DNA helicase RecQ1 regulates mutually exclusive expression of virulence genes in *Plasmodium falciparum* via heterochromatin alteration

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The *Plasmodium falciparum var* gene family encodes ∼60 surface antigens by which parasites escape the host immune responses via clonal expression of *var* genes. However, the mechanism controlling this mutual exclusivity, associated with alterations in chromatin assembly, is not understood. Here, we determined how expression of the *var* gene family is regulated by two RecQ DNA helicase family members, PfRecQ1 and PfWRN, in *P. falciparum*. Through genetic manipulation, we found that the complete *var* repertoire was silenced on *PfRecQ1* knockout, whereas their expression did not show noticeable changes when *PfWRN* was knocked out. More important, mutually exclusive expression of *var* genes could be rescued by complementation of PfRecQ1. In addition, knocking out either of these two helicase genes changed the perinuclear cluster distribution of subtelomeres and subtelomeric *var* genes. Whereas deletion of *PfRecQ1* increased the heterochromatin mark trimethylated (H3K9me3) at the transcription start site (TSS) of the *var* gene *upsC1*, that deletion had no effect on the global distribution of H3K9me3 over gene bodies, including those for the *var* genes. ChIP-seq assay showed that PfRecQ1 was enriched globally at the TSSs of all genes, whereas PfWRN-enriched regions occurred at the gene bodies of the *var* gene family, but not of other genes or at TSSs of all genes. On *PfRecQ1* deletion, the *upsC1 var* gene moved from the active perinuclear transcription region to a silenced region of the *upsC* type. These findings imply that PfRecQ1, but not PfWRN, is essential for maintaining the clonal expression of *var* genes.

DNA helicases | heterochromatin | virulence gene | mutually exclusive expression | Plasmodium falciparum

Malaria is an infectious disease that presents a serious threat to human life and health (1). The World Health Organization reported that ∼3.2 billion people are currently at risk for malaria (2), and among the human malaria parasites, *Plasmodium falciparum* is the deadliest. The surface variant antigen *P. falciparum* Erythrocyte Membrane Protein 1 (PfEMP1), encoded by ∼60 *var* genes, mediates binding of parasitized erythrocytes to endothelial surface and contributes to the major pathogenesis in severe malaria. *var* genes are classified into three major types, *upsA*, *upsB*, and *upsC*, depending on their promoter structures. Their chromosomal locations vary: *upsA* and most of *upsB var* genes are at the subtelomeric regions, whereas all *upsC* and a few *upsB var* genes are at the central regions of the chromosomes (3). Through mutually exclusive expression of *var* genes, *P. falciparum* escapes the host immune responses and parasite killing (4–8). The underlying molecular mechanism of *var* gene regulation is incompletely understood, but it is known to involve epigenetic factors such as specific alterations in histone modifications and chromatin assembly (8, 9).

The RecQ protein family is a highly conserved group of DNA helicases, the members of which play important roles in DNA replication, transcription, repair, recombination, and telomere

maintenance (10–12). Five family members have been identified in humans: RecQ1, RecQ2 (BLM), RecQ3 (WRN), RecQ4, and RecQ5 (11, 12). In human embryonic stem cells, WRN can associate with heterochromatin proteins SUV39H1, HP1α, and nuclear lamina-heterochromatin anchoring protein LAP2β, and WRN deficiency results in a global loss of trimethylated histone H3K9 (H3K9me3) associated with changes in heterochromatin architecture (13). *P. falciparum* contains only two members of the RecQ family: PfWRN, encoded by the PF3D7_1429900 gene, and PfRecQ1 (also named PfBLM), encoded by the PF3D7_0918600 gene (14). Both proteins possess an ATPase and a 3′–5′ direction-specific DNA helicase activity (15, 16). Recently, it has been reported that both PfRecQ1 and PfWRN may play roles in genome stability, gene expression patterns, and DNA replication dynamics (17). However, the molecular mechanisms of the two helicases in *var* gene regulation remain unclear.

Here we report on the effect of selectively deleting the *PfRecQ1* or *PfWRN* gene on epigenetic features of the *var* genes in *P. falciparum*. We find that transcription of the previously activated *var* gene was dramatically reduced in the *PfRecQ1*-knockout parasite strains (PfRecQ1Δ), but not in the *PfWRN*-knockout strain

(PfWRNΔ). The phenotype of the clonal *var* expression could be successfully rescued by complementation of PfRecQ1. We show that knockout of either helicase gene resulted in more accumulated perinuclear distribution of subtelomeric *var* genes, whereas PfRecQ1Δ led to increase of H3K9me3 at the transcription start site (TSS) of the upsC1 *var* gene, concomitant with the inactivation of this *var* gene. ChIP-seq assay showed that PfRecQ1 was enriched globally at the TSSs of all genes. In contrast, PfWRN-enriched regions were seen at the gene bodies of the *var* gene family, but not of other genes or at TSSs of all genes. In addition, on PfRecQ1 deletion, upsC1 *var* gene moved from the active perinuclear transcription region to a silenced region of the *upsC* type. This study implies an essential role for PfRecQ1 in the maintenance of *var* expression in clonal *P. falciparum* strains, possibly through decreasing the amount of silence mark H3K9me3 at the TSS and keeping a perinuclear localization of the active *var* gene in the active expression site.

