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Changes in Repeat Number, Sequence, and Reading Frame in S-Antigen Genes of *Plasmodium falciparum*

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The S antigens from different isolates of *Plasmodium falciparum* exhibit extensive size, charge, and serological diversity. We show here that the S-antigen genes behave as multiple alleles of a single locus. The size heterogeneity results from different numbers, lengths, and/or sequences of tandem repeat units encoded within the S-antigen genes. Two genes studied here encode antigenically different S antigens but nevertheless have closely related tandem repeat sequences. We show that antigenic differences can arise because repeats are translated in different reading frames.

In *Plasmodium falciparum*, the most pathogenic of the human malaria parasites, the S antigens exhibit the greatest degree of serological diversity known to date. These heat-stable proteins are found in the sera of infected individuals and in the supernatants of in vitro cultures (16, 17). Although S antigens from any one locality exhibit extensive serological diversity, individual S-antigen serotypes occur in very different parts of the world. In addition to serological diversity, the antigens exhibit size and charge heterogeneity and S antigens of the same serotype can exhibit size heterogeneity (1, 9, 19). S antigens are apparently secreted into the parasitophorous vacuole and are released into the plasma upon schizont rupture (4, 18), although the association of some of the S antigen with free merozoites cannot be excluded. The extreme serological diversity of S antigens suggests a role in immune evasion, although such a role is yet to be defined.

Recently we reported the isolation of a cDNA clone encoding a segment of the S antigen of the *P. falciparum* isolate FCQ27/PNG (FC27), obtained in Papua New Guinea (4). This cDNA clone (Ag16) was shown to consist entirely of tandem repeats of a 33-base-pair sequence. Antibodies raised against the protein product of the cDNA reacted with the native S antigen of FC27, but not with the S antigen of isolate NF7 from Ghana (4). Cowman et al. (5) described the isolation and complete nucleotide sequences of the chromosomal S-antigen genes from the *P. falciparum* isolates FC27 and NF7. In these clones, designated FC27.4.S and NF7.S, respectively, the coding regions contain a region of tandemly repeated sequences flanked by unique sequences. The unique sequences are relatively conserved between the two segments, whereas the tandem repeats are very different, consisting of 24- and 45-base-pair sequences in NF7 (5).

The availability of these chromosomal clones has allowed us to generate specific DNA and antibody probes for detailed studies on S-antigen diversity. We describe here the relationship of the S-antigen genes and their products in several independent isolates of *P. falciparum*. We show that the serological diversity and size heterogeneity of S antigens can result from changes in repeat number, sequence, and reading frame.

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MATERIALS AND METHODS

Reagents. Bacteriophage λgt10 and *Escherichia coli* RY1073 were the generous gifts of R. Young, T. Huynh, and R. Davis. Restriction endonucleases, DNA polymerase I, and T4 DNA ligase were from New England BioLabs or Boehringer Mannheim Biochemicals. Deoxynucleoside triphosphates were from Collaborative Research, Inc.; [α-^{32}P]dCTP (3,000 Ci/mmol) was from the Radiochemical Centre; and nitrocellulose filters were from Schleicher & Schuell, Inc.

Parasites. Isolates FCQ27/PNG (FC27), IMR143/PNG (IMR143), IMR144/PNG (IMR144), IMR147/PNG (IMR147), and MAD71/PNG (MAD71) were all obtained from Papua New Guineans living in the Madang Province of Papua New Guinea. FC27 clones G4, D10, and E12 are parasite lines cloned by limiting dilution (1). Isolates NF7 and K1 were obtained from Ghana and Thailand, respectively. The method of Trager and Jensen (15) was used to maintain all isolates in *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)-buffered RPMI 1640 medium containing 10% serum.

Clones and genomic blots. Chromosomal DNA was digested to completion with *Eco*RI and ligated to λgt10 DNA digested with *Eco*RI. The ligated DNA was packaged into phage and plated on *E. coli* RY1073 (20). The desired recombinants were detected by the method of Benton and Davis (2). The preparation of mRNA, synthesis of cDNA, and construction of cDNA clones of the NF7 isolate of *P. falciparum* were carried out as described by Kemp et al. (10) for isolate FC27.

