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Review

Protein farnesyl and *N*-myristoyl transferases: piggy-back medicinal chemistry targets for the development of antitrypanosomatid and antimalarial therapeutics

Michael H. Gelb[a,b,*], Wesley C. Van Voorhis[c], Frederick S. Buckner[c], Kohei Yokoyama[a], Richard Eastman[c], Elisabeth P. Carpenter[e], Chrysoula Panethymitaki[d,e], Katherine A. Brown[e], Deborah F. Smith[d,e,1]

[a] *Department of Chemistry, University of Washington, Seattle, WA 98195, USA*
[b] *Department of Biochemistry, University of Washington, Seattle, WA 98195, USA*
[c] *Department of Medicine, University of Washington, Seattle, WA 98195, USA*
[d] *Wellcome Trust Laboratories for Molecular Parasitology, Imperial College of Science, Technology and Medicine, London SW7 2AZ, UK*
[e] *Centre for Molecular Microbiology and Infection, Imperial College of Science, Technology and Medicine, London SW7 2AZ, UK*

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Abstract

To accelerate progress in the development of therapeutics for protozoan parasitic diseases, we are studying enzymes active in co- and post-translational protein modification that are already the focus of drug development in other eukaryotic systems. Inhibitors of the protein farnesyltransferases (PFT) are well-established antitumour agents of low cytotoxicity and known pharmokinetic properties, while inhibitors of *N*-myristoyl transferase show both selectivity and specificity in the treatment of fungal infections. Here, we summarise the current evidence that supports the targeting of these ubiquitous eukaryotic enzymes for drug development against trypanosomatid infections and malaria.

Keywords: Protein farnesyl transferase; *N*-Myristoyl transferase; Protein prenylation

1. Protein prenylation in eukaryotic cells

Attachment of the prenyl groups farnesyl and geranylgeranyl (Fig. 1) to specific eukaryotic cell proteins by protein prenyltransferases is required for the functioning of a number of cellular processes including signal transduction [1,2]. Mammalian protein farnesyltransferase (PFT) catalyzes the transfer of the 15-carbon farnesyl group from farnesyl pyrophosphate (FPP) to the cysteine residue in the C-terminal motif CaaX, where C is cysteine, a is usually but not necessarily an aliphatic residue, and X is glutamine, serine, methionine and a limited number of other residues (Fig. 1). Mammalian protein geranylgeranyltransferase-I (PGGT-I) transfers the geranylgeranyl group from geranylgeranyl phosphate to the cysteine of the C-terminal motif CaaL/F. Finally, protein geranylgeranyltransferase-II (PGGT-II) attaches two geranylgeranyl groups to proteins with C-terminal sequences CCXX, CXC, and CC. The prenyltransferases are heterodimeric proteins. PFT and PGGT-I have the same α-chain but have unique β-subunits, and PGGT-II contains a unique α/β-heterodimer and a third protein called Rep that delivers the protein substrate to the catalytic core [3].

The protein prenylation pathway for CaaX-containing proteins is shown in Fig. 1. After prenylation of the Cys residue, the aaX sequence is removed intact by a membrane-bound prenyl protein-specific endoproteinase. The prenylated-Cys now lies at the C-terminus of the protein, and its α-carboxyl group is methylated by a *S*-adenosylmethionine-dependent, membrane-bound prenyl protein-specific methyltransferase (PPMT). This series of post-translational modifications serves to anchor proteins to cell membranes (i.e. Ras proteins and heterotrimeric G proteins [4,5]). In addition, protein prenyl groups are recognized by hydrophobic binding sites on water-soluble proteins that extract

* Corresponding author. Tel.: +1-206-543-7142; fax: +1-206-685-8665.
E-mail addresses: gelb@chem.washington.edu (M.H. Gelb), d.smith@ic.ac.uk (D.F. Smith).
[1] Tel.: +44-20-7594-5282; fax: +44-20-7594-5283.

