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# A malaria protein exported into a new compartment within the host erythrocyte

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**A *Plasmodium falciparum* protein which is exported into a new compartment in the host erythrocyte has been located. This protein, exp-1, has a variable region recognized by a monoclonal antibody. Naturally occurring mutants of this region have been characterized. All mutants studied so far have the same A → G transition abolishing the target for the antibody. The exp-1 gene has a complex structure containing two introns. It is highly conserved in five independent, genetically defined parasite lines, suggesting that exp-1 has an important function. We discuss the possible role of exp-1 in *P. falciparum* infections.**

*Key words:* epitope mapping/exported protein/genomic clone/introns/*Plasmodium falciparum*

## Introduction

In man, the malaria parasite, *Plasmodium falciparum*, grows in two types of cell. The form first injected by a mosquito is called the sporozoite. It invades hepatocytes, multiplies and emerges to initiate cyclical growth in erythrocytes. In this asexual part of the life-cycle, the parasites released at the end of each cycle are called merozoites. They rapidly reinvade new red blood cells inside which they are contained in a vacuole. The parasitophorous vacuole membrane is believed to derive from that of the red cell (Aikawa and Seed, 1980). As it develops, the parasite extensively modifies the parasitophorous vacuole, the host cytoplasm and the erythrocyte membrane (Howard, 1982; Sherman, 1985). For example, metabolite transporters such as those for glutamine (Elford *et al.*, 1985) and general anions (Ginsburg *et al.*, 1985) are inserted into the eythrocyte membrane as well as electron-dense knob-associated proteins (Aikawa *et al.*, 1986).

The routing of ligands and transmembrane proteins to and from the intracellular malaria parasite is biologically very interesting. In normal eukaryotic cells, a protein intended for secretion is directed into the lumen of the rough endoplasmic reticulum and it is thereafter transported in vesicles to the cell surface and released. Exported proteins of blood stage parasites follow a more complex route. After leaving the parasite itself, they must pass the membrane of the parasitophorous vacuole, traverse the host cytoplasm and finally become inserted in the red cell plasmalemma.

However, not all such proteins reach the outside of the host cell. We describe here a protein, exp-1, which is addressed to a new compartment within the cytoplasm of the infected red cell. The protein, which also surrounds the parasite, probably in the parasitophorous vacuole membrane, has the primary structure typical of a transmembrane protein (Hope *et al.*, 1985).

Our interest in exp-1 was aroused by a monoclonal antibody, McAb 5.1, which recognized both it and the surface protein of sporozoites (CSP). Here, we characterize the peptide in exp-1 recognized by the antibody. These studies lead to an important conclusion. The peptide in question, the 5.1 epitope, mutates in nature to generate parasites no longer recognized by McAb 5.1. We have sequenced the gene of four such strains from different parts of the world. Despite their otherwise markedly different genotypes, these strains all have an identical base change leading to a single amino acid substitution in the 5.1 epitope. This strongly sugggests that exp-1 has an important biological role depending on the structure of this peptide. The remainder of the gene is also rigidly conserved.

Sequencing of genomic DNA reveals that the gene for exp-1 has a complex structure consisting of three exons.

## Results

### *Molecular characterization of the 5.1 epitope*

We adopted three approaches to elucidate the target for McAb 5.1 in the exp-1 protein. Firstly, since the antibody cross-reacts between two proteins, exp-1 and CSP, we compared their primary sequences. Computer searches (Devereaux *et al.*, 1984) reveal a single region in exp-1 similar in primary sequence to CSP, a peptide of 18 amino acids (Asp 120 to Pro 137 in Figure 1), most likely to contain the epitope.

This conclusion is supported by our second study. Parts of the exp-1 gene were expressed in *Escherichia coli* and their products tested for recognition by McAb 5.1. Subfragments of exp-1 cDNA were cloned in frame into M13 mp19 (Yanisch-Perron *et al.*, 1985). Three fragments were made (Figure 1): the N-terminal region (119 amino acids); a 22 amino acid peptide containing the putative epitope; and a C-terminal peptide (21 amino acids). A polyclonal rabbit antiserum raised against parasite exp-1 recognized protein in all the extracts (Table I). This confirms that each subclone was expressed and implies that epitopes from all the fragments are recognized by the serum. As expected, the entire gene (p-1) made protein reacting with McAb 5.1. In addi-

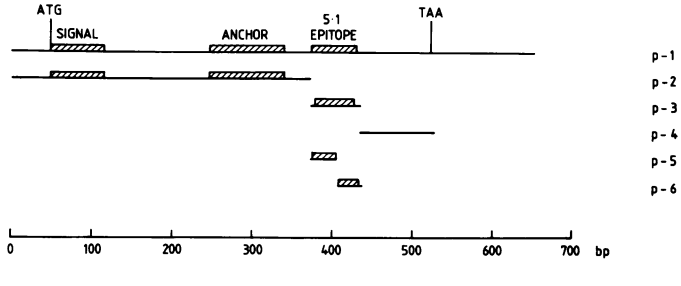


**Fig. 1.** Mapping of the 5.1 epitope by expression of exp-1 fragments in *E. coli*. Fragments of exp-1 cDNA (Hope *et al.*, 1985) were cloned in frame in M13 mp19 as described in Materials and methods. The plasmid p-1 contains the entire coding frame, p-2 has the signal and anchor sequences, p-3 has the 5.1 eiptope and p-4 the extreme C-terminal residues. The 69-bp insert in p-3 was cut with *Hin*fI generating the subclones p-5 and p-6. The signal, anchor and putative epitope sequences are shown boxed.

tion, the monoclonal recognized protein made by the subclone, p-3. This contained the 18 amino acid peptide, confirming our initial conclusion.

