Medical Faculty Heidelberg

An update on some virological aspects of HBV/HDV Biology

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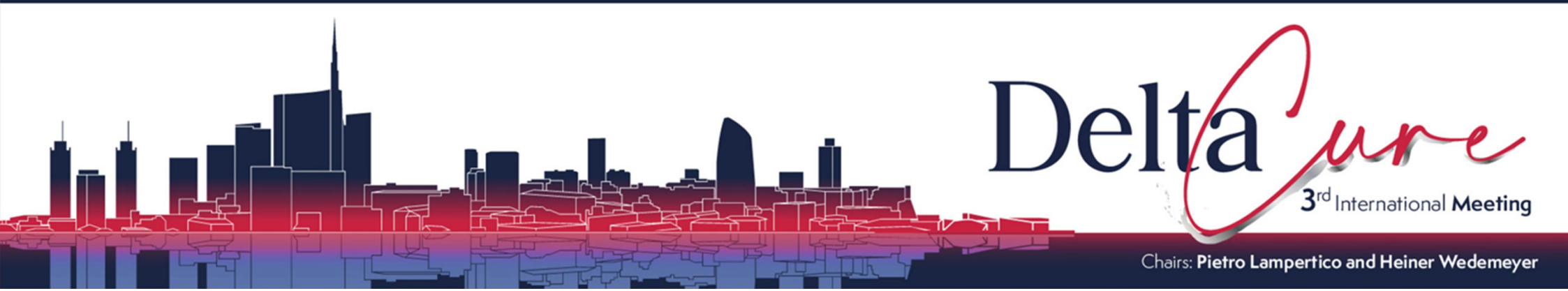
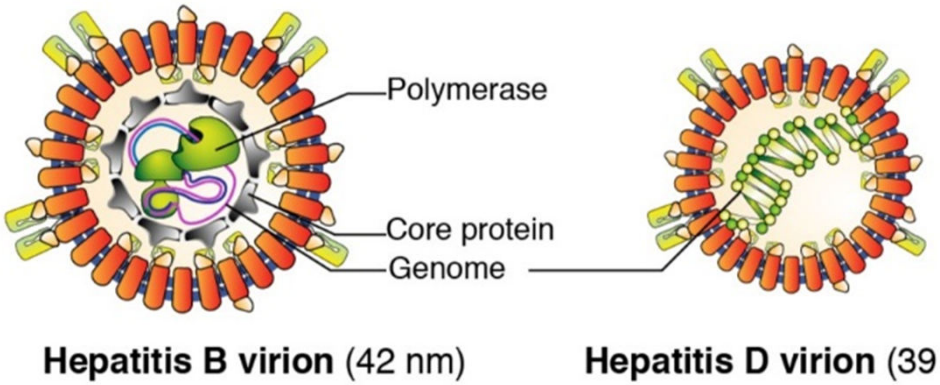
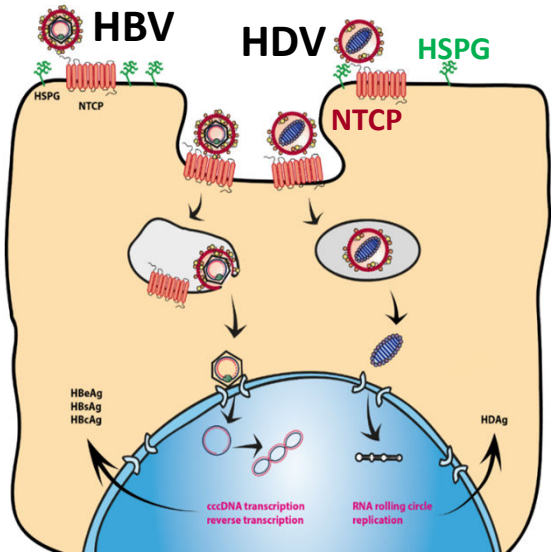


Disclosures of Stephan Urban

- *Opinions expressed do not reflect anyone's position but my own*
- *Assembly Biosciences, Gilead Sciences, Hepatera-Ltd, MYR-GmbH, VirBio*
- *I am patent holder and inventor on patents protecting Hepcludex/Bulevirtide*

HBV, HDV and their modes to establish persistent infections





- HBV and HDV share the same envelope proteins and use the same receptors
- Both viruses establish circular episomes (cccDNA, circRNA) in the nucleus of infected hepatocytes
- Maintenance of cccDNA and circRNA is crucial for persistence
- HBV cccDNA gets lost during mitosis (must be replenished by de novo entry or “amplification”)
- HDV can assemble and spread using HBV envelopes encoded in integrates (no cccDNA dependence)
- RNPs of HDV- and HDV-like agents efficiently spread via cell division

What are the consequences of HBV integration on HBV/HDV infection ?

Immediate integration of HBV ds-linear HBV DNA upon infection

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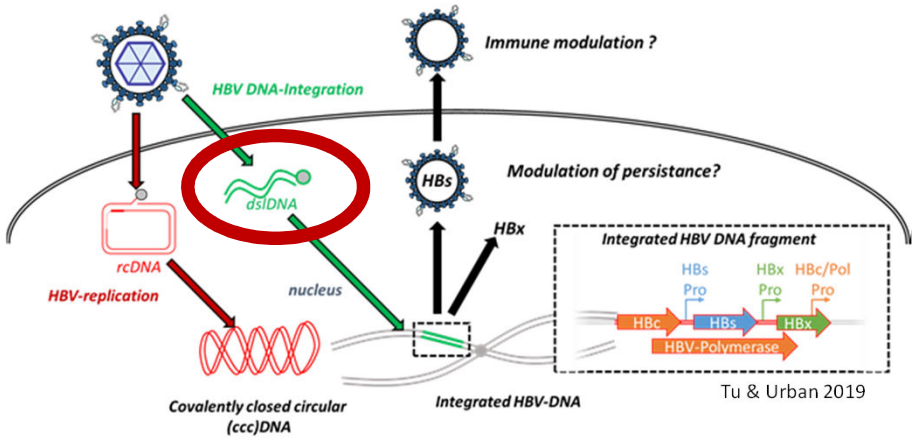
VIRUS-CELL INTERACTIONS

Hepatitis B Virus DNA Integration Occurs Early in the Viral Life Cycle in an *In Vitro* Infection Model via Sodium Taurocholate Cotransporting Polypeptide-Dependent Uptake of Enveloped Virus Particles

Thomas Tu,[a] Magdalena A. Budzinska,[b] Florian W. R. Vondran,[c,d] Nicholas A. Shackel,[b,e,f] Stephan Urban[a,g]

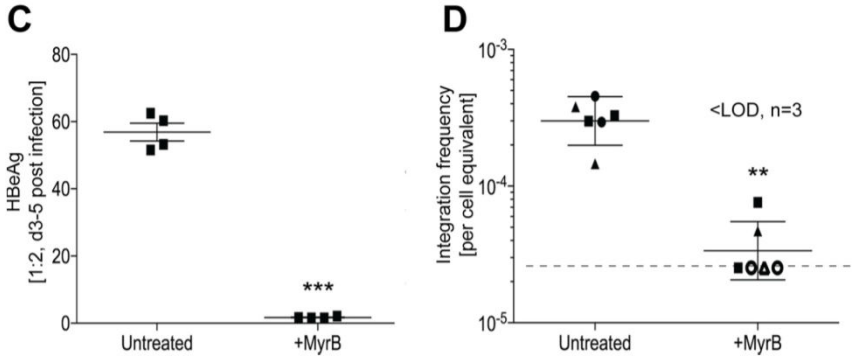
Chromosomal integration of dsl HBV DNA



Tu & Urban 2019

- 5-10 % of enveloped “virions” contain double stranded linear (dsl) HBV DNA.
- dslDNA containing particles enter hepatocytes via NTCP (blocked by BLV/MyrB).
- dslDNA instantly integrates into chromosomes.
- Integrated HBV DNA can propagate via cell division and clonal expansion.