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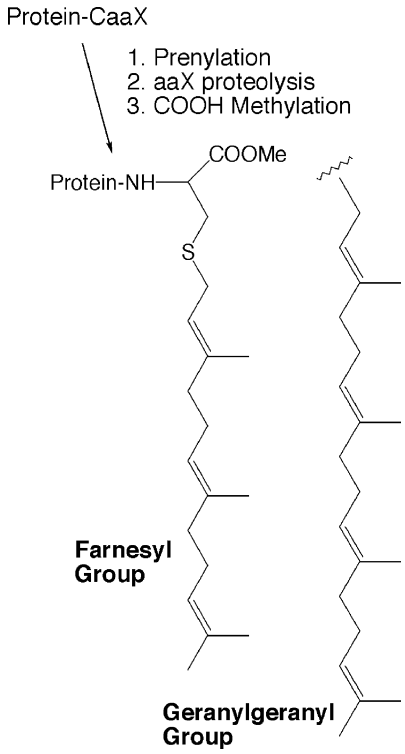

Fig. 1. The protein prenylation pathway in eukaryotic cells. Proteins containing a C-terminal CaaX sequence are farnesylated or geranylgeranylated (thioether linkage to the cysteine SH group) by PFT and PGGT-I, respectively. The aax tripeptide is subsequently removed by a membrane-bound, prenyl protein-specific endoprotease. The last step is the methylation of the α-carboxyl group of the prenylated cysteine, now at the C-terminus of the protein, by a membrane-bound, prenyl protein-specific methyltransferase. Some proteins contain a C-terminal CC, XXCC, or CXC motif, and each cysteine is geranylgeranylated by protein geranylgeranyltransferase-II (also known as Rab geranylgeranyltransferase).

prenylated proteins out of specific cellular membranes. One example of this phenomenon is the binding of Rab-GDI to prenylated Rab GTPases [6] which occurs in neurons during neurotransmitter release from synaptic vesicles. A second example is cGMP phosphodiesterase α-subunit binding to prenylated cGMP phosphodiesterase [7,8] which may regulate the sensitivity of the retina to light levels. These interactions between prenylated proteins and their binding proteins may be modulated by the methylation of the prenylated Cys residue [7,8].

1.1. Protein prenylation in trypanosomatid and malaria parasites

We, and independently the laboratory of Mark Field, showed that radiolabeled mevalonic acid, the precursor of isoprenoids in many organisms, is incorporated into *Trypanosoma brucei* proteins [9,10] and subsequent studies have shown that protein prenylation also occurs in *Trypanosoma cruzi* and *Leishmania mexicana* [11]. The genes coding for the two subunits of the enzyme that attaches farnesyl groups to proteins, PFT, have been identified in the *Plasmodium falciparum* genome, and PFT enzymatic activity has been detected in lysates prepared from *P. falciparum* intraerythrocytic stage parasites [12].

1.2. Protein farnesylation as a piggy-back medicinal chemistry drug target

PFT inhibitors have been extensively developed over the past decade because of their profound ability to arrest the growth of transformed cells and cause shrinkage of tumor xenographs in experimental animals (see for example [13,14]). There are currently >2000 primary publications on PFT inhibitors and >300 patents worldwide. Currently, three PFT inhibitors are showing promising results in Phase II clinical trials for the treatment of human malignancies [15]. Thus, PFT inhibitors are well developed in terms of medicinal chemistry and pharmacokinetics, and are toxic in man only at limiting doses that exceed the therapeutic dose. We reasoned that if PFT inhibitors are toxic to protozoan parasites, we would have a head start on development of novel agents for the treatment of devastating tropical diseases. Evidence to support this “piggy-back” approach is presented below.

1.3. Trypanosomatid and malaria PFTs

Our initial efforts led to the ∼60,000-fold purification of *T. brucei* PFT (TB-PFT) to apparent homogenity in ∼10% overall yield starting from procyclic (insect stage) parasites [11]. Partial amino acid sequence data led to the cloning of both subunits of TB-PFT [16]. Like PFTs from other eukaryotes [3], TB-PFT consists of an α- and β-subunit, with the active site formed at the interface between the two subunits. With the near completion of the *Leishmania major* and *T. cruzi* genome sequencing projects by the shotgun approach, we were able to find portions of the PFT subunits from these trypanosomatids, and the full-length genes were obtained by RACE PCR. All three trypanosomatid PFTs have been overexpressed in the baculovirus/Sf9 cell system, yielding milligrams amounts of fully active enzymes. Southern blot analysis indicates that the α- and β-subunits of all three trypanosomatids PFTs are encoded by single copy genes.

