

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

Joana Maria Da Silva Santos



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The iThree Insitute  
University of Technology, Sydney NSW,  
Australia

## **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.

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Joana Santos, October 2011

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## Contents

<b>Certificate of Authorship/Originality.....</b>	<b>ii</b>
<b>Acknowledgements .....</b>	<b>iii</b>
<b>Contents .....</b>	<b>iv</b>
<b>Figures.....</b>	<b>ix</b>
<b>Tables .....</b>	<b>x</b>
<b>Publications .....</b>	<b>xii</b>
<b>Abbreviations .....</b>	<b>xiii</b>
<b>Abstract.....</b>	<b>xvi</b>
<b>Chapter 1 .....</b>	<b>1</b>
<b>1.1 Preface.....</b>	<b>2</b>
<b>1.2 <i>Bacillus subtilis</i>: A study case .....</b>	<b>3</b>
1.2.1 The vegetative cell cycle .....	4
1.2.2 Sporulation and the spore outgrowth system .....	6
<b>1.3 Cell division .....</b>	<b>6</b>
1.3.1 The FtsZ protein.....	7
1.3.2 The Z ring.....	9
1.3.2.1 The biochemistry of the Z ring.....	9
1.3.2.2 Dynamics of the Z ring .....	11
1.3.3 Cellular localisation of the Z ring .....	13
<b>1.4 Regulation of cell division .....</b>	<b>15</b>
1.4.1 The Min system.....	16
1.4.2 Nucleoid occlusion.....	18
1.4.3 Positioning of Z ring at the division site in <i>B. subtilis</i> .....	19

---

1.4.3.1 The coordination between DNA replication and cell division .....	21
<b>1.5 Proteins affecting Z ring assembly .....</b>	<b>22</b>
1.5.1 ZapA .....	24
1.5.2 SepF .....	25
1.5.3 EzrA .....	26
<b>1.6 The FtsA protein .....</b>	<b>27</b>
1.6.1 FtsA structure and biochemistry .....	28
1.6.2 The interaction of FtsA with FtsZ .....	31
1.6.3 FtsA function in cell division .....	32
1.6.3.1 The role of FtsA in <i>E. coli</i> cell division .....	32
1.6.3.2 FtsA functions in <i>B. subtilis</i> cell division .....	34
<b>Chapter 2 .....</b>	<b>37</b>
<b>2.1 Chemicals, reagents and solutions .....</b>	<b>38</b>
<b>2.2 <i>B. subtilis</i> strains and growth conditions .....</b>	<b>39</b>
2.2.1 Testing the status of the <i>amyE</i> locus of <i>B. subtilis</i> .....	42
2.2.2 Depletion of FtsA using the P <sub>xyt</sub> inducible promoter .....	42
<b>2.3 Preparation and transformation of competent <i>B. subtilis</i> cells ...</b>	<b>43</b>
<b>2.4 Preparation and germination of <i>B. subtilis</i> spores .....</b>	<b>43</b>
<b>2.5 DNA methods .....</b>	<b>44</b>
2.5.1 Extraction and purification of DNA from <i>B. subtilis</i> .....	44
2.5.2 Agarose gel electrophoresis of DNA .....	45
2.5.3 Purification of DNA from agarose gels .....	45
2.5.4 Determination of DNA concentration .....	46
<b>2.6 Microscopy Methods .....</b>	<b>46</b>
2.6.1 Immunofluorescence microscopy (IFM) .....	46
2.6.2 Live cell fluorescence microscopy .....	47

