Les Pensières
Conference Center
Fondation Mérieux
Veyrier-du-Lac - France
2-4 May 2016

Mechanisms behind chronic infections (TB, HBV, HIV…): molecular and cellular mechanisms, evasion mechanisms, immunity,…

cccDNA biology and strategy to silence or degrade it

Massimo Levrero

CLNS@SAPIENZA, Istituto Italiano di Tecnologia (IIT), Rome, Italy
Dept of Internal Medicine - DMISM, Sapienza University, Rome, Italy
Cancer Research Center of Lyon (CRCL) and INSERM U1052, Lyon, France
Hopital de la Croix-Rousse - Hospices Civils de Lyon, Lyon, France

Disclosures

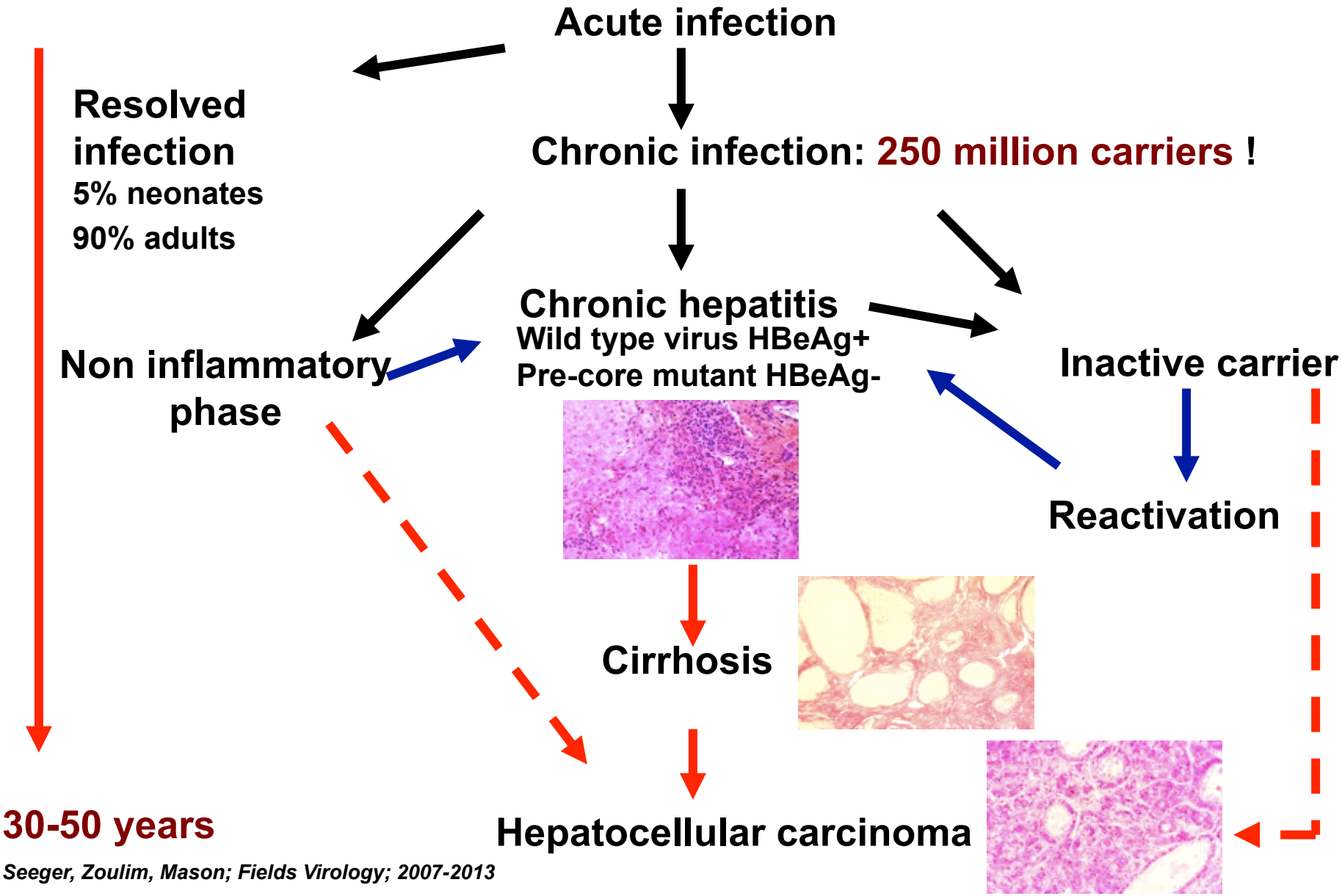
Advisory Committees or Review Panels:

- BMS
- Jansen
- Gilead
- Arbutus
- Galapagos
- Assembly Pharma
- Sanofi/Aventis

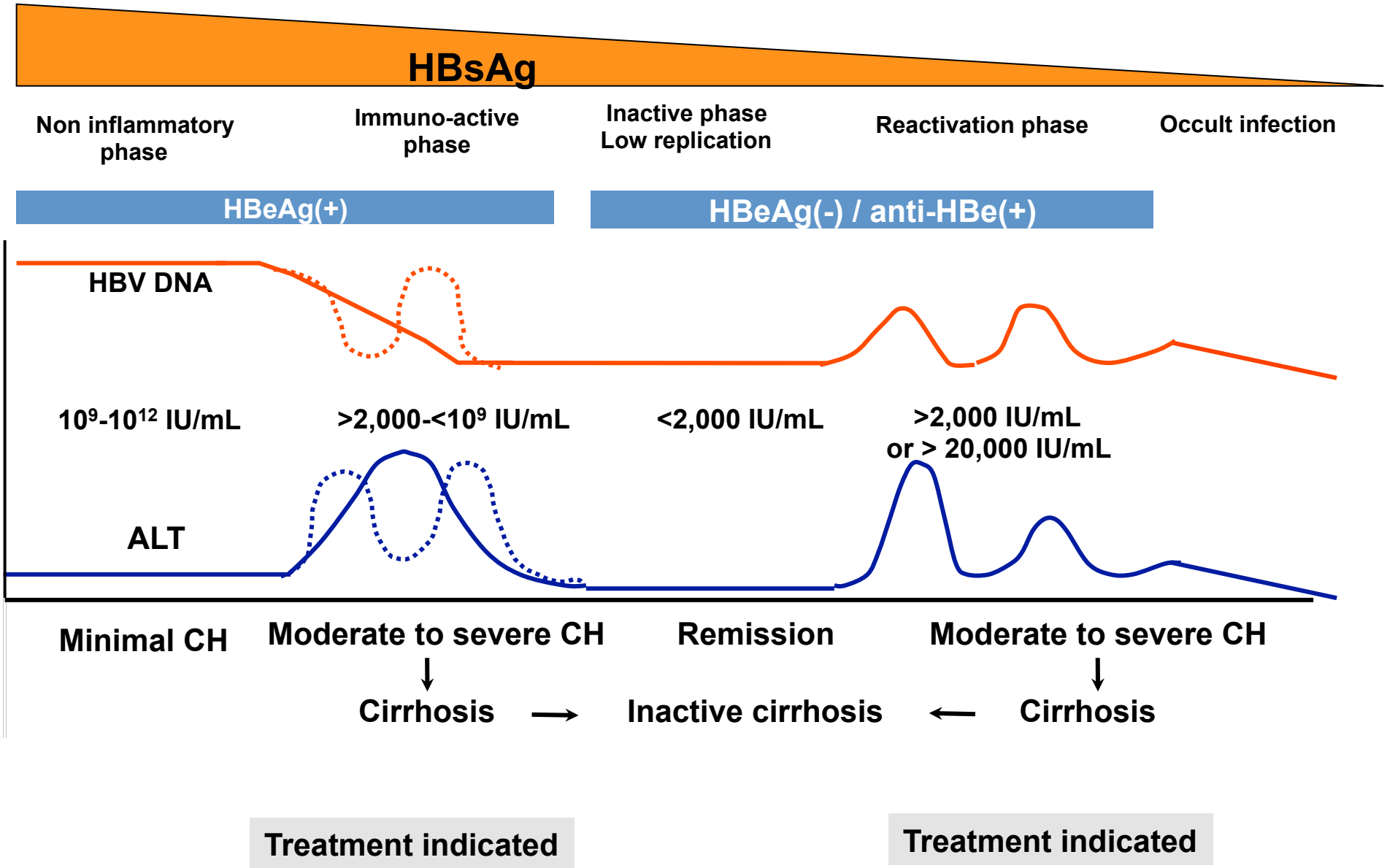
Speaking and Teaching:

- MSD
- Roche
- BMS
- Jansen
- Gilead

The burden of chronic HBV infections



Seeger, Zoulim, Mason; Fields Virology; 2007-2013



EASL Clinical Practice Guidelines, J Hepatol 2012

Current treatment indications

Patients in the immunoactive phase

AgHBeAg+, VL > 2,000 UI/mL, elevated ALT
HBeAg-, VL > 2,000 UI/mL, elevated ALT (fluctating)

EASL CPG 2012
AASLD CPG 2015

Inactive carriers

HBeAg-, VL < 2,000 UI/mL, normal ALT
If immune suppressive therapy / prevention of viral reactivation

Huang et al, JAMA 2014
Perrillo et al, JAMA 2015

« Immune tolerant » patients

HBeAg+, VL > 6log UI/mL, normal ALT
Familial history of cirrhosis or HCC
Why not all ?

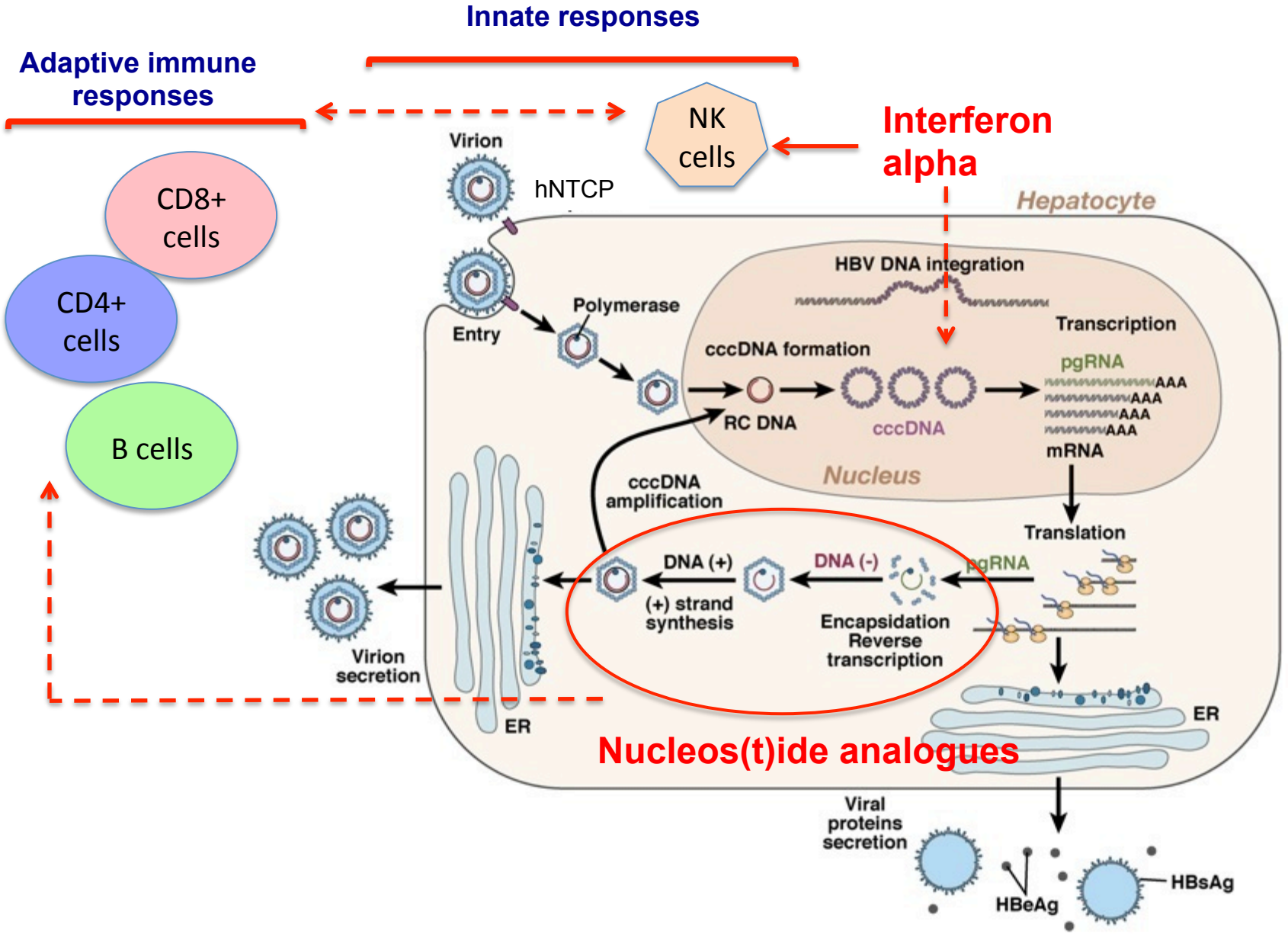
Chan et al,
Gastroenterology 2014

Pregnant women

If VL > 6 log UI/mL
Last trimester of pregnancy to prevent MTCT
With HBIG and vaccine in the newborn

Chen et al, Hepatology 2015
Brown et al, Hepatology 2016
Visvanathan et al, Gut 2016

Mode of action of antivirals for CHB



Zoulim & Locarnini, Gastroenterology 2009; Zoulim Antiviral Research 2012; Mico et al J Hepatol 2013; Boni et al Hepatology 2015

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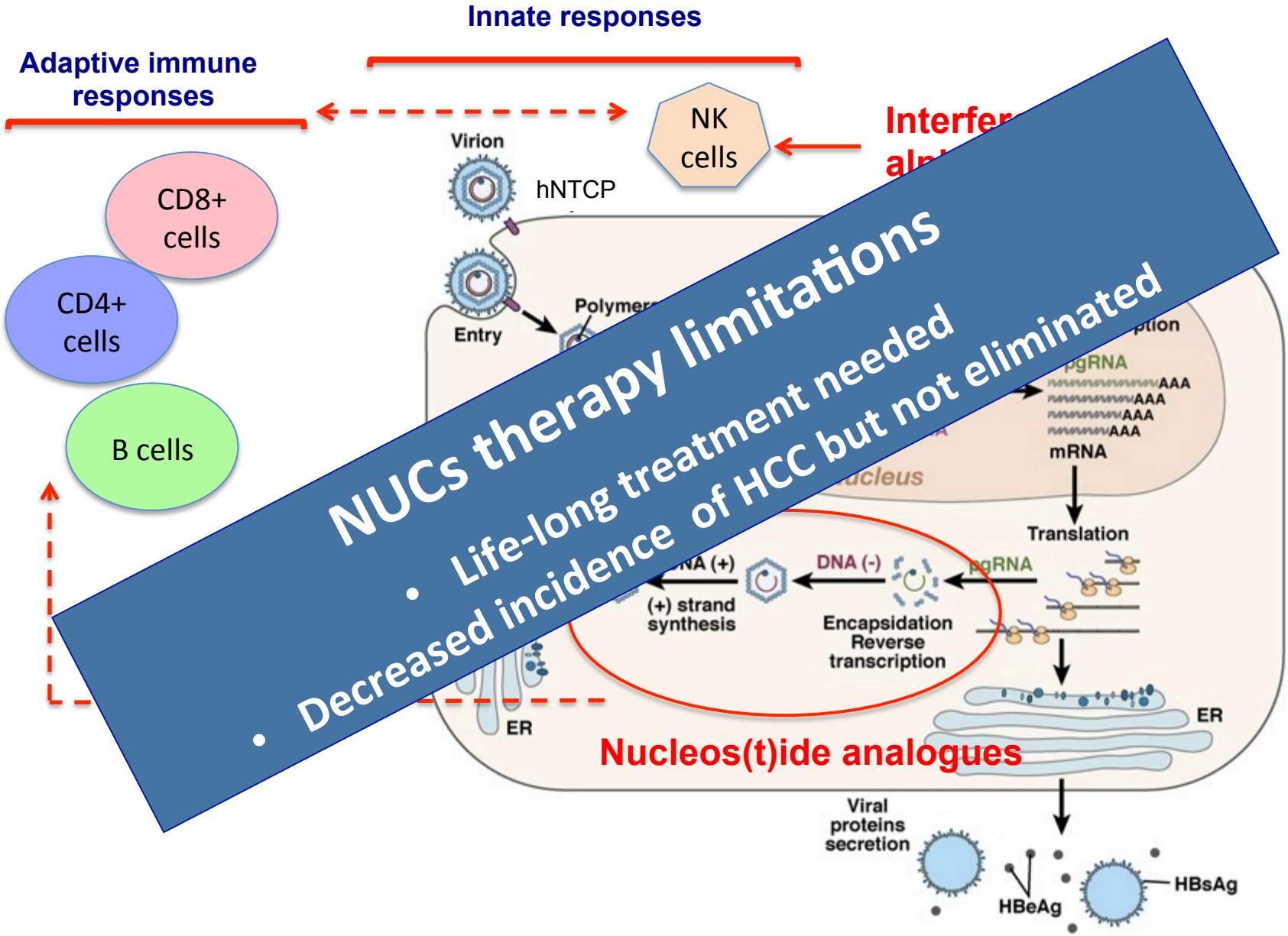

