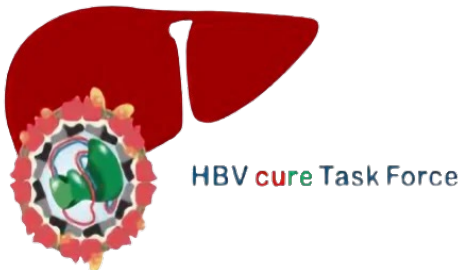


Novel insight in the regulation of cccDNA transcription

Cancer Research Center of Lyon, **Lyon, France**
The Lyon Hepatology Institute EVEREST, **Lyon, France**

Grants from Assembly Bio, Beam Therapeutics, BlueJay

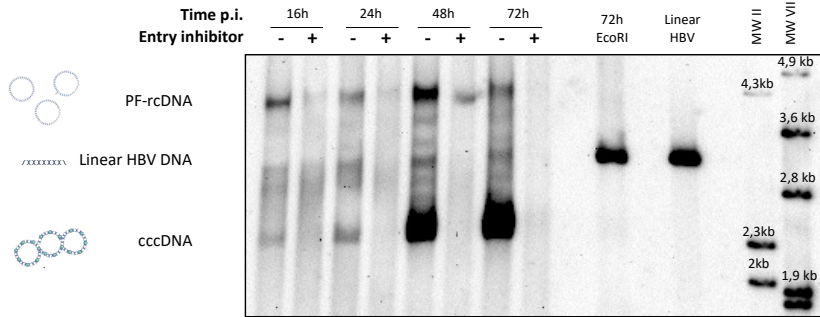


Endorsed by



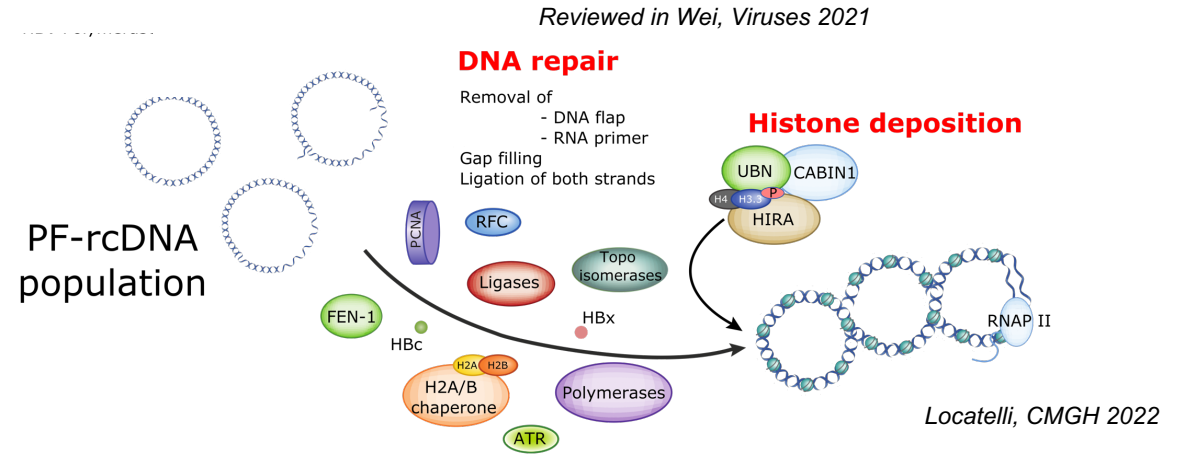
Formation of transcriptionally active cccDNA relies on H3.3 deposition

PHH

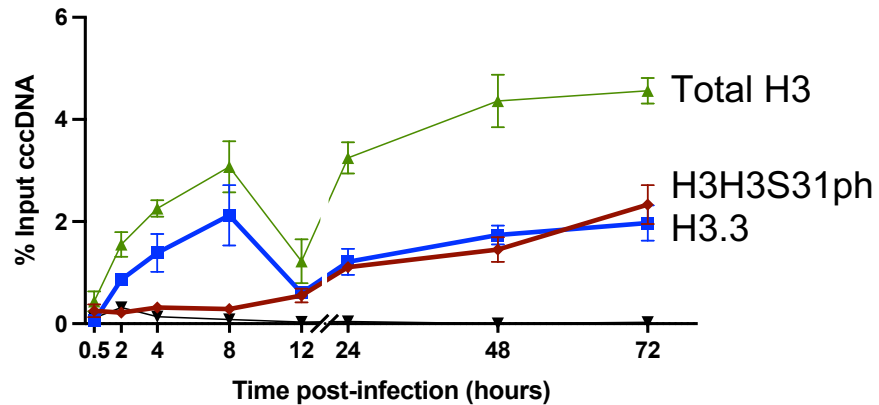


(Detectable within 2h p.i. by qPCR)

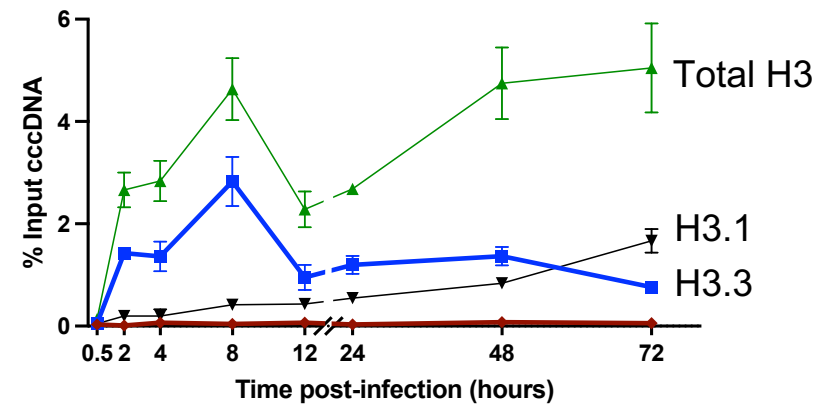
Locatelli, CMGH 2022
Ko, J Hepatol 2018
Luo, J Virol 2017



Transcriptionally active cccDNA

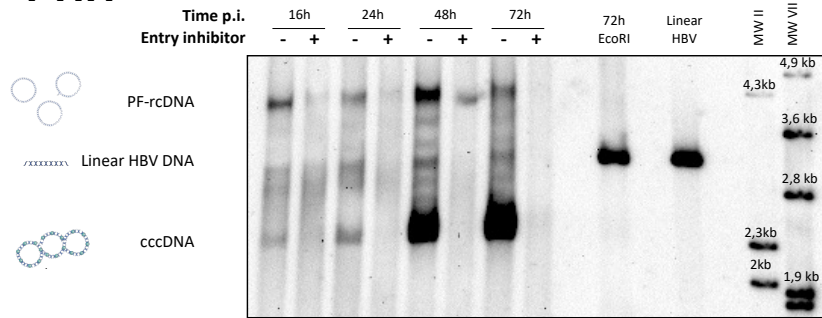


Transcriptionally inactive cccDNA

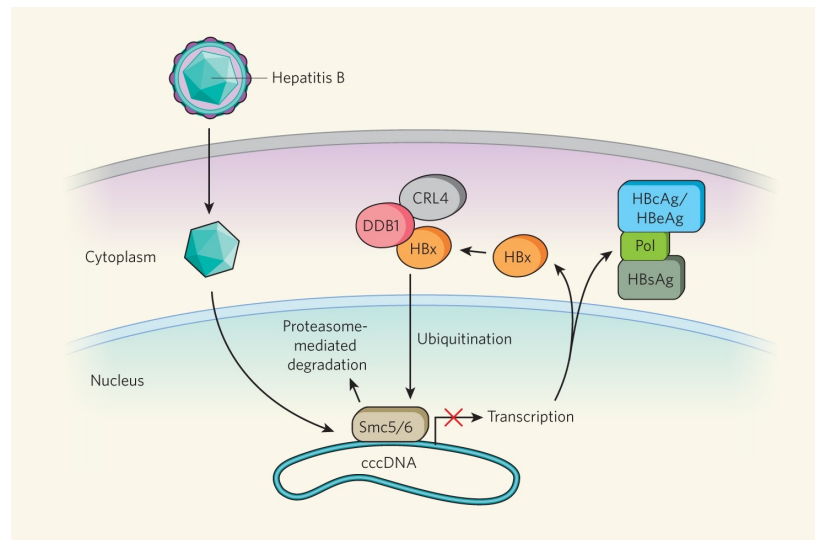


Smc5/6 topological recognition of +supercoiled extrachromosomal DNA

PHH



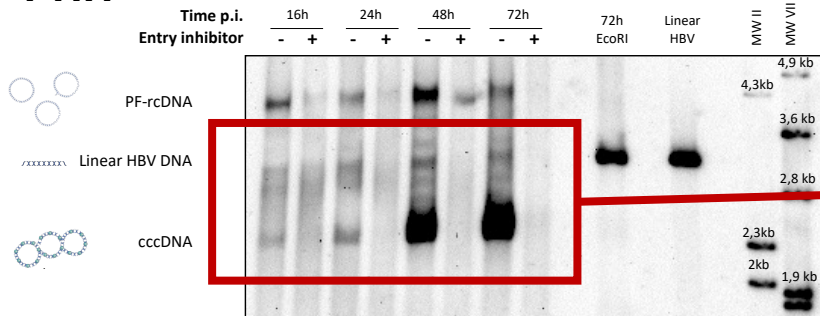
Locatelli, CMGH 2022



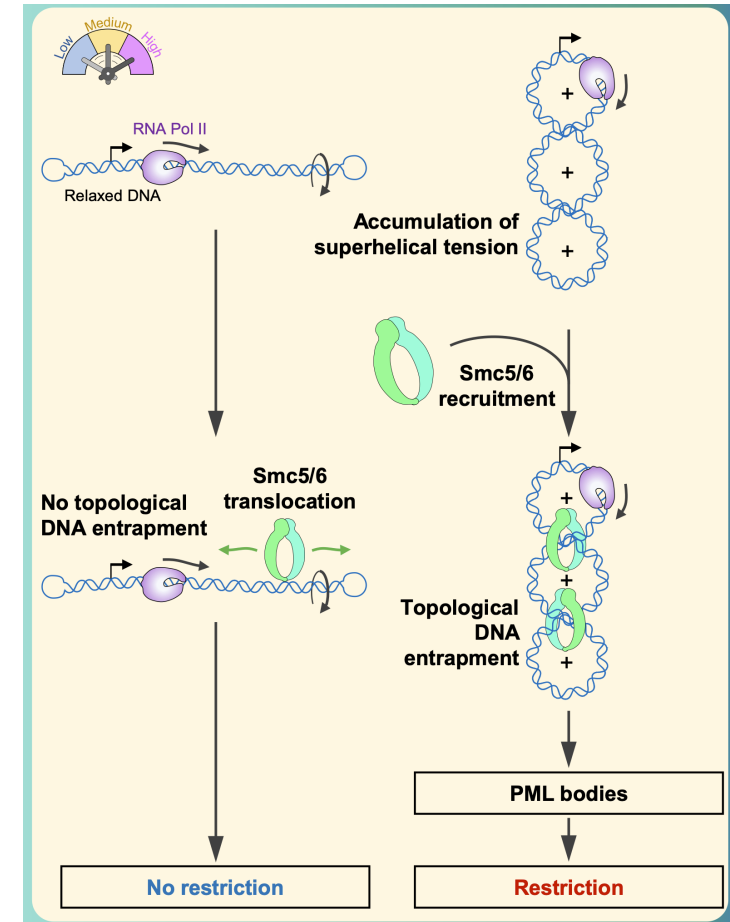
Decorsière, Science 2016
 Niu, Plos One 2017
 Abdul, Nat Struct&Mol Biol 2022

Smc5/6 topological recognition of +supercoiled extrachromosomal DNA

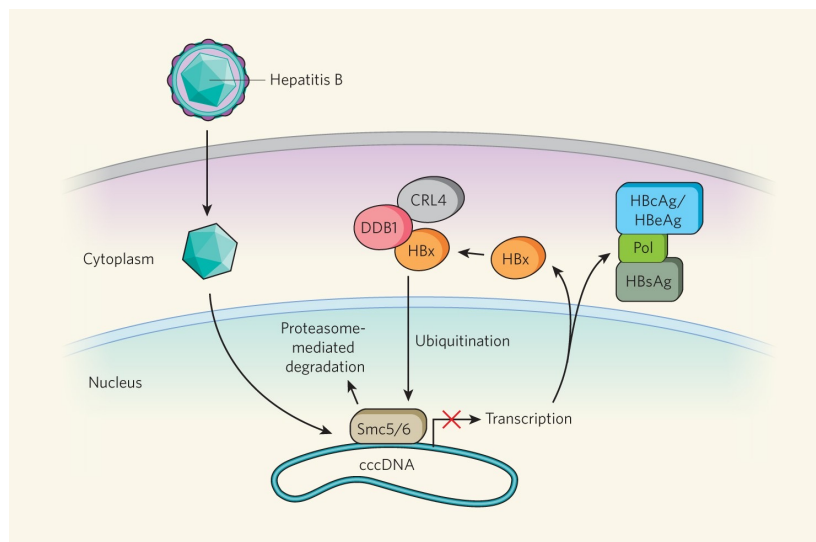
PHH



Locatelli, CMGH 2022



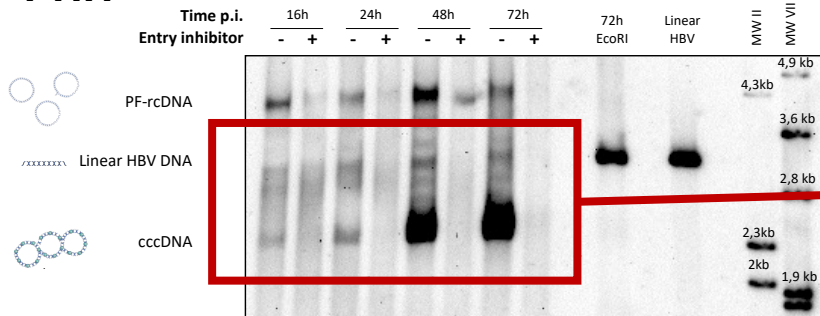
Modified from Diman, BioRxiv <https://doi.org/10.1101/2023.05.04.539344>



