Introduction to VIROLOGY

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&

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Nobel Prize for Medicine - 2008

Harald zur Hausen – human papilloma virus causes cervical cancer

Françoise Barre-Sinoussi and Luc Montagner
for the discovery of the HIV

H5N1

H5N1 Avian Strain vs 1918 Pandemic Strain

Recreating the 1918 Pandemic Influenza Virus

- Flu victim frozen in the Alaskan permafrost since 1918.
- Fragments of RNA were retrieved from lung tissue, converted into cDNA and sequenced.
- The overlapping sequences were pieced together to make the full genome sequence.
- 1918 strain – an avian virus (not a ‘re-assorted’ virus unlike the 1957 and 1968 pandemic strains)
- A DNA copy of the genome was synthesised in the laboratory
- The DNA was injected into human kidney cells which produced virus particles
- The virus was isolated from cells and used to infect mice.

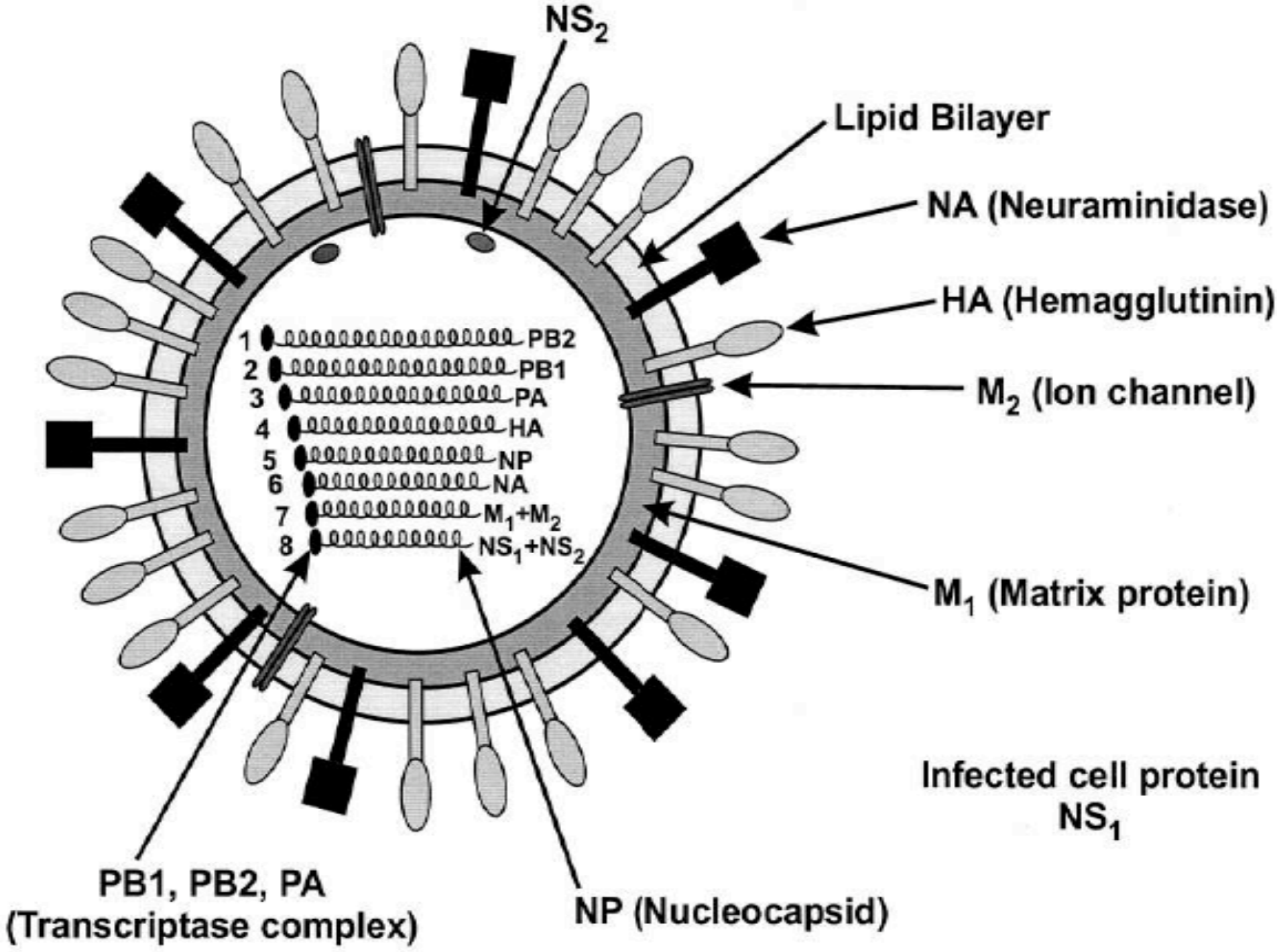
How Virulent is 1918 FLU?

50 times as many virus particles are released from human lung cells a day after infection with the 1918 virus compared to an “ordinary” influenza strain.

13% of body weight was lost by mice 2 days after infection with 1918 flu; weight loss is only transient in mice infected with the “ordinary” virus.

39,000 times more virus particles were found in mouse lung tissue 4 days after infection with 1918 flu than with “ordinary” flu virus.

All mice died within 6 days of infection with 1918 flu; none died from the “ordinary”virus.


NS2
Lipid Bilayer
NA (Neuraminidase)
HA (Hemagglutinin)
M2 (Ion channel)
M1 (Matrix protein)
1 PB2
2 PB1
3 PA
4 HA
5 NP
6 NA
7 M1+M2
8 NS1+NS2
Infected cell protein
NS1
PB1, PB2, PA
(Transcriptase complex)
NP (Nucleocapsid)

DEFINITION OF A VIRUS

Viruses are organised associations of macromolecules:-
nucleic acid contained within a protective shell of protein units.

On its own, a virus may be considered as an inert biochemical complex since it cannot replicate outside of a living cell. Once it has invaded a cell it is able to direct the host cell machinery to synthesise new intact infectious virus particles (virions).

Because viruses are non-motile, they are entirely dependent on external physical factors for chance movement and spread to infect other susceptible cells.

STRUCTURE

Viruses are very small in size (20 - 300 nanometers) and contain *either* DNA *or* RNA (not both as in higher forms of life)

The genome (DNA or RNA) codes for the few proteins necessary for replication.

- Some proteins are non-structural, eg. nucleic acid polymerases
- and some are structural, i.e. form part of the virion.

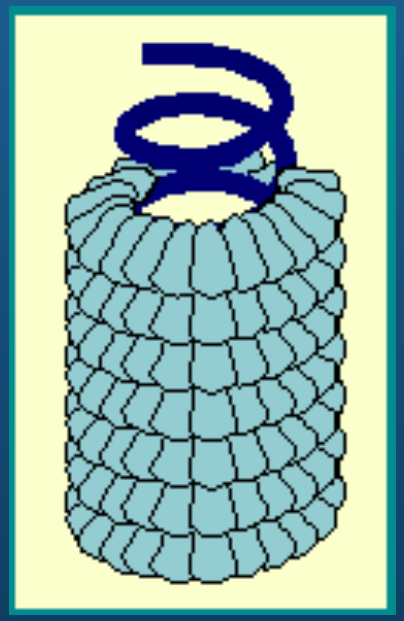
Protein building blocks are assembled to form a tight "shell" (capsid) inside which the nucleic acid genome lodges for protection.

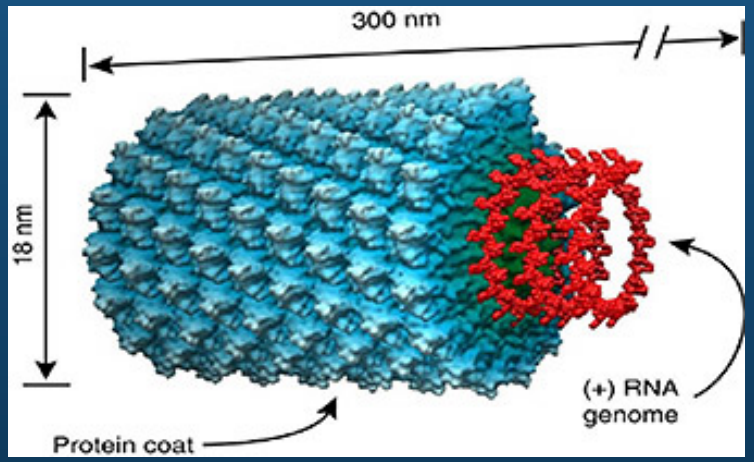
This shell may take the form of a polyhedron (usually icosahedral) or it may be spiral (helical symmetry).

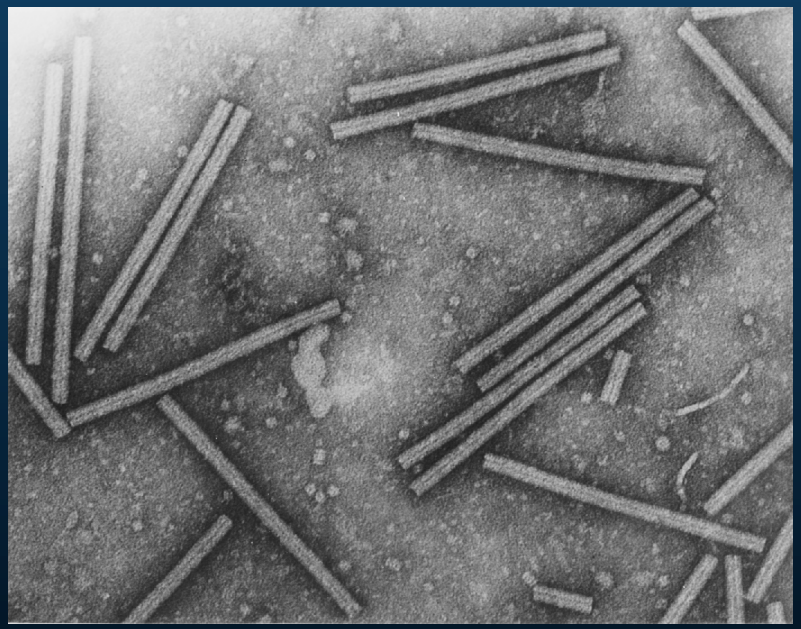
Some viruses acquire an outer lipoprotein coat by "budding" through the host cell membranes (nuclear membrane or cytoplasmic membrane) and are thus called enveloped viruses.

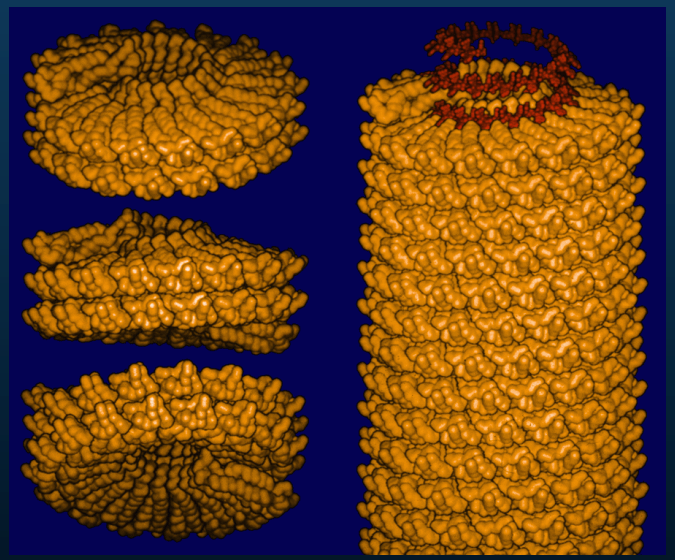
Helical Capsid Structure

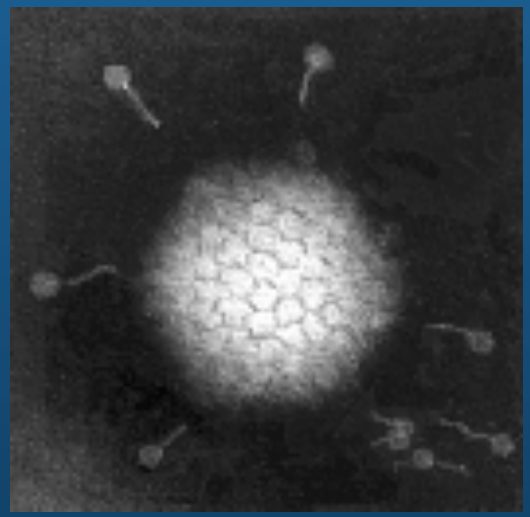
e.g. Tobacco Mosaic Virus (TMV)





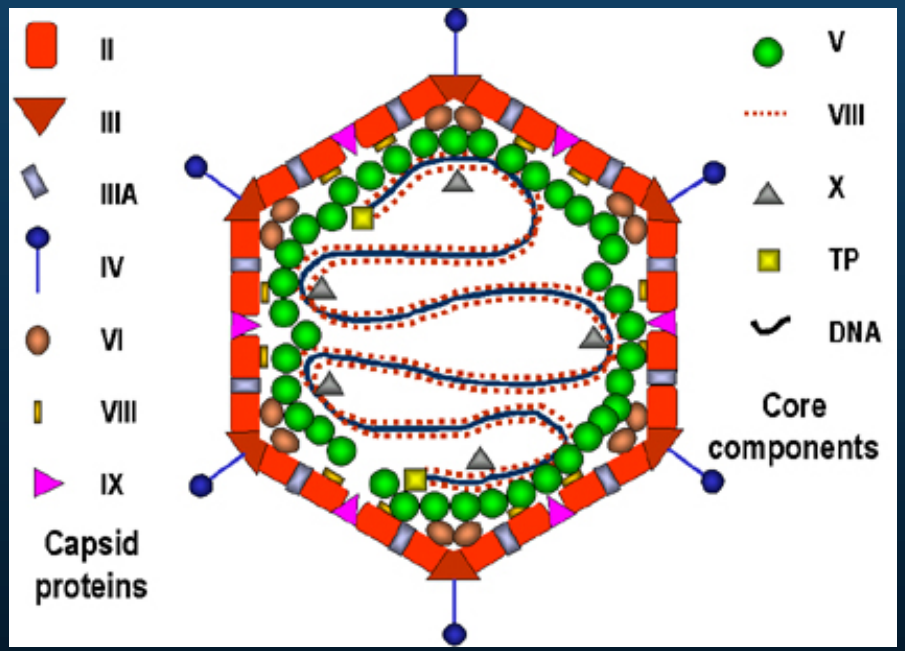


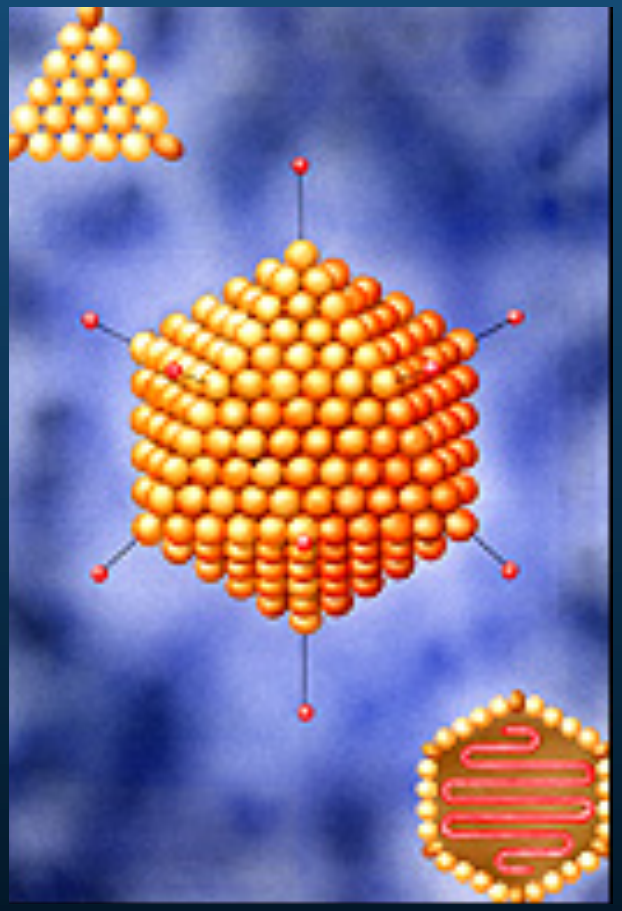




Icosahedral Capsid Structure

e.g. Adenovirus





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DNA Viruses

ss DNA, non-enveloped	Parvo viruses
ds DNA, non-enveloped	Papova-, Adenoviruses
ds DNA, enveloped	Herpes-, Pox-, Iridoviruses
ss/ds DNA, non-enveloped	Hepadnaviruses

RNA Viruses

ss RNA, positive-sense, non-segmented, non-enveloped	Picornaviruses, Caliciviruses
ss RNA, positive-sense, non-segmented, non-enveloped	Toga-, Flavi-, Coronaviruses
ss RNA, negative-sense, non-segmented, non-enveloped	Rhabdo-, Filo-, Paramyxoviruses
ss RNA, negative-sense, segmented, enveloped	Orthomyxoviruses

RNA Viruses

ss RNA, ambisense, segmented, enveloped	Bunya-, Arenaviruses
ds RNA, segmented, non-enveloped	Reo-, Birnaviruses
ss RNA, DNA step in replication, positive-sense, non-segmented, enveloped	Retroviruses

DNA VIRUSES

ADVANTAGES

- Genome is same structure as host cell chromosomes: replication resembles that of the host use of host enzymes

- Control elements that regulate gene expression are the same as those found in host cell

PROBLEMS

- Requires a primer to initiate DNA replication same problem faced by the host cell: DNA polymerase de novo initiate synthesis is on ss DNA templates, they only extend from double stranded regions (5’ - 3’)

- How faithfully replicates ends without losing information? solution: specialised structural features of genomes

RNA VIRUSES

ADVANTAGE

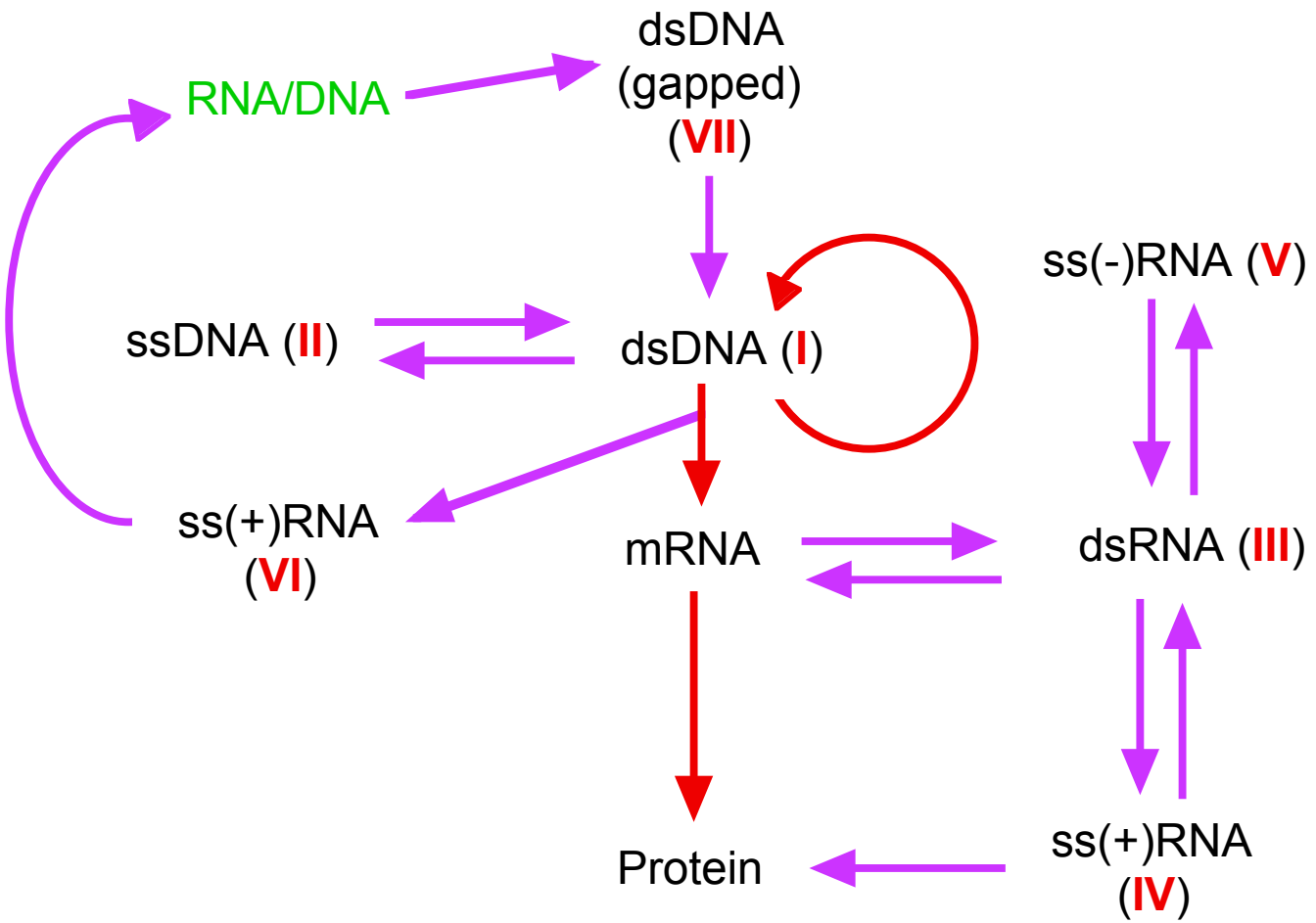
- RNA-dependent RNA polymerase do not require a primer
  replication can be initiated at the ends of a linear molecule

