

**New Approaches to Antivirals for HBV**  
**and HCV 1/02/12**

**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).**

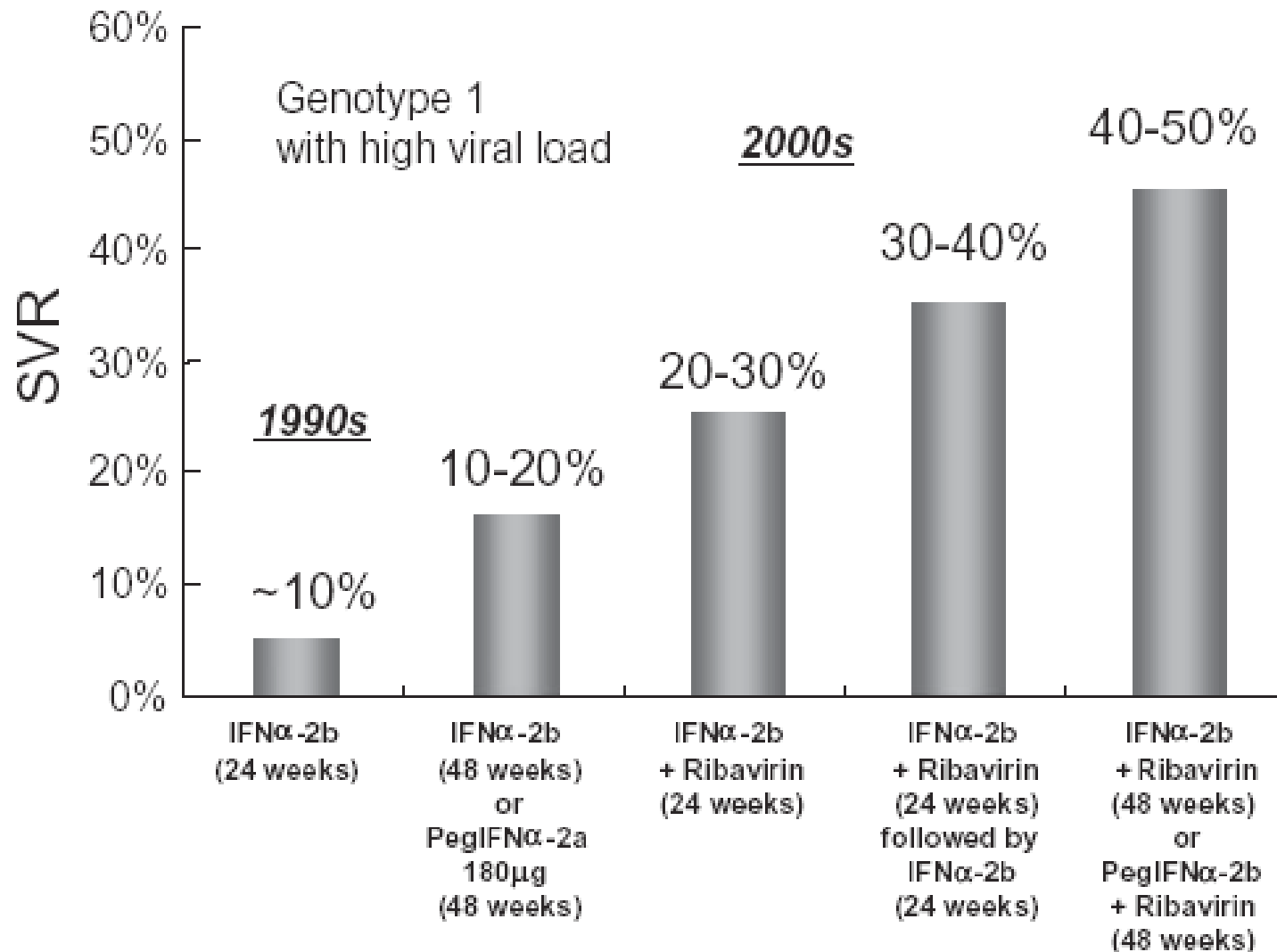
# **Current Therapy for Chronic HCV**

**Treat patients with viraemia and moderate/severe hepatitis;**

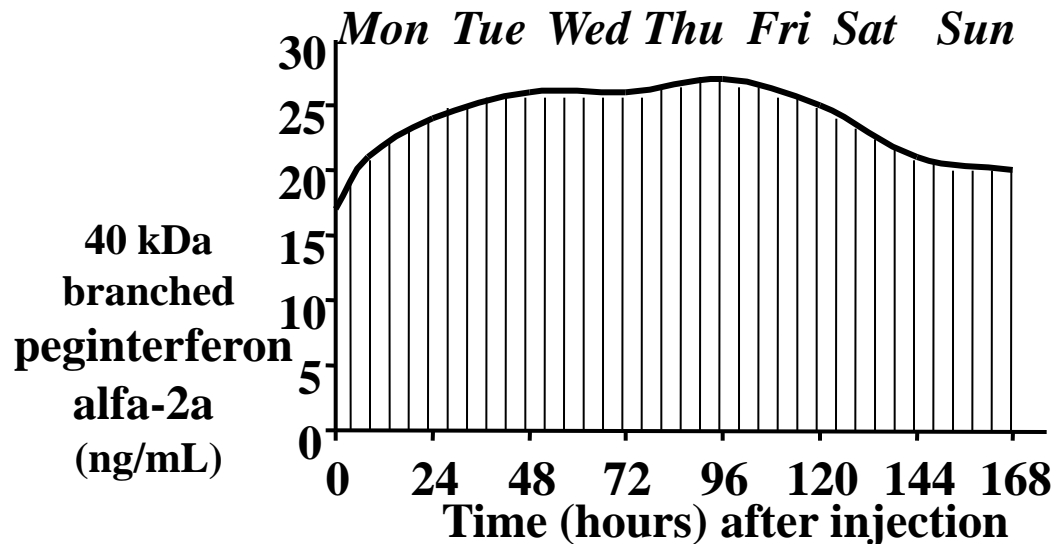
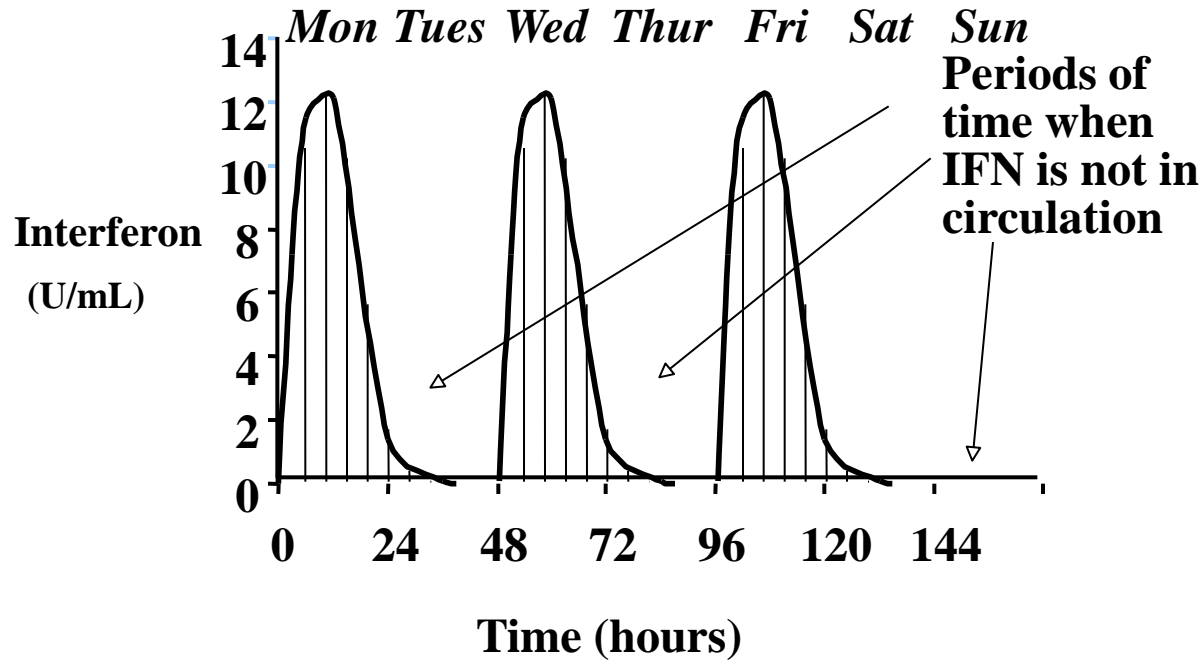
**Genotypes 1 and 4: 12 months interferon alpha (3miu tiw) and ribavirin (600-1200mg/day);**

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

# Evolving Therapy for Chronic HCV



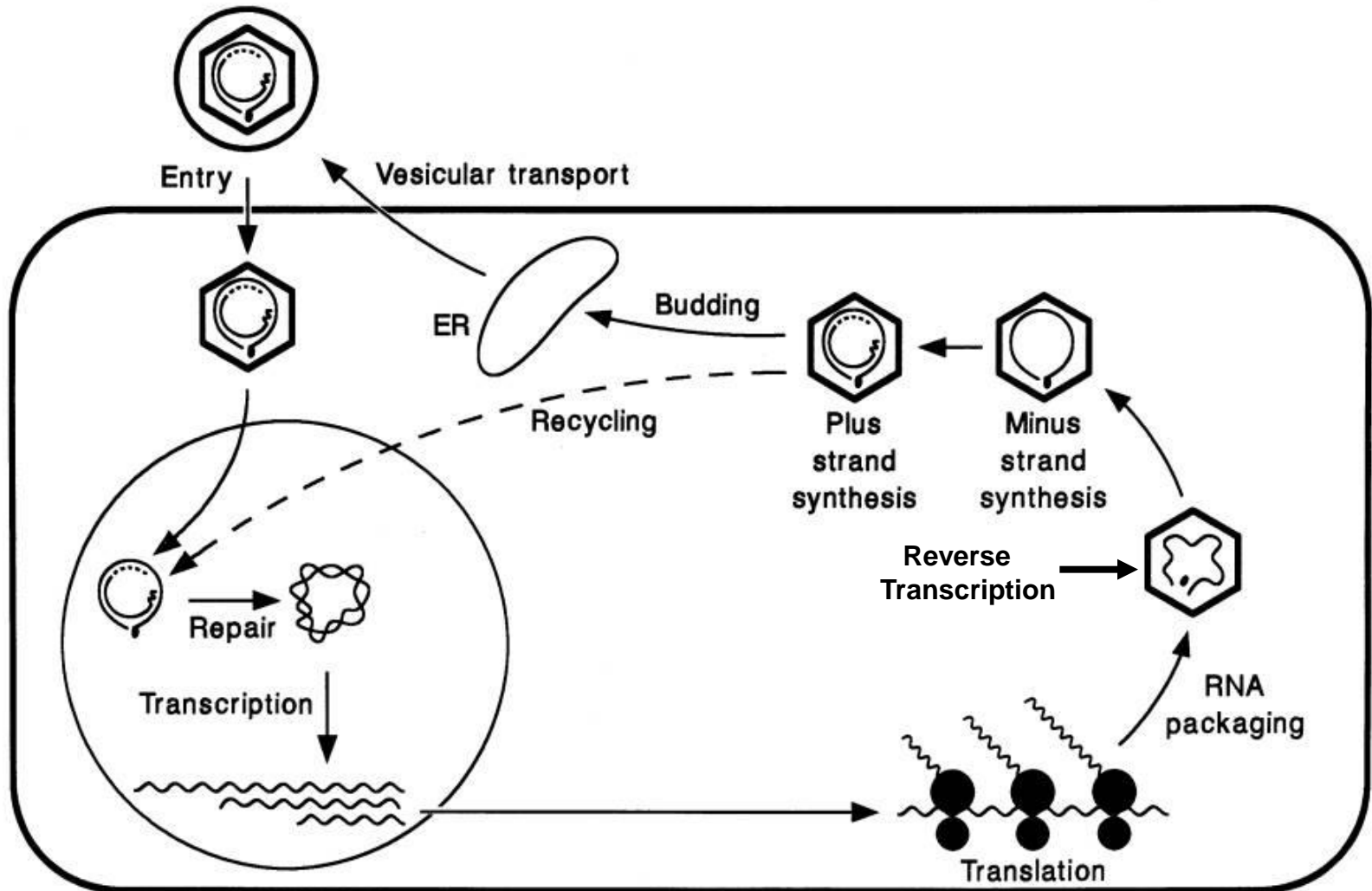
# Peg-IFN $\alpha$ -2a Blood Levels do not fluctuate



# New Antivirals for HCV - in Clinical Trials

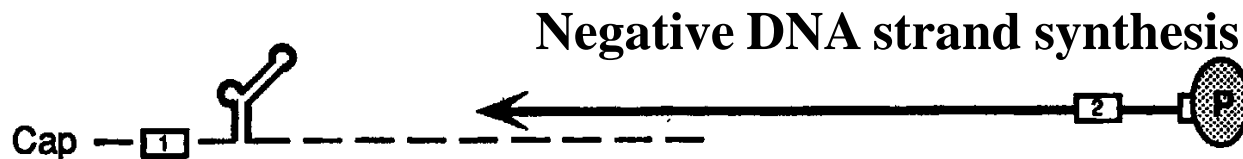
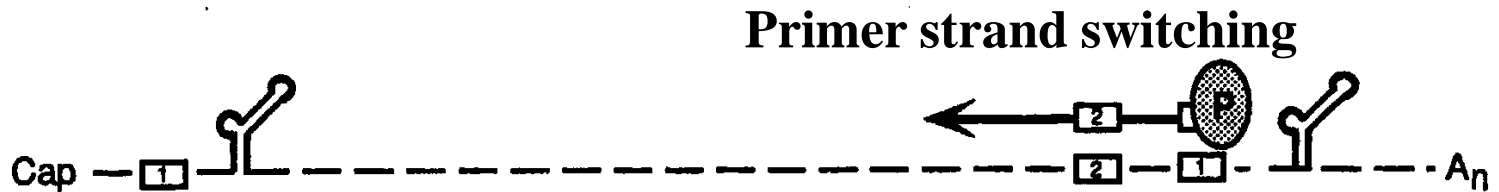
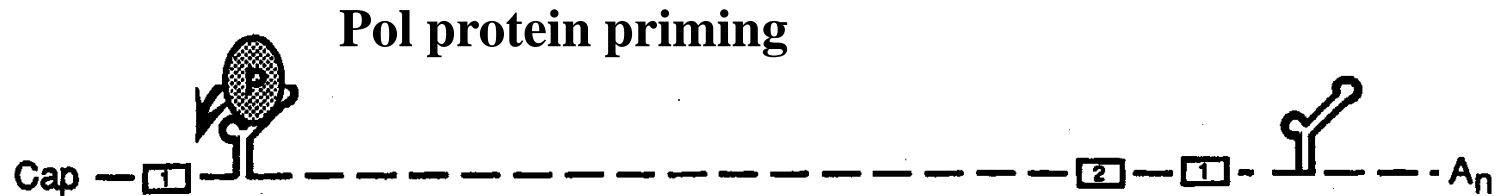
<b>Drug</b>	<b>Mechanism</b>	<b>Clinical trial</b>
<b>Albuferon</b>	<b>IFN with prolonged half-life</b>	<b>Phase 2</b>
<b>Omega interferon</b>	<b>IFN for continuous infusion</b>	<b>Phase 2</b>

# HBV Replication Cycle

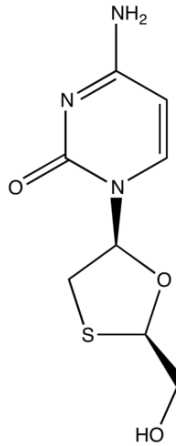




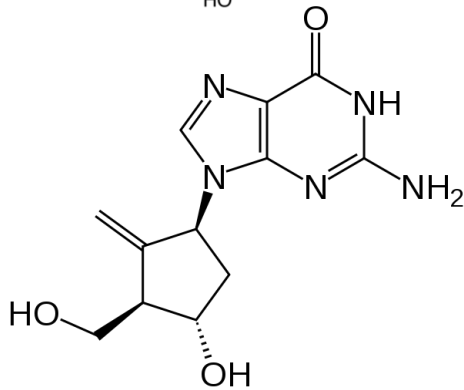
# HBV Genome Replication



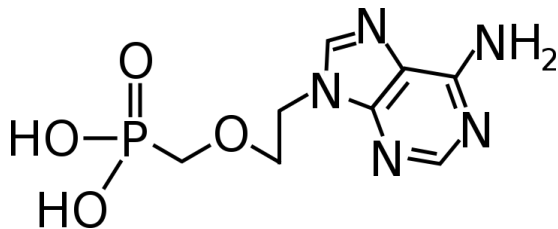
# Reverse Transcription Inhibitors (Nucleotide and Nucleoside Analogues) for Chronic HBV



