

Biochimica et Biophysica Acta 1491 (2000) 341-349

www.elsevier.com/locate/bba

BIOCHIMICA ET BIOPHYSICA ACTA

Short sequence-paper

Sexual stage-specific expression of a third calcium-dependent protein kinase from *Plasmodium falciparum*¹

Ji-Liang Li^{a,*}, David A. Baker^b, Lynne S. Cox^a

^a Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK ^b Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

Received 3 August 1999; received in revised form 15 November 1999; accepted 31 January 2000

Abstract

A third calcium-dependent protein kinase (CDPK) gene has been isolated from the human malaria parasite *Plasmodium falciparum* by vectorette technology. The gene consists of five exons and four introns. The open reading frame resulting from removal of the four introns encodes a protein of 562 amino acid residues with a predicted molecular mass of 65.3 kDa. The encoded protein, termed PfCDPK3, consists of four distinct domains characteristic of a member of the CDPK family and displays the highest homology (46% identity and 69% similarity) to PfCDPK2, the second CDPK of *P. falciparum*. The N-terminal variable domain is rich in serine/threonine and lysine and contains multiple consensus phosphorylation sites for a range of protein kinases. The catalytic domain possesses all conserved motifs of the protein kinase family except for the highly conserved glutamic acid residue in subdomain VIII, which is replaced by a glutamine residue. The sequence of the junction domain comprising 31 amino acid residues is less conserved. The calmodulin-like regulatory domain contains four EF-hand calcium-binding motifs, each consisting of a loop of 12 amino acid residues which is flanked by two α -helices. Southern blotting of genomic DNA digests showed that the *Pfcdpk3* gene is present as a single copy per haploid genome. A 2900 nucleotide transcript of this gene is expressed specifically in the sexual erythrocytic stage, indicating that PfCDPK3 is involved in sexual stage-specific events. It is proposed that PfCDPK3 may serve as a link between calcium and gametogenesis of *P. falciparum*.

Keywords: Malaria; Calcium-dependent protein kinase; Gametogenesis; Plasmodium falciparum

The resistance of *Plasmodium falciparum* to drugs and the resistance of mosquitos to insecticides have resulted in a resurgence of malaria in many parts of the world. Therefore, there is an urgent need for development of effective vaccines and new anti-malarial drugs. The identification of new targets for vaccine and drug development will be facilitated by a better understanding of the cellular and molecular processes at different stages of the parasite. The sexual erythrocytic stage is functionally very distinct from the asexual stage. It is responsible for trans-

Abbreviations: CDPK, calcium-dependent protein kinase; PfCDPK1, the first *P. falciparum* calcium-dependent protein kinase; PfCDPK2, the second *P. falciparum* calcium-dependent protein kinase; PfCDPK3, the third *P. falciparum* calcium-dependent protein kinase

^{*} Corresponding author. Fax: +44-1865-222431;

E-mail: lij@icrf.icnet.uk

¹ Nucleotide sequence data reported in this paper are available in the GenBank, EMBL and DDJB databases under the accession number AF106064.



Fig. 1. A schematic representation of a partial restriction map of the *Pfcdpk3* gene and the overlapping PCR fragments used to determine the nucleotide sequence of *Pfcdpk3*. A, *Acc*I; B, *BcI*I; H, *Hinc*II. The open boxes indicate the exon coding regions of the *Pfcdpk3* gene and the black boxes represent the introns. The fragments of CDB'-CD2 and CDB'-CDE' are derived from genomic DNA. The fragments of CD1-CD6, CD5-CDE' and CD1-CDE' are RT-PCR products.

mission of the parasite to the mosquito, where exflagellation and fertilisation occur sequentially. Therefore, it is conceivable that more active biochemical and molecular events could be involved in the sexual stage than in the asexual stage. However, the number of sexual stage-specific proteins identified so far is very limited. Most of the sexual stage-specific genes isolated encode membrane proteins which are being assessed for their potential as transmission blocking vaccine candidates. Little is known about their biological function in the sexual stage. We are interested in the signal transduction pathways involved in the sexual differentiation of *P. falciparum*. The first step toward this goal is to identify the components, mainly protein kinases and phosphatases, of the sig-

Table 1 Characteristics of the nucleotide sequence of the *Pfcdpk3* gene

nal transduction pathways. Recently, we have reported several sexual stage-specific genes encoding either protein serine/threonine phosphatases [1–3] or protein kinases (Li et al., unpublished). In this report, we describe the molecular cloning and characterisation of another novel gene encoding a third *P. falciparum* calcium-dependent protein kinase (CDPK) (PfCDPK3). PfCDPK3 is expressed specifically in the sexual stage, indicating that it may be important in regulating the processes of sexual stage development such as gametogenesis.

In the *P. falciparum* tag database, the nucleotide sequences of two DNA fragments (tag 0487m3 and tag 0532m3) were found encoding the same protein sequence with a high homology to the catalytic do-

Boundary sequence of exon/intron
• •
ATTGAGCATG:gtaataaaaa
attatatag:GTAAAGAAGG
ATTATCCAAG:gtttttatat
tttctttag:AAATTAATAT
ATTGGCCCAC:gtaataaaaa
tatttttag:ATACTTTATA
CGACGGAAAG:gtaatcataa
tttatttag:ATTGATTTTC

AACATTTTAT	GCAACGTTTT	AAAAATTTGT	CTTTTTTTTT	TTTAACATTT	AATGAAGGAT	60
АТААААТАТА	AACAGTGCTA	CTAATTTTTT	TTATCATTTT	AATTTTTTTA	ATTTTTTTA	120
TTTTTTTAAT	TTTTTTAATT	TTTTTATCTT	TTTAATTTTT	TTATTTATTT	TTTCTAAACG	180
AACATAAAAA	TTAAGCACAC	CATAAATATA	TTATATATAG	ΑΤΑΤΑΤΑΑΤΤ	TTTGTACGTA	240
GTCGAATCTC	AGAATGAATG	ATTTGATTAT	TAAGAATAAT	AAAAAGGGAA	GCTGCGATGT	300
GATTATAAAA	TATAAATGTA	AAAAGTCAGA	TGAGAATATA	AAAAGAAGAA	AGAGTTCACA	360
TAAATATATA	AAAAATAAGA	GTGTCGTATT	AGGTCGAAGC	ATAATGACAA	ATAAGAAGGA	420
K Y I GAAATTAAAA	K N K GGAGCTTTAA	S V V L AATACAAAGG	G R S ATCAAAAAAA	I M T GAGATAAAAA	N K K E TATGTAATAA	56 480
K L K GAAAAGTATG	G A L ATAAAAAATG	K Y K G ACAAAGATGA	S K K AAATACAACT	E I K TTAAAATCTA	I C N K TGAAAAGTGA	76 540
K S M CAATTTTAAA	I K N TTTTCAAGAA	D K D E GAGGATTTAT	N T T TCTGAGTTTT	L K S ACTGGTAATT	M K S D TAGAAGATTT	96 600
N F K TTATAATTTA	F S R TCAAAAGAAC	R G F I CATTAGGTAA	L S F AGGTACATAT	T G N GGATGTGTAT	L E D F ATAAAGCAAC	116 660
Y N L CGACAAATTA	S K E ΤΤΑΑΑΑΑΤΑΤ	P L G K CGAGAGCTGT	G T Y AAAAGTAGTA	G C V TCTAAAAAGA	Υ Κ Α Τ ΑΑΤΤΑΑΑGAA	136 720
D K L	L K I	S R A V	K V V	S K K	K L K N	156
I P R	F R Q	E I D I	M K N	L D H	P N V V	780 176
AAAATTGCTT K L L	GAAACGTTTG E T F	AAGATAGTAA E D S N	TCAAATATAT O I Y	TTAGTAATGG I. V M	AGTTATGTAC	840 196
GGGTGGGGAA	TTATTTGATA	AAATAGTAAA	AAAGGGTTGC	TTTGTAGAAA	CGTTTGCATC	900
G G E ATTTATTATG	L F D AAACAAATAT	K I V K TTTCTGTCTT	K G C AAATTATTTA	F V E CATATAAGAA	T F A S	216 960
FIM	KQI	FSVL	N Y L	HIR	N I C H	236
CAGAGATATT R D I	AAACCTGAGA K P E	ACTTTCTATT N F L F	CTATGATATG Y D M	ACACCTGAAT T P E	CGTTAATAAA S L I K	1020 256
AATTATAGAT	TTTGGATTGG	CTTCTTATTT	TACTCATAAT	AATTATGAAA	TGAAGACCAA	1080
AGCAGGGACT	F G L CCGTATTATG	A S Y F TAGCTCCTCA	T H N GGTATTAACC	N Y E GGTTCGTATA	M K T K ATTATAAATG	276 1140
A G T	P Y Y	V A P Q	V L T	G S Y	N Y K C	296
D M W	S S G	V L F Y	I L L Intron I	C G Y	P P F F	316
TGGAGAAAGT	GATCACGAAA	TATTGAGCAT	G GT AATAAAA	ААААААААА	ATAATAATAA	1260
G E S TAAAAGTGAA	D H E ATAATTTTAT	I L S M GGATACAACT	AATAATATAT	GAGTAAACAT	ATATATATAT	326 1320
ATATATATAT	ATATGTATAT	ATATATGTAC	ATATATATGT	ACATATGGTG	AGATTGTTTA	1380
TATGATAACA	AATGAATGTA	CATATTAACA	TTTTTGTGAC	ATCAATATTT	TGTTCTTCAT	1440
ATTATAT AG G	TAAAGAAGGG V K K G	GAAGTATCAA K Y O	TTTAAAGGAA FKG	AGGAGTGGAA K E W N	TAACATATCC	1500
GAAGAAGCAA	AAGATTTAAT	AAAAAGATGT	CTTACAATGG	ACGCTGATAA	AAGAATATGT	1560
E E A GCGAGTGAAG	K D L I CTTTACAACA	K R C TCCTTGGTTT	L T M	D A D K AATATGCTTT	R I C TAATATGGAT	363 1620
A S E	A L Q H	P W F	ККК	КҮАҒ	NMD	383
M K M	D I H V	L E N	TTTAAGAACT F K N	Y G L L	ATTAAAATTT L K F	1680 403
CAGAAATTAG	CTATGACGAT	AATAGCACAA	CAAAGTAATG	ATTATGATGT	TGAAAAATTA	1740
AAATCAACTT	TCTTAGTATT	AGATGAAGAT	GGAAAGGGAT	ATATAACTAA	e k l Agaacaatta	423
K S T	F L V L	D E D	G K G	Y I T K	E Q L	443
K K G	L E K D	G L K	L P Y	N F D L	L L D	463
CAAATCGATA	GTGATGGCAG	TGGGAAAATT G K I	GATTACACGG	AATTTATTGC	AGCTGCTTTA A A L	1920 483
¥ * 5	0 0 0 0	Intron II	5			100
GACAGAAAGC D R K	AATTATCCAA Q L S K	G GT TTTTATA	TATATATATA	TATAAAAAAA	АААААААААА	1980 490
АААААААААА	ААААААААА	ATTATATATT	AAATATATTA	TATATATTAT	ATATATTTAT	2040
ATATATAT <u>GT</u>	ATGTATGTAT Exon (GTATGTATGT	ATATATGTAT	GTATATATTT	TTTTTTTTTT	2100
TTTCTTTTTC	TTT AG AAATT K L	AATATATTGT I Y C	GCCTTTAGGG A F R	TCTTTGACGT V F D V	AGACAATGAC D N D	2160 505
GGGGAAATCA	CCACGGCAGA	ATTGGCCCAC	intron III GTAATAAAAA	AAAATATATA	TATATATATA	2220
G E I TATATACATA	T T A E TATATACATA	L A H CATGTGAGTA	AAACAAATGA	GCATTTTAAA	ATGTATGTAT	515 2280
ATGTATACAT	TTTATTTTT	Ex: ATTTTT AG AT	on D ACTTTATAAT	ggaaataaaa	AAGGCAACAT	2340
AACTCAAAGG	GACGTCAACA	I GGGTTAAAAG	L Y N GATGATTCGG	G N K GATGTTGACA	K G N I AAAACAACGA	526 2400
T Q R Int:	D V N ron IV	RVKR	MIR	D V D	K N N D	546
CGGAAAG GT A G K	ATCATAAGGA	ААСААААААА	AAAAAAAGAA	AAAAAAAAAA Ex	AAAATATATA on E	2460 548
TATATATATA	ΤΑΤΑΤΑΤΑΤΑ	TATGTATATT	TTGTTTTGTT	TTATTT AG AT I	TGATTTTCAT D F H	2520 552
GAATTTTCAG E F S	AAATGATGAA E M M K	GCTAAAATTT L K F	TAAA *			2554 562

