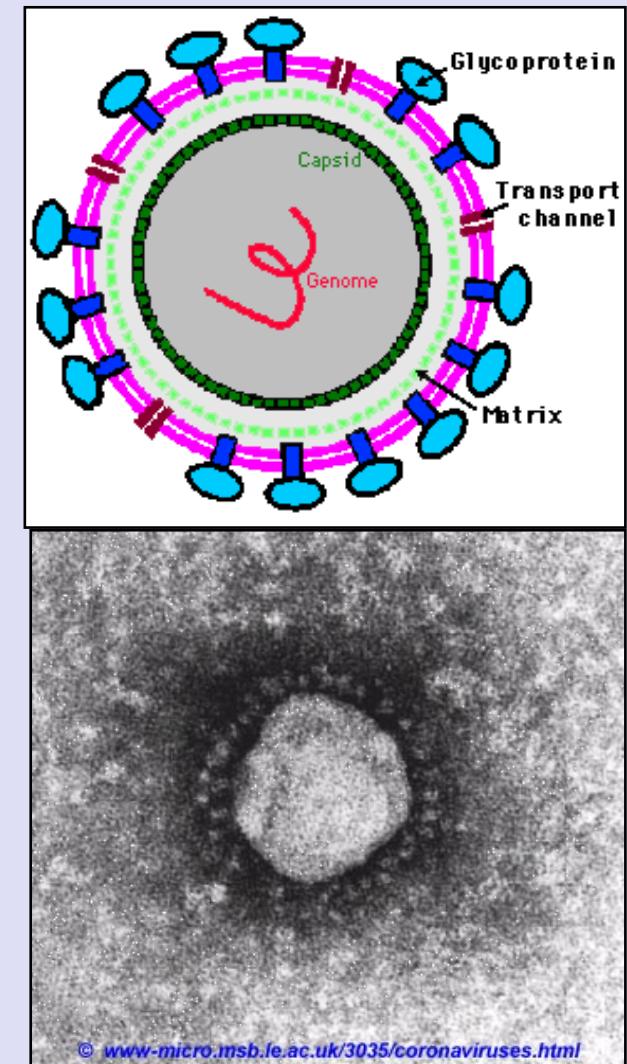
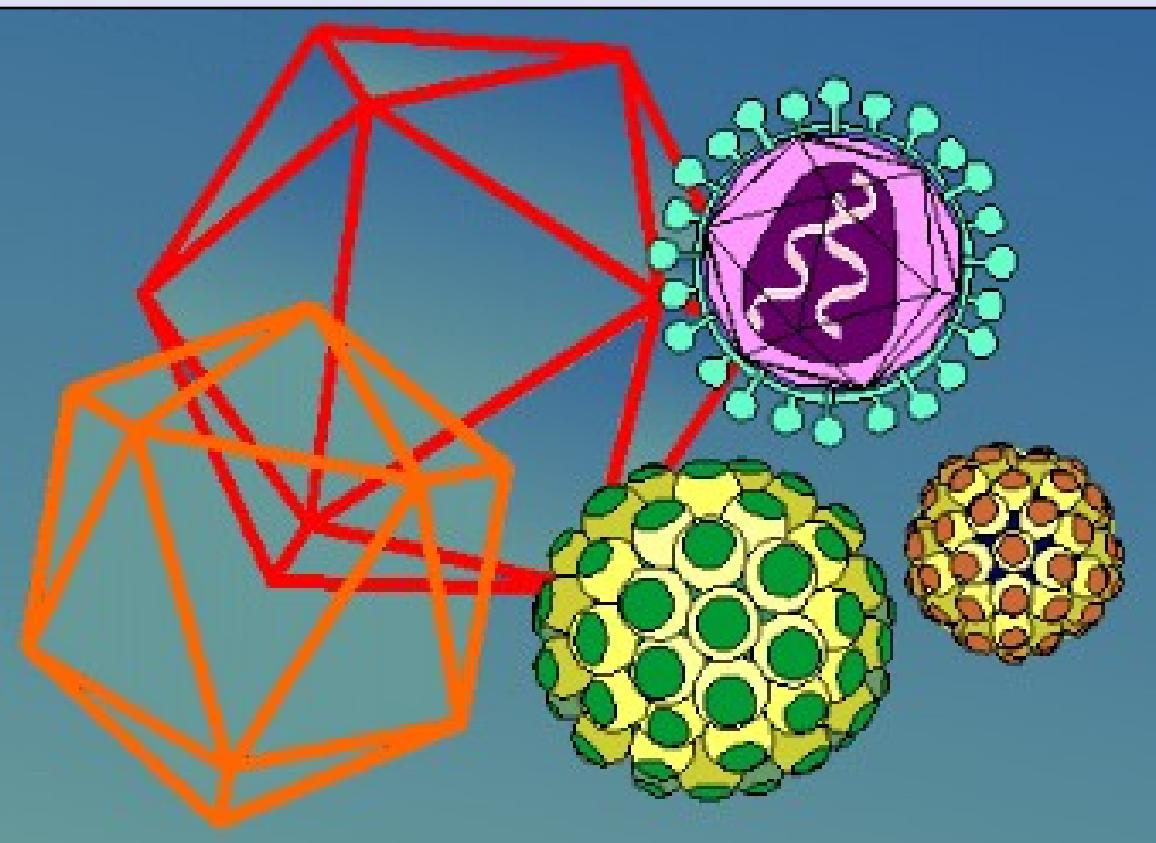


Scientific Practice IIA: An introduction to the world of viruses

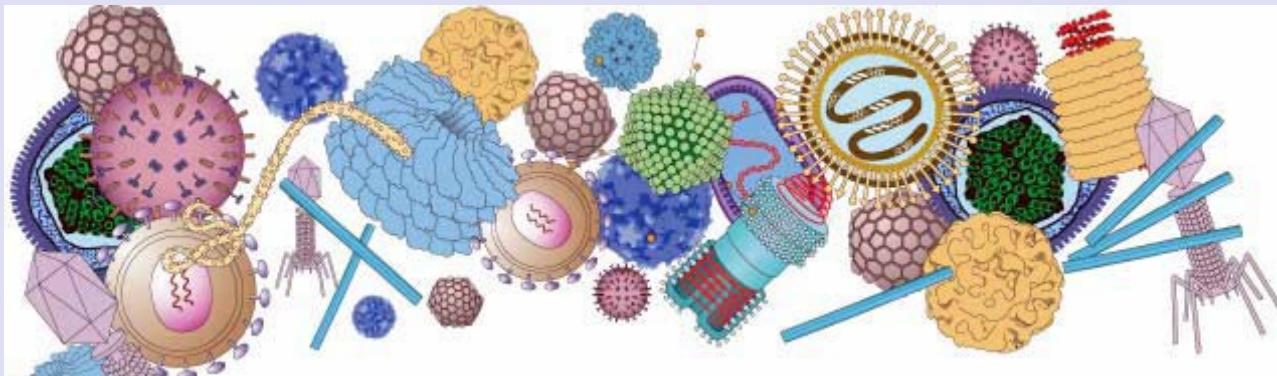
Professor M E C Rey
School of Molecular and Cell Biology



OUTLINE OF COURSE

- ***Introduction to Viruses***
- Structure of Viruses
- Virus Taxonomy and Classification
- Working with viruses in the laboratory
- Life cycles of viruses; replication and transcription
- ***Bacteriophages***
- ***Viruses and Diseases***
- ***Emerging viruses***
- Influenza
- Human Immunodeficiency virus (HIV)
- Zika virus
- Bat viruses

STRUCTURE AND TAXONOMY OF VIRUSES



Virus Sizes

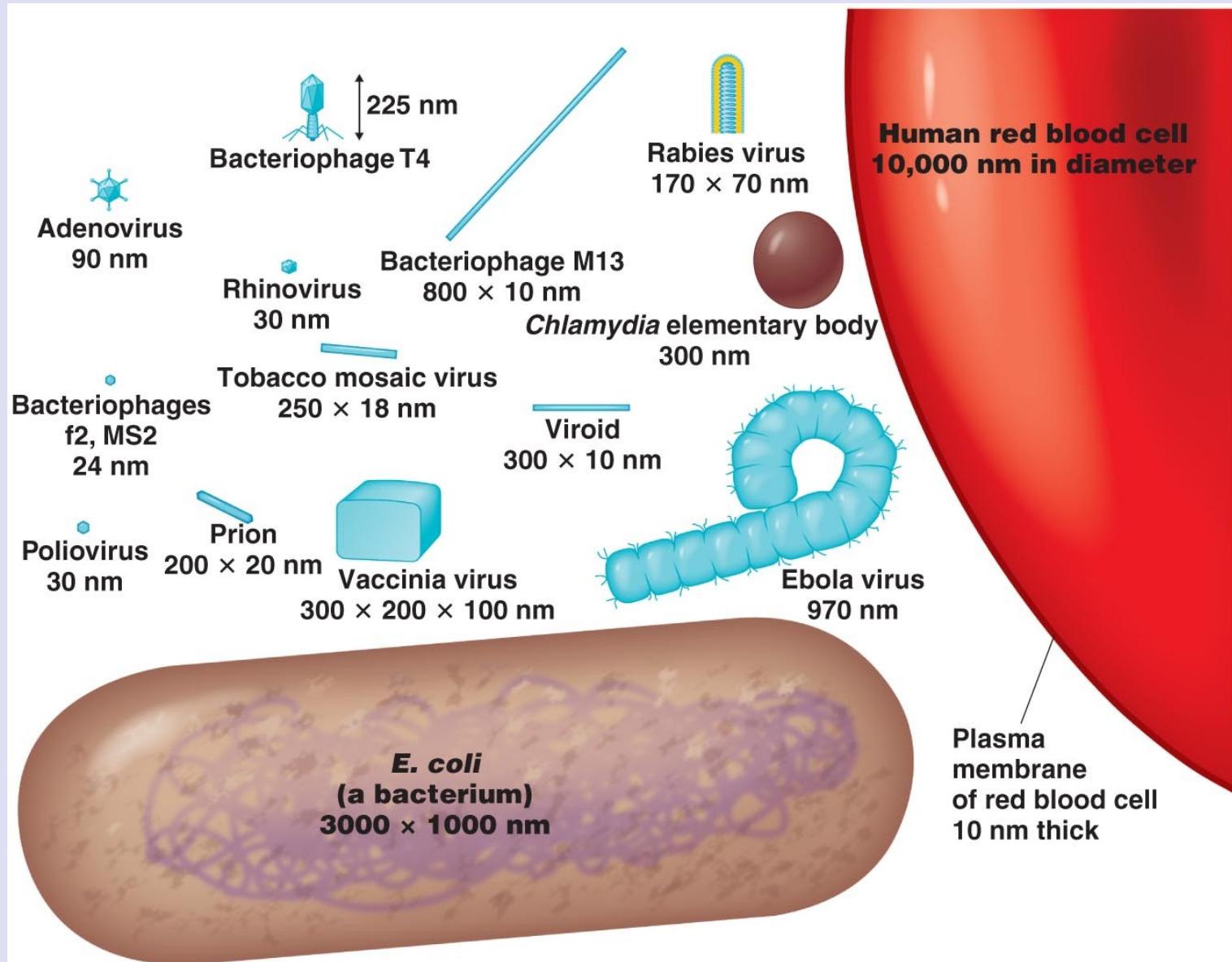


Figure 13.1

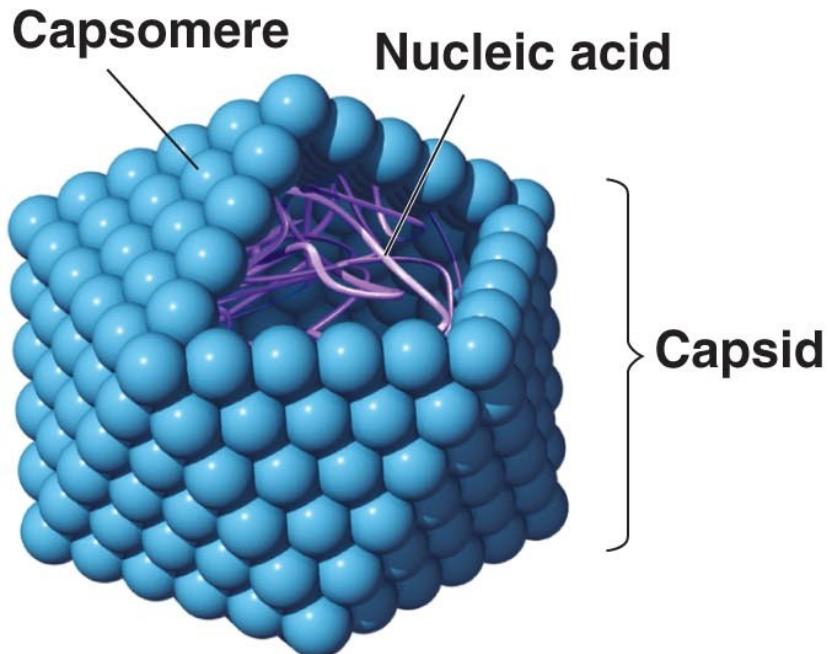
General Characteristics of Viruses

- **Obligatory intracellular parasites**
- Contain DNA or RNA
- Surrounded by a protein coat (capsid)
- Some are enclosed by an envelope
- Some viruses have spikes sticking out from envelope
- Most (not all) viruses infect only specific types of cells in one host
- **Host range** is determined by specific host attachment sites and cellular factors

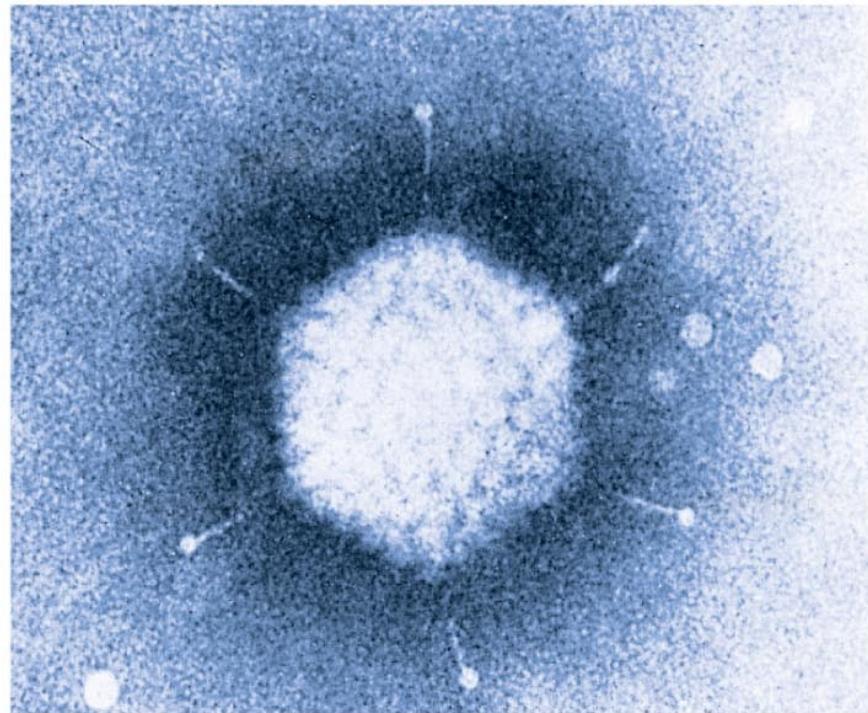
STRUCTURE AND SHAPES

- Two main classes of virus structures: *helical* and *icosahedral* (isometric) symmetry
- To form infectious particles viruses must assemble using information from the components and form regular geometric shapes even though capsid proteins are irregular in shape
- How? By using the rules of symmetry.
- Virus capsids are made up of multiple protein subunits or *capsomer* subunits which assemble through electrostatic and hydrophobic interactions
- Subunits can be made up of several smaller identical or non-identical polypeptides
- Subunits are arranged helically around the helical nucleic acid in *helical* viruses
- In *isometric* viruses the subunits are arranged around the vertices or faces of a cubic symmetrical structure such as an icosahedron which is made up of 20 equilateral triangles

Morphology of a Polyhedral (icosahedral) Virus



(a) A polyhedral virus



(b) *Mastadenovirus*

TEM
30 nm

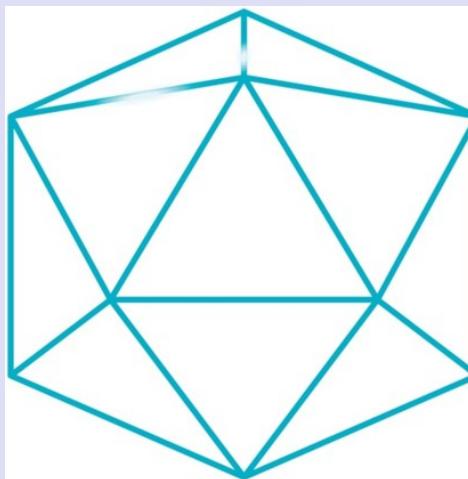


Figure 13.2

Icosahedron

- A triangular face can only have 3 protein subunits in symmetry. The simplest isometric virus is the icosahedron: has 20 triangles with 3 subunits/triangle = 60 subunits (fig. 3.3)
- Icosahedron has 5 triangles on top and 5 at the bottom and ten in the middle (fig. 3.4)
- Icosahedron has 12 vertices and three axes of symmetry: fivefold, threefold and twofold. Axes of symmetry meet in the middle of the icosahedron (fig. 3.5).
- Subunits can vary in size depending on size of virus
- Larger viruses are domes or spheres with their surfaces divided into triangular facets

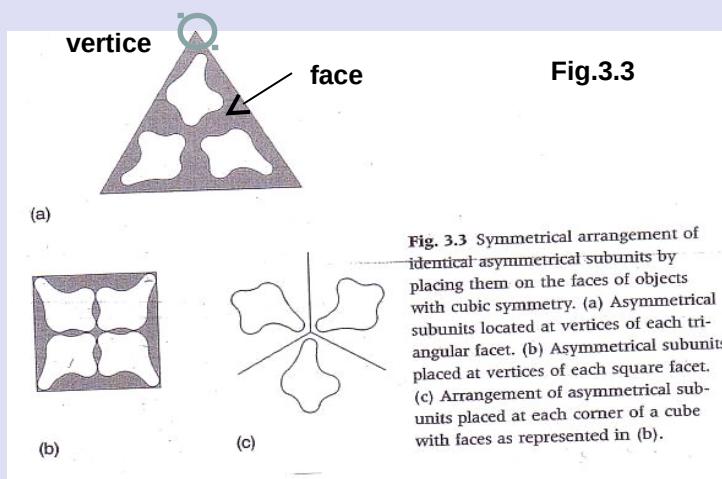
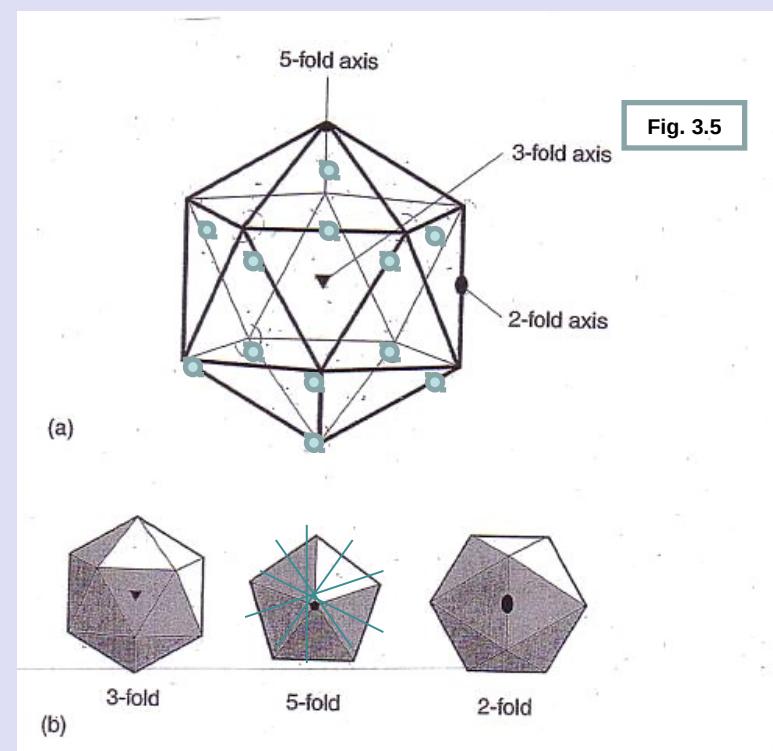


Fig. 3.4



What happens when a 60 SU icosahedron needs to increase its size to fit larger NA?

- We need to arrange more identical subunits so how do we do this?
- Subdivide the surface of a sphere into triangular facets which are arranged with icosahedral symmetry (which gives a minimum-energy structure); larger the virus NA the larger the circle, more triangles can be added or the subunit proteins are larger
- Each triangular face is divided into 4 smaller but identical, equilateral triangular facets (Fig. 3.7b), giving 240 subunits ($n=4$; 60×4)
- Note that at the vertices of the original icosahedron there are rings of 5 subunits called *pentamers*, but at the other vertices there are 6 subunits called *hexamers*
- The original icosahedron face can also be divided into 3 equilateral triangles and each new triangular facet can be further triangulated following laws of geometry (*Triangulation number T = Pf²* where f= the number of smaller identical equilateral triangles (1,2,3,4 etc. and P=1, 3 or 7)
- The smallest virus is T=1 (P, f are 1) with 60T (60 X1) subunits. T=3 means P=3 and f=1 which has $3 \times 60 = 180$ subunits (fig. 3.8)

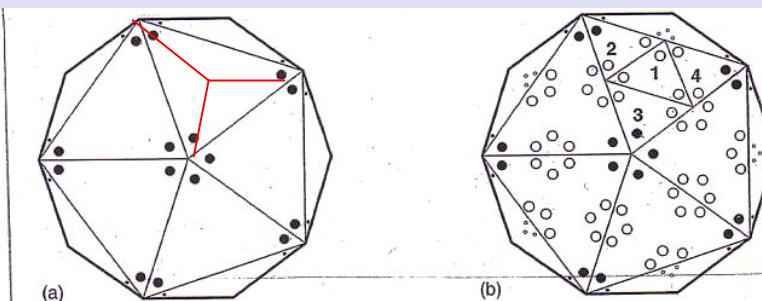


Fig. 3.7 Arrangement of $60n$ identical subunits on the surface of an icosahedron. (a) $n = 1$ and the 60 subunits are distributed such that there is one subunit at the vertices of each triangular face. Note that each subunit has the same arrangement of neighbours and so all the subunits are equivalently related. (b) $n = 4$. Each triangular face is divided into four smaller, but identical, equilateral triangular facets and a subunit is again located at each vertex. In total, there are 240 subunits. Note that, in contrast to the arrangements shown in Fig. 3.5, each subunit, whether represented by an open or closed circle, has the identical arrangement of neighbours—see the face in which triangles 1–4 have been drawn. However, as some subunits are arranged in pentamers and others in hexamers, the members of each set are only ‘quasi-equivalently’ related.

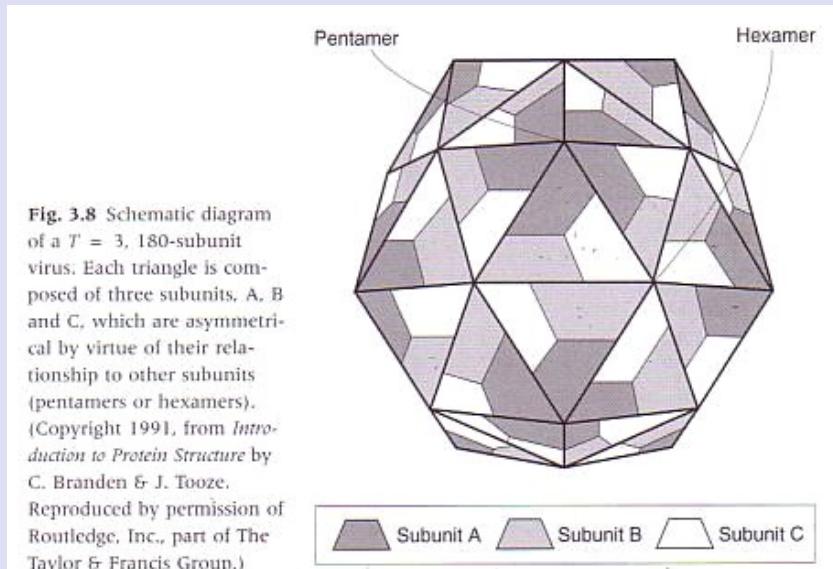


Fig. 3.8 Schematic diagram of a $T = 3$, 180-subunit virus. Each triangle is composed of three subunits, A, B and C, which are asymmetrical by virtue of their relationship to other subunits (pentamers or hexamers). (Copyright 1991, from *Introduction to Protein Structure* by C. Branden & J. Tooze. Reproduced by permission of Routledge, Inc., part of The Taylor & Francis Group.)

