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## Protein phosphatase $\beta$ , a putative type-2A protein phosphatase from the human malaria parasite *Plasmodium falciparum*

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Protein phosphatases play a critical role in the regulation of the eukaryotic cell cycle and signal transduction. A putative protein serine/threonine phosphatase gene has been isolated from the human malaria parasite *Plasmodium falciparum*. The gene has an unusual intron that contains four repeats of 32 nucleotides and displays a high degree of size polymorphism among different strains of *P. falciparum*. The open reading frame reconstituted by removal of the intron encodes a protein of 466 amino acids with a predicted molecular mass of approximately 53.7 kDa. The encoded protein, termed protein phosphatase  $\beta$  (PP- $\beta$ ), is composed of two distinct domains. The C-terminal domain comprises 315 amino acids and exhibits a striking similarity to the catalytic subunits of the type-2A protein phosphatases. Database searches revealed that the catalytic domain has the highest similarity to *Schizosaccharomyces pombe* Ppa1 (58% identity and 73% similarity). However, it contains a hydrophilic insert consisting of five amino acids. The N-terminal domain comprises 151 amino acid residues and exhibits several striking features, including high levels of charged amino acids and asparagine, and multiple consensus phosphorylation sites for a number of protein kinases. An overall structural comparison of PP- $\beta$  with other members of the protein phosphatase 2A group revealed that PP- $\beta$  is more closely related to *Saccharomyces cerevisiae* PPH22. Southern blots of genomic DNA digests and chromosomal separations showed that PP- $\beta$  is a single-copy gene and is located on chromosome 9. A 2800-nucleotide transcript of this gene is expressed specifically in the sexual erythrocytic stage (gametocytes). The results indicate that PP- $\beta$  may be involved in sexual stage development.

**Keywords:** *Plasmodium falciparum*; protein-serine/threonine-phosphatase; chromosome; mRNA; gametocyte.

Protein phosphatase 2A (PP-2A) accounts for a significant proportion of the total serine/threonine phosphatase activity in many tissues and cell types, and plays a critical role in signal transduction and the control of gene expression. It is involved in a number of cellular processes, such as metabolism, motility, cell division and cell-cycle progression (Wera and Hemmings, 1995). PP-2A is a heterotrimeric holoenzyme composed of a common core structure associated with different regulatory subunits. The core dimer consists of a 36-kDa catalytic subunit (C) and a 65-kDa regulatory subunit (A). The third variable subunit (B) (Mayer-Jackel et al., 1993; Csontos et al., 1996; Zolnierowicz et al., 1996) regulates phosphatase activity and specificity and generates a diversity of holoenzymes (Kamibayashi et al., 1994; McCright et al., 1996). In mammalian cells, at least two isoforms of the C subunit of PP-2A have been characterized

(Stone et al., 1987). PP-2A has been shown to be associated with microtubules and regulated during the cell cycle (Sontag et al., 1995). In yeasts, PP-2A is an essential enzyme encoded by at least two distinct genes (Kinoshita et al., 1990; Sneddon et al., 1990; Ronne et al., 1991). In *Schizosaccharomyces pombe*, disruption of both genes is lethal; in *Saccharomyces cerevisiae*, the double deletion causes slow growth and cell lysis with defects in the actin cytoskeleton and delayed entry into mitosis (Lin and Arndt, 1995). In *Drosophila*, mutations in genes encoding subunits of PP-2A cause defects in cell division (Mayer-Jackel et al., 1993; Snaith et al., 1996). PP-2A has also been implicated in the control of cyclin degradation in amphibian eggs (Lorca et al., 1991). It has been shown that PP-2A can bind to polyoma virus small and middle T antigens and simian virus 40 small t antigen (Pallas et al., 1990; Sontag et al., 1993); this interaction is thought to be necessary for their functions in transformation and viral replication (Mumby, 1995).

*Plasmodium falciparum* is a protozoan parasite responsible for the most severe form of human malaria, which remains one of the most prevalent infectious diseases in the world and causes 300–500 million clinical cases each year, resulting in up to 2.7 million deaths (Nussenzweig and Long, 1994). The parasite has a complex life cycle involving two hosts and interactions with multiple cell types. During the various stages of the life cycle the parasite undergoes several cycles of cell growth, division and differentiation. Sexual stage developments, gametocytogenesis in the host, and gametogenesis in the mosquito, are

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**Abbreviations.** PP-1, protein phosphatase 1; PP-2A, protein phosphatase 2A; PP-2B, protein phosphatase 2B; PP- $\beta$ , protein phosphatase  $\beta$ ; PFGE, pulse-field gel electrophoresis.

**Enzymes.** Protein kinase (EC 2.7.1.37); phosphoprotein phosphatase (EC 3.1.3.16); DNA-directed DNA polymerase (EC 2.7.7.7); type II site-specific deoxyribonuclease (EC 3.1.21.4).

**Note.** The novel nucleotide sequence data reported in this paper have been submitted to the GenBank/EMBL sequence data bank and are available under the accession number U89025.

accompanied by biochemical and morphological changes in the parasite. The signals involved in triggering cell growth, proliferation and the molecular events controlling sexual stage development of *P. falciparum* are unclear.

Protein kinases and phosphatases play a central role in regulation of cell-cycle progression and in signal-transduction pathways of eukaryotic cells (Hunter, 1995). In the malaria parasite, inhibitors of protein kinases and phosphatases can interfere with parasite growth (Ward et al., 1994), suggesting a requirement for phosphorylation/dephosphorylation in control of the parasite life cycle. Reversible phosphorylation has been shown to play an important role in invasion of erythrocytes by merozoites (Rangachari et al., 1986) and during intra-erythrocytic growth and development of malaria parasites (Yuthavong and Limpai-boon, 1987). Various inhibitors and activators of signal-transduction pathways have been reported to influence events such as exflagellation (Kawamoto et al., 1990; Ogwan'g et al., 1993). To reveal the signal-transduction pathways and cell-cycle events of *P. falciparum* regulated by reversible phosphorylation, the protein kinases and phosphatases involved have to be identified and characterized. Several protein kinases have been described in *Plasmodium berghei*, *Plasmodium chabaudi* or *P. falciparum* (Wiser and Schweiger, 1985; Wiser and Plitt, 1987; Read and Mikkelsen, 1990, 1991). Recently, a number of protein serine/threonine kinase genes have been isolated from *P. falciparum* (Zhao et al., 1992, 1993; Ross-MacDonald et al., 1994; Doerig et al., 1995; Kappes et al., 1995; Bracchi et al., 1996; Li et al., 1996; Lin et al., 1996). However, no information on protein phosphatases exists for malaria parasites. Here, we report the molecular characterization of a protein phosphatase from *P. falciparum*. This phosphatase, termed protein phosphatase  $\beta$  (PP- $\beta$ ), is most closely related to PP-2A of yeast but is distinct from it in several aspects. PP- $\beta$  not only contains an insert in the catalytic domain, but also has a large N-terminal extension. The gene, with one intron, is present on chromosome 9 as a single copy, and is specifically expressed in gametocytes, indicating that PP- $\beta$  may be important in regulating the processes of the sexual stage development and/or fertilization.

