New Approaches to Antivirals for HBV and HCV 16/01/13

Michael McGarvey

Overview of Lecture

- **Current Therapies**

- **Specifically Targeted Antiviral Therapies for - HCV (STAT-C)**
  - **Small Inhibitory Molecules**
  - **Gene Therapy**
  - **Antibody Reagents**

- **Host Targets**

Current Therapy for Chronic HBV

Treat patients with viraemia (HBe antigen positive and negative) and hepatitis;

HBe antigen positive:

- **3-6 months alpha interferon (5-10miu tiw);**
- **prolonged lamivudine (100mg/day).**

HBe antigen negative:

- **prolonged lamivudine (100mg/day).**

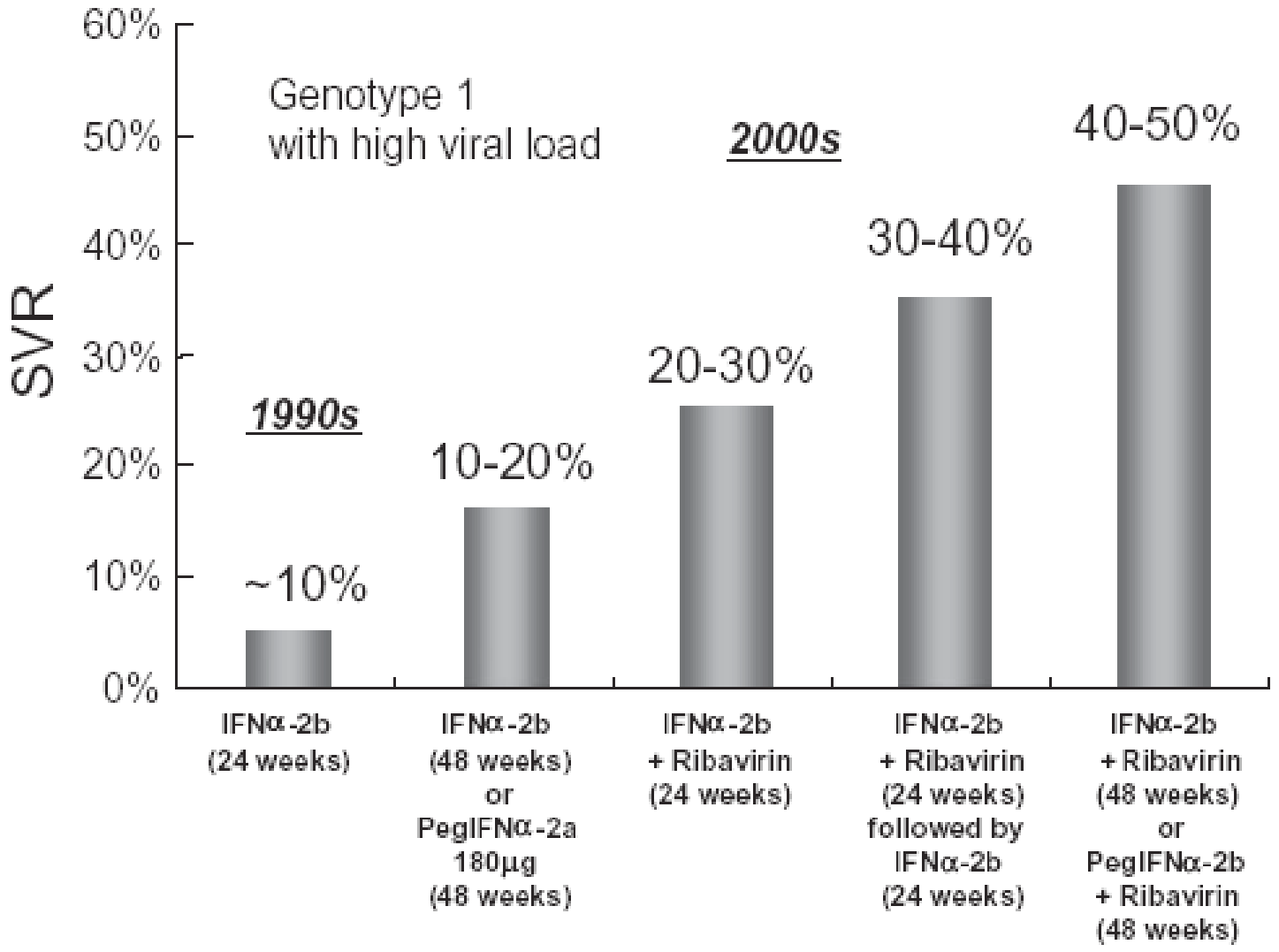
Current Therapy for Chronic HCV

Treat patients with viraemia and moderate/severe hepatitis;

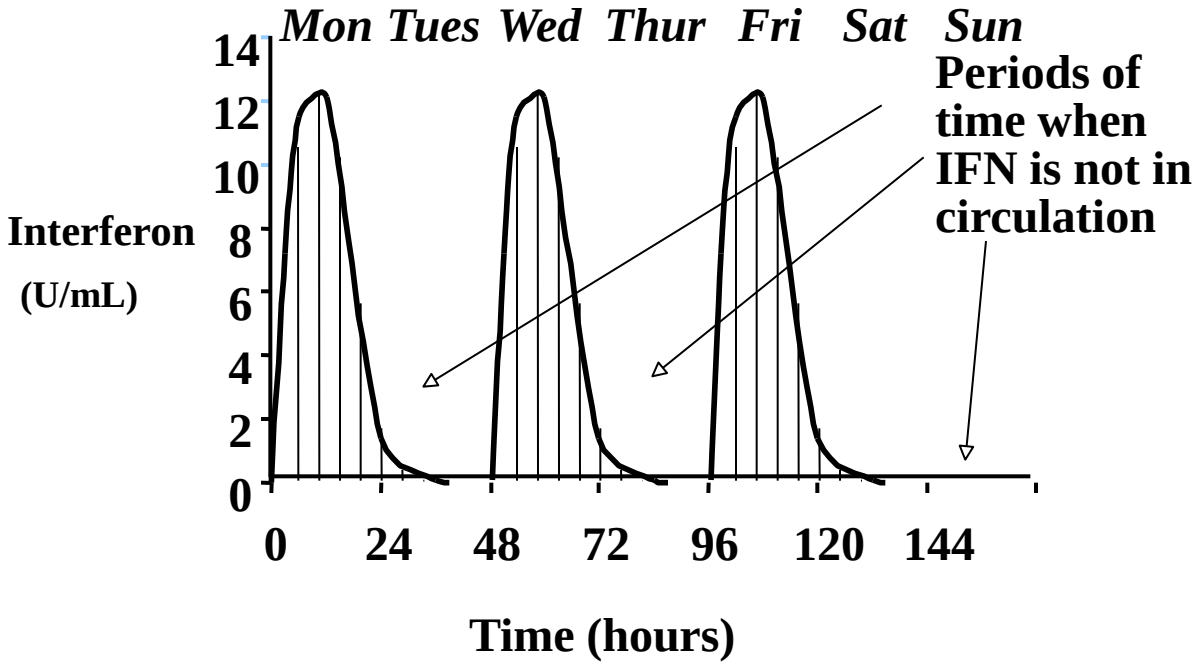
Genotypes 1 and 4: 12 months interferon alpha (3miu tiw) and ribavirin (600-1200mg/day);

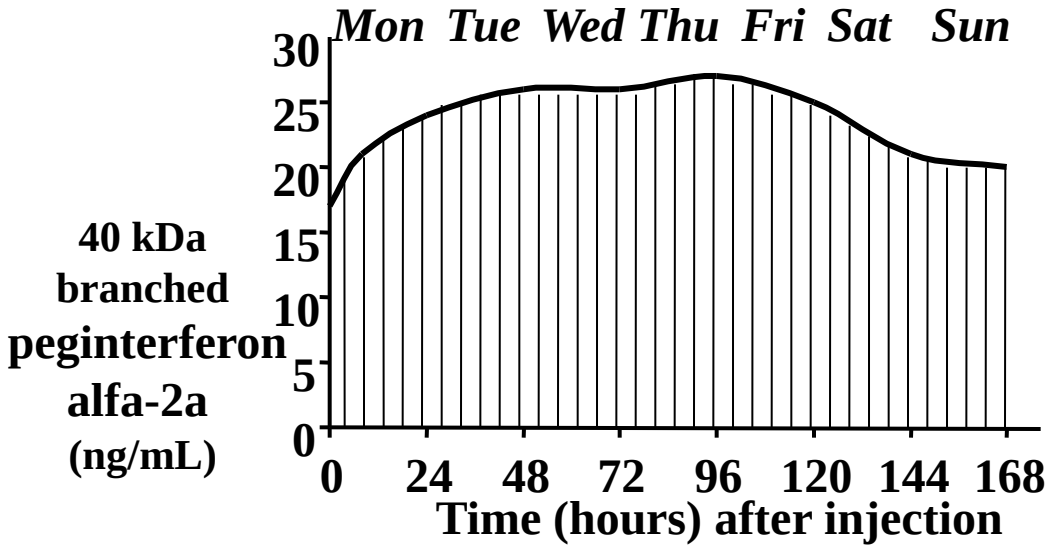
Genotypes 2 and 3: 6 months interferon alpha (3miu tiw) and ribavirin (600-1200mg/day).

Evolving Therapy for Chronic HCV



Peg-IFN α-2a Blood Levels do not fluctuate

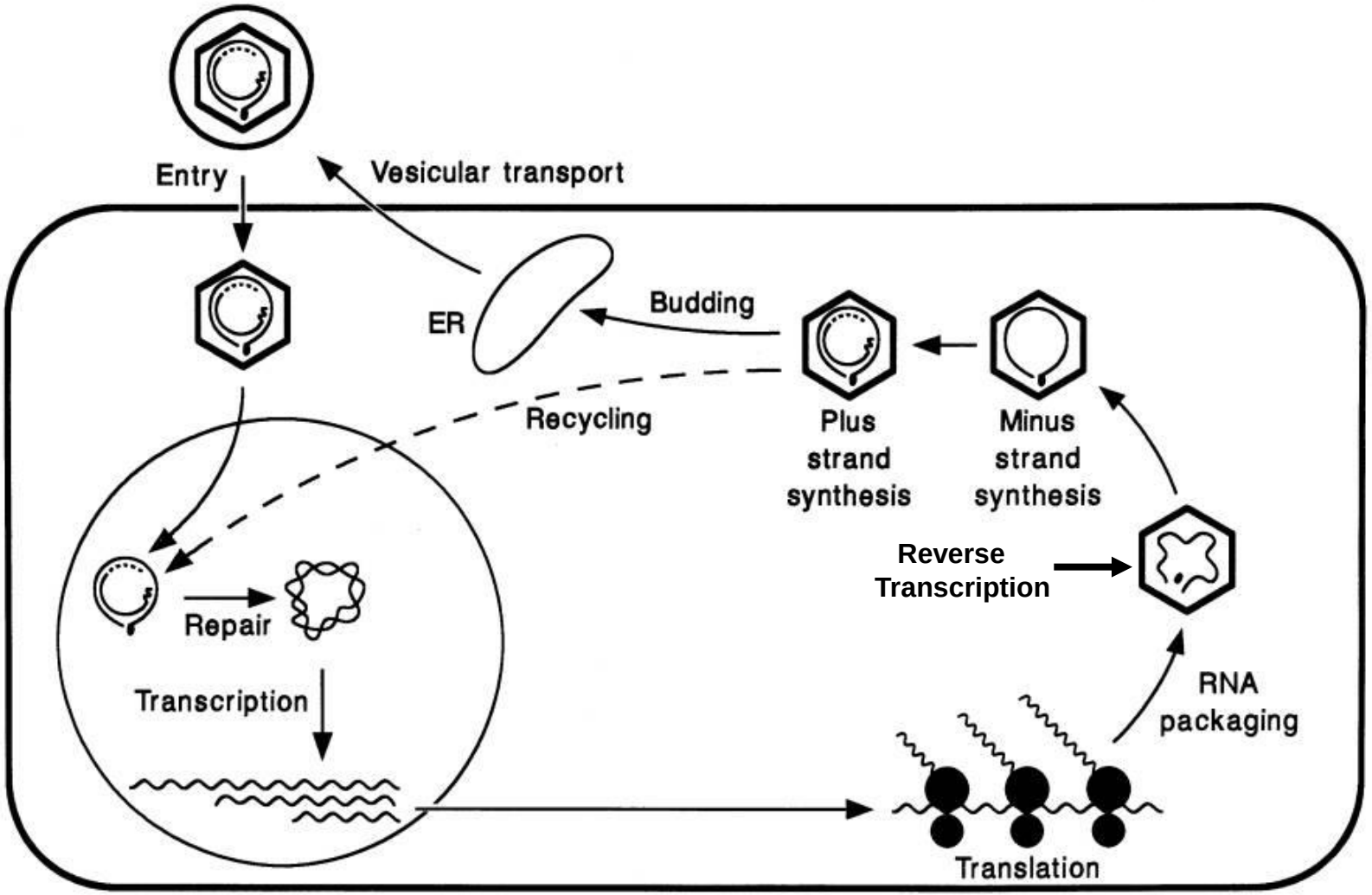




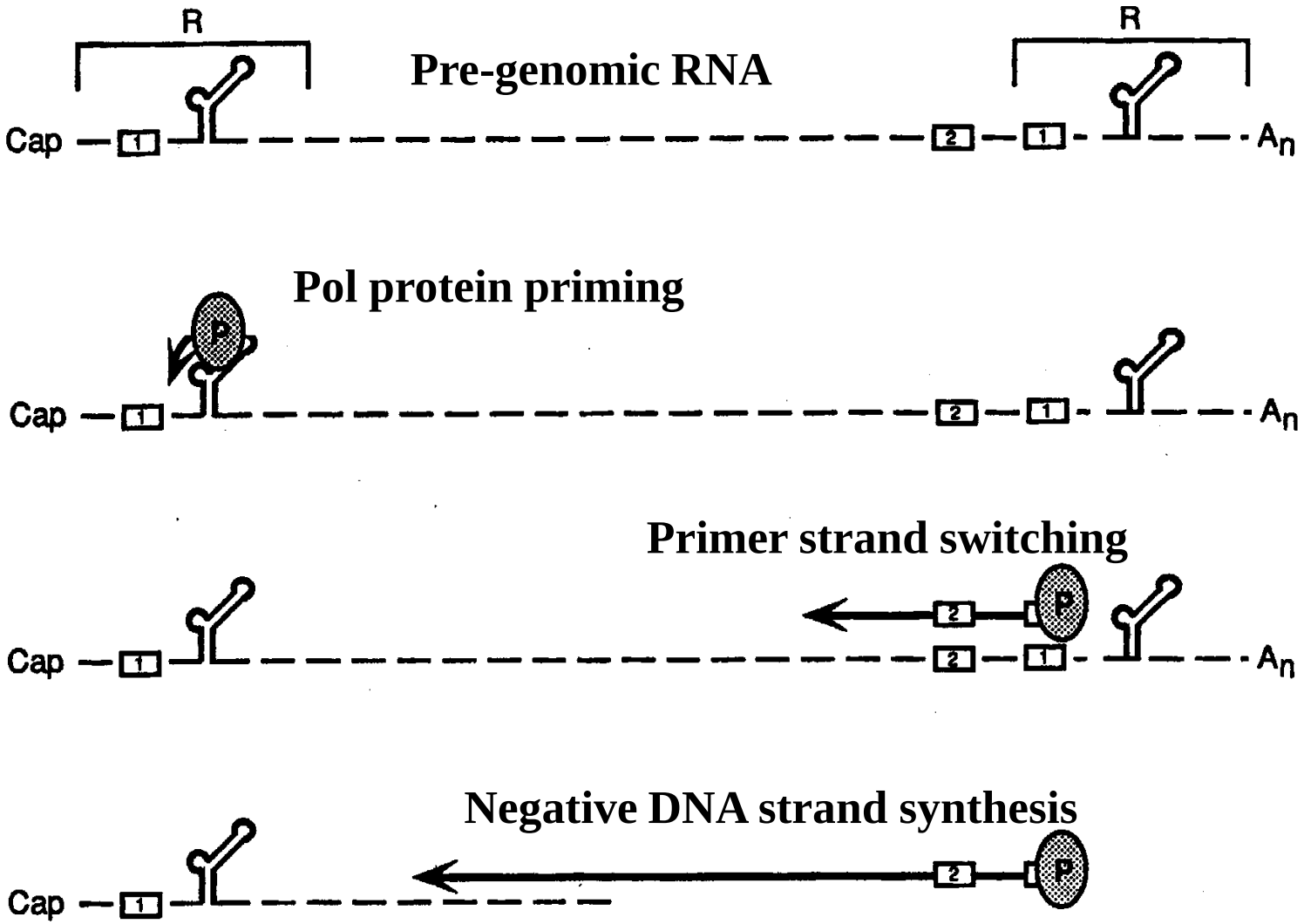
New Antivirals for HCV - in Clinical Trials

Drug	Mechanism	Clinical trial
Albuferon	IFN with prolonged half-life	Phase 2
Omega interferon	IFN for continuous infusion	Phase 2

