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Connecting the dots of the bacterial cell cycle: a potential role for Spo0J in Z ring positioning

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Certificate of Authorship/Originality

I, Isabella Veronica Hajduk, 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 thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Isabella Hajduk, February 2018

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Abstract

Cell division is of utmost importance for the propagation of all living organisms. In bacteria, the earliest stage of cell division is the formation of the cytokinetic Z ring at the division site at the cell centre (midcell), which must be tightly co-ordinated with chromosome replication and segregation to ensure that each newborn cell receives a full complement of the genetic material. What still eludes us is how bacterial cells position their division site so precisely at the cell centre to enable the production of two genetically identical daughter cells.

The current understanding of how bacterial cells position their division site is that it occurs via the combination of two negative regulators, the Min system and nucleoid occlusion mediated by the protein Noc. These two systems act by preventing the Z ring from forming at incorrect positions, either at the cell poles or within the vicinity of the chromosome, respectively. The overall result is that the two systems prevent the Z ring from forming anywhere other than the cell centre. However, as discovered recently, they do not define the division site, suggesting the existence of other regulatory mechanisms for midcell Z ring assembly. So what does define the division site? It has been shown in *Bacillus subtilis* that Z ring assembly may be coupled with the early stages of DNA replication and recent work in this area has led to the proposed Ready-Set-Go model.

The Ready-Set-Go model proposes a putative link between Z ring positioning and DNA replication such that the progress through the initiation phase of DNA replication promotes an increase in ability of the Z ring to assemble midcell. Specifically, mutants blocked at an early stage of initiation lead to fewer midcell Z rings than those blocked at later stages of initiation. Importantly, this correlation between DNA replication initiation progression and Z ring position is only observed in *noc* mutants. Interestingly the observations that led to this model also hinted at an alternative possibility: mechanisms linked to chromosome organisation may also impact Z ring positioning. Thus the primary objective of the work presented in this

thesis was to obtain a better understanding of the link between the early stages of DNA replication and cell division in the model organism *B. subtilis*, and how chromosome organisation plays into this link. To explore this possibility further, and ultimately test the validity of the Ready-Set-Go model, this thesis examined the role of Soj and Spo0J, two players with distinct roles in the regulation of DNA replication initiation and chromosome organisation, in Z ring positioning using the same conditions that led to the Ready-Set-Go model. Surprisingly, a *spo0J noc* double mutant, but not a *soj noc* double mutant, allows for wild-type levels of midcell Z ring assembly, regardless of the block imposed at the initiation stage of DNA replication. This suggests that the ability to assemble a Z ring at midcell is not linked to the progression of the initiation stage of DNA replication, thus challenging the idea of a link between DNA replication initiation and Z ring position. Importantly, this result and others, also suggest a role for Spo0J in the regulation of Z ring position.

To start to elucidate how Spo0J plays into the regulation of Z ring position, Z ring positioning was examined in cells blocked at an early event of DNA replication initiation that also harbor point mutants of Spo0J impacting its function in DNA replication initiation through *soj* or points mutants that impact its function in the recruitment of SMC (required to organise the chromosome). Interestingly, this data and others support two models for how Spo0J may function in Z ring positioning: Spo0J, like Noc, is a nucleoid occlusion protein or Spo0J-mediated chromosome organisation blocks midcell Z ring assembly by a generating a nucleoid morphology that inhibits midcell Z ring assembly. Both models are discussed and contrasted in detail in light of recent advances in the understanding of bacterial chromosome organisation.

Collectively, this thesis challenges the long-standing idea of a link between DNA replication initiation and Z ring positioning and creates a solid foundation for future studies examining how chromosome organisation impacts Z ring positioning.

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Publications

Journal articles

Monahan LG, Hajduk IV, Blaber SP, Charles IG, Harry EJ (2014) Coordinating Bacterial Cell Division with Nutrient Availability: a Role for Glycolysis. *mBio* **5**

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29th RNSH/UTS/USYD Scientific Research Meeting

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Abbreviations

AGRF	Australian Research Genome Facility
Ab	antibody
B.	*Bacillus*
bp	base pair(s)
BP	band pass
BSA	bovine serum albumin
cat	chloramphenicol
CFP	cyan fluorescent protein
cm	centimetres
CCD	charged coupled device
DAPI	4'6-diamidino-2-phenylindole
DNA	deoxyribonucleic acid
E.	*Escherichia*
erm	Erythromycin
et al.	and others
FRAP	fluorescence recovery after photobleaching
fts	filamentation temperature sensitive
g	centrifugal force
g	gram(s)
GFP	green fluorescent protein
GMD	germination medium defined
IFM	immunofluorescence microscopy
IgG	Immunoglobulin G
IPTG	isopropyl-1-thio-β-D-galactopyranoside
kan	Kanamycin
L	litre(s)
LP	long pass
M	moles per litre
MQW	Milli-Q purified water
MSA	mineral salts A
NA	numerical aperture
N/A	not applicable

neo	Neomycin
OD_x	optical density at (x refers to the wavelength in nm)
P	probability
P_{xyl}	xylose-inducible promoter
PAB	penassay antibiotic medium 3 broth
PAGE	polyacrylamide gel electrophoresis
PBP	penicillin binding protein
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pH	power of Hydrogen
phleo	phleomycin
RNA	ribonucleic acid
ROW	reverse osmosis purified water
rpm	revolutions per minute
RT	room temperature
SDS	sodium dodecyl sulfate
SEM	standard error of the mean
spp.	species
spc	spectinomycin
TBAB	tryptose blood agar base
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/v	weight per volume
YFP	yellow fluorescent protein
µ	micro- (10^{-6})