Nuclear Non-coding RNAs Are Transcribed from the Centromeres of Plasmodium falciparum and Are Associated with Centromeric Chromatin*

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Non-coding RNAs (ncRNAs) play an important role in a variety of nuclear processes, including genetic imprinting, RNA interference-mediated transcriptional repression, and dosage compensation. These transcripts are thought to influence chromosome organization and, in some cases, gene expression by directing the assembly of specific chromatin modifications to targeted regions of the genome. In the malaria parasite Plasmodium falciparum, little is known about the regulation of nuclear organization or gene expression, although a notable scarcity of identifiable transcription factors encoded in its genome has led to speculation that this organism may be unusually reliant on chromatin modifications as a mechanism for regulating gene expression. To study the mechanisms that regulate chromatin structure in malaria parasites, we examined the role of ncRNAs in the assembly of chromatin at the centromeres of P. falciparum. We show that centromeric regions within the Plasmodium genome contain bidirectional promoter activity driving the expression of short ncRNAs that are localized within the nucleus and appear to associate with the centromeres themselves, strongly suggesting that they are central characters in the maintenance and function of centromeric chromatin. These observations support the hypothesis that ncRNAs play an important role in the proper organizational assembly of chromatin in P. falciparum, perhaps compensating for a lack of both regulatory transcription factors and RNA interference machinery.

Recent years have seen many reports of non-coding RNAs (ncRNAs) and their involvement in chromatin assembly in eukaryotes. RNAs are non-coding if they are not translated into protein, and most do not have any substantial open reading frames. Examples of ncRNAs and their roles in chromatin assembly and modification are found in organisms as varied as dinoflagellates (1) and yeast, fruit flies, mice, and humans (2) and thus are likely conserved throughout a broad range of eukaryotic evolution. The most closely studied systems that employ ncRNAs to direct chromatin assembly and modification include examples of genetic imprinting (3), RNAi-based transcriptional repression (4), and dosage compensation in both mammals and fruit flies (5, 6). Although different aspects of these RNAs have been characterized, exactly how they execute their tasks is still a mystery. Several studies support the hypothesis that these nuclear, cis-acting ncRNAs act by recruiting chromatin-modifying enzymes to specific chromosomal regions, thereby influencing genome organization or gene expression.

The centromeres of eukaryotic chromosomes are examples of chromosomal elements that assemble a unique, specific chromatin architecture that is necessary for proper chromosome segregation during replication. Studies of ncRNAs found at the centromeres of various organisms underscore the influence these RNAs have on chromatin structure and function. There is strong evidence that epigenetic marks on chromatin, and not DNA sequence, are the basis for centromere identity, inheritance, and function. Convincing evidence from several species suggests that any piece of DNA may function as a centromere if it is flagged with the proper epigenetic marks (7, 8). Consistent with this notion, constitutive heterochromatin is strongly associated with centromeric and pericentromeric regions (9), and the only common trait of all centromeric chromatin studied so far is the presence of nucleosomes containing a centromere-specific histone H3 variant (10). Recent reports have shown that ncRNAs are involved in, or are at least associated with, the process of modifying centromeric chromatin in several organisms, including fission yeast (9, 11, 12), humans (13, 14), maize (15), and mice (16). The exact functions of these ncRNAs are unknown, and their interacting partners are just beginning to be discovered.