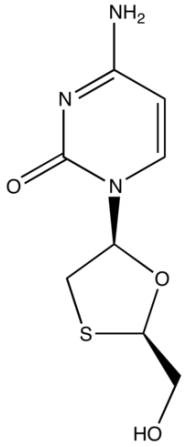
HBV Replication Cycle



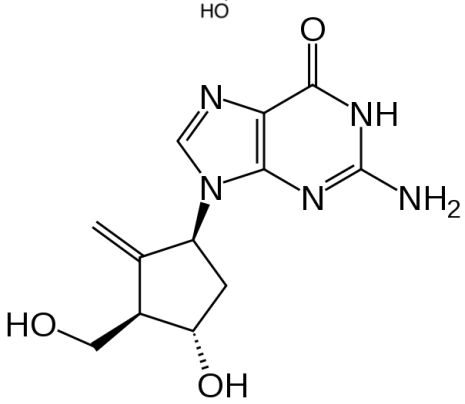
HBV Genome Replication



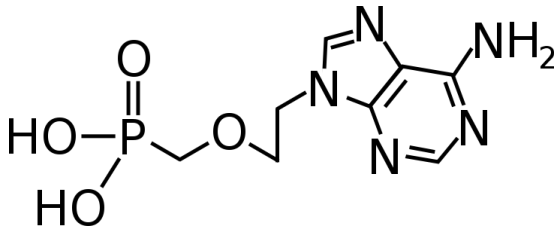
Reverse Transcription Inhibitors (Nucleotide and Nucleoside Analogues) for Chronic HBV



Lamivudine (2',3'-dideoxy-3'-thiacytidine, commonly called **3TC**)



Entacavir 2-Amino-9-[(1*S*,3*R*,4*S*)-4-hydroxy-3-(hydroxymethyl)-2-methylidenecyclopentyl]-6,9-dihydro-3*H*-purin-6-one

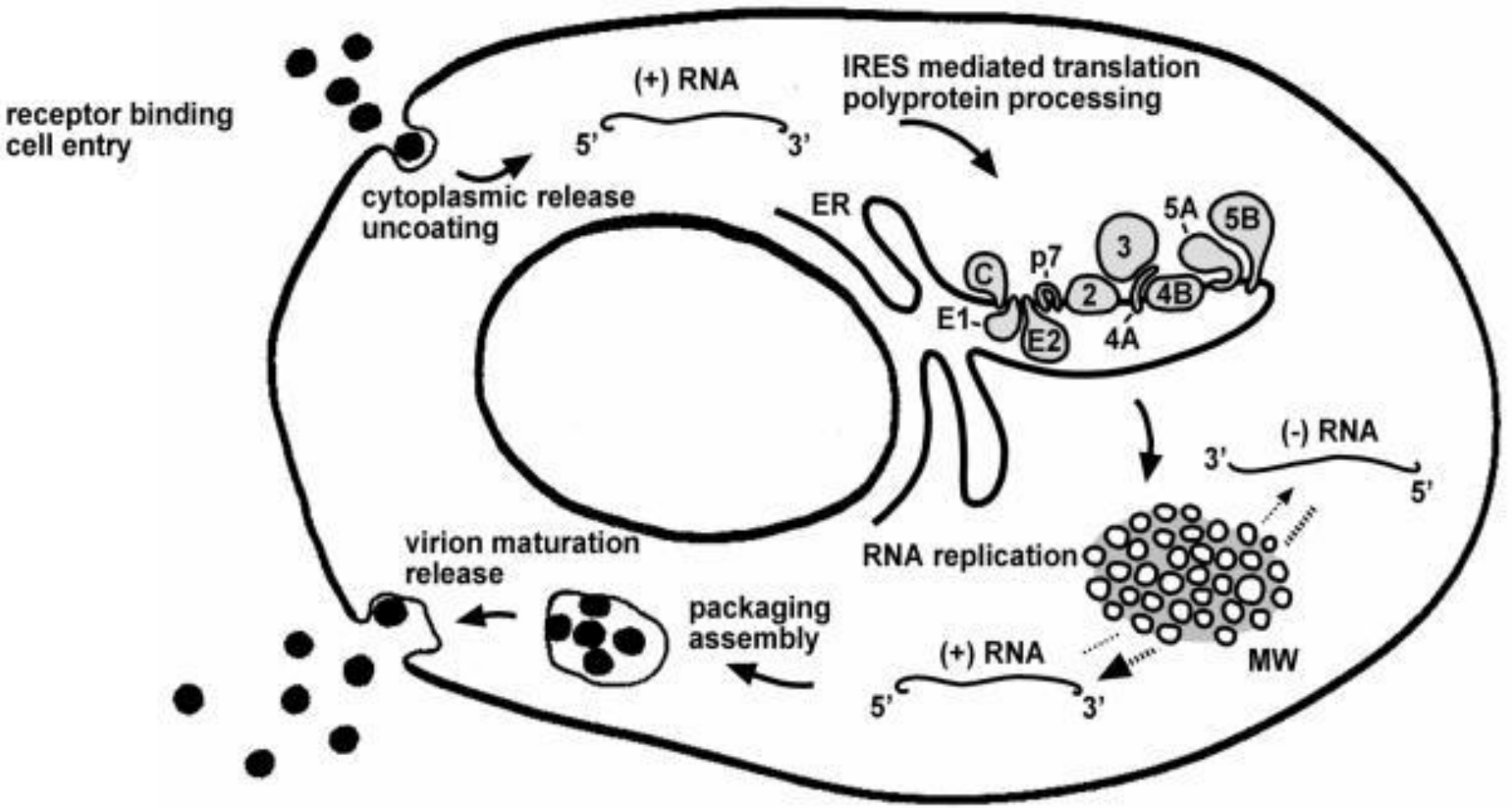


Adefovir 2-(6-amino-9*H*-purin-9-yl)ethoxy] methyl}phosphonic acid

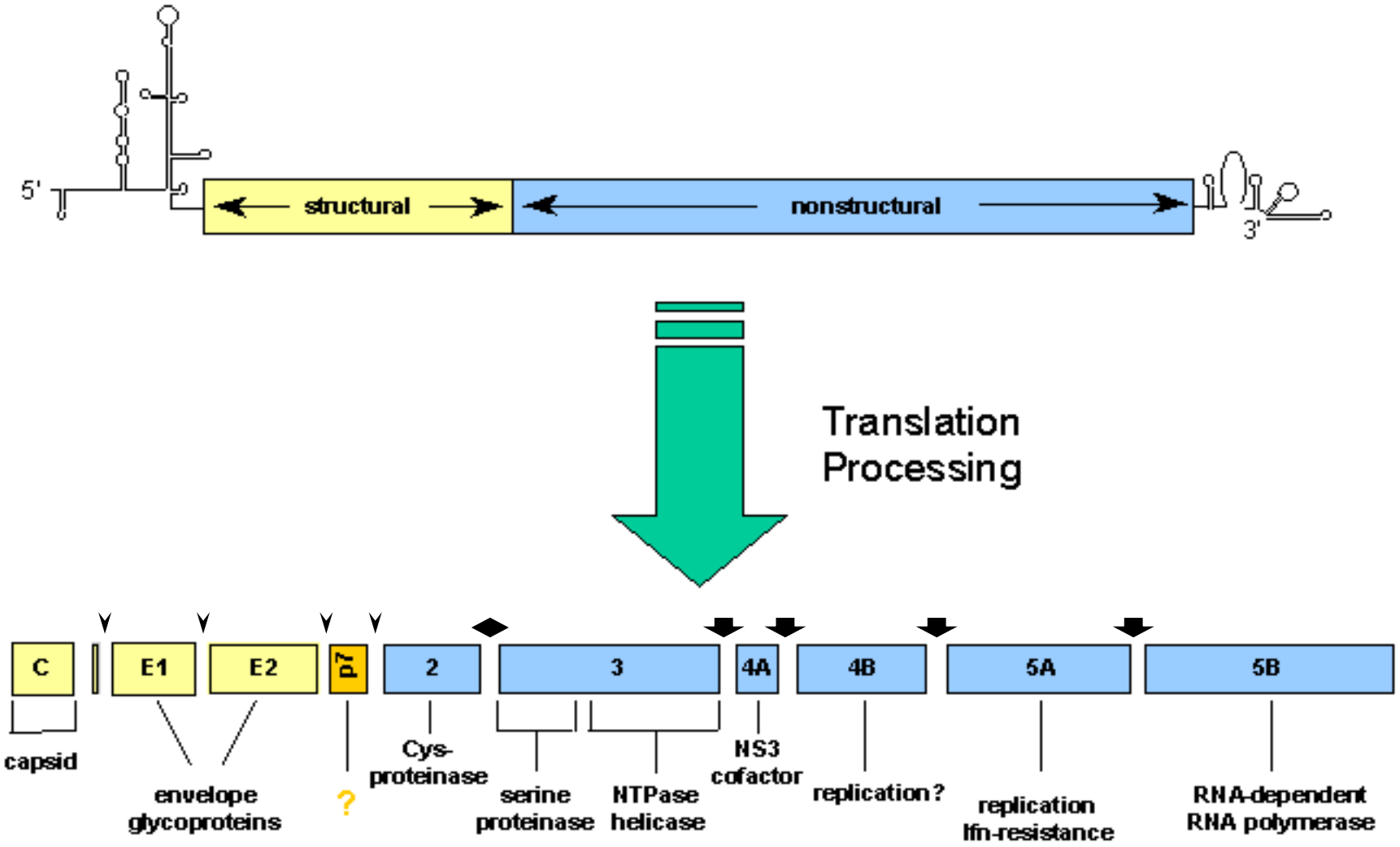
Reverse Transcription Inhibitors (Nucleotide and Nucleoside Analogues) for Chronic HBV

Compound	Status
Lamivudine	Approved
Adefovir	Approved
Entecavir	Approved (USA)
Emtricitabine	Phase III
Telbivudine	Phase III
Clevudine	Phase II

HCV RNA Replication



HCV RNA As a Target for RNAi

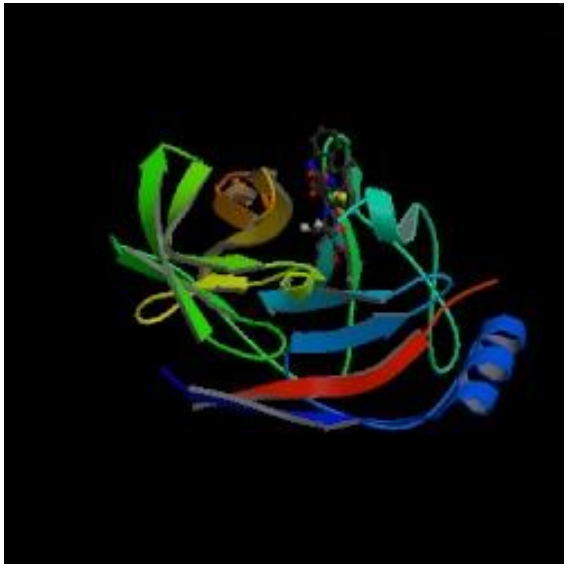


Properties of NS3

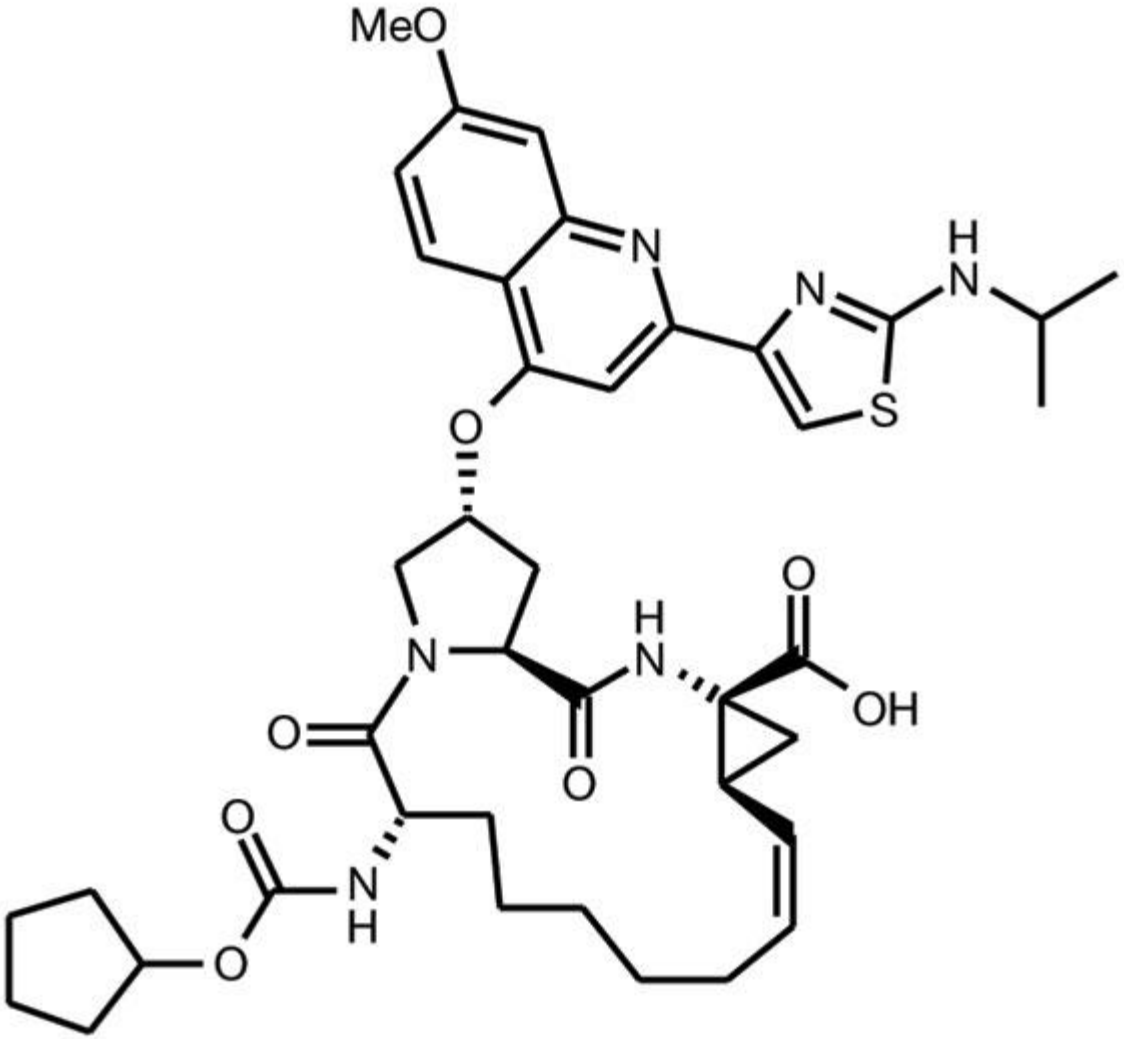
- **Protease**
- **Helicase in replication complex**
- **ATPase (associated with helicase activity)**
- **May contribute to viral persistence in HCV**

NS3 Protease

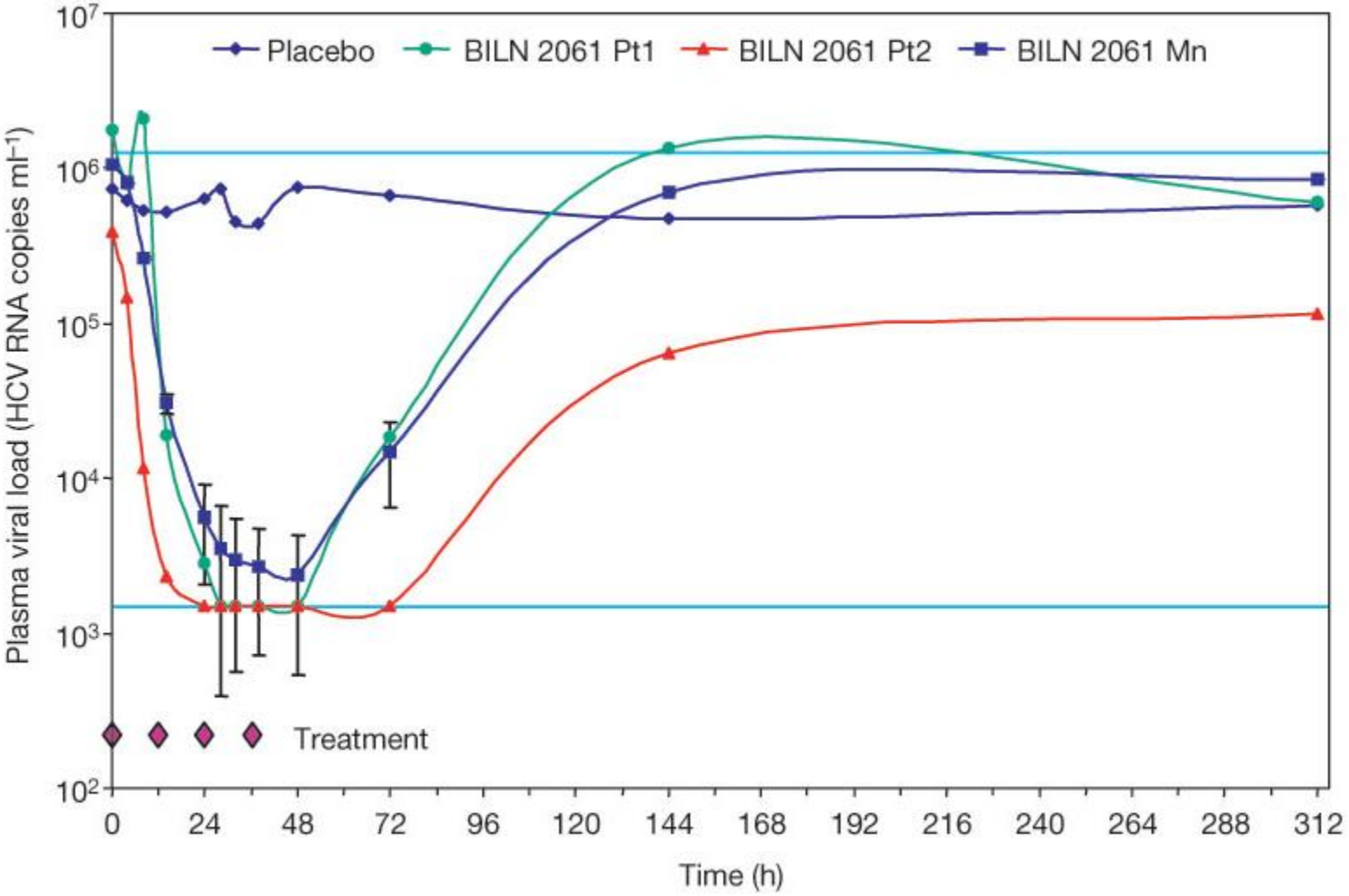
- **Amino 1/3 of NS3**
- **Serine protease with a catalytic triad of amino acids**
- **Cleaves cis and trans**