To pinpoint the epitope, we cleaved and further cloned the fragment in p-3 (Figure 1), to give two subclones, p-5 and p-6, encoding 8 and 11 amino acids respectively. Again, the polyclonal antibody recognized both peptides (Table I). Only the 11 amino acid peptide reacted with McAb 5.1 (p-6, Table I). This result suggests that McAb 5.1 recognizes a sequential epitope in the short fragment of exp-1 defined above.

**Table I.** Quantitative ELISA on exp-1 expression fragments

| Antibody | Activity[a] of exp-1 fragment | | | | | |
|---|---|---|---|---|---|---|
| | p-1 | p-2 | p-3 | p-4 | p-5 | p-6 |
| R α exp-1 | 1.31 | 0.99 | 0.20 | 0.15 | 0.12 | 0.11 |
| NRS | 0.02 | 0.01 | 0.02 | 0.01 | 0.01 | 0.01 |
| McAb 5.1 | 0.97 | 0.02 | 0.92 | 0.02 | 0.01 | 0.85 |
| NS-1 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |

[a]ELISA tests were performed on exp-1 fragments as described in Materials and methods. Equal amounts ($1 \times 10^{-11}$ mol) of each fragment (see Figure 1) were bound to wells and probed with the appropriate antibody: R α exp-1, rabbit anti exp-1 antiserum; NRS, non-immune rabbit serum; McAb 5.1, monoclonal antibody 5.1; NS-1, control; non-secreting myeloma. Control assays against extracts of cells containing the vector alone were in the range 0.02–0.04.

The primary sequence of CSP has multiple tandem repeats of a major (Asn-Ala-Asn-Pro) and a minor (Asn-Val-Asp-Pro) peptide. The 11 amino acid peptide of exp-1 has a similar tetrapeptide, Asn-Ala-Asp-Pro. Our final studies, presented below, confirm that this contains at least one amino acid crucial for McAb 5.1 recognition.

*Mutations in the 5.1 epitope*

Variants of exp-1 occur in the natural population. They can be detected by screening newly isolated parasites with McAb 5.1 — a rapid test for mutant parasites with an altered 5.1 epitope (McBride *et al.*, 1982). We decided to compare the sequence of several mutations. They occur in parasites which are already extensively characterized genetically (see Table II). Since the parasites differ in a wide range of characters and were isolated in different locations (SE Asia, Africa and Central America), it is virtually certain that each of their 5.1 epitope mutations arose independently.

We studied four different parasites not recognized by McAb 5.1 in immunofluorescence. First, we confirmed that each line does contain the exp-1 protein. In Western blots, each parasite gave a single band recognized by the rabbit polyclonal antibody. As expected, parallel blots were not recognized by McAb 5.1 (Figure 2). There was no variation in quantity or size of the exp-1 protein (23 kd) in any of the mutant lines compared with the 5.1-positive control (isolate K1). We conclude that they synthesize

**Table II.** Genetic characterization of *P. falciparum* lines containing exp-1 variants

| Parasite | Origin | Clone[a] | Enzyme isotype Ada[b] | Response to Pyr[c] | Antigens[d] | | | | | | | | Repetitive DNA pattern |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | PSA | | | | P55 | P155 | Exp-1 | | |
| | | | | | 7.3 | 9.2 | 12.1 | 12.2 | 13.4 | 12.3 | 5.1 | codon 160 | |
| K1 | Thailand | – | 1 | R | + | – | – | + | – | – | + | Pro | A |
| T9/100 | Thailand | * | 1 | R | – | + | + | NT | NT | + | – | Pro | B |
| Palo Alto 17 | Uganda | – | NT | NT | – | + | – | + | – | + | – | Thr | C |
| HB3 | Honduras | * | 2 | R | + | – | – | – | – | – | – | Pro | D |
| 3D7 | Netherlands | * | 1 | S | – | + | – | + | + | + | – | Thr | E |

[a]Some of the parasites used here (marked: *) are genetically pure clones obtained by limiting dilution from mixtures established in culture from malaria patients. The remainder (marked: –) have not been cloned out, although after many years in culture it is likely that a single type has been selected. In support of this view, only a single allele of each marker can be detected in these 'isolates'.
[b]Isozyme forms of adenosine deaminase (EC 3.5.4.4) are listed. Only two forms are ever found, ADA-1 and ADA-2.
[c]Sensitive (S) parasites are killed at $5 \times 10^{-9}$ M; resistant parasites survive $5 \times 10^{-5}$ M pyrimethamine.
[d]Antigens are defined by reactivity with a panel of monoclonal antibodies. PSA, polymorphic schizont antigen (McBride *et al.*, 1985; Mackay *et al.*, 1985). P55 and P155 are 55-kd and 155-kd antigens defined by monoclonal antibodies (Thaithong *et al.*, 1984). 5.1 = recognized by McAb 5.1 (+) or not (–). Codon 16; the residue at position 160 in exp-1 can be occupied by either proline or threnonine.
[e]Repetitive DNA pattern: DNA from each parasite line gives a distinctly different 'fingerprint' when probed with a repetitive DNA probe (rep 20; Oquendo *et al.*, 1985; Walker *et al.*, 1986).
NT, not tested.

