DNA replication during intra-erythrocytic stages of human malarial parasite *Plasmodium falciparum*

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***Plasmodium falciparum*, the causative agent of the most virulent form of human malaria, has deservingly held the candidature of being one of the most studied human pathogens. Here we attempt an overview of the studies probing one of the important aspects of DNA metabolism, that is, DNA replication in this parasite, focusing on the relatively well-characterized core components of chromosomal DNA replication in the asexual intra-erythrocytic stage.**

Keywords: DNA replication, origin recognition complex, *Plasmodium falciparum*, schizogony.

Introduction

PROTOZOAN parasite of genus *Plasmodium*, the causative agent of malaria, is responsible for an estimated 350–500 million clinical malarial episodes, most of which are caused by infection with *Plasmodium falciparum* and *Plasmodium vivax*. The former causes more than one million deaths each year, mainly in young children in synergy with other infections and illnesses. Approximately 60% of world's cases of malaria, including close to 75% of global *P. falciparum* malaria cases occur in sub-Saharan Africa[1]. Malaria has been a problem in India for centuries. Details of this disease can be found even in the ancient Indian medical literature like the *Charaka Samhita*. The annual incidence of malaria was estimated at around 75 million cases in 1953, with about 8 lakh deaths annually. To combat this menace, the Government of India launched the National Malaria Control Programme in April 1953. The programme proved highly successful[2] and within five years, the incidence dropped to 2 million. It was then that a series of setbacks was witnessed leading to a sharp rise in the number of reported cases. Currently, India accounts for two-thirds of all confirmed malaria cases reported from the South East Asia region, with Odisha, Jharkhand, West Bengal, Madhya Pradesh and Chhattisgarh contributing to the bulk (60%) of malaria[3]. Most of the malaria attributable mortality is reported from Odisha and other forested areas inhabited by ethnic tribes in the country[4].

Globally, *P. falciparum* malaria has become a matter of concern as it is increasingly showing resistance to the commonly used drugs like quinine, chloroquine, sulphadoxine, etc. It has become an urgent necessity to identify new drug targets for therapeutic intervention. DNA replication is a key biological process essential for both parasite survival and multiplication. Identification of a pathway or protein(s) of this important aspect of DNA metabolism that could be blocked or targeted offers a lot of scope and hope to combat this deadly parasitic disease.

Plasmodium life cycle

Of the human malaria species, *P. falciparum* is medically the most important and it is responsible for most of the mortality and morbidity associated with the disease. The life cycle of *P. falciparum* is complex, involving invasive, trophic and replicative forms in two different hosts – human and female *Anopheles* mosquito. Transmission between mosquitoes and humans involves a spectacular series of morphological transformations (Figure 1). The asexual stage begins with transmission of motile sporozoites into the blood stream of the human host during mosquito feeding. The sporozoites enter the hepatocytes where they multiply and then differentiate to generate numerous merozoites. Upon release, the merozoites proceed to invade the red blood cells (RBCs) of the host to initiate the blood stage of the infection. This is also the stage most successfully adapted into laboratory culture conditions. The intra-erythrocytic (IE) parasite matures through the ring, trophozoite and schizont stages, eventually bursting to release 16 or more daughter merozoites[5,6].

DNA replication, one of the fundamental biological processes, may be the key to these life-cycle changes. It appears that in spite of having multiple distinct developmental stages in the complex malaria life cycle where DNA replication occurs (Figure 1), the general features of eukaryotic DNA replication processes may be conserved in the parasite.

Cell biology and enzymology of the parasite DNA replication is not well understood, primarily because the cell

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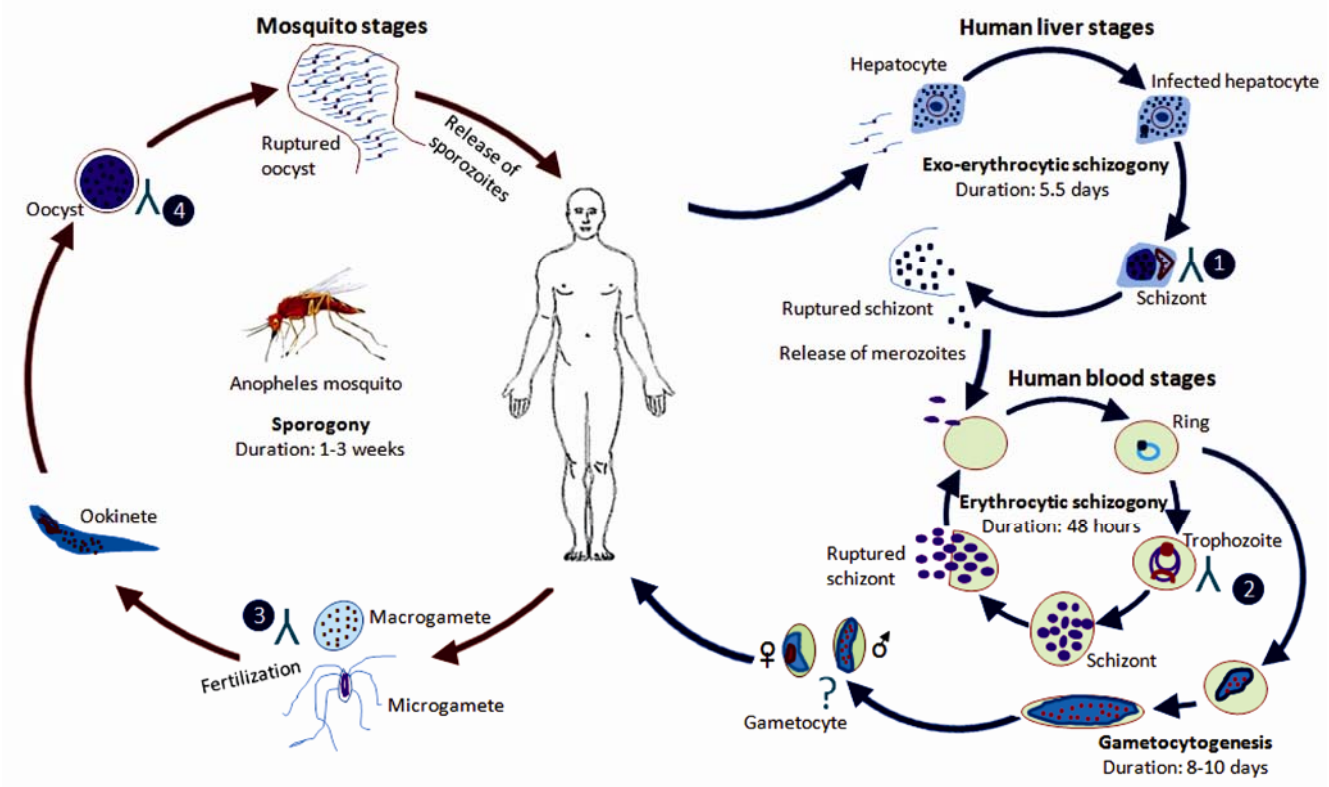

Figure 1. An illustrative diagram showing an overall *Plasmodium falciparum* life cycle in female anophiline mosquito and human hosts, depicting various stages and their approximate duration. Inverted forks (1–4) indicate the probable stages where DNA replication takes place. (The above figure is a modified form of the image available at http://www.dpd.cdc.gov/dpdx/HTML/Malaria.htm.)

cycle differs in several ways from that of better-understood models such as yeasts and mammalian cells[7]. However, for any organism, accurate and timely duplication of the chromosomes is indispensable. Failure to completely copy the genome has the potential to result in catastrophic genomic instability.

Chromosomal replication

Timing of replication

The overall organization of cell division in *Plasmodium* is unique compared to that observed in model organisms because DNA replicates more than once per cell cycle at several points of its life cycle[8]. DNA replication and cell-cycle studies in human malarial parasite *P. falciparum* in combination with those conducted in murine malarial parasite, *P. berghei* in the early 1980s gave insights into the DNA amplification at different stages of the parasite life cycle. In the mammalian host, DNA synthesis takes place when the parasite invades the hepatocytes (exo-erythrocytic schizogony) and during erythrocytic schizogony. In the anophiline host, parasite undergoes DNA replication during gametogenesis, once following fertilization prior to meiosis and during formation of sporozoites in oocysts[9–12]. One of the most interesting and unique features of DNA replication takes place during the activation of *P. berghei* microgamatocytes in the mosquito. Three successive rounds of DNA replication take place within 10 minutes raising the haploid nuclear content to octaploid level prior to exflagellation. This must be the highest rate of DNA replication observed in any known system. It is understood that the rate of DNA replication may not vary drastically among species[9]. Therefore, the usage of origins must be represented more frequently in this process. Erythrocytic schizogony is a relatively better characterized DNA replication event in the parasite life cycle, whereas others remain poorly understood. Timing and duration of DNA replication in synchronized parasite culture has been reported by different groups using radioactive nucleotides in the presence of DNA replication inhibitors like aphidicolin, hydroxyurea, etc.[11]. These results suggest that DNA replication initiates ~22–24 h following invasion, peaks at about 30–32 h and subsequently declines, continuing till 40–44 h post-invasion.

Replication initiation

During initiation of replication, in all species, initiator proteins recognize the origin DNA. This is then followed

by the recruitment of additional proteins facilitating the localized unwinding of the origin DNA in preparation for DNA synthesis. It involves organization of a pre-replicative complex (pre-RC) subsequently making way for the replication fork progression or elongation[13].

However, despite some initial clues, the question of what events trigger initiation of replication is unresolved and largely unexplored. It is also still unclear how initiation proteins mediate localized unwinding of DNA or how they recruit proteins involved in the elongation phase of replication.

Origin of replication

In eukaryotes, replication initiates in a coordinated manner from multiple locations, termed origins of replication, distributed across each of the chromosomes[14,15]. These sequences do not exhibit much uniformity across eukaryotes. The problem of replicating DNA in a reasonable time appears to have been solved in two ways during evolution, bacteria with rapid DNA synthesis rates can initiate multiple temporally overlapping rounds of replication from a unique origin, whereas eukaryotes and some archaea have acquired multiple origins. In the unicellular eukaryote *Saccharomyces cerevisiae*, three to four sequences of 10–15 base pairs (bp) spread over 100–150 bp consisting of highly conserved and essential A-element or autonomously replicating sequence (ARS) consensus sequence (ACS) and less well-conserved elements called B elements are sufficient to act as an origin[16,17]. Unlike the conserved ACS, 20–50 bp, AT-rich sequences spread across 800–1000 bp aid the replication initiation in *S. pombe*[18–20]. These sequences do not show strong sequence similarity just like the origins of replication in higher eukaryotes which are spread over thousands of base pairs and less well defined. For example, origins in early embryos of *Xenopus laevis* and *Drosophila melanogaster* do not exhibit any sequence specificity to facilitate rapid DNA amplification[17].

P. falciparum origin of replication is an unexplored territory. Rapid rates of DNA amplification (schizogony)[8,10] have been observed at various stages of the life cycle and requirement of multiple origins firing appears inevitable. The parasite genome is highly AT-rich and propensity of AT-rich sequences to act as initiator sequences along with high rate of genome duplication may lead us to speculate that there may not be sequence specificity like origins in early embryos of *X. laevis* and *D. melanogaster*. To confirm whether the speculations are true or indeed there are consensus sequences which may initiate and direct DNA synthesis, calls for an immediate attention for mapping and characterizing such sequences with the aid of available genome sequence and relatively well-characterized origin recognition complex (ORC) subunits.

ORC subunits

ORC, a six-protein complex, is one of the first molecules to arrive at the site of DNA replication initiation providing the landing pad for the subsequent components of pre-RC from yeast to mammals. ORC was first identified in *S. cerevisiae*, where it binds to the ACS[21].

In *P. falciparum*, ORC1 was the first subunit of the ORC complex to be identified, initially reported only to be expressing predominantly in sexual erythrocytic stage[22], but later established to be expressing in the asexual erythrocytic stages as well[23], substantiating a published microarray data[24]. PfORC1 (PFL0150w) shows 50% and 53% homology with the *S. cerevisiae* and human ORC1 proteins respectively. However, this homology is restricted to the C terminus only, whereas the long extended N-terminal harboured several aspargine and lysine repeats with little or no homology to any known ORC protein. The N-terminal contains a leucine heptad motif that might be involved in oligomerization and DNA-binding activity along with a putative nuclear localization signal (NLS) (Deshmukh and Dhar, unpublished data).

