Department of Microbiology and Molecular Genetics
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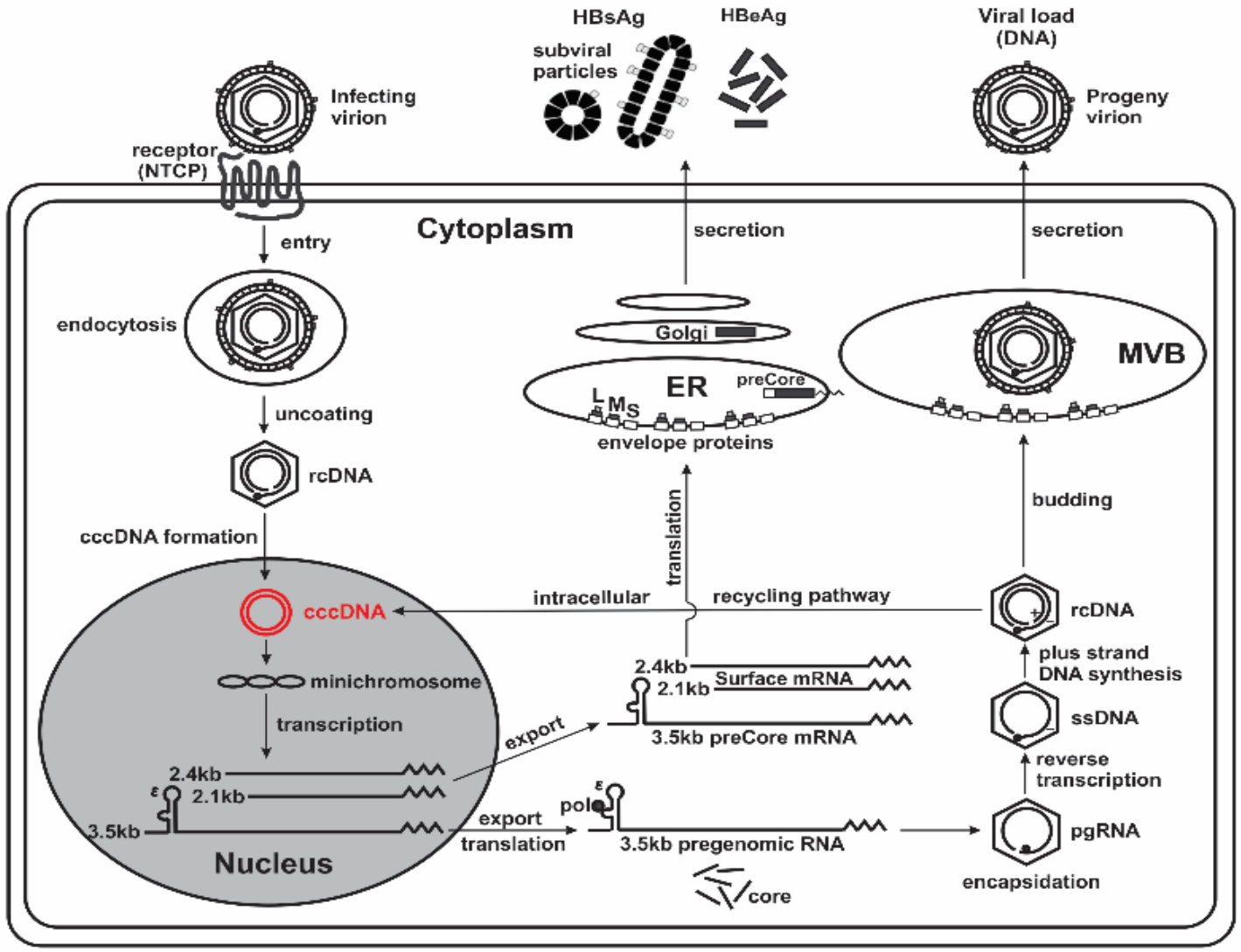
Pittsburgh Liver Research Center
University of Pittsburgh

ICE-HBV International Coalition to Eliminate HBV
HBV Cure Symposium 2021.09.30

Combinations of direct acting antivirals to decrease the cccDNA pool

Haitao Guo

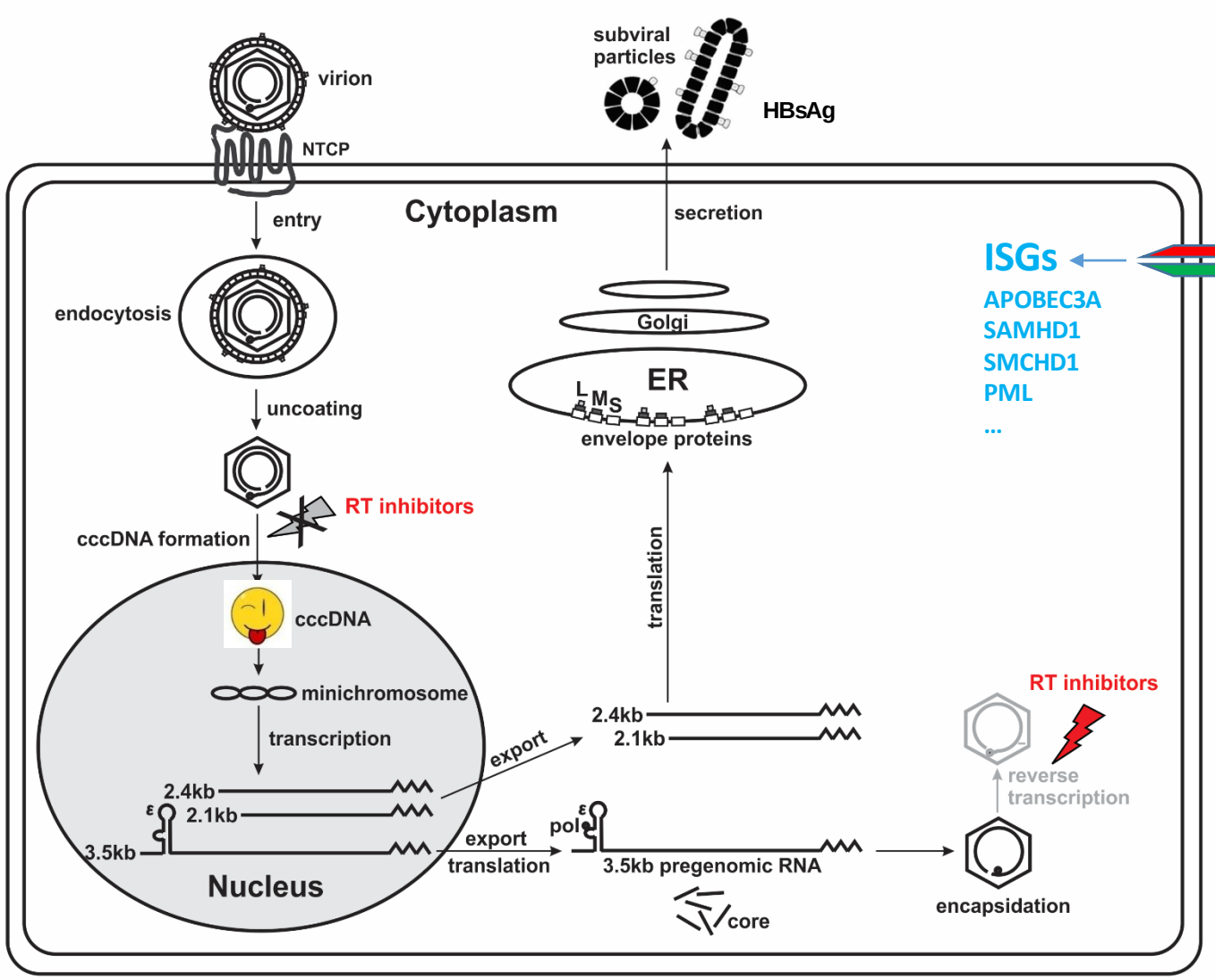
HBV life cycle centers on cccDNA



cccDNA basics

- cccDNA is the first viral product made by HBV upon infection.
- cccDNA is an episomal form of HBV DNA genome that serves as transcription template.
- cccDNA does not replicate, it is only made through conversion of HBV genomic RC DNA (de novo infection and intracellular recycling).
- cccDNA exists in low copy number (1-50 copies/cell).
- cccDNA is stable and may survive mitosis.
- cccDNA plays a key role in viral persistence, viral reactivation after treatment withdrawal, and drug resistance.
- cccDNA is not, or only indirectly, targeted by current antivirals.

cccDNA is refractory to IFN or NUC treatment



ISGs

APOBEC3A
SAMHD1
SMCHD1
PML
...

