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Biogenesis of *Shigella flexneri* IcsA Protein

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DECLARATION

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ABSTRACT

The IcsA autotransporter is a vital virulence factor for *Shigella flexneri*, a human-specific causative agent of bacillary dysentery that accounts for over a million global deaths annually. Ingested Shigellae invade and spread throughout the colonic epithelium. IcsA confers motility to intracellular bacteria by engaging host actin regulatory proteins to polymerise filaments of actin in a processes termed actin-based motility. This IcsA-dependent motility potentiates the intercellular spreading.

IcsA is displayed at one pole of the bacterium, thereby providing a functional focus for actin polymerisation that generated propulsive force. This work investigated the biogenesis of IcsA, seeking to identify factors that direct the cytoplasmic deliver of the nascent protein towards the pole. A refined polar targeting region, IcsA_{532–570} has been identified. Additionally, insertion mutant, i532 and i563, within the recognised targeting sequence IcsA_{506–620} that have been identified that are defective, though not entirely deficient, in polar targeting. GFP+ fusions to these mutated targeting sequences revealed rapid motion of fluorescent foci throughout the cytoplasm, a process that likely precedes polar targeting in the wild-type. The delivery of the polarly targeted IcsA_{506–620} region was shown to occur contemporaneously with segregating origins of chromosomal replication (*oriC*) and likely shares a common cell cycle cue.

The diffusive properties of exported IcsA in outer-membrane have also been investigated, addressing whether the protein diffuses in the outer membrane or is masked by LPS. To directly observe the behaviour of IcsA soon after it appears at the cell surface, a strategy exploiting metabolic biotinylation was developed and used to rapidly and specifically label nascent IcsA in the outer membrane. In further investigation of the IcsA-LPS interplay, the profile of polar LPS was shown to be uniform in comparison to the lateral cell body in *S. flexneri*. Reciprocal co-purification presented biochemical evidence that confirmed IcsA-IcsA interactions in the outer-membrane, supporting functional oligomerisation of IcsA in the outer-membrane.

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CONTENTS

| | |
|---|----------|
| I INTRODUCTION AND METHODS | 1 |
| 1 INTRODUCTION | 3 |
| 1.1 Introduction | 3 |
| 1.2 <i>Shigella</i> | 4 |
| 1.2.1 Pathogenesis | 4 |
| 1.3 The IcsA autotransporter | 8 |
| 1.3.1 Activation of N-WASP in actin-based motility | 9 |
| 1.3.2 A target of Autophagy | 10 |
| 1.4 IcsA structure and functional domains | 11 |
| 1.4.1 The extended IcsA signal sequence | 11 |
| 1.4.2 The effector domain | 12 |
| 1.4.3 N-WASP and vinculin binding regions | 13 |
| 1.4.4 IcsP cleavage site | 14 |
| 1.4.5 IcsB and Atg5 binding region | 15 |
| 1.4.6 Phosphorylation region | 16 |
| 1.4.7 Polarity determining regions | 16 |
| 1.4.8 Autochaperone regions | 16 |
| 1.4.9 The translocation domain | 17 |
| 1.5 IcsA synthesis and export | 17 |
| 1.5.1 Cytoplasmic translocation | 17 |
| 1.5.2 Periplasmic transit | 18 |
| 1.5.3 Crossing the outer membrane | 19 |
| 1.6 The interplay between lipopolysaccharide and IcsA | 22 |
| 1.6.1 Lipopolysaccharide | 22 |
| 1.6.2 The IcsA-LPS relationship | 22 |
| 1.7 Bacterial cell biology and the cell cycle | 26 |
| 1.7.1 Staying in shape: MreB, the actin homologue | 26 |
| 1.7.2 The FtsZ tubulin homologue | 28 |
| 1.7.3 Placing the Z ring: the oscillating Min system | 29 |
| 1.7.4 Chromosome replication | 32 |