PfORC1 is expressed at the protein level exclusively in the nucleus during the late ring/early trophozoite stages, just at the beginning of the DNA replication[25,26]. Moreover, the purified C-terminal catalytic domain, PfORC1C with nucleotide binding and hydrolysis domains, showed ATPase activity. The ATPase activity of ORC1 is essential for origin activity in yeast, *Drosophila*, and Xenopus[27–29]. It has been further demonstrated that PfORC1 shows ATP-independent DNA-binding activity, which is contrary to yeast ORC[23]. PfORC1 may show nonspecific ATP-independent DNA binding *in vitro*, whereas ATP may play a major role in binding active origins *in vivo.*

Moreover, at the replicating trophozoite stage of the parasite life cycle, PfORC1 was seen to be colocalized with PfPCNA in distinct punctate nuclear foci, as visualized by immunoflouresence microscopy. This result was further supported by direct physical interaction between PfORC1 and PfPCNA[30], as shown by coimmunoprecipitation of PfPCNA with PfORC1 from the replicating stage parasite. Interestingly, at late multinucleate schizont stage, nuclear PfORC1 staining disappeared and PfORC1 was completely degraded[25], in accordance with its stage-specific protein expression profile that might serve an important role in the regulation of DNA replication.

Further, with the aid of yeast genetic complementation system, PfORC1 was demonstrated to harbour functional PCNA interacting protein motif (PIP) at its C-terminus, essential for cell viability as well as physical interaction between PfORC1 and PfPCNA[30]. Conserved residues in the PIP motifs of both ScORC1 and PfORC1 were mutated and were subsequently tested for their ability to complement the yeast ORC1 function. PIP mutant forms failed to complement yeast ORC1 function, suggesting its essential role in DNA replication[30].

Analysis of PlasmoDB also revealed[31] the presence of an ORF (PFB0720c), which showed ~20% identity and ~43% homology with the yeast ORC5 homologue at the carboxy-terminal region. This gene consisting of 899 amino acid residues, has a long N-terminus extension containing aspargine/aspartic acid/lysine-rich repeat regions similar to the PfORC1 subunit and is not an exceptional phenomenon for *P. falciparum* as reported earlier[32].

This putative PfORC5 protein contains a functional NTP-binding domain at the N-terminus, a hallmark of ORC5 proteins[25]. Moreover, when tested for its ability to complement the yeast *ORC5* mutant strain, the chimera construct carrying the C-terminus of PfORC5 fused with the N-terminus of ScORC5, was able to functionally complement the yeast mutant strain, whereas the full length or the N-terminus alone could not do so[25]. *In vivo* analysis of this protein using specific antibody against the parasite culture shows its distinct nuclear localization in clear punctate foci along with PfPCNA and PfORC1 at the replicating asexual stages[25,30]. These foci dissociated from each other at the late schizont stage. Moreover, formation and progression of these replication stage foci were disrupted in the presence of hydroxyurea, a known replication inhibitor[25], confirming the role of PfORC1 and PfORC5 in parasite DNA replication.

A putative ORC2 ORF (MAL7P1.21) has been identified in the PlasmodB, which shares 36% and 42% homology with yeast and human counterparts respectively. Moreover, an unannotated ORF (Pf13_0189) sharing significant homology to yeast and trypanosomatid ORC4 has been found (unpublished data).

P. falciparum genomic database failed to identify a distinct homologue of Cdc6. Cdc6 proteins are members of the AAA+ protein family (ATPases associated with various cellular activities)[33,34]. In addition to the N-terminal AAA+ domain, Cdc6 proteins also contain a winged-helix DNA-binding motif towards the C-terminus and are essential for eukaryotic DNA replication[35]. Interestingly, PfORC1 shares significant sequence homology with the human and yeast Cdc6/Cdc18 protein. The identity and similarity of PfORC1 with HsCdc6 was found to be 36% and 55%, respectively. Other members of the ORC family, namely ORC3 and ORC6 have not been found so far in *P. falciparum.*

The dynamic nature of ORC proteins has been highlighted by recent studies where ORC has been shown to be involved in functions other than its pivotal role of DNA replication initiation. ScORC has been implicated in transcriptional gene silencing, while in *Drosophila*, ORC2 is known to be associated with heterochromatin[36–39]. Similar studies with PfORC1 showed its involvement in telomere position effect (TPE) where ORC1 was found to be localized to distinct perinuclear sub-compartments of the nucleolus and telomere along with SIR2 protein of *P. falciparum*[40]. PfSir2 is a histone deacetylase[41,42], that reversibly associates with the promoter regions of silent but not active subtelomeric *var* genes, which encode virulence genes of the parasite[43–45]. It seems that the N-terminal of PfORC1 has a specific role in parasite *var* gene silencing in coordination with PfSir2 (Deshmukh and Dhar, unpublished data).

MCM2-7 helicase subunit

The establishment of pre-RC requires recruitment of helicase ring which encircles the DNA, providing topologically stable interaction that allows efficient translocation and unwinding of DNA strands at the replication fork. The precise molecular mechanism of recruitment and unwinding remains elusive. In eukaryotes, six different MCM (mini-chromosome maintenance) subunits (MCM2–7) are required for replication fork progression[46]. Similar to other eukaryotes, the *Plasmodium* genome encodes six MCM subunits. All six MCM subunits contain the signature MCM motif G(IVT)$(LVAC)_2$(IVT)-D(DE)(FL)(DNST)KM along with a conserved WALKER A-type ATP-binding domain. In addition, a zinc-finger motif is located towards the N-terminus of MCM 2, 4, 6 and 7, as would be expected for these MCM proteins[47]. Patterson *et al.*[48] studied three out of these six subunits in the IE asexual cycle. The expression of these subunits shows a stage-specific pattern, peaking at the schizont stages with an increased localization in the nucleus at this stage. Interaction among these three MCM subunits was detectable in the ring and schizont stages of IDC. PfMCM6 was the only MCM among the three which remains bound to the chromatin throughout the asexual cycle[48].

PfMCM4 shows 58–62% similarity and 38–41% identity with the MCM family of proteins across the conserved region (residues 203–995). It was initially reported to be present at the RNA level only in the sexual stage[49], confirmed by proteome analysis data of sexual stage-specific parasite. However, subsequent studies revealed the presence of this protein in the nucleus during both asexual and sexual stages along with PfORC1, suggesting a functional role for MCM proteins in parasite DNA replication[50]. PfMCM3 and 5 still need to be characterized in detail.

CDT1, a protein required for loading MCMs and geminin that titrates CDT1 right at the beginning of the S phase to control re-initiation are yet to be identified and characterized in *P. falciparum*[51–53]. However, an unannotated ORF (PF13_237) having CDT1-like domain has been identified in the *Plasmodium* database. The absence of geminin is not surprising, as multiple rounds of DNA replication take place in the erythrocytic asexual stages. A model indicating the components of the pre-RC in eukaryotes and the corresponding proteins in *Plasmodium* is shown in Figure 2.

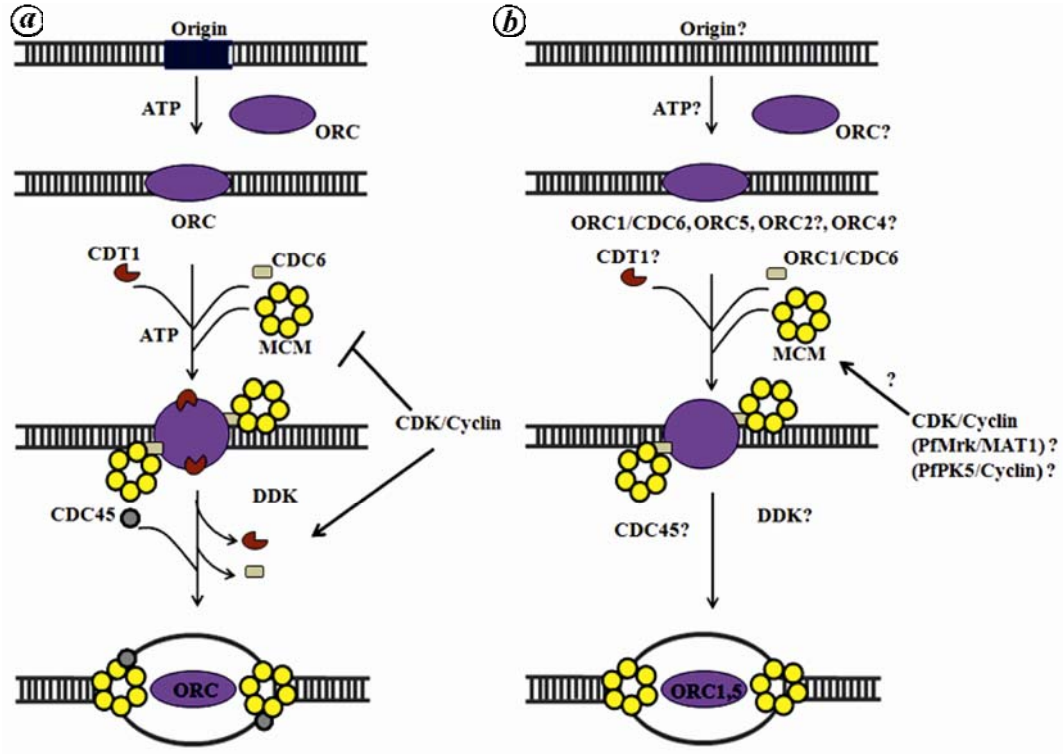

Figure 2. Comparison of models of pre-replication complex formation in eukaryotes and *Plasmodium*. ***a***, In eukaryotes, the pre-RC complex formation involves an ordered assembly of a number of components: origin recognition complex (ORC) composed of six subunits (1–6), Cdc6, Cdt1 and MCM2-7. Origin sequences are conserved in yeast *Saccharomyces cerevisiae*, but not in higher eukaryotes. Two proteins kinases, CDK and DDK (Dbf4-dependent kinase) are required for the firing of licenced origins for initiation. Activation by these kinases leads to changes in the pre-RC, causing the binding of Cdc-45 to the MCM complex. This results in an unwinding of replication origin. CDK activity may also be required for the phosphorylation of some pre-RC components leading to the inhibition of re-initiation. ***b***, *P. falciparum* ORC contains ORC1/CDC6 and ORC5. ORC2 and ORC4 may also exist. PfPK5 is a homologue of P34^{cd2} cyclin-dependent kinase required for both entry into S-phase and mitosis in fission yeast cells, but the exact function in firing of licenced origins is unclear. The role of PfMrk and Mat1 in replication initiation is not known, but they phosphorylate replication proteins like MCM *in vitro*. A Cdt1-like protein is found in *P. falciparum*, although its function is not known. Other key regulators of pre-replication complex formation, DDK and Cdc45, appear to be absent in *Plasmodium*. The above model suggests that the malaria parasite may have a minimum pre-RC (adapted from Patterson, S. *et al*.[48] and subsequently modified based on available data).

Components of the replication complex at the fork

After establishment of the pre-RC and onset of the signals marking the start of S phase of the cell cycle, further recruitment of several other proteins complete the building of mature DNA replication machines (‘replisomes’) for bidirectional DNA replication from each origin. The basic components of a replisome are the DNA replication fork itself, together with the primases, polymerases and processivity factors. Two classes of kinases, cyclin-dependent kinases and Dbf4-dependent kinases (DDK) have been known to be crucial at this point, primarily for the activation of the Pre-RC[54]. In *P. falciparum*, homologues of these kinases are being looked into. Further discussion on plasmodial S-phase kinases would be done in a later section.

At this juncture another factor which needs to be mentioned is Cdc45. Initially identified in yeast[55], Cdc45 is believed to play a crucial role in the transition of the pre-RC into an active replication fork. It is known to be associated with the MCM complex along with GINS (Go-Ichi-Ni-San, i.e. 5-1-2-3) complex, activating the replicative helicases bringing about unwinding of the DNA double helix[56–59]. Although plasmodial homologue of Cdc45 has not yet been identified, it would be interesting to see if any other known replication protein undertakes its function.

Primase

Primase is the ssDNA-dependent RNA polymerase which is absolutely essential for chromosomal DNA replication as it catalyses *de novo* synthesis of discrete length oligoribonucleotides (7–10 bp) that constitute the primers required for subsequent DNA synthesis by the polymerase alpha subunit[60–62]. Primase has the conserved features of the catalytic centres of DNA polymerases, RNA polymerases as well as that of reverse transcriptases. Originally, primase was identified as a heteroduplex, isolated as a part of DNA pol alpha complex[63,64].