IFN-α

Pleiotropic mechanism of action
Limited efficacy
Side effects

NUCs

Potent replication inhibitor
Less effect on antigenemia
Do not directly inhibit cccDNA metabolism or transcription

cccDNA longevity

cccDNA has a long half-life in vivo (30-50 days in duck and woodchuck, ~6 mos in human) under Nuc treatment *(Addison and Tyrrell, 2002; Zhu and Mason 2004)*

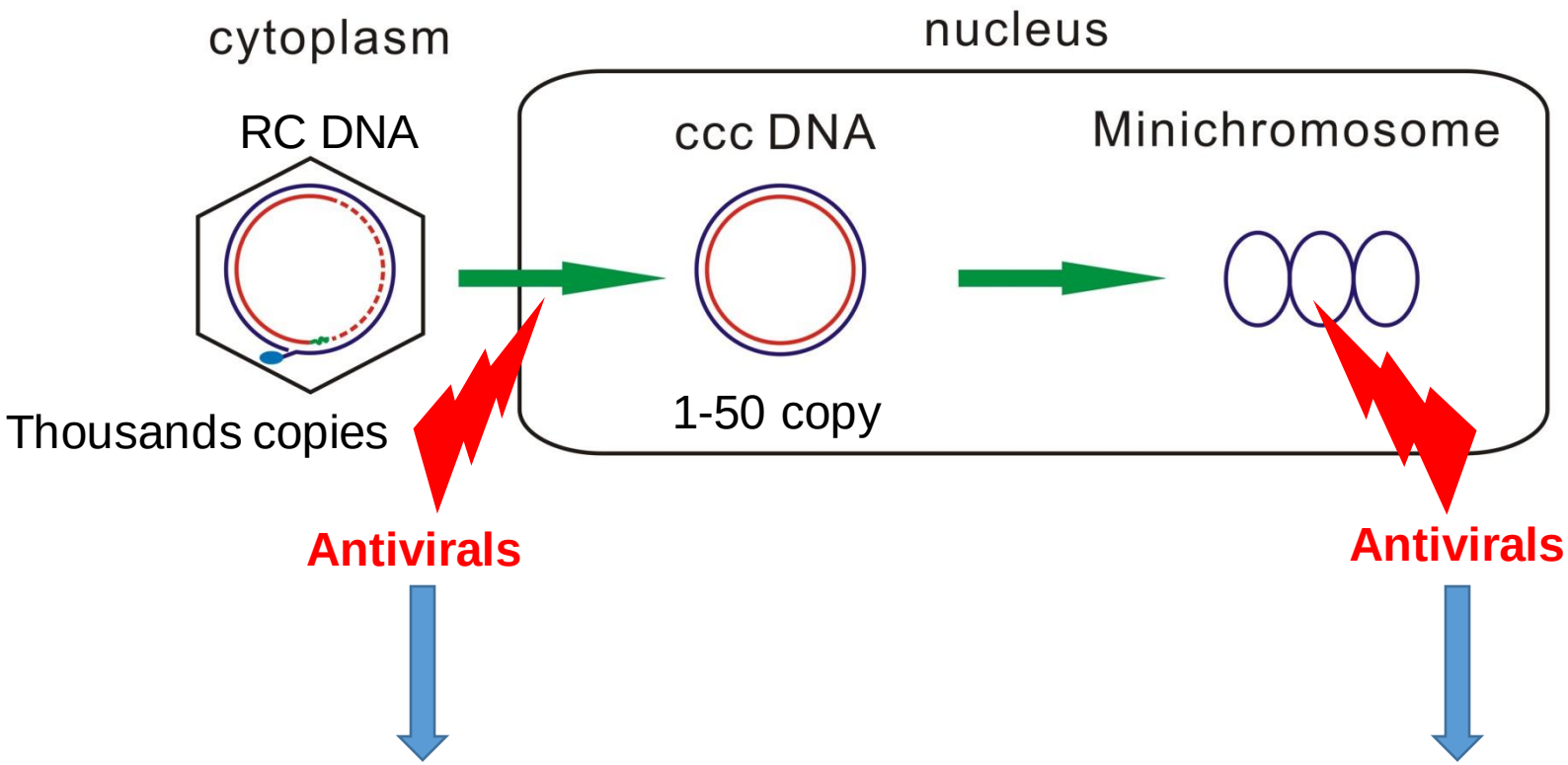
A complete loss of cccDNA solely by hepatocyte proliferation cannot be achieved in an immunodeficient mouse model without blocking virus spread *(Allweiss and Dandri, 2017)*

A kinetics study of cccDNA decay in CHB patients with long-term Nuc (ADV) treatment suggest that the level of cccDNA decreases less than 1 log in 48 weeks, and it would take 14.5 years to completely clear the intrahepatic cccDNA in a CHB patient. This is partly due to the incomplete inhibition of cccDNA replenishment by Nucs, even with potent drugs such as entecavir and tenofovir. *(Werle-Lapostolle and Zoulim, 2004; Boyd and Zoulim, 2016)*

By monitoring the dynamic turnover of Nuc-resistant mutants on serum HBV RNA (a marker for cccDNA), the half-life of cccDNA pool in LAM- or LdT-treated CHB patients was calculated as several months. *(Huang et al, Hepatology, 2021)*

cccDNA longevity is maintained by inherent stability and constant replenishment.
Both arms should be blocked in order to decrease and ultimately eliminate the cccDNA pool.

Strategies for pharmacological intervention of cccDNA

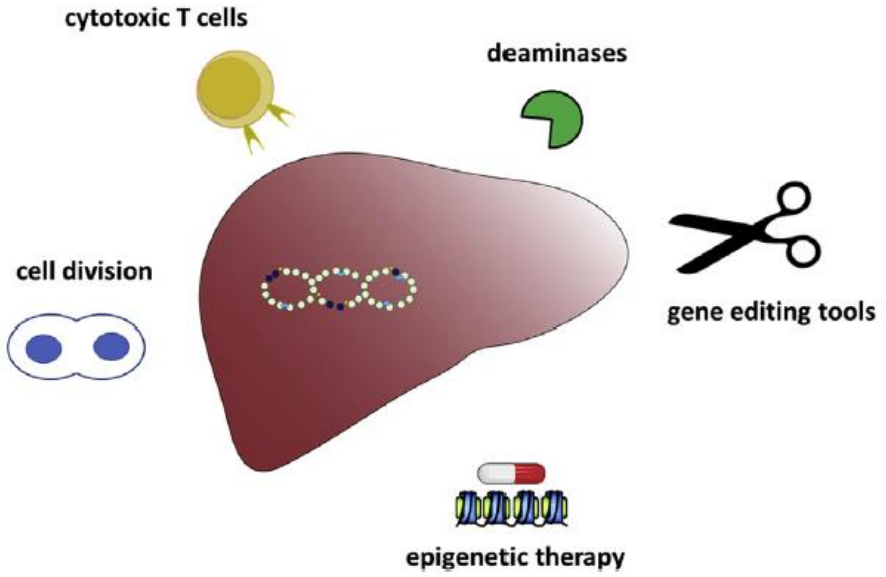


Direct antiviral agents that may directly or indirectly target cccDNA metabolism

1. Targeting cccDNA formation
   1) de novo cccDNA synthesis: neutralizing antibodies, entry inhibitors, nuclear import blockers…
   2) intracellular recycling:
      a: DNA replication inhibitors that reduce rcDNA production (pgRNA degrader, nucleocapsid assembly inhibitors, reverse transcriptase inhibitors, RNase H inhibitors…)
      b: Inhibitors targeting the production of cccDNA intermediates (DP-rcDNA, CM-rcDNA…)
      c: rcDNA nuclear import and uncoating inhibitors.

2. Targeting cccDNA stability
   1) DNA sequence targeted antivirals: deaminases, designer nucleases (ZFN, TALEN, CRISPR/Cas9), anti-sense oligos…
   2) cytokines-induced DNA deaminases (A3A/B, AID…)

3. Targeting cccDNA transcription
   1) HBx inhibitors
   2) Liver-enriched transcription factors
   3) Epigenetic modifiers (cytokines, chemicals, CRISPR/dCas9…)



(Xia and Guo, 2020)

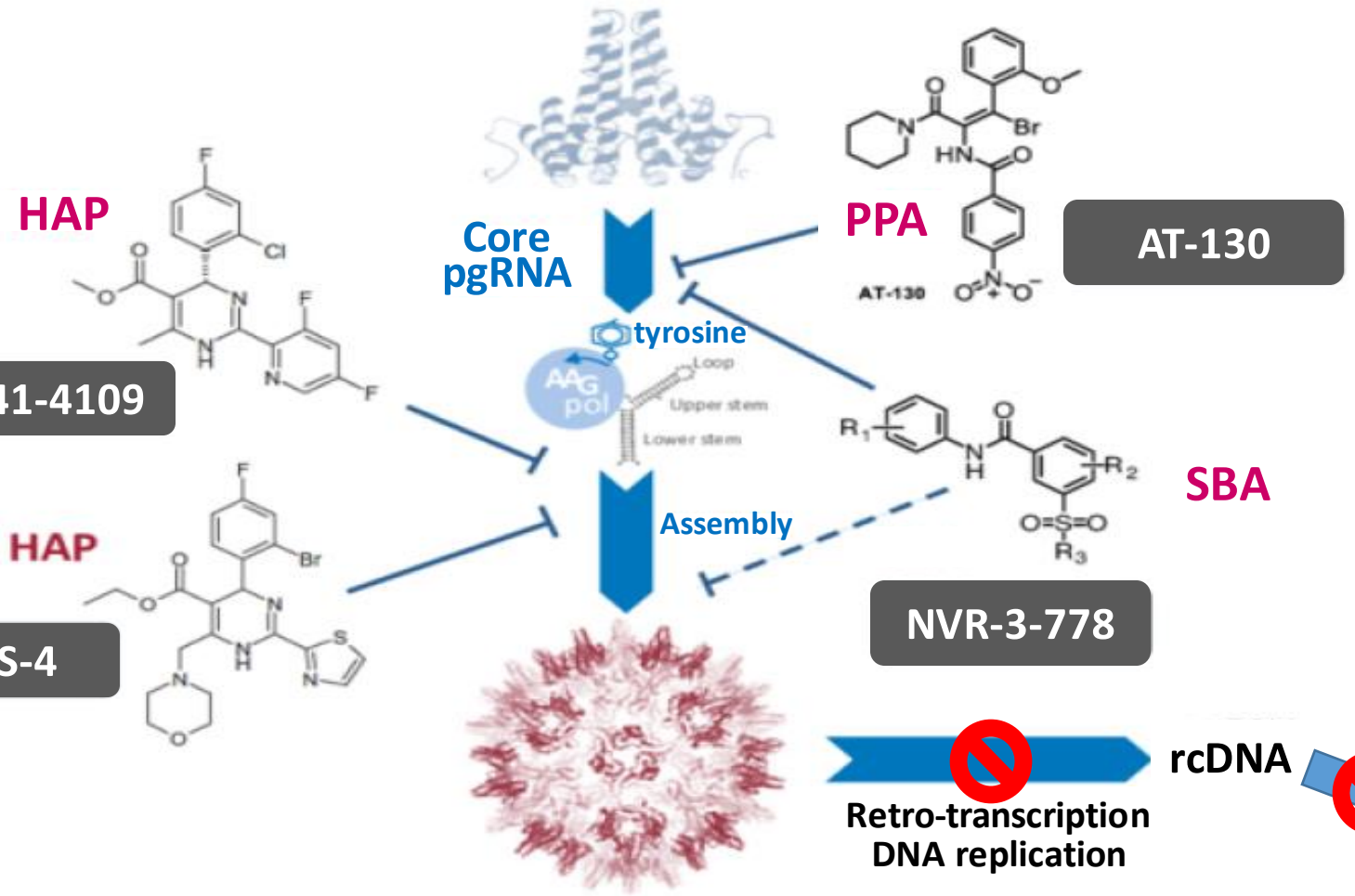
CpAMs: Capsid assembly/pgRNA encapsidation inhibitors

