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Genes for *Plasmodium falciparum* surface antigens cloned by expression in COS cells

(malaria/membrane proteins/mammalian cell expression)

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ABSTRACT **Two genes encoding membrane antigens of *Plasmodium falciparum* were isolated by transient expression in mammalian cells and selection with human immune sera from African adults exposed to *P. falciparum* malaria. COS-7 cells were transfected with a plasmid expression library constructed from *P. falciparum* genomic DNA, and cells expressing reactive malaria antigens on their surface were enriched by adherence to antibody-coated dishes. One of the genes isolated is distinctive in that it does not contain repeat sequences typical of many malarial genes cloned by immunoscreening of bacterial expression libraries. The second gene apparently encodes a polymorphic version of the *P. falciparum* merozoite surface antigen Ag513, since the two sequences are identical in the 5′ and 3′ coding regions but diverge completely in the center. The COS-7 expression system provides an alternate means for cloning genes encoding malarial membrane antigens by using those antibodies in complex immune sera that bind membrane-associated, nondenatured molecules.**

Proteins expressed on the surface of parasites constitute potential target antigens for vaccine development. These molecules are accessible to the immune system and also may mediate functions vital for parasite survival such as cell attachment, host-cell invasion, or membrane transport. In the case of *Plasmodium falciparum*, surface membrane antigens of the asexual blood stages are of special interest because these stages are responsible for the high morbidity and mortality of *P. falciparum* malaria. The *P. falciparum* genes characterized to date have been cloned largely in bacterial expression systems, typically by using λgt11 cDNA or genomic libraries in *Escherichia coli* and screening with human immune serum (1, 2). However, with these methods few genes for malarial surface antigens have been cloned, possibly due to poor expression of membrane proteins in *E. coli* and the fact that bacterial fusion proteins lack epitopes dependent on native secondary or higher order protein conformations. In this paper we show that genes encoding parasite membrane proteins can be isolated directly by expression in mammalian cells and selection for cells bearing heterologous surface antigens by using human immune serum from individuals exposed to the infection of interest. This method should be generally applicable to a number of parasitic diseases. Although the genes for a number of different human cell surface antigens have been cloned by transient expression in mammalian cells, these have been isolated exclusively from cDNA libraries, and immunological selection has depended on specific monoclonal antibodies (3–6). We demonstrate that genomic libraries can also be used in similar expression cloning and that polyspecific antibodies from human serum can be used for selection of transfected cells.

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MATERIALS AND METHODS

cDNA Expression Vector pJFE14. This simian virus 40 (SV40)-based COS cell expression vector is derived from pSSD2. The vector pSSD2 is similar to the original Okayama and Berg vector pcD-X (7), except that in pSSD2 the *Pst* I and *Xho* I sites that bracket the cDNA insertion site have been deleted, and the region lying between them has been replaced with a simple polylinker containing the restriction sites *Xba* I, *Not* I, *Sac* II, *Sfi* I, and *Eco*RI (D. Denny, personal communication). In pJFE14 the SV40 origin and early promoter region of pSSD2 (*Hin*dIII–*Xho* I fragment) was replaced with a modified SV40 origin/early promoter, which also contains portions of the R and U 5′ regions of human T-cell lymphotropic virus I (from the vector pcDL-SRα296; ref. 8). In addition, the polylinker of pSSD2 (*Xba* I to *Not* I) was modified by insertion of the *Xba* I–*Not* I polylinker fragment from pCDM8 (6), so that the polylinker in pJFE14 contains two *Bst* XI sites separated by 350 base pairs (bp). Complete details of the vector pJFE14 are available upon request.