| | | |
|-------|---|----|
| 1.8 | IcsA polar targeting | 35 |
| 1.8.1 | Multiple pathways for polar targeting | 36 |
| 1.8.2 | Scrutinising the role of the bacterial cytoskeleton | 37 |
| 1.8.3 | Other factors implicated in IcsA polar localisation | 38 |
| 1.8.4 | Genetic and proteomic approaches to polar targeting | 39 |
| 1.9 | Aims of this work | 39 |
| 2 | MATERIALS AND METHODS | 41 |
| 2.1 | Chemicals and reagents | 41 |
| 2.1.1 | Antibodies and antisera | 41 |
| 2.2 | Bacterial strains and plasmids | 41 |
| 2.3 | Bacterial growth media | 42 |
| 2.3.1 | Liquid growth media | 42 |
| 2.3.2 | Solid growth media | 42 |
| 2.3.3 | Antibiotics and Congo Red solution | 43 |
| 2.4 | Maintenance of bacterial strains | 43 |
| 2.4.1 | General | 43 |
| 2.5 | Nucleic acid methods | 43 |
| 2.5.1 | Isolation of bacterial DNA | 43 |
| 2.6 | Analysis of DNA | 44 |
| 2.6.1 | DNA quantitation | 44 |
| 2.6.2 | Restriction endonuclease digestion of DNA | 44 |
| 2.6.3 | Agarose gel electrophoresis | 44 |
| 2.6.4 | Calculation of DNA fragment length | 45 |
| 2.6.5 | DNA sequencing | 45 |
| 2.7 | DNA amplification | 46 |
| 2.7.1 | Synthesis of oligodeoxynucleotides | 46 |
| 2.7.2 | Polymerase chain reaction (PCR) | 46 |
| 2.8 | DNA purification | 46 |
| 2.8.1 | DNA gel extraction | 46 |
| 2.8.2 | Purification of PCR products | 47 |
| 2.9 | Manipulation of DNA | 47 |
| 2.9.1 | Oligodeoxynucleotide Annealing for insertions | 47 |
| 2.9.2 | Phosphorylation of Annealed Oligodeoxynucleotides | 48 |
| 2.9.3 | Shrimp Alkaline Phosphatase (SAP) treatment | 48 |

| | | |
|--------|---|----|
| 2.9.4 | Ligation of DNA fragments into cloning vectors | 48 |
| 2.10 | Transformation procedures | 49 |
| 2.10.1 | Preparation of chemically competent <i>E. coli</i> | 49 |
| 2.10.2 | Transformation of chemically competent <i>E. coli</i> | 49 |
| 2.10.3 | Preparation of electrocompetent <i>S. flexneri</i> and <i>E. coli</i> | 49 |
| 2.10.4 | Electroporation of <i>S. flexneri</i> and <i>E. coli</i> | 50 |
| 2.10.5 | Conjugation | 50 |
| 2.11 | Construction of chromosomal mutations | 50 |
| 2.11.1 | Allelic-exchange mutagenesis using the λ Red phage mutagenesis system | 50 |
| 2.11.2 | Transduction using P1vir phage | 51 |
| 2.12 | Protein techniques | 53 |
| 2.12.1 | Preparation of whole-cell lysates | 53 |
| 2.12.2 | SDS-PAGE | 53 |
| 2.12.3 | Coomassie blue staining | 53 |
| 2.12.4 | Western transfer and detection | 54 |
| 2.12.5 | Purification of outer membrane protein oligomers | 54 |
| 2.12.6 | Indirect immunofluorescence of whole bacteria | 56 |
| 2.13 | Minicell purification | 56 |
| 2.13.1 | Purification of minicells by sucrose gradients | 56 |
| 2.14 | Lipopolysaccharide techniques | 58 |
| 2.14.1 | Preparation of LPS samples | 58 |
| 2.14.2 | Analysis of LPS by silver-stained SDS-PAGE | 58 |
| 2.15 | Chemical cross-linking | 59 |
| 2.15.1 | DSP cross-linking | 59 |
| 2.16 | Tissue culture | 59 |
| 2.16.1 | Maintenance of cell lines | 59 |
| 2.16.2 | Plaque assays | 60 |
| 2.17 | Microscopy & imaging | 60 |
| 2.17.1 | Mounting medium | 60 |
| 2.17.2 | Mounting for live bacterial imaging | 61 |
| 2.17.3 | Microscopy | 61 |
| 2.17.4 | Automated image analysis | 62 |
| 2.17.5 | Flow cytometry | 62 |

