Rôle de FXR dans la réplication des virus des hépatites B et C

Patrice André, MD, PhD

Inserm U851
Université Claude Bernard Lyon1
Hospices Civils de Lyon
Lyon, France

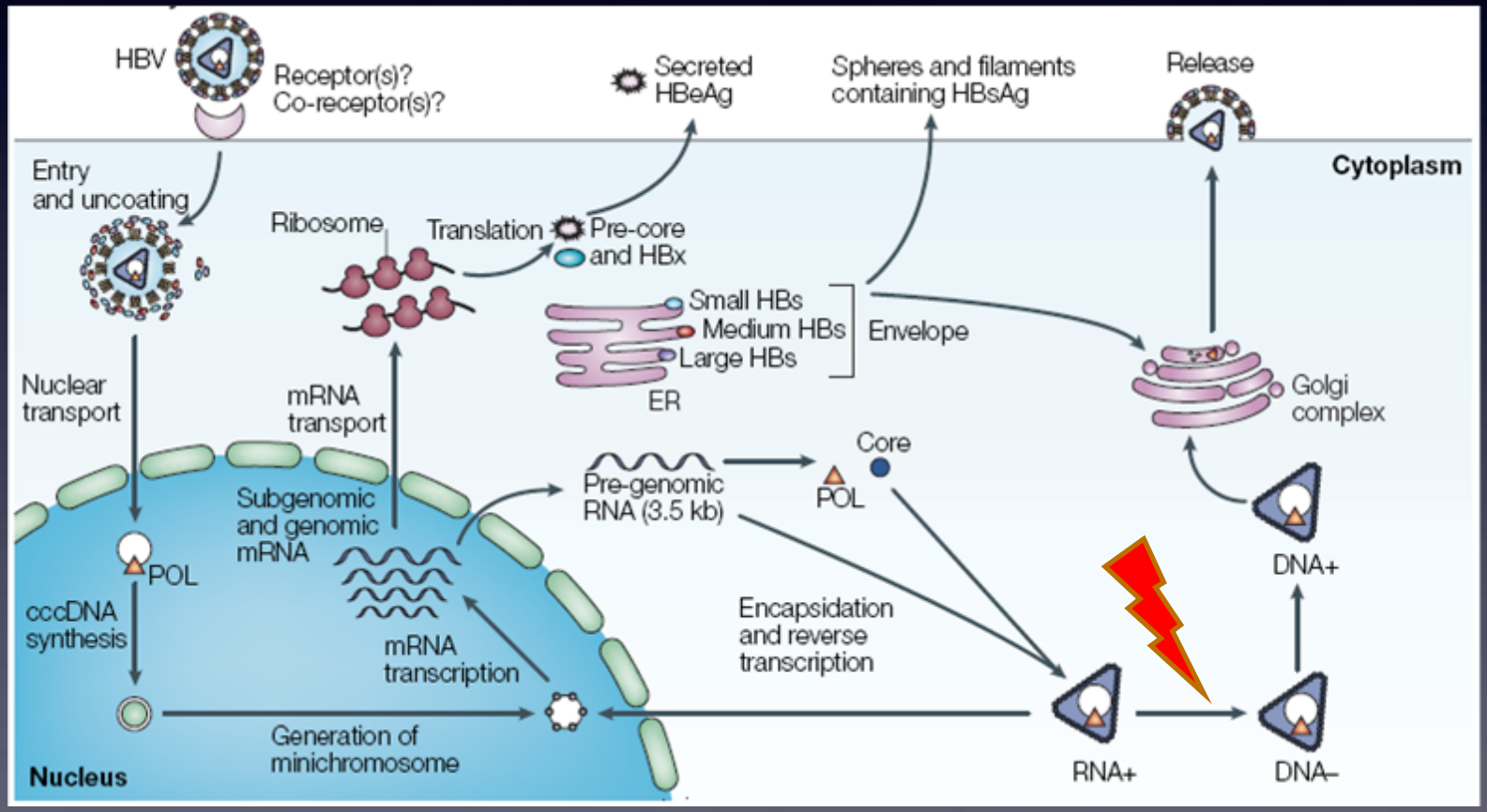
Journées Nationales d'Infectiologie
10-12 juin 2009
Lyon

Nuclear receptors and virus replication

- DNA virus
  Hepatitis B virus,
  a well known example of virus whose tropism depends at least partially on nuclear receptors

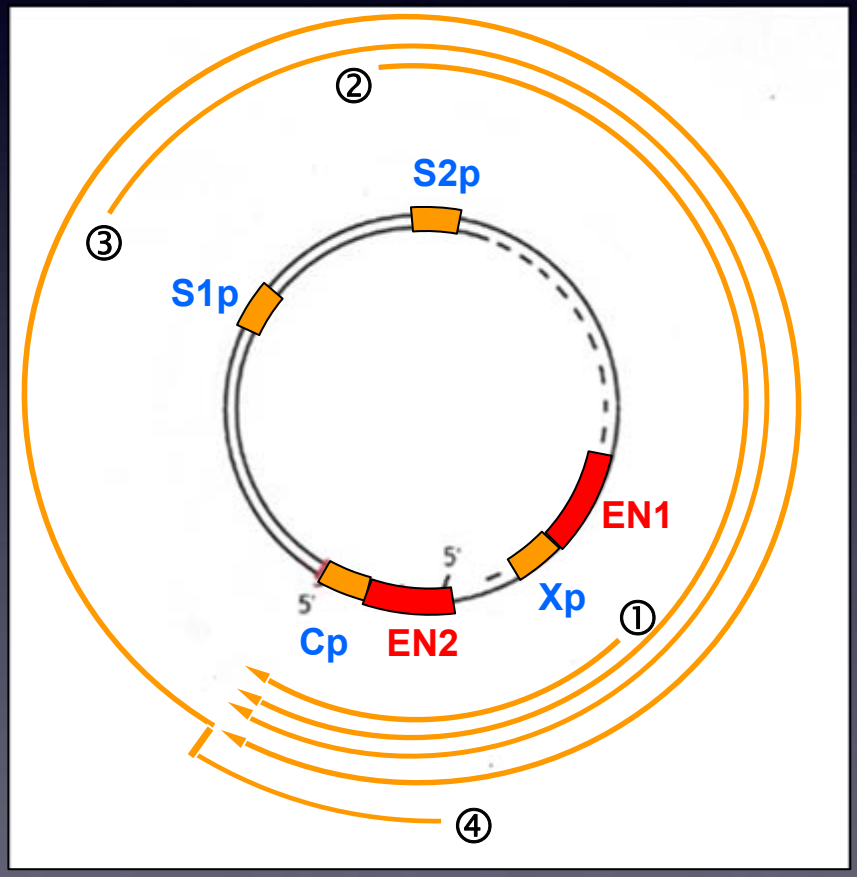
- RNA virus
  Hepatitis C virus

HBV replication cycle and therapeutic targets



Control of HBV genome transcription

- 3.2 kb circular DNA, partially double stranded
- 4 overlapping ORFs



① RNA 0.7 kb
→ protein X

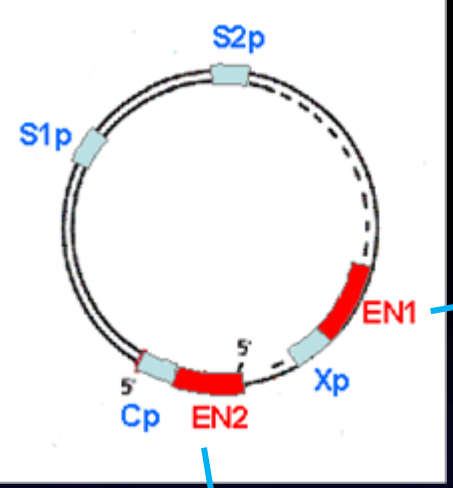
② RNA 2.1 kb

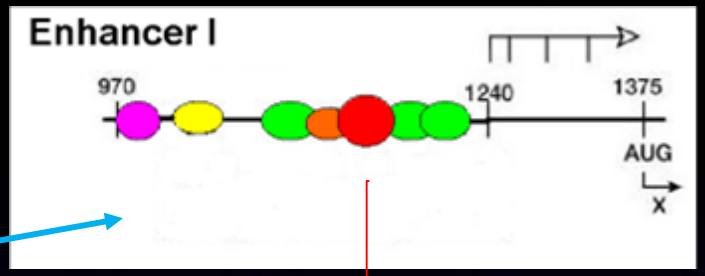
③ RNA 2.6 kb
→ envelope glycoproteins: AgHBs