Inhibition of infection and integration by BLV/MyrB

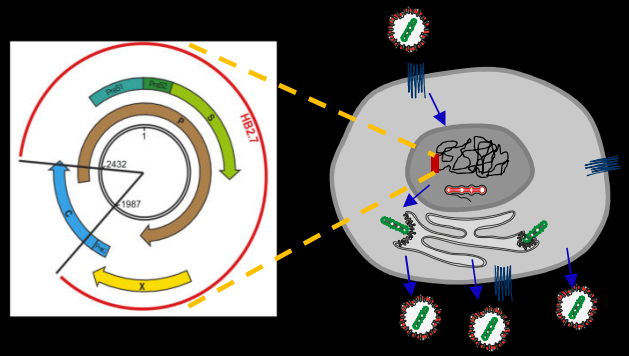


⇒ How does HBV integration affect HBV/HDV replication ?

Abrogation of HBV infection by integrated HBV DNA (encoding envelope proteins)

HepG2-NTCP
no integrate

HepNB2.7
artificial integrate



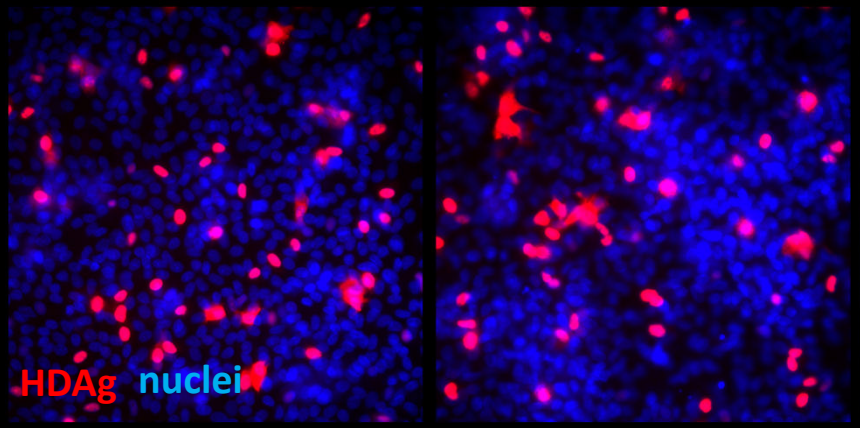
HepNB2.7

Integration of a 2.7 kb HBV + NTCP

Lempp et al., Nature Communications, 2019

Yi Ni unpublished

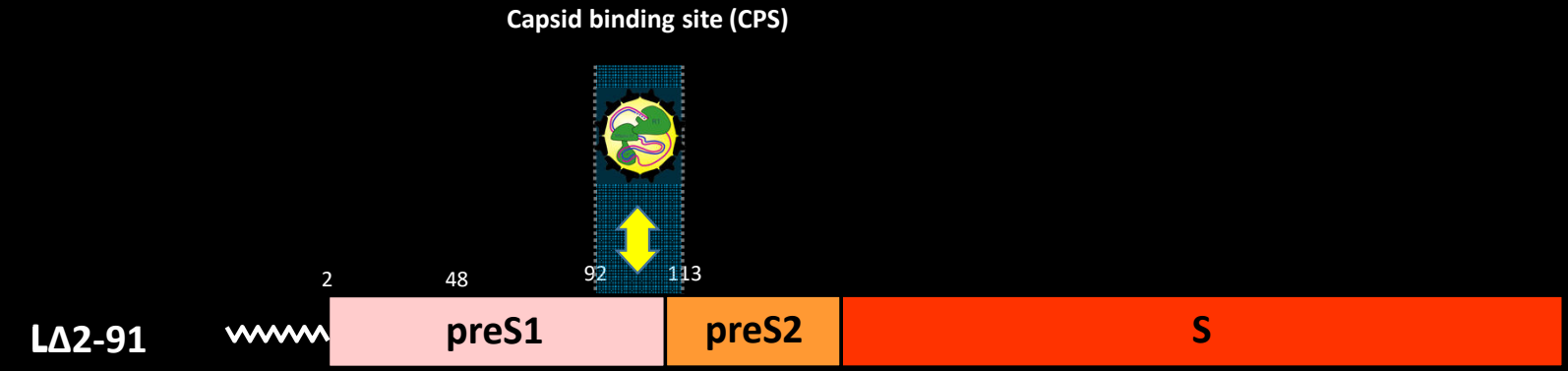
HDV

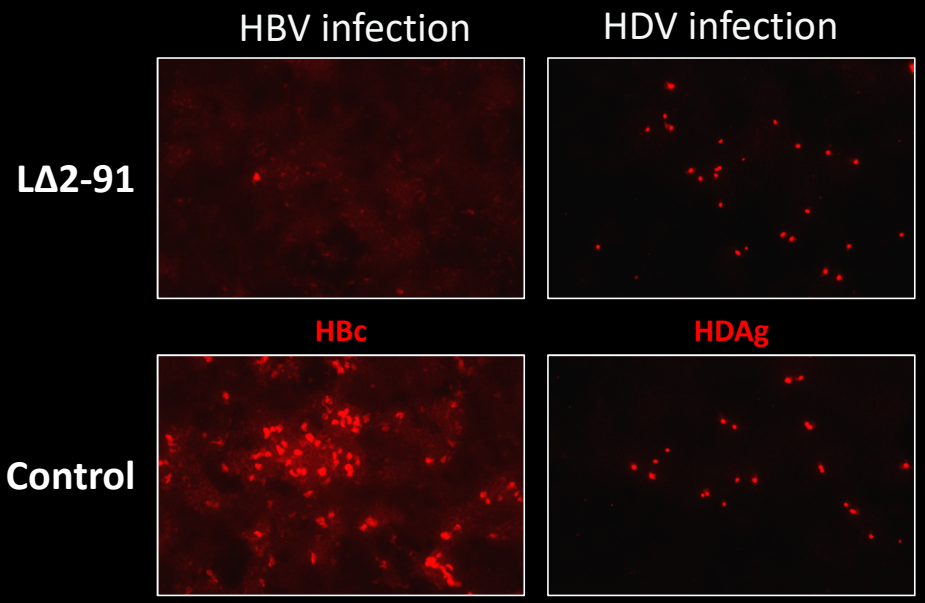


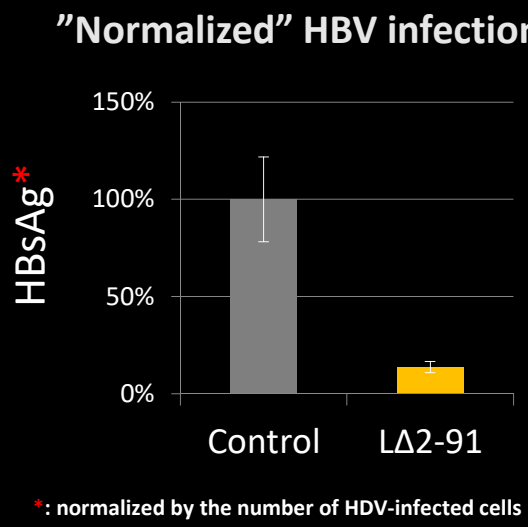
Cells expressing HBV proteins from integrated HBV-DNA support HDV infection but are resistant to HBV infection

