Review

# Identification and bioinformatics characterization of translation initiation complex eIF4F components and poly(A)-binding protein from *Plasmodium falciparum*

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Protein synthesis in eukaryotes initiates with binding of the multisubunit translation initiation complex eIF4F. This complex contains eIF4E, eIF4A and eIF4G. eIF4E directly interacts with the cap structure, eIF4A is an RNA helicase and eIF4G acts as a scaffold for the complex. eIF4G contains the binding sites for both the subunits i.e., eIF4A and eIF4E and it also interacts with poly(A)binding protein (PABP). In present study we have identified and characterized the main components of the eIF4F complex i.e., eIF4E, eIF4A and eIF4G and PABP from *Plasmodium falciparum*. Molecular modeling of PfeIF4E, PfeIF4G and PfPABP confirms that they contain all the characteristic conserved structural features. We have annotated some of the genes of *P. falciparum* and as a result these studies demonstrate that the components of translation initiation complex are highly conserved. Therefore these studies will contribute to understand the basic biology and components of translation complex in P. falciparum.

Generally eukaryotic mRNAs contain a 5' cap (m(7)GpppN) where N is any nucleotide and a 3' poly(A) tail (50–300 nucleotides), both of which are essential for efficient mRNA translation and are specifically recognized by the proteins. Eukaryotic translation initiation is a multistage process, which involves several sequential steps performed by a multisubunit protein complex termed as eukaryotic translation initiation factor 4F (eIF4F).<sup>1,2</sup> eIF4F is composed of multiple translation factors, which assemble at the monomethylated cap present on the 5'-end of the mRNA to promote the recruitment of the ribosome. eIF4F mainly consists of three subunits, eIF4A (eukaryotic translation initiation factor 4A), an ATP-dependent RNA helicase and a member of the DEAD-box protein family (DEAD corresponds to Asp-Glu-Ala-Asp), eIF4E (eukaryotic translation initiation factor 4E) which binds to the 5' cap structure of the

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Previously published online as a *Communicative & Integrative Biology* E-publication: http://www.landesbioscience.com/journals/cib/article/8843 mRNA and eIF4G (eukaryotic translation initiation factor 4G) that acts as a scaffold for the complex with binding sites for both eIF4A and eIF4E. eIF4G is also responsible for the circularization of the mRNA via interaction with poly(A) binding protein (PABP).<sup>3</sup> The 5' cap and the 3' poly(A) tail of eukaryotic mRNAs cooperate to synergistically increase translation.

The functions of the components of eIF4F complex have been well established by studies in various systems. eIF4A is believed to be involved in the unwinding of the inhibitory secondary structures present in the 5' untranslated region of the mRNA and its maintenance in a single stranded form to facilitate the binding and scanning of the ribosomes to the initiator AUG codon.<sup>4,5</sup> eIF4E mediates cap identification during initiation and is absolutely necessary for capdependent translation.<sup>6</sup> Most organisms contain a single gene, which encodes eIF4E but some organisms contain more than one eIF4E gene, which belong to different families.<sup>7</sup> eIF4G is an adapter protein with a modular structure, which is required to mediate the ribosome recruitment via the cap structure and poly(A) tail.<sup>1</sup> Mammals possess two functional isoforms of eIF4G eIF4GI and eIF4GII.<sup>2</sup> The binding sites for numerous interaction partners of eIF4G have been determined by mutation and deletion analysis and it has been suggested that eIF4G provides multiple contact points for interaction with other components of the initiation complex.<sup>2</sup> It has been shown that mammalian eIF4G contains two interaction domains for eIF4A, a C-terminal domain and a central domain and the interaction of eIF4A with the middle region of eIF4GI is necessary for translation, whereas the interaction of eIF4A with the C-terminal region plays a modulatory role.<sup>8,9</sup> PABPs usually bind poly(A) using one or more globular domains containing RNA-recognition motifs (RRMs) and therefore help in stabilization of mRNAs. PABPs are involved in interaction of the poly(A) tail with the translation initiation complex and simultaneous binding of eIF4E and PABP to eIF4G is essential for efficient translation.<sup>2,3,10</sup> The highly conserved PABP polypeptides are found only in eukaryotes; singlecelled eukaryotes each contains 1-2 PABP genes, while humans have five and Arabidopsis has up to eight PABP genes.<sup>3,11</sup> Several molecules of PABP cover the entire length of the poly(A) tail of the mRNA and this interaction occurs via RRMs of PABP.<sup>3,11</sup>

Malaria is a major parasitic infection caused by the protozoan parasites of the genus Plasmodium and *Plasmodium falciparum* causes

the most virulent form of malaria.<sup>12</sup> In order to understand the basic biology and specially the translation process in this protozoan parasite we have bioinformatically characterized various components of the eIF4F complex from *P. falciparum*. In previous studies we have reported the detailed characterization of an eIF4A homologue designated as PfH45 from P. falciparum.<sup>13,14</sup> In the present manuscript we present characterization of the other principal components of the eIF4F complex such as eIF4E, eIF4G and PABP from P. falciparum. Our studies reveal that P. falciparum contains only one gene each encoding eIF4E, eIF4G and PABP. The bioinformatics characterization of these proteins reveals that PfeIF4E, PfeIF4G and PfPABP all contain the characteristics features. PfPABP also contains the characteristic RRMs. A three-dimensional homology model of the middle domain of PfeIF4G shows that there is sufficient conservation to human eIF4G to make the models virtually super-imposable. The studies reported here show that despite the insignificant homology of PfeIF4G at the sequence level its structure is highly conserved. These studies will make an important contribution towards understanding the process of translation in the parasite.

## Sequence Analysis and Computer Based Prediction of Structure of eIF4E Homologue from P. falciparum

The search of the *P. falciparum* genome using eIF4E as query revealed that only one homologue of eIF4E is present in the genome. The 684 base-pair open reading frame of P. falciparum eIF4E (PfeIF4E) was analyzed. The deduced amino acid sequence of PfeIF4E revealed a protein consisting of 227 amino acids with a predicted molecular mass of approximately 27 kDa and it is a basic protein with a calculated isoelectric point of ~8.4. The sequence analysis confirmed the presence of characteristic motifs of this family including the conserved tryptophan residues.7 The 'PlasmoDB' (http://www.plasmodb.org) entry number for this gene is PFC0635c and it contains no introns. A multiple alignment of amino-acid sequence homology search using NCBI database revealed that PfeIF4E aligned contiguously and showed highest homology with its counterparts from *Plasmodium berghei* (~92%) and ~19-40% homology with eIF4E from other sources (Fig. 1A and B).

The 3 dimensional structures of the yeast eIF4E determined by NMR and mouse and human eIF4E proteins determined by X-ray crystallography have been reported.<sup>15-18</sup> The murine protein has a cupped hand shape and contains eight-stranded anti-parallel  $\beta$ -sheets, backed by three  $\alpha$ -helices on its convex side.<sup>16</sup> A thorough examination of the alignment of PfeIF4E revealed that the capbinding pocket is highly conserved. A comparison of the amino acids of human eIF4E and PfeIF4E is shown in Figure 2A. The interactions mediated by the amino acids required for specific binding to the 7-methyl-GDP are taken over by the amino acids Trp37, Asp102, Trp115, Glu116, Lys170, Arg180, His125 and Trp184 in PfE27,<sup>16</sup> (Fig. 2A, marked by red asterisk). Similarly the alignment showed that the amino acids His37, Pro38, Val69, Trp73, Leu128 and Leu135 of mammalian eIF4E,<sup>19</sup> which are responsible for binding to eIF4G, are replaced by the amino acids Leu31, Leu32, Val67, Trp71, Asn145 and Leu149 in PfeIF4E (Fig. 2A, marked by blue asterisk). For the location of the binding pocket on PfeIF4E, the complete protein sequence was submitted to the JIGsaw program (http://www. bmm.icnet.uk/servers/3djigsaw/).<sup>20-22</sup> A highly similar and superimposable model based on the crystal structure of human eIF4E was that PFL1170w (PfPABP) aligned contiguously and showed highest

obtained<sup>16</sup> [Fig. 2B (i)]. In these models the amino acids responsible for the binding of eIF4G have been marked using the VMD software (www.ks.uiuc.edu) and are shown in yellow color [Fig. 2B (ii) and (iii)]. These results further show that the binding pocket is present on the similar planes in PfeIF4E and human eIF4E.

