THE HCV LIFECYCLE: ACHIEVEMENTS AND CHALLENGES

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Apath, LLC: Shareholder
BMS: Consultant
Genentech, Inc: Consultant
GlaxoSmithKline: Scientific Advisor
iTherX: Scientific Advisor
Merck: Scientific Advisor
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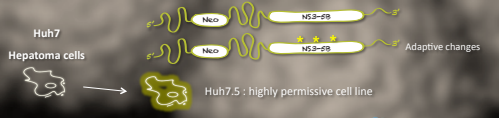
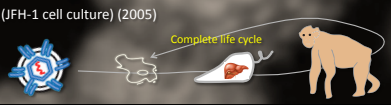
Evolution of HCV research tools

- Biochemical assays : NS3-4A protease, NS5B RNA dependent RNA polymerase
- Replicon systems (1999-2002)



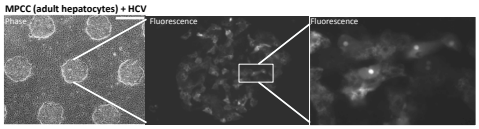
- HCVpp (pseudoparticle) (2003)

- HCVcc (JFH-1 cell culture) (2005)

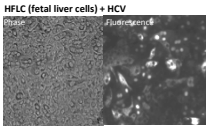


Hepatocyte-like culture systems supporting HCV replication

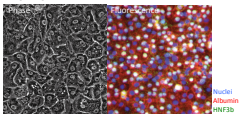
MPCC (adult hepatocytes) + HCV



HFLC (fetal liver cells) + HCV



iHLC + HCV



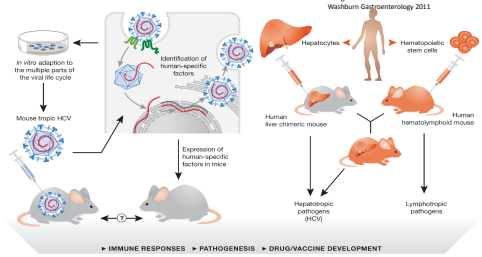
Schwartz, Trehan PNAS 2012
Wu PLoS Pathogen 2012
Andrus Hepatology 2011
Ploss, Khetani PNAS 2010
Si-Tayeb Hepatology 2009

Towards small animal models for HCV

Viral adaptation
Bitzegeio Plos Pathogen 2010

Genetic humanization
Dorner Nature 2011
Dorner Nature 2013

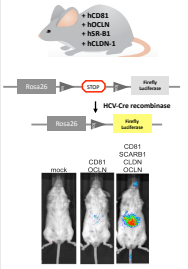
Xenotransplantation
Mercer Nat. Med. 2001
Mueleman Hepatology 2005
Bissig J Clin Invest 2010
Washburn Gastroenterology 2011



Ploss and Rice (2009) EMBO reports

Establishing genetically humanized mouse models

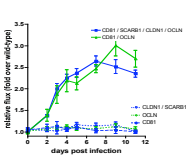
Adenovirus delivery



A genetically humanized mouse model for hepatitis C virus infection.

Dorner Nature 2011

Entry factor transgenic mice

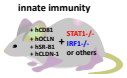


•*Transgenic* expression of human CD81 and OCLN is sufficient to render HCV infection in mice.

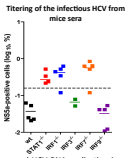
•*But do not readily support HCV replication in liver.*

Dorner Nature 2013

Entry factor Tg mice x Knockouts of innate immunity



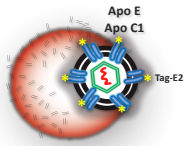
Titering of the infectious HCV from mice sera



•Improved HCV RNA replication in mice with blunting antiviral responses

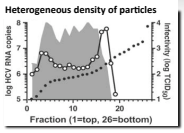
•*Assembly of infectious HCV in mice confirmed.*

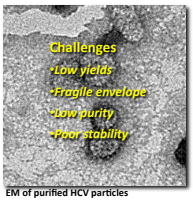
The HCV virion



Affinity EM grid for monolayer purification

Heterogeneous density of particles



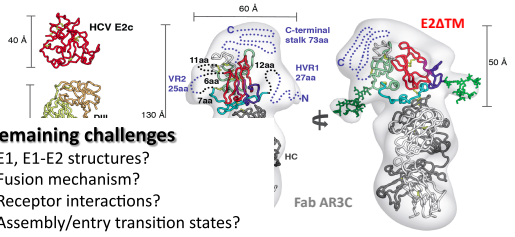


EM of purified HCV particles

Thomssen Med Microbiol Immunol. 1992
André JVI 2002
Chang JVI 2007
Huang PNAS 2007
Gastaminza JVI 2008
Catanese PNAS 2013
Merz JBC 2010
Gastaminza JVI 2010