Morphology of an Enveloped Virus

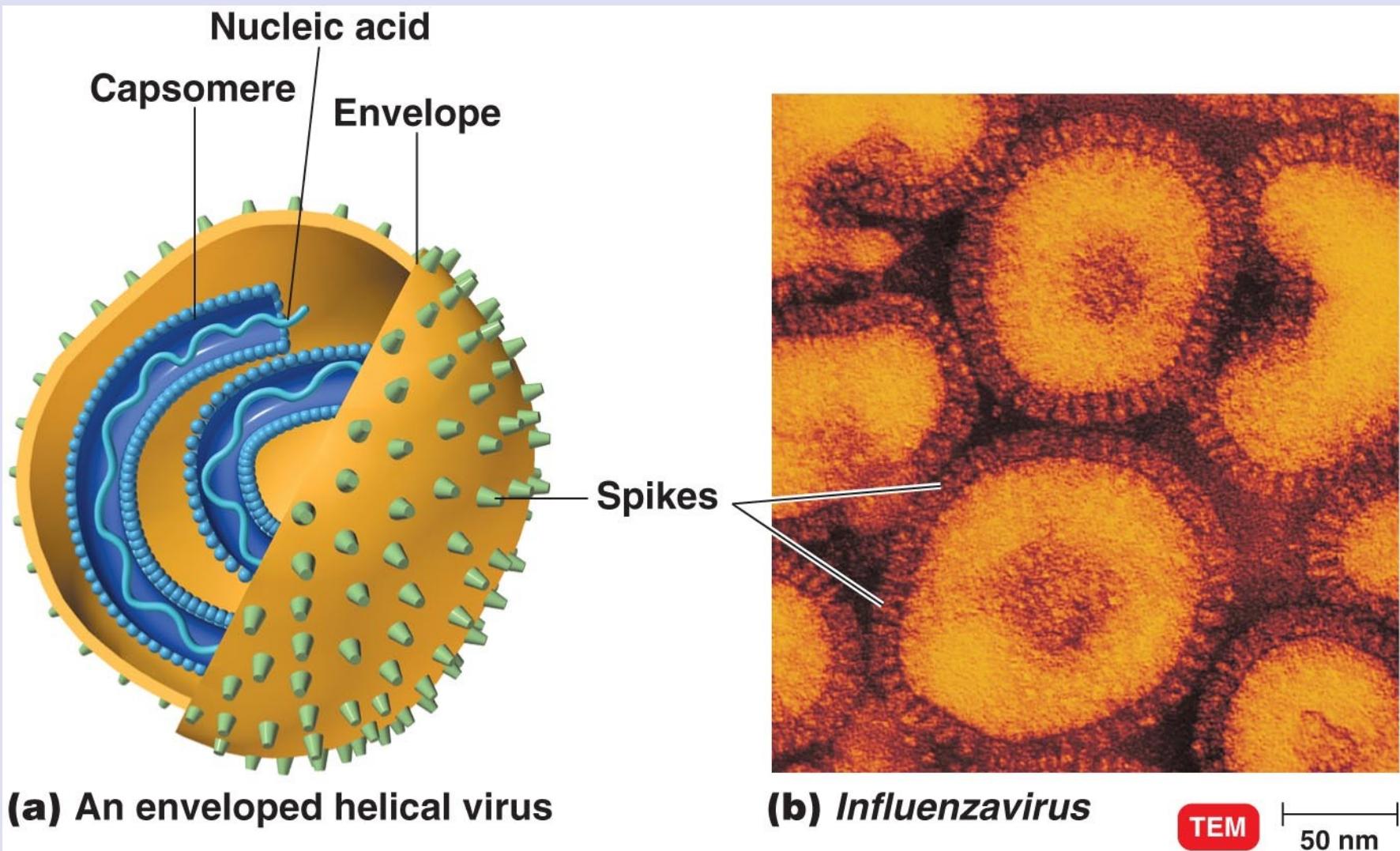
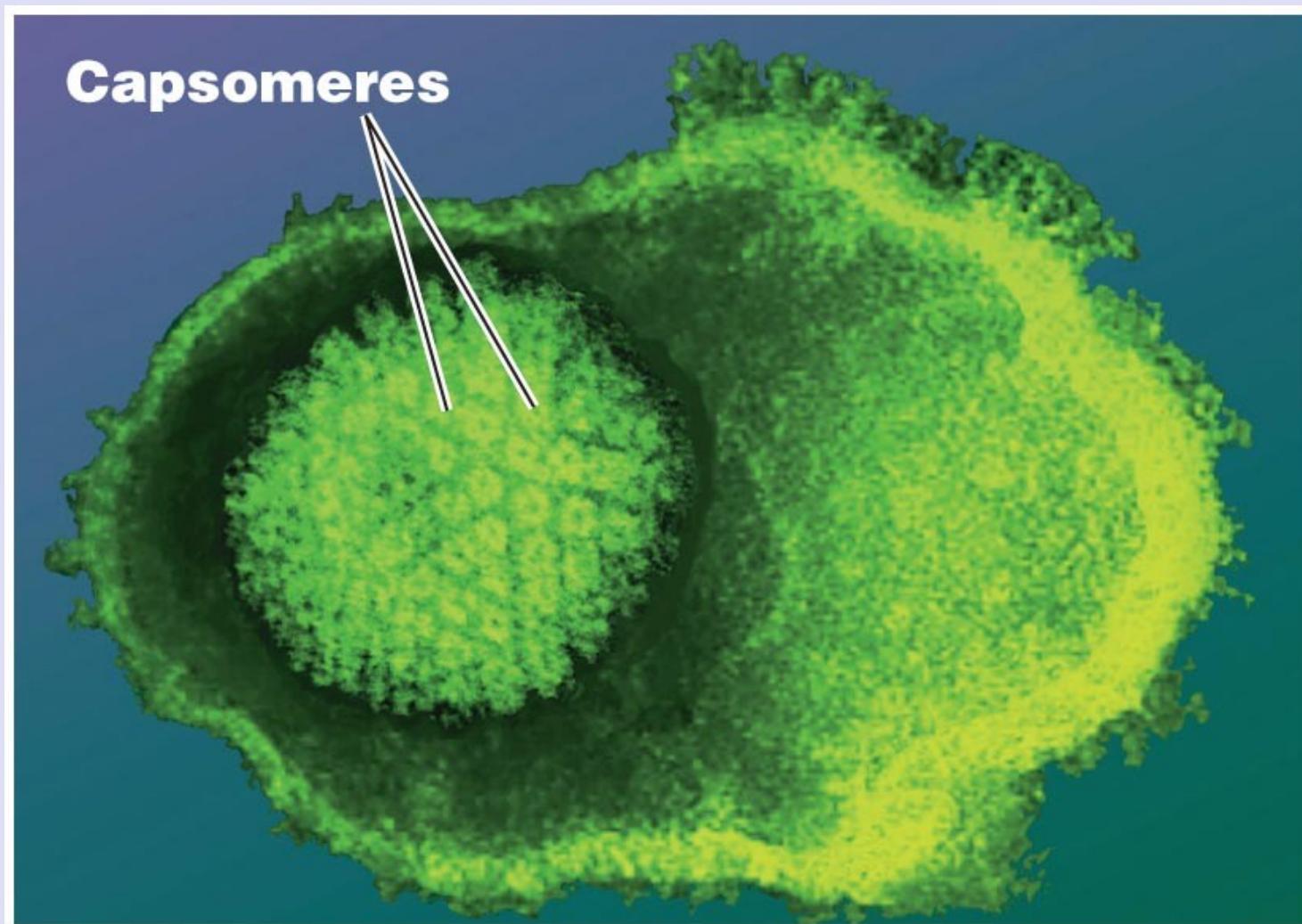


Figure 13.3

Enveloped Viruses



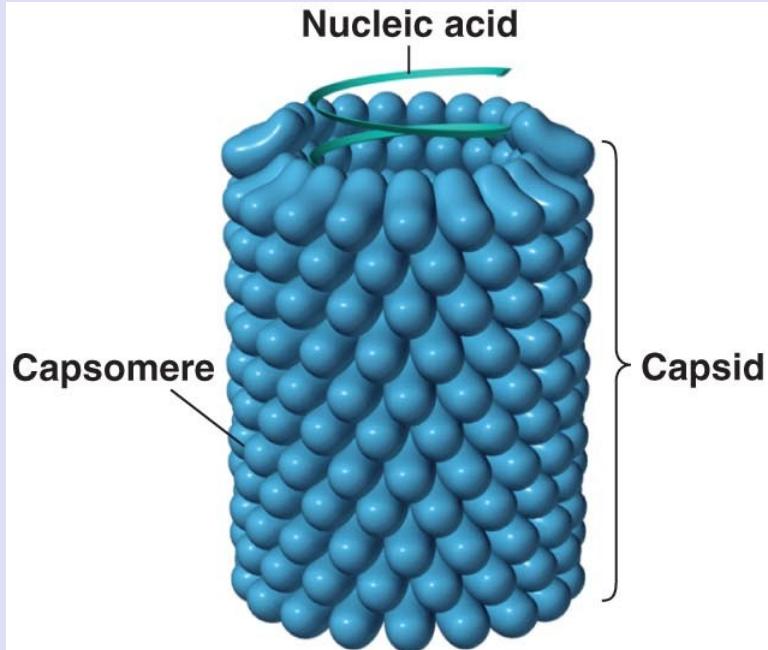
(b) *Herpesvirus*

TEM

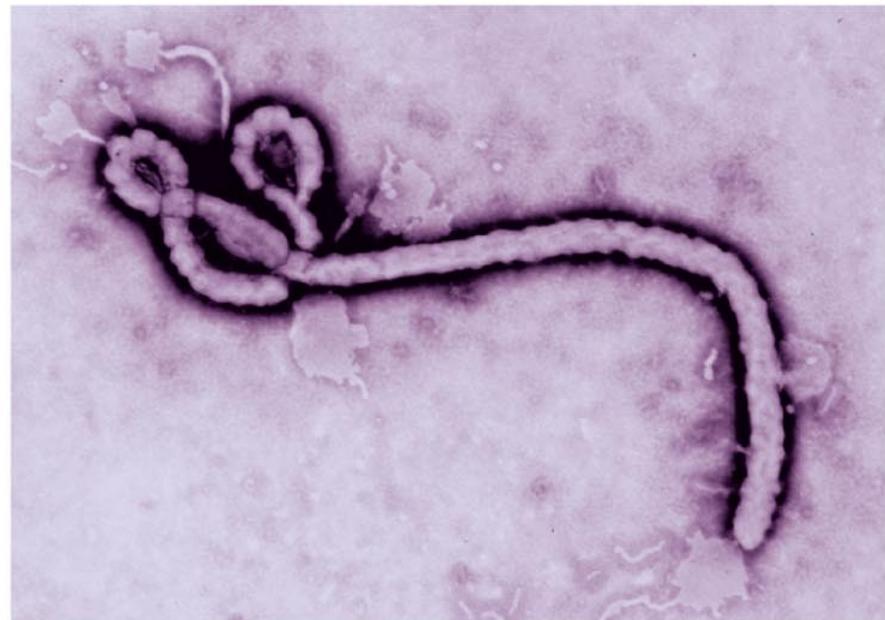
80 nm

Figure 13.16b

Morphology of a Helical Virus

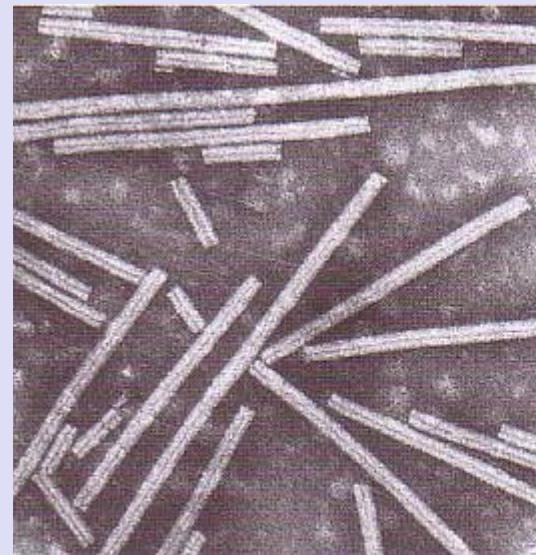
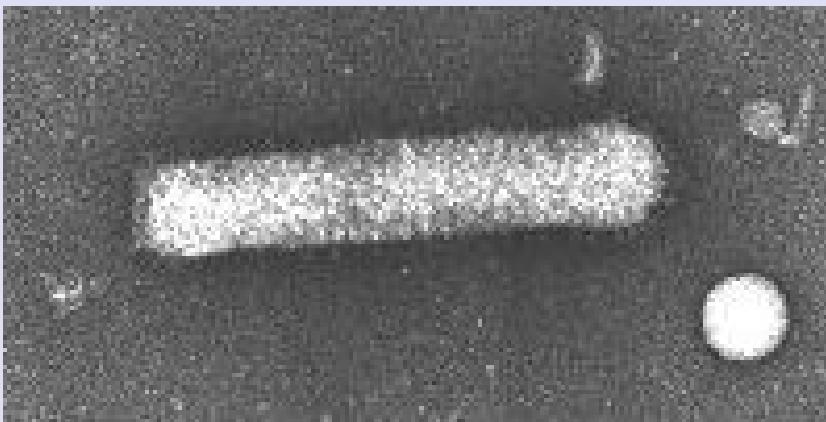


(a) A helical virus



(b) Ebola virus

TEM
100 nm



Rhabdoviridae

- Single-stranded RNA, – strand, one RNA strand
 - *Vesiculovirus*
 - *Lyssavirus*
(rabies virus)
 - Cause numerous animal diseases

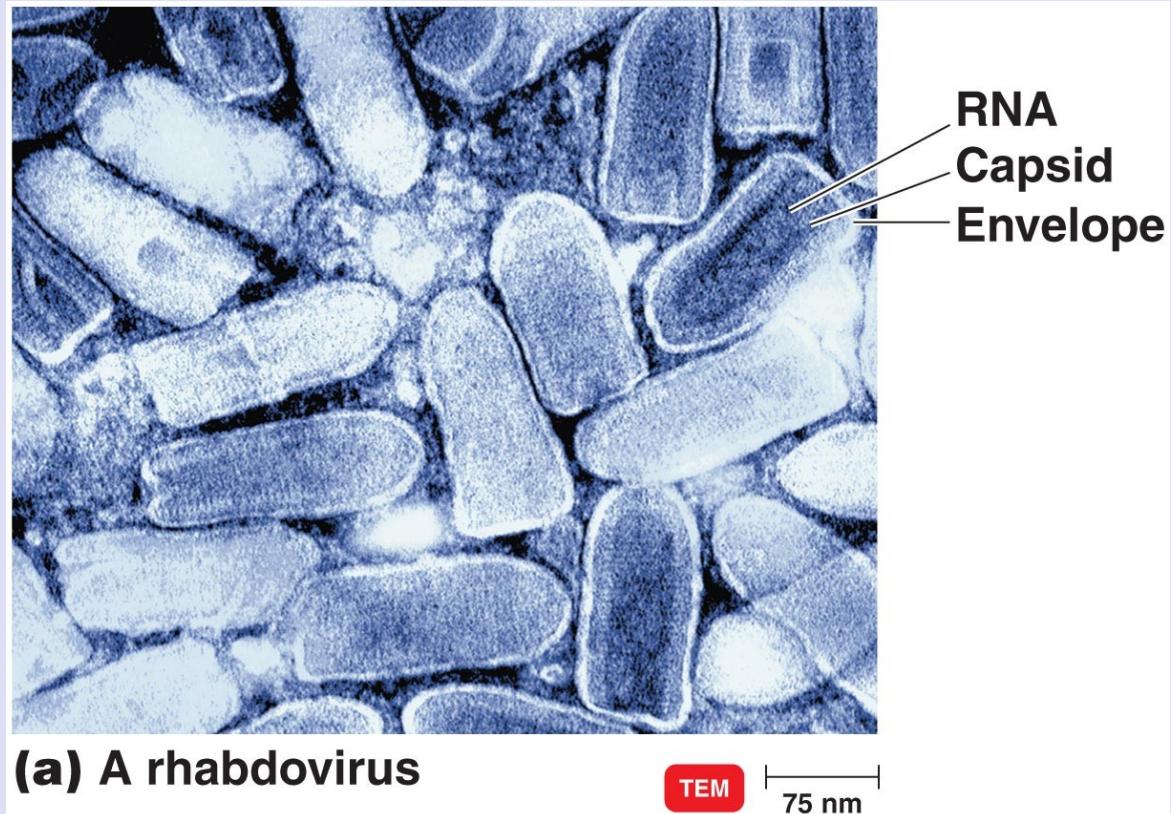


Figure 13.18a

Assembly of a helical virus

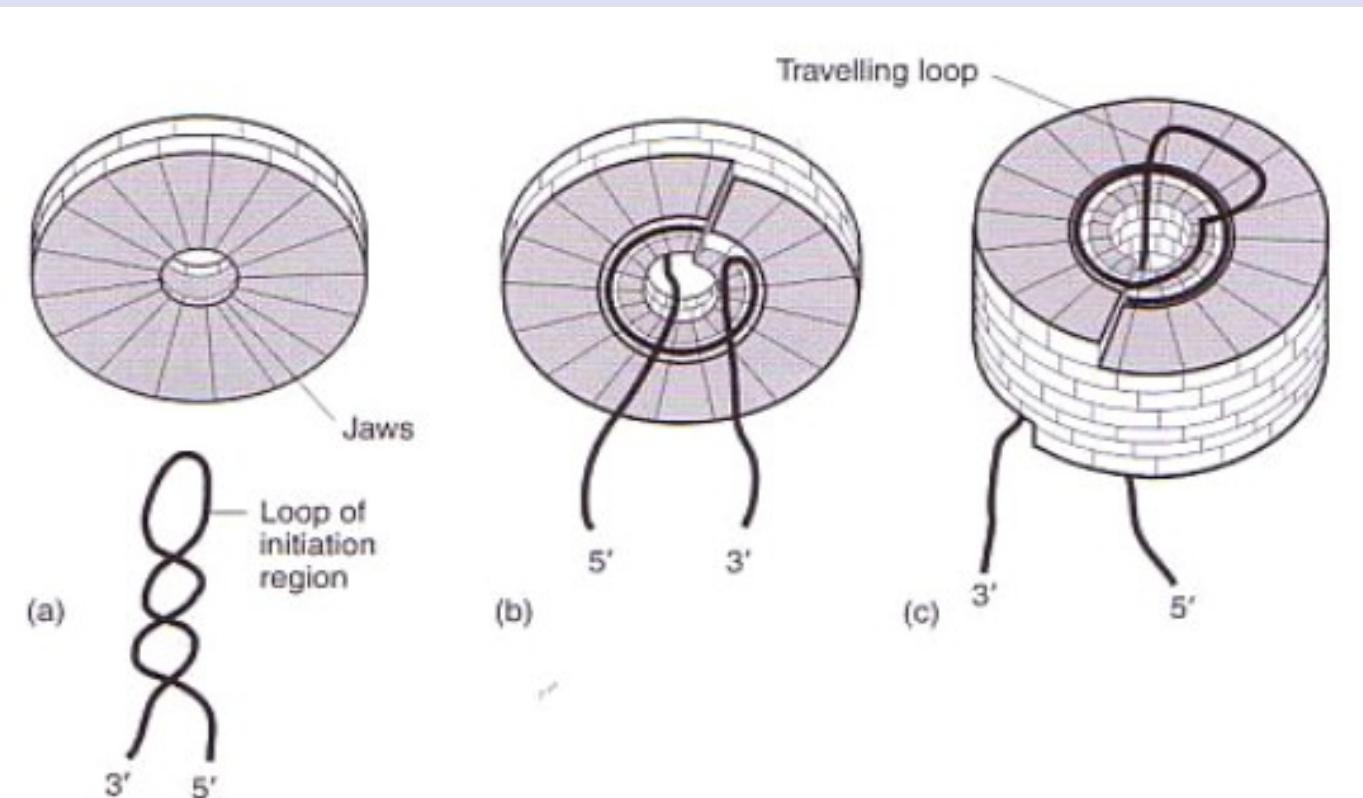


Fig. 11.5 The 'travelling loop' model for tobacco mosaic virus (TMV) assembly. (a) Nucleation begins with the insertion of the hairpin loop of the packaging region of TMV RNA into the central hole of the first protein disc. The loop intercalates between the two layers of subunits and binds around the first turn of the disc. (b) On conversion to the lock-washer, the RNA is trapped. (c) As a result of the mode of initiation, the longer RNA tail is doubled back through the central hole of the rod, forming a travelling loop to which additional discs are added rapidly.

Morphology of a Complex Virus

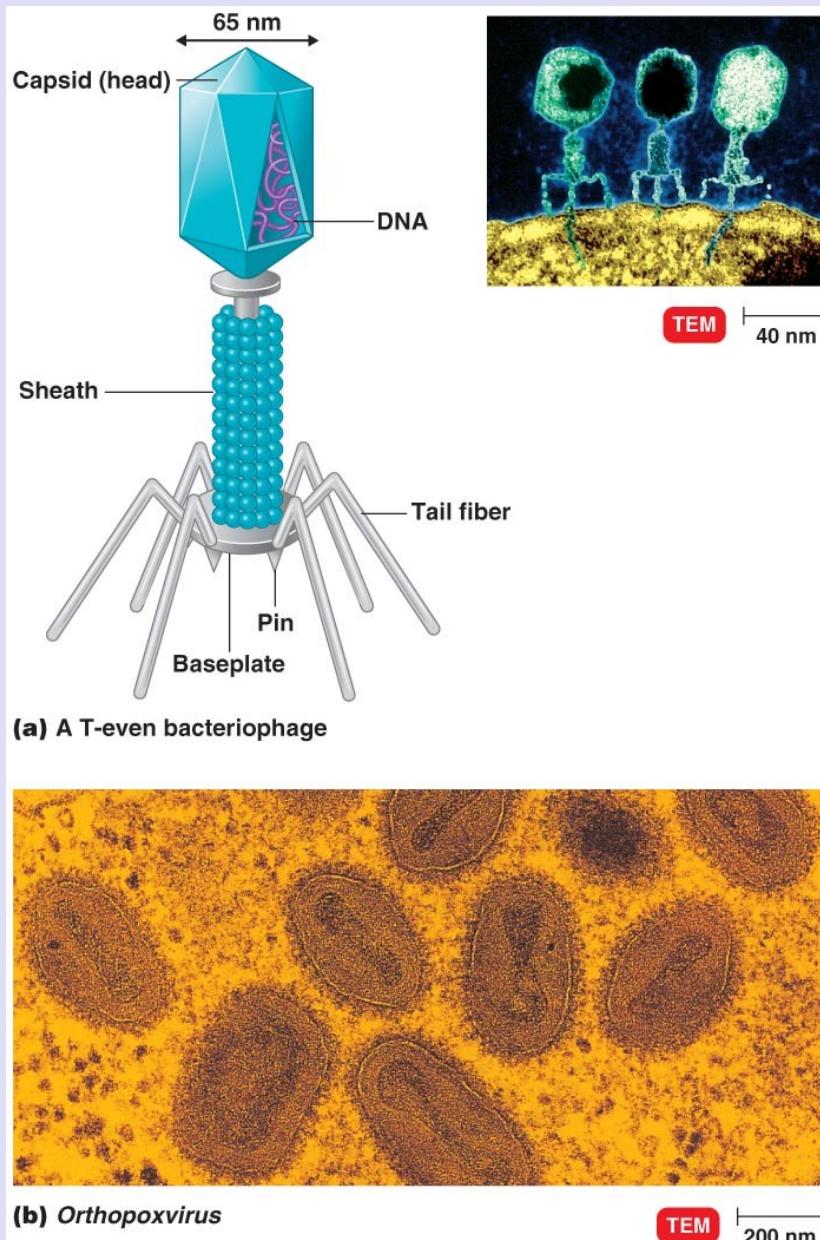


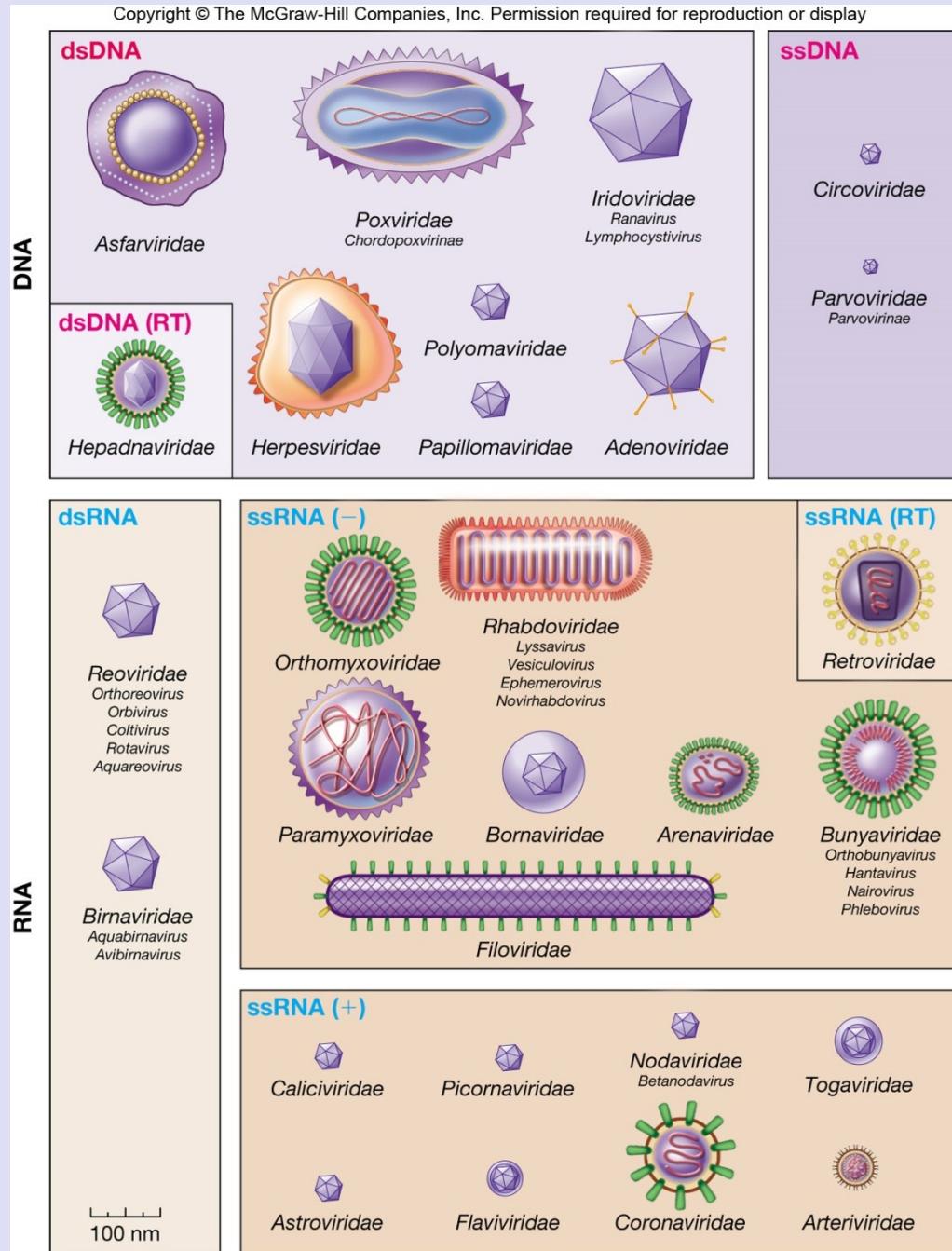
Figure 13.5

Taxonomy of Viruses

- Family names end in *-viridae*.
- Genus names end in *-virus*.
- **Viral species:** A group of viruses sharing the same genetic information and ecological niche (host). Common names are used for species.
- Subspecies are designated by a number.

