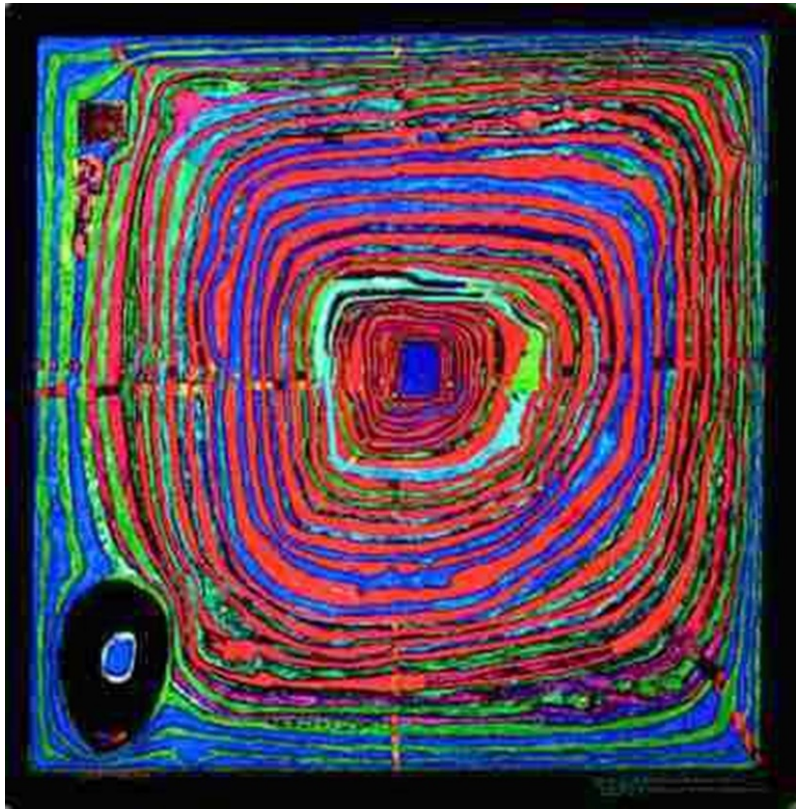
New insight on HBV particle trafficking

Eberhard Hildt,
Department of Virology, PEI



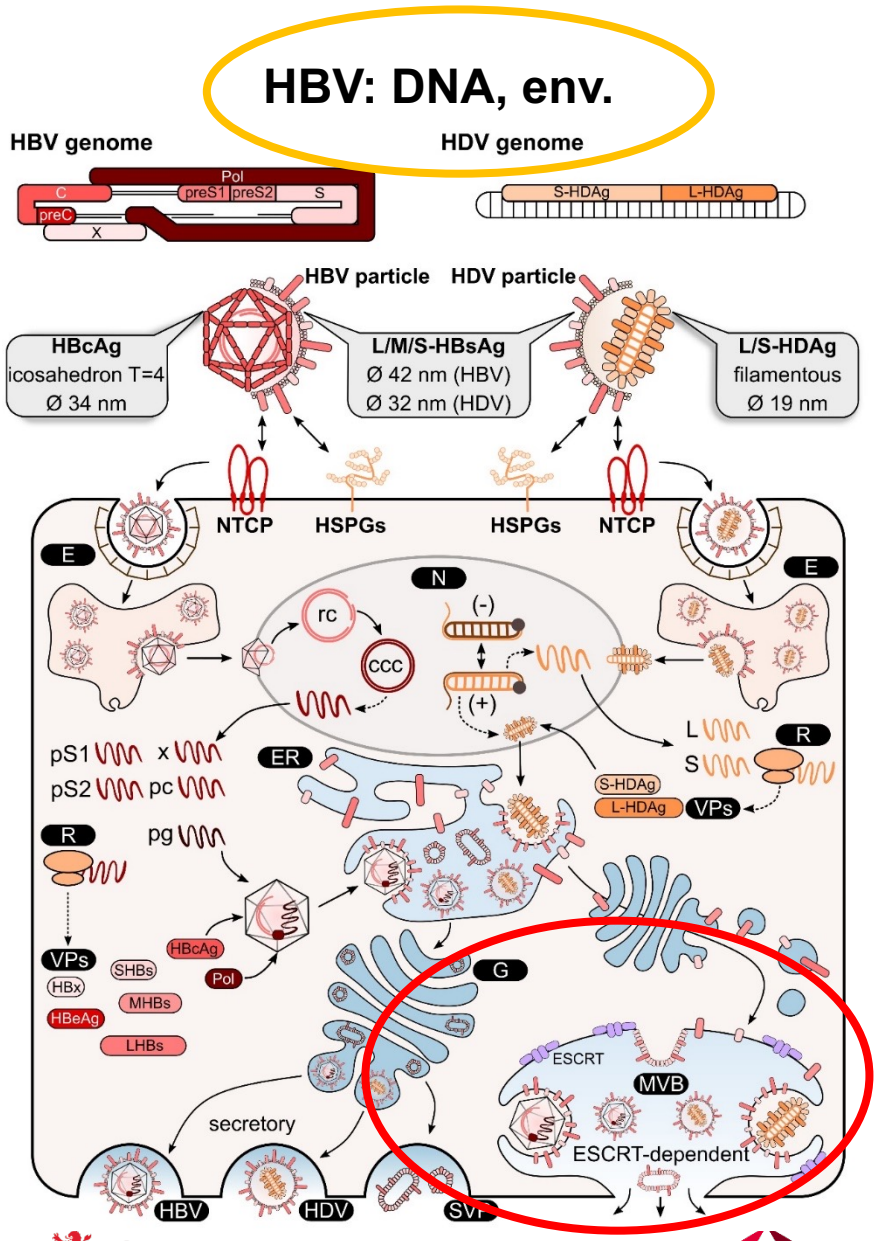
F. Hundertwasser: Der große Weg

Characterization of factors affecting egress of Hepatitis B virus

general aspects

Morphogenesis and release of viral and subviral particles

Exosomal release of HBV



specific aspects

Viral factors controlling formation of HBsAg

Chronic infection-sources of HBsAg

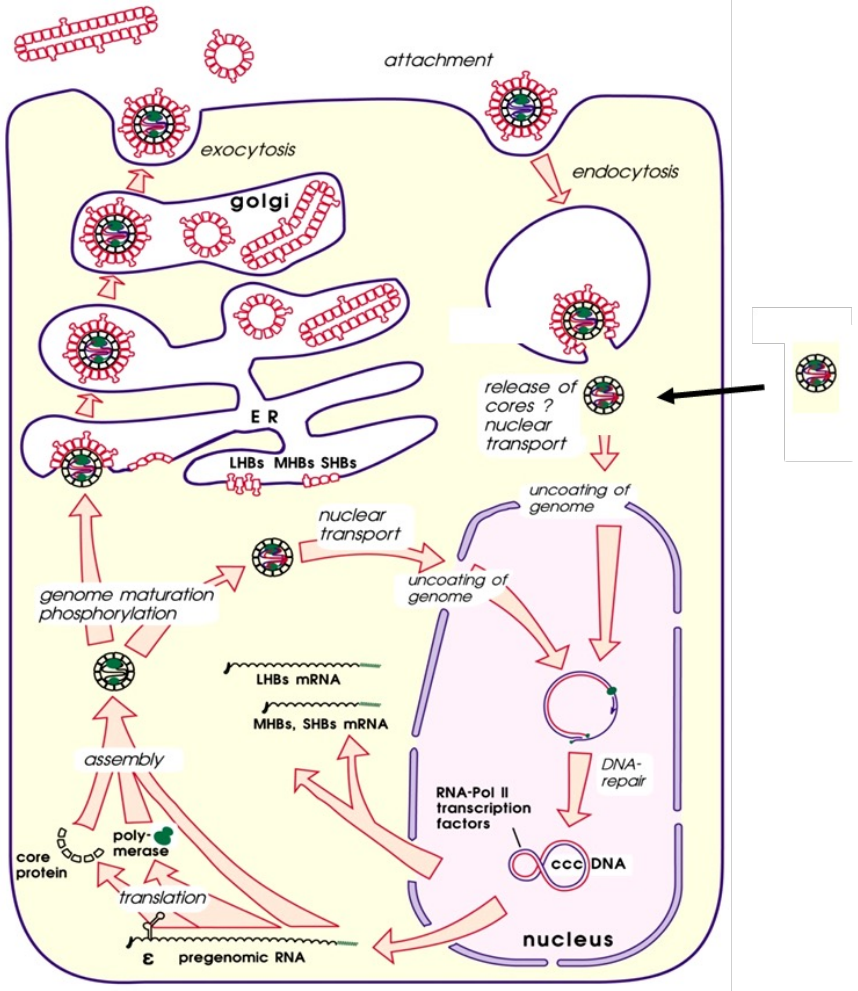
GOETHE UNIVERSITÄT FRANKFURT AM MAIN

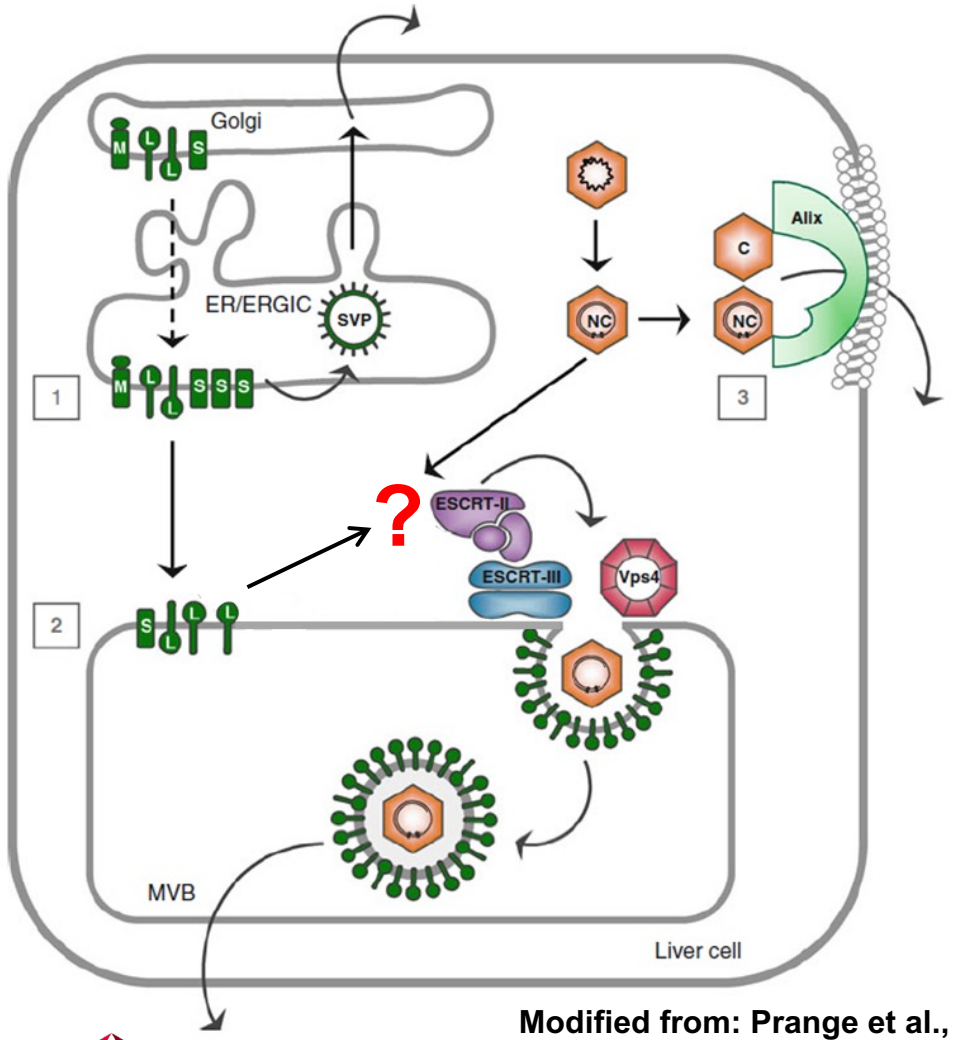
LOEWE Exzellente Forschung für Hessens Zukunft

DRUID

Paul-Ehrlich-Institut

HBV life cycle (old and new model): new: Viral particles, subviral particles and naked capsids leave the cell on different routes: MVB-dependent release of HBV

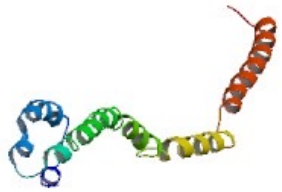




Modified from: Prange et al., 2012

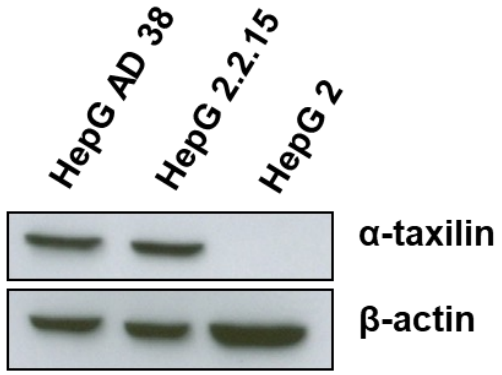
α-Taxilin

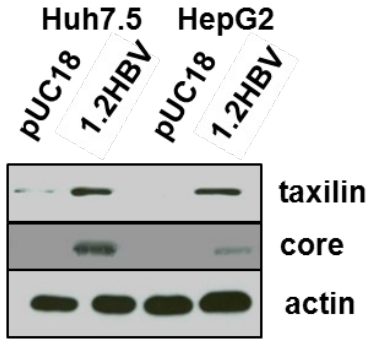
- Ubiquitously expressed protein of 62 kDa (app. MW 70 kD)
- C-terminal long coiled-coil domain
- No transmembrane domain
- Interacts with free Syntaxin 4 that is not part of the SNARE complex
- Increased expression in HCCs
- Decreased expression in HCV replicating cells

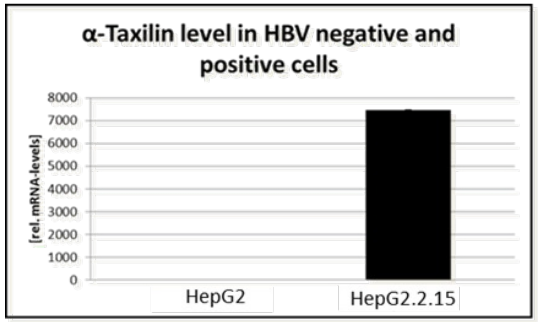


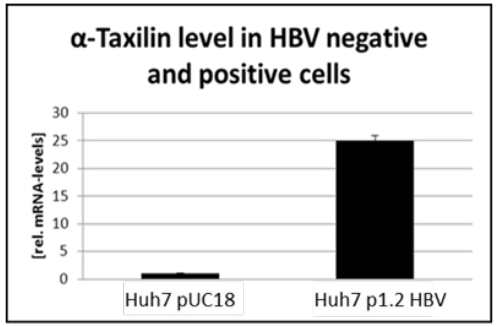
HBV expression increases the amount of α-Taxilin

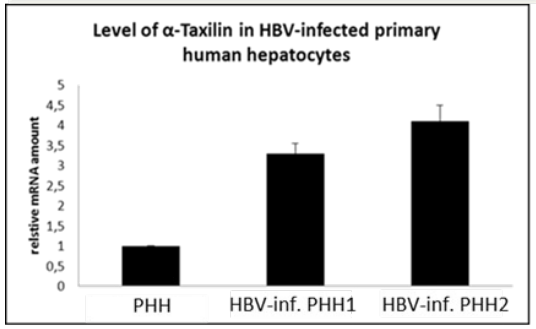
Western blot analysis and real-time PCR of HBV-expressing cells

