Biology of HBV infection

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Chronic Infection with hepatitis B Virus

HBV
- 95% Acute self-limiting infection
- 5% Persistent infection (but high rates in perinatal age)

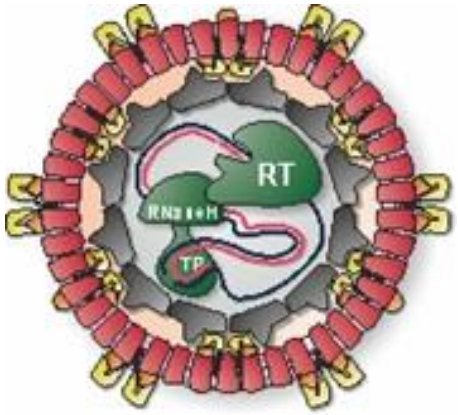
- Prevention of HBV infection is achievable (effective vaccine)
- ca. 250 million people are chronically infected with HBV worldwide
- HBV polymerase inhibitors efficiently suppress viral replication

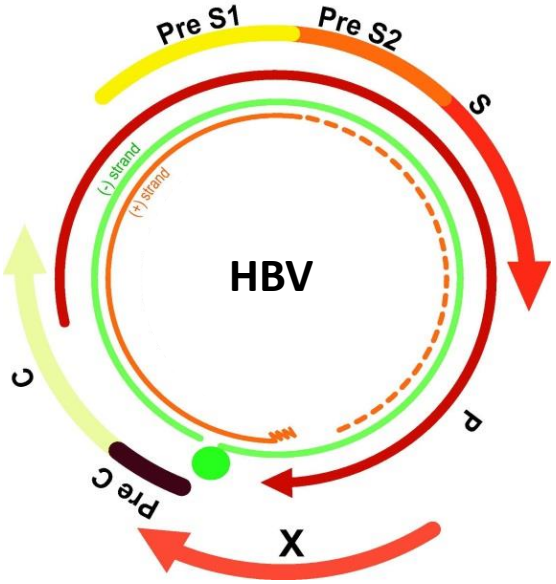
But no cure for CHB due to persistence of cccDNA & weak immune responses

Limited knowledge about
- mechanisms leading to infection establishment
- cccDNA formation, stability & regulation
- virus-host interactions (evasion mechanisms)

invisible or active evasion?

Hepatitis B Virus: structure





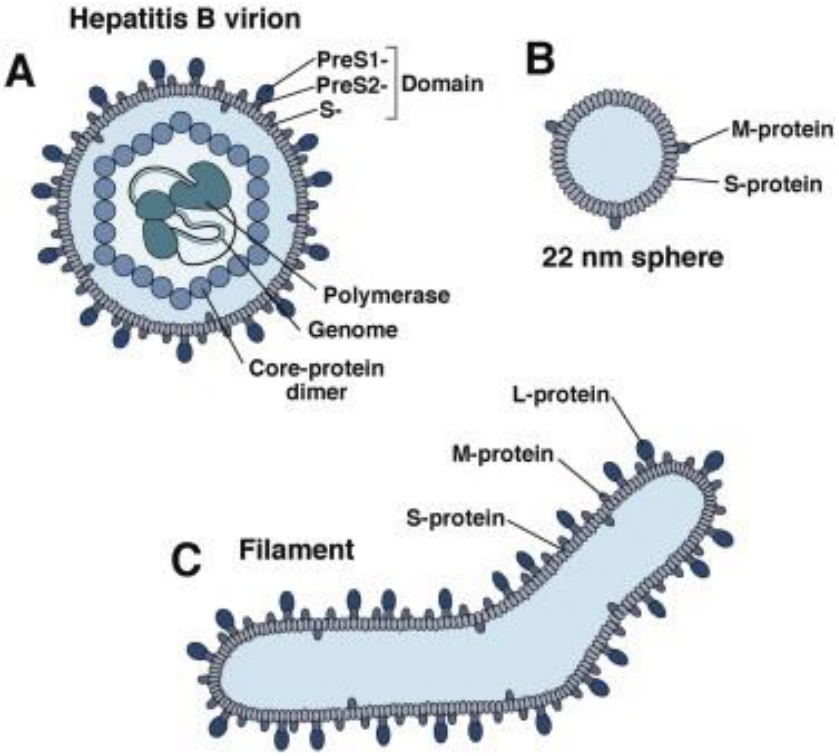
(adapted from Neuvet et al., J. Hepatol. 2010)

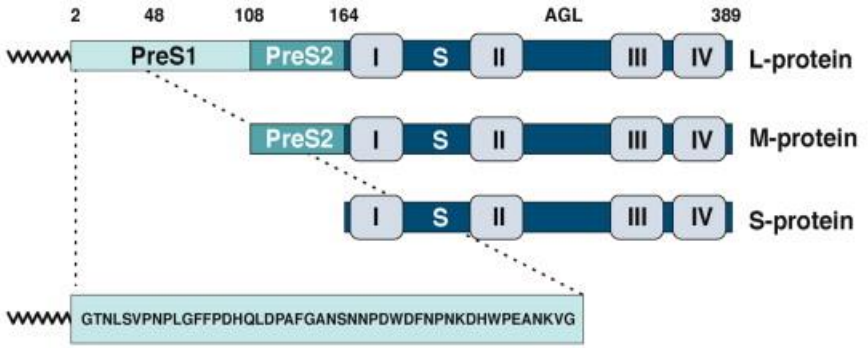
- **High species-specific: humans, chimpanzees (Tupaia)**
- **High tissue-specific: liver tropism**

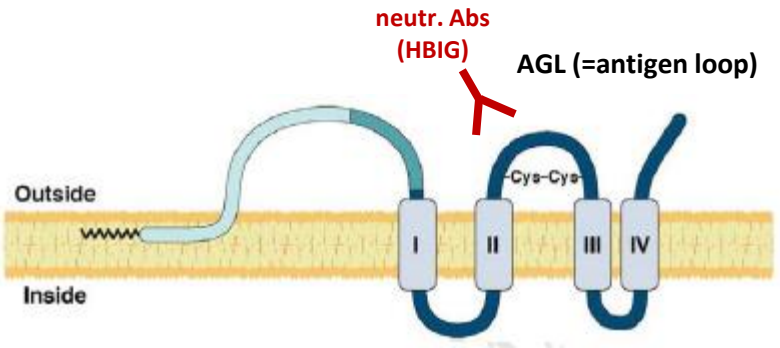
HBV extremely adapted to its host!

- smallest DNA enveloped animal virus (3,2 Kbp) (4 overlapping ORFs)
- **Genome** in circulating virions:
  - relaxed circular partially double-stranded DNA (**rcDNA**)
  - covalently linked to **viral polymerase (RT)**
- **Capsid**: core protein (HBcAg), icosahedral arrangement
- **Envelope**: Large (L), Middle (M), Small (S) proteins (host derived-lipids)

Hepatitis B Virus: structure

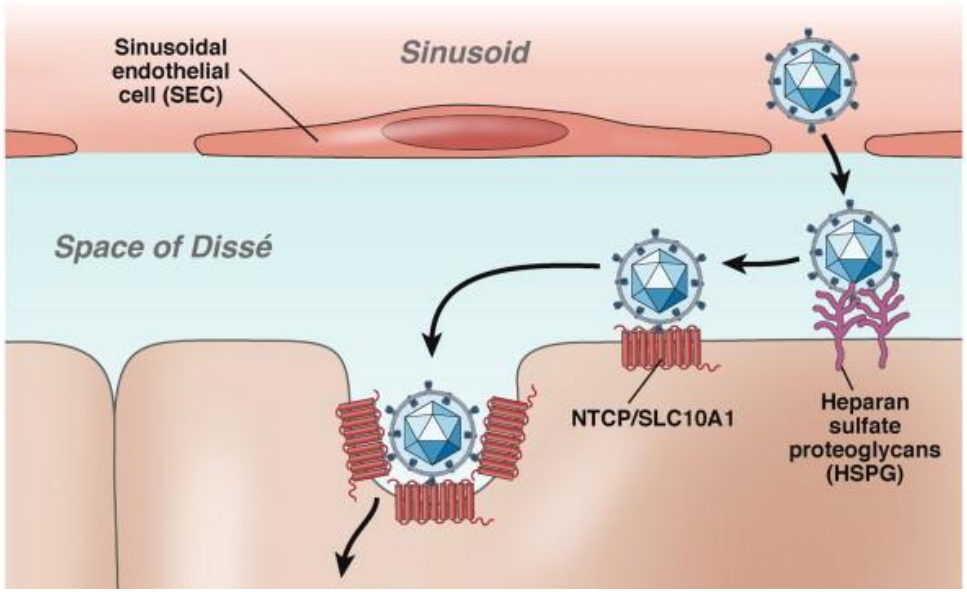






(S. Urban, R. Bartenschlager, R. Kubitz, F. Zoulim, Gastroenterol. 2014)

