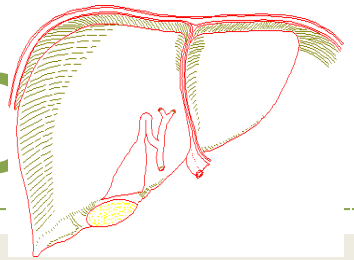
الجامعة السورية الخاصة
SYRIAN PRIVATE UNIVERSITY

HEPATITIS , C



الدكتور نزار الضاهر

استاذ مساعد (الأمراض الأنتانية)

HEPATITIS

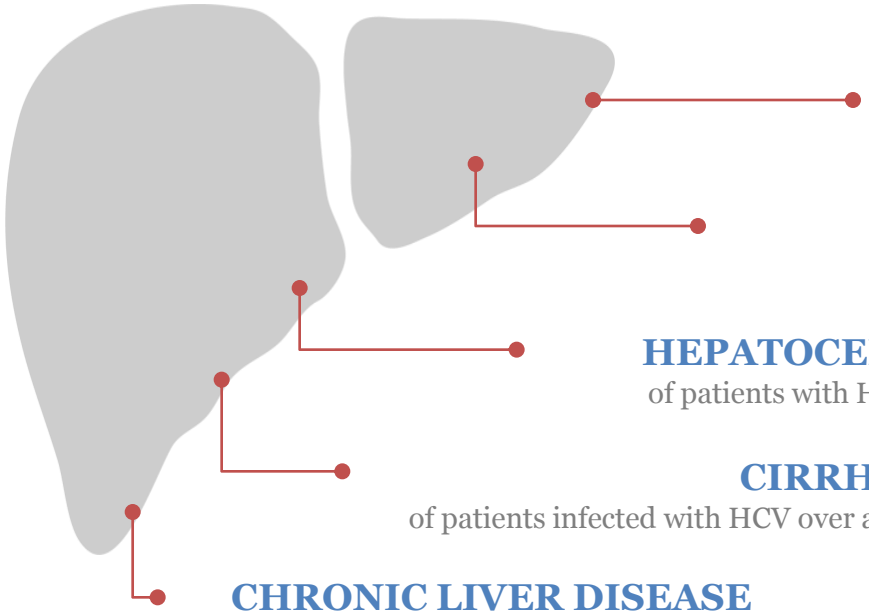
"INFLAMMATION OF THE LIVER"

CAUSED BY:

- VIRUSES- HEPATITIS A, B, C, D, E, G
- OTHER INFECTIONS (MONONUCLEOSIS)
- CHEMICALS
  - ALCOHOL
  - ACETAMINOPHEN

Why Screening for HCV is Important

Chronic HCV can progress to cirrhosis and eventually liver failure and/or HCC



DEATH ~1%–5%
of patients infected with HCV will die from the consequences of chronic HCV infection

LIVER TRANSPLANT #1
HCV is #1 cause of liver transplant

HEPATOCELLULAR CARCINOMA ~1%–5%
of patients with HCV-related cirrhosis will develop HCC annually

CIRRHOSIS 5%–20%
of patients infected with HCV over a period of 20–30 years

CHRONIC LIVER DISEASE
~60%–70%
of patients with HCV

Almost 20,000 deaths were associated with HCV in 2015

AbbVie Inc. AbbVie Standards of Care. The Importance of Screening to Help Manage the Growing Burden of Hepatitis C Virus. Sept 2017. Accessed March 2018

HEPATITIS C HISTORY

1973: NON A, NON B HEPATITIS IS DESCRIBED

1989: HEPATITIS C (HCV) GENONE IS CLONED; A SINGLE STRANDED, RNA VIRUS IN THE FLAVIVIRIDAE FAMILY

1989: HCV ANTIBODY TEST IS DEVELOPED (ELISA)

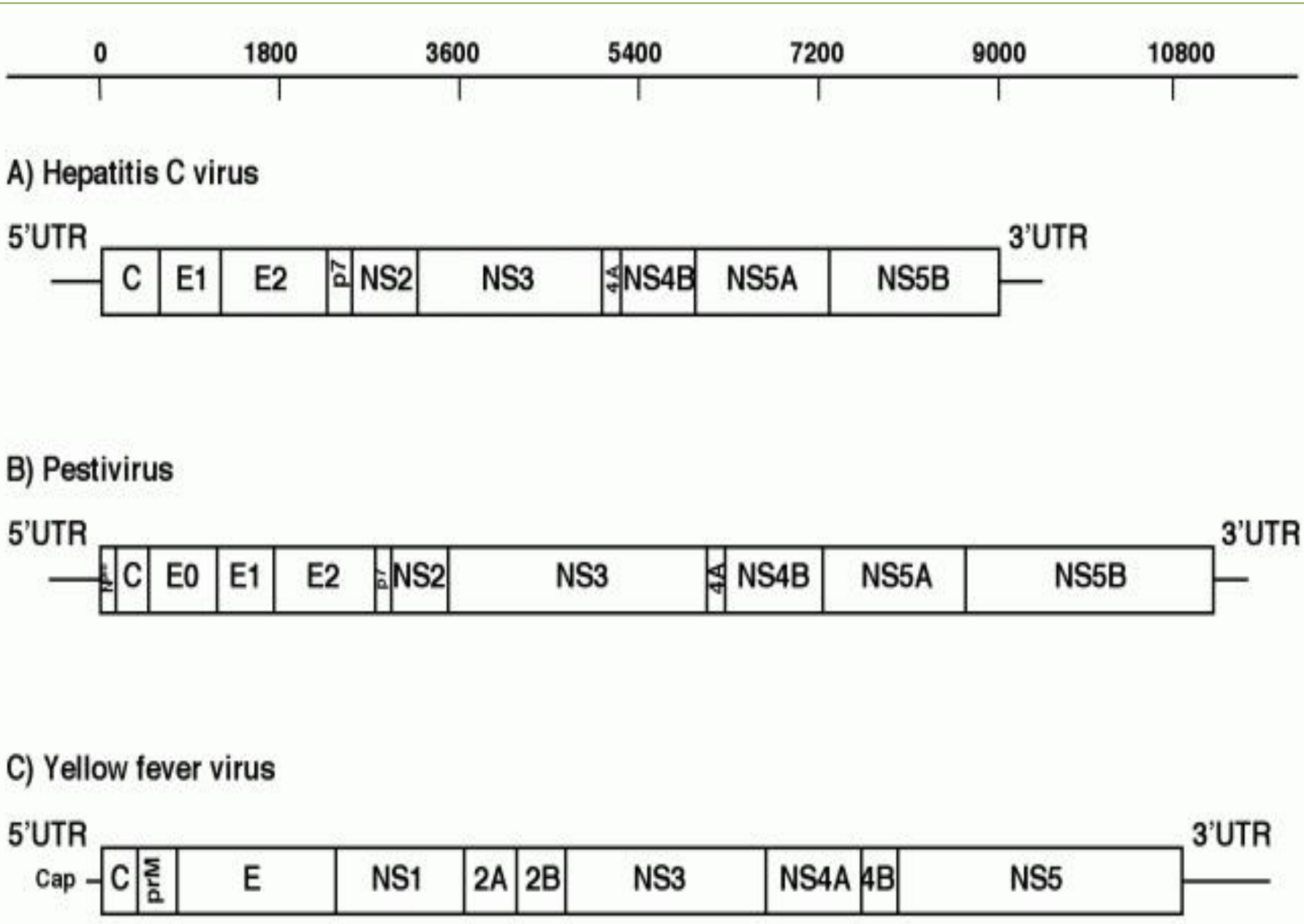
1990: HCV VIRAL LOAD TEST IS DEVELOPED TO DETECT HCV RNA IN SERUM (PCR TEST)

1998: COMBINATION THERAPY WITH INTERFERON AND RIBAVIRIN IS APPROVED BY THE FDA

- **The *Flaviviridae* family is divided into three genera:**
- **Flaviviruses : yellow fever virus, dengue fever virus, Japanese encephalitis virus, and Tick-borne encephalitis virus.**
- **Pestiviruses :bovine viral diarrhea virus, classical swine fever virus and Border disease virus.**
- **hepacivirus :**
- HCV, is a member of the hepacivirus genus,
- GB virus B (GBV-B) and is closely related to human virus GB virus C (GBV-C)

- The *Flaviviridae* genome is a positive-strand RNA molecule ranging in size from 9.6 to 12.3 thousand nucleotides (nt),
- with an **open reading frame** (ORF) encoding a polyprotein of 3000 **amino acids (aa)** or more.
- The structural proteins are encoded by the N-terminal part of the ORF, whereas the remaining portion of the ORF codes for the nonstructural proteins
- . similar locations in the polyproteins of all of the *Flaviviridae*).

- **The ORF is flanked in 5′ and 3′ by untranslated regions (UTR) of 95–555 and 114–624 nt in length**, respectively, which play an important role in polyprotein translation and RNA replication


0
1800
3600
5400
7200
9000
10800
A) Hepatitis C virus
5'UTR
C
E1
E2
p7
NS2
NS3
4A
NS4B
NS5A
NS5B
3'UTR
B) Pestivirus
5'UTR
C
E0
E1
E2
p7
NS2
NS3
4A
NS4B
NS5A
NS5B
3'UTR
C) Yellow fever virus
5'UTR
Cap
C
prM
E
NS1
2A
2B
NS3
NS4A
4B
NS5
3'UTR

HCV is transmitted exclusively between humans.
Flaviviruses are principally vectored by mosquitoes or ticks and can infect a broad range of vertebrate animals, with humans being a dead-end host that does not participate in the perpetuation of virus transmission.
No known pestivirus can infect humans

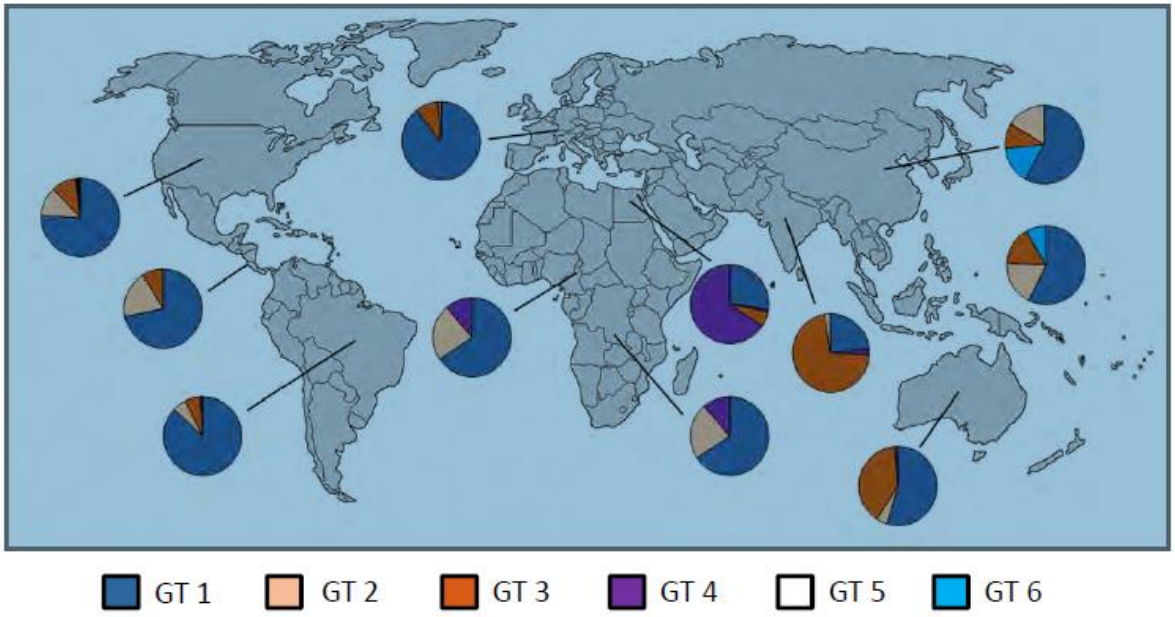
Infections by flaviviruses **are acute-limited in vertebrate animals,**
whereas HCV has a high chronicity rate in humans (50%–80%,).

Strong and adapted **humoral and cellular immune responses** have been shown to be involved in flavivirus and pestivirus infection recovery and protection.

Hepatitis C Genotypes

Worldwide Distribution of HCV Genotypes

Genotype	Seroprevalence (millions)
1	83.4
2	16.5
3	54.3
4	15.1
5	1.5
6	9.8
Total Prevalence	180.5



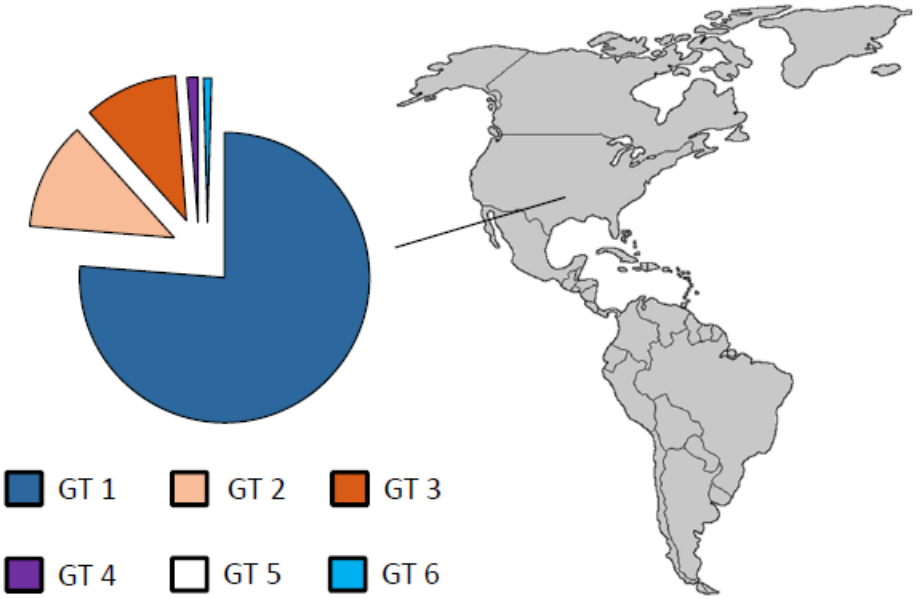
Messina J et al. *Hepatology*. 2015; 61:77-87.

Schafer J, Short W. Ask The Experts: Updates and Challenges in Managing Patients with Hepatitis C Virus Infection. 2018 American Society of Health-Systems Pharmacists. http://www.cemidday.com/ate-hepc18/. Accessed April 19, 2018.

Genotypes in North America

Distribution of HCV Genotypes In North America

Genotype	Seroprevalence	Percentage
1	3,595,000	75.8%
2	567,000	12%
3	492,000	10.4%
4	55,000	1.2%
5	6,000	0.1%
6	26,000	0.6%
Total Prevalence	4,742,000	100%

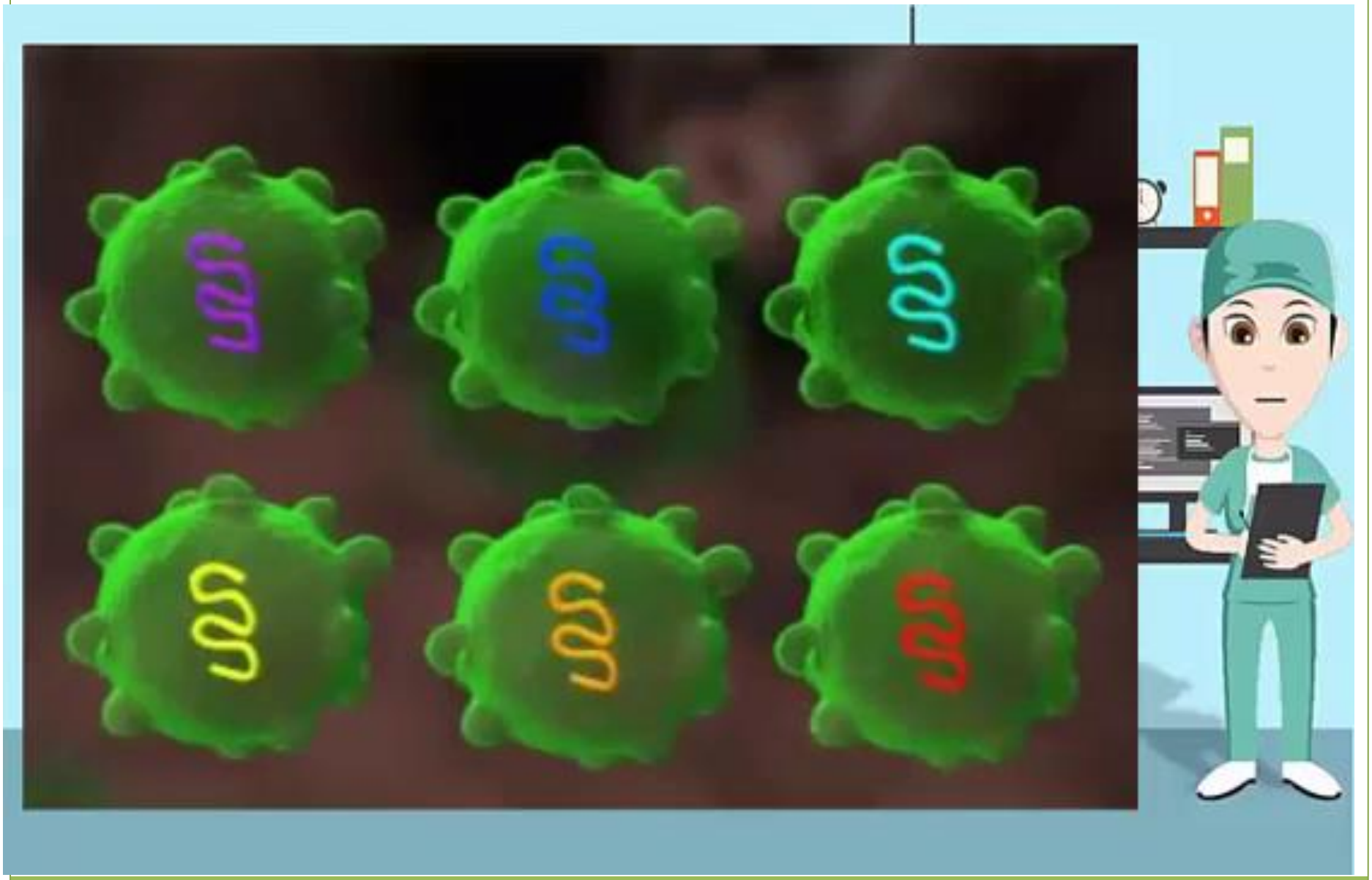


Messina J et al. *Hepatology*. 2015; 61:77-87.