④ RNA 3.5 kb
→ protein preCore

→ protein Core
polymerase

→ pregenomic RNA



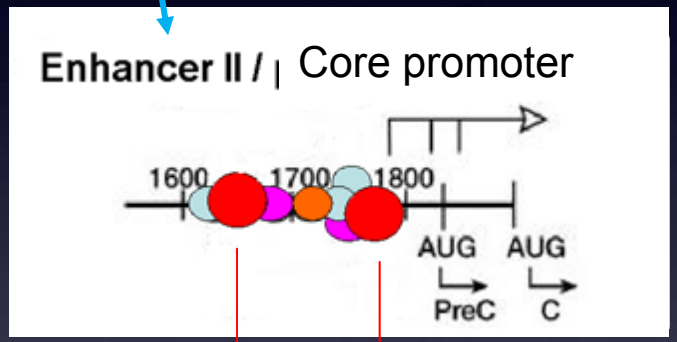


1067
GCATGTATACAATCTAAGCAGGCTTTCACT
TTCTCGCCAACTTATAAGGCCTTTCTGTGTA
AACAATATCTGAACCTTTACCCCGTTGCCC

$NRRE_{enhI}$

GGCAACGGTCAGGTCTCTGCCAAGTGTTTG
CTGACGCAACCCCCACTGGATGGGGCTTGG
1227

PPARα/RXRα
HNF4α
COUP-TF1



1623
TGAACGCCCACCAGGTCTTGCCCAAGGTCT
TACATAAGAGGACTCTTGGACTCTCAGCCAT

$NRRE_{enhII}$

1684

HNF4α

1686
GTCAACGACCGACCTTGAGGCATACTTCAAA
GACTGTGTGTTTAAAGACTGGGAGGAGTTGG
GGGAGGAGATTAGGTTAAAGGTCTTTGTACT

$NRRE_{preC}$

1779

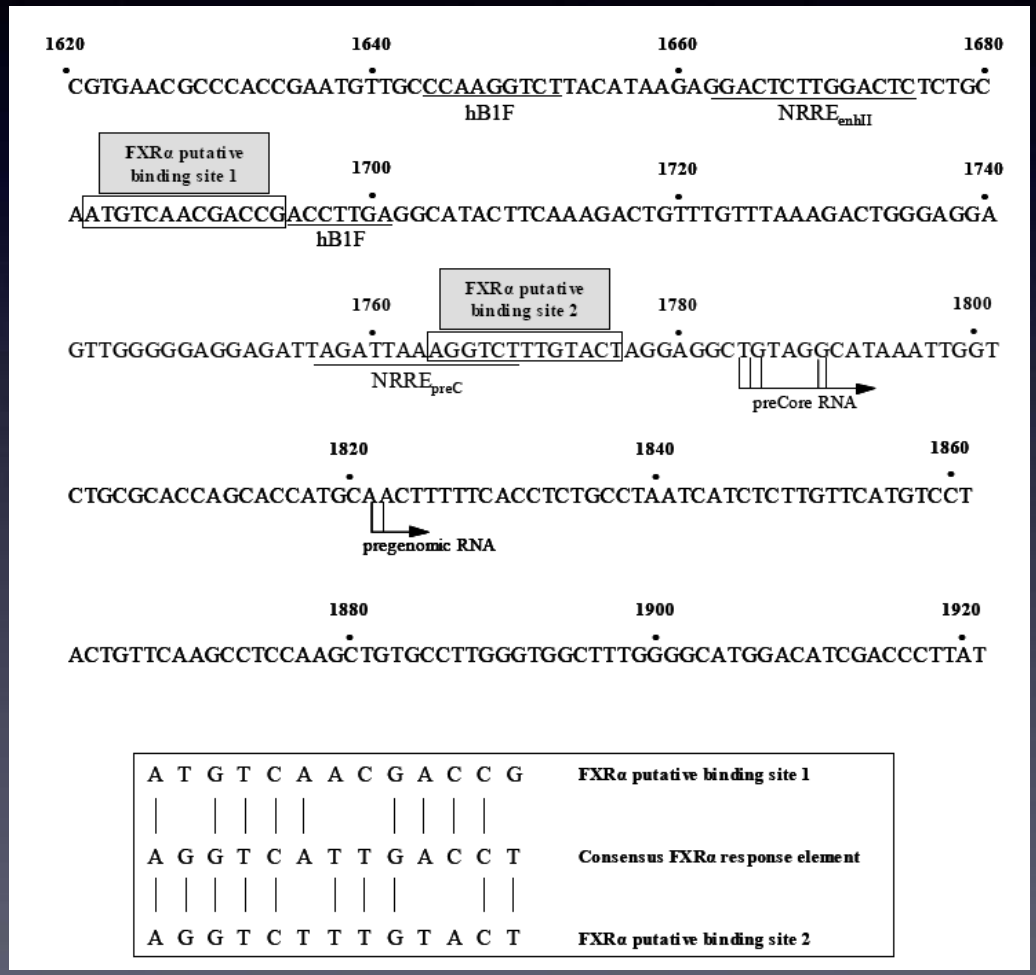
PPARα/RXRα
HNF4α
COUP-TF1

HBV : nuclear receptors binding sites

Study of FXRα on HBV replication

- **Farnesoid X Receptor FXR**
  - Belongs to the NR1 family of nuclear receptors
  - ligands = bile acids (CDCA)
  - Regulates many genes of biliary salts synthesis and transport as well as glucose and lipid metabolism in the liver and intestine
  - Forms heterodimer with RXRα
  - Consensus response element : AGGTCA .N.TGACCT (IR-1)

FXREs in the EN2/pCore of HBV



Two putative FXREs in the Enhancer 2 and the core promoter

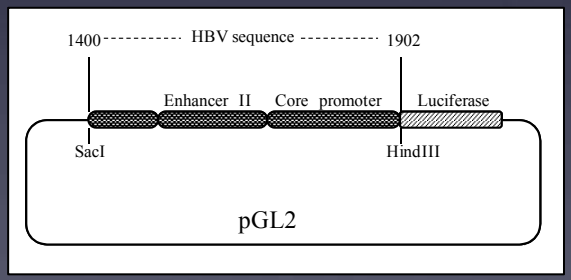
That may control the transcription of the pgRNA and HBV genome replication

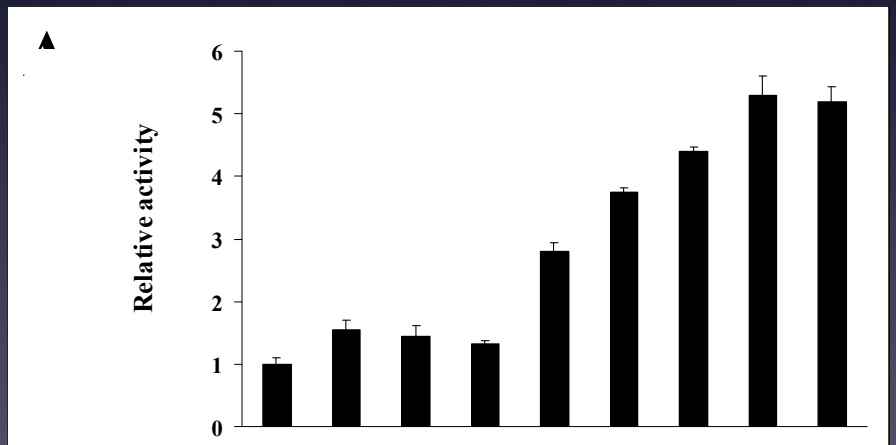
FXRα activates transcription from the HBV core promoter

