Characterization of *N*-myristoyltransferase from *Plasmodium falciparum*

Ruwani S. GUNARATNE^{1,2}, Mohammed SAJID³, Irene T. LING, Renu TRIPATHI⁴, Justin A. PACHEBAT and Anthony A. HOLDER

Division of Parasitology, The National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.

The gene coding for myristoyl-CoA:protein *N*-myristoyltransferase (NMT) has been cloned from the malaria parasite *Plasmodium falciparum*. The gene appears to be single copy and mRNA is expressed in asexual blood-stage forms. Comparison of cDNA and genomic sequences identified three small introns. The open reading frame codes for a 410-amino-acid protein and no evidence of forms with an extended N-terminal coding sequence was obtained. Residues important in substrate binding and in the catalytic mechanism in other species are conserved. The protein was expressed from a plasmid in *Escherichia coli*, partially purified and shown to have enzymic activity using a synthetic peptide substrate. Comparison of the malaria parasite

protein with that derived from the human gene showed a different pattern of inhibition by chemical modification. Human NMT activity was inhibited by diethylpyrocarbonate and partially inhibited by iodacetamide, whereas *P. falciparum* NMT activity was not inhibited by either pre-treatment. Since the enzyme in infectious fungi is a target for potential chemotherapeutic drugs, it should also be investigated in the context of parasitic infections such as that responsible for malaria.

Key words: malaria parasite, protein acylation, recombinant enzyme.

INTRODUCTION

Malaria is a debilitating disease transmitted by mosquitoes and caused by protozoa of the genus *Plasmodium*. *Plasmodium falciparum* causes the most serious illness and the asexual blood stage that replicates in erythrocytes may lead to coma and death within a few days. Due to emerging resistance against current anti-malarial drugs, there is an urgent need to identify new chemotherapeutic targets in malaria.

One candidate target is myristoyl-CoA:protein *N*-myristoyltransferase (NMT; EC 2.1.3.97). NMT catalyses the co-translational transfer of the C_{14:0} fatty acid from myristoyl-CoA to the N-terminal glycine of a variety of cellular proteins [1]. There are several reasons why NMT may be an attractive target for chemotherapy of infectious eukaryotes, and it has been investigated in the context of fungal infections [2]. The activity of this acyltransferase is essential for fungal viability [3,4] and in *Saccharomyces cerevisiae* NMT is an essential gene [5]. Although the binding site for the acyl-CoA is highly conserved, the protein binding site is variable [6,7], and peptide binding studies suggest that the protein substrate specificity has diverged in different species [8]. Significant differences between NMT in humans and fungi have led to the design of peptidomimetic inhibitors, which are highly selective against *Candida albicans* NMT compared with the human enzyme [9,10]. These studies suggest that NMT is a potential target for chemotherapeutic drugs against fungal infections and should be investigated in the context of parasitic infections.

Recently the structure of the *C. albicans* apoenzyme [11] and a ternary complex of the *S. cerevisiae* enzyme with bound myristoyl-CoA and peptide substrate analogues [12] have been reported, allowing the identification of the residues involved in

substrate binding and a proposed catalytic mechanism. It has been proposed that acyl transfer occurs through a direct nucleophilic attack of the glycine nitrogen on the carbonyl carbon of the thioester of myristoyl-CoA, following polarization of the carbonyl in an oxyanion hole and proton transfer from the glycine α -amino group to the C-terminal carboxylate of NMT [12].

The NMT enzyme activity is not dependent on its N-terminal sequence. The first 59 amino acids of the yeast enzyme appear to be unnecessary for enzymic activity [7]. In mammalian cells a number of different-sized forms have now been described, resulting from transcription from two loci [13], differential mRNA splicing [14] and proteolytic cleavage of the N-terminus [15]. The extended form of NMT appears to contain a ribosomal targeting sequence [16].

Although there have been few studies that directly demonstrate *N*-myristoylation of *P. falciparum* proteins, potential substrates such as the ADP-ribosylation factor 1 (ARF1) have been identified [17]. Metabolic labelling studies in pathogenic fungi have identified the ADP-ribosylation factors as major substrates for NMT [2], which require *N*-myristoylation for biological activity. Here we report the cloning and sequence analysis of the *P. falciparum* NMT, describe expression studies in *Escherichia coli* and carry out an initial comparison between the parasite and human enzymes.

