Dynamic Imaging of Hepatitis C Virus RNA Localisation and Traffic During Viral Replication

Guillaume Nicolas Fiches

Engineer in Biotechnology and Biochemistry

(National Institute of Applied Sciences, Lyon, France)

Department of Molecular and Cellular Biology

School of Biological Sciences

The University of Adelaide

A dissertation submitted to The University of Adelaide

in candidature for the degree of

Doctor of Philosophy in the Faculty of Science

March 2015

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Dear reader,

Please find on this DVD the movies relevant to my thesis. If you are experiencing any problems to play the files, please try installing the free multimedia player VLC, available for either windows (http://www.videolan.org/vlc/download-windows.html) and Mac OS (http://www.videolan.org/vlc/download-macosx.html) or contact Associate Professor Michael Beard (Michael.beard@adelaide.edu.au).

Thanks you for your time.

Best regards,

Guillaume Fiches

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Abstract

Much of our understanding of the HCV life cycle and host-viral interactions has evolved from the visualisation of fixed images of infected cells. However, the recent development of live cell imaging techniques now allows viral life cycles to be visualised in live cell cultures. We have tagged the NS5A protein of the infectious Jc1 chimera (J6/JFH-1) with fluorescent tags and shown that NS5A segregates into two distinct populations: one relatively static and one highly motile, although the role and composition of these structures is not well understood. To investigate HCV RNA dynamics throughout the viral life cycle and examine whether either or both sub-classes of NS5A-positive structures are enriched with HCV RNA we developed a system to simultaneously track HCV RNA and NS5A in living cells.

MS2 bacteriophage RNA stem loop sequences (6x /8x /12x /24x repeats) were inserted into the 3'UTR of the Jc1/5A-TCM virus (Jc1/5A-TCM+3'UTR:MS2) to allow indirect tracking of HCV RNA in Huh-7.5 cells via MS2.Coat-mCherry fusion protein that interacts specifically with MS2 stem loops. Jc1/5A-TCM+3'UTR:MS2 viruses replicated to significantly lower levels than the parent Jc1 as assessed by immunofluorescence analysis. However, long-term culture resulted in emergence of more efficient viral replication, with PCR and sequence analysis indicating at least partial retention of MS2 stem loops at 8 days post electroporation of HCV RNA. To further characterize and overcome the replication handicap induced by the insertion of the MS2 stem loop sequences we also generated Huh-7.5 cells that harbour the HCV subgenomic replicon featuring these MS2 stem loops insertions. Deep sequencing

analysis was conducted to identify emerging adaptive mutations. However none was found to be particularly predominant.

Most importantly, redistribution of the mCherry tagged-MS2 coat protein from a homogenous cytoplasmic distribution to a more punctate localisation was observed in the context of the full-length viral cultures indicating specific binding to HCV RNA. Using this approach we have simultaneously visualised HCV RNA (MS2.coat-mCherry) and NS5A traffic (FlAsH) in real-time during HCV replication. Both HCV RNA-positive small motile and larger static structures were enriched with NS5A. In contrast, a subset of the trafficking NS5A-positive structures was devoid of HCV RNA. We also investigated viral RNA traffic with respect to lipid droplets (LDs) and show that two sub-types of static HCV RNA-positive structures existed: one was closely juxtaposed to LDs while the second sub-class was localised away from LDs. Moreover the system enabled visualization of putative RNA delivery at the LD surface with examples of motile HCV RNA-enriched structures dynamically interacting with LDs. Finally performing co-imaging of HCV NS5A and Rab18, an NS5A-interacting host factor located at the LD surface, we were able to illustrate the often transient nature of NS5A interaction with the LD and putative sampling of the LD that may precede interaction with core and initiation of assembly steps of the viral life cycle. These studies reveal new insights into the dynamics of HCV RNA traffic and the interactions at play in the context of the HCV life cycle.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Acknowledgements

I first would like to thank my supervisor Associate Professor Michael R. Beard to give me the opportunity to conduct my PhD in his laboratory and for the continued mentoring and support he provided me throughout the years.

I also would like to sincerely thank my co-supervisor Dr Nicholas Eyre for his excellent teaching, encouragements and technical assistance during these years.