E2 ectodomain is globular in shape

Single-molecule EM analysis of E2ΔTM

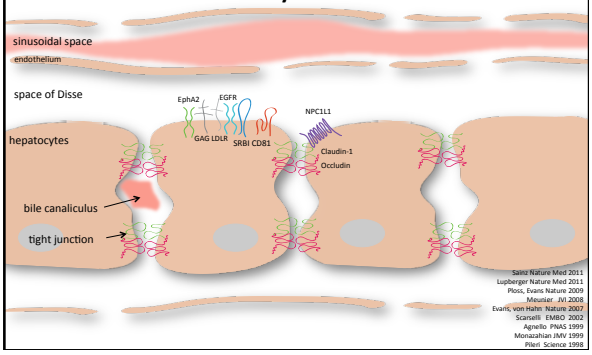


Remaining challenges

- E1, E1-E2 structures?
- Fusion mechanism?
- Receptor interactions?
- Assembly/entry transition states?

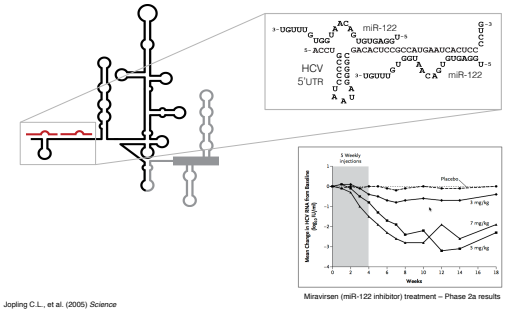
Kong *et al.* Science 2013

Entry factors



Sainz Nature Med 2011
Lupberger Nature Med 2011
Ploss, Evans Nature 2009
Meuleur , JVI 2008
Evans, von Hahn Nature 2007
Scarselli EMBO 2002
Agnello PNAS 1999
Monazahian JMV 1999
Pileri Science 1998

HCV and miR-122 – a critical host-viral interaction

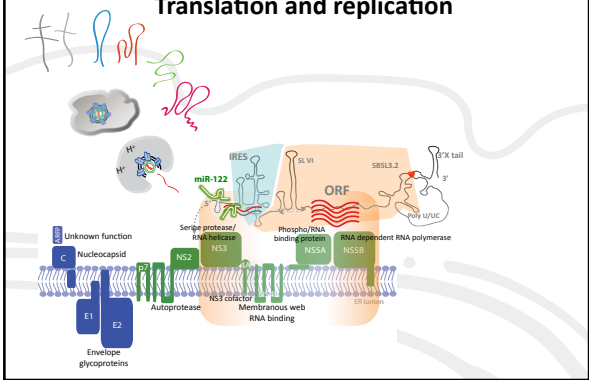


Miravirsen (miR-122 inhibitor) treatment – Phase 2a results

Jopling C.L., et al. (2005) *Science*
Jopling C.L., et al. (2008) *Cell Host & Microbe*
Machlin E.S., et al. (2011) *PNAS*

Janssen, H.L.A., et al. (2013) *NEJM*

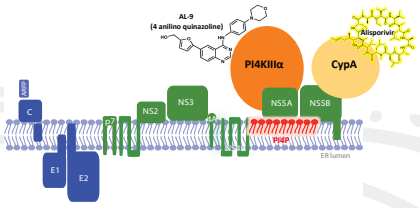
Translation and replication



Translation and replication

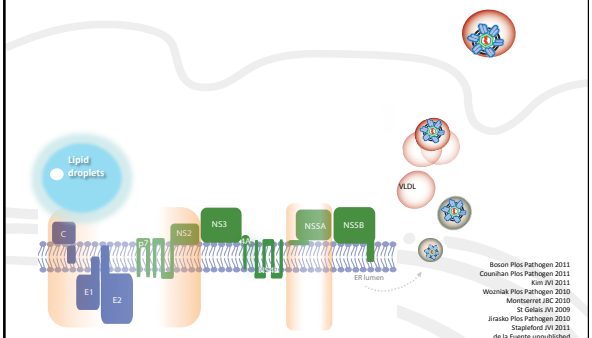
Important host proteins

- Regulation of genome RNA translation, replication, and packaging?
- Composition and structure of -/+ strand replicases?
- Roles of host factors, eg CypA and PI4KIIIa?



