Structural Virology

Lecture 4

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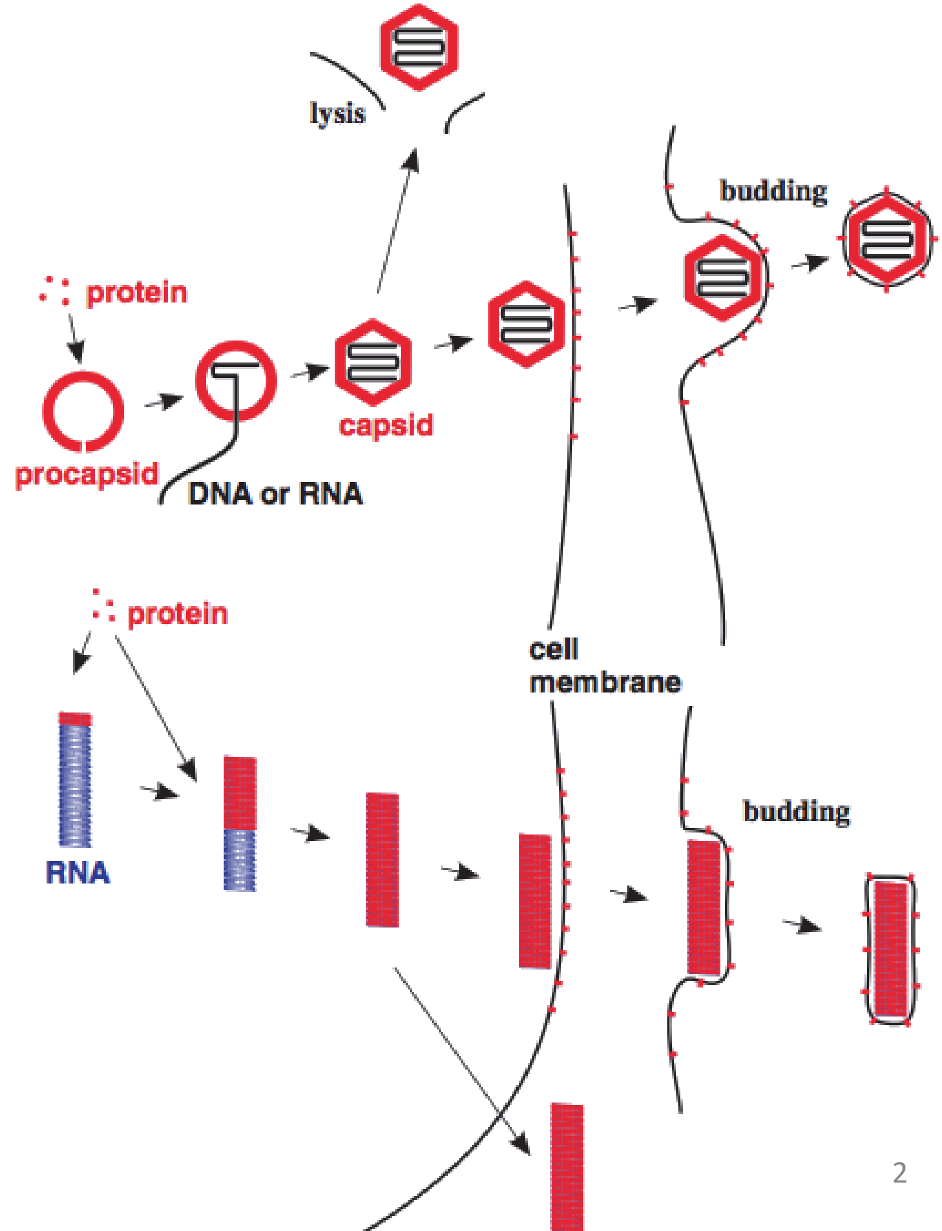
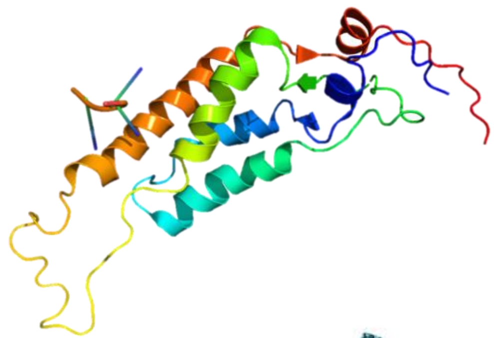
Assembly and exit of virions from cells

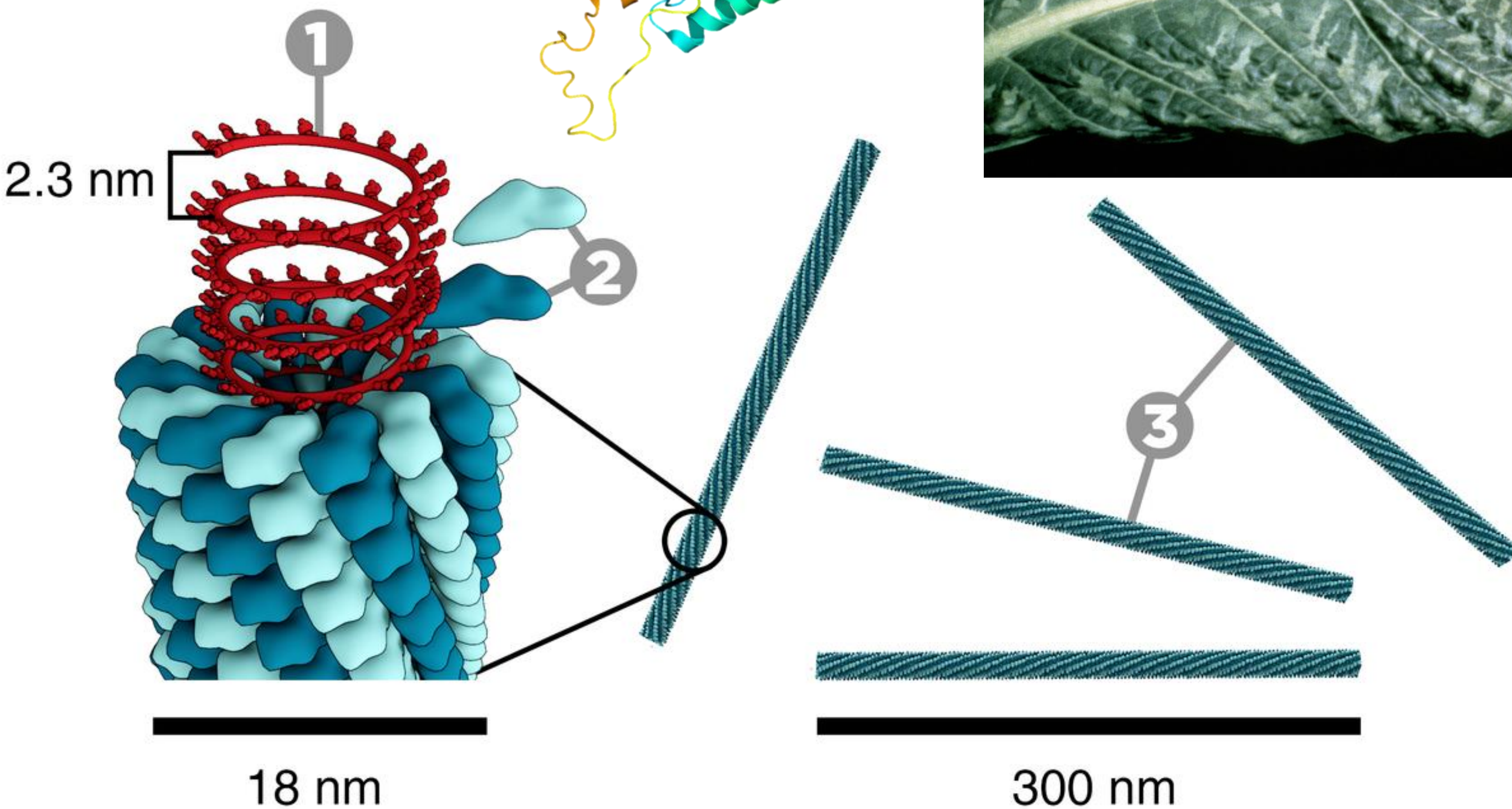
1. **A**ttachment
2. **E**ntry
3. **T**ranscription
4. **T**ranslation
5. **G**enome replication
6. **A**ssembly
7. **E**xit



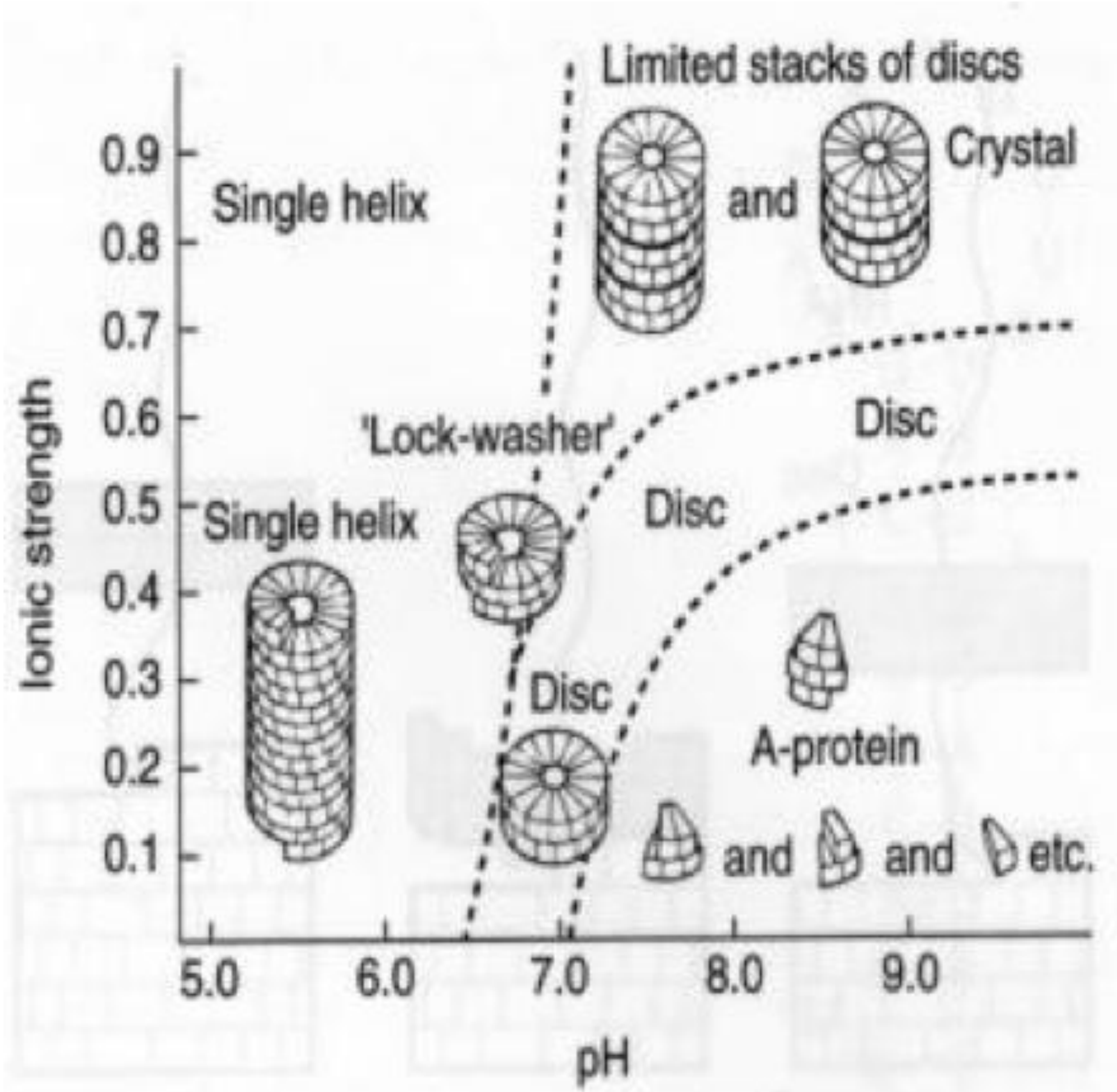
Assembly of helical viruses

Tobacco mosaic virus (TMV)

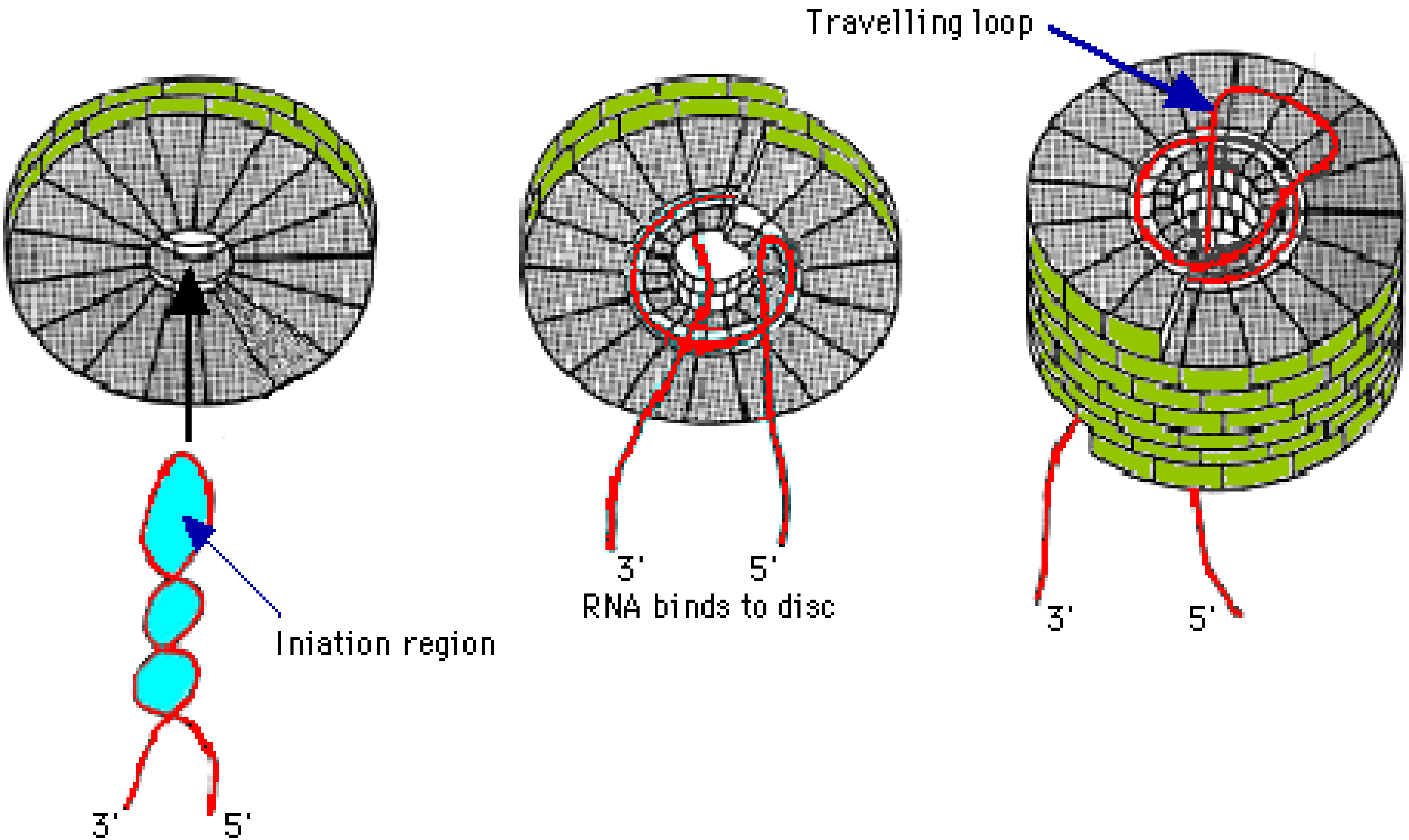




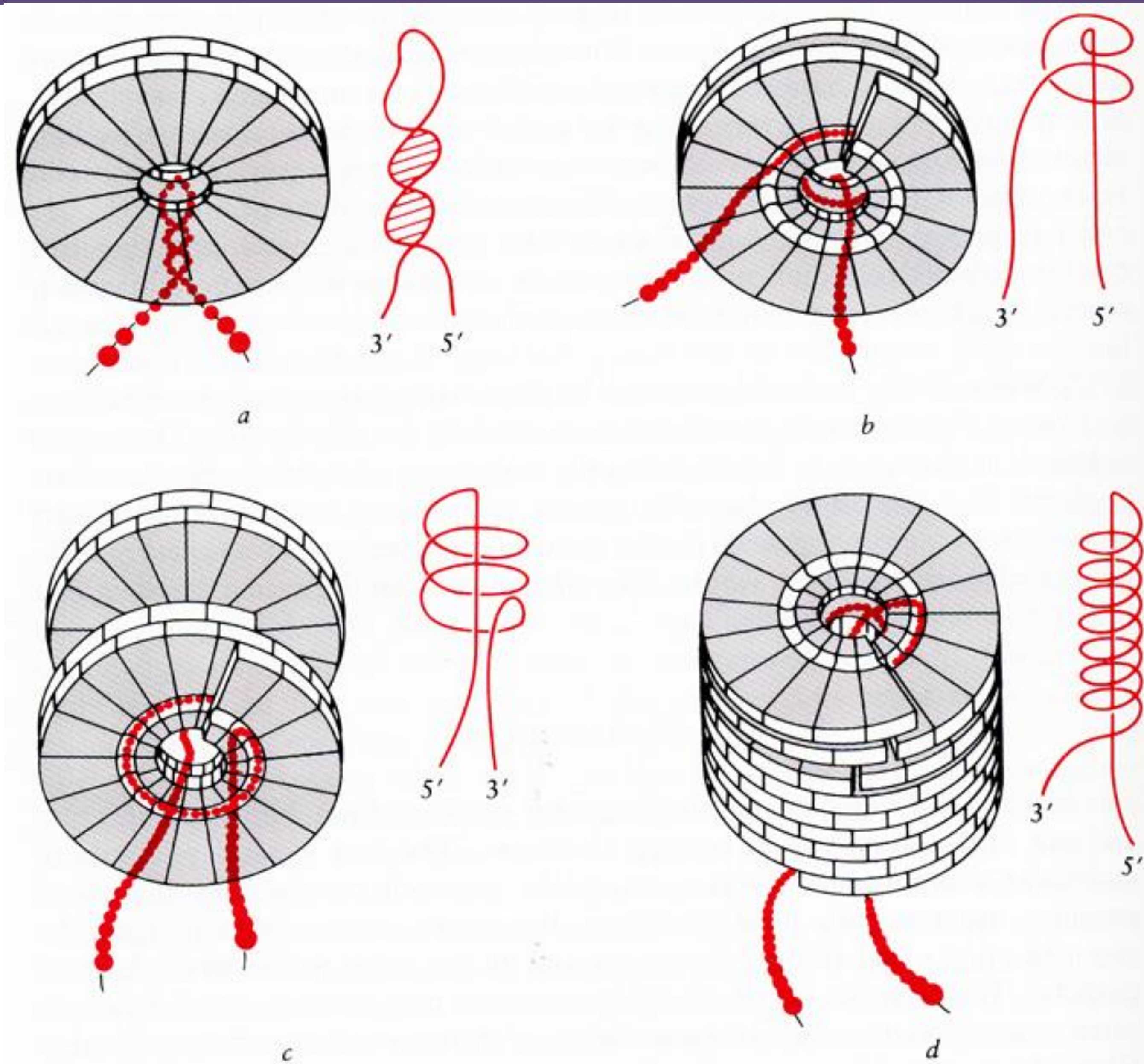
Effects of pH and ionic strength on formation of TMV capsid protein aggregates

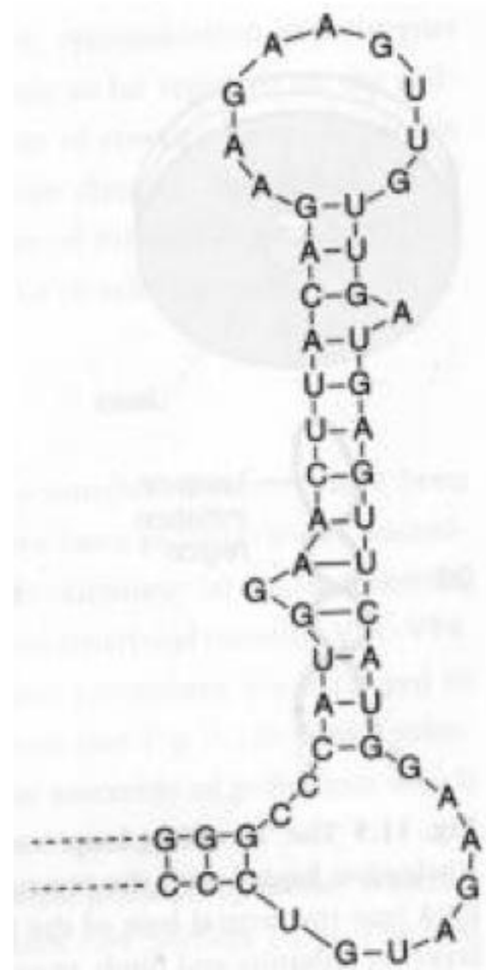


TMV initiation of assembly

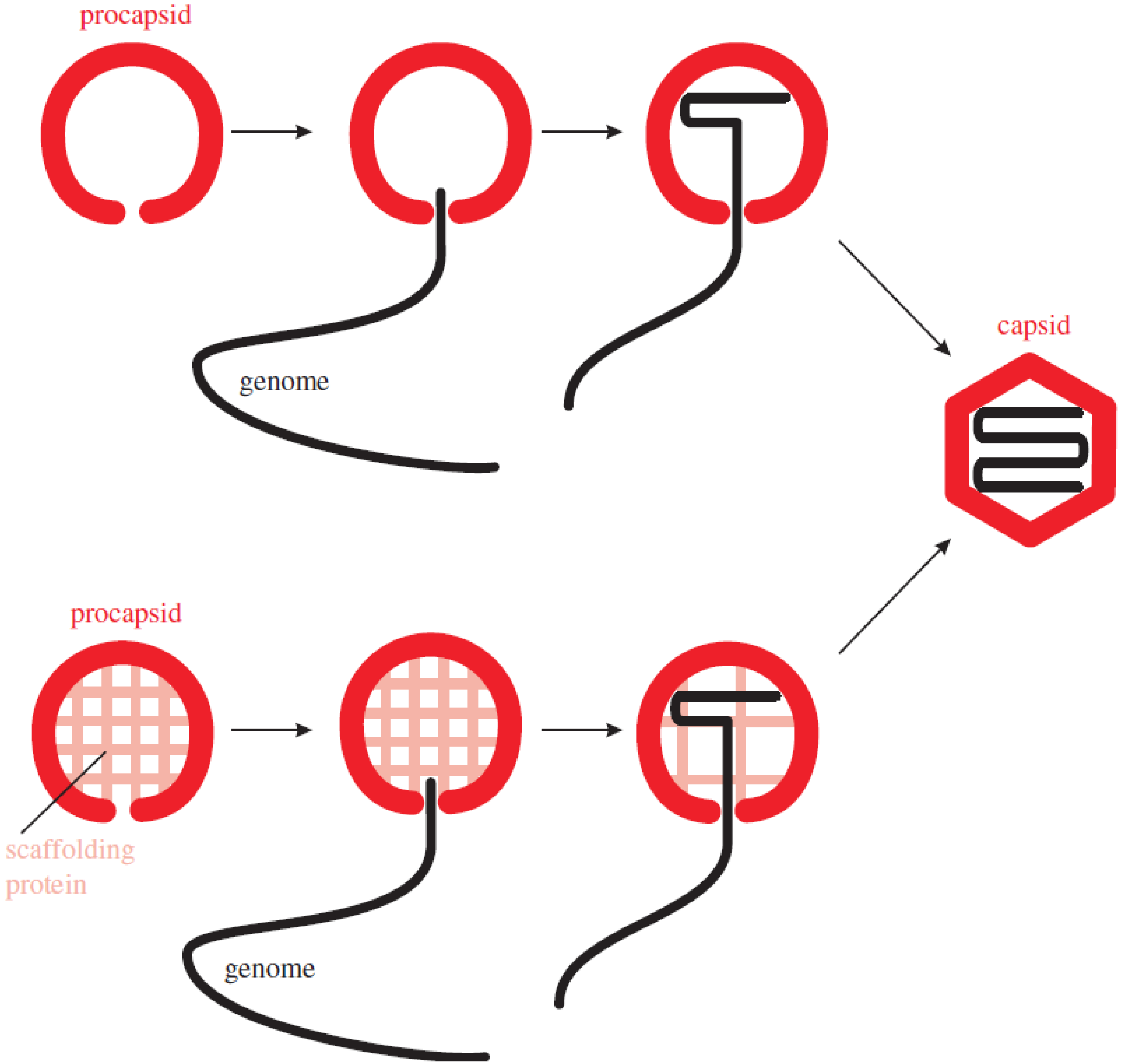


TMV assembly (GMO tobacco)