# **Sequence Analysis and Computer Based Prediction** of Structure of eIF4G Homologue from P. falciparum

On simple text-based data mining of PlasmoDB with the text term 'MIF4G' five genes are reported. These are PF11\_0086 (hypothetical protein of 3334 amino acid in PlasmoDB), PFI1265w (hypothetical protein of 1754 amino acid in PlasmoDB), PFL1855w (putative cell cycle control protein of 967 amino acid in PlasmoDB), PF14\_0113 (hypothetical protein of 943 amino acid in PlasmoDB) and MAL13P1.63 (asparagine-rich protein of 1192 amino acid in PlasmoDB). The detailed analysis of all of these proteins revealed that these contain MIF4G domain but all the others except MAL13P1.63 did not reveal any detectable homology to known proteins from other organisms. The detailed sequence analysis of MAL13P1.63 (~139 kDa protein) confirmed the presence of characteristic motifs of eIF4G. A multiple alignment of amino-acid sequence homology search using NCBI database revealed that PfeIF4G aligned contiguously and showed highest homology with its counterparts from Plasmodium yoelii (~49%) and ~6-13% homology with eIF4G from other sources (Fig. 3A and B).

Since PfeIF4G contained insignificant homology at amino acid level therefore to check the homology at structural level, the complete protein sequence of PfeIF4G was submitted to the 3D-JIGSAW program and a three-dimensional model was obtained, which was based on the X-ray structure of the template human eIF4G.<sup>23</sup> On careful analysis it was observed that this model was built by using only the phylogenetically conserved region i.e., amino acid 888 to amino acid 1125 in PfeIF4G (Fig. 4B (i)). It is interesting to note that this domain is composed of ten  $\alpha$  helices arranged as five antiparallel  $\alpha$  helical pairs or HEAT repeats (named for Huntington, Elongation factor 3, A subunit of protein phosphatase 2A [PP2A], and Target of rapamycin).<sup>24</sup> Similarly using the complete protein sequence of human eIF4G (AF104913) a three dimensional model was obtained [Fig. 4B (ii)]. This model spanned from amino acid 712 to 944 of human eIF4G. The alignment of amino acid 888 to 1125 of PfeIF4G and amino acid 712 to 944 of human eIF4G showed only 22% similarity and 27% identity (Fig. 4A). But it is interesting to note that the two structures of these regions are highly similar and largely super-imposable [Fig. 4B (iii)].

# **Sequence Analysis and Computer Based Prediction** of Structure of PABP Homologue from P. falciparum

On simple text-based data mining of PlasmoDB with the text term 'poly A binding protein' two genes are reported. These are MAL13P1.303 (putative polyadenylate binding protein of 414 amino acids) and PFL1170w (putative polyadenylate binding protein of 875 amino acids). MAL13P1.303 is small in size to be a bonafide PABP. The detailed sequence analysis of PFL1170w revealed a protein with a predicted molecular mass of ~97 kDa and it is a basic protein with a calculated isoelectric point of ~8.9. A multiple alignment of amino-acid sequence homology search using NCBI database revealed K LTFN:

W::WEQ:

18

40

24

58

72

98 98

DGSE 112

- - - - - - S

EILDE

Y

: D

L

: K S

S. cerevisiae

H. sapiens

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P. falciparum
                                  - MKYLTFNKNNRDAIDLS-
P. berghei
                 ----E
T. annulata
              MAESASVAVNGTKKCPSLTFNKNTEDHAKLS----- 832
              ----MTEKEVVSKYLSFNESIEPNLTLPTA----CS 28
C. parvum
             1
              MVVEDTPKSIITDDQITTNPNRVIEDDNNLEEGEILDBDD
P. sativum
             1
 S. cerevisiae
            1
              H. sapiens
                         - MATVEPETTPTPNPPTTEEEKTE
             1
              м
P. falciparum 19 K L B A T K I D L S N P L L L Q Y N W V I W E Q V S D N K I K Q S N N Y K D Y T
P. berghei 19 K I E A T K I D L S N P L L L Q Y N W V I W E Q V S D N K I K Q S N N Y K D Y T
            33 LFESTTINLDTPLSLKNKWVIWEQIVKMPEHSQNDYKEHT
T. annulata
            29 EVDLPEDLISRPIPLSHEWIVWEQLN-VETRKDLDYSNAT 67
41 SSATSKPVVHQPHLLENSWTFWFDTPAAK-SKQAAWGSSM 79
C. parvum
P. sativum
           S. cerevisiae
 H. sapiens
                : E : : : :
                               PL
                                   L:
P. falciparum 59 R P L A K F N S V Q K F W Q L W N R L P Q P S D L L A Q R S M T R F S E D G I F
P. berghei 59 R P L A K F N S V Q K F W Q L W N R L P Q P S D L L A Q R S M T R F S D D G I F
            73 KPLVSFDSVQAFWNLWFNIPQPSELATNKRLARECS
 T. annulata
C. parvum
P. sativum
              R P : : : F
                        svo
                               FW
                 VDA: FRD
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S. cerevisiae H. sapiens LWNNIPQPS:L: : MRS DG P. falciparum 99 RIVDALMIFRDNIQPMWBDPANSGGGHFEYKILPKDYPYS 138 P. berghei 99 RIVDALMIFRDNIQPMWEDPANAGGGHFEYKILPKDFPYS 138 113 H F V D A I M V F R D G V Q P M W E D P L N K D G G H F D Y R F R P T D V Q Q L 152 T. annulata C. parvum 108 SVVDAVILFKEGIOPMWEDPMNATGGHIHFRAWQSSVVPG 147 C. parvim 100 - - - FYC FK HK IE PK WEDP ICANGGKWSFQLRGK - - GA 124 H. sapiens 91 - - - YSL FKDGIE PM WEDEKNKRGGRWLITLNKQQ-RRS 124 IQPMWEDP:NA GGH: : : : P. falciparum 139 Q I D E F W N N L V L A I I G C S L K H Y D - L I T G I R L V D K L S T T R Y G 177 P. berghei 139 Q I D E F W N N L V L A I I G C S L K H Y D - L I T G I R L V D K L S T T R Y G 177 T. annulata 153 T V D E Y W N N I I L G L V G S T I P L C E - F I N G V R L V D K L S S - R F P 190 C. parvum 148 ELDTMWNNLVLAVIGGSLENSS-IVNGIRLVDKLGG-NKG185 140 K S D T S W L Y T S L A M I G E Q F D H G D - E I C G A V V N V R G R A - - - - 174 P. sativum s. cerevisiae 125 DIDELWLRTLLAVIGETIDEDDSQLNGVVLSIRKGG---- 160 H. sapiens 125 DLDRFWLETLLCLIGESFDDYSDDVCGAVVNVRAKG----160 G:RLVDKL : DE: WNN: : LA: IG s : : D I TR G P. falciparum 178 Y I R I E I W Y T T I T D E S V K N Y L R K D L E E H M C N R I D G S T I F P P 217 P. berghei 178 YIRIEIW YTTITDENVRNHLRKDLEEHMCNRIDGSHVYPP 217 191 V L R I E V W F K N L G E A N D P T Q L M K S V G S C M A R K L D G S V G T I P T. annulata 230 186 NIRVEIWFSDFSNQSAHQALLKEIETLMSSLLDGTSCEPP C. parvum 225 175 - EKISIWTKNASNEAAQVSIGKQWKEFPDYNEAMGFIFHD 213 P. sativum S. cerevisiae 161 - N K F A L W T K S E - D K E P L L R I G G K F K Q V L K L T D D G H L E F F P 198 H. sapiens 161 - D K I A I W T T E C E N R E A V T H I G R V Y K B R L G L P P K I V I G Y Q S 199 :RIEIW:: :::: : L:K : E м : DG: :: P P. falciparum 218 RV - KSLSHIHR - - - - - 227 P. berghei 218 RV-KSLSHVHK-----227 T. annulata 237 226 HF-EVKSHSKSKPDAN-- 240 C. parvum P. sativum 214 DARKLDRNAKNKYVV--- 228 s. cerevisiae 199 HSSANGRHPQPSITL -213 H. sapiens 200 HADTATKSGSTTKNRFVV 217 н:: : : : vv 0.358 P sativum 0.037 P. falciparum 0.24 0.035 0.038 P. berahei T. annulata 0.024 0.311 – C. parvum

Figure 1. (A) Comparison of amino acid sequences of P. falciparum eIF-4E protein (PlasmoDB No. PFC0635c; GenBank accession No. EF043517) with other elF-4E from Plasmodium berghei (XP\_674865), Theileria annulata (CAI74251), Cryptosporidium parvum (CAD98650), Pisum sativum (ABG35118), Saccharomyces cerevisiae (NP\_014502) and Homo sapiens (P06730). The accession numbers of the aligned sequences are written in brackets. (B) Phylogenetic guide tree using Clustal W program based on the multiple alignment. A value of 0.1 corresponds to a difference of 10% between two sequences.

