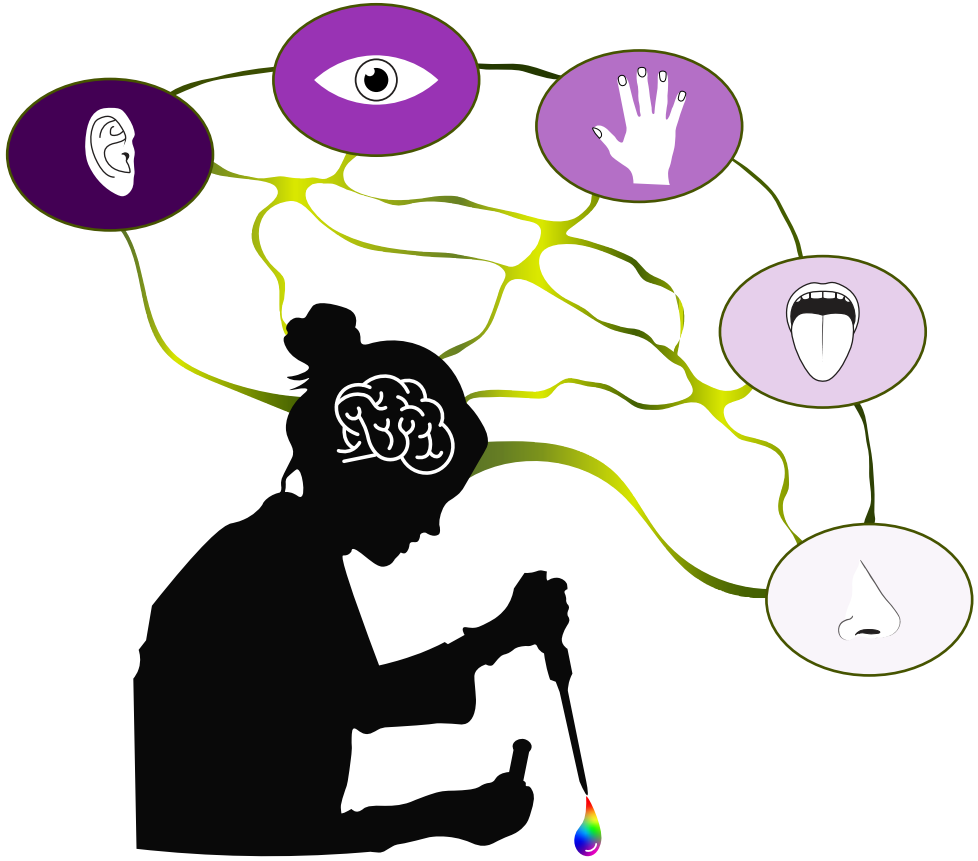
Vanesa Madan

Scientific Visual Communicator

Portfolio 2024



vmadanr@gmail.com

Hello!

I am Vanesa Madan, a biologist with a passion for scientific illustration. In my scientific journey I have had the pleasure to conduct challenging research projects in the cell and molecular biology fields in three different countries. An important part in my life as a scientist is to communicate not only orally but also visually my new findings. I have always enjoyed getting involved in the process of creating schematics and illustrations that give support and clarify a complex message in a presentation. I find very gratifying when the message reaches your audience.

In this portfolio, I have collected a series of *journal figures* (including schematics and models to explain virology concepts) that were published during my PhD and postdoctoral work in peer-reviewed journals. You can also find a *scientific poster* on "molecular motors" that I created and presented in a virtual Cell Biology conference. Finally, I show a recent project that includes a web design mockup and logo for a ficticious biopharmaceutical company.

Thank you very much for giving me the opportunity to show and share my portfolio.

Sincerely,

Vanesa Madan

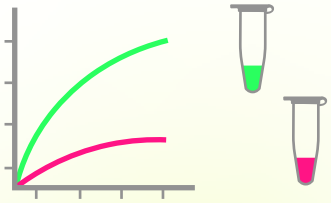
Scientific illustrations included in this portfolio were generated during my work in the following research institutes:

CBM Severo Ochoa — Madrid (Spain)

Universitätsklinikum Heidelberg — Molecular Virology Heidelberg — Heidelberg (Germany)

UKRI MRC Laboratory of Molecular Biology — Cambridge (UK)

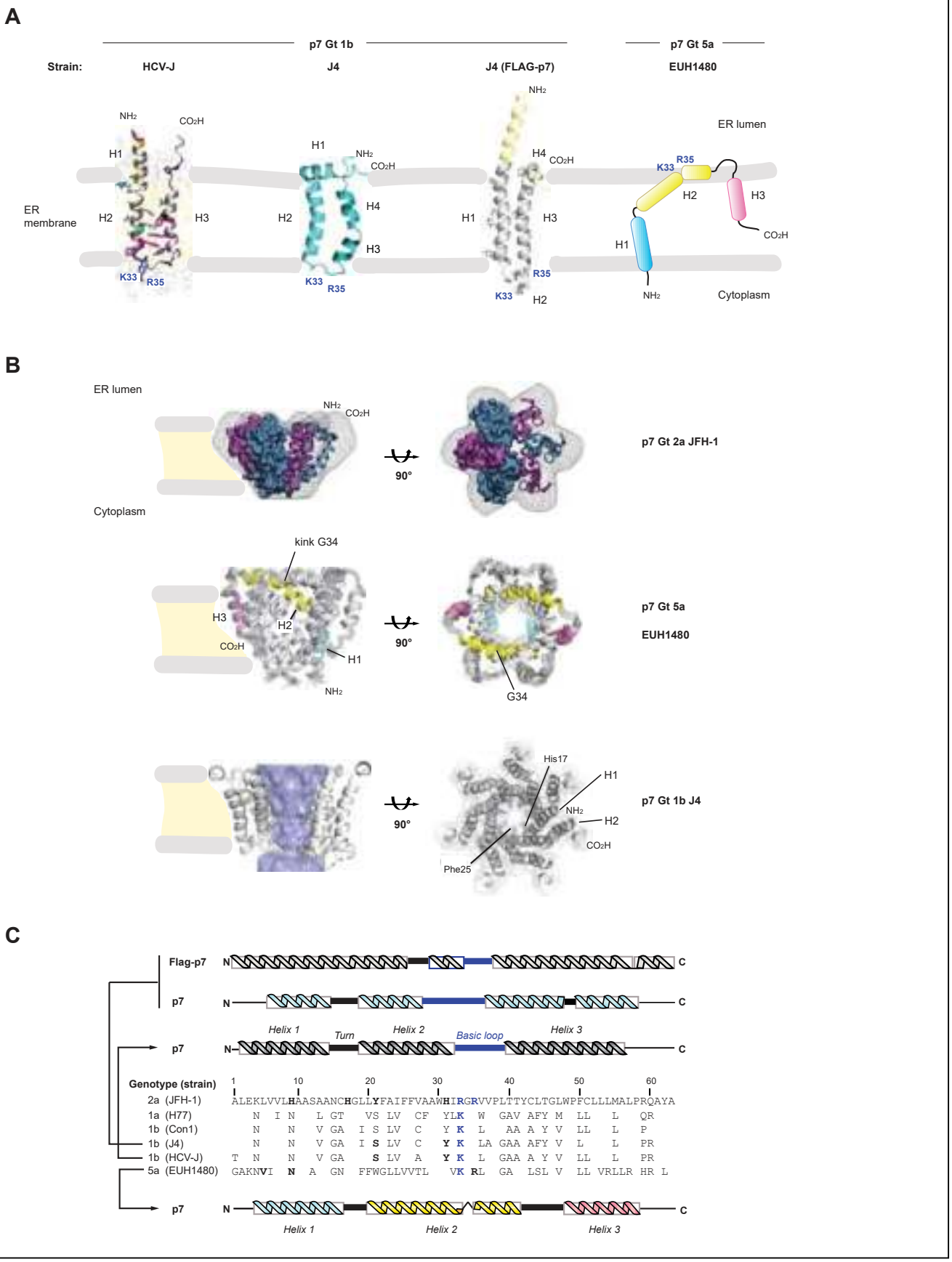
Journal Figures and Graphical Abstracts



Structural and Functional Properties of the Hepatitis C Virus p7 Viroporin

Published in (Journal/Editorial):

viruses MDPI



Journal figure from review: ***Viruses***. 2015 Aug; 7(8): 4461–4481: doi:10.3390/v7082826

Figure 2. Molecular structure of p7 monomer and hexamer.