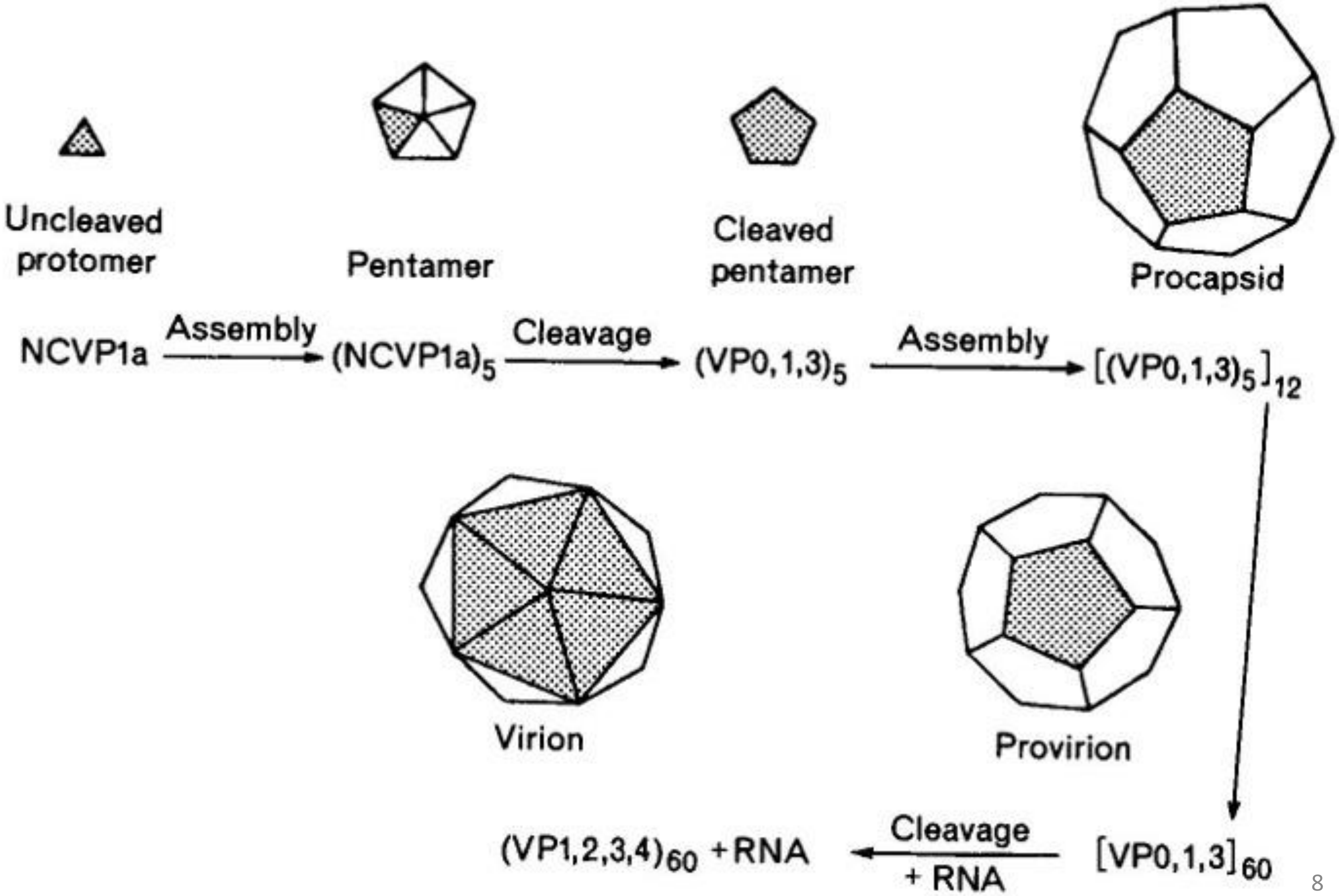




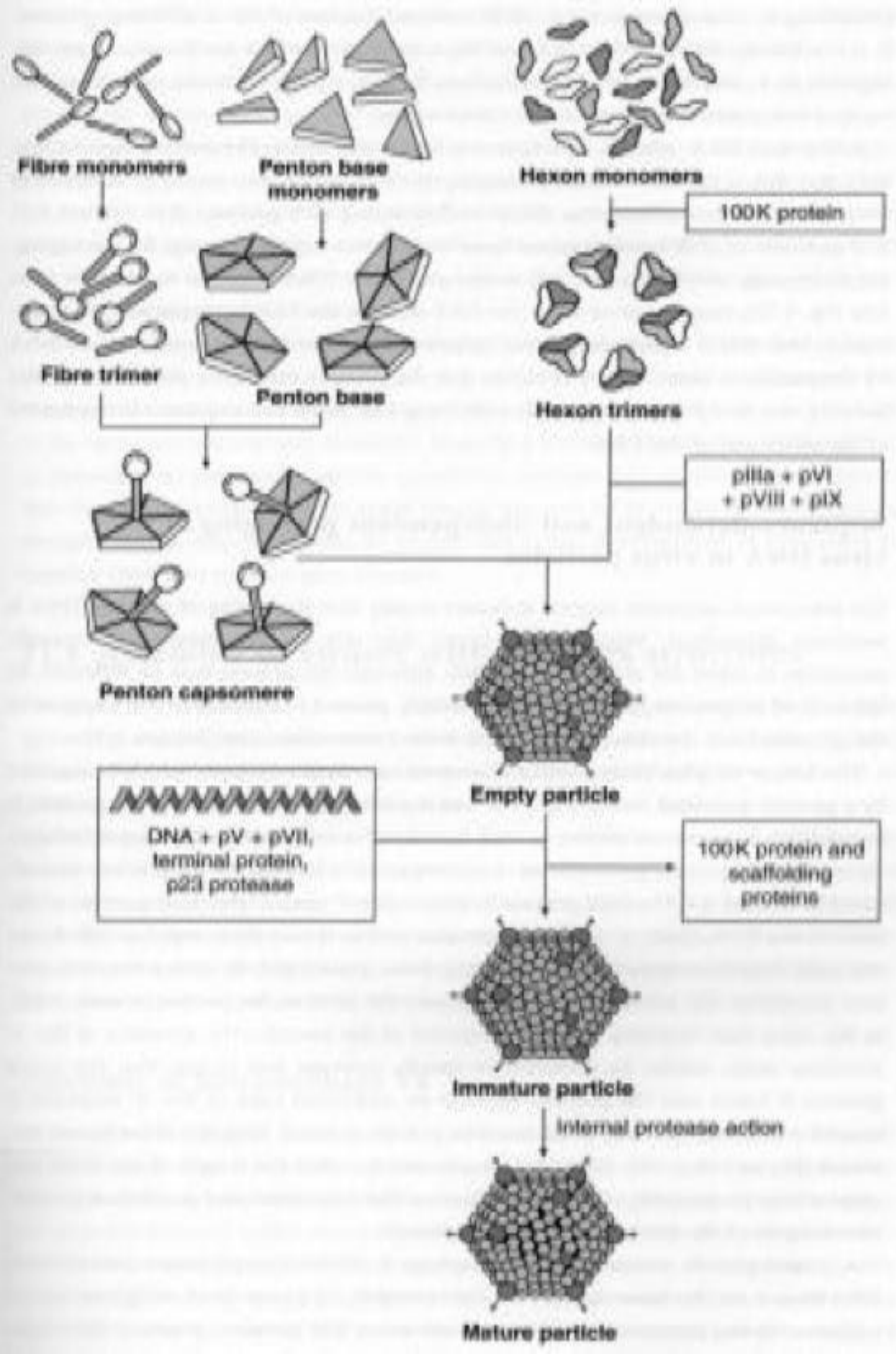
Assembly of icosahedral viruses

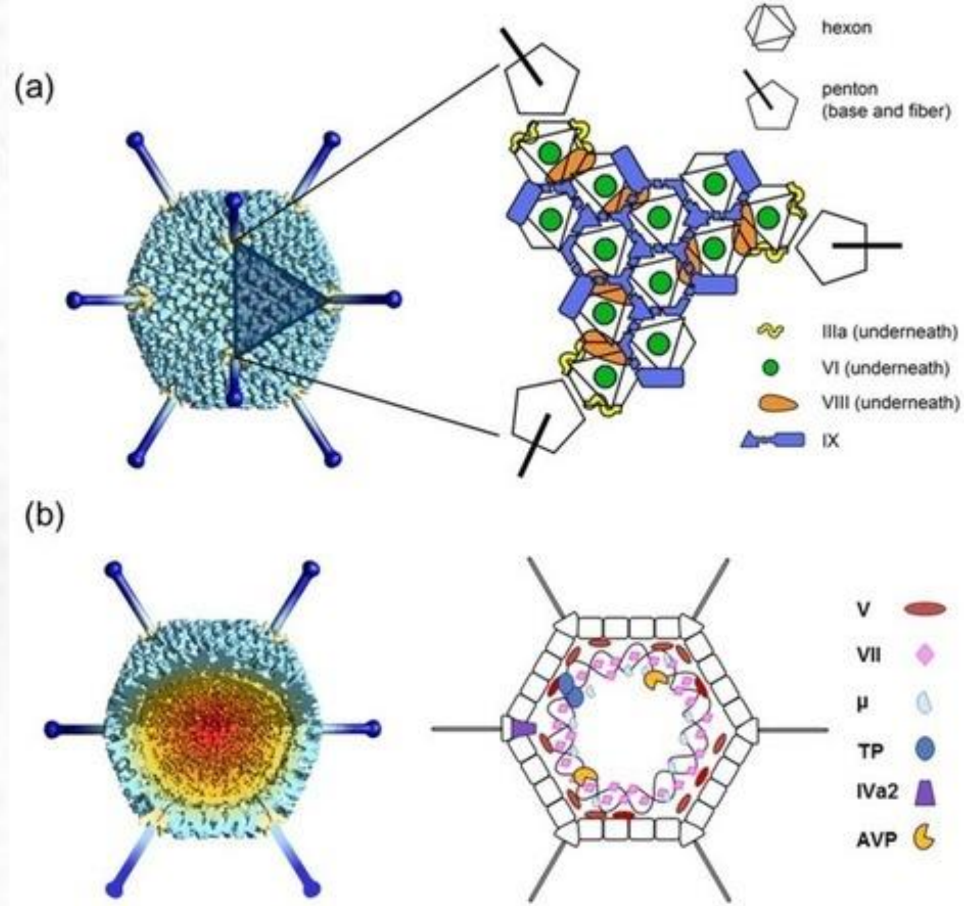


Picornavirus assembly

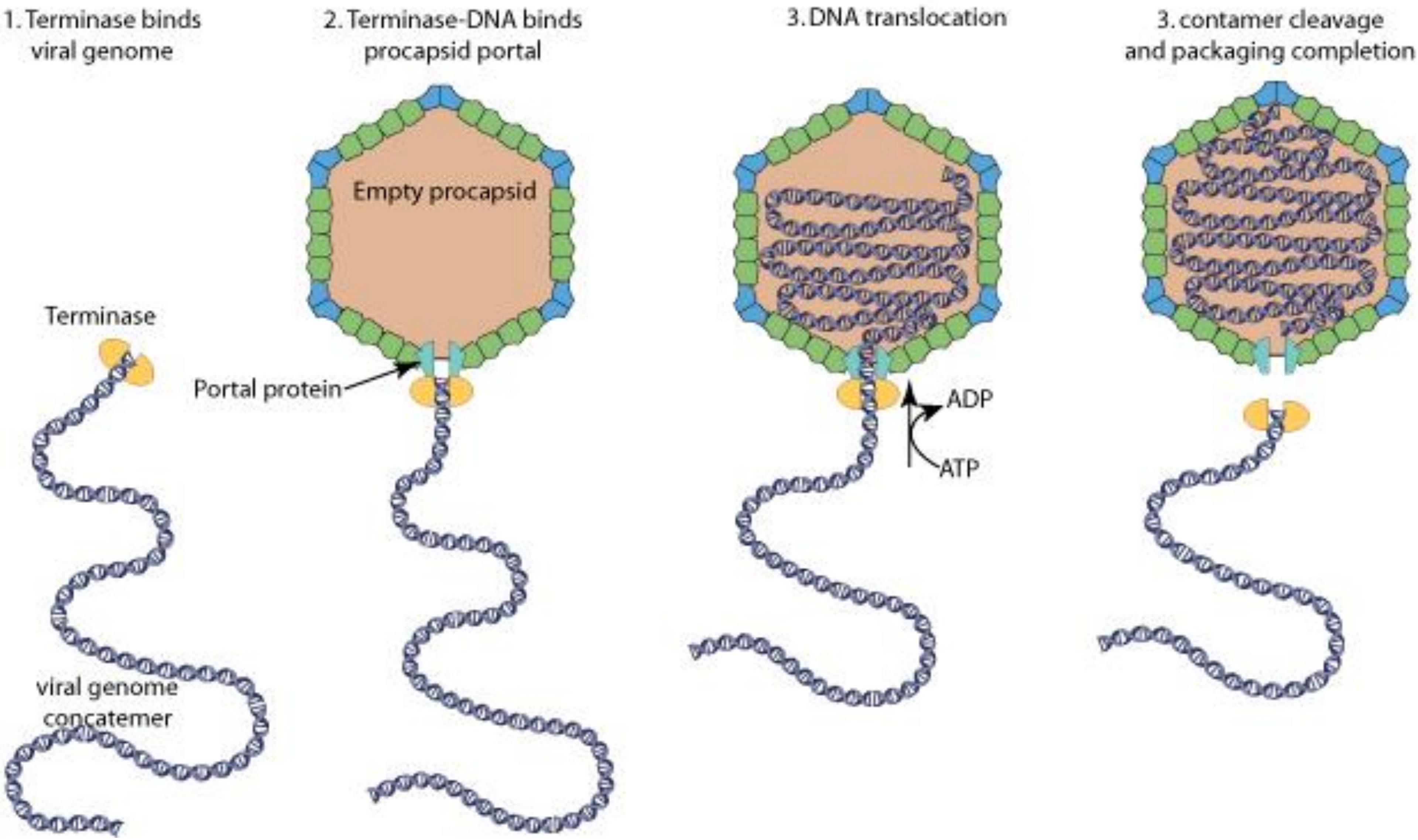


Adenovirus assembly

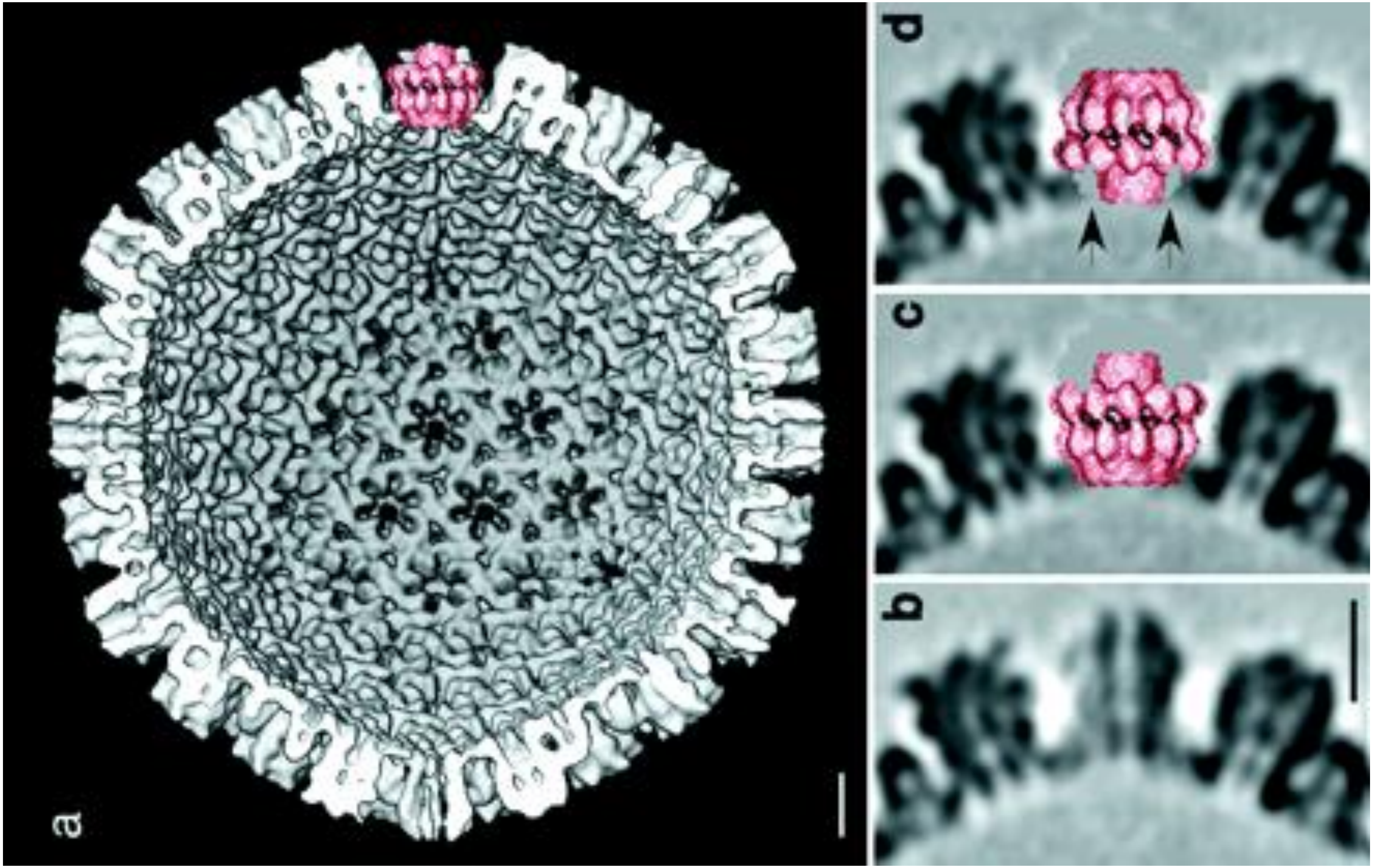




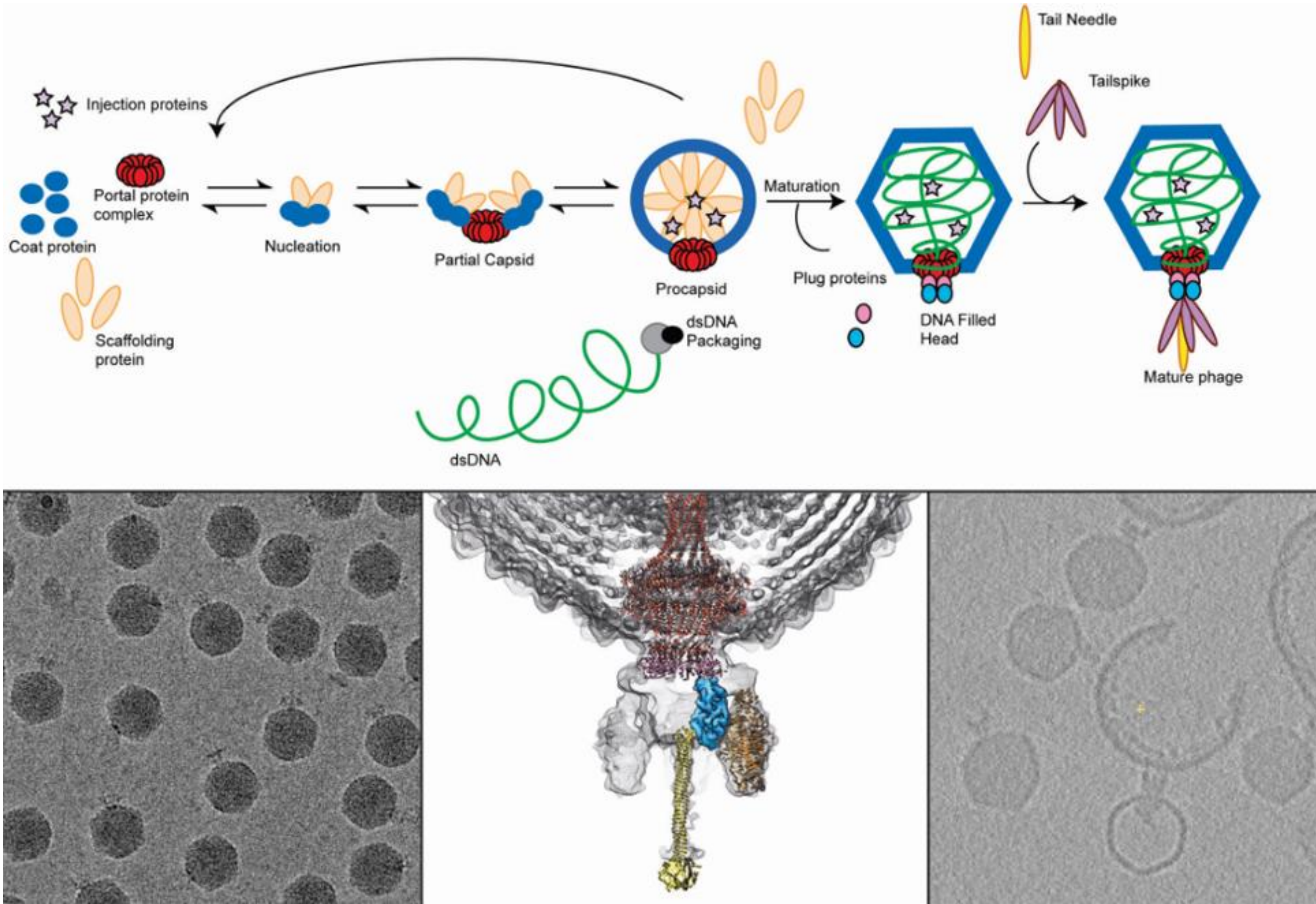
dsDNA virus genome packaging



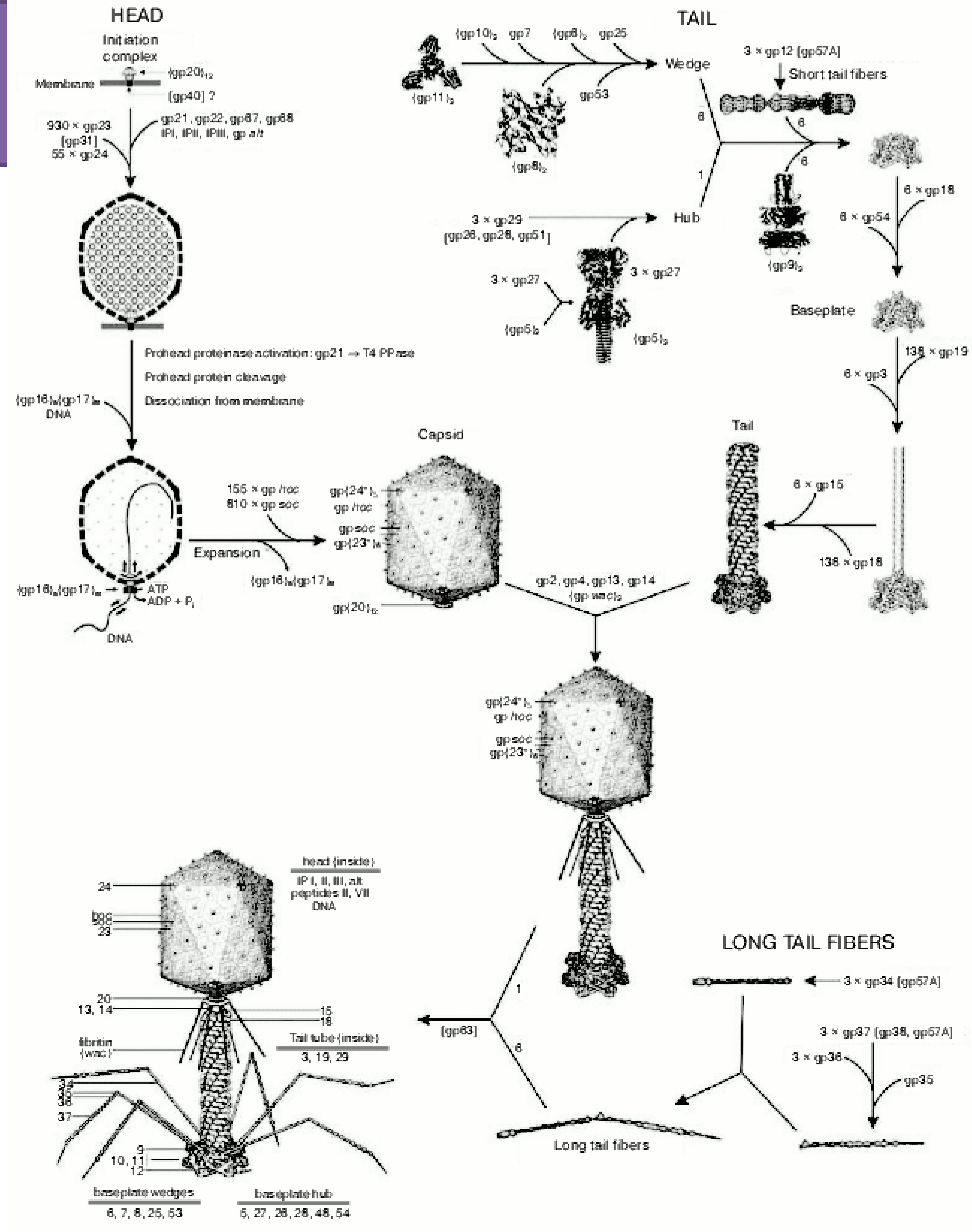
Herpesvirus portal structure



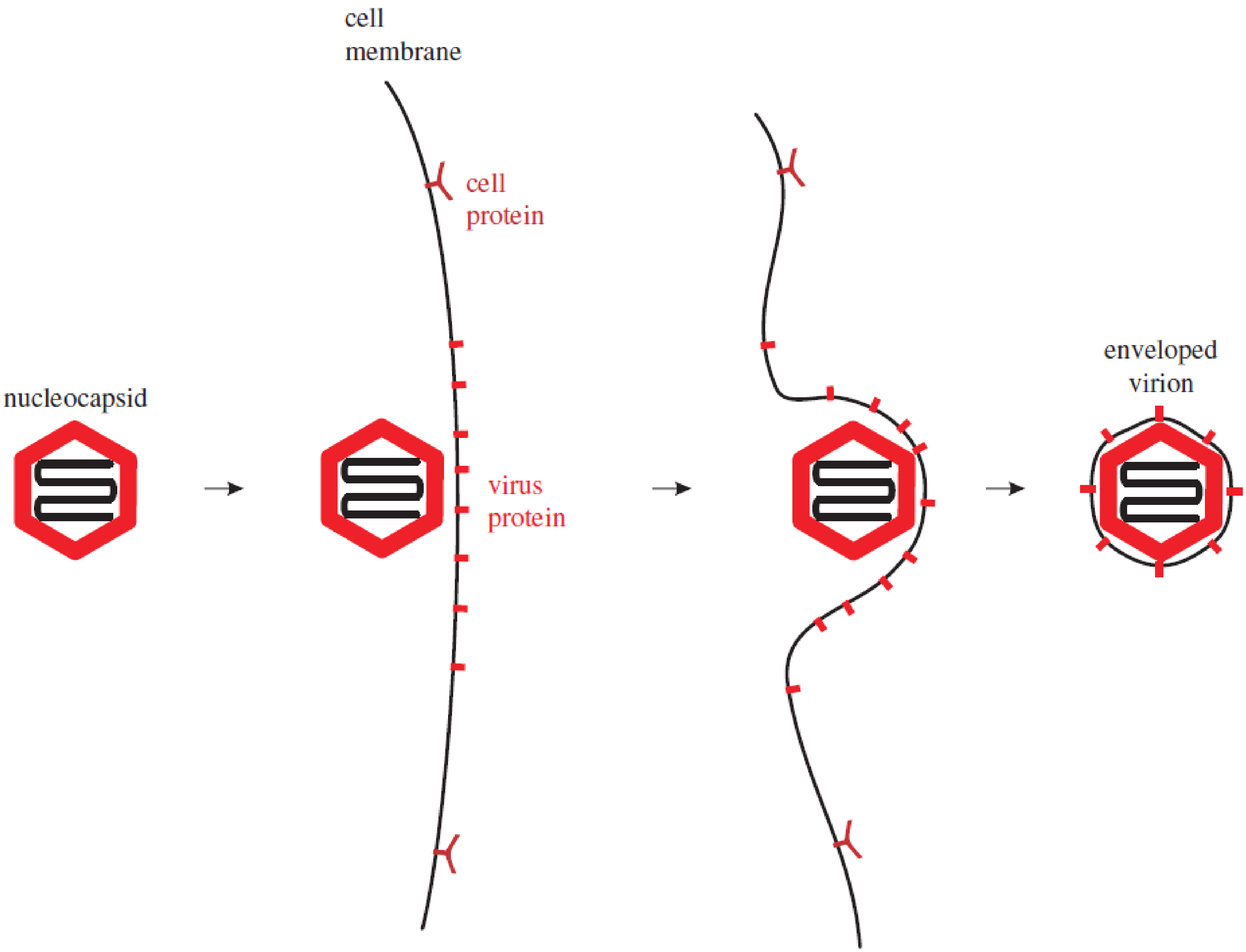
Podoviridae phage assembly



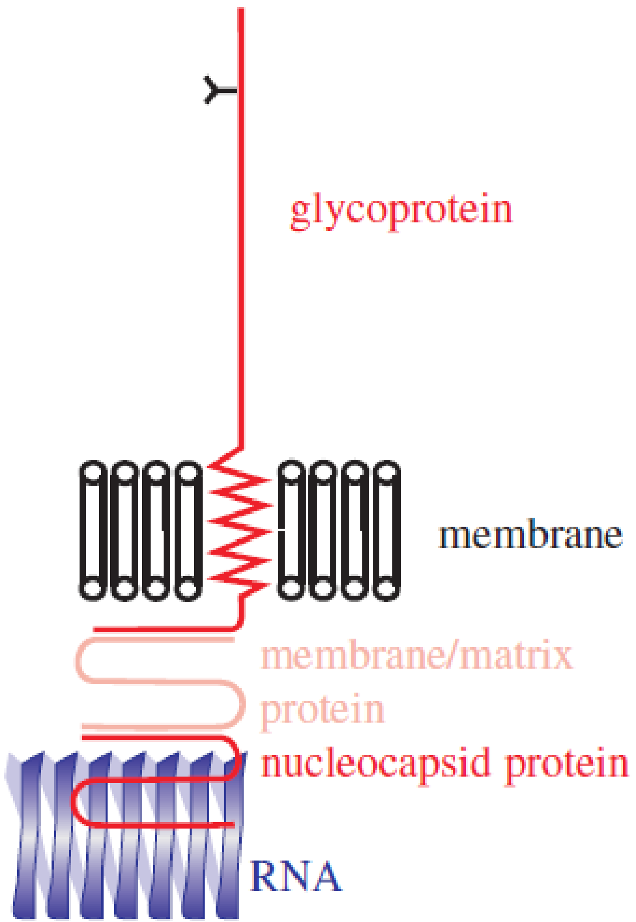
T4 assembly

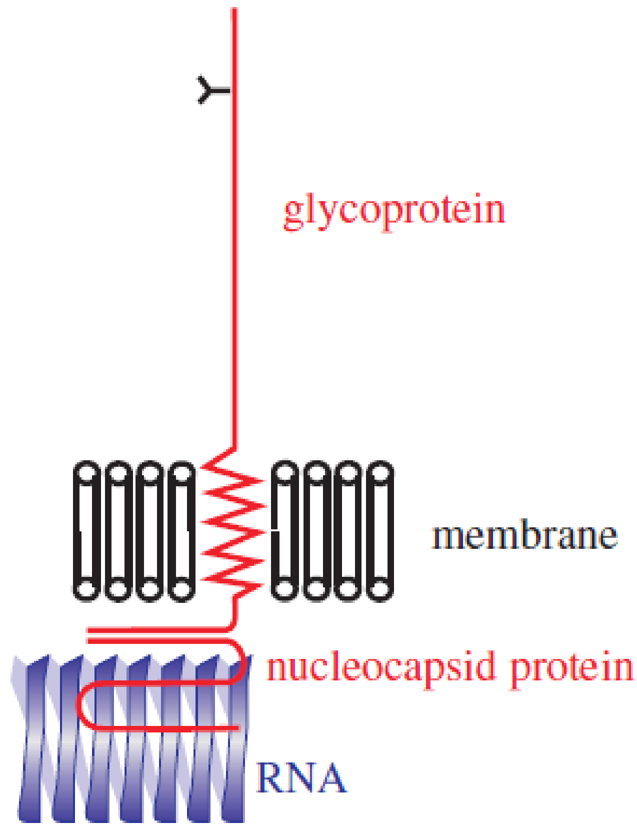

HEAD
Initiation complex
Membrane
930 × gp23
[gp31]
55 × gp24
gp21, gp22, gp67, gp68
IPI, IPII, IPIII, gp alt
Prohead proteinase activation: gp21 → T4 PPase
Prohead protein cleavage
Dissociation from membrane
DNA
ATP
ADP + Pi
155 × gp hoc
810 × gp soc
Expansion
Capsid
gp hoc
gp soc
TAIL
Wedge
3 × gp12 [gp57A]
Short tail fibers
gp53
Hub
3 × gp29
3 × gp27
6 × gp18
6 × gp54
Baseplate
138 × gp19
6 × gp3
Tail
6 × gp15
138 × gp18
gp2, gp4, gp13, gp14
LONG TAIL FIBERS
3 × gp34 [gp57A]
3 × gp37 [gp38, gp57A]
3 × gp36
gp35
Long tail fibers
[gp63]
head (inside)
IP I, II, III, alt
peptides II, VII
DNA
fibritin
(wac)
Tail tube (inside)
3, 19, 29
baseplate wedges
6, 7, 8, 25, 53
baseplate hub
5, 27, 26, 28, 48, 54