Chromosomal DNA from all isolates of *P. falciparum* was prepared as described by Coppel et al. (4). DNA portions (3 μg) were digested, subjected to electrophoresis in agarose, transferred to nitrocellulose, and hybridized in 50% formamide–5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1 mg of salmon sperm DNA per ml with appro-

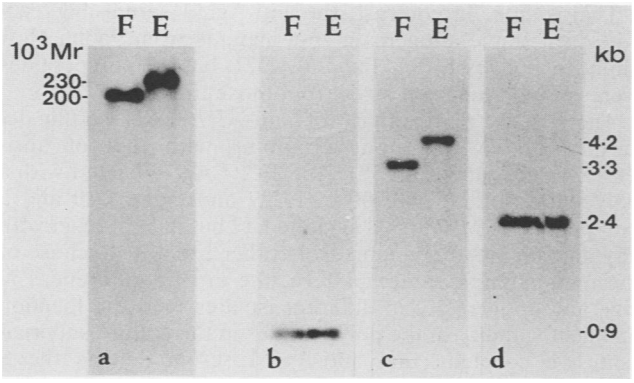

FIG. 1. Basis of the size variation in different S-antigen genes that encode antigenically cross-reacting S antigens. (a) Proteins from boiled culture supernatants of FC27 (F) or E12 (E) were fractionated by SDS-polyacrylamide gel electrophoresis, electroblotted to nitrocellulose, and reacted with antibodies to the fused polypeptide from cDNA clone FC27.Ag16. FC27.Ag16 is an S-antigen cDNA clone composed entirely of tandem repeats (4). Polypeptide sizes are indicated to the left. (b to d) Chromosomal DNA from FC27 (F) and E12 (E) was digested with *Eco*RI and *Bam*HI (b), *Bam*HI and *Sph*I (c), and *Eco*RI and *Sph*I (d), fractionated on a 1% agarose gel, blotted to nitrocellulose, and hybridized to the 5′ *Eco*RI-*Bam*HI fragment of FC27.4.S (panel b), to FC27.Ag16 (panel c), or to the 3′ *Sph*I-*Eco*RI fragment of FC27.4.S (panel d). The 5′ and 3′ fragments flanking the tandem repeats are indicated in Fig. 2 and described by Cowman et al. (5). Sizes are indicated to the right.

priate probes labeled by nick translation. Washing conditions are described in the figure legends.

DNA sequencing. Fragments used for sequencing were subcloned into the appropriate restriction sites of M13mp8 and M13mp9. Sequencing was done by the dideoxy procedure (12, 13).

Synthetic peptides. A 12-amino-acid peptide corresponding to the putative repeat in the K1 S antigen was synthesized by the Merrifield (11) solid-phase method on a chloromethylated polystyrene resin. The peptide was coupled to either keyhole limpet hemocyanin or bovine serum albumin by using glutaraldehyde. Freshly diluted 25 mM glutaraldehyde (0.5 ml) was added dropwise to 2 mg of peptide and 4 mg of carrier protein in 1 ml of 0.1 M phosphate buffer (pH 7.0). The mixture was kept at room temperature for 6 h with occasional stirring and then dialyzed extensively against phosphate-buffered saline (pH 7.3).

Antisera. Antisera to the S antigens of *P. falciparum* FC27 and NF7 were produced by immunizing rabbits and mice, respectively, with semipurified β-galactosidase fusion polypeptides produced by the appropriate λgt11-Amp3 clones. Antisera to the K1 S antigen were raised by immunizing BALB/c mice with the 12-amino-acid synthetic peptide coupled to keyhole limpet hemocyanin. Mice were initially immunized intraperitoneally and subcutaneously with 100 μg of keyhole limpet hemocyanin equivalents in complete Freund adjuvant. At 4 and 6 weeks after the primary immunization, the mice were boosted with the same amount of conjugate in incomplete Freund adjuvant. The mice were bled 10 to 14 days after each boost. Human serum sample with high-titered antibodies to various S antigens were taken from individuals living in the Madang region of Papua New Guinea.

Western blots. The S antigens in the supernatants of various *P. falciparum* cultures were detected by Western blotting (immunoblotting). The supernatants were heated (100°C for 10 min), centrifuged (1,300 × *g* for 10 min), and analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and Western blotting as previously described (1, 6).

RESULTS

Different-sized S antigens of the FC27 serotype. Size heterogeneity has been observed between serologically indistinguishable S antigens. The S antigens of isolate FC27 and the cloned line E12, derived from FC27 by limiting-dilution culture (1), differ in size by M_r ca. 30,000, although both react with rabbit antiserum to the FC27 repeat expressed by clone Ag16 (4) (Fig. 1a). This could result from recombination within the repeat region (for example, by unequal crossing over), leading to an increase or decrease in the number of repeats and hence to variations in the size of the S antigens and the genes encoding them. To test this explanation, we took advantage of the unique *Bam*HI and *Sph*I restriction sites at the 5′ and 3′ boundaries, respectively, of the tandem repeat region in FC27.4.S (5). Cleavage with the three enzymes *Eco*RI, *Bam*HI, and *Sph*I divided the gene into a 3′ unique region, the central region comprising tandem repeats and a 5′ unique region (Fig. 2). The fragment 5′ to the repeats of the FC27 and E12 S-antigen genes are identical in length (Fig. 1b), as are the corresponding 3′ ends of each gene (Fig. 1d). The length difference between the two S-antigen genes can be accounted for by the difference in the length of the *Bam*HI-*Sph*I fragment (Fig. 1c), which contains the repeats. The proposed structures of the *Eco*RI fragments encoding the FC27 and E12 S antigens are shown in Fig. 2.