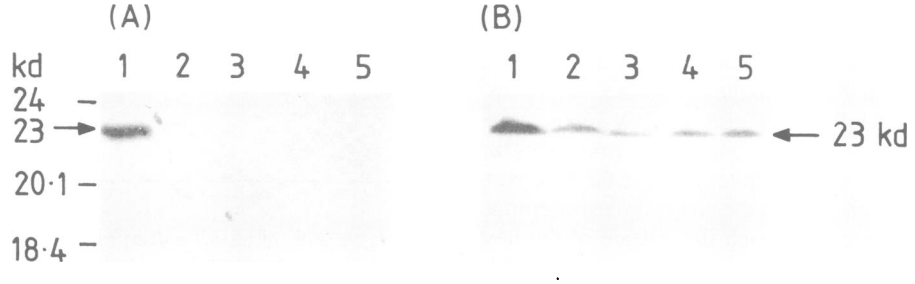


**Fig. 2.** Western blot analysis of exp-1 in *P. falciparum* lines. *P. falciparum* proteins from the lines described in Table II were separated by SDS–PAGE. Western blotted and probed with either (**A**) McAb 5.1 or (**B**) rabbit α exp-1. **Track 1**, K1 (5.1 positive control); **track 2**, T9/100; **track 3**, Palo Alto 17; **track 4**, HB3; **track 5**, 3D7. The size of exp-1 (23 kd) is indicated by an arrow. Molecular sizes were determined by maker proteins from Pharmacia.

exp-1 at normal levels. In addition, since we see no gross change in the apparent size of the protein, it is likely that only very minor changes, perhaps single amino acid substitutions, are responsible for absolishing binding or McAb 5.1.

To determine the structure of the 5.1 epitope, we made genomic libraries from each parasite out of which exp-1 clones were isolated and sequenced. The mutants, like the control, give a single 5.5-kb *Hin*dIII fragment hybridizing to the exp-1 cDNA probe. *Hin*dIII genomic libraries in the phage vector λ NM1149 (Murray, 1983) yielded clones containing the exp-1 gene from each parasite line. The inserts were digested with *Alu*I (Figure 4) and cloned into M13 mp18 and 19 for sequencing by dideoxy chain termination (Sanger *et al.*, 1977).

The nucleotide and translated amino acid sequences of the 5.1 epitopes are shown in Figure 3. Firstly, it can be seen that in each mutant a single base substitution has occurred. The original reading frame is thus conserved, allowing a normal-sized protein to be made, as we observed (Figure 2). Secondly, in each mutant the base change alters the Asp 136 residue contained in the tetrapeptide, Asn-Ala-Asp-Pro, inferred to the target for McAb 5.1. This result confirms our conclusion and demonstrates that the Asp 136 residue is crucial for antibody recognition.

Finally, each of the four different, independently isolated parasites acquire the same amino acid (Gly) in place of Asp 136 following an A → G transition mutation. This result suggests that the exp-1 protein may only tolerate limited changes at this

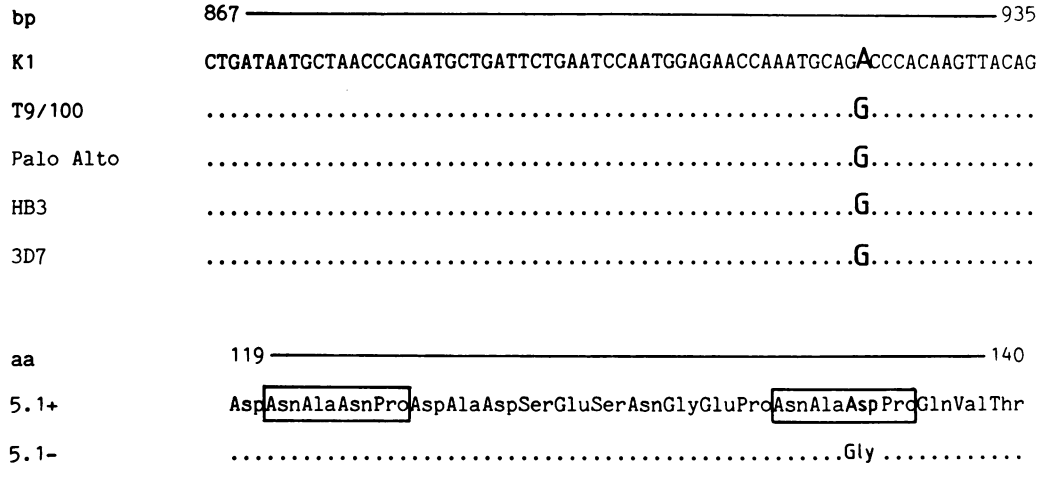


**Fig. 3.** Nucleotide and translated amino acid sequences of 5.1 epitope in five *P. falciparum* lines. All 5.1 negative lines have the same $A^{921}$ → G transition giving the amino acid sequence shown. Dots represent identical residues. bp: base pair scale (see Figure 4 for location of epitope in gene map). aa: amino acid scale of exp-1 protein. Boxed sequences are tetramers homologous to the circumsporozoite protein repeat units.