0.347

0.347

B

0.05

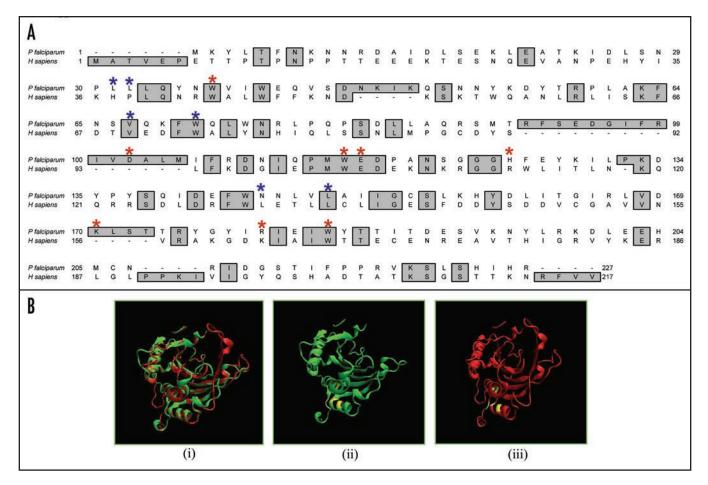


Figure 2. (A) Amino acid alignment of *P. falciparum* eIF4E (PlasmoDB No. PFC0635c, GenBank accession number EF043517) and human eIF4E (GenBank accession number P06730). The amino acids responsible for 7-methyl GDP-binding and eIF4G binding are marked by red and blue asterisk respectively. (B) A three-dimensional model for PfeIF4E was created as described in text, which was based on the crystal structure of human eIF4E.<sup>16</sup> The structures have been displayed using molecular visualization program for displaying, animating and analyzing large biomolecule systems using 3-dimensional graphics and built-in scripting (VMD software www.ks.uiuc.edu). (i) Super imposed image of the structure of human (green) and *P. falciparum* (red) eIF4E The amino acids responsible for the binding of eIF4G in (ii) human and (iii) *P. falciparum* eIF4E have been marked and are shown in yellow in the models.

homology with its counterparts from *Plasmodium berghei* (-71%) and -29–37% homology with PABP from other sources (Fig. 5). It was observed that similar to other PABPs, PfPABP also contains four RRMs, which span from amino acid 16–94; 104–176; 193–261 and 450–527 respectively (Fig. 6A).<sup>11</sup> It contains a few regions, which are Asn rich (amino acid 295–396; 617–799), Thr rich (amino acid 317–362) and Gln rich (amino acid 557–761) (Fig. 6A). The presence of homorepeats is a characteristic feature of proteins from *P. falciparum* and the most common is asparagines followed by lysines and glutamine.<sup>25,26</sup> These inserted residues encode non-globular domains of unidentified function, which are extruded from the core of the protein and probably form surface exposed structure that consequently in most of the proteins have no effect on the functional folding of the protein.<sup>25,26</sup>

The complete protein sequence of PfPABP was submitted to the 3D-JIGSAW program and a three-dimensional model was obtained, which was based on the X-ray structure of the template human PABP. These two structures were not similar and not super-imposable (data not shown). The amino acid alignment of full-length human and PfPABP revealed a similarity of ~14% and an identity of ~33% and it further showed that PfPABP contains an insertion of ~150

amino acids in between RRM3 and 4 (Fig. 6A). It is interesting to note that only the modeled structure of PfPABP from amino acids 1–180 (RRM1 and 2) superimposes with that of human PABP [Fig. 6B (i)] whereas the structures of PfPABP from amino acids 1–264 (RRM1-3) and from amino acids 1–531 (RRM1-4) are not superimposable with human PABP [Fig. 6B (ii) and (iii) respectively].

Our unpublished observations indicate that the middle and C-terminal domain of PfeIF4G is structurally conserved and is able to interact with PfH45, PfeIF4E and PfPABP but there was no detectable interaction between the PfeIF4G-N fragment and PfeIF4E or PfH45 or PfPABP. These interactions are shown schematically in Figure 7A and B shows the overall initiation complex of *P. falciparum*.

Protein synthesis is a complex process in eukaryotes and it requires numerous different macromolecules. The critical step of translation initiation is a coordinated action of a number of translation initiation factors. Most important of these is the heterotrimeric eIF4F complex, which is composed of eIF4A, eIF4E and the very large protein eIF4G, which mediates the interactions between eIF4F and other translation factors. Previously we have reported the char-

A P. falciparum P. yoelii S. pombe O. sativa H. sapiens	1 <u>М N S</u> N H N N N C E D K D N D W S Y L R S G A N T S F R N S I 31 1 M S C I D V K Q E N I Q N I D 15 1 <u>M S S K P P S N T P K F S Y A R A L A S S Q S N K S N S T 29</u> 1 0 1 <u>M N T P S Q P R Q H F Y P S R A Q P P S S A A S R V Q S A A P A R P G P A A H V 40</u>
P. falciparum P. yoelii S. pombe O. sativa H. sapiens	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$
P. falciparum P. yoelii S. pombe O. sativa H. sapiens	64 N N R N N S L N N N D M N N N N N N N N N N N N N N A N
0. sativa	92 L S F S R N F K N - S F N K G M N R N N S V T G N I K G I N N 121 75 R D N L I D - D N N R R L D K D G N L G N F R K K Y P N 101 105 P W R G D N T S K P S A N S S A E R T S S Q H Q K P E T S S Q I G K D N A A P V 144 54 P N F E V - R E R V R Y T R D Q L L E L R E I V D 77 121 A P V L M N Q P P - Q I A P K R E R K T I R I R D P N Q G K D I T E E I 156 N P N S S S S S S S S S S S S S S S S S
P. yoelii S. pombe O. sativa	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$
P. yoelii S. pombe O. sativa	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$
P. yoelii S. pombe O. sativa	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$
P. yoelii S. pombe O. sativa	204 Q N T N E F K D N K K N M N F K N Q Y N N N Y - K F D - E N M N N S N T M H S R 241 182 A N S E M N Q N N S K - M G N S S N Y N N L P - D G N - F N N N N N N N N N K M F F K 218 265 Q T T Q V S A S N S P A L S G S S T P A L S G S S N Y N N L P - D G N - F N N N N M N N N K M F F K 218 131 R T V Q P P A A B N E E K S W D N N I R
P. yoelii S. pombe O. sativa	242 N S N V E B H L R N N S I D M N N S N I N N Y T N Q Q T R F - 271 219 N N K F N K P G S Q N N T D S S N N F N N I N N N Q Q T R F - 247 305 D R Y G N S H P S Y N K Y S H Y Q H G F N Y N N S G N N R N R S G H 338 152 A A H A S S G R Q Q B Q 163 313 S H T V E I H B P N G M V P S E D L E P E V E S S P E L A P P A C P S B S P V 352 . H : S : N

Figure 3A. For figure legend, see page 253.

acterization of an eIF4A homologue (PfH45) from *P. falciparum* and have shown that PfH45 is localized in the cytoplasm and is essential for parasite survival.<sup>13</sup> In the present study we have isolated and characterized the other components i.e., eIF4E and eIF4G of eIF4F complex and PABP from *P. falciparum* using the bioinformatics approach. Previously it has been reported that PfeIF4E binds to mRNAs in *P. falciparum*.<sup>27</sup>

Previous studies have shown that the HEAT repeat proteins participate in a wide variety of cellular processes, which are dependent on accumulating large multiprotein complexes.<sup>23,24</sup> The overall similarity between human eIF4G and PfeIF4G is not very high

