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Transcriptional and nucleosomal characterization of a subtelomeric gene cluster flanking a site of chromosomal rearrangements in *Plasmodium falciparum*

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ABSTRACT

We have recently demonstrated that *Plasmodium falciparum* chromosomes are compartmentalized into different domains: conserved and polymorphic domains; transcriptionally active and silent domains. Here we have analyzed the transition between these domains at the structural and nucleosomal level. This study was conducted with the end of chromosome 2 that is associated with cytoadherence. At this end of the chromosome, the first set of erythrocytic genes has been mapped 85 kb from the telomere. These genes are monocistronically transcribed as revealed by nuclear run-on analysis. Two of these genes, *PfEMP3* and *KAHRP*, are deleted in cytoadherent negative mutants, whereas the third gene, *GLARP*, is expressed in all the strains investigated. The data indicate that the polymorphic domain at this end of chromosome 2 extends into the transcribed region. Analysis of the chromatin structure revealed that both the transcribed domain and the subtelomeric region are organized as nucleosomes with a periodicity of 155 ± 5 bp.

INTRODUCTION

Malaria is an important infectious disease that effects nearly half the world's human population. Malaria-related deaths are especially high among children, making malaria a major cause of death among infants in the tropics.

The most virulent form of human malaria is caused by the protozoan parasite *Plasmodium falciparum*. This parasite is transmitted to humans by the bite of an infected *Anopheles* mosquito. Within the human host, *P.falciparum* is haploid and multiplies asexually first within hepatocytes then within erythrocytes. The life cycle is completed when a mosquito ingests the sexual stages of the parasite during a blood meal.

The virulence of *P.falciparum*, is associated with the cytoadherence of parasitized erythrocytes to capillaries, which may result in organ damage and cerebral malaria (1–3). This phenomenon has been linked to electron dense protrusions on the surface of the erythroytic membrane (4,5). These structures are called knobs. Electron microscopy has demonstrated that these knobs establish a close contact with endothelial cells and probably are the sites for the parasite receptor for cytoadherence (4). A structural component of the knobs is the parasite encoded knob-associated histidine-rich protein, *KAHRP* (6,7).

The *KAHRP* gene is located at one end of the *P.falciparum* chromosome 2 (8). Terminal truncations of this end of chromosome 2 can extend into the *KAHRP* gene, resulting in *KAHRP*$^-$ parasite mutants (8,9). The *KAHRP*$^-$ mutants are knobless and exhibit reduced cytoadherence (1,3,10). Knobless mutants are cleared by the host's spleen from circulation (10). While the *KAHRP* gene product is an essential component for efficient cytoadherence, other parasite encoded proteins are also implicated in this process (11).

Since chromosome 2 contains an important *P.falciparum* virulence factor, it has been the subject of intense study. Cloning of the entire chromosome as an array of yeast artificial chromosomes (YAC) has demonstrated that the parasite's chromosomes are compartmentalized into different domains (12). The central domains contain unique sequences that are transcriptionally active, whereas the subtelomeric domains contain repetitive sequence elements that are silent (12,13). It has further been observed that the polymorphism associated with *P.falciparum* chromosomes are confined to chromosome ends (12). Interestingly, polymorphic domains are not only restricted to subtelomeric repeat sequences but extend into transcribed regions such as observed for chromosome 2 truncations that abrogate *KAHRP* gene expression (8).

To better define the transition between the different chromosomal domains, we have determined the transcriptional environment surrounding the *KAHRP* gene in both wild-type and mutant parasites. The knobless mutants also enable us to examine the effect of chromosomal truncations on gene expression.

Transcription mapping has indicated that the *KAHRP* gene is flanked by two erythrocytic genes, *PfEMP3* and *GLARP*. This cluster of genes is the most telomere proximal set of erythrocytic transcription units at this end of chromosome 2. In the knobless

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mutants, the *PfEMP3* gene is entirely deleted, while the *KAHRP* gene is truncated. This chromosomal rearrangement leaves exon 1 and the upstream region of the *KAHRP* gene intact but abrogates transcriptional activity (6,8). Transcriptional activity of the telomere distal gene, *GLARP*, is not affected by these chromosomal truncations. These data indicate that the transcribed domain of chromosome 2 extends into the polymorphic chromosome end.

Since the compartmentalization of *P.falciparum* chromosomes could also extend to the chromatin structure, the nucleosomal organization along chromosome 2 was determined. It has been found that the domains of chromosome 2 investigated are organized as nucleosomes with a phasing of 155 ± 5 bp.

