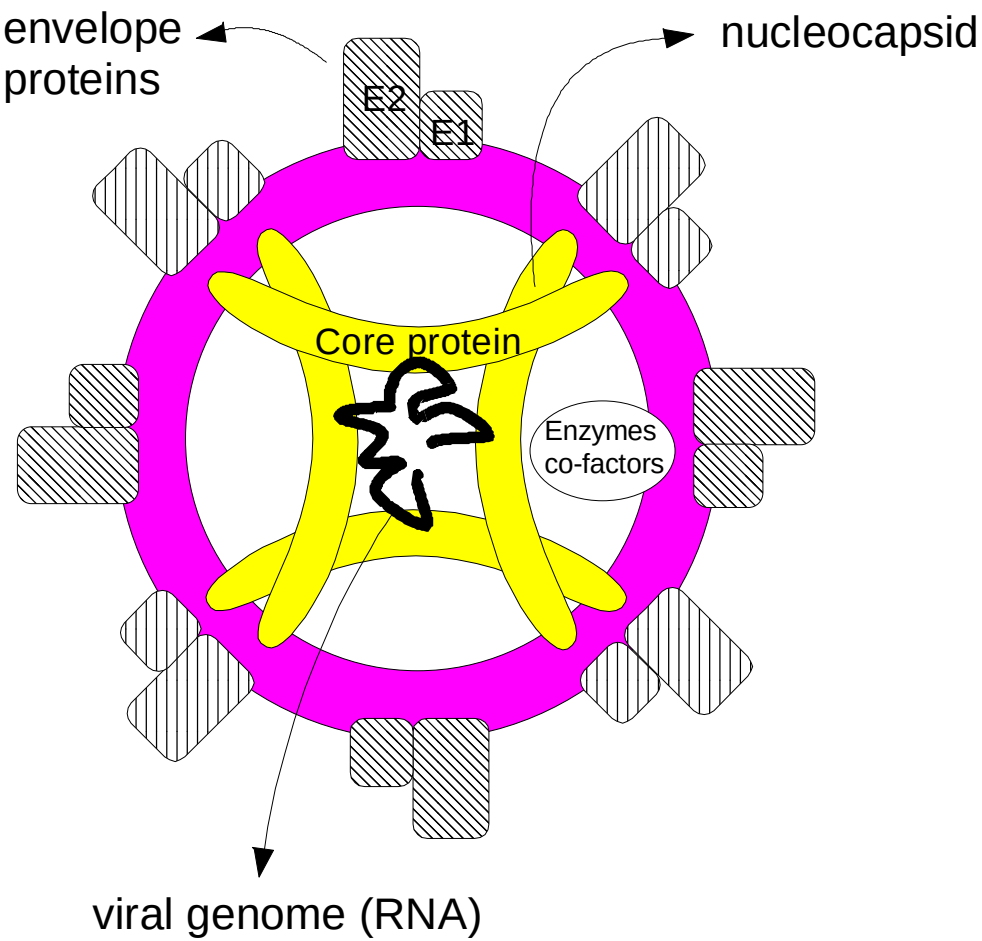
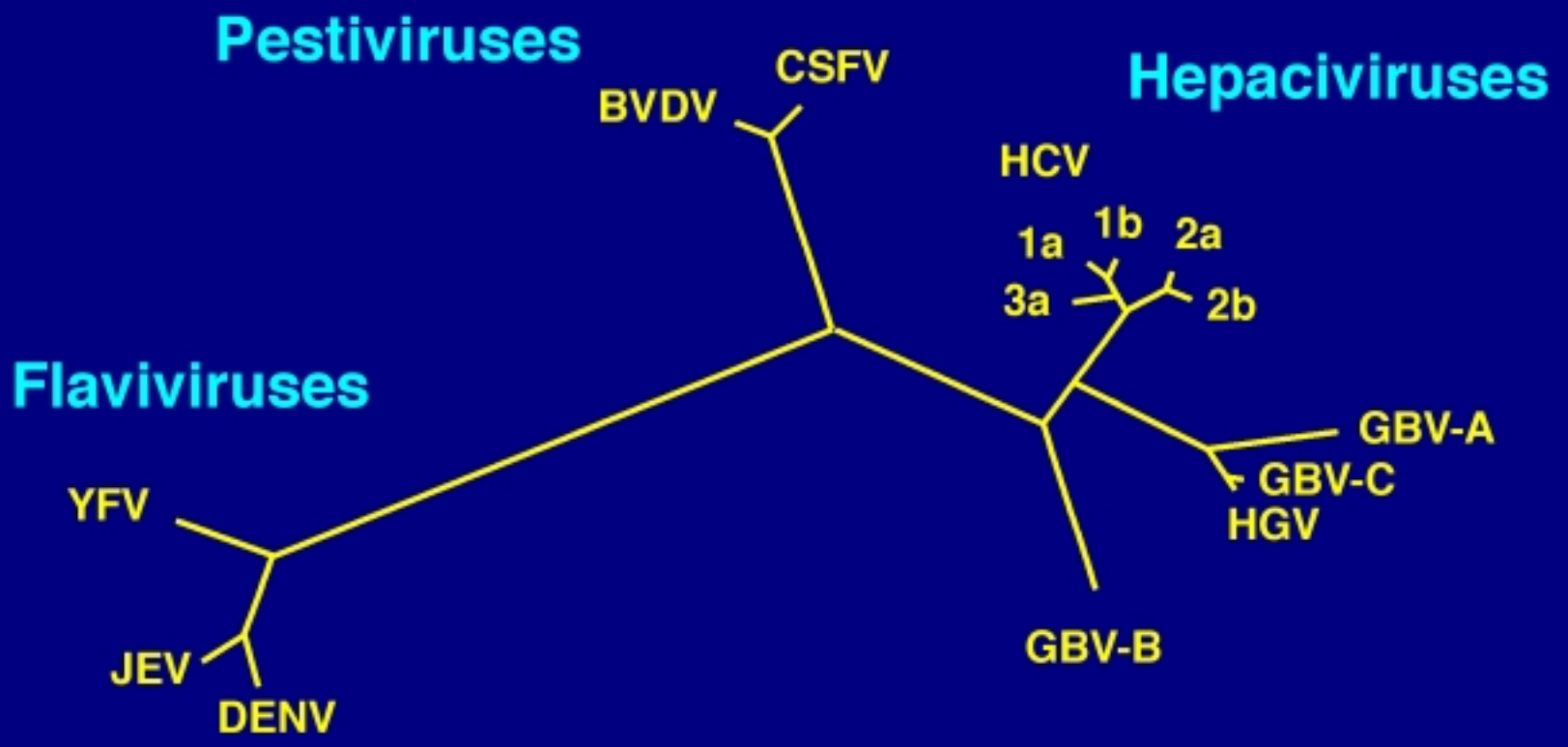
HCV structure



PHYLOGENETIC RELATIONSHIP OF THE *FLAVIVIRIDAE* FAMILY



HCV genome

- Single stranded RNA (positive polarity)
- 9600 nucleotides
- open reading frame (C, E1, E2, NS2-5) → encoding for polyprotein
- ev. small open reading frame © → encoding for AFR *
- 5’ non-coding region (NCR)
  → containing IRES (internal ribosomal entry site)
  → translation of RNA
- 3’ non-coding region (NCR)
  → co-regulates viral replication