The TB-PFT α-subunit is significantly larger than the human orthologue (70,113 versus 44,408 Da, respectively) due to the presence of five inserts of >25 residues scattered throughout its entire length. Three inserts occur in the β-subunit (65,432 versus 46,955 Da for *T. brucei* and human, respectively). Fig. 2 shows a homology model in which the TB-PFT sequences are overlayed on the X-ray structure of mammalian PFT [17]. Interestingly, all eight inserts map to turns between α-helices that lie on the enzyme’s surface away from the active site and from the subunit–subunit interface (Fig. 2). Thus, these inserts do not appear to disrupt the structural core of the PFTs. After removing these large inserts, TB-PFT and rat-PFT are found to be 21 and 36% identical for the α- and β-subunits,

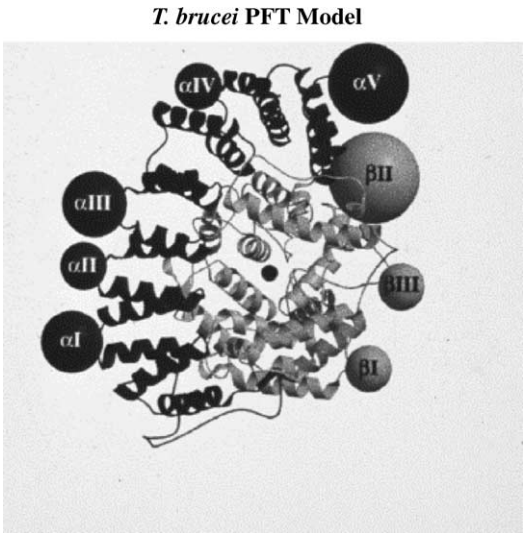

Fig. 2. Structural comparison of TB-PFT and rat-PFT. Ribbon diagram of rat-PFT α-subunit (dark shading) and β-subunit (light shading) is shown. The eight inserts of >25 amino acids (αI–αV in the α-subunit and βI–βIII in the β-subunit) present in TB-PFT are shown as spheres (larger diameter for larger inserts).

respectively. These inserts show no significant homology to proteins in the public databases. Hydropathy plot analysis does not reveal unusual hydrophobic/hydrophilic tendencies in these inserts compared to non-insert TB-PFT segments. The function of these loops, if any, is not known. TC-PFT and LM-PFT α- and β-subunits also contain a set of inserts in the same positions in the linear sequence as does TB-PFT, although the sequence homology within the insert regions among the three trypanosomatid enzymes is very low compared to homology in non-insert regions.

All of the residues that line the hydrophobic cavity of the FPP (the donor of the farnesyl group) are identical between mammalian and TB-PFT except for the modest change of Gly-250 in rat-PFT β-subunit being changed to an Ala in TB-PFT. The X-ray structure of the CaaX tetrapeptide CVIM (the acceptor of the farnesyl group, Fig. 1) bound to rat-PFT reveals the residues that contact the CaaX substrate. All residues surrounding the Caa portion are identical between rat- and TB-PFT except that Tyr-166 in the α-subunit of rat-PFT is replaced by a Phe in TB-PFT. This change probably has no effect on substrate binding since the Tyr hydroxyl does not contact aaX. There are significant residue changes in the enzyme pocket that binds the X portion of CaaX. Of the nine residues that contact the X side chain, five are different in TB-PFT versus the rat enzyme. The residue changes are presumably responsible for the difference in CaaX specificity for TB- versus rat-PFT previously described [16]. Also, we predict that it will be possible to prepare PFT inhibitors that are specific for the parasite versus the mammalian enzyme (see below). Similar differences between the active sites of parasite versus mammalian PFTs are predicted for TC-, LM- and PF-PFTs.

1.4. Inhibitors of TB-PFT and PF-PFT are lethal to bloodstream T. brucei and erythrocytic-stage P. falciparum, respectively

With purified TB-PFT in hand, we tested a selection of inhibitors of mammalian PFT against the parasite enzyme and against rat PFT in a side-by-side assay. Some compounds were found to be potent inhibitors (IC_{50} in the 1–10 nM range) of both enzymes (i.e. FTI-276 from A. Hamilton at Yale University and L-745,631 from Merck), whereas SCH-44342 (from Schering-Plough) was 630-fold more potent against rat-PFT than TB-PFT [11,16,18] (structures shown in Fig. 3). This was our first indication that parasite and mammalian PFTs share common active site features, but that the active sites are not identical.