(A) Membrane topology of Hepatitis C virus p7 proteins.

(B) Structure of p7 hexamer. Side orientation (in the membrane) and top views are represented.

(C) Multiple sequence aligment of p7 proteins and schematic representation of structured regions.

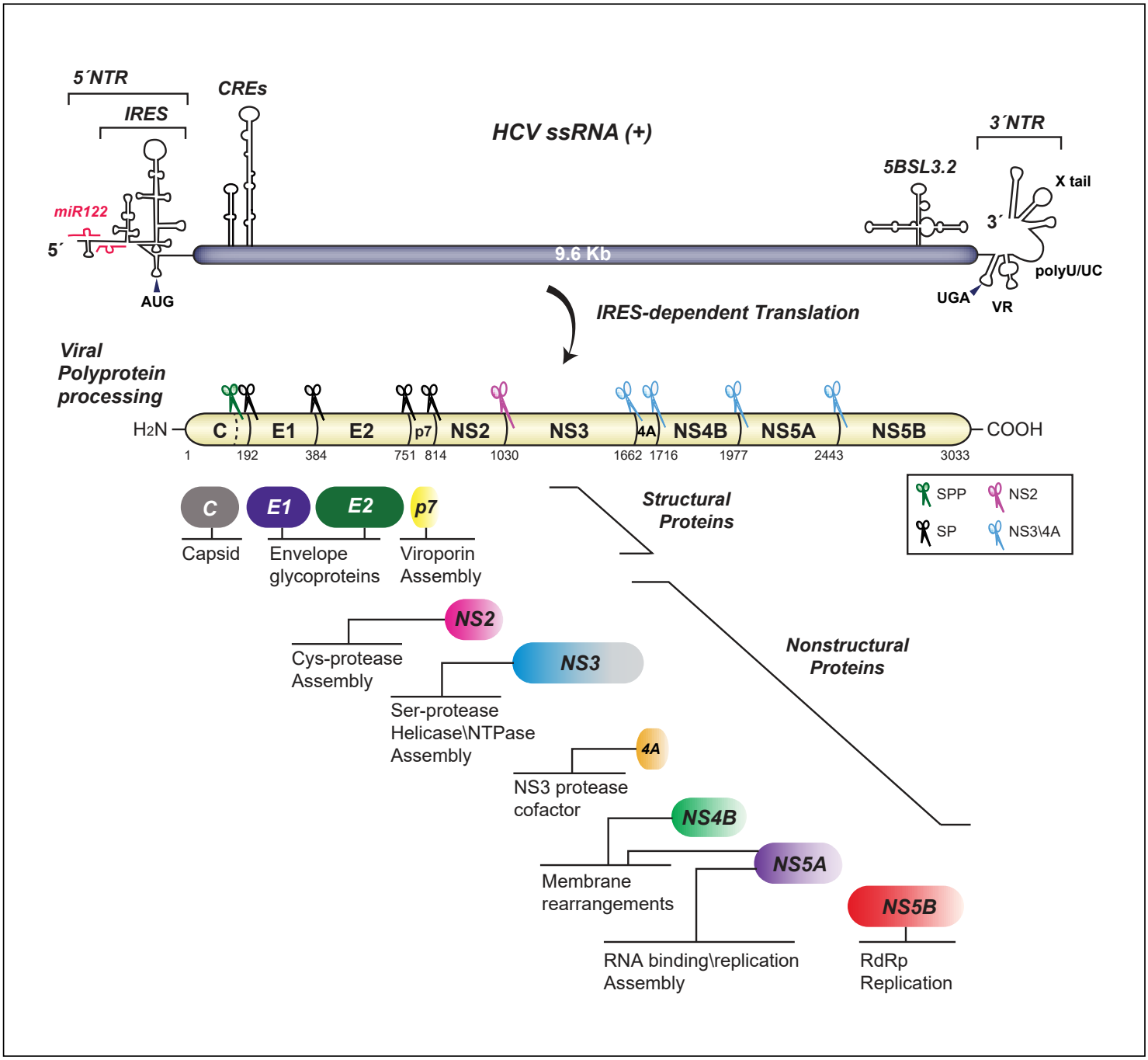
Created by Vanesa Madan. Review written by Vanesa Madan and Ralf Bartenschlager.

Hepatitis C Virus RNA Replication and Assembly: Living on the Fat of the Land

Published in (Journal/Editorial):

Cell Host & Microbe
Review

CellPress



Journal figure from review: ***Cell Host Microbe.*** 2014 Nov 12; 16(5): 569–579. http://dx.doi.org/10.1016/j.chom.2014.10.008

Figure 1. Schematic representation of Hepatitis C virus genome organisation, polyprotein processing and viral proteins function.
The single-strand (ss) HCV RNA genome is shown on the top.
The viral polyprotein is represented in yellow.
The cleavage of the viral polyprotein by cellular (SPP and SP) and viral proteases (NS4 and NS3/4A) is indicated by scissors.
Functions of cleavage products are indicated below each viral protein.

Created by Vanesa Madan.

Review written by Vanesa Madan, David Paul and Ralf Bartenschlager.