PROBLEMS

- Cells do not contain required to directly replicate RNA
  virus must provide its own RNA-dependent RNA polymerase
  (replicase, transcriptase)

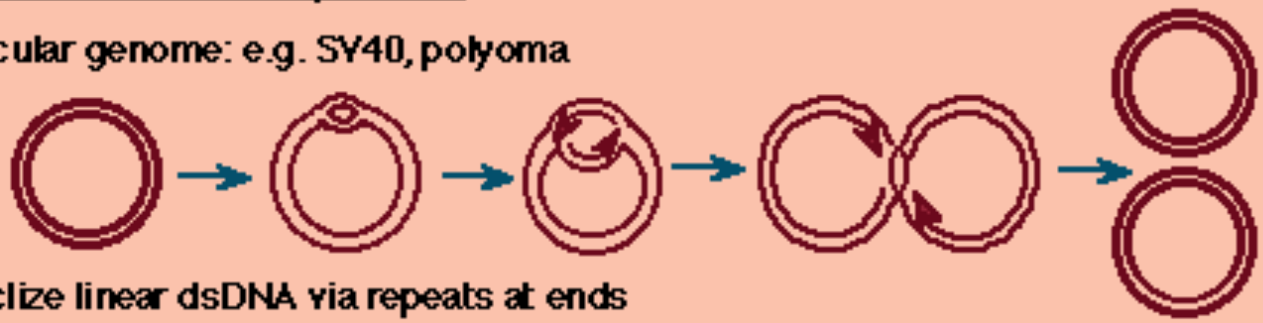
- Do not contain same controlling elements as the host to regulate gene expression have evolved some unique strategies to regulate their gene expression

Pathways of Information Flow in Virus Replication
Baltimore Classification



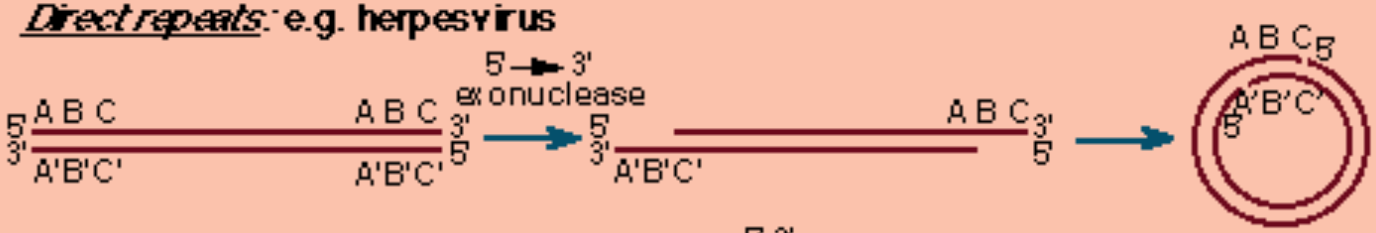
Modes of dsDNA virus replication

1. Circular genome: e.g. SV40, polyoma

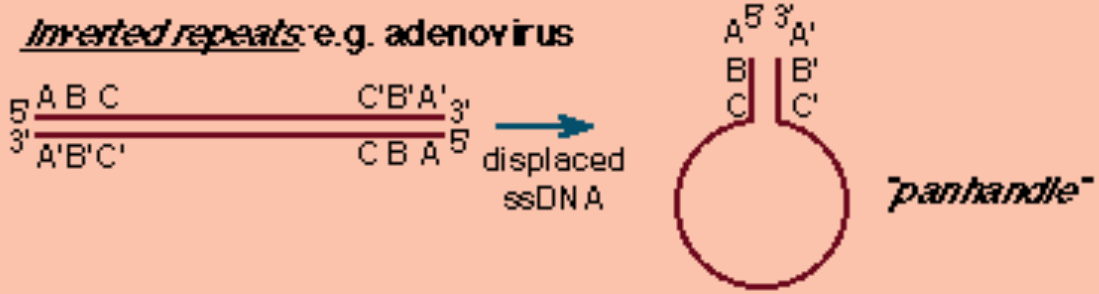


2. Cyclize linear dsDNA via repeats at ends

Direct repeats: e.g. herpesvirus



Inverted repeats: e.g. adenovirus



3. Terminal protein attached to ends

DNA viruses temporally regulate their gene expression

- Early genes: expressed prior to the onset of viral DNA replication
- Late genes: expressed following the onset of viral DNA replication

Early and Late genes may be clustered, thereby facilitating their coordinate regulation

Adenoviruses

• Adenoviruses are a frequent cause of acute upper respiratory tract (URT) infections, i.e. "colds".

• First isolated in 1953 bfrom adenoidal tissue of children (tonsillectomies) and from military recruits with febrile illness.

• In 1962, some Adenoviruses were shown to cause tumours in rodents - this caused a considerable panic ! (N.B. Adenovirus oncogenesis appears to be associated with abortive infections and has never been observed in humans.)

• During investigation of the Adenovirus genome and gene expression, many techniques were developed which were subsequently used to examine other viruses/cellular genes - these viruses are an important model system for the understanding of many other viruses.

• Widespread in nature, infecting birds, many mammals and man.
2 genera, Aviadenovirus (avian) and Mastadenovirus (mammalian)

• Several types have oncogenic potential.

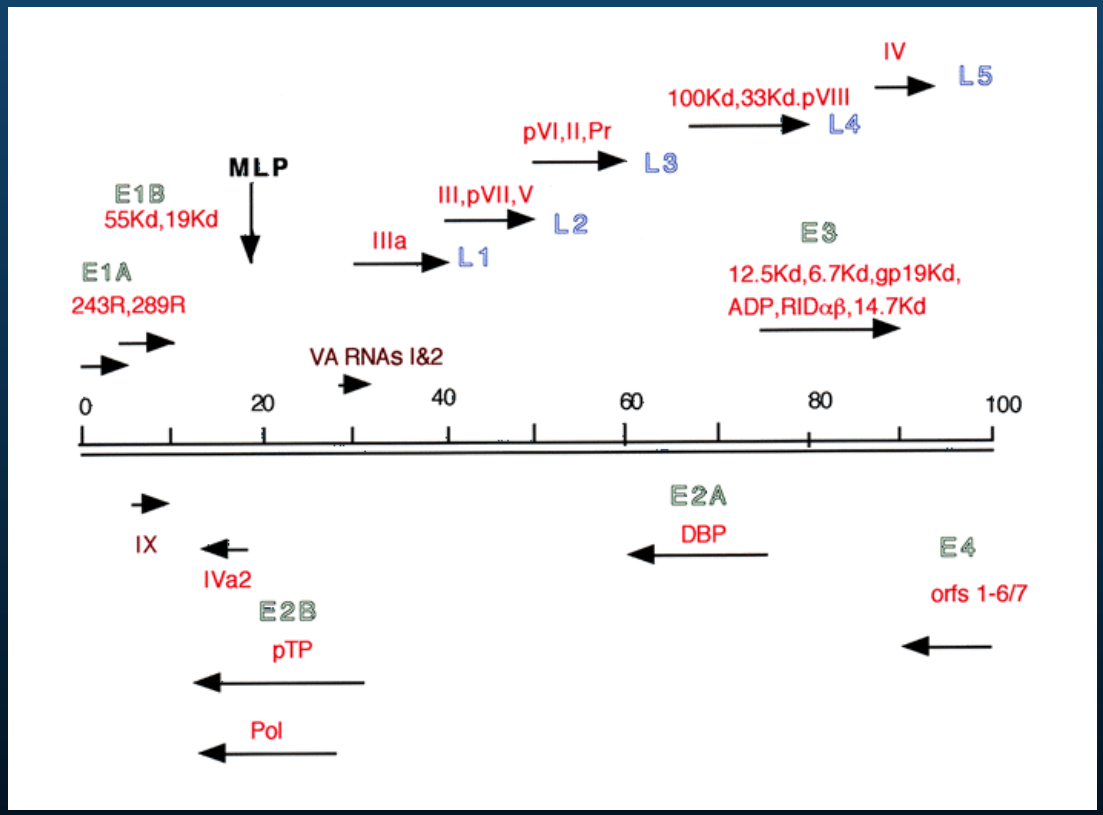
• There are at least 49 human adenovirus serotypes which have been classified into six subgenera (A to F). The serotypic origin of the E1A gene determines the oncogenic phenotype of adenovirus-transformed cells.

• All human adenoviruses studied so far can transform primary rodent cells in culture.

Adenovirus Genome

Linear, non-segmented, d/s DNA, 30 - 38 kbp (size varies from group to group) encodes 30 - 40 genes. Genome structure is one of the characters used to assign viruses to groups (70 - 95% similarity within groups, 5 - 20% between groups).

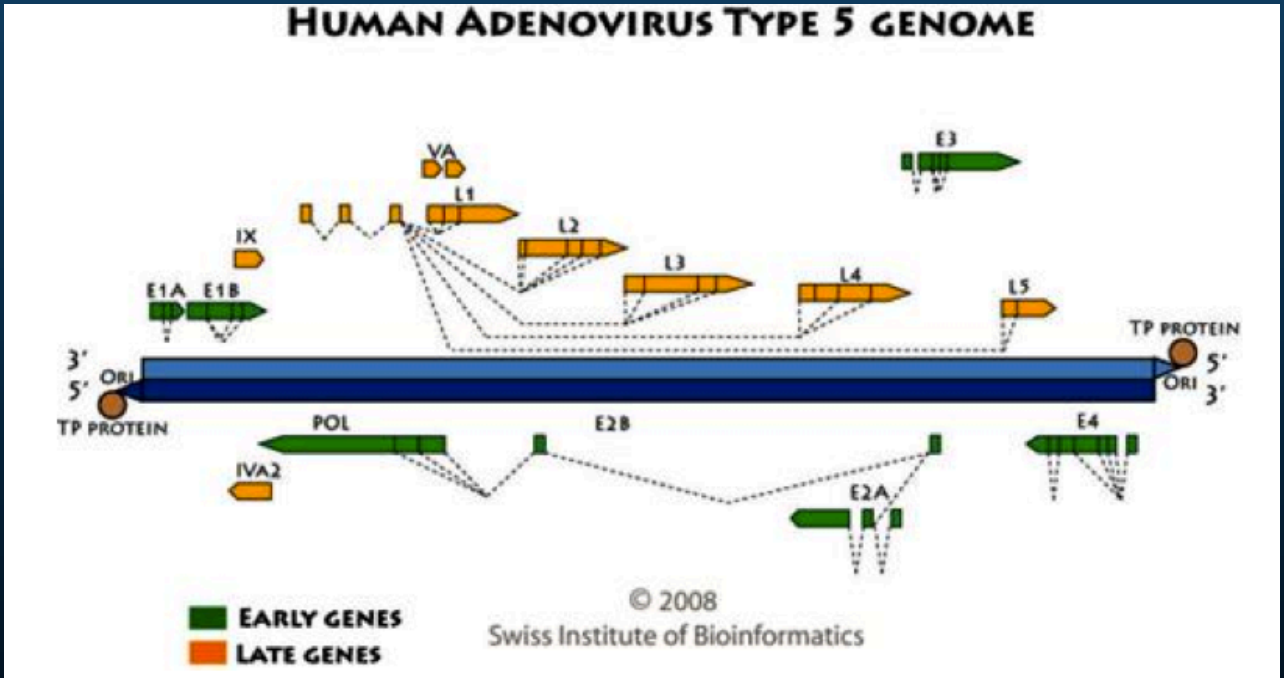
The terminal sequences of each strand are inverted repeats, hence the denatured single strands can form "panhandle" structures (100 - 140 bp). There is a 55 kD protein covalently attached to the 5' end of each strand.



Gene Expression

Phase:	Genes Transcribed:
Immediate early	E1A (~1 hour after infection)
Early	E1B, E2A, E2B, E3, E4, some virion proteins
Late	Late genes, mostly virion proteins

E1A is a trans-acting transcriptional regulatory factor whose precise mode of action is not known (not a DNA-binding "transcription factor") but is necessary for transcriptional activation of early genes.
The second protein made is E1B. E1A + E1B together (and independently of other virus proteins) are capable of transforming primary cells *in vitro* (especially Ad5, Ad12).



Herpesviruses

Name comes from the Greek *'Herpein'* - 'to creep' = chronic/latent/recurrent infections.
Epidemiology of the common Herpesvirus infections puzzled clinicians for many years.
In 1950, Burnet and Buddingh showed that HSV could become latent after a primary infection, becoming reactivated after later provocation.
~100 Herpesviruses have been isolated, at least one for most animal species.

To date, there are 8 known human Herpesviruses.

The family is divided into 3 Sub-families:

Alphaherpesvirinae:

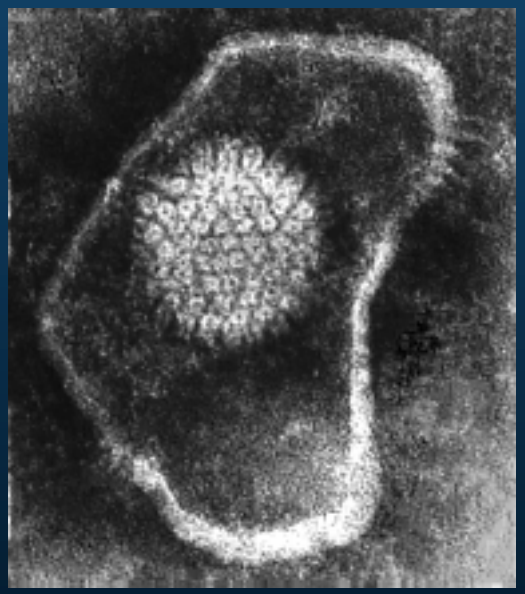
Simplexvirus	human herpesvirus 1, 2 (HSV-1, HSV-2)
Varicellovirus	human herpesvirus 3 (VZV)

Betaherpesvirinae:

Cytomegalovirus	human herpesvirus 5 (CMV)
Muromegalovirus	mouse cytomegalovirus 1
Roseolovirus	human herpesvirus 6, 7 (HHV-6, HHV-7)

Gammaherpesvirinae:

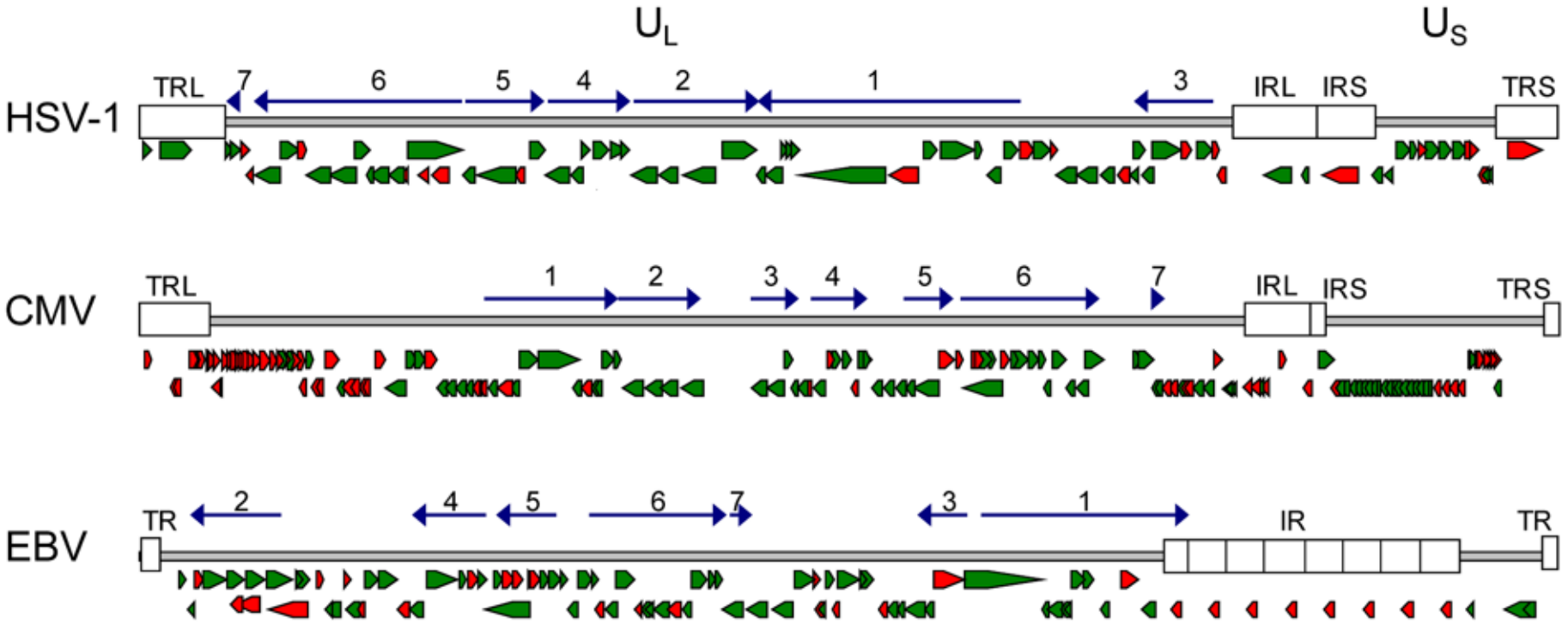
Lymphocryptovirus	human herpesvirus 4 (EBV)
Rhadinovirus	human herpesvirus 8 (HHV-8)



Large (genomes up to 235 kbp DNA) and complex viruses ~35 virion proteins.

All encode a variety of enzymes involved in nucleic acid metabolism, DNA synthesis and protein processing (protein kinase).

The Herpesviruses are widely separated in terms of genomic sequence and proteins, but all are similar in terms of virion structure and genome organization:



Herpesvirus Replication

Fusion deposits the capsid in the cytoplasm, where it migrates to the nucleus.
The core enters via a nuclear pore where the genome is circularized.
Transcription of the large, complex genome is sequentially regulated in a cascade fashion.

~50 mRNAs are produced by host cell RNA polymerase II.