MATERIALS AND METHODS

Preparation of *P. falciparum* parasites, and extraction of nucleic acids

P. falciparum clone T9/96 was maintained in erythrocytes in continuous culture, as described previously [18]. Infected ery-

Abbreviations used: ARF1, ADP-ribosylation factor 1; DEPC, diethylpyrocarbonate; ORF, open reading frame; NMT, myristoyl-CoA:protein *N*-myristoyltransferase; RT-PCR, reverse transcriptase PCR; Ni²⁺-NTA, Ni²⁺-nitrilotriacetate.

¹ Present address: Department of Biochemistry, University of Cambridge, Main Building, Downing Site, Tennis Court Road, Cambridge CB2 1QW, U.K.

² To whom correspondence should be addressed (e-mail rsg25@mole.bio.cam.ac.uk).

³ Present address: Tropical Disease Research Unit, University of California, San Francisco, VAMC 113B, 4150 Clement St., San Francisco, CA 94121, U.S.A.

⁴ Present address: Central Drug Research Institute, Chatter Manzil, Post Box No. 173, Lucknow-226001, India.

throcytes were harvested by centrifugation at 400 *g* for 5 min, washed twice with ice-cold PBS, and then lysed by the addition of 1% (v/v) acetic acid to release the intracellular parasites. After addition of a large volume of ice-cold PBS, the parasites were harvested by centrifugation at 400 *g* for 5 min, washed twice with ice-cold PBS, and then stored at -70°C .

Nucleic acids were prepared from the parasite material. The frozen extracts were thawed and genomic DNA prepared using the Blood and Cell Culture Genomic DNA Maxi Kit (Qiagen), according to the manufacturer's instructions. Total RNA was prepared from 3.8×10^9 infected red cells using the SNAPTM Kit (Invitrogen) according to the manufacturer's guidelines. Approx. 200 ng of RNA was used for cDNA synthesis using the cDNA Cycle Kit for reverse transcriptase PCR (RT-PCR; Invitrogen).

PCR amplification with degenerate primers, isolation of a specific DNA fragment, analysis of products from Vectorsite libraries, and assembly of the genomic sequence for *P. falciparum* NMT

In the first step to cloning *P. falciparum* NMT, degenerate oligonucleotide primers were synthesized, based on regions of amino acid sequence homology between NMT of six different organisms, and biased for *P. falciparum* codon usage. Two primers, NMT2F [CCA/T GTA/T T/CTA/T ATA/T AAA GAA A/GTA/T AC (forward PVLKEV/IT)] and NMT3R [CC ATC A/TCC NNN A/TCC AAA TTT (reverse KFGXGDG)], produced an approx. 630 bp product by PCR amplification from genomic DNA. This was confirmed to be part of the NMT gene by sequence analysis.

In the second step a specific probe was used to screen a Southern blot and clone a hybridizing fragment. A 480 bp sequence was amplified by PCR from the partial NMT sequence, and labelled with [α -³²P]dATP (Amersham), using the Prime-It II Random Labelling Kit (Stratagene), according to the manufacturer's guidelines. Southern blotting was carried out using 5 μg of *P. falciparum* genomic DNA digested with several restriction enzymes. The DNA was transferred to Hybond-N+ Nylon membranes (Amersham) overnight in $20 \times \text{SSC}$ (where $1 \times \text{SSC}$ is 0.15 M NaCl/0.015 M sodium citrate) and immobilized by UV cross-linking. After probe addition and washing under high-stringency conditions (final wash in $0.1 \times \text{SSC}/0.1\%$ SDS at 65°C for 15 min), hybridizing fragments were identified. Based on the result of the Southern blotting, a fragment hybridizing to the probe was cloned from a library prepared in λ -ZAP II (Stratagene) from size-fractionated *Bgl*II-digested genomic DNA. This fragment was sequenced and found to extend 3' from a *Bgl*II site within the NMT gene. At this stage a shotgun sequence (PF2MJ) read in The Institute for Genome Research *P. falciparum* genome database was found to overlap the sequence obtained and it was used to extend the sequence for an additional 150 bp in the 5' direction. These preliminary sequence data were obtained from The Institute for Genomic Research website (<http://www.tigr.org>). This sequencing was part of the International Malaria Genome Sequencing Project.