We suggest that these genes have undergone expansion or contraction, or both, in the number of tandem repeats and that size heterogeneity in S antigens in general is the sum of differences in the number of tandem repeats and differences

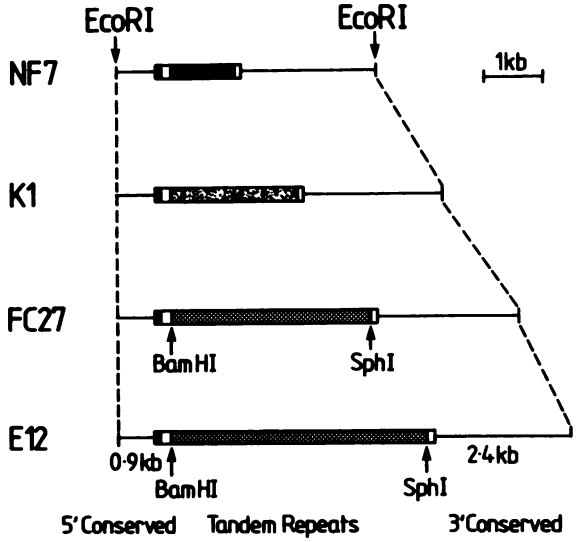

FIG. 2. Sequence organization in S-antigen genes. The structures deduced from the *Eco*RI fragments bearing the S-antigen genes of isolates NF7, K1, and FC27, as well as clone E12 are shown. Open regions at the 5′ and 3′ ends represent nonrepetitive regions, while the central shaded regions represent repeats. Different major repeats are shaded differently. Note that the open reading frames commence and terminate within the conserved regions but are mainly composed of the repeats (5).

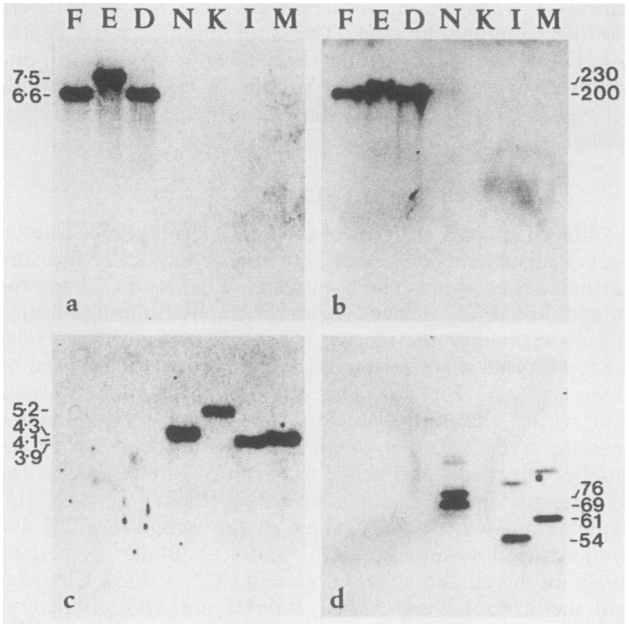

FIG. 3. Correlations in the patterns of specificity and size exhibited by S antigens and their genes in different isolates. (a and c) Chromosomal DNA from the *P. falciparum* isolates was digested with *Eco*RI, fractionated on 1% agarose gel, transferred to nitrocellulose, and hybridized to DNA from FC27.Ag16 (panel a) and NF7.Ag1 (panel c). The filters were washed in 5× SSC–0.1% SDS at 65°C prior to autoradiography. The isolates used were FC27 (F), E12 (E), D10 (D), NF7 (N), K1 (K), IMR143 (I), and MAD71 (M). Sizes are shown to the left, in kilobases. (b and d) proteins from boiled culture supernatants of the same isolates shown in panels a and c were fractionated by SDS-polyacrylamide gel electrophoresis, electroblotted to nitrocellulose, and reacted with antibodies to the proteins encoded by FC27.Ag16 (panel b) and NF7.Ag1 (panel d). Sizes are shown to the right in 10^3 M_r.

in the length of tandem repeat units encoded by the different S-antigen genes, although we cannot rule out the possibility that other sequences are present.

