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Identification of an MCM4 homologue expressed specifically in the sexual stage of *Plasmodium falciparum*☆

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Abstract

Mini-chromosome maintenance (MCM) proteins play an essential role in DNA replication initiation. We have isolated a novel gene encoding an MCM-like protein from the human malaria parasite *Plasmodium falciparum* using the vectorette technique. The gene has no introns and comprises an open reading frame encoding 1005 amino acid residues with a predicted Mr of 115 kDa. The encoded protein, termed PfMCM4, contains all conserved sequences in the MCM family and displays the highest homology to the Cdc54 (MCM4) of *Saccharomyces cerevisiae*. However, PfMCM4 possesses five unique amino acid inserts with sizes ranging from seven to 75 residues. Southern blotting of genomic DNA digests and chromosomal separations showed that the *Pfmcm4* gene is present as a single copy per haploid genome and is located on chromosome 13. A 4000-nucleotide transcript of this gene is expressed specifically in the sexual erythrocytic stage, indicating that PfMCM4 may be involved in gametogenesis in which DNA is quickly replicated.

Keywords: Malaria; Mini-chromosome maintenance protein; DNA replication initiation; *Plasmodium falciparum*

1. Introduction

Malaria continues to be a major health problem in large parts of the world. The lack of an effective vaccine and the development of *Plasmodium* resistance to many existing anti-malarial drugs have made the situation even worse. It is therefore imperative that our understanding of the fundamental biology and biochemical processes at different stages of the parasite be improved, to facilitate the identification of new targets for the development of novel drugs and vaccines. DNA replication represents such a key process of the parasite. There are at least five distinct points in the parasite life cycle when DNA replication occurs (White and Kilbey, 1996), two of which take place in the human host, i.e. in the hepatocytes and in the erythrocytes, and the remainder occur in the mosquito vector. The selective blocking of DNA synthesis in the parasite should inhibit both the disease itself and the parasite transmission. DNA replication proceeds in two continuous phases: initiation and elongation. To date, only four components involved in DNA elongation have been identified and isolated from *Plasmodium falciparum* (White and Kilbey, 1996). These include proliferating cell nuclear antigen (PCNA) (Kilbey et al., 1993) which, in other eukaryotic cells, acts as a clamp maintaining contact between DNA polymerase and its template at the primer terminus and increasing its processivity, DNA polymerase α (Abu-Elheiga et al., 1990; Choi and Mikkelsen, 1991; White et al., 1993) which is essential for initiation of DNA synthesis, DNA polymerase δ (Fox and Bzik, 1991; Ridley et al., 1991) which is thought to generate daughter DNA strands, and primase (Prasartkaew et al., 1996) which interacts with DNA polymerase α and synthesises the RNA primers in other eukaryotic cells. However, there is no information concerning the DNA replication initiation in the parasite. We are interested in DNA replication in *P. falciparum*, particularly at the initiation stage. The first step towards this goal has been to isolate the essential components of the initiation machinery from the parasite. In this paper, we report the molecular cloning and characterisation of a novel gene encoding a *P. falciparum* mini-chromosome maintenance protein 4 (PfMCM4). PfMCM4 contains five unique amino acid inserts and is expressed specifically in the sexual stage, indicating that it may be important in regulating the

☆ Note: Nucleotide sequence data reported in this paper are available in the GenBank™, EMBL and DDJB databases under the accession number AF083323.

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biochemical processes of sexual stage, such as gametogenesis (DNA replication).

