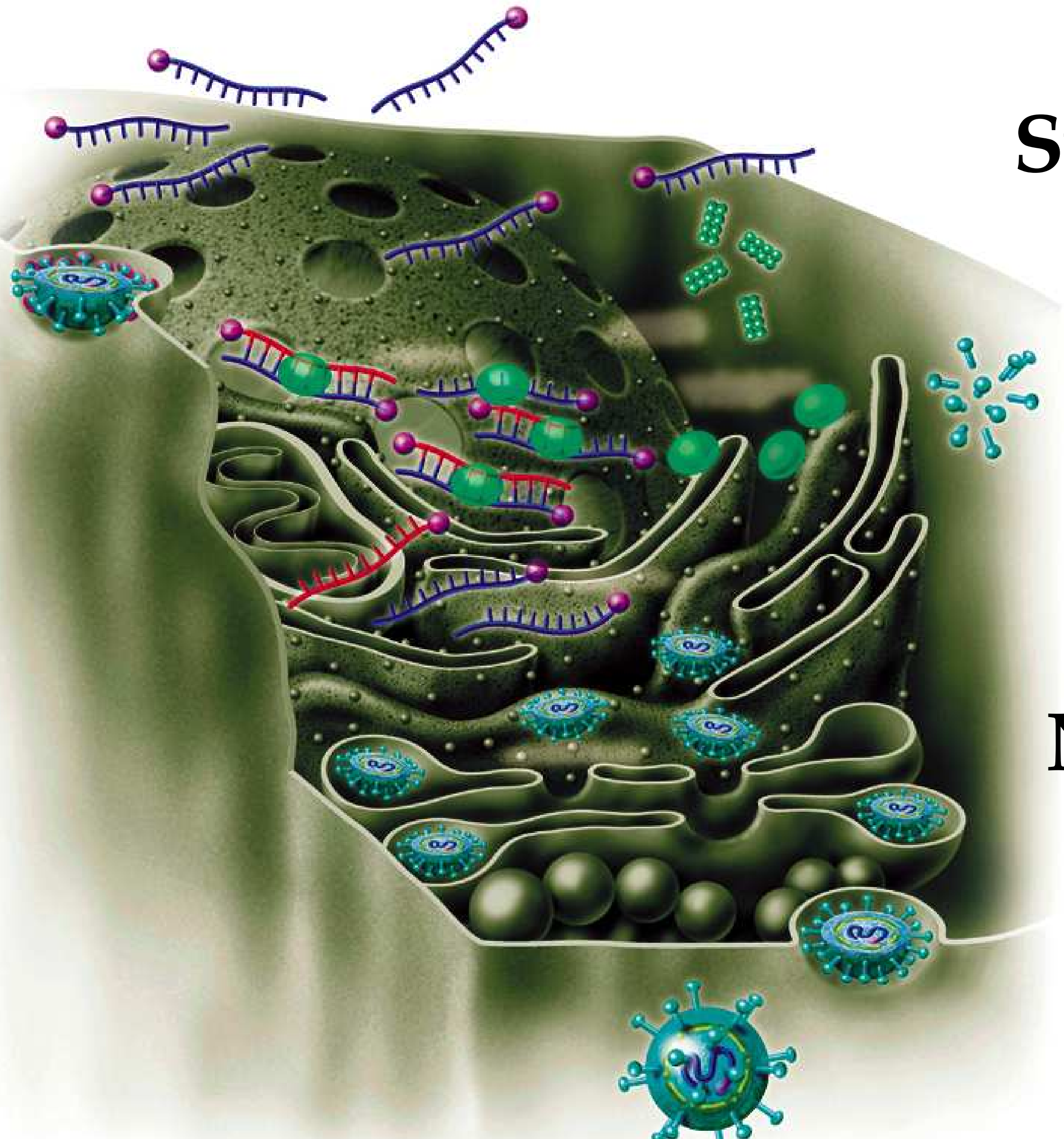


Synthetic RNA Virus Technology

Takaji Wakita

Dept. Virology II
National Institute of Infectious Diseases
Japan

Ancient Egyptian stone monument

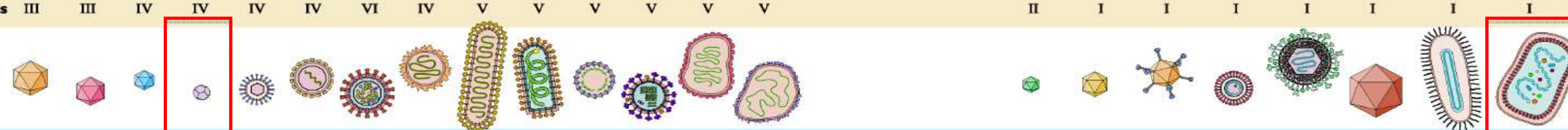
The oldest Polio patient?


MARINA

Virus Taxonomy

RNA virus

DNA virus



Nucleic acid	RNA													
Symmetry of capsid	Icosahedral							Helical						
Naked or enveloped	Naked				Enveloped			Enveloped						
Genome architecture	ds 10–18 seg.	ds 2 seg.	(+) ss cont.	(+) ss cont.	(+) ss cont.	(+) ss cont.	(+) ss 2 copies	(+) ss cont.	(–) ss cont.	(–) ss cont.	(–) ss 3 seg.	(–) ss 8 seg.	(–) ss cont.	(–) ss 2 seg.
Baltimore class	III	III	IV	IV	IV	IV	VI	IV	V	V	V	V	V	V
Family name	Reo	Birna	Calici	Picorna	Flavi	Toga	Retro	Corona	Filo	Rhabdo	Bunya	Ortho-myxo	Para-myxo	Arena
Virion polymerase	(+)	(+)	(–)	(–)	(–)	(–)	(+)	(–)	(+)	(+)	(+)	(+)	(+)	(+)
Virion diameter (nm)	60–80	60	35–40	28–30	40–50	60–70	80–130	80–160	80 × 790–14,000	70–85 × 130–380	90–120	90–120	150–300	50–300
Genome size (total in kb)	22–27	7	8	7.2–8.4	10	12	3.5–9	16–21	12.7	13–16	13.5–21	13.6	16–20	10–14

Nucleic acid	DNA							
Symmetry of capsid	Icosahedral						Helical	Complex
Naked or enveloped	Naked			Enveloped		Naked/Env. (cytoplasmic)	Enveloped	Enveloped (cytoplasmic)
Genome architecture	ss linear (+) or (–)	ds circular	ds linear	ds circle gapped	ds linear	ds linear	ds circular	ds linear (x linked)
Baltimore class	II	I	I	I	I	I	I	I
Family name	Parvo	Papova	Adeno	Hepadna	Herpes	Irido	Baculo	Pox
Virion polymerase	(–)	(–)	(–)	(+)	(–)	(–)	(–)	(+)
Virion diameter (nm)	18–26	45–55	70–90	42	150–200	125–300	60 × 300	170–200 × 300–450
Genome size (total in kb)	5	5–8	36–38	3.2	120–200	150–350	100	130–280