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2.6.2.1 Preparation of cells for nucleoid and membrane visualisation.....	48
2.6.2.2 Time-lapse of live fluorescence cells .....	48
2.6.3 Phase contrast and fluorescence microscopy .....	49
2.6.4 Cell scoring and statistics.....	49
2.6.5 Fluorescence Recovery after Photo-Bleaching (FRAP).....	50
<b>2.7 Protein methods .....</b>	<b>51</b>
2.7.1 Denaturing polyacrylamide gel electrophoresis (SDS-PAGE).....	51
2.7.2 Western blot analysis .....	51
2.7.2.1 Whole cell protein extraction for Western blot analysis .....	52
2.7.2.2 Western transfer .....	52
2.7.2.3 Immunodetection.....	52
<b>2.8 Suppliers of chemicals, reagents and equipment.....</b>	<b>53</b>
<b>Chapter 3 .....</b>	<b>56</b>
<b>3.1 Introduction.....</b>	<b>57</b>
3.1.1 Background on the <i>ftsA</i> story .....	57
3.1.2 Chapter Aims .....	59
<b>3.2 Results .....</b>	<b>60</b>
3.2.1 Characterisation of the <i>ftsA</i> null strain during vegetative growth.....	60
3.2.1.1 Analysis of cellular FtsA levels in <i>ftsA</i> -modified <i>B. subtilis</i> strains .....	64
3.2.2 Z ring formation in the absence of FtsA during spore outgrowth.....	65
3.2.3 Construction of an <i>ftsA</i> in-frame deletion strain (SU506).....	69
3.2.4 Construction of <i>ftsA</i> -complementation strains (SU630 and SU631) .....	73
3.2.5 Complementation of <i>ftsA</i> rescues cell division .....	73
3.2.5.1 <i>ftsA</i> null complementation strain (SU630).....	74
3.2.5.2 <i>ftsA</i> in-frame complementation strain (SU631) .....	74
3.2.6 Outgrowth spore system under FtsA-depletion conditions.....	75
3.2.6.1 Spore outgrowth of the <i>ftsA</i> in-frame complementation strain (SU631).....	75

3.2.7 The Z ring persists at midcell during transient FtsA depletion.....	78
<b>3.3 Discussion .....</b>	<b>81</b>
3.3.1 Why doesn't the absence of FtsA cause complete cell division inhibition? ..	83
3.3.2 The function of FtsA in later stages of cell division? .....	86
<b>Chapter 4 .....</b>	<b>88</b>
<b>4.1 Introduction.....</b>	<b>89</b>
4.1.1 Chapter Aims .....	90
<b>4.2 Results .....</b>	<b>90</b>
4.2.1 Construction of GFP-DivIB fusion strains.....	91
4.2.2 Characterisation of GFP-DivIB fusion strains .....	91
4.2.3 Recruitment and localisation of DivIB in the absence of FtsA.....	94
4.2.4 Septum formation in the absence of FtsA .....	98
<b>4.3 Discussion .....</b>	<b>102</b>
4.3.1 FtsA is not required for DivIB recruitment. What is the primary function of FtsA? .....	103
4.3.1.1 Role of FtsA in recruitment of other downstream divisome proteins? .....	103
4.3.1.2 Role of FtsA in recruitment of later proteins, after DivIB (and related proteins) assembly? .....	104
4.3.1.3 Role of FtsA in Z ring constriction through direct interaction with FtsZ?.....	105
<b>Chapter 5 .....</b>	<b>107</b>
<b>5.1 Introduction.....</b>	<b>108</b>
5.1.1 Overview of the FRAP technique .....	109
5.1.2 Chapter aims.....	110
<b>5.2 Results .....</b>	<b>111</b>
5.2.1 Introducing an FtsZ-GFP fusion protein into an FtsA-depletion background .....	111
5.2.2 Time-lapse microscopy of FtsZ-GFP in FtsA-depleted cells.....	113

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5.2.2.1 Different fates for the Z rings formed in the absence of FtsA .....	116
5.2.4 Z ring dynamics is decreased in the absence of FtsA .....	121
5.2.4.1 FtsZ turnover in the Z ring is slower in the absence of FtsA .....	122
<b>5.3 Discussion .....</b>	<b>125</b>
5.3.1 FtsA may directly affect Z ring constriction by influencing FtsZ dynamics within the ring .....	127
5.3.1.1 Affecting FtsZ turnover during constriction .....	127
5.3.1.2 Tethering the Z ring to the cell membrane during constriction.....	128
5.3.2 FtsA may affect Z ring constriction through effects on the conformation or activity of other divisome proteins.....	130
5.3.2.1 FtsA might be required to recruit MinC and thereby allow Z ring constriction.	130
<b>Chapter 6 .....</b>	<b>133</b>
<b>Supplementary Material .....</b>	<b>141</b>
<b>References.....</b>	<b>142</b>