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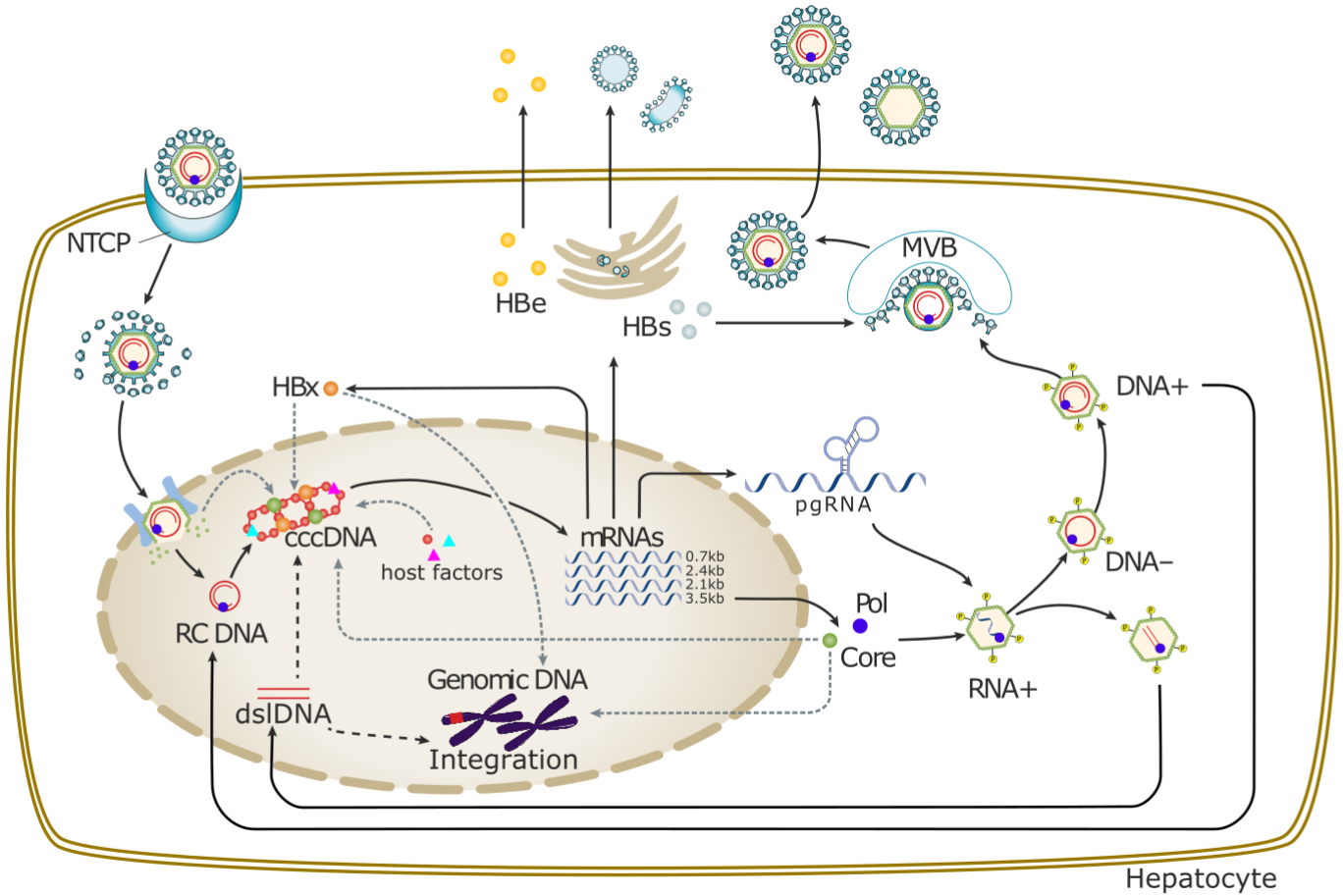
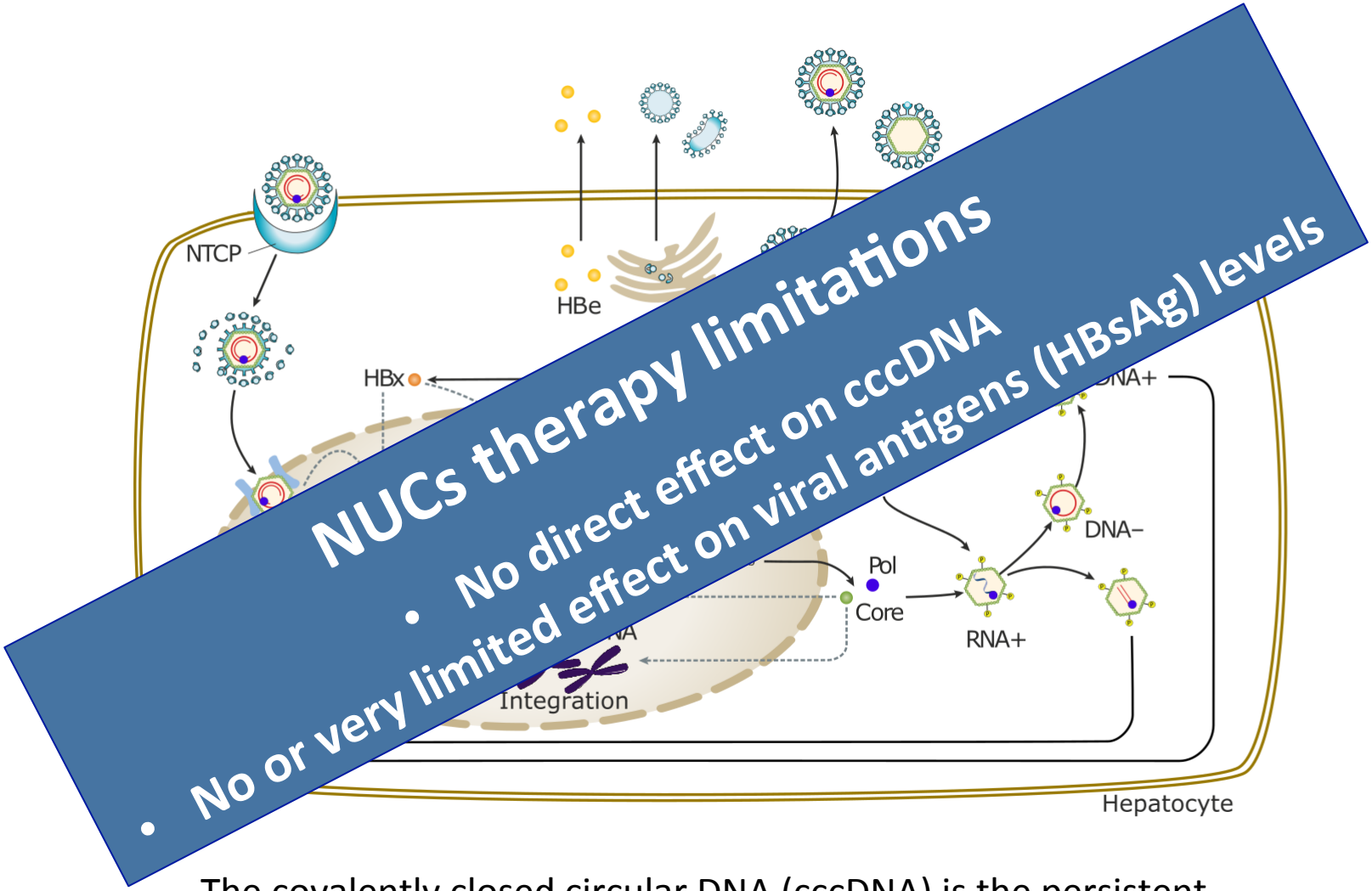
cccDNA is the key molecule of HBV lifecycle



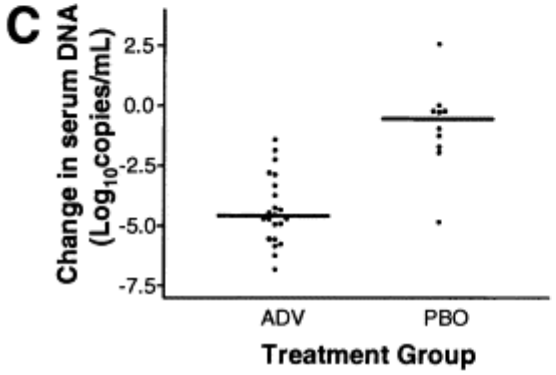
The covalently closed circular DNA (cccDNA) is the persistent, stable form of HBV genome, template for all viral mRNAs and pgRNA

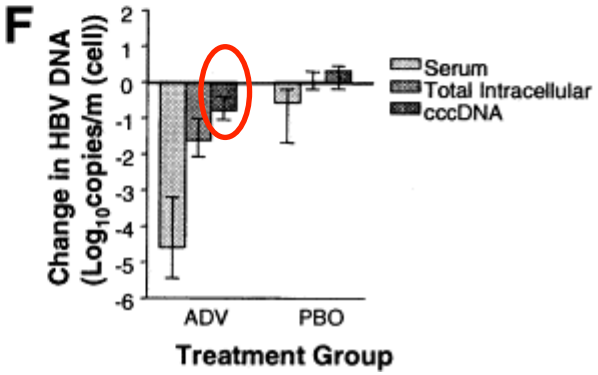
cccDNA is the key molecule of HBV lifecycle



The covalently closed circular DNA (cccDNA) is the persistent, stable form of HBV genome, template for all viral mRNAs and pgRNA

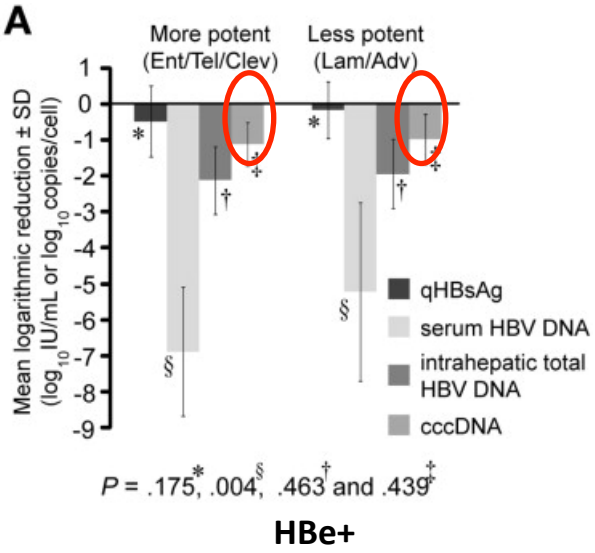
SOC therapy does not affect cccDNA reservoirs

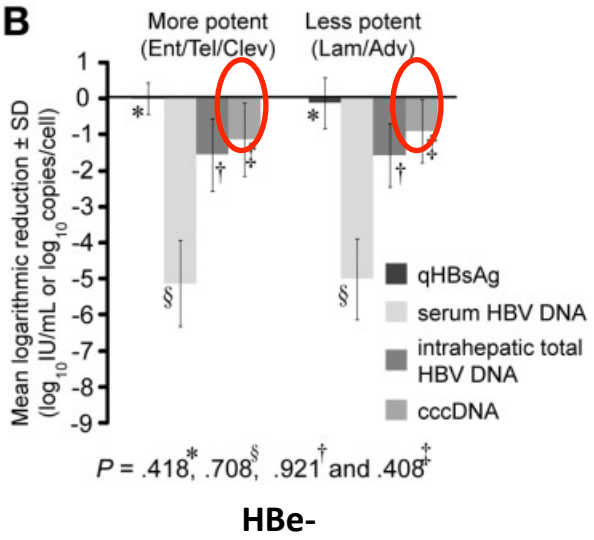




48-weeks ADV therapy

Werle-Lapostolle, Gastroenterology 2004





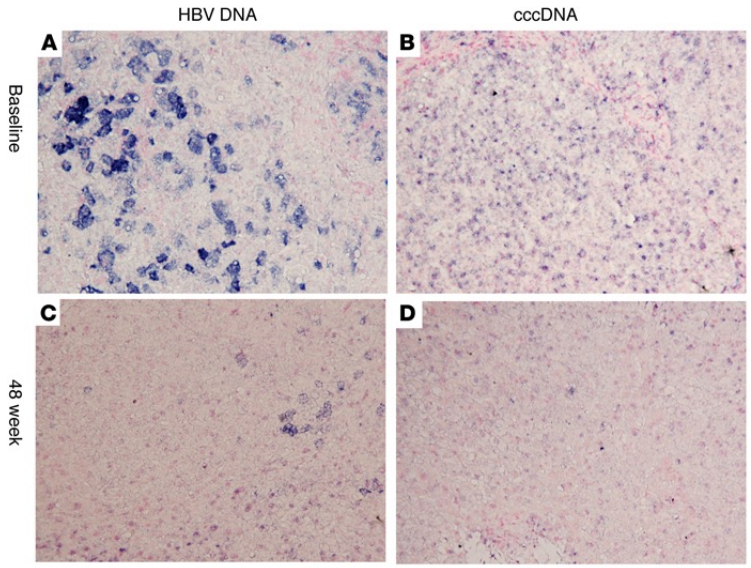
1 year NAs therapy

Wong, CGH 2013

Mathematical modeling suggests that >10 years therapy would be required to clear cccDNA!

SOC therapy does not affect cccDNA reservoirs

In situ analysis of intrahepatic viral DNA in 9 CHB patients before/after NUC therapy



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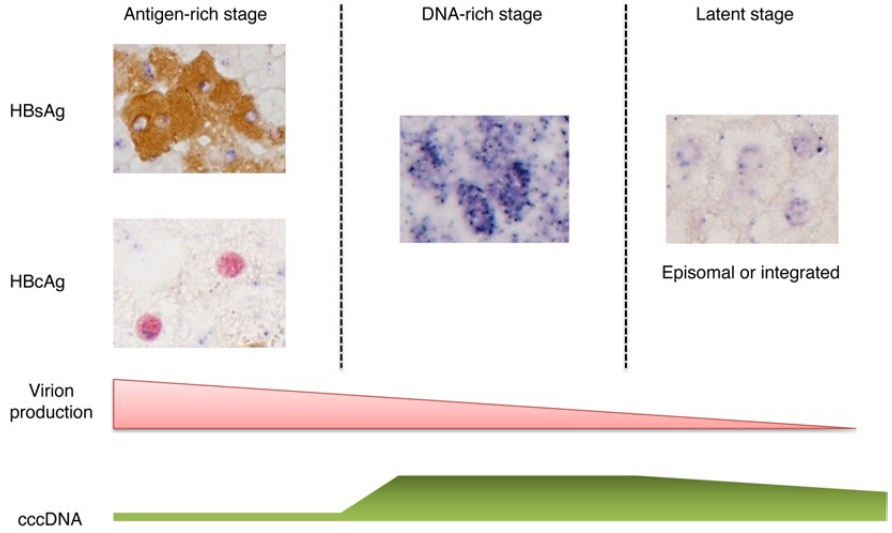
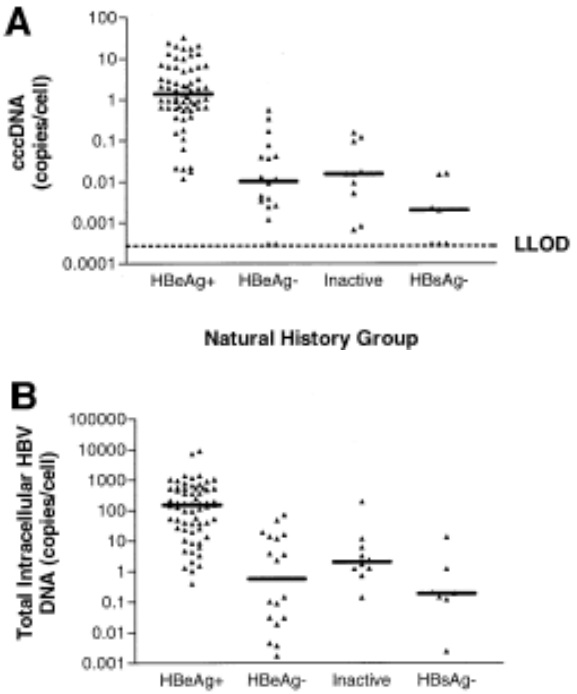


Zhang, JCI 2016

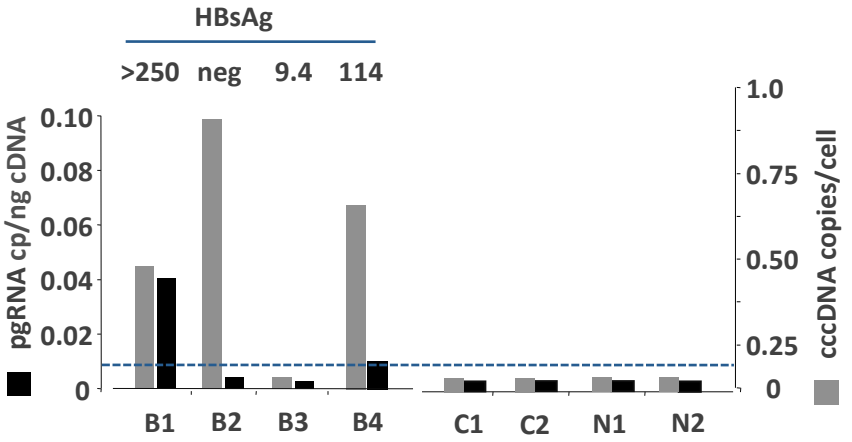
At a single cell level, a decline but not a disappearance of cccDNA is observed

Gradual transition from vigorous virion production to active stockpiling of genome copies and nuclear cccDNA reserves

Persistence of cccDNA



Werle Lapostolle 2004

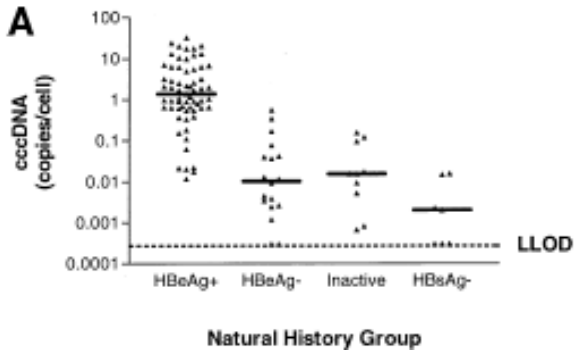


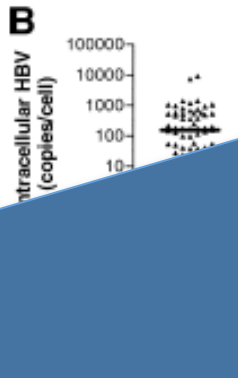
Persistence of cccDNA in 3 out of 4 patients with long term HBV suppression under lamivudine
In 2 out 3 patients cccDNA is inactive (no pgRNA)

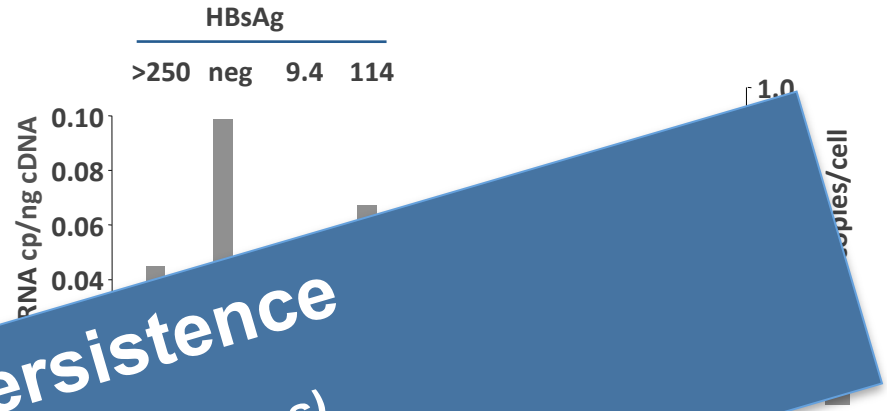
Belloni, Levrero, Gaeta unpublished

- **Detected in the liver of NUCs long-term suppressed patients after HBsAg to anti-HBs seroconversion** [Maynard, 2005; Belloni unpublished]
- **Detected in the liver of HBsAg negative patients (occult HBV infection)** [Werle-Lapostolle, 2004; Pollicino unpublished]
- **Present in 30 /30 patients with occult HBV infection and HCC** [Pollicino, 2004]

Persistence of cccDNA







cccDNA persistence

active cccDNA (pgRNA pos)
inactive cccDNA (pgRNA neg)

patients with long term HBV

cccDNA is inactive (no pgRNA)

Belloni, Levrero, Gaeta unpublished

2004

Detected in the liver of NUCs long-term suppressed patients after HBsAg to anti-HBs seroconversion [Maynard, 2005; Belloni unpublished]

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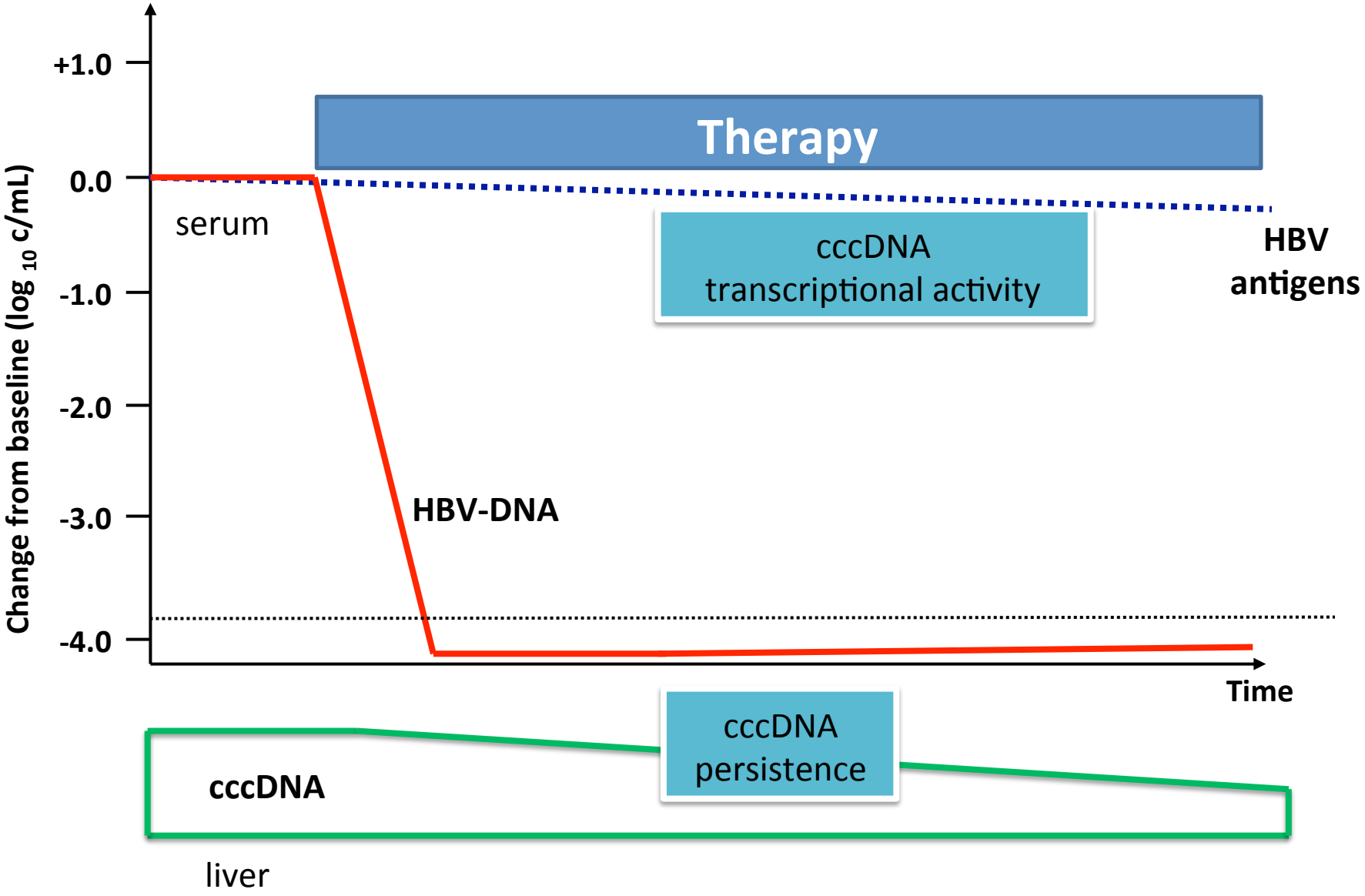
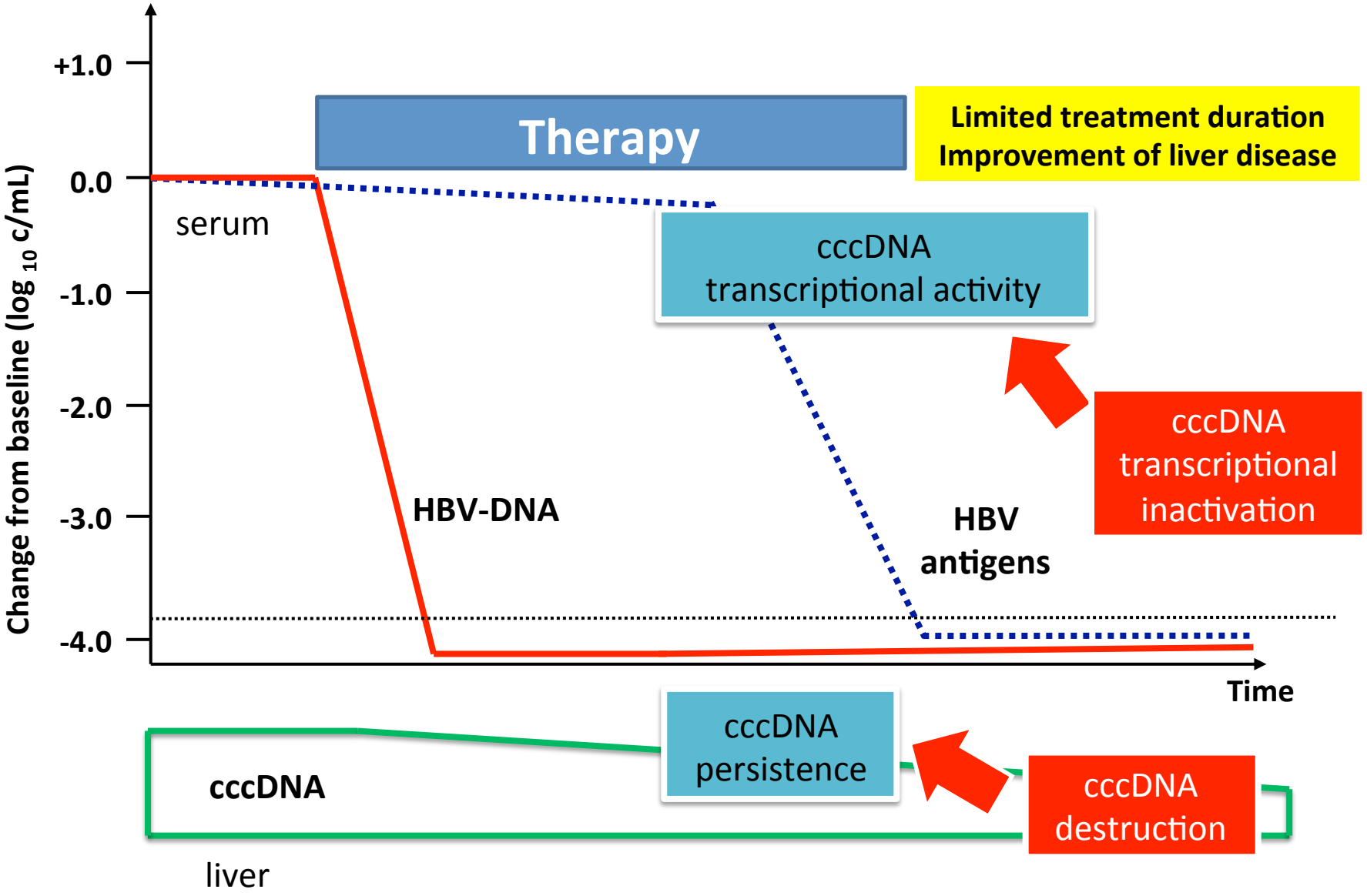
Strategies for an « HBV cure »