Class I

Forms aberrant non-capsid polymers for degradation

Class II

Forms empty capsid devoid of pgRNA/rcDNA

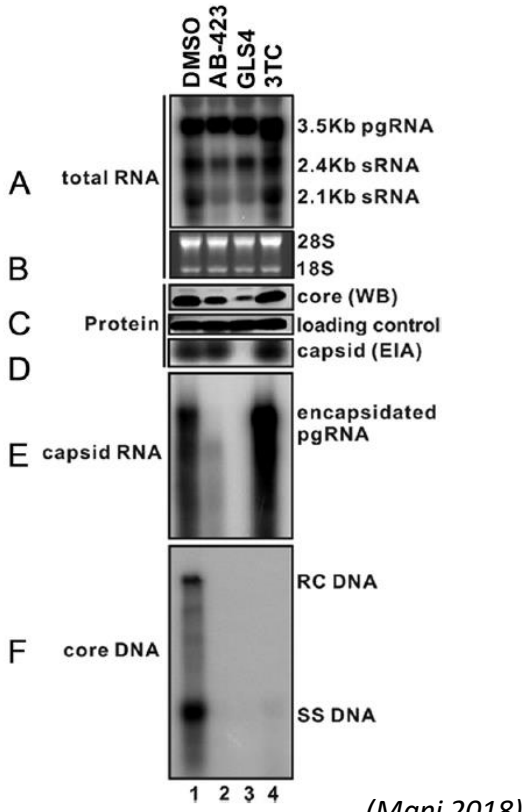


HAP: Heteroaryldihydropyrimidines; | **SBA:** Sulfamoylbenzamides; | **PPA:** phenylpropenamides

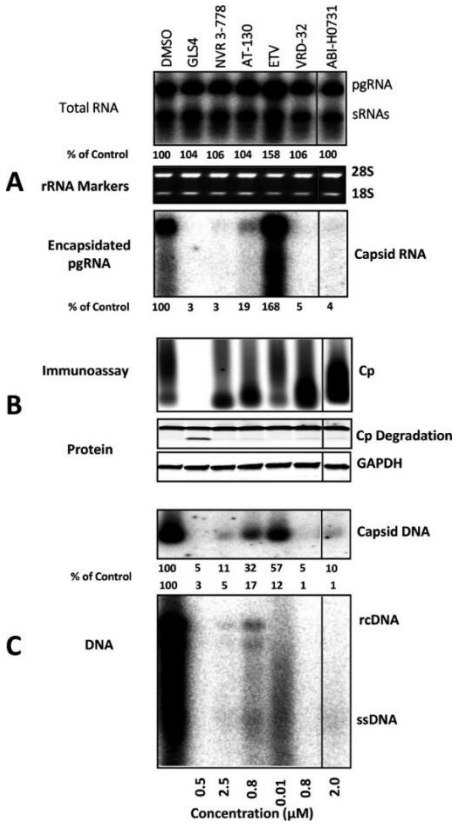
Mani Nagraj, 2017 AASLD #958 *Xia Y, et al. Gastroenterology 2019;156:311–24*

CpAM inhibits cccDNA formation via a dual mechanism

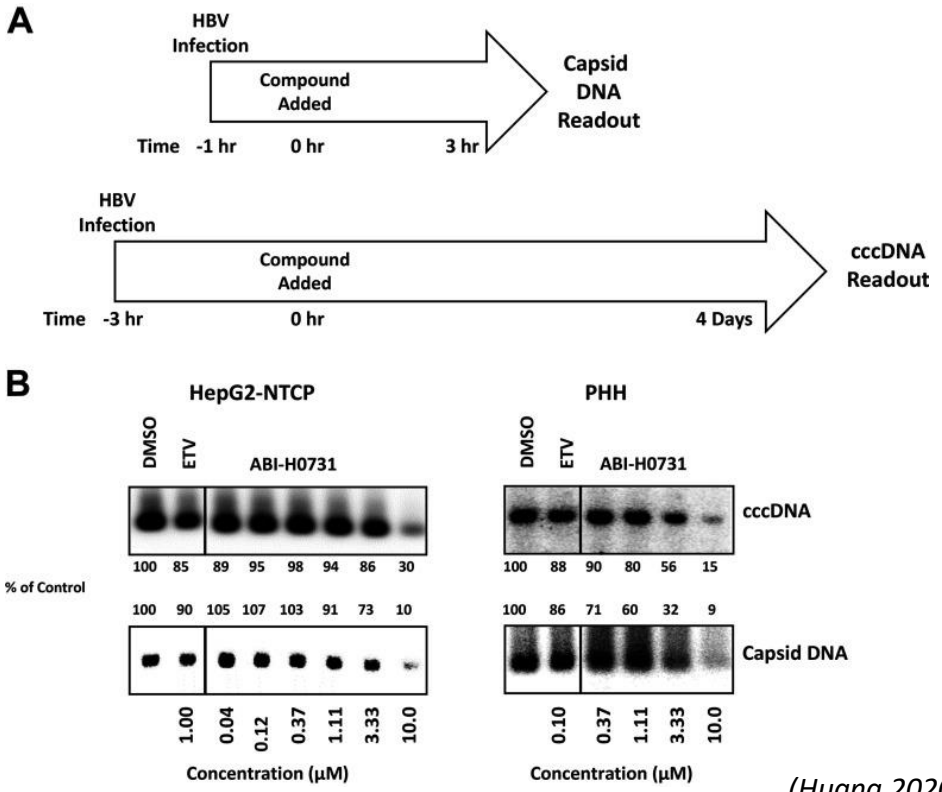
reduce de novo rcDNA synthesis



(Mani 2018)



Induce premature rcDNA uncoating-"Melting"

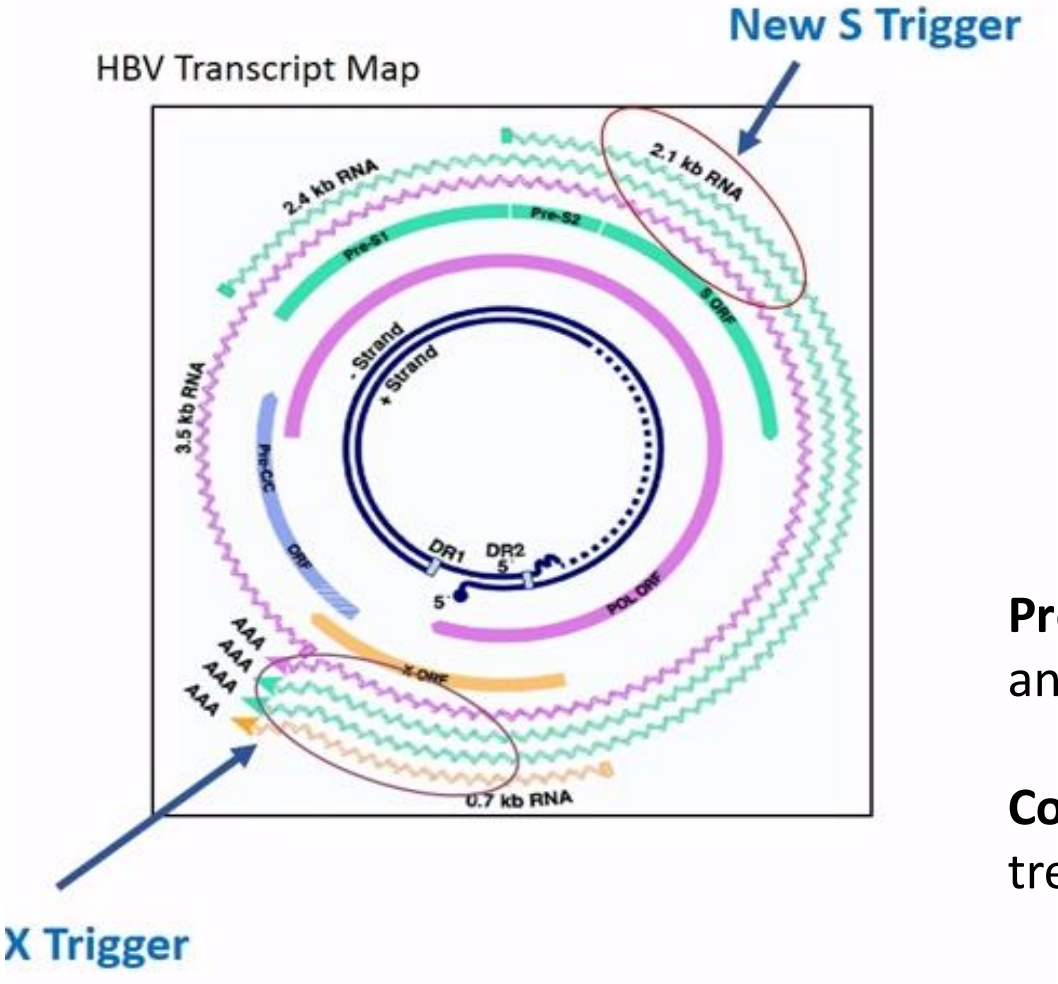


(Huang 2020)

So far, clinical trial data have demonstrated strong antiviral activity of CpAMs and synergy with Nuc or pegIFN, however, no cure or HBsAg seroconversion has been achieved, and virus relapsed after treatment cessation.