There is evidence indicating that ncRNAs may also play a substantial role in Plasmodium falciparum, the protozoan parasite that causes the most severe form of human malaria. The study of ncRNAs thus may be important for understanding the most basic aspects of this pathogen’s biology, such as genome organization, gene regulation, and mitosis. These cellular pro-
cesses are not well understood in this organism, and they likely do not occur in *P. falciparum* as they do in model eukaryotes. For example, there is a surprising lack of specific transcription factors and other transcription-associated proteins that bear any homology to those found in animals, plants, and fungi. In addition, although the degradation of double-stranded RNA molecules into short fragments (~25 nucleotides) has been reported (17), the key components of the RNAi pathway, including those required for RNAi-mediated transcriptional silencing, are missing from the genome (18). Significant control of gene expression is likely exerted at the level of transcription, but the effectors remain elusive. However, a notable exception to the paucity of specific transcription-associated proteins is the CCCH-type zinc finger RNA-binding domain, which, after adjusting for genome size, is twice as abundant in the *Plasmodium* genome when compared with other eukaryotes (19, 20). This finding may suggest a more widespread role for RNAs in the cellular processes of *P. falciparum* compared with other eukaryotes. In addition, several examples of ncRNAs have been described in the literature. For instance, Su et al. (21) showed that ncRNAs are abundantly transcribed from members of the *var* gene family, and Kyes et al. (22) subsequently revealed that expression of these transcripts is tightly regulated over the course of the cell cycle. Many sporozoite-specific genes (e.g. *starp*, *ctrp*, and *csp*) have also been shown to be actively transcribed within a narrow window of the intra-erythrocytic cell cycle, but remain untranslated into protein (23–25). In addition, both microarray analysis and studies using serial analysis of gene expression have detected transcripts that do not appear to encode proteins; however, the significance of these findings remains obscure (26, 27).

To study the potential role of ncRNAs in chromatin assembly in malaria parasites, we have examined the centromeres identified previously in *P. falciparum*. A recent report by Kelly et al. (28) provides experimental evidence to support the annotation by showing that the activity of topoisomerase II, a known kinetochore protein, localizes to the A/T-rich regions originally proposed to be centromeres. We show that centromeric regions of the *Plasmodium* genome contain bidirectional promoter activity, and we detect short ncRNAs of discrete length transcribed from these regions. These transcripts are tightly localized within the nucleus and appear to be associated with the sequences annotated as centromeres, strongly suggesting that they are central characters in the maintenance and function of centromeric chromatin. These data support the hypothesis that ncRNAs might play an important role in initiating the proper organizational assembly of chromatin throughout the genome.

**EXPERIMENTAL PROCEDURES**

*Parasite Culture*—The *P. falciparum* NF54 strain was used for transient and stable transfections as well as FISH experiments. For Northern blots, subclones of two isolates, IT (subclone A4) and NF54 (subclone NR13), were cultured and sorbitol-synchronized by standard techniques (29, 30). Parasites were grown as described previously (31). Briefly, parasites were reared at 5% hematocrit in RPMI 1640 medium and supplemented with either 0.5% Albimax II (Invitrogen) or 10% human serum, 0.25% sodium bicarbonate, and 0.1 mg/ml genetin. Parasites were incubated at 37 °C in an atmosphere of 5% oxygen, 5% carbon dioxide, and 90% nitrogen. For etoposide experiments, parasites were cultured to ~6% parasitaemia at ring stage; 200 μM etoposide (Sigma) was added; and parasites were incubated at 37 °C for ~5 h. Parasitaemia of parasites used in all experiments was between 6 and 8%.

*DNA Constructs*—The previously described plasmid pHLL (32) was modified into pHLRH. pHLRH was used as a plasmid backbone for cloning of pHLCsRH (Cs is the cen2 fragment) and pHLXRH (X being segments of the *cen2* fragment). The sequence of Cs corresponds to MAL2, 447,329–448,405, in the *P. falciparum* genome. The derivative fragments CsA, CsB, and CsC (see Fig. 2) correspond to MAL2, 447,329–447,717, 447,752–448,146, and 448,119–448,405, respectively. The primers used to amplify the Cs, CsA, CsB, and CsC fragments (see Fig. 2) from pHLCsRH were 5′-AAACTGCAAGCCCAGCGGTTTATAG-3′ and 5′-GTTGCGCGCCCTAGCATGCCAGAAC-3′. A control construct was made by amplifying the intron from PP0020 (a var-like gene) using primers 5′-CCCGGATCTCTACTATATATATATATT-3′ and 5′-GATCCTCAAAAGGTTATGGAT-3′. The control construct was then used to transfect parasites using 737-bp probe for *kahrp* (knob-associated histidine-rich protein) from NF54 genomic DNA were 5′-GGTTCAGAAGGTTATGGAT-3′ and 5′-CCCTCAGCAGCACATTGTC-3′.