Taxonomy of Viruses

- Herpesviridae
- *Herpesvirus*
- Human herpes virus HHV-1, HHV-2, HHV-3
- Retroviridae
- *Lentivirus*
- Human immunodeficiency virus HIV-1, HIV-2



Basic Classification Scheme

The problem with Virus Classification:

- Viruses are not directly related by evolutionary descent
- Probably have multiple origins; co-evolution with host
- no universal feature (e.g. rRNA)

Viruses are classified by:

The type of nucleic acid they possess (see figure);

Shape; enveloped or not;

Host;

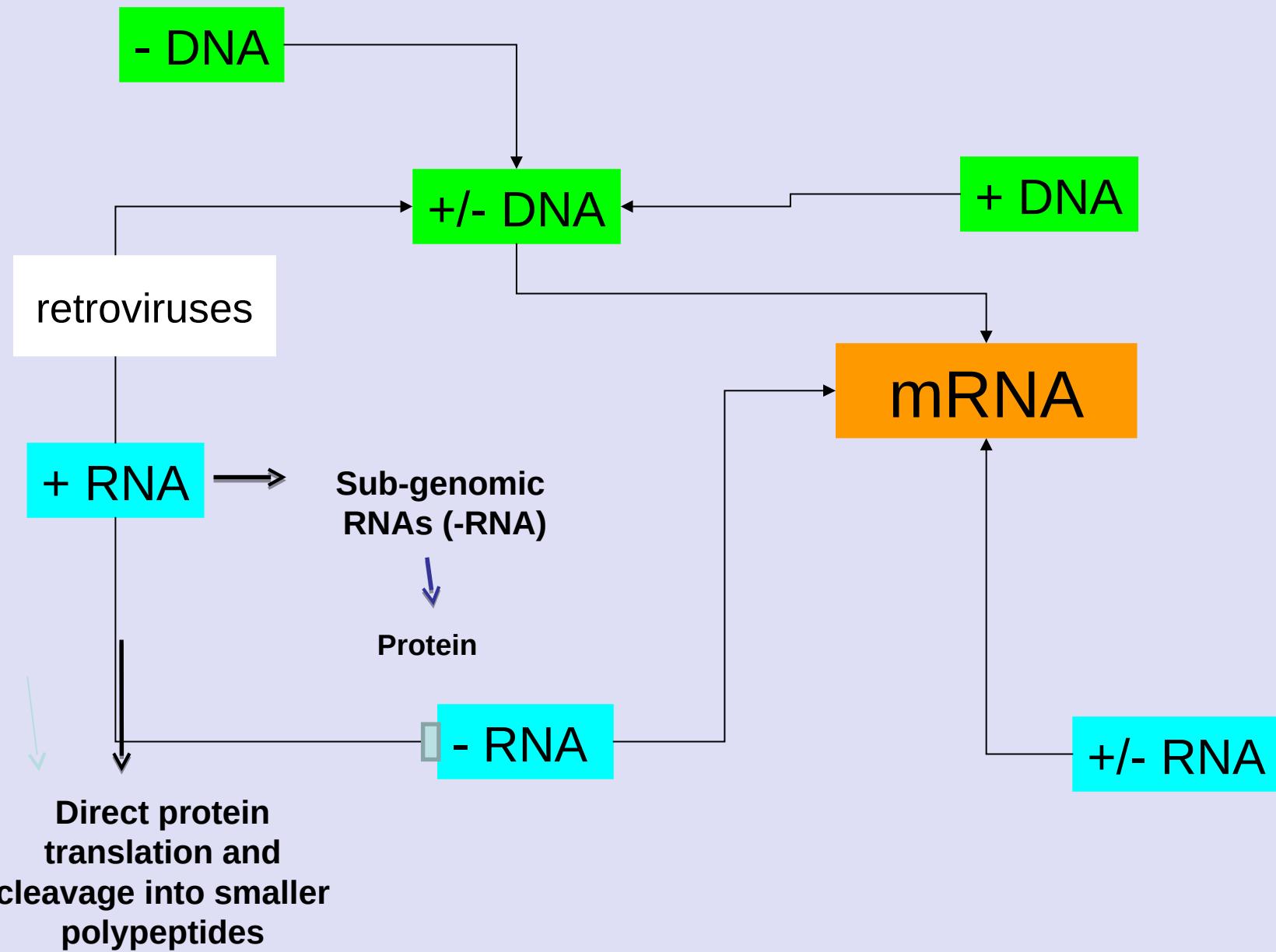
How they achieve transcription;

The Baltimore Classification Scheme

All viruses need to produce mRNA. David Baltimore developed a scheme to classify viruses using this criterion.

Are 7 groups: ssDNA; dsDNA; dsRNA; + sense ssRNA; -ve sense RNA; ss RNA reverse transcribing virus with DNA intermediate; dsDNA with RNA intermediate

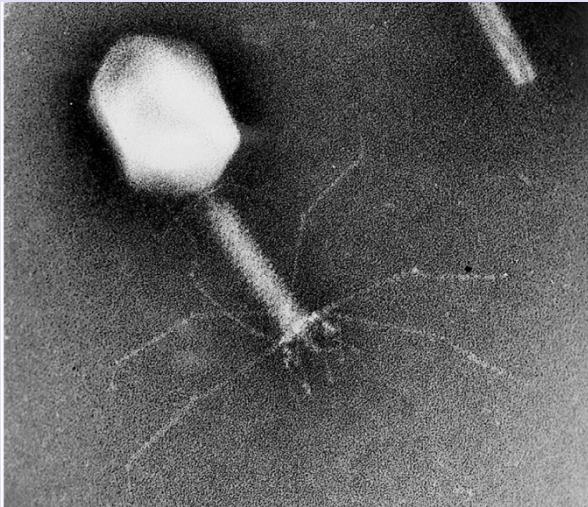
The Baltimore Classification Scheme



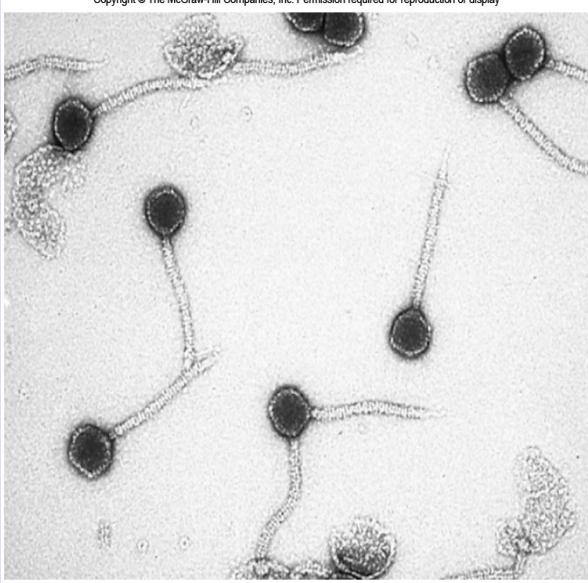
KEYPOINTS

- **Viruses are composed of protein, nucleic acids and sometimes lipid envelopes**
- **There are two basic symmetries, helical and icosahedral (polyhedral)**
- **Virus capsids are made up of protein subunits or capsomeres which fold to form specific structures**
- **Genomes within virus capsids are linear or circular RNA or DNA which are folded and interact with the capsid proteins through electrostatic or hydrophobic interactions**
- **Viable virus particles or virions are infectious**
- **Viruses have multiple origins and co-evolved with hosts**
- **Viruses are classified according to the expression (mRNA) of their genes**

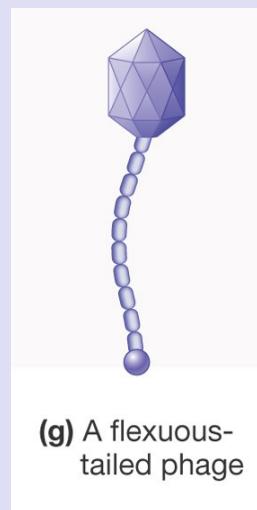
BACTERIOPHAGES



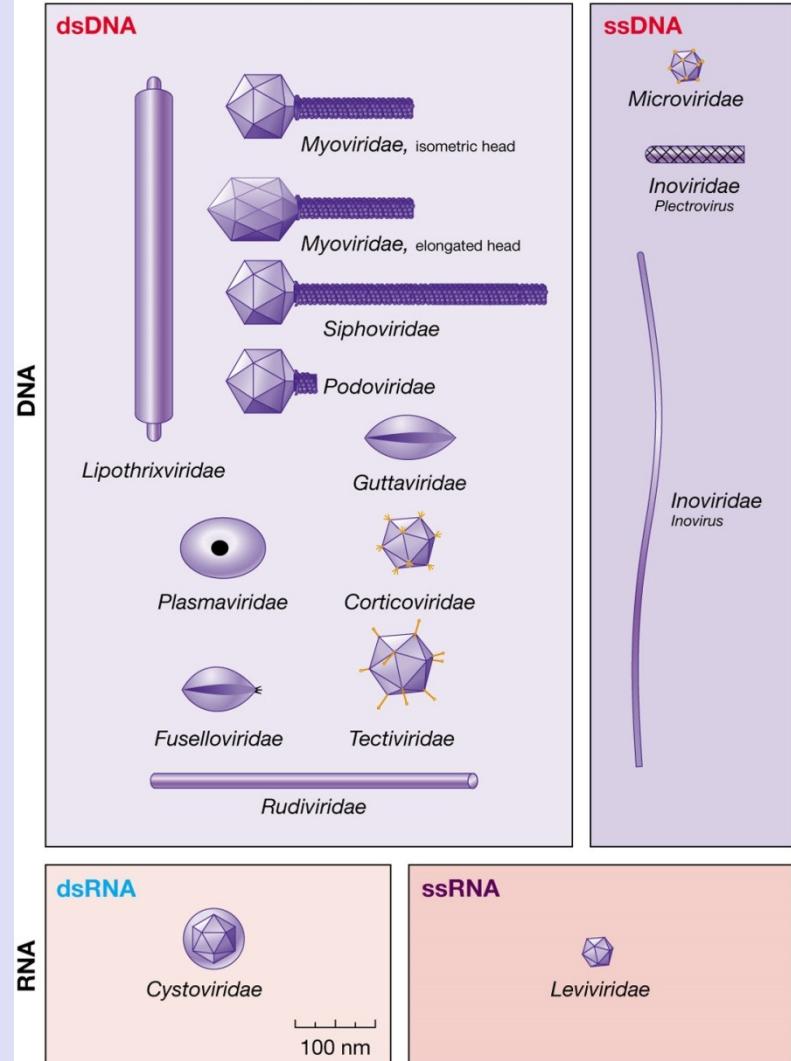
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The Lytic Cycle

- The **lytic cycle** is a phage replicative cycle that culminates in the death of the host cell; there is NO integration into the bacterial host genome
- The lytic cycle produces new phages and lyses (breaks open) the host's cell wall, releasing the progeny viruses and the cell dies
- A phage that reproduces only by the lytic cycle is called a **virulent phage**
- Bacteria have defenses against phages, including **restriction enzymes** that recognize and cut up certain phage DNA

A blue oval button with the word "PLAY" in white capital letters.

Animation: Phage T4 Lytic Cycle

The Lytic Cycle

- **Attachment:** Phage attaches by tail fibers to host cell
- **Penetration:** Phage lysozyme opens cell wall; tail sheath contracts to force tail core and DNA into cell
- **Biosynthesis:** Production of phage DNA and proteins
- **Maturation:** Assembly of phage particles
- **Release:** Phage lysozyme breaks cell wall

Lytic Cycle of a T-Even Bacteriophage

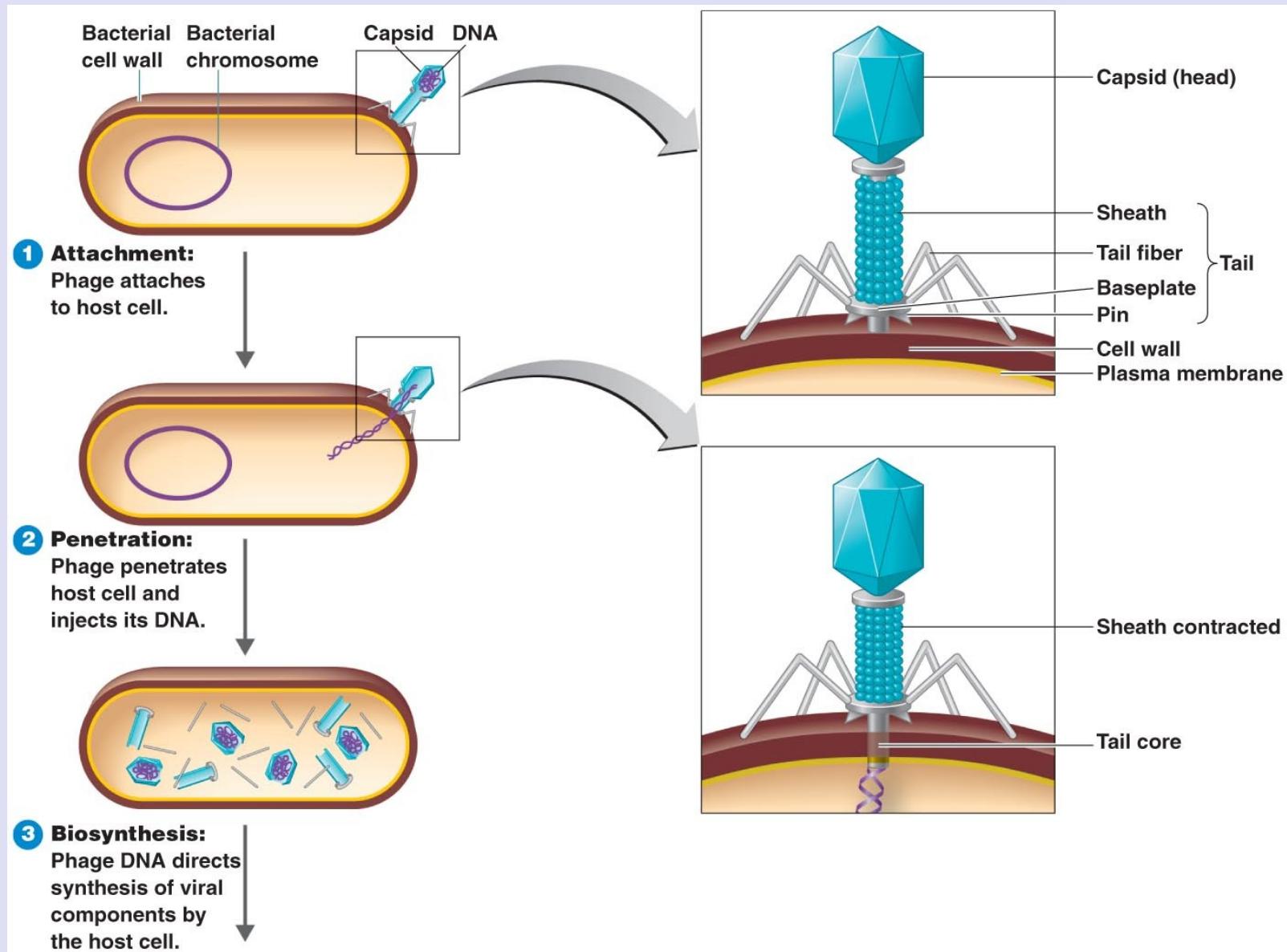


Figure 13.11

Lytic Cycle of a T-Even Bacteriophage cont.

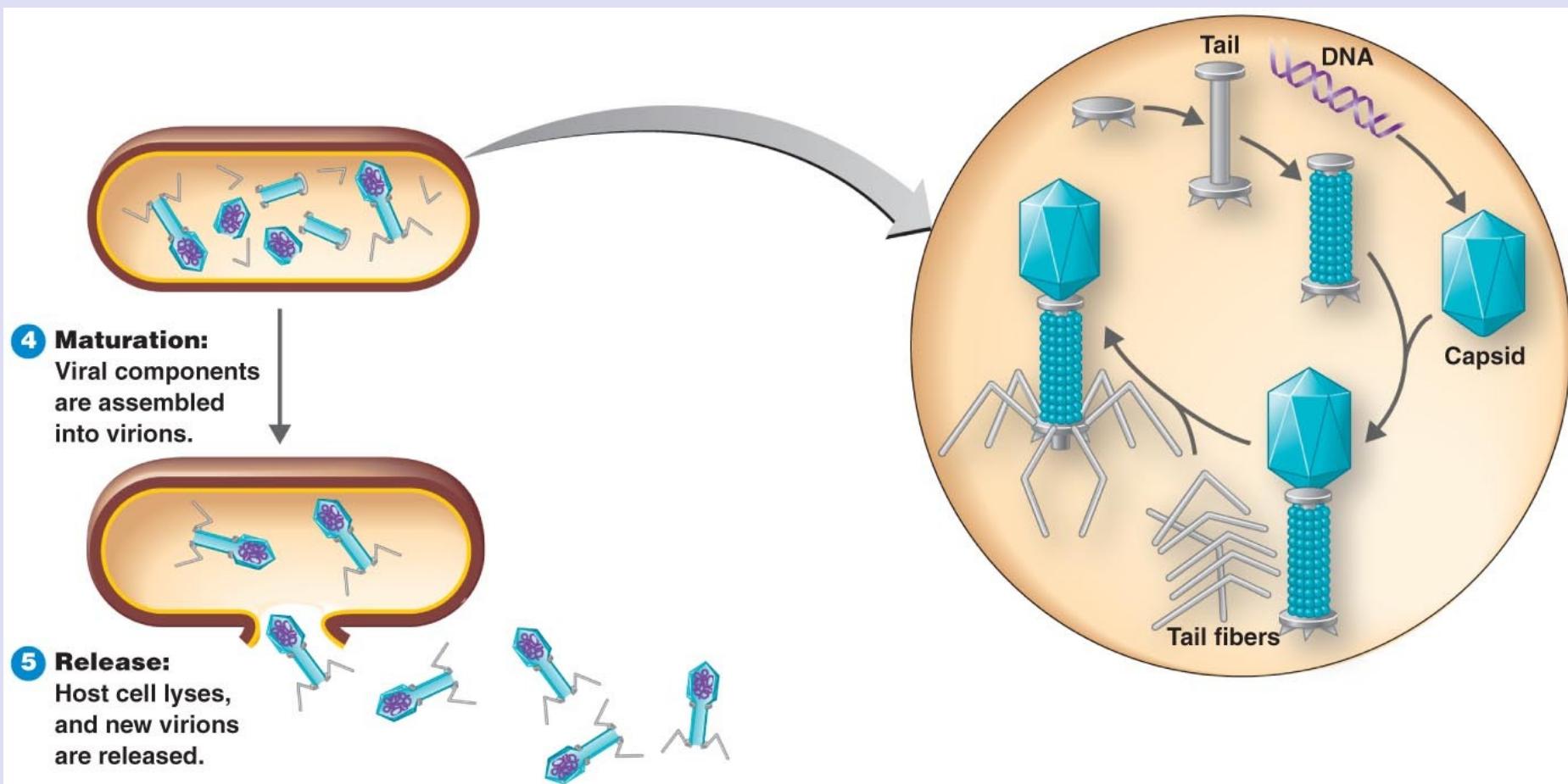


Figure 13.11

Generalized Transduction

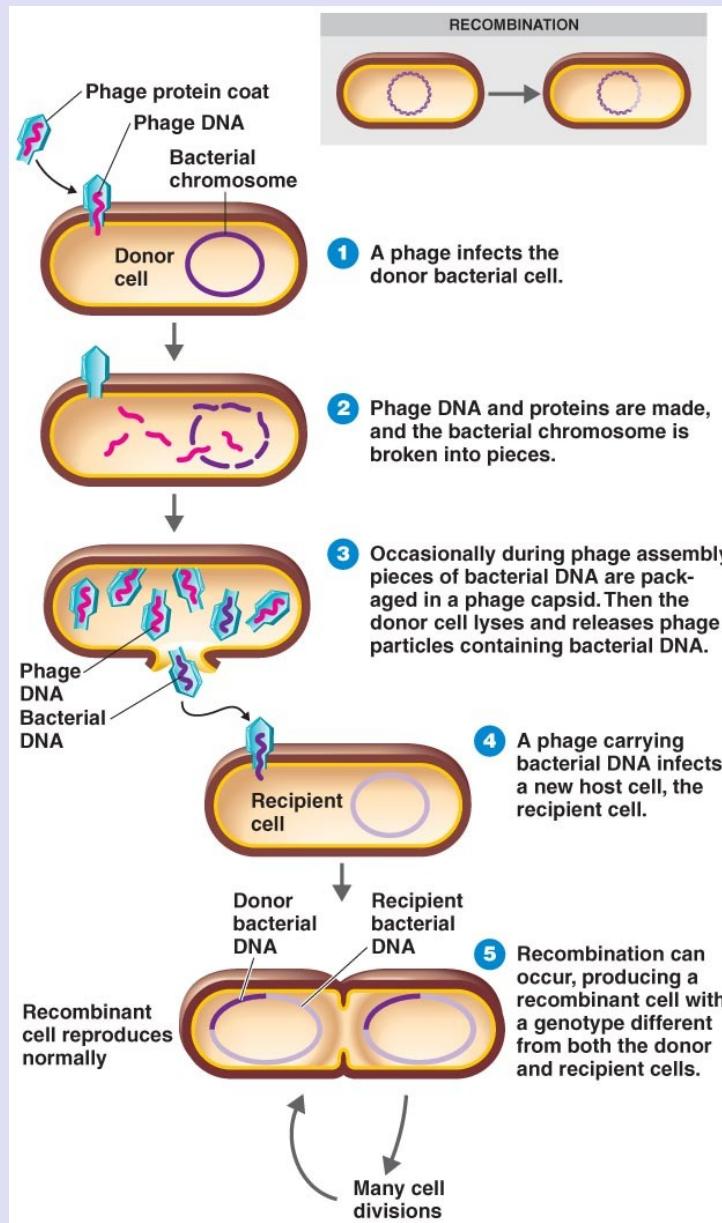
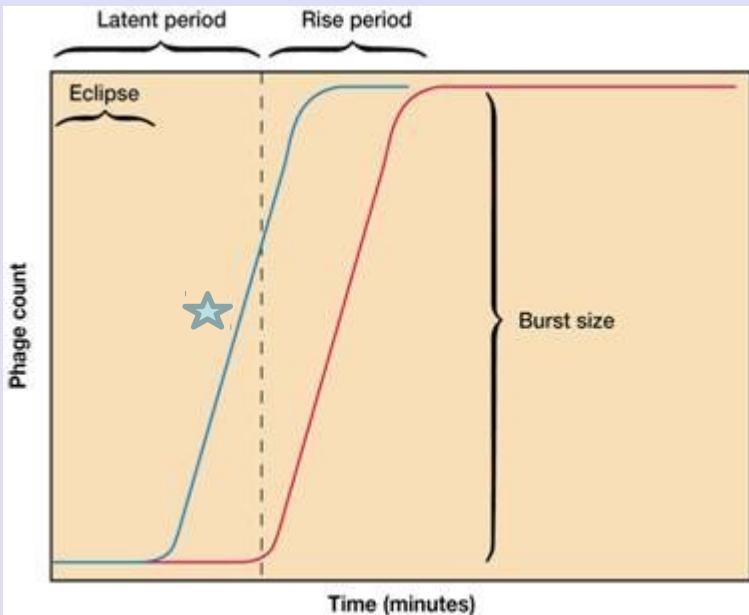
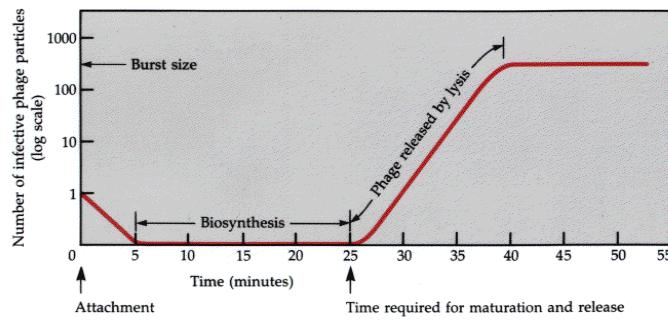


Figure 8.28



Blue line: total no. of complete virions
Red line: no. free phage (unabsorbed + lysed host cells)

A bacteriophage one-step growth curve (Figure 13.15)



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Note: Students to read practical manual for outline of procedure

One step growth curve: burst size determination

Bacteria mixed with phage for a short time; then **mixture is diluted**; samples removed at specific intervals and plated to quantify phage in culture
 Eclipse phase: attachment and penetration
 Latent phase: no phage virions yet (biosynthesis of phage enzymes; NAs etc.)

Rise phase: increase in plaque forming units (pfu) due to lysis of bacteria. At start of experiment plaque count is relatively constant as each bacterium will yield one plaque. In lab we synchronize phage population replication by chloroform lysing at time intervals. Due to dilution of adsorption mixture released phage fail to meet susceptible bacterium and do not infect them & remain free in culture medium. This leads to the plateau and no newly infected bacteria; at intervals during latent phase no. of assembled virions should go up until lysis.

