Ron, D. (2004). Translation reinitiation at alternative open reading frames regulates gene expression in an integrated stress response. *The Journal of Cell Biology* **167**, 27–33. doi: 10.1083/jcb.200408003.

Lüder, C. G., Giraldo-Velásquez, M., Sendtner, M. and Gross, U. (1999). *Toxoplasma gondii* in primary rat CNS cells: differential contribution of neurons, astrocytes, and microglial cells for the intracerebral development and stage differentiation. *Experimental Parasitology* **93**, 23–32. doi: 10.1006/expr.1999.4421.

Maier, T., Güell, M. and Serrano, L. (2009). Correlation of mRNA and protein in complex biological samples. *FEBS Letters* **583**, 3966–3973. doi: 10.1016/j.febslet.2009.10.036.

Markus, B. M., Waldman, B. S., Lorenzi, H. A. and Lourido, S. (2021). High-Resolution Mapping of Transcription Initiation in the Asexual Stages of *Toxoplasma gondii*. *Frontiers in Cellular and Infection Microbiology* **10**, 617998. doi: 10.3389/fcimb.2020.617998.

McLean, K. J. and Jacobs-Lorena, M. (2017). *Plasmodium falciparum* Maf1 Confers Survival upon Amino Acid Starvation. *mBio* **8**,. doi: 10.1128/mBio.02317-16.

Moir, R. D. and Willis, I. M. (2015). Regulating Maf1 Expression and Its Expanding Biological Functions. *PLoS Genetics* **11**, e1004896. doi: 10.1371/journal.pgen.1004896.

Mok, B. W., Ribacke, U., Rasti, N., Kironde, F., Chen, Q., Nilsson, P. and Wahlgren, M. (2008). Default Pathway of *var2csa* switching and translational repression in *Plasmodium falciparum*. *PloS One* **3**, e1982. doi: 10.1371/journal.pone.0001982.

Montoya, J. and Liesenfeld, O. (2004). Toxoplasmosis. *The Lancet* **363**, 1965–1976. doi: 10.1016/S0140-6736(04)16412-X.

Morris, D. R. and Geballe, A. P. (2000). Upstream Open Reading Frames as Regulators of mRNA Translation. *Molecular and Cellular Biology* **20**, 8635–8642. doi: 10.1128/MCB.20.23.8635-8642.2000.

Murata, Y., Sugi, T., Weiss, L. M. and Kato, K. (2017). Identification of compounds that suppress *Toxoplasma gondii* tachyzoites and bradyzoites. *PLOS ONE* **12**, e0178203. doi: 10.1371/journal.pone.0178203.

Narasimhan, J., Joyce, B. R., Naguleswaran, A., Smith, A. T., Livingston, M. R., Dixon, S. E., Coppens, I., Wek, R. C. and Sullivan, W. J. (2008). Translation regulation by eukaryotic initiation factor-2 kinases in the development of latent cysts in *Toxoplasma gondii*. *The Journal of Biological Chemistry* **283**, 16591–16601. doi: 10.1074/jbc.M800681200.

Oakley, M. S. M., Kumar, S., Anantharaman, V., Zheng, H., Mahajan, B., Haynes, J. D., Moch, J. K., Fairhurst, R., McCutchan, T. F. and Aravind, L. (2007). Molecular factors and biochemical pathways induced by febrile temperature in intraerythrocytic *Plasmodium falciparum* parasites. *Infection and Immunity* **75**, 2012–2025. doi: 10.1128/IAI.01236-06.

Pakos-Zebrucka, K., Koryga, I., Mnich, K., Ljujic, M., Samali, A. and Gorman, A. M. (2016). The integrated stress response. *EMBO reports* **17**, 1374–1395. doi: 10.15252/embr.201642195.