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



**Entecavir** 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

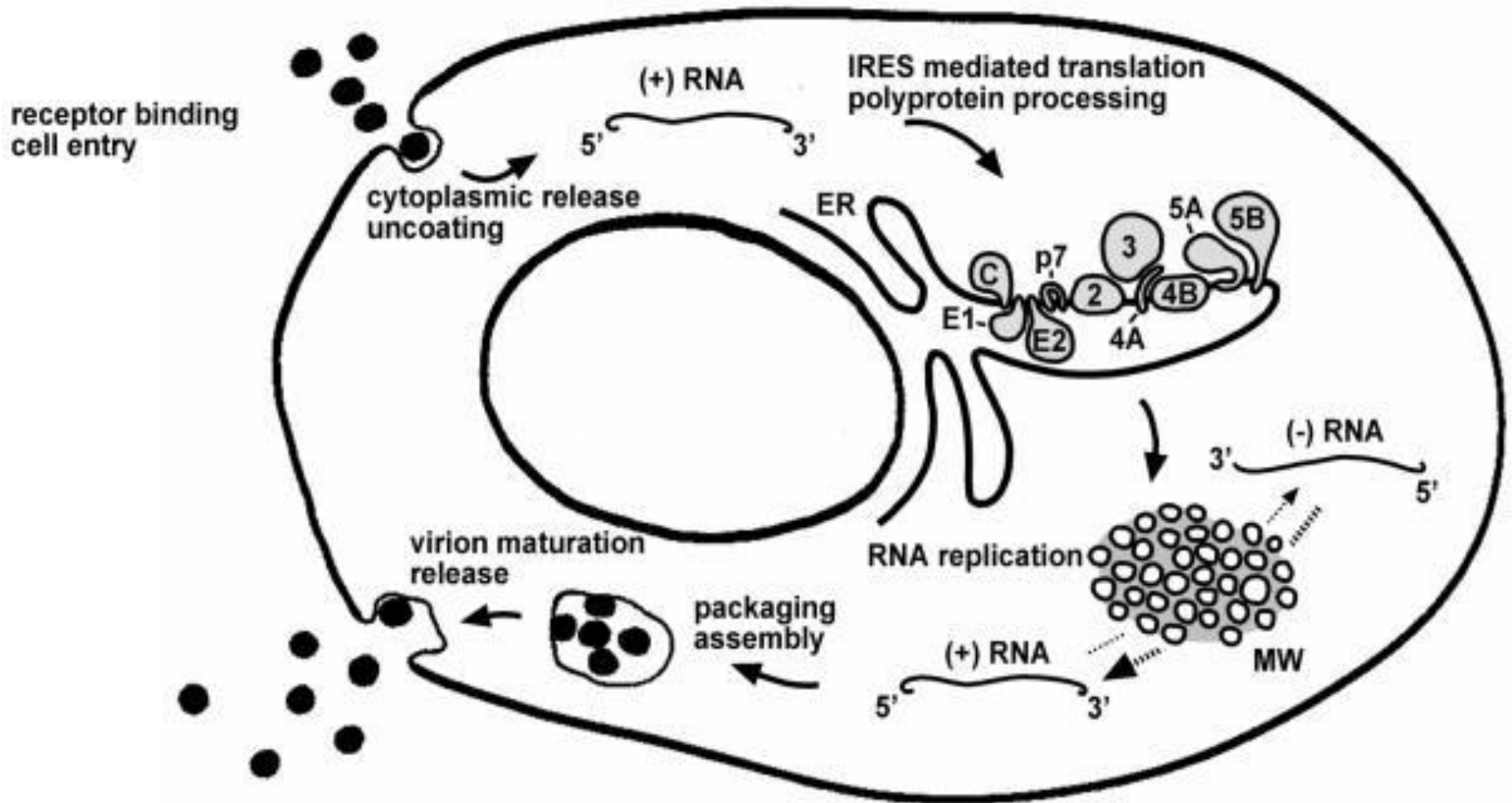
Telbivudine

Phase III

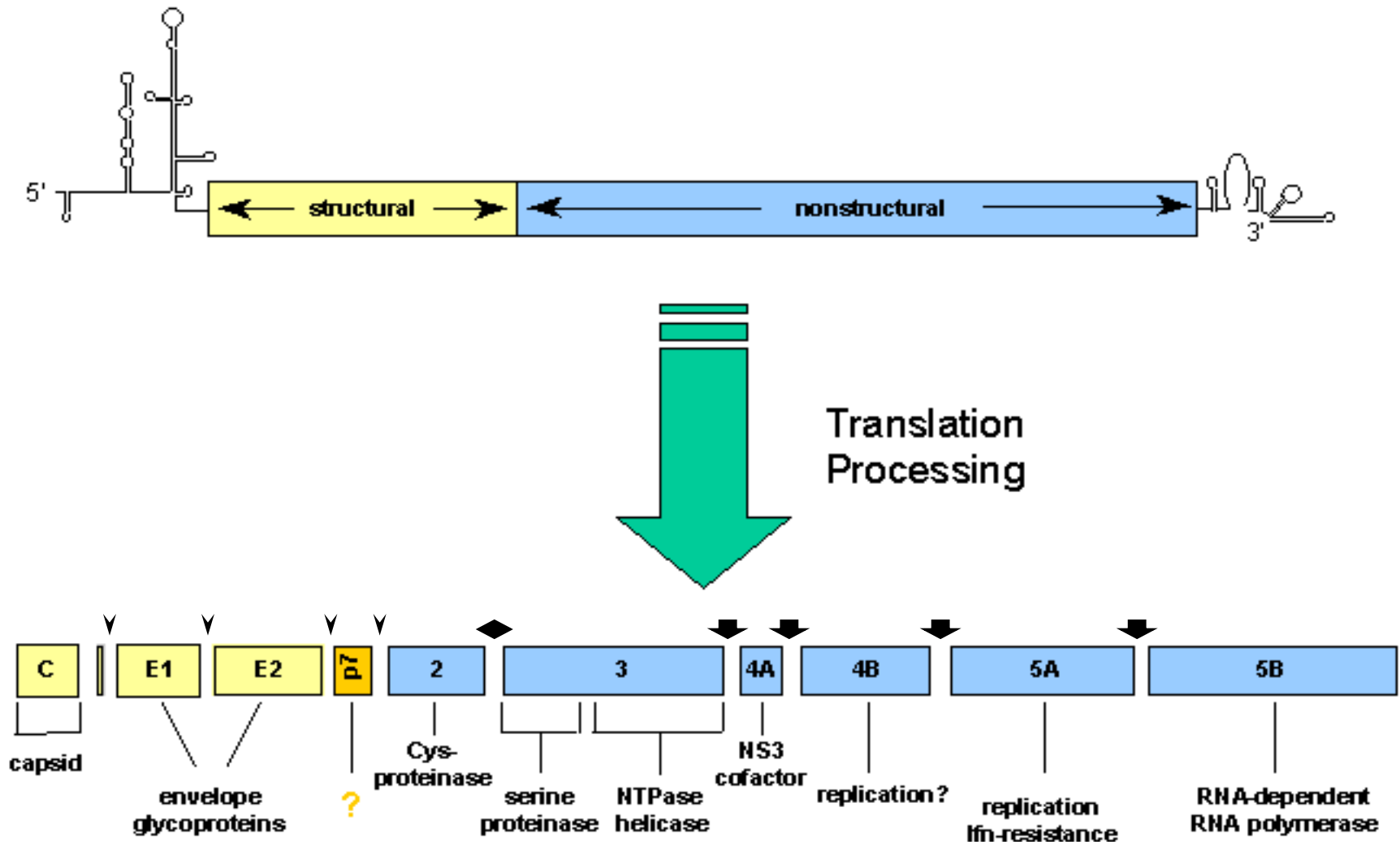
Clevudine

Phase II

# HCV RNA Replication



# HCV RNA As a Target for RNAi

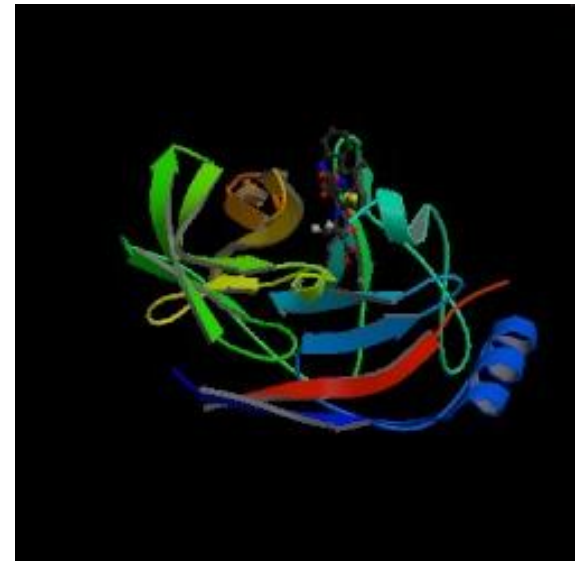


# Properties of NS3

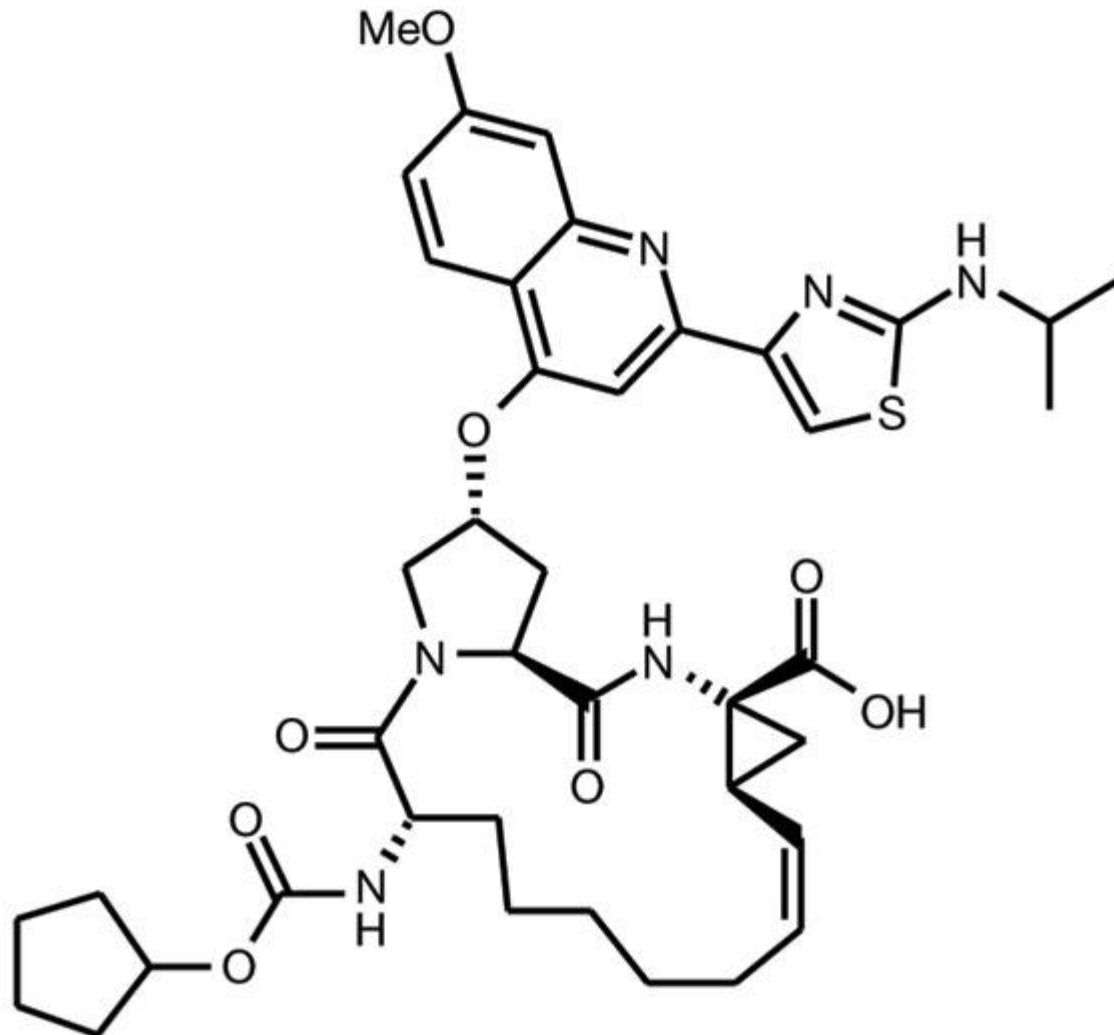
- **Protease**
- **Helicase in replication complex**
- **ATPase (associated with helicase activity)**
- **May contribute to viral persistence in HCV**

