

Hepatitis C Virus

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Hepatitis C Virus (HCV)

Discovery

- Pre-1988 - Non A, Non B hepatitis (NANBH) 2 -3 viruses ?
- 1988 expression cloning - HCV

Transmission

- Blood - transfusion, products, IVDU
- Sexual?
- Sporadic?

Characteristics of HCV

- **Only infects humans and chimps**
- **Icosahedral capsid, enveloped**
- **RNA genome**

HCV - Size of Problem

- **ca. 150 - 200 million chronic infections**
- **UK - ca. 50,000 - 100,000**
- **10- 20% cirrhosis**
- **primary liver cancer**

Clinical Consequences of HCV Infection

Course of Infection:

- Onset 5 - 12 weeks after infection**
- Acute - often asymptomatic, raised AL T levels**
- Chronic → 90% of cases**

Pathology:

- liver failure, cirrhosis, liver cancer (HCC)**
- HCC - 40% of Europeans have antibodies to HCV**
- Damage - Immunological, cytopathic**

Cirrhosis – Chronic HBV Infection



Normal Liver

Fatty Liver

Cirrhotic Liver

Therapies for HCV Infections

Prevention – testing blood and blood products
→ large reduction in transmission

Vaccine – None
→ Hypervariable sequence in envelope protein

Antivirals – Alpha Interferon
– Alpha Interferon + Ribavirin
→ 40% response
→ side effects

New Therapies

Virion Structure

Size

- 40 - 60 nm

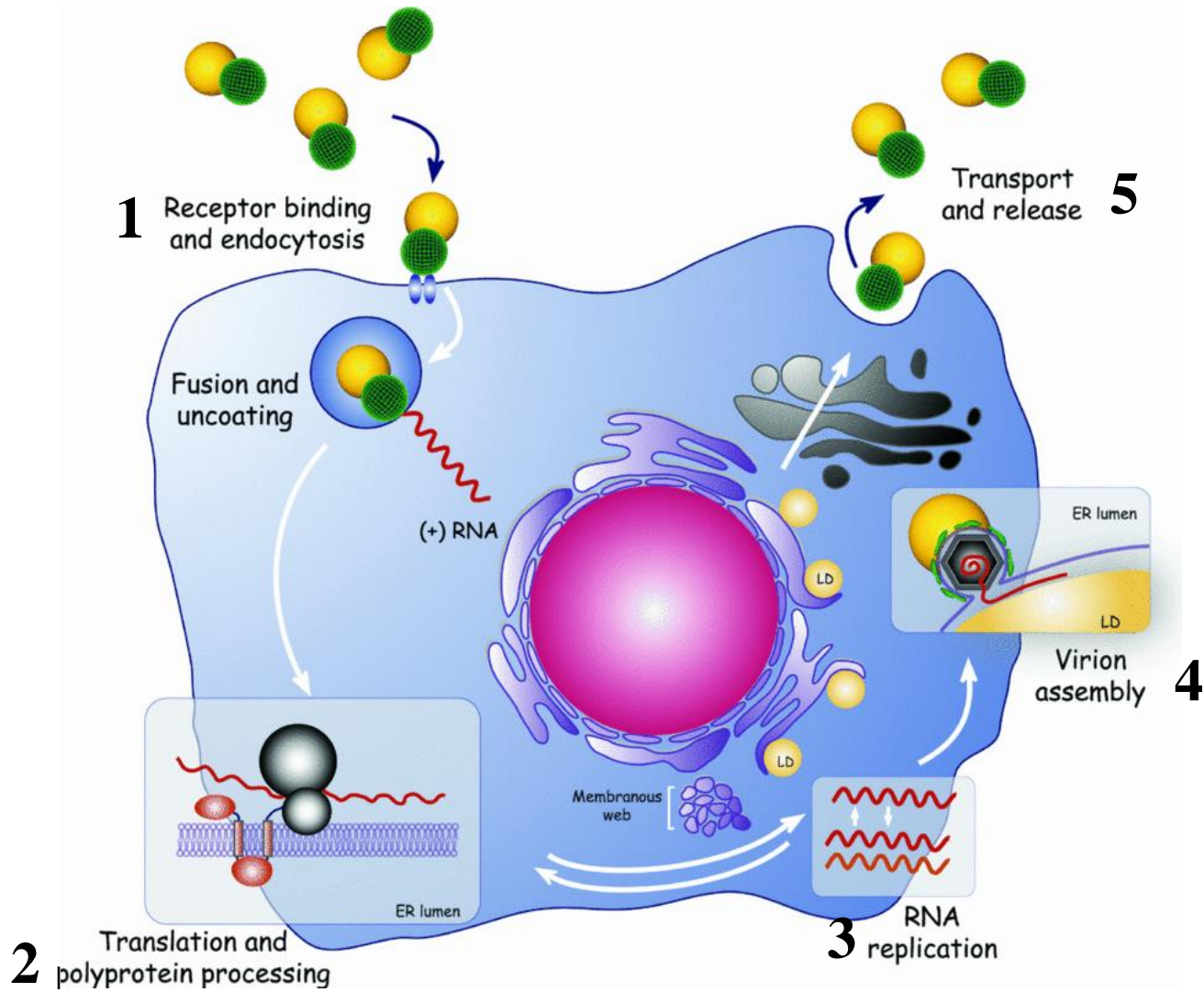
Capsid

- Icosahedral
- Single protein

Envelope

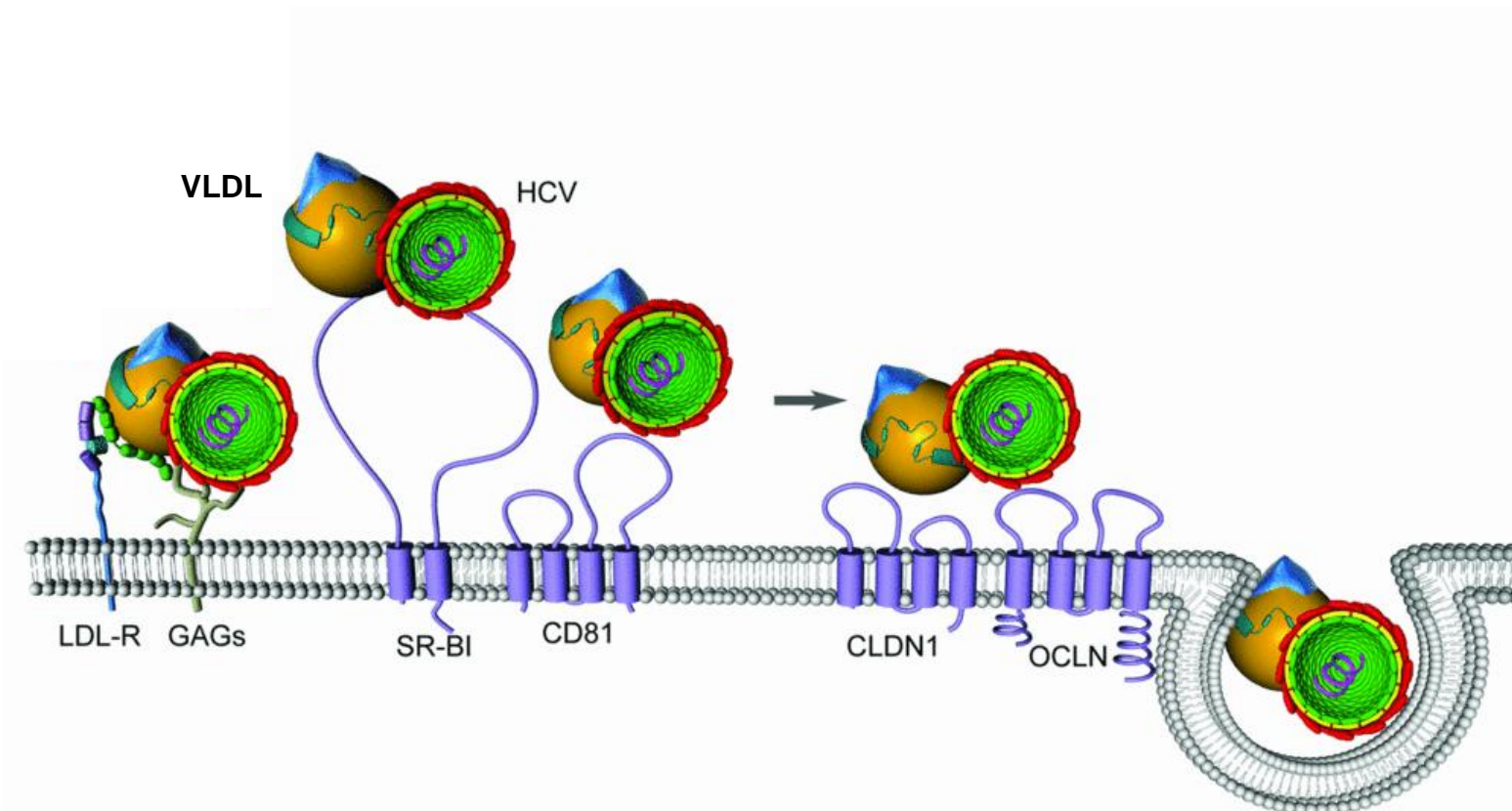
- Two envelope proteins E1 and E2
- Immune complexes?
- Complex with serum lipoproteins