Construction of Genomic Libraries. Genomic DNA (5 μg) from culture-adapted strain FMG *P. falciparum* was partially digested with *Dra* I and ligated to a 20-fold molar excess of non-self-complementary *Bst* XI linkers (pCTCTAGGG/pCCCTAGAGCACA). Excess linkers were removed by two serial spermine precipitations (9), and DNA fragments were size-selected by ultracentrifugation over a 5–20% (wt/vol) potassium acetate gradient as described (3). Individual gradient fractions (≈0.25 ml) were precipitated with 0.3 M sodium acetate/ethanol, and 1/10th of each fraction was analyzed by agarose gel electrophoresis. The fractions were pooled into two size classes, and each pool was reprecipitated with spermine. DNA fragments of 2000–8000 bp were used to construct one library (Fa3), and fragments of 800–2000 bp were used for another (Fa4). For each library the DNA fragments were ligated into *Bst* XI-cut pJFE14, from which the 350-bp *Bst* XI–*Bst* XI fragment had been removed (3), and ligation reaction mixtures were precipitated with sodium acetate/ethanol and transformed into *E. coli* strain WM1100 (Bio-Rad) by electroporation (10). After 1 hr of outgrowth without ampicillin selection, a small portion of each transformation mixture was used to determine library size, and the remainder was placed directly into liquid culture. Amplification took place overnight in the presence of

Abbreviations: ORF, open reading frame; SV40, simian virus 40; FACS, fluorescence-activated cell sorter.

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chloramphenicol, and plasmid DNA was isolated and purified. Library Fa3 contained ≈75,000 independent clones, and Fa4 contained ≈300,000.

Human Immune and Control Sera. Sera with high titers of anti-*P. falciparum* antibodies were collected from adults in rural Ghana or Tanzania where *P. falciparum* malaria is endemic. For antibody screening of transfected COS-7 cells, a pool of three Ghanian sera was used (OH, GB, BN). Control sera were from European and American adults who had never been exposed to malaria. All sera were incubated at 56°C for 45 min to inactivate complement.

Isolation of Genomic Clones by "Panning." For the first round of screening, COS-7 cells were transformed by electroporation. For each of the libraries (Fa3 and Fa4) 40 μg of purified plasmid DNA was mixed with 4×10^7 COS-7 cells in 1.8 ml of Dulbecco's modified Eagle's medium, and electroporation was accomplished by discharging across a 4-mm cuvette with the Bio-Rad gene pulsar at 250 V and 960 μF. After 72 hr of culture, transfected COS cells were detached and treated essentially as described by Seed and Aruffo (4), except that (*i*) primary incubation was with the pool of human immune serum (diluted 1:5; total volume, 10 ml) rather than monoclonal antibody and (*ii*) after primary incubation, cells were washed by pelleting through a cushion of fetal bovine serum instead of 2% Ficoll. The washed cells were distributed into 14 (10 cm) plates that had been coated with goat anti-human IgG Fc antibodies (Cappel Laboratories), and cells were panned and plasmids were recovered as described (4). One-tenth of the DNA recovered from the first round of panning was used to transform *E. coli* WM1100 by electroporation, and the transformed plasmids were amplified by growth of cells in liquid culture (400 ml). The resulting bacteria were converted to spheroplasts and fused with COS-7 cell monolayers in 30 6-cm dishes (4×10^5 COS cells per dish; ≈70% confluent) by using polyethylene glycol as described (11). Panning of these transformed COS-7 cells with human immune sera (diluted 1:4; total volume, 4 ml) and recovery of plasmid DNA was carried out as before. DNA recovered from the second round of panning was used to transform WM1100 by electroporation, and COS-7 cells (24 6-cm dishes) again were transformed by spheroplast fusion and subjected to immunoselection by panning with the human immune sera (diluted 1:10; total volume, 10 ml). DNA from this third and final round of selection was transformed into WM1100 cells, and plasmid DNA from individual colonies and from the total pool was prepared by the alkaline miniprep method and characterized by restriction enzyme analysis. Selected DNA inserts were sequenced (both strands) by the dideoxy method (12).

Fluorescent Staining and Fluorescence-Activated Cell Sorter (FACS) Analysis. COS-7 cells were transformed by electroporation and detached from monolayers as described above. The cell suspension was washed with Dulbecco's modified phosphate-buffered saline (J.R. Scientific) containing 0.5% bovine serum albumin (PBS+a) and was incubated at 4°C for 30 min with the pool of human immune sera used for panning (1:6 dilution in PBS+a) or control nonimmune human sera (1:6 dilution as above). After three washes with PBS+a at 4°C, secondary staining was performed with a 1:50 dilution of fluorescein-conjugated rabbit anti-human γ-chain Fc antibody (Calbiochem; 30-min incubation at 4°C). Cells (10^4 per sample) were analyzed on a FACStar^{Plus} (Becton Dickinson) with a coherent I-90 argon laser at 200 mW.