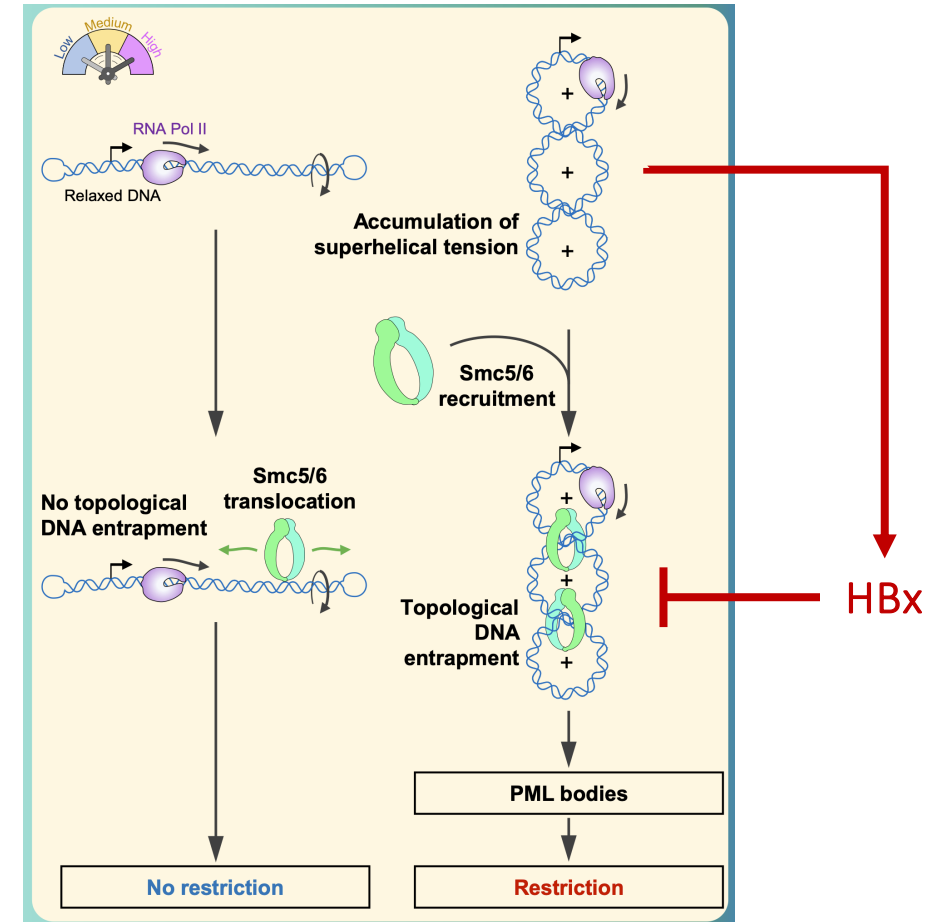
Decorsière, Science 2016
 Niu, Plos One 2017
 Abdul, Nat Struct&Mol Biol 2022

Smc5/6 topological recognition of +supercoiled extrachromosomal DNA

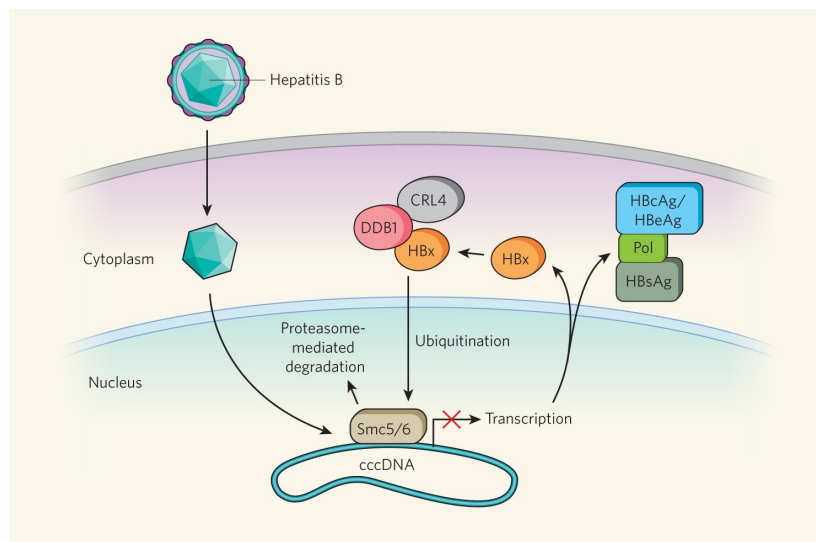
PHH



Locatelli, CMGH 2022



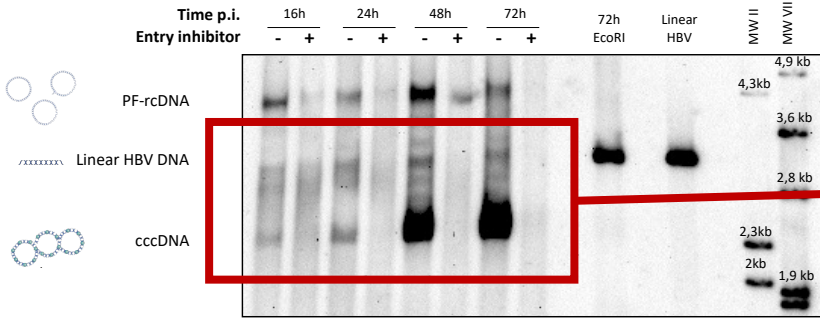
Modified from Diman, BioRxiv <https://doi.org/10.1101/2023.05.04.539344>



Decorsière, Science 2016
 Niu, Plos One 2017
 Abdul, Nat Struct&Mol Biol 2022

Smc5/6 topological recognition of +supercoiled extrachromosomal DNA

PHH

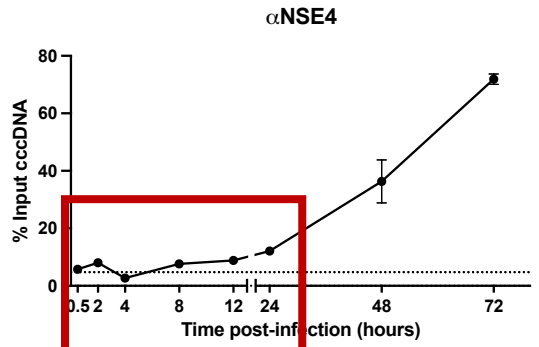
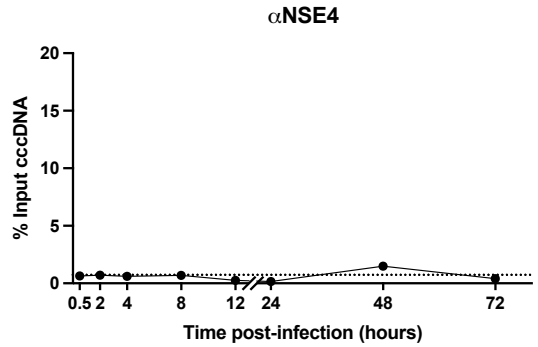


Locatelli, CMGH 2022

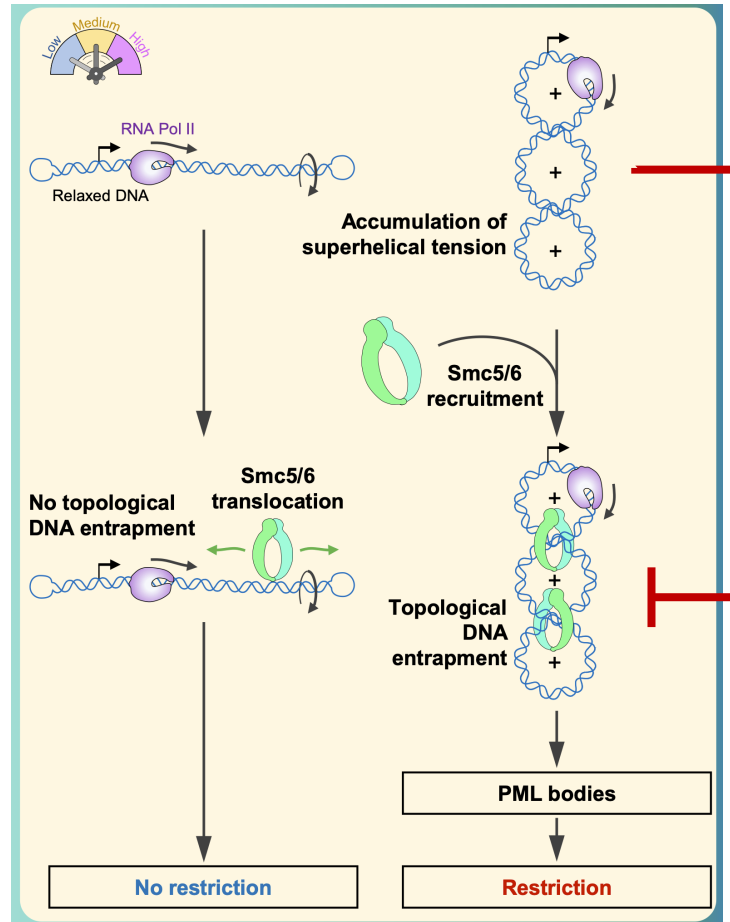


Transcriptionally active cccDNA

Transcriptionally inactive cccDNA



Locatelli, CMGH 2022



Modified from Diman, BioRxiv <https://doi.org/10.1101/2023.05.04.539344>

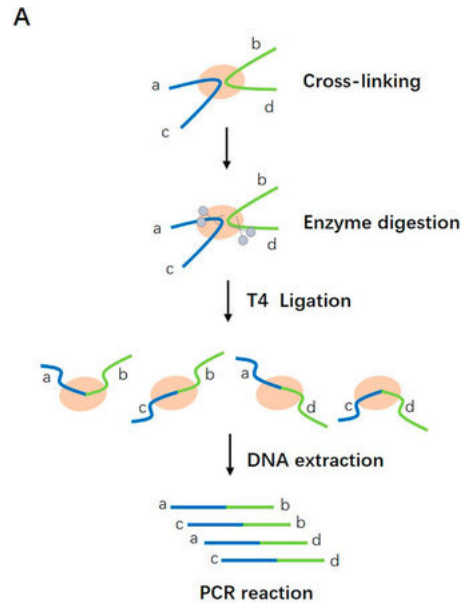
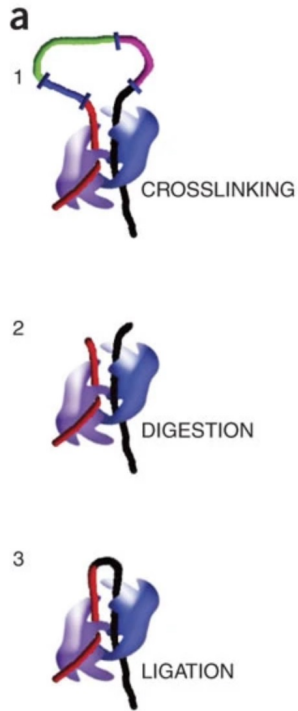
cccDNA permeates the hepatocyte nucleus

Chi-C:HBV capture coupled to 3C *Moreau, Nat Comm 2018*

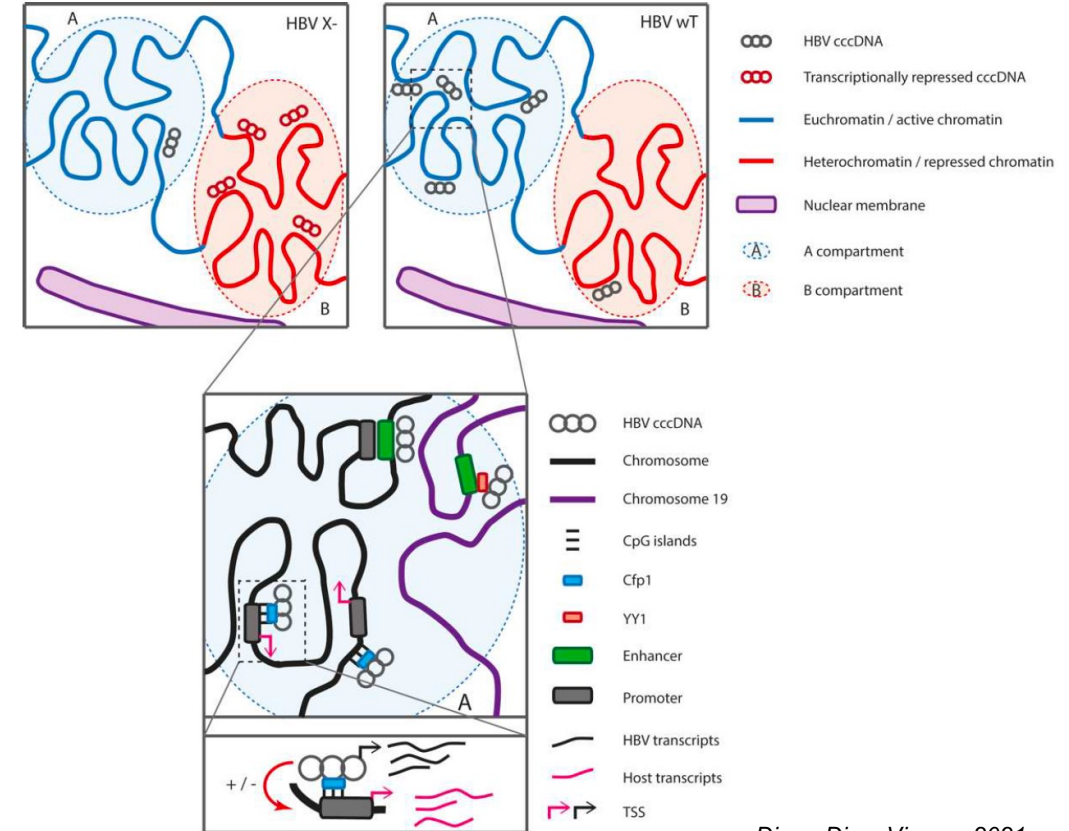
HTGTS-3C: high throughput wide translocation sequencing, coupled to 3C *Yang, Cell Discov 2020*