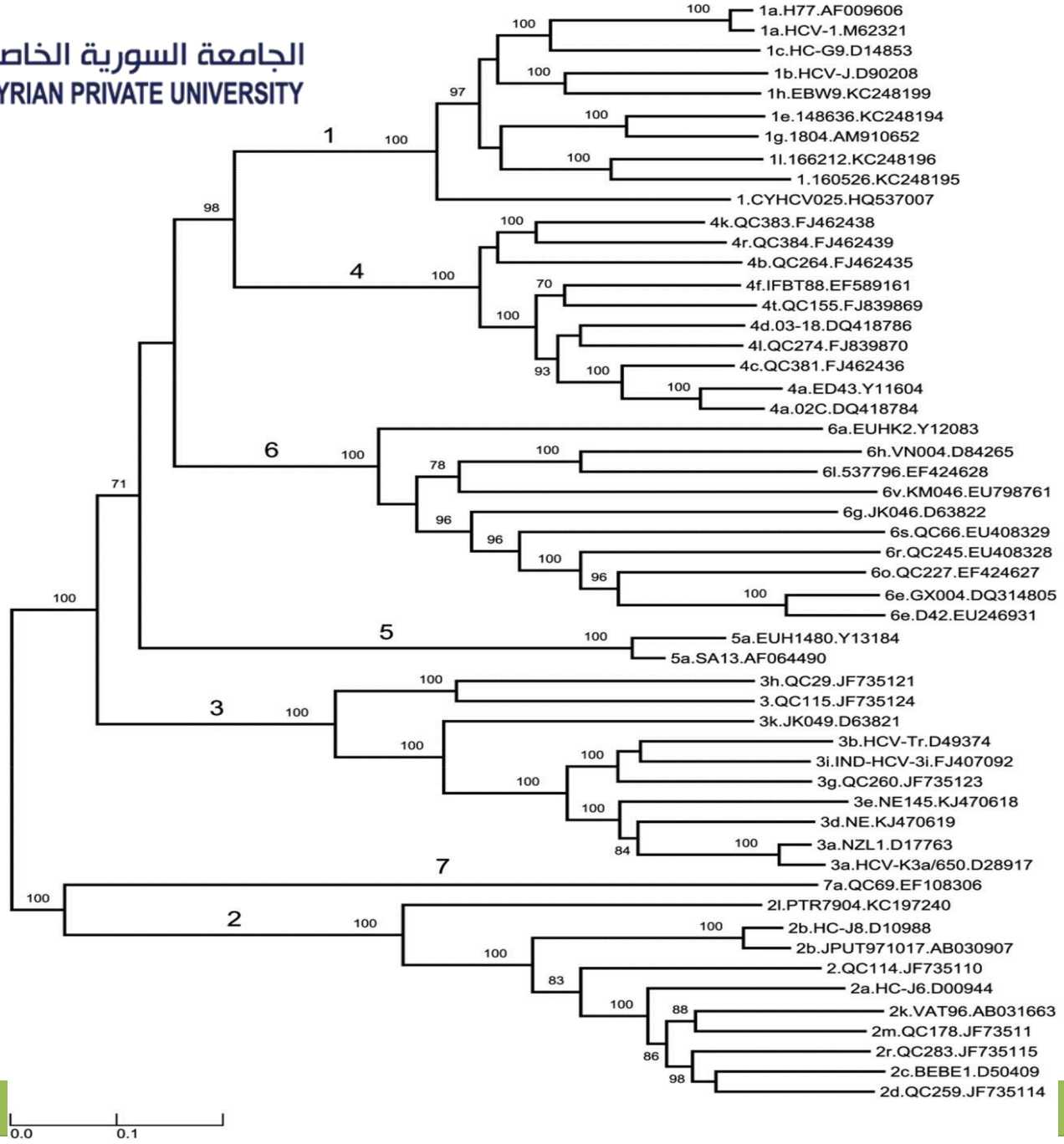
Schafer J, Short W. Ask The Experts: Updates and Challenges in Managing Patients with Hepatitis C Virus Infection. 2018 American Society of Health-Systems Pharmacists. http://www.comidday.com/ate-hepc18/. Accessed April 19, 2018.

Geographical distribution of HCV subtypes

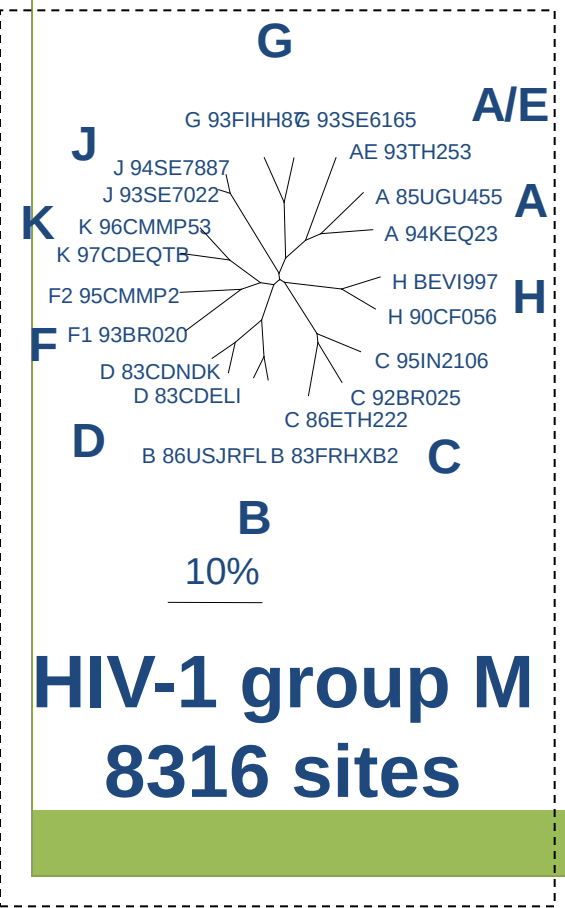
HCV genotypes	Subsets	Geographical distribution[20]
Genotype 1	1a, 1b	North America, Central Africa, Europe
Genotype 2	2a, 2b, 2c, 2d	Western Africa
Genotype 3	3a, 3b, 3c, 3d, 3e, 3f	Southeast Asia
Genotype 4	4a, 4b, 4c, 4d, 4e, 4f, 4g, 4h, 4i, 4j	Central Africa
Genotype 5	5a	South Africa and Asia
Genotype 6	6a	Southeast Asia



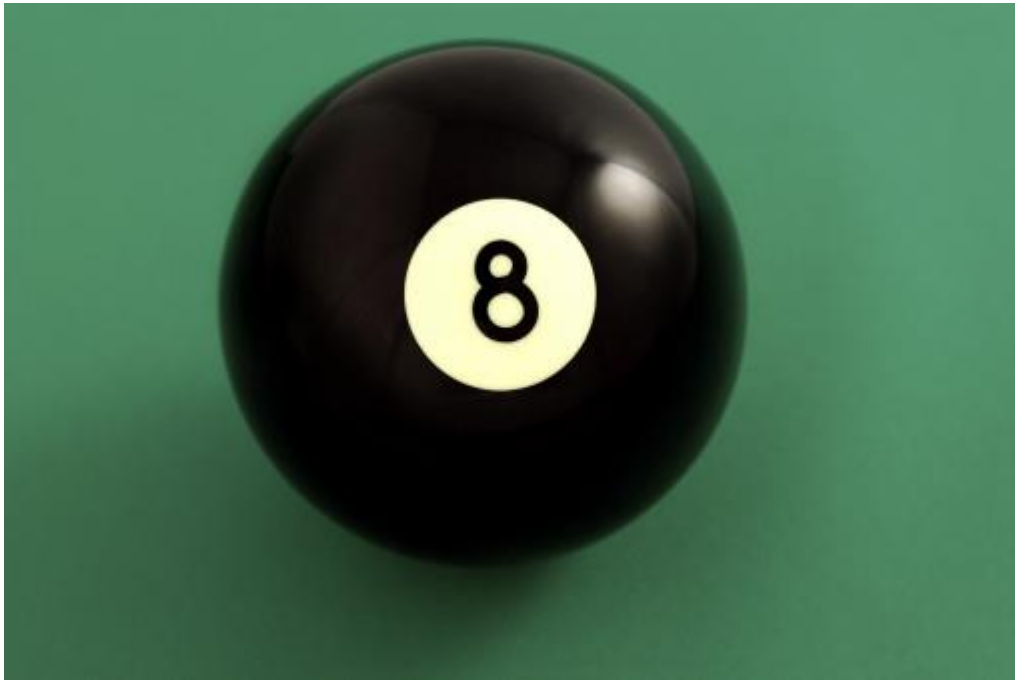
- the virus may remain in latent phase due to suppression by host immune system. However, during its infectious phase,
- **the replication of HCV is very robust, and around 10 trillion virion particles can be produced per day7.**
- May 2013. **On the basis of genomic variability, HCV is classified into seven major genotypes and 67 subtypes.**


1a.H77.AF009606
1a.HCV-1.M62321
1c.HC-G9.D14853
1b.HCV-J.D90208
1h.EBW9.KC248199
1e.148636.KC248194
1g.1804.AM910652
1l.166212.KC248196
1.160526.KC248195
1.CYHCV025.HQ537007
4k.QC383.FJ462438
4r.QC384.FJ462439
4b.QC264.FJ462435
4f.IFBT88.EF589161
4t.QC155.FJ839869
4d.03-18.DQ418786
4l.QC274.FJ839870
4c.QC381.FJ462436
4a.ED43.Y11604
4a.02C.DQ418784
6a.EUHK2.Y12083
6h.VN004.D84265
6l.537796.EF424628
6v.KM046.EU798761
6g.JK046.D63822
6s.QC66.EU408329
6r.QC245.EU408328
6o.QC227.EF424627
6e.GX004.DQ314805
6e.D42.EU246931
5a.EUH1480.Y13184
5a.SA13.AF064490
3h.QC29.JF735121
3.QC115.JF735124
3k.JK049.D63821
3b.HCV-Tr.D49374
3i.IND-HCV-3i.FJ407092
3g.QC260.JF735123
3e.NE145.KJ470618
3d.NE.KJ470619
3a.NZL1.D17763
3a.HCV-K3a/650.D28917
7a.QC69.EF108306
2l.PTR7904.KC197240
2b.HC-J8.D10988
2b.JPUT971017.AB030907
2.QC114.JF735110
2a.HC-J6.D00944
2k.VAT96.AB031663
2m.QC178.JF73511
2r.QC283.JF735115
2c.BEBE1.D50409
2d.QC259.JF735114
1
4
6
5
3
7
2
0.0
0.1

HCV
9213 sites



HIV-1 group M
8316 sites



Hepatitis C Genotype 8

This brings the current total to *8 genotypes and 84 subtypes*

Four patients, previously classified as GT5 by RealTime polymerase chain reaction assays, were identified as infected with a novel HCV GT.

This novel HCV GT, GT8, is genetically distinct from previously identified HCV GT1-7 with >30% nucleotide sequence divergence to the established HCV subtypes. All 4 patients were originally from Punjab, India, but now reside in Canada

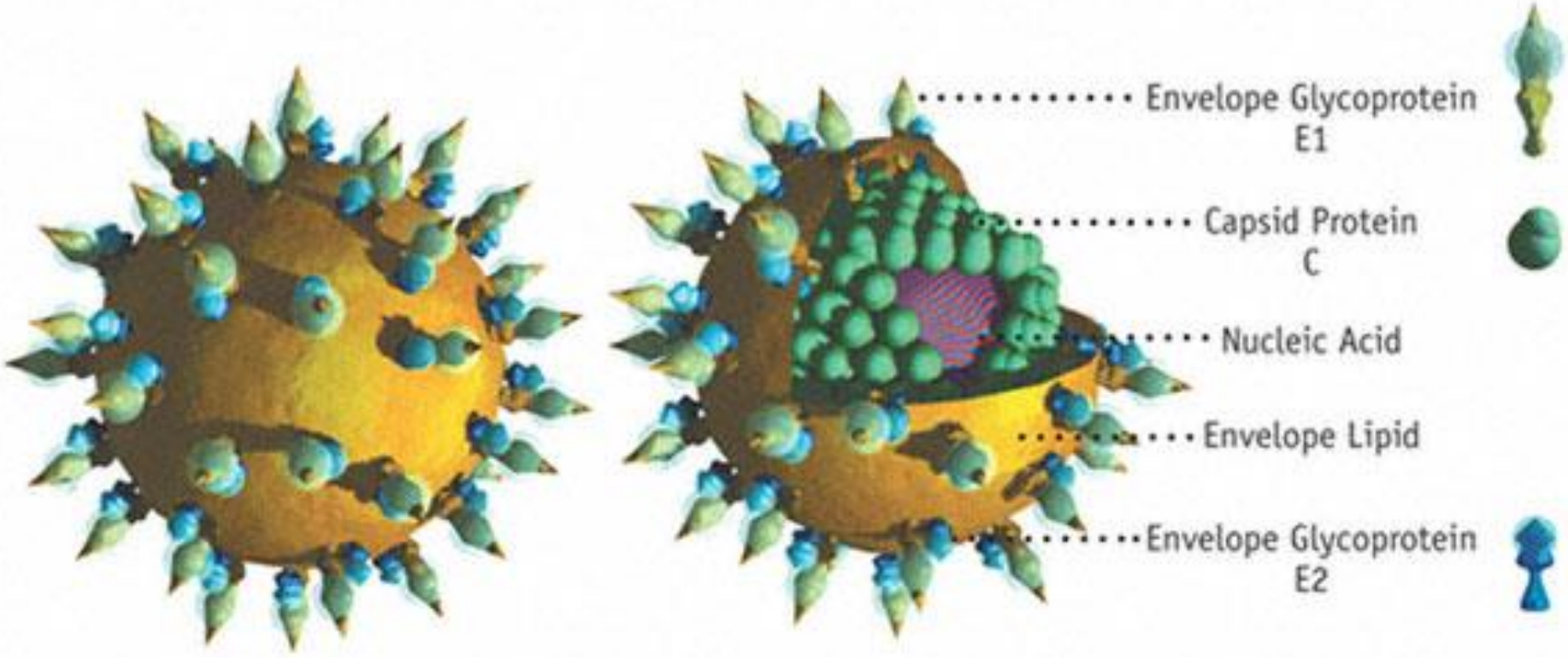
all patients achieved a sustained virologic response; 2 treated with sofosbuvir/velpatasvir/voxilaprevir for 8 weeks, 1 with sofosbuvir/ledipasvir plus ribavirin for 24 weeks and 1 with sofosbuvir plus daclatasvir for 12 weeks.

HEPATITIS C

- AT LEAST 9 MAJOR GENOTYPES OF THIS VIRUS EXIST
  - GENOTYPE 1 - 4 HAVE SUBTYPES
  - IN U.S. GENOTYPE 1A AND 1B ACCOUNT FOR 70 - 80 % OF ALL CASES
  - IN SOUTH AFRICA AND SE ASIA PREDOMINANT TYPES ARE 5 AND 6
  - IN THE MIDDLE EAST AND CENTRAL AFRICA IT IS TYPE 4

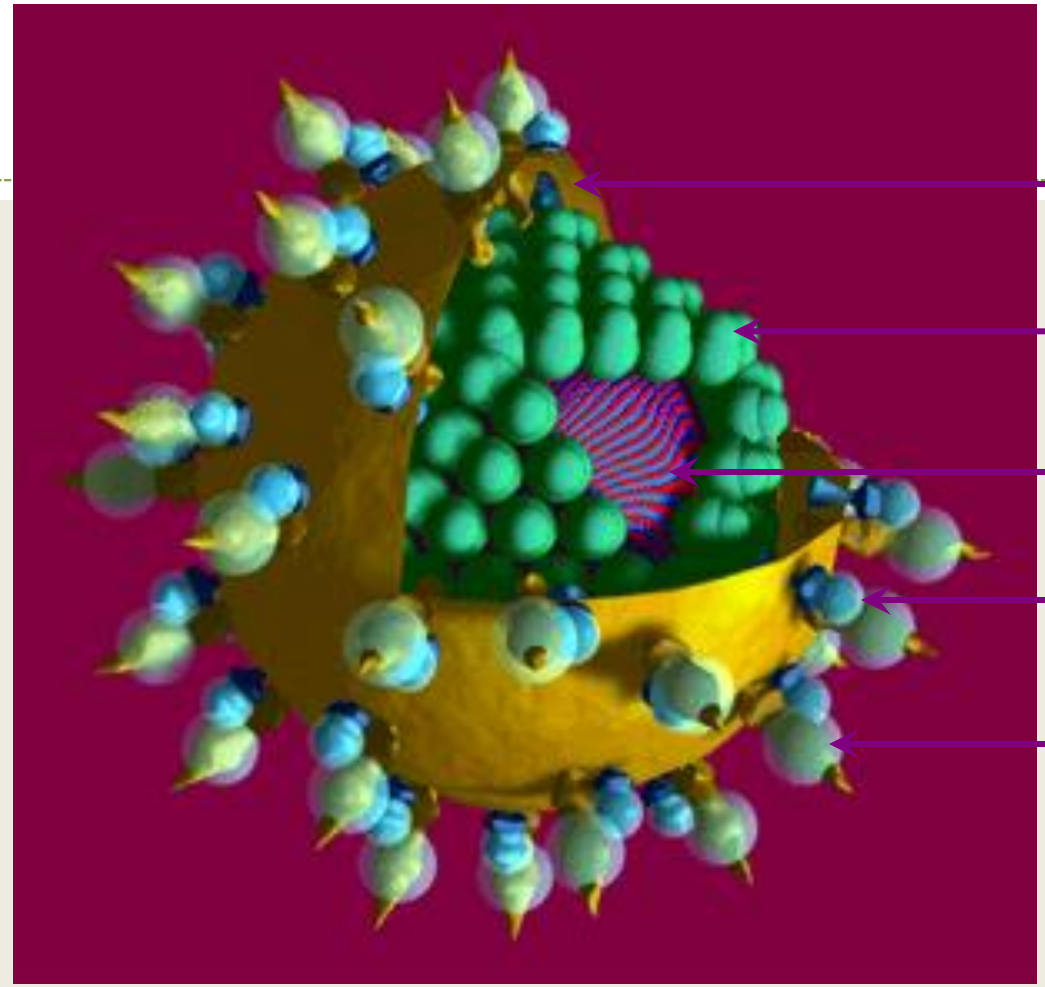

Lipo-viro-particle

Model of the Human Hepatitis C Virus



Published in The PRN Notebook, Volume 6, Number 1, March 2001 and The PRN Notebook Online at www.prn.org.
Three-dimensional model of HCV created by Louis E. Henderson, Ph.D., Frederick Cancer Research Center.