Fig. 2. Nucleotide and deduced amino acid sequences of the Pfcdpk3 gene (single letter code). The nucleotides and the amino acid residues are numbered to the right of the sequence. The conserved dinucleotides of the exon-intron boundary sites are in bold. The repeated nucleotide sequence within intron II is underlined. The asterisk indicates the termination codon.

main of the CDPK family. Nucleotide sequence analvsis revealed that the A+T content and codon usage of the tag sequences are typical of the coding region of P. falciparum genes. To isolate a full-length of the gene, two specific primers, CD1 (to obtain further sequence in the 3' direction) and CD2 (to obtain further sequence in the 5' direction), were constructed on the basis of the tag sequences and used in PCR to screen vectorette libraries [4]. Two fragments (CD1-TaqI and CD2-DraI) (Fig. 1) were obtained and sequenced. The sequence data permitted construction of the CD3 and CD4 primers and subsequent screening of vectorette libraries. Two overlapping PCR products (CD4-RsaI and CD4-HincII) were amplified with CD4, both containing a putative ATG start codon, whereas only one fragment (CD3-DraI) was obtained with CD3. However, the sequence data of CD3-DraI made it possible to construct the CD5 primer that gave rise to the CD5-AluI fragment. Based on the sequence of CD5-AluI, the CD7 primer was designed to produce the CD7-DraI fragment. Analysis of the sequence revealed a putative TAA stop codon in CD7-DraI. In order to confirm the sequence obtained from the overlapping fragments, a pair of primers, CDB' and CDE', were used to amplify the full-length gene from genomic DNA and the PCR product was sequenced in both strands (see Fig. 1).

The sequence derived from overlapping fragments consists of 2554 bp and contains five exons, four introns and a 5' untranslated region (Fig. 2). The proposed coding region of the gene starts with an ATG codon at nucleotide 254 and terminates with a TAA codon at nucleotide 2551. The sequence and codon usage in the coding region are typical for a *P. falciparum* gene. The A+T contents of the 5' flanking (253 bp) and four putative intron noncoding regions are characteristically higher than those of the exon coding regions (Table 1). The four proposed introns, ranging from 101 to 218 nucleotides in length, interrupt the coding region from

the middle to the C-terminus. Intron I is located in the middle of the gene, corresponding to subdomain X of the catalytic domain of the deduced kinase, whereas introns II, III and IV are located towards the 3' end of the gene, corresponding to the calmodulin-like domain of the kinase. The highly conserved dinucleotides GT and AG, found at eukaryotic intron boundaries [5], define these intervening sequences. The sequences around the 5' splice sites of these introns (GTA/TA/TT) show more conserved nucleotides, while the sequences around the 3' splice sites (TA/TTAG) are fairly consistent with the consensus sequence found at the 3' end of eukaryotic introns [5]. Long runs of poly(AT), poly(T) and poly(A) are present in the introns. A four nucleotide repetitive sequence, consisting of GTAT, occurs within intron II and repeats nine times. To verify the existence of these introns, reverse transcription (RT-) PCRs [2] were performed and the products sequenced (see Figs. 1 and 2). The data confirmed the precise exon-intron boundaries.

The open reading frame resulting from removal of the four introns encodes a protein of 562 amino acids (see Fig. 2) with a predicted molecular mass of approximately 65.3 kDa. Database searches revealed that the amino acid sequence of PfCDPK3 shares 50–69% similarity and 32–46% identity with kinases in the CDPK family. PfCDPK3 has the highest homology to PfCDPK2 (46% identity, 69% similarity), the second CDPK of *P. falciparum* [6], but only 40% identity and 64% similarity to PfCDPK1 (PfCPK), the first CDPK of the malaria parasite [7]. Fig. 3A shows a sequence alignment of all three *P. falciparum* CDPKs. PfCDPK3 is composed of four distinct domains characteristic of a member of the CDPK family: (1) a variable N-terminal segment, (2) a highly conserved protein kinase catalytic domain, (3) a junction domain, and (4) a calmodulin-like domain. The N-terminal domain, consisting of 116 amino acid residues, is not related to any previously described protein serine/threonine kinase and has several interesting features. Firstly, it is rich in serine/threonine (14% (16/116)) and lysine (24% (28/116)). Secondly, the N-terminal region contains multiple potential phosphorylation sites for a range of known protein kinases [8], suggesting that the PfCDPK3 activity may also be regulated by reversible phosphorylation of the N-terminal segment. Thirdly, in contrast to those of the known kinases in the CDPK family, the N-terminal segment is among the larger extensions of the CDPKs yet described. The kinase catalytic domain of PfCDPK3 is composed of 264 residues and contains all 11 conserved subdomains of the protein kinase family. PfCDPK3 has almost all of the characteristic features of a kinase [9,10]. These include (corresponding to the residue numbers for the bovine cAPK- α catalytic subunit): the glycine loop G50-X-G52-X-X-G55, forming part of the ATP-binding site; D166, N171 and D184, which are also thought to be a sequence motif implicated in ATP-binding; the triad composed of the side chain of K72, D184 and E91, that is close to the ATP γ-phosphate and plays a critical role in recognition of the phosphate; the catalytic loop R165-D166-X-X-X-N171, involved in catalysis and in guiding the peptide substrate into the proper orientation for catalysis; D220 and R280, involved in the stabilisation of kinases; and A206, which is diagnostic of the catalytic domain of protein kinases. In addition, PfCDPK3 also contains almost invariant amino acids corresponding to F185, G186, W222 and G225 whose functions are still unclear. The se-

Fig. 3. (A) Alignment of the predicted amino acid sequence of PfCDPK3 with those of PfCDPK1 and PfCDPK2. The GenBank/ EMBL database accession numbers are as follows: PfCDPK1, X67288; PfCDPK2, X99763; PfCDPK3, AF106064. Sequences were aligned with the CLUSTAL W (1.60) multiple sequence alignment programme. The amino acid residues are numbered to the left of the sequence. Identical residues are highlighted with solid black and conservative changes shaded with grey. The 11 canonical subdomains of protein kinases [11] are indicated by roman numerals. The residues conserved in the catalytic domain of the protein kinase family are underlined with bold in PfCDPK3. The boundaries of the variable, kinase, junction and calmodulin-like domains are shown. The four calcium-binding EF-hand motifs are boxed. (B) Alignment of amino acid sequences in junction regions of the protozoan CDPKs. EmCDPK, *E. maxima* [24]; EtCDPK, *E. tenella* [24]; TgCDPK, *T. gondii* (accession no. AF043629); PCaPK- α and PCaPK- β , *P. tetraurelia* [14]; and PfCDPK1, PfCDPK2 and PfCDPK3, *P. falciparum* [6,7]. AtCPK1 from *A. thaliana* [17] acts as a reference. Sequences were aligned with the CLUSTAL W (1.60) multiple sequence alignment programme. Identical residues in more than half of the CDPKs are highlighted with solid black and conservative changes shaded with grey.