Therefore **burst size** (no. of phage released per bacterium) relates to the no. plaques at the plateau (one bacterium=one plaque=one phage)

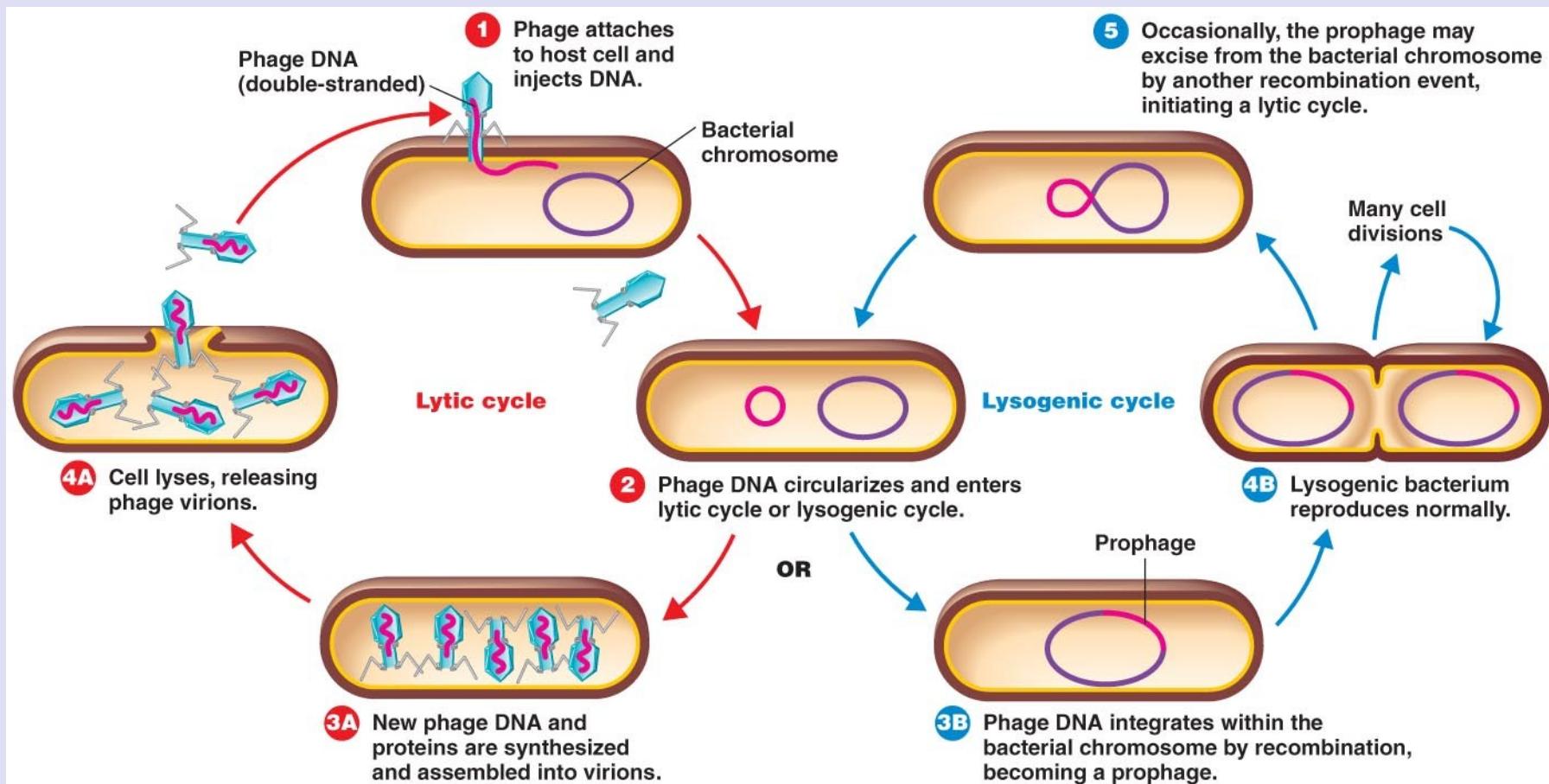
The Lysogenic Cycle

- The **lysogenic cycle** replicates the phage genome without destroying the host
- The viral DNA molecule is incorporated into the host cell's chromosome
- This integrated viral DNA is known as a **prophage**
- Every time the host divides, it copies the phage DNA and passes the copies to daughter cells
- Some phages can switch to lytic cycle and can take some host DNA with it (specialized transduction)

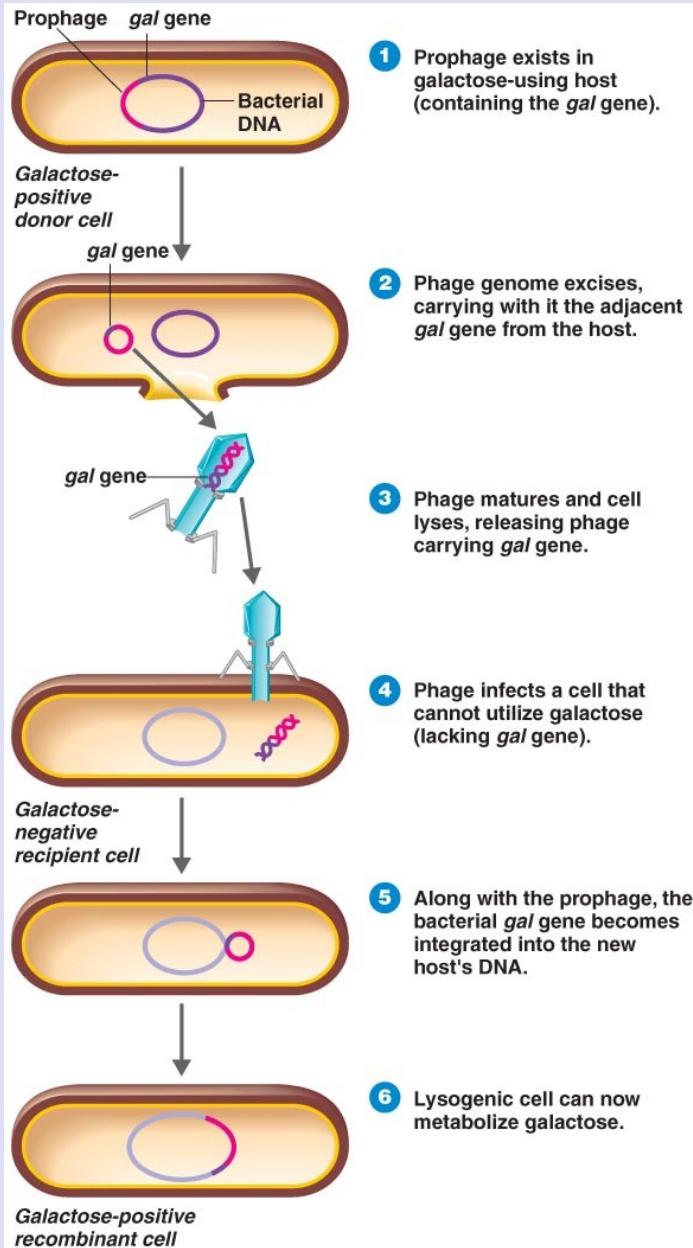
PLAY

Animation: Phage Lambda Lysogenic and Lytic Cycles

The Lysogenic Cycle



Specialized Transduction



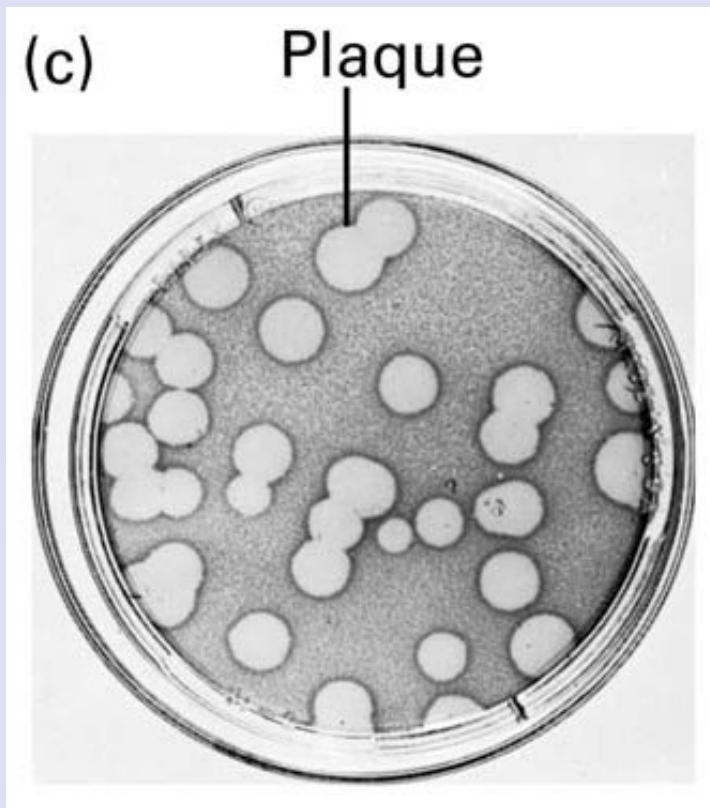
WORKING WITH VIRUSES IN THE LABORATORY: CULTIVATION, ISOLATION AND DETECTION OF VIRUSES

CULTURE OF VIRUSES

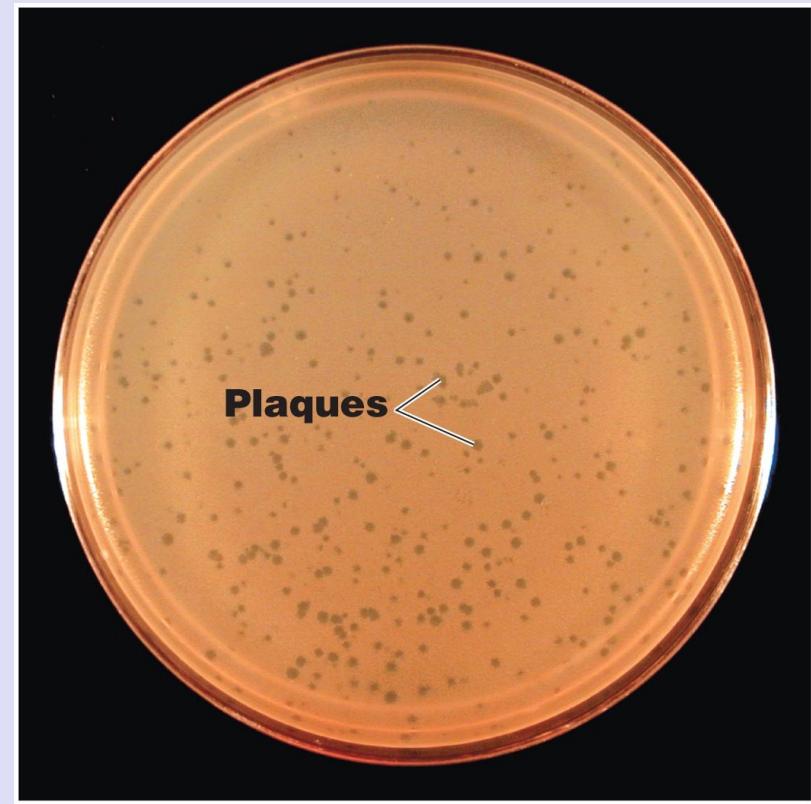
- Began in the early 20th century with whole organ then progressed to individual cells involving *primary* cell cultures or *immortalized* dedifferentiated cell lines
- *Plaque assay* to quantify animal or bacteriophages using virus dilutions and monolayers of cultured cells
- Viruses cause *cytopathic* effects in infected cultured cells
- Some viruses e.g. HIV do not replicate in culture and do not produce plaques e.g. HIV
- However investigations of natural infections is best done in natural hosts; not always possible so use experimental animals e.g. rats
- Some viruses e.g. influenza is grown in *embryonated* eggs
- Plant viruses grown in host plants or can infect protoplasts

Viruses must be grown in living cells

Plaques formed by poliovirus plated on HeLa cells (c)

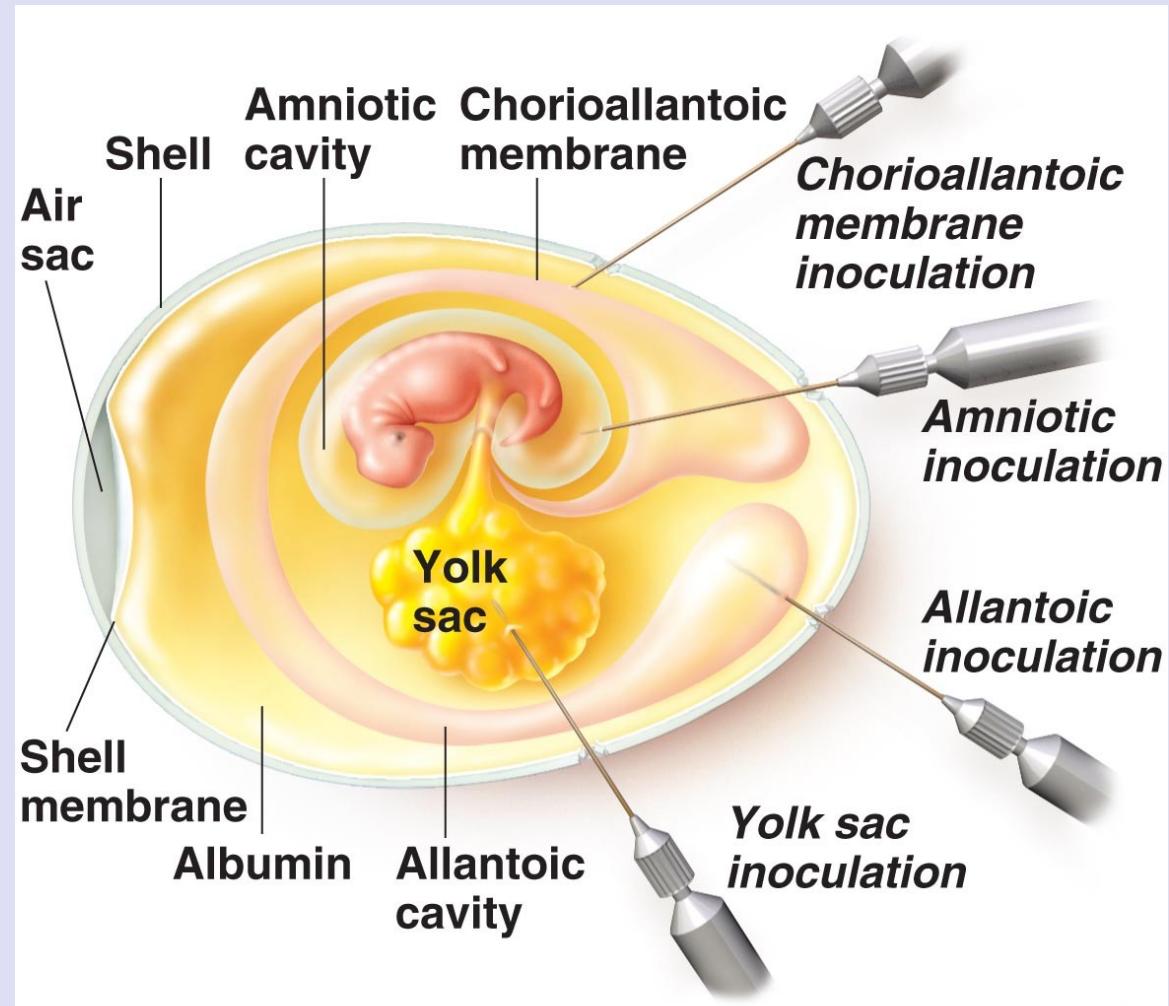


Bacteriophages form plaques on a lawn of bacteria



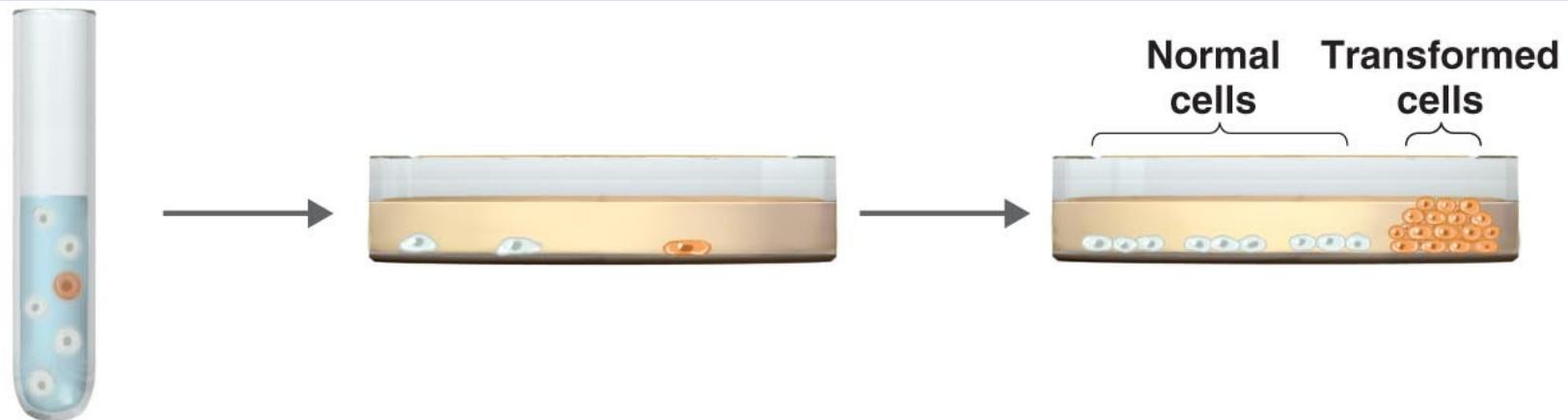
Growing Viruses

- Animal viruses may be grown in living animals or in embryonated eggs



Growing Viruses

- Animal and plant viruses may be grown in cell culture (cells derived from human tissue such as liver, epidermis or kidneys)
 - Continuous cell lines may be maintained indefinitely



1 A tissue is treated with enzymes to separate the cells.

2 Cells are suspended in culture medium.

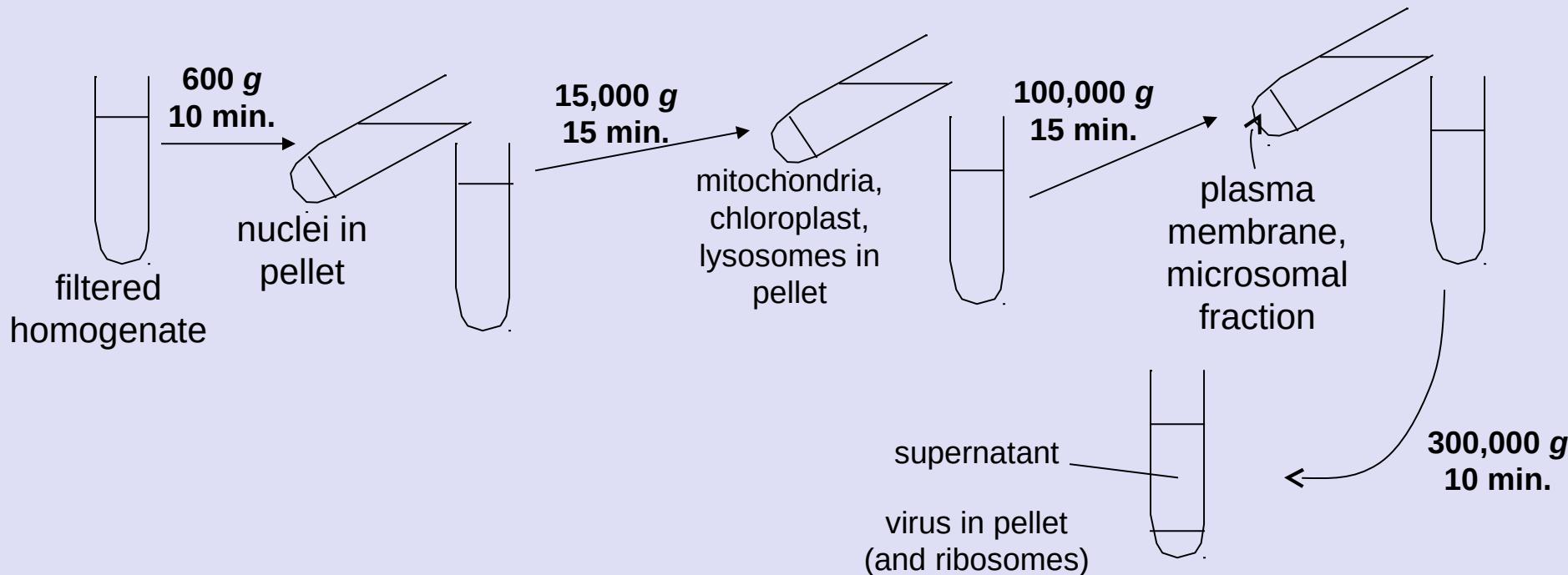
3 Normal cells or primary cells grow in a monolayer across the glass or plastic container. Transformed cells or continuous cell cultures do not grow in a monolayer.

Isolation of viruses

- Biological macromolecular material can often be separated according to it's size, density or charge
- Enveloped viruses (and other cellular organelles) can often be separated according to their buoyant density (e.g. viruses with large membranes are less dense than those with smaller envelopes). Proteins or nucleic acid will be much denser.
- Separation based on density is accomplished by generating an equilibrium density gradient of, generally sucrose, in an ultracentrifugal field
- The virus particles will band / float according to their respective equilibrium buoyant density

Rate Zonal Centrifugation

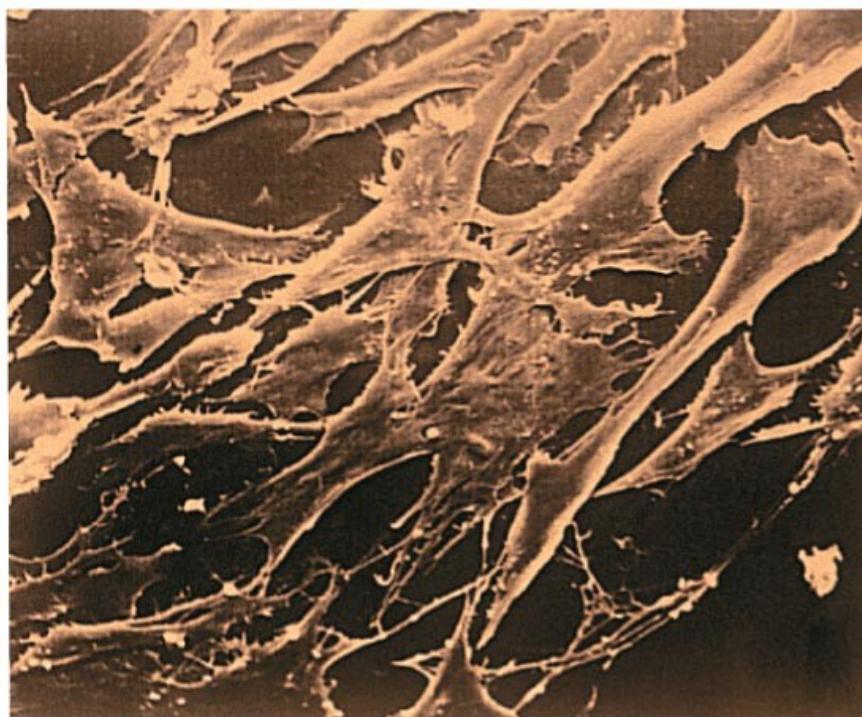
- Form of **differential centrifugation** based on **size** of particles
- Allows rapid **fractionation** and purification of subcellular particles (e.g. organelles; virions)
- Largest particles will **sediment most rapidly** or under the *least force*
- e.g. In an infected cell – organelles e.g. mitochondria will sediment first, then virus, then ribosome



Virus Identification

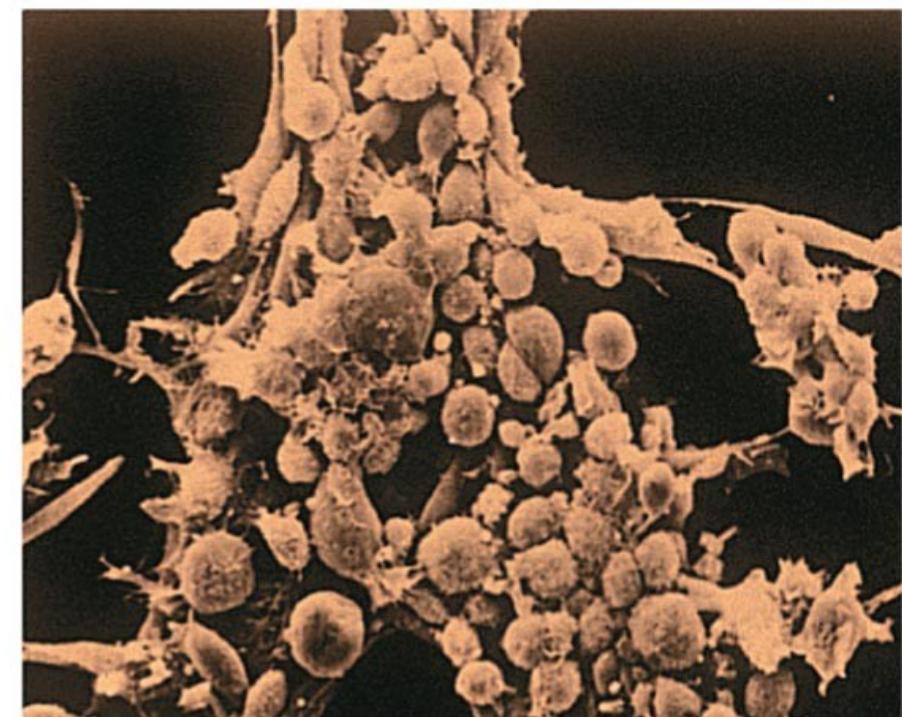
- Cytopathic effects (microscopy)
- Serological tests (proteins)
 - Based on the principal of the specificity of binding of an antibody (Ab) to a specific antigen/viral antigen
 - Detect antibodies against viruses, or virus-infected cells expressing virus antigens on surface, in a patient
 - Or use antibodies to identify viruses in neutralization tests such as viral hemagglutination or complement fixation tests, ELISAs (ENZYME LINKED IMMUNOSORBENT ASSAY); **ISEM** or Western blots
- Nucleic acids
 - Hybridization (Southern Blot) based on the principal that two complementary strands will bind to each other
 - Polymerase chain reaction (PCR) based on the exponential amplification of DNA by a heat stable DNA polymerase

Virus Identification: cytopathic effects



(a)

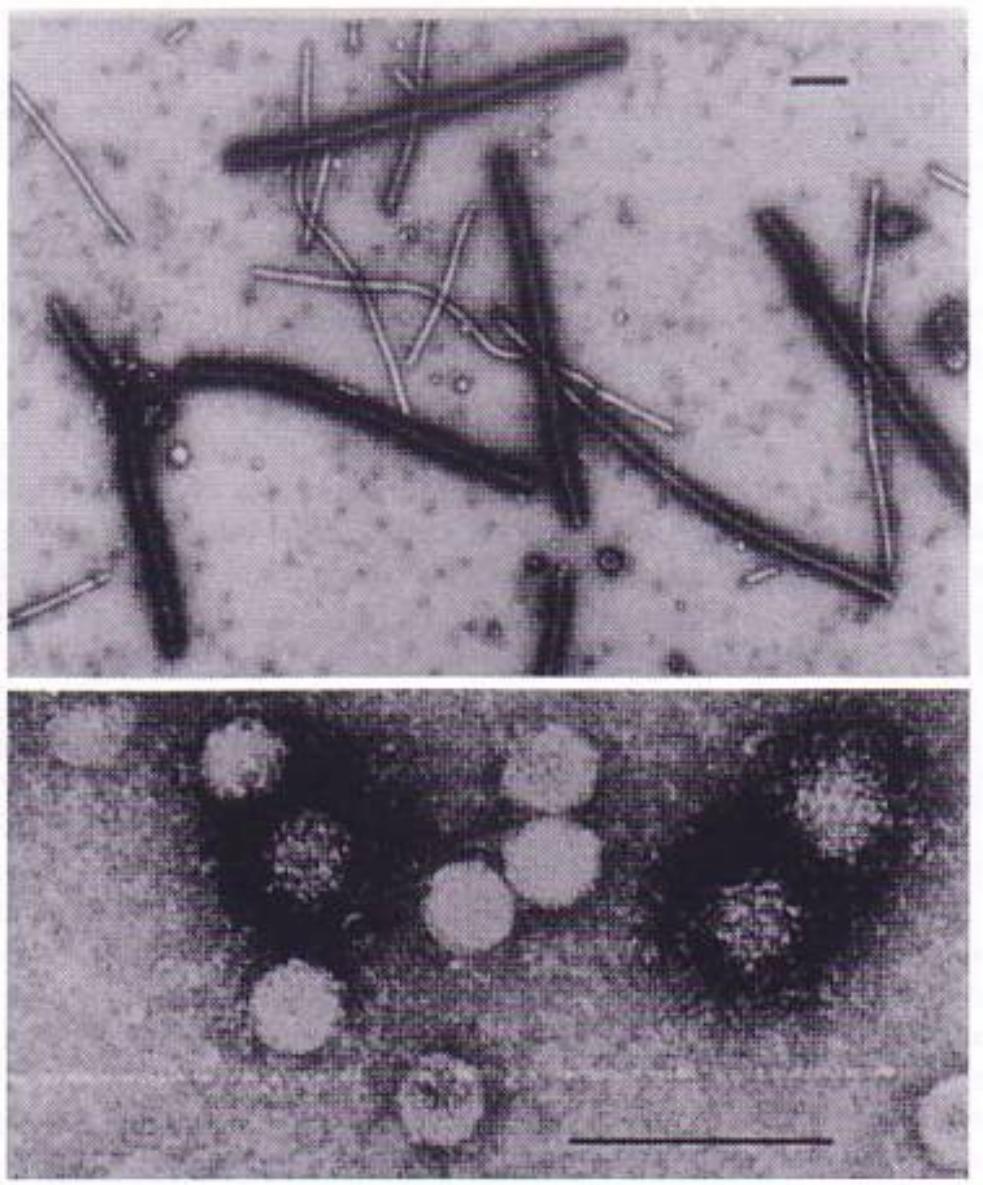
SEM
40 μ m



(b)