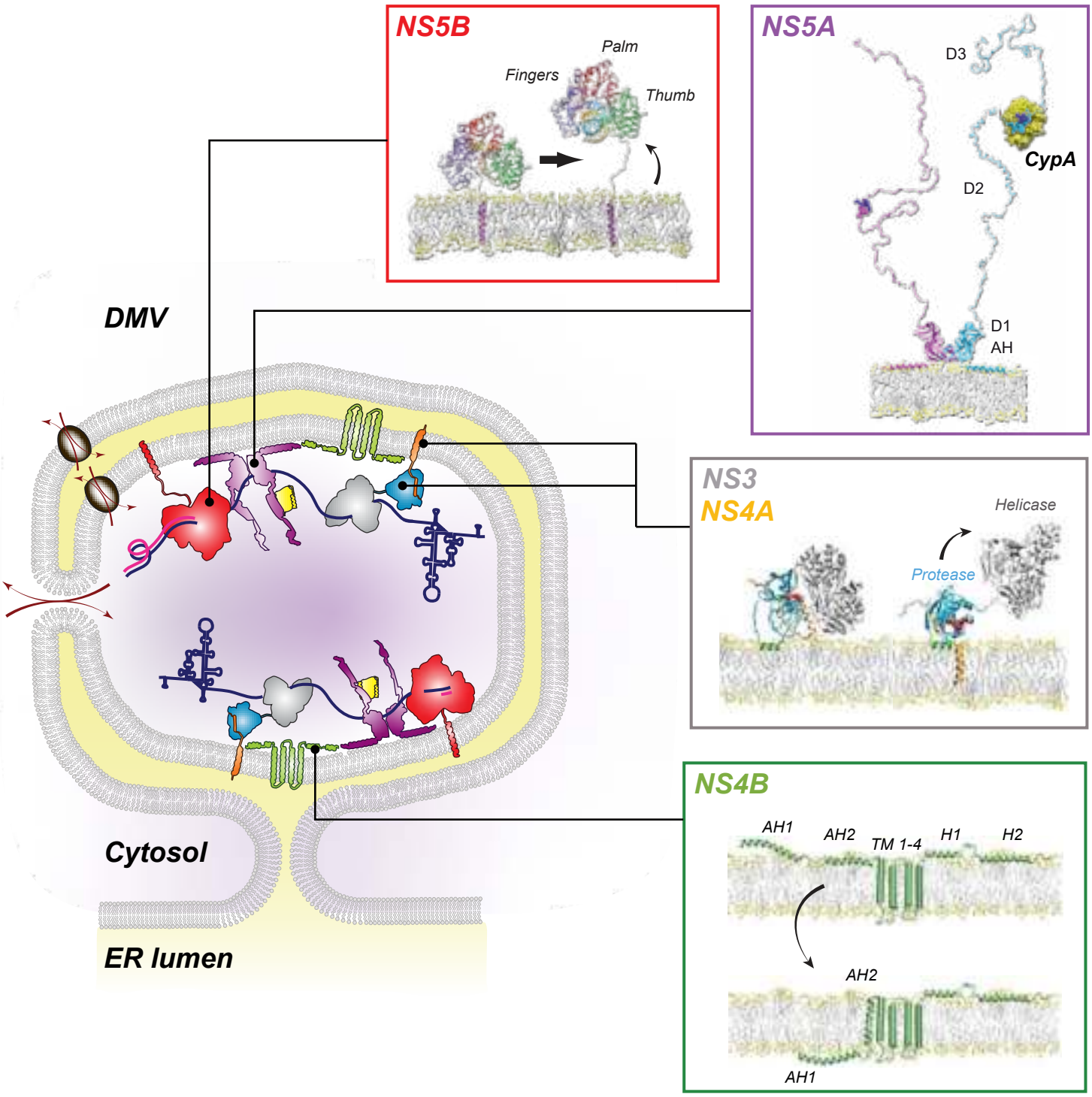


Figure 2. Model of Hepatitis C Virus-Induced Double-Membrane Vesicle (left) and Hypothetical 3D Structures of Membrane-Associated Hepatitis C virus proteins (in boxes).

Virus-induced double-membrane vesicles (DMVs) contain viral nonstructural proteins and RNA. Viral DMVs are sites of active viral RNA replication.

Created by Vanesa Madan.

Hepatitis C Virus RNA Replication and Assembly: Living on the Fat of the Land

Cell Host & Microbe
Review
CellPress

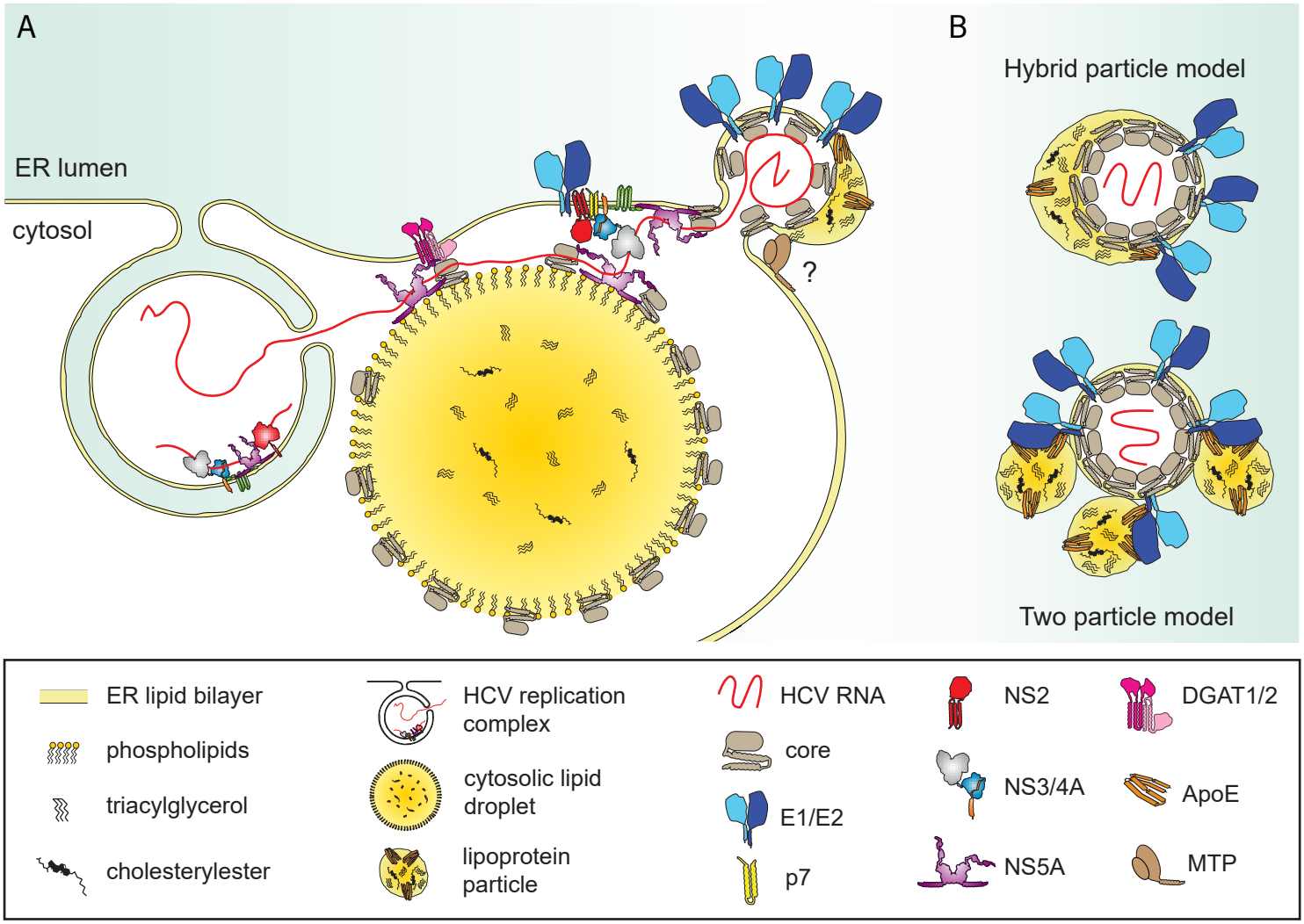


Figure 3. Model of Hepatitis C Virus Particle Production.

