OptiPrep™ Mini-Review MV06

Purification and analysis of hepatitis C virus and other Group IV viruses

- This Mini-Review principally provides in **Section 2** a bibliography of all those papers reporting the use of OptiPrep™ in the purification and analysis of Group IV viruses. A short **Section 1** briefly summarizes the gradient strategies that have been used and the advantages of using OptiPrep™.
- **Section 1** concludes with a list of all the relevant OptiPrep™ Application Sheets that are currently available for research on hepatitis C virus and other Group IV viruses, and how this information is accessed.
- The format for **Section 2** is decribed in the introduction to the section.

1. Introduction

1-1. Technical background to the use of OptiPrep™

In all comparative studies between CsCl and iodixanol, the recovery of virus infectivity is much higher and the particle:infectivity ratio much lower when viruses are purified in iodixanol. Although sucrose is generally less deleterious to viral infectivity than CsCl, it can nevertheless also have serious effects on certain important aspects of viral function; in particular the loss of surface glycoproteins from retroviruses has been noted [1]. This may be related to its viscosity, which, in solutions of the same density, is much higher than that of iodixanol. Most iodixanol gradients can also be made isoosmotic over the entire density range.

Like CsCl, sucrose must be dialyzed before infectivity can be measured. In contrast both infectivity measurements using cultured cells and many add-on techniques can be performed without dialysis of iodixanol. Combined with the availability of OptiPrep™ as a sterile solution, this makes the use of OptiPrep™ for virus purification and assembly analysis much more convenient than the use of either CsCl or sucrose. The only analytical technique for which removal of the iodixanol is essential is electron microscopy. Consequently iodixanol is being increasingly used for the purification of hepatitis C virus particles from lysed cultured cells, from conditioned culture medium or from plasma samples from infected patients.

1-2. Solution preparation

Isoosmotic solutions for making gradients may be prepared simply by diluting OptiPrep™ with either Tris- or HEPES-buffered sucrose [2] or NaCl [3] solutions. Sometimes bovine serum albumin is included [3].

1-3. Gradient strategy

A wide range of density gradient strategies is available; most of the separations are based on buoyant density and both sedimentation and flotation formats have been reported. Continuous 10-40% (w/v) iodixanol gradients, with the crude virus-containing sample top-loaded [3] or discontinuous gradients of 10, 20, 30 and 40% (w/v) iodixanol, with the crude virus in the densest layer [4] have been run in routine swinging-bucket rotors. Centrifugation is normally carried out for at least 6 h at 150-200,000 *g* but lower *g*-forces for longer times are not uncommon. The flotation mode offers the advantage of easy handling of virus that has been concentrated on to a cushion of a dense iodixanol solution [5]. Virus particle size has also been estimated by sedimentation through 4-24% (w/v) iodixanol gradients [6].

Hepatitis C virus particles have also been analyzed in self-generated gradients of iodixanol [7]. This very simple technique, which has been used for a number of other viruses, requires only adjustment of the crude virus suspension to 25% (w/v) iodixanol. The formation of a self-generated gradient is most efficiently carried out in a vertical or near-vertical rotor at approx 350,000 *g* for 2-3 h but a fixed-angle rotor (approx. 10 ml tube size) may be substituted for longer times. The low virus concentration at the start of the centrifugation and the lack of any liquid/liquid interfaces may optimize resolution of virus particles from contaminants.

- Large-scale production of virus has been addresses in ref 8

1-4. References

1. **Palker, T.J.** (1990) *Mapping of epitopes on human T-cell leukemia virus type 1 envelope glycoprotein* In: Human Retrovirology: HTLV (ed. Blattner, W.A.) Raven Press, NY, pp 435-445
2. **Nielsen, S.U.**, Bassendine, M.F., Burt, A.D., Martin, C., Pumeechockchai, W. and Toms, G.L. (2006) *Association between hepatitis C virus and very-low-density lipoprotein (VLDL)/LDL analyzed in iodixanol density gradients* J. Virol., **80**, 2418-2428
3. **Lindenbach, B.D.**, Evans, M.J., Syder, A.J., Wolk, B., Tellinghuisen, T.L., Liu, C.C., Maruyama, T., Hynes, R.O., Burton, D.R., McKeating, J.A. and Rice, C.M. (2005) *Complete replication of hepatitis C virus in cell culture* Science, **309**, 623-626
4. **Steinmann, E.**, Brohm, C., Kallis, S., Bartenschlager, R. and Pietschmann, T. (2008) *Efficient trans-encapsidation of hepatitis C virus RNAs into infectious virus-like particles* J. Virol., **82**, 7034-7046
5. **Nielsen, S.U.**, Bassendine, M.F., Burt, A.D., Martin, C., Pumeechockchai, W. and Toms, G.L. (2006) *Association between hepatitis C virus and very-low-density lipoprotein (VLDL)/LDL analyzed in iodixanol density gradients* J. Virol., **80**, 2418-2428
6. **Romero-Brey, I.**, Merz, A., Chiramel, A., Lee, J-Y., Chlanda, P., Haselman, U., Santarella-Mellwig, R., Habermann, A., Hoppe, S., Kallis, S., Walther, P., Antony, C., Krijnse-Locker, J. and Bartenschlager, R. (2012) *Three-dimensional architecture and biogenesis of membrane structures associated with hepatitis C virus replication* PLoS Pathog., **8**: e1003056
7. **Nielsen, S.U.**, Bassendine, M.F., Martin, C., Lowther, D., Purcell, P.J., King, B.J., Neely, D., Toms, G.L. (2008) *Characterization of hepatitis C RNA-containing particles from human liver by density and size* J. Gen. Virol., **89**, 2507-2517
8. **Earnest-Silveira, L.**, Christiansen, D., Herrmann, S., Ralph, S.A., Das, S. Gowans, E.J. and Torresi, J. (2016) *Large scale production of a mammalian cell derived quadrivalent hepatitis C virus like particle vaccine* J. Virolog. Meth., **236**, 87–92

1-5. OptiPrep™ Application Sheets

Detailed protocols for the isolation of the hepatitis C virus (*Flaviviridae*) may be accessed from the OptiPrep™ Applications flash-drive or from the following website: www.axis-shield-density-gradient-media.com, click on "Methodology" then "Viruses" to open up the Virus Index. Other relevant OptiPrep™ Application Sheets may also be accessed from the top of the Index.

♦ *Flaviviridae* - pre-formed gradient	OptiPrep™ Application Sheet V19
♦ *Flaviviridae* - self-generated gradient	OptiPrep™ Application Sheet V20
♦ Other Group IV viruses	OptiPrep™ Application Sheets V21 & V22
♦ Preparation of density gradient solutions	OptiPrep™ Application Sheet V01
♦ Preparation of continuous and discontinuous gradients	OptiPrep™ Application Sheet V02
♦ Preparation of self-generated gradients	OptiPrep™ Application Sheet V03
♦ Harvesting gradients	OptiPrep™ Application Sheet V04
♦ Analysis of gradients	OptiPrep™ Application Sheet V05
♦ Concentration of virus samples	OptiPrep™ Application Sheet V06

2. Bibliography

- This bibliography provides a comprehensive reference list of all the papers reporting the use of OptiPrep™ for purification of Group IV viruses, published before the end of November 2017.
- Viruses are listed alphabetically according to **family and virus type**
- References are also divided alphabetically into **"Research topic" sections** and **subsections.**
- All references are listed alphabetically according to **First Author**
- To aid selection, key words in the titles are highlighted in light blue.

2-1. Arteriviridae

Porcine reproductive and respiratory syndrome virus

Delputte, P.L., Meerts, P., Costers, S. and Nauwynck, H.J. (2004) *Effect of virus-specific antibodies on attachment, internalization and infection of porcine reproductive and respiratory syndrome virus in primary macrophages* Vet. Immunol. Immunopathol., **102**, 179-188

Li, J. and Murtaugh, M.P. (2012) *Dissociation of porcine reproductive and respiratory syndrome virus neutralization from antibodies specific to major envelope protein surface epitopes* Virology, **433**, 367–376

Li, J. and Murtaugh, M.P. (2015) *Functional analysis of porcine reproductive and respiratory syndrome virus N-glycans in infection of permissive cells* Virology, **477**, 82–88

Li, J., Tao, S., Orlando, R. and Murtaugh, M.P. (2015) *N-glycosylation profiling of porcine reproductive and respiratory syndrome virus envelope glycoprotein 5* Virology **478**, 86–98
Van Noort, A., Nelsen, A., Pillatzki, A.E., Diel, D.G., Li, F., Nelson, E. and Wang, X. (2017) *Intranasal immunization of pigs with porcine reproductive and respiratory syndrome virus-like particles plus 2', 3'-cGAMP VacciGrade™ adjuvant exacerbates viremia after virus challenge* Virol. J., **14**: 76

2-2. Caliciviridae

Bertolotti-Ciarlet, A., White, L.J., Chen, R., Prasad, B.V.V. and Estes, M.K. (2002) *Structural requirements for the assembly of Norwalk virus-like particles* J. Virol., **76**, 4044-4055

2-3. Coronaviridae

2-3-1. Middle East respiratory syndrome virus

De Wit, E., Prescott, J., Baseler, L., Bushmaker, T., Thomas, T., Lackemeyer, M.G., Martellaro, C., Milne-Price, S., Haddock, E., Haagmans, B.L., Feldmann, H. and Munster, V.J. (2013) *The Middle East respiratory syndrome coronavirus (MERS-CoV) does not replicate in Syrian hamsters* PLoS One, **8**: e69127

2-3-2. SARS-Coronavirus

Beniac, D.R., deVarennes, S.L., Andonov, A., He, R. and Booth, T.F. (2007) *Conformational reorganization of the SARS Coronavirus spike following receptor binding: implications for membrane fusion* PLoS ONE, **10:e1082**
Berry, J.D., Jones, S., Drebot, M.A., Andonov, A., Sabara, M., Yuan, X.Y., Weingartl, H., Fernando, L. et al (2004) *Development and characterization of neutralizing monoclonal antibody to the SARs-coronavirus* J. Virol. Methods, **120**, 87-96
Gubbins, M. J., Plummer, F.A., Yuan, X.Y., Johnstone, D., Drebot, M., Andonova, M., Andonov, A. and Berry, J.D. (2004) *Molecular characterization of a panel of murine monoclonal antibodies specific for the SARS-coronavirus* Mol. Immunol., **42**, 125-136
Hatakeyama, S., Matsuoka, Y., Ueshiba, H., Komatsu, N., Itoh, K., Shichijo, S., Kanai, T., Fukushi, M., Ishida, I., Kirikae, T., Sasazuki, T. and Miyoshi-Akiyama, T. (2008) *Dissection and identification of regions required to form pseudoparticles by the interaction between the nucleocapsid (N) and membrane (M) proteins of SARS coronavirus* Virology, **380**, 99-108
Huang, Y., Yang, Z-Y., Kong, W-P. and Nabel, G.J. (2004) *Generation of synthetic severe acute respiratory syndrome coronavirus pseudoparticles: implications for assembly and vaccine production* J. Virol., **78**, 12557-12565
Kuo, L., Hurst-Hess, K.R., Koetzner, C.A. and Masters, P.S. (2016) *Analyses of coronavirus assembly interactions with interspecies membrane and nucleocapsid protein chimeras* J. Virol., **90**, 4357-4368
Milewska, A., Zarebski, M., Nowak, P., Stozek, K., Potempa, J. and Pyrca, K. (2014) *Human coronavirus NL63 utilizes heparan sulfate proteoglycans for attachment to target cells* J. Virol., **88**, 13221–13230
Milewska, A., Kaminski, K., Ciejka, J., Kosowicz, K., Zeglen, S., Wojarski, J., Nowakowska, M., Szczubiałka, K. and Pyrc, K. (2016) *HTCC: broad range inhibitor of coronavirus entry* PLoS One, **11**: e0156552
Tseng, Y-T., Wang, S-M., Huang, K-J., Lee, A.I-R., Chiang, C-C. and Wang, C-T. (2010) *Self-assembly of severe acute respiratory syndrome coronavirus membrane protein* J. Biol. Chem., **285**, 12862–12872
Tseng, Y-T., Wang, S-M., Huang, K-J. and Wang, C-T. (2014) *SARS-CoV envelope protein palmitoylation or nucleocapid association is not required for promoting virus-like particle production* J. Biomed. Sci., **21**: 34
Yang, Z-Y., Huang, Y., Ganesh, L., Leung, K., Kong, W-P., Schwartz, O., Subbarao, K. and Nabel, G.J. (2004) *pH-dependent entry of severe acute respiratory syndrome coronavirus is mediated by the spike glycoprotein and enhanced by dendritic cell transfer through DC-sign* J. Virol., 78, 5642-5680