Poliovirus

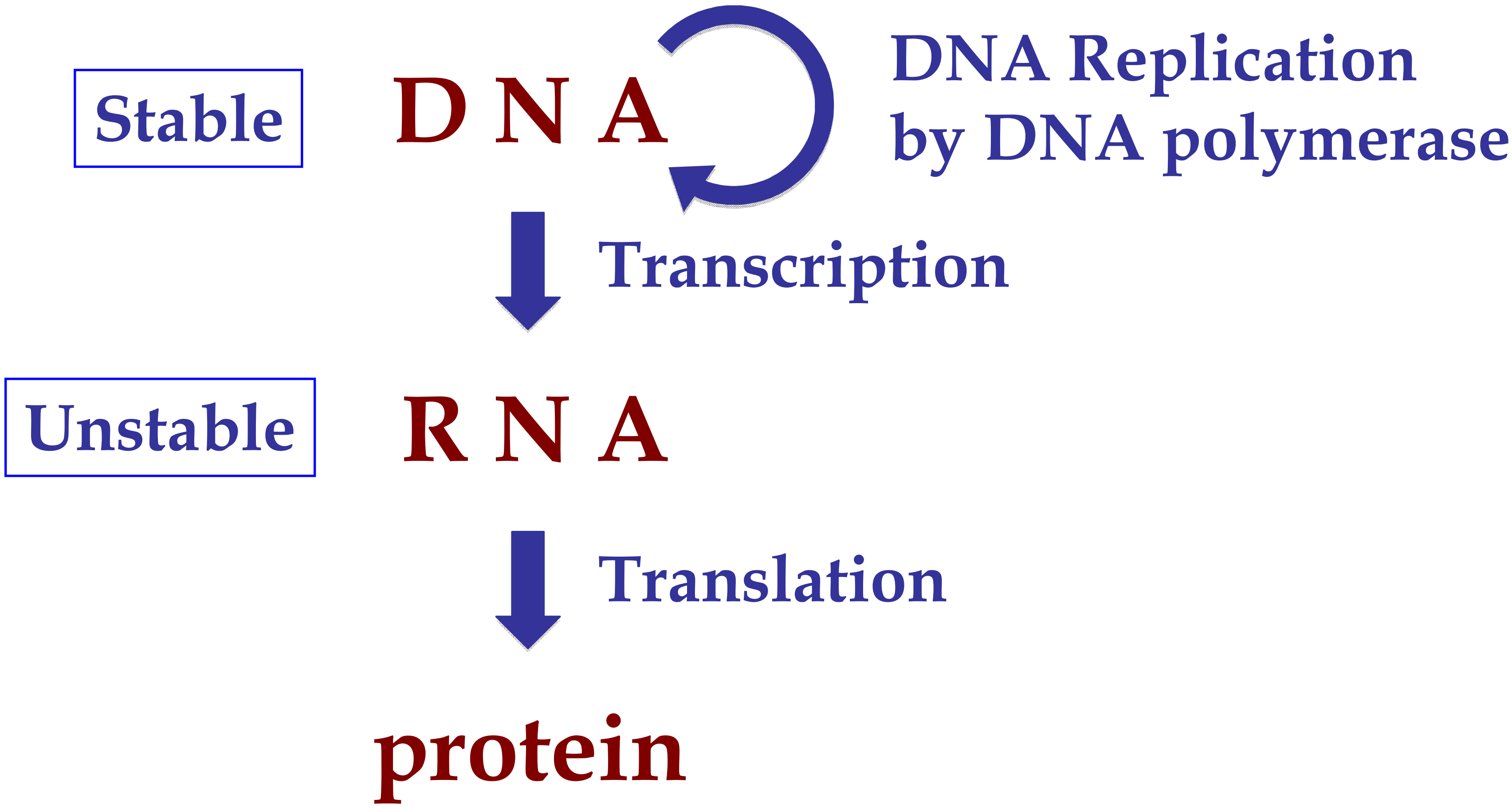
Variola virus

RNA viruses

Calici:	Norovirus, Sapovirus
Flavi:	JEV, YEV, HCV
Picorna:	Polio, Coxsackie, HAV
Orthomyxo:	Influenza virus
Paramyxo:	Mumps, Measles
Rhabdo:	VSV
Filo:	Ebola, Marburg
Retro:	HIV, HTLV1

