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Biogenesis of *Shigella flexneri* IcsA Protein

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Declaration

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Marcin Grabowicz

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

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