PowerPoint® Lecture Presentations

CHAPTER 5

Viruses and Their Replication

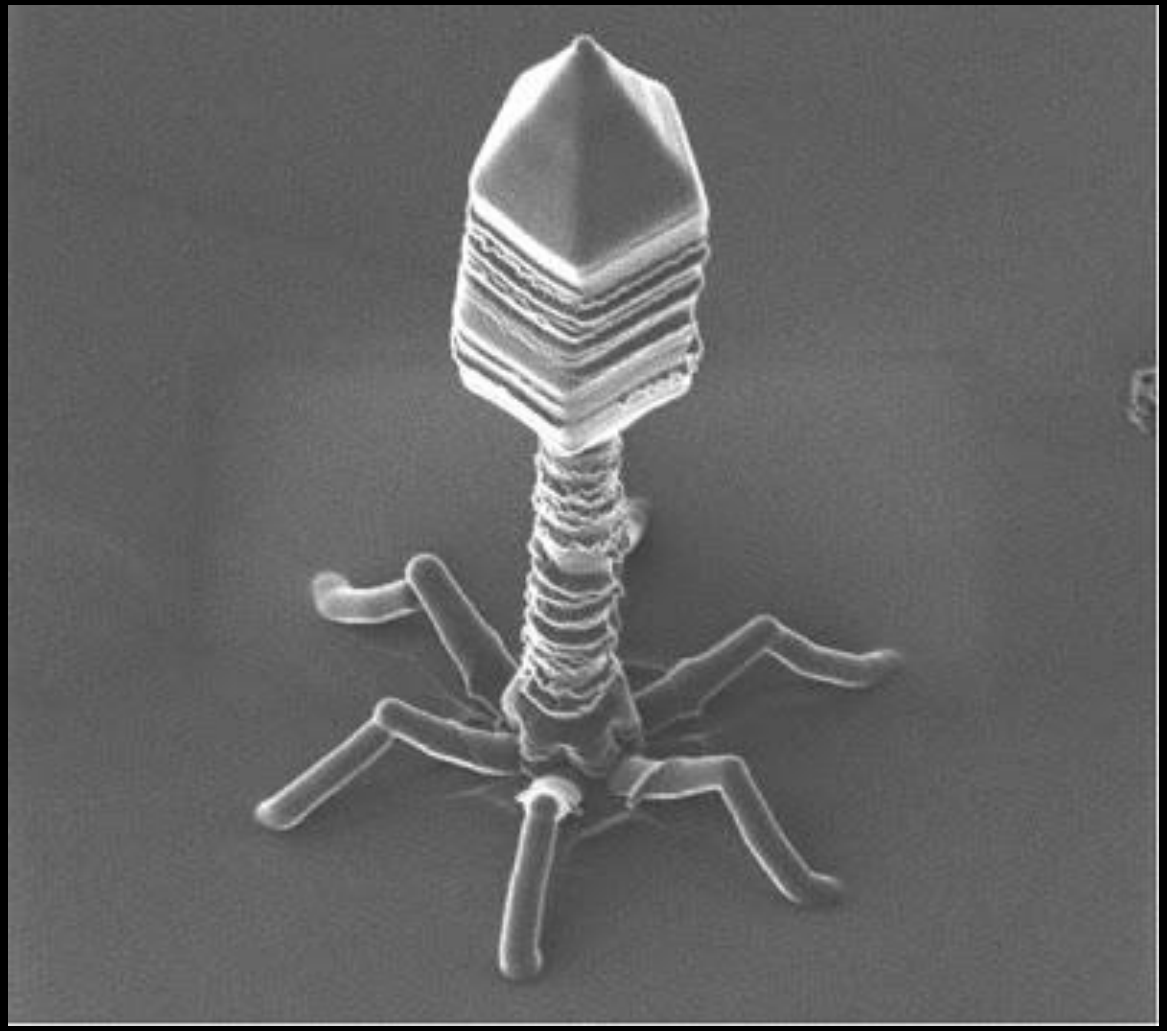
Schema Micro2

Les	Hoofdstuk	Paragraaf
1	7	7.1, 7.2, 7.3, 7.8
2	7	7.5, 7.6, 7.7
3	7	7.9, 7.10, 7.11
4	7	7.12, 7.13, 7.14, 7.15
5	5	5.1, 5.2, 5.3, 5.4, 5.5, 5.6
6	5 en 11	5.7, 5.8, 11.1, 11.2
7	11	11.6, 11.7, 11.8 (MS2 niet)
8	11	11.9, 11.11,
9	11	11.13, 11.15, 11.16
10	24	24.1, 24.2, 24.5
11	25	25.1, 25.2, 25.3, 25.5
12	25	25.6, 25.7, 25.8
13	28 en 8	28.10, 28.11, 28.12, 8.10
14	Oefententamen	Alles

NB: Hfdstnrs niet accuraat

Microbiologie 2: Les 5

I. The Nature of Viruses



I. The Nature of Viruses

- 5.1 What Is a Virus?
- 5.2 Structure of the Virion
- 5.4 Overview of the Viral Replication Cycle
- 5.3 Culturing, Detecting, and Counting Viruses

5.1 What Is a Virus?

- Virus: genetic element that cannot replicate independently of a living (host) cell
- Virus particle (virion): extracellular form of a virus