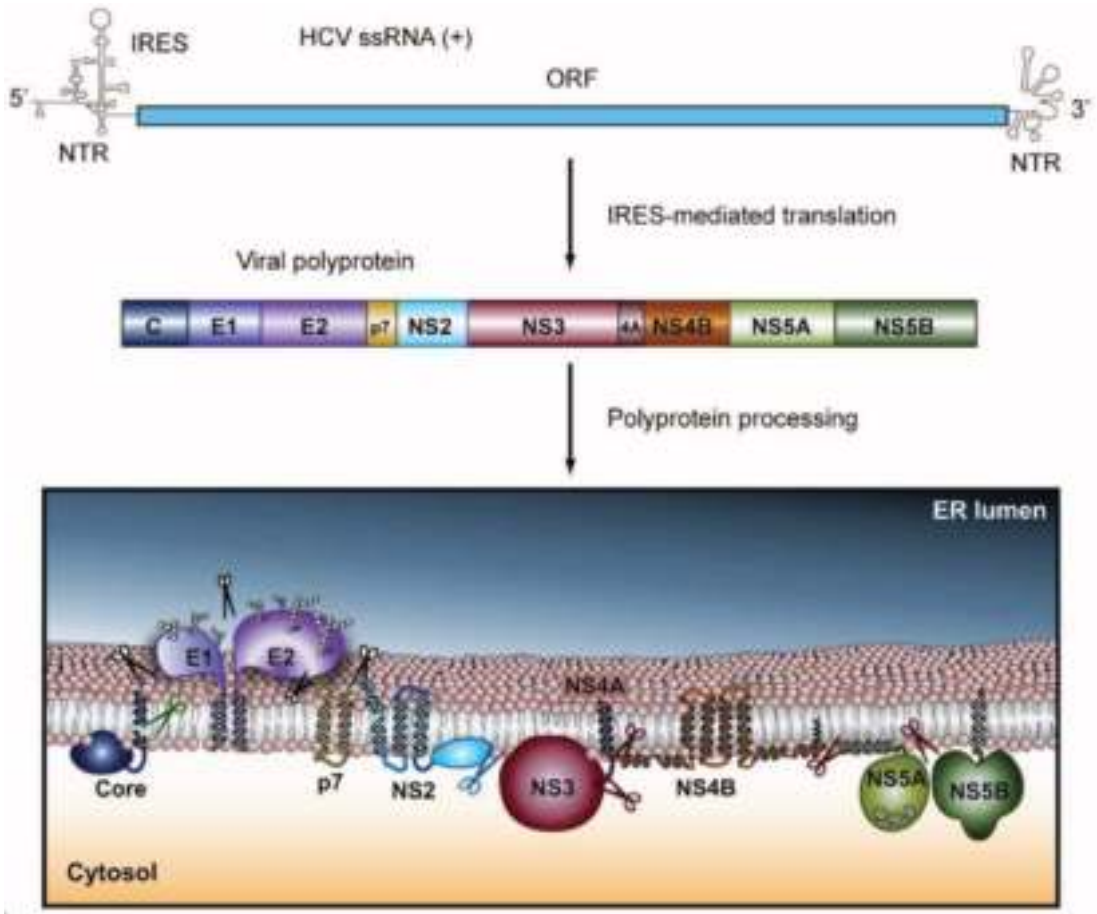
(A) Hypothetical model of Hepatitis C virus assembly (left side).
Viral RNA (HCV RNA in red) is shuttled from replication sites to cytosolic lipid droplets.
Core protein on lipid droplets and at the ER membrane (lipid bilayer) is thought to trigger nucleocapsid formation and budding into the ER (endoplasmic reticulum) lumen.

(B) Models of viral particle lipidation (right side).
In the hybrid model, cellular lipoproteins are incorcoparated to nascent virions during budding.
In the two particle model, lipidation occurs during virus eggress via interaction between the virion and lipoprotein particles.

Created by Vanesa Madan and David Paul.

Hepatitis C virus and host cell lipids: An intimate connection

Published in (Journal/Editorial):
RNA Biology
Taylor & Francis



Journal figure from review: **RNA Biology.** 2011. 8:2, 258-269, DOI: 10.4161/rna.8.2.15011.
Link to this article: https://doi.org/10.4161/rna.8.2.15011

Figure 1. Schematic of Hepatitis C virus (HCV) genome organisation, polyprotein and membrane topology of viral proteins.

Schematic representation of HCV RNA genome in blue is shown at the top.

Genome translation produces a viral polyprotein precursor (middle) which is cleaved by viral and cellular proteases releasing 10 viral proteins.

Membrane topology of the viral proteins is depicted showing protein helices inserted in the membrane and globular regions facing the ER (endoplasmic reticulum) lumen and the cytosol.

Black and green scissors indicate cellular proteases. Blue and red scissors indicate viral proteases.

Glycosylation sites in E1 and E2 are depicted as sugar chains.

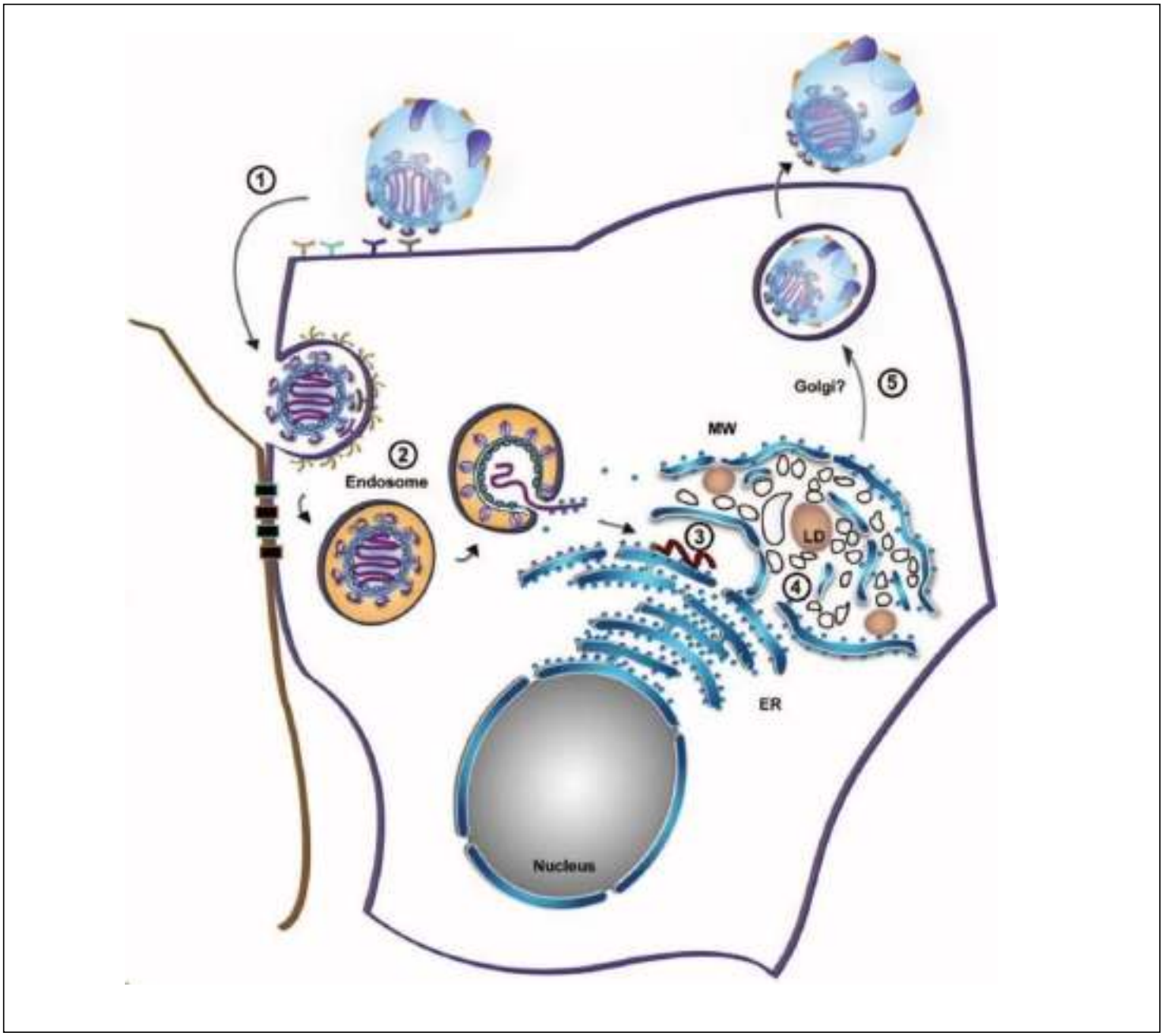
Palmitoylation of NS4B is represented by a black zig-zag line.

Phosphorylation of NS5A is represented by encircled "P".

Created by Vanesa Madan.
Review written by Vanesa Madan, Gualtiero Alvisi and Ralf Bartenschlager.