*Parasite Transfection and Luciferase Assays*—Parasites were transfected using “DNA-loaded” red blood cells as described previously (33). Briefly, 350 μL of red blood cells (at 50% hematocrit) was combined with 50 μg of plasmid DNA in a total volume of 400 μL. This was then transferred to a 0.2-cm electroporation cuvette, chilled on ice, and electroporated using a BioRad Gene Pulser apparatus under conditions of 0.31 kV and 960 microfarads. The erythrocytes were washed with complete medium, and parasites were added for invasion. 4.5 ml of culture medium containing DNA-loaded erythrocytes at 5% hematocrit was combined with 0.5 ml of a standard parasite culture. For stable transfections, parasites were grown in the presence of 5 nM WR99210 as described (34). Luciferase assays were performed on 5-ml cultures at selected times after transfection (33).

*RNA Extraction and Northern Blot Analysis*—RNA and Northern blot hybridizations were performed as described previously (35). RNA was extracted from synchronized parasites at various time points after red blood cell invasion, size-separated by electrophoresis on 1% agarose, and then capillary-transferred to a Hybond N+ membrane (GE Healthcare). DNase treatment was performed for indicated samples using Amobion Turbo-DNase following the manufacturer’s instructions. Short RNA was resolved on 15% acrylamide, 7 M urea, 1 × TBE (0.089 M Tris, 0.089 M boric acid, 2 mM EDTA) gels, which were electrophoresed for 15 min (50 V) in 0.5 × TBE onto Hybond N+. RNA sizes were estimated with size markers (0.24–9.5-kb RNA probes).
Centromeric Non-coding RNAs in Plasmodium

A. Chromosome 2

B. Chromosome 3

FIGURE 1. ncrRNAs transcribed from centromeres on chromosomes 2 and 3. A, schematic representation of the centromeric regions of *P. falciparum* chromosomes 2 and 3. The very A/T-rich core of each centromere is shown by a black box, and the nearest annotated genes are displayed as gray boxes with gene annotation numbers. The stippled boxes show the sequences used to generate single-stranded RNA probes. The portion of the centromere used in the promoter assays is shown by the hatched box and labeled CenSub, with the different subfragments labeled A, B, and C. B. Northern blot detection of ncrRNAs transcribed from the centromeres on chromosomes 2 and 3. Probes were specific to either the c or w strand as indicated.

RESULTS

The Centromeres of *P. falciparum* Produce 75–175-Nucleotide Non-coding Transcripts—In the *P. falciparum* genome data base, centromeres have been annotated in all but 1 of the 14 chromosomes (28). These sequences were designated as centromeres based on their length (2–3 kb) and their A/T content (~97%, compared with ~82% across the genome). They are devoid of open reading frames and contain numerous multiple tandem repeats not conserved between chromosomes (38). The function of these sequences as centromeres was experimentally validated recently (28). To determine whether ncrRNAs were associated with these centromeres in a manner similar to other eukaryotes, Northern blotting was performed using total RNA extracted from cultured, asexual-stage parasites. To avoid making probes from the extremely A/T-rich, repetitive sequence found within the center of the centromeres, the DNA sequence from the boundaries of the A/T-rich cores of the centromeres on chromosomes 2 and 3 was amplified by PCR and subcloned into plasmids that enabled the production of strand-specific RNA probes using *in vitro* transcription (Fig. 1A).
Hybridization of probes on both sides of the centromere on chromosome 2 (Cen2L and Cen2R) to Northern blots containing RNA extracted from trophozoite-stage parasites identified transcripts of \( \sim 75 \) and \( \sim 175 \) nucleotides (Fig. 1B). The Cen2R probes detected RNA using both c and w strand probes, indicating that both strands of the genomic region are actively transcribed. Hybridization with probes from one side of the chromosome 3 centromere (Cen3Rw) also identified an \( \sim 175 \)-nucleotide transcript. Probes located farther from the centromere (Cen3Rb), as well as probes from elsewhere in the genome, did not give a strong hybridization signal. DNase treatment did not change the strength of the hybridization signals, verifying that the bands detected on the Northern blots are, in fact, the result of hybridization to RNA and not contaminating genomic DNA (data not shown).