Central Dogma of Molecular Biology

by Francis Click in 1958



Central Dogma of Molecular Biology

But it is not always the case…

DNA

RNA

protein

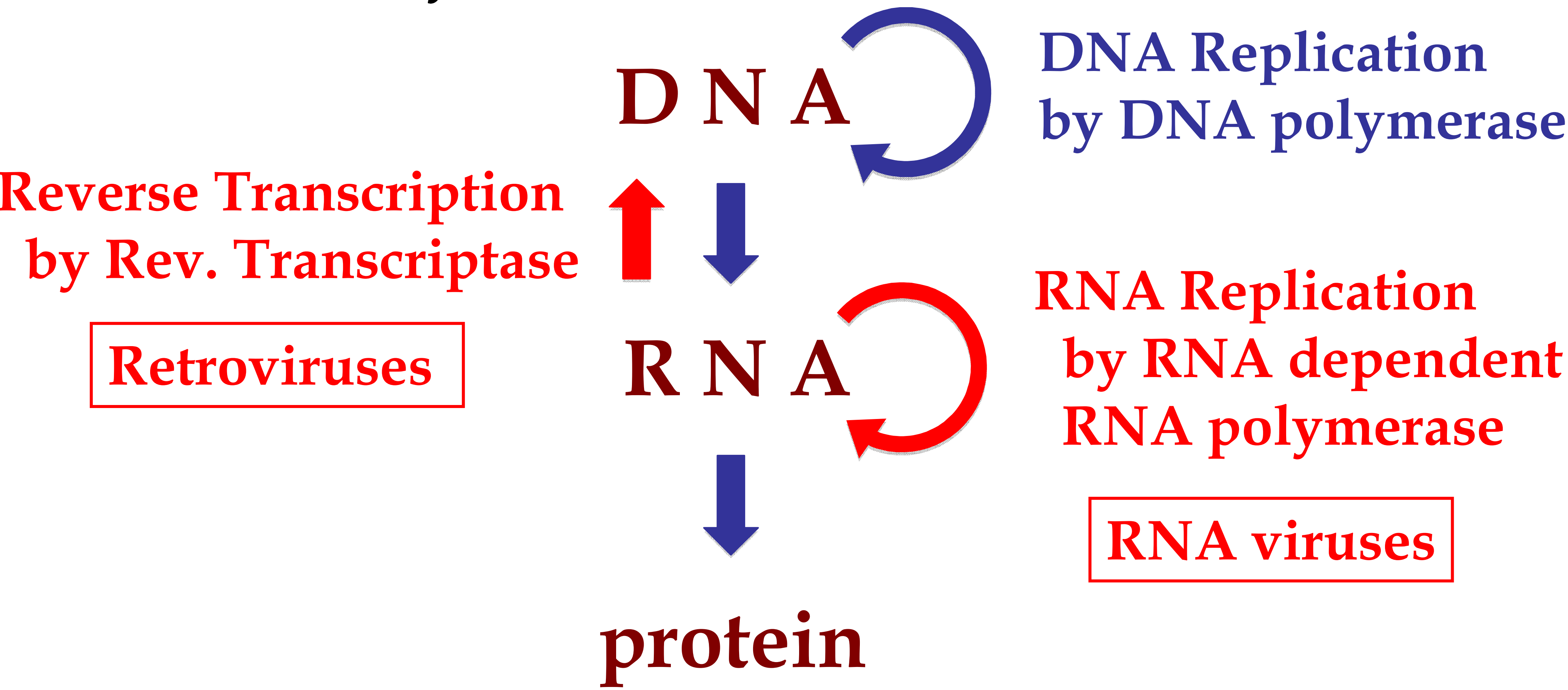
DNA Replication by DNA polymerase

RNA Replication by RNA dependent RNA polymerase

RNA viruses

Central Dogma of Molecular Biology

But it is not always the case…



Central Dogma of Molecular Biology

Innovation

D N A

Reverse Transcription
by Rev. Transcriptase

↑ ↓

Unstable RNA information
can be stored by DNA form
by reverse transcription

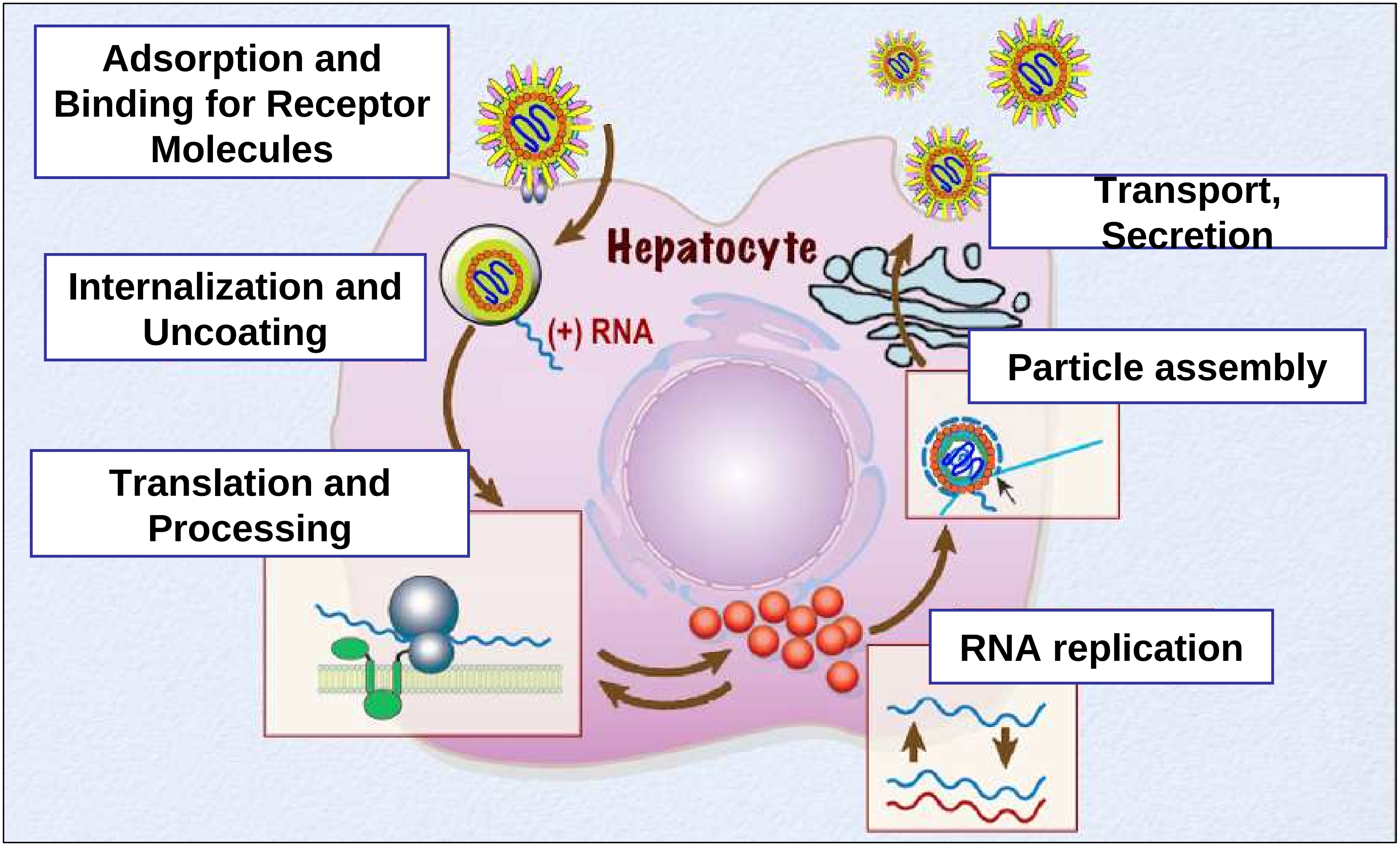
Retroviruses

R N A

↓

protein

Life Cycles of RNA Viruses



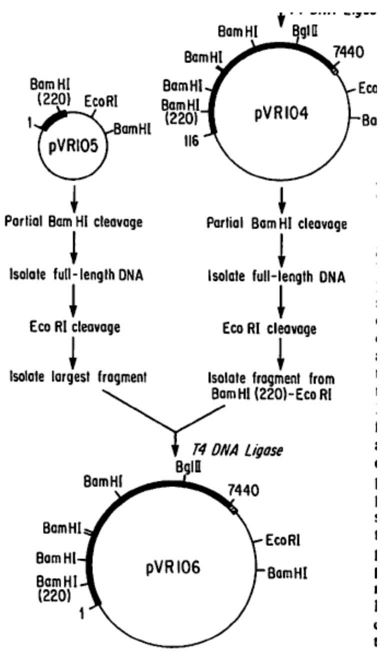
First infectious cDNA clone of RNA virus

Science. 1981 214(4523):916-9.

Cloned poliovirus complementary DNA is infectious in mammalian cells.

Racaniello VR, Baltimore D.

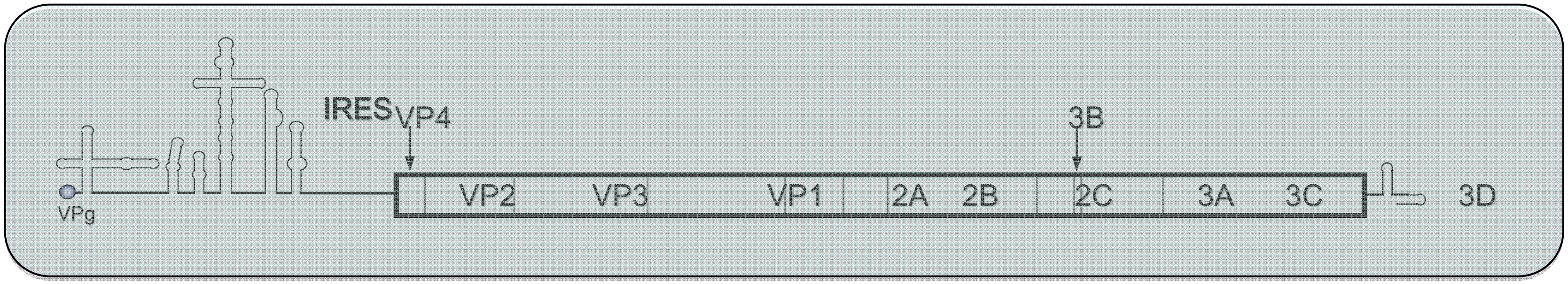
A complete, cloned complementary DNA copy of the RNA genome of poliovirus was constructed in the bacterial plasmid pBR322. Cultured mammalian cells transfected with this hybrid plasmid produced infectious poliovirus.



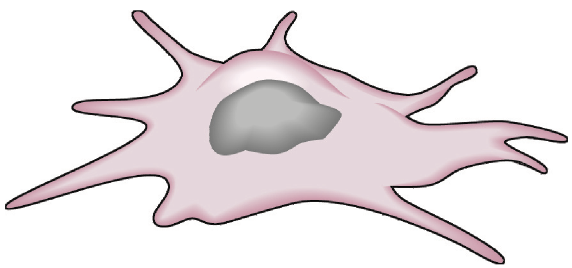
Nucleic acid	Plaque-forming units per milliliter in medium	Number of plaques on transfected cell monolayer
CV-1 cells		
pVR106	1.2×10^9	22
pVR106 + Hinf I	0	0
pVR106 + ribonuclease	1.3×10^9	10
pVR106, phenol extracted	1×10^9	22
pVR106, phenol extracted, then ribonuclease	1.4×10^9	26
pVR104	0	0
pBR322	0	0
Viral RNA	1.5×10^9	71
Viral RNA + ribonuclease	0	0
Viral RNA + Hinf I	1.4×10^9	20
HeLa cells		
pVR106	3.7×10^8	69
pBR322	0	0

Simple system to produce infectious poliovirus

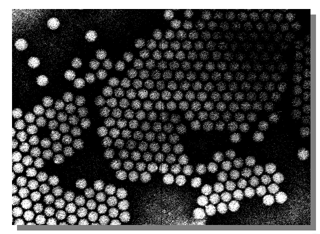
Cloned poliovirus cDNA or synthesized RNA

