L'infezione Occulta da Virus dell'Epatite B

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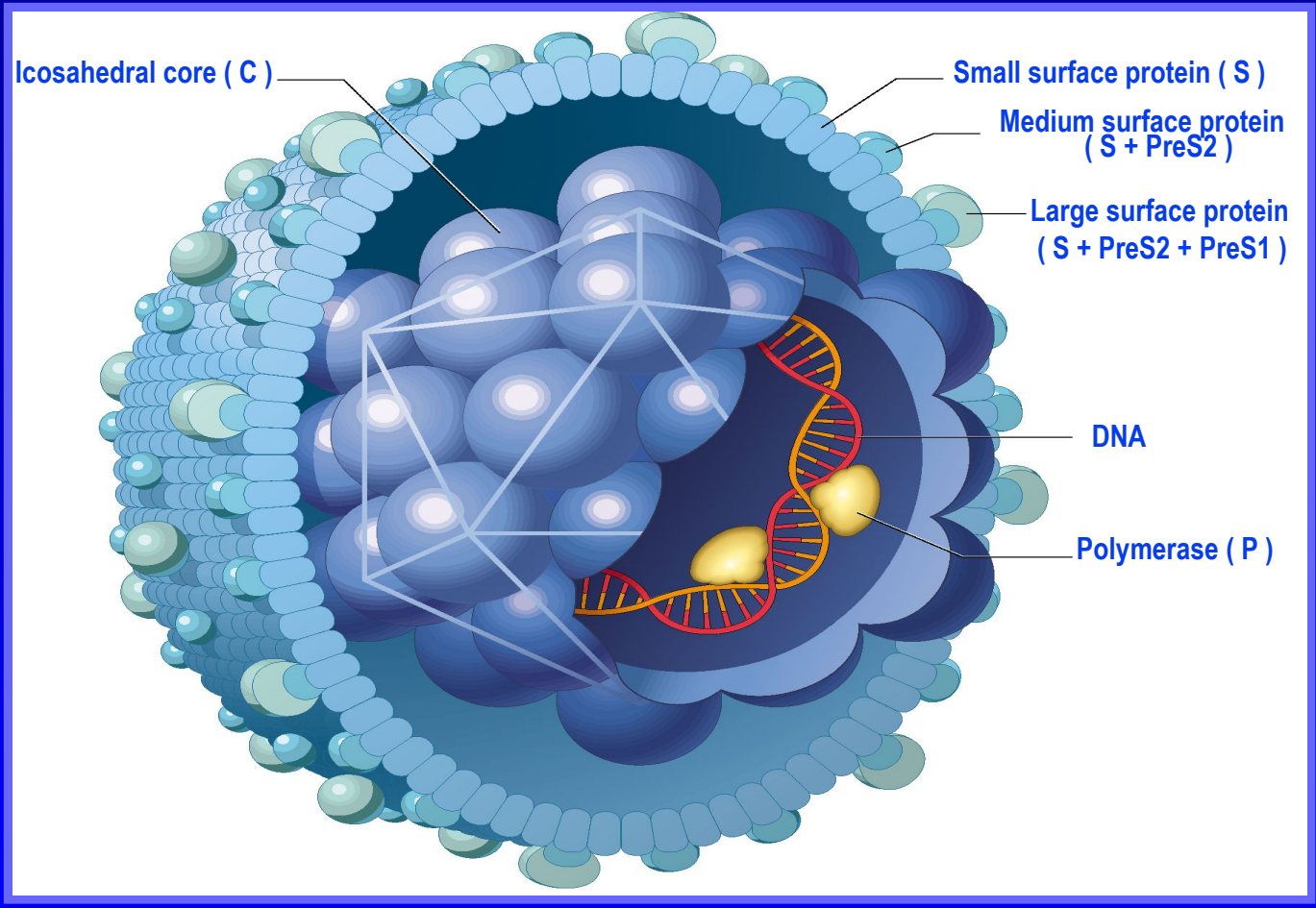
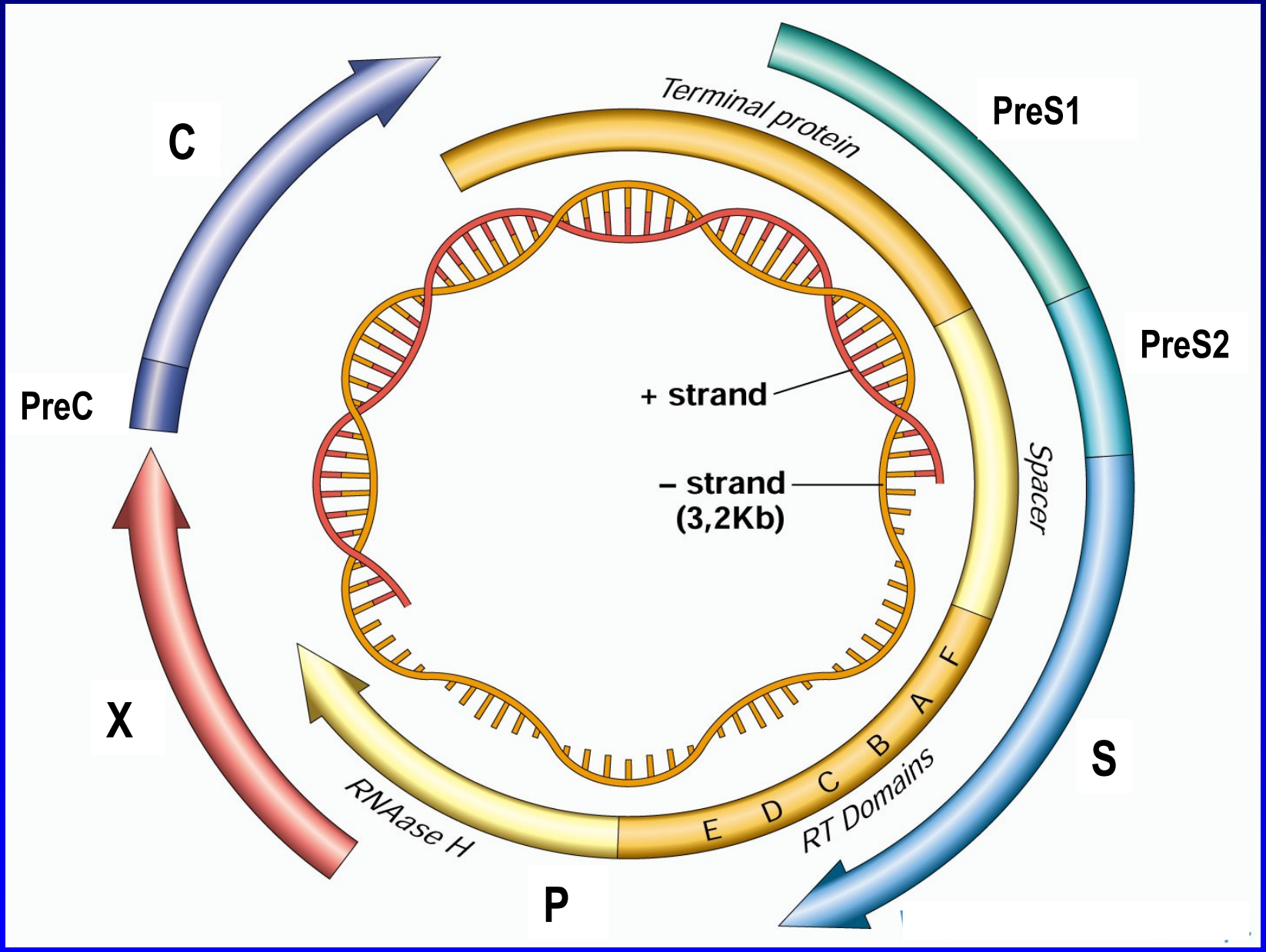
Definition of Occult hepatitis B virus Infection

- Presence of HBV DNA without detectable HBsAg
  - outside the window period
  - with or without anti-HBs
  - with or without anti-HBe
  - with or without anti-HBc
- Found in
  - Hepatocellular carcinoma
  - Chronic hepatitis B, healthy HBV carriage
  - Recovered infection
  - No HBV marker
  - Chronic HCV infection