## MATERIALS AND METHODS

**Parasite culture and parasite materials.** *P. falciparum* clones T996 and 3D7A were cultivated at 37°C in RPMI-1640 medium (Gibco) supplemented with 25 mM Hepes, pH 7.2, 10% (by vol.) human serum (A<sup>+</sup>) and filter-sterilized gas (96% N<sub>2</sub>, 3% CO<sub>2</sub> and 1% O<sub>2</sub>) in a semi-automated continuous-flow apparatus. Mixed asexual stages of the parasite were collected at a parasitaemia of 5–10%; sexual stages of the parasite (gametocytes) were harvested after 14–18 days and purified by Percoll-gradient centrifugation. The purified gametocytes were mainly of stages III–V.

Parasites were recovered from infected erythrocytes by treatment with 0.1% (by vol.) saponin and washed with NaCl/P, (140 mM NaCl, 2.7 mM KCl, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.4). Parasite DNA and total RNA were then isolated as described previously (Li et al., 1996).

**Oligonucleotide design and synthesis.** The following oligonucleotides were synthesized and used for PCR and sequencing reactions. P1 (5'-GGIGATTAC<sup>T</sup>GTIGAT<sup>T</sup>AGIGG-3', nucleotides 1099–2018), P2 (5'-TA<sup>T</sup>GGITT<sup>T</sup>TA<sup>T</sup>GA<sup>T</sup>GA<sup>T</sup>TG-3', nucleotides 1228–1247) and P3 (5'-GGATCIIICCAIA<sup>A</sup>IA<sup>A</sup>TC-3', nucleotides 1441–1460) were used to identify the PP- $\beta$  gene, and correspond to the consensus sequence motifs GDYVDRG, YG-FYDEC and DLLWSDP, respectively, in the catalytic domain of protein-serine/threonine phosphatases (Cohen et al., 1990). P4

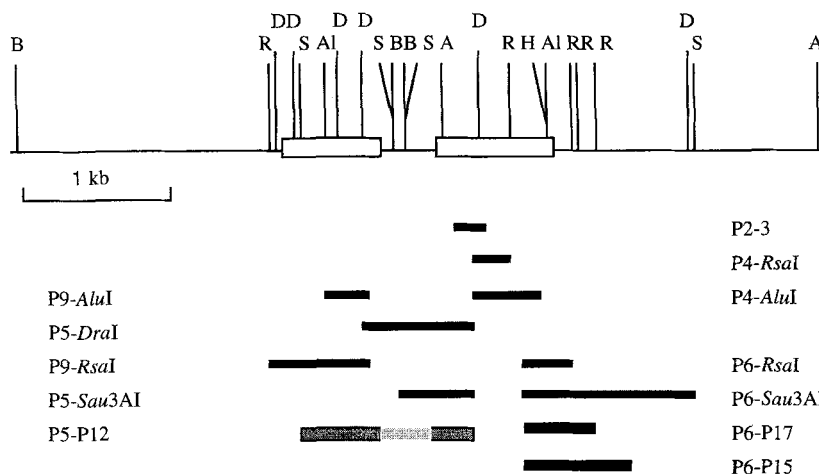
(5'-GTGACCATGGTGGTATATCGCC-3', nucleotides 1346–1367), P5 (5'-GGCGATATACCACCATGGTCAC-3', nucleotides 1346–1367), P6 (5'-AGTGGCCCAATTATTGTTA-TCG-3', nucleotides 1648–1670) and P9 (5'-ACAGGGACAT-TAATTCTGACGCAG-3', nucleotides 622–645) were used in Vectorette PCR to obtain further sequences in both directions. P10 (5'-TGTTGCTGGAGATATTCATGGAC-3', nucleotides 649–671), P12 (5'-GAACTAGATTGTGGACAATCAG-3', nucleotides 206–227), P15 (5'-GGTAAGTCAATTGCACAAA-TTAG-3', nucleotides 2499–2522) and P17 (5'-CCATAAATG-TAAACATGAATAAC-3', nucleotides 2079–2101) were used in reverse transcription/PCR or for sequence analysis. All these oligonucleotides were synthesized by Pharmacia. The Vectorette I primer, Vectorette I nested primer and Vectorette I sequencing primer were obtained from Cambridge Research Biochemicals.

**Construction and screening of DNA libraries.** A  $\lambda$ GEM-12 genomic library was constructed using a commercial kit (Promega) and screened as described previously (Li et al., 1996). A number of Vectorette libraries were constructed from *P. falciparum* 3D7A genomic DNA (Li et al., 1996). 1  $\mu$ g genomic DNA was digested with several restriction enzymes, and approximately 200 ng of the digested DNA was ligated directly onto Vectorette adaptors (Cambridge Research Biochemicals). Based on the known sequence and Southern blot information, a number of specific primers were synthesized, and PCR was performed using a specific primer and the Vectorette I primer. The resulting PCR fragments were sequenced directly or cloned into the pGEM-T vector (Promega) for sequence analysis.

**Southern and northern blotting.** For Southern blotting, approximately 4  $\mu$ g genomic DNA (*P. falciparum* clone 3D7A) was digested overnight with several restriction enzymes and then fractionated on a 1.0% agarose gel. For northern blotting, approximately 10  $\mu$ g total RNA extracted from asexual and sexual erythrocytic stages of *P. falciparum* 3D7A were separated on 1.0% agarose gels under denaturing conditions using formamide and formaldehyde in Mops buffer (Li et al., 1996). DNA gels were then denatured in 0.5 M NaOH, 1.5 M NaCl and neutralized in 1.5 M NaCl, 1 M Tris/HCl, pH 8.0. The nucleic acids were transferred to Hybond N<sup>+</sup> nylon membranes (Amersham) in 20 $\times$ NaCl/Cit (1 $\times$  = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0). The membranes were dried at room temperature and the nucleic acids covalently cross-linked (Ultraviolet Cross-linker, Stratagene) with ultraviolet light prior to hybridization.

**DNA and RNA hybridization.** Southern, northern and pulse field gradient electrophoresis (PFGE) blots or plaque-lift filters were prehybridized in 50% formamide, 6 $\times$ NaCl/Cit, 5 $\times$ Denhardt's solution, 0.5% SDS and 100  $\mu$ g/ml denatured herring sperm DNA at 42°C for 4–6 h. Hybridization with a radiolabeled DNA probe (random-hexamer-primed DNA-labeling kit, Boehringer Mannheim) was carried out overnight under the same conditions. Filters were washed at 52–65°C once in NaCl/Cit/0.1% SDS for 30 min and twice in 0.5 $\times$ NaCl/Cit/0.1% SDS for 40 min. If necessary, the filters were washed further at the same temperature in 0.2 $\times$ NaCl/Cit/0.1% SDS. All filters were autoradiographed at –70°C.

