

# **Hepatitis B and D Viruses**

**Michael McGarvey 15/1/13**

# **Hepatitis B Virus: Scope of Lecture**

- **Disease pathogenesis**
- **Structure of virion**
- **Structure of genome**
- **Transcription and translation of viral proteins**
- **Genome replication, including reverse transcription**
- **HBx antigen**

# **Hepatitis D Virus: Scope of Lecture**

- **Structure of virion**
- **Structure of genome**
- **Transcription and genome replication**
- **Delta antigens**
- **Pathogenesis**

# Hepatitis

## **Inflammation of the liver:**

- **Fibrosis (scarring), Cirrhosis, Liver failure, Cancer**

## **Types of Hepatitis:**

- **Self Limiting, Acute, Chronic, Fulminant (rare)**

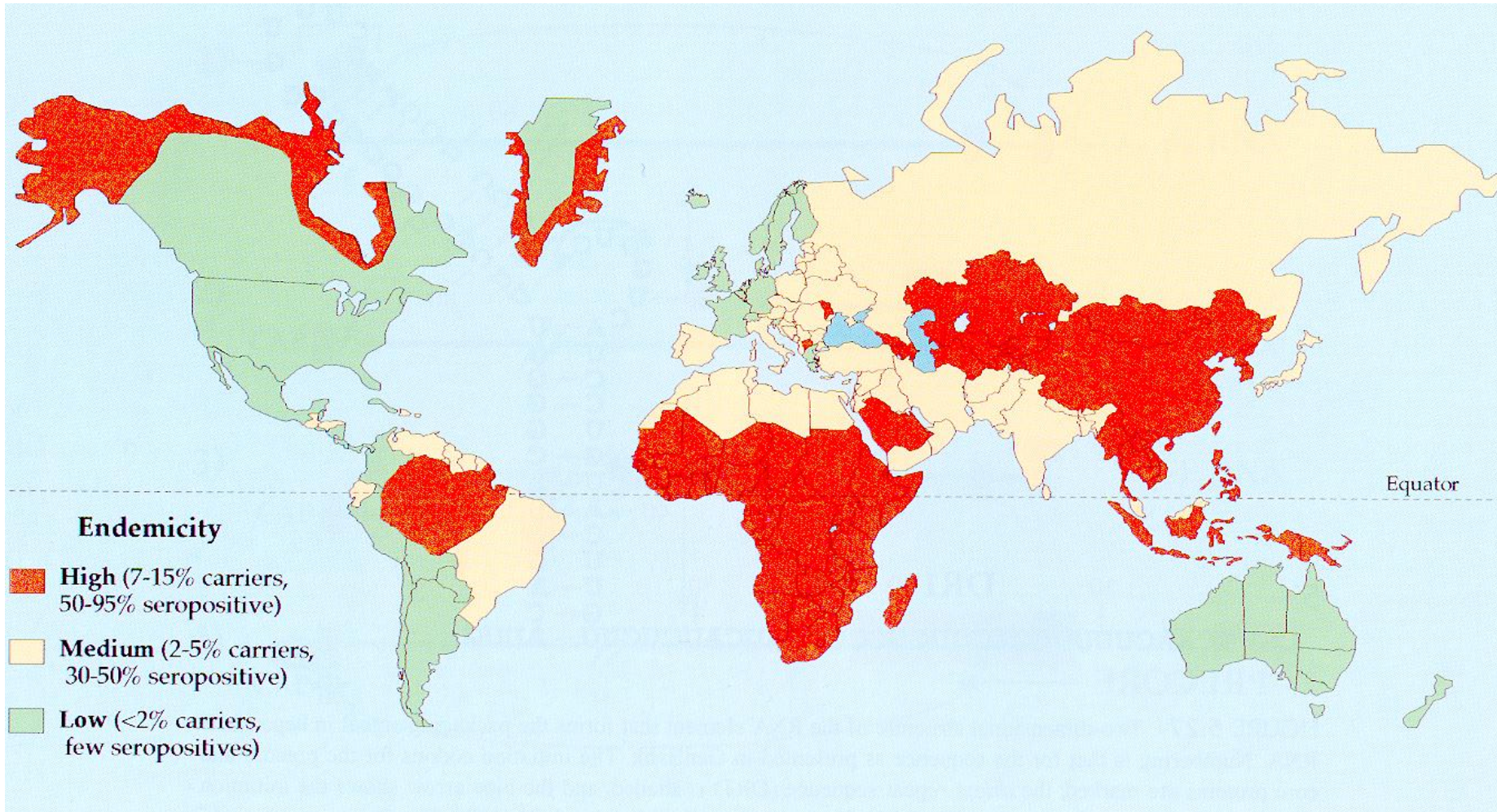
## **Caused by:**

- **Alcohol, Drugs, Toxins, Metabolic Disorders, Autoimmunity, Viruses**

# **HBV - Size of Problem**

- **ca. 200 million chronic infections**
- **10- 20% cirrhosis**
- **primary liver cancer**