Hepatitis C virus and host cell lipids: An intimate connection

Published in (Journal/Editorial):
RNA Biology
Taylor & Francis



Journal figure from review: **RNA Biology.** 2011. 8:2, 258-269, DOI: 10.4161/rna.8.2.15011.
Link to this article: https://doi.org/10.4161/rna.8.2.15011

Figure 2. The Hepatitis C virus (HCV) replication cycle.

(1) Attachment of HCV to the cell surface.
(2) Receptor-mediated endocytosis. Internalisation of HCV in a clathrin-dependent manner. Low pH in the endosome triggers fusion of viral and endosome membranes, and consequent release of viral RNA genome into the cytoplasm.
(3) Viral proteins synthesis at the endoplasmic reticulum (ER).
(4) HCV genome replication at the membranous web (virus-induced vesicles in black).
(5) Virus release through the secretory pathway.

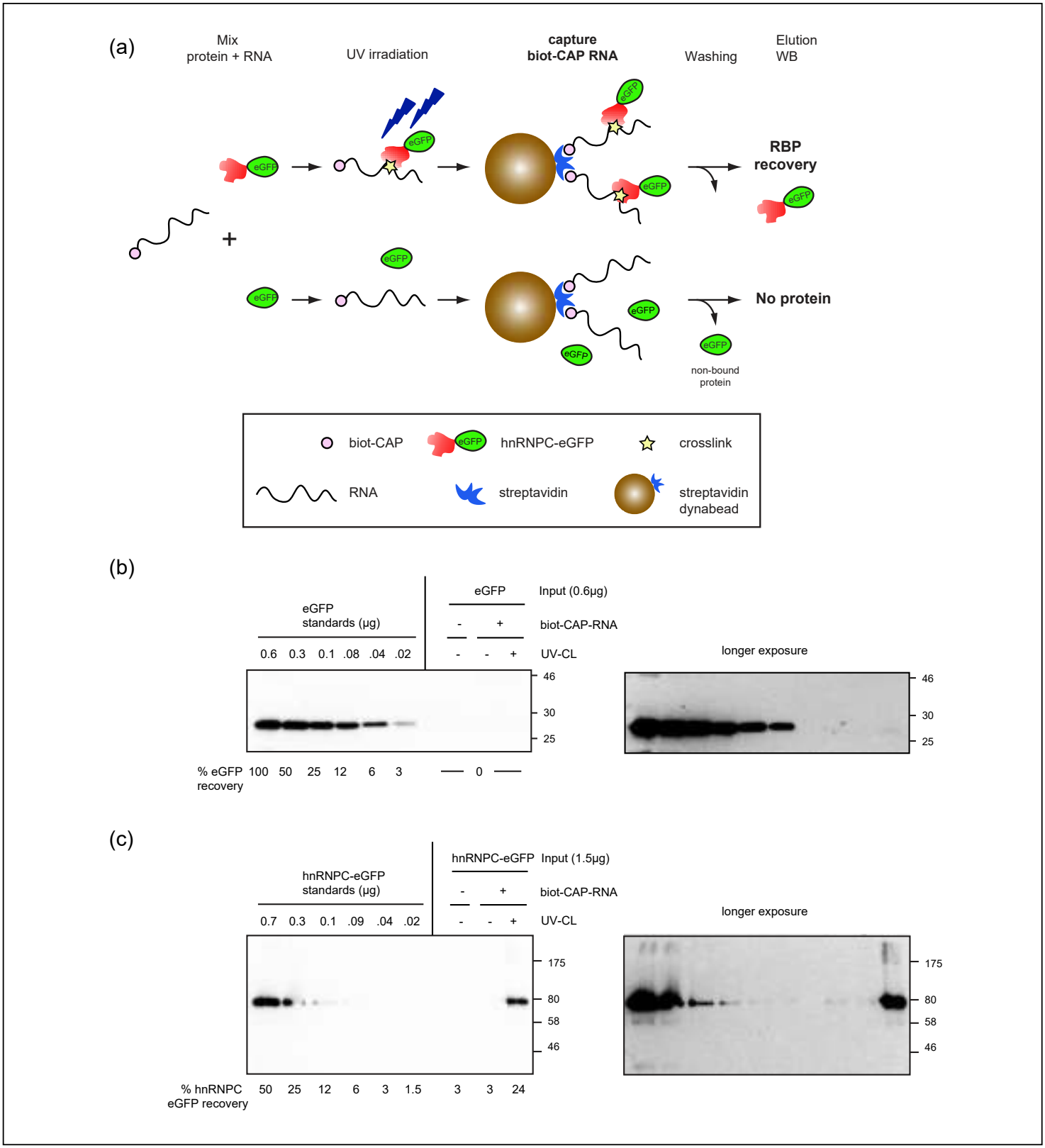
Created by Vanesa Madan.
Review written by Vanesa Madan, Gualtiero Alvisi and Ralf Bartenschlager.

mRNAs biotinylated within the 5′ cap and protected against decapping: new tools to capture RNA - protein complexes

Published in (Journal/Editorial):

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

THE ROYAL SOCIETY PUBLISHING



Journal figure from article: **Philos Trans R Soc Lond B Biol Sci.** 2018 Dec 19; 373(1762): 20180167.
http://dx.doi.org/10.1098/rstb.2018.0167

Figure 7. Study of protein-RNA interactions by UV-cross-linking and affinity capture of single-biotin-capped RNA.

(a) Schematic of RNA-binding protein recovery using biotinilated RNA.

(b) eGFP (control) or hnRNPC-eGFP (c) interaction with biotinylated RNA measured by western blot.

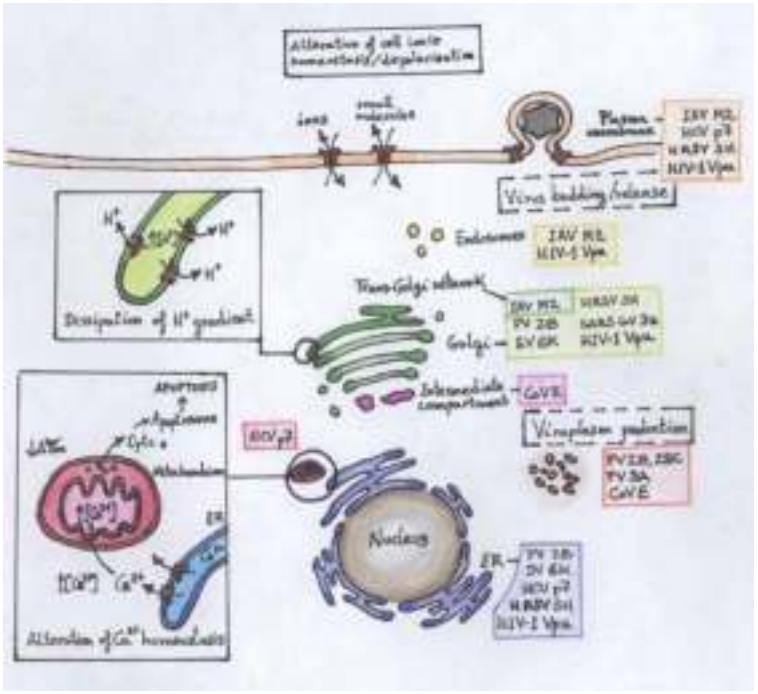
Created by Vanesa Madan.