**PCR.** Reaction mixtures (50  $\mu$ l total volume) contained 10 mM Tris/HCl pH 8.3, 2.5 mM MgCl<sub>2</sub>, 50 mM KCl, 0.01% gelatin, 200  $\mu$ M each dNTP, 0.5  $\mu$ M each primer, 1–20 ng DNA as template and 2.5 U *Taq* DNA polymerase (Promega). Samples were overlaid with sterile mineral oil (Sigma) and subjected to 36 cycles of denaturation for 50 s (4 min for the first cycle) at 94°C, annealing for 1 min at 48–68°C, and extension for 2 min at 72°C (10 min for the final cycle). For reverse transcription/PCR, first-strand cDNA was generated from 1  $\mu$ g total RNA using the (dT)<sub>12–18</sub> primer or random hexamer in the rapid-amplification-of-5'-cDNA-ends system of synthesis (Gibco) with or



**Fig. 1.** A schematic representation of a partial restriction map of the *PP- $\beta$*  gene and the overlapping PCR fragments used to determine the nucleotide sequence of the *PP- $\beta$*  gene. A, *AccI*; Al, *AluI*; B, *BclII*; D, *DraI*; H, *HindIII*; R, *RsaI*; and S, *Sau3AI*. The open boxes indicate the coding regions of the *PP- $\beta$*  gene, and the shaded box represents the reverse transcription/PCR fragments (P5–P12). Primers P5, P9, P15 and P17 are in the 5' direction, P4, P6 and P12 in the 3' direction.

without RNase A and DNase I. PCR were carried out on template cDNA as above. PCR products were separated on agarose gels, purified using a GeneClean kit (Bio 101, Inc.) and cloned into a plasmid vector for sequence analysis.

**Subcloning of DNA fragments and sequence analysis.** Inserts from the  $\lambda$ GEM-12 library and restriction-endonuclease-generated fragments isolated from it were subcloned into the pUC19, pBluescript or pGEX-1 $\lambda$ T plasmid vectors. PCR fragments were cloned into the pGEM-T vector (Promega). DNA sequence analysis was carried out with a Sequenase Version 2.0 kit (Amersham) on double-stranded or single-stranded plasmid DNA. Single-stranded DNA was generated by treatment with T7 gene 6 exonuclease (Amersham). Both strands were sequenced using vector-derived primers and a number of specific primers spanning the whole sequence. Sequence data derived from PCR were verified using independent fragments. Sequence data analysis was carried out using the DNA Inspector IIe programme (Textco Inc.) and the MRC Human Genome Mapping Project Resource Centre computer facilities.

**PFGE.** Preparation of agarose blocks containing chromosomal DNA, and PFGE with a Bio-Rad CHEF DRII system were performed as described previously (Li et al., 1996). After electrophoresis, the gel was deproteinized, denatured, neutralized and blotted onto a Hybond N<sup>+</sup> nylon membrane. The membrane was fixed with ultraviolet light. Prehybridization and hybridization of the PFGE blot were performed as described above.

## RESULTS

**Identification and isolation of the gene encoding *P. falciparum* PP- $\beta$ .** Phosphatases of the PP-1/PP-2A/PP-2B family have a catalytic domain of approximately 32 kDa, within which some sequences are remarkably conserved amongst all members of the family. Three degenerate oligonucleotide primers, P1, P2 and P3, were designed on the basis of the highly conserved sequences GDYVDRG, YGFYDEC and DLLWSDP, respectively. By means of semi-nested PCR (P1 and P3 followed by P2 and P3) a single band of approximately 230 bp was amplified from *P. falciparum* genomic DNA (3D7A; data not shown). The band was purified and cloned into the pGEM-T vector. A positive clone, termed P2-3, was obtained and sequenced. The sequence data showed that the P2-3 clone contains a 232-bp insert, encoding 77 amino acids of a protein-serine/threonine-phosphatase-

like enzyme. A database search revealed that this protein is most closely related to PP-2A. Nucleotide sequence analysis revealed an A+T content of 70.3%, typical of the coding region of a *P. falciparum* gene (Weber, 1988). Codon usage is consistent with other *P. falciparum* sequences. Southern blotting results confirmed that P2-3 originates from *P. falciparum* DNA (data not shown).

To isolate the remainder of the gene, a  $\lambda$ GEM-12 genomic DNA library was screened with a P2-3 radiolabeled probe. A positive clone, termed  $\lambda$ PP1, was isolated and confirmed the presence of the gene by PCR using the phage DNA as template. Restriction digestion of  $\lambda$ PP1 DNA with *EcoRI*, *BamHI* and *NotI* generated a fragment of approximately 2.3 kb, which was shown by hybridization to contain the target gene. However, the fragment was unclonable. Accordingly, Vectorette libraries were constructed and screened by PCR. Restriction analysis and Southern blotting using the P2-3 probe enabled development of a partial restriction map of the gene (Fig. 1). PCR amplifications of Vectorette libraries using the P4 and P5 primers and restriction sites known from the initial sequence were used to walk outwards in both directions. The sequence data thus acquired permitted synthesis of the P6 and P9 primers and subsequent complete sequencing across the gene (Fig. 1). There were slight variations in the numbers of TA repeats within the nucleotide region 1810–1837 and of T repeats in the region 1845–1859 in different PCR products, but use of P15 and P17 in PCR with P6 led to the consensus sequence shown in Fig. 2.

**Characteristics of the *PP- $\beta$*  gene.** The sequence derived from overlapping PCR clones comprises 2838 bp (Fig. 2). The proposed coding region of the *PP- $\beta$*  gene, beginning with a putative ATG codon at nucleotide 38 and terminating with a TAA at nucleotide 1795, is interrupted by a single intron. The sequence and codon usage in the coding regions are typical for a *P. falciparum* gene. Several lines of evidence indicate that the whole coding region of the *PP- $\beta$*  gene may have been obtained. First, there is one in-frame stop codon located at nucleotide position –12, upstream of the putative ATG initiation codon and at least three in-frame stop codons, at nucleotide positions +7, +92 and +104, downstream of the TAA termination codon. In addition, the A+T content of the coding region is about 70.5%, while the A+T contents of the 3' flanking (1041 bp) and intron non-coding regions are 85.9% and 85.0%, respectively, higher than that