# HBV – Global Distribution



# **HBV Liver Disease**

**Immunopathic:**

**-Host immune response destroys infected liver cells**

# HBV Liver Disease

## Types of Infection

**Acute - 5-6 months duration**

**- Self limiting (~ 90%)**

**- Fulminant (rare) – very extensive  
liver damage**

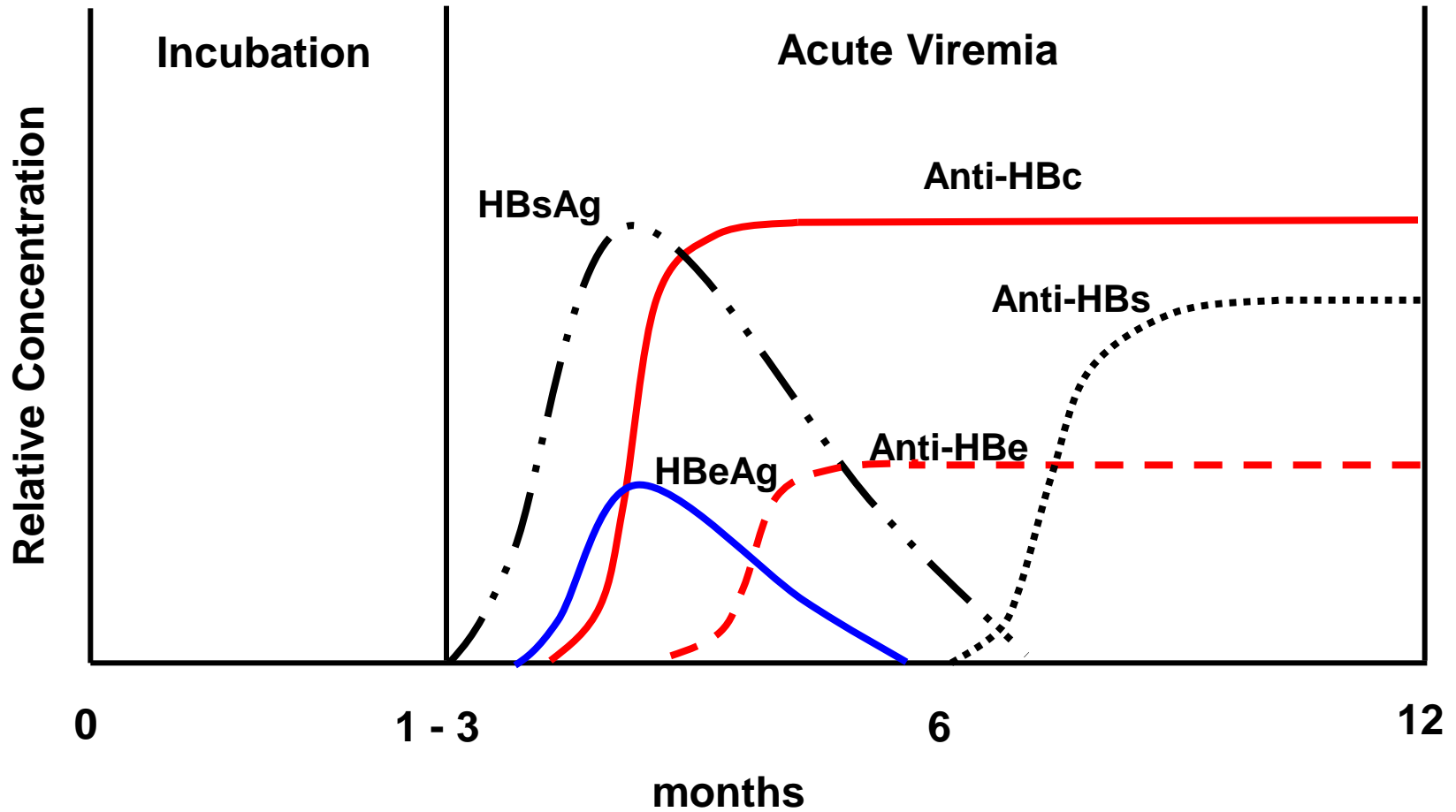
**Chronic (~10%)**

**- Quiescent**

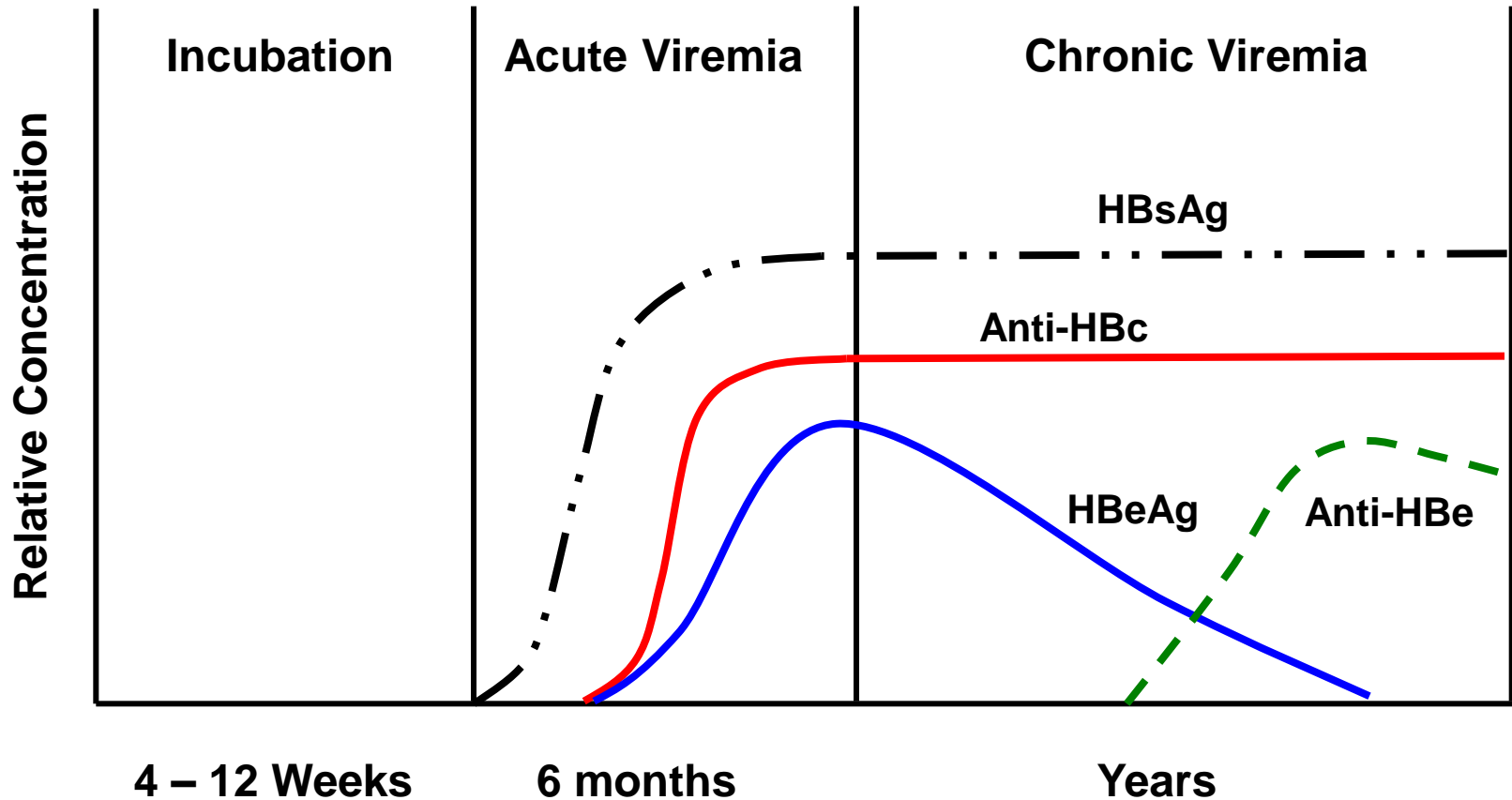
**- Active → Cirrhosis**



# HBV Liver Disease



# HBV Liver Disease



# HBV Liver Disease

## Types of Infection

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liver damage**

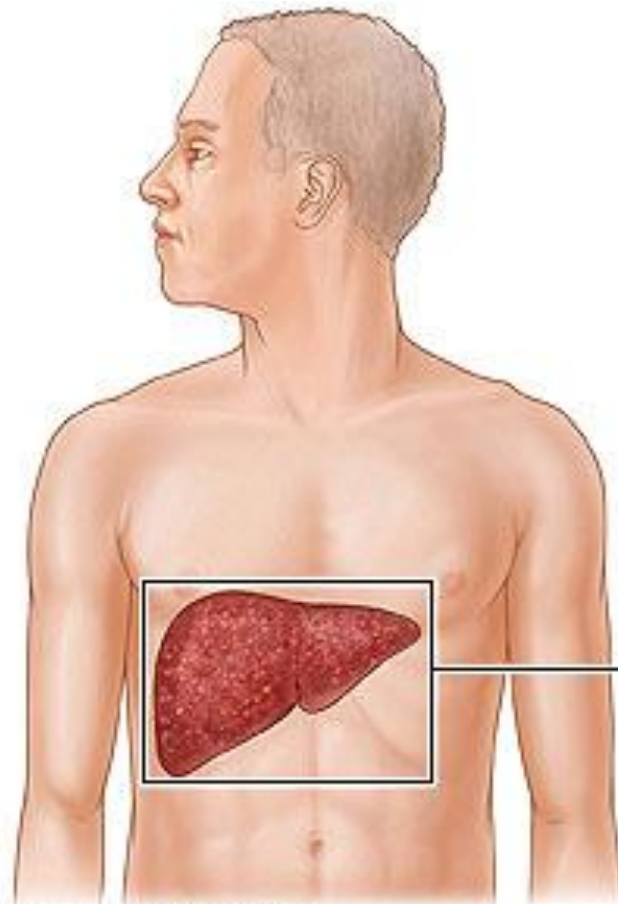
**Chronic (~10%)**

**- Quiescent**

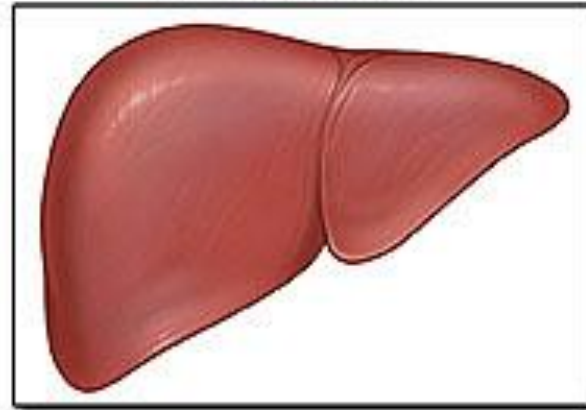
**- Active → Cirrhosis**

**→ Liver Cancer**

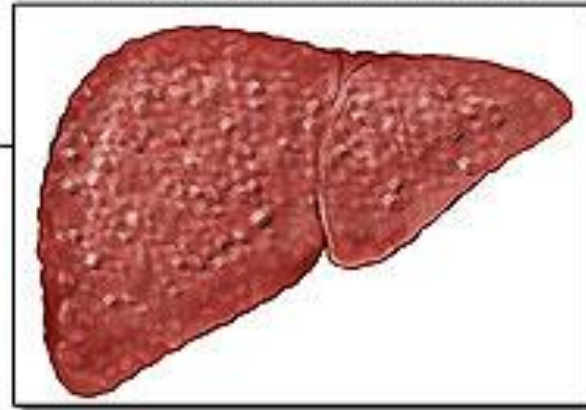
# Cirrhosis – Chronic HBV Infection



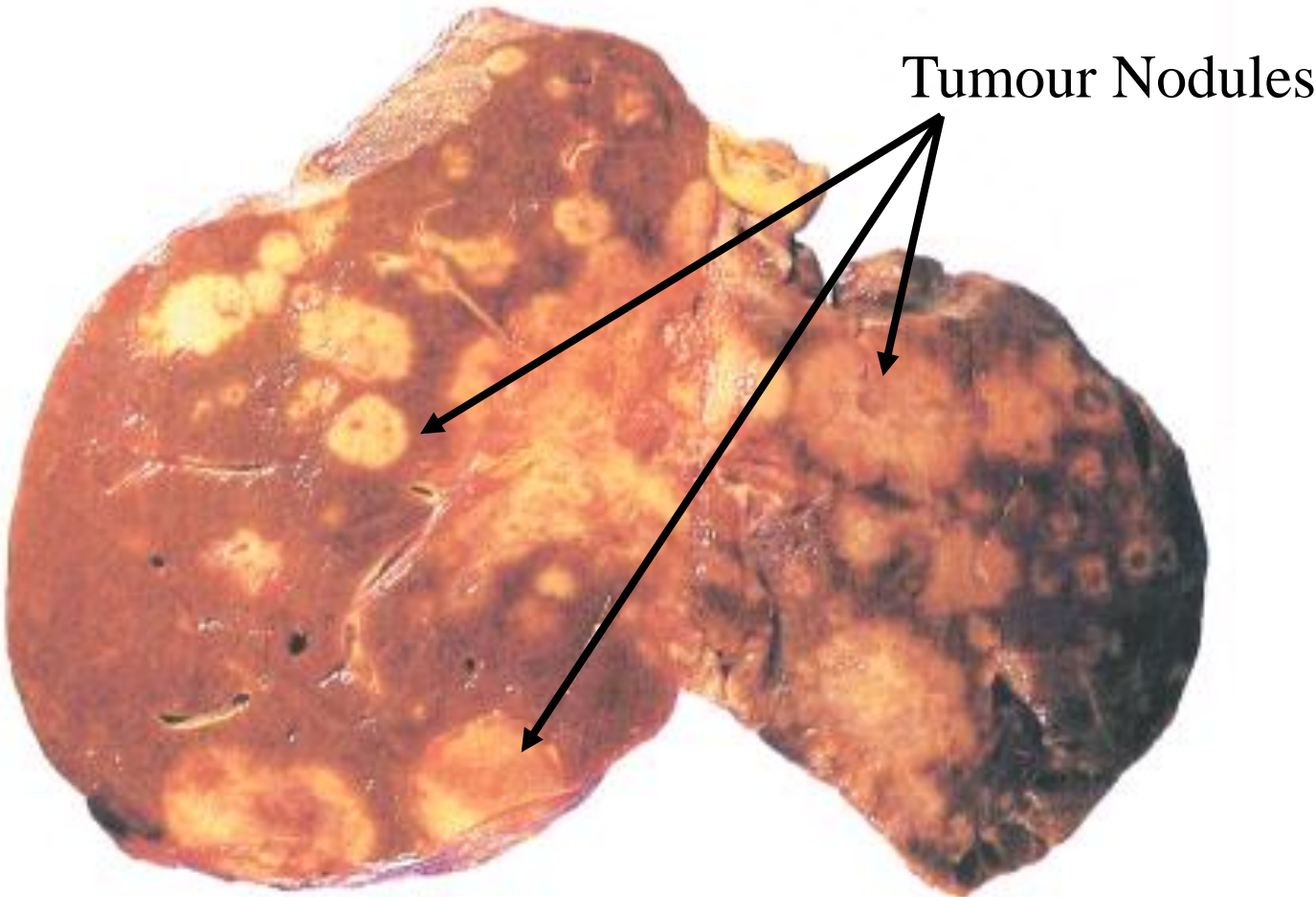
Normal liver



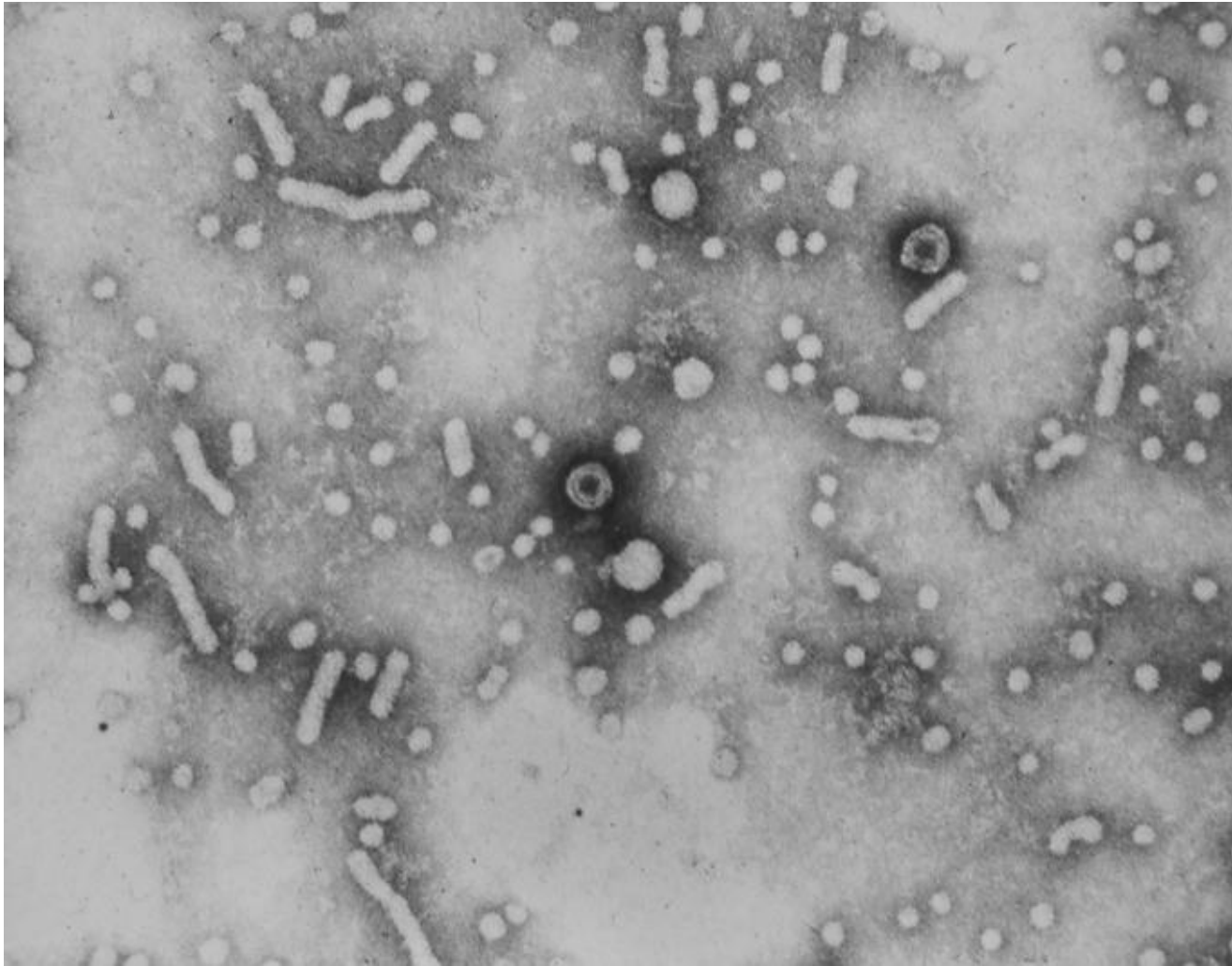
Liver with cirrhosis



# Chronic HBV Infection - Hepatocellular Carcinoma

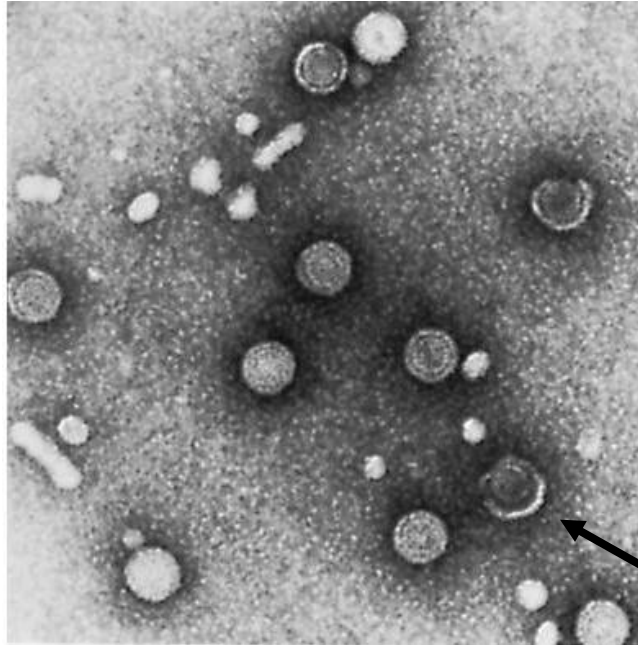


# HBV Virions

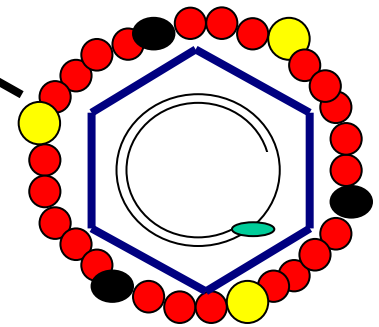


Transmission EM of Serum for HBV Infected Individual