Different-sized S antigens of the NF7 serotype. To analyze S antigens of the NF7 serotype in further detail, we generated antibodies specific for the NF7 repeat. Sonicated fragments of DNA from chromosomal clone NF7.S were expressed in the *Eco*RI site of λgt11-Amp3 (10). Serum samples from mice immunized with one of these clones, designated NF7.Ag661, gave strong fluorescent staining on NF7-infected erythrocytes in an indirect immunofluorescence test, in a pattern identical to that reported previously for monospecific antibodies reacting with the S antigen of FC27 (4). Hybridization studies demonstrated that NF7.Ag661 consists entirely of repeat sequences from NF7.S and does not contain either 5′ or 3′ unique sequences (data not shown).

The mouse anti-NF7.Ag661 serum was used to probe immunoblots of NF7 culture supernatants separated by SDS-polyacrylamide gel electrophoresis. The serum gave a strong reaction with a polypeptide doublet of M_r 76,000 and 69,000 (Fig. 3d). In addition to this prominent antigen doublet, there were two minor doublets present in the NF7 culture supernatant, one larger and one smaller. As expected, this anti-NF7.Ag661 serum exhibited isolate specificity (Fig. 3d). No antigens were detected in supernatants from FC27, from the FC27 clones E12 and D10, or from K1, a Thai isolate. In contrast, the anti-Ag661 serum did react with heat-stable antigens in supernatants from Papua New Guinean isolates IMR143 and MAD71, but the polypeptides were of quite different sizes from those in NF7.

The pattern of reactivity of anti-NF7.Ag661 serum described above was nonoverlapping with that of anti-FC27.Ag16 serum (Fig. 3b). Anti-FC27.Ag16 reacted with a polypeptide of M_r 200,000 in FC27 and clone D10 and a polypeptide of M_r 230,000 in clone E12 but did not react with any antigen in supernatants from other isolates. Because of the immunofluorescence pattern, the drastic differences in size and antigenicity in different isolates, and the location and heat stability of the polypeptides in the culture supernatant, we conclude that anti-Ag661 serum detects the S antigens of NF7, IMR143 and MAD71.

S-antigen genes behave as allelic variants in different *P. falciparum* isolates. To investigate the number of S-antigen genes in the *P. falciparum* genome, the S-antigen cDNA clones FC27.Ag16 and NF7.Ag1 (4, 5) were hybridized to DNA from the same *P. falciparum* isolates used in the immunoblotting experiments above. FC27.Ag16 consists entirely of 33-base-pair repeats (4), while NF7.Ag1 contains both 24- and 45-base-pair repeats (5) but could not be completely sequenced because of its extreme instability. *Eco*RI-digested chromosomal DNA from each of the isolates and cloned lines examined encodes sequences homologous to either FC27.Ag16 or NF7.Ag1 but not both (Fig. 3a and c). FC27.Ag16 hybridized to DNA from the cloned lines D10 and E12 as well as to DNA from isolate FC27, whereas NF7.Ag1 hybridized to DNA from the Papua New Guinean isolates IMR143 and MAD71 as well as to DNA from isolate NF7, from which it was derived. A striking feature that is evident in Fig. 3 is that with one exception, the pattern of immunological reactivity and size of S antigens in this panel of isolates closely parallels the pattern of hybridization with two different repeats. The exception was isolate K1, which hybridized with NF7.Ag1 even though the mouse anti-NF7.Ag661 antibodies did not react with K1.

The 2.4-kilobase (kb) nonrepetitive 3′ *Sph*I-*Eco*RI fragment of FC27.4.S (Fig. 2) was hybridized to *Eco*RI restriction digests of chromosomal DNA prepared from the same isolates. In each case a single *Eco*RI fragment hybridized to the FC27.4.S fragment (Fig. 4). This was also true of isolate MAD71 from Papua New Guinea (result not shown). The 0.9-kb nonrepetitive 5′ *Eco*RI-*Bam*HI fragment of FC27.4.S (Fig. 2) also hybridized to the same set of restriction fragments in these isolates (results not shown). The most important point is that in all isolates tested, the *Eco*RI fragments that hybridized to the tandem repeats of either FC27.Ag16 or NF.Ag1 were the same size as those that hybridized to the conserved nonrepeat portion of FC27.4.S. The same conclusion was obtained from *Hin*dIII and *Aha*III digests (data not shown). There is, therefore, no evidence for a copy of the tandem repeats other than that found within the conserved flanking sequences of the S-antigen genes.

