

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

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## 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 these 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	DNA	deoxyribonucleic acid
A	Alanine	dNTP	deoxynucleoside triphosphate
Å	angstrom(s)	dsDNA	double stranded DNA
aa	amino acid	dUTP	deoxyuridine triphosphate
AIDS	acquired immune deficiency syndrome	E	glutamic acid
AK	auto-activated protein kinase	EDTA	ethylenediaminetetraacetic acid
AMV	avian myeloblastosis virus	EIAV	equine infectious anaemia virus
ARV	AIDS-associated retrovirus	ELISA	enzyme-linked immunosorbent assay
ATP	adenosine triphosphate	EM	electron microscopy
AZT	Azidothymidine	ER	endoplasmic reticulum
B	Beta	ERK2	extracellular-signal regulated kinase
βME	β-mercaptoethanol	ERT	endogenous reverse transcription
bp	base pair(s)	FAK	focal adhesion kinase
BSA	bovine serum albumin	FCS	foetal calf serum
°C	degrees Celsius	FDA	Food and Drug Administration
CA, p24	Capsid	FeLV	feline leukaemia virus
cAMP	cyclic AMP	FMDV	foot and mouth disease virus
CaMV	cauliflower mosaic virus	FPLC	fast phase liquid chromatography
CCR5	chemokine (C-C motif) receptor 5	G	gram(s)
CDC	Centers for Disease Control and Prevention	x g	g force
cDNA	coding DNA	G	glycine
CEL	core envelope linkage	g/mL	grams per millilitre
CK-II	casein kinase II	GDP	guanosine diphosphate
CO <sub>2</sub>	carbon dioxide	GFP	green fluorescent protein
CPK	cytosolic protamine kinase	GTP	guanosine triphosphate
C-PKA	cyclic AMP-dependent protein kinase	HAART	highly active anti-retroviral therapy
CTD	carboxyl terminal domain	HBV	hepatitis B virus
CTL	cytotoxic T lymphocyte	HCV	hepatitis C virus
CXCR4	chemokine (C-X-C motif) receptor 4	HIV	human immunodeficiency virus
CypA	cyclophilin A	HLA-DR	human leukocyte antigen DR
D	aspartic acid	HPV	human papilloma virus
Da	dalton(s)	hr	hour(s)
DHBV	duck hepatitis B virus	HRP	horse radish peroxidase
DMEM	Dulbecco's Modified Eagle's medium	Hsp70	heat shock protein 70
DMSO	dimethyl sulfoxide	HSV	herpes simplex virus

HTLV	human T-cell leukaemia virus	N	Asparagine
ICTV	International Committee on the Taxonomy of Viruses	NC	Nucleocapsid
IDAV	immunodeficiency-associated virus	NDR	nuclear Dbf2 related kinase
IL	Interleukin	Nef	negative replication factor
IN	Integrase	NERT	natural endogenous reverse transcription
K	Lysine	NIH	National Institute of Health
kb	kilobase(s)	NMR	nuclear magnetic resonance
kDa	kilodalton(s)	NNRTI	non-nucleoside reverse transcriptase inhibitor
L	Leucine	NPC	nuclear pore complex
LAV	lymphadenopathy-associated virus	NRTI	nucleoside reverse transcriptase inhibitor
LB	Luria-Bertani broth	nt	Nucleotide
Lck	lymphocyte-specific protein tyrosine kinase	NTD	amino terminal domain
LTR	long terminal repeat	OD	optical density
M	Methionine	OH	hydroxyl group
M	molar (moles/litre)	P	Proline
MA	Matrix	p.i.	post infection
MAPK	mitogen activated protein kinase	PAGE	polyacrylamide gel electrophoresis
MBPK	myelin basic protein kinase	PBS	phosphate buffered saline
MEK	MAPK ERK2 kinase	PBS	primer binding site
mg	milligram(s)	PCR	polymerase chain reaction
MgCl <sub>2</sub>	magnesium chloride	pI	isoelectric point
MHC	major histocompatibility complex	PI	protease inhibitor
MHR	major homology region	PIC	preintegration complex
min	minute(s)	PKC	protein kinase C
MIP	macrophage inflammatory protein	PKR	RNA dependent protein kinase
mL	millilitre(s)	PO <sub>4</sub>	Phosphate
MLV	murine leukaemia virus	PPT	polypurine tract
mm	millimetre(s)	PR	protease inhibitor
mM	Millimolar	PVA	potato virus A
MMTV	mouse mammary tumour virus	PVDF	polyvinylidene fluoride
M-	moloney murine leukaemia virus	pyk2	proline-rich tyrosine kinase 2
MuLV	mouse mammary tumour virus	Q	Glutamine
MOI	multiplicity of infection	R	Arginine
mRNA	messenger RNA	R	repeat region
MTOC	microtubule-organising centre	Rev	regulator of virion expression
MWCO	molecular weight cut-off	RIPA	radio immunoprecipitation assay
ng	nanogram(s)	RNA	ribonucleic acid
nm	nanometer(s)	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		
TRIM5 $\alpha$	tripartite motif protein 5 alpha		
$\mu$ g	microgram(s)		
$\mu$ L	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 3<sup>rd</sup> 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)