PCODEX2 1	A		
PICUPK3 1 1 MUDITIKANKAS SUDVITELOVAS DENIAKANS SIMILAKES VIEWS INTERACES PICUPK1	PfCDPK2	. 1	MCNHLSVNKLKRKKKKKSFL-NIYCKNTNEN-TSKQSN
N-terminal domain PfCDPK2 37 DYKYDINTSOBS	PICDPK3 PfCDPK1	1	MNDLIIKNNKKGSCDVIIKIKKKASDENIKKKASSHATINNSVVLGASIMINAALALAG MG-CSQSSNVKDFKTRRSKFT-NGN-NYCKSGNNKNSEDLAI
PfCDPK2 37 DYKYDINTSCHGFGTTILERKLLICHESKLEDYTIL PfCDPK3 10 ALKYKSKELIKCNKKSMINDDZDDNTILSMKSDNFFFSRRFILSTENLEDYYTIL PfCDPK1 40 NPGMYVRKEGKEG			N-terminal domain
H10183 0.1 HIALASSMERIA PHCDEN 0. NEGMYURKKEGK[C	PfCDPK2	37 61	DYKYDINTSCISREGTTTLERKNLILCHSCKLEDKY-II
PFCDPK2 75 DEKLGE TYGCVYKATDKILKISKA VKVSKKKLENINGE FEIT SEKLDHEN VK VE PFCDPK1 74 REKHGE GRÄTKVIKKSGEDENKYSTINKIGEDDIHEE VNESLE SELHEN VK VE PFCDPK1 74 REKHGE GRÄTKVIKKSGEDENKYSTINKIGEDDIHEE VNESLE SELHEN VK VE PFCDPK1 74 REKHGE GRÄTKVIKKSGEDENKYSTINKIGEDDIHEE VNESLE SELHEN VK VE PFCDPK2 135 TYGOVYKATDKILKISPEDENKYSTINKIGEDDIHEE VNESLE SELHEN VK VE PFCDPK2 135 TYGOVYKATDKILKISPEDENKYSTINKIGEDDIHEE VNESLE SELHEN VK VE PFCDPK2 135 TYGOVYKATDKILKISPEDENKYSTINKIGEDDIHE VK	PfCDPK1	40	NPGMYVRKKEGKIGESYFKVRKLGSGAYGEVL-LC
PICDPK2 75 DEALESTTYGEVYKGIDWYNOLYAKEDKURURUN UNNETSER 20 SEMENTAL WYNYKKUN UN PERFEDEL 2008 MUDHIN VICULE PFCDPK1 74 REKHESERAIKVIKKSSFDRAKYSTINKIECDDIHEEYNSISUUSISUUHIN IN INFORMEDING UN SEMENTAL PFCDPK2 135 TYDNDNY TU MA CSERELDSE INNGSTEKNAAT UNKOLSSIE FUNNI EN INTO PFCDPK2 135 TYDNDNY TU MA CSERELDSE INNGSTEKNAAT UNKOLSSIE FUNNI INTO PFCDPK2 135 TYDNDNY TU MA CSERELDSE INNGSTEKNAAT UNKOLSSIE FUNNI INTO PFCDPK2 135 TYDNDNY TU MA CSERELDSE INNGSTEKNAAT UNKOLSSIE FUNNI INTO PFCDPK2 135 TYDNDNY TU MA CSERELDSE INNGSTEKNAAT UNKOLSSIE FUNNI INTO PFCDPK1 134 VEDKAYFMUTTEYEESELESE SKUKKGEVETT-TKAETFYN A CYU DEKNAKED INTO PFCDPK2 195 EEN EU OSENKDSILKI I DEGISKNI GTGEFTT-TKAETFYN A CYU DEKNAKED INTO PFCDPK2 134 VEDKKYFMUTTEYEESEN STENNISTER VUTAKEN INKOLSKED PFCDPK2 134 STENY WTESELLIN TEN I DEGISKNI GEGESTENNISTER VUTAKEN INKOLSKED PFCDPK2 254 SOTIAT LLCOYPETYETTETSSHELSEN KAURTENNISTER VUTAKEN INKOLSKED PFCDPK2 135 GYTENT LLCOYPETYETTESSHELSEN KAURTENNISTER VUTAKEN INFORMATING VU	5 6 6 5 5 7 6		Kinase domain
PFCDPK1 74 REKHEREKÄIKVIKKSQFDKMKYSTINKIECDDSIHEETYNESSILSSILLASIIIVKOISSATEVAN PFCDPK2 135 TYDENDNYTVITMELCSERBILGSILLASIA III IIII IIIII IIIIIII IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	PICDPK2 PfCDPK3	/5 121	DEKLEGGTYGCVYKGIDKVTNQLYAIKEEKKDRLKNINRFYGELEIMKKLDHPNIVKLYE KEPLCKCTYGCVYKATDKLLKISRAVKVVSKKKLKNIPRFROBIDIMKNLDHPNVVKLLE
PfCDPK2 135 TTONDNYLVI MOLCSERBINGS DENGS TEKNATTIKKOLFGATFYLKSLI VIRTIA PfCDPK3 181 TTODSNOTYLVI MELCTGGLIGOK VKKGOVETFASTIKKOLFGATFYLKSLI VIRTIA PfCDPK1 134 VEDEKKYLVI VTETYEGGLIGOLIGAK VKKGOVETFASTIKKOLFGOVETAKAT VII VII PfCDPK2 195 EENTLEQSENKDSLIKI IDEGLSKNIGTGEFTT-TKASTEYV VEOUTDGKNEKKENKOM PFCDPK3 241 EENTLEQSENKDSLIKI IDEGLSKNIGTGEFTT-TKASTEYV VEOUTDGKNEKKENKOM VII VII PfCDPK2 194 EENTLEQSENKDSLIKI IDEGLSKNIGTGEFTT-TKASTEYV VEOUTDGKNEKKENKOM PFCDPK1 194 EENTLEQSENKDSLIKI IDEGLSKNIGTGEFTT-TKASTEYV VEOUTDGKNEKKENKOM VII VIII VIII PfCDPK2 254 SOVINTTLEQSENKDSLIKI IDEGLSKNIGTGEFTT-TKASTEYV VEOUTDGKNEKKENKOM VII VIII VIII VIII PfCDPK2 254 SOVINTTLEQSENKDSLIKI IDEGLASY THANYEKKAGTCG VENKGSSEBADLIKKOM VADDK VIII VIII VIII VIII III PfCDPK2 254 SOVINTTLEQSENKDSLIKI IDEGLASY THANYEKKAGTCG VENKGKAGAN SEEANDLIKKOM VADDK VIII VIII VIII VIII III IX PfCDPK2 254 SOVINTTLEQCYPHYCEN DAKKKAGTCG VENKGKAGTG VENKGKAGAN SEEANDLIKKOM VADDK VIII VIII VIII III IX PfCDPK2 314 GTCTEFAI NHPAITOWKANAN KKYAF Junction domain ++++ ++++ <td< td=""><td>PfCDPK1</td><td>74</td><td>REKHCHCEKAIKVIKKSQFDKMKYSITNKIECDDKIHEEIYNEISLIKS<mark>LDHPN</mark>IIKLFD I II III IV</td></td<>	PfCDPK1	74	REKHCHCEKAIKVIKKSQFDKMKYSITNKIECDDKIHEEIYNEISLIKS <mark>LDHPN</mark> IIKLFD I II III IV
PfCDPK3 181 THE DSNOT VIEWELCT GELLEK KUKKGGEVET FRSPINKOUSSYENNUH IRRECEDED IN PfCDPK1 134 VEDKKY SVILVTEYYESGLEGO INSKKEGEVET FRSPINKOUSSYENNUH IRRECED IN PfCDPK2 195 SENED SQSENKDSLEKT DEGLESVINGTGEFTT-TKACH PHYVAFOVIDGKUKKGT IN PfCDPK1 194 SENED SQSENKDSLEKT DEGLESVITHIN YEMKTKACH PHYVAFOVIDGKUKKON KOUNS PfCDPK1 194 SENED SQSENKDSLEKT DEGLESVITHIN YEMKTKACH PHYVAFOVIDGKUKKON KOUNS PfCDPK2 254 SQVIDY LLCGYPP YEOTONEVIKKYKREFE GYENDGSISS SPAROTIKKIN KOUNS PfCDPK2 254 SQVIDY LLCGYPP FGUSDETISMUKKREFE GYENDGSISS SPAROTIKKIN KECHWARK PfCDPK2 254 SQVIDY LLCGYPP FGUSDETISMUKKREFE GYENDGSISS SPAROTIKKIN THERAKULOWS PfCDPK2 254 SQVIDY LLCGYPP FGUSDETISMUKKREFE GYENDGSISS SPAROTIKKIN THERAKULOWS PfCDPK1 253 CSVIDY LLCGYPP FGUSDETISMUKREFE GYENDGSISS SPAROTIKKIN THERAKULOWS PfCDPK2 254 SQVIDY LLCGYPP FGUSDETISMUKREFE GYENDGSISSSAROTIKKIN THERAKULOWS Y VII VIII VIII Y VIII VIII VIII Y VIII VIII VIIII Y VIII VIII VIII Y VIIII VIIII VIIII	PfCDPK2	135	TYENDNY IYLIMELCSGRELEDS I LENGSETEKNAAT IYKOT ISAL FYIHSLNIVHRDLK
PFCDPR1 134 VHDUKKYFULVTERYEGEDDEGUINRERK DECDANNEKCTEGEGUCYFEKENWYRITE V VI VI PFCDPK2 195 PENEL QSENKDSLIKI DEGLSKNIGTGEFT-TKAGEFTVAFOUTGSKUKKOTINS PfCDPK3 241 PENEL QSENKDSLIKI DEGLSSFESKDKLR-DELGTAM TEEVIRKENEKOTINS PfCDPK1 194 PENEL QSENKDSLIKI DEGLSSFESKDKLR-DELGTAM TEEVIRKENEKOTINS PfCDPK2 254 SQUENTLLCGYPEFTSESDIETISMUKKEKSEGEGTENENSEENKETKENEKOTINS PfCDPK3 301 SQUENTLLCGYPEFTSESDIETISMUKKEKSTOBKSESSBARNEITKLATYNNE PfCDPK3 302 SQUENTLLCGYPEFTSESDIETISMUKKEKSTOBKSESSBARNEITKLATYNNE VII VII VIII IX PfCDPK3 SQUENTLLCGYPEFTSESDIETISMUKKEKSTOBKEENNEGEIKKLIKLIKUNKENTRENTKELIKUMENTERSTIKKUNKENTRENTKELIKUMENTERSTIKKUNKENTRENTKELIKUMENTERSTIKKUNKENTRENTKELIKUMENTKENTKELIKUMENTRENTKELIKUMENTKE VIII PfCDPK2 314 RCTIEGAMENTETTATESSTILKUNKEKSTERSTIKKUNKENTRENTKELIKUMENTERSTIKKUNKENTRENTKELTIKKUNKENTRENTKENTERSTIKKUNKENTRENTKELTIKKUNKENTRENTKENTETTATESSTILKUNKENTRENTKENTETTATESSTIKKUNKENTRENTKENTETTATESSTIKKUNKENTRENTKENTENTETTATESSTIKKUNKENTRENTKENTENTETTATESSTIKKUNKENTRENTKENTENTETTATESSTIKKUNKENTRENTKENTENTTETTATESSTIKKUNKENTRENTKENTENTETTATESSTIKKUNKENTETTATESSTIKKUNKENTETTATESSTIKKUNKENTRENTKENTETTATESSTIKKUNKENTRENTKENTETTATESSTIKKUNKENTRENTKENTETTATESSTIKKUNKENTETTATESSTIKKUNKENTRENTKENTETTATESSTIKKUNKENTRENTKENTETTATESSTIKKUNKENTETTATESSTIKKUNKENTETTATESSTIKKUNKENTRENTKENTETTATESSTIKKUNKENTRENTKENTETTATESSTIKKUNKENTRENTKENTETTATESSTIKKUNKENTRENTKENTETTATESSTIKKUNKENTRETATESSTIKKUNKENTRENTKENTRENTKENTRENTKENTRENTKENTETTATESST	PfCDPK3	181	TFEDSNQIYLVMELCTGGELFDKIVKKGCFVETFASFIMKQIFSVLNYLHIRNICHRDIK
PfCDPK2 195 PENDLEQSENKDSLLKI DFGLSKNLGTGEFTT-TKAGTPYVAFOVDGKNDKKCLINS PfCDPK3 241 PENDLFYDMTPESLLKI DFGLSSYFTHIN YEMKTKAGTPYVAFOVDGKNDKKCLINS PfCDPK1 194 PENDLENKHSLLNTKIVDEGLSSYFTHIN YEMKTKAGTPYVAFOVDGKNDKKCLINS PfCDPK2 254 SVIINTTLENKHSLLNTKIVDEGLSSYFTHIN YEMKTKAGTPYVAFOVDGKNDKKCHWCDWS PfCDPK2 254 SVIINTTLENKHSLLNTKIVDEGLSSYFTHIN YEMKTKEEP PfCDPK2 254 SVIINTTLEOGYPEFESDUETISMVKKKEEPC PfCDPK2 254 SVIINTTLEOGYPEFESDUETISMVKKKEEPC PfCDPK2 254 SVIINTTLEOGYPEFESDUETISMVKKKEEPC 9fCDPK1 253 GVIINTLEOGYPEFESDUETISMVKKKEEPC 9fCDPK2 314 CCTTEFANINFØTTOMTKS HEHVELSSTLIKNKKKEEPLKKENAMTTAKCTIMADAK 9fCDPK3 361 RICASPALOHPRIKKKKYAF-NMMMMDIHVENKKENEKKKLANTTAKLEGSKLAMTTAKLOSONDY 9fCDPK2 314 RITAKPAINSKNTKYANINKSDOKTLCGANSNKKEEGSOKIACAATLEGSKLITTES 9fCDPK2 372 INN-FRINFITAMIVNSKTSSOTLOGLKRDGKIPYPFILLOGTSDG 9fCDPK3 420 VEK-KSTSTLVHDEDGKYTTKELKKGLKRDGKIPYPFILLOGTSDG 9fCDPK3 372 INN-FRINFITAATHKOTYKKKEUSE FEF-Hand 3 9fCDPK3 420 VEK-KSTSTLVHDEDGKVLKKEVCLIPKKEUSENTKKEENKUEKVEVDTKKKENTO <td>PfCDPK1</td> <td>134</td> <td>VEBEKKYEYIVTEFYEGGDIGEQUINRHKEDECDAANIMKQIESGICYIHKHNIVHRDIK V VI</td>	PfCDPK1	134	VEBEKKYEYIVTEFYEGGDIGEQUINRHKEDECDAANIMKQIESGICYIHKHNIVHRDIK V VI