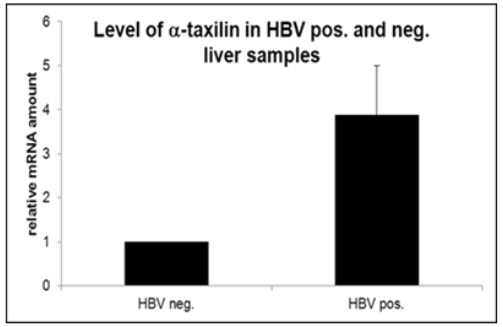






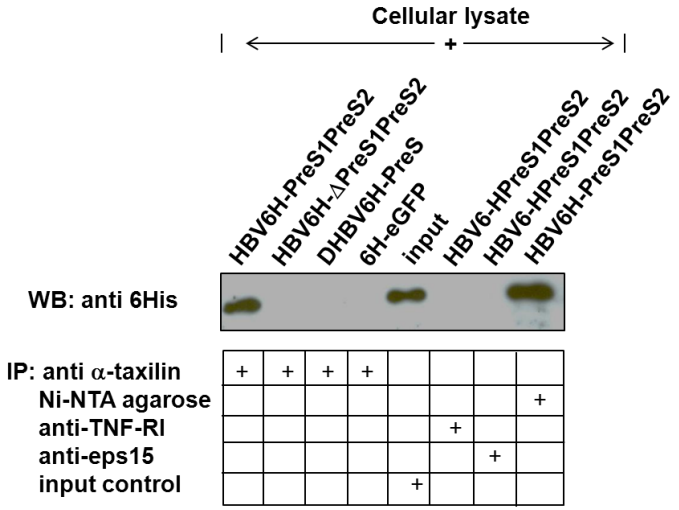


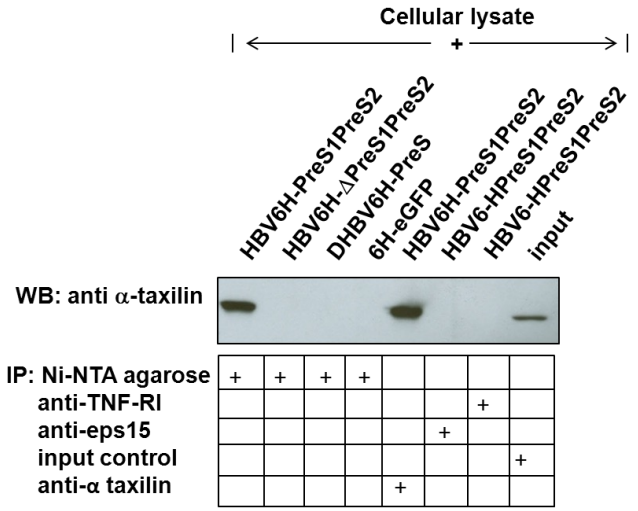


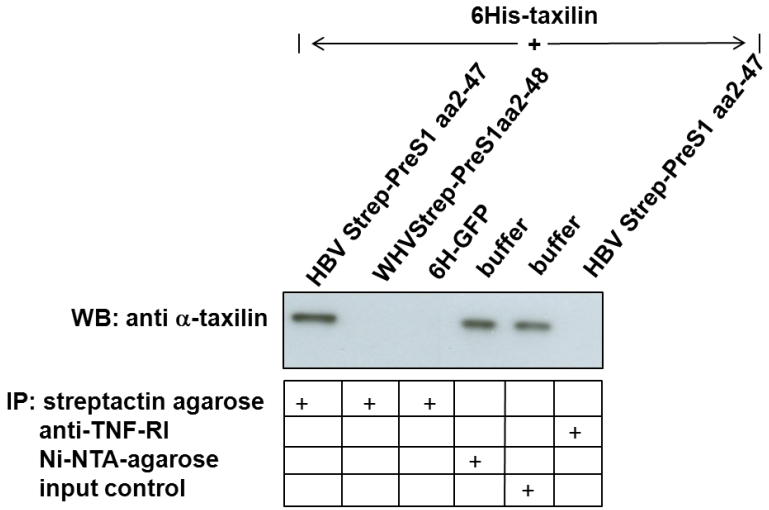


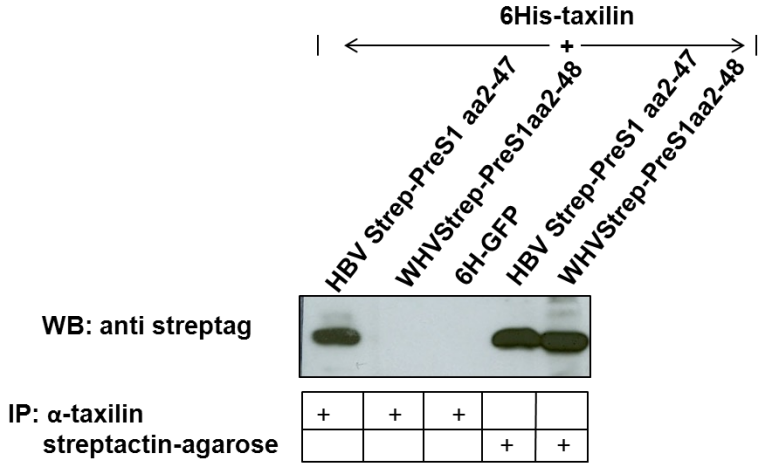
Human α-taxilin interacts specifically with the receptor-binding-domain of LHBs from HBV, but not from WHV or DHBV

CoIPs of human a-taxilin with various PreS-specific peptides









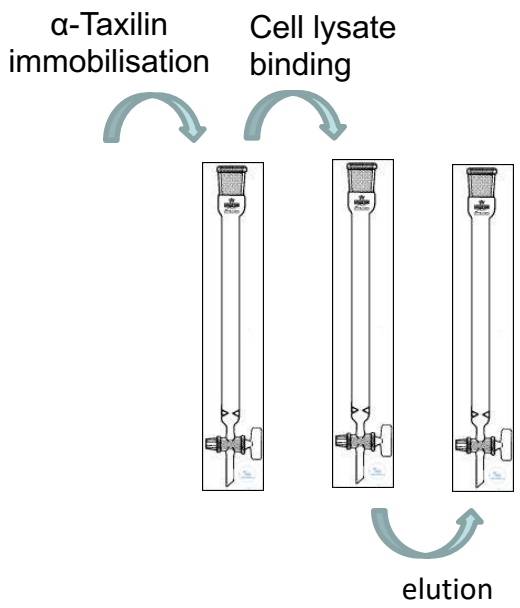
Taxilin harbours PXXP-motives and a YXXL-motive

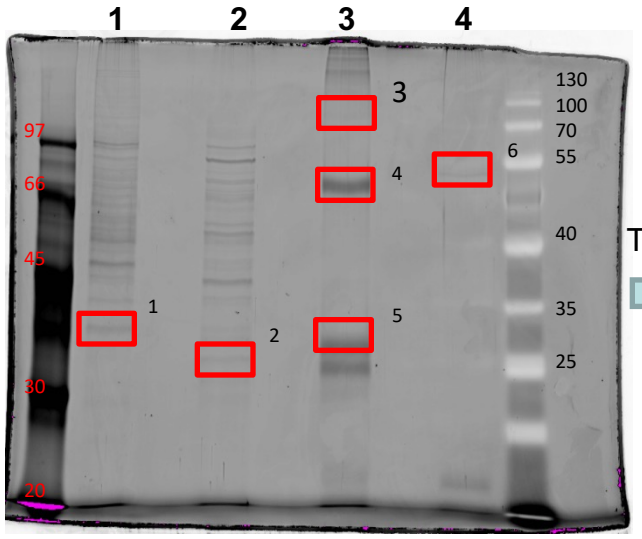
The YAEL motive is homologous to the late domain identified in EIAV

MKNQDKKNGA AKQSNPKSSP GQPEAGPEGA QERPSQAAPA VEAEGPGSSQ APRKPEGAQA
RTAQSGALRD VSEELSRQLE DILSTYCVDN NQGGPGEDGA QGEPAEPEDA EKSRTYVARN
GEPEPTPVVN GEKEPSKGDP NTEEIRQSDE VGDRDHRRPQ EKKKAKGLGK EITLLMQTLN
TLSTPEEKLA ALCKKYAELL EEHRNSQKQM KLLQKKQSQL VQEKDHLRGE HSKAVLARSK
LESLCRELQR HNRSLKEEGV QRAREEEEKR KEVTSHFQVT LNDIQLQMEQ HNERNSKLRQ
ENMELAERLK KLIEQYELRE EHIDKVFKHK DLQQQLVDAK LQQAQEMLKE AEERHQREKD
FLLKEAVESQ RMCELMKQQE THLKQQLALY TEKFEEFQNT LSKSSEVFTT FKQEMEKMTK
KIKKLEKETT MYRSRWESSN KALLEMAEEK TVRDKELEGL QVKIQRLEKL CRALQTERND
LNKRVQDLSA GGQGSLTDSG PERRPEGPGA QAPSSPRVTE APCYPGAPST EASGQTGPQE
PTSARA

The ESCRT component tsg101 is binding to α-Taxilin

Highly purified a-taxilin was immobilized on a column.
Specifically binding proteins were analyzed by mass spectrometry.



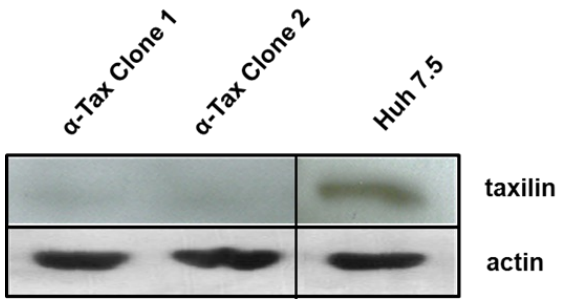


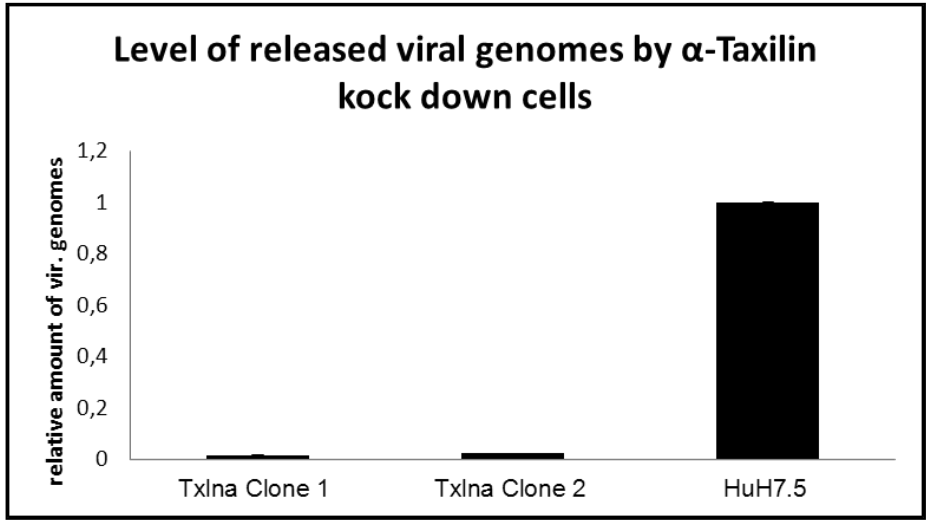
Trypsin digestion → Mass spec.

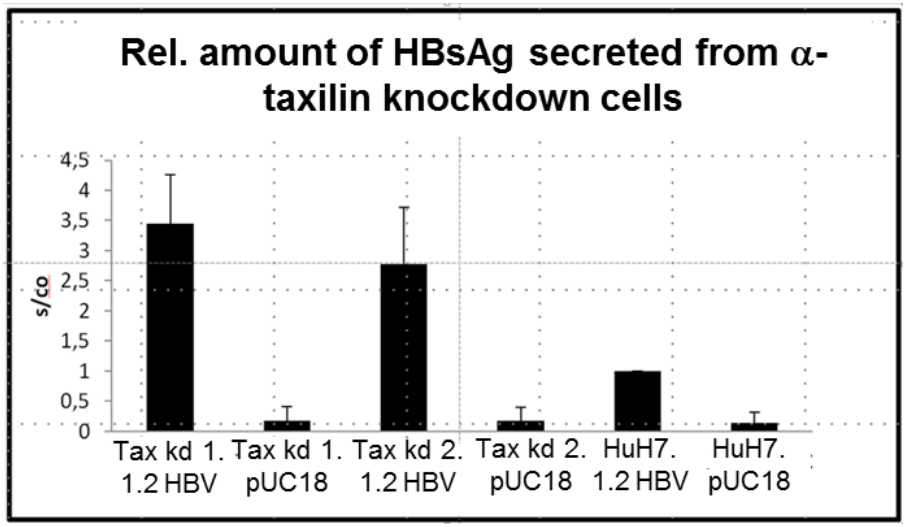
→ **Identification of tsg101 as a specific binding partner**

α-Taxilin knock down results in impaired release of viral particles, but increased secretion of HBsAg

Expression of α-taxilin was silenced. The effect on HBV replication was analyzed by HBsAg-specific ELISAs and by real-time PCR for quantification of released viral genomes.