Need more potent CpAM or combination with other replication inhibitors?

siRNA



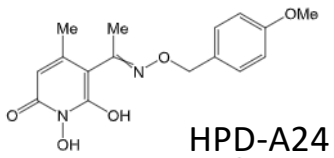
- **siRNA in X gene theoretically can reduce all HBV mRNAs.**
- **However, miss integrated HBV DNA derived mRNAs**
- **Target abolish by mutation in X gene**
- **Combined X plus S siRNAs mitigate such risk**

Pros: sequence specific, theoretically reduce viremia and all antigenemia, liver-specific delivery tools available.

Cons: virus mutations and quasi species, viral rebound after treatment withdrawal.

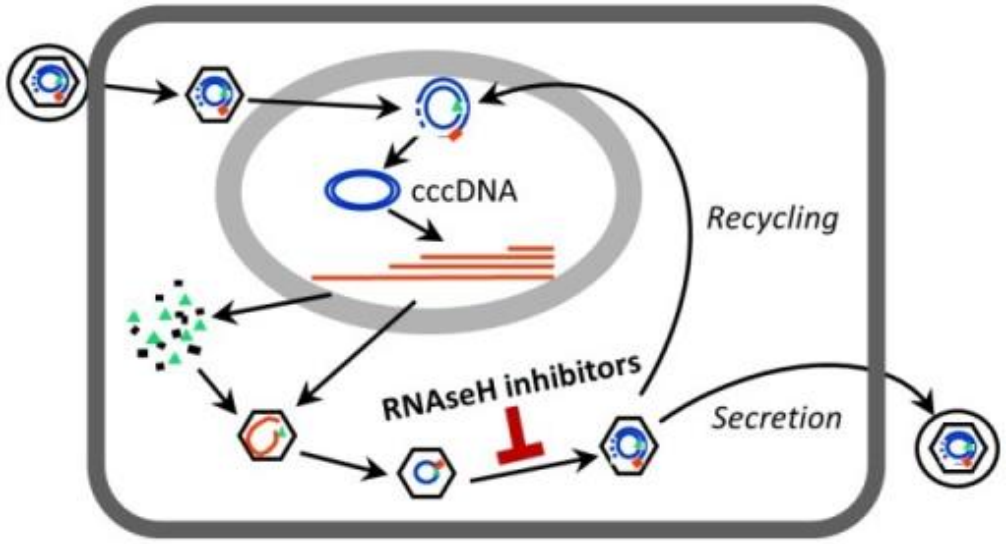
(by courtesy of S. Locarnini)

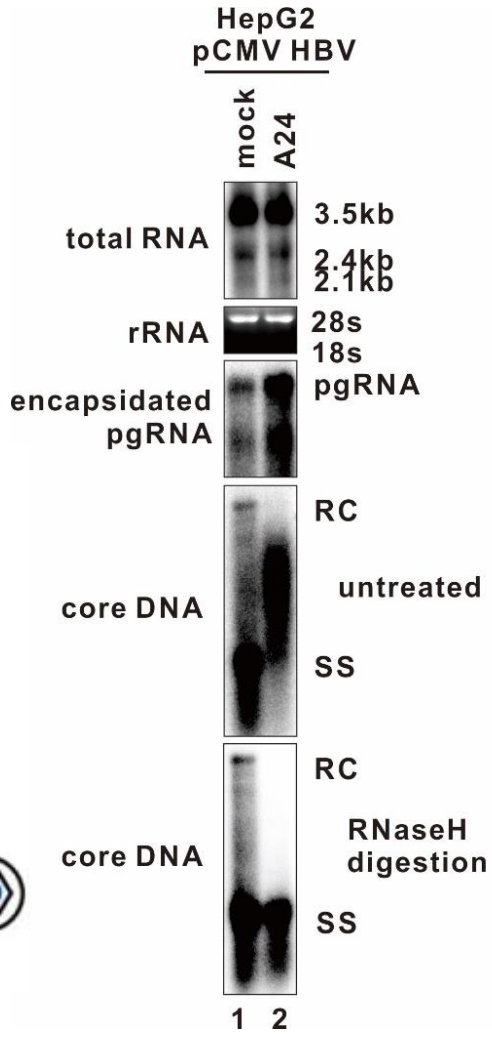
RNase H Inhibitors



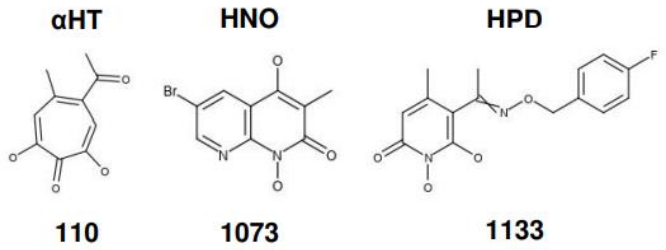
HPD-A24

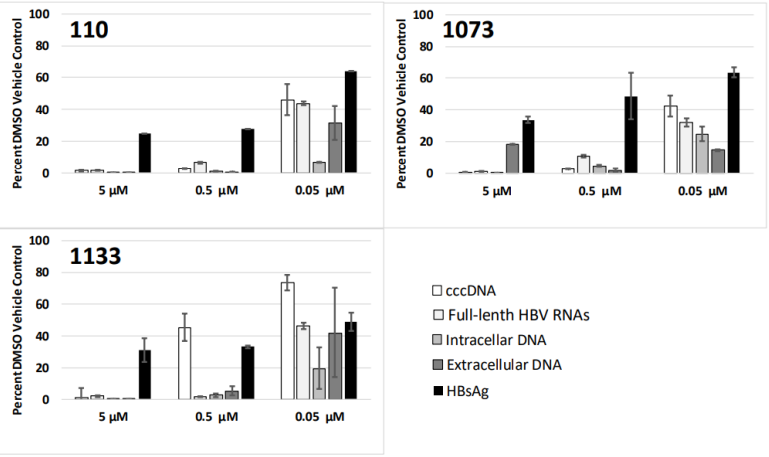
(Edwards 2019)





(Shen, unpublished)

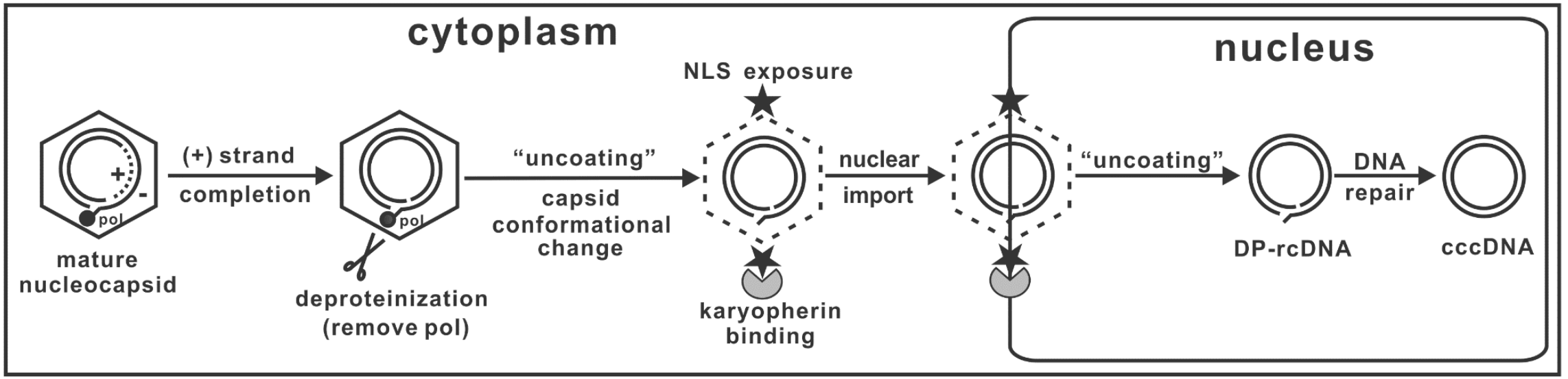




(Chauhan 2021)

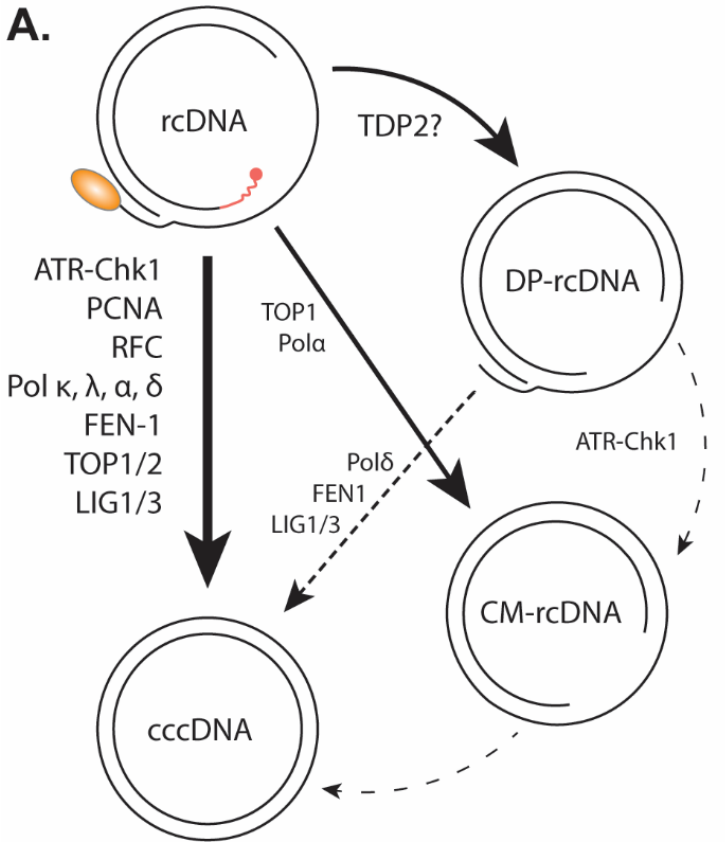
Proposed Model of HBV cccDNA Formation

1. Deproteination (removal of pol, DP) of mature viral DNA is an intermediate step in HBV cccDNA formation;
2. Deproteinization occurs in cytoplasmic mature nucleocapsid with capsid structure shift;
3. DP-rcDNA containing capsid delivers viral genome into nucleus through NLS-mediated nuclear importation;
4. DP-rcDNA is converted into cccDNA in nucleus by host DNA repair apparatus.



