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

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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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Contents

Abbreviations

$A_{(x)}$	absorbance (where x = wavelength in nanometres)
Ap^R	ampicillin resistance marker
AR	analytical reagent
ATP	adenosine 5′-triphosphate
B.	*Bacillus*
BP	band pass
bp	base pair(s)
BSA	bovine serum albumin
C.	*Caulobacter*
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
E.	*Escherichia*
ECL	enhanced chemi-luminescence
ECT	electron cryotomography
EDTA	ethylenediaminetetraacteic acid
EGTA	ethyleneglycol-bis(2-aminoethyl ether)-tetraacetic acid
Em^R	erythromycin resistance marker
FRAP	fluorescence recovery after photobleaching
fts	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-(*p*-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})
M.	*Methanococcus*
M	moles per litre
MCS	multiple cloning site
min	minute(s)
mol	mole(s)
MOPS	3-(*N*-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})
P	probability
P.	*Prosthecobacter*
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
S.	*Streptomyces*

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.

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Peters, P. C., **Monahan, L. G.**, and Harry, E. J. (March, 2008) Targeting cell division: The Z ring assembles from a dynamic helical-like structure. ***SEMINAR***, *Gordon Research Conference on Antibacterial Discovery and Development,* Il Ciocco, Italy.

Monahan, L. G., Peters, P. C., and Harry, E. J. (February, 2008) Bacterial cell division: Molecules and mechanisms. ***SEMINAR***, *Pasteur-Walter and Eliza Hall Institute Workshop on Imaging Infectious Diseases at the Molecular, Cellular and Tissue Level,* Melbourne, Australia.

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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 *ts1*, 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 *ts1*). 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 *ts1* thermosensitivity. Using insertional mutagenesis, a total of four unique genes were identified that rescue *ts1* to temperature resistance when inactivated. Given that the *ts1* 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.