In the final step to obtain the full sequence of *P. falciparum* NMT two rounds of Vectorsite-II PCR were used to clone the remainder of the gene. Vectorsite-II libraries were made according to the manufacturer's guidelines (Genosys) using 0.1 μg of T9/96 genomic DNA digested with either *Alu*I, or *Dra*I. PCR reactions were carried out in 100 μl reaction volumes, using 1 μl of each Vectorsite-II library, 250 nM of Universal Vectorsite Primer, 250 nM of NMT-specific primer, either NMTVEC2 (AAC ACA TAA AAA ATT CAC CAC AGC) or NMTVEC4 (GAT ATA AAT CCC TAC CAA CAA A), and 5 units of AmpliTaq Gold (Perkin-Elmer). Thermal cycling was as follows:

94°C for 2 min; 94°C for 1 min, 60°C for 2 min, 72°C for 3 min for 40 cycles, followed by final extension step of 72°C for 10 min. In the first round a 984 bp fragment was produced from the *Dra*I library with the NMTVEC2 primer, cloned and sequenced; and in the second round a 702 bp fragment was produced from the *Alu*I library using the NMTVEC4 primer, cloned and sequenced.

Cloning full-length *P. falciparum* NMT from genomic DNA and cDNA and confirmation of the putative translational start site

To confirm the full-length NMT sequence, and to allow insertion of the DNA into an expression vector, it was amplified from both genomic DNA and cDNA using two primers, termed NMTORF1A and NMTORF2A. NMTORF1A (GGATCCCCG AAT GAT GAT AAG AAA GAT TTT GTT GG) contained an artificial *Bam*HI restriction site (underlined) and NMTORF2A (G GAATTC AAG ATG GTG GTT ATA AAC TTA TAA TAA AAC) contained an artificial *Eco*RI restriction site. PCR reactions used 100 ng of genomic DNA or 50 ng of cDNA and KlenTaq Polymerase (Clontech). Cycling conditions were 94°C for 5 min for 1 cycle, 94°C for 1 min, 64°C for 30 s, 68°C for 2 min, for 30 cycles, followed by a final extension step of 68°C for 10 min. PCR products were ligated into the TA vector used to transform *E. coli* TOP10, and the sequence of chosen inserts was obtained by automatic sequencing using an ABI 377 machine.

The absence of an additional intron 5' of the putative translational initiation site was confirmed by amplification using both genomic and cDNA as DNA templates using the primers NMTUP4 (CGT TAA AAT AAA ATA AAC C) and NMTVEC4. Products were analysed by agarose gel electrophoresis and cloned and sequenced.

Expression of *P. falciparum* NMT in *E. coli*

The NMT open reading frame (ORF) was ligated into the expression plasmid pTrcHis-C (a vector that directs expression of the inserted sequence as a polypeptide fused to six histidine residues and the XpressTM epitope at the N-terminus), the DNA was used to transform *E. coli* TOP10 cells, and the DNA sequence was confirmed in a chosen clone. Bacteria from a single colony were grown overnight at 37°C in Terrific Broth, the culture was diluted 20-fold, and after a further 2 h (D_{600} , 0.6–1.0) protein expression was induced by addition of 1 mM isopropyl β -D-thiogalactoside and overnight incubation at 37°C . Bacterial cells were harvested by centrifugation and resuspended in 1 ml of lysis buffer (50 mM sodium phosphate, pH 8.0, 300 mM NaCl, 10 mM imidazole and CompleteTM Protease Inhibitor Cocktail EDTA-free from Boehringer Mannheim). They were then lysed by addition of lysozyme to a final concentration of $1 \text{ mg} \cdot \text{ml}^{-1}$ on ice for 30 min followed by sonication in a Sonics and Materials VibraCell Sonicator, for 4×15 s bursts on maximum deflection. The lysate was cleared by centrifugation (147000 *g*, 1 h at 4°C) and loaded on to a Ni^{2+} -nitrilotriacetate (Ni-NTA) column (Qiagen), equilibrated with lysis buffer. The column was washed twice with wash buffer (50 mM sodium phosphate, pH 8.0, 300 mM NaCl and 20 mM imidazole), and then the bound protein was eluted from the column using elution buffer (50 mM sodium phosphate, pH 8.0, 300 mM NaCl and 1.0 M imidazole). The expressed protein was detected by Western blotting, using the Anti-Penta His (Qiagen) or the Anti-XpressTM Antibodies (Invitrogen). The blot was developed using a secondary antibody conjugated to horseradish peroxidase and ECL[®] reagents (Pierce), and exposed to X-Omat film (Kodak) for 10–30 s. Protein concentration was estimated using the BCA (bicin-