PfCDPK3 241 PENDEFYDMTPESLIKIUDECLASYTTHNYEMTKAGHPYVAR COUTGSDNYRCDWS VII PfCDPK1 194 PENDELENKHSLLNIKIUDECLASYTTHNYEMTKAGHPYVAR COUTGSDNYRCDWS VII PfCDPK2 254 SVIMUTLLOKYPEFESDHEISSFFKDNKLR-DELGAYIAPEVIRKKNNECDWS VII PfCDPK3 301 SOLRVILLOKYPEFESDHEISMYRKKYOGKGEDECTYENDGSDSDAWNIKLEYNPNE PfCDPK3 253 CSVINVILLOGYPEFESSONDDDIIKKVEKGENECTYENDGSDSDAWNIKLEPNRCHTMADK PfCDPK2 314 PfCDPK2 314 CTTEPANNENTTOMYKS-HEHVELSSTIKKVEKEELKKINNTTAACOSINYYNK Minase domain Junction domain PfCDPK2 314 CTTEPANNENTTOMYKS-HEHVELSSTIKKVEKEESSKIAAATLEGSSKIATTEAGOSINY 9fCDPK3 Junction domain PfCDPK2 314 CTTEPANNENTROMKSTKSSORTLOGASSNYKEGSSKIAAATLEGSSKIATTEAGSSKIATE XI EF-hand 1 Calmodulin-like domain PfCDPK3 372 INN-IRNIFIAMUNNSTISSORTLOGLKRIGY-OKIANTHACKINKSNA YI EF-hand 2 EF-hand 3 PfCDPK3 420 VEK-KSTELVHDEGKEYTKECLKKELKRIGY-OKIANTHACHNIKENEFEK YI XI EF-hand 3 PfCDPK3 420 VEK-KSTELVHDEGKEYTKECLKKE	PfCDPK2	195	PENFLFQSENKDSLLKIIDFGLSKNLGTGEFTT-TKAGTPYYVAFQVLDGKYDKKCDIWS
PICDPK1 174 EMANDMENT FOR STATE AND ALL PLACE AND AL	PfCDPK3	241	PENFLIFYDMTPESLIKIIDFGLASYFTHNNYEMKTKAGTPYYVAPOVLTGSYNYKCDMWS
PfCDPK2 254 SVILNTILCGYPPTYEDTERSPECTIONEVIKKVKKEFECEVENDIGSISSDAKNUTTKLUTYNPNE PfCDPK3 301 SCHEYTILLCGYPPTFESPHEITSMVKKKYCFEKEKNNISEAKDLIKKUTYNYNK PfCDPK1 253 CVILNTLLCGYPPTFESPHEITSMVKKKYCKEKYDFNDKKNISEAKDLIKKUTYNYNK PfCDPK1 253 CVILNTLLCGYPPTFESPHEITSMVKKKKYKKEKEKNNISEAKDLIKKUTYNYNK PfCDPK2 314 RCTIEALNHPTTOWTKSHEHVELSSTLKNLKNFKKENELKKIALTTAKHLCDVE PfCDPK2 314 RCTIEALNHPTTOWTKSHEHVELSSTLKNLKNFKKENELKKIALTTAKHLCDVE PfCDPK2 314 RCTIEALNHPTTOWTKSHEHVELSSTLKNLKNFKKENELKKIALTTAKHLCDVE PfCDPK2 314 RCTIEALNHPTTOWTKSHEHVELSSTLKNLKNFKKENELKKIALTTAKHLCDVE PfCDPK2 314 RCTIEALNHPTTOWTKSHEHVELSSTLKNLKNFKKENELKKIALKULOVE 313 RITAKEALNSKTIKKYANNINKSDOKTLCGALSMMKKFEGSOKLAGAALLFIGSKLTTLE YICDFX3 312 CAlmodulin-like domain PfCDPK2 372 INN-TRNIFIALTVONSTISSOLILDELKKIFYPJIHOVRDELTOSSO PfCDPK3 372 INN-TRNIFIALTVONSTISSOLILDELKKIFYPJHPYPJHPVRDLIDOIDSSO PfCDPK2 423 SOCHTTDEAATTKKNTKKELKKERDOOIDKKTIFEYNILRSFKNELEELKNVEEVDNIKKENFTK PfCDPK2 423 SOCHTDEAATTKKTKKERLERERERERERERERERERERERERERERERERERE	TIODINI	T	VII VIII IX
PFCOPK3 301 Sciency ILLCGYPPERGESDIELISM WRKCKY CHKCKEDNIN SEPAKDLIKKCHMADADK PFCDPK1 253 CVILY ILLCGYPPEGGON ODIIKKVSKGKYY DENDANN SEPAKDLIKKCHMADADK YK X X Kinase domain Junction domain Y Y PfCDPK2 314 RCTEEAD NHP TOWTKSHENVELSSTLKNLKNEKKENELKTATTTAKHLCDVE PfCDPK1 313 RITAKEAINSKOTKKYANNINKSDCKTLCGALSMMRKFEGSOKIACAATLFICSKLTTLE XI EF-hand 1 Calmodulin-like domain PfCDPK3 372 Y INN-ERNT IALEVDNSTISSOITLDGLKKIGY-OKIPPDIHQVERDISSNA PfCDPK3 420 VEK-LKSTELVLDEDGKSYTTKECLKKELKKIGY-OKIPPDIHQVERDISSNA PfCDPK3 373 EREETDIKKLEKNGDCOLDKKTITE YNTLRSFKNELGELKNVEEEVNILKENDEN KENDET Y EF-Hand 2 EF-Hand 3 PfCDPK1 473 SCH PYTETAAALDR-OLSKKLIYCARVERVDNDCETTAETAHLINNGKKGNTO PfCDPK1 433 NGY EYSEISVCMKGILESERIDHBENLMSKKK- PfCDPK1 433 NGY EYSEISTVCMKGEDTOKGEEDHBERLENNELKF- PfCDPK1 489 QMWNEVIGEADKWRDWKNMGKUDHBERLENNELKF- PfCDPK1 489 QMWNEVIGEADKWRDWKNMG	PfCDPK2	254	SGVIMYTLLCGYPPFYGDTDNEVLKKVKKGEFCFYENDWGSISSDAKNLTKLLTYNPNE
X X Minase domain ↓ Junction domain PfCDPK2 314 CTIEPALNHPNITOMTKSHEHVELSSTLKKIKNEKKENELKKIEUTIAAKLCDVE PfCDPK1 313 RITAKPALNEKKYAF-MMOMMMDIHVESPKNYGLLLKEOKLAMTILAQOSNDYD PfCDPK1 313 RITAKPALNSKAIKKYAF-MMOMMMDIHVESPKNYGLLLKEOKLAMTILAQOSNDYD PfCDPK1 313 RITAKPALNSKAIKKYAF-MMOMMDIHVESPKNYGLLLKEOKLAMTILAQOSNDYD PfCDPK2 372 INN-ERNIFIAHDYDNSGTSSOFILDG	PICDPK3 PfCDPK1	253	SGVLFYILLCGYPPFFGESDHEILSMVKKGKYQFKGKEWNNISERAKBLIKIMIADK CGVILYILLCGYPPFGGONDODIIKKWEKCKYYFDFNDWKNISERAKBLIKIMITYDYNK
PfCDPK2 314 RCTIEEATNHPWITOMTKSHERVELSSTIEKNEKNYKENELKKIALTITAKHLCDVE PfCDPK3 361 RICASEATOHPWEKKKYAF-NMDMKMDIHUENEKNYGLLKFOKLAMTTIAQOSNDYD PfCDPK1 313 RITAKEAINSKWIKKYANNINKSDOKTLCGAISNMEKFEGSOKLAGAAILEDGSKITTLE XI EF-hand 1 Calmodulin-like domain PfCDPK2 372 INN-ERNIETALDVENSETISSOEILDELKKIEY-QKIPPDIHQVIRDISSNA PfCDPK3 420 VEK-IKSTELVLDELGKEYTTKECLKKGLKKIEY-QKIPPDIHQVIRDISSOE PfCDPK1 373 ERKEETDIEKKLDKNGEOOLDKKEITESYNILRSFKNELGELKNVEEEVDNIKKEVDFDK PfCDPK1 373 ERKEETDIEKLDKNGEOOLDKKEITESYNILRSFKNELGELKNVEEEVDNIKKEVDFDK PfCDPK2 423 SOCHMYDDLAATDKOTYLKKEVCLIPEKFEDIEONSKISVEELKRIFGRDDIENPLID PfCDPK3 420 VEK-IKSTEIVLOEVGKUILESERLRDAENLEDTKSSKITKEETANIEGLTSISE PfCDPK1 423 SOCHMYDDLAATDKOTYLKKEVCLIPEKFEDIEONSKISVEELKRIFGRDDIENPLID PfCDPK3 423 SOCHMYDGLAATDKOTYLKKEVCLIPEKFEDIENKISSETTAEIAHILYNGNKKGNTO PfCDPK4 433 NGYLEYEEISVCMEKQILESERLRDAENLEDTKSSKKLIYCARVEDVDNDKKKGNTO PfCDPK3 433 NGYLEYEEISVCMEKQILESERTHDAENLEDTKSSKKLIYCARVEDVDVDNDKETTENANIEGETTAEIAHILYNGNKKGNTO PfCDPK4 483 KAIDSLLQEVDLNGGEIDHEESENKLKF- PfCDPK1			Kinase domain
PICDPR3 361 PICASEAL OHPERKKKYAF-NMDMMMDIHVUENENNYGLLIKPOKLAMTTIAQOSNDYD PfCDPK1 313 RITAKEALNSKTIKKYANNINKSDOKTLCGALSNMRKFEGSOKLAOAATLFTGSKLTTLE XI EF-hand 1 Calmodulin-like domain PfCDPK2 372 INN-ERNIFIALUVDNSCTISSOETLDGLKKIGY-OKIPPDIHQVIRDTSNA PfCDPK3 420 VEK-USSTELVEDEDGKEYITKECLKKCLKKIGY-OKIPPDIHQVIRDTSNA PfCDPK1 373 ERKELTDIEKKLDKNGDGOLDKKELIEGYNILRSFKNELGELKNVEEEVDNILKEVEFDK PfCDPK2 423 SQUHYDELAATIEKKLDKNGDGOLDKKELIEGYNILRSFKNELGELKNVEEEVDNILKEVEFDK PfCDPK3 470 SCKIDYTE FIAAALER -OLSKLIJVCARVEDVENDEETTAAELAHLIVNGNKKONTO PfCDPK1 433 NGYLEYSE FISVCMENDEGEDHEEMLMMSKKK- PfCDPK2 483 KAIDSLIQEVELNGGEDHEEMLMMSKKK- PfCDPK3 529 RDVNRVKRMIRVEKNDCKIDHEESEMLKDENLEDTDKSCKTKEETANLEGLTSISE B 1 11 21 31 AtCPK1 A89 OMWNEVIGEAEKNKDNMIDEDEFVNMMHKICDNKSS B 1 11 21 31 AtCPK1 APEKELDSAV SSMKQESAM NKFKKMALLY ECDPK SINLESLEST ILNIRQEQGT OKLAAAALLY M ECCDFK <td>PfCDPK2</td> <td>314</td> <td>RCTIE EALNHPWITQMTKSHEHVELSSTLIKNLKNFKKENELKKIALTIIAKHLCDVE</td>	PfCDPK2	314	RCTIE EALNHPWITQMTKSHEHVELSSTLIKNLKNFKKENELKKIALTIIAKHLCDVE
XI EF-hand 1 Calmodulin-like domain PfCDPK2 372 INN-DRNIFIALDYDNSGTISSOELLDGLKKIGY-OKIPPIHQVIRDISNA PfCDPK3 420 VEK-UKSTBLVLDEDGKGYTTKECLKKGLKKIGY-OKIPPIHQVIRDISNA PfCDPK1 373 ERKEUTDIEKKLIKNGDCOLDKKELIEGYNILRSFKNELGELKNVEEEVDNILKEVFFD EF-Hand 2 PfCDPK2 423 SCOHMTDELAAIDRKOISKELIEGYNILRSFKNELGELKNVEEEVDNIKKEVFFD PfCDPK3 470 SCKIDYTETAALDRK-OISKKLIYCABRV9DVDNDGETTABLAHTLYNGNKKGNITO PfCDPK1 433 NGYLEVSEFISVCMDKOILESEERLDABNLEDTDKSKKITKEELANLEGLTSISE EF-Hand 4 PfCDPK2 483 KAIDSLQEVFLNGGEFIDHHEMLMMSKKK- PfCDPK2 483 KAIDSLQEVFLNGGEFIDHHEMLMMSKKF- PfCDPK3 529 RDVNRVKRMIRDVDKNNDGKIDHHESEVMKLKF- PfCDPK1 489 ØMWNEVIGEAKNKDNMIDEDEFVNMMHKICDNKSS B 1 11 21 31 AtCPK1 APPKELDSAV ISRMKQESAM NKFKKMAILY M EmCDPK SINUESLEST ILNIRQEOFT OKLAAAALLY M TgCDPK SINUESLEST ILNIRQEOFT OKLAAAALLY M PCAPK-α KVDK0IVQCR IKNIVNRAE ØKIQAALLY M PCAPK-α KVDK0IVQCR IKNIVNRAE ØKIQAALLY M PCAPK-A KVDK0IVQCR IKNIVNRAE ØKIQAALLY M PCAPK-A KVDK0IVQCR IKNIVNRAE ØKIQAALLY M PCAPK-A KVDK0IVQCR IKNIVNRAE ØKIQAALLY M PCAPK-B APNGIDMKA IKNISSEFGA NKVRAMOF I PfCDPK1 KSOKTLCGA ISNMRKEGS ØKIAQAALLY M PCAPK-B APNGIDMKA IKNISSEFGA NKVRAMOF I PfCDPK1 KSOKTLCGA ISNMRKEGS ØKIAQAALLY M PCAPK-B APNGIDMKA IKNISSEFGA NKVRAMOF I PfCDPK3 NMMKDHV ENFKNGLL LKFQKLAMTI I	PICDPK3 PfCDPK1	361 313	RICASEALQHPWEKKKKYAF-NMDMKMDIHVLENFKNYGLLLKFQKLAMTIHAQQSNDYD RITAKEALNSKWIKKYANNINKSDOKTLCGALSNMRKFEGSOKLAGAATLFIGSKLTTLF
PfCDPK2 372 INN-ERNIFIALDVONSGTLSSORILDGLKKIGY-OKIPPDIHOVERDIDSNA PfCDPK3 420 VEK-EKSTELVEDDGKGYITKECLKKGLEKDGLKLPYNFDLLEDOTDSDG PfCDPK1 373 ERKETTDIFKKEDKNGDGOLDKKEITEGYNILRSFKNELGELKNVEEEVDNIKKEVDFDK PfCDPK2 423 SQTHMTDFLAATIDKQTYLKKEVCLIPEKFFDIGONGKISVEELKNVEEEVDNIKKEVDFDK PfCDPK3 470 SGKIDYTEFIAAALDRK-QLSKKLIYCAFRVEDVDNDGEITTAELAHTLYNGNKKGNITO PfCDPK1 433 NGYLEYSEFISVCMDKQILFSEERLRDAENLFDTKKSCKITKEELANLFGLTSISE EF-Hand 4 PfCDPK2 483 KAIDSLLQEVDLNGGGEIDFHEMLMMSKKK- PfCDPK3 529 RDVNRVKRMTRDVDKNNGKIDHDEDVNMMHKICDNKSS B 1 11 21 31 AtCPK1 APDKRLDAV SRMKQESAM NKFKKMALRV I EmcDPK SINLESLEST INTROPOCT OKLAAAALLY M TgCDPK SVVPSLDNA INTROPOCT OKLAQAALLY M PCaPK-a KVUKQIVQCR KKNLVNERAE OKLQAALLY M PCAPK-B APOKELDSAV SRMKQESAM NKFKKMALRV I PCCPK SINLESLEST INTROPOCT OKLAAAALLY M			XI EF-hand 1 Calmodulin-like domain