# NS3 Protease

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

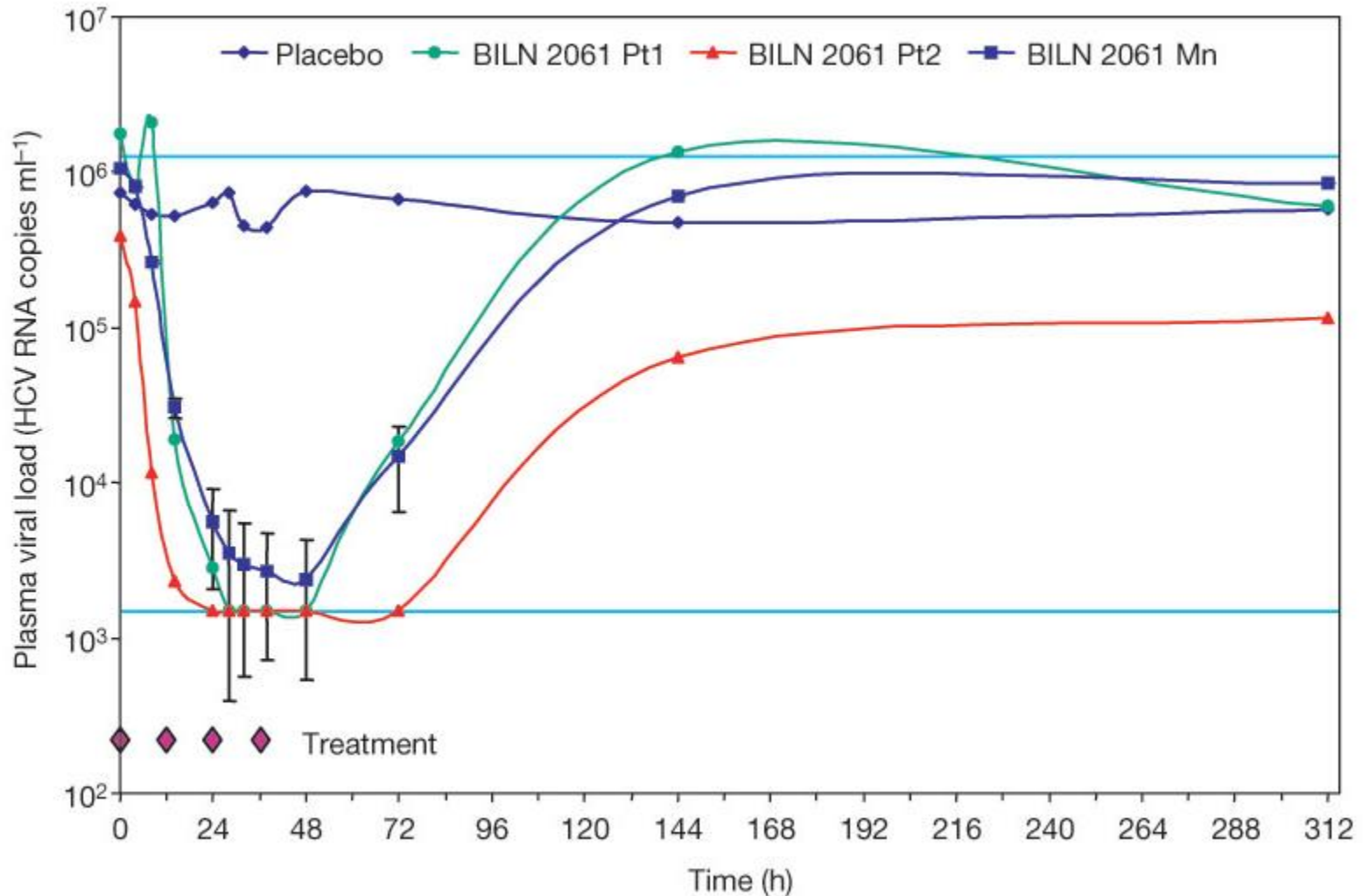


# HCV Protease Inhibitor: BILN 2061





# BILN 2061: HCV Infected Human Patients



# New Antivirals for HCV - in Clinical Trials

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

# Gene Therapy

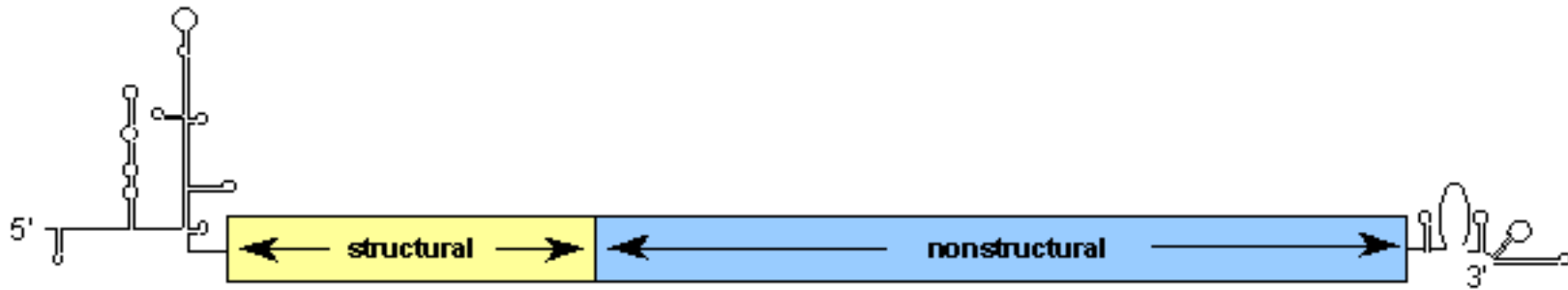
# HCV

- **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**

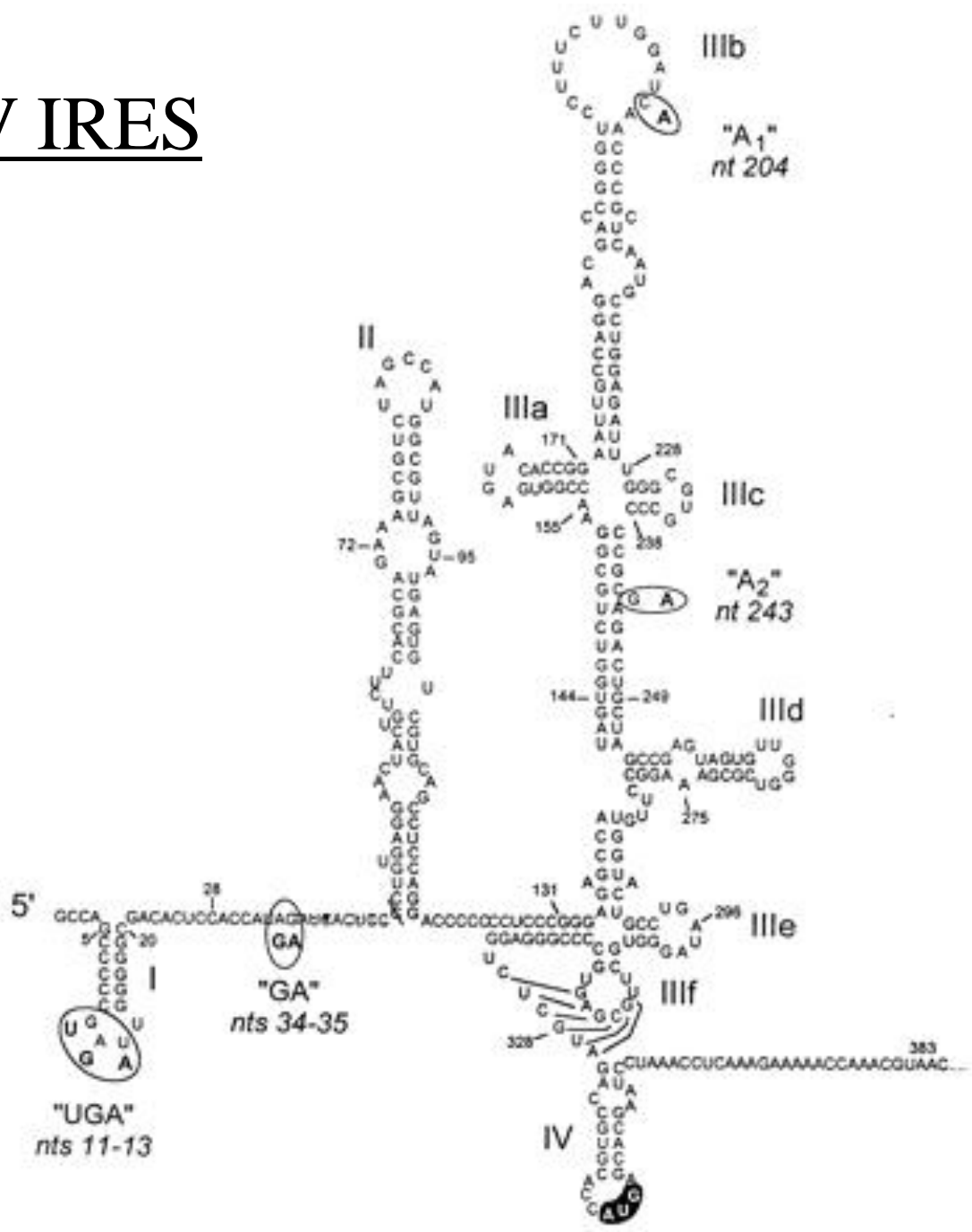
# HCV Genome

- **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

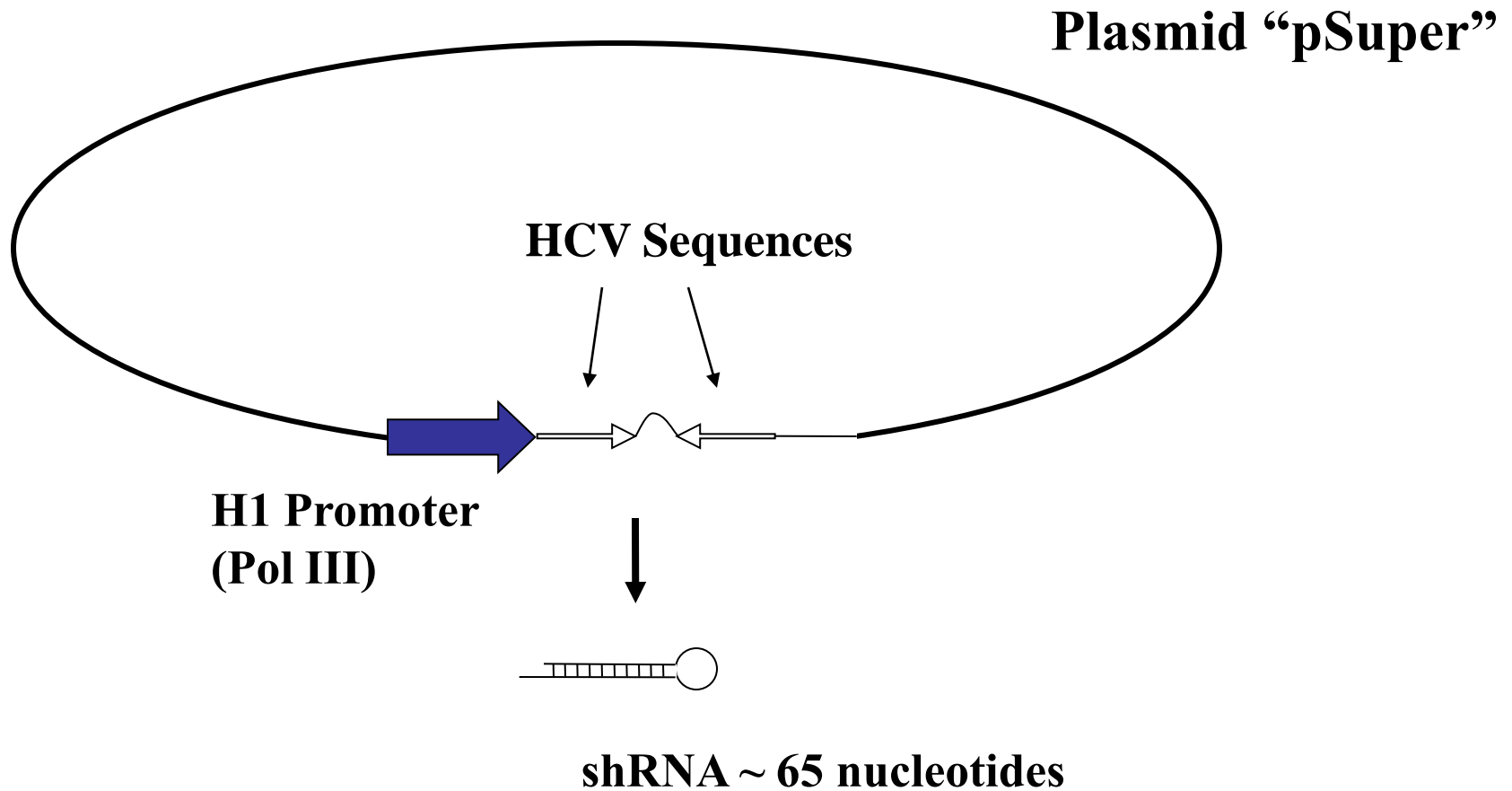


# RNA Interference (RNAi)

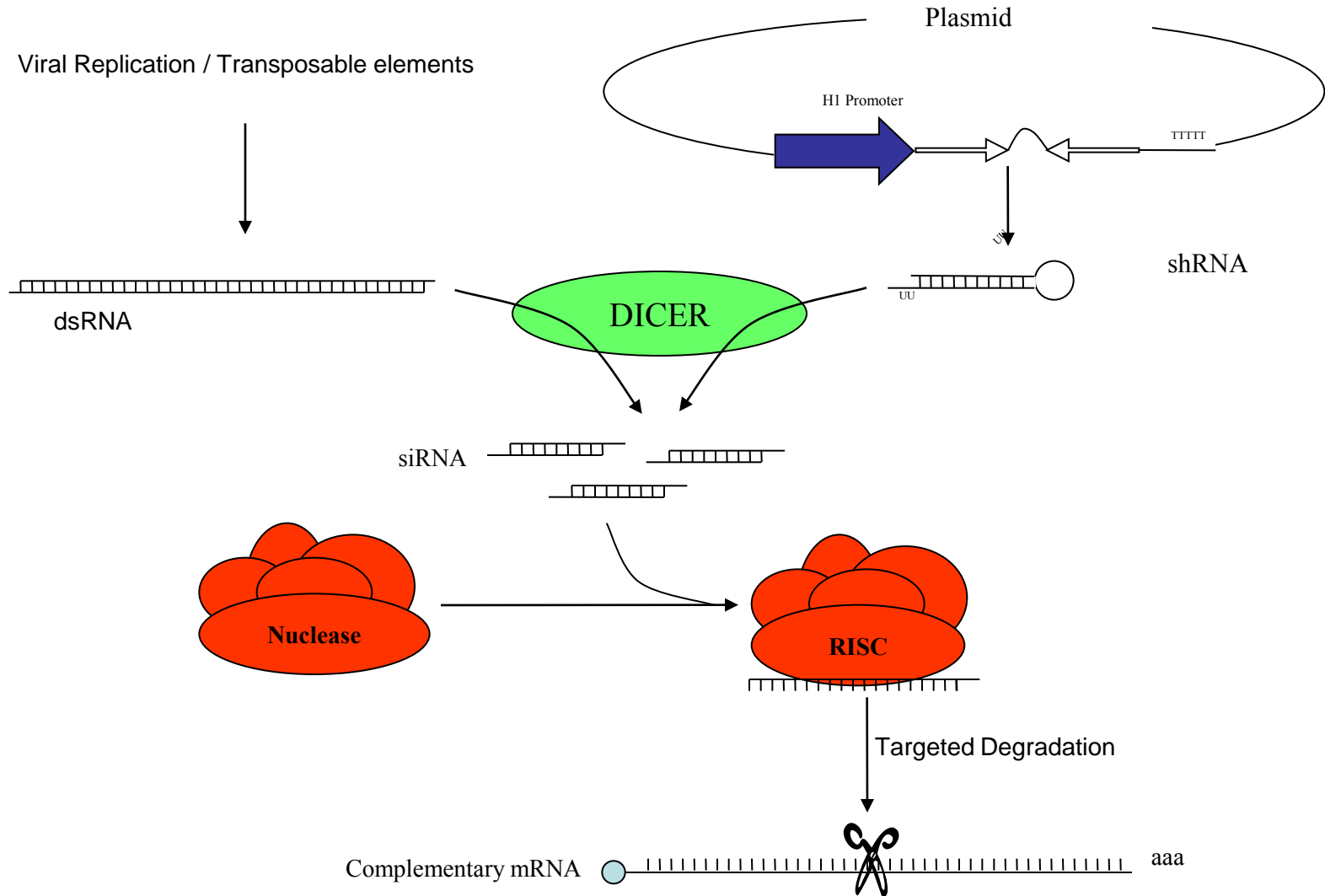
- **RNAi: Introduction of dsRNA into a cell inhibits gene expression in a sequence dependent fashion.**
- ***C. elegans* (Fire *et al*, 1998, Nature, 391: 806-811).**
- **This phenomenon resulted in sequence-specific gene silencing.**



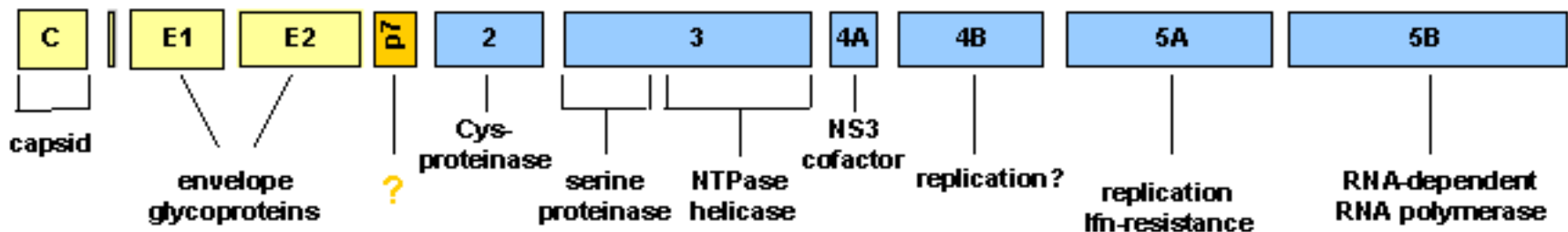
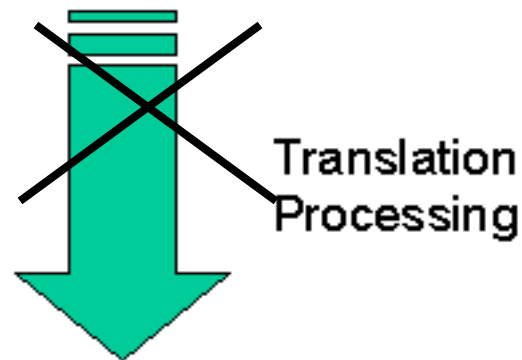
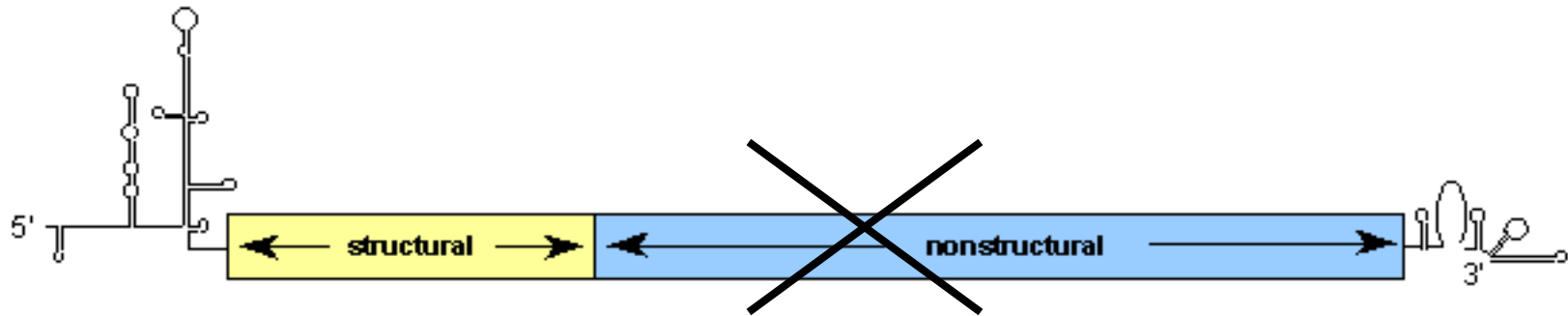
# si RNA Expression from DNA Plasmids



# 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**

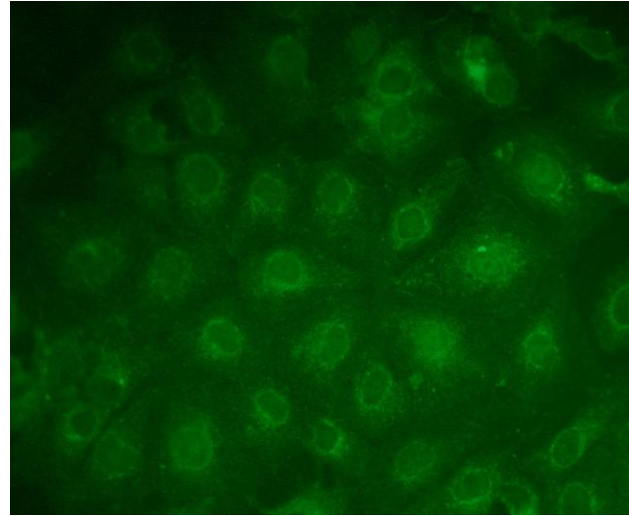
- **pSUPER-HCV3 reduced NS3 expression by more than 90%.**