HCV Replication Cycle



Infection of Host Cells

- Cell receptor(s) - CD81, SR-B1, CLDN1 and OCLN
- Receptor mediated endocytosis



Infection of Host Cells

- **Cell receptor(s) - CD81, SR-B1, CLDN1 and OCLN**
- **Receptor mediated endocytosis**
- **Coated vesicles - pre-lysosomal vesicles (low pH)**
- **Conformational change in E protein revealing a fusogenic domain**
- **Fusion of virus envelope with cellular membranes**
- **Translation in the cytoplasm**

HCV Genome



Single Stranded RNA Genome

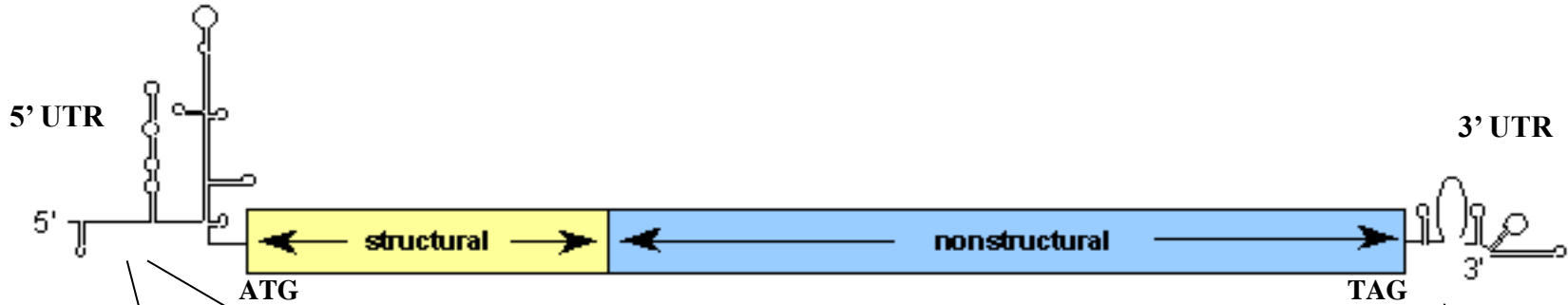
Approx 9,600 nucleotides

Positive Polarity = large mRNA

5' and 3' UnTranslated Regions (UTRs)

Single large Open Reading Frame (ORF)

HCV Genome



Internal Ribosome Entry Site (IRES)

→ Translational Control

RNA replication

Structure of HCV 3'-UTR

200 - 240 nucleotides

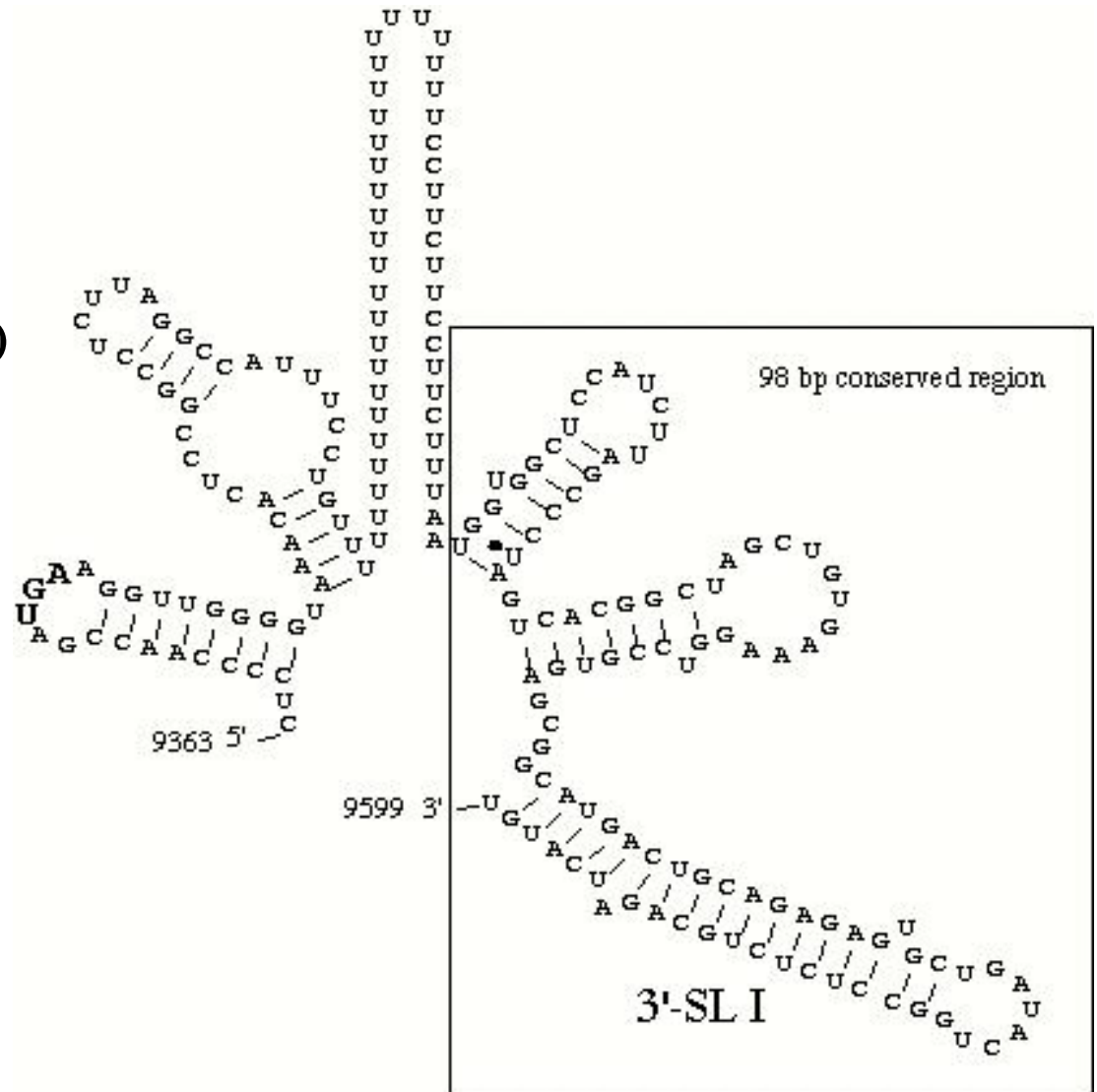
PolyU tract (30-90 nucleotides)