Chromosome Conformation Capture (3C) *Shen, Emerg Microbes Infect 2020*

Circular Chromosome Conformation Capture (4C) *Tang, Cell Rep 2021*



Dekker, Science 2022

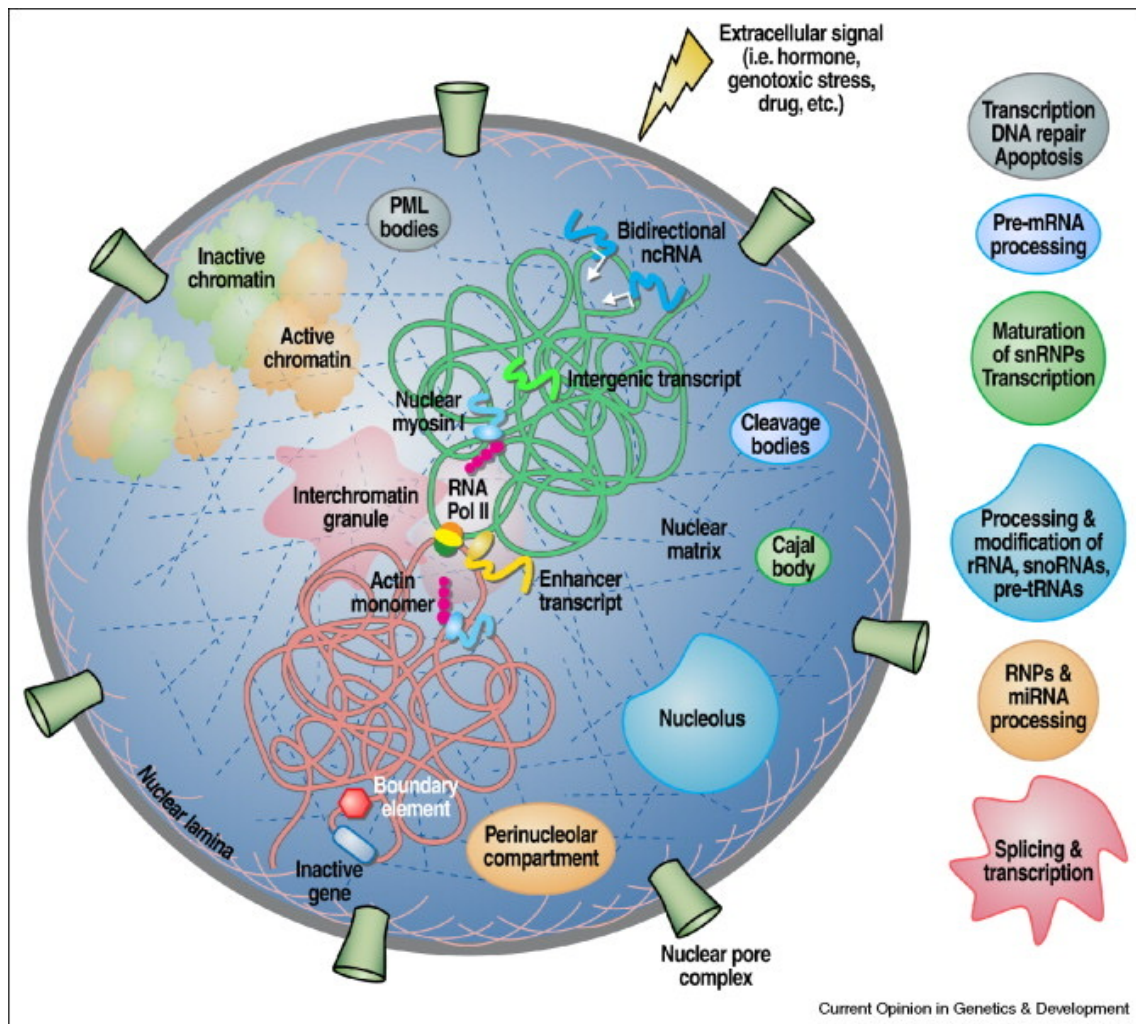


Not randomly localized: TSS and regions enriched with CpG islands

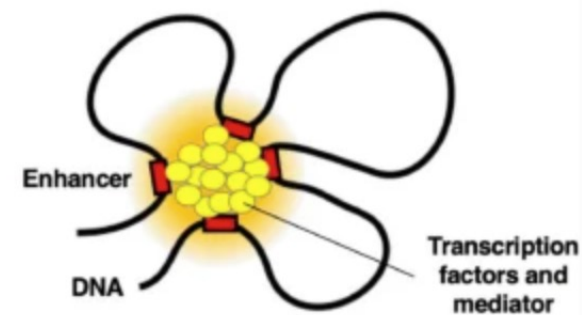
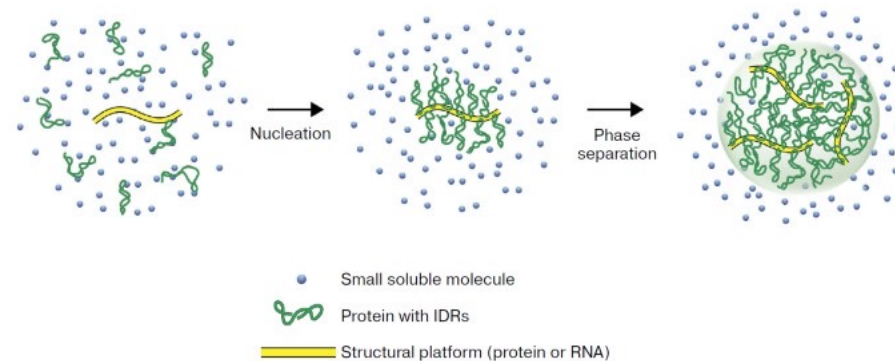
Transcriptional status of cccDNA may impact on its localization (or the opposite?)

Spatial segregation promoted by nuclear compartments

3d organization impacts nuclear functions

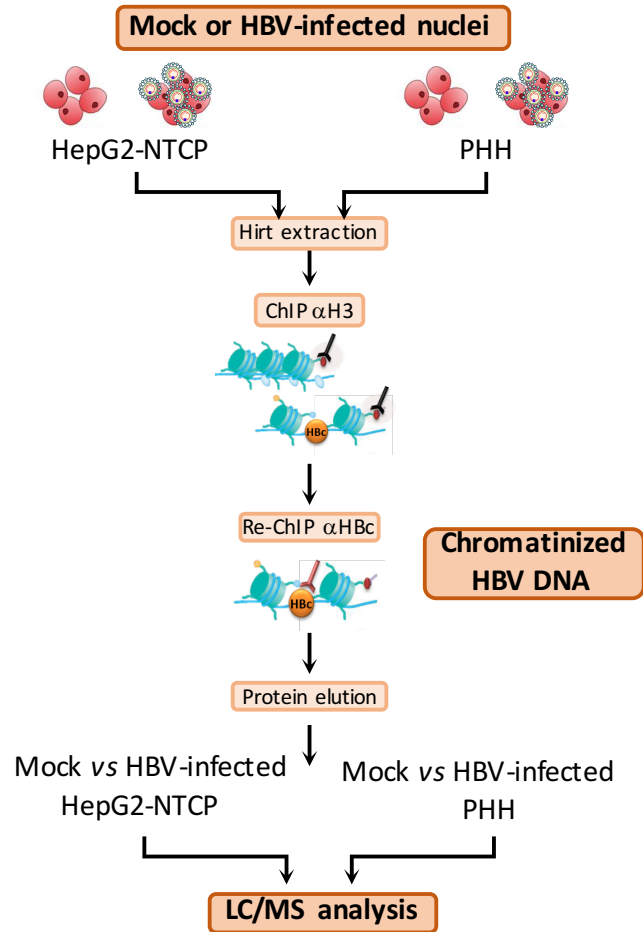


Liquid-liquid phase separation



Yang, *Front Genetics* 2020

Transcriptionally active cccDNA is associated to RNA/ssDNA binding proteins in vivo



Testoni, International HBV Meeting 2017

Transcriptionally active cccDNA is associated to RNA/ssDNA binding proteins in vivo

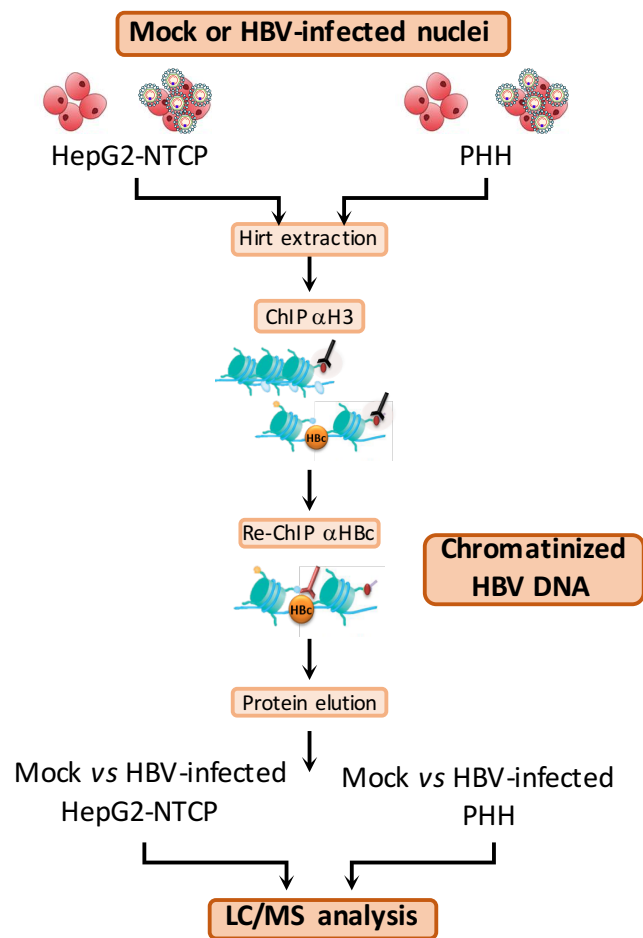


Table 2. Domains represented in RNA/DNA hybrid-binding proteins

Domain	Proteins	FDR
Alpha-beta plait	SRSF1, RBM3, TRMT2A, U2AF2, U2SURP, RBM5, SYNCRIP, SLIRP, SART3, NONO, RAVR1, DDX21, PTBP1, G3BP2, NCL, TAF15, CPSF7, MATR3, FUS, SRSF10, IGF2BP1, NIFK, IGF2BP3, HNRNPL, HNRNPA3, HNRNPM, SAFB, HNRNPC, TNRC6B, RBM27, RBM25, EWSR1, RBM26, RPS24, HNRNPAB, ALYREF, ELAVL1, RPL23A, SSB, HNRNPDL, LARP4B, HNRNPA0, UPF3B, SRSF7, SFPQ, PSPC1, CIRBP, HNRNPH1, RBM14, RBM15	10^{-30}
P-loop triphosphate hydrolase	KIFC1, SEPT2, HBS1L, EIF5B, RNF213, MTHFD1L, DDX17, DDX18, DHX38, DDX23, DDX21, DHX36, DHX30, MX1, GNL3, MYO6, RAN, DDX39B, EIF2S3, EEF2, MYH9, HNRNPU, RFC5, RFC3, AQR, DHX29, ASCC3, SMARCAL1, SMARCA5, EEFSEC, SEPT7, SMARCA4, SEPT9, RAB7A, GNAI2, CTPS1, MOV10, DDX46, DDX3X, DDX41, HELLS, EEF1A1, DHX9, MYO1C, MYO1E, MYO1G, DDX1, YTHDC2, DDX5, DDX6, ARF4, SMC1A, DAP3, ABCF1, ABCF2, SKIV2L2, MTHFD1, LONP1, MCM7, DYNC1H1, KIF2A, SRPRB, MCM2, MCM3, MCM4, RAD50, MCM5, MCM6, EIF4A3, RECQL, EIF4A1, SNRNP200, RUVBL2, RUVBL1, MYO5A, TUFM, SKIV2L, TAP1, DHX15, RAB11A, DHX16, EHD1, CHD4, EHD4, UPF1, MSH3, MSH2, SMC6, DRG1, SMC3, SMC4, HNRNPUL2, SAMD9	10^{-23}
Nucleic acid-binding, OB-fold	NARS, PNPT1, YBX1, KARS, RPA1, MCM7, CSDE1, CCAR1, YARS, PDCD11, DARS, AIMP1, EXOSC2, LIG3, EXOSC3, MCM2, DIS3L, MCM3, MCM4, MCM5, MCM6, DIS3, EIF2S1, RUVBL2, RPS11, RUVBL1, SRBD1	10^{-17}
DNA/RNA helicase, DEAD/DEAH box type, N-terminal	SKIV2L, SKIV2L2, DDX17, DDX46, DDX18, DDX23, DHX38, DDX3X, DHX15, DHX16, DDX21, DHX36, DDX41, DHX30, DHX9, DDX39B, DDX1, YTHDC2, DDX5, DDX6, EIF4A3, RECQL, DHX29, ASCC3, SNRNP200, EIF4A1	10^{-15}
K homology domain	KHDRBS1, HDLBP, PNPT1, PNO1, SF1, EXOSC2, EXOSC3, IGF2BP1, ANKHD1, IGF2BP3, FXR1, FUBP1, ANKRD17, PCBP2, KHSRP	10^{-8}

Wang, *Genome Res* 2018

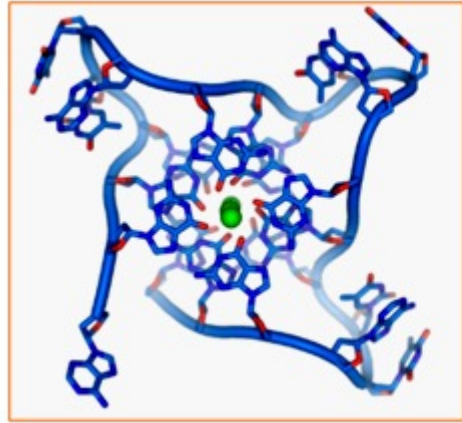
G-quadruplex structures

Testoni, *International HBV Meeting* 2017

G4s regulate multiple steps in gene expression

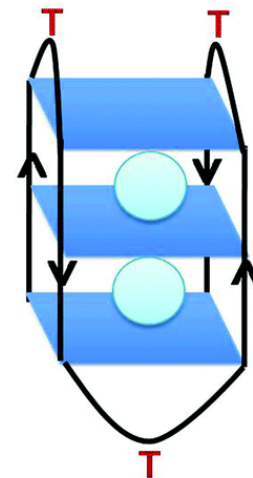


$x \geq 3$



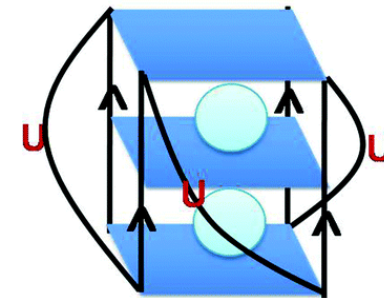
four guanine bases form a flat plate and these flat four-base units then stack on top of each other, to form a stable G-quadruplex structure

DNA G-quadruplex
(less stable)



•Both antiparallel and parallel topologies possible

RNA G-quadruplex
(more stable)



- 2' hydroxyl group in ribose sugar
- Less hydration
- increased intramolecular bonding
- Only parallel topology possible

Transcription regulator
Initiation

*Chromatin Looping between
Enhancer and promoter*

mRNA splicing

**3' end mRNA
processing**

Translation

Epigenome and Epitranscriptome

Does cccDNA contain G4 sequences?

> [Nucleic Acids Res.](#) 2017 Nov 2;45(19):11268-11280. doi: 10.1093/nar/gkx823. 

A G-quadruplex motif in an envelope gene promoter regulates transcription and virion secretion in HBV genotype B

Banhi Biswas ¹, Manish Kandpal ¹, Perumal Vivekanandan ¹

Editorial > [FEBS J.](#) 2017 Apr;284(8):1184-1203. doi: 10.1111/febs.14050. Epub 2017 Mar 17.

