# Hepatitis B and D virus

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Internal Medicine II / Molecular Biology

## The alphabet of hepatitis viruses

**Hepatitis Viruses:** Primarily hepatotropic viruses causing hepatitis

**Hepatitis:** Inflammation of the liver parenchyma, destruction of hepatocytes

*(Symptoms: Jaundice [Ikterus], elevated transaminases in the blood)*

<table>
<thead>
<tr>
<th>Hepatitis A Virus (HAV)</th>
<th>Picornaviridae, 7.5 kb ss (+) RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B Virus (HBV)</td>
<td>Hepadnaviridae, 3 kb RC-DNA</td>
</tr>
<tr>
<td>Hepatitis C Virus (HCV)</td>
<td>Flaviridae, 9.5 kb ss (+) RNA</td>
</tr>
<tr>
<td>Hepatitis D (Delta) Virus (HDV)</td>
<td>&quot;virusoid-like&quot;, 1.68 kb circular ss (-) RNA</td>
</tr>
<tr>
<td>Hepatitis E Virus (HEV)</td>
<td>Calicivirus-like, 7.5 kb ss (+) RNA</td>
</tr>
</tbody>
</table>

**NO proof for hepatitis:**

[Hepatitis G Virus (GBV-C)]

GBV-A,B: likely monkey viruses;
B: closest to HCV; C: human virus
2010: GBV-D in bats
[Transfusion transmitted virus (TTV) and SEN virus]

Flaviridae, 9.5 kb ss (+) RNA

Circoviridae, 3.6 - 3.8 kb ss DNA
## Hepatitis viruses - pathogenicity overview

<table>
<thead>
<tr>
<th>Transmission</th>
<th>acute hepatitis</th>
<th>severe sequelae of acute inf.</th>
<th>chronic hepatitis</th>
<th>cirrhosis, HCC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HAV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oral-faecal (enteral)</td>
<td>YES</td>
<td>rare</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>(travel hepatitis)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HBV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>parenteral (serum hepatitis)</td>
<td>YES</td>
<td>rare</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>(blood, blood products, other body fluids)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HDV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>as HBV/together with HBV (HBV envelope!)</td>
<td>YES</td>
<td>increased</td>
<td>YES</td>
<td>YES increased</td>
</tr>
<tr>
<td><strong>HCV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>parenteral (transfusion hepatitis)</td>
<td>YES</td>
<td>rare</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>(blood, blood products, etc.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HEV</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>oral-faecal</td>
<td>YES</td>
<td>rare</td>
<td>NO</td>
<td></td>
</tr>
</tbody>
</table>

*Zoonosis - in DE >10% of wild boars are HEV+*

## Hepatitis viruses - pathogenicity

### Chronic hepatitis: >400 million carriers worldwide

- Chronic hep B: ~250 million *(possibly more - proper estimates difficult!)*
- Chronic hep C: ~180 million

- Healthy liver
- Cirrhosis: functional hepatocytes replaced by connective tissue
- Primary liver carcinoma *(hepatocellular carcinoma, HCC)*

**Immunopathogenesis,**

- Viruses are not cytotoxic
  - 15 - 25% of chronic carriers within 20 - 40 years;
  - hepB: 1 million deaths per year
HBV - prophylaxis and therapy

Prophylactic active immunization (effective in >95% of vaccinees):
- early on: HBsAg (empty viral envelopes) from chronic carriers
- later: recombinant HBsAg from yeast

Prophylactic passive immunization:
- human anti-HBsAg immunoglobulin (from vaccinees)
- post-exposition-, post-transplantation-prophylaxis (needlestck injuries!)

For prevention of perinatal transmission: combined active + passive immunization

Therapy of chronic hep B:
- Pegylated (polyethyleneglycol-linked) type I - interferon (IFN-α)
- 5 approved chemotherapeutics: all RT-inhibitors (nucleot(s)ide analogs)
  - Lamivudine, Adefovir; newer: Telbivudine, Entecavir, Tenofovir

Overall sustained viral responses in <40% of treated patients

Major problems with current therapies

Type I - interferon
- only a fraction of patients eligible
- effective in only a fraction of patients
- very severe side-effects > reduced compliance
- limited duration of therapy (24 or 48 weeks)

