A New Noncoding RNA Arranges Bacterial Chromosome Organization

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RESEARCH ARTICLE

ABSTRACT Repeated extragenic palindromes (REPs) in the enterobacterial genomes are usually composed of individual palindromic units separated by linker sequences. A total of 355 annotated REPs are distributed along the Escherichia coli genome. RNA sequence (RNaseq) analysis showed that almost 80% of the REPs in E. coli are transcribed. The DNA sequence of REP\textsubscript{325} showed that it is a cluster of six repeats, each with two palindromic units capable of forming cruciform structures in supercoiled DNA. Here, we report that components of the REP\textsubscript{325} element and at least one of its RNA products play a role in bacterial nucleoid DNA condensation. These RNA not only are present in the purified nucleoid but bind to the bacterial nucleoid-associated HU protein as revealed by RNA IP followed by microarray analysis (RIP-Chip) assays. Deletion of REP\textsubscript{325} resulted in a dramatic increase of the nucleoid size as observed using transmission electron microscopy (TEM), and expression of one of the REP\textsubscript{325} RNAs, nucleoid-associated noncoding RNA 4 (naRNA4), from a plasmid restored the wild-type condensed structure. Independently, chromosome conformation capture (3C) analysis demonstrated physical connections among various REP elements around the chromosome. These connections are dependent in some way upon the presence of HU and the REP\textsubscript{325} element; deletion of HU genes and/or the REP\textsubscript{325} element removed the connections. Finally, naRNA4 together with HU condensed DNA \textit{in vitro} by connecting REP\textsubscript{325} or other DNA sequences that contain cruciform structures in a pairwise manner as observed by atomic force microscopy (AFM). On the basis of our results, we propose molecular models to explain connections of remote cruciform structures mediated by HU and naRNA4.

IMPORTANCE Nucleoid organization in bacteria is being studied extensively, and several models have been proposed. However, the molecular nature of the structural organization is not well understood. Here we characterized the role of a novel nucleoid-associated noncoding RNA, naRNA4, in nucleoid structures both \textit{in vivo} and \textit{in vitro}. We propose models to explain how naRNA4 together with nucleoid-associated protein HU connects remote DNA elements for nucleoid condensation. We present the first evidence of a noncoding RNA together with a nucleoid-associated protein directly condensing nucleoid DNA.

Noncoding RNAs (ncRNAs) present in both prokaryotic and eukaryotic cells do not function as mRNA, tRNA, or rRNA (1). Although many ncRNAs of different sizes and different functions have been widely reported (2–6), new ncRNAs with new functions are still being discovered. Recently, we discovered a novel ncRNA, transcribed from a specific repeated extragenic palindromic element, REP\textsubscript{325}, in the chromosome of Escherichia coli by RNA IP followed by microarray analysis (RIP-Chip) assays of the nucleoid-associated HU protein (7). In this paper, we termed it as nucleoid-associated ncRNA (naRNA). REP elements in the enterobacterial genomes, first reported 30 years ago, contain independent palindromes separated by linkers (8–10). The functions of these REPs have been speculated to be related to transcription termination signals, binding sites for proteins, cleavage sites for DNA gyrase, and, possibly, manipulation of nucleoid structures (11–14). In this study, we investigated potential functions of the REP\textsubscript{325} element, which is located between genes yjdM (phnA) and yjdN (phnB), and its RNA products. REP\textsubscript{325} contains six homologous units (Fig. 1A). Each repeat is composed of two palindromic cruciform-generating motifs, Y and Z\textsubscript{2}, connected by a short linker, l. Cells deleted for the REP\textsubscript{325} segment and/or hup genes encoding the nucleoid-associated HU protein showed a decondensed nucleoid structure, suggesting that these two factors participate in nucleoid condensation (7). RNA sequencing (RNaseq) analysis and nucleoid RNA tiling array clearly showed the existence of RNA species transcribed from each unit of REP\textsubscript{325}, named naRNA1 to naRNA6 (Fig. 1A). Multialignment of DNA sequences of the 6 repeats in REP\textsubscript{325} showed high homology (Fig. 1B). Each naRNA contains two potential hairpins, corresponding to Y and Z\textsubscript{2} motifs (Fig. 1C). It is unknown whether these six RNAs are transcribed independently or are the result of processing of a larger RNA transcribed from a common promoter (the promoter of the upstream gene, yjdM). One of these RNAs is naRNA4, which binds two dimeric forms of HU, HU\textalpha and HU\textbeta. In this report, we show that the expression of naRNA4 from a plasmid restores the decondensed morphology of the nucleoid caused by

Received 8 July 2015 Accepted 3 August 2015 Published 25 August 2015

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We also show intersegmental connections in vivo between different remote cruciform-containing DNA structures like those present in REP elements; the connections are affected by deletion of hup genes encoding HU subunits and/or of REP325. We also demonstrate the connections between cruciforms in DNA in vitro in the presence of nRNA4 and HU. We propose that these connections are a major part of the cellular nucleoid architecture and help its condensation.