(only ~12%) because it is well established that the HEAT repeat proteins do not share any absolutely conserved amino acids.<sup>23</sup> It has been reported that although *Leishmania major* contains five eIF4G homologues but most of them show similarity to the human sequence only at the level of central HEAT domain.<sup>28</sup> The comparison of human and *P. falciparum* eIF4G suggests that specifically the middle region of PfeIF4G might be involved in the interaction with PfH45 as reported for human eIF4G and eIF4A.<sup>24</sup> Due to the presence of HEAT domain, the conserved middle domain of PfeIF4G most likely serves as a central assembly platform for its translation initiation machinery. It has been reported previously that mammalian eIF4A

P. falciparum P. yoelii S. pombe O. sativa H. sapiens	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
P. falciparum P. yoelii S. pombe O. sativa H. sapiens	300       K Y D N       N       N       -       -       S       S       M K N T D N N K       T       -       -       D       T       S       Y       N M K G T I N N D       N N N D Y       335         276       M E N E       N N N       -       -       -       -       -       -       -       -       -       296       N M K G T I N N D N N N D Y       335         372       P Q N V       N N N G S       T       P       V S L Q       T       P Y G Q V Y G Q P Q Y I V D P N       -       -       -       -       -       296         377       K A Q V G P -       T       T       V S L Q       T       P Y G Q V Y G Q P Q Y I V D P N       -
P. falciparum P. yoelii S. pombe O. sativa H. sapiens	336       L R N I N N I N E Y K G S A K N K F Y T N Y M N K N       N L K F T Q N N N D N - 372         299
P. falciparum P. yoelii S. pombe O. sativa H. sapiens	373       M N T N E D N N N N N N N N N N N G V F S N Y Q N 336         314       I K Y Y G N N H N S P N N N N L P P N V N M H N 336         448       Q V V E S P N S S T L S P R N G F A P I V K Q Q K K S S A L K 476         210       V K G I L N K L T P E K F D L L K G Q L M 236         473       K R R       K K E A V G D L L D A F K E A N P A V P E V E N Q P A G S 512         K R R       :       N
P. falciparum P. yoelii S. pombe O. sativa H. sapiens	397       -       -       N       M       N       N       N       N       N       N       N       N       N       N       N       M       N       M       N       363
P. falciparum P. yoelii S. pombe O. sativa H. sapiens	433 <u>S</u> N N N F Y M N Y N Y Q N R K N S M N N N M N N N M N N N M N N N M N N M N N M N N N M N N N M N N M N N N N M N N N M N N N M N N N M N N N M N N N M N N N M N N N M N
P. falciparum P. yoelii S. pombe O. sativa H. sapiens	464       H N M N N N M N       H N M N N M N M N M N M N M N N N M N H N M N N M N H N       M N       491         378       E N M N Q P N N       T Y M Q N N Y N D V N N Y S N I N I S       405         547       R K A E E K A R L E A E E N A K R E A E E Q A K R E A E       574         262       A Q L C S D L N E K L P S F P S E E P G G K E I T       286         593       L P H I S D V V L D K A N K T P L R P L D P T R L Q G I N C G P D F T P S F A N       632         :<
P. falciparum P. yoelii S. pombe O. sativa H. sapiens	492       N N M N N I N S L D S D M S P N Y H A H V K M S M M N Y N N N E S N T A N P       529         406       N N Y M N D N N L K K N I D S S N L P H M N V M R G N N N D D G 437         575       E K A K R E A E E K A K R E A E K A K R E A E E N A K R E A E E K A K R E
P. falciparum P. yoelii S. pombe O. sativa H. sapiens	530       N Q M N F E Q T N N D N - M K R E N N N M N N Y G Y D D N T V H       560         438       D F V K R E F T N E N - N N Q N N Q N N V T K N T       462         613       A E E K A K R E A E K K E K E E F K A K R E A E E K A K R E A E K K E K E K E K E K E K E K E K E

Figure 3A (continued). For figure legend, see page 253.

binds to the central region of eIF4G via its HEAT domain and it also binds to its C-terminal domain.<sup>29</sup> Similarly in yeast it has been shown that eIF4G and eIF4A physically associate and this association is required for translation and is essential for yeast cell viability.<sup>30</sup> The binding site for eIF4E in human eIF4G has been reported previously<sup>31,32</sup> and the alignment of the human and PfeIF4G shows that this site in PfeIF4G is between amino acid 464 to 473 and these amino acids are present in the middle domain of PfeIF4G.

It is interesting to note that *P. falciparum* contains only one PABP, which is localized in the cytoplasm and as reported earlier evolutionarily PfPABP is closer to PABP from *L. major.*<sup>3</sup> Multiple PABPs, both nuclear and cytoplasmic have been reported in human.<sup>33</sup> It has been reported previously that the amino acid sequences of the PABP-binding site in eIF4G are not conserved between yeast<sup>31</sup> and human.<sup>32</sup> The interaction between PABP and eIF4G is conserved in many species and this interaction can be imparted to RRM 1 and 2 of PABP.<sup>33</sup> Previously it has been reported that the interaction of eIF4G with PABP requires RRM1 and 2 in animal and yeast systems<sup>3,32,34</sup> but in wheat PABP RRM1 was sufficient to interact with eIF4G.<sup>35</sup> The structural modeling of PfPABP suggests that the RRM1 and 2 are structurally conserved and PfPABP is also able to interact with the middle and C-terminal domain of PfeIF4G.

Overall in this study we have reported the bioinformatics characterization of important components of translation initiation complex

P. falciparum 561 VNNNTPSTDFFSRAVGYNNNYLNNNNNNSAVNNNSSNGN 600 463 T G N M D A S T Y I M N A F A A F N N N S T N N - - - - - - - - - - - S N T N 647 E K A K R E A E E K A K R E A E E N A K R E A E E K A K R - - - - E A E E N A K P. voelii 490 s. pombe 682 sativa 338 LIGELLKQKMVPEKIVHHIVQELLG-----366 - - - - S G P D 713 ADGSKTQDLFRRVRSILNKLTPQMFQQLMKQVTQLAIDTE H. sapiens 752 : : : P. falciparum 601 NMKNENSE - - - NKNVADN - - - NDSLNNNKNNNN - NI - NMN 632 491 NTNNDN----DKNVIN----DSSNNNSNDKR-SD-HMN P. yoelii 518 s. pombe 718 sativa 381 753 ERLKGVIDLIFEKAISEPNFSVAYANMCRCLMALKVPTTE H. sapiens 792 EKNV NFS 