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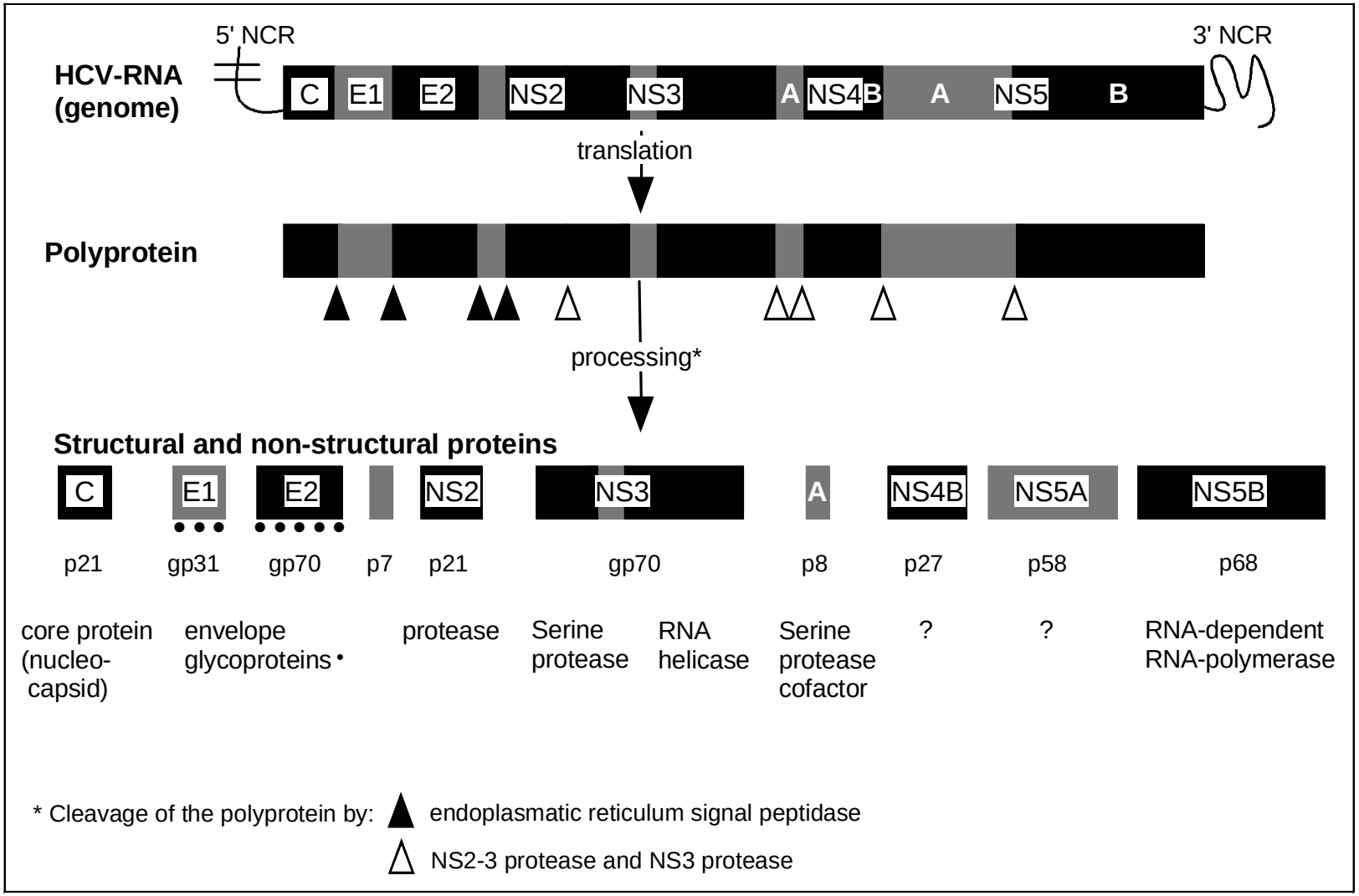
* ARF = alternative reading frame protein/frameshift: 160AA

Genetic organisation of HCV



Cleavage products of HCV polyprotein

- Structural proteins

HCV envelope *

- composed of 2 glycoproteins E1 (gp31) and E2 (gp70) which associate to noncovalent heterodimers
- only limited sequences are highly conserved

- E2 contains 2 hypervariable regions: HVR1 and 2
- E2 also contains the binding site for CD81
- little or no surplus production of HCV envelope proteins

HCV nucleocapsid *

- core protein (p21)
- fairly conserved sequences
- core protein might interact with a variaty of cellular proteins

* processed from the HCV polyprotein by the host's endoplasmatic reticulum signal peptidase

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- non-structural, regulatory proteins

NS2/3 autoprotease

NS3 serine protease + NS4A co-factor

→ both proteases process polyprotein (non-structural part)

RNA helicase (NS3)

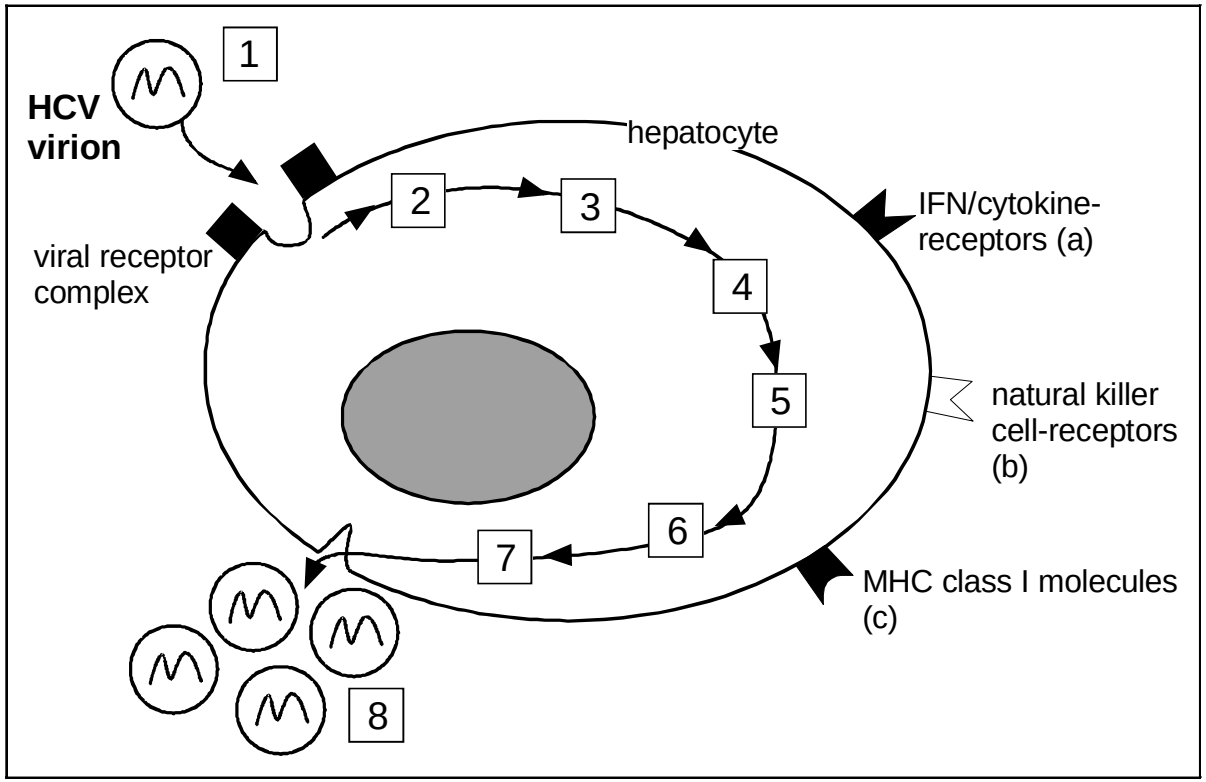
RNA dependent RNA-protease

→ essential for viral replication

NS5A encoded protein

→ interferon sensitivity

HCV life cycle



Legend

Life cycle

1. binding of HCV to a cell surface receptor
2. cytoplasmic release and uncoating of the viral RNA genome
3. IRES-mediated translation
4. polyprotein processing by cellular and viral proteases
5. RNA replication
6. packaging and assembly
7. virion maturation
8. release from the host cell