chonic acid) assay according to the manufacturer's guidelines and the reactions were incubated at 65 °C for 1 h.

NMT activity and inhibition assays

The assay was based on that of King and Sharma [19] that measures the ability of active NMT to transfer [³H]myristate from [³H]myristoyl-CoA, to the N-terminal glycine of a synthetic peptide *in vitro*. The assay was performed using human NMT (kindly provided by Dr Jeffrey McIlhinney [20]) and *P. falciparum* NMT expressed in *E. coli*. A peptide, termed PfG8, was designed based on the N-terminal sequence of the *P. falciparum* ARF1 protein. This peptide contained the sequence Gly-Leu-Tyr-Val-Ser-Arg-Leu-Phe and conformed to the consensus sequence required for the N-myristoylation of proteins by NMT. The peptide sequence was verified after reversed-phase HPLC and provided as a freeze-dried powder (Peptide Synthesis Service, National Institute for Medical Research, London, U.K.), which was resuspended in DMSO to a final stock concentration of 10 mM and used subsequently in the NMT assays.

The NMT assay was carried out in a final volume of 100 µl in 30 mM Tris/HCl, pH 7.4, containing 0.5 mM EGTA, 40 mM peptide, 1% (w/v) Triton X-100 and partially purified *P. falciparum* NMT (615 ng/assay). The reaction was initiated by the addition of 0.55 µCi of [³H]myristoyl-CoA (Amersham) and incubation at 37 °C. Samples were removed at various time points and spotted on to P81 phosphocellulose paper (Whatman), which was then washed in three changes of 40 mM Tris/HCl at pH 7.3. The level of incorporation of [³H]myristoyl-CoA was quantified in 5 ml of Ready Safe Liquid Scintillation mixture (Beckman), in a Liquid Scintillation Counter (Beckman LKB Wallac 1216 Rackbeta).

NMT enzyme assays were also carried out after preincubation of the partially purified enzyme (370 ng/assay) in the presence or absence of two known human-NMT inhibitors, 10 mM iodoacetamide and 10 mM diethylpyrocarbonate (DEPC), at room temperature for 15 min. The reaction was then initiated by addition of the peptide substrate and [³H]myristoyl-CoA and transferred to 37 °C for a further 4 h in a reaction volume of 50 µl. The assays were carried out in parallel with human NMT (100 ng/assay) as a positive control at 37 °C for 30 min, unless otherwise stated.

RESULTS AND DISCUSSION

Isolation and sequencing of the full-length *P. falciparum* NMT gene

The full *NMT* gene sequence was amplified from both genomic and cDNA. Initially, degenerate oligonucleotide primers based on conserved sequences in NMTs of six other organisms were used to amplify a genomic DNA fragment. This fragment was used to clone a *Bgl*II fragment extending beyond the 3' end of the ORF, and sequences extending in the 5' direction were obtained largely from overlapping Vectorette clones. Finally the full genomic and RT-PCR gene sequences were amplified and analysed. Southern-blotting experiments suggested that *NMT* is a single-copy gene in *P. falciparum* (results not shown). The amplification of *P. falciparum* *NMT* as an RT-PCR product indicates that the mRNA for this protein is expressed in the asexual blood stages of the malarial parasite's life cycle.