Three distinct classes of mRNAs are made:
Alpha - immediate early (IE) mRNAs (trans-acting regulators of virus transcription)
Beta - (delayed) Early mRNAs (non-structural regulatory & minor structural proteins)
Gamma - Late mRNAs Major structural proteins

Gene expression is co-ordinately regulated:

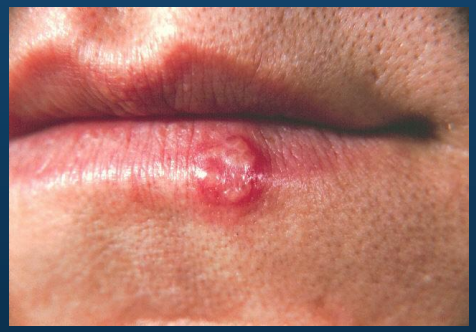
The capacity to establish latency is a characteristic feature of all herpesviruses, and involves three separable phases:

- establishment
- maintenance
- reactivation

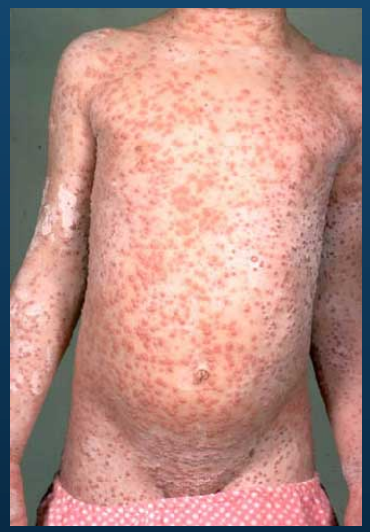
Pathogenesis:

Herpes simplex (HSV/ HHV-1/2):
HSV-1: Primarily associated with oral (cold sores) and ocular lesions
HSV-2: Primarily associated with genital and anal lesions

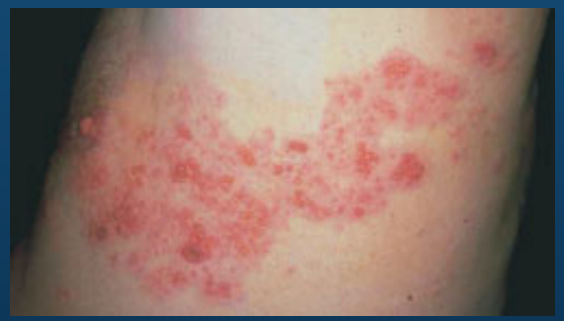
Varicella Zoster Virus (VZV / HHV-3): *Varicella (Chicken pox) -Zoster (Shingles)*



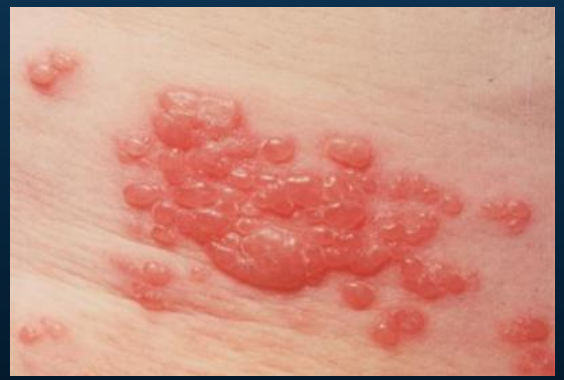
HSV-1 - cold sore



VZV - varicella (chickenpox)

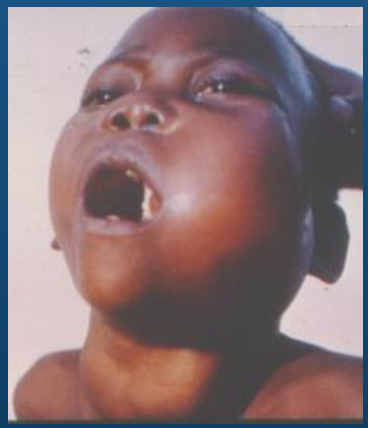


VZV - zoster (shingles)



Cytomegalovirus (CMV / HHV-5):

CMV infection is common; 60% of the UK population infected by the age of 40.
Most infections are asymptomatic. CMV infection only occurs in people with immune defects, specifically T-cell defects, e.g. AIDS patients and immunosuppressed transplant patients.

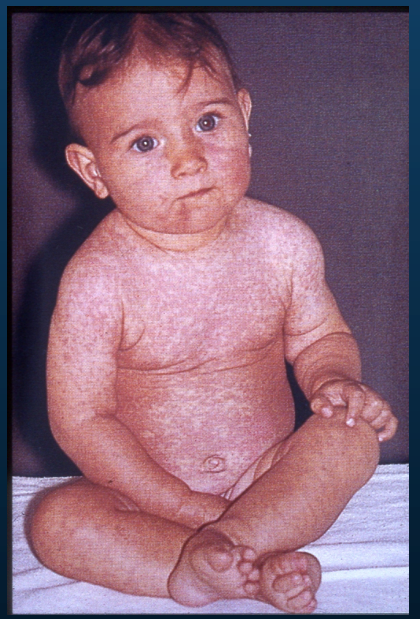


Epstein-Barr Virus (EBV / HHV-4):

The usual outcome of infection is polyclonal B-cell activation and benign proliferation, which may be sub-clinical or produce infectious mononucleosis (glandular fever).
There is a well-established relationship with oncogenesis - Burkitts lymphoma and nasopharyngeal carcinoma.

Human Herpesvirus 6 (HHV-6):

Primary infection in childhood causes "roseola infantum"



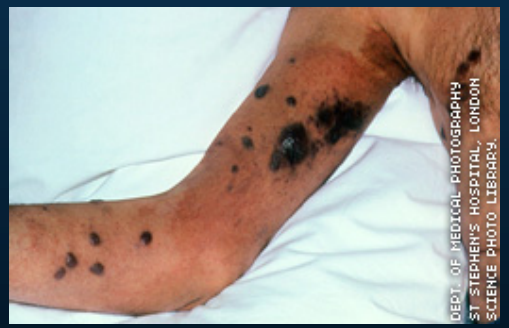
roseola infantum

Human Herpesvirus 7 (HHV-7):

At present, there is no clear evidence for the direct involvement of HHV-7 in any human disease

Human Herpesvirus 8 (HHV-8):

There is a strong correlation (>95%) with Kaposi's sarcoma in HIV+ *and* HIV- patients



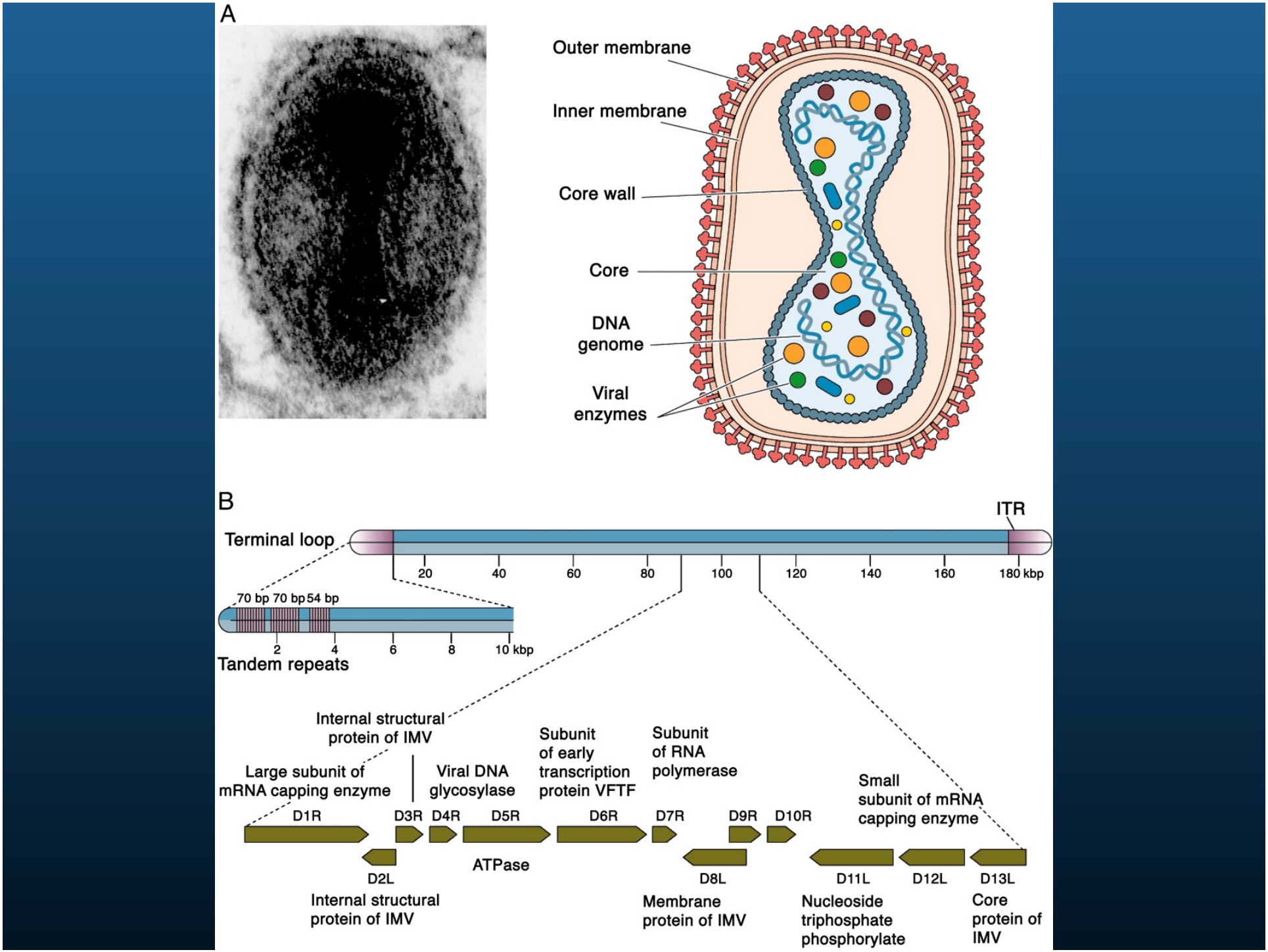
Poxviruses

The largest and most complex viruses known! Have been known about for centuries - the characteristic "pocks" produced by variola virus (Smallpox) giving their name to all forms of infectious disease "a dose of the pox".

Smallpox first appeared in China and the Far East at least 2,000 years ago.

The Pharaoh Ramses V (left) died of smallpox in 1157 B.C. The disease reached Europe in 710 A.D. and was transferred to America by Hernando Cortez in 1520 - 3.5 million Aztecs died in the next 2 years.

In the cities of 18th century Europe, smallpox reached plague proportions and was a feared scourge - highly infectious. Five reigning European monarchs died from smallpox during the 18th century


A
Outer membrane
Inner membrane
Core wall
Core
DNA genome
Viral enzymes
B
ITR
Terminal loop
20
40
60
80
100
120
140
160
180 kbp
70 bp 70 bp 54 bp
2
4
6
8
10 kbp
Tandem repeats
Internal structural protein of IMV
Large subunit of mRNA capping enzyme
Viral DNA glycosylase
Subunit of early transcription protein VFTF
Subunit of RNA polymerase
Small subunit of mRNA capping enzyme
D1R
D3R
D4R
D5R
D6R
D7R
D9R
D10R
D2L
ATPase
D8L
D11L
D12L
D13L
Internal structural protein of IMV
Membrane protein of IMV
Nucleoside triphosphate phosphorylate
Core protein of IMV

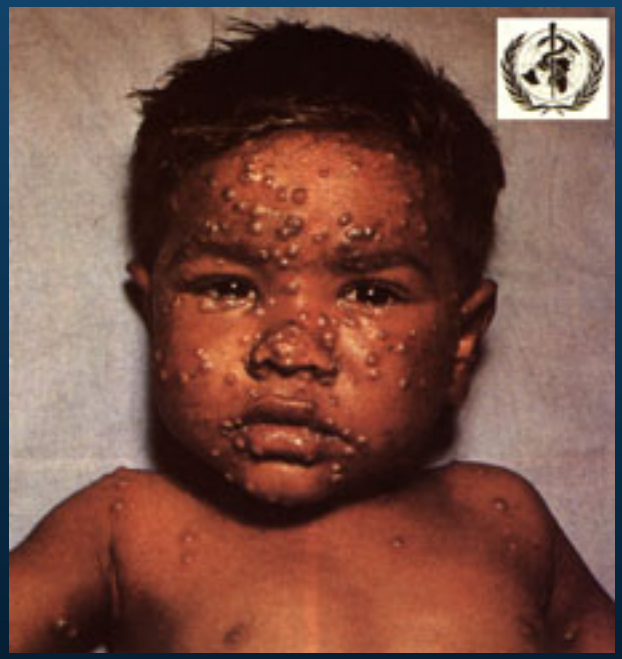
Vaccination

- 1798, Edward Jenner realized that milkmaids tended to catch a mild form of "the pox," from cows

- They were then protected against the typical disfiguring ravages of ordinary smallpox.

Variola and Vaccinia

At least 9 different poxviruses cause disease in humans, variola virus (VV) and vaccinia best known. VV strains are divided into variola major (25 - 30% fatalities) and variola minor (same symptoms but less than 1% death rate). "Variolation" = the administration of material from known smallpox cases to protect recipients - practiced for at least 1000 years (Chinese) but risky - Jenner was nearly killed by variolation in 1756!



The precise origins of vaccinia virus are uncertain:

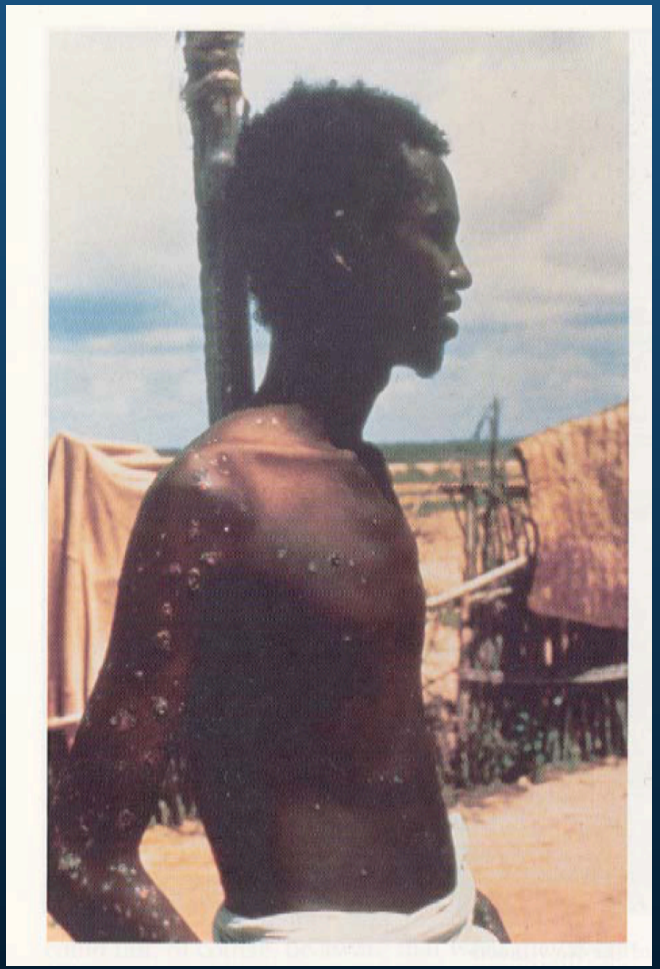
Edward Jenner, 14th May 1796, used cowpox to "vaccinate" 8 year old James Phipps, who he later challenged with VV (ethical?) and showed that he was protected.

For more than 100 years, the "vaccine strains" (many origins) were propagated from arm-to-arm, but for at least the last 50 years, Vaccinia has been a distinct virus from cowpox - origin?

(In molecular terms, vaccinia is most similar to buffalopox - did the two viruses somehow become mixed at some stage?).

Smallpox: Eradication

- WHO Vaccination Programme
- Last case (non-laboratory) 1977, Somalia
  None since October 26, 1977
- Last person to die from smallpox?
- Remaining stocks of virus
  - USA
  - Russia
  - ?



Picornaviruses

Picornaviruses are among the most diverse (more than 200 serotypes) and 'oldest' known viruses (temple record from Egypt ca. 1400 B.C.). FMDV was one of the first viruses to be recognised - Loeffler and Frosch 1898. Poliomyelitis as a viral disease was first recognised by Landsteiner and Popper, 1909 (though the virus was not isolated until the 1930's).

Name: 'Pico *(Greek very small)* RNA Viruses'.

Classification:

Originally based on physical properties (particle density & pH-sensitivity) & serological relatedness, more recently based on nucleotide sequence. There are 5 genera:

Aphthovirus	7 serotypes
Cardiovirus	2 serotypes
Enterovirus	111 serotypes
Hepatovirus	2 serotypes (1 human, 1 simian)
Rhinovirus	105 serotypes
Unassigned	3 serotypes
Total:	~230 viruses

Genome

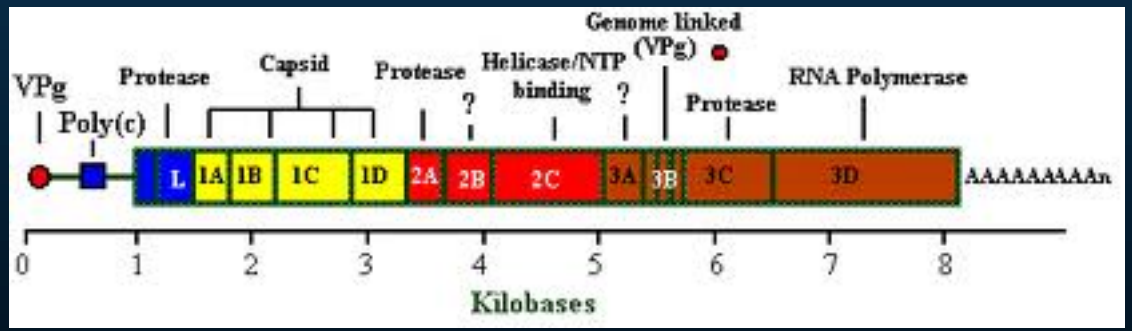
The genome consists of one s/s (+)sense RNA molecule of between 7.2 kb (HRV14) to 8.5 kb (FMDV). A number of features are conserved in all Picornaviruses:

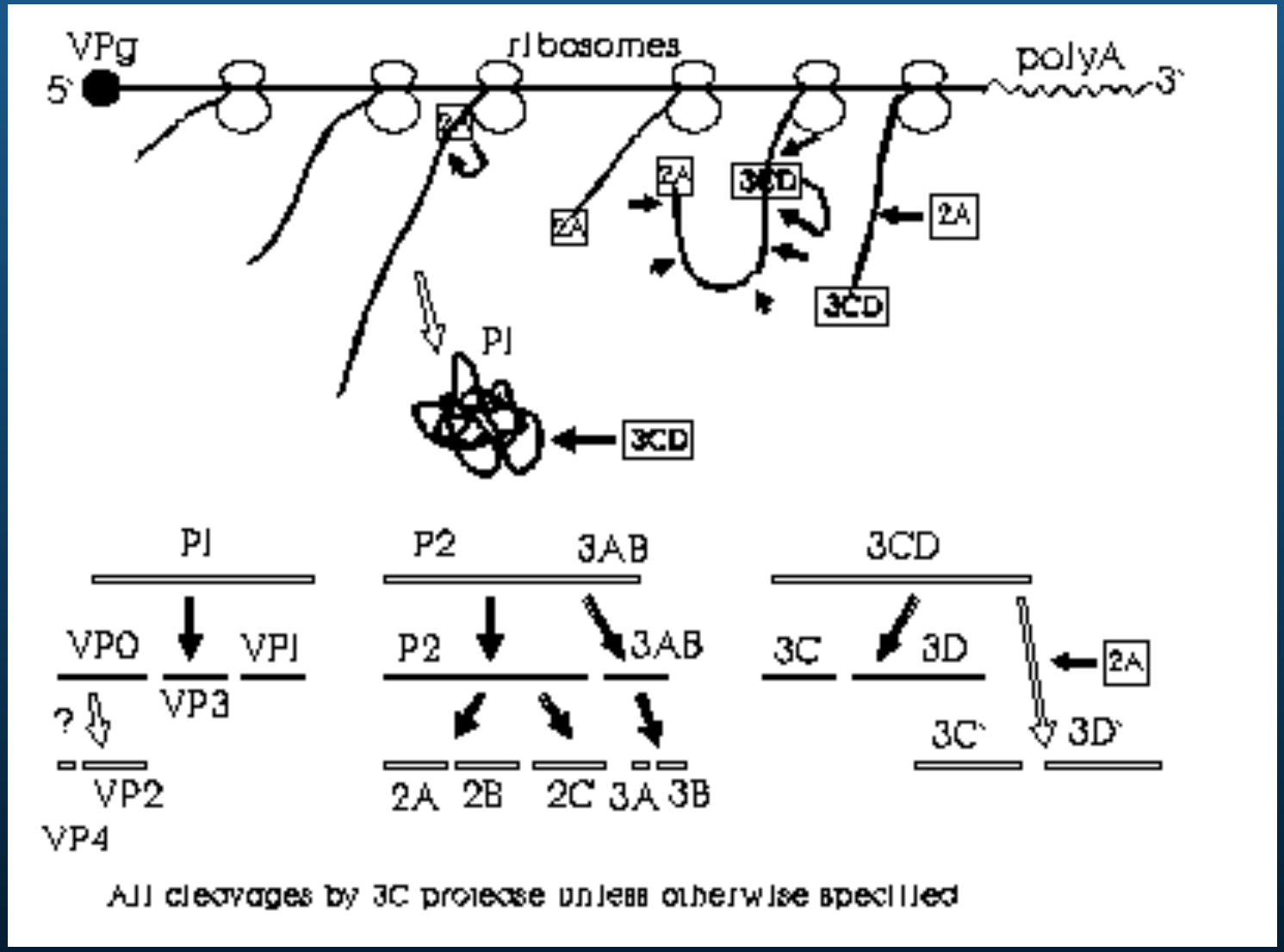
Genomic RNA is infectious (~1x10^6-fold less infectious than intact particles).