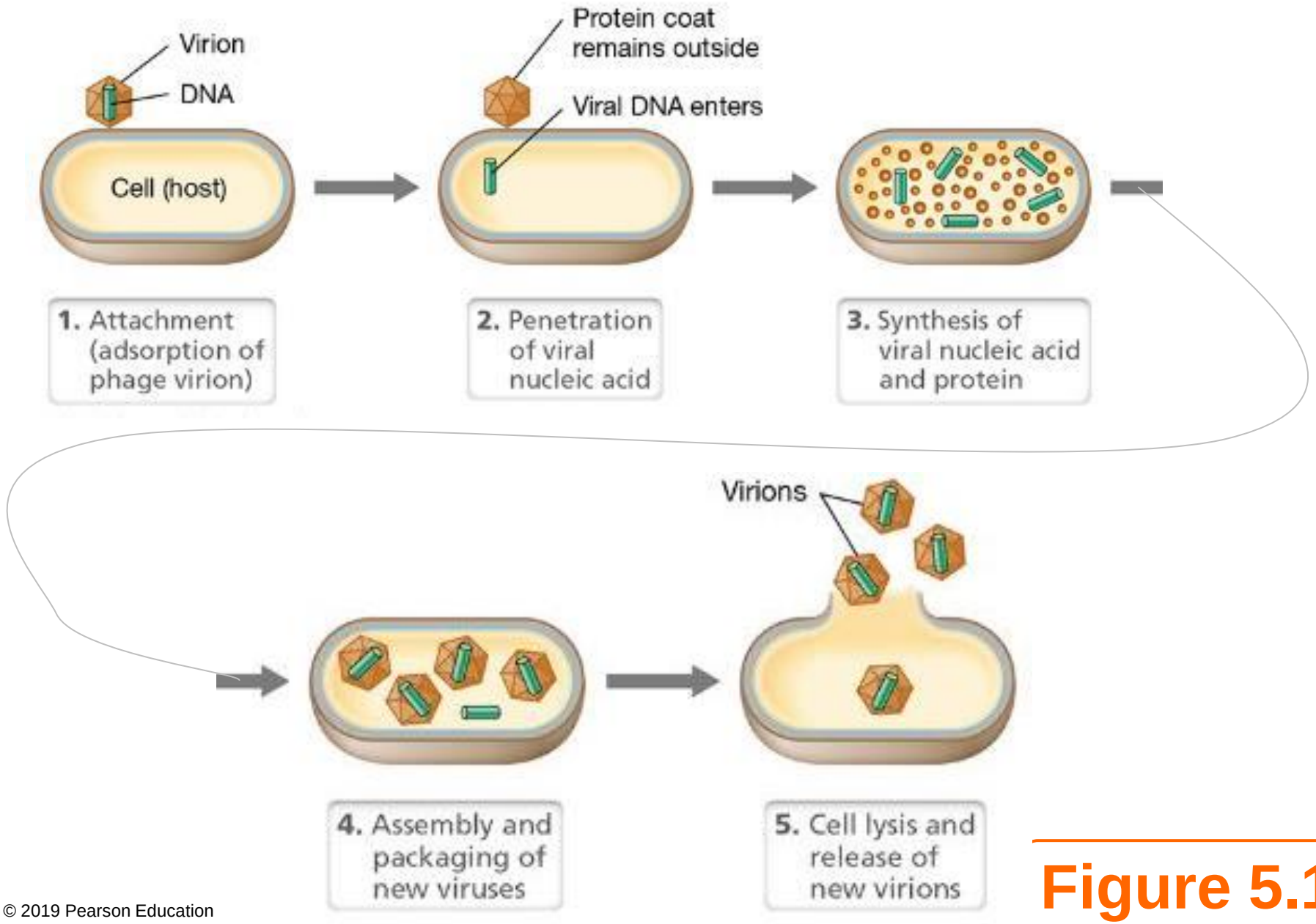


Figure 5.12

5.1 What Is a Virus?

- Viral components and activities
  - capsid: the protein shell surrounding the viral the genome
  - *Enveloped* viruses: outer phospholipid bilayer (from host cell membrane) and viral proteins.
    - Nucleocapsid: nucleic acid + protein in enveloped viruses
  - *Naked* viruses: no other layers.

5.1 What Is a Virus?

- Classification based on host specificity.
  - bacterial viruses (bacteriophages; model systems)
  - archaeal viruses
  - animal viruses (extensively studied)
  - plant viruses (less well studied)

5.2 Structure of the Virion

- Viruses come in many shapes and sizes.
  - Most viruses are smaller than prokaryotic cells; range from 0.02 to 0.3 μm.