HBV infection: early entry steps

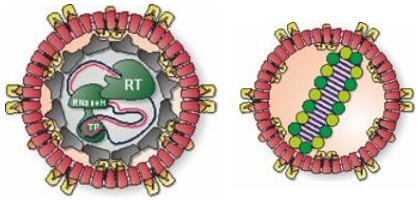


(Urban et al. Gastroenterology 2014)

Attachment:

1) **Reversible interaction with HSPGs**
   (Schulze, Hepatol. 2007)

2) **Irreversible binding to NTCP**
   (Yan, Elife 2012)



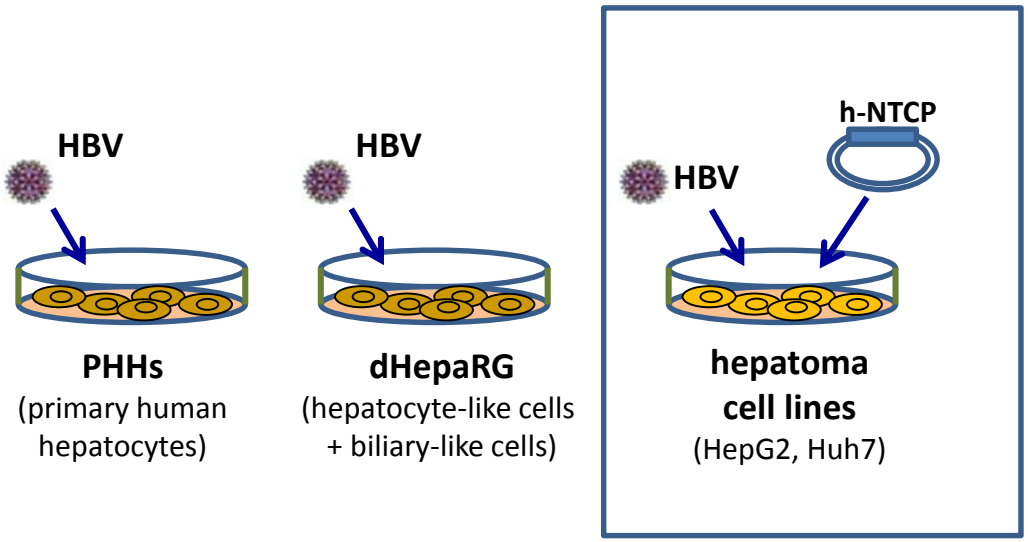
HBV **HDV**

NTCP: sodium taurocholate co-transporting polypeptide

- **is localized to the basolateral membrane of highly differentiated primary hepatocytes**
- **it mediates most of the hepatocellular uptake of bile salts**
- **Binding is mediated by the preS1 domain of the HBV envelope protein**

HBV infection: early entry steps

The recognition of NTCP as the *bona fide* HBV and HDV entry receptor has enabled the establishment of human cell lines supporting HBV and HDV infection

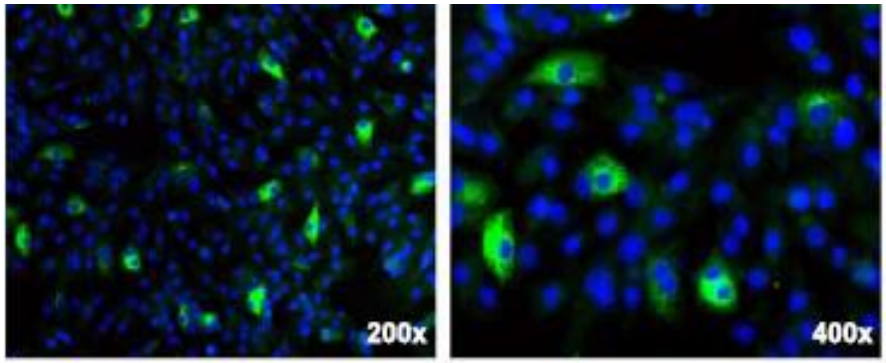


Pre-screening of new antiviral compounds & elucidation of unclear infection steps ☺

Implications of HBV receptor discovery

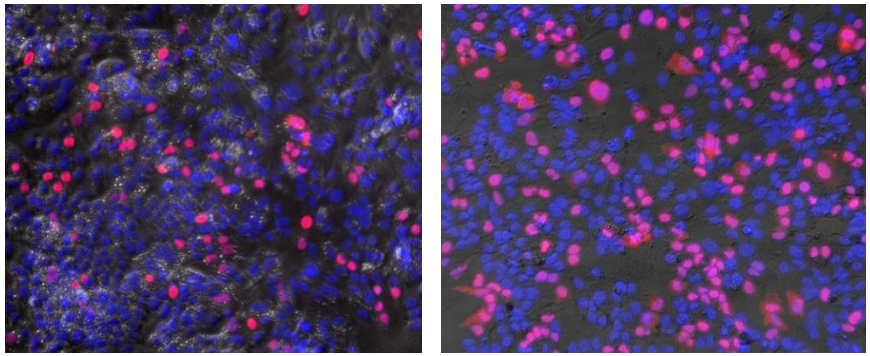
Improved in vitro infection systems

HBV-HBsAg in HepG2-hNTCP



(Yan et al, Elife 2012)

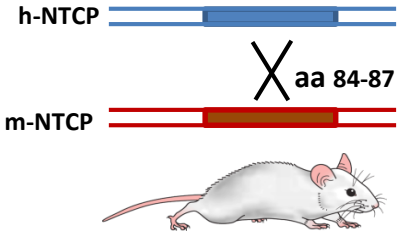
HDV-δAg in HepG2-hNTCP



(Bhadra et al. unpublished)

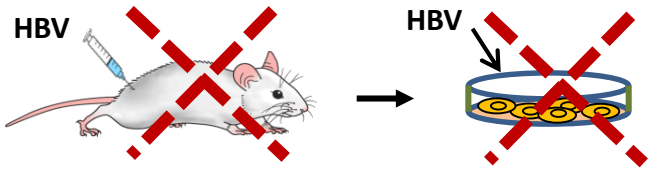
HDV displays higher infection efficacy!