Composition of Hepatitis C Virus



Lipid Envelope

Capsid Protein

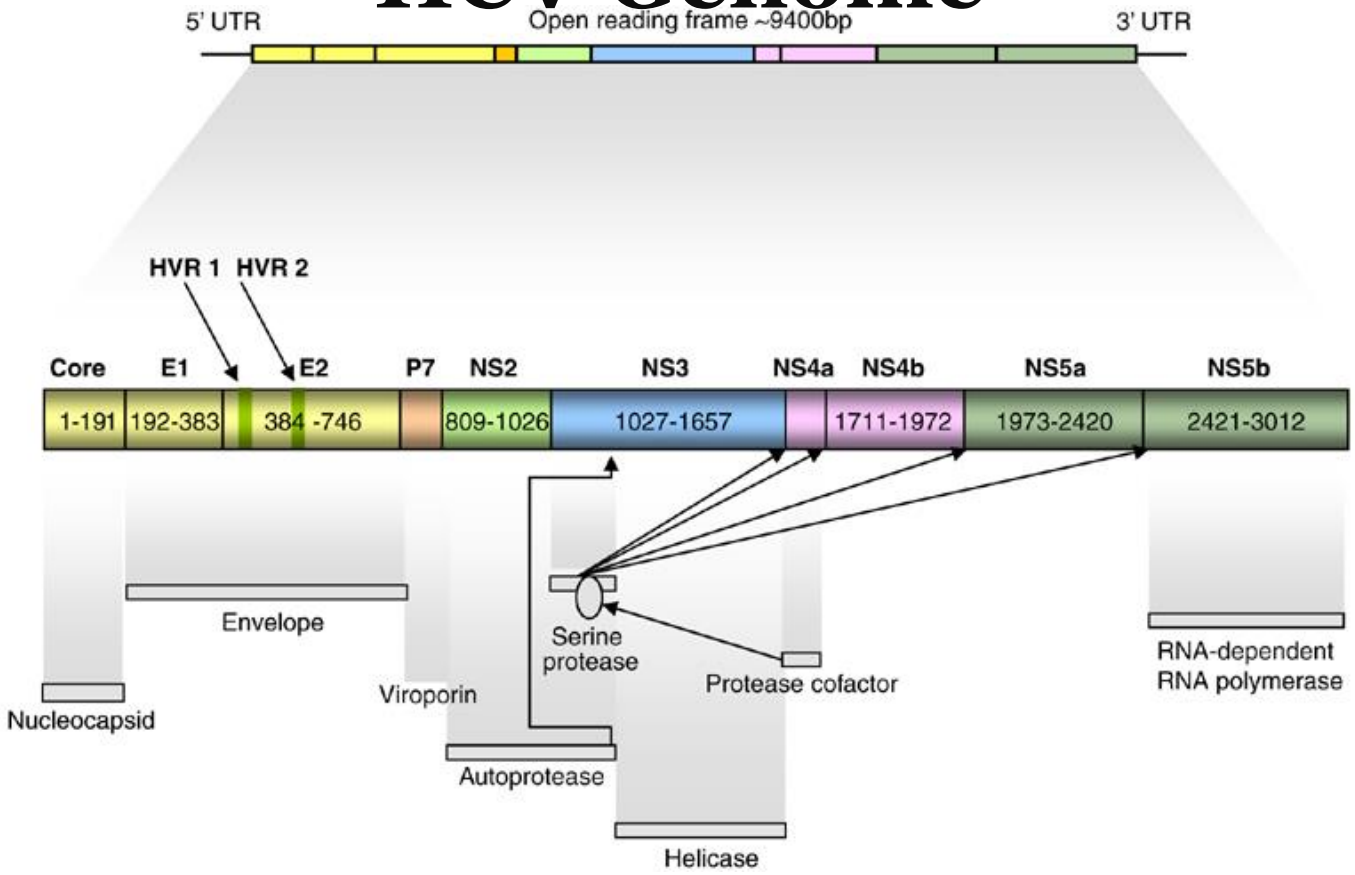
Nucleic Acid

Envelope Glycoprotein E2

Envelope Glycoprotein E1

Courtesy Michael Gale via Tenille Gale

HCV Genome



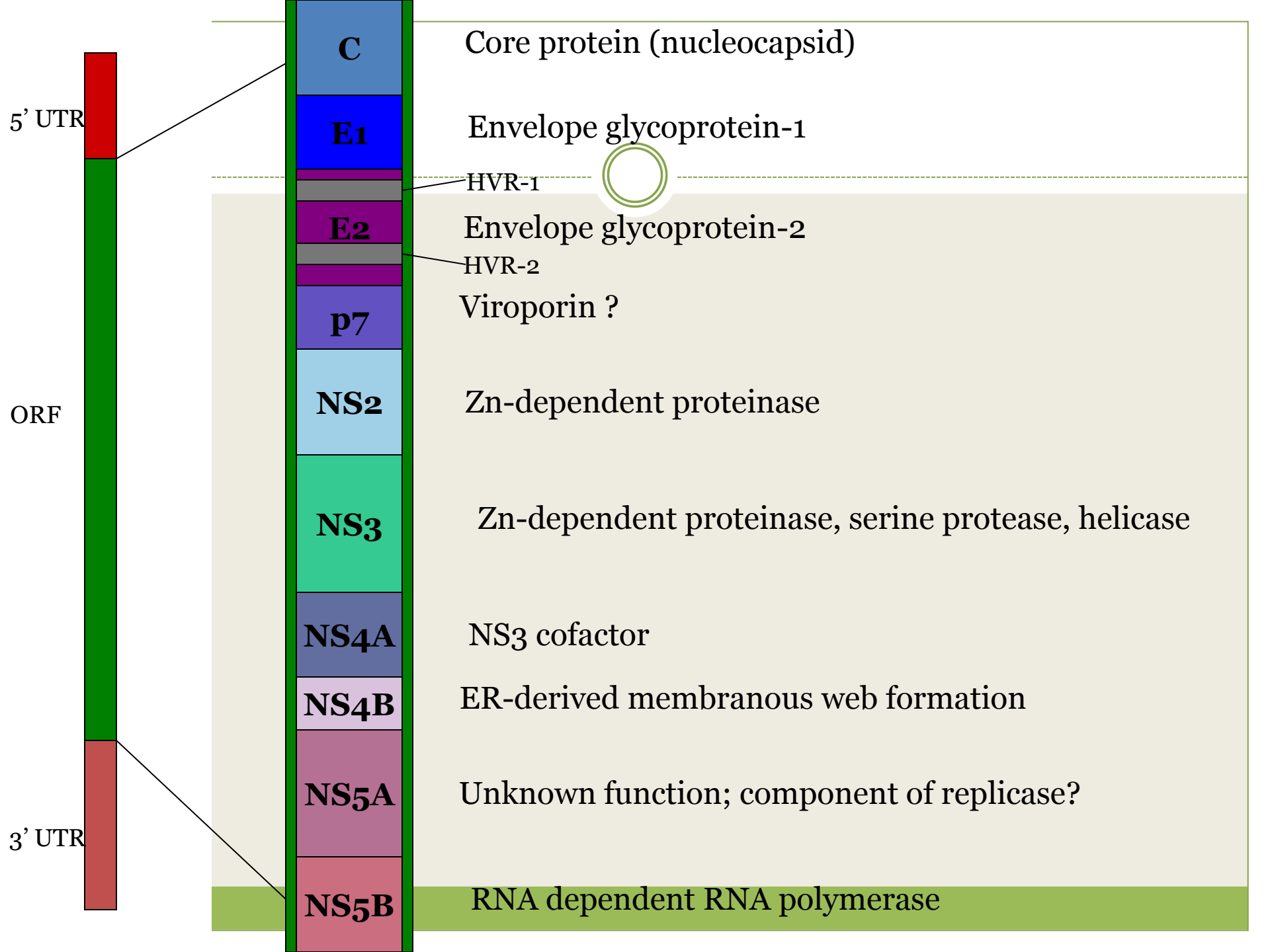
-HCV genome is about 9400 nucleotides long, it is ssRNA and positive sense

-the 1O viral proteins are first made as a large polyprotein

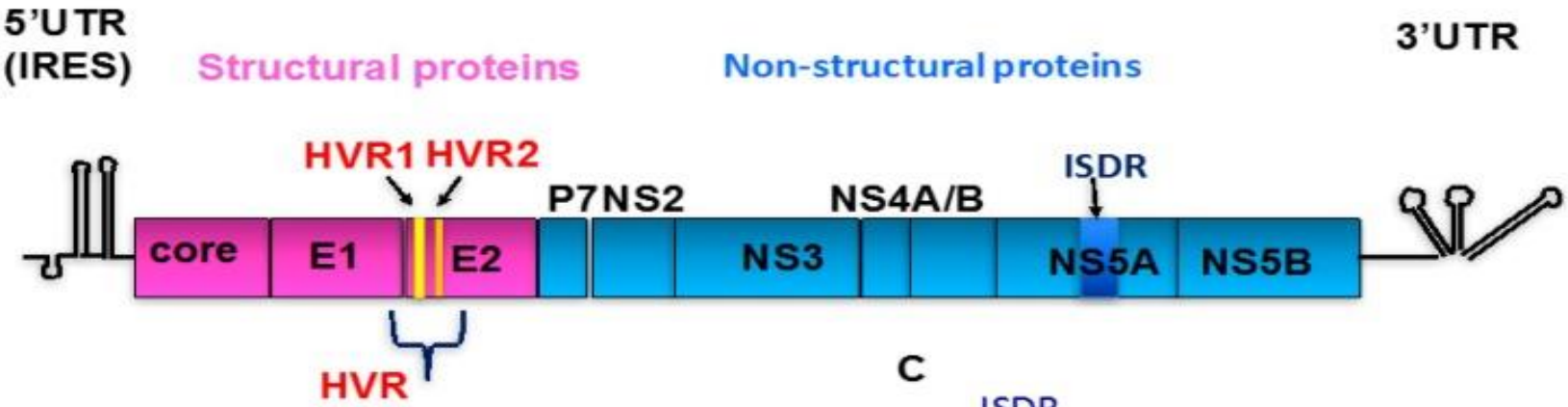
-individual proteins are released from polyprotein by cellular and viral proteases

-core, E1 and E2 are the structural proteins which form the virus particle

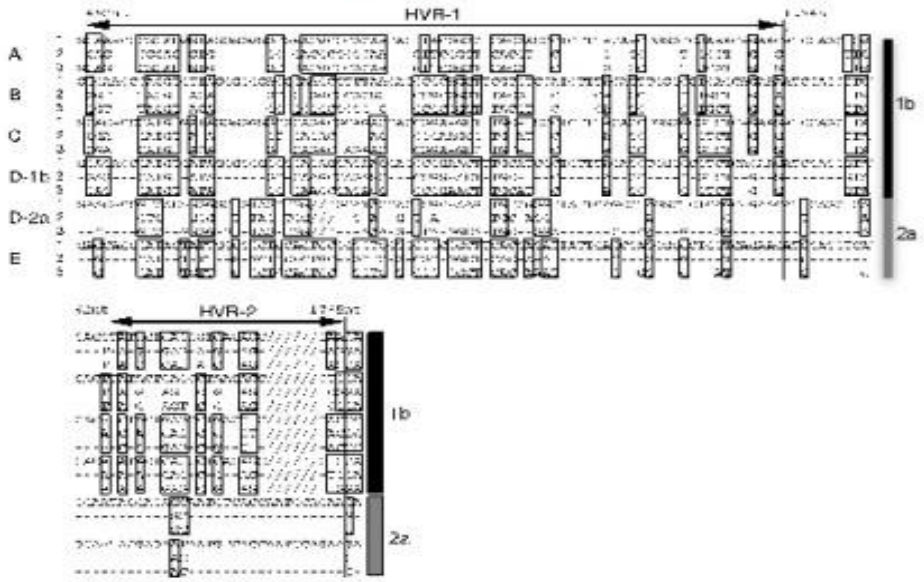
-remaining proteins are nonstructural and have roles in viral replication


5' UTR
ORF
3' UTR
C
E1
HVR-1
E2
HVR-2
p7
NS2
NS3
NS4A
NS4B
NS5A
NS5B
Core protein (nucleocapsid)
Envelope glycoprotein-1
Envelope glycoprotein-2
Viroporin ?
Zn-dependent proteinase
Zn-dependent proteinase, serine protease, helicase
NS3 cofactor
ER-derived membranous web formation
Unknown function; component of replicase?
RNA dependent RNA polymerase

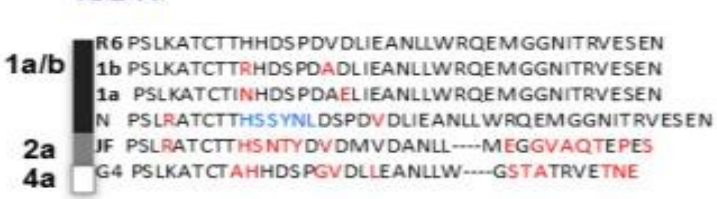
A

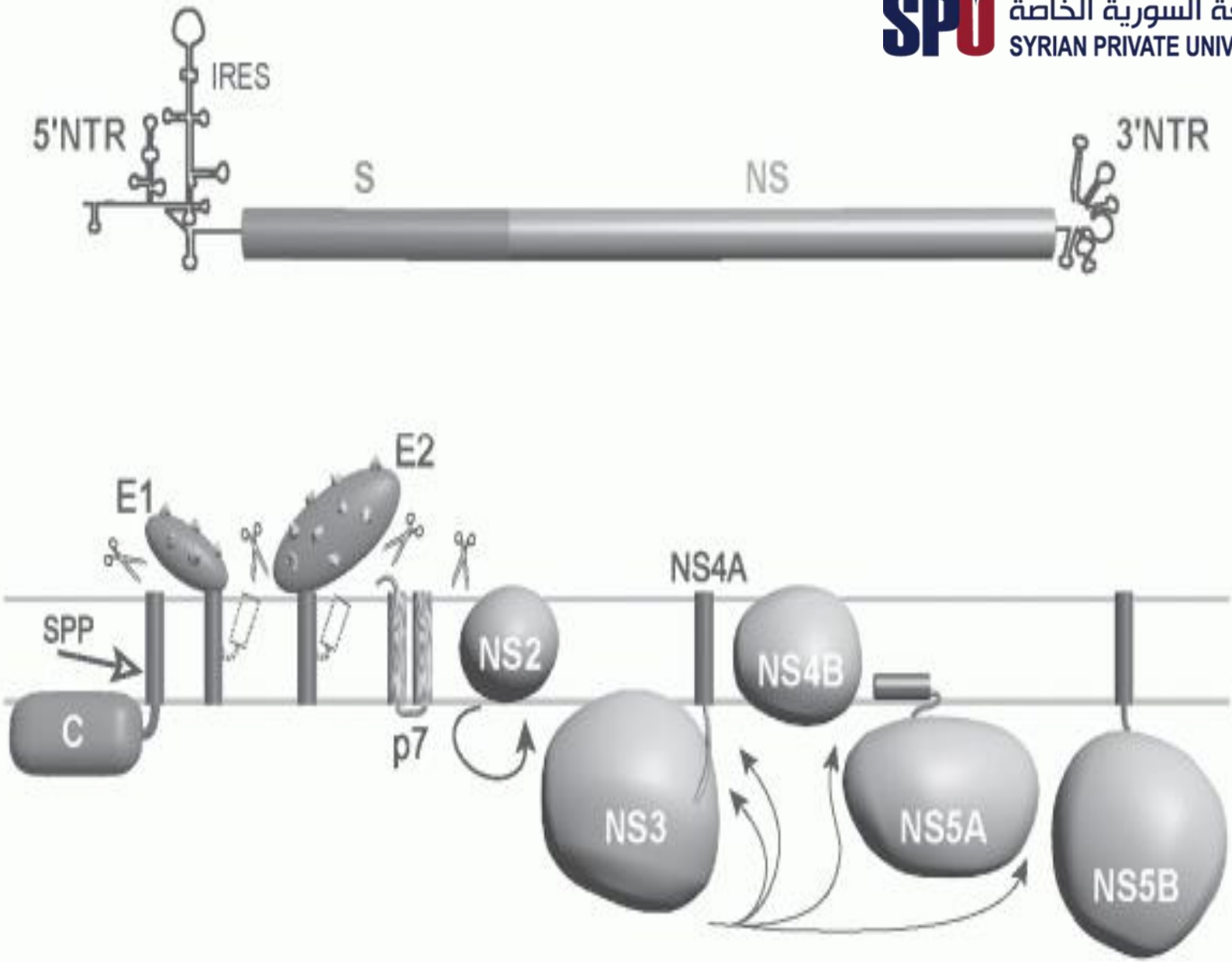

5'UTR
(IRES)
Structural proteins
Non-structural proteins
3'UTR
HVR1 HVR2
P7NS2
NS4A/B
ISDR
core
E1
E2
NS3
NS5A
NS5B
HVR