⇒ **Clonal expansion of integrants reduces HBV replication space but provides additional space for HDV replication !**

The capsid binding domain of L-protein is responsible for abrogation of HBV infection







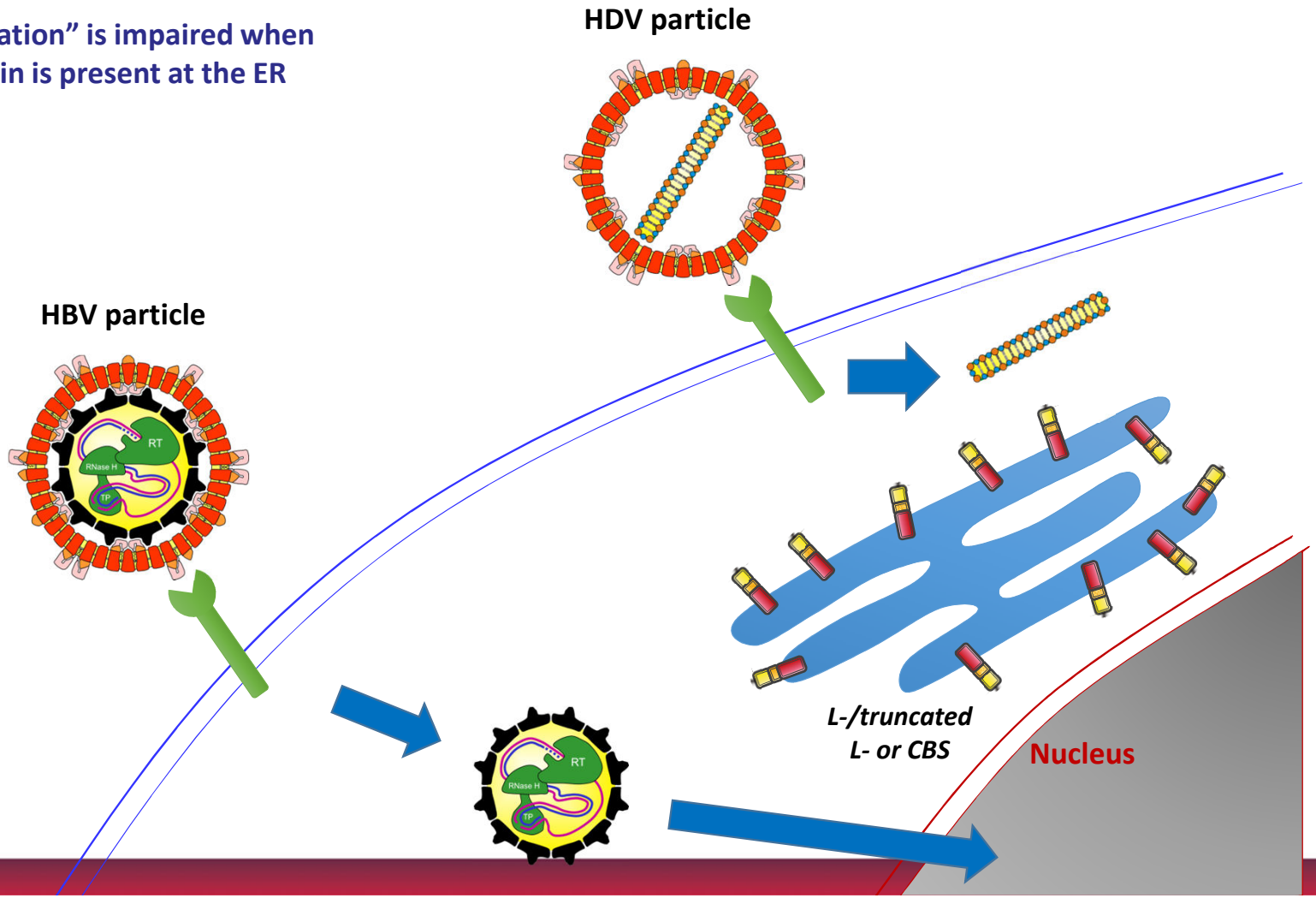
*: normalized by the number of HDV-infected cells

Yi Ni, unpublished

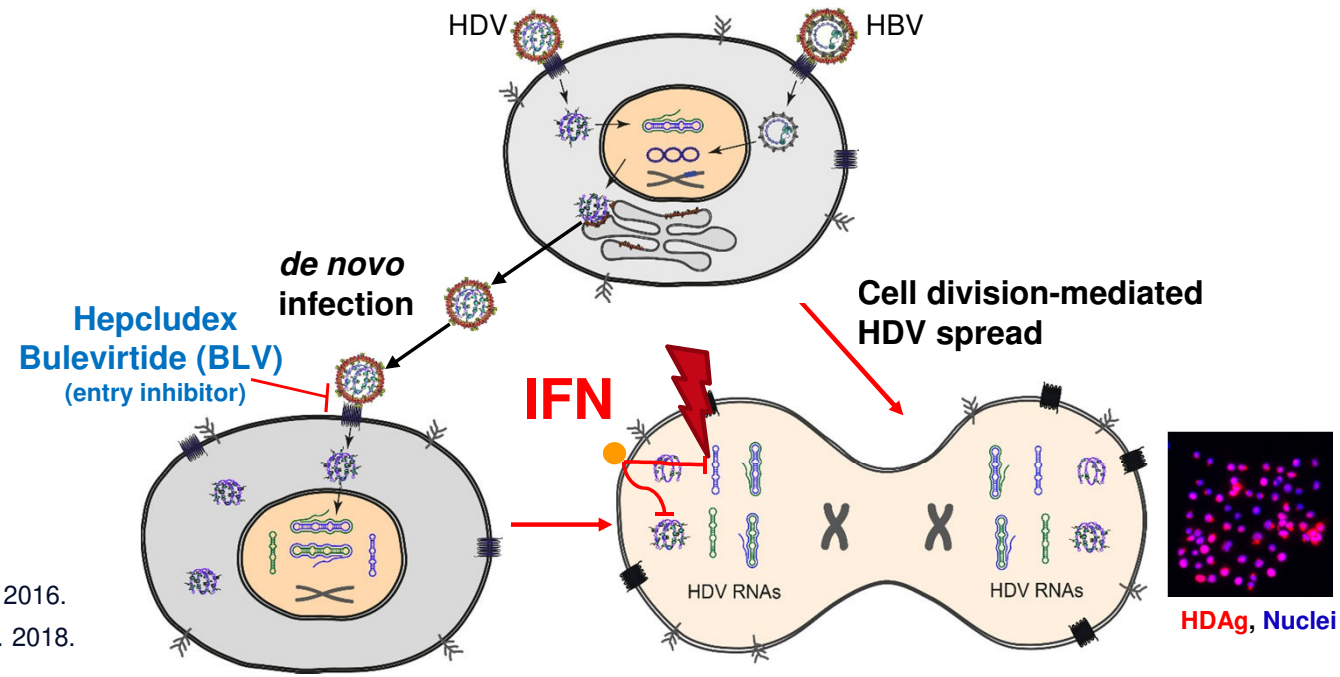
⇒ **Intracellular/cytoplasmic expression of the HBV Capsid binding site blocks establishment of HBV infection**