Further evidence for a single S-antigen gene was obtained by assaying the number of copies of the nonrepeat region of the S-antigen gene in the FC27 genome. A standard amount of chromosomal DNA was restricted, fractionated, and hybridized with the 0.9-kb 5′ *Eco*RI-*Bam*HI fragment of FC27.4.S or with two other unrelated cDNA clones which, on the basis of restriction analysis, were encoded by single-copy sequences. The extent of hybridization to the 5′ S-antigen probe was intermediate between those of the other two presumed single-copy probes (data not shown). As the S-antigen gene is present in one copy per haploid genome for

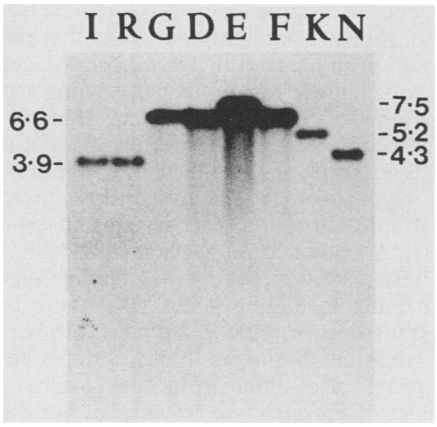

FIG. 4. Hybridization of *P. falciparum* chromosomal DNA to the nonrepeat portion of FC27.4.S. Chromosomal DNA from *P. falciparum* isolates and cloned lines was restricted with *Eco*RI, fractionated, transferred to nitrocellulose, and hybridized to the 3′ *Sph*I-*Eco*RI fragment of FC27.4.S shown in Fig. 2. This fragment is adjacent to, but does not contain, the tandemly repeated sequences (5). DNA was prepared from IMR143 (I), IMR147 (R), G4 (G), D10 (D), E12 (E), FC27 (F), K1 (K), and NF7 (N). The filter was washed in 5× SSC–0.1% SDS at 65°C prior to autoradiography.

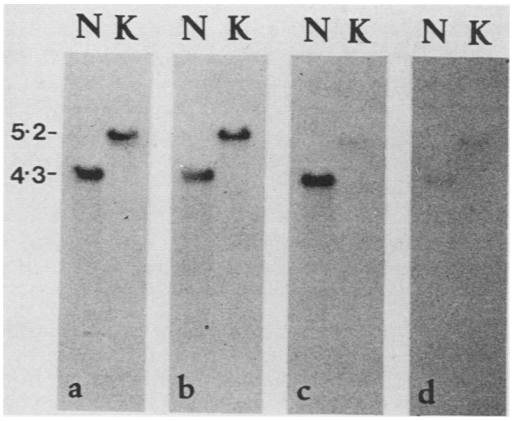

FIG. 5. Thermal stabilities of hybrids formed with related S-antigen repeats. Chromosomal DNA from NF7 (N) and K1 (K) was digested with *Eco*RI, fractionated, transferred to nitrocellulose, and hybridized with NF7.Ag1 DNA. The filters were washed at 65°C in 1.0× SSC (a), 0.3× SSC (b), 0.1× SSC (c), or 0.03× SSC (d) prior to autoradiography.

all the isolates tested, we conclude that the S-antigen genes make up an allelic series of variants that occupy a single locus in the *P. falciparum* genome.

Antigenically different S antigens encoded by genes with closely related tandem repeat sequences. Because there are a large number of different S-antigen serotypes (17), it was surprising that all isolates and cloned lines examined hybridized with either the NF7 or the FC27 S-antigen repeat sequence. However, it is quite possible that these two groups of cross-hybridizing sequences encode related repeat sequences that are antigenically distinct. Indeed, antibodies against NF7.Ag661 reacted with NF7 but not with K1, even though K1 cross-hybridizes with the NF7 repeat.

We therefore examined the degree of sequence mismatch between the NF7 and K1 repeats, as determined by the relative thermal stabilities of hybrids formed with NF7.Ag1 and chromosomal DNA from these two isolates. Under low-stringency conditions, the degree of hybridization obtained with NF7 and K1 was very similar (Fig. 5a and b). However, at higher stringency (0.1× SSC, 65°C), the K1 hybrids melted, while those obtained with NF7 remained stable (Fig. 5c). We conclude that these two antigenically different S antigens are encoded by genes with related but nonidentical tandem repeat sequences.