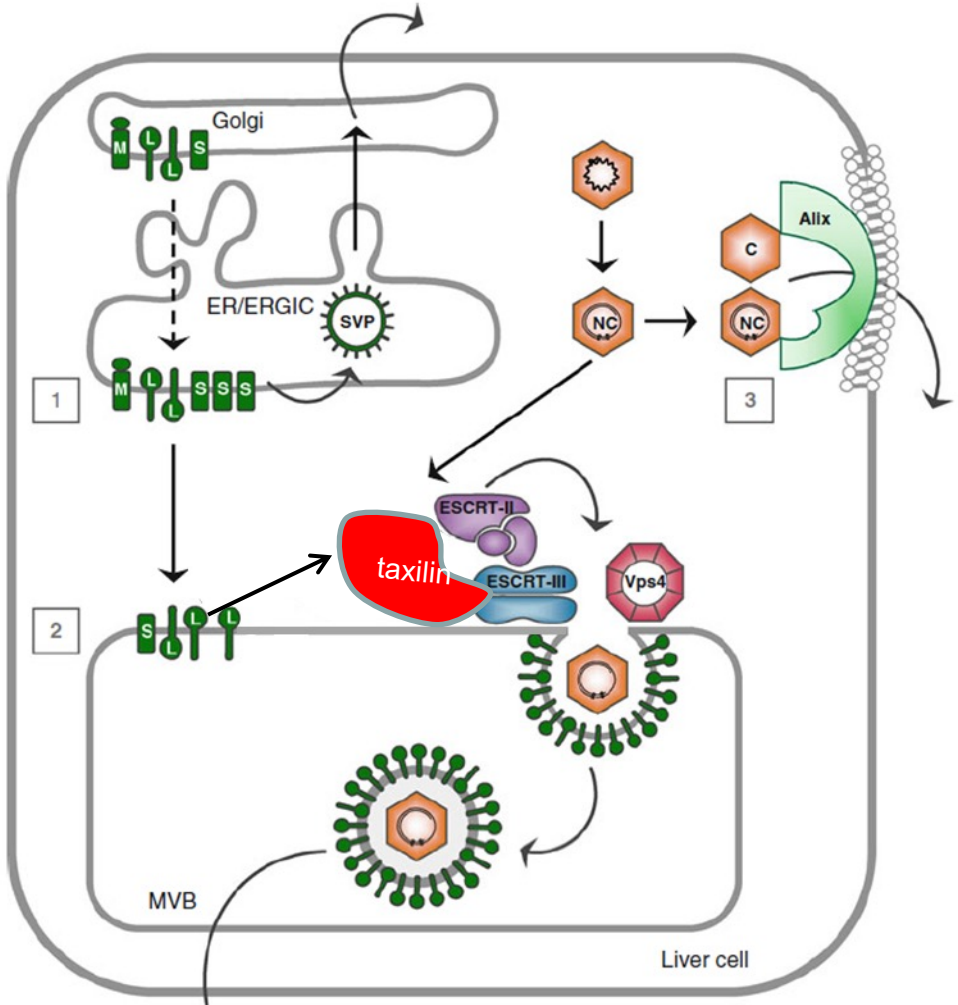






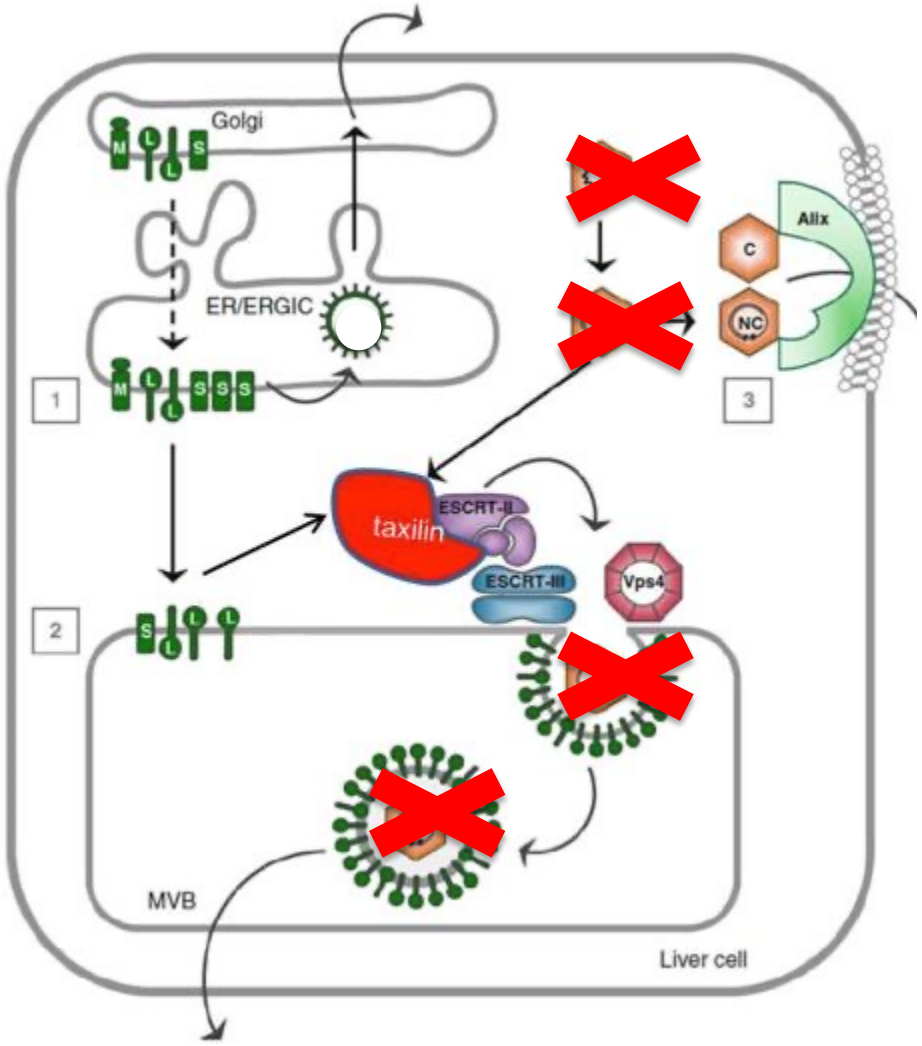
Summary I

By binding to the PreS1 domain of LHBs and to the ESCRT I component tsg101, α-Taxilin enables the MVB-dependent release of HBV



Does the release of filaments depend on the functionality of MVBs?

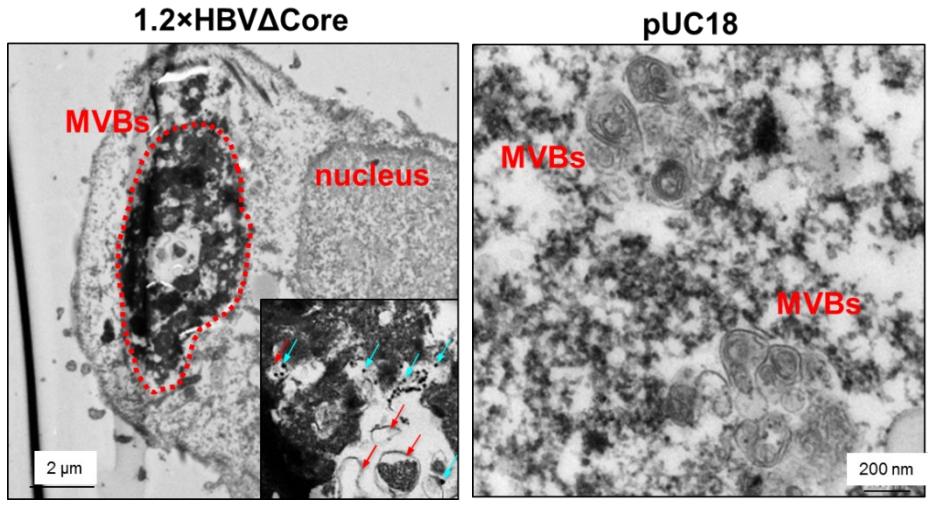
Experimental strategy



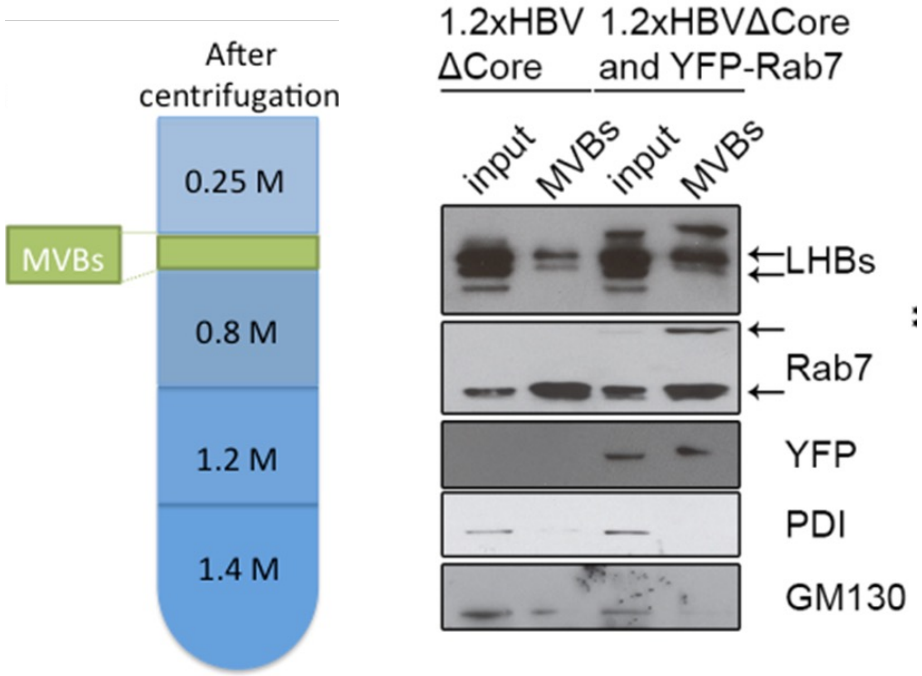
- The core protein is a major component of the viral particle.
- The formation of core protein is destroyed.
- No viral particles will be assembled.
- The release of HBV filaments can be traced by LHBs in the absence of virion production.

LHBs enters MVBs in the absence of virion formation

Immun electronmicroscopy of ultrathin sections from p1.2×HBVΔcore or pUC18-transfected Huh7.5 cells. Gold particles (blue arrows) representing the LHBs were predominantly found in the large dilated MVBs (red dashed circle), characterized by the presence of many small intraluminal vesicles (red arrows).

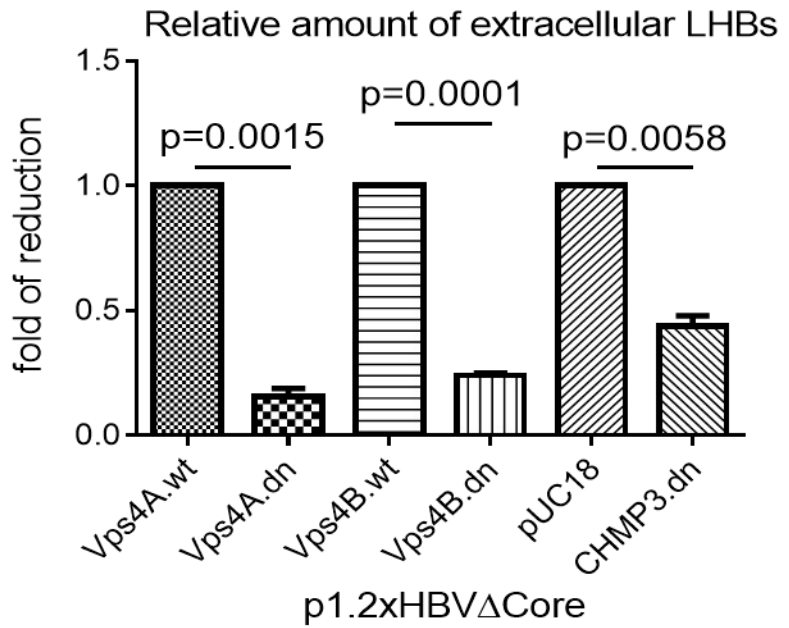


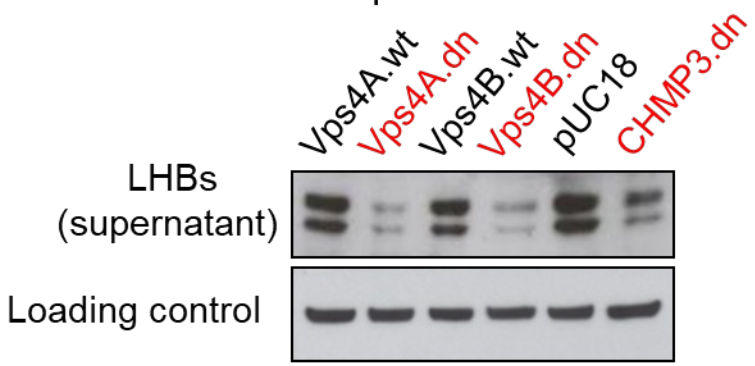
Homogenates from cells expressing 1.2×HBVΔCore or coexpressing 1.2×HBVΔCore and eYFP-Rab7 were subfractionated by sucrose density gradient ultracentrifugation and the fractions were analyzed by Western blot.

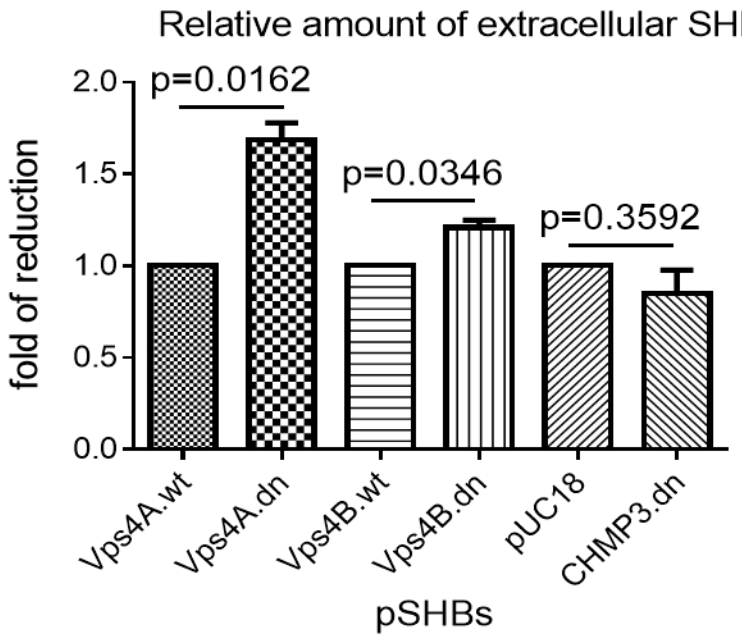


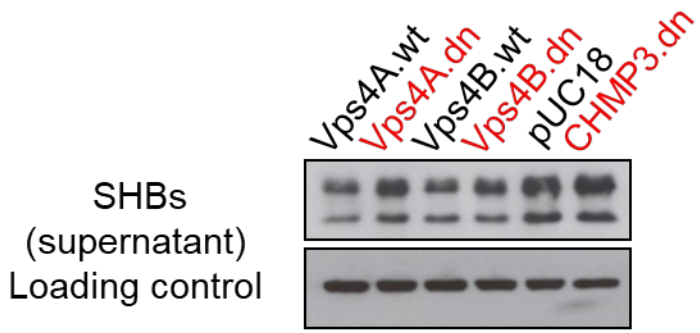
Dominant negative mutants of Vps4A, Vps4B and of CHMP3 inhibit the release of filaments but not spheres

Western blot of supernatants derived from HBVΔcore- (left panel) or SHBs- (right panel) expressing Huh7.5 cells cotransfected with wt or tdn mutants of Vps4A/B or CHMP3 were using LHBs- or SHBs-specific antisera