2. Results and discussion

Database searches revealed that in the *P. falciparum* tag database, there were four cDNA fragments (tags 0159C3, 0381C3, 1313C3 and 1328C3) encoding four protein fragments all with a high homology to the yeast MCM4 protein (Cdc54 of *Saccharomyces cerevisiae* and CDC21 of *Shizosaccharomyces pombe*). Nucleotide sequence analysis showed that the A + T content and codon usage of the fragments are typical of the coding region of *P. falciparum* genes (Weber, 1988). To isolate the full-length gene encoding the malaria MCM4, two specific primers, MC1 (5′-GTAAACCTAGGACAAACCTCTCAAC-3′, 3226–3250) (walking in the 3′ direction) and MC2 (5′-GTTGTCTTGGACTAGCTGTTGG-3′, 3049–3070) (walking in the 5′ direction) were synthesised on the basis of 0381C3 cDNA sequence and used in PCR to screen Vectorette libraries (Li et al., 1996). Two fragments (MC1-*Alu*I and MC2-*Dra*I) were obtained (Fig. 1). As expected, MC2-*Dra*I contains all sequence information obtained from the 1313C3, 1328C3 and 0159C3 tags. The sequence data of MC1-*Alu*I and MC2-*Dra*I permitted synthesis of MC3 (5′-CATGAAACTATAATGAACGATCCAC-3′, 3340–3364) and MC6 (5′-TAGAAATTGACTGTGGCGTTTCTCC-3′, 1645–1669), respectively and subsequent screening of Vectorette libraries. Two overlapping PCR products (MC3-*Dra*I and MC3-*Rsa*I) were amplified with MC3, both containing a putative TAA stop codon, whereas only one fragment (MC6-*Taq*I) was obtained with MC6 (Fig. 1). However, the sequence data of MC6-*Taq*I made it possible to construct the MC8 primer (5′-TGGTAAGGTCCTAATTTTCTAGGGG-3′, 1331–1355) that gave rise to the MC8-*Dra*I fragment. Based on the sequence of MC8-*Dra*I, the MC10 primer (5′-CTACATTCATTTTGCCTTCCTGTTTC-3′, 985–1010) was designed and used to produce MC10-*Rsa*I which contains all sequence information of the 0622C3 tag. On the basis of MC10-*Rsa*I, MC12 (5′-CTTCCAAATATCTCATTGTTCGACC-3′, 536–560) was synthesised to produce the MC12-*Alu*I fragment. MC12-*Alu*I permitted construction of MC14 (5′-TCCTAATCTTCTTCTTGGTGTACCC-3′, 456–480) that consequently gave rise to the MC14-*Taq*I fragment (see Fig. 1). In order to confirm the sequence obtained from the overlapping fragments, several independent PCR fragments (MCm-MCE, MCB-MC2 and MCB-MCE) were amplified from the parasite (3D7A) genomic DNA and sequenced in both strands (Fig. 1). The sequence derived from overlapping fragments comprises 3570 bp and