Results

PfRecQ1Δ Completely Abolishes Expression of the *var* Gene Family.
PfRecQ1 and PfWRN are two known PfRecQ helicase members.

Knockout strains of these two genes were generated separately, using the CRISPR/Cas9 system (*SI Appendix*, Fig. S1A), and confirmed by PCR (*SI Appendix*, Fig. S1B) and DNA sequencing of the PCR products. Transcriptomic analysis of the knockout parasites at the ring stage by RNA sequencing revealed that the deletion of either PfRecQ1 or PfWRN altered the expression of many genes (*SI Appendix*, Fig. S1 C–H and Tables S1 and S2). Notably, the transcription of genes implicated in the DNA replication pathway dramatically increased (*SI Appendix*, Fig. S1 G and H), consistent with the roles of both PfRecQ gene members in DNA replication. With regard to down-regulated genes, there was no particular gene family except the *var* genes. PfRecQ1Δ, in particular, markedly inhibited the dominantly expressed *var* gene (PF3D7_1240600, upsC1) in the 3D7C8 (WT) strain, leading to the overall silencing of the entire *var* gene family (*SI Appendix*, Fig. S1E). Such a drastic silencing effect was not observed in the PfWRNΔ strain (*SI Appendix*, Fig. S1F).

To confirm that the silencing of the *var* gene family was the direct result of *PfRecQ1* knockout, RT-qPCR for each *var* gene was carried out on multiple clones from different transfection
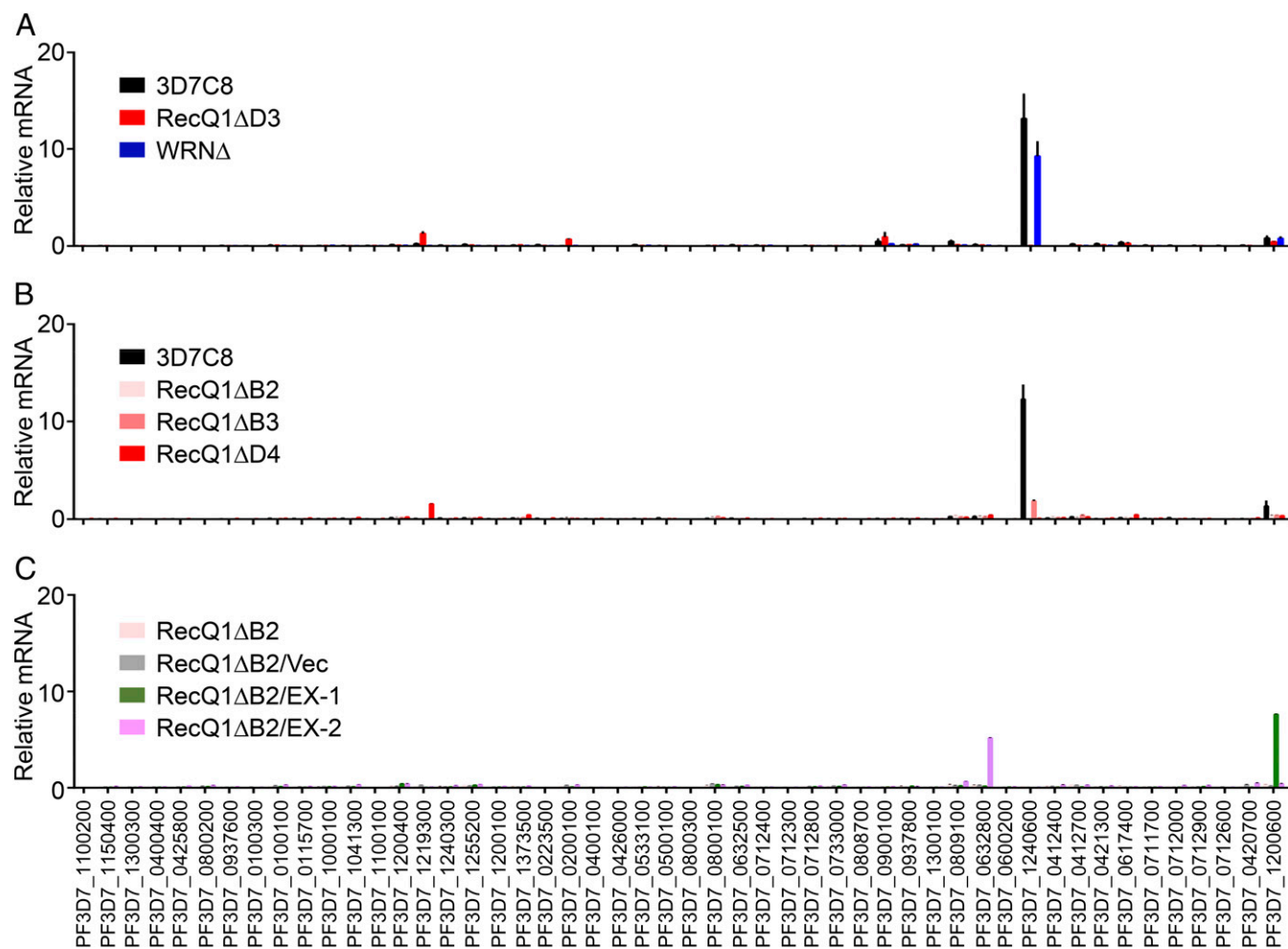