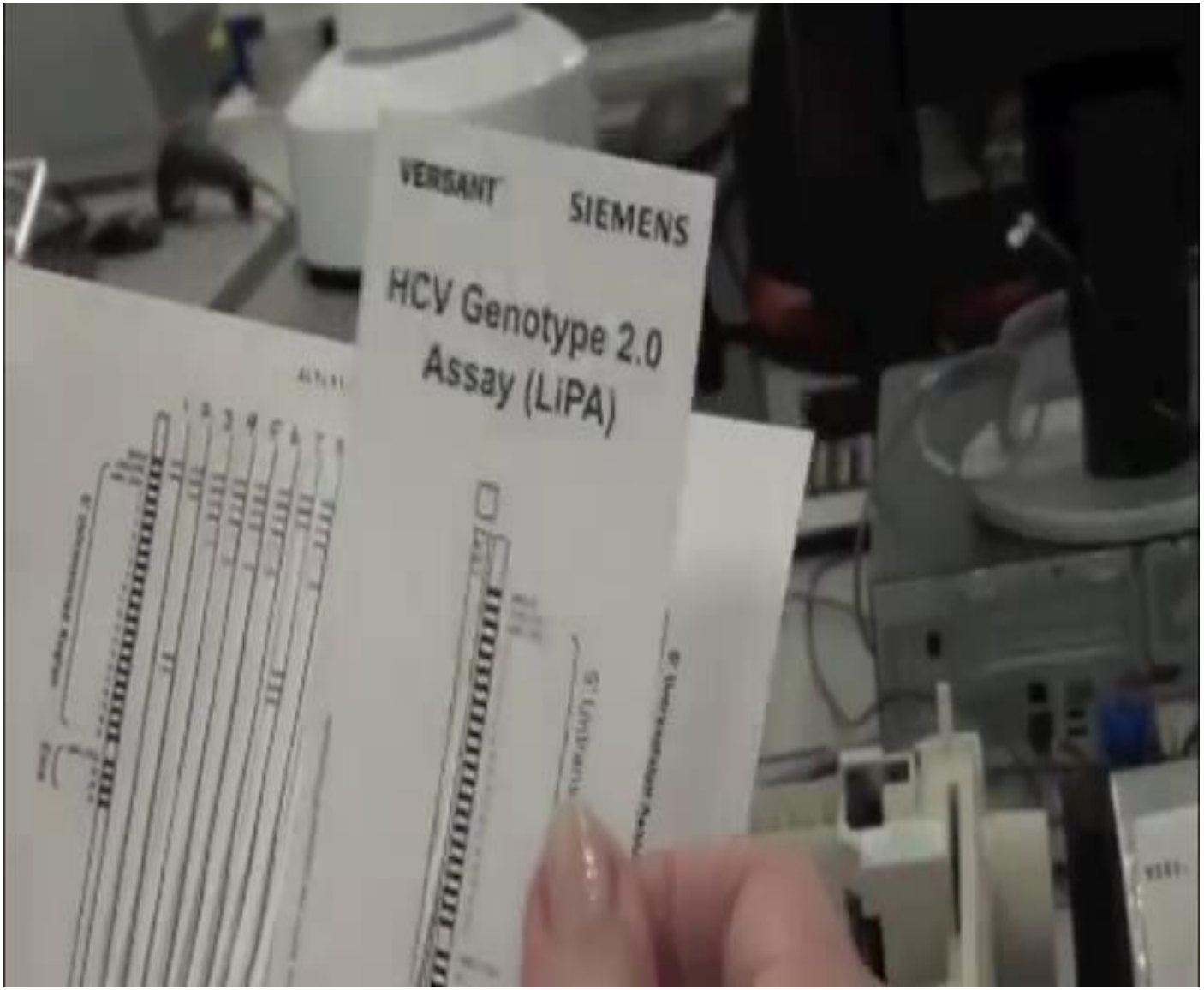
B

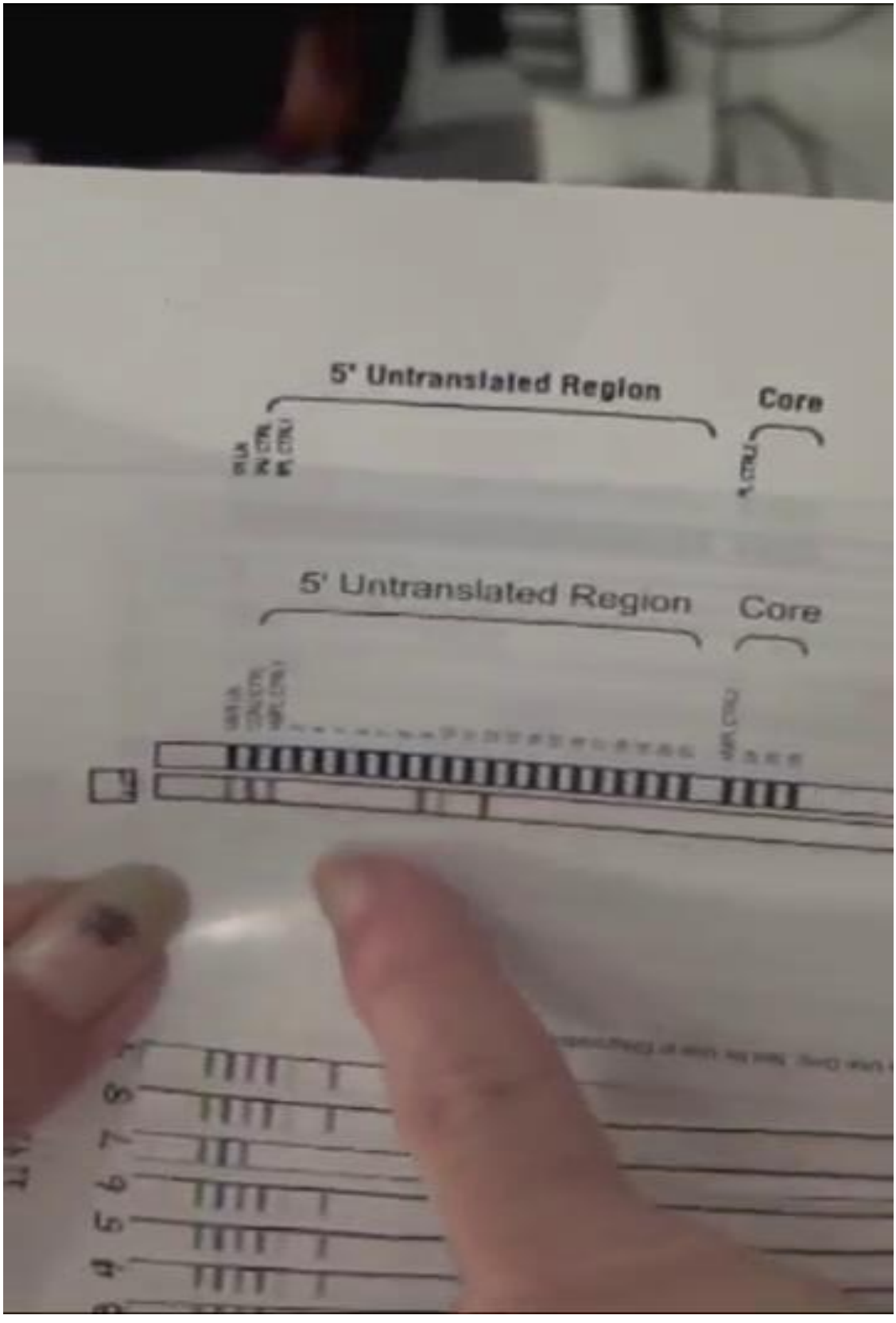

HVR-1
A
B
C
D-1b
D-2a
E
1b
2a
HVR-2
1b
2a

C


ISDR
1a/b
2a
4a
R6 PSLKATCTTHHDSPDVDLIEANLLWRQEMGGNITRVESEN
1b PSLKATCTTRHDSPDADLIEANLLWRQEMGGNITRVESEN
1a PSLKATCTINHDSPDAELIEANLLWRQEMGGNITRVESEN
N PSLRATCTTHSSYNLDSPDVDLIEANLLWRQEMGGNITRVESEN
JF PSLRATCTTHSNTYDVDMVDANLL----MEGGVAQTEPES
G4 PSLKATCTAHHDSPGVDLLEANLLW----GSTATRVETNE

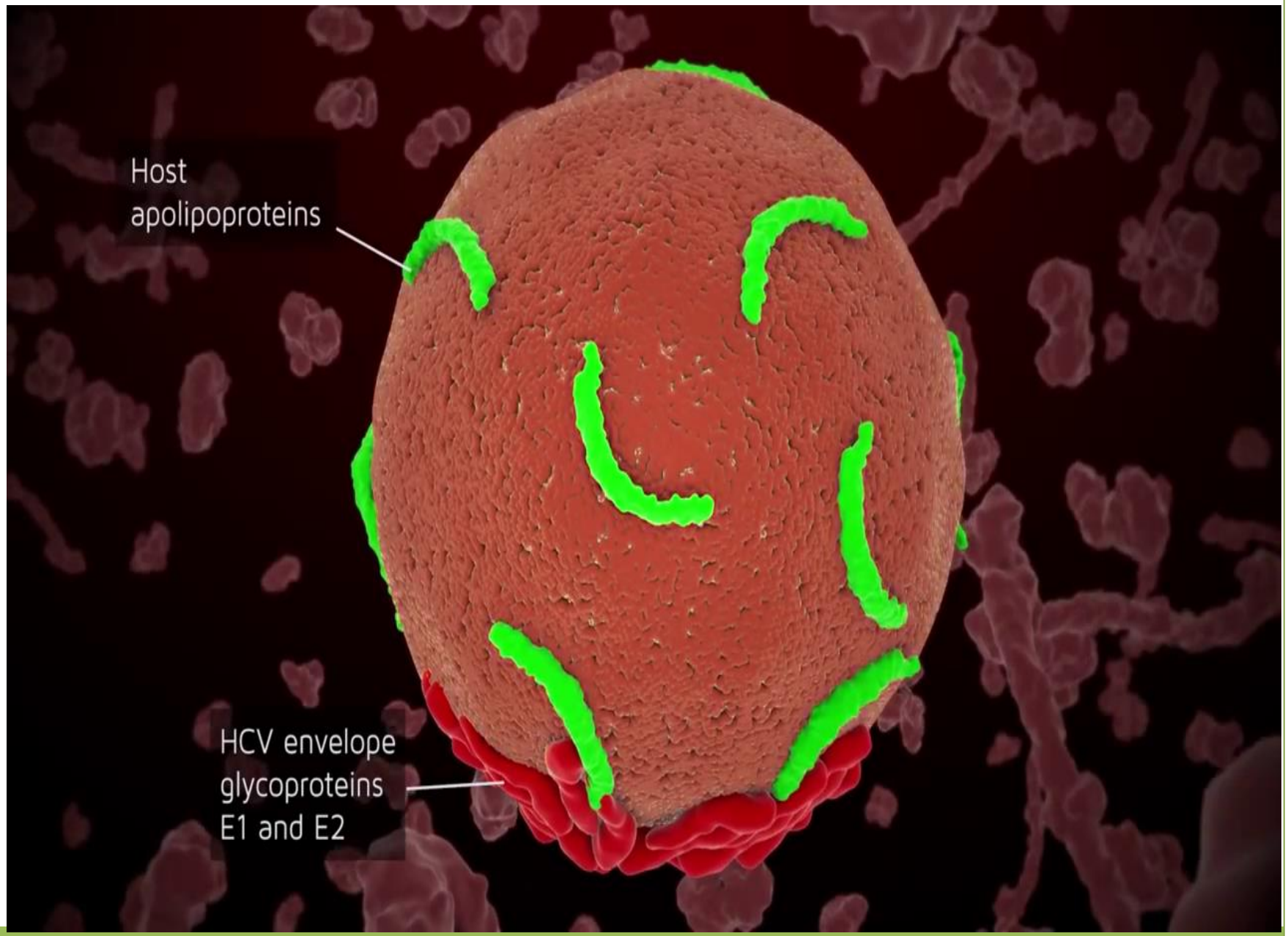

IRES
5'NTR
S
NS
3'NTR
E2
E1
NS4A
SPP
NS2
NS4B
C
p7
NS3
NS5A
NS5B

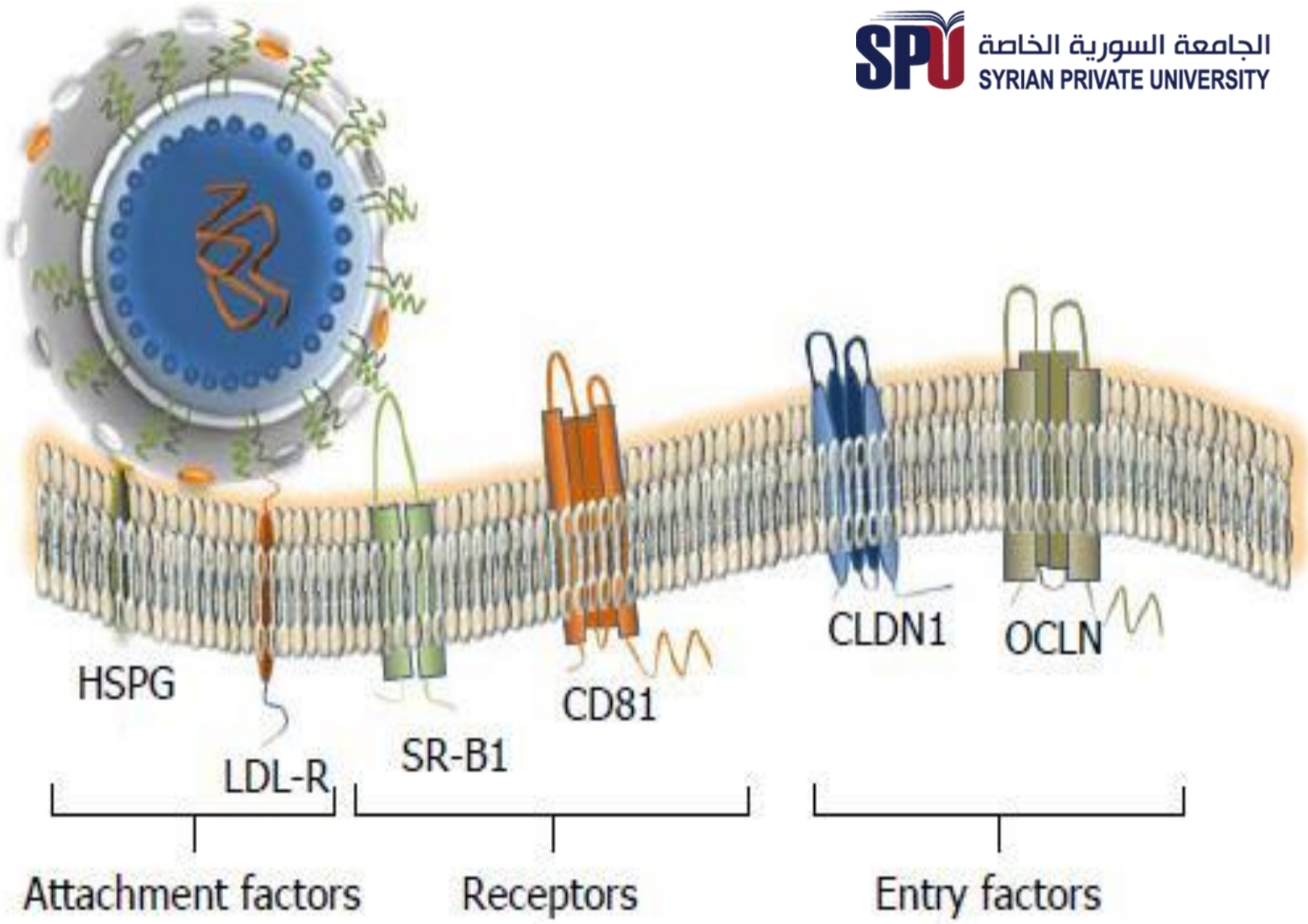

VERSANT
SIEMENS
HCV Genotype 2.0
Assay (LiPA)


5' Untranslated Region
Core
5' Untranslated Region
Core

Protein F

- Newly discovered protein F
- Produced by ribosomal frameshift mutation around codon 11 of Core protein
- Infected individuals contain antibodies
- Function unknown


Host
apolipoproteins
HCV envelope
glycoproteins
E1 and E2


HSPG
LDL-R
SR-B1
CD81
CLDN1
OCLN
Attachment factors
Receptors
Entry factors

HCV receptors/entry factors

GAGs

Linear polysaccharides on proteins of all human cell surfaces

Provides a way for the virus to stick to cells

binding factors

CD81

Tetraspanin superfamily member

Expressed in all nucleated cells

Part of B-cell receptor complex

Expression confers susceptibility

SR-BI

Scavenger receptor class B member I

HDL receptor (multiple other ligands)

Expressed in **hepatocytes**, adrenal cortex, gonads (less elsewhere)

Ligands influence infection

Claudin-1

Form the backbone of tight junction strands in epithelial tissues

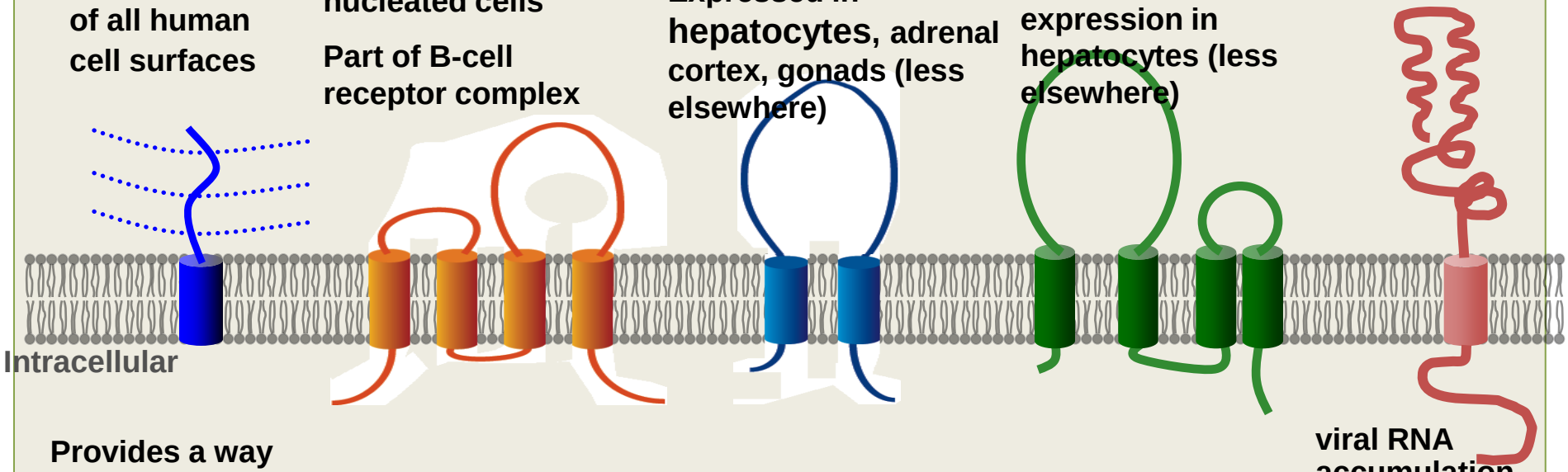
Highest expression in hepatocytes (less elsewhere)

Expression confers susceptibility

LDL-R

Low density lipoprotein receptor

viral RNA accumulation increased or decreased in parallel with LDLR mRNA expression and LDL entry