RNA Blot Analyses. Total cytoplasmic RNA was isolated from parasites by lysis of infected erythrocytes with 0.8% Nonidet P-40 in the presence of 10 mM vanadyl–ribonucleoside complexes, phenol extraction, and sodium acetate/ethanol precipitation as described (13). Samples enriched ≈10-fold for mRNA by one passage over oligo(dT)-cellulose (14) were denatured by heating to 55°C in the presence of formamide and formaldehyde, electrophoresed through a 1% agarose/formaldehyde gel, and transferred to nitrocellulose (13). DNA fragments used as probes were radiolabeled directly in low-melting-point agarose gel slices by random hexamer priming (15). Blots were hybridized in 1 M NaCl containing 50 mM NaH_2PO_4 (pH 6.5), 50% formamide, 100 μg of salmon sperm DNA per ml, 100 μg of poly(A) per ml, 1× Denhardt's solution (0.2 mg each of Ficoll, polyvinylpyrrolidone, and bovine serum albumin per ml), and 10% dextran sulfate at 42°C and were washed in 30 mM NaCl/2 mM NaH_2PO_4/0.2 mM EDTA, pH 7.4, at 55°C, as described (13).

DNA and Protein Data Base Searches. The DNA sequences[‖] were compared with all *P. falciparum* sequences published to June 1989, as well as against the EMBL and GenBank data bases to September 1989. The Protein Identification Resource and Swiss-Prot Protein Data Bank were searched with the Pf12 protein sequence in conjunction with the FASTDB program of IntelliGenetics and a PAM-256 (1-tuple) protein matrix. For proteins, significant homology was considered ≥12% match of amino acids.

Cell Surface Labeling and Immunoprecipitation. COS-7 cells were transformed by electroporation and cultured for 60 hr. Monolayers were washed twice with PBS, and 2 ml of PBS was added to each 10-cm plate of confluent cells, followed by 300 μl containing lactoperoxidase (Sigma) at 1 mg/ml, 20 μl of 0.1 mM KI, 1 mCi (1 Ci = 37 GBq) of carrier-free $Na^{125}I$ (100 mCi/ml), and 20 μl of 0.03% H_2O_2 to initiate the reaction at 22°C. At 1-min intervals, an additional five 10-μl aliquots of H_2O_2 were added. The cells were washed twice with 5 ml of ice-cold PBS, and each plate was extracted by vigorous pipetting for 5 min with 2.5 ml of 1% wt/vol Triton X-100 (Boehringer Mannheim) in ice-cold PBS. Extracts were centrifuged at 12,000 × *g* for 10 min at 4°C, and 100-μl samples of supernatant were mixed with 100 μl of 10% SDS electrophoresis sample buffer (16). The remaining supernatants were diluted with an equal volume of 50 mM Tris (pH 7.2)/150 mM NaCl/5 mM EDTA/0.02% sodium azide/1% Triton X-100/1% bovine serum albumin (16). Immunoprecipitation was accomplished by addition of 20 μl of human serum to 1 ml of diluted extract and incubation at 4°C for 18 hr, followed by addition of 150 μl of protein A-Sepharose (50% suspension; Pharmacia) and incubation at 23°C for 1 hr. SDS sample buffer was added to the washed sepharose beads, and samples were analyzed by electrophoresis on SDS/5–12% acrylamide gradient gels and autoradiography (16).

RESULTS AND DISCUSSION

The expression libraries (Fa3 and Fa4) constructed from *P. falciparum* genomic DNA were taken through three cycles of panning to enrich for plasmids encoding parasite antigens that appeared on the COS cell surface. In comparison to the original libraries, when amplified DNA from the final round of immunoselection was characterized by restriction enzyme analysis, two individual plasmids with insert sizes of ≈1400 bp (designated Pf12) and ≈2200 bp (designated Pf7) appeared to be highly enriched. Each of these accounted for one-quarter of the plasmids present in the final selected pool, and because their inserts did not cross-hybridize, they were assumed to contain unrelated parasite genes.