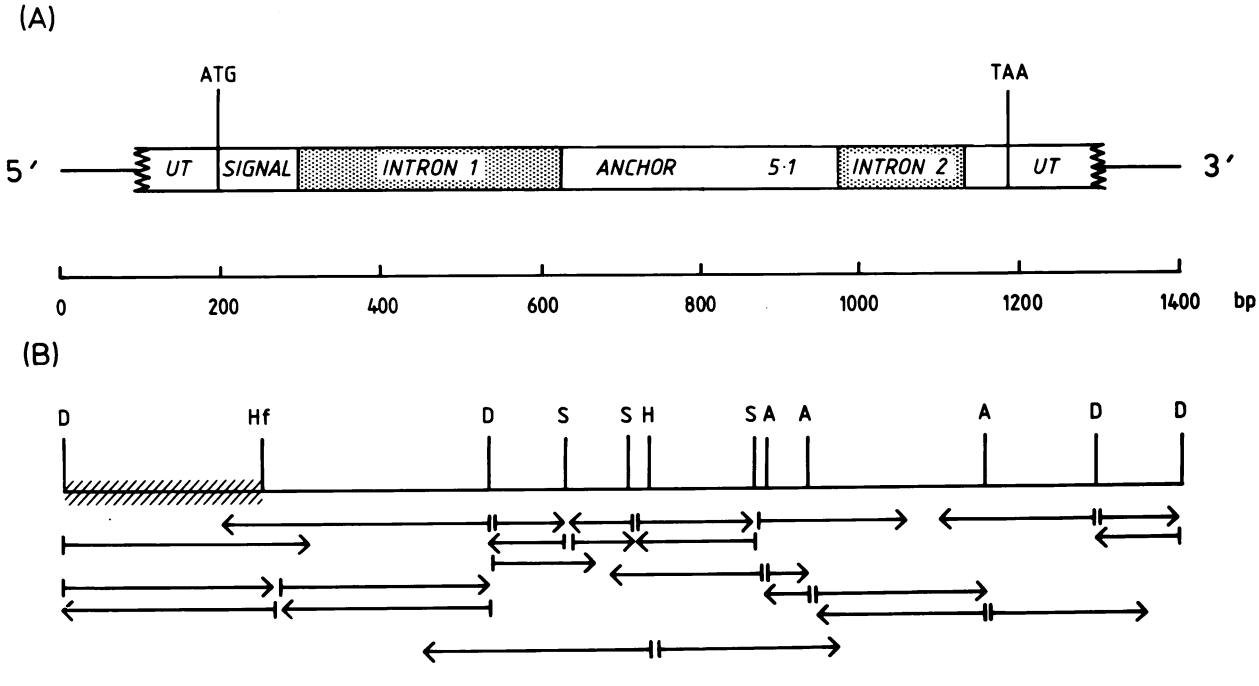


**Fig. 4.** Map of exp-1 gene and sequencing strategy. (**A**) Map of exp-1 gene determined from genomic and cDNA sequences. UT are untranslated sequences found in the cDNA. The precise limits of the exp-1 transcript have not yet been mapped. The putative signal and anchor peptides inferred from the DNA sequence are indicated. (**B**) Sequencing strategy (see Materials and methods). The hatched sequence defines a *Dra*I–*Hin*fI fragment containing the exp-1 signal sequence used to probe for multiple copies in genomic DNA (see Results). A = *Alu*I, D = *Dra* I, H = *Hin*fI.

