Chapter-1

Introduction
(Review of Literature)
1.1. Malaria World wide

Malaria is caused by genus *Plasmodium* and distributed throughout the world. 215 million malaria cases have been identified in the year 2015 and out of which 438000 deaths occurred. Most malaria cases are found in African region (88%) followed by south East Asia region (10%). Malaria causes 4th highest deaths in children. Prevention of malaria is difficult because of unavailability of vaccine (WHO, 2015). However incidence of malarial deaths are reduced by house hold chemical spray (Okumu and Moore, 2011), insecticide treated mosquito nets and artemisinin and chloroquine combination therapy (Malik *et al.*, 2006). To eradicate malaria there has been concerted efforts by governments to educate, develop vaccines and drugs, but still large area of globe are at risk. Members of genus *Plasmodium* cause different types of malaria in various organisms. In humans, malaria is caused by four species of *Plasmodium*, *P. falciparum*, *P. ovale*, *P. malariae* and *P. vivax*, and is transmitted by female *Anopheles* mosquito. Out of 400 species of *Anopheles* mosquitoes, only 30 species are of importance for transmission of malaria (Lives *et al.*, 2004).

Major population under the risk of *P. falciparum* transmission is African (52% of the global) or Central, South and East Asia (46%). Second most prevalent species is *P. vivax*. Around one third of earths land are endemically under risk, half of area located in Africa (51%) and other area equally shared by Asia (27%) and America (22%) (Antinori *et al.*, 2012).

1.2. The life cycle of Plasmodium falciparum

Phylum Apicomplexa contain group of infectious parasitic protozoa which causes un-counted morbidity and mortality in humans and agricultural animals. Members of Apicomplexa such as *Plasmodium spp.* cause malaria, *Toxoplasma gondii* cause Toxoplasmosis in immune-compromised humans. Animal infecting parasites are *Eimeria spp.* pathogen of cattle and chicken, *Theileria spp.*, pathogen of cattle in Africa and certain member of Apicomplexa like *Cryptosporidium* infect
both cattle and humans. There are certain parasites such as *Gregarines* which inhabit guts of invertebrates including cockroaches and shrimp (Morrissette and Sibley, 2002). This group of organism contains unique organelle; Apicoplast which has its own genome that codes for few proteins and also is a part of lipid synthesis system, although most of the organelle proteins are encoded by parasite genome (Waller and Mcfadden, 1997).

Members of genus *Plasmodium* undergo a complex and well conserved life cycle which is bi-organismal, a vertebrate host like human and the invertebrate host mosquito. In vertebrate host, the parasite remain in haploid state and completes the asexual stages by infecting liver hepatocytes (Exo-Erythrocytic) and blood (intra-erythrocytic), while in the invertebrate host the diploid sexual development takes place in mosquito midgut and salivary gland (sporogonic). *P. falciparum* is a human pathogen that undergoes a complex life cycle; infections start when mosquito takes blood meal: sporozoites from salivary gland enters into human blood, this sporozoite now move to the liver and infect the hepatocytes. After 5-14 days the schizont rupture and merozoites released into blood stream, each hepatocyte releases around 40,000 merozoites in the blood stream which can then infect the erythrocytes. Merozoite infected erythrocytes undergo three major stages of development, Ring stage (named because of its morphology), G-phase called trophozoite which is metabolically active stage, consumes most of erythrocytes cytosolic material and finally S-phase, schizont in which several round of nuclear division happens and eventually releasing 16-32 merozoites (Boyle *et al.*, 2010). In addition to asexual cycle certain infected erythrocytes undergo alternative pathway and develop into single microgametocyte and megagametocytes known to be sexual cycle of erythrocytic development which take about 12-14 days. There are no evidences to tell how this asexual to sexual stage transition happens, when and how the decision is taken (Dixon *et al.*, 2008). These gametocytes are then taken up by mosquito during subsequent blood meal. Once inside the mosquito midgut the parasite is exposed to altered...
environmental factors like low temperature, high pH and Xanthurenic acid causes activation and differentiation of gametocytes followed by fertilization to form zygote (Cloning and Jr, 1998). The zygote develops into motile ookinete in 24 hrs and traverses through midgut epithelium beneath basal lamina and further differentiated in to oocysts. After maturation oocyst release thousands of sporozoite into mosquito’s body cavity, infecting mosquito salivary gland and remain there till released in human bloodstream (Aly, Vaughan and Kappe, 2010). Different species of Plasmodium has different preference towards host cells. P. falciparum prefers mature erythrocytes for its infection but other species such P. vivax, P. khowlesi and P. ovale has strong preference to reticulocytes. These host cell preferences leads to high severity of infection and parasitemia in P. falciparum. The percentage of reticulocytes in blood are quite low, this explains why P. falciparum are more dangerous over other species of Plasmodium (Tarun et al., 2007).

