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

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

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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.

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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*.

TABLE OF CONTENTS

Chapter 2: Materials and methods

Chapter 4: *In vivo* studies of the functional importance of the hydrophobic cavity and the membrane anchor of *B. subtilis* ResA

Chapter 5: *In vivo* Studies of the TDOR BdbD from *B. subtilis*.

Chapter 6: Studies of the Res system in *S. coelicolor*

LIST OF FIGURES

Chapter 3:

Chapter 4:

Chapter 5:

Chapter 6:

LIST OF TABLES

Chapter 6:

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	*Bacillus subtilis*
caa_3	Cytochrome *c* oxidase
Da	Daltons
DTNB	5,5'-dithio-bis(2-nitrobenzoic acid)
DTT	Dithiothreitol
ε	Extinction coefficient (M^{-1} cm^{-1})
EDTA	Ethylene diamide tetraacetic acid
GST	Glutathione S-transferase
IPTG	Isopropyl-β-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	*Staphylococcus aureus*
Sc	*Streptomyces coelicolor*
SDS	Sodium dodecyl sulphate
TBAB	Tryptose blood agar base
TCA	Trichloroacetic acid
TIM	Inner membrane transport machinary
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-β-D-galactopyranoside