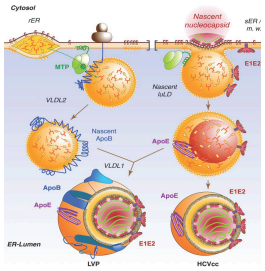
Reiss Cell Host Microbe 2011
Bianco Plos Pathogens 2012
Grise JVI 2012
Kaul Plos Pathogen 2009
Chatterji JBC 2009
Coelmont AAC 2009
Many others

Assembly and release



Boson Plos Pathogen 2011
Counihan Plos Pathogen 2011
Kim JVI 2011
Wozniak Plos Pathogen 2010
Montserret JBC 2010
St Gelais JVI 2009
Jirasko Plos Pathogen 2010
Stapleford JVI 2011
de la Fuente unpublished

Models of the biogenesis of HCV particles



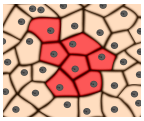
Lipoviroparticles
1.06 - 1.25 g/ml
Lower density = more infectious

Cell culture derived HCV
~ 1.1 g/ml
ApoB negative

Bartenschlager, Penin, Lohmann & André Trends in Microbiology 2011

What's going on in vivo?

HCV infection is clustered



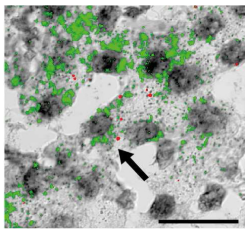
HCV infected cell

		% infected
2PE-microscopy	Liang et al.	7-20
LCM/PCR	Kadathil et al.	21-45
FISH	Wieland et al.	1-54

Liang et al. Gastro 2009
Kadathil et al. Gastro 2013
Wieland et al. Hepatology 2013

HCV and innate immune activation

HCV infected cells upregulate ISGs *in vivo*



Fluorescent *in situ* hybridization (FISH)

HCV RNA

IFI-27

Figure 4C Wieland et al. Hepatology 2013

Take home lessons

Achievements

- HCVcc, hepatocyte cultures, animal models
- Production of virus particles
- Many entry factors defined
- Identification of cis RNA elements & miR-122
- Viral and cellular factors in RNA replication
- S and NS protein interplay in assembly
- Role of lipid droplets, lipid metabolism
- Requirement for ApoE
- HCV activation of innate immunity

•Bottom line: Many advances, important therapeutic targets revealed

Challenges

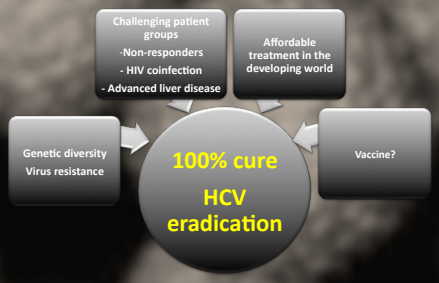
- Phenotyping clinical isolates?
- Composition, structure, heterogeneity?
- Entry pathway in polarized cells?
- How do they work?
- Composition & structure of the replicase?
- Regulation of genome RNA packaging?
- Sites of NC assembly, budding mechanism?
- Site of lipoprotein association?
- Contribution to control vs persistence?

•Bottom line: Deeper mechanistic insights needed at all levels

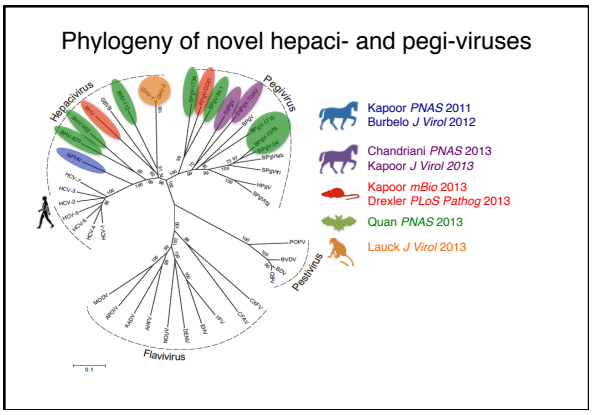
HCV 2014

- Great research tools
- Growing understanding of the virus lifecycle
- Many unanswered questions but…
- Curative IFN-free therapies with >90% efficacy
- Still no approved vaccine—is one needed?
- What's next?

But challenges still remain…



And you never know what's coming next…


Phylogeny of novel hepaci- and pegi-viruses
Hepacivirus
Pegivirus
Pestivirus
Flavivirus
Kapoor PNAS 2011
Burbelo J Virol 2012
Chandriani PNAS 2013
Kapoor J Virol 2013
Kapoor mBio 2013
Drexler PLoS Pathog 2013
Quan PNAS 2013
Lauck J Virol 2013

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