Acquisition of a virion envelope by budding

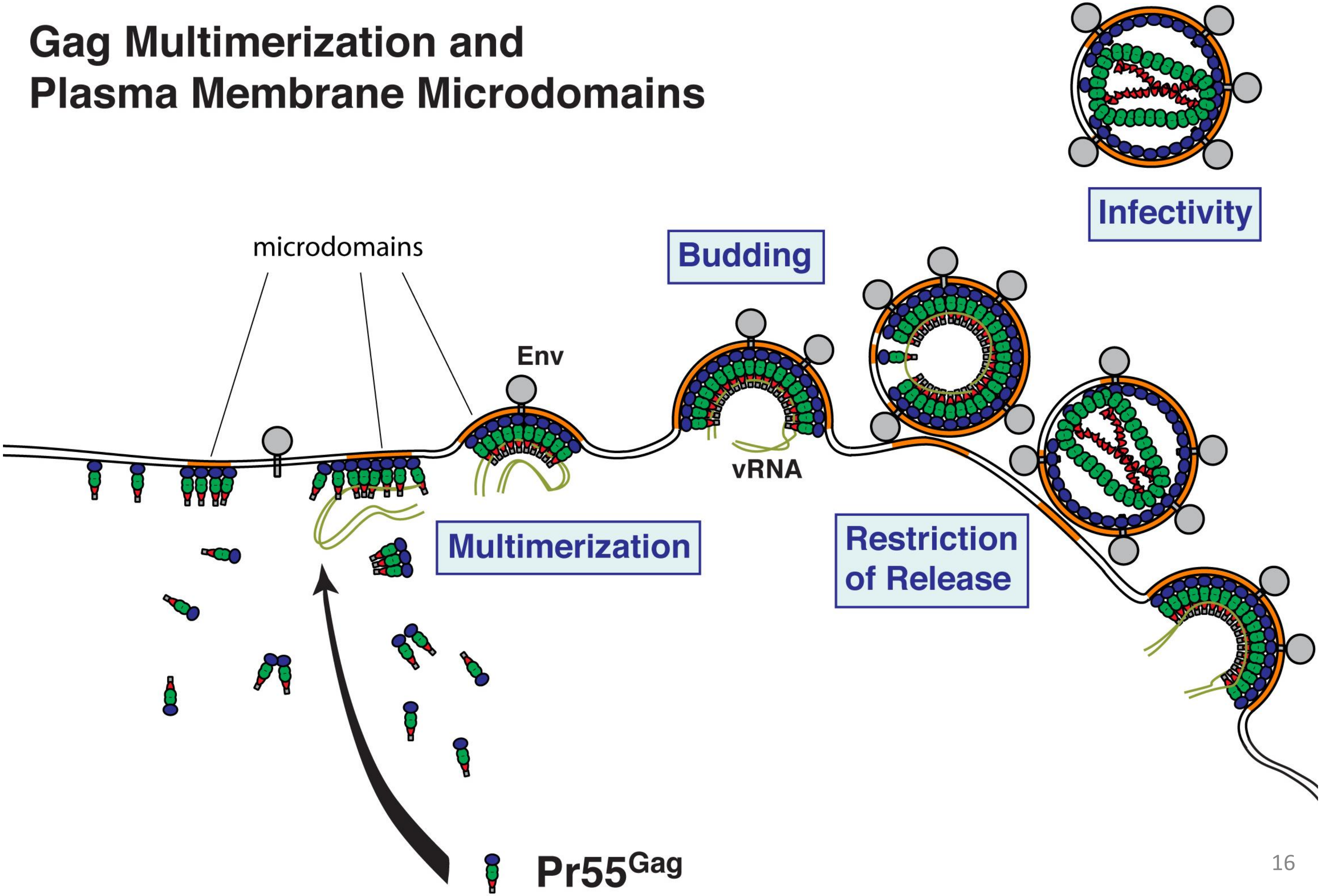


Complementation of enveloped viruses

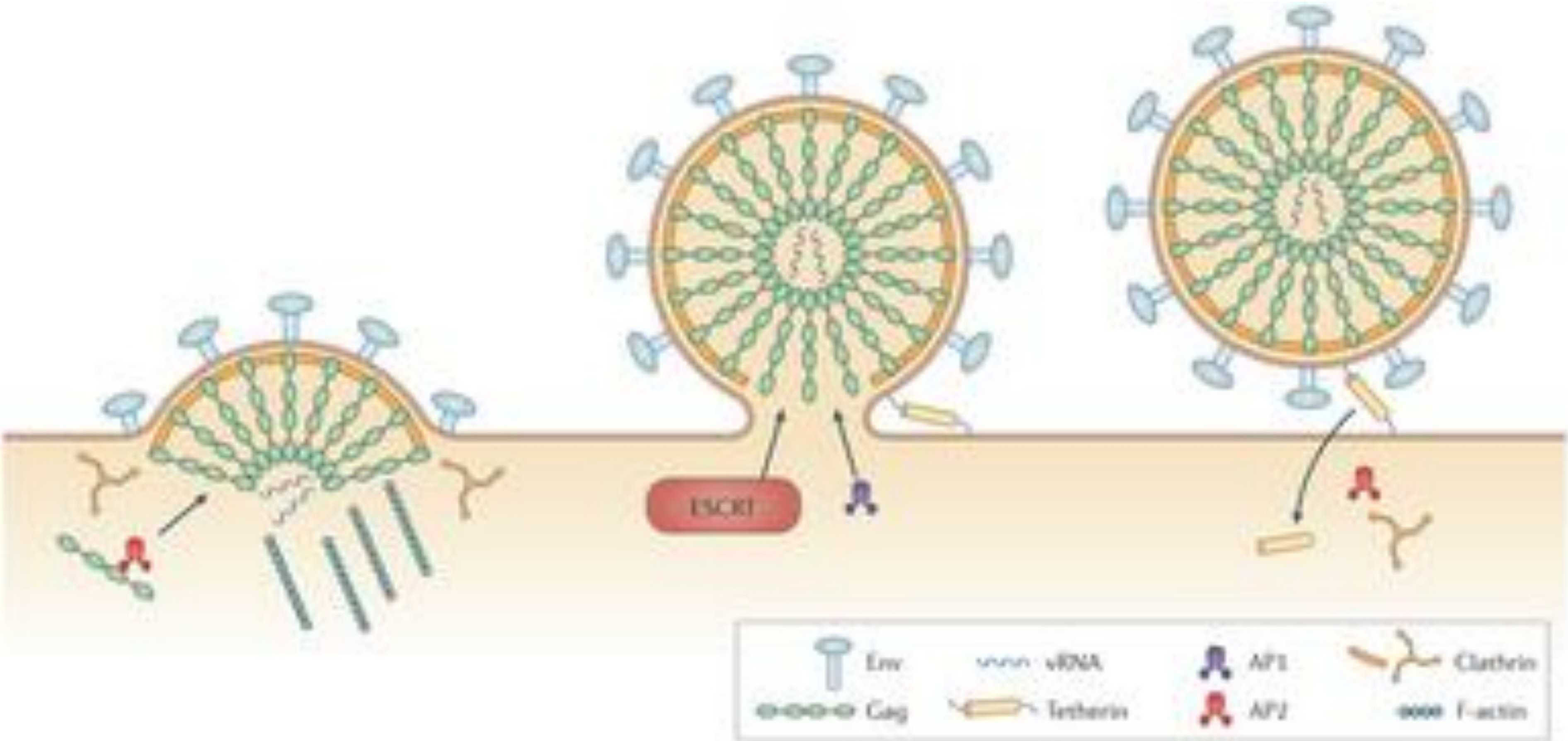




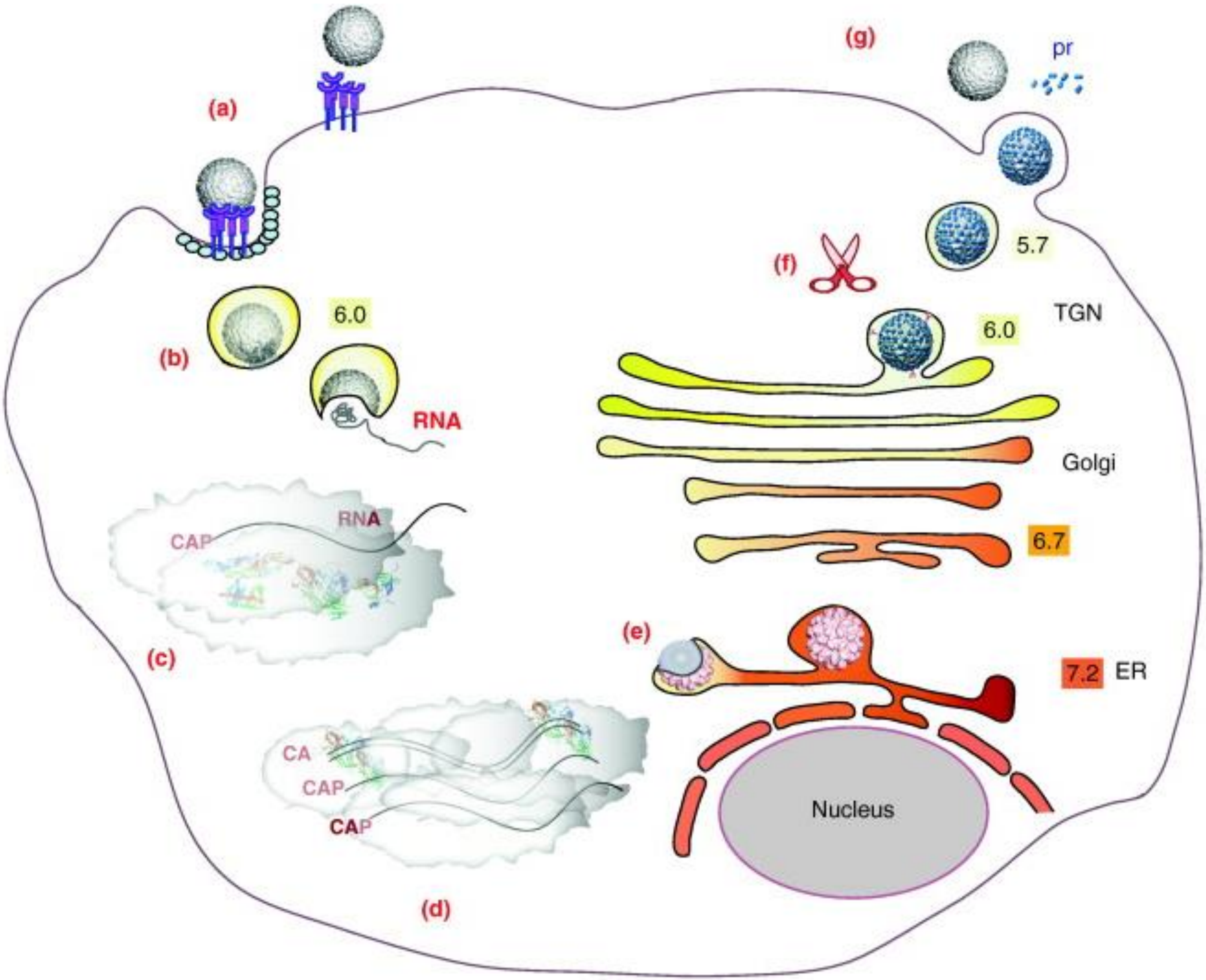
HIV assembly and maturation



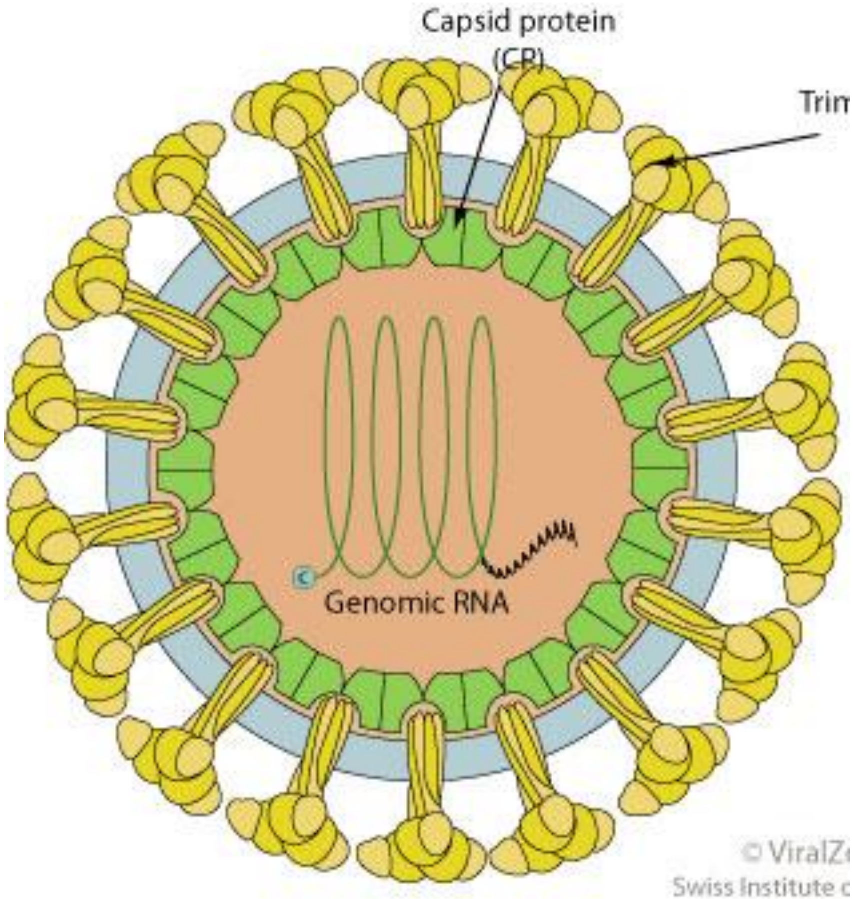
Retrovirus budding

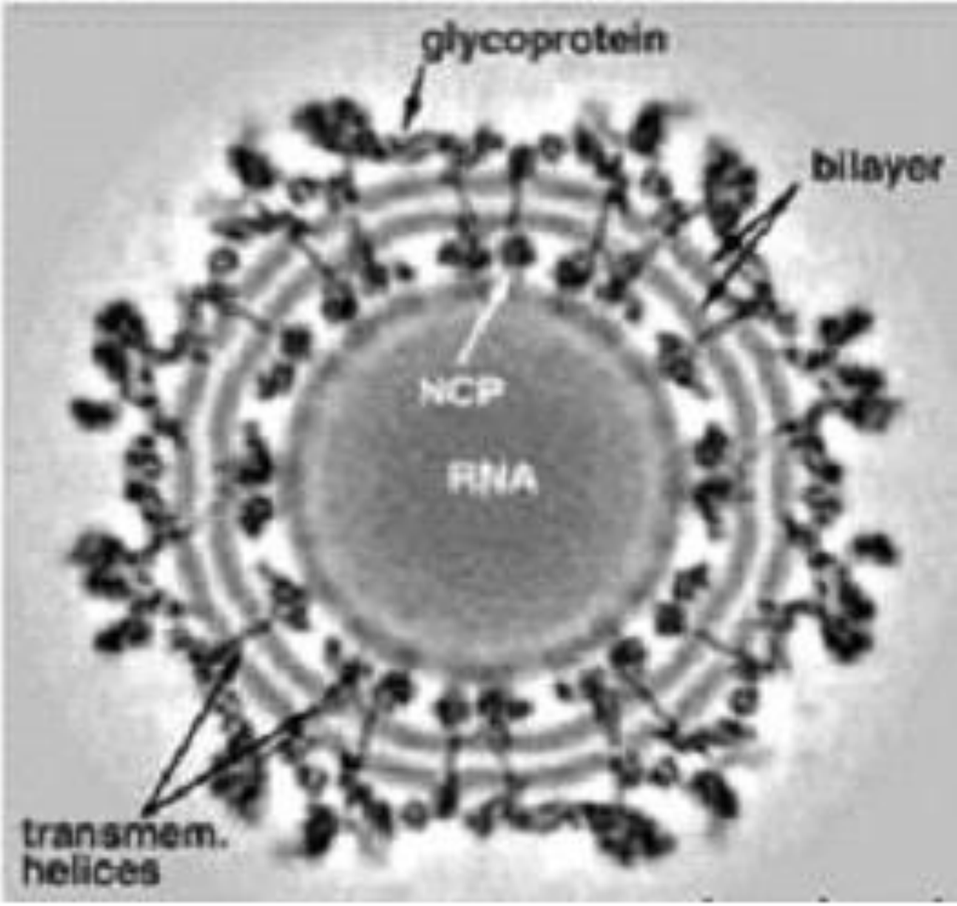


Assembly and maturation of dengue virus

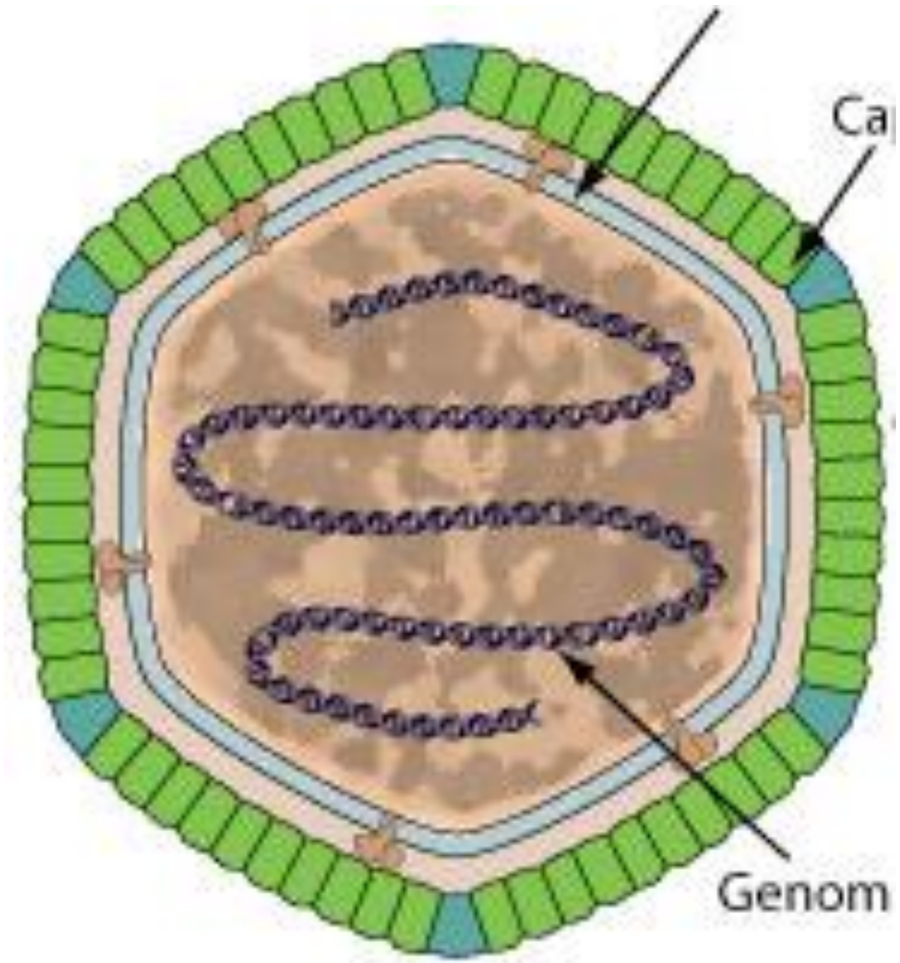


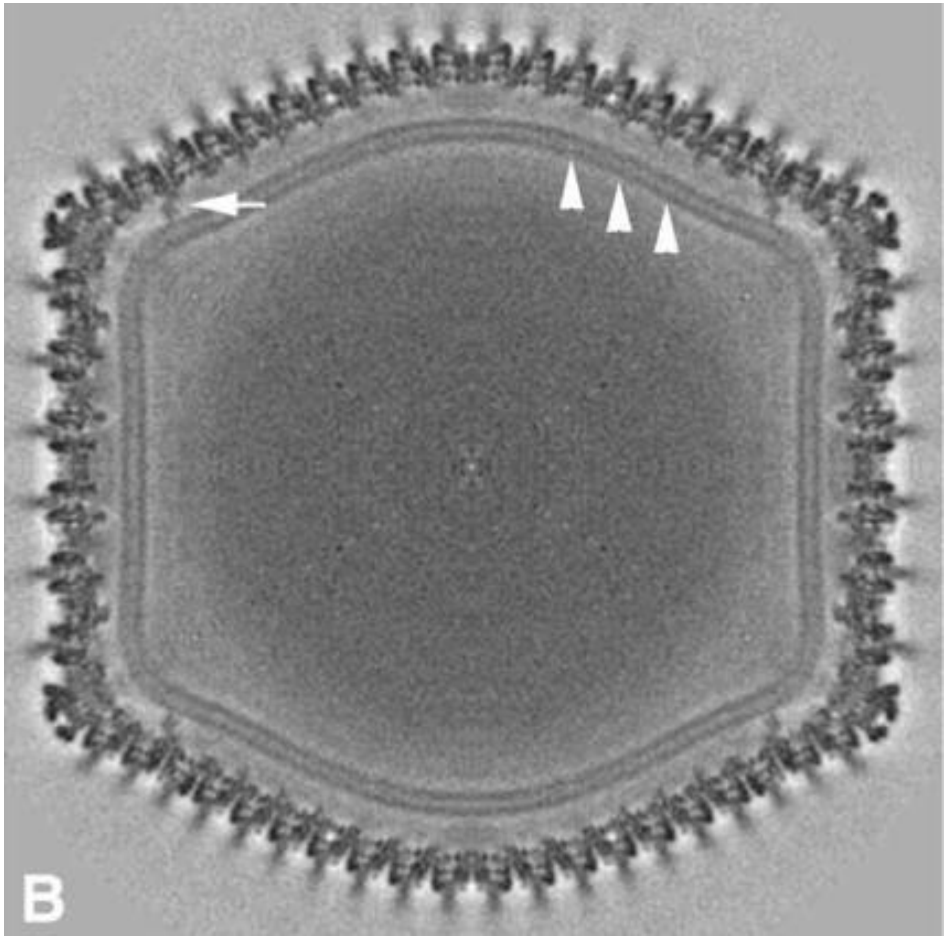
Structure of alphavirus





Structure of iridovirus





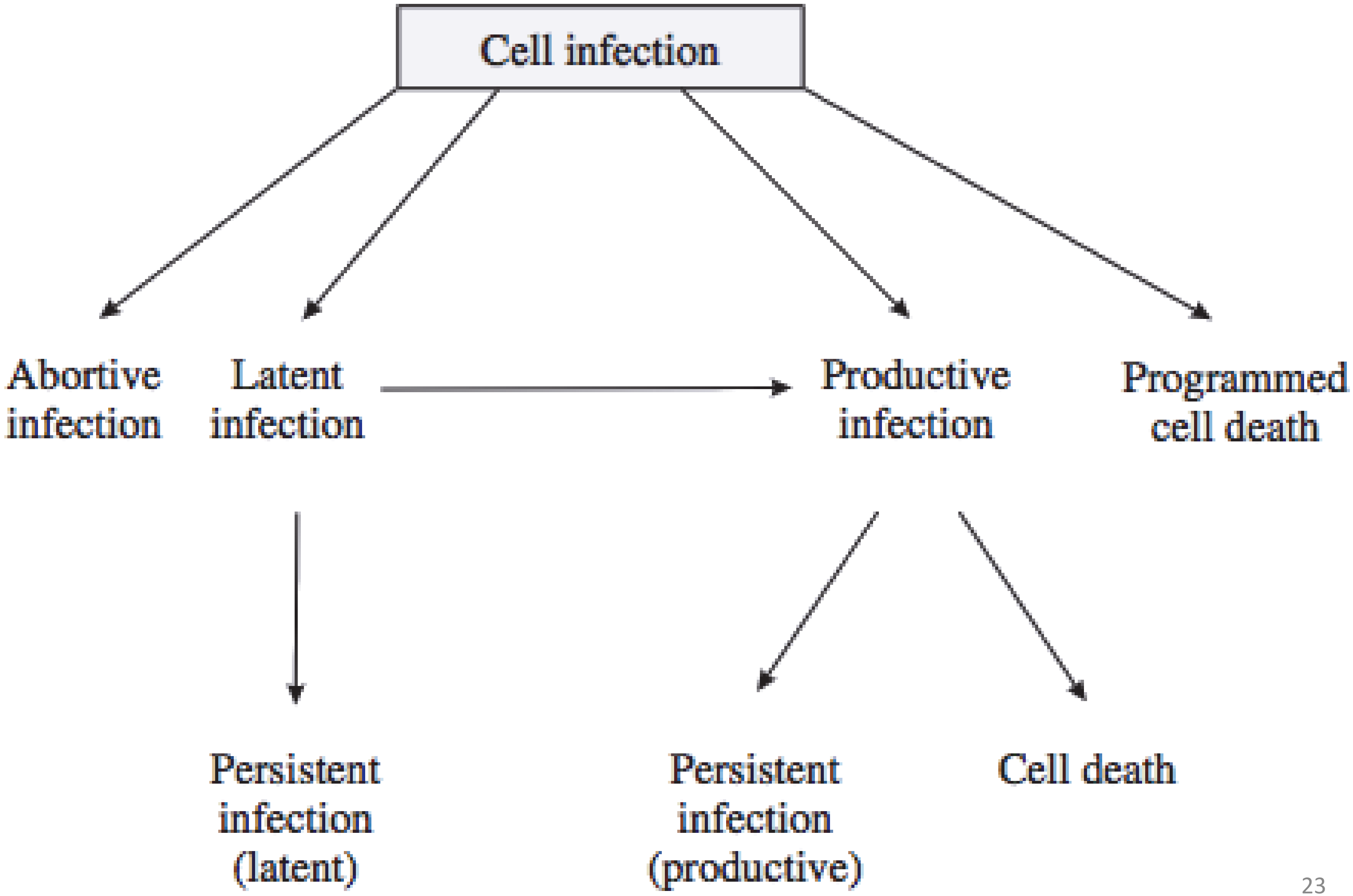
Summary of virus membrane acquisition

Site of virion assembly	Origin of virion membrane	Examples	
		Virion envelope	Internal virion membrane
Eukaryotic cell cytoplasm	Plasma membrane	Most retroviruses Most rhabdoviruses	
	Post-RER membrane	Hepadnaviruses	
	De novo synthesis	Poxviruses	Iridoviruses
Eukaryotic cell nucleus	Inner nuclear membrane	Nucleorhabdoviruses	
	De novo synthesis	Baculovirus virions that will be occluded	
Prokaryotic cell	Cell membrane	*Acholeplasma laidlawii* virus L2	
	De novo synthesis	*Pseudomonas* phage φ6	*Alteromonas* phage PM2

Learning outcomes

- describe the assembly mechanisms for nucleocapsids with (a) helical symmetry and (b) icosahedral symmetry
- discuss the origins of internal virion membranes and of virion envelopes
- explain the roles played by membrane/matrix proteins in the budding of some enveloped viruses