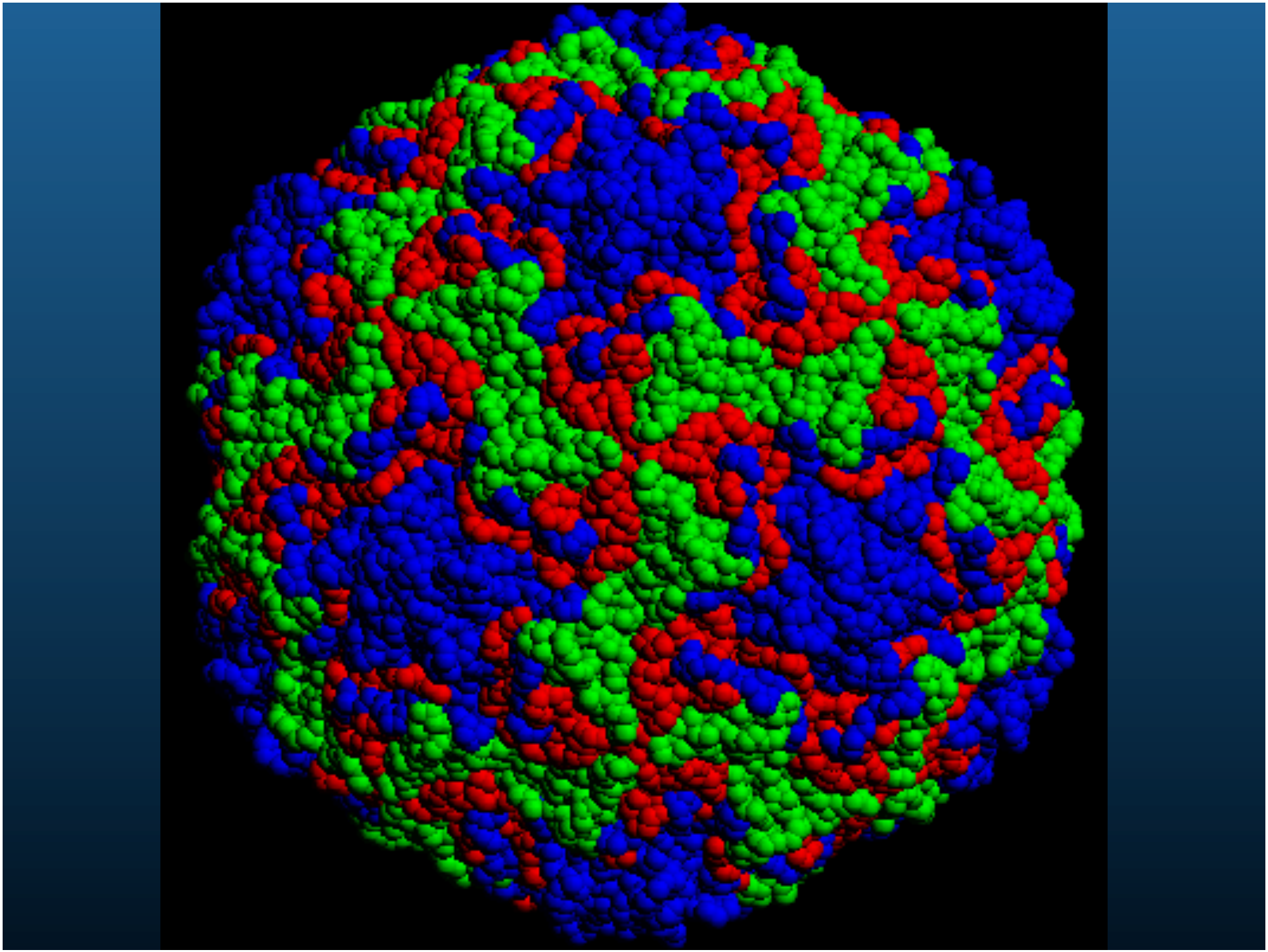
There is a long (600 - 1200 base) untranslated region at the 5' end (important in translation, virulence and possibly encapsidation and a shorter 3' untranslated region (50 - 100 bases) - important in (-)strand synthesis. The 5' UTR contains a 'clover-leaf' secondary structure known as the IRES: Internal Ribosome Entry Site.

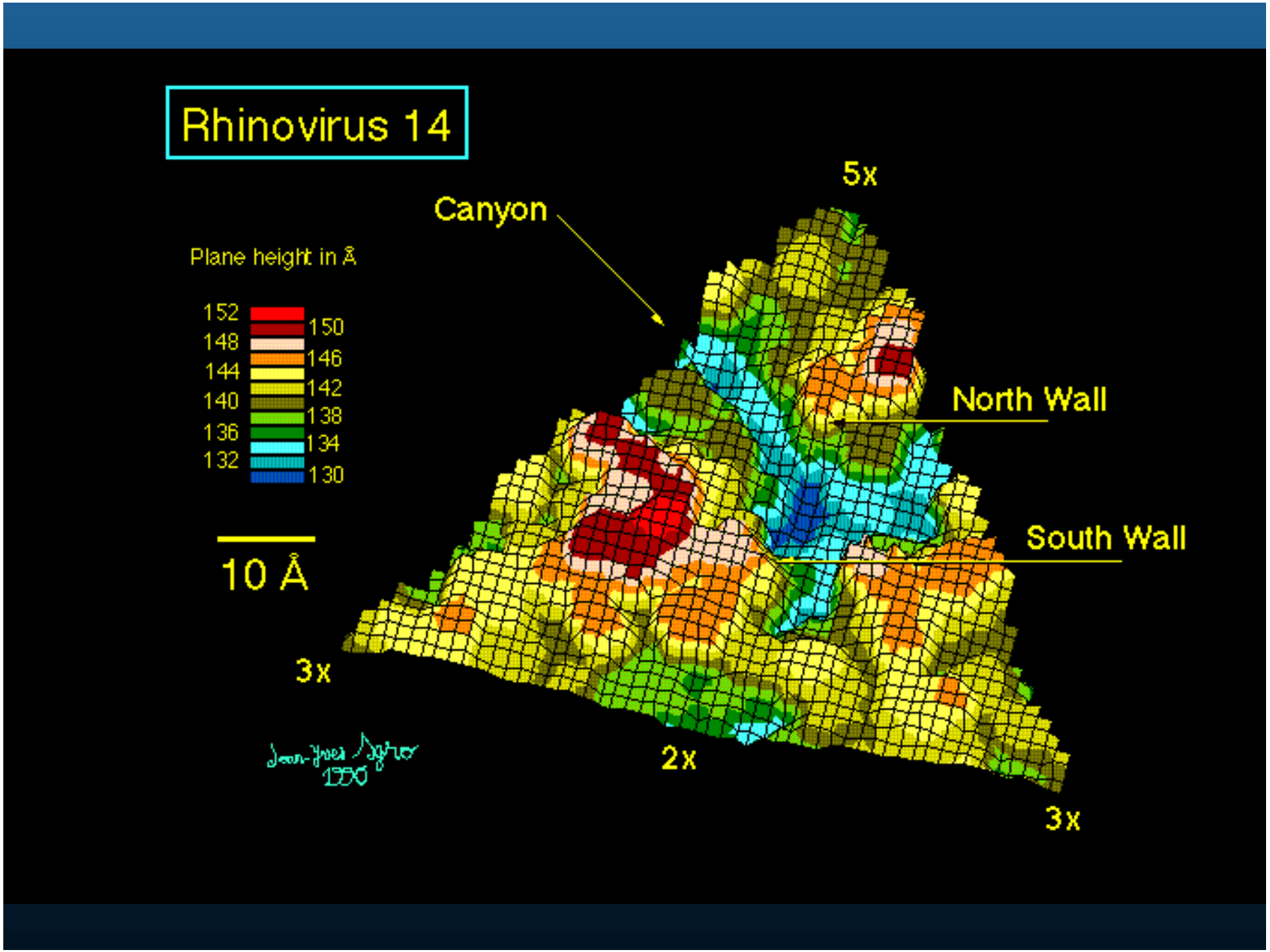
The rest of the genome encodes a single 'polyprotein' of between 2100 - 2400 aa's.

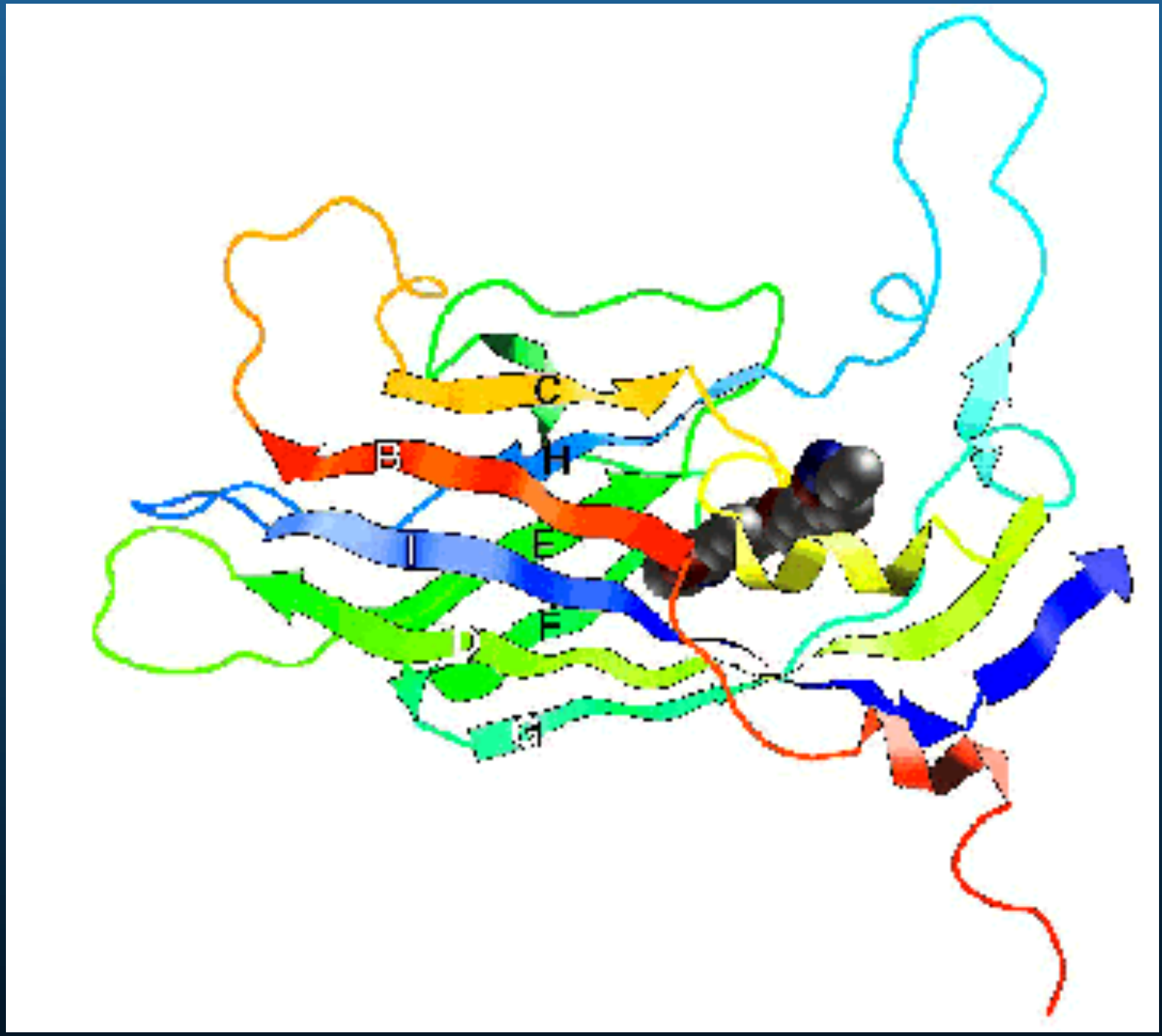
Both ends of the genome are modified, the 5' end by a covalently attached small, basic protein VPg (~23 aa's), the 3' end by polyadenylation (polyadenylic acid sequences are not genetically coded, there is a 'polyadenylation signal' upstream of the 3' end as in eukaryotic mRNAs):




VPg
5`
ribosomes
polyA
3`
2A
3CD
P1
P1
VP0
VP1
VP3
?
VP2
VP4
P2
3AB
P2
3AB
2A
2B
2C
3A
3B
3CD
3C
3D
2A
3C`
3D`
All cleavages by 3C protease unless otherwise specified

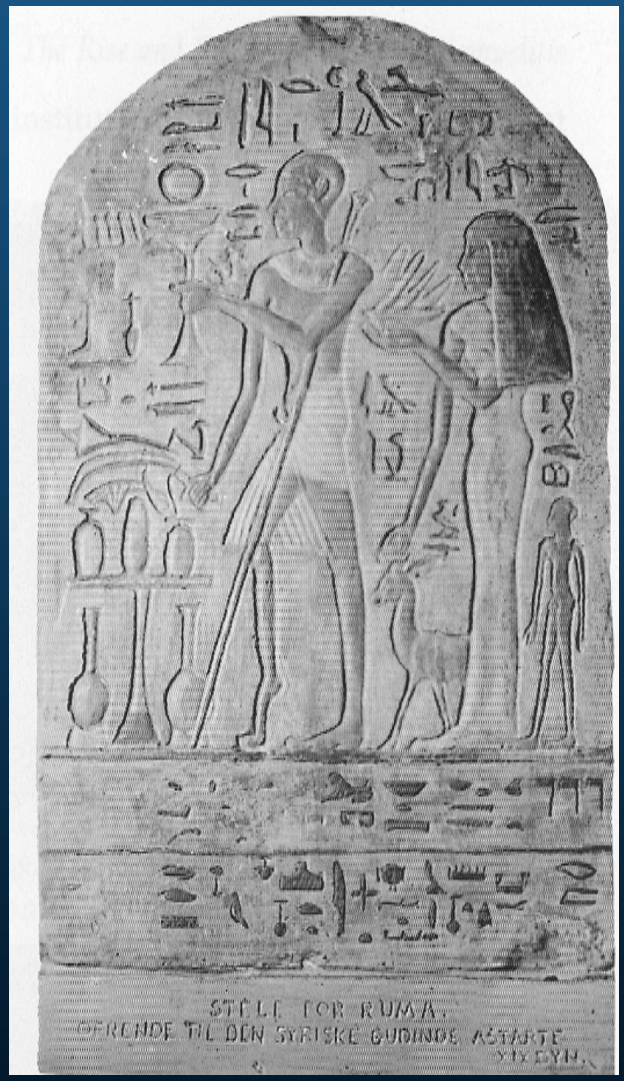



Rhinovirus 14
Plane height in Å
152
148
144
140
136
132
150
146
142
138
134
130
10 Å
Canyon
5x
North Wall
South Wall
3x
2x
3x


C
B
H
I
E
F
D
G

Polio

- Bas-relief, Egypt 1500 B.C.
- Egyptian priest -shrivelled leg
- Appearance is consistent with the consequences of paralytic polio.



Polioviruses

They cause poliomyelitis (flaccid muscular paralysis).

Transmitted by the faecal-oral route.

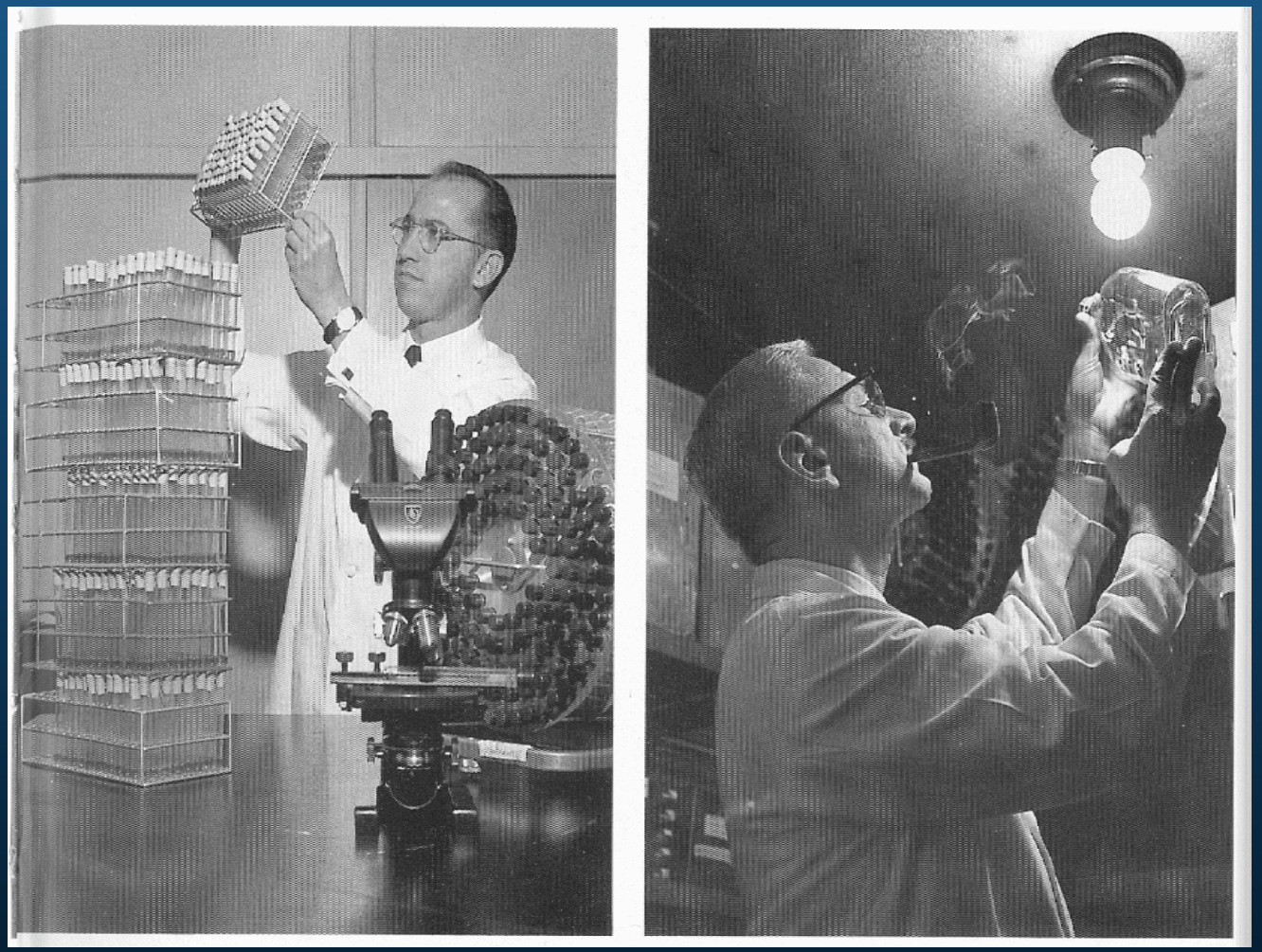
Primary site of infection is lymphoid tissue associated with the oropharynx and gut.