Improved in vivo infection systems ?



hNTCP Transgenic mice (immune competent HBV/HDV infection models)

Implications of HBV receptor discovery



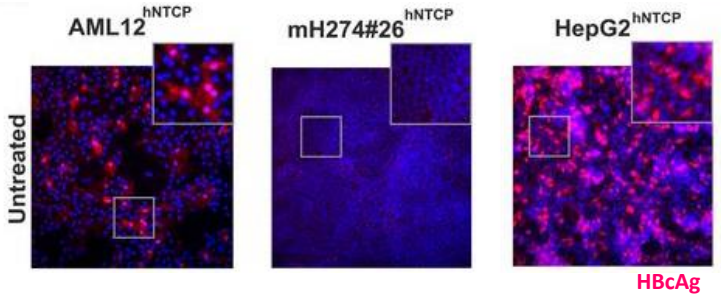
hNTCP transgenic primary murine hepatocytes (in vivo & in vitro) are not HBV permissive
(Li et al. Cell. & Mol. Immunol.2014, Yan et al. J.Virol.2013)

Low efficiency and Temporary HDV infection could be shown in hNTCP transgenic mice
(He et al. PLOS Pathogens 2015)

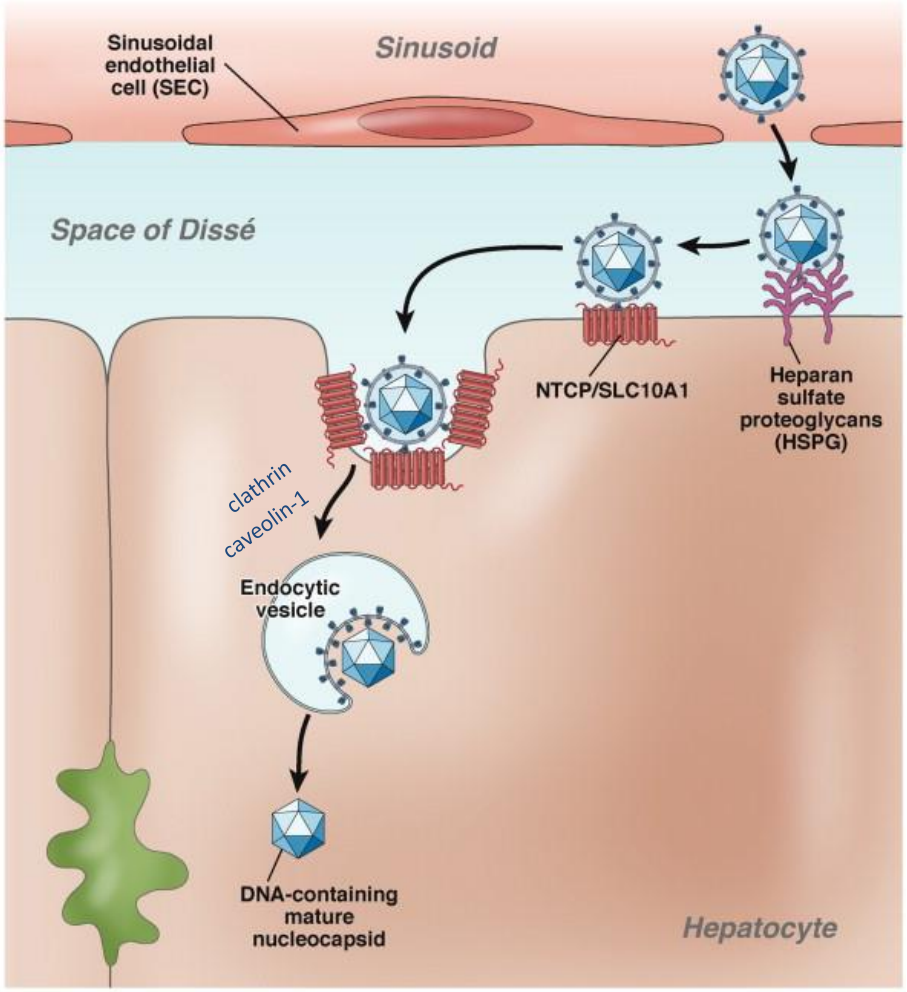
Unknown species-specific factors hinder HBV infection establishment in mice

A mouse liver cell line (AML12) supports HBV entry upon hNTCP expression
(HBV RNA and proteins production,
rcDNA – virion productivity – barely detectable)

(Lempp J Virol 2016)



Steps involved in HBV infection establishment



(Urban et al. Gastroenterology 2014)

Attachment:

1) Interaction with HSPGs (Schulze, Hepatol. 2007)
2) Binding to NTCP (Yan, Elife 2012)

Internalization:

By endocytosis mediated by host factors

- caveolin-1 (Macovei, J.Virol.2010)
- clathrin
- Rab proteins (GTPases) (Macovei, J.Virol.2013)

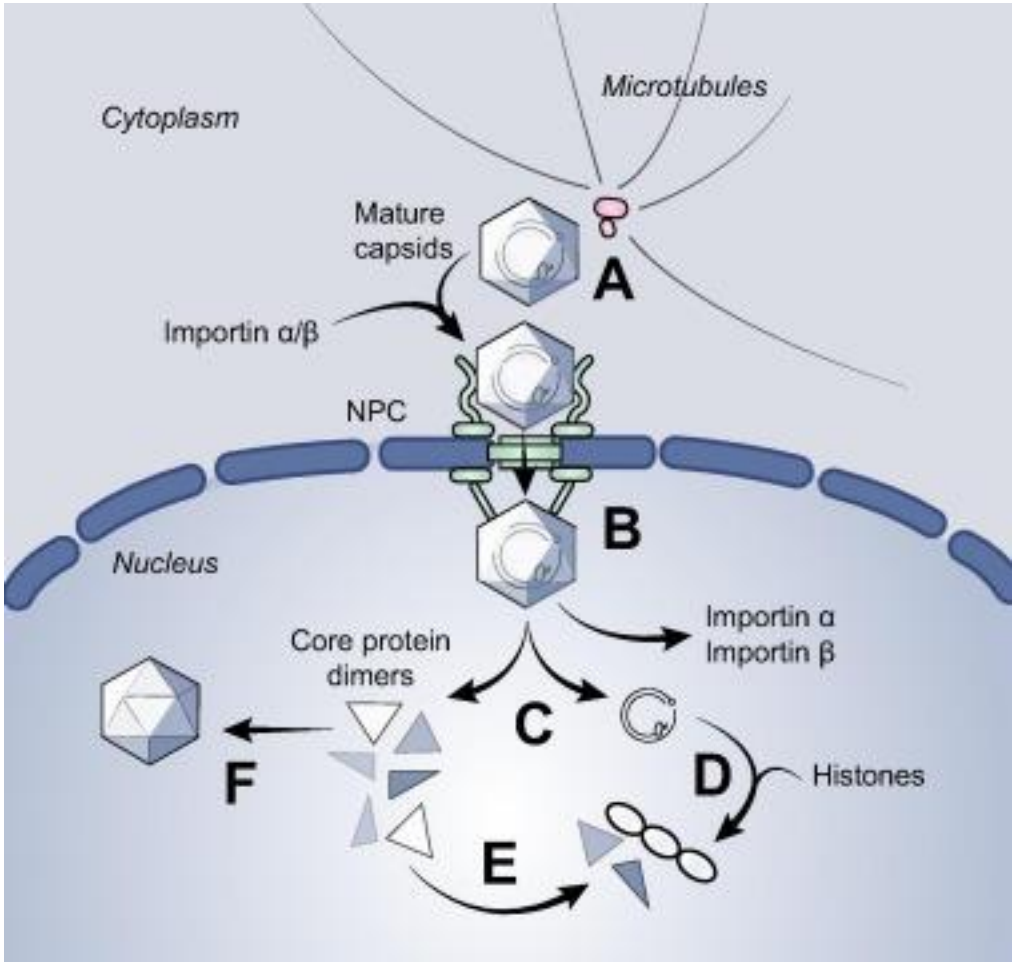
Uncoating

Transport to the nucleus along microtubules

?