(Guo, 2007; Guo 2010; Guo 2012; Guo and Guo, 2015; Long 2017; Cai 2020)

host DNA replication/repair factors involved in cccDNA formation



(Marchetti and Guo, Cells 2020)

ATR-Chk1 *(Luo et al, mBio 2020)*

UV-DDB *(Marchetti et al, JVI in revision)*

Cellular polymerases (κ, λ, α). *(Qi et al, Plos Path, 2016) (Tang et al, Plos Path, 2019)*

TOP1 and **TOP2** *(Sheraz et al, JVI 2019)*

Flap endonuclease I (**FEN1**) *(Kitamura et al, Plos Path, 2018)*

MUS81 flap nuclease *(Zhang and Long et al #O25)*

Lig I/III *(Long et al, Plos Path, 2017)*

Core components of lagging strand DNA synthesis machinery (**PCNA, RFC, pol δ, FEN1, Lig I**) *(Wei and Ploss, Nat Micro, 2020)*

cccDNA formation as antiviral target

Pros:

In combination with NUCs and other replication inhibitors:

1. Block de novo HBV infection;
2. Serve as an additional layer of blocking intracellular cccDNA recycling pathway;
3. Block cccDNA repair, if any;
4. Inhibitors of DNA repair enzymes are being used in cancer therapy.

Cons:

1. No direct effect on preexisting cccDNA;
2. Uncertain long-term efficacy and toxicity, including genotoxicity;
3. Redundant functions among DNA repair factors.

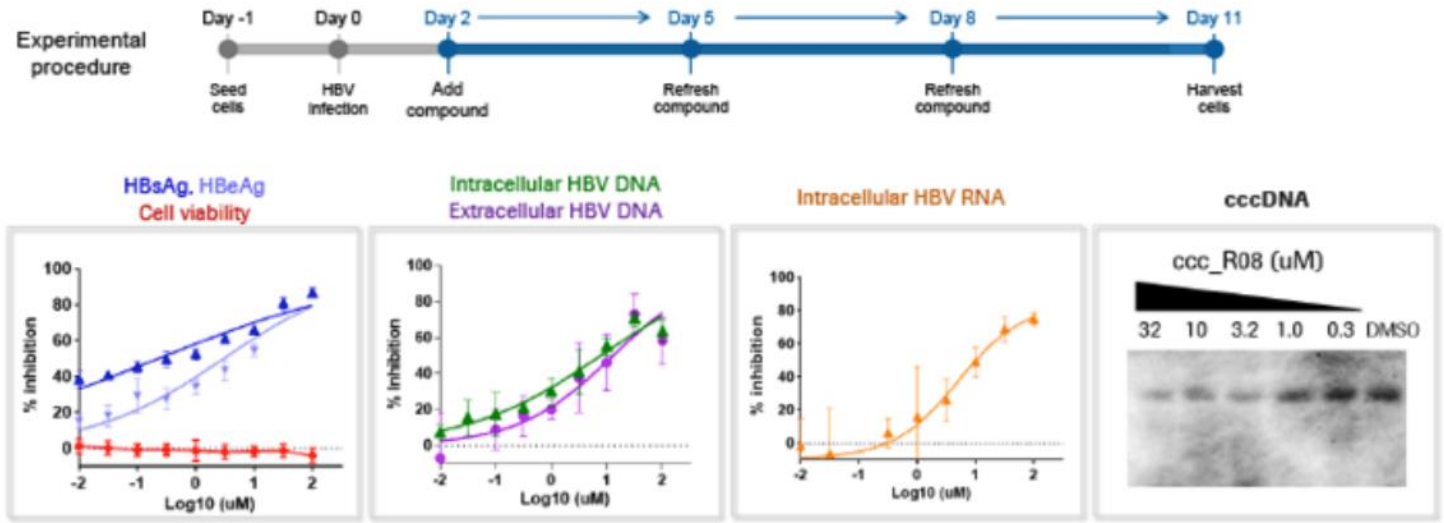
cccDNA destabilizer

A first-in-class orally available HBV cccDNA destabilizer ccc_R08 achieved sustainable HBsAg and cccDNA reduction in the HBVcircle mouse model

[1] Li Wang, [1] Qihui Zhu, [1] Jing Zeng, [1] Zhipeng Yan, [1] Song Feng, [2] John A. T. Young, and [1] **Lu Gao**

1. Roche Innovation Centre Shanghai, Shanghai, China; 2. Roche Innovation Centre Basel, Basel, Switzerland

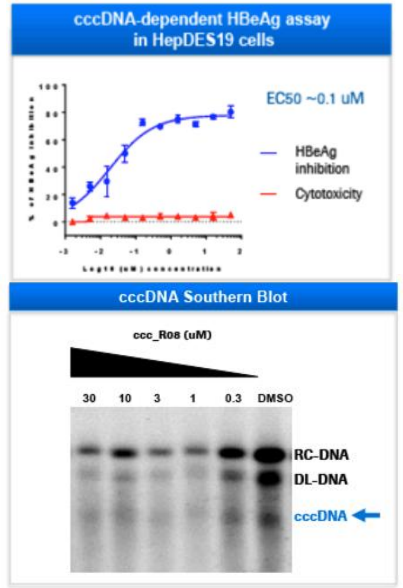
ccc_R08 Reduces HBsAg, HBeAg, HBV DNA, HBV RNA, and cccDNA in HBV infected primary human hepatocyte (PHH)



- No significant change in mitochondria DNA levels in PHH cells observed

6

HepDES19



Unknown Mechanism

EASL 2019

HBx maintains a transcriptionally active state of cccDNA

Research Article

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Hepatitis B virus X protein is essential to initiate and maintain virus replication after infection

Julie Lucifora[1,†], Silke Arzberger[1,†], David Durantel[2,3,4], Laura Belloni[6,7], Michel Strubin[5], Massimo Levrero[6,7], Fabien Zoulim[2,3,4], Olivier Hantz[2,3], Ulrike Protzer[1,*]

PNAS

Nuclear HBx binds the HBV minichromosome and modifies the epigenetic regulation of cccDNA function

Laura Belloni[a,b,1], Teresa Pollicino[c], Francesca De Nicola[d,e], Francesca Guerrieri[a,e], Giuseppina Raffa[c], Maurizio Fanciulli[d,e], Giovanni Raimondo[c], and Massimo Levrero[a,b,e,1]

doi:10.1038/nature17170

Hepatitis B virus X protein identifies the Smc5/6 complex as a host restriction factor

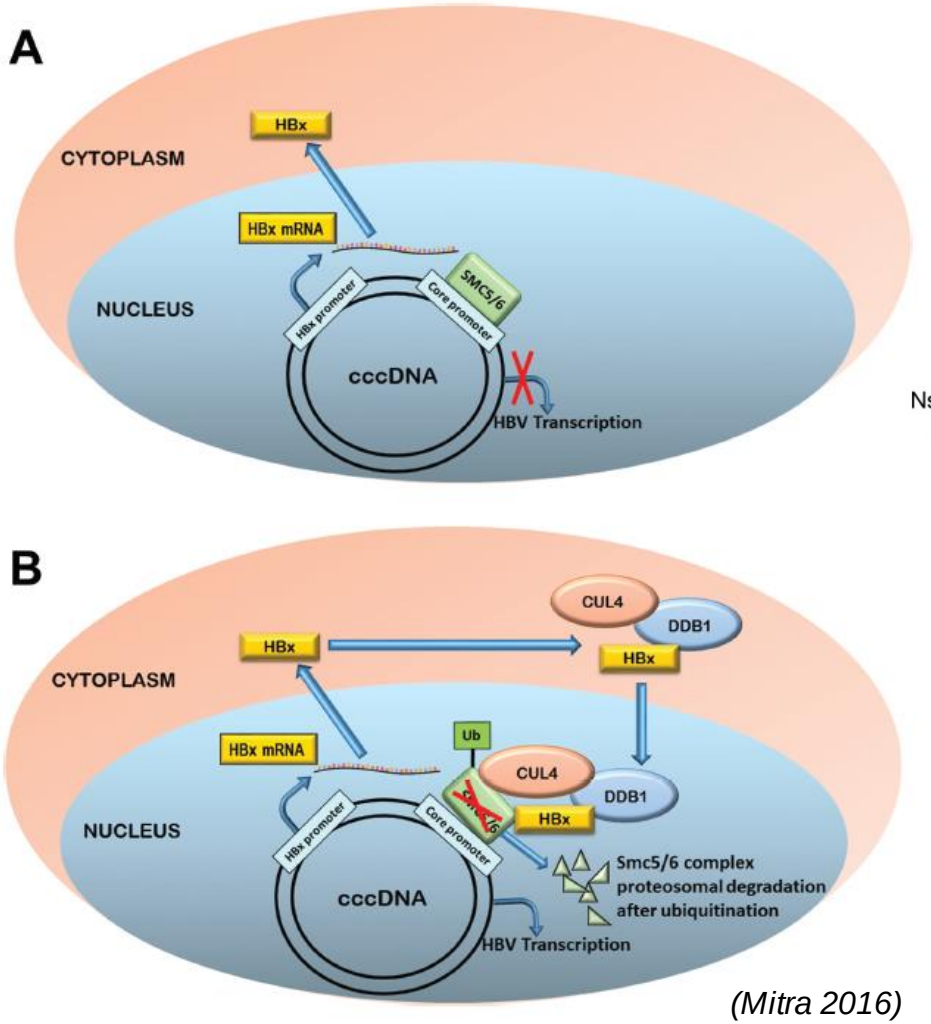
Adrien Decorsière[1]*, Henrik Mueller[1]†*, Pieter C. van Breugel[1]†*, Fabien Abdul[1]*, Laetitia Gerossier[2], Rudolf K. Beran[3], Christine M. Livingston[3], Congrong Niu[3], Simon P. Fletcher[3], Olivier Hantz[2] & Michel Strubin[1]