Huh-7 Transfection
- pGL2-EN2/PC
- pRL-SV40
- pSG5-FXRα

Biliary salts

Luciferase activity
- firefly
- renilla



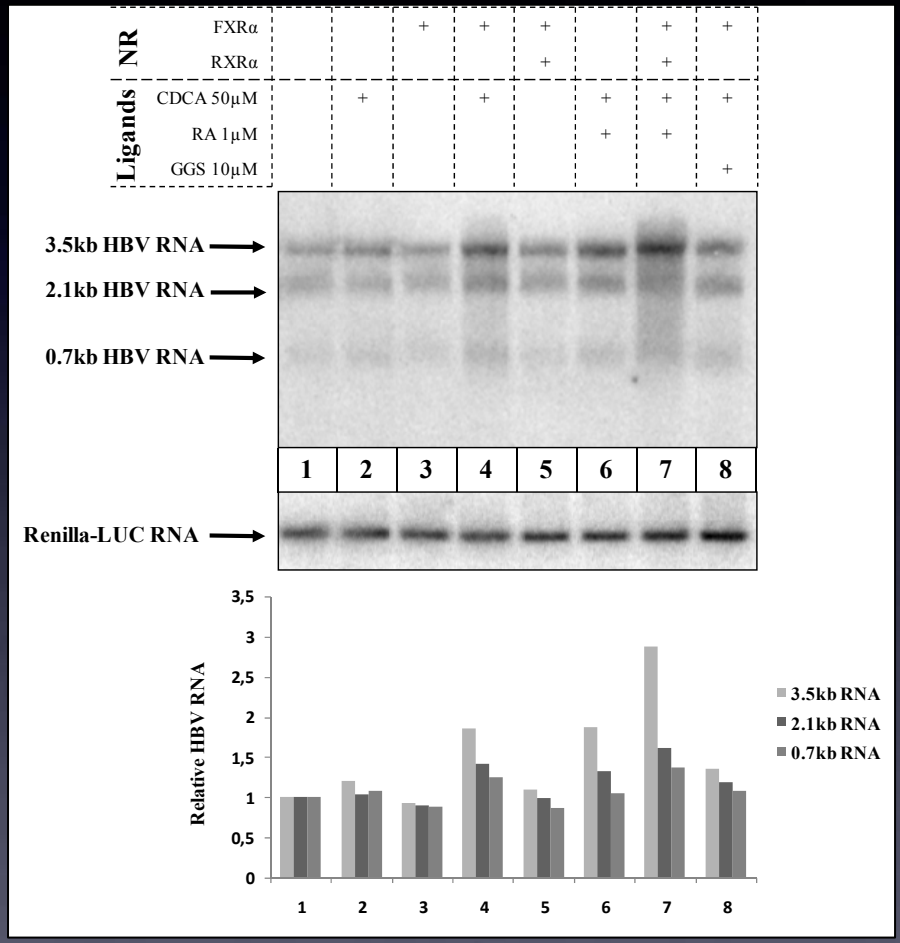


FXRα					+	+	+	+	+
CDCA	10µM					+			
	25µM						+		
	50µM		+	+				+	
	100µM							+	+
GGS 10µM				+					+

Effect of FXRα and RXRα on viral RNA synthesis

- Transfection of Huh-7
  p1.3xHBVwt
  pSG5-FXRα and/or –RXRα
- Ligands of FXRα and/or RXRα

FXR/RXR increases synthesis of the pregenomic RNA and viral DNA



Conclusions for HBV

Ramière C. J Virol 2008

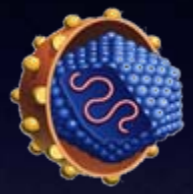
- FXRα can be added to the list of liver NR with PPARα and HNF4α that activate HBV replication

- Role of biliary salts in the natural history of hepatitis B ?

- Screening for small molecules modulating FXR activity

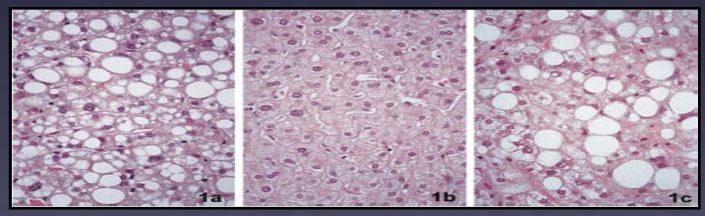
Hepatitis C virus replication and FXRα

- HCV
  - positive strand RNA
  - *Flaviviridae* family



d = 1.20 g/mL

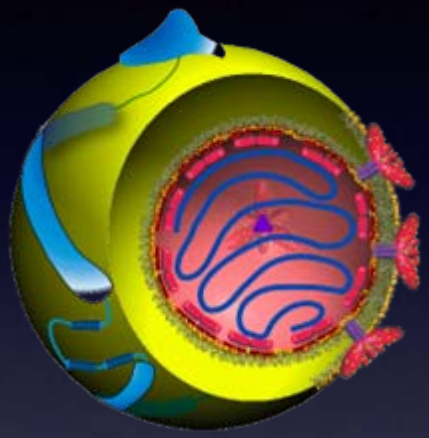
- Hepatitis C induces a specific metabolic syndrome:
  - insulin resistance
  - liver steatosis
  - hypo-betalipoproteinemia



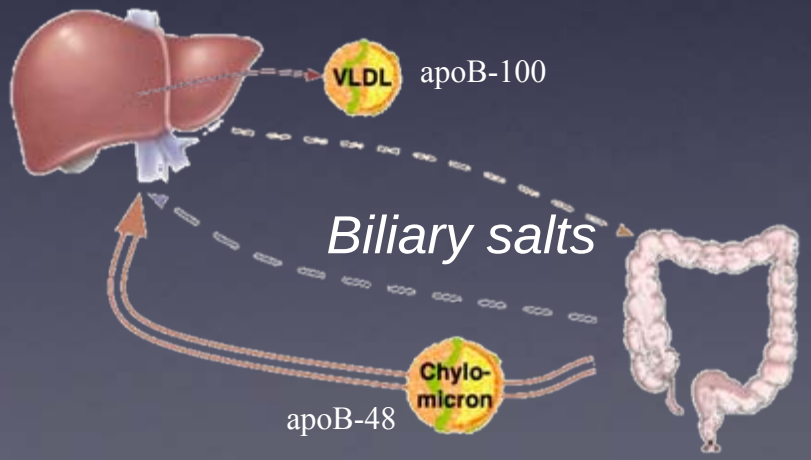
- Hepatitis C viral particles in the blood of chronically infected patients are associated with lipoproteins

RUBBIA-BRANDT L, J Hepatol 2001
André P, J Virol 2002, André P, Sem Liv Dis. 2005

Lipo-viral-particles (LVP)



- Hybrid viral / lipoprotein particles :
  - Viral capside
  - HCV RNA
  - Viral envelope proteins
  - Globular
  - Triglyceride rich
  - apoB-100 or apoB-48
- Liver and intestine : replication sites



d <1,06 g/mL

Andre et al. J Virol 2002
Andre et al. Semin Liver Dis 2005
Diaz et al. J Gen Virol 2006
Nielsen et al. J Virol 2006
Icard et al PLoSOne 2009

Desforges et al. J Gen Virol 2004

Bile acids and HCV

- **Enterohepatic circulation of BAs between the liver and intestine**

  the two replication sites of HCV

  (Fischer et al. Clin Chim Acta 1996)