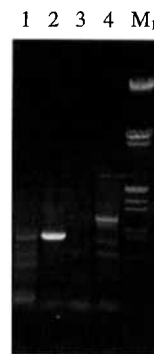
ACGAAGACAT AATACATTAA TATTTTAAAA GGGAAGGATG AAAAAATTGT AATTATCAAA 60  
 M K N C E L S K 8  
 ACGTGATTTA AITTTTAATA ATGTAATAAA TACAAGAAT AATTTAAAGC GTCTGAAATA 120  
 R D L I F N N V I N T K N N L K R L N K 28  
 AAACGTAATA GGAGATCTTA CTTATGCCCA AAAAAACCA AAGATACCGT CTTTAAATPT 180  
 N V K G D L T Y A Q K K P K D T S L I I 48  
 TGAAGAAGAT AAGATGAGG ATAGTGAECT AGATTGTGGA CAATCGAGAA AGAATACAA 240  
 E E D K D E D S E L D C G Q S E K K Y K 68  
 AATATCAAT GGGAAGAAA TAAATAATC ATTTCCAAAT TTTAATAAAG AAGTAAATTA 300  
 I S N G E E I N N T F P N F N K E G N Y 88  
 TAATATATAT GATAATTTAT CCGTAAAAGA ATTAAGCGAT GAAGAAGTAA GCTCCGAAAA 360  
 N I Y D N L S V K E L S D E E V S S E N 108  
 TGGATATAT AAAAAATAA AAAAAAATGT TTTAAACAAC CCAATAAATA ATCAAAACTC 420  
 G Y N K K I K K N V L N N P I N N Q N S 128  
 CATAGAACTA TATACTAAAA CGTCAATAAA CGATGAACAA AATTTATAAA ATATTAATGA 480  
 I E L Y T K T S I N D E Q N Y K N I N E 148  
 ACAAAACAAT AATAGTTCCG GCAAAATCTC TTATAATGTT GATGAATGGA TTTGTAAATT 540  
 Q N N N S S G K I S Y N V D E W I C K L 168  
 GTTAAATGTT GAATTTGCTAA AAATCGAAGA AGTCAAAATTA ATGTGTGACC TCTTAATGG 600  
 L K C E L L K I E E V K L M C D L L I G 188  
 TATTTAAAA AATGAAGAAA ACTGCCTCAG AATTAATGTC CCTGTAACCT TTGCTGGAGA 660  
 I L K N E E N C V R I N V P V T V A G D 208  
 TATTCATGGA CAATTTTTCG ACCTTTTGGG ATTATCCCAT ATCGGTATAT TTTTATATAT 720  
 I H G O F F D L L E L F H I 222  
 GTAGCAACA CAAATGCAT TGAAGGTTT TAATTTTAT TACCTGATC ATGTCATAA 780  
 TATATAGATA TATATATATA TATATATATA TATATATAT TAGATATTT TACCTGATCA 840  
 TGTTCAATAT ATATAGATAT ATGTTTATGA TTTTACCTGA ACATGTTCAAT AATATATACA 900  
 TATTAATTTA TGATTTTACC TGAACATTTA CATAATATAT ATATATATAT ATATATATAT 960  
 ATATATATAT ATAGTTTATC ACCTTGACAC ATATAAAAA ATATATATAT ATATATATAT 1020  
 ATATATATAT TTTTCTGTTT TTTTFTTTTT TTTTFTTTTT TAGGAGGTTT ACCACCTGAT 1080  
 G G L P P D 228  
 GTGAATTTAT TATCTTACG CGATACGTA GACAGAGGT ACTATTCTGT TGAGTGTGTT 1140  
 V N Y L F L G D Y V D R G Y Y S C E C F 248  
 TGCTTAGTTG CTTGTTTAAA GATAAATAAC CCCAGCAGG TGACACTATT GAGAGGTAAT 1200  
 C L V A C F K I K Y P S R V U T L L R G N 268  
 CATGAGAGCA GACAGATAAC CAAGGTATAT GGTTTTATG ATGAATGAT AAGAAAATAT 1260  
 H E S R Q I T K V Y G F Y D E C I R K Y 288  
 AATAATAACA ACATAGTATG GAAATATTTA ACGGATGAT TTGATTTAT GCCTTTAACA 1320  
 N N N I V W K Y L T D V F D Y L P L T 308  
 GCAATAATA ATGATAGAT ATTTTGTGAC CATGGTGGTA TATCGCCATG TTTGCCAACA 1380  
 A I I N D E I F C D H G G I S P C L Q T 328  
 ATAGATGAGA TAAATAAGTT GGATAGGTTT AAAGAGATAC CTCAGGATGG AGCAATATGT 1440  
 I D E I N K L D R F K E I P Q D G A I C 348  
 GATTATTTGT GGAGTGACCC GCCTGGTCTT GAGGACGAAA TAACAGAGGG TTGGAACCTT 1500  
 D L L W S D P A G P E D E I T E G W K P 368  
 TCTCTAGAG GAGCAGGAGT TTTATTTAGT GAAGAAAAA CCGAGAGTTT TTTACGTATG 1560  
 S P R G A G V L F S E E K T E S F L R M 388  
 AATAAACITTT CTTGTATATG TAGGGCTCAT CAGTTATAC CAGTTGATG TCAGTGGATG 1620  
 N K L S C I C R A H Q L V Q D G F Q W M 408  
 CATAATGATA AAGTTGTTAC AATTTTATG GCGCCCAATT ATTTGTTATG TTGTTGGTAA 1680  
 H N D K V V T I F S A P N Y C Y R C G N 428  
 TGTCACCTT TAATGCTTGT AGATGAATTT ATGGAAGAGG ATTTTATAAC GTTTACACAT 1740  
 C A S L M L V D E P M E K D F I T F N T 448  
 GCTCCATTA GAGCAATATG TCAAGCTTTA AGAAGAAATG TCAATATAT GTTGTAAATC 1800  
 A P L R A N A Q A L R R N V Q Y M L \* 466  
 TTTGAAAA ATATATATAT ATATATATAT ATATATATAT CCACCTTTTT TTTTFTTTTT 1860  
 TATTTATTTT ATTCATTTT ATTTTCTAAA AGAAATATAT AATACATACA TTACTTTCAA 1920  
 TTTCTAGTTT TTTTACAC CTTTTCTTA TGAAGATCC TTTTATATA AAGATATATG 1980  
 TACATAAAT ACTTACCATA TATATATAT ATATATATAT GTATTTATAC ATATGTACAT 2040  
 TATTTATTTA TATTTTTTT TTTTAAITTA ACTTTTATG PATTCATGTT TACATTTATG 2100  
 GTATTTAAA ATTTTAAATTT TCCCCATTT CTATTTTFTT CGIACCTCAT TATATATATA 2160  
 TATAAATAT GTGTATGAT GTATTTATTT ATTTATTTTT TTTTFTTCC TTTTGTGTTT 2220  
 TAATAAGTCA TATATATATA TATATATATA TATATATATA TATAATCTTT TCCCTCTTTC 2280  
 TTTCTTTTT CATTTTATTT AATTTTPTGG GATTAATATT AAAGACTATT ATAAAATAAA 2340  
 CTFACACACA TGAATATTA TATTATATG TATTATATA TATATATATA TGGAAAAATT 2400  
 TATTTGTTTT GATGATTTAA ATTAGGTTAG CGAATATATT ATACCCTGAA GAAAAAATGA 2460  
 AAATCTGCA TATTTTAAAG TTTTATTAAT ATATAAATCT AATTTTGTGC AATTGCATTA 2520  
 CCATATATAT TTATATAAAT TTATTTATAG TATTTATAAA GAACAAGAGG TCGTAAATAT 2580  
 AAATATATG TATTTTAAATA TTTTATACAT ATATATTTAT TATTTCCCTT TTTATATATT 2640  
 TTTTTCATT TTATTTATACA TTTGATATAT TTTTATATAT TGTATATTT GTTTTATTTT 2700  
 TATAAAAAA GAAGAAAAA AATGAGAAAA GGAAGAGTTT CATTTTATTT ATAATGTTAA 2760  
 TTAAGATAT AATTTTAAAT GGTTTATTTA TCAATGATA TTATCATTTA AATTGGTTAA 2820  
 TTTAAAAAG AAGAGATC 2838

**Fig. 2. Nucleotide and deduced amino acid sequences of the PP-β gene.** The nucleotides and the amino acids are numbered on the right. The amino acid sequences used to synthesize PCR primers for identification of this gene are underlined. The in-frame start and stop codons are in bold. A potential polyadenylation signal, the G+T-rich region, and the conserved nucleotides of the exon-intron boundary sites are bold and underlined. The repeated nucleotide sequence within the intron is underlined. The asterisk indicates the termination codon.