Pfprimase small subunit is encoded by an ORF of 1356 nucleotides with a calculated molecular weight of

53 kDa. The study by Prasartkaew *et al.*[65] has shown that the genomic sequence encoding Pfprimase is unusually interrupted by 15 introns. Amino acid sequence analysis of PfPrimase small subunit across eukaryotic species revealed several conserved regions. Moreover, the features of the catalytic centre of DNA polymerase, reverse transcriptase and RNA polymerase are shared by this parasite primase small subunit. Further, similarity with DNA polymerase alpha, RNA polymerase II and Herpes virus primase was identified at the C-terminal region. The protein purified from a recombinant baculovirus vector overexpressed in Sf9 insect cells demonstrated its ability to initiate *de novo* primer formation[65]. *In vivo* expression pattern and localization of the protein has not yet been studied in detail.

DNA polymerases

One of the most important components of the replisome is the DNA polymerase that facilitates the incorporation of the correctly base paired deoxyribonucleoside monophosphate onto a growing primer/template duplex, with the exception of some of the translesion polymerases[66]. Several different polymerases have been identified and characterized so far. They are categorized into five types, namely alpha (α), beta (β), gamma (γ), delta (δ) and epsilon (ε)[66]. DNA polymerase α, δ and ε are sensitive to aphidicolin, whereas DNA polymerase γ and β are resistant to aphidicolin, but sensitive to nucleotide analogues[67–69]. DNA polymerase β can be differentiated from DNA polymerase γ by its resistance to *N*-ethylmaleimide[68,69]. In addition, DNA polymerase γ is more processive than Polβ[68–70]. In *P. falciparum* asexual-stage parasite, several different polymerase activities have been reported[71–78]. The most recent being the study by Nunthawarasilp *et al.*[75], which identified and partially purified a DNA polymerase beta-like enzyme from large-scale *P. falciparum* parasite culture. This enzyme was found to be highly resistant to aphidicolin and *N*-ethylmaleimide as in other eukaryotic enzymes, but was also resistant to 2,3-dideoxythymidine-5-triphosphate and to other synthetic nucleoside analogues. Like other eukaryotic Polβ, this Polβ-like enzyme too showed possible involvement in base excision repair pathway[67]. Previously, in *P. falciparum*, DNA polymerase α had been purified and characterized from crude extract[77], whereas DNA polymerase γ had been detected in both crude extract and parasite mitochondrion[78]. The genes encoding *P. falciparum* DNA polymerases α and δ have been fully sequenced[74,73]. Polα shows an overall low (14–17%) amino acid sequence identity with Polα from other species, while PfPol δ shows very high degree of conservation when compared with Pol δ homologues from other eukaryotes. Horrocks *et al.*[79] have probed the expression pattern of the two most important components of the replisome, that is, DNA Pol δ along with that of the processivity factor (PCNA) in the asexual erythrocytic phase. It shows that both the genes are expressed predominantly at the trophozoite/schizont stage, both at transcript and protein level. Promoter activity analysis of these two genes showed that the PCNA promoter was active throughout the asexual stage, whereas Pol δ promoter activity was only detectable at the trophozoite stage.

Processivity factors

Isolated polymerases have a low affinity for DNA, resulting in frequent dissociation during synthesis. To increase processivity within the replisome, the polymerase is coupled to a specialized sliding clamp that encircles DNA, but does not bind tightly to it, and effectively tethers polymerase to the DNA. The two sliding clamps at each replication fork are positioned behind the polymerases on each strand, such that they encircle the newly formed double-stranded DNA (dsDNA). PCNA aids in a range of DNA-processing events[80,81]. All of the PCNA-interacting factors were found to contain short, highly conserved sequence motifs (PCNA interacting protein motif, PIP) that were important for the interactions[82–84]. PCNA was first identified by Miyachi *et al.*[85] in 1978 and later Bravo and Celis[86] characterized it and called it 'cyclin'. The PCNA/cyclin was directly implicated in DNA replication using SV40 *in vitro* replication system[87] and was demonstrated to be an auxiliary factor for DNA pol delta[88,89]. Using SV40 *in vitro* replication system, it has been shown that PCNA associates with replication factor C (RF-C) and ATP at the 3′ OH terminus of the first Okazaki fragment to be synthesized from the origin, thus facilitating a switch from alpha polymerase to delta polymerase-mediated DNA synthesis[90,91].

P. falciparum, unlike most eukaryotes, harbours two PCNAs. Certain organisms, including the thermoacidophilic archaeon *Sulfolobus solfataricus*[92], *Aeropyrum pernix*[93], the chlorella virus *Paramecium bursaria*[94,95], the carrot plant *Daucus carota*[96], the maize plant *Zea mays*[97,98], the plant *Arabidopsis thaliana*[99,100] and the fellow protozoan *Toxoplasma gondii*[101] also contain multiple PCNAs.

The first PCNA (PfPCNA1) identified in *P. falciparum* was encoded by chromosome 13, consisting approximately 270 amino acids with a predicted molecular mass of 30.168 kDa and shared an overall identity of 34% and 31% with human and yeast PCNA respectively[102]. It contained all the conserved residues of a processivity factor. PfPCNA1 was demonstrated to be expressing predominantly at the late trophozoite and schizont stages coinciding with the expression of polymerase delta[79]. More recently, PfPCNA1 was demonstrated to form distinct nuclear punctate foci co-localizing with subunits of origin recognition complex (1 and 5) during replicating trophozoite stages of the asexual cycle[25]. PfPCNA1 was also

shown to functionally interact with PfORC1 most likely through its PIP motif[30]. Together these data support the involvement of PfPCNA1 in DNA replication in the asexual erythrocytic stage. Additionally, PfPCNA1 has been studied as a model to understand the temporal control of genes during *P. falciparum* asexual stages[103]. Previous studies also characterized the minimal promoter (including mapping of the transcription start site) and demonstrated (using a transient transfection system) a role for flanking sequences in directing the absolute level of gene expression[79] of PfPCNA1.

Later, two groups independently reported the identification of a second PCNA in the *P. falciparum* genome[104,105]. PfPCNA2 located on chromosome 12, codes for a protein of 264 amino acids with a predicted molecular weight of 30.2 kDa. It shares an overall similarity of 43–56% with other members of the PCNA family and a 29% identity with PfPCNA1. Molecular modelling studies based on structures of human and yeast PCNA suggest that PfPCNA2 may form a trimeric ring with a six-fold axis of symmetry[106,107]. Northern blot analyses revealed two transcripts with size of 1800 and 2500 nucleotides respectively. The former is expressed in both asexual and sexual stages, whereas the latter is specifically expressed in the sexual stage, suggesting that PfPCNA2 may play an essential role in DNA metabolism at different stages of the parasite.

Replication factor complex

During DNA replication *in vivo*, the sliding clamp requires a specialized clamp loader complex to open and shut the ring around dsDNA whenever DNA synthesis is initiated at a primer. Since lagging strand synthesis requires frequent reloading of the polymerase and sliding clamp, the clamp loader complex is an integral component of the replisome[108]. The loading of PCNA onto DNA by the replication factor complex (RFC) is an important step in DNA replication and increases the processivity of DNA polymerase[109].

Most RFCs are composed of five subunits highly conserved in all eukaryotes. *Plasmodium* genome mining has identified an RFC5 (PF11 0117) homologue that displays an overall sequence similarity of 30% to replication factor complex subunits from *Drosophila* and yeast. RFC5 contains: (i) the Walker A nucleotide-binding motif (consensus sequence, GxxGxGKS/T) encompassing residues 40–47, (ii) the Walker B nucleotide hydrolysis motif consensus containing four aliphatic residues followed by two negatively-charged residues, encompassing residues 128–133, and (iii) an arginine finger. The arginine finger is an AAA+-family specific motif that senses ATP-binding and hydrolysis, and transmits conformational changes[110]. The RFC clamp loader has been shown to require the arginine finger sensors (emanating from each of the subunits) to drive DNA binding and loading of the PCNA[111]. *In vivo* expression profiling reveals that PfRFC5 peaks in late trophozoites through early schizont stages, where most of the DNA replication is thought to occur[24].

PfRFC5 has been identified along with another pre-RC protein, PfMCM6, to be interacting with CDK Pfmrk in a bacterial two-hybrid screen. Pfmrk was found to phosphorylate both PfRFC-5 and PfMCM6 *in vitro*[112]. Like other eukaryotes, whether their phosphorylation status affects their subcellular localization and their ability to bind DNA and other subunits requires further studies[113–145].

Replication protein A

Replication protein A (RPA) is a eukaryotic, single-stranded (ss) DNA-binding protein which plays essential roles in various aspects of DNA metabolism, including replication, recombination and repair[116]. The protein has much higher propensity to bind ssDNA[117–119] than towards dsDNA and RNA[120,121]. Eukaryotic RPA exists as a heterotrimeric complex and consists of subunits of size 70, 34 and 14 kDa respectively.

P. falciparum replication protein A large subunit (PfRPA1) was isolated and identified in the parasite extract through affinity purification and mass spectral analysis. A 55 kDa factor displaying a major single-strand binding activity was identified from the parasite extract. Subsequent sequence analysis revealed homology varying from ~34% to 39% with other eukaryotic RPA large subunits, but restricted only to the C-terminal 466 amino acids. Interestingly, the large RPA subunits in higher eukaryotes have molecular masses ranging between 67 and 73 kDa, while PfRPA1 similar to large RPA subunits identified in fellow protozoan *Cryptosporidium parvum*[122] and *Crithidia fasciculata*[123] has molecular mass between 51 and 55 kDa. This has been attributed to the absence of a N-terminal protein interaction domain found in all other RPA large subunits. PfRPA1 located on chromosome 4 (GenBank™ accession number AL035475) is encoded by an unusual 6.5 Kb transcript, indicating the presence of extensive 5′ and 3′ untranslated regions. Protein expression profile of PfRPA1 in IE asexual stages coincides with the replication phase in late trophozoites and schizonts[124], which is in agreement with the expression profile of other DNA replication genes. Other subunits of this heterotrimeric complex in *P. falciparum* are still to be identified and characterized.

Topoisomerases

Topoisomerases are critical because of their ability to relieve torsional stress and resolve topological constraints arising as a by-product of DNA metabolism. They are termed as type-I if one strand of the dulpex DNA is cleaved, whereas the type-II class cleaves both the strands[125,126].

Studies with SV40 cell-free system shows that either of the Topos (I or II) is sufficient for DNA replication, whereas Topo II is required for daughter molecule decatenation in yeast[127]. While Topo I has been shown to be involved in gene transcription in yeast[128] and *Drosophila*[129]. Topo II has been reported to be present in high levels in rapidly dividing cells[130,131], and a number of anti-cancer drugs are known which act as Topo II poisons[126]. Moreover, Topo II plays a critical role at the centromeres during mitosis and has also been implicated in efficient chromosomal segregation across species[132,133].

P. falciparum homologue of Topo II was identified by Cheesman *et al.*[134]. Sequence analysis revealed an overall identity of 47.4% and a similarity of 65.4% with human Topo II, whereas with *S. cerevisiae* homologue, 44.4% identity and 62.2% similarity is shared indicating a relatively higher degree of conservation compared to other *P. falciparum* replication factors. The degree of conservation is generally greater in the amino terminal two-thirds of the coding sequences and falls off markedly towards the carboxy-termini. A study showed the presence of Topo II throughout the IE cycle by immunofluorescence analysis using specific antibodies[134]. Any direct correlation of PfTopo II expression with DNA replication is still awaited.

