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Genome wide comparative comprehensive analysis of *Plasmodium falciparum* MCM family with human host

Abulaish Ansari and Renu Tuteja*

Malaria Group; International Centre for Genetic Engineering and Biotechnology; Aruna Asaf Ali Marg, New Delhi India

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Mini chromosome maintenance (MCM) proteins 2–7, a subgroup of the large AAA ATPase family are critically required for eukaryotic DNA replication. These proteins are most likely responsible for unwinding DNA at the replication forks. Besides this function, some MCMs are also involved in other chromosome transactions such as transcription, chromatin remodeling and genome stability. All the MCMs contain a conserved region of ~200 amino acids responsible for nucleotide binding. The importance of MCM proteins is evident by the fact that deregulation of the activity of MCM family of proteins appears to be directly linked to human carcinogenesis. This article will focus on members of this important family of proteins from the malaria parasite *Plasmodium falciparum* and their comparison with the human host.

Introduction

The minichromosome maintenance (MCM) genes were first identified in the yeast *Saccharomyces* cerevisiae by a genetic screen for minichromosome maintenance-defective mutants.[1] The mutants defective in each of these genes exhibit high rates of mitotic chromosome loss and recombination. MCM4 (CDC54) and MCM7 (CDC47) were isolated as cell cycle division mutants and MCM6,[2] as a chromosome segregation mutant (mis5) was originally isolated in *Schizosaccharomyces pombe*.[3] The MCM proteins are essential replication initiation factors consisting of six sequence-related subunits MCM2 to 7, which are evolutionally conserved in all eukaryotes and archaea. Because of their involvement in a fundamental cellular process, all six of these genes are essential for viability[4,5] and renamed MCM2 through MCM7,[6] the name MCM1 had already been categorized as a transcription factor and it does not belong to the MCM2-9 family.[7] It has been suggested that MCM family in human is constituted of eight members, falling into two different groups, one constituted by the MCM2-7 complex and the other by MCM8 and MCM9, which are present only in higher eukaryotes. Each MCM protein is essential for DNA replication because deletion of any one of the six subunits results in cell death in yeast. The six eukaryotic MCM proteins share significant sequence similarity with one another, concentrating on a nearly 250- amino-acid region that encodes the ATPase active site (AAA domain).[8] MCMs drive the formation of prereplicative complexes (PRCs) during the G1 phase of the cell cycle. It has been well established that the conversion from the cell cycle to G0 phase (quiescence) is due to the downregulation of the MCM2-7 protein complexes.

AAA or AAA+ is an abbreviation for ATPases associated with diverse cellular activities. They have a common conserved module of approximately 230–250 amino acid residues. They contain ring-shaped P-loop NTPases, which exert their activity through the energy-dependent remodeling or translocation of macromolecules.[9,10] MCM family is a large and functionally diverse protein family belonging to the AAA+ superfamily. AAA proteins form ATPase active sites at clefts between two subdomains: one containing a series of parallel β-strands (P loop) and a second called the lid.[11] Both subdomains contain conserved active-site motifs. The P loop contains motifs involved in binding ATP (Walker A box) and positioning the nucleophilic water molecule (Walker B box and sensor 1), while the lid domain contains motifs that contact the γ-phosphate of ATP (arginine finger and sensor 2).[6] Three indispensable motifs known as Walker A or motif I, Walker B or motif II and arginine finger are characteristic of all of the 8 MCM proteins.

During the G1 phase of the cell cycle, MCM2-7 is carried to origins of replication in an inactive state by Cdt1 to form the prereplication complex (pre-RC),[12] a well ordered assembly that contains the AAA+ proteins Cdc6 and the hetero-hexameric origin recognition complex (Orc1-6).[12,13] All the six MCM subunits 2 to 7 co-localize to origins of replication during pre-RC formation.[13-15] The inactivation or loss of any of the six MCM subunits during G1 phase inhibits pre-RC formation in vivo in yeast.[16] The loading of MCM2-7 onto DNA requires ATP hydrolysis by both Orc1-6 and Cdc6.[17,18] Once the loading has been achieved, Orc1-6 and Cdc6 are no more required for MCM2–7 retention at the origin[17,19] and they are unnecessary for subsequent DNA replication.[20,21]

*Correspondence to: Renu Tuteja; Email: renu@icgeb.res.in
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Table 1. MCM family of *Plasmodium falciparum*

S.No	Protein name	PlasmoDB No.	Location (chrom.No/Position)	Size in Kda (Pf/Hs)	Exon/intron	Identity with HsMCM (%)
1	MCM2	PF14_0177	14/ 753,388 to 756,439	111/91	2/1	42
2	MCM3	PFE1345c	5/1,125,094 to 1,128,141	110/91	2/1	41
3.	MCM4	PF13_0095	13/ 712,146 to 715,163	115/80	1/0	43
4	MCM5	PFL0580w	12/521,681 to 524,464	86/82	4/3	41
5	MCM6	PF13_0291	13/2,183,768 to 2,186,557	106/92	1/0	39
6	MCM7	PF07_0023	7/320,354 to 322,819	94/81	1/0	43
7	MCM8	PFL0560c	12/ 498,518 to 502,223	133/81	3/2	36
8	MCM9	PFD0790c	4/ 718,075 to 722,472	171/127	1/0	49

MCM proteins are related to cell proliferation therefore they serve as useful biomarkers for cancer screening, surveillance and prognosis. The deregulation of MCM expression disrupts genetic stability and the overexpression of MCM subunits has been reported in various premalignant dysplastic lesions and cancers.[22] It has been reported that MCM2, MCM3 and MCM7 expression are frequently deregulated in medulloblastoma (MB).[23] MCM family of proteins was reported to be upregulated in meningiomas and it was suggested that these can be used as diagnostic markers. Significant increase in expression in meningiomas was reported for MCM2 (8-fold), MCM3 (5-fold), MCM4 (4-fold), MCM5 (4-fold), MCM6 (3-fold), and MCM7 (5-fold).[24] Although the roles of many proteins involved in the initiation of replication are understood, the role of MCM10 remains controversial.[25,26] A key process of replication initiation is to convert inactive MCM2-7 to active Cdc45-MCM-GINS (CMG) replicative helicase. MCM10 is supposed to have an essential role in functioning of the CMG replicative helicase independent of assembly of a stable CMG complex at origins.[26]