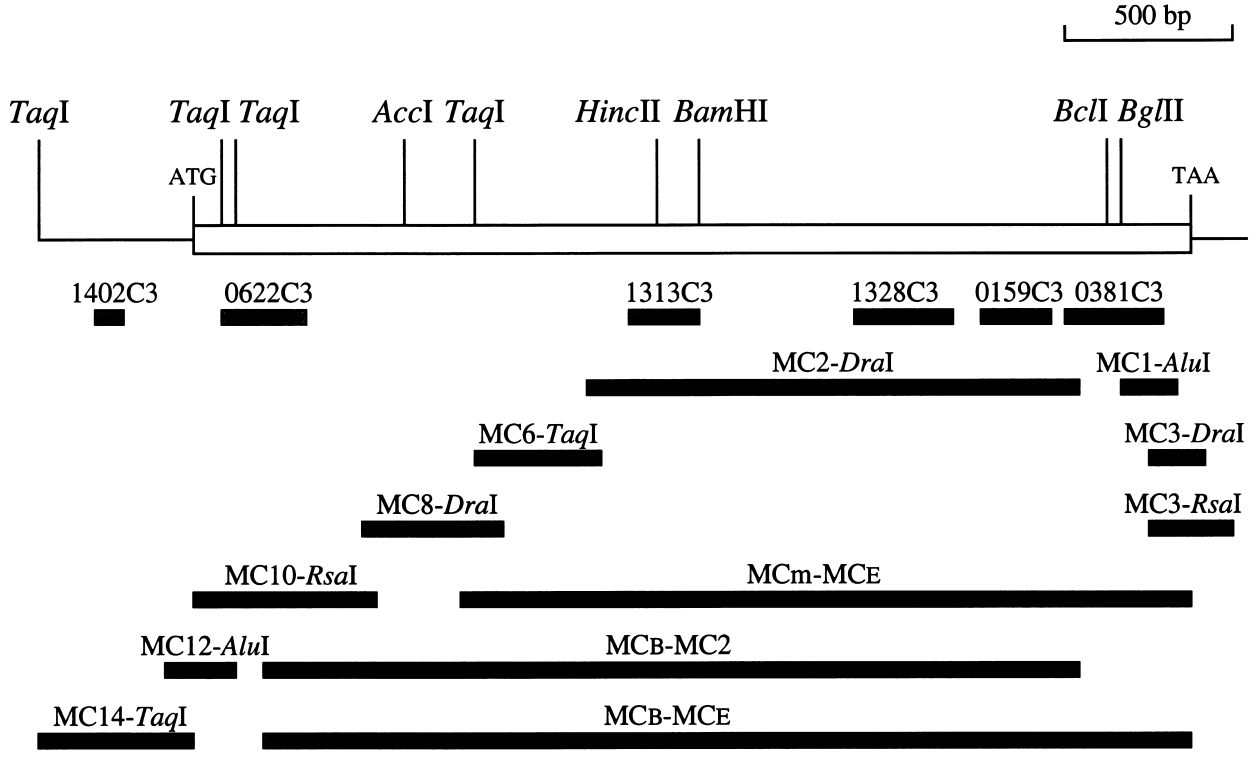

Fig. 1. A schematic representation of a partial restriction map of the *Pfmcm4* gene and the overlapping fragments used to determine its nucleotide sequence. The open box represents the coding region of the *Pfmcm4* gene. The fragments of 1402C3, 0622C3, 1313C3, 1328C3, 0159C3 and 0381C3 are obtained from the cDNA tag database. The fragments of MC1-*Alu*I, MC2-*Dra*I, MC3-*Dra*I, MC3-*Rsa*I, MC6-*Taq*I, MC8-*Dra*I, MC10-*Rsa*I, MC12-*Alu*I and MC14-*Taq*I are derived from the Vectorette PCRs. MCm-MCE, MCB-MC2 and MCB-MCE are amplified from genomic DNA.

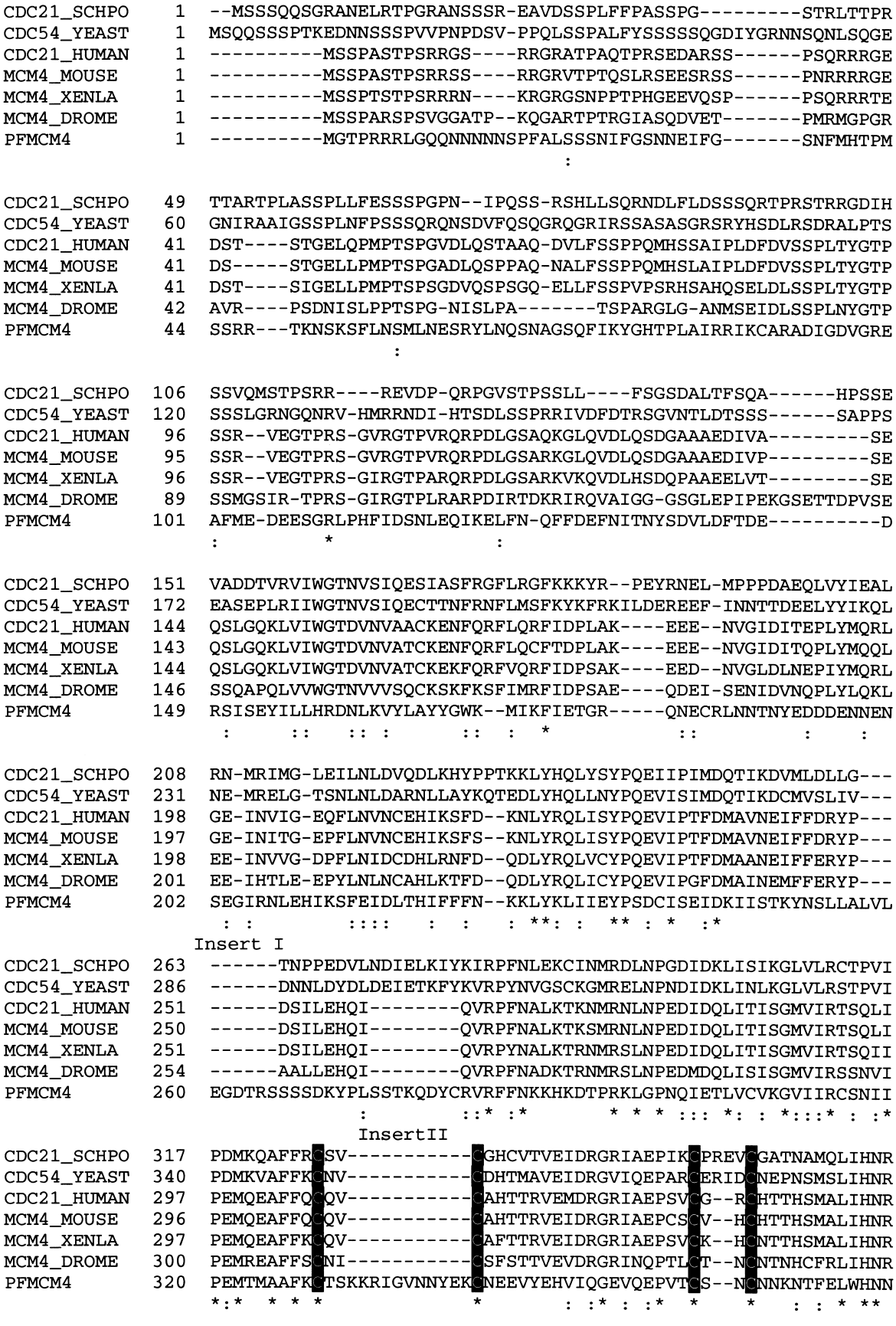

Fig. 2. (*continued overleaf*)