HCV Protease Inhibitor: BILN 2061



BILN 2061: HCV Infected Human Patients



New Antivirals for HCV - in Clinical Trials

Drug	Mechanism	Clinical trial
Albuferon	IFN with prolonged half-life	Phase 2
Omega interferon	IFN for continuous infusion	Phase 2
NM 283 (valopicitabine)	**NS5B polymerase inhibitor**	**Phase 2b**
VX-950	**NS3 protease inhibitor**	**Phase 1b**
SCH 503034	**NS3 protease inhibitor**	**Phase 2a**
Viramidine	**Ribavirin prodrug**	**Phase 3**

Gene Therapy

HCV

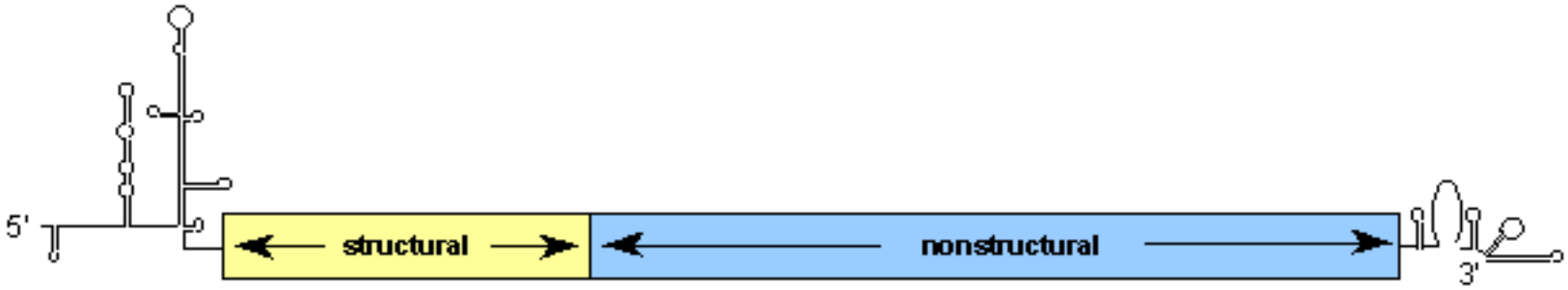
- **Therapy: 60% of patients show no response to the combination of Interferon and Ribavirin**

- **Development of antivirals has been hampered by the lack of a cell culture system for propagating the virus**

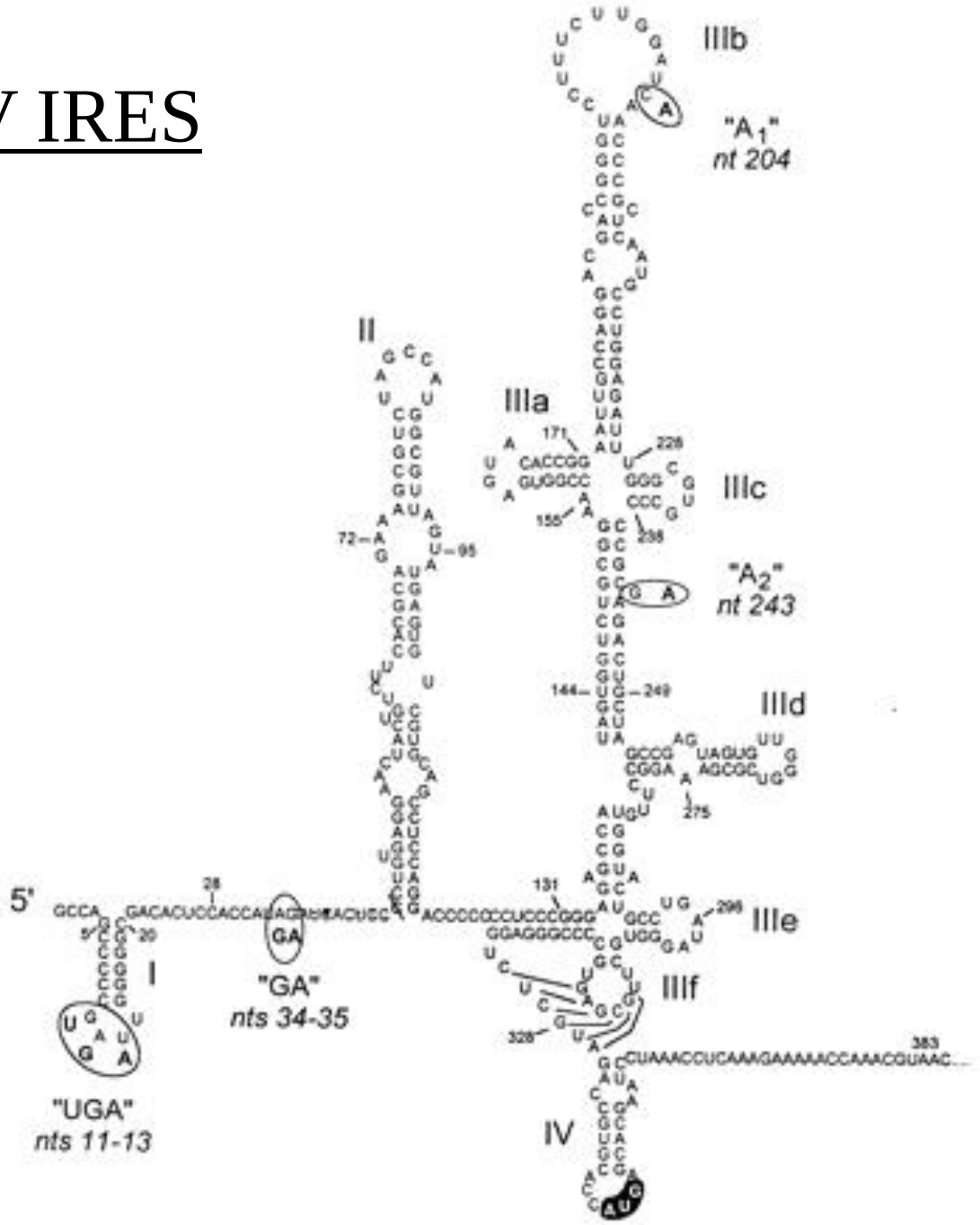
HCV Genome

- **Single stranded RNA of positive polarity and Length of 9600 nt**
- **Genome contains a single ORF encoding a 3010-3033 aa polyprotein**
- **Translation of the polyprotein is controlled by an IRES**
- **A good candidate for RNA interference (RNAi)**

HCV RNA As a Target for RNAi



HCV IRES


IIIb
"A1"
nt 204
II
IIIa
IIIc
"A2"
nt 243
IIId
IIIe
IIIf
IV
5'
I
"GA"
nts 34-35
"UGA"
nts 11-13

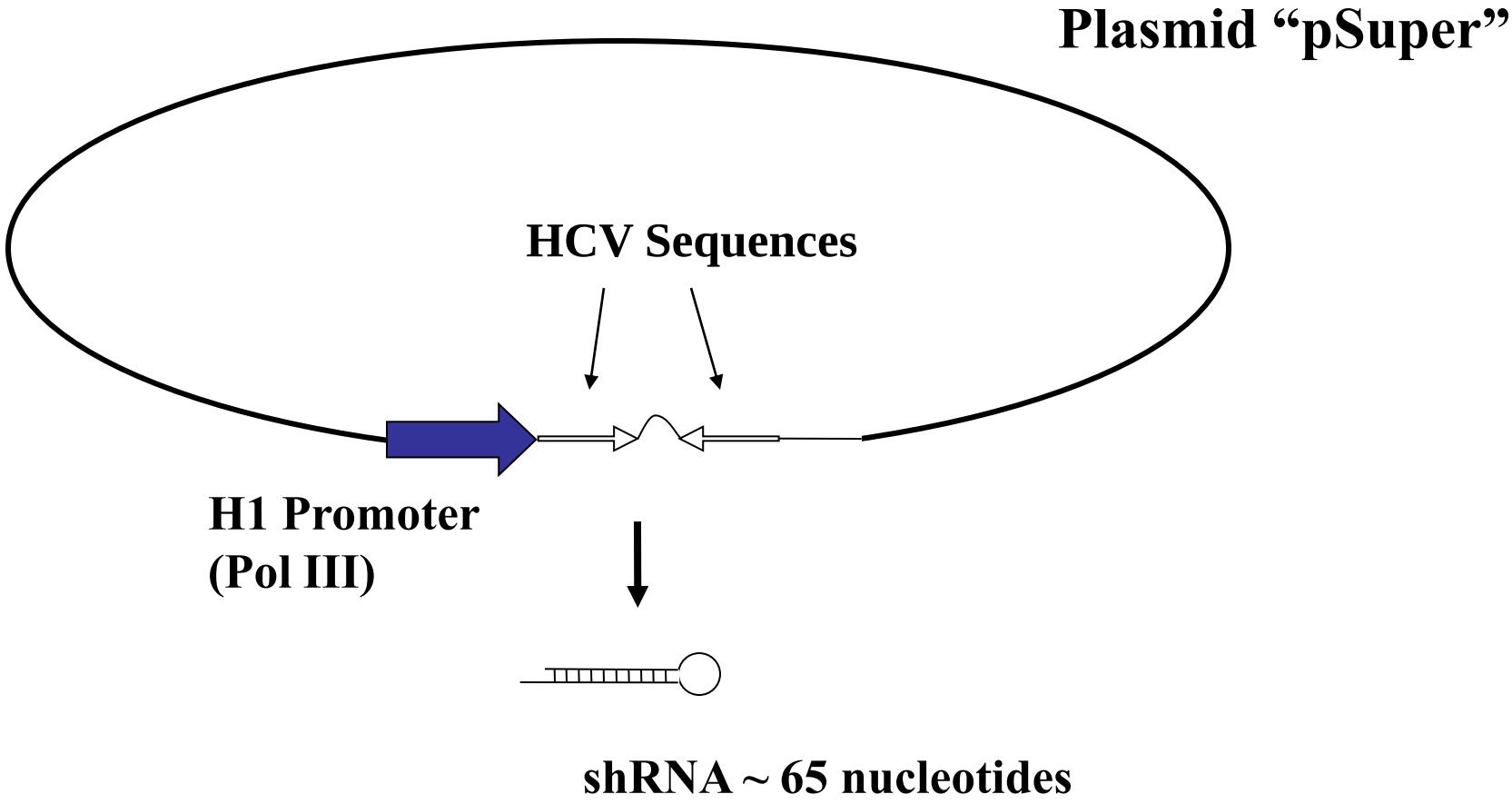
RNA Interference (RNAi)

- **RNAi: Introduction of dsRNA into a cell inhibits gene expression in a sequence dependent fashion.**

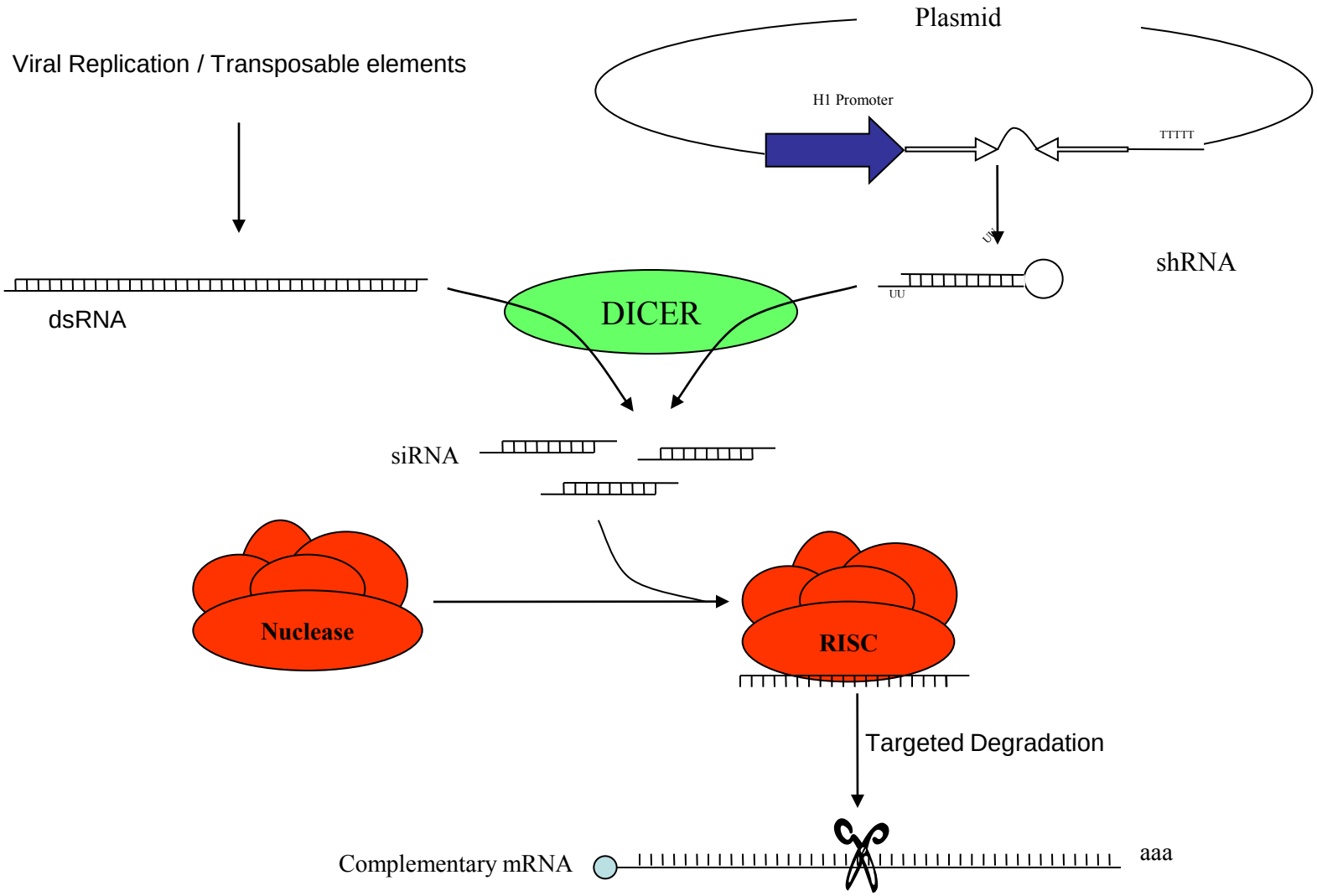
- ***C. elegans* (Fire *et al*, 1998, Nature, 391: 806-811).**

- **This phenomenon resulted in sequence-specific gene silencing.**

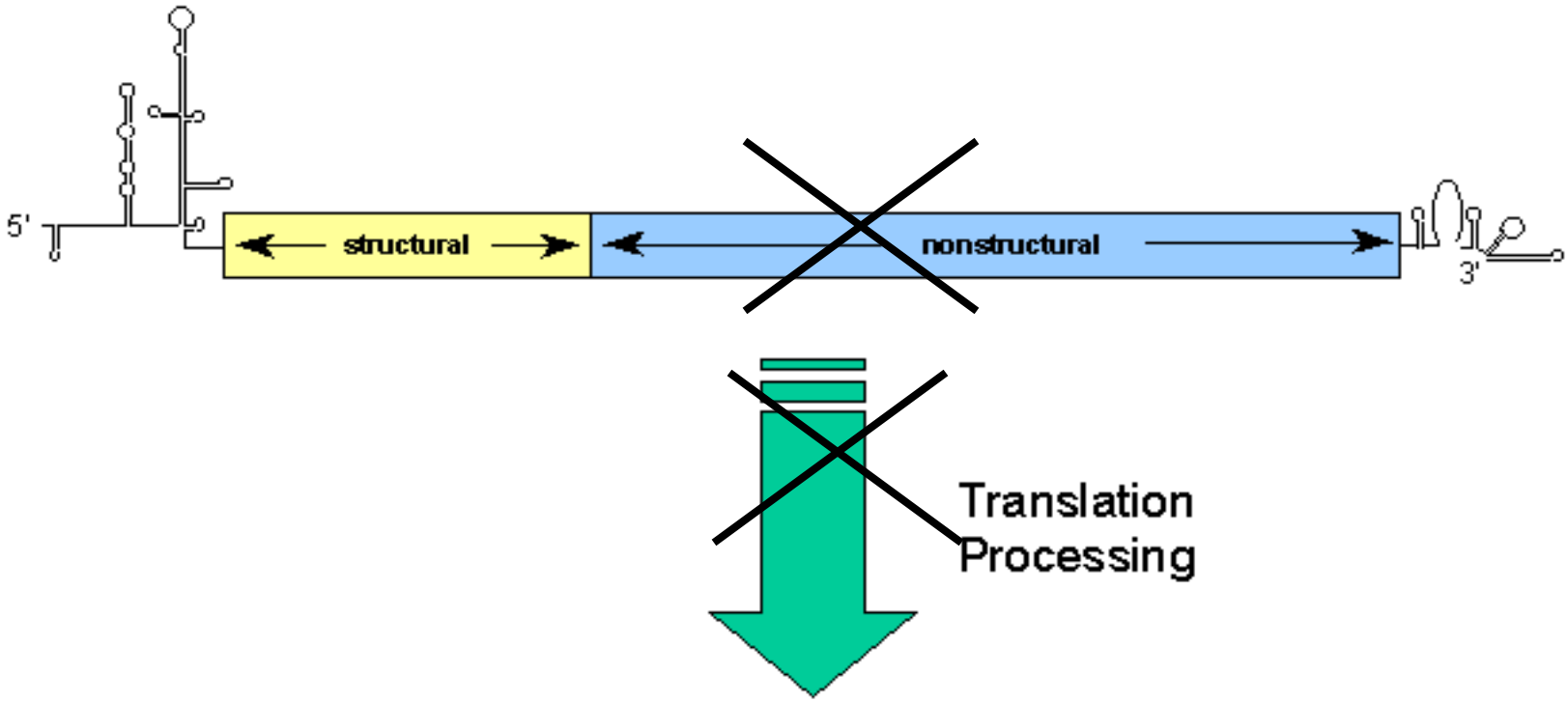
si RNA Expression from DNA Plasmids

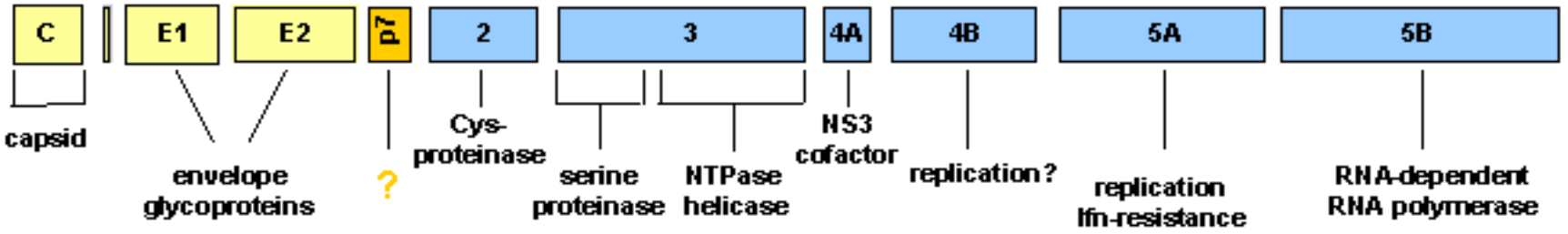


RNAi: Mechanism



HCV RNA As a Target for RNAi





HCV RNAi

- **Different regions of the HCV NS3 (1b) coding sequence were chosen as target for potential RNAi oligonucleotides.**