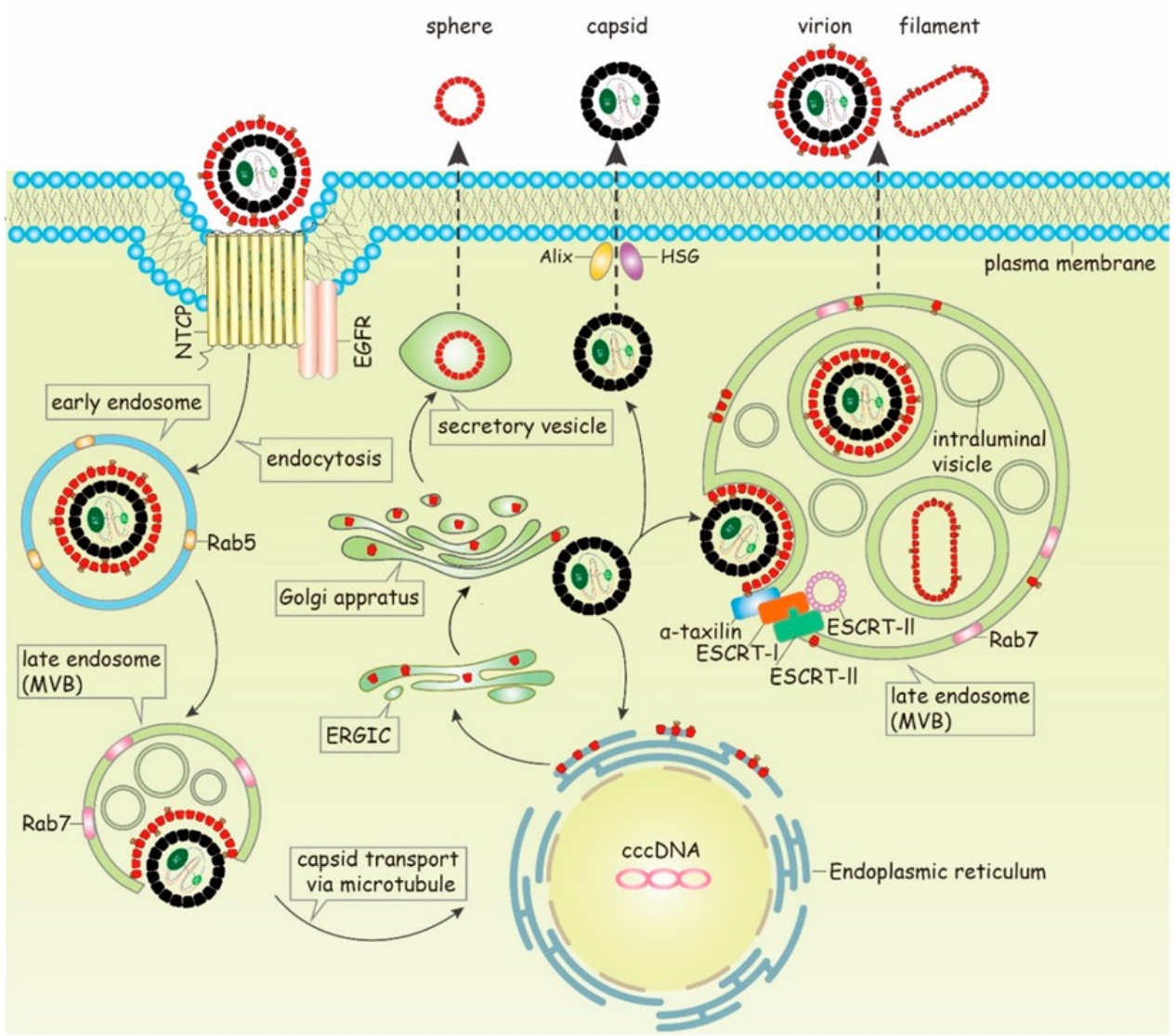
GOETHE UNIVERSITÄT FRANKFURT AM MAIN

LOEWE Exzellente Forschung für Hessens Zukunft

DRUID

Paul-Ehrlich-Institut

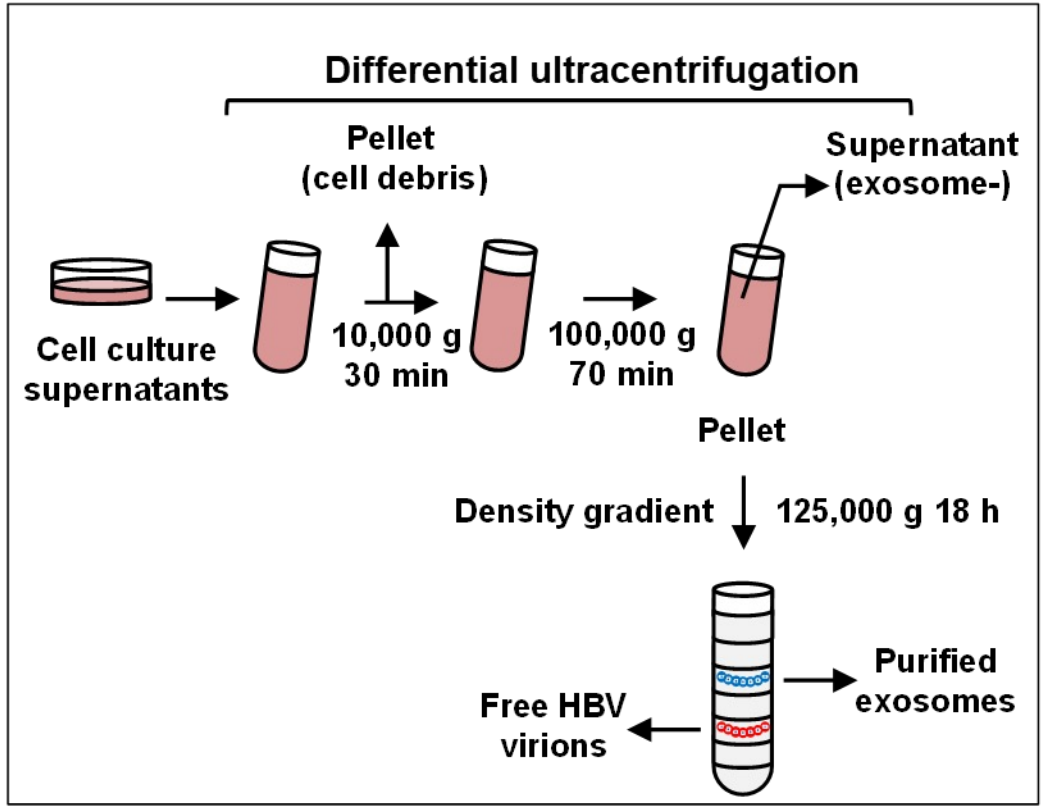
Summary II
Intracellular trafficking of HBV



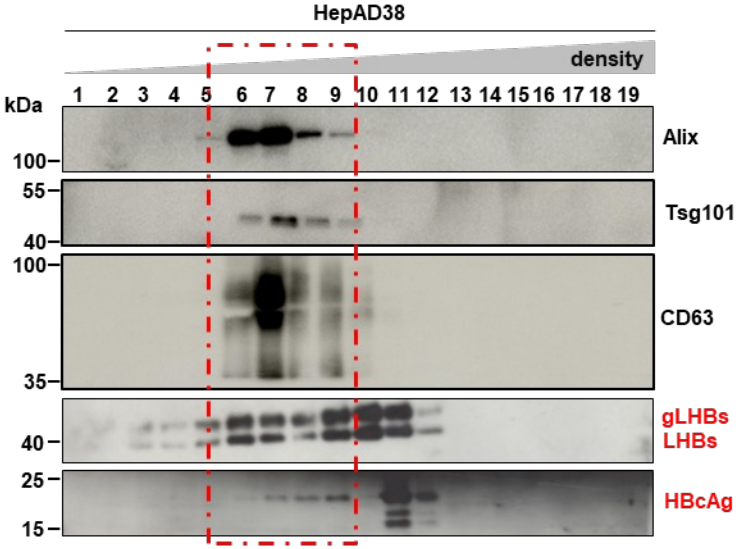
- α-taxilin binds to LHBs and via a late domain to the ESCRT component tsg101
- Inhibition of MVBs biogenesis or functionality disrupts the release of virions and filaments, but not of spheres
- HBV virions and HBV filaments leave the cell via MVBs

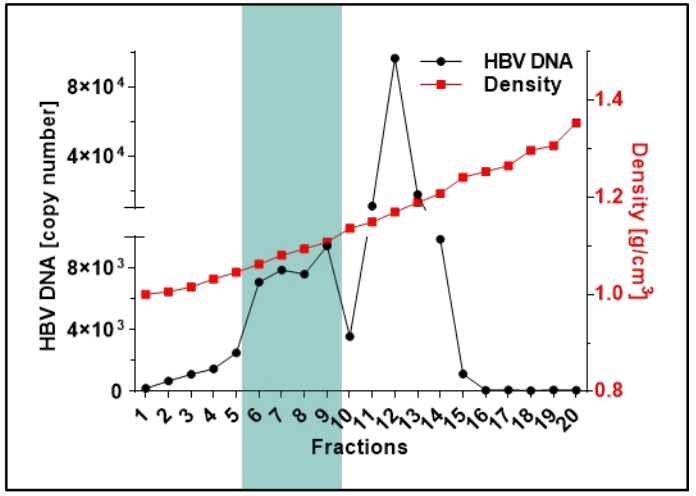
Presence of intact Hepatitis B virus in exosomes

1. Robust method for efficient separation of exosomes from virions were established



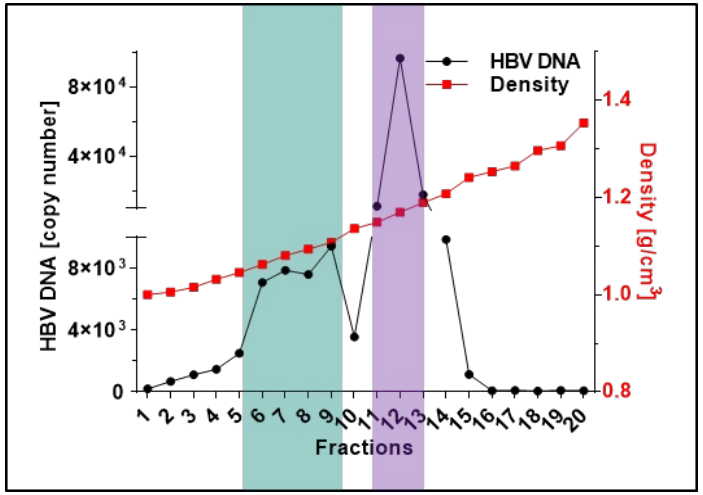
In the exosomal fractions,
HBcAg, LHBs, SHBs, and viral DNA are detectable

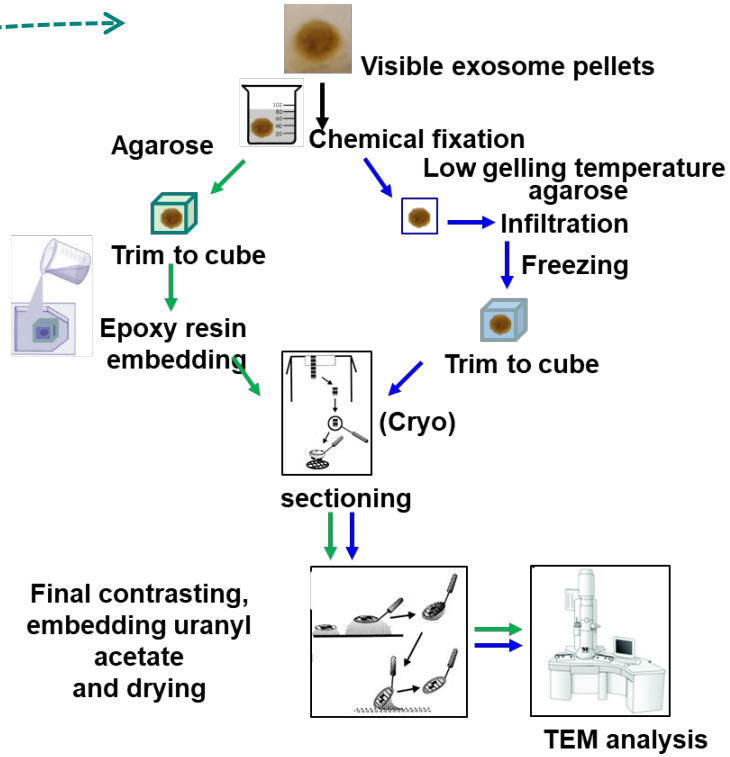


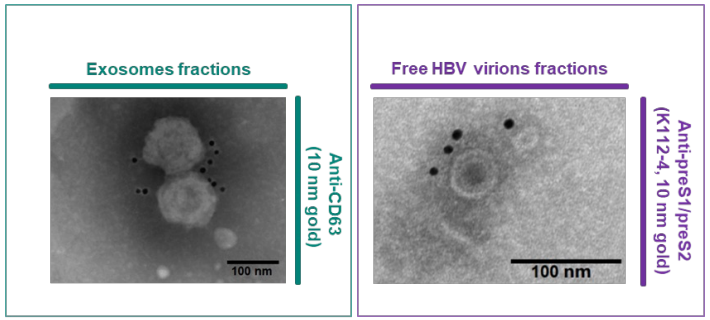


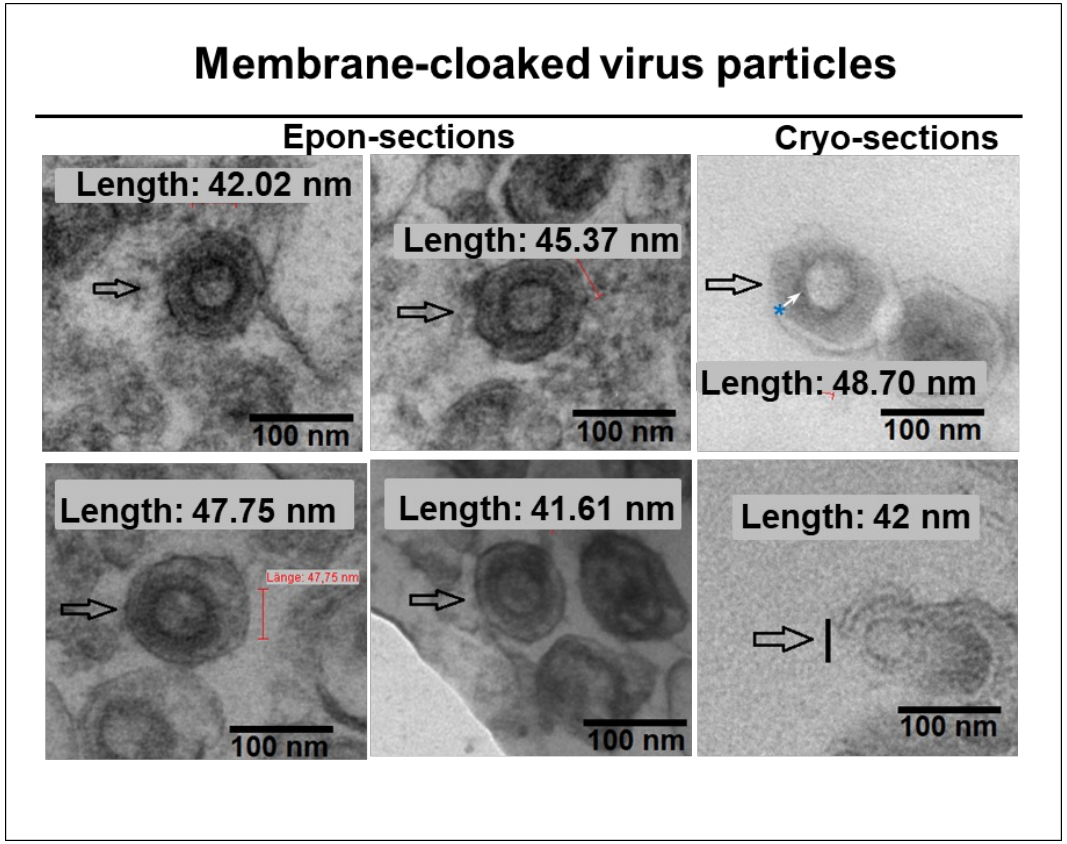
Presence of intact Hepatitis B virus in exosomes