# HCV RNAi: RNA Replicon Cell Line

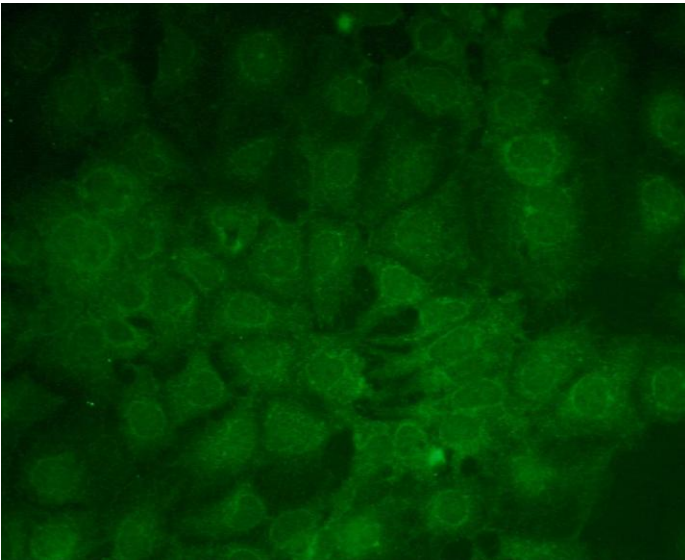
Huh7  
cells



S1179I  
Replicon



pSUPER-  
Control

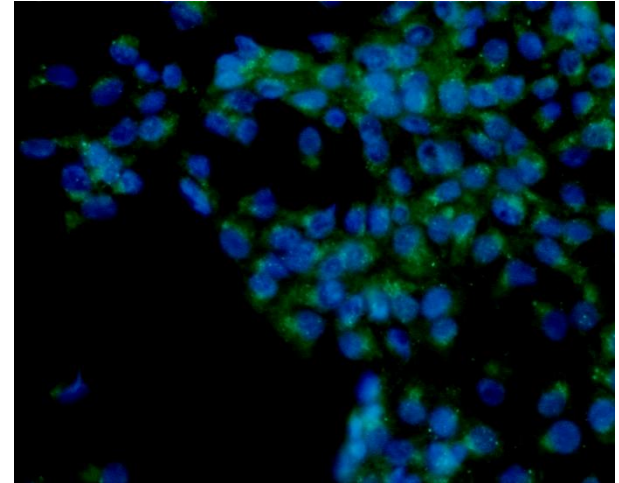
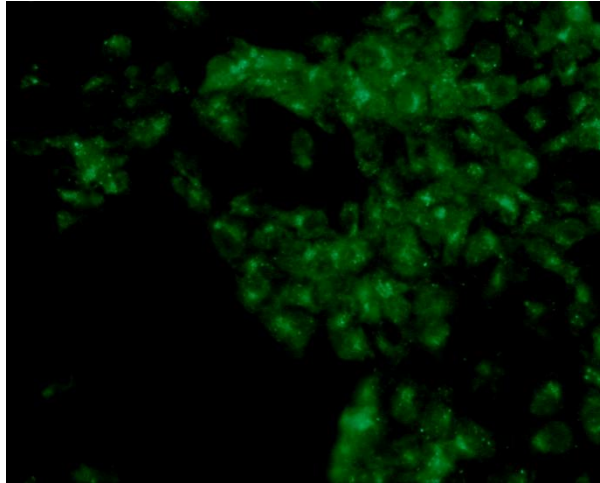


siNS3-3

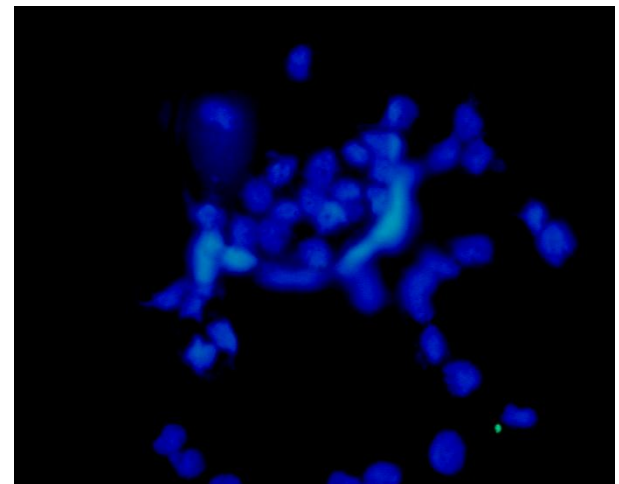
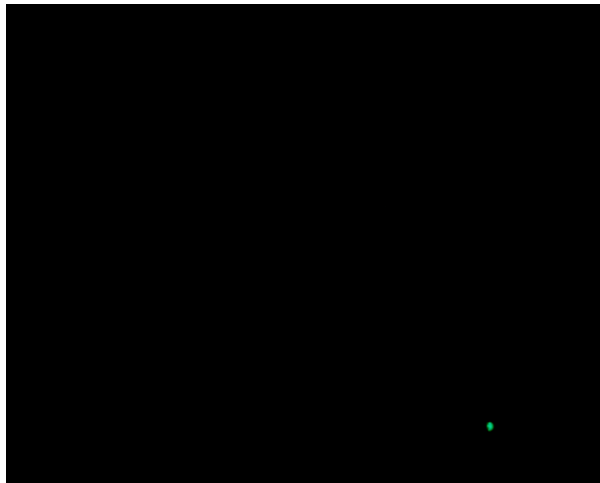


# siRNA-Adenovirus: HCV Infected Cells

Ad-siRNA-C



Ad-siNS3-3



Anti-NS3 Antibodies

DAPI / Anti-NS3 Antibodies

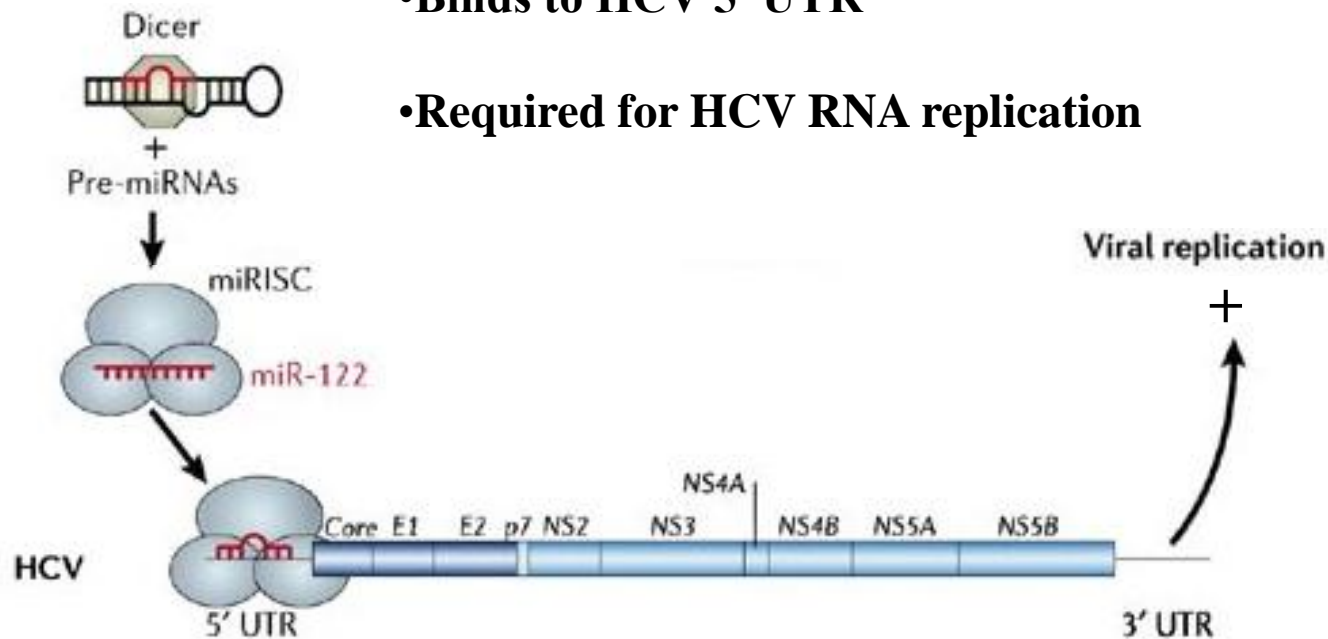
# HCV RNAi: RNA Replicon Cell Line

- **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.**

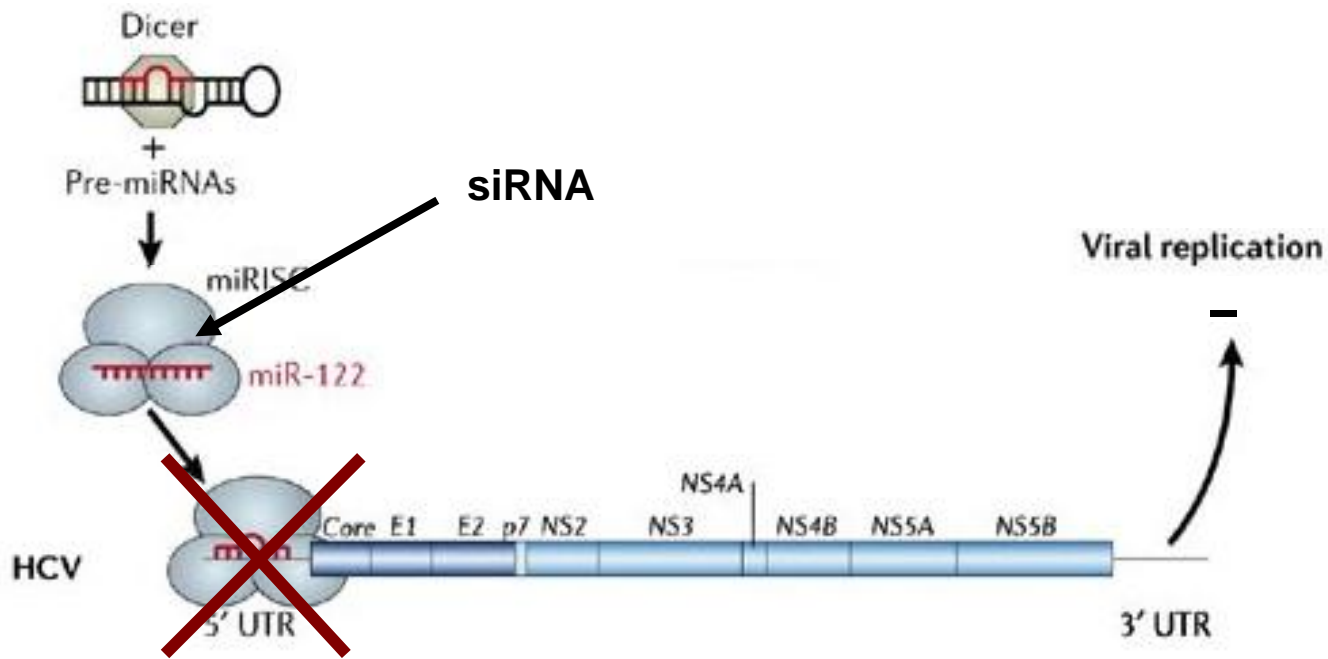


# MicroRNA 122 (miR-122) and HCV

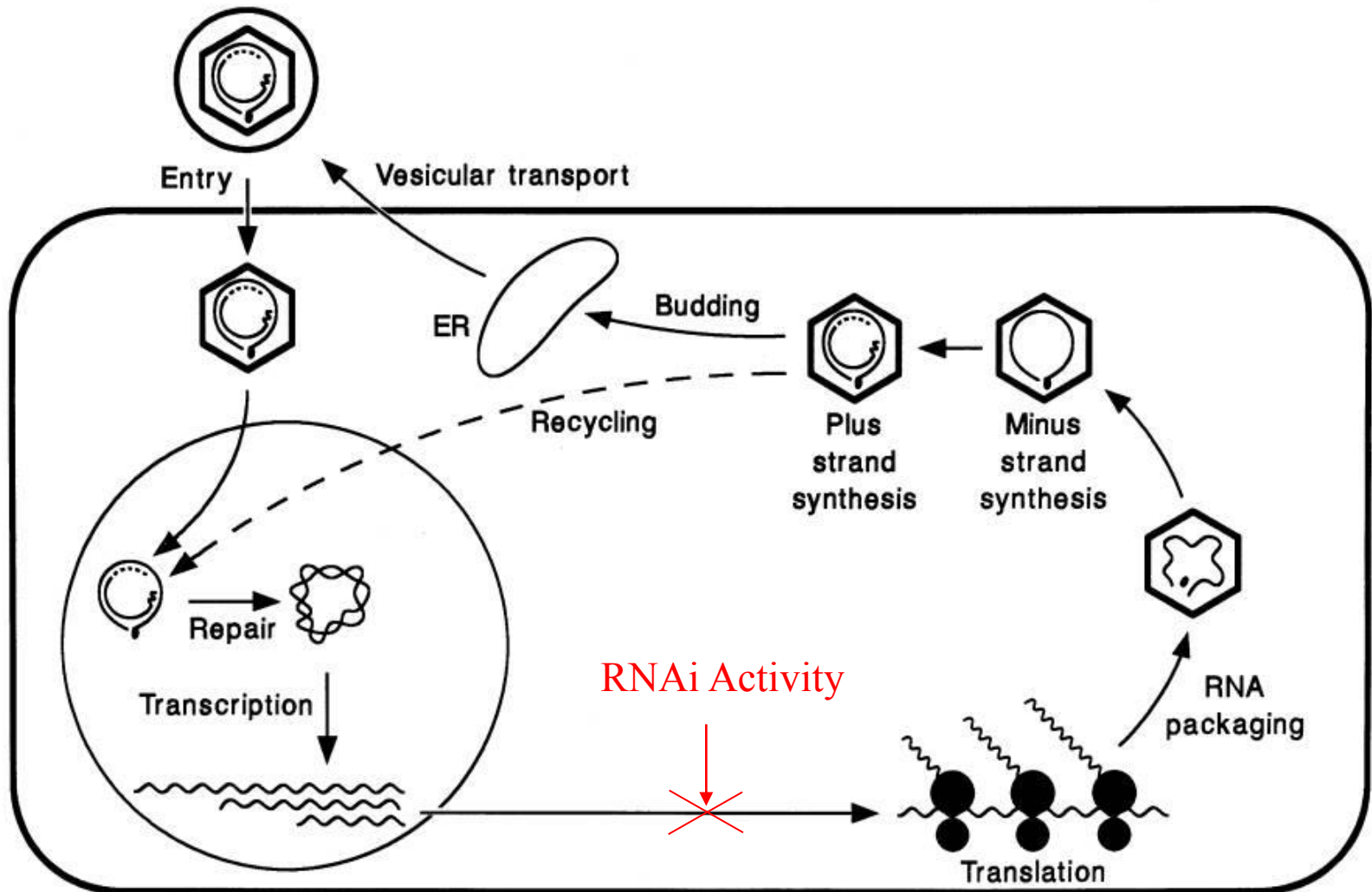
- Liver specific
- Binds to HCV 5' UTR
- Required for HCV RNA replication