SEM
20 μ m

Figure 13.9

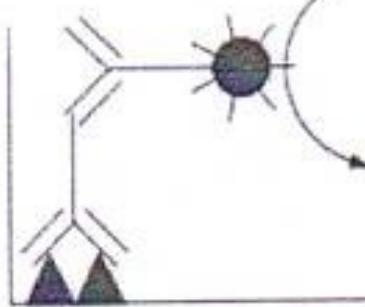


IMMUNOSORBENT ELECTRON MICROSCOPY (ISEM)

Enzyme-linked immunosorbent assay (ELISA)

(c) ELISA

Colourless substrate
O—O
Coloured product

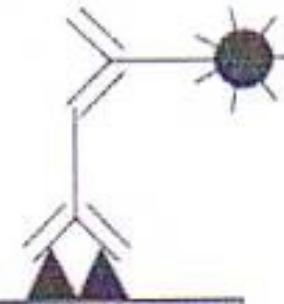


(d) Western blot

Direct:



Indirect:



Key:



Virus antigen



Secondary antibody

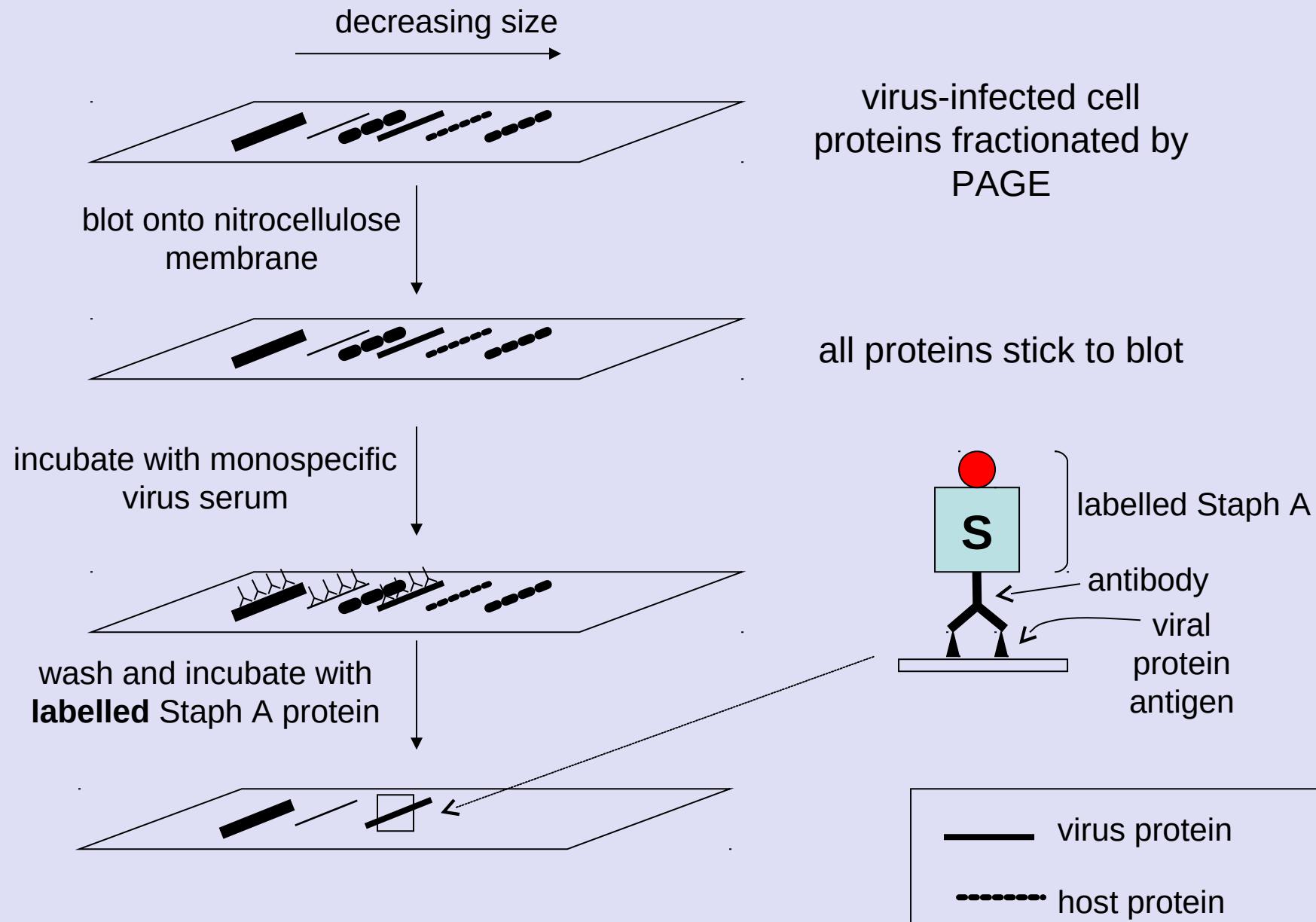


Primary antibody



'Detector' molecule: complement, enzyme,
radioisotope, or fluorescent dye

Using Staph A protein to detect antibody – antigen complex in a Western blot



Southern Blot

- SB is based on the principal of hybridization of two complementary homologous DNA strands (figure below)
- ss DNA test fragments are run on a gel; transferred to a nitrocellulose membrane; and then incubated in a solution containing the *probe* (known complementary ssDNA sequence) labelled with radioactive or fluorescent nucleotides e.g. Alpha -³²P-dCTP
- Unbound nucleotides are washed off and if the two strands hybridize they are detected using an autoradiography film; if no hybridization takes place you know your “test” sample DNA is not related to your known probe

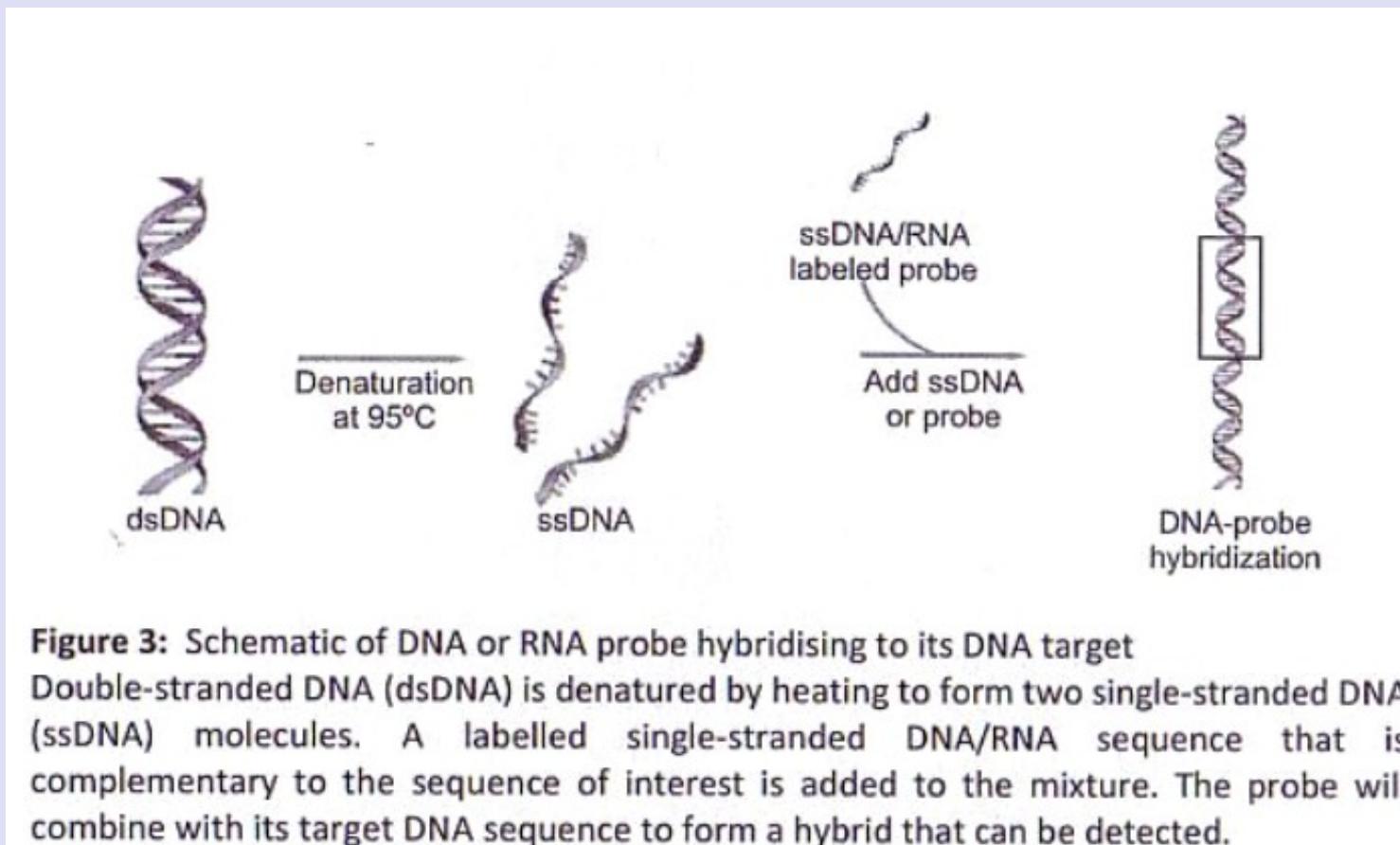
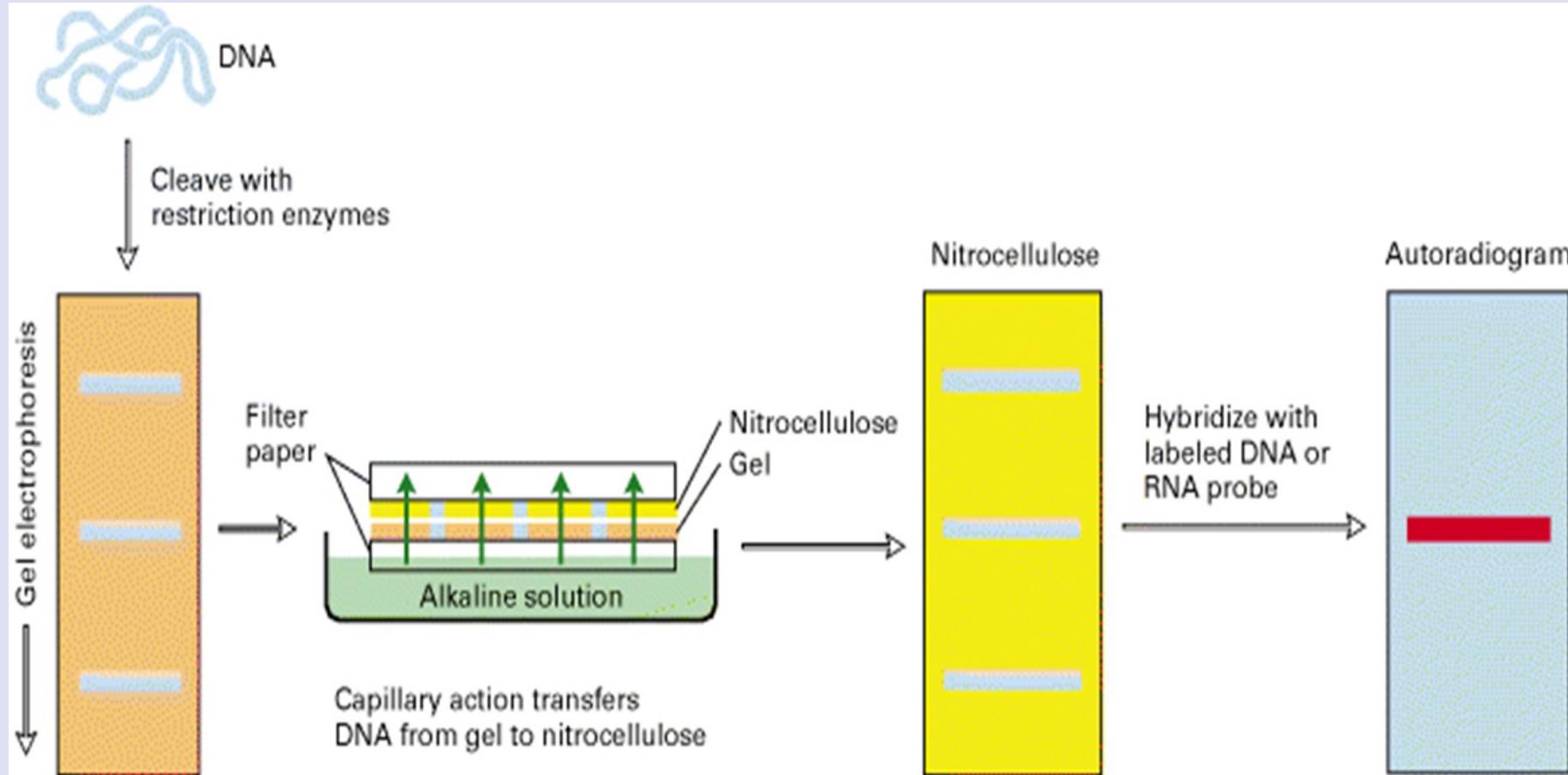


Figure 3: Schematic of DNA or RNA probe hybridising to its DNA target

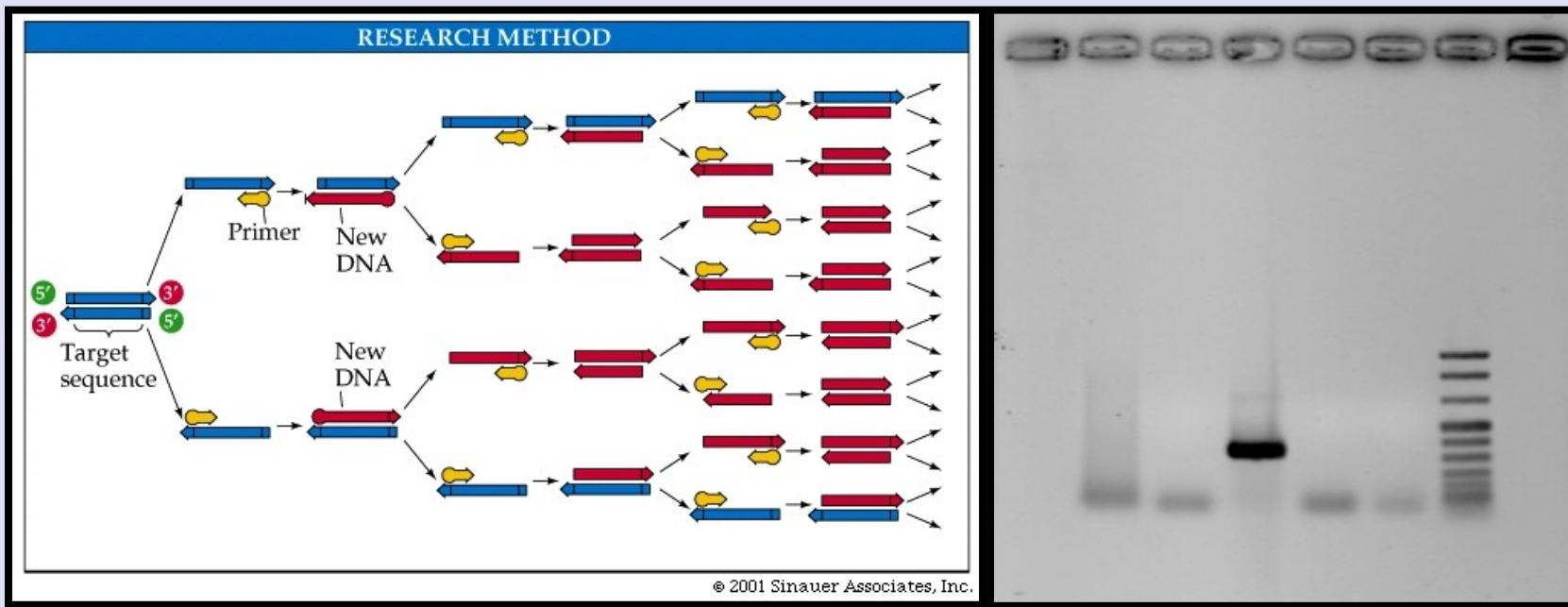
Double-stranded DNA (dsDNA) is denatured by heating to form two single-stranded DNA (ssDNA) molecules. A labelled single-stranded DNA/RNA sequence that is complementary to the sequence of interest is added to the mixture. The probe will combine with its target DNA sequence to form a hybrid that can be detected.

Southern Blot Procedure



Virus detection/diagnosis

Nucleic acid hybridization: Detection by PCR (Polymerase chain reaction): **exponential amplification**



Design primer

Steps

Denature DNA 92 °C

Anneal primer (45 – 60 °C)

Extension 72°C

Repeat the 3 cycles ~ 35 times

LIFE CYCLES, REPLICATION AND TRANSCRIPTION OF VIRUSES

Stages in life cycles of animal/human viruses

- **Attachment:** Viruses attach to cell membrane receptors
- **Penetration** by endocytosis or fusion (env); translocation of nucleocapsid (non-env); injection of NA (phages)
- **Uncoating** by viral or host enzymes (or both); pH
- **Biosynthesis:** Production of viral nucleic acid (replication) and mRNA & proteins (transcription and translation)
- **Assembly & maturation:** Nucleic acid and capsid proteins assemble to form complete virion
- **Release** by budding out (exocytosis) (enveloped viruses) or rupture (lysis) by non-enveloped viruses

Attachment by injection (bacteria)

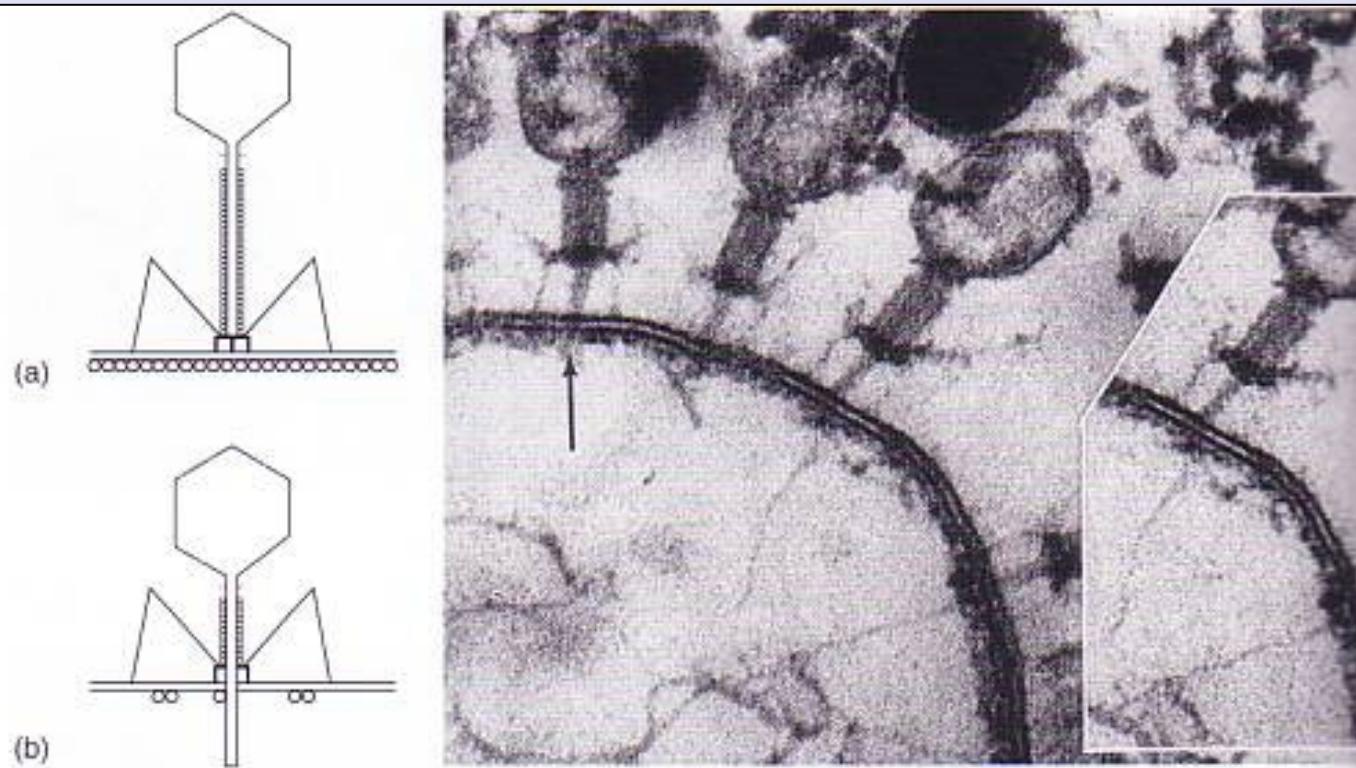
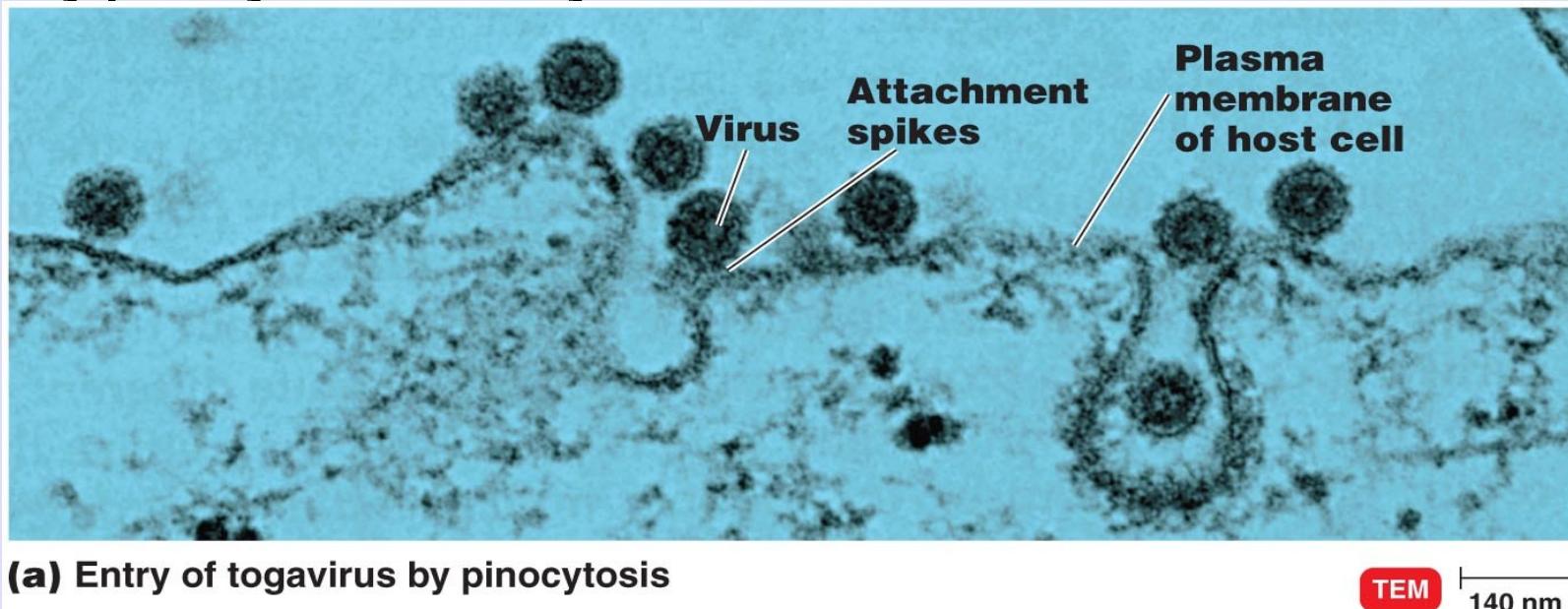


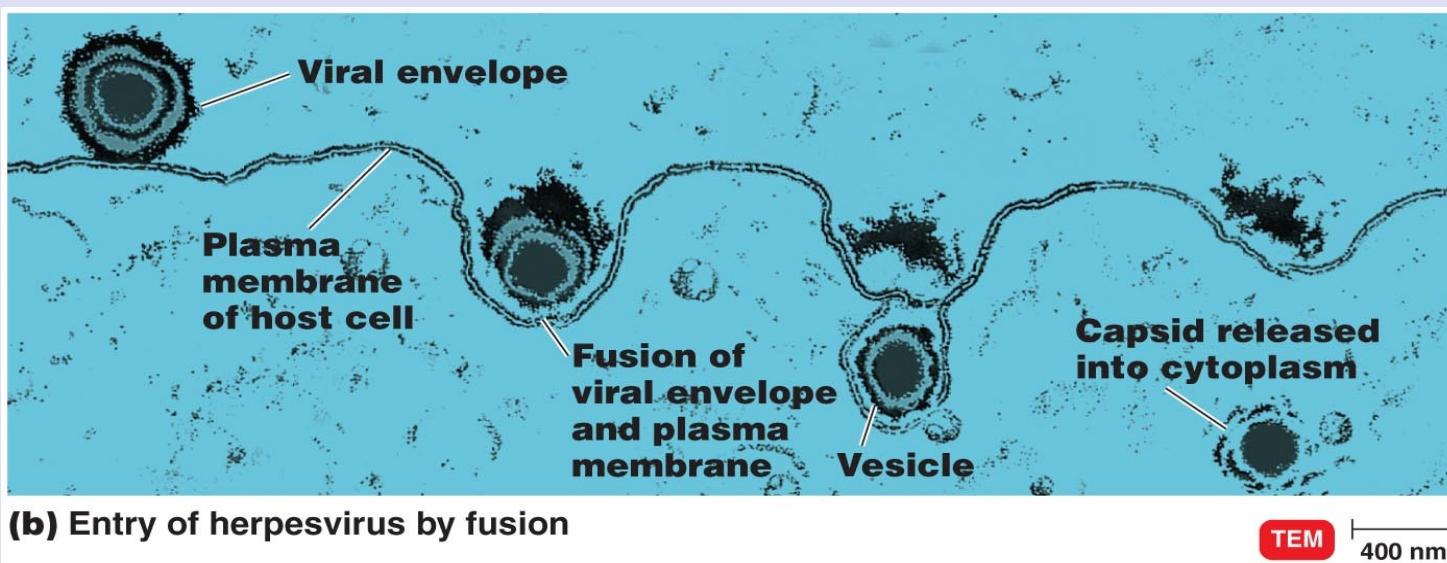
Fig. 5.7 Schematic representation of the mechanism of entry of the phage T4 genome into the bacterial cell wall. (a) The phage tail pins are in contact with the cell wall and the sheath is extended. (b) The tail sheath has contracted and the phage core has penetrated the cell wall; phage lysozyme has digested away the cell beneath the phage. (c) Electron micrograph of T4 attached to an *E. coli* cell wall, as seen in thin section. The core of one of the phages can be seen to penetrate just through the cell wall (arrow). Thin fibrils extending on the inner side of the cell wall from the distal tips of the needles are probably DNA.