FEN-1

Flap endonuclease-1 (FEN-1) is a divalent, cation-dependent, dual-function nuclease operating as a 5′–3′ exonuclease in the processing of Okazaki fragments during lagging-strand DNA synthesis and a structure-specific endonuclease involved in long patch BER[135,136]. FEN-1 is responsible for general genomic maintenance, and loss of FEN-1 has resulted in increased trinucleotide repeat expansion, destabilization of telomeres and general genomic instability[137–140]. Previously it has been demonstrated that *P. falciparum* repairs AP sites in DNA predominantly via long-patch base excision repair (BER) where the damaged DNA strand is displaced by a DNA polymerase. As ~10–12 nucleotide repair patch is synthesized, a ssDNA flap is generated, and subsequently acted upon by FEN-1 to endonucleolytically resolve the DNA flap. As a result, a single-stranded nick is created that can be ligated by DNA ligase I[141]. In contrast, mammalian cells show preference for short-patch repair pathway, referring to the fewer nucleotides length of the repair patch. Moreover, most of the components required in the long-patch pathway could be identified in the *Plasmodium* genomic database. Casta *et al.*[142], first identified PfFenI which was localized to chromosome 4, encoding 2019 bp long gene, weighing 76.6 kDa. The study reported that PfFEN1 has enzymatic activities similar to other species, but contains extended C-termini and a more internally located PCNA-binding site, while its N-terminus domain and intermediate domain show high degree of conservation[143–145]. In this study, PfFEN1 along with murine FenI was demonstrated to possess DNA structure-specific flap endonuclease and 5′–3′ exonuclease activities, similar to FEN1s from other species. Additionally, endonuclease activity was stimulated by Mg^{2+} or Mn^{2+} and inhibited by monovalent ions (>20.0 mM). Interestingly, a PfFEN1 C-terminal truncation mutant lacking the terminal 250 amino acids had endonuclease activity that was ~130-fold greater than full-length PfFEN1 *in vitro*[142]. Also PfFEN1 generated a nicked DNA substrate that was ligated by recombinant Pf DNA ligase I (PfLigI)[146] using an *in vitro* DNA repair synthesis assay. This study suggests PfFenI as a critical component of both DNA replication and repair machinery, although further *in vivo* studies are required to substantiate these claims.

DNA ligase I

DNA ligase I constitutes the primary ligase utilized in DNA replication within the eukaryotic nucleus by joining the Okazaki fragments, and plays a critical role in DNA repair and recombination by nick sealing during long-patch BER. Many eukaryotes express three DNA ligase isoforms, DNA ligase I, III and IV. DNA ligase III, associated only with vertebrate cells is also involved with DNA repair in the nucleus and mitochondria[147]. DNA ligase IV has been identified in lower eukaryotes such as yeast and is involved in repairing DNA double-strand breaks as well as V(D)J recombination and non-homologous end-joining (NHEJ)[148]. Ligase I knockouts are lethal in mice[149], while its overexpression has been linked to various human cancers and induced genetic instability in yeast[150,151].

Buguliskis *et al.*[146] identified and characterized codon-optimized recombinant PfLigI. This is localized to chromosome 13 and is a 2.7 kb gene encoding a 912 amino acid protein with a probable molecular weight of 104 kDa. It showed a 30% overall sequence identity to human DNA ligase I and 60% identity in the C-terminal region. Curiously missing from the N-terminus of PfLigI is a PCNA binding domain, which is replaced by a putative apicoplast targeting signal. The kinetic parameters of PfLigI are similar to other eukaryotic ATP-dependent DNA ligases and displays a divalent metal cofactor dependence. All the replication proteins as described above and their status in *P. falciparum* are shown in Table 1.

Cyclin-dependent kinase-like proteins in *P. falciparum*

The regulation of DNA replication by cell cycle-regulated kinases (cyclin-dependent kinases, CDKs) and their regulatory counterparts has been established in eukaryotes[152].

Table 1. Details of proteins involved in nuclear DNA replication

Protein	PLASMODB No.	Reference
Origin recognition complex1 (ORC1)	PFL0150w	23, 25, 30
Origin recognition complex5 (ORC5)	PFB0720c	25
Origin recognition complex2 (ORC2)	MAL7P1.21	–
Origin recognition complex4 (ORC4)	PF13_0189	–
Mini-chromosome maintenance (MCM) proteins	PFE1345c, PF07_0023, PFL0580w, Pf13_0095, PF13_0291, Pf14_0177	48–50
CDT1	PF13_0237	–
Replication protein A (RPA) large subunit	PFD0470c	124
Replication factor C (RFC)	PFA0545c	24, 112
Proliferating cell nuclear antigen 1 (PCNA1)	Pf13_0328	47, 49, 50
Proliferating cell nuclear antigen 2 (PCNA2)	PFL1285c	104–107
Primase	PF14_0366	65
DNA polymerase alpha	PFD0590c	74, 77
DNA polymerase epsilon	PFF1470c	–
DNA polymerase delta	PF10_0165	73, 79
DNA polymerase beta	?	75
FLAP endonuclease-I (FenI)	PFD0420c	142–146
DNA ligase I	MAL13P1.22	146
Topoisomerase II	PF14_0316	134

Bioinformatic analysis indicated the presence of several CDK-like kinases and their effectors in *P. falciparum*, and their biochemical characterization is being done[153–155]. Among these kinases, the most prominent ones are Pfmrk, PfPK5 and PfPK6[156–159].

Pfmrk shows 46% homology to human CDK7. This protein along with its effector PfMAT1 phosphorylated two components (namely PfRFC5 and PfMCM6) of the replication fork as its substrate *in vitro*[112]. Although the *in vivo* phosphorylation status of these DNA replication components remains to be investigated, it is possible that Pfmrk-mediated phosphorylation of the above proteins may regulate the initiation of DNA replication[112]. Another malarial CDK-like kinase, PfPK5, has been proposed to play a role in DNA replication[155,157]. PfPK5 expression and kinase activity levels peak 36 h post-invasion and PfPK5 has been shown to co-localize with DNA in the early trophozoite stages or just at the onset of DNA synthesis. Elevated PfPK5-associated kinase activity was detected in parasites treated with the DNA synthesis inhibitor aphidicolin, whereas treatment of the parasite with the PfPK5 inhibitors flavopiridol and olomoucine resulted in reduced DNA synthesis[158].

Analysis of the amino acid sequences of few of the replication proteins like PfORC1 revealed the presence of conserved CDK-like consensus sequence[25] (SPTK and TPKK) at the N-terminus (Deshmukh and Dhar, unpublished data). Further, preliminary results indicate phosphorylation of PfORC1 at the late trophozoite/schizont stage followed by its degradation at the very late schizont stage just before the release of the merozoites[25] (Deshmukh and Dhar, unpublished data). It will be interesting to see whether *Plasmodium* CDK-like kinases are responsible for PfORC1 phosphorylation and its regulation.

Few *Plasmodium* cyclin-like proteins have been characterized *in vitro*[160]. Their putative role in *Plasmodium* DNA replication *in vivo* needs to be studied further.

A model for *Plasmodium* DNA replication and overall cell cycle progression during the IE stage

Endoreduplication is a process that takes place during differentiation of mammalian trophoblasts and megakaryoblasts[161]. This process is a manifestation of rapid DNA replication where re-initiation may take place before the end of one replication cycle. Considering the rapid DNA replication during erythrocytic trophozoite stage where 1N may give rise to 16N to 32N nuclear content, it is possible that *P. falciparum* undergoes endoreduplication, although it needs to be verified experimentally.

The duration of DNA replication and mitotic phases in *P. falciparum* IE stages is not clearly defined. Recent studies[157] demonstrated the asynchronous division of centriolar plaques (CPs), eukaryotic equivalent of centrosomes and correlated its significance with mitotic progression. There is still speculation over the cell-cycle phases of the parasite IE stage, specifically surrounding the timing of nuclear envelope division following genomic division[25,162,163]. An accurate model of *P. falciparum* IE cell-division cycle requires detailed analysis of events and timings of mitotic progression.

There is a cyclic pattern in the expression of most of the characterized core replication proteins in the IE asexual cycle, where there is distinct upregulation in the expression in the mid/late trophozoite to early schizont stages coinciding with the active replication window. This is demonstrated by the foci dynamics study involv-

ing ORC subunits (1 and 5) along with PCNA1[25]. Based on the above observations along with other published data, we propose a simplified model of cell-cycle progression of IE schizogony (Figure 3) whereby the ring and early trophozoite stage (0–18 h PI) carrying a single nucleus could be representative of the G1 phase, while the onset of the synthetic phase (S-phase) is marked by the assembly of ORC proteins (subunits 1 and 5) in distinct nuclear foci along with the marker of active DNA replication PCNA1. This model suggests that the S-phase (18–36 h PI) may be characterized by coordinated processing of clustered replication forks and as we move towards the end of this phase, the foci disassemble and fulfil their independent fates as demonstrated by dissociation of ORC (1 and 5) and PCNA1 foci and subsequent degradation of ORC1 at the late schizont stage. The degradation of ORC1 may involve phosphorylation by CDK-like kinase, PfPK5. This is followed by a post-replicative phase, including completion of chromosome segregation, cytokinesis and formation of daughter nuclei (merozoites), which may be representative of the G2/M phase. Invasion of fresh RBCs by these newly formed merozoites marks the beginning of the next cycle. It is important to note that there may be certain overlap between the S phase and G2/M phase as segregation of chromosome and nuclear division take place along with the DNA replication, which is contrary to the eukaryotic systems in general.

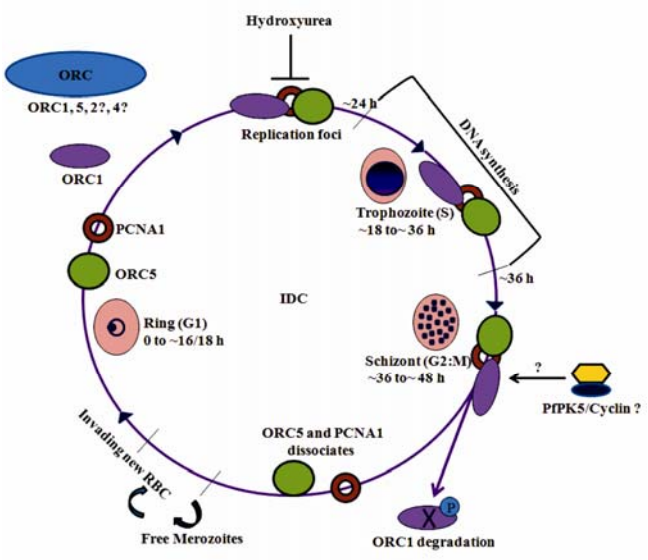

Figure 3. Model of replication foci formation and progression in *P. falciparum*. *Plasmodium* ORC consists of ORC1, ORC5 and putative ORC2 and ORC4 subunits. During intraerythrocytic developmental cycle (IDC), ORC1 is expressed at the late-ring stage, whereas PCNA1 and ORC5 are present through all stages. At the onset of DNA replication, during early trophozoite stage, these proteins co-localize with each other and form replication foci. These foci persists till the late trphozoite/early schizont stage. During late schizont stage, ORC5 and PCNA1 slowly dissociate from each other. However, ORC1 is completely degraded at the late schizont stage possibly via phosphorylation mediated by PfPK5 (unpublished data). G1, S and G2/M phases are indicated according to parasite developmental stages. There may be considerable overlap between different stages, especially between the S and G2/M phase, which is not indicated in the model. This model suggests a critical role for PfORC1 in the regulation of parasite DNA replication (adapted from Gupta, A. *et al*.[25] and subsequently modified based on available data).

Organelle DNA replication

The phylum Apicomplexa which includes the malaria parasites, contains two organelles containing extrachromosomal DNA: mitochondria (mtDNA) and plastid-like apicoplast (plDNA).

Mitochondria are essential organelles required for energy transduction and cellular functions, and are ubiquitous in almost all eukaryotic cells. Like nuclear genome, mitochondrial (mt) genome exhibits remarkable variation in structure and size[164]. The largest mt genome is found in land plants[165], in which the size ranges from 180 to 2400 kb. The *Plasmodium* mt genome has the smallest, multi-copy, linear and tandemly repeated 6-kb element[166]. The mitochondrial genome is highly conserved among *Plasmodium* species[167]. The genome of mitochondria has a concatemeric structure[168] present in *S. cerevisiae* and other fungi[169,170]. The *Plasmodium* mt genome encodes only three protein-coding genes: cytochrome *c* oxidase subunit I (coxI), coxIII and cytochrome *b* (cob). It also contains large- and small-subunit ribosomal RNA (rRNA) genes, which are highly fragmented[171]. The apicoplast is believed to be the site for type-II fatty-acid biosynthesis[172,173], the non-mevalonate pathway of isoprenoid biosynthesis[174,175], as well as synthesis of heme-intermediates within the parasite[176]. The apicoplast contains a ~35 kb, closed, circular, dsDNA genome of unusually high A/T content (86%) that carries genes for large- and small-subunit rRNAs and accompanying ribosomal proteins, 25 species of tRNA, an RNA polymerase and several ORFs coding for chaperones as well as other proteins of unknown function[177].