2-4. Flaviviridae

2-4-1. Bovine diarrhea virus

Fredericksen, F., Delgado, F., Cabrera, C., Yáñez, A., Gonzalo, C., Villalba, M. and Olavarría, V.H. (2015) *The effects of reference genes in qRT-PCR assays for determining the immune response of bovine cells (MDBK) infected with the Bovine Viral Diarrhea Virus 1 (BVDV-1)* Gene, **569**, 95–103
Fredericksen, F., Carrasco, G., Villalba, M. and Olavarría, V.H. (2015) *Cytopathic BVDV-1 strain induces immune marker production in bovine cells through the NF-κB signaling pathway* Mol. Immunol., **68**, 213–222
Maurer, K., Krey, T., Moennig, V., Thiel, H-J. and Rümenapf, T. (2004) *CD46 is a cellular receptor for bovine viral diarrhea virus* J. Virol., **78**, 1792-1799

2-4-2 Dengue virus

Alayli, F. and Scholle, F. (2016) *Dengue virus NS1 enhances viral replication and pro-inflammatory cytokine production in human dendritic cells* Virology, **496**, 227–236

Ayala-Nuñez, N.V., Wilschut, J. and Smit, J.M. (2011) *Monitoring virus entry into living cells using DiD-labeled dengue virus particles* Methods **55**, 137–143

Briggs, C.M., Smith, K.M., Piper, A., Huitt, E., Spears, C.J., Quiles, M., Ribeiro, M., Thomas, M.E., Brown, D.T. and Hernandez, R. (2014) *Live attenuated tetravalent dengue virus host range vaccine is immunogenic in African green monkeys following a single vaccination* J. Virol., **88**, 6729–6742

Hacker, K., White, L. and de Silva, A.M. (2009) *N-Linked glycans on dengue viruses grown in mammalian and insect cells* J. Gen. Virol., **90**, 2097–2106

Hadjilaou, A., Green, A.M., Coloma, J. and Harris, E. (2015) *Single-cell analysis of B cell/antibody cross-reactivity using a novel multicolor FluoroSpot assay* J. Immunol., **195**, 3490–3496

Heaton, N.S., Perera, R., Berger, K.L., Khadka, S., LaCount, D.J., Kuhn, R.J. and Randall, G. (2010) *Dengue virus nonstructural protein 3 redistributes fatty acid synthase to sites of viral replication and increases cellular fatty acid synthesis* Proc. Natl. Acad. Sci. USA, **107**, 17345–17350

Raheel, U., Jamal, M. and Zaidi, N.U.S.S. (2015) *A molecular approach designed to limit the replication of mature DENV2 in host cells* Viral Immunol., **28**, 378–384

Rodenhuis-Zybert, I.A., van der Schaar, H.M., da Silva Voorham, J.M., van der Ende-Metselaar, H., Lei, H-Y., Jan Wilschut, J. and Smit, J.M. (2010) *Immature dengue virus: a veiled pathogen?* PLoS Pathogens, **6**:e1000718

Smith, K.M., Nanda, K., McCarl, V., Spears, C.J., Piper, A., Ribeiro, M., Quiles, M., Briggs, C.M., Thomas, G.S., Thomas, M.E., Brown, D.T. and Hernandez, R. (2012) *Testing of novel dengue virus 2 vaccines in African green monkeys: safety, immunogenicity, and efficacy* Am. J. Trop. Med. Hyg., **87**, 743–753

Vancini, R., Kramer, L.D., Ribeiro, M., Hernandez, R. and Brown, D. (2013) *Flavivirus infection from mosquitoes in vitro reveals cell entry at the plasma membrane* Virology **435**, 406–414

Wahala, W.M.P.B., Kraus, A.A., Haymore, L.B., Accavitti-Loper, M.A. and de Silva, A.M. (2009) *Dengue virus neutralization by human immune sera: Role of envelope protein domain III-reactive antibody* Virology **392**, 103–113

Wahala, W.M.P.B., Donaldson, E.F., de Alwis, R., Accavitti-Loper, M.A., Baric, R.S. and de Silva, A.M. (2010) *Natural strain variation and antibody neutralization of dengue serotype 3 viruses* PLoS Pathogens, **6**: e1000821

White, L.J. Parsons, M.M., Whitmore, A.C., Williams, B.M., de Silva, A. and Johnston, R.E. (2007) *An immunogenic and protective alphavirus replicon particle-based Dengue vaccine overcomes maternal antibody interference in weanling mice* J. Virol., **81**, 10329-10339

Zaitseva, E., Yang, S-T., Melikov, K., Pourmal, S., Chernomordik, L.V. (2010) *Dengue virus ensures its fusion in late endosomes using compartment-specific lipids* PloS Pathogens, **6**: e1001131

Zicari, S., Arakelyan, A., Fitzgerald, W., Zaitseva, E., Chernomordik, L.V., Margolis, L. and Grivel, J-C. (2016) *Evaluation of the maturation of individual Dengue virions with flow virometry* Virology, **488**, 20–27

Zybert, I.A., van der Ende-Metselaar, H., Wilschut, J. and Smit, J.M. (2008) *Functional importance of dengue virus maturation: infectious properties of immature virions* J. Gen. Virol., **89**, 3047–3051

2-4-3. Hepatitis C

2-4-3-1. Antimicrobial peptides

Kato, T., Sugiyama, N., Murayama, A., Matsumura, T., Shiina, M., Asabe, S., Wakita, T. and Imawari, M. (2013) *Antimicrobial peptide LL-37 deteriorate infectivity of hepatitis C virus* Hepatology, **58** (suppl), 443A-444A

Matsumura, T., Sugiyama, N., Murayama, A., Yamada, N., Shiina, M., Asabe, S., Wakita, T., Imawari, M. and Kato, T. (2016) *Antimicrobial peptide LL-37 attenuates infection of hepatitis C virus* Hepatol. Res., **46**, 924–932

2-4-3-2. Anti-scavanger receptor (B type)

Vercauteren, K., Van Den Eede, N., Mesalam, A.A., Belouzard, S., Catanese, M.T. et al (2014) *Successful anti-scavenger receptor class B type I (SR-BI) monoclonal antibody therapy in humanized mice after challenge with HCV variants with in vitro resistance to SR-BI-targeting agents* Hepatology, **60,** 1508-1518

2-4-3-3. Assembly and cellular release of virus particles

2-4-3-3a. Apolipoprotein/lipoprotein, influence of

Bankwitz, D., Doepke, M., Hueging, K., Weller, R., Bruening, J., Behrendt, P., Lee, J-Y., Vondran, F.W.R. et al (2017) *Maturation of secreted HCV particles by incorporation of secreted ApoE protects from antibodies by enhancing infectivity* J. Hepatol., **67**, 480–489

Benga, W.J.A., Krieger, S.E., Dimitrova, M., Zeisel, M.B., Parnot, M., Lupberger, J., Hildt, E., Luo, G., McLauchlan, J., Baumert, T.F. and Schuster, C. (2010) *Apolipoprotein E interacts with hepatitis C virus nonstructural protein 5A and determines assembly of infectious particles* Hepatology, **51**, 43-53
Fukuhara, T., Tamura, T., Ono, C., Shiokawa, M., Mori, H., Uemura, K., Yamamoto, S., Kurihara, T. et al (2017) *Host-derived apolipoproteins play comparable roles with viral secretory proteins E^{rns} and NS1 in the infectious particle formation of Flaviviridae* PLoS Pathog., **13**: e1006475
Hueging, K., Doepke, M., Vieyres, G., Bankwitz, D., Frentzen, A., Doerrbecker, J., Gumz, F., Haid, S., Wölk, B., Kaderali, L. and Pietschmann, T. (2014) *Apolipoprotein E co-determines tissue tropism of hepatitis C virus and is crucial for viral cell-to-cell transmission by contributing to a post-envelopment step of assembly* J, Virol., **88**, 1433–1446
Icard, V., Diaz, O., Scholtes, C., Perrin-Cocon, L., Ramière, C., Bartenschlager, R., Penin, F., Lotteau, V. and André, P. (2009) *Secretion of hepatitis C virus envelope glycoproteins depends on assembly of apolipoprotein B positive lipoproteins* PLoS One **4**: e4233
Long, G., Hiet, M-S., Windisch, M.P., Lee, J-Y., Lohmann, V. and Bartenschlager, R. (2011) *Mouse hepatic cells support assembly of infectious hepatitis C virus particles* Gastroenterology **141**, 1057–1066
Puig-Basagoiti, F., Fukuhara, T., Tamura, T., Ono, C., Uemura, K., Kawachi, Y., Yamamoto, S., Mori, H. et al (2016*) Human cathelicidin compensates for the role of apolipoproteins in hepatitis C virus infectious particle formation* J. Virol., **90**, 8464-8477

2-4-3-3b. Lipid droplets

Rösch, K., Kwiatkowski, M., Hofmann, S., Schöbel, A., Grüttner, C., Wurlitzer, M., Schlüter, H. and Herker, E. (2016) *Quantitative lipid droplet proteome analysis identifies annexin A3 as a cofactor for HCV particle production* Cell Rep., **16**, 3219–3231
Salloum, S., Wang, H., Ferguson, C., Parton, R.G. and Tai1, A.W. (2013) *Rab18 binds to hepatitis C virus NS5A and promotes interaction between sites of viral replication and lipid droplets* PLoS Pathog., **9**: e1003513

2-4-3-3c. Lipids, influence of

Liefhebber, J.M.P., Hague, C.V., Zhang, Q., Wakelam, M.J.O. and McLauchlan, J. (2014) *Modulation of triglyceride and cholesterol ester synthesis impairs assembly of infectious hepatitis C virus* J. Biol. Chem., **289**, 21276-21288

2-4-3-3d. Intracellular membrane compartments

Bayer, K., Banning, C., Bruss, V., Wiltzer-Bach, L. and Schindler, M. (2016) *Hepatitis C virus is released via a noncanonical secretory* route J. Virol., **90**, 10558-10573
Pène, V., Lemasson, M., Harper, F., Pierron, G. and Rosenberg, A. (2017) *Role of cleavage at the core-E1 junction of hepatitis C virus polyprotein in viral morphogenesis* PLoS One, **12**: e0175810