1.3. Clinical symptoms of malaria

The pre-erythrocytic stages in the liver remain asymptomatic. The clinical symptoms of malaria are exclusively caused by the asexual multiplication of P. falciparum in erythrocytes. The common symptom of all malaria infections is high fever, headache, malaise, fatigue, and muscle aches. Fever induced by rupture of infected red blood cells after every cycle of asexual reproduction. The pyrogenic compounds released after rupture of RBCs are grouped together as malaria toxins. Glycosylphosphatidylinositol (GPI) and haemozoin are the substances to act as pyrogens. GPI can directly upregulate surface receptors like ICAM1 and VCAM1 and induce TNF and IL1 secretion from macrophages (Carlton et al., 2002). Haemozoin induces endogenous pyrogens like TNF-alpha (Olivier et al., 2014) and IL-1B (Gardner et al., 2002). Cerebral malaria, a severe form of malaria is induced by extensive TNF alpha release (Carlton et al., 2008).

The parasite remodels the surface of the erythrocytes and this causes erythrocyte clumping and oxygen deprivation of tissues that can lead to
organ failure. The integration of parasite proteins, especially Erythrocyte Membrane Protein 1 (PfEMP1) into the erythrocyte plasma membrane gives it the ability to bind to the vascular endothelium and to uninfected RBCs. This effect is severe because clumped blood cells clog the thin blood vessels at the time of clearing iRBCs from blood circulation during spleen passage. A special case of infection is the pregnancy associated malaria (PAM). Placenta is often heavily infected with sequestered parasites, high load of parasite in placenta is dangerous for fetus and may lead to growth restriction, decreased birth weight or preterm delivery (Buffet et al., 1999) (Rogerson et al., 1995) (Menendez et al. 2000) apart from inducing severe anaemia in the mother. Rossettes caused by sequestering of iRBCs by uninfected RBCs leads to embolism like obstruction and may be a cause of coma.

1.4. Intra-Erythrocytic stages gene regulation in *P. falciparum*

Eukaryotic gene regulation is well coordinated and controlled process; it can be broadly classified into transcriptional and post-transcriptional regulation. Gene regulation at transcriptional level involves multiple check point and different pathways such as nucleosome assembly, chromatin remodelling, cis-regulatory elements, transcription factors, RNA pol II phosphorylation and through non-coding RNA (Venters and Pugh, 2010) (Barrett, Fletcher and Wilton, 2012).

*P. falciparum* genome was sequenced in 2002 (Gardner et al., 2002), later on other species of *Plasmodium* namely *P. yoelii*, *P. vivax* and *P. Berghei* were also sequenced (Carlton et al., 2002) (Carlton et al., 2008) (Berry et al., 2008). *P. falciparum* nuclear genome consists of 14 linear chromosomes. Other than chromosomal DNA *P. falciparum* also have 6 kb linear mitochondrial DNA and 35 kb circular apicoplast DNA (Gardner et al., 2002). In total, *P. falciparum* encodes a total of 6000 genes (Otto et al., 2010) (Lasonder, Ishihama and Andersen, 2002) during its life cycle and half of its total predicted open reading frame (ORFs) does not show nucleotide sequence complementarity towards other organism genome. The high AT content of *P. falciparum* genome
(80.7%) create challenge for prediction of gene, with almost 50% of mRNAs still unannotated (Florens et al., 2002).