MATERIALS AND METHODS

Cultivation of parasites and preparation of DNA

The *P.falciparum* strains FCR3 (knobby) and FVO$^-$ (knobless) were grown and maintained as described (14). Different intra-erythrocytic stages were separated by percoll/sorbitol gradient centrifugation (15). *P.falciparum* genomic DNA was prepared and analyzed as previously described (16). Conditions for the separation of intact *P.falciparum* chromosomes by pulse-field gel electrophoresis have been described (12,16).

Analysis of YAC clones and transcription mapping

The cloning and analysis of *P.falciparum* YAC clones has been described (16). The identification of transcription units on YAC clones using complex cDNA probes has been described (12,17).

Cloning of transcribed regions

The artificial chromosome, EC3, containing the *KAHRP* locus, was purified by pulse-field gel electrophoresis (16). The agarose plug containing the isolated EC3 YAC DNA was subjected to digestion with the restriction endonuclease *Dra*I. The digested fragments were purified by phenol–chloroform extraction followed by ethanol precipitation. This material was then cloned into a *Hin*cII linearized pUC18 plasmid. Colonies were then screened with radio-labelled, complex cDNA probes derived from total mRNA from the *P.falciparum* strain FCR3.

Northern analysis

Total cellular RNA was isolated by the acidic guanidinium–phenol chloroform method (18). 1.5 μg of total cellular RNA was fractionated on a 0.8% agarose–formaldehyde gel, transferred to nitrocellulose and hybridized with nick-translated probes (17).

Preparation of nuclei

P.falciparum nuclei were prepared as follows. All steps were carried out on ice. The content of twenty 10 cm petri dishes was collected at a parasitemia of 10% and washed once in ice cold Trager's buffer. Erythrocytes were lysed by the addition of an equal volume of 0.1% saponin, followed by three washes in E buffer (250 mM sucrose, 20 mM PIPES pH 6.3, 0.5 mM $CaCl_2$, 1 mM PMSF, 2 μg/ml aprotinin, 2 μg/ml leupeptin, 1 μg/ml pepstatin A, 2 μg/ml antipain, 0.1 mM benzamidine, 0.1 mM sodium metabisulfite). The free parasites were then resuspended in 3 ml of E buffer and subjected to N_2 cavitation at 1050 psi for 30 min (Parr Instruments). Nuclei were collected (10000 rpm, 10 min, Sorvall SM24 rotor), washed three times in E buffer and resupended in S buffer (1.0 M sorbitol, 20 mM Tris–HCl pH 7.4, 0.5 mM $CaCl_2$, protease inhibitors as indicated above) for storage at −80°C.

Nuclear run-on analysis

Nascent, radio-labelled RNA was generated from 5×10^9 nuclei as described (17).

Micrococcal nuclease digestion

$(0.5-1)\times10^9$ nuclei were subjected to limited digestion with 3 units/ml of micrococcal nuclease (purchased from Pharmacia) as described (19). At the time points indicated aliquots were withdrawn. The reaction was terminated by the addition of stop buffer to a final concentration of 1% SDS, 0.8 M NaCl. The DNA was purified by phenol–chloroform extraction and ethanol precipitated prior to Southern analysis. The size of the DNA fragments used for Southern analysis are: STS 19, 980 bp; KAHRP, 1200 bp; R-CG7, 103 bp; Rep 20, 180 bp.

Nucleotide sequences

A genomic sequence of the *GLARP* gene has been submitted to the EMBL data library.

RESULTS

Identification of a subtelomeric gene cluster

A *P.falciparum* YAC library, constructed from the parasite strain FCR3, was screened for YAC clones containing the *KAHRP* gene, as described (16). Two YAC clones, designated EC3 and GG3, were selected for further analysis. As the *KAHRP* gene has been mapped near the end of chromosome 2, an additional YAC clone, GC6, which overlaps EC3 and extends to the telomere, has been isolated. The YAC clones were mapped with several restriction endonucleases in comparison to genomic FCR3 DNA (Fig. 1). This analysis revealed that the YAC clones were stable and unrearranged.

In order to identify transcription units more telomere proximal than the *KAHRP* gene, the three YAC clones were digested with the restriction endonuclease *Eco*RI, transferred to nitrocellulose and hybridized with a complex cDNA probe derived from total poly A$^+$ RNA. The poly A$^+$ RNA was prepared from asynchronous erythrocytic parasite cultures from both a *KAHRP*$^+$ wild-type and a *KAHRP*$^-$ mutant. In addition to the *KAHRP* gene, two novel transcription units were identified in the wild-type (Fig. 1A). No transcripts were observed on the telomere YAC clone GC6. Thus, the transcription units identified represent the most telomere proximal erythrocytic gene cluster, with the first gene mapping 85 kb from the telomere.