Structures for defense (viral clearance)

a) occupation of receptor leads to signal transduction (anti-viral status)
b) binding of NK cells leads to destruction of infected cell
c) T cell epitopes of HCV presented on the MHC molecules target the infected cells for the attack by HCV-specific cytotoxic T-cells

HCV life cycle

8 Steps

1. Binding of HCV to a cell surface receptor complex
→ internalisation
• components of surface receptor
- CD81 protein, a tetraspanin
- low density lipoprotein receptor
- other candidates

2. Cytoplasmatic release and uncoding of viral genome
• interaction of HCV-IRES with 40S ribosomal unit

3. IRES mediated translation
→ polyprotein

4. Processing of polyprotein
• Host cell proteases → envelope glycoproteins E1, E2, core protein
• viral proteases → regulatory enzymes/co-factors

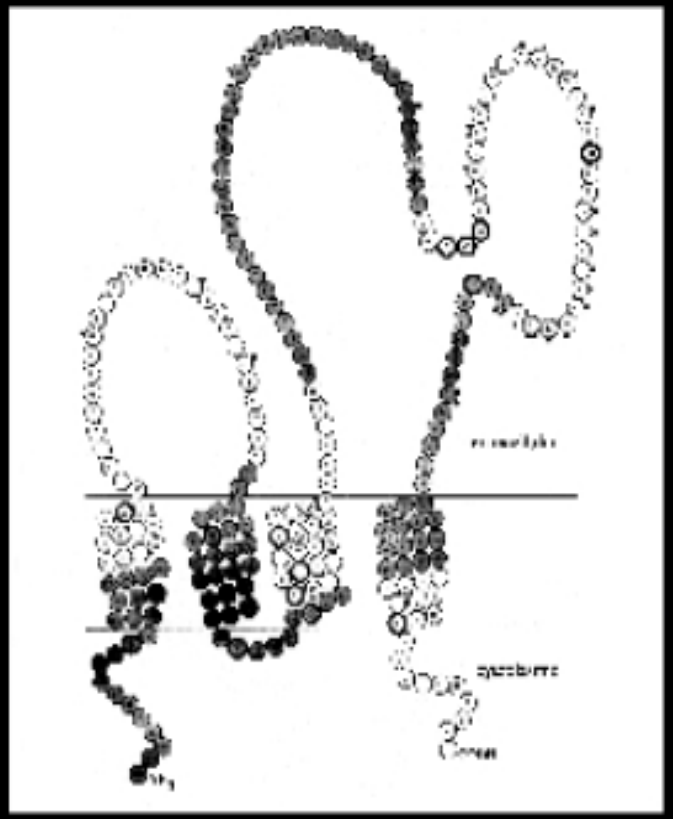
5. RNA replication

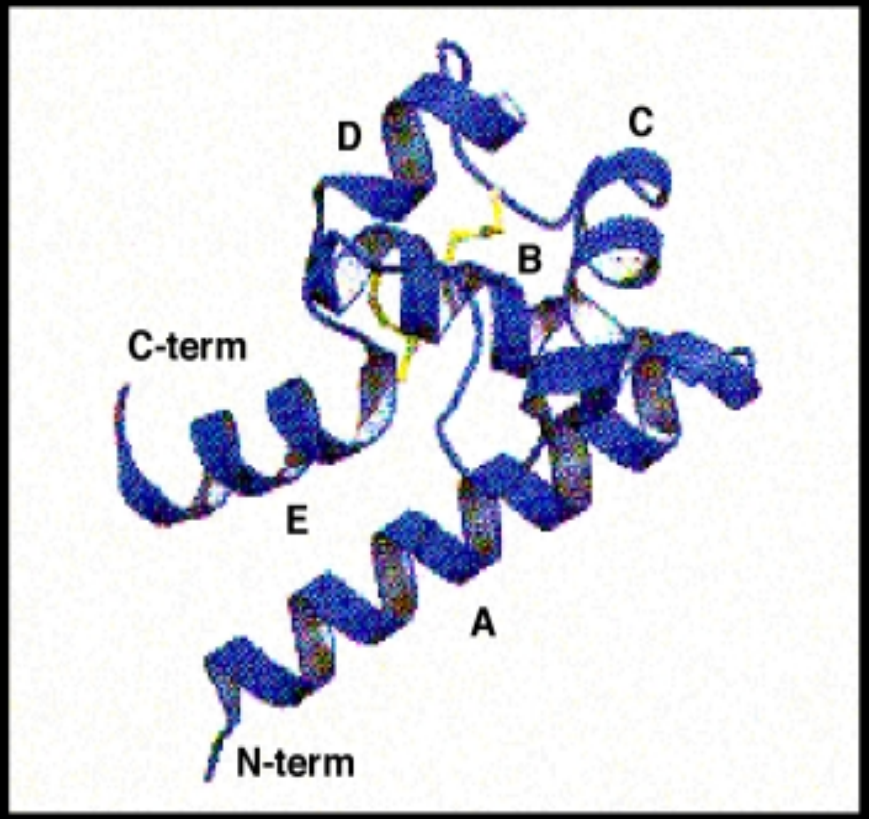
6. Packaging and assembly

7. Virion maturation

8. Virion release from the host cell

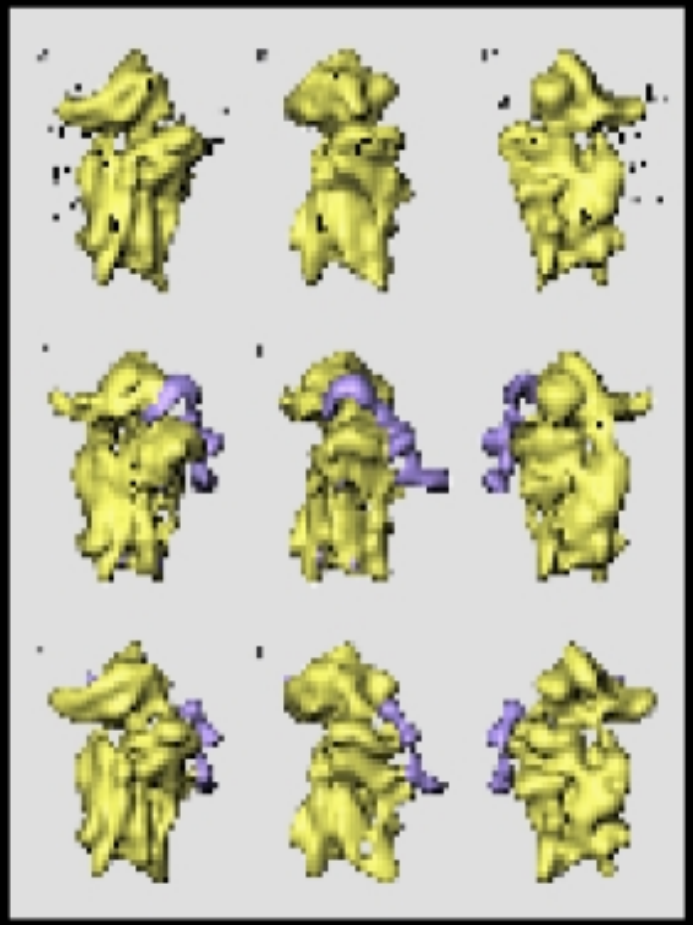
CD81 - a Binding Partner for E2

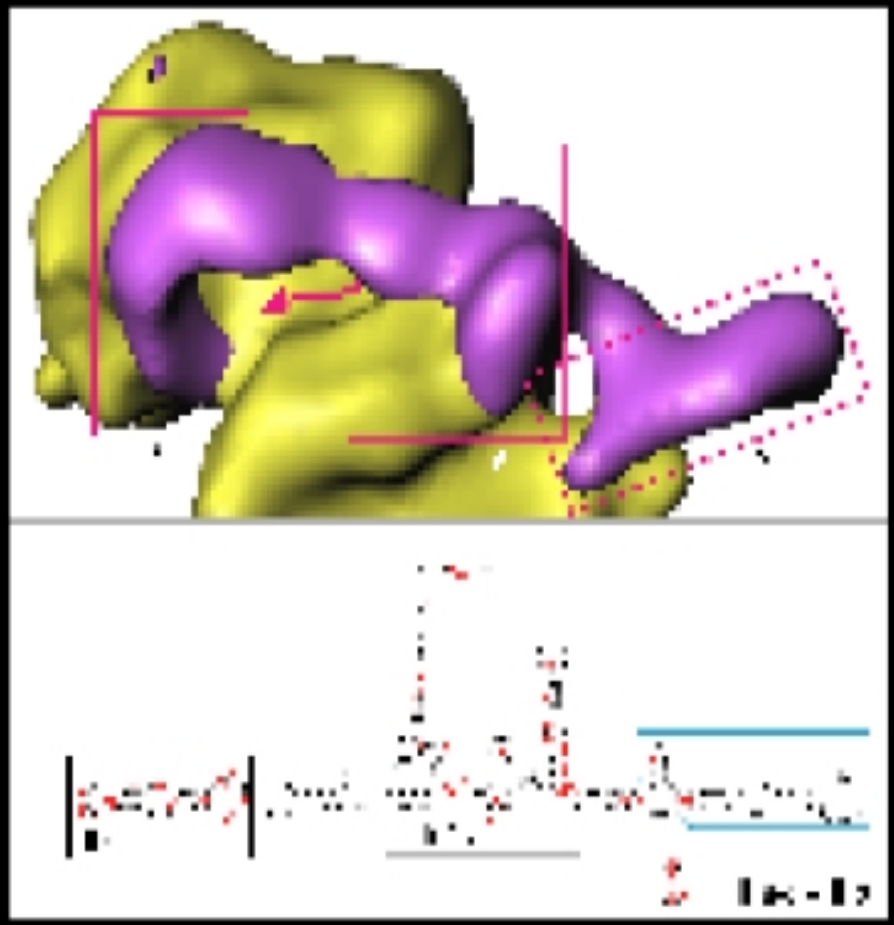




Kitadokoro K et al. EMBO J 2001;20:12-18.

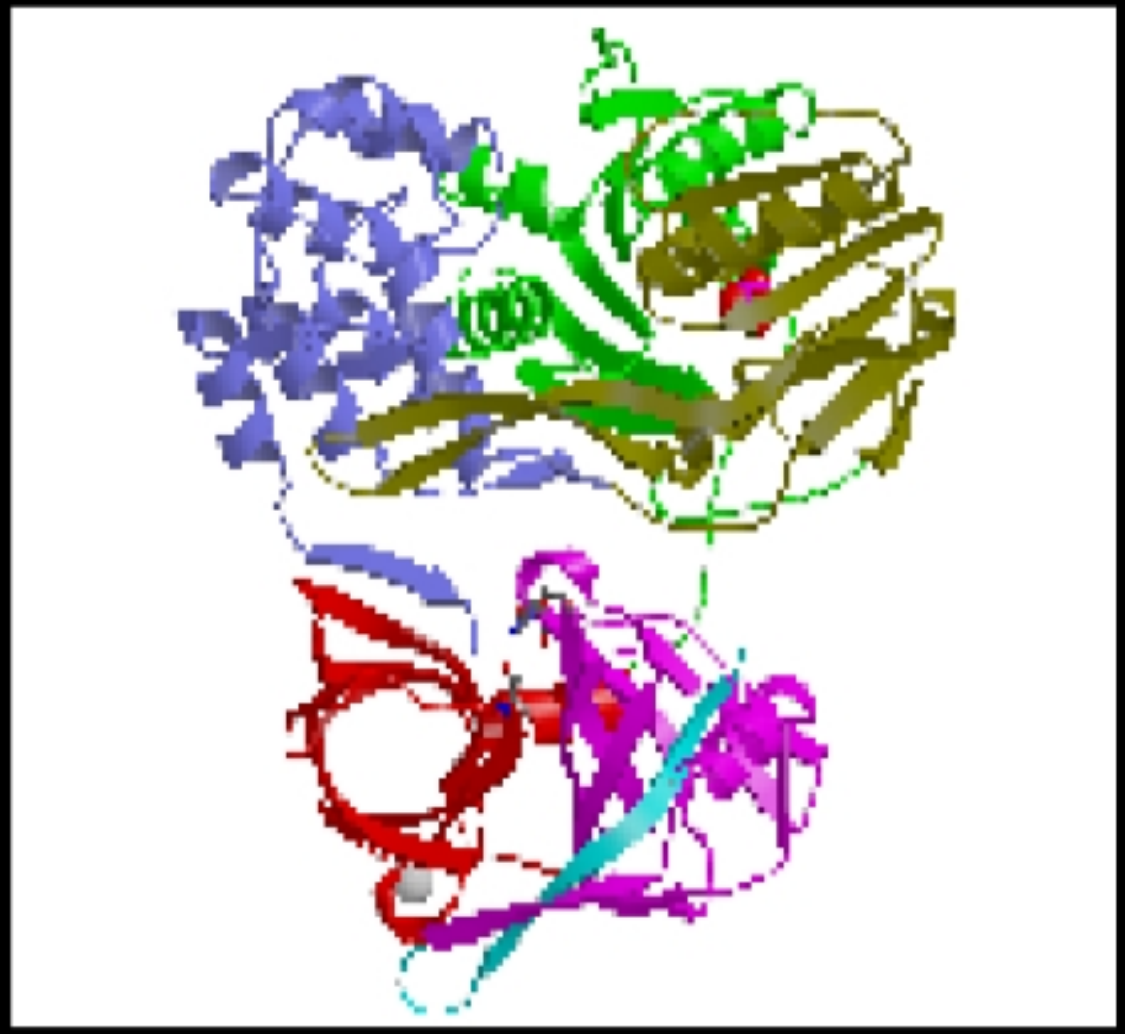
Interaction of the HCV IRES with the 40*S* Ribosomal Subunit