Fig. 1. Expression status of *var* genes on knock-out of *PfRecQ1* or *PfWRN*. *Var* genes expression level (A) in WT 3D7C8, PfRecQ1Δ (strain D3), and PfWRNΔ were detected by RT-qPCR with five repeats, and (B) in different strains of PfRecQ1Δ with at least two repeats. PfRecQ1ΔD4 and PfRecQ1ΔD3 were clones from first transfection into 3D7C8 (PF3D7_1240600, dominantly expressed); PfRecQ1ΔB2 and PfRecQ1ΔB3 were clones from second transfection into 3D7C8. 3D7C8 is the WT parasite strain. (C) In PfRecQ1ΔB2, PfRecQ1ΔB2/vec, PfRecQ1ΔB2/EX-1, and PfRecQ1ΔB2/EX-2. PfRecQ1ΔB2/vec was PfRecQ1ΔB2 transfected with PCC4-G418 vector, as control of complementation transfection. PfRecQ1ΔB2/EX-1 and PfRecQ1ΔB2/EX-2 were PfRecQ1ΔB2 transfected with PCC4-TY1-FlagRecQ1-G418 plasmid to episomally express TY1-FlagPfRecQ1 to rescue the functions of PfRecQ1. PfRecQ1ΔB2/EX-1 and PfRecQ1ΔB2/EX-2 were results from twice-independent complementation transfections. Experiments were repeated three times. The *y* axis gives the relative abundance of *var* genes mRNA; *var* genes IDs are shown below the *x* axis. Arginyl-tRNA synthetase (PF3D7_0913900), a housekeeping gene, is used as internal control. Error bars represent SEM.

experiments. PfRecQ1Δ D4 and D3 are clones from the first transfection of 3D7C8, which dominantly expressed the *upsC1 var* gene PF3D7_1240600. B2 and B3 are clones from another transfection of 3D7C8, whereas D9 is a clone from transfection of 3D7G4, which dominantly expressed another *upsC var* gene, PF3D7_0711700. PfRecQ1Δ consistently silenced the entire *var* repertoire in all these strains (Fig. 1 *A* and *B* and *SI Appendix*, Fig. S2).

To further confirm the specific silencing effect of PfRecQ1Δ on the *var* gene family, phenotypic rescue experiments were performed, where the PCC4-^{TY1-Flag}RecQ1-G418 plasmid was transfected into one PfRecQ1Δ strain to episomally express

^{TY1-Flag}PfRecQ1 (*SI Appendix*, Fig. S3). In two independent transfection experiments (named PfRecQ1ΔB2/EX-1 and PfRecQ1ΔB2/EX-2), we observed rescue of clonal *var* expression in the complementation strains (Fig. 1C). As expected, this phenotypic rescue was not observed in the control parasite PfRecQ1ΔB2/Vec that carries the plasmid PCC4-G418 lacking *PfRecQ1* (Fig. 1C). Interestingly, the *var* genes rescued in these two complementation experiments were different (Fig. 1C), with one being PF3D7_0632800 (a *upsB* type) and the other PF3D7_1200600 (a *upsE* type). These results demonstrated that PfRecQ1 was essential for maintaining expression of the active
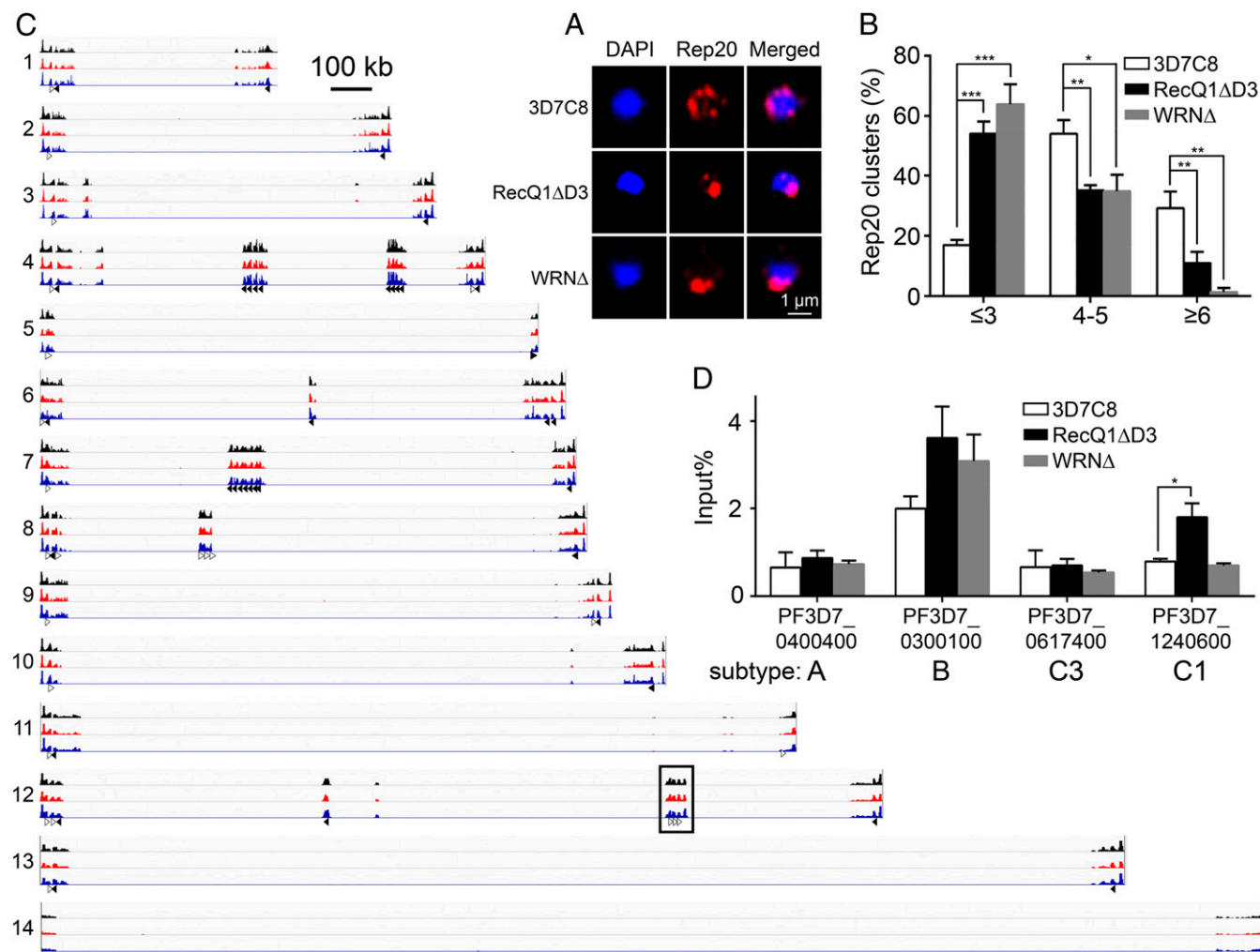