- describe mechanisms used by viruses to exit from cells

Outcomes of virus infection for the host



Outcomes of virus infection for the host

Reasons for non-productive infection:

- latent infection
- abortive infection

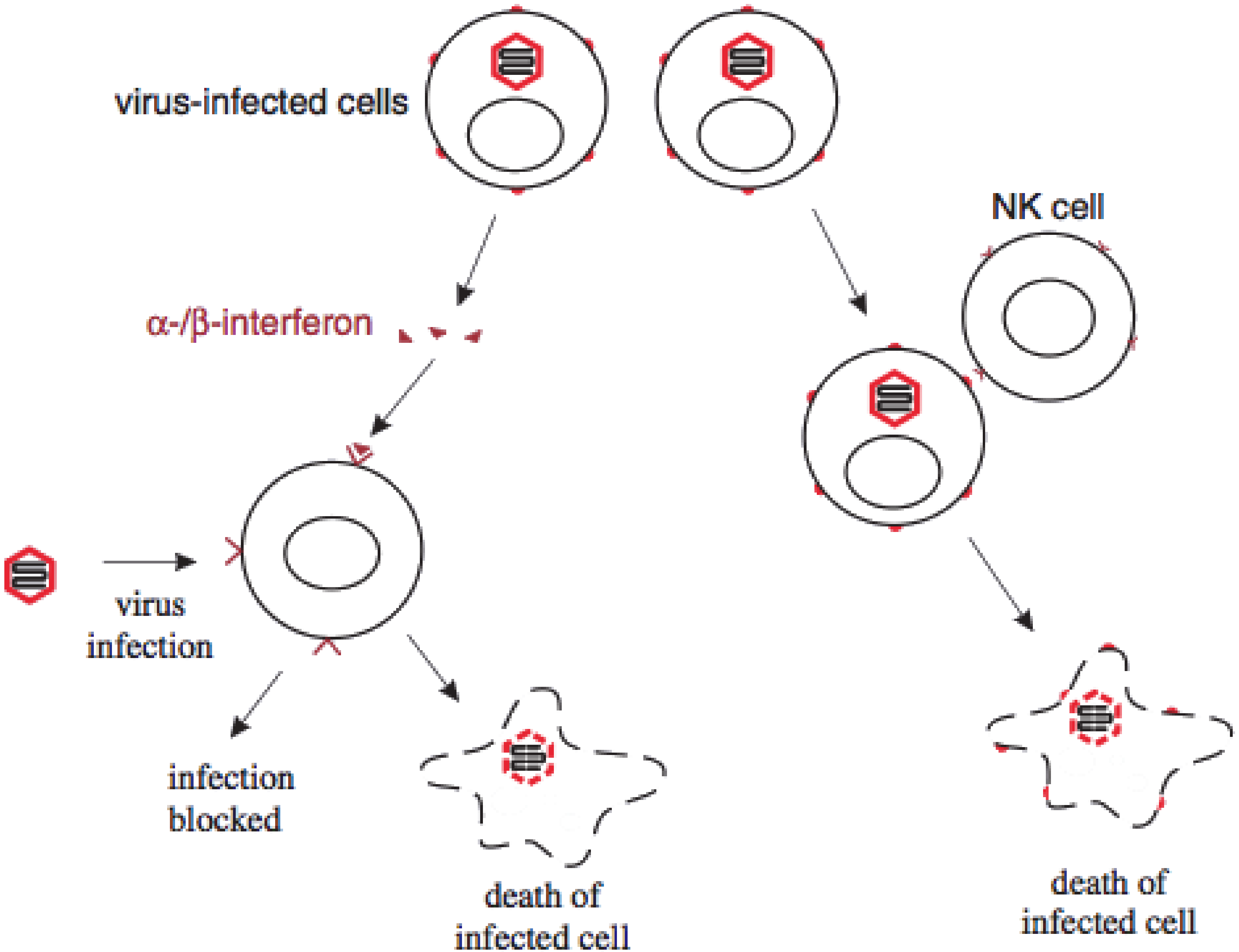
Persistent infections:

- productive (HIV)
- latent (herpesviruses)

Factors affecting outcomes of infection:

- host immune system
- "quality" of the virus (mutations, suitable host)

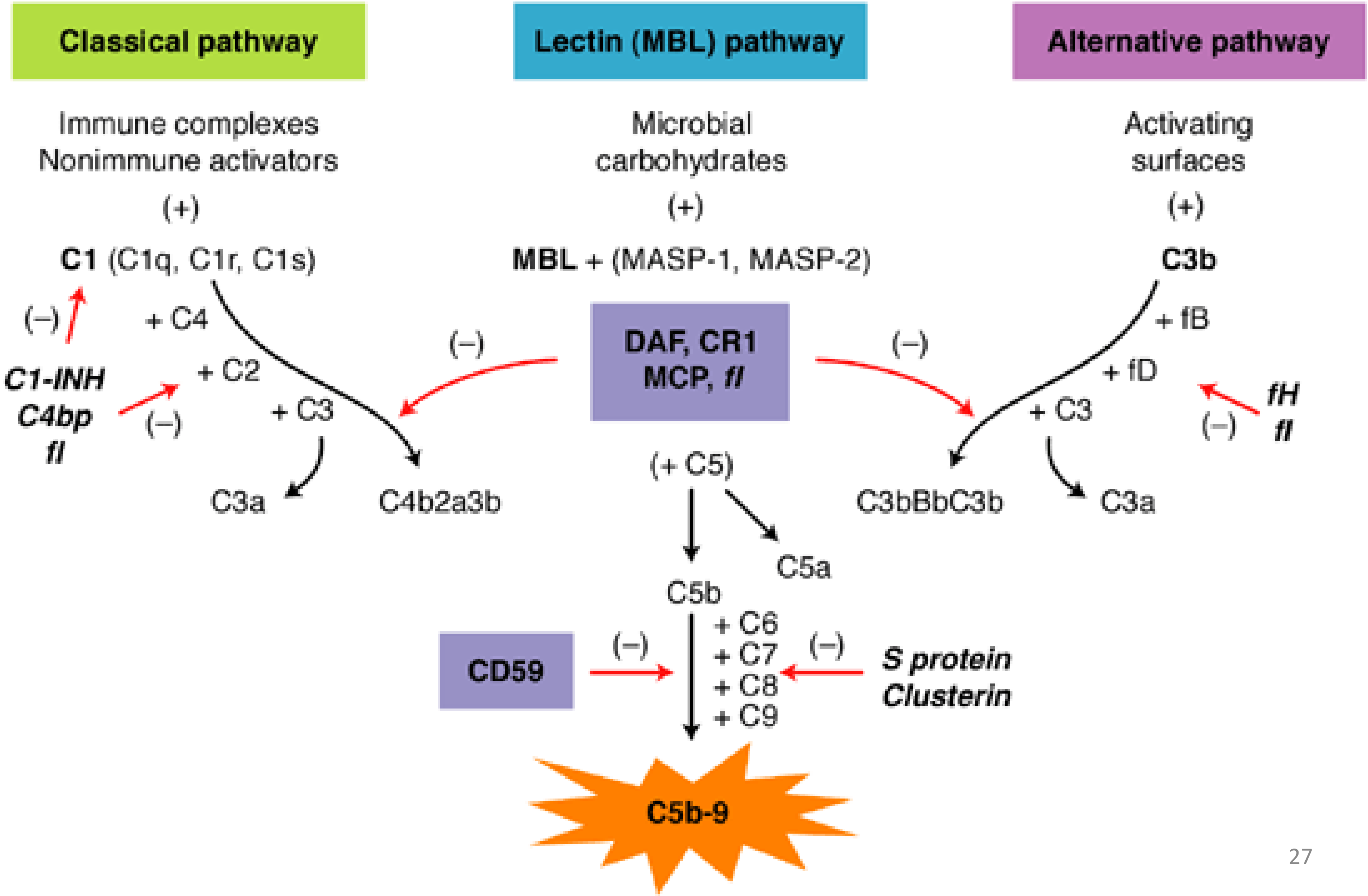
Innate immunity



Components of innate immunity

- Complement
- Interferons
- Natural Killer (NK) cells
- APOBEC3 protein complex
- tetherin

Activation and regulation of complement system



Complement effector system

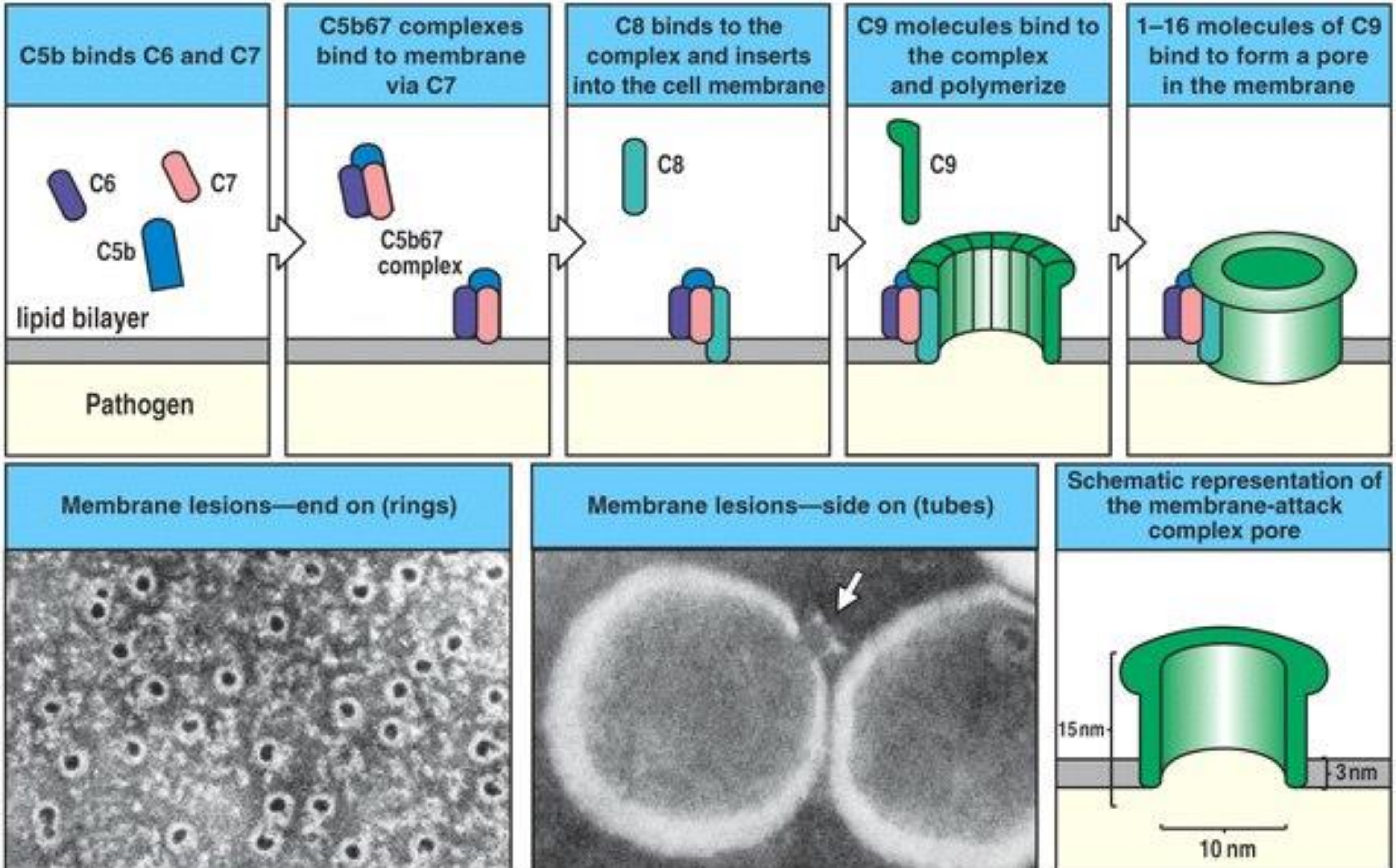
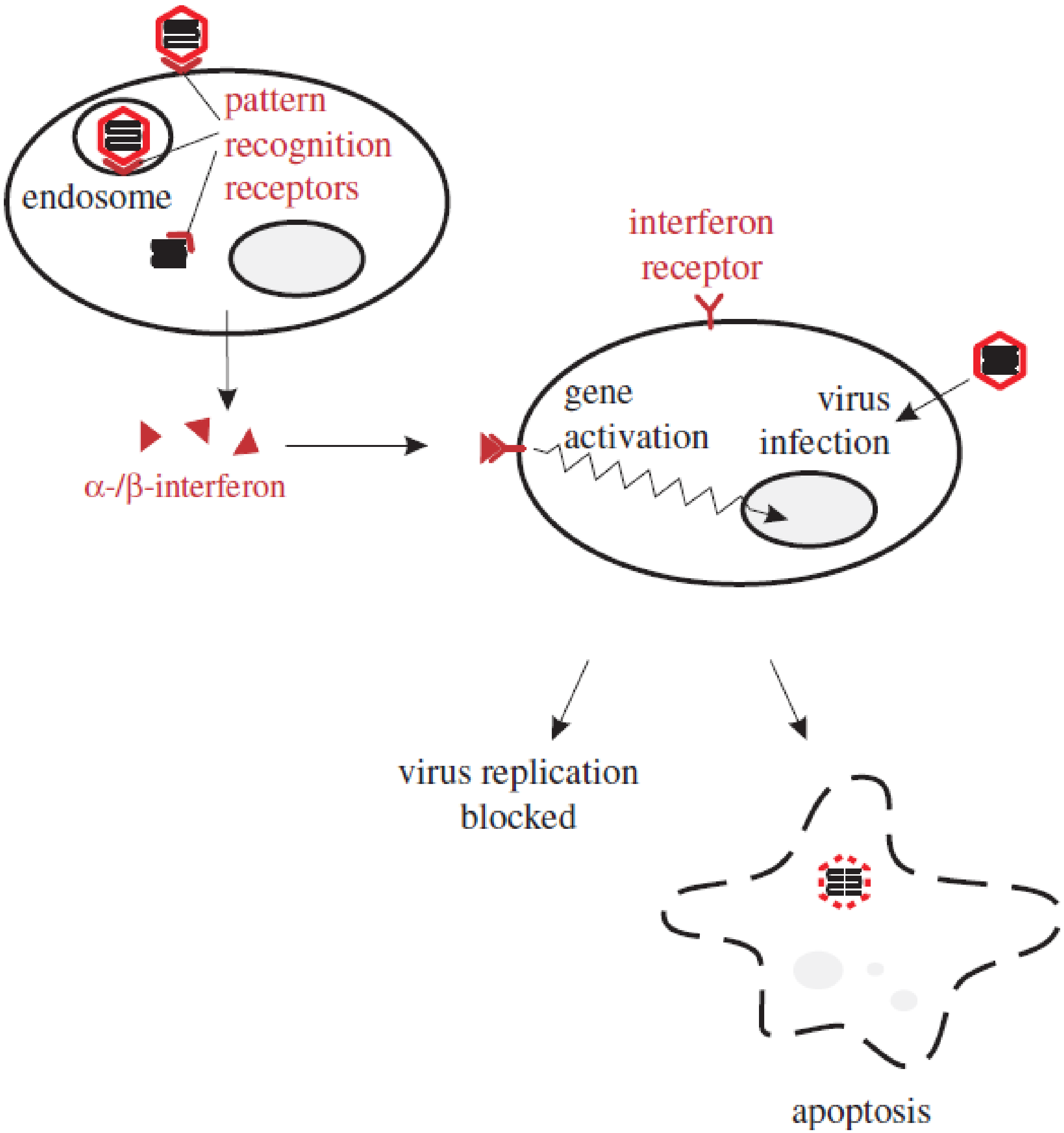


Figure 2-35 Immunobiology, 6/e. (© Garland Science 2005)

Interferon action



Interferon effects:

Alpha and beta

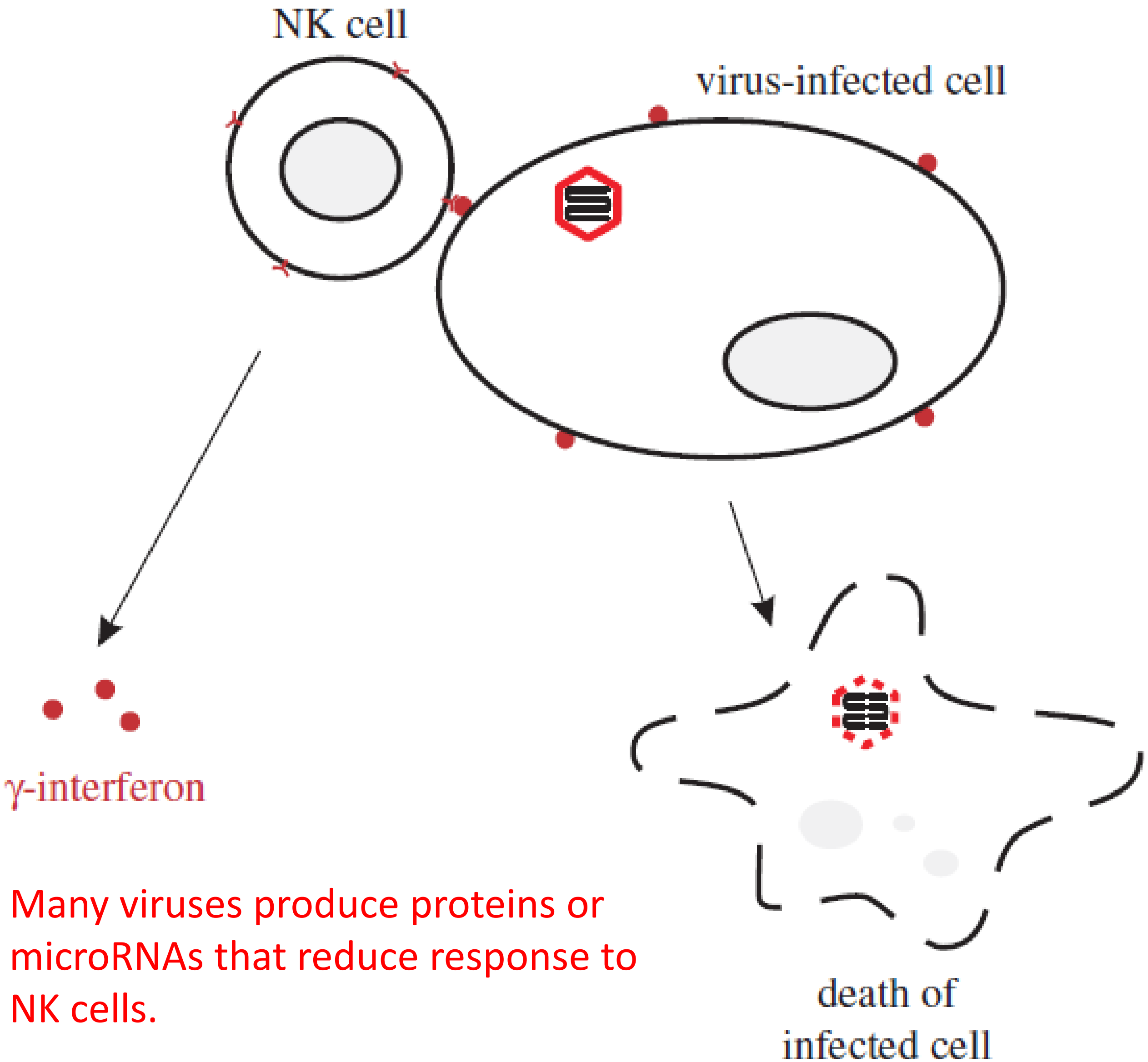
- activation of expression of antiviral proteins: dsRNA dependent protein kinase R; tetherin
- production of MHC I and proteasome components (presentation of peptides for control by T cells)
- activation of NK cells
- induction of apoptosis

Gamma

- produced by cells of immune system
- activation of phagocytes and NK cells

Many viruses inhibit production of interferons in infected cells.