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CDC21_SCHPO  366  SEFADKQVIKLQETPDVVPDGQTPHSVSLCVYDELVDSARAGDRIEVTGIFRCVPVRLNP
CDC54_YEAST  389  CSFADKQVIKLQETPDFVPDGQTPHSISLCVYDELVDSCRAGDRIEVTGTFRSIPIRANS
CDC21_HUMAN  344  SLFSDKQMIKLQESPEDMPAGQTPHTVILFAHNDLVDKVQPGDRVNVTGIYRAVPIRVNP
MCM4_MOUSE   343  SFFSDKQMIKLQESPEDMPAGQTPHTIVLFAHNDLVDKVQPGDRVNVTGIYRAVPIRVNP
MCM4_XENLA   344  SMFSDKQMIKLQESPEDMPAGQTPHTTILYGHNDLVDKVQPGDRVNVTGIYRAVPIRVNP
MCM4_DROME   347  SEFTDKQLVKLQESPDDMAAGQTPHNVLLYAHNDLVDKVQPGDRVTVTGIYRATPLKTGG
PFMCM4       378  CCFSSKQLIKLSEVTEHLKQGETPQSISIYAYDDLIDYTKPGDTVELTGILKASPVRLNP
                    *: **::** *  : :  *:**:   :  :::*:*  : ** : :**  :  *::
                                                Insert III
CDC21_SCHPO  426  RMRTVKSLFKTYVDVVHIKKQDKRRLGTDPST---------LESDIAEDAALQ--IDEVR
CDC54_YEAST  449  RQRVLKSLYKTYVDVVHVKKVSDKRLDVDTST---------IEQELMQNKVDHNEVEEVR
CDC21_HUMAN  404  RVSNVKSVYKTHIDVIHYRKTDAKRLHG--------------LDEEAEQKLFS-------
MCM4_MOUSE   403  RVSNVKSVYKTHIDVIHYRKTDAKRLHG--------------LDEEAEQKLFS-------
MCM4_XENLA   404  RVRNVKSVYKTHIDVIHYRKTDSKRLHG--------------IDEDTEQKLFT-------
MCM4_DROME   407  LSSSVKSVYKTHVDVVHFRKVDNKRLY---------------EEEEGKDHIFP-------
PFMCM4       438  RSRCYNSVHRTYINVIHIKKENKQKMKLTEQNDTANIILKRNEDGTVEENFEKLNEQGNL
                       :*: :*:::*:* :*   :::                        ::