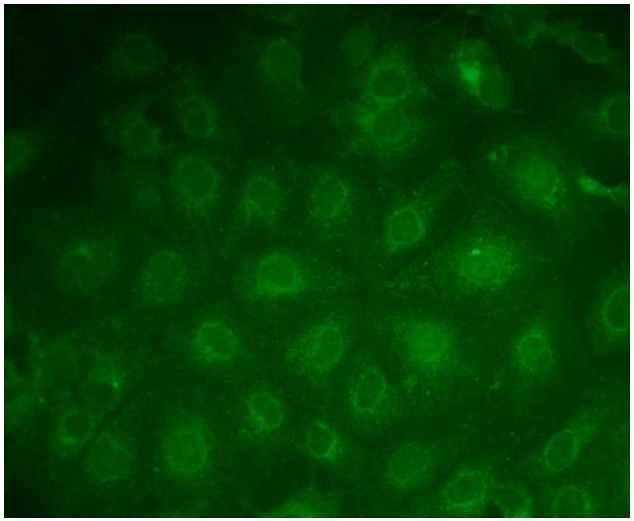
- **siNS3-3 reduced NS3 expression by more than 90%.**

HCV RNAi: Transient Transfection

- **pSUPER-HCV3 reduced NS3 expression by more than 90%.**

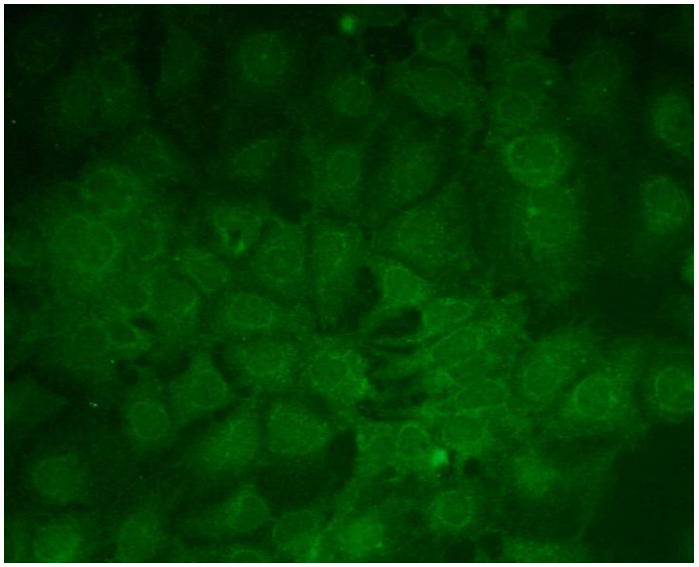
HCV RNAi: RNA Replicon Cell Line

Huh7 cells



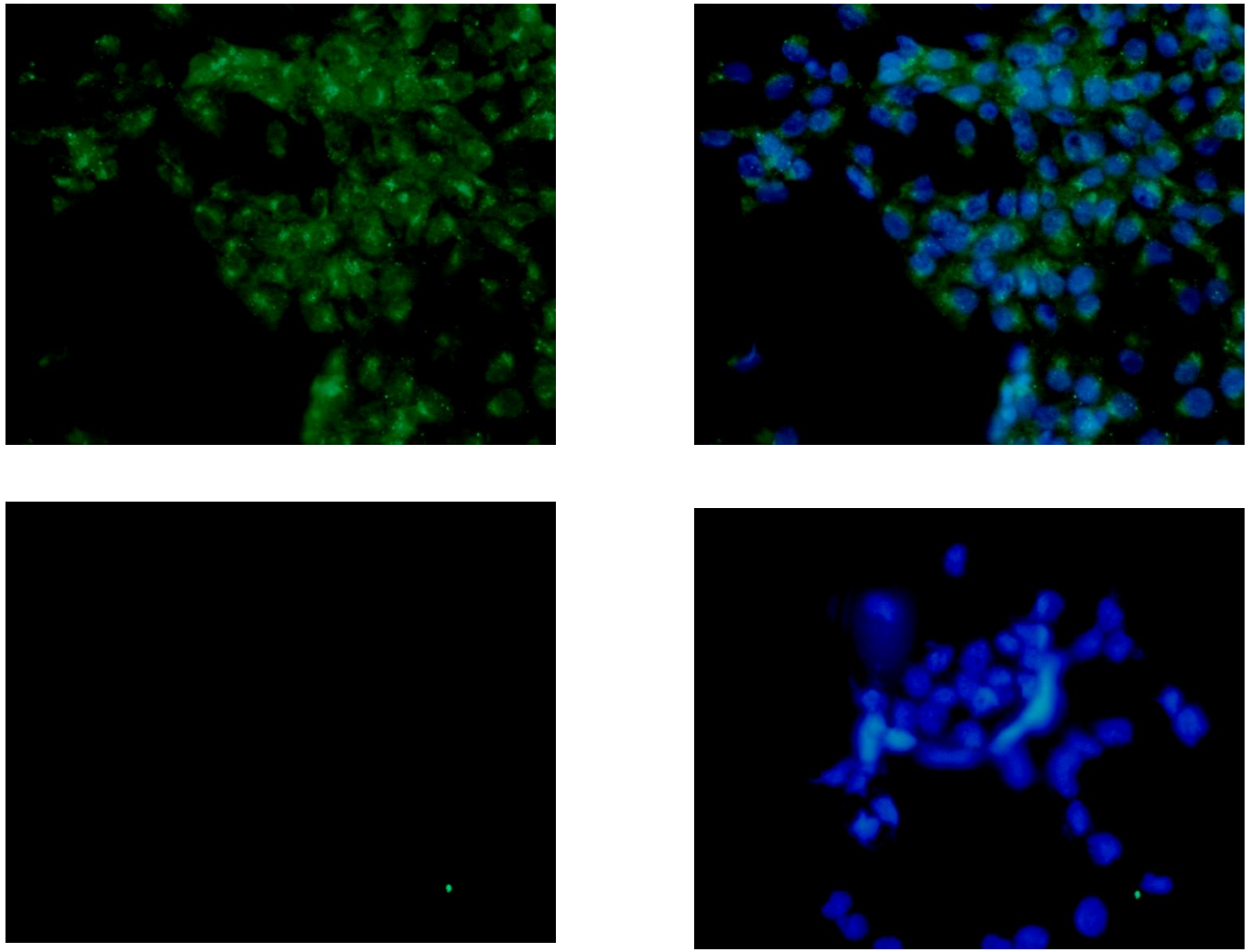
S1179I Replicon

pSUPER-Control



siNS3-3

siRNA-Adenovirus: HCV Infected Cells

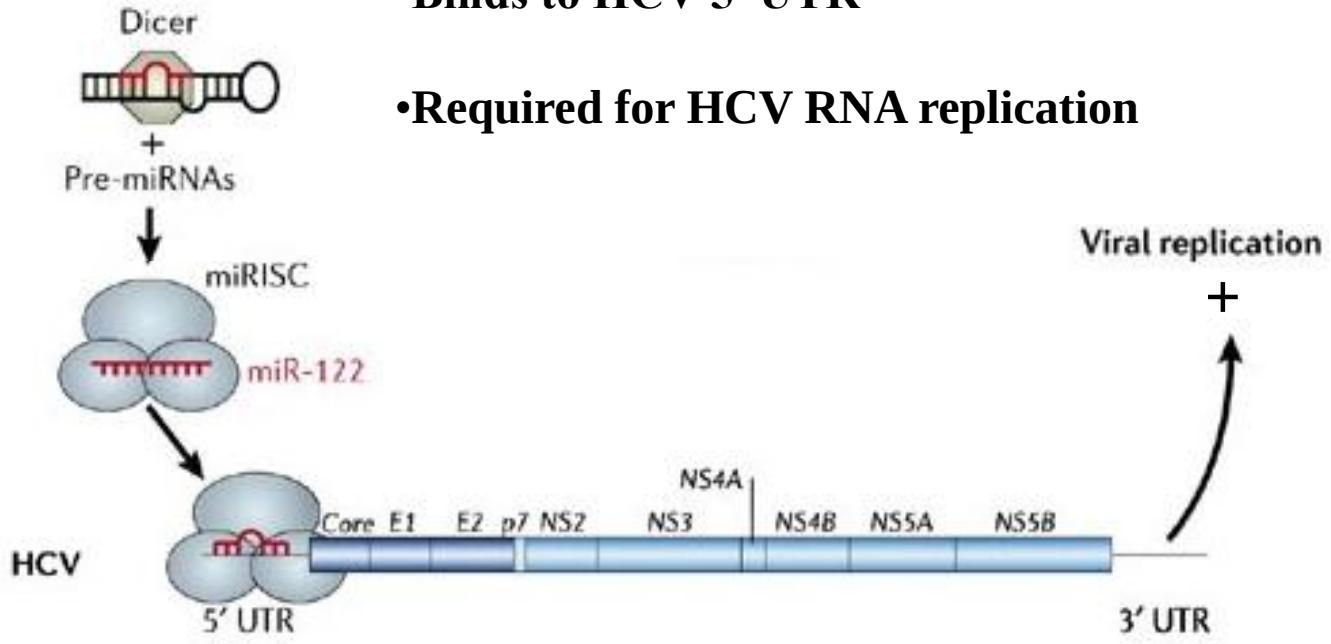


HCV RNAi: RNA Replicon Cell Line

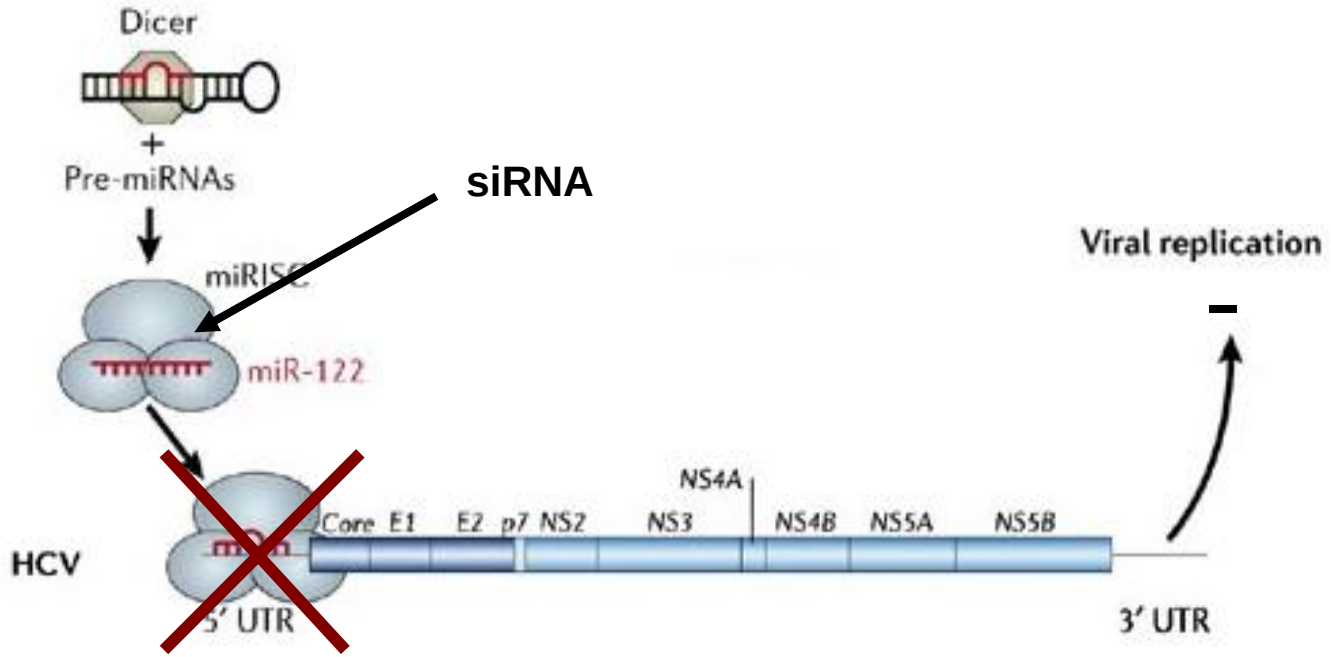
- **RNAi inhibited NS3 expression in transient transfection (FACS analysis).**
- **siRNAs against HCV degrade HCV RNA.**
- **siRNAs against HCV inhibited NS3 expression in HCV infected cells.**
- **siRNAs can be delivered to liver cells by viral and non-viral means.**

MicroRNA 122 (miR-122) and HCV

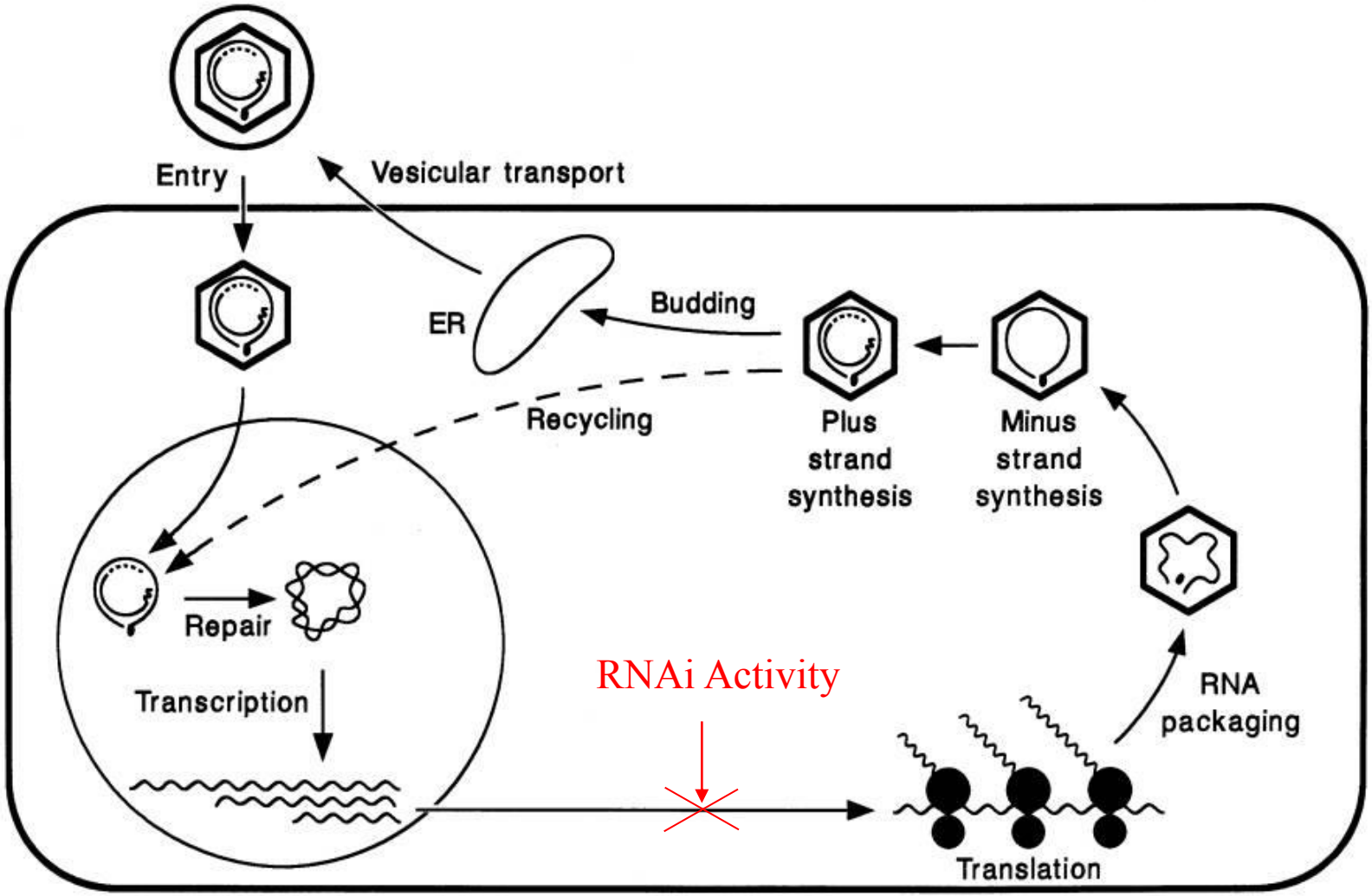
- **Liver specific**
- **Binds to HCV 5' UTR**
- **Required for HCV RNA replication**



