Plasmodium telomeres: a pathogen's perspective
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New data on the organization of plasmodial telomeres has recently become available. Telomeres form clusters of four to seven heterologous chromosome ends at the nuclear periphery in asexual and sexual parasite stages. This subnuclear compartment promotes gene conversion between members of subtelomeric virulence factor genes in heterologous chromosomes resulting in diversity of antigenic and adhesive phenotypes. This has important implications for parasite survival.

Introduction
Linear eukaryotic chromosomes present three problems that do not exist in circular genomes. First, chromosome extremities need to be protected from degradation. Second, they have to avoid end-to-end fusion by DNA repair mechanisms. Third, they have to compensate for the gradual loss of DNA. How do eukaryotic organisms solve these problems? The answer lies in a specialized structure, found at the physical termini of chromosomes, called the telomere. For the vast majority of eukaryotes, telomeres consist of G-rich repetitive DNA and specific associated proteins. A variety of other functions have been assigned to telomeres, such as transcriptional silencing [1], chromo-proteins. A variety of other functions have been assigned to telomeres, such as transcriptional silencing [1], chromosomal positioning in the nucleus [2] and homologous and telomere-like structure that prevents telomere elongation and reversibly represses transcription of subtelomeric genes [15,16*]. The formation of such telomere loops in P. falciparum could contribute to the epigenetic regulation of adjacent genes.

Most models for telomere length regulation take into account mechanisms that act in trans on each telomere via specific telomere-binding proteins (for a review, see [17*]). In a recent study, it was shown that P. falciparum chromosome ends that have lost the telomere-associated sequences (TASs) harbour longer telomeres (an increase in length of up to threefold), suggesting that telomere maintenance mechanisms in P. falciparum are sensitive to the genomic environment upstream of the telomere. This is the first evidence to suggest that telomere length regulation depends not only on trans-acting factors, but also on the genomic environment adjacent to the telomere (LM Figueiredo, LH Freitas-Junior, E Bottius, A Scherf, unpublished data).

Telomere repeat similarity has been shown in several malaria species by DNA cross-hybridization [18]. Additionally, internal chromosome regions appear to display a high degree of gene synteny in plasmodial species [19]. This is in striking contrast to the TASs, which are highly species-specific in their organisation. P. falciparum TASs consist of both a coding and a noncoding region (Figure 1a,b). The noncoding regions are adjacent to the telomere repeats and are composed of a mosaic of six different polymorphic repetitive elements called telomere-associated repetitive

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Abbreviations
FISH fluorescent in situ hybridization
t-loop telomere loop
TARE telomere-associated repetitive element
TAS telomere-associated sequence

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Plasmodium chromosome extremities: not just the end
The extremely AT-rich nuclear genome of P. falciparum (~80% AT) is organized into 14 linear chromosomes. Telomeric DNA has been cloned from several malaria parasites and found to consist of degenerate canonical G-rich tandem repeats, with GGGTT(T/C)A being the most frequent motif [5–7]. The mean length of the telomeric array shows significant interspecies variation (for example, 1.2 kb for P. falciparum and 6.7 kb for Plasmodium vivax), but is relatively conserved between P. falciparum strains (LM Figueiredo, LH Freitas-Junior, E Bottius, A Scherf, unpublished data).

The terminal tract of P. falciparum telomeric DNA is assembled into a non-nucleosomal chromatin structure, called the telosome [8], as has been described in Saccharomyces cerevisiae, human and Trypanosoma cruzi telomeres [9,10,11**]. Proteins of the telosome complex mediate specific properties associated with telomeres. For example, a telomere structure has been reported for humans, ciliates and African trypanosomes, and found to form a terminal telomere loop (t-loop) at the chromosome end [12**,13,14]. T-loops are thought to be formed by invasion of the 3’ telomeric overhang into the duplex telomeric repeat array. It has been proposed that this structure protects chromosomal termini from degradation and recognition as broken ends. On the other hand, the short telomere tracts of yeast (around 0.3 kb) seem to fold back and interact with an adjacent region via specific protein–protein interactions, establishing a heterochromatin-like structure that prevents telomere elongation and reversibly represses transcription of subtelomeric genes [15,16*].