MCM proteins have been isolated from a number of plants but their straightforward role is yet to be established.[27] Their abundant presence in plants is still ambiguous. It has been suggested that other than their established role in DNA unwinding, they might have the additional roles most likely in stress tolerance.[27] It has been reported recently that pea MCM6 transcript is upregulated in pea plant in response to high salinity and cold stress but not with ABA, drought and heat stress. The overexpression of pea MCM6 single subunit in tobacco plant promotes salinity stress tolerance without affecting its yield.[28]

Basic understanding of the molecular basis of cell growth and differentiation in the malaria parasite is essential for the development of novel chemotherapeutic agents. The malaria parasite has a complex life cycle in mosquito and human hosts.[29] There are five points in the life cycle of the parasite where cell division/replication occurs.[30,31] The MCM complex is recruited to the pre-replication complex (pre-RC) before DNA synthesis. Although the parasite has a tight control mechanism for cell proliferation and differentiation during various points of its life cycle, however very little is known about the molecular machinery that regulates this process. Different studies have reported the involvement of only ORC1 and MCM4 in pre-replication formation during replication and both have been reported to be expressed only in gametocytes.[32,33] However presence of all the six subunits of the MCM complex in the *P. falciparum* genome has been reported.[33] In the present manuscript we present a comprehensive in silico analysis of MCM family of proteins from *P. falciparum* and their comparison with human host.

Results and Discussion

The genome of *P. falciparum*, available at www.plasmodb.org was investigated using 'MCM' as query. The results of this search presented in **Table 1** showed a total of 8 hits. The PlasmoDB number for MCM29 and other details are listed in **Table 1**. All the 8 MCM members from this list were used to BLAST with *H. sapiens* and the comparative analysis are presented under different categories in the following sections. MCM proteins show considerable sequence conservation particularly in a 200 amino acid residue domain, which is located almost in the center of these large proteins. Walker A motif contains the consensus sequence [(G)xxxxGK[S/T], where x is any residue and lysine residue is present which is characteristic of all the ATP-binding proteins. It has been observed that the Walker A motif sequence in all the MCMs is slightly deviated from the consensus and the glycines in the motif GK(S/T) are substituted by serine or alanine.[8] The Walker B motif [hhhh(D/E)] has conserved nucleotide phosphate-binding motif (where h is a hydrophobic residue). These are the characteristic feature of members of the P-loop NTPase domain superfamily. The Walker A motif binds the β-γ phosphate moiety of the bound nucleotide (typically ATP or GTP) and Walker B motif binds with the Mg^{2+} cation.[34] All the MCMs contain a Zn finger and another short motif SRFD, which is present approximately 70 residues after the Walker B motif and defines an arginine finger motif.

MCM2. It has been well established that HsMCM2 contributes to a variety of nuclear functions in addition to DNA replication. The detailed biochemical characterization of HsMCM2 showed that the C-terminal region of HsMCM2 contains ssDNA- binding activity that inhibits the DNA helicase activity.[35] On the other hand using pulldown analysis it was reported that two fragments from the central region were mainly responsible for the interaction between HsMCM2 and HsMCM4.[35]

The gene with PlasmoDB number PF14_0177 is a homolog of human MCM2 and is located on chromosome 14. PfMCM2 protein is slightly larger in size as compared with its human