 P. falciparum
 633
 E S I N N N N T L N N N N E Y N N Q N N N E D E D D D D - - - - - - - W G
 662

 P. yoelii
 519
 A P N N K S - - - - - - - N N N G D E E F D B - - - - - - W G
 536

 S. pombe
 719
 A P L A S S - E A N V D T S KQ T N A T E P E V V D K T K V E KL K - A S E G
 755

 sativa H. sapiens N N DE: 663 - - - ELGE - - - - DKYIDINSIMKKN - - - - - V 537 - - - ELGE - - - - DKYIDINSIIKQKN - - - - - V 756 KSTSSLSPSHSTSSKRDLLSGLESLSLKTNPKSEQCLES P. falciparum 681 P. yoelii 555 s. pombe 795 sativa 410 H. sapiens 856 PSHSTSS ELG D:: D: SI : K : N P K S E O C L E V 682 I L N - Q L E A D L N D L S K K G N D G K - - N K K K N K M K K D D - - - - L F 556 I L N - Q L G A N L N D M T K K G N D N K - - N K K I K S K K D N P F T P D N 796 L L N S Q F I T D F S A L V Y P S T I K P P S T E E A L K A G K Y E Y - - D V P P. falciparum 714 P. yoelii 592 s. pombe 833 411 TTNLQLAPRLRFMVRDVVDLR---SNNWVPRRE---0. sativa 441 857 LGNIKFIGELFKLKMLTEAIMHDCVVKLLKNHDEE-SLEC H. sapiens 895 LN 0 L : : : L : D:: т. : к : к KDE 715 VLPHTN<u>T</u>LLVDKKKNKKNKNAKNNNTNSNNNNNNNN P. falciparum 754 P. yoelii 593 LNIPTTSLASDKKKNKRNKNKNGKNDNNTKENNNMIDKEK 632 834 FLLQFQSVYTDKPMKGWDBRMKETVASAFSDKSSRGMYSS s. pombe 873 442 IKAKTISEIHDEAIKTLGLRPGATGLTRNGRNAPGGPLSP 481 0. sativa H. sapiens 896 LCRLLTTIGKDLDFEKAKPRMDQYFNQMEKIIKEKKTSSR 935 : : T S : : DK p. falciparum 755 NNNNNNNNNNNSNILKK-DNDANDKNSKQ-IVNASGVNKKKGK 792 633 NKQN----AN---NLANKKKTEK\_TVNTTNNSTQ--P. yoelii 658 482 G G F P - - - - M N - - - R P G T G G M M P G - M P G T P G M P G S - - 507 936 I R F M L Q D V L D L R G S N W V P R R G D Q G P K - T I D Q I H K B A B M B E 974 s. pombe 0. sativa H. sapiens SN: : K : : P : P. falciparum 793 KTGVEANSKNVEQKGDTKTSTKVD---EEVKTDEVNNKKK 829 - 0 K P. yoelii 659 - - - N - - - E E K Q L D N I N N - - -671 - SGAGFGSGSNYKSAPSRGVSHHG---HGGMSGSHRGSQR948 s. pombe 913 0. sativa 508 975 HREHIKVQQLMAKGSDKRRGGPPGPPISRGLPLVDDGGWN 1014 H. sapiens 830 DLIEDKN - - ANNVNDVDTKPDGDDDNNNNNKGEEDNKE 867 P. falciparum P. yoelii 672 ----N--QSNIPQNNETVEGAKEVENTENK----NEE 698 949 GSRRGGG--ERDKPDPSSLTIPVDQVAPLQLS---ANRW S. pombe 982 ----NWEVPRSKSMPRGDSLRNQGPLL---NK- sativa 520 544 1015 TVPISKGSRPIDTSRLTKITKPGSIDSNNQLFAPGGRLSW 1054 H. sapiens SR : : P : G : N :

Figure 3A (continued). For figure legend, see page 253.

eIF4F and PABP from *P. falciparum*. We have shown that the scaffold protein PfeIF4G contains the characteristic features for binding to PfeIF4E, PfeIF4A (PfH45) and PfPABP and thus provides the assembly platform for the components of the initiation complex. In order to control malaria, it is essential to understand the basic biology of malaria parasite. Therefore this study makes an important valuable contribution towards better understanding of the function of main components of one of the basic metabolic pathways, the translation, in the malaria parasite.

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P. falciparum       868
P. falciparum       897       Y       N       R       Q       V       K       S       L       L       N       K       T       V       E       N       P       P       I       T       E       K       C       Q       I       N       T       D       -       -       -       932         P. yoelii       724       F       M       K       Q       I       K       L       N       K       T       V       E       N       P       P       I       T       E       K       C       Q       I       N       T       D       -       -       -       759         S. pombe       1007       V       Q       R       K       K       M       M       T       L       K       F       D       K       I       L       N       N       T       L       K       N       N       N       D       S       R       N       D       S       R       N       D       S       R       N       N       N       N       N       D       G       -       -       1044         O. sativa       563       S </td
P. falciparum 933 E I Q T V V N Q V I D K A V L B H D W S E M Y A D L C Q T L K W 964 P. yoelii 760 E I Q T V V N E V I N K A V L B H D W S E M Y A D L C Q T L K W 971 S. pombe 1045 R T L K Q V I Q L T F E K A T D B P N P S N M Y A D L C Q A L K W 791 O. sativa 600 P K P V S A A P A V V P V T D K A A G S S H E M P A A V Q K K T 631 H. sapiens 1135 L D R A R T P A T K R S F S K E V B E R S R E R P S Q P E G L R K A A S L T E D 1174 L D R : V : : : : K A V : E R S R E R : S E M Y A : A K
P. falciparum 965 R S P N F E M K K K T S F E I A L L K K I Q E Q Y E N L P S T 995 P. yoelii 792 R S P N F E M K K K S S F E I A L L K K I Q E Q Y E N L P S T 822 S. pombe 1078 S I D D S I R D E G V L D K N N Q P V R G G L L F R K Y L L S R - C Q E D 1113 O. sativa 632 V S L L E E Y F G I R I L D E A Q Q C I E E L Q C P E Y 659 H. sapiens 1175 R D R G R D A V K R E A A L P P V S P L K A A L S E E E L E K K S K A I I E E Y 1214 R S G R D : K F I A L L : Q E E : P E
P. falciparum       996       FE       S T M K E K L K S D - E N E E E L S FV E Q K Q K K R L L G I 1027         P. yoelii       823       FE S T M K E K L K N D - E N E E E L S FA E Q K Q K K R L L G I 1027         P. yoelii       823       FE S T M K E K L K N D - E N E E E L S FA E Q K Q K K R L L G I 1027         S. pombe       1114       FE R G W K A N L P S G K A G E A E I M S D E Y Y V A A A I K R R G L G L 1150         O. sativa       660       Y S E I V K E A I N L A - L D K G P N FI D P L V R L L E H L H 690         H. sapiens       1215       L H L N D M K E A V Q C V Q E L A S P S L L F I FV R H G V E S T L E R S A I A 1254         FE       K E L       E E : S       FV : : : K S T L : R : : G :
P. falciparum 1028 V K L I G E L F Q R Q I V S I S I V I S I A H D L L I A Y 1056 P. yoelii 855 V K L I G E L F Q R Q I V S I S I V I S I A H D L L I A Y 1056 S. pombe 1151 V R F I G E L F K L S M L S E K I M H E C I K R L L G N V 1179 O. sativa 691 T K K I F K T E D L K T G C L L Y A A L L E D I G I D L 718 H. sapiens 1255 R E H M G Q L L H Q L L C A G H L S T A Q Y Y Q G L Y E I L E L A E D M E I D I 1294 V K I G E L F H Q L L C A G H L S T : S : I : : : : D L L I :
P. falciparum 1057 E E P K E Y C I E A F L Q L I Y S T G F F I D K I E K Y K N V L 1088 P. yoelii 884 D E P K E Y C I E A F L Q L I Y S T G F F I D K K E K Y K N V L 1088 S. pombe 1180 T D P E E E E I E S L C R L L M T V G V N I D A T E K Y K N V L 915 S. sativa 719 P L A P A L F G E V V A R L S L S C S L S F E V V E E I L K A V E 751 H. sapiens 1295 P H V W L Y L A E L V T P I L Q E G V P M G E L F R E I T K P L R L G K A A 1334 : P E Y I E : : L : S G V P M G E : F I D : E K R P : K V :
P. falciparum 1089       D T W F G R L K E L Q R K K M Y S K R I K F V - I Q D V       1115         P. yoelii       916       D T W F G R L K E L Q R K K M Y S K R I K F V - I Q D V       1115         S. pombe       1212       D T V V L R M B T I T K I P N L P S R I K F M - L N D V       942         O. sativa       752       D T Y F R R R
P. falciparum 1116       F D L R S S E W R K K T H K D T - A K G L N E L R - 1139         P. yoelii       943       F D L R L S E W R K K T H K D T - A K G L N E L R - 966         S. pombe       1239       M D S R K N G W A V K N E V E K G P K T I A E I H E A B R K K A L A E S Q R P 1278         O. sativa       760       F D A V

Figure 3A (continued). For figure legend, see page 253.