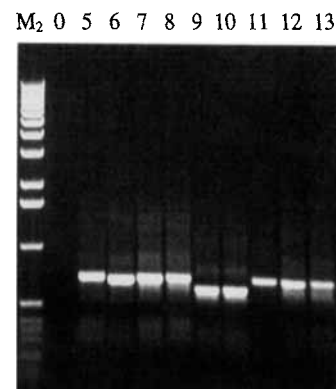
of the coding region. Moreover, the C-terminal region of the predicted protein is comparable to the PP-2A homologues of other organisms and the N-terminal region is larger than any known phosphatases of the PP-2A group. A putative polyadenylation signal AATAAA is found at nucleotide +537, downstream of the TAA termination codon. A diffuse G+T-rich sequence is also found at nucleotide +62, downstream of the polyadenylation signal (Lanzer et al., 1993).

The proposed intron interrupts the coding region midway, extending from nucleotide 705–1063. It begins at GT and ends at AG, the highly conserved dinucleotides forming intron boundaries. The sequence around the 5' splice site of the intron (GTA-TAT) includes two more conserved nucleotides, while the sequence around the 3' splice site (T<sub>n</sub>AG) is fairly consistent with

**A**



**B**



**Fig. 3. Confirmation and characterization of an intron in the PP-β gene by PCR.** (A) An agarose-gel analysis of reverse transcription/PCR products corresponding to the P5–P12 region of PP-β amplified from sexual-stage cDNA of *P. falciparum*. Lane 1, a PCR product using the oligo(dT) primer in the cDNA-synthesis step; lane 2, a PCR product using the random primer in the cDNA-synthesis step; lane 3, a PCR product using no reverse transcriptase in the cDNA synthesis step; lane 4, a PCR product from *P. falciparum* genomic DNA; M<sub>1</sub>, λ DNA digested with *EcoRI*+*HindIII* (Promega). (B) An agarose-gel analysis of PCR products corresponding to the P5–P10 region of PP-β amplified from different strains/clones DNA of *P. falciparum*. Lanes 5–13, the PCR products from K1, T996, T994R, FC27, Honduras, H1, V1S, T994, 3D7A, respectively; lane 0, a negative control without template DNA; M<sub>2</sub>, a 1-kb DNA ladder (BRL).

the consensus sequence found at the 3' end of eukaryotic introns (Padgett et al., 1986). Long runs of poly(AT) and poly(T) are found in the intron. A 32-nucleotide repetitive sequence occurs within the PP-β intron, which is comprised of TTTACCTGAT-CATGTTTATAATATATAGATAT and repeats four times. The repeat unit contains a relatively high proportion of C+G nucleotides (7 of 32). The repetitive sequences represent over 35% of the total intron length. In the third repeat, T10 is replaced by A and G28 replaced by C; whereas in the fourth repeat, four nucleotides, T, G, T and G, at positions 10, 14, 16 and 28, are replaced by A, T, A and T, respectively (Fig. 2). To investigate the existence of the intron, reverse transcription/PCR was performed using the P5 and P12 primers (Fig. 3A) and the product sequenced directly. The sequence results confirmed the presence of the intron and the precise intron-exon boundaries. PCR analysis on genomic DNA from various *P. falciparum* strains using P5 and P10 primers revealed that the intron sizes are highly variable in different parasite strains (Fig. 3B). This, together