2-4-3-3e. Protein/glycoprotein interactions

Adair, R., Patel, A.H., Corless, L., Griffin, S., Rowlands, D.J. and McCormick, C.J. (2009) *Expression of hepatitis C virus (HCV) structural proteins in trans facilitates encapsidation and transmission of HCV subgenomic RNA* J. Gen. Virol., **90**, 833–842
Counihan, N.A., Rawlinson, S.M. and Lindenbach, B.D. (2011) *Trafficking of hepatitis C virus core protein during virus particle assembly* PLoS Pathog., **7**: e1002302
Eyre, N.S., Aloia, A.L., Joyce, M.A., Chulanetra, M., Tyrrell, D.L. and Beard, M.R. (2017) *Sensitive luminescent reporter viruses reveal appreciable release of hepatitis C virus NS5A protein into the extracellular environment* Virology, **507**, 20–31
Haddad, J.G., Rouillé, Y., Hanoulle, X., Descamps, V., Hamze, M., Dabboussi, F., Baumert, T,F., Duverlie, G., Lavie, M. and Dubuisson, J. (2017) *Identification of novel functions for hepatitis C virus envelope glycoprotein E1 in virus entry and assembly* J. Virol., **91**: e00048-17
Jones, D.M., Atoom, A.M., Zhang, X., Kottilil, S. and Russell, R.S. (2011) *A genetic interaction between the core and NS3 proteins of hepatitis C virus is essential for production of infectious virus* J. Virol., **85**, 12351–12361
Nielsen, S.U., Bassendine, F., Burt, A.D., Bevitt, D.J. and Toms, G.L. (2004) *Characterization of the genome and structural proteins of hepatitis C virus resolved from infected human liver* J. Gen. Virol., **85**, 1497-1507
Wahid, A., Helle, F., Descamps, V., Duverlie, G., Penin, F. and Dubuisson, J. (2013) *Disulfide bonds in hepatitis C virus glycoprotein E1 control the assembly and entry functions of E2 glycoprotein* J. Virol., 87, 1605-1617
Zayas, M., Long, G., Madan, V. and Bartenschlager, R. (2016) *Coordination of hepatitis C virus assembly by distinct regulatory regions in nonstructural protein* 5A PLoS Pathog., **12**: e1005376

2-4-3-3f. Proton channel

Bentham, M.J., Foster, T.L., McCormick, C. and Griffin, S. (2013) *Mutations in hepatitis C virus p7 reduce both the egress and infectivity of assembled particles via impaired proton channel function* J. Gen. Virol., **94**, 2236–2248

2-4-3-3g. Release inhibition

Shimakami, T., Honda, M., Shirasaki, T., Takabatake, R., Liu, F., Murai, K., Shiomoto, T. et al (2014) *The acyclic retinoid Peretinoin inhibits hepatitis C virus replication and infectious virus release in vitro* Sci. Rep., **4**: 4688

2-4-3-3h. RNA-related effects

Ma, Y., Yates, J., Liang, Y., Lemon, S.M. and Yi, MK. (2008) *NS3 helicase domains involved in infectious intracellular hepatitis C virus particle assembly* J. Virol., **82**, 7624-7639

Ndongo, N., Selliah, S., Berthillon, P., Raymond, V-A., Trépo, C., Bilodeau, M. and Petit, M-A. (2011) *Expression of E1E2 on hepatitis C RNA-containing particles released from primary cultured human hepatocytes derived from infected cirrhotic livers* Intervirology, **54**, 1–9

Steinmann, E., Brohm, C., Kallis, S., Bartenschlager, R. and Pietschmann, T. (2008) *Efficient trans-encapsidation of hepatitis C virus RNAs into infectious virus-like particles* J. Virol., **82**, 7034-7046

Yi, M., Ma, Y., Yates, J. and Lemon, S.M. (2007) *Compensatory mutations in E1, p7, NS2, and NS3 enhance yields of cell culture-infectious intergenotypic chimeric hepatitis C virus* J. Virol., **81**, 629-638

Yi, M-K., Ma, Y., Yates, J. and Lemon, S.M. (2009) *Trans-complementation of an NS2 defect in a late step in hepatitis C virus (HCV) particle assembly and maturation* PLoS Pathog., **5**:e1000403

2-4-3-4. Cell entry/virus fusion/virus transmission

2-4-3-4a. Inhibitors/markers

Anggakusuma, Colpitts, C.C., Schang, L.M., Rachmawati, H., Frentzen, A., Pfaender, S., Behrendt, P. et al (2014) *Turmeric curcumin inhibits entry of all hepatitis C virus genotypes into human liver cells* Gut, **63**, 1137–1149

Calland, N., Albecka, A., Belouzard, S., Wychowski, C., Duverlie, G., Descamps, V., Hober, D., Dubuisson, J., Rouill, Y. and Séron, K. (2012) *(-)-Epigallocatechin-3-gallate is a new inhibitor of hepatitis C virus entry* Hepatology, **55**, 720-729

Calland, N., Sahuc, M.E., Belouzard, S., Pène, V., Bonnafous, P., Mesalam, A.A., Deloison, G., Descamps, V. et al (2015) *Polyphenols inhibit hepatitis C virus entry by a new mechanism of action* J. Virol., **89**, 10053-10063

Ciesek, S. von Hahn, T., Colpitts, C.C., Schang, L.M., Friesland, M., Steinmann, J., Manns, M.P. et al (2011) *The green tea polyphenol, epigallocatechin-3-gallate, inhibits hepatitis C virus entry* Hepatology, **54**, 1947-1955

Fénéant, L., Potel, J., François, C., Sané, F., Douam, F., Belouzard, S., Calland, N., Vausselin, T. et al (2015) *New insights into the understanding of hepatitis C virus entry and cell-to-cell transmission by using the ionophore monensin A* J. Virol., **89**, 8346–8364

Grigorov, B., Reungoat, E., Gentil dit Maurin, A., Varbanov, M., Blaising, J., Michelet, M., Manuel, R., Parent, R., Bartosch, B. et al (2017) *Hepatitis C virus infection propagates through interactions between Syndecan-1 and CD81 and impacts the hepatocyte glycocalyx* Cell. Microbiol., **19**: e12711

Maillard, P., Walic, M., Meuleman, P., Roohvand, F., Huby, T., Le Goff, W., Leroux-Roels, G., Pécheur, E.I. and Budkowska, A. (2011) *Lipoprotein lipase inhibits hepatitis C virus (HCV) infection by blocking virus cell entry* PLoS One, **6**: e26637

Qian, X-J., Zhang, X-L., Zhao, P., Jin, Y-S., Chen, H-S., Xu, Q-Q., Ren, H., Zhu, S-Y. et al (2016) *A schisandra-derived compound schizandronic acid inhibits entry of pan-HCV genotypes into human hepatocytes* Sci. Rep., **6**: 27268

Sabahi, A. (2009) *Hepatitis C virus entry: the early steps in the viral replication cycle* Virol. J., **6**:117

Vausselin, T., Calland, N., Belouzard, S., Descamps, V., Douam, F., Helle, F., François, C. et al (2013) *The antimalarial ferroquine is an inhibitor of hepatitis C virus* Hepatology, **58**, 86-97

2-4-3-4b. Lipid composition

Haid, S., Pietschmann, T. and Pécheur, E.I. (2009) *Low pH-dependent hepatitis C virus membrane fusion depends on E2 integrity, target lipid composition, and density of virus particles* J. Biol. Chem., **284**, 17657–17667

2-4-3-4c. Lipoprotein association

Apolipoproteins

Diedrich, G. (2006) *How does hepatitis C virus enter cells?* FEBS J., **273**, 3871-3885

Nielsen, S., Sheridan, D., Bridge, S., Felmlee, D., Neely, D. Toms, G. and Bassendine, M. (2009) *Characterization of hepatitis C virus particles in human plasma: association with immunoglobulins G1, G3 and M and apolipoproteins A-I, A-II, B, C-I and E* J. Hepatol., **50** (Supp. 1) S316-S317
Oliveira, C., Fournier, C., Descamps, V., Morel, V., Scipione, C.A., Koschinsky, M.L., Boullier, A., Marcelo, P. et al (2016) *Apolipoprotein(a) inhibits hepatitis C virus entry* J. Clin. Virol., **82S**, S82-S83

Apolipoprotein(a) association

Oliveira, C., Fournier, C., Descamps, V., Morel, V., Scipione, C.A., Romagnuolo, R., Koschinsky, M.L., Boullier, A., Marcelo, P. et al (2017) *Apolipoprotein(a) inhibits hepatitis C virus entry through interaction with infectious particles* Hepatology, **65**, 1851-1864

Apolipoprotein B association

Bridge, S.H., Sheridan, D.A., Felmlee, D.J., Nielsen, S.U., Thomas, H.C., Taylor-Robinson, S.D., Neely, R.D.G., Toms, G.L. and Bassendine, M.F. (2011) *Insulin resistance and low-density apolipoprotein B-associated lipoviral particles in hepatitis C virus genotype 1 infection* Gut, **60**, 680-687
Bridge, S., Sheridan, D., Felmlee, D., Crossey, M., Thomas, H., Taylor-Robinson, S., Toms, G., Neely, D. and Bassendine, M. (2011) *P50 Apolipoprotein E and low-density, apolipoprotein B associated lipoviral particles in chronic hepatitis C infection: evidence for genotype-specific modulation of lipid pathways* Gut, **60**, A24
Icard, V., Diaz, O., Scholtes, C., Perrin-Cocon, L., Ramière, C., Bartenschlager, R., Penin, F., Lotteau, V. and André, P. (2009) *Secretion of hepatitis C virus envelope glycoproteins depends on assembly of apolipoprotein B positive lipoproteins* PLoS One **4**: e4233
Sheridan, D., Bridge, S., Sheridan, D.A., Felmlee, D., Thomas, H., Taylor-Robinson, S., Dermot, R., Neely, G., Toms, G.L. and Bassendine, M.F. (2010) *Measurement of low density apolipoprotein B associated hepatitis C virus lipoviral particles in genotype 1 infection is more clinically relevant than total viral load* Gut, **59** Suppl 2, A6

Apolipoprotein E association

Bridge, S., Sheridan, D., Felmlee, D., Crossey, M., Thomas, H., Taylor-Robinson, S., Toms, G., Neely, D. and Bassendine, M. (2011) *P50 Apolipoprotein E and low-density, apolipoprotein B associated lipoviral particles in chronic hepatitis C infection: evidence for genotype-specific modulation of lipid pathways* Gut, **60**, A24
Bridge, S.H., Sheridan, D.A., Felmlee, D.J., Crossey, M.M.E., Fenwick, F.I., Lanyon, C.V., Dubuc, G., Seidah, N.G., Davignon, J. et al (2015) *PCSK9, apolipoprotein E and lipoviral particles in chronic hepatitis C genotype 3: Evidence for genotype-specific regulation of lipoprotein metabolism* J. Hepatol., **62**, 763−770
Fauvelle, C., Felmlee, D.J., Crouchet, E., Lee, JY., Heydmann, L., Lefèvre, M., Magri, A., Hiet, M-S., Fofana, I., Habersetzer, F. ey al (2016) *Apolipoprotein E mediates evasion from hepatitis C virus neutralizing antibodies Gastroenterology* **150**, 206–217
Fénéant, L., Potel, J., François, C., Sané, F., Douam, F., Belouzard, S., Calland, N., Vausselin, T. et al (2015) *New insights into the understanding of hepatitis C virus entry and cell-to-cell transmission by using the ionophore monensin A* J. Virol., **89**, 8346–8364
Hishiki, T., Shimizu, Y., Tobita, R., Sugiyama, K., Ogawa, K., Funami, K., Ohsaki, Y., Fujimoto, T. et al (2010) *Infectivity of hepatitis C virus is influenced by association with apolipoprotein E isoforms* J. Virol., **84**, 12048-12057
Hueging, K., Doepke, M., Vieyres, G., Bankwitz, D., Frentzen, A., Doerrbecker, J., Gumz, F., Haid, S., Wölk, B., Kaderali, L. and Pietschmann, T. (2014) *Apolipoprotein E co-determines tissue tropism of hepatitis C virus and is crucial for viral cell-to-cell transmission by contributing to a post-envelopment step of assembly* J, Virol., **88**, 1433–1446
Lee, J-Y., Acosta, E.G., Stoeck, I.K., Long, G., Hiet, M-S., Mueller, B., Fackler, O.T., Kallis, S. and Bartenschlager, R. (2014) *Apolipoprotein E likely contributes to a maturation step of infectious hepatitis C virus particles and interacts with viral envelope glycoproteins* J. Virol., **88**, 12422–12437
Li, Z., Li, Y., Bi, Y., Zhang, H., Yao, Y., Li, Q., Cun, W., Dong, S. (2017) *Extracellular interactions between hepatitis C virus and secreted apolipoprotein* E J. Virol., **91**: e00227-16
Owen, D.M., Huang, H., Ye, J. and Gale, M. (2009) *Apolipoprotein E on hepatitis C virion facilitates infection through interaction with low-density lipoprotein receptor* Virology **394**, 99–108
Sheridan, D.A., Bridge, S.H., Felmlee, D.J., Crossey, M.M.E., Thomas, H.C., Taylor-Robinson, S.D., Toms, G.L., Neely, R.D.G. and Bassendine, M.F. (2012) *Apolipoprotein-E and hepatitis C lipoviral particles in genotype 1 infection: Evidence for an association with interferon sensitivity* J. Hepatol., **57**, 32–38
Yang, Z., Wang, X., Chi, X., Zhao, F., Guo, J., Ma, P., Zhong, J., Niu, J., Pan, X. and Long, G. (2016) *Neglected but important role of apolipoprotein E exchange in hepatitis C virus infection* J. Virol., **90**, 9632-9643