## Figures

<b>Figure 1.1</b> Electron micrograph of a <i>B. subtilis</i> cell undergoing cell division.....	3
<b>Figure 1.2</b> <i>B. subtilis</i> cell division.....	5
<b>Figure 1.3</b> Z ring localisation .....	9
<b>Figure 1.4</b> FtsZ polymerisation and assembly of the Z ring. ....	10
<b>Figure 1.5</b> Model for FtsZ polymerisation and assembly into a Z ring, during the cell cycle of <i>B. subtilis</i> .....	15
<b>Figure 1.6</b> The Min system in <i>B. subtilis</i> .....	17
<b>Figure 1.7</b> The combined action of the Min system and nucleoid occlusion in Z ring positioning.....	20
<b>Figure 1.8</b> Divisome assembly pathways in <i>B. subtilis</i> and <i>E. coli</i> .....	22
<b>Figure 1.9</b> Network of stabilisers and destabilisers of Z ring formation.....	23
<b>Figure 1.10</b> Alignment sequence of FtsA.....	29
<b>Figure 1.11</b> Crystal structure of FtsA protein from <i>Thermotoga maritima</i> .....	30
<b>Figure 1.12</b> Visualisation of FtsA in <i>B. subtilis</i> cells.....	35
<b>Figure 3.1</b> Genetic constructs of <i>ftsA</i> -modified strains .....	62
<b>Figure 3.2</b> Z ring localisation in the absence of FtsA .....	63
<b>Figure 3.3</b> Western analysis of native FtsA presence in <i>B. subtilis</i> .....	65
<b>Figure 3.4</b> Z ring localisation in the absence of FtsA in outgrown spores.....	68
<b>Figure 3.5</b> Z ring localisation in the new <i>ftsA</i> in-frame deletion strain.....	70
<b>Figure 3.6</b> Z ring localisation in the absence of FtsA in outgrown spores.....	72
<b>Figure 3.7</b> Z ring localisation in <i>ftsA</i> in-frame complementation (SU631) cells during spore outgrowth.....	77
<b>Figure 3.8</b> Z ring localisation in <i>ftsA</i> null complementation after depletion of FtsA. ...	80
<b>Figure 3.9</b> Z ring formation and constriction in wild-type and <i>ftsA</i> mutant <i>B. subtilis</i> cells .....	82
<b>Figure 4.1</b> GFP-DivIB localisation visualised by live cell microscopy .....	94
<b>Figure 4.2</b> GFP-DivIB localisation in SU636 outgrown spores.....	95
<b>Figure 4.3</b> DivIB localisation to midcell is delayed in the absence of FtsA .....	97
<b>Figure 4.4</b> Frequency of cell lengths for septum formation .....	98
<b>Figure 4.5</b> Septum formation in SU636 outgrown spores.....	101
<b>Figure 5.1</b> Schematic illustrating the FRAP technique .....	110
<b>Figure 5.2</b> FtsZ-GFP localisation in the absence of FtsA .....	113

<b>Figure 5.3</b> Time-lapse microscopy of FtsZ–GFP localisation in <i>B. subtilis</i> SU570 ( <i>ftsZ-gfp</i> ) cells. ....	116
<b>Figure 5.4</b> Time-lapse microscopy of FtsZ–GFP localisation in <i>B. subtilis</i> SU638 (FtsA-depleted) cells. ....	117
<b>Figure 5.5</b> Time-lapse microscopy of FtsZ–GFP localisation in <i>B. subtilis</i> SU638 (FtsA-depleted) cells. ....	119
<b>Figure 5.6</b> Time-lapse microscopy of FtsZ–GFP localisation in <i>B. subtilis</i> SU638 (FtsA-depleted) cells. ....	120
<b>Figure 5.7</b> FRAP of a Z ring in control FtsZ-GFP <i>B. subtilis</i> cell .....	122
<b>Figure 5.8</b> Overall FRAP in Z ring fluorescence intensity for <i>B. subtilis</i> cells ( <i>ftsZ-gfp</i> ). .....	123
<b>Figure 5.9</b> FRAP of a Z ring in <i>ftsA</i> -depleted FtsZ-GFP <i>B. subtilis</i> cell. ....	124
<b>Figure 5.10</b> Overall FRAP in Z ring fluorescence intensity for <i>B. subtilis</i> cells ( <i>ftsA</i> - depleted <i>ftsZ-gfp</i> ). ....	125

