

**CELL DIVISION IN *BACILLUS SUBTILIS*:
NEW INSIGHTS FROM AN OLD
MUTANT**

Leigh Graham Monahan

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requirements for the degree of
Doctor of Philosophy



Institute for the Biotechnology of Infectious Diseases
University of Technology, Sydney
NSW, Australia

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Certificate of Authorship/Originality

I certify that the work presented in this thesis has not previously been submitted for a degree, nor has it been submitted as part of the 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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Leigh Monahan

April 2008

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Contents

Certificate of Authorship/Originality	ii
Acknowledgements	iii
Abbreviations	x
Publications	xiii
Abstract	xv
Chapter 1. Introduction	1
1.1 Preface	2
1.2 <i>B. subtilis</i> : An overview	3
1.2.1 Features of the <i>B. subtilis</i> cell	4
1.2.2 The vegetative cell cycle	4
1.2.3 Sporulation	5
1.3 The genes and proteins of bacterial cell division	6
1.4 FtsZ and the Z ring	7
1.4.1 Conservation of FtsZ	9
1.4.2 FtsZ biochemistry	10
1.4.2.1 FtsZ polymerisation	10
1.4.2.2 Higher-order FtsZ polymer formation	12
1.4.3 Z ring architecture and the role of lateral interactions <i>in vivo</i>	13
1.4.4 Cellular localisation of FtsZ	15
1.4.5 FtsZ polymer dynamics, Z ring constriction and the role of GTP	17
1.4.6 A new role for FtsZ in cell morphogenesis	21
1.5 Regulation of Z ring assembly	21
1.5.1 Spatial regulation of Z ring assembly	22
1.5.1.1 The Min system	22
1.5.1.2 The Min system of <i>E. coli</i>	23
1.5.1.3 The Min system of <i>B. subtilis</i>	24
1.5.1.4 Nucleoid occlusion	25
1.5.2 Temporal regulation of Z ring assembly	29
1.5.2.1 Coordination of midcell Z ring assembly with chromosome replication	30
1.5.3 What defines the cell centre?	32

1.5.4	Regulation of Z ring assembly under stress: SulA and the SOS response.....	33
1.5.5	Control of Z ring formation under high nutrient conditions	34
1.5.6	Regulation of Z ring assembly in <i>C. crescentus</i>	35
1.5.7	Other proteins affecting FtsZ assembly	36
1.5.7.1	FtsA	37
1.5.7.2	ZipA	39
1.5.7.3	SepF (YlmF)	40
1.5.7.4	ZapA.....	41
1.5.7.5	EzrA	41
1.5.7.6	ClpX	42
1.5.8	FtsZ accessory proteins form a robust regulatory network in bacteria.....	42
1.6	Project aims.....	43
Chapter 2.	Materials and Methods.....	46
2.1	Chemicals, reagents and solutions	47
2.2	<i>B. subtilis</i> strains and growth conditions	47
2.3	<i>E. coli</i> strains and growth conditions	50
2.4	Plasmids.....	51
2.5	Preparation and transformation of competent cells.....	52
2.5.1	Preparation of competent <i>B. subtilis</i>	52
2.5.2	Transformation of <i>B. subtilis</i>	53
2.5.3	Preparation of competent <i>E. coli</i>	53
2.5.4	Transformation of <i>E. coli</i>	53
2.6	DNA methods	54
2.6.1	Extraction and purification of DNA from bacterial cultures.....	54
2.6.1.1	Small-scale plasmid purification from <i>E. coli</i>	54
2.6.1.2	Large-scale plasmid purification from <i>E. coli</i>	54
2.6.1.3	Purification of chromosomal DNA from <i>B. subtilis</i>	55
2.6.2	Agarose gel electrophoresis of DNA	55
2.6.3	Purification of DNA from agarose gels	56
2.6.4	Determination of DNA concentration.....	56
2.6.5	Enzymatic modification and synthesis of DNA	56
2.6.5.1	Polymerase chain reaction (PCR) and oligonucleotides	56