Virus production at this site leads to a transient viraemia, following which the virus may infect the CNS.

~1% of people infected with the most virulent strains experience paralysis (99% asymptomatic infections). Death is usually due to respiratory failure by paralysis of the intercostal muscles and diaphragm.

Effective polyvalent vaccines are available against polioviruses. In 1988, the World Health Assembly established the year 2000 for achieving global poliomyelitis eradication. By 1994, the Americas were certified as polio-free. All other regions are making steady progress towards this goal. The WHO reports that global eradication of poliomyelitis is still on course for the year 2000.

Polio Vaccine



Salk: “Killed”

Sabin: “Live”

Hand-foot-and-mouth disease (HFMD) is most commonly caused by coxsackievirus A16, a member of the enterovirus family

Rhinoviruses

Cause of 'the common cold' (but not the only one!). ~105 serotypes (hence repeated infections). Relatively fragile viruses with optimum growth temperature of 33°C

A major economic pest worldwide (number of lost working days).

No effective prophylaxis or treatment

Cardioviruses

One serotype. Includes encephalomyocarditis virus (EMCV) (model infection of mice), mengovirus, Maus-Elberfield virus, Columbia virus - all considered to be strains of EMCV (really a mouse virus, but can infect man, elephants, squirrels).

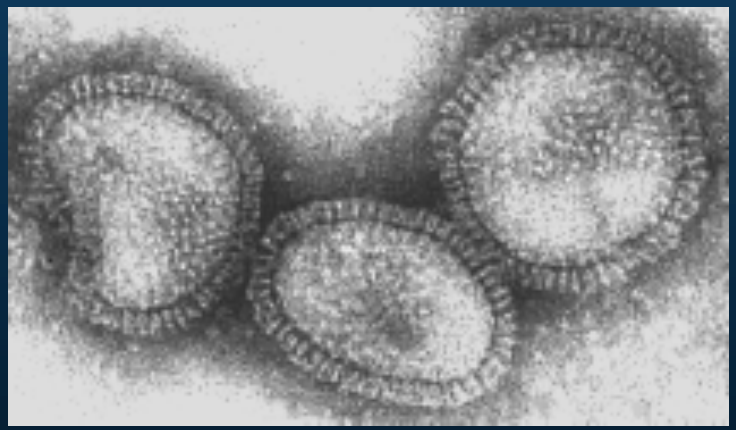
Genome size ~7.8 kb; 5' non-translated region contains poly-C tract of ~100 - 170 bases.

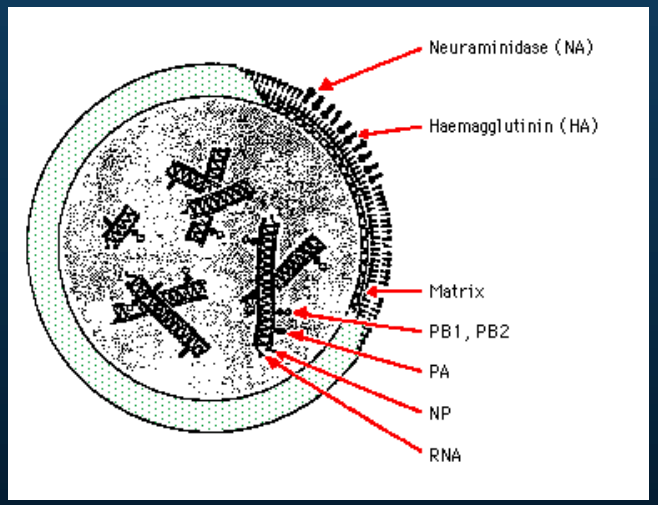
Orthomyxoviruses

Host Range:

Influenza A viruses infect a wide variety of mammals, including man, horses, pigs, ferrets and birds. Pigs and birds are believed to be particularly important reservoirs, generating pools of genetically/antigenically diverse viruses which get transferred back to the human population via reassortment (close contact between pigs and man in the far east; ducks - migration!).

The main human pathogen, associated with epidemics and pandemics.

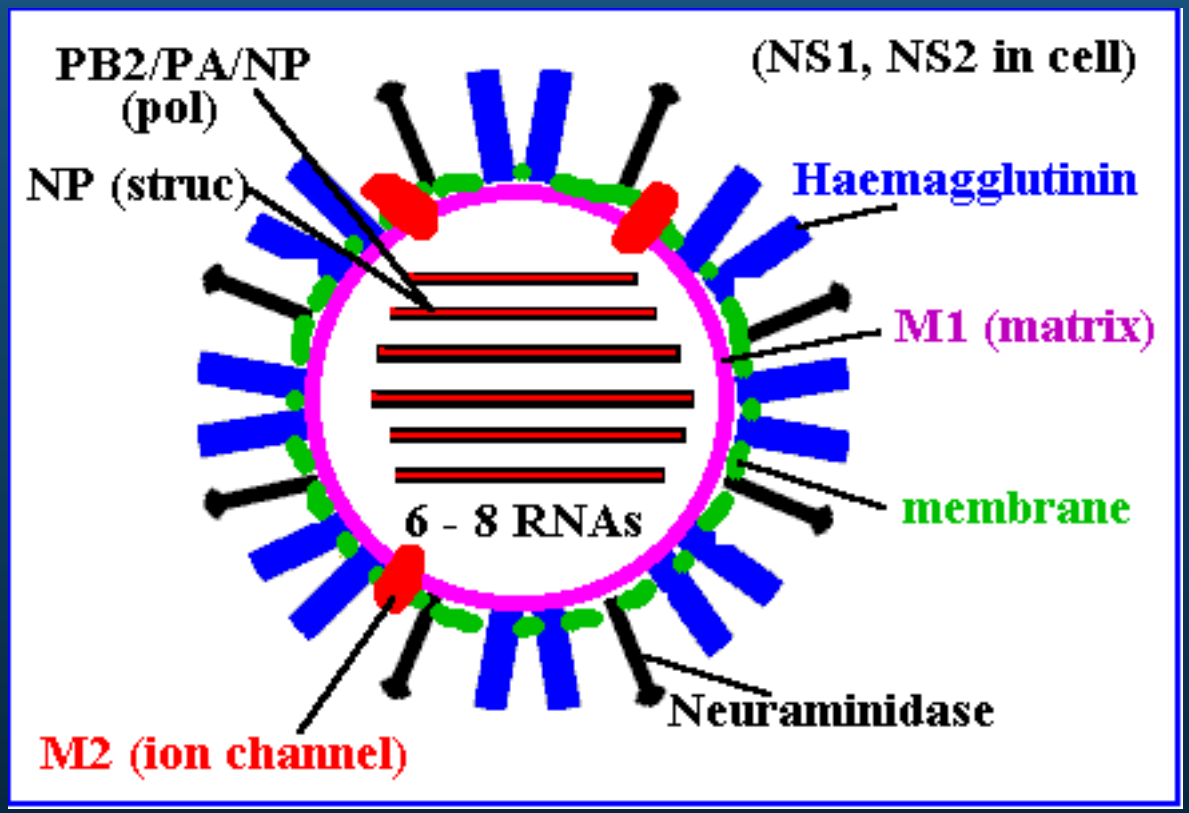


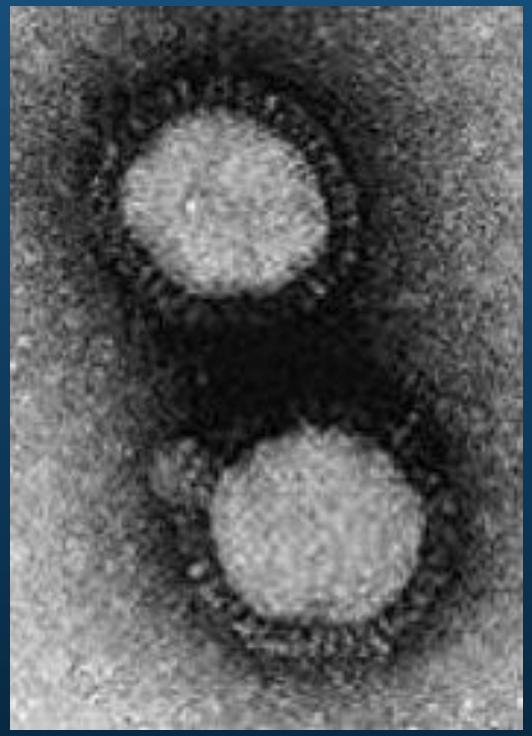


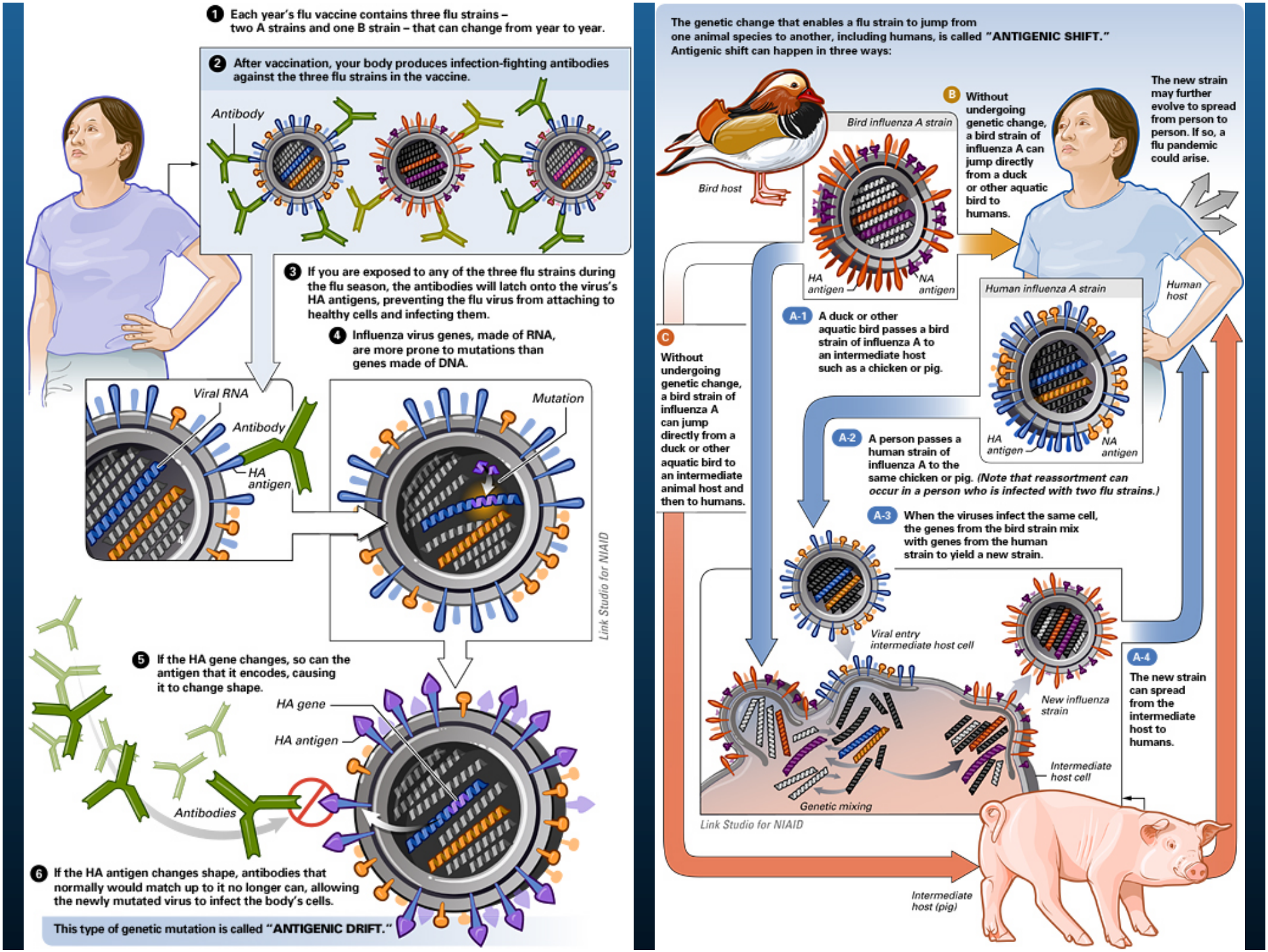
Orthomyxoviruses Genome

Consists of s/s (-)sense RNA in 8 segments. The 5' and 3' terminal sequences of all the genome segments are highly conserved:

Segment:	Size(nt)	Polypeptide(s)	Function
1.	2341	PB2	Transcriptase: cap binding
2.	2341	PB1	Transcriptase: elongation
3.	2233	PA	Transcriptase: protease activity (?)
4.	1778	HA	Haemagglutinin
5.	1565	NP	Nucleoprotein: RNA binding; part of transcriptase complex;
6.	1413	NA	Neuraminidase: release of virus
7.	1027	M1	Matrix protein: major component of virion
		M2	Integral membrane protein - ion channel
8.	890	NS1	Non-structural: nucleus; effects on cellular RNA transport, splicing, translation
		NS2	Non-structural: nucleus+cytoplasm, function unknown


PB2/PA/NP
(pol)
NP (struc)
(NS1, NS2 in cell)
Haemagglutinin
M1 (matrix)
membrane
6 - 8 RNAs
Neuraminidase
M2 (ion channel)



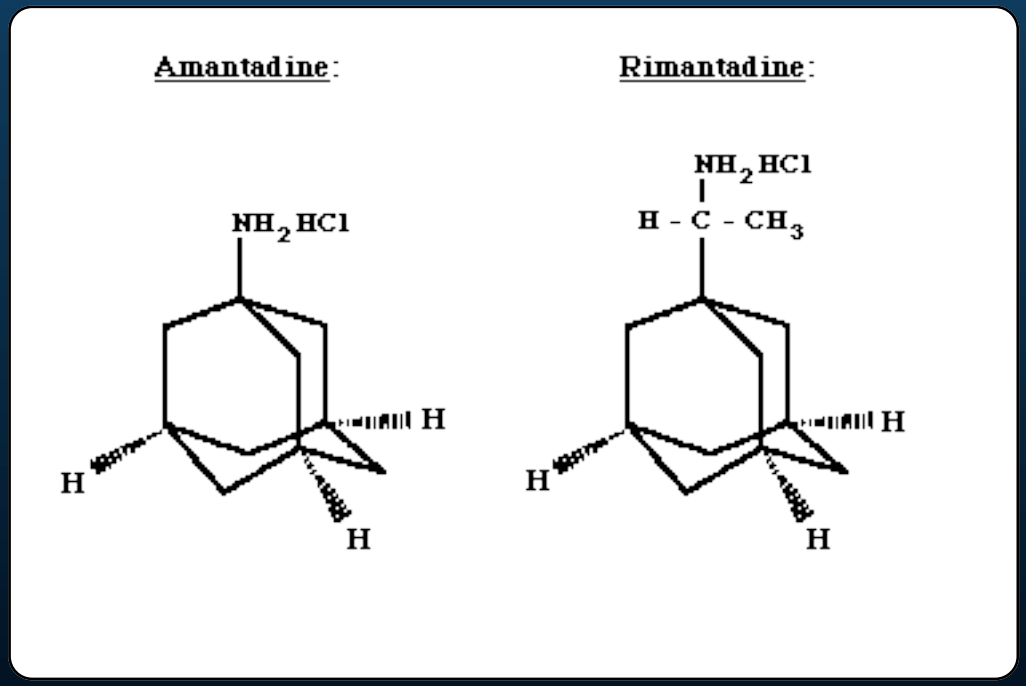

1 Each year's flu vaccine contains three flu strains – two A strains and one B strain – that can change from year to year.
2 After vaccination, your body produces infection-fighting antibodies against the three flu strains in the vaccine.
Antibody
3 If you are exposed to any of the three flu strains during the flu season, the antibodies will latch onto the virus's HA antigens, preventing the flu virus from attaching to healthy cells and infecting them.
4 Influenza virus genes, made of RNA, are more prone to mutations than genes made of DNA.
Viral RNA
Antibody
HA antigen
Mutation
Link Studio for NIAID
5 If the HA gene changes, so can the antigen that it encodes, causing it to change shape.
HA gene
HA antigen
Antibodies
6 If the HA antigen changes shape, antibodies that normally would match up to it no longer can, allowing the newly mutated virus to infect the body's cells.
This type of genetic mutation is called "ANTIGENIC DRIFT."
The genetic change that enables a flu strain to jump from one animal species to another, including humans, is called "ANTIGENIC SHIFT." Antigenic shift can happen in three ways:
Bird host
Bird influenza A strain
HA antigen
NA antigen
B Without undergoing genetic change, a bird strain of influenza A can jump directly from a duck or other aquatic bird to humans.
The new strain may further evolve to spread from person to person. If so, a flu pandemic could arise.
Human host
Human influenza A strain
HA antigen
NA antigen
A-1 A duck or other aquatic bird passes a bird strain of influenza A to an intermediate host such as a chicken or pig.
C Without undergoing genetic change, a bird strain of influenza A can jump directly from a duck or other aquatic bird to an intermediate animal host and then to humans.
A-2 A person passes a human strain of influenza A to the same chicken or pig. (Note that reassortment can occur in a person who is infected with two flu strains.)
A-3 When the viruses infect the same cell, the genes from the bird strain mix with genes from the human strain to yield a new strain.
Viral entry intermediate host cell
New influenza strain
Intermediate host cell
Genetic mixing
Link Studio for NIAID
A-4 The new strain can spread from the intermediate host to humans.
Intermediate host (pig)

Prevention/Treatment

Several anti-influenza drugs already exist. Amantadine and rimantadine are active against influenza A viruses. The action of these closely related agents is complex and incompletely understood, but they are believed to block cellular membrane ion channels.

The target for both drugs is the matrix protein (M2), but resistance to the drug maps to the haemagglutinin (HA) gene.

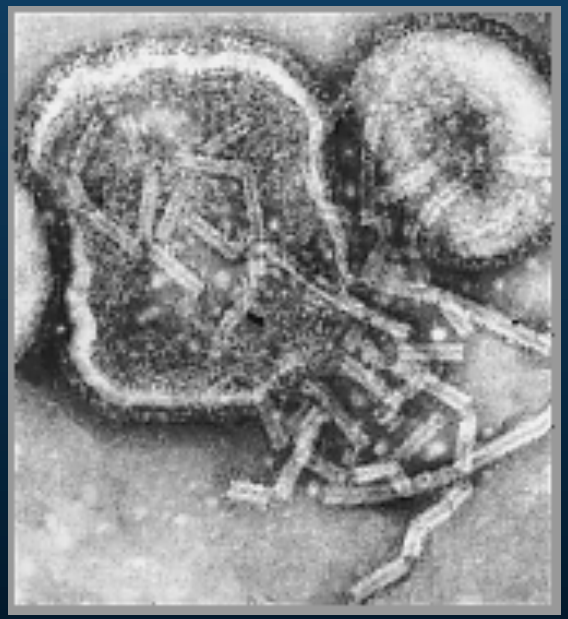
This biphasic action results from the inability of drug-treated cells to lower the pH of the endosomal compartment (a function normally controlled by the M2 gene product), a process which is essential to induce conformational changes in the HA protein to permit membrane fusion.