Strategies for an « HBV cure »



Definition of HBV cure

« Functional cure »

- Situation where antiviral therapy could be stopped with a minimal risk of viral reactivation
- HBsAg loss with anti-HBsAb seroconversion
- cccDNA inactivation and/or control by host mechanisms

« Complete cure »

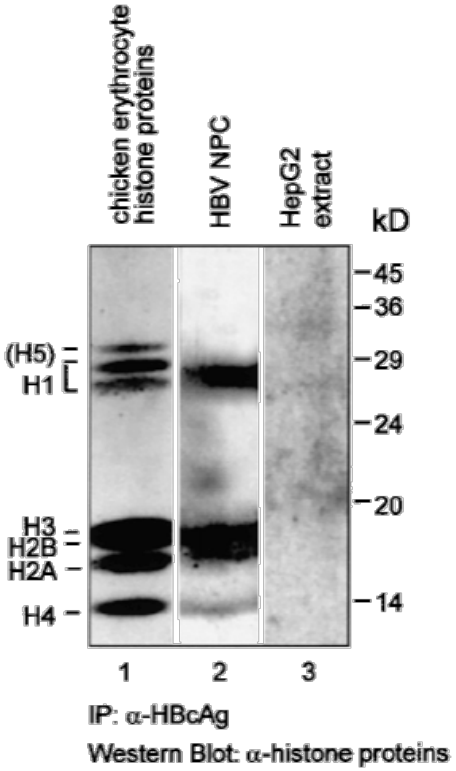
- HBsAg clearance and cccDNA eradication

In all cases, «HBV cure » associated with **clinical benefit:** regression in the risk of disease progression and HCC

The impact of molecular damage and **integrated viral sequences** in infected hepatocytes will need to be addressed.

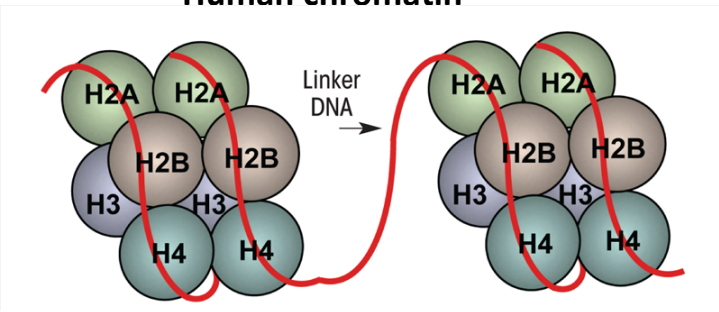
Zeisel, Lucifora et al, Gut 2015;
Revill et al, Nature Reviews Gastroenterol Hepatol 2016
Levrero, Testoni, Zoulim Curr Opin Virology 2016

cccDNA is a minichromosome



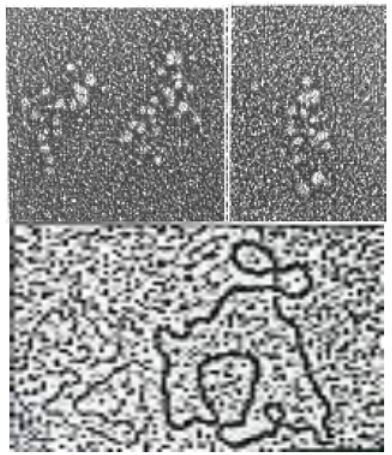
cccDNA is associated to histones and non-histone proteins and to HBV Core

Human chromatin

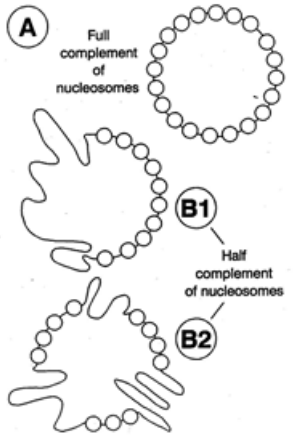


~ 215bp

cccDNA



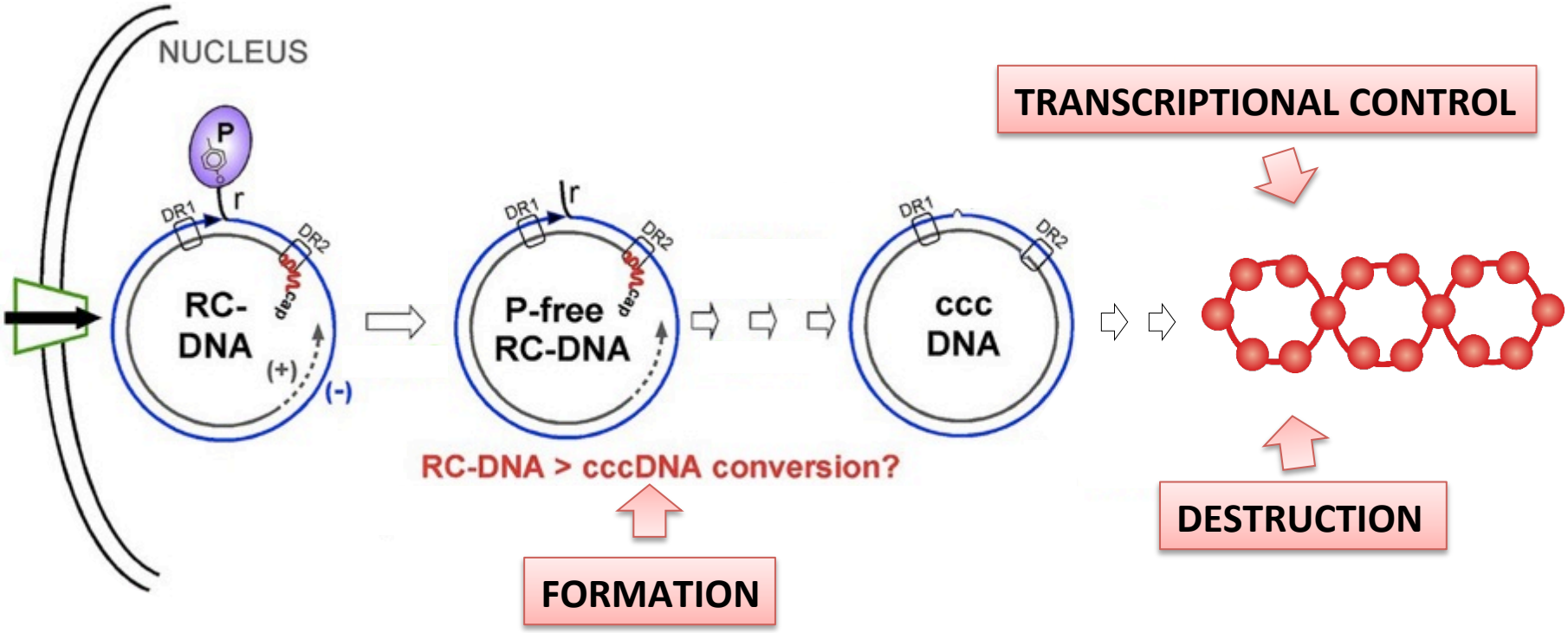
16 nucleosomes with a spacing ~ 200bp



10% spacing reduction respect to cellular chromatin

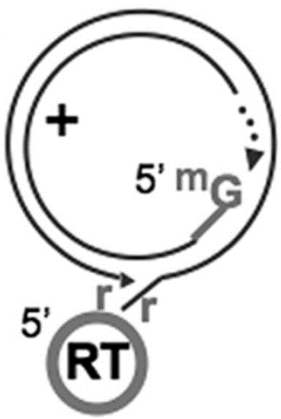
Bock, Virus Gen 1994 and JMB 2001; Newbold, 1995

Targeting cccDNA



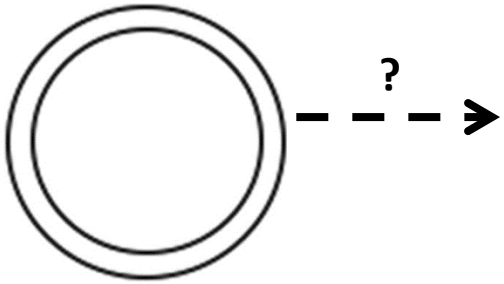
Modified from Nassal, Gut 2015

cccDNA formation: a black box



Viral RT? Host DNA repair?

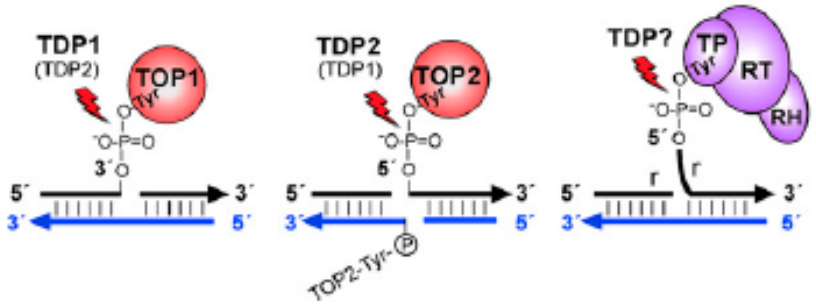
1. Removal of RT
2. Removal of r
3. Ligation of (-) DNA
4. Completion of (+) DNA
5. Removal of capped RNA
6. Ligation of (+) DNA



DP-rcDNA

minichromosome

Gao and Hu, J Virol 2007



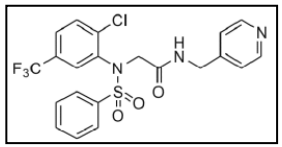
Tdp2 Knockdown does not block but ENHANCES HBV infection with increased cccDNA formation?!

Cortes Ledesma, Nature 2009
Koeniger, PNAS 2014
Cui, Plos One 2015

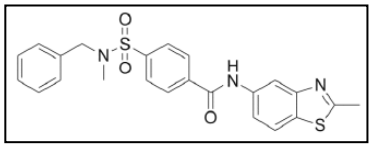
Inhibitors of rcDNA to cccDNA conversion

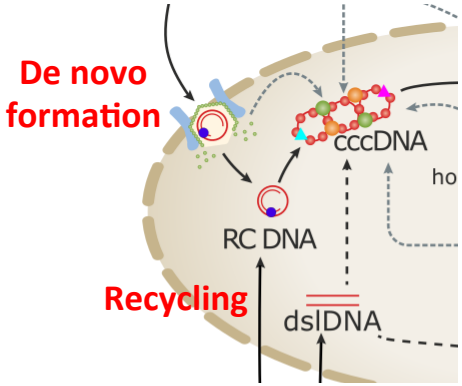
Disubstituted sulfonamides (DSS)

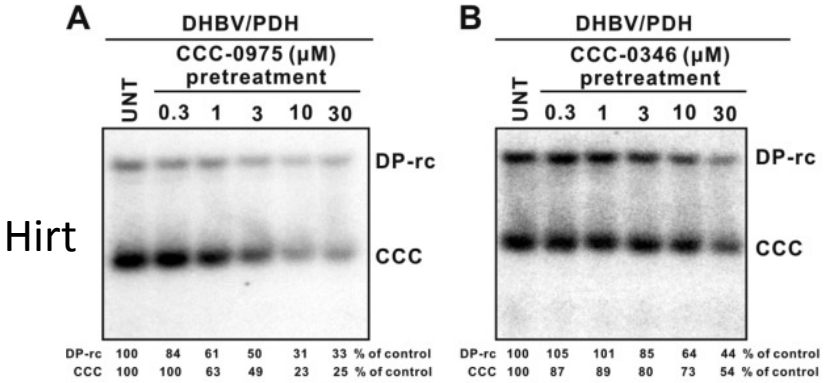
CCC-0975

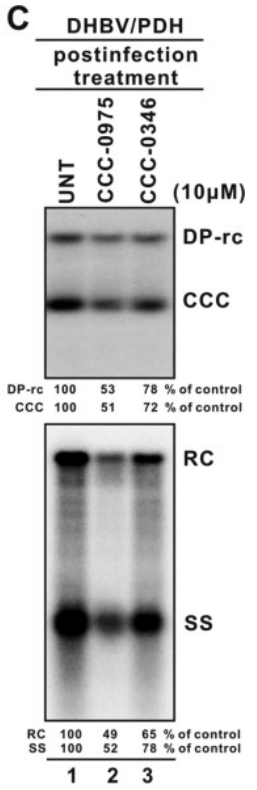


CCC-0946









Molecular mechanism still unknown

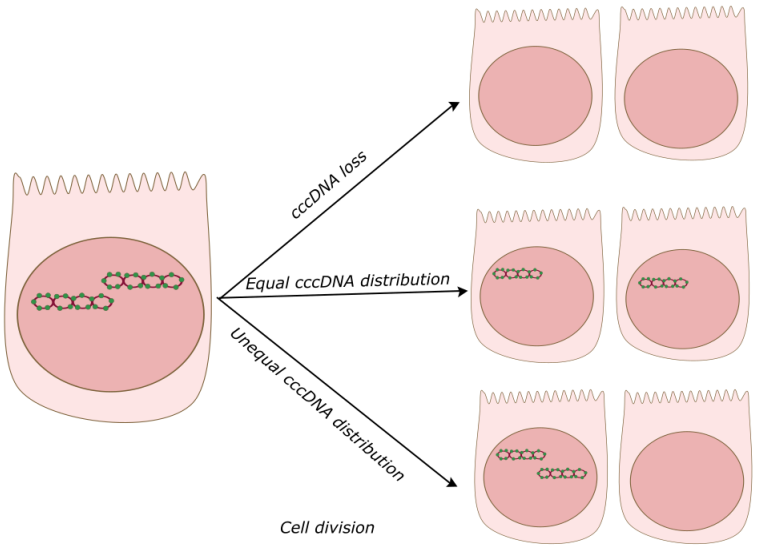
Effect only during establishment of infection or during phases of high hepatocytes turnover

Cai, Antimic Agents & Chemother, 2012

cccDNA destruction

- Selective killing of infected cells
- **Hepatocytes turn-over**
- Non-cytotoxyc degradation

Do *in vivo* proliferation of hepadnavirus-infected hepatocytes affect cccDNA levels?

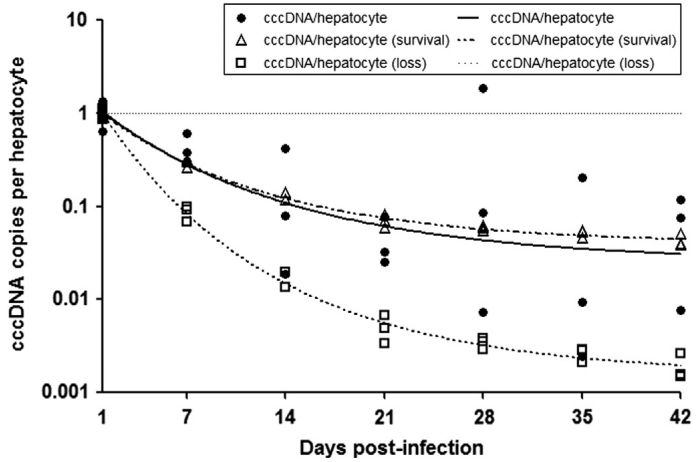


No origin of replication

No nuclear retaining signal

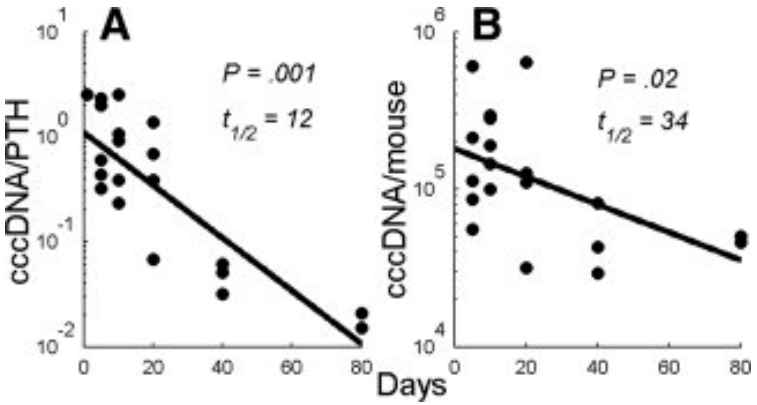
Does cccDNA survive mitosis?

