STUDIES INTO THE ROLE OF CAPSID SERINE/THREONINE RESIDUES IN HIV CORE STABILITY AND VIRUS REPLICATION

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TABLE OF CONTENT

ABSTRACT

Disassembly of the HIV viral core describes the rearrangement and release of capsid (CA) from the core following entry into the host cell. In this process, while the conical shaped core may be lost, some CA remains associated with the resulting reverse transcription (RTC) and preintegration complexes (PIC).

What triggers release of CA from the core is unknown. Cores from virus containing mutations in CA that show altered core stability, and release CA from the core at rates faster or slower than wild type (WT) virus demonstrate blocks in replication during reverse transcription and nuclear translocation. How the CA protein affects theses process is not understood, but intrinsic stability of the core is instrumental in regulating interactions with cellular factors. Evidence suggests that core disassembly is critical for the early steps in HIV replication and it may regulate replication in a cell type dependent fashion.

Mutation of charged residues throughout CA results in viruses displaying altered core stability. Regulation of charge in the core, possibly by phosphorylation of CA, is one potential mechanism that may control core disassembly. Substitution of serine residues within CA illustrates five viruses, including three representing the major phospho-acceptor sites (S109, S149 and S178) that show altered replication profiles.

To explore the role of these residues in core disassembly, the present study investigated the *in vitro* stability and the intracellular disassembly of the cores from these viruses. Chapter 3 describes the characterisation of viruses with mutations in CA at S41A, S109A, S146A, S149A, S178A and T188V to analyse the effect of substitution at these sites on viral replication. Substitution at S109, S149, S178 and T188 reduced replication competence and altered the production of reverse transcription intermediates. S41A and S146A demonstrated altered reverse transcription, but did not result in blocks in replication.

Chapter 4 describes modification of an assay to examine viral core stability. Using this assay, CA mutant viruses (S109A, S149A and S178A) demonstrated reduced *in vitro* stability of the viral core in comparison with WT NL4-3 virus. Analysis of core disassembly following cell infection (Chapter 5) could not identify defects in core disassembly inside the cell, but suggested progressive changes occurred to viral complexes following infection.

The results in this thesis suggest that substitution in CA at S109, S149 and S178 alters *in vitro* core stability in these viruses, and may impact on core disassembly during HIV replication.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Sarah Martin 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.

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ABBREVIATIONS

A	Alpha
A	Alanine
Å	angstrom(s)
aa	amino acid
AIDS	acquired immune deficiency syndrome
AK	auto-activated protein kinase
AMV	avian myeloblastosis virus
ARV	AIDS-associated retrovirus
ATP	adenosine triphosphate
AZT	Azidothymidine
B	Beta
βME	β-mercaptoethanol
bp	base pair(s)
BSA	bovine serum albumin
°C	degrees Celsius
CA, p24	Capsid
cAMP	cyclic AMP
CaMV	cauliflower mosaic virus
CCR5	chemokine (C-C motif) receptor 5
CDC	Centers for Disease Control and Prevention
cDNA	coding DNA
CEL	core envelope linkage
CK-II	casein kinase II
CO2	carbon dioxide
CPK	cytosolic protamine kinase
C-PKA	cyclic AMP-dependent protein kinase
CTD	carboxyl terminal domain
CTL	cytotoxic T lymphocyte
CXCR4	chemokine (C-X-C motif) receptor 4
CypA	cyclophilin A
D	aspartic acid
Da	dalton(s)
DHBV	duck hepatitis B virus
DMEM	Dulbecco's Modified Eagle's medium
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
dsDNA	double stranded DNA
dUTP	deoxyuridine triphosphate
E	glutamic acid
EDTA	ethylenediaminetetraacetic acid
EIAV	equine infectious anaemia virus
ELISA	enzyme-linked immunosorbent assay
EM	electron microscopy
ER	endoplasmic reticulum
ERK2	extracellular-signal regulated kinase
ERT	endogenous reverse transcription
FAK	focal adhesion kinase
FCS	foetal calf serum
FDA	Food and Drug Administration
FeLV	feline leukaemia virus
FMDV	foot and mouth disease virus
FPLC	fast phase liquid chromatography
G	gram(s)
x *g*	g force
G	glycine
g/mL	grams per millilitre
GDP	guanosine diphosphate
GFP	green fluorescent protein
GTP	guanosine triphosphate
HAART	highly active anti-retroviral therapy
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HLA-DR	human leukocyte antigen DR
HPV	human papilloma virus
hr	hour(s)
HRP	horse radish peroxidase
Hsp70	heat shock protein 70
HSV	herpes simplex virus

