

## CHARACTERIZATION OF A *PLASMODIUM FALCIPARUM* MUTANT THAT HAS DELETED THE MAJORITY OF THE GAMETOCYTE-SPECIFIC Pf11-1 LOCUS

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*We identified a gametocyte-specific protein of Plasmodium falciparum called Pf11-1 and provide experimental evidence that this molecule is involved in the emergence of gametes of the infected erythrocyte (gametogenesis). A mutant parasite clone, which has deleted over 90% of the Pf11-1 gene locus, was an important control to establish the gametocyte-specific expression of the Pf11-1. Molecular analysis of the Pf11-1 deletion indicates that it is presumably due to a chromosome breakage with subsequent 'healing' by the addition of telomeric heptanucleotides. Moreover, similar DNA rearrangements are observed in most of the laboratory isolates during asexual propagation in vitro.*

Key words: chromosome breakage and healing – gametocyte specific antigen – telomere – HRPI – HRP II deletions

Over the last decade, the antigenic structure of *Plasmodium falciparum* has been subjected to intense investigation using hybridoma and recombinant DNA technologies. One motivation for these investigations has been the desire to identify protective antigens for inclusion in a subunit vaccine for *falciparum* malaria. A major problem complicating the antigenic analysis of *P. falciparum* has been the remarkable degree of immunological cross-reactivity among many malarial proteins. This cross-reactivity appears to be due to two motifs that occur frequently in the sequence of *P. falciparum* proteins: glutamic acid richness, especially the occurrence of gluglu dipeptides, and the presence of one or more domains of tandemly repeated, degenerate amino acid sequence that are strongly immunogenic in animals and humans. The degeneracies within the individual repeats of these domains appear to increase the probability of shared epitopes among *P. falciparum* proteins. Thus, the immune response to *P. falciparum* antigens often generates anti-

bodies that recognize several different malarial proteins.

One example of the immunological cross-reactivity described above is the Pf11-1 antigen of *P. falciparum*. The Pf11-1 gene is a large locus (> 30 kb) with a nucleotide sequence that predicts a glutamic acid rich polypeptide containing three blocks of tandem repeats of 3, 6 and 9 amino acids. Due to extensive cross-reactivity with other glu-rich, repetitive antigens, antibodies to Pf11-1 synthetic peptides or recombinant proteins react with multiple *P. falciparum* proteins (Mercereau-Puijalon et al., 1987; Mattei et al., 1989). This cross-reactivity has made it difficult to unambiguously identify the Pf11-1 gene product.

In the course of our studies on the Pf11-1 locus, we identified and characterized a mutant parasite with a large deletion in the Pf11-1 gene. The availability of this Pf11-1 deletion mutant provided an important negative control, allowing us to circumvent the problems of immunological cross-reactivity described above and to establish the gametocyte-specific expression of the Pf11-1 gene product. Although deletions

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within asexual stage genes of *P. falciparum* have been previously described, the Pf11-1 deletion appears to be the first example of deletion involving a sexual stage gene. We compare the Pf11-1 deletion with deletions in asexual stage genes and show that they have several characteristics in common, including their subtelomeric location and the presence of a "CA" dinucleotide at the chromosomal breakpoint site. Finally, we speculate on a possible mechanism for the healing of these deletions.

## RESULTS AND DISCUSSION

*Characterization of a mutant parasite with a large deletion in the Pf11-1 gene* – During a survey of Pf11-1 gene structure in several *P. falciparum* strains and clones, we identified a single subclone of the D6 strain (C9H12) which failed to hybridize with an oligonucleotide probe specific for the Pf11-1 27 bp repeated sequence. As all known Pf11-1 genes contain approximately 28 kb of this sequence organized in a single tandem repeat, it was of considerable interest to determine whether the observed lack of hybridization of C9H12 was due to an alteration in the sequence of the 27 bp repeats or, more probably, to deletion of, at least, that portion of the gene containing the repeated 27 bp motif. Genomic Southern and electrophoretic karyotype analysis using a unique sequence probe specific for the 5' terminus of Pf11-1 (11-1.5', Scherf et al., 1988) clearly indicated that C9H12 parasites had deleted approximately 100 kb of chromosome 10 including most of the Pf11-1 gene and that what remained of the Pf11-1 gene now resided in a telomere proximal location.

In order to isolate and clone DNA fragments spanning the deletion breakpoint we employed a polymerase chain reaction-based strategy (Fig. 1). Pf11-1 and telomere-specific synthetic oligonucleotides were used to prime PCR with C9H12 genomic DNA. A 1.4 kb fragment amplified by PCR was cloned in pUC13 and sequenced by the dideoxy technique. The sequence of the amplified fragment was identical to that of the 5' region of the Palo Alto Pf11-1 gene (Scherf et al., 1988) to nucleotide 1686 at which point it diverged completely; the remainder of the sequence was composed entirely of degenerate repetitions of the canonical *P. falciparum* telomere repeat sequence, AGGGTTC (Vernick & McCutchan, 1988). These results clearly indicate that approximately 28 kb of the Pf11-1 locus has been

deleted in C9H12 parasites, presumably due to a chromosome break within the gene which was "healed" by the addition of telomeric heptanucleotide repeats (Scherf et al., submitted).