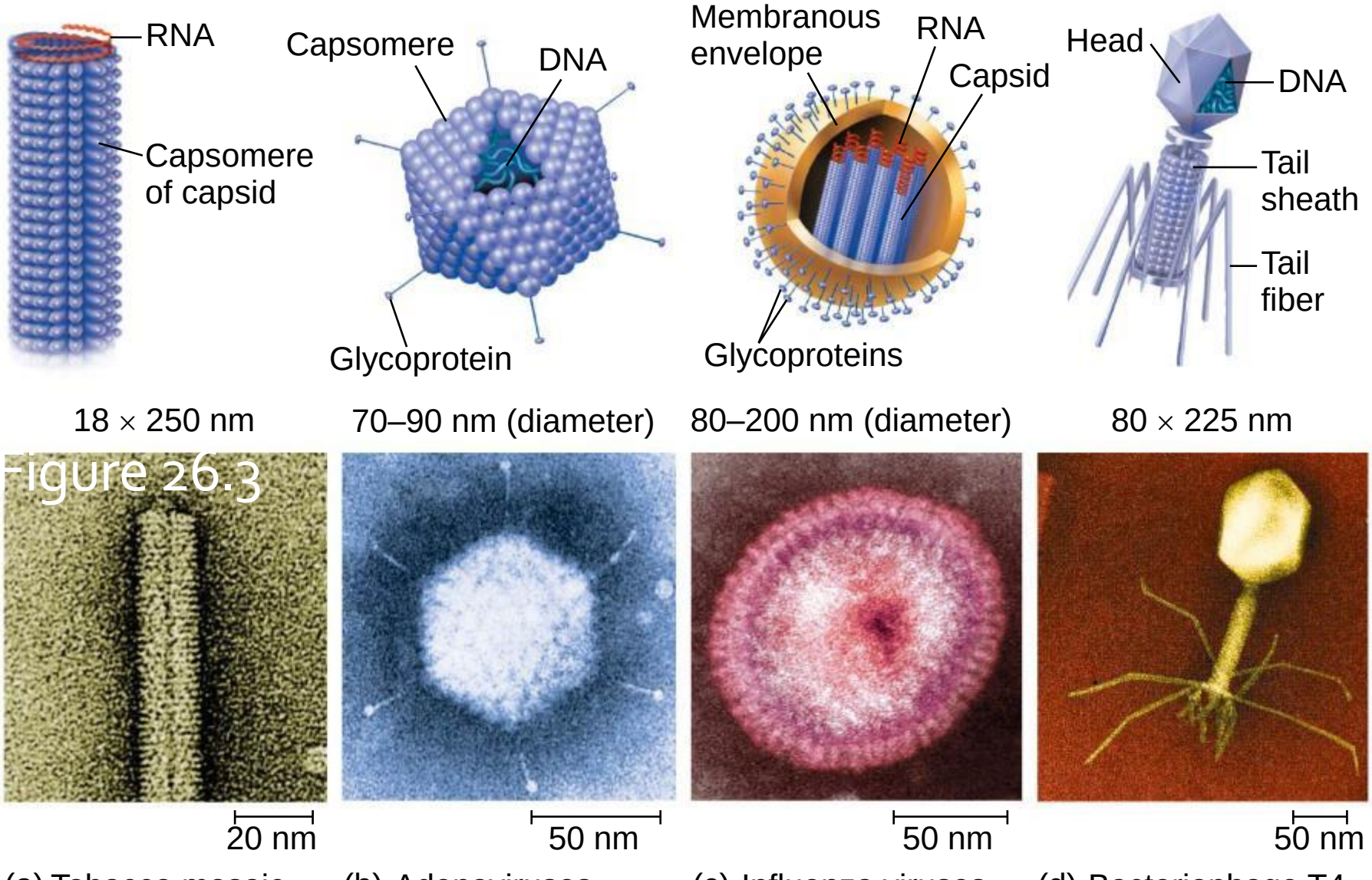


Figure 26.3

(a) Tobacco mosaic virus

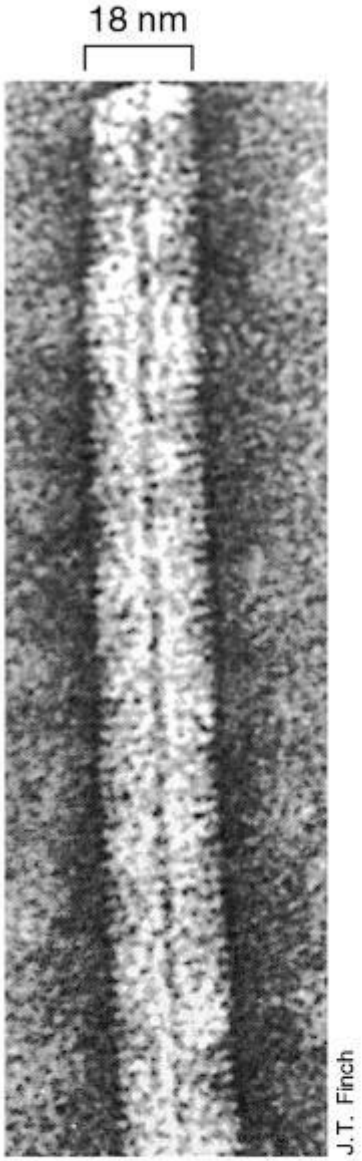
(b) Adenoviruses

(c) Influenza viruses

(d) Bacteriophage T4

5.2 Structure of the Virion

- Virion structure
  - capsomere: individual protein molecules arranged in a precise and highly repetitive pattern around the nucleic acid making up the capsid (Figure 5.5)
  - Capsids can be put together through *self-assembly* (spontaneous) or require host cell folding assistance.



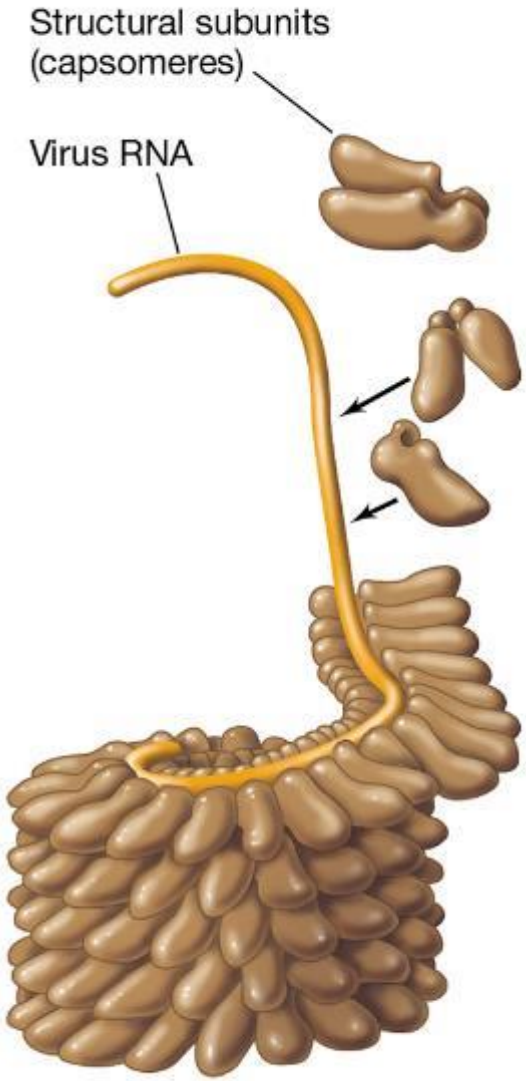


Figure 5.5

5.2 Structure of the Virion

- Virus symmetry
  - *helical* symmetry: rod-shaped viruses (*e.g.*, tobacco mosaic virus or TMV)
    - length of virus determined by length of nucleic acid
  - *icosahedral* symmetry: spherical viruses (*e.g.*, human papillomavirus; Figure 5.6)
    - most efficient arrangement of subunits in a closed shell
    - requires fewest capsomeres