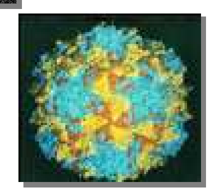


Transfection



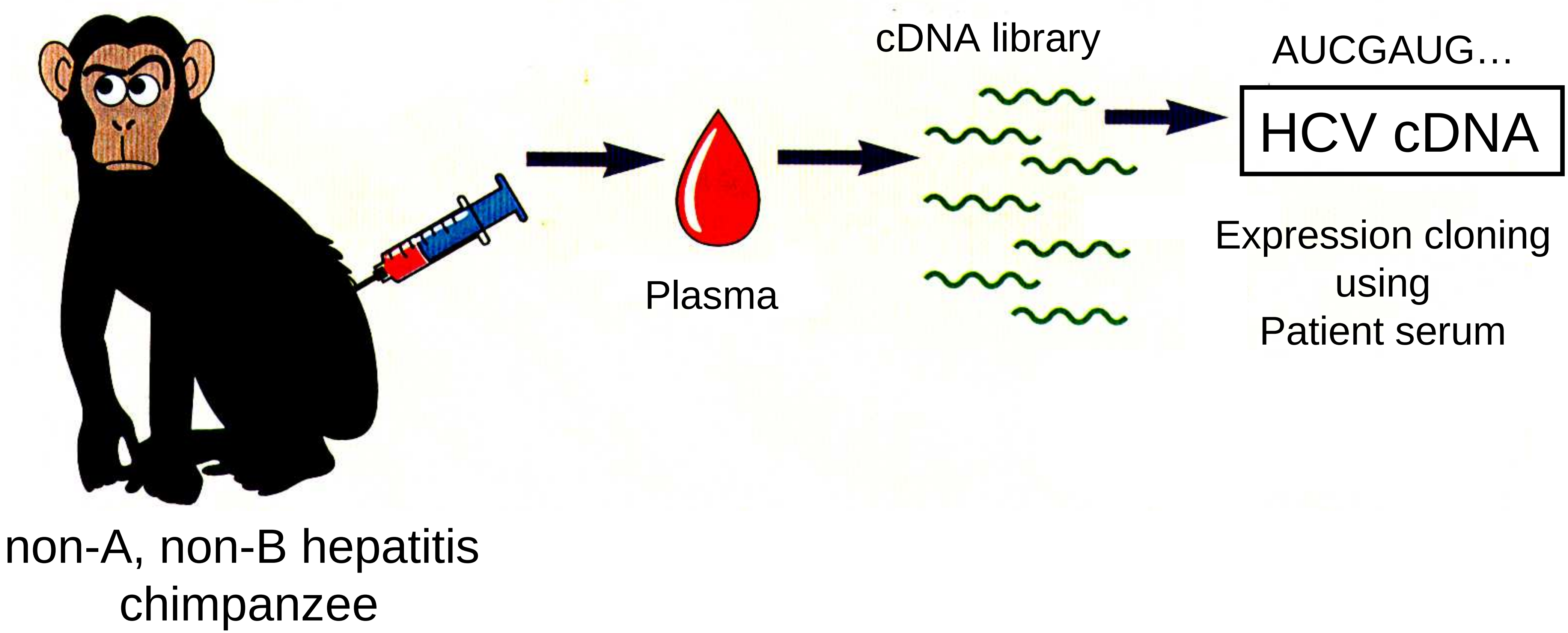
Cultured cells



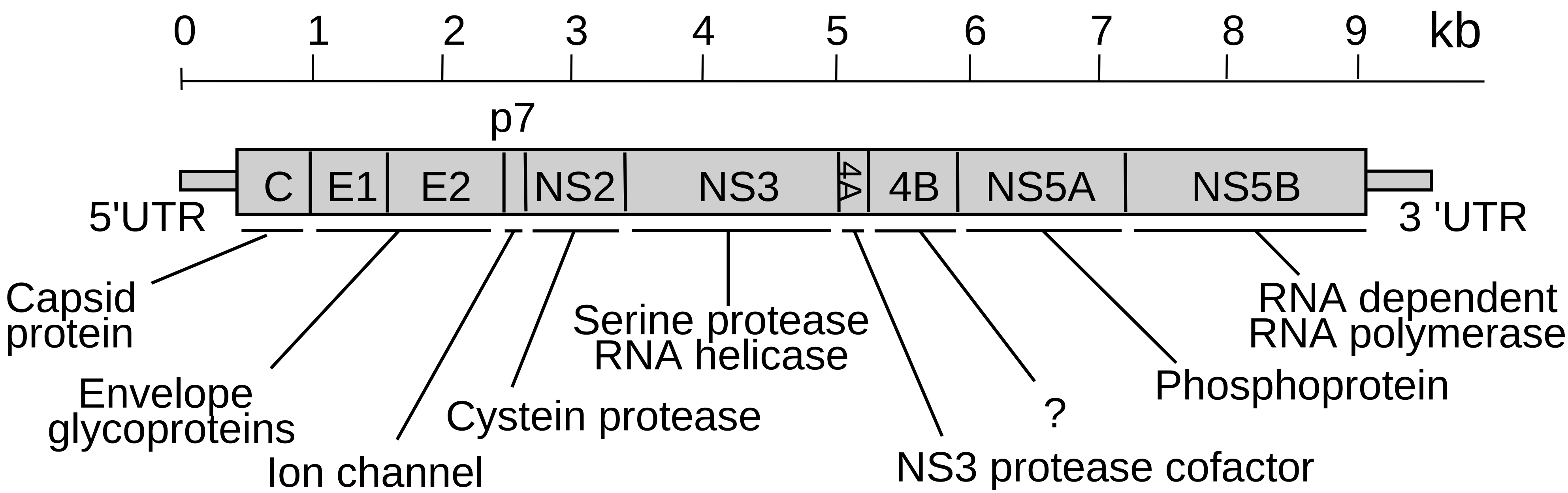


Infectious polio virus

Isolation of HCV cDNA

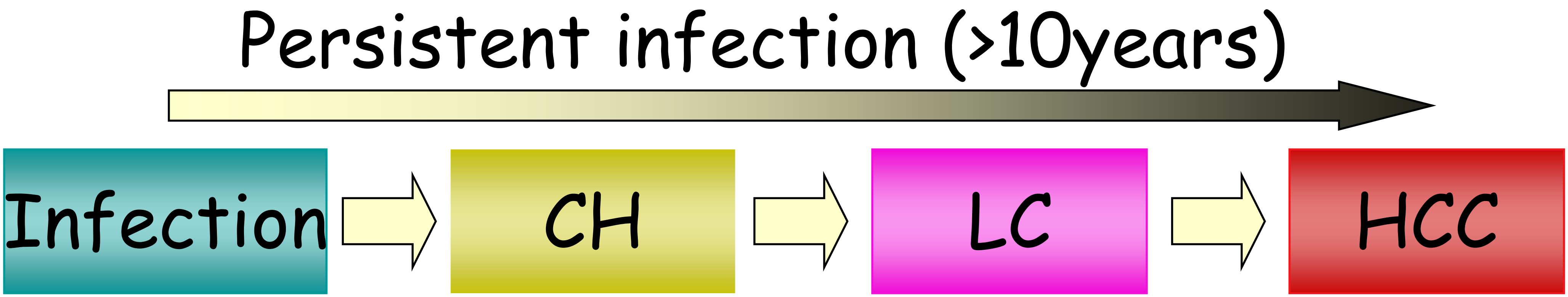


Structure of HCV RNA Genome

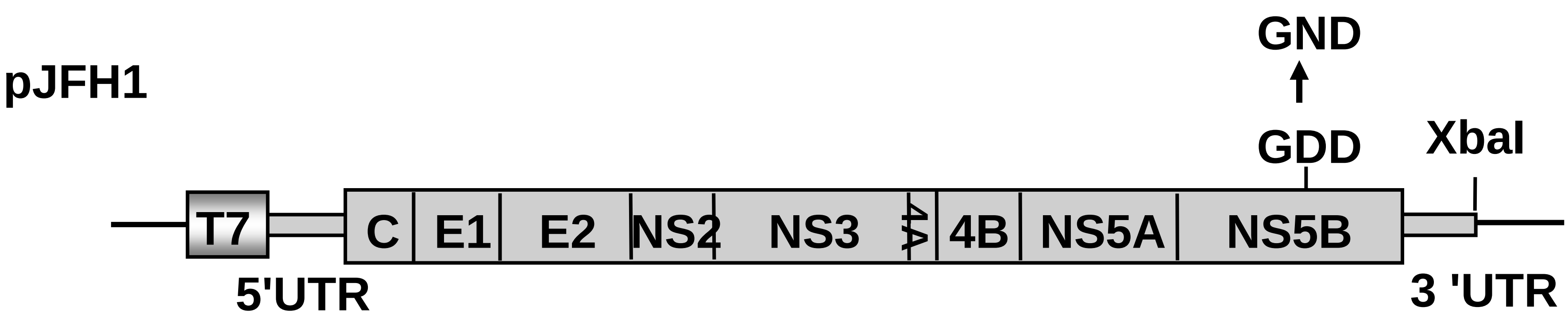


Clinical Course of HCV Infection

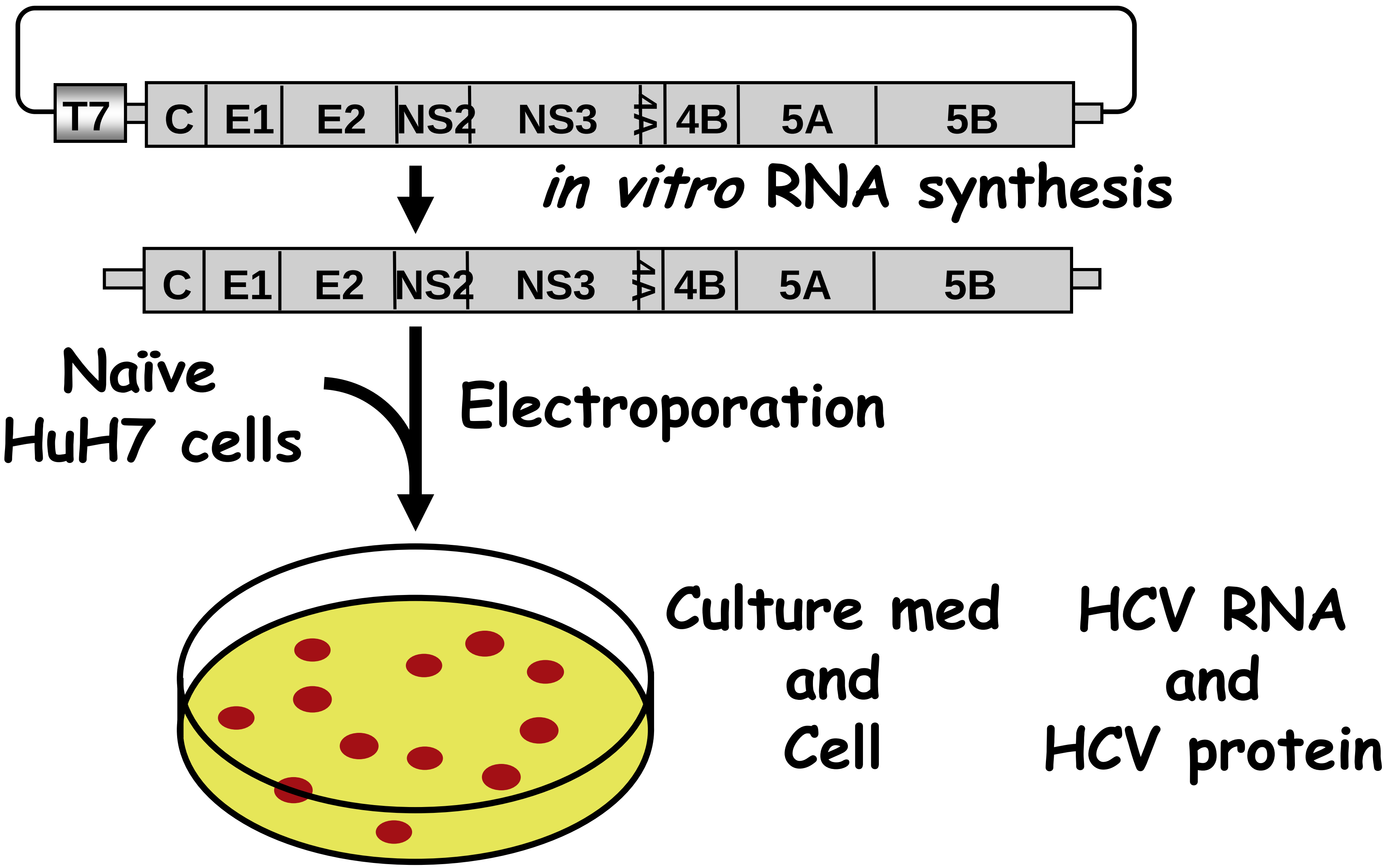
- 2 million HCV carriers in Japan
- 170 million world wide