Cell Reports

Report

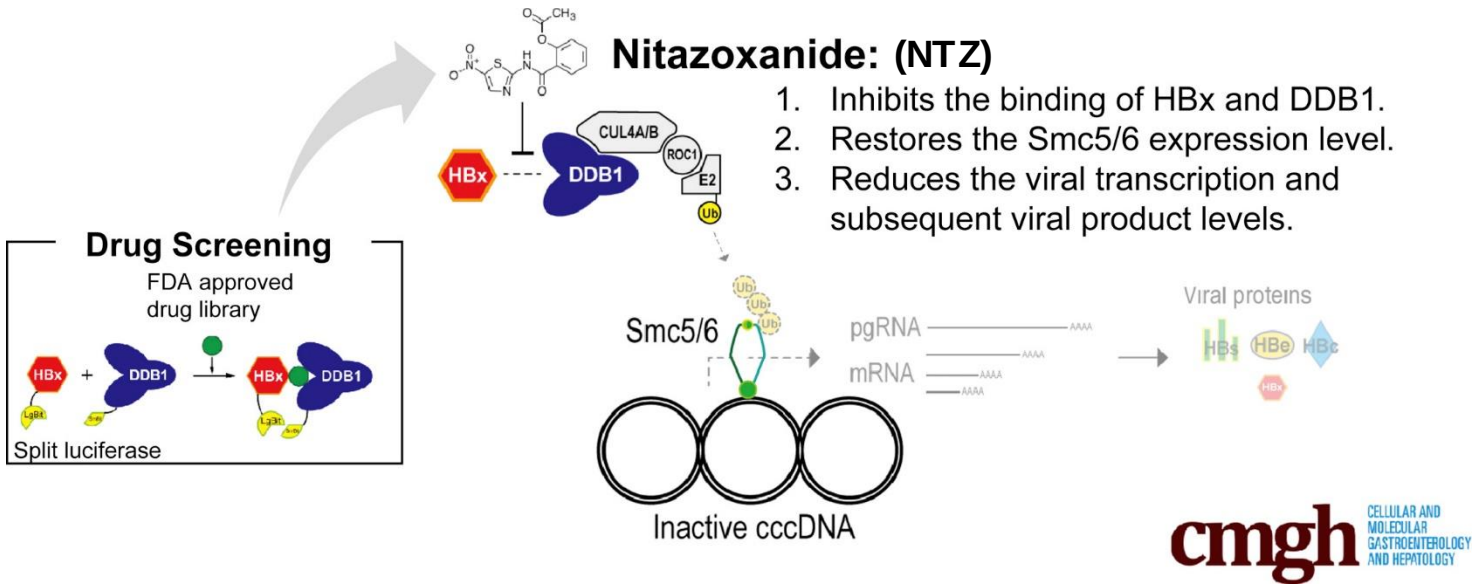
Hepatitis B Virus X Protein Promotes Degradation of SMC5/6 to Enhance HBV Replication

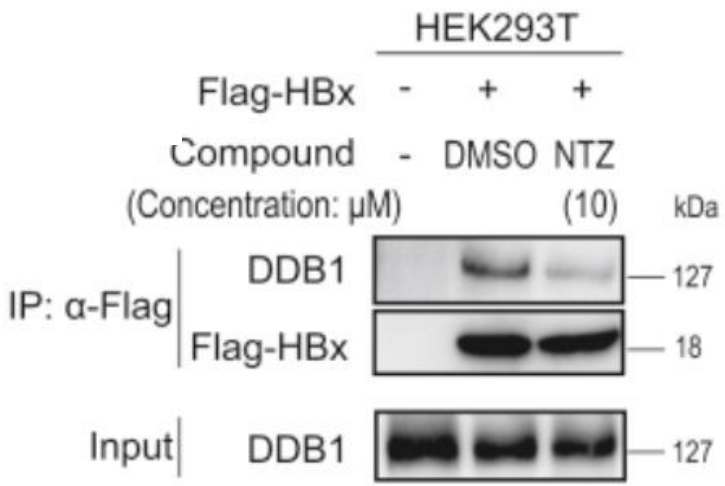
Christopher M. Murphy,[1,2,5] Yanping Xu,[1,3,5] Feng Li,[1,2,5] Kouki Nio,[1,2] Natalia Reszka-Blanco,[1,2] Xiaodong Li,[1,2] Yaxu Wu,[1,2] Yanbao Yu,[4] Yue Xiong,[1,3,*] and Lishan Su[1,2,6,*]

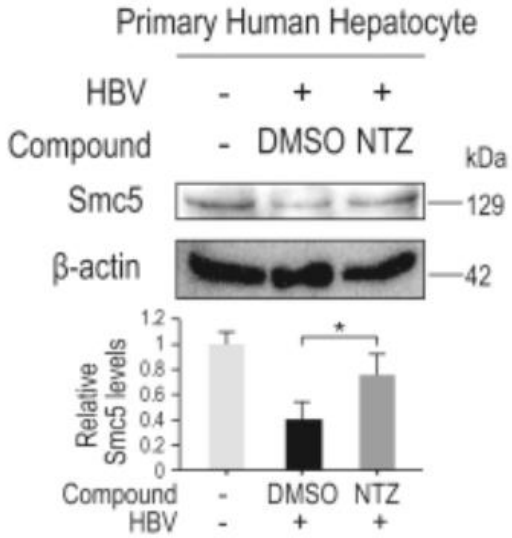


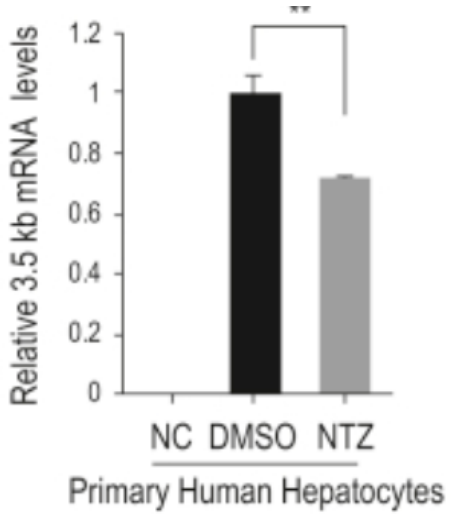
(Mitra 2016)

Inhibitors of HBx-DDB1 interaction may silence cccDNA









(Sekiba 2019)

HEPATOLOGY
HEPATOLOGY, VOL. 69, NO. 5, 2019

Pevonedistat, a Neuronal Precursor Cell-Expressed Developmentally Down-Regulated Protein 8–Activating Enzyme Inhibitor, Is a Potent Inhibitor of Hepatitis B Virus

Kazuma Sekiba, Motoyuki Otsuka, Motoko Ohno, Mari Yamagami, Takahiro Kishikawa, Takahiro Seimiya, Tatsunori Suzuki, Eri Tanaka, Rei Ishibashi, Kazuyoshi Funato, and Kazuhiko Koike

MLN4924

NEDD8

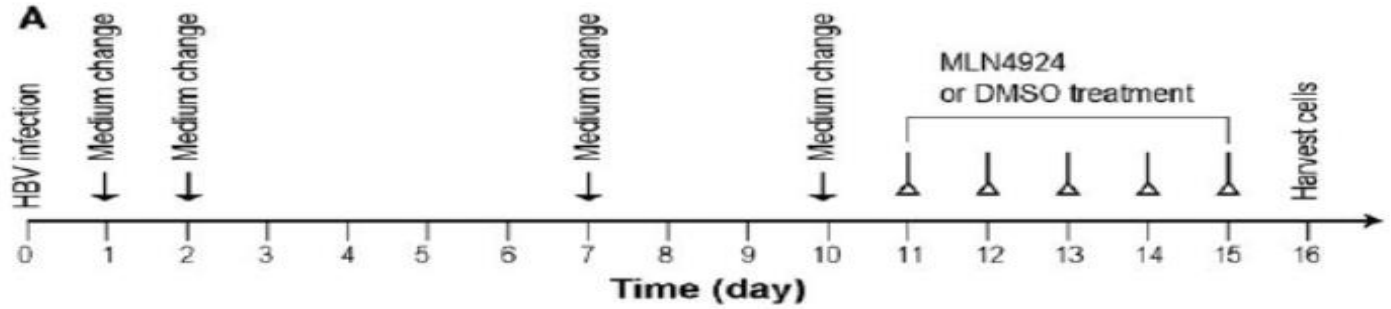
NEDD8-activating enzyme E1 (NAE)