Hybridization, or alternatively, that the promoter activity depends more on the secondary structure of the DNA than on its primary sequence. In this case, shortening the DNA fragment might alter its structure and, consequently, its promoter activity. In either case, these data confirm that this centromere contains robust promoter activity and suggest that this promoter activity is likely responsible for the production of the transcripts detected by Northern blotting.

To further validate the promoter activity contained in the centromeric sequence and to investigate its activity over the course of the cell cycle, the Renilla coding region in the expression vector was replaced by the human dihydrofolate reductase gene (hdhfr) to allow for selection of stably transformed parasites. In addition, a stage-specific Northern blot was hybridized with the Cen2R probe. The stage specificity of the RNA on this blot was verified...
The centromeric non-coding RNAs localize within the nucleus. A, first two columns, strand-specific RNA probes from cen2 and cen3 as shown in Fig. 1A. The probes were labeled with either fluorescein (Cen2Rc) or biotin (Cen3Rw) and used in FISH. Nuclei of ring-stage parasites are stained with DAPI. Single, nuclear clusters are seen in 90% of cells probed with the Cen2R probe (first column) or the Cen3R probe (second column). Third column, two-color FISH allows simultaneous display of both probes. Fourth column, hybridization with a probe specific for kahrp mRNA. B, quantification of the percentage of nuclei in which the hybridization signal is found within the DAPI-stained nucleus for either cen2 (90.5%; n = 85) or kahrp (36.1%; n = 72).

Previously (22), the Northern blot easily detected the transcripts in all stages tested, indicating that these RNAs are present in the nuclei throughout the cell cycle (Fig. 2C). The reporter construct displayed, however, a stark increase in expression levels late in the cell cycle (Fig. 2D), when the genome is being replicated 24–36 times. These data might suggest that the centromeric promoters actively transcribe these non-coding RNAs when the chromosomes are replicating and segregating or during merozoite formation and that the RNAs then persist throughout the rest of the cell cycle, as detected by Northern blotting.

The Centromeric Non-coding RNAs Are Retained within the Nucleus—Non-coding RNAs involved in chromatin assembly in other systems are typically found within the nucleus and are associated with chromatin. To address the question of where the centromeric ncRNAs of *P. falciparum* are located, FISH was utilized. Ring-stage parasites were fixed and hybridized to strand-specific, fluorescently labeled ∼300-nucleotide RNA probes from the boundaries of both cen2 and cen3. The hybridization images clearly show that the non-coding RNAs produced from the centromeres on chromosomes 2 and 3 (cen2 and cen3) are nuclear, localizing to distinct foci within the region of DAPI-stained genomic DNA (Fig. 3A). Although the cen RNAs were almost exclusively nuclear (>90%), hybridization to kahrp mRNA gave signals that were primarily outside the nucleus (Fig. 3B). To determine whether centromeric RNAs colocalized into a specific position within the nucleus, two-color RNA FISH was performed using RNA probes for the centromeres on both chromosomes 2 and 3 (Fig. 3A, third column). There was no discernible pattern to where in the nucleus the non-coding RNAs were located, suggesting that there is no specific subnuclear location in which the centromeres reside.

Subnuclear Localization of the Centromeric Non-coding Transcripts Is Protein-dependent—The localization of the centromeric ncRNAs within the nucleus may be the result of base pairing of the transcripts with the chromosomal DNA or, alternatively, incorporation into the protein structure of the chromatin assembled within this region of the chromosome. To determine whether disruption of the protein structure of the chromatin altered or eliminated the localization of the ncRNAs, RNA FISH was carried out with or without the addition of SDS/Triton X-100 as described previously (39). The smears were probed again with the Cen2R probe. RNA FISH performed without the addition of SDS/Triton X-100 resulted in positive signals as expected (Fig. 4). However, with the addition of the detergent the signal was lost. SDS/Triton X-100 treatment did not disrupt hybridization using DNA FISH, and there was no change in signal intensity between the slides treated or untreated with detergent, indicating the treatment did not disrupt the chromosomal DNA. In addition, DNA FISH typically resulted in smaller, sharper spots, suggesting that the larger, more diffuse spots observed by RNA FISH may be the result of hybridization to several RNA molecules at each centromere.