Zhu, W., Pei, R., Jin, R., Hu, X., Zhou, Y., Wang, Y., Wu, C., Lu, M. and Chen, X. (2014) *Nuclear receptor 4 group A member 1 determines hepatitis C virus entry efficiency through the regulation of cellular receptor and apolipoprotein E expression* J. Gen. Virol., **95**, 1510–1521

HCV E2 protein-lipoprotein association

Calattini, S., Fusil, F., Mancip, J., Thi, V.L.D., Granier, C., Gadot, N., Scoazec, J-Y., Zeisel, M.B. et al (2015) *Functional and biochemical characterization of hepatitis C virus (HCV) particles produced in a humanized liver mouse model* J. Biol. Chem., **290**, 23173–23187

Grove, J., Nielsen, S., Zhong, J., Bassendine, M.F., Drummer, H.E., Balfe, P. and McKeating, J.A. (2008) *Identification of a residue in hepatitis C virus E2 glycoprotein that determines scavenger receptor BI and CD81 receptor dependency and sensitivity to neutralizing antibodies* J. Virol., **82**, 12020–12029

Koutsoudakis, G., Dragun, J., Pérez-del-Pulgar, S., Coto-Llerena, M., Mensa, L., Crespo, G., González, P., Navasa, M. and Forns, X. (2012) *Interplay between basic residues of hepatitis C virus glycoprotein E2 with viral receptors, neutralizing antibodies and lipoproteins* PLoS One, **7**: e52651

2-4-3-4d. Receptors

Barth, H. Schnober, E.K., Neumann-Haeflin, C., Thumann, C., Zeisel, M.B., Diepolder, H.M., Hu, Z. et al (2008) *Scavenger receptor class B is required for hepatitis C virus uptake and cross-presentation by human dendritic cells* J. Virol., **82**, 3466-3479

Sainz Jr., B., Barretto, N., Martin, D.N., Hiraga, N., Imamura, M., Hussain, S., Marsh, K.A., Yu, X., Chayama, K., Alrefai1, W.A., and Uprichard, S.L. (2012) *Identification of the Niemann-Pick C1–like 1 cholesterol absorption receptor as a new hepatitis C virus entry factor* Nat. Med., **18**, 281-285

2-4-3-4e. Protein/glycoprotein/proteoglycan interactions

Bitzegeio, J., Bankwitz, D., Hueging, K., Haid, S., Brohm, C., Zeisel, M.B., Herrmann, E., Iken, M.. Ott, M., Baumert, T.F. and Pietschmann, T. (2010) *Adaptation of hepatitis C virus to mouse CD81 permits infection of mouse cells in the absence of human entry factors* PLoS Pathogens, **6**, e:1000978

Carlsen, T.H.R., Scheel, T,K,H, Ramirez, S., Foung, S.K.H. and Bukha, J. (2013) *Characterization of hepatitis C virus recombinants with chimeric E1/E2 envelope proteins and identification of single amino acids in the E2 stem region important for entry* J. Virol., **87**, 1385-1399

Haid, S., Pietschmann, T. and Pécheur, E.I. (2009) *Low pH-dependent hepatitis C virus membrane fusion depends on E2 integrity, target lipid composition, and density of virus particles* J. Biol. Chem., **284**, 17657–17667

Lavie, M., Sarrazin, S., Montserret, R., Descamps, V., Baumert, T.F., Duverlie, G., Séron, K., Penin, F. and Dubuissona, J. (2014) *Identification of conserved residues in hepatitis C virus envelope glycoprotein E2 that modulate virus dependence on CD81 and SRB1 entry factors* J. Virol., **88**, 10584–10597

Xu, Y., Martinez, P., Séron, K., Luo, G., Allain, F., Dubuisson, J. and Belouzard, S. (2015) *Characterization of hepatitis C virus interaction with heparan sulfate proteoglycans* J. Virol., 89, 3846-3858

2-4-3-4f. Cholesterol transport

Bocchetta, S., Maillard, P., Yamamoto, M., Gondeau, C., Douam, F., Lebreton, S., Lagaye, S., Pol, S. et al (2014) *Up-regulation of the ATP-binding cassette transporter A1 inhibits hepatitis C virus infection* PLoS One, **9**: e92140

Elgner, F., Ren, H., Medvedev, R., Ploen, D., Himmelsbach, K., Boller, K. and Hildt, E. (2016) *The intracellular cholesterol transport inhibitor U18666A inhibits the exosome-dependent release of mature hepatitis C virus* J. Virol., **90**, 11181-11196

2-4-3-4g. Cultured cell infection

Belouzard, S., Danneels, A., Fénéant, L., Séron, K., Rouillé, Y. and Dubuisson, J. (2017) *Entry and release of hepatitis C virus in polarized human hepatocytes* J. Virol., 91: e00478-17

Bridge, S.H., Sheridan, D.A., Felmlee, D.J., Nielsen, S.U., Neely, R.D.G., Toms, G.L. and Bassendine, M.F. (2010) *Insulin resistance correlates with low density hepatitis C virus particles in genotype 1 infection* J. Hepatol., **52**, S319–S457

Buck, M. (2008) *Direct infection and replication of naturally occurring hepatitis C virus genotypes 1, 2, 3 and 4 in normal human hepatocyte cultures* PLoS One, **3:**e2660

Da Costa, D., Turek, M., Felmlee, D.J., Girardi, E., Pfeffer, S., Long, G., Bartenschlager, R., Zeisel, M.B. and Baumert, D.F. (2012) *Reconstitution of the entire hepatitis C virus life cycle in nonhepatic cells* J. Virol., **86**, 11919-11925

Doerrbecker, J., Friesland, M., Riebesehl, N., Ginkel, C., Behrendt, P., Brown, R.J.P., Ciesek, S., Wedemeyer, H., Sarrazin, C., Kaderali, L. et al (2014) *Incorporation of primary patient-derived glycoproteins into authentic infectious hepatitis C virus particles* Hepatology, **60,** 508-520
Haid, S., Windisch, M.P., Bartenschlager, R. and Pietschmann, T. (2010) *Mouse-specific residues of claudin-1 limit hepatitis C virus genotype 2a infection in a human hepatocyte cell line* J. Virol., **84**, 964-975
Kato, T., Matsumura, T., Heller, T., Saito, S., Sapp, R.K., Murthy, K., Wakita, T. and Liang, T.J. (2007) *Production of infectious hepatitis C virus of various genotypes in cell cultures* J. Virol., **81**, 4405-4411
Lindenbach, B.D., Meuleman, P., Ploss, A., Vanwolleghem, T., Syder, A.L., McKeating, J.A., Lanford, R.E. et al (2006) *Cell culture-grown hepatitis C virus is infectious in vivo and can be recultured in vitro* Proc. Natl. Acad. Sci. USA, **103**, 3805-3809
Long, G., Hiet, M-S., Windisch, M.P., Lee, J-Y., Lohmann, V. and Bartenschlager, R. (2011) *Mouse hepatic cells support assembly of infectious hepatitis C virus particles* Gastroenterology 2011;141:1057–1066
Mathiesen, C.K., Jensen, T.B., Prentoe, J., Krarup, H., Nicosia, A., Law, M., Bukh, J. and Gottwein, J.M. (2104) *Production and characterization of high-titer serum-free cell culture grown hepatitis C virus particles of genotype 1* Virology **458-459**, 190–208
Molina-Jimenez, F., Benedicto, I., , Dao Thi, V.L., Gondar, V., Lavillette, D., Marin, J.J., Briz, O. et al (2012) *Matrigel-embedded 3D culture of Huh-7 cells as a hepatocyte-like polarized system to study hepatitis C virus cycle* Virology, **425**, 31–39
Ndongo-Thiam, N., Berthillon, P., Errazuriz, E., Bordes, I., De Sequeira, S., Trépo, C. and Petit, M-A. (2011) *Long-term propagation of serum hepatitis C virus (HCV) with production of enveloped HCV particles in human HepaRG hepatocytes* Hepatology, **54**, 406-417
Pietschmann, T., Zayas, M., Meuleman, P., Long, G., Appel, N., Koutsoudakis, G., Kallis, S., Leroux-Roels, G., Lohmann, V. and Bartenschlager, R. (2009) *Production of infectious genotype 1b virus particles in cell culture and impairment by replication enhancing mutations* PLoS Pathog., **5**:e1000475
Podevin, P., Carpentier, A., Pène, V., Aoudjehane, L., Carriere, M., Zaïdi, S., Hernanadez, C., Calle, V. et al (2010) *Production of infectious hepatitis C virus in primary cultures of human adult hepatocytes* Gastroenterology, **139**, 1355-1364
Podevin, P., Carpentier, A., Pène, V., Aoudjehane, L., Hernandez, C., Calle, V., Demignot, S., Scatton, O. et al (2010) *Culture of hepatitis C virus (HCV) in primary human adult hepatocytes: a physiological model for the production of authentic infectious particles* J. Hepatol., **52**, S183–S317
Skardasi, G. and Michalak, T.I. (2013) *Hepatitis C virus propogation in human CD4$^+$ and CD8$^+$ T lymphocytes* J. Hepatol., 58, S477-S578
Sugiyama, N., Murayama, A., Suzuki, R., Watanabe, N., Shiina, M., Liang, T.J., Wakita, T. and Kato, T. (2014) *Single strain isolation method for cell culture-adapted hepatitis C virus by end-point dilution and infection* PLoS One, **9**: e98168
Yi, M., Villanueva, R.A., Thomas, D.L., Wakita, T. and Lemon, S.M. (2006) *Production of infectious genotype 1a hepatitis C virus (Hutchinson strain) in cultured human hepatoma cells* Proc. Natl. Acad. Sci. USA, **103**, 2310-2315

2-4-3-4h. Envelope glycoproteins (E1/E2)

Atoom, A.M., Jones, D.M. and Russell, R.S. (2013) *Evidence suggesting that HCV p7 protects E2 glycoprotein from premature degradation during virus production* Virus Res., **176**, 199– 210
Carlsen, T.H.R., Scheel, T,K,H, Ramirez, S., Foung, S.K.H. and Bukha, J. (2013) *Characterization of hepatitis C virus recombinants with chimeric E1/E2 envelope proteins and identification of single amino acids in the E2 stem region important for entry* J. Virol., **87**, 1385-1399
Catanese, M.T., Uryu, K., Kopp, M., Edwards, T.J., Andrus, L., Rice, W.J., Silvestry, M., Kuhn, R.J. and Rice, C.M. (2013) *Ultrastructural analysis of hepatitis C virus particles* Proc. Natl. Acad. Sci. USA, **110**, 9505–9510
Doerrbecker, J., Friesland, M., Riebesehl, N., Ginkel, C., Behrendt, P., Brown, R.J.P., Ciesek, S. et al (2014) *Incorporation of primary patient-derived glycoproteins into authentic infectious hepatitis C virus particles* Hepatology, **60,** 508-520
Felmlee, D.J., Fauvelle, C., Heydmann, L., Hiet, M-S., Fofana, I., Bartenschlager, R., Stoll-Keller, F., Zeisel, M.B.. Fafi-Kremer, S. and Baumert, T.F. (2013) *Hepatitis C virus liver transplantation escape variant is characterized by both enhanced triglyceride-rich lipoprotein association and sensitivity to apoE antibodies* J. Hepatol., **58**, S468
Fénéant, L., Potel, J., François, C., Sané, F., Douam, F., Belouzard, S., Calland, N., Vausselin, T. et al (2015) *New insights into the understanding of hepatitis C virus entry and cell-to-cell transmission by using the ionophore monensin A* J. Virol., **89**, 8346–8364
Grove, J., Nielsen, S., Zhong, J., Bassendine, M.F., Drummer, H.E., Balfe, P. and McKeating, J.A. (2008) *Identification of a residue in hepatitis C virus E2 glycoprotein that determines scavenger receptor BI and CD81 receptor dependency and sensitivity to neutralizing antibodies* J. Virol., **82**, 12020–12029

