Identification of host cell proteins involved in *Shigella flexneri* pathogenesis

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April 2014
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Abstract

*Shigella flexneri* is the etiological agent of bacillary dysentery (shigellosis). It is transmitted via the faecal-oral route and is a significant human pathogen due to the high morbidity among children <5 years in developing countries. The key pathogenic features of *Shigella* include cell death induction in myeloid immune cells and circumventing cell death in colonic epithelial cells, the site of bacterial infection. *Shigella* also interact with host proteins to initiate *de novo* actin synthesis to facilitate its intra- and intercellular spread to disseminate in the host.

In this thesis, the role of three host proteins: myosin IIA, dynamin II, and dynamin-related protein 1 (Drp1) during *Shigella* cell-to-cell spreading was examined. The myosin IIA specific kinase, myosin like chain kinase (MLCK), was previously shown to be important for *Shigella* plaque formation. Myosin IIA and MLCK have also been implicated in septin caging of non-motile *Shigella* which are targeted for degradation. Chemical inhibition and siRNA knockdown of myosin IIA reduced *Shigella* plaque formation. Curiously HeLa cells infected with *Shigella* mutants defective in cell-to-cell spreading have significantly reduced myosin IIA levels when quantified by immunofluorescence microscopy.

Dynamin II and Drp1 are members of the dynamin superfamily. Both proteins have self-assembly driven GTPase activation. Dynamin II is important for clathrin-mediated endocytosis and pinches the budding clathrin-coated vesicle, and Drp1 is essential for mitochondrial fission. It was hypothesized that *Shigella* protrusion formation into adjacent host cells resembles endocytic and exocytic processes, and components of these processes may facilitate *Shigella* dissemination. When dynamin II GTPase was inhibited with dynasore and dynamin II was knocked down with siRNA, *Shigella* cell-to-cell spreading was significantly reduced. The *in vivo* efficacy of dynasore was tested in a murine Sereny model. No significant reduction in inflammation was observed but mice were protected against weight loss during infection. Further experimentation suggested dynasore protected mice against cytotoxic effects from the three secretion system (TTSS) effectors expressed by *Shigella* during infection.

Drp1 was investigated in this thesis as dynasore also inhibits the GTPase of this mitochondrial fission protein. Mitochondrial fission is important in maintaining mitochondrial
dynamics and also in events downstream of intrinsic apoptosis and programmed necrosis pathways activation. Loss of mitochondrial function in Shigella-induced epithelial cell death has been reported previously. Hence the role of Drp1 in Shigella plaque formation and HeLa death was examined with the Drp1-specific inhibitor, Mdivi-1, and siRNA knockdown. HeLa cell death was significantly reduced; suggesting loss of mitochondrial function observed previously may now be attributed to Drp1 and subsequent Drp1-mediated mitochondrial fission. The impairment in Shigella cell-to-cell spreading in the absence of Drp1 suggests maintaining an intact mitochondrial network is essential for Shigella lateral spread since loss of Drp1 function would result in excessive mitochondrial fusion, leading to formation of net-like or perinuclear structures.

The outcomes of this thesis highlight the importance of host proteins during different stages of Shigella infection. By improving our understanding on the host and bacteria interaction, future work on novel approaches to prevent Shigella dissemination can be developed.
Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Adelaide, Australia, April 2014

________________________________________
Mabel Yuen Teng Lum
Publications


**Lum M & Morona R (2014).** Dynamin-related protein Drp1 and mitochondria are important for *Shigella flexneri* infection. *Int J Med Microbiol* 304, 530-541.

Acknowledgements

First and foremost, I would like to thank the Australian Government for my scholarship.

I would like to thank my supervisor A/Prof Renato Morona for what has been a challenging project. Thank you for all the support and guidance over the past few years. I would also like to thank Luisa Van Den Bosch for the preliminary experiments and for imparting all her TC skills. I am grateful to Dr Stephen Attridge who was instrumental in setting up the animal model. I have enjoyed working with you. I would also like to thank members of the Morona laboratory for proof reading bits of my thesis, helpful suggestions, assistance with experiments, kind words of encouragement and putting up with my whinging.

Thank you to all my friends in the MLS building: Min, Pratiti, Donald, Rethish, Zarina, Alex, and Long, for always lending an ear. Also a big thank you to Donald, Min and Pratiti for the yummy cakes, desserts, snacks, dinner dates and shopping sprees. A shout-out to Paul who is my partner in crime in annoying Pratiti. To all my friends outside the lab, thank you all for your unwavering moral support. To my boss (and old friend) at AGRF, thank you for keeping me employed for so many years and for your many funny stories and words of encouragement when I needed them.

I would also like to thank my family who is very supportive in spite of not knowing what I actually do. To my sister in Adelaide, her husband and lovely children, thank you for always lending a hand and for silly times. A special thank you goes to my partner who is always there when I needed him. I look forward to a fun and exciting journey ahead with you!

Lastly I would like to dedicate this thesis to my late German teacher. I miss your passion for life and for learning. Thank you for always reminding me not to give up and that I can do better.
# Abbreviations

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MEFs
mouse embryonic fibroblasts

Mdivi-1
mitochondrial division inhibitor-1

MYH9
myosin, heavy chain 9, non-muscle gene
(myosin IIA heavy chain gene)

min
minute(s)

MLCK
myosin light chain kinase

moi
Multiplicity of infection

MOMP
mitochondrial outer membrane permeabilisation

myosin IIA / B / C
non-muscle myosin IIA / B / C

N-WASP
Neural Wiskott-Aldrich syndrome protein

NF-κB
nuclear factor-κB

NMP
N-methyl-2-pyrrolidone

NPF(s)
nucleation promoting factor(s)

OM
outer membrane

PEG / PEG300
polyethylene glycol 300

PGN
peptidoglycan

PH
pleckstrin homology

PMN(s)
polymorphonuclear cell(s)

PRD(s)
proline-rich domain(s)

PtK2
Potorous tridactylis kidney epithelial (cells)

R-LPS
rough LPS

ROS
reactive oxygen species

S-LPS
smooth LPS

siRNA
small interfering RNA

STS
staurosporine

t
time

TJ(s)
tight junction(s)

TTSS
type three secretion system

VP
virulence plasmid

VP− / VP-
virulence plasmid-cured

WT
wild type

xviii