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- - SOLETEEM MGG SІН P . falciparum 1140 - - - - - -- -T. ΑQ 1156 --SQLETEEM-- M G G -P. voelii 967 - SIH L AQ 983 1279 SSGRMHGRDMNRGDSRMGGRGSNPPFSSSDWSNNKDGYAR 1318 s. pombe - - - - - - M G G - - - N S 774 sativa 767 - - s G 0 - - - EGS - - SNQ LNRQLEKLLK---IEANLSEQQ 1433 H. sapiens 1404 R DW : MNRGDSRMGGRGSN QLE PF DW ΑQ IVIVfalciparum 1157 LGN-LR NES 1174 ₽. G ER -L G N - I V I V - - G E R - - H N - - - - - - I R N - -L G Q G I R G L K S G T Q G S H G P T S L S S M L K G G S - - 1001 984 ΝE s P. voelii VSRTP s. pombe 1319 - - 1353 s o. sativa A - - - ILSS - - - - - - -H A -775 781 IVSNTLVRALMTAVC H. sapiens 1434 **Y**SAIIFE LRVD v VLKA RAKLLQ 1473 ΤP А LG: I : : : G н L R AKLLQ s s кхмд 1182 falciparum 1175 ₽. Q P. yoelii 1002 ктмбеб 1009 RQN SALRREQ 1354 VRAP V A v 1374 s. pombe ₽ SN o. sativa 782 VVIDA 788 K Y L C D E Q K E L Q A L Y A L Q A L V V CN H. sapiens LEQPPNLLRMF DALYDE 1513 1474 т KYLCDEQKELQALYA Q s : : ΕQ PNLL ₽. falciparum 1183 - EKLSKQ - - - N QΚ 1192 P. yoelii 1010 ERLSKL-N QК 1019 s. pombe 1375 TSANSFELLEEHDHDNDGGQKD s NSKTSS 1403 O. sativa 789 - - - - K L L K -792 1514 DVVKEDAFYSWESSKDPABQQGKGVALKSVTAFFKWLRBA 1553 H. sapiens LS N ĸ V TAFFKWLREA QК s falciparum 1193 1192 - - - - - - -- - - - - - -P. yoelii 1020 1019 - - - - - 1403 s. pombe 1404 sativa 793 792 H. sapiens 1554 EEESDHN 1560 EEESDHN B 0.244 P. falciparum S. pombe O. sativa 0.047 H. sapiens P. yoelii 0.1

Figure 3. (A) Comparison of amino acid sequences of *P. falciparum* eIF-4G protein (PlasmoDB No. MAL13P1.63) with other eIF4G from *Plasmodium yoelii* (XP\_727991), *Schizosaccharomyces pombe* (Q10475), *Oryza sativa* (AAO72569) and *Homo sapiens* (AF104913). The accession numbers of the aligned sequences are written in brackets. (B) Phylogenetic guide tree using Clustal W program based on the multiple alignment. A value of 0.1 corresponds to a difference of 10% between two sequences.

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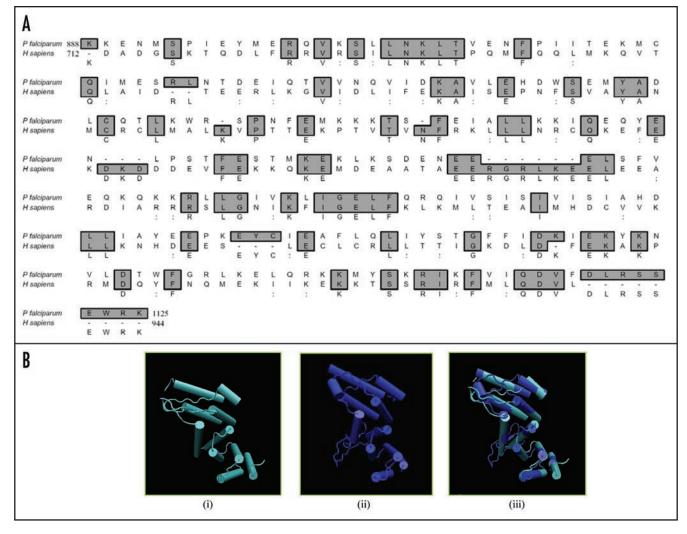


Figure 4. (A) Comparison of amino acid sequence (from amino acid 888–1125) of *P. falciparum* elF4G protein (PlasmoDB no. MAL13P1.63) with elF4G (from amino acid 712–944) of *H. sapiens* (GenBank accession number AF104913). (B) Three-dimensional models for (i) human elF4G and (ii) *P. falciparum* elF4G were created as described in text. The structures have been displayed using the same program as described in legend to Figure 1B. This model has been built by using only the phylogenetically conserved middle domain i.e., amino acid 888 to amino acid 1125 in PfelF4G. A super-imposed image is shown in (iii).

۸	
P. faloiparum	1 0
P. berghei T. annulata	1 0
C. parvum	10
S. pombe O. sativa	1 M P S T D L K K Q A D A A V E S D V N T N N E A V E S S T K E E S S N T P S T E 40
H. sapiens	1 0
	MPSTDLKKQADAAVESDVNTNNEAVESSTKEESSNT
P. faloiparum	1 HIATGTNMMHP8 F8 14
P. berghei T. annulata	1
C. parvum	1 M T S N N V V P V 10
S. pombe O. sativa	41 ΤΟ ΡΕΚΚΑΕΕΡΕΑΑΑΕΡΒΕΒΤΒΤΡΤΝΑΒΒΥΑΤΡΒΟΤΑΡΤ 78 5 V Ο ΑΥΡΑΑΕΓΟΓΑΡΡΟΑΝΟΥΎΑΑΟΒΑΑΑΑΑΤΓΟ 37
H. sapiens	1
-	ο λε λ ν : : 🗗 ουγε
-	15 TASLYVGDLNEDVTEAVLYEIFNTVGHVLSIRVCRDSVTR 54 15 TASLYVGDLSEDVTEAVLYEIFNTVGHVLSIRVCRDSVTR 54
P. berghei T. annulata	15 T A S L Y V G D L S E D V T E A V L Y E I F N T V G H V L S I R V C R D S V T R 54 26 S A S L Y V G D L K P D V T E A V L Y E V F N T V G P V A S I R V C R D S V T R 65
C. parvum	11 SASLYVGDLDADVTETMLYEIFNSVAVVSSVRTCRDALTR 50
S. pombe O. sativa	79 SASLYVGELD PSVTEAM LFELFNSTGPVASTRVCRDAVTR118 38 ATSLYVGELD VSVGDAOLED VESQVGSVVSVGSVCPDVNTP177
H. sapiens	38 A T B L Y V G D L D V S V Q D A Q L F D V F S Q V G S V V S V R V C R D V N T R 77 10 M A S L Y V G D L H S D V T E A M L Y E K F S P A G P V L S I R V C R D M I T R 49
	: ASLYVGDL DVTEA LYE: FN: VG V: SIRVCRD VTR
-	55 KBLGYAYVNYHNLADAERALDTLNYTNIKGOPARLMWSHR 94
P. berghei T. annulata	55 K S L G Y A Y V N Y H N L A D A E R A L D T L N Y T N I K G Q P A R L M W S H R 94 66 K S L G Y A Y V N Y Y S T Q D A E A A L E S L N Y I E I K G H P T R I M W S N R 105
C. parvum	51 R S L G Y A Y W Y W S V A D A E R A L D T L W FTC I R G R P C R I M W C L R 90
	51 R S L G Y A Y V N Y N S VÂD A E RALDTLNETCIRC R C RIM WCL R 90 119 R S L G Y A Y V N F H N MEDGERALDELNYTLIK G R P C RIM WSQ R 158
0. sativa H. sapiens	78 L S L G Y A Y V N F S S P A D A A R A L E M L N F T P I N G K P I R I M Y S N R 117 50 R S L G Y A Y V N F Q Q P A D A E R A L D T M N F D V I K G K P I R I M W S Q R S9
	SLGYAYVNY : ADAERALDTLNYT IKG: P:RIMWS: R
-	95 D P 8 L R K 8 G T G N I F V K N L D K 8 I D N K A L F D T F 8 M F G N I L 8 C K 134
P. berghei T. annulata 1	95 D P 8 L R K 8 G T G N I F V K N L D K T I D N K A L F D T F 8 M F G N I L 8 C K 134 106 D P 8 L R 8 G A G N I F V K N L D K 8 I D T K 8 L Y D T F 8 H F G P I L 8 C K 145
C. parvum	91 D P A S R R N N D G N V F V K N L D K S I D N K T L F D T F S L F G N I M S C K 130
	159 D P 8 L R R M G T G N V F I K N L D P A I D N K A L H D T F 8 A F G K I L 8 C K 198
O. sativa 1 H. sapiens	118 D P 8 S R R S G A A N T F I K N L D K S I D N K A L Y D T F S V F G N I L S C K 157 90 D P 8 L R K S G V G N V F I K N L D K S I D N K A L Y D T F S A F G N I L S C K 129
	DPSLRKSG GNIFVKNLDKSIDNKAL:DTFS:FGNILSCK
-	135 VATDEFGRBRSYGFVHYEDEEBAKEAIEKVNGVQLGSKNV 174
	135 VATDEFCKSKNYGFVHYEDEESAKEAIEKVNCMOLGSKNV 174 146 VAVDASCASKRYGFVHYENEESAREAIEKVNCMLICCKRV 185
C. parvum 1	131 IAT DVECKSLCYGFIHFEHADSAKEAISELNCAVLCDEPI 170
	199 VAVDELGNARGYGFVHFD3VEBANAAIEHVNGMLLNDRRV 238
	158 V A T E M 8 C E 8 K C Y C F V Q F E L E E A A Q N A I 8 K L N C M L L N D K K V 197 130 V V C D E N C - 8 K C Y A F V H F E T Q E A A D K A I E K M N C M L L N D R K V 168
	VATDE C SKCYGFVHFE EESA:EAIEKVNCMLLCDK:V
P. faloiparum 1	175 YVCPFIKKSERATHDTKFTNLYVKNFPDSVTETHLR 210
P. berghei 1 T. annulata 1	175 Y V G H F I K K S E R A T N D T K F T N L Y V K N F P D T V T E A H L K 210 186 E V A P F L R K Q D R E G E E V F T N L Y V R N F P A D W N E E A L R 220
C. parvum 1	171 YVGKFQKKAERFSEKDKTFTNVYVKHIPKSWTEDLLY 207
S. pombe 2	239 YVGHHVSREERQSKVEALKANFTNVYIKNLDTEITEQEFS 278
	198 YV CPFV RKOEREN VSCN PKFNN VYVKN LSESTTEDN LK 235 169 FV CRFK SRKERENELCAKN KEFTN VYIKN FGEEVDDESLK 208
	YVG:F::K ER : A : FTNVYVKNFP:::TE: L:
•	