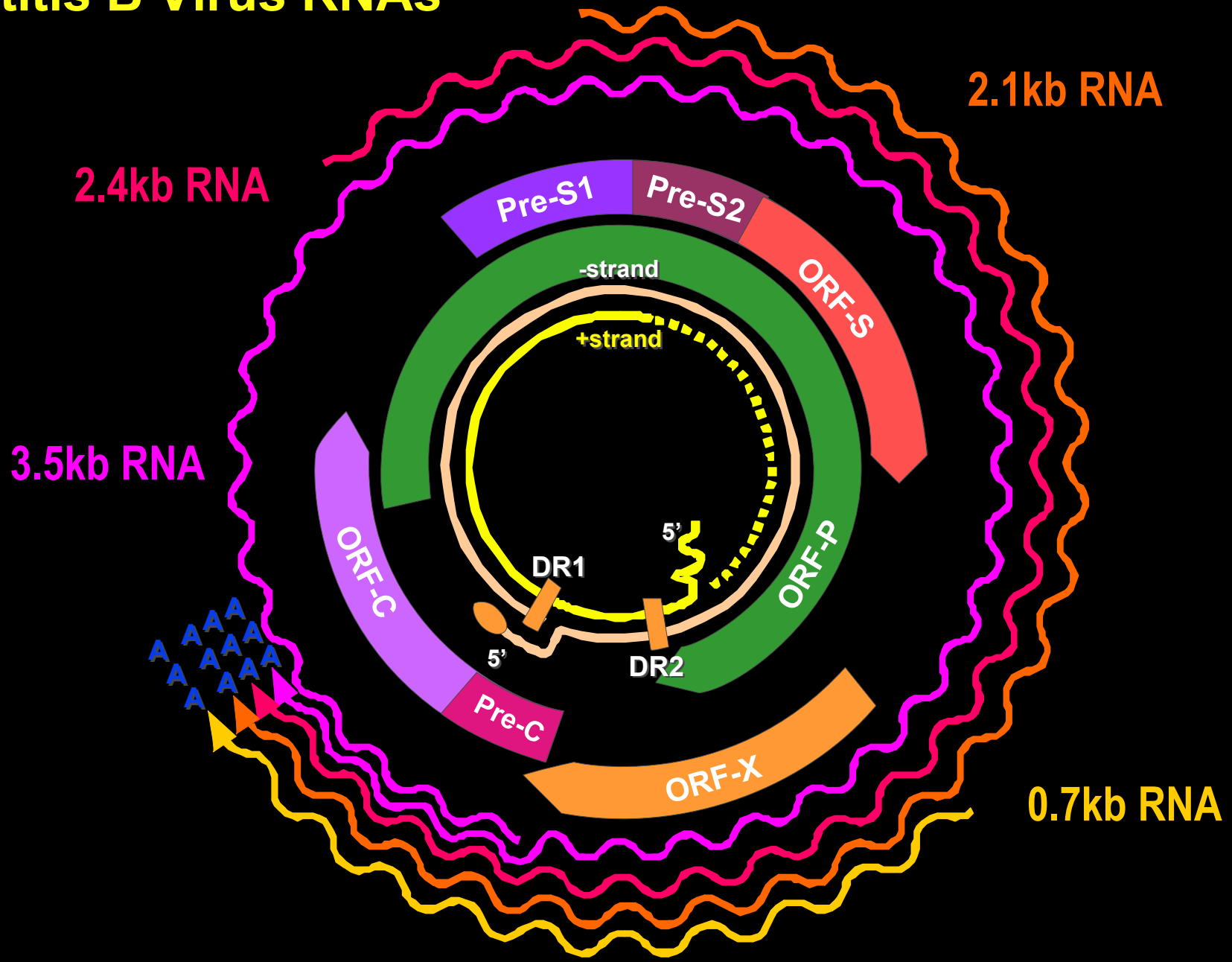
Virus dell'Epatite B



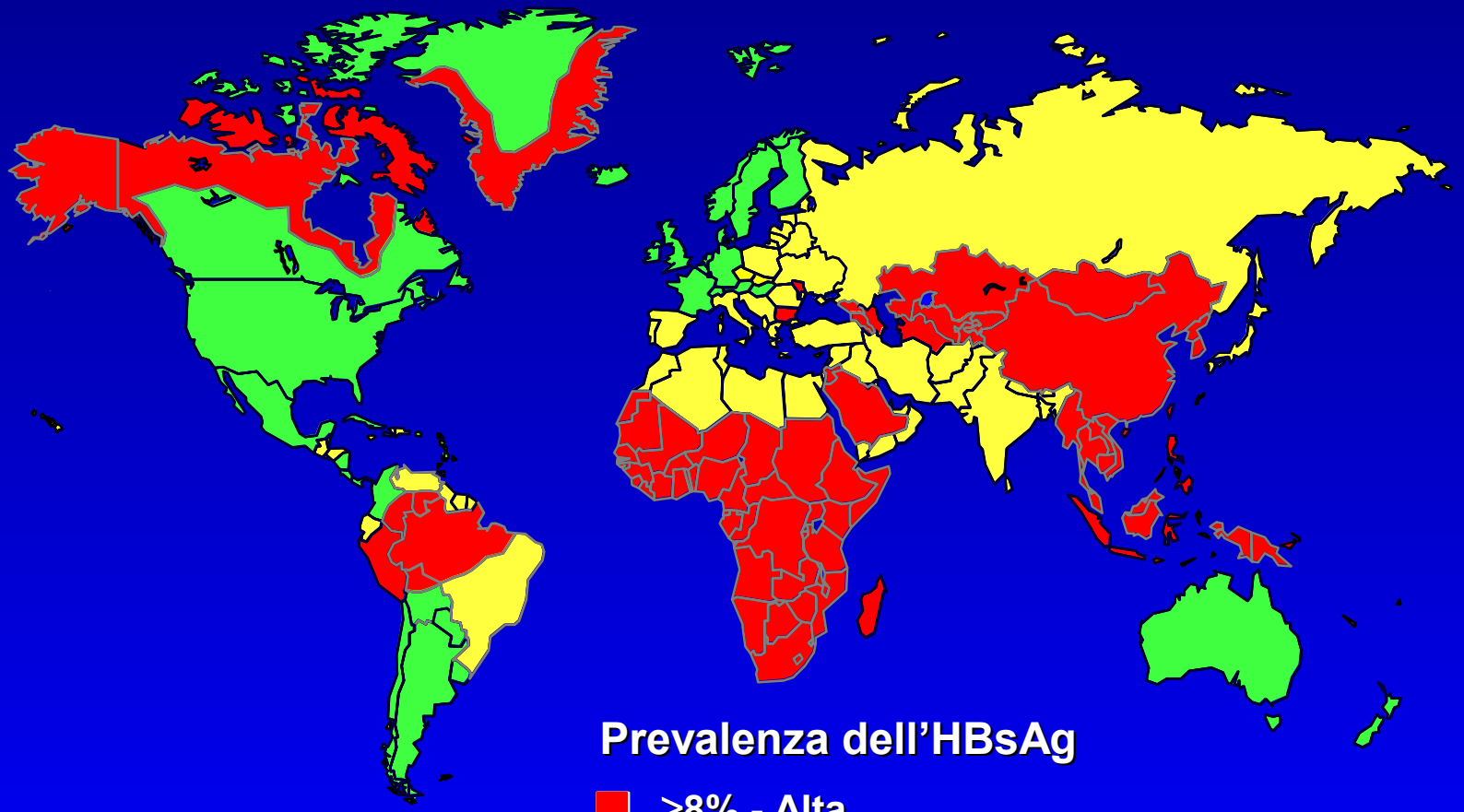
HBV Genome Organization



Hepatitis B Virus RNAs

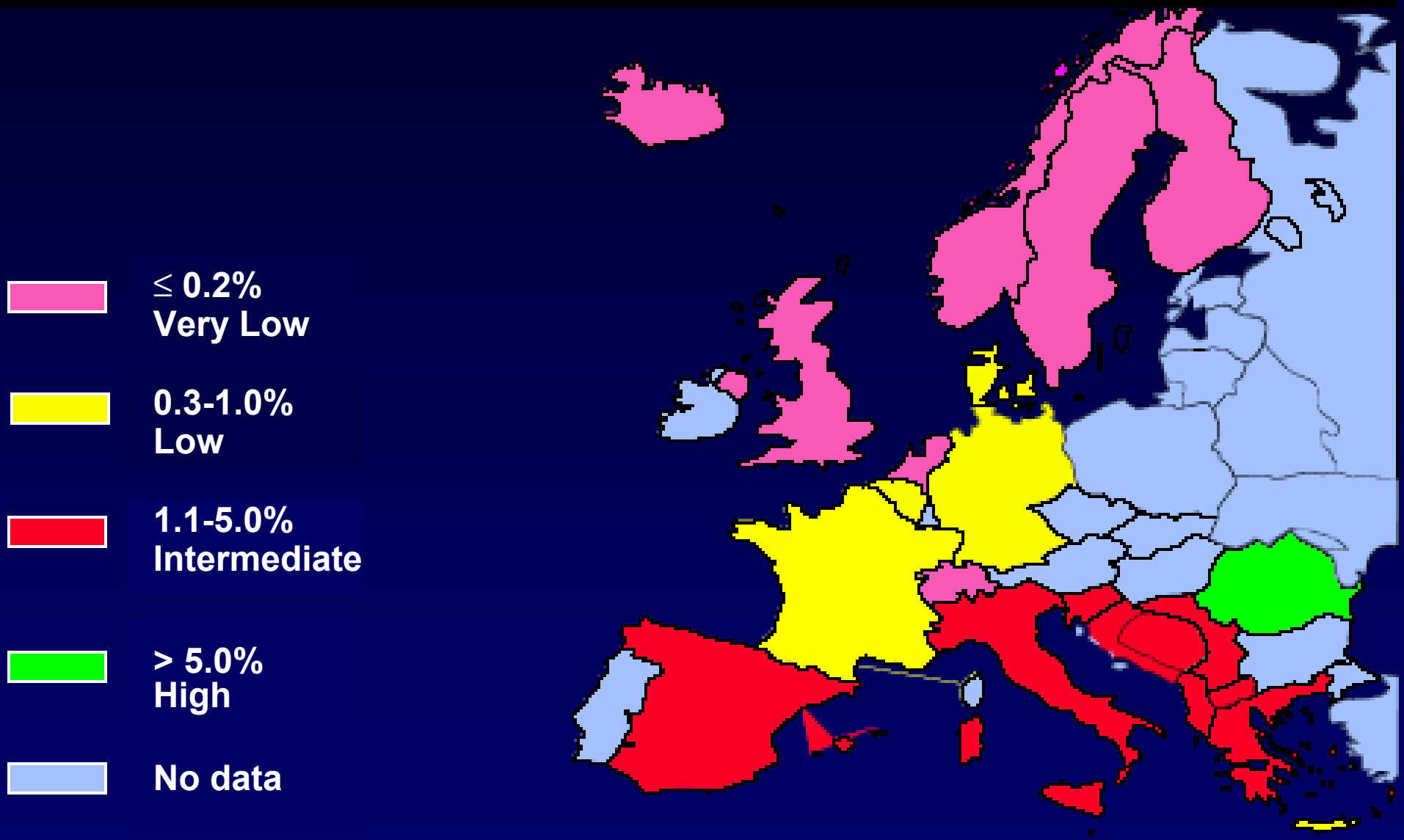


Distribuzione Geografica dell'Epatite Cronica B



Fonte: CDC and Prevention

Prevalence of HBsAg Positivity in Europe

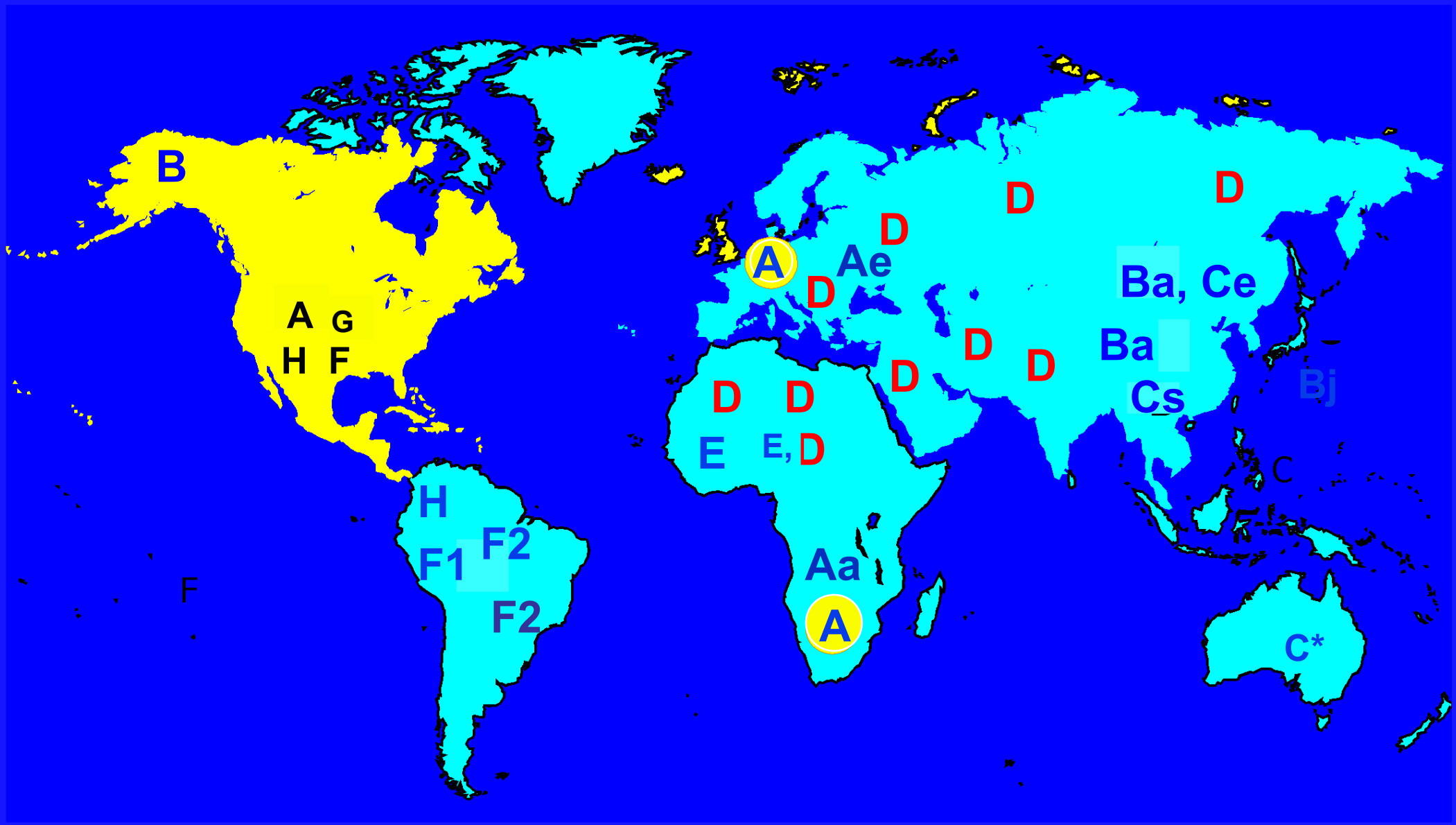


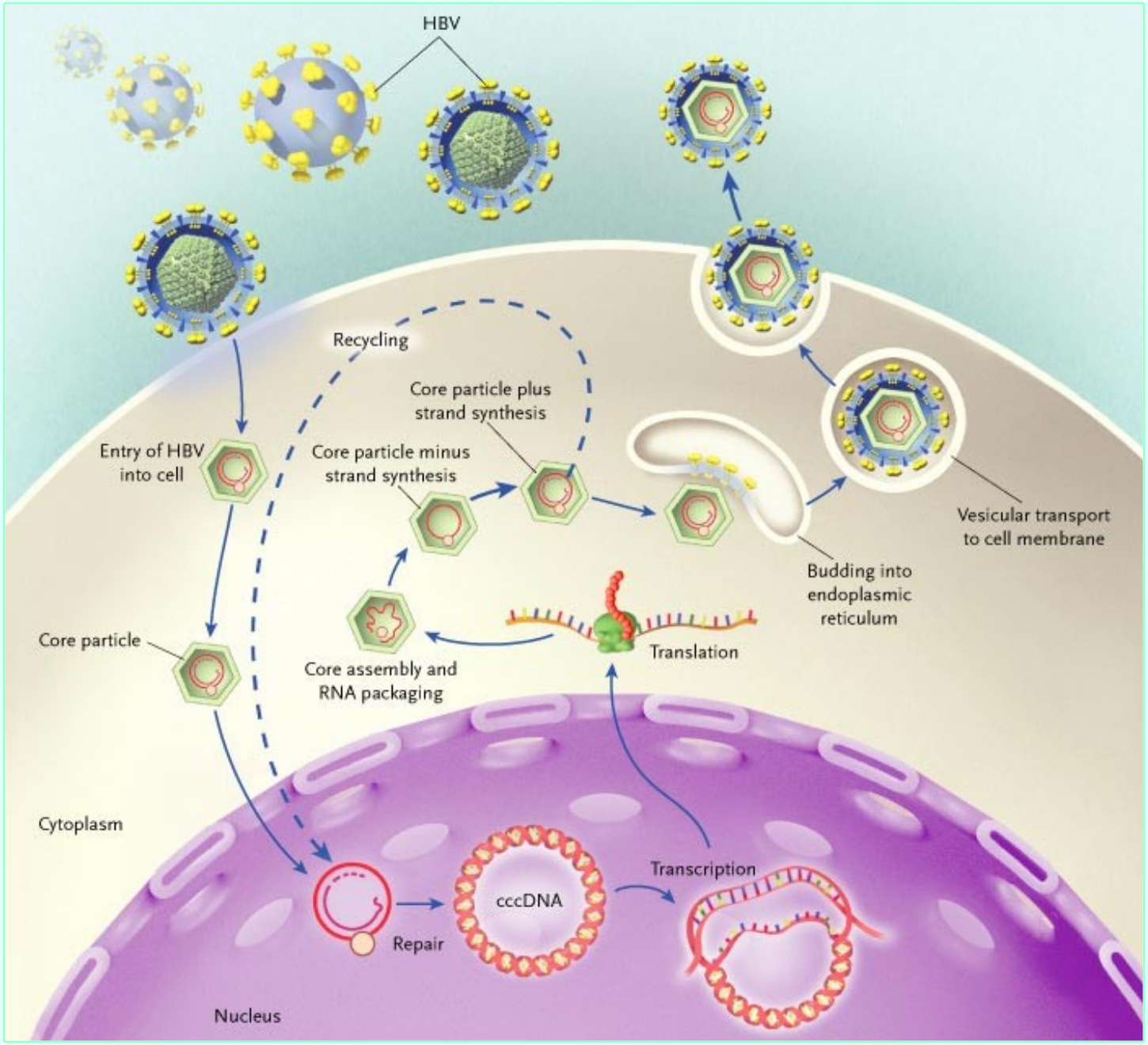
Genotipi dell'HBV

8 Genotipi A ⟶ H

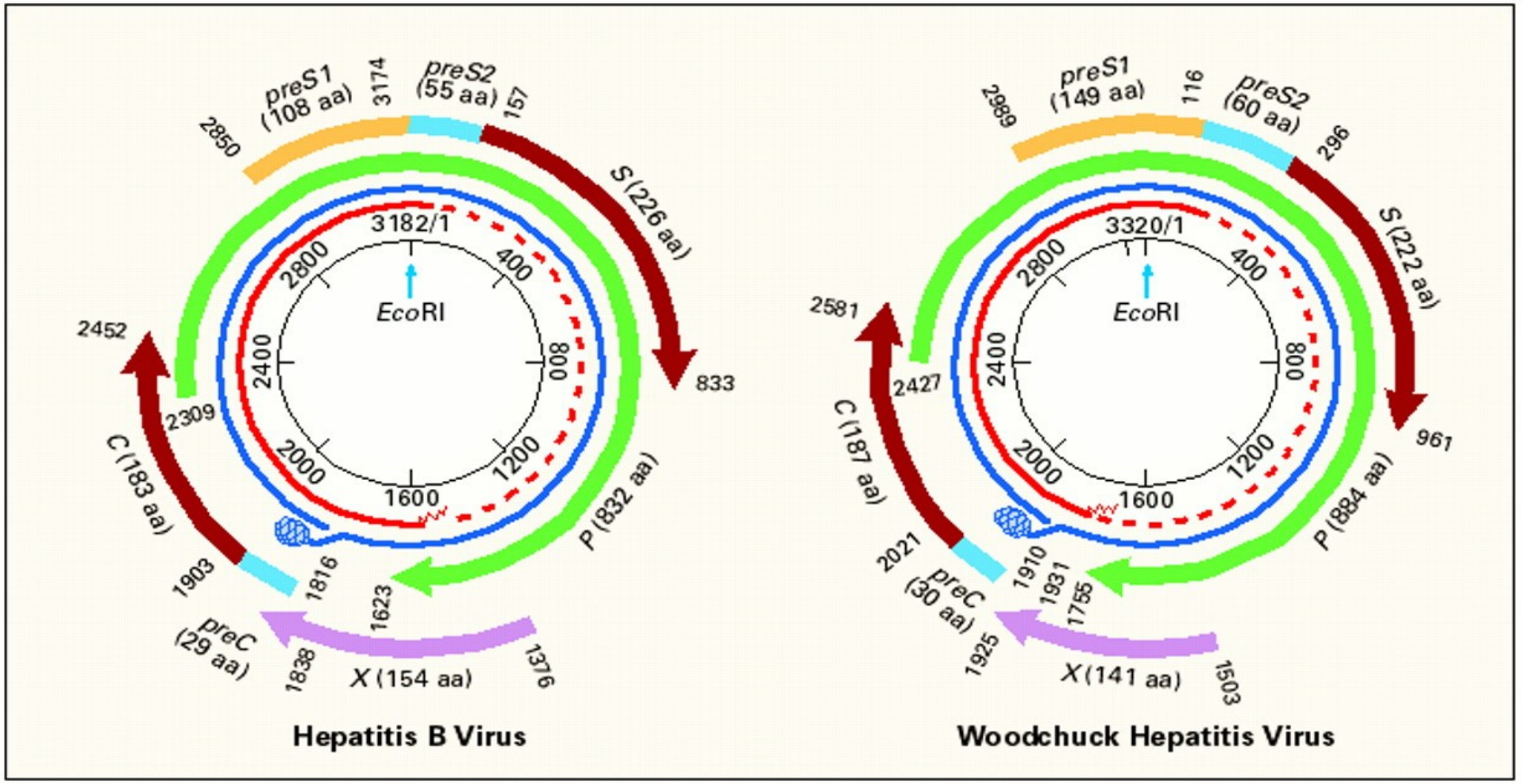
- Classificati in base ad una differenza ≥ 8 % sull'intera sequenza nucleotidica
- Differente distribuzione geografica
- Associati con differenti frequenze e profili di mutazioni dell'HBV
- Contribuiscono in differenti parti del mondo a variazioni di : epidemiologia, storia naturale, esito clinico, risposta al trattamento

Distribuzione Geografica dei Genotipi dell'HBV A - H




HBV
Recycling
Core particle plus
strand synthesis
Entry of HBV
into cell
Core particle minus