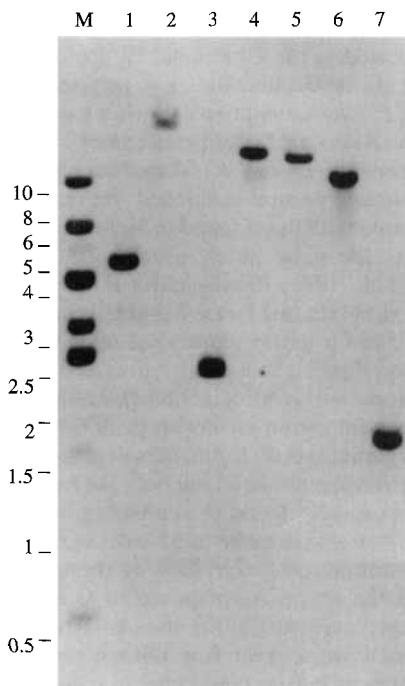
PfPP- $\beta$	MKNCELSKRDLIFNNVINTK	NNLRLNKNVKGDLTYAQKK	PKDTSLEIIEEDKDESELDC	60
ScPPH22	-----	-----	-----	
SpPpa1	-----	-----	-----	
HuPP-2A $\beta$	-----	-----	-----	
RbPP-2A $\beta$	-----	-----	-----	
PfPP- $\beta$	GQSEKKYKISNGEEINNTFP	NFNKEGNYNIYDNLVSKELS	DEEVSSSENGYNKKIKKNVLN	120
ScPPH22	-----M	DMEIDDFMHGSDQLSPTL	DEDMNSDDGKNNTKARSNDE	41
SpPpa1	-----	-----	-----	
HuPP-2A $\beta$	-----	-----	-----	
RbPP-2A $\beta$	-----	-----	-----	
PfPP- $\beta$	NPINNQNSIELYTKTSINDE	QNYKNINEQNNSSGKISYN	VDEWICKLLKCELLKIEEVK	180
ScPPH22	DTDEELEDNFNFKPGSSGIAD	---HKSSKPLKLTNTNINQ	LDQWIEHLSKCEPLSEDDVA	97
SpPpa1	-----	-----MSVSGKIGE	VDRWIEQLSRCEPLSEEDVI	29
HuPP-2A $\beta$	-----	-----MDDKAFTKE	LDQWVEQLNECKQLNENQVR	29
RbPP-2A $\beta$	-----	-----MDDKTFTKE	LDQWVEQLNECKQLNENQVR	29
			* * * * *	
PfPP- $\beta$	LMCDLLIGILKNEENCVRIN	VPVTVAGDIHGQFFDLLELF	HIGGLPPDVNYLFLGDYVDR	240
ScPPH22	RLCKMAVDVLQFEENVKPIN	VPVTICGDVHGQFHDLELF	KIGGPCDNTNLYLFGDYVDR	157
SpPpa1	QMCDLAKEVLSVESNVQSVR	CPVTVCGDVHGQFHDLMELF	NIGGPSPTNLYLFGDYVDR	89
HuPP-2A $\beta$	TLCEKAKEILTKESENQVEVR	CPVTVCGDVHGQFHDLMELF	RIGGKSPDTNLYLFGDYVDR	89
RbPP-2A $\beta$	TLCEKAKEILTKESENQVEVR	CPVTVCGDVHGQFHDLMELF	RIGGKSPDTNLYLFGDYVDR	89
	* * *	*** ** * ** * * * *	*** ** * ** * * * *	
PfPP- $\beta$	GYYSCECFCLVACFKIKYPS	RVTLRLGNHESRQITKVYGF	YDECIRKYNMNNIVWKYLT	300
ScPPH22	GYYSVETVSYLVAMKVRYPH	RITILRGNHESRQITQVYGF	YDECLRKYG-SANVWKMFTD	216
SpPpa1	GYHSVETVSLLIAFKIRYPQ	RITILRGNHESRQITQVYGF	YDECLRKYG-NANVWQYFTD	148
HuPP-2A $\beta$	GYYSVETVTLVALKVRYPE	RITILRGNHESRQITQVYGF	YDECLRKYG-NANVWYFTD	148
RbPP-2A $\beta$	GYYSVETVTLVALKVRYPE	RITILRGNHESRQITQVYGF	YDECLRKYG-NANVWYFTD	148
	* * * * *	* * * * * * * * * * * * *	* * * * * * * * * * * *	
PfPP- $\beta$	VFDYLPLTAIINDEIFCDHG	<del>GLSPCLQCTIDEINKLDRFKE</del>	IPQDGAICDLLWSDPAGPED	360
ScPPH22	LFDYFPVTALVDNRI FCLHG	GLSPMIETIDQVRDLNRIQE	VPHEGPMCDLLWSDPDD---	273
SpPpa1	LFDYLPLTALIEDRIFCLHG	GLSPSIDTLDHVRLDRVQE	VPHEGPICDLLWSDPDD---	205
HuPP-2A $\beta$	LFDYLPLTALVDGQIFCLHG	GLSPSIDTLDHIRALDRLQE	VPHEGPMCDLLWSDPDD---	205
RbPP-2A $\beta$	LFDYLPLTALVDGQIFCLHG	GLSPSIDTLDHIRALDRLQE	VPHEGPMCDLLWSDPDD---	205
	* * * * * * * * * * * *	* * * * * * * * * * * *	* * * * * * * * * * * *	
PfPP- $\beta$	EITEGWKPSPRGAGVLFSEE	KTESFLRMNKLSCICRAHQ	VQDGFQWMHNDKVVVTFISAP	420
ScPPH22	--RGGWGISPRGAGTFGQD	ISEQFNHTNDLSLARAHL	VMEGYSWSHQQNVVTFISAF	331
SpPpa1	--RPGWGISPRGAGYTFGPD	IAEAFNHNGLDLARAHL	VMEGYNWTNHNVVVTFISAF	263
HuPP-2A $\beta$	--RGGWGISPRGAGYTFGQD	ISETFNHANGLTLVSRHQ	VMEGYNWCNDRNVVTFISAP	263
RbPP-2A $\beta$	--RGGWGISPRGAGYTFGQD	ISETFNHANGLTLVSRHQ	VMEGYNWCNDRNVVTFISAP	263
	** * * * * * *	* * * * * * * * * * * *	* * * * * * * * * * * *	
PfPP- $\beta$	NYCYRCGNQAAIMELDDTLK	KDFITFNTAPLRANAQALRR	NVQYML	466
ScPPH22	NYCYRCGNQAAIMEVDENHN	RQFLQYDPSVRPGEPTVTRK	TPDYFL	377
SpPpa1	NYCYRCGNQAAIMGIDDHIN	YAFIQYDTPRKEELHVTRR	TPDYFL	309
HuPP-2A $\beta$	NYCYRCGNQAAIMELDDTLK	YSFLQYDPAAPRRGEPHVTRR	TPDYFL	309
RbPP-2A $\beta$	NYCYRCGNQAAIMELDDTLK	YSFLQYDPAAPRRGEPHVTRR	TPDYFL	309
	* * * * * * *	* * * * * * * * * * * *	* * * * *	

**Fig. 4.** Alignment of the predicted amino acid sequence of PP- $\beta$  with those of *S. cerevisiae* PPH22, *S. pombe* Ppa1, human PP-2A $\beta$  and rabbit PP-2A $\beta$ . The GenBank/EMBL database accession numbers are as follows: *P. falciparum* PP- $\beta$  (PfPP- $\beta$ ), U89025; *S. cerevisiae* PPH22 (ScPPH22), M60317; *S. pombe* Ppa1 (SpPpa1), M58518; human PP-2A $\beta$  (HuPP-2A $\beta$ ), M23591; and rabbit PP-2A $\beta$  (RbPP-2A $\beta$ ), Y00763. The sequences were aligned with the CLUSTAL W (1.60) multiple-sequence-alignment programme. Identical amino acids are indicated by asterisks and conservative changes by dots.

with the unique repeats in the intron, gives the PP- $\beta$  gene an unusual feature distinct from other *P. falciparum* genes.

**Characteristics of the predicted PP- $\beta$  protein.** The open reading frame resulting from removal of the nucleotides between the 5' and 3' splice sites of the intron encodes a protein of 466 amino acids (Fig. 2) with a predicted molecular mass of approximately 53.7 kDa. PP- $\beta$  consists of two distinct domains: the C-terminal domain; and the N-terminal segment. The C-terminal portion, comprising 315 amino acid residues, has a striking similarity to the catalytic subunits of phosphatases in the PP-1/PP-2A/PP-2B family and contains all 45 invariant amino acid residues conserved in this family (Barton et al., 1994). Some of these residues have been determined to be involved in metal cation binding, substrate recognition, catalysis and toxin binding by genetic mutagenesis (Kinoshita et al., 1990; Zhuo et al., 1994; Zhang et al., 1994a,b) and more recently by crystallographic studies of rabbit and human PP-1 (Goldberg et al., 1995;

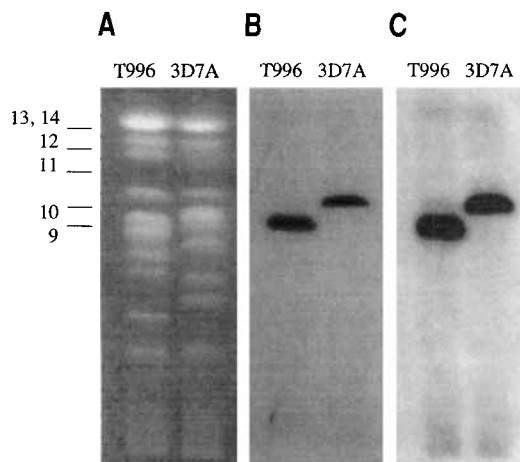
Egloff et al., 1995). These include (relative to the residue numbers of rabbit muscle PP-1 $\alpha$ ; Goldberg et al., 1995) Asp64, His66, Asp92, Asn124, His173 and His248, which coordinate Mn<sup>2+</sup> and Fe<sup>2+</sup> binding, Asp95, Arg96, Asn124, His125, Asp208 and Tyr272, forming part of the substrate-binding and catalytic sites, Tyr272, involved in binding to toxins such as tautomycin and okadaic acid, Arg246, which is essential for the function of the phosphatase proteins, and Leu121-Arg122-Gly123-Asp124-His125-Glu126, a diagnostic motif of the catalytic domain of serine/threonine-specific protein phosphatases. However, PP- $\beta$  contains an unusual hydrophilic insert consisting of five amino acid residues in the region corresponding to the  $\beta$ 7 and  $\beta$ 8 strands of rabbit muscle PP-1 $\alpha$ . The N-terminal portion of PP- $\beta$  consists of 151 amino acid residues and is relatively rich in charged amino acids (32.5% Lys, Arg, Glu or Asp). The N-terminal portion contains a high level of Asn (19.9%), much higher than that of the C-terminal domain (6.3%). In addition, in the N-terminal region there are several potential phosphoryla-