<u>The model</u>: Presence of the nucleocapsid binding site within preS prevents nuclear import of capsids and redirects both, incoming and newly synthesized NCs into the secretion pathway

Implications: "cccDNA formation" is impaired when L-protein/truncated L-protein is present at the ER



HDV spreading pathways and persistence



Bogomolov *et al*. J Hepatol. 2016.
Yurdaydin *et al*. Hepatology. 2018.

Giersch *et al*. Gut. 2019.
Zhang *et al*. J Hepatol. 2022.
Zhang, *et al*. Viruses 2020.

- HBV-env-dependent *de novo* infection.
- Cell division-mediated HDV spread.

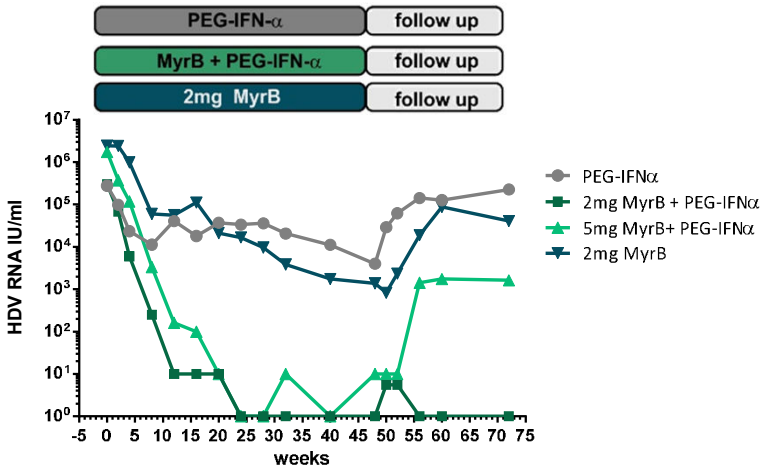
Cell division mediated spread is inhibited by IFNs

Extracellular spread is inhibited by BLV or mAbs

Therapeutic interference with both pathways result in synergistic effects (Myr-203)

Clinical findings: Myr-203 and 204 study (HDV serum RNA)

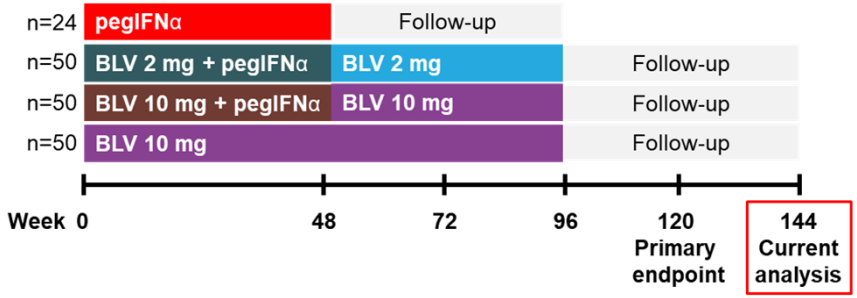
Myr-203: Median HDV RNA levels from 15 patients



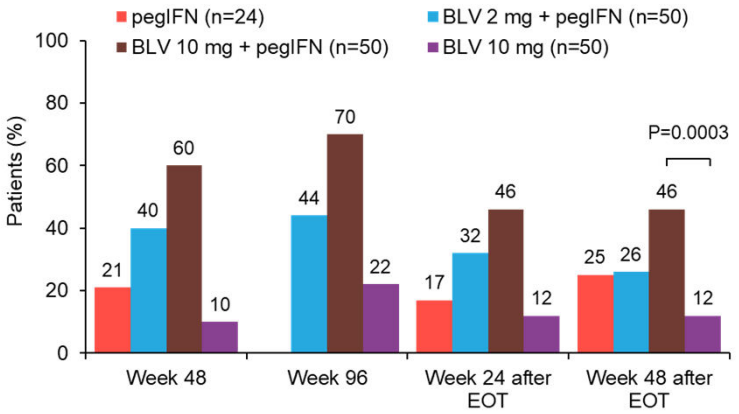
Strong synergism of IFNα and BLV on HDV serum RNA

Wedemeyer, et al. EASL ILC 2020.

Myr-204: study design



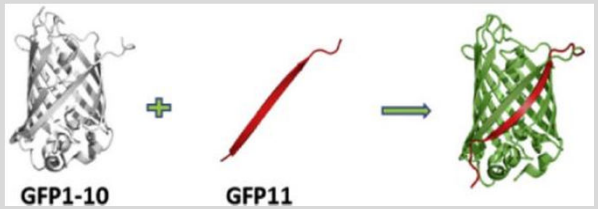
Undetectable HDV RNA through 48 weeks after EOT

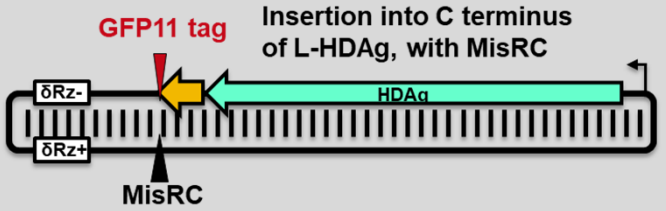


46% of patients remain HDV RNA negative 48w after EOT (10 mg BLV + pegIFNα)

Asselah T, et al. EASL ILC 2024. GS-002.