Nucleos(t)ide analogs
- usually well tolerated
- resistance
- control - but no cure (life-long treatment required)
  - (therapy withdrawal: virus rebound virtually inevitable)

HBV cccDNA as persistence reservoir
HBV: Some "premolecular" history

1965 "Australia antigen"

Initial concept: host-encoded antigen overexpressed due to disease (leukemia)
Later: patient sera are infectious for chimps => Virus

1970 HB Virions visualized by EM

**Dane particles and subviral filaments and spheres (HBsAg)**

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HBV, the virus: Some molecular history

1978  First cloning and sequencing of HBV genomes (only ~3 kb!)

1984  Recombinant HBsAg vaccine from yeast
       first commercially important recombinant protein, first recombinant vaccine

1980  Duck HBV (DHBV) discovered as first avian hepatitis B virus
       > hepatotropic DNA viruses = Hepadnaviruses

1982  Hepadnaviruses replicate via REVERSE TRANSCRIPTION
       Summers J, Mason WS. Replication of the genome of a hepatitis B-like virus

       Nearly all basic discoveries in molecular HB virology were made with DHBV!

1987  Human HepG2, Huh7 hepatoma cells support HBV replication - but are NOT infectable!

2002/3  HepaRG: the first infectable cell line (yet no net amplification of virus!)

       HBV can not be propagated/amplified in cell culture!

2013/14  a change is at the horizon... see below
HBV’s narrow host-range prompted search for animal models

Ortho-Hepadnaviruses  
(\textit{mammalian hosts})

Avi-Hepadnaviruses  
(\textit{avian hosts})

\textit{woodchuck}

\textit{Pekin-duck}

\textit{Grey heron}

+ snow goose, stork, crane...

But NOT mouse, rat, chicken!

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... surprise from 2013: HBV in bats

PNAS | October 1, 2013 | vol. 110 | no. 40 | 16151–16156

Bats carry pathogenic hepadnaviruses antigenically related to hepatitis B virus and capable of infecting human hepatocytes

Drexler et al.

\textit{Uroderma bilobatum}\n
"tent-making bat"  
[Panama; 18% of sera + for HBsAg]

first-hand from  
Jan Felix  
Drexler!

... possibly new theories for the origin and evolution of HBV required ...
From host organisms to molecules: HB virions and subviral particles

Empty envelopes = bulk of HBsAg
(>10,000 x excess over virions; vaccine)

A "zipped" genome: unusually small, exceptionally compact

3.2 kb: one of the smallest animal virus genomes
partially double-stranded
complete (-)-strand
incomplete (+)-Strang (distribution!)
circular, but not covalently closed:
Relaxed Circular (RC)-DNA (vs. cccDNA)

5’-end of (-)-strand is COVALENTLY linked with P protein!
Prime example for "genetic economy"

- each nt has coding function
- overlapping ORFs: > 50% of the nt are coding in 2 different ORFs
- all regulatory elements (promoters, enhancers, poly-A-signal, replication-cis-elements) overlap with ORFs
- ORFs are modularly organized: PreC/C, PreS1/PreS2/S > C and S modul are used in different proteins

The HBV replication cycle: an overview

- Primary hepatocyte, "proper" host species
A new era coming up: NTCP (SLC10A1) - the (?) HBV receptor

Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus

Yan et al. eLife 2012;1:e00049.

Hepatitis B and D Viruses Exploit Sodium Taurocholate Co-transporting Polypeptide for Species-Specific Entry into Hepatocytes

Yi Ni,1 Florian A. Lemp,1 Stefan Mehrle,1 Shirin Nkongolo,1 Christina Kaufman,1 Maria Fälth,2 Jan Stindt,3 Christian König,2 Michael Nossal,2 Ralf Kubitz,2 Holger Sültmann,2 and Stephan Urban1

some hepatoma cell lines transfected with NTCP support HBV infection!

