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

by

Tracy Leonora Meiring

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:
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My family and friends for their encouragement and understanding
SUMMARY

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

by

Tracy Leonora Meiring

Supervisor: Dr Vida van Staden
Department of Genetics
University of Pretoria

Co-supervisor: Prof Henk Huismans
Department of Genetics
University of Pretoria

For the degree PhD

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 α, β and γ. 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 (γ NS3), AHSV-3 (β NS3) and AHSV-4 (α 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 γ, β and α 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.
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<thead>
<tr>
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<tr>
<td>aa</td>
<td>amino acid</td>
</tr>
<tr>
<td>AHS</td>
<td>African horsesickness</td>
</tr>
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<td>AHHSV</td>
<td>African horsesickness virus</td>
</tr>
<tr>
<td>amp</td>
<td>ampicillin</td>
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<td>BHK</td>
<td>baby hamster kidney cells</td>
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<td>BTV</td>
<td>bluetongue virus</td>
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<td>complementary deoxyribonucleic acid</td>
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<td>Curie</td>
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<td>core-like particle</td>
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<td>EEV</td>
<td>equine encephalosis virus</td>
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<td>eGFP</td>
<td>enhanced green fluorescent protein</td>
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<td>EHDV</td>
<td>epizootic haemorrhagic disease virus</td>
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<td>HD</td>
<td>hydrophobic domain</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
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<td>Hyg B</td>
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<td>i.e.</td>
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<td>NS</td>
<td>non-structural</td>
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<td>OD&lt;sub&gt;600&lt;/sub&gt;</td>
<td>optical density at 600 nm</td>
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<td>OIE</td>
<td>Office International des Epizooties</td>
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<td>open reading frame</td>
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<td>Onderstepoort Veterinary Institute</td>
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</tr>
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<td>polyethylene glycol</td>
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<tr>
<td>pfu</td>
<td>plaque-forming units</td>
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<td>p.i.</td>
<td>post infection</td>
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<td>PMSF</td>
<td>phenylmethylsulphonyl fluoride</td>
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<td>rif</td>
<td>rifampicin</td>
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<td>&lt;sup&gt;35&lt;/sup&gt;S</td>
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<td>Sf9</td>
<td><em>Spodoptera frugiperda</em> insect cells</td>
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<td>small interfering RNA</td>
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<td>ss</td>
<td>single-stranded</td>
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<td>TEMED</td>
<td>N,N,N',N'-tetramethylethylene diamine</td>
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<td>tet</td>
<td>tetracycline</td>
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<td>TM</td>
<td>transmembrane</td>
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<td>TMHMM</td>
<td>TransMembrane Hidden Markov Model</td>
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<td>Tris</td>
<td>Tris hydroxymethyl aminomethane</td>
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<td>UHQ</td>
<td>ultra high quality water</td>
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<tr>
<td>µg</td>
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<td>University of Pretoria</td>
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<td>ultraviolet</td>
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<td>V</td>
<td>volts</td>
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<td>VIB</td>
<td>viral inclusion body</td>
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<td>VLP</td>
<td>virus-like particle</td>
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<td>VMP</td>
<td>viral membrane protein</td>
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<td>VP</td>
<td>virus protein</td>
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<td>VPS</td>
<td>vacuolar protein sorting</td>
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<td>volume per weight</td>
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<td>5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside</td>
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LIST OF BUFFERS

Hypotonic buffer:
10 mM Tris, 0.2 mM MgCl$_2$ [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$_2$HPO$_4$.7H$_2$O, 1.4 mM KH$_2$PO$_4$ [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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