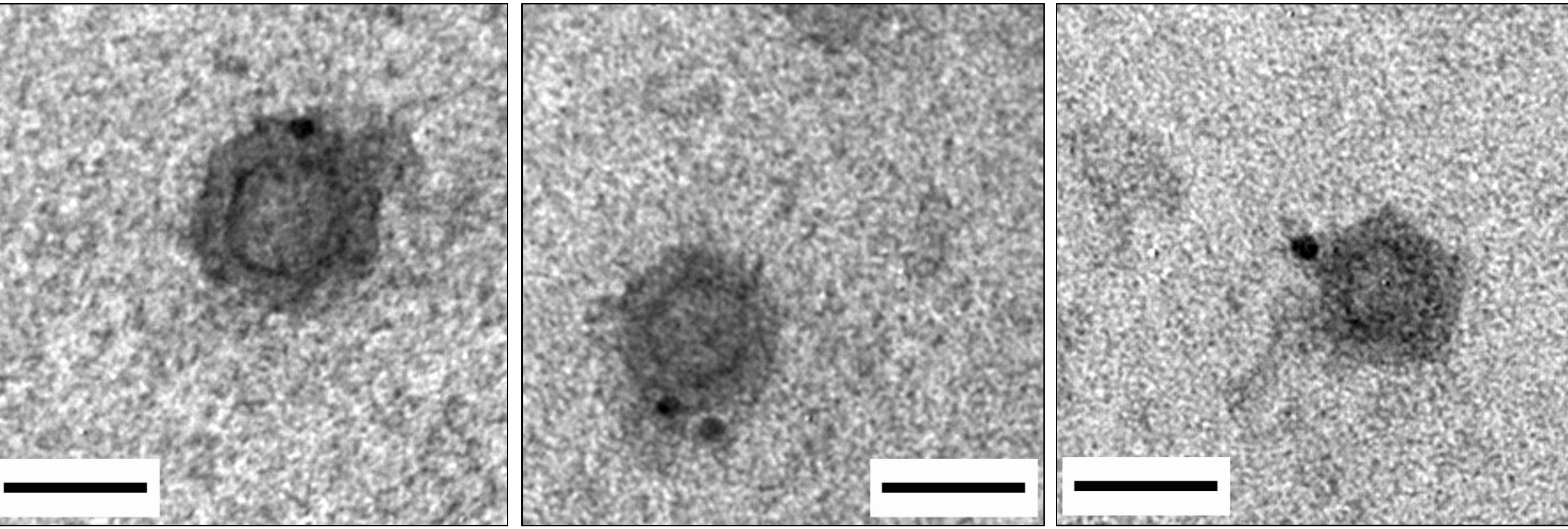
Construction of Full-Length HCV cDNA



Production of infectious HCV from cloned cDNA



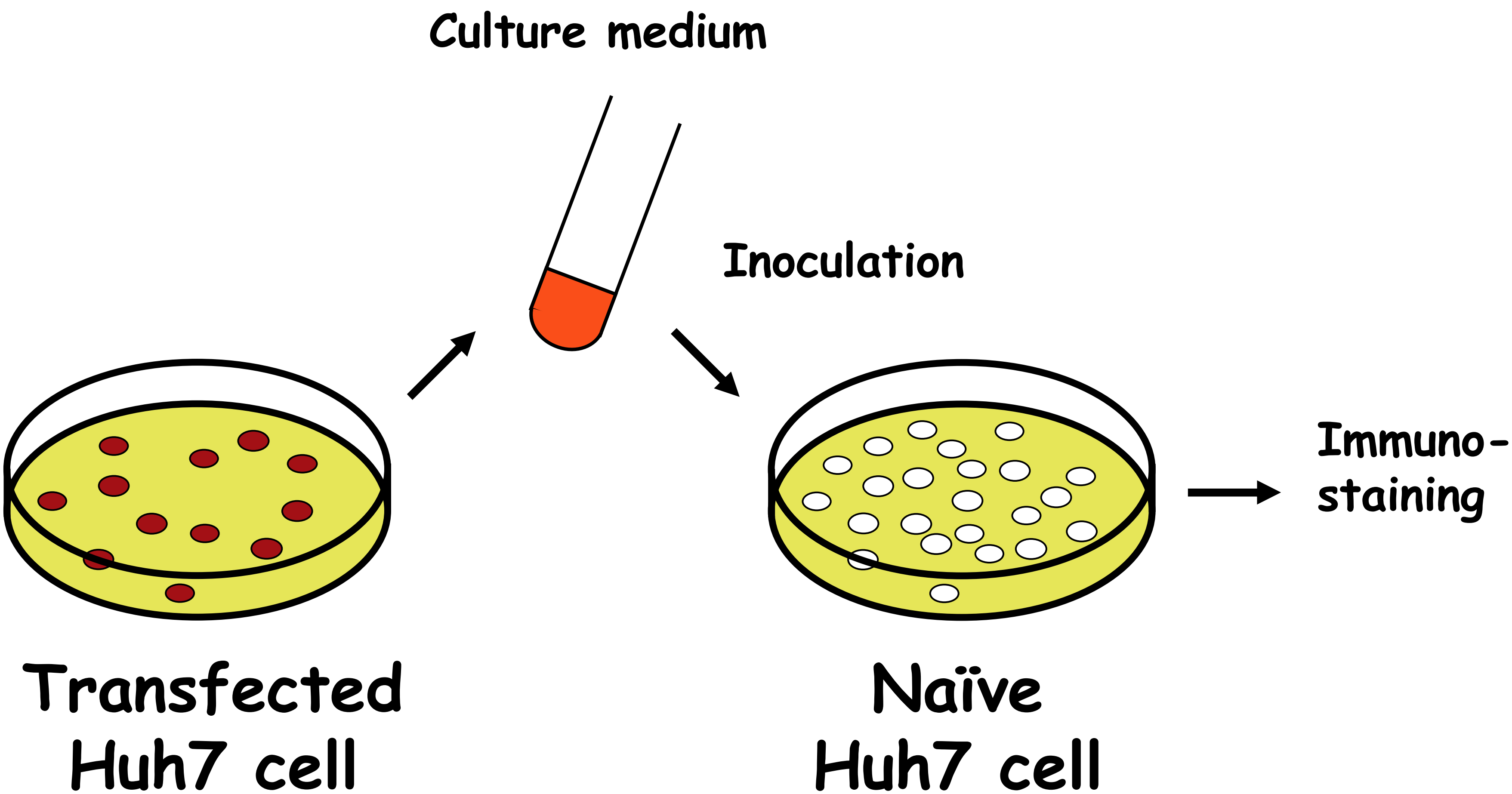
Virus particles visualized by Immuno EM



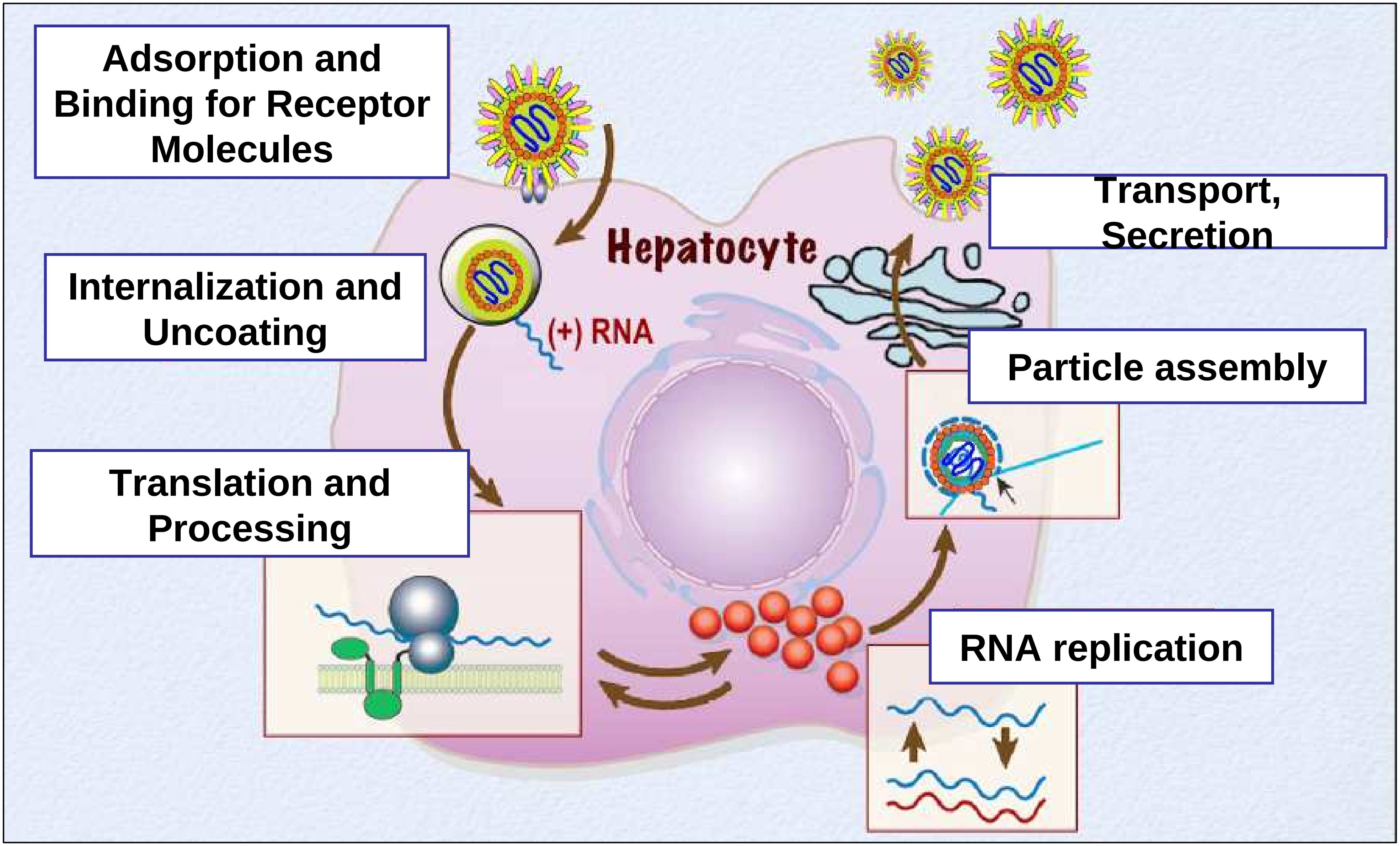
By Dr. Ralf Bartenschlager

Wakita T, Nat Med. 2005.11:791