To demonstrate that Pf7 and Pf12 encode malarial antigens expressed on the COS cell surface, FACS analysis of COS-7 cells transfected with Pf7, Pf12, or the vector alone (pJFE14) was performed after staining with either the pool of human immune serum used for immunoselection or with nonimmune

[‖]The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M28890 for Pf7 and M28889 for Pf12).

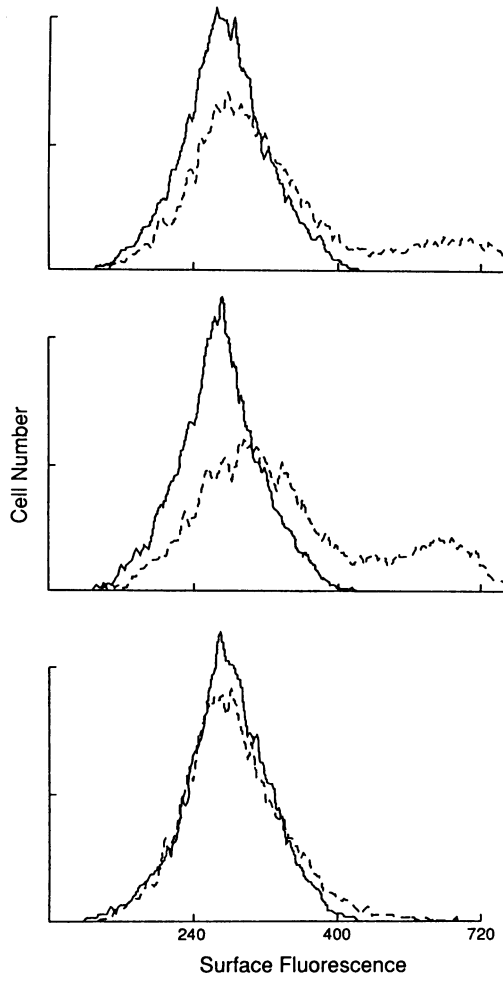

FIG. 1. Flow cytofluorometry analysis of transfected COS-7 cells. Cells were transfected with plasmid Pf7 (*Top*), Pf12 (*Middle*), or the vector pJFE14 alone (*Bottom*) and were stained with control sera (solid line) or with malaria immune sera (broken line).

normal human serum. Only those cells transfected with Pf7 or Pf12 gave surface staining, and staining was observed only with serum from individuals who had been infected with *P. falciparum* (Fig. 1). Indirect immunofluorescence microscopy of nonfixed transfected COS cells using these and other antibodies confirmed that Pf7 and Pf12 encode antigens that appear on the COS cell surface and that react only with malaria immune sera (results not shown).

Since the malaria parasite grows in human blood, it is possible that human DNA could be present as a contaminant in the original libraries, Fa3 and Fa4. To show that the selected plasmids Pf7 and Pf12 contain malaria sequences, the DNA inserts were used to probe Southern blots containing *P. falciparum* and human genomic DNA (results not shown). In constructing these Southern blots, the ≈100-fold larger size of the human genome and the corresponding difference in gene copy number per microgram of DNA was taken into account. Both inserts showed negligible cross-hybridization to human DNA but very strong cross-hybridization to one or a few bands present only in the *P. falciparum* digests, confirming that both genes originated from parasite DNA.

The DNA inserts of Pf7 and Pf12 were further characterized by DNA sequencing (see Figs. 2 and 3). For each insert, only one long open reading frame (ORF) was found when the DNA sequences were translated in both directions and in all six reading frames. In both clones these ORFs are oriented so that a sense-strand message will be transcribed from the SV40 promoter present in the vector. For Pf7 (insert size, 2172 bp) the ORF begins with a methionine codon at nucleotide 46 and ends with a stop codon at nucleotide 907. For Pf12 (insert size, 1385 bp) the ORF begins with a methionine codon at nucleotide 7 and ends with a stop codon at nucleotide 1048. Both ORFs show a codon usage characteristic for malaria (17–19) and both DNA inserts show a lower A+T content in the coding regions than in flanking regions, also typical of malarial genes (17–20).