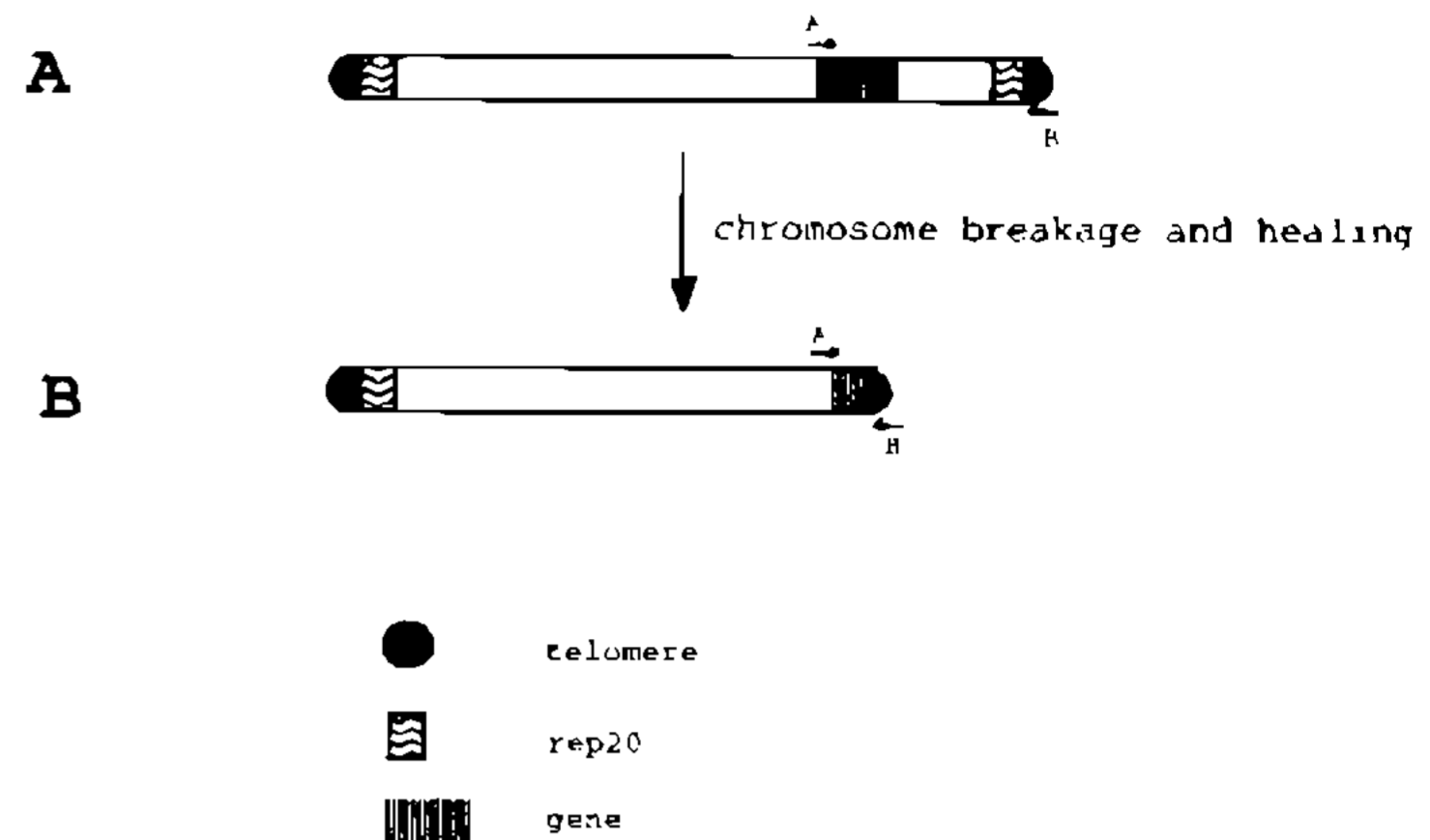


Fig. 1-A: schematic of a *Plasmodium falciparum* chromosome; telomeres are indicated by the region shaded black, subtelomeric rep20 repeats by wavy lines and a subtelomeric gene by a hatched box. B: chromosome breakage within the subtelomeric gene leads to the loss of the distal portion of the right chromosome arm and healing by addition of telomere repeats. Sense (gene) and antisense (telomere) PCR primers are indicated by arrows labeled A and B.