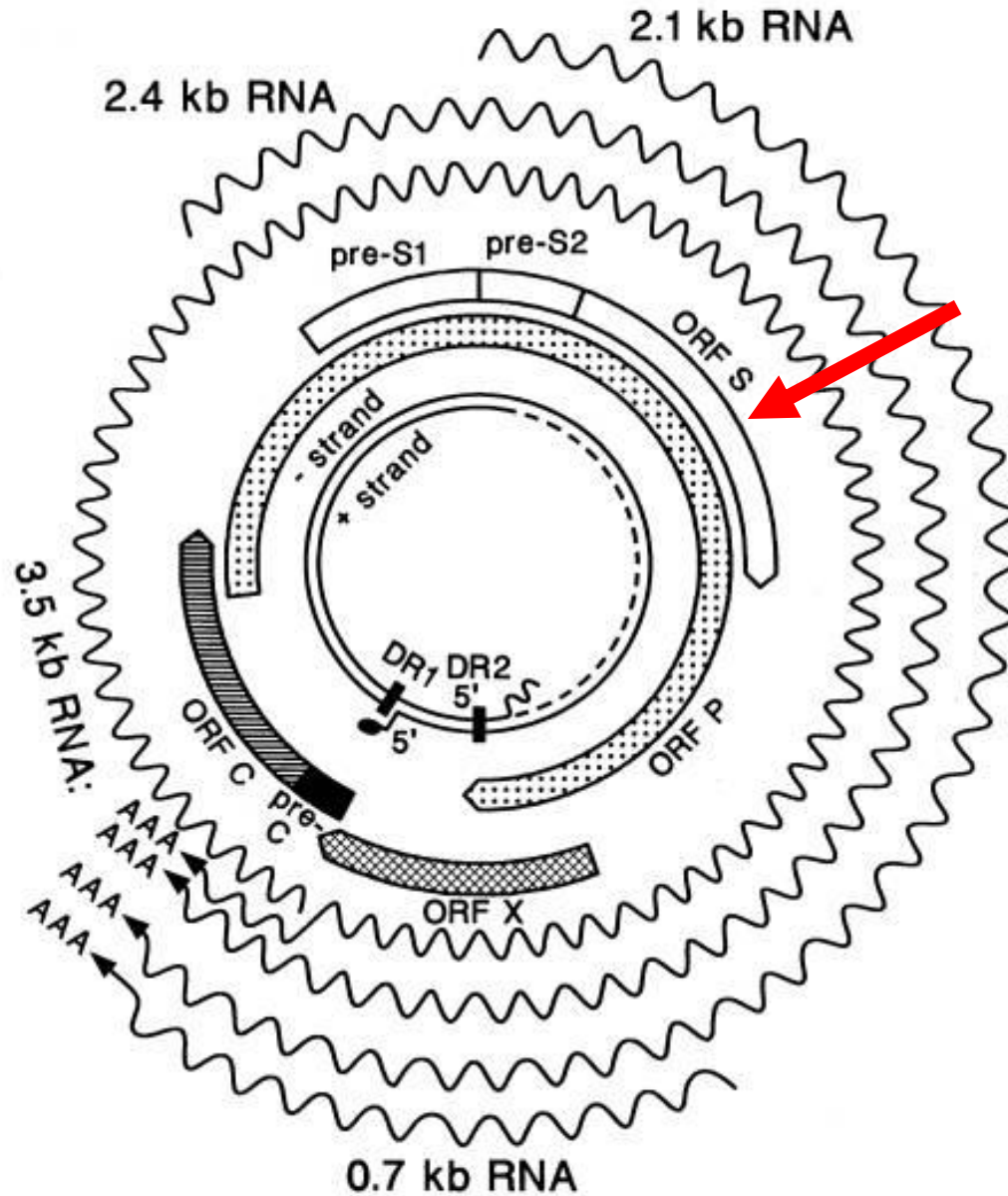
# MicroRNA 122 (miR-122) and HCV



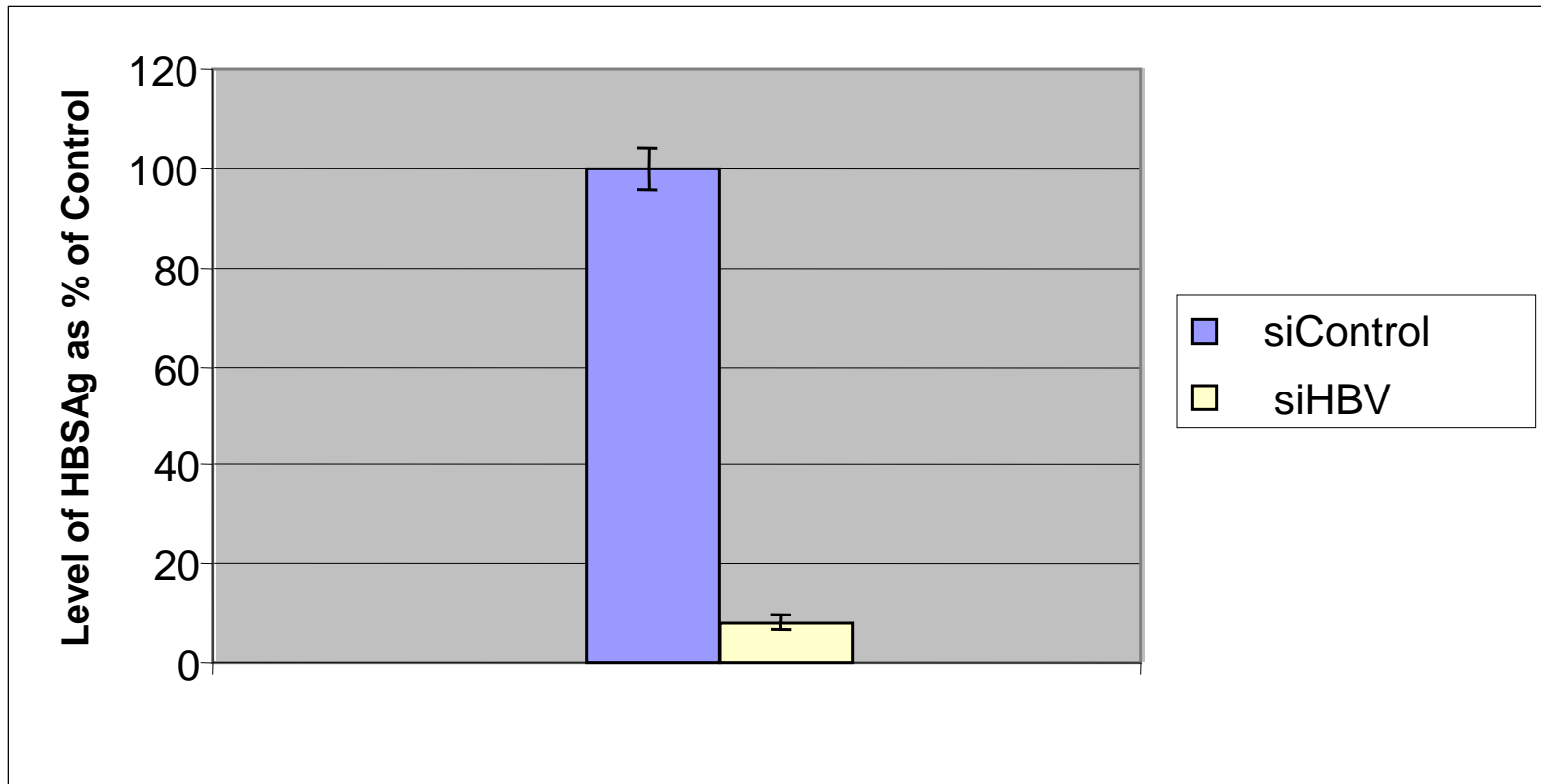
# HBV Replication Cycle



# HBV Transcripts



# Effect of siHBV on HBV Expression in

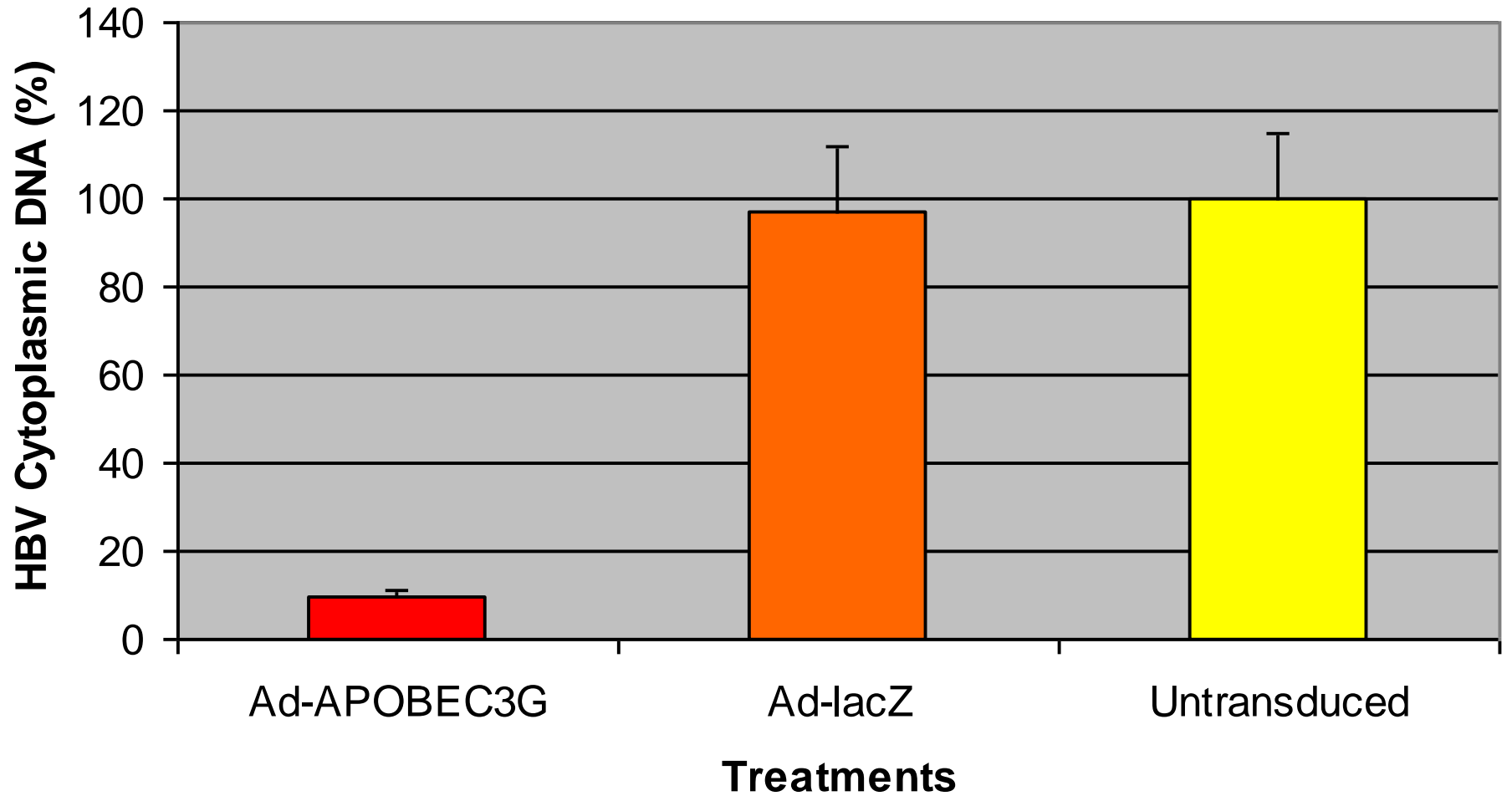


**APOBEC3G**

# 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





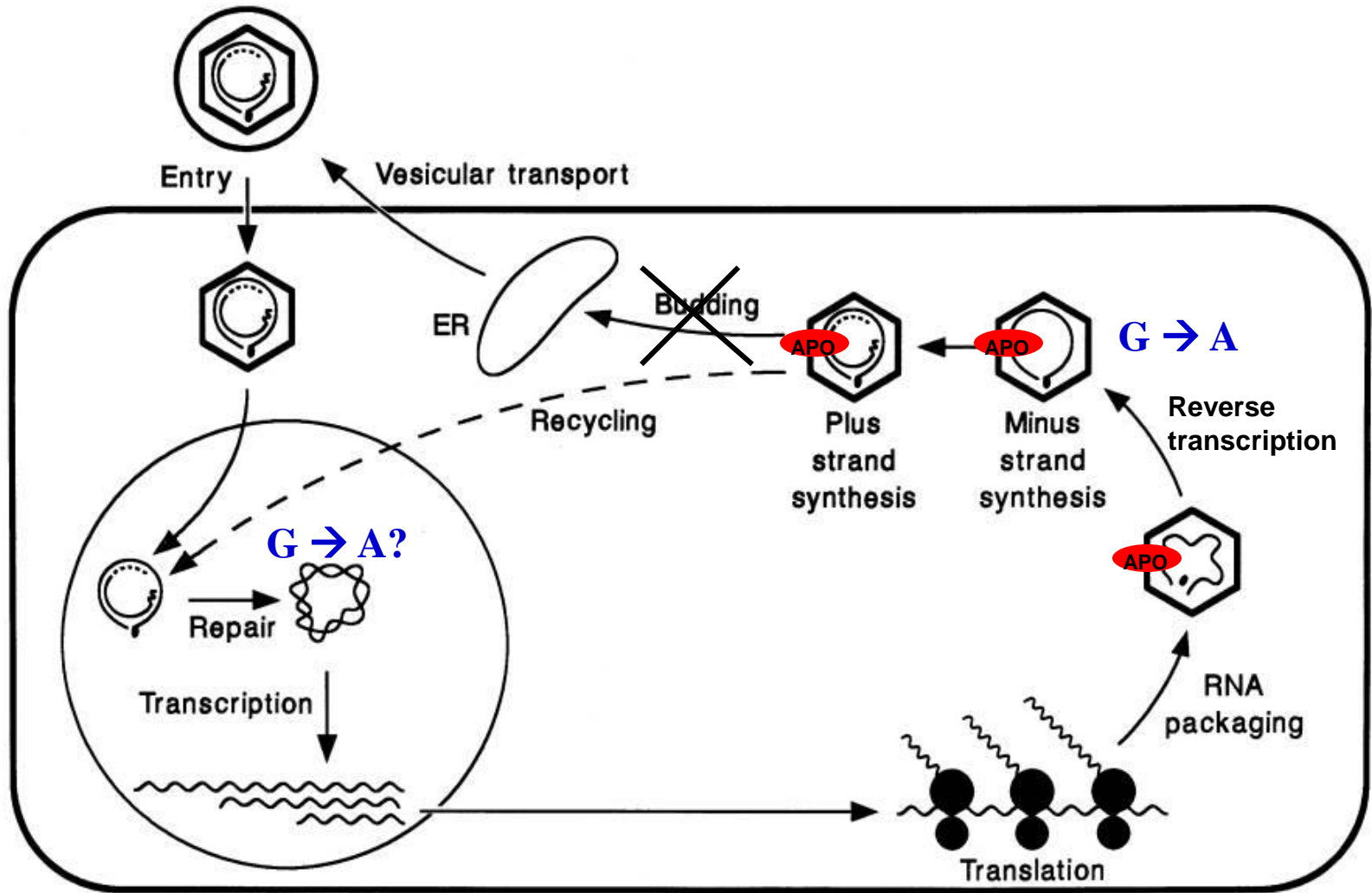
# Cytosine Deamination of HBV Pre-core Region by APOBEC3G