RESULTS

The existence of nucleoid-associated RNA: tiling array and RNAseq analysis. Ohniwa et al. showed that the E. coli nucleoid is a 40-nm-thick fibrous structure as observed by atomic force microscopy (AFM); the fibers assume 10-nm-thick structures in cells devoid of the nucleoid-associated protein (NAP) HU (15). They also showed the existence of 10-nm-thick nucleoid fibers after RNase treatment, suggesting a role of some RNA and HU in nucleoid architecture. Pettijohn and Hecht also suggested that the E. coli nucleoid contains RNAs which are important for the structural integrity of the nucleoid (16). We isolated RNA from the E. coli nucleoid, which was purified from cells cultured in minimal medium, and identified structural elements by the use of a DNA tiling array. It is clear that the nucleoid RNA contains fragments of rRNAs, tRNAs, a few mRNAs, and many ncRNAs, many of which are also present among HU binding RNAs (7). It is noticeable that more than 30 ncRNAs are transcribed from the REP elements (see Text S1 in the supplemental material).

In order to analyze the transcription profile of the REP elements in E. coli, we analyzed RNAseq data that was previously published and deposited in the NCBI Sequence Read Archive (17). According to the gene annotation of E. coli MG1655, there are a total of 355 REP elements, 152 of which are transcribed in cells cultured in defined minimal medium (see Text S3 and Text S4 in the supplemental material). In our previous study, two overlapping RNA reads, which were part of transcripts from REP325 and identified in RIP-Chip assays of HU protein, were assumed to originate from a single RNA, named nc5 RNA (7).

Exploring the RNAseq data with unique alignments revealed RNA sequences matched to six repeated structures (Y-Z2) in the REP325 (see Fig. S1A in the supplemental material). It appears to generate six RNA species, now named naRNA1 to naRNA6 (Fig. 1A). The DNA repeats are connected by five unknown motifs with identical sequences (named U motifs), which are also transcribed. We have not determined if the entire REP325 segment is cotranscribed and processed into six naRNAs. But the abundance of matched readings suggests a potential direction of transcription of the entire REP325 from the promoter of the upstream yjdM (phnA) gene to the position upstream of the yjdN (phnB) gene. Furthermore, sequence analysis of the unmatched gaps for the REP325 segment showed high sequence similarity between the repeats. Multisequence alignments of DNA sequences encoding the naRNA1 gene to the naRNA6 gene indicated that these six repeats share high sequence homology and that the sequence of the naRNA4 gene is identical to that of the naRNA2 gene (Fig. 1B). Multiple-sequence alignments also showed that the entire REP325 is transcribed (see Fig. S1B). RNA secondary structure analysis confirmed that RNAs from the repeats have similar structures and}

**FIG 1** Description of the REP<sub>325</sub> element. (A) Genetic map of the REP<sub>325</sub> element. REP<sub>325</sub>, located in the intergenic region of yjdM and yjdN, is composed of 6 highly homologous repeats separated by 5 unknown spacers with exactly the same DNA sequence (U in blue). The transcripts from the 6 repeats are named naRNA1 to naRNA6, as shown. Each unit is 77 bp long and contains two different palindromes (Y and Z<sub>2</sub> in diamonds) separated by a constant linker (l in black). Each naRNA contains a Z<sub>2</sub> motif and a Y motif, connected by a linker (l). (B) Multisequence alignment analysis of the 6 repeats in REP<sub>325</sub>. Each repeat contains a Y motif and a Z<sub>2</sub> motif connected by the linker l (boxed). Analysis was done by ClustalW. The corresponding RNA products are marked on the left. (C) Typical secondary RNA structure of an naRNA (naRNA4) as predicted by Mfold with modifications.
contain the Y and Z₂ potential hairpins. The typical structure of naRNA4 is shown in Fig. 1C.