A data base search of existing malaria sequences revealed that the DNA sequence of Pf7 is identical to that for a previously cloned merozoite surface antigen called Ag513 (21) over the first 174 nucleotides and then is completely different until nucleotide 638, where it is again identical except for minor differences (Fig. 2 and legend). The Ag513 and Pf7 proteins share the same hydrophobic N-terminal signal sequence and also the same C-terminal hydrophobic sequence, the latter probably acting as a signal for cleavage and crosslinking of the mature protein to glycosyl phosphatidylinositol (21–23). Both proteins contain blocks of tandemly repeated amino acids, a characteristic of many malarial proteins (1, 2, 19). Ag513 has two identical copies of a 32-amino acid sequence in tandem (21), whereas Pf7 has 12 tandem copies of the repeat Gly-Gly-Ser-Ala (Fig. 2). The cloning and identification of Pf7 as an apparently polymorphic form of the gene for Ag513 is in agreement with results

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                                                M  K  V  I  K  T  L  S  I  I  N  F  F  I  F
AAAAGAATTGTATTTATTAATTCTTAACATATTATATTAGTCAAAATGAAGGTAATTAAAACATTGTCTATTATAAATTTCTTTATTTTT    90

V  T  F  N  I  K  N  E  S  K  Y  S  N  T  F  I  N  N  A  Y  N  M  S  I  R  R  S  M  T  E
GTTACCTTTAATATTAAAAATGAAAGTAAATATAGCAACACATTCATAAACAATGCTTATAATATGAGTATAAGGAGAAGTATGACAGAA   180

S  N  P  P  T  G  A  S  G  S  A  (G  G  S  A)  G  G  S  A  G  G  S  A  G  G  S  A  G  G  S
AGTAATCCTCCTACTGGTGCTAGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGT   270

A  G  G  S  A  G  G  S  A  G  G  S  A  G  G  S  A  G  G  S  A  G  G  S  A  G  G  S  A  G
GCTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGT   360

S  G  D  G  N  G  A  N  P  G  A  D  A  E  R  S  P  S  T  P  A  T  T  T  T  T  T  T  T  N
TCTGGTGATGGTAATGGTGCTAATCCTGGTGCAGATGCTGAGAGAAGTCCAAGTACTCCCGCTACTACCACAACTACCACAACTACTAAT   450

D  A  E  A  S  T  S  T  S  S  E  N  P  N  H  N  N  A  E  T  N  Q  A  N  K  E  T  Q  N  N
GATGCAGAAGCATCTACCAGTACCTCTTCAGAAAATCCAAATCATAATAATGCCGAAACAAATCAAGCAAATAAAGAAACTCAAAATAAC   540

S  N  V  Q  Q  D  S  Q  T  K  S  N  V  P  P  T  Q  D  A  D  T  K  S  P  T  A  Q  P  E  Q
TCAAATGTTCAACAAGACTCTCAAACTAAATCAAATGTTCCACCCACTCAAGATGCAGACACTAAAAGTCCTACTGCACAACCTGAACAA   630

A  E  N  S  A  P  T  A  E  Q  T  E  S  P  E  L  Q  S  A  P  E  N  K  G  T  G  Q  H  G  H
GCTGAAAATTCTGCTCCAACAGCCGAACAAACTGAATCCCCCGAATTACAATCTGCACCAGAGAATAAAGGTACAGGACAACATGGACAT   720

M  H  G  S  R  N  N  H  P  Q  N  T  S  D  S  Q  K  E  C  T  D  G  N  K  E  N  C  G  A  A
ATGCATGGTTCTAGAAATAATCATCCACAAAATACTTCTGATAGTCAAAAAGAATGTACCGATGGTAACAAAGAAAACTGTGGAGCAGCA   810