> Stephan Urban on HBV/HDV entry
HBV transcription: Multiple transcripts from internal promoters

**Template:** nuclear cccDNA
- All known transcripts by host RNA pol 2
- All with 5´ cap and 3´ poly-A
- 4 internal promoters
  - (<> one promoter in non-coding 5´-LTR of most retroviruses)
- RNA initiation sites in front of the respective ORF
  - > just the FIRST cistron is translated (BUT: P!?)
- PreC/C and PreS/S:
  - Heterogeneous 5´ ends (+/- Pre-ATG)
- All 3´ ends colinear (just one poly-A-signal)
- Splicing is not essential (but occurs)

**General strategy for subgenomic RNAs: OWN promoters**
(vs. splicing & polyproteins in retroviruses; NO polyprotein / polyprotein processing in HBV)

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**HBV transcripts**

- Precore RNA
- Pregenomic RNA

- **2.4 kb RNA**
  - preS1/preS2/S \(\Rightarrow\) L

- **2.1 kb RNAs**
  - preS2/S \(\Rightarrow\) M
  - S \(\Rightarrow\) S

- **0.7 kb RNA**
  - X \(\Rightarrow\) X
**HBV gene products: Structural proteins - envelope**

3 envelope / surface proteins: L, M, S - all have S domain with 4 TM helices
- all partially N-glykosylated (N-X-S/T); also O-glykosylated

<table>
<thead>
<tr>
<th>Protein</th>
<th>PreS1</th>
<th>PreS2</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>L (large)</td>
<td>myr-</td>
<td>108 aa</td>
<td>55 aa</td>
</tr>
<tr>
<td>M (middle)</td>
<td></td>
<td></td>
<td>N4</td>
</tr>
<tr>
<td>S (small)</td>
<td></td>
<td></td>
<td>(N146)</td>
</tr>
</tbody>
</table>

S = major component of subviral S particles und viral envelope = HBsAg

M = S with externally located PreS2 domain

L = S with PreS1 + PreS2 domains; partially external, partially internal!
PreS is N-terminally myristoylated

myristoylated PreS-peptides inhibit infection!
"Myrcludex" as a novel entry inhibitor!

**Dual topology of PreS1: Nucleocapsid envelopment + infection**

in fact more complicated, involves ESCRT machinery!

ER lumen = virion exterior

NTCP on host cell

cytosol = virion interior
Structural proteins: Core protein - the capsid building block

Core protein

- preC
- C

Precore RNA

pgRNA

1 149 183

assembly domain nucleic acid binding domain

Capsid = HBcAg

Maria Teresa Catanese
& Francois Penin

3D structure of the assembly domain

Precore protein: alternative secretory core gene product

Precore protein = precursor to (secreted) HBeAg

Precore protein/p25

HBeAg / p17e

secretory antigen (signal sequence: 1-19 of PreC aa 1-29); DIAGNOSTICS!
almost identical to core protein - yet distinct antigenicity, no particle formation

Function unclear - possibly "tolerogen" for HBcAg = increased chance for chronicity?

Precore/HBeAg deficient HBV is viable => not essential!
**HBx protein: a longstanding mystery** *(about to be solved...)*

| X | 154 aa |

"X" because of unknown function - no homologies to known proteins/evolutionary origin??

**Avian HBVs have no X!**

**in vivo relevance:**

(woodchuck) HBV without intact X gene is not infectious (Fabien Zoulim 1994!)

=> HBx helps establishing infection - but HOW?

**in vitro:**

- moderate trans-activator for diverse promoters
- interacts with numerous diverse cellular factors (proteasome, DNA-repair...)
- Ca²⁺-release from ER, activation of diverse kinase-pathways (PKA, PKC, Src...)
- involved in cancerogenesis? - controversial!

**THE experimental drawback:** HBV plasmid transfected cells do not usually respond to HBx!

**since 2011:** HepaRG infection with "real" HBV:

- transcripational activity of cccDNA appears to be epigenetically blocked (DNA methylation / histone acetylierung/deacetylation / microRNAs?)
- X counteracts this block - HOW?

Stephan Urban & Massimo Levrero

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**P protein: an unusual Reverse Transcriptase**

**Translation:**

- no separate P mRNA - 2nd cistron on pgRNA
  (leaky scanning? reinitiation? shunting?)
- no core-P polyprotein analogous to retroviral Gag-Pol

![Diagram of P protein domains](image)

**Domains:**

- conserved motifs in Pol/RT and RH ~ retroviral RT
- But: Extra TP domain has NO known homologs/orthologs!
From pgRNA to RC-DNA: The special role of pregenomic RNA

1. Bicistronic mRNA for core- and P protein translation

   ![Diagram of bicistronic mRNA]

   - C
   - P
   - cap
   - pA

2. RNA precursor for the RC-DNA form of the HBV genome

   Nearly a self-sustaining system:
   pgRNA and its gene products core and P (+ cellular factors)
   are sufficient for genome replication

   Summers 1990:
   Chicken hepatoma cells transfected with DHBV pgRNA produce infectious DHBV!