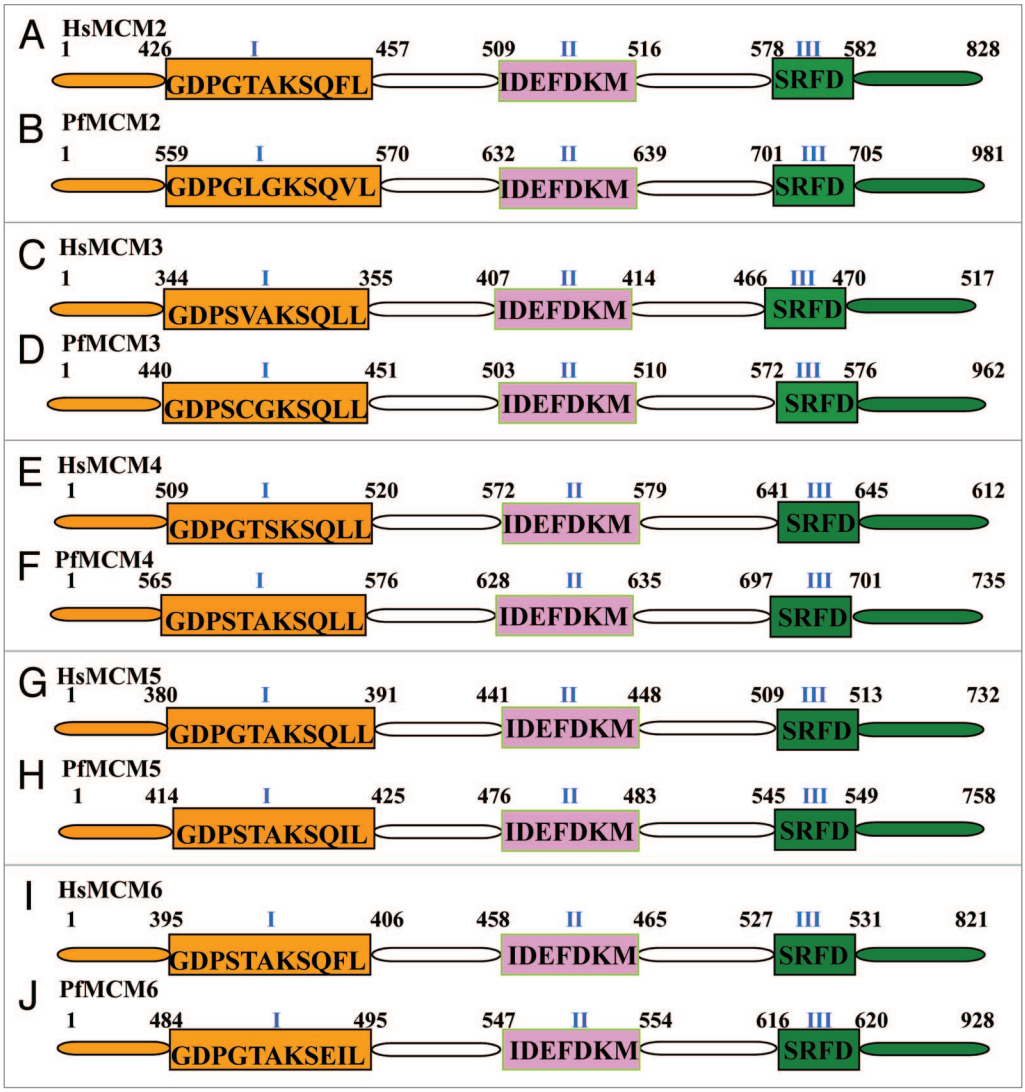


Figure 1. Comparison of MCM2-6 of *Plasmodium falciparum* with their human homologs. All the sequence data used in the analysis were downloaded from PlasmoDB (www.plasmodb.org). The downloaded sequences were used as query to match with the human homolog using BLAST search (www.ncbi.nlm.nih.gov). The corresponding human sequence was retrieved and the domain analysis was done. The results were further checked manually to confirm that all the domains are present and then used in the figures. Similarly the *P. falciparum* sequence was also analyzed and the results are presented in figures. The conserved sequences of each domain are written inside the boxes. The text in blue refers to the names of various conserved domains and the numbers refer to the amino acids positions of various domains. The number I, II and III refer to Walker A or motif I, Walker B or motif II and arginine finger motif respectively. This figure is not drawn to scale.

homolog (**Table 1**). It contains additional 163 amino acids in comparison to the human counterpart. The difference in size mainly lies in the N-terminal extension (**Fig. 1A** and **B**). The sequence of all the domains and the comparison with the human counterpart shows that Walker A motif of PfMCM2 follows the consensus sequence but in human counterpart A is present instead of G (**Fig. 1A** and **B**). In motif II i.e., Walker B motif PfMCM2 and HsMCM2 obey the consensus sequence, although there is little sequence dissimilarity (**Fig. 1A** and **B**). Similar to other eukaryotic MCMs, PfMCM2 also contains a putative C4-type zinc finger domain at its N-terminal region. The zinc finger domain may have a role in the binding of PfMCMs to chromatin because these domains are known to be responsible for protein-DNA and protein-protein interactions and therefore contribute to complex assembly. The arginine finger motif of MCM2 of *P. falciparum* and human are highly conserved (**Fig. 1A** and **B**). The expression of PfMCM2 predominantly in late trophozoites and during schizont maturation,[32] concur with DNA replication in Plasmodium.[33]

MCM3. The phosphorylation of HsMCM3 has been well studied. It was reported that cyclin B–CDK1 catalyzes phosphorylation of HsMCM3 at Ser-112, thereby regulating HsMCM3 association with other HsMCM2–7 subunits and loading of HsMCM3 onto chromatin.[36] In a recent study it has been reported that MCM3 is a substrate of cyclin E/Cdk2 and can be phosphorylated by cyclin E/Cdk2 at Thr-722.[37] The HsMCM3 T722A mutant binds chromatin much less efficiently as compared with wild type HsMCM3 suggesting that

this phosphorylation site is involved in HsMCM3 loading onto chromatin. The knockdown of HsMCM3 does not affect the S phase entry and progression, indicating that a small fraction of HsMCM3 is sufficient for normal S phase completion.[37] These studies strongly suggest that HsMCM3 phosphorylation is a critical posttranslational modification that regulates assembly and activity of the HsMCM2-7 complex.

The gene with PlasmoDB number PFE1345c is a homolog of human MCM3 and is located on chromosome 5 and is slightly larger in size as compared with its human homolog (**Table 1**). It has additional 154 amino acids; ~100 at N-terminal and 50 amino acids at its C-terminal region respectively (**Fig. 1C** and **D**). The sequence comparison of all the domains with human counterpart indicates that except Walker A motif (motif I) all the other domains are identical (**Fig. 1C** and **D**).

MCM4. The importance of phosphorylation for the activity of HsMCM4 has been well studied. The phosphorylation at sites 3 and 32 of HsMCM4 required CDK2 in HeLa cells and this phosphorylated HsMCM4 had several distinct and site-specific roles in MCM function.[38] It was reported that the central region of HsMCM2, which contains zinc finger and ATPase motif interacts with HsMCM4.[35] In a recent study the first human mutation in *MCM4* gene has been reported and it has been shown to be associated with adrenal insufficiency, short stature, and natural killer (NK) cell deficiency.[39] In a related study it was reported that the partial HsMCM4 deficiency results in a genetic syndrome of growth retardation with adrenal insufficiency and selective NK deficiency.[40]