Palam, L. R., Baird, T. D. and Wek, R. C. (2011). Phosphorylation of eIF2 facilitates ribosomal bypass of an inhibitory upstream ORF to enhance CHOP translation. *The Journal of Biological Chemistry* **286**, 10939–10949. doi: 10.1074/jbc.M110.216093.

Pandey, A. V., Bisht, H., Babbarwal, V. K., Srivastava, J., Pandey, K. C. and Chauhan, V. S. (2001). Mechanism of malarial haem detoxification inhibition by chloroquine. *Biochemical Journal* **355**, 333–338. doi: 10.1042/bj3550333.

Pluta, K., Lefebvre, O., Martin, N. C., Smagowicz, W. J., Stanford, D. R., Ellis, S. R., Hopper, A. K., Sentenac, A. and Boguta, M. (2001). Maf1p, a negative effector of RNA polymerase III in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology* **21**, 5031–5040. doi: 10.1128/MCB.21.15.5031-5040.2001.

Qiao, H., Lu, N., Du, E., Yao, L., Xiao, H., Lu, S. and Qi, Y. (2011). Rare codons in uORFs of baculovirus p13 gene modulates downstream gene expression. *Virus Research* **155**, 249–253. doi: 10.1016/j.virusres.2010.10.016.

Rajendran, E., Hapuarachchi, S. V., Miller, C. M., Fairweather, S. J., Cai, Y., Smith, N. C., Cockburn, I. A., Bröer, S., Kirk, K. and van Dooren, G. G. (2017). Cationic amino acid transporters play key roles in the survival and transmission of apicomplexan parasites. *Nature Communications* **8**, 14455. doi: 10.1038/ncomms14455.

Rajendran, E., Clark, M., Goulart, C., Steinhöfel, B., Tjhin, E. T., Smith, N. C., Kirk, K. and van Dooren, G. G. (2019). *Substrate-mediated regulation of the arginine transporter of Toxoplasma gondii*, preprint. Microbiology doi: 10.1101/798967.

Rao, S. J., Chatterjee, S. and Pal, J. K. (2017). Untranslated regions of mRNA and their role in regulation of gene expression in protozoan parasites. *Journal of Biosciences* **42**, 189–207. doi: 10.1007/s12038-016-9660-7.

Rawat, M., Srivastava, A., Gupta, I. and Karmodiya, K. (2019). *Single Cell RNA-sequencing reveals cellular heterogeneity, stage transition and antigenic variation during stress adaptation in synchronized Plasmodium falciparum*, preprint. Microbiology doi: 10.1101/752543.

Reynolds, K., Zimmer, A. M. and Zimmer, A. (1996). Regulation of RAR beta 2 mRNA expression: evidence for an inhibitory peptide encoded in the 5'-untranslated region. *The Journal of Cell Biology* **134**, 827–835. doi: 10.1083/jcb.134.4.827.

Rocamora, F., Zhu, L., Liong, K. Y., Dondorp, A., Miotto, O., Mok, S. and Bozdech, Z. (2018). Oxidative stress and protein damage responses mediate artemisinin resistance in malaria parasites. *PLoS pathogens* **14**, e1006930. doi: 10.1371/journal.ppat.1006930.

Rowe, J. A., Claessens, A., Corrigan, R. A. and Arman, M. (2009). Adhesion of *Plasmodium falciparum*-infected erythrocytes to human cells: molecular mechanisms and therapeutic implications. *Expert Reviews in Molecular Medicine* **11**, e16. doi: 10.1017/S1462399409001082.

Ruan, H., Shantz, L. M., Pegg, A. E. and Morris, D. R. (1996). The upstream open reading frame of the mRNA encoding S-adenosylmethionine decarboxylase is a polyamine-responsive translational control element. *The Journal of Biological Chemistry* **271**, 29576–29582. doi: 10.1074/jbc.271.47.29576.