The epsilon motif of hepatitis B virus RNA exhibits a potassium-dependent ribonucleolytic activity

Dibyajnan Chakraborty ¹, Sagarmoy Ghosh ¹

> [Sci Rep.](#) 2021 Dec 1;11(1):23243. doi: 10.1038/s41598-021-02689-y.

Characterization of a G-quadruplex from hepatitis B virus and its stabilization by binding TMPyP4, BRACO19 and PhenDC3

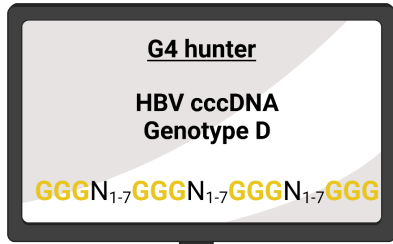
Orsolya Réka Molnár ^{# 1}, András Végh ^{# 1 2}, Judit Somkuti ¹, László Smeller ³

> [J Biol Chem.](#) 2021 Jan-Jun;296:100589. doi: 10.1016/j.jbc.2021.100589.  Epub 2021 Mar 24.

Identification and characterization of a G-quadruplex structure in the pre-core promoter region of hepatitis B virus covalently closed circular DNA

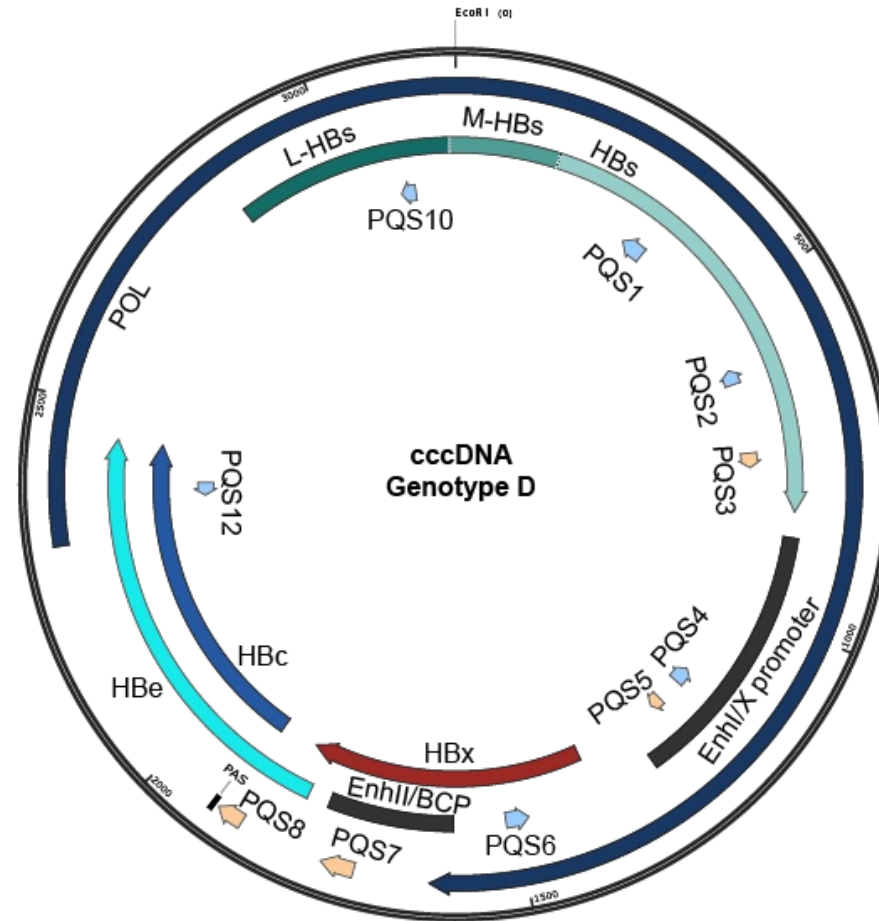
Vanessa Meier-Stephenson ¹, Maulik D Badmalia ², Tyler Mrozowich ², Keith C K Lau ³, Sarah K Schultz ², Darren L Gemmill ², Carla Osiowy ⁴, Guido van Marle ³, Carla S Coffin ⁵, Trushar R Patel ⁶

Both cccDNA and HBV RNAs contain G4 sequences (PQS)



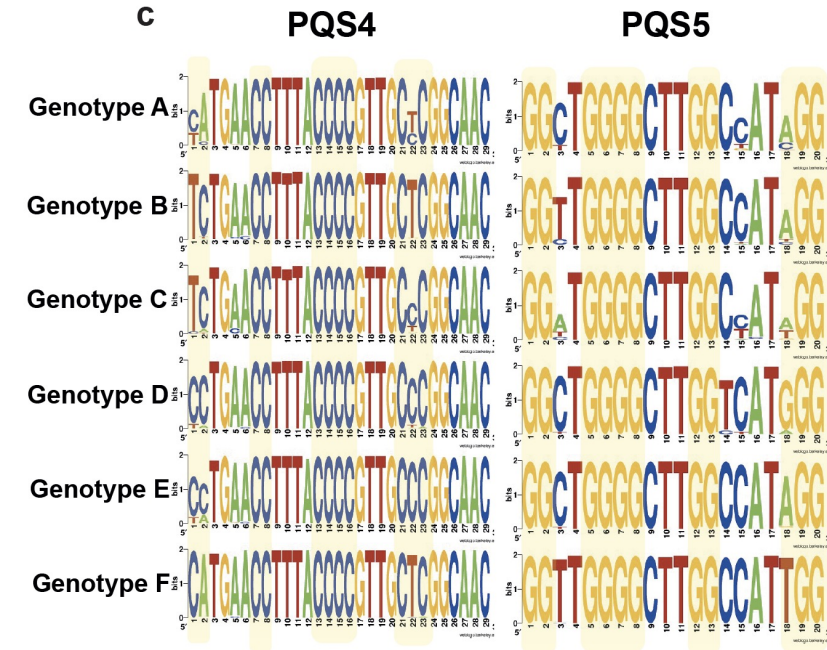
12 potential G4 sequences

In collaboration with Prof. JL Mergny

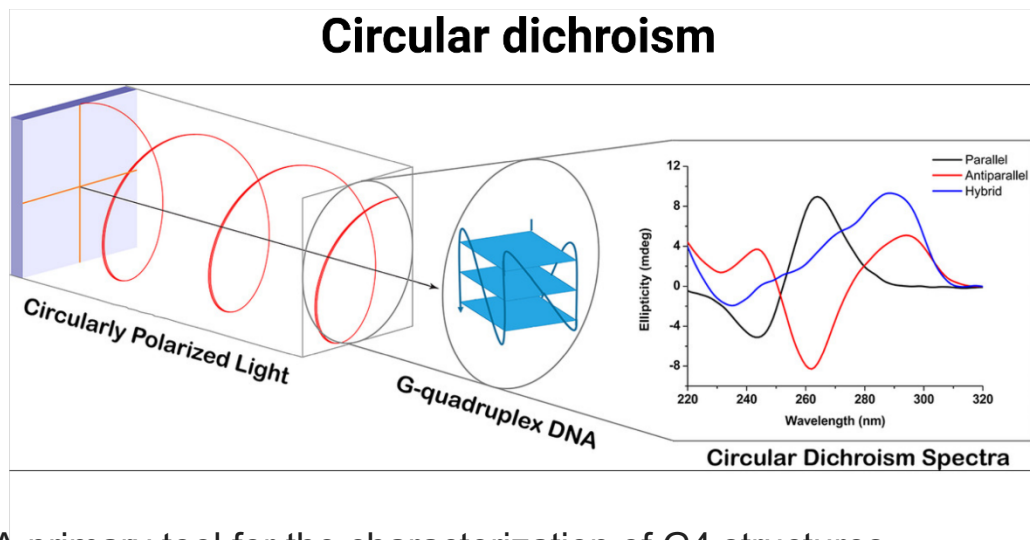


⇒ DNA & RNA G4

⇐ DNA G4

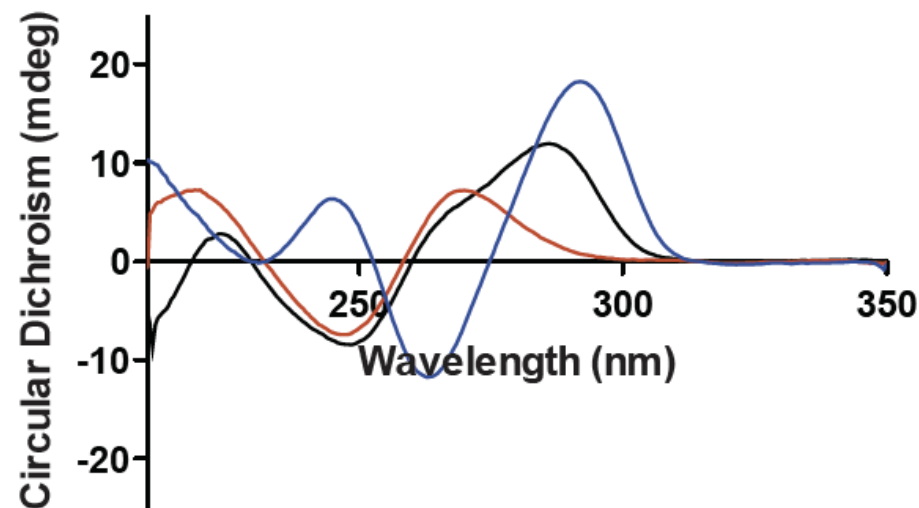


Both cccDNA and HBV RNAs contain G4 forming sequences



A primary tool for the characterization of G4 structures

PQS-containing oligonucleotides were incubated 10 minutes at 95 °C in 100 mM KCl and 10 mM Sodium cacodylate and progressively cooled down until reaching room temperature



PQS5 WT: **GGCTGGGGCTTGGTCATGGG**

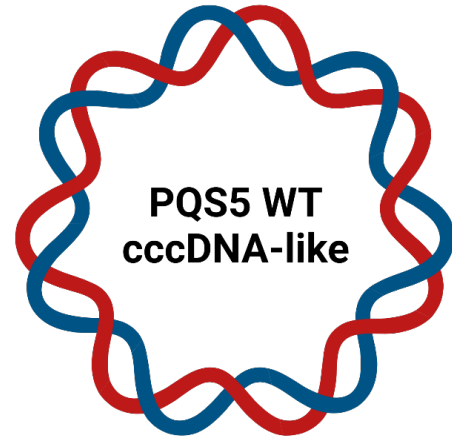
PQS5mut: **ATATGGATAATTAGTCATGAT**

10/12 predicted cccDNA PQS were confirmed by circular dichroism
The 2 PQS in HBV RNA were also confirmed

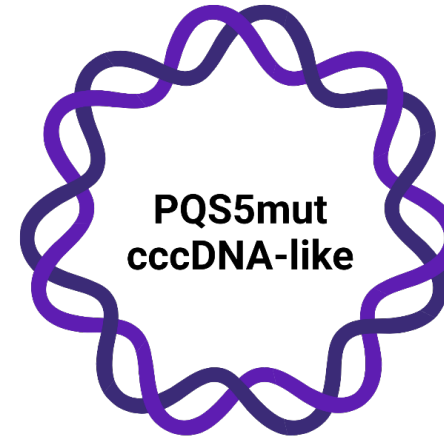
Bedrat, NAR, 2016

Del Villar-Guerra, Angew Chem Int Ed Engl., 2018

Functional investigation of PQS4/5 sequences role in HBV replication

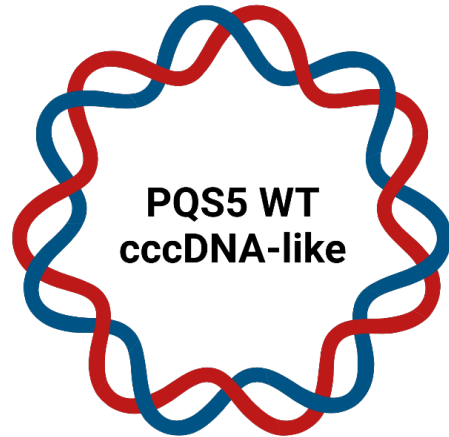


PQS5 WT: GGCTGGGGCTTGGTCATGGG

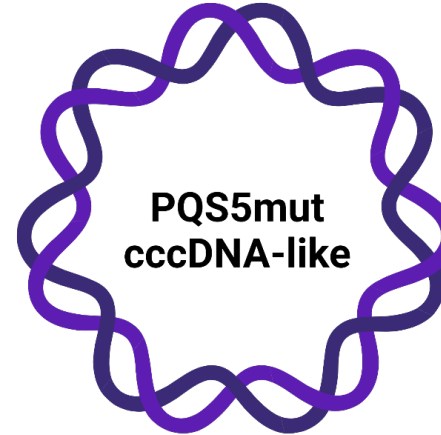


PQS5mut: ATATGGATATTAGTCATGAT
POL: G694I, G696I, G700I

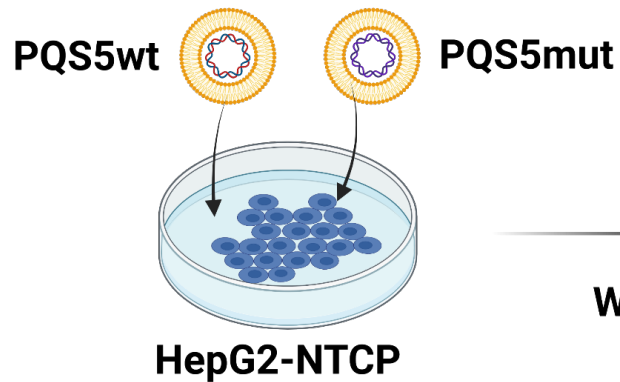
Functional investigation of PQS4/5 sequences role in HBV replication