Disruption of Centromeres Using Etoposide Results in Loss of Centromeric ncRNA—To confirm that the ncRNAs transcribed from the centromeres are in fact associating with centromeric DNA sequences, we utilized the fact that treatment of cells with...
the topoisomerase II inhibitor etoposide results in cleavage of chromosomal DNA and disruption of centromeres (28). RNA FISH was performed on parasites grown in complete medium with or without etoposide using Cen2R RNA probes labeled with fluorescein. As a control, a probe to a small nuclear RNA (PFC0358c) that should not be affected by etoposide was used. As shown in Fig. 5, the centromeric non-coding RNAs were easily detectable in parasites prior to etoposide treatment; however, this signal was lost after treatment. The presence of a small nuclear RNA signal in both the untreated and treated slides indicates that the etoposide treatment specifically disrupts either the localization or the production of the centromeric non-coding RNAs while not affecting other RNAs found in the nucleus. These results indicate that the ncRNAs transcribed from the centromeres of *P. falciparum* likely bind to the centromeres themselves, consistent with previously proposed models of the role of ncRNAs in the assembly of specific chromatin structure (2, 4).

**DISCUSSION**

In protozoans, little is known about chromosomal organization, including centromere identity and structure. In this report, we lay the groundwork for the study of ncRNAs and their association with centromeric chromatin in *P. falciparum*. We have identified short ncRNAs of discrete length that are transcribed from centromeric sequences and are found exclusively in the nucleus. Additionally, we have demonstrated that the ncRNAs associate with centromeric chromatin likely via a protein interaction. This is the first time the cellular localization and chromatin associations of ncRNAs have been described in *P. falciparum*.

It is noteworthy that all of the ncRNAs appear as short, discrete bands of similar sizes (either ~75 or 175 nucleotides in length). Although the apparent consistent, discrete sizes of the detected transcripts suggest that the *P. falciparum* centromeric RNAs are processed, the genome does not contain homologues to any of the known factors involved in the micro-RNA or RNAi pathways. Our data therefore might provide the first indication that this protozoan may have a different, yet analogous system of non-coding RNA processing.

The observation that transcripts from centromeric repeats localize to the nucleus and associate with nuclear proteins is consistent with studies of centromeric ncRNAs from other organisms. In fission yeast, maize, and mammals, the ncRNAs associate with the centromeric regions where the centromere-specific histone H3 is recruited. This association, in turn, has been shown to result in methylation of histone H3K9 and formation of pericentromeric heterochromatin in *Schizosaccharomyces pombe* (40). The common theme among our results and the other reports of centromeric ncRNAs is that these RNAs most likely act via protein and/or RNA-RNA interactions, rather than by base pairing with DNA. This distinction is notable, for there are examples of repetitive non-coding transcripts (for example, those that initiate Ig class-switch recombination in humans and mice) that form RNA-DNA hybrids and higher order chromatin structures (41).

Even in cases where the existence of centromeric ncRNAs has been established, the mechanisms that lead to transcription of the centromeric repeats are not clear. There is an apparent paradox in that centromeres and pericentromeric repeats consist of heterochromatin, an environment that is defined as repressive to transcription. Nonetheless, transcription is necessary for the establishment and maintenance of this heterochromatic state (11, 16, 42, 43). How are the transcribed regions defined, and how is transcription initiated in presumably heterochromatic areas of such low sequence complexity (~97% A/T content in the centromeric regions of *P. falciparum*? The results of our reporter genes assays suggest that the centromeric RNAs are primarily transcribed at a point in the cell cycle when the chromosomes replicate and segregate into dividing nuclei and then persist throughout the remainder of the cell cycle, probably associated with the centromeres themselves. Thus the centromeric promoters would be highly active only when the structure of the chromosomes and the organization of the dividing nuclei are being organized. In fission yeast, a heterochromatin-associated protein was shown to promote RNA polymerase II accessibility to centromeric sequences (44), resulting in the production of transcripts that go on to be processed by the RNAi machinery and are necessary for the recruitment of the heterochromatin structural protein Swi6/HP1 (11, 43). The *P. falciparum* genome, although lacking in specific transcription factors and RNAi components, does contain homologues to histone-modifying enzymes, chromodomain proteins (such as HP1), and chromatin assembly factors, and therefore malaria parasites are likely to use a similar mechanism to maintain their centromeres.

