Coordination between Chromosome Translocation and Peptidoglycan Remodeling during Spore Development

by

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the degree of
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under the supervision of

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Certificate of Original Authorship

I, Ahmed Mohamed, declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the iThree Institute, Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis. This document has not been submitted for qualifications at any other academic institution.

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Abstract

In all cells, including bacteria, coordination between different molecular processes is fundamental for successful growth, division and differentiation. In sporulating bacteria, two fundamental processes, peptidoglycan remodeling and chromosome segregation, occur at the same time and are essential for the early stages of spore development. However, it remains unclear if and how they are coordinated. This thesis addresses this question using the model organism *Bacillus subtilis*.

Upon starvation, some bacteria enter a developmental process called sporulation to produce highly-resistant and dormant cells known as spores. Initially, the starving cell divides asymmetrically in two compartments of different size: the larger one is called the mother cell and the smaller one is called the forespore. Asymmetric division triggers compartment-specific transcription controlled by sigma factors, with σ^F in the forespore and σ^E in the mother cell. Interestingly, the asymmetric septum also traps ~30% of the forespore chromosome in the forespore, while the remaining ~70% resides in the mother cell. Through the septum, A DNA transporter called SpoIIIE translocates the remaining ~70% of the chromosome into the forespore. Concurrently with chromosome translocation, the peptidoglycan within the asymmetric septum undergoes remodeling by hydrolytic and synthetic enzymes, which drive the internalization of the forespore into the mother cell, through a process called engulfment.

During engulfment, two forespore enzymes that function to synthesize a new layer of peptidoglycan are suggested to be functionally redundant, PbpG and PbpF. However, previous observations suggest that PbpG and PbpF could function in separate pathways and thus have specialized roles during sporulation. To investigate this hypothesis, stemming from a genetic screen, this thesis identified SpoIIIM (formerly YqfZ) as being required for efficient sporulation in cells lacking PbpG. Through the phenotypic characterization of cells lacking SpoIIIM and PbpG, multiple lines of evidence led to the conclusion that SpoIIIM, PbpG and SpoIIIE coordinate peptidoglycan remodeling and chromosome translocation at a septal pore. This coordination is required to ensure septal pore stability and its closure upon complete chromosome translocation. Interestingly, other data revealed an important role for the SpoIIIAH-SpoIIQ interaction in the stabilization of the septal pore. Furthermore, the coordination between peptidoglycan remodeling and chromosome translocation was shown to happen through direct interactions between SpoIIIM, PbpG and SpoIIIE. Collectively, this thesis reveals that peptidoglycan remodeling and chromosome translocation are coordinated at

a septal pore, to ensure septal pore stability, successful chromosome translocation and transcriptional compartmentalization during spore development.

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Publications

Journal article

Ahmed Mohamed, Helena Chan, Johana Luhur, Elda Bauda, Benoit Gallet, Cecile Morlot, Louise Cole, Milena Awad, Simon Crawford, Dena Lyras, David Z. Rudner, Christopher D. A. Rodrigues (2021). "Chromosome Segregation and Peptidoglycan Remodeling Are Coordinated at a Highly Stabilized Septal Pore to Maintain Bacterial Spore Development." *Developmental Cell*, 56, 1–16 January 11; https://doi.org/10.1016/j.devcel.2020.12.006

Conferences

Ahmed Mohamed*, Helena Chan, Johana Luhur, Elda Bauda, Benoit Gallet, Cecile Morlot, Louise Cole, Milena Awad, Simon Crawford, Dena Lyras, David Z. Rudner, Christopher D. A. Rodrigues (2020). "Chromosome Segregation and Peptidoglycan Remodeling Are Coordinated at a Highly Stabilized Septal Pore to Maintain Bacterial Spore Development." **Bugs By The Beach**, Newcastle, Australia (virtual oral presentation), 20th November 2020.

*the talk presenter

Abbreviations

α	alpha
A	alanine
amp	ampicillin
β	beta
B.	*Bacillus*
cat	chloramphenicol resistance gene
CFP	cyan fluorescent protein
cfu	colony-forming unit
D	aspartic acid
DNA	deoxyribonucleic acid
DNaseI	deoxyribonuclease I
DSM	Difco Sporulation Medium
DTT	1,4-Dithiothreitol
FM4-64	N-(3-Triethylammoniumpropyl)-4-(6-(4-(Diethylamino) Phenyl) Hexatrienyl) Pyridinium Dibromide
E	Glutamic acid
E.	*Escherichia*
EDTA	ethylenediaminetetraacetic acid
et al.	and others
erm	erythromycin resistance gene
γ	gamma
g	gram (s)
GCW	germ cell wall
GFP	green fluorescent protein
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
His	histidine
hr	hour

IPTG	isopropyl-1-thio-β-D-galactopyranoside
kan	kanamycin resistance gene
L	litre (s)
LB	Luria-Bertani broth (Lennox)
m	milli (10^{-3})
M	moles per litre
min	minutes
MQW	milli-Q purified water
mypet	monomeric yellow fluorescent protein for energy transfer
n	nano (10^{-9})
neo	neomycin resistance gene
ODx	optical density at (x refers to the wavelength in nm)
opt	optimized
p	probability
$P_{hyperspank}$	IPTG-hyper-inducible promoter
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PBP	penicillin binding protein
PCR	polymerase chain reaction
pH	power of Hydrogen
phleo	phleomycin resistance gene
PMSF	phenylmethylsulfonyl fluoride
RBS	ribosome binding site
RNase	ribonuclease A
rpm	revolutions per minute
SD	standard deviation
S	serine
SDS	sodium dodecyl sulfate
σ	sigma

spec	spectinomycin resistance gene
tet	tetracycline resistance gene
TMA-DPH	TMA-DPH (1-(4-Trimethylammoniumphenyl)-6-Phenyl-1,3,5-Hexatriene p-Toluenesulfonate)
Tn-seq	transposon sequencing
Tris	tris(hydroxymethyl)methylamine
U	units (enzyme activity)
UV	ultraviolet
V	volt(s)
v/v	volume per volume
W	watt
w/v	weight per volume
X-Gal	5-Bromo-4-Chloro-3-Indolyl β-D-Galactopyranoside
YFP	yellow fluorescent protein
µ	micro (10^{-6})