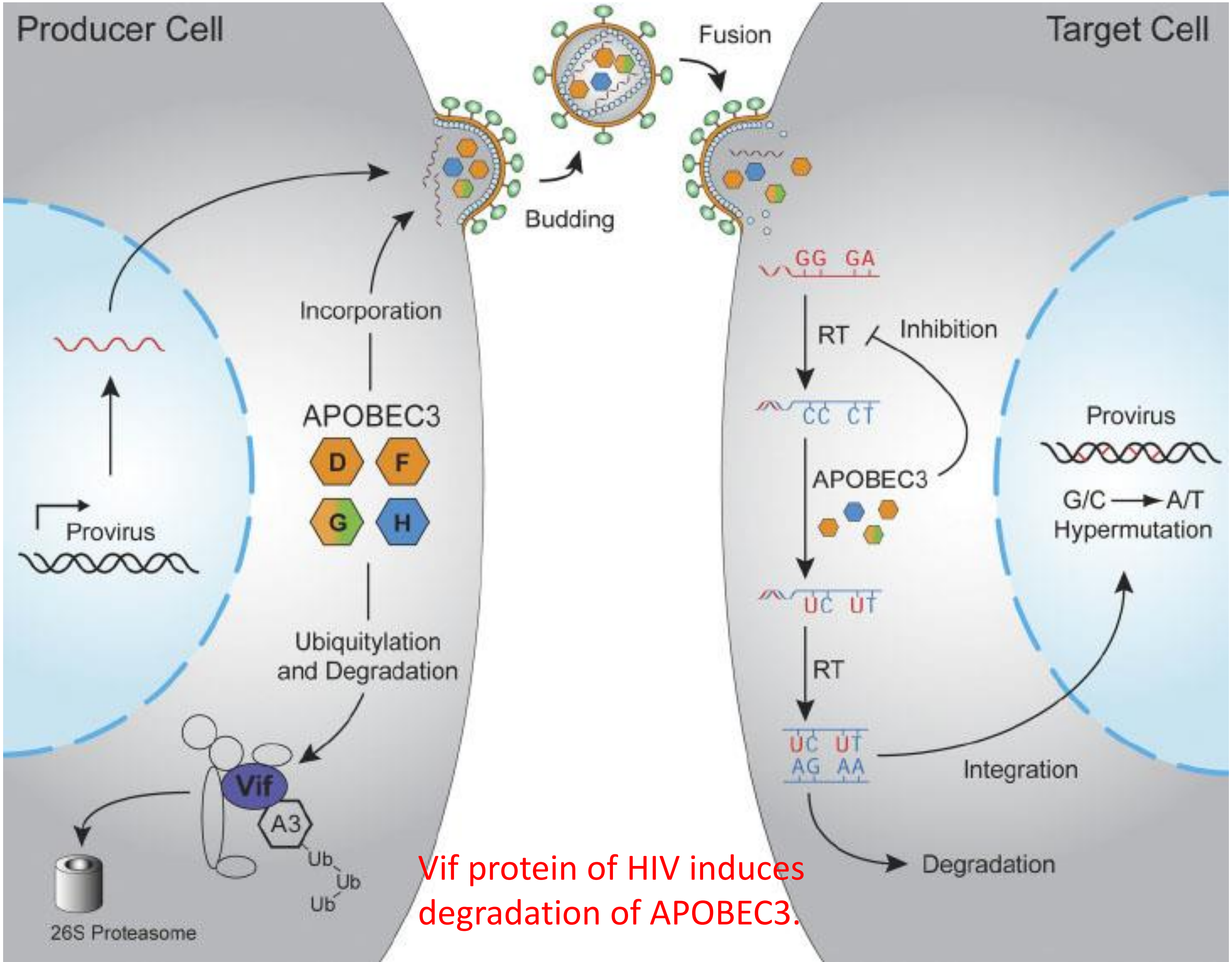
Activities of Natural Killer (NK) cells



Recognize infected cells
Use perforins to kill cells
Induce apoptosis

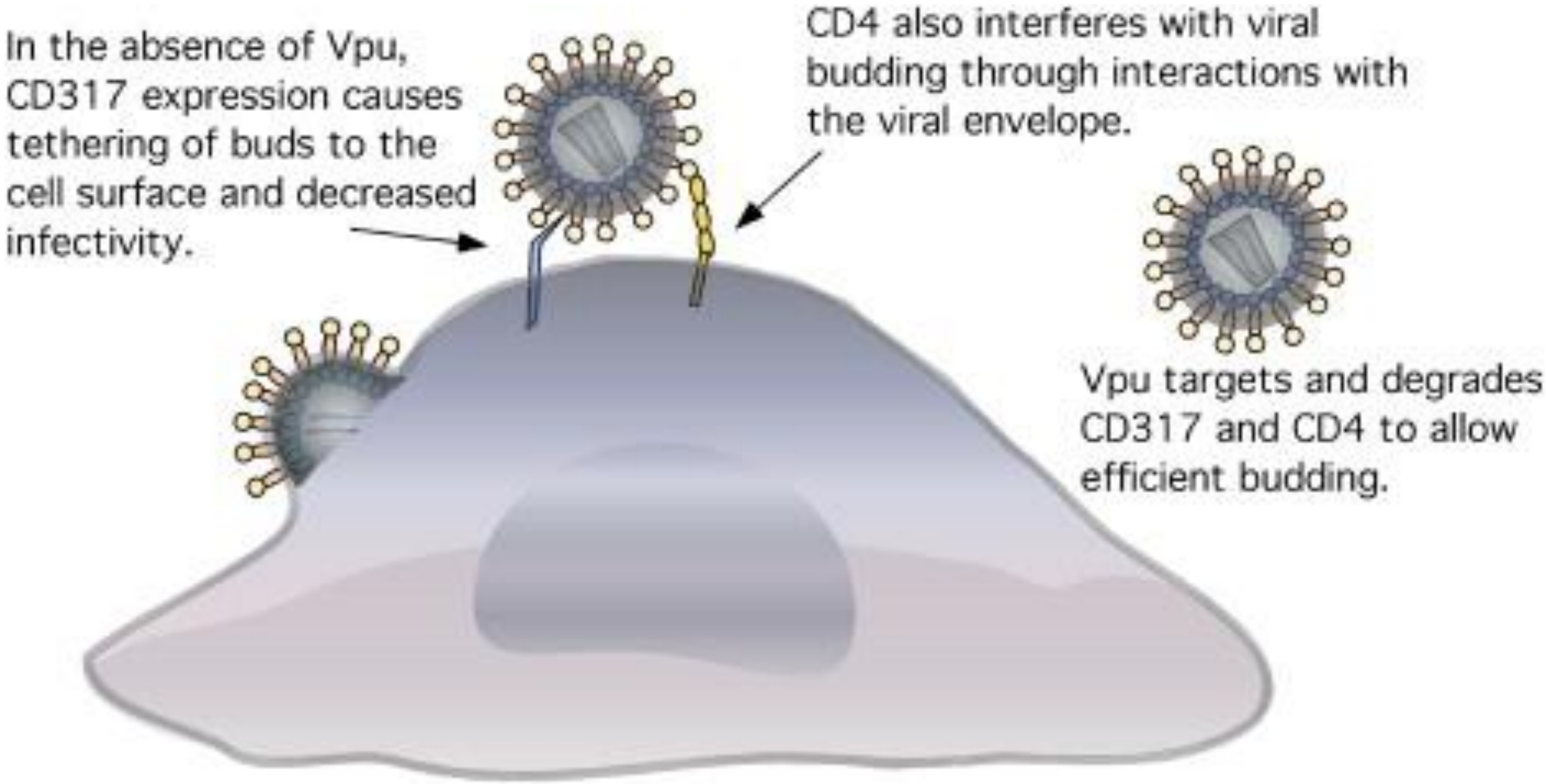
Many viruses produce proteins or microRNAs that reduce response to NK cells.

APOBEC3 proteins

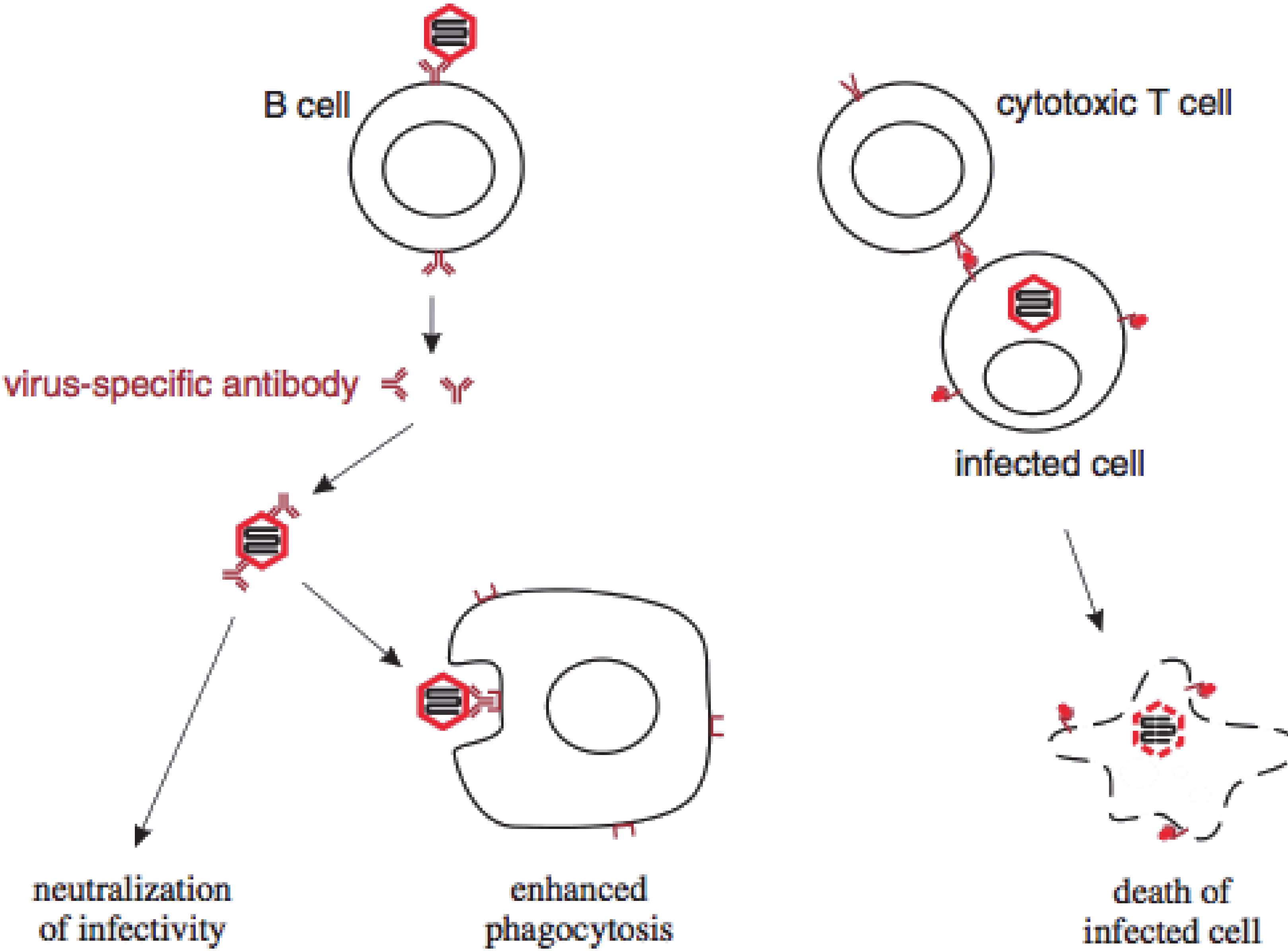


Vif protein of HIV induces degradation of APOBEC3.

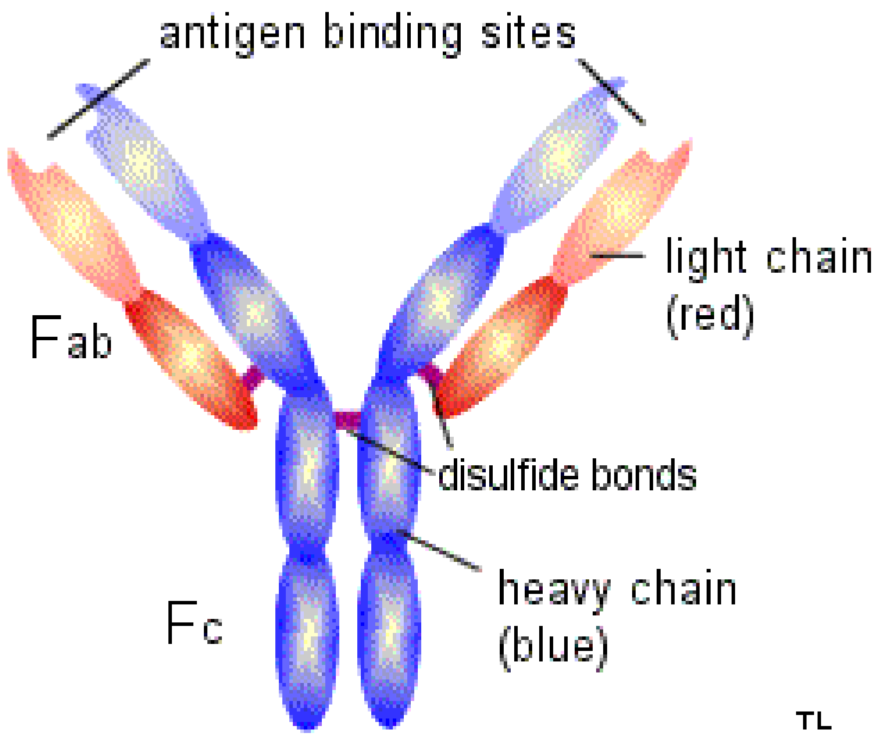
Tetherin


In the absence of Vpu, CD317 expression causes tethering of buds to the cell surface and decreased infectivity.
CD4 also interferes with viral budding through interactions with the viral envelope.
Vpu targets and degrades CD317 and CD4 to allow efficient budding.