Infection Assay of Full-Length HCV RNA Transfected Cell Supernatant



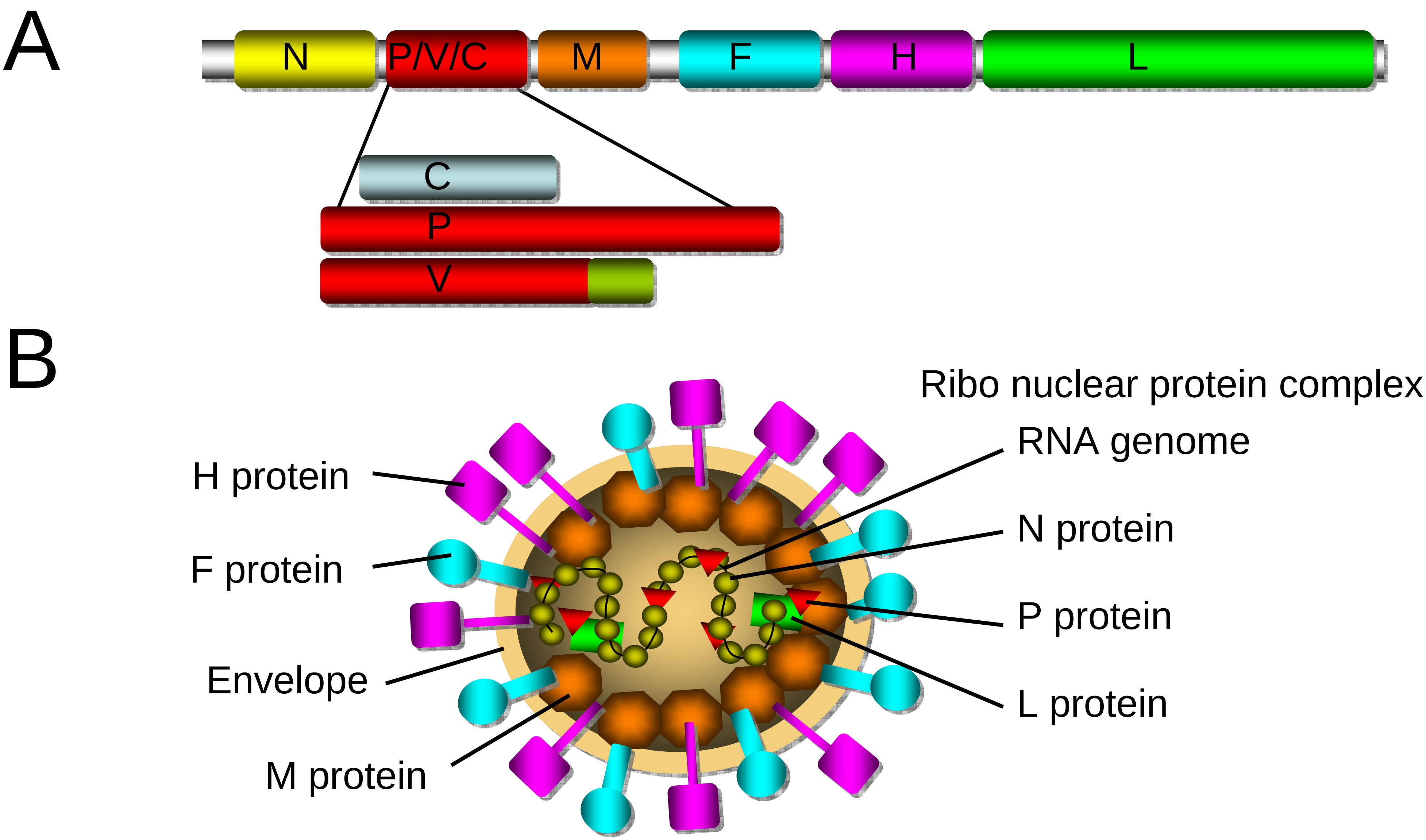
Life Cycles of HCV



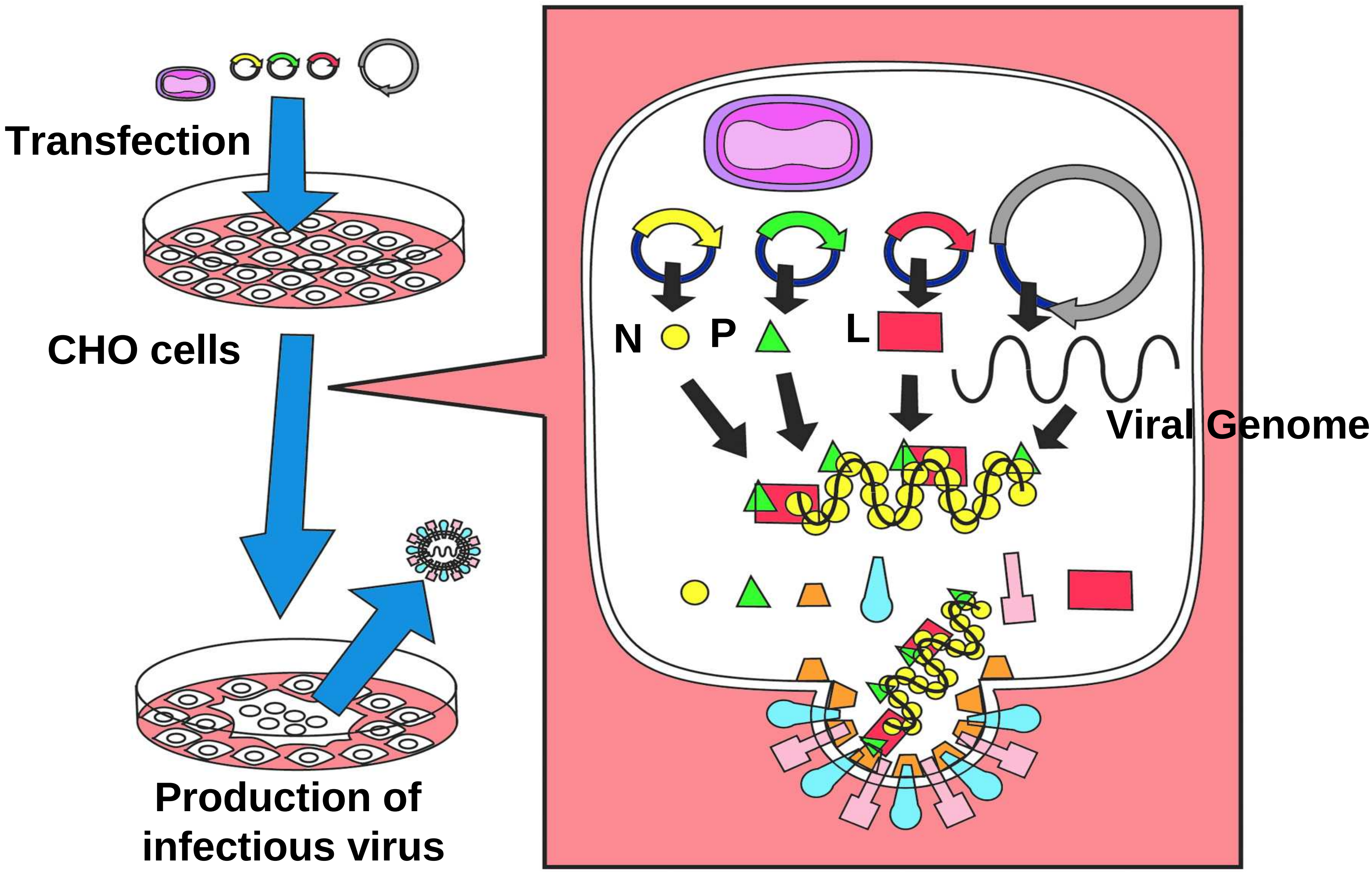
Research Progress with Infectious HCV System

1. **Virus entry**
2. **Virus replication**
3. **Virus particle formation and secretion**
4. **Anti-viral drug and Vaccine development**