PfCDPK3 420 VEK-EKSTELVEDEGKGYTTKECLEKGLEEDGLKLPYNFDLEDOTDSDG PfCDPK1 373 ERKETDIEKKLEKNGDGOLDKKELTEGYNTLRSFKNELGELKNVEEEVDNTEKEVDFDK PfCDPK2 423 SCOTHYTDELAATIEKCTYLKKEVCLIPEKFEDIEGNEKESVEELKRTFGRDDIENPLID PfCDPK3 470 SCKTDYTETAAALERK-QLSKKLIYCAERVEDVEDVEDVELKRTFGRDDIENPLID PfCDPK3 470 SCKTDYTETAAALERK-QLSKKLIYCAERVEDVEDVEDGETTAELAHTLIYNGNKKGNITO PfCDPK1 433 NGYEENSEETSVCMEKQILFSEERLEDAENLEDTEKSCKTTKEELANLFGLTSISE PfCDPK1 483 KAIDSLOEVOLNGEGETDEHEEMLAMSKKK- PfCDPK3 529 RDVNRVKMIRDVDKNNDGKIDEHEESEMKLKF- PfCDPK1 489 OMWNEVIGEAEKKENMIDEDEEVNMMHKICDNKSS B 1 21 31 AtCPK1 APDKELDSAV SRMKOFSAM NKFKKMALRV EmCDPK SINLSLEST INTROPOET OKLAAAALLY MKFKKMALRV EcCDPK SIDVISLEST INTROPOET OKLAAAALLY MKVRAALLY MKVRAALLY PCaPK-a KVDKQIVQCR KKNLVNERAE OKLQQATLY MKVRAALMOF F PCAPK-a APNGIDMAA IKNLSSEFGA NKVRAALMOF F F PCAPK-a	PfCDPK2	372	INN-LRNIHIALDVDNSGTLSSQEILDGLKKIGY-QKIPPDIHQVIRDIDSNA
EF-Hand 2 EF-Hand 3 PfCDPK2 423 SGQHMTDBLAATIDKQTYLKKEVCLIPEKFEDIDGNGKISVEELKRIFGRDDIENPLID PfCDPK3 470 SGKUDYTEFTAAALDRK-QLSKKLIYCAFRVEDVDNDGEITTABLAHLLYNGNKKGNITO PfCDPK1 433 NGYLEYSEFTSVCMDKQILFSERLRDAENLFDTDKSGKITKEELANLFGLTSISE EF-Hand 4 PfCDPK2 483 KAIDSLLQEVDLNGDGEIDFHERMLMNSKKK- PfCDPK3 529 RDVNRVKMIRDVDKNNDGKIDFHERSEMKLKF- PfCDPK1 489 QMWNEVIGEADKNKDNMIDFDEHVNMHKLCDNKSS B 1 11 21 31 AtCPK1 APDKELDSAV SSMKQESAM NKFKKMAIRV I EmCDPK SINLPSLEST ILNIRQEQGT OKLAAAALLY M GCDPK-a KVDVPSLDNA ILNIRGEQGT OKLQAALLY M PCaPK-a KVDKOIVQCR KNLVNERAE OKLQAALLY M PCAPK-a APNGEIDMKA IKNLSSEFA NKVRALMOF I PCAPK-B APNGEIDMKA KNLSSEFA NKVRALMOF I PCAPK-B APNGEIDMKA KNLSSEFA NKVRALMOF I PCAPK-B APNGEIDMKA KNLSSEFA NKVRALMOF<	PICDPK3 PfCDPK1	420 373	VEK-LKSTELVEDEDGKGYLTKEQLKKGLEKDGLKLPYNFDLLEDQIDSDG ERKELTDIEKKLDKNGDGQLDKKELIEGYNILRSFKNELGELKNVEEEVDNILKEVDFDK
PfCDPK2 423 SGOTHATDELAATIDKOTYLKKEVCLIPEKFEDIDGNGKISVEELKRIEGRDDIENPLID PfCDPK3 470 SGKIDYTEFIAAALDRK-QLSKKLIYCAHRVFDVDNDGEITTAELAHILYNGNKKGNITO PfCDPK1 433 NGYLEYSEBISVCMDKQILFSEERLRDAENLFDTDKSGKITKEELANLFGLTSISE PfCDPK2 483 KAIDSLLQEVDLNGDGEITHHEAMLMNSKKK- PfCDPK3 529 RDVNRVKRMTRDVDKNNDGKIDHHEASEMNKLKF- PfCDPK1 489 QMWNEVLGEADKNKDNMIDFDEHVNMMHKICDNKSS B 1 11 21 31 AtCPK1 APDKELDSAV SSRMKQDSAM NKFKKMALRV I EmCDPK SINLPSLEST ILNIRQEQGT OKLAAAALLY M PCaPK-a KVDKQIVQCR KNLVNERAE OKLQAALLY M PCaPK-B APNGBIDMKA IKNLSSEFA NKVRALMOF I PCaPK-A KVDKQIVQCR KNLVNERAE OKLQAALLY M PCAPK-B APNGBIDMKA IKNLSSEFA NKVRALMOF I PCAPK-B APNGBIDMKA IKNLSSEFGA NKVRALMOF I PCCDPK1 KSDQKTLCGA SNMKFEGS OKLQAAILF I PCOPK2 HEHVELSSTL KNLKNEKKE			EF-Hand 2 EF-Hand 3
PfCDPK3 470 SCKLDWIEGLAAALDRK-QLSKKLIYCAERVEDVDNDGELTTADJAHLIYNGNKKGNITO PfCDPK1 433 NCYLEYSEEISVCMDKQILFSEERLRDAENLEDTDKSGKITKEELANLEGLTSISE EEF-Hand 4 PfCDPK2 483 KAIDSLIQEVDLNGDGEIDFHEFSEMMKSKKK- PfCDPK3 529 RDVNRVKRMIRDVDKNNDGKIDFHEFSEMMKLKF- PfCDPK1 489 QMWNEVIGEADKNKDNMIDFDEFVNMMHKICDNKSS B 1 11 21 31 AtCPK1 APDKPLDSAV SSRMKQESAM NKFKKMALRV I EmCDPK SINLPSLEST ILNIRQEQGT QKLAAAALLY M EtCDPK SIDVPSLEST ILNIRQEQGT QKLAQAALLY M PCaPK-α KVDKQIVQCR KNLNSEFGA NKVRALMUF I PCaPK-β APNGEIDMKA IKNLSSEFGA NKVRALMUF I PCAPK-β APNGEIDMKA IKNLSSEFGA NKVRALMUF I PCCDFK1 KSDQKTLCGA SKMKREGS OKLQQATLIF I PCCDFK2 HEHVELSSTL KKIKNEKKE NELKKATITI I	PfCDPK2	423	SCQIHYTDELAATIDKQTYLKKEVCLIPEKFFDIDGNGKISVEELKRIFGRDDIENPLID
EF-Hand 4 PfCDPK2 483 KAIDSLIQEVDLNGDGEIDHHEMLMMSKKK- PfCDPK3 529 RDVNRVKRMTRDVDKNNDGKIDHHEMSMMKLKF- PfCDPK1 489 QMWNEVLGEADKNKDNMIDEDEBVNMMHKICDNKSS B 1 11 21 31 AtCPK1 APDKPLDSAV SSMKQESAM NKFKKMALRV I EmCDPK SINLPSLEST ILNIRQEOGT OKLAAAALLY M EtCDPK SIDVPSLEST ILNIRQEOGT OKLAQAALLY M PCaPK-α KVDKQIVQCR KNLVNERAE OKLAQAALLY M PCaPK-β APNGPIDMKA INISSEFGA NKVRALMVF I PfCDPK1 KSDQKTLCGA SNMRKEEGS OKLAQAALLF I PfCDPK1 KSDQKTLCGA SNMRKEEGS OKLAQAALLF I PfCDPK1 KSDQKTLCGA SNMRKEEGS OKLAQAALLF I PfCDPK2 HEHVELSSTL KNLKNEKKE NELKKIALTI I PfCDPK3 NMDMKMDIHV ENFKNYGLL LKFQKLAMTI I	PICDPK3 PfCDPK1	470	SGKUDYTETAAALDRK-QLSKKLIYCARRVFDVDNDGEITTAELAHILYNGNKKGNITQ NGYTEYSEFISVCMDKOILFSEERLRDAENLEDTDKSGKUTKEELANLEGLTSISE
PfCDPK2 483 KAIDSLIQEVDINGDGEIDEHEMIMMSKKK- PfCDPK3 529 RDVNRVKMIRDVDKNNDGKIDEHEFSEMMKLKF- PfCDPK1 489 QMWNEVIGEADKNKDNMIDEDEHVNMHHKICDNKSS B 1 11 21 AtCPK1 APDKPLDSAV SSRMKQESAM NKFKKMAIRV I EmCDPK SINLPSLEST ILNIRQEOGT OKLAAAALLY M EtCDPK SIDVPSLEST ILNIRQEOGT OKLAAAALLY M PCaPK-α KVDKQIVQCR ILNIRQEOGT OKLAQAALLY M PCaPK-a KVDKQIVQCR IKNLSSEFA NKVRALMOF I PCaPK-a KVDKQIVQCR IKNLSSEFA NKVRALMOF I PCAPK-B APNGBIDMKA IKNLSSEFA NKVRALMOF I PCAPK-B APNGBIDMKA IKNLSSEFA NKVRALMOF I PCCDPK1 KSDQKTLCGA JSMMKFEGS OKLQAAILF I PfCDPK2 HEHVELSSTL KKIKNKKKK NEKKIALTI I PfCDPK3 NMMMKDIHV ENFKNYGLL LKFQKLAMTI I			FF-Hand A
PfCDPK3 529 RDVNRVKRMIRDVEKNNDGKIDEHERSEMMKLKF- PfCDPK1 489 QMWNEVIGEADKNKDNMIDEDEFVNMMHKICDNKSS B 1 11 21 31 AtCPK1 APDKPLDSAV SRMKQESAM NKFKKMALRV I EmCDPK SINLPSLEST ILNIRQEQGT QKLAAAALLY M FtCDPK SIDVPSLEST ILNIRQEQGT QKLAAAALLY M PGCAPK-α KVDKQIVQCR IKKLVNERAE QKLQQATLMF I PGCAPK-β APNGPIDMKA IKNISSEFGA NKVRAALMQF I PfCDPK1 KSDQKTLCGA ISNMRKEEGS QKLAQAALLF I PfCDPK2 HEHVELSSTL IKNIKNEKKE NELKKIALTI I PfCDPK3 NMDMKMDIHV IENFKNYGLL LKFQKLAMTI I	PfCDPK2	483	KAIDSLLQEVDLNGDGEIDFHERMLMMSKKK-
B 1 11 21 31 AtCPK1 APDKPLDSAV SRMKQFSAM NKFKKMALRV I EmcDPK SINLPSLEST ILNIRQFQGT OKLAAAALLY M EtcDPK SIDVPSLEST ILNIRQFQGT OKLAAAALLY M TGCDPK SVDVPSLDNA ILNIRQFQGT OKLAAAALLY M PCaPK-α KVDKQIVQCR IKNLVNERAE OKLQAALLY M PCaPK-β APNGBIDMKA IKNLSSFGA NKVRALMOF I PfcDPK1 KSDQKTLCGA ISNMKFEGS OKLAQAALLF I PfCDPK2 HEHVELSSTL IKNLKNEKKE NELKKIALTI I PfcDPK3 NMDMKMDIHV LENFKNYGLL LKFQKLAMTI I	PfCDPK3 PfCDPK1	529 489	
B11/2131AtCPK1APDKPLDSAVSSRMKQFSAMNKFKKMALRVIEmCDPKSINLPSLESTILNIRQFQGTOKLAAALLYMEtCDPKSIDVPSLESTILNIRQFQGTOKLAAALLYMTgCDPKSVDVPSLDNAILNIRQFQGTOKLAQAALLYMPCaPK-αKVDKQIVQCRKKILVNFRAEOKLQQATLMFIPfCDPK1KSDQKTLCGAISNMRKFEGSOKLAQAALLFIPfCDPK2HEHVELSSTLIKNLKNFKKENELKKIATTIIPfCDPK3NMDMKMDIHVIENFKNYGLLIKFQKLAMTII	TICDINI	409	WWW
AtCPK1APDKPLDSAVSRMKQESAMNKFKKMALRVIEmCDPKSINLPSLESTILNIRQEQGTQKLAAAALLYMEtCDPKSIDVPSLESTILNIRQEQGTQKLAAAALLYMTgCDPKSVDVPSLDNAILNIRQEQGTQKLAQAALLYMPCaPK-αKVDKQIVQCRKKNLVNERAEQKLQQATLMFIPCaPK-βAPNGPIDMKAIKNLSSEFGANKVRAALMQFIPfCDPK1KSDQKTLCGAISNMRKEGSQKLQQATLFIPfCDPK2HEHVELSSTLIKNLKNEKKENILKKIALTIIPfCDPK3NMDMKMDIHVIENFKNYGLLLKFQKLAMTII	В	1	11 21 31
EtCDPK SINDESLEST INNECTOR QKLAAAALI TgCDPK SVDVPSLEST ILNIRQFQGT QKLAQAALLY M TgCDPK SVDVPSLDNA ILNIRQFQGT QKLAQAALLY M PCaPK-a KVDKQIVQCR IKNLVNERAE QKLQQATLMF I PCaPK-β APNGPIDMKA IKNLSSEFGA NKVRAALMQF I PfCDPK1 KSDQKTLCGA ISNMRKEEGS QKLAQAAILF I PfCDPK2 HEHVELSSTL IKNLKNEKKE NELKKIALTI I PfCDPK3 NMDMKMDIHV LENFKNYGLL LKFQKLAMTI I	AtCPK1	AP et	DKPLDSAV ISRMKQISAM NKFKKMALRV I Nidsiest tintoogoft oklaaaatiy
TgCDPKSVDVESLDNALINIRQEQGTQKLAQAALLYMPCaPK-αKVDKQIVQCRKKNUVNERAEQKLQQATLMFIPCaPK-βAPNGEIDMKAIKNUSSEFGANKVRAALMQFIPfCDPK1KSDQKTLCGAISNMRKEEGSQKLAQAAILFIPfCDPK2HEHVELSSTLIKNLKNEKKENELKKIALTIIPfCDPK3NMDMKMDIHVIENKKNGLLLKFQKLAMTII	EtCDPK	SI	DVPSLEST ILNIRGEOGT OKLAAAALLY M
PCaPK-β APNGPIDMKA IKNISSEFGA NKVRAALMQF I PCCPK1 KSDQKTLCGA ISNMRKEGS OKLAQAAILF I PfCDPK2 HEHVELSSTI IKNIKNEKKE NELKKIAITI I PfCDPK3 NMDMKMDIHV IENFKNYGLI IKFQKLAMTI I	TgCDPK	SV	DVPSLDNA ILNIRQEOGT OKLAQAALLY M
PfCDPK1 KSDOKTLCGA LSNMRKHEGS OKLAQAALLF I PfCDPK2 HEHVELSSTL LKNLKNEKKE NELKKIALTI I PfCDPK3 NMDMKMDIHV LENHKNYGLL LKFOKLAMTI I	ΡCaPK-α PCaPK-β	r v AP	NGPIDMKA IKNISSEFGA NKVRAALMQF I
PICDEKZ HEHVELSSIL UKNIKNUKKE NEDKKIAUTI I PfCDPK3 NMDMKMDIHV LENFKNYGLL LKFQKLAMTI I	PfCDPK1	KS	DQKTLCGA LSNMRKFEGS QKLAQAAILF I
	PICDPK2 PfCDPK3	не NM	DMKMDIHV LENFKNYGLL L <mark>K</mark> FQKLAMTI I