FTI-277 is the methyl ester of FTI-276 (Fig. 3) and is able to penetrate mammalian cells, undergo intracellular conversion to FTI-276, and block protein prenylation [18]. We cultured *T. brucei* bloodstream form in the presence of mevalonolactone and simvastatin (to suppress endogenous mevalonate synthesis) and visualized the prenylated proteins after fluorography of an SDS–PAGE gel. Sub-micromolar concentrations of FTI-277 blocked the radiolabeling of some, but not all, parasite prenylated proteins (Fig. 4) [16]. We suspect that the radiolabeled proteins that are sensitive to the PFT inhibitor are farnesylated proteins, whereas those that are insensitive are likely to be doubly-geranylgeranylated Rab GTPases. These latter

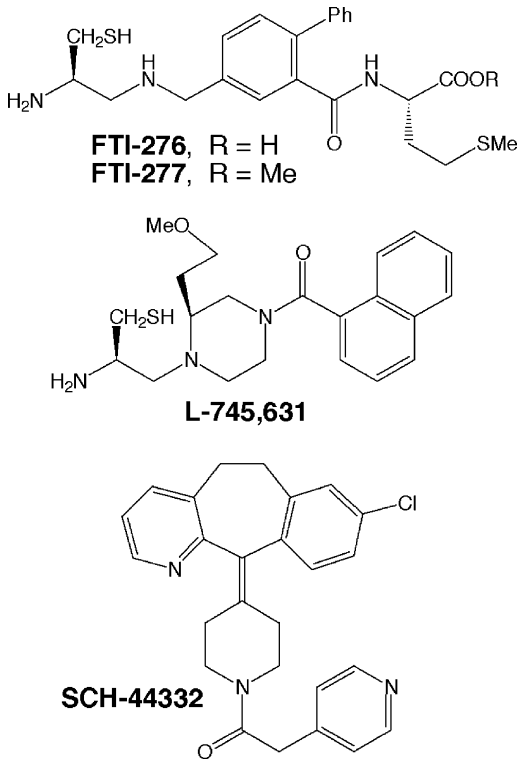

Fig. 3. Structures of PFT inhibitors. See text for a discussion of these compounds.

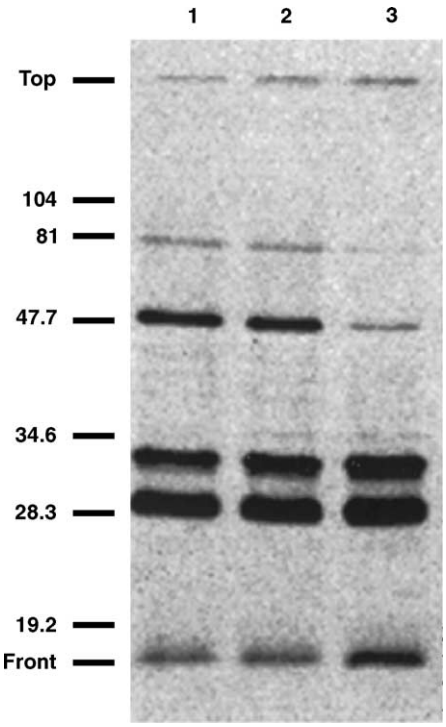

Fig. 4. PFT inhibitors block protein prenylation in *T. brucei*. *T. brucei* bloodstream form was cultured in the presence of mevalonolactone and simvastatin (to suppress endogenous mevalonate production). Proteins were resolved by SDS–PAGE. Lanes 1–3 contain 0, 0.2, and 5 M, respectively, of the PFT inhibitor FTI-277 (Me ester prodrug form of FTI-276). MW markers are shown on the left.

proteins have apparent MWs in the 28–32 kDa range, typical of Rab GTPases. A number of Rab GTPase homologues can be found in the *T. brucei* genome databases [19], and we have been able to detect Rab geranylgeranyltransferase (also known as protein geranylgeranyltransferase type II) in lysates prepared from insect stage *T. brucei* (using mammalian Rab 6 and geranylgeranyl pyrophosphate as substrates). Furthermore, the radiolabeled protein bands that decreased in intensity after treatment with the PFT inhibitor were selectively detected by Western blot analysis using an antiserum that reacts with *S*-farnesyl-cysteine methyl ester but not with the geranylgeranylated amino acid [20]. This study clearly shows that potent inhibitors of TB-PFT suppress protein farnesylation in parasites. FTI-277 is cytotoxic to bloodstream *T. brucei* grown in vitro (EC_{50} = 0.5 M). Parasite lysis begins about 2–3 h after addition of PFT inhibitor to the culture medium. Among ~130 Hamilton-lab CaaX mimetic PFT inhibitors, the lowest ED_{50} values for cytotoxicity to bloodstream *T. brucei* and *T. brucei rhodesiense* are in the 1–10 nM range. Efforts are underway to test a large variety of previously characterized PFT inhibitors, including compounds in clinical development for the treatment of cancer. Many of these compounds have optimized pharmacokinetic properties, thus we anticipate testing them in *T. brucei*-infected mice. Studies are also underway to evaluate PFT inhibitors as anti-*T. cruzi* and anti-*Leishmania* spp. agents.