HTLV	human T-cell leukaemia virus
ICTV	International Committee on the Taxonomy of Viruses
IDAV	immunodeficiency-associated virus
IL	Interleukin
IN	Integrase
K	Lysine
kb	kilobase(s)
kDa	kilodalton(s)
L	Leucine
LAV	lymphadenopathy-associated virus
LB	Luria-Bertani broth
Lck	lymphocyte-specific protein tyrosine kinase
LTR	long terminal repeat
M	Methionine
M	molar (moles/litre)
MA	Matrix
MAPK	mitogen activated protein kinase
MBPK	myelin basic protein kinase
MEK	MAPK ERK2 kinase
mg	milligram(s)
$MgCl_2$	magnesium chloride
MHC	major histocompatibility complex
MHR	major homology region
min	minute(s)
MIP	macrophage inflammatory protein
mL	millilitre(s)
MLV	murine leukaemia virus
mm	millimetre(s)
mM	Millimolar
MMTV	mouse mammary tumour virus
M-MuLV	moloney murine leukaemia virus
MOI	multiplicity of infection
mRNA	messenger RNA
MTOC	microtubule-organising centre
MWCO	molecular weight cut-off
ng	nanogram(s)
nm	nanometer(s)
N	Asparagine
NC	Nucleocapsid
NDR	nuclear Dbf2 related kinase
Nef	negative replication factor
NERT	natural endogenous reverse transcription
NIH	National Institute of Health
NMR	nuclear magnetic resonance
NNRTI	non-nucleoside reverse transcriptase inhibitor
NPC	nuclear pore complex
NRTI	nucleoside reverse transcriptase inhibitor
nt	Nucleotide
NTD	amino terminal domain
OD	optical density
OH	hydroxyl group
P	Proline
p.i.	post infection
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PBS	primer binding site
PCR	polymerase chain reaction
pI	isoelectric point
PI	protease inhibitor
PIC	preintegration complex
PKC	protein kinase C
PKR	RNA dependent protein kinase
PO_4	Phosphate
PPT	polypurine tract
PR	protease inhibitor
PVA	potato virus A
PVDF	polyvinylidene fluoride
pyk2	proline-rich tyrosine kinase 2
Q	Glutamine
R	Arginine
R	repeat region
Rev	regulator of virion expression
RIPA	radio immunoprecipitation assay
RNA	ribonucleic acid
RNaseH	ribonuclease H
rpm	revolutions per minute

RRE	Rev response element	>	greater than
RT	reverse transcriptase	≥	equal to or greater than
RTC	reverse transcription complex		
S	serine		
<	less than		
SDS	sodium dodecyl sulfate		
sec	second(s)		
SIV	simian immunodeficiency virus		
ssDNA	single stranded DNA		
T	Threonine		
TAR	trans-activating response region		
TAS	temperature arrested state (of fusion)		
Tat	trans-activator protein		
TCA	trichloroacetic acid		
TEM	transmission electron microscopy		
TNF	tumour necrosis factor		
TRIM5α	tripartite motif protein 5 alpha		
µg	microgram(s)		
µL	microlitre(s)		
U3 or U5	untranslated region (3' or 5')		
UNAIDS	Joint United Nations Programme on AIDS		
UV	Ultraviolet		
V	Valine		
V	volt(s)		
v/v	volume per volume		
VAPK	virus-associated protein kinase		
Vif	virion infectivity factor		
VLP	virus-like particles		
Vpr	viral protein R		
Vpu	viral protein U		
VSV-G	vesicular stomatitis virus glycoprotein		
W	Tryptophan		
WHO	World Health Organisation		
WT	wild type		
[3H]-dTTP	tritiated thymidine		

PRESENTATIONS ARISING

Conference Presentations

Oral Presentations:

Oct 2006
Australian Centre for Hepatitis Virology & HIV Virology Interest Group 3rd National Scientific Workshop in Lorne, Victoria:
HIV-1 capsid phosphorylation, capsid disassembly and reverse transcription

Poster Presentations:

Dec 2005
The 3rd Scientific Meeting of the Australian Virology Group at Cowes, Phillip Island, Victoria:
Investigating a role for HIV-1 Capsid Phosphorylation in Virus Uncoating and Reverse Transcription (abstract #253)

July 2007
Annual Scientific Meeting of the Australian Society for Microbiology, in Adelaide, South Australia:
HIV-1 Capsid Phosphorylation and Core Disassembly

Dec 2007
The 4th Scientific Meeting of the Australian Virology Group at Fraser Island, Queensland:
Investigating the role of HIV-1 capsid phosphorylation in capsid disassembly and reverse transcription (abstract #143)