Steps involved in HBV infection establishment

Intracellular transport



(Blondot, Bruss, Kann, J Hepatol. 2016

Intracytoplasmic transport via microtubules

capsids dissociate from microtubules (unknown mechanisms)

attach to importin α/β

pass the Nuclear Pore (NPC)

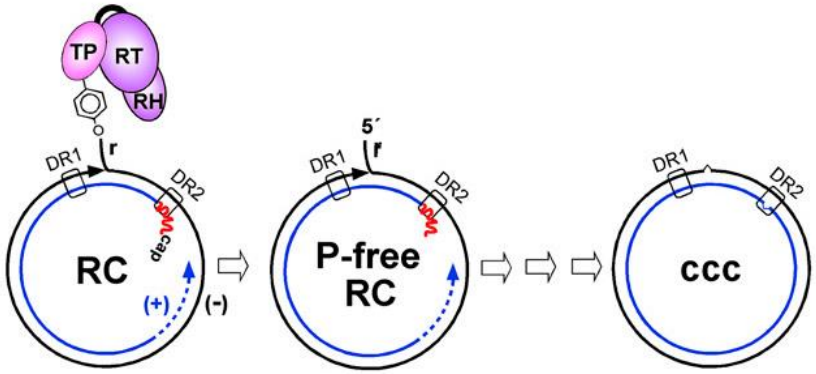
Capsids dissociate to core protein dimers

Genome is released

cccDNA formation

cccDNA formation

Conversion of rcDNA to cccDNA is a largely unknown multistep process involving different host enzymes

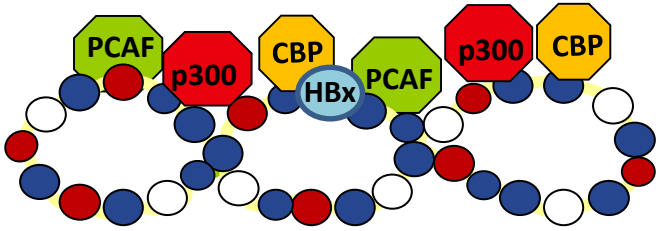


(≈ damaged dsDNA) **(≈ plasmid)**

- **HBV Polymerase removal mediated by host DNA repair enzymes like TDP2** (tyrosyl-DNA-phosphodiesterase)
- **Removal of a short terminal redundancy**
- **& small RNA primer on + strand**
- **Completion of the plus strand**
- **Ligation**

(adapted from Königer et al. PNAS, 2014)

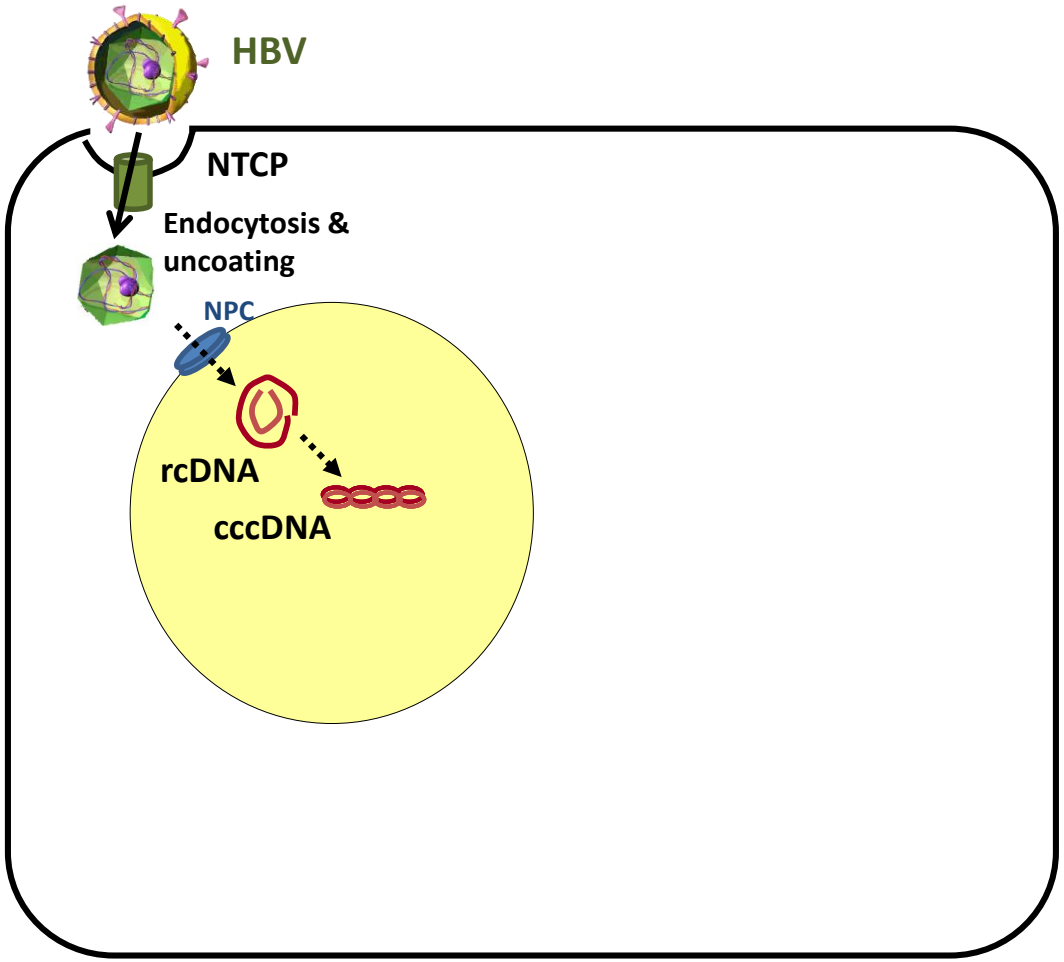
The cccDNA assembles with histone and non-histone proteins to form a stable minichromosome in hepatocyte nuclei



acetylated histones **core protein**

(Newbold; Levrero et al. J.Hepatol.2009; Lucifora et al. J.Hepatol. 2011; Tropberger PNAS 2015)

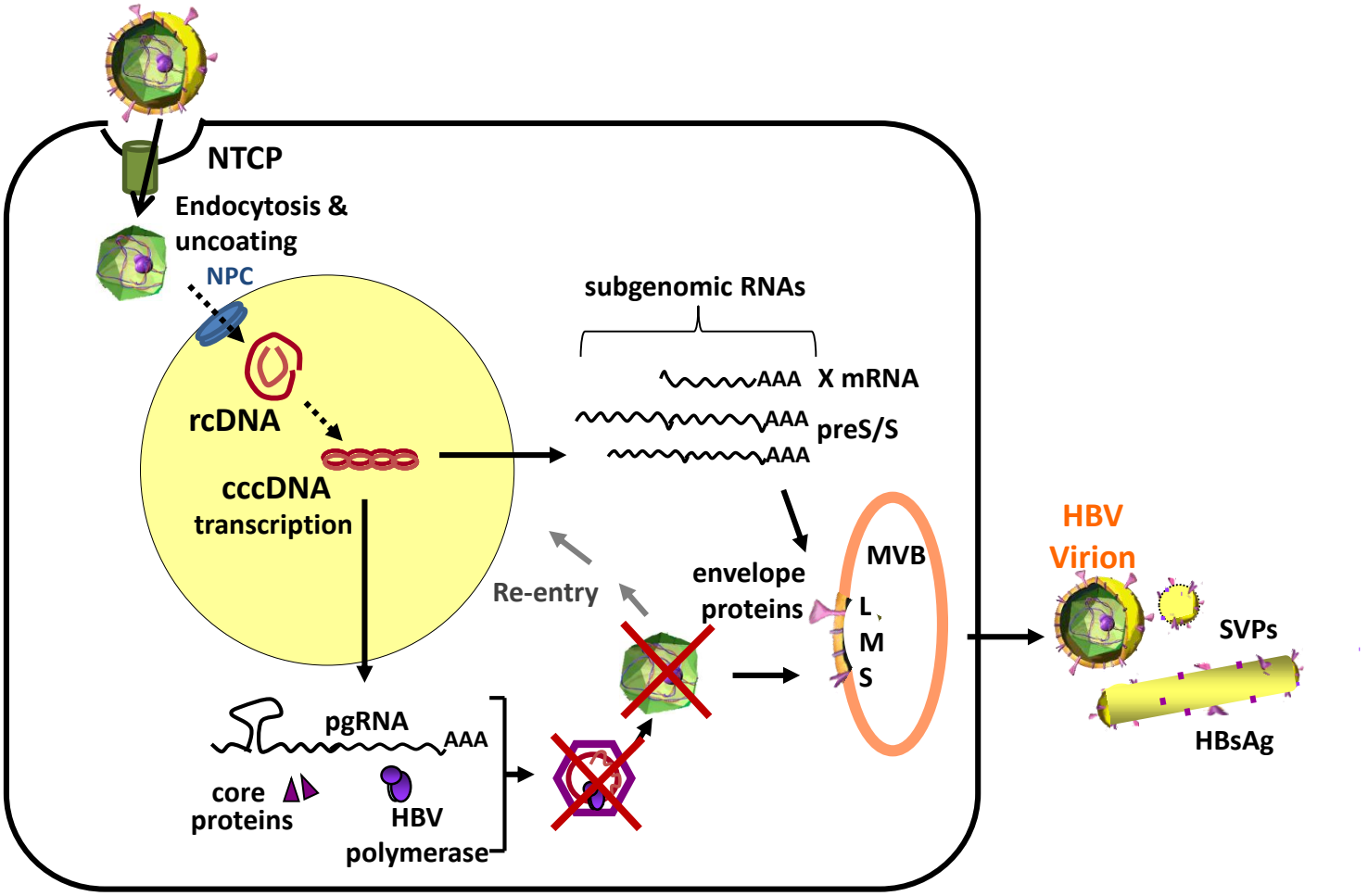
HBV productive infection = cccDNA establishment & transcription