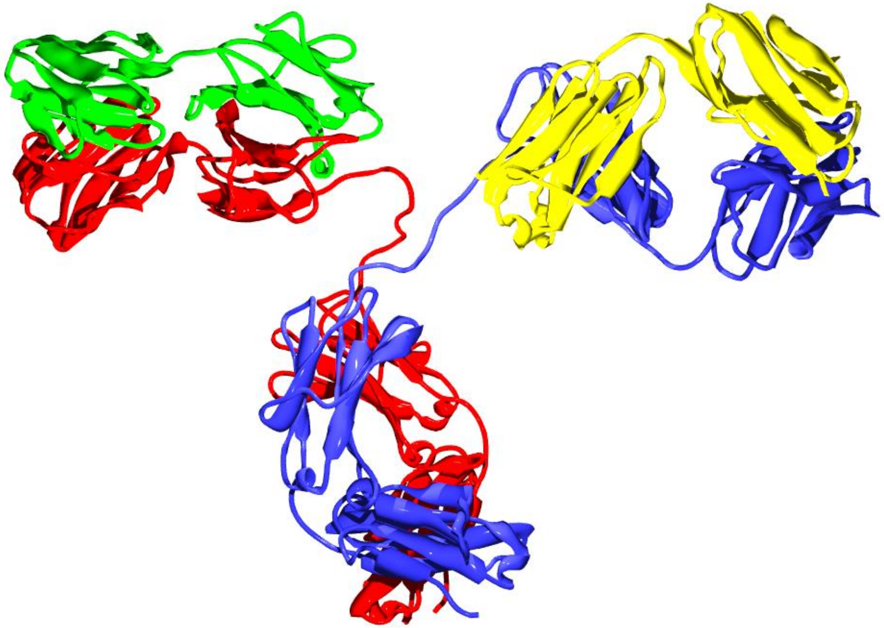
Adaptive immunity



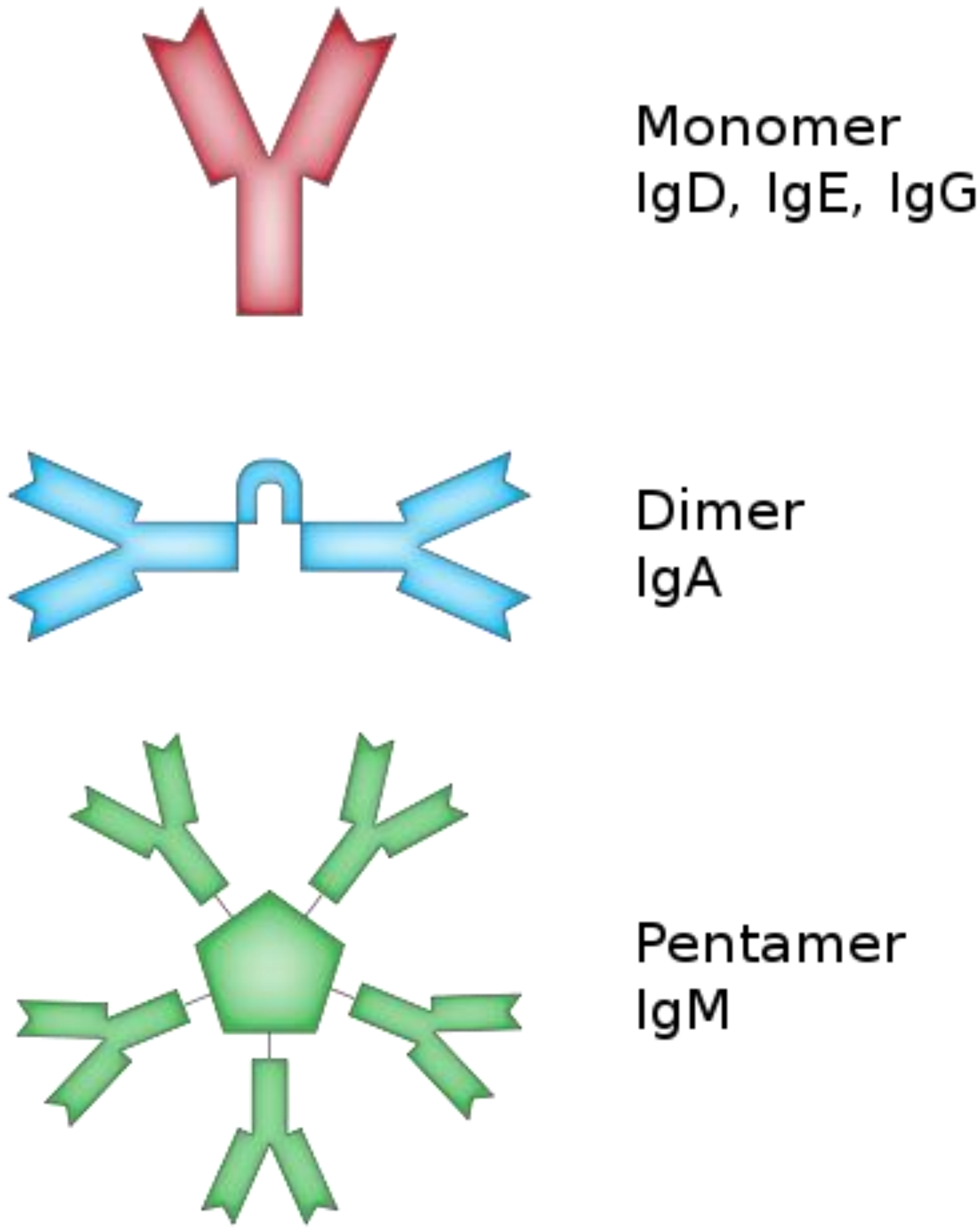
Antibody structure



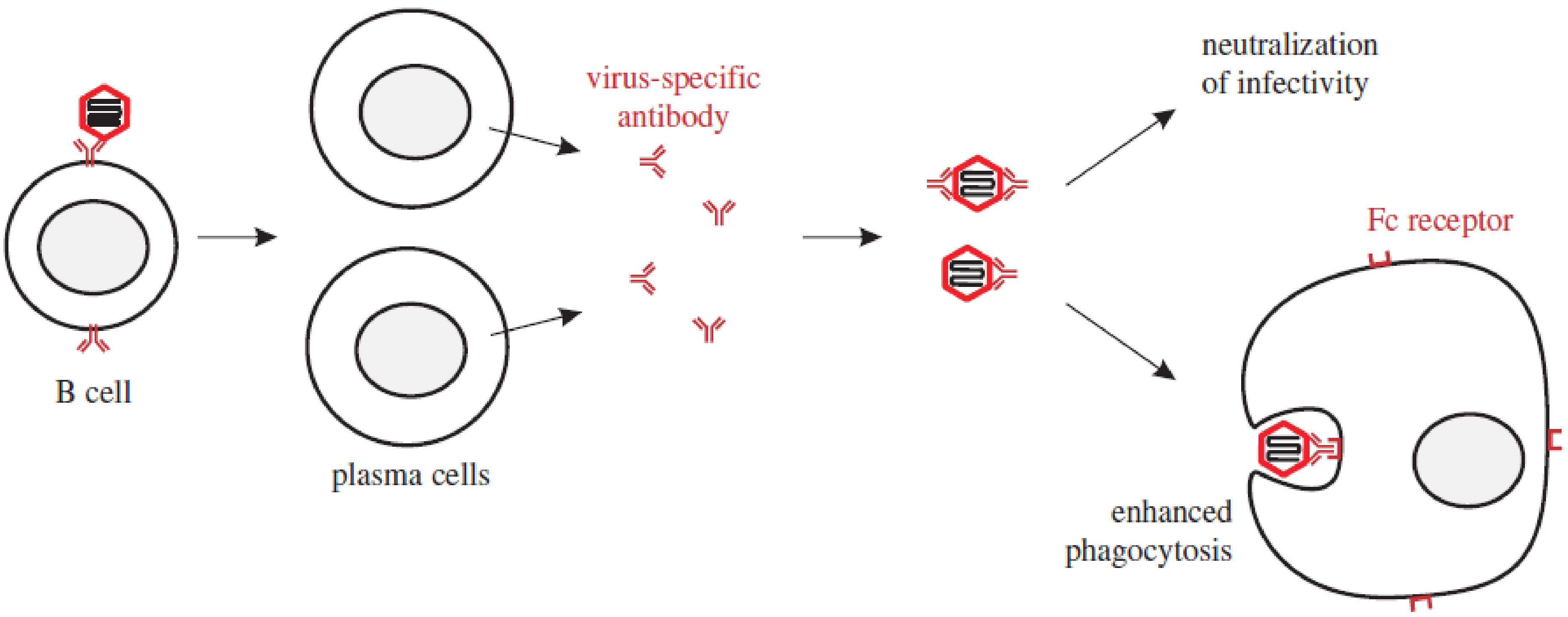
TL



Antibody structures



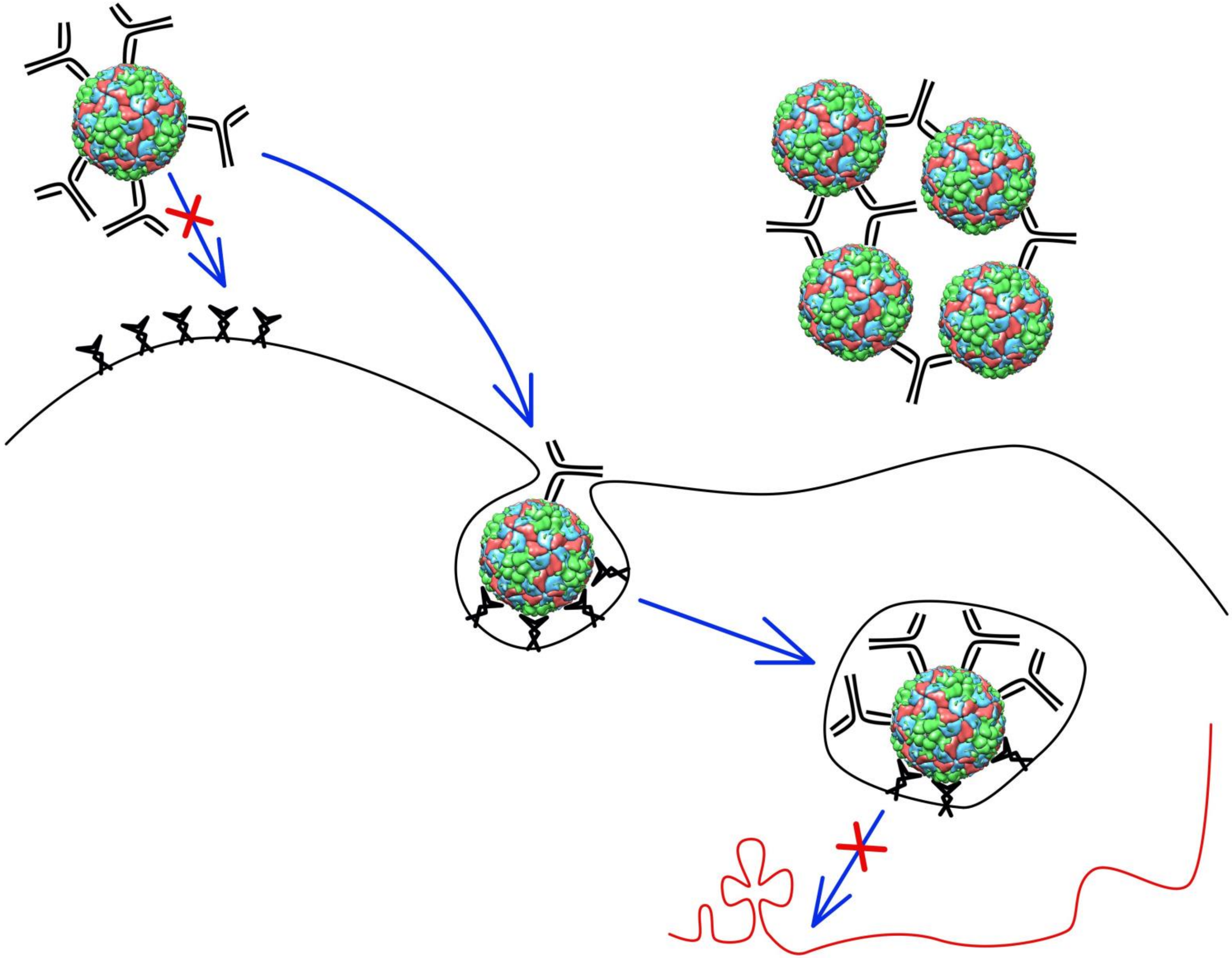
Production of virus-specific antibodies

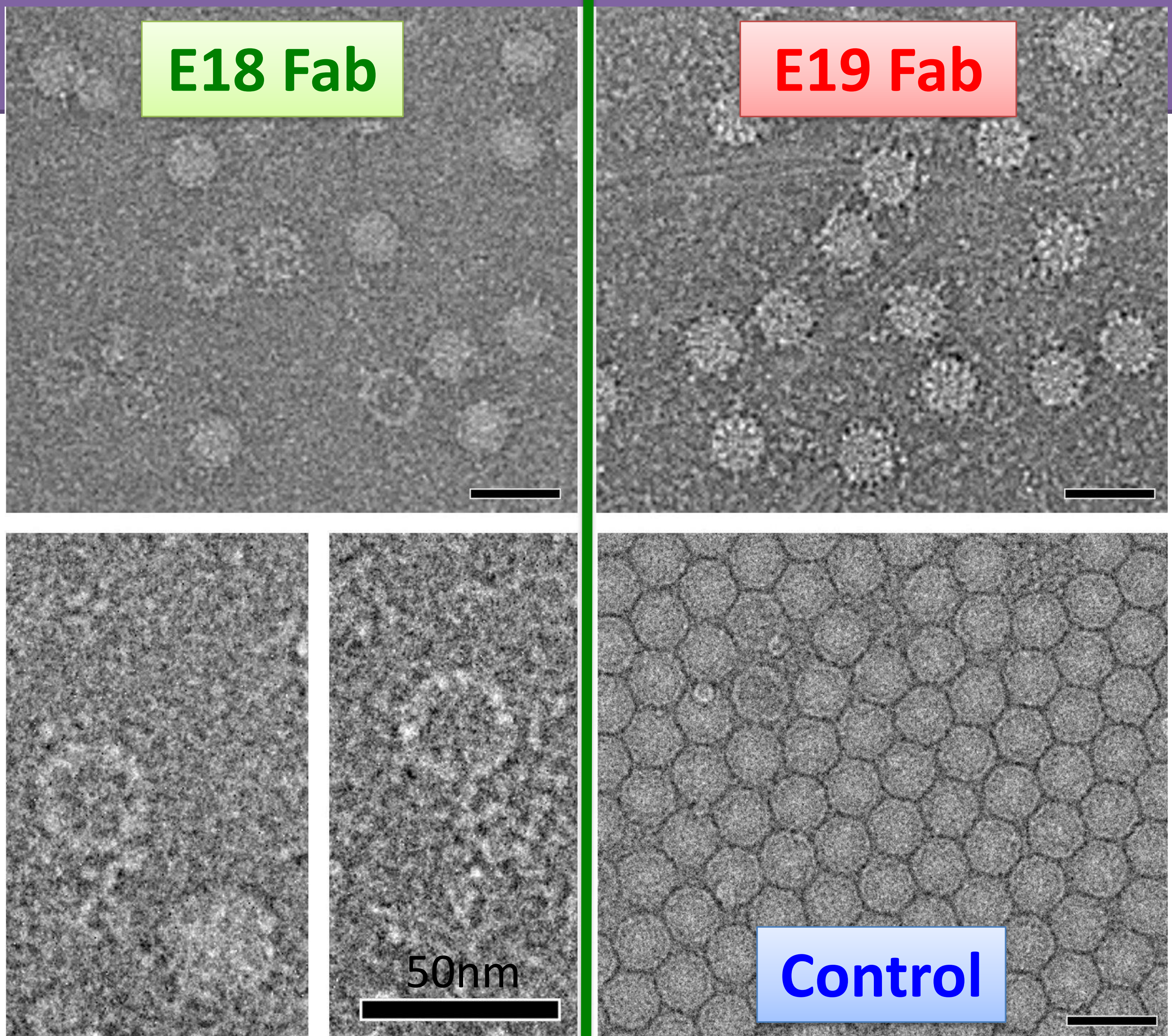


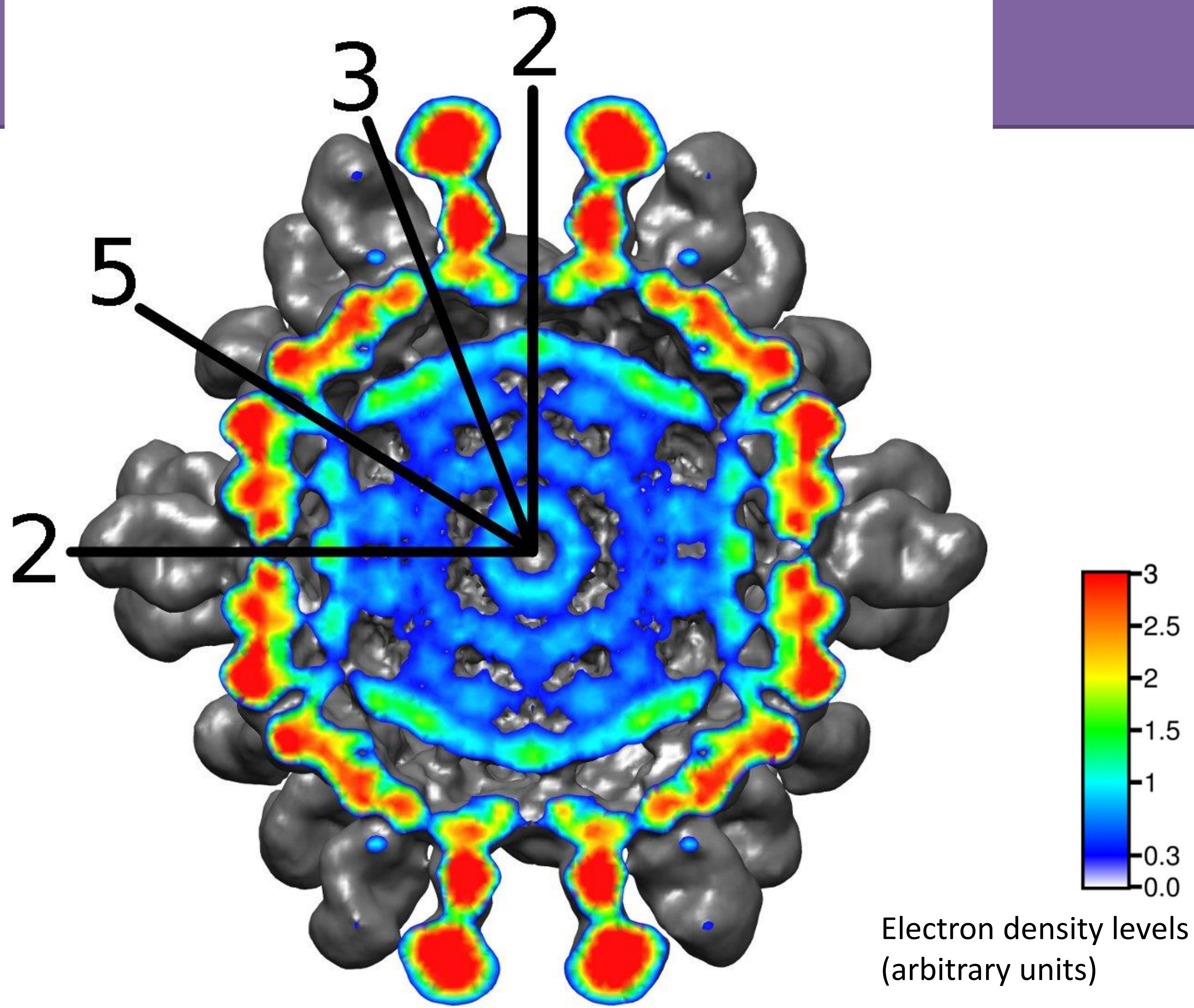
Antiviral effects of antibodies

- Neutrophils and macrophages have IgG Fc receptors -> phagocytosis of infected cells and induction of apoptosis
- NK cells have IgG Fc receptors -> killing of infected cells.
- Antibodies can induce genome release from virions (poliovirus, EV71).
- Prevention of receptor binding
- Release of virions attached to cells
- Inhibition of cell entry (fusion proteins)
- Inhibition of genome uncoating
- Activation of complement

Neutralization of viruses by antibodies

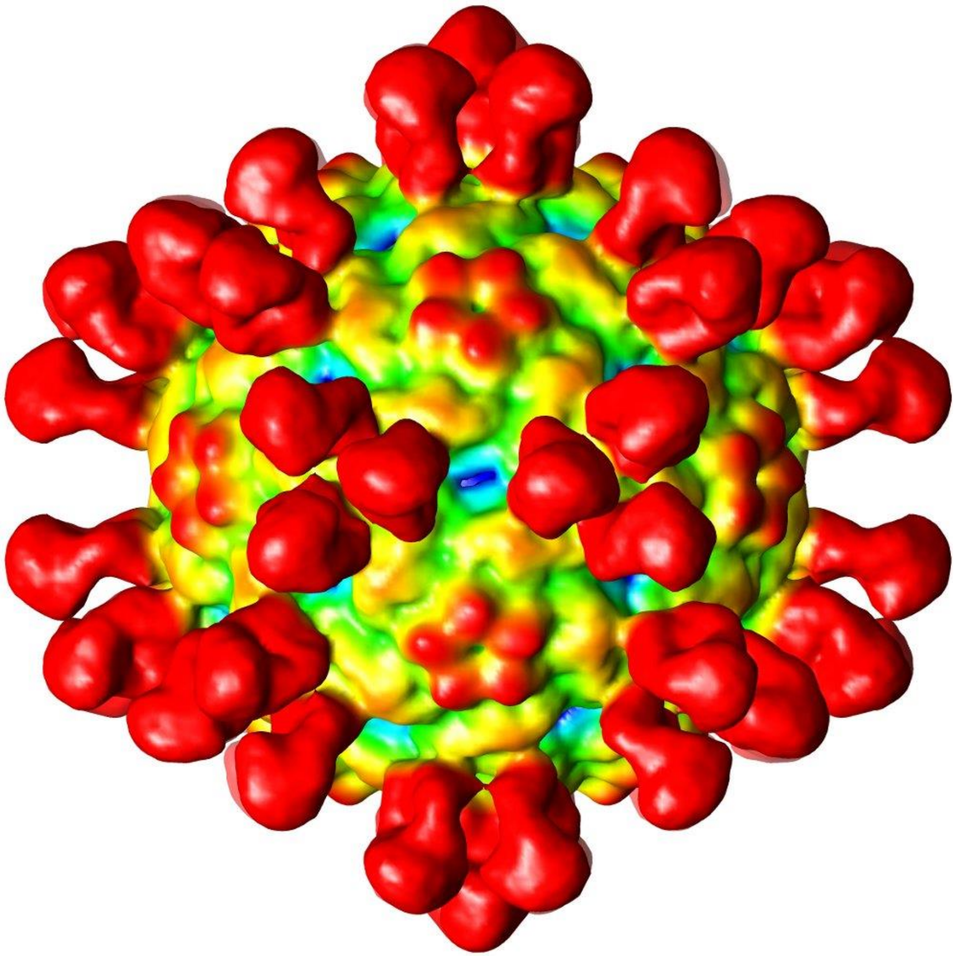



E18 Fab
E19 Fab
50nm
Control

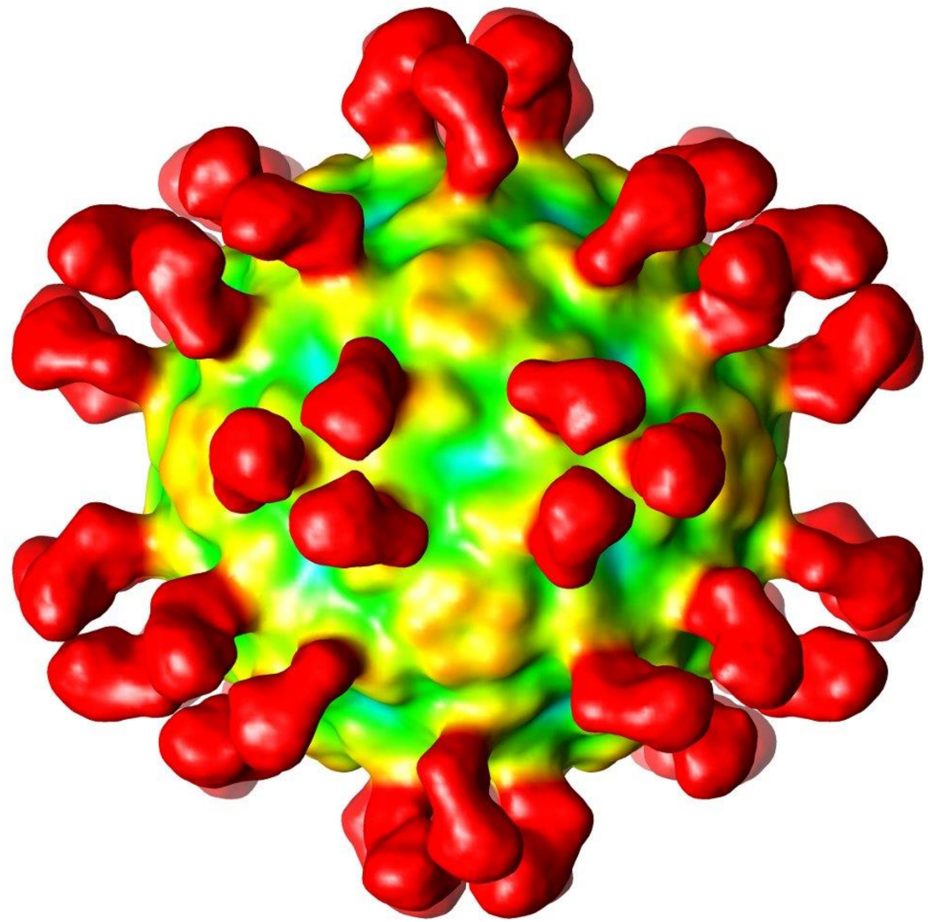


Electron density levels
(arbitrary units)

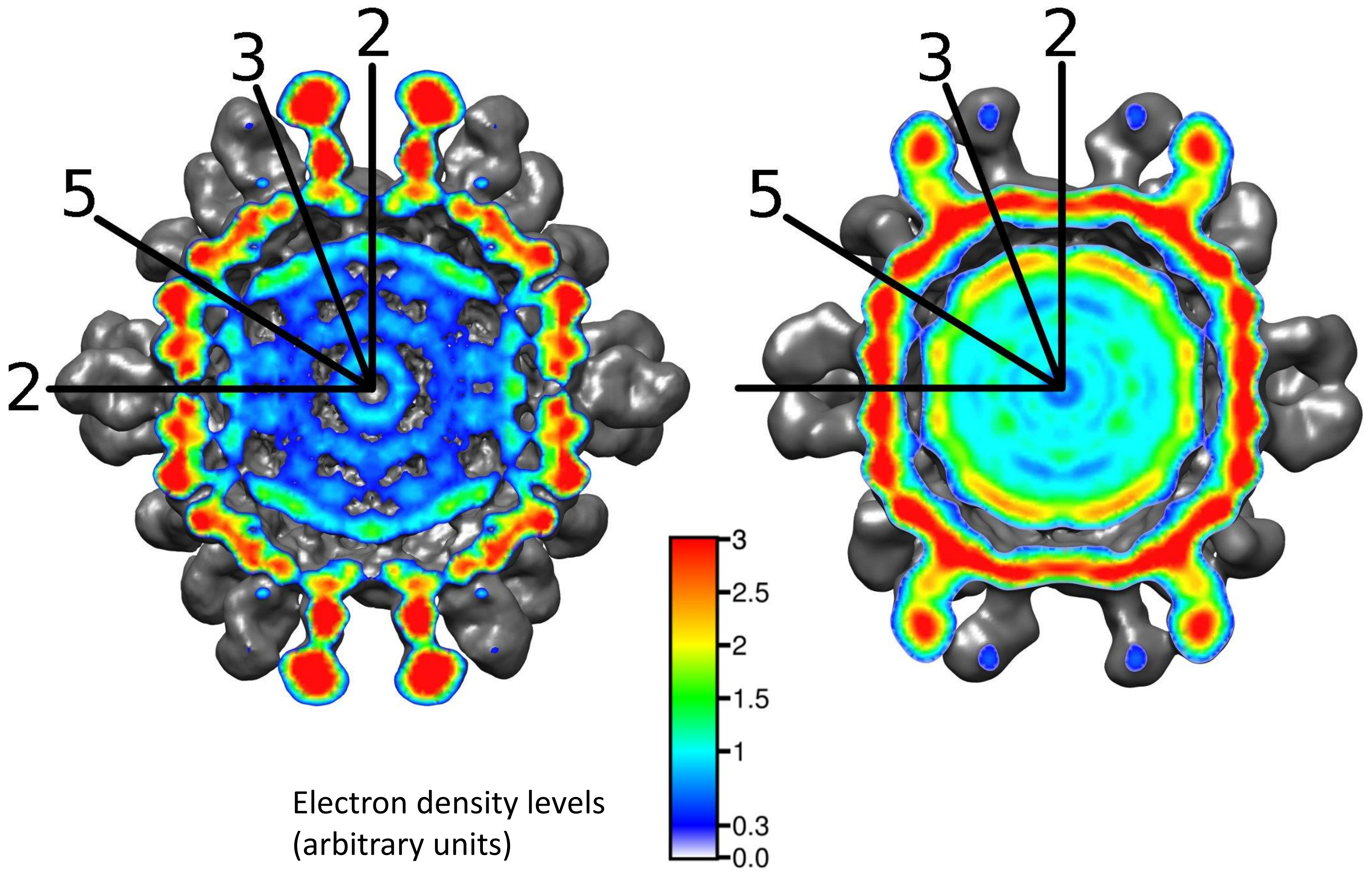
E18



E19

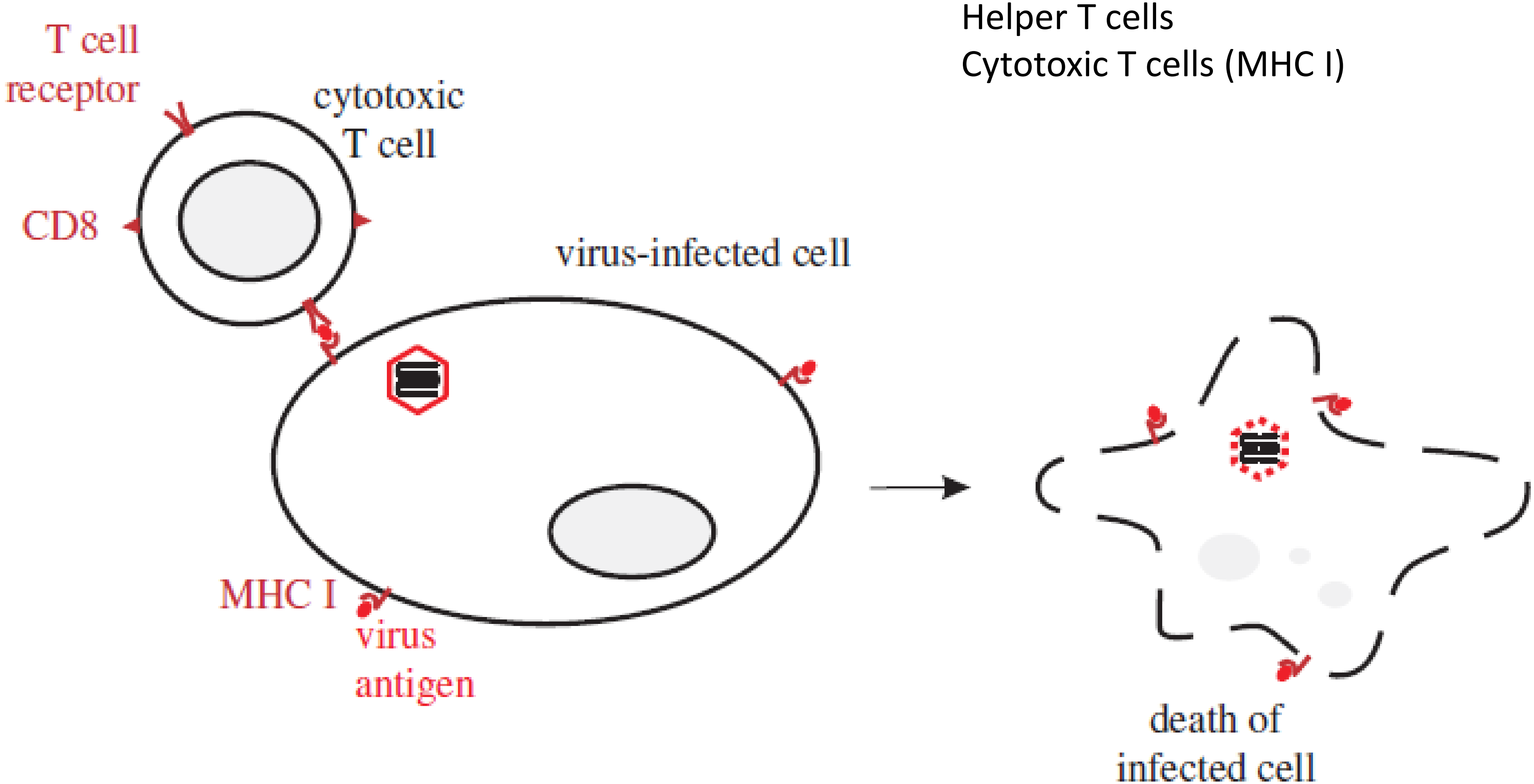


E18 Fab **E19 Fab**



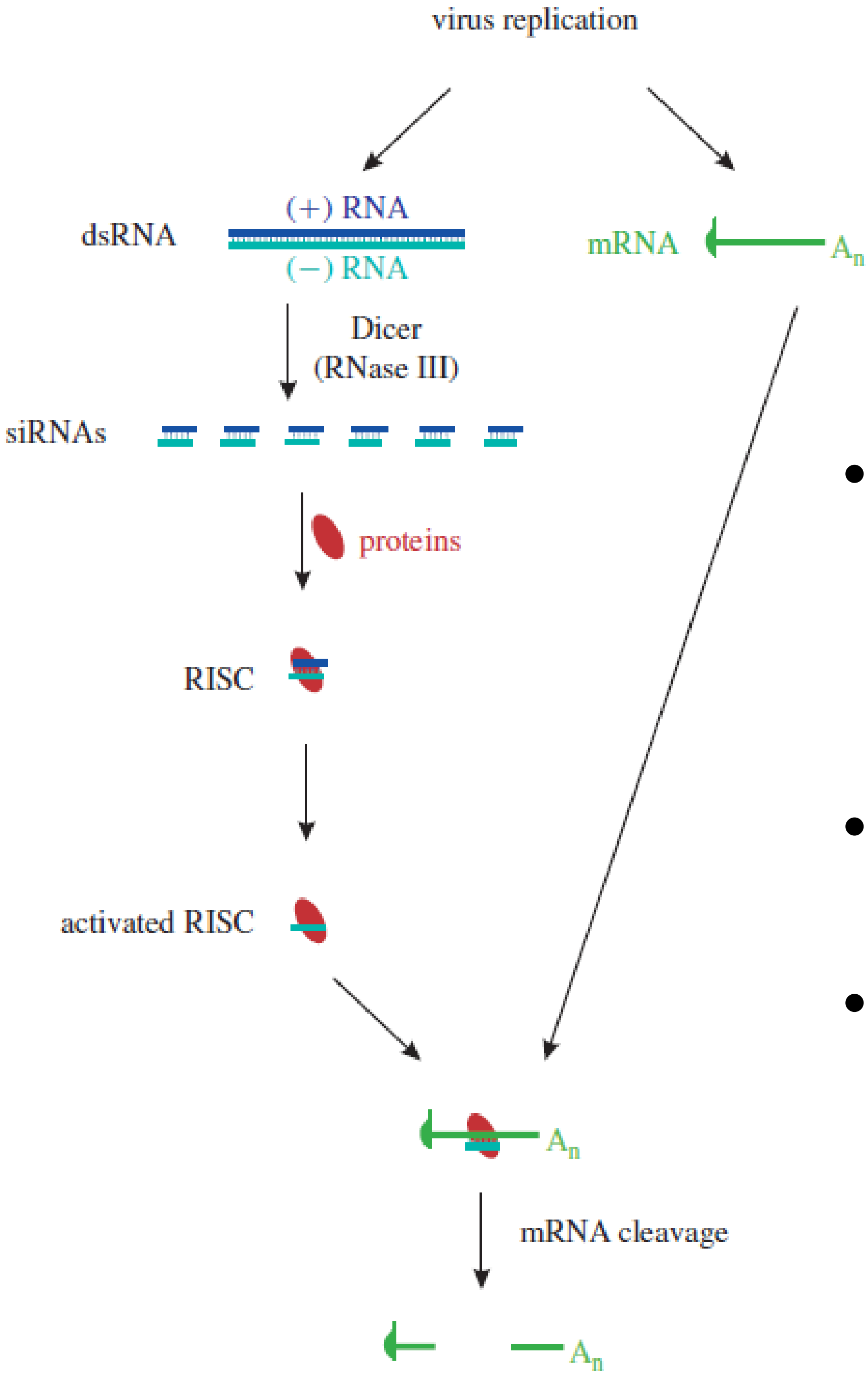
Electron density levels
(arbitrary units)

Recognition and killing of infected cell by cytotoxic T-cell



Helper T cells
Cytotoxic T cells (MHC I)

RNA silencing

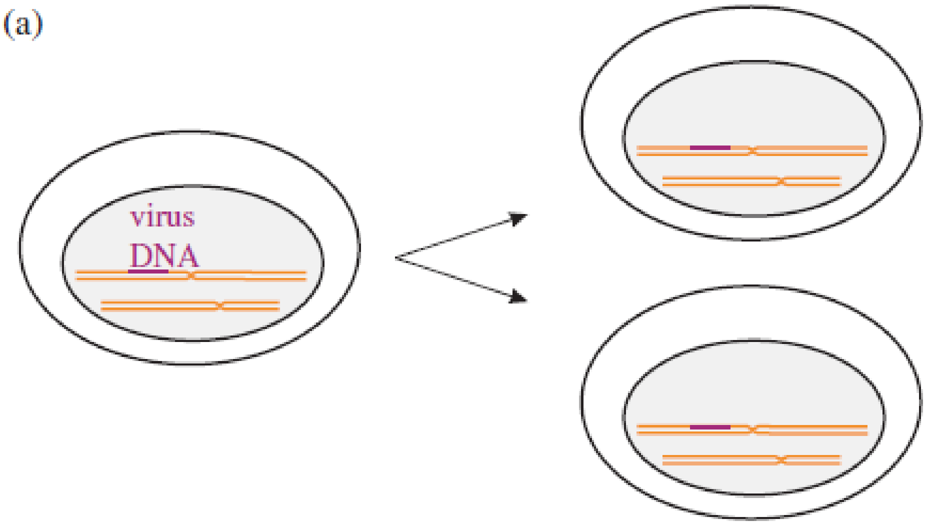


- Fragmenting of dsRNA by DICER (production of 21-25bp fragments with 2-3nt 3’ overhangs)
- Activation of RISC (RNA – induced silencing complex)
- Destruction of mRNAs

Many plant viruses produce proteins that inhibit RNA silencing.