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elements (TAREs 1–6), which are always found at the chromosome ends in the same order and span 20–40 kb [8]. TARE-6, which is also known as rep20, is responsible for most of the chromosome-end size polymorphism. However, large subtelomeric deletions, which occur relatively frequently in laboratory-cultured strains and have been observed in clinical isolates, generate viable truncated chromosomes with no TAREs [20]. Surprisingly, noncoding TASs are as AT-rich as coding regions (70% AT) (Figure 1c), instead of as rich as noncoding regions (>90% AT). The noncoding region of TAS may have evolved from coding regions, as has been proposed for isochores in vertebrates [21]. We cannot, however, discard the possibility that the maintenance of such a relatively G-rich noncoding region results from functional constraints, as is probably the case for telomere repeat sequences.

The species-specificity of the subtelomeric compartment is not limited to the noncoding region of the TAS. Systematic sequence analysis of several *P. falciparum* chromosomes has revealed that gene families coding for species-specific virulence factors involved in antigenic variation (*var* and *rifin* gene families) and cytoadhesion (*car* gene family) are found adjacent to the noncoding region [22,23]. Undoubtedly, a number of genes implicated in parasite–host-specific molecular interactions have evolved in this chromosome compartment.

Telomeres—are they the ‘Achilles heel’ of *Plasmodium*?
In single-celled organisms, telomere length needs to be maintained within a minimal size range to ensure the survival of the cell. However, because of the incomplete replication of linear chromosomes, there is a net loss of telomeric DNA with each successive cell division. To compensate for this loss, new telomeric repeats are added onto chromosome ends in a reaction catalysed by a specialized reverse transcriptase called telomerase. Telomerase is a holoenzyme, in which the catalytic core is composed of a protein, called TERT, and an RNA component. Telomerase RNA serves as the template for the addition of telomeric repeats. Interaction with other proteins may be important for the regulation of telomerase activity (for review, see [17•]).

In the absence of telomerase activity, telomeres shrink with each replication cycle and the erosion is so extensive (approximately 50 to 200 bp per replication in human somatic cells) that, with time, the telomere capping function becomes severely compromised, leading to growth arrest and, eventually, senescence [24]. The mean telomere length of *P. falciparum* is maintained at a constant average size during blood-stage proliferation, and telomerase activity has been detected in protein extracts of cells at this stage [25•]. Telomerase activity has also been identified in three kinetoplastid species: *Trypanosoma brucei*, *Leishmania major* and *Leishmania tarentolae* [26]. Drugs that interfere with telomerase maintenance may induce fatal shortening of telomeres, leading to the death of the parasite. Thus, telomeres can be considered as the ‘Achilles heel’ of highly proliferating protozoan pathogens. However, in the absence of telomerase, it is possible that parasites may make use of alternative mechanisms to maintain a functional telomere. Recombination-mediated telomere maintenance has been described in yeast [27–29••], and chromosome
Circulization has been observed in *S. pombe* [29]. A candidate for the *P. falciparum* telomerase catalytic subunit has been identified in the genome database. Knockout experiments are in process to verify that this is an essential gene for the survival of the parasite and that it can be considered as a new target for the development of antiparasite therapies (LM Figueiredo, A Scherf, unpublished data). Telomerase has also been considered an attractive target for cancer therapeutics, as more than 90% of tumors show telomerase activity. Inhibition of telomerase activity in human cancer cells arrests their growth in vitro and in vivo [24].

**The role of telomeres in the nuclear architecture**

Recent technological advances such as FISH (fluorescent in situ hybridization) have provided new insights into the three-dimensional chromatin organization of the nucleus, even in relatively small organisms such as yeast and *Plasmodium*. Telomeres have been shown to form clusters and to anchor chromosomes to the nuclear periphery, thus forming distinct nuclear compartments in yeast. These nuclear subdomains are thought to have an important role in epigenetic gene regulation, RNA recombination and telomeric silencing (reviewed in [30,31,2]). The telomere position effect (TPE) brings about a heritable yet variegated...
repression of genes placed near telomere repeats [1]. This effect has been associated with chromatin organization, as proteins essential for telomeric silencing are found to be concentrated at the telomeres and/or subtelomeric regions. In yeast, a number of telomere-associated proteins involved in telomere-length regulation, cluster formation, telomere anchoring to the periphery and gene silencing have been described and characterized [32]. P. falciparum orthologues to most yeast telomere-associated proteins have been identified (LH Freitas-Junior, A Scherf, unpublished data; for more details, see Figure 2c), implying that yeast can serve as a model for investigating the role of biologically relevant aspects in P. falciparum, such as the importance of virulence factor localization at chromosome ends.