strand synthesis
Vesicular transport
to cell membrane
Budding into
endoplasmic
reticulum
Core particle
Core assembly and
RNA packaging
Translation
Cytoplasm
Transcription
cccDNA
Repair
Nucleus

Genomes of HBV and Woodchuck Hepatitis Virus



Lee W. N Engl J Med 1997;337:1733-1745

HBV – INFEZIONE OCCULTA SECONDARIA (RESIDUA)

Modello animale - WHV

- **Infezione residua persistente**
  - **in animali completamente guariti dopo epatite acuta sperimentale**
  - **che sviluppano anticorpi verso l'antigene di superficie**
  - **con anticorpi anti-core persistenti per tutta la vita**

- **DNA virale è presente nel siero, cellule linfoidi (PMBC) e tessuto di fegato**
- **Intermedi replicativi, cccDNA e mRNA virali sono presenti in cellule del sistema linfatico e nel fegato**

HBV – INFEZIONE OCCULTA SECONDARIA (RESIDUA)

Modello animale - WHV

- Modesta, intermittente necroinfiammazione del fegato nella maggioranza degli animali completamente guariti dopo epatite acuta sperimentale
- 20% degli animali sviluppa epatocarcinoma 3-5 anni dopo la guarigione nonostante la presenza di anticorpi verso l'antigene di superficie
- Il virus residuo negli animali con infezione occulta secondaria può essere riattivato dopo trattamento con ciclosporina A, con la comparsa

HBV – INFEZIONE OCCULTA SECONDARIA (RESIDUA)

Modello animale - WHV

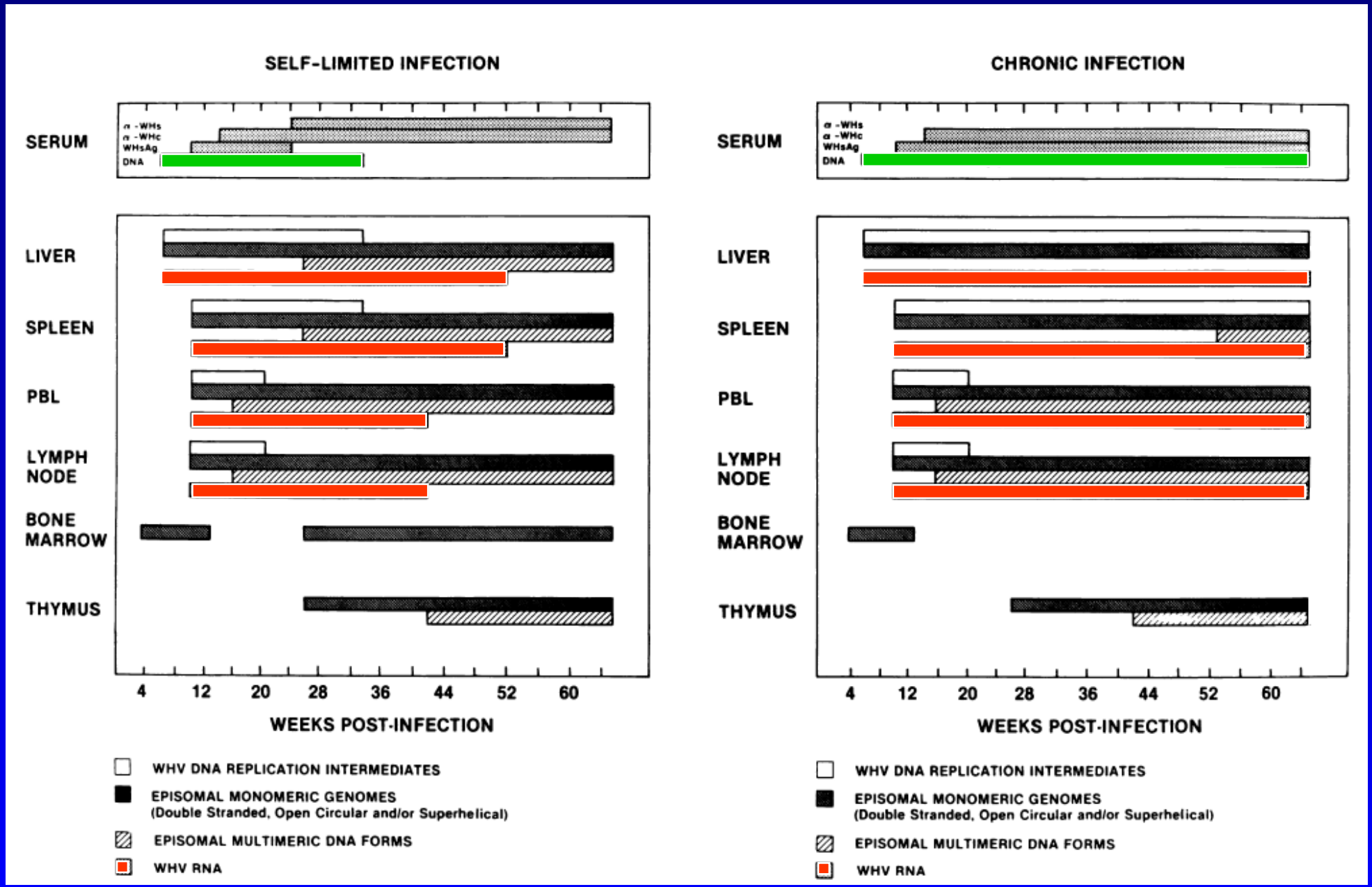
- La presenza di anticorpi anti-core in assenza di altri marcatori sierologici di infezione è un indicatore affidabile di persistenza di virus occulto
- Questa condizione, analogamente all'esistenza di risposte linfocitarie HBV- specifiche (cytotoxic T cell and T helper) a distanza di anni dalla guarigione da un'epatite acuta B è con ogni probabilità la conseguenza di una prolungata ristimolazione del sistema immune con una proteina virale prodotta durante da HBV
- La presenza di anti-HBc è importante per identificare l'infezione occulta