Salanti, A., Staalsoe, T., Lavstsen, T., Jensen, A. T. R., Sowa, M. P. K., Arnot, D. E., Hviid, L. and Theander, T. G. (2003). Selective upregulation of a single distinctly structured *var* gene in chondroitin sulphate A-adhering *Plasmodium falciparum* involved in pregnancy-associated malaria. *Molecular Microbiology* **49**, 179–191. doi: 10.1046/j.1365-2958.2003.03570.x.

Salanti, A., Dahlbäck, M., Turner, L., Nielsen, M. A., Barfod, L., Magistrado, P., Jensen, A. T. R., Lavstsen, T., Ofori, M. F., Marsh, K., Hviid, L. and Theander, T. G. (2004). Evidence for the involvement of VAR2CSA in pregnancy-associated malaria. *The Journal of Experimental Medicine* **200**, 1197–1203. doi: 10.1084/jem.20041579.

Sarrazin, S., Starck, J., Gonnet, C., Doubeikovski, A., Melet, F. and Morle, F. (2000). Negative and translation termination-dependent positive control of FLI-1 protein synthesis by conserved overlapping 5' upstream open reading frames in Fli-1 mRNA. *Molecular and Cellular Biology* **20**, 2959–2969. doi: 10.1128/mcb.20.9.2959-2969.2000.

Scherf, A., Hernandez-Rivas, R., Buffet, P., Bottius, E., Benatar, C., Pouvelle, B., Gysin, J. and Lanzer, M. (1998). Antigenic variation in malaria: in situ switching, relaxed and mutually exclusive transcription of *var* genes during intra-erythrocytic development in *Plasmodium falciparum. The EMBO journal* **17**, 5418–5426. doi: 10.1093/emboj/17.18.5418.

Schlüter, G., Boinska, D. and Nieman-Seyde, S. C. (2000). Evidence for translational repression of the SOCS-1 major open reading frame by an upstream open reading frame. *Biochemical and Biophysical Research Communications* **268**, 255–261. doi: 10.1006/bbrc.2000.2109.

Schneider-Poetsch, T., Ju, J., Eyler, D. E., Dang, Y., Bhat, S., Merrick, W. C., Green, R., Shen, B. and Liu, J. O. (2010). Inhibition of eukaryotic translation elongation by cycloheximide and lactimidomycin. *Nature Chemical Biology* **6**, 209–217. doi: 10.1038/nchembio.304.

Serfontein, J., Nisbet, R. E. R., Howe, C. J. and de Vries, P. J. (2010). Evolution of the TSC1/TSC2-TOR signaling pathway. *Science Signaling* **3**, ra49. doi: 10.1126/scisignal.2000803.

Sharma, J., Rodriguez, P., Roy, P. and Guiton, P. S. (2020). Transcriptional ups and downs: patterns of gene expression in the life cycle of *Toxoplasma gondii*. *Microbes and Infection* **22**, 525–533. doi: 10.1016/j.micinf.2020.09.001.

Sherman, I. W. (1977). Transport of amino acids and nucleic acid precursors in malarial parasites. *Bulletin of the World Health Organization* **55**, 211–225.

Shor, B., Wu, J., Shakey, Q., Toral-Barza, L., Shi, C., Follettie, M. and Yu, K. (2010). Requirement of the mTOR kinase for the regulation of Maf1 phosphorylation and control of RNA polymerase III-dependent transcription in cancer cells. *The Journal of Biological Chemistry* **285**, 15380–15392. doi: 10.1074/jbc.M109.071639.

Silva, J., Fernandes, R. and Romão, L. (2019). Translational Regulation by Upstream Open Reading Frames and Human Diseases. *Advances in Experimental Medicine and Biology* **1157**, 99–116. doi: 10.1007/978-3-030-19966-1_5.

Skabkin, M. A., Skabkina, O. V., Hellen, C. U. T. and Pestova, T. V. (2013). Reinitiation and other unconventional posttermination events during eukaryotic translation. *Molecular Cell* **51**, 249–264. doi: 10.1016/j.molcel.2013.05.026.