**Fig. 5. Southern blot analysis of the *PP-β* gene.** 4 μg genomic DNA from 3D7A *P. falciparum* was digested with restriction enzymes, separated on a 1.0% agarose gel, transferred onto a nylon membrane, and probed with the P9-*RsaI* fragment of the *PP-β* gene. Lanes 1–7, digests with *AccI*, *BamHI*, *BclI*, *EcoRI*, *EcoRV*, *HincII* and *RsaI*, respectively. The sizes of the DNA markers (M) are given (in kb) on the left.

tion sites for cAMP-dependent protein kinase and protein kinase C (Kennelly and Krebs, 1991). These include SKR at amino acid positions 7–9, KDTS at 42–45, SEK at 63–65, SVK at 95–97, KELS at 97–100, and KTS at 134–136 (Fig. 2).

**Amino-acid-sequence alignment and comparison.** Database analysis revealed that the catalytic domain of *PP-β* shares 60–73% similarity and 50–58% identity with phosphatases of the *PP-2A* group, in comparison with the 50–59% similarity and 41–49% identity with members of the *PP-1* group, and 38–49% similarity and 33–39% identity with enzymes of the *PP-2B* group from other species. Sequence comparison of *PP-β* with *PP-2A* (Fig. 4) shows that *PP-β* contains 52 of the 61 amino acid residues highly conserved in the *PP-2A* family (Barton et al., 1994). These include (relative to the residue numbers of human *PP-2Aβ*) Cys266-Tyr267-Arg268-Cys269-Gly270, required for the binding of okadaic acid and contributing to the increased sensitivity of *PP-2A* to the toxin compared with other protein serine/threonine phosphatases (Zhang et al., 1994b; Shima et al., 1994; Kaneko et al., 1995), Tyr307, responsible for the inactivation of *PP-2A* when phosphorylated by the protein-tyrosine kinases *p60<sup>v-src</sup>*, *p56<sup>lck</sup>*, epidermal-growth-factor receptor, and insulin receptor (Chen et al., 1992, 1994), and Leu309, the terminal residue responsible for the regulation of the phosphatase activity by reversible methylation (Lee and Stock, 1993; Favre et al., 1994; Xie and Clarke, 1994; Lee et al., 1996). Despite the similarity to *PP-2A*, *PP-β* is different in three respects, as mentioned above: a longer N-terminal extension; a five-residue insert between the *β7* and *β8* strands; and nine changes to conserved residues (five conservative changes). The changes are (relative to human *PP-2Aβ*) Glu25 (to Ile), Cys55 (to Ala), Leu100 (to Val), Ile111 (to Val), Gly138 (to Asn), Leu170 (to Ile), Gly221 (to Ser), Asp306 (to Gln) and Phe308 (to Met) (Fig. 4).

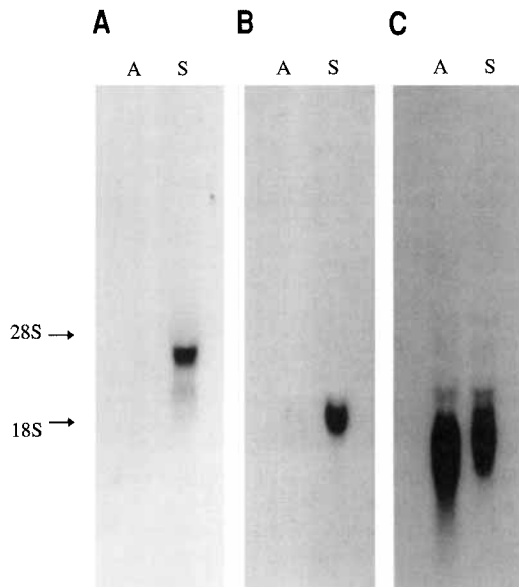


**Fig. 6. Chromosome localization of the *PP-β* gene.** Parasite chromosomes from *P. falciparum* T996 and 3D7A were separated by PFGE, stained with ethidium bromide, blotted onto a nylon membrane and hybridized with radiolabeled probes. According to the yeast chromosome markers and hybridization of several *P. falciparum* chromosome marker genes, the position of chromosome 9–14 was identified on the ethidium-bromide-stained gel (A). The *PP-β* gene (B) and the *P. falciparum* *MSP-1* gene (C) hybridized to chromosome 9.

**Determination of the copy number of the *PP-β* gene.** To investigate the copy number of the *PP-β* gene in the *P. falciparum* genome, 3D7A genomic DNA was digested with a variety of restriction enzymes and analyzed by Southern blotting. Hybridization of the P2-3 probe (data not shown) revealed a single band in digests with *AccI*, *BamHI*, *BclI*, *BglII*, *EcoRI*, *EcoRV*, *HaeIII*, *HincII*, *HindIII*, *HpaII* and *PvuII*. However, at least three bands (one predominant band and others faint) were detected in digests with *AluI*, *DraI*, *RsaI* and *Sau3AI*, inconsistent with the restriction map (Fig. 1). These faint bands disappeared after washing under higher stringency conditions. To confirm the above results, a separate Southern blot was hybridized with the P9-*RsaI* probe. The results (Fig. 5) are consistent with the restriction map (Fig. 1), suggesting strongly that *PP-β* is encoded by a single-copy gene/haploid genome. The several bands detected in the *AluI*, *DraI*, *RsaI* or *Sau3AI* digestions may indicate the presence of several P2-3-related genes in the *P. falciparum* genome.

**Chromosomal localization.** To establish the chromosomal location of the *PP-β* gene, hybridization of the P9-*RsaI* clone was performed using a PFGE blot containing 3D7A and T996 chromosomes. A single band was detected corresponding to chromosome 9 (Fig. 6). The result was repeated by hybridization of the same probe with an independent PFGE blot (data not shown), and further confirmed by probing these two blots with a control probe derived from the *MSP-1* gene, which is known to be located on chromosome 9. The single band on PFGE blots is consistent with the conclusion that the *PP-β* gene is a single-copy gene/haploid genome.