PQS5 WT: GGCTGGGGCTTGGTCATGGG



PQS5mut: ATATGGATATTAGTCATGAT
POL: G694I, G696I, G700I



Day 1

Wash cells

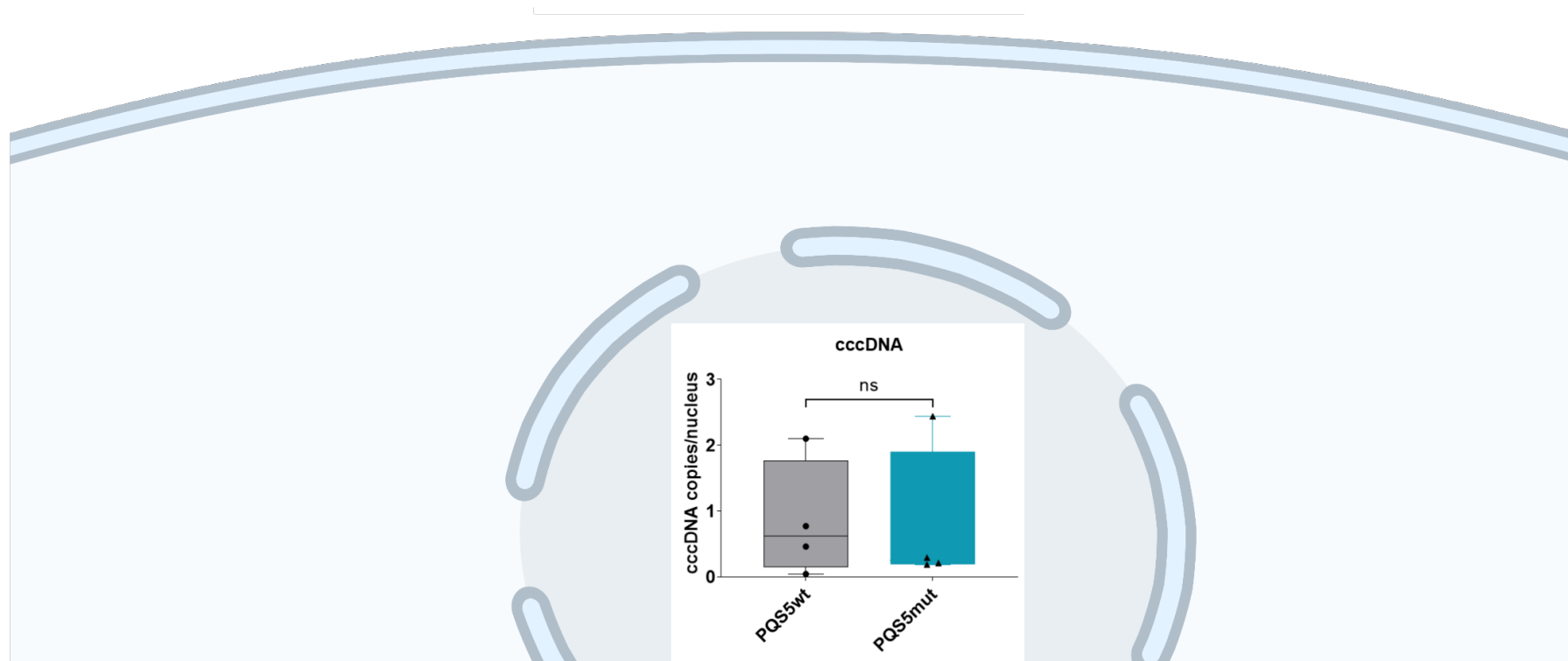
Day 3

Quantification of:

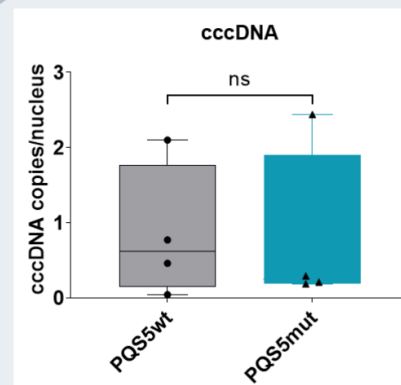
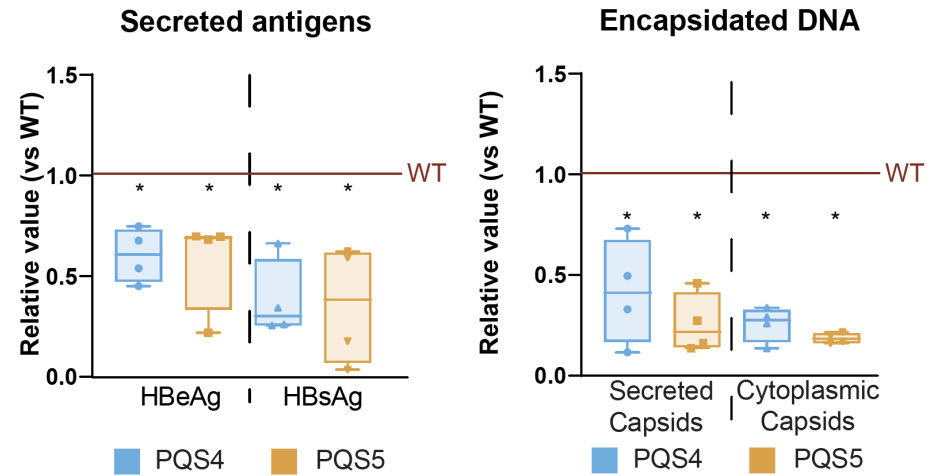
- Secreted antigens
- Secreted encapsidated DNA
- Intracellular encapsidated DNA
- HBV RNAs
- cccDNA

Data normalized to cccDNA levels
and normalized to WT

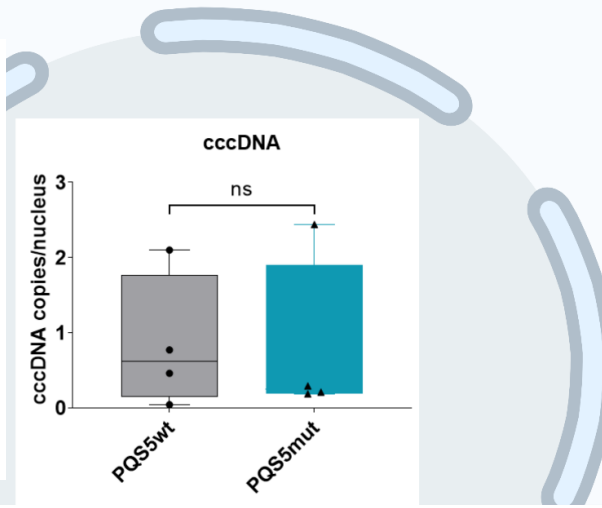
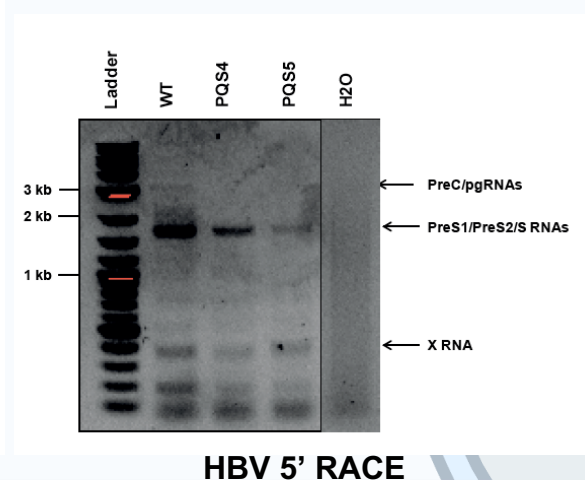
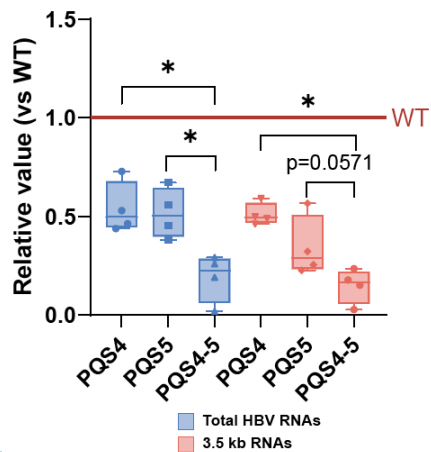
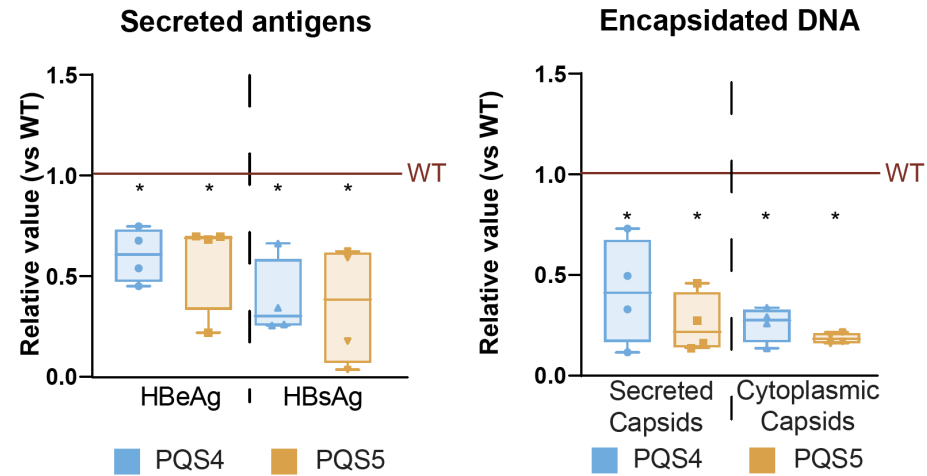
PQS4/5 mutations decrease all viral replicative parameters



PQS4/5 mutations decrease all viral replicative parameters



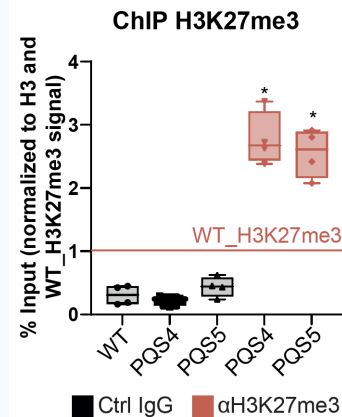
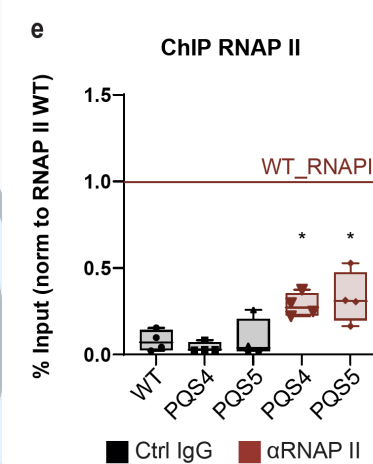
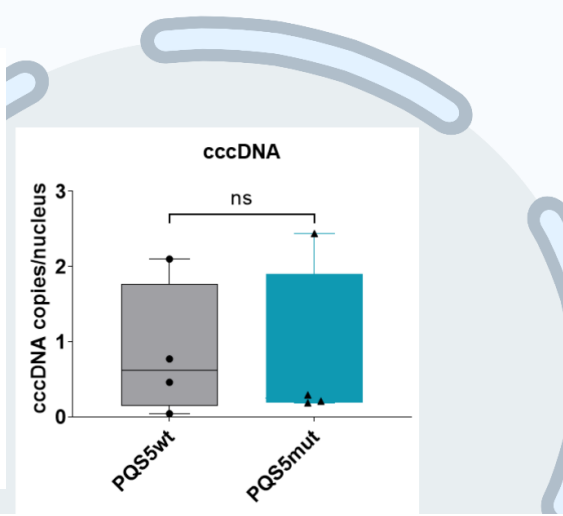
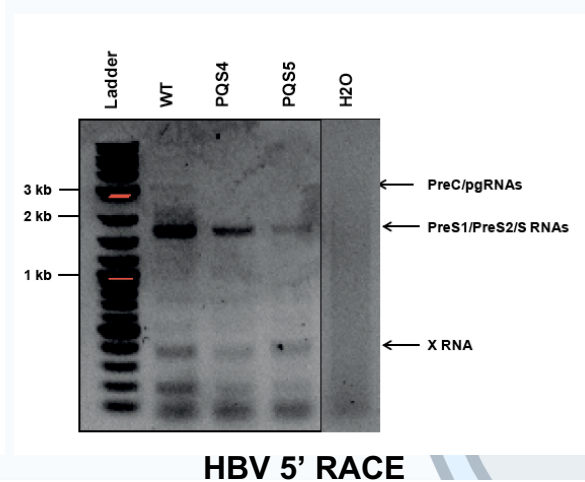
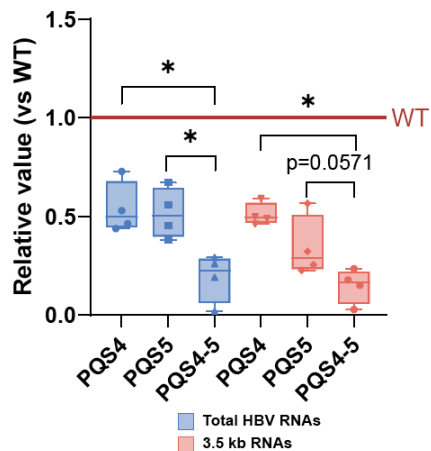
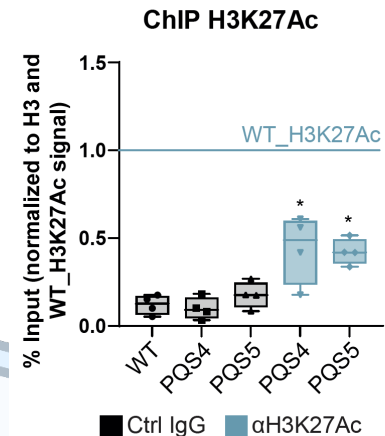
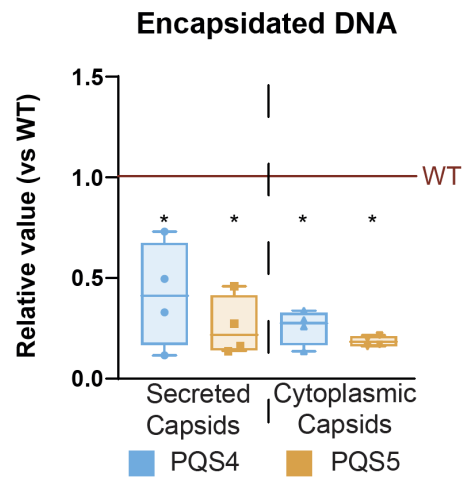
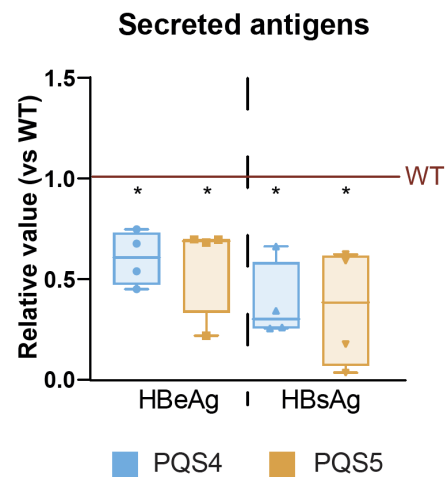
PQS4/5 mutations decrease all viral replicative parameters



