The role of the FtsA protein in *Bacillus subtilis* cell division

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Certificate of Authorship/Originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the written preparation of the thesis, and all experimental work associated with it has been carried out solely by me, unless otherwise indicated.

Finally, I certify that all information sources and literature used are acknowledged in the text.

Joana Santos, October 2011

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Publications

J. Santos and E. J. Harry (2012). The role of the FtsA protein in *Bacillus subtilis* cell division (manuscript in preparation).

Conference proceedings

J. Santos and E. J. Harry – July, 2010 – Annual Scientific Meeting & Exhibition of the Australian Society of Microbiology – Sydney, Australia – **Oral Presentation** – Bacterial Cell Division: an "early" protein acting at a "late" stage.

J. Santos and E. J. Harry – November, 2009 – Light in Life Sciences Conference of Fluorescent Applications in Biotechnology and Life Sciences' (FABLS) Network – Melbourne, Australia – **Poster Presentation –** Life cell imaging: a fluorescent look into the function of a bacterial protein, in *Bacillus subtilis*.

J. Santos and E. J. Harry – July, 2009 – Prokaryotic Development Conference of the American Society of Microbiology – Cambridge, Massachusetts, USA – **Poster Presentation** – Unravelling the function of the bacterial cell division protein FtsA, in *Bacillus subtilis*.

J. Santos and E. J. Harry – July, 2008 – Annual Scientific Meeting & Exhibition of the Australian Society of Microbiology – Melbourne, Australia – **Oral Presentation** – The role of FtsA protein in *Bacillus subtilis* cell division.

J. Santos, A. Porta Cubas, and E. J. Harry – November, 2007 – Royal North Shore Hospital Annual Meeting – Sydney, Australia – **Poster Presentation** – Unravelling the role of an Actin-like bacterial cell division protein.

Abbreviations

A(x)	absorbance (where x = wavelength in nanometres)
A	alanine
aa	amino acid
Ab	antibody
B.	*Bacillus*
β	beta
bp	base pair(s)
BP	band pass
BSA	bovine serum albumin
cm	centimetres
Cm^R	chloramphenicol resistance
DAPI	4'6-diamidino-2-phenylindole
DNA	deoxyribonucleic acid
DTT	dithiothreitol
dTTP	deoxythymidine 5‟-triphosphate
E.	*Escherichia*
ECT	electron cryotomography
ECL	enhanced chemiluminescence
ermC	erythromycin resistance gene
et al.	and others
FITC	fluorescein isothiocyanate
FRAP	fluorescence recovery after photobleaching
FRET	fluorescence energy resonance transfer
fts	filamentation temperature sensitive
g	centrifugal force
g	gram(s)
GFP	green fluorescent protein
GMD	germination medium defined
GTP	guanosine 5′-triphosphate
h	hour(s)
IFM	immunofluorescence microscopy

Ig	Immunoglobulin
IPTG	isopropyl-1-thio-β-D-galactopyranoside
kD	kilo Dalton(s)
L	litre(s)
LP	long pass
M	milli- (10-3)
M	moles per litre
min	minute(s)
MQW	Milli-Q purified water
MSA	mineral salts A
MTS	membrane targeting sequence
N	nano- (10-9)
NA	numerical aperture
N/A	not applicable
Neo	neomycin resistance gene
NO	nucleoid occlusion
ODx	optical density at (x refers to the wavelength in nm)
P	probability
Pspac	IPTG-inducible promoter
Pxyl	xylose-inducible promoter
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
Phleo	phleomycin resistance gene
PCR	polymerase chain reaction
pH	power of Hydrogen
PSF	point spread function
RNA	ribonucleic acid
RNase	ribonuclease A
ROW	reverse osmosis purified water
rpm	revolutions per minute
S.	*Streptomyces*
sec	second(s)
SDS	sodium dodecyl sulfate
SEM	standard error of the mean

SMM	spizizen minimal medium
spp.	species
spec	spectinomycin
T	thymine
TBAB	tryptose blood agar base
TDE	2,2'-thiodiethanol
TEMED	N,N,N‘,N‘-tetramethyl-ethylenediamine
tet	tetracycline
thy-	thymine auxotroph
Tris	tris(hydroxymethyl)methylamine
Trp	L-Tryptophan
ts	temperature sensitive
U	units (enzyme activity)
UV	ultraviolet
V	volt(s)
v/v	volume per volume
W	watt
w/v	weight per volume
YFP	yellow fluorescent protein
2D	2-dimensional
3D	3-dimensional
µ	micro- (10-6)

Abstract

Bacterial cell division involves the invagination of the membrane and the cell wall to form a septum at midcell, between two replicated chromosomes. From a molecular perspective, the main event in cell division is the formation of a circumferential structure, the Z ring, formed by polymerisation of the tubulin-like FtsZ protein. The Z ring recruits a multi-protein complex to the division site, forming a division apparatus that eventually constricts as the septum forms. FtsA, a eukaryotic actin homologue, is another division protein, known to interact directly with FtsZ. It has been proposed that FtsA promotes Z ring formation; however its exact role has remained unknown. This thesis investigateshow FtsA affects the Z ring and cytokinesis in the Gram-positive model organism, *Bacillus subtilis*.

Interestingly, FtsA is essential in *Escherichia coli*, the Gram-negative model organism, but not in *Bacillus subtilis*. Rather, deletion of the *ftsA* gene in vegetatively-growing *B. subtilis* cells causes a significant reduction in Z ring formation and cell division is severely diminished while cell growth is maintained, resulting in cell filamentation (long cells without septa). To confirm that this phenotype is due to the inability of FtsZ to efficiently form rings, Z ring formationwas examined in the absence of FtsA, during the first round of cell division following *B. subtilis* spore germination. Surprisingly the Z rings formed with wild-type efficiency. However, unlike wild-type cells that showed subsequent constriction of these Z rings leading to septum formation, Z rings did not constrict immediately in the *ftsA* mutant and persisted into the second cycle of division. These results reveal for the first time that, unlike *E. coli*, FtsA is not required for Z ring formation in *B. subtilis*.

To understand the delay in Z ring constriction, further experiments were conducted to determine if the recruitment of downstream division proteins to the Z ring is affectedin the absence of FtsA. The live-cell microscopy data confirmed that the recruitment of DivIB, and presumably other downstream division proteins that are co-recruited with DivIB, is delayed in *ftsA*-mutant cells, but occurs with wild-type efficiency. However, after recruitment of DivIB, Z ring constriction and septation are still inefficient in the absence of FtsA. These observations indicate a primary role for FtsA in *B. subtilis* in the

later stages of division, that is, after the division apparatus has assembled. This work reveals a novel perspective on the function of this protein.

In an attempt to further explore how Z ring constriction is affected by FtsA, microscopy studies were designed to analyse this cell process. Different Z ring constriction defects were observed in *ftsA*-mutant cells. Importantly, it was shown that, in the absence of FtsA, constriction is either significantly delayed or never occurs, resulting in destabilisation of the Z ring, indicating that FtsA is required for efficient Z ring constriction in *B. subtilis*. This finding raised the possibility that FtsA may be affecting the dynamics of the Z ring during cytokinesis. To verify this, the rate of FtsZ turnover in Z rings of *ftsA*-mutant cells was investigated. The results demonstrated a decrease in the rate of the FtsZ turnover in the Z ring in the absence of FtsA, possibly enough to cause an effect on Z ring constriction.