Fig. 2. H3K9me3 modification and DNA-FISH of subtelomeric *var* genes on PfRecQ1Δ or PfWRNΔ. (*A*) Rep20 DNA-FISH in WT 3D7C8, PfRecQ1ΔD3, and PfWRNΔ. Rep20 probe was labeled with Biotin and then incubated with streptavidin (Alexa Fluor 568 conjugate, Thermo Fisher, red); chromosomal DNA was stained with DAPI (blue), representing the nuclear area. Parasites synchronized at ring stage were used for this DNA-FISH. Resolution of *A* is 600 dpi. (*B*) Graphic representation of the statistical analysis of Rep20 DNA-FISH result. The experiment was repeated three times, each with a count of 100 nuclei at ring stage. Nuclei were classified as type 1 (1–3 foci/nucleus), type 2 (4–5 foci/nucleus), or type 3 (≥6 foci/nucleus). The percentage of each type per 100 nuclei was calculated. (Scale bars of all pictures of DNA-FISH, 1 μm.) Here all error bars were SEM, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. (*C*) Integrative genomic view of ChIP-seq result of H3K9me3 at ring stage in *P. falciparum*. In parasites of WT-3D7C8 (black), RecQ1ΔD3 (red), and WRNΔ (blue) at ring phase, H3K9me3 bound specially to sequences of telomeres and *var* genes. *Var* genes are categorized as being in the forward orientation (unfilled arrows) or reverse orientation (filled arrows). Telomere sequences were at the end of chromosomes, which were not labeled. ChIP was performed with the anti-H3K9me3 antibody twice, and the average was used for this IGV. Each read was normalized by the total number of uniquely mapped ChIP-seq reads. Values on the *y* axis were calculated by ChIP reads divided by input reads, representing the net signal of ChIP. Chromosomal numbers are shown to the left. Two *var* genes in the boxed region are amplified for a detailed view in *SI Appendix*, Fig. S4C. A scale bar representing 100 kilobases (kb) is shown to the right of chromosome 1. The data range on the *y* axis used for IGV here was 0–40. (*D*) ChIP-qPCR of H3K9me3. The primers at the TSS of four *var* genes were used for this assay. PF3D7_0400400 is a *upsA* type, also used as *A1 var* gene for paired DNA-FISH. PF3D7_0300100 is a *upsB* type *var* gene. PF3D7_0617400 is a *upsC* type, named as *C3*. PF3D7_1240600, is another *upsC* type, also used as *C1 var* gene for paired DNA-FISH. *Var A1*, *B*, *C3* genes are silent in WT, PfRecQ1Δ, and PfWRNΔ; *Var C1* is dominantly transcribed in WT or PfWRNΔ, but silent in PfRecQ1Δ. Primers for ChIP-qPCR are shown in *SI Appendix*, Table S3. The experiment was repeated five times. The *P* value of *upsC1* between WT and PfRecQ1Δ was 0.013, whereas the others were >0.05 (no significant difference).

var genes in clonal parasites, and its effect was not specific to the *upsC* type. In contrast to PfRecQ1Δ, PfWRNΔ did not affect *var* gene expression, and the dominantly expressed *upsC1 var* gene in WT 3D7C8 was still transcribed (Fig. 1*A*).