Decondensation of nucleoid in vivo: TEM analysis. Transmission electron microscopy (TEM) observations previously showed that deletion mutants of HU genes (ΔhupA ΔhupB strain) and/or of the REP₃₂₅ element (ΔREP₃₂₅ Bne strain) decondensed the E. coli nucleoid in both growing and nongrowing cells compared to a compacted nucleoid observed in the wild-type strain under similar conditions, suggesting that HU and part or all of REP₃₂₅ DNA and/or its RNA product affect nucleoid architecture (7). We extended the TEM observations further by investigating the details of REP₃₂₅ participation in the nucleoid structure. We first confirmed that in growing cells, compared to wild-type results, deletion of HU genes or of REP₃₂₅ decondensed the nucleoid size (Fig. 2A; nucleoids are outlined in red). Moreover, the REP₃₂₅ deletion strain carrying a plasmid vector showed no change in decondensed nucleoid morphology. But expression of naRNA4 from the plasmid reproducibly condensed the nucleoid. We observed some overcondensation that was most likely due to overexpression of naRNA4. The expression of an unrelated RNA from the same plasmid had no effect on the decondensed nucleoid. We also tested the effect of expression of derivatives of naRNA4 containing only a Z₂ or Y motif in the same REP₃₂₅-deleted cells. The expression of RNA containing only the Z₂ or Y motif alone, unlike that of the intact naRNA4, did not restore the nucleoid morphology to wild type, although we do not know anything about the relative stability of naRNA4 or its truncated derivatives under the conditions of the experiments. TEM analysis performed with non-growing cells in the same set of strains gave identical results; only the presence of an intact naRNA4 caused nucleoid condensation (Fig. 2B). We note that, in the absence of any simple way to quantify the nucleoid volume in TEM observations, we estimated the size of the nucleoid in two-dimensional (2-D) analysis of the thin sections (shown by red outlines in Fig. 2). Although these observations show the involvement of HU and naRNA4 in nucleoid condensation, a direct participation of any part of the REP₃₂₅ element in the process is not apparent. If DNA is involved in the condensation, as seems very likely, other DNA sequences homologous to REP₃₂₅ may fulfill the same role. In summary, this is first demonstration of a direct involvement of an ncRNA in DNA condensation at the molecular level.

Intersegmental chromosomal interactions in vivo: 3C analysis. We hypothesized that one plausible mechanism of nucleoid structural organization is that of facilitating contacts between REP elements around the chromosome by naRNA4 and the nucleoid protein HU. To test the idea, we employed the chromosome conformation capture (3C) approach, the use of which has been established in studies of distal intrachromosomal interactions in vivo in both eukaryotes and prokaryotes (18–20). We designed primers for 23 randomly selected REP segments, including
REP325 around the chromosome to investigate potential connections between REP sites (primer sequences are listed in Table S1 in the supplemental material). Of 253 pairs tested, 27 combinations showed positive PCR amplifications, suggesting that the corresponding DNA segments may be connected to each other (Fig. 3). These pairs were further tested by 3C in the following mutants: the /H9004 hup mutant, the /H9004 REP325 mutant, and /H9004 hup /H9004 REP325 mutant.

We measured the ratio of PCR signals in each mutant compared to that in the wild-type strain after normalization to an internal control. In interpreting the positive amplification results in 3C experiments in the following discussion, we assume that an observed contact involves the REP elements and not another DNA sequence present nearby in the chromosome. Similarly, when a contact signal observed in the wild-type strain is missing in the /H9004 REP325 deletion strain, we assume that it is because of the absence of naRNA4. The effect of the HU and naRNA4 deletions on the observed intrachromosomal interactions were grouped into four classes. (i) HU and naRNA4 independent. Deletion of either HU and/or naRNA4 has no effect on the interactions suggesting perhaps other NAPs and RNAs are involved in DNA contacts (4 out of 27). (ii) HU dependent. Deletion of the HU gene significantly affected the interactions while deletion of REP325 did not (6 out of 27). In these cases, HU together with other RNA may be involved in bringing DNA contacts. (iii) HU and naRNA4 dependent. Deletion of either the HU gene or REP325 significantly affected the interactions (4 out of 27). In these cases, both HU and naRNA4 are specifically involved in DNA contacts. (iv) HU or naRNA4 dependent. Only deletion of both HU and REP325 significantly affected the interactions while removal of either HU or RNA4 did not (13 out of 27). In this group, HU collaborates with another RNA or naRNA4 collaborates with another protein for DNA-DNA interactions.