Smith, J. D., Subramanian, G., Gamain, B., Baruch, D. I. and Miller, L. H. (2000). Classification of adhesive domains in the *Plasmodium falciparum* erythrocyte membrane protein 1 family. *Molecular and Biochemical Parasitology* **110**, 293–310. doi: 10.1016/s0166-6851(00)00279-6.

Smith, J. D., Rowe, J. A., Higgins, M. K. and Lavstsen, T. (2013). Malaria's deadly grip: cytoadhesion of *Plasmodium falciparum* -infected erythrocytes: *Plasmodium falciparum* cytoadhesion and *var* genes. *Cellular Microbiology* **15**, 1976–1983. doi: 10.1111/cmi.12183.

Soete, M., Fortier, B., Camus, D. and Dubremetz, J. F. (1993). *Toxoplasma gondii*: kinetics of bradyzoite-tachyzoite interconversion in vitro. *Experimental Parasitology* **76**, 259–264. doi: 10.1006/expr.1993.1031.

Soeters, P. B., van de Poll, M. C. G., van Gemert, W. G. and Dejong, C. H. C. (2004). Amino acid adequacy in pathophysiological states. *The Journal of Nutrition* **134**, 1575S-1582S. doi: 10.1093/jn/134.6.1575s.

Song, K. Y., Hwang, C. K., Kim, C. S., Choi, H. S., Law, P.-Y., Wei, L.-N. and Loh, H. H. (2007). Translational repression of mouse mu opioid receptor expression *via* leaky scanning. *Nucleic Acids Research* **35**, 1501–1513. doi: 10.1093/nar/gkm034.

Spevak, C. C., Ivanov, I. P. and Sachs, M. S. (2010). Sequence requirements for ribosome stalling by the arginine attenuator peptide. *The Journal of Biological Chemistry* **285**, 40933–40942. doi: 10.1074/jbc.M110.164152.

Srinivas, V., Kumar, M., Noronha, S. and Patankar, S. (2016). ORFpred: A Machine Learning Program to Identify Translatable Small Open Reading Frames in Intergenic Regions of the *Plasmodium falciparum* Genome. *Current Bioinformatics* **11**, 000–000.

Sullivan, W. J., Narasimhan, J., Bhatti, M. M. and Wek, R. C. (2004). Parasite-specific eIF2 (eukaryotic initiation factor-2) kinase required for stress-induced translation control. *The Biochemical Journal* **380**, 523–531. doi: 10.1042/BJ20040262.

Tennen, R. I., Haye, J. E., Wijayatilake, H. D., Arlow, T., Ponzio, D. and Gammie, A. E. (2013). Cell-cycle and DNA damage regulation of the DNA mismatch repair protein Msh2 occurs at the transcriptional and post-transcriptional level. *DNA repair* **12**, 97–109. doi: 10.1016/j.dnarep.2012.11.002.

Upadhya, R., Lee, J. and Willis, I. M. (2002). Maf1 Is an Essential Mediator of Diverse Signals that Repress RNA Polymerase III Transcription. *Molecular Cell* **10**, 1489–1494. doi: 10.1016/S1097-2765(02)00787-6.

van der Heyde, H. C., Nolan, J., Combes, V., Gramaglia, I. and Grau, G. E. (2006). A unified hypothesis for the genesis of cerebral malaria: sequestration, inflammation and hemostasis leading to microcirculatory dysfunction. *Trends in Parasitology* **22**, 503–508. doi: 10.1016/j.pt.2006.09.002.

Vannini, A., Ringel, R., Kusser, A. G., Berninghausen, O., Kassavetis, G. A. and Cramer, P. (2010). Molecular basis of RNA polymerase III transcription repression by Maf1. *Cell* **143**, 59–70. doi: 10.1016/j.cell.2010.09.002.