**98 nucleotide conserved
sequence no polyadenylation**

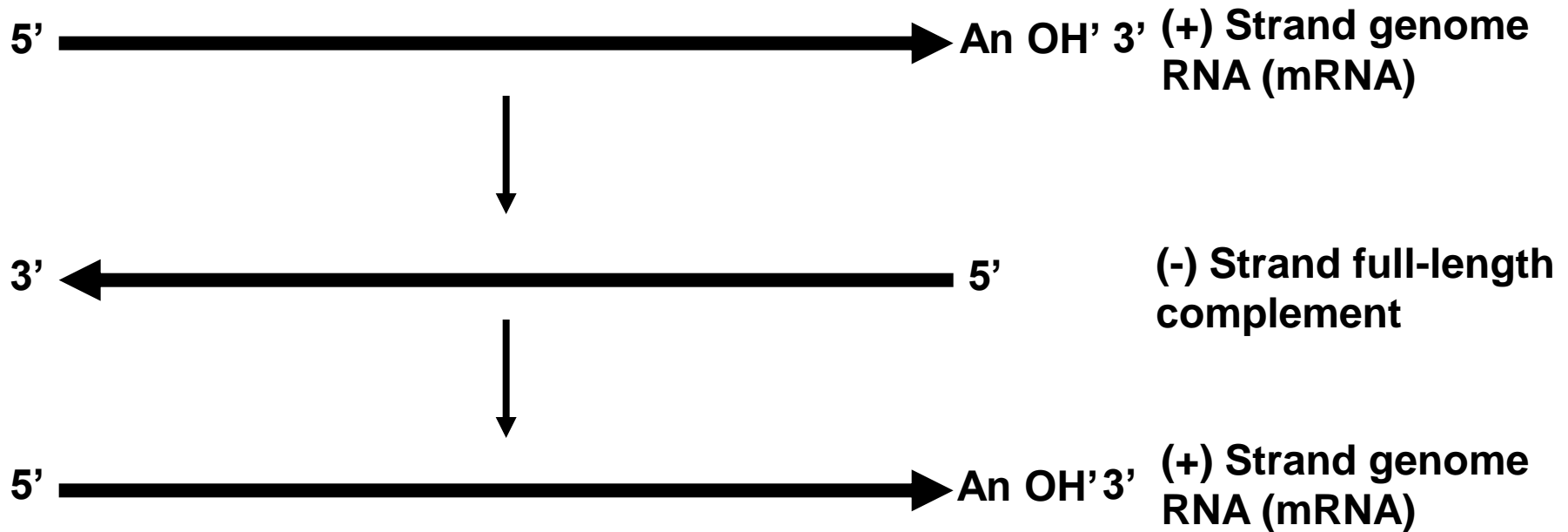
Stem Loop Structures

Role in replication?

Binds NS3



HCV Genome Replication



HCV Proteins

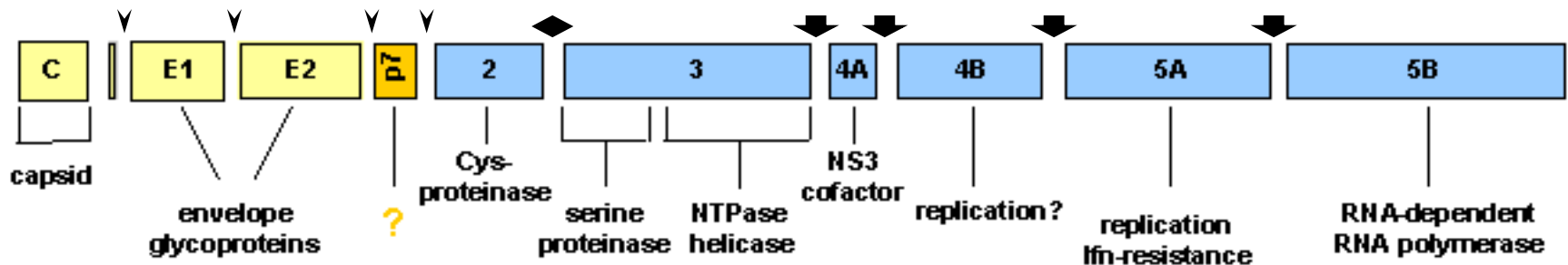


Translation

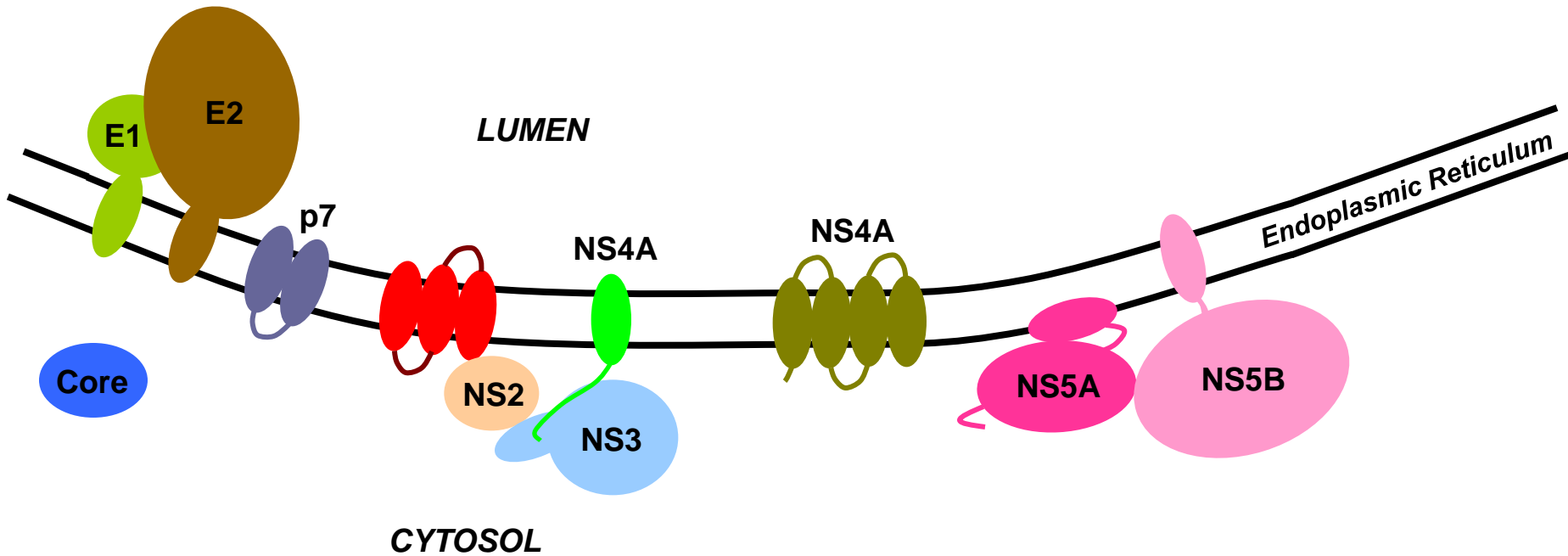


Polyprotein (~ 3,011 Amino acids)

Processing



HCV Proteins –ER Association



Capsid Protein

Highly basic

- Binds RNA

- Associates with lipid droplets

Nucleocapsid monomer

- binds to E 1

Associates at cytoplasmic side of ER

Envelope Proteins

Structure

- **E1 - 35 kd**
- **E2 - 72 kd**
- **Glycosylated**
- **Homology regions**
- **Divergence regions (HVR) N-terminus of E2**