(Modified from Dandri, Locarnini, Gut 2012)

The cccDNA minichromosome serves as transcription template for all HBV RNAs

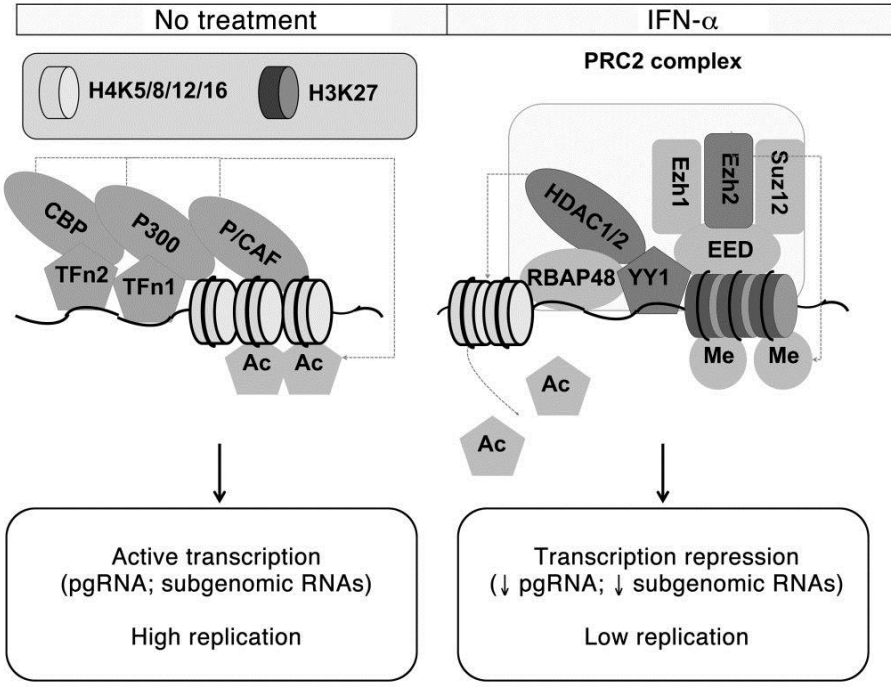
HBV replication cycle



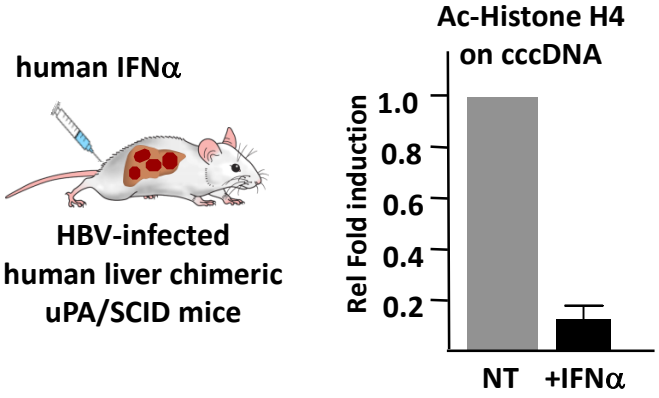
(Modified from Dandri, Locarnini, Gut, 2012)

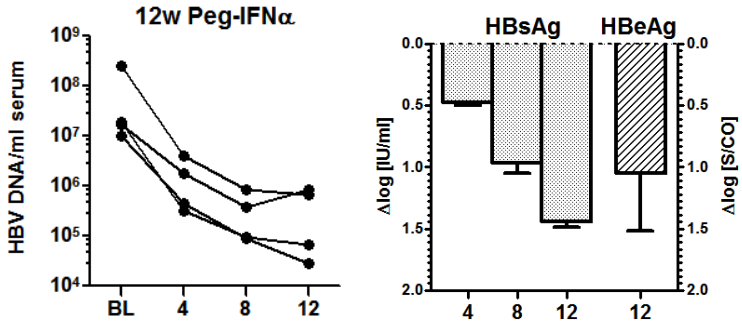
NUCs efficiently suppress HBV replication but not cccDNA-driven RNA transcription (no reduction of viral proteins and circulating HBsAg)

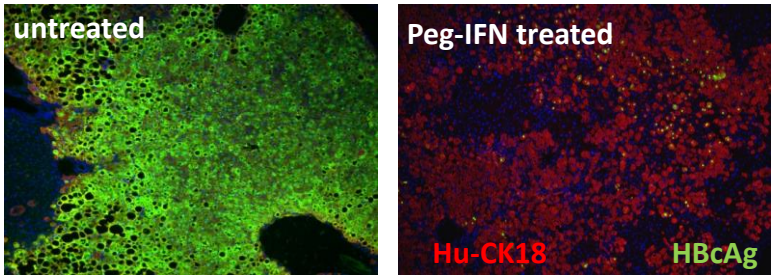
cccDNA transcription can be reduced by IFNα



(Belloni et al., J.Clin.Invest. 2012)







IFNα can inhibit cccDNA transcription by reducing the acetylation status of histones bound to the cccDNA

12 weeks of peg-IFNα treatment in humanized mice lacking adaptive immune responses led to significant suppression of viremia and antigen production

(Allweiss et al., J. Hepatology 2014)

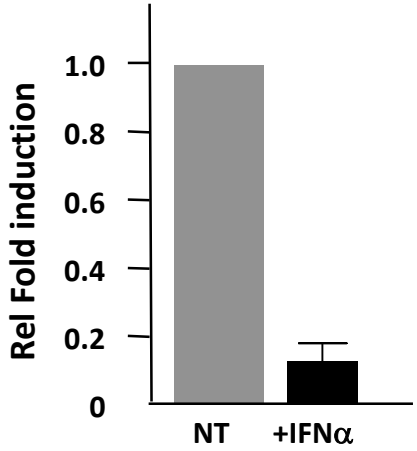
IFNα & LTβR activation can promote cccDNA destabilization

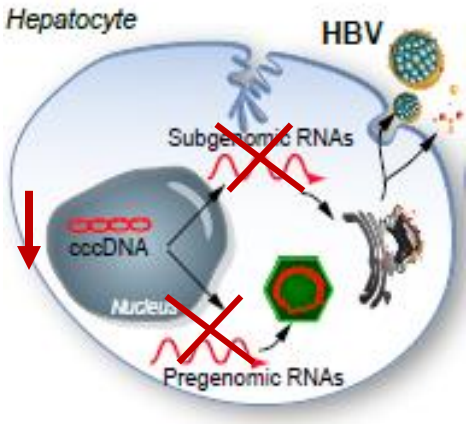
peg-IFNα

IFN-α induced epigenetic suppression of the cccDNA and strong reduction of viral antigen production

(Belloni, JCI 2012; Allweiss, J.Hepatology2014)

