CLONING, SEQUENCING AND EXPRESSION ANALYSIS OF THE GENE ENCODING THE PUTATIVE RNA POLYMERASE OF AFRICAN HORSE SICKNESS VIRUS.

By

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But He said to me,
"My grace is sufficient for you,
for my power is made perfect in weakness".
(2 Corinthians 12 v 9a)

dedicated to, and in loving memory of, my father
FA Vreede (1922 – 1998)

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SUMMARY

Cloning, sequencing and expression analysis of the gene encoding the putative RNA polymerase of African horse sickness virus.

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The aim of this investigation was to clone, characterize by sequencing and express the gene that encodes the minor core protein VP1 of African horse sickness virus (AHSV), with a view to the analysis of this protein as the putative viral RNA-dependent RNA polymerase.

A generally applicable technique for the amplification and cloning of large dsRNA genome segments was developed. The approach is both sequence-independent, not requiring any prior knowledge of the gene to be cloned, and convenient, introducing terminal restriction enzyme sites for subsequent subcloning. The full-length VP1 gene of AHSV-1 was cloned.

The VP1 gene of AHSV-9, previously cloned as incomplete cDNA fragments, was assembled and sequenced in its entirety. This represents the first AHSV VP1 gene sequence, and completes molecular characterization of the AHSV genome. AHSV-9 genome segment 1 is 3965 nucleotides in length, encoding a protein of 1305 amino acids with a predicted molecular weight of 150.3K. The amino acid sequence was shown to possess conserved motifs specific for RNA-dependent RNA polymerases. Comparisons with VP1 of other orbiviruses revealed high conservation, confirming the evolutionarily imposed functional constrictions.

The AHSV VP1 gene was furthermore transcribed and expressed in a system that enables the *in vivo* generation of authentic viral RNA. This system utilises recombinant vaccinia virus-expressed T7 RNA polymerase to synthesise transcripts *in vivo* that are autolytically cleaved by ribozyme activity to yield authentic 3' termini. However, expression of AHSV VP1, alone or in combination with other potentially fundamental AHSV proteins, yielded no detectable VP1-specific replicase activity on authentic viral RNA templates in RNA-dependent RNA polymerase assays. AHSV VP1 was subsequently also expressed in a baculovirus system, yielding high levels of insoluble protein.

The results serve as the basis for future investigation of the molecular biology of AHSV and specifically into RNA-dependent RNA polymerase activity of VP1.

ABBREVIATIONS

#	-	number
A	-	adenosine
Å	-	angstrom
AHSV	-	African horse sickness virus
AHSV-1	-	African horse sickness virus serotype 1
AMV	-	avian myeloblastosis virus
ATCC	-	American type culture collection
ATP	-	adenosine-5'-triphosphate
BaMV	-	bamboo mosaic virus
bp	-	base pairs
BSA	-	bovine serum albumin
BTV	-	bluetongue virus
BVDV	-	bovine viral diarrhea virus
C	-	cytidine
°C	-	degrees Celsius
cDNA	-	complementary DNA
CER	-	chicken embryo rabbit
cf.	-	confer (compare)
Ci	-	Curie
CLP	-	core-like particle
dA	-	deoxyadenosine
dATP	-	2'-deoxyadenosine-5'-triphosphate
dC	-	deoxycytidine
dCTP	-	2'-deoxycytidine-5'-triphosphate
dG	-	deoxyguanosine
dGTP	-	2'-deoxyguanosine-5'-triphosphate
DI	-	defective interfering particle
DMEM	-	Dulbecco's modified Eagles' medium
DMSO	-	dimethyl sulfoxide

DNA	-	deoxyribonucleic acid
dNTP	-	2'-deoxynucleoside-5'-triphosphate
ds	-	double-strand
dT	-	deoxythymidine
DTT	-	1,4-dithiothreitol
dTTP	-	2'-deoxythymidine-5'-triphosphate
EDTA	-	ethylenediaminetetra-acetic acid
EEV	-	equine encephalosis virus
EHDV	-	epizootic hemorrhagic disease virus
et al.	-	et alii (and others)
fg	-	femtogram
FCS	-	foetal calf serum
FHV	-	flock house virus
FLUAV	-	Influenza A virus
g	-	gram / gravitational acceleration
G	-	guanosine
h	-	hour
HA	-	haemaglutinin
HDV	-	hepatitis delta virus
HEPES	-	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPRI	-	human placental ribonuclease inhibitor
IBDV	-	infectious bursal disease virus
i.e.	-	id est (that is)
IPTG	-	isopropyl-ß-D-thiogalactopyranoside
ISVP	-	infectious sub-viral particle
K	-	kilodalton
kb	-	kilobase pairs
lacZ	-	β-galactosidase gene
M	-	molar
mA	-	milliampere
mCi	-	millicurie
min	-	minute

ml	-	millilitre
mM	-	millimolar
MMOH	-	methylmercuric hydroxide
mmol	-	millimole
MOI	-	multiplicity of infection
mol	-	mole
M_r	-	molecular weight
mRNA	-	messenger RNA
m/v	-	mass per volume
NLRV	-	*Nilaparvata lugens* reovirus
NaAc	-	sodium acetate
ng	-	nanogram
NS	-	non-structural
OD_{550}	-	optical density at 550nm
orf	-	open reading frame
PAGE	-	polyacrylamide gel electrophoresis
PCR	-	polymerase chain reaction
pfu	-	plaque forming units
pmol	-	picomole
PNK	-	polynucleotide kinase
PPO	-	2,5-diphenyloxazole
PV	-	poliovirus
RABV	-	rabies virus
RDV	-	rice dwarf virus
RNA	-	ribonucleic acid
RNP	-	ribonucleoprotein
rpm	-	revolutions per minute
RRSV	-	rice ragged stunt virus
RT-PCR	-	reverse transcriptase polymerase chain reaction
s	-	second
SDS	-	sodium dodecyl sulphate
SeV	-	Sendai virus
Sf9	-	*Spodoptera frugiperda*

ss	-	single-strand
SV40	-	simian virus 40
TEMED	-	N,N,N',N'-tetramethylethylenediamine
Tris	-	Tris(hydroxymethyl)-aminomethane
U	-	uridine
µCi	-	microcurie
µg	-	microgram
µl	-	microlitre
µM	-	micromolar
UV	-	ultraviolet
V	-	volts
VACV	-	vaccinia virus
VLP	-	virus-like particle
VP	-	viral protein
vRNA	-	viral RNA
VSV	-	vesicular stomatitis virus
v/v	-	volume per volume
X-gal	-	5-bromo-4-chloro-3-indolyl-ß-D-galactopyranoside

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