The gene with PlasmoDB number PF13_0095 is a homolog of human MCM4 and is located on chromosome 13 and is larger in size as compared with its human homolog (**Table 1**). It contains 197 additional amino acids in comparison to human homolog. The N-terminal and C-terminal of PfMCM4 has 56 and 38 amino acid more than the human counterpart (**Fig. 1E** and **F**). The conserved motif of PfMCM4 showed difference in sequences in Walker A motif (motif I) while Walker B (motif II) and arginine finger motifs (motif III) are identical to HsMCM4 (**Fig. 1E** and **F**). PfMCM4 contains the zinc finger domain at the N-terminal region. This domain may have a role in the protein-DNA interactions. In the case of Walker A motif, the consensus sequence i.e., GXXXXGK(S/T) is not followed by both PfMCM4 and HsMCM4 (**Fig. 1E** and **F**). In a previous study it has been reported that PfMCM4 contains some unique features such as it is the largest of all the MCM4 and contains insertions at few places within its entire sequence including the zinc finger domain.[31] It was also reported that PfMCM4 is expressed specifically in the sexual erythrocytic stages indicating that PfMCM4 may be involved in gametogenesis where DNA is replicated.[31]

MCM5. Cyclin E has been shown to directly interact with and colocalize on centrosomes with HsMCM5 in a centrosomal localization sequence (CLS)-dependent but Cdk2-independent manner.[41] The HsMCM5 domain responsible for interaction with cyclin E is reported to be highly conserved in MCM5 proteins from yeast to mammals. The expression of HsMCM5 or its cyclin E-interacting domain significantly inhibits over-duplication of centrosomes indicating that proteins involved in DNA replication might also regulate centrosome duplication.[41] In a related study it was reported that cyclins E and A sequentially prevent centrosome reduplication throughout interphase by recruitment of HsMCM5 and Orc1.[42] HsMCM-5 expression has been reported to be associated with clinicopathological parameters in gastric adenocarcinoma.[43]

The gene with PlasmoDB number PFL0580w is a homolog of HsMCM5. PfMCM5 is almost similar in size and is located on chromosome number 12 (**Table 1**). The comparative analysis of their motifs showed that only Walker A motifs (motif I) are identical and other two motifs have little dissimilarity in their sequences (**Fig. 1G** and **H**).

MCM6. MCM proteins are essential for DNA replication and MCM6 is one subunit of HsMCM2-7 complex that serves as the replicative helicase in DNA replication. It is well established that Cdt1 physically interacts with the MCM complex and this interaction mainly occurs between Cdt1 and MCM6 in human cells. The detailed analysis indicated that the C-terminal 79 residues of hCdt1 interact with the C-terminal 113 residues of HsMCM6 while the large N-terminal Orc6-binding domain recruits Cdt1/MCM2-7 to ORC complex.[44] HsMCM6 has been suggested to be a suitable marker for evaluation of the proliferation potential of craniopharyngioma. The study of expression of HsMCM6 and its correlation with recurrence of the tumor in craniopharyngiomas suggested no difference in HsMCM6 expression between the primary and recurring tumors.[45]

The gene with PlasmoDB number PF13_0291 is a homolog of HsMCM6. PfMCM6 is larger by 108 amino acids (**Table 1**). The difference in size lies in the N-terminal region of PfMCM6 (**Fig. 1I** and **J**). The comparative analysis of the conserved motifs showed slight differences in their amino acid sequences in all three motifs (**Fig. 1I** and **J**). The zinc finger domain in PfMCM6 is also present at its N-terminal region and may play a role in the binding of PfMCM6 to chromatin. Walker A motifs of both MCM6 do not obey the consensus sequences (**Fig. 1I** and **J**). In a previous study the expression of PfMCM6 was observed predominantly in late trophozoites and during schizont maturation.[33] During the trophozoite stage, the antibody against PfMCM6 pulls down PfMCM2 very inefficiently.[33] It was also reported that only PfMCM6 is associated with the chromatin fraction at all stages of growth and PfMCM6 is also present in both the nucleosolic and cytoplasmic fractions.[33] It might be possible that the PfMCM6 subunit makes direct contact with chromatin while other subunits are associated through protein–protein interactions.

MCM7. Recent studies suggest that in a variety of human malignancies MCM7 is amplified and overexpressed. It has been reported that dysregulation of MCM7 expression is observed in various types of cancer and it correlates with a negative outcome in patients with non-small cell lung cancer after surgical resection. Therefore MCM7 can be explored as a potential therapeutic and prognostic target in lung and other cancers.[46]

The gene with PlasmoDB number PF07_0023 is a homolog of HsMCM7 and it is located on chromosome 7 (**Table 1**). PfMCM7 contains additional 102 amino acids as compared