In its complex life cycle *Plasmodium* parasites undergo different infective, propagative, sexual and asexual stages, which are well timed in a vertebrate and mosquito host. To efficiently propagate in both the hosts, *Plasmodium* parasite requires a wide range of specific proteins in different conditions to accompany the morphological changes and also to adapt to various host environments requiring extensive regulation (Kooij and Matuschewski, 2007).

Search for transcription factors, domain homology among *Plasmodium* proteins identified transcription factors that correspond to only 1.3% of total predicted proteome, which is significantly lower than what is expected (5.7%) for the genome size of *P. falciparum* suggesting paucity of transcription factors in *P. falciparum*. In *P. falciparum* genome study it is found that both transcriptional regulator proteins as well as sequence needed for transcriptional regulation are not very abundant as compared to other eukaryotes, instead a CCCH-type zinc finger (common in proteins modulating mRNA decay and translation rates) was found to be the most abundant in the *P. falciparum* genome. C2H2 is classical DNA binding motif, which is mostly found in several transcription factors. The eukaryote genome carries average 130 C2H2 motifs per 10,000 genes, in contrast the *P. falciparum* genome has only 11 per 10,000 genes, on other hand CCCH type zinc finger is average 17 genes every 10,000 genes, *P. falciparum* carries nearly double of this motifs containing proteins (Coulson, Hall and Ouzounis, 2004).

The detailed analysis of *Plasmodium* transcriptome and proteome has divided parasite gene expression into four categories namely, i. Housekeeping (136 proteins) ii. Host related expression iii. Strategy specific expression and iv. Stage specific expression. More than half of the analysed proteins (948 out of 1836) were identified in specific stages, suggesting regulation of gene expression in a stage specific manner (Blair et al., 2002) (Waters et al., 1997) (Sims et al., 2009). More recently,
transcriptome profiling of three different strains of *P. falciparum* at intra erythrocytic developmental cycle (IDC) (3D7, Dd2, HB3 strains) suggests significant temporal regulation at transcriptional level (Pulliam et al. 2003) (Bozdech et al., 2006). Even clinical field isolates of *P. falciparum* shows differential transcriptional profile (Mackinnon et al., 2009) and response to drugs also alter gene expression (Gunasekera et al., 2007) and gene expression data has demonstrated dramatic alterations in steady-state levels of many genes in response to various drugs (Gunasekera et al., 2007). Expression of *P. falciparum*, erythrocyte membrane protein 1 (PIEMP1), encoded by var gene is regulated through chromatin modification (Guizetti, Scherf and Biologie, 2013).

### 1.4.1. Significance of translation regulation in eukaryotes

Gene expression is regulated at multiple levels, including at the step of translation of mRNAs into proteins. Translational regulation is a process in which control of existing mRNAs allows the cells to adapt to more subtle and acute changes in extra-cellular signals by altering the cellular concentrations of the encoded proteins. This leads to maintaining the homeostasis by such modulation either by temporary or by permanent changes in cell physiology or fate. Many physiological conditions have been reported wherein the regulation at the translation step is required like during development, differentiation, stress, disease conditions etc. (Sonenberg and Hinnebusch, 2009). The untranslated region (UTR) of mRNA play an important role at various stages of mRNA life cycle like translational efficiency, mRNA stability and mRNA localization (Chatterjee and Pal, 2009). Regulatory elements present in these UTR’s have been shown to interact with various cytoplasmic factors including proteins and microRNA.