Haddad, J.G., Rouillé, Y., Hanoulle, X., Descamps, V., Hamze, M., Dabboussi, F., Baumert, T,F., Duverlie, G., Lavie, M. and Dubuisson, J. (2017) *Identification of novel functions for hepatitis C virus envelope glycoprotein E1 in virus entry and assembly* J. Virol., **91**: e00048-17
Icard, V., Diaz, O., Scholtes, C., Perrin-Cocon, L., Ramière, C., Bartenschlager, R., Penin, F., Lotteau, V. and André, P. (2009) *Secretion of hepatitis C virus envelope glycoproteins depends on assembly of apolipoprotein B positive lipoproteins* PLoS One **4**: e4233
Koutsoudakis, G., Dragun, J., Pérez-del-Pulgar, S., Coto-Llerena, M., Mensa, L., Crespo, G., González, P., Navasa, M. and Forns, X. (2012) *Interplay between basic residues of hepatitis C virus glycoprotein E2 with viral receptors, neutralizing antibodies and lipoproteins* PLoS One, **7**: e52651
Lavie, M., Sarrazin, S., Montserret, R., Descamps, V., Baumert, T.F., Duverlie, G., Séron, K., Penin, F. and Dubuissona, J. (2014) *Identification of conserved residues in hepatitis C virus envelope glycoprotein E2 that modulate virus dependence on CD81 and SRB1 entry factors* J. Virol., **88**, 10584–10597
Lee, J-Y., Acosta, E.G., Stoeck, I.K., Long, G., Hiet, M-S., Mueller, B., Fackler, O.T., Kallis, S. and Bartenschlager, R. (2014) *Apolipoprotein E likely contributes to a maturation step of infectious hepatitis C virus particles and interacts with viral envelope glycoproteins* J. Virol., **88**, 12422–12437
Lee, M., Yang, J., Jo, E., Lee, J-Y., Kim, H-Y., Bartenschlager, R., Shin, E-C., Bae, Y-S. and Windisch, M.P. (2017) *A novel inhibitor IDPP interferes with entry and egress of HCV by targeting glycoprotein E1 in a genotype-specific manner* Sci. Rep., **7**: 44676
Pécheur, E-I., Diaz, O., Molle, J., Icard, V., Bonnafous, P., Lambert, O. and André, P. (2010) *Morphological characterization and fusion properties of triglyceride-rich lipoproteins obtained from cells transduced with hepatitis C virus glycoproteins* J. Biol. Chem., **285**, 25802–25811
Prentoe, J., Jensen, T.B., Meuleman, P., Serre, S.B.N., Scheel, T.K.H., Leroux-Roels, G., Gottwein, J.M. and Bukh, J. (2011) *Hypervariable region 1 differentially impacts viability of hepatitis C virus strains of genotypes 1 to 6 and impairs virus neutralization* J. Virol., **85**, 2224-2234
Wahid, A., Helle, F., Descamps, V., Duverlie, G., Penin, F. and Dubuisson, J. (2013) *Disulfide bonds in hepatitis C virus glycoprotein E1 control the assembly and entry functions of E2 glycoprotein* J. Virol., 87, 1605-1617

2-4-3-4i. Exosome association

Elgner, F., Ren, H., Medvedev, R., Ploen, D., Himmelsbach, K., Boller, K. and Hildt, E. (2016) *The intracellular cholesterol transport inhibitor U18666A inhibits the exosome-dependent release of mature hepatitis C virus* J. Virol., **90**, 11181-11196
Liu, Z., Zhang, X, Yu, Q. and He, J.J. (2014) *Exosome-associated hepatitis C virus in cell cultures and patient plasma* Biochem. Biophys. Res. Comm., **455**, 218–222

2-4-3-4j. Genome/genome manipulation

Chan, K., Cheng, G., Beran, R.K.F., Yang, H., Appleby, T.C., Pokrovskii, M.V., Mo, H., Zhong, SW., Delaney IV, W.E. (2012) *An adaptive mutation in NS2 is essential for efficient production of infectious 1b/2a chimeric hepatitis C virus in cell culture* Virology, **422**, 224–234
Nielsen, S.U., Bassendine, F., Burt, A.D., Bevitt, D.J. and Toms, G.L. (2004) *Characterization of the genome and structural proteins of hepatitis C virus resolved from infected human liver* J. Gen. Virol., **85**, 1497-1507
Vassilaki, N., Friebe, P., Meuleman, P., Kallis, S., Kaul, A., Paranhos-Baccalà, G., Leroux-Roels, G., Mavromara, P. and Bartenschlager, R. (2008) *Role of the hepatitis C virus core+1 open reading frame and core cis-acting RNA elements in viral RNA translation and replication* J. Virol., **82**, 11503-11515

2-4-3-4k. Hepatic nuclear factor α

Vallianou, I., Dafou, D., Vassilaki, N., Mavromara, P., Hadzopoulou-Cladaras, M. (2016) *Hepatitis C virus suppresses Hepatocyte Nuclear Factor 4 alpha, a keyregulator of hepatocellular carcinoma* Int. J. Biochem. Cell Biol., **78**, 315–326

2-4-3-4l. Host primate restriction

Marnata, C., Saulnier, A., Mompelat, D., Krey, T., Cohen, L., Boukadida, C., Warter, L., Fresquet, J., Vasiliauskaite, I., Escriou, N. et al (2015) *Determinants involved in hepatitis C virus and GB virus B primate host restriction* J. Virol., **89**, 12131-12144

2-4-3-4m. Immune responses

Bankwitz, D., Steinmann, E., Bitzegeio, J., Ciesek, S., Friesland, M., Herrmann, E., Zeisel, M.B., Baumert, T.F. et al (2010) *Hepatitis C virus hypervariable region 1 modulates receptor interactions, conceals the CD81 binding site, and protects conserved neutralizing epitopes* J. Virol., **84**, 5751–5763

Garrone, P., Fluckiger, A-C., Mangeot, P.E., Gauthier, E., Dupeyrot-Lacas, P., Mancip, J., Cangialosi, A. et al (2011) *A prime-boost strategy using virus-like particles pseudotyped for HCV proteins triggers broadly neutralizing antibodies in Macaques* Sci. Transl. Med., **3**: 94ra71
Grove, J., Nielsen, S., Zhong, J., Bassendine, M.F., Drummer, H.E., Balfe, P. and McKeating, J.A. (2008) *Identification of a residue in hepatitis C virus E2 glycoprotein that determines scavenger receptor BI and CD81 receptor dependency and sensitivity to neutralizing antibodies* J. Virol., **82**, 12020–12029
Johnson, D.F., Chin, R., Earnest-Silveira, L., Zentgraf, H., Bock, T., Chua, B., Jackson, D.C. and Torresi, J. (2010) *Recombinant mammalian cell derived hepatitis C virus-like particles induce neutralizing antibody responses to hepatitis C virus* Clin. Microbiol. Infect., **16**, S319
Lambotin, M., Baumert, T.F. and Barth, H. (2010) *Distinct intracellular trafficking of hepatitis C virus in myeloid and plasmacytoid dendritic cells* J Virol., **84**, 8964–8969

2-4-3-4n. Immunization

Elmowalid, G.A., Qiao, M., Jeong, S-H., Borg, B.B., Baumert, T.F., Sapp, R.K., Hu, Z., Murthy, K. and Liang, T.J. (2007) *Immunization with hepatitis C virus-like particles results in control of hepatitis C virus infection in chimpanzees* Proc. Natl. Acad. Sci. USA, **104**, 8427-8432

2-4-3-4o. Infectivity/infection control

Angus, A.G.N., Loquet, A., Stack, S.J., Dalrymple, D., Gatherer, D., Penin, F. and Patela, A.H. (2012) *Conserved glycine 33 residue in flexible domain I of hepatitis C virus core protein is critical for virus infectivity* J. Virol., **86**, 679-690
Bentham, M.J., Foster, T.L., McCormick, C. and Griffin, S. (2013) *Mutations in hepatitis C virus p7 reduce both the egress and infectivity of assembled particles via impaired proton channel function* J. Gen. Virol., **94**, 2236–2248
Bitzegeio, J., Bankwitz, D., Hueging, K., Haid, S., Brohm, C., Zeisel, M.B., Herrmann, E., Iken, M.. Ott, M., Baumert, T.F. and Pietschmann, T. (2010) *Adaptation of hepatitis C virus to mouse CD81 permits infection of mouse cells in the absence of human entry factors* PLoS Pathogens, **6**, e:1000978
Blanchet, M., Sureau, C., Guévin, C., Seidah, N.G. and Labonté, P. (2015) *SKI-1/S1P inhibitor PF-429242 impairs the onset of HCV infection* Antiviral Res., **115**, 94–104
Bocchetta, S., Maillard, P., Yamamoto, M., Gondeau, C., Douam, F., Lebreton, S., Lagaye, S., Pol, S. et al (2014) *Up-regulation of the ATP-binding cassette transporter A1 inhibits hepatitis C virus infection* PLoS One, **9**: e92140
Brault, C., Lévy, P., Duponchel, S., Michelet, M., Sallé, A., Pécheur, E-I., Plissonnier, M-L., Parent, R., Véricel, E. et al (2016) *Glutathione peroxidase 4 is reversibly induced by HCV to control lipid peroxidation and to increase virion infectivity* Gut, **65**, 144–154
Bridge, S.H., Sheridan, D.A., Felmlee, D.J., Nielsen, S.U., Neely, R.D.G., Toms, G.L. and Bassendine, M.F. (2010) *Insulin resistance correlates with low density hepatitis C virus particles in genotype 1 infection* J. Hepatol., **52**, S319–S457
Bush, C.O., Pokrovskii, M.V., Saito, R., Morganelli, P., Canales, E., Clarke, M.O., Lazerwith, S.E., Golde, J. et al (2014) *A small-molecule inhibitor of hepatitis C virus infectivity* Antimicrob. Agents Chemother., **58**, 386–396
Carlsen, T.H.R., Scheel, T,K,H, Ramirez, S., Foung, S.K.H. and Bukha, J. (2013) *Characterization of hepatitis C virus recombinants with chimeric E1/E2 envelope proteins and identification of single amino acids in the E2 stem region important for entry* J. Virol., **87**, 1385-1399
Farquhar, M.J., Harris, H.J., Diskar, M., Jones, S., Mee,, C.J., Nielsen, S.U., Brimacombe C.L. et al (2008) *Protein kinase A-dependent step(s) in hepatitis C virus entry and infectivity* J. Virol., **82**, 8797-8811
Gastaminza, P., Kapadia, S.B. and Chisari, F. (2006) *Differential biophysical properties of infectious intracellular and secreted hepatitis C virus particles* J. Virol., **80**, 11074-11081
Haid, S., Windisch, M.P., Bartenschlager, R. and Pietschmann, T. (2010) *Mouse-specific residues of claudin-1 limit hepatitis C virus genotype 2a infection in a human hepatocyte cell line* J. Virol., **84**, 964-975
Jones, D.M., Atoom, A.M., Zhang, X., Kottilil, S. and Russell, R.S. (2011) *A genetic interaction between the core and NS3 proteins of hepatitis C virus is essential for production of infectious virus* J. Virol., **85**, 12351–12361
Owen, D.M., Huang, H., Ye, J. and Gale, M. (2009) *Apolipoprotein E on hepatitis C virion facilitates infection through interaction with low-density lipoprotein receptor* Virology **394**, 99–108
Podevin, P., Carpentier, A., Pène, V., Aoudjehane, L., Carriere, M., Zaïdi, S., Hernanadez, C., Calle, V. et al (2010) *Production of infectious hepatitis C virus in primary cultures of human adult hepatocytes* Gastroenterology, **139**, 1355-1364