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TTTAAATGTTCATGTAATAAAATAAATATGTAAATAAAATTCTTTATATTTTTTGAGAAATGTTATATATA   71
TATATATATATATATATAGAATATATTTATAAAGCATGTATAACAATTTTAATCAATTTTCTATAGAAA   142
CCTTTTTTTTCTTCTTCTCTTATAGTTTGTAGATTTTTAATTTATTTAATATATTCAAA ATG AAA ATC  210
                                                          Met Lys Ile     3
TTA TCA GTA TTT TTT CTT GTT CTT TTC TTT ATC ATT TTC AAT AAA GAA TCC TTA  264
Leu Ser Val Phe Phe Leu Ala Leu Phe Phe Ile Ile Phe Asn Lys Glu Ser Leu   21
GCC GAA AAA ACA AAC AAA GAA ACT GGA AGT GGT GTT AGC AGC AAA AAA AAA AAT  318
Ala Glu Lys Thr Asn Lys Glu Thr Gly Ser Gly Val Ser Ser Lys Lys Lys Asn   39
AAA A GTAAGTCATTTTGATATATATATATATATATGTATTGCGTTAGAAATGTATAATATGTATACAAT  387
Lys                                                                      40
ATATTTATGTAATGATTTTATTATATATATGAATACCTTTTTTTTTTTATTATGTTATGTAAAAATCATAT  458
ACATATATATATATATATGTATTATGTTTAAATGTGGACTTTTATTCATTATAAATTTATATATGACGAAT  529
TGAAGTATTTATTTTTTTTTGAAGTGAAATATATATTTTTGTATAATAATGTTGTGTGATATATATGTGTA  600
TGATATATAATATTTTATTTTCTTTTTGTTAATAG  AA GGA TCA GGT GAA CCA TTA ATA GAT  661
                                    Lys Gly Ser Gly Glu Pro Leu Ile Asp    49
GTA CAC GAT TTA ATA TCT GAT ATG ATC AAA AAA GAA GAA GAA CTT GTT GAA GTT  715
Val His Asp Leu Ile Ser Asp Met Ile Lys Lys Glu Glu Glu Leu Val Glu Val   67
AAC AAA AGA AAA TCC AAA TAT AAA CTT GCC ACT TCA GTA CTT GCA GGT TTA TTA  769
Asn Lys Arg Lys Ser Lys Tyr Lys Leu Ala Thr Ser Val Leu Ala Gly Leu Leu   85
GGT GTA GTA TCC ACC GTA TTA TTA GGA GGT GTT GGT TTA GTA TTA TAC AAT ACT  823
Gly Val Val Ser Thr Val Leu Leu Gly Gly Val Gly Leu Val Leu Tyr Asn Thr  103
GAA AAA GGA AGA CAC CCA TTC AAA ATA GGA TCA AGC GAC CCA GCT GAT AAT GCT  877
Glu Lys Gly Arg His Pro Phe Lys Ile Gly Ser Ser Asp Pro Ala Asp Asn Ala  121
                                                  *
AAC CCA GAT GCT GAT TCT GAA TCC AAT GGA GAA CCA AAT GCA GAC CCA CAA GTT  931
Asn Pro Asp Ala Asp Ser Glu Ser Asn Gly Glu Pro Asn Ala Asp Pro Gln Val  139
ACA GCT CAA GAT GTT ACA CCA GAG GTATGATTTTTTTTTTTTTTTTATAAATTATATATAT  994
Thr Ala Gln Asp Val Thr Pro Glu                                         147
ATATATATTAATATATTTTTTACATTTTCCTTTTAATAATATATCTGTGTATCCAAATGTCTATATATTTA 1065
TTTTATTTTTCTTTATTTTTATAG    CAA CCA CAA GGT GAC GAC AAC AAC CTC GTA AGT  1122
                           Gln Pro Gln Gly Asp Asp Asn Asn Leu Val Ser    158
GGC CCT GAA CAC TAAACAGCTGTAAACTTTTTTGTTAATGGGTTTTTTTGAAACACGTGAAAATAAT 1189
Gly Pro Glu His                                                         162
TTTTATTTATGATTATATTATATATATTGCTATTTTAAAAAAAAAAAAAAATAAAATAAAATAAAAAAAAA 1260
AAAAAATGAATAAAATAAAAAAAAAAAAAAAAAGAAATATATTTAAAAAATTCTAAATTCTAAAATATATTA 1331
AGTATTATATATATATATATATATATATATATATATATATTATATATATATATATATAT             1390
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**Fig. 5.** Nucleotide sequence and translated amino acid sequence of exp-1. The numbers on the right indicate nucleotide (top) and amino acid (bottom) positions. The putative signal sequence lies between Met 1 and Ala 22, the putative anchor sequence between Val 80 and Tyr 101 and the 5.1 epitope between Asn 129 and Thr 140. Asp 136 (box with asterisk) lies within the 5.1 eiptope and changes to Gly 136 in 5.1 negative parasites (see Figure 3). Pro 160, boxed, becomes Thr 160 in Palo Alto 17 and 3D7. Underlined nucleotides vary in the five lines as follows: $A^{65}T$ is absent from T9/100, $T^{425}$ is absent from Palo Alto and $C^{1047}$ changes to a T in T9/100 and HB3. Overlined nucleotides have homology with metazoan splice signals.

site, implying that its continued function is important for survival.

### *Organization of the exp-1 gene*

To extend previous studies on cDNA clones for exp-1 (Hope *et al.*, 1985; Coppel *et al.*, 1985), we decided to obtain the full sequence of the gene at the genomic level. By using subclones of the 5.5-kb *Hin*dIII genomic fragment (see above), we sequenced the gene and flanking regions for each of five different parasite lines (Table II). Our strategy and a detailed map obtained from these studies appears in Figure 4.

Comparison of the cDNA sequence (Hope *et al.*, 1985) with the genomic sequence (Figure 5) shows that the exp-1 gene contains two introns. To date, few introns have been documented in *Plasmodium*. One is in the gene for a histidine-rich protein (HRP) in the duck parasite, *P. lophurae* (Ravetch *et al.*, 1984). The existence of an intron in the RESA gene of *P. falciparum* has also been reported (Kemp *et al.*, 1986).