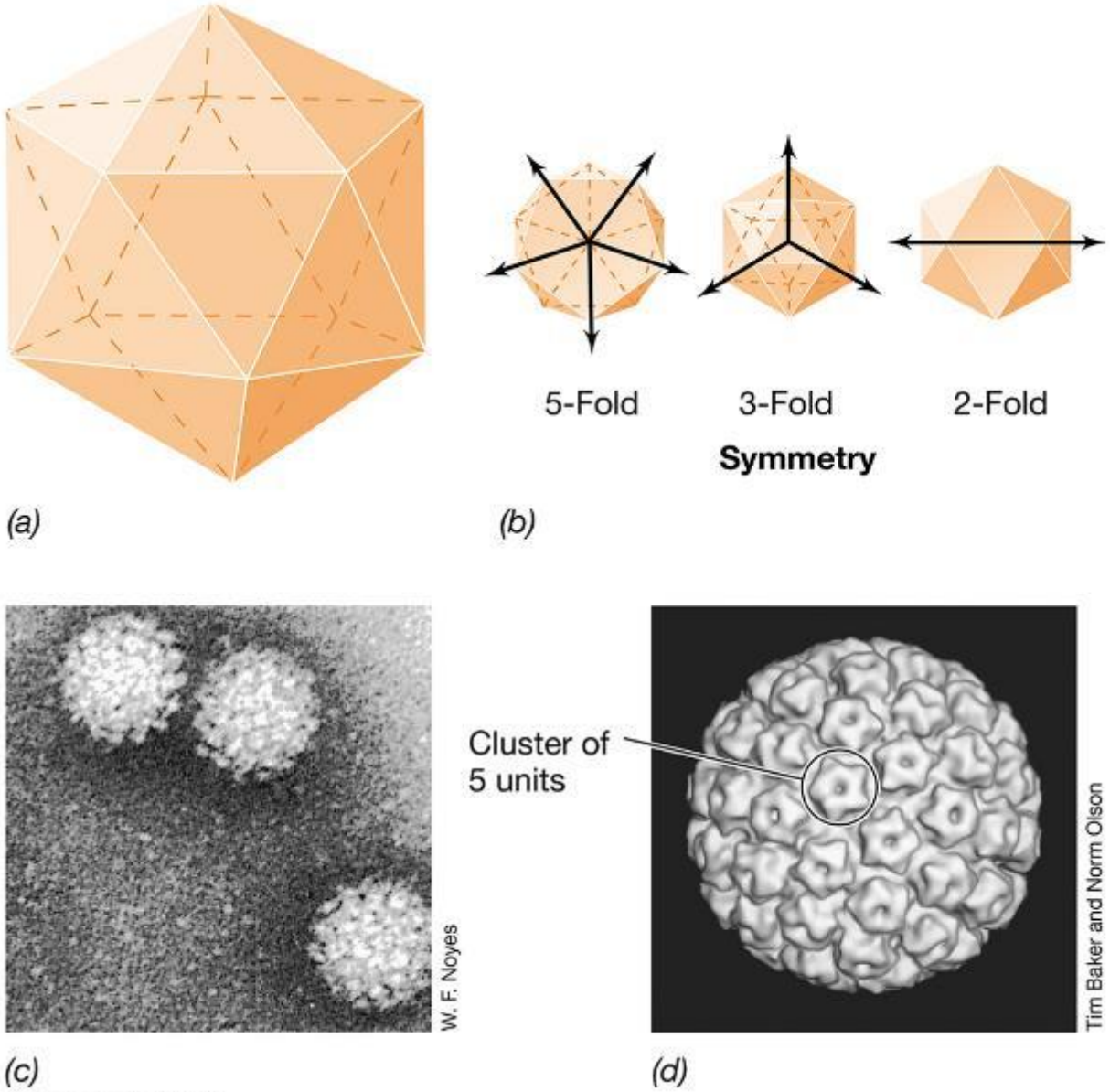


Figure 5.6

5.2 Structure of the Virion

- Enveloped viruses (Figure 5.8)
  - lipoprotein membrane surrounding
  - RNA or DNA genome
  - Envelop and infect animal host cell.
  - enveloped plant or bacterial viruses because of cell walls surrounding cell membrane
  - Entire virion enters animal cell during infection.
  - Enveloped viruses exit more easily.

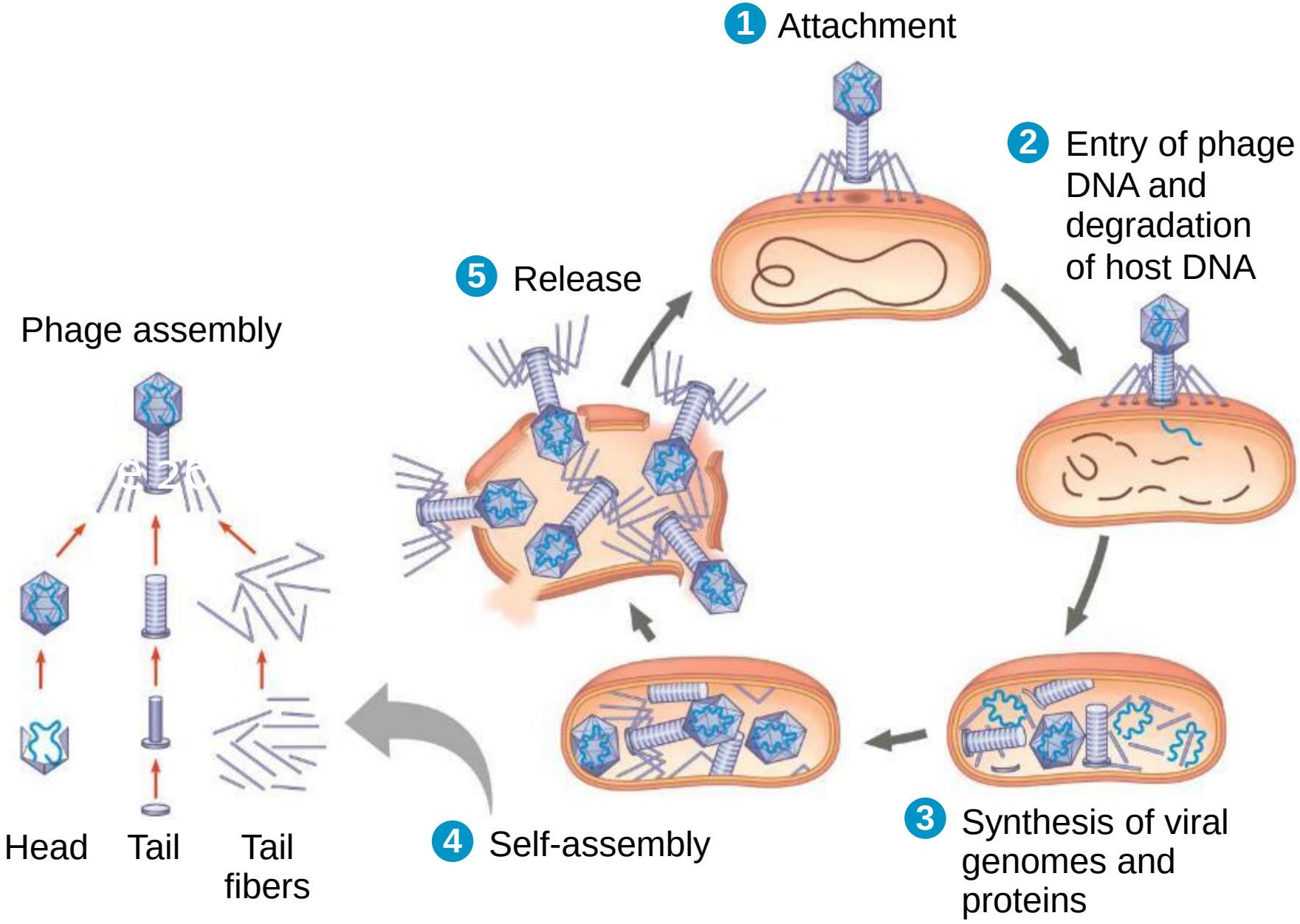
Komen we later op terug!