Reverse transcription of pgRNA inside intact nucleocapsids

   ![Diagram of reverse transcription]

   - cap
   - pgRNA
   - Immature RNA nucleocapsid
   - Mature RC-DNA nucleocapsid

Requirements

- a mechanism that ensures specific encapsidation of pgRNA AND P protein

- a mechanism enabling a primer-dependent DNA polymerase like HBV P to initiate DNA synthesis on the RNA template

   both are closely related and guided by RNA structure!
Co-packaging of P and pgRNA into newly forming nucleocapsids

- pgRNA packaging is highly specific
- no pgRNA packaging w/o P
- no pgRNA packaging w/o intact 5' end
- Fusing the pgRNA 5' end to heterologous RNAs induces their packaging

An RNA stem-loop (ε) at the pgRNA 5' end serves as encapsidation signal recognized by P protein

Initiation of reverse transcription by PROTEIN-PRIMING

**Early observations**

- For HBV P no hint of any nucleic acid primer (as e.g. a host tRNA in retroviruses)
- 5'-nt of (-)-DNA is covalently linked to the TP domain of P > "Protein-priming" (phenol extraction: (-)-DNA partitions with P into organic phase!)
- P accepts no other template RNA than pgRNA (<- cDNA synthesis!)

- 5' end of (-)-DNA maps to 3' proximal DR1* (direct repeat 1)

P required at 5'ε for pgRNA packaging yet also at 3' DR1*??
**Genetic proof for $\varepsilon$ as first-strand DNA replication origin**

The UUCA motif in DR1 is also present in the $\varepsilon$ bulge - the complementary DNA sequence 3´-AAG- 5´ may be copied from 5´ $\varepsilon$?

Proof: UUC in $\varepsilon$ > UUG, sequence at (-)-DNA 5´ end: AAC, not AAG!  

$\varepsilon$ contains the replication origin for (-)-DNA. This "priming" reaction establishes the covalent linkage of P via Tyr to RC-DNA.

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**Towards a mechanistic understanding of (D)HBV replication**

The basic "in vitro priming assay" in Rabbit Reticulocyte Lysate (RRL; in vitro translation extract)

Initial improvements
- $\varepsilon$ RNA stem-loop is sufficient
- Bacterially expressed DHBV (but not HBV) P protein shows activity in RRL

> Mutational analysis of variations in $\varepsilon$ RNA and P protein > SAR
> RRL must provide important factors other than translational machinery - WHAT ARE THEY?
Cell chaperones are required for in vitro priming activity

Complete in vitro reconstitution

> the essential cell factors are Hsp70+Hsp40+ATP - Hsp90+Hop stimulate activity

Stahl, Beck, MN
NAR 2007

Priming is dynamic - with alterations in P protein and ε RNA

Chaperone activation transiently opens an ε RNA binding pocket between TP and RT domains

Productive interaction with P restructures the RNA - only then can it serve as template

Pb²⁺ probing of free vs. protein-bound RNA
A mechanism for replication initiation - based on DHBV in vitro priming

DHBV in vitro priming has yielded lots of basic information on hepadnaviral P protein and ε RNA, their interactions, and contributed potentially clinically relevant findings ... *(priming inhibitors)*

*Is this relevant for *human* HBV?*

*Since 1990ies no progress in getting *human* HBV to show in vitro priming...*

*New concept:* Higher stability of human HBV ε prevents RNA rearrangement

The HBV ε stem-loop is much more stable than that of DHBV ε

*Destabilizing the upper stem may facilitate rearrangement into the priming-active conformation - and enable HBV in vitro priming??*
Two destabilizing mutations in $\varepsilon >$ HBV in vitro priming activity

Pool of HBV vectors with partially randomized $\varepsilon$ upper stem

Transfect Huh7 cells; reamp virion DNA

in vitro priming

23 non-wt replication-competent, with 3 to 7 mutations

65.000

the closest to wt, 2 mutations

K. Dümbrack, PhD thesis 2014

The high stability of the HBV $\varepsilon$ upper stem prevents in vitro priming

WT HBV $\varepsilon$

upper stem-destabilized $\varepsilon$

by mutation, or by physical disruption of the RNA backbone (synthetic "split $\varepsilon$")