Attachment and Penetration

- By pinocytosis/endocytosis



- By fusion



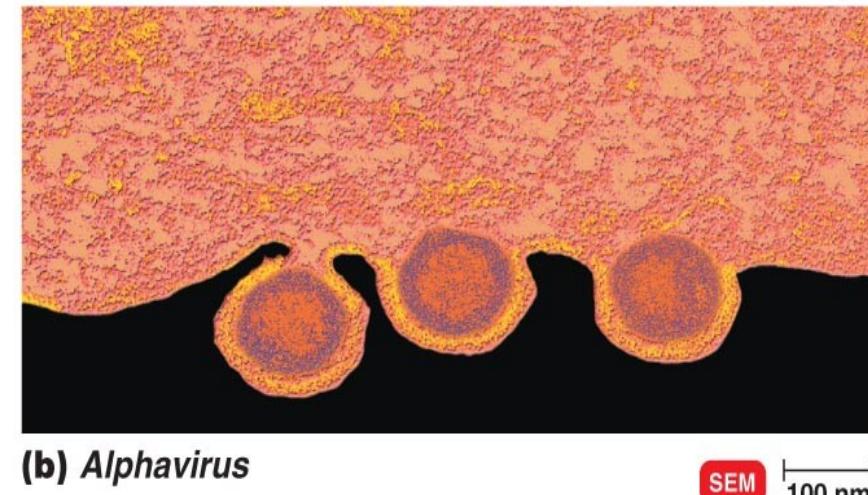
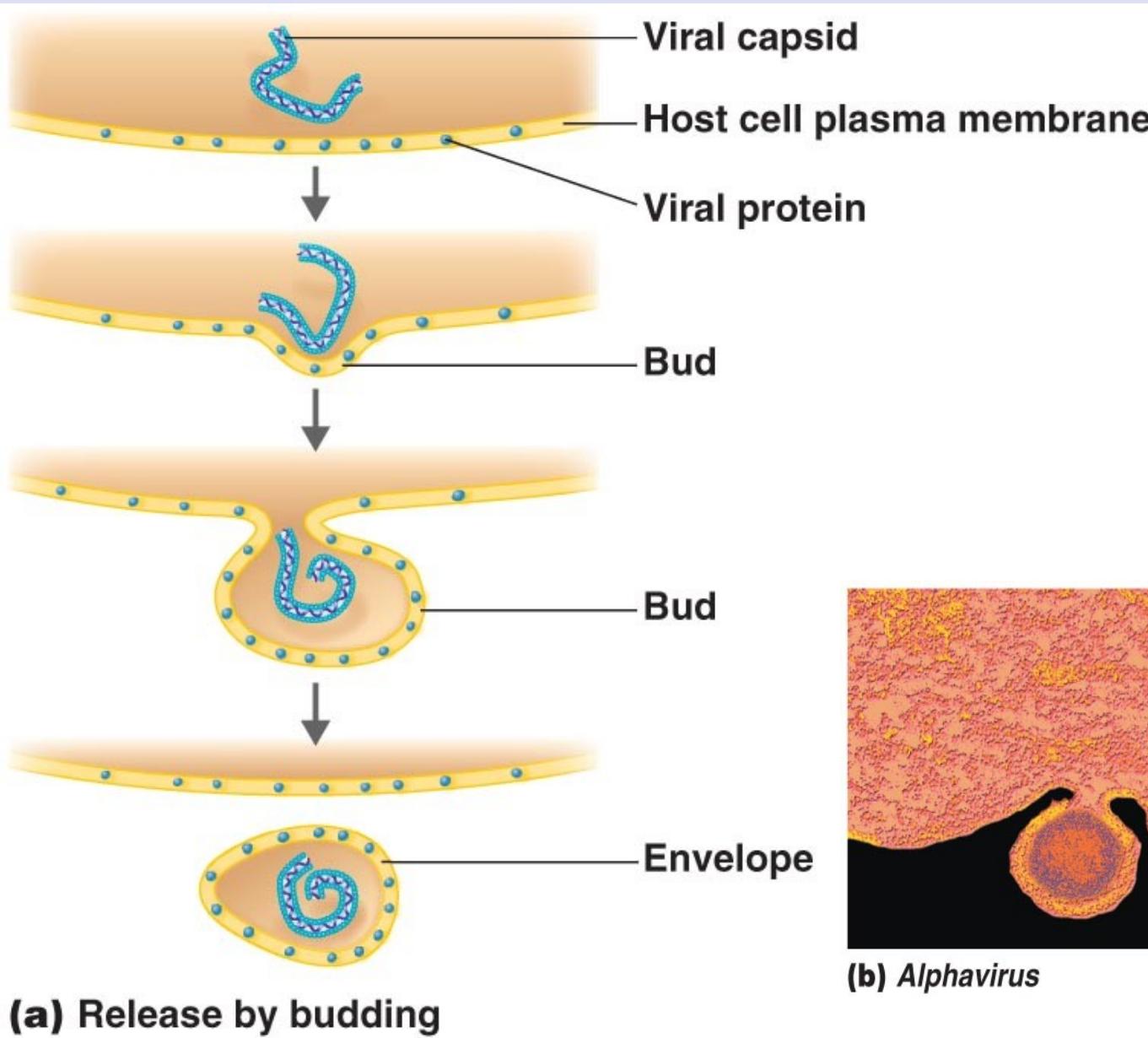
Early transcription of viral genome

Protein translation of viral mRNA (by host)

Replication of viral genome

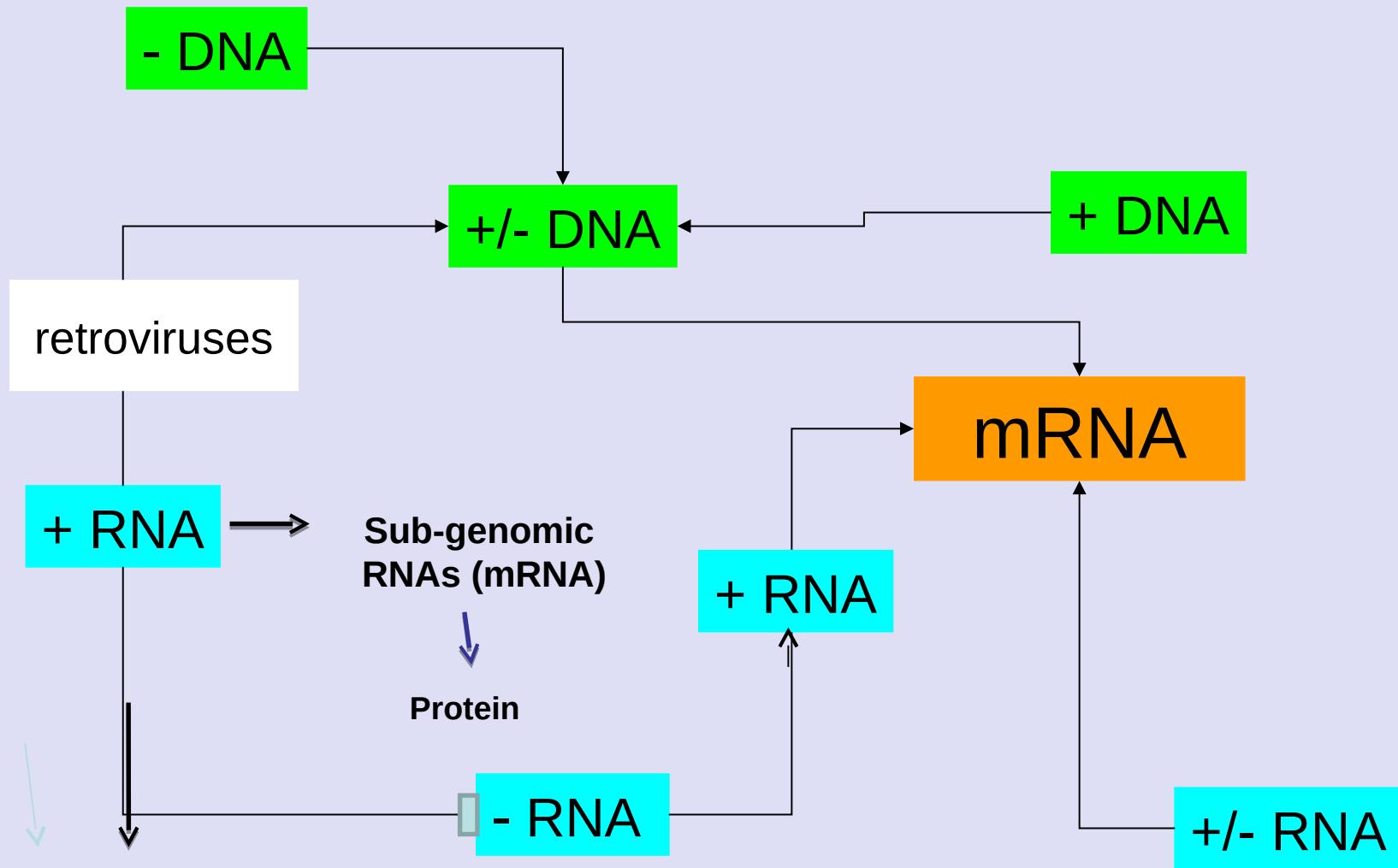
Late transcription & translation

Budding of an Enveloped Virus



SEM 100 nm

Transcription



Direct polyprotein translation and cleavage into smaller polypeptides

Note: mRNA must be capped and polyA-tailed, using mainly host enzymes, before translated by **HOST machinery** in viruses
All proteins are translated from mRNA by host cellular ribosomal machinery and cellular factors

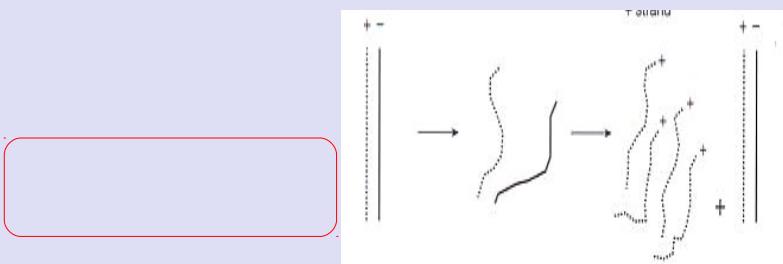
RNA viruses

Replication

DNA viruses:

Replicate (with exception) in the nucleus)

Use host-encoded DNA-dep DNA polymerase (with exception) to replicate



RNA Viruses:

Use RNA- dep RNA polymerase (replicase) to copy RNA (note same RNA pol to transcribe but if transcribe call this transcriptase)

RNA viruses replicate in cytoplasm

ssRNA (-ve or +sense) use a dsRNA intermediate

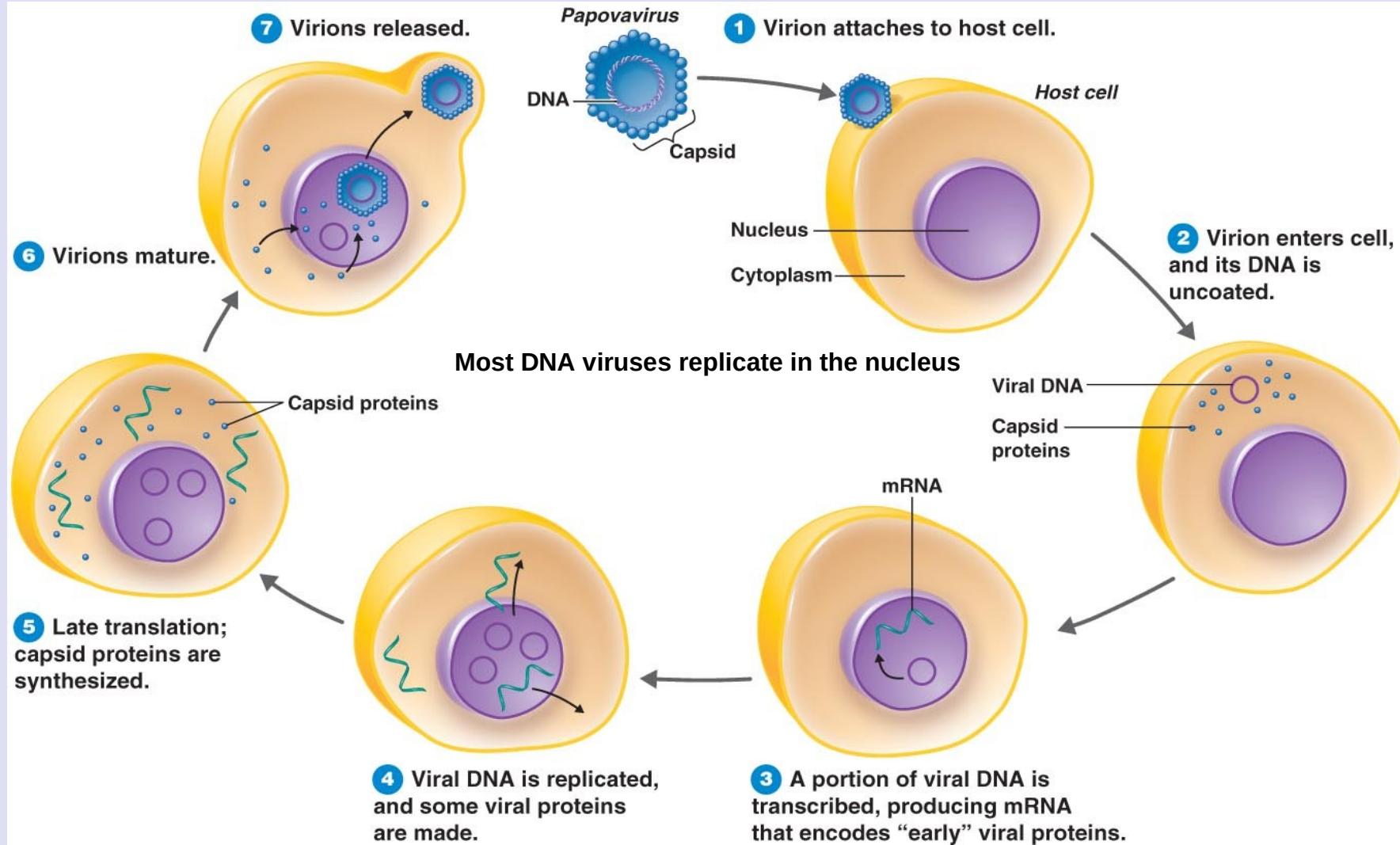
(replicative form) to replicate
dsRNA viruses copy both strands which then pair up

(note that the +ve RNA copied from -ve strand is also used as mRNA)

Retroviruses (ssRNA) use **host RNA polymerase** to replicate (& transcribe)

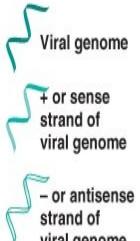
Life cycles of a ss DNA and +ssRNA virus

Replication and transcription of DNA Virus



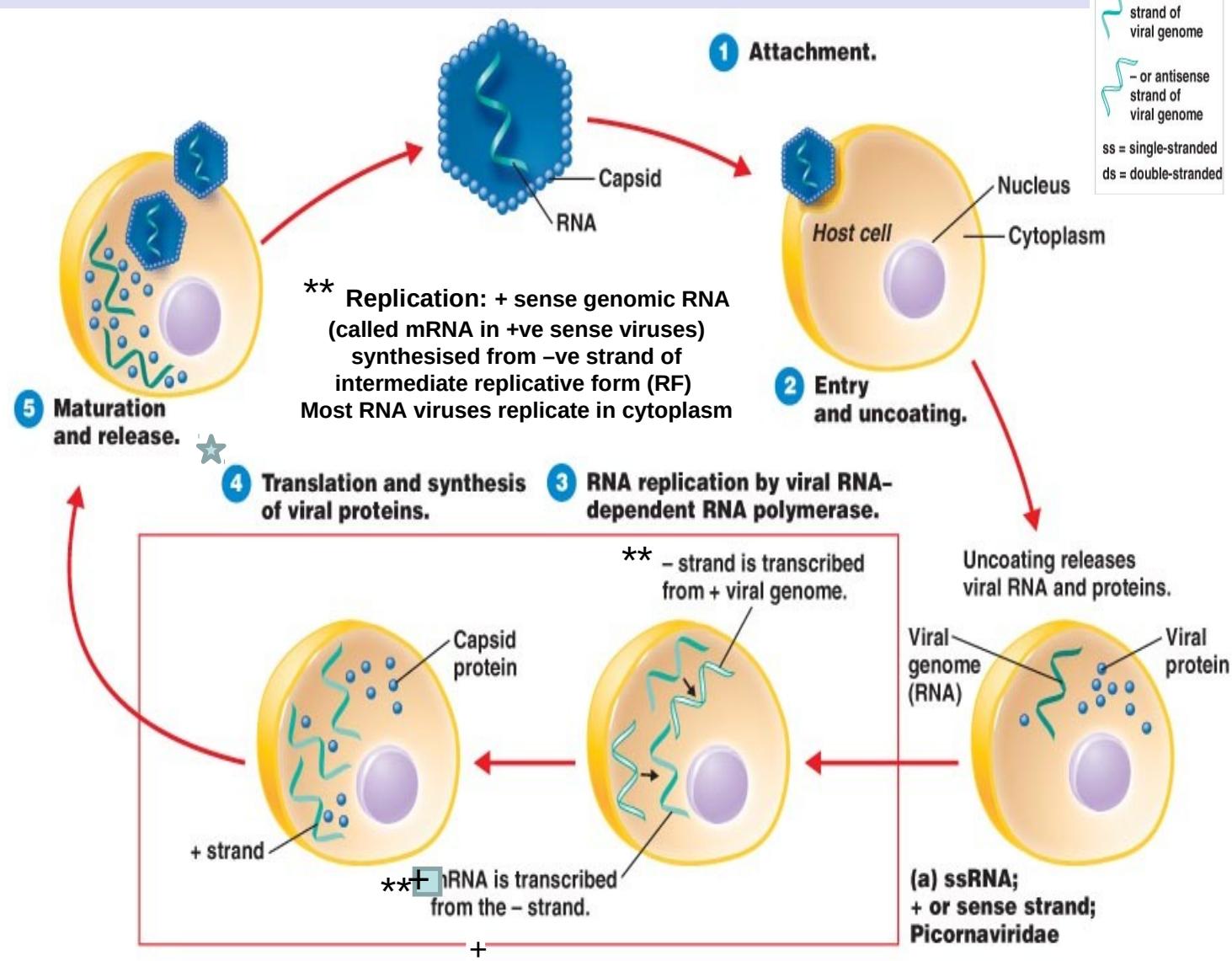
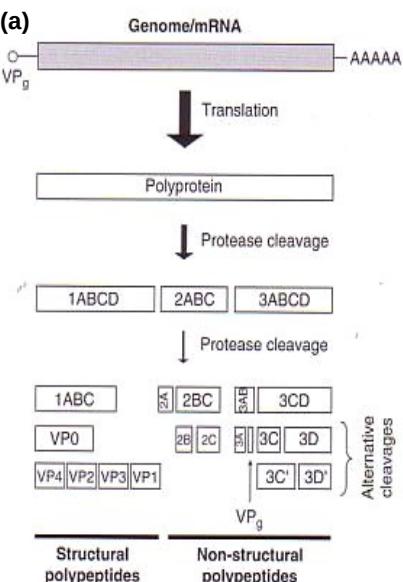
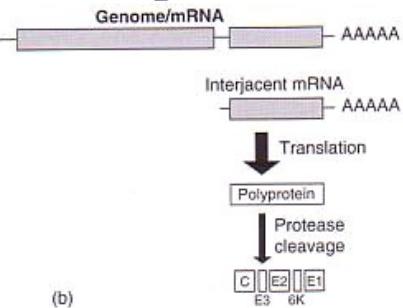
ssDNA viruses (+ or – sense) copy a complementary strand (dsDNA form) which is copied back to original viral genomic strand. The complementary strand of the ds DNA form is the template for mRNA synthesis

dsDNA viruses use either strand to make mRNA and copy both strands in a semi-conservative way like host DNA

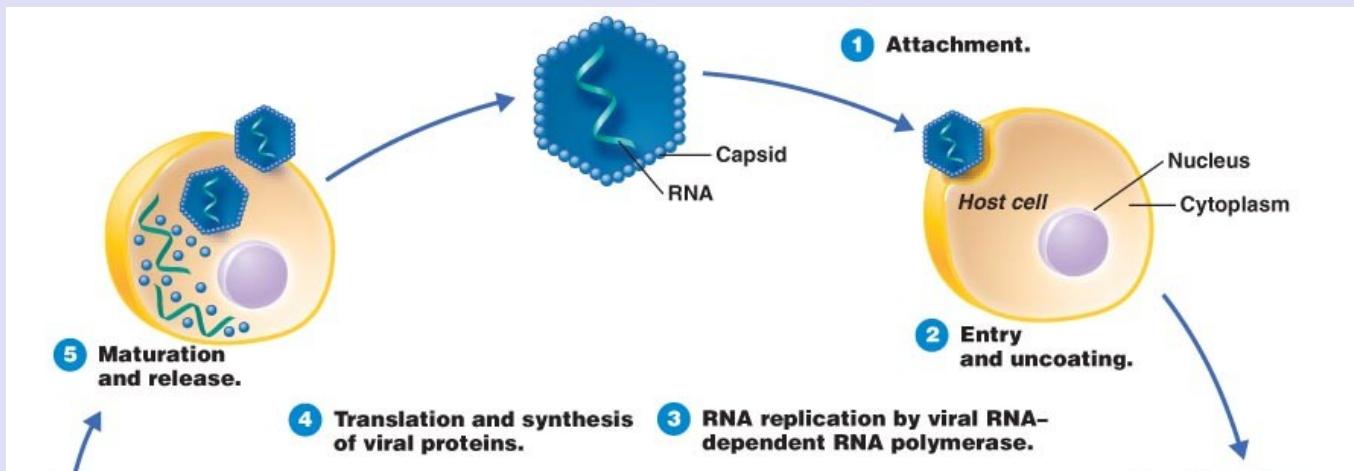


Sense Strand (+ Strand) RNA Virus

By sub-genomic mRNA from virus genomic + RNA (b) or by polyproteins directly translated from RNA (with Vpg or caps at 5" end and poly A tailed)



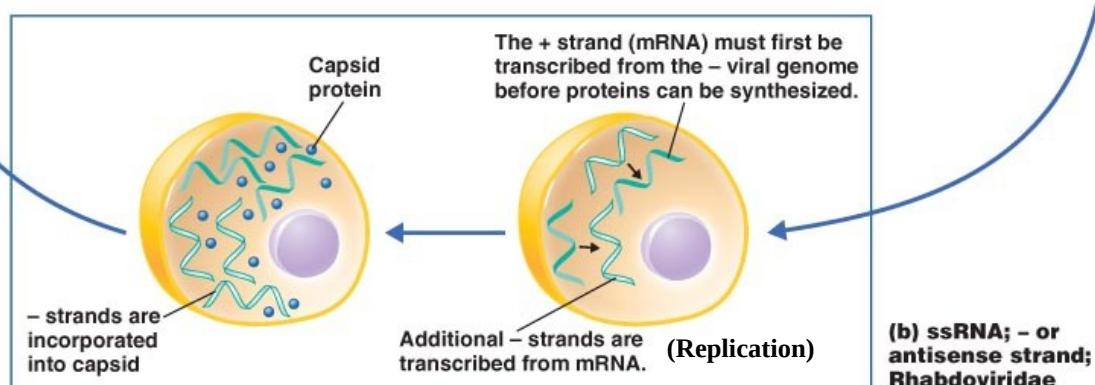
Antisense Strand (- Strand) ss RNA Virus



Note: mRNA (+) synthesized by virus-encoded RNA -dep RNA polymerase present in virus particle; this +sense RNA is template for replication and transcription

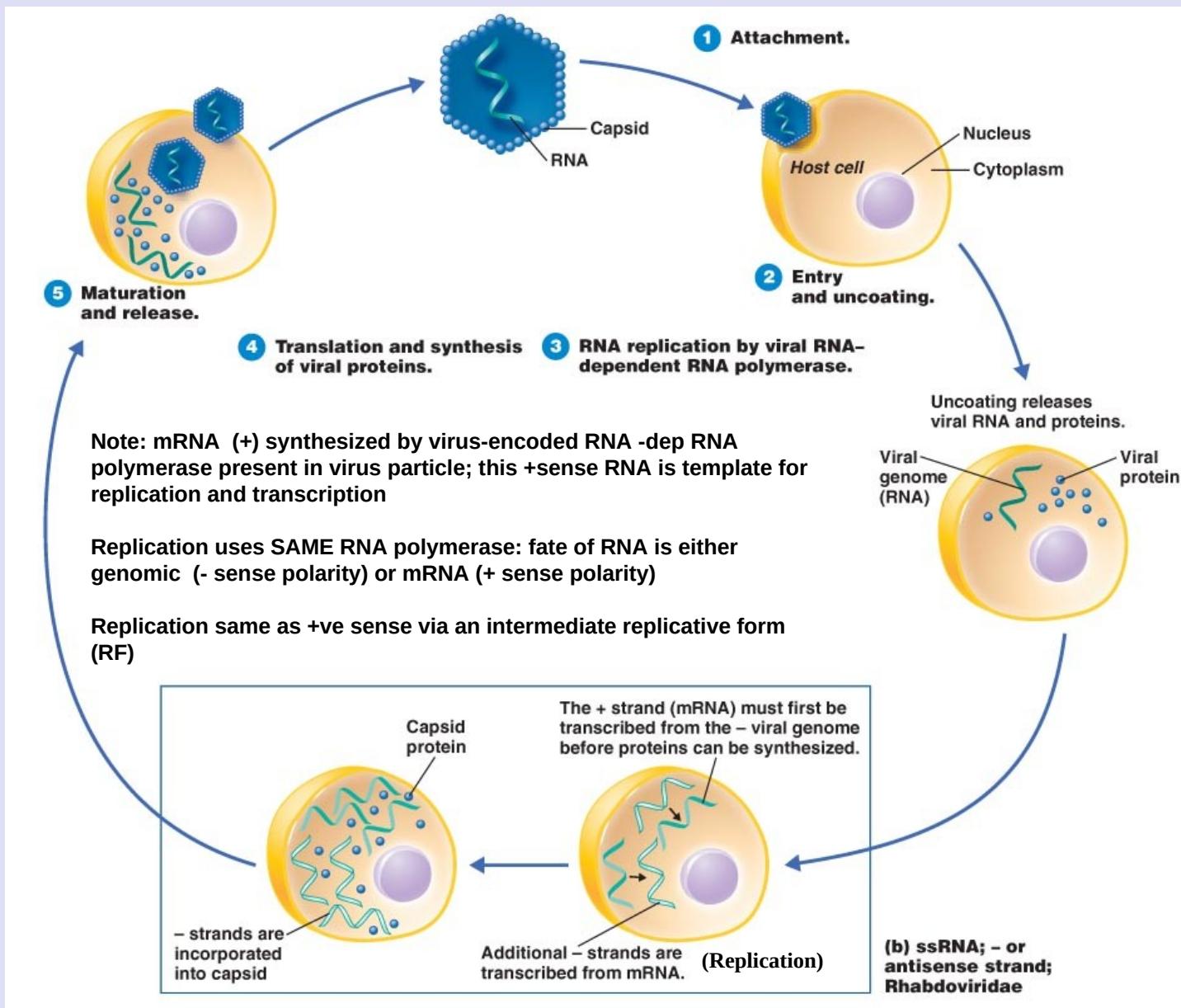
Replication uses SAME RNA polymerase: fate of RNA is either genomic (- sense polarity) or mRNA (+ sense polarity)