- **High BA levels during hepatitis are marker of poor response to anti-HCV therapy**

  (Lebovisc et al. Dig Dis Sci 1997, *Jorquera et al.* J Gastroenterol Hepatol 2005)

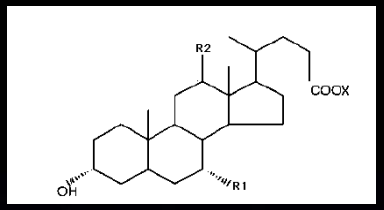
- **BAs are needed for *in vitro* replication of porcine Calicivirus**

  (Chang et al. PNAS 2004)

hypothesis

BAs might not be only consequence of hepatitis but rather metabolic factors favoring HCV replication and resistance to antiviral therapy

Bile acids

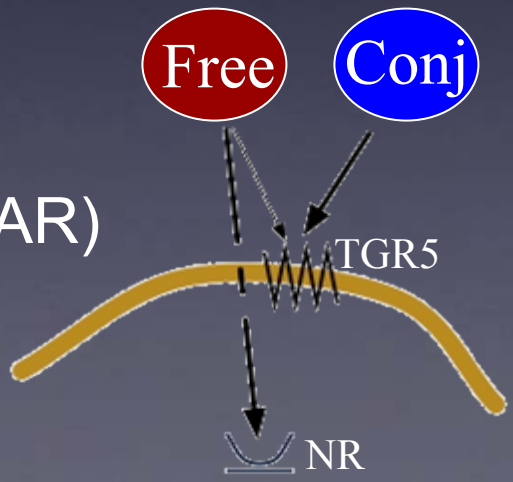


- **Metabolic by-products of cholesterol**,

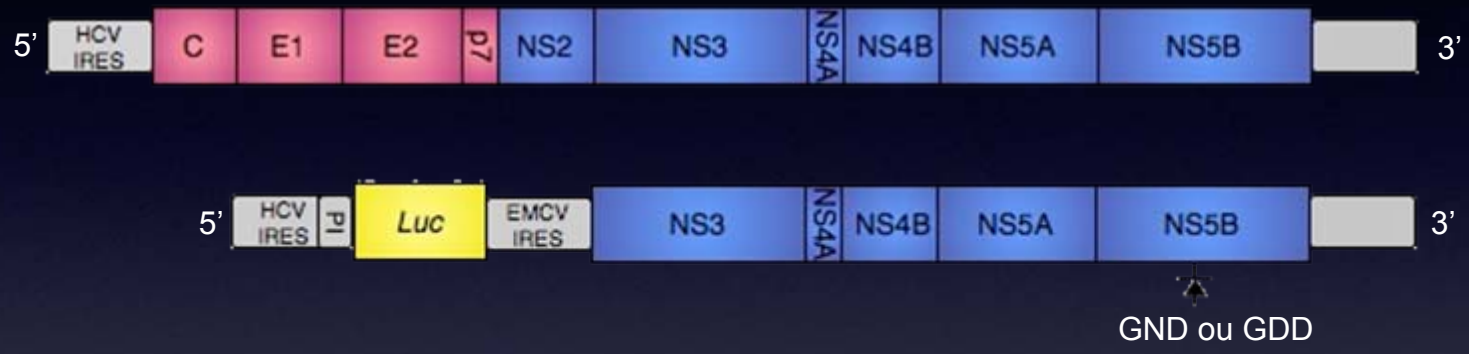
  dietetary lipids and fat-soluble vitamins absorption

- **Hormones,** 2 kinds of receptors:

  - **Membrane receptor** (TGR5)
    - *Rapid intracellular signaling (cAMP)*
      - Inhibition of IFN pathway *(Calicivirus)*
  - **Nuclear receptors** (FXR, PXR, VDR, CAR)
    - *Control gene expression*

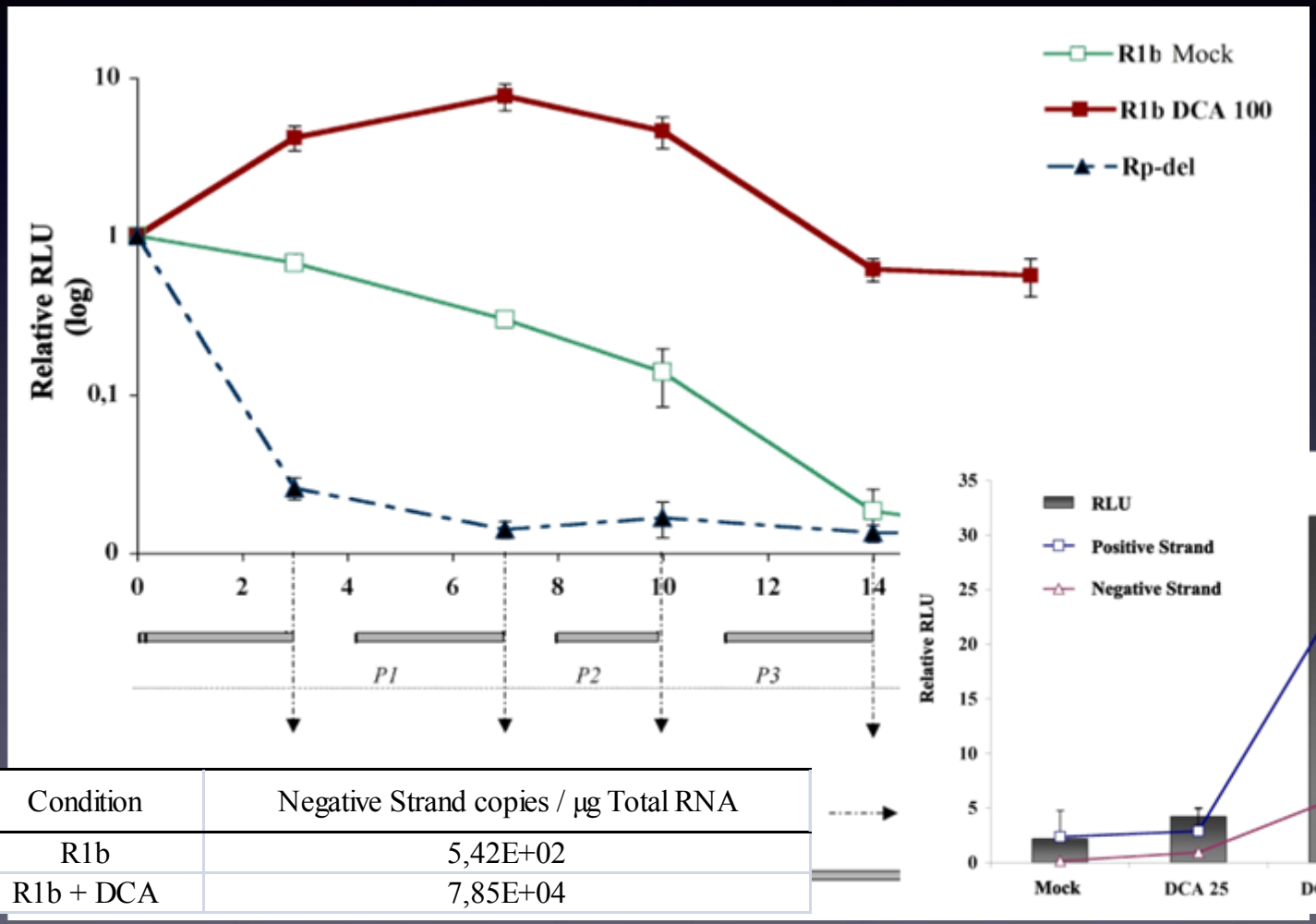


HCV replicon system



- **Luciferase replicons**
  - ✓ Genotypes 1b (Con1), 1a (H77), 2a (JFH1)
  - ✓ negative control : mutation in pol (Rp-del)
- **Self replicating HCV RNA without production of viral particles in Huh-7**