$\rightarrow$ human HBV P-$\varepsilon$ structure-activity relations

$\rightarrow$ P-$\varepsilon$ complex structure by chemical biology

$\rightarrow$ HBV priming inhibitors

What makes WT HBV $\varepsilon$ work in cells? Helicase? RNA chaperone? Milieu?
From protein-priming to full-length (-)strand DNA

**Initiation**

1. template switch to 3’DR1
5’ end of (-)DNA templated by ε

(-)DNA synthesis

extension of (-)-DNA to 5’ end of pgRNA template
Degradation of copied pgRNA by RNase H, except very 5’ end with DR1

(+)DNA synthesis

remaining 5’ end of pgRNA as conventional nucleic acid primer

w/o 2nd template switch:
ds linear DNA as by-product*

in situ priming
ds linear DNA

cells don’t like free DNA ends - degradation or repair:
circularization (with loss of some nt) or integration (> pathogenesis...)

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From single-stranded (-) DNA to partially double-stranded RC-DNA

**Proper (+)-DNA synthesis**

2. template switch:
RNA primer from DR1 to DR2
(+)-DNA extension to the 5’ end of the (-)-DNA template

“r” small redundancy at 5’ & 3’ end of (-)-DNA

3. template switch:
(+)-DNA 3’ end from (-)-DNA 5’r to 3’r

relaxed circular partially ds DNA
with covalently linked P protein
= RC-DNA

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for reviews see:
Beck & Nassal, World J Gastro 2007
Nassal, Virus Research 2008
How from nucleocapsid-borne RC-DNA to episomal cccDNA?

Why is cccDNA so important?

- cccDNA is the template for all viral transcripts
- cccDNA is not directly targeted by current anti-HBV drugs
- a few copies of cccDNA per liver can re-activate full infection

CONTROL - but no CURE

- any CURE of chronic hepatitis B will require elimination of cccDNA
- understanding cccDNA formation is mandatory for a cure!
Why is elucidating RC-DNA to cccDNA conversion difficult?

- Nearly complete lack of pre-existing mechanistic knowledge
- Little if any cccDNA formation in HBV-transfected cells
- Multiple steps must be involved
- Given HBV’s tiny genome, most/all activities must come from the CELL

What must happen for RC- to cccDNA conversion?

RC- vs. cccDNA structure predicts *multiple* steps:

- removal of non-DNA moieties:
  - P protein
  - RNA primer
- generation of *exactly one* equivalent of unit length ds-DNA (no extra nt, no nt missing)
  of ligation-compatible ends: 5′ phosphate, 3′ OH
- ligation of the ends of both strands
RC-DNA has several features of "damaged" (= non-perfect ds-) DNA

Non-perfect dsDNA provokes host DNA damage repair

DNA damage response involves >250 known factors which:
- sense DNA anomalies
- signal to activate repair, temporarily halt the cell cycle, or eventually induce apoptosis
- remove the lesions + restore perfect double-stranded DNA structure ("core repair factors")

~20-25 core repair factors are conceptually relevant for RC > cccDNA conversion
P protein-linked RC-DNA: a multi-target DNA repair substrate

Analogies to repair of cellular DNA anomalies suggest numerous candidate repair factors

1st focus on P protein release!

Are there structural look-alikes amongst cellular DNA damage products?

Trapped topoisomerase cleavage complexes - analogs of RC-DNA?

One type of repair for TOP1 & TOP2 cleavage complexes is **nucleolytic**
(various repair nucleases, incl. MRE11 in the MRN complex)

another involves **hydrolysis** of the Tyr-3’ / Tyr-5’ DNA phosphodiester bond by Tyrosyl-DNA-phosphodiesterases (TDPs)

**Literature:**
TDP1 for Tyr-3’-DNA bonds - but yeast TDP1 reportedly active on Tyr-5’-DNA
TDP2 (only discovered in 2009) preferentially for Tyr-5’-DNA - but also some activity on Tyr-3’-DNA

> investigate both
A functional role for TDP2 (but not TDP1) in P protein release from RC-DNA

A) In vitro 5' vs. 3' substrate specificity of recombinant human, chicken, yeast TDP1 and TDP2
- on synthetic model substrates, on 32P in vitro primed P protein > TDP1 out
- on nucleocapsid-borne RC-DNA

RNAi knockdown of huTDP2 impairs RC-DNA to cccDNA conversion in cells

THE problem:
How to determine a reduction in cccDNA when there is (almost) none to begin with?