DNA condensation mediated by HU and naRNA4 in vitro: AFM analysis. Both TEM and 3C analyses showed the involvement of HU and naRNA4 in nucleoid organization. However, they did not reveal any mechanistic details. We used AFM to monitor any condensing effects of HU protein and naRNA4 on naked supercoiled DNA in vitro to get some insights about the mecha-
nism(s) of their action. A plasmid containing one REP<sub>325</sub> (pQZ080) was used as the template for AFM (Fig. 4). The addition of naRNA4 or of different HU dimers to the plasmid did not noticeably change its supercoiled morphology. The absence of any effect of HU in this experiment is consistent with previous reports (15, 21). However, the presence of either HU<sub>αα</sub> or HU<sub>ββ</sub> together with naRNA4 dramatically condensed the DNA, apparently because of the presence of multiple intersegmental contacts, thus demonstrating that naRNA4 collaborates with HU in condensing DNA (Fig. 4A). Note that either HU<sub>αα</sub> or HU<sub>ββ</sub> dimer works but not HU<sub>αβ</sub> dimer (αα, αβ, and ββ). Since the plasmid DNA contained only one REP<sub>325</sub> element, the multiple contacts very likely involve either nonspecific DNA binding or some other sequences in the plasmid that allow DNA contacts. The REP<sub>325</sub> palindromic repeats generate cruciform structures in a supercoiled state. We believe that several transcription terminators (22) that are present in the plasmid and which also generate cruciform structures perform the role of REP<sub>325</sub>. Consistent with this idea, the addition of HU and naRNA4 to the parental plasmid (pSA508) containing no authentic REP element but several transcription terminators also resulted in DNA condensation (Fig. 4B). To investigate the features of naRNA4 required in DNA condensation, we first tested whether an intact naRNA4 is needed to condense DNA. We performed AFM analysis with three 77-nucleotide (nt) RNAs: a nonspecific control RNA, an RNA containing only the Y motif, and an RNA containing only the Z<sub>2</sub> motif (see Table S2 in the supplemental material). Compared to naRNA4, none of these RNAs condensed DNA (Fig. 4B), which is in agreement with the TEM data (Fig. 2). We also asked whether the exact sequences or only hairpin features of Z<sub>2</sub> and Y motifs are involved. We synthesized a 77-nt-long RNA whose sequence was the exact complement of naRNA4. This anti-naRNA4 molecule has an RNA sequence completely different from that of naRNA4 but should contain two hairpin structures. Figure 4B shows that the anti-naRNA4 also condensed DNA in the presence of HU protein, indicating that it is the secondary structure of naRNA4 and not the sequence of the RNA itself that is important in DNA condensation.

Because of the large sizes of pQZ080 and pSA508 (3.9 kb and 3.5 kb, respectively), we could not discern the precise organization of the DNA contact points involved in the observed DNA condensation. To simplify the condensed DNA structure, we tested a number of minicircle DNAs which contain 0, 1, or 2 potential condensation sites (cruciform structures originated from REP<sub>325</sub>...
or one of the transcription terminators, rpoC, present in the parental plasmid, pSA508) at marked positions: mini103, mini104, mini105, mini106, mini107, and mini120 (Fig. 4C; see also Fig. S2 in the supplemental material). Any looping generated using the marked cruciform sites can be discerned by measuring the size of the DNA loops between contact points. As elaborated below, consistent with our proposal, HU- and RNA4-mediated DNA condensation in vitro requires only a cruciform structure in DNA and not other parts of the multipalindromic unit and the presence of both HU and naRNA4 on minicircle DNAs (Fig. 4C). “Figure 8” structures, which are caused by either random crossover of DNA or specific bridging of DNA due to the presence of HU and naRNA4, were observed in all minicircle DNAs. To determine whether the looping was random or specific, we measured each loop from the figure 8 structures in the minicircle DNAs in the presence of naRNA4 and HU protein. We observed that the frequency of figure 8 structures with the expected loop sizes resulting from interactions between any two marked cruciform structures was significantly higher in all minicircle DNAs except in mini103, which has no cruciform structure. For example, we found 79 figure 8 structures in mini103 in the presence of naRNA4 and HU, and none was found with an expected loop size. In mini106, mini107, and mini20 containing two cruciforms, the ratios of the numbers of observed figure 8 structures with expected loop sizes to the total numbers of counted structures were 24/49, 48/101, and 28/53, respectively. These results suggest that HU and naRNA4 form bridges between two cruciforms present in a minicircle DNA.