The full-length *NMT* cDNA ORF contains 1230 bp, with the potential to encode a 48 kDa protein. The corresponding genomic DNA contains 1617 bp consisting of four exons; the first exon codes for the first five amino acids in the ORF. The three introns (all of which have the GT/AG boundary consensus) are located

towards the 5' end of the coding region and span 117, 126 and 144 bp, respectively. It has been shown that human NMT exists in two forms as a result of differential mRNA splicing, the longer form containing a lysine-rich N-terminal extension [14]. Comparison of both genomic and cDNA sequences upstream of the proposed ATG initiation codon, based on the size of amplified products and sequence analysis, provided no evidence for a similar extended form of the protein in *P. falciparum*.

An alignment of orthologous NMT sequences showed that the *P. falciparum* protein conforms to the pattern of extensive sequence conservation in the central and C-terminal regions of the protein (an alignment with three other sequences is shown in Figure 1), with most diversity at the N-terminus. The deduced *P. falciparum* NMT amino acid sequence is approx. 50% identical to human NMT and approx. 40% identical to the two fungal enzymes. The N-terminal sequence divergence is most evident in both the *Histoplasma capsulatum* NMT [2] and the human NMT [13,14]. *H. capsulatum* contains an apparent N-terminal extension of 71 residues and human NMT can also contain an N-terminal extension resulting from differential mRNA splicing. This N-terminal extension is postulated to direct the normally soluble enzyme to the ribosomal fraction [16]. Previous studies using *S. cerevisiae* NMT have suggested that the first 59 amino acids are not required for catalytic activity [7]. The data presented here indicate that there is no alternative start site for *P. falciparum* NMT and that the protein does not contain an N-terminal extension.

All the residues known to participate directly in the enzymic mechanism or play critical regulatory roles in yeast NMT are conserved in the *P. falciparum* enzyme with the exception of Ile¹⁸⁰, which is replaced by Val, and His⁴⁶, which is Tyr as in other non-yeast enzymes. A conserved cysteine residue (Cys²²⁹ in Figure 1) is replaced by Ala in *P. falciparum* NMT. The two PROSITE signature sequences that uniquely identify NMT proteins, (KFGXGDG) and ([E/D](I/V)NFLC(V/I)HK), are present.

Expression of active *P. falciparum* NMT in *E. coli*

To determine whether or not the protein could be expressed in an enzymically active form, the 1230 bp *NMT* cDNA obtained by RT-PCR was cloned into the plasmid pTrcHis-C and used to transform *E. coli* TOP10 cells. *E. coli* has no endogenous NMT activity [21]. The recombinant protein was expressed as a N-terminal hexa-histidine and Xpress[™] epitope-tagged fusion protein and partially purified by affinity chromatography using a nickel-chelate (Ni-NTA) column. After induction of protein expression the NMT fusion protein was detectable by Western blotting using monoclonal antibodies anti-pentaHis (results not shown) and anti-Xpress[™] directed at the N-terminal tag coded by the expression vector (Figure 2). The fusion protein was purified partially by binding to a Ni-NTA column and eluted with 1.0 M imidazole. The antibodies recognized a 47 kDa protein, in good agreement with the predicted size, in the crude lysate of bacteria expressing *P. falciparum* NMT (Figure 2, lane 1) and material eluted from the nickel column (Figure 2, lane 3), but did not recognize proteins in the lysate of bacteria containing the expression vector alone (Figure 2, lanes 4–6).