CDC21_SCHPO  475  KISDEEVEKIQQVSKRDDIYDILSRSLAPSIYEMDDVKKGLLLQLFGGTNKSFHKGASPR
CDC54_YEAST  500  QITDQDLAKIREVAAREDLYSLLARSIAPSIYELEDVKKGILLQLFGGTNKTFTKGG--R
CDC21_HUMAN  443  ---EKRVELLKELSRKPDIYERLASALAPSIYEHEDIKKGILLQLFGGTRKDFSHTGRGK
MCM4_MOUSE   442  ---EKRVKLLKELSRKPDIYERLASALAPSIYEHEDIKKGILLQLFGGTRKDFSHTGRGK
MCM4_XENLA   443  ---EERVAMLKELAAKPDIYERLAAALAPSIYEHEDIKKGILLQLFGGTRKDFSHTGRGK
MCM4_DROME   445  ---PERVELLQLLAKKPDIYDRLARAIAPSIYENDDIKKGILLQLFGGTKKKHATLGRQN
PFMCM4       498  LFTTEVIQKMEQLSKDPNIYQRLVDSIAPSIYGRGDIKKGLLCQLFGGSKITDKYNN--K
                      : :  :  ::   ::*  *  ::*****   *:***:* *****:
                         Walker A
CDC21_SCHPO  535  YRGDINILMCGDPSTSKSQILKYVHKIAPRGVYTSGKGSSAVGLTAYITRDQDTKQLVLE
CDC54_YEAST  558  YRGDINILLCGDPSTSKSQILQYVHKITPRGVYTSGKGSSAVGLTAYITRDVDTKQLVLE
CDC21_HUMAN  500  FRAEINILLCGDPGTSKSQLLQYVYNLVPRGQYTSGKGSSAVGLTAYVMKDPETRQLVLQ
MCM4_MOUSE   499  FRAEINILLCGDPGTSKSQLLQYVYNLVPRGQYTSGKGSSAVGLTAYVMKDPETRQLVLQ
MCM4_XENLA   500  FRAEVNILLCGDPGTSKSQLLQYVFNLVPRGQYTSGKGSSAVGLTAYVMKDPETRQLVLQ
MCM4_DROME   502  FRSEIHLLLCGDPGTSKSQMLQYVFNLVPRSQYTSGRGSSAVGLTAYVTKDPETRQLVLQ
PFMCM4       556  YRSEIHILLRGDPSTAKSQLLHYVHKLSPRGIYTSGKGSSSVGLTAFISKDSETKEYILE
                  :* ::::*: *** *:***:*:** :: **  ****:***:*****:: :* :*:: :*:
                    Walker B
CDC21_SCHPO  595  SGALVLSDGGICCIDEFDKMSDATRSILHEVMEQQTVTVAKAGIITTLNARTSILASANP
CDC54_YEAST  618  SGALVLSDGGVCCIDEFDKMSDSTRSVLHEVMEQQTISIAKAGIITTLNARSSILASANP
CDC21_HUMAN  560  TGALVLSDNGICCIDEFDKMNESTRSVLHEVMEQQTLSIAKAGIICQLNARTSVLAAANP
MCM4_MOUSE   559  TGALVLSDNGICCIDEFDKMNESTRSVLHEVMEQQTLSIAKAGIICQLNARTSVLAAANP
MCM4_XENLA   560  TGALVLSDNGICCIDEFDKMNESTRSVLHEVMEQQTLSIAKAGIICQLNARTSVLAAANP
MCM4_DROME   562  TGALVLADNGVCCIDEFDKMNDSTRSVLHEVMEQQTLSIAKAGIICQLNARTSILAAANP
PFMCM4       616  SGAVVLSDKGICCIDEFDKMDDSARAILHEVMEQQTVTIAKAGIVATLNARTSILASANP
                  :**:**:* *:********* :::*::*********:::*****:  ****:*:**:***
                                                                  Insert
CDC21_SCHPO  655  IGSKYNPDLPVTKNIDLPPTLLSRFDLVYLILDRVDETLDRKLANHIVSMYMEDTP----
CDC54_YEAST  678  IGSRYNPNLPVTENIDLPPPLLSRFDLVYLVLDKVDEKNDRELAKHLTNLYLEDKP----
CDC21_HUMAN  620  IESQWNPKKTTIENIQLPHTLLSRFDLIFLMLDPQDEAYDRRLAHHLVALYYQS------
MCM4_MOUSE   619  IESQWNPKKTTIENIQLPHTLLSRFDLIFLMLDPQDEAYDRRLAHHLVSLYYQS------
MCM4_XENLA   620  VESQWNPKKTTIENIQLPHTLLSRFDLIFLMLDPQDEAYDRRLAHHLVALYYQS------
MCM4_DROME   622  AESQWNKRKNIIDNVQLPHTLLSRFDLIFLVLDPQDEIFDKRLASHLVSLYYVT------
PFMCM4       676  INSRYDKNKAVVENINLPPSLFSRFDLIYLVIDQANEDEDRKLATVLCKNFSYNPEEEED
                    *:::       *::**  *:*****::*::*  :*  *: **  :   :
                  IV                                    Insert V
CDC21_SCHPO  711  ---EHATDME-------------------------------------------------
CDC54_YEAST  734  ---EHISQDD-------------------------------------------------
CDC21_HUMAN  674  ---EEQAEEE-------------------------------------------------
MCM4_MOUSE   673  ---EEQVEEE-------------------------------------------------
MCM4_XENLA   674  ---EEQMKEE-------------------------------------------------
MCM4_DROME   676  ---RHEEEDT-------------------------------------------------
PFMCM4       736  EDQEDQEEDEPNYITQQRARKSKGTSRKNERENYYNDGDNDDDDDISNYLNDSNDAQNKR
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CDC21_SCHPO 718  -------------------------VFSVEFLTSYITYARNNINPVISEEAAKELVNAYV
CDC54_YEAST 741  -------------------------VLPVEFLTMYISYAKEHIHPIITEAAKTELVRAYV
CDC21_HUMAN 681  -------------------------LLDMAVLKDYIAYAHSTIMPRLSEEASQALIEAYV
MCM4_MOUSE  680  -------------------------FLDMAVLKDYIAYAHSTIMPRLSEEASQALIEAYV
MCM4_XENLA  681  -------------------------HLDMAVLKDYIAYARTYVNPRLSEEASQALIEAYV
MCM4_DROME  683  -------------------------MFDMSVLRDYIAYAREHLSPTLSDEAQQRLIQAYV
PFMCM4      796  GSWANVNISYDEYNNSSNKKTSKNYLIDSNTLALYIAYCRITCNPIISLESKKIIIEEYI
                                          :    *  **:* :    * ::  :   ::  *:

CDC21_SCHPO 753  GMR-KLGEDVRASEKRITATTRQLESMIRLSEAHAKMHLRNVVEVGDVLEAARLIKTAIK
CDC54_YEAST 776  GMR-KMGDDSRSDEKRITATTRQLESMIRLAEAHAKMKLKNVVELEDVQEAVRLIRSAIK
CDC21_HUMAN 716  DMR-KIG----SSRGMVSAYPRQLESLIRLAEAHAKVRLSNKVEAIDVEEAKRLHREALK
MCM4_MOUSE  715  NMR-KIG----SSRGMVSAYPRQLESLIRLAEAHAKVRFSNKVEAIDVEEAKRLHREALK
MCM4_XENLA  716  SMR-KIG----SGRGMVSAYPRQLESLIRRAEAHAKVRFSNKVETIDVEEAKRLHREALK
MCM4_DROME  718  DMR-KVG----AGRGQISAYPRQLESLIRLSEAHAKVRLSNQVELLDVEEAWRLHREALK
PFMCM4      856  KMRCKEG------TKSPTASPRQLEGLVRLSQSLAKMKLKRVVSPEEANEAVRLMNIATF
                  ** * *          :*  **** ::* ::: **:::   *   :  ** **   *

CDC21_SCHPO 812  DYATDPATGKISLDLIYVNE--RETLVPEDMVKELAN---LISNLTVGGKTMLVSQLLTR
CDC54_YEAST 835  DYATDPKTGKIDMNLVQTGKSVIQRKLQEDLSREIMN---VLKDQAS--DSMSFNELIKQ
CDC21_HUMAN 771  QSATDPRTGIVDISILTTGMSATSRKRKEELAEALKK---LILSKGK-TPALKYQQLFED
MCM4_MOUSE  770  QSATDPRTGIVDISILTTGMSATSRKRKEELAEALRK---LILSKGK-TPALKYQQLFED
MCM4_XENLA  771  QSATDPRTGIVDISILTTGMSATARKRKEELAQVLKK---LIQSKGK-TPALKYQQLFED
MCM4_DROME  773  QSATDPLSGKIDVGILTTGLSTAARKKRADLVAAIKE---NLKKKGK-VLTVPYQKLFSD
PFMCM4      910  QSLIDPLSGRIDFDQVNLGQT-SQHKKKSDLIKDIIMNALVLKNMTK-DELLTHCHETIM
                 :   ** :* :    :             ::   :      :         :

CDC21_SCHPO 867  FREQSSTRLDASDFEACLGALERRGRIKVITMLATHCTFNCTD-
CDC54_YEAST 890  INEHSQDRVESSDIQEALSRLQQEDKVIVLGEGVRRSVRLNNRV
CDC21_HUMAN 827  IRGQSDIAITKDMFEEALRALADDDFLTVTGK-TVRLL------
MCM4_MOUSE  826  IRGQSDTAITKDMFEEALRALADDDFLTVTGK-TVRLL------
MCM4_XENLA  827  LRGQSDAAITKDMFDEALHALADDDYLTVTGK-TVRLL------
MCM4_DROME  829  IKEGSQIMITREQFEDALKEVQDEGAIVVMGKNTIRIC------
PFMCM4      968  NDPQHTTSMDRKSFEEAFYDLEKSQEITRLCSGLYKKK------
                        :    ::  :  :     :        :
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Fig. 2. Sequence alignment of MCM4 proteins from different species. The GenBank™/EMBL/DDJB database accession numbers are as follows: *Shizosaccharomyces pombe* CDC21, P29458; *Saccharomyces cerevisiae* Cdc54, P30665; human MCM4, P33991; mouse MCM4, P49717; *Xenopus* MCM4, P30664; *Drosophila* MCM4, Q26454; and *Plasmodium falciparum* MCM4, AF083323. Sequences were aligned with the CLUSTAL W (1.60) multiple sequence alignment programme. The amino acid residues are numbered to the left of the sequence. Identical residues are indicated with asterisks and conservative changes indicated with dots. Inserts I–V are labelled on the top of sequence. The zinc finger motif is highlighted with solid black and the Walker-type A and B motifs are indicated by overhead thick lines.

contains an open read frame beginning with a putative start ATG codon at nucleotide 454 and terminating with a TAA at nucleotide 3471. The sequence and codon usage in the coding region are typical for a *P. falciparum* gene. The A + T contents of both flanking regions are characteristically higher than that of the coding region (Weber, 1988). To exclude the possibility of the existence of intron(s) in the coding region, RT-PCRs (Li and Baker, 1997; Li et al., 2000; Li and Cox, 2000) were performed using a number of primer pairs that cover the whole coding region and no intron was detected.