The two novel transcripts labelled *GLARP* and *PfEMP1* in Figure 1A were cloned by subcloning EC3 YAC DNA into the *E.coli* pUC18 vector. Clones containing transcribed DNA fragments were identified by hybridization with radio-labelled, complex cDNA probes derived from total *P.falciparum* FCR3 mRNA.

The most telomere proximal gene was found to be identical to the previously described *PfEMP3* gene (data not shown) (20). The telomere distal gene represents a novel *P.falciparum* erythrocytic gene, which is designated *GLARP* (glutamine–lysine–arginine-rich protein). Screening of protein data bases with the *GLARP* sequence has revealed no homologies to other known proteins. Thus, the biological function of this gene remains obscure.

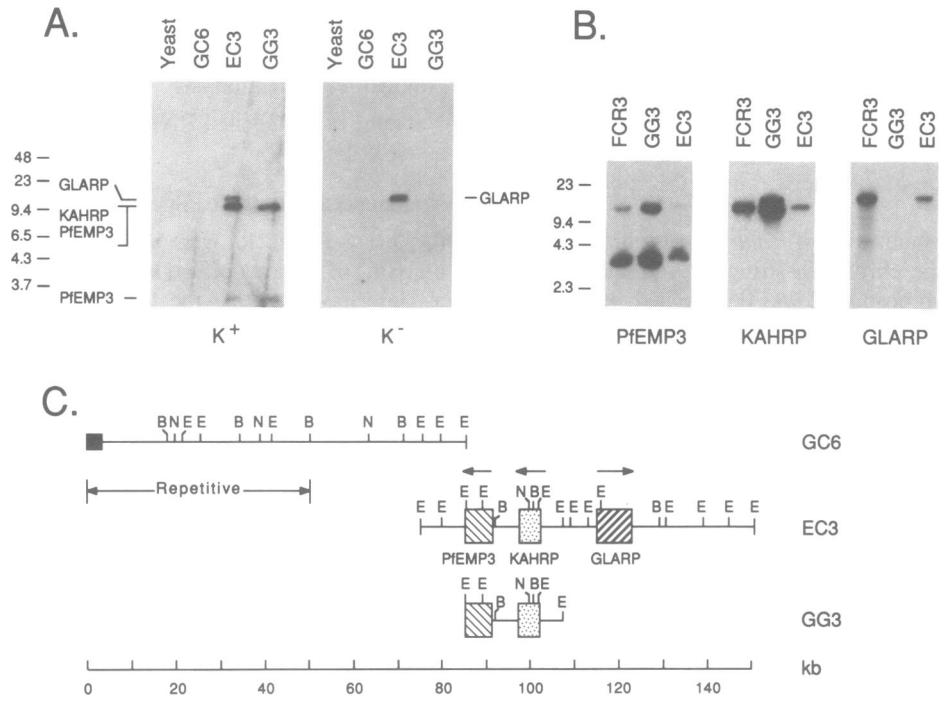

Figure 1. Identification of a subtelomeric gene cluster of chromosome 2. (**A**) DNA from the YAC clones GC6, EC3, GG3 was digested with the restriction endonuclease *Eco*RI, size-fractionated by pulse-field gel electrophoreses and transferred to nitrocellulose. The filter was then hybridized with complex cDNA probes that were obtained from total poly A^+ RNA from asynchronous, erythrocytic cultures of the knobby *P.falciparum* strain FCR3 (K^+) or the knobless isolate FVO^- (K^-). The hybridization signals corresponding to the genes *GLARP*, *KAHRP* and *PfEMP3* are indicated. A DNA size standard is indicated in kilo base pairs. (**B**) DNA from the *P.falciparum* strain FCR3 and the YAC clones GG3 and EC3 was digested with the restriction endonuclease *Eco*RI, size fractionated by pulse-field gel electrophoreses and transferred to nitrocellulose. The filters were hybridized with probes to the genes *PfEMP3*, *KAHRP* and *GLARP*. (**C**) Schematic summary. The YAC clones GC6, EC3 and GG3 are indicated. The positions of the genes *PfEMP3*, *KAHRP*, and *GLARP* are indicated by rectangles with different shadings. The transcriptional orientation of these genes is indicated by arrows. A filled square at the left end of YAC clone GC6 symbolizes telomere repeat sequences. Cleavage sites of the following restriction endonucleases are indicated: B, *Bam*HI; N, *Nco*I; E, *Eco*RI.