Once again: DHBV to the rescue

Well known:
- DHBV in transfected avian hepatoma cells => >10 copies cccDNA/cell
- preventing virion secretion by env-ko => >100 copies cccDNA/cell

surprisingly, this works also with DHBV in human hepatoma cells

Stable shRNA knockdown of TDP2 in cccDNA producing cells

- stably anti-TDP2 shRNA expressing HepG2 cell lines: 80-90% less TDP2

cccDNA kinetics in naive HepG2 vs. TDP2 knockdown cells

<table>
<thead>
<tr>
<th></th>
<th>day 2</th>
<th>day 3</th>
<th>day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>HepG2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ccc</td>
<td></td>
<td></td>
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</table>

Königer, Wingert, Marsmann, Rössler, Beck, MN. Involvement of the host DNA repair enzyme TDP2 in formation of the cccDNA persistence reservoir of hepatitis B viruses. *PNAS*, 2014 Oct 7;111(40):E4244-53

TDP2 knockdown-dependent delay is highly significant

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TDP2 - the beginning of uncovering HBV’s interaction with DNA repair

Why is RC>cccDNA conversion delayed but not completely blocked?

- residual cccDNA formation due to residual TDP2?
- or an alternative repair (perhaps nucleolytic) pathway steps-in for TDP2?

> hepatoma cells with TDP2 knock-out rather than knock-down

- preliminary evidence: alternative repair pathways CAN CONTRIBUTE to P protein release
- the other steps in RC to cccDNA conversion require alternative repair factors anyway!

... more work to come (and to be funded...)
Major open issues in basic HBV research

Persistence vs. HBV cure
- What is the complete pathway from RC to cccDNA? Which host factors involved?
- How does HBx regulate cccDNA transcriptional activity?
- Can cccDNA be directly targeted / destroyed?
- Why do innate and adaptive immune responses fail in chronic hep B? Saturday talks!

Mechanisms of entry, nucleocapsid release and genome uncoating
- Is NTCP the only receptor for HBV (and HDV)?
- What are potential co-receptors and intracellular dependency and/or restriction factors that determine HBV host-range?
- How, where, does the HB virion loose its envelope for cytoplasmic release of the nucleocapsid? What is the "fusion protein / fusion peptide"?
- What triggers nucleocapsid disassembly for release of RC-DNA into the nucleoplasm?

Small animal model(s) for human HBV infection? Marcus Dorner

HDV comes (also here) into play =>

HDV - a mammalian virusoid
Hepatitis D virus - pathogenicity overview

- >5% of all chronic HBV carriers are coinfected with HDV
- NO monoinfection with HDV because HDV requires HBV for infectivity
- HBV/HDV coinfection exacerbates clinical consequences of hep B
  - more severe course of acute infection, more fulminant cases
  - higher incidence of cirrhosis, HCC
  - poorer response to therapy
- HDV usurps the envelope of HBV:
  - vaccination against HBV protects from HDV infection!
- There is NO HDV-specific therapy yet

Basic molecular features of Hepatitis D (Delta) Virus

- 1.7 kb circular (-)-RNA, rod-shaped due to ~75 % base-pairing
- The only known "virusoid-like" agent in mammals
  (virusoids = satellite RNAs that depend on a helper virus are common in plants;
  vs. viroids - noncoding, naked, autonomously replicating RNAs
- HDV encodes only ONE protein: H\(\delta\)Ag
- H\(\delta\)Ag occurs in 2 forms: H\(\delta\)Ag-S vs. H\(\delta\)Ag-L, generated by RNA editing
- HDV usurps the envelope of HBV to form infectious particles
- Once in a cell, HDV does NOT require HBV for replication
- Replication via rolling-circle > concatemeric linear ss-RNA
- Unit-length RNAs via built-in self-cleaving ribozyme
  (only known mammalian virus ribozyme)
Schematic comparison HB vs. HD virion