Live cell imaging of HDV-spread by cell division

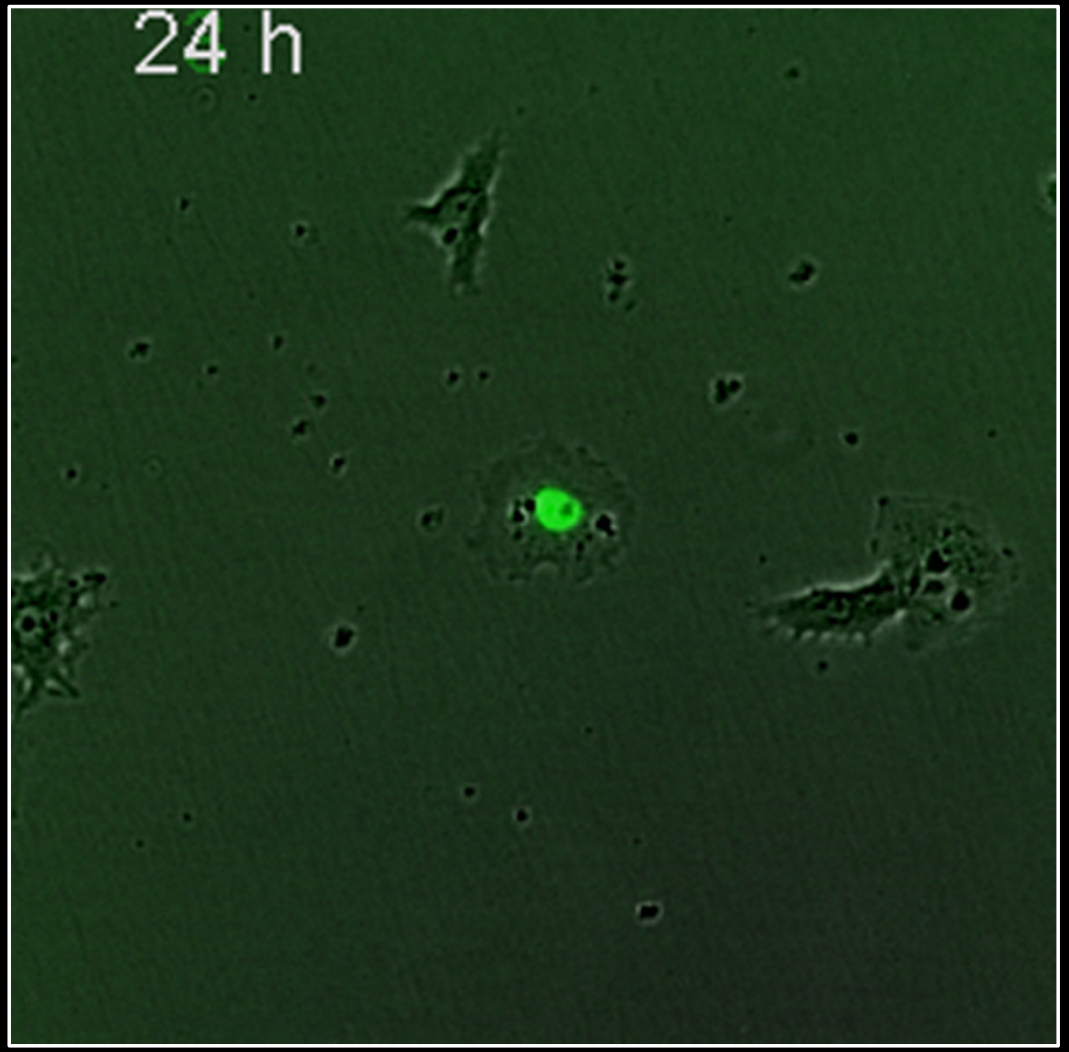




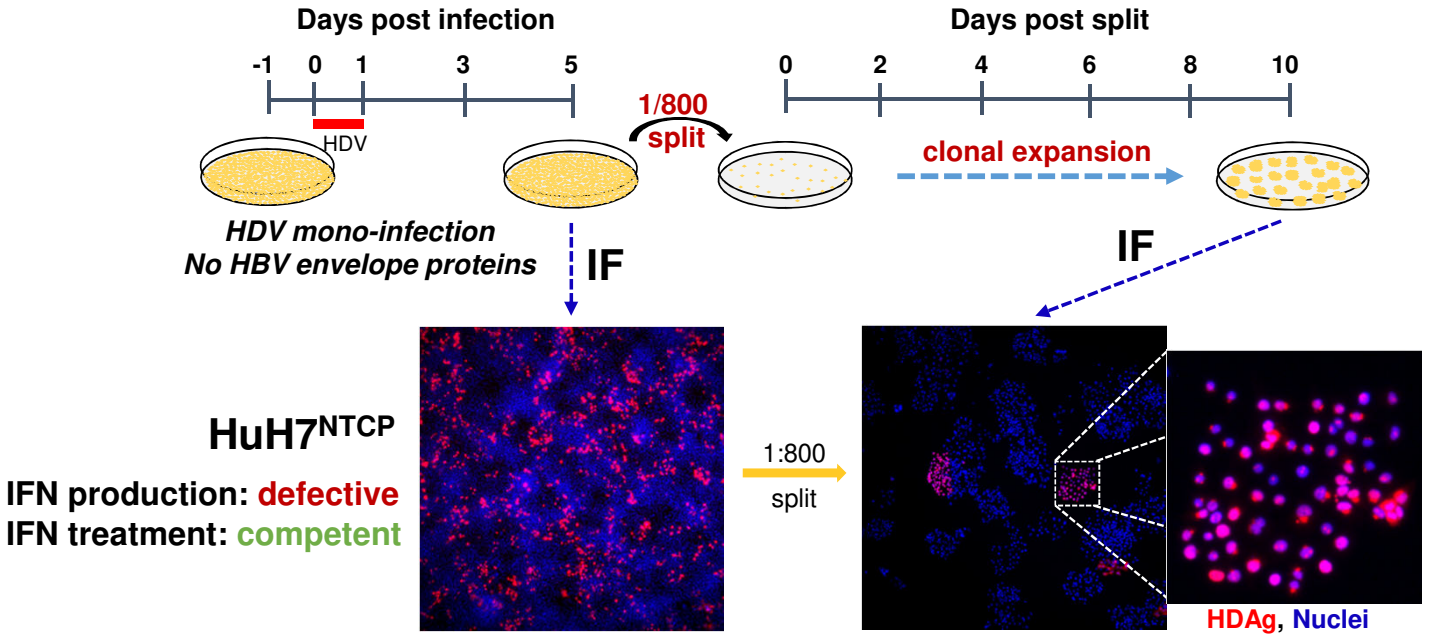
214-GFP11: *WDILFPADPPFSPQSCRPQ*
210-GFP11: *WDILFPADPPFSPQS*
208-GFP11: *WDILFPADPPFSP*
204-GFP11: *WDILFPADP*
202-GFP11: *WDILFPA*
200-GFP11: *WDILF*
199-GFP11: *WDIL*
198-GFP11: *WDI*
196-GFP11: *W*

C termianal truncation of L-HDAg

Zhenfeng Zhang, unpublished



Efficacy of cell-division-mediated HDV spread depends on the innate immune competence of cells



The HDV-induced IFN response "self-restricts" cell division-mediated HDV spread in HepaRG cells