The transcription map was confirmed by Southern analysis of the YAC and FCR3 genomic DNAs, using probes derived from the genes *PfEMP3*, *KAHRP* and *GLARP* (Fig. 1B). We also established the orientation of these genes in relation to the telomere. Both *PfEMP3* and *KAHRP* are transcribed towards the telomere, while the *GLARP* gene has the opposite orientation (Fig. 1C).

Transcription mapping using poly A^+ RNA derived probes from the *KAHRP*$^-$ mutant FVO^- revealed only the *GLARP* gene (Fig. 1A). In the *KAHRP*$^-$ mutant FVO^-, chromosome breakage and healing events have deleted the *PfEMP3* gene and truncated the *KAHRP* gene as demonstrated by Southern analysis (Fig. 2) (8,9). In addition to the mutant FVO^-, several other *P.falciparum* strains investigated revealed chromosome 2 truncations since these strains also failed to hybridize to *KAHRP* and *PfEMP3* derived probes (Fig. 2). The *GLARP* gene, however, is present in all the strains investigated (Fig. 2). This finding was also confirmed by a more expanded study, examining 22 geographically different parasite isolates (data not shown). Thus, the *GLARP* gene seems to essential for parasite growth and development.

The chromosomal structure of the various parasite isolates correlates with the expression of these three genes. Northern analysis revealed that the *GLARP* mRNA of 5 kb is expressed in all the strains investigated, whereas the *PfEMP3* and the *KAHRP* genes are transcribed only in wild-type parasites (Fig. 3A). Stage-specific Northern analysis performed with RNA from

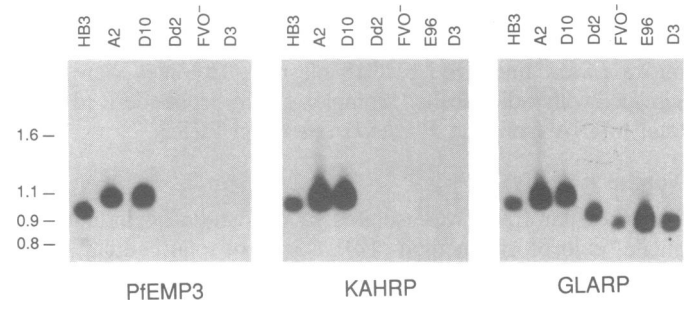

Figure 2. Subtelomeric gene cluster is subject to chromosomal truncations. Intact chromosome 2 DNA from different *P.falciparum* isolates was tested by Southern analysis for the presence or absence of the genes *PfEMP3*, *KAHRP*, *GLARP*. The geographic origin of the *P.falciparum* isolates are: HB3, Honduras; A2, D3, The Gambia; D10, Papua New Guinea; Dd2, Indochina; FVO^-, Vietnam.

the wild-type parasite line FCR3 indicated that the *GLARP* gene is present only in the ring stage (Fig. 3B). The *PfEMP3* transcript of 10 kb is detectable during both the ring and trophozoite stages (Fig. 3B). The *KAHRP* transcript of 4.2 kb accumulates during the ring stage as previously described (21).

To determine whether chromosomal polymorphism at this side of chromosome 2 has an effect on the developmental expression of the *GLARP* gene, stage-specific Northern analyses were conducted using RNA from the knobless parasite clones Dd2 and