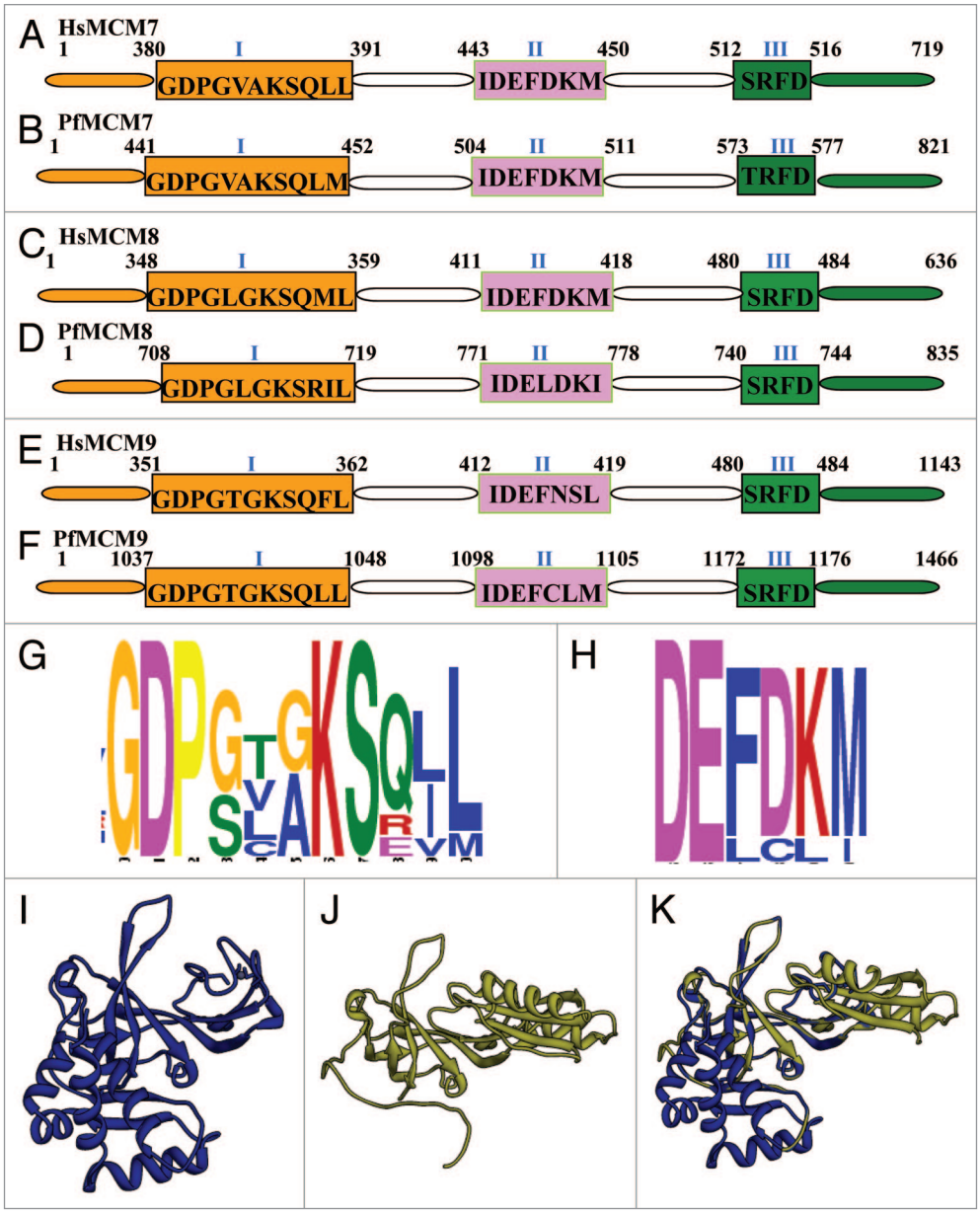


Figure 2. (A-F) Comparison of MCM7-9 of *Plasmodium falciparum* with their human homologs. All the sequence data used in the analysis were downloaded from PlasmoDB (www.plasmodb.org). The downloaded sequences were used as query to match with the human homolog using BLAST search (www.ncbi.nlm.nih.gov). The corresponding human sequence was retrieved and the domain analysis was done. Other details are as in Figure 1. This figure is not drawn to scale. **(G and H)** Distribution of amino acids at various positions in **(G)**. 'Walker motif A; **(H)** 'Walker motif B' of MCM2-9 of *P. falciparum*. Single letter code for amino acids has been used. This analysis was done using MEME program. **(I-K)** The modeled structure of **(I)**. template **(J)**. PfMCM9 and **(K)**. superimposition are shown. The PfMCM9 sequence was submitted to Swissmodel server and the structure was obtained. The molecular graphic images were produced using the UCSF Chimera package from the resource for Biocomputing, Visualization, and Informatics (www.cgl.ucsf.edu/chimera) at the University of California, San Francisco (supported by NIH P41 RR-01081). The details are described in text.

with its human counterpart. These amino acids are distributed on both the N-terminal and C-terminal sides of the protein (**Fig. 2A** and **B**). Walker A motifs (motif I) in both Pf and HsMCM7 do not agree with the consensus sequences (**Fig. 2A** and **B**). The comparison of HsMCM7 with PfMCM7 indicates that Walker B motifs (motif II) are identical but the arginine finger motif (motif III) displays variation in one amino acid (**Fig. 2A** and **B**). Similar to other MCMs, PfMCM7 contains putative C4-type zinc finger domain at its N-terminal region. This domain probably has a role in the binding of PfMCM7 to DNA. It has been shown that during the trophozoite stage, the antibody against PfMCM6 does not pull-down PfMCM7.[33] The expression of PfMCM7 polypeptides was predominantly observed in late trophozoites and during schizont maturation[33,47] and decreased in the ring stages, which is in agreement with DNA replication in Plasmodium. It was also reported that

PfMCM7 is found in the nucleosolic and cytoplasmic fractions throughout the intraerythrocytic life cycle and increased in both fractions from ring to schizont stages.[33]

MCM8. MCM8 is a new evolutionarily conserved family member but its homolog is not present in yeast.[48] It may function alone or with other family members in DNA metabolism. HsMCM8 mRNA accumulates during the G1/S to G2/M phase, while its protein product is detectable throughout the normal cell cycle.[49] MCM8 is a crucial component of the pre-RC and the interaction between HsMCM8 and hCDC6 is required for pre-RC assembly.[49] In this study MCM8 appears to participate in pre-RC formation in human cells, although this is not the case in other systems. It has been reported that MCM8, like MCM7, colocalizes on a specific DNA segment of the c-Myc replication initiation zone (c-Myc replicator) with Cdc6.[50] The association of both MCM proteins with Cdc6 continues even after DNA replication is complete. It was reported that MCM8 may play a role in elongation and was strongly suggested that MCM8 functions with other known replication proteins in processes which accompany initiation of DNA replication.[50]

The gene with PlasmoDB number PFL0560c is a homolog of HsMCM8 and PfMCM8 is located on chromosome 12 (**Table 1**). There is a large difference in their size; PfMCM8 is larger by 400 amino acids in comparison to HsMCM8. The difference in size is mainly attributable to a longer N-terminal region in PfMCM8 (**Fig. 2C** and **D**). The comparative analysis of the conserved motifs showed that only arginine finger motif (motif III) is highly conserved and identical in HsMCM8 and PfMCM8 but there are some differences in Walker A (motif I) and Walker B motifs (motif II) of HsMCM8 and PfMCM8 (**Fig. 2C** and **D**).