HBV – INFEZIONE OCCULTA PRIMARIA

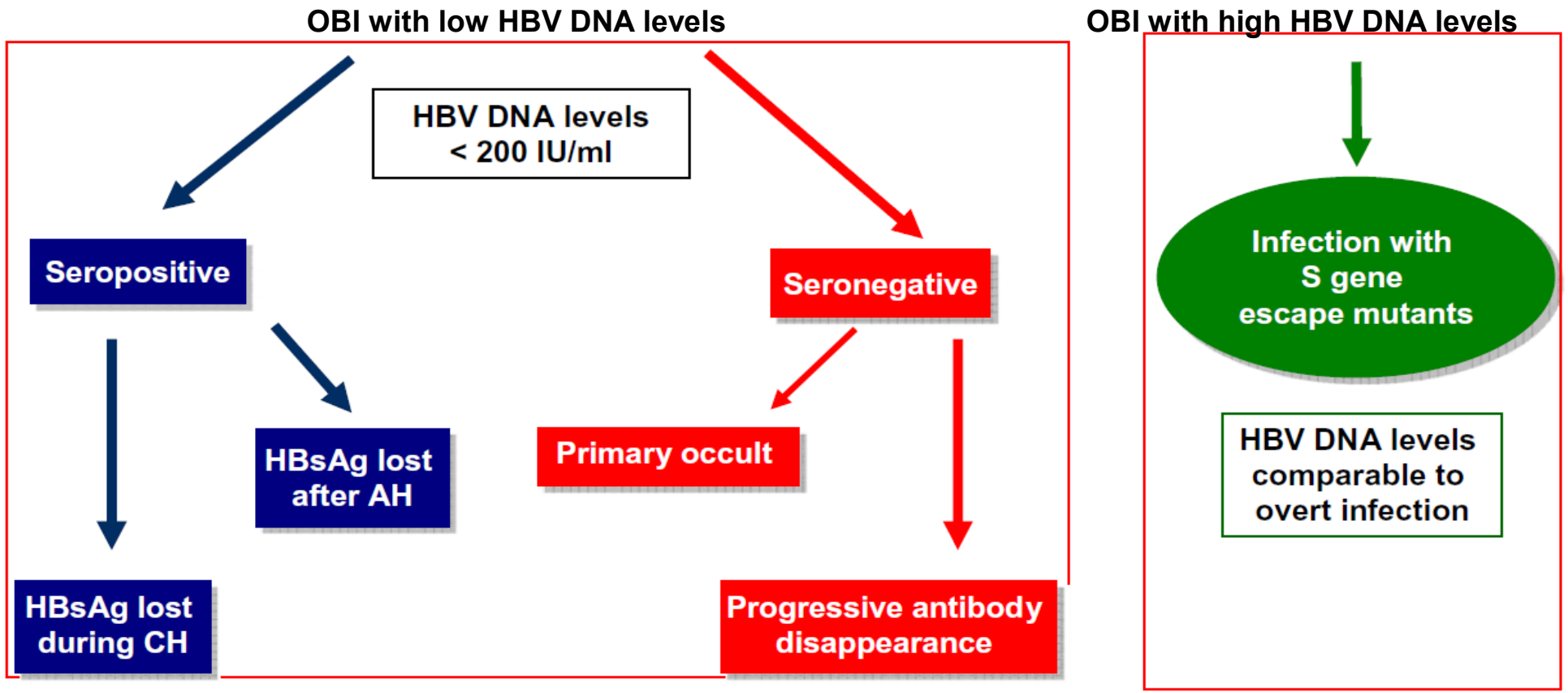
Modello animale - WHV

- DNA virale & cccDNA sono presenti solo nel sistema linfatico, non nel fegato, in assenza di marcatori sierologici di infezione, inclusi gli anticorpi anticore
- L'infezione occulta è stata indotta in animali adulti per iniezione E.V. con una dose virale < 1.000 virioni
- Dosi virali > 1.000 virioni, oltre ad infettare il sistema linfatico, provocano l'epatite acuta classica – sono patogeni per il fegato
- Nell'uomo, la presenza di un'infezione HBV DNA positiva, anti-HBc negativa è suggestiva di un'infezione occulta primaria

Natural history of WHV infections during the course of experimental viral infection - (*J Virol 1989*)



Schematic representation of HBV profile in OBI



Raimondo et al , Statements from the Taormina Expert meeting on OBI - J Hepatol 2008

Natural History of Chronic HBV Carriers in Northern Italy

(Manno et al - Gastroenterology Sep 2004)

- In Modena between 1972 and 1977 296 HBsAg + blood donors

  were excluded from donation

- Re-evaluated after 30 years, 32.2% became HBsAg negative

  (yearly incidence, 1%)

- Antibodies to HBsAg were present in 45.7% of carriers who

  cleared HBsAg

- 10.1% remained HBV DNA positive by PCR

  (detection limit, 10^2 - 10^3 genome equivalents / mL)

HBsAg seroclearance in chronic hepatitis B in Asian patients

Yuen et al - Gastroenterology Oct 2008 (Hong Kong)

After HBsAg seroclearance	Serum HBV DNA +	Serum HBV DNA Median level (range)
Within 1 year (n=142)	13.4 %	7.0 IU/mL (1.1 – 396.8 IU/mL)
Between 5 and 10 years (n=99)	6.1 %	13.9 IU/mL (1.6 – 169.5 IU/mL)
> 10 years (n=27)	3.7 %	53.6 IU/mL

Of the 29 patients who underwent liver biopsies between 1 and 10 years after HBsAg seroclearance, all (100 %) had detectable intrahepatic HBV DNA, and 79.3 % had detectable

Occult hepatitis B virus in liver tissue of individuals without hepatic disease

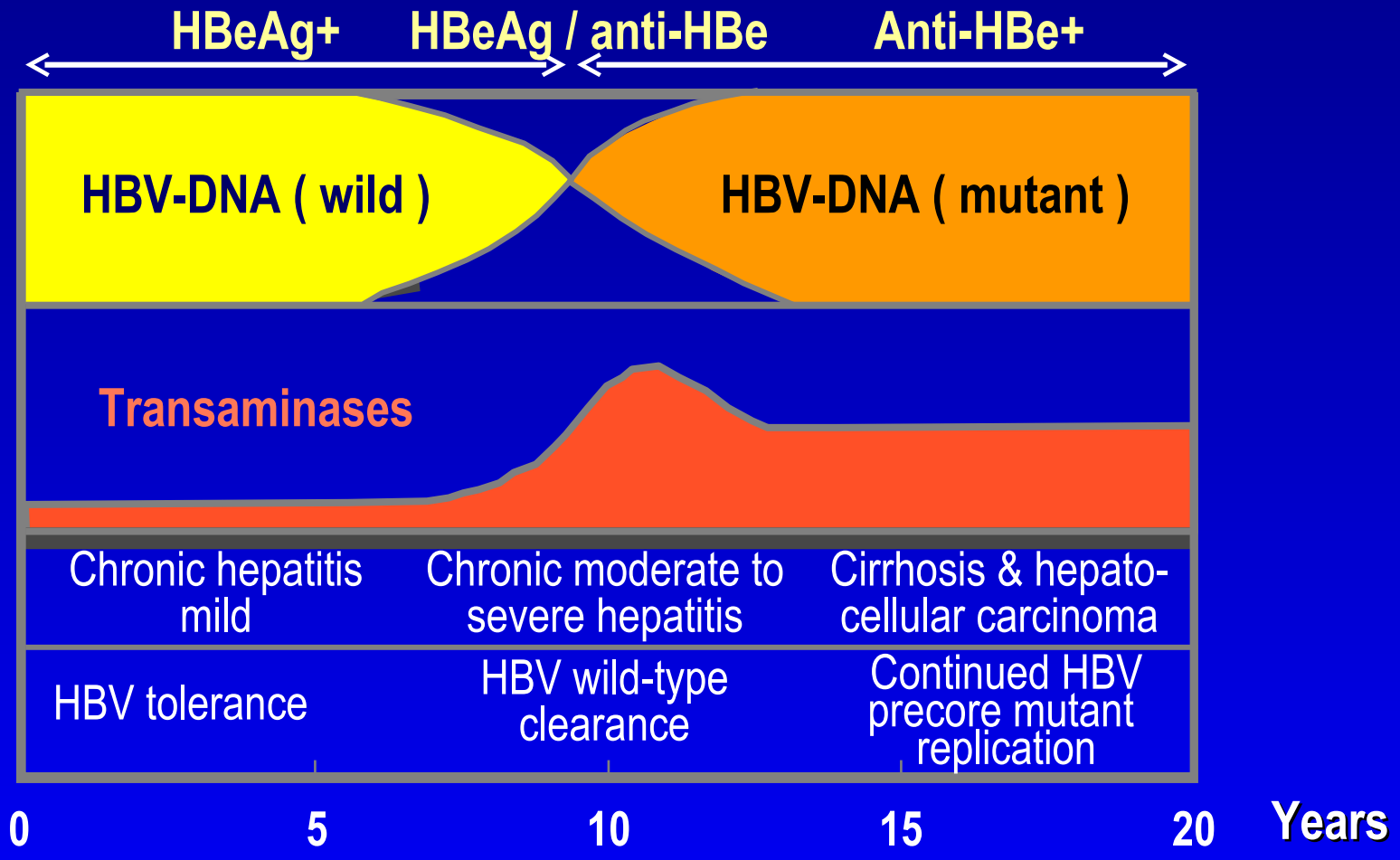
(G. Raimondo et al - J Hepatol May 2008)