Ducks (NAs + rapid liver growth):



Reaiche-Miller, Virology 2013

HuHep mice undergoing serial engrafments:



Lutgehetmann, Hepatology 2010

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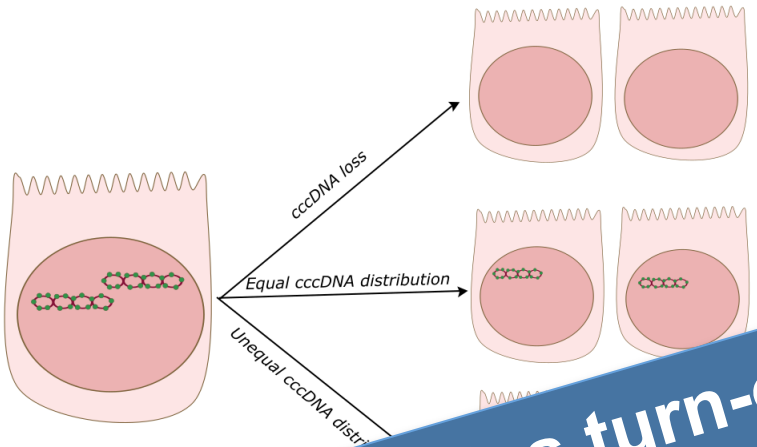

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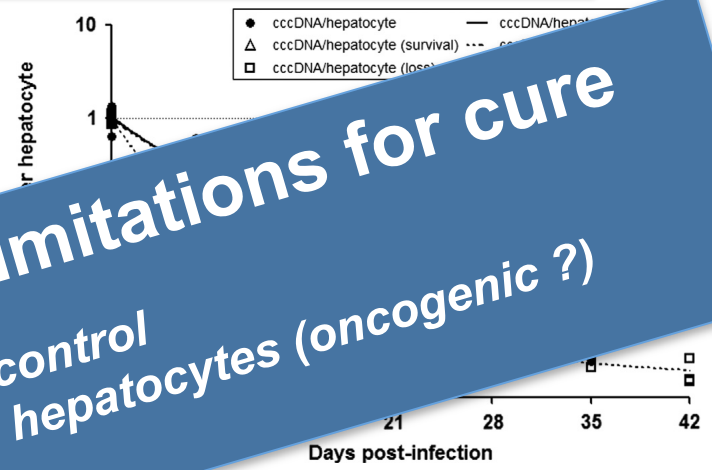
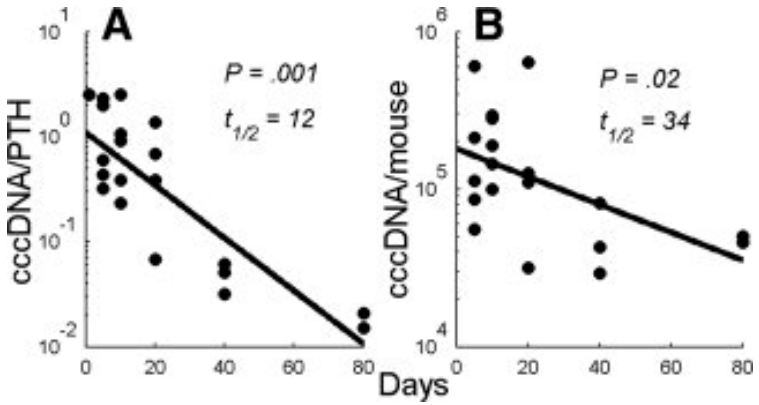


Reaiche-Miller, Virology 2013

Hepatocytes turn-over: limitations for cure

- Difficult to control
- May trigger clonal selection of hepatocytes (oncogenic ?)

HuHep mice undergoing serial engrafments:



Lutgehetmann, Hepatology 2010

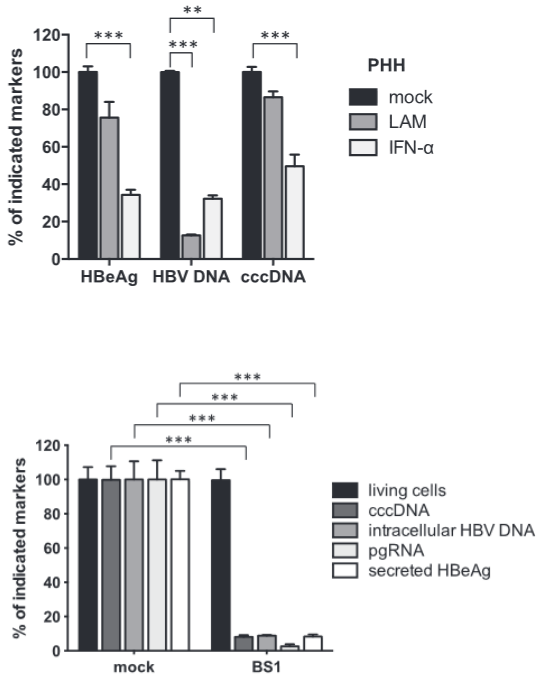
cccDNA destruction

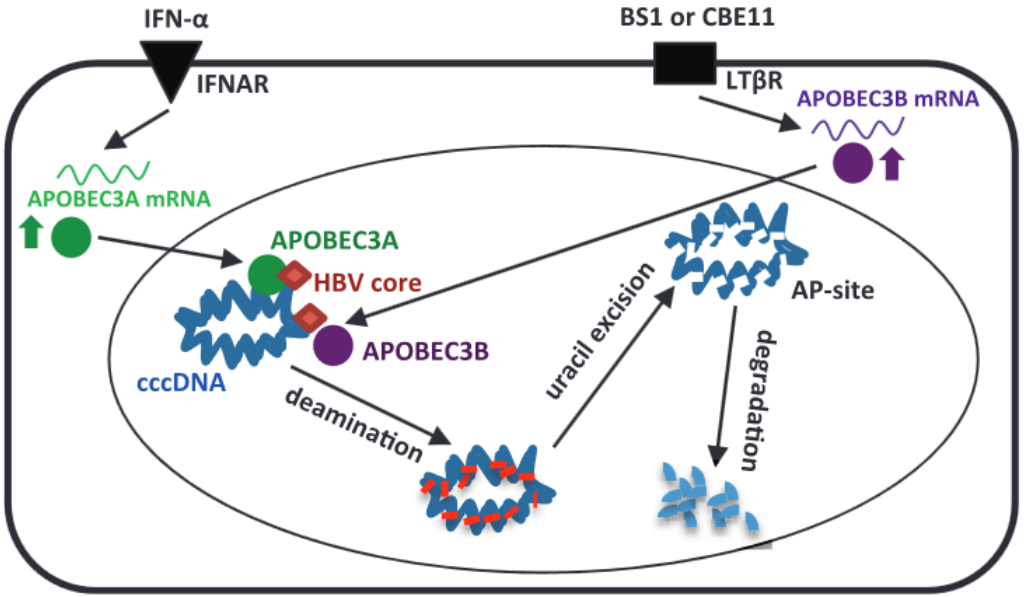
- Selective killing of infected cells
- Hepatocytes turn-over
- **Non-cytotoxyc degradation**

cccDNA destruction: deamination

IFNα, Lymphotoxin β agonists, IFNγ, TNFα can induce

APOBEC3A/B dependent degradation of HBV cccDNA





Lucifora, Science 2014; Shlomai & Rice, Science 2014; Xia, Gastroenterology 2015

cccDNA destruction: deamination

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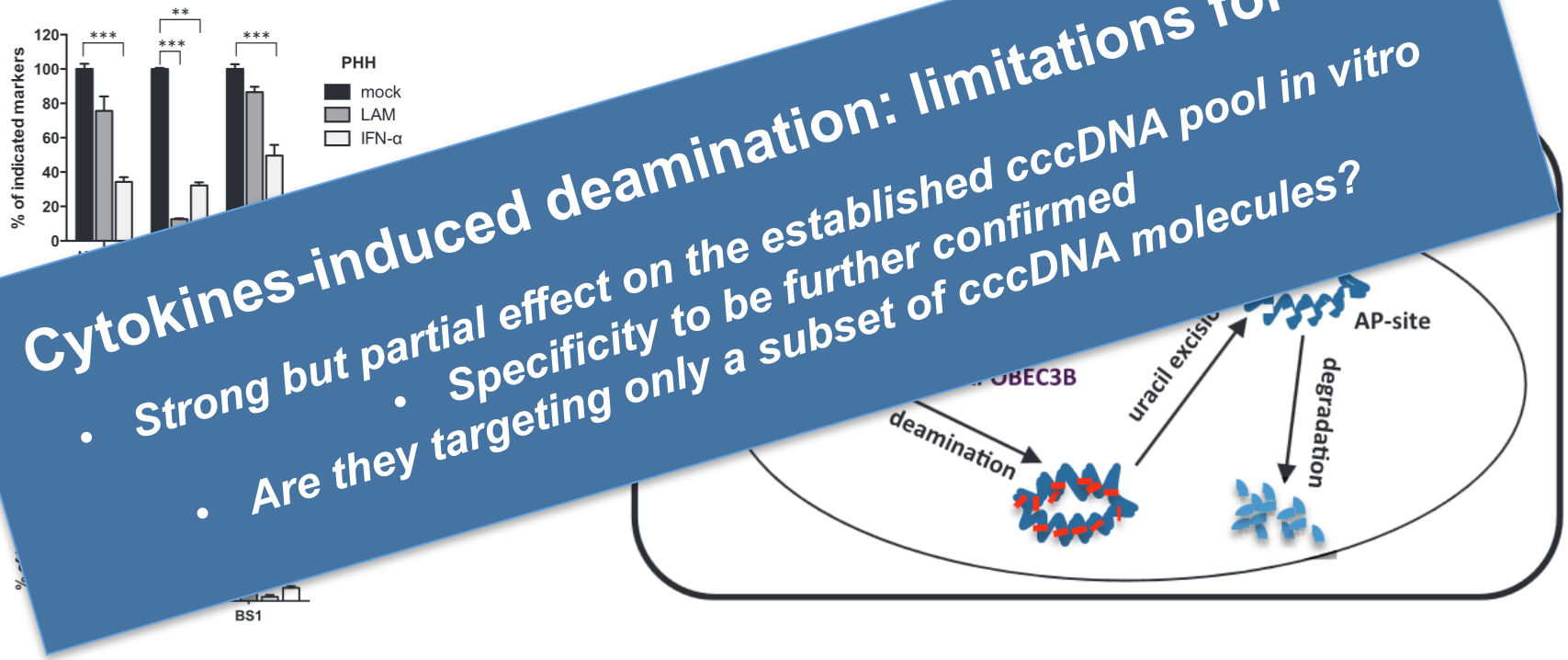
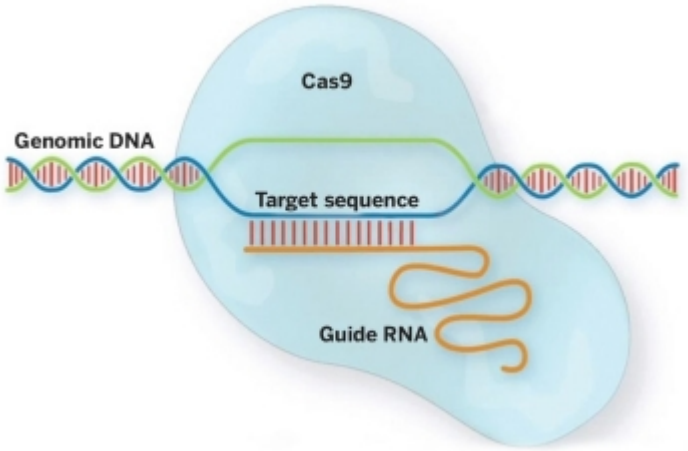


Cytokines-induced deamination: limitations for cure

- Strong but partial effect on the established cccDNA pool *in vitro*
- Specificity to be further confirmed
- Are they targeting only a subset of cccDNA molecules?

Lucifora, Science 2014; Shlomai & Rice, Science 2014; Xia, Gastroenterology 2015

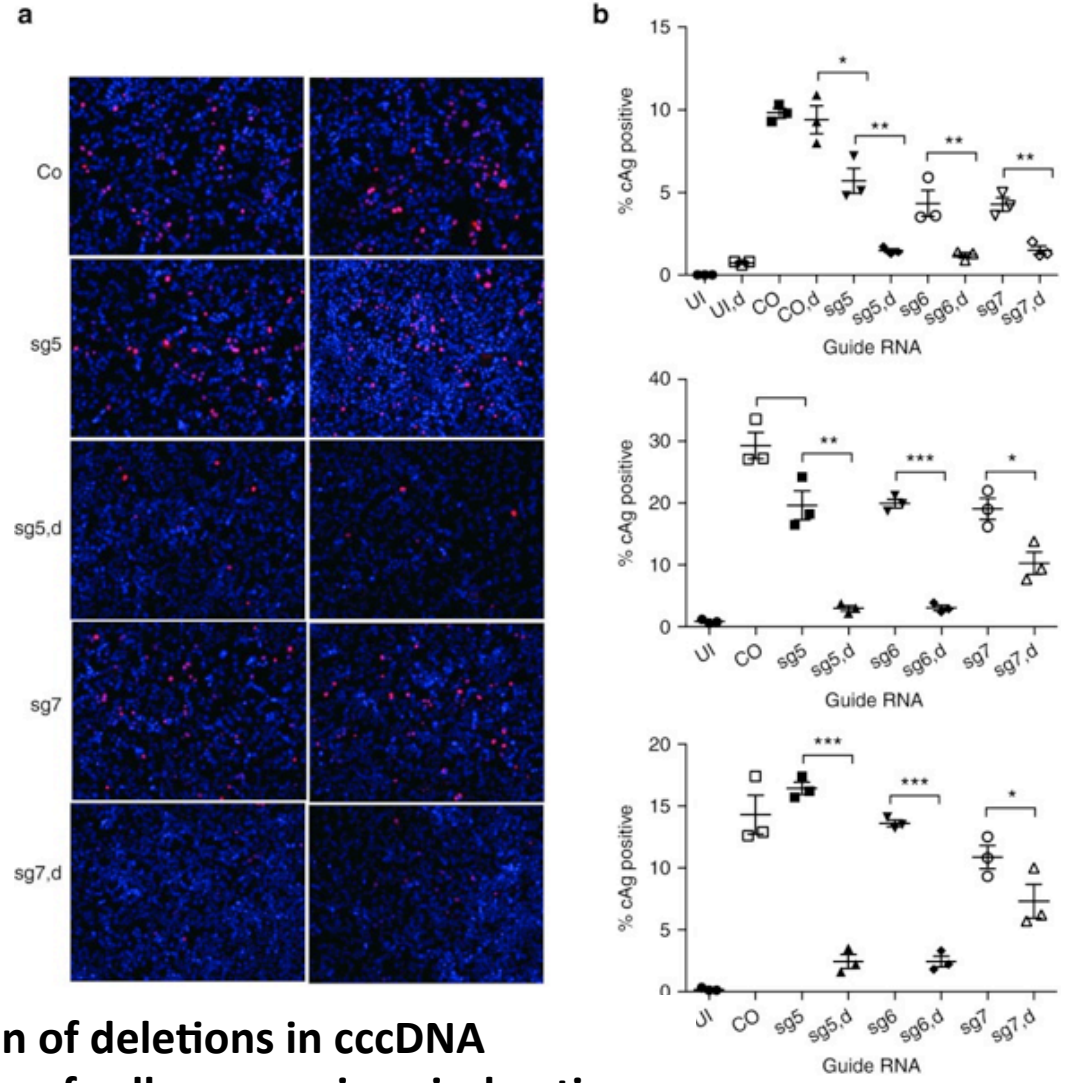
cccDNA destruction: CRISPR/Cas9



Double strands breaks

NHEJ DNA repair

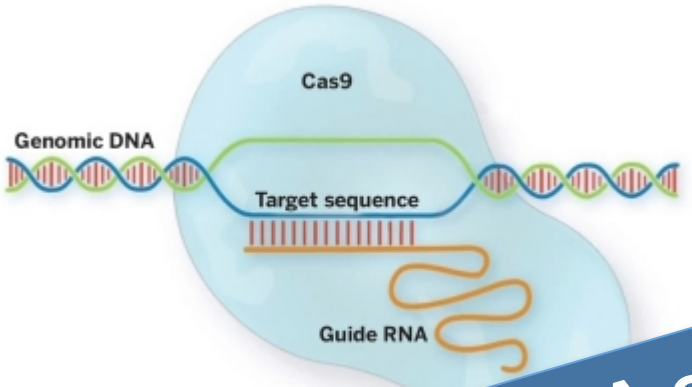
Deletions/large mutations



Induction of deletions in cccDNA
Decreased number of cells expressing viral antigens

Seeger, Mol Therapy Nucl Acids, 2014; Lin, Mol Therapy Nucl Acids, 2014; Kennedy, Virology, 2015; Kennedy, Antiviral Res 2015

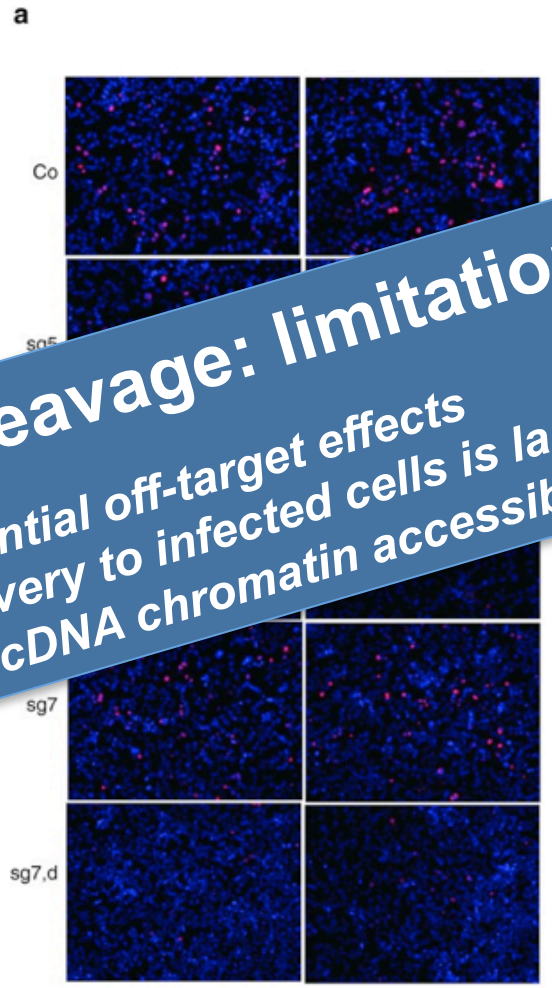
cccDNA destruction: CRISPR/Cas9

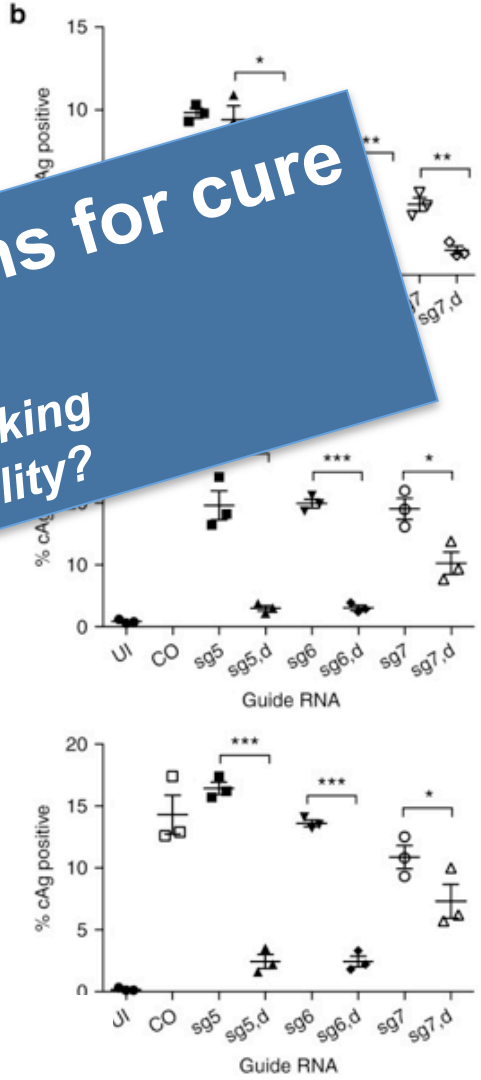


Targeted cccDNA cleavage: limitations for cure

- Potential off-target effects
- Specific delivery to infected cells is lacking
- Effect of cccDNA chromatin accessibility?