The translation regulation in eukaryotic cells can be divided into two types, viz. global or general translation control in which the translation of most mRNAs in the cell is regulated; and mRNA transcript-specific control, where only a subset of the mRNA pool is under the translational control while the overall cellular protein biosynthesis as a whole remains
unaffected or only marginally affected. Global regulation mainly occurs by the modification of translation-initiation factors, while mRNA specific regulation is mostly attributed to trans-acting regulatory protein complexes that recognize particular elements that are usually present in the 5’ and/or 3’ un-translated regions (UTRs) of the transcript under regulation. Furthermore, the relative paucity of transcription factors (Painter, Campbell and Llinás, 2012) coupled with the high number of putative RNA-binding proteins suggests that post-transcriptional regulation plays a major role in parasite gene regulation.

1.4.2. Post-transcriptional gene regulation of Plasmodium

In the absence of expected proportion of transcription factors and miRNA pathway members, translation regulation could be a possible mechanism for Plasmodium gene regulation (Baum et al., 2009) (Xue et al., 2008). When the initial transcriptomic and proteomic studies of P. falciparum were completed, a “just-in-time” transcription model for gene regulation was proposed (Roch et al., 2003a). However, with the numerous genomic technology applied to the study of P. falciparum, significant inconsistencies have been noted between the steady-state transcriptome (Pulliam et al. 2003) (Otto et al., 2010) and proteome (Florens et al., 2002) (Lasonder, Ishihama and Andersen, 2002) (Lasonder et al., 2008). Translation delay was observed in case of about 30% genes in each blood stage (Bunnik et al., 2013). These studies highlight post-transcriptional regulation as potentially key regulatory steps in Plasmodium. Recent studies have revealed the importance of gene splicing (Saenz et al., 2008), mRNA stability (Shock et al. 2007) and gene silencing (The, 2001) (Duraisingh et al., 2005) (Roch et al., 2003b) in various Plasmodium species. Global analysis of RNA decay in P. falciparum indicates a dramatic increase in the half-life of mRNA as the asexual stage progress. The average mRNA half-life is approximately 9.5 minutes during the ring stage, extending to average of 65 minutes during the late schizont stage of development (Shock et al. 2007).
Extensive post-transcriptional and post-translational modifications have also been noted during the erythrocytic stage of development (Mair et al., 2007). Apart from common view of translation regulation in *Plasmodium* few studies have identified transcript specific translation regulation in *Plasmodium*. RNA binding proteins have been implicated in cell-fate choice between the asexual and sexual stage of development. Translation repression is well studied in eukaryotic oocyte, where transcripts are translationally repressed and are activated during zygote development (Kronja and Orr-weaver, 2011) (Qin et al., 2007). A similar translation regulation was reported in *Plasmodium* asexual to sexual transition (Mair et al., 2007). For example P25 and P28 are ookinete surface proteins and are required for motility of ookinete from mosquito midgut to salivary gland. Transcripts of p25 and p28 remain translationally repressed during gametocyte stage in human host (Tomas et al., 2001)(Vermeulen et al, 1985). Once these gametocytes are in mosquito translation of these mRNA begins, leading to the synthesis corresponding proteins. DEAD box RNA helicase DOZI was shown to play a role in this regulatory process. DOZI forms mRNA complex with p25/p28 transcripts and does the cytoplasmic partitioning and prevent the translation (Mair et al., 2007). Another example is the PbPuf2, a *P. berghei* homolog of the pumilio family of RNA-binding protein which was shown to be involved in increased gametocytogenesis and is required for sporozoite invasion of hepatocytes (Gomes et al., 2011). Taken together, these results highlight the importance and complexity of post-transcriptional, and in particular translational regulation in *Plasmodium*.