Replication same as +ve sense via an intermediate replicative form (RF)



KEY
Viral genome
+ or sense strand of viral genome
- or antisense strand of viral genome
ss = single-stranded
ds = double-stranded

Antisense Strand (- Strand) ss RNA Virus



VIRUSES AND DISEASE

- ACUTE, LATENT & PERSISTENT INFECTIONS
- EMERGING AND RE-EMERGING VIRUSES
 - ❖ INFLUENZA
 - ❖ HUMAN IMMUNODEFICIENCY VIRUS (HIV)
 - ❖ Zika virus
 - ❖ Bat viruses

VIRUSES AND INFECTION

- Virus infection of a higher organism is the cumulative result of all the processes of replication and gene expression in cells of tissues & immune response or activation (**innate and adaptive immunity**) by host
- Can last for a brief time or may encompass the entire life of the host
- Can be regarded as interaction of viruses and *intact organisms* or viruses and *cells*
- Virus infection induces 3 classes of antibodies (**humoral response**) i.e. IgG, IgM and IgA whose function is to neutralize the virus and prevent spread. Secretory IgA is important at mucosal surfaces while IgG is most NB for neutralisation of virus particles in the blood.
- **Cell-mediated immunity** is very NB in combating viral infections and is effected through 3 main mechanisms:
 - Non-specific killing of infected cells by natural killer (NK) cells

(active in early stages of viral infection & stimulated by interferon)

- Specific killing by cytotoxic T-lymphocytes (CD+8 CTLs)
- Antibody-dependent cellular cytotoxicity (mediated by NK & CTLs but requires Ab bound to virus antigen and IgG bound to CTL by Fc receptor); NK & CTLs can induce *apoptosis* of infected cells

VIRUS-HOST INTERACTIONS

- Site of entry of virus influences course of infection: egs. Skin; mucosal membranes of eye and genito-urinary (e.g. retroviruses; herpesviruses); alimentary canal (e.g. herpesviruses); respiratory tract (e.g. orthomyxoviruses; coronaviruses)
- Virus spread in the environment: some viruses are heat; UV -resistant such as water or food-borne viruses but many use a secondary vector for transmission between the primary hosts:
 - Horizontal transmission: direct host-to-host
 - e.g. human-human via aerosol is influenza and relies on high rate of infection.
 - e.g. animal-human (**direct**) such as *rabies virus*; animal-human (by vector such as mosquitoes), for example *bunyaviruses*
 - Vertical transmission: of virus from one generation of hosts to next e.g. mother-child via breastfeeding (e.g. HIV).
- Virus initiates infection by entering susceptible cell and can remain localized e.g. in dermis (papillomaviruses) or spread systemically e.g. herpesviruses primary infect genital or urinary tracts and secondary spreadreplication in central nervous system and lymphoid cells.
- Spread through host is (I) via cell-to-cell or (ii) via direct replication in the blood plasma in association with cells (**viraemia**) or (iii) nervous system
- Spread is controlled by virus's cell or tissue **tropism**

VIRUS-HOST INTERACTIONS cont.

- The immune response plays a large role in determining on the amount of secondary replication hence spread of virus
- IS also plays a large part in determining amount of cell or tissue damage; outcome of any infection is balance between IS clearing virus and putting pressure on virus to alter its antigenic composition e.g. influenza
- Viruses may replicate widely without significant cell death or damage e.g. retroviruses. Conversely picornaviruses for e.g lyse cells leading to fever and with poliovirus CNS damage and respiratory failure
- Another factor that determines outcome of virus infection is ability of virus to *persist* in the host (immunosuppression; downregulation of MHC1 proteins etc.)
- There are different types or patterns of virus infections:

Abortive: virus infects cell but cannot complete full replication cycle

Acute: virus replicates before symptoms; rapid onset; IS clears infection quickly

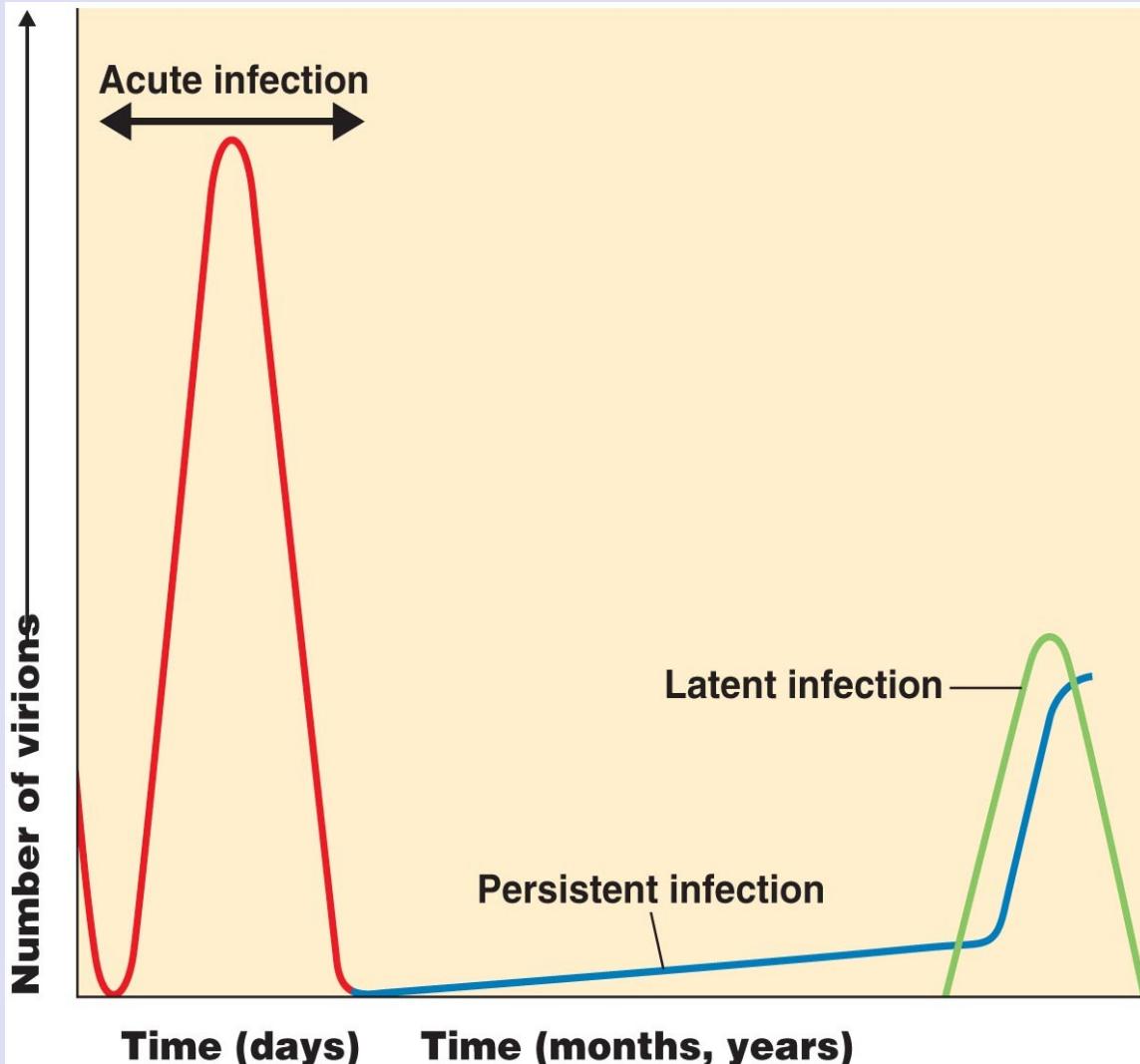
Chronic: Ongoing virus replication but virus adjusts pathogenicity to avoid killing host; virus eventually cleared by the host (exceptions Hepatitis B; 10% carriers)

Persistent: Same as chronic but virus persist in host for its entire lifetime. Not infrequently persistent infections may result from DIs that modify pathogenesis

Latent: Persist but virus able to downregulate its gene expression & establish an inactive state e.g. HSV travels via axons & hides in dorsal root ganglia of NS

Emerging and re-emerging diseases

Acute, Latent and Persistent Viral Infections

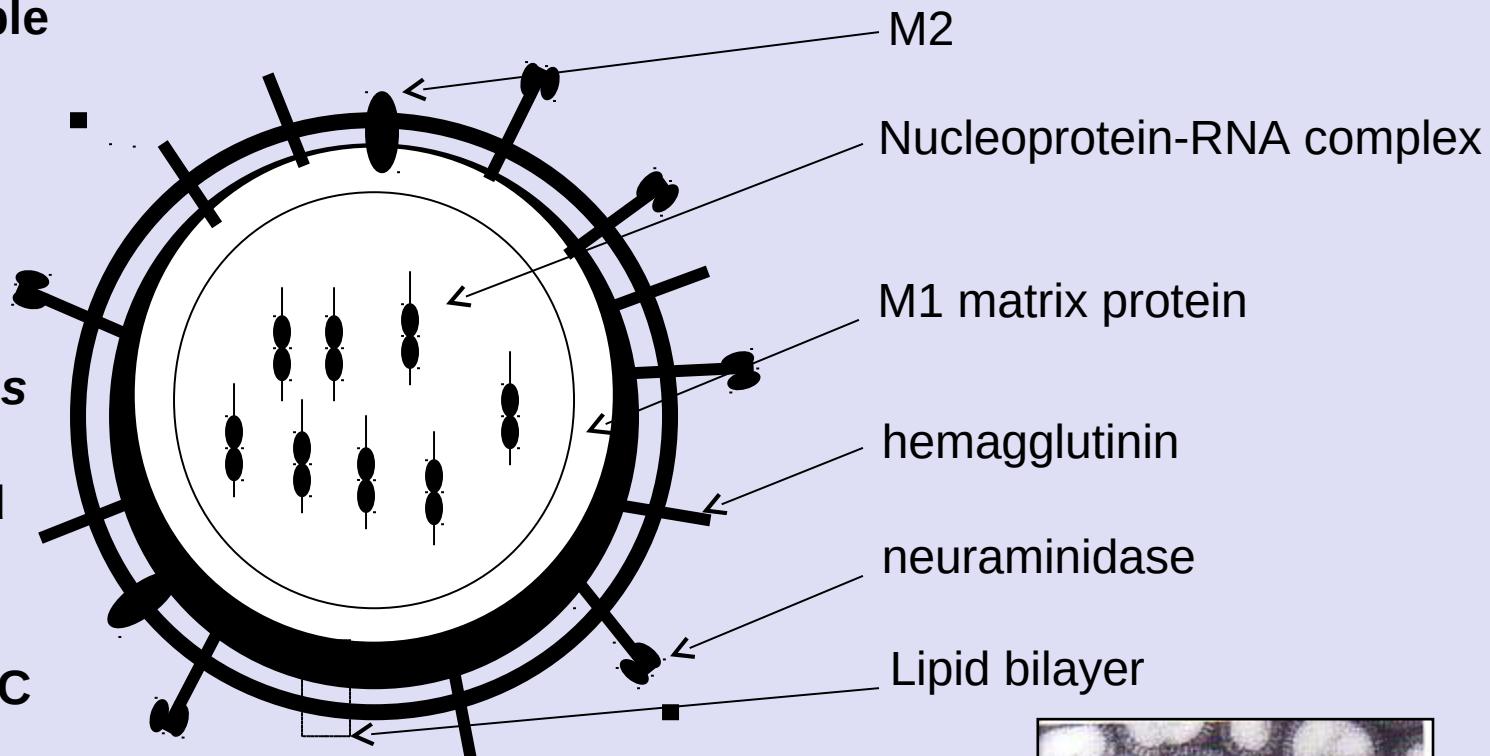


- **Acute:** Virus multiplies rapidly; acute symptoms for short time; virus cleared from body by immune system
- **Latent:** Virus remains in asymptomatic host cell for long periods; IS does not clear
 - Cold sores, shingles
- **Persistent:** Disease processes occurs over a long period (generally lifetime of host); IS does not clear;
 - can be fatal e.g. HIV

Orthomyxoviridae

- Single-stranded RNA, -ve sense strand, multiple RNA strands
- Envelope spikes can agglutinate RBCs
- *Influenza virus* (influenza viruses A and B have 8 segments)
 - Influenza C virus (7 segments)

Schematic diagram of Influenza A virion structure



All RNA segments replicate separately and are required for a productive infection

Influenza Epidemics

- flu considered to be a mild disease
- Major killer of the aged and immune compromised
- able to build up effective immune response
- periodic outbreaks where prior protection ineffective
- due to virus broad host range; natural reservoir of influenza A is wild birds
- genome segment *re-assortment* where viral mutations able to effectively disseminate
- *antigenic drift*: gradual accumulation of mutations in genome (neutralizing Ab pressure), affecting surface proteins (antigenicity) & leading to decrease in recognition by IS; occurs in all viruses but at different rates. IS in response adapts .
- Genome segment re-assortment between diff antigenic types can lead to sudden dramatic changes in the antigenic properties – *antigenic shift* (problem exacerbated by sharing hosts e.g. pig virus – human virus sharing a cell) leading to worldwide epidemics (*pandemics*); *have been 4 major shifts*; shift accompanied by abrupt change in HA and NA subtype
- Reassortment between pig and avian varieties often cause of antigenic shift leading to new outbreak strains

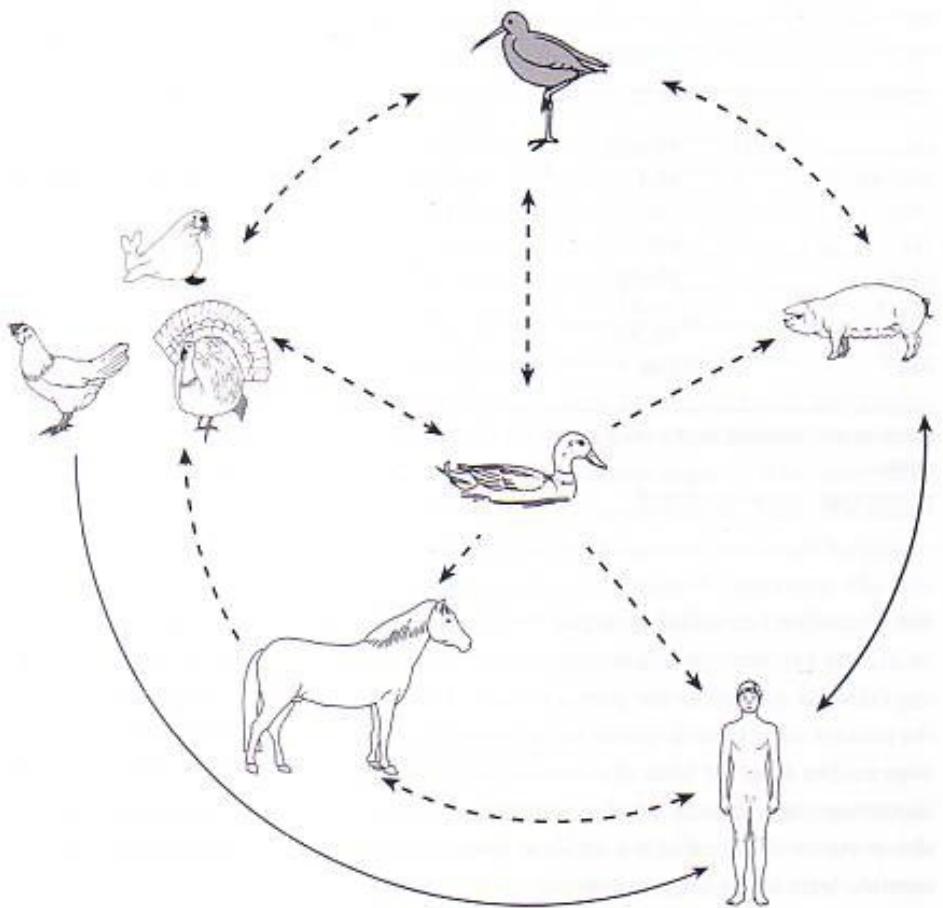
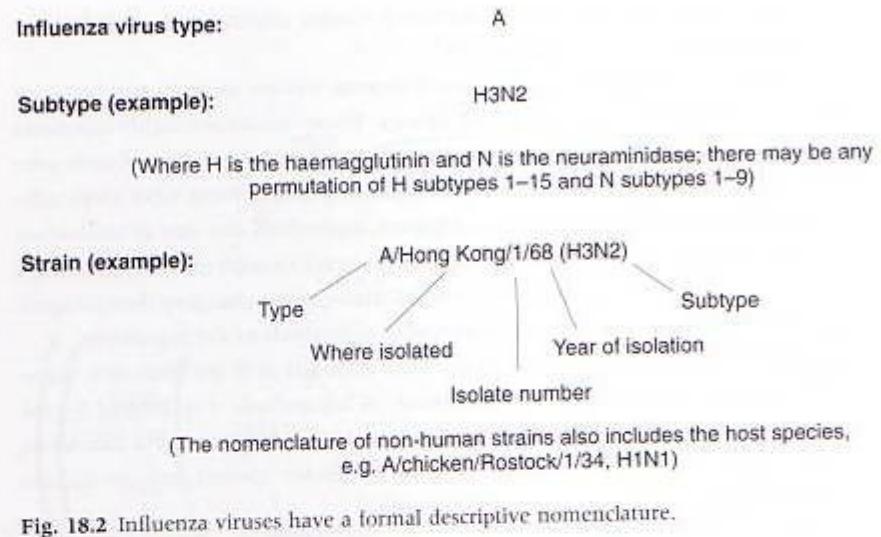
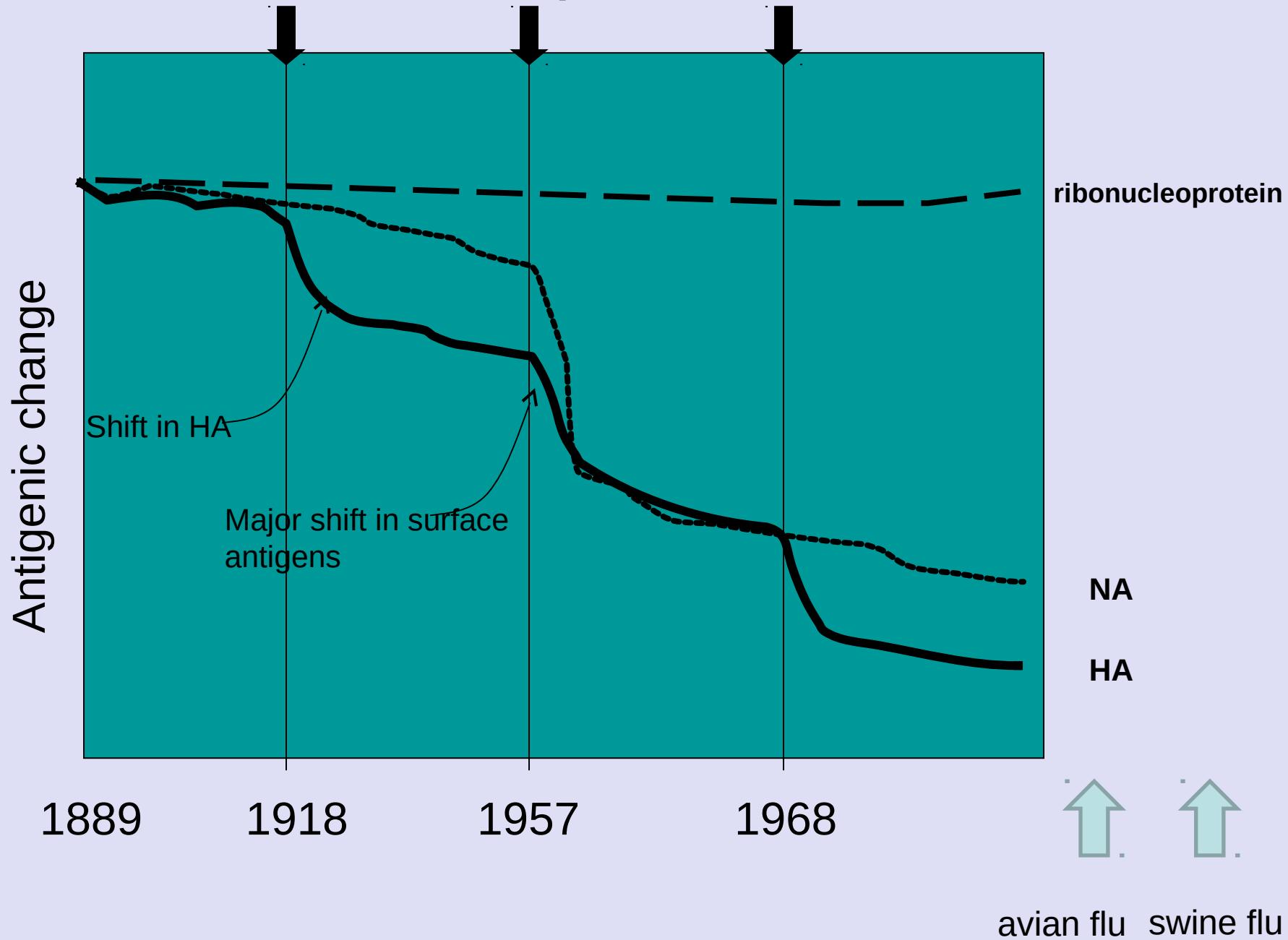


Fig. 18.1 Animal species that are naturally infected with influenza A viruses. Wild birds of the sea and shore form the natural reservoir (top, shaded). Known routes of transmission are indicated by continuous arrows and probable routes of transmission by dashed arrows.



Influenza epidemics



INFLUENZA & ANTIGENIC SHIFT AND DRIFT

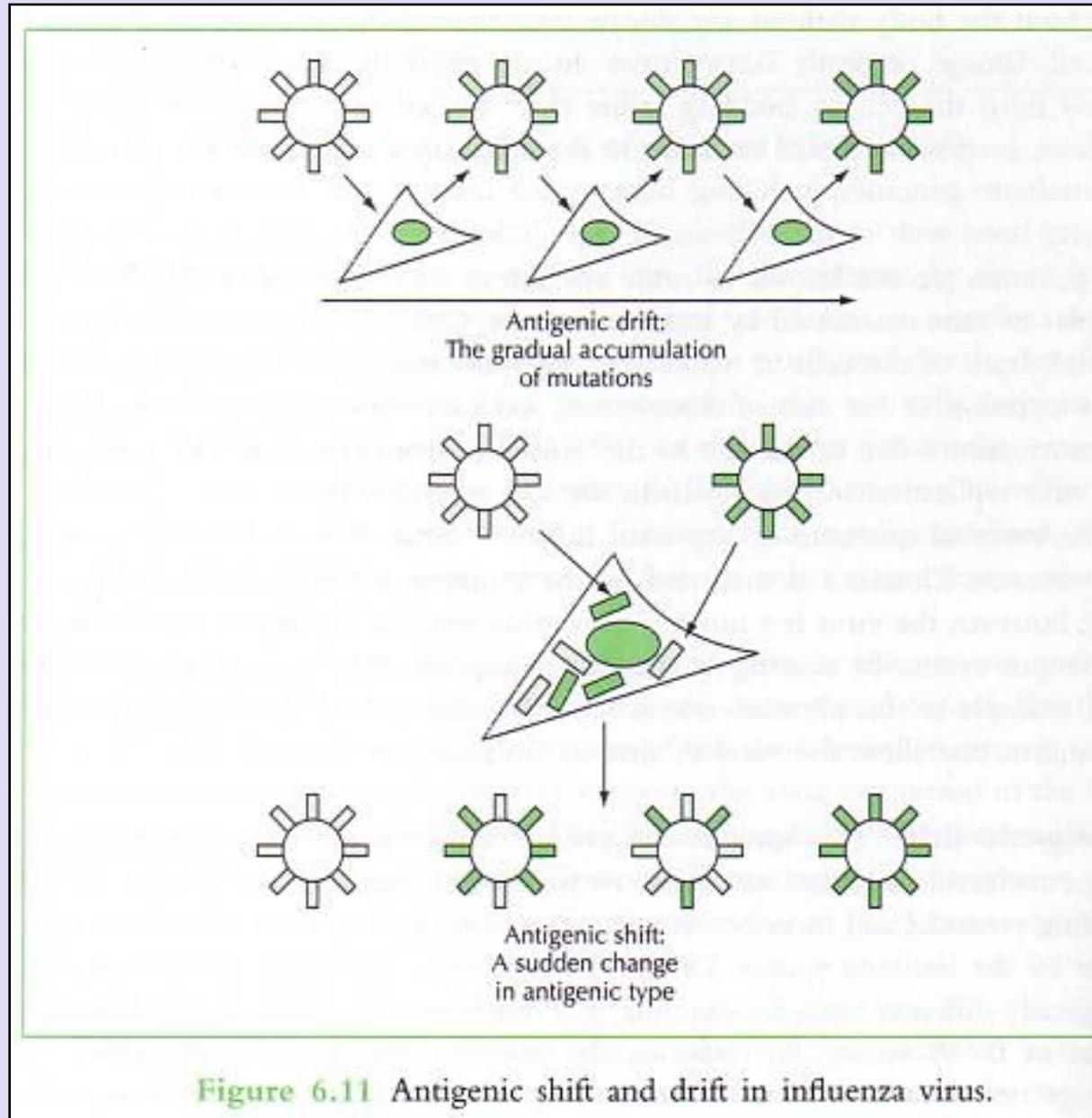
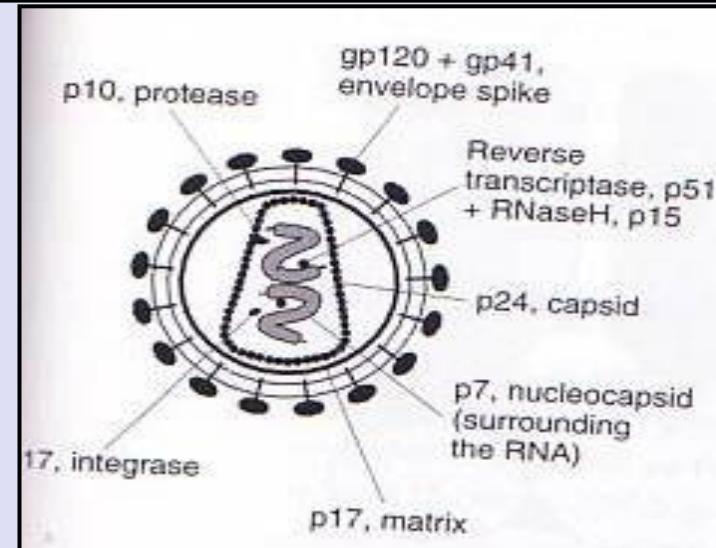
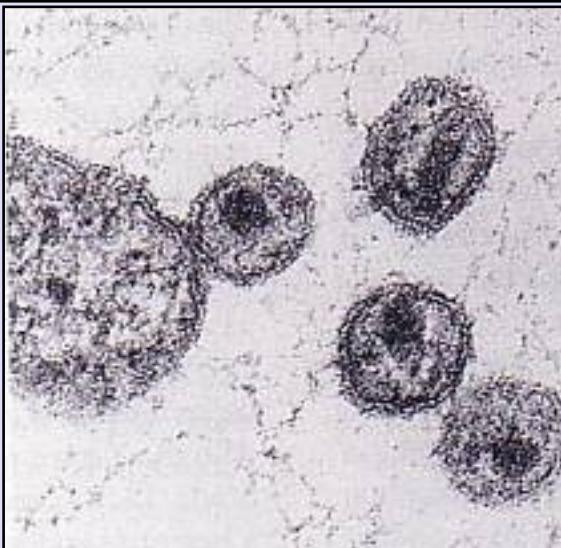