I would like to extend my sincere gratitude to all the other members past and present of the Hepatitis C Virus research laboratory and especially Dr Amanda Aloia and Dr Kylie Van Der Hoek for their assistance and friendship.

I would also like to thank Dr Fabio Luciani and Dr Brigit Betz-Stablein (UNSW, Sydney) for their assistance in interpretating the deep sequencing data.

I would also like to thank The University of Adelaide for providing me with an ASI scholarship enabling me to conduct my PhD here in Adelaide.

Finally I would like to thank my family and especially my parents Marie-Christine and Bernard and my brother Anthony for their continued encouragements and support from afar. I would also like to thank my grand-pa Georges for his encouragements but who sadly passed away during the last year of my studies.

Presentations Arising From PhD

International:

20th International Symposium on Hepatitis C and related Viruses, Melbourne – October 2013: Dynamic imaging of HCV Viral RNA traffic and localisation *(Oral presentation)*

19th International Symposium on Hepatitis C and related Viruses, Venice - October (2012). Dynamic imaging of HCV Viral RNA traffic and localisation *(Poster presentation)*

National:

The Australian Society for Microbiology Annual Scientific Meeting, Adelaide, July 2013: Dynamic imaging of HCV Viral RNA traffic and localisation *(Oral presentation)*

Australian Centre for HIV and Hepatitis Virology Research Workshop, Adelaide - June 2012: Dynamics of HCV Viral RNA traffic and localisation *(Oral presentation)*

Australian Centre for HIV and Hepatitis Virology Research Workshop, Maroochydore - June 2011: The influence of Hepatitis C Virus genotype on NS5A protein localisation and traffic *(Oral presentation)*

Publication Arising During PhD

Directly related to thesis:

Fiches, G.N., Eyre, N.S., Aloia, A., Van Der Hoek, K., Betz-Stablein, B., Luciani, F., Chopra, A., Beard. M.R., 2015. Dynamic imaging of HCV RNA traffic in living cells. *(In preparation).*

Eyre, N.S., **Fiches, G.N**., Aloia, A.L., Helbig, K.J., McCartney, E.M., McErlean, C.S., Li, K., Aggarwal, A., Turville, S.G., Beard, M.R., 2014. Dynamic imaging of the hepatitis C virus NS5A protein during a productive infection. Journal of virology 88, 3636-3652.

Unrelated to thesis:

Helbig, K.J., Carr, J.M., Calvert, J.K., Wati, S., Clarke, J.N., Eyre, N.S., Narayana, S.K., **Fiches, G.N**., McCartney, E.M., Beard, M.R., 2013. Viperin is induced following dengue virus type-2 (DENV-2) infection and has anti-viral actions requiring the C-terminal end of viperin. PLoS neglected tropical diseases 7, e2178.

Helbig, K.J., Eyre, N.S., Yip, E., Narayana, S., Li, K., **Fiches, G.**, McCartney, E.M., Jangra, R.K., Lemon, S.M., Beard, M.R., 2011. The antiviral protein viperin inhibits hepatitis C virus replication via interaction with nonstructural protein 5A. Hepatology 54, 1506-1517.

Awards Received During PhD

2013 20th International Symposium on Hepatitis C and related Viruses, Melbourne, student Travel grant – 450$

2013 The Australian Society for Microbiology Annual Scientific Meeting, Adelaide, Bursary Award – 250$

2012 Australian Centre for HIV and Hepatitis Virology Research Workshop, Adelaide, Robert Dixon award – 500$

Abbreviations Used

A	adenosine
aa	amino acids
AP2M1	clathrin adaptor protein complex 2, µ1 sub-unit
ApoE	apolipoprotein E
ATP	adenosine triphosphate
BFP	blue fluorescent protein
bp	base pair
BrUTP	5-bromouridine 5’-triphosphate
BSA	bovine serum albumin
°C	degrees Celsius
C	cytosine
cDNA	complimentary deoxyribosenucleic acid
CHC	chronic hepatitis C
cLD	cytoplasmic lipid droplet
CLDN	claudin
CMV	cytomegalovirus
cPLA	cytosolic phospholipase A2
C_T	threshold cycle
DAPI	4’, 6-Diamidino-2-pheylinodole
dH_2O	deionised water
DAA	direct acting antiviral
DGAT1	diacylglycerol O-acyltransferase 1