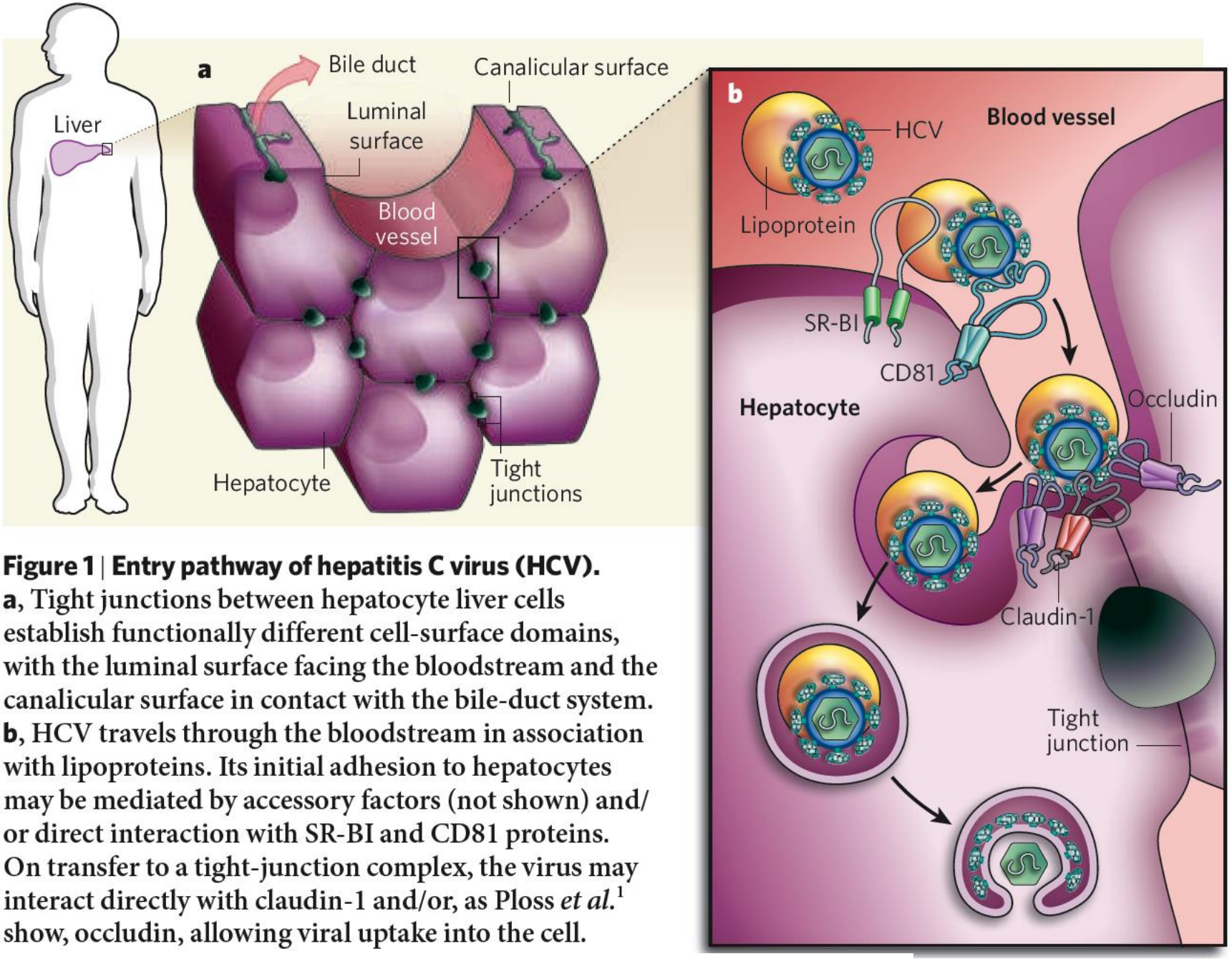
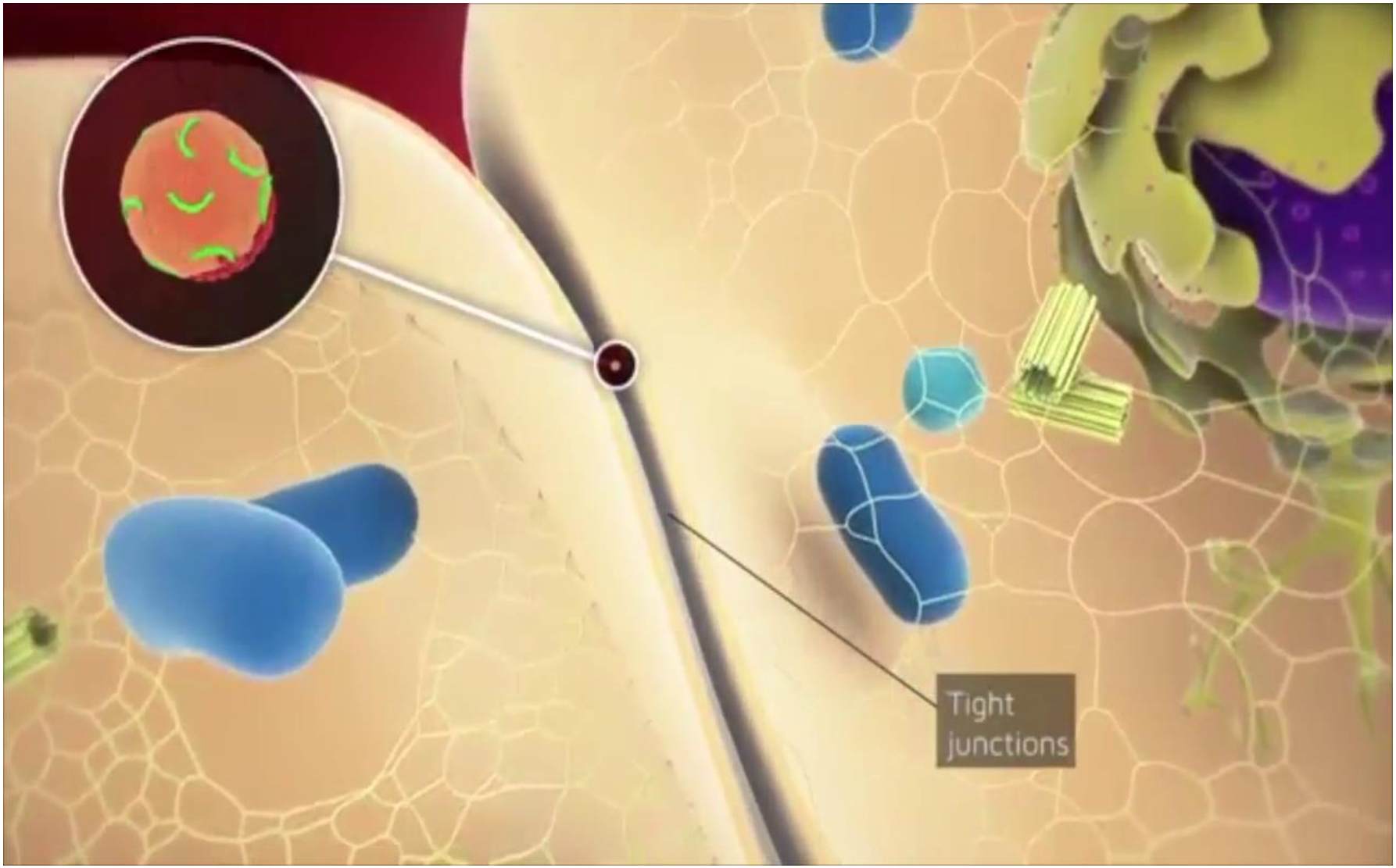
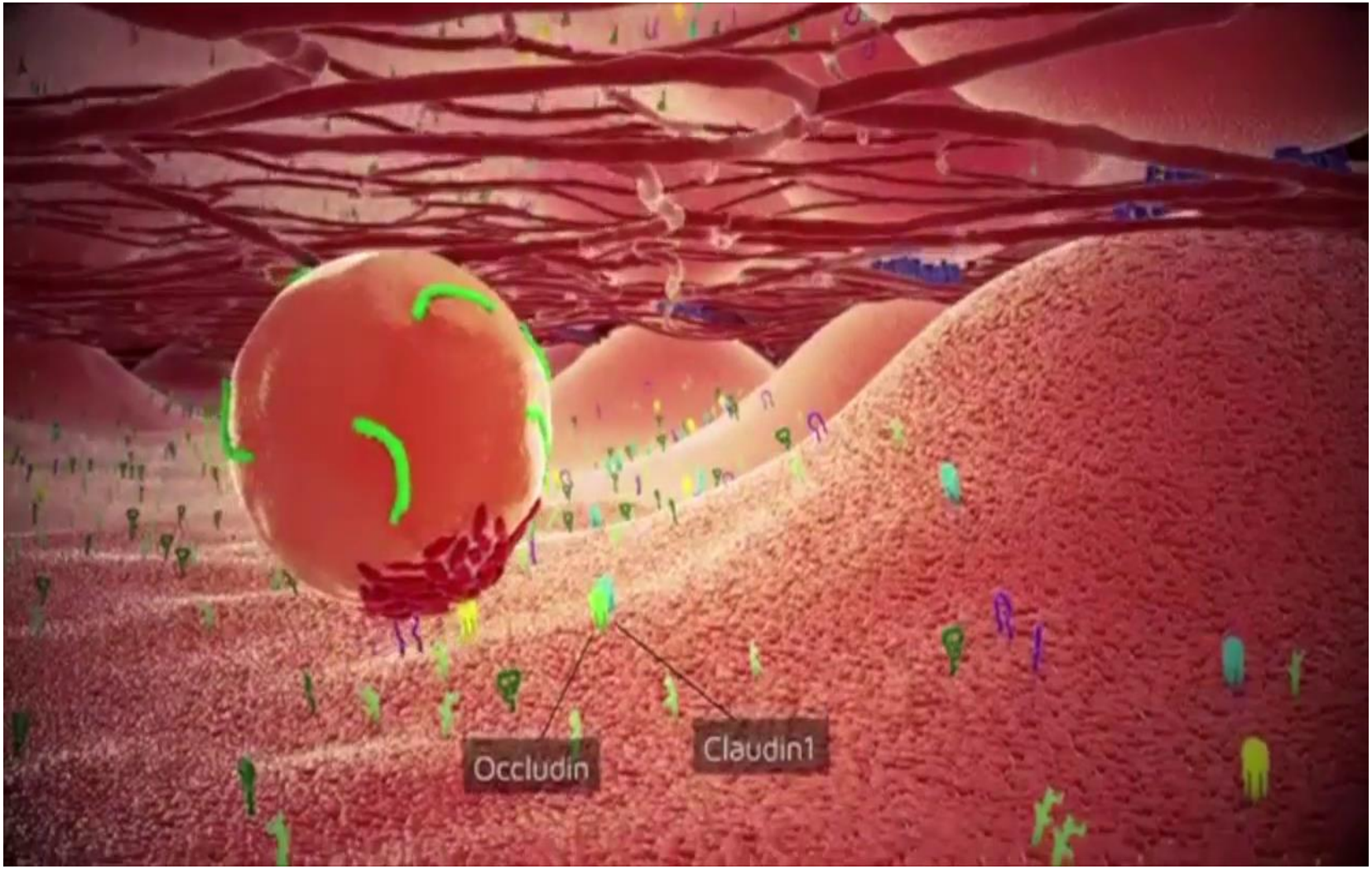
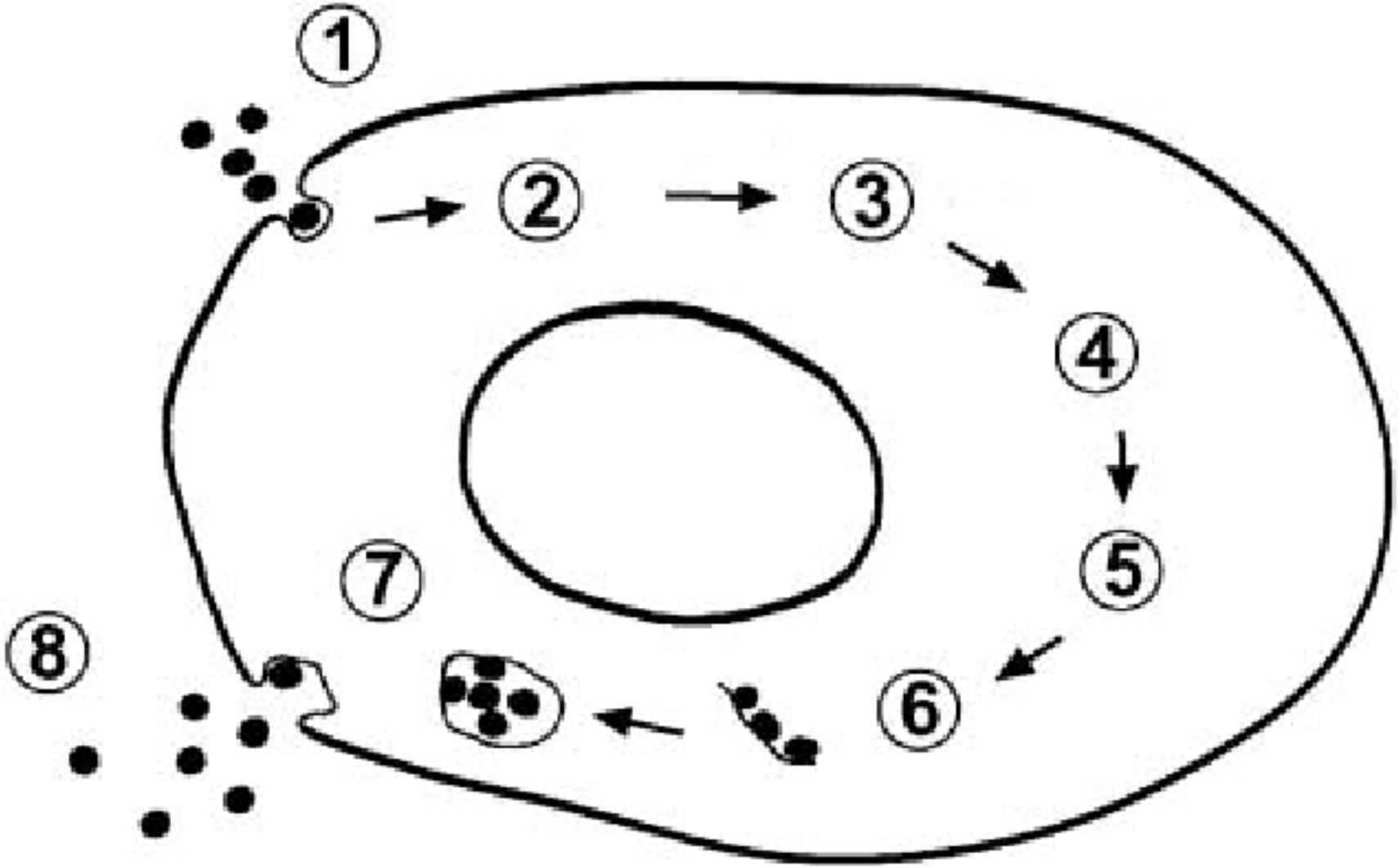
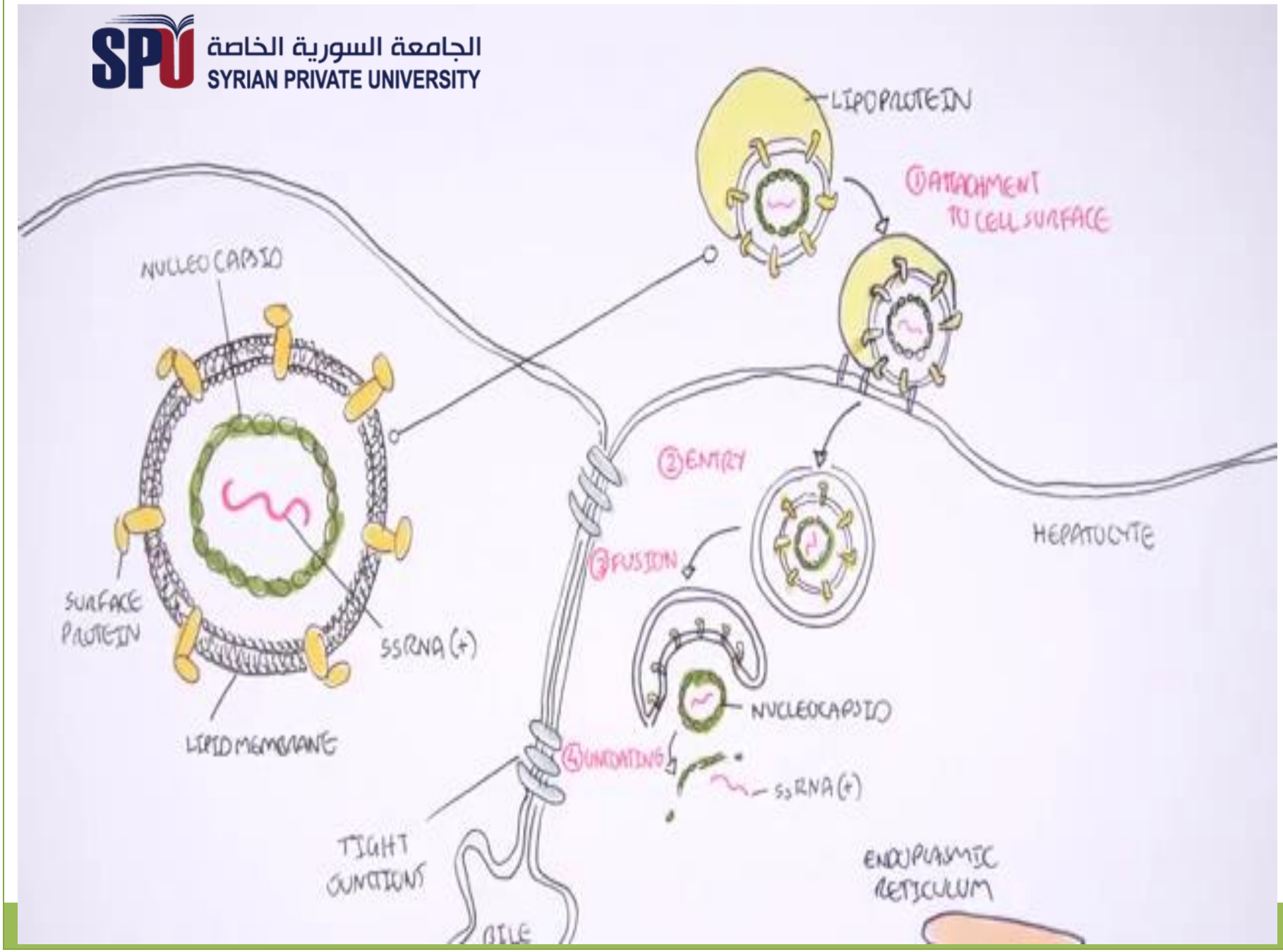


