

# **Role of African horsesickness virus protein NS3 in cytotoxicity and virus induced cytopathology**

by

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Submitted in partial fulfilment of the requirements for the degree  
Philosophiae Doctor

in the Faculty of Natural & Agricultural Science  
University of Pretoria

Pretoria  
February 2009

## DECLARATION

I, Tracy Leonora Meiring declare that the thesis/dissertation, which I hereby submit for the degree Philosophiae Doctor at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature:

Date:

## ACKNOWLEDGEMENTS

I would like to express my sincere appreciation and thanks to the following people:

Dr Vida van Staden for her valuable guidance, motivation, and support throughout this study

Prof Henk Huismans for his leadership, support and critical advice

Flip Wege for his excellent technical assistance with cell culture

Alan Hall and Chris van der Merwe at the Laboratory for Microscopy and Microanalysis at the University of Pretoria (UP)

Renate Zipfel, Gladys Shabangu and Mia Beyleveld at the Sequencing facility (UP)

Dr Marco Romito and Dr A.C. Potgieter at the Onderstepoort Veterinary Institute

The Department of Biochemistry (UP) for the use of their facilities

My fellow colleagues in the Orbivirus Research group and Department of Genetics (UP) for their interest and support

The National Research Foundation for financial assistance

My family and friends for their encouragement and understanding

## SUMMARY

### **Role of African horsesickness virus protein NS3 in cytotoxicity and virus induced cytopathology**

by

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The viral determinants of African horsesickness virus (AHSV) cytopathology are not well understood. Several AHSV proteins may play a role, including non-structural protein NS3, a cytotoxic membrane protein that localises to sites of virus release and plasma membrane disorganisation in infected cells. AHSV NS3 is highly variable and clusters into three phylogenetic groups, termed  $\alpha$ ,  $\beta$  and  $\gamma$ . In chapter 2 we examined the role of NS3 in determining the phenotypic characteristics observed during AHSV infection of cells. Three AHSV strains, AHSV-2 ( $\gamma$  NS3), AHSV-3 ( $\beta$  NS3) and AHSV-4 ( $\alpha$  NS3), were shown to have quantitatively different phenotypes in Vero cells. To investigate the contribution of NS3 to these differences, reassortants were generated between these strains in which the S10 genome segment encoding NS3 was exchanged, alone or in combination with other segments. Exchange of NS3 resulted in changes in virus release and membrane permeability, indicating an important role for NS3 in these viral properties. The cytopathic effect and decreased viability of infected cells was not associated with NS3 alone and it is likely that a number of viral and host factors contribute to these complex phenotypes.

In chapter 3 the cytolytic effect of the NS3 proteins of the orbiviruses AHSV, bluetongue virus (BTV) and equine encephalosis virus (EEV) were compared. Inducible expression in *Escherichia coli* (*E. coli*) showed differences in cytotoxicity, with EEV NS3 having a greater lytic effect than

AHSV and BTV NS3. Cytotoxicity was linked to increased membrane permeability of the cells as confirmed by an increased uptake of membrane impermeant compounds. When expressed in insect cells however all three NS3 proteins caused a marked but equivalent decrease in cell viability. Although the orbivirus NS3 proteins have similar predicted secondary structures, differences could lie in structural stability and association with membranes of specific cell types, which impacts on cytotoxicity. To determine the regions within AHSV NS3 that mediate cytotoxicity, a series of truncated mutants of NS3 were constructed and expressed in *E. coli*. The combined presence of both hydrophobic domains of AHSV NS3 was found to be critical for membrane permeabilisation and cytotoxicity.

In chapter 4 the AHSV-2, AHSV-3 and AHSV-4 NS3 proteins (from the  $\gamma$ ,  $\beta$  and  $\alpha$  NS3 clades) were compared to examine the impact of sequence variation in NS3 on structure and function. The proteins were expressed in the baculovirus expression system as both wild-type proteins and C-terminal eGFP (enhanced green fluorescent protein) fusions. Exogenous addition of the baculovirus expressed proteins to Vero cells resulted in different permeabilisation levels that could be linked to that induced by the AHSV strains. Cell viability and membrane association assays in insect cells showed that all three proteins were equivalently cytotoxic and membrane associated. The subcellular localisation of the eGFP-NS3 fusion proteins was examined by confocal fluorescent imaging of live cells. NS3 localised to the plasma membrane, and as distinct punctuate foci in the perinuclear region. This suggests localisation to the internal membrane systems of cells and has important implications for the function of this membrane permeabilising protein.