The timing of replication of both plDNA and mtDNA in blood-stage cultures is well established. It is known that total DNA synthesis starts in trophozoite and continues into schizogony[9,10,12,178,179]. Both organelle DNAs start replicating at about the same time, a few hours before the first nuclear division that marks the onset of schizogony[180,181]. Each sporozoan cell of the parasite carries a single apicoplast[182,183] with estimated copy number of plDNA varying from 1 to 15.

The plDNA is replicated by a bidirectional theta mechanism and segregated into daughter cells[184–188]. The mtDNA replication involves both a rolling circle mechanism and a recombination-associated process resembling that of bacteriophage T4[178,186,187].

The replication of plDNA initiates within the inverted repeat (IR) region that covers a ~10 kb segment and carries genes for large- and small-subunit rRNA and several

Table 2. Details of proteins involved in organelle DNA replication

Protein	PLASMODB No.	Organelle	Reference
Gyrase A	PFL1120c	Apicoplast	193
Gyrase B	PFL1915w	Apicoplast	192, 193
Single-strand DNA-binding (SSB) protein	PFE0435c	Apicoplast	194
Topoisomerase VI	MAL13P1.328	Apicoplast	–
Plastid replication–repair enzyme	PF14_0112	Apicoplast	195, 196
Topoisomerase IV	Pf10_0412	Apicoplast	–
Apicoplast genome replication DNA polymerase Pfprex (primase, helicase and exonuclease/polymerase)	PF14_0112	Apicoplast	195, 196

tRNAs[188–190]. The plDNA replication proceeds via two mechanisms. The first one follows the theta mode with replication initiating within the IR region, while a minor population of plDNA molecules may follow the rolling circle mode possibly utilizing as yet unidentified initiation sites outside the IR. The multiple *ori* within each IR sector have been identified and differential utilization of these initiation sites during plDNA replication has been suggested[190]. The biochemical and cellular processes involved in plDNA replication are poorly understood. The apicoplast genome itself does not encode any proteins known to be involved in DNA replication. However, several proteins that are involved in apicoplast DNA metabolic processes are encoded by the nuclear genome, synthesized in the cytoplasm and transported to the apicoplast. These include bacteria-like gyrase, ssDNA-binding (SSB) protein, apicoplast genome replication DNA polymerase, Pfprex and several unclassified ORFs that are homologous to DNA repair enzymes[191–195]. Pfprex is a large (2016 aa), multifunctional peptide that contains three distinct domains that exhibit DNA primase, DNA helicase and DNA polymerase activities respectively[195,196]. The KPom1 (Klenow-like polymerase of malaria 1) DNA polymerase domain of Pfprex exhibits a strongly biased mutational spectrum, and differs significantly[196] from the closely related *Escherichia coli* DNA polymerase I. Table 2 shows the list of proteins involved in apicoplast DNA replication.

This description has underlined our limited understanding of the replication of organelle DNAs in the malaria parasites and there is a clear need for progress on both the regulation and enzymology of the process(es) in each of the apicomplexan organelles. Apart from any intrinsic value, knowledge of the genes involved in mediating organellar DNA replication is doubly valuable. The essentially prokaryotic nature of the two organellar compartments suggests that they offer potential as drug targets.

Concluding remarks

While some progress has been made in identification and characterization of individual *P. falciparum* DNA replication proteins, it is clear that considerable challenges lie ahead. Both replication initiation and replication fork assemblies are complex nucleoprotein machines, with highly defined architectures and regulation. Efforts must now be made for the molecular dissection of these fundamentally important and conserved processes in one of the most important human pathogens.

Moreover, there are different stages in the complex life cycle of the parasite where DNA replication takes place[10] for which there is little or no information available. Although it is beyond the scope of this review which primarily focuses on the IE stage, it is imperative to mention the need and the importance of probing the DNA replication event in the other stages of the *P. falciparum* life cycle.

Unprecedented success of this parasite in adapting to challenges of the host environment like immune pressure and antimalarial drugs may be due to the dynamic nature of its DNA metabolism. In spite of the overall conservation in the DNA replication machinery, there are certain peculiarities unique to the parasite which may be used to evaluate the possibility of using the replication apparatus as a drug target for malaria therapy. Efforts are already being channelized in this regard. Topoisomerase II poisons have been evaluated as antimalarial agents. In this regard, 9-anilinoacridines, which are structurally related to pyronaridine have been extensively studied[197,198].

The other important aspect is that many of the replication proteins contain long N-terminal extension with no homology with their host counterparts. The members of the origin recognition complex (namely ORC1 and ORC5) are worth mentioning in this regard. Some of them also contain important motifs or signatures that can be used as potential targets against malaria therapy. As DNA replication is central to the parasite proliferation and pathogenesis, continuous efforts to combat these processes will be helpful amidst the growing incidences of drug resistance and lack of an effective vaccine.

1. World Health Organization, WHO World Malaria Report 2008, WHO, Geneva, Switzerland, 2008.
2. Sharma, R. S., Sharma, G. K. and Dhillon, G. P. S., Intervention measures for transmission control in epidemiology and control of malaria in India, National Malaria Eradication Programme, New Delhi, 1996, pp. 218–224.
3. World Health Organization, WHO World Malaria Report 2010, WHO, Geneva, Switzerland, 2010.
4. Kumar, A., Urban malaria and its control in India. *J. Parasit. Dis*., 2007, **21**, 83–88.

5. Bannister, L. H., Hopkins, J. M., Fowler, R. E., Krishna, S. and Mitchell, G. H., A brief illustrated guide to the ultrastructure of *Plasmodium falciparum* asexual blood stages. *Parasitol. Today*, 2000, **16**, 427–433.
6. Garcia, C. R., de Azevedo, M. F., Wunderlich, G., Budu, A., Young, J. A. and Bannister, L., *Plasmodium* in the post genomic era: new insights into the molecular cell biology of malaria parasites. *Int. Rev. Cell. Mol. Biol*., 2008, **266**, 85–156.
7. Arnot, D. E. and Gull, K., The *Plasmodium* cell-cycle: facts and questions. *Ann. Trop. Med. Parasitol*., 1998, **92**(4), 361–365.
8. White, J. H. and Kilbey, B. J., DNA replication in malaria parasite. *Parasitol. Today*, 1996, **12**(4), 151–155.
9. Janse, C. J., van der Klooster, P. F., van der Kaay, H. J., van der Ploeg, M. and Overdulve, J. P., DNA synthesis in *Plasmodium berghei* during asexual and sexual development. *Mol. Biochem. Parasitol*., 1986, **20**(2), 173–182.
10. Janse, C. J. *et al.*, DNA synthesis in gametocytes of *Plasmodium falciparum*. *Parasitology*, 1988, **96**, 1–7.
11. Inselburg, J. and Banyal, H. S., *Plasmodium falciparum*: synchronization of asexual development with aphidicolin, a DNA synthesis inhibitor. *Exp. Parasitol*., 1984, **57**(1), 48–54.
12. Inselburg, J. and Banyal, H. S., Synthesis of DNA during the asexual cycle of *Plasmodium falciparum* in culture. *Mol. Biochem. Parasitol*., 1984, **10**(1), 79–87.
13. Dutta, A. and Bell, S. P., Initiation of DNA replication in eukaryotic cells. *Annu. Rev. Cell Dev. Biol*., 1997, **13**, 293–332.
14. Lundgren, M., Andersson, A., Chen, L., Nilsson, P. and Bernander, R., Three replication origins in *Sulfolobus* species: synchronous initiation of chromosome replication and asynchronous termination. *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 7046–7051.
15. Robinson, N. P., Dionne, I., Lundgren, M., Marsh, V. L., Bernander, R. and Bell, S. D., Identification of two origins of replication in the single chromosome of the archaeon *Sulfolobus solfataricus*. *Cell*, 2004, **116**, 25–38.
16. Bell, S. P., Eukaryotic replicators and associated protein complexes. *Curr. Opin. Genet. Dev.*, 1995, **5**(2), 162–167.
17. Bielinsky, A. K. and Gerbi, S. A., Where it all starts: eukaryotic origins of DNA replication. *J. Cell. Sci.*, 2001, **114**, 643–651.
18. Dubey, D. D., Zhu, J., Carlson, D. L., Sharma, K. and Huberman, J. A., Three ARS elements contribute to the ura4 replication origin region in the fission yeast, *Schizosaccharomyces pombe*. *EMBO J*., 1994, **13**(15), 3638–3647.
19. Dubey, D. D., Kim, S. M., Todorov, I. T. and Huberman, J. A., Large, complex modular structure of a fission yeast DNA replication origin. *Curr. Biol.*, 1996, **6**(4), 467–473.
20. Clyne, R. K. and Kelly, T. J., Genetic analysis of an ARS element from the fission yeast *Schizosaccharomyces pombe*. *EMBO J*., 1995, **14**(24), 6348–6357.
21. Bell, S. P. and Stillman, B., ATP-dependent recognition of eukaryotic origins of DNA replication by a multiprotein complex. *Nature*, 1992, **357**, 128–134.
22. Li, J. L. and Cox, L. S., Characterization of a sexual stage-specific gene encoding ORC1 homologues in the human malaria parasite *Plasmodium falciparum*. *Parasitol. Int*., 2003, **52**, 41–52.
23. Mehra, P., Biswas, A. K., Gupta, A., Gourinath, S., Chitinis, C. E. and Dhar, S. K., Expression and characterization of human malaria parasite *Plasmodium falciparum* origin recognition complex subunit1. *Biochem. Biophys. Res. Commun*., 2005, **337**, 955–966.
24. Le Roch, K. G. *et al.*, Discovery of gene function by expression profiling of the malaria parasite life cycle. *Science*, 2003, **301**, 1503–1508.
25. Gupta, A., Mehra, P. and Dhar, S. K., *Plasmodium falciparum* origin recognition complex subunit 5: functional characterization and role in DNA replication foci formation. *Mol. Microbiol*., 2008, **69**, 646–665.
26. Gritzmacher, C. A. and Reese, R. T., Protein and nucleic acid synthesis during synchronized growth of *Plasmodium falciparum*. *J. Bacteriol*., 1984, **160**, 1165–1167.
27. Bowers, J. L., Randell, J. C., Chen, S. and Bell, S. P., ATP hydrolysis by ORC catalyzes reiterative Mcm2–7 assembly at a defined origin of replication. *Mol. Cell*, 2004, **16**, 967–978.
28. Chesnokov, I., Remus, D. and Botchan, M., Functional analysis of mutant and wild-type *Drosophila* origin recognition complex. *Proc. Natl. Acad. Sci. USA*, 2001, **98**, 11997–12002.
29. Giordano-Coltart, J., Ying, C. Y., Gautier, J. and Hurwitz, J., Studies of the properties of human origin recognition complex and its Walker A motif mutants. *Proc. Natl. Acad. Sci. USA*, 2005, **102**, 69–74.
30. Gupta, A., Mehra, P., Deshmukh, A., Dar, A., Mitra, P., Roy, N. and Dhar, S. K., Functional dissection of the catalytic carboxyl-terminal domain of human malaria parasite *Plasmodium falciparum* origin recognition complex subunit 1 (PfORC1). *Eukaryot. Cell*, 2009, **8**, 1341–1351.
31. The Plasmodium Genome Database Collaborative, PlasmoDB: An integrative database of the *Plasmodium falciparum* genome. Tools for accessing and analyzing finished and unfinished sequence data. *Nucleic Acids Res*., 2001, **29**, 66–69.
32. Singh, G. P., Chandra, B. R., Bhattacharya, A., Akhouri, R. R., Singh, S. K. and Sharma, A., Hyper-expansion of asparagines correlates with an abundance of proteins with prion-like domains in *Plasmodium falciparum*. *Mol. Biochem. Parasitol*., 2004, **137**, 307–319.
33. Davey, M. J., Jeruzalmi, D., Kuriyan, J. and O'Donnell, M., Motors and switches: AAA+ machines within the replisome. *Nature Rev. Mol. Cell Biol*., 2002, **3**(11), 826–835.
34. Hanson, P. I. and Whiteheart, S. W., AAA+ proteins: have engine, will work. *Nature Rev. Mol. Cell Biol*., 2005, **6**(7), 519–529.
35. Giraldo, R., Common domains in the initiators of DNA replication in Bacteria, Archaea and Eukarya: combined structural, functional and phylogenetic perspectives. *FEMS Microbiol. Rev*., 2003, **26**(5), 533–554.
36. Bell, S. P., The origin recognition complex: from simple origins to complex functions. *Genes Dev.*, 2002, **16**, 659–672.
37. Pak, D. T. *et al.*, Association of the origin recognition complex with heterochromatin and HP1 in higher eukaryotes. *Cell*, 1997, **91**, 311–323.
38. Gerbi, S. A. and Bielinsky, A. K., DNA replication and chromatin. *Curr. Opin. Genet. Dev*., 2002, **12**, 243–248.
39. Prasanth, S. G., Juan Me´ndez, Prasanth, K. V. and Stillman, B., Dynamics of pre-replication complex proteins during the cell division cycle. *Philos. Trans. R. Soc. London Ser. B*, 2004, **359**, 7–16.
40. Mancio-Silva, L., Rojas-Meza, A. P., Vargas, M., Scherf, A. and Hernandez-Rivas, R., Differential association of Orc1 and Sir2 proteins to telomeric domains in *Plasmodium falciparum*. *J. Cell Sci.*, 2008, **121**, 2046–2053.
41. Chakrabarty, S. P., Saikumari, Y. K., Bopanna, M. P. and Balaram, H., Biochemical characterization of *Plasmodium falciparum* Sir2, a NAD+-dependent deacetylase. *Mol. Biochem. Parasitol.*, 2008, **158**(2), 139–151.
42. Prusty, D., Mehra, P., Srivastava, S., Shivange, A. V., Gupta, A., Roy, N. and Dhar, S. K., Nicotinamide inhibits *Plasmodium falciparum* Sir2 activity *in vitro* and parasite growth. *FEMS Microbiol. Lett.*, 2008, **282**, 266–272.
43. Duraisingh, M. T. *et al.*, Heterochromatin silencing and locus repositioning linked to regulation of virulence genes in *Plasmodium falciparum*. *Cell*, 2005, **121**, 13–24.
44. Freitas-Junior, L. H. *et al.*, Telomeric heterochromatin propagation and histone acetylation control mutually exclusive expression of antigenic variation genes in malaria parasites. *Cell*, 2005, **121**, 25–36.