Destruction of the exosomal membrane-accessibility for HBsAg-specific antibodies









GOETHE UNIVERSITÄT FRANKFURT AM MAIN

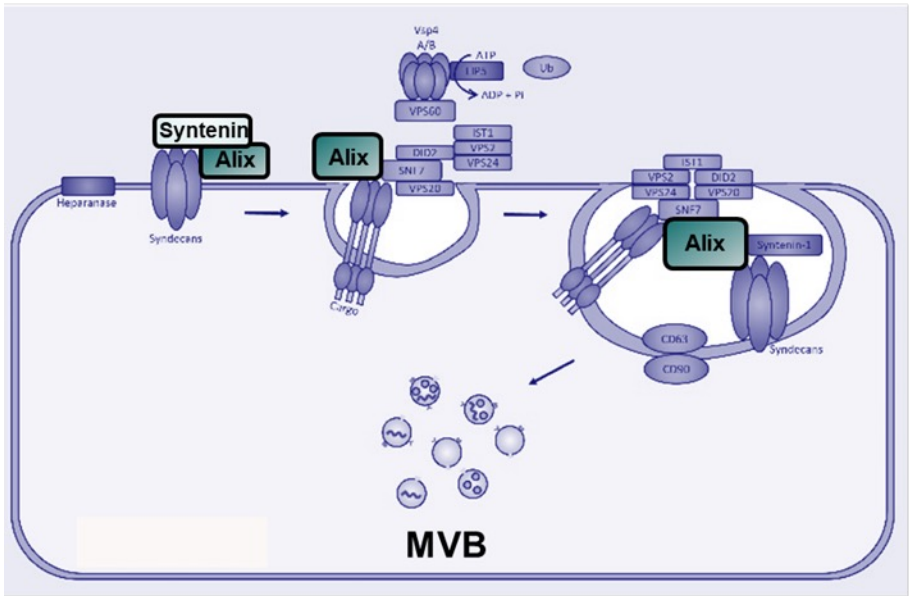
LOEWE Exzellente Forschung für Hessens Zukunft

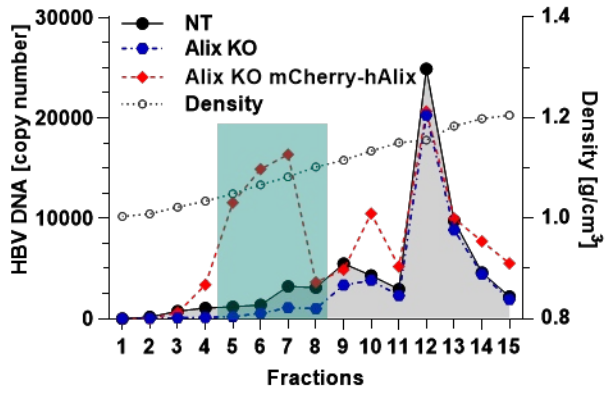
DRUID

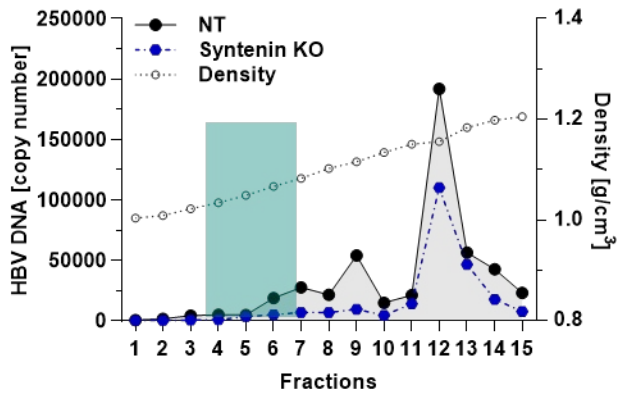
Paul-Ehrlich-Institut

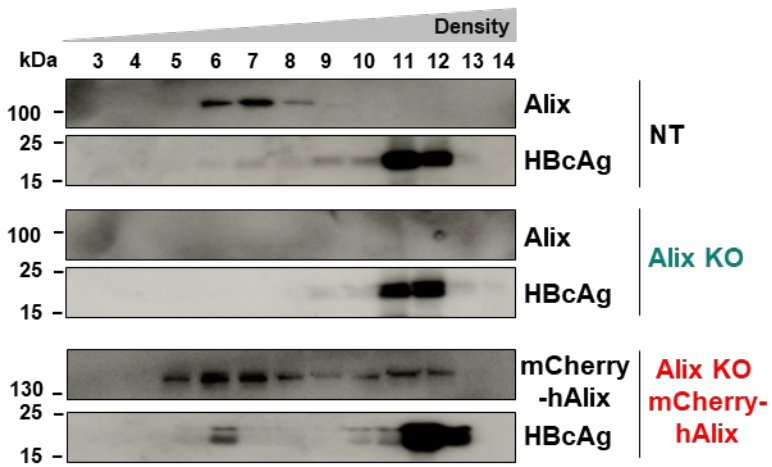
Crispr/Cas-mediated knockout of Alix- or Syntenin-expression impaires the release of exosomal HBV

MVB Cargo sorting / Endosome maturation (Exosome biogenesis)

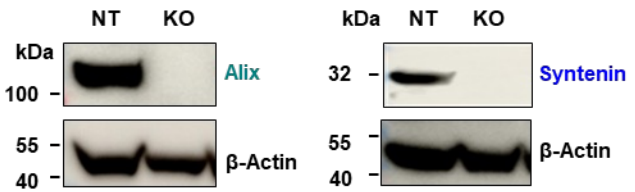








Alix-KO and Syntenin KO



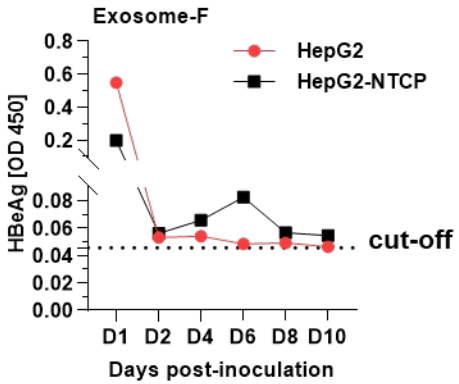
GOETHE UNIVERSITÄT FRANKFURT AM MAIN

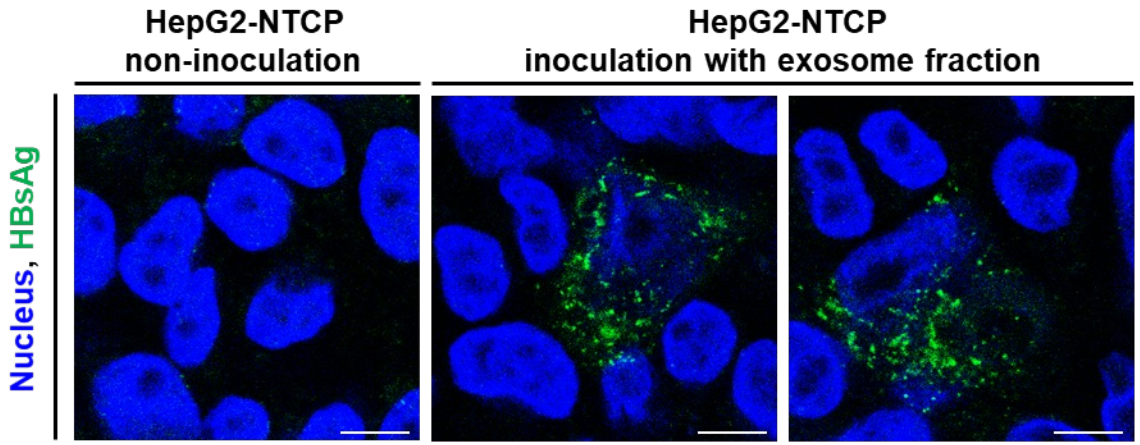
LOEWE Exzellente Forschung für Hessens Zukunft

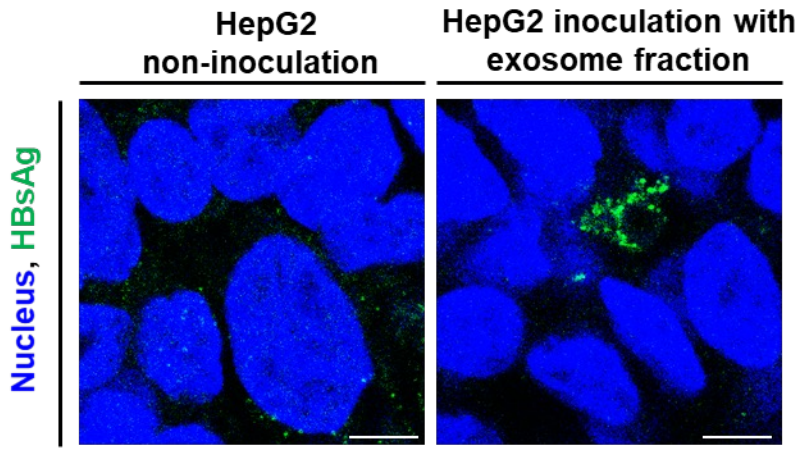
DRUID

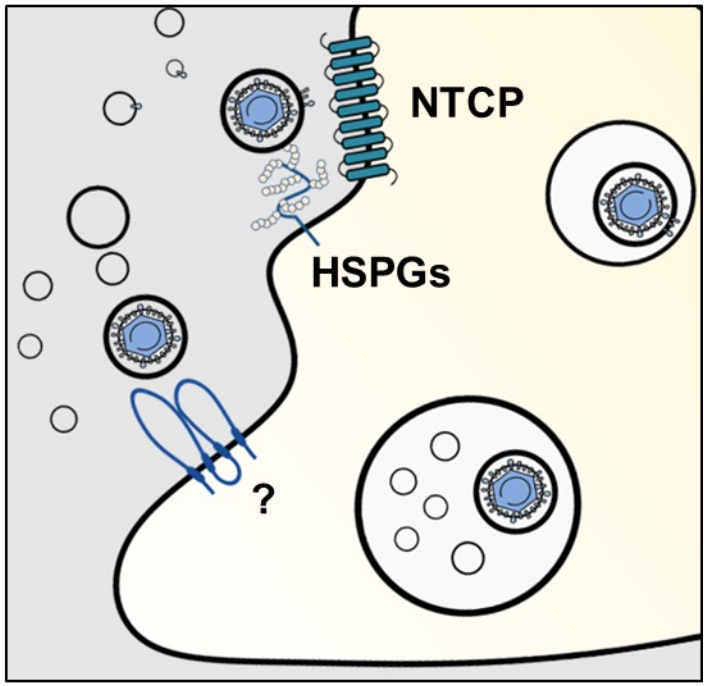
Paul-Ehrlich-Institut

Infection of non-permissive cells (HepG2) by exosomal HBV







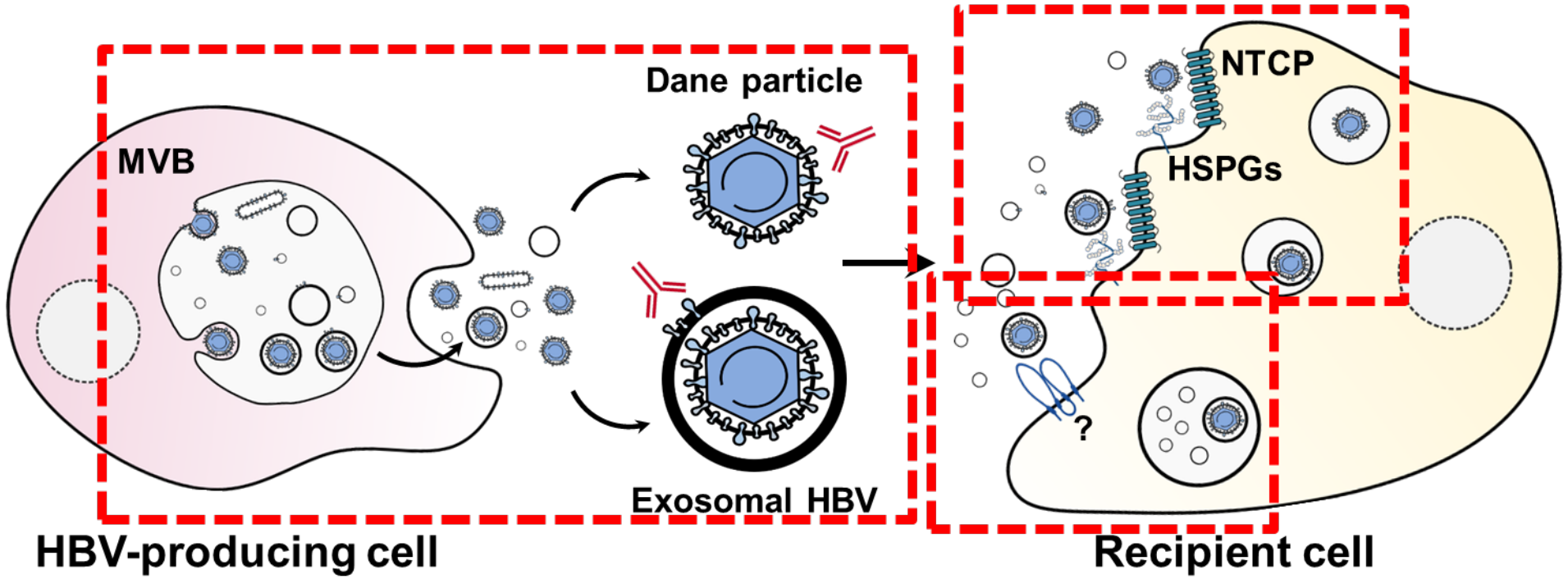


GOETHE UNIVERSITÄT FRANKFURT AM MAIN

LOEWE Exzellente Forschung für Hessens Zukunft

DRUID

Paul-Ehrlich-Institut



Summary III

Intact HBV virions can be released as exosomes. A so far not described release pathway for HBV.

LHBs on the surface of exosomal HBV mediates LHBs/NTCP-dependent infection of susceptible

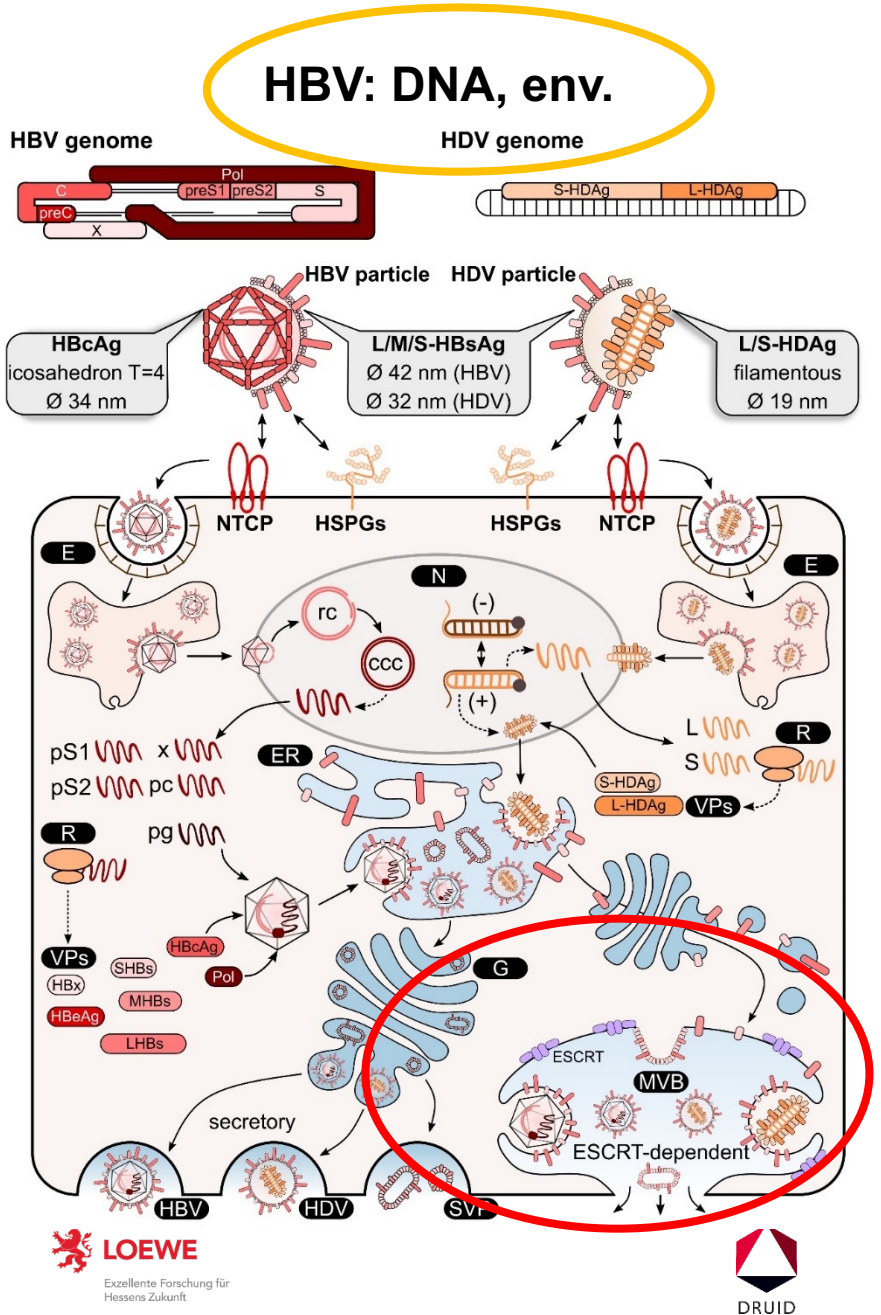
HBV harbouring exosomes can deliver HBV to non-susceptible tissues with low efficiency.