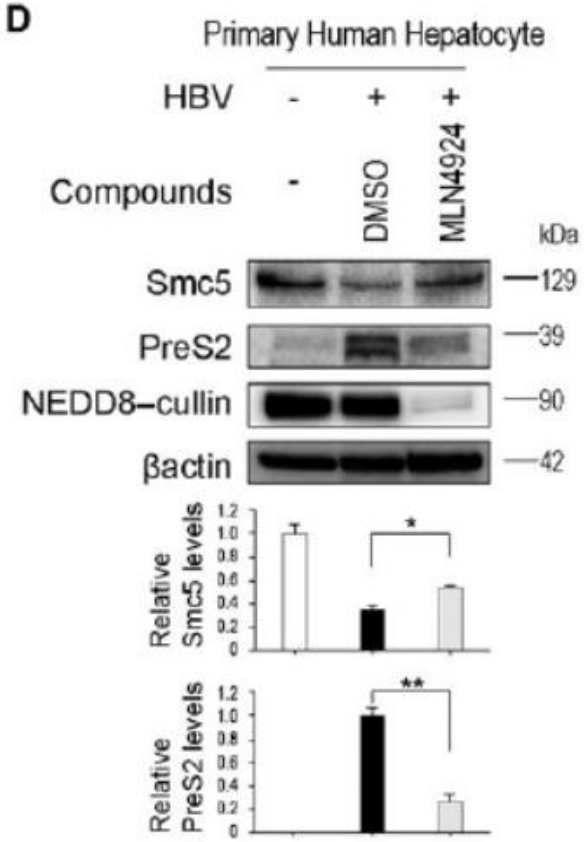
Cullin-RING E3 ligases (CRLs)

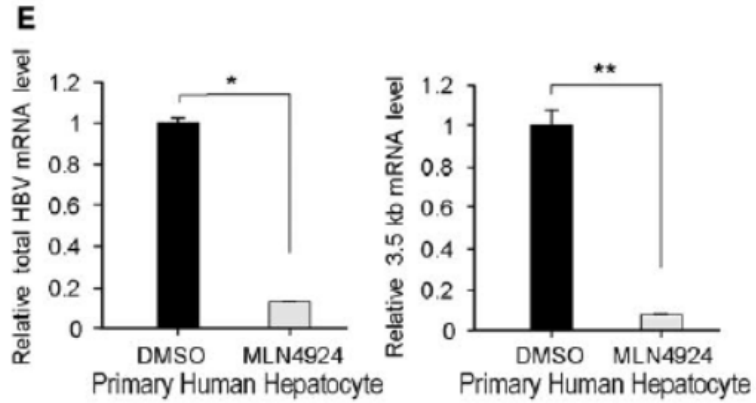
HBx-DDB1

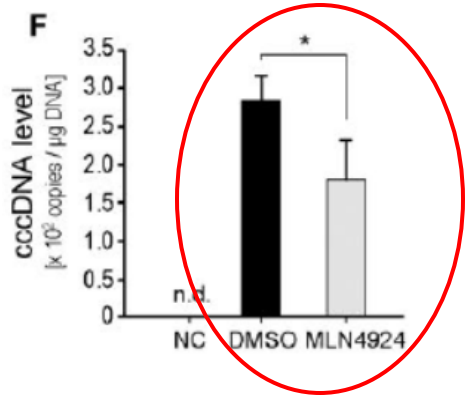
Smc5/6 degradation

cccDNA transcription



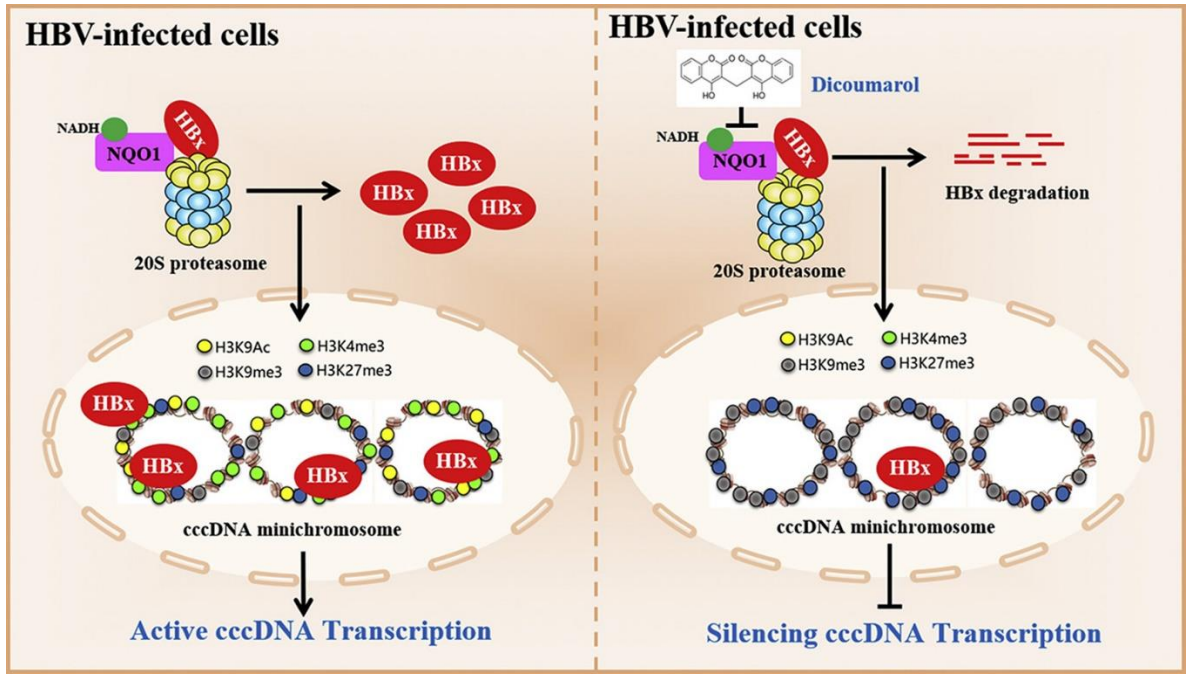


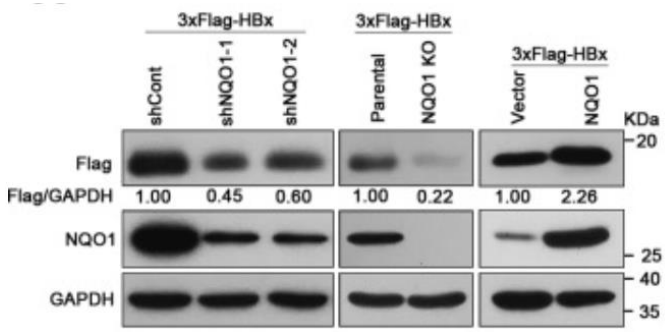


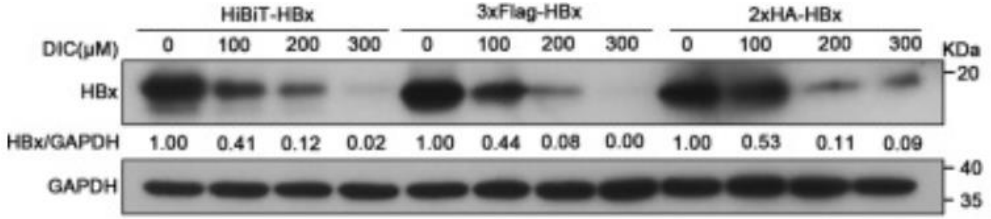


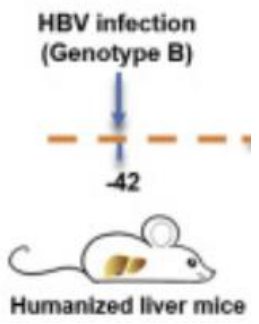
Inhibiting cccDNA transcription may lead to cccDNA destabilization?

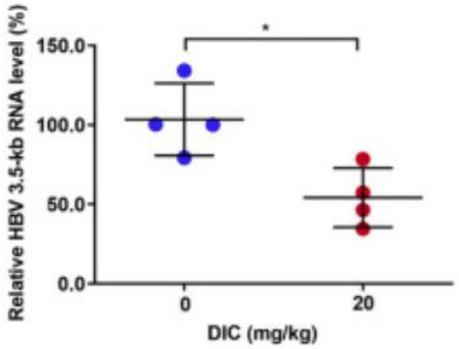
Dicoumarol, an NQO1 inhibitor, blocks cccDNA transcription by promoting HBx degradation