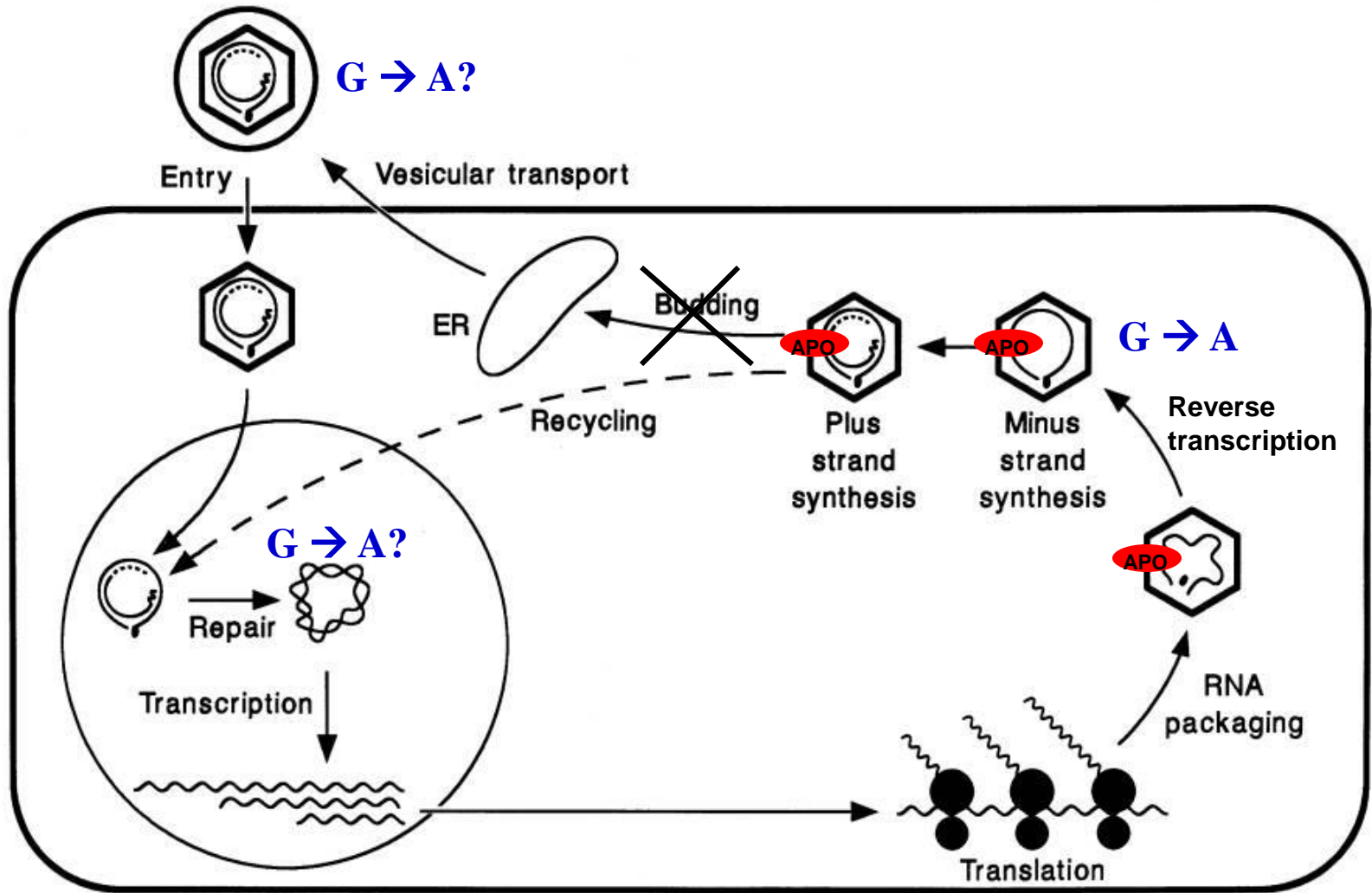
Figure 3

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

# HBV Replication Cycle: APOBEC3G



# HBV Replication Cycle: APOBEC3G



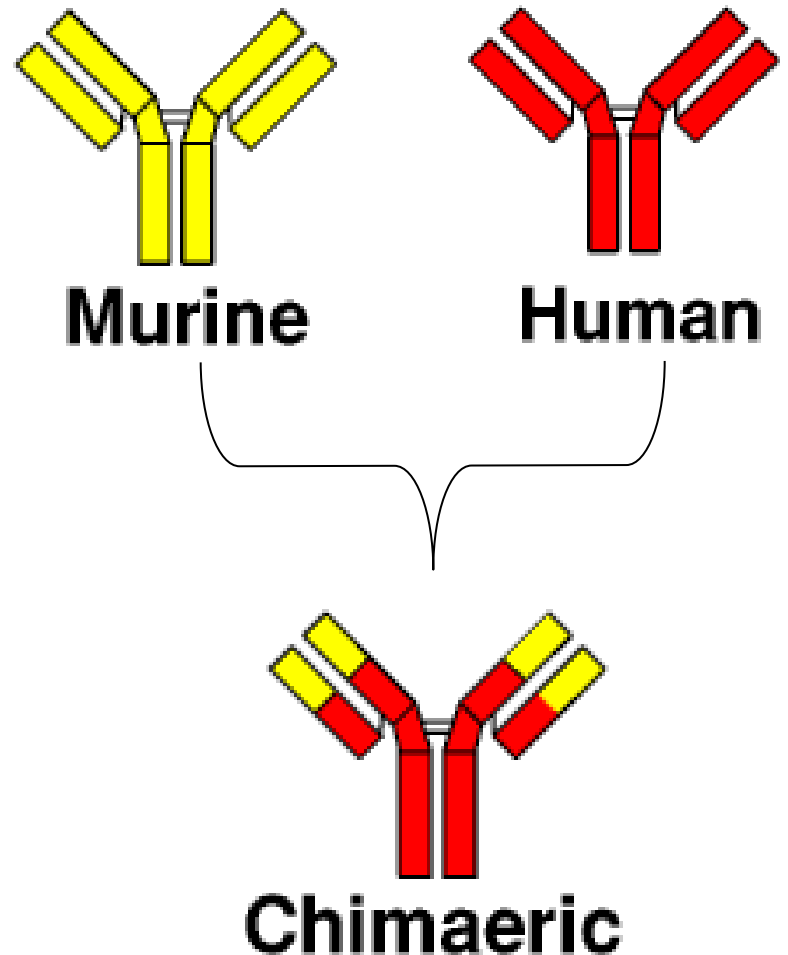
# **Antibody Reagents**

# **Monoclonal Antibodies**

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

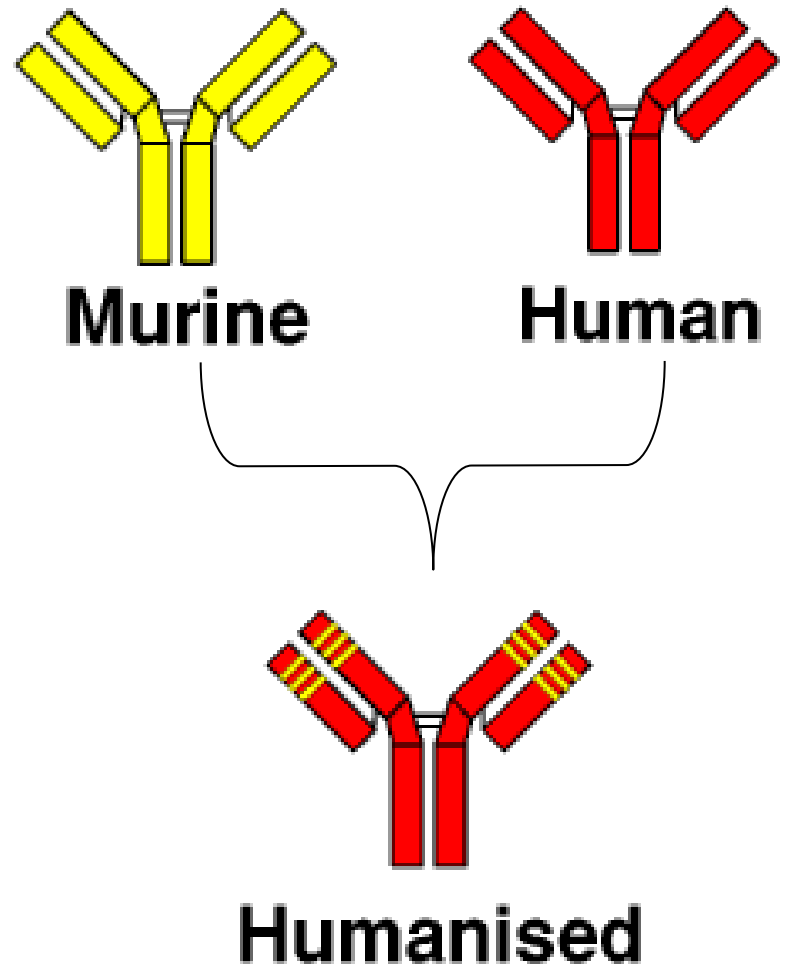
# Chimaeric Antibodies

- **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.**

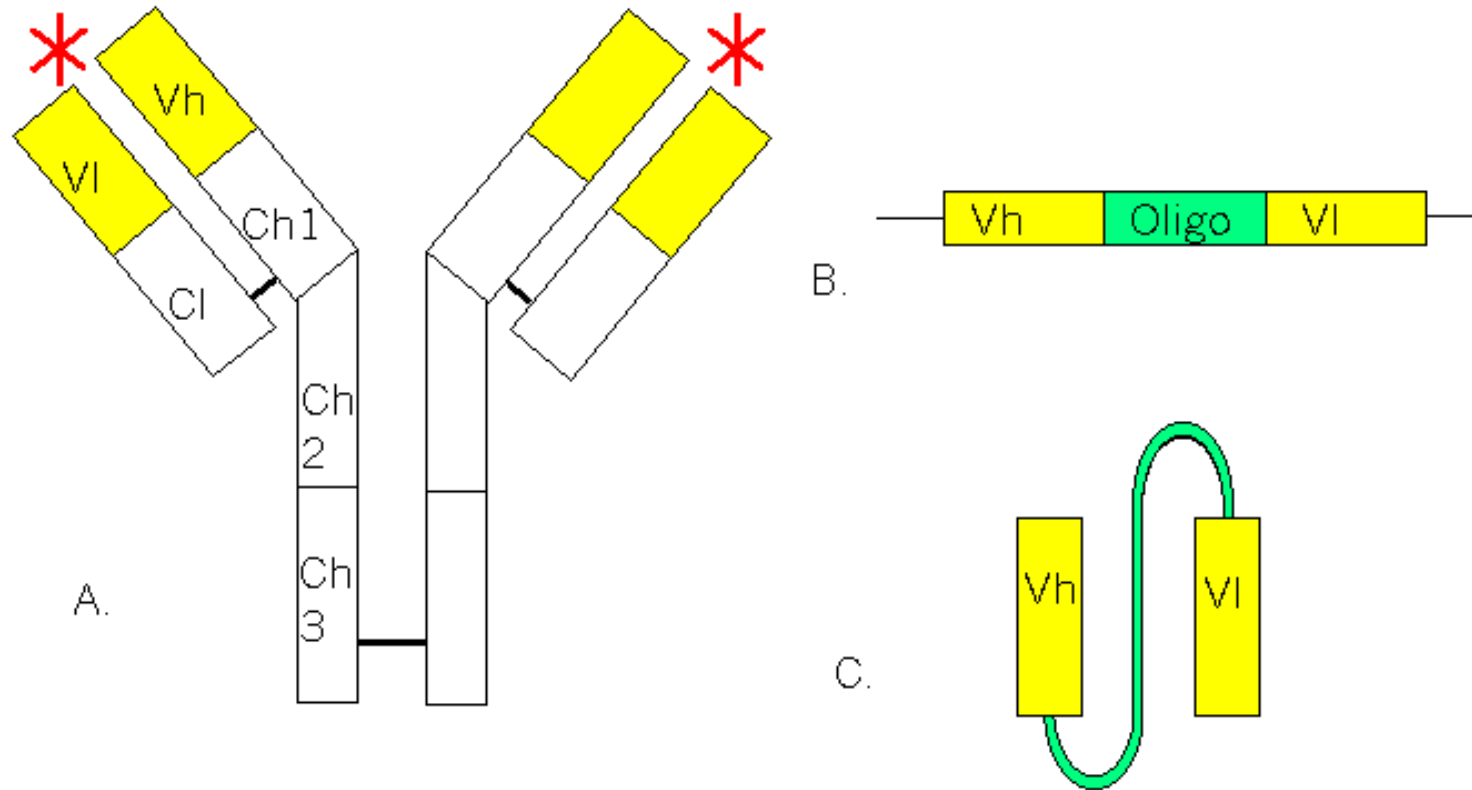


## **Single-chain antibodies (scFvs)**

- **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)



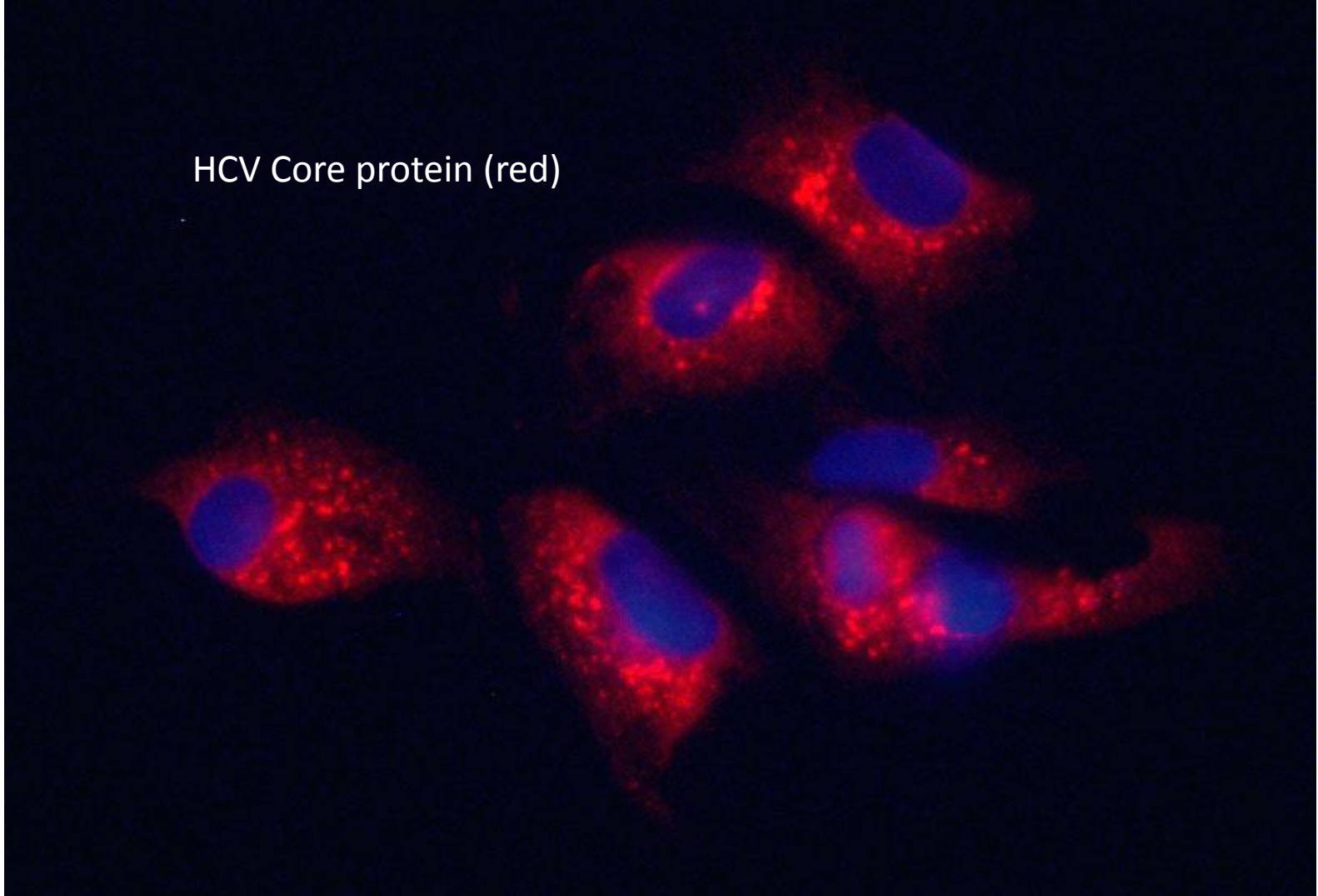
A. Antibody molecule

B. Sequences for variable domains spliced together

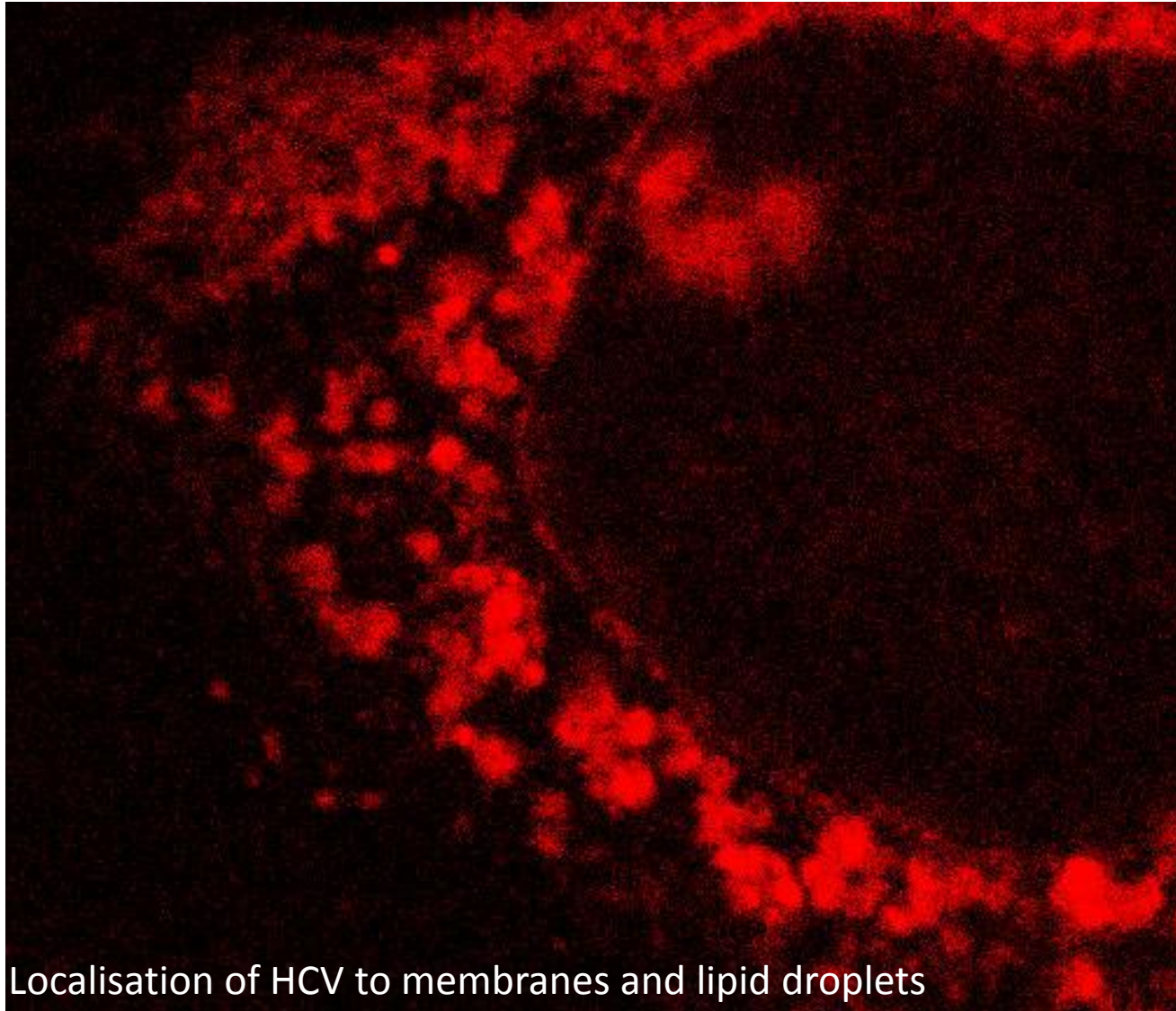
C. Single-chain antibody (scFv)