The assay for NMT activity is based on the transfer of the [³H]myristoyl group from myristoyl-CoA to a synthetic peptide and the binding of the [³H]myristoylated peptide to phosphocellulose paper [19]. In the complete reaction mixture containing *P. falciparum* NMT and a peptide substrate based on the N-terminal amino acid sequence of the *P. falciparum* ARF1 protein

Figure 1 The deduced amino acid sequence of *P. falciparum* NMT aligned with that of two fungal and a corresponding human sequence

The *P. falciparum* NMT (Pf) sequence is aligned with those of *S. cerevisiae* (Sc; accession no. M23726) and *C. albicans* (Ca; accession no. M80544), two fungal enzymes that have been used for X-ray crystallographic studies, and with the short form of human NMT1 (Hs; accession no. M86707). Residues identical in the *P. falciparum* sequence to one or more of the other three sequences are shaded; gaps inserted to improve the alignment are shown (.). Residues discussed in the text are indicated (*), together with the two PROCITE NMT signature sequences (underlined). The alignment was made using the program PileUp (Genetics Computer GroupTM).

Figure 2 Expression of *P. falciparum* NMT in *E. coli* and a partial purification

A lysate was prepared from bacterial cells and the soluble fraction applied to a Ni-NTA column. After washing the column, bound material was eluted with 1.0 M imidazole. Samples of each fraction were resolved by SDS/PAGE analysis on a 12.5% gel and the proteins analysed by Western blotting using the Anti-XpressTM monoclonal antibody. In lane 1, the total lysate from bacteria containing the *P. falciparum* NMT plasmid construct; lane 2, the proteins not bound to the column; lane 3, proteins bound to the column and eluted with imidazole; lane 4, the total lysate from control bacteria containing the plasmid without the NMT sequence; lane 5, proteins in the control lysate not bound to the column; lane 6, proteins in the control lysate bound to the column and eluted with imidazole. The mobilities of the molecular-mass markers are shown on the left. In lanes 1 and 3 a 47 kDa band corresponds to the full-length *P. falciparum* NMT fusion protein.

[17], enzyme activity was detected, whereas in the absence of either peptide or NMT expression only background levels of radiolabel incorporation were observed (Figure 3).

Figure 3 Recombinant *P. falciparum* NMT is enzymically active

The time course for *P. falciparum* NMT activity of protein purified from *E. coli* using the Ni-NTA column. Samples were assayed as described in the Materials and methods section. The results of a representative experiment from three performed are shown as the means of duplicate samples (which consistently differed by less than 10%). ■, *P. falciparum* NMT with synthetic peptide substrate; ○, *P. falciparum* NMT without peptide substrate; □, synthetic peptide substrate without NMT; ▲, control eluate with synthetic peptide substrate; ●, control eluate without peptide substrate; 100% activity corresponds to an incorporation of 16297 c.p.m. ³H.

Although the level of expression in *E. coli* was not high (12 µg·l of bacterial culture⁻¹), the protein could be detected by Western blotting and by enzymic activity. In view of the skewed codon usage, typical of *P. falciparum* genes, it is likely that expression levels would be improved by re-synthesis of the gene using *E. coli*-preferred codons. Furthermore, additional purification steps will be required to obtain homogeneous enzyme. The sequence of the peptide used is consistent with the consensus

Table 1 A comparison of the sensitivity of the malarial and human enzymes to chemical inhibition

The inhibition of human and *P. falciparum* NMT activities by treatment with 10 mM iodoacetamide or 10 mM DEPC for 20 min at 37 °C was measured. Samples were assayed as described in the Materials and methods section using an incubation period of 4 h to allow maximal incorporation. The results of a representative experiment are shown as the means of duplicate samples (which differed consistently by less than 10%). In this assay 100% activity corresponds to incorporations of 8645 and 6950 c.p.m. ³H for the human and *P. falciparum* enzymes, respectively.

Assay	Activity (%)	
	Human NMT	<i>P. falciparum</i> NMT
With peptide substrate	100	100
Without peptide substrate	11.3	2.9
Iodoacetamide pre-treated	53.0	113.2
DEPC pre-treated	22.7	85.5

requirement for *N*-myristoylation and the specific incorporation of radiolabel due to NMT-dependent transfer of [³H]myristate to the peptide indicated that *P. falciparum* NMT is expressed as an enzymically active form in *E. coli*.