The open reading frame encodes a protein of 1005 amino acids with a predicted Mr of 115 kDa. Database analysis showed that the encoded protein shares 58–62% similarity and 38–41% identity with the MCM4 proteins in the MCM family (Tye, 1999) across the conserved region (residue positions 203–995) and displays the highest homology to the Cdc54 (MCM4) of *S. cerevisiae* (Whitbread and Dalton, 1995). Accordingly, we designated it as PfMCM4. Fig. 2 shows a sequence alignment of MCM4 proteins from diverse organisms. The largest and most conserved was found in the core region encompassing the Walker-type A motif (GDPSTAKS) and B motif (GAVVLSDKGICCIDEF) (Koonin, 1993). This region shows moderate similarity with the NtrC family of bacterial transcription factors, which are putative ATPases that facilitate DNA melting at promoters (Wedel and Kustu, 1995). Close inspection of the amino acid sequence revealed that PfMCM4 contains a number of potential phosphorylation sites for casein kinase II (CKII), protein kinase C (PKC), cAMP-dependent protein kinase (PKA) and cyclin-dependent protein kinase (Cdk), suggesting that the activity of PfMCM4 may be regulated by reversible phosphorylation. Although the parasite CKII and PKC have not yet been described, PKA and Cdk have

already been demonstrated in *P. falciparum* (Ross-MacDonald et al., 1994; Li et al., 1996; Li and Cox, 2000). Indeed, it has been proven that Cdc2/cyclin B, Cdk2/cyclin A and Cdk2/cyclin E are able to phosphorylate human and *Xenopus* MCM4 proteins, and consequently inhibit helicase activity of the MCM4,6,7 complex (Coue et al., 1996; Hendrickson et al., 1996; Ishimi et al., 2000).

Despite the similarity to the MCM4 proteins from other species, PfMCM4 also contains several unique features. Firstly, among members of the MCM4 subfamily, PfMCM4 is the largest protein containing 1005 amino acid residues. Secondly, the zinc finger motif (C-X2-C-X18-C-X2-4-C), a characteristic of DNA-binding domains which is conserved in the MCM4 subfamily (Tye, 1999), is interrupted in PfMCM4 by the insertion of 11 amino acid residues into the first two cysteines (C-X13-C-X18-C-X2-C). Finally, PfMCM4 contains five unique amino acid inserts with sizes ranging from seven to 75 residues. These amino acid inserts may represent unique targeting points for the development of new anti-malarial drugs.

To investigate the structural organisation of the *Pfmcm4* gene in the parasite genome, clone 3D7A genomic DNA was digested with a number of restriction enzymes and analysed by Southern blotting. Hybridisation of the MCm-MCE probe revealed a single band in digests with *Acc*I, *Eco*RI or *Eco*RV, for which there is no restriction site in the MCm-MCE fragment, and two bands on digestion with *Bcl*I, for which only one restriction site exists in MCm-MCE (data not shown). The results suggest strongly that PfMCM4 is encoded by a single copy gene in the parasite genome.

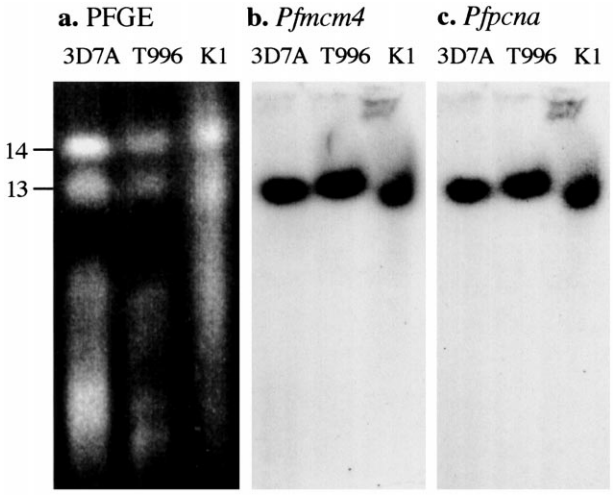

Fig. 3. Chromosomal localisation of the *Pfmcm4* gene. Parasite chromosomes from *Plasmodium falciparum* 3D7A, T996 and K1 were separated by pulse-field gel electrophoresis, stained with ethidium bromide, blotted onto a nylon membrane and hybridised with radiolabelled probes. Based on the yeast chromosome markers and hybridisation of several *P. falciparum* chromosome marker genes, the positions of chromosome 13 and 14 were identified on the ethidium bromide-stained gel. *Pfmcm4* and *Pfpcna* both hybridised to chromosome 13.

To determine the chromosome location of the *Pfmcm4* gene, *P. falciparum* (3D7A, T996 and K1) chromosomes were separated on the CHEF gel system, blotted onto a nylon membrane and hybridised with the MCm-MCE probe. A single band was detected corresponding to chromosome 13 (Fig. 3b). The result was repeated by hybridising another blot with the same probe (data not shown) and further confirmed by probing these blots with a control probe derived from the *Pfpcna* gene (Fig. 3c), which is known to be located on chromosome 13 (Kilbey et al., 1993).