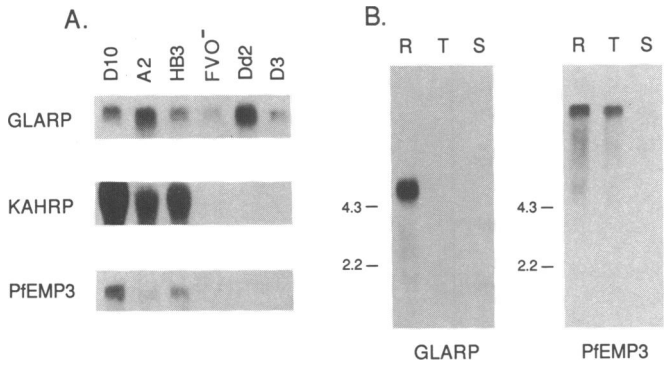

Figure 3. Developmental expression of the chromosome 2 gene cluster. (**A**) Total RNA from asynchronous erythrocytic cultures of the *P.falciparum* strains indicated was size fractionated by gel electrophoresis and transferred to nitrocellulose. The Northern filters were hybridized with probes to the genes *GLARP*, *KAHRP*, and *PfEMP3*. The sizes of the transcripts are: *GLARP*, 5.0 kb; *KAHRP*, 4.2 kb; *PfEMP1*, 10 kb. (**B**) Total erythrocytic RNA was prepared from the ring (R), trophozoite (T) and schizont (S) stages of the *P.falciparum* isolate FCR3. The Northern filters were hybridized with probes to the *GLARP* and *PfEMP3* transcripts. A RNA size standard is indicated in kilo bases.

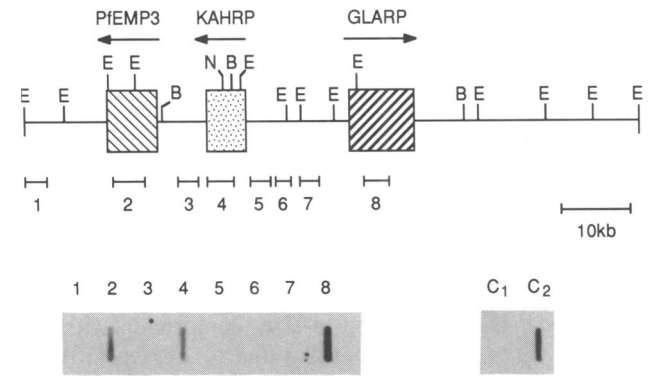

Figure 4. Transcriptional activity of a chromosome 2 gene cluster. DNA fragments spanning the entire gene cluster were immobilized on nitrocellulose and hybridized with nascent radio-labelled RNA. The nuclei used in this study were prepared from an asynchronously growing erythrocytic culture of FCR3. The position of the DNA fragments used are indicated. The position and orientation of the three genes investigated are indicated (E, *Eco*RI; B, *Bam*HI; N, *Nco*I). As a control, a non-plasmodial A/T rich fragment (C1) and the *P.falciparum P195* gene were analyzed in parallel.

FVO⁻ (data not shown). No changes in the developmental expression of the *GLARP* mRNA were observed in comparison to the wild-type (data not shown). In both knobby and knobless parasites, the *GLARP* gene is expressed during the ring stage. Thus, a relation between chromosomal polymorphism and *GLARP* gene expression was not found.

Monocistronic transcription of erythrocytic genes

To determine whether the genes of this cluster are transcribed in a monocistronic or polycistronic fashion, a nuclear run-on analysis was conducted. Probes spanning the entire 50 kb gene cluster were immobilized on nitrocellulose and hybridized with nascent, radio-labelled RNA derived from purified ring stage nuclei. Ring stage nuclei were prepared from a synchronized FCR 3 parasite culture. Hybridization of nascent RNA was observed

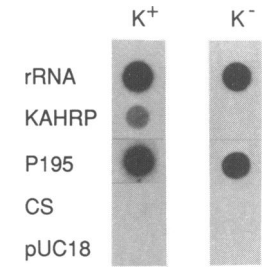

Figure 5. Strain-dependent transcriptional activity of the *KAHRP* gene. The transcriptional activity of the *KAHRP* gene was examined by nuclear run-on analysis using nuclei from the knobby parasite strain FCR 3 (K^+) and the knobless strain FVO⁻ (K^-). The *KAHRP* specific cDNA fragment, LP 20, used in the study is present in both strains (8). The transcriptional activity of the ribosomal RNA genes (rRNA), the major merozoite surface antigen (P195), the circumsporozoite antigen (CS) were analyzed in parallel. pUC18 plasmid DNA served as a negative control in this study.

only to probes containing the coding regions of the genes investigated (Fig. 4). No hybridization was detected to probes derived from intergenic regions. This indicates that the genes *PfEMP3*, *KAHRP*, and *GLARP* are monocistronically transcribed.

A similar nuclear run-on analysis was performed with nascent, radio-labelled mRNA prepared from FVO⁻ ring-stage nuclei (Fig. 5). The *KAHRP*⁻ mutant, FVO⁻, contains a truncation within 200 bp of exon 2 of the *KAHRP* gene, leaving exon 1 and the upstream region intact (8,9). The *KAHRP* probe used in the nuclear run-on analysis is derived from a region that abuts the site of truncation and extends 1 kb upstream. No hybridization of FVO⁻ derived nascent RNA was seen to this *KAHRP* probe, in contrast to a control nuclear run-on analysis using nascent RNA from the wild-type clone FCR3. These data indicate that the chromosomal rearrangement abrogates transcriptional activity of the truncated *KAHRP* gene during the ring stage.