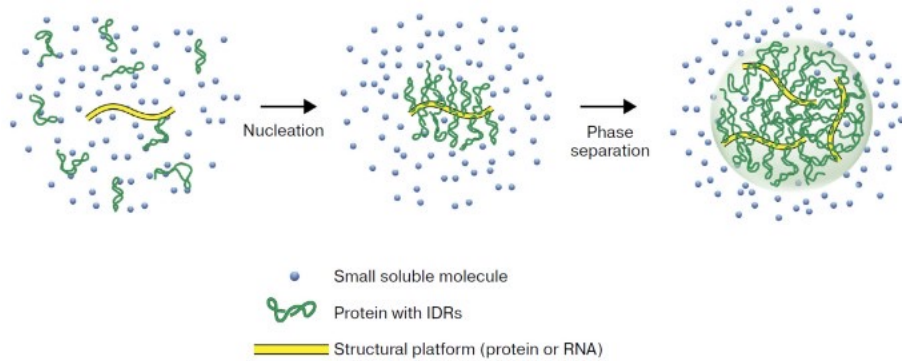
PQS4/5 mutations decrease cccDNA transcriptional activity

No effect on HBV RNA stability and translation

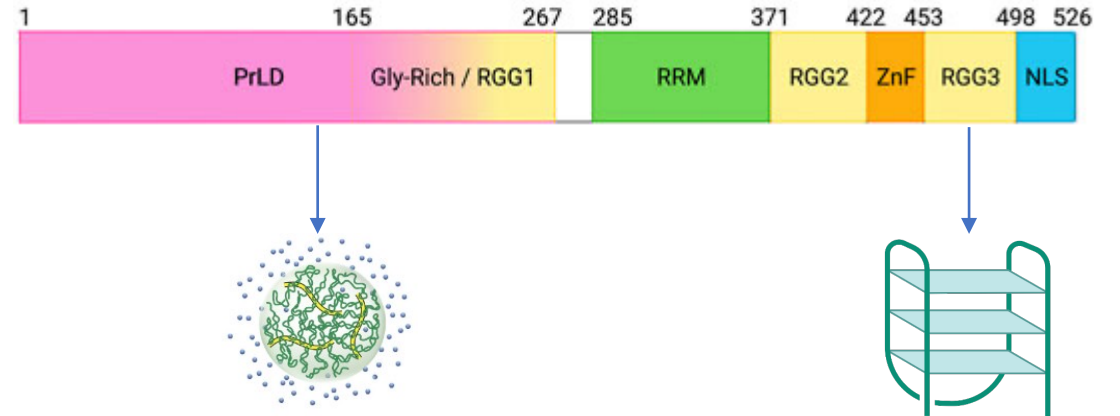
No effect on cccDNA transcription factor binding landscape



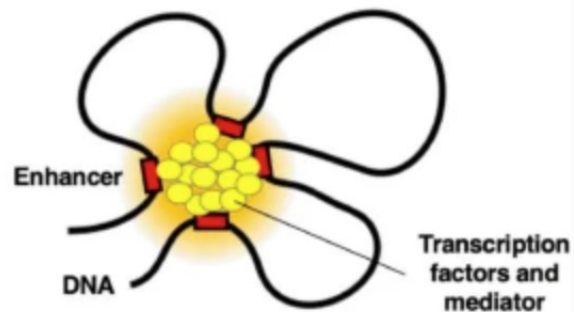
G4s promote liquid–liquid phase separation (LLPS)



FUS



b

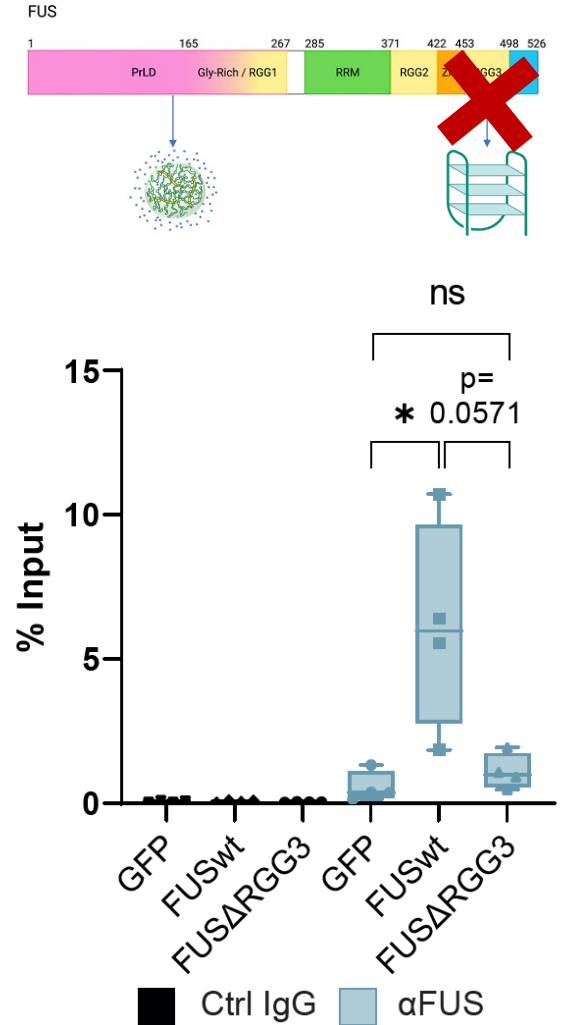
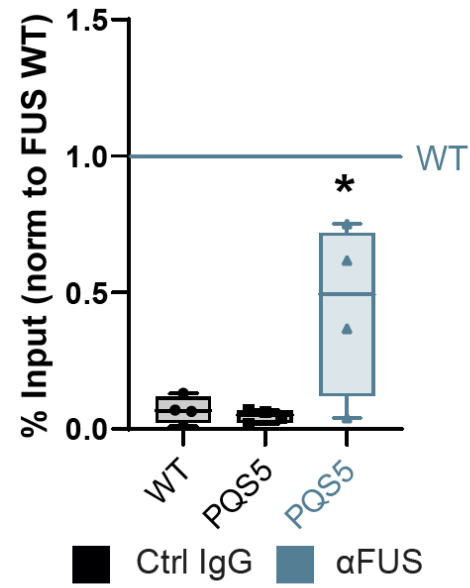
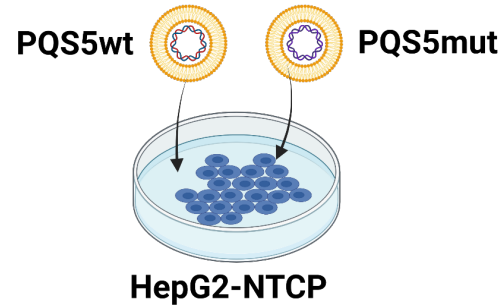
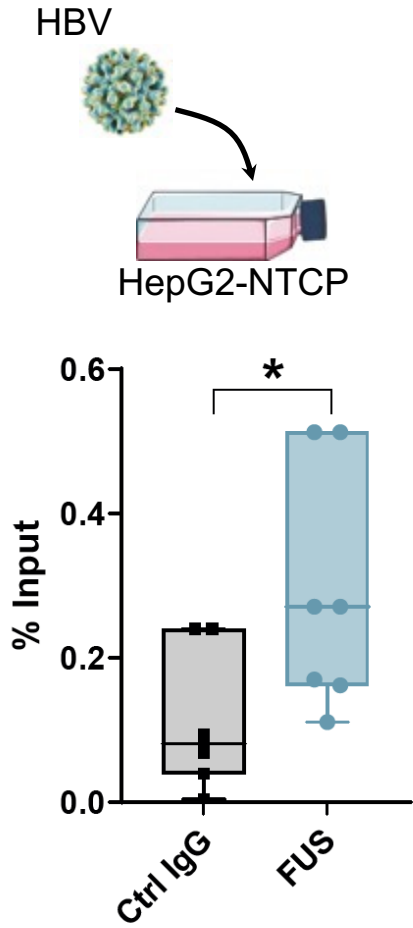


Involved in many steps of gene expression including transcription

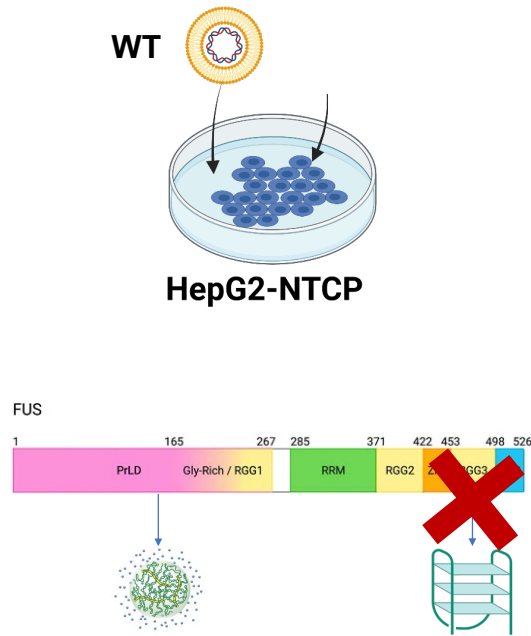
Yang, *Front Genetics* 2020

FUS binding to cccDNA is G4-dependent

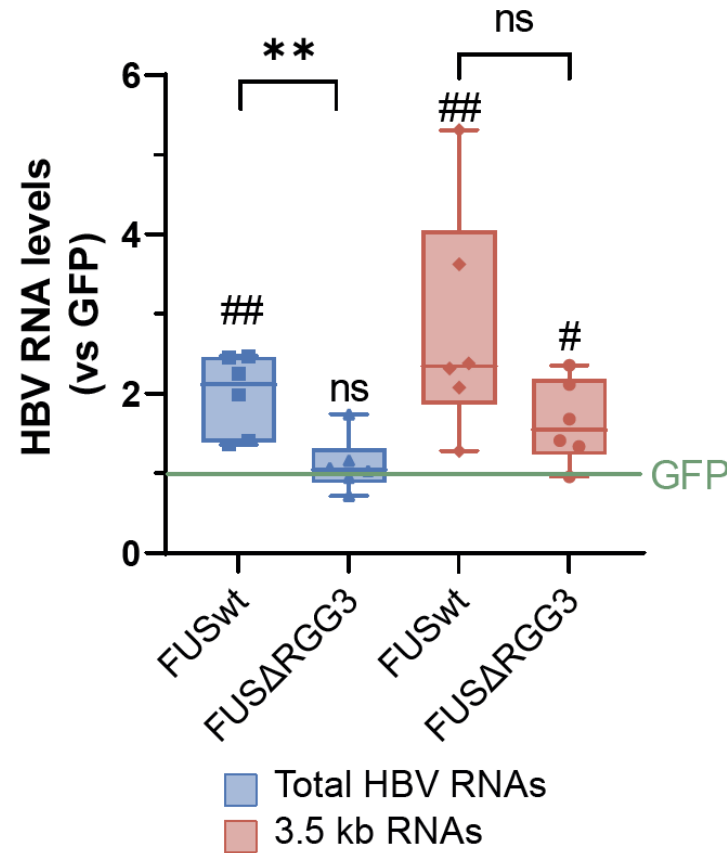
cccDNA ChIP-qPCR



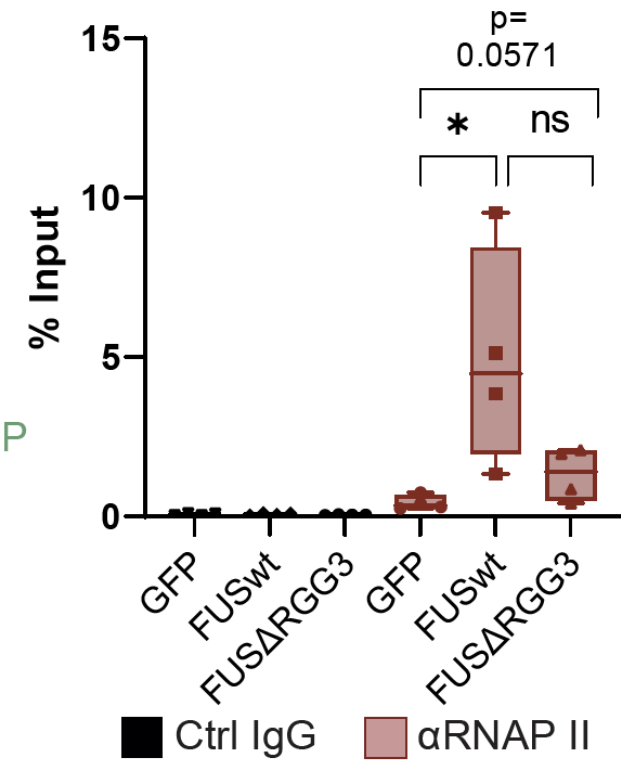
FUS binding is required for cccDNA transcription