45. Tonkin, C. J. *et al.*, Sir2 paralogues cooperate to regulate virulence genes and antigenic variation in *Plasmodium falciparum*. *PLoS Biol*., 2009, **7**, e84.
46. Labib, K., Tercero, J. A. and Diffley, J. F., Uninterrupted MCM2-7 function required for DNA replication fork progression. *Science*, 2000, **288**, 1643–1647.
47. Young, M. R. *et al*., Nuclear accumulation of *Saccharomyces cerevisiae* Mcm3 is dependent on its nuclear localization sequence. *Genes Cells*, 1997, **2**, 631–643.
48. Patterson, S., Robert, C., Whittle, C., Chakrabarti, R., Doerig, C. and Chakrabarti, D., Pre-replication complex organization in the atypical DNA replication cycle of *Plasmodium falciparum*: characterization of the mini-chromosome maintenance (MCM) complex formation. *Mol. Biochem. Parasitol*., 2006, **145**(1), 50–59.
49. Li, J. L. and Cox, L. S., Identification of an MCM4 homologue expressed specifically in the sexual stage of *Plasmodium falciparum*. *Int. J. Parasitol*., 2001, **31**(11), 1246–1252.
50. Gupta, A., Mehra, P., Nitharwal, R., Sharma, A., Biswas, A. K. and Dhar, S. K., Analogous expression pattern of *Plasmodium falciparum* replication initiation proteins PfMCM4 and PfORC1 during the asexual and sexual stages of intraerythrocytic developmental cycle. *FEMS Microbiol. Lett.*, 2006, **261**(1), 12–18.
51. Tada, S., Cdt1 and geminin: role during cell cycle progression and DNA damage in higher eukaryotes. *Front. Biosci*., 2007, **12**, 1629–1641.
52. Saxena, S. and Dutta, A., Geminin-Cdt1 balance is critical for genetic stability. *Mutat. Res.*, 2005, **569**, 111–121.
53. Feng, H. and Kipreos, E. T., Preventing DNA re-replication – divergent safeguards in yeast and metazoa. *Cell Cycle*, 2003, **2**(5), 431–444.
54. Pollock, S., Stoepel, J., Bauerschmidt, C., Kremmer, E. and Nasheuer, H. P., Regulation of eukaryotic DNA replication at the initiation step. *Biochem. Soc. Trans*., 2003, **31**, 266–269.
55. Hardy, C. F. J., Identification of Cdc45p, an essential factor required for DNA replication. *Gene*, 1997, **187**, 239–246.
56. Blow, J. J. and Dutta, A., Preventing re-replication of chromosomal DNA. *Nature Rev. Mol. Cell Biol*., 2005, **6**, 476–486.
57. Machida, Y. J., Hamlin, J. L. and Dutta, A., Right place, right time, and only once: replication initiation in metazoans. *Cell*, 2005, **123**, 13–24.
58. Nasheuer, H. P., Pospiech, H. and Syva¨oja, J., Progress towards the anatomy of the eukaryotic DNA replication fork. In *DNA Polymerases: Encyclopaedic Reference of Genomics and Proteomics in Molecular Medicine*, Springer Verlag, Berlin, 2006, pp. 27–68.
59. Moyer, S. E., Lewis, P. W. and Botchan, M. R., Isolation of the Cdc45/Mcm2–7/GINS (CMG) complex, a candidate for the eukaryotic DNA replication fork helicase. *Proc. Natl. Acad. Sci. USA*, 2006, **103**, 10236–10241.
60. Chang, L. M., Rafter, E., Augl, C. and Bollum, F. J., Purification of a DNA polymerase-DNA primase complex from calf thymus glands. *J. Biol. Chem*., 1984, **259**, 14679–14687.
61. Kuchta, R. D., Reid, B. and Chang, L. M., DNA primase. Processivity and the primase to polymerase alpha activity switch. *J. Biol. Chem*., 1990, **265**, 16158–16165.
62. Kitani, T., Yoca, K. and Okazaki, T., Discontinuous DNA replication of *Drosophila melanogaster* is primed by octaribonucleotide primer. *Mol. Cell. Biol*., 1984, **4**, 1591–1596.
63. Wong, S. W., Paborsky, L. R., Fisher, P. A., Wang, T. S. and Korn, D., Structural and enzymological characterization of immunoaffinity-purified DNA polymerase alpha DNA primase complex from KB cells. *J. Biol. Chem.*, 1986, **261**, 7958–7968.
64. Lehman, I. R. and Kaguni, L. S., DNA polymerase alpha. *J. Biol. Chem*., 1989, **264**, 4265–4268.
65. Prasartkaew, S., Zijlstra, N. M,, Wilairat, P., Overdulve, J. P. and de Vries, E., Molecular cloning of a *Plasmodium falciparum* gene interrupted by 15 introns encoding a functional primase 53 kDa subunit as demonstrated by expression in a baculovirus system. *Nucleic Acid Res*., 1996, **24**, 3934–3941.
66. Woodgate, R., A plethora of lesion-replicating DNA polymerases. *Genes Dev*., 1999, **13**, 2191–2195.
67. Hubscher, U., Nasheuer, H. P. and Syvaoja, J. E., Eukaryotic DNA polymerases, a growing family. *Trends Biochem. Sci*., 2000, **25**, 143–147.
68. Fry, M. and Loeb, L., Multiplicity of DNA polymerases animal cells. In *Animal Cells DNA Polymerases*, Boca Raton, FL, CRC Press, 1986.
69. Wang, T. S., Eukaryotic DNA polymerases. *Annu. Rev. Biochem*., 1991, **60**, 513–552.
70. DePamphilis, M. L. and Wassarman, P. M., Replication of eukaryotic chromosome: a close-up of the replication fork. *Annu. Rev. Biochem*., 1980, **49**, 627–666.
71. Abu-Elheiga, L., Spira, D. T. and Bachrach, U., *Plasmodium falciparum*: properties of an a-like DNA polymerase, the key enzyme in DNA synthesis. *Exp. Parasitol*., 1990, **71**, 21–26.
72. Choi, I. and Mikkelsen, R. B., Cell cycle-dependent biosynthesis of *Plasmodium falciparum* DNA polymerase a. *Exp. Parasitol*., 1991, **73**, 93–100.
73. White, J. H. *et al*., The gene encoding DNA polymerase alpha from *Plasmodium falciparum*. *Nucleic Acids Res.*, 1993, **21**, 3643–3646.
74. Ridley, R. G. *et al*., DNA polymerase delta: gene sequences from *Plasmodium falciparum* indicate that this enzyme is more highly conserved than DNA polymerase alpha. *Nucleic Acids Res*., 1991, **19**, 6731–6736.
75. Nunthawarasilp, P., Petmitr, S. and Chavalitshewinkoon-Petmitr, P., Partial purification and characterization of DNA polymerase beta-like enzyme from *Plasmodium falciparum*. *Mol. Biochem. Parasitol*., 2007, **154**, 141–147.
76. Fox, B. A. and Bzik, D. J., The primary structure of *Plasmodium falciparum* DNA polymerase d is similar to drug sensitive d-like viral DNA polymerases. *Mol. Biochem. Parasitol*., 1991, **49**, 289–296.
77. Chavalitshewinkoon, P., de Vries, E., Stam, J. G., Franssen, F. F., van der Vliet, P. C. and Overdulve, J. P., Purification and characterization of DNA polymerases from *Plasmodium falciparum*. *Mol. Biochem. Parasitol*., 1993, **61**, 243–253.
78. Chavalitshewinkoon-Petmitr, P., Chawprom, S., Naesens, L., Balzarini, J. and Wilairat, P., Partial purification and characterization of mitochondrial DNA polymerase from *Plasmodium falciparum*. *Parasitol. Int*., 2000, **49**, 279–288.
79. Horrocks, P., Jackson, M., Cheesman, S., White, J. H. and Kilbey, B. J., Stage-specific expression of proliferating cell nuclear antigen and DNA polymerase delta from *Plasmodium falciparum*. *Mol. Biochem. Parasitol*., 1996, **79**, 177–182.
80. Kelman, Z., PCNA: structure, functions and interactions. *Oncogene*, 1997, **14**(6), 629–640.
81. Jónsson, Z. O. and Hübscher, U., Proliferating cell nuclear antigen: more than a clamp for DNA polymerases. *Bioessays*, 1997, **19**(11), 967–975.
82. Dionne, I., Nookala, R. K., Jackson, S. P., Doherty, A. J. and Bell, S. D., A heterotrimeric PCNA in the hyperthermophilic archaeon *Sulfolobus solfataricus*. *Mol. Cell*, 2003, **11**, 275–282.
83. Dionne, I. and Bell, S. D., Characterization of an archaeal family 4 uracil DNA glycosylase and its interaction with PCNA and chromatin proteins. *Biochem. J.*, 2005, **387**, 859–863.
84. Dalrymple, B. P., Kongsuwan, K., Wijffels, G., Dixon, N. E. and Jennings, P. A., A universal protein–protein interaction motif in the eubacterial DNA replication and repair systems. *Proc. Natl. Acad. Sci. USA*, 2001, **98**, 11627–11632.
85. Miyachi, K., Fritzler, M. J. and Tan, E. M., Autoantibody to a nuclear antigen in proliferating cells. *J. Immunol*., 1978, **121**, 2228–2234.