T  S  L  L  N  N  S  S  N  I  A  S  I  N  K  F  V  V  L  I  S  A  T  L  V  L  S  F  A  I
ACATCCCTCTTAAATAACTCTAGTAATATTGCTTCAATAAATAAATTTGTTGTTTTAATTTCAGCAACACTTGTTTTATCTTTTGCCATA   900

F  I  •                                                                              #
                                                                                     1
TTCATATAATTCTCTTCATTTTACAACATTGACTTATATAATATATATATATATATATATATATATATATATATATATGTATATATATATATGT   990
ACATATAGATGTGTTTATTTTTTTATATAATAAAATGCCATATATACATTCGAATTTTTTATGTCACCACATTGATTATGTATACGTTTG  1080
AATATTTATTATAATGCATGGTTTTTACATATGAAATAAATAATGCACGCATTGAAAAAACTAAAAAATAACATTATATATGACAATGTT  1170
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FIG. 2. Nucleotide sequence of Pf7, along with the deduced amino acid sequence in single-letter code for the ORF. Only the first 1170 bp of the 2172-bp Pf7 insert are shown. The sequence Gly-Gly-Ser-Ala is repeated in tandem 12 times in the protein, and the first of these is indicated in parentheses. The DNA sequence of Ag513 (21) is identical to that of Pf7 from position 1 to 174 and from position 683 to 1154, except for those bases that are underlined and at the position (#) where ATATAT is inserted in the Ag513 sequence. One of the nucleotide differences results in a change in codon (serine to asparagine), also underlined. For bases 175–682 inclusive, the nucleic acid sequence of Pf7 is completely different from that of Ag513.

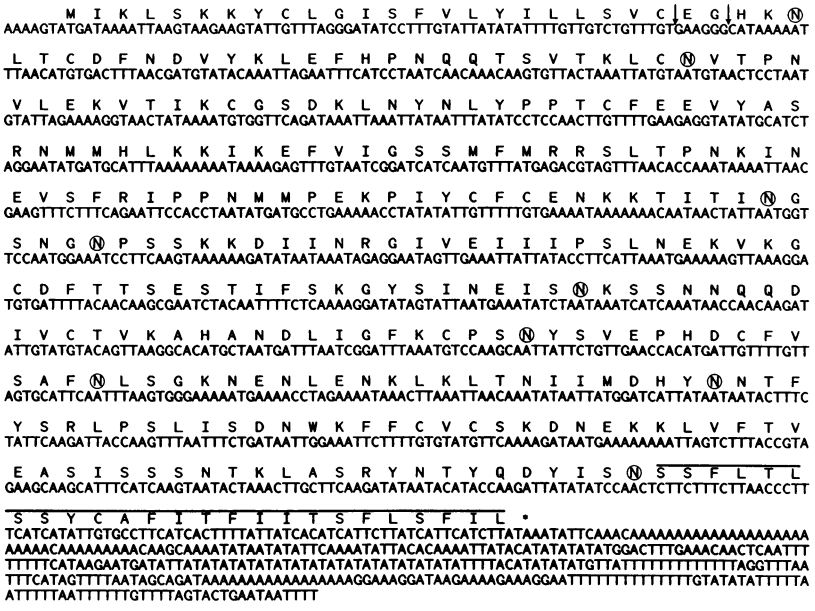

FIG. 3. Nucleotide sequence of Pf12, along with the deduced amino acid sequence for the ORF. The nine potential N-linked glycosylation sites in the protein are circled, and two potential cleavage sites for the N-terminal signal sequence (27) are indicated by arrows. The line above the 25 C-terminal amino acids delineates a hydrophobic segment of the protein, which very likely acts as a signal for covalent attachment of the mature protein to glycosyl phosphatidylinositol.

described in a recent review (24). The 4-amino acid repeat identified in Pf7 was also identified in this gene from an Indonesian parasite isolate (12 tandem copies as for Pf7) and a culture-adapted clone 3D7 (5 copies) (24). These workers have designated this polymorphic gene MSA2 ("merozoite surface antigen 2"). MSA2 is a potential vaccine molecule because the protein is expressed on the parasite surface membrane and specific monoclonal antibodies against it can block invasion of erythrocytes by merozoites (24–26).