quences (DIKPEN) in subdomain VI and (GTPYY-VAPO) in subdomain VIII indicate that PfCDPK3 is a serine/threonine kinase rather than a tyrosine kinase [11]. However, a highly conserved E residue in the APE motif of subdomain VIII is replaced by a Q residue in PfCDPK3. A similar change was also found in PfCDPK2 [6]. It is not clear how this change would influence the kinase activity. The junction domain of PfCDPK3 comprises 31 amino acid residues and separates the catalytic and calmodulinlike domains. Sequence comparison of all known protozoan CDPKs showed less conservation in this region for PfCDPK3 (Fig. 3B). The junction region is thought to contain an autoinhibitory motif which acts as a pseudosubstrate, interacting with the normal substrate-binding region of the enzyme to block entry of the substrate and, therefore, inhibiting kinase activity [12,13]. The binding of Ca^{2+} to the calmodulin-like domain is believed to release the pseudosubstrate from the active site of the catalytic domain by a mechanism that involves binding of the calmodulin-like domain to the autoinhibitory motif, therefore activating the kinase [14,15]. However, the basic-X-X-S/T consensus motif recognised by CDPKs [16] does not exist in the junction region of PfCDPK3. The sequences of the three P. falciparum kinases in the junction region are identical in only four of 31 residues, and all differ in at least 22 of 31 residues from the highly conserved junction sequences of plant CDPKs. This divergence of junction sequences within P. falciparum is similar to the situation in Paramecium [17] but is in sharp contrast to that in Arabidopsis, in which the junction region is strongly conserved; many Arabidopsis CDPKs are more than 90% identical in this region [18]. The LRVI sequence is thought to mediate an intramolecular interaction between the calmodulin-like domain and the junction region of the Arabidopsis CPK1 (AK1) [14]. However, only the I residue exists in PfCDPK3, although there are two residues (L and I) conserved in PfCDPK2 (see Fig. 3B). The calmodulin-like regulatory domain of PfCDPK3 consists of 151 residues and contains four putative EF-hand (helix-loop-helix) calcium-binding motifs, which are very similar to the EF-hands of calmodulin and other calcium-binding proteins. Each EF-hand of PfCDPK3 contains nearly all the requirements of such calcium-binding sites: six oxygen-containing ligands at positions 1, 3, 5, 7, 9 and 12, an invariant G residue at position 6 and a conserved aliphatic residue at position 8. The only exception is the highly conserved E residue at position 12 in the first EFhand, which is replaced by a O residue (see Fig. 3A). The refinement of the crystal structure of calmodulin has delineated the crucial role of this conserved E residue in calcium-binding [19]. In PfCDPK1, mutation of the conserved E residue to either K or Q in EF-hand 1 is deleterious and dramatically reduces the sensitivity of the Ca²⁺-induced conformational change and the Ca^{2+} -dependent activation [20]. Therefore, it would be of great interest to examine whether the replacement in EF-hand 1 of PfCDPK3 would influence the Ca^{2+} -binding and the Ca^{2+} -dependent enzyme activity. Each of the four calciumbinding sites is flanked by residues predicted to form α -helices (data not shown), as expected in a calciumbinding EF-hand. The presence of four EF-hands suggests that calcium would directly bind to PfCDPK3 and regulate its activity.