# HBV Virions

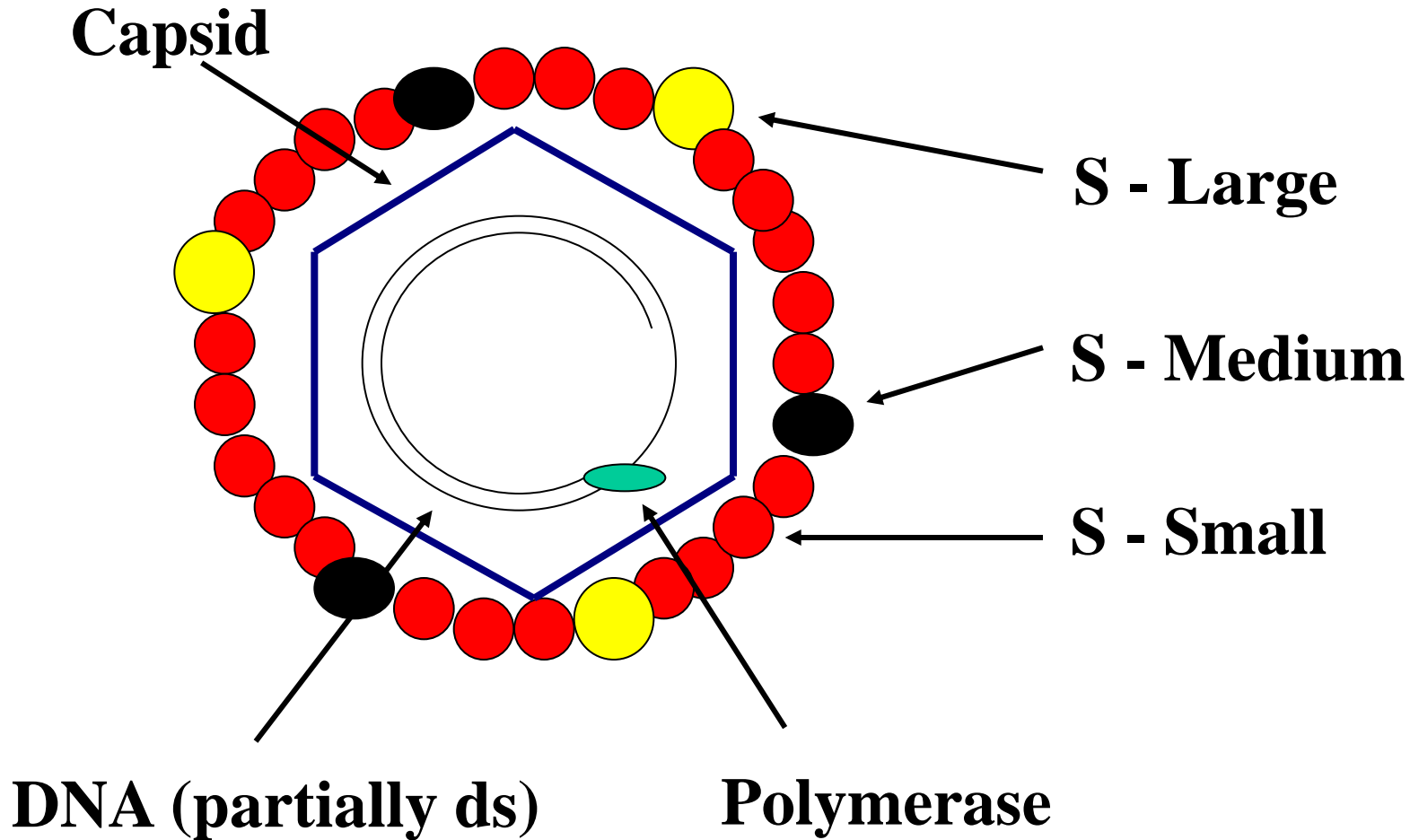


**Transmission electron  
micrograph of HBV  
infected human serum**



**HBV virions**

# HBV Virion





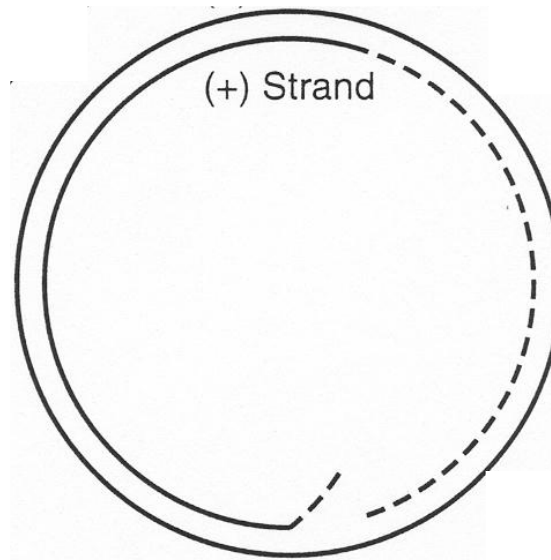
# HBV Genome

**DNA (partially ds)**

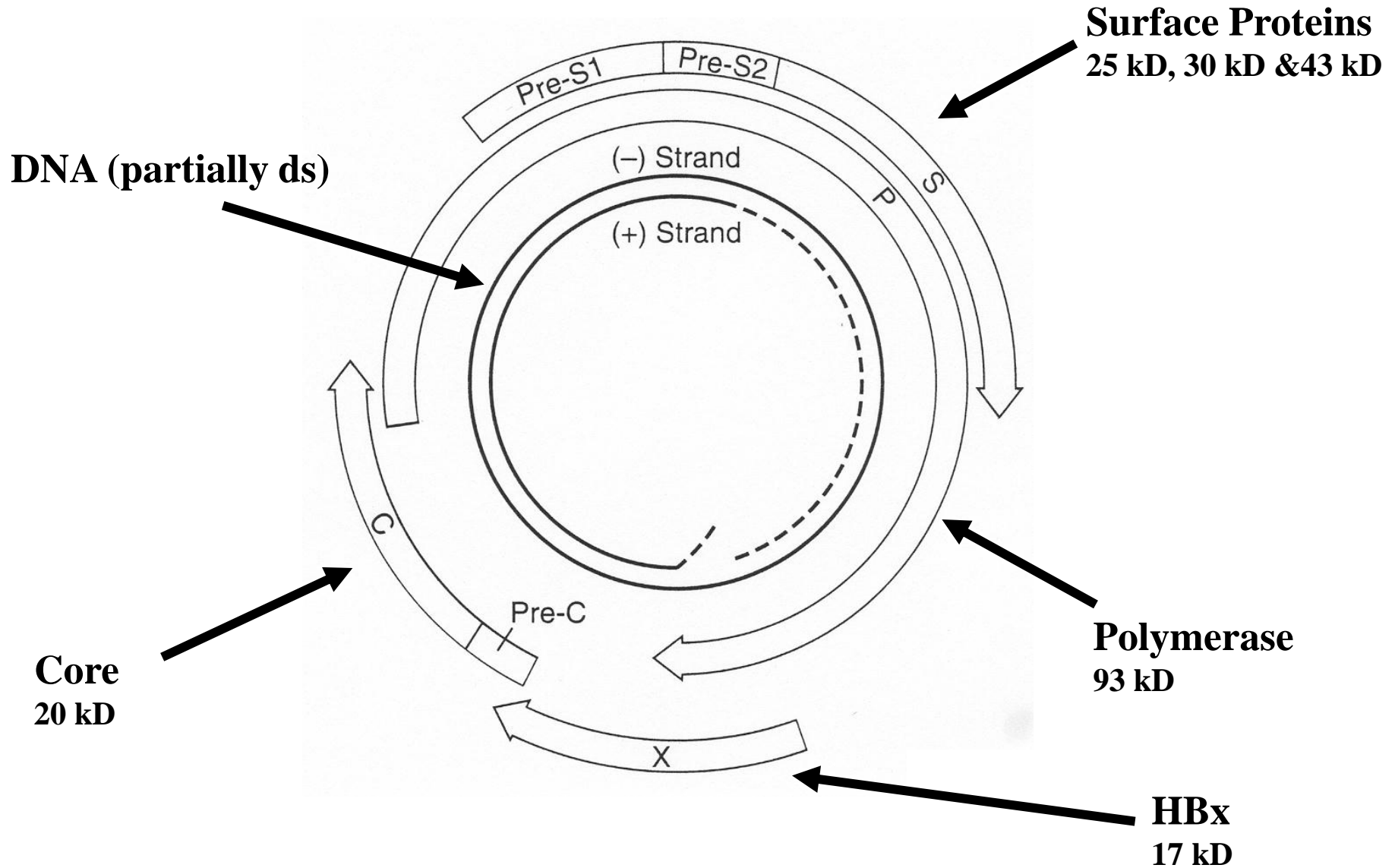
**3,200 nucleotides**

**4 open reading frames (ORFs),**

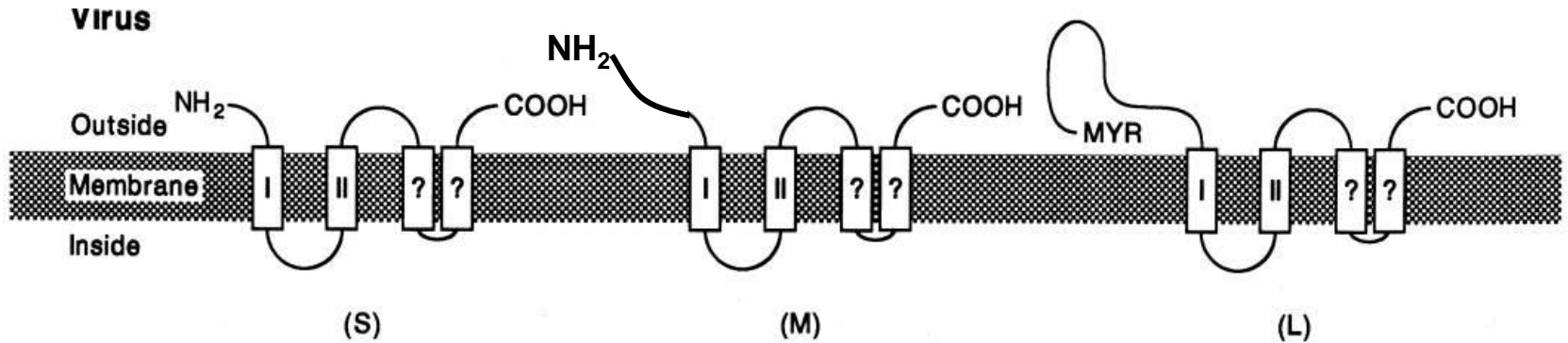
**Partial overlap of ORFs**



# HBV Genes



# Surface Antigen Proteins



**Small**

**226 aa**

**~25 kD**

**Medium**

**281aa**

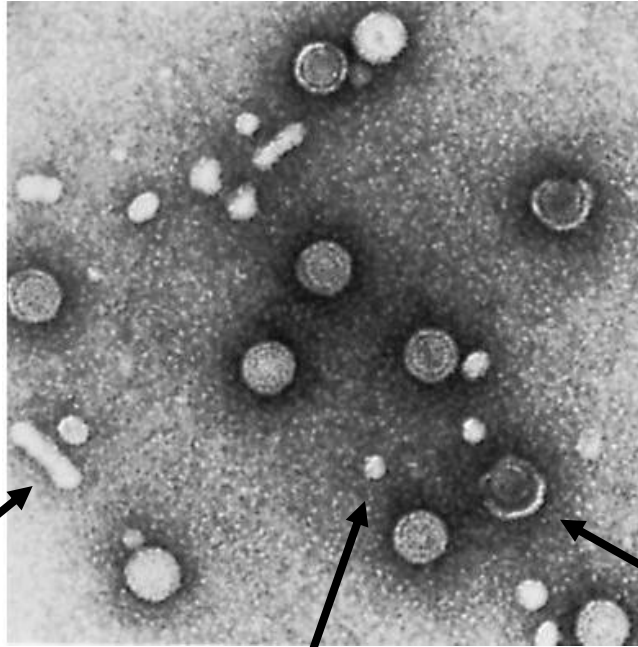
**~30 kD**

**Large**

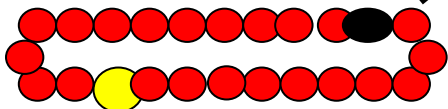
**389 aa**

**~43 kD**

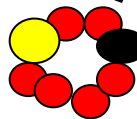
# HBV Virions



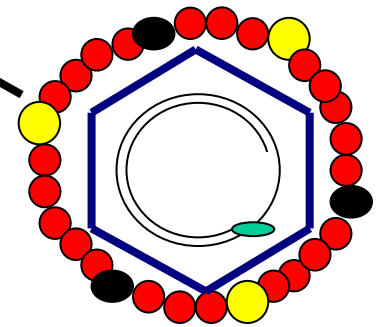
Transmission electron micrograph of HBV infected human serum



S protein tubules

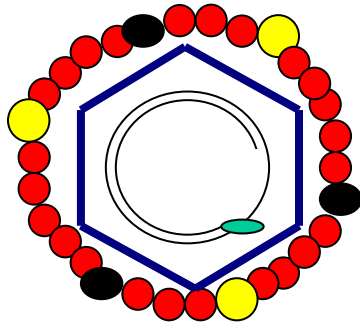


S protein spheres



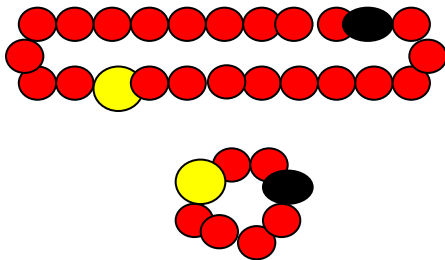
HBV virions

# HBV and SVPs



HBV virions 42nm diameter

- Outer envelope , lipid and HBs-S, M and L
- 27nm nucleocapsid
- 180 copies of core protein
- Pol enzyme and HBV DNA

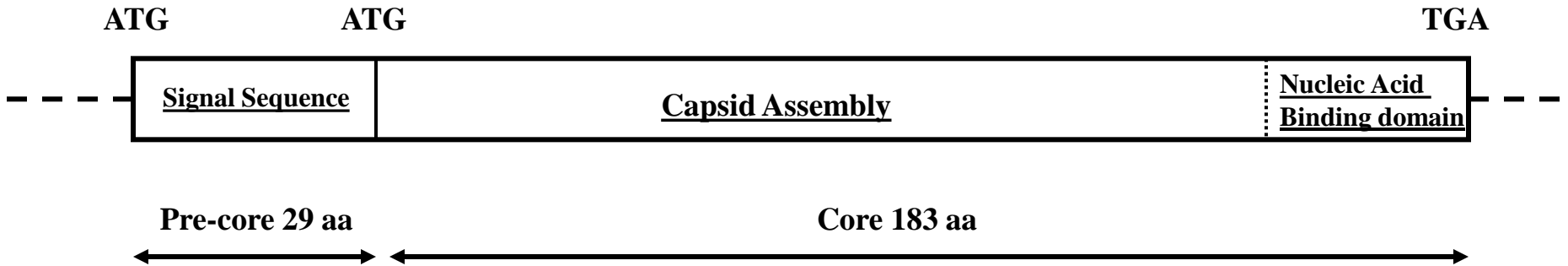


Empty non-infectious subviral particles (SVP)