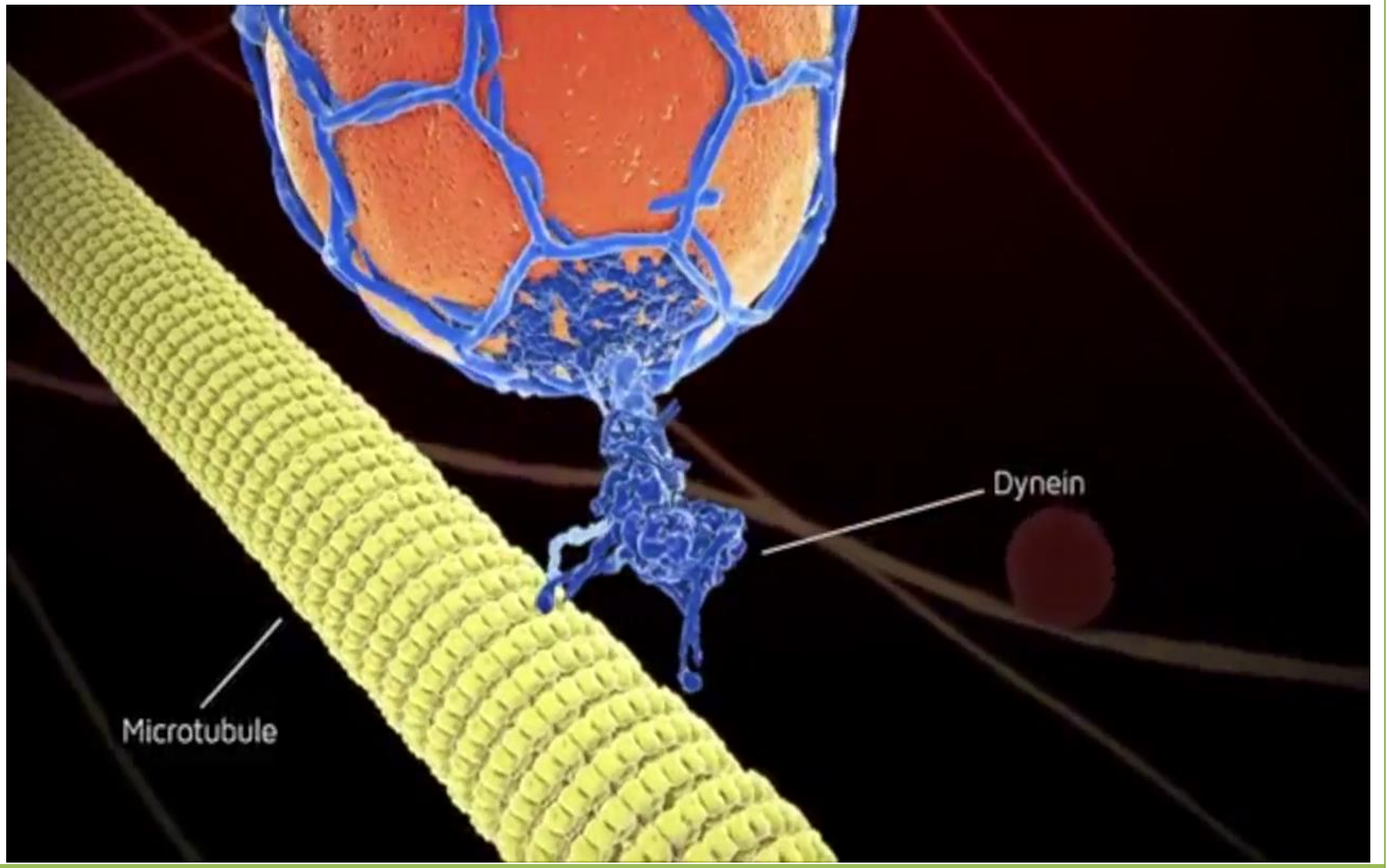
Figure 1 | Entry pathway of hepatitis C virus (HCV). **a**, Tight junctions between hepatocyte liver cells establish functionally different cell-surface domains, with the luminal surface facing the bloodstream and the canalicular surface in contact with the bile-duct system. **b**, HCV travels through the bloodstream in association with lipoproteins. Its initial adhesion to hepatocytes may be mediated by accessory factors (not shown) and/or direct interaction with SR-BI and CD81 proteins. On transfer to a tight-junction complex, the virus may interact directly with claudin-1 and/or, as Ploss *et al.*[1] show, occludin, allowing viral uptake into the cell.

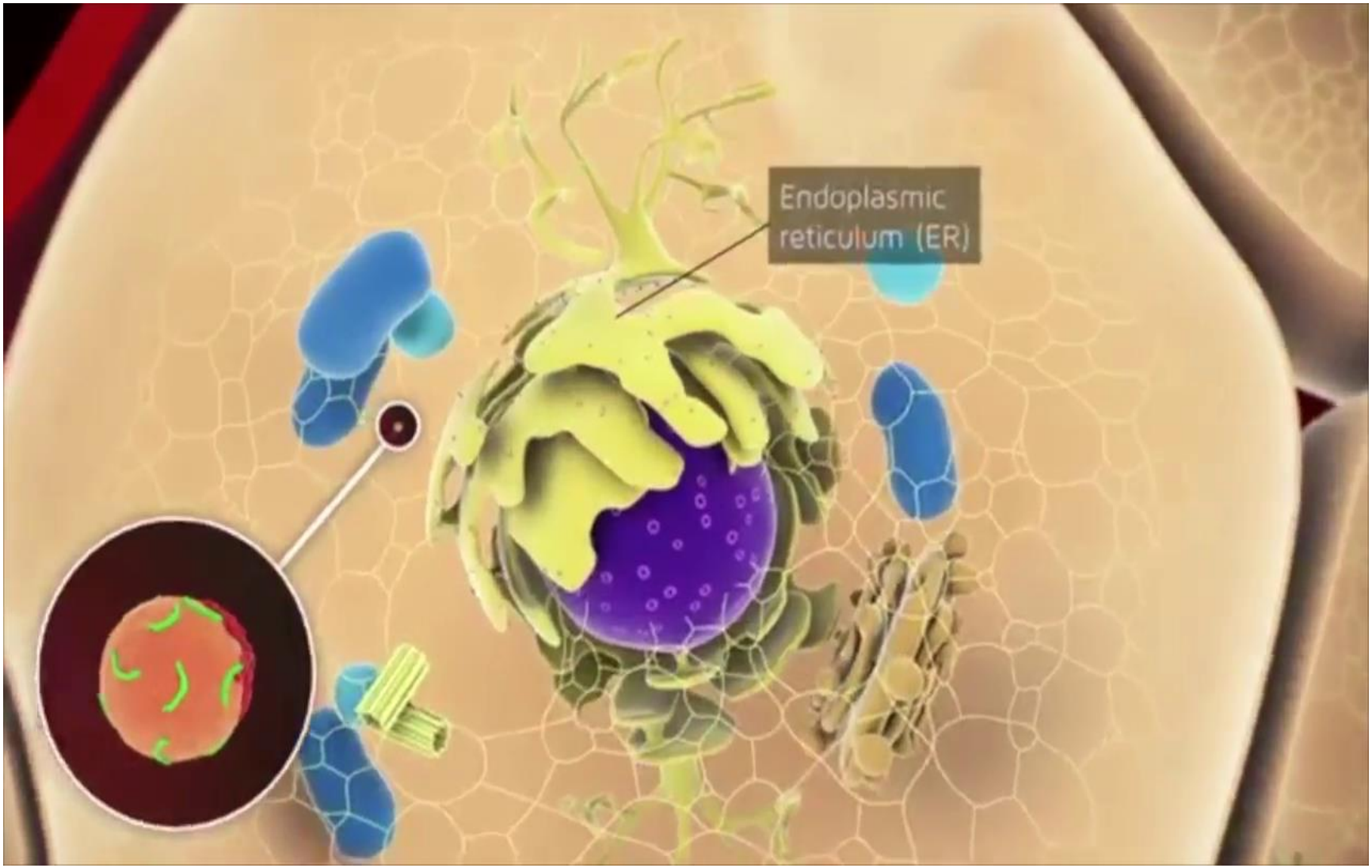

Tight
junctions

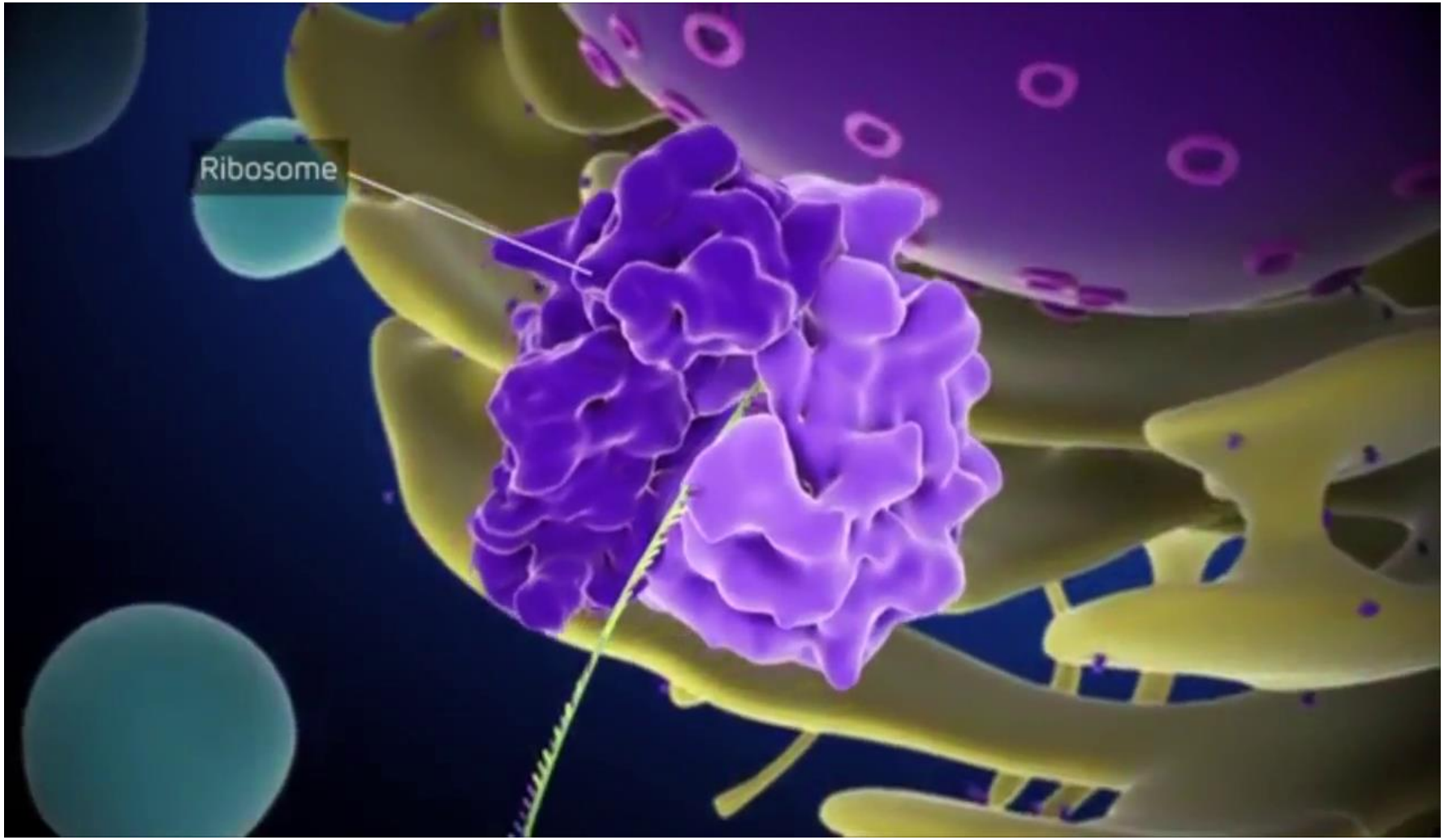

Occludin
Claudin1

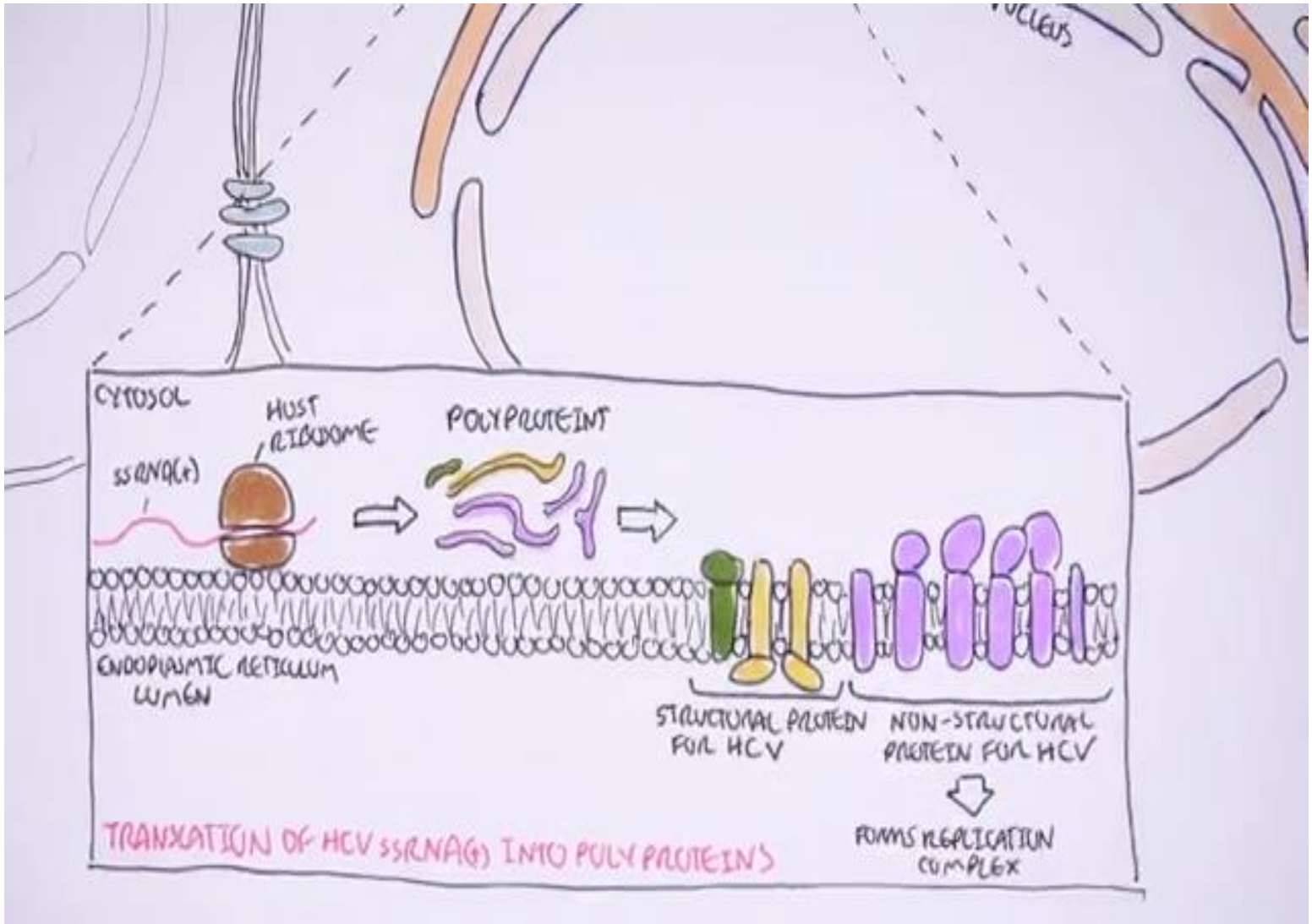

1
2
3
4
5
6
7
8

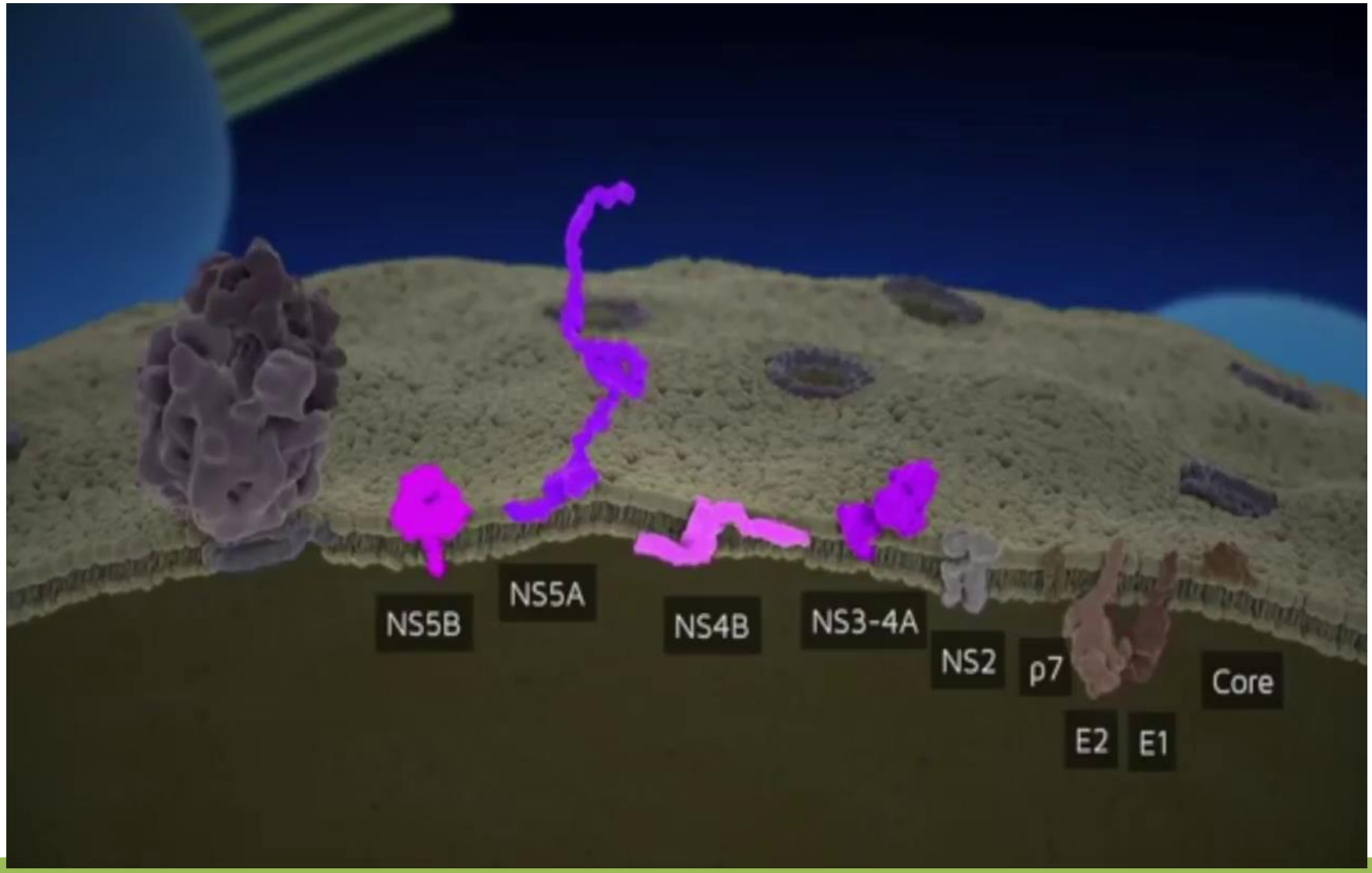

LIPOPROTEIN
① ATTACHMENT TO CELL SURFACE
NUCLEOCAPSID
② ENTRY
③ FUSION
HEPATOCYTE
SURFACE PROTEIN
ssRNA (+)
LIPID MEMBRANE
NUCLEOCAPSID
④ UNCOATING
ssRNA (+)
TIGHT JUNCTIONS
ENDOPLASMIC RETICULUM
BILE

