The targets and role of palmitoylation in *Plasmodium* parasites

Chwen Ling Tay (BSc Hons)

Christ's College, University of Cambridge
Wellcome Trust Sanger Institute

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This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically stated in the text.

This dissertation does not exceed the word limit of 60000 words for the Degree Committee for the Faculty of Biology.

Abstract

Palmitoylation is the post-translational reversible addition of the acyl moiety, palmitate, to cysteine residues of proteins, and has been shown to be important in regulating protein trafficking, localisation, stability and function. Palmitoylation is wide-spread in all eukaryotes, and recent work revealed the presence of more than 400 palmitoylated proteins in the *Plasmodium falciparum* intraerythrocytic schizont stages, including proteins involved in key aspects of malaria biology and pathogenesis. The work described in this dissertation advances our understanding of protein palmitoylation in *Plasmodium* by developing a novel method to specifically identify palmitoylated cysteines within the *P. falciparum* palmitome, and characterising for the first time, the *Plasmodium* DHHC and MBOAT proteins, which are thought to mediate protein palmitoylation.

In the first section of work, a quantitative mass spectrometry based approach was developed to identify palmitoylation sites, resulting in the identification of over 100 putative palmitoylation sites in the *P. falciparum* schizont palmitome. These potential palmitoylation sites can be used to guide further experiments into the role of palmitoylation in the function of specific proteins. Pilot experiments were also carried out with related parasites, *P. berghei* and *Toxoplasma gondii*, and revealed palmitoylation sites that were conserved across Apicomplexan species.

The *Plasmodium* DHHC protein family was characterised in *P. falciparum* and *P. berghei,* establishing that individual DHHC proteins are localised to distinct organelles, including specialised parasite-specific organelles such as the rhoptries and the IMC. DHHC protein localisation may therefore play some role in substrate specificity. Knock-out studies identified individual DHHC proteins that were essential for blood stage growth, as well as proteins that could be successfully disrupted, suggesting that a subset of DHHCs is functionally redundant. Lastly, an assay was developed to demonstrate the palmitoyl transferase activity of the *Plasmodium* DHHC proteins, confirming for the first time that these proteins are responsible for protein palmitoylation in *Plasmodium* parasites. This assay further revealed that different *P. falciparum* DHHC proteins could palmitoylate the same target protein, further confirming the existence of overlapping functionality for these proteins in *Plasmodium.*

The occurrence of palmitoylation on so many *Plasmodium* proteins, as well as the existence of a repertoire of *Plasmodium* proteins shown to demonstrate palmitoyl transferase activity, indicate that this post-translational modification may have an important role in the normal cellular function of the parasite. Further study of palmitoylation in *Plasmodium* may thus result in the discovery of potential therapeutic drug targets, and the assays developed here could assist in achieving this goal.

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List of Abbreviations

2-BMP – 2-bromopalmitate

3-HA – triple-haemagglutinin

5FC – 5-fluorocytosine

6-FAM – 6-carboxyfluorescein

17-ODYA – 17-octadecynoic acid

ABE – Acyl-biotinyl exchange

AMPA – α-amino-3-hydroxy-5-methyl-4-isoxazole propionate

APT – Acyl-protein thioesterase

ARO – Armadillo repeats-only

β-ME – β-mercaptoethanol

BSA – Bovine serum albumin

CDPK1 – Calcium-dependent protein kinase 1

CRT – Chloroquine resistance transporter

DGAT – Diacylglycerol acyltransferase

DHHC – Aspartate-Histidine-Histidine-Cysteine

DMSO – Dimethyl sulphoxide

eNOS – Endothelial nitric oxide synthase

ER – Endoplasmic reticulum

FBS – Foetal bovine serum

GAP45 – Glideosome-associated protein 45

GO – Gene ontology

GPI – glycosylphophotidylinositol

HEK293 – Human embryonic kidney 293

Hh – Hedgehog

Hhat – Hedgehog acyltransferase

HRP – Horse radish peroxidase

IAA - Iodoacetamide

IMC – Inner membrane complex

KAHRP – Knob-associated histidine-rich protein

MBOAT – Membrane-bound O-acyl transferase

MESA – Mature-parasite-infected erythrocyte surface antigen

MSP1 – Merozoite surface protein 1

MTIP – myosin A tail domain-interacting protein

MWCO – Molecular weight cut-off

NEM – N-ethylmaleimide

PAT – Protein acyl transferase

PBS – Phosphate buffered saline

PBST – PBS with 0.1% Tween-20

PCR – Polymerase chain reaction

PEI – Polyethylenimine

PfEMP1 – *P. falciparum* erythrocyte membrane protein 1

PFGE – Pulsed field gel electrophoresis

PPT – Protein palmitoylthioesterase

PSD-95 – Post synaptic density protein-95

PTM – Post-translational modification

RAP1 – Rhoptry associated protein 1

RT-PCR – Reverse transcription-PCR

SDS – Sodium dodecyl sulphate

SDS-PAGE – SDS-polyacrylamide gel electrophoresis

Shh – Sonic Hedgehog

SILAC – Stable isotope labelling with amino acids in cell culture

SNARE – Soluble NSF attachment protein receptor

TBTA – Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine

TCEP – Tris(2-carboxyethyl)phosphine

TCR – T cell receptor

TM – Transmembrane

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