Figure 5A. For figure legend see page 258.

P. faloiparum 211       Q       L       F       N       P       G       E       I       T       S       M       I       V       K       M       D       -       N       K       N       R       F       C       F       I       N       A       D       D	M 248 V 258 V 245 V 318 V 275
p. faloiparum 249       D N L N G K K I T       - D D G Q I D E T Y D P K K E E A T A S T S G A A N Q T T G         p. berghei       249       E N L N G K K I T       - E D G K I D Y N Y D P K K E	- 271 - 272 - 270 - 330 - 287 - 260
P.       faloiparum 288       D & D & K & T D & K & N & K & A D & K & M & E & K & G D & S & N & A & M & N & A & T & A & T & G & A & T & T & D & T          P.       berghei       272       E & S & E & K & A & N & E & N & Y & N & N & N & T & T & G & A & T & T & T & D & T          P.       berghei       272       E & S & E & K & A & N & E & N & Y & N & N & N & T & T & G & A & T & T & T & D & T          P.       berghei       272       E & S & E & K & A & N & E & N & Y & N & N & N & T & T & S & E & I & I & T & T & T & T & T & T & T & T	E 296 - 272 0 292
P. faloiparum 328 T T P G E T T T T T A N A D S T G A N N N N O C L S P N T N T S N T T T G S S N P. berghei 207 T P A E K K T T D S E S A T N K D A T P - G E D Q T S A N G T T T T V T S T T. annulata 273	T 334 - 272
	8 364
p.       faloiparum 408       S D C A D       T P N I L Y V G P H Q       S R A R R H A I L K A K F D N L N V E N K N K         p.       berghei       365       G E S T E T P N I L Y V G P H Q       S R A R R H A I L K A K F D N L N V E N K N K         r.       annulata       273       L L V G P H Q       D K A K R Q N L L A Q F N N S S M G Q E D K         c.       parvum       326       V Q P N R L Y V S R A Q K K N E R Q V V L K S Q H E A V - K E S H Q R         s.       pombe       331       L Y V G R A Q K K N E R E V L K S Q H E A V - K E S H Q R         o.       sativa       288       L Y V G R A Q K K N E E R E M E L K E K F E K N I K E A D K         H.       sapiens       261       I F V G R A Q K K V E R Q A L K R K F E Q L K Q E I S R	H 404 R 303 Y 360 Y 361 N 318
P. faloiparum 448       Q G V N L Y I K N L D D G I D D - I M L R E L F E P F G T I T S A K V M R D E         P. berghei       405       P G V N L Y I K N L D D S M N D - Q T L K E L F E P Y G T I T S A K V M K D D         T. annulata       304       V T S N L Y I K N L D D S F D D - E S L G E L F K P F G T I T S A K V M K D D         C. parvum       361       Q G V N L Y V K N L A D S I N E - E D L R S M F E P F G T I T S A K V M L D A         S. pombe       362       Q G V N L F I K N L Q D S I D D - E R L K A E F S A F G T I T S A K I M T D E         O. sativa       319       Q G T N L Y L K N L D D S I D D D E K L K E I F A D F G T I T S A K V M R D L         H. sapiens       292       Q G V N L Y I K N L D D T I D D E K L R K E F S P F G S I T S A K V M L E D	K 443 N 342 S 399 Q 400 N 358

Figure 5A (continued). For figure legend see page 258.

P.faloiparum487EQ8XGFGFVCFABQEEANKAVTEMHLKIINGKPLYVGLAEP.berghei444DQ8KGFGTHEANKAVTEMHLKIINGKDLYGLAT.annulata343NH8RGFGFTNPQANHLXVGKDLYGLACCADQAXCADQATNANHLXVGKDLACADDATKADDATKADDATNDDATNANHBACSDDATKADDDATKANDDDATNDDDATNDDDATNDDD	483 382 439 440 398 309
P.       faloiparum 527       K R E Q R L & R L Q Q R F R M H P - I R H H M N N P L N T P M Q Y A & P Q & P Q         P.       berghei       484       K R E H R L & R L Q Q R F R M N P - I R H H M N N P L N T P M Q Y A & P Q & P Q         P.       berghei       484       K R E H R L & R L Q Q R F R M N P - I R H H I N N & L & S P I Q Y P N N Q T P Q         T.       annulata       383       K R D Q R M M R M Q Q R & S F E P H & R D V L F & E L P & Q & L Y P R P F P A         C.       parvum       440       R F E Q R A L R L Q Q R I R G G A V P P V L R P G A I P R G P - P -         S.       pombe       441       R F E V R R & Q L E A Q I R Q R N Q F R L Q Q Q V A A A A         O.       satira       309       R F E E R R A R L Q A Q F S Q M R P M V M P P S V A P R M M M M P Y G V         H.       sapiens       370       R F E E R R A H L T N Q Y M Q R V M C M R A E P A N A I L N Q F Q P A A         R K E       R       R L Q Q R F R M       : R       :       L       :	522 422 471 469 434 405
p.       faloiparum 566       LQF       SQNTLSYGR       PVITAFNQNNLISWRHQQAA       QQQAVHQQ         p.       berghei       523       LQF       NQNTLNYCR       PVITAFNQNNLISWRHQQAA       QQQAVHQQ         p.       berghei       523       LQF       NQNTLNYCR       PVITTFNQNNLISWRHQQAA       QQQAVHQQ         r.       annulata       423       PSF       NNSVAGPNKKF       PDNNFTTN       NQNNLISWRHQQAA       A         c.       parvum       472       - GVHGAPMQFGV       PVGTGTGT       PQNYFT       PQ       -       -       NND       -       -       ND       -	558 445 493 486 486 456 427
P.       faloiparum       606       A       H       Q       Q       A       A       Q       Q       L       N       F       N       T       N       L       Q       N       <	598 475 528 509 495
P. faloiparum 646 K P N A Q L H H N Q Q Y V P N A L A Q N G Q Q Q P N L N A A G Q H N A Q Q L Q Q         p. berghei       599 K A S Q Q L H H N Q Q Y S M G D N D Q H Q Q T N L N A D G Q A N D Q Q L Q Q         T. annulata       476 A P S A G N A R N M S P M S S A N I P S N I P H Y P S V V P P S M A H V V A         C. parvum       529 P Y T A G V P P Q M T G G P Q M T A Y N G N V I Q Q N G V S P N G A A N A T G         S. pombe       510 M P M Y P G M P T Q F P - A G G P A P G Y P G M N A R G P V P A Q         O. sativa       496 A G T G G I Q Q M P M M G H Q Q M L P - R G S R G G Y R Y A S G R G M D D N A F         H. sapiens       466 A P T G - N A P A S R G L P T T T Q R V G S E C P D R L A M D F G G A G A A Q	636 513 568 541 534
P. faloiparum 686       Q       Q       -       -       N	675 545 601 569 559 536
P. faloiparum 723       IN - N K Q Q N A A S Q A N Q M N H Q A Q P Q G A Q A Q Q K N P Q         P. berghei       676       M I G A K Q T N T Q Q N Q P Q G Q P Q G Q P Q G Q P Q A Q P Q V Q S Q Q K T G Q         T. annulata       546       M V P G A V G I R R D N N N P K S	715 562 625 569 559

Figure 5A (continued). For figure legend see page 258.

