Molecular & Biochemical Parasitology 187 (2013) 121–128

Contents lists available at SciVerse ScienceDirect

Molecular & Biochemical Parasitology

Review

Impact of chromosome ends on the biology and virulence of *Plasmodium falciparum*

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ARTICLE INFO

Article history:
Received 12 May 2012
Received in revised form 15 January 2013
Accepted 17 January 2013
Available online 24 January 2013

Keywords:
Telomere
TAREs
Telosome complex
Heterochromatin
lncRNA
Nuclear architecture

ABSTRACT

In recent years, many studies have focused on heterochromatin located at chromosome ends, which plays an important role in regulating gene expression in many organisms ranging from yeast to humans. Similarly, in the protozoan *Plasmodium falciparum*, which is the most virulent human malaria parasite, the heterochromatin present in telomeres and subtelomeric regions exerts a silencing effect on the virulence gene families located therein. Studies addressing *P. falciparum* chromosome ends have demonstrated that these regions participate in other functions, such as the formation of the T-loop structure, the replication of telomeric regions, the regulation of telomere length and the formation of telomeric heterochromatin. In addition, telomeres are involved in anchoring chromosome ends to the nuclear periphery, thereby playing an important role in nuclear architecture and gene expression regulation. Here, we review the current understanding of chromosome ends, the proteins that bind to these regions and their impact on the biology and virulence of *P. falciparum*.

Contents

1. Introduction

Despite more than a century of efforts to control or eradicate malaria, this disease remains a growing threat to public health [1]. One of the factors that have contributed to the spread of malaria is the generation of antigenic diversity that allows malaria parasites to evade host immune responses, rendering it impossible to develop effective long-term vaccines against them. This antigenic diversity reflects the frequent duplication events that occur within the ends of the linear chromosomes harboring the parasite genome, where most of the genes implicated in cytoadherence and antigenic variation are located. These duplication events have facilitated the generation of large families of genes, which often encode variable surface proteins [2,3]. In addition,

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http://dx.doi.org/10.1016/j.molbiopara.2013.01.003

recombination events that occur in these regions also promote genotypic diversity because *P. falciparum's* telomeres are anchored to the nuclear periphery forming cluster-type structures of three to seven heterologous chromosome ends [4,43]. This compartment might facilitate ectopic recombination events in genes, such as *var*, *rif* and *stevor* (adjacent to telomere-associated repeat elements, TAREs), which confer enhanced genotypic and phenotypic diversity on antigenic determinants and cytoadherence proteins to promote chronic parasite infection in the host [43]. Moreover, telomeres and subtelomeric regions also participate in the formation and extension of telomeric heterochromatin, which regulates the expression of genes that encode virulence factors through a mechanism similar to the telomere position effect (TPE) [4,5]. These data, along with the recent discovery that the telomeric and subtelomeric regions in parasite genomes are both transcribed [6–8], justify a revision of the current concept that chromosome ends are purely structural regions. Accordingly, we describe some of the functions recently attributed to chromosome ends and their associated proteins in the biology and virulence of *P. falciparum*.

2. Chromosomal structure of *P. falciparum*

Sequencing of the haploid genome of *P. falciparum* revealed that the 23 Mb genome is organized into 14 linear chromosomes varying in size from 0.7 (chromosome 1) to 3.4 (chromosome 14) Mb [9]. Analyses of the physical and genetic maps of the parasite chromosomes demonstrated that they are compartmentalized into highly conserved central regions containing "housekeeping" genes and highly polymorphic terminal regions that harbor genes encoding immunodominant antigens [2,10]. The terminal regions contain simple G-rich telomeric tandem repeats, followed by subtelomeric regions composed of telomere-associated sequences (TASs) [11]. The TASs are species-specific and include noncoding and coding regions. The noncoding region is composed of six different blocks of repetitive sequences located between the telomere and coding regions. These elements are referred to as telomere-associated repetitive elements (TAREs 1-6) and span 20–40 kb. The six elements are positioned in the same orientation and relative order at all *P. falciparum* chromosome ends, although the size and DNA sequence of each TARE is polymorphic [11,12]. Coding regions flank most TAREs, forming part of the TAS. These coding regions contain members of multigene families (*e.g.*, *var*, *rifin* and *stevor*) that encode the proteins involved in antigenic variation and cytoadherence [11–13].