5.2 Structure of the Virion

- Enzymes inside virions
  - lysozyme
    - makes hole in cell wall to allow nucleic acid entry
    - also lyses bacterial cell to release new virions
  - *neuraminidases*
    - destroy glycoproteins and glycolipids
    - allows liberation of viruses from cell
  - nucleic acid polymerases (*RNA replicases:* RNA-dependent RNA polymerases)
    - *RNA replicases:* RNA-dependent RNA polymerases
    - *Reverse transcriptase:* RNA-dependent DNA polymerase in retroviruses

5.4 Overview of the Viral Replication Cycle

- Major difference between prokaryotic and eukaryotic viruses is nucleic acid entry in prokaryotes and virion entry in eukaryotes.
- Phases of viral replication in a *permissive* (supportive) host (Figure 5.12)
  - *attachment* (adsorption) of the virion
  - *penetration* (entry, injection) of the virion nucleic acid
  - *synthesis* of virus nucleic acid and protein by host cell metabolism as redirected by virus
  - *assembly* of capsids and *packaging* of viral genomes into new virions
  - *release* of mature virions from host cell


1 Attachment
2 Entry of phage DNA and degradation of host DNA
3 Synthesis of viral genomes and proteins
4 Self-assembly
5 Release
Phage assembly
Head
Tail
Tail fibers

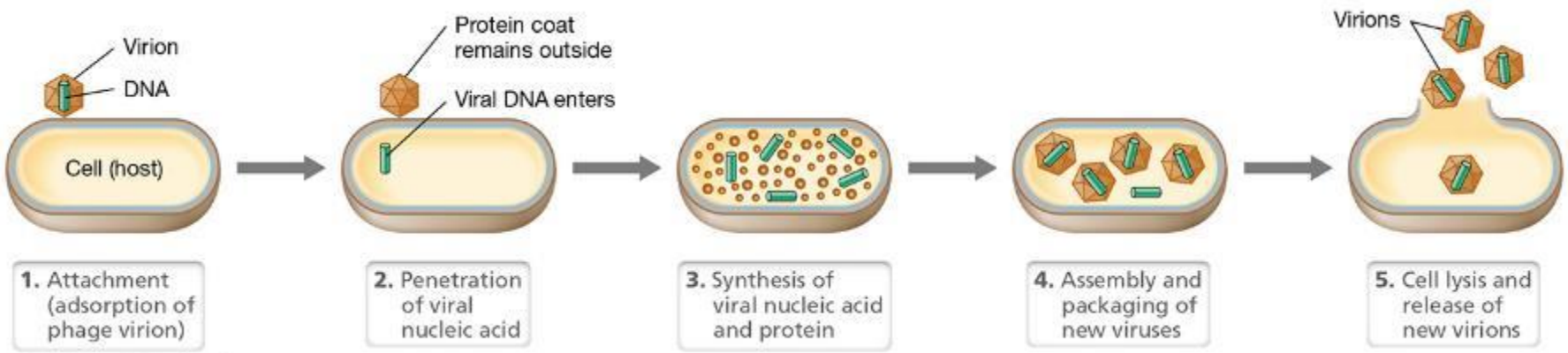


Figure 5.12

5.4 Overview of the Viral Replication Cycle

- Virus replication is typically characterized by a *one-step growth curve:* Increase occurs when cells burst. (Figure 5.13)
- *Eclipse:* genome replicated and proteins translated
- *Maturation:* packaging of nucleic acids in capsids
- *Latent period:* eclipse + maturation
- Release: cell lysis, budding, or excretion
  - *burst size:* number of virions released