**HBV**
- ENVELOPE
- CORE-particle

**HDV**
- H\(\delta\)Ag - anti-genome RNP complex
  \(-70\) molecules \(\delta\)Ag/RNA

HBV SVPs + HD virions

HBV L is not required for HDV morphogenesis but for infectivity

HDV RNAs: Genome, anti-genome, H\(\delta\)Ag mRNA

- **Genome (in virions):** (-)-polarity
  - Replication
  - Transcription
  - self-cleaving ribozyme
  - \(~300,000\) copies per cell

- **Anti-genome:** (+)-polarity
  - Replication
  - Transcription
  - \(~50,000\) copies per cell

- **H\(\delta\)Ag 0.8 kb mRNA**
  - Replication
  - Transcription
  - poly-A Signal
  - \((A)\_n\)
  - \(~500\) copies per cell
**HDV RNA amplification via rolling circle replication**

**Rolling-circle:**

- Circular genomic RNA (-)
- 3' → 5' self-cleavage by built-in ribozyme
- Concatemeric, linear anti-genome RNA
- 3' → 5' end ligation
- Circular anti-genomic RNA (+)

**HDV genome replication vs. HδAg mRNA transcription**

- Circular genomic RNA
- mRNA initiation as for anti-genome
- BUT: polyA-signal is observed > termination and poly-adenylation!

**Regulation mRNA transcription vs. replication: 2 models**

1) HδAg-S is essentiell for replication:
   HδAg-S suppresses polyA-signal during anti-genome synthesis via cellular RNA pol II

2) RNA pol II (for mRNAs) respects polyA-Signal, RNA pol I (for rRNA) does not:
   anti-genome by RNA pol I, mRNA by RNA pol II... (M. Lai vs. J. Taylor)
Peculiarities of HDV vs. "normal" RNA virus replication

1. RNA > RNA

*Common RNA viruses:* have their own RNA-dependent RNA Polymerase (RdRP)
(no RdRP in mammalian cells)

HDV: encodes only HδAg; HBV-"helper" has no RdRP (RT=RdDP)

➢ HDV usurps cell DNA-dependent RNA pol to use HDV-RNA as template

**HDV RNA mimics a ds-DNA Promoter!**

2. Concatemeric linear HDV RNA cleaves itself

**HDV-RNAs (genome & anti-genome) contain a RIBOZYME!**

3. Ligation of the unit-length HDV-RNAs is likely by cellular ligase activity
   (not yet identified)

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**HDV Ribozyme: Complex 3D structure from RNA, not protein**
**HDV RNA Editing: Two distinct proteins from ONE ORF**

- HδAg-S: 195 aa - essential for replication (early)
- HδAg-L: 214 aa - essential for virion formation (late)
  - inhibitory for replication

Codon 196 = UAG = STOP

- HδAg-S: Codon 196 = UAG = STOP
- HδAg-L: Codon 196 = UGG = W

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**HDV RNA Editing: ADAR1 (Adenosine Deaminase Acting on RNA)**

ADAR1 deaminates the A in UAG to UIG; I is read as G in the next round

The ADAR1 substrate is the HDV anti-genome

- UAG anti-genome
- UIG anti-genome
- "ugg" genome
- UGG mRNA

Given the opposing functions of HδAg-S vs. -L editing must be well regulated - how is not finally settled
HDV as a tool in HBV research

Beyond HDV’s relevance as a pathogen "on its own":

HDV as a surrogate model for HBV infection

uses the envelope of HBV, needs PreS1 and human (hu) NTCP for infectivity like HBV

>100,000x amplification of HDV RNA upon cell entry > unmatched sensitivity

but from recent results

huNTCP+ HepG2 cells are well infectable by HBV and HDV

huNTCP+ Huh7 cells are well infectable by HDV but not HBV

huNTCP tg mice are apparently infectable by HDV but not HBV

After the initial events the pathways for HBV vs HDV entry likely diverge

more probably by Stephan Urban and Marcus Dornen

Acknowledgements

Former and current lab members

Jürgen Beck
Katharina Dönmbrack
Christian Königer
Ida Wingert
Christine Rösler
Kai Dallmeier

Colleagues from DFG FOR 1202 "Persistence of hepatotropic viruses"

Colleagues from "other places"

Bettina Böttcher, Edinburgh
Adam Zlotnick, Bloomington
Dan Loeb, Madison

& Hubert Blum

Funding

DFG, Infect-ERA

... and Fabien & Darius for inviting me