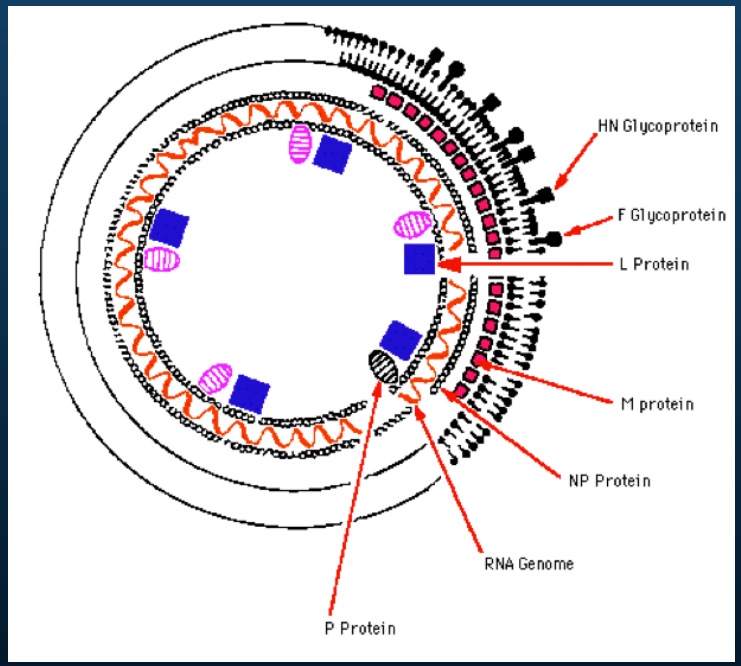


Paramyxoviruses

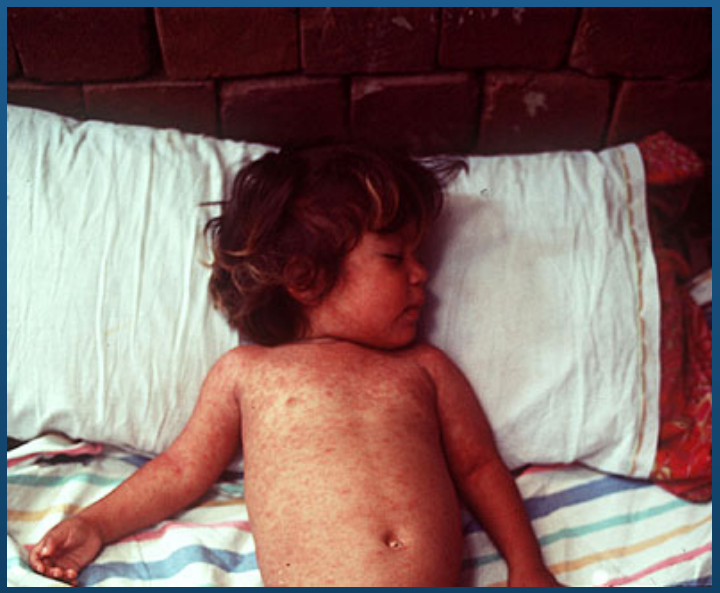
The family is divided into 3 genera:
Paramyxovirus: Parainfluenzavirus 1-4; Mumps
Pneumovirus: Respiratory Syncytial Virus (RSV)
Morbillivirus: Measles; Canine Distemper Virus.

Parainfluenzaviruses and RSV produce acute respiratory diseases (c.f. influenza), Morbilliviruses and Mumps systemic disease - diversity!
They also differ from orthomyxoviruses genetically - non-segmented genome with little genetic variation (c.f. influenza).

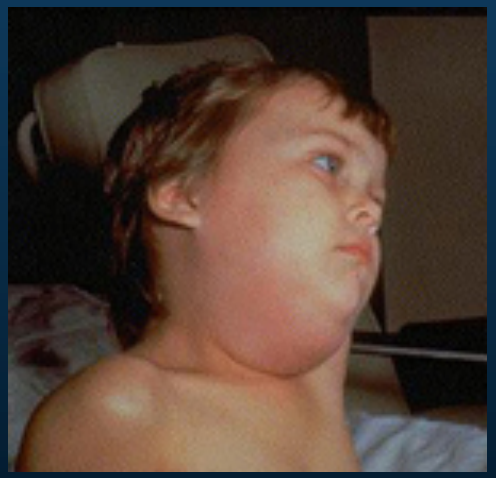




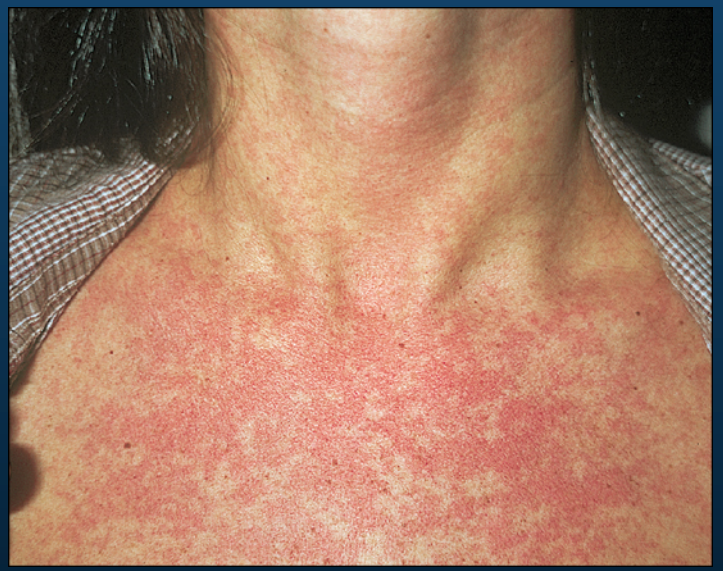
MMR



Measles



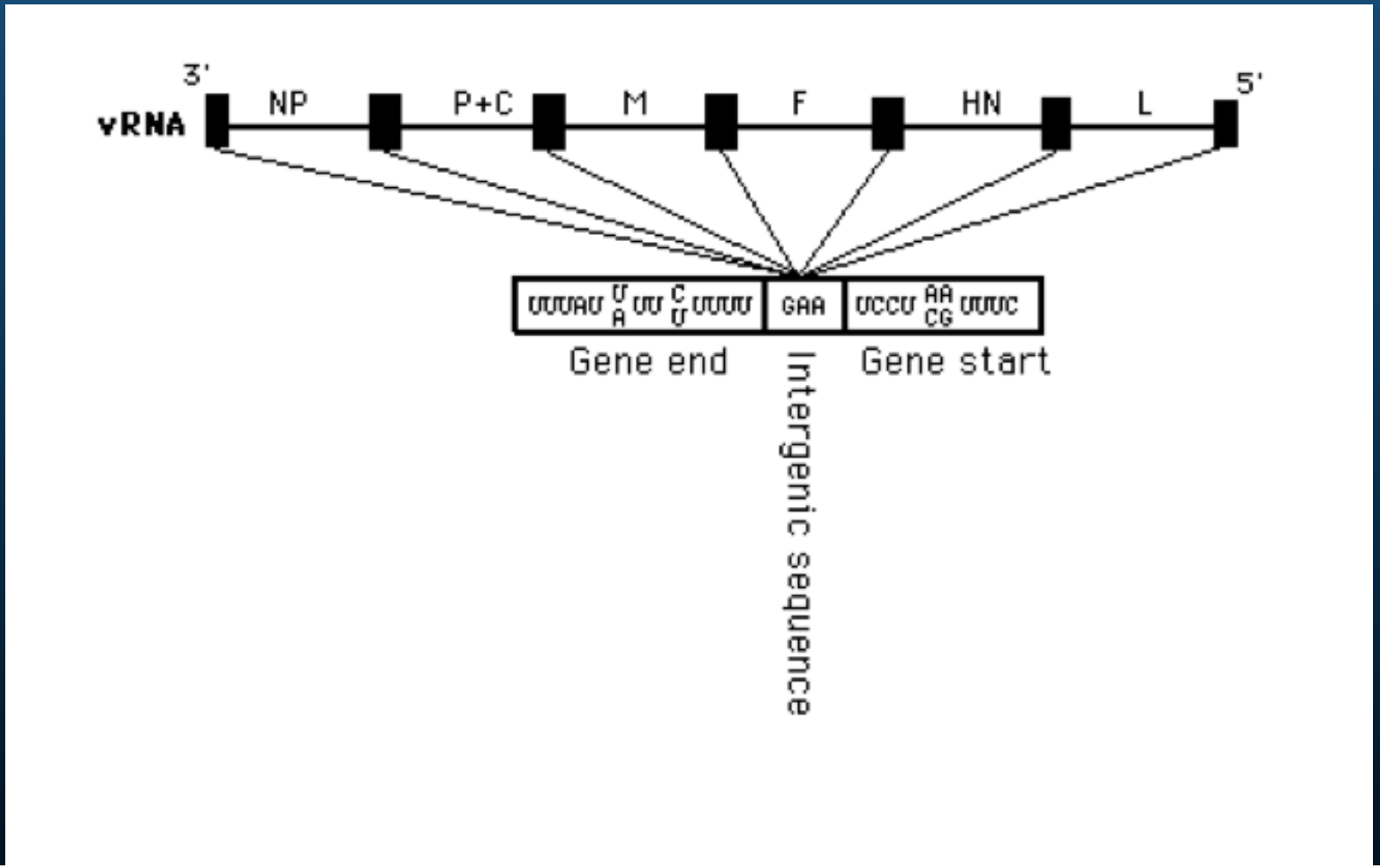
Mumps



Rubella

Paramyxoviruses Genome

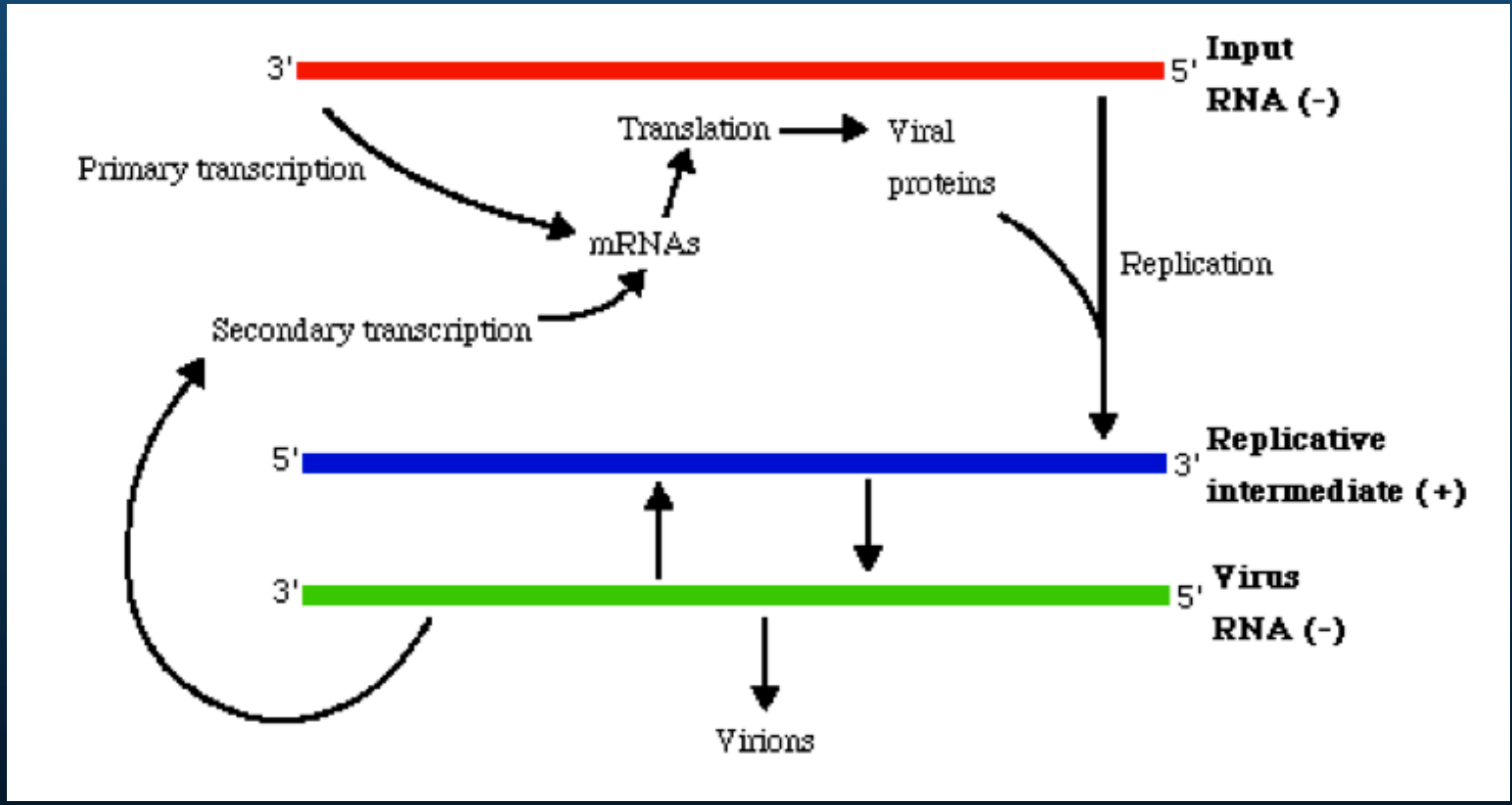
Non-segmented (-)sense RNA, 17 - 20 kb. The linear arrangement of genes (6) are separated by repeated sequences, a polyadenylation signal at the end of the gene, the intergenic sequence GAA followed by a translation start signal at the beginning of the next gene.



Replication

Very similar for all viruses in this group. Unlike influenza, all the action occurs in the cytoplasm. However, the overall strategy very similar to influenza.

A large excess of nucleocapsids are produced in infected cells, which form characteristic cytoplasmic inclusion bodies. Syncytium formation is quite common (F glycoprotein).



Retroviruses

1904: Ellerman and Bang, searching for an infectious cause (bacterium) for leukaemia, studied leukaemia in chickens and succeed in transferring the disease from one to another by cell-free tissue filtrates.

1911: Peyton Rous transmitted solid tumours of chickens by transplanting tissue, but also isolated the infectious agent (RSV).

1960's: Howard Temin knew that retrovirus genomes were composed of RNA and observed that replication was inhibited by actinomycin D (inhibits DNA synthesis therefore he proposed the concept of reverse transcription (Nobel prize awarded to Baltimore and Temin, 1975).

1969: Huebner and Todaro proposed the viral oncogene hypothesis - the transmission of viral and oncogenic information as genetic elements (rather than as a pathogenic response to a virus) - explains the vertical (germ line) transmission of 'cancers', first observed by Gross, 1951.

1981: Human T-cell leukaemia virus discovered, the first pathogenic human retrovirus.

1983: Human immunodeficiency virus discovered.

Genome

All retrovirus genomes consist of **two molecules of RNA**, which are s/s, (+)sense and have 5' cap and 3' poly-(A) (equivalent to mRNA). These vary in size from ~8-11kb. Retrovirus genomes have 4 unique features:

They are the only viruses which are truly diploid.

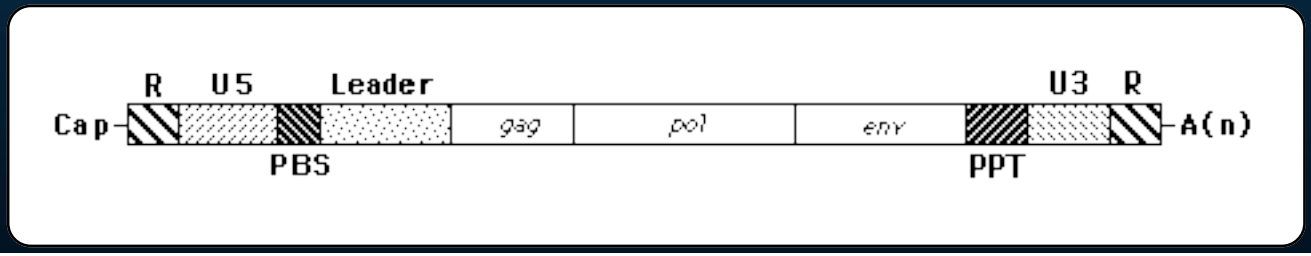
They are the only RNA viruses whose genome is produced by cellular transcriptional machinery (without any participation by a virus-encoded polymerase).

They are the only viruses whose genome requires a specific cellular RNA (tRNA) for replication.

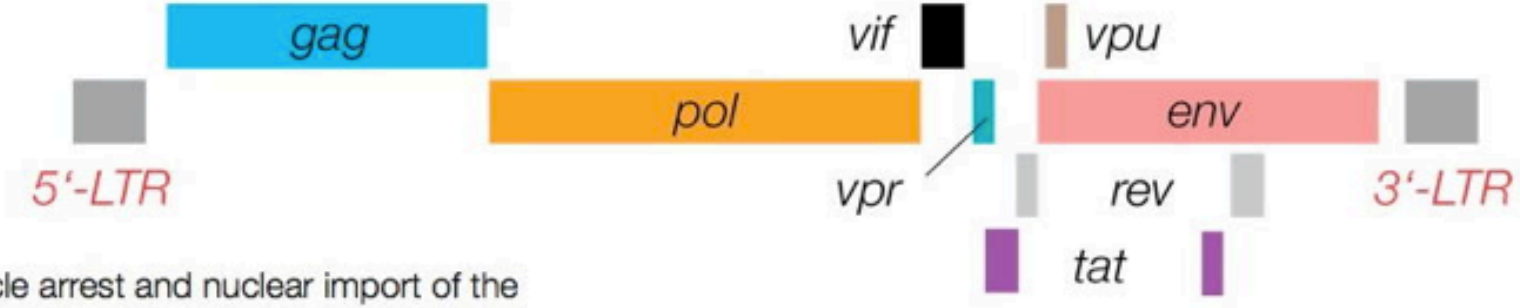
They are the only (+)sense RNA viruses whose genome does **not** serve directly as mRNA immediately after infection.

These two molecules are physically linked as a dimer by hydrogen bonds (co-sediment). In addition, there is a specific type of tRNA (usually trp, pro or lys) - required for replication.