## Tables

<b>Table 2.1</b> Commonly used aqueous buffers and solutions. ....	38
<b>Table 2.2</b> <i>B. subtilis</i> strains. ....	40
<b>Table 2.3</b> <i>B. subtilis</i> growth media. ....	41
<b>Table 2.4</b> Antibiotics used for selection in <i>B. subtilis</i> . ....	41
<b>Table 2.5</b> Antibodies used for primary and secondary detection for both IFM and western blot analysis. ....	47
<b>Table 2.6</b> Suppliers of chemicals, reagents and equipment. ....	53
<b>Table 3.1</b> Quantitative analysis of FtsA <sup>+</sup> (SU456), <i>ftsA</i> null (SU457), wild-type (SU5) and <i>ftsA</i> in-frame (SU506) cell lengths during mid-exponential vegetative growth. ....	61
<b>Table 3.2</b> Quantitative analysis of FtsA <sup>+</sup> (SU456) and <i>ftsA</i> null (SU457) cells average cell lengths during spore outgrowth. ....	66
<b>Table 3.3</b> Quantitative analysis of wild-type (SU5) and <i>ftsA</i> in-frame deletion (SU506) average cell lengths during spore outgrowth .....	71
<b>Table 3.4</b> Cell lengths and Z ring/ $\mu\text{m}$ at several time points after FtsA depletion, during vegetative growth. Quantitative analysis of wild-type (parental strain, SU456) and <i>ftsA</i>	

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null complementation (SU630) cell lengths ( $\mu\text{m}$ ), with standard error of the mean values (SEM), for sequential time points after depletion of FtsA.....	79
<b>Table 4.1</b> Average cell lengths of SU633 ( <i>gfp-divIB</i> ) and SU636 ( <i>gfp-divIB ftsA</i> compl.) strains.....	93
<b>Table 4.2</b> Frequency of DivIB localisation and septum formation in <i>gfp-divIB ftsA</i> complementation strain (SU636).....	96
<b>Table 5.1</b> Average cell lengths of SU570 ( <i>ftsZ-gfp</i> ) and SU638 ( <i>ftsZ-gfp ftsA::cat P<sub>xyl</sub>-ftsA</i> ) strains.....	112
<b>Table 5.2</b> Frequency of Z ring fates in FtsA-depleted cells (SU638; <i>ftsZ-gfp ftsA::cat P<sub>xyl</sub>-ftsA</i> ), during time-lapse fluorescence microscopy.....	117

## 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
<i>B.</i>	<i>Bacillus</i>
$\beta$	beta
bp	base pair(s)
BP	band pass
BSA	bovine serum albumin
cm	centimetres
Cm <sup>R</sup>	chloramphenicol resistance
DAPI	4'6-diamidino-2-phenylindole
DNA	deoxyribonucleic acid
DTT	dithiothreitol
dTTP	deoxythymidine 5''-triphosphate
<i>E.</i>	<i>Escherichia</i>
ECT	electron cryotomography
ECL	enhanced chemiluminescence
<i>ermC</i>	erythromycin resistance gene
<i>et al.</i>	and others
FITC	fluorescein isothiocyanate
FRAP	fluorescence recovery after photobleaching
FRET	fluorescence energy resonance transfer
<i>fts</i>	filamentation temperature sensitive
<i>g</i>	centrifugal force
g	gram(s)
GFP	green fluorescent protein
GMD	germination medium defined
GTP	guanosine 5'-triphosphate
h	hour(s)
IFM	immunofluorescence microscopy

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Ig	Immunoglobulin
IPTG	isopropyl-1-thio- $\beta$ -D-galactopyranoside
kD	kilo Dalton(s)
L	litre(s)
LP	long pass
M	milli- (10 <sup>-3</sup> )
M	moles per litre
min	minute(s)
MQW	Milli-Q purified water
MSA	mineral salts A
MTS	membrane targeting sequence
N	nano- (10 <sup>-9</sup> )
NA	numerical aperture
N/A	not applicable
<i>Neo</i>	neomycin resistance gene
NO	nucleoid occlusion
OD <sub>x</sub>	optical density at (x refers to the wavelength in nm)
<i>P</i>	probability
<i>Pspac</i>	IPTG-inducible promoter
<i>Pxyl</i>	xylose-inducible promoter
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
<i>Phleo</i>	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
<i>S.</i>	<i>Streptomyces</i>
sec	second(s)
SDS	sodium dodecyl sulfate
SEM	standard error of the mean

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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 <sup>-6</sup> )

## 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 investigates how 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 formation was 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 affected in 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.