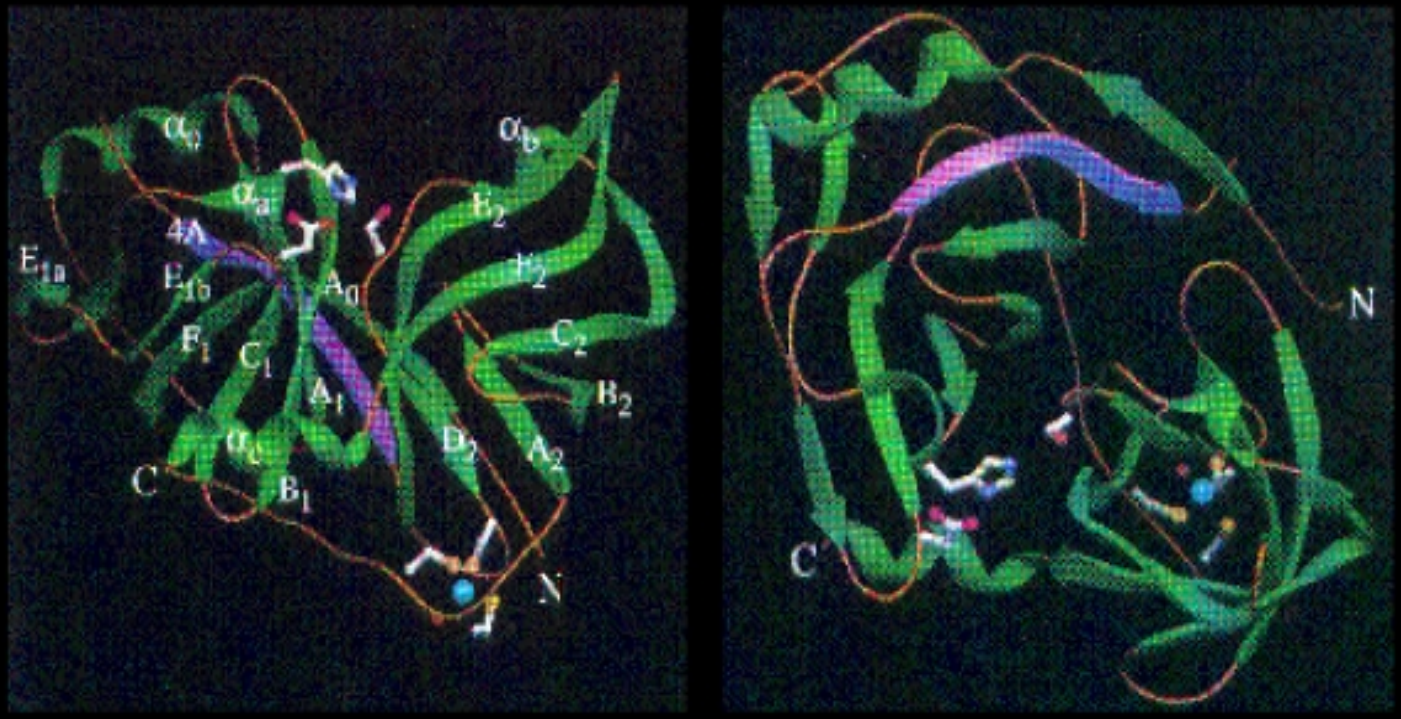
Spahn CMT et al. Science 2001;291:1959-1962.

Structure of the HCV NS3-4A Complex



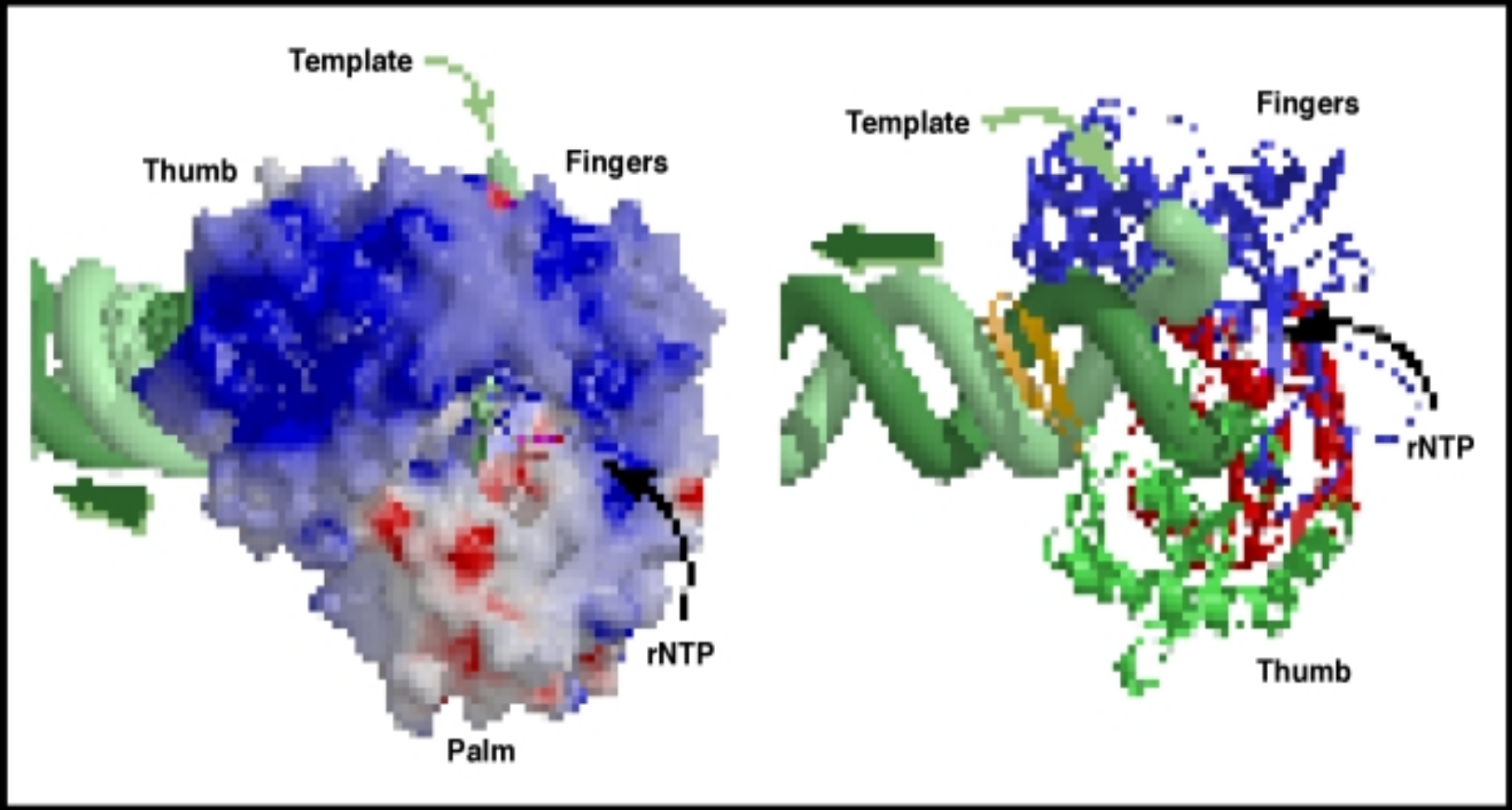
Yao N et al. Structure 1999;7:1353-1363.