Inhibition of *P. falciparum* NMT activity by chemical modification

The sensitivity of both human and *P. falciparum* NMT to inhibition by iodoacetamide and DEPC was investigated. Using these inhibitors, the recombinant *P. falciparum* NMT could be distinguished from the human enzyme. Human NMT is partially inhibited by 5 mM iodoacetamide and completely inhibited by 10 mM DEPC [20]. *P. falciparum* NMT expressed in *E. coli* was not inhibited by 10 mM iodoacetamide or 10 mM DEPC (Table 1), conditions under which human NMT activity was inhibited by 50% and > 75%, respectively.

Although it had been suggested previously that one or more of the conserved cysteine and histidine residues are involved directly in the catalytic transfer mechanism [22], this is now considered unlikely based on the structural studies [12]. Whether or not the replacement of the conserved cysteine residue (residue 229 in Figure 1) by Ala in *P. falciparum* NMT results in insensitivity to iodoacetamide inhibition remains to be determined. Further studies are required of the effects of other NMT inhibitors on the purified enzyme and on parasite cultures to determine whether or not anti-*N*-myristoyltransferase drugs are anti-parasitic.

We thank Dr Jeffrey McIlhinney for the generous gift of the recombinant human enzyme. R.G. was supported by an MRC postgraduate studentship; R.T. was supported by a Royal Society-Indian National Scientific Academy Exchange Fellowship; the work undertaken by J.A.P. was in part fulfilment of an M.Sc. degree from the London School of Hygiene and Tropical Medicine. Support from the British Council Deutsche Akademische Austauschdienst (DAAD) Academic Research Collaboration (ARC) programme is gratefully acknowledged. Some preliminary sequence data were obtained from The Institute for Genomic Research website (<http://www.tigr.org>); this sequencing was part of the International Malaria Genome Sequencing Project and was supported by awards from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, the Burroughs Wellcome Fund and the U. S. Department of Defense.