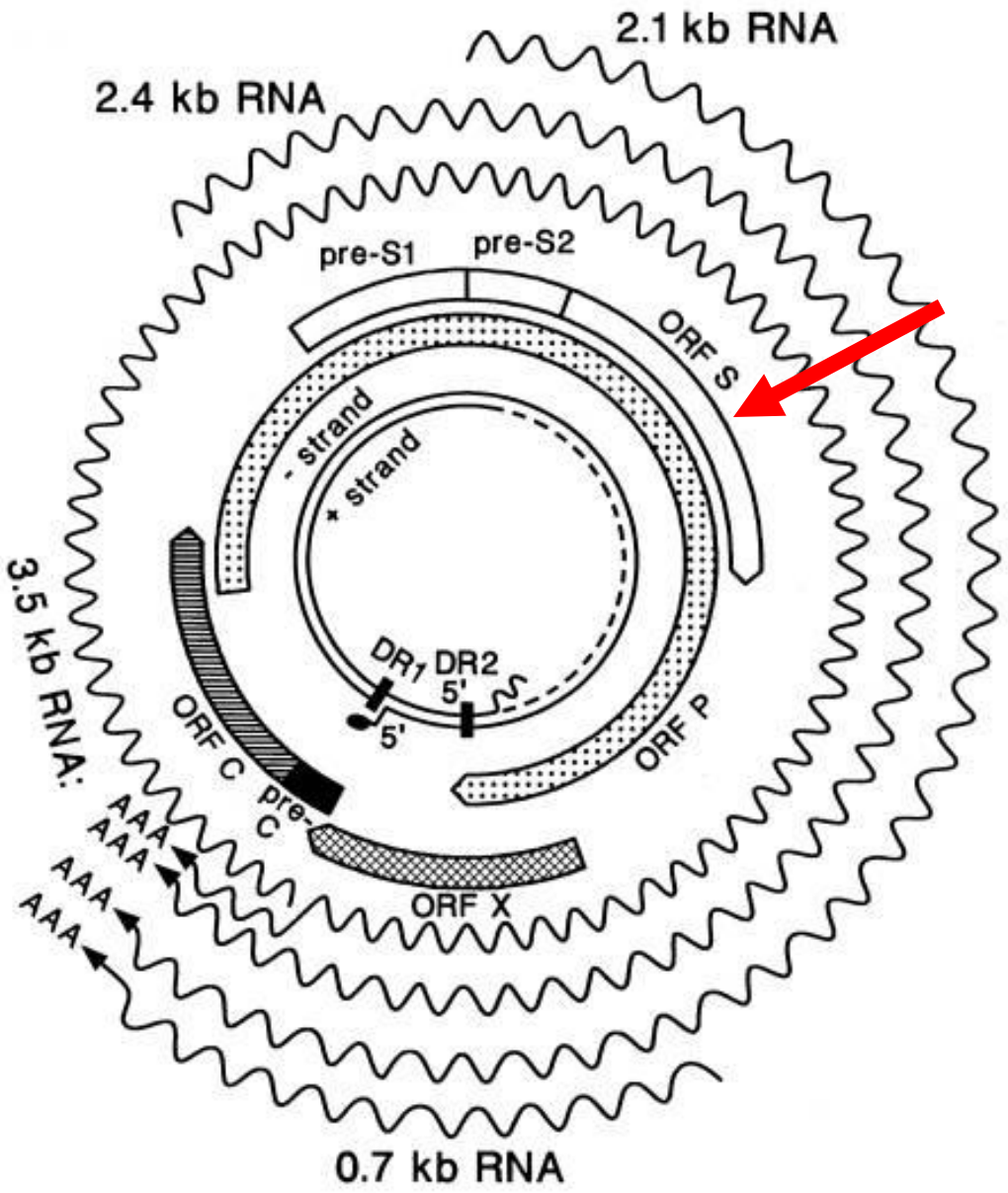
MicroRNA 122 (miR-122) and HCV



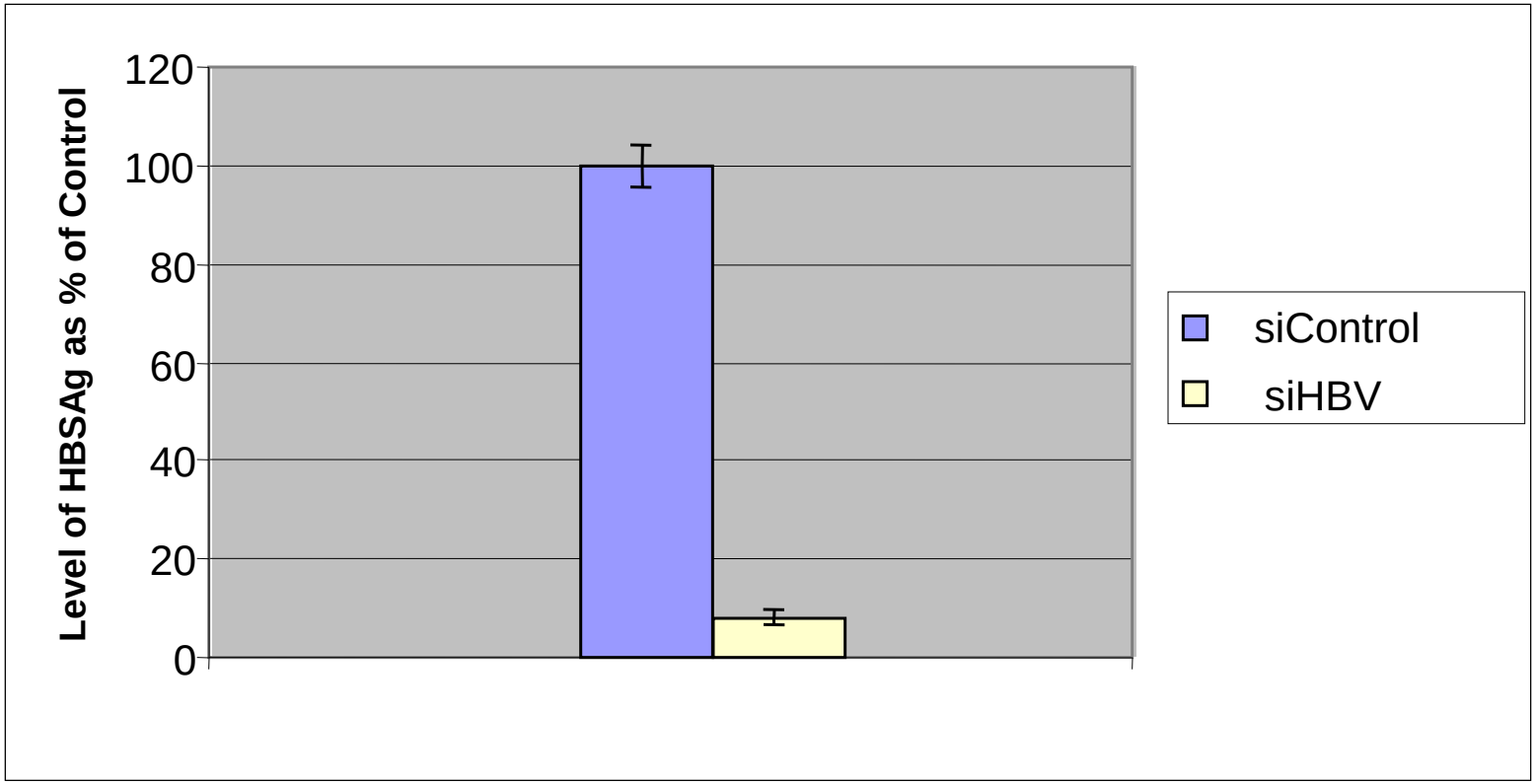
HBV Replication Cycle



HBV Transcripts



Effect of siHBV on HBV Expression in

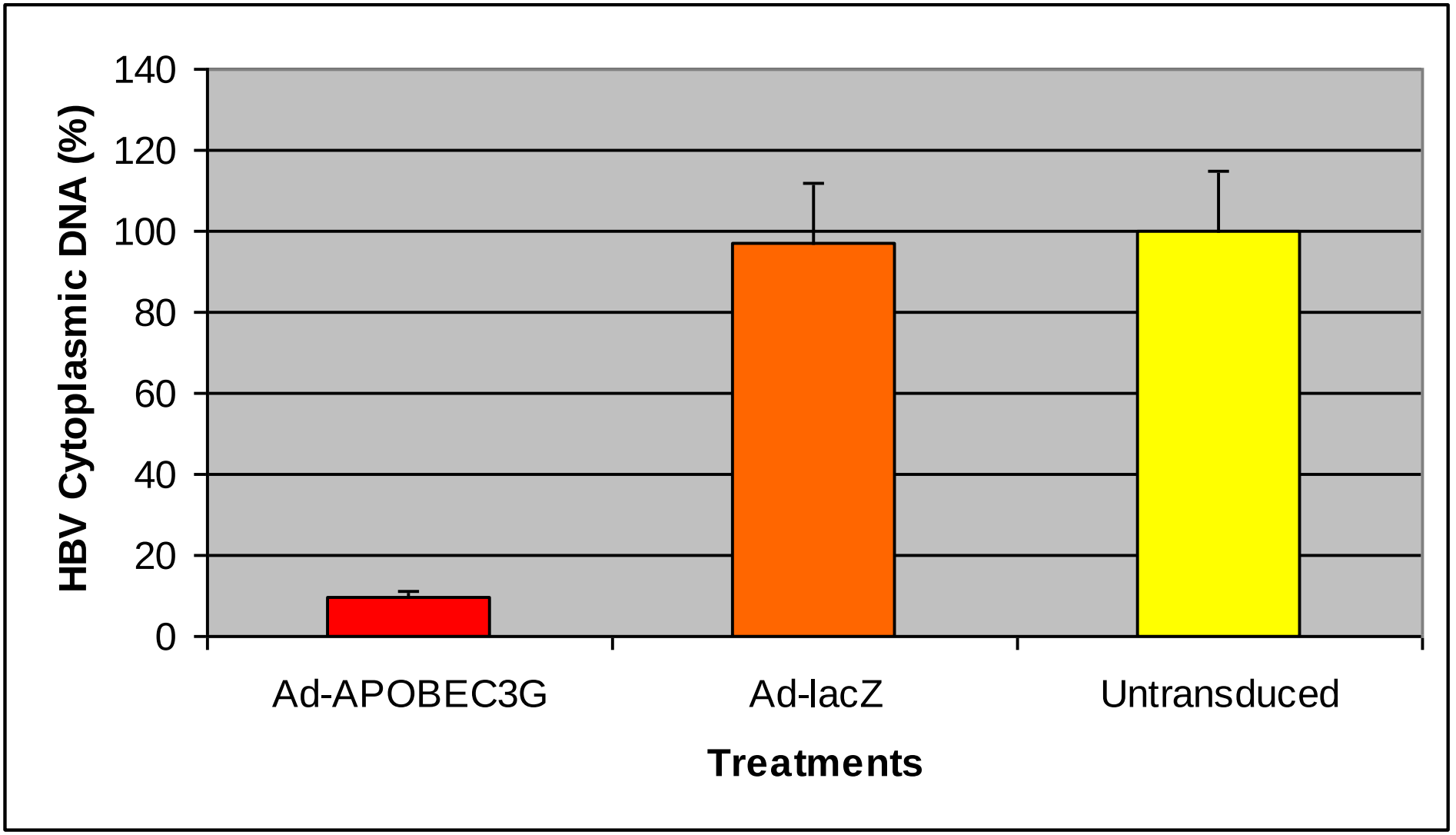


APOBEC3G

APOBEC3G

- **A member of a family of seven related genes which may have antiviral activity (particularly A3B, A3C, A3G and A3F)**
- **APOBEC3G is a DNA deaminase enzyme that deaminates 2'-deoxycytidines (C) to 2'-deoxyuridines (U).**
- **APOBEC3G can produce a high frequency of G-to-A substitutions in the plus strand of HIV-1 & other retroviral DNA.**
- **APOBEC3G inhibits HBV replication**