In contrast to the majority of malarial genes characterized (1, 2, 19), the DNA sequence of Pf12 does not exhibit any blocks of tandemly repeated elements (Fig. 3). The predicted Pf12 protein has a typical N-terminal hydrophobic signal sequence (28) and a C-terminal hydrophobic segment. The latter sequence probably acts as a signal for cleavage and covalent attachment of the mature protein to glycosyl phosphatidylinositol (22, 23). The Pf12 protein has a calculated M_r of 39,500, but because the sequence contains nine potential N-linked glycosylation sites, the mature glycoprotein may be considerably heavier (see below). We were unable to find any significant homology between the sequence of Pf12 (DNA or protein) and other known sequences using the data bases and search routines described in *Materials and Methods*.

The use of genomic expression libraries may result in the isolation of genes expressed during any one of the various stages in the parasite life cycle. Human immune serum potentially contains antibodies against the sporozoite, hepatic, asexual, and sexual blood stages. Smythe *et al.* (21) found that the Ag513 protein was present in the asexual blood cycle during the trophozoite and schizont stages. Similarly, these same stages contained a 1550-base Pf7 transcript (Fig. 4, lanes 1–3; note that lanes 1 and 3 contain RNA from a parasite strain other than FMG, so that the transcript seen may represent a polymorphic form of Pf7). The Pf12 gene also appears to be transcribed (and probably expressed) during the trophozoite and schizont stages, since we were able to detect a 1750-base transcript in mRNA from these stages

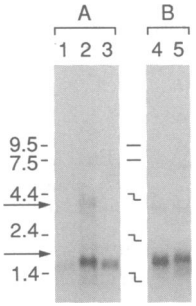

FIG. 4. Northern blot analysis of RNA from erythrocytic stages of *P. falciparum*. (*A*) Pf7 probe (first 730 nucleotides of insert). (*B*) Pf12 probe (entire DNA insert). The RNAs are from parasite strains Malayan Camp/adherent to C32 cells (lane 1), FMG (lane 2), and Malayan Camp/nonadherent to C32 cells (lanes 3–5). Lanes 1–4 contain RNA from trophozoite and schizont stages (without any sexual stage parasites), whereas lane 5 contains RNA from a mixed parasite population, which includes all erythrocytic stages including some gametocytes. The following approximate quantities of poly(A)$^+$ RNA were transferred: 0.5 μg (lane 1), 2 μg (lane 2), 1 μg (lane 3), 5 μg (lane 4), and 5 μg (lane 5). The location of the large and small ribosomal RNA bands are shown by arrows. RNA size markers (BRL) are indicated, with fragment sizes in kilobases.

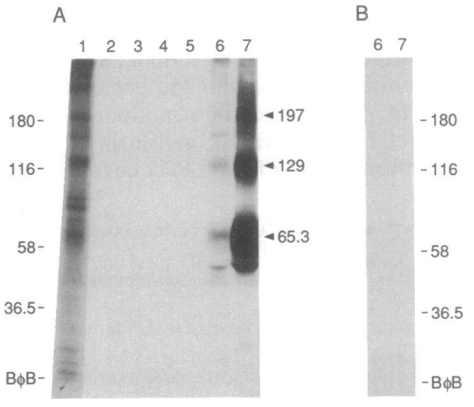

FIG. 5. Immunoprecipitation analysis of COS-7 cells transfected with plasmid Pf12 or Pf7. (*A*) The total Triton X-100 extract from COS-7 cells transfected with Pf12 is shown in lane 1. The same extract was immunoprecipitated with individual control sera (lanes 2–5) or with African sera (lanes 6 and 7)—either the pool of Ghanaian sera used for immunoselection (lane 6) or an individual Tanzanian sera (lane 7). ^{125}I-labeled bands specifically immunoprecipitated in lanes 6 and 7 are identified with arrowheads. (*B*) Immunoprecipitates of Triton X-100 extract from Pf7-transfected COS cells with the same African sera as in lanes 6 and 7 of *A*. For clone Pf7 no bands were evident with these or any of the other sera shown in *A*. COS cells transfected with pJFE14 vector alone gave the same results as for Pf7. Results in *A* and *B* are from 1-day autoradiography at −70°C. BϕB, bromophenol blue dye-front.