Liver specimens from 98 liver-disease-free individuals who underwent liver resection or needle biopsy during abdominal surgery were tested for the presence of HBV DNA

OBI was revealed in 16 of the 98 cases (16.3%) :

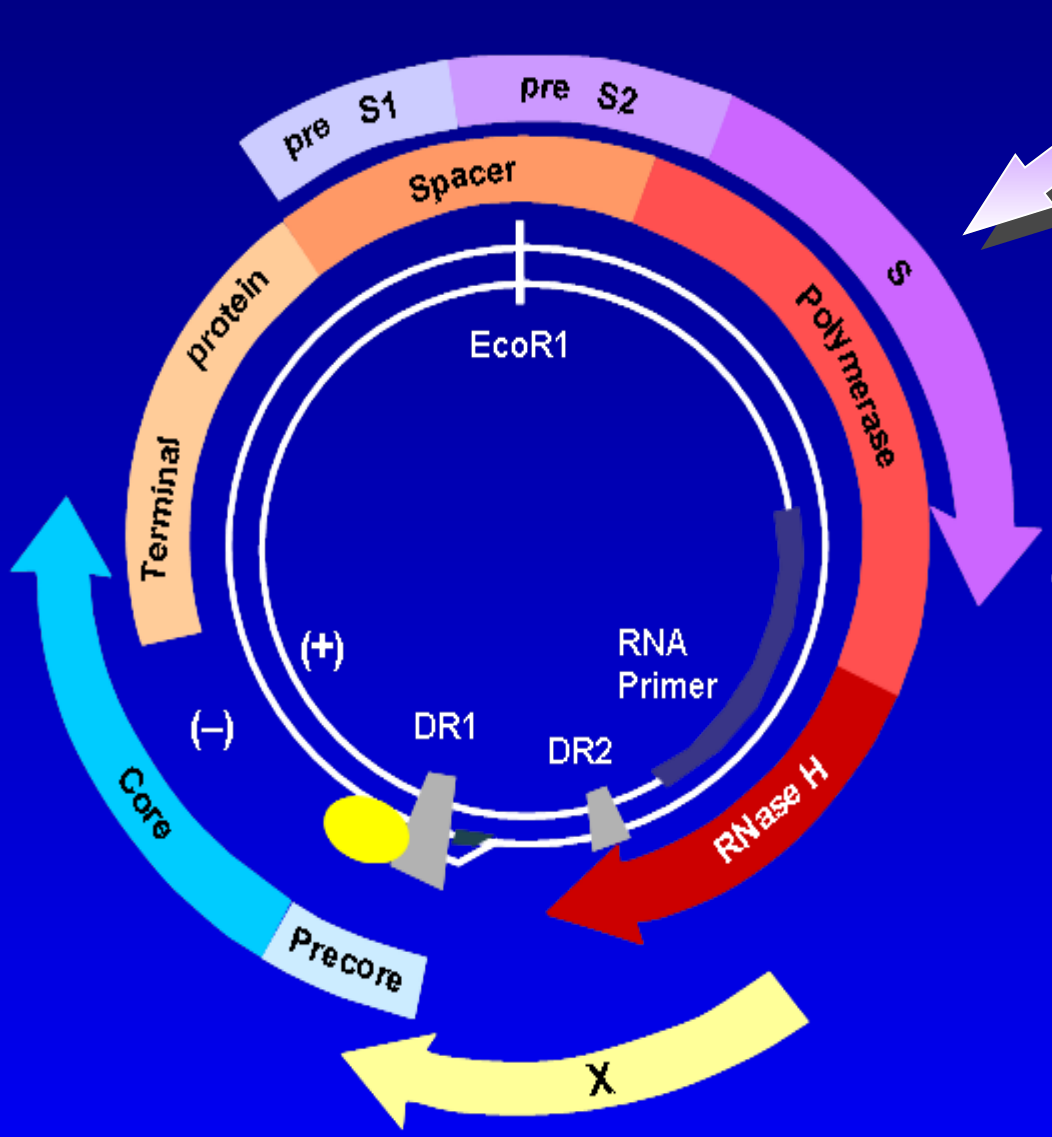
- **10 / 16 anti-HBc positive (62.5%)**
- **6 / 82 negative for all HBV markers (7.3%)**

Emergence of the HBeAg-Negative Precore Mutant



Carman WF et al Viral Hepatitis. Scientific Basis and Clinical Management. NY: Churchill Livingstone; 1993:121.

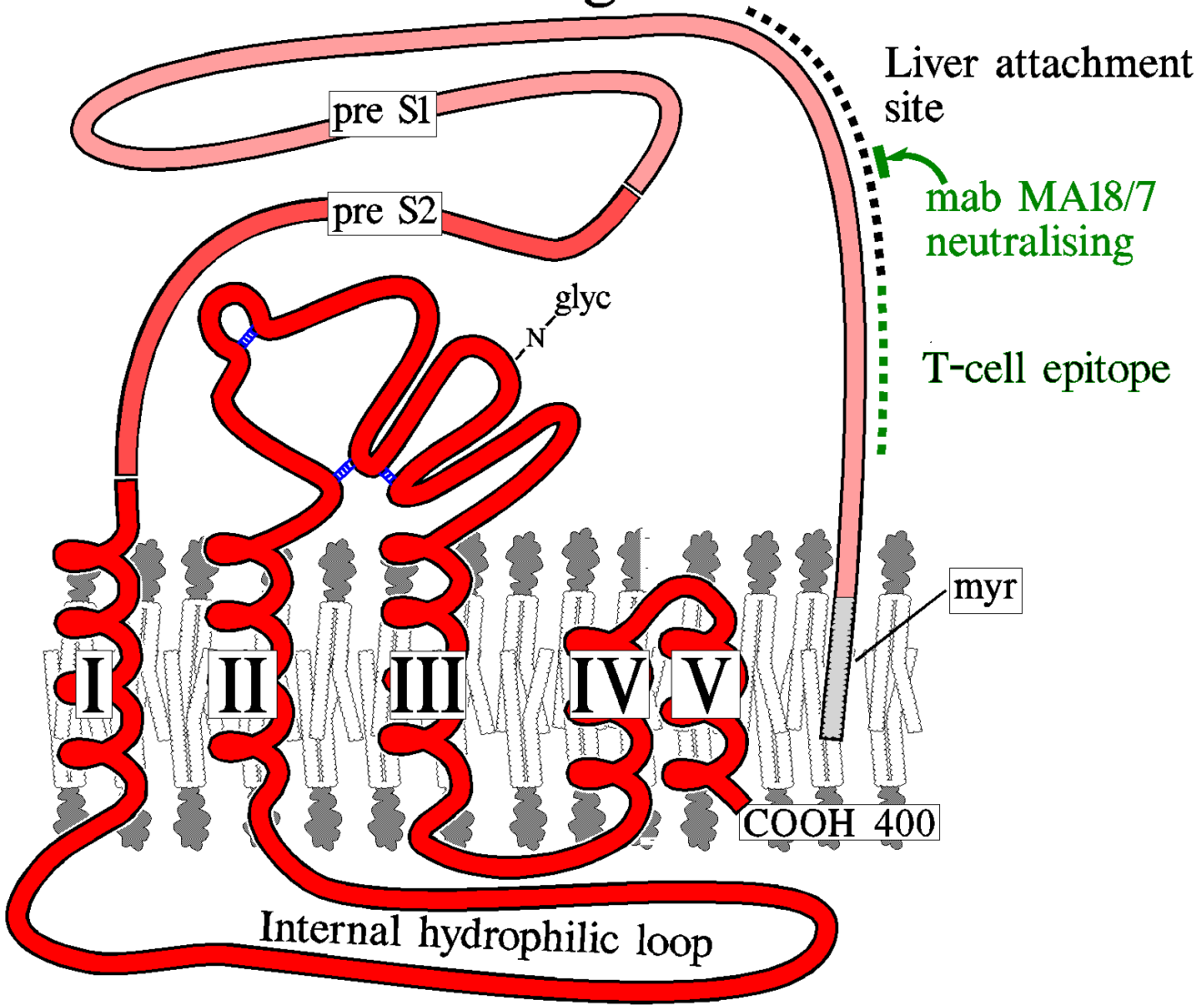
Determinante “a” del gene S dell’HBV



determinante “a”

porzione del dominio idrofilico di HBsAg (aa 101-160)