2.6.5.2	Restriction endonuclease cleavage	57
2.6.5.3	5'-Dephosphorylation of digested vectors	57
2.6.5.4	Vector-insert ligation	58
2.6.6	DNA sequencing	58
2.7	Construction of <i>B. subtilis</i> strains	58
2.7.1	Construction of strains for FtsZ localisation	60
2.7.2	Construction of strains for ZapA overproduction	61
2.7.3	Construction of strains for ZapA localisation	61
2.7.4	Construction of strains for FtsA overproduction	62
2.7.5	Construction of strains for SepF overproduction	62
2.7.6	Construction of <i>ezrA</i> deletion strains	62
2.7.7	Construction of <i>clpX</i> and <i>rho</i> deletion strains	63
2.7.8	Construction of <i>hprT</i> , <i>tig</i> and <i>pyk</i> mutants	63
2.8	Microscopy	64
2.8.1	Preparation of cells for microscopic analysis	64
2.8.1.1	Preparation for the study of cellular morphology and length	64
2.8.1.2	Preparation for live cell fluorescence microscopy	64
2.8.1.3	Preparation for immunofluorescence microscopy	65
2.8.1.4	Preparation of cells for nucleoid visualisation	66
2.8.2	Phase-contrast and fluorescence microscopy	66
2.8.3	Cell scoring and statistics	67
2.9	Protein methods	67
2.9.1	Denaturing polyacrylamide gel electrophoresis (SDS-PAGE)	67
2.9.2	Western blot analysis	68
2.9.2.1	Whole cell protein extraction for Western blot analysis	68
2.9.2.2	Western transfer	68
2.9.2.3	Immunodetection	69
2.10	Quantitation of colony forming units (CFUs) in <i>B. subtilis</i> cultures	69
2.11	Plating efficiency assays	70
2.12	Isolation of <i>tsI</i> suppressor mutants	70
2.13	Suppliers of chemicals, reagents and equipment	72

Chapter 3. Cytological investigation of a temperature sensitive *ftsZ* mutant of

<i>B. subtilis</i>	74
3.1 Introduction	75
3.1.1 A history of the <i>tsI</i> mutant	75
3.1.2 Chapter aims	80
3.2 Results	80
3.2.1 Construction of FtsZ-YFP fusion strains	80
3.2.2 Characterisation of FtsZ-YFP strains.....	83
3.2.2.1 SU489 [FtsZ(Ts1)-YFP] cells are temperature sensitive for division in the absence of inducer	83
3.2.2.2 Optimising the conditions for FtsZ-YFP visualisation	85
3.2.2.3 FtsZ-YFP induction has no discernable phenotypic effects	86
3.2.3 FtsZ localisation in live <i>tsI</i> cells: FtsZ(Ts1) forms helical structures at 49°C ..	87
3.2.4 FtsZ(Ts1) helices are real, not an artefact of the YFP fusion	91
3.2.5 The FtsZ(Ts1) protein is able to interact with wild-type FtsZ at high temperatures and has a dominant effect on its polymerisation	94
3.2.5.1 Analysis of the cellular FtsZ(Ts1)-YFP concentration required for dominance at 49°C	99
3.2.6 FtsZ(Ts1) helices can reorganise into rings upon a shift down to permissive temperatures	101
3.2.7 The localisation of FtsZ(Ts1) helices at 49°C is dictated by nucleoid occlusion	104
3.3 Discussion	110
3.3.1 Dynamic helices and rings: A new model for Z ring assembly	111
3.3.2 The FtsZ(Ts1) helix: A trapped intermediate in the Z ring assembly pathway?	114
3.3.3 An insight into nucleoid occlusion	117
3.3.4 Biochemical analysis of the FtsZ mutation responsible for <i>tsI</i> thermosensitivity	118
3.3.5 What is the specific defect of the FtsZ(Ts1) protein <i>in vivo</i> ?	119
3.3.5.1 Is FtsZ(Ts1) abnormal in end-to-end polymerisation?	120
3.3.5.2 Is FtsZ(Ts1) deficient in membrane attachment?	120