Viroporins: structure and biological functions

Published in (Journal/Editorial):
nature reviews microbiology
nature

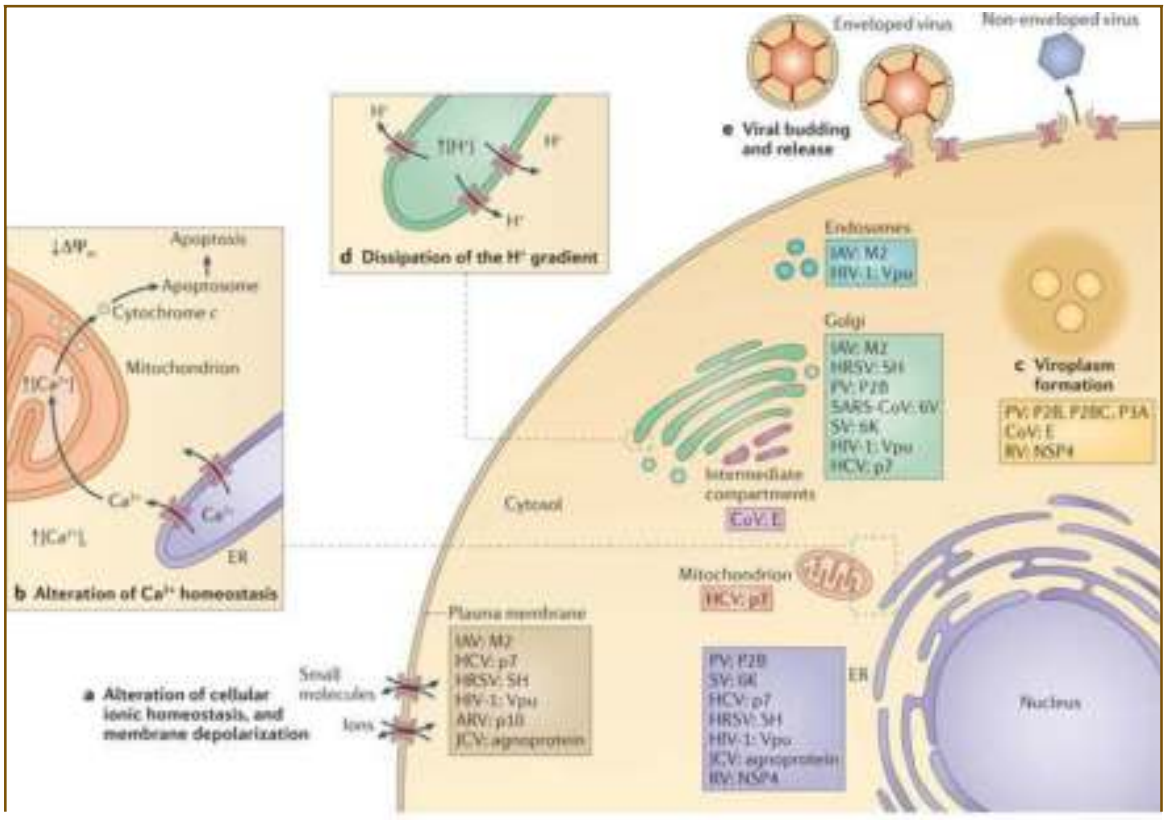
Original Sketch

Initial concepts drawn on paper



Final version

Digital illustration

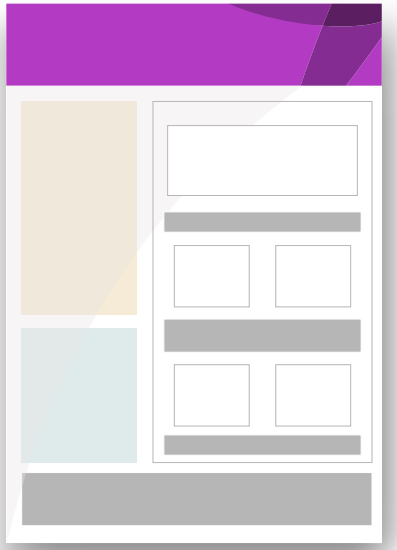


Visual concept and Sketch for figure design and illustration in: **Nature Reviews Microbiology.** 2012 Jul 2;10(8):563-74. doi: 10.1038/nrmicro2820.

Figure 2. Cytophatic effects of viroporins and their functions during the viral life cycle.
The main host cell organelles targeted by viroporins, and cytopathic effects and viroporins functions are represented.
(a) Alteration of plasma membrane potential (b) Alteration of calcium homeostasis (c) Virus-induced membranes (viroplasm) (d) Dissipation of proton gradient in the Golgi apparatus (e) Virus budding and release.

Sketch created by Vanesa Madan. Digital illustration created by Nature creative team.
Review written by Vanesa Madan, JL Nieva and Luis Carrasco.

Scientific Poster Design



Heatr5b is a new dynactin-associated protein that mediates AP-1 vesicle transport

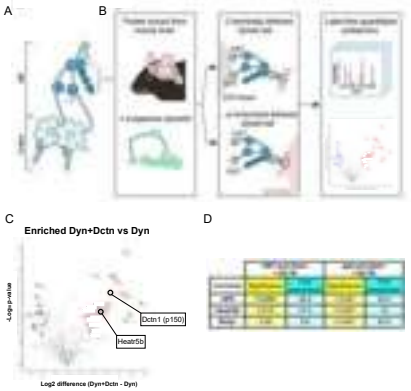
Vanesa Madan, Li Jin*, Lucas Albacete*, Joseph Watson, Emmanuel Derivery and Simon Bullock
MRC Laboratory of Molecular Biology, Cambridge, UK. vmadan@mrc-lmb.cam.ac.uk
*Equal contribution

INTRODUCTION

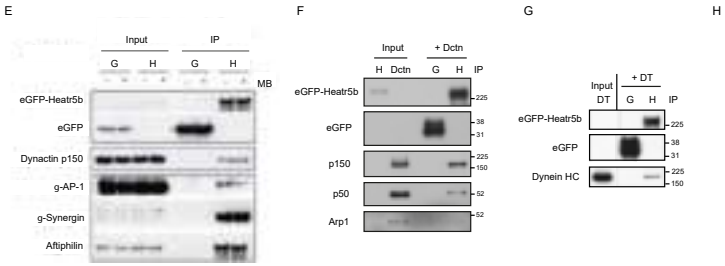
The dynein motor and its essential cofactor dynactin mediate microtubule minus-end-directed transport of a wide variety of cellular cargoes including organelles, macromolecules, viruses and vesicles.
Dynein heavy chain contains a motor domain, which has force-generating ATPase activity as well as a microtubule-binding site, and a tail domain that mediates self-dimerisation and recruits cargoes (Figure.1).
The activating adaptors and cargo-associated proteins that link these cargoes to dynein and dynactin have been defined in only a small number of cases. Identifying these links is a prerequisite for understanding the principles of cargo recognition by this motor, as well as for dissecting the functional consequences of specific dynein-based transport processes in the cell. To address these issues, we combined pulldowns with a recombinant dynein tail complex, which interacts with dynactin and cargo adaptors, with label free quantitative proteomics.
We found many new tail interactors in mammalian cell extracts, including several whose binding is enhanced by dynactin. We focused on one dynactin-stimulated interactor called Heatr5B, which is predicted to be comprised entirely of HEAT repeats (AlphaFold2 v2.0.).
Heatr5B interacts with the clathrin adaptor AP-1 by binding two AP-1 accessory proteins, Aftiphilin and γ-Synergin (Hirst et al. 2005 Mol Biol Cell). Our functional analysis of Heatr5b reveals that this protein promotes AP-1 vesicle transport to the trans-Golgi network in mammalian cells. In vivo, we show using *Drosophila* genetics that the Heatr5b orthologue is essential for organismal survival and promotes dynein-mediated long-distance apical transport of AP-1 vesicles in the early embryo.
Our study uncovers a novel and conserved role of Heatr5b as a key component of the dynein-dynactin machinery for AP-1 vesicle transport.