- ...ns/large mutations





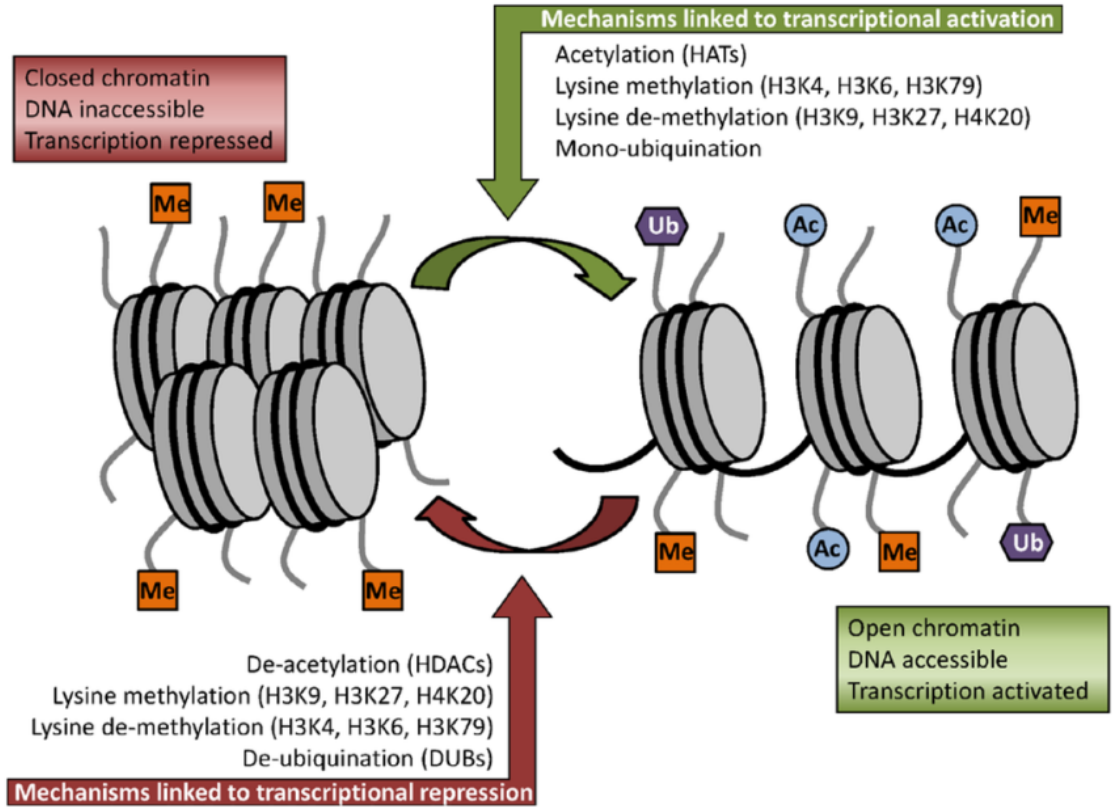
Induction of deletions in cccDNA
Decreased number of cells expressing viral antigens

Seeger, Mol Therapy Nucl Acids, 2014; Lin, Mol Therapy Nucl Acids, 2014; Kennedy, Virology, 2015; Kennedy, Antiviral Res 2015

cccDNA transcriptional control

- **cccDNA epigenetic modifications**
- Viral/host Transcription factors/cofactors associated to cccDNA

cccDNA is subjected to the « histone code »



Histone tails ACETYLATION -> TRANSCRIPTIONAL ACTIVATION

METHYLATION [H3K9, H3K27 -> REPRESSION

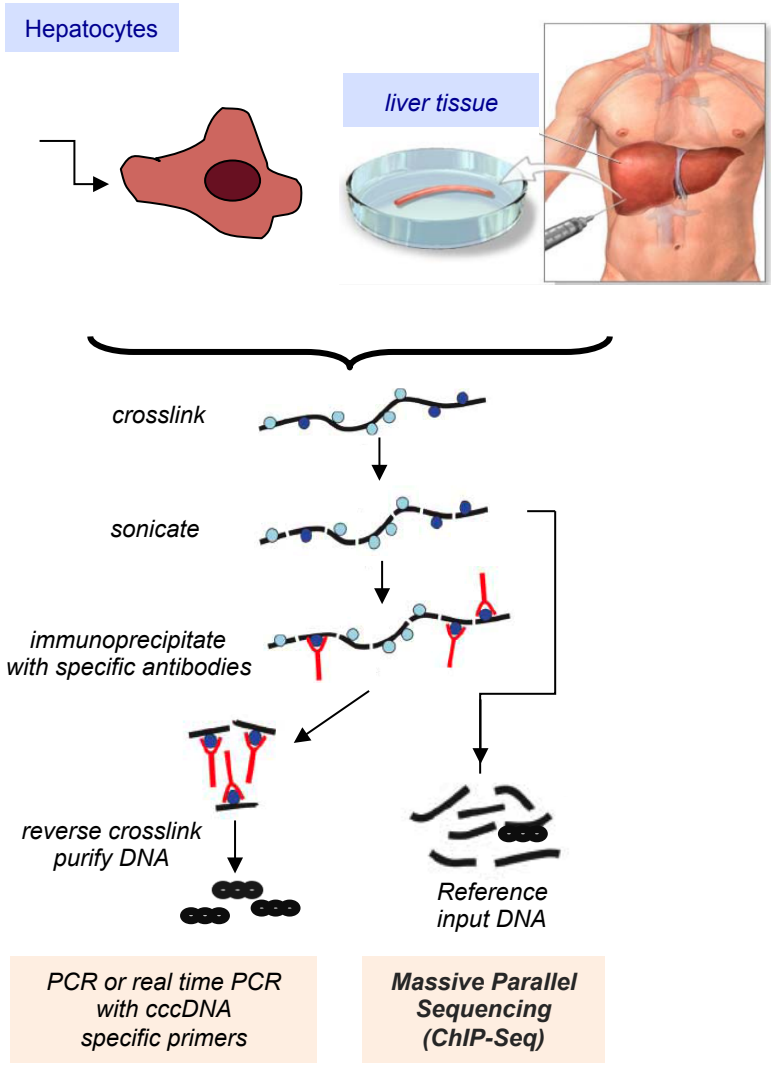
METHYLATION [H3K4] -> ACTIVATION

The cccDNA-ChIP assay

A methodology to study cccDNA function

[HBV minichromosome structure]
[modifications of cccDNA bound histones]
[binding of TF and coregulators]

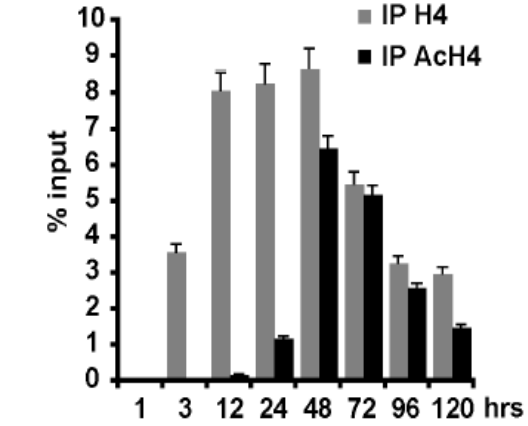
in vitro,
in animal models
ex vivo (liver samples/biopsies)

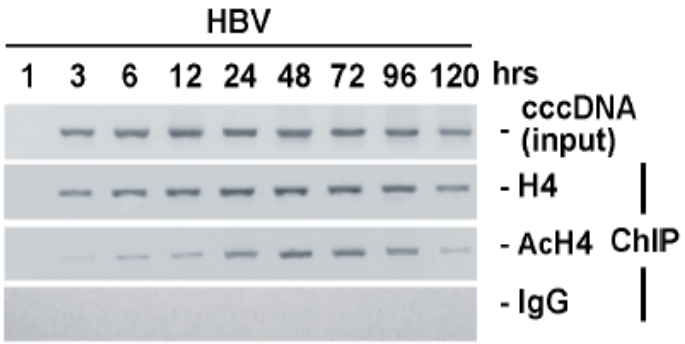


Pollicino et al. Gastroenteroplogy 2006
Levrero et al. J Hepatol, 2009
Belloni, PNAS 2009
Belloni, JCI 2012

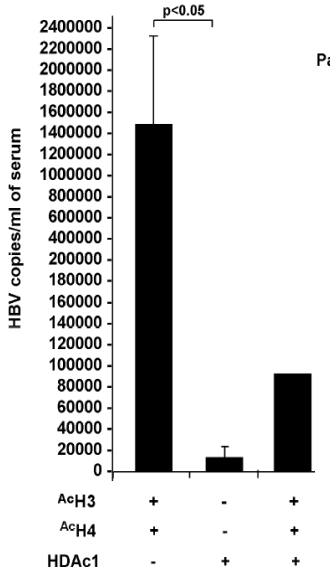
cccDNA acetylation parallels HBV replication *in vitro* and *in vivo*

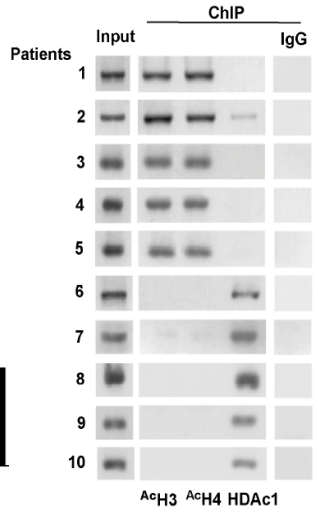
HuH7 cells replicating HBV



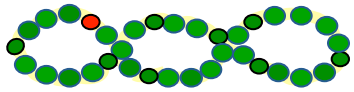


Liver biopsies of CHB patients



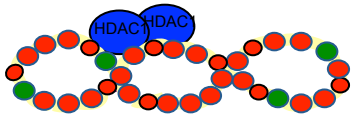


High Replication



Acetylated Histones

Low Replication

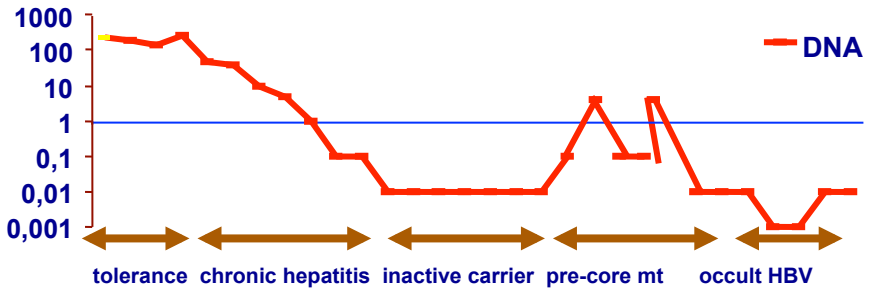


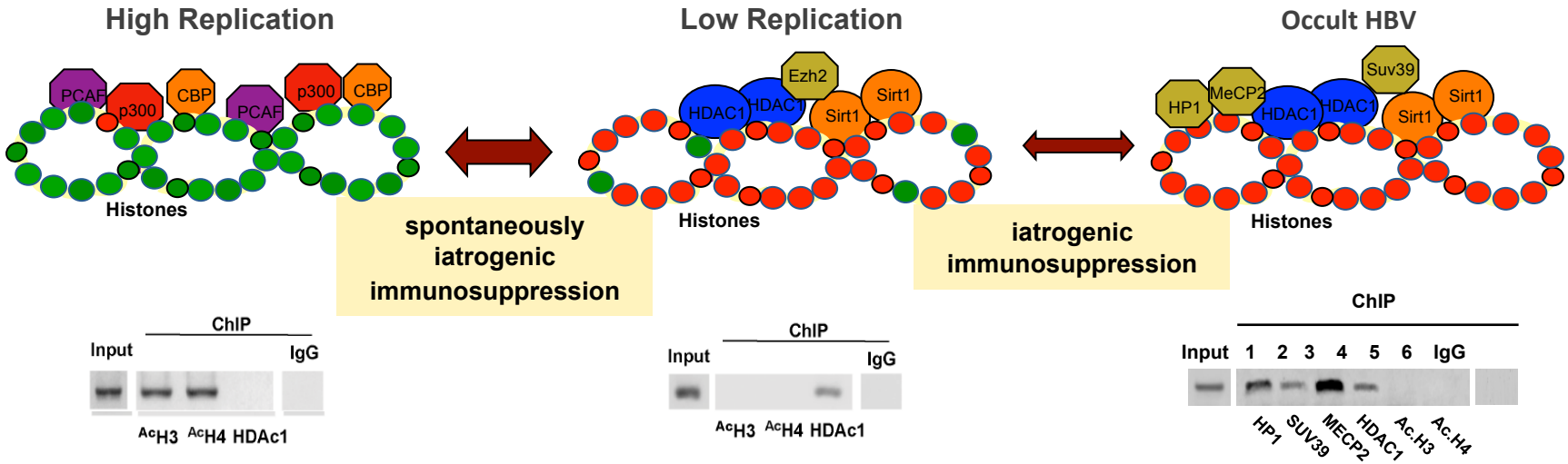
Hypoacetylated Histones

HBV replication is regulated by the acetylation status of cccDNA-bound H3/H4 histones

Pollicino, Gastroenterology 2006
Levrero, Hepatology 2009

cccDNA epigenetic status in patients



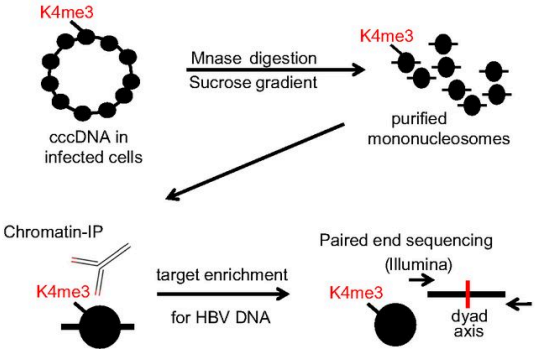


Low-replicative to latent infection

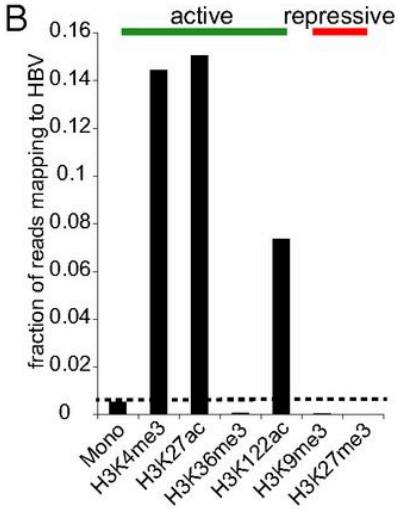
Epigenetic control

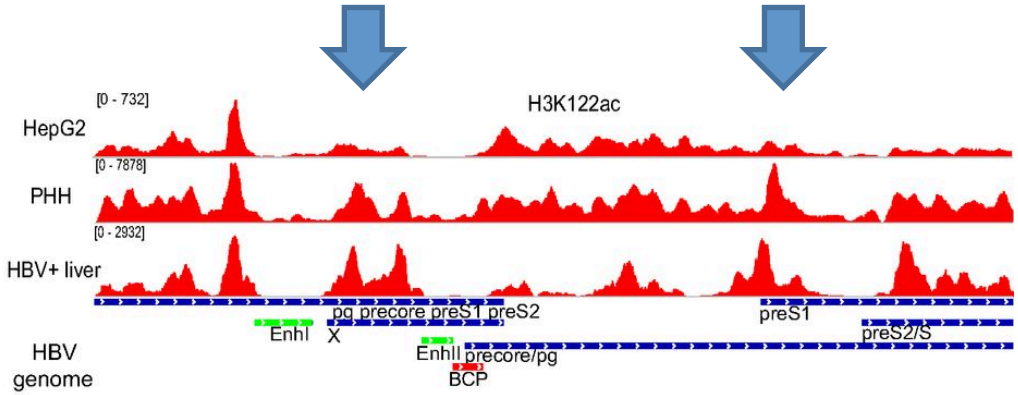
Pollicino et al., Gastroenterology 2006;
Belloni et al., HBV meeting 2006
Pollicino, unpublished

Mapping cccDNA nucleosomes and histones PTMs: ChIP-Seq



PTM	localisation
H3K4me3	TSS
H3K27ac	TSS, Enhancer
H3K36me3	gene body
H3K122ac	TSS, Enhancer
H3K9me3	heterochromatin
H3K27me3	TSS, intergenic





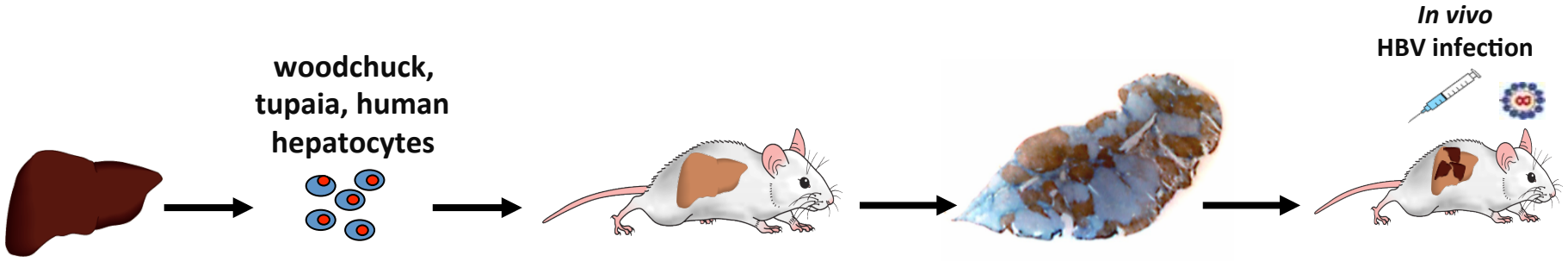
HBV chromatin shares some basic features with cellular chromatin

Different HBV regulatory regions shows specific histone PTMs profiles

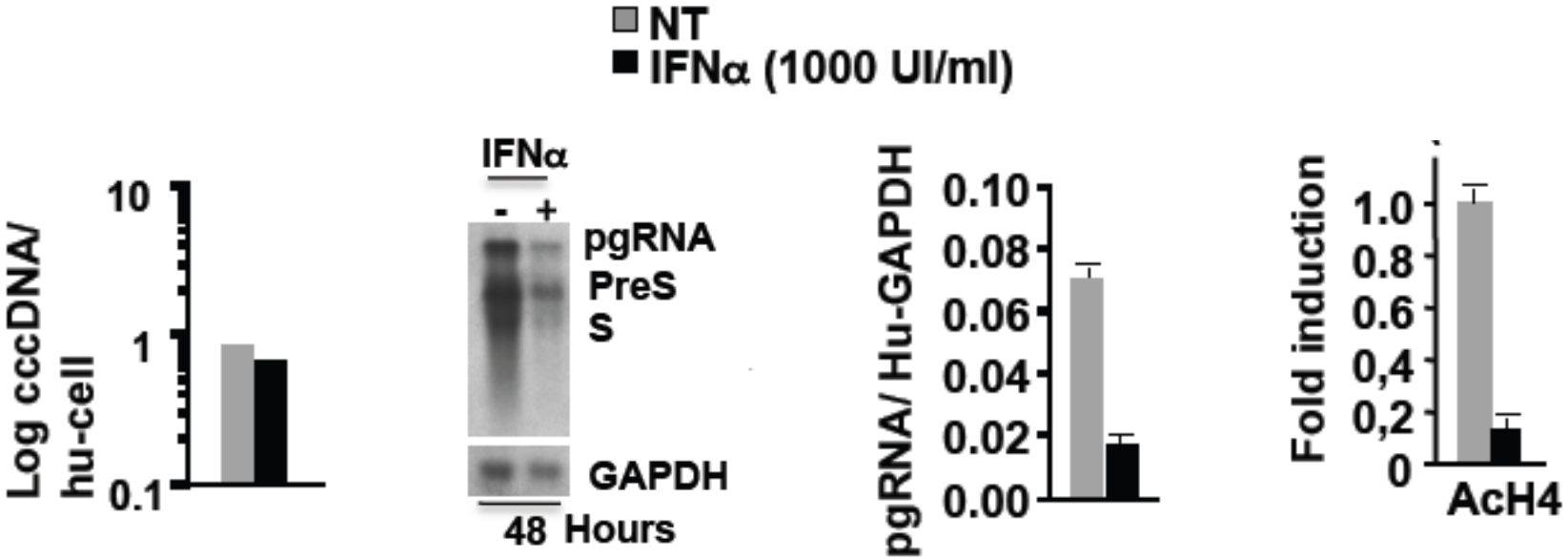
cccDNA molecules coming from infected HepG2-NTCP, PHH and human liver share a very similar, but not identical, histone PTMs profile

Tropberger, PNAS 2015

Effects of IFNα treatment on HBV replication and transcription in HuHep mice



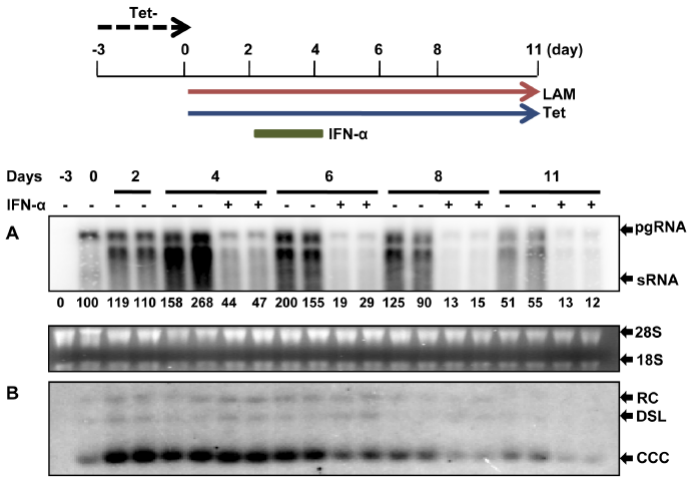
Petersen, PNAS 1998, Dandri, Hepatology 2001, 2002, 2008, J Hepatol 2005, Petersen, Nature Biotech.2008