1.4.3 Role of UTRs in Plasmodium Translation regulation

In higher eukaryotes, gene regulation dominates at translation level during many specialized processes such as fertilization, differentiation, cell cycle and stress response (Chatterjee and Pal, 2009). Untranslated regions (UTRs) of mRNA play a significant role in global as well as mRNA-specific translation regulation. UTRs are sequences present upstream of start codon AUG called as 5’ UTR and downstream of stop codon named as 3’UTR of a transcript that do not code for protein(s) but
may have features to allow differential regulation of a gene. These cis-acting elements interact with other non-coding RNA and different transacting factors that decides stability and translation efficiency of transcripts. In eukaryotic organism, perturbations in the 5’ and 3’ UTRs result in deregulation of genes, leading to various diseases or susceptibility to diseases (Chatterjee and Pal, 2009). Although UTR are untranslated, they mean a lot in deciding the fate of a transcript, including transcript stability, localization and translation (Szostak, 2012). Interaction of RNA binding proteins with transcript help regulating translation process, this binding can be sequence dependent or secondary structure dependent. CPEB, CPSF, and PUF are proteins that interact with conserved primary sequence present in the UTRs, while the GAIT (Interferon-γ- Activated Inhibitor of Translation) complex on 3’ UTR of Ceruloplasmin mRNA recognizes both sequence and structure of the RNA (Sampath et al., 2003). Intra-erythrocyte stage of Plasmodium parasite also shows 5’ and 3’ UTR mediated gene expression control in case of var gene family (Dahlbäck et al., 2007) and for P25/P28 protein in gametocyte stage. The var gene family consists of 60 different genes arranged on various chromosomes and located on different chromosomal regions. A var gene encodes surface protein pfEMP1 (Plasmodium falciparum erythrocyte membrane protein 1) enabling cytoadhesion of parasite infected erythrocytes and only single var gene expression takes place during parasite life cycle (Kraemer and Smith, 2006). UTR mediated translation regulation in Plasmodium parasite is also reported during malarial transmission stage gametocytes as discussed previously for p28 and p25 transcripts in many of the Plasmodium species like P. falciparum, P. berghei, P. gallinaceaum (Golightly et al., 2000) (Cann et al., 2004). Another instance for the role of UTR in Plasmodium gene expression is the regulation of phosphoglucomutase 2 (PFD0660w), deletion of 661 bases in 5’UTR decreases protein expression level (Hasenkamp et al., 2013). Proteomic and transcriptomic analysis of Plasmodium in different stages revealed translation regulation of 9 transcripts in gametocytes, including p25 and p28. Using sequence comparison and MEME motif search programme in all the 9 repressed
transcripts a consensus motif of  was identified in 3’ UTR of the transcripts, however a similar conserved motif was not detected in P. falciparum transcripts undergoing translation regulation (Hall et al., 2005).

*P. falciparum* genome has two genes for Puf proteins (Pumilio and FBF homology protein), PfPuf1 (PFE0935c) and PfPuf2 (PFD0825c) coding 224kDa and 61.4kDa respectively, with conserved UUGU motif RNA binding activity (Cui, Fan and Li, 2002) (Liebert, 2004). In *P. falciparum*, PUF proteins PfPuf1 and PfPuf2 are expressed in intra-erythrocytic stages as well as male and female gametocytes, with highest level during gametocyte maturation and salivary gland sporozoite stage. Later a PBE (puf binding element) was identified and characterized in maintenance of gametocyte repressed transcripts p25 and p28 within 5’ UTR and 3’UTR respectively (Miao et al., 2013). This indicates that Puf proteins can regulate UTR mediated translation regulation in gametocyte stage of *Plasmodium* parasite. Beyond the UTRs, certain factors interacts coding region of mRNA and regulates its gene expression by gene specific manner.

### 1.5. Host pathogen interaction in *Plasmodium* erythrocyte stage

Parasite take over the host system for its growth and progression and this process is well programmed. Parasite modulates host cells using various strategies, for example virus hijacks host translation machinery to predominantly synthesize its own proteins (Kash et al., 2006). In case of *Plasmodium* parasites at intra-erythrocytic stages, active transcription and translation is absent in the host because of lack of nucleus in erythrocytes. *Plasmodium* extensively modulates host environment by exporting various proteins into erythrocyte and they do various functions such as parasite sequestration in blood vessels using pfEMP proteins and KHRP proteins (Maier et al., 2009). This sequestration and attachment helps and protects parasite from immune clearance in spleen (Ombrain et al., 2007) (Biology, Pasternak and Dzikowski, 2009). A signal sequence named *Plasmodium* export element (PEXEL) or HT element (host targeting) has
been identified in proteins that are exported to host cell (Beck et al., 2015) (Riglar et al., 2013). This signal consists of signature sequence RxLxE/D/Q, which binds with phosphoinositide-3-phosphate (PI3P). *Plasmodium* translocon complex PTEX (*Plasmodium* translocon of exported proteins) was identified and shown to be responsible for exporting/targeting parasite proteins to host.