Functions

Receptor binding

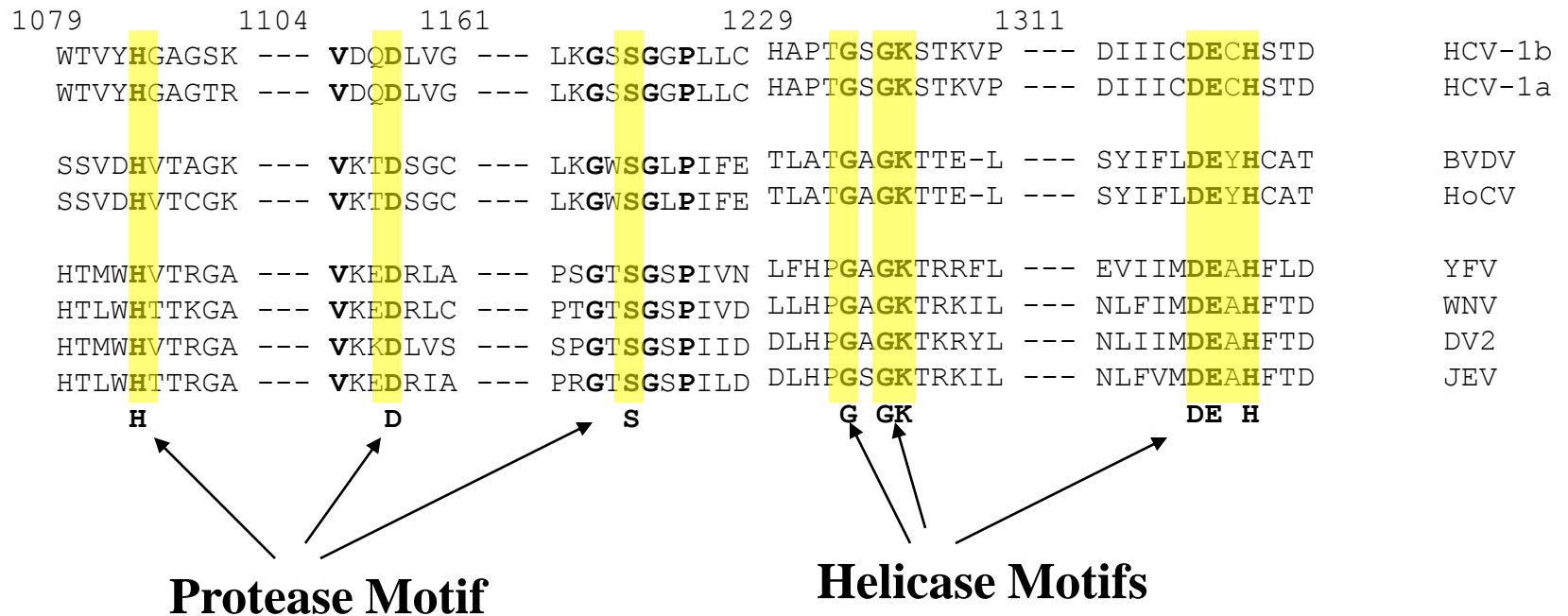
Membrane fusion - Fusogenic unit probably a trimer

Virion assembly

Properties of NS3

- **Protease**
- **Helicase in replication complex**
- **ATPase (associated with helicase activity)**

Comparison of Flaviviridae NS3 Proteins



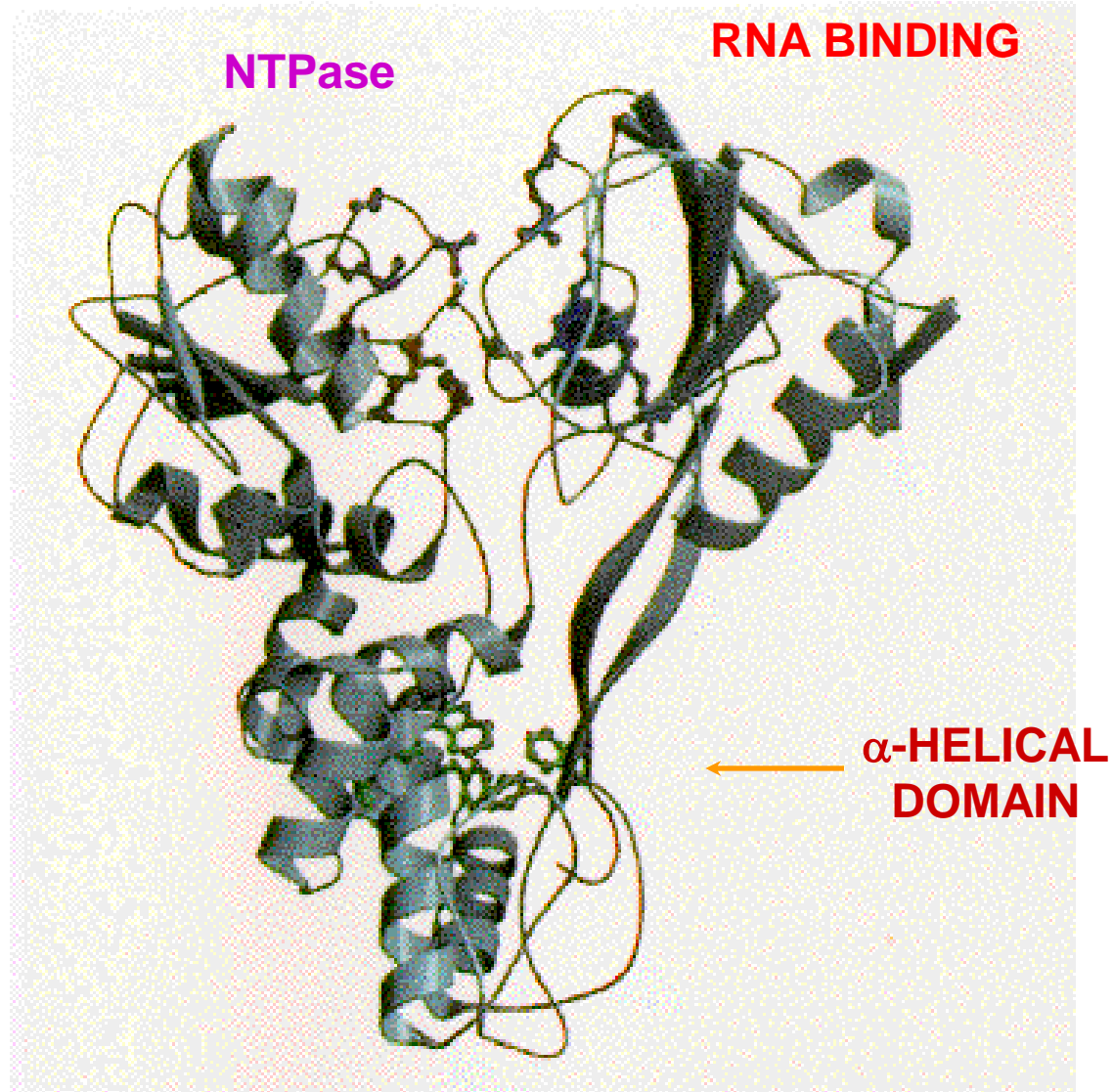
NS3 Protease

- **Amino 1/3 of NS3**
- **Serine protease with a catalytic triad of amino acids**
- **Cleaves HCV polyprotein both cis and trans**
- **Targets host interferon stimulation pathway proteins**
→ **viral persistence in HCV**