86. Bravo, R. and Celis, J. E., A search for differential polypeptide synthesis throughout the cell cycle of HeLa cells. *Cell Biol*., 1980, **84**, 795–802.
87. Prelich, G. and Stillman, B., Coordinated leading and lagging strand synthesis during SV40 DNA replication *in vitro* requires PCNA. *Cell*, 1988, **53**, 117–126.
88. Prelich, G., Tan, C.-K., Kostura, M., Mathews, M. B., So, A. G., Downey, K. M. and Stillman, B., Functional identity of proliferating cell nuclear antigen and a DNA polymerase-delta auxiliary protein. *Nature*, 1987, **326**, 517–520.
89. Bravo, R., Frank, R., Blundell, P. A. and MacDonald-Bravo, H., Cyclin/PCNA is the auxiliary protein of DNA polymerase-delta. *Nature*, 1987, **326**, 515–517.
90. Tsurimoto, T. and Stillman, B., Replication factors required for SV40 DNA replication *in vitro*. I. DNA structure-specific recognition of a primer–template junction by eukaryotic DNA polymerases and their accessory proteins. *J. Biol. Chem*., 1991, **266**, 1950–1960.
91. Tsurimoto, T. and Stillman, B., Replication factors required for SV40 DNA replication *in vitro*. II. Switching of DNA polymerase alpha and delta during initiation of leading and lagging strand synthesis. *J. Biol. Chem*., 1991, **266**, 1961–1968.
92. De Felice, M., Sensen, C. W., Charlebois, R. L., Rossi, M. and Pisani, F. M., Two DNA polymerase sliding clamps from the thermophilic archeon *Sulfolobus solfataricus*. *J. Mol. Biol.*, 1999, **291**, 47–57.
93. Daimon, K., Kawarabayasi, Y., Kikuchi, H., Sako, Y. and Ishino, Y., Three proliferating cell nuclear antigen-like proteins found in the hyperthermophilic archeon *Aeropyrum pernix*: interactions with the two DNA polymerases. *J. Bacteriol*., 2002, **184**, 687–694.
94. Lu, Z., Li, Y., Zhang, Y., Kutish, G. F., Rock, D. L. and Van Etten, J. L., Analysis of 45 kb of DNA located at the left end of the chlorella virus PBCV-1 genome. *Virology*, 1995, **206**, 339–352.
95. Li, Y., Lu, Z., Sun, L., Ropp, S., Kutish, G. F., Rock, D. L. and Van Etten, J. L., Analysis of 74 kb of DNA located at the right end of the 330-kb chlorella virus PBCV-1 genome. *Virology*, 1997, **237**, 360–377.
96. Hata, S., Kouchi, H., Tanaka, Y., Minami, E., Matsumoto, T., Suzuka, I. and Hashimoto, J., Identification of carrot cDNA clones encoding a second putative proliferating cell-nuclear antigen, DNA polymerase delta auxiliary protein. *Eur. J. Biochem*., 1992, **203**, 367–371.
97. Lo´pez, I., Khan, S., Va´zquez-Ramos, J. and Hussey, P. J., Molecular cloning of a maize cDNA clone encoding a putative proliferating cell nuclear antigen. *Biochim. Biophys. Acta*, 1995, **1260**, 119–121.
98. Lopez, I., Khan, S., Vazquez, J. and Hussey, P. J., The proliferating cell nuclear antigen (PCNA) gene family in *Zea mays* is composed of two members that have similar expression programmes. *Biochim. Biophys. Acta*, 1997, **1353**, 1–6.
99. Lin, X. *et al*., Sequence and analysis of chromosome 2 of the plant *Arabidopsis thaliana*. *Nature*, 1999, **402**, 761–768.
100. Theologis, A. *et al*., Sequence and analysis of chromosome 1 of the plant *Arabidopsis thaliana*. *Nature*, 2000, **408**, 816–820.
101. Guerini, M. N., Que, X., Reed, S. L. and White, M. W., Two genes encoding unique proliferating-cell-nuclear-antigens are expressed in *Toxoplasma gondii*. *Mol. Biochem. Parasitol*., 2000, **109**, 121–131.
102. Kilbey, B. J., Fraser, I., McAleese, S., Goman, M. and Ridley, R. G., Molecular characterisation and stage-specific expression of proliferating cell nuclear antigen (PCNA) from the malarial parasite *Plasmodium falciparum. Nucleic Acids Res*., 1993, **21**, 239–243.
103. Wong, E. H., Hasenkamp, S. and Horrocks, P., Analysis of the molecular mechanisms governing the stage-specific expression of a prototypical housekeeping gene during intraerythrocytic development of *P. falciparum*. *J. Mol. Biol.*, 2011, **408**(2), 205–221.
104. Li, J. L., Warren, A. V. and Cox, L. S., Identification of a second proliferating cell nuclear antigen in the human malarial pathogen *Plasmodium falciparum*. *Int. J. Parasitol*., 2002, **32**, 1683–1692.
105. Patterson, S., Whittle, C., Robert, C. and Chakrabarti, D., Molecular characterization and expression of an alternate proliferating cell nuclear antigen homologue, PfPCNA2 of *Plasmodium falciparum*. *Biochem. Biophys. Res. Commun*., 2002, **298**, 371–376.
106. Krishna, T. S., Kong, X. P., Gary, S., Burgers, P. M. and Kuriyan, J., Crystal structure of the eukaryotic DNA polymerase processivity factor PCNA. *Cell*, 1994, **79**, 1233–1243.
107. Gulbis, J. M., Kelman, Z., Hurwitz, J., O'Donnell, M. and Kuriyan, J., Structure of the C-terminal region of p21(WAF1/CIP1) complexed with human PCNA. *Cell*, 1996, **87**, 297–306.
108. Johnson, A. and O'Donnell, M., Cellular DNA replicases: components and dynamics at the replication fork. *Annu. Rev. Biochem*., 2005, **74**, 283–315.
109. Mossi, R. and Hubscher, U., Clamping down on clamps and clamp loaders – the eukaryotic replication factor. *Eur. J. Biochem*., 1998, **254**, 209–216.
110. Snider, J. and Houry, W. A., AAA+ proteins: diversity in function, similarity in structure. *Biochem. Soc. Trans*., 2008, **36**, 72–77.
111. Johnson, A., Yao, N. Y., Bowman, G. D., Kuriyan, J. and O'Donnell, M., The replication factor C clamp loader requires arginine finger sensors to drive DNA binding and proliferating cell nuclear antigen loading. *J. Biol. Chem.*, 2006, **281**, 35531–35543.
112. Jirage, D. *et al.*, The malarial CDK Pfmrk and its effector PfMAT1 phosphorylate DNA replication proteins and co-localize in the nucleus. *Mol. Biochem. Parasitol*., 2010, **172**, 9–18.
113. Tye, B. K., MCM proteins in DNA replication. *Annu. Rev. Biochem*., 1999, **68**, 649–686.
114. Brown, G. W. and Kelly, T. J., Purification of Hsk1, a minichromosome maintenance protein kinase from fission yeast. *J. Biol. Chem.*, 1998, **273**, 22083–22090.
115. Honey, S., Schneider, B. L., Schieltz, D. M., Yates, J. R. and Futcher, B., A novel multiple affinity purification tag and its use in identification of proteins associated with a cyclin-CDK complex. *Nucleic Acids Res*., 2001, **29**, 1–9.
116. Wold, M. S., Replication protein A: a heterotrimeric, single-stranded DNA-binding protein required for eukaryotic DNA metabolism. *Annu. Rev. Biochem*., 1997, **66**, 61–92.
117. Fairman, M. P. and Stillman, B., Cellular factors required for multiple stages of SV40 DNA replication *in vitro*. *EMBO J*., 1988, **7**, 1211–1218.
118. Wobbe, C. R., Weissbach, L., Borowiec, J. A., Dean, F. B., Murakami, Y., Bullock, P. and Hurwitz, J., Replication of simian virus 40 origin-containing DNA *in vitro* with purified proteins. *Proc. Natl. Acad. Sci. USA*, 1987, **84**, 1834–1838.
119. Wold, M. S. and Kelly, T., Purification and characterization of replication protein A, a cellular protein required for *in vitro* replication of simian virus 40 DNA. *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 2523–2527.
120. Kim, C., Snyder, R. O. and Wold, M. S., Binding properties of replication protein A from human and yeast cells. *Mol. Cell Biol.*, 1992, **12**, 3050–3059.
121. Wold, M. S., Weinberg, D. H., Virshup, D. M., Li, J. J. and Kelly, T. J., Identification of cellular proteins required for simian virus 40 DNA replication. *J. Biol. Chem.*, 1989, **264**, 2801–2809.
122. Zhu, G., Marchewka, M. J. and Keithly, J. S., *Cryptosporidium parvum* possesses a short-type replication protein A large subunit that differs from its host. *FEMS Microbiol. Lett*., 1999, **176**, 367–372.
123. Brown, G. W., Hines, J. C., Fisher, P. and Ray, D. S., Isolation of the genes encoding the 51-kilodalton and 28-kilodalton subunits of *Crithidia fasciculata* replication protein A. *Mol. Biochem. Parasitol.*, 1994, **63**, 135–142.

124. Voss, T. S., Mini, T., Jenoe, P. and Beck, H. P., *Plasmodium falciparum* possesses a cell cycle-regulated short type replication protein A large subunit encoded by an unusual transcript. *J. Biol. Chem*., 2002, **277**, 17493–17501.
125. Liu, L. F., DNA topoisomerase poisons as antitumor drugs. *Annu. Rev. Biochem.*, 1989, **58**, 351–375.
126. Schneider, E., Hsiang, Y. H. and Liu, L. F., DNA topoisomerases as anticancer drug targets. *Adv. Pharmacol*., 1991, **21**, 149–183.
127. Yang, L., Wold, M. S., Li, J. J., Kelly, T. J. and Liu, L. F., Roles of DNA topoisomerases in simian virus 40 DNA replication *in vitro*. *Proc. Natl. Acad. Sci. USA*, 1987, **84**(4), 950–954.
128. Brill, S. J., DiNardo, S., Voelkel-Meiman, K. and Sternglanz, R., DNA topoisomerase activity is required as a swivel for DNA replication and for ribosomal RNA transcription. *NCI Monogr*., 1987, **4**, 11–15.
129. Gilmour, D. S., Pflugfelder, G., Wang, J. C. and Lis, J. T., Topoisomerase I interacts with transcribed regions in *Drosophila* cells. *Cell*, 1986, **44**, 401–407.
130. Sullivan, D. M., Glisson, B. S., Hodges, P. K., Smallwood-Kentro, S. and Ross, W. E., Proliferation dependence of topoisomerase II mediated drug action. *Biochemistry*, 1986, **25**, 2248–2256.
131. Hwang, J., Shyy, S., Chen, A. T., Juan, C-C. and Whang-Peng, J., Studies of topoisomerase-specific antitumor drugs in human lymphocytes using rabbit antisera against recombinant human topoisomerase II polypeptide. *Cancer Res*., 1989, **49**, 958–962.
132. Hayama, R. and Marians, K. J., Physical and functional interaction between the condensing MukB and the decatenase topoisomerase IV in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA*, 2010, **107**, 18826–18831.
133. Obado, S. O., Bot, C., Echeverry, M. C., Bayona, J. C., Alvarez, V. E., Taylor, M. C. and Kelly, J. M., Centromere-associated topoisomerase activity in blood stream form *Trypanosoma brucei*. *Nucleic Acids Res*., 2011, **39**(3), 1023–1033.
134. Cheesman, S., McAleese, S., Goman, M., Johnson, D., Horrocks, P., Ridley, R. G. and Kilbey, B. J., The gene encoding topoisomerase II from *Plasmodium falciparum*. *Nucleic Acids Res*., 1994, **22**, 2547–2751.
135. Harrington, J. J. and Lieber, M. R., The characterization of a mammalian DNA structure-specific endonuclease. *EMBO J*., 1994, **13**, 1235–1246.
136. Harrington, J. J. and Lieber, M. R., DNA structural elements required for FEN-1 binding. *J. Biol. Chem*., 1995, **270**, 4503–4508.
137. Liu, Y., Kao, H. I. and Bambara, R. A., Flap endonuclease 1: a central component of DNA metabolism. *Annu. Rev. Biochem*., 2004, **73**, 589–615.
138. Liu, Y. and Bambara, R. A., Analysis of human flap endonuclease 1 mutants reveals a mechanism to prevent triplet repeat expansion. *J. Biol. Chem*., 2003, **278**, 13728–13739.
139. Matsuzaki, Y., Adachi, N. and Koyama, H., Vertebrate cells lacking FEN-1 endonuclease are viable but hypersensitive to methylating agents and H_2O_2. *Nucleic Acids Res*., 2002, **30**, 3273–3277.
140. Ruggiero, B. L. and Topal, M. D., Triplet repeat expansion generated by DNA slippage is suppressed by human flap endonuclease 1. *J. Biol. Chem*., 2004, **279**, 23088–23097.
141. Haltiwanger, B. M. *et al*., DNA base excision repair in human malaria parasites is predominantly by a long-patch pathway. *Biochemistry*, 2000, **39**, 763–772.
142. Casta, L. J., Buguliskis, J. S., Matsumoto, Y. and Taraschi, T. F., Expression and biochemical characterization of the *Plasmodium falciparum* DNA repair enzyme, flap endonuclease-1 (PfFEN-1). *Mol. Biochem. Parasitol*., 2008, **157**, 1–12.
143. Warbrick, E., PCNA binding through a conserved motif. *Bioessays*, 1998, **20**, 195–199.
144. Gary, R., Kim, K., Cornelius, H. L., Park, M. S. and Matsumoto, Y., Proliferating cell nuclear antigen facilitates excision in long-patch base excision repair. *J. Biol. Chem*., 1999, **274**, 4354–4363.
145. Sakurai, S. *et al*., Structural basis for recruitment of human flap endonuclease 1 to PCNA. *EMBO J*., 2005, **24**, 683–693.
146. Buguliskis, J. S., Casta, L. J., Butz, C. E., Matsumoto, Y. and Taraschi, T. F., Expression and biochemical characterization of *Plasmodium falciparum* DNA ligase I. *Mol. Biochem. Parasitol*., 2007, **155**, 128–137.
147. Martin, I. V. and MacNeill, S. A., ATP-dependent DNA ligases. *Genome Biol*., 2002, **3**, 1–7.
148. Timson, D. J., Singleton, M. R. and Wigley, D. B., DNA ligases in the repair and replication of DNA. *Mutat. Res*., 2000, **460**, 301–318.
149. Bentley, D. J. *et al*., DNA ligase I null mouse cells show normal DNA repair activity but altered DNA replication and reduced genome stability. *J. Cell Sci*., 2002, **115**, 1551–1561.
150. Subramanian, J., Vijayakumar, S., Tomkinson, A. E. and Arnheim, N., Genetic instability induced by overexpression of DNA ligase I in budding yeast. *Genetics*, 2005, **171**, 427–441.
151. Sun, D. *et al*., Elevated expression of DNA ligase I in human cancers. *Clin. Cancer Res*., 2001, **7**, 4143–4148.
152. Henneke, G., Koundrioukoff, S. and Hubscher, U., Multiple roles for kinases in DNA replication. *EMBO Rep*., 2003, **4**, 252–256.
153. Doerig, C., Endicott, J. and Chakrabarti, D., Cyclin-dependent kinase homologues of *Plasmodium falciparum*. *Int. J. Parasitol*., 2002, **32**, 1575–1585.
154. Halbert, J. *et al*., A *Plasmodium falciparum* transcriptional cyclin-dependent kinase-related kinase with a crucial role in parasite proliferation associates with histone deacetylase activity. *Eukaryot. Cell*, 2010, **9**, 952–959.
155. Ross-MacDonald, P. B., Graeser, R., Kappes, B., Franklin, R. and Williamson, D. H., Isolation and expression of a gene specifying a cdc2-like protein kinase from the human malaria parasite *Plasmodium falciparum*. *Eur. J. Biochem*., 1994, **220**, 693–701.
156. Li, J. L., Robson, K. J. H., Chen, J. L., Targett, G. A. T. and Baker, D. A., Pfmrk, a MO15-related protein kinase from *Plasmodium falciparum*: gene cloning, sequence, stage-specific expression and chromosome localization. *Eur. J. Biochem*., 1996, **241**, 805–813.
157. Graeser, R., Franklin, R. M. and Kappes, B., Mechanisms of activation of the cdc2-related kinase PfPK5 from *Plasmodium falciparum*. *Mol. Biochem. Parasitol*., 1996, **79**, 125–127.
158. Graeser, R., Wernli, B., Franklin, R. M. and Kappes, B., *Plasmodium falciparum* protein kinase 5 and the malarial nuclear division cycles. *Mol. Biochem. Parasitol*., 1996, **82**, 37–49.
159. Bracchi-Ricard, V., Barik, S., Delvecchio, C., Doerig, C., Chakrabarti, R. and Chakrabarti, D., PfPK6, a novel cyclin-dependent kinase/mitogen-activated protein kinase related protein kinase from *Plasmodium falciparum*. *Biochem. J*., 2000, **347**, 255–263.
160. Merckx, A. *et al*., Identification and initial characterization of three novel cyclin-related proteins of the human malaria parasite *Plasmodium falciparum*. *J. Biol. Chem*., 2003, **278**, 39839–39850.
161. Ullah, Z., Lee, C. Y., Lilly, M. A. and DePamphilis, M. L., Developmentally programmed endoreduplication in animals. *Cell Cycle*, 2009, **8**, 1501–1509.
162. Arnot, D. E., Ronander, E. and Bengtsson, D. C., The progression of the intra-erythrocytic cell cycle of *Plasmodium falciparum* and the role of the centriolar plaques in asynchronous mitotic division during schizogony. *Int. J. Parasitol*., 2011, **41**, 71–80.
163. Leete, T. H. and Rubin, H., Malaria and the cell cycle. *Parasitol. Today*, 1996, **12**, 442–444.
164. Gray, M. W., Lang, B. F. and Burger, G., Mitochondria of protists. *Annu. Rev. Genet*., 2004, **38**, 477–524.