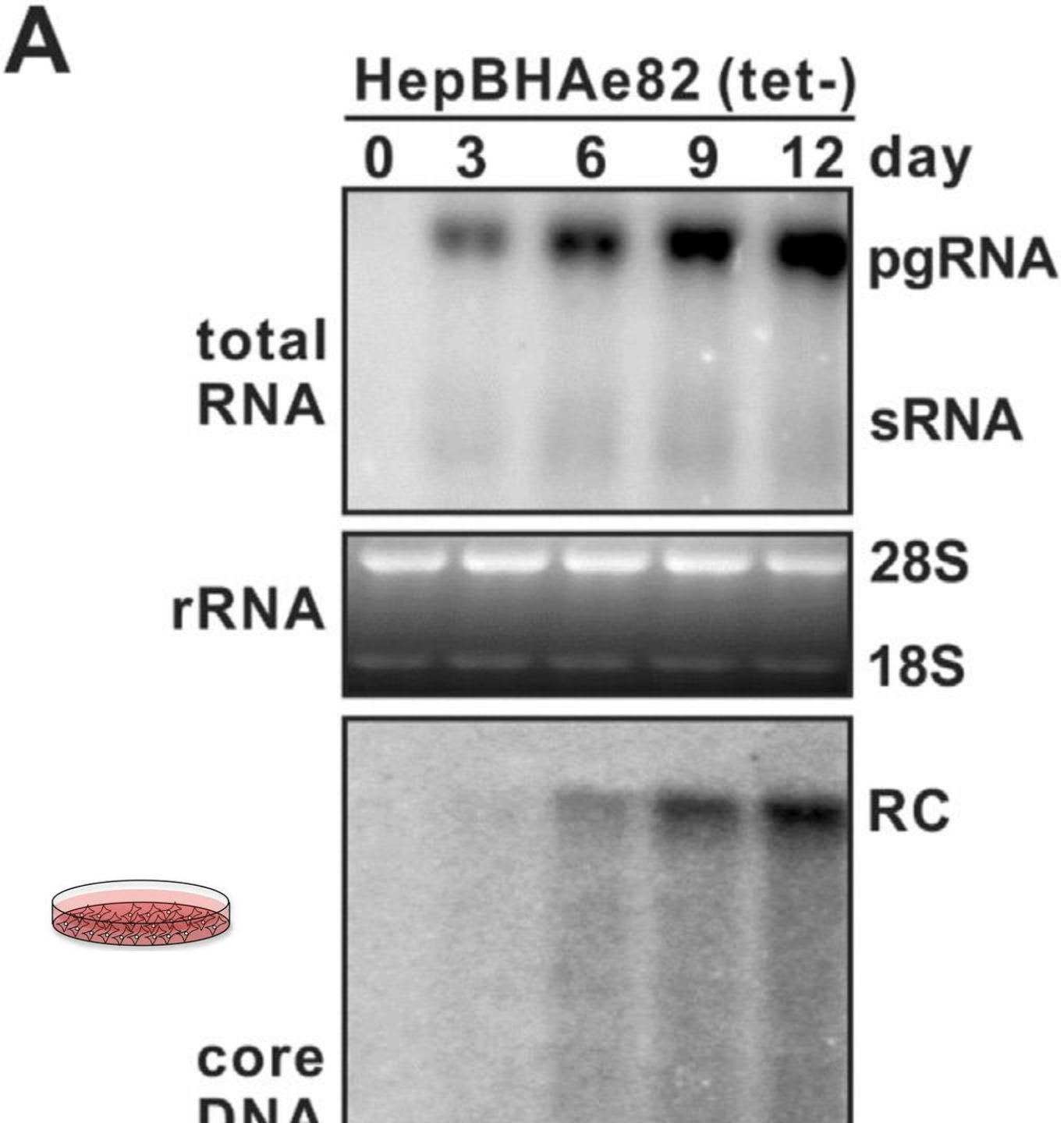


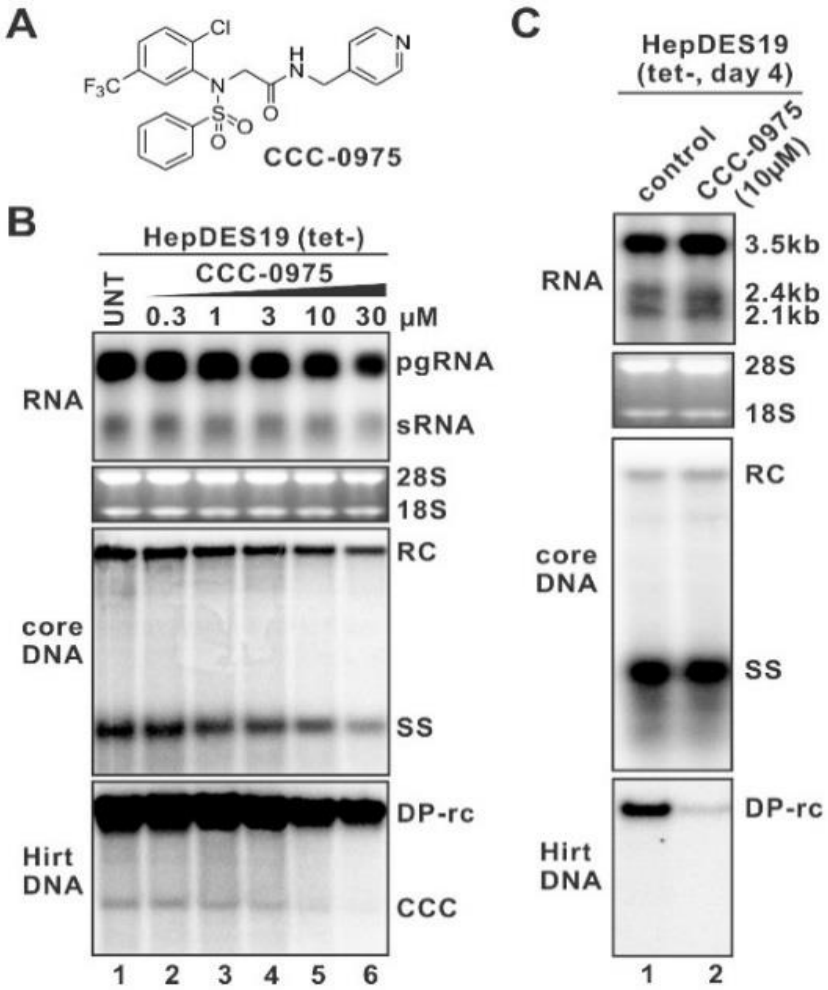


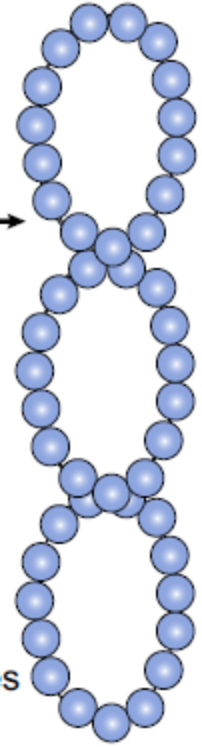
Proof-of concept:
HBx can be targeted to silence cccDNA

(Cheng 2021)

ry of cccDNA inhibitors







Summary & Outlook

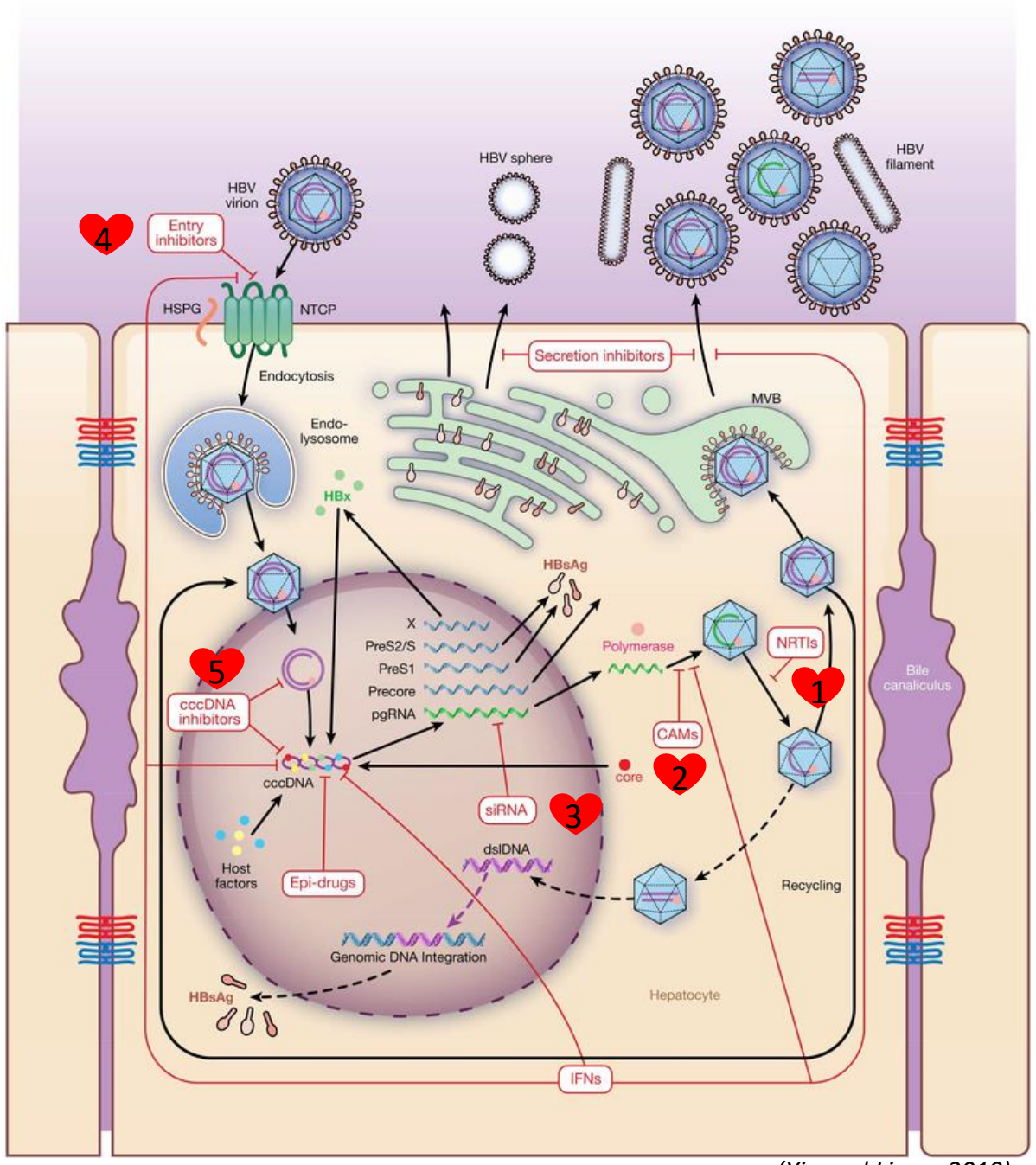
C C C DNA

cure of hepatitis B requires the elimination or permanent inactivation of cccDNA .

Need more basic research on cccDNA metabolism and transcription to identify specific antiviral targets.

Combination of cccDNA inhibitor and other inhibitors targeting different steps in HBV replication cycle is an ideal strategy to achieve the cure.

Control, Combination, Cure!



(Xia and Liang, 2019)

My picks ❤ for combination therapy

1. Nuc: ETV or TAF	oral	backbone
2. CpAM	oral	
3. siRNA	injectable	bases
4. MyrB	injectable	
5. cccDNA inhibitor	N/A	

Criteria:

Effective

Synergistic

Safe

Affordable