3. *P. falciparum* telomeres

Similar to all organisms with linear chromosomes, *P. falciparum* exhibits chromosomes that are capped with specialized nucleoprotein complexes known as telomeres. In model organisms, telomeres have been shown to impede chromosomal fusion (end-to-end joining) and degradation. Therefore, the absence of telomeres in these organisms results in genetic instability and loss of cellular viability [14,15].

Telomeric DNA is arranged in short tandem sequence repeats containing groups of three or four guanines (*e.g.*, TTGGGG in *Tetrahymena* [16] and TTAGGG in humans [17]). *P. falciparum* telomeres are composed of tandem GGGTT(T/C)A repeats, which are highly conserved among distinct *Plasmodium* species. However, the average length of telomeres varies among the different parasite species, ranging from 1.2 kb in *P. falciparum* to 6.7 kb in *P. vivax*; thus, telomere length is considered to be species specific [18].

Studies using micrococcal nuclease (MNase) have revealed that each of the *P. falciparum* telomeres is organized in its most proximal region into three or four consecutive nucleosomes (nucleosomal organization). However, the most distal telomeric region is not associated with nucleosomes, constituting to a non-nucleosomal structure known as the telosome [11].

Several studies conducted in yeast have associated the telosome with numerous proteins constituting the telosome complex. In mammals, the telosome has been associated with shelterin, which is a complex containing six telomere-specific proteins: Telomeric repeat-binding factors 1 and 2 (TRF1 and TRF2), Protection of telomeres 1 (POT1), TRF1-Interacting nuclear protein 2 (TIN2), the human ortholog of the yeast Repressor/Activator Protein 1 (Rap1) and TPP1 (formerly referred to as PTOP/PIP1/TINT1) [19]. These associated proteins participate in many of the functions formerly attributed to telomeres, such as formation of the T-loop structure, replication of telomeric regions, regulation of telomere length, formation of telomeric heterochromatin and anchoring of telomeres to the nuclear periphery [20].

In *P. falciparum*, some of the above-mentioned functions have been attributed to proteins that constitute the telosome complex. However, our knowledge of these proteins is limited [21]. Therefore, we will discuss the manner in which the telosome complex, the telomere and the subtelomeric regions participate in these functions.

3.1. The *P. falciparum* telosome complex

In 2001, Scherf et al. performed an *in silico* analysis of the *P. falciparum* database (PlasmoDB) and identified several orthologs of proteins that contribute to the telosome complex in *S. cerevisiae*. These identified proteins include the histone deacetylase ySir2A (PfSir2A), yeast telomerase (PfTel), a protein similar to myosin 1 (PfMlp1), yRap1 (PfRap1), yRif1 (PfRif1) and Taz1 (PfTaz1), which is a protein found in *Schizosaccharomyces pombe* [21]. Subsequently, Mancio-Silva and colleagues performed gel-shift assays using a DNA probe containing 25 *P. falciparum* telomeric repeat sequences (simulating a telosome) with nuclear parasite extracts and demonstrated that telomeres specifically bind nuclear proteins [22]. These data suggest that *P. falciparum* possesses a telosome complex, and these proteins might participate in the functions attributed to telomeres. However, despite the *in silico* identification of orthologs of various yeast telosome proteins in *P. falciparum*, experimental verification has only been provided for telomerase (PfTERT) [23], the histone deacetylase Sir2 (PfSir2A) [4] and the origin recognition complex subunit 1 protein (PfOrc1) [22]. Additionally, a member of the Alba protein family (PfAlba3) was demonstrated *via* ChIP assays to bind to telomeric and subtelomeric regions [24]. These findings indicated that the proteins that constitute the *P. falciparum* telosome complex might differ from those of the yeast and/or human telosome complex.

Although the proteins constituting the telosome complexes of different organisms might perform the same functions, these proteins differ in every organism [25]. For example, Rap1 and Taz1 are found in *S. cerevisiae* and *S. pombe*, respectively, where they perform the same functions. However, these two proteins exhibit little sequence similarity, although both proteins recognize telomeric repeats in a sequence-specific manner [25]. This observation suggests the utility of identifying and characterizing the proteins comprising the *P. falciparum* telosomic complex. If these proteins are distinct from those of other species, they could represent promising novel therapeutic drug targets.

3.2. Participation of telomeric and subtelomeric regions in the formation of the T-loop structure

Structurally, telomeric DNA is composed of a region of double-stranded DNA, together with a single-stranded 3′ end. Therefore, the strands are asymmetric with respect to their composition and