

Polarity and Secretion of *Shigella flexneri*

IcsA: A Classical Autotransporter

MATTHEW THOMAS DOYLE,
B. SC. (BIOTECHNOLOGY)



THE UNIVERSITY
of ADELAIDE

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Department of Molecular and Cellular Biology

School of Biological Sciences

The University of Adelaide

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Declaration

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Abstract

The classical autotransporter IcsA is an essential virulence factor for the enteropathogen *Shigella flexneri* as it provides adherence properties and allows intra- and intercellular spreading in the colonic mucosa. IcsA is an outer membrane surface protein that specifically hijacks host-cell actin recruiting and polymerising complexes allowing actin polymerisation as a form of actin-based motility (ABM). Importantly, since IcsA is localised specifically at one end of the bacterium (the pole), the resulting ABM is unidirectional which is a requirement for efficient *S. flexneri* dissemination. However, the molecular mechanisms that generate IcsA polarity remain poorly understood. Furthermore, IcsA is a member of the autotransporter family of secreted virulence factors (Type Va). Although many steps in the autotransporter pathway have been elucidated, it is still poorly understood how these diverse proteins are efficiently translocated to the bacterial cell surface. As such, this thesis investigates the two arms of IcsA biogenesis: (1) polar targeting and (2) autotransport.

Regarding IcsA polarity, it was identified here that the IcsA-specific outer membrane protease IcsP localises to the septa of dividing *S. flexneri* and to the opposing pole relative IcsA. The concentration of IcsP was higher at the septum than the pole showing a life cycle dependent distribution of IcsP. This provides the basis of a model where IcsP is important during division of *S. flexneri* for setting up (and the continued maintenance of) IcsA polarity by the proteolysis of misdirected IcsA. Further, multiple previous reports have suggested that the *S. flexneri* lipopolysaccharide O-antigen surface structure can influence IcsA polarity by augmenting membrane fluidity or by asymmetric masking of IcsA surface exposure. These notions were tested here resulting in data that clearly refutes these models and argues simply that IcsA exposure is masked symmetrically over the bacterial cell surface. Finally, IcsA itself contains polar targeting (PT) regions that direct it to the pole by an, as yet, unclear mechanism. Examination of these regions revealed that the central PT (cPT) is most important in polarity augmentation and contains critical polarity targeting function residues.

Regarding IcsA autotransport, a highly conserved but uncharacterised autotransporter motif was scrutinized for potential biogenesis functions and was designated in this work as the passenger-associated transport repeat (PATR). It was found that the PATR plays a critical role in the efficient secretion of IcsA to the cell surface. Strikingly, bioinformatics analyses revealed that the PATR delineates an important separate autotransporter sub-type with unique functions, composition, and architecture.

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For Mum, who protected me’

Thesis Style and Layout

This Thesis is submitted in the style of a ‘Thesis by Publication’. As such, the results chapters are replaced by four Research Articles (In chronological publishing order Chapters 3 to 6). In Chapter 1: ‘Introduction’, the research is framed and the Aims of my Doctoral studies are outlined. Chapter 2 outlines the base ‘Materials and Methods’ used throughout candidature, with nuances and changes specific to each article described in the methods subsection of the respective publication.

Author contributions for each publication are stated in preceding ‘Statements of Authorship’, and the objectives of the article with respect to my thesis are outlined in ‘Purpose of Article’ subheadings. Article chapters retain the section order layout style of the journal publisher. Subheadings starting with ‘Article ...’ indicate subdivisions that were included in the publication. Article supplementary data is included in this thesis (and [S] after the figure or table number). Please note that formatting may be slightly different between articles (i.e. US versus Australian spelling). This is a result of complying with the specific journal requirements. Important data that was collected in relation to a publication (but was not included in the article due to its peripheral nature) are marked with ‘Additional Results’ subheadings (and [A] after the figure number).

Chapter 7: ‘Conclusions’ draws together the outcomes of the presented publications, argues how the data aligns with the current mechanisms and models, and describes how this research leads to intriguing questions for future investigations.