PfRecQ1Δ Alters Heterochromatin. Subtelomeric regions of the *P. falciparum* chromosomes are clustered into several foci at the perinuclear space, where the *var* genes locate. The transcription status of *var* genes is related to their spatial distribution in the perinuclear area (18). To obtain a mechanistic understanding of the influence of PfRecQ proteins on *var* gene expression, we first studied the perinuclear distribution of the subtelomer *var* genes. For this analysis, we used DNA-FISH, targeting Rep20, a conserved repetitive sequence present at the subtelomeric regions of all *P. falciparum* chromosomes. Because Rep20 flanks subtelomeric *var* genes, its spatial distribution mimics that of subtelomeric *var* genes (19). Consistent with earlier observations (20), chromosome ends in WT parasites at the ring stage typically formed 4–7 Rep20 clusters. Deletion of either PfRecQ DNA helicase resulted in significant clumping of the chromosomal ends. Parasites containing 4–5 and ≥6 Rep20 foci greatly

decreased, whereas the proportions of parasite nuclei containing 1–3 Rep20 foci increased from 17% in WT to 54% and 64% in PfRecQ1Δ and PfWRNΔ, respectively (Fig. 2 *A* and *B*). This result suggests that each of the two helicases might, directly or indirectly, regulate the spatial distribution of subtelomeric *var* genes, reminiscent of our hypothesis that PfRecQ1's regulation on *var* genes is not specific to *upsC* type.

In humans, the WRN helicase is important for maintaining global distribution of H3K9me3 and heterochromatin architecture (13). In *P. falciparum*, H3K9me3 is known to repress *var* gene expression (21). Therefore, we next wanted to determine whether the two helicase genes in *P. falciparum* were also involved in maintaining chromatin structure. ChIP and sequencing (ChIP-seq) analysis showed that H3K9me3 at the ring stage is specifically enriched at telomeric sequences and *var* genes in the WT 3D7C8 strain (Fig. 2*C*). In the PfRecQ1Δ and PfWRNΔ strains, there were no changes of H3K9me3 distribution on gene bodies globally (*SI Appendix*, Fig. S4 *A–C*). Given that H3K9me3 alteration at the TSS is generally more critical for gene silencing (8, 22), we further evaluated H3K9me3 at TSS of *var* genes. ChIP-qPCR was used to circumvent the problem
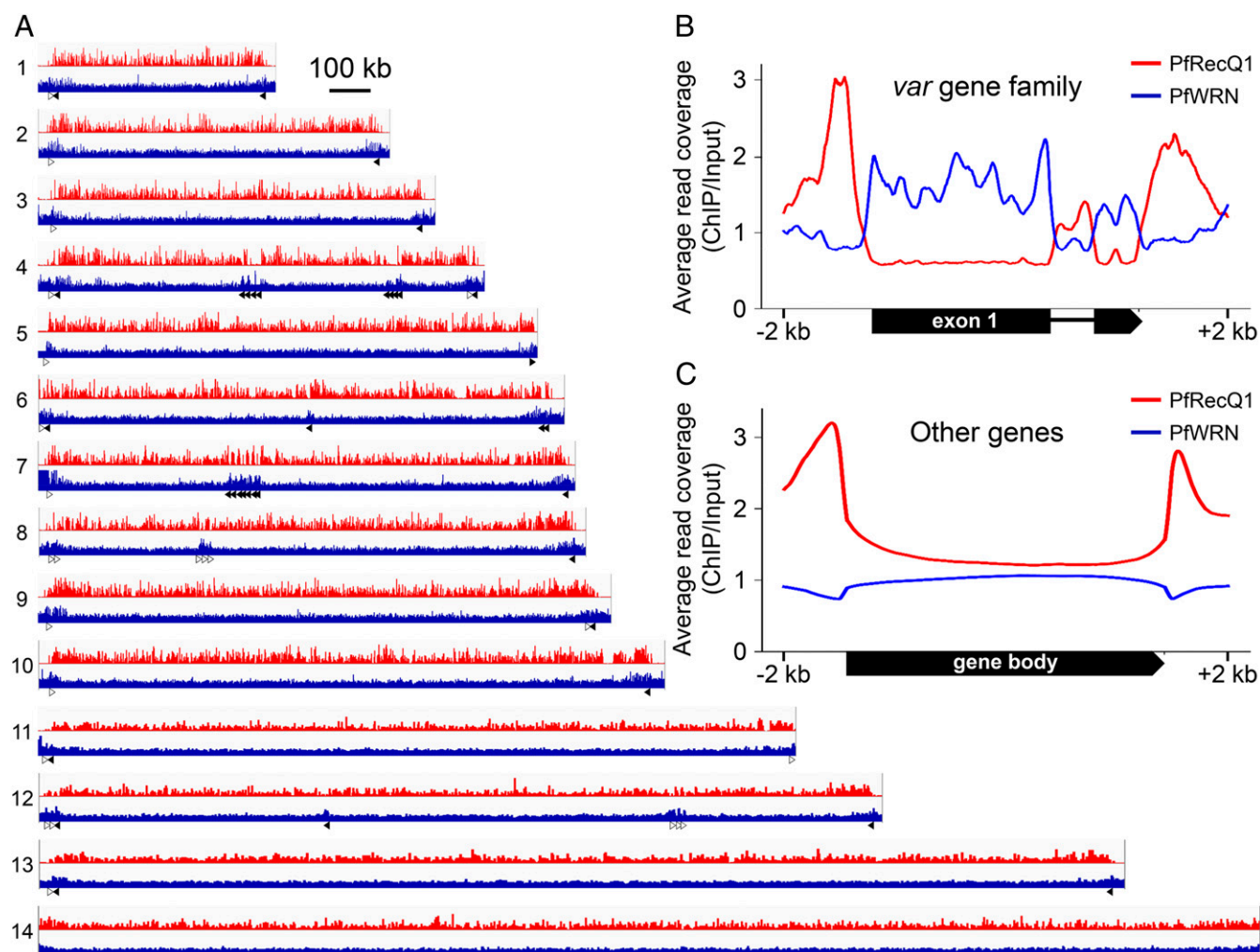