Sabahi, A., Marsh, K.A., Dahari, H., Corcoran, P., Lamora, J.M., Yu, X., Garry, R.F. and Uprichard, S.L. (2010) *The rate of hepatitis C virus infection initiation in vitro is directly related to particle density* Virology, **407**, 110–119
Sheridan, D.A., Hajarizadeh, B., Fenwick, F.I., Matthews, G.V., Applegate, T., Douglas, M., Neely, D., Askew, B. Dore, G.J., et al (2016) *Maximum levels of hepatitis C virus lipoviral particles are associated with early and persistent infection* Liver Int., **36**, 1774–1782
Shimizu, Y., Hishiki, T., Sugiyama, K., Ogawa, K., Funami, K., Kato, A., Ohsaki, Y., Fujimoto, T., Takaku, H. and Shimotohno, K. (2010) *Lipoprotein lipase and hepatic triglyceride lipase reduce the infectivity of hepatitis C virus (HCV) through their catalytic activities on HCV-associated lipoproteins* Virology, **407**, 152-159
Vausselin, T., Séron, K., Lavie, M., Mesalam, A.A., Lemasson, M., Belouzard, S., Fénéant, L., Danneels, A., Rouillé, Y. et al (2016) *Identification of a new benzimidazole derivative as an antiviral against hepatitis C virus* J. Virol., **90**, 8422-8434

2-4-3-4p. Insulin resistance

Bridge, S.H., Sheridan, D.A., Felmlee, D.J., Nielsen, S.U., Neely, R.D.G., Toms, G.L. and Bassendine, M.F. (2010) *Insulin resistance correlates with low density hepatitis C virus particles in genotype 1 infection* J. Hepatol., **52**, S319–S457
Bridge, S.H., Sheridan, D.A., Felmlee, D.J., Nielsen, S.U., Thomas, H.C., Taylor-Robinson, S.D., Neely, R.D.G., Toms, G.L. and Bassendine, M.F. (2011) *Insulin resistance and low-density apolipoprotein B-associated lipoviral particles in hepatitis C virus genotype 1 infection* Gut, **60**, 680-687
Das, G.C. and Hollinger, F.B. (2012) *Molecular pathways for glucose homeostasis, insulin signaling and autophagy in hepatitis C virus induced insulin resistance in a cellular mode*l Virology, **434**, 5–17

2-4-3-4q. Interferon sensitivity

Meredith, L.W., Farquhar, M.J., Tarr, A.W. and McKeating, J.A. (2014) *Type I interferon rapidly restricts infectious hepatitis C virus particle genesis* Hepatology, **60**, 1891-1901
Sheridan, D.A., Bridge, S.H., Felmlee, D.J., Crossey, M.M.E., Thomas, H.C., Taylor-Robinson, S.D., Toms, G.L., Neely, R.D.G. and Bassendine, M.F. (2012) *Apolipoprotein-E and hepatitis C lipoviral particles in genotype 1 infection: Evidence for an association with interferon sensitivity* J. Hepatol., **57**, 32–38

2-4-3-4r. LDL receptor

Albecka, A., Belouzard, S., Op de Beeck, A., Descamps, V., Goueslain, L., Bertrand-Michel, J., Tercé, F., Duverlie, G., Rouillé, Y. and Dubuisson, J. (2012) *Role of low-density lipoprotein receptor in the hepatitis C virus life cycle* Hepatology, **55**, 998-1007

2-4-3-4s. Lipid analysis

Felmlee, D.J., Fauvelle, C., Heydmann, L., Hiet, M-S., Fofana, I., Bartenschlager, R., Stoll-Keller, F., Zeisel, M.B.. Fafi-Kremer, S. and Baumert, T.F. (2013) *Hepatitis C virus liver transplantation escape variant is characterized by both enhances triglyceride-rich lipoprotein association and sensitivity to apoE antibodies* J. Hepatol., **58**, S468
Merz, A., Long, G., Hiet, M-S., Brügger, B., Chlanda, P., Andre, P., Wieland, F., Krijnse-Locker, J. and Bartenschlager, R. (2011) *Biochemical and morphological properties of hepatitis C virus particles and determination of their lipidome* J. Biol. Chem., **286**, 3018-3032
Shirasaki, T., Honda, M., Shimakami, T., Horii, R., Yamashita, T., Sakai, Y., Sakai, A. et al (2013) *MicroRNA-27a regulates lipid metabolism and inhibits hepatitis C virus replication in human hepatoma cells* J. Virol., **87**, 5270–5286

2-4-3-4t. Lipoprotein lipase

Maillard, P., Walic, M., Meuleman, P., Roohvand, F., Huby, T., Le Goff, W., Leroux-Roels, G., Pécheur, E.I. and Budkowska, A. (2011) *Lipoprotein lipase inhibits hepatitis C virus (HCV) infection by blocking virus cell entry* PLoS One, **6**: e26637
Shimizu, Y., Hishiki, T., Sugiyama, K., Ogawa, K., Funami, K., Kato, A., Ohsaki, Y., Fujimoto, T., Takaku, H. and Shimotohno, K. (2010) *Lipoprotein lipase and hepatic triglyceride lipase reduce the infectivity of hepatitis C virus (HCV) through their catalytic activities on HCV-associated lipoproteins* Virology, **407**, 152-159

2-4-3-4u. Lipoprotein metabolism

Caldwell, S., Hoehn, K.K. and Hahn, Y.S. (2013) *The strange and critical intersection of hepatitis C and lipoprotein metabolism: ''C-zing'' the Oil* Hepatology, 57, 1684-1687
Shimizu, Y., Hishiki, T., Ujino, S., Sugiyama, K., Funami, K. and Shimotohno, K. (2011) *Lipoprotein component associated with hepatitis C virus is essential for virus infectivity* Curr. Opin. Virol., **1**, 19–26

2-4-3-4v Scavenger receptor

Thi, V.L.D., Granier, C., Zeisel, M.B., Guérin, M., Mancip, J., Granio, O., Penin, F. et al (2012) *Characterization of hepatitis C virus particle subpopulations reveals multiple usage of the scavenger receptor BI for entry steps* J. Biol., Chem., **287**, 31242–31257

2-4-3-4w Triglyceride-rich particles

Felmlee, D.J., Sheridan, D.A., Bridge, S.H., Nielsen, S.U., Milne, R.W., Packard, C.J., Caslake, M.J. et al (2010) *Intravascular transfer contributes to postprandial increase in numbers of very-low-density hepatitis C virus particles* Gastroenterology **139**, 1774–1783

Felmlee, D., Sheridan, D., Bridge, S., Packard, C., Caslake, M., Toms, G., Neely, D. and Bassendine, M. (2011) *Use of Intralipid infusion to analyse apolipoprotein B (apoB) and HCV RNA kinetics in chronic infection* Gut, **60**, A21

Felmlee, D.J., Fauvelle, C., Heydmann, L., Hiet, M-S., Fofana, I., Bartenschlager, R., Stoll-Keller, F., Zeisel, M.B.. Fafi-Kremer, S. and Baumert, T.F. (2013) *Hepatitis C virus liver transplantation escape variant is characterized by both enhanced triglyceride-rich lipoprotein association and sensitivity to apoE antibodies* J. Hepatol., **58**, S468

2-4-3-4x VLDL association

Andreo, U., Scull, M.A., De Jong, Y.P., Ramanan, V., Flatley, B., Schwartz, R.E., Ng, S., Chen, A.A., Fisher, E.A., Bhatia, S. and Rice, C.M. (2014) *Novel in vitro models for assembly of VLDL and low-density hepatitis C virus particles* Hepatology, 60 (Suppl), 1050A-1051A

Bridge, S.H., Sheridan, D.A., Felmlee, D.J., Toms, G.L., Neely, R.D.G. and Bassendine, M.F. (2010) *Low density Hepatitis C virus particles (lipoviral particles) associate with insulin resistance in genotype 1 infection* Atherosclerosis **213**, e4

Jammart, B., Michelet, M., Pécheur, E-I., Parent, R., Bartosch, B., Zoulim, F. and Durante, D. (2013) *Very-low-density lipoprotein (VLDL)-producing and hepatitis C virus-replicating HepG2 cells secrete no more lipoviroparticles than VLDL-deficient Huh7.5 cells* J. Virol., **87**, 5065–5080

Nielsen, S.U., Bassendine, M.F., Burt, A.D., Martin, C., Pumeechockchai, W. and Toms, G.L. (2006) *Association between hepatitis C virus and very-low-density lipoprotein (VLDL)/LDL analyzed in iodixanol density gradients* J. Virol., **80**, 2418-2428

Nielsen, S.U., Bassendine, M.F., Martin, C., Lowther, D., Purcell, P.J., King, B.J., Neely, D., Toms, G.L. (2008) *Characterization of hepatitis C RNA-containing particles from human liver by density and size* J. Gen. Virol., **89**, 2507-2517

Pene, V., Hernandez, C., Blanc, E., Aoudjehane, L., Le Grand, B., Carpentier, A., Méritet, J-F., Conti, F. et al (2015) *Alcohol increases the production of hepatitis C virus (HCV) lipo-viro-particles in primary human hepatocytes* Hepatology, **62** (Suppl) 221A-222A

Podevin, P., Carpentier, A., Pène, V., Aoudjehane, L., Hernandez, C., Calle, V., Demignot, S., Scatton, O. et al (2010) *Culture of hepatitis C virus (HCV) in primary human adult hepatocytes: a physiological model for the production of authentic infectious particles* J. Hepatol., **52**, S183–S317

2-4-3-5. Lipid peroxidation

Brault, C., Lévy, p., Duponchel, s., Michelet, M., Sallé, A., Pécheur, E-I., Plissonnier, M-L., Parent, R., Véricel, E. et al (2016) *Glutathione peroxidase 4 is reversibly induced by HCV to control lipid peroxidation and to increase virion infectivity* Gut, **65**, 144–154

Yamane, D., McGivern, D.R., Wauthier, E., Yi, M., Madden, V.J., Welsch, C., Antes, I., Wen, Y., Chugh, P.E., McGee, C.E. et al (2014) *Regulation of the hepatitis C virus RNA replicase by endogenous lipid peroxidation* Nature Med., **20**, 927-935

2-4-3-6. Liver transplantation

Vercauteren, K., Van Den Eede, N., Mesalam, A.A., Belouzard, S., Catanese, M.T. et al (2014) *Successful anti-scavenger receptor class B type I (SR-BI) monoclonal antibody therapy in humanized mice after challenge with HCV variants with invitro resistance to SR-BI-targeting agents* Hepatology, **60,** 1508-1518

2-4-3-7. Molecular structure

Yu, X., Qiao, M., Atanasov, I., Hu, Z., Kato, T., Liang, T.J. and Zhou, Z.H. (2007) *Cryo-electron microscopy and three-dimensional reconstructions of hepatitis C virus particles* Virology, **126**, 126-134

2-4-3-8. Morphogenesis

Lussignol, M., Kopp, M., Molloy, K., Vizcay-Barrena, G., Fleck, R.A., Dorner, M., Bell, K.L., Chait, B.T., Rice, C.M. and Catanese, M.T. (2016) *Proteomics of HCV virions reveals an essential role for the nucleoporin Nup98 in virus morphogenesis* Proc. Natl. Acad. Sci. USA, **113**, 2484-2489