Vattem, K. M. and Wek, R. C. (2004). Reinitiation involving upstream ORFs regulates ATF4 mRNA translation in mammalian cells. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 11269–11274. doi: 10.1073/pnas.0400541101.

Vembar, S. S., Scherf, A. and Siegel, T. N. (2014). Noncoding RNAs as emerging regulators of *Plasmodium falciparum* virulence gene expression. *Current Opinion in Microbiology* **20**, 153–161. doi: 10.1016/j.mib.2014.06.013.

Vembar, S. S., Macpherson, C. R., Sismeiro, O., Coppée, J.-Y. and Scherf, A. (2015). The PfAlba1 RNA-binding protein is an important regulator of translational timing in *Plasmodium falciparum* blood stages. *Genome Biology* **16**, 212. doi: 10.1186/s13059-015-0771-5.

Vembar, S. S., Droll, D. and Scherf, A. (2016). Translational regulation in blood stages of the malaria parasite *Plasmodium* spp.: systems-wide studies pave the way. *Wiley interdisciplinary reviews. RNA* **7**, 772–792. doi: 10.1002/wrna.1365.

von Arnim, A. G., Jia, Q. and Vaughn, J. N. (2014). Regulation of plant translation by upstream open reading frames. *Plant Science: An International Journal of Experimental Plant Biology* **214**, 1–12. doi: 10.1016/j.plantsci.2013.09.006.

Waldman, B. S., Schwarz, D., Wadsworth, M. H., Saeij, J. P., Shalek, A. K. and Lourido, S. (2020). Identification of a Master Regulator of Differentiation in *Toxoplasma*. *Cell* **180**, 359-372.e16. doi: 10.1016/j.cell.2019.12.013.

Wang, X.-Q. and Rothnagel, J. A. (2004). 5'-untranslated regions with multiple upstream AUG codons can support low-level translation *via* leaky scanning and reinitiation. *Nucleic Acids Research* **32**, 1382–1391. doi: 10.1093/nar/gkh305.

Warnakulasuriyarachchi, D., Ungureanu, N. H. and Holcík, M. (2003). The translation of an antiapoptotic protein HIAP2 is regulated by an upstream open reading frame. *Cell Death and Differentiation* **10**, 899–904. doi: 10.1038/sj.cdd.4401256.

Wei, J., Wu, C. and Sachs, M. S. (2012). The arginine attenuator peptide interferes with the ribosome peptidyl transferase center. *Molecular and Cellular Biology* **32**, 2396–2406. doi: 10.1128/MCB.00136-12.

Weiss, L. M., Laplace, D., Takvorian, P. M., Tanowitz, H. B., Cali, A. and Wittner, M. (1995). A cell culture system for study of the development of *Toxoplasma gondii* bradyzoites. *The Journal of Eukaryotic Microbiology* **42**, 150–157. doi: 10.1111/j.1550-7408.1995.tb01556.x.

Weiss, L. M., Ma, Y. F., Takvorian, P. M., Tanowitz, H. B. and Wittner, M. (1998). Bradyzoite development in *Toxoplasma gondii* and the hsp70 stress response. *Infection and Immunity* **66**, 3295–3302. doi: 10.1128/IAI.66.7.3295-3302.1998.

Wek, R. C. (2018). Role of eIF2α Kinases in Translational Control and Adaptation to Cellular Stress. *Cold Spring Harbor Perspectives in Biology* **10**,. doi: 10.1101/cshperspect.a032870.

White, M. W., Radke, J. R. and Radke, J. B. (2014). *Toxoplasma* development - turn the switch on or off? *Cellular Microbiology* **16**, 466–472. doi: 10.1111/cmi.12267.

Wu, C., Wei, J., Lin, P.-J., Tu, L., Deutsch, C., Johnson, A. E. and Sachs, M. S. (2012). Arginine changes the conformation of the arginine attenuator peptide relative to the ribosome tunnel. *Journal of Molecular Biology* **416**, 518–533. doi: 10.1016/j.jmb.2011.12.064.