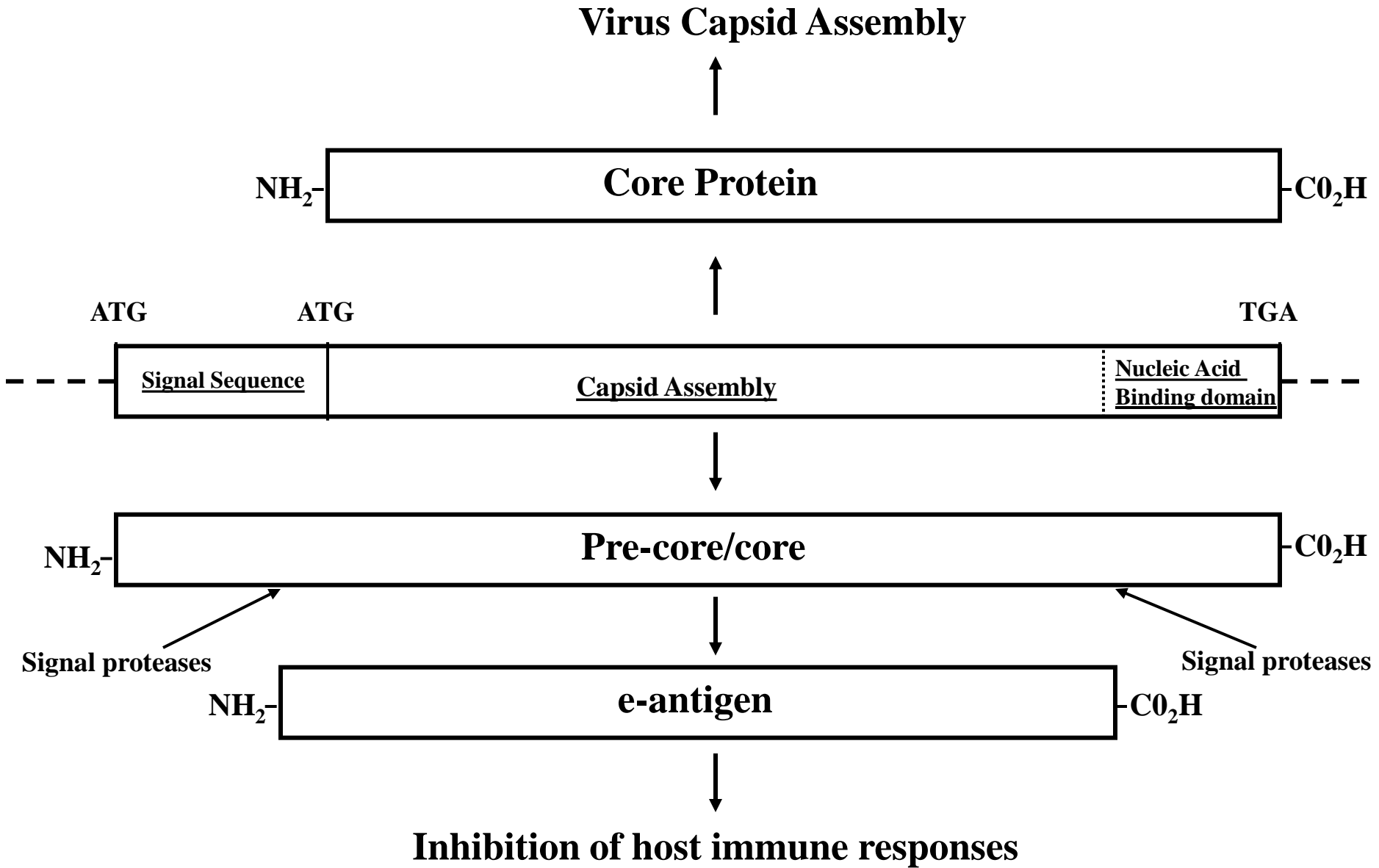
- 22nm tubules and spheres
- Contain lipid
- Mainly HBs-S
- 100 – 100,000 times more than HBV virion

# HBV Core Protein

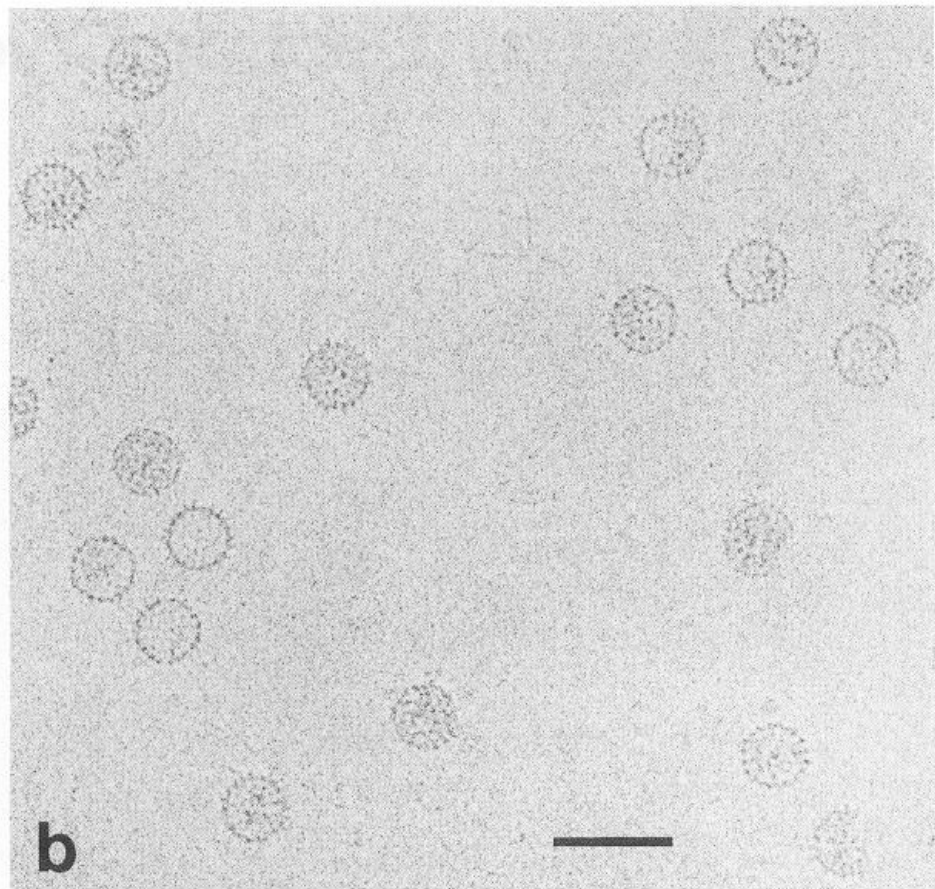
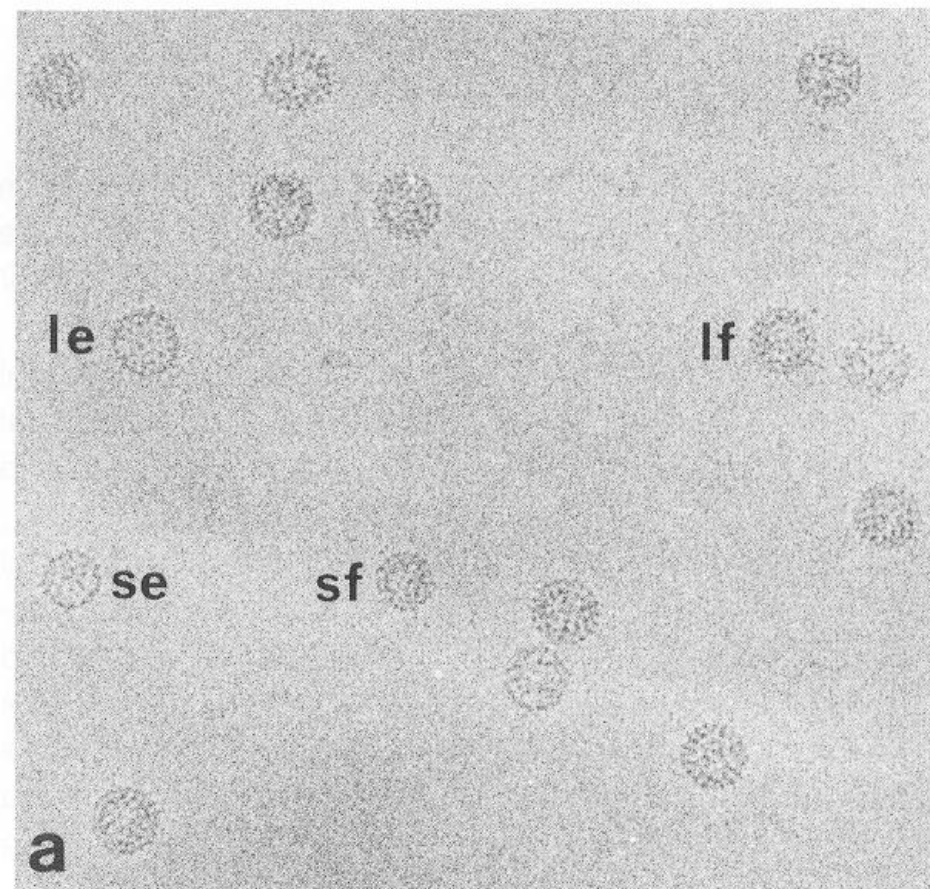
## Core Gene