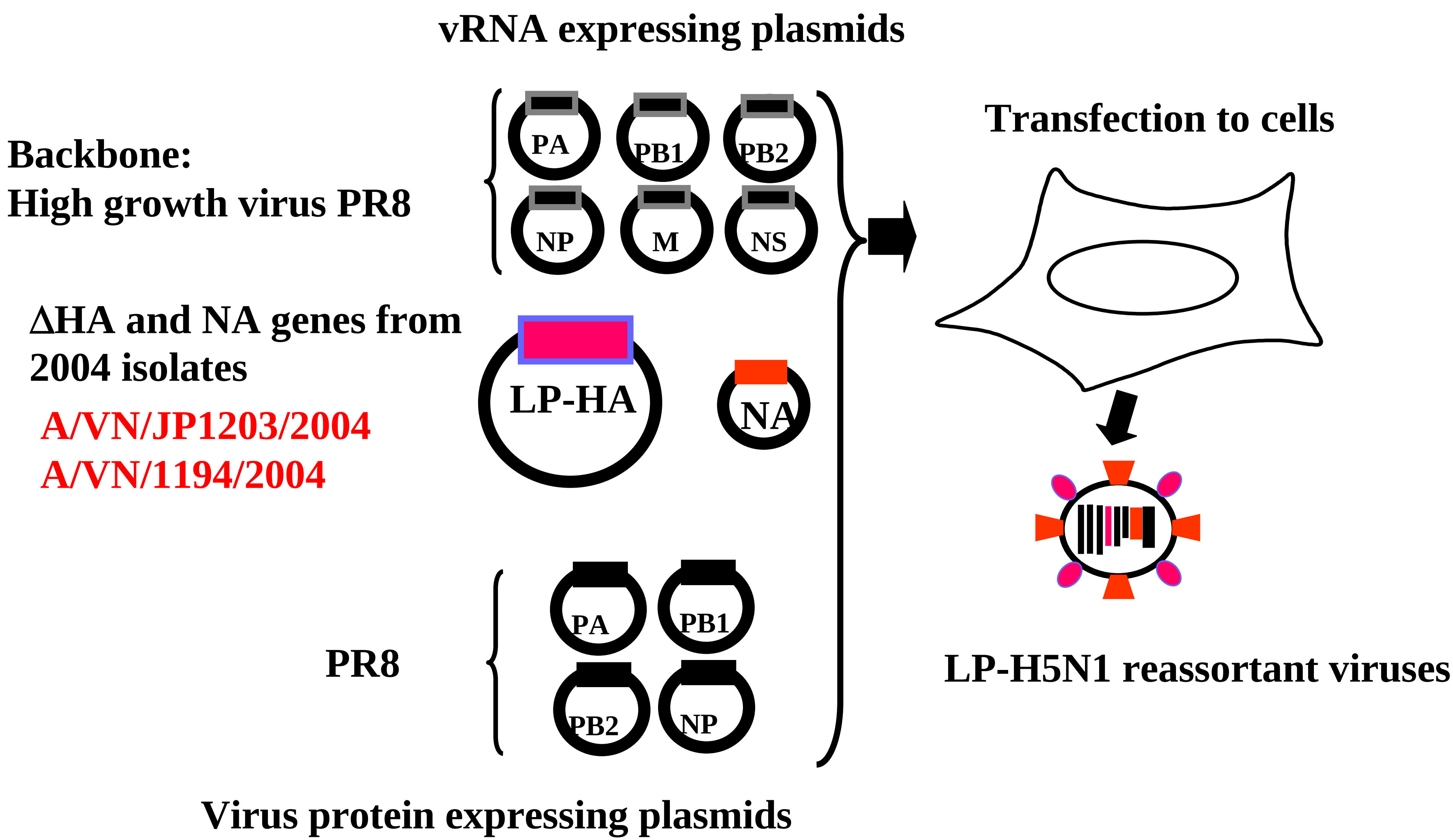
Viral Genome and Particle Structure of Measles Virus



Generation of Measles Virus by Reverse Genetics System



Generation of LP-H5N1 reassortant virus by reverse genetics



Neuman & Kawaoka (1999)

Benefits and Risks of Reverse Genetics System of RNA viruses

Benefits

- Production of recombinant viruses
- Analysis of viral life cycles and pathogenesis
- Anti-viral and vaccine development

Risks

- Spread of artificial viruses to nature
- Production of artificial virulent virus with or without intention
- Concern for bioterrorism

Small Pox	Polio
Declaration of eradication in 1980	Still endemic in 4 countries
Vaccine termination in 1976	After eradication and containment
Preservation of virus stock only in USA and Russia	All over the world
Increase younger generation without vaccination	High Ab titer in all generation
Increased concern for Bioterrorism	
Difficult artificial synthesis of giant DNA genome of variola	Easy to synthesize its small RNA genome

First chemical synthesis of poliovirus infectious cDNA

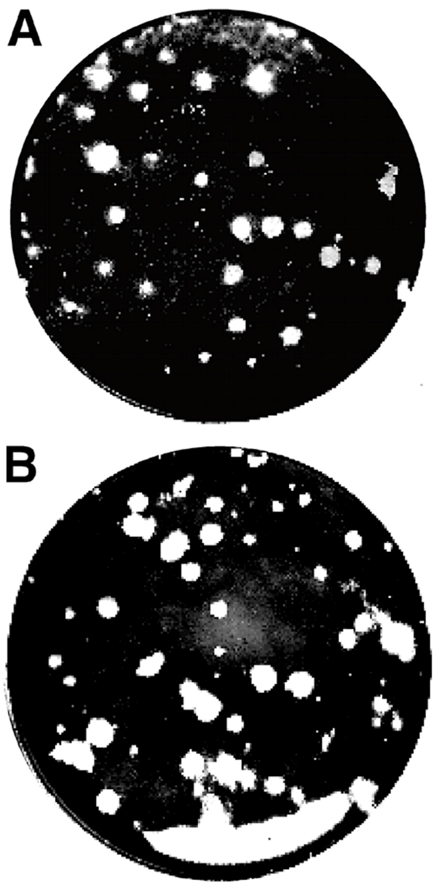
Science. 2002. 297(5583):1016-8.

Chemical synthesis of poliovirus cDNA: generation of infectious virus in the absence of natural template.

Cello J, Paul AV, Wimmer E.