Characterization of factors affecting egress of Hepatitis B virus

general aspects

Morphogenesis and release of viral and subviral particles

Exosomal release of HBV



specific aspects

Viral factors controlling formation of HBsAg

Chronic infection-sources of HBsAg

GOETHE UNIVERSITÄT FRANKFURT AM MAIN

LOEWE Exzellente Forschung für Hessens Zukunft

DRUID

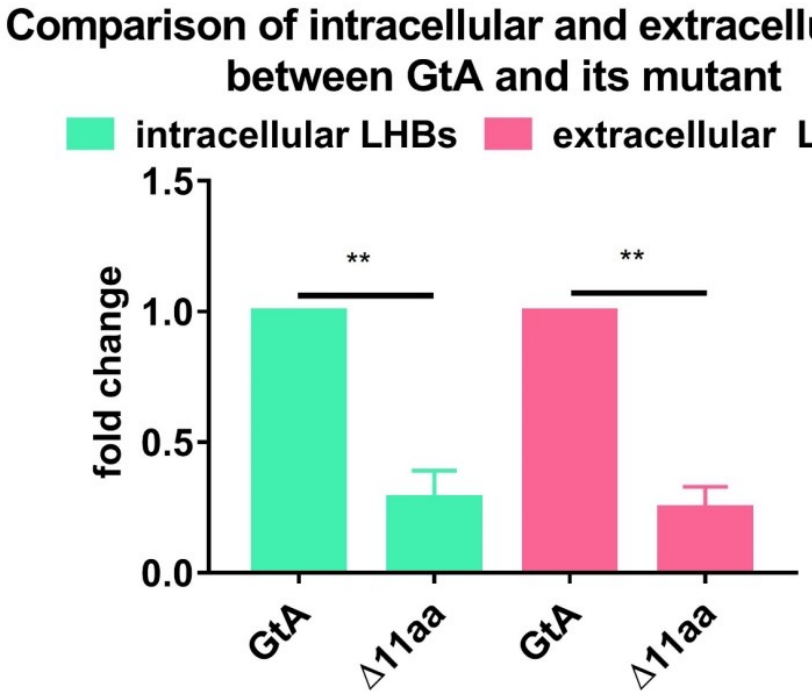
Paul-Ehrlich-Institut

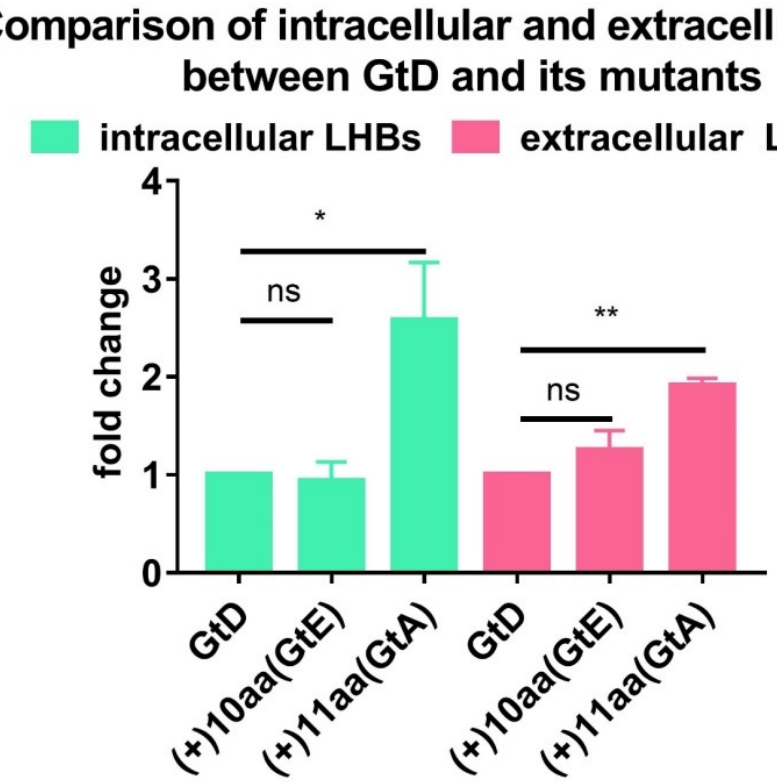
HBV genotypes differ with respect to the N-terminaus of the PreS1-domain

	1	2	3	4	5	6	7	8	9	10	11	12	13
GtD_(+)11aa(GtA)	M	G	G	W	S	S	K	P	R	K	G	M	G
GtD												M	G
GtD_(+)10aa(GtE)	M	G	L	S	W	T	V	P	L	E	W		G

The N-terminal 11 aa of the PreS1 domain affect the amount of LHBs

Lysates and supernatants from Huh7.5 cells transfected with GtA, GtD and the mutants were analyzed by Western blot using a LHBs-specific serum MA18/7.

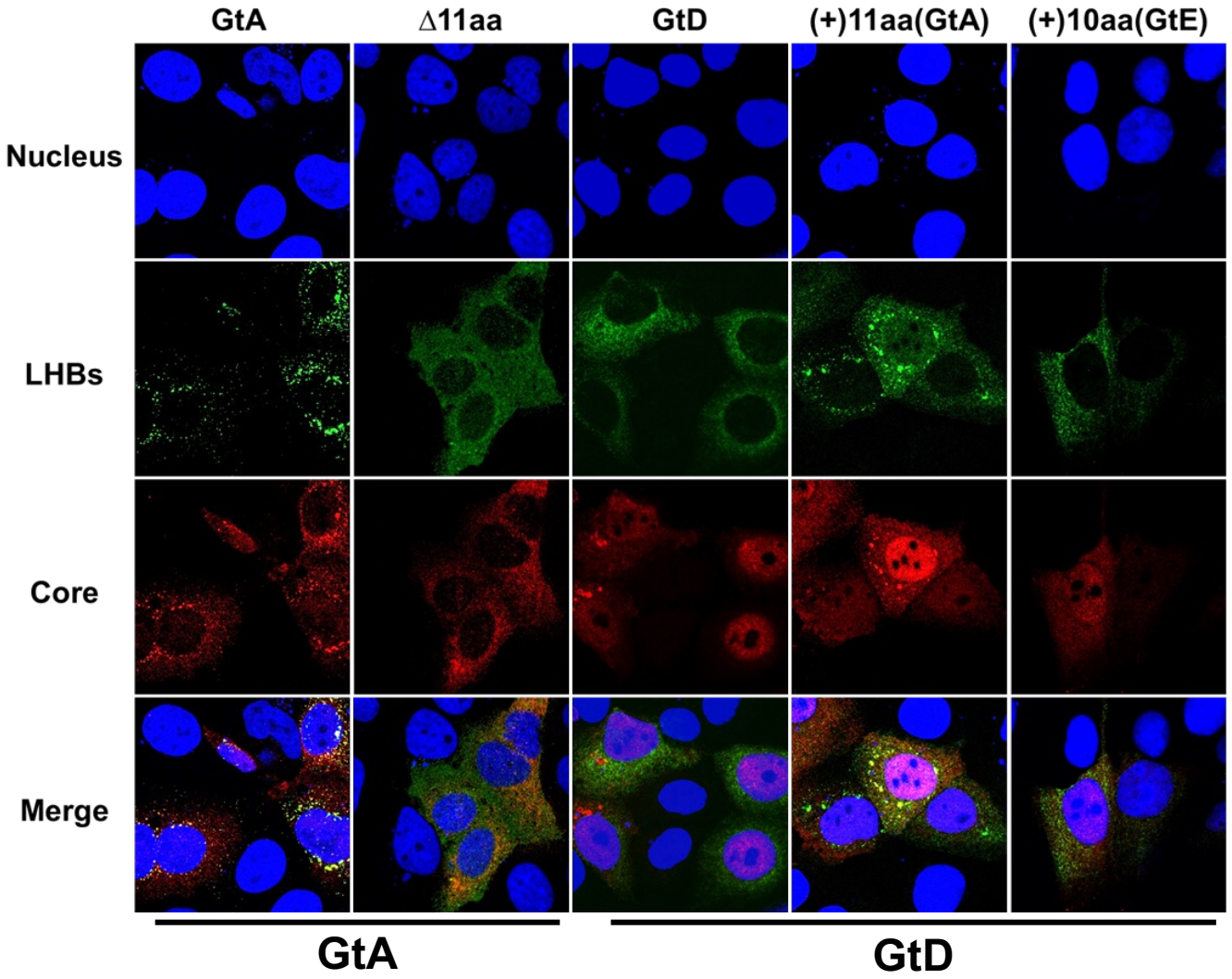




- Deletion of 11 aa in GtA reduced dramatically the amounts of intra/extracellular LHBs.
- Fusion of 11 aa (GtA) to GtD increased significantly the amount of intra/extracellular LHBs.
- Fusion of 10 aa (GtE) to GtD did not change the amount of intra/extracellular LHBs.

The N-terminal 11 aa of the PreS1 domain affect the subcellular distribution of LHBs

CLSM analysis using the LHBs-specific serum MA18/7 and a core-specific serum (Dako).



Summary IV
N-terminal 11 aa (GtA)

- increase intracellular LHBs
  - GtA-like distribution pattern (significant dot-like accumulation of LHBs)
- increase extracellular LHBs
  - higher filament/virion ratio
  - long filaments

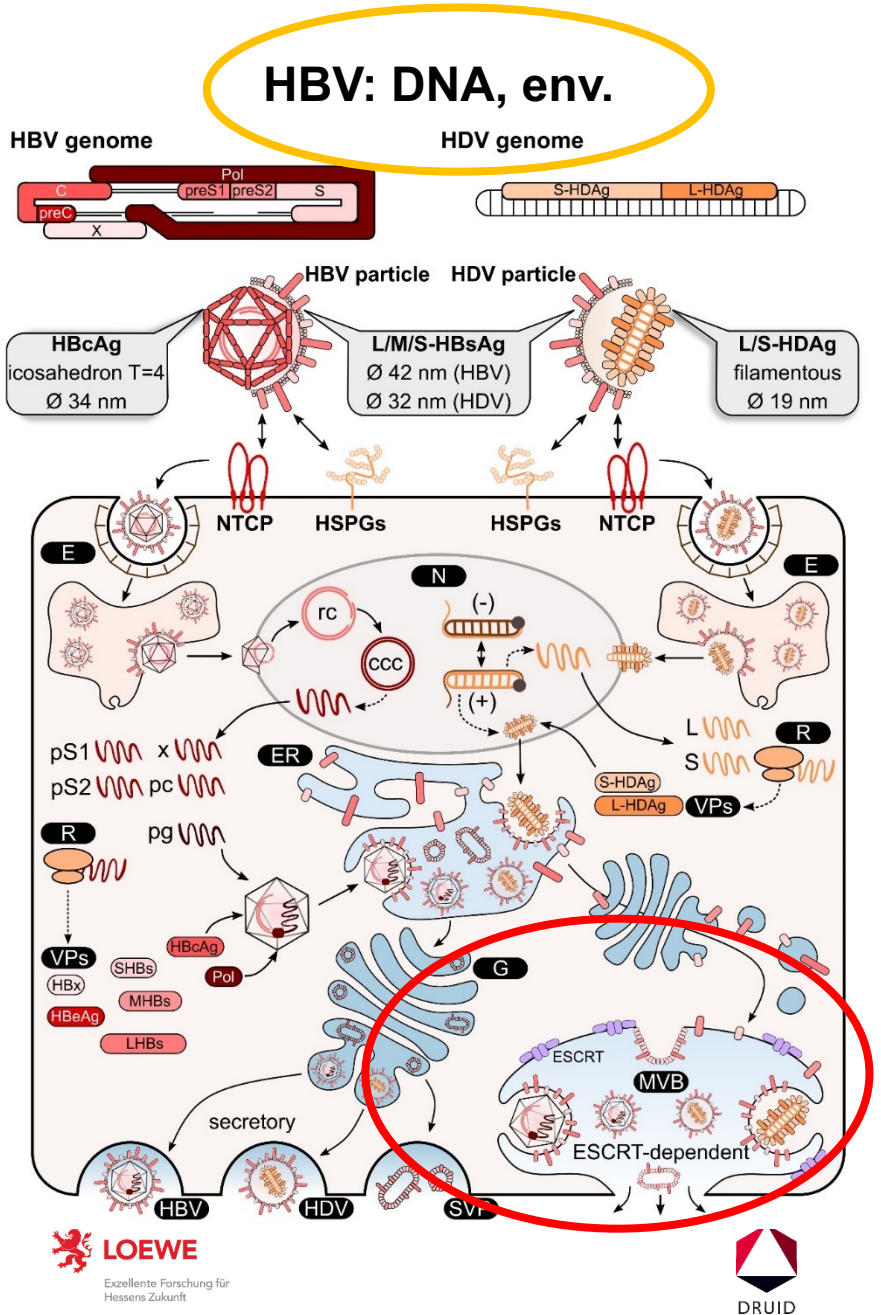
- have no impact on
  - core protein
  - released viral genome
  - the viral particle size