# HBV Core Protein

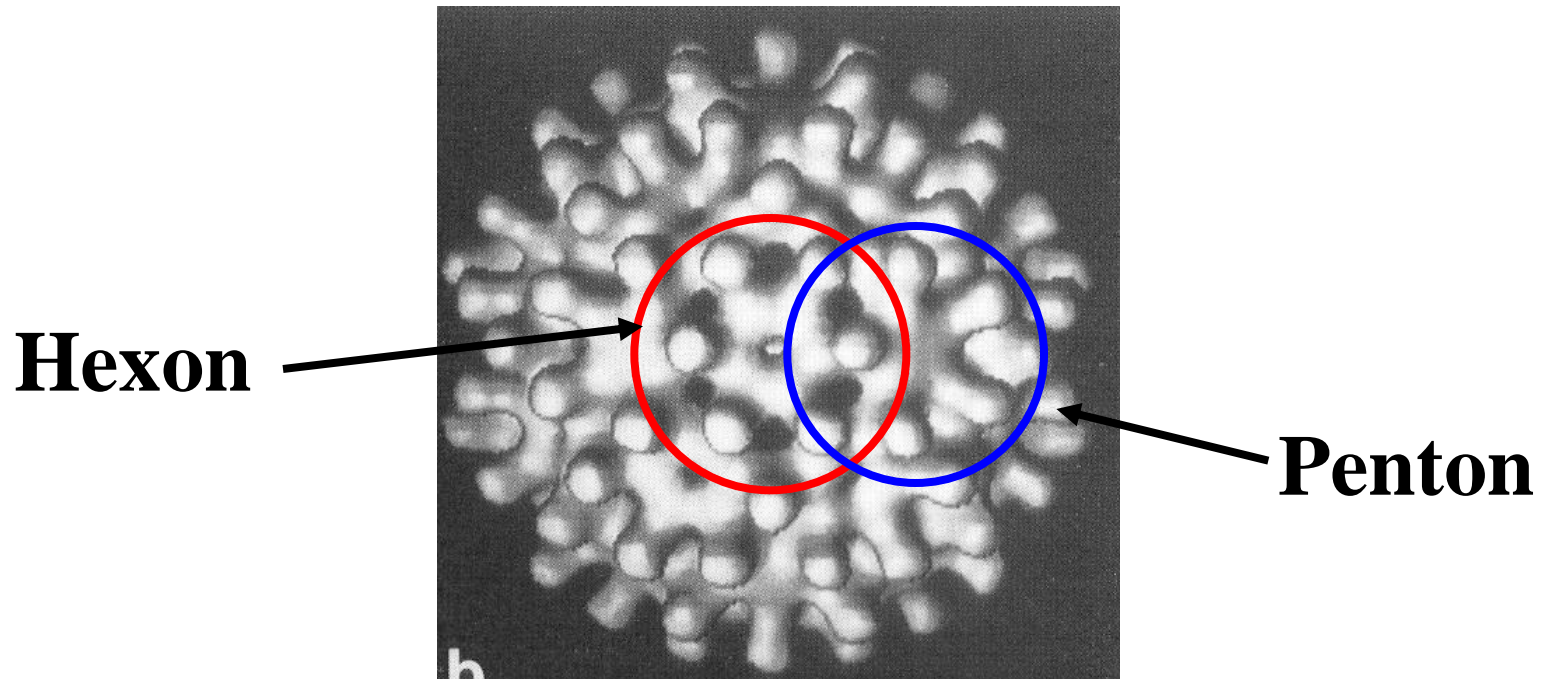


# Cryo-Electron Micrographs of HBV – Capsids

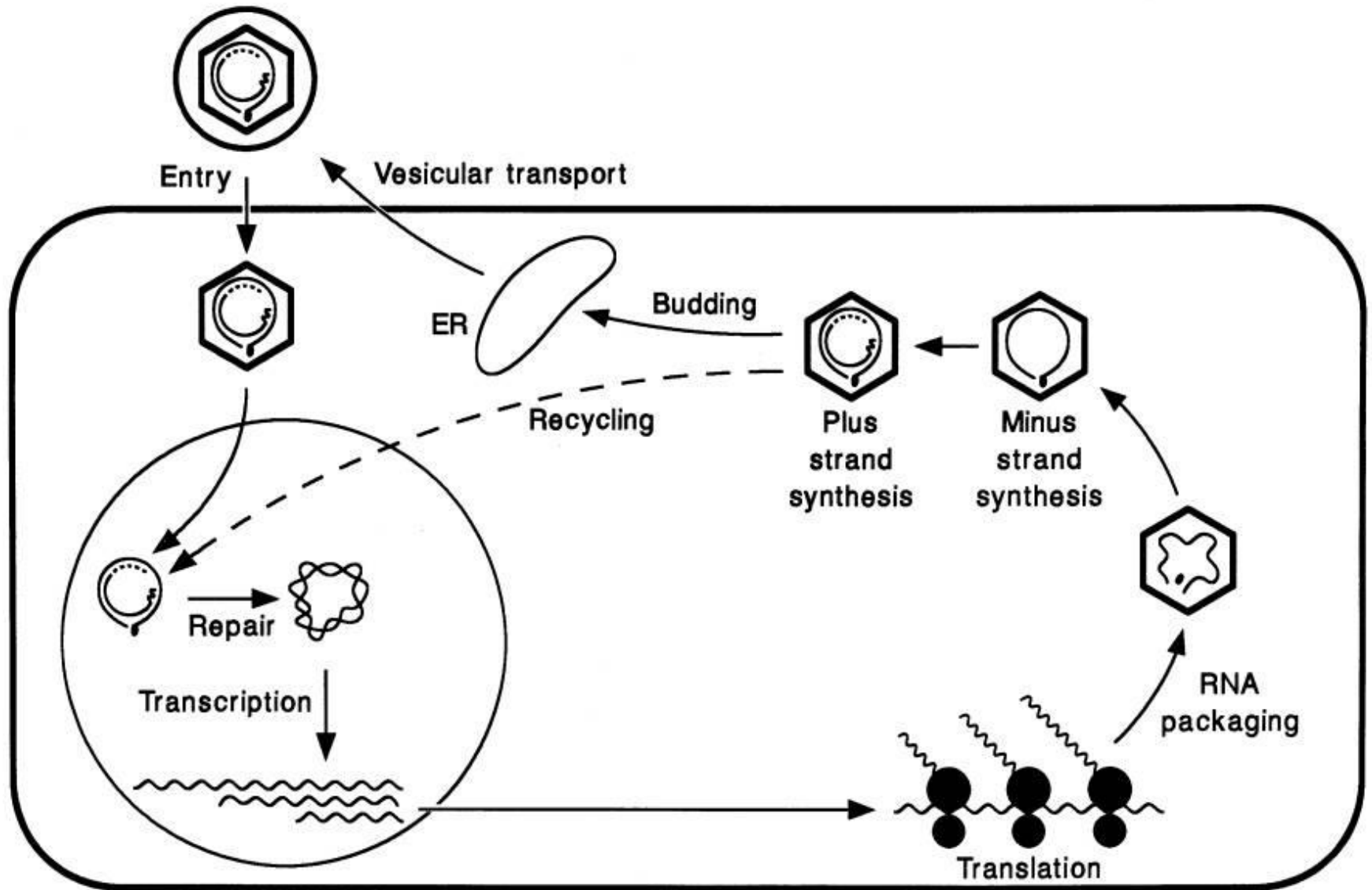




# 3-D Structure of HBV Capsids – Cryo EM



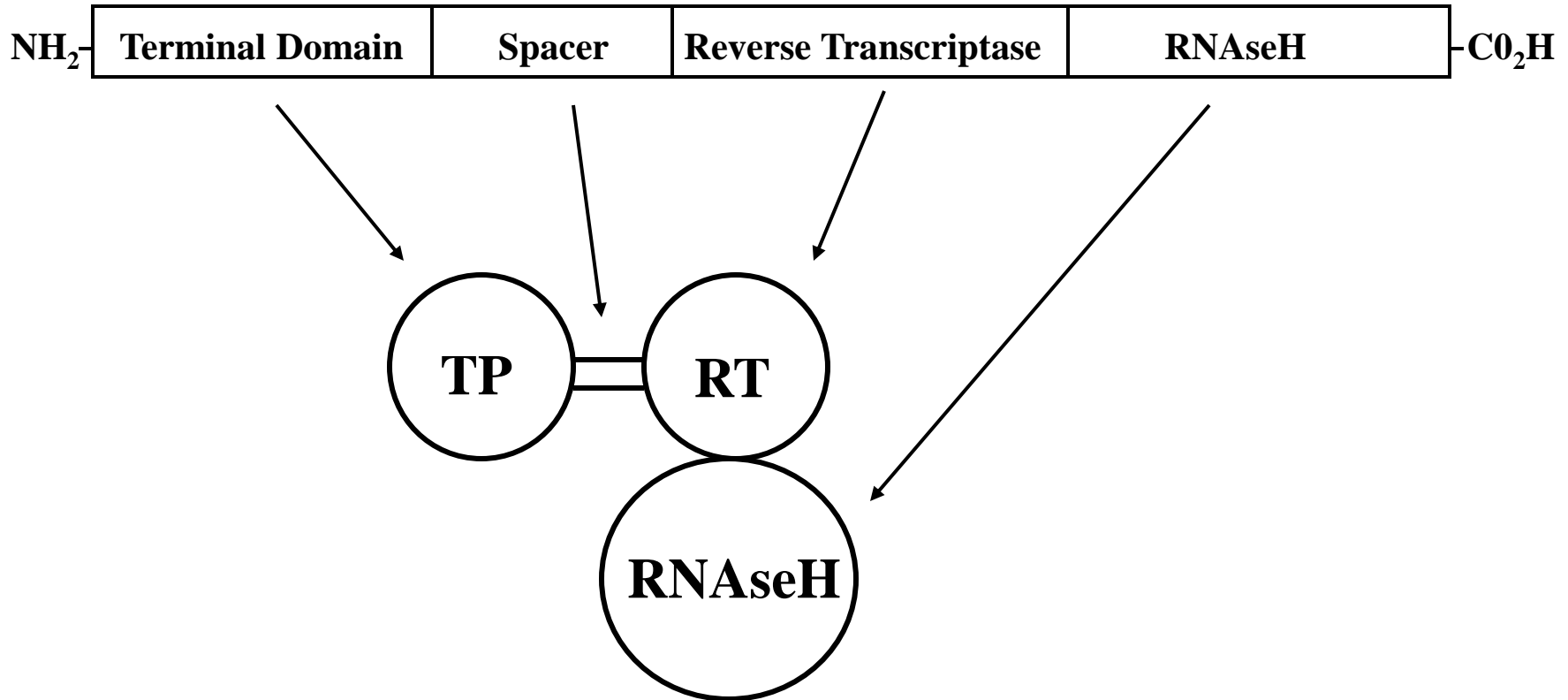
# HBV Replication Cycle



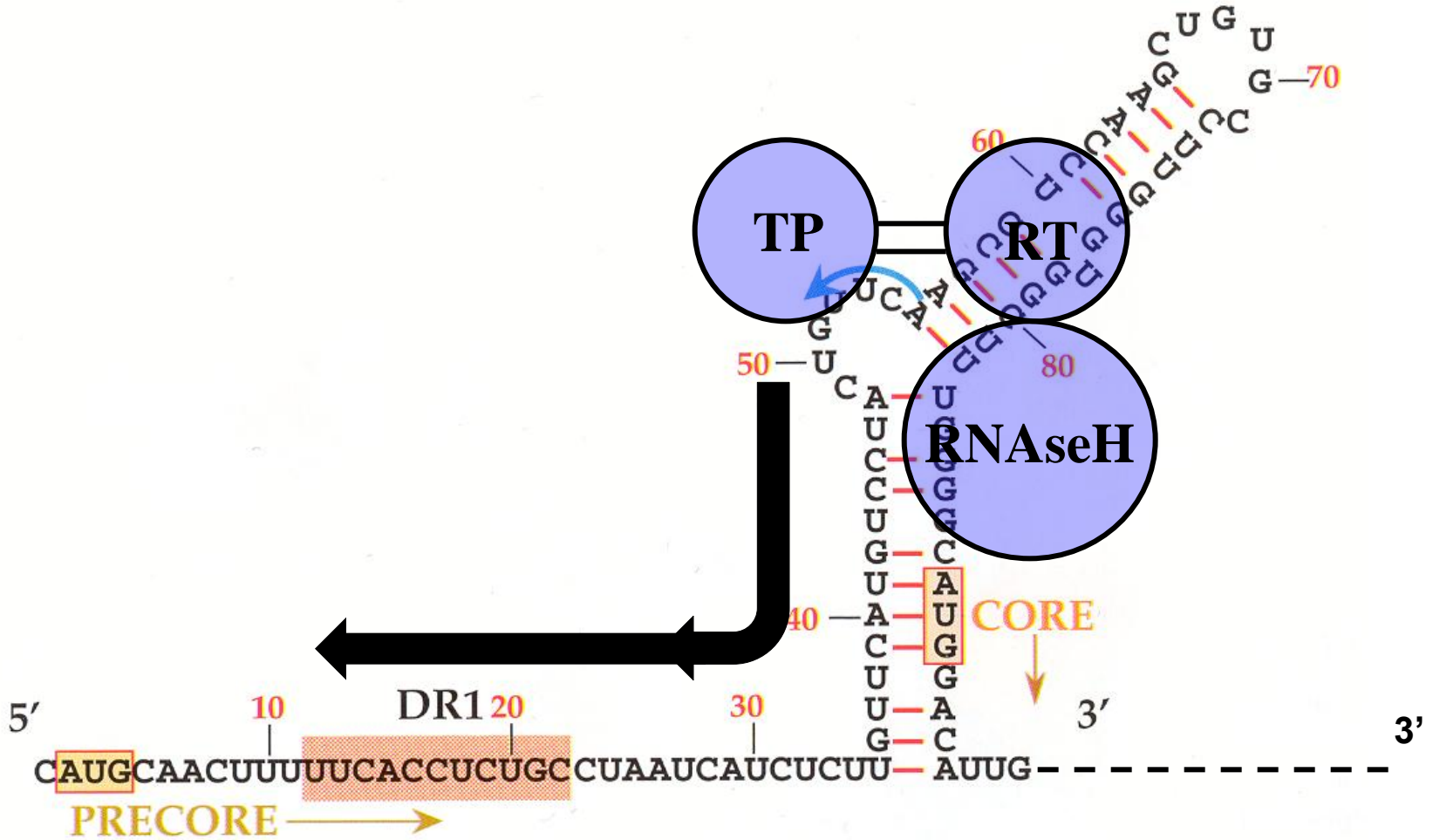
# HBV Transcripts

- Core** → **3.5 kb RNA (greater than genome length)**
- Translation → **core, e-antigen, polymerase**
  - **Template for reverse transcription**
- S1** → **2.4 kb RNA**
- Translation → **Pre-S1 (Large S)**
- S2** → **2.1 kb RNA**
- Translation → **Pre-S2 (Medium S)**
  - **S (Small S)**
- X** → **0.7 kb RNA**
- Translation → **X protein**

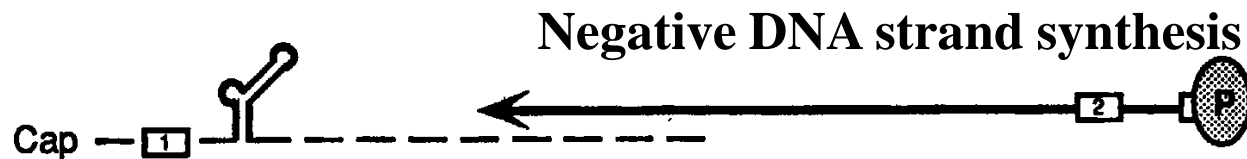
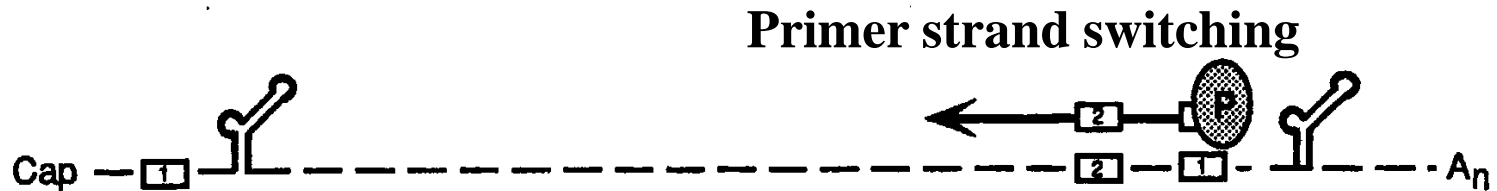
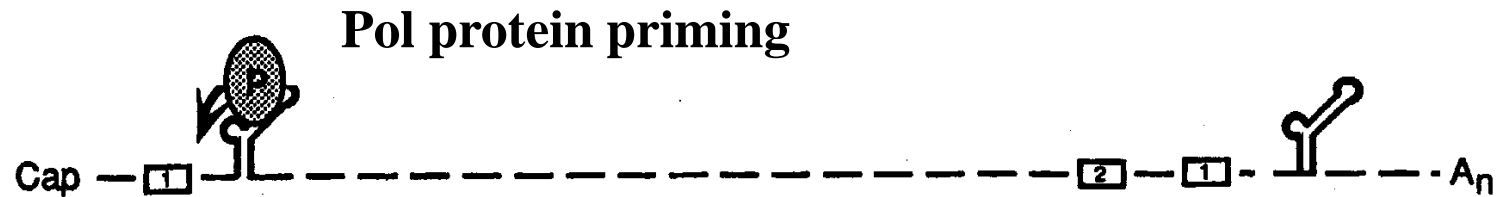
# Model for HBV Polymerase Structure



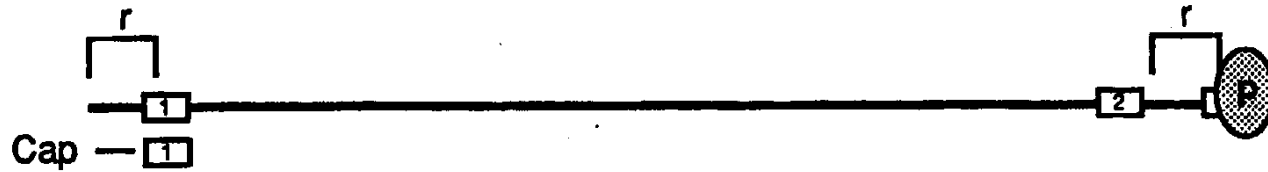
# Stem-Loop RNA Recognition Site



# HBV Genome Replication 1



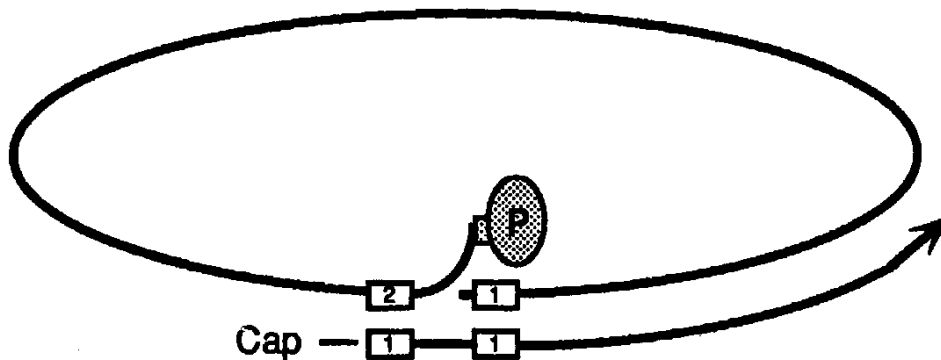
# HBV Genome Replication 2



Positive DNA primer



Positive DNA strand priming



Positive DNA strand synthesis

# **HBV X Protein**

**Has a transactivating activity**

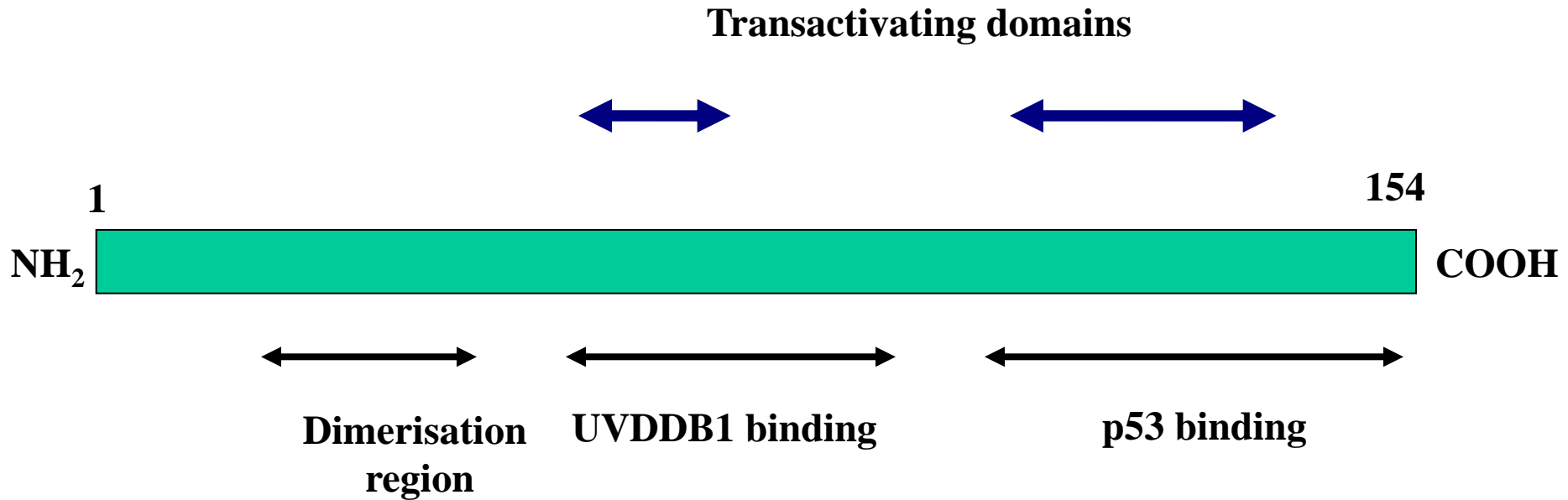
- a variety of promoters (no common elements)**

