
We Are What We Eat: Identifying a Regulatory Crosstalk between Central Carbon Metabolism and Cell Division in Bacteria

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Certificate of Authorship/ Originality

I, Riti Mann, 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.

This research is supported by an Australian Government Research Training Program.

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Riti Mann, June 2019

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Abstract

Cell division is crucial to the survival and propagation of all living organisms. In bacteria, the process of cell division is initiated by the formation of the cytokinetic Z ring, made up of the tubulin-like protein FtsZ, at the cell centre (midcell). Research into the control of Z ring positioning in the well-studied rod-shaped bacteria has focused on two main regulatory systems, the Min system and nucleoid occlusion. However, an aspect of cell division control that remains often overlooked is the need to coordinate cell cycle events with the nutrient availability.

Central Carbon Metabolism (CCM), being the entry point for almost all the raw materials required for cellular growth and proliferation, forms the hub for coordinating nutrient availability to cell growth, size and the division process. Our lab recently identified a novel link between the central carbon metabolic pathway of glycolysis and cell division, wherein disrupting glycolysis by deleting the pyruvate kinase (*pyk*) enzyme was shown to affect the correct positioning of the Z ring at the cell centre, such that 1/3rd of the Z rings formed at acentral positions. Interestingly, the addition of pyruvate (the end product of the pyruvate kinase reaction), alleviated this Z ring positioning defect of Δpyk cells.

Pyruvate is a metabolite that lies at the intersection of many metabolic pathways, raising the possibility of any of the potential metabolic fates of pyruvate being actually responsible for the Z ring positioning rescue of Δpyk cells. The experimental results presented in this thesis confirm that pyruvate, and not any of its potential metabolic fates, is the key metabolite coordinating CCM to cell division in *Bacillus subtilis*. Furthermore, this thesis also provide evidence for the role of DNA replication and nucleotide synthesis in the Z ring positioning defect of Δpyk cells. Collectively, the work presented here demonstrates the importance of one specific metabolite - pyruvate, in maintaining the coordination between the cellular events of

division, metabolism and DNA replication in *B. subtilis*; which leads to the hypothesis that pyruvate might be acting as a signaling molecule to synchronize cell cycle events with the nutrient availability.

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Publications and Awards

Awards

New Horizons Young Investigator **Popular Choice award** 2018

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Australian Society of Microbiology **Becton Dickinson award** 2017

Journal articles

Mann R, Mediati DG, Duggin IG, Harry EJ and Bottomley AL (2017) Metabolic Adaptations of Uropathogenic *E. coli* in the Urinary Tract. *Front. Cell. Infect. Microbiol.* 7:241

Mann R, Monahan L, Harry E and Bottomley A (2017) We Are What We Eat: True for Bacteria Too. *Front Young Minds.* 5:54

Kusrini E, Hashim F, Gunawan C, **Mann R**, Noor Azmi WNNW, Amin NM (2018) Anti-amoebic activity of acyclic and cyclic-samarium complexes on *Acanthamoeba*. *Parasitol. Res.*, 117(5):1409-1417

Mann R (2017) We are what we eat: Metabolic control of the division process in bacteria. *ASM Syntrophy.* 18:4

Conference proceedings*

Mann R, Bottomley A, Monahan L and Harry E - *New Horizons 2018 (November 19-20), Sydney, Australia* - **Oral presentation** - “We are what we eat: Identifying a regulatory crosstalk between central carbon metabolism and cell division in bacteria”

Mann R, Bottomley A, Monahan L, Sonenshein A and Harry E - *ComBio 2018 (September 23-26), Sydney, Australia* – **Poster teaser oral and poster presentation** - “We are what we eat: Connecting central carbon metabolism to cell division in *Bacillus subtilis*”

Mann R, Bottomley A, Monahan L, Sonenshein A and Harry E - *Gordon Research Conference on Microbial stress response 2018 (July 15-20), Massachusetts, United States* – **Poster presentation** - “We are what we eat: Identifying a regulatory crosstalk between central carbon metabolism and cell division in bacteria”

Mann R, Bottomley A, Monahan L, Sonenshein A and Harry E - *Gordon Research Seminar on Microbial stress response 2018 (July 14-15), Massachusetts, United States* – **Oral and poster presentation** - “We are what we eat: Identifying a regulatory crosstalk between central carbon metabolism and cell division in bacteria”