Ac-Histone H4

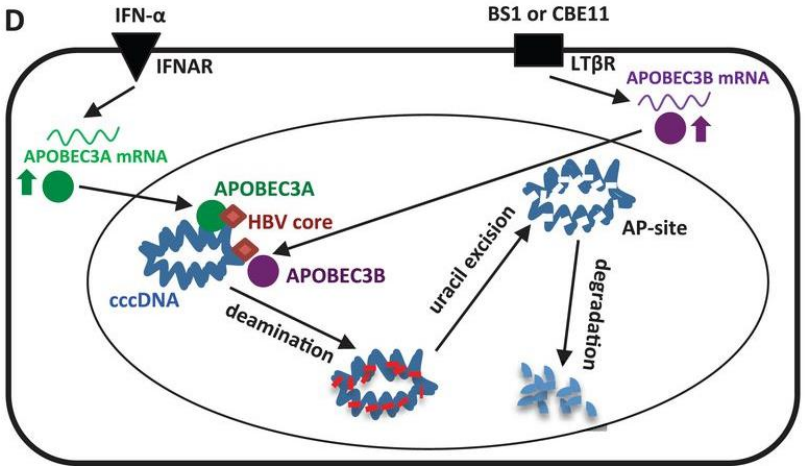




(Adapted from Thimme & Dandri, J.Hepatol. 2012)

IFNα-treatment & activation of the lymphotoxin β receptor can induce APOBEC-mediated cccDNA deamination and partial cccDNA degradation

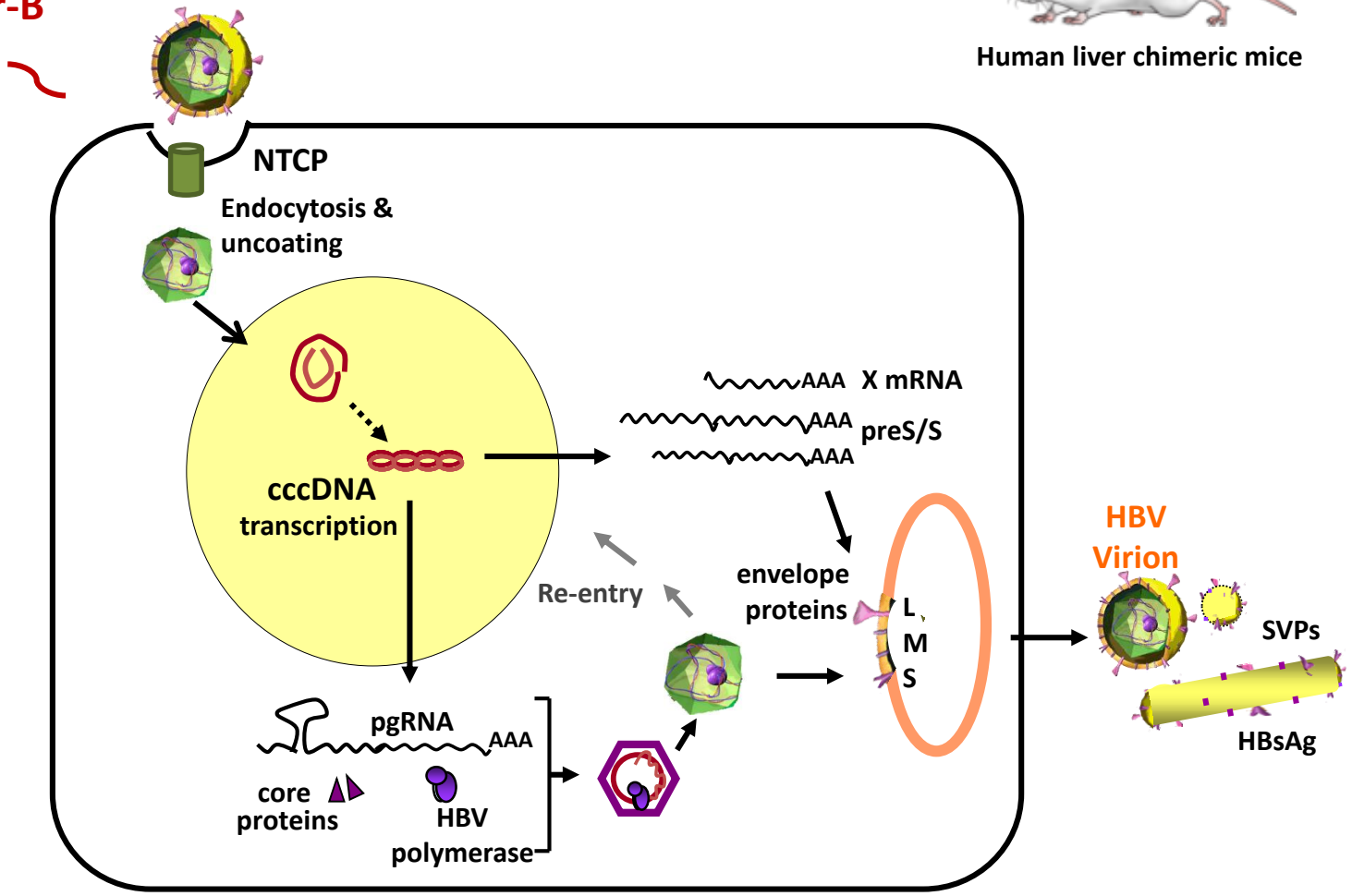
(Lucifora, et al. Science 2014)



Studies aiming at understanding cccDNA biology / dynamics & HBV-host interactions

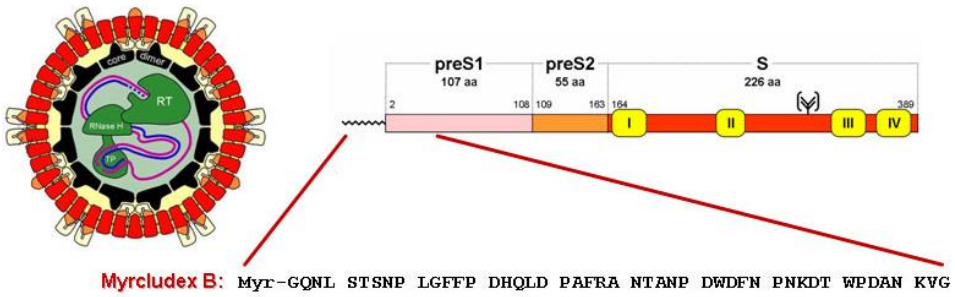
Use of preS1-derived Lipopeptide Myr-B

Human liver chimeric mice



(Modified from Dandri, Locarnini, Gut, 2012)

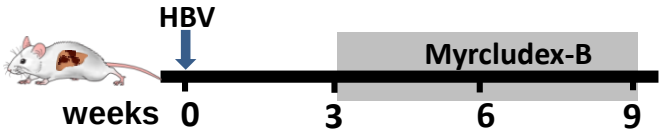
Preclinical studies with HBV entry inhibitors

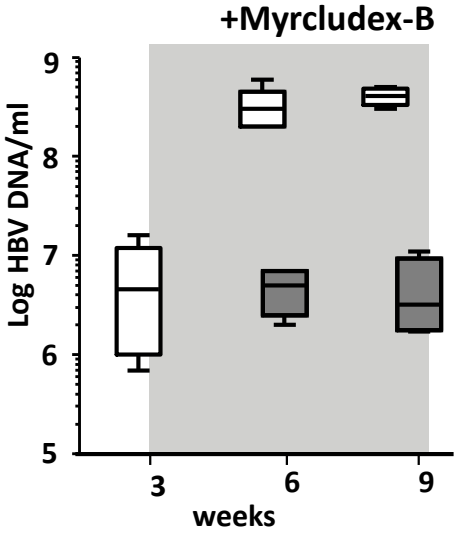


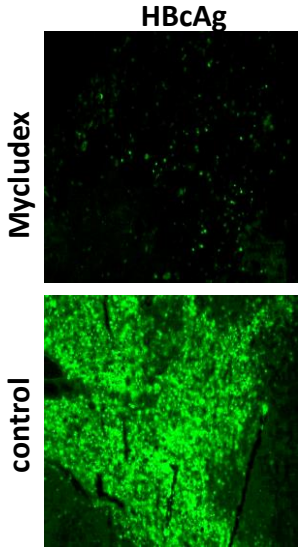
Chemically synthesized lipopeptides derived from the preS1 domain of HBV envelope block de novo HBV infection in vitro and in vivo

