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Cellular trafficking of rotavirus NSP4 in epithelial cells

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A thesis submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy
July 2007
Abstract

Rotavirus is a non-enveloped dsRNA virus that belongs to the family Reoviridae and is associated with severe dehydrating diarrhea in infants and young children throughout the world. Rotavirus targets the fully differentiated epithelial cells lining the tips of the small intestinal villi. Although different aspects of the rotavirus replication cycle have been researched extensively, the mechanism underlying the assembly of mature viral particles and the precise method by which rotavirus is able to induce diarrheal disease have not been fully elucidated.

The rotavirus replication cycle involves budding of immature progeny virus particles, termed DLPs, from the site of replication and assembly into the lumen of ER via an interaction of the particle with the cytoplasmic domain of a virally encoded non-structural protein, NSP4. Recent studies confirm subcellular localisation of this protein in distinct membrane lipid microdomains at the later stages of the viral replication cycle in polarised epithelial cells and further classify these structures as a final assembly location for mature viral particles. Overall aim of the study presented in Chapter 3 was to characterise the domain of NSP4 responsible for such interaction and to further elucidate the significance of proposed interaction in the context of the rotavirus replication cycle.

In order to assess this interaction in vitro, separate domains of the NSP4 protein, expressed by several different recombinant protein expression systems, were applied to a Caco-2 cell lysate and examined by floatation gradient and subsequent Western blot analysis. The NSP4-lipid microdomain interaction was mapped to a part of a coiled-coil domain including amino acids 85 to 122. The potential involvement of NSP4 in translocation progeny DLPs to the site of final viral assembly was also examined.

Recent studies suggest involvement of this protein in the pathophysiology of rotavirus-induced diarrhea by acting as an enterotoxin. To mediate enterotoxic effects in vivo, NSP4 needs to be secreted or released from rotavirus-infected cells in a soluble form, but studies indicate that this is a transmembrane glycoprotein located
in the ER membrane. Overall aim of the studies outlined in Chapter 4 was to identify and further characterise secretion of NSP4 from rotavirus-infected fully polarised Caco-2 cells.

These studies reveal that NSP4 is actively secreted into the culture medium of polarised, but not non-polarised cells. The secretion was detected preferentially from the apical surface and was found to be dramatically inhibited by addition of BFA and monensin, suggesting a Golgi-dependent pathway is involved in release of this protein. In accordance with this finding, the secreted NSP4 isoform was found to undergo additional post-translational modification and is partially resistant to deglycosylation by EndoH and PNGaseF. Furthermore, apical secretion was not blocked by addition of glucosidase- or cholesterol- inhibitors, and no evidence was found that NSP4 was packaged in exosomes for its release.

Taken together, these studies outline the pool of NSP4 that is able to actively participate in an assembly of mature progeny virus particles through its interaction with lipid microdomains and further identify a novel, soluble form of NSP4 secreted from rotavirus-infected polarised cells.
Acknowledgments

My sincere gratitude goes to my supervisor, Dr John Taylor. A special thanks to Dr Judith O’Brien and Prof Dick Bellamy for all advice and constant encouragement. The understanding and support over the years were very much appreciated. Thanks especially to Dr Taylor for assistance in the final preparation of this thesis.

Thank you to Hillary Holloway and Jacquie Ross from Bioimaging Research Unit, University of Auckland for guidance with confocal microscopy.

Extreme thanks to all the members of Molecular Virology lab, both past and present. Special thanks to Jan Meyer for kind words, interesting discussions and her brilliant proof reading skills. To my good friends Sarah Greig and Julie Hill - certain things seemed so much better after a good talk and a few laughs. Thank you for always being there! Thank you to Kevin Ke, for all your words of encouragement and endless DIY skills. Thank you also to Kristy Manning and Carol Wang, for help when the help was needed.

Thanks to the many people in SBS who have helped me over the years. In particular, Adrian Turner and Iain MacDonald, for help with the EM and graphics. A special thanks to members of various labs that made certain things more bearable. A very special thanks to my friends Bronwen Jongbloed, Shanthi Jayawaradena, Mel Ragget, David and Rachel Goldstone and Jo Dodd for both practical advice and infinite capacity to make me laugh. Thank you for being my friends even when that seemed not to be reciprocated. A special thanks to Leo Payne, Haylyn Wong and Peter Brown.