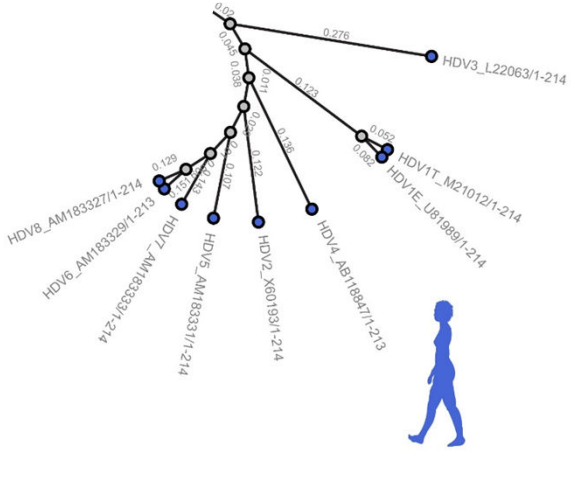
HDV evolution and discovery of HDV-like agents

Gnimah Eva Gnouamozi

HDV and HDV-like agents (DLA)

- Encode homologous small delta antigens (SDAg)

- No (farnesylated) large delta antigen expression
- No evidence for hepadnaviral helper function / no liver tropism



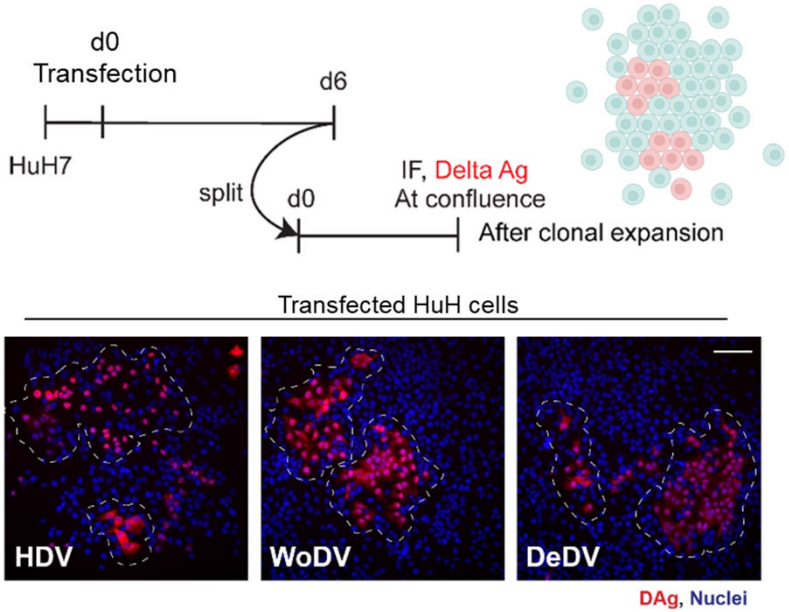
Wille, M. et al., 2018
Hetzel, U. et al., 2019
Chang, W.S. et al.,2019
Paraskevopoulou S. et al. 2020
Bergner, M. et al., 2021
Iwamoto et al., 2021
Khalfi et al. 2024
Gnouamozi et al., 2024

Replication, spreading pathways and host counteractions are still unknown

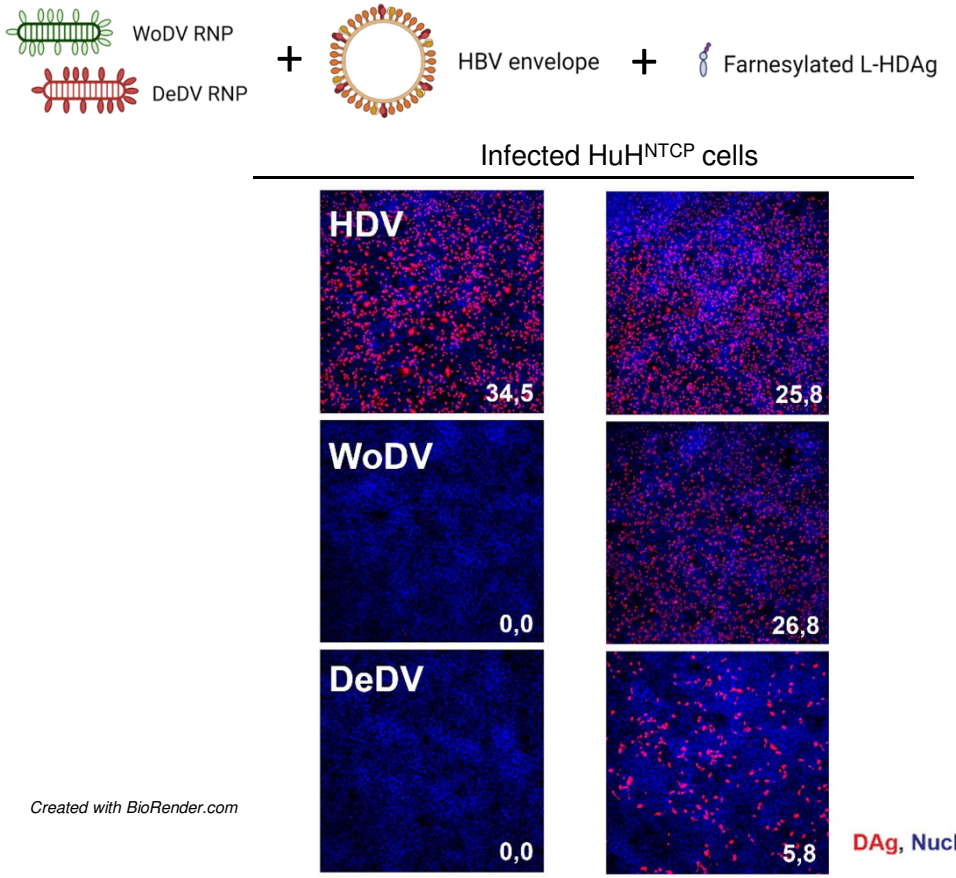
Woodchuck and Deer delta-like agents: persistence and pseudotyping

Woodchuck delta agent (WoDV)

Deer delta agent (DeDV)



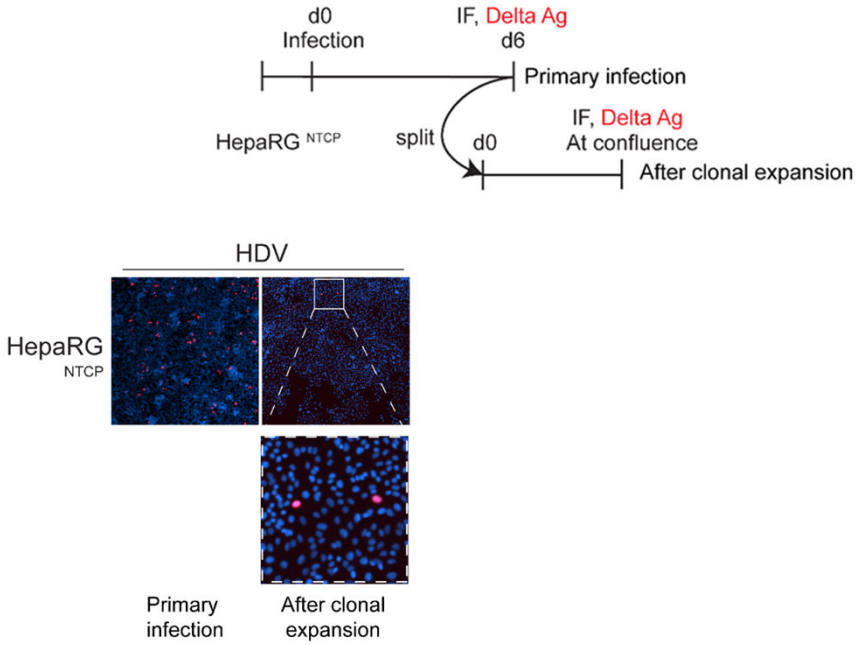
Cell division-mediated spread is a conserved propagation pathway for DLA in *vitro*