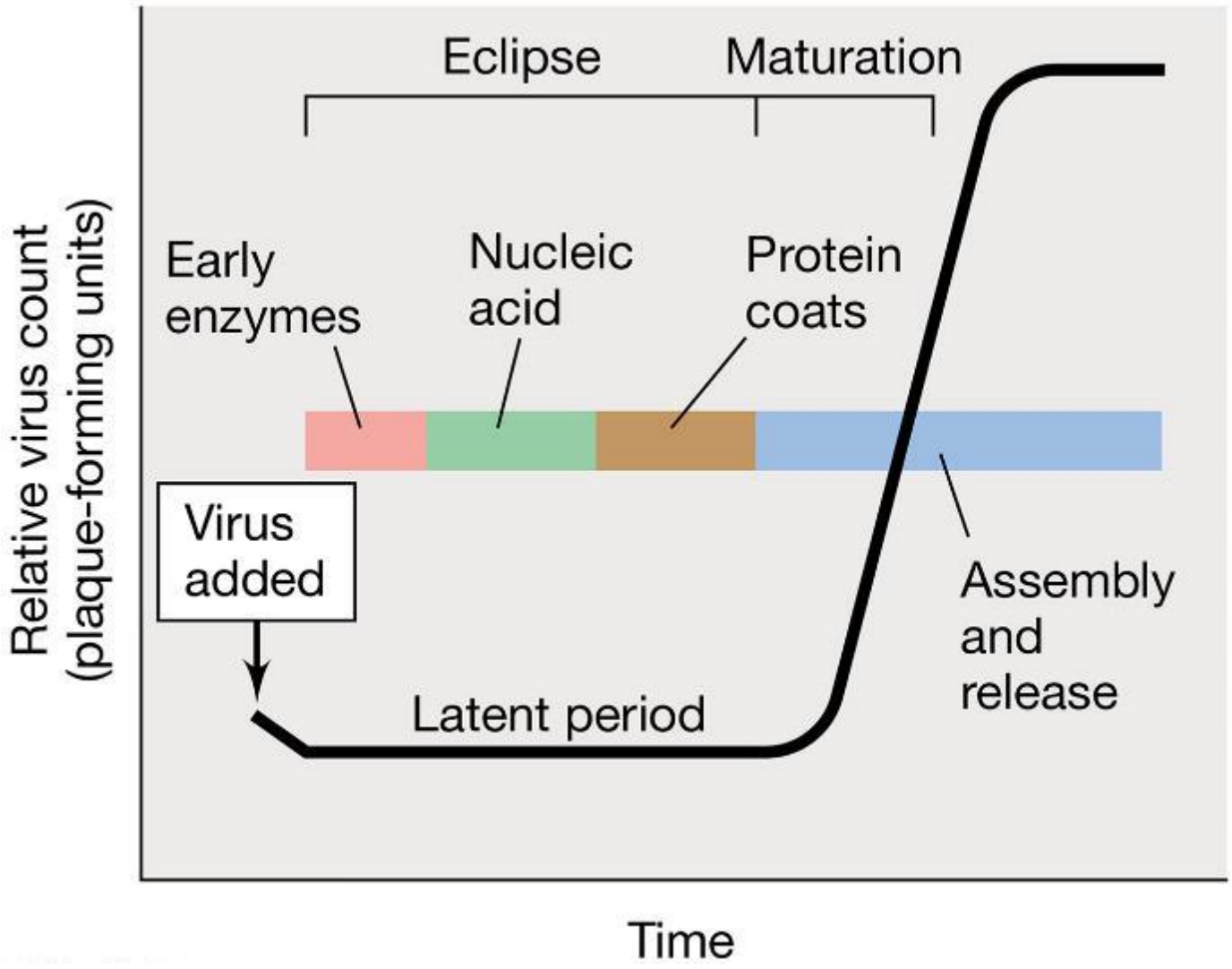
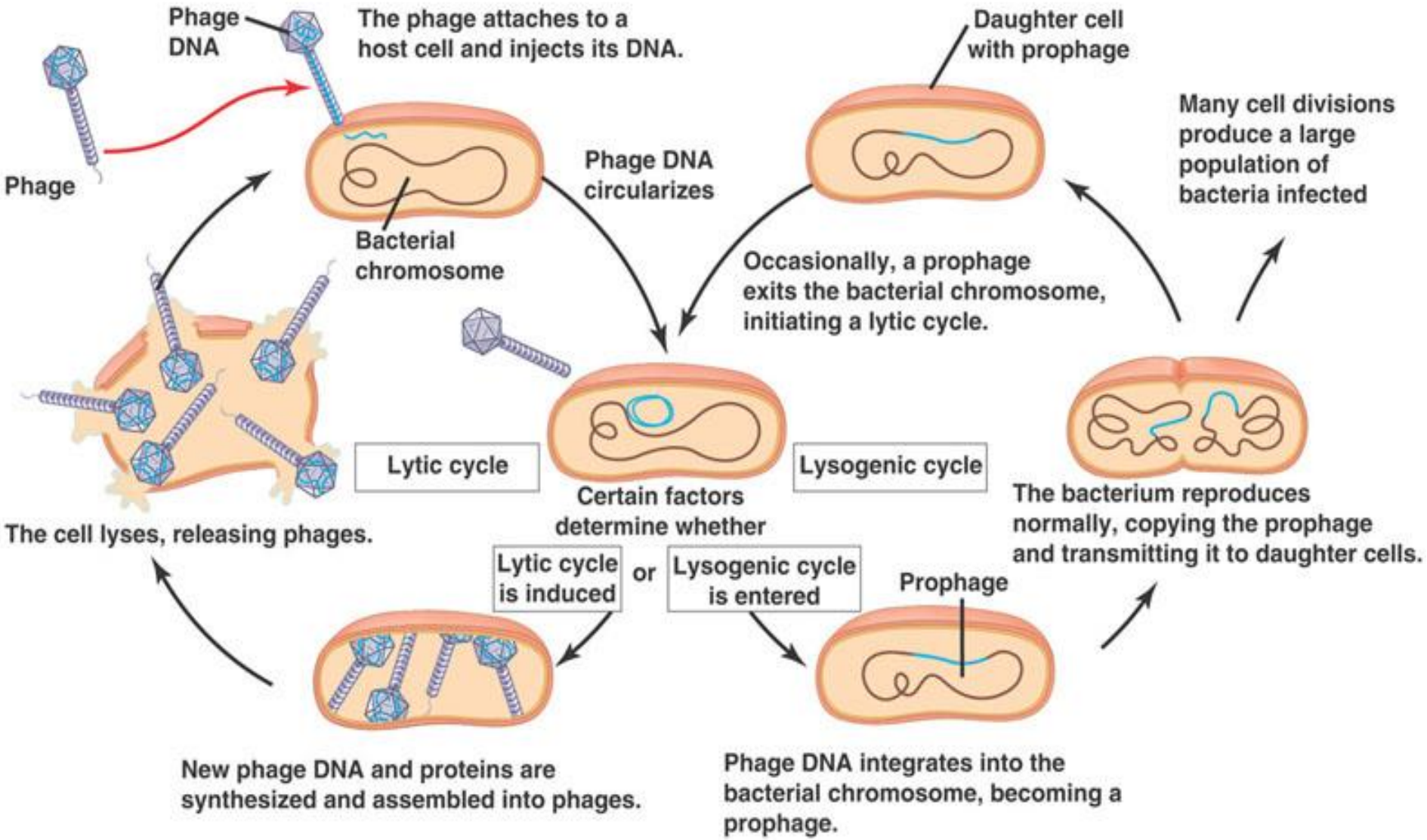


Figure 5.13

5.1 What Is a Virus?

- Viral components and activities
  - *virulent* (lytic) *infection:* replicates and destroys host
  - *lysogenic* infection: host cell genetically altered because viral genome becomes part of host genome

Lytisch vs lysogeen

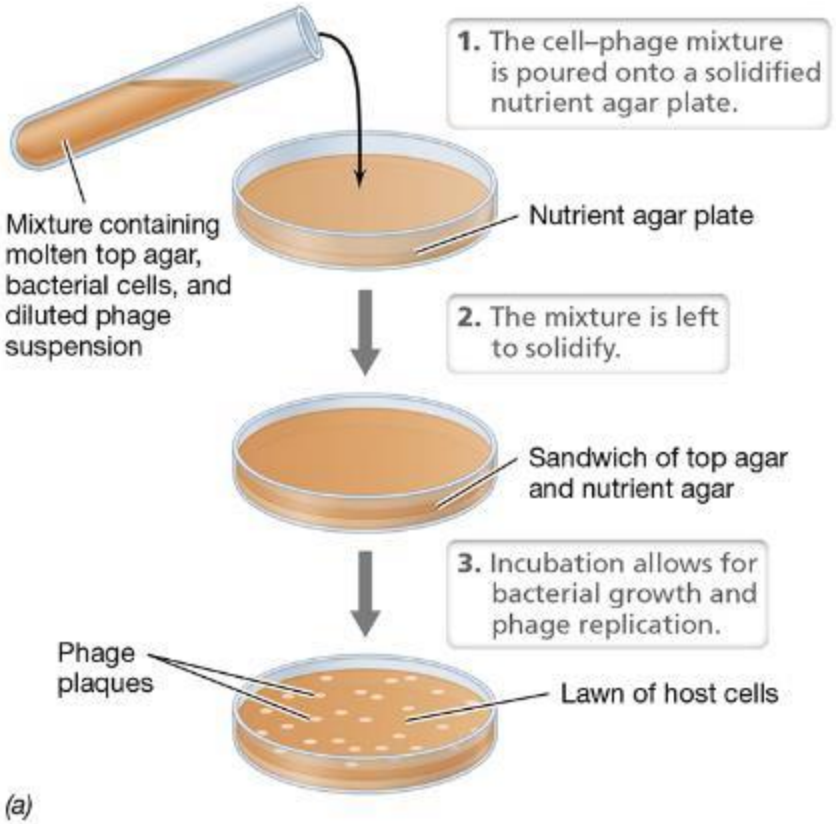


5.3 Culturing, Detecting, and Counting Viruses

- Bacterial viruses are easiest to grow (hosts in liquid medium or spread as “lawns” on agar and inoculated with virus).
- Animal viruses (and some plant viruses) can be cultivated in *tissue cultures* (from animal organ in culture medium).