Pène, V., Lemasson, M., Harper, F., Pierron, G. and Rosenberg, A. (2017) *Role of cleavage at the core-E1 junction of hepatitis C virus polyprotein in viral morphogenesis* PLoS One, **12**: e0175810

2-4-3-9. Mutants

Mathiesen, C.K., Prentoe, J., Meredith, L.W., Jensen, T.B., Krarup, H., McKeating, J.A., Gottwein, J.M. and Bukha, J. (2015) *Adaptive mutations enhance assembly and cell-to-cell transmission of a high-titer hepatitis C virus genotype 5a core-NS2 JFH1-based recombinant* J. Virol., **89**, 7758-7775

Sugiyama, N., Murayama, A., Suzuki, R., Watanabe, N., Shiina, M., Liang, T.J., Wakita, T. and Kato, T. (2014) *Single strain isolation method for cell culture-adapted hepatitis C virus by end-point dilution and infection* PLoS One, **9**: e98168

2-4-3-10. Neutralization/neutralizing antibodies

Fauvelle, C., Felmlee, D.J., Crouchet, E., Lee, JY., Heydmann, L., Lefèvre, M., Magri, A., Hiet, M-S., Fofana, I., Habersetzer, F. ey al (2016) *Apolipoprotein E mediates evasion from hepatitis C virus neutralizing antibodies* Gastroenterology **150**, 206–217

Koutsoudakis, G., Dragun, J., Pérez-del-Pulgar, S., Coto-Llerena, M., Mensa, L., Crespo, G., González, P., Navasa, M. and Forns, X. (2012) *Interplay between basic residues of hepatitis C virus glycoprotein E2 with viral receptors, neutralizing antibodies and lipoproteins* PLoS One, **7**: e52651

Prentoe, J. and Bukh, J. (2011) *Hepatitis C virus expressing flag-tagged envelope protein 2 has unaltered infectivity and density, is specifically neutralized by flag antibodies and can be purified by affinity chromatography* Virology **409**, 148–155

Prentoe, J., Jensen, T.B., Meuleman, P., Serre, S.B.N., Scheel, T.K.H., Leroux-Roels, G., Gottwein, J.M. and Bukh, J. (2011) *Hypervariable region 1 differentially impacts viability of hepatitis C virus strains of genotypes 1 to 6 and impairs virus neutralization* J. Virol., **85**, 2224-2234

2-4-3-11. Pathogenesis

Eyre, N.S., Aloia, A.L., Joyce, M.A., Chulanetra, M., Tyrrell, D.L. and Beard, M.R. (2017) *Sensitive luminescent reporter viruses reveal appreciable release of hepatitis C virus NS5A protein into the extracellular environment* Virology, **507**, 20–31

2-4-3-12. Patient treatment

Sheridan, D.A., Bridge, S.H., Crossey, M.M.E., Felmlee, D.J., Fenwick, F.I., Thomas, H.C., Neely, R.D.G., Taylor-Robinson, S.D. and Bassendine, M.F. (2014) *Omega-3 fatty acids and/or fluvastatin in hepatitis C prior non-responders to combination antiviral therapy – a pilot randomized clinical trial* Liver Int., **34**, 737–747

2-4-3-13. Peretinoin

Shimakami, T., Honda, M., Shirasaki, T., Takabatake, R., Liu, F., Murai, K., Shiomoto, T. et al (2014) *The acyclic retinoid Peretinoin inhibits hepatitis C virus replication and infectious virus release in vitro* Sci. Rep., **4**: 4688

2-4-3-14. Permissivity

Helle, F., Brochot, E. Fournier, C., Descamps, V., Izquierdo, L., Hoffmann, T.W., Morel, V. et al (2013) *Permissivity of primary human hepatocytes and different hepatoma cell lines to cell culture adapted hepatitis C virus* PLoS One, **8**: e70809

Shiokawa, M., Fukuhara, T., Ono, C., Yamamoto, S., Okamoto, T., Watanabe, N., Wakita, T. and Matsuura, Y. (2014) *Novel permissive cell lines for complete propagation of hepatitis C virus* J. Virol., **88**, 5578–5594

2-4-3-15. Phospholipase A2 lipolysis

Menzel, N., Fischl, W., Hueging, K., Bankwitz, D., Frentzen, A., Haid, S., Gentzsch, J.et al (2012) *MAP-kinase regulated cytosolic phospholipase A2 activity is essential for production of infectious hepatitis C virus particles* PLoS Pathog., **8**: e1002829

2-4-3-16. Physical properties

Simmonds, P., Becher, P., Collett, M.S., Gould, E.A., Heinz, F.X., Meyers, G., Monath, T., Pletnev, A. et al (2012) *Hepacivirus* In Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses International Committee on Taxonomy of Viruses. Elsevier Inc., pp 1003-1020

2-4-3-17. Proteins

2-4-3-17a. Channel p7 protein

Bentham, M.J., Foster, T.L., McCormick, C. and Griffin, S. (2013) *Mutations in hepatitis C virus p7 reduce both the egress and infectivity of assembled particles via impaired proton channel function* J. Gen. Virol., **94**, 2236–2248

2-4-3-17b. Envelope protein 2

Prentoe, J. and Bukh, J. (2011) *Hepatitis C virus expressing flag-tagged envelope protein 2 has unaltered infectivity and density, is specifically neutralized by flag antibodies and can be purified by affinity chromatography* Virology **409**, 148–155

2-4-3-17c. Proteomics

Lussignol, M., Kopp, M., Molloy, K., Vizcay-Barrena, G., Fleck, R.A., Dorner, M., Bell, K.L., Chait, B.T., Rice, C.M. and Catanese, M.T. (2016) *Proteomics of HCV virions reveals an essential role for the nucleoporin Nup98 in virus morphogenesis* Proc. Natl. Acad. Sci. USA, **113**, 2484-2489

2-4-3-17d. Rab18

Salloum, S., Wang, H., Ferguson, C., Parton, R.G. and Tai1, A.W. (2013) *Rab18 binds to hepatitis C virus NS5A and promotes interaction between sites of viral replication and lipid droplets* PLoS Pathog., **9**: e1003513

2-4-3-17e. Structural proteins

Adair, R., Patel, A.H., Corless, L., Griffin, S., Rowlands, D.J. and McCormick, C.J. (2009) *Expression of hepatitis C virus (HCV) structural proteins in trans facilitates encapsidation and transmission of HCV subgenomic* RNA J. Gen. Virol., **90**, 833–842

Nielsen, S.U., Bassendine, F., Burt, A.D., Bevitt, D.J. and Toms, G.L. (2004) *Characterization of the genome and structural proteins of hepatitis C virus resolved from infected human liver* J. Gen. Virol., **85**, 1497-1507

2-4-3-18. Replication

Bankwitz, D., Steinmann, E., Bitzegeio, J., Ciesek, S., Friesland, M., Herrmann, E., Zeisel, M.B., Baumert, T.F., Keck, Z-y., Foung, S.K.H., Pécheur, E.I. and Pietschmann, T. (2010) *Hepatitis C virus hypervariable region 1 modulates receptor interactions, conceals the CD81 binding site, and protects conserved neutralizing epitopes* J. Virol., **84**, 5751–5763

Jammart, B., Michelet, M., Pécheur, E-I., Parent, R., Bartosch, B., Zoulim, F. and Durante, D. (2013) *Very-low-density lipoprotein (VLDL)-producing and hepatitis C virus-replicating HepG2 cells secrete no more lipoviroparticles than VLDL-deficient Huh7.5 cells* J. Virol., **87**, 5065–5080

Lindenbach, B.D., Evans, M.J., Syder, A.J., Wolk, B., Tellinghuisen, T.L., Liu, C.C., Maruyama, T., Hynes, R.O., Burton, D.R., McKeating, J.A. and Rice, C.M. (2005) *Complete replication of hepatitis C virus in cell culture* Science, **309**, 623-626

Pietschmann, T., Zayas, M., Meuleman, P., Long, G., Appel, N., Koutsoudakis, G., Kallis, S., Leroux-Roels, G., Lohmann, V. and Bartenschlager, R. (2009) *Production of infectious genotype 1b virus particles in cell culture and impairment by replication enhancing mutations* PLoS Pathog., **5**:e1000475

Romero-Brey, I., Merz, A., Chiramel, A., Lee, J-Y., Chlanda, P., Haselman, U., Santarella-Mellwig, R. et al (2012) *Three-dimensional architecture and biogenesis of membrane structures associated with hepatitis C virus replication* PLoS Pathog., **8**: e1003056

Salloum, S., Wang, H., Ferguson, C., Parton, R.G. and Tai1, A.W. (2013) *Rab18 binds to hepatitis C virus NS5A and promotes interaction between sites of viral replication and lipid droplets* PLoS Pathog., **9**: e1003513

Shimakami, T., Honda, M., Shirasaki, T., Takabatake, R., Liu, F., Murai, K., Shiomoto, T., Funaki, M. et al (2014) *The acyclic retinoid Peretinoin inhibits hepatitis C virus replication and infectious virus release in vitro* Sci. Rep., **4**: 4688

Shiokawa, M., Fukuhara, T., Ono, C., Yamamoto, S., Okamoto, T., Watanabe, N., Wakita, T. and Matsuura, Y. (2014) *Novel permissive cell lines for complete propagation of hepatitis C virus* J. Virol., **88**, 5578–5594

Shirasaki, T., Honda, M., Shimakami, T., Horii, R., Yamashita, T., Sakai, Y., Sakai, A., Okada, H. et al (2013) *MicroRNA-27a regulates lipid metabolism and inhibits hepatitis C virus replication in human hepatoma cells* J. Virol., **87**, 5270–5286

Vassilaki, N., Friebe, P., Meuleman, P., Kallis, S., Kaul, A., Paranhos-Baccalà, G., Leroux-Roels, G., Mavromara, P. and Bartenschlager, R. (2008) *Role of the hepatitis C virus core+1 open reading frame and core cis-acting RNA elements in viral RNA translation and replication* J. Virol., **82**, 11503-11515

2-4-3-19. miRNAs

Bourhill, T., Arbuthnot, P. and Ely, A. (2016) *Successful disabling of the 5'UTR of HCV using adeno-associated viral vectors to deliver modular multimeric primary microRNA mimics* J. Virol. Meth., **235**, 26–33

Shirasaki, T., Honda, M., Shimakami, T., Horii, R., Yamashita, T., Sakai, Y., Sakai, A., Okada, H. et al (2013) *MicroRNA-27a regulates lipid metabolism and inhibits hepatitis C virus replication in human hepatoma cells* J. Virol., **87**, 5270–5286

2-4-3-20. Serum, detection in

Bartolomé, J., López-Alcorocho, J.M., Castillo, I., Rodriguez-Iñigo, E., Quiroga, J.A., Palacios, R. and Carreño, V. (2007) *Ultracentrifugation of serum samples allows detection of hepatitis C virus RNA in patients with occult hepatitis C.* J. Virol., **81**, 7710-7715

2-4-3-21. Signal peptidase

Pène, V., Lemasson, M., Harper, F., Pierron, G. and Rosenberg, A. (2017) *Role of cleavage at the core-E1 junction of hepatitis C virus polyprotein in viral morphogenesis* PLoS One, **12**: e0175810

2-4-3-22. Subpopulations of

Andreo, U., de Jong, Y.P., Scull, M.A., Xiao, J.W., Vercauteren, K., Quirk, C., Mommersteeg, M.C., Bergaya, S. et al (2017) *Analysis of hepatitis C virus particle heterogeneity in immunodeficient human liver chimeric fah-/- mice* Cell. Mol. Gastroenterol. Hepatol., **4**, 405–417

Thi, V.L.D., Granier, C., Zeisel, M.B., Guérin, M., Mancip, J., Granio, O., Penin, F. et al (2012) *Characterization of hepatitis C virus particle subpopulations reveals multiple usage of the scavenger receptor BI for entry steps* J. Biol., Chem., **287**, 31242–31257