Hamilton-lab CaaX mimetics are also cytotoxic to *P. falciparum* cultured in human erthryocytes [21]. The most potent compounds are active in the sub- to low micromolar range. Preliminary studies indicate that some of the compounds in the Bristol-Myers Squibb series are active against *P. falciparum* with ED_{50} values down to 0.1 μM. Studies with parasite-infected rodents are underway.

1.5. Trypanosomatid Rho GTPase is farnesylated

The studies described above, showing that PFT inhibitors are much more toxic to *T. brucei* and *P. falciparum* than to mammalian cells, are encouraging and nicely illustrate the piggy-back approach for the rapid development of antiparasitic agents. Our working hypothesis is that the selective toxicity of PFT inhibitors to these parasites is due to the apparent lack of protein geranylgeranyltranferase type I (PGGT-I). In other eukaryotic cells, PGGT-I attaches a single geranylgeranyl group to a number of proteins including a large number of signaling GTPases such as the Rho GTPase [22]. We have not been able to detect PGGT-I activity in crude lysates prepared from *T. brucei*, *T. cruzi*, and *L. mexicana*. PGGT-I and PFT share a common α-subunit but have distinct β-subunits. By searching public DNA sequence databases, we have not been able to find an open reading frame in trypanosomatid and *Plasmodium* genomes that shows convincing homology to known PGGT-I β-subunits. We hypothesized that a number of proteins that are geranylgeranylated in mammals and that are required for cell survival are farnesylated in trypanosomatids and in malarial species. This could be the basis for the selective toxicity of PFT inhibitors to these parasites.

The laboratory of U. Lopes (Federal University of Rio de Janeiro) recently cloned TcRHO1, the *T. cruzi* orthologue of the Rho GTPase [20]. Rho proteins have a well-established role in the control of the cytoskeleton. In mammals, Rho GTPase is geranylgeranylated, but recent evidence has shown that TcRho1 is farnesylated [20]. It is interesting to note that TcRho1 contains the C-terminal CaaX, sequence, CQLF. In mammals, CaaX sequences with X = phenylalanine are typically preferred substrates for PGGT-I. On the other hand RcRho1 is a good substrate for TC-PFT, and no PGGT-I activity could be found when *T. cruzi* cell lysate was analyzed with TcRHO1 and radiolabeled geranylgeranyl pyrophosphate [20]. We have initiated a study to systematically identify most of the prenylated proteins in these parasites, which should lead to conclusive evidence about the existence of PGGT-I in these organisms.

1.6. T. brucei prenyl protein-specific methyltransferase

Using partial sequence information from the *T. brucei* genome databases, we have cloned the full-length prenyl protein-specific methyl transferase (TB-PPMT) by RACE-PCR. The enzyme displays 24–28% identity to known

orthologues from other species (mammalian, yeast, and others). We have expressed the enzyme in active form using the baculovirus/Sf9 cell system. Membranes from infected cells display high PPMT activity. Interestingly, TB-PPMT shows dramatic specificity for farnesylated versus geranylgeranylated cysteine substrates (40-fold preference for the C-15 substrate based on k_{cat}/K_M). In contrast, mammalian PPMT acts on farnesylated and geranylgeranylated substrates with similar efficiency. These data further suggest that trypanosomatids lack mono-geranylgeranylated proteins due to the proposed lack of PGGT-I as described above. Studies are underway to characterize the prenyl protein-specific endoprotease in trypanosomatids that removes the C-terminal aaX sequence subsequent to the prenylation of the cysteine residue of the CaaX motif (Fig. 1).

2. Protein *N*-myristoylation in eukaryotic cells

An alternative modification that facilitates protein membrane-anchoring involves covalent attachment, via an amide bond, of the C_{14} saturated fatty acid, myristate, to the N-terminal glycine of target proteins ([23]; for a recent review, see [24]; Fig. 5). Like farnesylation, *N*-myristoylation increases the hydrophobicity of polypeptides, thereby enhancing associations with both membranes and other proteins. However, a myristate anchor alone is often insufficient to support stable membrane association and may require either accompanying palmitoylation or the presence of a neighbouring region of basic residues to enhance membrane attraction [25,26]. Myristoylation can also support reversible membrane binding via conformational changes associated with so-called "myristoyl switch" mechanisms [27]. In view of these various roles, it is not surprising that, like farnesylation, the addition of myristate can be essential for biological function in a range of cellular and viral proteins, including signaling pathway components, e.g. the Src family of tyrosine protein kinases and α-subunits of heterotrimeric G proteins [24,25].