5.3 Culturing, Detecting, and Counting Viruses

- Detecting and counting viruses: the plaque assay
  - *titer:* number of infectious units per volume of fluid
  - *Plaque assay:* Plaques are clear zones that develop on lawns of host cells where successful viral infection occurs. (Figures 5.10 and 5.11)



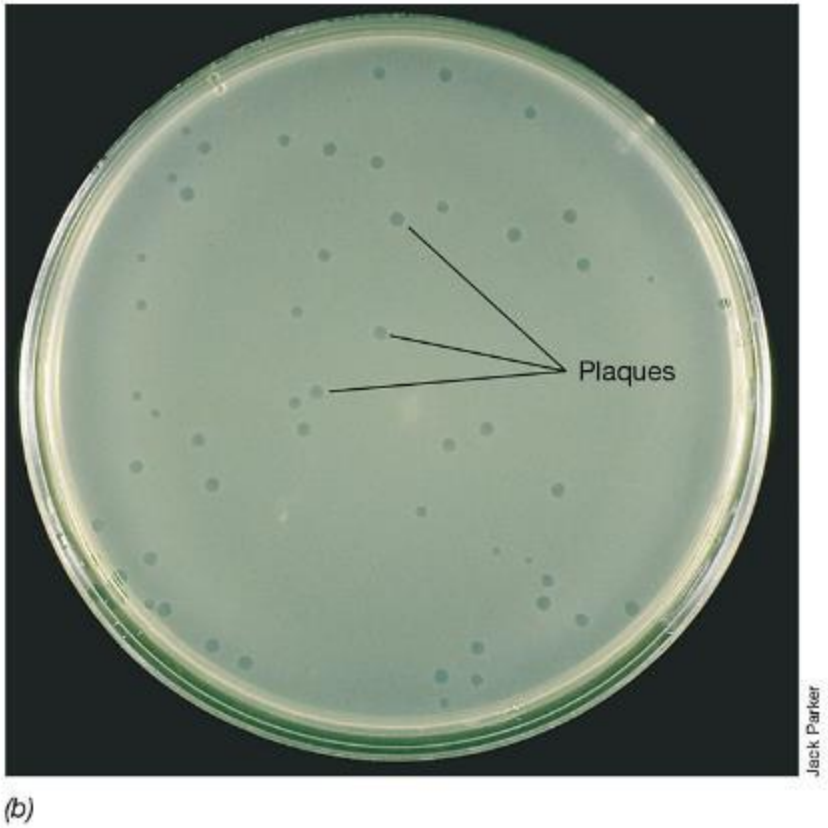
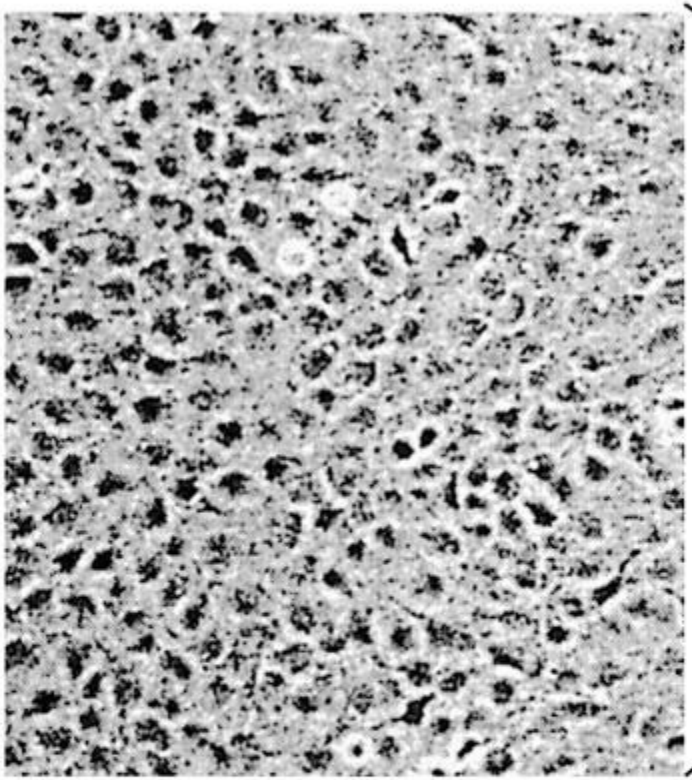