Because the cDNA sequence for exp-1 is known, we can locate

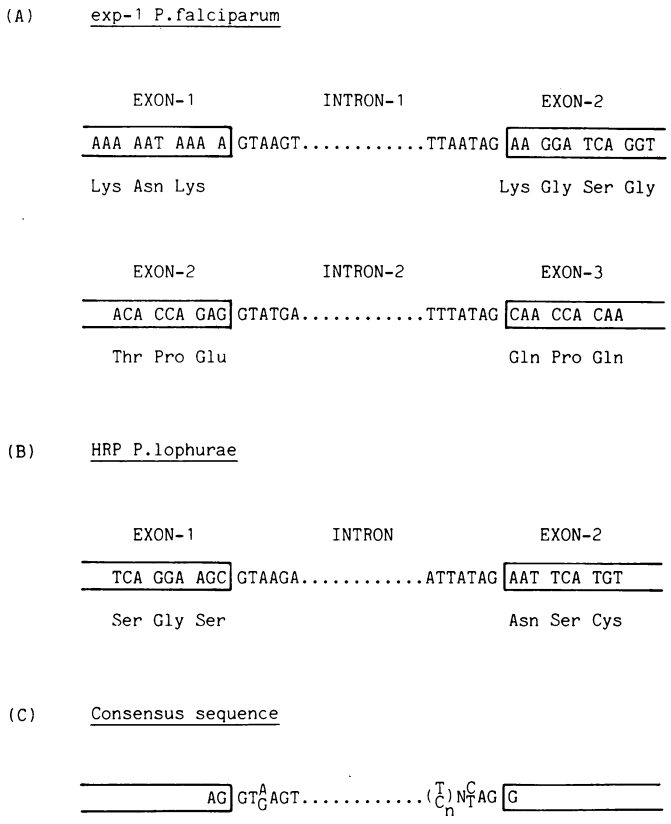


**Fig. 6.** Intron–exon junctions in *Plasmodium* genes. (**A**) exp-1 from *P. falciparum*. (**B**) Histidine-rich protein of *P. lophurae*. (**C**) Consensus sequences of 5′ and 3′ splice junctions from Mount (1982).

exactly the splice junctions used to excise the introns from the primary transcript of the gene. In Figure 6, their sequence is compared with those found in other organisms. Like all introns, they begin with GT and end with AG. In addition, adjacent bases fall within the concensus sequences deduced (Mount, 1982) from a wide range of organisms (see Figure 6) and in particular are very similar to those at the junctions of the *P. lophurae* intron (Ravetch *et al.*, 1984).

Both fungal and metazoan introns have an additional sequence, thought to participate in formation of the lariat intermediate in the splicing mechanism. In yeast, this 3′ splice signal, TACTAAC, is located near the 3′ splice site (Langford and Gallwitz, 1983; Pikielny *et al.*, 1983). In metazoans, a related, but more variable consensus sequence, PxYPyTPuAPy, occurs between 10 and 50 bases upstream from the 3′ splice site (Ruskin *et al.*, 1984; Keller and Noon, 1984). The exp-1 introns do not have the TACTAAC sequence. Candidate sequences related to the metazoan consensus do occur; two in intron 1 (2 and 288 bases upstream) and two in intron 2 (59 and 84 bases upstream). However, the location of these sequences differs from those in other organisms (Figure 5).

The introns have two other features worthy of comment. Firstly, they have stop codons in all reading frames. The coding sequences between them are short and have no significant homology with known intron maturases (Jacq *et al.*, 1980). Secondly, unlike other introns, their base composition is very rich in A–T (90%). This result is not surprising since the DNA of *P. falciparum* overall has 80% A–T and even the coding sequences have 65% A–T or more. On the other hand, it may mean that the splice

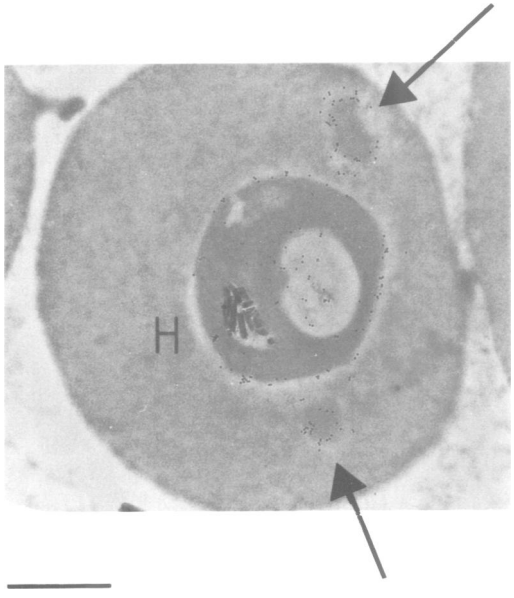


**Fig. 7.** Immunoelectron micrograph showing location of exp-1. The micrograph shows a section of a parasitized erythrocyte. It was reacted first with McAb 5.1, then with goat anti-mouse gold conjugate. Control sections treated with the gold-conjugated antibody alone revealed negligible staining (1–2 gold particles per erythrocyte). Note: the micrograph presented here is one of many obtained from this sectioned material. The majority of the structures labelled by the monoclonal antibody are separate from both parasitophorous vacuole and plasma membrane. A few appear to be connected to the parasitophorous vacuole. Final magnificiation: × 13 800. The bar represents 1 μm.

signal in *P. falciparum* diverges from the concensus discussed above.

The genomic DNA contains three exons. The first contains the start codon previously inferred to be used by the parasite (Hope *et al.*, 1985). The more extensive genomic sequence confirms this view; it is the only AUG before a stop codon, UAG, 10 triplets upstream in this frame.

The first exon contains the sequence encoding the N-terminal functional domain of exp-1 — the signal peptide. Recent studies on the LDL receptor reveal a close concordance between exons and functional domains of the protein (Südhof *et al.*, 1985). In addition, the first exon of the *P. lophurae* HRP gene also contains the signal peptide (Ravetch *et al.*, 1984).

The second exon encodes the transmembrane domain and the 5.1 epitope; the third encodes the short remaining C-terminal sequence (15 amino acids). Until more is known about exp-1, we cannot judge whether these parts of the protein are functionally distinct.

Ravetch *et al.* (1984) showed that exon 1 of the *P. lophurae* HRP gene had homology with several genomic fragments suggesting that it could be a conserved sequence encoding leaders for several proteins. However, the first exon of exp-1 (a *Dra*I–*Hin*fI fragment; see Figure 4) only hybridized to a single fragment of genomic DNA from *P. falciparum* (data not shown).