DMEM	Dulbecco’s Modified Eagle Medium
DMSO	dimethyl sulfoxide
DMV	double-membrane vesicle
DNA	deoxyribosenucleic acid
dNTP	deoxyribosenucleotide triphosphate
ds	double stranded
dsRNA	double stranded RNA
DTT	dithiothreitol
EASL	European Association for the Study of the Liver
ECMV	encephalomyocarditis virus
EDTA	ethylene diamine tetra acetic acid
ER	endoplasmic reticulum
FACS	fluorescence-activated cell sorting
FCS	foetal calf serum
FDA	Food and Drug Administration
ffu	focus forming units
FISH	fluorescent *in situ* hybridization
FITC	fluorescein isothiocyanate
g	grams
g	G-force
G	guanosine
GAG	glycosaminoglycan
GFP	green fluorescent protein
HBV	hepatitis B virus
HCC	hepatocellular carcinoma

HCV	hepatitis C virus
HCVcc	cell-culture propagated hepatitis C virus
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIV	human immunodeficiency virus
HRP	horse radish peroxidase
HuH	human hepatoma
h-VAPA	human vesicle-associated membrane protein-associated protein A
h-VAPB	human vesicle-associated membrane protein-associated protein B
HVR1	hyper variable region 1
IFN-α	interferon alpha
Ig	Immunoglobulin
IRES	internal ribosome entry site
ISH	*in situ* hybridization
IV	intravenous
kb	kilobase
kDa	kilo Dalton
L-Agar	LB + agar
LB	Luria Bertani broth
LD	lipid droplet
LDL	low density lipoproteins
LDLR	low density lipoprotein receptor
LRA	long-range annealing
Luc	luciferase
LVP	lipoviral particle
µg	micrograms

μL	microlitres
μM	micromolar
mA	milliamps
mg	milligrams
mL	millilitres
mM	millimolar
MCL	monoclonal cell line
MCP	MS2 bacteriophage coat protein
MCS	multiple cloning site
min	minute
miR-122	micro RNA 122
MLV	murine leukemia virus
MMV	multi-membrane vesicle
MOI	multiplicity of infection
mRNA	messenger RNA
MW	membranous webs
MW	molecular weight
NANBH	non-A, non-B hepatitis
NCR	non coding region
ng	nanograms
nM	nanomolar
NPC1L1	Niemann-Pick C1 like 1
NS	non-structural
NTR	non translated region
nts	nucleotides

OCLN	occludin
OD	optical density
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCL	polyclonal cell line
PCR	polymerase chain reaction
PEG	pegylated
PI4KIIIα	phosphatidylinositol 4-kinase III alpha
PI4P	phosphatidylinositol 4-phosphate
RBV	Ribavirin
RC	replication complex
RdRp	RNA-dependent RNA polymerase
RNA	ribonucleic acid
rpm	revolutions per minute
RT	room temperature
RT-PCR	reverse transcriptase polymerase chain reaction
sd	standard deviation
SDS	sodium dodecyl sulphate
sec	second(s)
SL	stem loop
SMV	single-membrane vesicle
SNR	signal-to-noise ratio
SOC	super optimal broth with catabolite repression
SRB1	scavenger receptor class B1

ss	single stranded
SV40	simian virus 40
T	thymidine
TAE	tris, acetic acid, EDTA (TAE) buffer
TBEV	tick-borne encephalitis virus
tk	thymidine kinase
Tris	tris(hydroxymethyl)aminomethane
U	unit(s)
UTR	untranslated region
UV	ultraviolet
V	volt(s)
VLDL	very low density lipoprotein
v:v	volume per volume
w:v	weight per volume
WHO	World Health Organization
WT	wild-type

Materials Providers

Abcam	Cambridge, UK
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Amresco	Ohio, USA
Applied Biosystem	Massachusetts, USA
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Thermo Scientific	Massachusetts, USA
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