FISH analysis using a chromosome-end-specific fluorescent probe recently demonstrated that the 28 chromosome ends of P. falciparum are not randomly distributed in the nucleus. The telomeres form physical associations demonstrated by four to seven spots at the nuclear periphery of asexual and sexual blood stage parasites, suggesting that each cluster contains between four and seven distinct chromosome ends (Figure 2a) [33]. The molecular components involved in cluster formation are as yet unknown. The extent of chromosome-end alignment is also unclear. However, several lines of evidence support the idea that it spreads beyond the telomere repeats by a distance of 30 to 40 kb, including the subtelomeric region that encodes variant-surface-antigen families. DNA sequences in this region undergo ectopic (non-allelic) recombination at a much higher rate than expected for homologous recombination [34]. We assume that the physical alignment of heterologous chromosome ends brings together homologous sequences from different chromosomes allowing efficient DNA recombination (Figure 2b). Also, it was recently shown that the P. falciparum subtelomeric region is crucial for the maintenance of chromosome termini in clusters (LM Figueiredo, LH Freitas-Junior, E Bottins, A Scherf, unpublished data). In parasites that have lost the subtelomeric domain because of spontaneous chromosome breakage, the large majority of chromosome ends are not associated with clusters. We speculate that specific TAS-binding proteins exist that crosslink chromosome termini and stabilize the cluster. Thus, deletion of chromosomal TAS would explain the observed dissociation of truncated chromosome termini from the cluster. We predict that chromosome ends not associated with clusters have a significantly lower frequency of ectopic recombination between subtelomeric gene families than do normal chromosome ends. This hypothesis is supported by recent reports showing that mutant fission yeast with defective telomere cluster formation have reduced meiotic recombination between chromosome termini [35], and ectopic recombination is no longer restricted to telomere-associated regions [36••].

The subtelomeric region and antigenic variation

What are the biological implications of the P. falciparum nuclear organization on genes located close to telomeres? It is now well established that plasmodial chromosome ends form a highly dynamic chromosome compartment and most of the chromosome polymorphism is due to DNA rearrangements occurring in the subtelomeric region. The high recombination frequencies observed in subtelomeric regions seem to create an environment that allows the expansion and diversification of gene families located at chromosome ends [37]. Thus, telomeres provide an ideal setting for genes involved in mechanisms such as antigenic variation. In P. falciparum, antigenic variation and cytoadhesion

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**Figure 3**

Model showing the subtelomeric organisation in several human pathogens. The gene families that express variant cell-surface molecules on T. brucei [39] and P. carinii [43], or on the surface of infected erythrocytes in P. falciparum [22,23] and P. vivax [7] are indicated. The P. vivax chromosome end model has been derived from the sequence analysis of a telomeric yeast artificial chromosome (YAC) clone. ESAG, expression-site-associated gene; MSG, major surface glycoprotein; VSG, variant surface glycoprotein.
are mediated by the var gene family, which consists of approximately 50 members per haploid genome. Most var genes are localized in the subtelomeric region of the chromosomes (Figure 1b) and expression of these genes occurs in a mutually exclusive manner. Programmed DNA rearrangement is not necessary to switch expression from one variant to another.