Sequence of the tandem repeat of K1. To examine the relationship of the NF7 and K1 S-antigen repeats, the 5.2-kb *Eco*RI fragment corresponding to the K1 S antigen was isolated, by hybridization to the NF7.Ag1 cDNA, from a library of K1 chromosomal fragments cloned in λgt10. As found with all other S-antigen clones that we studied, a deletion in *E. coli* removed many of the repeats from this segment. Restriction mapping of this clone demonstrated that the nonrepeated flanking regions are identical in size to those of FC27 (Fig. 2) (data not shown). A *Dra*I fragment that spanned the entire remaining coding sequence was inserted into M13 and completely sequenced by the dideoxy procedure (Fig. 6). By comparing this sequence with those of FC27.4.S and NF7.S (7), we deduced that the sequence was closely homologous to the NF7 and FC27 S-antigen genes, but the deletion had removed all but four repetitive elements. At the 3′ end there were two 45-base-pair repeats, identical to those located in precisely the same position in NF7, at the 3′ end of the long array of 24-base-pair repeats (5) labeled D in Fig. 6. We demonstrated previously that sequences A and D show some homology: although they differ in length they are identical at 16 of 24 comparable positions (7). In K1, a third incomplete copy of the same sequence (labeled B in Fig. 6), lacking the first 18 bases, was located immediately 5′ to the two 45-base-pair repeats. Between this 27-base sequence and the 5′ nonrepeat sequences, K1, but not NF7, contains a 36-base-pair sequence (labeled C in Fig. 6) homologous with the other three repetitive elements; it included a stretch in which 26 of 27 bases were identical with a sequence within the 45-mer (Fig. 6). Remarkably, however, the deduced reading frame through this 36-base-pair sequence was different from that of the 45-mer present in K1 and NF7. Because of the deletion, we could not be certain whether this 36-base-pair sequence

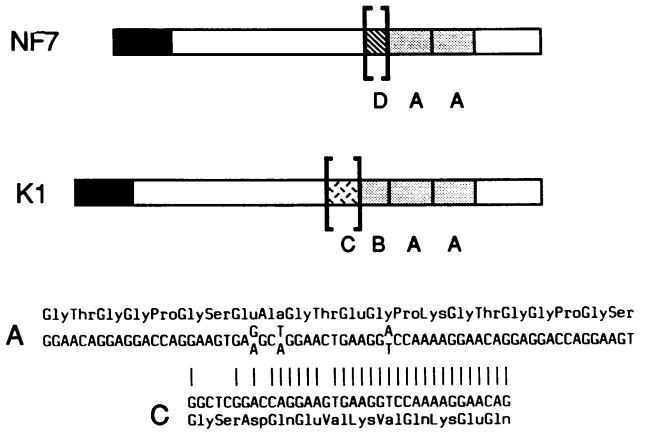

FIG. 6. Nucleotide and amino acid sequences of the tandem repeat units of the K1 S antigen. The sequence of the NF7 repeat units are taken from Cowman et al. (5). The K1 15-mer (top) and K1 12-mer (bottom) are shown aligned but out of frame as predicted from the sequence and confirmed in the text. The 15-mers of K1 and NF7 (5) are identical. Region D corresponds to the 24-base-pair repeat unit of the NF7 S antigen (5).

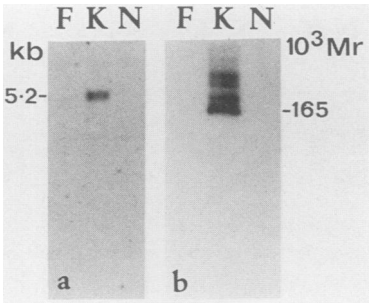

FIG. 7. The 12-mer sequence is highly repeated in the K1 S-antigen and is read in the predicted frame. (a) A synthetic oligonucleotide (see text) was hybridized to *Eco*RI-digested, fractionated DNA from isolates FC27, NF7, and K1 at 37°C in 5× SSC. The filter was washed at 65°C in 2× SSC prior to autoradiography. (b) Proteins from boiled culture supernatants of the *P. falciparum* isolates were analyzed by immunoblotting as for Fig. 3a and b. The antibodies were rabbit antibodies to a synthetic peptide and were prepared as described in Materials and Methods.

represented a highly repeated sequence. To examine this, we hybridized the corresponding ^{32}P-labeled synthetic oligonucleotide (GGC TCG GAC CAG GAA GTG AAG GTC CAA AAG GAA CA) to chromosomal fragments of NF7 and K1 DNA. The oligonucleotide hybridized strongly to a 5.5-kb *Eco*RI fragment corresponding to the S-antigen repeat of K1, but not to NF7 DNA (Fig. 7a). An oligonucleotide corresponding to the 45-mer also hybridized strongly to the S-antigen repeat of K1 and faintly to that of NF7 (data not shown). Because NF7 contains two copies of the related 45-nucleotide sequence, we conclude that the 36-mer or a closely related sequence must be present in many additional copies in the K1 gene and thus represents a major repeat in this S antigen. An oligonucleotide corresponding to the 24-mer (sequence D in Fig. 6) hybridized strongly to the chromosomal fragment containing the NF7 S-antigen repeat, but not detectably to that of K1 (data not shown). Hence the hybridization of the NF7.Ag1 probe observed in Fig. 3 and 5 results from cross-hybridization of sequence C in the K1 genome to sequence A in the probe.