| | |
|---|------------|
| II RESULTS | 65 |
| 3 POLAR TARGETING DOMAINS OF ICSA | 67 |
| 3.1 Introduction | 67 |
| 3.2 GFP+ fusions | 67 |
| 3.3 Polar targeting mutants | 71 |
| 3.3.1 Protein concentration-dependent defective polar targeting | 73 |
| 3.4 Refining the polar targeting region | 75 |
| 3.5 Summary | 79 |
| 4 POLAR TARGETING AND THE REPLICATING CHROMOSOME | 81 |
| 4.1 Constructing a plasmid for visualising IcsA polarity and <i>oriC</i> -motion in live <i>E. coli</i> | 81 |
| 4.2 Examining polar targeting and <i>oriC</i> -motion | 83 |
| 4.3 Verifying the specificity of IcsA and <i>oriC</i> co-localisation | 84 |
| 4.3.1 Filamented cells | 88 |
| 4.3.2 MreB inhibition | 89 |
| 4.4 Overexpressing the centromere-like <i>migS</i> sequence | 90 |
| 4.5 Summary | 92 |
| 5 SURFACE DISTRIBUTION OF LPS MODAL LENGTHS IN <i>shigella flexneri</i> | 95 |
| 5.1 Introduction | 95 |
| 5.2 Creation of a <i>S. flexneri</i> minicell mutant | 96 |
| 5.3 Minicell purification and analysis | 99 |
| 5.3.1 Distribution of Wzz proteins | 99 |
| 5.3.2 Distribution of LPS | 101 |
| 5.4 Summary | 102 |
| 6 ICOSA BEHAVIOUR IN THE OUTER MEMBRANE | 105 |
| 6.1 Tagging IcsA by metabolic biotinylation | 105 |
| 6.2 Unipolarity is preserved in BIO-tagged IcsA | 108 |
| 6.3 BIO-tagged IcsA remains functional | 110 |
| 6.4 Controlled expression of BIO-tagged IcsA | 110 |
| 6.5 Creation of smooth UT5600 for expression of BIO-tagged IcsA | 112 |
| 6.6 Improving biotinylation of BIO-tagged IcsA | 114 |
| 6.7 Detection of nascent BIO-tagged IcsA in the outer-membrane | 116 |
| 6.8 Rapid single molecule detection of nascent BIO-tagged IcsA | 117 |
| 6.9 Summary | 118 |

| | | |
|-------|--|-----|
| 7 | FUNCTIONAL OLIGOMERISATION OF EXPORTED ICsA | 121 |
| 7.1 | Surface IcsA expression in negative dominant strains | 122 |
| 7.2 | Reciprocal co-purification confirmed IcsA oligomerisation | 123 |
| 7.3 | Defining the region mediating IcsA-IcsA interaction | 125 |
| 7.4 | Summary | 128 |
| | | |
| III | DISCUSSION | 129 |
| 8 | DISCUSSION | 131 |
| 8.1 | The polar targeting of IcsA | 132 |
| 8.1.1 | The amino terminal proximal polar targeting region | 132 |
| 8.1.2 | The amino terminal distal polar targeting region and its mutants | 133 |
| 8.1.3 | A model: aggregating towards the pole | 133 |
| 8.1.4 | Refinement of the polar targeting domain | 135 |
| 8.2 | IcsA polarity and the replicating chromosome | 136 |
| 8.3 | IcsA within the outer membrane | 139 |
| 8.3.1 | The LPS modal length distribution | 139 |
| 8.3.2 | A strategy for tracking nascent IcsA | 140 |
| 8.3.3 | IcsA oligomerisation | 141 |
| 8.4 | Concluding remarks | 143 |
| | | |
| IV | APPENDICES | 145 |
| A | APPENDIX A | 147 |
| B | APPENDIX B | 151 |
| C | APPENDIX C | 153 |
| D | APPENDIX D | 155 |
| E | APPENDIX E | 157 |
| F | APPENDIX F | 159 |
| G | APPENDIX G | 161 |
| G.1 | List of donated and laboratory stock bacterial strains | 161 |
| G.2 | List of Bacterial strains generated during this work | 162 |
| | | |
| | BIBLIOGRAPHY | 177 |

ABBREVIATIONS

| | |
|--------|-------------------------------------|
| ABM | actin-based motility |
| ADP | adenosine diphosphate |
| ATP | adenosine triphosphate |
| ATPase | adenosine triphosphatase |
| cDNA | complementary DNA |
| CFU | colony-forming units |
| Da | Dalton |
| DNA | deoxyribonucleic acid |
| DNase | deoxyribonuclease |
| dsDNA | double-stranded DNA |
| ECFP | enchanced cyan fluorescent protein |
| EYFP | enhanced yellow fluorescent protein |
| GFP | green fluorescent protein |
| GTP | guanosine triphosphate |
| GTPase | guanosine triphosphatase |
| HRP | horse radish peroxidase |
| kb | kilobase |
| mRNA | messenger RNA |
| nt | nucleotide |
| Oag | O-antigen |

| | |
|-------|---------------------------------|
| PCR | polymerase chain reaction |
| PFU | plaque-forming units |
| RNA | ribonucleic acid |
| RNase | ribonuclease |
| rRNA | ribosomal RNA |
| Tris | tris(hydroxymethyl)aminomethane |
| U | units |