Characterization of factors affecting egress of Hepatitis B virus

general aspects

Morphogenesis and release of viral and subviral particles

Exosomal release of HBV



specific aspects

Viral factors controlling formation of HBsAg

Chronic infection-sources of HBsAg

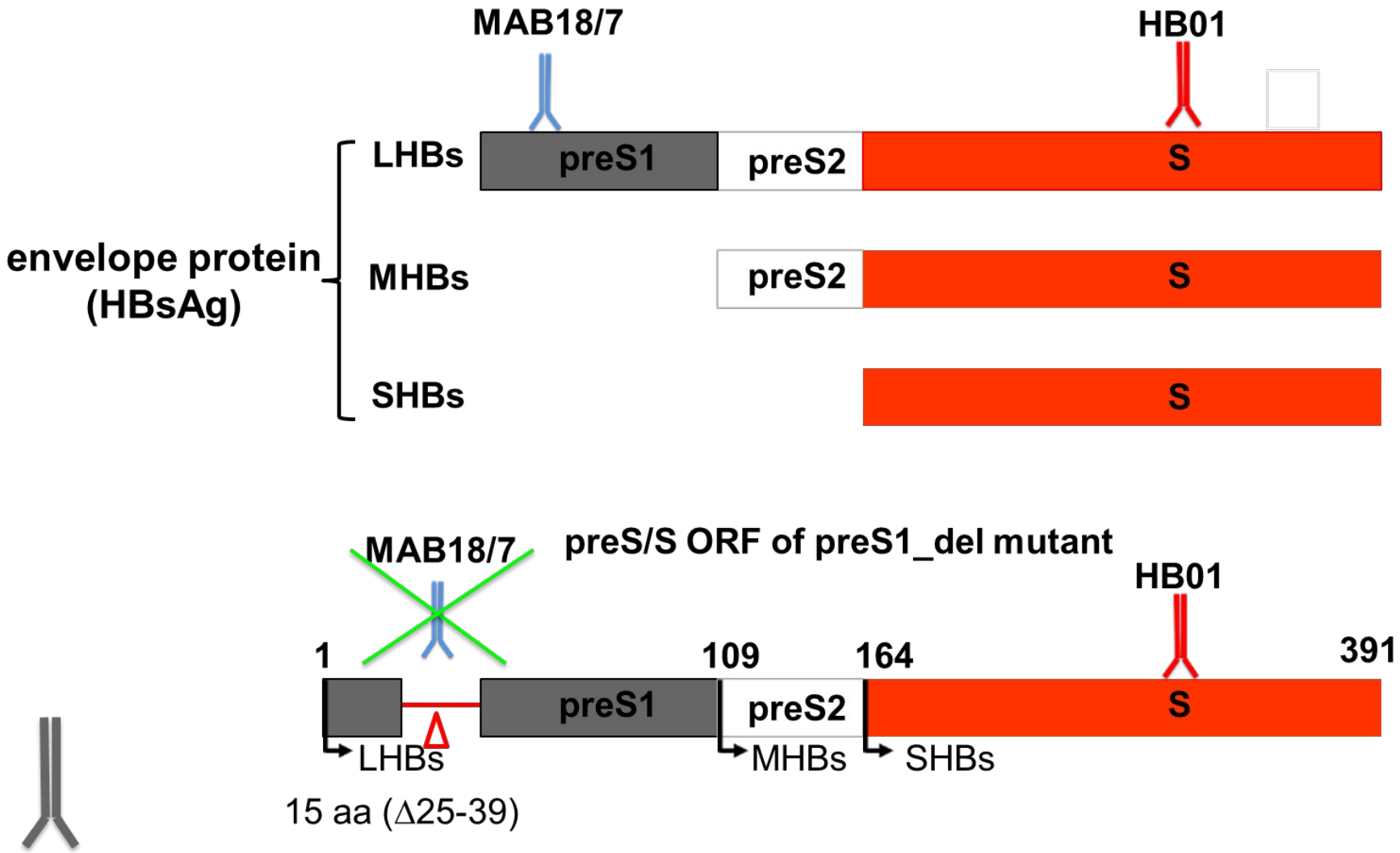
GOETHE UNIVERSITÄT FRANKFURT AM MAIN

LOEWE Exzellente Forschung für Hessens Zukunft

DRUID

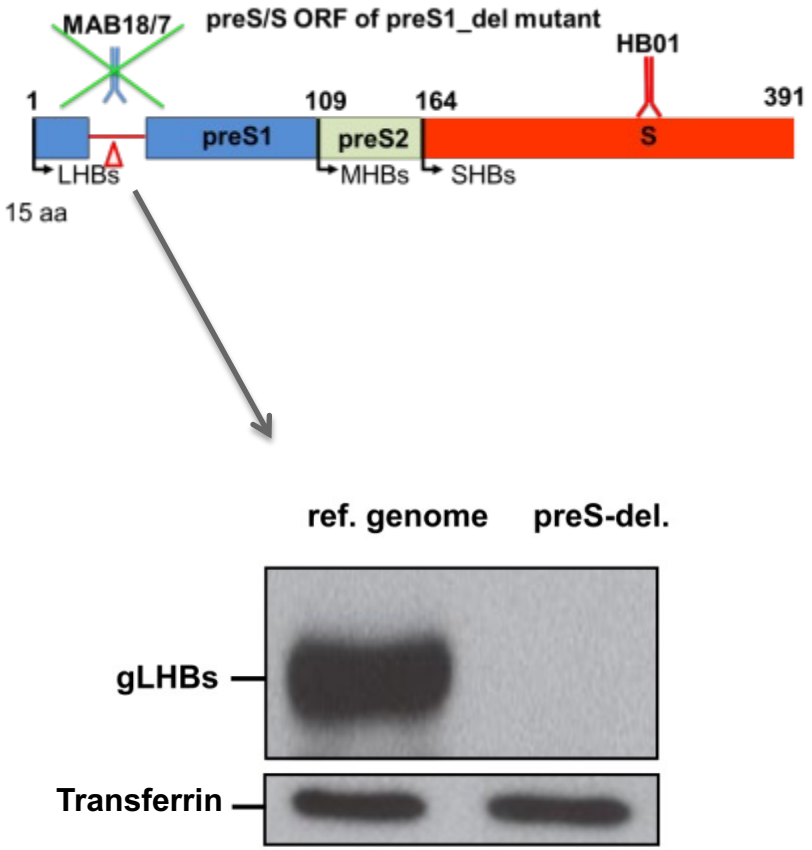
Paul-Ehrlich-Institut

Characterization of a PreS1-deletion mutant isolated from a patient suffering from chronic hepatitis B infection

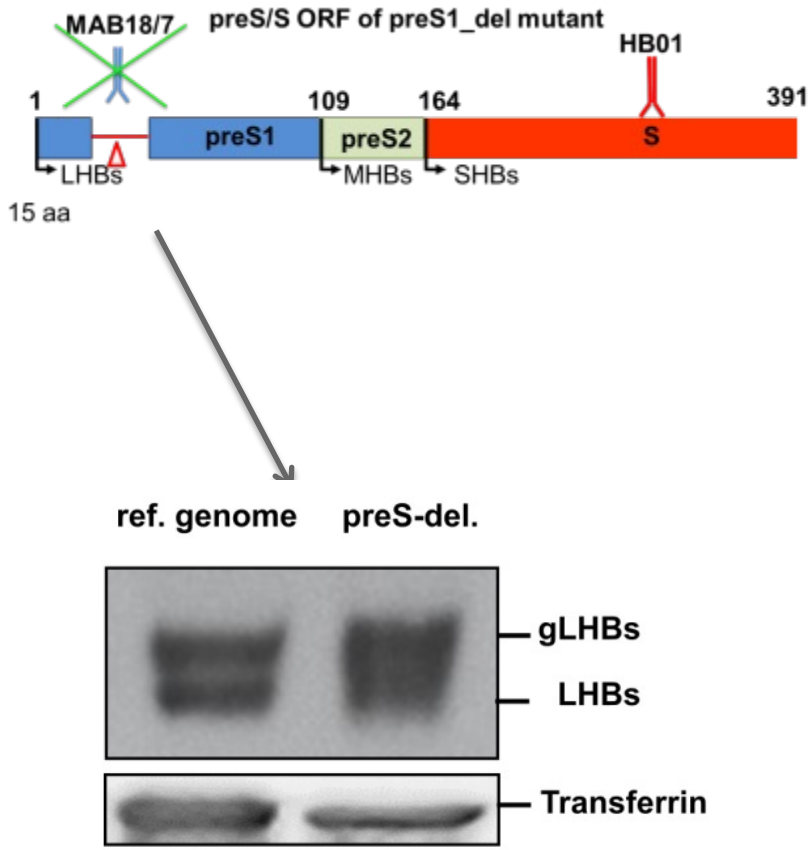


Transcomplementation of a defective HBV genome from integrated HBV-DNA

Western blot analysis of the cell culture supernatant derived from cells expressing the defective genome

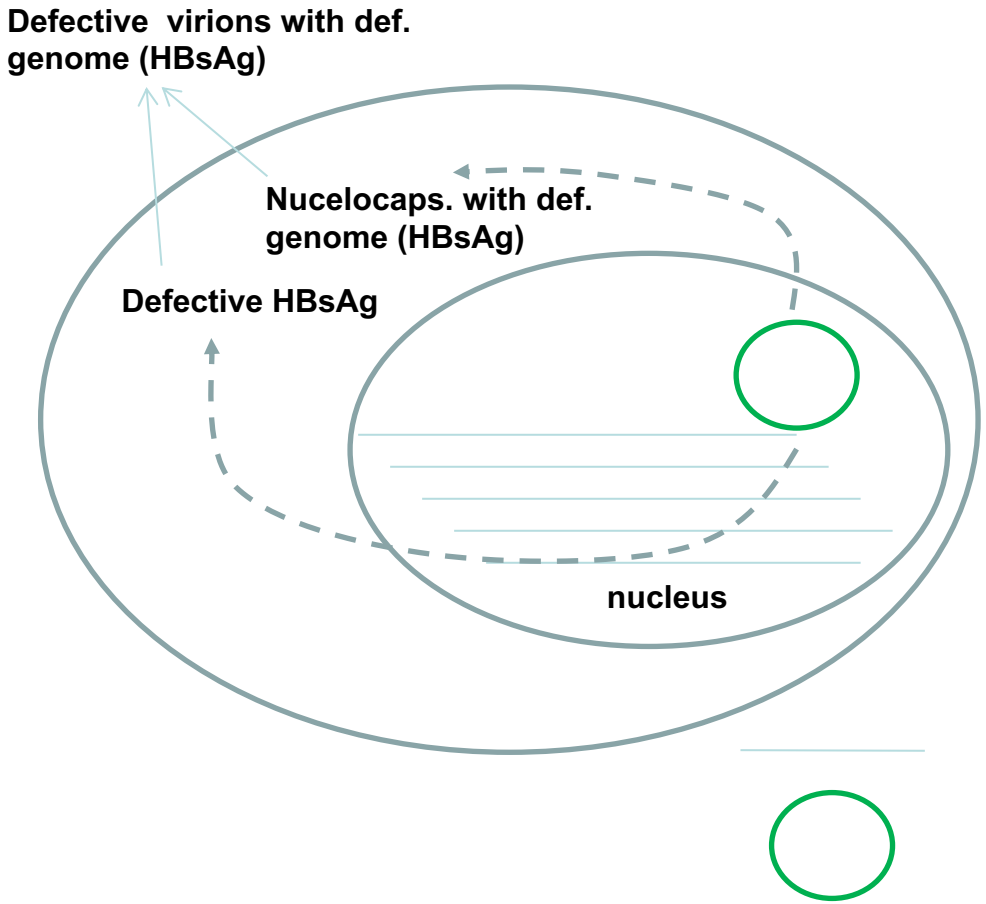


Western blot analysis of serum samples derived from patients chronically infected with HBV deletion mutants