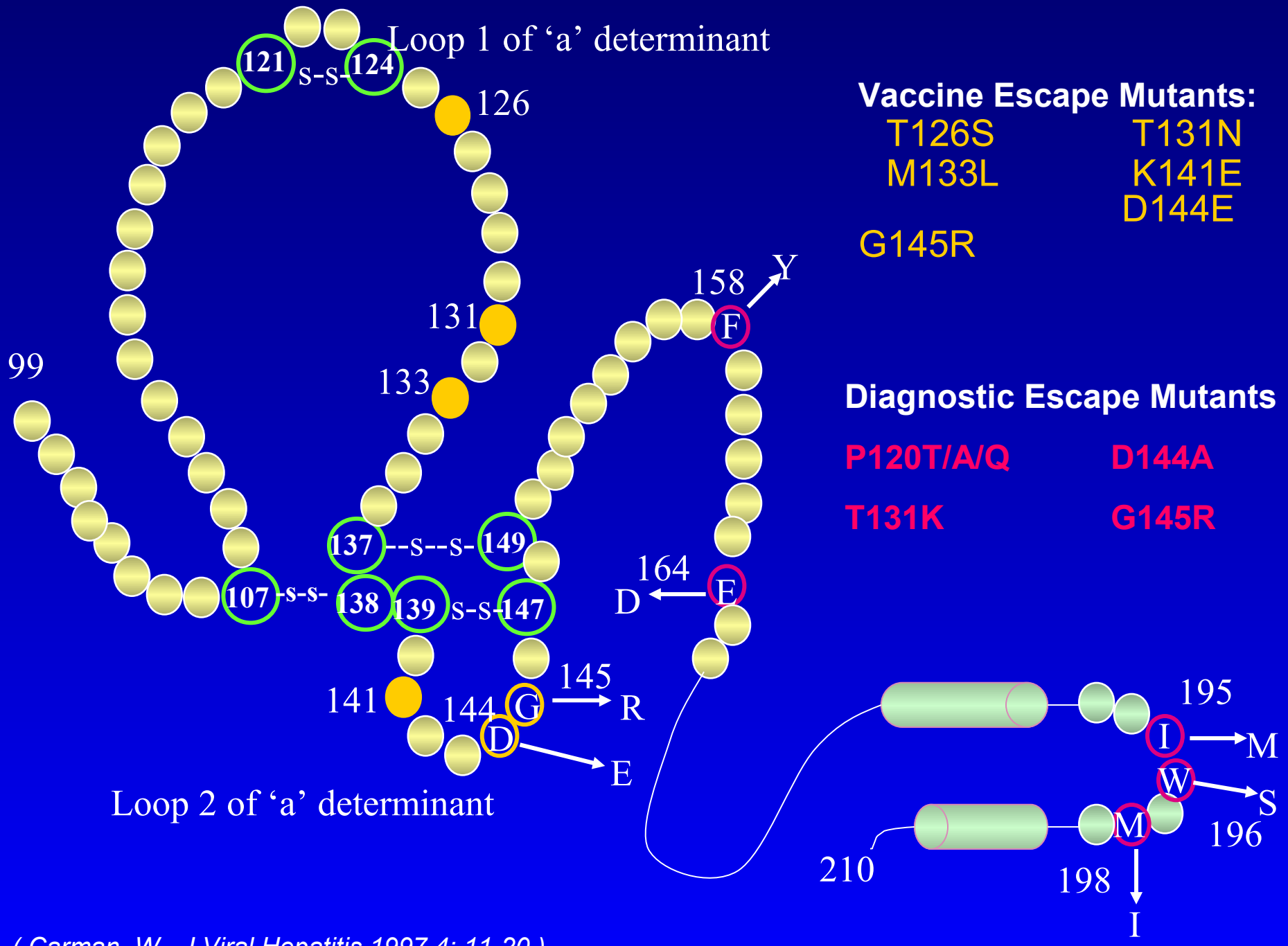
Structural elements of Large HBs Protein (LHBs)



Origine dei Mutanti del Gene S dell'HBV

- **Escape mutants indotti da vaccino / HBIg**
  - Bambini nati da madre HBsAg +
  - Riceventi trapianto di fegato

- **Escape mutants naturali indotti dalla pressione immunitaria durante l'infezione cronica da HBV**
  - Portatori positivi per HBsAg e anti-HBs, anticorpi diretti verso epitopi non più presenti sugli antigeni circolanti
  - Infezione occulta da HBV



Vaccine Escape Mutants:

T126S T131N
M133L K141E
D144E
G145R

Diagnostic Escape Mutants

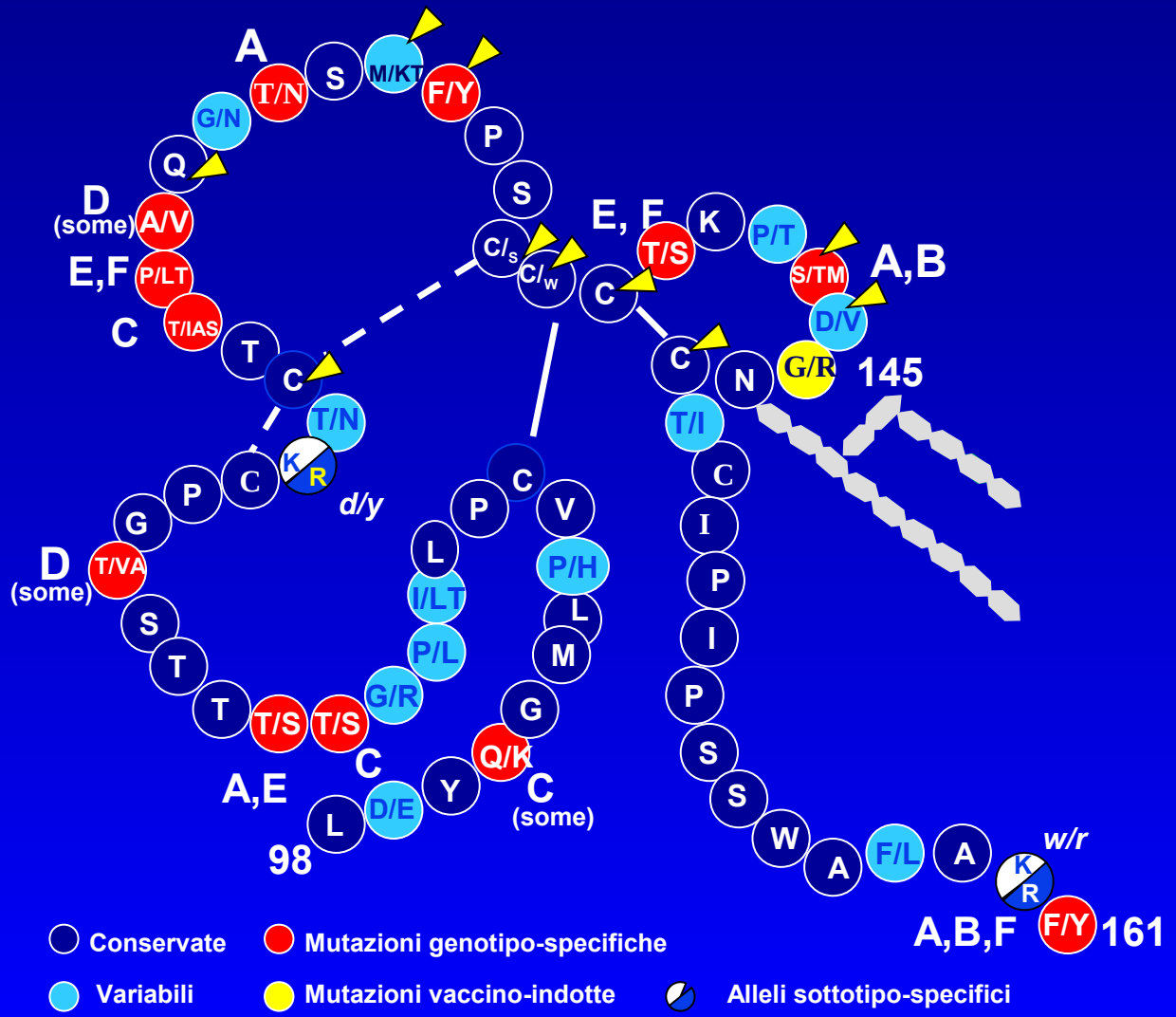
P120T/A/Q **D144A**
T131K **G145R**

(Carman, W. J Viral Hepatitis 1997 4: 11-20)

Mutazioni Genotipo-specifiche e Escape Mutants da Vaccino nel Determinante "*a*" del Gene S dell'HBV

(S.Schaefer, modificato)

Schaefer, 2001



aa (n=11) Genotipo-specifici nella Regione Immunodominante del Gene S dell'HBV, in base alla Sequenza Consensus

Determinante "a"

Genotipo	115	124	139	147	167
D (n=72)	TTSTGPCRT	CTTPAQGTSMYPSCCCTKPSDGNC			TCIPISSWAFGKFLWEWAS
H (n=7)	. . . . . K.	. . .L . . . . . F.			. . . . . Y. . . . .
G (n=10)	. . . . . K.	. . . . . N			. . . . . A.Y. . . . .
F (n=31)	. . . . . K.	. . .L . . . . . F. . . . S			. . . . . L . . Y. . . . .
E (n=6)	. . . . .	. . .L . . . . . F. . . . S			. . . . .
C (n=165)	. . . . . K.	. .I . . . . . F			. . . . . AR. . . . .
B (n=117)	. . . . . K.	. . . . . F. . . . . T. . . .			. . . . . A.Y. . . . .
A (n=90)	. . . . . K.	. . ↕ ↕ . . . ↕ . F. . . . ↕ . . T. . . .			. . . . . ↕ ↕ ↕ . . . . .
	122	126-127	131 134	140 143	158-161