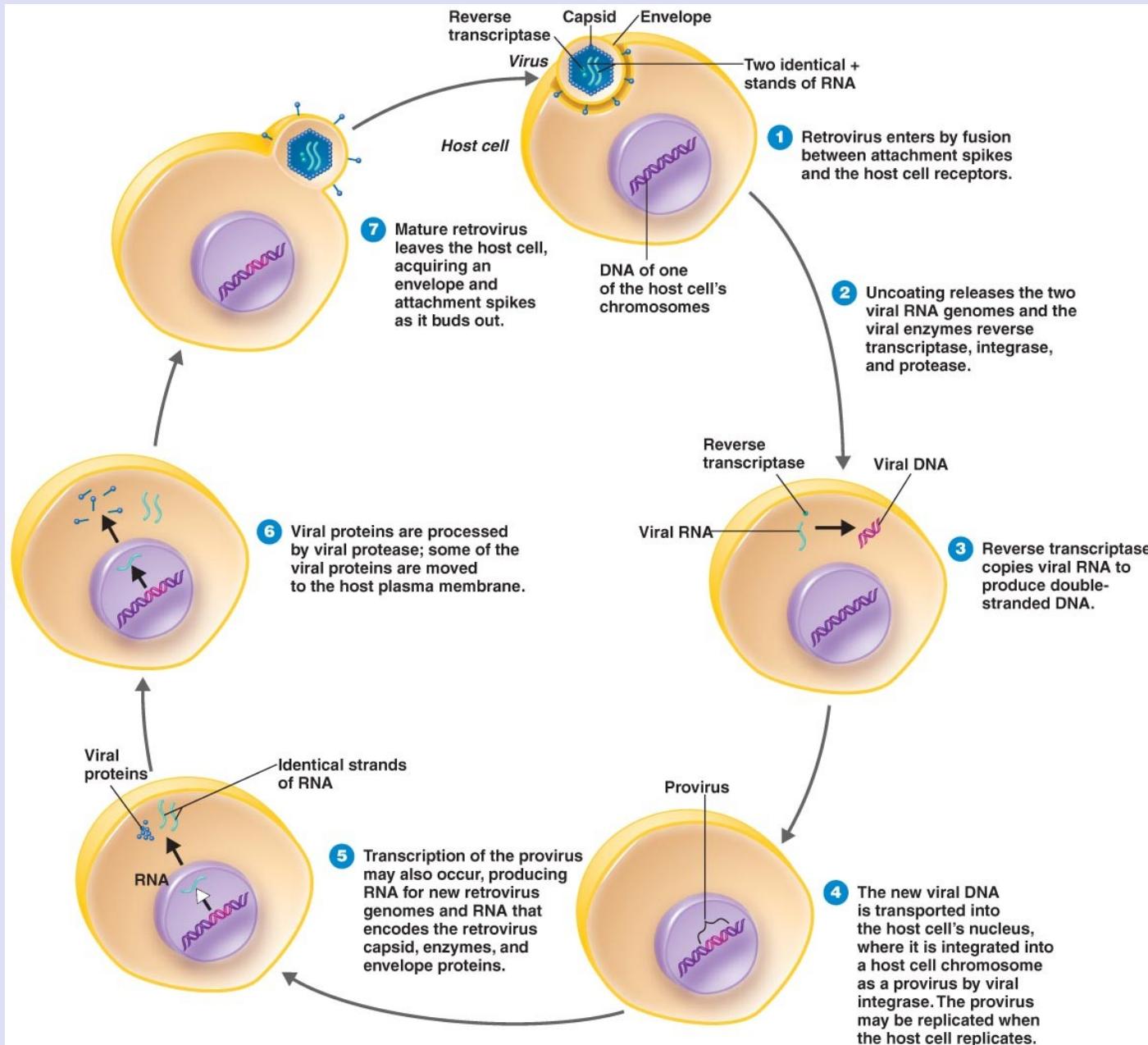
Figure 6.11 Antigenic shift and drift in influenza virus.

HUMAN IMMUNODEFICIENCY VIRUS (HIV)



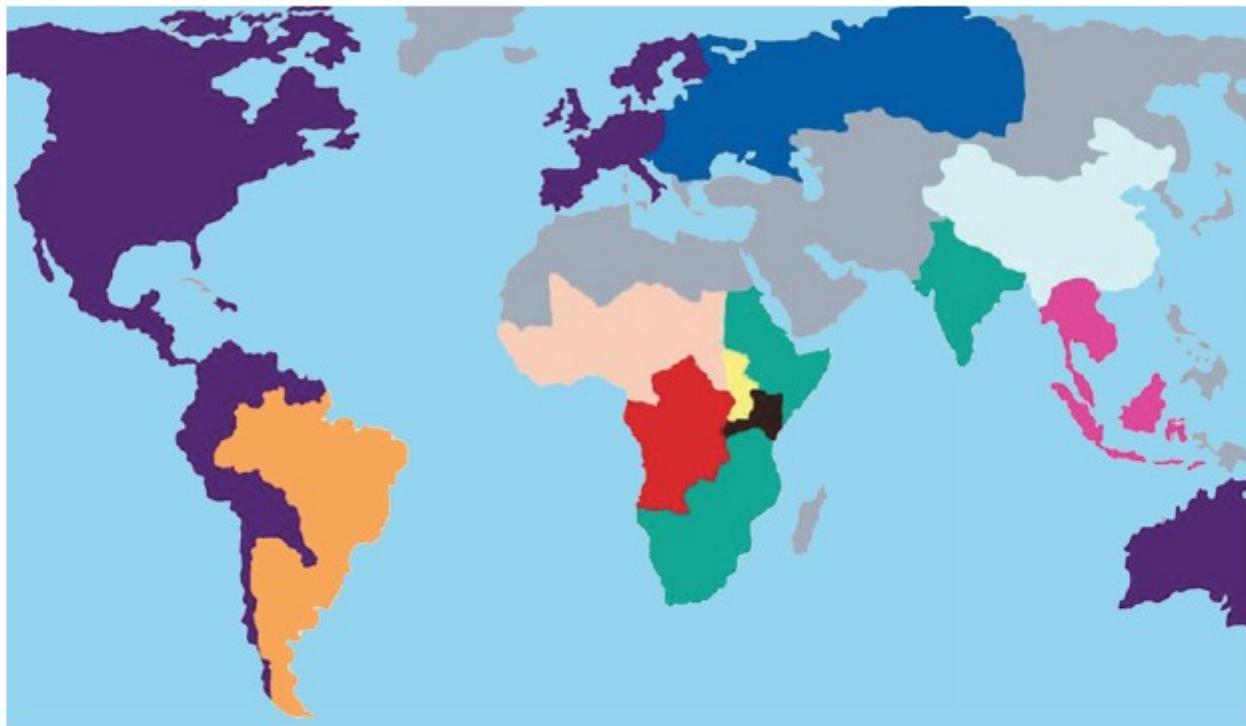
- Lentivirus; highly pathogenic
- HIV-1 is a collection of evolutionary linked clades based on nt sequence of *gag* or *env* genes.
- 9719 nt long; 16 proteins
- Are diploid (two identical ssRNA molecules); 3 ORFs: *gag*, *pol* and *env* which are transcribed and translated into polyproteins which are cleaved by proteases; regulatory proteins expressed from spliced RNAs
- ssRNA is reverse transcribed into dsDNA which integrates into host genome
- Require cellular tRNA for replication primer; ss viral RNA genome transcribed/replicated by host RNA pol II from provirus (integrated dsDNA)
- The provirus is under host cell control and is transcribed as are other cellular genes. Cellular transcription factors bind to promoter sites in LTRs and promote virus gene transcription.
- HIV can however positively or negatively regulate expression of their genetic information using Tat, Rev, Vif, Vpr, Vpu & Nef proteins; e.g. Tat proteins stimulate virus gene transcription and Rev proteins regulates balance of expression between virion structural proteins and regulatory proteins

Life cycle of a Retrovirus e.g. HIV



- The viral DNA that is integrated into the host genome is called a **provirus**
- Unlike a prophage, a provirus remains a permanent resident of the host cell
- The **host RNA polymerase** transcribes the proviral DNA into mRNA molecules for synthesis of viral proteins and full length RNA molecules (genomes) for new virus particles released from the cell

Global distribution of HIV-1 subtypes and recombinants



B

B, BF recombinant

CRF02_AG, other recombinants

F, G, H, J, K, CRFO1 other recombinants

A

C

D

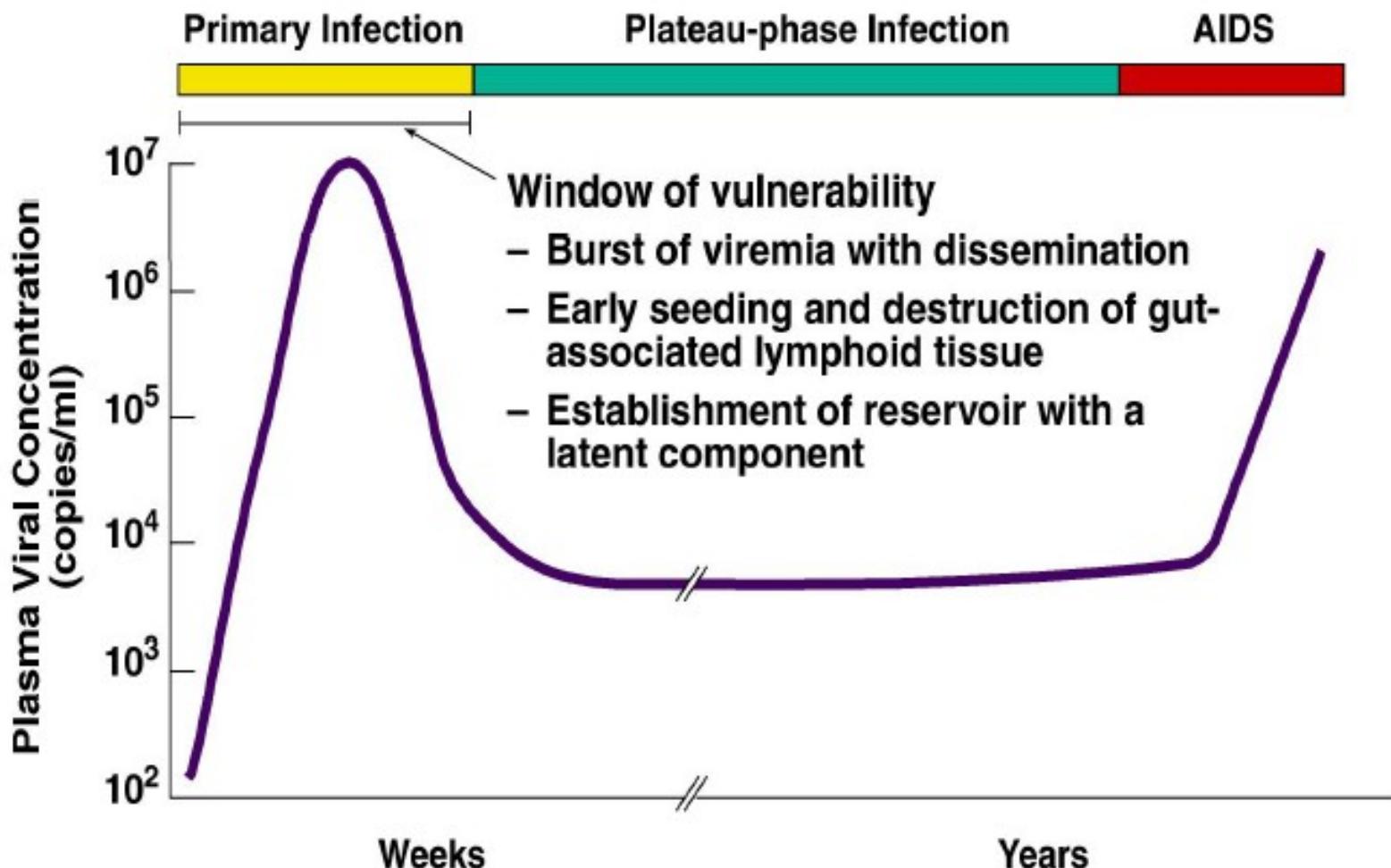
A, B, AB recombinant

CRFO1_AE, B

B, C, BC recombinant

Insufficient data

Course of HIV Infection: Without Intervention



HIV PREVENTION & TREATMENT

- **PREVENTION**
 - Sexual behaviour: condoms; testing; no. of partners; education
- **TREATMENT**

Antiretroviral drugs (HAART)

- Nucleotide Reverse transcriptase inhibitors
- Non-nucleotide RT inhibitors
- Protease inhibitors

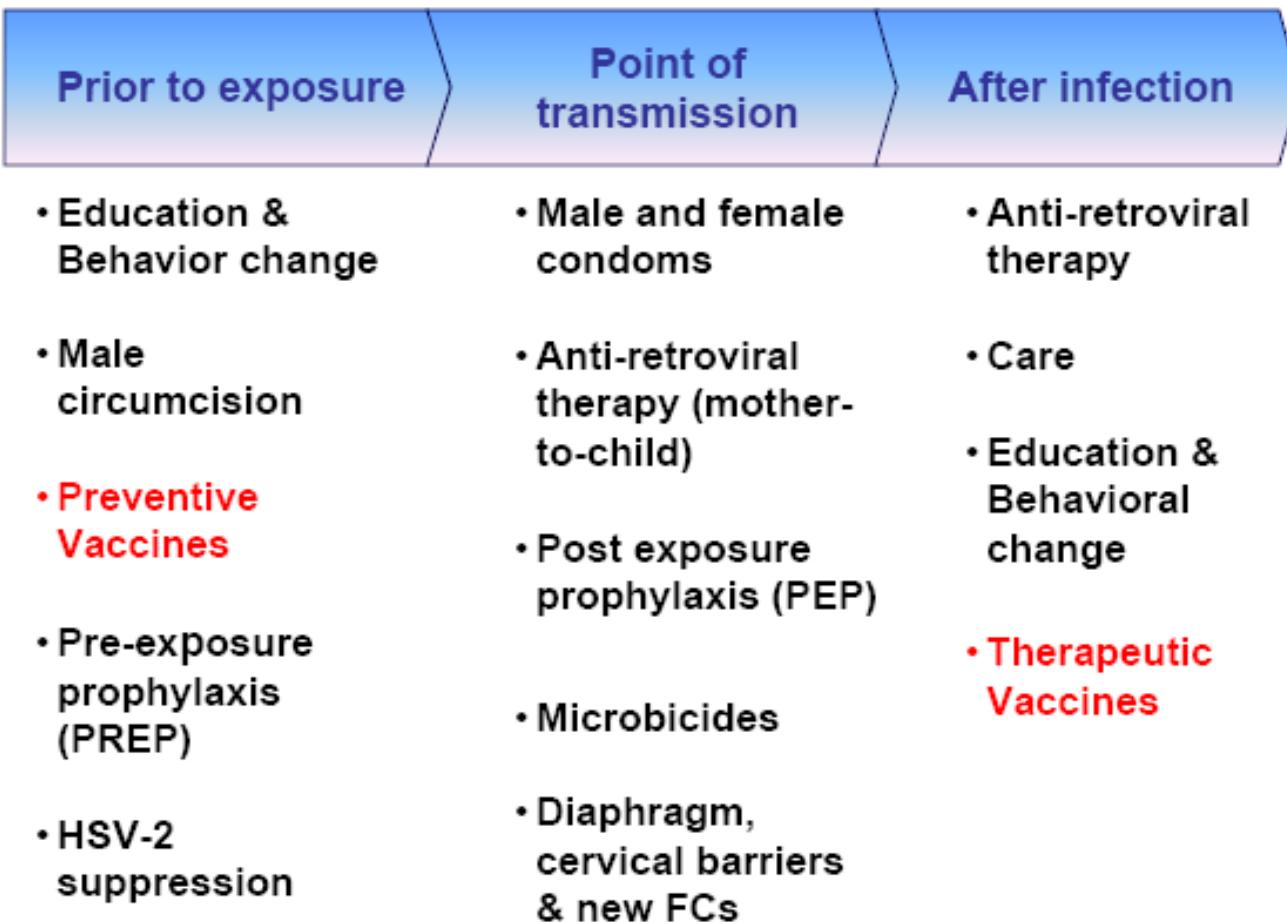
New strategies

- Integrase inhibitors
- Receptor blocking

Vaccines

Prevention better than cure!

HIV/AIDS Prevention Toolkit





HIV VACCINE UPDATE

What is a vaccine?



- A vaccine is a substance that stimulates an immune response that can either prevent an infection or create a resistance to an infection.
- No vaccine is 100% effective - Most are between 70% and 95% effective
- Vaccines provide both an **individual benefit** and a **public health benefit**

Why is it so difficult to make an HIV vaccine?

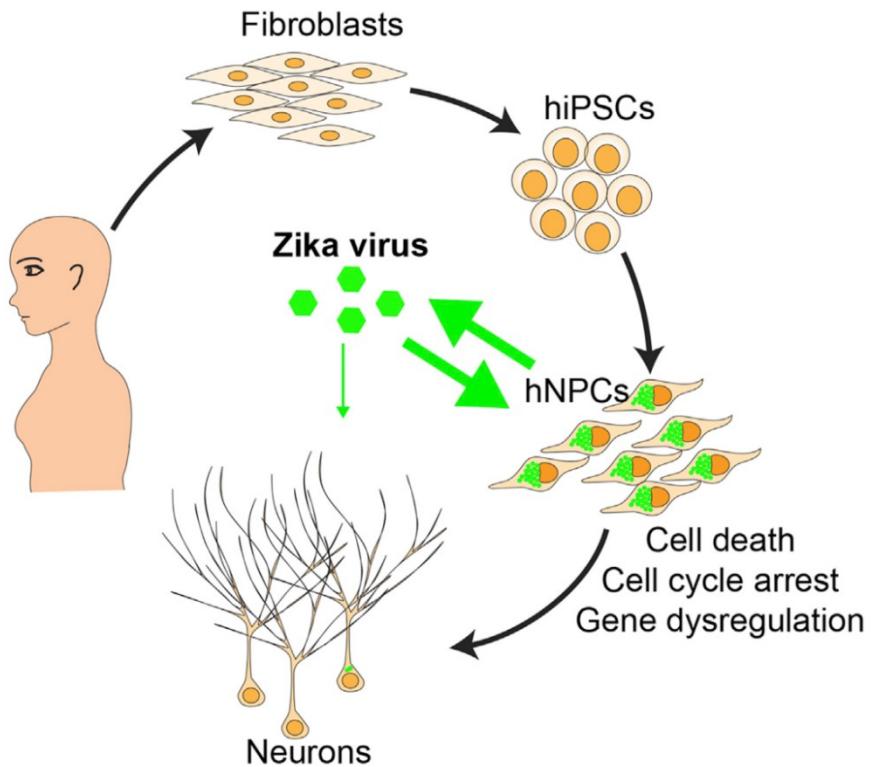
- Nobody has ever recovered from HIV infection, so there is not natural mechanism to imitate
 - HIV destroys the immune system cells that are meant to fight against it
 - HIV inserts its genetic material into human cells, where it remains hidden
 - HIV RNA mutates: proteins are highly variable and constantly changing to avoid IS
 - There are no good animal models to test vaccines
 - Antibody neutralization-sensitive sites on HIV are masked
-

Facts about Zika virus



- The Zika virus belongs to the family *Flaviviridae* family and the genus *Flavivirus*, thus is related to the dengue, yellow fever, Japanese encephalitis, and West Nile viruses.
- Like other flaviviruses, Zika virus is enveloped and **icosahedral** and has a **nonsegmented**, single-stranded, 10-kilobase, **positive-sense** RNA genome.
- ✓ The Zika virus is carried by infected mosquitos of the *Aedes* genus and spreads aggressively
- ✓ Following the first report in Brazil in May 2015, the virus has now spread to at least 25 countries.
- ✓ While symptoms for the virus are relatively mild, the risk to unborn children is more severe, and can lead to significant birth defects.
- ✓ Interestingly, the virus had gone years without an outbreak of this proportion, so why the sudden change? A recent report, currently under peer review, suggests that **mutations to the viral genome** may have contributed to its ability to infect cells so easily.
- ✓ Specifically, **NS1 codon usage adaptation (in neural cells)** may have enabled viral replication

Zika virus infection of neural progenitor cells

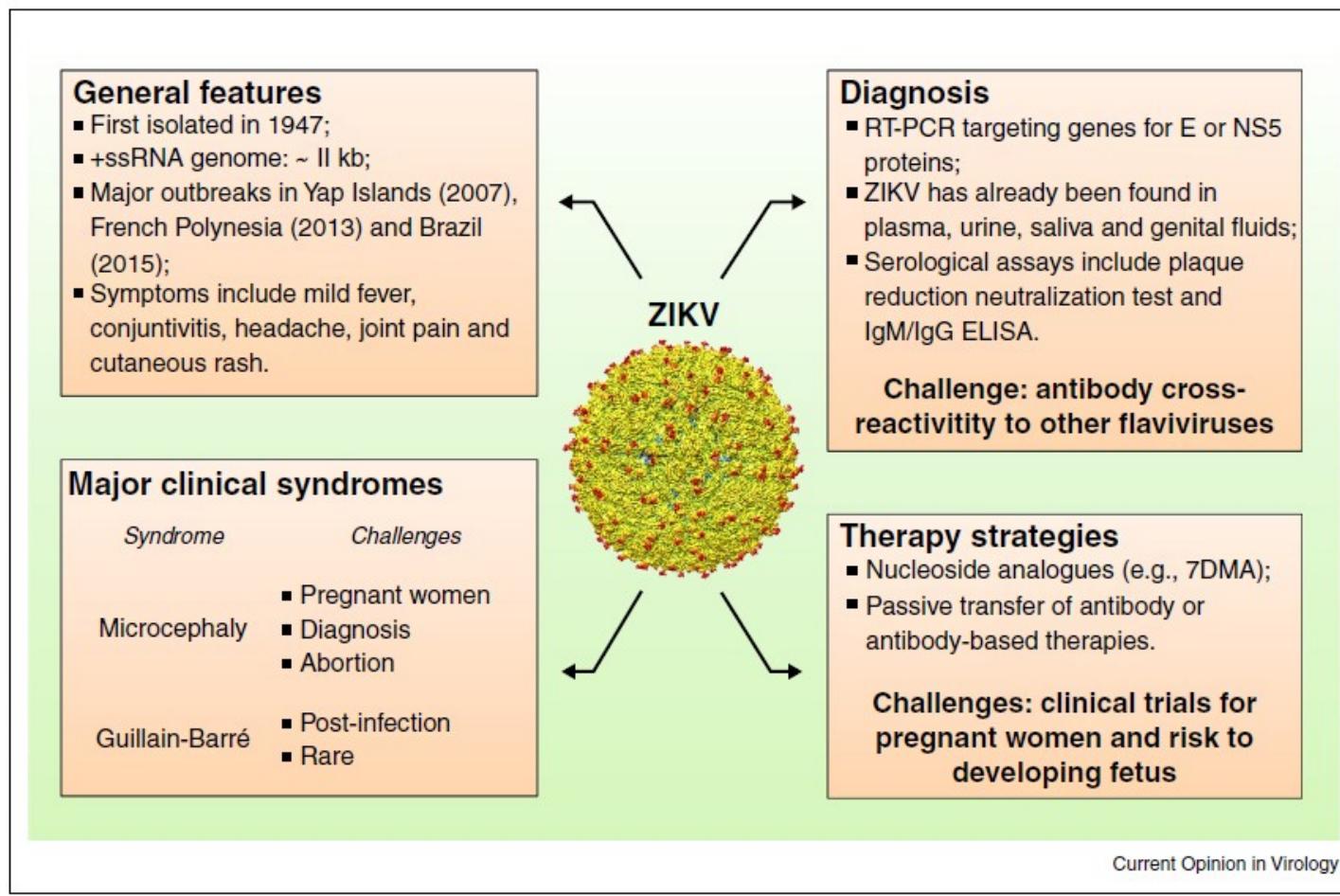


Zika virus (ZIKV) infects human embryonic cortical **neural progenitor cells** (hNPCs)

ZIKV-infected hNPCs produce infectious ZIKV particles

ZIKV infection leads to increased cell death of hNPCs

ZIKV infection dysregulates cell cycle and transcription in hNPCs



ZIKV features and challenges in ZIKV research. General features of ZIKV biology and ZIKV infection are listed, together with major clinical syndromes observed during the epidemic in Brazil. The main challenges faced by the research community are the development of reliable diagnostic tests, and the development of an adequate treatment for pregnant women. The three-dimensional structure of ZIKV was adapted from Sirohi *et al.* (2016) [4].

Zika crisis in Brazil: challenges in research and development

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Amélia Maria Ribeiro de Jesus³, Roque Pacheco de Almeida³
and Mauro Martins Teixeira²