Artificial expression of L-HDAg allows HBsAg-dependent pseudotyping of WoDV and DeDV ribonucleoproteins

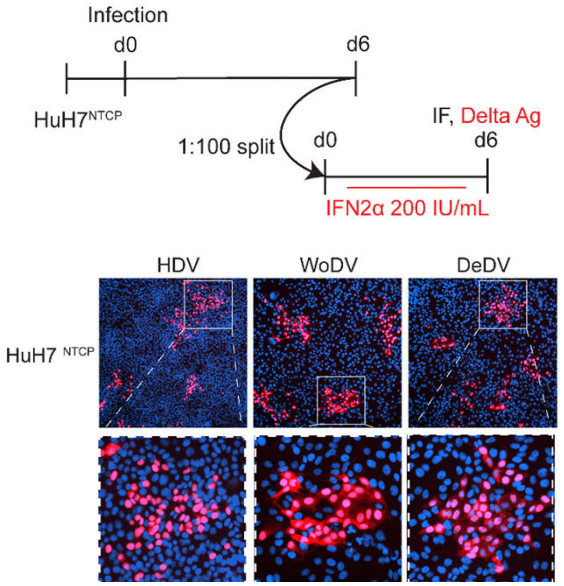
Gnouamozi et al. Viruses 2024

Effect of intrinsic and exogenous interferon stimulation on spread



Delta antigen , Nuclei, Scale bar : 200 µm

WoDV and DeDV efficiently spread in innate immune competent HepaRG^{NTCP} cells



Delta antigen , Nuclei, Scale bar : 200 µm

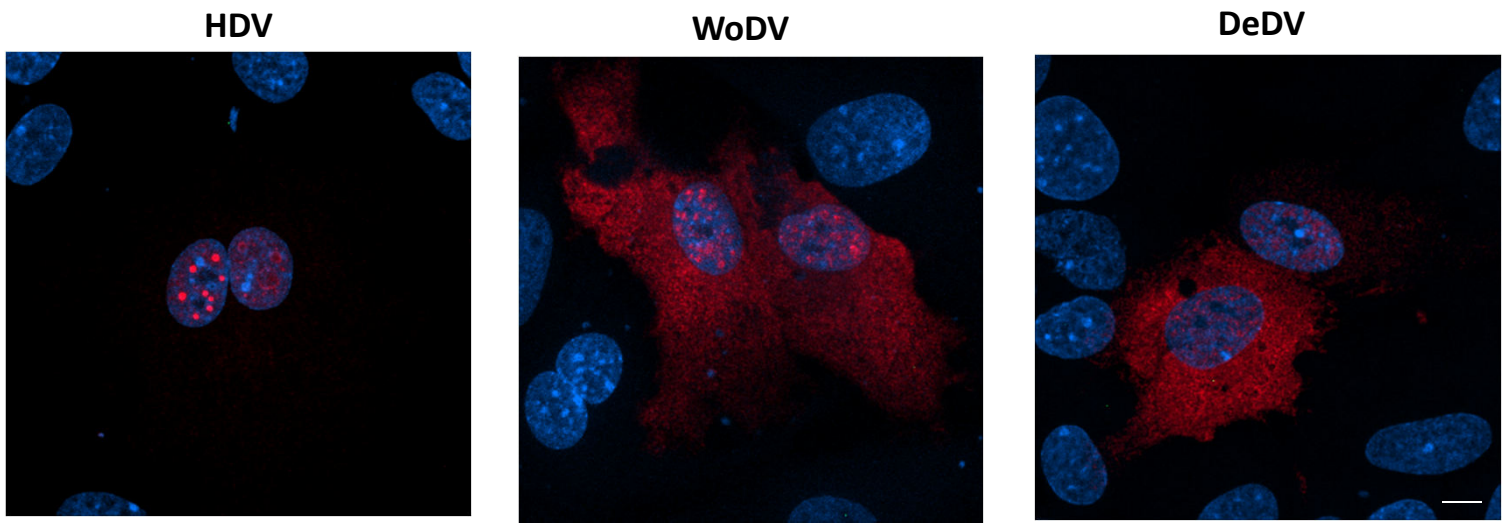
IFN does not inhibit spread in HuH7^{NTCP} cells

Gnouamozi *et al., HBV meeting 2023, Kobe*

Gnouamozi *et al., In preparation*

Viral antigen subcellular localization in infected HepaRG^{NTCP} cells

HepaRG^{NTCP} , d6 post-infection



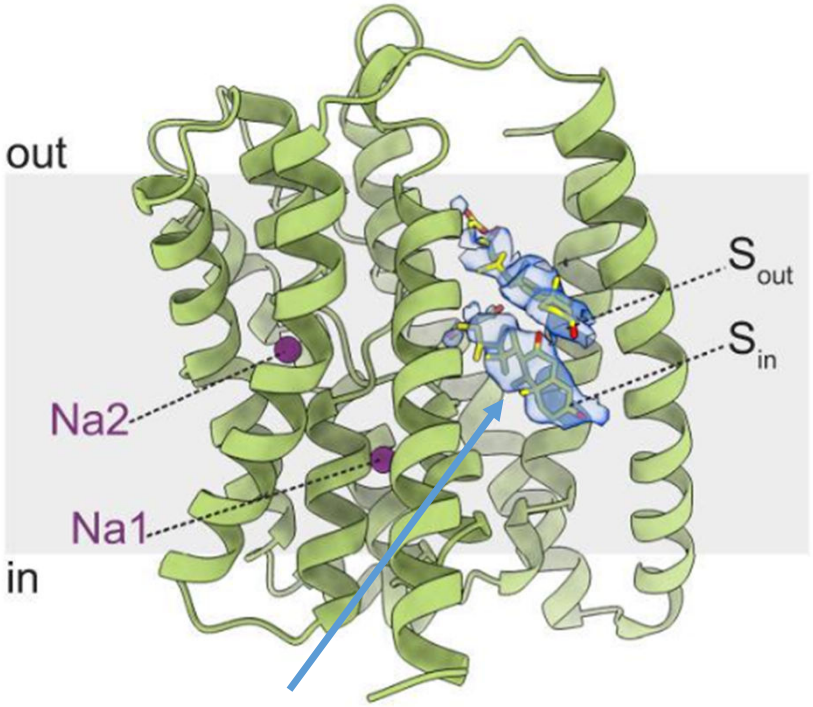
Delta antigen Nuclei Scale bar: 10 µm

- The HDV HDAg exhibits predominantly nuclear staining in infected cells
- WoDV and DeDV DAg are also widely distributed throughout the cytoplasm

A possible role for DAg in counteracting the innate immune system.