REFERENCES

- Rudnick, D. A., McWherter, C. A., Gokel, G. W. and Gordon, J. I. (1993) Myristoyl CoA:protein N-myristoyltransferase. *Adv. Enzymol. Relat. Areas Mol. Biol.* **67**, 375–430
- Lodge, J. K., Johnson, R. L., Weinberg, R. A. and Gordon, J. I. (1994) Comparison of myristoyl-CoA:protein N-myristoyltransferases from three pathogenic fungi: *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Candida albicans*. *J. Biol. Chem.* **269**, 2996–3009
- Lodge, J. K., Jackson-Machelski, E., Toffaletti, D. L., Perfect, J. R. and Gordon, J. I. (1994) Targeted gene replacement demonstrates that myristoyl-CoA:protein N-myristoyltransferase is essential for viability of *Cryptococcus neoformans*. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 12008–12012
- Weinberg, R. A., McWherter, C. A., Freeman, S. K., Wood, D. C., Gordon, J. I. and Lee, S. C. (1995) Genetic studies reveal that myristoyl CoA:protein N-myristoyltransferase is an essential enzyme in *Candida albicans*. *Mol. Microbiol.* **16**, 241–250
- Duronio, R. J., Towler, D. A., Heuckeroth, R. O. and Gordon, J. I. (1989) Disruption of the yeast *N*-myristoyl transferase gene causes recessive lethality. *Science* **243**, 796–800
- Duronio, R. J., Reed, S. I. and Gordon, J. I. (1992) Mutations of human myristoyl-CoA:protein N-myristoyltransferase cause temperature-sensitive myristic acid auxotrophy in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 4129–4133
- Rudnick, D. A., Johnson, R. L. and Gordon, J. I. (1992) Studies of the catalytic activities and substrate specificities of *Saccharomyces cerevisiae* myristoyl-coenzyme A:protein N-myristoyltransferase deletion mutants and human/yeast Nmt chimeras in *Escherichia coli* and *S. cerevisiae*. *J. Biol. Chem.* **267**, 23852–23861
- Towler, D. A., Adams, S. P., Eubanks, S. R., Towery, D. S., Jackson-Machelski, E., Glaser, L. and Gordon, J. I. (1988) Myristoyl CoA:protein N-myristoyltransferase activities from rat liver and yeast possess overlapping yet distinct peptide substrate specificities. *J. Biol. Chem.* **263**, 1784–1790
- Devadas, B., Freeman, S. K., Zupiec, M. E., Lu, H. F., Nagarajan, S. R., Kishore, N. S., Lodge, J. K., Kuneman, D. W., McWherter, C. A., Vinjamoori, D. V. et al. (1997) Design and synthesis of novel imidazole-substituted dipeptide amides as potent and selective inhibitors of *Candida albicans* myristoyl-CoA:protein N-myristoyltransferase and identification of related tripeptide inhibitors with mechanism-based antifungal activity. *J. Med. Chem.* **40**, 2609–2625
- Devadas, B., Freeman, S. K., McWherter, C. A., Kishore, N. S., Lodge, J. K., Jackson-Machelski, E., Gordon, J. I. and Sikorski, J. A. (1998) Novel biologically active nonpeptidic inhibitors of myristoyl CoA:protein N-myristoyltransferase. *J. Med. Chem.* **41**, 996–1000
- Weston, S. A., Camble, R., Colls, J., Rosenbrock, G., Taylor, I., Egerton, M., Tucker, A. D., Tunnicliffe, A., Mistry, A., Mancía, F. et al. (1998) Crystal structure of the anti-fungal target N-myristoyl transferase. *Nat. Struct. Biol.* **5**, 213–221
- Bhatnagar, R. S., Futterer, K., Farazi, T. A., Korolev, S., Murray, C. L., Jackson-Machelski, E., Gokel, G. W., Gordon, J. I. and Waksman, G. (1998) Structure of N-myristoyltransferase with bound myristoyl CoA and peptide substrate analogs. *Nat. Struct. Biol.* **5**, 1091–1097
- Giang, D. K. and Cravatt, B. F. (1998) A second mammalian N-myristoyltransferase. *J. Biol. Chem.* **273**, 6595–6598
- McIlhinney, R. A. J., Young, K., Egerton, M., Camble, R., White, A. and Soloviev, M. (1998) Characterization of human and rat brain myristoyl-CoA:protein N-myristoyltransferase: evidence for an alternative splice variant of the enzyme. *Biochem. J.* **333**, 491–495
- Glover, C. J. and Felsted, R. L. (1995) Identification and characterization of multiple forms of bovine brain N-myristoyltransferase. *J. Biol. Chem.* **270**, 23226–23233
- Glover, C. J., Hartman, K. D. and Felsted, R. L. (1997) Human N-myristoyltransferase amino-terminal domain involved in targeting the enzyme to the ribosomal subcellular fraction. *J. Biol. Chem.* **272**, 28680–28689
- Stafford, W. H. L., Stockley, R. W., Ludbrook, S. B. and Holder, A. A. (1996) Isolation, expression and characterization of the gene for an ADP-ribosylation factor from the malarial parasite, *Plasmodium falciparum*. *Eur. J. Biochem.* **242**, 104–113
- Blackman, M. J., Chappell, J. A., Shai, S. and Holder, A. A. (1993) A conserved parasite serine protease processes the *Plasmodium falciparum* merozoite surface protein-1 (MSP1). *Mol. Biochem. Parasitol.* **62**, 103–114
- King, M. J. and Sharma, R. K. (1991) N-myristoyl transferase assay using phosphocellulose paper binding. *Anal. Biochem.* **199**, 149–153
- McIlhinney, R. A. J., Patel, P. B. and McGlone, K. (1994) Characterization of a polyhistidine-tagged form of human myristoyl-CoA:protein N-myristoyltransferase produced in *Escherichia coli*. *Eur. J. Biochem.* **222**, 137–146
- Duronio, R. J., Jackson-Machelski, E., Heuckeroth, R. O., Olins, P. O., Devine, C. S., Yonemoto, W., Slice, L. W., Taylor, S. S. and Gordon, J. I. (1990) Protein N-myristoylation in *Escherichia coli*: reconstitution of a eukaryotic protein modification in bacteria. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 506–510
- Peseckis, S. M. and Resh, M. D. (1994) Fatty acyl transfer by human N-myristoyl transferase is dependent upon conserved cysteine and histidine residues. *J. Biol. Chem.* **269**, 30888–30892