Structure of the Hepatitis C Virus NS3 Serine Protease Domain



Kim JL et al. Cell 1996;87:343-355.

Structure of the HCV RNA-Dependent RNA Polymerase



Lesburg CA et al. Nat Struct Biol 1999;6:937-943.

Viral dynamics

• viral half-life	few hours - 1 day
• average daily production and clearance rate	up to 10^{12} copies
• surplus liver cell death/ replacement rate	?

Peripheral viral load

- measured by RNA/DNA amplifying methods
- results given as HCV-RNA copies/ml
- rough statistics of Zurich
  <u>untreated patients</u> (more than 10'000 measurements)

500-1000	< 5%
1000-10'000	5-20%
10^5-10^6	60-75%
10^7 and more	< 5%

Total viral mass

- multiple of viral load ?

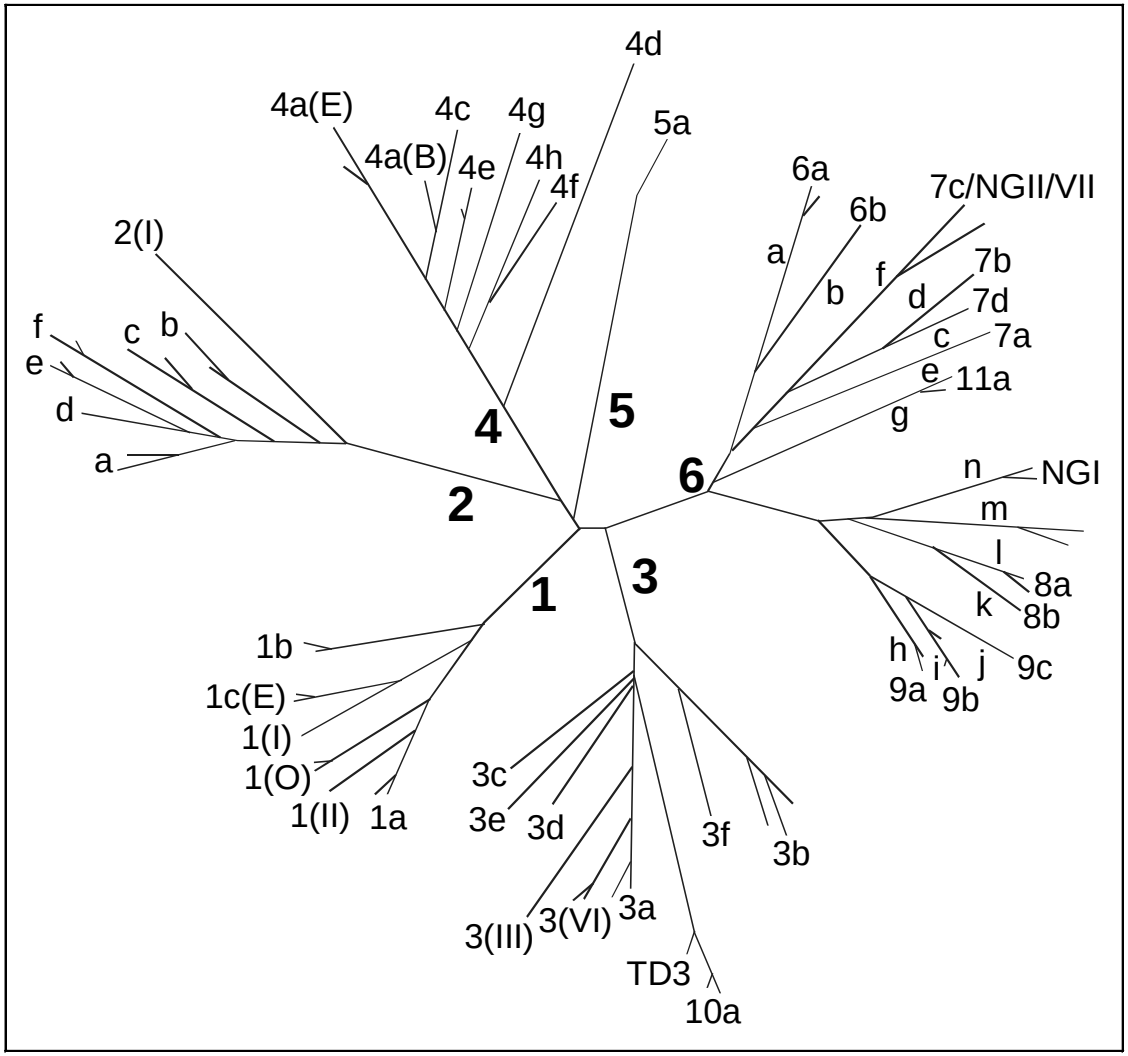
Viral reservoir

- hepatocytes, B-lymphocytes, ev. other cells with

- latent infection ?
- abortive infection ?

HCV genotypes and subtypes

(according to the nucleotide sequences of the HCV NS5B region; according to P. Simmonds)



PG, AKI, Zürich 2002

World wide distribution of HCV genotypes

Country	**Main genotypes**
USA and Canada	1a, 1b, 2a, 2b, 3a
South America	1a, 1b, 2, 3a
Nordern Europe	1a, 1b, 2b, 3a
Western Europe	1a, 1b, 2a, 2b, 3a
Southern Europe	1b, 2c (Italien, Span)
Eastern Europe	1b
Asia	
-Turkey	1b
-Middle East	4
China	1b, 2a, 2b
Africa	
- parts Northern Central Africa	4
- Egypt	4a
- South Africa	1, 2, 3, 5a
Pacific	
-Australia	1a, 1b, 2a, 2b, 3a.
-Taiwan	1b, 2a, 2b
-Japan	1a, 2a, 2b
-Hong Kong	6a, 1b, 2a, 2b
-Thailand	1b, 2, 3, 6
-Malaysia	1b, 2, 3
-Vietnam	1b, 2, 6

According P. Simmonds, P. Marcellin

Distribution of HCV genotypes in Switzerland

HCV genotypes	Zürich [1)]	Geneva [2)]
1	172 (51.9%) [3)]	185 (52.9%)
2	35 (10.6%) [4)]	35 (10.0%)
3	100 (30.2%)	92 (26.3%)
4	22 (6.7%)	34 (9.7%)
5	1 (0.3%)	2 (0.6%)
6	1 (0.3%)	0
mixed types	0	2 (0.6%)
Total	**331**	**350**

1) Tested by the Clinical immunology, University Hospital Zürich between Aug. 99 and Jan. 2000 using the "line-probe assay" [INNO-LiPA, Innogenetics, Ghent, Belgium]

2) Tested by the Gastroenterology and Hepatology, University Geneva between June 98 and Jan. 2000 using „restriction fragment length polymorphism"

3) Subtypes 1a: 64, 1b: 98, other subtypes 1: 10

4) Subtypes 2a/2c: 29, other subtypes 2: 6

Co-infections with multiple HCV-geno-/subtypes

- most infected individuals: 1 geno-/subtype
- < 1-3% 2 or more geno-/subtypes

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in multiple infections

- 1 geno-/subtype often prevails

- genotype 1 over the others
- subtype 1a over 1b

- all infected individuals develop quasi-species

Measurable markers for HCV infection

- **anti-HCV** (against biogenetically produced antigens)
  - screening test formats
  - confirmatory test formats