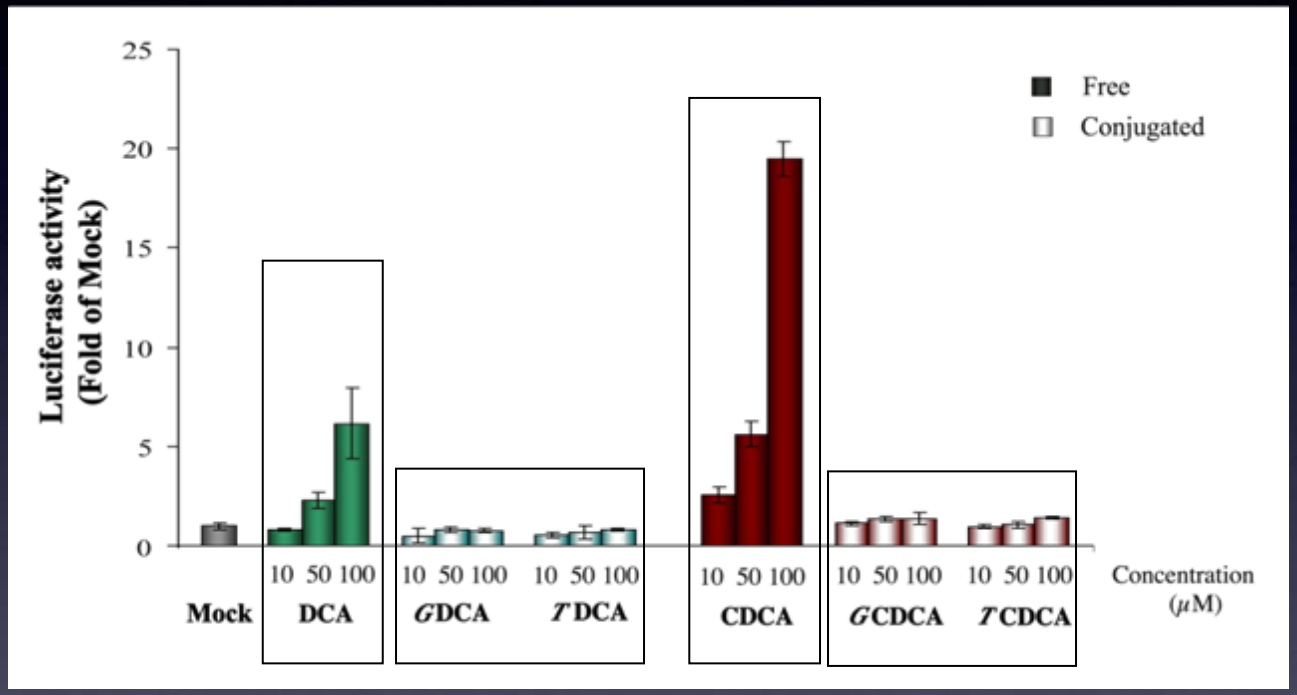
Bile acids enhance R1b replication

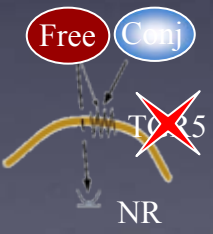


Condition	Negative Strand copies / µg Total RNA
R1b	5,42E+02
R1b + DCA	7,85E+04

- Prolonged and sustained replication induced by DCA

FREE bile acids enhance HCV replication

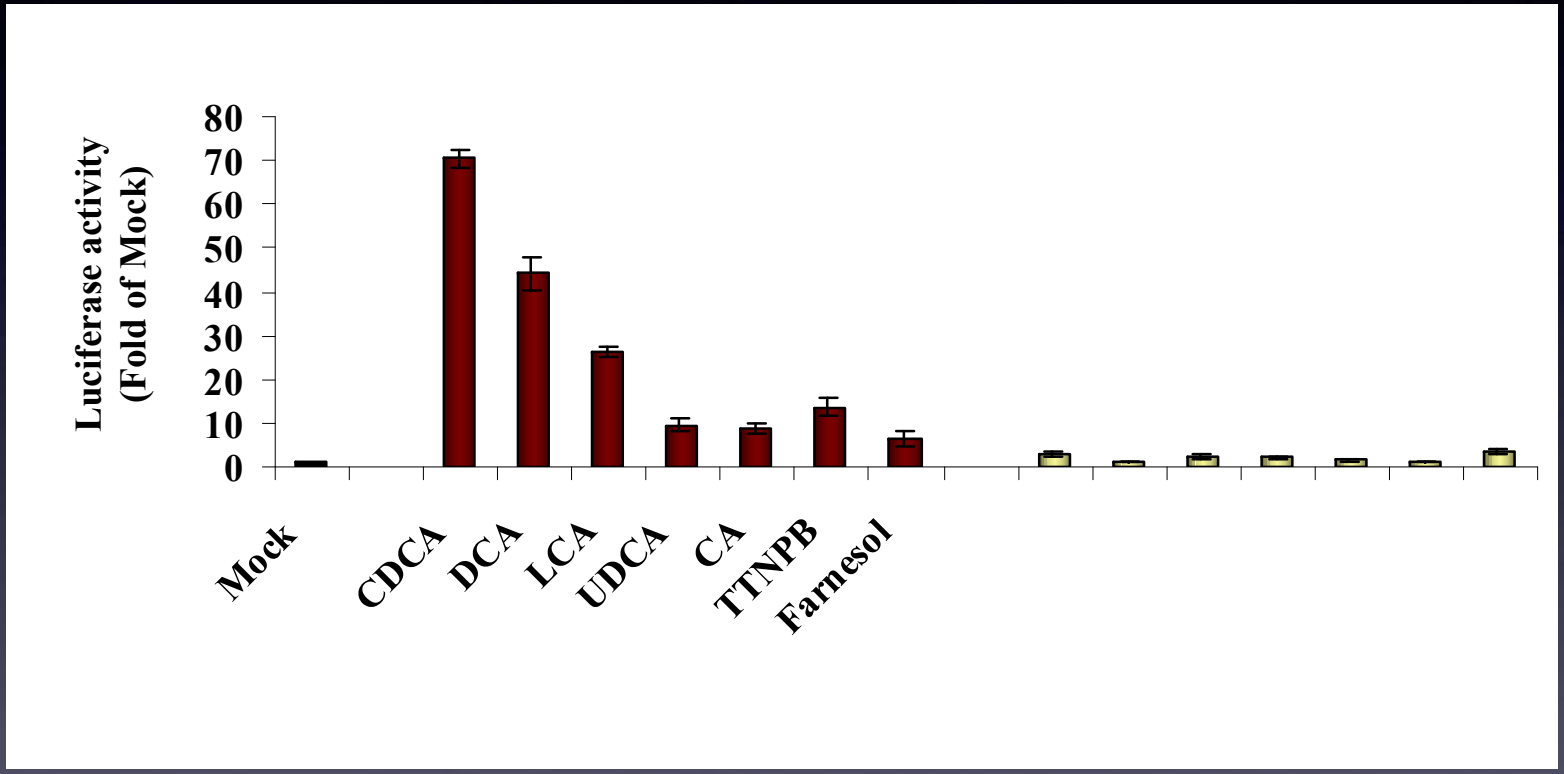




- Free bile acids induce a dose-dependent increase of the replication
- No effect of conjugated bile acids

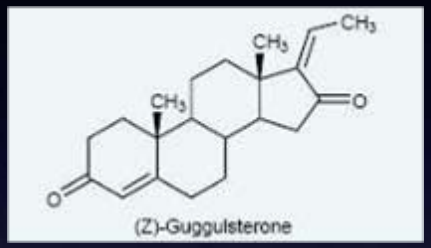
Effect of bile acids on HCV RNA replication is not mediated by the membrane receptor TGR5