Fig. 3. Localization of PfRecQ1 and PfWRN proteins to *P. falciparum* chromatins. (*A*) Integrative genomic view of ChIP-seq result of [HA-Flag]RecQ1 (red) and [GFP]WRN (blue). Anti-Flag and anti-GFP were used for ChIP of PfRecQ1 and PfWRN, respectively. *y* axis values were calculated using each ChIP signal as the numerator and the respective signal input as the denominator. Sixty *var* genes distributed along *P. falciparum* chromosomes 1–13 are indicated by forward orientation (unfilled) and reverse orientation (filled) arrows. The data range of values on the *y* axis used for RecQ1 and WRN was 0–15 and 0–3, respectively. (*B* and *C*) Average reads coverage profile of [HA-Flag]RecQ1 (red line) and [GFP]WRN (blue line) at gene bodies of (*B*) *var* gene family and (*C*) other genes (nonvar genes). The curves were normalized per million mapped reads. *y* axis values were calculated as each ChIP signal divided by its respective input signals.

associated with the high sequence similarity in the untranslated regions of the *var* genes (8). The result revealed that the H3K9me3 level at the TSS of *upsC1 var* gene was markedly increased when this gene became silenced on PfRecQ1Δ (Fig. 2D), whereas we saw no significant changes of the H3K9me3 levels at the TSS of the other three selected *var* genes. In contrast, the H3K9me3 levels at the TSS of four representative *var* genes did not change in the PfWRNΔ line compared with those in the WT parasites.

PfRecQ1 and PfWRN Display Disparate Chromosomal Localizations.

To determine whether the observed differential global effects of the helicase gene knockouts on *var* genes were a result of their distinct associations with chromosomal regions, we established parasite lines with PfRecQ1 and PfWRN tagged at their N termini with 3×HA-Flag and GFP, respectively, in 3D7 parasites (named [HA-Flag]RecQ1 and [GFP]WRN), using the CRISPR/Cas9 system (*Methods* and *SI Appendix*, Fig. S5A). Tagging of these two genes was confirmed by PCR (*SI Appendix*, Fig. S5B) and sequencing on the PCR products, as well as by Western blotting to detect the tagged proteins (*SI Appendix*, Fig. S5C). ChIP-seq analysis of the [HA-Flag]RecQ1 strain showed genomewide enrichment of PfRecQ1 at the TSS sequences of all genes, including the *var* genes, suggesting the involvement of PfRecQ1 in transcription. In contrast, PfWRN was not associated with the TSS of *P. falciparum* genes, but instead was enriched only at the gene bodies of the *var* gene family (Fig. 3).

The Active *var* Gene Is Relocated to a Silent Perinuclear Site on PfRecQ1Δ.

In light of the spatial clumping of chromosomal ends in the knockout strains, we further aimed to investigate whether the relative positions of different *var* gene types were also changed. For this purpose, we selected different *var* gene types for paired DNA-FISH analysis, including *upsC1*, the predominantly expressed *var* gene in WT 3D7 but silenced in PfRecQ1Δ, PF3D7_0400400 (*upsA1*), PF3D7_0115700 (*upsB1*), and PF3D7_0412400 (*upsC2*) (Fig. 4A). The result showed that *upsC1* was predominantly not colocalized with those of *upsA1* or *upsB1* in both WT 3D7 and PfRecQ1Δ, suggesting the spatial distribution of *upsC1* relative to the *upsA* or *upsB* type was unaffected by deletion of PfRecQ1 (Fig. 4 B and C). Interestingly, *upsC1* became predominantly colocalized with *upsC2* in PfRecQ1Δ (Fig. 4D). As expected, the perinuclear distribution of *upsC1* relative to other *var* genes was not changed in PfWRNΔ; *upsC1* was largely separated from the other three *var* genes tested in both WT 3D7 and PfWRNΔ (Fig. 4 B–D), consistent with its remaining dominant expression after *PfWRN* was knocked out. These results indicated that PfRecQ1 might regulate heterochromatin at TSS of *upsC1*, leading to its relocation from an active transcription region to a silenced region of the *upsC* type.