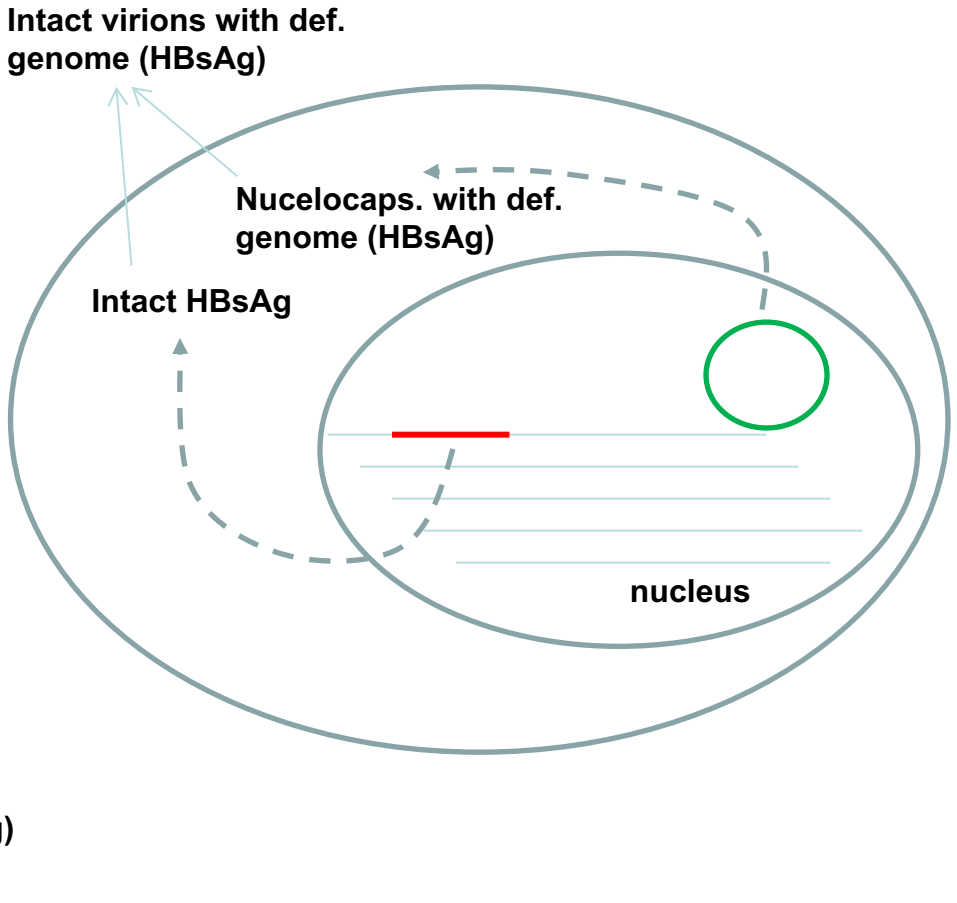


Transcomplementation of a defective HBV genome from integrated HBV-DNA

Transfection exp. cell culture



Chronically infected patient

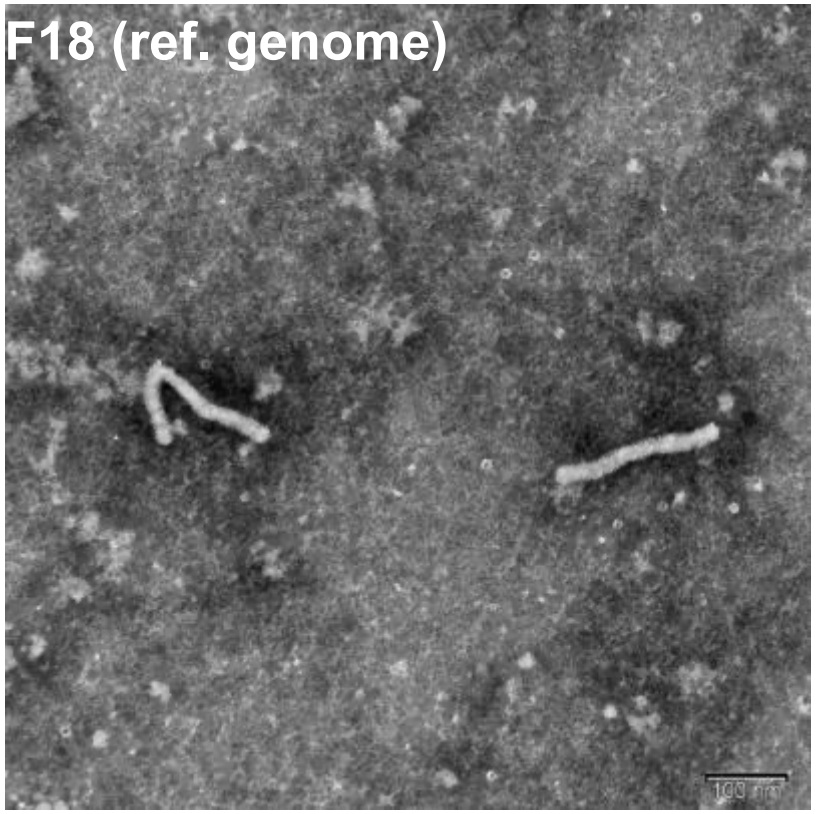


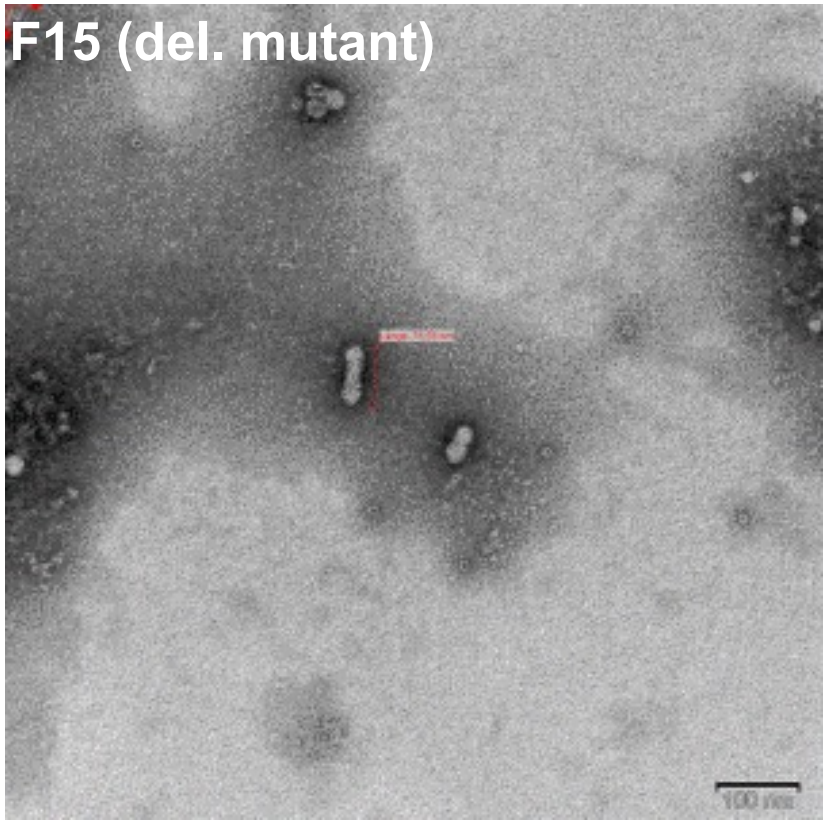
Chromos. DNA

def. genome (HBsAg)

Integrate encoding intact HBsAg

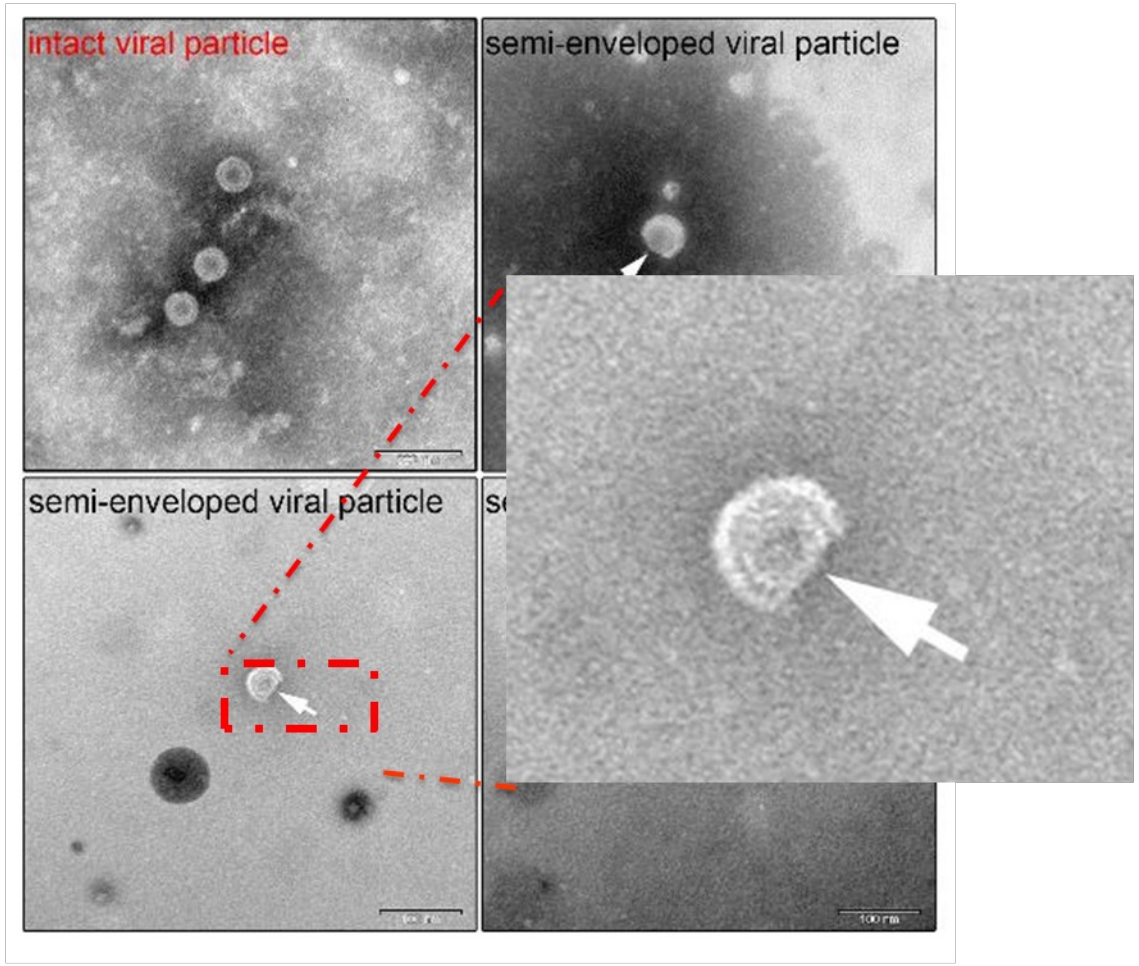
Shorter HBV filaments are released from cells expressing the PreS1 deletion mutant ∆aa25-39





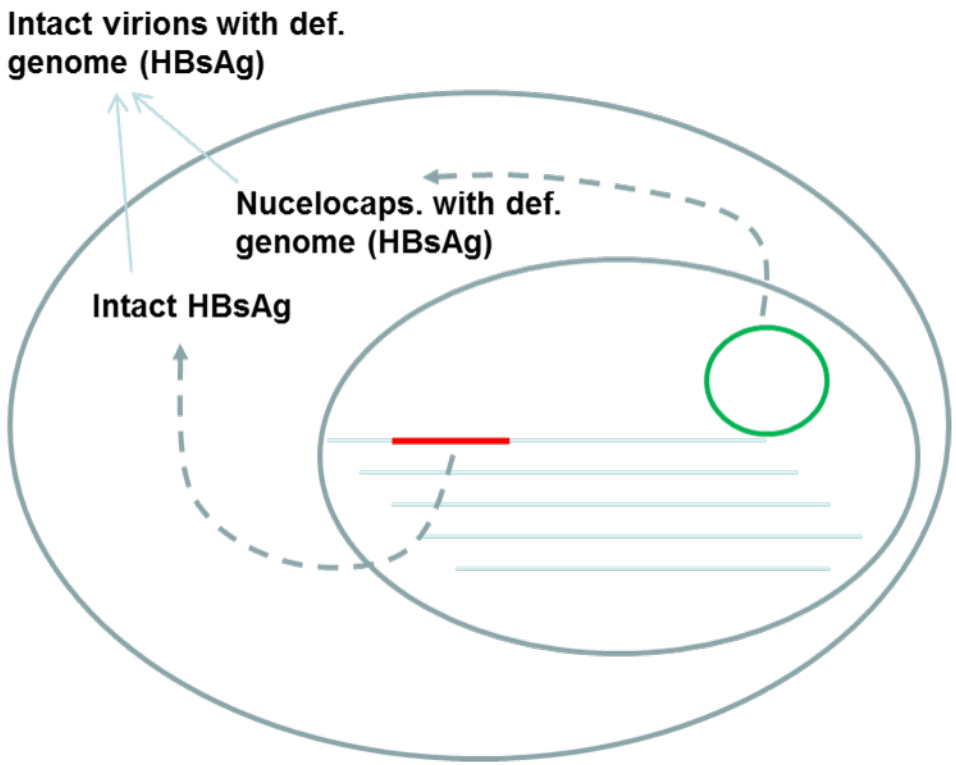
Cells expressing the PreS1 deletion mutant ∆aa25-39 release significant amounts of semi-enveloped HBV virions

LHBs-rich fractions (F22) concentrated from supernatant of reference construct- or a preS1_∆aa25-39 mutant-transfected cells were analyzed by electron microscopy after negative staining.



Summary V

- Transcomplementation of a defective HBV genome from integrated HBV-DNA
- Cells overexpressing preS1 del. mutant ∆aa25-39 release higher amounts of shorter filaments and semi enveloped viral particles.
- The semi-enveloped viral particles were confirmed by immunogold EM and native agarose gel electrophoresis.
- The 15aa deletion (25F-N39) doesn't alter the subcellular distributions and release pathways of viral and subviral particles.



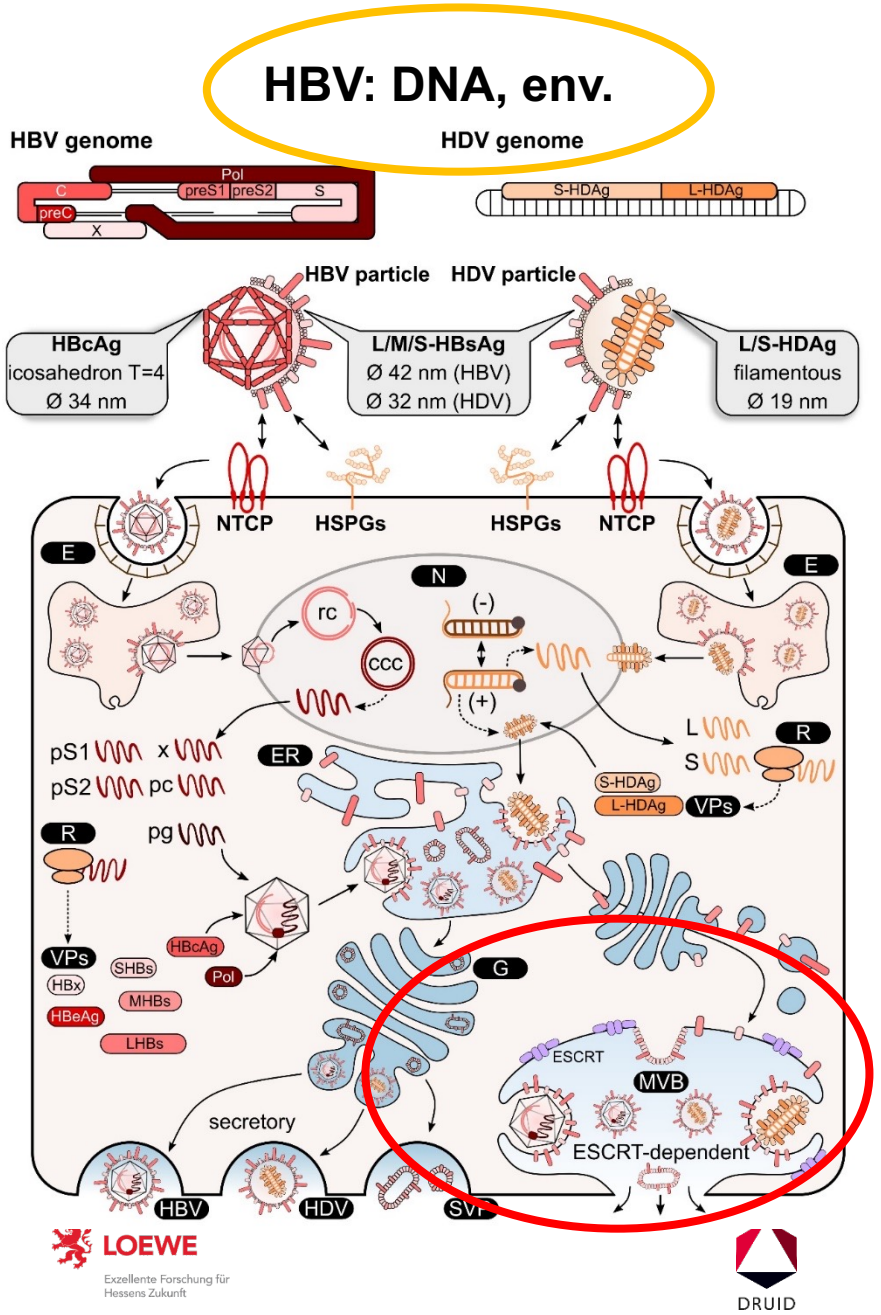
Characterization of factors affecting egress of Hepatitis B virus

Viral factors controlling formation of HBsAg

The N-terminus of the PreS1 domain affects amount and distribution of LHBs

Chronic infection-sources of HBsAg

Transcomplementation of a defective HBV genome by HBsAg from an integrate



Morphogenesis and release of viral and subviral particles

Taxilin is crucial for the MVB-dependent release of HBV and filaments

Exosomal release of HBV

Infection of non-permissive cells (HepG2) by exosomal HBV

GOETHE UNIVERSITÄT FRANKFURT AM MAIN

LOEWE Exzellente Forschung für Hessens Zukunft

DRUID

Paul-Ehrlich-Institut

Acknowledgements

Collaborators:
Sabine Werner, ETH Zurich
Reimar Johne, BfR, Berlin
Matti Sällberg, Karolinska, Stockholm
Thomas Baumert and Catherine Schuster, Inserm Strassbourg
Severin Gudima, University of Kansas Medical Center
Dieter Glebe, University of Giessen
Thomas Weiss, University of Regensburg
Kai-Henrik Peiffer, Stephan Zeuzem, University Hospital Frankfurt
Ute Helmich, University of Mainz/Jena
Arnold Grünweller, Thorsten Steinmetzer University of Marburg
Felix Hausch, Technical University of Darmstadt

Department of Virology-research group

Department of Virology-research group

Most of the presented data were generated by :

Michael Basic –patient isolates

Daniela Bender- microscopy

Mirco Glitscher –kinome analysis

Bingfu Jiang- svp release

Quingyan Wu-HBV and exosomes

Jasmin Hofman-MVB/ESCRT

Bundesministerium für Gesundheit

DZIF Deutsches Zentrum für Infektionsforschung

DFG Deutsche Forschungsgemeinschaft

GOETHE UNIVERSITÄT FRANKFURT AM MAIN

DRUID

LOEWE Exzellente Forschung für Hessens Zukunft

Thank you for your attention!