**Several biochemical activities?**

- but not DNA binding**



# Structure of HBx Protein



# Actions of HBx Protein

**Activation of signalling cascades**

**Interaction with cellular transcription factors**

**Interaction with p53**

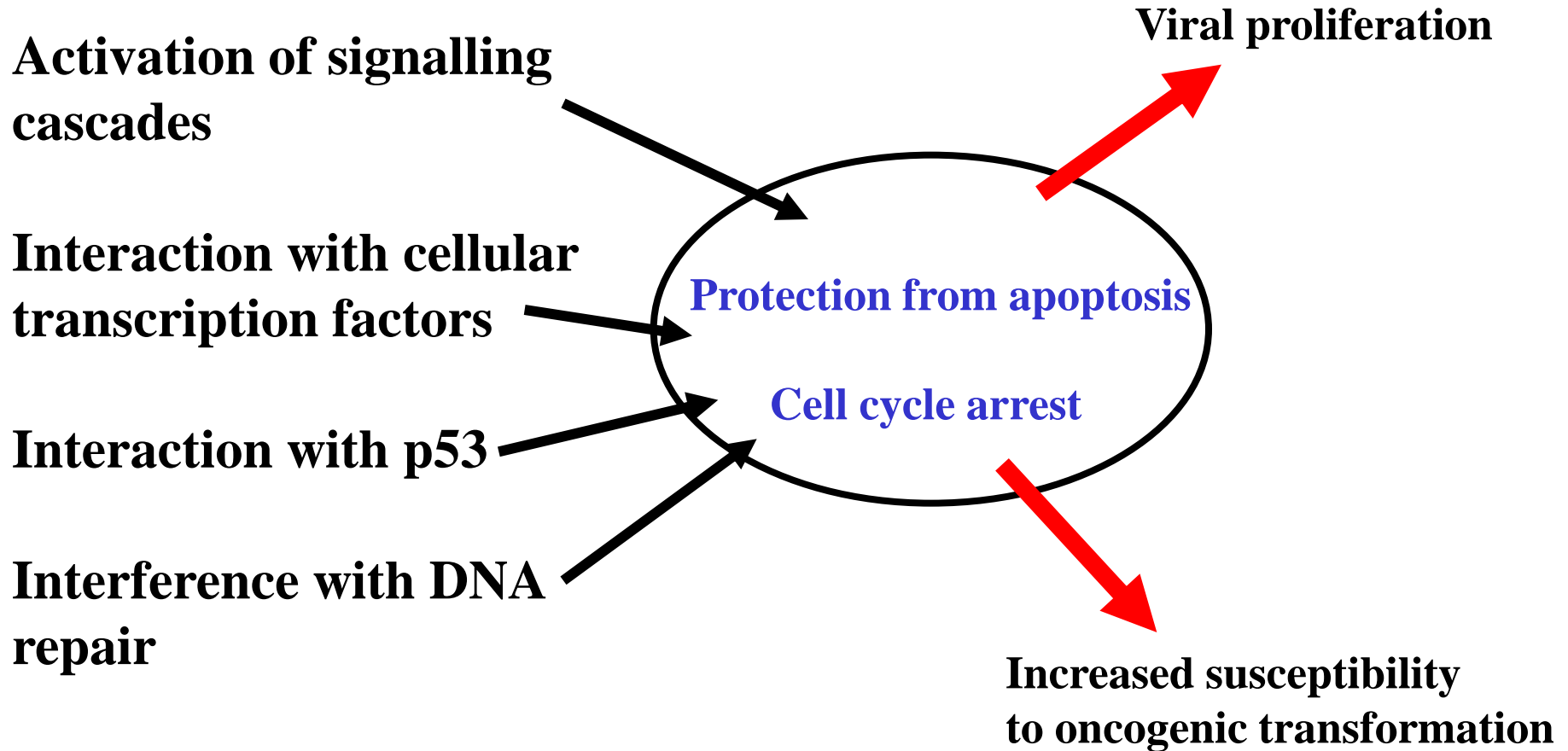
**Interference with DNA repair**

**Protection from apoptosis**

**Cell cycle arrest**

**Viral proliferation**

**Increased susceptibility to oncogenic transformation**



# Treatment for HBV

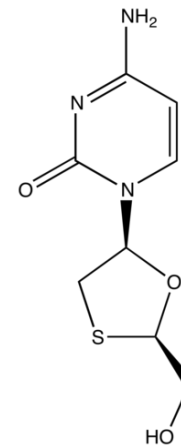
## Vaccine

- HBsAg
- Recombinant protein synthesised in yeast
- 3 Doses per patient over six months

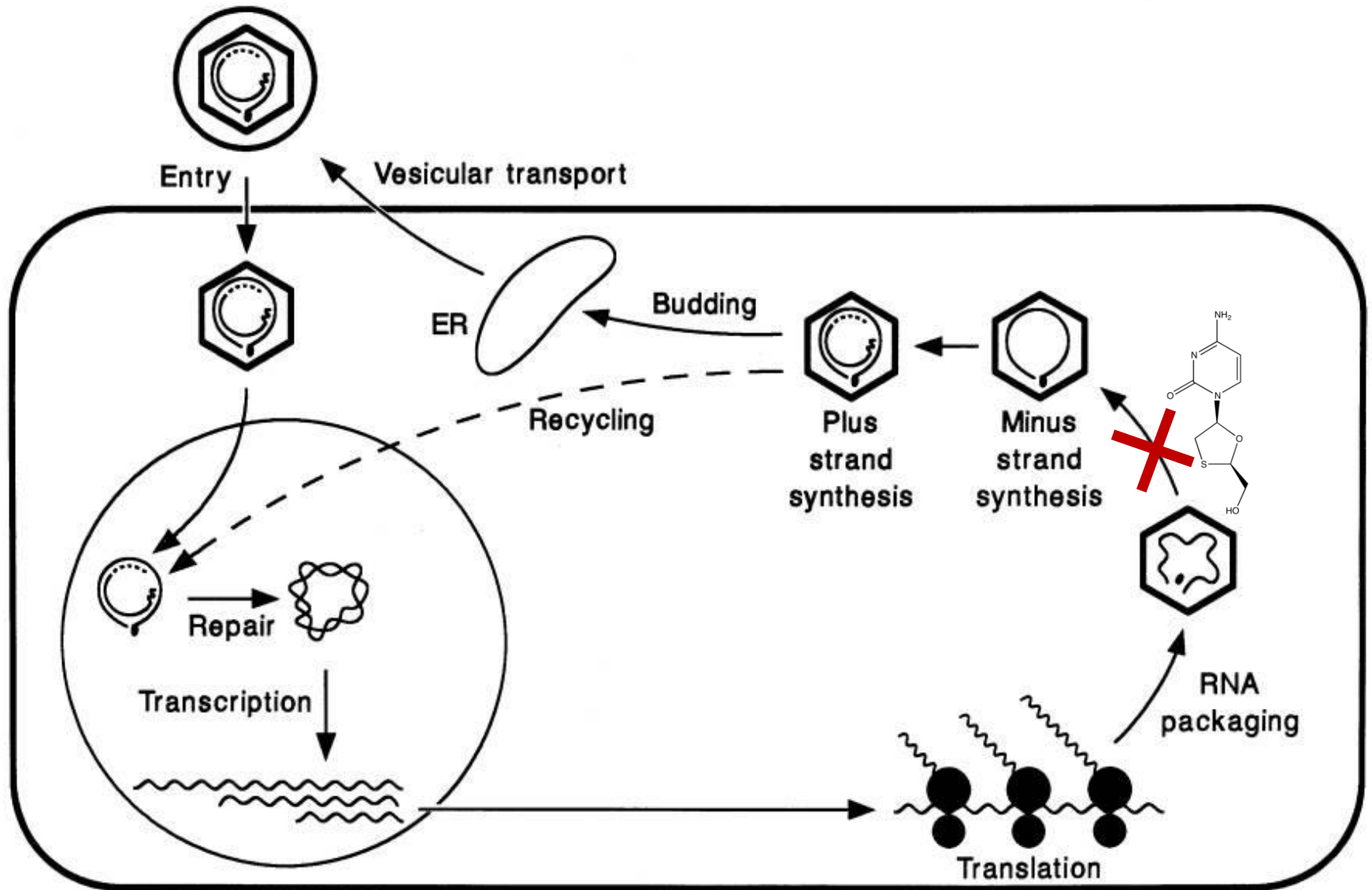
## Antivirals

- alpha interferon
- lamivudine (targets reverse transcription)
- Daily treatment over 3-6 months

lamivudine



# HBV Replication Cycle



# **Hepatitis D Virus: Scope of Lecture**

- **Structure of virion**
- **Structure of genome**
- **Transcription and genome replication**
- **Delta antigens**
- **Pathogenesis**

# **Hepatitis D Virus: Background:**

- Mid-1970s, a new nuclear antigen detected in hepatocytes of some chronic HBV carriers, initially thought to be another HBV antigen**
- 1977, named delta antigen (HDAg)**
- 1980, Transmission to chimpanzees confirmed the existence of a transmissible pathogen that was defective and required HBV**
- A radioimmunoassay developed for detection of anti-delta, showed high prevalence in haemophiliacs and drug-addicts**
- Early 1980s, delta antigen shown to be an internal component of virus-like particles, that contained RNA and had an outer envelope consisting of HBsAg**

# **Hepatitis D Virus: Classification**

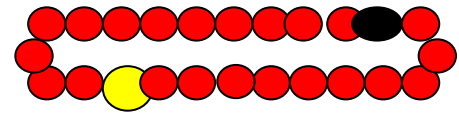
- **1983, delta agent was designated as a distinct hepatitis virus, named hepatitis delta virus (HDV)**
- **1986, HDV cloned and sequenced, - similarities to plant viroids**
- **No family assigned yet**
- **HDV, the only member of Deltavirus genus,**

# Structure

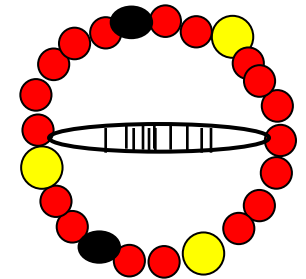
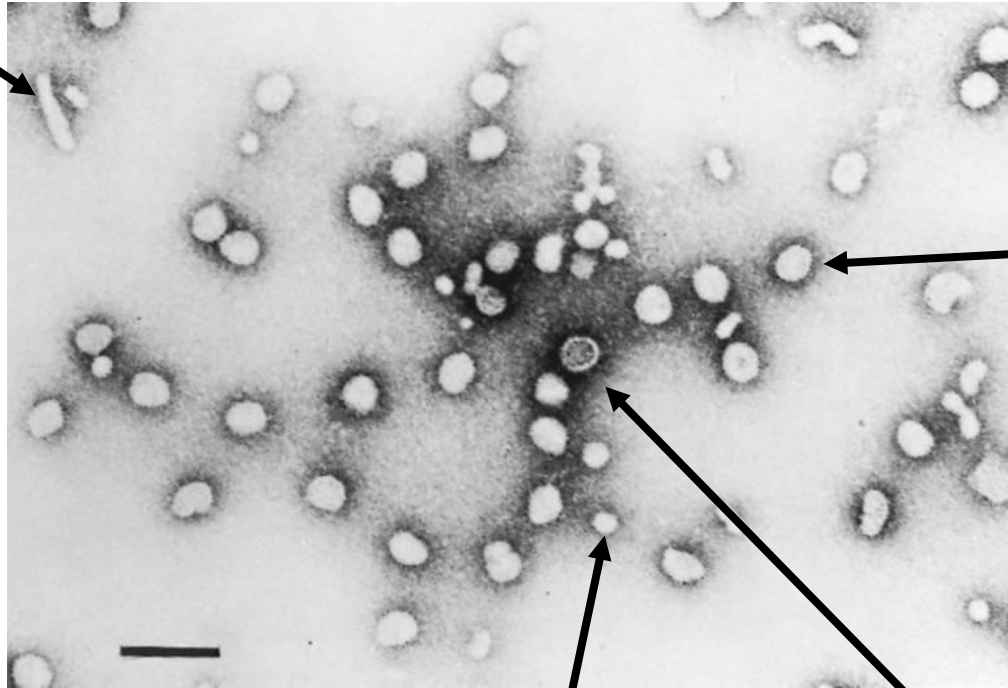
- **36nm virus particle**
- **Outer envelope consists of HBsAg, from the helper virus**
- **Nucleocapsid: 70 copies of hepatitis delta antigen (HDAg)**
- **Encloses the RNA genome, single stranded, circular and of negative polarity, 1.7kb in length**
- **It forms a rod-like structure, due to internal base-pairing, involving ca 74% of the molecule**
- **Genome smallest known human pathogens, encoding only HDAg**
- **Structure and mode of RNA replication resembles that of plant viroids and virusoids**