Structure model and CryoEM-structure of the HBV/HDV receptor NTCP *(SLC10A1)*

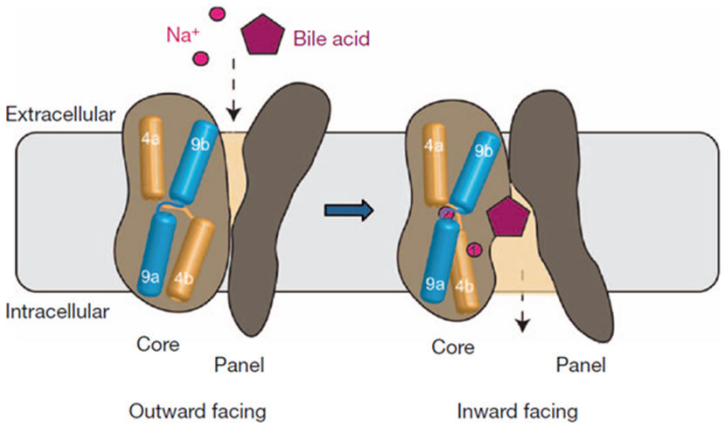
Model of the structure of **sodium Taurocholate Co-transporting Polypeptide NTCP:**
with the location of a double bile salt pocket



Two bile salt molecules

NTCP

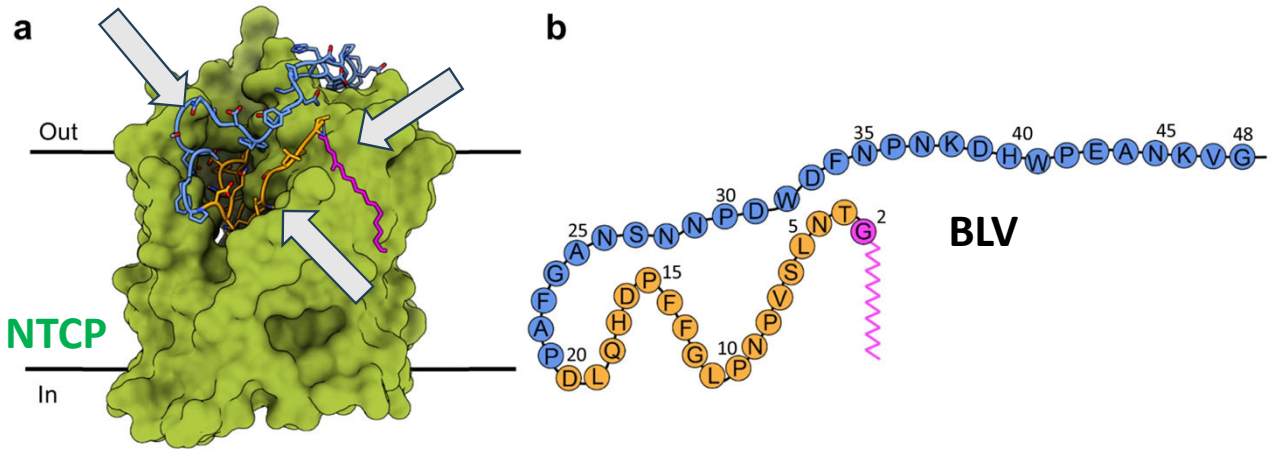
- Integral transmembrane protein (9 TM helices)
- Transports bile salts through PM into hepatocytes
- Conformational change driven by sodium Ion gradient



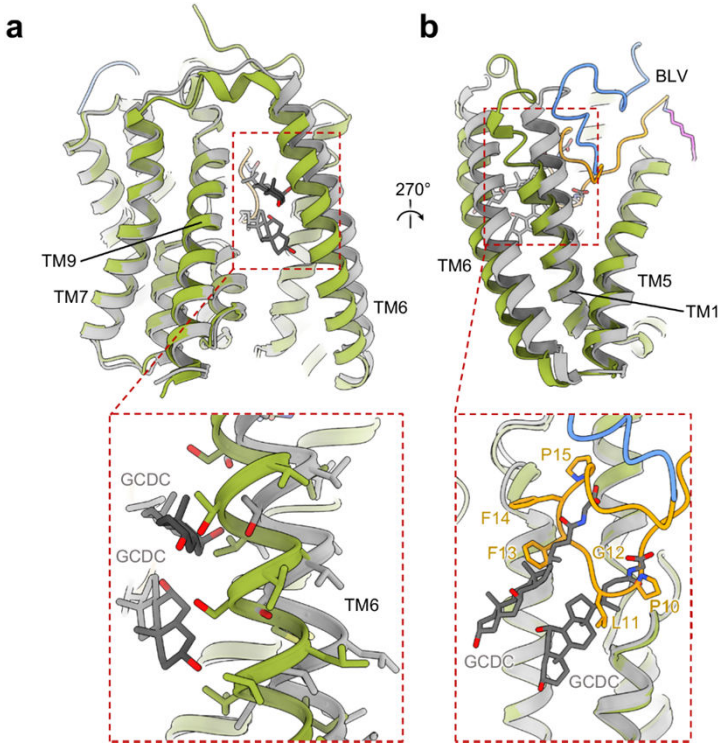
Liu et al., Cell Res 2022

Goutam et al., Nature 2022; Park et al., Nature 2022; Asami et al., Nature 2022; Liu et al., Cell Res 2022

Cryo-EM structure of bulevirtide/Hepcludex bound to NTCP



- **Bulevirtide: myristoyl anchor, plug domain and string domain**
- The **myristoyl anchor** locates sidewise intruding the lipid bilayer and positioning Glycin-2 at the external entrance of the tunnel.
- The **plug domain (Gly2-Asp20)** wedges inside the translocation tunnel, deeply intruding the protein by making several specific contacts.
- **The string domain (Pro21-Gly48)** covers the surface of the plug and bridges the extracellular surface of NTCP ("clasping" the molecule)



Superposed BLV-bound NTCP (green) and substrate-bound NTCP (gray).

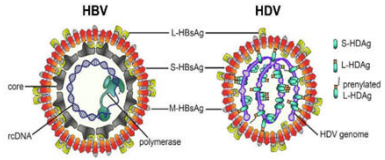
Binding of BLV to NTCP displaces the core alpha helices

→ Resulting in a shift of the panel domain.

→ blocking bile acid and sodium ion binding.

Liu et al., Nat Communication 2024

Summary, conclusions, take home messages



- HBV and HDV share the same envelope proteins which are provided by HBV integrates or cccDNA.
- HBV DNA readily integrates into host chromosomes and mediates superinfection exclusion of HBV but not HDV
- Expression of envelope proteins restricts the replenishment of cccDNA by preventing NC-(re)import
- HDV genomes disseminate by an extracellular route (intrahepatic and between hosts) and by cell-division mediated spread (intrahepatic).
- Cell division mediated spread of HDV but not WoDV and DeDV are sensitive to IFNs.

Clinical implications:

In addition to entry inhibition with BLV (or upcoming neutralizing antibodies), acceleration of HDV clearance by inhibitors that prevent cell-division mediated spread may allow elimination of HDV even in the presence of HBsAg.

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Groups **Lohmann and Ruggieri**

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