HBV Cell Culture Model

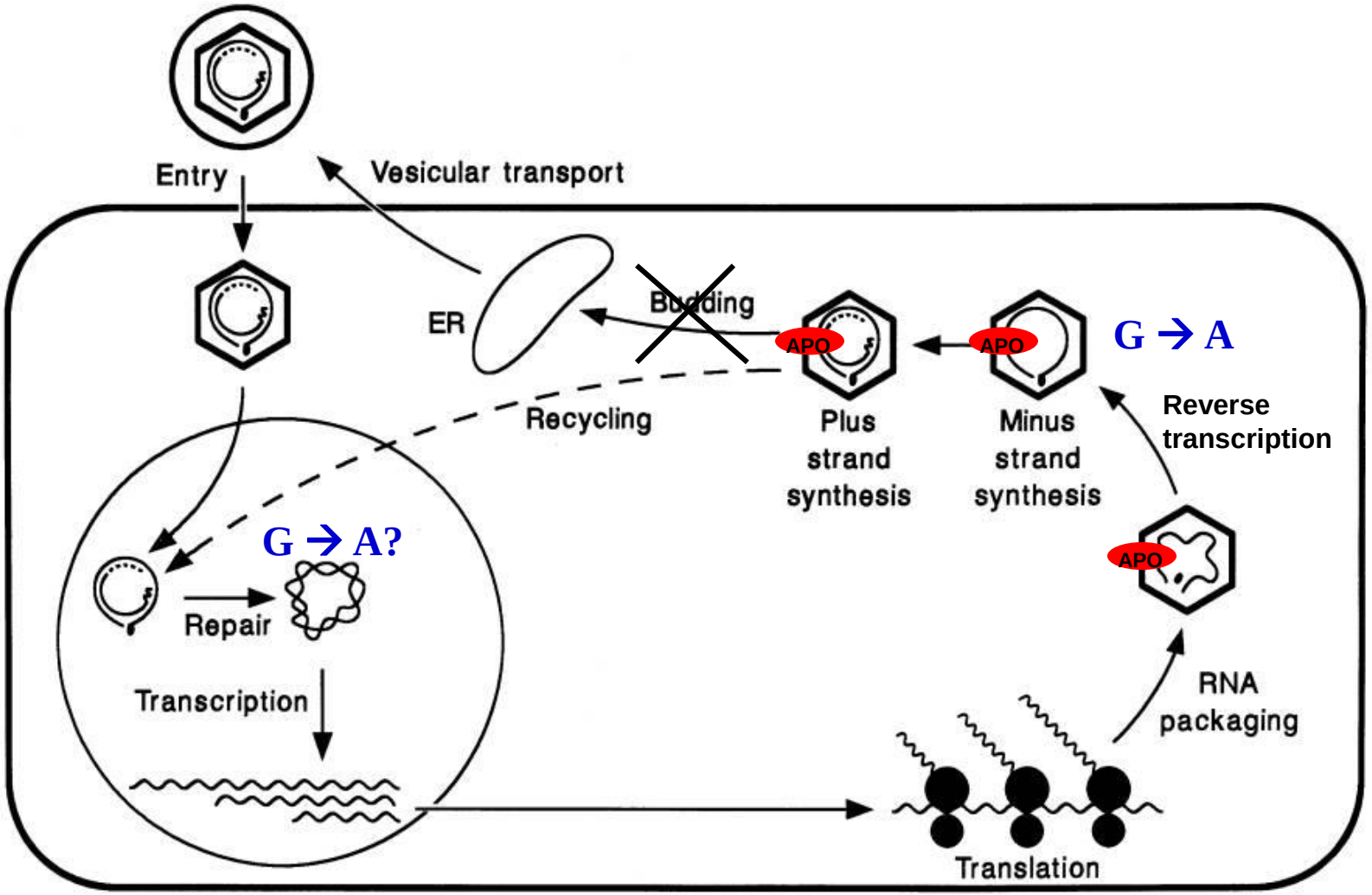


Cytosine Deamination of HBV Pre-core Region by APOBEC3G

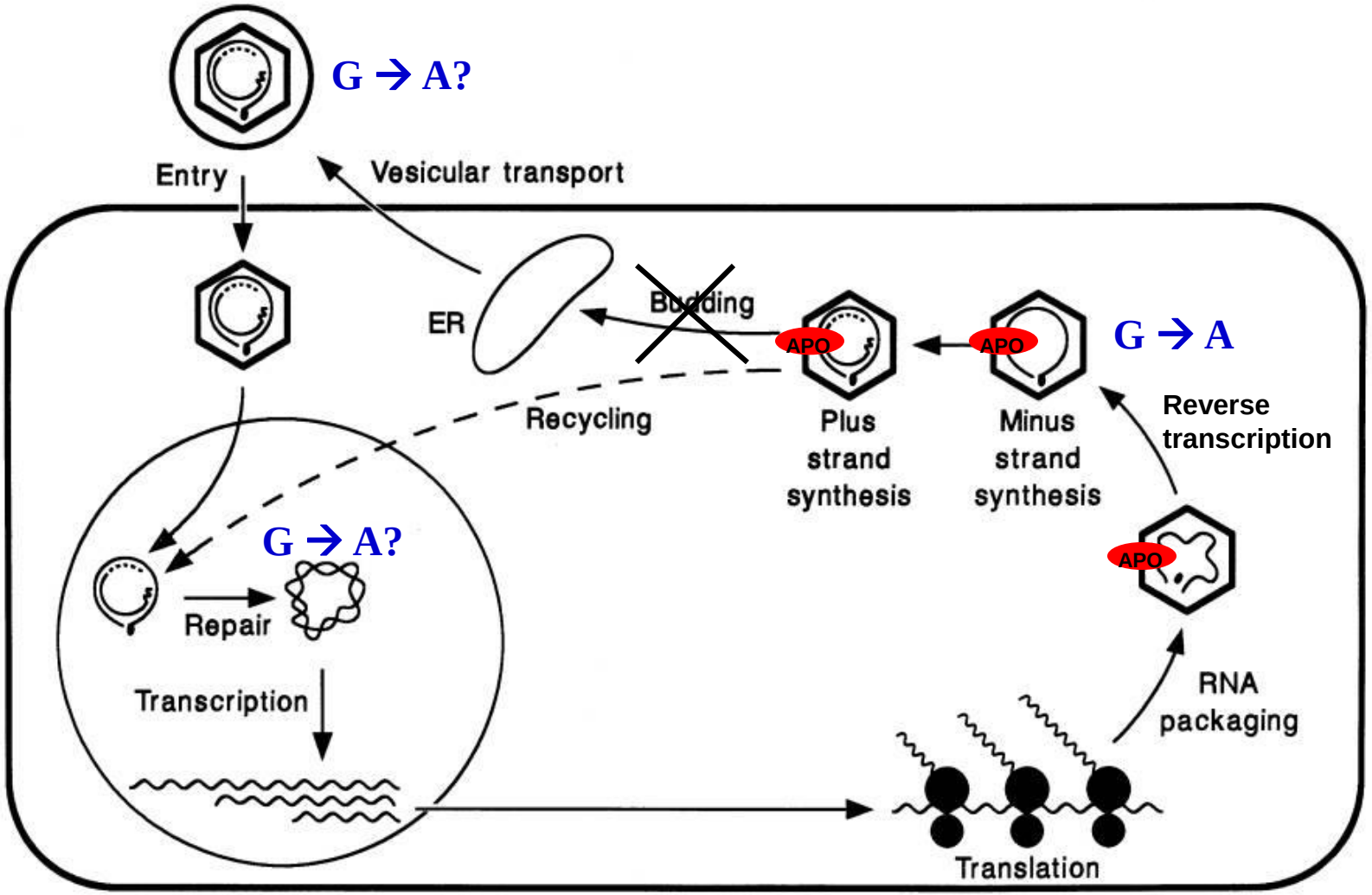
Figure 3

*** The arrow in Figure 3 indicates the position of the G-A changes at position 1896**

HBV Replication Cycle: APOBEC3G



HBV Replication Cycle: APOBEC3G



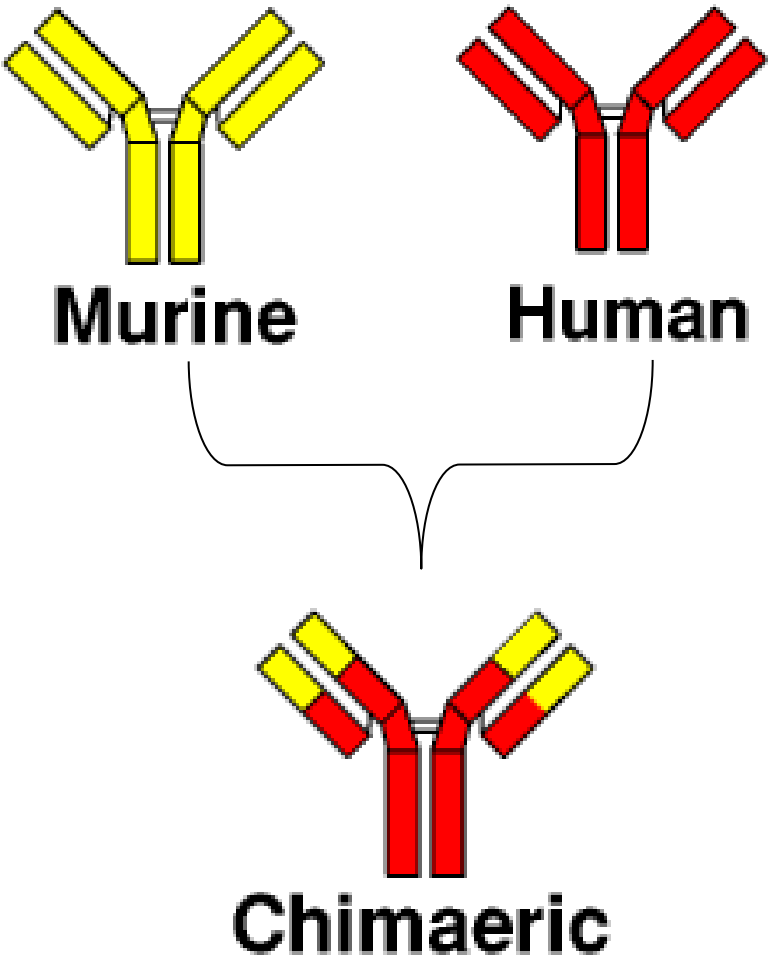
Antibody Reagents

Monoclonal Antibodies

- **Unlimited source of antibody from cell lines**
- **Good for research, however immunogenic**
- **Immunogenicity can be reduced by replacement of mouse sequences**

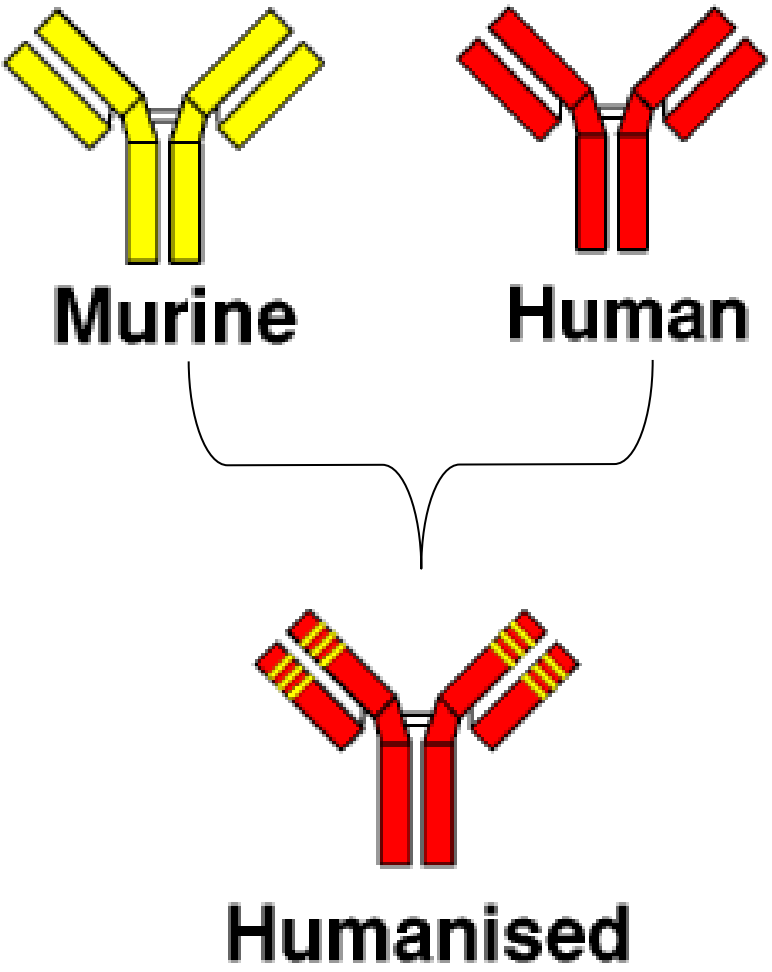
Chimaeric Antibodies

- **Consist of human and mouse sequences**
- **Variable regions from mouse Ab. Constant regions from human Ab.**
- **Still immunogenic**



Humanised Antibodies

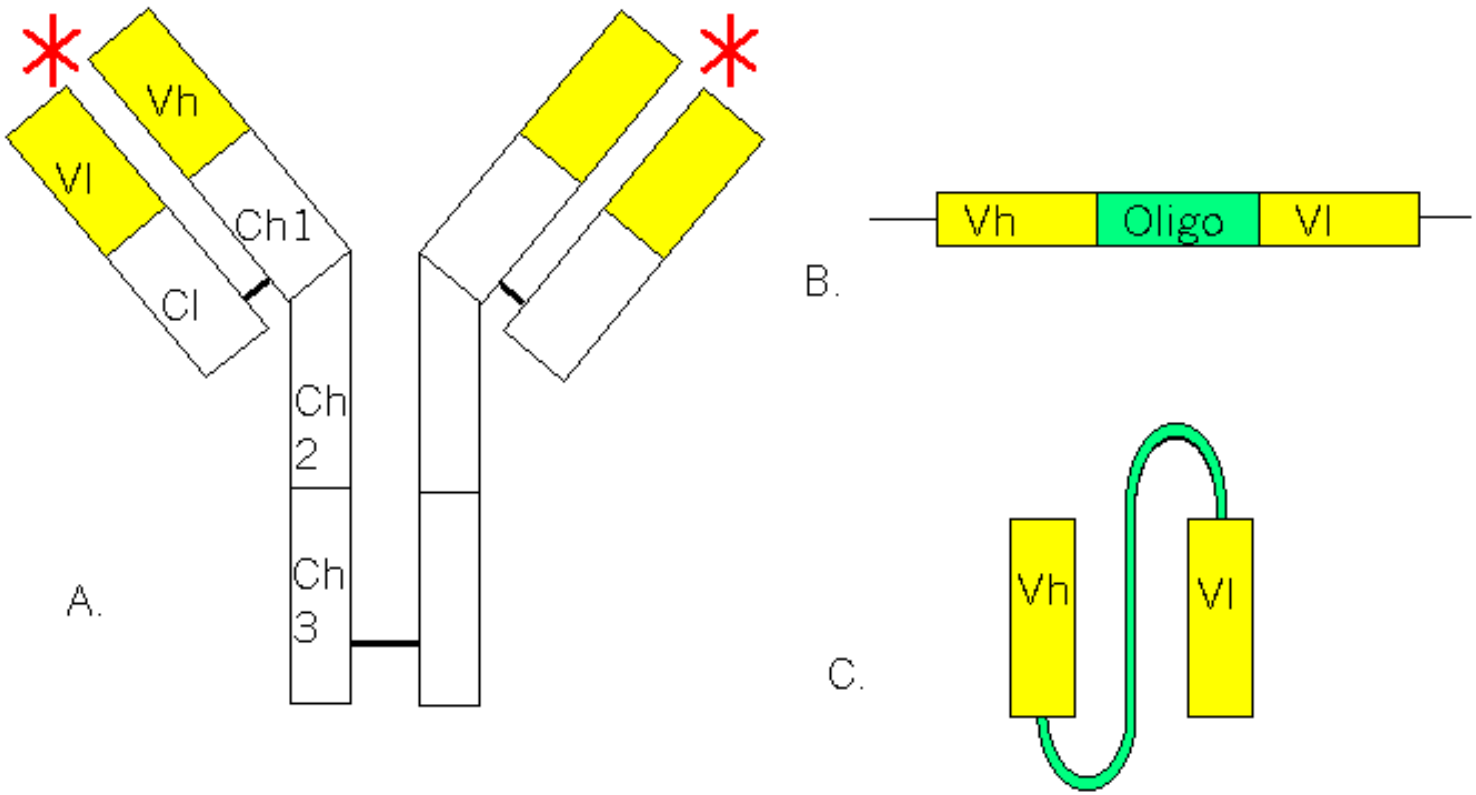
- **Consist of human and mouse sequences**
- **Complementarity determining regions (CDRs) from mouse Ab. Rest from human Ab.**



Single-chain antibodies (scFvs)

- **Consist of the antigen binding portion of antibodies**
- **Variable domains spliced together**
- **Have been shown to inhibit virus replication when expressed intracellularly**

Single-chain antibodies (scFvs)