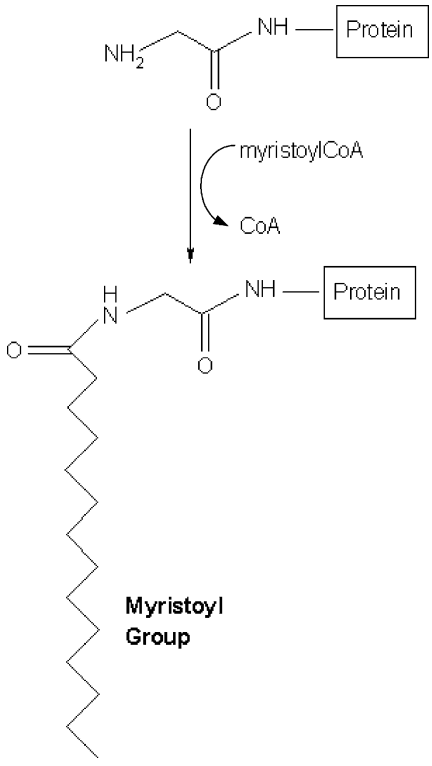

Fig. 5. Protein *N*-myristoylation in eukaryotic cells. Myristoyl CoA:protein *N*-myristoyltransferase (NMT) catalyses the covalent attachment of the fatty acid myristate to the N-terminal glycine of a range of proteins. The fatty acid moiety is provided by myristoyl CoA, which binds to the apo-enzyme first, forming a binary complex. This complex then binds the substrate protein and reaction occurs. Subsequently, CoA is released, followed by release of the myristoylated protein product.

N-Myristoylation is catalysed by myristoyl CoA:protein *N*-myristoyltransferase (NMT), a ubiquitous eukaryotic enzyme that was first purified to homogeneity from *Saccharomyces cerevisiae*. This monomeric protein has an ordered Bi Bi reaction mechanism, in which the apo-enzyme binds myristoyl CoA to form a binary complex that can acquire peptide substrates [28]. These substrates must have an N-terminal glycine (position 2), with serine or threonine preferred at position 6 and lysine or arginine preferred at positions 7 and/or 8 (reviewed in [25]). Subsequent conversion of the enzyme–substrate complex to the enzyme–product complex causes the release of CoA followed by the myristoylated peptide. NMT is highly selective for myristoyl CoA both in vitro and in vivo but is divergent in its peptide substrate specificity [23]. Recent X-ray crystallographic studies have enabled identification of key residues involved in catalysis that will lead to revised strategies for inhibition of NMT activity [24,29,30].

2.1. NMT is an essential enzyme

Genetic studies have shown that the *NMT* gene is essential for viability in a range of species, including *Drosophila melanogaster* [31], *S. cerevisiae* [32] and *Candida albicans* and *Cryptococcus neoformans* (pathogenic fungi that are causative agents of systemic infections in immunocompromised individuals [33,34]). In these organisms, NMT is encoded by a single copy gene, while in several mammalian species (including human) and *Arabidopsis thaliana*, there are two *NMT* genes coding for isoforms that may localize differently in cells [35,36]. In *A. thaliana*, down-regulated expression of one isoform also causes reduced survival. Hence, it is likely that for most, if not all, eukaryotes, NMT activity is indispensable. It therefore follows that this enzyme could be an attractive drug target for human pathogens such as *C. albicans* and *C. neoformans*, if specific reagents selective for the pathogen rather than the human enzyme can be developed. To verify this prediction, several classes of NMT inhibitors have been identified and tested, including myristate, myristoyl CoA and histidine analogues and myristoylpeptide derivatives (e.g. [37–39]). In this latter class, peptidomimetic compounds generated

from the N-terminal octapeptide of a known NMT substrate, ADP-ribosylation factor 1 (ARF 1), have been shown to be >500-fold selective for *C. albicans* NMT over human NMT and are fungistatic, an effect correlating with <50% reduction in ARF *N*-myristoylation [40]. Similar inhibitors are fungicidal against *C. neoformans*, an effect shown to be NMT-dependent [40,41]. These and other studies with fungal anti-NMT agents provide evidence for both selectivity and specificity in inhibition of this essential enzyme. Following the piggy-back approach used for development of farnesyltransferase inhibitors, can NMT inhibitors therefore provide an alternative source of therapeutic agents for trypanosomatid parasitic infections and malaria?