Subtelomeric domains are organized as nucleosomes

The structural compartmentalization of *P.falciparum* chromosomes into various domains suggests that differences between these domains could extend to the chromatin structure. To examine this possibility, we have analyzed the nucleosome structure of *P.falciparum* chromosomes. Purified parasite nuclei prepared from an asynchronously growing FCR3 culture were subjected to limited digestion with micrococcal nuclease. This endonuclease preferentially cleaves the linker regions between nucleosomes. The digested DNA was size-fractionated by gel electrophoresis and transferred to nitrocellulose. The nitrocellulose filters were then hybridized with a series of probes derived from various parts of chromosome 2 (Fig. 6).

Hybridization with either subtelomeric or internal probes, including probes derived from the erythrocytic gene cluster, revealed the characteristic phasing of nucleosomes (Fig. 6). The phasing is absent in a control experiment, in which purified genomic FCR3 DNA was subjected to digestion with micrococcal nuclease and analyzed by hybridization with a probe to the *KAHRP* gene (Fig. 6B). Thus, the phasing observed is indicative of the *P.falciparum* chromatin organization.

To determine the size of the *P.falciparum* nucleosome repeat unit, the autoradiogram for the probes R-CG7 and *KAHRP* were analyzed by densitometry. This study was conducted with the 2 min time points. The nucleosome numbers were then plotted

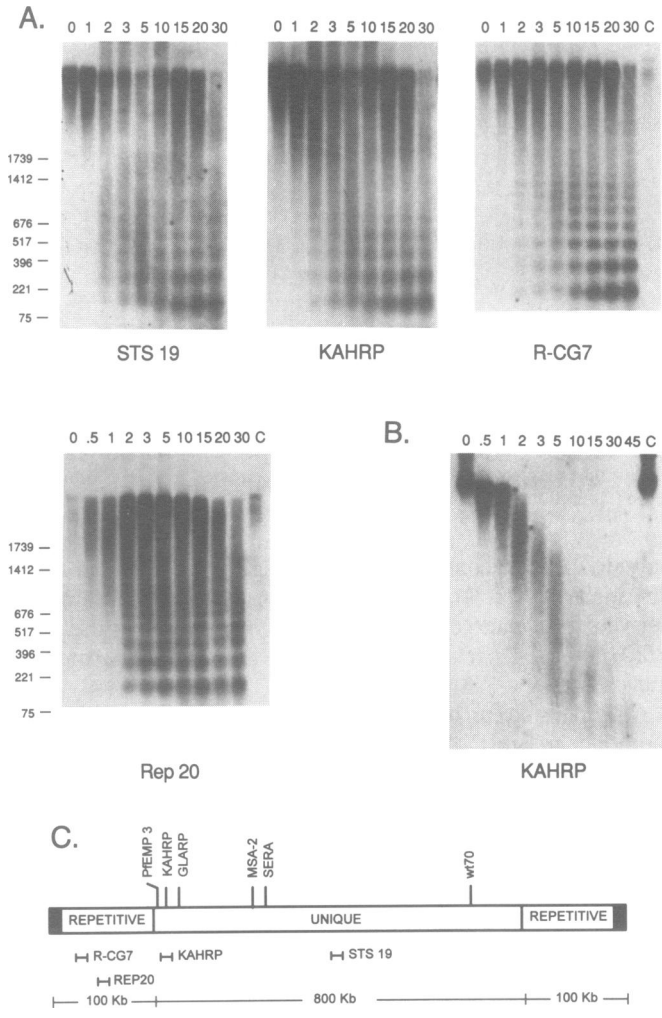

Figure 6. Chromatin organization of chromosome 2. (**A**) *P.falciparum* FCR3 nuclei were isolated and subjected to limited digestion with micrococcal nuclease. Aliquots were withdrawn at the time points indicated (min). As a control (C), nuclei were incubated for 30 min at 37°C without micrococcal nuclease. The DNA was extracted and examined by Southern analysis using probes to central (STS 19, *KAHRP*) and subtelomeric regions (REP 20, R-CG7) of chromosome 2. DNA size standards are indicated in base pairs. (**B**) Purified genomic DNA from the parasite strain FCR3 was subjected to limited digestion with micrococcal nuclease and analyzed by Southern hybridization using a probe to the KAHRP gene. (**C**) Structural organization of the *P.falciparum* Chromosome 2. Chromosome 2 is compartmentalized into a central domain that contains unique sequences, and subtelomeric ends that contain repetitive sequences (12,13). The position of probes used to examine the nucleosomal organization are indicated.