Wu, H.-Y., Guan, B.-J., Su, Y.-P., Fan, Y.-H. and Brian, D. A. (2014). Reselection of a genomic upstream open reading frame in mouse hepatitis coronavirus 5'-untranslated-region mutants. *Journal of Virology* **88**, 846–858. doi: 10.1128/JVI.02831-13.

Wu, H.-Y. L., Song, G., Walley, J. W. and Hsu, P. Y. (2019). The Tomato Translational Landscape Revealed by Transcriptome Assembly and Ribosome Profiling. *Plant Physiology* **181**, 367–380. doi: 10.1104/pp.19.00541.

Yang, Y. and Wang, Z. (2019). IRES-mediated cap-independent translation, a path leading to hidden proteome. *Journal of Molecular Cell Biology* **11**, 911–919. doi: 10.1093/jmcb/mjz091.

Young, S. K. and Wek, R. C. (2016). Upstream Open Reading Frames Differentially Regulate Gene-specific Translation in the Integrated Stress Response. *The Journal of Biological Chemistry* **291**, 16927–16935. doi: 10.1074/jbc.R116.733899.

Zach, L., Braunstein, I. and Stanhill, A. (2014). Stress-induced start codon fidelity regulates arsenite-inducible regulatory particle-associated protein (AIRAP) translation. *The Journal of Biological Chemistry* **289**, 20706–20716. doi: 10.1074/jbc.M114.547828.

Zhang, Z. and Dietrich, F. S. (2005). Identification and characterization of upstream open reading frames (uORF) in the 5' untranslated regions (UTR) of genes in *Saccharomyces cerevisiae*. *Current Genetics* **48**, 77–87. doi: 10.1007/s00294-005-0001-x.

Zhang, S., Chen, H. and Gerhard, G. S. (2010). Heme synthesis increases artemisinin-induced radical formation and cytotoxicity that can be suppressed by superoxide scavengers. *Chemico-Biological Interactions* **186**, 30–35. doi: 10.1016/j.cbi.2010.03.021.

Zhang, M., Gallego-Delgado, J., Fernandez-Arias, C., Waters, N. C., Rodriguez, A., Tsuji, M., Wek, R. C., Nussenzweig, V. and Sullivan, W. J. (2017). Inhibiting the *Plasmodium* eIF2α Kinase PK4 Prevents Artemisinin-Induced Latency. *Cell Host & Microbe* **22**, 766-776.e4. doi: 10.1016/j.chom.2017.11.005.

Zhang, H., Dou, S., He, F., Luo, J., Wei, L. and Lu, J. (2018). Genome-wide maps of ribosomal occupancy provide insights into adaptive evolution and regulatory roles of uORFs during Drosophila development. *PLoS biology* **16**, e2003903. doi: 10.1371/journal.pbio.2003903.

Zhang, H., Wang, Y. and Lu, J. (2019). Function and Evolution of Upstream ORFs in Eukaryotes. *Trends in Biochemical Sciences* **44**, 782–794. doi: 10.1016/j.tibs.2019.03.002.

Zhao, C., Datta, S., Mandal, P., Xu, S. and Hamilton, T. (2010). Stress-sensitive regulation of IFRD1 mRNA decay is mediated by an upstream open reading frame. *The Journal of Biological Chemistry* **285**, 8552–8562. doi: 10.1074/jbc.M109.070920.

Zuzarte-Luís, V. and Mota, M. M. (2018). Parasite Sensing of Host Nutrients and Environmental Cues. *Cell Host & Microbe* **23**, 749–758. doi: 10.1016/j.chom.2018.05.018.