2.2. *N*-Myristoylation in trypanosomatid and *Plasmodium* species

Trypanosomatids mainly use myristate (which can be both synthesized de novo and salvaged from the bloodstream [42]) for incorporation into glyco-phosphatidylinositol (GPI) anchors, that tether the major surface molecules (glycoproteins and glyconconjugates) to the external surface of the plasma membrane. In *T. brucei*, non-specific myristate analogues are toxic, probably due to a cumulative effect on GPI anchor, phospholipid and *N*-myristoylated protein synthesis [43,44]. Few *N*-myristoylated trypanosomatid proteins have yet been characterized although data from the genome projects have demonstrated the presence of orthologues of mammalian *N*-myristoylated proteins (e.g. ARF 1), as well as novel proteins predicted to be *N*-myristoylated by virtue of their N-terminal sequences. Metabolic labeling studies in *L. major* have identified at least 10 ^{3}[H] myristate-labeled proteins (presumably both *N*- and *S*-myristoylated molecules), including ARF 1 and the infective stage, hydrophilic acylated surface proteins (HASPs) [45,46]. HASPB, a candidate vaccine for visceral leishmaniasis [47], requires N-terminal myristoylation and palmitoylation for translocation to the parasite plasma membrane [48]. A similarly dual acylated protein in *T. cruzi*, the flagellar calcium-binding protein (FcaBP), associates with the flagellar plasma membrane via a calcium-myristoyl switch mechanism [49]. FcaBP is one of a relatively small number of myristate-labeled proteins in *T. cruzi*, that include a novel *N*-myristoylated phosphatidylinositol-phospholipase C, expressed and membrane localized during parasite differentiation [50]. The *T. brucei* cytoskeleton-associated calpain-related protein, CAP5.5, also requires N-terminal myristate and palmitate anchors for intracellular localization within procyclic stages of the parasite [51].

2.3. *Plasmodium* NMT

Despite the lack of characterized *N*-myristoylated substrates, *P. falciparum* NMT was the first protozoan NMT to be cloned and characterized [52]. The single copy *NMT* gene is expressed in asexual blood-stages of the parasite and codes for a protein that is 50% identical with human NMT. Although sharing most of the key residues implicated in catalysis, recombinant *Plasmodium* NMT could be distinguished from its human homologue by the differential effects of chemical modification in an in vitro activity assay. Further analysis is required to determine whether NMT inhibitors are antiparasitic.

2.4. Trypanosomatid NMTs

NMT genes from *Leishmania* species have recently been cloned and characterized [53], together with those from *T. brucei* and *T. cruzi* (thanks to the genome projects). In *L. major*, the single copy *NMT* gene is constitutively expressed as a protein that shares 97% identity across the genus *Leishmania*, but is less well-conserved in *T. brucei* (54%) and *T. cruzi* (44%). Interestingly, identity with human NMT is comparable to that determined for these other trypanosomatid species. Both the *L. major* and *T. brucei* enzymes have parasite-specific domains and these have been shown to be important for optimal enzyme activity in an *E. coli* co-expression assay system [53]. Gene targeting experiments (by homologous recombination in *L. major* and RNAi assay in *T. brucei*) have demonstrated that trypanosomatid *NMT* genes are essential for viability, at least in extracellular parasite stages [53]. These observations correlate with the inhibitory effects of myristoyl CoA inhibitors on *Leishmania* [53] and *T. brucei* [43,44] growth in vivo, despite their lack of specificity.

2.5. Modeling the structure of *L. major* NMT

In contrast to the PFT structures described earlier, the NMT proteins from *L. major* and *S. cerevisiae* have similar molecular weights (48,524 and 52,837 Da, respectively). Comparison of these sequences shows that the more compact *L. major* NMT lacks the N-terminal 26 residues and three short sections in the C-terminal half of the protein. The *L. major* enzyme does however have three insertions of 3, 21 and 4 residues, all present in the N-terminal 250 residues. We have built a homology model of the *L. major* NMT structure based on the *S. cerevisiae* structure [24], using the modeling program MODELLER [54]. From this analysis, it is clear that the insertions and deletions found in the NMT structure are on the surface of the protein and do not disrupt the active site (Fig. 6). The known NMT sequences (>20) vary considerably in the lengths of their N-termini (regions that are implicated in cellular localization [55]). The known NMT crystal structures show varying degrees of disorder in this region, the *S. cerevisiae* and *C. albicans* NMT structures having the N-terminal 33 and 59 residues disordered, respectively [24,29,30]. The *L. major* sequence lacks these disordered regions and can be modeled from the known structure of *S. cerevisiae* from Ser-8.