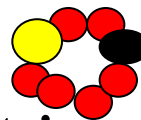
# HBV and HDV and SVP



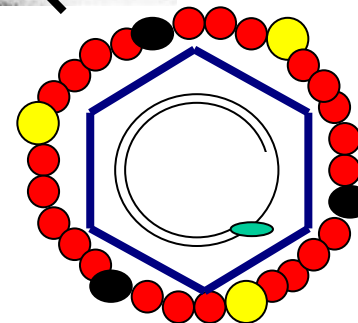
S protein tubules



HDV virions

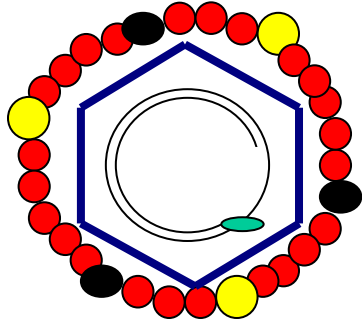


S protein spheres



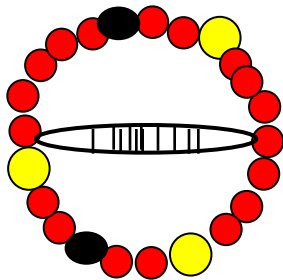
HBV virions

# HBV and HDV and SVP



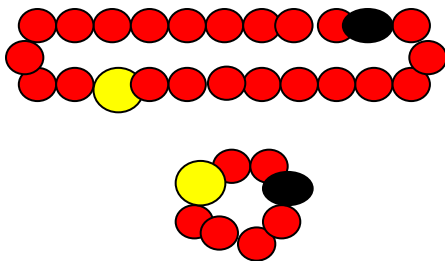
**HBV virions 42nm diameter**

- **Outer envelope , lipid and HBs-S, M and L**
- **27nm nucleocapsid**
- **180 copies of core protein**
- **Pol enzyme and HBV DNA**



**HDV virions 36 -43nm diameter**

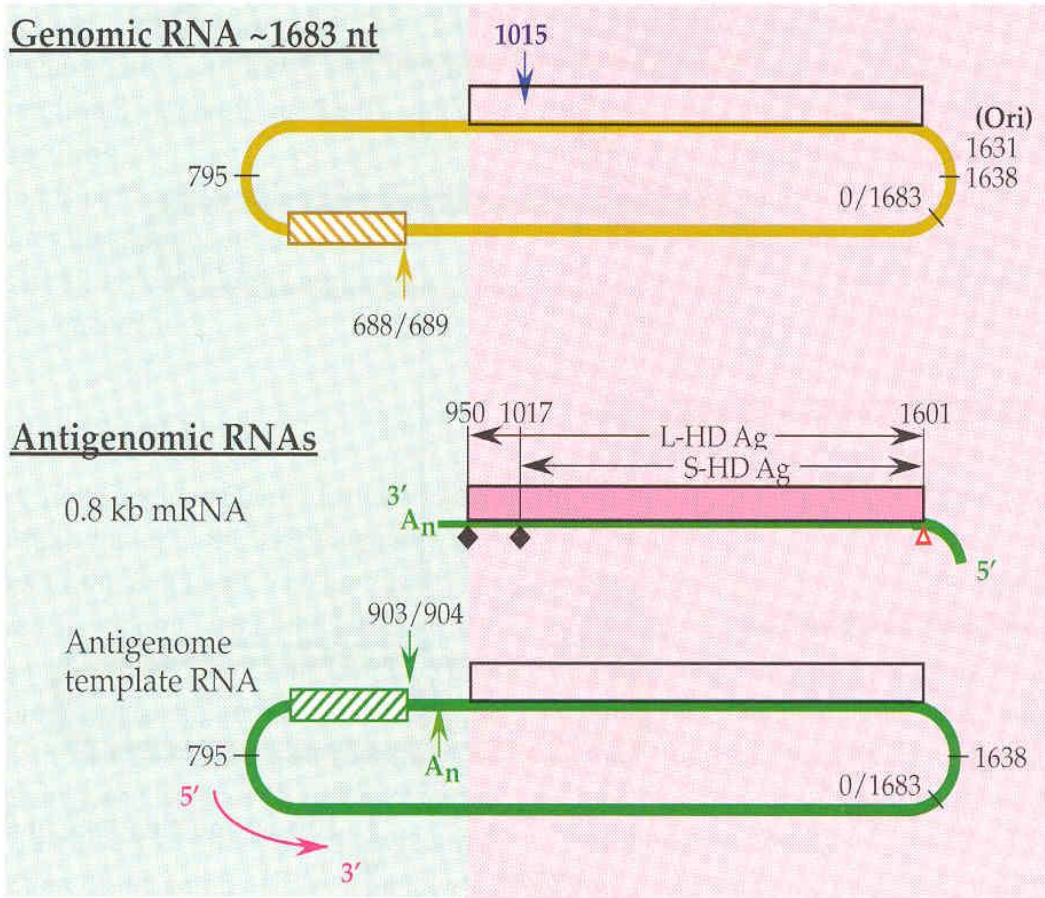
- **Outer envelope , lipid and HBs-S, M and L**
- **19 nm nucleocapsid**
- **160copies of core delta antigen**
- **HDV RNA**



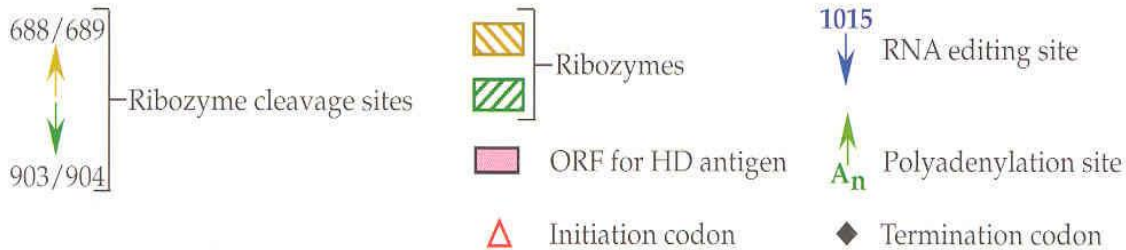
**Empty non-infectious subviral particles (SVP)**

- **22nm tubules and spheres**
- **Contain lipid**
- **Mainly HBs-S**
- **100 – 100,000 times more than HBV virion**

A. Viroid Domain HD-Ag Coding Domain



# GENOMIC ORGANISATION AND ANTIGENOMIC RNAs



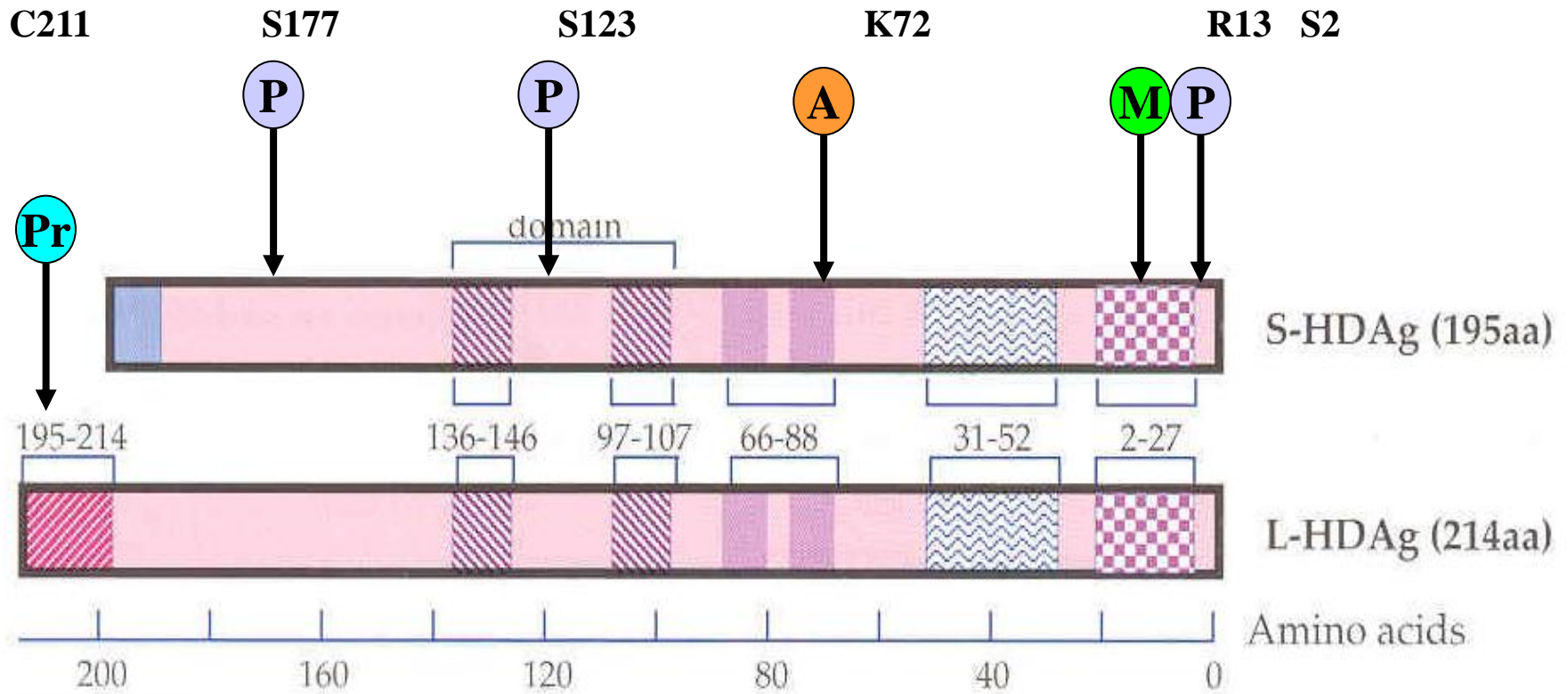
# Transcription/Replication

- **RNA transferred to the nucleus following uncoating**
- **Replication of the genome and synthesis of mRNA carried out by host RNA polymerase II**
- **Genomic RNA is used as the template for production of**
  - **an antigenomic mRNA (0.8kb) that is translated into HDAg**
  - **an antigenomic RNA that is full-length, and is produced from greater-than-full-length transcripts**
- **The antigenomic RNA is also covalently circular and single stranded**
- **Three key features of the genome are necessary for transcription:**
  - **an origin for the start of RNA synthesis**
  - **a polyadenylation site downstream of the ORF encoding HDAg**
  - **a self-cleavage site that is capable of self-ligation**

# HDAg Translation

- The nucleocapsid consists of two peptides 24 and 27kd in size
- Both are serine-linked phosphoproteins, and are encoded by the mRNA
- The mRNA for HDAg is exported to the cytoplasm
- Translated into a polypeptide of 195 aa, known as the small (S)-HDAg (24kd)
- (S)-HDAg is required for replication
- The second peptide, known as L-HDAg (27kd), is identical to the short form, with the exception that it has 19 additional aa (214aa long)
- L-HDAg is produced later in the infection cycle
- Suppresses RNA replication and promotes encapsidation of RNA

# FUNCTIONAL DOMAINS OF HDAgs



**Pr** = isoprenylation    **P** = phosphorylation    **A** = acetylation    **M** = methylation

# **HDAg Translation (cont)**

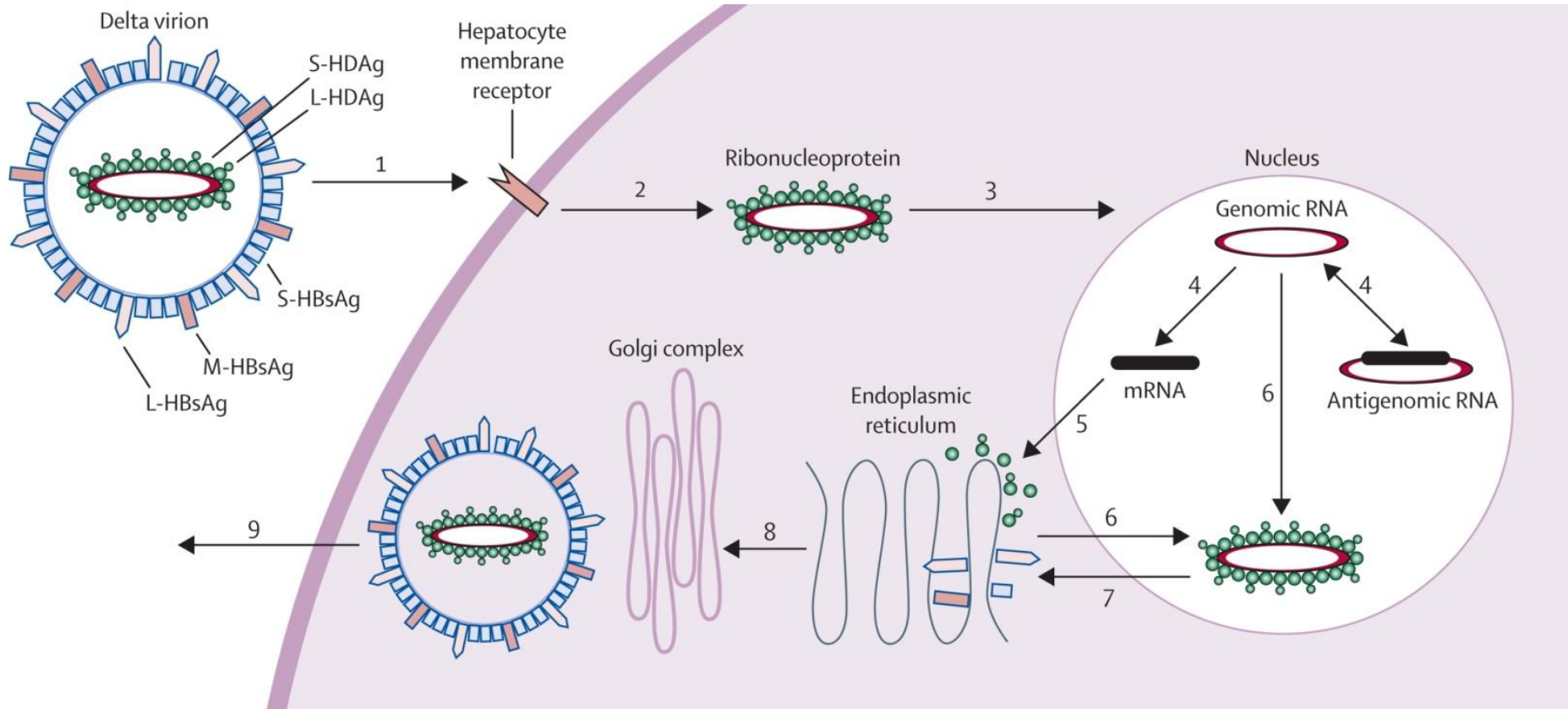
- **L-HDAg is produced from a distinct RNA species arising from a unique RNA editing event**
- **Editing occurs in about 1/3rd of the antigenome templates**
- **The termination codon, UAG at position 196 of the ORF for S-HDAg, is changed to UGG, for tryptophan**
- **This A to G change is effected by deamination, in the antigenome, of the adenosine to produce inosine**
- **This is performed by a cellular adenosine deaminase 1 (ADAR 1)**
- **Inosine pairs as Guanosine, thus introducing a C in the genomic strand, which in the antigenomic strand is a G**
- **Thus translation continues to the next stop codon, 19 aa downstream**