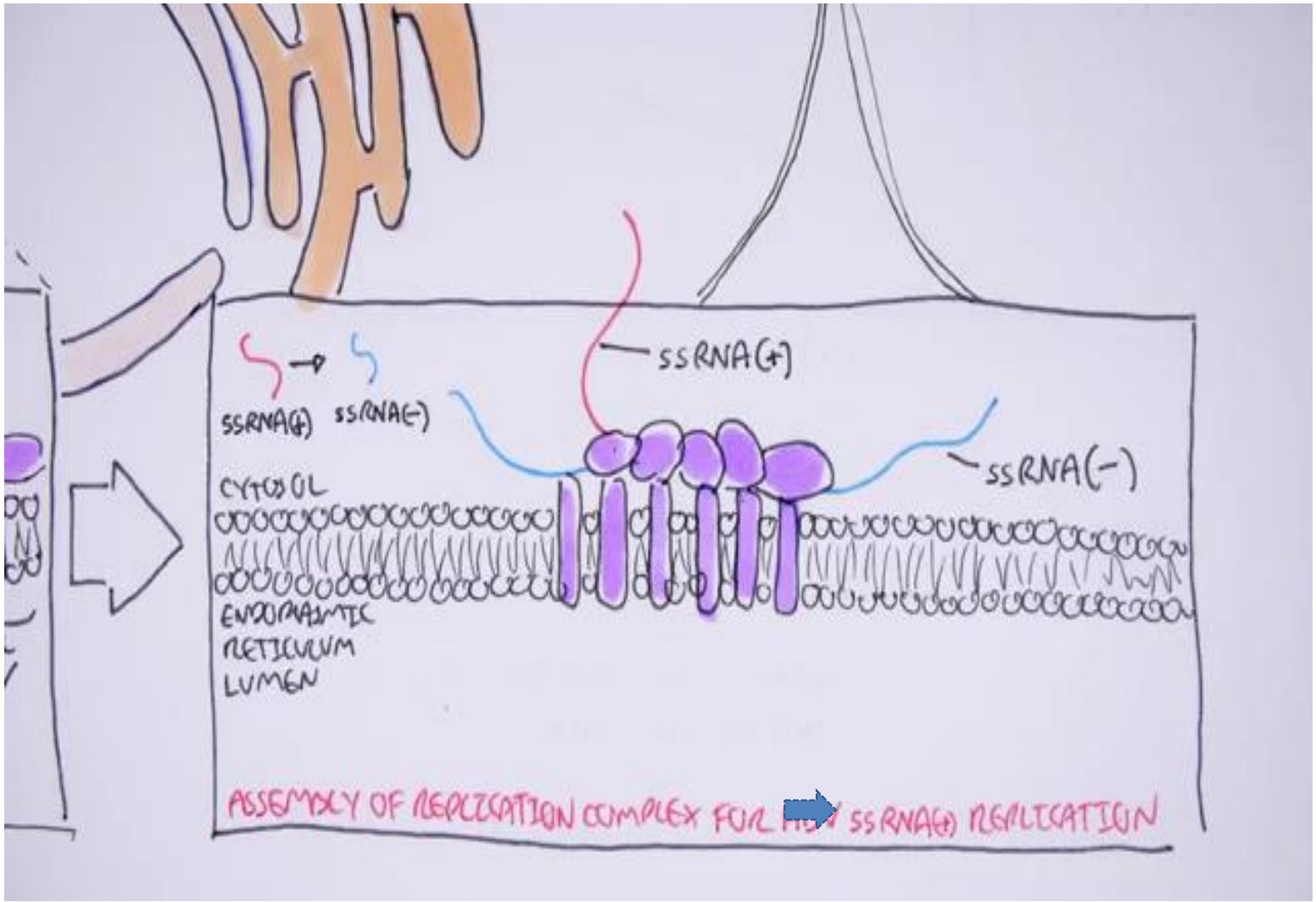

Dynein
Microtubule

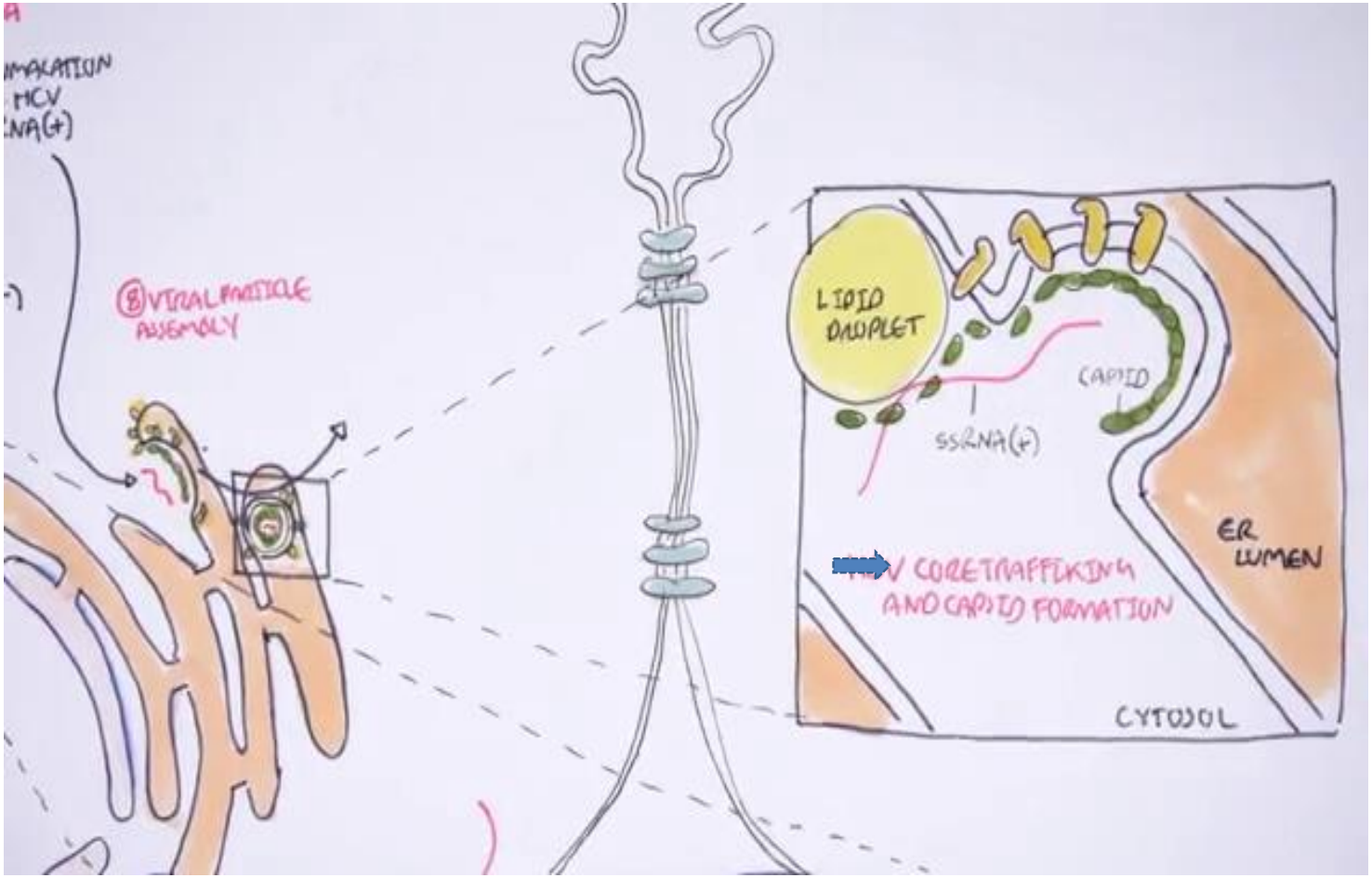

Endoplasmic
reticulum (ER)

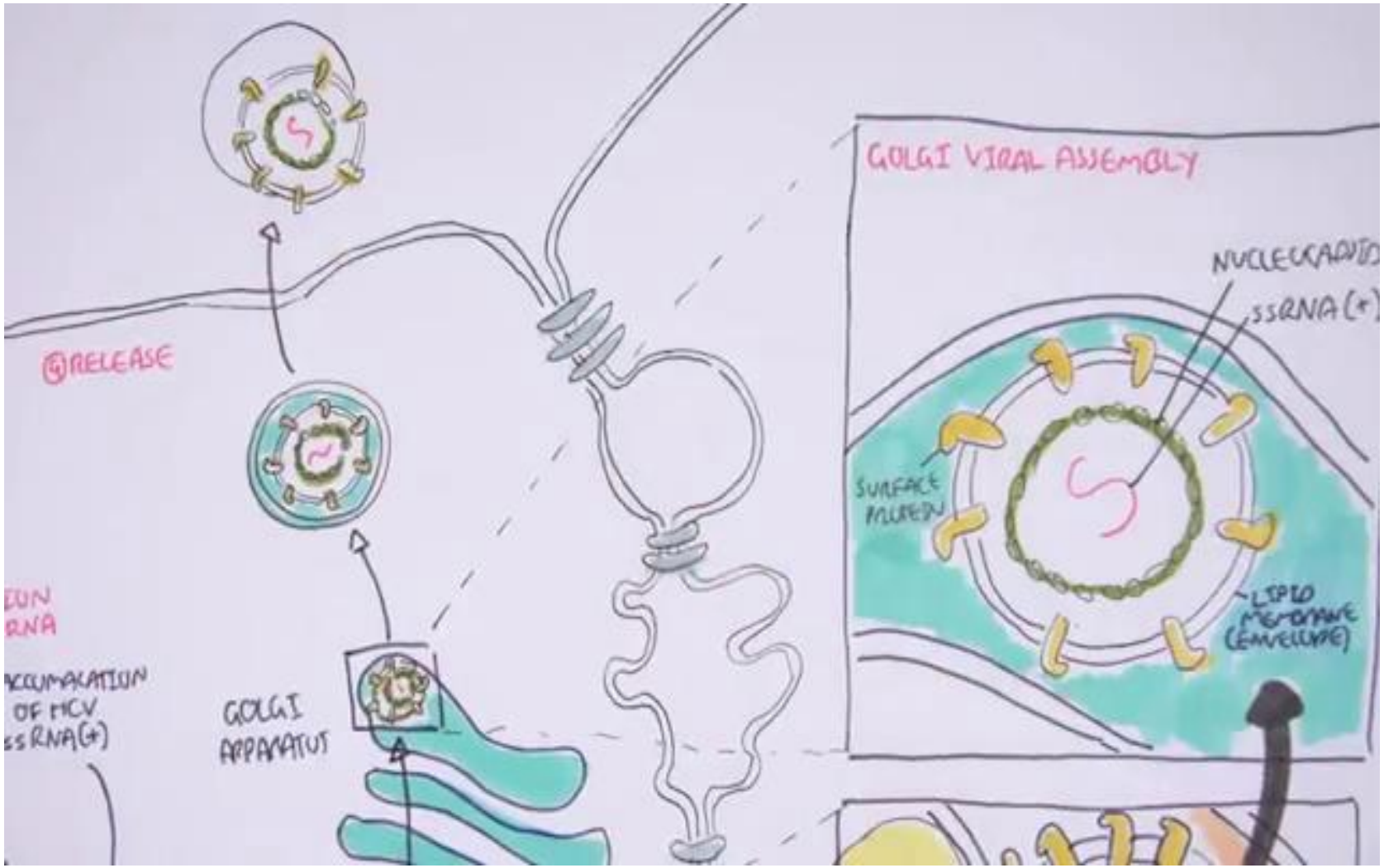

Ribosome

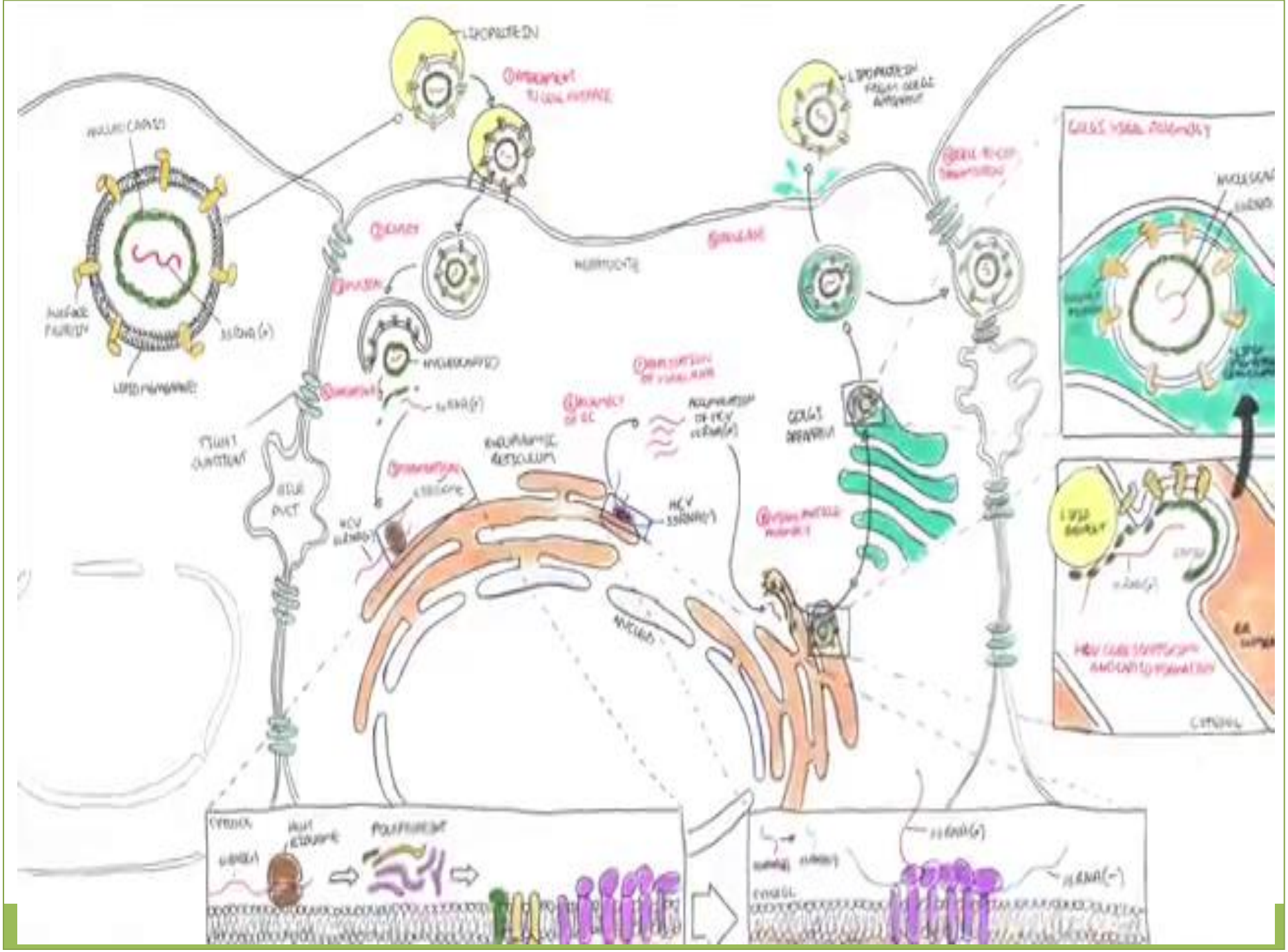

CYTOSOL
HOST RIBOSOME
POLYPROTEINT
ssRNA(+)
ENDOPLASMIC RETICULUM LUMEN
STRUCTURAL PROTEIN FOR HCV
NON-STRUCTURAL PROTEIN FOR HCV
FORMS REPLICATION COMPLEX
TRANSLATION OF HCV ssRNA(+) INTO POLY PROTEINS