Figures

Figure 1: Evidence of uORF-mediated gene regulation
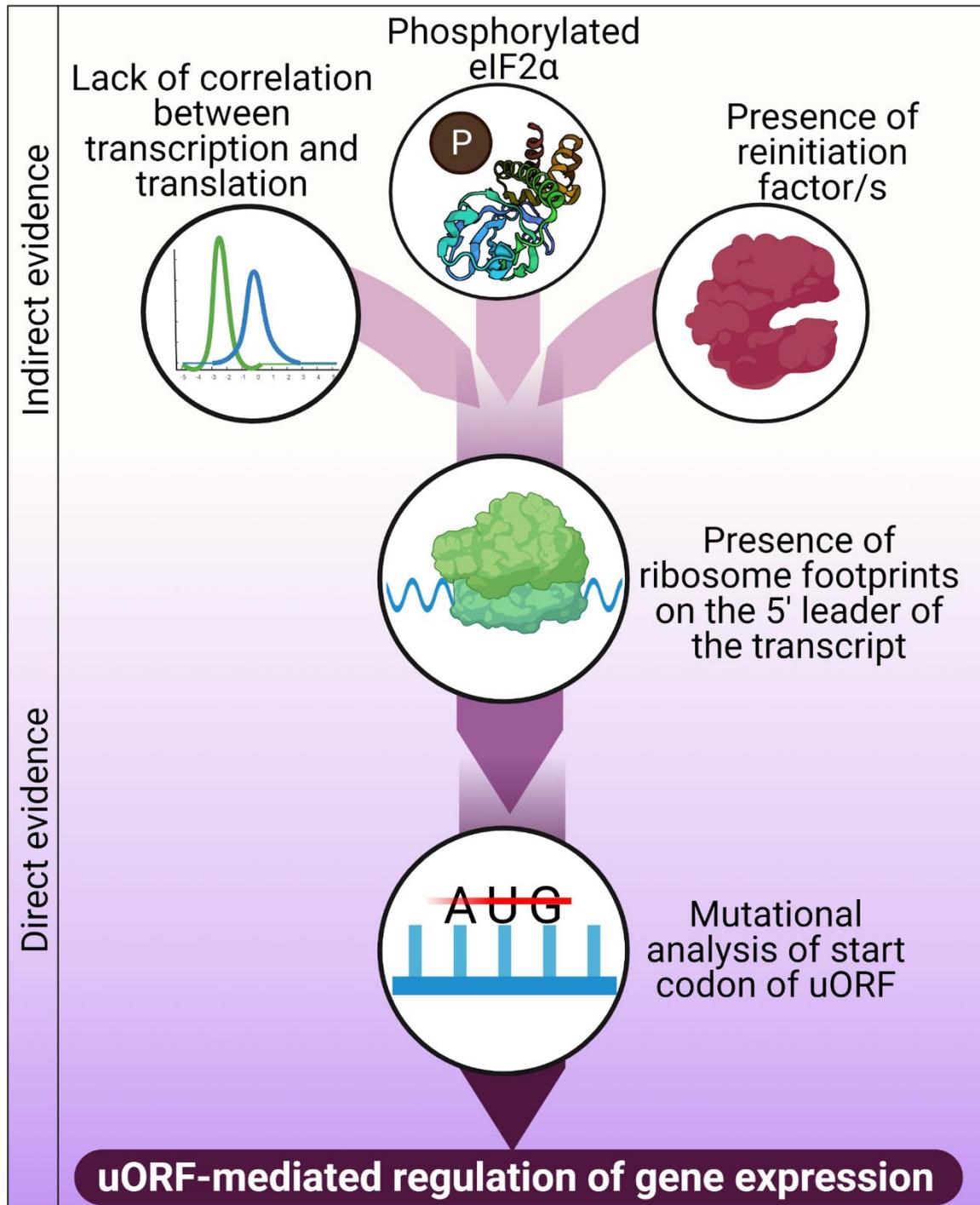
Figure 2: Examples of genes in *P. falciparum* and *T. gondii* that are regulated *via* uORFs

Last updated: 26 April 2021