Lastly, a very special thank you to my family – my boys, Marko and Nikola, my husband, Mum, Dad and my sisters – Daša, Jaca and Brana. All of this would not be possible without your endless support, encouragement and most of all love.

This project was supported by Auckland Medical Research Foundation Senior PhD and University of Auckland PhD scholarships.
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Abbreviations

S.I. (Système Internationale) abbreviations for units and standard notations for chemical elements, formulae and chemical abbreviations are used throughout this work. Other abbreviations used in the text are defined below.

2D........................................two-dimensional
3D..........................................three-dimensional
α-...........................................anti-
A..............................................absorbance
aa..............................................amino acid
Å................................................angstroms
ATP...........................................adenosine-5'-triphosphate
BCA...........................................bicinchoninic acid
BGN...........................................benzyl-N-acetyl-α-galactosamine
bp.............................................base pair
BSA...........................................bovine serum albumin
BTV...........................................bluetongue virus
C-terminal ..................................carboxyl terminal
C90...........................................C-terminal 90 amino acids of NSP4
cryoEM......................................cryo-electron microscopy
CV.............................................column volume
DLP...........................................double layered particle of rotavirus
DMEM......................................Dulbecco’s modified Eagle’s medium
DMJ...........................................deoxymannojirimycin
DMSO.......................................dimethyl sulphoxide
DNA..........................................deoxyribonucleic acid
DRM..........................................detergent-resistant membranes
dsRNA.....................................double-stranded RNA
E. coli ...................................Escherichia coli
ECL...........................................enhanced chemiluminescence
EDTA........................................ethylenediaminetetraacetic acid
ELISA.......................................enzyme-linked immunosorbent assay
EM..........................................electron microscopy
ECM........................................extracellular matrix
ENS..........................................enteric nervous system
ER.............................................endoplasmic reticulum
FBS..........................................foetal bovine serum
FFT..........................................fast Fourier transform
G serotype ................................glycoprotein serotype
GST..........................................glutathione-S-transferase
HBV..........................................hepatitis B virus
HDL..........................................high density lipoprotein
HIV..........................................human immunodeficiency virus
hpi.........................................hours post infection
Hsc70 ..................................................heat shock cognate protein 70
HSV ..................................................herpes simplex virus
ICP ...................................................inner core particle
IPTG ..................................................isopropyl-\(-\)D-thiogalactopyranoside
kDa ..................................................kilodaltons
moi ...................................................multiplicity of infection
mAb ..................................................monoclonal antibody
mRNA ................................................messenger RNA
MW ...................................................molecular weight
MPa ..................................................mega pascals
N-terminal .........................................amino terminal
NA ..................................................neuraminidase
NSP ..................................................non-structural protein of rotavirus
\(^\circ\)C ..................................................degrees Celsius
P serotype ..........................................protease-sensitive serotype
PAGE ...............................................polyacrylamide gel electrophoresis
pfu ..................................................plaque-forming units
RNA ..................................................ribonucleic acid
RNase ..............................................ribonuclease
RNAi ...............................................RNA interference
rpm ..................................................revolutions per minute
RV ..................................................rotavirus
SA ..................................................sialic acid
SDS ..................................................sodium dodecyl sulphate
Sf9 ..................................................Spodoptera frugiperda
SG ..................................................subgroup
siRNA ...............................................small interfering RNA
ssRNA ...............................................single-stranded RNA
SV40 ...............................................simian vacuolating virus 40
SW ..................................................swainsonine
T .....................................................triangulation number
TCA ..................................................trichloroacetic acid
TEM ..................................................transmission electron microscopy
TEMED ............................................N,N,N\(,\)N\(,\) tetramethylmethylenediamine
TGN ..................................................trans Golgi network
TLP ..................................................triple layered particle of rotavirus
Tris ..................................................2-amino-2-(hydroxymethyl)-1,3
.................................propanediol
TX-100 .............................................Triton X-100
U .....................................................units of enzyme (defined by
.................................manufacturer)
UV ..................................................ultraviolet light
v/v ..................................................volume per volume
VLP ..................................................virus-like particle
VP ..................................................structural viral protein
w/v ..................................................weight per volume
w/w ..................................................weight per weight
xg ..................................................... relative centrifugal force
ρ ............................................................ density