For mini104 and mini105 plasmids, which contain one rpoC transcription terminator of pSA508 and one REP325 repeat, respectively, we observed only random figure 8 structures and, occasionally, two or more minicircle DNAs bridged together by a complex core (red arrows in Fig. S2 in the supplemental material), suggesting that two cruciform structures present in different DNA molecules can connect.

**DISCUSSION**

It has become clear that the organization of the chromosome in *E. coli* is not random. The chromosome is not merely a disordered aggregate of randomly coiled DNA. Instead, it is a dynamic but spatially organized defined entity that undergoes strictly controlled and reproducible changes when they are needed (23). Structural models of elements such as “macro domains,” “supercoiled topological loops,” “filaments,” and “remote connections” are suggested to represent structural constituents of chromosomes from observations using different approaches (19, 24–27). A number of NAPs, such as the HU, Fis, IHF, H-NS, and SMC proteins, modulate chromosome structure. We focused on HU, which binds to DNA nonspecifically but prefers distorted DNA structures such as nicks, gaps, bends, and cruciforms (28, 29). Due to its high abundance and growth-phase-dependent subunit compositions (HUαα, HUαβ, and HUββ), HU is believed to modulate chromosome structure in accordance with the growth phase of the cell (30). We confirmed that HU binds to naRNA4 and to several other RNAs by electrophoretic mobility shift assay (EMSA) (see Fig. S3 in the supplemental material), but not all HU-RNA bindings could help DNA condensation both in vivo (Fig. 2) and in vitro but bound to those which contained two hairpin structures (Fig. 4). Thus, an HU-naRNA4 interaction may be somewhat unusual and specific; the presence of at least two hairpin motifs, such as Z2 and Y, in the RNA is needed for DNA condensation. We conclude that two cruciform structures in DNA, not yet completely defined, interact with each other in a pairwise fashion for DNA condensation, which needs both HU and naRNA4. We propose four mechanisms for interactions between two DNA cruciforms mediated by HU and naRNA4 (Fig. 5). (i) For DNA-naRNA-HU-naRNA-DNA interactions, each cruciform structure binds to one hairpin of naRNA and two DNA-bound RNAs are bridged together by an HU dimer using the other hairpins of the two RNAs (Fig. 5A). (ii) For DNA-naRNA(HU)-DNA interactions, the model is similar to model i, but the stoichiometry of HU and naRNA in the complex is 1:1. HU binding to naRNA makes the latter amenable to interaction with two cruciforms (Fig. 5B). (iii) For DNA-HU-naRNA-HU-DNA interactions, HU binds to cruciform DNA; two bound-HU dimers are then connected by a molecule of naRNA through the two hairpins (Fig. 5C). (iv) For DNA-HU(naRNA)-DNA interactions, the model is similar to model iii, but the stoichiometry of HU and naRNA in the complex is 1:1. naRNA binding to HU makes the latter potent for interactions with two cruciform structures. We note here that in models ii and iv, it is possible that the roles of naRNA and HU, respectively, could be only catalytic and that they are not involved in the complex. At this stage, we are unable to prefer one model to the others except that a specific interaction between HU and a DNA cruciform structure has been previously established (31, 32), which would support models iii and iv. Cross-linking of the condensed DNA complexes followed by fragmentation and chemical identification of the products may distinguish between the different models.

**MATERIALS AND METHODS**

**Construction of strains and plasmids.** Wild-type *E. coli* MG1655 and the ΔhupA ΔhupB mutant were previously described (7). The ΔREP325 strain was constructed by mini-λ recombineering, in which REP325 was replaced by a Cat-SacB cassette (33, 34). Plasmid pQZ080 was constructed with the insertion of REP325 into pSA508 (35) at SacI to BamHI sites. Minicircle DNA was purified according to the method of Choy and Adhya (35). Plasmid pNM12 was from Nadim Majdalani (NIH, USA). DNA fragments encoding the naRNA4 gene and Con, Y-Con, and Con-Z2 genes were amplified using chemically synthesized single-stranded DNAs (ssDNAs) as templates and inserted into pNM12 at MscI to HindIII sites. All recombinant plasmids and pNM12 were transformed into the ΔREP325 strain.