MCM9. HsMCM9 is a novel member of MCM family.[51,52] Similar to HsMCM8, HsMCM9 is only present in the genome of higher eukaryotes. It showed 24–31% total amino acid identity with HsMCM2–MCM8 proteins and contains a unique C-terminal domain which has only weak homology to MCM2-7 and MCM8 but is conserved within MCM9 homologs.[53] The phylogenetic analysis on MCM family members indicated that MCM9 was most closely related to MCM8.[52] Further studies reported that HsMCM9 is expressed and required for loading the HsMCM2-7 helicase onto chromatin during pre-RC formation.[53]

PFD0790c is the PlasmoDB number of the homolog of HsMCM9 (**Table 1**). PfMCM9 has additional 322 amino acids as compared with HsMCM9. PfMCM9 has extra 685 amino acid at its N-terminal while its C-terminal is shorter by 321 amino acid as compared with HsMCM9 (**Fig. 2E** and **F**). The motif comparison showed that only the arginine finger motif (motif III) is identical in HsMCM9 and PfMCM9 but there are some variations in Walker A (motif I) and Walker B (motif II) motifs of HsMCM9 and PfMCM9 (**Fig. 2E** and **F**).

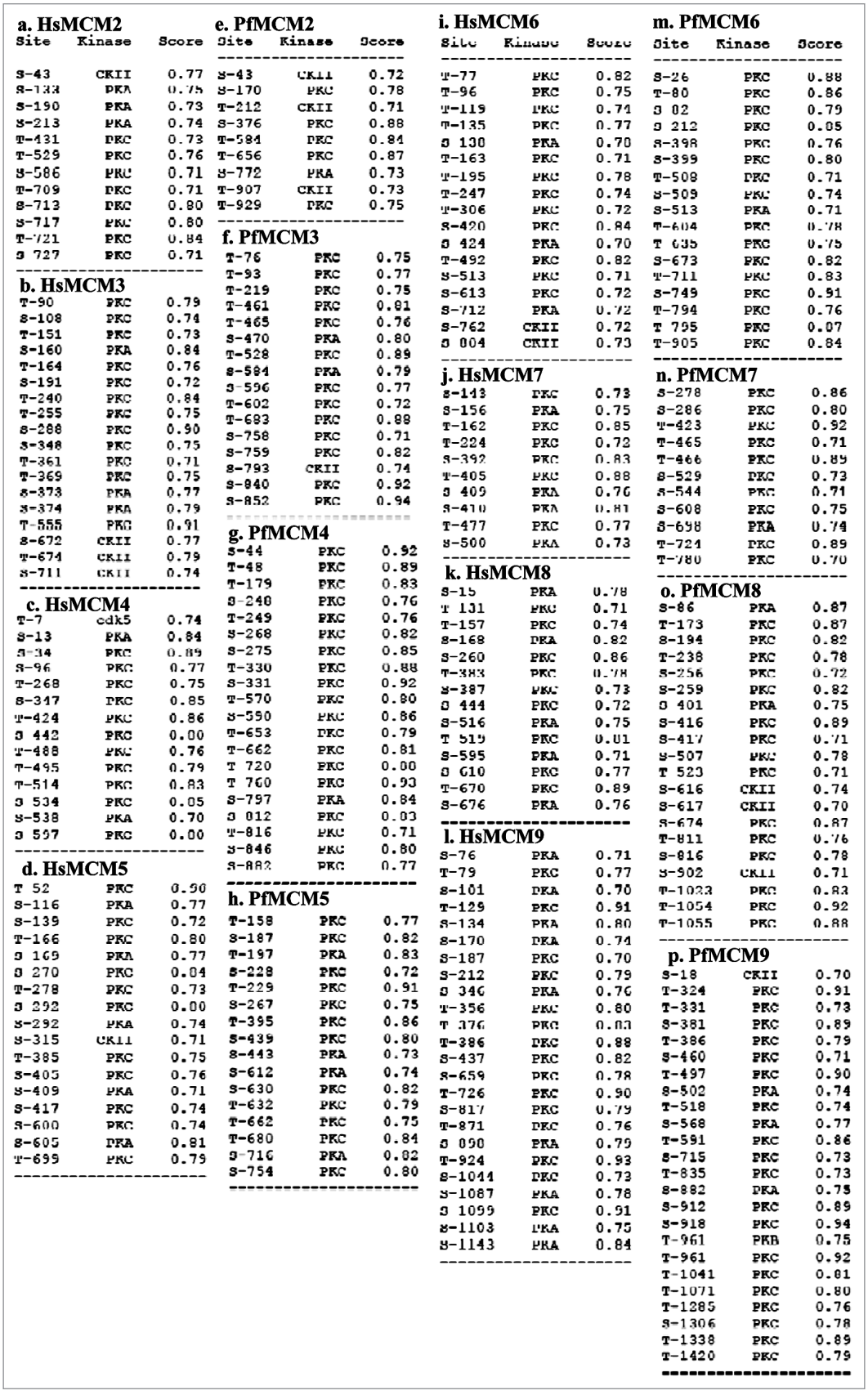
To further analyze the conserved motifs in *P. falciparum* protein family, MEME Suite-GLAM2 version 4.8.0 was used. This analysis indicated that in *P. falciparum* MCM family in Walker A motif (motif I), 5 out of 11 amino acids are highly conserved and others are variable to some extent (**Fig. 2G**). On the other hand in Walker B motif (motif II), two out of six amino acids are highly conserved and others are slightly variable (**Fig. 2G**).