RESULTS

Biochemical screen for dynein tail (DT) interactors



Heatr5b interacts directly with dynein tail and dynactin



Heatr5b is located on AP-1 vesicles

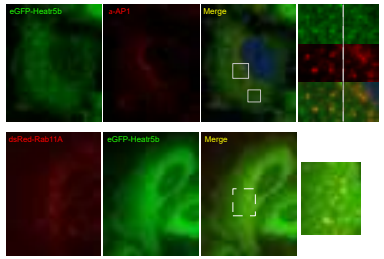
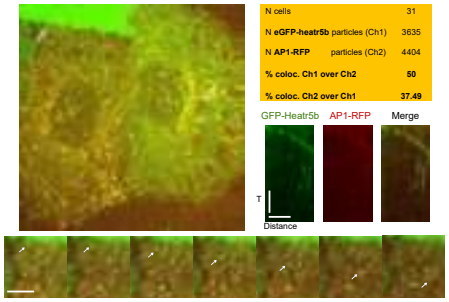
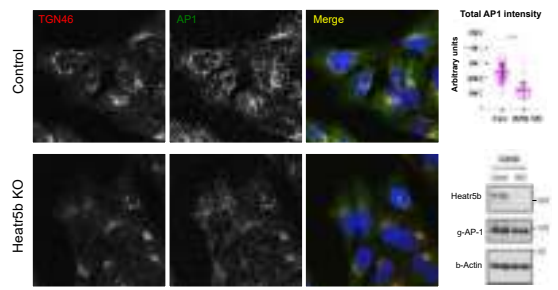


Figure 1. (A) Scheme of dynein complex. (B) Pulldown scheme. Mouse brain extracts were used as source of DT interactors. Dynactin complex purified from pig brain was added or not to the extracts to facilitate capture of tail interactors involved in cargo transport processes. Recombinant dynein tail tagged with GST at the C-terminus and ZZ domain of protein A at the N-terminus was either coupled to glutathione or IgG beads, and incubated with mouse brain extracts in presence or absence of exogenous dynactin. The samples were digested and processed by LC-MS/MS. Captured proteins were analysed with label free quantitative proteomics. (C) High confidence specific interactors of DT (IgG pulldown) enriched in presence of exogenous dynactin are showed in red (p <0.05, 5% FDR). This strategy allows detection of interactors of the dynein-dynactin complex such as BICD2 and JIP3. New interactors of DT-dynactin complex such as Heatr5b were found. (D) The three top proteins found independently from N-terminal and C-terminal DT pull downs are listed in the table. (E) Immunoprecipitations of eGFP or eGFP-Heatr5b from Hek293 cell lines using GFP-Trap beads. Specific interactions of eGFP-Heatr5b with dynactin (p150), AP-1 complex (γ-adaptin), and two accessory AP-1 proteins: Aftiphilin and γ-synergin were detected. (F) Human recombinant eGFP-Heatr5b purified from insect cells or eGFP alone were coupled to GFP-Trap beads and incubated either with purified dynactin or recombinant DT (G). Specific interaction with the dynactin complex and DT was detected using antibodies to dynactin subunits or dynein HC, respectively. (H) GFP-Heatr5b stably expressed in Hela cells colocalises with endogenous AP-1 complex and transiently expressed Rab11 (recycling endosome marker). Magnifications of square areas are shown on the right.

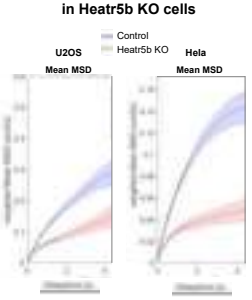
Heatr5b is co-transported with AP1 vesicles



AP-1 membrane association is stabilised by Heatr5b



Motility of AP-1 is impaired in Heatr5b KO cells



Heatr5b recruits dynactin to the recycling endosome

Figure 2. (A) Live imaging in eGFP-Heatr5b Hela cells expressing AP1-RFP transiently. eGFP-Heatr5b is present in static structures and motile vesicles containing AP1-RFP. Kymographs from a vesicle containing eGFP-Heatr5b and AP1-RFP (indicated by a white arrow) are shown. Scale bars, 10 s and 5 mm. Image series of vesicle dynamics are shown below. Scale bar, 5 mm (B) Knock out (KO) of Heatr5b in U2OS cells reduces total AP-1 intensity at the trans-Golgi network (TGN) and in cytoplasmic vesicles (graph) whereas the total amount of AP-1 in the cell is not significantly affected. Absence of Heatr5b in KO cells was confirmed by western blot. (C) Mean square displacement of AP1-positive particles was analysed in control and Heatr5b KO cells. Overall motion and transport events were significantly reduced in KO cells. D) Heatr5b recruits dynactin to the recycling endosome compartment. U2OS cells overexpressing eGFP-Heatr5b were incubated with Alexa 647-Transferrin and treated with digitonin to visualise proteins associated with the membrane. No p150 recruitment to the RE was senn without eGFP-Heatr5b overexpression.

Loss of Heatr5b impairs trafficking to the TGN

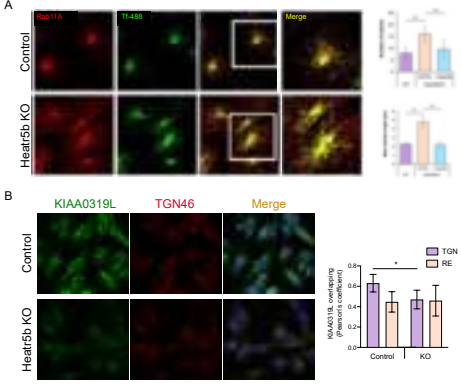


Figure 3. (A) Absence of Heatr5b induces tubulation of the recycling endosome compartment. U2OS control and Heatr5b KO cells were incubated with Alexa488-Transferrin and immunostained with Rab11A antibodies to visualise the recycling endosome compartment. B) Trafficking of KIAA0319L (an AP-1 cargo protein) from the recycling endosome to the TGN (Hirst et al. 2012. Curr Biol) is reduced in Heatr5b KO cells.

Heatr5 promotes dynein-mediated transport of AP-1 *in vivo*

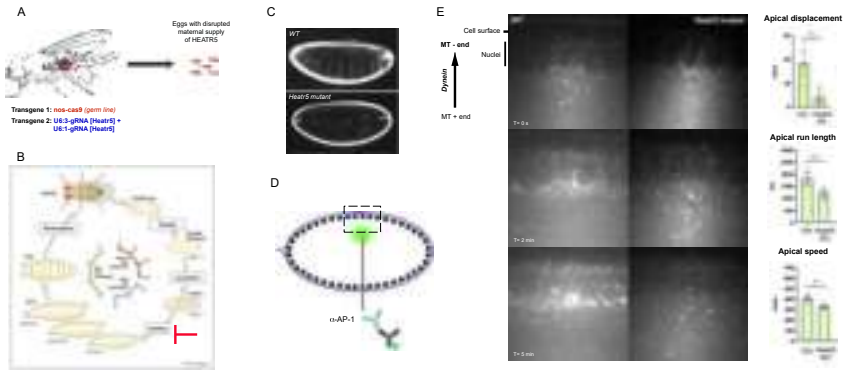
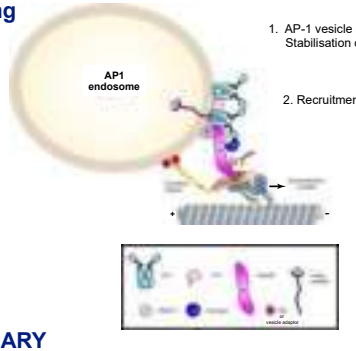


Figure 4. (A) CRISPR/Cas9 was used to generate an out-of frame mutation in the single *Drosophila* orthologue of Heatr5b (CG2747 or Heatr5 hereafter). (B) Development of homozygous embryos are arrested at late embryo/1st instar stages. (C) Heatr5 mutant embryos are abnormal and do not have denticle hairs. (D and E) The *Drosophila* syncytial blastoderm is an excellent model to study polarised trafficking. Embryo microinjection with a fluorescent complex of AP-1 antibodies allows AP-1 dynamics to be followed (square region) as the microtubule cytoskeleton is highly polarised with minus ends above the nuclei, and plus ends extended basally. E) Images series of injected control embryos shows fast apical transport of AP-1 puncta, which accumulate or fuse beneath the nuclei. In Heatr5b mutant embryos, the rate of apical movement of AP1 puncta is strongly impaired. Blocking antibodies revealed that the apical AP-1 transport is dynein dependent (not shown).