FXR agonists enhance HCV replication

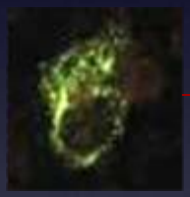


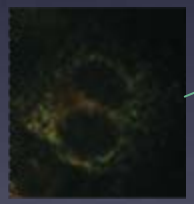
- Within NR1 agonists, only FXR agonists enhance HCV RNA replication

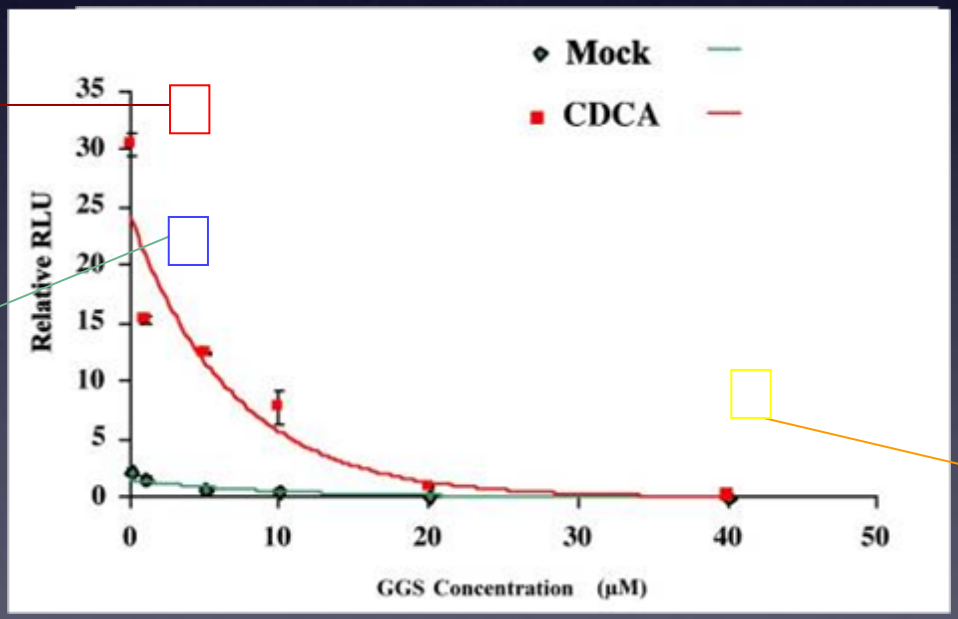
FXR antagonism inhibits HCV RNA replication

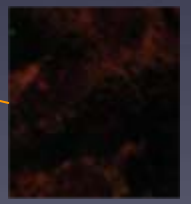


natural FXR antagonist



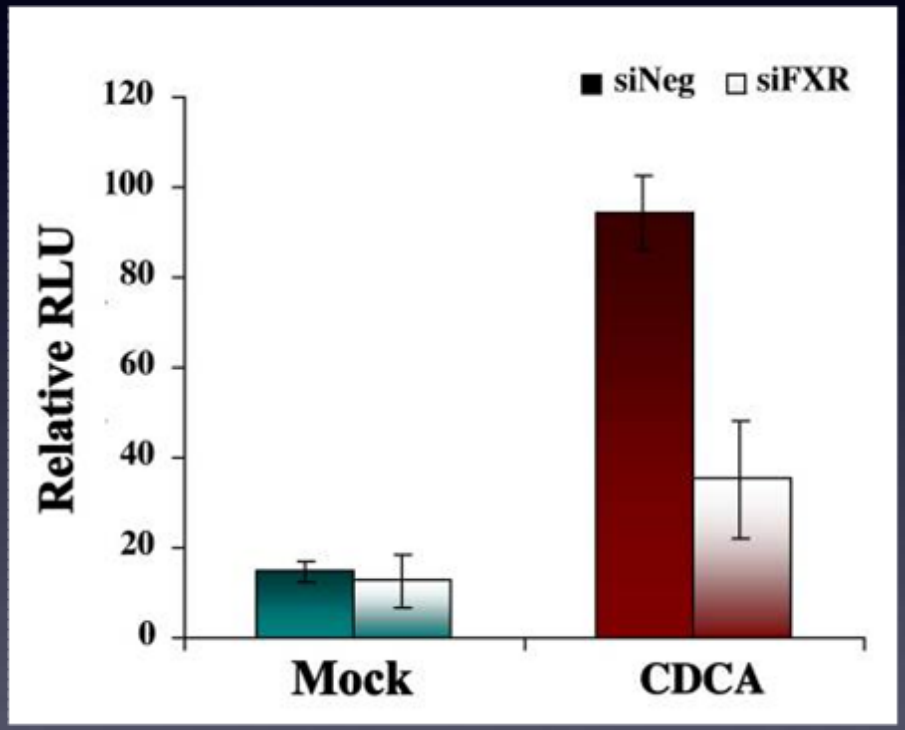


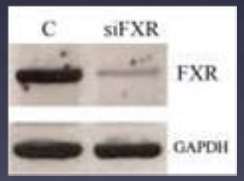




- GGS inhibits replication when bile acid is added
- Bile acids induce replication in a dose-dependent fashion

FXR silencing inhibits HCV RNA replication





No effect of bile acids in the absence of FXR

Summary

Scholtes, J Hepatol 2008

- **Free bile acids enhance HCV RNA replication**
  - Dose-dependent effect
  - Mediated by FXR
  - Activation and inhibition of FXR modulates accordingly HCV RNA replication
  - Key factor for the growth of HCV

Perspectives

- **Molecular mechanisms downstream of FXR**
  - role of PGC1 and coregulators ?
  - what metabolic pathways regulated by FXR are essential for HCV and why ?
  - Is the activity of FXR modified by HCV ?

FXR, a therapeutic target for treating hepatitis C ?

- Several clinical trials targeting FXR are or have been conducted for metabolic diseases with GGST or synthetic molecule.

- A proof of concept clinical trial that FXR modulate in vivo HCV replication is scheduled early this summer with GGST in non responder patients (HCL; Service hépatologie, C. Trépo, F. Zoulim and CIC Lyon, F. Gueyffier).