To confirm that this 36-mer indeed encodes a major repeat present in the S-antigen of K1, in the frame predicted, we immunized rabbits with a synthetic peptide corresponding to the predicted sequence (GSDQEVKVQKEQG) and used the antibodies in immunoblotting studies on polypeptides from supernatants of *P. falciparum* cultures. The antibodies reacted most strongly with a polypeptide of M_r 165,000, but they also reacted with several other polypeptides of higher M_r, presumably aggregates, which were present in the K1 supernatant (Fig. 7b). There was no reaction at all with polypeptides in the supernatants from any of the other isolates tested (Fig. 7b). The dominant polypeptide was identical in size to one of two polypeptides identified as K1 S antigens by immunoblotting with human serum (data not shown). We conclude that the S-antigen repeat of K1 is translated in *P. falciparum* in the frame predicted, a result that has important implications for the generation of S-antigen diversity.

DISCUSSION

The observations reported in this paper bear directly on the nature of the generation of S-antigen diversity in *P. falciparum*. First, the S antigens behave as an allelic series of variant genes at a single locus. No evidence was obtained for unique or repeat S-antigen sequences other than those at the single S-antigen locus in the *P. falciparum* genome, and so there is no evidence for sequence rearrangement models in the generation of S-antigen diversity. Hence the generation of S-antigen diversity is very different from the generation of diversity in, for example, the variant surface glycoprotein of trypanosomes (3). In contrast to the variant surface glycoprotein and other systems in which a programmed DNA sequence rearrangement occurs, e.g., *Salmonella* phase variation (21), yeast mating type (8), and mammalian immunoglobulin genes (14), there is no silent (nonexpressed) copy of the S-antigen variable region in isolates of different S-antigen types. In this respect, the S-antigen genes also differ from the surface protein (i-antigen) genes of *Paramecium tetraurelia*. Several i-antigen genes are present in all strains examined, but can apparently switch expression in the absence of rearrangements (7).

Although immunoblotting and immunoprecipitation experiments involving the use of monospecific antibodies identify several polypeptides in culture supernatants as S antigens, there is only one S-antigen gene in each of these isolates of *P. falciparum*. Thus the different polypeptides must derive from the one translation product by proteolysis or, possibly, aggregation. The putative S antigens described by others (11, 19) may reflect the same processes but may also result from the designation as S antigens of unrelated gene products which are also heat stable.

The simplest explanation of the S-antigen characteristics is that the different S-antigen types arose from a common ancestral gene by sequence divergence that gave rise to a series of alleles maintained as a polymorphism in natural populations of the parasite. Our data support the proposition that S-antigen diversity is generated by the spreading of mutations throughout the region of tandem repeats, presumably by recombinational mechanism such as unequal crossing over or unequal gene conversion or both (5). Such mutations that alter the sequence and consequently the antigenic nature of the repeat portion of the S antigen would result in novel S-antigen serotypes. Our observation that immunologically indistinguishable S antigens can differ in size because they have different numbers of tandem repeats is entirely in accord with the notion that unequal crossing over is occurring within the repeat region of the S-antigen genes. The existence of S antigens that are closely related in sequence but antigenically different was a prediction made on the basis of this model for the generation of S-antigen diversity (5). The discovery that the antigenically different S antigens of NF7 and K1 are nevertheless related in sequence fulfills that prediction and so adds further support to the model. Indeed, the K1 repeat sequence presented here contains two copies of a 15-amino-acid repeat that are identical to the 15-amino-acid repeats found previously in NF7. As described in detail previously (5), this sequence is related to the 11-amino-acid repeat of FC27, albeit much more distantly. This 15-amino-acid sequence may therefore be most closely related to the ancestral sequence from which all S-antigen repeats have derived.

One novel feature of the 15- and 12-amino-acid repeats of the K1 S antigen is that they are homologous at the nucleotide level but differ in reading frame. Hence in addition to the mechanism of codon mutation, addition, and deletion that we noted previously, frame change by the deletion or addition of nucleotides can result in an even more dramatic change in the polypeptide sequence encoded by a repetitive array. Such changes would totally change the antigenic

properties of the polypeptide. Several other repetitive antigens of *P. falciparum* also have more than one open reading frame throughout the repeat arrays, and so it is possible that this mechanism operates in a number of genes. From these observations, it would seem likely that the repeated sequences in proteins such as the S antigen are advantageous to the parasite because of the enhanced rate of antigenic change that can result from a variety of genetic processes acting on the repeated sequences.