## LIST OF ABBREVIATIONS

aa	amino acid
AHS	African horsesickness
AHSV	African horsesickness virus
amp	ampicillin
BHK	baby hamster kidney cells
bp	base pairs
BTV	bluetongue virus
°C	degrees Celsius
cDNA	complementary deoxyribonucleic acid
Ci	Curie
CLP	core-like particle
cm <sup>2</sup>	centimetre squared
CPE	cytopathic effect
CPM	counts per minute
Da	Dalton
DEPC	diethylpyrocarbonate
DLP	double-layered particle
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
ds	double-stranded
DTT	dithiothreitol
EC	endothelial cells
EDTA	ethylenediaminetetra-acetic acid
EEV	equine encephalosis virus
eGFP	enhanced green fluorescent protein
EHDV	epizootic haemorrhagic disease virus
ER	endoplasmic reticulum
<i>et al.</i>	<i>et alibi</i>
EtBr	ethidium bromide
FCS	foetal calf serum
Fig.	figure
g	gravitational force
G	gauge
gal	galactosidase
gent	gentamycin
GFP	green fluorescent protein
GST	glutathione S-transferase
h	hour/s
HD	hydrophobic domain
HIV	human immunodeficiency virus
Hyg B	hygromycin B
i.e.	that is
IgG	immunoglobulin class G
IgY	immunoglobulin class Y
IPTG	isopropyl-β-D thiogalactopyranoside
k	kilo
kan	kanamycin
kb	kilobase pairs
kDa	kilodalton
LB	Luria-Bertani medium
M	molar
MEM	minimal essential medium Eagle
min	minute/s
ml	millilitre



mM	millimolar
MMOH	methyl mercuric hydroxide
MOI	multiplicity of infection
mRNA	messenger RNA
NaCl	sodium chloride
ND	not done
ng	nanograms
NLS	nuclear localisation signal
nm	nanometers
NS	non-structural
OD <sub>600</sub>	optical density at 600 nm
OIE	Office International des Epizooties
ORF	open reading frame
OVI	Onderstepoort Veterinary Institute
P	Particulate
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PEG	polyethylene glycol
pfu	plaque-forming units
p.i.	post infection
PMSF	phenylmethylsulphonyl fluoride
PSB	protein solvent buffer
rif	rifampicin
RNA	ribonucleic acid
rpm	revolutions per minute
S	supernatant
S1-S10	segments 1 to 10 (refers to orbiviruses)
<sup>35</sup> S	radiolabelled sulphur
SD	standard deviation
SDS	sodium dodecyl sulphate
sec	second/s
Sf9	<i>Spodoptera frugiperda</i> insect cells
siRNA	small interfering RNA
ss	single-stranded
TEMED	N,N,N',N'-tetramethylethylene diamine
tet	tetracycline
TM	transmembrane
TMHMM	TransMembrane Hidden Markov Model
Tris	Tris hydroxymethyl aminomethane
UHQ	ultra high quality water
µg	micrograms
µl	microlitres
µm	micrometers
U	units
UP	University of Pretoria
UV	ultraviolet
V	volts
VIB	viral inclusion body
VLP	virus-like particle
VMP	viral membrane protein
VP	virus protein
VPS	vacuolar protein sorting
v/v	volume per volume
v/w	volume per weight
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

## LIST OF BUFFERS

Hypotonic buffer:

10 mM Tris, 0.2 mM MgCl<sub>2</sub> [pH 7.4]

NTE:

100 mM NaCl, 10 mM Tris, 1 mM EDTA [pH 7.4]

PBS:

137 mM NaCl, 2.7 mM KCl, 4.3 mM Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O, 1.4 mM KH<sub>2</sub>PO<sub>4</sub> [pH 7.3]

PSB (2x):

125 mM Tris-HCl [pH 6.8], 4% SDS, 20% glycerol, 10% 2-mercaptoethanol

TGS:

25 mM Tris-HCl [pH 8.3], 192 mM glycine, 0.1% SDS

Transfer buffer:

25 mM Tris, 192 mM glycine

Tris-glycine buffer:

25 mM Tris, 250 mM glycine



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