To investigate the structural organisation of the

1 2 3 4 5 6



Fig. 4. Southern blot analysis of the *Pfcdpk3* gene. Four µg of genomic DNA from *P. falciparum* clone 3D7A was digested with restriction enzymes, electrophoresed on a 1.0% agarose gel, transferred onto a nylon membrane and probed with the CDB'-CD2 fragment of *Pfcdpk3*. Lanes 1–6 correspond to digests with *AccI*, *Bam*HI, *BcII*, *Eco*RI, *Eco*RV and *HincII*. The sizes of 1 kb DNA markers are given in kb to the left.

Pfcdpk3 gene in the P. falciparum genome, 3D7A genomic DNA was digested with a number of restriction enzymes and analysed by Southern blotting. Hybridisation of the CDB'-CD2 (see Fig. 1) probe revealed a single band in digests with AccI, BamHI, BclI and EcoRV, respectively, consistent with the restriction map (Fig. 4), suggesting strongly that Pfcdpk3 is encoded by a single copy gene in the parasite genome. However, two bands (one predominant band and the other faint) were detected in digests with EcoRI and HincII, respectively, contradictory to the restriction map (lanes 4 and 6 in Fig. 4). The intensity of the faint bands decreased with higher stringency washing conditions (data not shown), indicating the presence of CDB'-CD2-related gene(s) in the P. falciparum genome. Indeed, two genes encoding PfCDPK1 and PfCDPK2, respectively, have recently been isolated from P. falciparum [6,7]. Whether the extra bands detected on the Southern blot represent these or other related gene(s) is unknown. Therefore, it is concluded that CDPKs exist as a multigene family in P. falciparum. A similar situation has been found in some plant species [21] and in the ciliated protozoan Paramecium [17]. So far, at least 20 CDPKs have been found in Arabidopsis thaliana [18,21], nine in maize [21,22], three in rice [23,24], three in soybean [25,26] and three in Paramecium tetraurelia [17]. CDPKs have also been isolated from other plants including mungbean, carrot, sweet potato and zucchini [21] and protozoans such as Eimeria tenella and Eimeria maxima [27] and Toxoplasma gondii (accession no. AF043629) as well as the unicellular algae Chlamydomonas moewusii [21].