To obtain some information on how *Pfmcm4* transcript levels are regulated during parasite development and differentiation, a Northern blot containing equal amounts of total RNA prepared from cultures enriched in stage III–V gametocytes and from mixed asexual erythrocytic stages was probed with the MCm-MCE fragment. A single transcript of approximately 4000 nucleotides in size was detected only in the lane containing the sexual stage RNA, migrating behind the 28S rRNA band (Fig. 4a). As internal controls for hybridisation to the sexual and asexual stage mRNA, the same blot was hybridised with *Pflammer*, a sexual stage-specific gene (Li et al., 2001) and *Pfpkac*, an asexual stage-specific gene (Li and Cox, 2000) (Fig. 4b,c). The results suggest that PfMCM4 may be involved in regulating biochemical processes of the sexual stage development.

It has been shown that the quantity of DNA in the mature gametocyte of *P. falciparum* reaches the diploid value

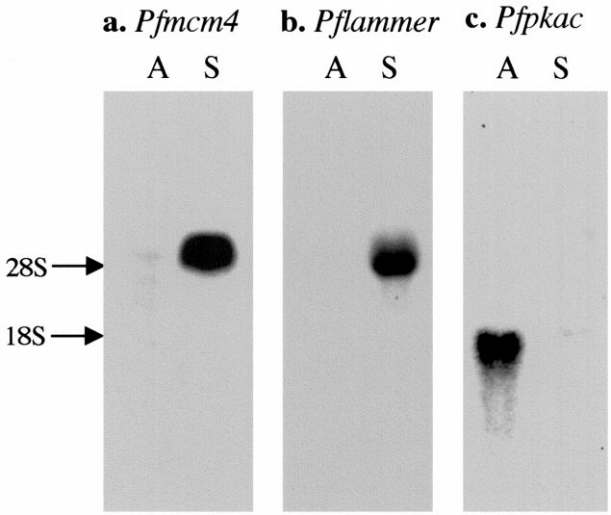

Fig. 4. Northern blot analysis of the *Pfmcm4* gene. Ten micrograms of total RNA extracted from asexual erythrocytic stage (A) and sexual erythrocytic stage (S) of *Plasmodium falciparum* (3D7A) were fractionated in a denaturing formaldehyde gel, blotted onto a nylon membrane and hybridised to radiolabelled probes. The positions of *P. falciparum* rRNA subunits (18S and 28S) are indicated by arrows. Autoradiographs of the membrane probed with: (a), the *Pfmcm4* gene (the MCm-MCE fragment); (b), the *Pflammer* gene; and (c), the *Pfpkac* gene, and exposed for 3, 44 and 1 h, respectively. The *Pfmcm4* probe detected a transcript of approximately 4000 nucleotides in the sexual stage, *Pflammer* hybridised with a band of approximately 3800 nucleotides in the sexual stage and *Pfpkac* hybridised with a transcript of approximately 1800 nucleotides in the asexual stage.

(Janse et al., 1988), implying either complete genome duplication or selective gene amplification per haploid genome, consistent with results of electron microscopic studies (Sinden, 1982). However, in *Plasmodium berghei*, the mature gametocytes have DNA contents only between haploid and diploid levels (Janse et al., 1986), suggesting amplification of a fraction of the genome. Upon activation, the microgametocyte develops rapidly in the mosquito midgut (gametogenesis). Three successive rounds of genome replication are completed within 10 min, raising the DNA contents to octaploid values just before exflagellation, indicating that the genome duplication rate of the malaria parasite is extremely high, probably among the highest recorded. Assuming that the rate of replication fork movement in *Plasmodium* is similar to that in other eukaryotes, at least 1300 origins of replication would be needed to achieve this rate of replication (Janse et al., 1986).

An attractive model for the core components of replication initiation in yeast has been proposed (reviewed by Leatherwood, 1998): the origin recognition complex (ORC) is the replication initiator, MCMs, which consist of six different subunits (MCM2–MCM7), form the helicase, and Cdc6 (*S. cerevisiae*)/Cdc18 (*S. pombe*) as well as Cdt1 (Nishitani et al., 2000) are the helicase loading factors. The high level of specific expression of PfMCM4 in the sexual stage of *P. falciparum*, therefore, is consistent with its potential role in replication initiation during the sexual stage development, particularly during gametogenesis. Identification of other MCM subunits and other components, including ORC, Cdc6/Cdc18 and Cdt1 homologues, in the parasite will help afford new insight on the regulatory mechanisms of gametogenesis.

Acknowledgements

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