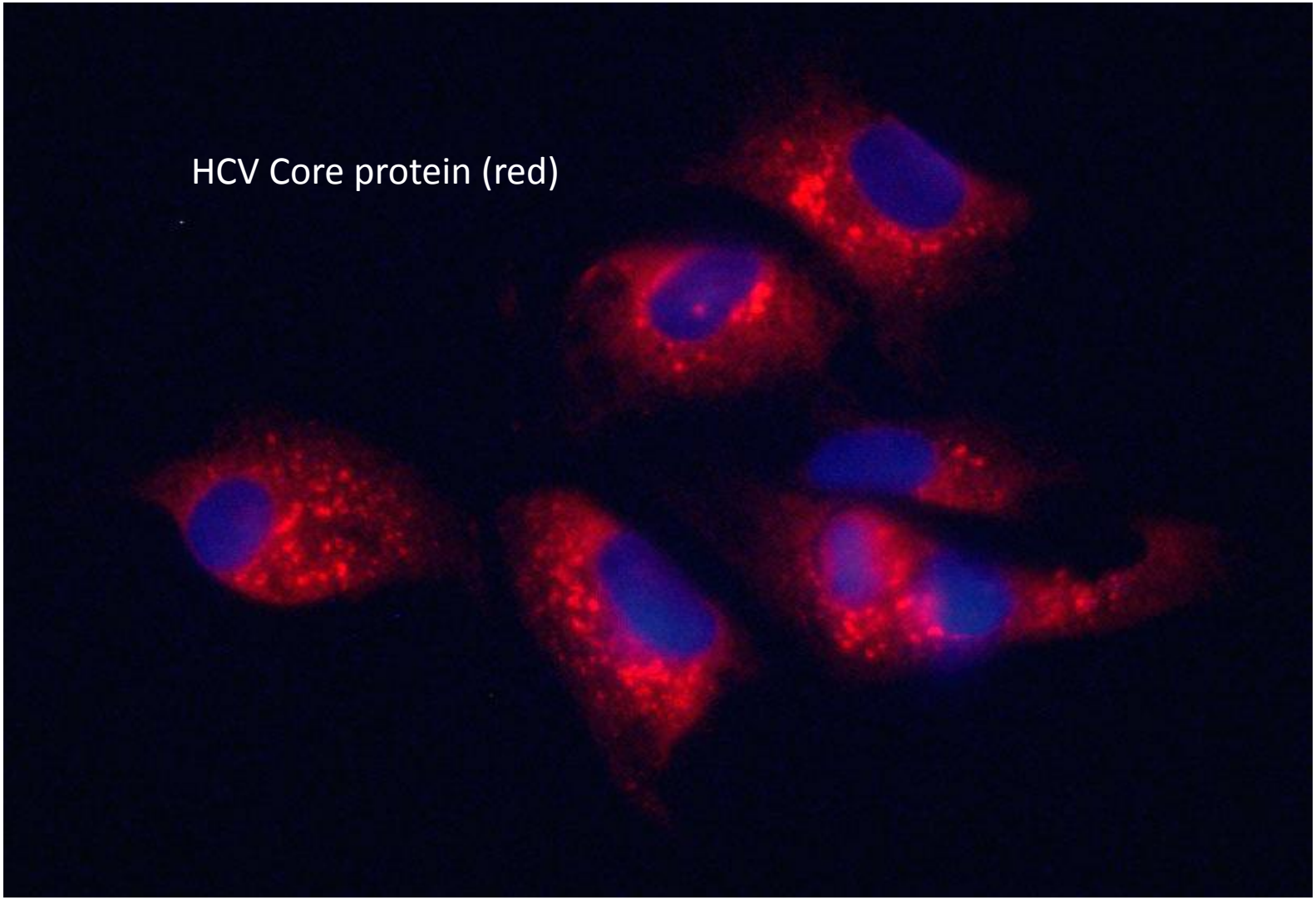


A. Antibody molecule

B. Sequences for variable domains spliced together

C. Single-chain antibody (scFv)

Human Liver Cells Infected with HCV



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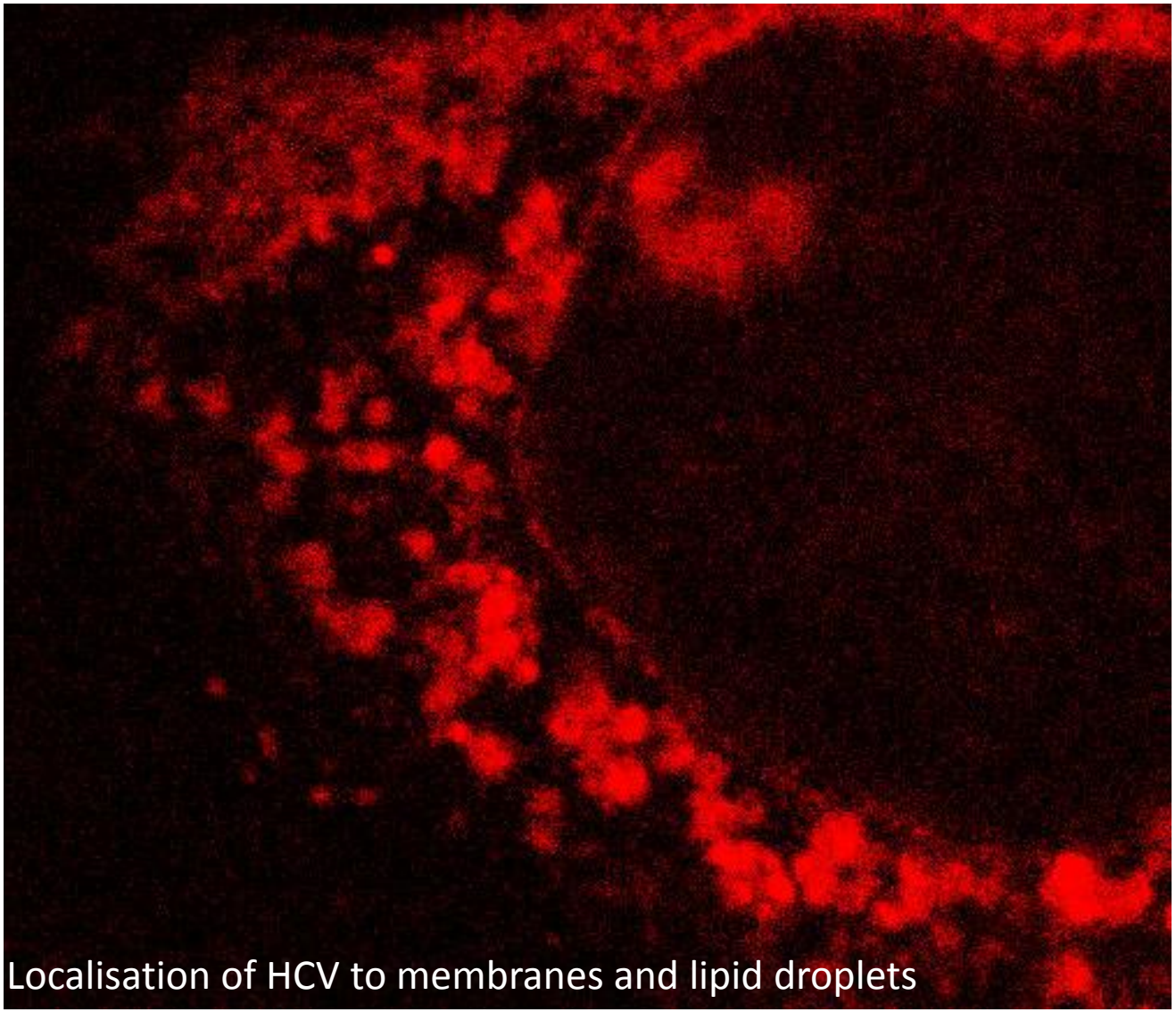
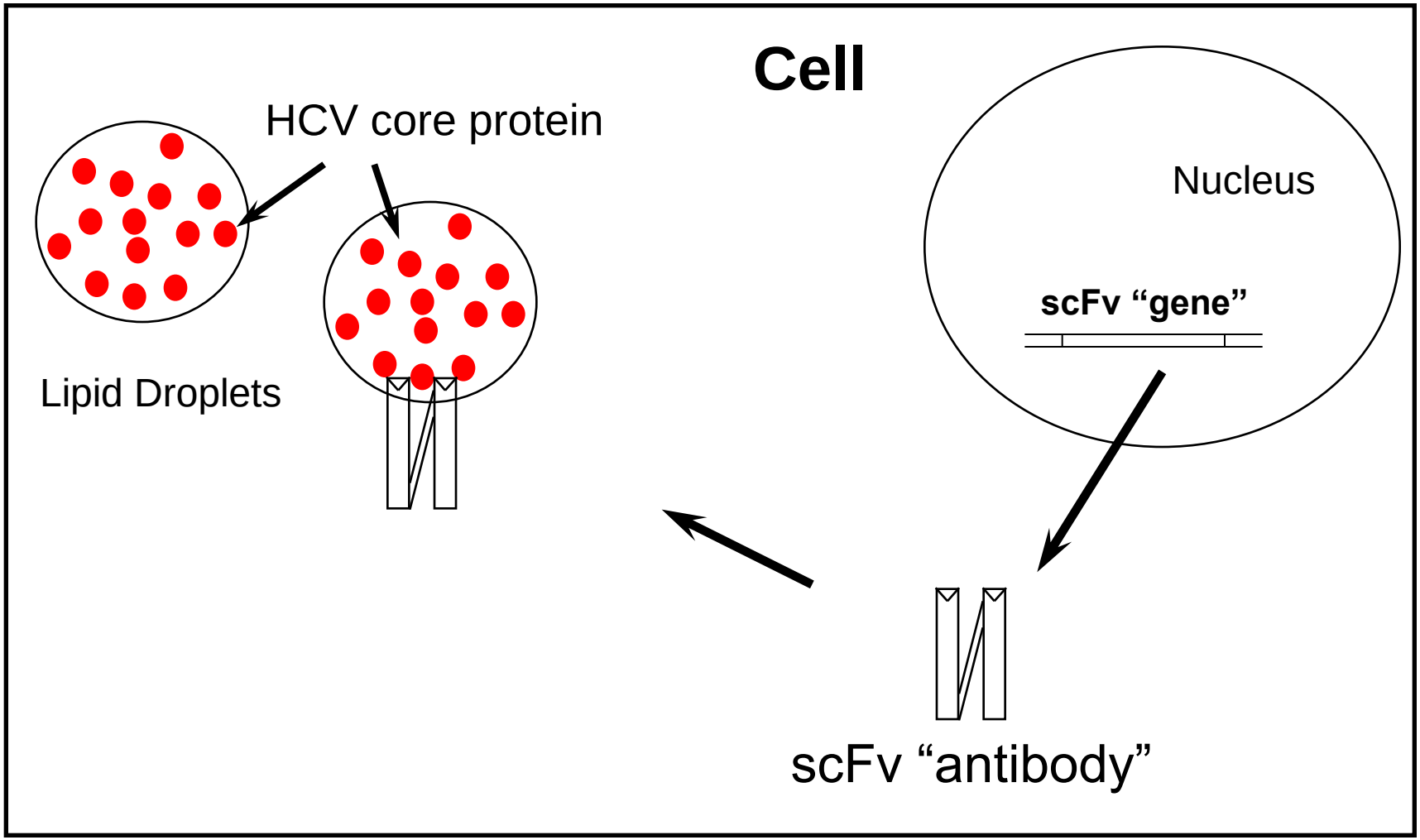
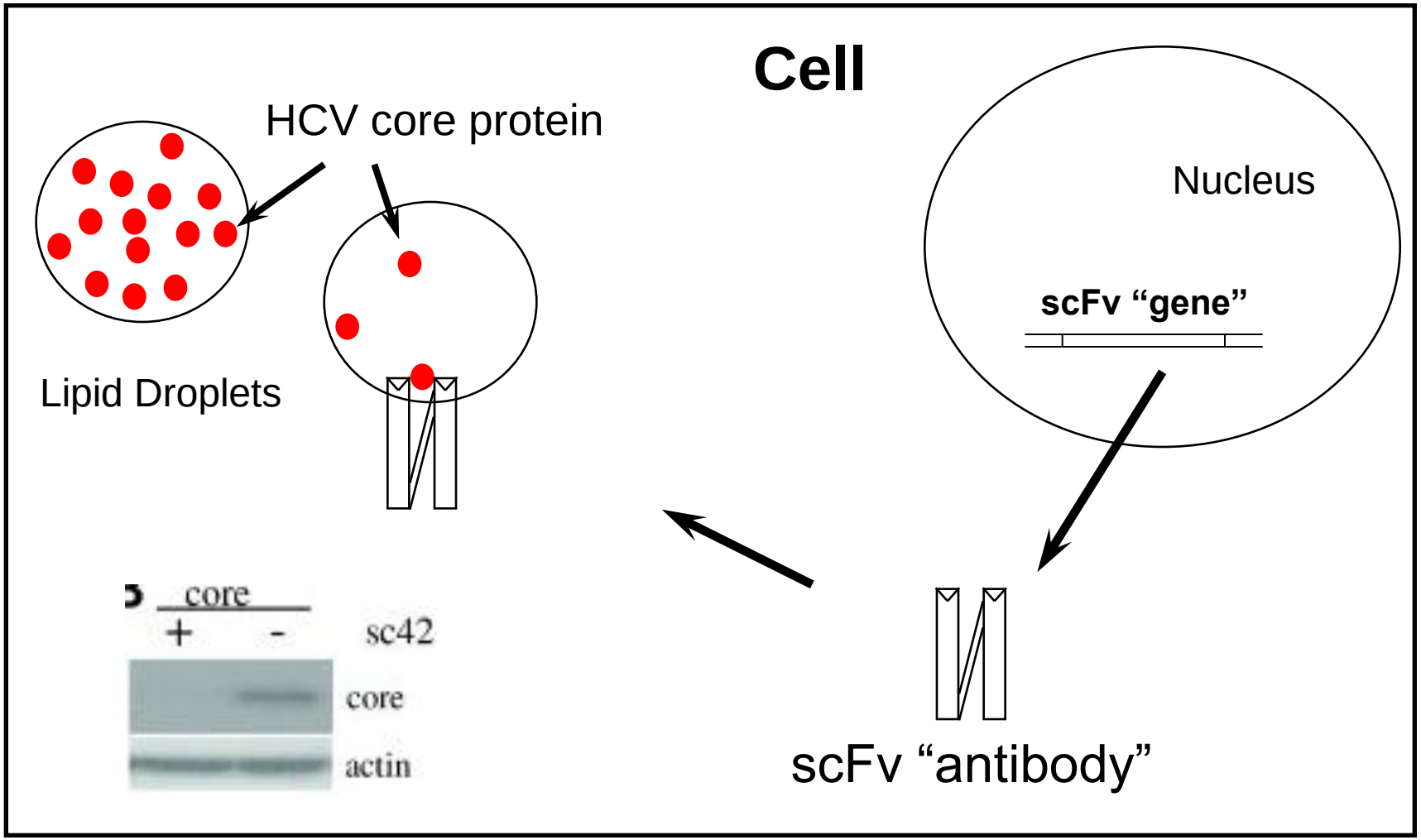