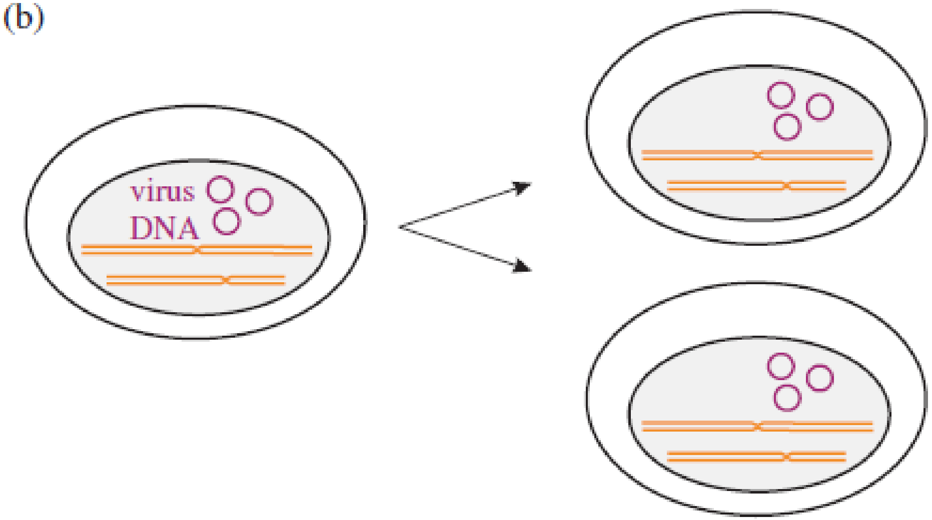
Maintenance of genomes during latent infection

(a)



Retroviruses

(b)



Polyoviruses,
Papillomaviruses

Signals that induce activation of latent infections

- A eukaryotic host cell moves into another phase of the cell cycle
- The host cell is irradiated with ultra-violet light. (phages, herpes simplex virus)
- A host organism becomes immunocompromised. (herpes simplex virus)
- The host cell becomes infected with a second virus that provides a function that the first virus lacks. (satellite virus and helper virus)

Productive infections in plants

Mode of transport through plasmodesmata	Virus examples
Virus RNA–MP complexes transported	Tobacco mosaic virus Cowpea chlorotic mottle virus
Virus RNA–coat protein–MP complexes transported	Cucumber mosaic virus
Virions transported through tubules composed of MP	Cowpea mosaic virus

Disease

Virulence of a virus strain
Dose of virus

Effectiveness of immune system (age, nutritional status, previous training)
Human interventions

Virus elimination x latent infections

Learning outcomes

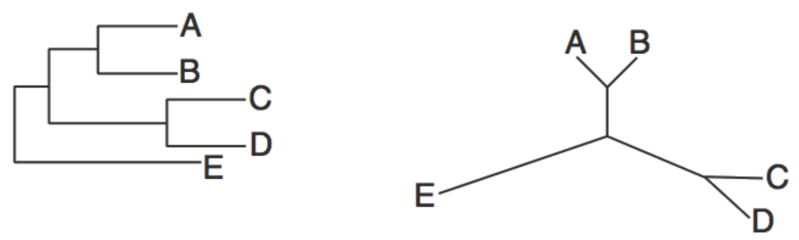
- describe the major components of innate and adaptive immunity in vertebrates
- outline the process of RNA silencing
- explain programmed cell death
- explain the terms
    - productive infection
    - non-productive infection
    - latent infection
    - abortive infection
    - defective virus
- discuss the spread of virus infections within animal bodies and within plants
- discuss the factors that determine whether virus infection results in disease

Virus classification

Authority: International Committee on Taxonomy of Viruses
http://www.ncbi.nlm.nih.gov/ICTVdb/index.htm

Taxonomic groups: Order
Family
Subfamily
Genus
Species

Phylogenetic trees - based on genome sequences
- indicate relationships between viruses:



Baltimore Classification: - seven classes of viruses
- based on genome type and transcription.

Phyogenetics trees

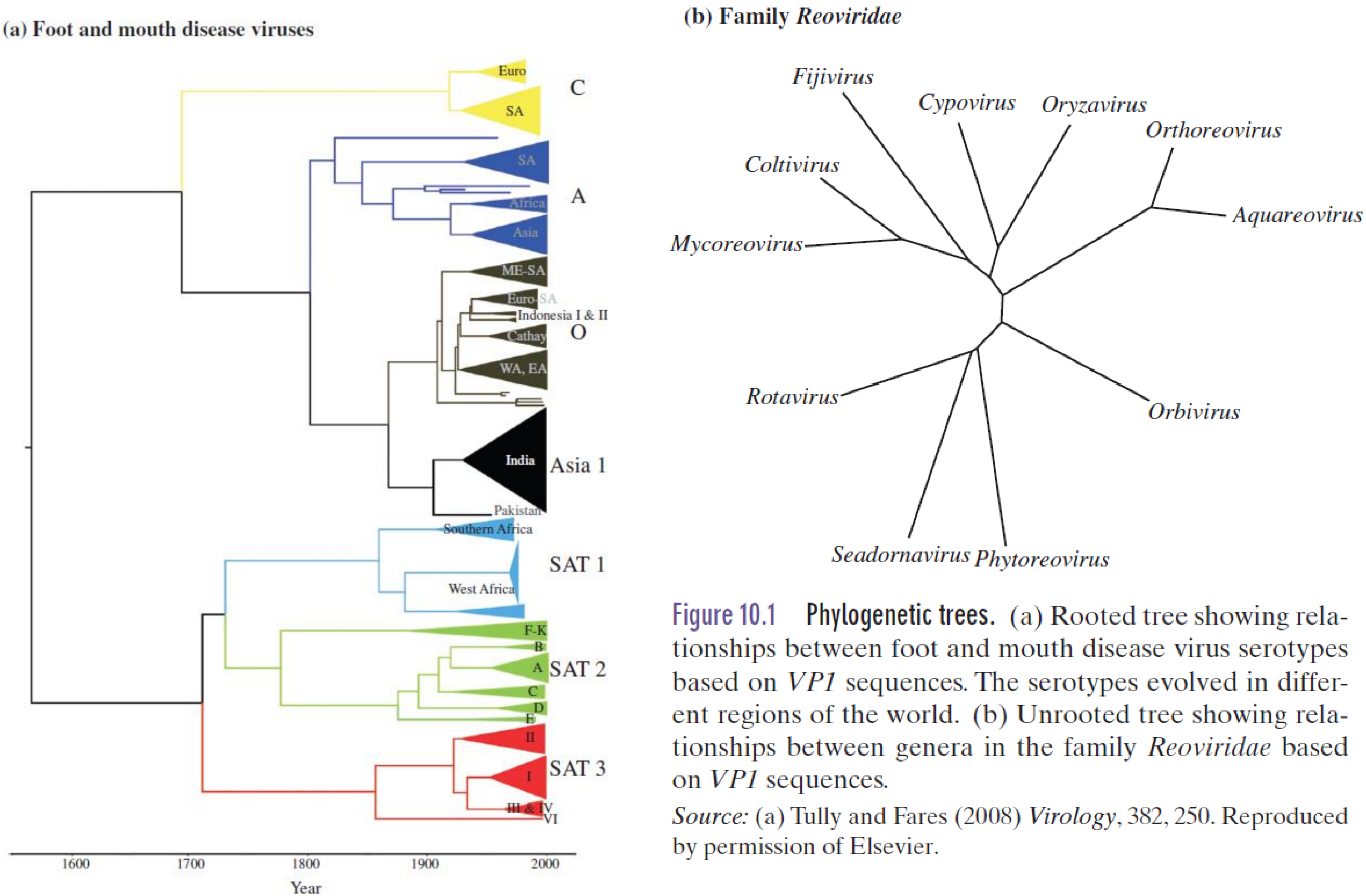


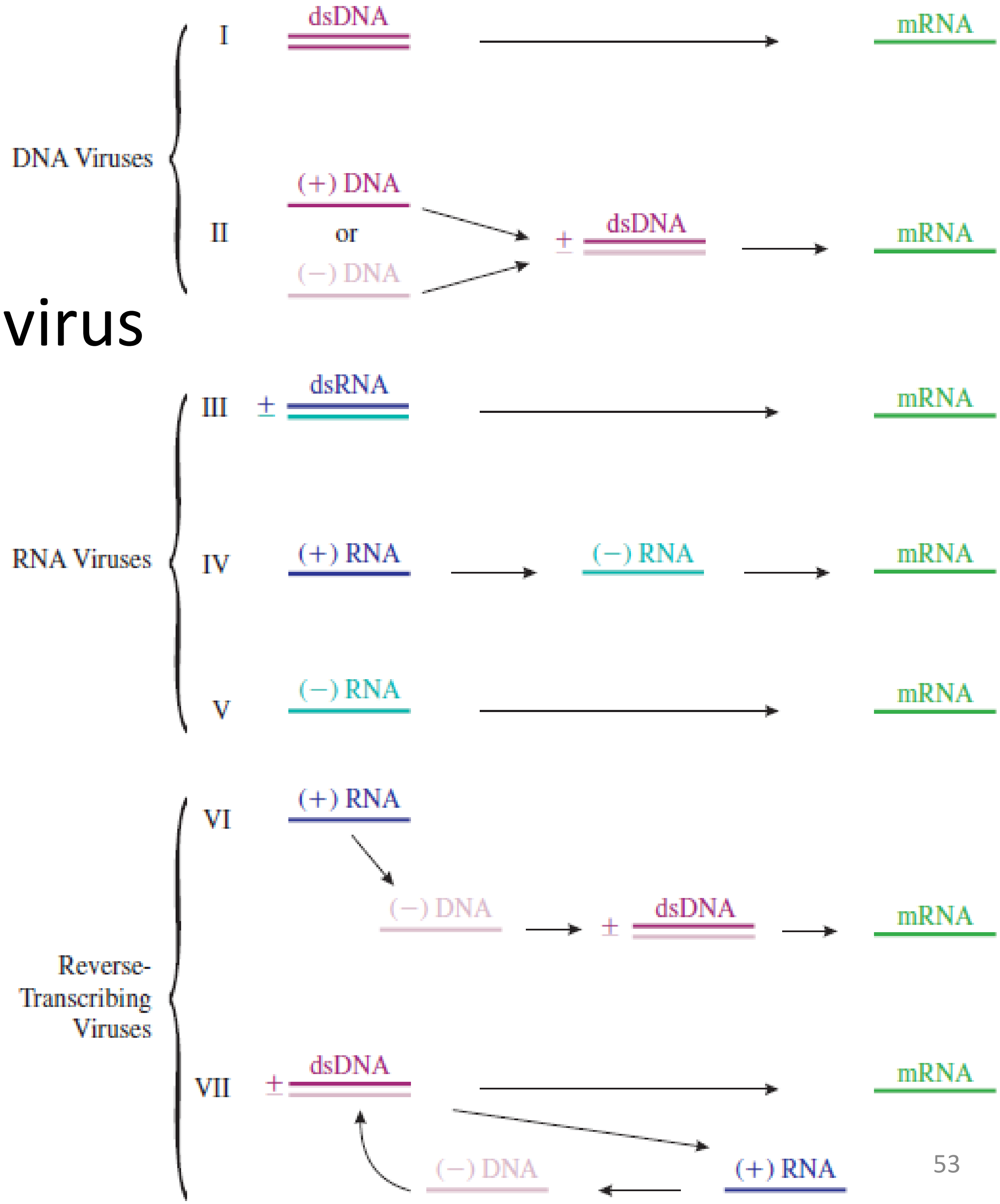
Figure 10.1 Phylogenetic trees. (a) Rooted tree showing relationships between foot and mouth disease virus serotypes based on *VP1* sequences. The serotypes evolved in different regions of the world. (b) Unrooted tree showing relationships between genera in the family *Reoviridae* based on *VP1* sequences.

Source: (a) Tully and Fares (2008) *Virology*, 382, 250. Reproduced by permission of Elsevier.

Nomenclature

Taxonomic group	Suffix	Example 1	Example 2	Example 3
Order	*-virales*	*Caudovirales*	*Mononegavirales*	*Nidovirales*
Family	*-viridae*	*Myoviridae*	*Paramyxoviridae*	*Coronaviridae*
Subfamily	*-virinae*	–	*Paramyxovirinae*	–
Genus	*-virus*	*T4-like viruses*	*Morbillivirus*	*Coronavirus*
Species	–	*Enterobacteria phage T4*	*Measles virus*	*Severe acute respiratory syndrome virus*

(David) Baltimore virus classification



Learning outcomes

- evaluate the traditional criteria used to classify viruses into families and genera
- write family and genus names in the correct format
- explain how genome sequence data are used to classify viruses
- evaluate phylogenetic trees
- explain the basis of the Baltimore classification of viruses

Herpesviruses

Figures
Chapter 11

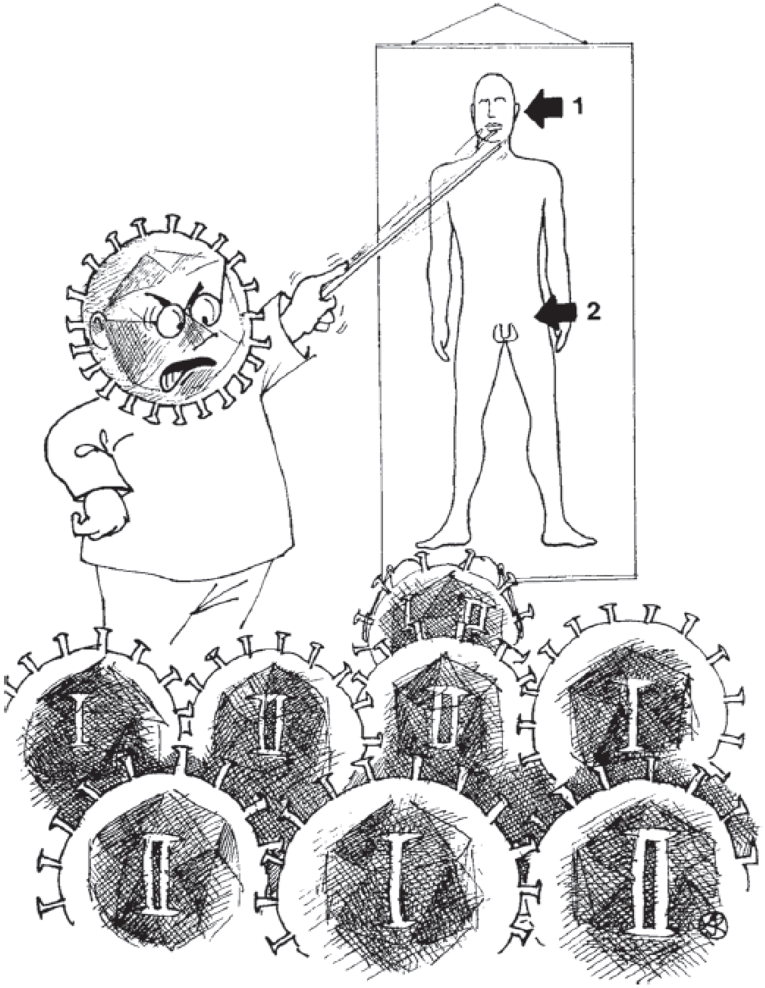
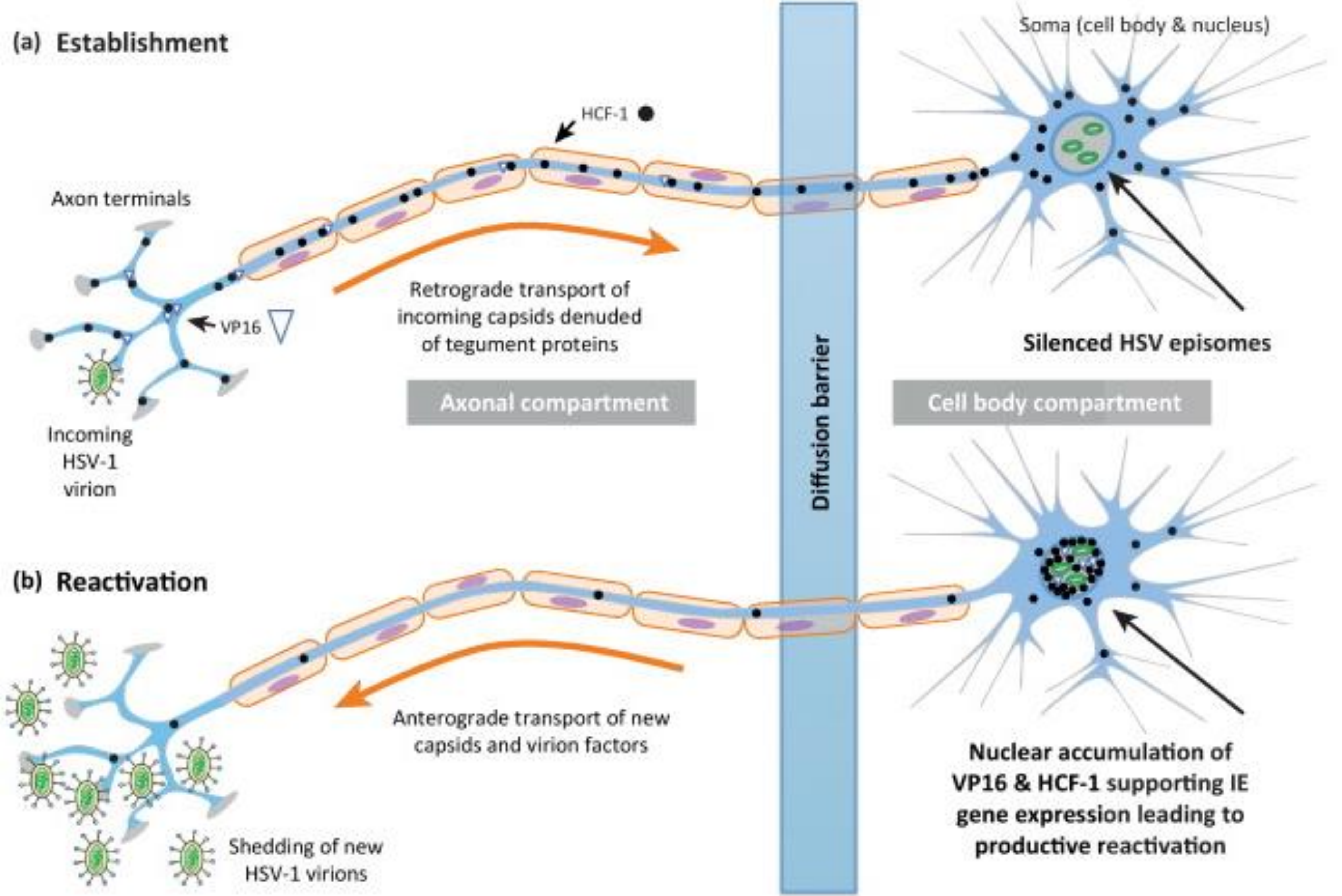


Figure 11.1 "Get your priorities right."

Source: Haaheim, Pattison, and Whiteley (2002) *A Practical Guide to Clinical Virology*, 2nd edition. Reproduced by permission of John Wiley & Sons.

Latent infection



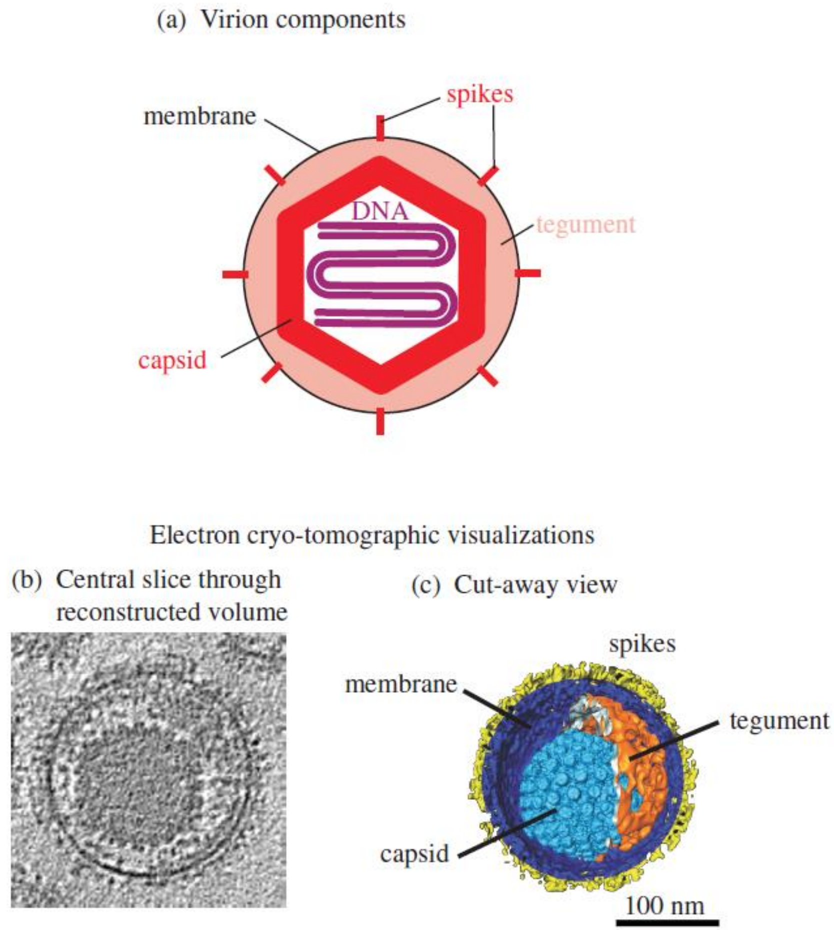


Figure 11.2 The herpesvirus virion.

Source: (b) and (c) are images of HSV-1, from Grünewald and Cyrklaff (2006) *Current Opinion in Microbiology,* 9, 437. Reproduced by permission of the authors and Elsevier Limited.

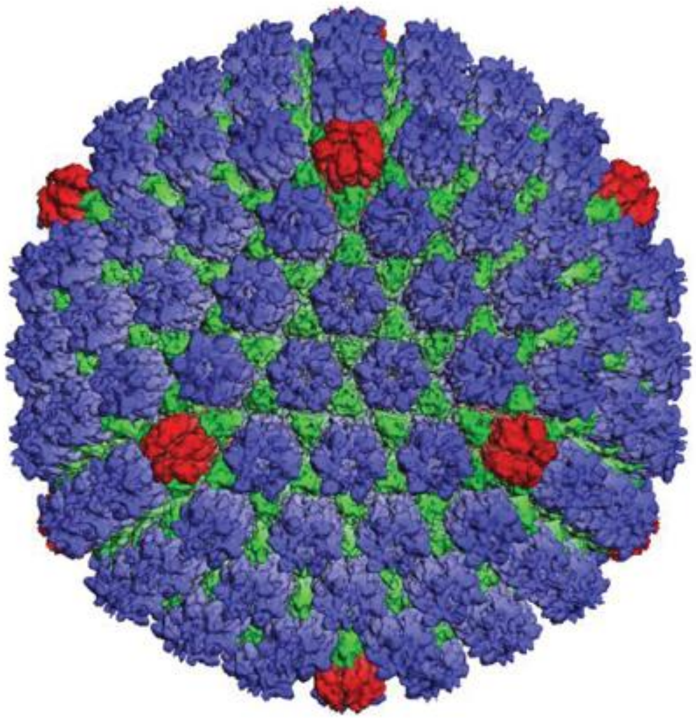


Figure 11.3 The HSV-1 capsid. Reconstructed image from cryo-electron microscopy.

Source: Courtesy of Professor Wah Chiu, Baylor College of Medicine, Houston, TX. Reinterpretation of data from Zhou *et al*. (2000) *Science*, 288, 877, with permission of the American Association for the Advancement of Science.