Gene order in all retroviruses is invariant:



The HIV genome



vpr: G2 cell cycle arrest and nuclear import of the preintegration complex

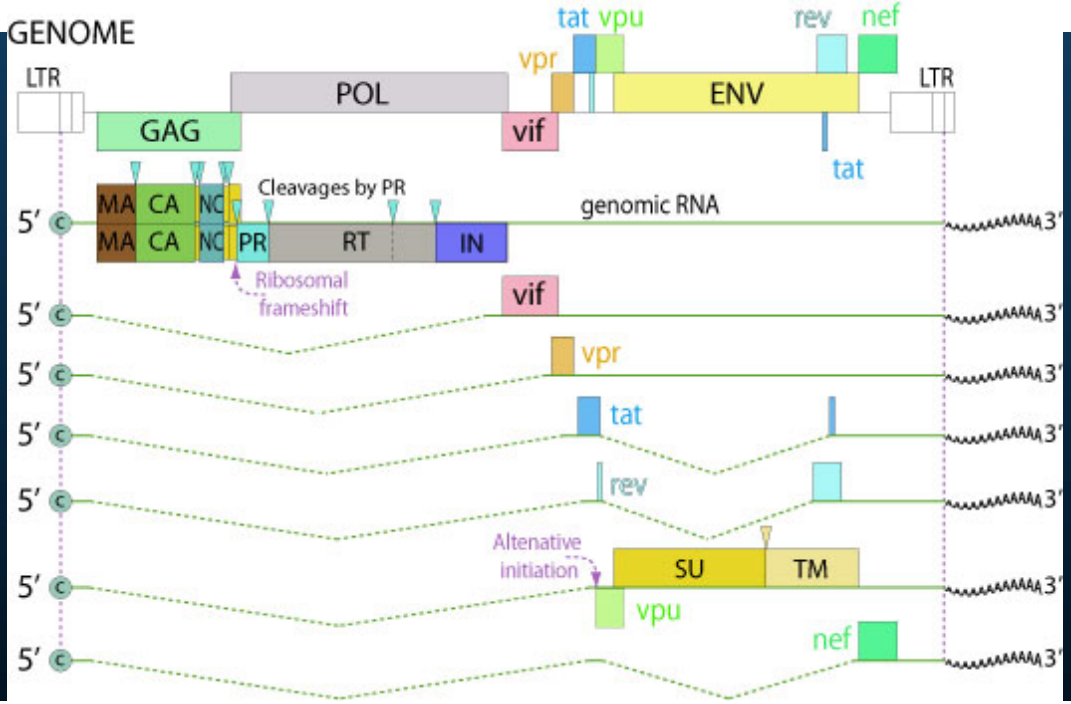
nef: down-regulation of cell surface CD4 and MHC class I

vif: virion infectivity factor

vpu: enhancement of virion release and CD4 degradation

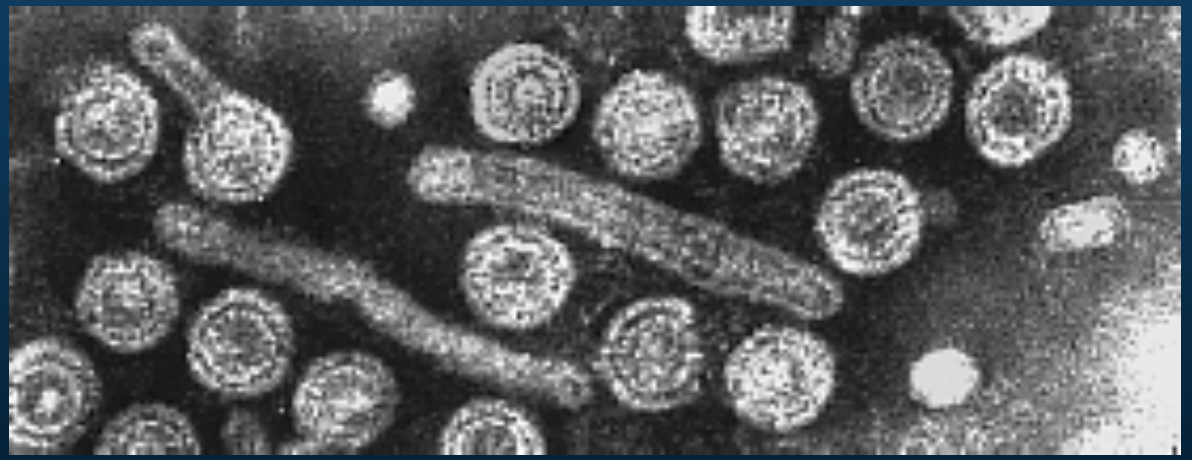
tat: trans-activator of HIV promoter

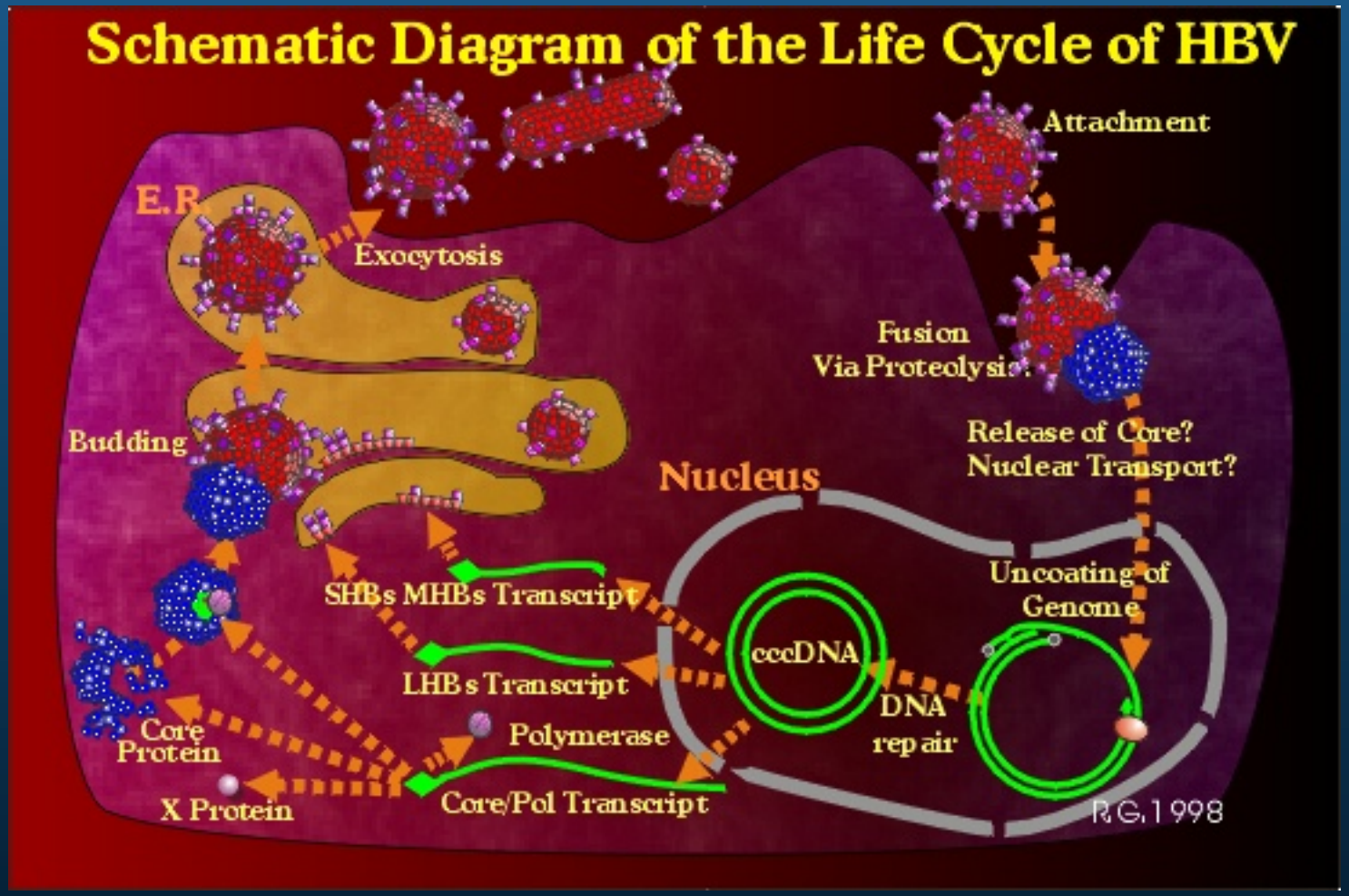
rev: nuclear export of late, unspliced RNA

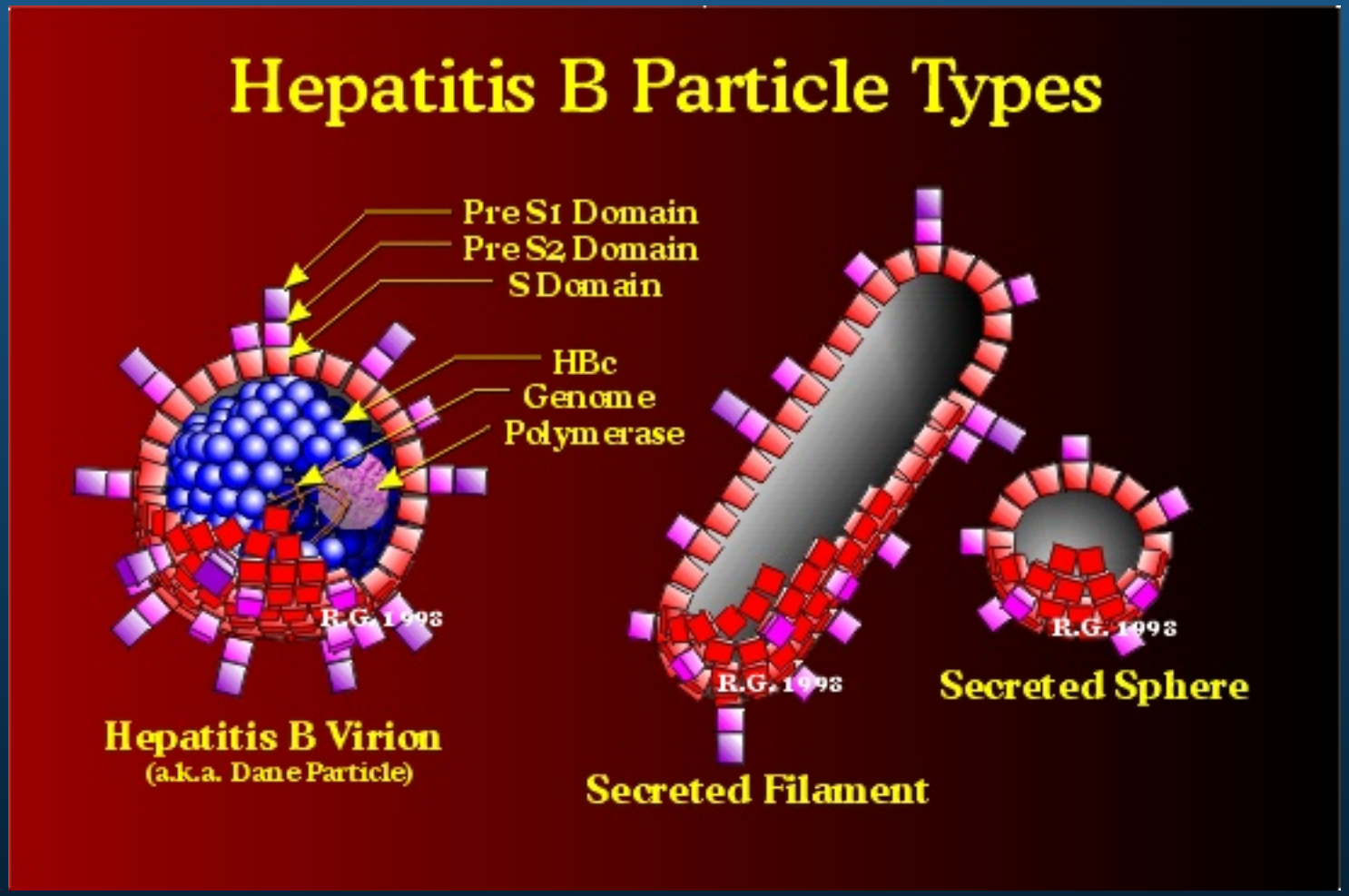


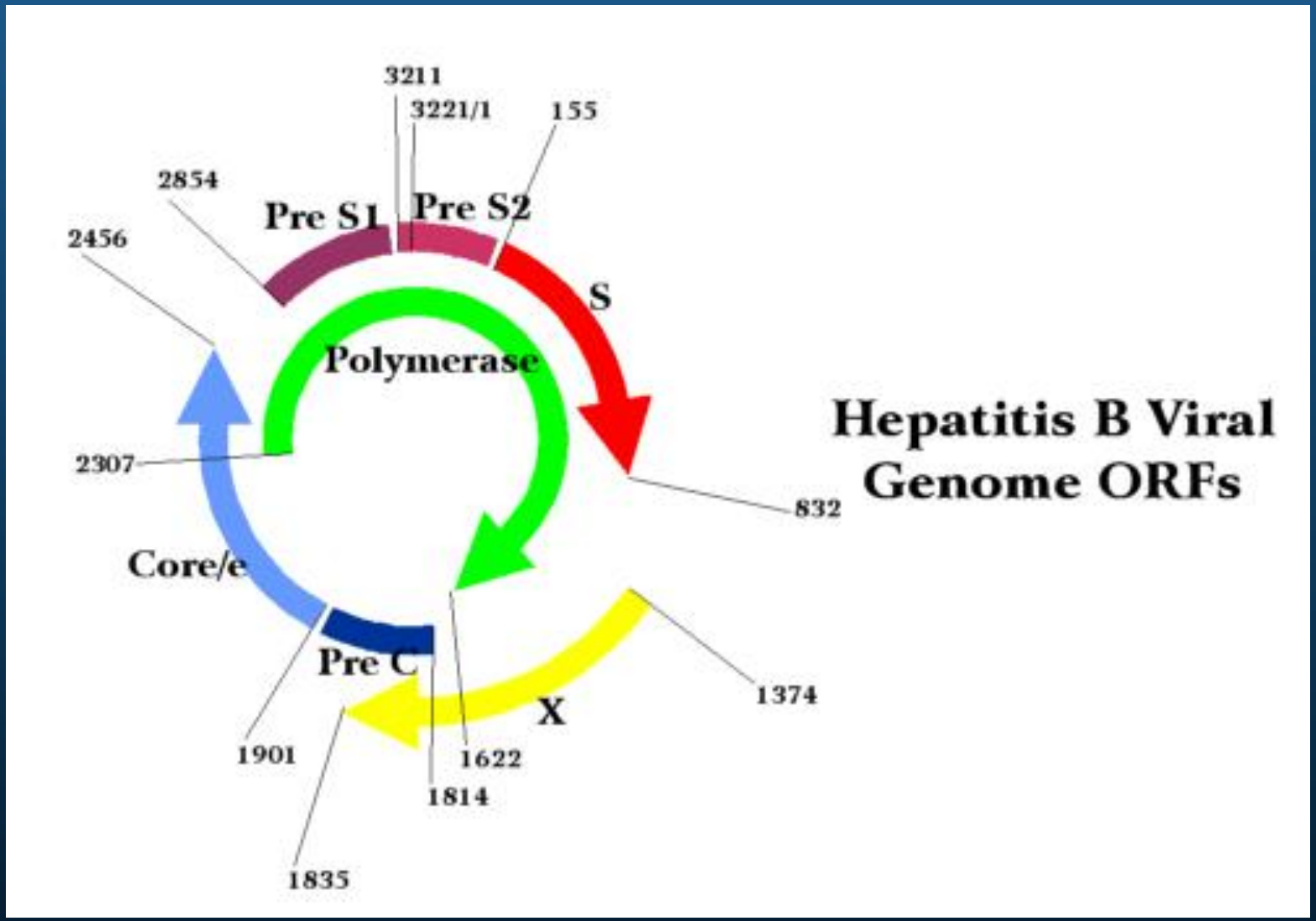
Hepatitis B Virus (HBV)

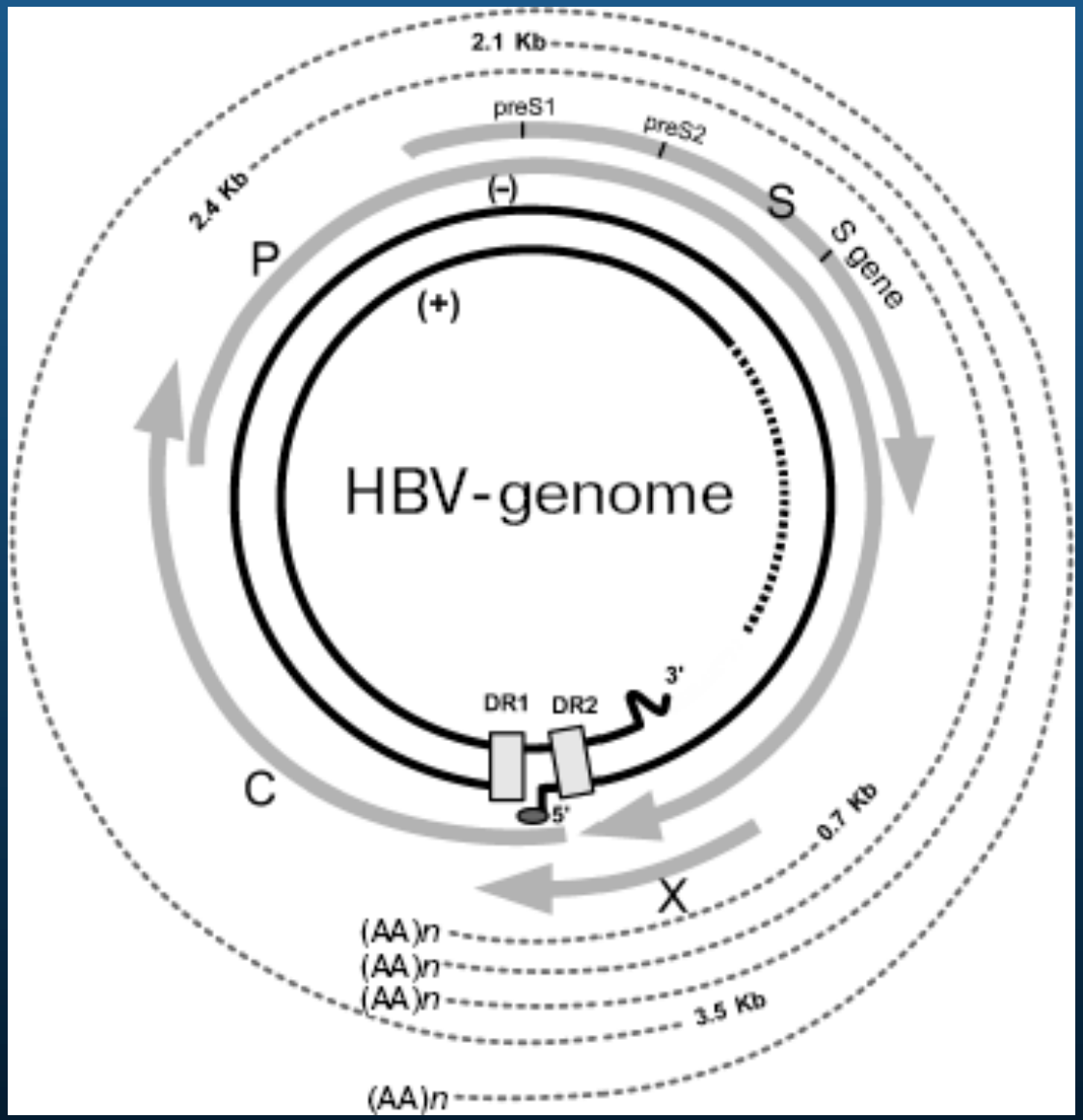
'Serum hepatitis' was distinguished clinically from 'infectious hepatitis' in the 1930's indicating that at least at least 2 different infectious agents were responsible for hepatitis. Infection often results from inoculation with human serum - blood transfusions, transplants or passive immunization (common among IVDAs). However, the virus is also transmitted sexually, by ingestion and from mother to child (transplacenta and breast milk) - accounting for familial clusters. All blood/organ/tissue donations in developed countries are now tested for HBV and risk of transmission is extremely low. Incubation period 45 - 120 days. HBV does not grow in tissue culture and this has hindered investigations.




