

***In vitro* and *in vivo* studies of the thiol:disulphide  
oxidoreductase ResA from *Bacillus subtilis* and  
*Streptomyces coelicolor***

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A thesis submitted in part fulfilment of the degree of Doctor of  
Philosophy at the University of East Anglia 2010

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## **DECLARATION**

I declare that the work contained in this thesis, submitted by me for the degree of Ph.D., is my own work, except where due reference is made to other authors, and has not been previously submitted by me for a degree at any other university.

Christopher T. C. Hodson

April 2010

## **PUBLICATIONS ARISING FROM WORK IN THIS THESIS**

Hodson, C. T. C., Lewin, A., Hederstedt, L., and Le Brun, N. E. (2008) “The active-site cysteinyls and hydrophobic cavity residues of ResA are important for cytochrome *c* maturation in *Bacillus subtilis*” *Journal of Bacteriology* **190** 4697-4705

Lewin, A., Crow, A., Hodson, C. T. C., Hederstedt, L., and Le Brun, N. E. (2008) “Effects of substitutions in the CXXC active-site motif of the extracytoplasmic thioredoxin ResA” *Biochemical Journal* **414** 81-91

Many of the ideas discussed in this thesis are also discussed in these papers. Reference to these papers in the main text has been deliberately restricted to a minimum, not out of any wish to diminish the importance of these works, but rather, to prevent the need to cite these works recurrently.

## ACKNOWLEDGEMENTS

Throughout the course of my research I have received help, advice and support, both professionally and more informally, from many people, all of whom I wish to sincerely thank here.

In particular I would like to thank my supervisor, Nick Le Brun who has somehow managed to put up with me for the last four years and always found the time to support, advise and tutor; Allison Lewin who has offered advise and tuition long after she was contractually obliged to; Lars Hederstedt (and his laboratory) for much needed help with BdbD; Matt Hutchings and Dave Widdick for guiding me through *Streptomyces* genetics; Nick Cull for technical support; all my friends and colleagues in both CAP and BIO that I've not already mentioned (Jason Crack, John Holmes, Allister Crow, Alisa Gaskil, Liang Zhou, Tamara Lawson, Chloe Singleton, Oliver Hecht, Angelo Figueiredo, Rose Marie Doyle, Kate Haynes, Gaye White, Matt Bawn, Myles Cheesman, Julea Butt, Ben Thompson and sorry to anyone I have forgotten); My girlfriend Nicola; Dad, Mum, Jim, Tim and Granny, I can not thank you enough for the love and support you have all offered me over the years to get me this far; finally I would like to thank my late Grandad, Gerald Lucian Baldit, and dedicate this thesis to his memory.

This Ph.D. thesis was funded by the Engineering and Physical Sciences Research Council (EPSRC).

## ABSTRACT

Thiol:disulphide oxidoreductases (TDORs) are essential in many organisms for the correct insertion and/or removal of disulphide bonds into and from peptides and proteins. One process for which TDORs have been shown to be integral is cytochrome *c* maturation (CCM). In the Gram positive soil bacterium *Bacillus subtilis* the membrane bound TDOR ResA is involved in the removal of a disulphide bond from the CXXCH haem binding motif of apo-cytochromes *c* in order to allow correct haem insertion by ResBC. The majority of TDORs contain a CXXC active site in which the sulphur residues of the cysteine side chain shuffle between the oxidised (disulphide) and reduced (thiol) forms. It is demonstrated here that both cysteines of the ResA CXXC active site are essential for protein function and that other residues, Pro141, Glu80 and Glu75, are important for stability, recognition and maintaining the reducing power of the active site, respectively. Studies of the membrane anchor domain of ResA reveal that it is important but not essential for CCM. Further to this, a homologue of *B. subtilis* ResA found in *Streptomyces coelicolor* was shown to play a similar role *in vivo* with regard to CCM; and *in vitro* studies of a purified soluble form of the protein revealed that although it has a similar low reduction potential to *B. subtilis* ResA it also has some interesting differences. Finally, *in vivo* studies of an oxidising TDOR, BdbD, from *B. subtilis* have provided some insight to the delicate balance of the redox state of proteins on the outside of the cytoplasmic membrane as well as future perspective on how to study this protein *in vivo*.

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## LIST OF ABBREVIATIONS

aa	Amino acid
Abs, A	Absorbance
Amp	Ampicillin
Badan	6-Bromoacetyl-2-dimethylaminonaphthalene
B&W	Bott and Wilson solution
BCA	Bicinchononic acid
bp	Base pairs
Bs	<i>Bacillus subtilis</i>
<i>caa</i> <sub>3</sub>	Cytochrome <i>c</i> oxidase
Da	Daltons
DTNB	5,5'-dithio-bis(2-nitrobenzoic acid)
DTT	Dithiothreitol
$\epsilon$	Extinction coefficient ( $M^{-1} \text{ cm}^{-1}$ )
EDTA	Ethylene diamide tetraacetic acid
GST	Glutathione S-transferase
IPTG	Isopropyl- $\beta$ -D-thiogalactopyranoside
Kan	Kanamycin
nt	Nucleotide
dNTPs	Deoxynucleotide 5'-triphosphates
LB	Luria-Bertani broth
LBA	Luria-Bertani agar
MG	Minimal glucose
MOPS	3-(N-morpholino)-propanesulfonic acid
NSMP	Nutrient sporulation medium with phosphate

OD	Optical density
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PDB	Protein data bank
PMSF	Phenylmethanesulphonylfluoride
Sa	<i>Staphylococcus aureus</i>
Sc	<i>Streptomyces coelicolor</i>
SDS	Sodium dodecyl sulphate
TBAB	Tryptose blood agar base
TCA	Trichloroacetic acid
TIM	Inner membrane transport machinery
TMPD	N,N,N',N'-tetramethyl-p-phenylenediamide
TOM	Outer membrane transport machinery
Tris	Tris(hydroxymethyl)aminomethane
Trx	Thioredoxin
UV	Ultraviolet
% v/v	ml per 100 ml
% w/v	grams per 100 ml
WT	Wild type
X-gal	5-bromo-4-chloro-3-indonyl- $\beta$ -D-galactopyranoside