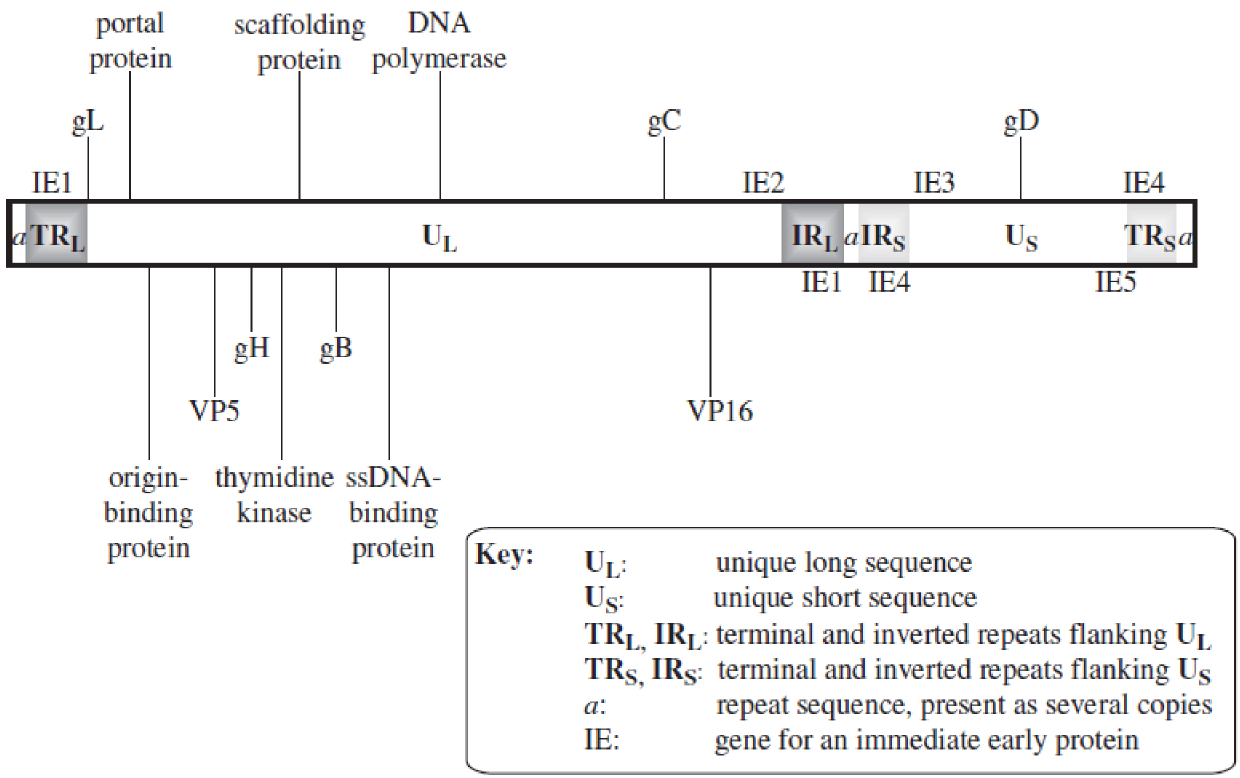


Figure 11.4 The HSV-1 genome.

The locations of some genes are indicated. Those shown above the genome are in ORFs read left to right; those shown below the genome are in ORFs read right to left.

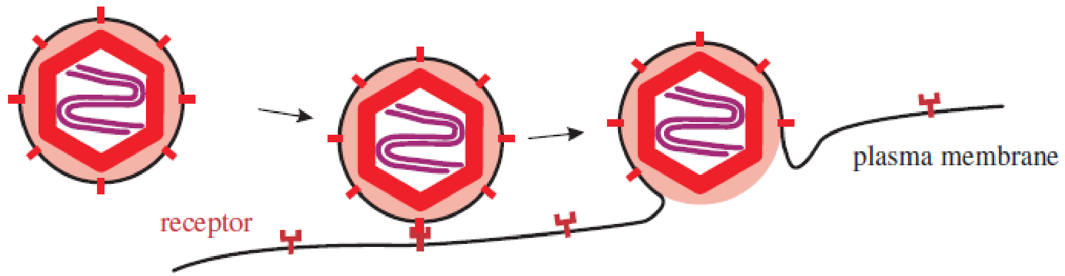


Figure 11.5 **HSV-1 attachment and entry into the cell.** Virus glycoproteins bind to receptors on the cell, then the virion envelope fuses with the plasma membrane.

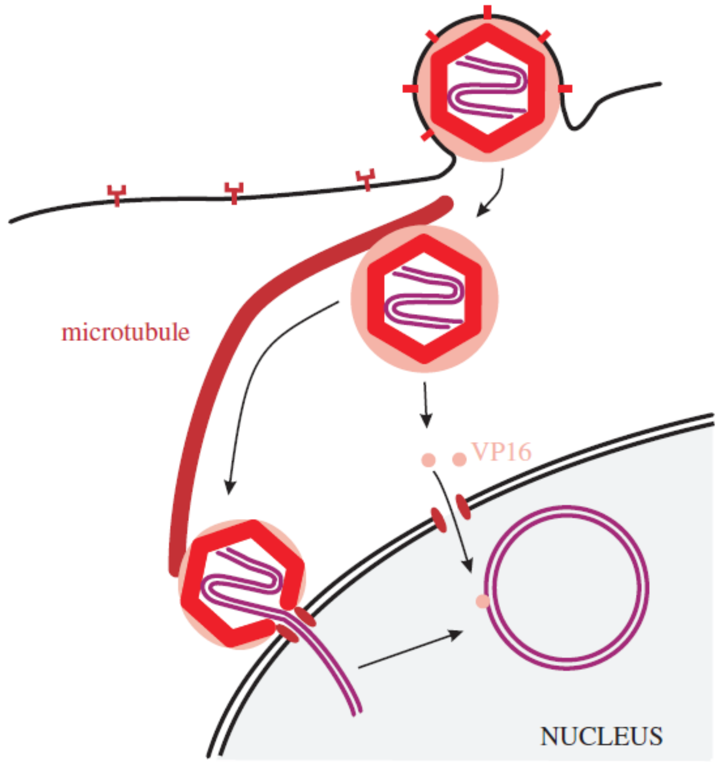


Figure 11.6 Transport of HSV-1 DNA and VP16 into the nucleus. The nucleocapsid is transported along a microtubule to a site close to the nucleus. Docking of the nucleocapsid at a nuclear pore is followed by release of the genome into the nucleus. Molecules of VP16 released from the tegument are also transported into the nucleus.

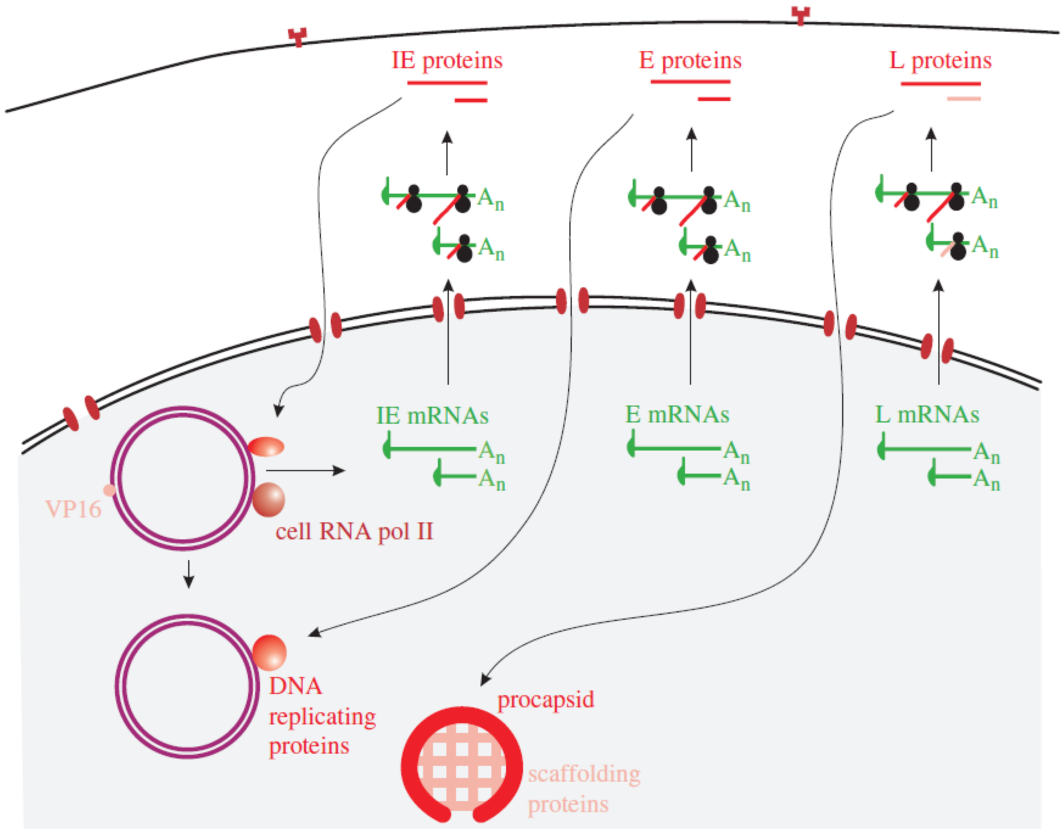


Figure 11.7 **HSV-1 transcription and translation.**

There are three phases of transcription and translation:

IE: immediate early

E: early

L: late.

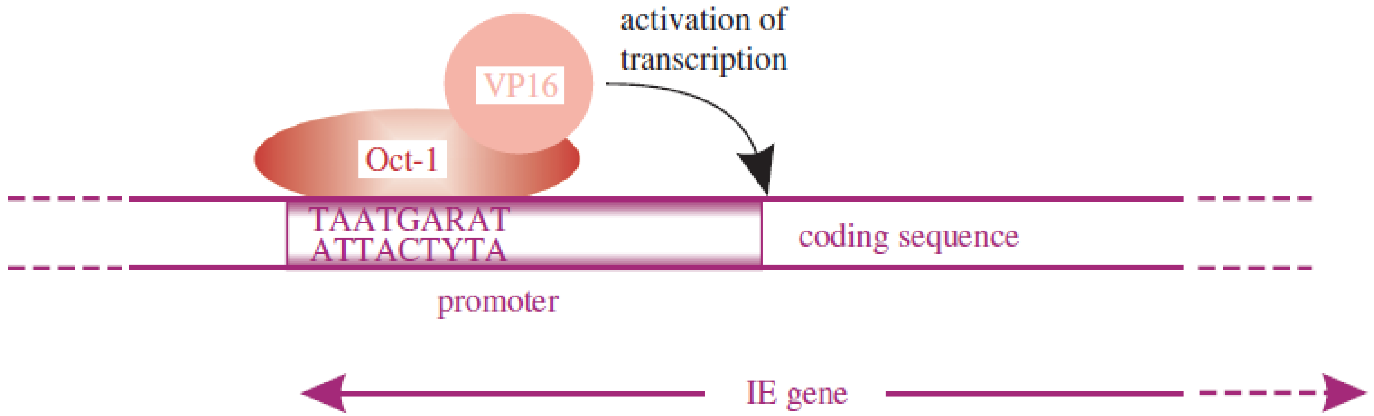


Figure 11.8 Activation of transcription of HSV-1 IE genes by VP16.
R: purine
Y: pyrimidine

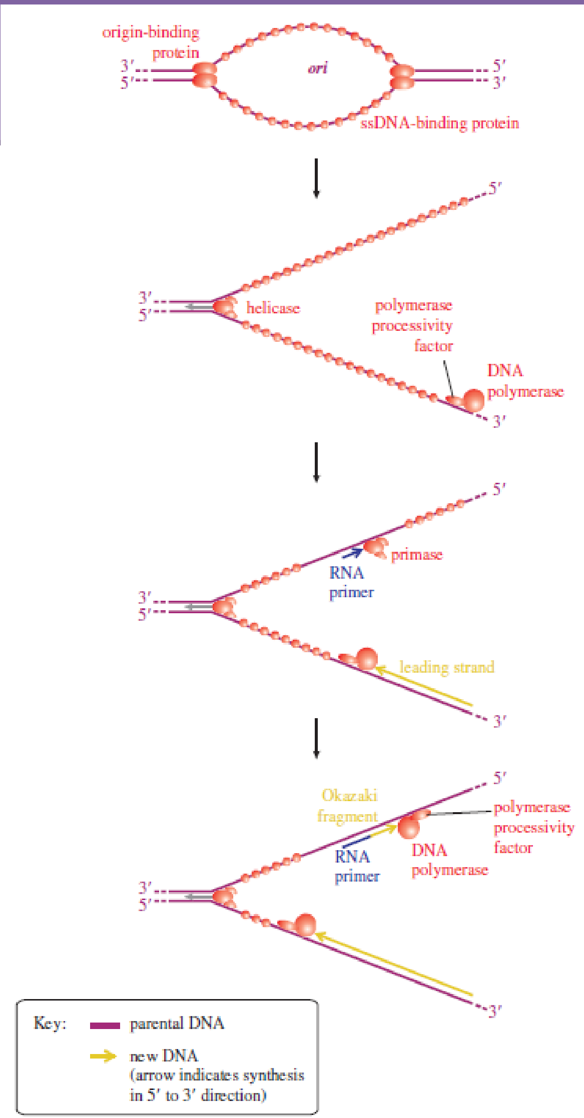


Figure 11.9 Roles of HSV-1 proteins in DNA replication. The roles of the seven virus proteins essential for DNA replication are shown. The helicase and the primase are complexes of the same three proteins. Please see text for details.

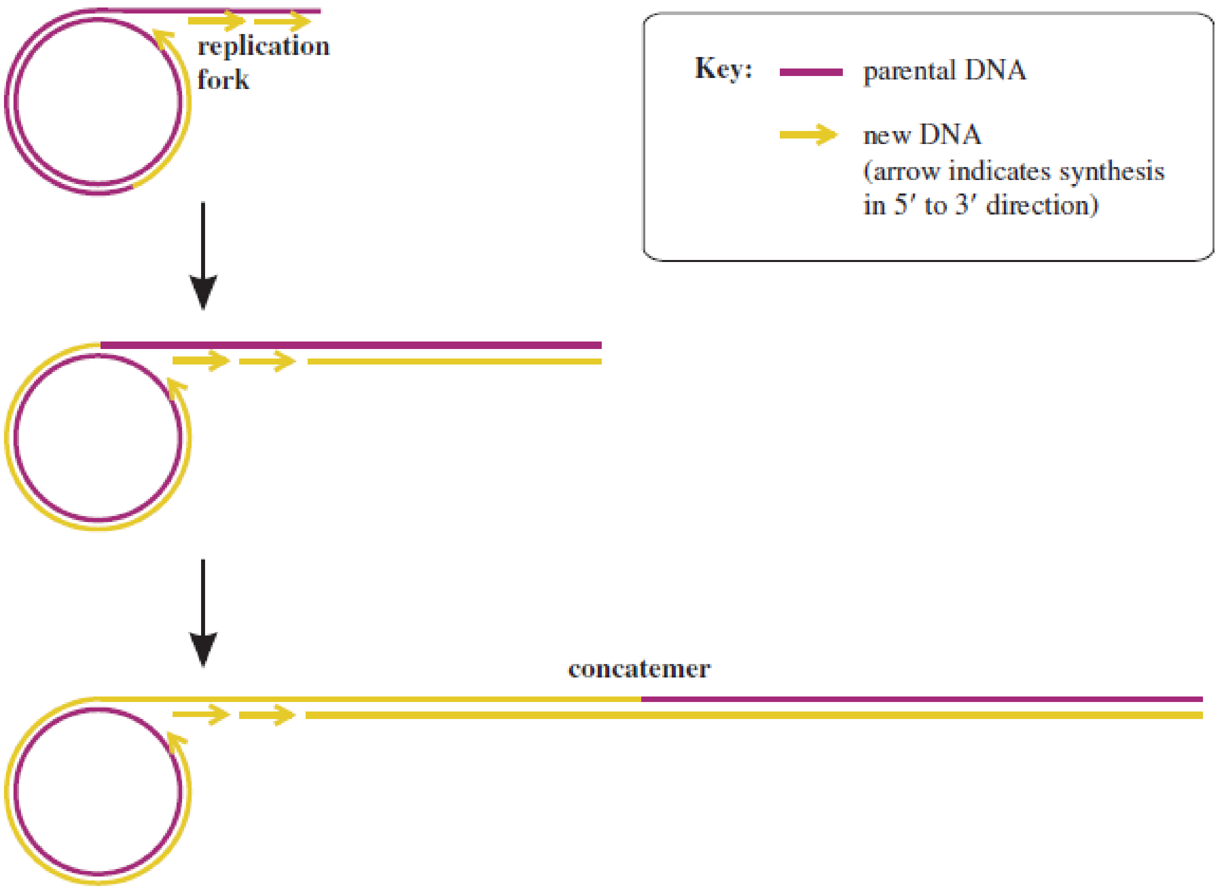


Figure 11.10 Formation of a concatemer. See Figure 7.5 for earlier stages of rolling circle replication of DNA. Color coding indicates the fates of the two parental strands of DNA.

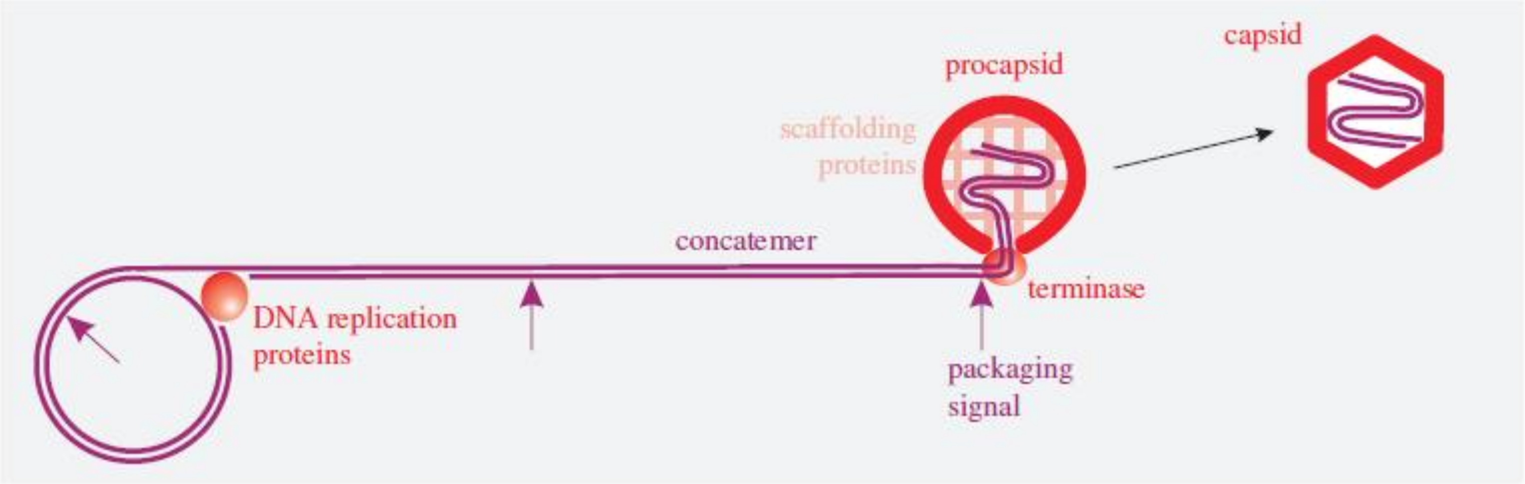


Figure 11.11 **Packaging HSV-1 DNA into a procapsid.** A genome-length of DNA enters a procapsid and is cleaved by a terminase at a packaging signal.

(a) Transport of nucleocapsids to the cytoplasm, envelopment, and exit from the cell

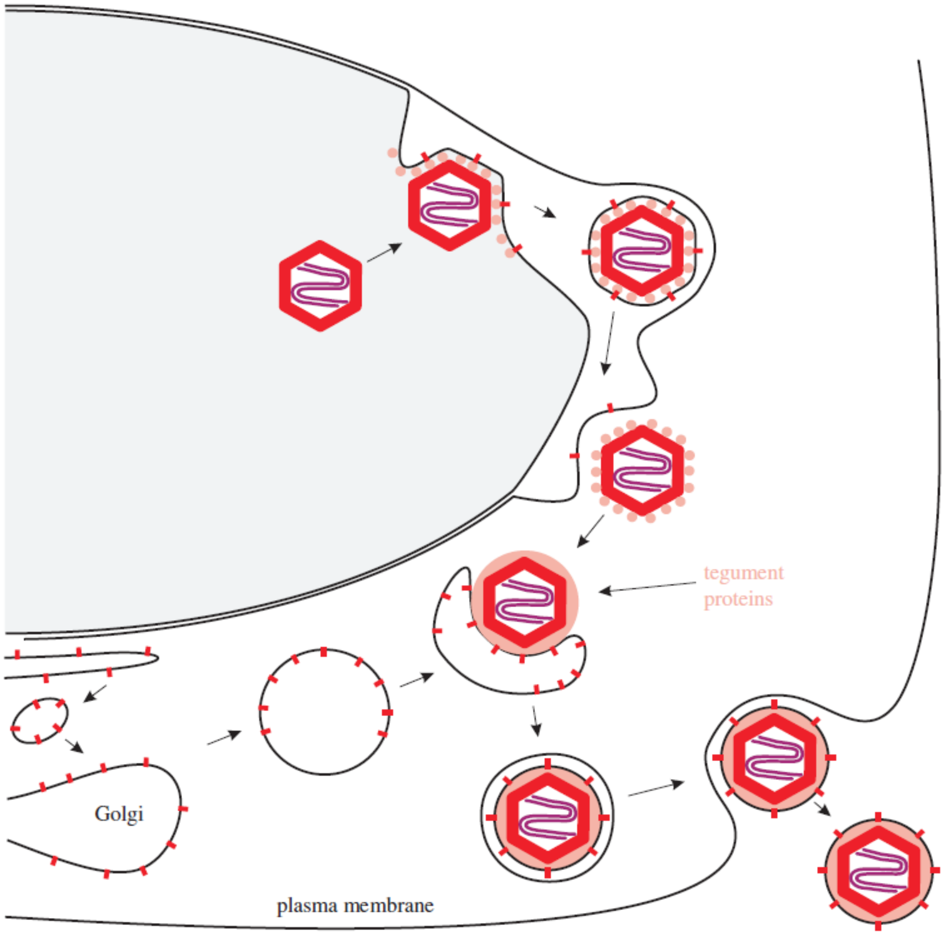


Figure 11.12 Final stages of HSV-1 assembly.

(b) Nucleocapsid with temporary envelope in the perinuclear space

(c) Nucleocapsid budding into a vesicle in the cytoplasm

(d) Enveloped virion within a vesicle during transport to the plasma membrane

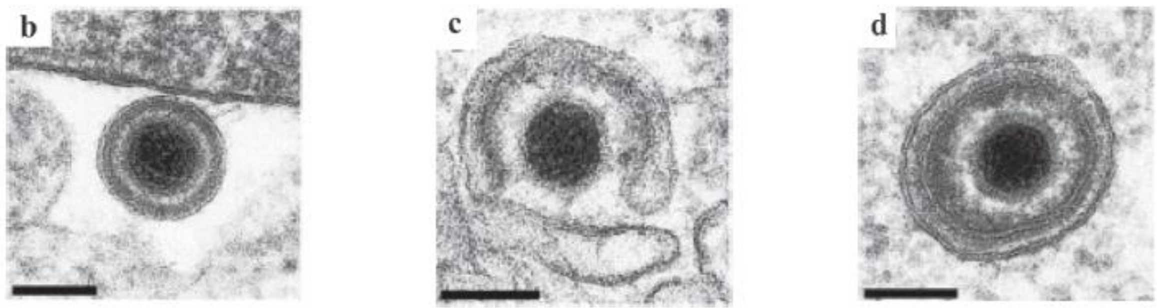
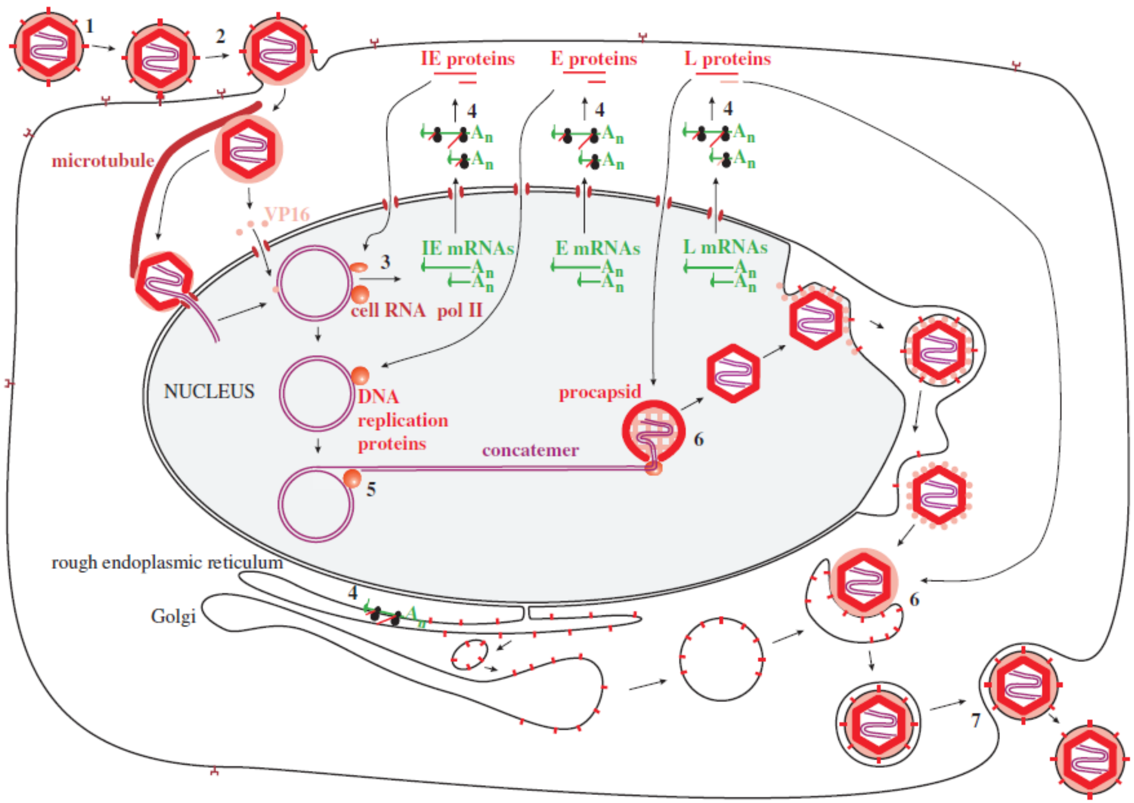


Figure 11.12 (*continued*)

Source: (b)–(d) from Mettenleiter, Klupp, and Granzow (2009) *Virus Research*, 143, 222. Scale bar: 100 nm. Reproduced by permission of Elsevier and the authors.



1. Attachment
2. Entry
3. Transcription
4. Translation
5. Genome replication
6. Assembly
7. Exit

Figure 11.13 **The HSV-1 replication cycle.** Stages in virion assembly include procapsid construction, packaging a copy of the genome, and acquisition of the envelope by budding into a vesicle within the cytoplasm.