Mann R, Bottomley A, Monahan L, Sonenshein A and Harry E - *Gordon Research Conference on Bacterial Cell Surfaces 2018 (June 24-29), Mount Snow, West Dover, United States* – **Oral and poster presentation** - “We are what we eat: Identifying a regulatory crosstalk between central carbon metabolism and cell division in bacteria”

Mann R, Bottomley A, Monahan L, Sonenshein A and Harry E - *Gordon Research Seminar on Bacterial Cell Surfaces 2018 (June 23-24), Mount Snow, West Dover, United States* – **Oral and poster presentation** - “We are what we eat: Identifying a regulatory crosstalk between central carbon metabolism and cell division in bacteria”

Bottomley A, Mann R, Sonenshein A, Monahan L and Harry E - *ComBio 2017 (October 2-5), Adelaide, Australia* – **Poster presentation** - “We are what we eat: Identifying a regulatory crosstalk between central carbon metabolism and cell division in bacteria”

Mann R, Bottomley A, Monahan L and Harry E - *Australian Society of Microbiology Annual Scientific Meeting 2017 (July 2-5), Hobart, Australia* – **Oral presentation** - “We are what we eat: Identifying a regulatory crosstalk between central carbon metabolism and cell division in bacteria”

Bottomley A, Mann R, Sonenshein A, Monahan L and Harry E – *19th International Conference on Bacilli & Gram-Positive Bacteria 2017 (June 11-15), Berlin, Germany* – **Oral presentation** - “We are what we eat: Identifying a regulatory crosstalk between central carbon metabolism and cell division in bacteria”

Mann R, Bottomley A, Monahan L and Harry E – *6th Annual JAMS (Joint Academic Microbiology Seminars) Symposium 2017 (March 22), Australian Museum, Sydney, Australia* – **Poster presentation** - “We are what we eat: Identifying a regulatory crosstalk between central carbon metabolism and cell division in bacteria”

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Monahan L, Mann R, Bottomley A, Hajduk I, Harry E - *New Horizons 2015 (November 23-25), Sydney, Australia* – **Poster presentation** - “Coordinating bacterial cell division with nutrient availability: a role for glycolysis”

*The presenting author is underlined

Abbreviations

AGRF	Australian Genome Research Facility
Ab	Antibody
<i>B.</i>	<i>Bacillus</i>
bp	Base pair (s)
BP	Band pass
BSA	Bovine serum albumin
CAA	Casamino acids
<i>cat</i>	Chloramphenicol
cm	Centimetres
CCD	Charged coupled device
DAPI	4'6-diamidino-2-phenylindole
DNA	Deoxyribonucleic acid
<i>E.</i>	<i>Escherichia</i>
EDTA	ethylenediaminetetraacetic acid
<i>erm</i>	Erythromycin
<i>et al.</i>	and others
<i>fts</i>	Filamentation temperature sensitive
<i>g</i>	Centrifugal force
g	Gram (s)
GFP	Green fluorescent protein
GTP	guanosine 5'-triphosphate
h	Hour (s)
IFM	Immunofluorescence microscopy
IgG	Immunoglobulin G
IPTG	Isopropyl-1-thio- β -D-galactopyranoside
<i>kan</i>	Kanamycin
kb	Kilobase pair (s)
L	Litre (s)
LP	Long pass
M	Moles per litre
min	Minute (s)

MQW	Milli-Q purified water
MSA	Mineral salts A
NA	Numerical aperture
N/A	Not applicable
OD _x	Optical density at (x refers to the wavelength in nm)
P _{<i>xyl</i>}	Xylose-inducible promoter
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
pH	Power of hydrogen
RNA	Ribonucleic acid
RNase	Ribonuclease A
ROW	Reverse osmosis purified water
rpm	Revolutions per minute
RT	Room temperature
SDS	Sodium dodecyl sulfate
SEM	Standard error of the mean
spp.	Species
<i>spec</i>	Spectinomycin
TBAB	Tryptose blood agar base
TE	Tris-EDTA buffer
TES	Tris-EDTA-salt buffer
<i>tet</i>	Tetracycline
Tris	Tris(hydroxymethyl)methylamine
ts	Temperature sensitive
UV	Ultraviolet
V	Volt (s)
v/v	Volume per volume
w/v	Weight per volume
YFP	Yellow fluorescent protein
μ	Micro (10 ⁻⁶)