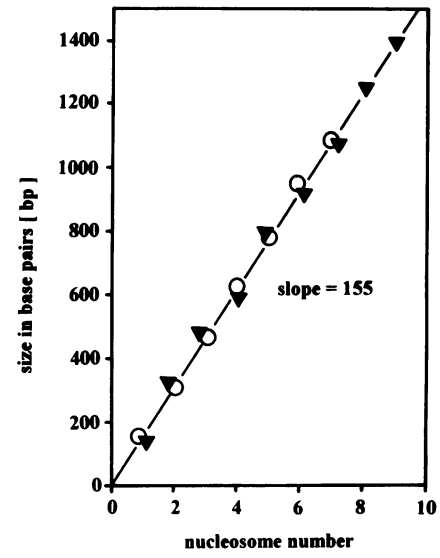

Figure 7. Determination of the *P.falciparum* nucleosomal repeat unit. The autoradiograms for the probes, R-CG7 and *KAHRP*, were quantified by densitometry. For each probe, the 2 min point was analyzed. R-CG7 is derived from the transcriptionally-silent subtelomeric region of chromosome 2, whereas *KAHRP* represents a transcribed domain. The nucleosome numbers were plotted versus the DNA fragment size (▼, R-CG7; ○, KAHRP). The slope of the linear regression is 155 ± 6 bp, which corresponds to the *P.falciparum* nucleosome repeat unit.

versus the DNA fragment size (Fig. 7). A linear regression emerged with a slope of 155 ± 5 bp, corresponding to the size of the *P.falciparum* nucleosome repeat unit. No differences regarding the size of the nucleosome repeat unit were observed between central and subtelomeric domains of chromosome 2. However, probes to the subtelomeric region detect higher order nucleosomes than do probes to transcribed regions. The subtelomeric probe, R-CG7, revealed up to 13 consecutive nucleosomes, while a probe to the *KAHRP* gene revealed only up to six consecutive nucleosomes (Fig. 6A). This may indicate that the nucleosomes in subtelomeric repeat regions are more tightly positioned than in other domains, consistent with a silent region.

Prolonged digestion of *P.falciparum* chromatin with micrococcal nuclease revealed the size of the DNA fragment that is associated with a histone octamer in a core particle (Fig. 6). This fragment is 148 ± 6 bp in size, conforming within the margins of error to the canonical value of 146 bp (15). The *P.falciparum* nucleosome linker region is 8 ± 3 bp in size.

The data indicate that the central and subtelomeric domains of chromosome 2 investigated are organized as nucleosomes with a phasing of 155 ± 5 bp.

DISCUSSION

We have recently shown that *P.falciparum* chromosomes are functionally compartmentalized (12). The central domains are transcribed and contain the parasite's genes (12). The subtelomeric domains are transcriptionally silent and may function as an initiator of homologous and non-homologous recombination (12,13,22).

The functional compartmentalization of *P.falciparum* chromosomes extends to the structural level. The transcribed central domains of a chromosome contain unique sequences unlike the transcriptionally silent chromosome ends that are composed of multiple repetitive sequence elements (13).

It has further been shown that *P.falciparum* chromosomes are polymorphic (23). This polymorphism is confined to chromosome ends, whereas central domains are conserved (12). Here we have defined the junction between the various chromosomal domains at the structural and nucleosomal level. The study was conducted with that end of chromosome 2 which is associated with cytoadherence.

Fine scale transcription mapping of a 150 kb terminal fragment of chromosome 2 has identified the most telomere proximal erythrocytic gene cluster, thereby defining the subtelomeric domain as an 85 kb region. This 85 kb subtelomeric region is transcriptionally inactive during the erythrocytic parasite cycle as has also previously been observed (12).

The erythrocytic gene cluster identified consists of three genes, *PfEMP3*, *KAHRP* and *GLARP*. These genes are closely linked, spanning 37 kb. The distance between the genes *PfEMP3* and *KAHRP* is 7.5 kb, between *KAHRP* and *GLARP* 11.5 kb. All three genes are expressed during early parasite development and are transcribed into single RNA species.