# Human Liver Cells Infected with HCV

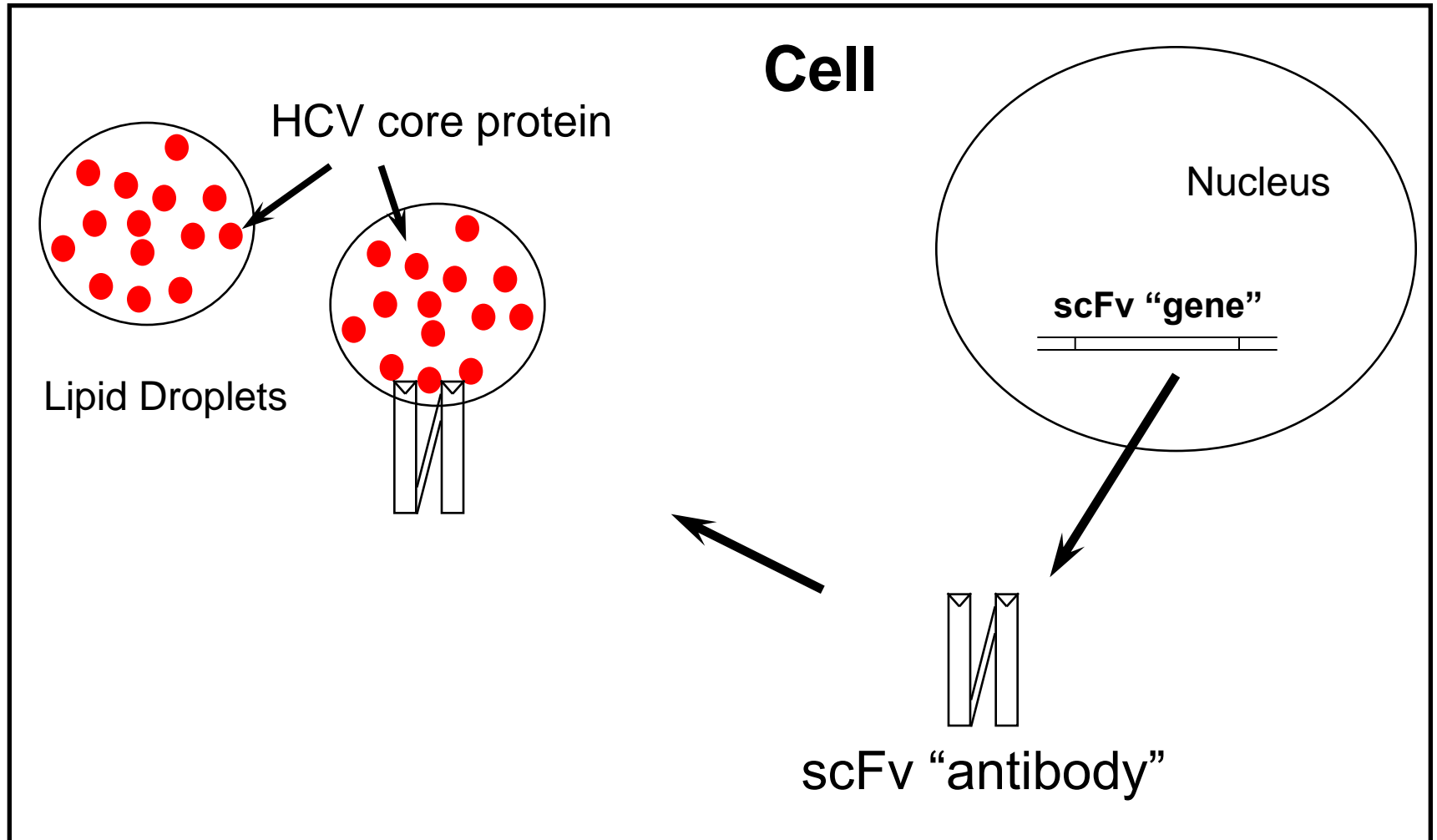
HCV Core protein (red)



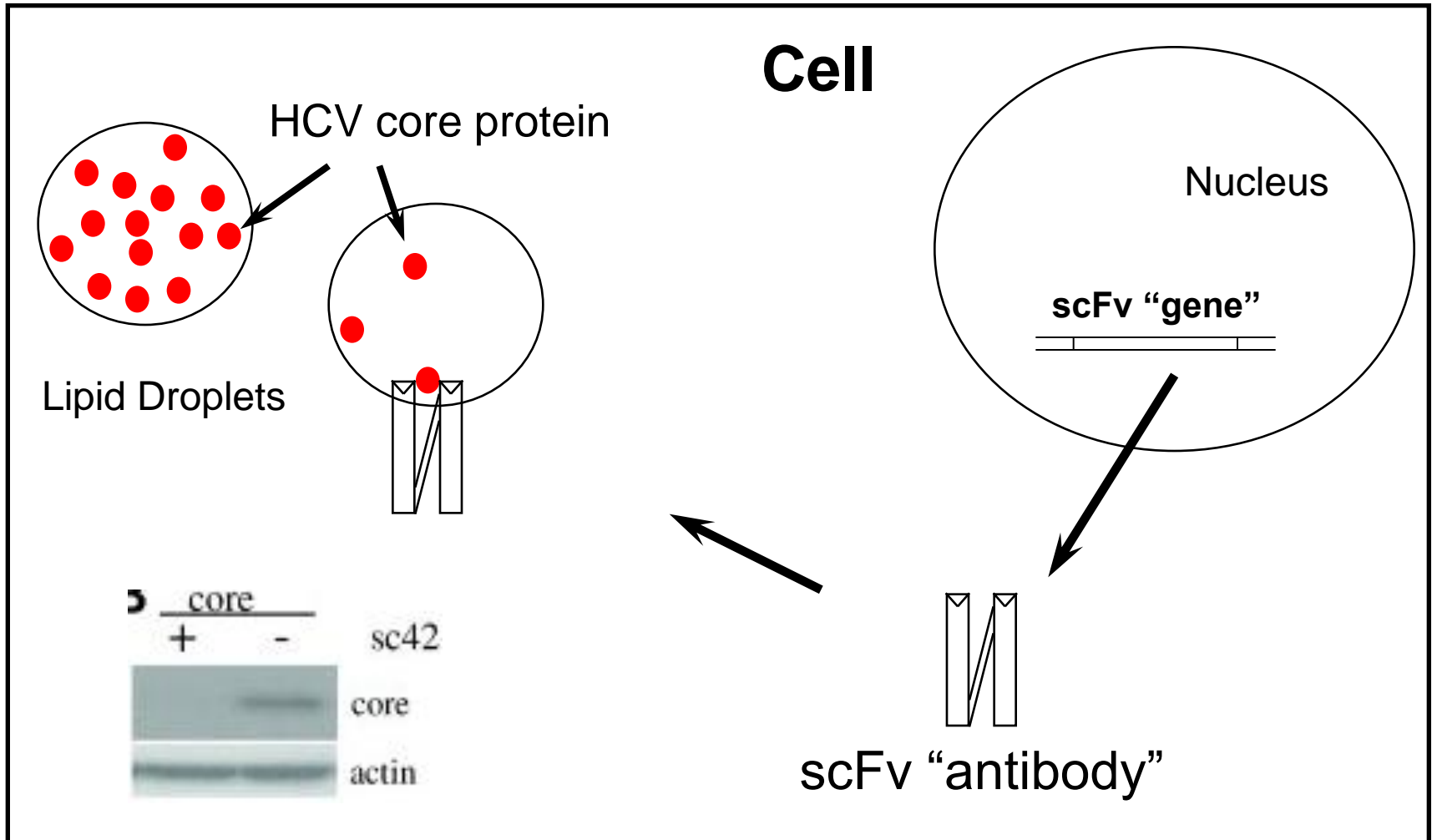
# Human Liver Cells Infected with HCV



# Targeting of HCV core protein by an intracellular scFv



# Targeting of HCV core protein by an intracellular scFv



# Inhibition of Virus Replication by scFvs

**HIV-1 - gp120**  
**- gp41**  
**- integrase**

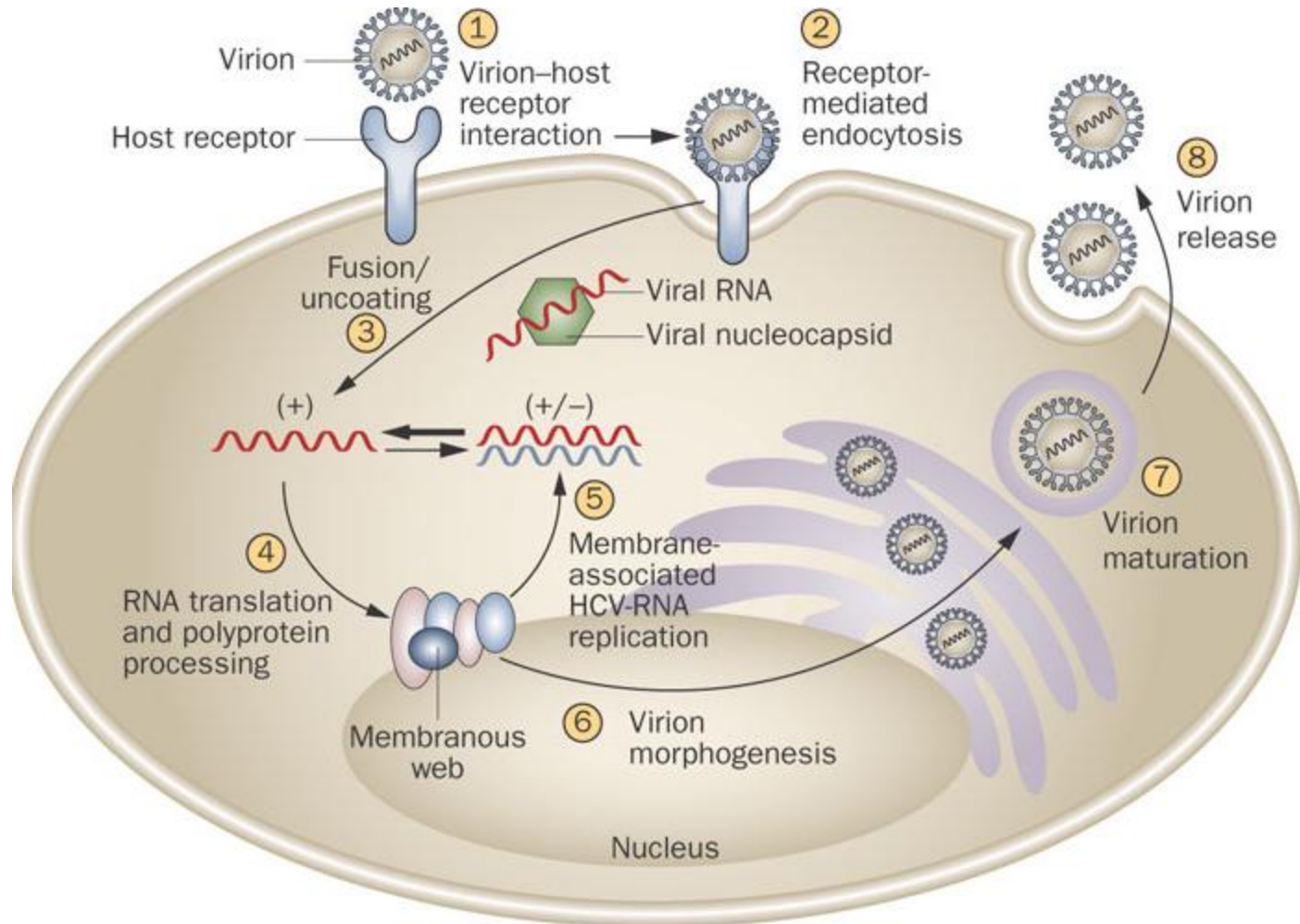
**TBE – Envelope protein**

**VSV – Coat protein**

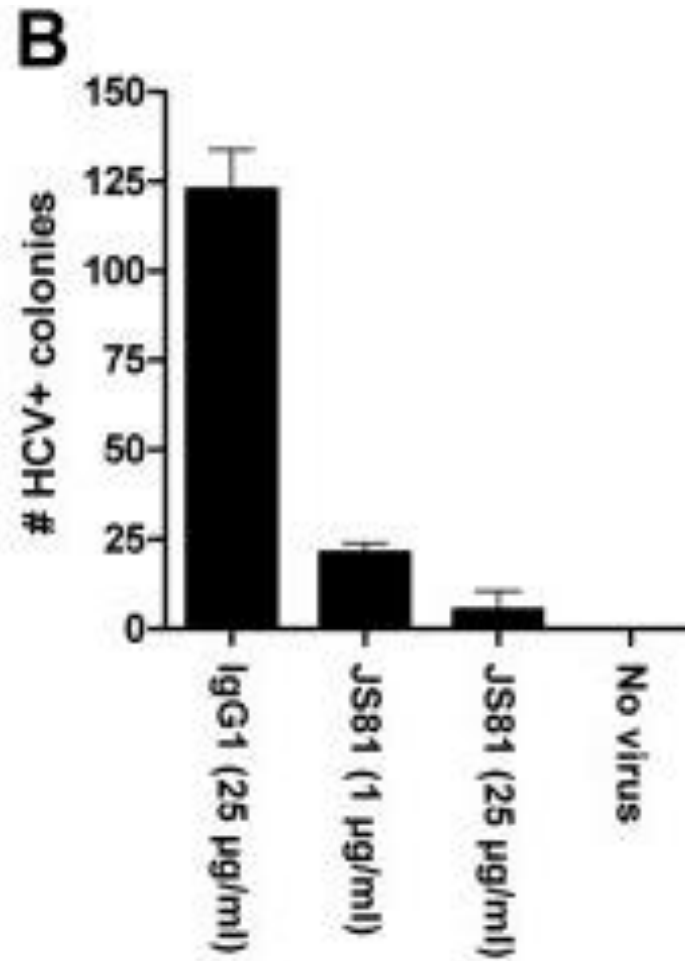
**RSV – Fusion protein**

**HCV – NS3 protease, core protein**

# Host Targets of HCV Replication



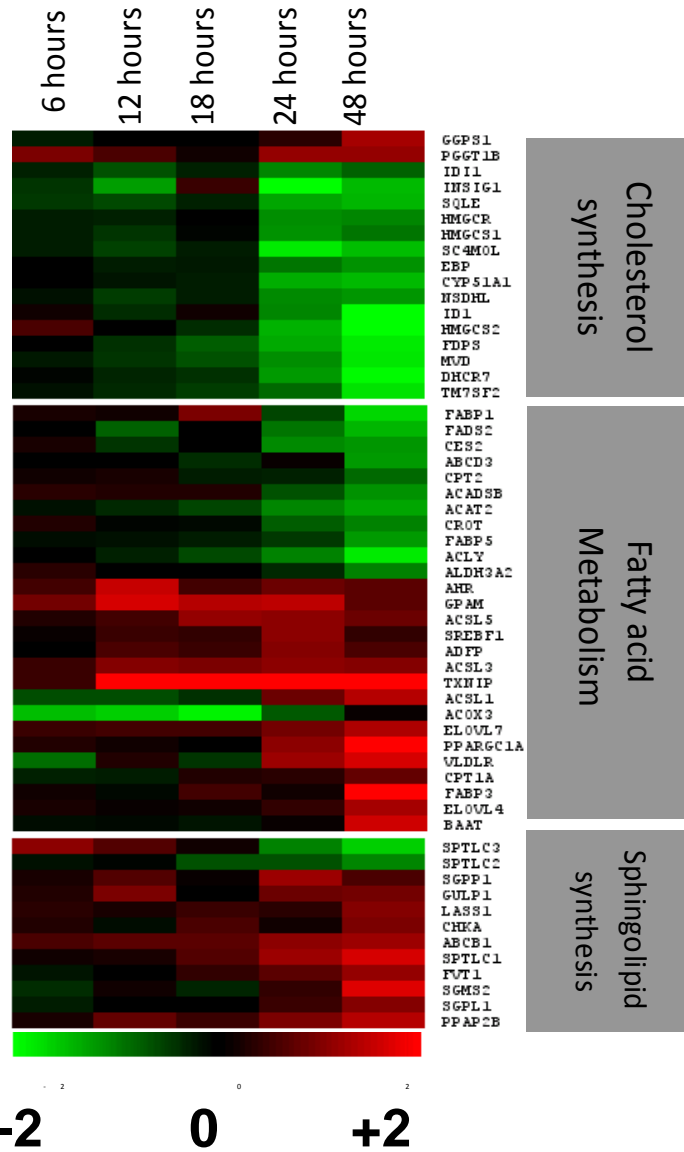
# Inhibition of HCV with anti-CD81 Antibodies