Impact of NAT on the safety of blood supply in Italy : a 6-year survey (SIMTI)

Between 2001 and 2006 in 93 Italian transfusion centers : *(Velati et al - Transfusion June 2008)*

10,776,288 units were tested for HCV RNA

7,932,430 for HIV RNA, and

27 (2.5 per million) were HCV RNA +

3,405,497 for HBV DNA

14 (1.8 per million) were HIV RNA +

197 (57.8 x million) were HBV DNA +

OBI in hematopoietic stem cells donors in a hepatitis B virus endemic area (Hong Kong)

(J Hepatol June 2005)

Serum samples from 124 HBsAg negative HSC donors were tested for HBV DNA

		OBI +
Negative for all HBV markers	**(n=26) ……**	**3 (11.5 %)**
Positive for anti-HBc alone	**(n=17) ……..**	**2 (11.8 %)**
Positive for anti-HBc and anti-HBs	**(n=77) ……**	**14 (18.2 %)**
Positive for anti-HBs but negative for anti-HBc	**(n=4)……**	**0**

Total (n=124) ……19 (15.3%)

Occult HBV in Blood Donors : genotype A2 (n=14) and genotype D (n=38)

(Candotti et al J Hepatol 2008)

OBI Prevalence in Blood Donations

	OBI	HBsAg +
Poland - total	1 : 14,717	
1st time donors	1: 7,630	1 : 107
Italy – Rome - total	1 : 4,500	1 : 1,755
Turin - total	1 : 13,111	
1st time donors	1 : 1,756	1 : 230
Spain – Barcelona - total	1 : 1,943	1 : 2,591
Valencia - total	1 : 9,819	1 : 2,904
1st time donors	1 : 23, 563	1 : 501
Germany – Frankfurt – total	1: 192,680	1 : 310,214
1st time donors	0	1 : 674

Occult HBV in Blood Donors : genotype A2 (n=14) and genotype D (n=38)

(Candotti et al J Hepatol 2008)

POLAND – ITALY – SPAIN - GERMANY

- Compared to HBsAg + samples, genotype D was significantly more frequent than genotype A2 in OBIs from Poland or Italy ($p < 0.04$)

- Amino acid substitutions were concentrated in the immunologically active parts of the pre-S/S proteins ($p < 0.0001$)

- affecting both cellular CD8 T-cell epitopes and B-cell neutralizing Major Hydrophilic Region epitopes

Occult HBV in Blood Donors : genotype A2 (n=14) and genotype D (n=38)

(Candotti et al J Hepatol 2008)

POLAND – ITALY – SPAIN - GERMANY

Amino acid substitutions were <u>more frequent</u>

- **in OBI than HBsAg+ strains of both genotype D (p<0.001) and A2 (p<0.01)**
- **in OBI of genotype D than A2 in the "a" determinant (p<0.001)**

HYPOTHESIS:

HUMORAL AND CELLULAR IMMUNE PRESSURE ON THE HBV ENVELOPE PROTEINS ARE MAJOR MECHANISMS GENERATING OBI

- **in anti-HBs+ than anti-HBs neg (p<0.001)**

A blood donor with acute OBI transmitted HBV to immunodeficient patient

Wendel et al - Transfusion Aug 2008 (Brasil)

A 9-year old female child with high-grade acute lymphoblastic leukemia developed acute hepatitis B (viral load 2.7 x 10^8 IU/L and ALT level 1744 IU/L) 13 months after transfusion of red cells.

The viral load of index donation was about 500 IU/mL.

She had been vaccinated to HBV and anti-HBs+.

6 weeks after the index donation, the donor seroconverted to anti-HBc, but no HBsAg or anti-HBs were detectable.

Phylogenetic analysis of the pre-S/S region showed that recipient and donor sequences were identical (except for one ambiguity) and they clustered with HBV genotype A1 reference sequences of African origin.

An anti-HBs + blood donor with OBI transmitted HBV to 2 immunocompetent transfusion recipients

Levicnik-Stezinar et al (Slovenia) - J Hepatol 2008

OBI donor : anti-HBc, anti-HBs (12 IU/mL) and 180 IU/mL HBV DNA

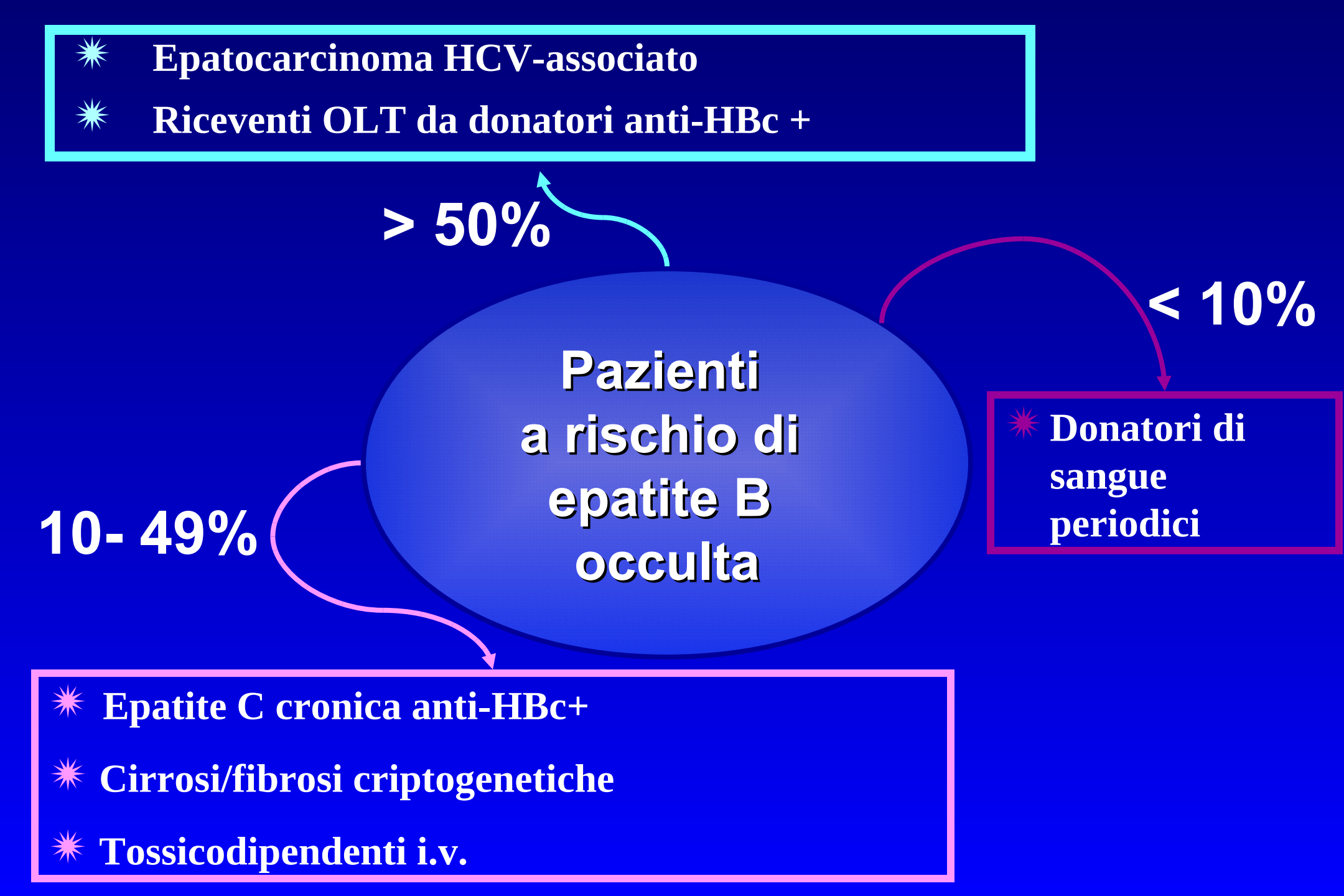
Patient 1 : 59-year-old recipient (cardiac arterial bypass) of 5 units of fresh frozen plasma and 3 units of red cell concentrate - developed acute hepatitis B (ALT level 1821 IU/L) 4 months after transfusion

Patient 2 : 71-year-old patient (orthopedic surgery) transfused with 2 units of red cell concentrate - had early (anti-HBc neg) HBV infection (ALT level 566 IU/L) 7 months after transfusion

Recipients carried genotype D strains with sequences identical to the donor strain

2 previous donations did not cause HBV infection

Previous and subsequent samples contained 3-10 times lower viral load and slightly variable anti-HBs


Epatocarcinoma HCV-associato
Riceventi OLT da donatori anti-HBc +
> 50%
< 10%
Pazienti a rischio di epatite B occulta
Donatori di sangue periodici
10- 49%
Epatite C cronica anti-HBc+
Cirrosi/fibrosi criptogenetiche
Tossicodipendenti i.v.