Schematic Diagram of the Life Cycle of HBV
Attachment
E.R.
Exocytosis
Fusion
Via Proteolysis?
Budding
Release of Core?
Nuclear Transport?
Nucleus
Uncoating of
Genome
SHBs MHBs Transcript
cccDNA
LHBs Transcript
DNA
repair
Core
Protein
Polymerase
X Protein
Core/Pol Transcript
R.G. 1998


Hepatitis B Particle Types
Pre S1 Domain
Pre S2 Domain
S Domain
HBc
Genome
Polymerase
R.G. 1993
Hepatitis B Virion
(a.k.a. Dane Particle)
R.G. 1993
Secreted Filament
R.G. 1993
Secreted Sphere

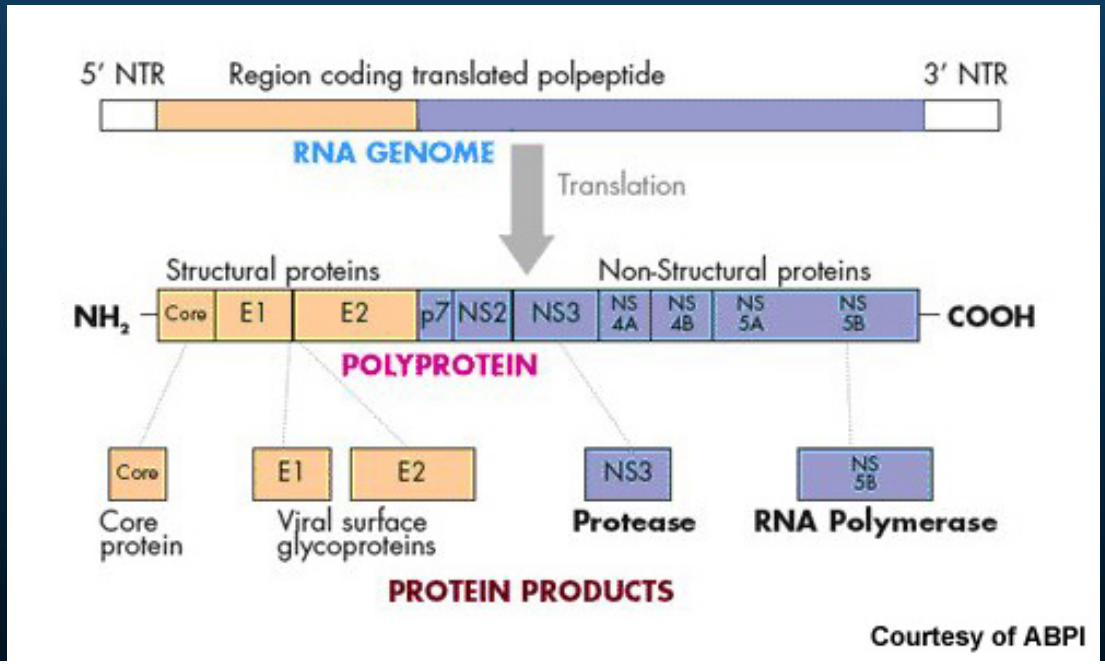

3211
3221/1
155
2854
Pre S1
Pre S2
2456
S
Polymerase
Hepatitis B Viral
Genome ORFs
2307
832
Core/e
Pre C
1374
X
1901
1622
1814
1835


2.1 Kb
preS1
preS2
2.4 Kb
(-)
S
S gene
P
(+)
HBV-genome
DR1
DR2
3'
5'
C
0.7 Kb
X
(AA)n
(AA)n
(AA)n
3.5 Kb
(AA)n

Hepatitis C Virus (HCV)

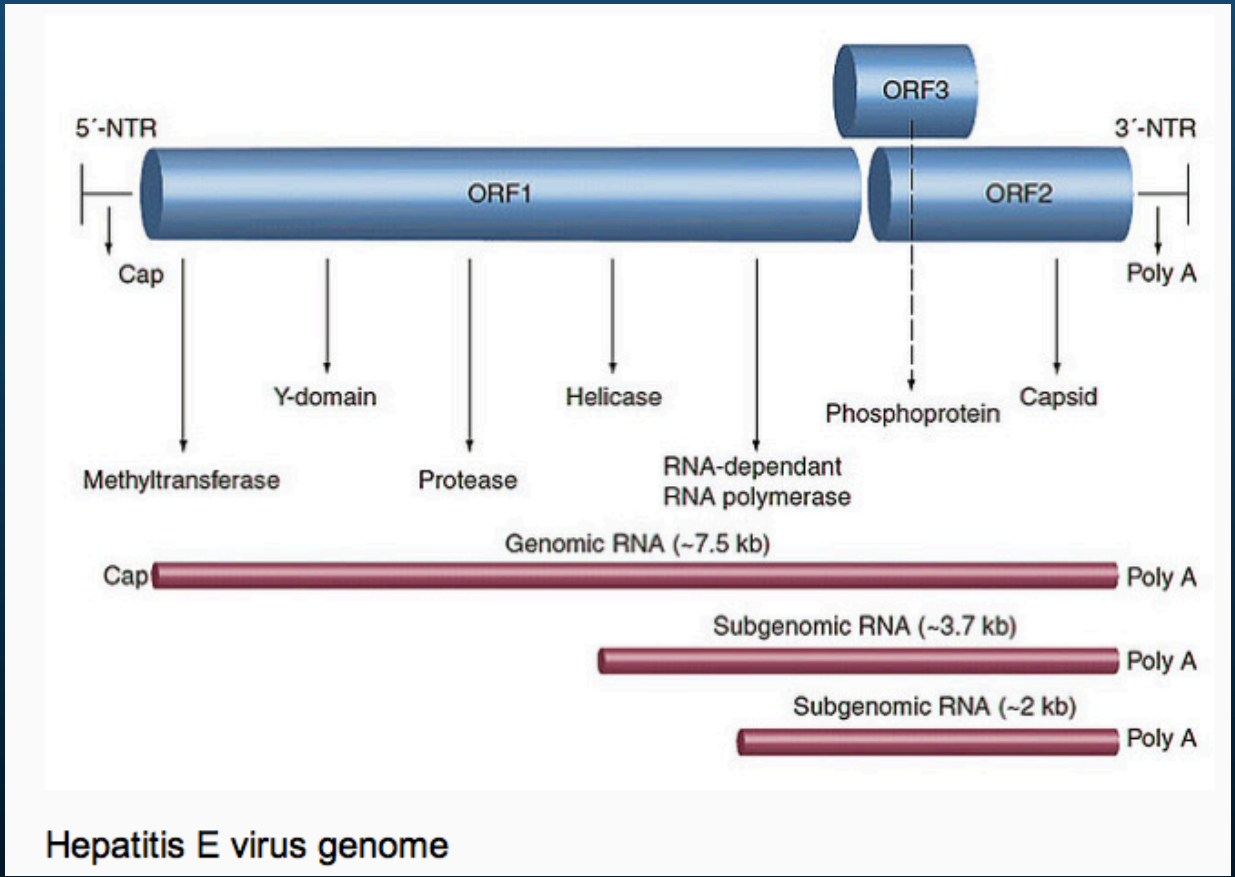
Originally one source of NANBH (~90%?), hepatitis C virus (HCV) was first definitively identified by molecular cloning of the virus genome in 1989 (Choo et al, *Science* 244: 359 - 362, 1989). The virus cannot be cultured in vitro & this has hampered investigations. The HCV genome consists of a positive-sense RNA molecule approximately 9.5 kb in length:

In terms of genome organization, HCV is a member of the Flaviviridae and has now been placed in a new monotypic genus in this family. The genome contains a single long open reading frame which encodes a polyprotein of about 3,000 amino acids. There is a non-coding region (NCR) of 324 - 341 nucleotides at the 5' end & a 3' NCR of variable length including a poly(U) tract. The 5' NCR contains an IRES apparently similar in function (but not structure) to that of picornaviruses.



Hepatitis E Virus (HEV)

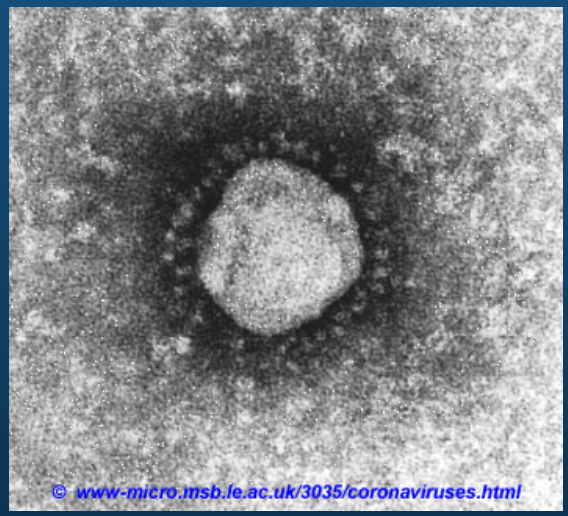
HEV has now been cloned and sequenced. The virion is a 30 - 32 nm non-enveloped particle containing a s/s (+)sense RNA genome of ~7.5 Kb. Genetic organization similar (not identical) to Caliciviruses:



Hepatitis E virus genome

SARS - Severe Acute Respiratory Syndrome virus

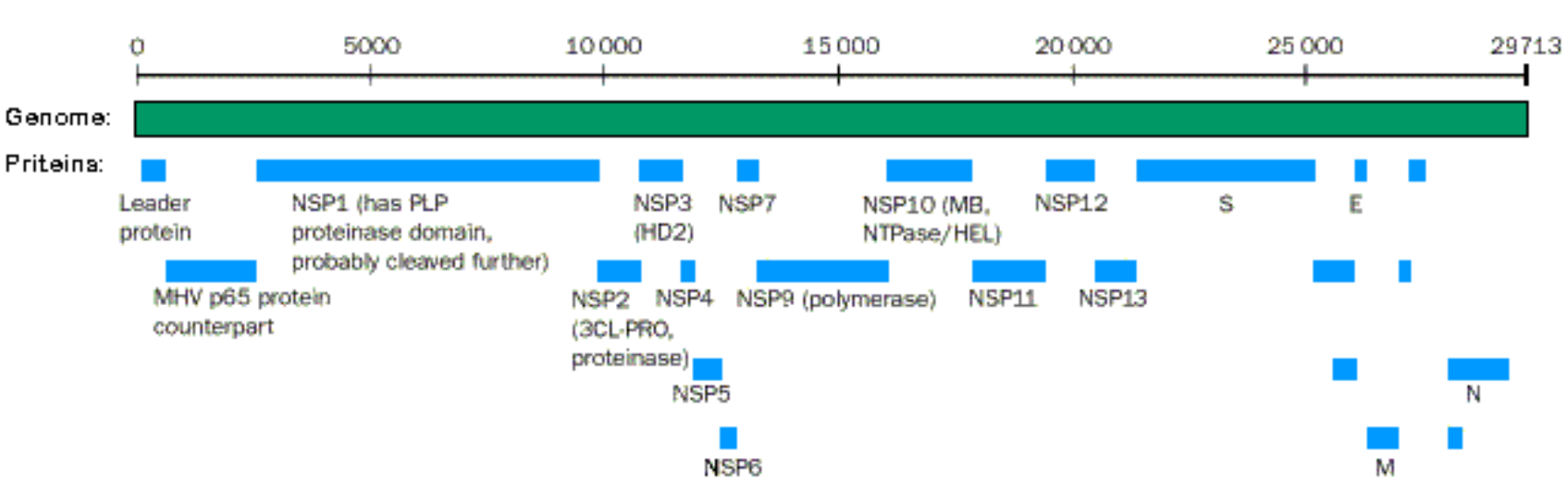
SARS - viral pneumonia, with symptoms including fever, a dry cough, dyspnea (shortness of breath), headache, and hypoxaemia (low blood oxygen concentration). Typical laboratory findings include lymphopaenia (reduced lymphocyte numbers) and mildly elevated aminotransferase levels (indicating liver damage).



Death may result from progressive respiratory failure due to alveolar damage. The typical clinical course of SARS involves an improvement in symptoms during the first week of infection, followed by a worsening during the second week.

Studies indicate that this worsening may be related to patient's immune responses rather than uncontrolled viral replication

SARS Genome Structure



SARS

The outbreak is believed to have originated in February 2003 in the Guangdong province of China, where 300 people became ill, and at least five died. After initial reports that a paramyxovirus was responsible, the true cause appears to be a novel coronavirus with some unusual properties.

For one thing, the SARS virus can be grown in Vero cells (a fibroblast cell line isolated in 1962 from a primate) - a novel property for HCoV's, most of which cannot be cultivated.

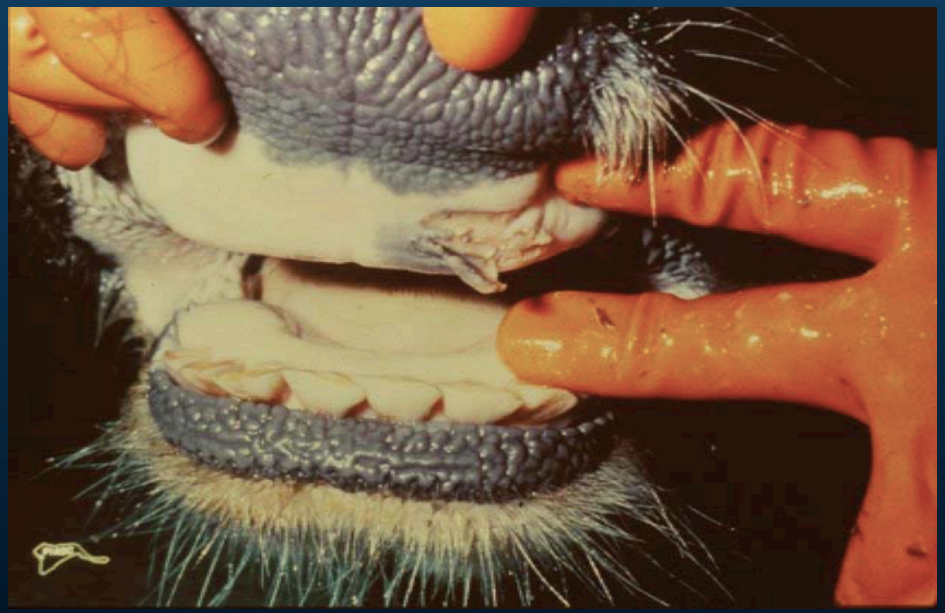
In these cells, virus infection results in a cytopathic effect, and budding of coronavirus-like particles from the endoplasmic reticulum within infected cells.

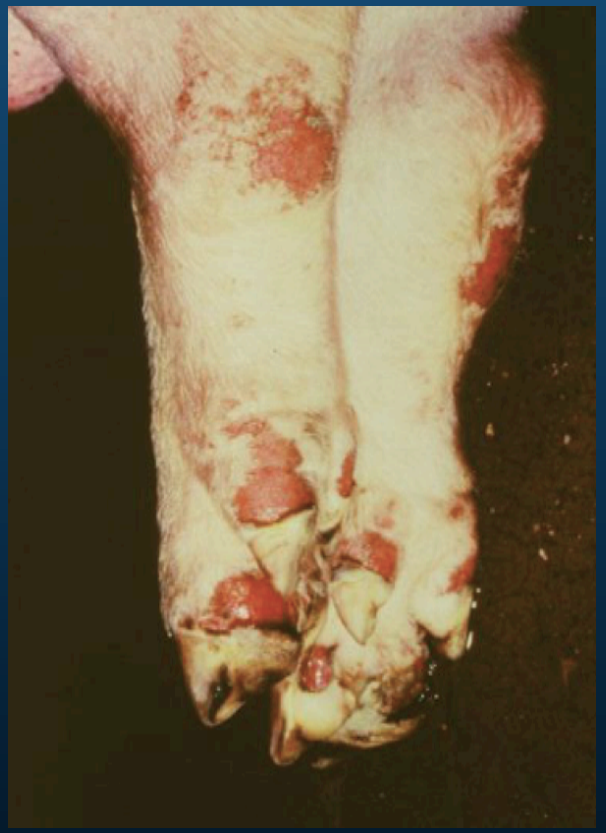
Where did the SARS virus come from?

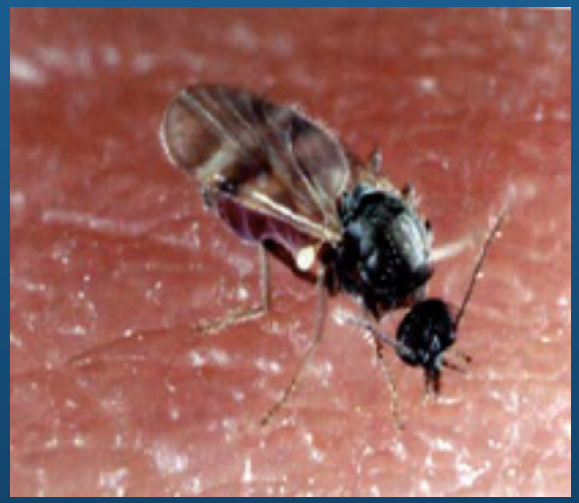
Coronaviruses with 99% sequence similarity to the surface spike protein of human SARS isolates have been isolated in Guangdong, China, from apparently healthy masked palm civets (*Paguma larvata*), a cat-like mammal closely related to the mongoose. The unlucky palm civet is regarded as a delicacy in Guangdong and it is believed that humans became infected as they raised and slaughtered the animals rather than by consumption of infected meat.

2001 Foot & Mouth Outbreak

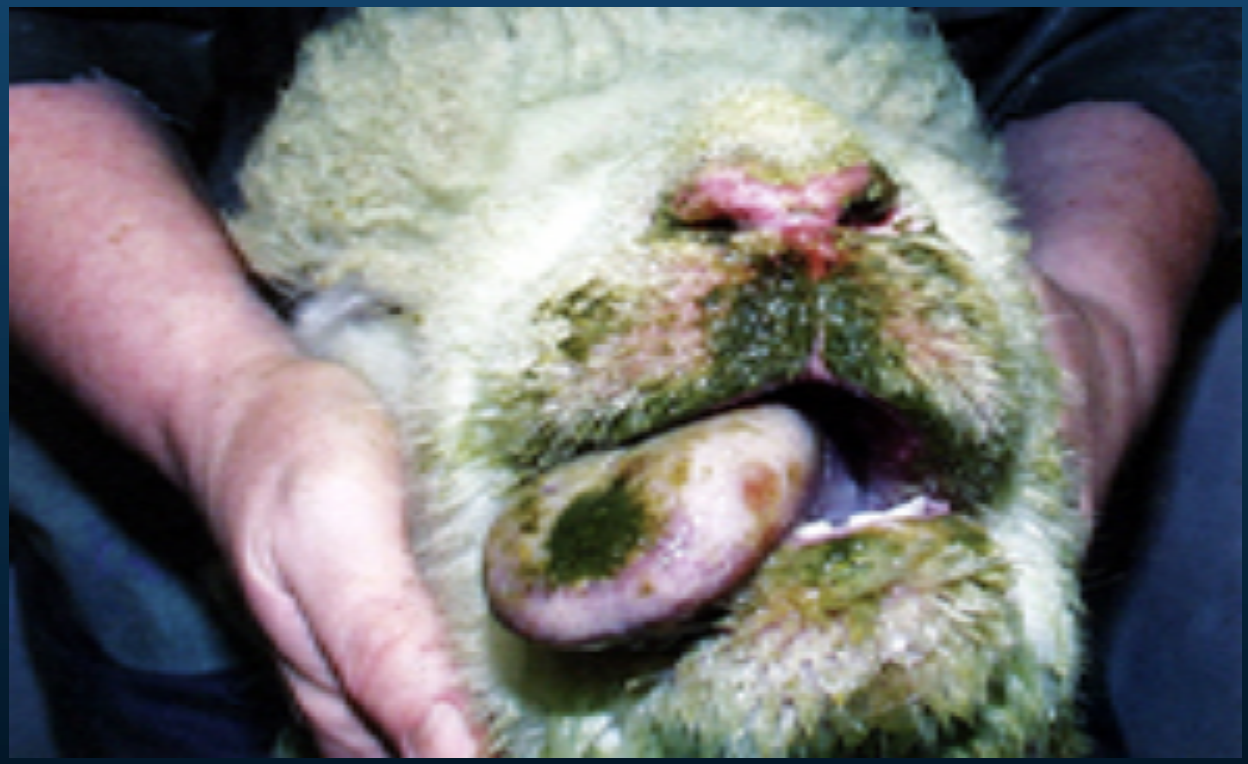
2007







Bluetongue Virus



Journal Club Paper

A Hybrid Vector for Ligand-Directed Tumor Targeting and Molecular Imaging

Hajitou *et al.* (2006) *Cell*, **125**, 385 - 398