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**Biochemical and Molecular
Characterisation of FliI and FliH from
*Helicobacter pylori***

**A thesis presented in partial fulfilment
of
Doctor of Philosophy in Microbiology**

**at the Institute of Molecular BioSciences,
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Abstract

The bacterium *Helicobacter pylori* is a human pathogen that infects a large proportion of the world's population and is associated with serious diseases such as gastric ulcers and adenocarcinoma. The motility of this organism, by virtue of sheathed polar flagella is essential to colonisation and persistence in the human host.

The sequencing of the *H. pylori* genome in 1996 identified homologues of the majority of the flagellar genes found in *S. enterica* serovar *typhimurium*. These included genes encoding the flagellum ATPase, FliI and FliH a presumptive inhibitor, the primary focus of this study. Sequencing did not originally identify an *H. pylori* homologue of the flagellar chaperone FliJ, and this is also considered in this study.

Bioinformatic analysis and modeling suggests a structural and functional relationship between FliI and homologues such as F₁-ATPase α - and β -subunit. In particular, residues 2-91 of FliI resemble the N-terminal domain of the F₁-ATPase α - and β -subunits. Biochemical analyses reported in this thesis showed that a truncated FliI-(2-91) protein was folded, although the N-terminal 18 residues were likely unstructured. Furthermore, deletion mutagenesis showed that this disordered segment of the protein mediates interaction with FliH and very likely forms an amphipathic α -helix upon forming of the FliI-FliH complex. The scanning mutagenesis of this interaction segment of FliI identified a cluster of conserved hydrophobic residues that was critical for the interaction with FliH. Thus, the interaction between FliI and FliH has similarities to the interaction between the N-terminal α -helix of the α -subunit and the globular domain of the δ -subunit of the F₁-ATPase. This similarity suggests that FliH, by analogy with the δ -subunit of the F₁-ATPase, may function as a molecular stator of the flagellum. The findings presented above have been published (96).

The function of a putative *H. pylori* FliJ homologue, HP0256, was also investigated by knock-out mutagenesis. Disruption of this gene does not abolish flagellar assembly, however further research continued beyond this thesis showed that the knock-out mutant results in impaired motility.

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List of Abbreviations

ADP	adenosine diphosphate
AMP	adenosine monophosphate
AMP-PNP	adenylyl imidodiphosphate
Ap	Ampicillin
APS	ammonium persulphate
ATP	adenosine triphosphate
BLAST	Basic Local Alignment Search Tool
BSA	bovine serum albumin
CAPS	3-(cyclohexylamino)-1-propanesulfonic acid
CD	Circular dichroism
Cm	Chloramphenicol
<i>colE1</i>	colicin E1
DLS	Dynamic light scattering
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
FP	forward primer
FPLC	Fast protein liquid chromatography
FSB	final sample buffer
GSP	general secretory pathway
GEP	general export pathway
GST	Glutathione-S-transferase
HEPES	N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid
HIC	Hydrophobic interaction chromatography
IEC	Ion exchange chromatography
IPTG	isopropyl- β -D-galactoside
Kan	Kanamycin
LB	Luria-Bertani broth
LBA	Luria-Bertani broth agar
MALT	mucosa-associated lymphoid tissue
MCS	multiple cloning site
na	not applicable
NCBI	National Centre for Biotechnology Information
OD	Optical density
PBS	Phosphate buffered saline
PCR	polymerase chain reaction
PHI-BLAST	Pattern Hit Initiated BLAST
pI	isoelectric point
RP	reverse primer
Rpm	revolutions per minute
PSI-BLAST	Position Specific Iterative BLAST
RT	room temperature

SDS sodium dodecyl sulphate
SDS-PAGE Sodium dodecyl sulphate-Polyacrylamide gel electrophoresis
SEC Size exclusion chromatography
T3SSs Type III secretion systems
T4SSs Type IV secretion systems
TEMED N,N,N',N'-Tetramethylethylenediamine
TIGR The Institute for Genomic Research
Tris Tris(hydroxymethyl)aminomethane hydrochloride
TSB tryptic soy broth
U unit
w/v weight per volume

Amino acid abbreviations used:

A, Ala, Alanine
C, Cys, Cysteine
D, Asp, Aspartic acid
E, Glu, Glutamic acid
F, Phe, Penylalanine
G, Gly, Glycine
H, His, Histidine
I, Ile, Isoleucine
K, Lys, Lysine
L, Leu, Leucine
M, Met, Methionine
N, Asn, Asparagine
P, Pro, Proline
Q, Gln, Glutamine
R, Arg, Arginine
S, Ser, Serine
T, Thr, Threonine
V, Val, Valine
W, Trp, Tryptophan
Y, Tyr, Tyrosine

Deoxyribonucleosides: A, deoxyadenylate, C, deoxycytidylate, G, deoxyguanylate, T, deoxythymidylate

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