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LITERATURE CITED

1. **Anders, R. F., G. V. Brown, and A. Edwards.** 1983. Characterization of an S antigen synthesized by several isolates of *Plasmodium falciparum*. Proc. Natl. Acad. Sci. USA **80**:6652–6656.
2. **Benton, W. D., and R. W. Davis.** 1977. Screening λgt recombinant clones by hybridization to single plaques *in situ*. Science **196**:180–182.
3. **Borst, P., and G. A. M. Cross.** 1982. The molecular basis for trypanosome antigenic variation. Cell **29**:291–303.
4. **Coppel, R. L., A. F. Cowman, K. R. Lingelbach, G. V. Brown, R. B. Saint, D. J. Kemp, and R. F. Anders.** 1983. Isolate-specific S-antigen of *Plasmodium falciparum* contains a repeated sequence of eleven amino acids. Nature (London) **306**: 751–756.
5. **Cowman, A. F., R. B. Saint, R. L. Coppel, G. V. Brown, R. F. Anders, and D. J. Kemp.** 1985. Conserved sequences flank variable tandem repeats in two S-antigen genes of *Plasmodium falciparum*. Cell **40**:775–783.
6. **Crewther, P. E., A. E. Bianco, G. V. Brown, R. L. Coppel, H. D. Stahl, D. J. Kemp, and R. F. Anders.** 1986. Affinity purification of human antibodies directed against cloned antigens of *Plasmodium falciparum*. J. Immunol. Methods **86**:257–264.
7. **Forney, J. D., L. M. Epstein, L. B. Preer, B. M. Rudman, D. J. Widmayer, W. H. Klein, and J. R. Preer.** 1983. Structure and expression of genes for surface proteins in *Paramecium*. Mol. Cell. Biol. **3**:466–474.
8. **Hicks, J. B., J. M. Strathern, and I. Herskowitz.** 1977. The cassette model of mating-type interconversion, p. 457–462. *In* A. Bukhari, J. Shapiro, and S. Adhya (ed.), DNA insertion elements, plasmids and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **Howard, R. J., L. J. Panton, K. Marsh, I. T. Ling, E. J. Winchell, and R. J. M. Wilson.** 1986. Antigenic diversity and size diversity of *Plasmodium falciparum* antigens in isolates from Gambian patients. I. S-antigens. Parasite Immunol. **8**:39–55.
10. **Kemp, D. J., R. L. Coppel, A. F. Cowman, R. B. Saint, G. V. Brown, and R. F. Anders.** 1983. Expression of *Plasmodium falciparum* blood-stage antigens in *Escherichia coli*: detection with antibodies from immune humans. Proc. Natl. Acad. Sci. USA **80**:3787–3791.
11. **Merrifield, R. B.** 1965. Solid-phase peptide synthesis. Endeavour **24**:3–7.
12. **Messing, J., and J. Vieira.** 1982. A new pair of M13 vectors for selecting either DNA strand of double digest restriction fragments. Gene **19**:269–276.
13. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
14. **Tonegawa, S.** 1983. Somatic generation of antibody diversity. Nature (London) **302**:575–581.
15. **Trager, W., and J. B. Jensen.** 1976. Human malaria parasites in continuous culture. Science **193**:673–675.
16. **Wilson, R. J. M., and R. K. Bartholomew.** 1975. The release of antigens by *Plasmodium falciparum*. Parasitology **71**:183–192.
17. **Wilson, R. J. M., I. A. McGregor, P. Hall, K. Williams, and R. Bartholomew.** 1969. Antigens associated with *Plasmodium falciparum* infections in man. Lancet **ii**:201–205.
18. **Wilson, R. J. M., I. A. McGregor, and K. Williams.** 1975. Occurrence of S-antigens in serum in *Plasmodium falciparum* infections in man. Trans. R. Soc. Trop. Med. Hyg. **69**:453–459.
19. **Winchell, E. J., I. T. Ling, and R. J. M. Wilson.** 1984. Metabolic labelling and characterization of S-antigens, the heat-stable, strain-specific antigens of *Plasmodium falciparum*. Mol. Biochem. Parasitol. **10**:287–296.
20. **Young, R. A., and R. W. Davis.** 1983. Efficient isolation of genes by using antibody probes. Proc. Natl. Acad. Sci. USA **80**: 1194–1198.
21. **Zieg, J., M. Silverman, M. Hilmen, and M. I. Simon.** 1978. The mechanism of phase variation, p. 411–423. *In* J. H. Miller and W. S. Reznikoff (ed.), The operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.