Other than exporting proteins *Plasmodium* also imports various factors in intra-erythrocytic stage. A quantitative and time dependent protein profile study revealed 24 human proteins that are imported during intra-erythrocytic development of *P. falciparum*. Some of these proteins play an important role in *Plasmodium* development. Immunofluorescence and immunoblotting experiments have shown that human proteins are imported by *P. falciparum* for example Catalase, Peroxiredoxin 2, Superoxide dismutase, GAPDH, hemoglobin etc (Foth et al., 2011). An interesting example of import of host protein is the case of enzymes involved in heme metabolism. Heme is necessary for *Plasmodium* development and parasite utilizes host haemoglobin derived heme as well as de novo synthesised heme (Nagaraj, Sundaram and Varadarajan, 2013). All the enzymes required for heme synthesis has been identified in *Plasmodium* parasite, still *Plasmodium* parasite import heme synthesis pathway enzymes like δ-aminolevulinate dehydrase (ALAD) from host erythrocyte (Of et al., 1997). Taken together the evidences of erythrocyte proteins import and parasite proteins export in host environment suggests importance of host (erythrocytes) pathogen (*Plasmodium*) interaction. Some of the imported proteins perform important function like Catalase is imported for protection of *Plasmodium* against oxidant stress while imported peroxiredoxin2 activity help in peroxide detoxification (Koncarevic et al., 2009). Certain erythrocyte proteins play important role in parasite proliferation and development even without their import in parasite, examples include host protease Calpain I and Gs protein (erythrocyte guanine nucleotide regulatory protein). *Plasmodium* utilises host protein Calpain I for egress out of host erythrocytes. Inhibition or depletion of Calpain I in host cells cause arrest at schizogamy in *P.
falciparum and Toxoplasma Gondii and inhibition of merozoites (Chandramohanadas et al., 2012). Similarly, inhibition of Gs protein and receptor interaction with preloaded Gs peptide into erythrocyte affects parasite maturation and proliferation (Murphy et al., 2006). Taken together, these examples all highlight the importance and complexity of post-transcriptional, and in particular translational regulation in Plasmodium. Apart from import of proteins, other host erythrocyte factors like miRNA has been reported in recent years and their role in Plasmodium development has been reported (Lamonte et al., 2013).

1.6. Micro RNA

1.6.1. Micro RNA Biogenesis

miRNAs are a class of regulatory non-coding RNAs, 19-22nt long, and expressed in most eukaryotes (Krol, Loedige and Filipowicz, 2010). More than 500 miRNA coding genes are present on mammalian genome (Soumillon et al., 2013). Approximately 50% of miRNA are expressed from non-protein coding genes (Wirth and Crespi, 2017) and remaining miRNA are expressed from intronic region of protein coding transcripts (Lin, Miller and Ying, 2006). Size of primary miRNA transcript can be several kilobases. Pri-miRNA is converted to small hairpin pre-miRNA (around 60nt in length) after cleavage by the microprocessor complex which has RNase III enzymatic activity. Microprocessor complex consist of several proteins, including Drosha and DGCR8 (Gregory, Yan and Amuthan, 2004). The pre-miRNA is then transported from the nucleus by exportin-5 mediated process, once within the cytosol, the hairpin loop of the pre-miRNA is recognized by Dicer, which cleaves the loop, leaving a ~21nt dsRNA, with 2nt overhangs on both 3’ ends (Agrawal et al., 2003). These overhangs are recognized by the RISC (RNA Induced Silencing Complex) and dsRNA get separated, the guide strand (which is a part of RISC complex and bind to un-translated region of mRNA) and the passenger strand, released and degraded (Pratt and Macrae, 2010).