2-4-3-23. Vaccines

Garrone, P., Fluckiger, A-C., Mangeot, P.E., Gauthier, E., Dupeyrot-Lacas, P., Mancip, J., Cangialosi, A. et al (2011) *A prime-boost strategy using virus-like particles pseudotyped for HCV proteins triggers broadly neutralizing antibodies in Macaques* Sci. Transl. Med., **3**: 94ra71

Mathiesen, C.K., Prentoe, J., Meredith, L.W., Jensen, T.B., Krarup, H., McKeating, J.A., Gottwein, J.M. and Bukha, J. (2015) *Adaptive mutations enhance assembly and cell-to-cell transmission of a high-titer hepatitis C virus genotype 5a core-NS2 JFH1-based recombinant* J. Virol., **89**, 7758-7775

2-4-3-24. Viral receptor association

Koutsoudakis, G., Dragun, J., Pérez-del-Pulgar, S., Coto-Llerena, M., Mensa, L., Crespo, G., González, P., Navasa, M. and Forns, X. (2012) *Interplay between basic residues of hepatitis C virus glycoprotein E2 with viral receptors, neutralizing antibodies and lipoproteins* PLoS One, **7**: e52651

2-4-3-25. Vitamin D

Kato, T., Sugiyama, N., Murayama, A., Matsumura, T., Shiina, M., Asabe, S., Wakita, T. and Imawari, M. (2013) *Antimicrobial peptide LL-37 deteriorate infectivity of hepatitis C virus* Hepatology, **58** (suppl), 443A-444A

2-4-4. West Nile virus

Thompson, B.S., Moesker, B., Smit, J.M., Wilschut, J., Diamond, M.S. and Fremont, D.H. (2009) *A therapeutic antibody against West Nile virus neutralizes infection by blocking fusion within endosomes* PLoS Pathog., **5**:e1000453

Vancini, R., Kramer, L.D., Ribeiro, M., Hernandez, R. and Brown, D. (2013) *Flavivirus infection from mosquitoes in vitro reveals cell entry at the plasma membrane* Virology **435**, 406–414

Vogt, M.R., Moesker, B., Goudsmit, J., Jongeneelen, M., Austin, K., Oliphant, T., Nelson, S., Pierson, T.C., Wilschut, J., Throsby, M. and Diamond, M.S. (2009) *Human monoclonal antibodies against West Nile Virus induced by natural infection neutralize at a post-attachment step* J. Virol., **83**, 6494–6507

2.5 Group IV Hepeviridae

Hepatitis E

Allweiss, L., Gass, S., Giersch, K., Groth, A., Kah, J., Volz, T., Rapp, G., Schöbel, A. et al (2016) *Human liver chimeric mice as a new model of chronic hepatitis E virus infection and preclinical drug evaluation* J. Hepatol., **64**, 1033–1040

Behrendt, P., Bremer, B., Todt, D., Brown, R.J.P., Heim, A., Manns, M.P., Steinmann, E. and Wedemeyer, H. (2016) *Hepatitis E virus (HEV) ORF2 antigen levels differentiate between acute and chronic HEV infection* J. Infect. Dis., **214**, 361–368

Chapuy-Regaud, S., Dubois, M., Plisson-Chastang, C., Bonnefois, T., Lhomme, S., Bertrand-Michel, J., You, B., Simoneau, S. et al (2017) *Characterization of the lipid envelope of exosome encapsulated HEV particles protected from the immune response* Biochimie, **141**, 70-79
Emerson, S.U., Nguyen, H.T., Torian, U., Burke, D., Engle, R. and Purcell, R.H. (2010) *Release of genotype 1 hepatitis E virus from cultured hepatoma and polarized intestinal cells depends on open reading frame 3 protein and requires an intact PXXP motif* J. Virol., **84**, 9059–9069
Feng, Z. and Lemon, S.M. (2014) *Peek-a-boo: membrane hijacking and the pathogenesis of viral hepatitis* Trends Microbiol., **22**, 59-64
Izopet, J., Lhomme, S., Chapuy-Regaud, S., Mansuy, J-M., Kamar, N. and Abravanel, F. (2017) *HEV and transfusion-recipient risk* Transfusion Clinique et Biologique, **24**, 176–181
Von Nordheim, M., Boinay, M., Leisi, R., Kempf, C. and Ros, C. (2016) *Cutthroat trout virus—towards a virus model to support hepatitis E research* Viruses, **8**: 289
Yin, X., Ambardekar, C., Lu, Y. and Feng, Z. (2016) *Distinct entry mechanisms for nonenveloped and quasi-enveloped hepatitis E viruses* J. Virol., **90**, 4232-4242

2.6 Group IV Picornaviridae

2.6.1 Coxsackie virus

Petrik, J. (2016) *Immunomodulatory effects of exosomes produced by virus-infected cells* Transfus. Apher. Sci., **55**, 84–91
Robinson, S.M., Tsueng, G., Sin, J., Mangale, V., Rahawi, S., McIntyre, L.L., Williams, W., Kha, N. et al (2014) *Coxsackievirus B exits the host cell in shed microvesicles displaying autophagosomal markers* PLoS Pathog., **10**: e1004045

2.6.2 Hepatitis A

Das, A., Hirai-Yuki, A., González-López, O., Rhein, B., Moller-Tank, S., Brouillette, R., Hensley, L., Misumi, I. et al (2017) *TIM1 (HAVCR1) is not essential for cellular entry of either quasi-enveloped or naked hepatitis A virions* mBIO, **8**: e00969-17
Feng, Z. and Lemon, S.M. (2014) *Peek-a-boo: membrane hijacking and the pathogenesis of viral hepatitis* Trends Microbiol., **22**, 59-64
Feng, Z., Hensley, L., McKnight, K.L., Hu, F., Madden, V., Ping, L-F., Jeong, S-H., Walker, C., Lanford, R.E. and Lemon, S.M. (2013) *A pathogenic picornavirus acquires an envelope by hijacking cellular membranes* Nature **496**, 367-371
Hirai-Yuki, A., Hensley, L., Whitmire, J.K. and Lemon, S.M. (2016) *Biliary secretion of quasi-enveloped human hepatitis A virus* mBio, **7**: e01998-16
Hofer, U. (2013) *Cloak and dagger* Nat. Rev. Microbiol., **11**, 3026
Kapsch, A-M., Farcet, M.R., Antoine, G. and Kreil, T.R. (2017) *A nonenveloped virus with a lipid envelope: hepatitis A virus as used in virus-reduction studies* Transfusion **57**, 1433–1439
McKnighta, K.L., Xiec, L., González-Lópeza, O., Rivera-Serrano, E.E., Chen, X. and Lemon, S.M. (2017) *Protein composition of the hepatitis A virus quasi-envelope* Proc. Natl. Acad. Sci. USA, **114**, 6587–6592
Vaughan, G., Goncalves Rossi, L.M., Forbi, J.C., de Paula, V.S, Purdy, M.A., Xia, G. and Khudyakov, Y.E. (2014) *Hepatitis A virus: Host interactions, molecular epidemiology and evolution* Infect. Genet. Evol., **21**, 227–243

2.6.3. Rhinovirus

Simpson, J.L., Carroll, M., Yang, I.A., Reynolds, P.N., Hodge, S., James, A.L., Gibson, P.G. and Upham, J.W. (2016) *Reduced antiviral interferon production in poorly controlled asthma is associated with neutrophilic inflammation and high-dose inhaled corticosteroids* Chest, **149**, 704-713
Xi, Y., Finlayson, A., White, O.J., Carroll, M.L., and Upham, J.W. (2015) *Rhinovirus stimulated IFN-α production: how important are plasmacytoid DCs, monocytes and endosomal pH?* Clin. Translat. Immunol., **4**, e46

2.7 Group IV Togaviridae

2.7.1 Alphavirus

Akahata, W. and Nabel, G. J. (2012) *A specific domain of the Chikungunya virus E2 protein regulates particle formation in human cells: implications for alphavirus vaccine design* J. Virol., **86**, 8879-8883
Jose, J., Przybyla, L., Edwards, T.J., Perera, R., Burgner II, J.W. and Kuhn, R.J. (2012) *Interactions of the cytoplasmic domain of Sindbis virus E2 with nucleocapsid cores promote alphavirus budding* J. Virol., 86, 2585-2599

Snyder, J.E., Azizgolshani, O., Wu, B., He, Y., Lee, A.C., Jose, J., Suter, D.M., Knobler, C.M., Gelbart, W.M. and Kuhn, R.J. (2011) *Rescue of infectious particles from preassembled alphavirus nucleocapsid cores* J. Virol., **85**, 5773–5781
Snyder, J.E., Berrios, C.J., Edwards, T.J., Jose, J., Perera, R. and Kuhn, R.J. (2012) *Probing the early temporal and spatial interaction of the Sindbis virus capsid and E2 proteins with reverse genetics* J. Virol., **86**, 12372-12383
Snyder, J.E., Kulcsar, K.A., Schultz, K.L.W., Riley, C.P., Neary, J.T., Marr, S., Jose, J., Griffin, D.E. and Kuhn, R.J. (2013) *Functional characterization of the alphavirus TF protein* J. Virol., **87**, 8511–8523
Sokoloski, K.J., Snyder, A.J., Liu, N.H., Hayes, C.A., Mukhopadhyay, S. and Hardy, R.W. (2013) *Encapsidation of host-derived factors correlates with enhanced infectivity of Sindbis virus* J. Virol., **87**, 12216–12226
Tang, J., Jose, J., Chipman, P., Zhang, W., Kuhn, R.J. and Baker, T.S. (2011) *Molecular links between the E2 envelope glycoprotein and nucleocapsid core in sindbis virus* J. Mol. Biol., **414**, 442–459
Urakami, A., Sakurai, A., Ishikawa, M., Yap, M.L., Flores-Garcia, Y., Haseda, Y., Aoshi, T., Zavala, F.P. et al (2017) *Development of a novel virus-like particle vaccine platform that mimics the immature form of alphavirus* Clin. Vacc. Immunol., **24**: e00090-17

2.7.2 Rubella virus

Battisti, A.J., Yoder, J.D., Plevka, P., Winkler, D.C., Prasad, V.M., Kuhn, R.J., Frey, T.K., Steven, A.C. and Rossmann, M.G. (2012) *Cryo-electron tomography of rubella virus* J. Virol., **86**, 11078-11085

2.7.3. Semliki Forest virus

Hammarstedt, M., Wallengren, K., Pedersen, K.W., Roos, N. and Garoff, H. (2000) *Minimal exclusion of plasma membrane proteins during retrovirus envelope formation* Proc. Natl. Acad. Sci. USA, **97**, 7527-7532
Kalvodova, L., Sampaio, J.L., Cordo, S., Ejsing, C.S., Shevchenko, A. and Simons, K. (2009) *The lipidomes of vesicular stomatitis virus, Semliki Forest virus and the host plasma membrane analyzed by quantitative shotgun mass spectrometry* J. Virol., **83**, 7996-8003
Sjøberg, M. and Garoff, H. (2003) *Interactions between the transmembrane segments of the alphavirus E1 and E2 proteins play a role in virus budding and fusion* J. Virol., **77**, 3441-3450

Sindbis virus – see 2.7.1 Alphavirus

2.7.5 Venezuelan equine encephalitis virus

Jurgens, C.K., Young, K.R., Madden, V.J., Johnson, P.R. and Johnston, R.E. (2012) *A novel self-replicating chimeric lentivirus-like particle* J. Virol., **86**, 246-261
Lamb, K., Lokesh, G.L., Sherman, M. and Watowich, S. (2010) *Structure of a Venezuelan equine encephalitis virus assembly intermediate isolated from infected cells* Virology **406**, 261–269
Porta, J., Jose, J., Roehrig, J.T., Blair, C.D., Kuhn, R.J. and Rossmann, M.G. (2014) *Locking and blocking the viral landscape of an alphavirus with neutralizing antibodies* J. Virol., **88**, 9616–9623

Mini-Review MV06: 4th edition, December 2017