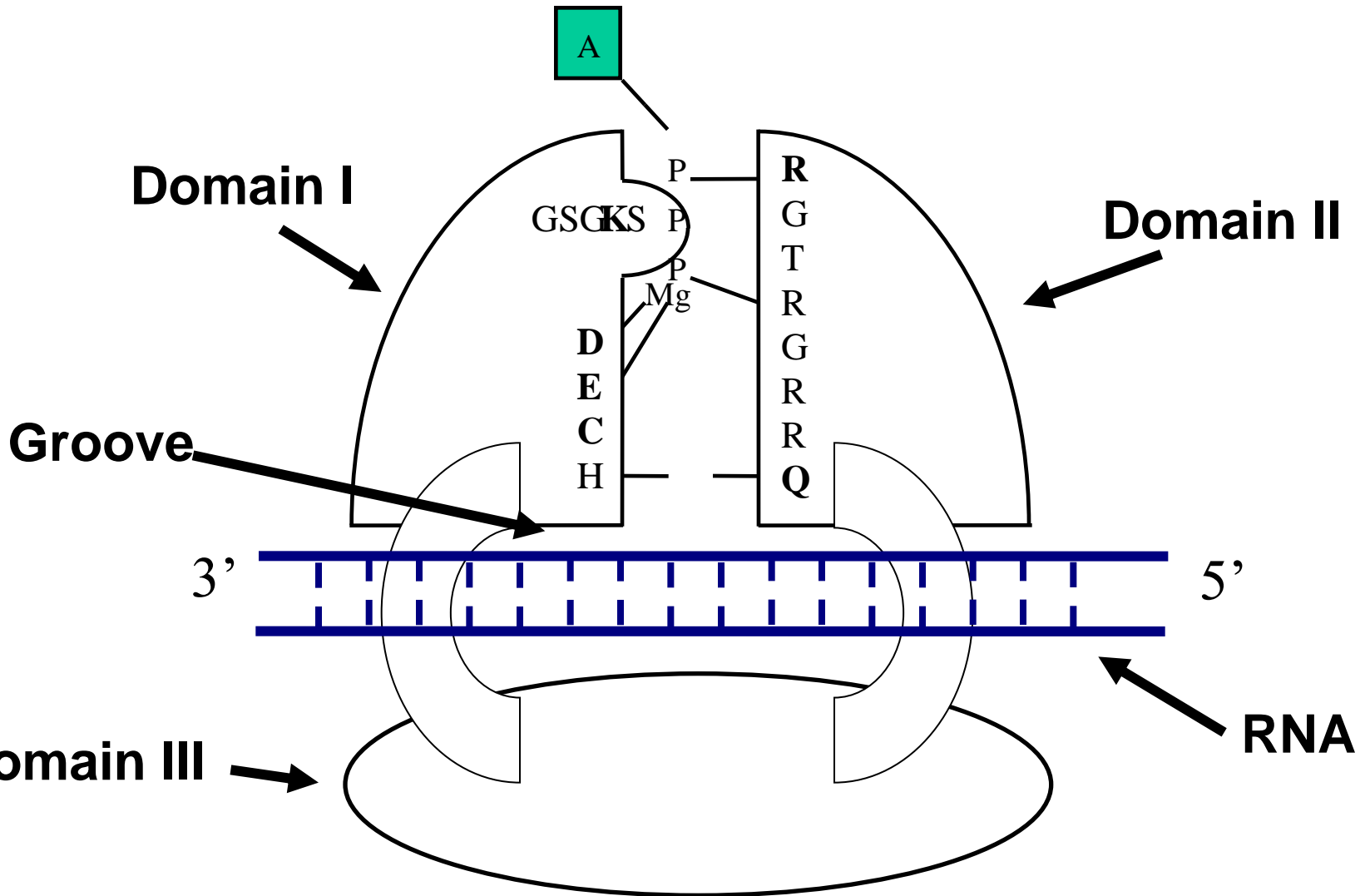
NS3 Helicase/NTPase

- **Helicase at carboxy 2/3 of NS3**
- **Unwinds double stranded RNA which is produced during virus replication**
- **Essential part of the replication complex**

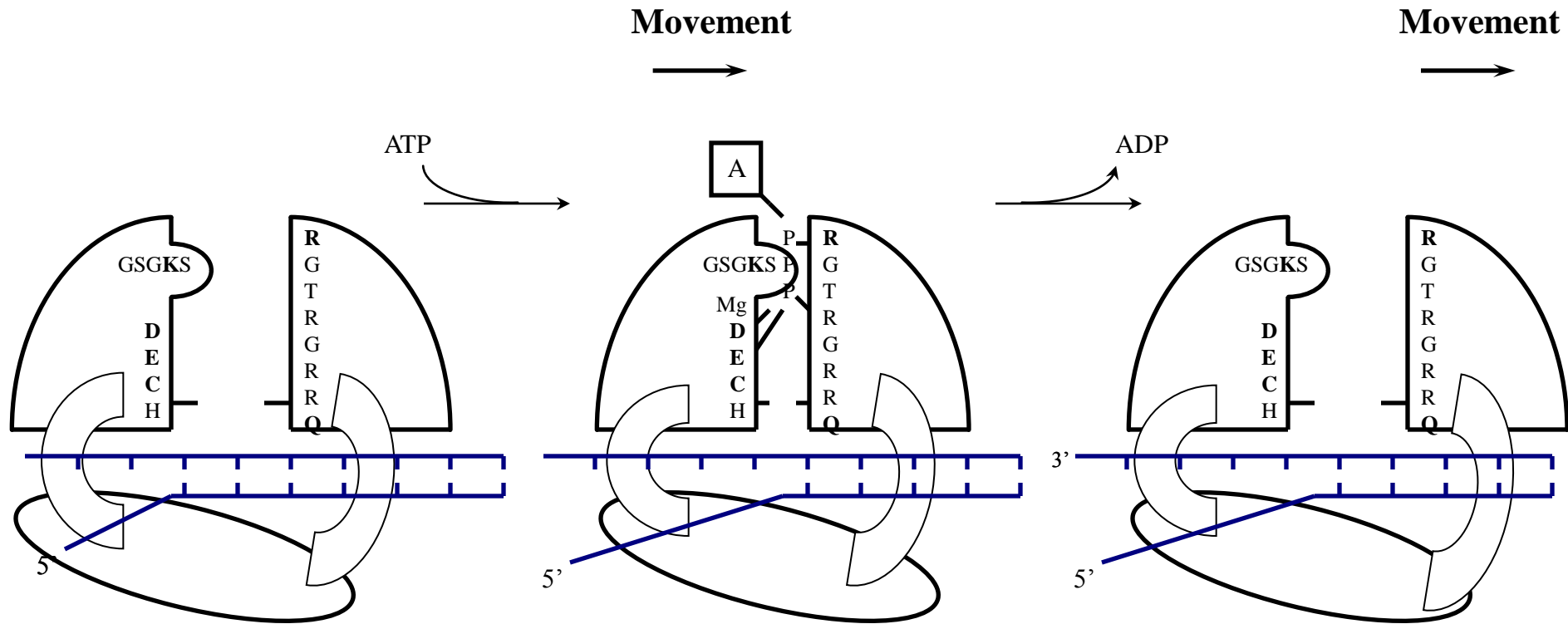
NS3 RNA Helicase



NS3 RNA Helicase: Model



Ratchet Model for HCV RNA Helicase



NS5B

RNA dependent RNA polymerase (RdRp)

GDD motif (characteristic of RdRps)