165. Dahl, E. L. and Rosenthal, P. J., Apicoplast translation, transcription and genome replication: targets for antimalarial antibiotics. *Trends Parasitol*., 2008, **24**, 243–284.
166. Levine, N. D., Progress in taxonomy of the apicomplexan protozoa. *J. Protozool*., 1988, **35**, 518–520.
167. Hikosaka, K., Watanabe, Y., Kobayashi F., Waki, S., Kita, K. and Tanabe, K., Highly conserved gene arrangement of the mitochondrial genomes of 23 *Plasmodium* species. *Pasitol. Int*., 2011, **60**, 175–180.
168. Hikosaka, K. *et al*., Concatenated mitochondrial DNA of the coccidian parasite *Eimeria tenella*. *Mitochondrion*, 2011, **11**, 273–278.
169. Malesza, R., Skelly, P. J. and Clarke-Walker, G. D., Rolling circle replication of DNA in yeast mitochondria. *EMBO J*., 1991, **10**, 3923–3929.
170. Malesza, R. and Clarke-Walker, G. D., *In vitro* conformation of mitochondrial DNA in fungi and zoosporic moulds. *Curr. Genet*., 1991, **22**, 341–344.
171. Feagin, J. E., Mericle, B. L., Werner, E. and Morris, M., Identification of additional rRNA fragments encoded by the *Plasmodium falciparum* 6 kb element. *Nucleic Acids Res*., 1997, **25**, 438–446.
172. Waller, R. F. *et al*., Nuclear-encoded proteins target to the plastid in *Toxoplasma gondii* and *Plasmodium falciparum*. *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 12352–12357.
173. Surolia, N. and Surolia, A., Triclosan offers protection against blood stages of malaria by inhibiting enoyl-ACP reductase of *Plasmodium falciparum*. *Nature Med*., 2001, **7**, 167–173.
174. Jomaa, H. *et al*., Inhibitors of the nonmevalonate pathway of isoprenoid biosynthesis as antimalarial drugs. *Science*, 1999, **285**, 1573–1575.
175. Wilson, R. J. M. (Iain), Progress with parasite plastids. *J. Mol. Biol*., 2002, **319**, 257–274.
176. Dhanasekaran, S., Chandra, N. R., Sagar, K. C., Rangarajan, P. N. and Padmanaban, G., Aminolevulinic acid dehydrase from *Plasmodium falciparum* indigenous vs imported. *J. Biol. Chem*., 2004, **279**, 6934–6942.
177. Wilson, R. J. M. (Iain) *et al*., Complete gene map of the plastid-like DNA of the malaria parasite *Plasmodium falciparum*. *J. Mol. Biol*., 1996, **261**, 155–172.
178. Smeijsters, L. J. J. W. *et al*., The effect of (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl) adenine on nuclear and organellar DNA synthesis in erythrocytic schizogony in malaria. *Mol. Biochem. Parasitol*., 1994, **67**, 115–124.
179. Rojas, M. *et al*., Temporal relationships on macromolecular synthesis during the asexual cycle of *Plasmodium falciparum*. *Trans. R. Soc. Trop. Hyg*., 1985, **79**, 792–796.
180. Preiser, P. R. *et al*., Recombination associated with replication of malarial mitochondrial DNA. *EMBO J*., 1996, **15**, 684–693.
181. Williamson, D. H., Preiser, P. R. and Wilson, R. J. M. (Iain), Organelle DNAs: the bit players in malaria parasite DNA replication. *Parasitol. Today*, 1996, **12**, 357–362.
182. Matsuzaki, M., Kikuchi, T., Kojima, S. and Koroiwa, T., Large amounts of apicoplast nucleoid DNA and its segregation in *Toxoplasma gondii*. *Protoplasma*, 2001, **218**, 180–191.
183. Fichera, M. E. and Roos, D. S., A plastid organelle as a drug target in apicomplexan parasites. *Nature*, 1997, **390**, 407–409.
184. He, H. Y., Shaw, M. K., Pletcher, C. H., Striepen, B., Tilney, L. G. and Roos, D. S., A plastid segregation defect in the protozoan parasite *Toxoplasma gondii*. *EMBO J*., 2001, **20**, 330–339.
185. Stanway, R. R., Witt, T., Zobiak, B., Aepfelbacher, M. and Heussler, V. T., GFP-targeting allows visualization of the apicoplast throughout the life cycle of live malaria parasites. *Biol. Cell*, 2009, **101**, 415–430.
186. Brewer, B. J., Sena, E. P. and Fangman, W. L., Analysis of replication intermediates by two-dimensional agarose gel electrophoresis. *Cancer Cells*, 1988, **6**, 229–234.
187. Mosig, G., In *Baceriophage T4* (eds Mathew, C.), American Society for Microbiology, 1983, pp. 120–130.
188. Williamson, D. H., Preiser, P. R., Moore, P. W., McCready, S., Strath, M. and Wilson, R. J. M. (Iain), The plastid DNA of the malaria parasite *Plasmodium falciparum* is replicated by two mechanisms. *Mol. Microbiol*., 2002, **45**, 533–542.
189. Singh, D., Chaubey, S. and Habib, S., Replication of the *Plasmodium falciparum* apicoplast DNA initiates within the inverted repeat region. *Mol. Biochem. Parasitol*., 2003, **126**, 9–14.
190. Singh, D., Kumar, A., Raghu Ram, E. V. S. and Habib, S., Multiple replication origins within the inverted repeat region of the *Plasmodium falciparum* apicoplast genome are differently activated. *Mol. Biochem. Parasitol*., 2005, **139**(1), 99–106.
191. Kumar, A., Tanveer, A., Biswas, S., Ram, E. V. S. R., Gupta, A., Kumar, B. and Habib, S., Nuclear encoded DnaJ homologue of *Plasmodium falciparum* interacts with replication ori of the apicoplast genome. *Mol. Microbiol*., 2010, **75**, 942–956.
192. Ram, E. V. S. R., Kumar, A., Biswas, S., Kumar, A., Chaubey, S., Siddiqi, M. I. and Habib, S., Nuclear gyrB encodes a functional subunit of the *Plasmodium falciparum* gyrase that is involved in apicoplast DNA replication. *Mol. Biochem. Parasitol*., 2007, **154**, 30–39.
193. Dar, M. A. *et al*., Molecular cloning of apicoplast-targeted *Plasmodium falciparum* DNA gyrase genes: unique intrinsic ATPase activity and ATP-independent dimerization of PfGyrB subunit. *Eukaryot. Cell*, 2007, **6**, 398–412.
194. Prusty, D. *et al*., Single-stranded DNA binding protein from human malarial parasite *Plasmodium falciparum* is encoded in the nucleus and targeted to the apicoplast. *Nucleic Acids Res*., 2010, **38**, 7037–7053.
195. Seow, F. *et al*., The plastidic DNA replication enzyme complex of *Plasmodium falciparum*. *Mol. Biochem. Parasitol*., 2005, **141**, 145–153.
196. Kennedy, S. R. *et al*., The biochemistry and fidelity of synthesis by the apicoplast genome replication DNA polymerase Pfprex from the malaria parasite *Plasmodium falciparum*. *J. Mol. Biol*., 2011, **410**, 27–38.
197. Fu, S. L. and Xiao, S. H., Pyronaridine: A new antimalarial drug. *Parasitol. Today*, 1991, **7**, 310–313.
198. Chevalitskewinkoon, P., Wilairat, P., Gamage, S., Denny, W., Figgitt, D. and Ralph, R., Structure–activity relationships and modes of action of 9-anilinoacridines against chloroquine-resistant *Plasmodium falciparum in vitro*. *Antimicrob. Agents Chemother*., 1993, **37**, 403–406.

ACKNOWLEDGEMENTS. Research in our laboratory is supported by a Swarnajayanti Fellowship awarded to S.K.D. by Department of Science and Technology, New Delhi and European Commission (MALSIG). P.M. thanks the Indian Council of Medical Research, New Delhi; and A.S.D. thanks the Council of Scientific and Industrial Research, New Delhi for fellowships.