Although the details of ncRNA function at centromeres may differ widely among species, the various mechanisms may converge at the level of higher order chromatin-organizing factors. Employing RNA-protein and RNA-RNA interactions to specify centromere structure may have allowed eukaryotic cells to cir-
cumvent problems implied in coding functional elements in the medium of rapidly evolving centromeric DNA. In *P. falciparum*, this idea is supported by the fact that the centromeric regions do not share DNA sequence homology, but they are characterized by A/T-rich tandem repeat sequences unique to each chromosome. It is possible that the secondary structure of the transcripts from these sequences is more important than the sequence itself. The transcripts may then function as beacons for the recruitment of chromatin-modifying complexes.

The extent to which ncRNAs and alterations in chromatin structure affect gene expression, chromosome segregation, and other nuclear processes in *P. falciparum* is only just beginning to be discovered. The centromeres may provide a useful model for deciphering the mechanisms underlying these phenomena. By gaining insights into how parasites organize their genomes and control gene expression, it may be possible to design strategies to better manipulate these systems and potentially control some aspects of the disease. In addition, knowledge of how these processes are regulated in lower eukaryotes will shed light on the evolutionary conservation of fundamental aspects of genome organization.

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REFERENCES

1. Moreno Diaz de la Espina, S., Alverca, E., Cuadrado, A., and Franca, S. (2005) *Eur. J. Cell Biol.* 84, 137–149
2. Morey, C., and Avner, P. (2004) *FEBS Lett.* 567, 27–34
3. Lewis, A., and Reik, W. (2006) *Curr. Top. Microbiol. Immunol.* 310, 117–140
4. Cooke, H. J. (2004) *Trends Biotechnol.* 22, 319–321
5. Karpen, G. H., and Allshire, R. C. (1997) *Trends Genet.* 13, 89–96
6. Kirk, C. W., and Allshire, R. C. (2005) *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 360, 569–579
7. Carroll, C. W., and Straight, A. F. (2006) *Trends Cell Biol.* 16, 70–78
8. Volpe, T., Schramke, V., Hamilton, G. L., White, S. A., Teng, G., Martienssen, R. A., and Allshire, R. C. (2003) *Chromosome Res.* 11, 137–146
9. Chen, E. S., Saitoh, S., Yanagida, M., and Takahashi, K. (2003) *Mol. Cell* 11, 175–187
10. Nakano, M., Okamoto, Y., Ohzeki, I., and Masumoto, H. (2003) *J. Cell Sci.* 116, 4021–4034
11. Valgardsdottir, R., Chiodi, I., Giordano, M., Cobianchi, F., Riva, S., and Biamonti, G. (2005) *Mol. Biol. Cell* 16, 2597–2604
12. Topp, C. N., Zhong, C. X., and Dawe, R. K. (2004) *Proc. Natl. Acad. Sci. U. S. A.* 101, 15986–15991
13. Maison, C., Bailly, D., Peters, A. H., Quivy, J. P., Rocche, D., Taddei, A., Lachner, M., Jenuwein, T., and Almouzni, G. (2002) *Nat. Genet.* 30, 329–334
14. Malhotra, P., Dasaradhi, P. V., Kumar, A., Mohmmed, A., Agrayal, N., Bhatnagar, R. K., and Chauhan, V. S. (2002) *Mol. Microbiol.* 45, 1245–1254