3.3.5.3 Is FtsZ(Ts1) deficient in accessory protein interactions?.....	121
3.3.5.4 Is FtsZ(Ts1) deficient in lateral protofilament interactions?.....	121
Chapter 4. Lateral FtsZ interactions and the assembly mechanism of the Z ring ...	122
4.1 Introduction.....	123
4.2 Results	124
4.2.1 ZapA overproduction suppresses the temperature sensitivity of <i>tsI</i>	124
4.2.2 ZapA overproduction rescues Z ring formation in <i>tsI</i>	127
4.2.3 Some non-ring FtsZ(Ts1) assemblies can also facilitate cell division at 49°C upon ZapA overproduction	128
4.2.4 Wild-type cells are unaffected by an increase in cellular ZapA levels.....	130
4.2.5 ZapA co-localises with FtsZ(Ts1) at 49°C, whether or not cell division is rescued	130
4.2.6 Time-lapse analysis of Z ring formation and division in ZapA overproducing <i>tsI</i> cells.....	133
4.2.7 <i>tsI</i> cannot be rescued by other FtsZ accessory proteins	136
4.3 Discussion.....	137
Chapter 5. A genetic screen for extragenic suppressors of <i>tsI</i>	145
5.1 Introduction.....	146
5.2 Results	148
5.2.1 Isolation of <i>tsI</i> suppressors by transposon-mediated insertional mutagenesis	148
5.2.1.1 Properties of the transposon delivery plasmid pHV1249.....	148
5.2.1.2 Isolation of suppressor mutants	150
5.2.2 Mapping suppressor mutations onto the <i>tsI</i> chromosome	153
5.2.3 Inactivation of the <i>tig</i> gene alone is sufficient for suppression of <i>tsI</i>	158
5.2.4 Operon structure of the <i>tsI</i> suppressor genes.....	160
5.2.4.1 Operon structure of <i>hprT</i>	161
5.2.4.2 Operon structure of <i>tig</i>	163
5.2.4.3 Operon structure of <i>pyk</i>	164
5.2.4.4 Operon structure of <i>rho</i>	164
5.2.5 Deletion of the <i>rho</i> coding sequence suppresses <i>tsI</i> thermosensitivity	164
5.2.6 Validation of suppressor mutations in the <i>hprT</i> , <i>tig</i> and <i>pyk</i> genes.....	165

5.3 Discussion	168
5.3.1 Proteins encoded by the <i>hprT</i> , <i>tig</i> , <i>pyk</i> and <i>rho</i> genes may have more than one <i>in vivo</i> function	169
5.3.2 How do the suppressor mutations rescue the <i>tsI</i> defect?	170
5.3.3 Alternative mechanisms for the suppression of <i>tsI</i> thermosensitivity	171
5.3.3.1 Suppression of <i>tsI</i> via effects on gene expression or macromolecular crowding?	171
5.3.3.2 Suppression of <i>tsI</i> through the physiological response to stress?	172
5.3.3.3 <i>tsI</i> , glycolysis, and the regulation of the bacterial cell cycle	173
5.3.4 Future directions	175
Chapter 6. General Discussion	177
References	185

Abbreviations

A _(x)	absorbance (where x = wavelength in nanometres)
Ap ^R	ampicillin resistance marker
AR	analytical reagent
ATP	adenosine 5'-triphosphate
<i>B.</i>	<i>Bacillus</i>
BP	band pass
bp	base pair(s)
BSA	bovine serum albumin
<i>C.</i>	<i>Caulobacter</i>
CCD	charge-coupled device
CFU	colony forming unit
Cm ^R	chloramphenicol resistance marker
Da	dalton(s)
DAPI	4'6-diamidino-2-phenylindole
dATP	deoxyadenosine 5'-triphosphate
dCTP	deoxycytosine 5'-triphosphate
DEAE	diethylaminoethyl
dGTP	deoxyguanosine 5'-triphosphate
DNA	deoxyribonucleic acid
dTTP	deoxythymidine 5'-triphosphate
<i>E.</i>	<i>Escherichia</i>
ECL	enhanced chemi-luminescence
ECT	electron cryotomography
EDTA	ethylenediaminetetraacetic acid
EGTA	ethyleneglycol-bis(2-aminoethyl ether)-tetraacetic acid
Em ^R	erythromycin resistance marker
FRAP	fluorescence recovery after photobleaching
<i>fts</i>	filamentous temperature sensitive
g	centrifugal force (× unit gravitational field)
g	gram(s)

GDP	guanosine 5'-diphosphate
GFP	green fluorescent protein
GTP	guanosine 5'-triphosphate
h	hour(s)
HPUra	6-(<i>p</i> -hydroxyphenylazo)-uracil
IFM	immunofluorescence microscopy
IPTG	isopropyl-1-thio- β -D-galactopyranoside
k	kilo- (10^3)
kb	kilobase(s)
LP	long pass
m	milli- (10^{-3})
<i>M.</i>	<i>Methanococcus</i>
M	moles per litre
MCS	multiple cloning site
min	minute(s)
mol	mole(s)
MOPS	3-(<i>N</i> -morpholino)propanesulfonic acid
MQW	Milli-Q [®] purified water (Millipore)
mRNA	messenger ribonucleic acid
N	any nucleotide
n	nano- (10^{-9})
NA	not applicable
p	pico- (10^{-12})
<i>P</i>	probability
<i>P.</i>	<i>Prostheobacter</i>
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
P_{spac}	IPTG-inducible promoter
P_{xyl}	xylose-inducible promoter
RNA	ribonucleic acid
ROW	reverse osmosis purified water
<i>S.</i>	<i>Streptomyces</i>

SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEM	standard error of the mean
TBAB	tryptose blood agar base
TBS	tris-buffered saline
TEMED	N,N,N',N'-tetramethyl-ethylenediamine
Tris	tris(hydroxymethyl)methylamine
U	units (enzyme activity)
UV	ultraviolet
v/v	volume per volume
w/v	weight per volume
YFP	yellow fluorescent protein
μ	micro- (10^{-6})

Publications

Journal article

Michie, K. A., **Monahan, L. G.**, Beech, P. L., and Harry, E. J. (2006) Trapping of a spiral-like intermediate of the bacterial cytokinetic protein FtsZ. *Journal of Bacteriology* **188**: 1680-1690.

Book chapter

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Michie, K. A., **Monahan, L. G.**, and Harry, E. J. (September, 2004) Insights into FtsZ from a temperature sensitive mutant of *Bacillus subtilis*. *POSTER*, 46th Annual Meeting of the Australian Society for Microbiology, Sydney, Australia.

Abstract

In bacteria, cell division is mediated by a macromolecular complex consisting of numerous proteins that act together to split the cell into two. The earliest event in this process is the formation of a polymeric ring, composed of the tubulin-like protein FtsZ, at the future site of division. This so-called 'Z ring' plays a pivotal role in the cell division mechanism, at least in part because it serves as a scaffold for the assembly of the division apparatus. Importantly, Z ring formation establishes both when and where the cell will divide, and is therefore subject to stringent spatiotemporal control. This thesis is concerned with the molecular mechanism of Z ring assembly and its regulation in the gram-positive model organism *Bacillus subtilis*. It involves the use of powerful fluorescence microscopy techniques in combination with molecular biological and genetic methods to examine the specific effects of a temperature sensitive FtsZ mutation on Z ring formation *in vivo*. The *B. subtilis* strain harbouring this mutation is known as *tsI*, while the mutant protein itself has been designated FtsZ(Ts1).

Initial experiments examined the intracellular localisation pattern of the FtsZ(Ts1) protein in live cells growing at 49°C (the non-permissive temperature for *tsI*). This work revealed that while FtsZ(Ts1) is unable to form Z rings under non-permissive conditions, it retains the capacity to polymerise *in vivo* and instead assembles into short helical-like structures. Interestingly, these helices were observed to reorganise into fully functional Z rings following a shift from 49°C down to permissive temperatures. These and other observations suggest an exciting new model for Z ring assembly in wild-type bacterial cells, involving a regulated helix-to-ring remodelling of FtsZ polymers. The work also suggests that at non-permissive temperatures, the FtsZ(Ts1) protein is unable to complete the final stages of this remodelling process, and becomes trapped as a short helical intermediate of Z ring formation *in vivo*.

To explore how the FtsZ helix-to-ring assembly mechanism is orchestrated within the cell, further experiments aimed to identify exactly why FtsZ(Ts1) is unable to complete this process at 49°C. During this work, it was discovered that in the presence of elevated levels of the FtsZ-binding protein ZapA, FtsZ(Ts1) regains the capacity to form functional Z rings

at 49°C via the normal assembly pathway. The ZapA protein has previously been shown to promote Z ring assembly in the cell, and to stimulate the association of simple FtsZ polymers (protofilaments) into higher-order polymeric structures *in vitro*. These and other results suggest that FtsZ(Ts1) is specifically defective in its ability to support higher-order polymer association *in vivo* under non-permissive conditions. This enables FtsZ(Ts1) to polymerise into a helix, while preventing the helix from undergoing the structural changes required for it to reorganise into a stable ring. These findings have important implications regarding the molecular mechanism of the FtsZ helix-to-ring transition in wild-type cells.

Other work presented in this thesis involved a genetic screen for extragenic suppressors of *tsI* thermosensitivity. Using insertional mutagenesis, a total of four unique genes were identified that rescue *tsI* to temperature resistance when inactivated. Given that the *tsI* strain is specifically defective in FtsZ function, and taking into account other findings in the literature, this strongly suggests that these genes act in some capacity to control FtsZ activity *in vivo*. Further characterisation of the gene products promises to uncover novel insights into the regulation of Z ring assembly in bacteria.