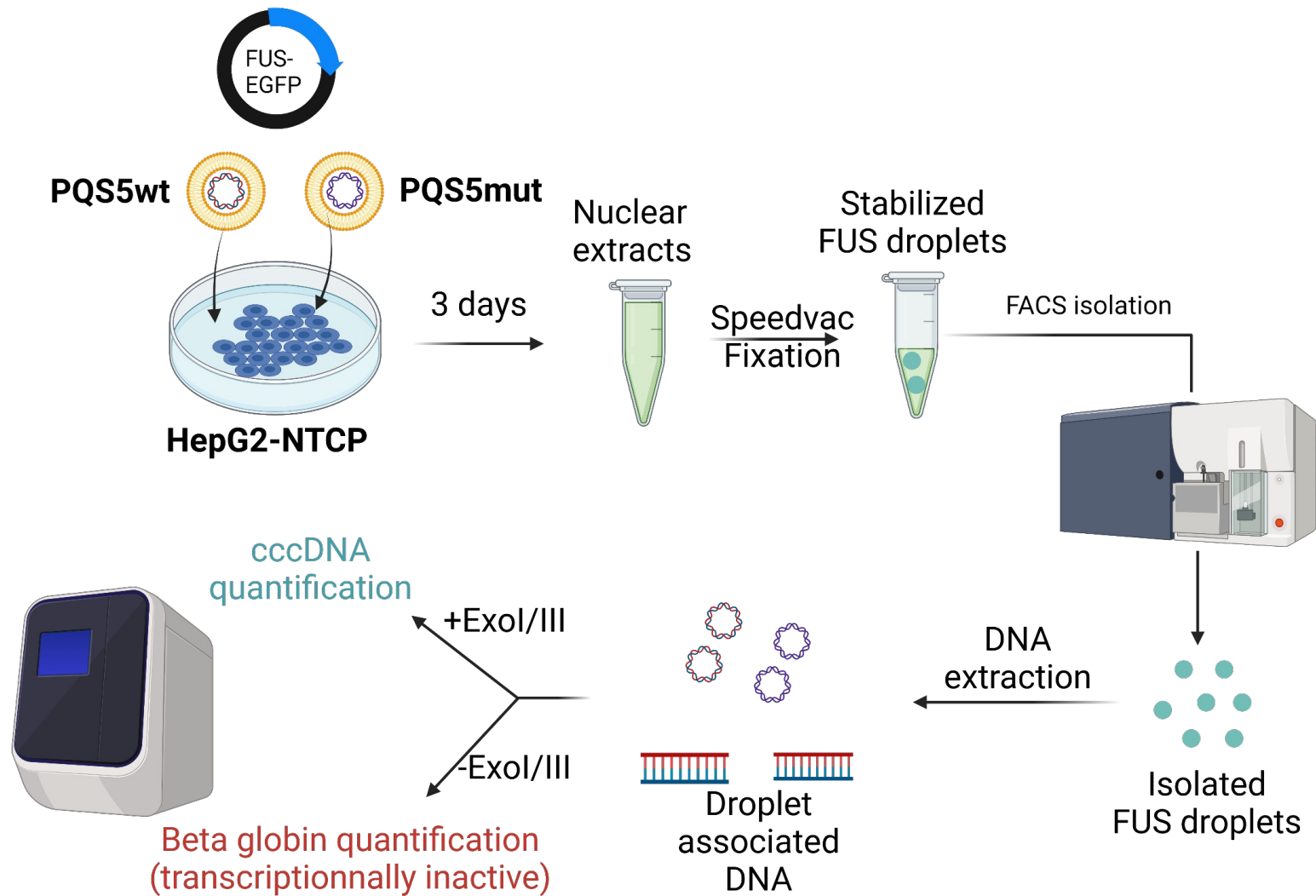
RNA RT-qPCR



cccDNA ChIP-qPCR

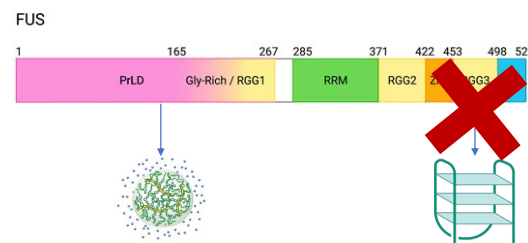
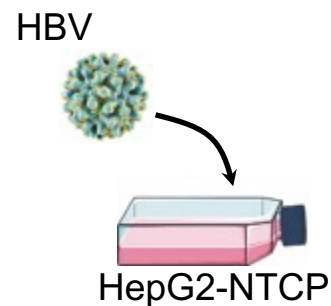
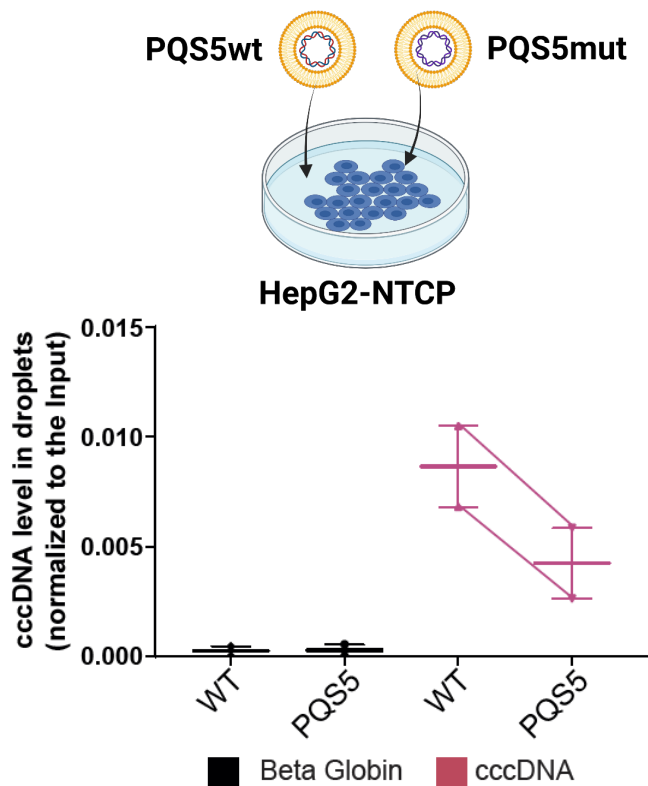


LLPS Isolation of FUS droplets–associated DNA

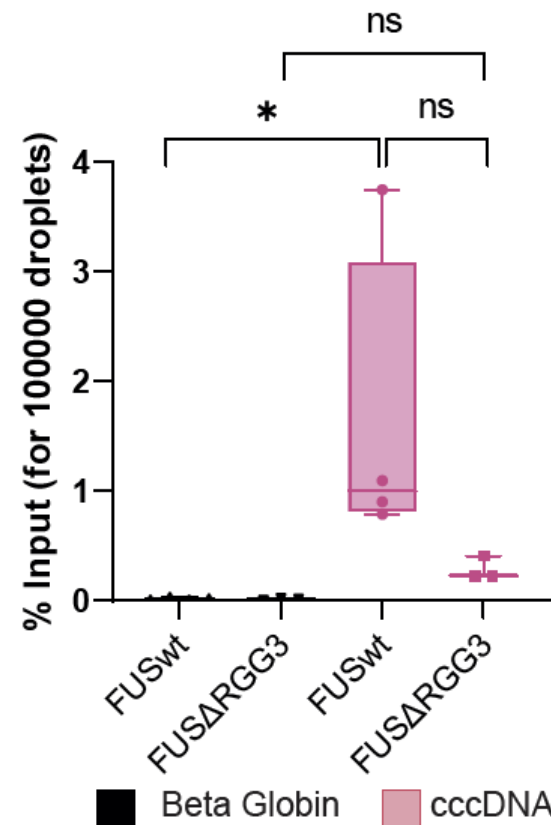


cccDNA undergoes LLPS in a G4- and FUS-dependent manner

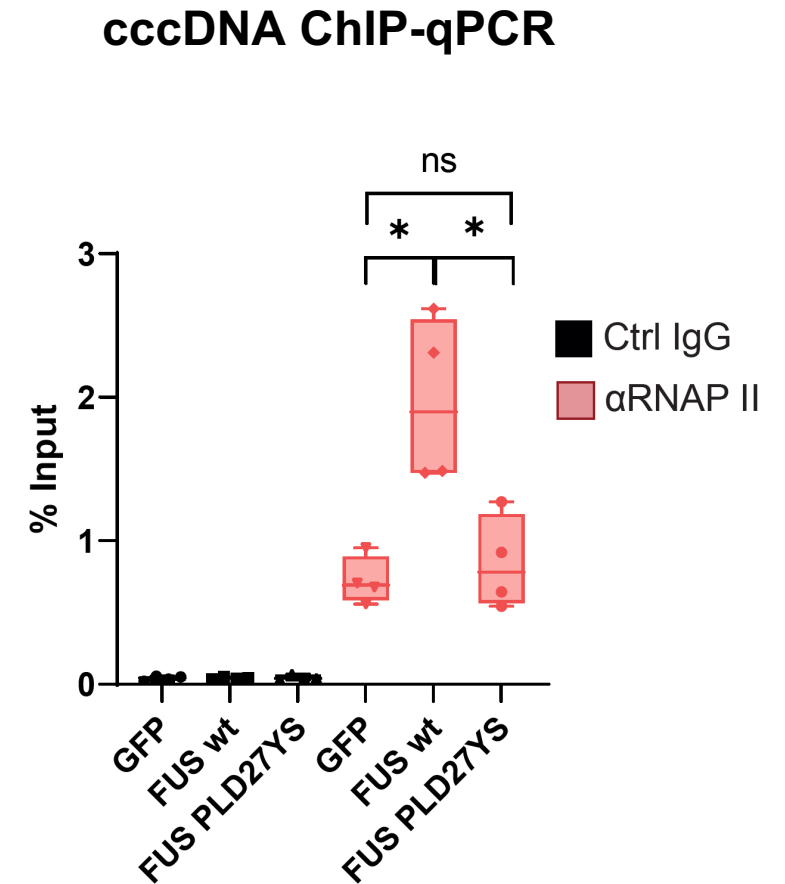
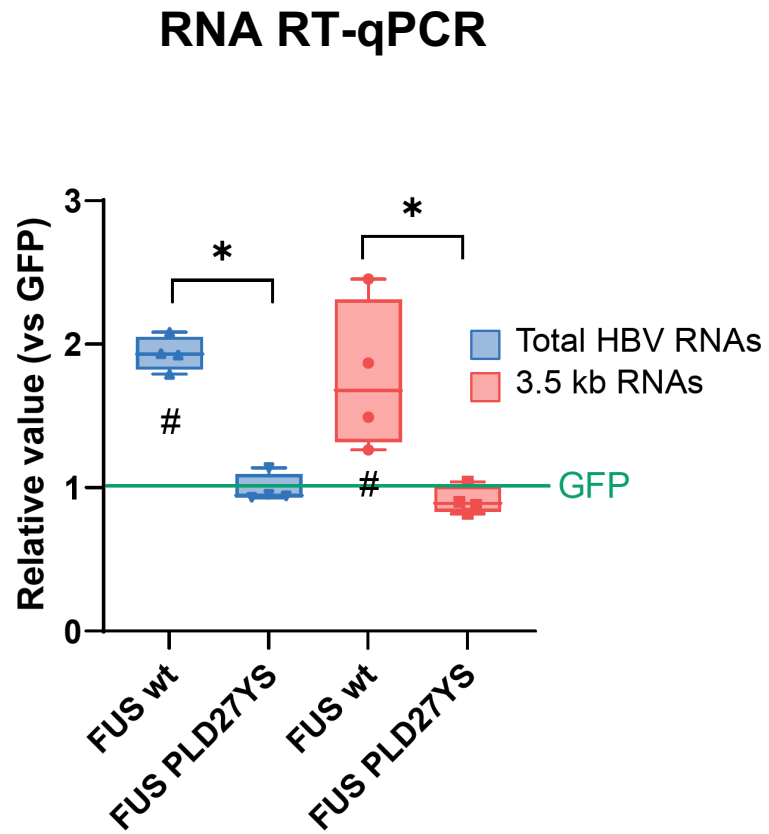
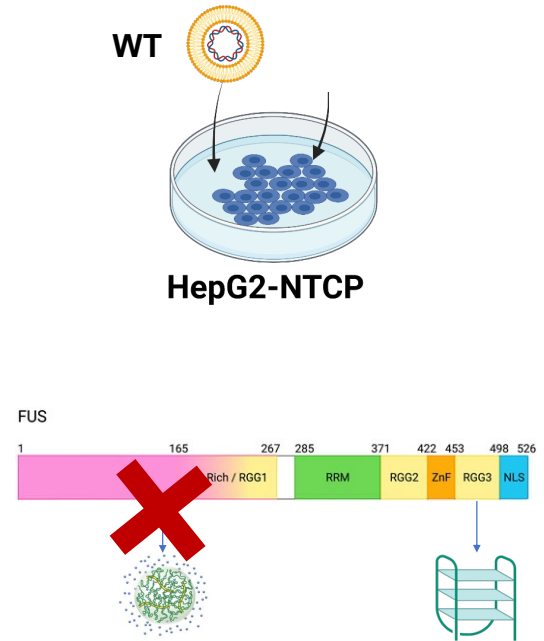
cccDNA level in droplets