# **HDAg Translation (cont)**

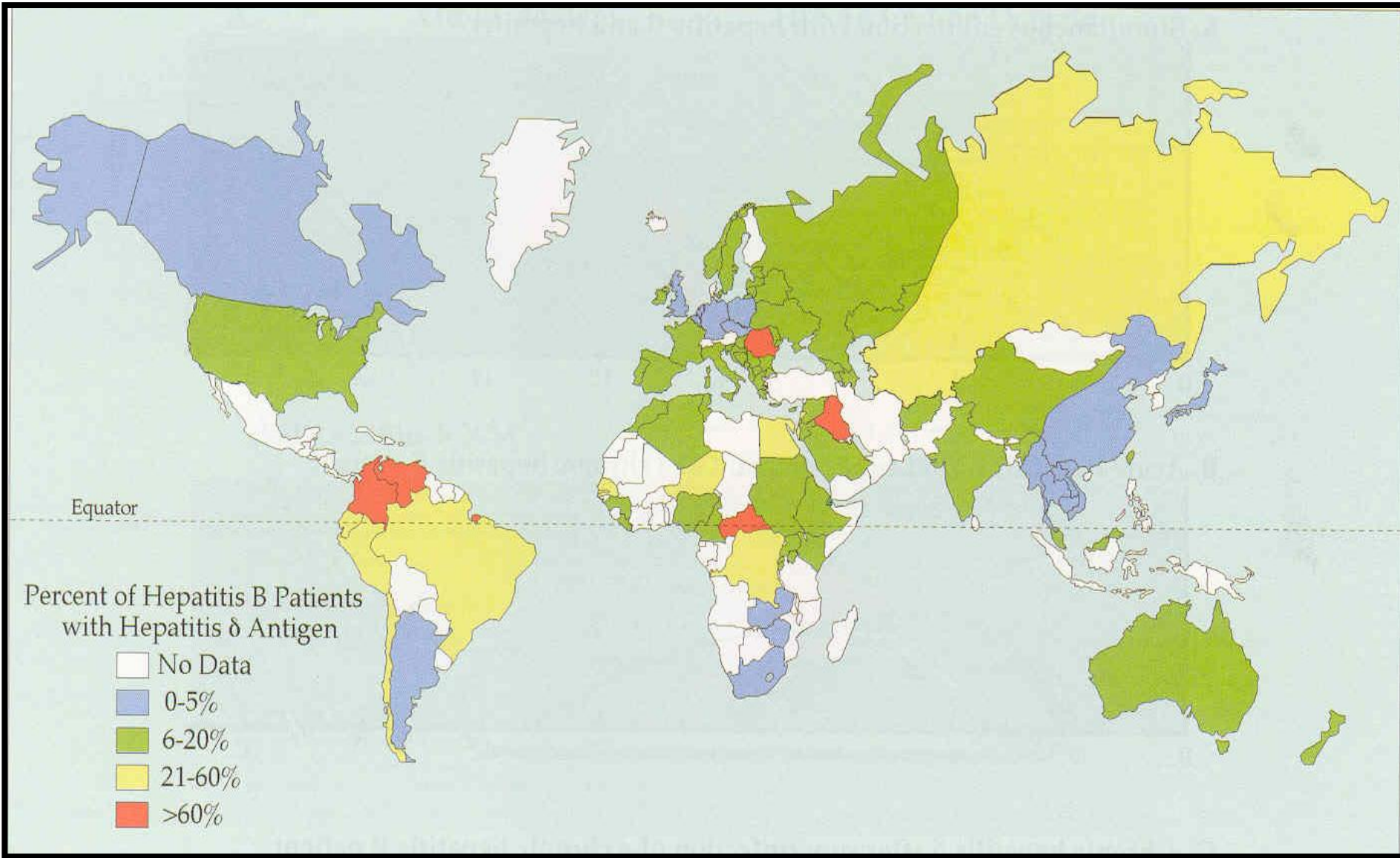
- **L-HDAg is produced later in the infection**
- **It suppresses RNA replication and promotes encapsidation**
- **Extent of editing is probably controlled by S-HDAg**
- **Only genomes that are not edited are encapsidated**
- **An isoprenylation signal at the C-terminus of L-HDAg, is required for interaction between L-HDAg and HBsAg**
- **HDV capsids contain both HDAGs, and bud off the ER membrane picking up their HBsAg envelope in the process**



# Replication cycle of HDV



# WORLDWIDE DISTRIBUTION OF HDV INFECTION



# Acquisition of Infection

## **Co-infection**

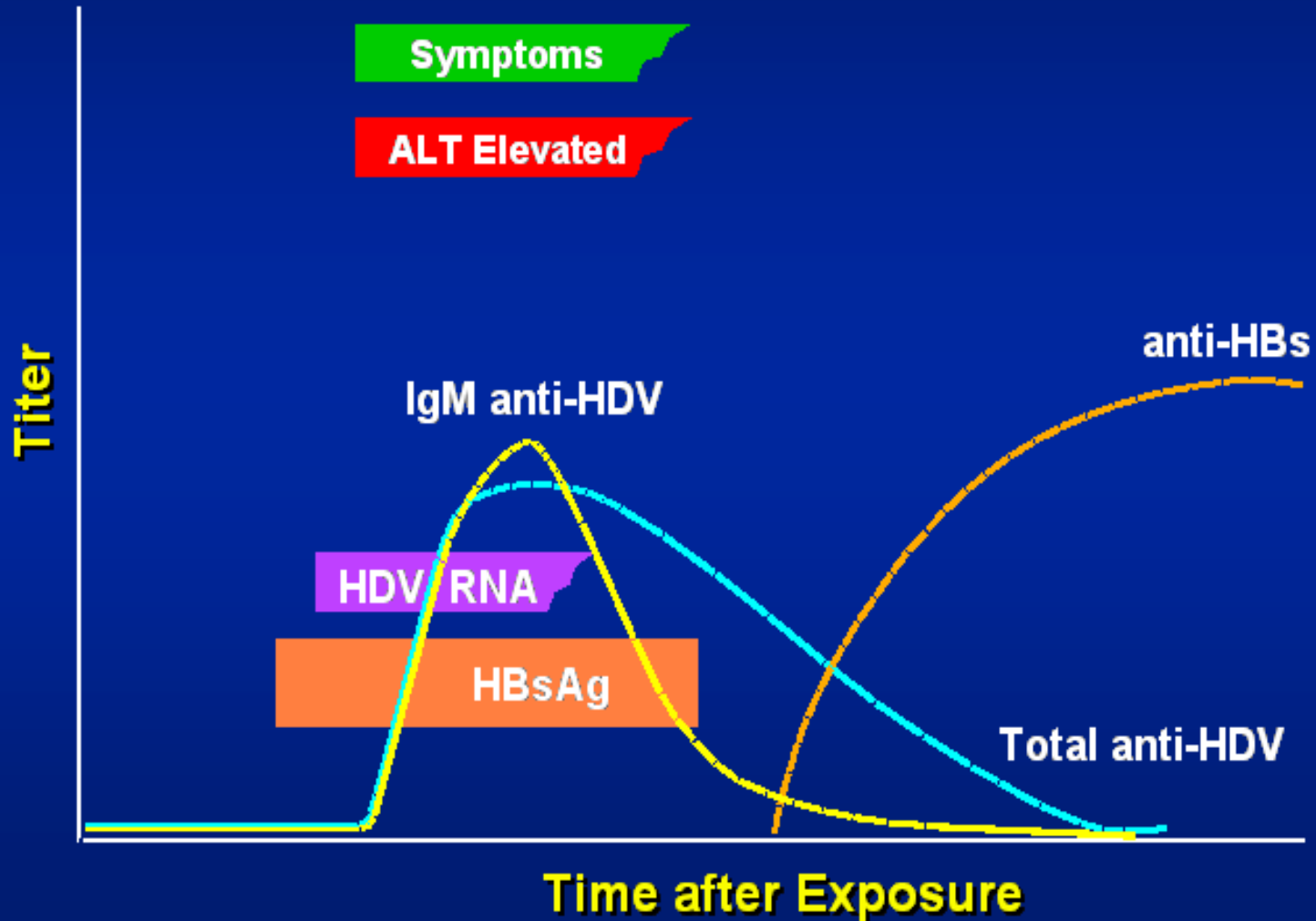
- **Simultaneous introduction of HV and HDV**

## **Superinfection**

- **Introduction of HDV into HBV positive host**

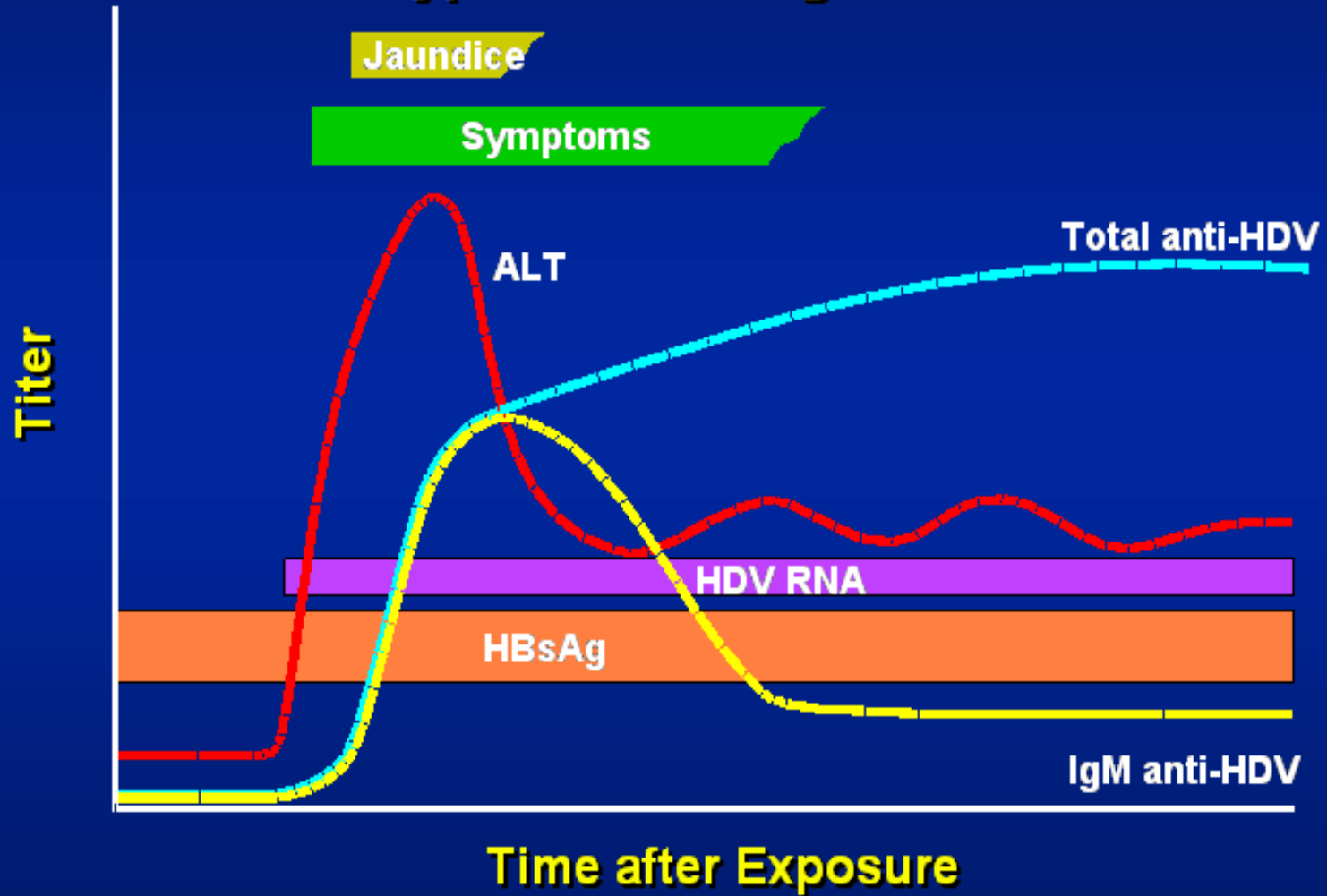
# HBV – HDV Coinfection

## Typical Serologic Course



# HBV – HDV Superinfection

## Typical Serologic Course



# Disease Outcome

- **60-79% of patients with chronic HDV infection develop cirrhosis**
- **HCC also occurs**
- **Co-infection of HBV and HDV are usually acute, self-limited  
Infections leading to chronic infection in 1-3% of cases**
- **Superinfections cause generally a severe acute hepatitis, which  
Leads to chronic infection in 60-70% of patients**
- **Disease is usually more serious than that caused by HBV alone**

# **Hepatitis D – Prevention**

## **HBV-HDV Coinfection**

**Pre or post-exposure prophylaxis to prevent HBV infection**

## **HBV-HDV Superinfection**

**Education to reduce risk behaviour among persons with chronic HBV infection**

# References

## HBV:

Seeger et al, “Hepadnaviruses” Chapter 76, Fields Virology (2007) Fifth Edition (Lippincott-Williams, Wilkins, Philadelphia) p2977-3030.

Yokosuka O and Makoto A (2006) Molecular biology of hepatitis B virus. Med. Mol. Morphol. 39: 113-120.

Shadler S and Hildt E (2009) HBV Life cycle : entry and Morphogenesis. Viruses 1:185-209.

## HDV:

Hughes SA, Wedemeyer H and Harrision PM (2011). Hepatitis delta virus. Lancet 378:73-85.

Taylor J and Pelchat M (2010). Origin of hepatitis delta virus Future Microbiol. 5:393-402.

Taylor JM, Farci P and Purcell RH, “Hepatitis D Virus” Chapter 77, Fields Virology (2007) Fifth Edition (Lippincott-Williams, Wilkins, Philadelphia) p3031-3046.