**Expression pattern of the *PP-β* gene in the erythrocytic stages of the life cycle.** Northern blots containing equal amounts of total RNA prepared from gametocytes (mainly stages III–V) or mixed asexual erythrocytic stages of *P. falciparum* were probed with the P9-*RsaI* fragment. A transcript of approximately 2800 nucleotides was detected in the sexual-stage preparation, migrating between the 28S and 18S ribosomal RNA species (Fig. 7). Based on the assumptions that the mRNA of *PP-β* con-



**Fig. 7. Northern blot analysis of the *PP-β* gene.** 10 μg total RNA extracted from the asexual erythrocytic stages (A) and sexual erythrocytic stages (S) of *P. falciparum* (clone 3D7A) were fractionated on a denaturing formaldehyde gel, blotted onto a nylon membrane, and hybridized to radiolabeled probes. The positions of *P. falciparum* rRNA subunits (18S and 28S) are indicated by arrows. (A), (B) and (C) are autoradiographs of the membrane probed with the *PP-β* gene, the *Pfs16* sexual-stage-specific gene, and the calmodulin housekeeping gene (*PfCaM*), and exposed for 96 h, 50 min and 96 h, respectively. The *PP-β* probe detected a transcript of approximately 2800 nucleotides in the sexual erythrocytic stages, *Pfs16* hybridized with a band of approximately 1400 nucleotides in the sexual erythrocytic stages, and *PfCaM* hybridized with two bands of 1300 and 1000 nucleotides from the parasite.

tains a 100–200-residue poly(A) tail (Levitt, 1993) and that the polyadenylation signal AATAAA starting at nucleotide 2335 is meaningful, the result suggests that the mature *PP-β* transcript may contain approximately 700 bp of 5′ untranslated sequences. An extensive 5′ untranslated region is not unusual in *P. falciparum* genes (Levitt, 1993; Lanzer et al., 1993; Li et al., 1996). To evaluate the quality of the RNA, probes corresponding to *Pfs16*, a sexual-stage-specific gene (Bruce et al., 1990) and calmodulin, a house-keeping gene (Robson, 1993), were used as controls (Fig. 7).

## DISCUSSION

PP-2A has remained remarkably constant through evolution and represents one of the most conserved of all known enzymes (Cohen et al., 1990). In the budding yeast, *Saccharomyces cerevisiae*, at least five PP-2A and PP-2A-like phosphatases have been characterized (Arndt et al., 1989; Sneddon et al., 1990; Sutton et al., 1991; Ronne et al., 1991; Posas et al., 1993). In this study, a PP-2A-like gene was isolated on the basis of the amino acid sequences conserved in the catalytic subunits of the PP-1/PP-2A/PP-2B family from *P. falciparum*.

Reverse transcription/PCR and nucleotide sequence analysis revealed that the *PP-β* gene contains a single intron. In contrast to higher eukaryotes, few introns have been found in the *P. falciparum* genes sequenced. Data from early work showed that when a *P. falciparum* gene was interrupted it was usually by a single intron, which was commonly located near the 5′ end of the gene (Weber, 1988). However, the present intron is located in the middle of the coding region where amino acid sequences

are highly conserved in the PP-1/PP-2A/PP-2B family. In Ca<sup>2+</sup>-regulated protein serine/threonine kinase of *P. falciparum*, four introns are located in the C-terminal EF hand Ca<sup>2+</sup>-binding region (Zhao et al., 1993). In addition, in protein kinase 5, a Cdc2-like kinase of *P. falciparum*, four introns are distributed over the coding region (Ross-MacDonald et al., 1994). Moreover, protein kinase 1, a recently reported *P. falciparum* protein kinase, contains a C-terminal-portion-positioned intron (Kappes et al., 1995). Compared with those found in higher eukaryotes, *P. falciparum* introns are quite short, usually 100–220 bp (Weber, 1988; Zhao et al., 1993; Ross-MacDonald et al., 1994; Kappes et al., 1995). However, the house-keeping gene calmodulin contains a 479–546-bp intron (dependent upon the *P. falciparum* strain; Robson, 1993). The longest intron identified is of 868 bp in the chaperonin-60 gene of *P. falciparum* (Holloway et al., 1994). The present intron is 359 bp in 3D7A and is therefore among the larger introns of *P. falciparum* genes described. More strikingly, the *PP-β* gene intron not only possesses the AT repeat motif, which is usually found in non-coding regions of *P. falciparum* genes, but also contains a 32-base sequence that repeats four times and represents over 35% of the total intron length. Over 21% of the repeat is composed of C and G. This is an unusual feature, particularly for the A+T-rich and relatively short introns of *P. falciparum*. It is still not known what role the repeat sequences of introns could play in regulating the splicing of transcripts or the significance of any secondary structures that they might form. In addition, as revealed here by PCR, the intron sizes are variable between different parasite strains (Fig. 3B). The polymorphic intron could provide a useful marker for looking at sequence variation within the malaria genome.

Analysis of the deduced protein sequence (466 amino acids) showed that the C-terminal domain of PP-β is more closely related to mammalian and yeast PP-2A than to PP-1 and PP-2B, with the highest similarity (58% identity and 73% similarity) to *S. pombe* Ppa1 (Kinoshita et al., 1990). Disruption of the *ppa1*<sup>+</sup> gene, together with the *ppa2*<sup>+</sup> gene (encoding another mammalian PP-2A-like phosphatase in fission yeast), has been shown to be lethal to *S. pombe* (Kinoshita et al., 1990). PP-β contains most (52 of 61) of the conserved residues of the PP-2A family, including the sequence CYRCG, suggesting that PP-β may be highly sensitive to okadaic acid. However, the presence of a unique hydrophilic insert composed of five amino acids between the β7 and β8 strands and the nine changed amino acids (five conservative changes) make it difficult to define PP-β as a true homologue of mammalian PP-2A. The presence of an N-terminal extension argues against a close relationship between the proteins. Database searches revealed that part of the N-terminal sequence, amino acids 53–99, exhibits 44% similarity and 27% identity to the N-terminal sequence, amino acids 22–68, of *S. cerevisiae* PPH22, a homologue of mammalian PP-2A. Therefore, the overall sequence structure of PP-β seems to be most closely related to yeast PPH22. It has been described that PPH22 and another phosphatase, PPH21, are essential for the growth of budding yeast (Sneddon et al., 1990; Ronne et al., 1991). Several potential phosphorylation sites occurred in the N-terminal segment for cAMP-dependent protein kinase and protein kinase C, indicating that the activity of PP-β may be regulated by reversible phosphorylation of the N-terminal segment. Although a parasite protein kinase C has not been described, the cAMP-dependent protein kinase genes have been cloned from *P. falciparum* (Li, J.-L., unpublished data) and *Plasmodium yoelii* (Saito-Ito et al., 1995).

Southern blots of genomic DNA digests and chromosomal separations showed that *PP-β* is present as a single-copy gene on chromosome 9. It was thought that chromosome 9 might be involved in gametocytogenesis (Day et al., 1993). Northern blot



analysis revealed a single transcript of approximately 2800 nucleotides only in the sexual erythrocytic stages. These results imply that PP- $\beta$  may regulate specific gametocyte development and function. Work indicated that okadaic acid does not influence early gametocyte development *in vitro* (Hall, B. S. and Targett, G. A. T., unpublished data), suggesting that PP- $\beta$  may be involved in late sexual-stage events, such as regulation of exflagellation, fertilization, and post-fertilization development. The increasing numbers of protein kinases and phosphatases found specifically in gametocytes indicate the importance of reversible phosphorylation in the control of this key stage in the parasite life cycle. However, okadaic acid is highly toxic to asexual stage parasites (Hall, B. S. and Targett, G. A. T., unpublished data), suggesting the presence of other PP-2A homologues in the parasite. These may be represented in the weakly detectable signals on the Southern blots at low stringency. Identification of the target(s) of okadaic acid and the native substrates of PP- $\beta$  may help development of more specific anti-malarial drugs.

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