Thanks

Inserm unit 851,
Lyon

Caroline Scholtes
Christophe Ramière
Olivier Diaz
Martine Carreras

Vincent Lotteau

University of Heidelberg
Ralf Bartenschlager

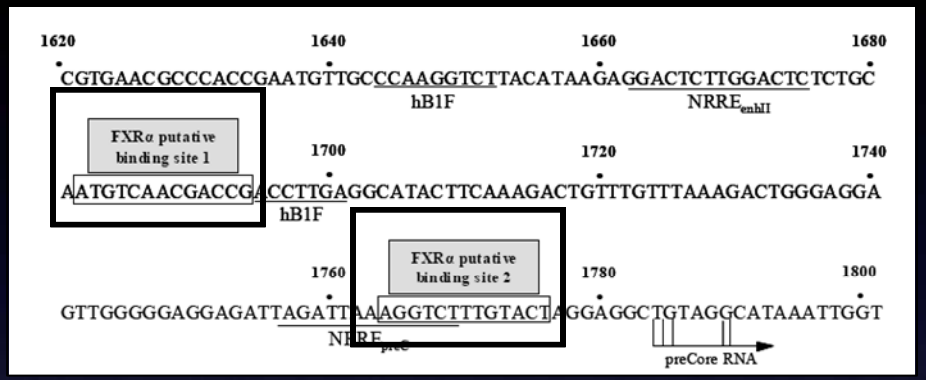
Institut Pasteur Lille
Bart Staels

FXRα binds as a heterodimer with RXRα to 2 REs in the EN2/core promoter region

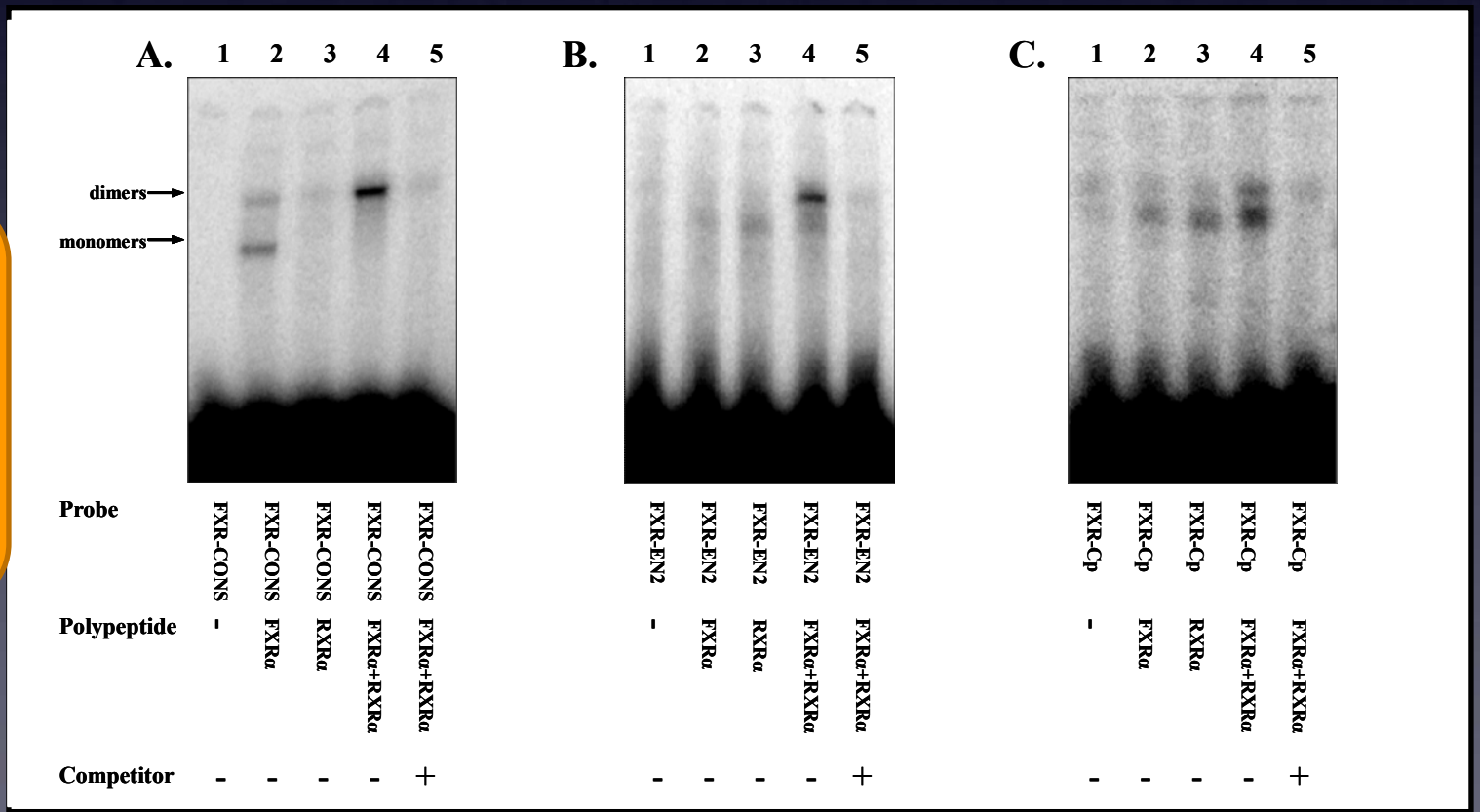
CONS probe :
5'-GATCTCAAGAGGTCATTGACCTTTTTG – 3'

EN2 probe :
5'- GATCTCTGCAATGTCAACGACCGACCTTGA – 3'

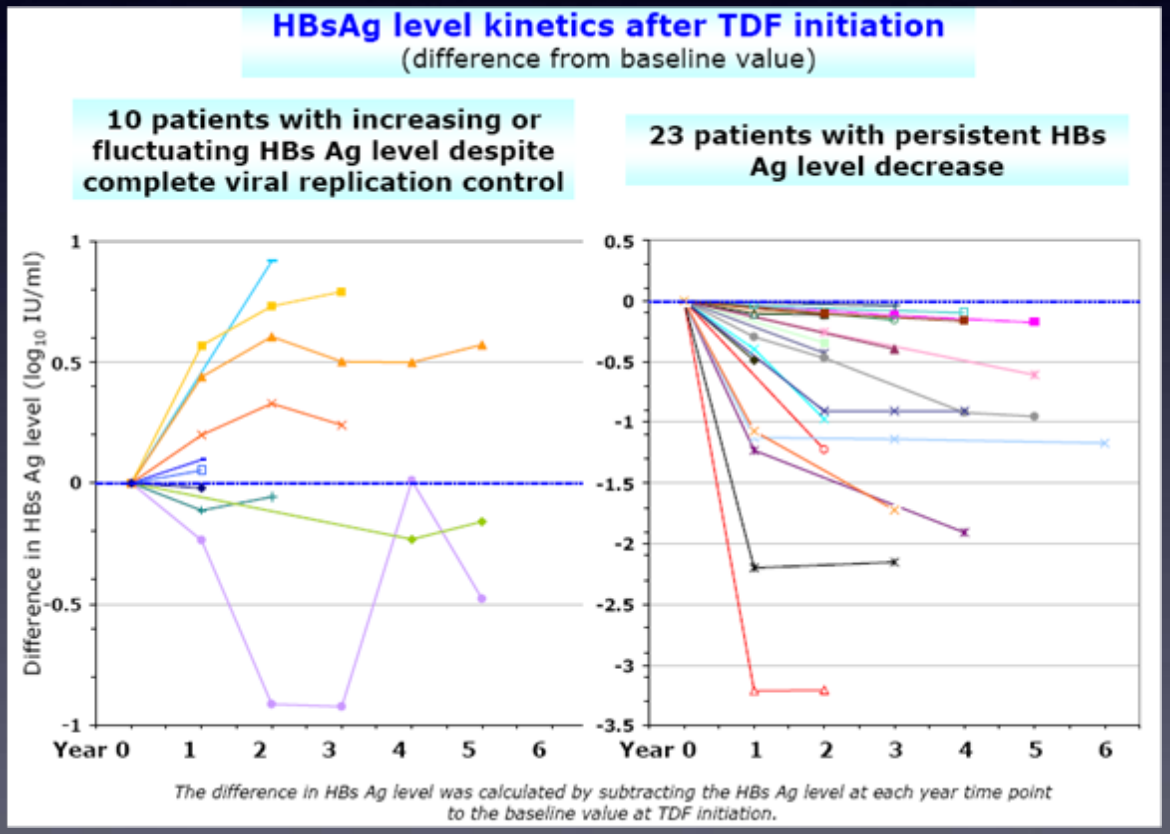
Cp probe:
5'- GATCGATTAGATTAAAGGTCTTTGTACTAGGA – 3'