Α					
P. berghei 716 T. annulata 563		арана и и и и и и и и и и и и и и и и и и	 		
P. berghei 751 T. annulata 587 C. parvum 661 S. pombe 570 O. sativa 560	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	8 A P P 8 M Q K Q V L G E N L F V D D V A M Q K Q M I G E R L F A A 3 P 8 M Q K Q V L G E R L F A A 9 P E 8 R K Q V L G E L L Y N 8 P P D Q Q R L M L G E 8 L Y	D         L         V         N         Y         H         D         790           D         I         I         A         R         D         N         D         621           P         I         I         A         R         D         N         D         621           P         I         I         Q         F         Q         D         602           D         L         D         Q         L         E         505           P         L         I         Q         T         M         H         600		
P. berghei 791 T. annulata 622 C. parvum 699 S. pombe 603 O. sativa 596	$ \begin{array}{c} T \\ L \\ A \\ G \\ K \\ I \\ I$	$\begin{array}{c c} \mathbf{E} \ \mathbf{L} \ \mathbf{L} \ \mathbf{I} \ \mathbf{L} \ \mathbf{L} \ \mathbf{E} \ \mathbf{N} \ \mathbf{E} \ \mathbf{D} \ \mathbf{Q} \ \mathbf{L} \ \mathbf{K} \ \mathbf{K} \ \mathbf{K} \ \mathbf{I} \ \mathbf{I} \\ \mathbf{E} \ \mathbf{L} \ \mathbf{L} \ \mathbf{Q} \ \mathbf{L} \ \mathbf{L} \ \mathbf{E} \ \mathbf{D} \ \mathbf{N} \ \mathbf{D} \ \mathbf{Q} \ \mathbf{L} \ \mathbf{K} \ \mathbf{A} \ \mathbf{K} \ \mathbf{I} \\ \mathbf{E} \ \mathbf{L} \ \mathbf{L} \ \mathbf{E} \ \mathbf{L} \ \mathbf{L} \ \mathbf{E} \ \mathbf{L} \ \mathbf{L} \ \mathbf{E} \ \mathbf{D} \ \mathbf{N} \ \mathbf{D} \ \mathbf{Q} \ \mathbf{L} \ \mathbf{K} \ \mathbf{A} \ \mathbf{K} \ \mathbf{I} \\ \mathbf{E} \ \mathbf{L} \ \mathbf{L} \ \mathbf{E} \ \mathbf{L} \ \mathbf{L} \ \mathbf{E} \ \mathbf{L} \ \mathbf{L} \ \mathbf{E} \ \mathbf{D} \ \mathbf{D} \ \mathbf{S} \ \mathbf{A} \ \mathbf{L} \ \mathbf{K} \ \mathbf{K} \ \mathbf{K} \ \mathbf{K} \ \mathbf{N} \ \mathbf{V} \\ \mathbf{E} \ \mathbf{L} \ \mathbf{L} \ \mathbf{E} \ \mathbf{L} \ \mathbf{L} \ \mathbf{E} \ \mathbf{L} \ \mathbf{L} \ \mathbf{E} \ \mathbf{D} \ \mathbf{D} \ \mathbf{S} \ \mathbf{A} \ \mathbf{L} \ \mathbf{K} \ \mathbf{A} \ \mathbf{K} \ \mathbf{V} \\ \mathbf{E} \ \mathbf{L} \ \mathbf{L} \ \mathbf{E} \ \mathbf{L} \ \mathbf{L} \ \mathbf{E} \ \mathbf{L} \ \mathbf{L} \ \mathbf{E} \ \mathbf{S} \ \mathbf{P} \ \mathbf{D} \ \mathbf{S} \ \mathbf{A} \ \mathbf{L} \ \mathbf{K} \ \mathbf{A} \ \mathbf{K} \ \mathbf{V} \\ \mathbf{E} \ \mathbf{L} \ \mathbf{K} \ $	D E A L V V L Q 830 D E A I K V L K 661 D E A M V V L E 738 N E A I G V L Q 642 A E A M E V L 2 635		
T. annulata 662 C. parvum 739 S. pombe 643 O. sativa 636	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	ALTLSDGVVS 660			
В	0.285				
	0.291	S. pomb	oe O. sativa		
0.02	0.12	0.133	— P. falciparum — P. berghei		
0.016	0.3	0.283	T. annulata		
C. parvum					
H. sapiens					

Figure 5. (A) Comparison of amino acid sequences of *P. falciparum* PABP protein (PlasmoDB No. PFL1170w; GenBank accession No. EF116593) with other PABP from *Plasmodium berghei* (XP\_677383), *Theileria annulata* (CAI73150), *Cryptosporidium parvum* (CAD98589), *Schizosaccharomyces pombe* (AAA35320), *Oryza sativa* (XP\_481529) and *Homo sapiens* (CAI12298). The accession numbers of the aligned sequences are written in brackets. (B) Phylogenetic guide tree using Clustal W program based on the multiple alignment. A value of 0.1 corresponds to a difference of 10% between two sequences.

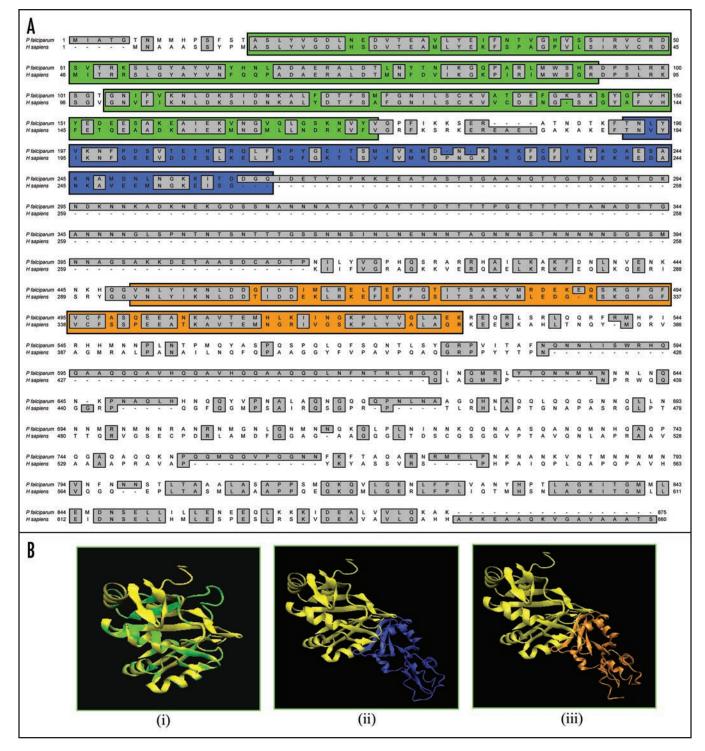


Figure 6. (A) Comparison of amino acid sequence of *P. falciparum* (PlasmoDB no. PFL1170w, GenBank accession number EF116593) and human PABP (GenBank accession number CAI 12298). The RNA recognition motifs (RRM) 1–2, 3 and 4 have been highlighted with green, blue and orange color respectively. (b) Three-dimensional models for human PABP and various fragments of PfPABP were created as described in text. The structures have been displayed using the same program as described in legend to Figure 1B. (B) (i)–(iii) Show the modeled structure of human (in yellow) and PfPABP (in green from amino acid 1–180), PfPABP (in blue from amino acid 1–264) and PfPABP (in orange from amino acid 1–531) respectively.

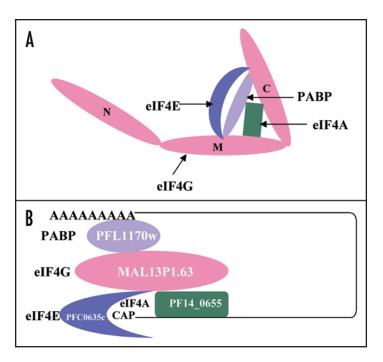


Figure 7. (A) Schematic diagram showing the interaction of various components (eIF4E, eIF4G and PABP) with the N-terminal, Middle and C-terminal domains of eIF4G. (B) Schematic diagram of translation initiation complex in *P. falciparum*. The name and PlasmoDB number of each component is written.