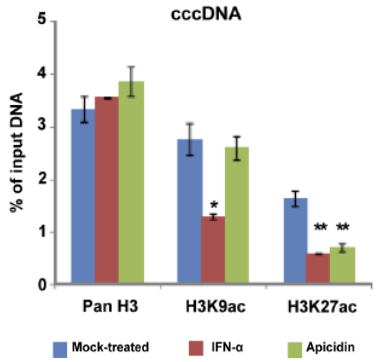


Belloni, JCI 2012

Alteration of cccDNA epigenetic modifications: IFNα

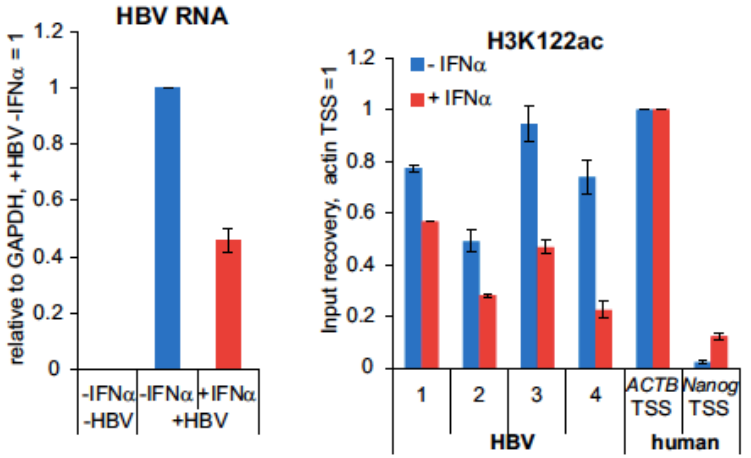
Dstet5 with Tet-on DHBV





Liu, PLosPath 2013

HBV-infected HepG2-NTCP

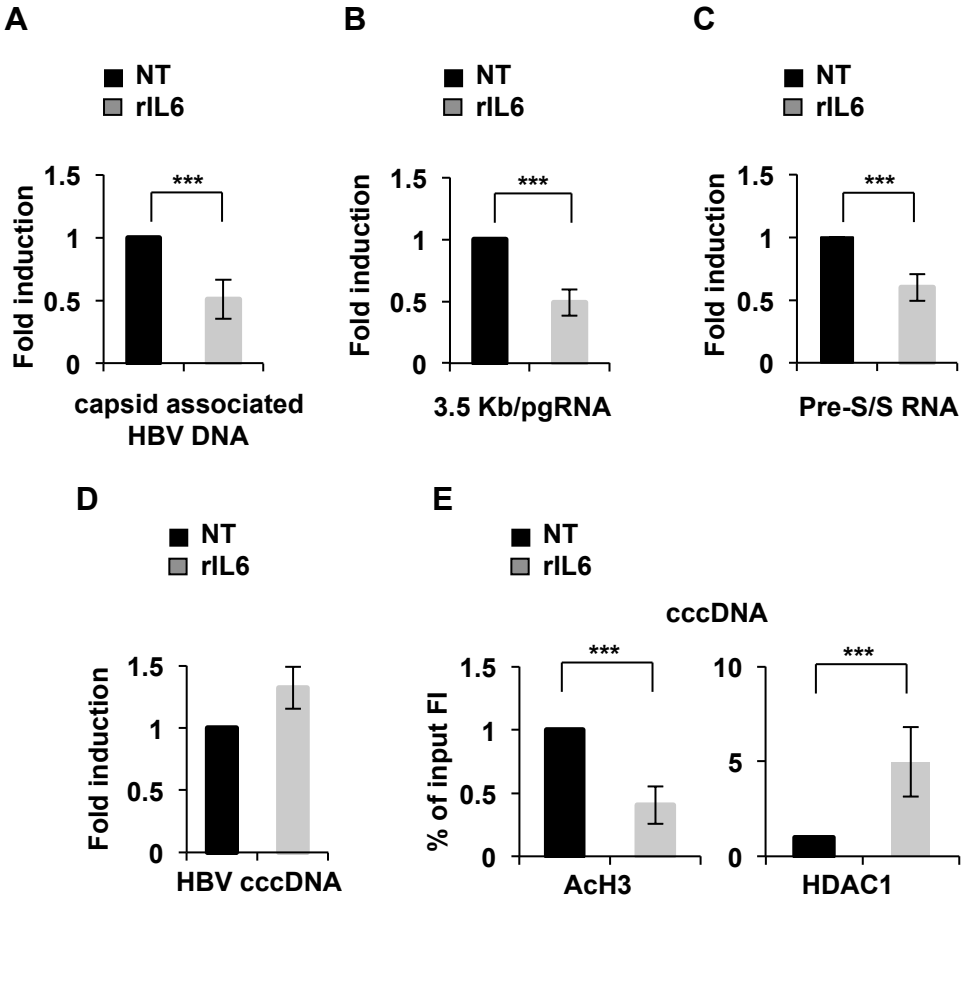


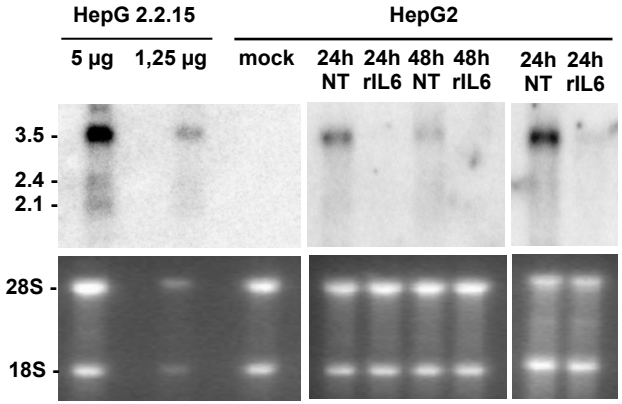
Tropberger, PNAS 2015

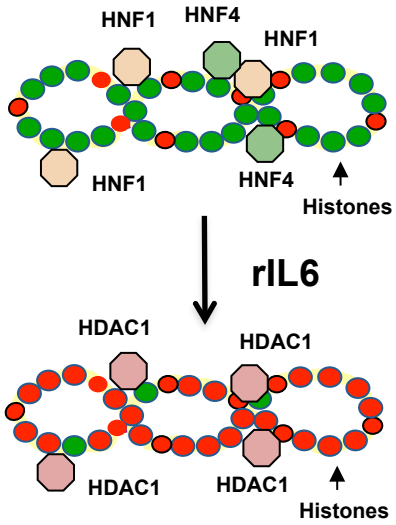
Alpha-IFN:

- **inhibits cccDNA transcription**
- **reduces the acetylation of cccDNA-bound histones**

Alteration of cccDNA epigenetic modifications: IL-6



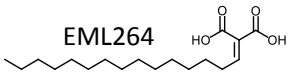




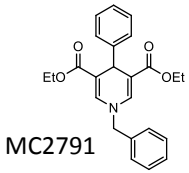
Hosel, Hepatology 2009
Palumbo, PlosOne 2015

Alteration of cccDNA epigenetic modifications: epigenetic small molecules

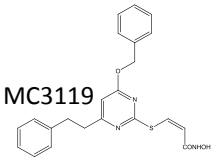
PCAF/p300 inhibitor

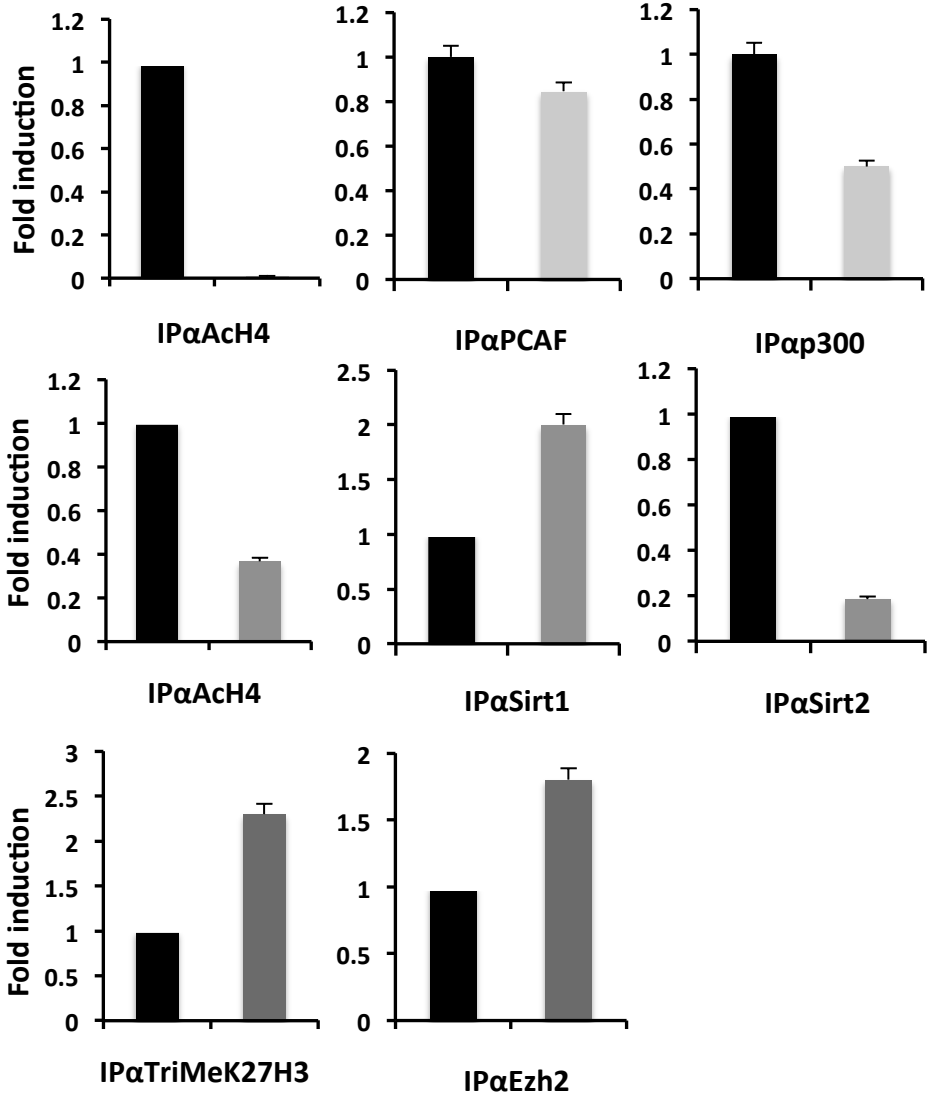


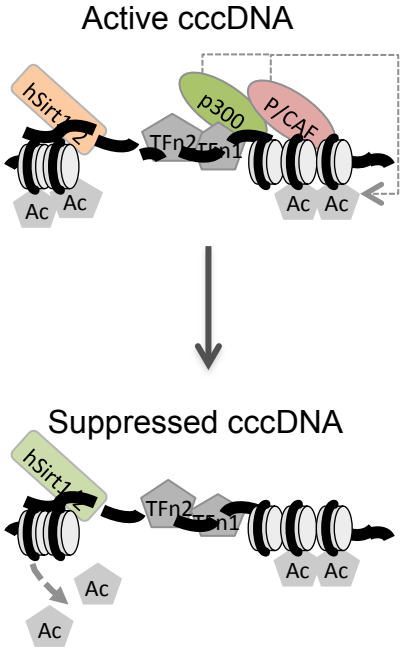
Sirt1/2 stimulator



JMD3 inhibitor







EMs modify chromatin remodelling enzymes recruitment onto HBV minichromosome

Palumbo ACS 2016

Alteration of cccDNA epigenetic modifications: epigenetic small molecules

PCAF/p300 inhibitor

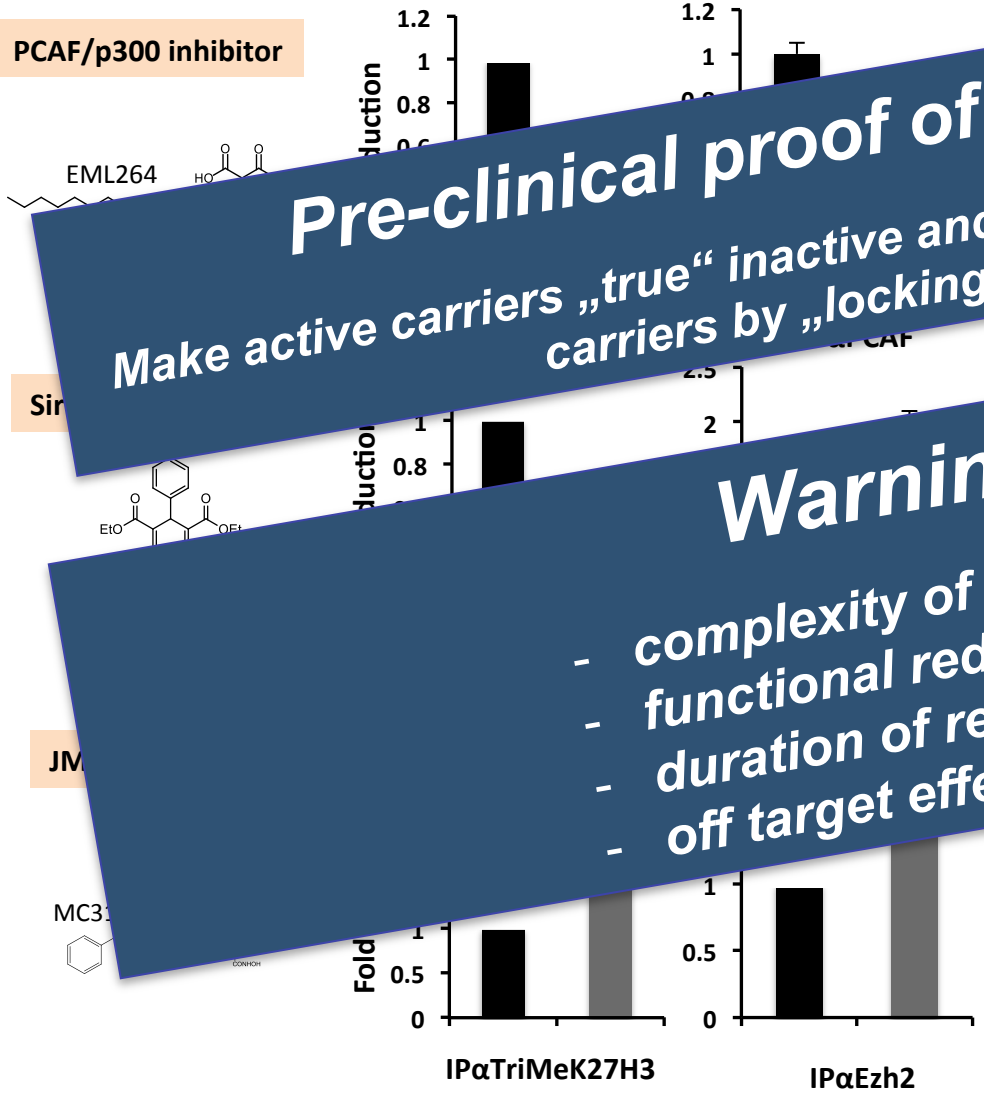
EML264

Pre-clinical proof of concept stage

Make active carriers „true“ inactive and, eventually, over time „occult“ carriers by „locking“ the cccDNA

Warnings

- complexity of responses
- functional redundancy
- duration of response
- off target effects



EMs modify chromatin remodelling enzymes recruitment onto HBV minichromosome

Palumbo ACS 2016

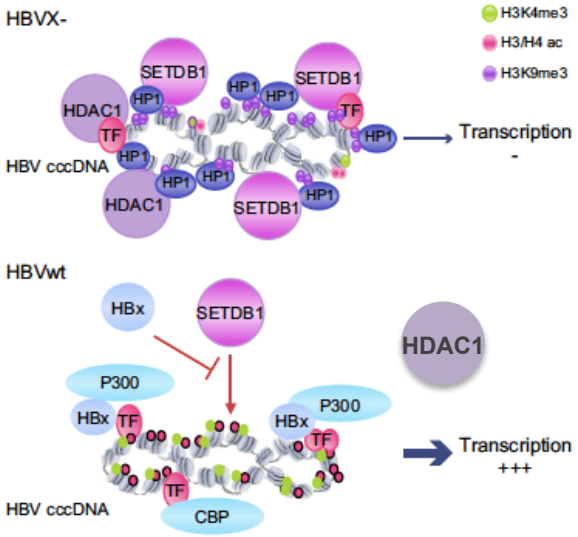
cccDNA transcriptional control

- cccDNA epigenetic modifications
- **Viral/host Transcription factors/cofactors associated to cccDNA**

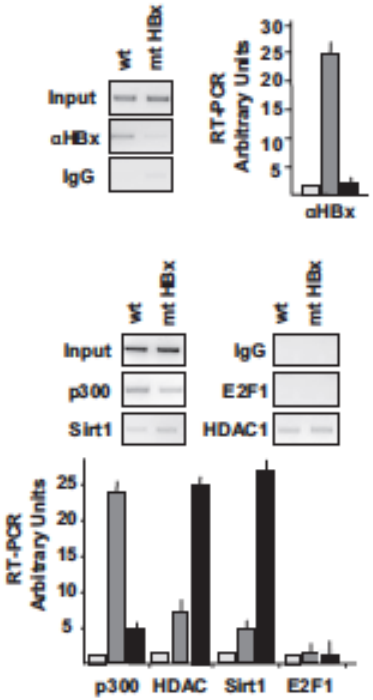
HBx protein impacts on cccDNA transcription

HBx binds to and is required for full cccDNA transcription and viral replication
(Belloni, PNAS 2009; Lucifora, JHepatol 2011)

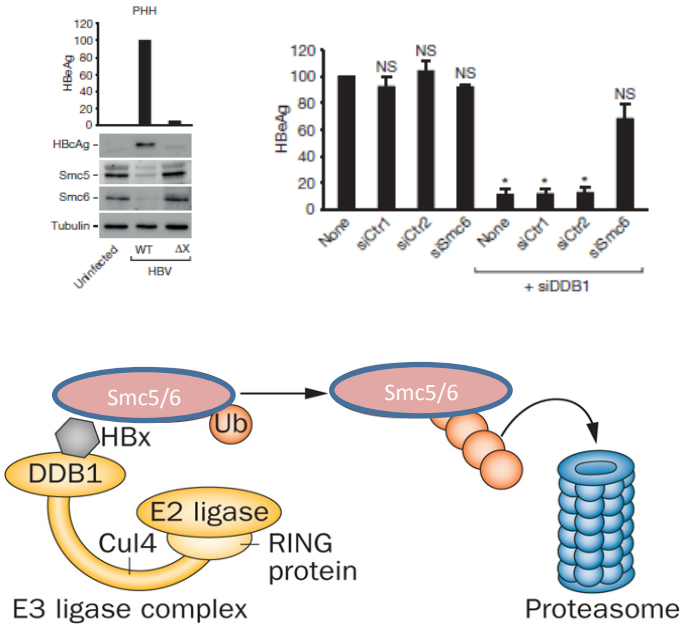
HBx prevents recruitment of negative regulators on cccDNA
(Belloni, 2009; Benhenda, 2013; Ducroux 2014, Rivière 2015, Decorsière, 2016)



Modified from Riviere, J Hepatol 2015



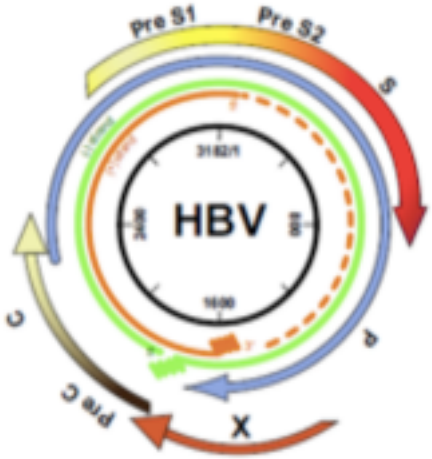
Belloni, PNAS 2009

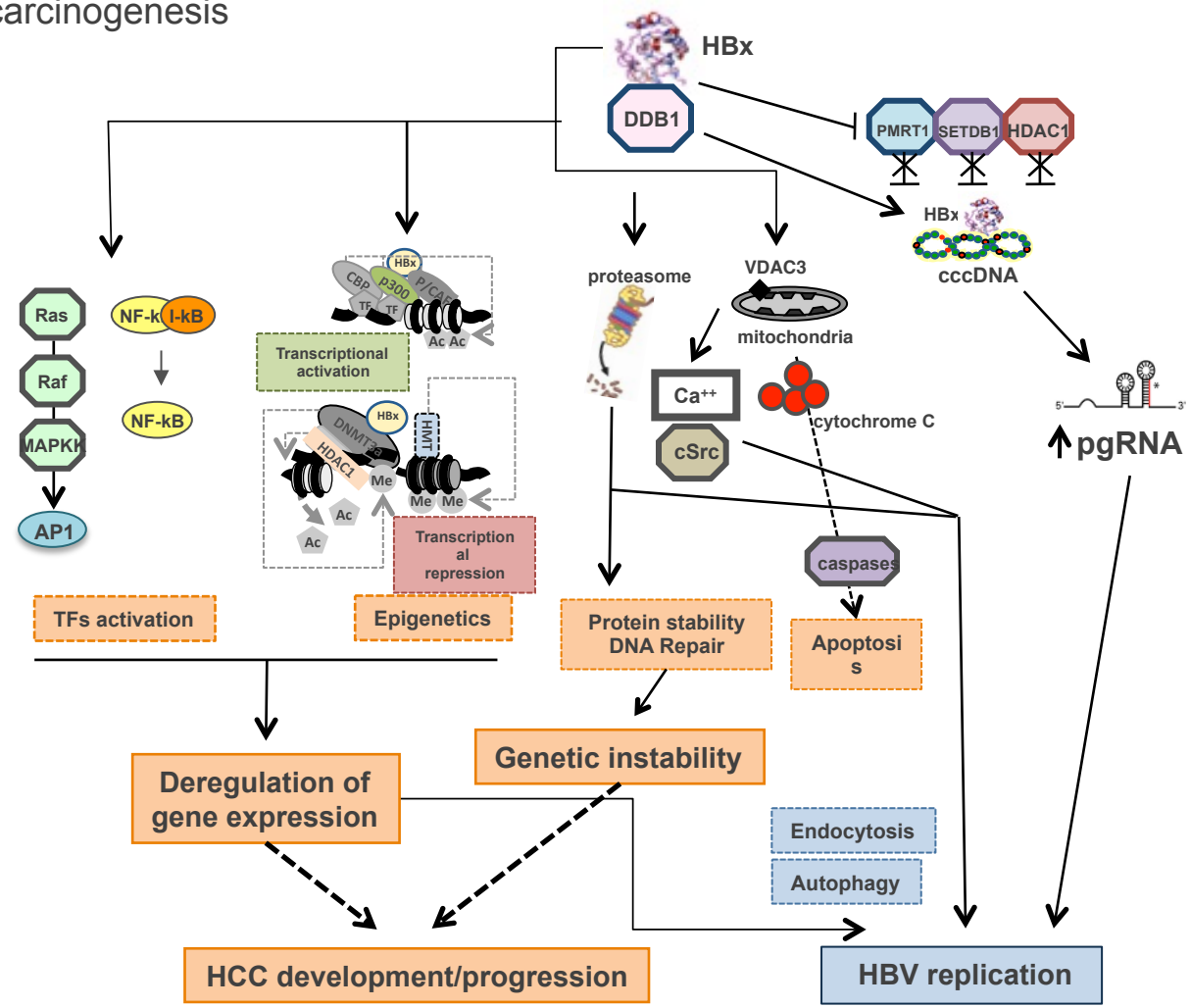


Decorsière, Nature 2016

HBx protein, HBV replication and HBV pathogenesis

- HBx binds to and is required for transcription from the viral cccDNA minichromosome and viral replication
- HBx binds to cellular promoters and modulates the epigenome by relocating chromatin regulators
- HBx contributes to hepato-carcinogenesis