Mutations in either FXRE abolish the binding and activity of FXR

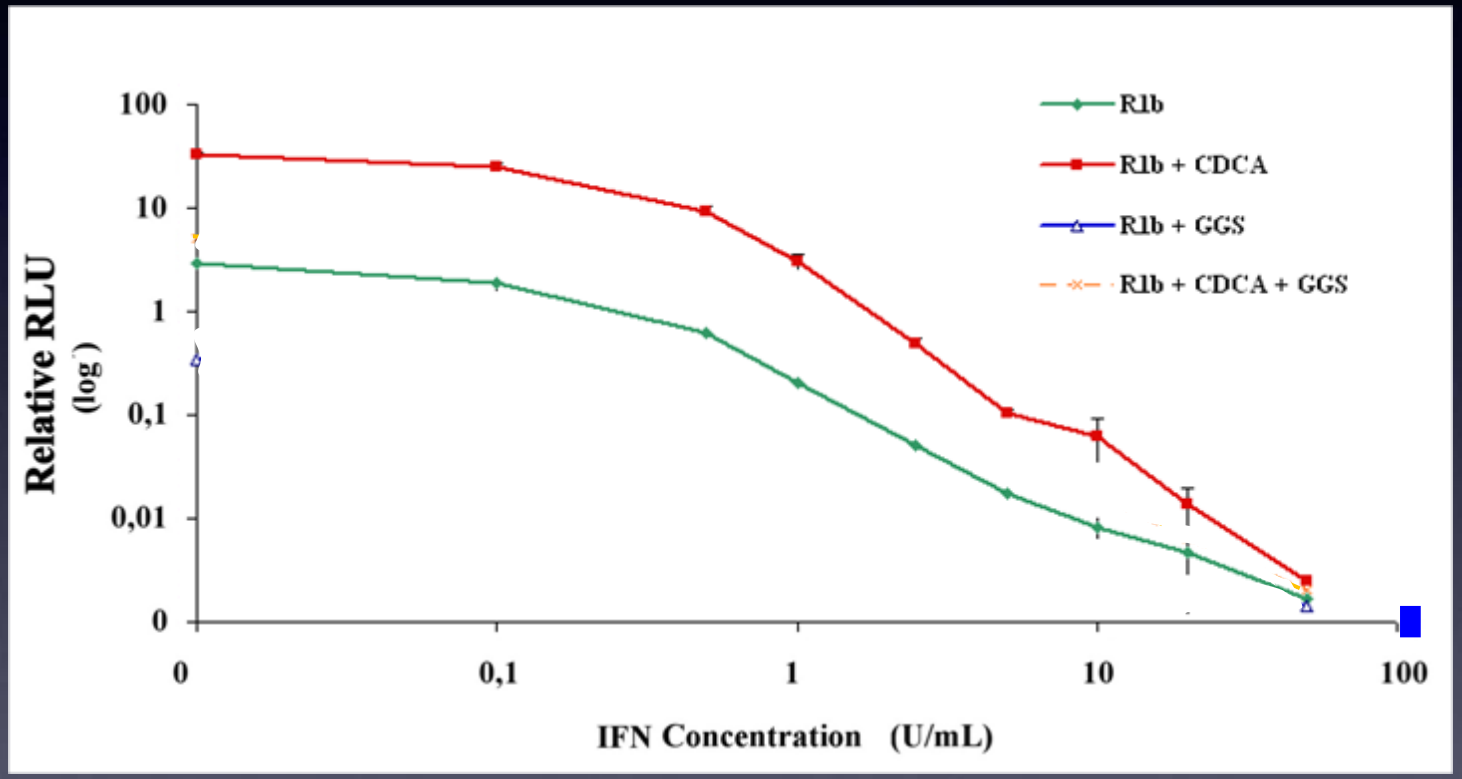


HBsAg persistence under effective anti-pol therapy



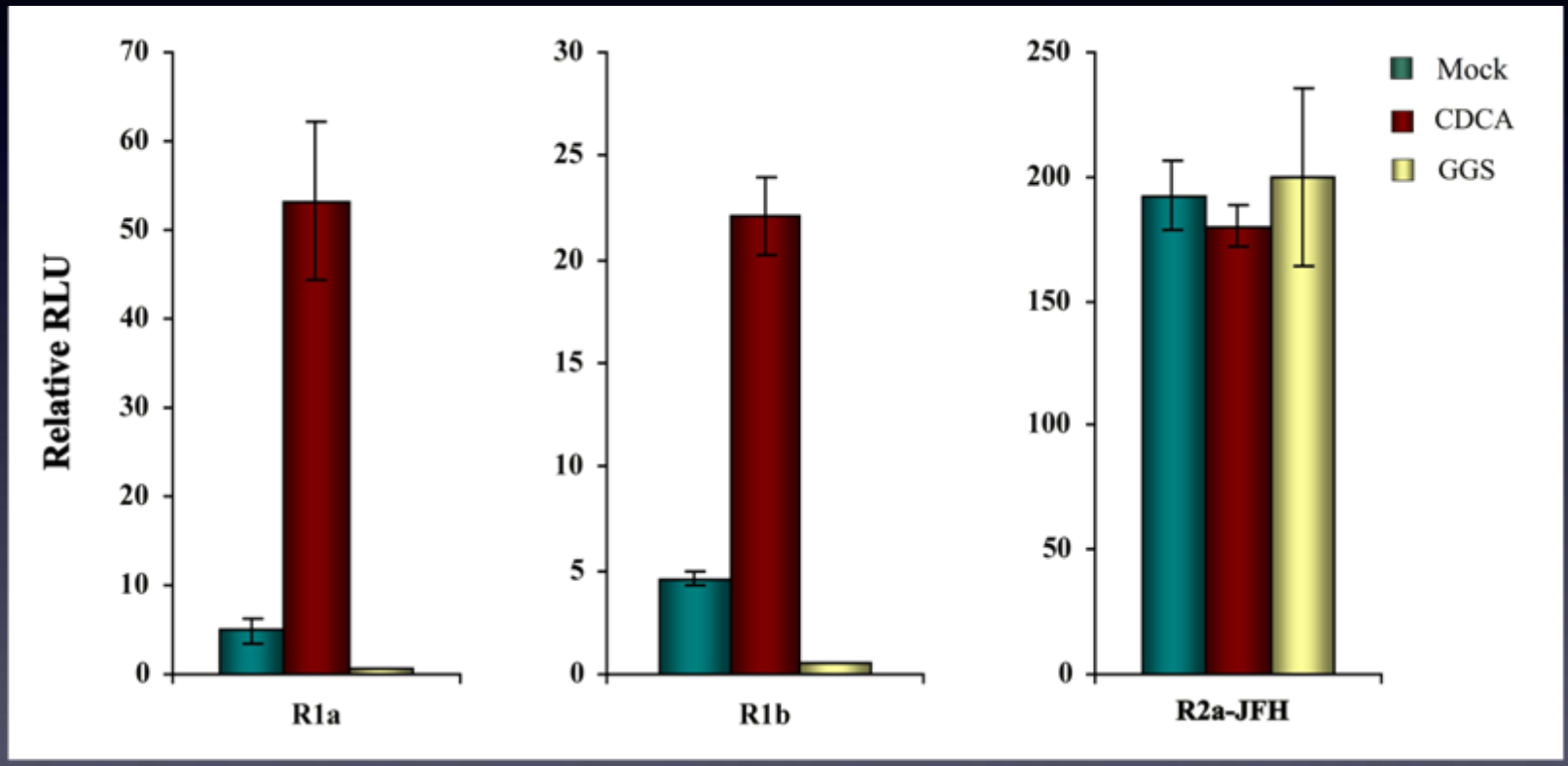
Thibault V. CROI 09

Modulation of HCV RNA replication by FXR is independent of IFN pathway



- Replication is still sensitive to IFN inhibition with similar IC50
- GGS has an additive effect to IFN

FXR modulation on different HCV genotypes



- FXR controls the replication of both genotypes 1a and 1b
- No modulation of JFH-genotype 2a replication

Perspectives 2

- **HCV and metabolism crosstalk**

  - Host metabolism influences HCV replication: FXR major metabolic regulator, HCV as a "metabolovirus"

  - HCV influences host metabolism:
    If HCV modifies FXR activity, what could be the impact on glucose and lipid metabolism as well as on liver injury ?

    Expression of PPARα and γ, LXR, SREBPs are modified by HCV (core, NS2).