For structural modeling the sequence of PfMCM9 was submitted to the Swissmodel homology-modeling server (swissmodel.expasy.org/). PfMCM9 primary sequence residues 405 to 650 showed only:16.4% sequence identity to a MCM protein homolog from archaeal *Methanobacterium thermoautotrophicum*.[54] The structural modeling of the PfMCM9 was therefore done using the known crystal structure of this homolog as the template (PDB number 1LTL at www.rcsb.org). The ribbon diagram of the template is shown in **Figure 2I** and the predicted structure of PfMCM9 is shown in **Figure 2J** respectively. When the modeled structure of PfMCM9 was superimposed, it is clear that these structures superimpose partially (**Fig. 2K**). Molecular graphic images were produced using the UCSF Chimera package (www.cgl.ucsf.edu/chimera) from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR-01081).[55]

It is well established that post translational modification especially phosphorylation plays a very important role in regulating the function of various MCMs. The phosphorylation of MCM2, MCM3, MCM4, MCM6 and MCM7 has been observed in vivo and in vitro in different eukaryotic cells[56,57] however the same is not reported in case of PfMCMs.[33] Therefore the phosphorylation potential of all the HsMCMs and PfMCMs was analyzed using NetphosK at www.cbs.dtu.dk/services/NetPhosK.[58] The results of this analysis suggested that all of these proteins are prone to phosphorylation and contain the recognition sites mostly for PKC, followed by PKA, CKII and only few contain the sites for phosphorylation by PKB or cdk5 (**Fig. 3**). This analysis suggested that although there is a large variation in the size of PfMCMs and HsMCMs, the phosphorylation sites are almost equal in Hs and PfMCM3, MCM5, MCM6, MCM7 and MCM9 (**Fig. 3b** and **f, d** and **h, i** and **m, j** and **n, l** and **p** respectively). Furthermore it is interesting to note that PfMCM2 contains only 9 sites as opposed to HsMCM2, which contains 12 sites (**Fig. 3a** and **e**) and PfMCM4 and PfMCM8 each contain 20 phosphorylation sites as compared with HsMCM4 and HsMCM8, which contain only 14 sites each (**Fig. 3c** and **g, k** and **o** respectively). Overall in this manuscript, we have presented a comparative systematic analysis of MCM family from *P. falciparum* and human. These studies pave the way for further studies on this important family of proteins.

Materials and Methods

All the sequence data used in the analysis were downloaded from Plasmodium genome database available at www.plasmodb.org. The downloaded sequences were used as query and then matched with the human homolog using BLAST search (www.ncbi.nlm.nih.gov). The corresponding human sequences were retrieved and then their conserved domains were searched by using online CD search tool available on www.ncbi.nlm.nih.gov/Structure/



a. HsMCM2

Site	Kinase	Score
S-43	CKII	0.77
S-133	PKA	0.75
S-190	PKA	0.73
S-213	PKA	0.74
T-431	PKC	0.73
T-529	PKC	0.76
S-586	PKC	0.71
T-709	PKC	0.71
S-713	PKC	0.80
S-717	PKC	0.80
T-721	PKC	0.84
S 727	PKC	0.71

b. HsMCM3

T-90	PKC	0.79
S-108	PKC	0.74
T-151	PKC	0.73
S-160	PKA	0.84
T-164	PKC	0.76
S-191	PKC	0.72
T-240	PKC	0.84
T-255	PKC	0.75
S-288	PKC	0.90
S-348	PKC	0.75
T-361	PKC	0.71
T-369	PKC	0.75
S-373	PKA	0.77
S-374	PKA	0.79
T-555	PKC	0.91
S-672	CKII	0.77
T-674	CKII	0.79
S-711	CKII	0.74

c. HsMCM4

T-7	cdk5	0.74
S-13	PKA	0.84
S-34	PKC	0.89
S-96	PKC	0.77
T-268	PKC	0.75
S-347	PKC	0.85
T-424	PKC	0.86
S 442	PKC	0.00
T-488	PKC	0.76
T-495	PKC	0.79
T-514	PKC	0.83
S 534	PKC	0.05
S-538	PKA	0.70
S 597	PKC	0.00

d. HsMCM5

T 52	PKC	0.90
S-116	PKA	0.77
S-139	PKC	0.72
T-166	PKC	0.80
S 169	PKA	0.77
S 270	PKC	0.04
T-278	PKC	0.73
S 292	PKC	0.00
S-292	PKA	0.74
S-315	CKII	0.71
T-385	PKC	0.75
S-405	PKC	0.76
S-409	PKA	0.71
S-417	PKC	0.74
S-600	PKC	0.74
S-605	PKA	0.81
T-699	PKC	0.79

e. PfMCM2

Site	Kinase	Score
S-43	CKII	0.72
S-170	PKC	0.78
T-212	CKII	0.71
S-376	PKC	0.88
T-584	PKC	0.84
T-656	PKC	0.87
S-772	PKA	0.73
T-907	CKII	0.73
T-929	PKC	0.75

f. PfMCM3

T-76	PKC	0.75
T-93	PKC	0.77
T-219	PKC	0.75
T-461	PKC	0.81
T-465	PKC	0.76
S-470	PKA	0.80
T-528	PKC	0.89
S-584	PKA	0.79
S-596	PKC	0.77
T-602	PKC	0.72
T-683	PKC	0.88
S-758	PKC	0.71
S-759	PKC	0.82
S-793	CKII	0.74
S-840	PKC	0.92
S-852	PKC	0.94

g. PfMCM4

S-44	PKC	0.92
T-48	PKC	0.89
T-179	PKC	0.83
S-240	PKC	0.76
T-249	PKC	0.76
S-268	PKC	0.82
S-275	PKC	0.85
T-330	PKC	0.88
S-331	PKC	0.92
T-570	PKC	0.80
S-590	PKC	0.86
T-653	PKC	0.79
T-662	PKC	0.81
T 720	PKC	0.00
T 760	PKC	0.93
S-797	PKA	0.84
S 812	PKC	0.03
T-816	PKC	0.71
S-846	PKC	0.80
S-882	PKC	0.77