(Fig. 4, lane 4). The Pf12 gene may be transcribed also during other stages of the parasite life cycle, since an approximately equal signal was seen when we probed RNA from a mixed culture containing all asexual blood stages and sexual stages (Fig. 4, compare lanes 4 and 5).

To confirm that the Pf7 and Pf12 proteins were expressed on the transfected COS-7 cell surface, we immunoprecipitated a detergent extract of ^{125}I-surface-labeled cells (Fig. 5). The recombinant Pf12 protein reacted with antibodies present in the sera of individuals exposed to *P. falciparum* but was not immunoprecipitated by four different control sera (Fig. 5*A*). The pool of three immune sera from Ghana used for immunoselection and serum from a Tanzanian adult specifically immunoprecipitated three ^{125}I-labeled bands at M_r 65,300, 129,000, and 197,000. These bands were not identified after immunoprecipitation of COS cells transfected with Pf7 (Fig. 5*B*) or with the pJFE14 vector alone (not shown). The Pf12 protein was also specifically immunoprecipitated as a tritiated band after [^{3}H]glucosamine labeling (not shown), confirming that carbohydrate is present in the recombinant molecule. We suggest that the predominant M_r 65,300 band represents a heavily glycosylated Pf12 monomer (core polypeptide of M_r 39,468) and that the M_r 129,000 and 197,000 bands are the dimer and trimer, respectively.

Parallel experiments with Pf7 identified a specific ^{125}I-surface-labeled band in the total Triton X-100 extract at M_r 74,000 (not shown). However, this protein could not be immunoprecipitated as readily as Pf12, and we failed to detect it in this series of gels. The Ag513 protein has on SDS/PAGE an M_r of ≈45,000 after biosynthetic labeling of the malaria parasite (21). The calculated molecular weight from the gene sequence of Pf7 is 28,555 (Fig. 2). The high apparent molecular weight of the Pf7 protein expressed in COS cells (M_r 74,000) indicates additional glycosylation or possibly dimer formation.

In this work we have expressed a malarial genomic library in mammalian cells and selected two *P. falciparum* genes based on transient expression of the corresponding gene product at the cell surface and its binding to antibodies found in human immune serum. One of the genes codes for an unusual malarial protein lacking repeat sequences, while the other is a variant of an already identified gene. Both genes encode surface membrane antigens with hydrophobic sequences at the N and C termini. For both genes, the genomic DNA fragment isolated appears to consist of a single coding exon, which is not surprising when one considers the limitations of the method used to construct the libraries (a *Dra* I digestion is likely to cut within introns). It is possible that the full-length versions of Pf7 and Pf12 may contain additional noncoding or perhaps even coding exons, although most malaria genes characterized to date appear to be contained within a single exon (19). In any case, it is very likely that this method of genomic expression cloning can also be used to clone parasite genes whose coding segments are interrupted by introns, since the signals used for RNA splicing in malaria are apparently identical with those used in higher eukaryotes (19, 29).

Adults who are repeatedly exposed to *P. falciparum* malaria infection acquire a nonsterile but protective immunity to the disease. Eukaryotic expression cloning by immunoselection with sera from such individuals may allow the isolation of precisely those genes encoding epitopes that would be useful in a vaccine against malaria. Mammalian cell expression also offers the possibility of mutagenic analysis and precise structure–function mapping of malarial genes. Although malarial genes cannot yet be transfected into the malaria parasite, they could readily be submitted to epitope-mapping, receptor-site-mapping, or other functional studies by transfecting mammalian cells with an appropriate recombinant expression plasmid. Any limitations of these methods because of differences in protein glycosylation between the parasite and mammalian cells remain to be explored. Our results also show that specific monoclonal antibodies against the surface antigen of interest are not obligatory for mammalian cell expression cloning. Important membrane antigens defined by antibodies in human sera from diverse diseases may also be amenable to gene cloning from transiently transfected mammalian cells.

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