1.6.2. Expression pattern of miRNA
A single miRNA can often target multiple mRNAs and conversely multiple miRNA can target a single mRNA (Kim, 2005). Micro RNA are involved in various cellular functions including lineage differentiation (miR-181, miR-223, miR-99) (Garzon et al., 2006), erythropoesis (miR-451) (Pase et al., 2017), neurogenesis (miR-124) (Yoo et al., 2011), specific miRNAs have been shown to regulate host immune responses against pathogens (Arora et al., 2017) (Xiao and Rajewsky, 2008) etc. Expression pattern of miRNAs are of wide range with many miRNA being expressed in cell specific or tissue specific manner. miR-451 is expressed in erythrocytes and miR-122 is liver-specific (Barad et al., 2004)(Landgraf et al., 2009) while let-7 is expressed ubiquitously.

1.6.3. Mode of action

MicroRNA-mRNA interaction is largely mediated by complementarity within the “seed sequence,” a 7 base pair sequence close to the 5’ end (nucleotides 2-7/8 following the 1st base pair at the 5’ end, which is often an adenosine) (Pairing, 2005). MicroRNA typically have their target in the 3’ UTR of mRNA however some miRNA can also have their target sequence at the 5’ UTR of mRNA as well as within the coding region of mRNAs (Krol, Loedige and Filipowicz, 2010). Interestingly, the miRNA target site density in the 5’ UTR and coding region is more than in 3’ UTR in all organisms (Zhou et al., 2009). But it has been reported that miRNAs targeting to the 5’ UTR or coding region are less effective than those targeting 3’ UTR (Gu et al. 2009).

1.6.4. Micro RNA function

MicroRNAs usually have target site in the 3’UTR of mRNA and inhibit their expression (Guo et al., 2010). It has been reported that miRNA induces deadenylation and degradation and at the same time it inhibits the translation of the target mRNA (Yekta, Shih and Bartel, 2004) (Mathonnet et al., 2007). MicroRNA typically functions via two mechanisms. First, perfect complementarity between miRNA and target mRNA, transcripts will be cleaved by RISC complex. This phenomenon often found in plants but rarely observed in other eukaryotic organisms.
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(Bartel, 2013). Second, most common pathways seen in mammalian systems is translation repression by incomplete complementarity (Martin et al., 2014). Mechanism of translational repression is not universal, however it has been shown that eventually most targeted mRNAs are degraded following the removal of cap or polyA tail (Behm-ansmant et al., 2006). Role of miRNA in inhibiting gene expression is well established, however a role for miRNA in translation activation of target mRNA have also been reported. The miR369 targets the 3’UTR along with the Ago2 complex and fragile X-related protein1 (FXR1) and activates the translation in serum deprived condition. Also, tethering of Ago2 or FXR1 to the reporter mRNA activates the translation during cell cycle arrest (Vasudevan, Tong and Steitz, 2008). miRNA-10a targets the 5’UTR of ribosomal protein and activates its translation in response to amino acid deprivation (Ørom, Nielsen and Lund, 2008). Thus miRNA can affect the translation in either way by targeting the mRNA at any position with different mechanisms of action.