Levrero & Zucman Rossi – J Hepatology 2016

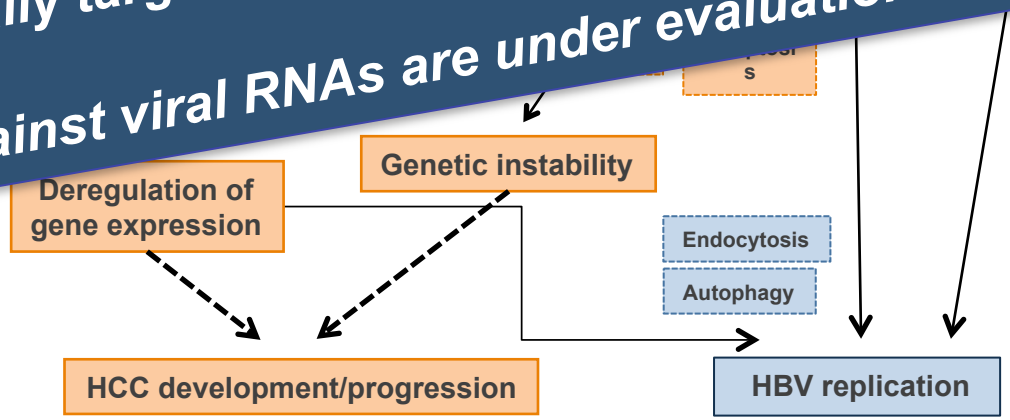
HBx protein, HBV replication and HBV pathogenesis

- HBx binds to and is required for transcription from the viral cccDNA minichromosome
- HBx binds to cellular promoters and modulates the epigenome
- HBx contributes to hepato-carcinogen

HBx
a very attractive target for HBV cure

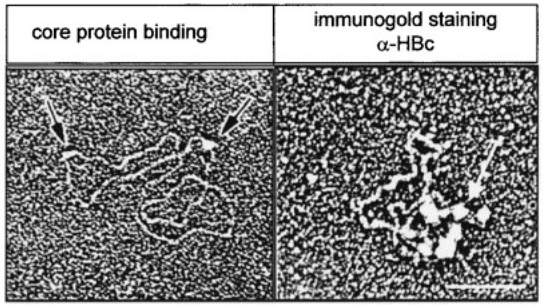
but

- **Difficult task: HBx has no enzymatic activity**
- **Try to specifically target HBx-host factors interactions?**
- **siRNA against viral RNAs are under evaluation**



Levrero & Zucman Rossi – J Hepatology 2016

HBV Core binds to cccDNA and arranges nucleosome spacing

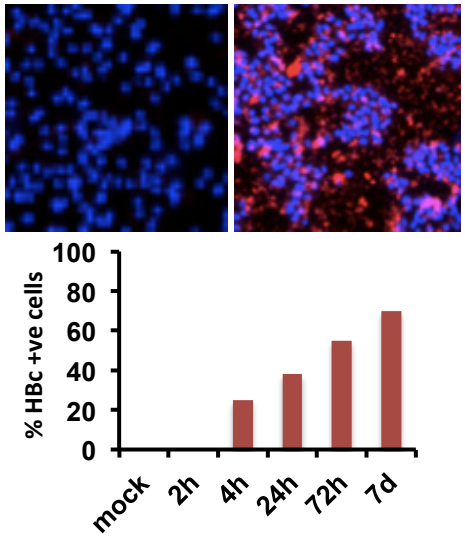


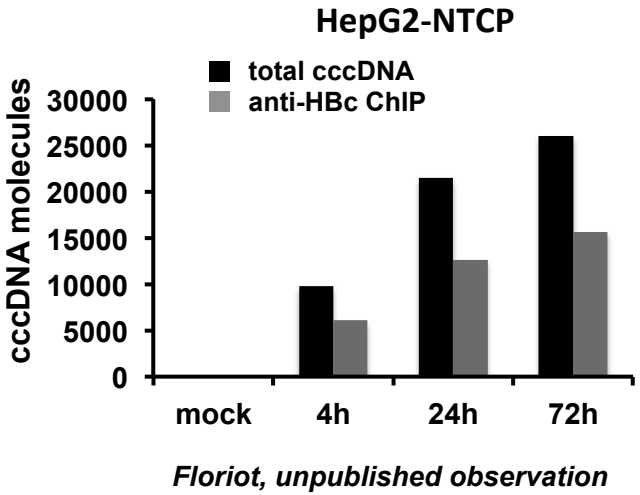
Hatton et al, J Virol 1992; Bock et al, JMB 2001

157-SPRRRT-162

8 times more specific for dsDNA than pdsDNA

SPXX family motif = bind to DNA minor groove
(like histones and RNA Pol II)





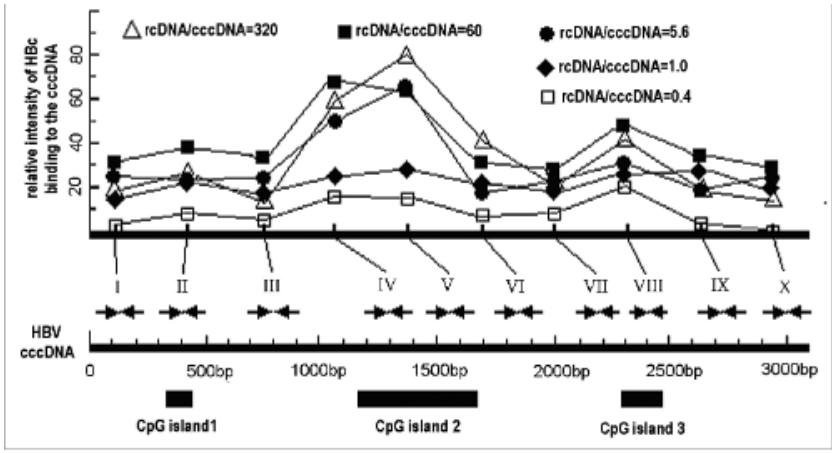
Floriot, unpublished observation

HBc is bound to cccDNA EARLY POST-INFECTION and is associated to positive histone PTMs

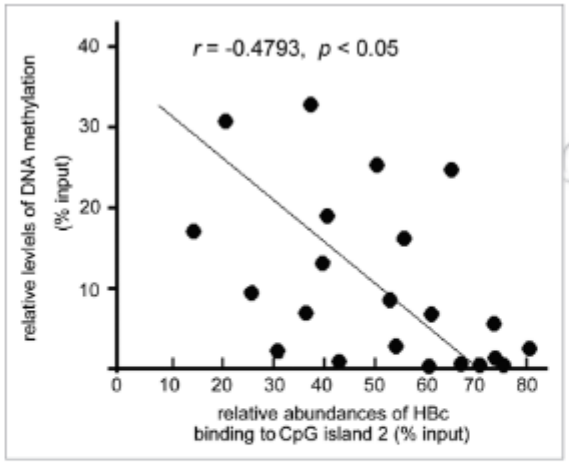
HBx-independent

Role of cccDNA-bound HBc

HBc binds to the CpG islands of HBV cccDNA and promotes an epigenetic permissive state

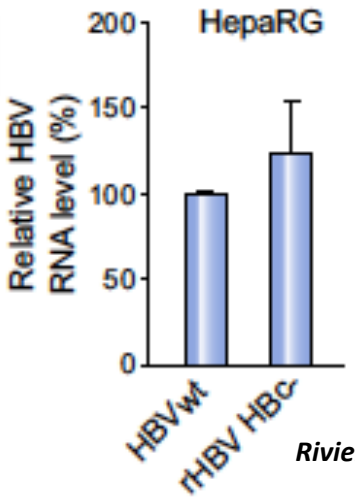


22 liver biopsies CHB patients

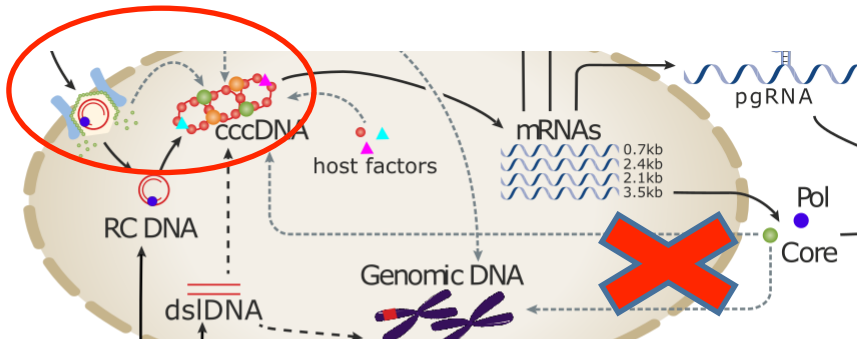


Guo, Epigenetics 2011

ΔHBc virus

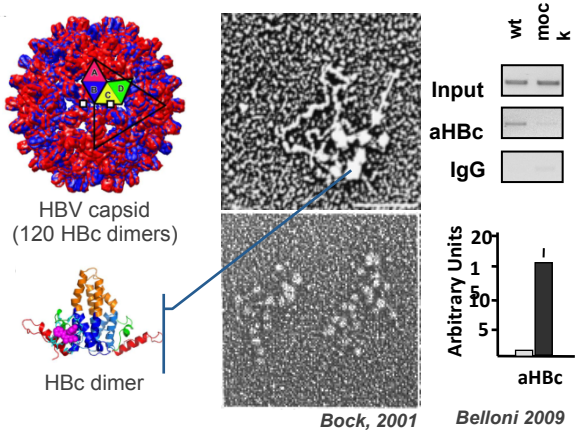


Riviere, J Hepatol 2015



Role of incoming HBc protein

Core inhibitors drugs

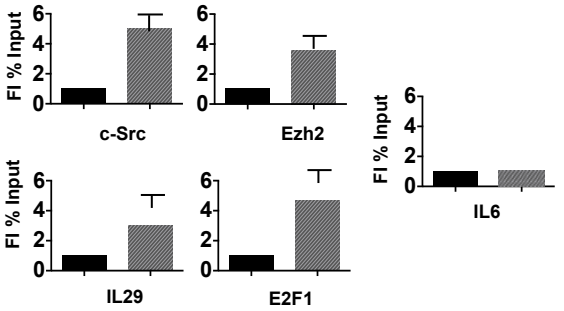


Bock, 2001

Belloni 2009

- HBc binds the cccDNA and modifies cccDNA nucleosome spacing
- HBc binds to cellular promoters and regulates gene expression
- HBc binds to (and represses) the IFN-b, IL-29 and OAS1 cellular promoters

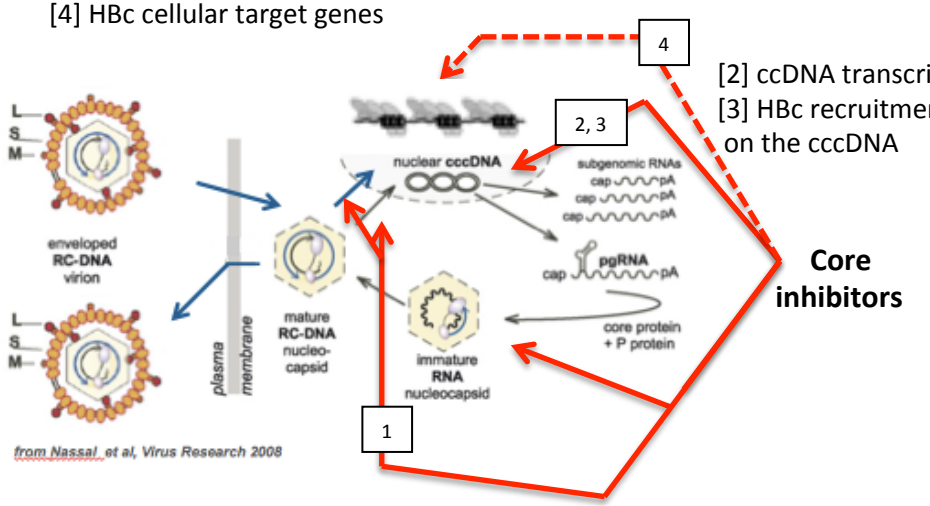
1. Bock T. et al., JMB 2001; 2. Belloni L. et al. PNAS USA 2009; 3. Guo, BMC genomics, 2013; 4. Durantel D. et al., AASLD 2013; Lupacchini and Floriot et al. unpublished



Lupacchini and Floriot, (unpublished)

Core inhibitors are the first "viral specific" compounds that potentially target cccDNA

[4] HBc cellular target genes

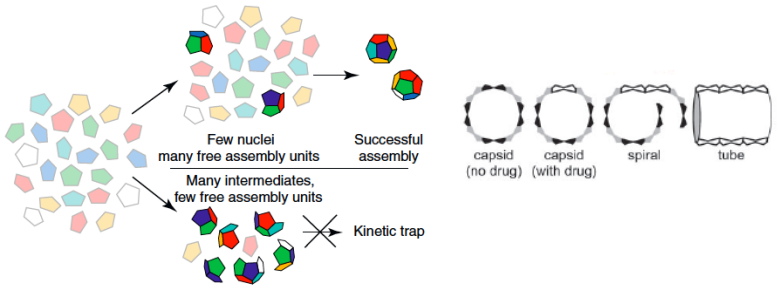


[2] ccDNA transcription
[3] HBc recruitment on the cccDNA

Core inhibitors

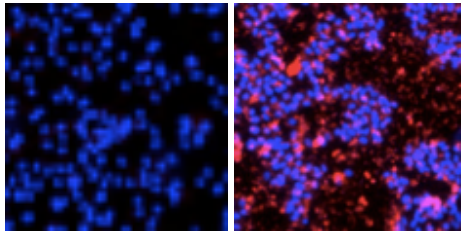
[1] cccDNA accumulation
(Rc-DNA delivery and/or core particles recycling)

- Phenylpropenamide derivatives (AT61, AT130) [Gilead]
- Heteroaryldihydropyrimidines (HAP-1 and Bay 41-4109)
- Sulfamoylbenzamide derivatives (DVR-23, DVR-56 and Novira Therapeutics NVR-1221, NVR 3-378)
- BCM-599 [2-amino-N-(2,6-dychloropyridin-3-yl) acetamide family]
- Iso-thiafludine (pg-RNA packaging)

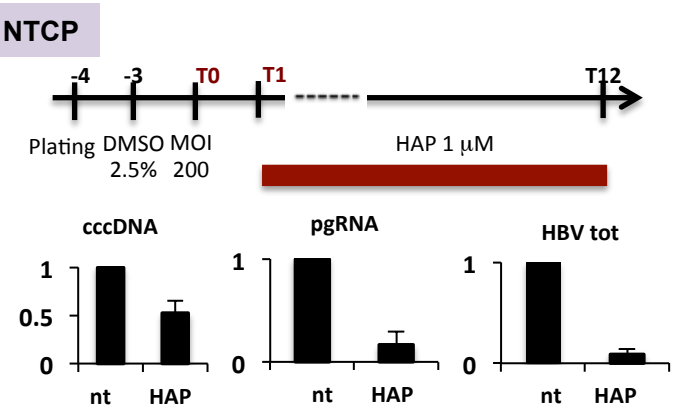


HAP12 affects cccDNA in HepG2 NTCP cells and PHHs

a.



b.



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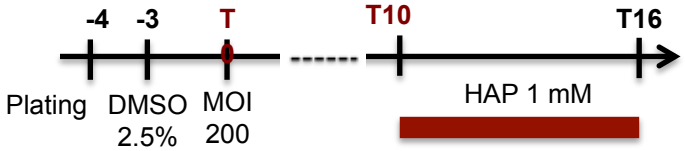

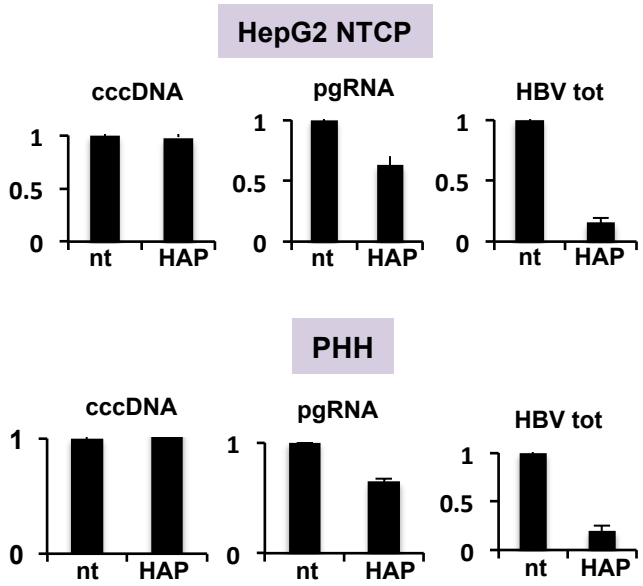

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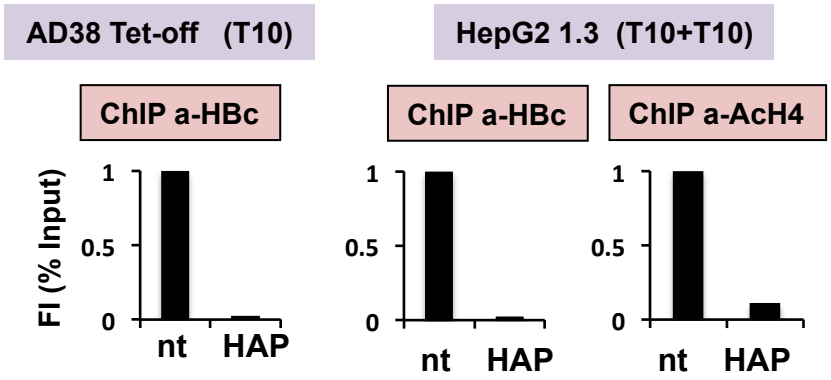

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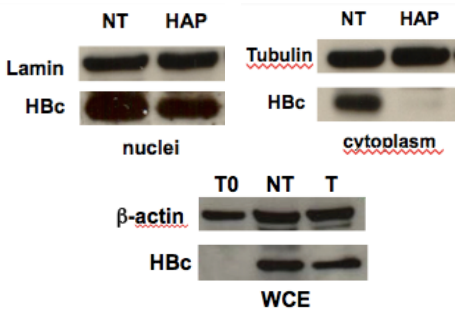

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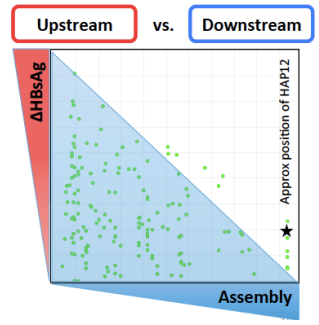
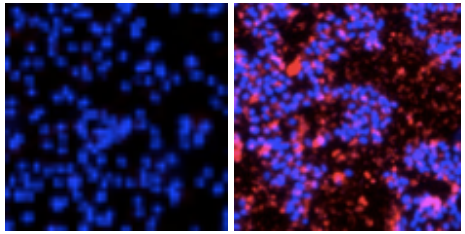


Zlotnick, HBV cure workshop 2015

HAP12 affects cccDNA in HepG2 NTCP cells and PHHs

a.