- No distinction between ongoing and past infection
- Anti-HCV might disappear decades after end of infection (under estimation of HCV prevalence)
- Immuno-compromised individuals with ongoing HCV infection might have no anti-HCV

- **HCV-RNA** (measured by RNA/DNA amplifying methods)
  - commercial tests available with lowest detection limits
    - 600 copies/ml (quantitative test format)
    - 50 copies/ml (qualitative test format)

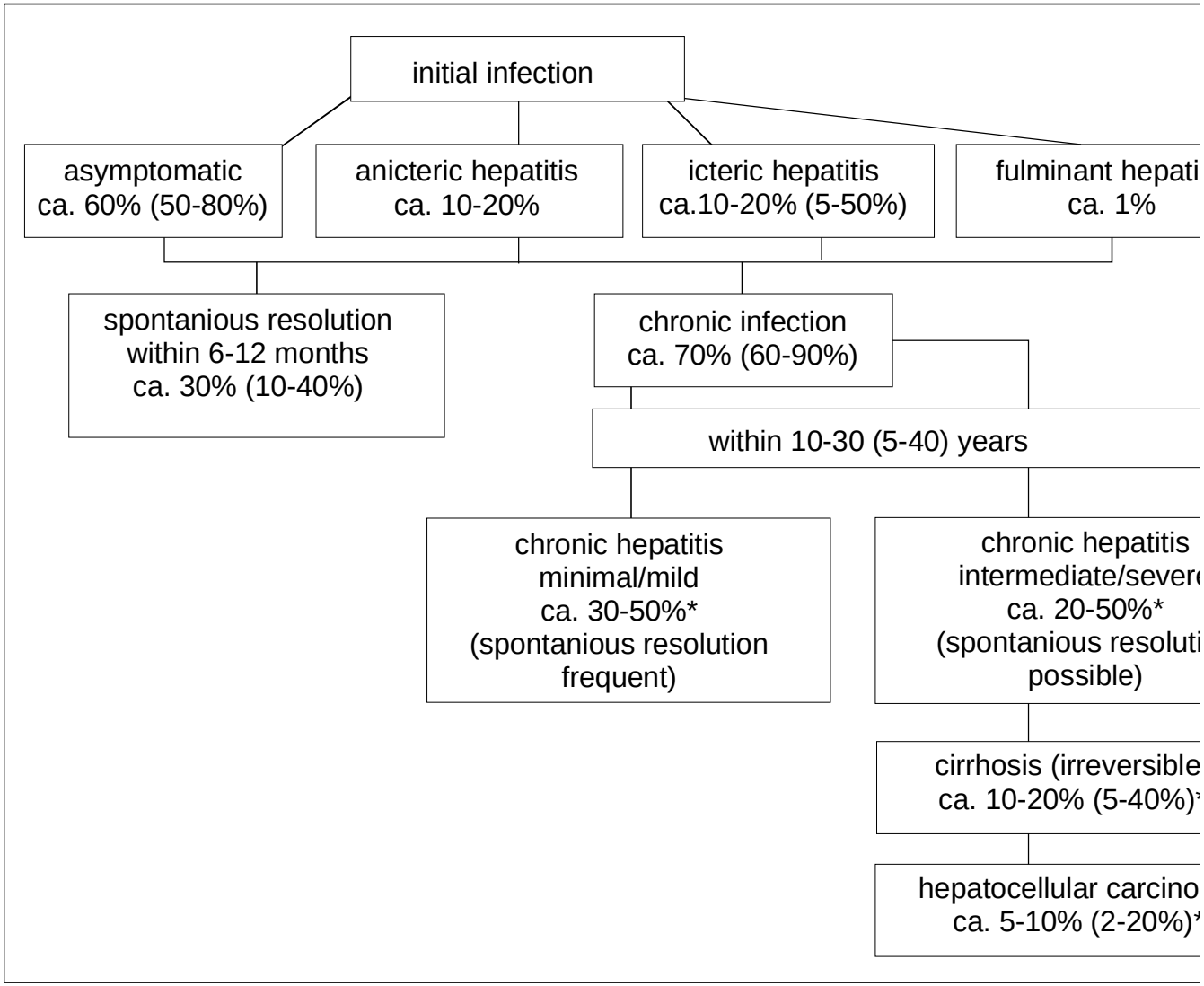
Low-grade HCV infection is not detectable

- **HCV core antigen**

- **HCV components in cryoglobulins**

- Autoantibodies (against cell nuclei, mitochondria etc)

Natural disease course of HCV infection



* of those individuals with chronic infection

Infection course and pathogenic mechanisms

- The course of infection and the eventuell clinical sequelae are very variable, the clinical sequelae being
  - surplus liver cell replacement/turnover *
  - liver inflammation *
  - liver fibrosis *, liver cirrhosis *, HCC
  - extrahepatic manifestations *: cryoglobulinopathy/vasculitis

* These events do often take an independent course.
  The individual course of infection is not predictable.

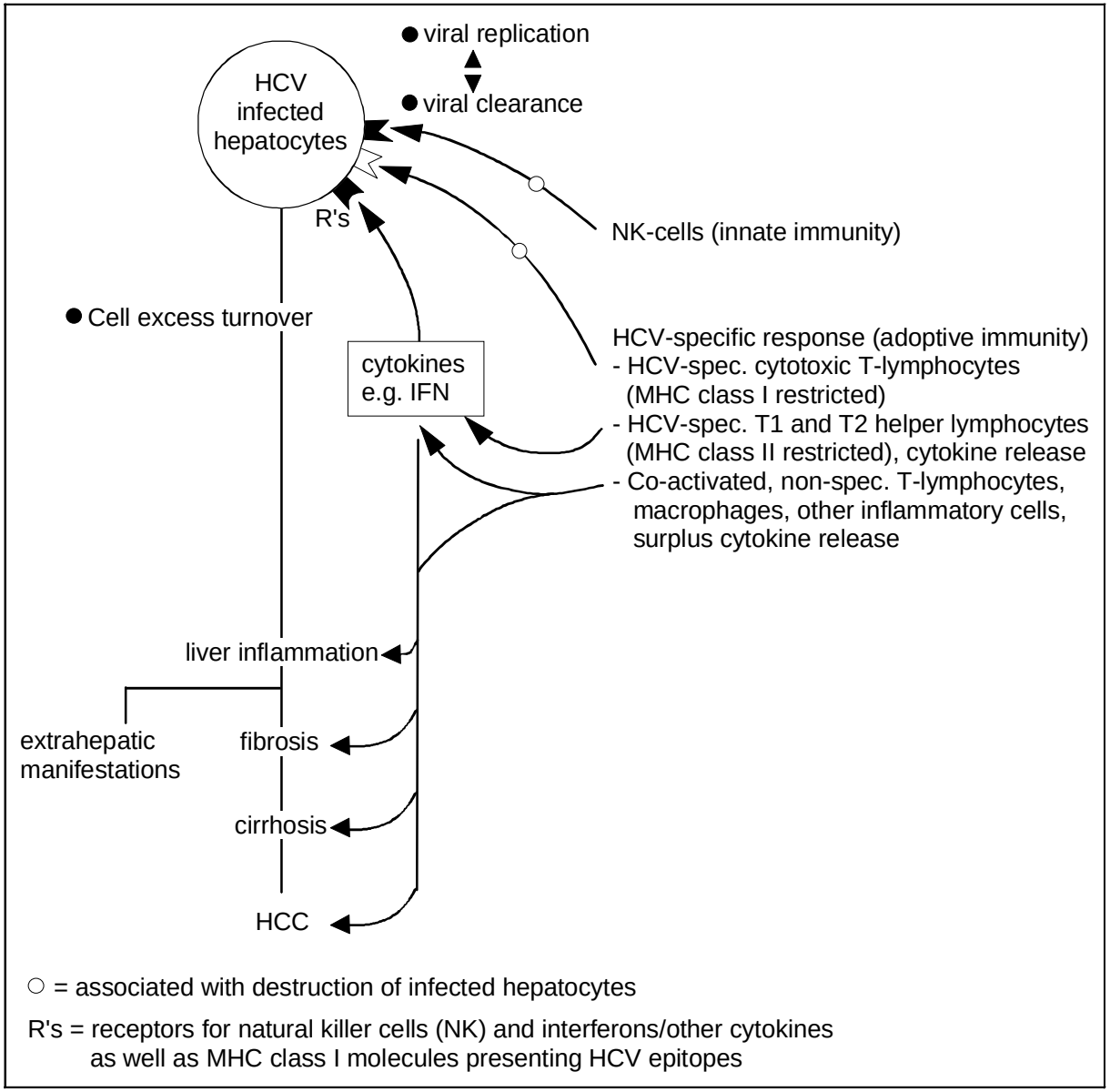
- Also the crucial events of infection must have complex self-tuning and interaction mechanisms, the crucial events being:
  - viral replication, viral clearance