Transcription of these genes is monocistronic as demonstrated by nuclear run-on analysis. Monocistronic transcription has also been observed for the *P.falciparum* GBP130 gene (17). Thus, the intergenic regions of these genes must contain the minimal necessary elements for transcription initiation and termination. However, this does not exclude the possibility of common regulators, such as enhancers or silencers, that could control the developmental expression of the entire gene cluster. The analysis of transcription signals in *P.falciparum* is currently hampered by the absence of functional assays, such as transfection or *in vitro* transcription.

The telomere proximal genes *PfEMP3* and *KAHRP* are polymorphic and subject to chromosomal deletions and truncations (Fig. 2) (8,20). Especially, the *KAHRP* gene represents a hot spot for double-stranded chromosome breaks in *P.falciparum* as has previously been demonstrated (27). Additional chromosome 2 breakpoints have also been identified more telomere proximal than the *KAHRP* gene, whereas none were found towards the centromere (12). In contrast to the genes *KAHRP* and *PfEMP3*, the *GLARP* gene is conserved among all 28 parasite strains tested. Thus, the junction between the polymorphic and conserved domains at this side of chromosome 2 maps to the *KAHRP* gene.

The chromosome 2 truncations observed in the knobless parasite strains Dd2 and FVO$^-$ delete the 3′ region of the *KAHRP* gene but leave exon 1 and the upstream region intact (8,9). Nuclear run-on data using nascent RNA from the *KAHRP*$^-$ mutant FVO$^-$ indicates that transcription of the truncated *KAHRP* gene is abrogated. It is conceivable that the deletion event could have removed essential transcription signals that either stimulate promoter activity or effect messenger stability. Alternatively, the *KAHRP* promoter could have been silenced by a telomere position effect, a phenomenon that is observed in yeast when a gene is positioned in the vicinity of telomere repeat sequences (24).

Truncations of chromosome 2 had no effect on the transcription of the *GLARP* gene. In both knobby and knobless parasites, the *GLARP* gene is expressed during the ring stage (Fig. 2B). Furthermore, this gene was found to be highly conserved among all 28 parasite strains investigated. This may suggest an essential, albeit unknown function for the *GLARP* gene product for parasite growth and development. Screening of protein data bases has revealed no homologies to other known proteins. The analysis of the primary amino acid sequence revealed the presence of repetitive amino acid sequences: K E K D K E D (D/N). Since most other *P.falciparum* encoded surface proteins contain repetitive sequences, it is conceivable that the *GLARP* protein is also membrane-associated (23).

The chromosome 2 truncation results in parasite mutants that reveal a knobless phenotype and a deficiency to cytoadhere (1,3,10,20). This phenotype has so far solely been linked to the *KAHRP* gene since its gene product is a structural component of the knobs (6–8). It has also been suggested that the *KAHRP* protein binds in a complex with thrombospondin to endothelial cells (6).

Whether the *PfEMP3* protein is also involved in knob formation or cytoadherence is speculative. However, it has recently been shown that the *PfEMP3* gene product is located at the membrane of infected erythrocytes but, in contrast to the *KAHRP* gene product, is not only found in knobs but also in areas in between (20). A parasite mutant with a truncated *PfEMP3* gene and a functional *KAHRP* gene would be helpful in discriminating between the different biological functions of these two genes.

The functional and structural compartmentalization of *P.falciparum* chromosomes suggests that possibly supramolecular differences exist as well between subtelomeric and central chromosome domains. To examine this possibility, we have analyzed the nucleosome organization of different chromosome 2 domains. Both, the transcribed domains investigated and the transcriptionally inactive subtelomeric regions are organized as nucleosomes with a phasing of 155 ± 5 bp (Fig. 7). While this value is only indicative of the average *P.falciparum* nucleosome repeat unit, the result has been confirmed by an indirect end-labelling study in which the precise nucleosome positioning along the *KAHRP* gene was determined (27). In comparison to the transcribed domains investigated, the subtelomeric regions of chromosome 2 seem to reveal a tighter positioning of nucleosomes, consistent with transcriptionally silent regions. Transcriptional activity results in disruption of the nucleosome organization as has recently been demonstrated (28,29).

Preliminary data suggest that *P.falciparum* nucleosomes contain the histones H1, H3, H2A, H2B and H4 (25). The *P.falciparum* histone H2A has recently been cloned (26). Thus, the *P.falciparum* histone constitution and the chromatin organization seem to conform to other eukaryotes.

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