1.7. Argonaute Proteins and RISC complex

The Argonaute proteins are one of the important members of the RNA induced silencing complex and plays a vital role in mediating the miRNA mediated gene regulation. These proteins were first identified in plants. Different organism carries varied number of Argonaute genes: Schizosaccharomyces pombe has one gene, while Caenorhabditis elegans has 27. This protein family is divided into Ago and Piwi subfamily (Carmell et al., 2002) (Meister and Tuschl, 2004). The expression of Piwi proteins is restricted to the germ line in most of the organism, including zebrafish, drosophila and mouse, where they interacts with Piwi-interacting RNA (piRNA). The human Piwi subfamily comprises HIWI1, HIWI2, HIWI3 and HILI; they are located on chromosome 12, 11, 22 and 8 respectively. In contrast, Ago proteins are ubiquitously expressed in many organisms. Human has four Ago genes; Ago1, Ago3 and Ago4 genes are in clustered on chromosome 1, whereas Ago2 gene is located on chromosome 8 (Höck and Meister, 2008). Ago proteins has two characteristic and functional domain, PAZ and PIWI. PAZ domain
contain specific binding pocket that recognize two nucleotides from the 3’ overhang of miRNA and siRNA in sequence independent manner. PIWI domain has structural homology to RNase H and is required for the essential function of target cleavage. Active site of PIWI domain has catalytic triad aspartate–aspartate–histidine. Although Ago protein is integral part of RISC complex but it’s a multi-protein complex (Hall and Carolina, 2005). In cytoplasm RISC loading complex members are transactivation response (TAR) RNA binding protein (TRBP), Dicer, GW182 and Ago proteins etc, and Protein kinase RNA activator (PACT) is also integral to the RISC complex. Role of TRBP and PACT are critical in miRNA processing, depletion of these results in decreased mature miRNA level (Redfern et al., 2013). Apart from cytoplasmic post-transcriptional gene silencing role of Ago proteins, non-canonical function of transcriptional gene silencing (TGS) in nucleus is also reported. Ago2 protein guided TGS with small RNAs facilitates transcriptome surveillance, suppression by long non-coding RNA, maintenance of epigenetic inheritance. In nucleus Ago2-miRNP with histone methyltransferases (HMTs) or histone deacetylases (HDACs) inhibit the transcription by modifying histones on the promoter region of target genes by blocking entry of RNAP II on promoter (Hawkins and Morris, 2010). RISC was also shown to perform gene regulation through splicing events. Several reports indicates specific alternative splicing through TGS by synthetic siRNAs. These siRNA target exonic or intronic sequence near to the splicing junction of mRNA thereby regulating the splicing of specific introns. Intrinsic siRNAs trigger alternative splicing by inducing histone H3K9 dimethylation and subsequently inhibiting transcription through lysine methyl transferase (KMT) activity resulting in the slowdown of RNAP II (Huang and Li, 2014). Resulting in increased availability of time for splicing factors to recognize splice sites and influences splicing decisions (Kalantari, Chiang and Corey, 2016). All these evidences show that Argonaute 2 functions as epigenetic modifier or post-transcriptional regulator. Argonaute 2 functions depend on the localization in cellular compartments and its interacting partners.
1.8. Micro RNA in Host-Pathogen interactions

miRNA plays an important role in the host-pathogen interactions of many pathogens, including numerous parasites and viruses. For example, enhanced replication of HIV-1 was reported when key components in miRNA biogenesis like Dicer or Drosha were inhibited (Triboulet et al., 2007). While there are many examples of miRNAs involved in viral replication and host-immune responses, miRNAs also play a similar role against complex parasitic pathogens. Some prokaryotes (Archaea and Bacteria) also possess Argonaute like proteins and their ability to utilize miRNA is quite widespread (Makarova et al., 2006). *Trypanosomes* and *Entamoeba* possess several endogenous miRNA and several proteins similar in function and sequence to Dicer and Argonaute 2 (Shi, Tschudi and Ullu, 2006) (Shi, Tschudi and Ullu, 2007). *Entamoeba histolytica* also encode several miRNAs and their target have been identified and validated (Abed and Ankri, 2005) (De, Pal and Ghosh, 2006). *Giardia lamblia* possesses small RNA similar to miRNA that are processed from snoRNA and has a homolog of Dicer and Ago2 to make these small RNA function like miRNA (Saraiya and Wang, 2008). *Toxoplasma*, the closest relative to *Plasmodium*, has been shown to alter the levels of several host miRNA during its infection (Zeiner et al., 2010) (Zeiner and Boothroyd, 2010). In addition, *Toxoplasma* appears to possess an array of small RNA which functions via an Argonaute-like protein complex (Sautel et al., 2010). Micro RNAs have been identified from within mature erythrocytes, which is one of the host cells for *P. falciparum*, which led us to wonder whether those miRNA might play a role in malaria infection.