In a recent study, it was shown that chimeric var forms were created by gene conversion involving two var members located at different chromosome ends [33]. Thus, telomere clusters appear to provide a platform for ectopic recombination between virulence factors from different chromosomes. The continuous generation of variant-surface-antigen diversity is particularly important for P. falciparum [33,38] and for other pathogens whose survival in the vertebrate host depends on antigenic variation. This is demonstrated by the localization of variant-surface-molecule genes at subtelomeric chromosome regions in numerous human pathogens (Figure 3). P. falciparum contains a relatively small set of ~50 var genes, compared to other organisms that express variant surface antigens. African trypanosomes, for example contain around 1000 VSG genes [39] and P. vivax has between 600 and 1000 vir genes [7]. Nevertheless, P. falciparum populations harbor many var forms, whose diversity and continual renewal support the success of this species against host immunity. We speculate that parasite isolates carrying different var repertoires are more likely to initiate new infections than isolates that have previously infected the same host.

Telomeric location of variant gene families may provide a special chromatin environment for reversible, mutually exclusive gene regulation. The mechanisms that control the regulation of var and rifin gene expression in P. falciparum are still unresolved, but many lines of evidence point to epigenetic factors being critically involved in the transcription of a single var gene family member at a time [40,41]. In analogy to the yeast system, we suggest that P. falciparum telomeres could mediate reversible silencing of var genes. The fact that P. falciparum telomeres closely resemble yeast telomere organization should facilitate the identification of the molecules that contribute to the control of epigenetic var gene transcription. However, further studies are needed to find a concept explaining how one subtelomeric var gene can be transcribed while the remaining members are repressed.

Conclusions

For many years, telomere biology was of interest to only a handful of specialists. Recently, the subject has gained a much broader interest, partly because of the fact that telomeres play a central role in cell senescence, and thus provide a new target for therapy development not only against human cancer but also against protozoan pathogens.

The recent discovery of telomere clustering at the nuclear periphery and its role in ectopic recombination suggests that P. falciparum subtelomeric regions define a specific subcompartment able to generate continual diversity in virulence factor genes. Other protozoan pathogens may follow the same blueprint. Another important feature of the telomere is its potential involvement in epigenetic gene regulation. The recent principles established for the telomere position effect in yeast [42–] may also be applicable to transcriptional control mechanisms of antigenic variation in Plasmodium, which remains, at the moment, enigmatic.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
•• of outstanding interest


This study demonstrates that telomeres form clusters containing four to seven heterologous chromosome ends at the nuclear periphery of P. falciparum asexual and sexual stages. This spatial organisation creates a highly recombinogenic compartment, in which the genes coding for adhesive proteins are located. Cluster formation is thought to be important for generating diversity in molecules responsible for tissue tropism and pathogenesis of malaria in the human host.


Electron microscopy has shown that telomeres in mammalian cells form large terminal loops. In these so-called t-loops, the 3′ G strand extension is tucked into the double-stranded telomeric tract, thus protecting the chromosome terminus. This process appears to be mediated by telomeric-repeat binding factor 2 (TRF2). This telomere structure provides a totally new perspective on the understanding of telomere function.
In this study, telomerase activity was identified in cell extracts of telomeric repeat amplification protocol (TRAP) assay. The authors show that telomeric repeats are added not parum, together with chromatin immunoprecipitation experiments, strongly agrees with previous suggestions that yeast telomeres form back-folding loops. The formation of these structures is dependent on the activity of Sir2 and Sir3 proteins. The authors suggest that such folded structures may be a characteristic of the heterochromatin in general.

McEachem MJ, Krauskopf A, Blackburn EH: Telomeres and their control. Annu Rev Genet 2000, 34:331-358. This is a comprehensive review on the current knowledge of the telomere components and the telomerase functional complex. Terminal DNA replication and elongation, homologous recombination as an alternative method for telomere maintenance and non-homologous end-joining of telomeric DNA are focused. A summary of current knowledge on the regulation of these processes is also described.


In this study, telomerase activity was identified in cell extracts of P. falciparum for the first time, using a modified telomeric repeat amplification protocol (TRAP). The authors show that telomeric repeats are added not only to telomeric DNA oligonucleotides, but also to primers based on natural P. falciparum chromosome breakpoints. This suggests that telomerase contributes to chromosome maintenance and de novo telomere formation on spontaneously broken chromosomes. It is also shown that telomidal telomerase can be efficiently inhibited in vitro by reverse transcriptase inhibitors.