(Petersen, Dandri, Urban, Nature Biotech.2008)

Myrcludex-B efficiently blocked HBV spreading post-infection in the setting of constant viral exposure

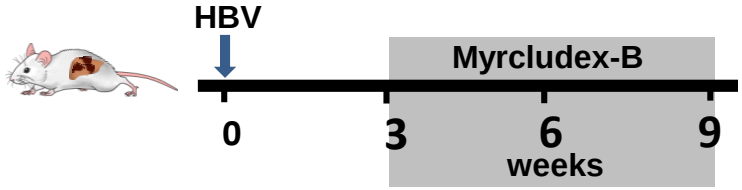


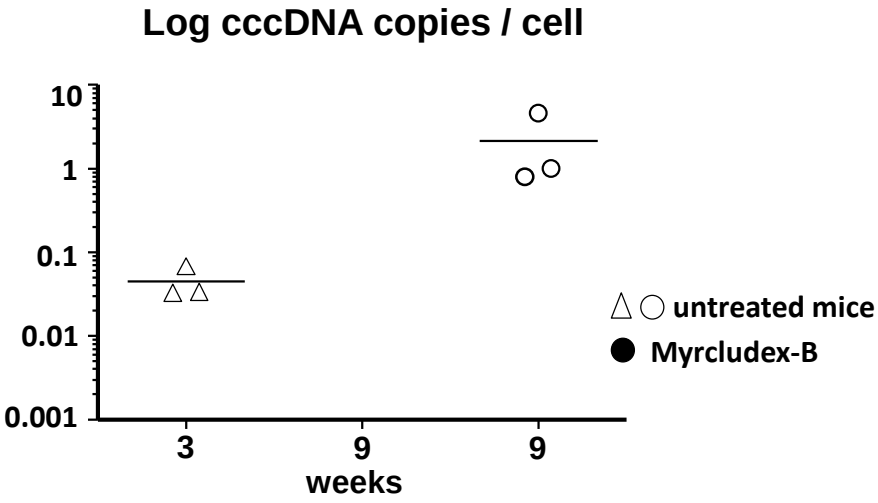


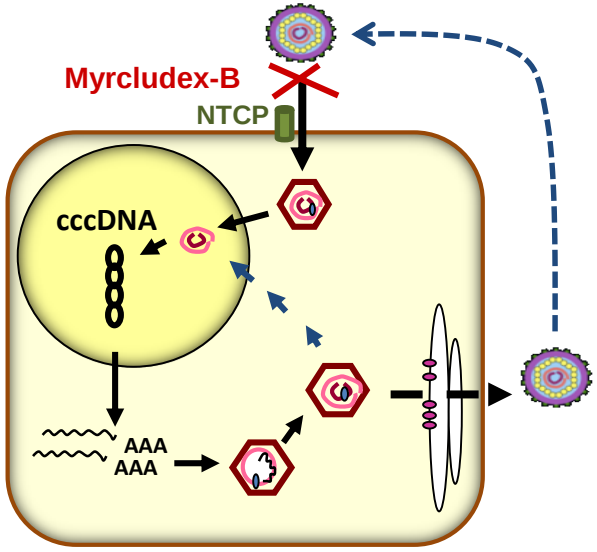


(Volz et al. J. Hepatology 2013)

Antiviral efficacy of Myrcludex-B administration on intrahepatic cccDNA amplification







Myrcludex-B hindered the increase of intrahepatic / intracellular cccDNA loads

(Volz et al. J.Hepatol 2013)

Intracellular cccDNA amplification seems to be inefficient in PHHs, explaining lower cccDNA/cell found in patients

Virus-specific differences among hepadnaviruses (DHBV vs. HBV)

(Kock et al. PloS Pathpgens 2010

HBV and host metabolism

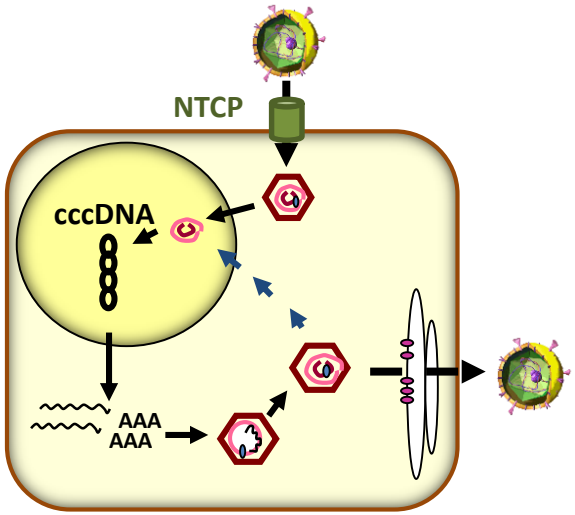
Use of preS1-derived lipopeptide to explore virus-host interactions

NTCP mediates 80% of the hepatocellular uptake of bile acids

(Stieger, Handb. Exp. Pharmacol.2011)

Chronic HBV infection has been associated with alterations in lipid and cholesterol metabolism

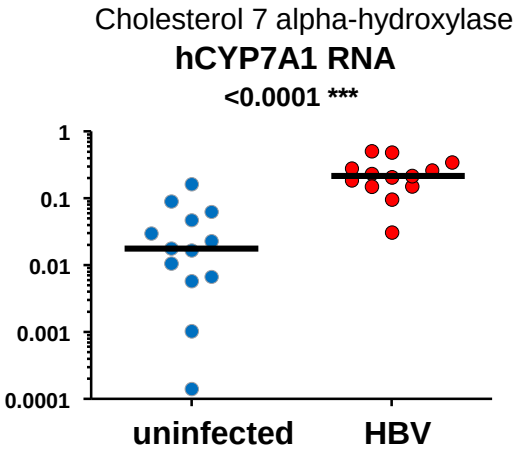
(Bar-Yishay Liver Int 2011; Hsu et al., J. Viral Hepatitis 2012)

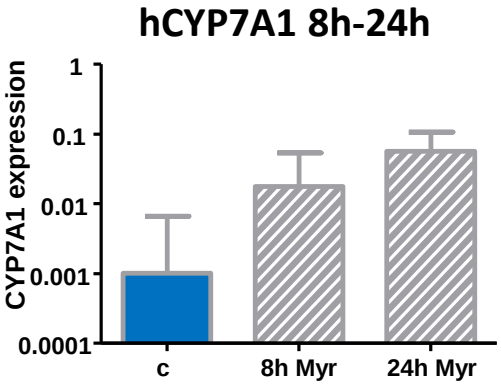


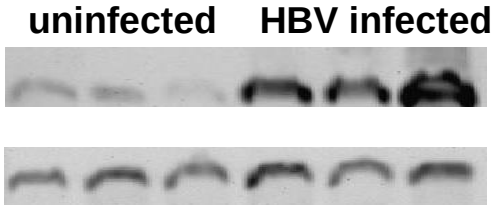
Studies in humanized mice indicated that HBV alters the profile of various genes related to host metabolism

(Oehler, et al. Hepatol.2014)

CYP7A1 is strongly enhanced in HBV- infected mice







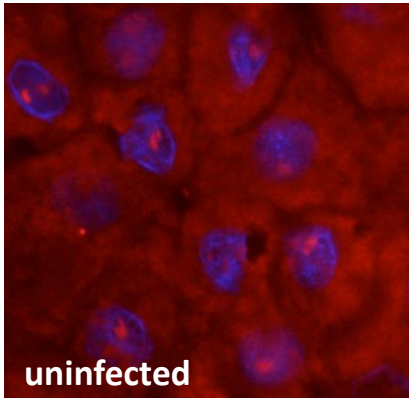
Strong enhancement of CYP7A1, the rate-limiting enzyme promoting conversion of cholesterol to bile acids