- HCV is not essentially cytopathogenic. Immune reactions are thought to be essential for
  - viral elimination
  - pathogenic events leading to the clinical sequelae

- The essential immune reactions are
  - HCV-specific reactions:
    - cytotoxic T lymphocytes, · T1 and T2 helper lymphocytes (cytokine release),
    - B lymphocytes/plasma cells producing anti-HCV
  - non-specific reactions (co-activated lymphocytes, macrophages, other inflammatory cells) leading to a surplus production of cytokines

- The immune reactions leading to the pathogenic events seem antigen/HCV driven.

HCV and speculative defense mechanisms



PG, AKI, Zürich 2002

Factors decisive for the outcome of a HCV-infection

Influencing factors	
virus dependent	• infection dose
	• HCV replication rate
	• “aggressivity” of HCV
	• escape mutations (e.g. quasi-species)
	• viral reservoir (abortive and latent infections)
	• resistance to anti-virals
host dependent	• innate immunity (natural killer cells, complement [alternative pathway]) etc
	• specific immunity to HCV (antigen presentation on MHC class I and II molecules, HCV-specific cytotoxic and helper [type 1 and 2] T-cells, B-lymphocytes [antibody formation], cytokine production
	• non-specific immune response (co-activated T and B cells, macrophages, dentritic cells, surplus cytokine release etc)
	• genetics (e.g. MHC class I, II dependent) at various levels
	• sex, age at infection
	• risk behaviour e.g. alcohol intake
	• viral co-infections e.g. with HIV, HBV, GBV-C/HGV, HAV
environmental	• nutritive etc

HCV - Superinfection with HAV

Patients with chronic HCV infection, superinfected with HAV
→ increased risk of fulminant hepatitis

- negative reports: Leino et al. 1997, Battegay et al. 1998
  Helbling et al. 1998, Mele et al. 1998

- confirmation: Pramoolsinsap et al. 1999 (Thailand)

HCV - Co-infection with HBV

- Fulminant hepatitis: HBV-related fulminant hepatitis;
  HCV co-infection might often be implicated (Feray et al. 1993)

- **Chronic co-infections HBV/HCV**:
  viral level:
  - HBsAg is lost → „anti-HBc alone“ (HBV-DNA pos.: 2-80%)
    (Jilg et al. 1995, Grob et al. 2000)
  - HBsAg and anti-HBc are lost (HBV-DNA pos.) = occult HBV-infection
    (Cacciola et al. 1999)
  - HBV-DNA and HCV-RNA levels are lower in single than
    in double infections (Jardi et al. 2001)

  clinical level:
  Patients with double infections
  - more aggressive liver disease
  - HCC is more frequent
  - less response to therapy
    (Brechot et al. 1998, Chiaramonte et al. 1999, Tagger et al. 1999)

HCV and HBV

Co-infections with HBV of patients with chronic HCV-infection might be underestimated (Cacciola 1999).

200 patients with chronic HCV-infection
- HBsAg neg.
→ 100 patients with „anti-HBc alone“ → 46 (46%) HBV-DNA pos.
→ 100 patients without any HBV markers → 20 (20%) HBV-DNA pos.

Total: 200 patients → 66 (33%) HBV-DNA pos.

HCV-RNA	HBV-DNA		
+	+	n = 66	→ 22 (33%) cirrhosis
+	-	n = 134	→ 26 (20%) cirrhosis

HCV and HIV

- Simultaneous infection with HCV and HIV
  (Eyster et al. 1993; 223 hemophiliacs, Yee et al. 2000; 310 hemophiliacs,
  Garcia-Samaniego 1997, Soto 1997)
  - accelerates the progression of liver disease including HCC
  - liver disease develops earlier
  - liver related death is more frequent

- Simultaneous infection with HCV and HIV (and low CD4 counts)
  (Di Martino et al. 2001)
  - worsened outcome of liver demage
  - HCC occurs earlier
  - increased level of HCV-RNA
  - decreased response to interferon therapy

GBV-C/HGV and HIV infection

• Tillmann et al., New Engl. J Med 2001; 345;10: 715-724

197 HIV-infected patients
- 33 (16,8%) GBV-C/HGV-RNA pos.
- 112 (56,9%) anti-E2 pos.
- 52 (26,4%) no markers

• Xiang et al., New Engl. J Med 2001; 345;10: 707-714

362 HIV-infected patients
- 144 (39,8%) GBV-C/HGV-RNA pos.

41/144 (28,5%) GBV-C/HGV-RNA pos. patients died
123/218 (56,4%) GBV-C/HGV-RNA neg. patients died

Main conclusions of both papers
GBV-C/HGV-infection of HIV-infected individuals results in:
- reduced mortality
- slower progression to AIDS
- longer survival with AIDS
- lower viral load, higher CD4 lymphocytes

Independent of age, sex, risks, and concentrations of CD4 lymphoycytes

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• Data remained controversial. Very preliminary results: HCV infection is mandatory

Experimental systems

- Chimpanzees, only animal susceptible to HCV infection. Limitations/protection
- Newer test systems

  - HCV-infection in immunodeficient mize reconstituted with human hepatocytes (Lechner 2000)

  - Replicon system (Blight 2000, Lohmann 1999)
    In vitro transcribed HCV-RNA „plasmid“ constructs containing IRES is transfected into HuH-7 human hepatoma cells. Clones with replicating subgenomic HCV-RNA are then selected.

Future therapeutics and vaccines

- Therapeutics: e.g. phase I and phase II clinical trials with inhibitors of
  - NS3 serine protease
  - HCV RNA helicase
  - HCV RNA-dependent polimerase

- New vaccines
  - peptide and protein vaccines
  - dentritic cell based vaccines
  - vaccines with virus-like particles
  - DNA vaccines

A phase II clinical trial (therapeutic vaccination) is currently ongoing with a HCV E1 recombinant vaccine