**Validation of expression of REP325 by analysis of RNAseq data.** Raw RNAseq data for *E. coli* MG1655 obtained from the NCBI Sequence Read Archive (accession no. SRP006793) (17) were mapped onto the *E. coli* genome using Novoalign software and allowing up to two mismatches between a 36-nt read and the genome sequence. Two different strategies, using unique map reads and total map reads, were applied, and the unique map read results and total map read results were preserved separately, with multiple alignments of up to 50 different locations in the genome. The alignment files (sorted by bam format) were used for visualization in tracks in the genome browser at the University of Southern California, Santa Cruz (UCSC) (36).

**Synthesis of RNA used in AFM analysis.** A series of complementary ssDNAs that contain a T7 promoter sequence (5'-TATAGGACTCAT ATAGGGAGA-3') followed by experimental sequences and their complements, listed in Table S2 in the supplemental material, were chemically synthesized. The double-stranded DNAs (dsDNAs) were obtained by annealing the appropriate complementary ssDNAs (7). Synthesis and purification of RNAs were completed by the use of an AmpliScribe T7-Flash transcription kit according to the manufacturer’s instructions (Epicentre, Madison, WI). The quality and quantity of RNAs were determined by the
use of an agarose gel and a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE), respectively. For RNAs used in the gel shift assay, \(^{32}\)P-UTP was used instead of the unlabeled UTP provided in the kit.

**Electrophoretic mobility shift assay.** Electrophoretic mobility shift assays in gels were done as described before (37, 38) with modifications. Radioactively labeled RNAs were incubated with increasing amounts (0 to 1.6 mM) of HU protein in binding buffer containing 20 mM Tris-HCl (pH 7.5), 0.2 M NaCl, and 10% glycerol at 37°C for 20 min. The mixtures were separated by the use of 8% prerun native polyacrylamide gels and 100 mM Tris-borate-EDTA (TBE) buffer. Gels were finally exposed to X-film at 80°C.

**TEM analysis of nucleoid structure in E. coli.** Strains used in TEM analysis were inoculated from plates with appropriate antibiotics into M63 minimal medium with 0.2% fructose, 0.05% Casamino Acids, and proper antibiotics and incubated at 37°C overnight. The cultures were diluted into fresh medium as mentioned above with 0.1% arabinose and grown to log or stationary phase for harvest. One milliliter of fresh cultures was mixed with an equal volume of Fixation buffer (8% formaldehyde– 4% glutaraldehyde– 0.2 M cacodylate buffer or 20 mM phosphate-buffered saline [PBS]) and kept at room temperature for 2 h. The fixed cell solutions were stored at 4°C until TEM analyses were performed. Cells were spun down to form a small pellet and then processed for EM analysis of thin sections. Briefly, the pellet was postfixed in 1% osmium tetroxide (Electron Microscopy Sciences, Ft. Washington, PA)–0.1 M cacodylate buffer for 1 h at room temperature, stained in 0.5% uranyl acetate–0.1 M acetate buffer for 1 h, and then dehydrated in a series of ethanol (35%, 50%, 75%, 95%, and 100%) and propylene oxide (100%) solutions. The pellets were infiltrated into 100% propylene oxide and epoxy resin (1:1) overnight and embedded in pure resin the following day. The epoxy resin was cured in a 55°C oven for 48 h, followed by quenching with ethanol at a final concentration of 0.125 M for 5 min at room temperature.

Images were preprocessed using the instrument image processing software and then exported for further analysis with the NIH ImageJ image processing software package. The lengths of DNA loops observed in minicircle DNA were measured by tracing.

**SUPPLEMENTAL MATERIAL**


Text S1, DOC file, 0.1 MB.
Text S2, XLSX file, 0.04 MB.
Text S3, XLSX file, 0.02 MB.
Text S4, XLSX file, 0.02 MB.
Figure S1, PDF file, 0.2 MB.
Figure S2, PDF file, 0.1 MB.
Figure S3, PDF file, 0.05 MB.
Table S1, PDF file, 0.05 MB.
Table S2, PDF file, 0.05 MB.

ACKNOWLEDGMENTS
This research was supported by the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research.
We thank our colleagues Dale Lewis, Amlan Dhar, Phuoc Le, Sangmi Lee, and Andrei Trostel for assistance.

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