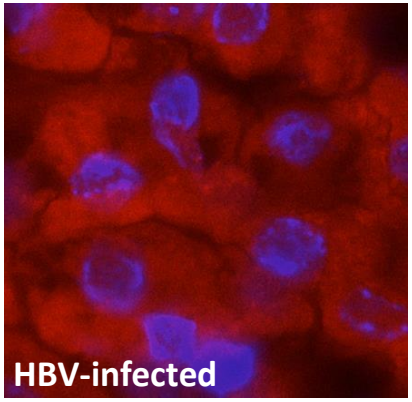
CYP7A1 increase was confirmed also in liver biopsies from CHB patients

Myrcludex-B enhanced *CYP7A1* expression in uninfected mice, thus identifying the preS1-domain as the viral component triggering CYP7A1 induction

(Oehler, et al. Hepatol.2014)

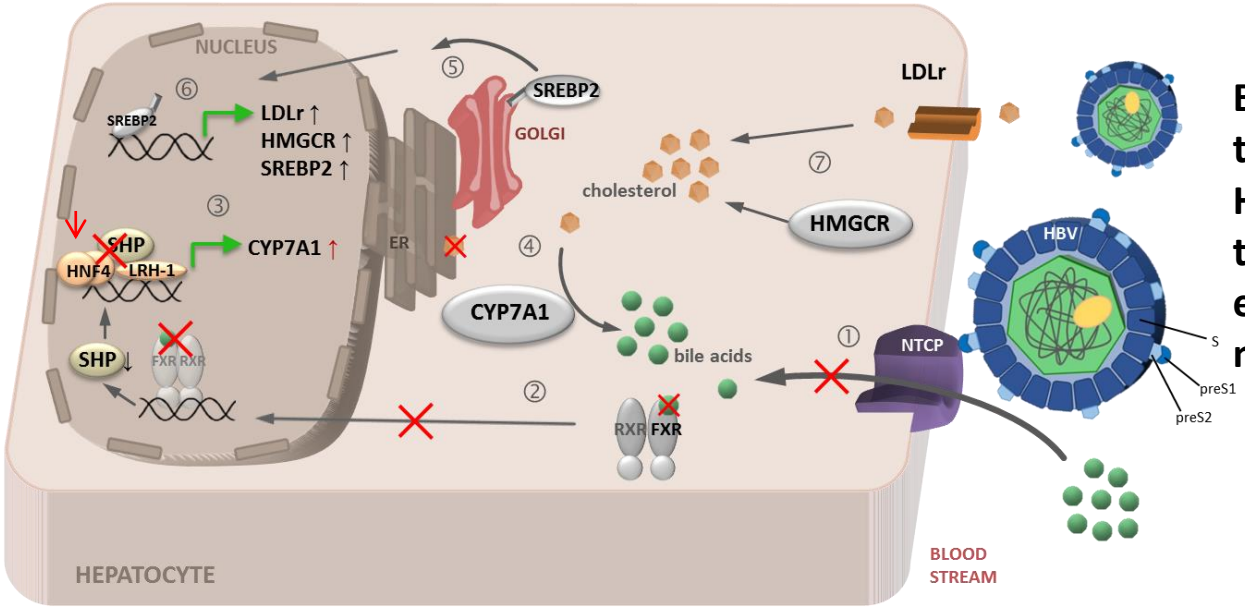
HBV and host metabolism





Nuclear localization of the bile acid sensor FXR was strongly reduced in HBV-infected cells

(Oehler, et al. Hepatology 2014)



Binding of HBV to NTCP limits the uptake of bile salts, HBV hinders FXR nuclear translocation and alters the expression of bile acid metabolic genes

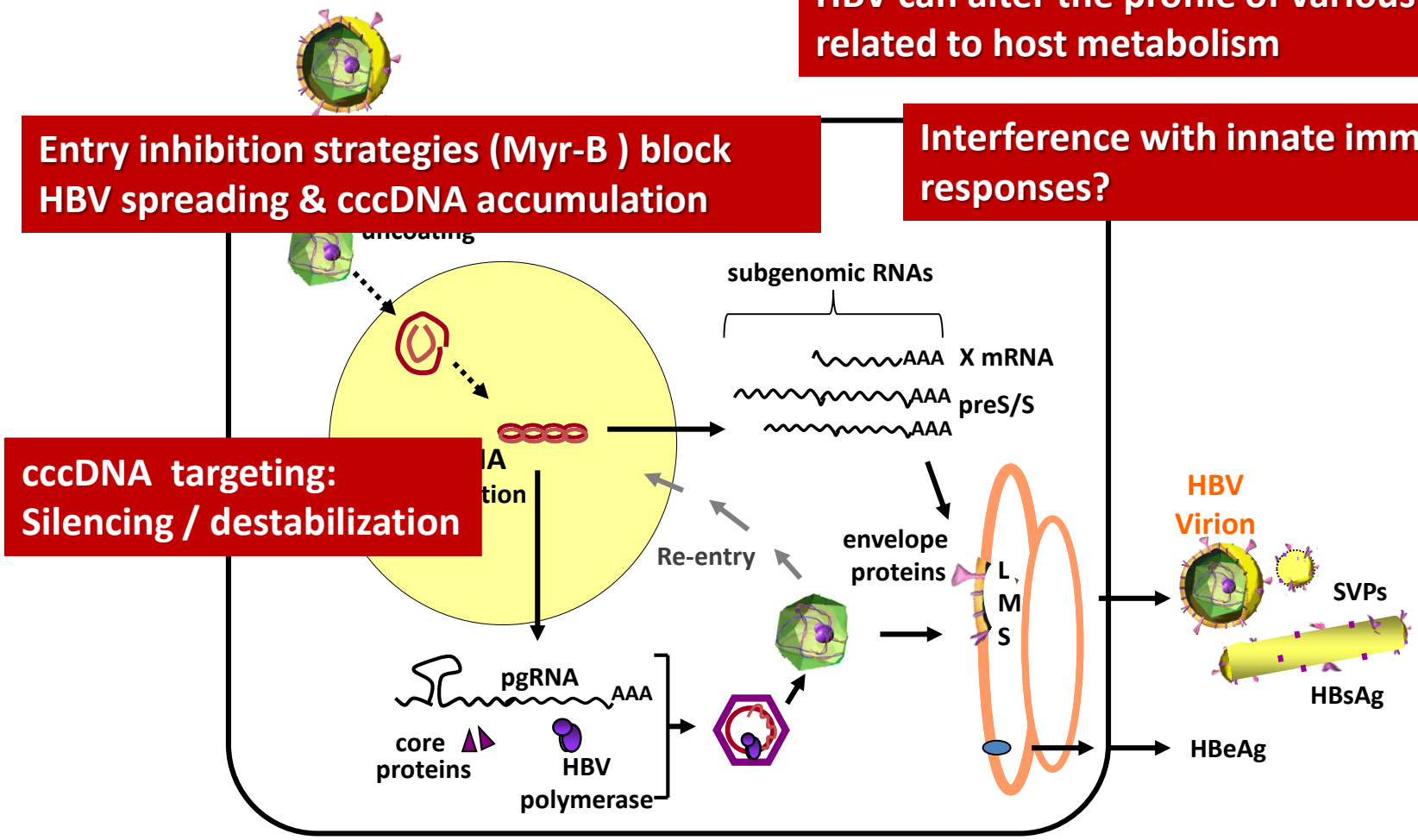
(Ni et al. Gastroenterology 2014; Oehler, et al. Hepatology 2014)

SUMMARY

HBV can alter the profile of various genes related to host metabolism

Entry inhibition strategies (Myr-B) block HBV spreading & cccDNA accumulation

Interference with innate immune responses?



cccDNA targeting: Silencing / destabilization

(Modified from Dandri, Locarnini, Gut, 2012)

Acknowledgement

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