The structure of the *S. cerevisiae* enzyme includes the myristoyl CoA cofactor and a peptide analogue of a pro-

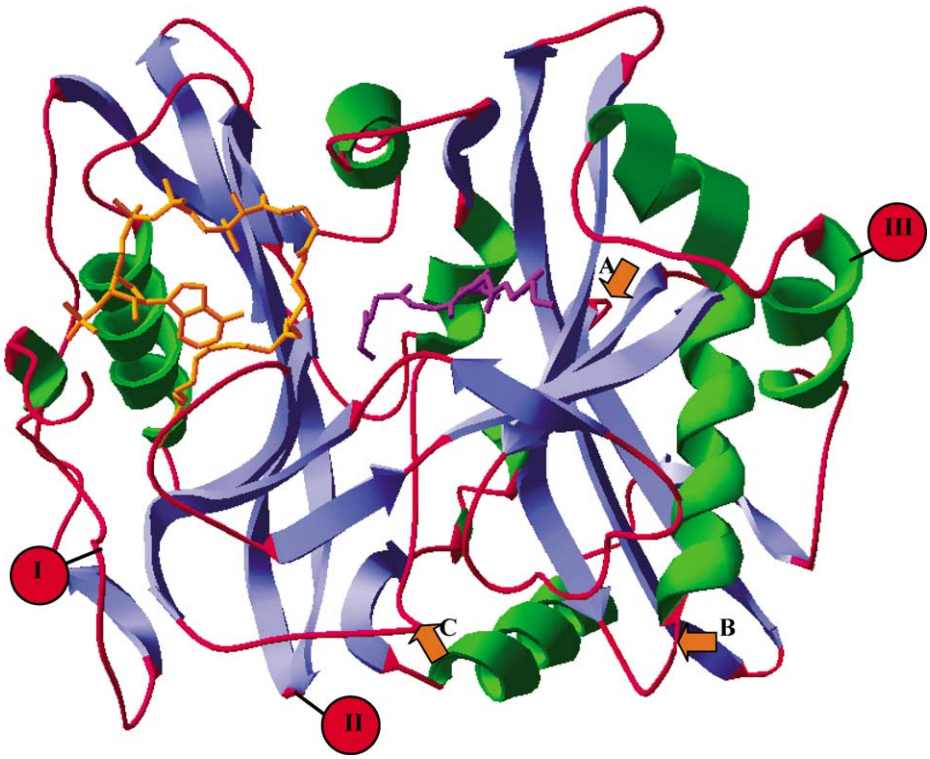

Fig. 6. Structural comparison of NMTs from *L. major* and *S. cerevisiae*. The model of the *L. major* NMT structure is based on the *S. cerevisiae* structure, bound to the myristoyl CoA analogue [*S*-(2-oxo)pentadecylCoA] (orange) and an octapeptide substrate GLYASKLA (magenta). Alpha helices are shown in green, strands in blue and loops in red. Regions where deletions occur in *L. major* compared to *S. cerevisiae* NMT are indicated by orange arrows (A–C) and regions where insertions are present in *L. major* are indicated by red spheres (I–III).

tein substrate, GLYASKLA [24]. The residues surrounding the binding sites are largely unchanged between the *S. cerevisiae* and *L. major* sequences, with the exception of Thr-183, Phe-334 and Ile-347, which are changed to Ala-181, Ser-330 and Ala-343, respectively. We do not believe that these changes are likely to have any major effects on substrate binding or catalysis. Five residues are directly involved in catalysis: the side chains of Asn-169 and Thr-205, the backbone nitrogens of Leu-171 and Phe-170, and the C-terminal carboxylate of Leu-455 [24]. All of these residues, except the C-terminal Leu-455, are conserved in the other known NMT sequences.

Based on the wealth of existing data from the study of fungal NMTs, it is likely that NMT activity in the trypanosomatid and *Plasmodium* species can be selectively blocked with high specificity, when compared to the human enzyme. The testing of specific inhibitors, in the form of peptide or peptidomimetic agents, on the viability of intracellular parasite stages will confirm whether NMT is a valid target for drug development in these species.

3. Concluding remarks

The “piggy-back” approach to drug development for protozoan parasitic diseases is a logical way around the perennial problems associated with meeting the costs of de novo drug design. These methods are clearly starting to pay off in the case of the PFT inhibitors while the evidence to date suggests that NMT may be another good candidate for this approach. Other potential targets will be generated by the parasite genome project data. In the long term, perhaps these generic methods may prove to be the most cost-effective for the development of new antiparasitic therapeutics.

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