Localisation of HCV to membranes and lipid droplets

Targeting of HCV core protein by an intracellular scFv



Targeting of HCV core protein by an intracellular scFv



(Karthe et al, Hepatology, 2008, 48(3):**702-712.)**

Inhibition of Virus Replication by scFvs

HIV-1 - gp120
- gp41
- intergrase

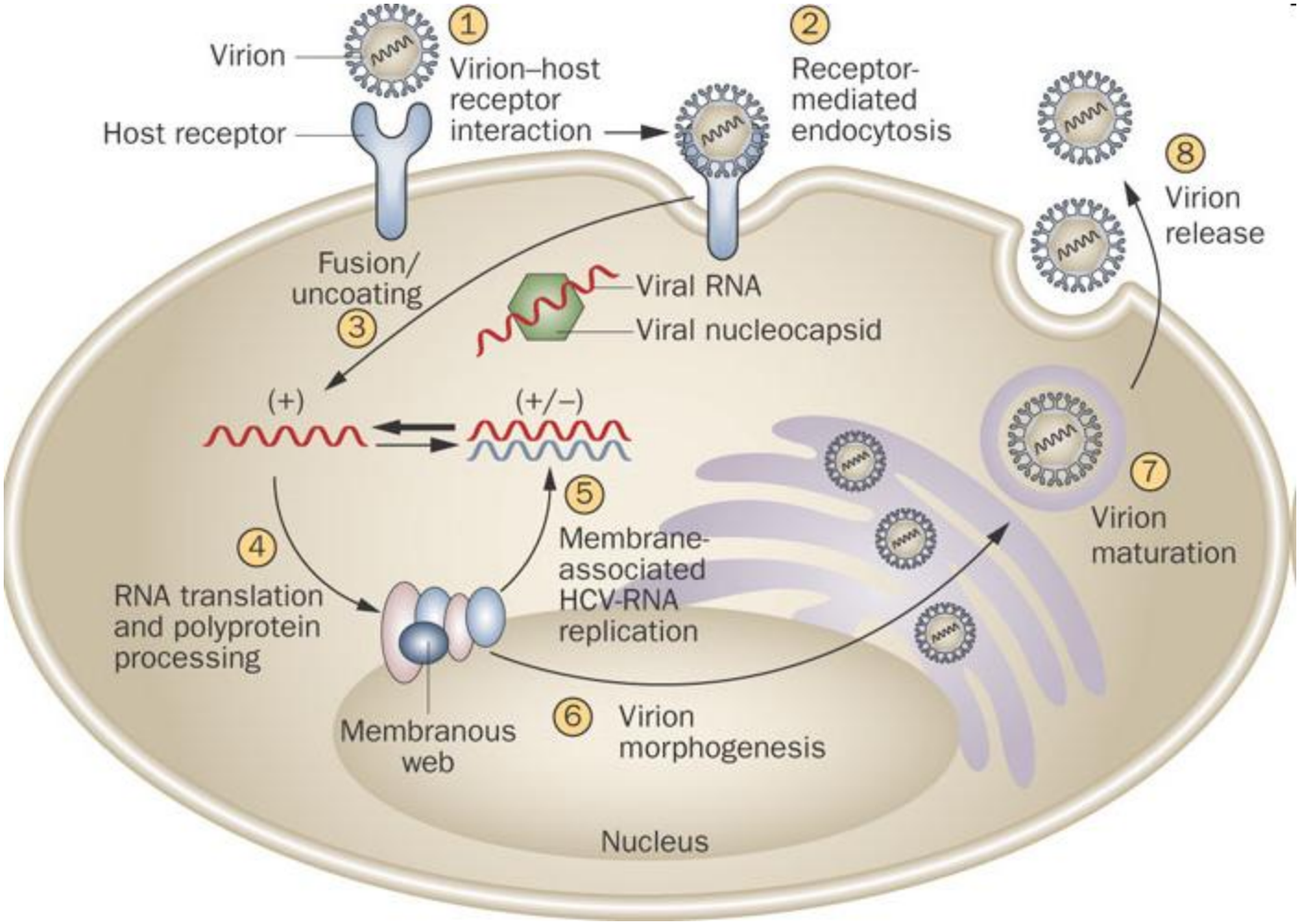
TBE – Envelope protein

VSV – Coat protein

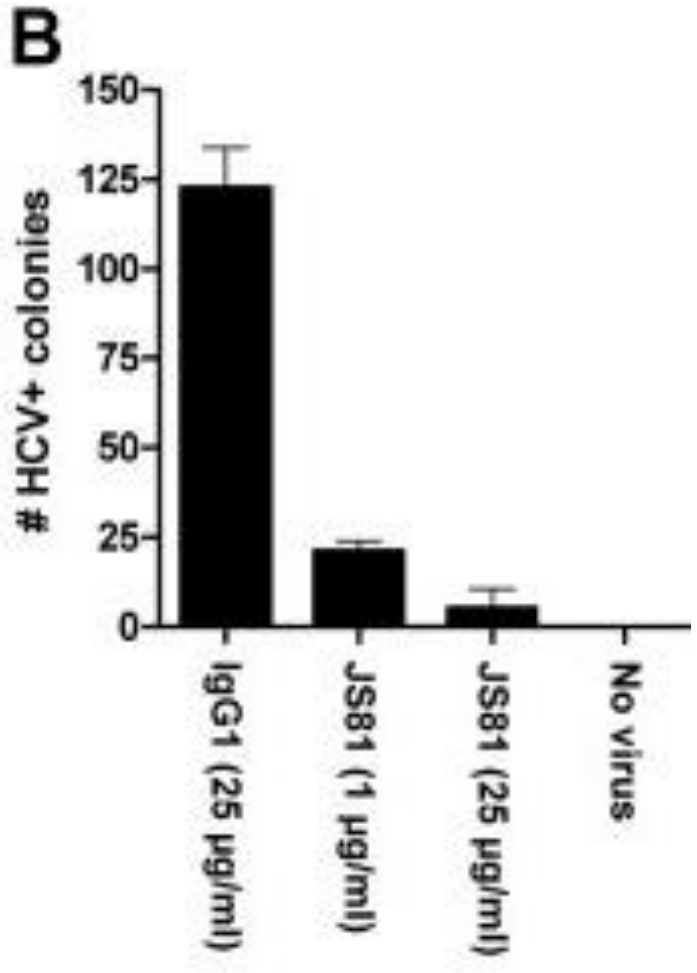
RSV – Fusion protein

HCV – NS3 protease, core protein

Host Targets of HCV Replication

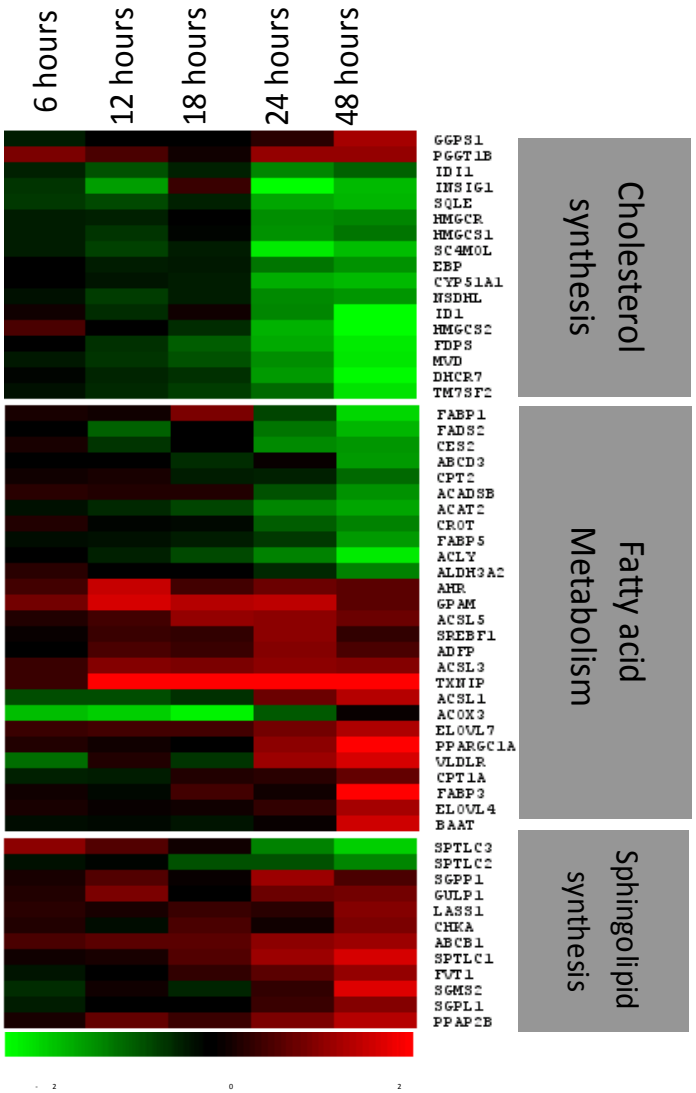


Inhibition of HCV with anti-CD81 Antibodies



Effects of HCV on Host Gene Expression

Gene Microarray Analysis

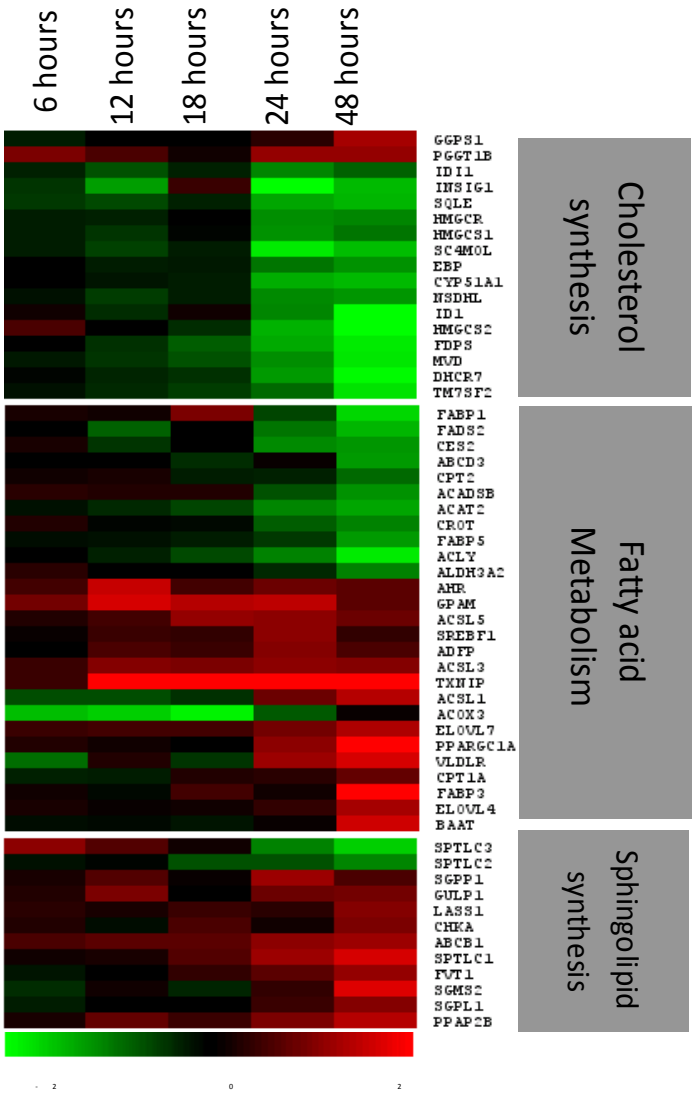


Fold Changes **-2** **0** **+2**

Effects of HCV on Host Gene Expression

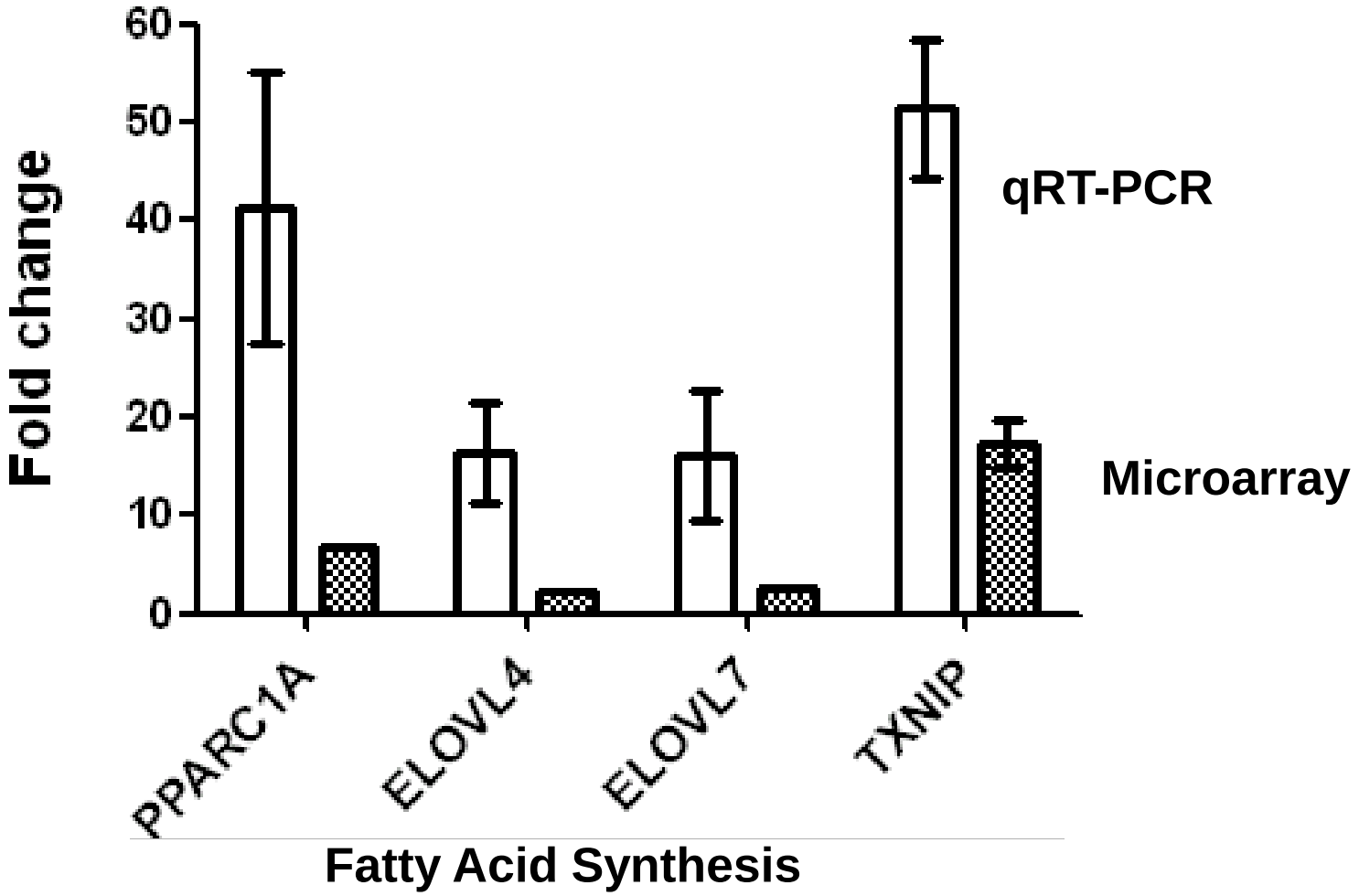
Gene Microarray Analysis

“TXNIP” →

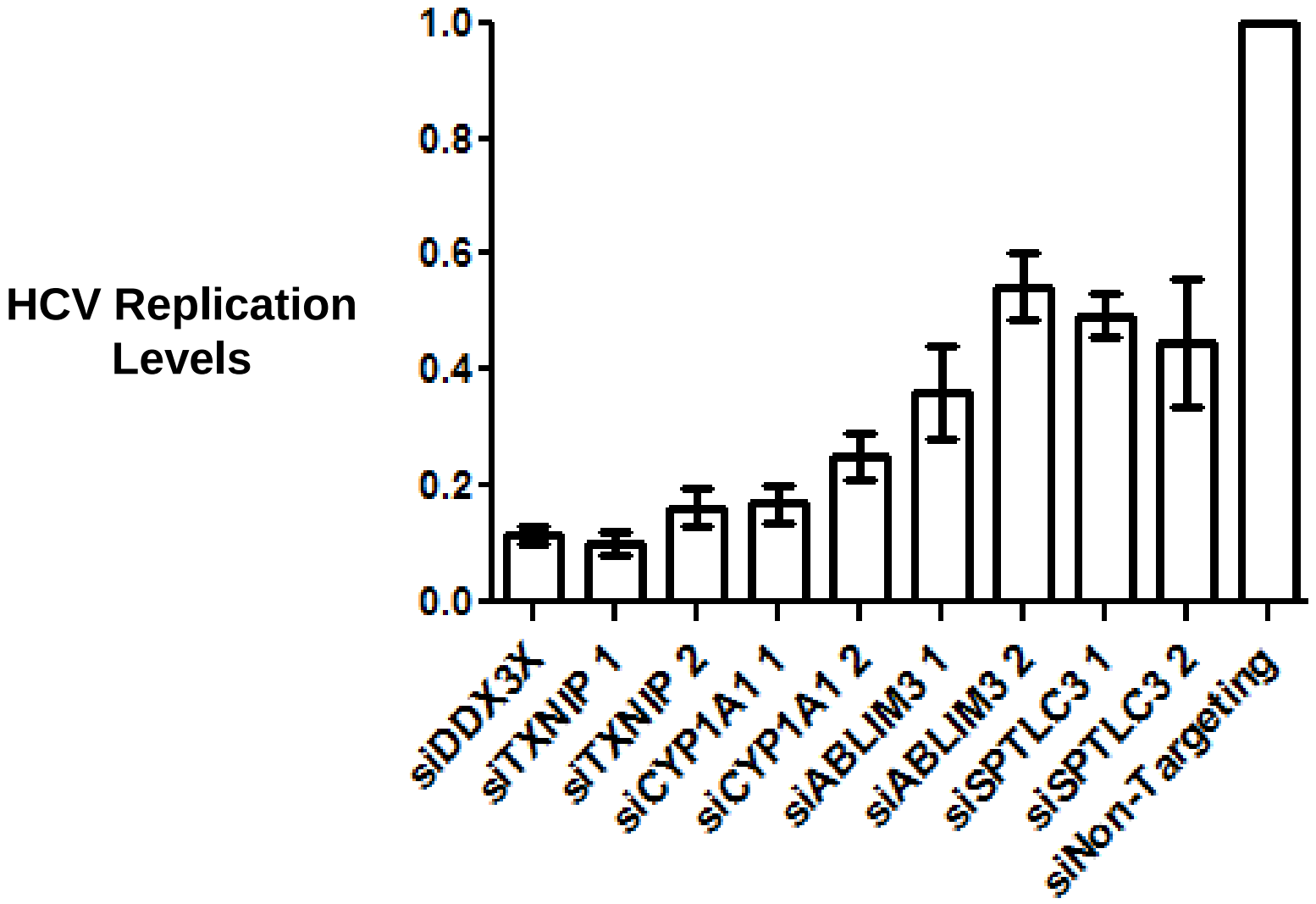


Fold Changes **-2** **0** **+2**

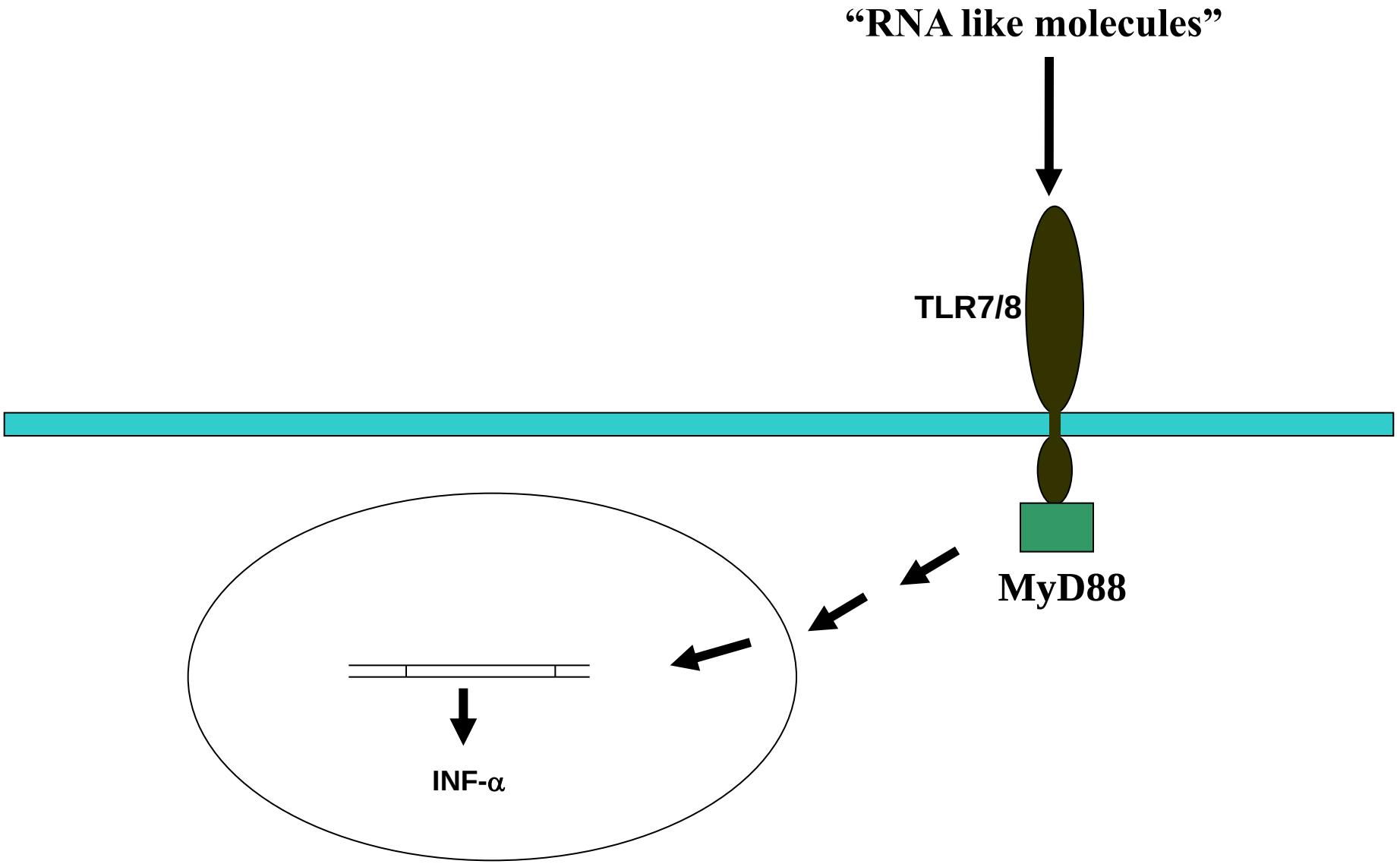
Effects of HCV on Host Gene Expression



Effects of HCV on Targeting Host Genes



Toll-Like Receptors Agonists



Summary New Therapies for HBV

- **Inhibitors of Viral Enzymes – Reverse Transcriptase**
- **Immunomodulators**
- **RNA Interference**
- **Antibody Reagents**
- **APOBEC3G?**

Summary: New Approaches to Antiviral for HCV

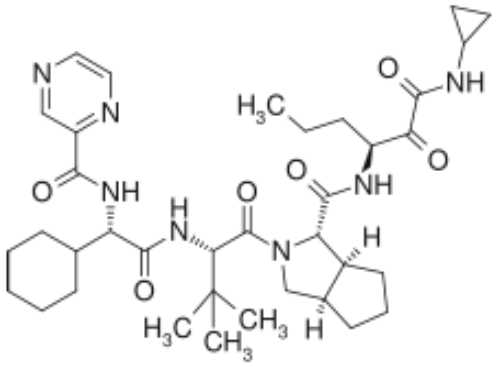
- **Inhibitors of Viral Enzymes**
  - **RNA Polymerase,**
  - **Protease, Helicase**

- **Virus Targets (viral proteins and nucleic acid)**
  - **Antiviral Gene Therapy (siRNAs, miRNAs)**
  - **Antibody Reagents (scFvs)**

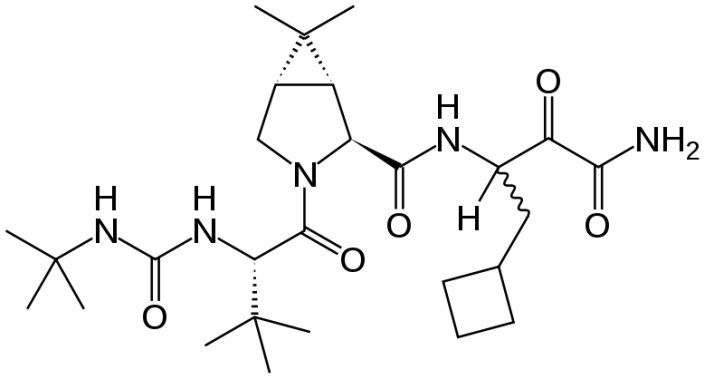
- **Host Targets**
  - **Immunomodulators**
  - **Metabolic enzymes**
  - **Signal regulatory proteins**

New Antivirals for HCV

Inhibitors of Viral NS3 Protease



Telaprevir (VX-950),



Boceprevir

New Antivirals for HCV - in Clinical Trials

Drug	Mechanism	Clinical trial
Albuferon	IFN with prolonged half-life	Phase 2
Omega interferon	IFN for continuous infusion	Phase 2
NM 283 (valopicitabine)	NS5B polymerase inhibitor	Phase 2b
VX-950	NS3 protease inhibitor	Phase 1b
SCH 503034	NS3 protease inhibitor	Phase 2a
Viramidine	Ribavirin prodrug	Phase 3
VX-497 (Merimepodib)	**IMPDH inhibitor**	**Phase 2**
Histamine dihydrochloride	**Immunomodulatory/antioxidant**	**Phase 2**
Thymalfasin	**Prodrug of thymosin-α1 (immunomodulatory)**	**Phase 2**
Isatoribine	**TLR7 agonist**	**Phase 1**
Etanercept	**TNF blocker**	**Phase 2**

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