In order to obtain some information on how Pfcdpk3 mRNA levels are regulated during parasite development and differentiation, a Northern blot containing equal quantities of total RNA prepared from cultures enriched in stage III to stage V gametocytes and from mixed asexual erythrocytic stages was probed with the CDB'-CD2 fragment. A single transcript of approximately 2900 nucleotides in size was detected only in the lane containing the sexual stage RNA, migrating between the 28S and 18S ribosomal RNA bands (Fig. 5A). The result suggests that PfCDPK3 is involved in sexual stage-specific events. To exclude the possibility that *Pfcdpk3* could crossreact with the other two related genes (*Pfcdpk1* total RNA extracted from asexual erythrocytic stages (A) and sexual erythrocytic stage (S) of P. falciparum (3D7A) was fractionated in a denaturing formaldehyde gel, blotted onto a nylon membrane and hybridised 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 Pfcdpk3 gene (CDB'-CD2), the Pfcdpk1 gene and the Pfcdpk2 gene, and exposed for 18 h, 24 h and 24 h, respectively.

and Pfcdpk2) on the Northern blotting, the same blot was hybridised with the PCR fragment (equivalent to the coding region of the CDB'-CD2 fragment for PfCDPK3) of Pfcdpk1 and Pfcdpk2, respectively (Fig. 5B,C). Interestingly, PfCDPK1 expressed not only in the asexual stage as described previously [7] but also in the sexual stage (see Fig. 5B), whereas PfCDPK2 expressed predominantly in the sexual stage (see Fig. 5C) rather than in the asexual stage [6]. In maize, it has been shown that a CDPK is specifically and developmentally expressed in pollen and required for germination and pollen tube growth [22]. In rice, expression of a CDPK gene has also been reported to be spatially and temporally regulated during seed development [23]. Interestingly, treatment of Plasmodium berghei and P. falciparum with Ca^{2+} antagonists such as TMB-8 (an inhibitor of intracellular Ca²⁺ release) and W-7 (a calmodulin inhibitor) strongly inhibited exflagellation, but

Fig. 5. Northern blot analysis of the Pfcdpk3 gene. Ten µg of



A. Pfcdpk3 B. Pfcdpk1 C. Pfcdpk2

EGTA (a Ca^{2+} chelator) and nicardipine (a Ca^{2+} channel inhibitor) had no effect, indicating that mobilisation of the parasite internal resources of Ca^{2+} is a prerequisite for exflagellation [28]. It has also been shown that DNA synthesis and axoneme formation in male gametocytes may be regulated by Ca²⁺/calmodulin [29]. Taken together, we propose that PfCDPK3 (probably PfCDPK2 as well) may serve as a link between Ca^{2+} and gametogenesis of *P. fal*ciparum. Identification of the upstream regulators and downstream substrates of PfCDPK3 will afford new insight on the regulatory mechanisms of sexual stage-specific processes. In addition, CDPK does not seem to exist in vertebrates, PfCDPK3 may, therefore, represent a promising target for development of new anti-malarial drugs.

Acknowledgements

We are very grateful to Mr. Richard J. Carrier and Mr. Phillip J. Howard for their critical reading of the manuscript. This work was supported in part by an equipment grant from the Royal Society (to L.S. Cox). D.A. Baker was supported by the Wellcome Trust. J.-L. Li was supported by the Cancer Research Campaign.

References

- D.A. Baker, J.L. Li, A family of PP2 phosphatases in *Plasmodium falciparum* and parasitic protozoa: reply, Parasitol. Today 15 (1999) 124.
- [2] J.L. Li, D.A. Baker, Protein phosphatase β, a putative type-2A protein phosphatase from the human malaria parasite *Plasmodium falciparum*, Eur. J. Biochem. 249 (1997) 98–106.
- [3] J.L. Li, D.A. Baker, A putative protein serine/threonine phosphatase from *Plasmodium falciparum* contains a large N-terminal extension and five unique inserts in the catalytic domain, Mol. Biochem. Parasitol. 95 (1998) 287–295.
- [4] J.L. Li, K.J.H. Robson, J.L. Chen, G.A.T. Targett, D.A. Baker, Pfmrk, a MO15-related protein kinase from *Plasmodium falciparum*: gene cloning, sequence, stage-specific expression and chromosome localization, Eur. J. Biochem. 241 (1996) 805–813.
- [5] R.A. Padgett, P.J. Grabowski, M.M. Konarska, S. Seiler, P.A. Sharp, Splicing of messenger RNA precursors, Annu. Rev. Biochem. 55 (1986) 1119–1150.
- [6] P.M. Farber, R. Graeser, R.M. Franklin, B. Kappes, Mo-

lecular cloning and characterization of a second calcium-dependent protein kinase of *Plasmodium falciparum*, Mol. Biochem. Parasitol. 87 (1997) 211–216.

- [7] Y. Zhao, B. Kappes, R.M. Franklin, Gene structure and expression of an unusual protein kinase from *Plasmodium falciparum* homologous at its carboxyl terminus with the EF hand calcium-binding proteins, J. Biol. Chem. 268 (1993) 4347–4354.
- [8] P.J. Kennelly, E.G. Krebs, Consensus sequences as substrate specificity determinants for protein kinases and protein phosphatases, J. Biol. Chem. 266 (1991) 15555–15558.
- [9] D.R. Knighton, J. Zheng, L.F. TenEyck, F.A. Ashford, N.H. Xuong, S.S. Taylor, J.M. Sowadski, Crystal structure of the catalytic subunit of cyclic adenosine monophosphatedependent protein kinase, Science 253 (1991) 407–414.
- [10] H.L. DeBondt, J. Rosenblatt, J. Jancarik, H.D. Jones, D. Morgan, S.H. Kim, Crystal structure of cyclin-dependent kinase 2, Nature 363 (1993) 595–602.
- [11] S.K. Hanks, A.M. Quinn, T. Hunter, The protein kinase family: conserved features and deduced phylogeny of the catalytic domains, Science 241 (1988) 42–52.
- [12] A.C. Harmon, B.C. Yoo, C. McCaffery, Pseudosubstrate inhibition of CDPK, a protein kinase with a calmodulinlike domain, Biochemistry 33 (1994) 7278–7287.
- [13] J.F. Harper, J.F. Huang, S.J. Lloyd, Genetic identification of an autoinhibitor in CDPK, a protein kinase with a calmodulin-like domain, Biochemistry 33 (1994) 7267–7277.
- [14] J.F. Huang, L. Teyton, J.F. Harper, Activation of a Ca²⁺dependent protein kinase involves intramolecular binding of a calmodulin-like regulatory domain, Biochemistry 35 (1996) 13222–13230.
- [15] B.C. Yoo, A.C. Harmon, Intramolecular binding contributes to the activation of CDPK, a protein kinase with a calmodulin-like domain, Biochemistry 35 (1996) 12029–12037.
- [16] D.M. Roberts, A.C. Harmon, Calcium-modulated proteins: targets of intracellular calcium signals in higher plants, Annu. Rev. Plant Physiol. Plant Mol. Biol. 43 (1992) 375– 414.
- [17] K. Kim, L.A. Messinger, D.L. Nelson, Ca²⁺-dependent protein kinases of *Paramecium*. Cloning provides evidence of a multigene family, Eur. J. Biochem. 251 (1998) 605–612.
- [18] E.M. Hrabak, L.J. Dickmann, J.S. Satterlee, M.R. Sussman, Characterization of eight new members of the calmodulinlike domain protein kinase gene family from *Arabidopsis thaliana*, Plant Mol. Biol. 31 (1996) 405–412.
- [19] Y.S. Babu, C.E. Bugg, W.J. Cook, Structure of calmodulin refined at 2.2 A resolution, J. Mol. Biol. 204 (1988) 191–204.
- [20] Y. Zhao, S. Pokutta, P. Maurer, M. Lindt, R.M. Franklin, B. Kappes, Calcium-binding properties of a calcium-dependent protein kinase from *Plasmodium falciparum* and the significance of individual calcium-binding sites for kinase activation, Biochemistry 33 (1994) 3714–3721.
- [21] J.S. Satterlee, M.R. Sussman, Unusual membrane-associated protein kinases in higher plants, J. Membr. Biol. 164 (1998) 205–213.
- [22] J.J. Estruch, S. Kadwell, E. Merlin, L. Crossland, Cloning

and characterization of a maize pollen-specific calcium-dependent calmodulin-independent protein kinase, Proc. Natl. Acad. Sci. USA 91 (1994) 8837–8841.

- [23] T. Kawasaki, N. Hayashida, T. Baba, K. Shinozaki, H. Shimada, The gene encoding a calcium-dependent protein kinase located near the *sbe1* gene encoding starch branching enzyme I is specifically expressed in developing rice seeds, Gene 129 (1993) 183–189.
- [24] D. Breviario, L. Morello, S. Giani, Molecular cloning of two novel rice cDNA sequences encoding putative calcium-dependent protein kinases, Plant Mol. Biol. 27 (1995) 953–967.
- [25] J.F. Harper, M.R. Sussman, G.E. Schaller, C. Putnam-Evans, H. Charbonneau, A.C. Harmon, A calcium-dependent protein kinase with a regulatory domain similar to calmodulin, Science 252 (1991) 951–954.
- [26] J.Y. Lee, B.C. Yoo, A.C. Harmon, Kinetic and calcium-

binding properties of three calcium-dependent protein kinase isoenzymes from soybean, Biochemistry 37 (1998) 6801–6809.

- [27] P.P.J. Dunn, J.M. Bumstead, F.M. Tomley, Sequence, expression and localization of calmodulin-domain protein kinases in *Eimeria tenella* and *Eimeria maxima*, Parasitology 113 (1996) 439–448.
- [28] F. Kawamoto, R. Alejo-Blanco, S.L. Fleck, R. Kawamoto, R.E. Sinden, Possible roles of Ca²⁺ and cGMP as mediators of the exflagellation of *Plasmodium berghei* and *Plasmodium falciparum*, Mol. Biochem. Parasitol. 42 (1990) 101–108.
- [29] F. Kawamoto, H. Fujioka, R.I. Murakami, Syafruddin, M. Hagiwara, T. Ishikawa, H. Hidaka, The roles of Ca²⁺/cal-modulin- and cGMP-dependent pathways in gametogenesis of a rodent malaria parasite, *Plasmodium berghei*, Eur. J. Cell Biol. 60 (1993) 101–107.