*Similar DNA rearrangements occur at several P. falciparum loci during asexual propagation in vitro* – To examine if DNA rearrangements of the type described above occur commonly during *in vitro* culture, we used PCR to reveal chromosome breakage and healing events within the Pf11-1, HRP I and HRP II genes. The wild type genes reside in subtelomeric regions of chromosome 2, 8 and 10 respectively and each gene is located at least 100 kb from the end of the chromosome. Oligonucleotide primers based on the gene of interest and a trimer of the *P. falciparum* telomere repeat were used to prime PCR with the genomic DNA of several laboratory strains and the resulting PCR-amplified fragments were analyzed by Southern hybridization with the respective gene-specific probe. This methodology, should only detect that subpopulation of Pf11-1, HRP I and HRP II genes that have undergone a rearrangement which moves them suitably close to a telomere for efficient amplification by PCR. In most of the laboratory strains examined a multitude of heterogeneously-sized fragments were observed to hybridize with each of the probes and the hybridization patterns differed among each of the strains. Conversely, no hybridizing fragments were observed in control experiments using single oligonucleotides to prime PCR (Scherf et al., submitted).

TABLE  
Inactivation of genes of *Plasmodium falciparum* by genome rearrangements

Gene	Type of DNA rearrangement	References
HRP I	chromosome breakage and healing	Pologe & Ravetch, 1988 this work
HRP II	chromosome breakage and healing	Pologe & Ravetch, 1988
HRP III	not characterised	Wellems et al., 1987
MESA	not characterised	Petersen et al., 1989
RESA	partial inversion of the first exon	Cappai et al., 1987
Pf11-1	chromosome breakage and healing	Scherf et al., 1992

#### Comparison of deletion breakpoint sequences

– Those telomere-proximal genes that have been shown to undergo deletional inactivation are summarized in Table. Pologe & Ravetch (1988) analyzed deletions in the genes encoding histidine rich proteins HRP I and HRP II and showed that they were accompanied by DNA rearrangements similar to those described for the Pf11-1 gene in this investigation. Cappai et al. (1989) reported that inversion of a 5' segment of the Pf155/RESA gene caused deletion of the chromosome fragment located upstream and that the resulting DNA rearrangement inactivated the Pf155/RESA gene. Remarkably, the dinucleotide sequence "CA" is found precisely at the wild-type side of the breakpoint in each of these cases. Although such a simple sequence motif is unlikely to function as a site for programmed DNA rearrangement, its presence at the identical position in each of these deletions is striking.

#### Gametocyte-specific expression of Pf11-1

– As described in the Introduction, our attempts to identify and characterize the Pf11-1 gene product have yielded ambiguous results due to immunological cross-reactivity between putative Pf11-1 proteins and other malarial proteins. We previously reported the immunoprecipitation of a megadalton protein from asexual parasites using antibodies to Pf11-1 synthetic peptides and recombinant proteins (Petersen et al., 199). However, a subsequent immunoprecipitation analysis with the same Pf11-1 antibodies and metabolically-labeled proteins from asexual stages of the mutant parasite identified the same

megadalton protein, despite the fact that over 90% of the Pf11-1 gene was deleted. This discrepancy of a positive immunoprecipitation result with the mutant parasite indicated that the immunoprecipitation of the megadalton protein was due to immunological cross-reactivity and led us to reexamine the identification of the Pf11-1 gene product, using the mutant parasite as a negative control. Specific antibodies against nondegenerate amino acid repeats of Pf11-1 reacted strongly by immunofluorescence with mature gametocytes of parasites carrying the Pf11-1 gene, but failed to react with gametocytes of the mutant parasite or with asexual parasites. Northern blot analysis showed that a large Pf11-1 gene-specific transcript (> 10 kb) is present in gametocytes but not in asexual blood stage parasites. Taken together, the results indicate that the immunoprecipitation of the megadalton protein of asexual parasites by Pf11-1 specific antibodies was due to immunological cross-reactivity and that the bona fide product of the Pf11-1 gene is a gametocyte-specific protein (Scherf et al., submitted).

#### CONCLUSIONS

In most *P. falciparum* laboratory strains examined in this study we have detected parasite subpopulations that have undergone chromosome breakage and healing within the HRP I, HRP II, and Pf11-1 genes. With the exceptions of the knob-phenotype/HRP I-genotype frequently observed with cultured parasites and the Pf11-1 genotype described for C9H12 parasites in this report, these deletions were undetectable by con-

ventional genomic. Southern analysis. This suggests that the mutant subpopulations are numerically small and have no selective advantage over wild-type parasites. When we applied the same PCR-Southern methodology to parasite DNA isolated directly from infected patients we were unable to detect deletions in any of these genes. Thus, it would appear that the corresponding gene products are functionally important, if not crucial, to the natural malaria life cycle. If this is true, any parasites harboring deletions in these genes would be subject to severe negative selection, at least *in vivo*.

Both microbial and multicellular eukaryotes have been shown to contain a "telomerase" enzyme activity that synthesizes repetitive telomere sequences *de novo* and adds them to pre-existing telomeres (Blackburn, 1991). It is possible that a similar, but less stringent, enzyme activity is responsible for the events described in this report. Since all *P. falciparum* chromosome healing events documented to date are initiated from a "CA" dinucleotide one might speculate that only those chromosome breaks occurring precisely at this sequence are repairable by telomere addition. Alternatively, an exonuclease might digest broken chromosomes until it uncovers a CA dinucleotide to which telomeric repeats can be added.

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