15. Aravind, L., Iyer, L. M., Wellems, T. E., and Miller, L. H. (2003) *Cell* 115, 771–785
16. Couolson, R. M., Hall, N., and Ouzounis, C. A. (2004) *Genome Res.* 14, 1548–1554
17. Cook, C. R., Kung, G., Peterson, F. C., Volkman, B. F., and Lei, M. (2003) *J. Biol. Chem.* 278, 36051–36058
18. Su, X., Heatwole, V. M., Wertheimer, S. P., Guinet, F., Herrfeldt, J. V., Peterson, D. S., Ravetch, J. V., and Wellems, T. E. (1995) *Cell* 82, 89–100
19. Kyes, S. A., Christidoulou, Z., Raza, A., Horrocks, P., Pinches, R., Rowe, J. A., and Newbold, C. I. (2003) *Mol. Microbiol.* 48, 1339–1348
20. Le Roch, K. G., Zhou, Y. Y., Blair, P. L., Grainger, M., Moch, J. K., Haynes, J. D., De la Vega, P., Holder, A. A., Batalov, S., Carucci, D. J., and Winzeler, E. A. (2003) *Science* 301, 1503–1508
21. Fidock, D. A., Bottius, E., Brhimi, K., Moelans, I. I., Aikawa, M., Konings, R. N., Certa, U., Olafsson, P., Kaidoh, T., Asavanchich, A., Guerinmarchand, C., and Drulhe, P. (1994) *Mol. Biochem. Parasitol.* 64, 219–232
22. Chen, Q., Fernandez, V., Sundstrom, A., Schlichterle, M., Datta, S., Hagblom, P., and Wahlgren, M. (1998) *Nature* 394, 392–395
23. Gunasekera, A. M., Patankar, S., Schug, J., Eisen, G., Kissing, J., Roos, D., and Wirth, D. F. (2004) *Mol. Biochem. Parasitol.* 136, 35–42
24. Patankar, S., Munasinghe, A., Shoaib, A., Cummings, L. M., and Wirth, D. F. (2001) *Mol. Cell Biol.* 21, 3114–3125
25. Kelly, J. M., McRobert, L., and Baker, D. A. (2006) *Proc. Natl. Acad. Sci. U. S. A.* 103, 6706–6711
26. Trager, W., and Jensen, J. B. (1976) *Science* 193, 673–675
27. Lambros, C., and Vanderberg, J. P. (1979) *J. Parasitol.* 65, 418–420
28. Trager, W., and Jensen, J. B. (1977) *Bull. W. H. O.* 55, 363–365
29. Wu, Y., Sifri, C. D., Lei, H.-H., Su, X., and Wellems, T. E. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 973–977
30. Deitsch, K. W., Driskill, C. L., and Wellems, T. E. (2001) *Nucleic Acids Res.* 29, 850–853
31. Fidock, D. A., and Wellems, T. E. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 10931–10936
32. Kyes, S. Pinches, R., and Newbold, C. (2000) *Mol. Biochem. Parasitol.* 105, 311–315
33. Kelly, L. H., Bottius, E., Pirrit, J. A., Deitsch, K. W., Scheidig, C., Guinet, F., Nezhbass, U., Wellems, T. E., and Scherf, A. (2000) *Nature* 407, 1018–1022
34. Thompson, J. (2002) *Methods Mol. Med.* 72, 225–233
35. Wickstead, B., Ersfeld, K., and Gull, K. (2003) *Microbiol. Mol. Biol. Rev.* 67, 360–375
36. Marty, A. J., Thompson, J. K., Duffy, M. F., Voss, T. S., Cowman, A. F., and Crabb, B. S. (2006) *Mol. Microbiol.* 62, 72–83
37. Ekbakk, K. (2004) *Chromosome Res.* 12, 535–542
38. Mizuta, R., Mizuta, M., and Kitamura, D. (2005) *J. Electron Microsc.* 54, 403–408
39. Guenat, M., Bailly, D., Maisen, C., and Almouzni, G. (2004) *J. Cell Biol.* 166, 493–505
40. Volpe, T. A., Kidner, C., Hall, I. M., Teng, G., Grewal, S. I., and Martienssen, R. A. (2002) *Science* 297, 1833–1837
41. Zofall, M., and Grewal, S. I. (2006) *Mol. Cell* 22, 681–692
42. Gerbi, S. A., and Lange, T. S. (2002) *Mol. Biol. Cell* 13, 3123–3137