Replicase

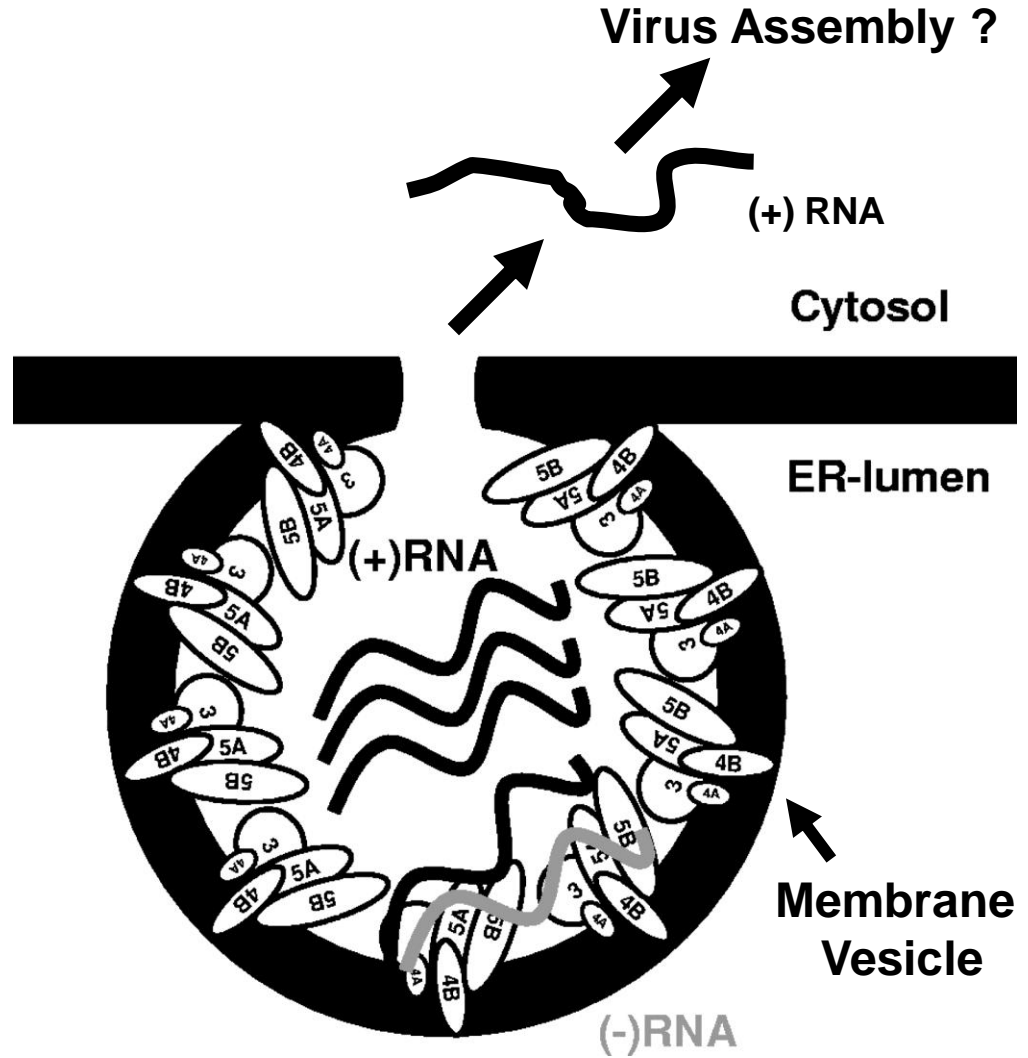
NS5B + NS3 + (NS4A ?) + (Host proteins ?)

Membrane associated

ER / perinuclear

Double stranded RNA localisation

HCV Replication Complexes - Model



Other Non-structural Proteins

NS4A

- **Protease complex with NS3**
- **Localisation of RNA replication machinery to membranes**

NS2

- **NS2/NS3 protease**

NS5A

- **Interferon inhibition - PKR**
- **p53 (inhibition?)**

NS4B – Induces membranous web - site of replication

- **Inhibits cellular translation?**

Assembly and Release of Virus

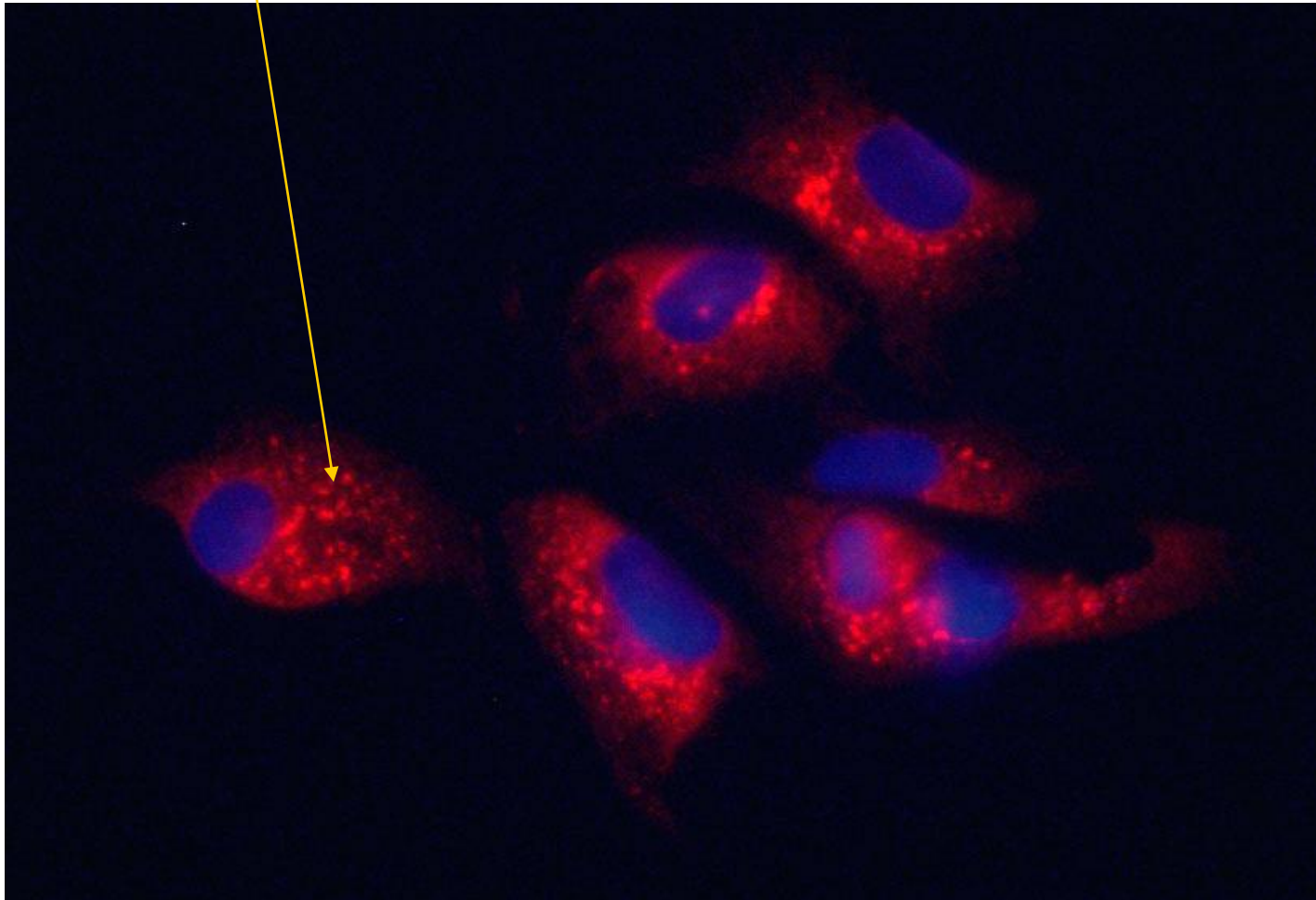
**C - protein interaction with RNA - nucleocapsid
(cytoplasm)**