Discussion

RecQ family members play vital roles in many aspects of DNA metabolism. The deadliest malarial parasite, *P. falciparum*, possesses just two PfRecQ family DNA helicases, PfRecQ1 and PfWRN. In this work, we show that PfRecQ1 participates in the regulation of *var* gene expression; deletion of PfRecQ1 abolishes the monoallelic expression pattern of the *var* genes and results in complete silencing of *var* gene family, which could also be rescued by complementation of PfRecQ1. This role is restricted to PfRecQ1, as PfWRNΔ does not affect *var* expression, although both of these DNA helicase members are associated with parasite chromosomes.

The involvement of PfRecQ1 in *var* gene regulation appears not be restricted to a particular *var* gene. Regardless of which individual *var* genes were active in the parasite strains tested, PfRecQ1Δ resulted in their repression. Complementation studies confirmed the role of PfRecQ1 in the maintenance of *var* gene expression, as

episomally expressed PfRecQ1 restored the monoallelic expression pattern of *var* genes. Interestingly, the two complementation strains expressed two different *var* genes of the *upsB* and *upsE* types, respectively, instead of the original *upsC1* gene, further implying that PfRecQ1's role applies to the whole *var* gene family.

To elucidate the mechanisms by which PfRecQ family proteins regulate gene expression, we determined the association of PfRecQ proteins with chromosomal regions and their potential effects on chromatin structures. Both PfRecQ proteins were shown to regulate the spatial distribution of chromosomal ends; their deletion led to enhanced clustering of the subtelomeric regions containing *var* genes in perinuclear areas. Active and silent *var* genes are known to occupy distinct perinuclear sites, and *var* gene activation requires relocation to the active perinuclear site (18). Moreover, an active *var* from a telomere region could move to the silent sites enriched for telomeric *var*s when it is silenced. Consistently, our results showed that silencing of the active *upsC1 var* gene on PfRecQ1Δ is coupled with the redistribution of this gene from a transcriptionally active site to a
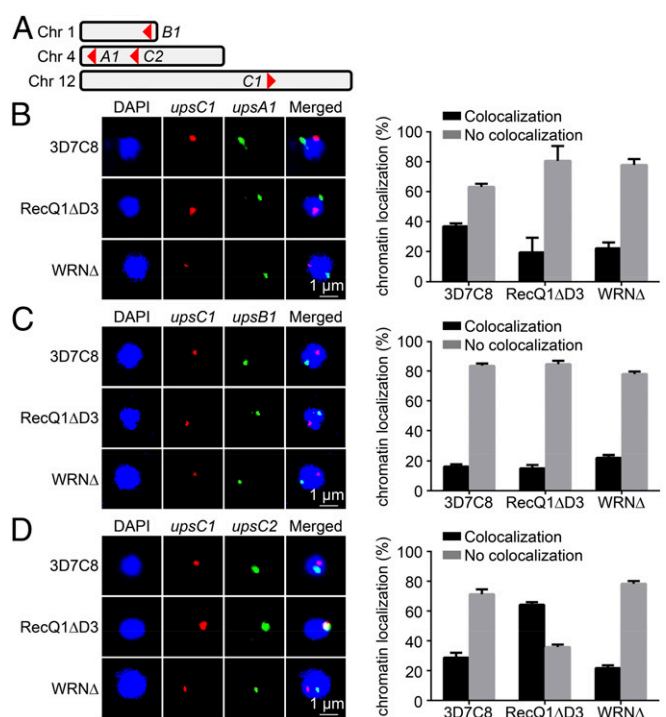

Fig. 4. *Var* gene DNA redistribution correlates with expression status on RecQ1Δ. (A) Localizations of four picked *var* genes for paired DNA-FISH in chromosomes. (B) Representative image of paired DNA-FISH of *upsA1* (PF3D7_0400400, green) with *upsC1* (PF3D7_1240600, red). In 3D7C8 and RecQ1Δ, *upsA1* was labeled with fluorescein, whereas *upsC1* was labeled with biotin. In WRNΔ, *upsA1* was labeled with biotin, whereas *upsC1* was labeled with fluorescein. (C) Representative image of paired DNA-FISH of *upsB1* (PF3D7_ 0115700, green) with *upsC1* (red). In all strains, *upsB1* was labeled with biotin, whereas *upsC1* was labeled with fluorescein. (D) Representative image of paired DNA-FISH of *upsC2* (PF3D7_0412400, green) with *upsC1* (red). In 3D7C8 and RecQ1Δ, *upsC2* was labeled with fluorescein and *upsC1* was labeled with biotin. In WRNΔ, *upsC2* was labeled with biotin and *upsC1* was labeled with fluorescein. Parasites synchronized at ring stage were used for this DNA-FISH. Biotin labeling was visualized by incubating with streptavidin-Alexa Fluor 568. DAPI staining (blue) was used to locate nuclear area. Graphs at right are statistical analyses of paired DNA-FISH results. Experiments were repeated three times, each with a count of ≥50 nuclei. The percentage of colocalization or no colocalization were calculated. Error bars were SEM. Scale bars of all pictures of DNA-FISH were 1 μm. In DNA-FISH of *upsC1* and *upsC2*, the P value of RecQ1Δ to 3D7C8 was 0.006. Resolution of B, C, and D is 600 dpi.