The entire gene for exp-1 was sequenced for five independent lines of *P. falciparum*. As discussed above, four of these have the same base change by which Asp 136 is changed to Gly. Otherwise, all five sequences are totally identical with the exception that two lines, Palo Alto 17 and 3D7, have the same transversion (C → A) changing Pro 160 to Thr. These results are therefore consistent with the conclusion, drawn from sequencing of 5.1 epitope variants, that the protein sequence is conserved.

*Untranslated regions*

The nucleotide sequence extending 200 bp upstream of the exp-1 coding region is identical in all five strains sequenced. Like other *P. falciparum* genes (Dame *et al.*, 1984; Cowman *et al.*, 1985; Mackay *et al.*, 1985), it is very AT rich. Characteristically, the 5′ sequence has interspersed runs of two, three and four T and A residues and ladders of alternating A and T. The same features appear in the 3′ untranslated region. For this reason, it is unprofitable to search the untranslated sequences for the transcription signals defined in other systems.

*The location of the exp-1 protein in the infected erythrocyte*

It has been possible to locate the exp-1 protein in sections of parasitized red cells labelled wtih McAb 5.1 and visualized for the electron microscope with a gold-conjugated second antibody. A typical section appears in Figure 7. It shows a parasite in mid-development containing malaria pigment (H). Some antibody is bound inside the parasite, presumably reflecting synthesis of the exp-1 protein. The remainder is bound to the periphery of the parasite. In addition, the monoclonal labels the outside of two bodies (arrows) located in the cytoplasm of the erythrocyte. There is no gold on the outer surface of the infected cell. We infer that the exp-1 protein is used inside the infected cell. In addition, we are led to the conclusion that under parasite control, new compartments develop in the erythrocyte to which protein(s) such as exp-1 are addressed.

## Discussion

Infection of erythrocytes by *P. falciparum* has long been known to cause structural changes in the host cell cytoplasm (Maurer, 1902; Aikawa and Seed, 1980). The immunogold studies presented here provide the first evidence for a defined compartment in this part of the infected red cell and show that a parasite-encoded protein is addressed to it.

The function of this structure is of great interest. Since we know that the parasite radically alters erythrocyte behaviour, it is reasonable to suppose that the new body participates in this process. We envisage two main areas in which it could play a role. The structure could serve as a metabolic outpost for the parasite. It could be a centre for proteolysis. The parasite does actively degrade haemoglobin. However, the evidence suggests that degradation occurs inside the parasite itself (Sherman, 1979; Aikawa and Seed, 1980). Carbohydrate metabolism in the erythrocyte is greatly stimulated by parasite infection. In particular, glucose uptake increases 20-fold and the sugar is largely catabolized to lactate which is excreted (Zolg *et al.*, 1984). It is quite possible that part of this pathway occurs in the new compartment. Otherwise, it could contribute to nucleotide metabolism providing a centre regulating utilization of host purines and hypoxanthine (Sherman, 1979).

Infected erythrocytes also develop a secretory pathway for parasite proteins. This novel activity must require vesicles or their functional equivalent which are not normally found in red cells. We favour the view that the compartment containing the exp-1 protein is part of this machinery. It makes the strong prediction that proteins in transit to the erythrocyte surface should pass through the new structure.

These questions relate to the function of exp-1. The coding sequence leads us to expect that it is a transmembrane protein. Further electron microscope studies should help us to decide how it is oriented in the membrane. It could be a receptor for a ligand molecule or for vesicles in transit through the red cell cytoplasm under parasite control. Alternatively, it could be used in

metabolite transport between intracellular compartments. The apparent absence of genetic variation in the coding sequence suggests that structure and function of exp-1 are important in the parasite.

The structure of the 5.1 epitope itself does vary. Nevertheless, isolates studied to date have only Asp or Gly at position 136 in the protein. Thus, it remains possible that here also structure is important and the protein can only function with Asp or Gly at this site on the chain.

We were interested to find two introns in this small gene. Their sequence points to the possible mechanism of gene splicing in *P. falciparum*. The highly conserved junctions of the exp-1 introns indicate that splicing follows a similar path to that in metazoans (Sharp, 1985), involving formation of an intramolecular lariat. Consistent with this view, it is possible to find sequences homologous to the splice signal located within metaozoan introns (Keller and Noon, 1984). However, examination of the predicted RNA sequence for both introns reveals recurring AT-rich motifs which can readily form extensive secondary structures. In this regard, they differ markedly from other introns, including that described in another *Plasmodium* species. Perhaps some of these structures are functionally important, playing a role in the splicing process.

## Materials and methods

*Parasites*

All *P. falciparum* lines were obtained from Dr D.Walliker (Edinburgh University); the uncloned isolates were from Thailand (K1, Thaithong and Beale, 1981) and Uganda (Palo Alto 17, McBride *et al.*, 1982), the parasite clones were from Thailand (T9/100, Rosario, 1981; Thaithong *et al.*, 1984), Honduras (HB3, Walliker *et al.*, 1986) and from an original case of airport malaria in the Netherlands (3D7, Walliker *et al.*, 1986). All parasites were cultured in the Hepes-buffered RPMI 1640 medium containing 10% human serum by a modification of the method of Trager and Jensen (1976).