Publications

Peer Reviewed Research Articles

Tran ENH*, **Doyle MT***, Morona R (2013) LPS unmasking of *Shigella flexneri* reveals preferential localisation of tagged outer membrane protease IcsP to septa and new poles. *PLoS ONE* 8(7): e70508. (* equal authorship)

Doyle MT, Grabowicz M, May KL, Morona R (2015) Lipopolysaccharide surface structure does not influence IcsA polarity. *FEMS Microbiology Letters* 362 (8), fnv042

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Doyle MT, Grabowicz M, Morona R (2015) A small conserved motif supports polarity augmentation of *Shigella flexneri* IcsA. *Microbiology-SGM* doi: 10.1099/mic.0.000165.

Abbreviations

a.a.	amino acid	PAP2	type 2 phosphatidic acid phosphatase
ABM	actin-based motility	PATR	passenger-associated transport repeat
Ap ^R	ampicillin resistance cassette	PbH1	parallel β -helix type 1
AT	autotransporter	PBS	phosphate buffered saline
BAM	β -Barrel Assembly Machinery	PCR	polymerase chain reaction
BSG	buffered saline gelatin	PGN	peptidoglycan
CM	cytoplasmic membrane	PK	proteinase K
CmI ^R	chloramphenicol resistance cassette	PL	pertactin-like
cPT	central polar targeting region	PLL	pectin lyase like
DMEM	Dulbecco's modified eagles medium	PMBN	polymixin B nonapeptide
DNA	deoxyribonucleic acid	PMN	polymorphonuclear leukocytes
dNTP	deoxyribonucleotide triphosphates	PMSF	phenylmethanesulfonyl fluoride
D-PBS	Dulbecco's phosphate buffered saline	POMP	polymorphic outer membrane protein
DSP	dithiobis(succinimidyl propionate)	POTRA	polypeptide transport associated
DTRM	domain targeted random mutagenesis	PT	polar targeting region
ESS	extended signal sequence	QC	QuikChange mutagenesis
F-actin	filamentous actin	QD	quantum dot
FAE	follicle-associated epithelia	RBS	ribosome binding site
FCS	foetal calf serum	RDA	randomly distributed aggregate
<i>g</i>	force of gravity	R-LPS	rough lipopolysaccharide
GA	Gibson assembly	RNA	ribonucleic acid
GFP	green fluorescent protein	SD	standard deviation
GL	grey level	SDS	sodium dodecyl sulfate
GRR	glycine rich repeats	SEM	standard error of the mean
GST	glutathione S-transferase	ShET	<i>Shigella</i> enterotoxin
HRP	horse radish peroxidase	^S LPS	short type lipopolysaccharide
HS	hot-spot	S-LPS	smooth lipopolysaccharide
IF	immunofluorescence	SOE	splicing by overlap extension
IM	inner membrane	SPATE	serine protease AT of the enterobacteriae
IPTG	isopropyl- β -D-thiogalactopyranoside	SRL	<i>Shigella</i> resistance locus
kb	kilobase	SRP	signal recognition particle
kDa	kilodalton	SS	signal sequence
Km ^R	kanamycin resistance cassette	T3SS	Type III secretion system
LB	Luria Bertani broth / Lysogeny broth	TBS	tris buffered saline
LPA	large polar aggregate	TCA	trichloroacetic acid
LPS	lipopolysaccharide	Tc ^R	tetracycline resistance cassette
MEM	modified eagles medium	TS	tryptic soy
MQ	MilliQ H ₂ O	TSP	tail-spike protein
mRNA	messenger RNA	TTBS	tris buffered saline plus tween
Mw	molecular weight	v/v	volume per volume
NC	non-cleavable	VC	vacuolating cytotoxin
nPT	N-terminal polar targeting region	^{VL} LPS	very long type lipopolysaccharide
ns	not significant	VP	virulence plasmid
N-WASP	Neural Wiskott Aldrich Syndrome Protein	w/v	weight per volume
OA	oligo annealing	WIP	WASP inhibiting protein
Oag	O-antigen	WM	whole membrane
OD ₆₀₀	optical absorbance at 600 nm	WT	wild-type
OM	outer membrane	X-gal	5-bromo-5-chloro-3-indolyl- β -D-galactoside
OMP	outer membrane protein	β -ME	β -mercaptoethanol
PAGE	polyacrylamide gel electrophoresis		

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