# Effects of HCV on Host Gene Expression

Gene Microarray Analysis



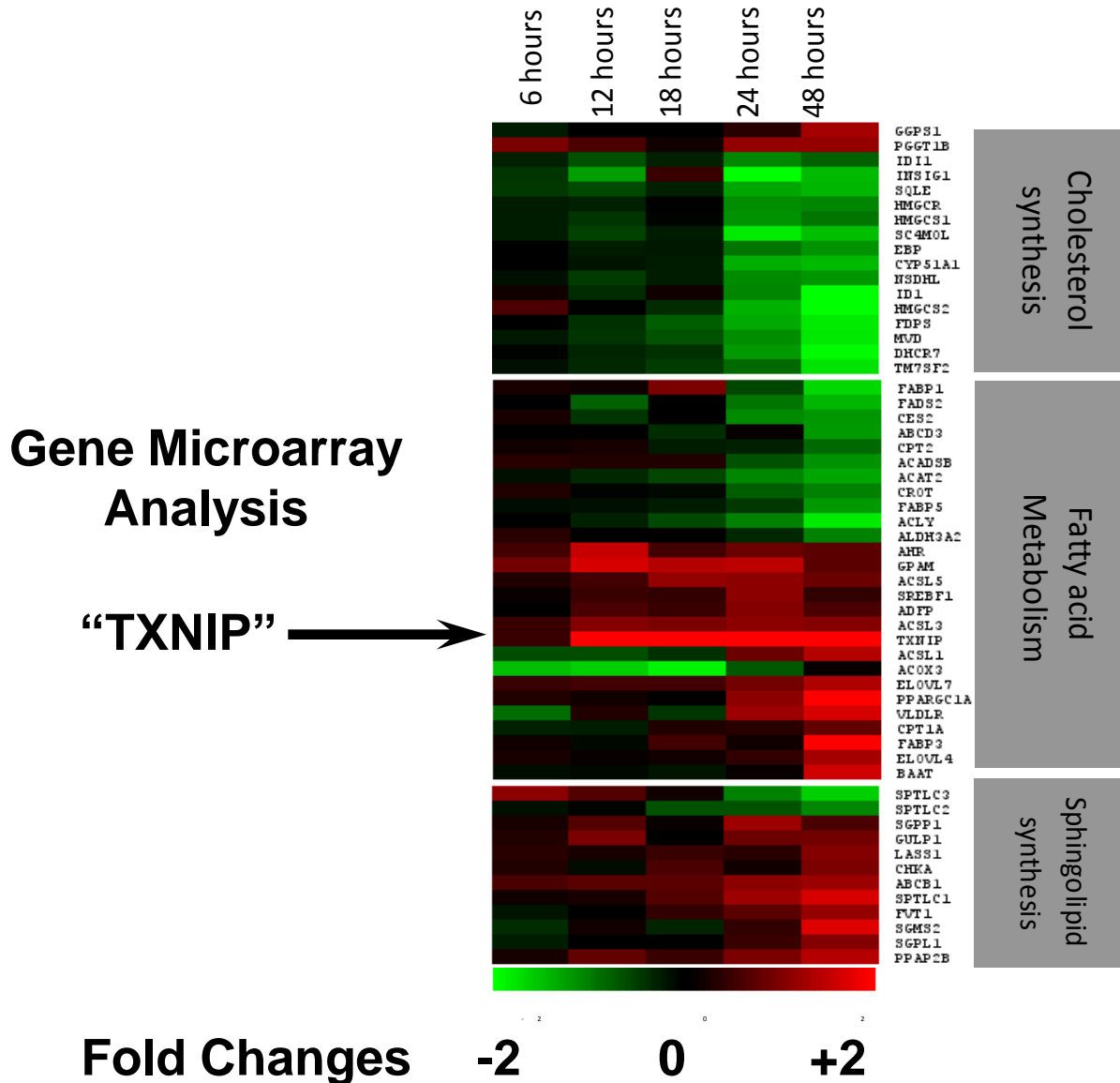
Fold Changes

-2

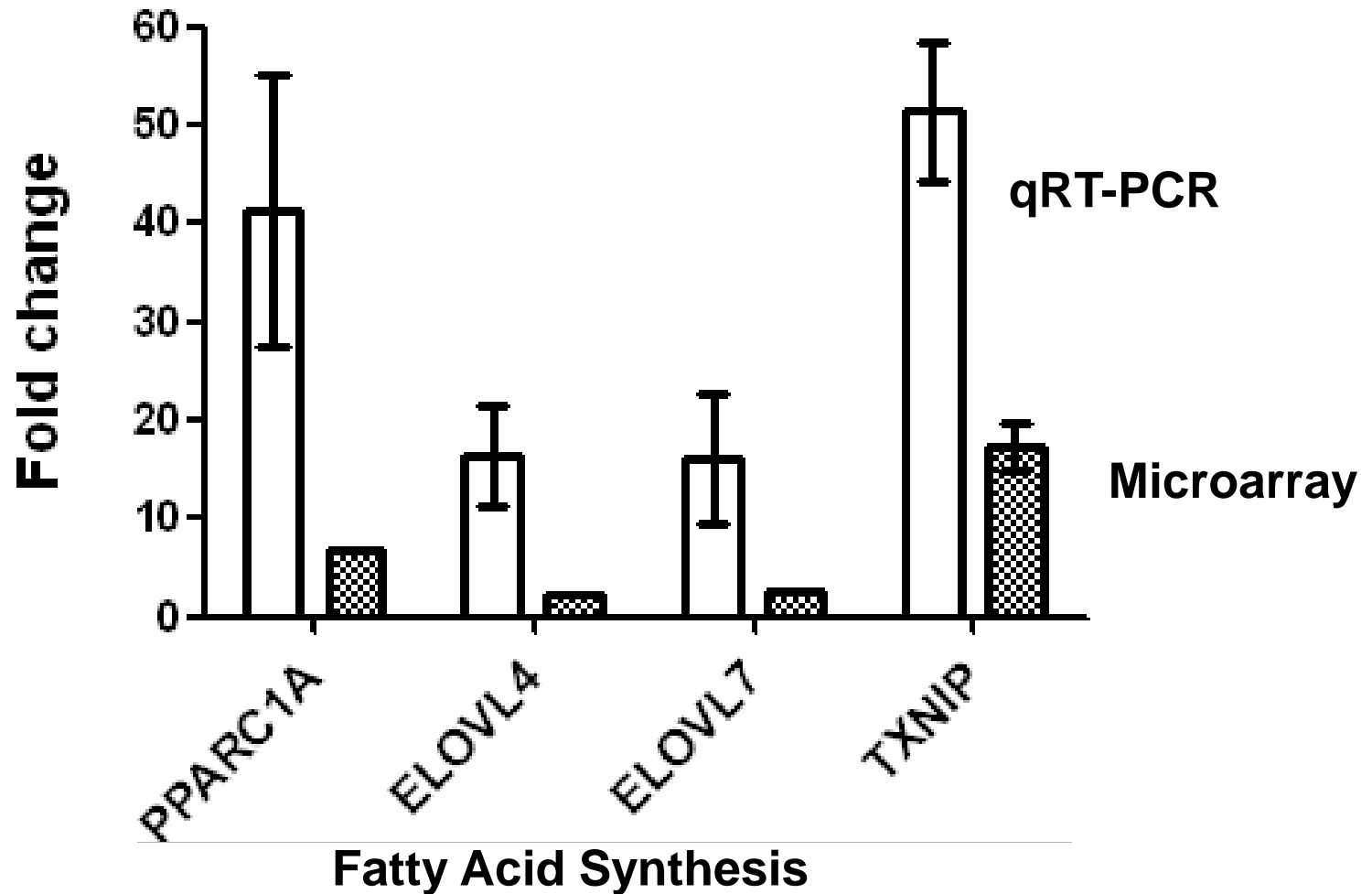
0

+2

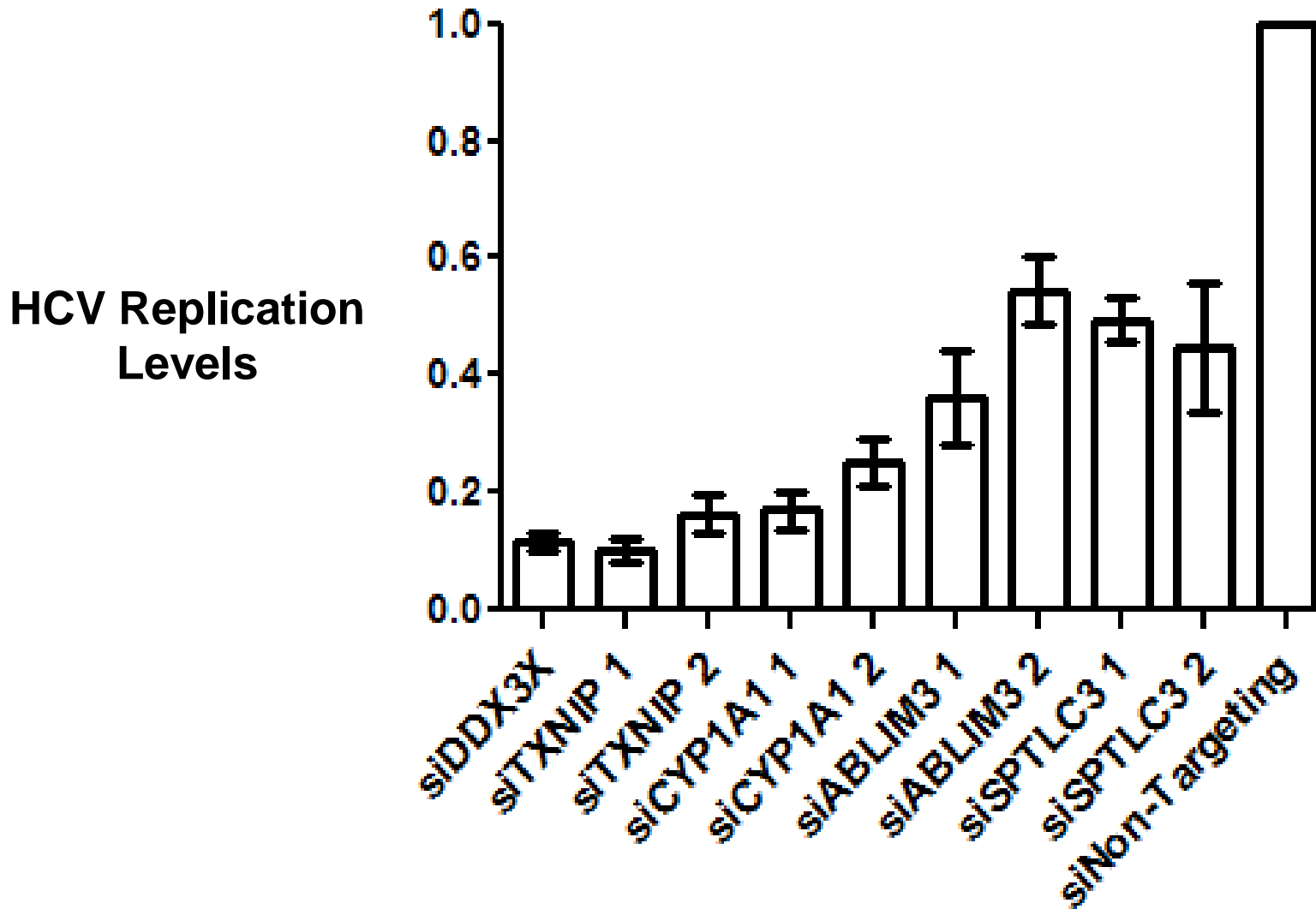
# Effects of HCV on Host Gene Expression



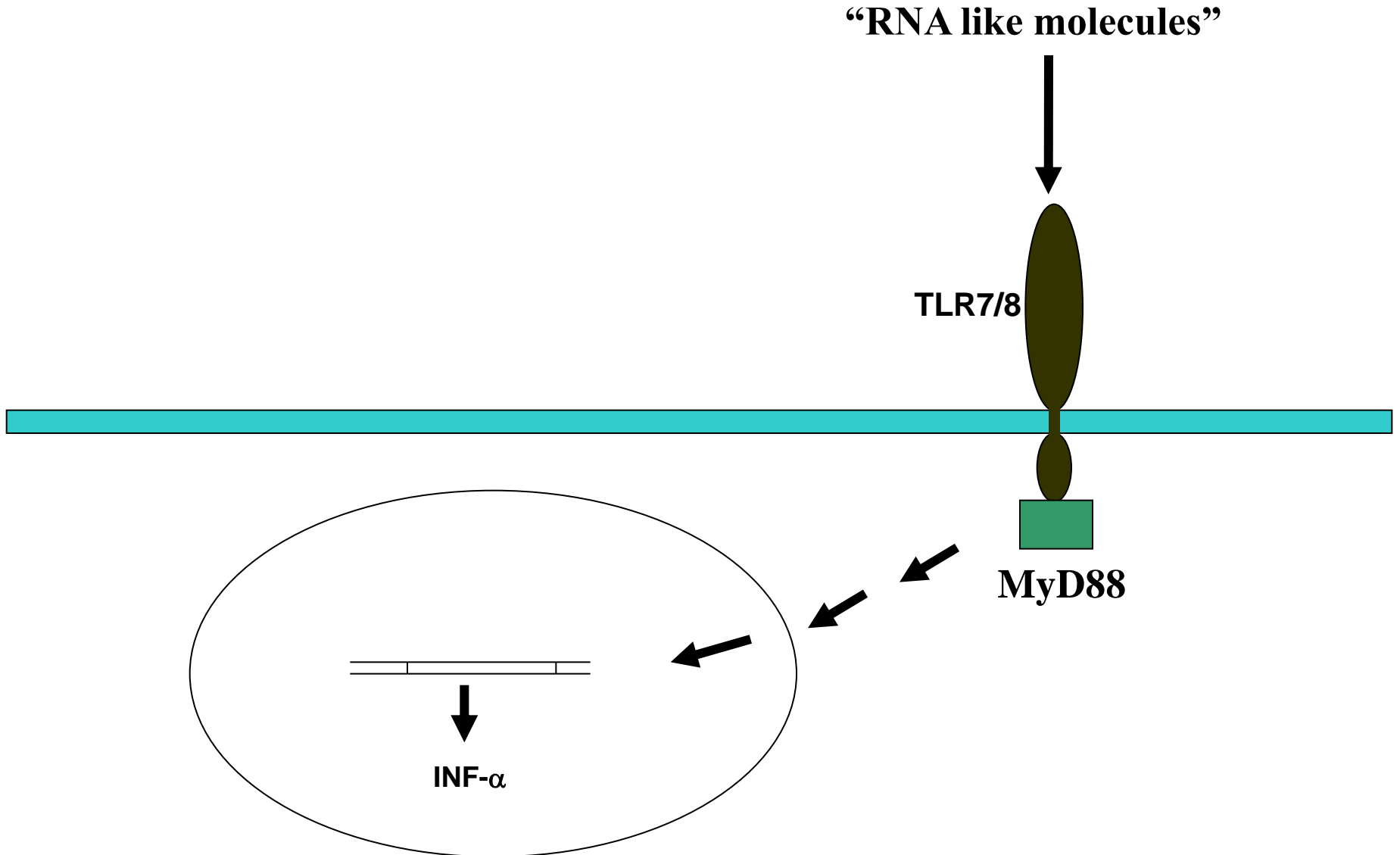
# Effects of HCV on Host Gene Expression



# 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)**

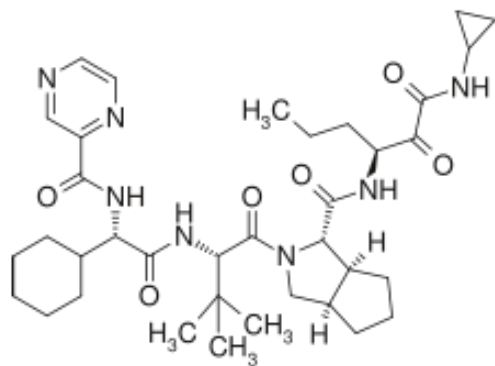
- Antiviral Gene Therapy (siRNAs, miRNAs)
- Antibody Reagents (scFvs)

## **•Host Targets**

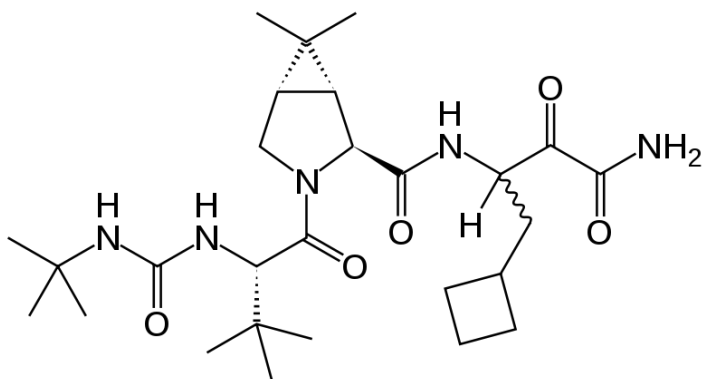
- Immunomodulators
- Metabolic enzymes
- Signal regulatory proteins

# New Antivirals for HCV

## Inhibitors of Viral NS3 Protease



**Telaprevir (VX-950),**



**Boceprevir**



# New Antivirals for HCV - in Clinical Trials

<b>Drug</b>	<b>Mechanism</b>	<b>Clinical trial</b>
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
<b>VX-497 (Merimepodib)</b>	<b>IMPDH inhibitor</b>	<b>Phase 2</b>
<b>Histamine dihydrochloride</b>	<b>Immunomodulatory/antioxidant</b>	<b>Phase 2</b>
<b>Thymalfasin</b>	<b>Prodrug of thymosin-<math>\alpha</math>1 (immunomodulatory)</b>	<b>Phase 2</b>
<b>Isatoribine</b>	<b>TLR7 agonist</b>	<b>Phase 1</b>
<b>Etanercept</b>	<b>TNF blocker</b>	<b>Phase 2</b>

# References

**Matskevich AA and Strayer DS (2003). Exploiting hepatitis C virus activation of NF $\kappa$ B to deliver HCV-responsive expression of interferons  $\alpha$  and  $\gamma$ . Gene Therapy 10 1861-1873.**

**Grimm D and Kay MA (2006). Therapeutic short hairpin RNA expression in the liver viral targets and vectors. Gene Therapy 13 563-575.**

**Wu G-Y and Chen H-S (2007). Novel Approaches towards conquering hepatitis B infection. World J Gastroenterol 14 830-836.**

**Pereira AA and Jacobson IA (2009) New and experimental therapies for HCV. Nature Rev in Gastro & Hepatol 6 403-411.**

# References

**Matskevich AA and Strayer DS (2003). Exploiting hepatitis C virus activation of NF $\kappa$ B to deliver HCV-responsive expression of interferons  $\alpha$  and  $\gamma$ . Gene Therapy 10 1861-1873.**

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**Rice CM (2011). New Insights Into HCV Replication: Potential Antiviral TargetsDrug. Top Antivir Med 19 117-120.**