Interactions between E1 and C

**Acquire envelope by budding into the ER lumen
and lipid droplet (LD)**

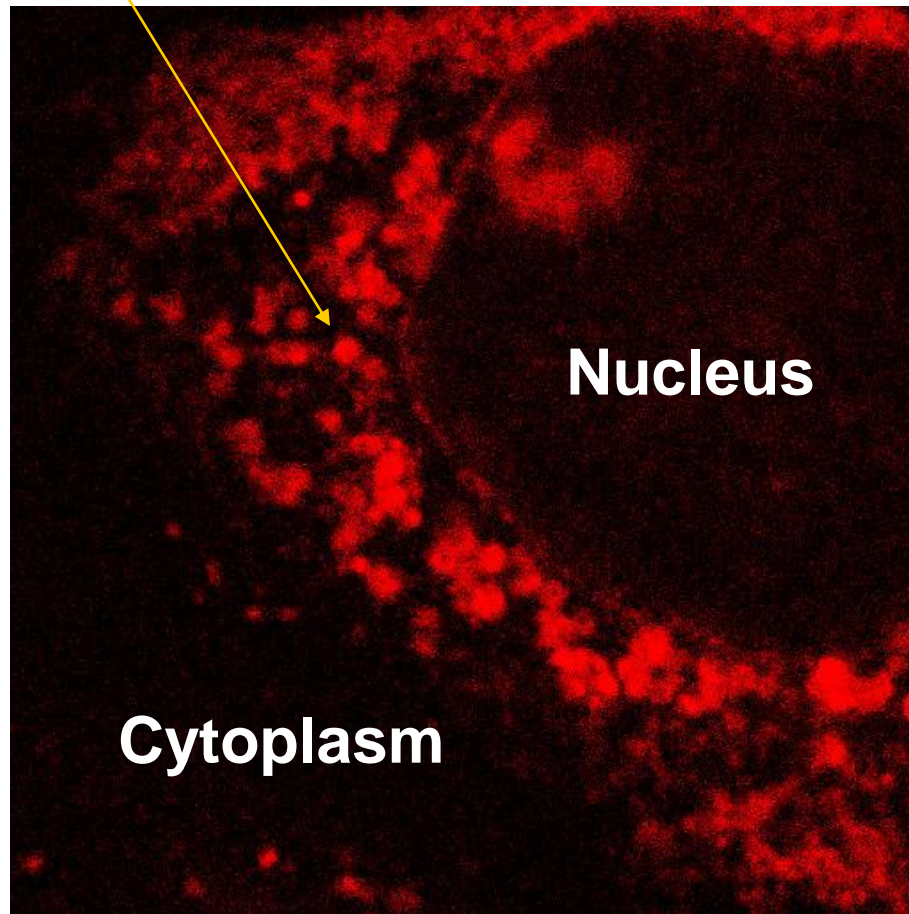
Liver Cells Infected with HCV

Core protein

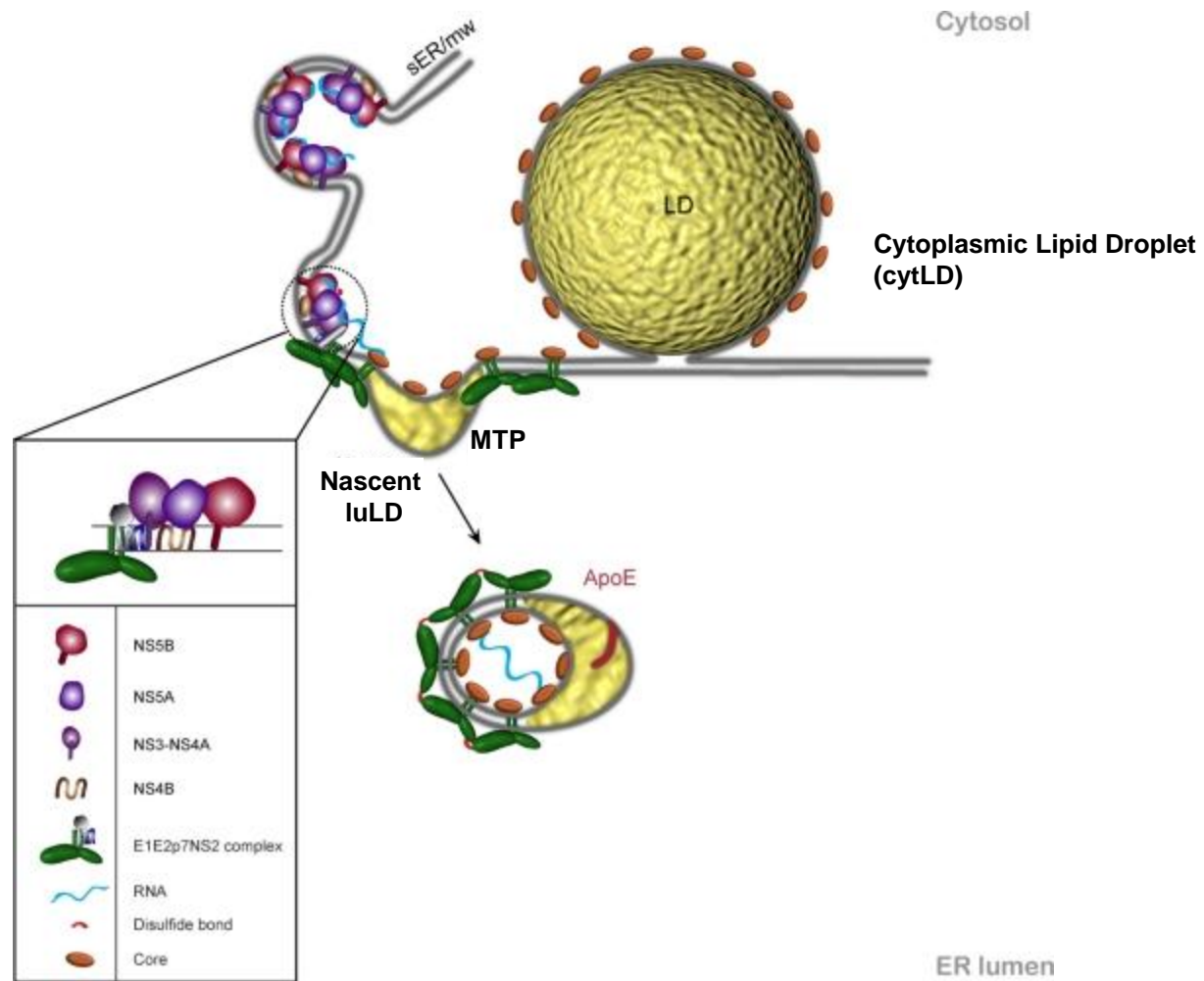


Liver Cells Infected with HCV: Confocal Microscopy

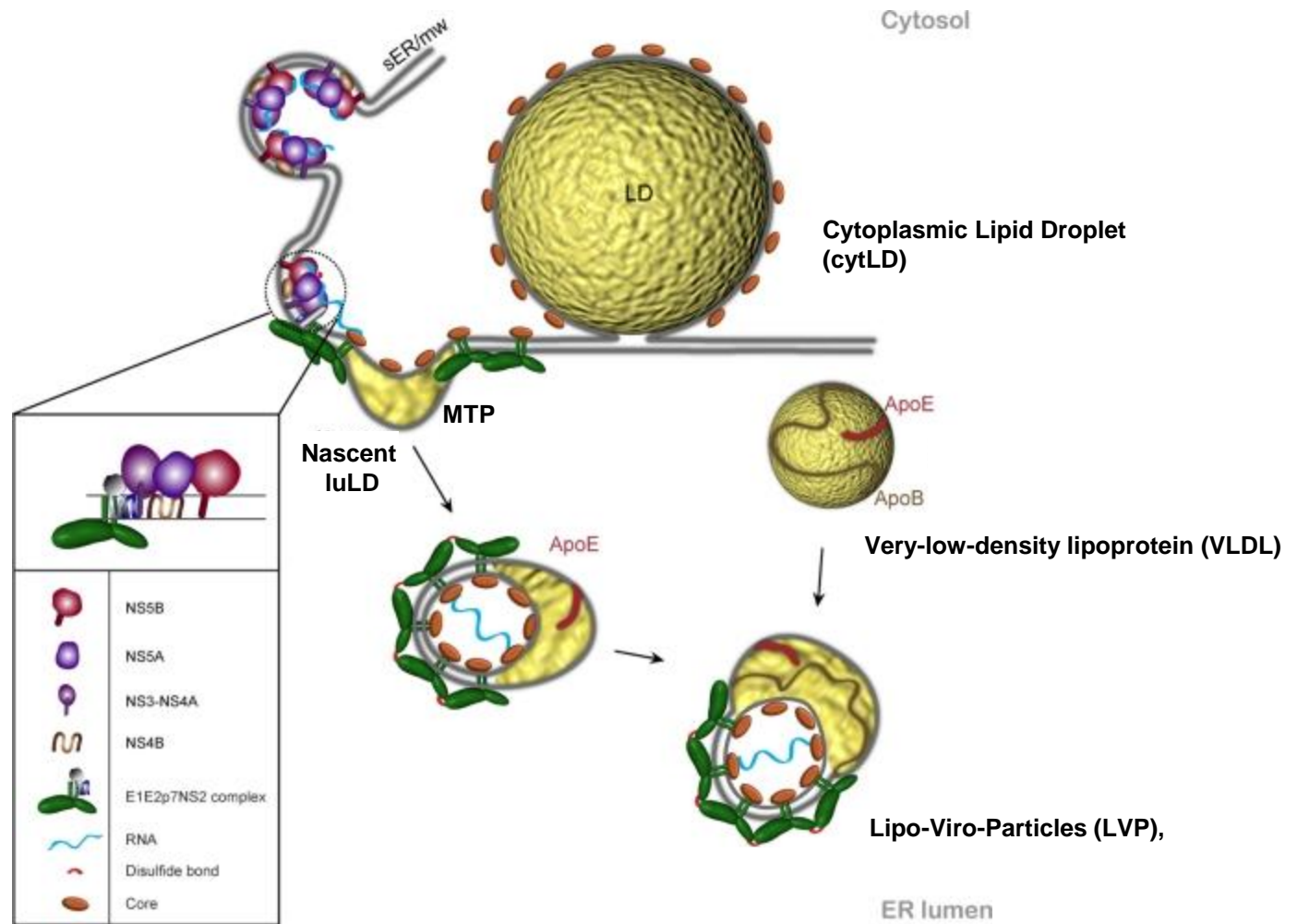
Core protein/Lipid Droplets



HCV Assembly – Lipid Droplet Model



HCV Assembly – Lipid Droplet Model



Assembly and Release of Virus

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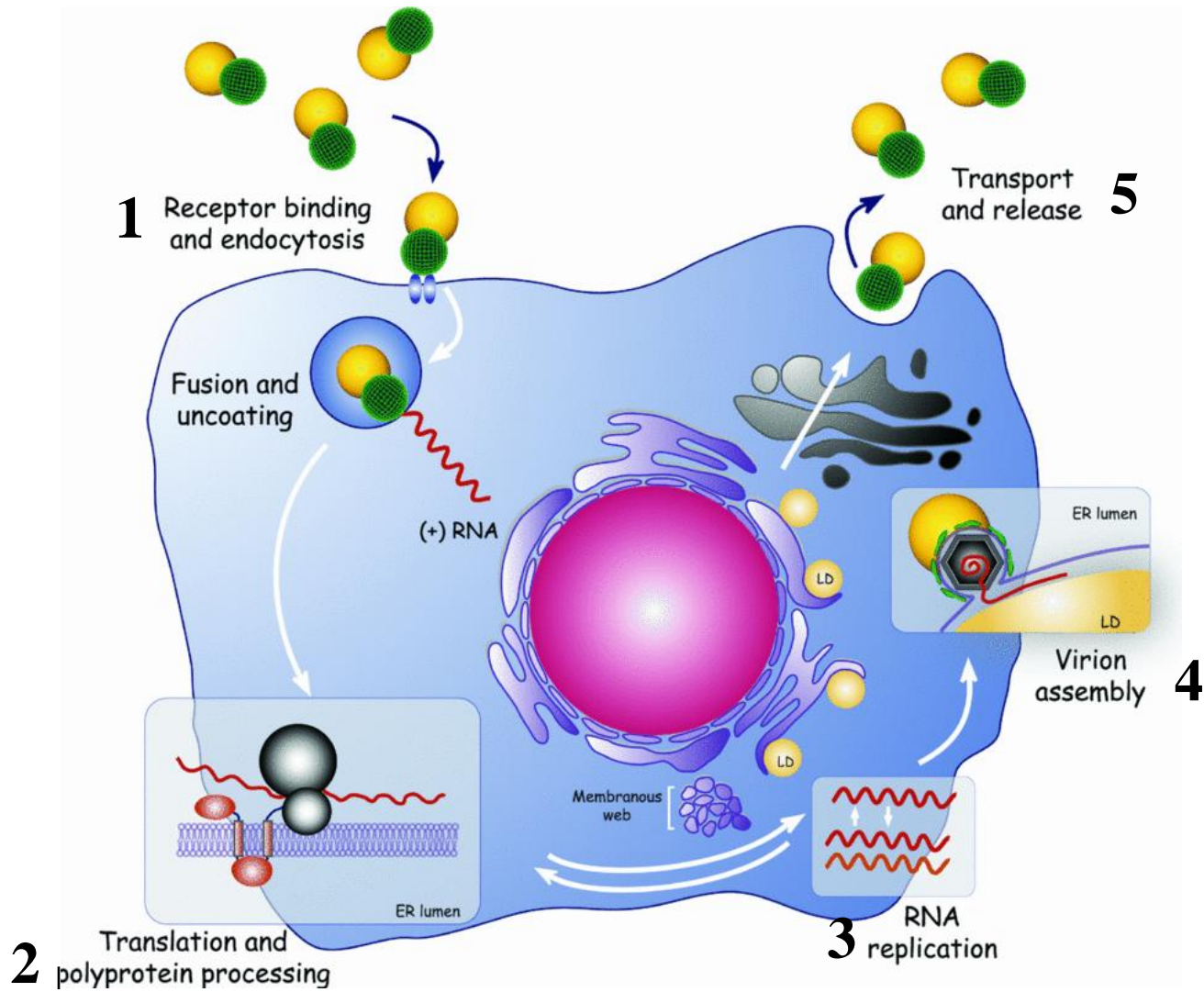
Interactions between E1 and C

**Acquire envelope by budding into the ER lumen
and lipid droplet (LD)**

Movement through exocytosis pathway

**Vesicles fuse with the plasma membrane
and release the virions from the cells?**

HCV Replication Cycle



Persistence of HCV Infections

Variation in genome/protein structure – quasispecies

Site of RNA replication – ER vesicles

Assembly – transfer of new genomes to virions??

Non-structural protein inhibition of host defences

NS5A –Inhibits interferon responses

NS3 – Inhibits cell monitoring of virus molecules

References

Lemon et al, “Hepatitis C Virus” Chapter 35, Fields Virology (2007) Fifth Edition (Lippincott-Williams, Wilkins, Philadelphia) p1253-1304.

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