silent site. This silent site colocalized with the silent *upsC2* gene, but differed from the sites that the silent *upsA* and *upsB* genes occupied. This relocation could be directly related to the loss of PfRecQ1 protein on the loci, or indirectly to the absence of other active factors. Fine global mapping of the two helicases revealed that PfRecQ1 and PfWRN were associated with different chromosomal regions. Whereas PfRecQ1 had a global association with the TSS of all genes (including *var* genes), PfWRN had a more restricted role, enriched only in the gene bodies of *var* genes. This is consistent with the *var* gene silencing on deletion of PfRecQ1, but not PfWRNΔ. The silencing of *var* genes in PfRecQ1Δ was also associated with the enrichment at its TSS of H3K9me3, a mark of heterochromatin.

On the basis of these findings, we speculate that PfRecQ1 functions in the maintenance of clonal *var* expression. With its DNA helicase activity, PfRecQ1 may regulate the DNA conformation of *var* genes and be essential for keeping related genes within such active expression regions at the nuclear periphery, providing *var* gene access to transcription machinery in this active region to maintain its active transcription. Thus, it may be that if PfRecQ1 protein is deficient, the *var* gene DNA reverts to an inactive conformation and is relocated to silenced region at nuclear periphery. In such conformation, the originally active *var* gene cannot escape silencing because of the heterochromatin environment.

PfEMP1 is known to mediate cyto-adherence of infected red blood cells to host receptors, which is essential for the survival of the parasite (1). With regard to the global silencing of all *var* genes in PfRecQ1Δ parasites, PfRecQ1 could serve as a target for therapeutic development against malaria. Furthermore, the finding that PfRecQ1 is involved in *var* gene expression identifies another layer of the complex regulatory mechanisms of antigenic switching in malaria parasites.

Materials and Methods

Parasite Culture and Transfection. *P. falciparum* was cultured and synchronized following standard protocols (23). Ring-stage parasites were synchronized with Percoll-sorbitol and transfected with 100 μg plasmid DNA by electroporation (24). Transfected parasites were subjected to selection with the BSD and WR99210 drugs, which respectively targeted blasticidin S deaminase and human dihydrofolate reductase, to select parasites carrying both pL6CS and pUF1-BSD-Cas9. After resistant parasites emerged, genomic DNA was extracted for PCR validation, and parasites were cloned by limiting dilution.

The knockout and tagging of the *PfRecQ*1 and *PfWRN* genes were carried out separately, using the CRISPR/Cas9 system (*SI Appendix*, Figs. S1A and S5A), and was confirmed by PCR. For gene knockout, the entire 1,042-bp functional domain (210 bp away from ATG to 1,252 bp) of *PfRecQ*1 or 1,828 bp functional domain (167 bp away from ATG to 1,995 bp) of *PfWRN* was replaced by 5′-UTR-hdhfr-3′-UTR. Individual PCR was performed with genomic DNA as templates, and primer pairs P1/P2 or P3/P4 (*SI Appendix*, Fig. S1A). The knockout was confirmed by sequencing of the PCR products. For tagging, the coding sequences of HA-Flag or GFP were, respectively, fused to the N terminus of PfRecQ1 or PfWRN. Tagged parasites [HA-Flag]RecQ1 and [GFP]WRN were confirmed by PCR with genomic DNA and primer pairs P5/P6 and P7/P8, respectively (*SI Appendix*, Fig. S5 A and B), by sequencing of the PCR products, and by Western blotting (*SI Appendix*, Fig. S5C). All primers used are shown in *SI Appendix*, Table S3.

Complementation Assay. The PCC4-[TY1-Flag]RecQ1-G418 and PCC4-G418 plasmids were transfected into PfRecQ1ΔB2 strain to express [TY1-Flag]PfRecQ and be used as control, respectively. Two independent transfection experiments were performed for PCC4-[TY1-Flag]RecQ1-G418, named RecQ1ΔB2/EX-1 and RecQ1ΔB2/EX-2. Transfected parasites were subjected to G418 (neomycin) selection, which targets aminoglycoside phosphotransferase, to select parasites carrying the episomes of PCC4-[TY1-Flag]RecQ1-G418 or PCC4-G418. When resistant parasites emerged, ring-stage parasites were collected, and episomal expression of [TY1-Flag]PfRecQ1 was checked by RT-qPCRs of PfRecQ1 with primers P9/P10 (*SI Appendix*, Fig. S3 and Table S3) and confirmed by Western blotting with anti-TY1 antibody (Genscript).

Detailed Methods. RNA-seq and RT-qPCR, Western blot analysis, DNA-FISH, ChIP, next-generation sequencing, and data analysis are described in detail in *SI Appendix, Supplementary Materials and Methods*. All the raw data of next-generation sequencing in this manuscript have been deposited in NCBI's Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) and can be accessed through accession no. PRJNA506122 (25).

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