h. PfMCM5

T-158	PKC	0.77
S-187	PKC	0.82
T-197	PKA	0.83
S-228	PKC	0.72
T-229	PKC	0.91
S-267	PKC	0.75
T-395	PKC	0.86
S-439	PKC	0.80
S-443	PKA	0.73
S-612	PKA	0.74
S-630	PKC	0.82
T-632	PKC	0.79
T-662	PKC	0.75
T-680	PKC	0.84
S-710	PKA	0.82
S-754	PKC	0.80

i. HsMCM6

Site	Kinase	Score
T-77	PKC	0.82
T-96	PKC	0.75
T-119	PKC	0.74
T-135	PKC	0.77
S 130	PKA	0.70
T-163	PKC	0.71
T-195	PKC	0.78
T-247	PKC	0.74
T-306	PKC	0.72
S-420	PKC	0.84
S 424	PKA	0.70
T-492	PKC	0.82
S-513	PKC	0.71
S-613	PKC	0.72
S-712	PKA	0.72
S-762	CKII	0.72
S 804	CKII	0.73

j. HsMCM7

S-143	PKC	0.73
S-156	PKA	0.75
T-162	PKC	0.85
T-224	PKC	0.72
S-392	PKC	0.83
T-405	PKC	0.88
S 409	PKA	0.76
S-410	PKA	0.81
T-477	PKC	0.77
S-500	PKA	0.73

k. HsMCM8

S-15	PKA	0.78
T 131	PKC	0.71
T-157	PKC	0.74
S-168	PKA	0.82
S-260	PKC	0.86
T-383	PKC	0.78
S-387	PKC	0.73
S 444	PKC	0.72
S-516	PKA	0.75
T 519	PKC	0.81
S-595	PKA	0.71
S 610	PKC	0.77
T-670	PKC	0.89
S-676	PKA	0.76

l. HsMCM9

S-76	PKA	0.71
T-79	PKC	0.77
S-101	PKA	0.70
T-129	PKC	0.91
S-134	PKA	0.80
S-170	PKA	0.74
S-187	PKC	0.70
S-212	PKC	0.79
S 340	PKA	0.76
T-356	PKC	0.80
T 376	PKC	0.80
T-386	PKC	0.88
S-437	PKC	0.82
S-659	PKC	0.78
T-726	PKC	0.90
S-817	PKC	0.79
T-871	PKC	0.76
S 890	PKA	0.79
T-924	PKC	0.93
S-1044	PKC	0.73
S-1087	PKA	0.78
S 1099	PKC	0.91
S-1103	PKA	0.75
S-1143	PKA	0.84

m. PfMCM6

Site	Kinase	Score
S-26	PKC	0.88
T-80	PKC	0.86
S 82	PKC	0.79
S 212	PKC	0.05
S-398	PKC	0.76
S-399	PKC	0.80
T-508	PKC	0.71
S-509	PKC	0.74
S-513	PKA	0.71
T-604	PKC	0.78
T 635	PKC	0.75
S-673	PKC	0.82
T-711	PKC	0.83
S-749	PKC	0.91
T-794	PKC	0.76
T 795	PKC	0.07
T-905	PKC	0.84

n. PfMCM7

S-278	PKC	0.86
S-286	PKC	0.80
T-423	PKC	0.92
T-465	PKC	0.71
T-466	PKC	0.89
S-529	PKC	0.73
S-544	PKC	0.71
S-608	PKC	0.75
S-698	PKA	0.74
T-724	PKC	0.89
T-780	PKC	0.70

o. PfMCM8

S-86	PKA	0.87
T-173	PKC	0.87
S-194	PKC	0.82
T-238	PKC	0.78
S-256	PKC	0.72
S-259	PKC	0.82
S 401	PKA	0.75
S-416	PKC	0.89
S-417	PKC	0.71
S-507	PKC	0.78
T 523	PKC	0.71
S-616	CKII	0.74
S-617	CKII	0.70
S-674	PKC	0.87
T-811	PKC	0.76
S-816	PKC	0.78
S-902	CKII	0.71
T-1023	PKC	0.83
T-1054	PKC	0.92
T-1055	PKC	0.88

p. PfMCM9

S-18	CKII	0.70
T-324	PKC	0.91
T-331	PKC	0.73
S-381	PKC	0.89
T-386	PKC	0.79
S-460	PKC	0.71
T-497	PKC	0.90
S-502	PKA	0.74
T-518	PKC	0.74
S-568	PKA	0.77
T-591	PKC	0.86
S-715	PKC	0.73
T-835	PKC	0.73
S-882	PKA	0.75
S-912	PKC	0.89
S-918	PKC	0.94
T-961	PKB	0.75
T-961	PKC	0.92
T-1041	PKC	0.81
T-1071	PKC	0.80
T-1285	PKC	0.76
S-1306	PKC	0.78
T-1338	PKC	0.89
T-1420	PKC	0.79

Figure 3. Phosphorylation sites in Hs and PfMCMs. The sequence of each MCM was submitted to www.cbs.dtu.dk/services/NetPhosK for predictions of kinase specific eukaryotic protein phosphorylation sites. A threshold of 0.7 was used and the sites predicted for each MCM are presented in the figure. CKII- casein kinase II, PKA-protein kinase A, PKB-protein kinase B, PKC-protein kinase C.

cdd/wrpsb.cgi. All the motifs in conserved domains were manually assigned. Similarly the motifs were manually assigned in *P. falciparum* sequence and the data are presented in figures. To identify conserved motifs within MCM family of *P. falciparum*, MEME Suite-GLAM2 version 4.8.0 was employed (meme.nbcr.net/meme/intro.html) using default settings.[59]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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