NS5B
NS5A
NS4B
NS3-4A
NS2
p7
Core
E2
E1

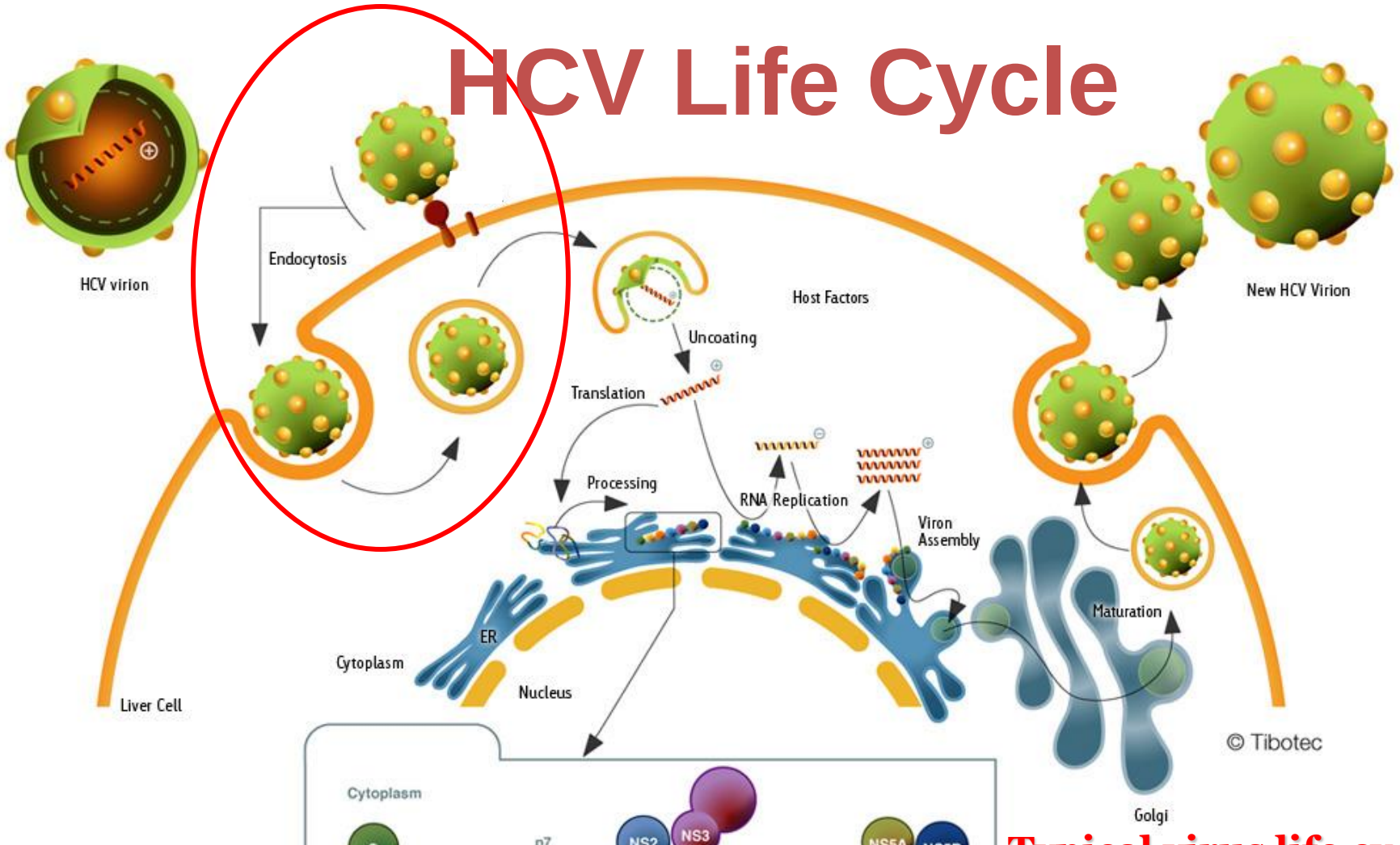

SSRNA(+)
SSRNA(+)
SSRNA(-)
SSRNA(+)
SSRNA(-)
CYTOSOL
ENDOPLASMIC
RETICULUM
LUMEN
ASSEMBLY OF REPLICATION COMPLEX FOR
SSRNA(+) REPLICATION


LIPID DROPLET
CAPSID
ssRNA(+)
ER LUMEN
CYTOSOL


GOLGI VIRAL ASSEMBLY
NUCLEOCAPSID
ssRNA (+)
SURFACE PROTEIN
LIPID MEMBRANE (ENVELOPE)
RELEASE
GOLGI APPARATUS

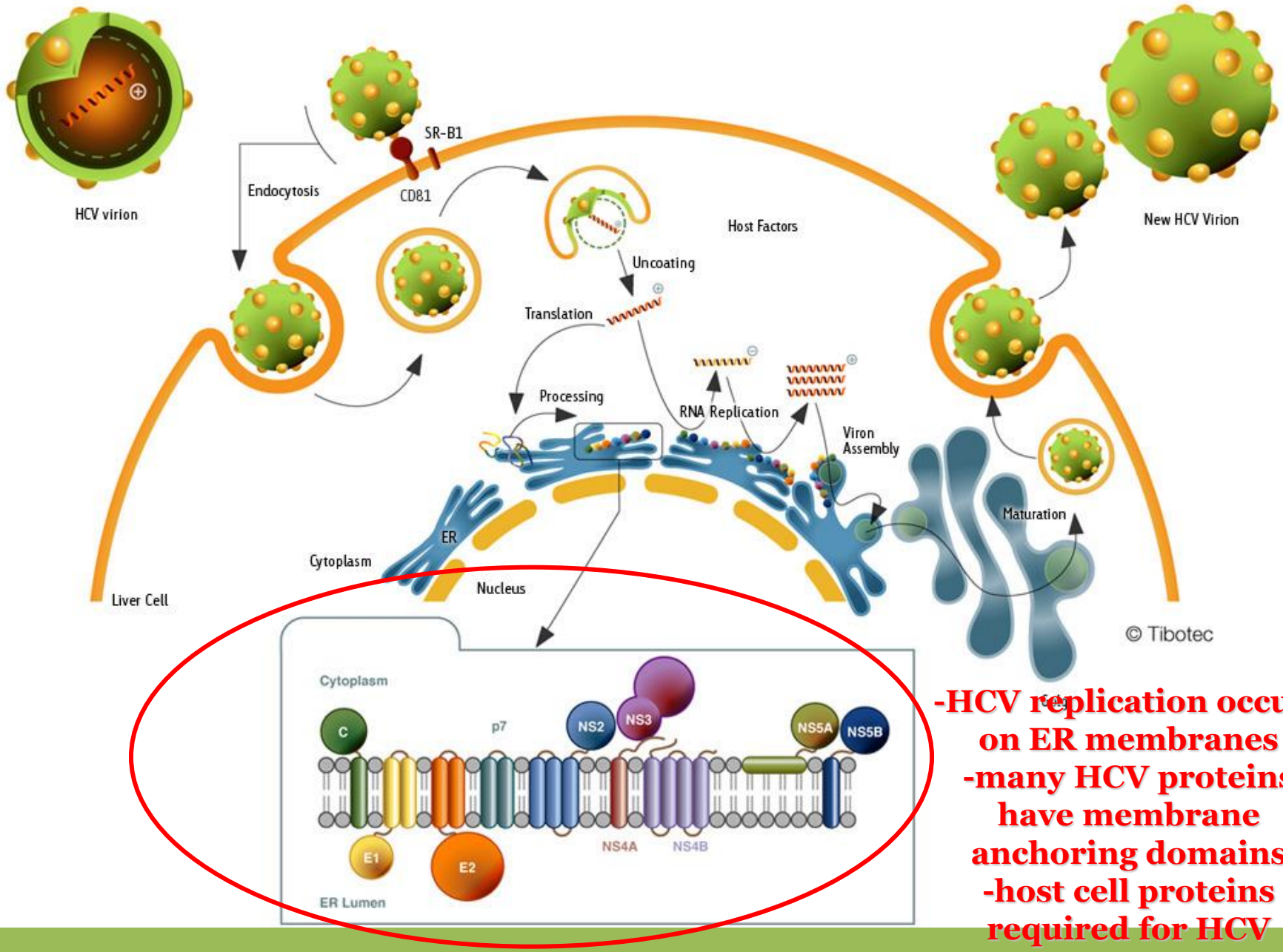


HCV Life Cycle

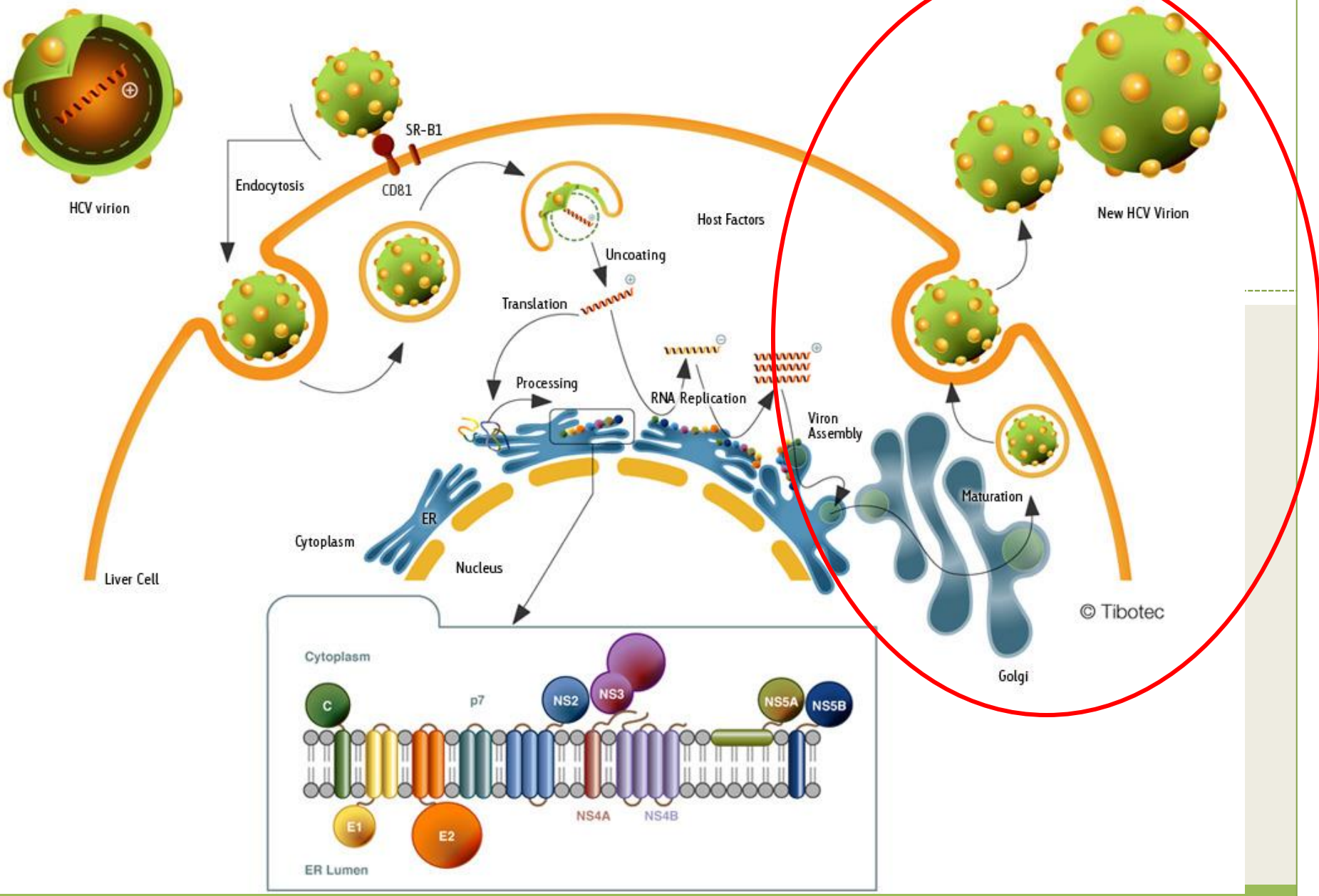


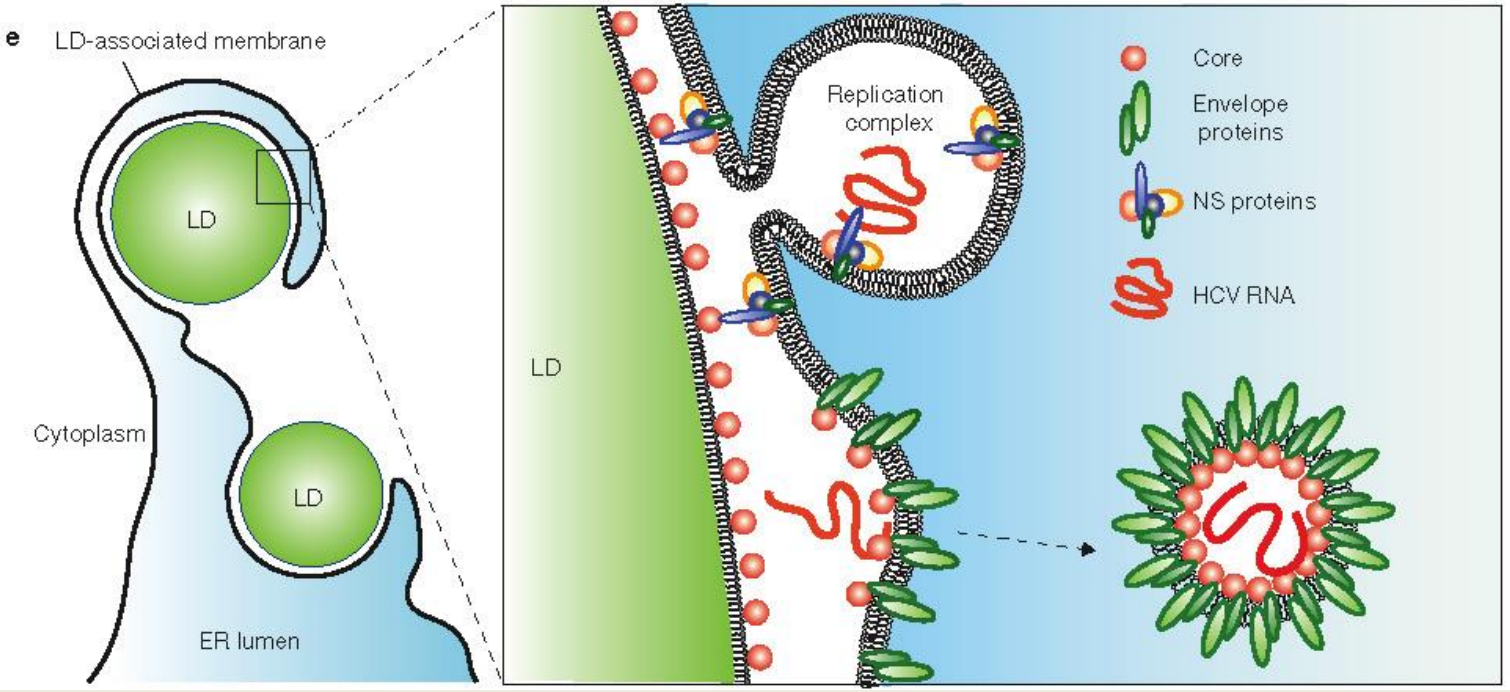
Typical virus life cycle:

Binding, internalization, uncoating, protein expression/replication, assembly, release



-HCV replication occurs on ER membranes
-many HCV proteins have membrane anchoring domains
-host cell proteins required for HCV replication


HCV virion
SR-B1
CD81
Endocytosis
Host Factors
Uncoating
Translation
Processing
RNA Replication
Viron Assembly
Maturation
New HCV Virion
Golgi
ER
Cytoplasm
Nucleus
Liver Cell
© Tibotec
Cytoplasm
C
p7
NS2
NS3
NS5A
NS5B
NS4A
NS4B
E1
E2
ER Lumen



-HCV core protein drives assembly at the lipid droplet

-LD is bound by core, then NS5A and other NS proteins

HCV Genotypes and Quasispecies

Term	Definition	Nucleotide Similarity
Genotype	Heterogeneity among different viruses	66% – 69%
Subtype	Closely related viruses within each genotype	77% – 80%
Quasispecies	Complex of genetic variants within individual viruses	91% – 99%

Innate Immune Response

- Regardless of infection outcome
- Viral resistance
- Targeting by HCV proteins?
  - **NS5A and E2 (PKR)**

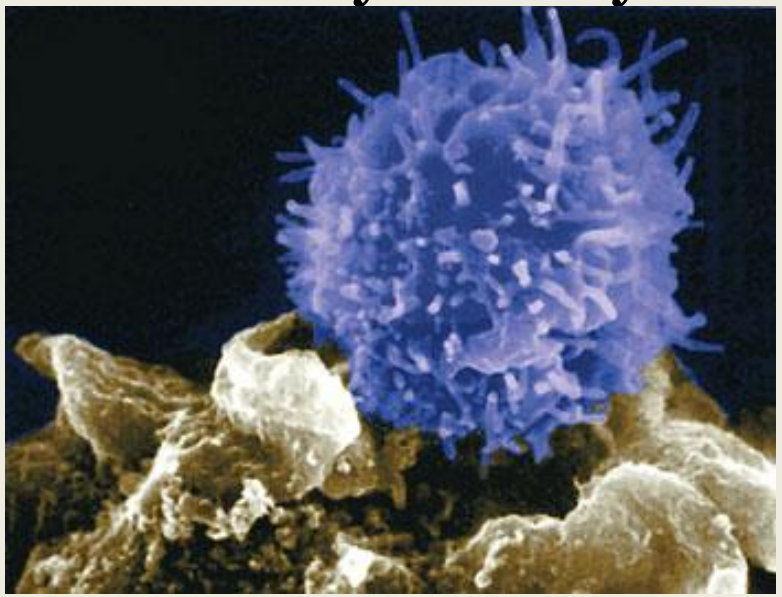
    inhibit the double-stranded (ds) RNA-dependent protein kinase (PKR), which is involved in the cellular antiviral response induced by interferon (IFN).
  - **Core (JAK-STAT pathway)**
- would modify the Janus kinase (JAK)-signal transducer and activator transcription factor (STAT) pathway under interleukin-6 (IL-6) and interferon (IFN)-gamma stimuli. **NS3/4A (phosphorylated IRF-3**
- Interferon regulatory factor 3)

CD8+ and CD4+ T cells

- More vigorous CD8+ and CD4+ T cell responses in all individuals that controlled infection
- Chronic infections occur when
  - unable to mount HCV-specific T cell responses
  - strong response that results in viral RNA clearance, followed by contraction in CD8+/CD4+ and rebound in viremia

Chronic HCV infection

- Low frequencies and reduced capacity of HCV-specific CD8+ cells
- Dendritic cells do not mature normally and have impaired stimulatory activity
- CD4+ cells have reduced IL-2 production and proliferation



Antibodies

- Role of antibodies unclear and poorly studied

antibodies are thought to play a minimal role, however, as the Virus can be cleared in absence of detectable antibody responses

- Neutralizing antibodies target E2, which is highly variable and able to evade