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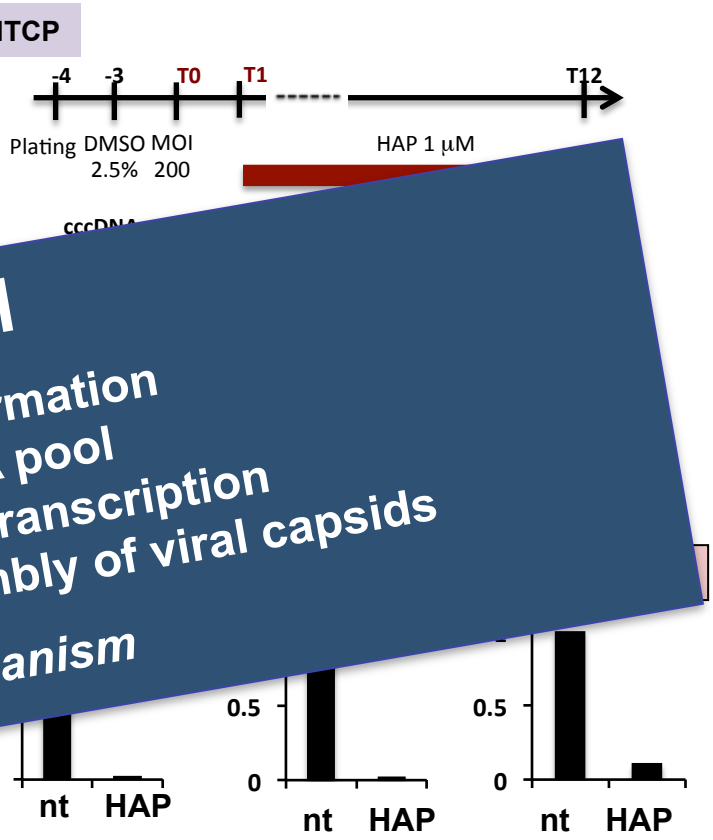
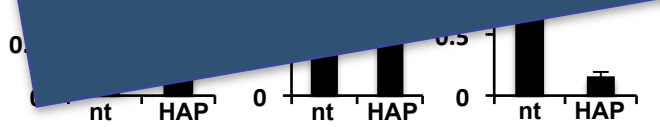


Hap12 CpAM

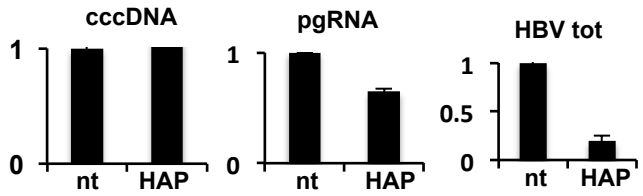
- blocks new cccDNA formation
- reduces the cccDNA pool
- repress residual cccDNA transcription
- leads to an inappropriate assembly of viral capsids

virus specific mechanism

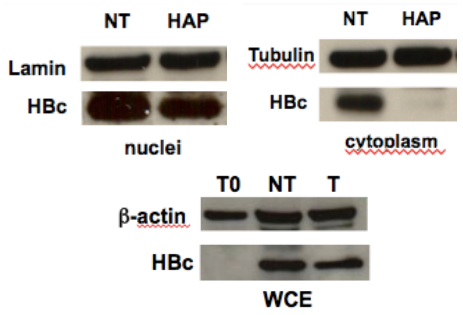
c.



PHH



e.



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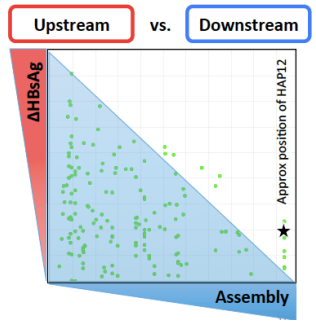


Zlotnick, HBV cure workshop 2015

HAP12 affects cccDNA in HepG2 NTCP cells and PHHs

Core inhibitors are the first "viral specific" compounds capable to target the cccDNA

Preclinical and Early Clinical Profile of NVR 3-778, a Potential First-In-Class HBV Core Inhibitor

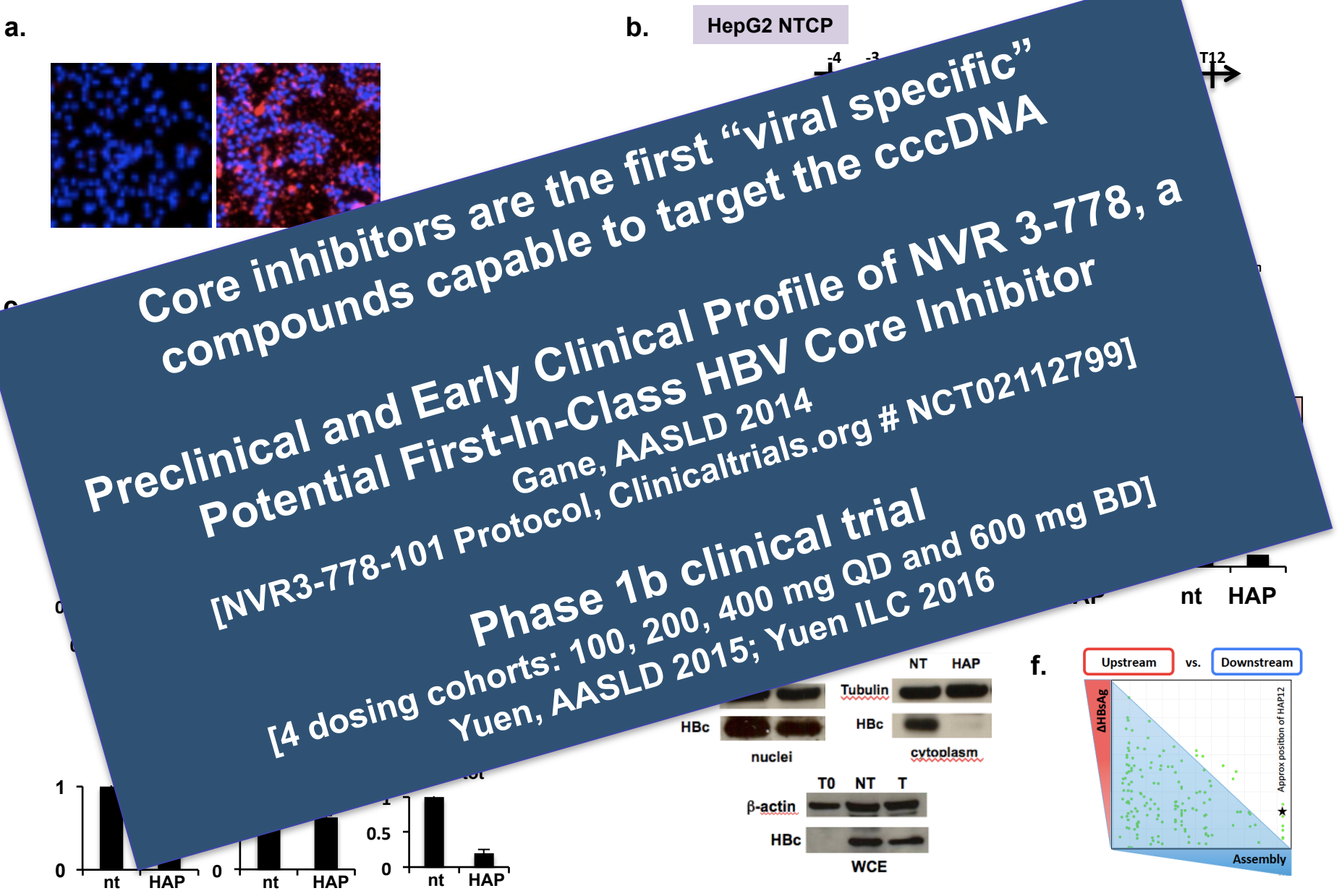
Gane, AASLD 2014

[NVR3-778-101 Protocol, Clinicaltrials.org # NCT02112799]

Phase 1b clinical trial

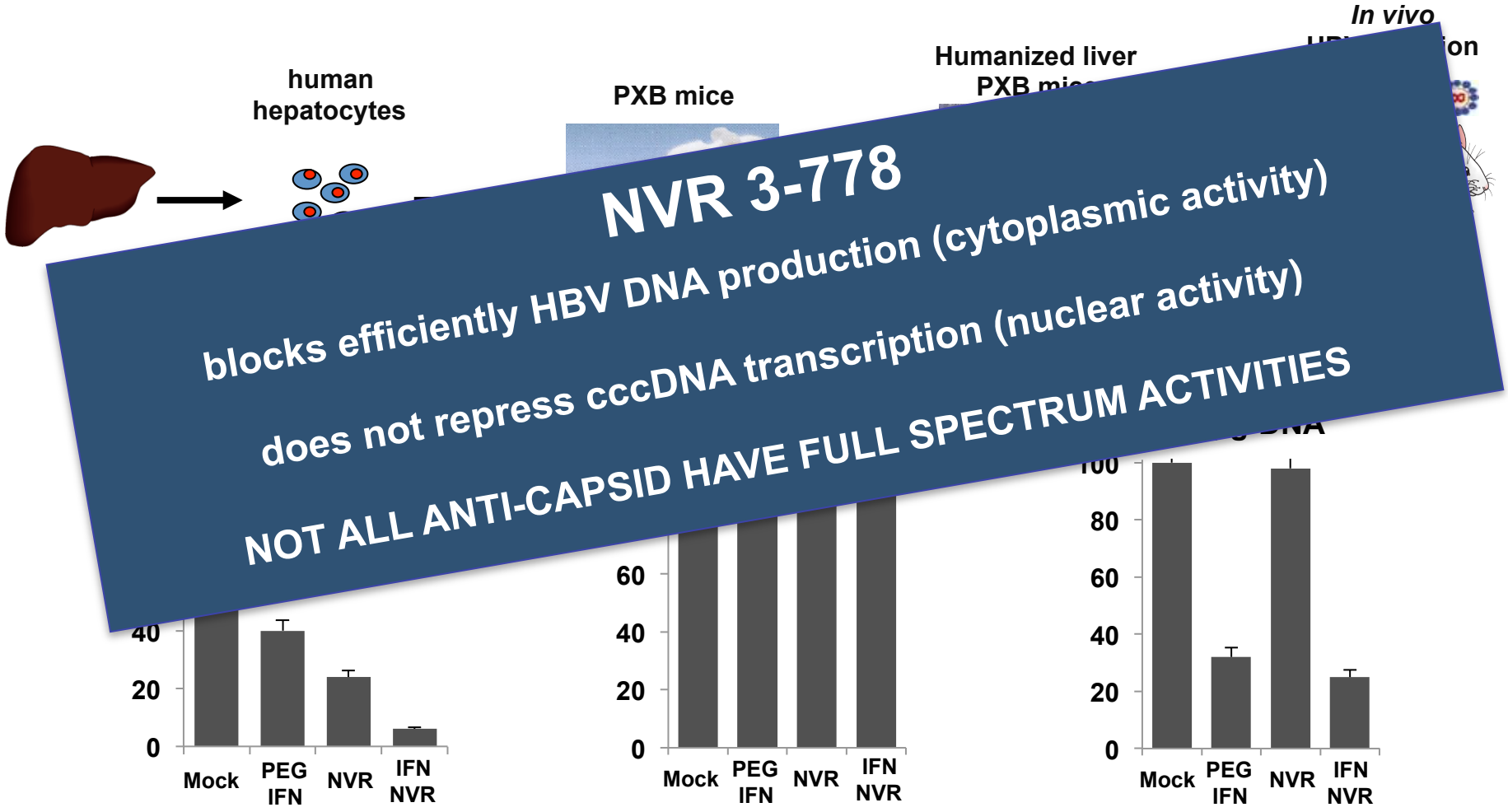
[4 dosing cohorts: 100, 200, 400 mg QD and 600 mg BD]

Yuen, AASLD 2015; Yuen ILC 2016



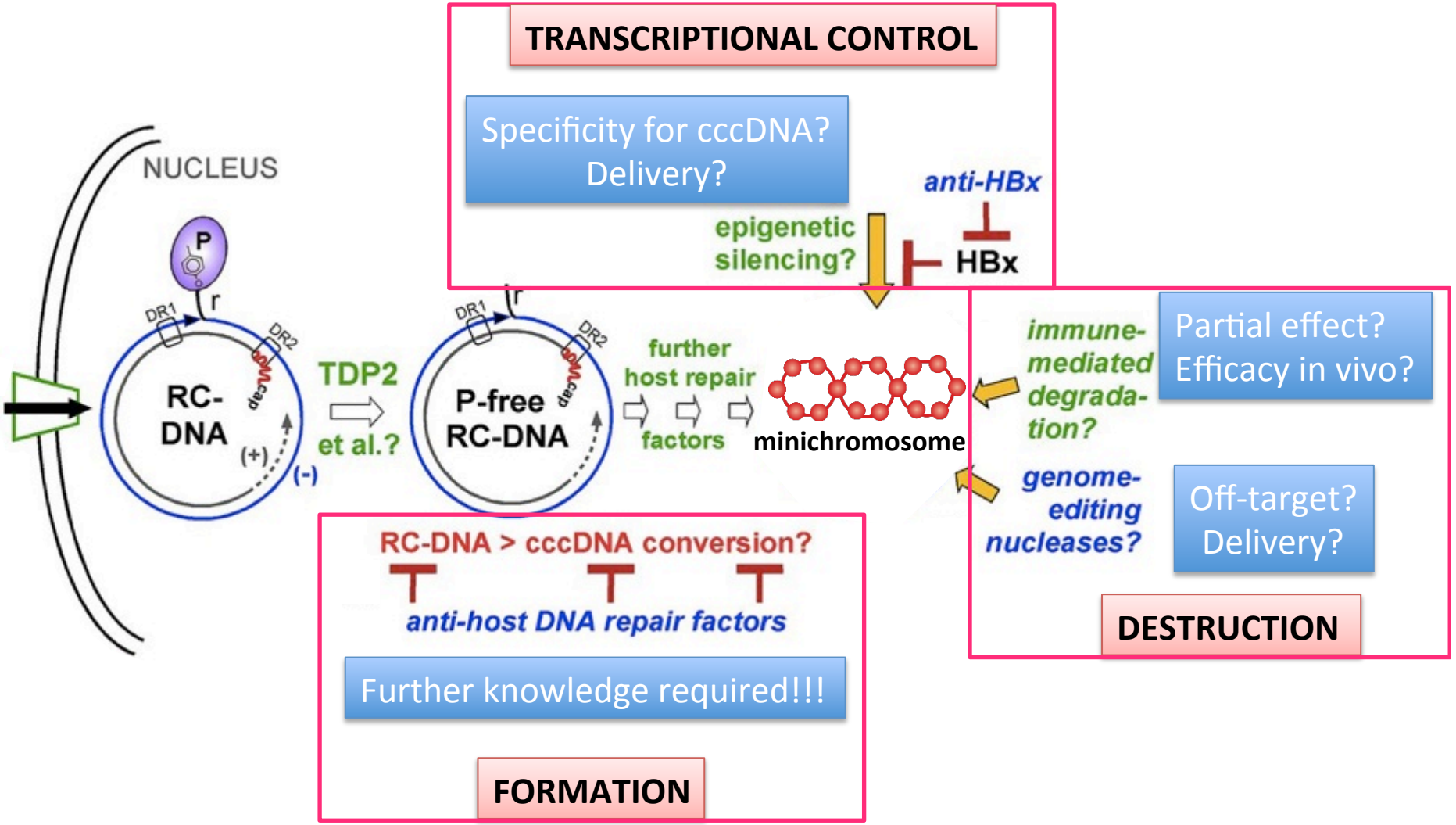
Zlotnick, HBV cure workshop 2015

Preclinical characterization of the antiviral activity of NVR 3-778, a Potential First-In-Class HBV Core Inhibitor, *in vivo*



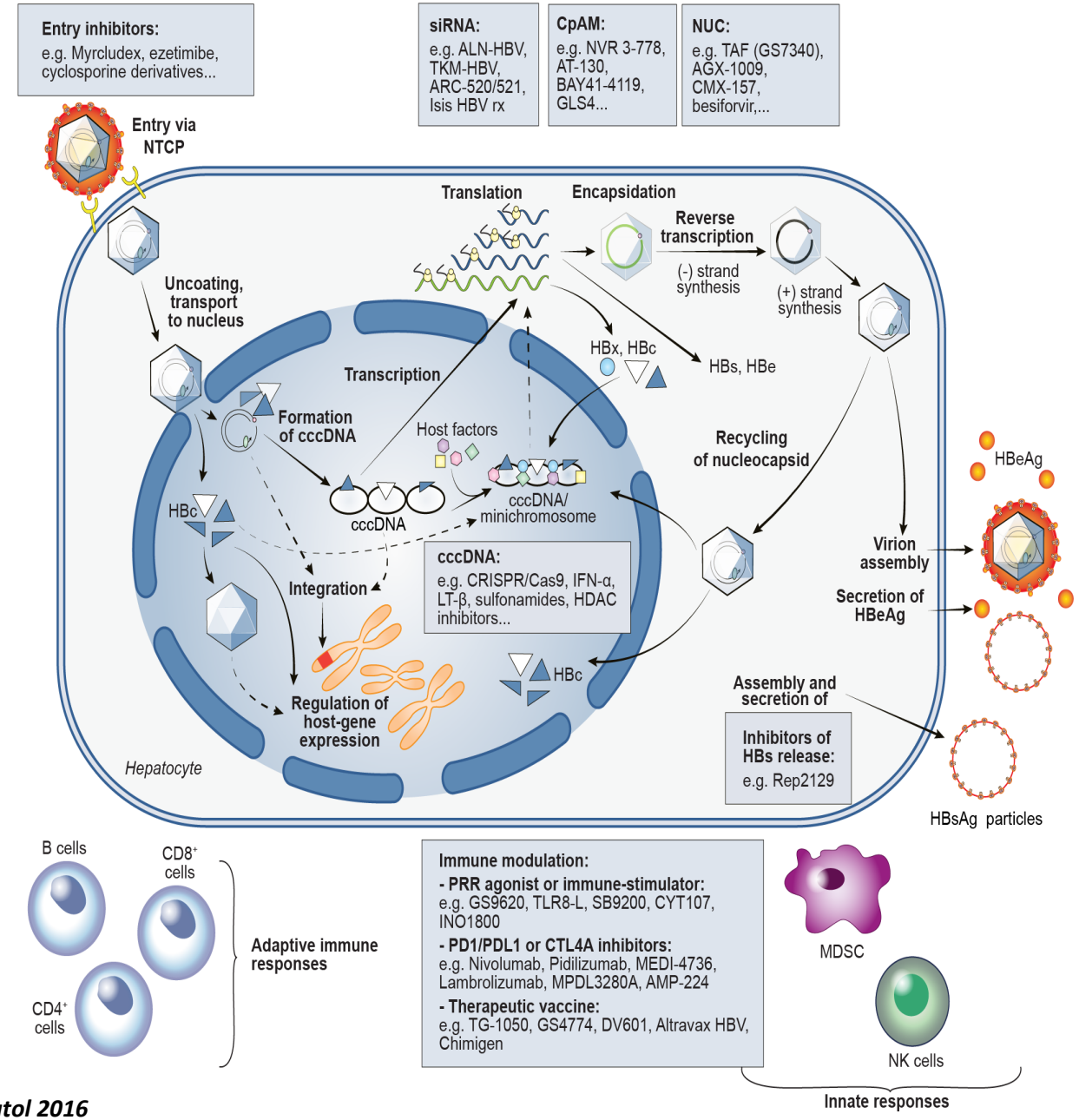
Klumpp et al. EASL 2015

Conclusions



Modified from Nassal, Gut 2015

HBV cure - A highly dynamic drug discovery effort



Durantel & Zoulim, J Hepatol 2016

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Collaborations:

Adam Zlotnick
Lichun Li
Indiana University, Bloomington, USA

Uri Lopatin
Assembly Pharmaceuticals

Maura Dandri
Lena Alweiss
University Medical Hospital Hamburg

Ulrike Protzer
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Agence autonome de l'Inserm

"Save the date"

Third ANRS "HBV cure" Workshop
HBV pathobiology and target discovery

Scientific coordination: Fabien Zoulim

Tuesday, May 31st, 2016
Union internationale des chemins de fer (UIC)
16, rue Jean Rey - 75015 PARIS

HBV cure 2014: Zeisel, M. B. *et al.* Towards an HBV cure: state-of-the-art and unresolved questions-report of the ANRS workshop on HBV cure. *Gut*, doi:10.1136/gutjnl-2014-308943 (2015).

HBV cure 2015: http://www.anrs-hbvcure2015.com/