Working model



1. AP-1 vesicle recognition
   Stabilisation of AP-1 membrane association
2. Recruitment/activation of dynein machinery

SUMMARY

Heatr5b is a new interactor of the dynein-dynactin complex

Heatr5b functions as a cargo-associated adaptor/regulator that interacts with the motor machinery and two accessory proteins of the clathrin adaptor AP-1.

Absence of Heatr5b impairs the motion of AP-1 vesicles in mammalian cells and induces a dramatic tubulation of the recycling endosome compartment.

Heatr5b orthologue in *Drosophila melanogaster* is essential for survival. Loss of this protein arrests embryo development and disrupts dynein-driven transport of AP-1 vesicles.

SCIENTIFIC POSTER DESIGN

Scientific poster presented at the virtual Dynein 2021 International Workshop hold online on 8th/9th September 2021.
Poster title: "Heatr5b is a new dynactin-associated protein that mediates AP-1 vesicle transport".

Research topic: Molecular motors (Cell Biology).

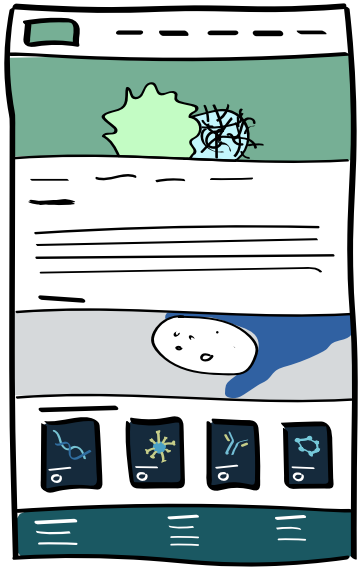
The molecular motor dynein/dynactin is a protein complex that transports different cell components in our cells.
Intracellular transport is crucial for many cellular functions, and defects in transport can cause neurological diseases.
Dynein/Dynactin uses adaptors to link the motor to different cargos. Many of these adaptors have not been identified yet.
In this work we identified a new adaptor that allows dynein/dynactin motor transport a specific cell organelle called AP-1 vesicle.

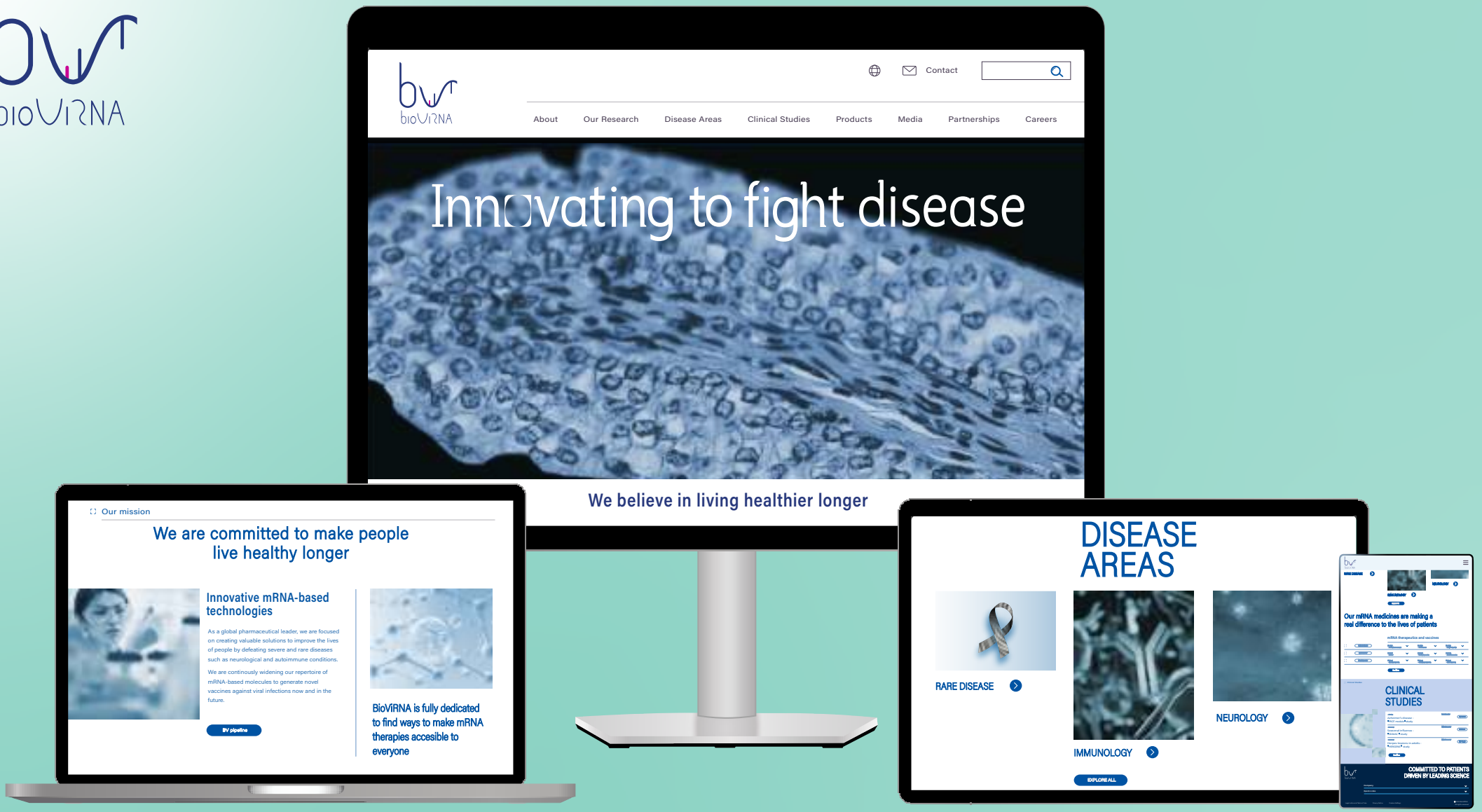
The poster shows research data obtained from biochemical assays, fluorescence microscopy, live cell imaging and experiments using the fruit fly, *Drosophila melanogaster*.

Poster created by Vanesa Madan.

Experiments conducted by: Vanesa Madan, Li Jin, Lucas Albacete, Joseph Watson, Emmanuel Derivery and Simon Bullock in the MRC Laboratory of Molecular Biology, Cambridge, UK.

Web layout





Web design (content and layout) **mockup** for fictitious biopharmaceutical company "BioViRNA" (2024).
Created using Photoshop (image generation, image editing) and Illustrator (layout, mockup and logo).

"BioViRNA" represents a leader pharmaceutical company that develops new medicines based on messenger ribonucleic acid (mRNA) for use as protein replacement therapies for rare diseases, as immunotherapies, and as vaccines against infectious diseases.

Created by Vanesa Madan.

Vanesa Madan

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Cambridge 2024