*Construction of exp-1 subclones and expression assays*

The 660-bp insert encompassing the entire coding region of exp-1 (Hope *et al.*, 1985) was digested with *Alu*I and the fragments subcloned into M13 mp19 (Yanisch-Perron *et al.*, 1985) generating in-frame fusions with β-glactosidase. Similarly, the 69-bp fragment encoding the 5.1 epitope was gel-purified, cut with *Hin*fI and two fragments subcloned in-frame into M13 mp19.

*Escherichia coli* JM105 infected with M13 recombinant phages were grown to saturation overnight. Infected cells were then diluted 1:4 in fresh Luria broth, to a final volume of 10 ml, induced by adding IPTG to 1 mM and incubated at 37°C for 90 min before harvesting. Induced cells were lysed in 0.1 M Tris, pH 8.0, 5 mg/ml lsyozyme and 2 mg/ml DNase I on ice for 30 min. The extracts were sonicated briefly to ensure total cell lysis and the exp-1 fusion proteins affinity purified by passage through a rabbit anti (α)-exp-1 Sepharose column (Pharmacia). After thorough washing, specifically bound proteins were eluted with 50 mM diethylamine, pH 11.0, according to the method of Hall *et al.* (1984).

Quantitative enzyme-linked immunosorbant assays (ELISA) were performed by binding equal amounts of each of the exp-1 protein fragments ($5 \times 10^{-11}$ mol in 100 μl in PBS) to COSTAR micro ELISA plates overnight at 4°C. Plates were washed three times in PBS/0.1% Tween 20 (PBST), blocked with PBS/1% BSA for 30 min at 37°C and incubated with antibody probes; McAb 5.1 (1:1000 dilution), control myeloma NS-1 (1:1000), rabbit α exp-1 (1:500) and non-immune rabbit serum (1:500) for 1 h at 37°C. After three washes in PBST, the appropriate second antibody conjugated to horse-radish peroxidase (HRP) was added and incubated for a further 1 h at 37°C (affinity purified rabbit α mouse HRP or goat α rabbit HRP, Sigma). Finally, after three washes in PBST, plates were developed in 0.1 M phosphate/0.1 M citrate buffer, pH 5.0, containing *o*-dianisidine-HCl (2.5 μg/ml) and $H_2O_2$ (0.02%), in the dark for 15 min. Reactions were stopped by addition of 50 mM sodium metabisulphite, pH 2.0, and absorbance at 450 nm measured on a Dynatech Minireader II.

*Analysis of parasite proteins by SDS–PAGE and Western blotting*

Parasites were concentrated by saponin treatment modified from Zuckerman *et al.* (1967). We lysed infected erythrocytes with 0.1% saponin (10 min, 4°C) in the presence of 200 μg/ml phenylmethyl sulphonyl fluoride (PMSF; Sigma), centrifuged for 10 min at 12 000 *g* and the parasite pellet washed four times with PBS at 4°C. Parasite proteins were analysed on 10% SDS–polyacrylamide gels (Laemmli, 1970) and Western blotted (Towbin *et al.*, 1979). Monoclonal antibody 5.1 (Hope *et al.*, 1983) was used at 1:500 dilution. The rabbit α exp-1 antiserum was used at 1:100 dilution.

*Preparation of genomic DNA, genomic blots and construction of libraries*

Chromosomal DNA from each parasite line was prepared as described by Goman *et al.* (1982). DNA aliquots (3 μg) were digested, subjected to electrophoresis in 0.7% agarose, transferred to nitrocellulose (Southern, 1975) and hybridized to an exp-1 cDNA probe in 50% formamide, 5 × SSC 0.1 mg/ml salmon sperm DNA at 37°C. The filters were washed in 0.1 × SSC, 0.1% SDS at 37°C.

For construction of genomic libraries, DNA was digested to completion with *Hin*dIII, ligated to the phage λ vector NM1149 (Murray, 1983) and packaged. Exp-1 recombinants were detected by hybridization with the exp-1 cDNA probe (Benton and Davis, 1977).

*DNA sequencing*

Exp-1 genomic clones were digested with a variety of restriction enzymes (*Alu*I, *Dra*I, *Hin*fI, *Hpa*I and *Sau*3A) and subcloned into phages M13 mp18 and 19. They were sequenced by the dideoxy chain termination method (Sanger *et al.*, 1977).

*Immunoelectron microscopy*

Samples of parasite culture were fixed for 24 h at 4°C in 0.75% (w/v) paraformaldehyde in 0.1 M phosphate buffer, centrifuged at 2000 r.p.m. for 10 min and resuspended very gently in 4% (w/v) paraformaldehyde in the same buffer. After a further 24 h in 4°C, the cells were transferred to 10% (w/v) paraformaldehyde, left for 1 week at 4°C, washed extensively in phosphate buffer, dehydrated in ethanol and embedded in L.R. white resin, medium grade (London Resin Co. Ltd). Selections were first treated at room temperature with 2% bovine serum albumin in 0.05 M phosphate, pH 7.4, containing 0.25% Tween 20 (P. Tween) for 5 min. They were then transferred to a drop of McAb 5.1 (diluted 1:5) in P. Tween containing 1% bovine serum albumin, for 30 min at room temperature. The sections were washed in P. Tween, transferred to goat anti-mouse conjugated to 10-nm gold particles (Janssen Life Sciences), diluted 1:15 in P. Tween for 60 min, washed four times in P. Tween and twice in water.

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