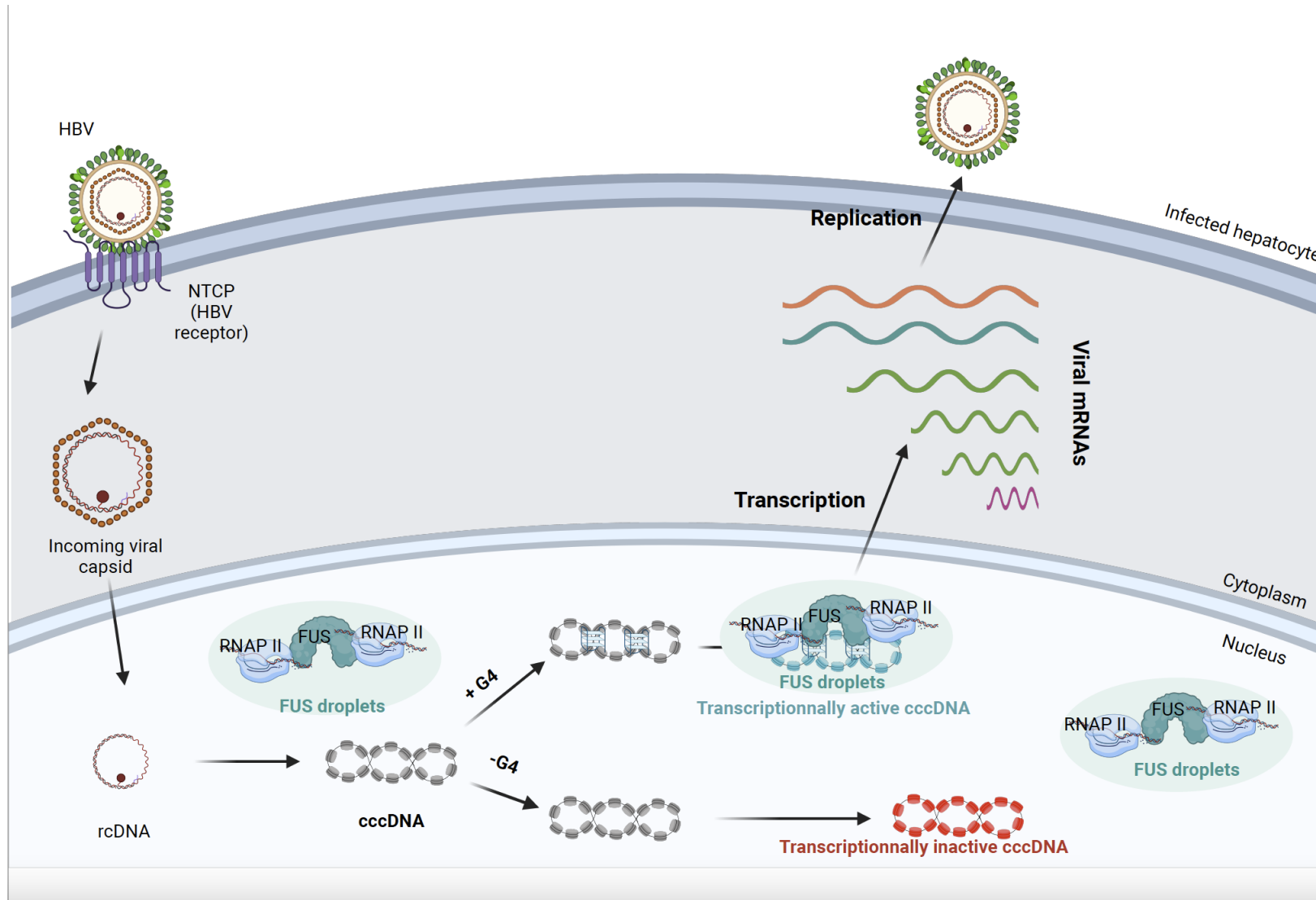
cccDNA level in droplets



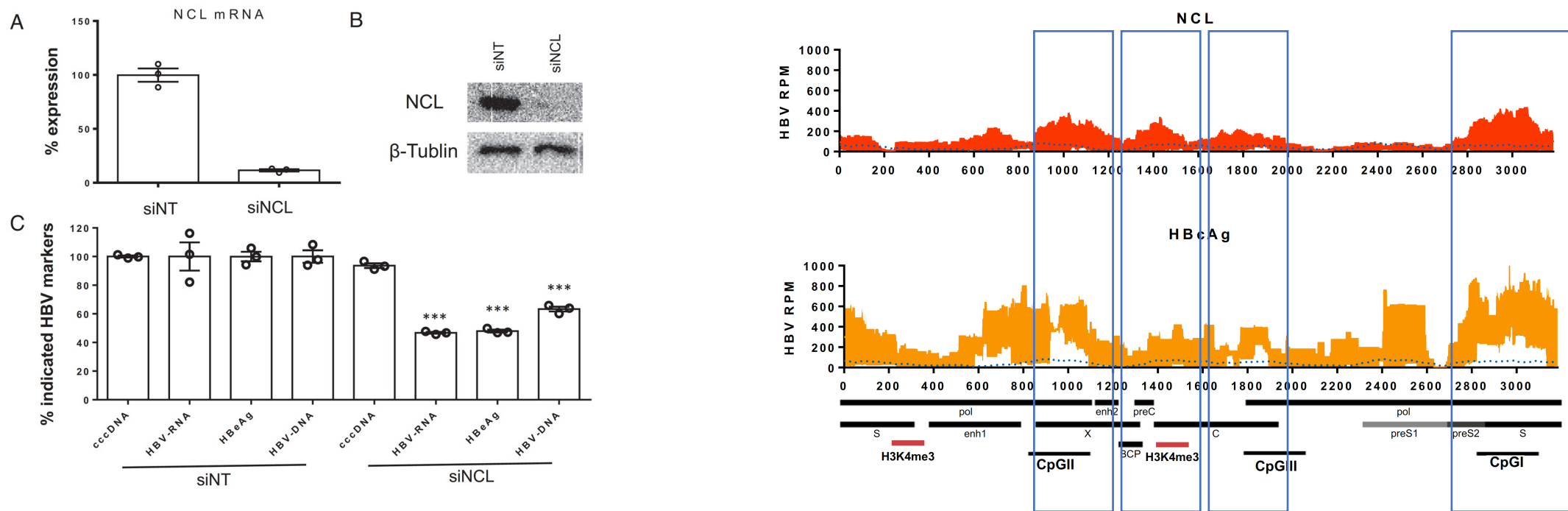
FUS-dependent LLPS is required for full cccDNA transcriptional activity



Conclusions



Nucleolin regulates cccDNA transcription via G4s?

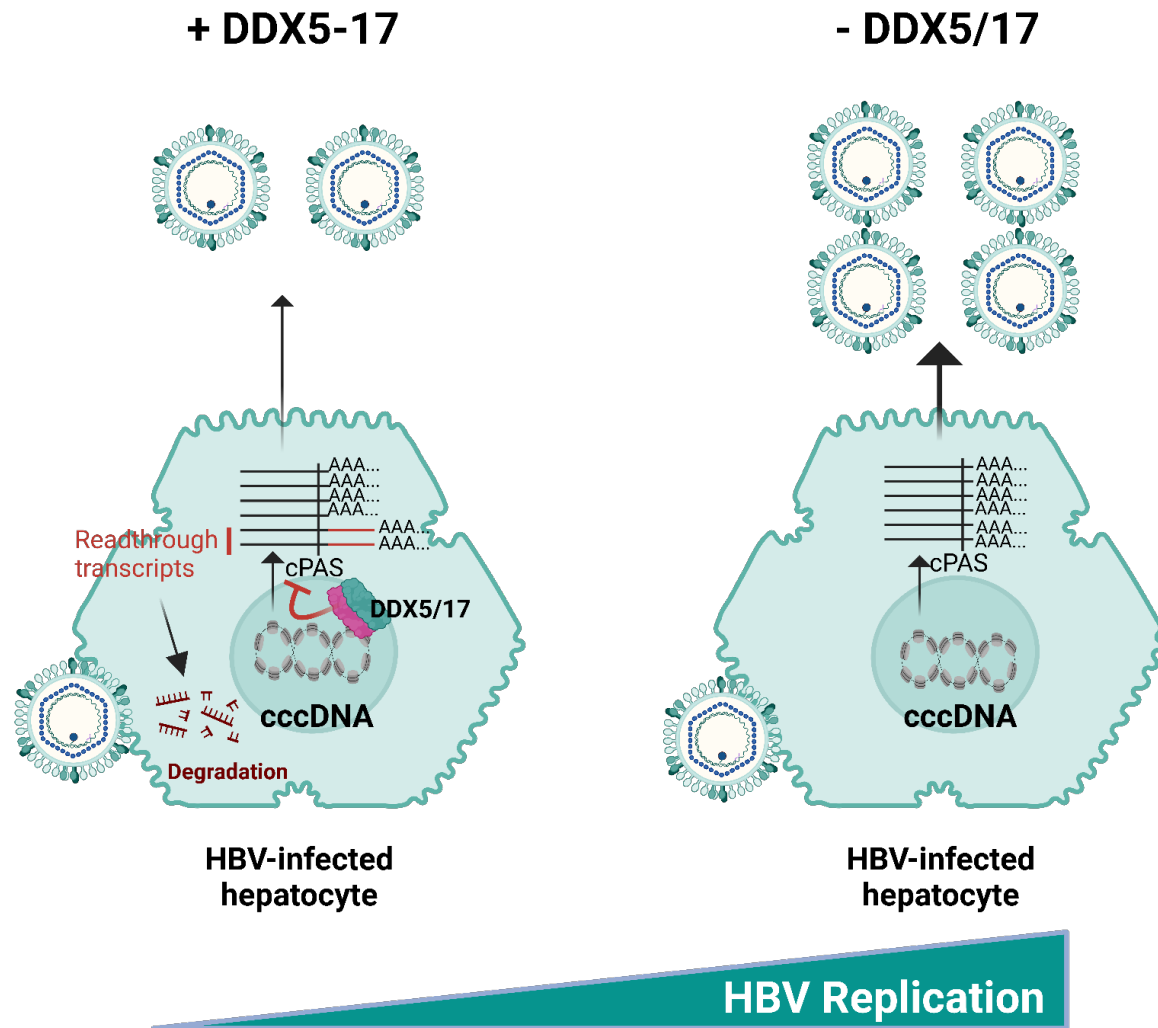


Xia, PNAS 2023

Nucleolin contains a RGG domain: G4-binding LLPS inducer!

Reviewed in Santos, Trends in Cell Biol 2022

DDX5/17 regulate HBV RNA transcriptional termination



DDX5 is also a G4-binding LLPS driver!

Reviewed in Pavlova, Genes 2023

DDX5/17 act as restriction factors for HBV replication

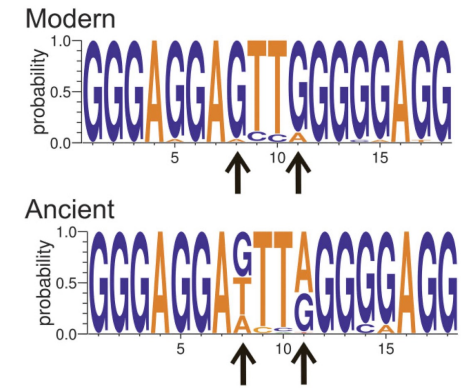
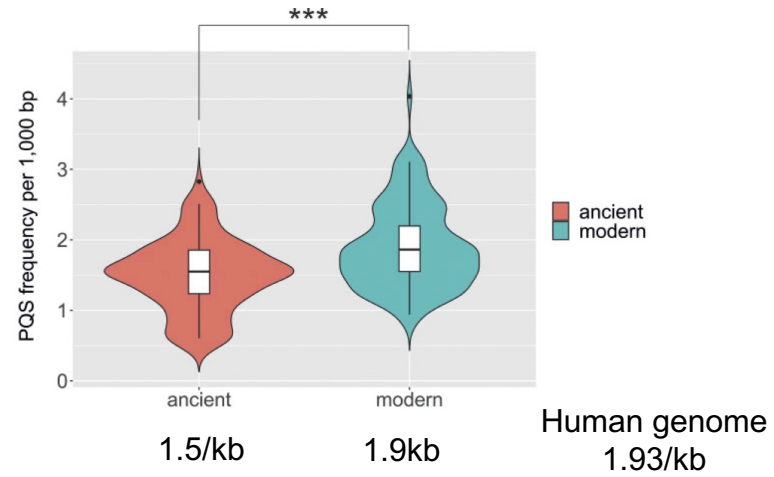
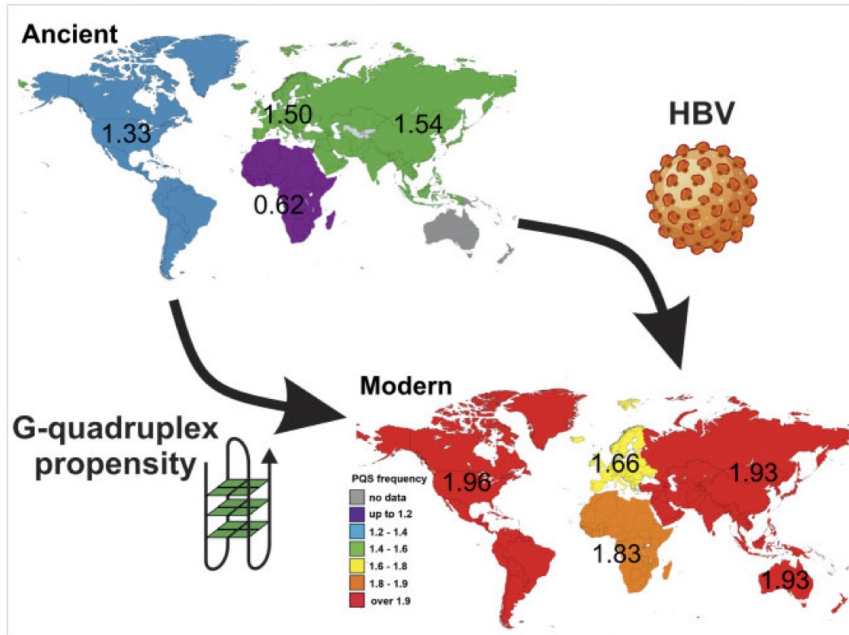
Chapus, Giraud*, J Hepatol 2024*
Zhang, Hepatology 2016
Mao, J Virol 2021

Is this antiviral effect mediated by G4s?

Chapus, Giraud*, J Hepatol 2024*

G4s in the evolution of HBV

232 HBV genomes from samples covering a more than 10-thousand-year history

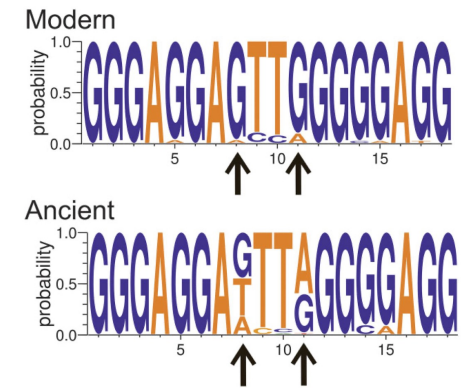
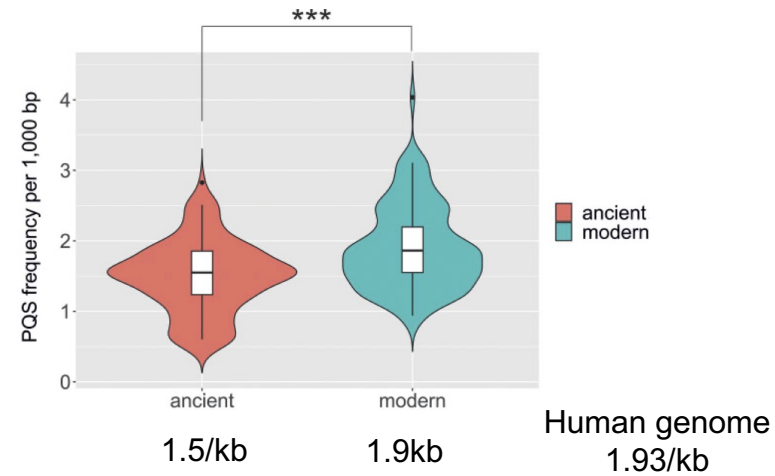
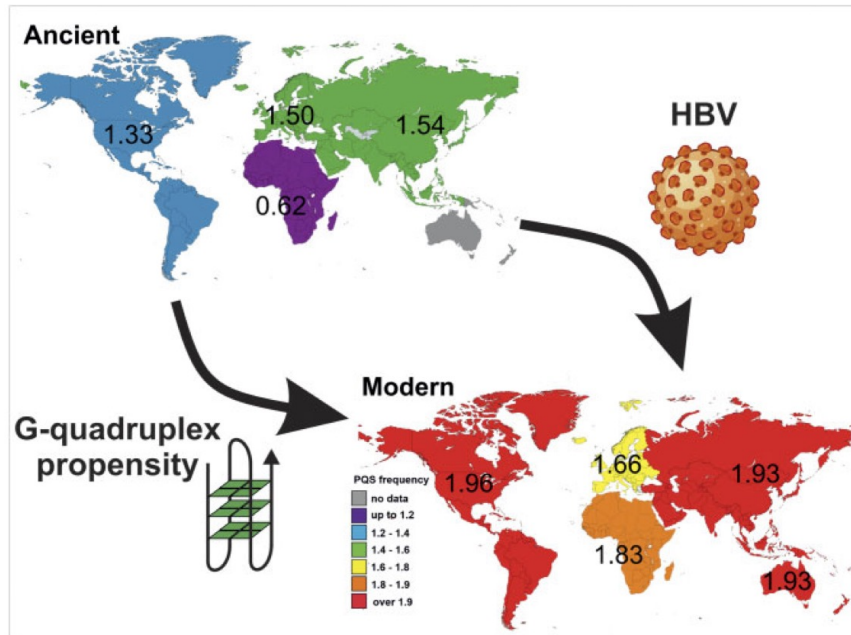


evolutionary shift for an increased number of G4s in recent HBV viruses

Brazda, NAR 2024

G4s in the evolution of HBV

232 HBV genomes from samples covering a more than 10-thousand-year history



evolutionary shift for an increased number of G4s in recent HBV viruses

Brazda, NAR 2024

For viruses causing chronic infections, G4s frequencies tend to converge evolutionarily with those of their hosts

Bohálová, Biochimie 2021 and Int J Mol Sci 2021

'Genetic camouflage' to both hijack host cell transcriptional machinery and avoid recognition as foreign material?

THANKS!



Viral hepatitis Team & Hepatology Dept



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M Michelet
S Maadadi
R Parent
C Combet



R Montserret



D Jutzi
M-D Ruepp



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