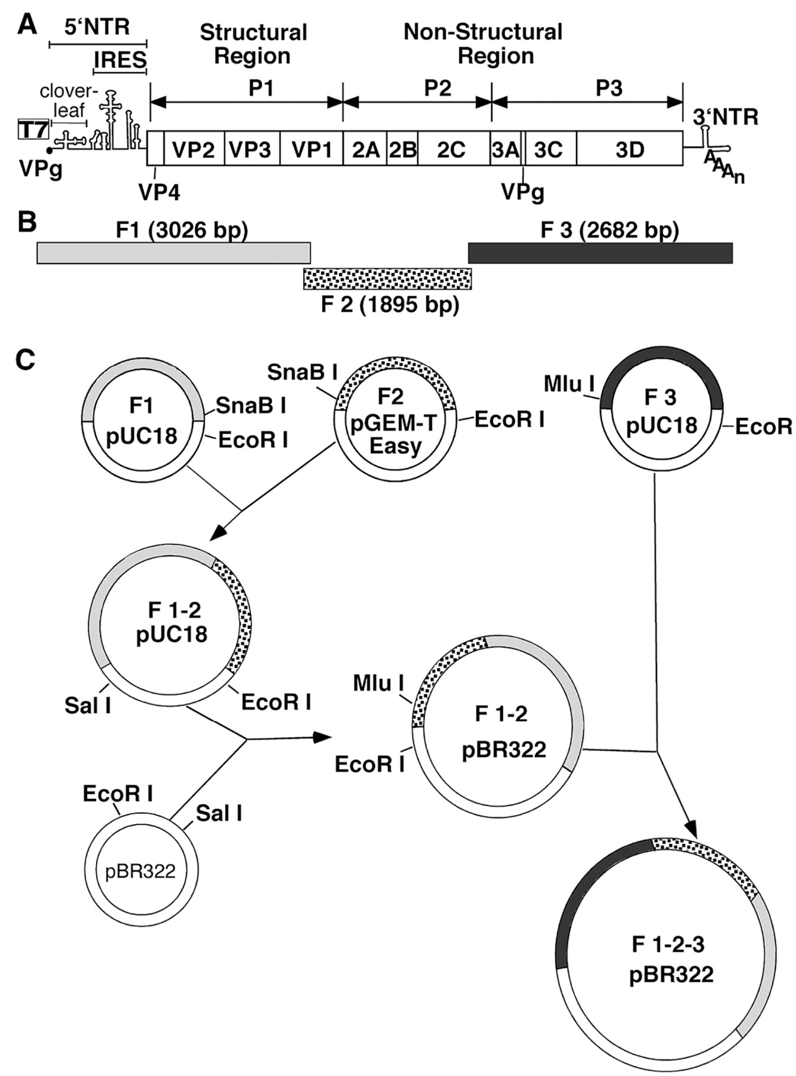
A

B



sPV

wPV

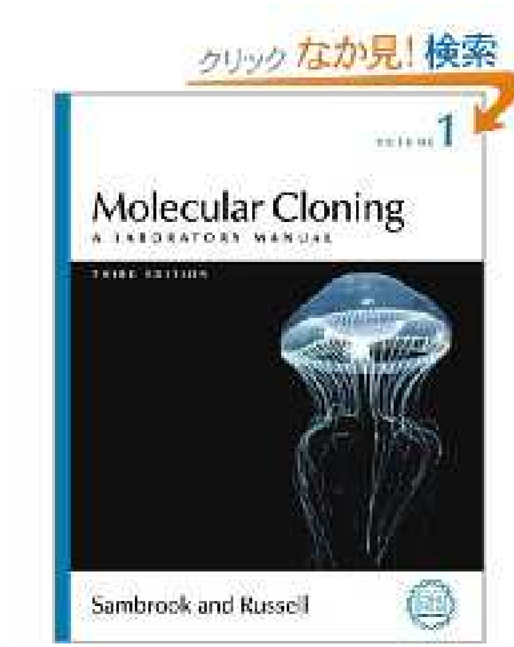


Calculated cost for poliovirus cDNA and infectious virus production

Chemical synthesis of poliovirus cDNA

7500nt length x 2 = 15,000nt

Oligonucleotide synthesis 1nt = ~$0.5



$250

Most common text book

15,000nt x $0.5 = $7,500

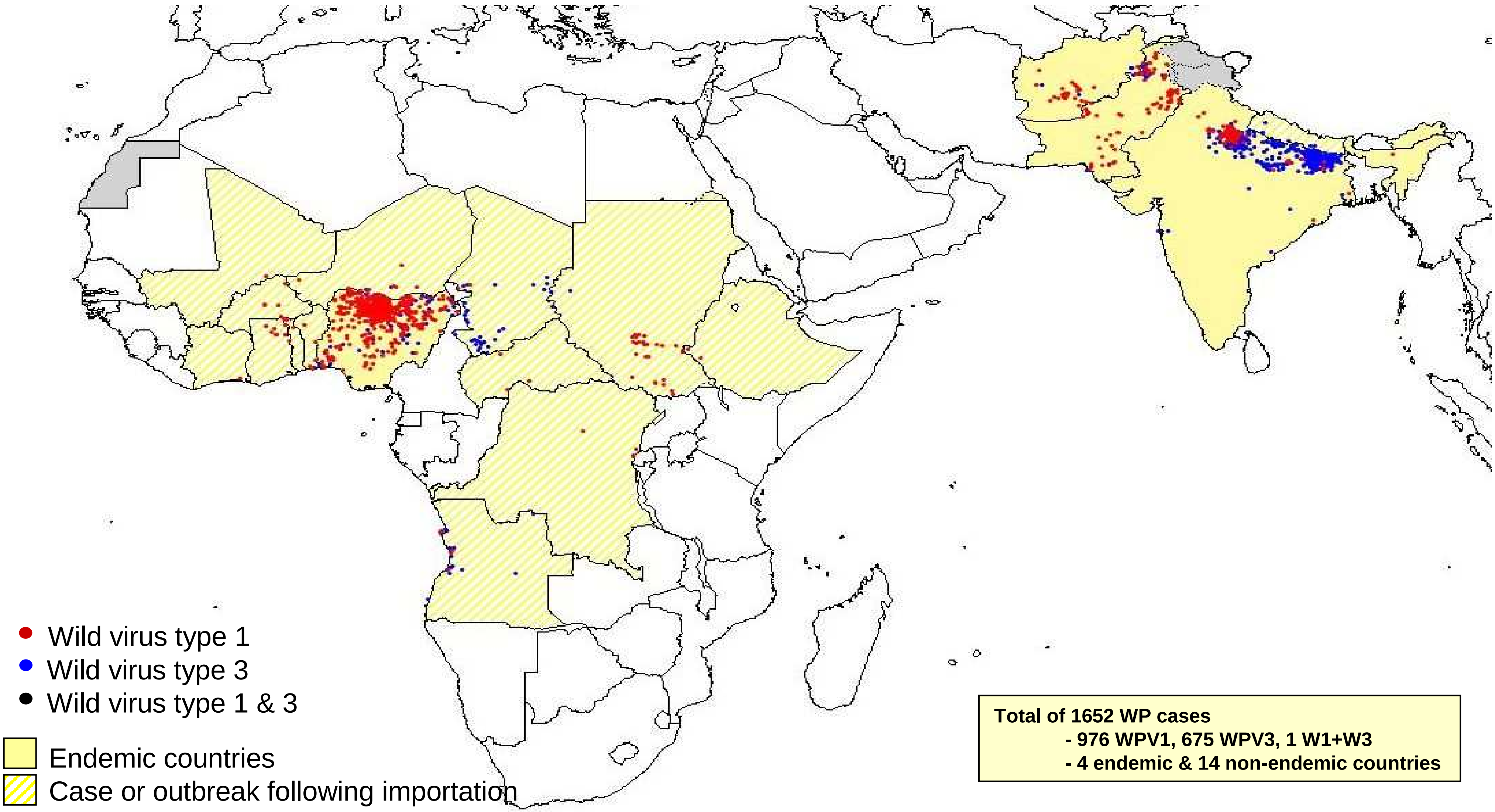
plus basic molecular biology technique and cell culture facility (easily found in the most universities or institutes)

Risk of reintroducing viruses

- Smallpox (UK, 1978)
- SARS (Singapore 2003; China, 2004)
- Tularemia (USA, 2004)
- Polio:
  - 1941 to 1976 – USA at least 12 documented cases of lab associated poliomyelitis;
  - 1992 – Netherlands WPV introduction from a vaccine production facility;
  - 2003 – India identification of a WPV laboratory strain (MEF-1) circulating in general population.

- **Wild poliovirus type 2 eradicated**
- **Polio reportable under new IHR**

Wild Poliovirus*, 2008

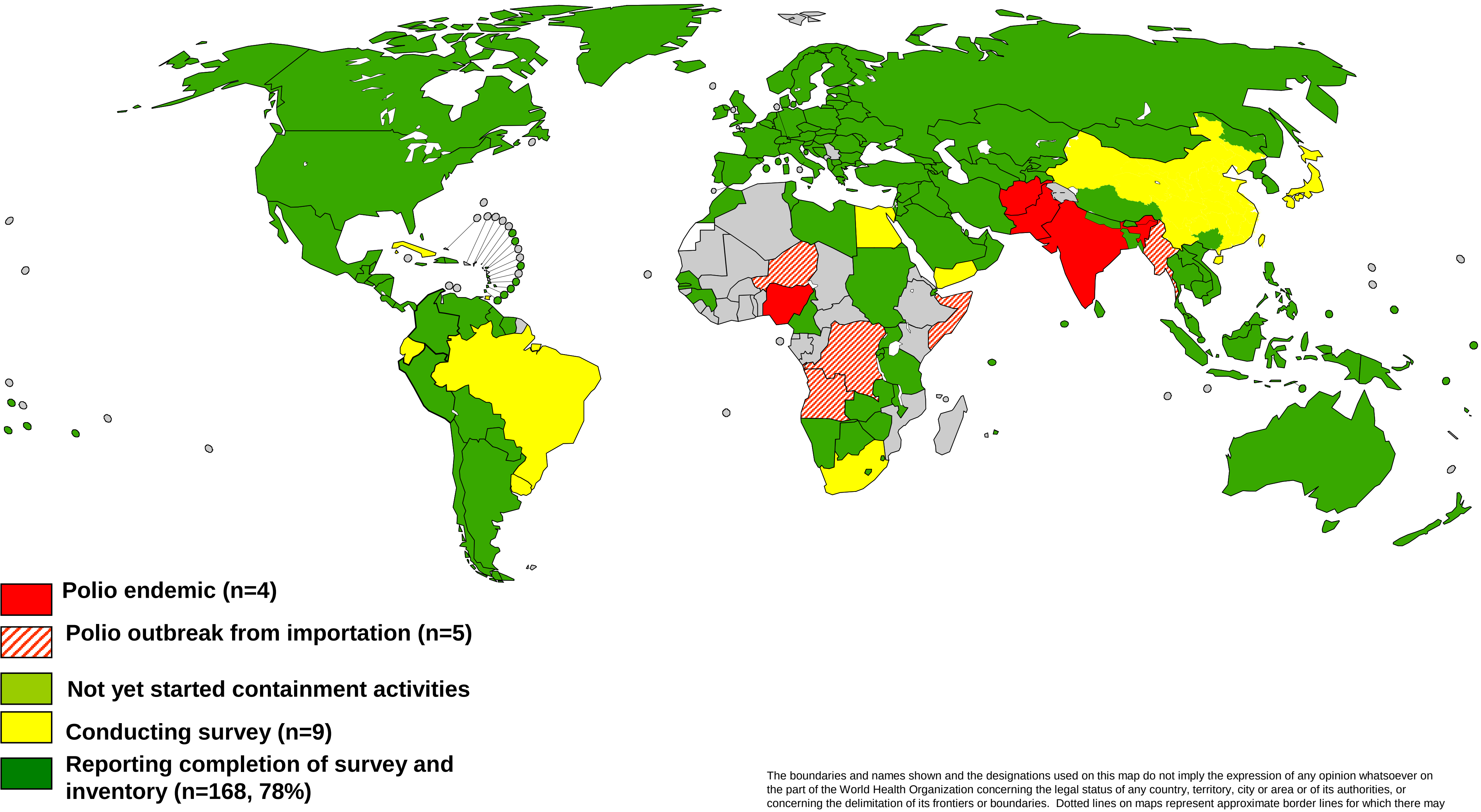


*Excludes viruses detected from environmental surveillance and vaccine derived polioviruses

Data in WHO HQ as of 16 Jun 09

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

Progress towards phase I of global polio virus containment



Data in WHO HQ as of 10 Jul 2007

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

Conclusions

Reverse genetics system has been developed for RNA virus analysis
and is obviously important for progress of medicine

On the other hand, these kinds of experiments should
be regulated by the law and monitored by the authority

Poliovirus eradication program progressed and retain
only 4 endemic countries of wild polio

Appropriate containment should be necessary for the highly virulent
or spreading virus