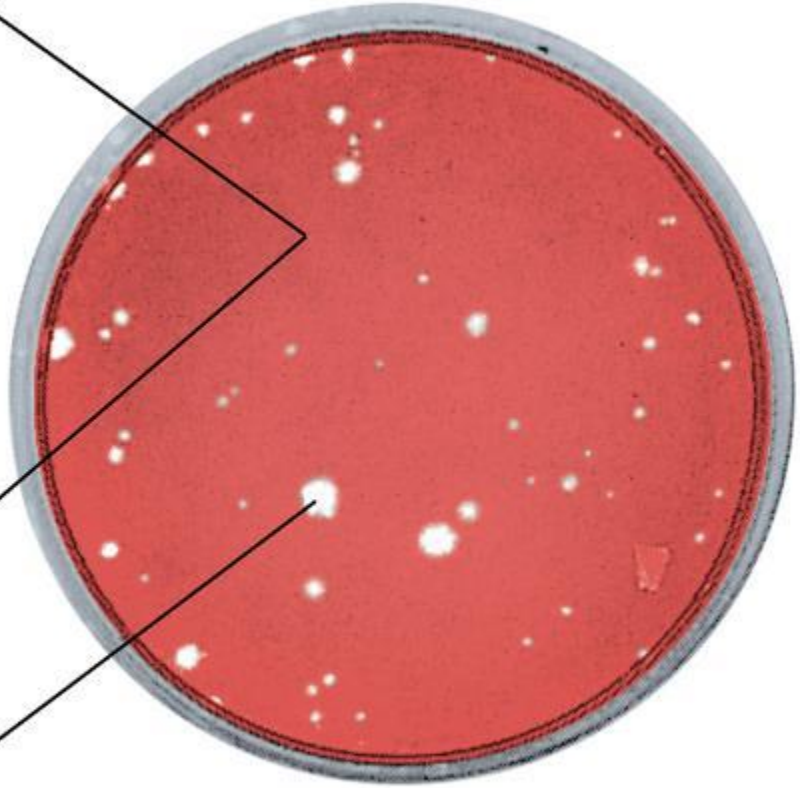
Figure 5.10



Paul Kaplan

Confluent monolayer of tissue culture cells

Viral plaques



T.D. Brock

Figure 5.11

5.3 Culturing, Detecting, and Counting Viruses

- *Plating efficiency* is used in quantitative virology.
  - The number of plaque-forming units is always lower than direct counts by electron microscopy.
    - efficiency of infection usually much less than 100 percent
    - inactive virions or conditions inappropriate for infectivity

II. The Viral Replication Cycle

- 5.5 Bacteriophage T4: A Model Lytic Virus
- 5.6 Temperate Bacteriophages and Lysogeny
- 5.7 An Overview of Viruses of Eukaryotes

5.5 Bacteriophage T4: A Model Lytic Virus

- Host: *Escherichia coli*
- double-stranded DNA genome is about 169 kbp long[3] and encodes 289 proteins.
- Only lytic, no lysogenic cycle.

Wiki

5.5 Bacteriophage T4: A Model Lytic Virus

- Attachment
  - major factor in host specificity
  - requires complementary receptors on the surface of a susceptible host for its infecting virus
  - Receptors include proteins, carbohydrates, glycoproteins, lipids, lipoproteins, or other cell structures. (Figure 5.14)
  - Receptors on host cell carry out normal functions for cell (*e.g.*, uptake proteins, cell-to-cell interaction, flagella, pili).

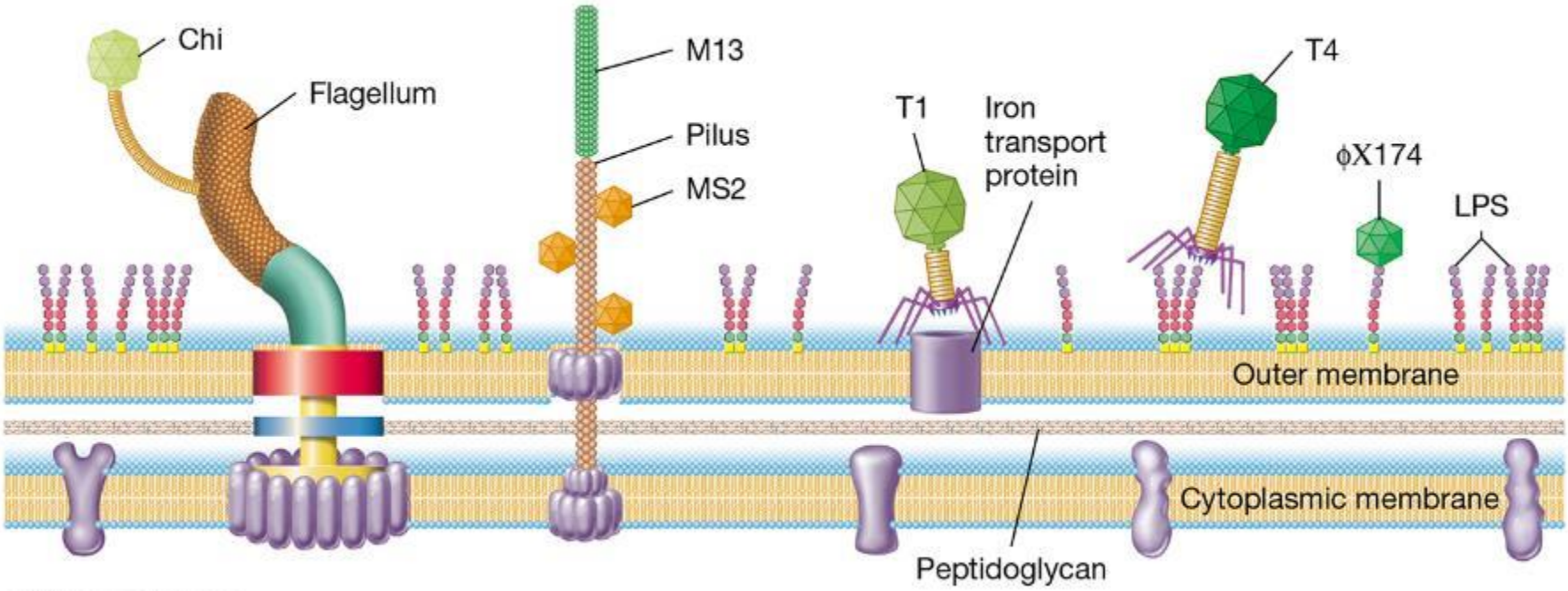


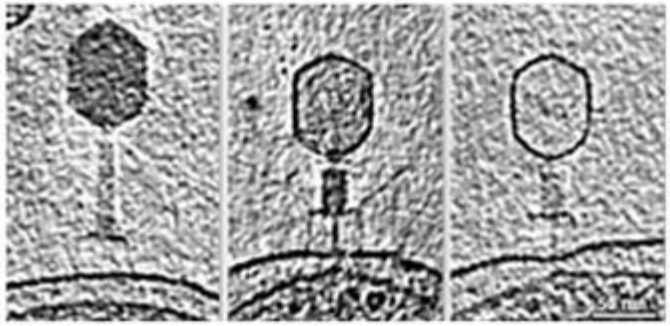
Figure 5.14

5.5 Bacteriophage T4: A Model Lytic Virus

- Penetration
  - capside left outside cell
  - Viral genome and viral proteins (for some viruses) enter host cell.

5.5 Bacteriophage T4: A Model Lytic Virus

- Most complex penetration mechanisms found in tailed bacteriophages (*e.g.*, T4) (Figure 5.15)
  - Virions attach to cells via tail fibers that interact with polysaccharides on *E. coli* LPS layer.
  - Tail fibers retract, and tail pins contact cell wall.
  - T4 lysozyme forms small pore in peptidoglycan.
  - Tail sheath contracts, and viral DNA passes into cytoplasm.
  - Capside stays outside.



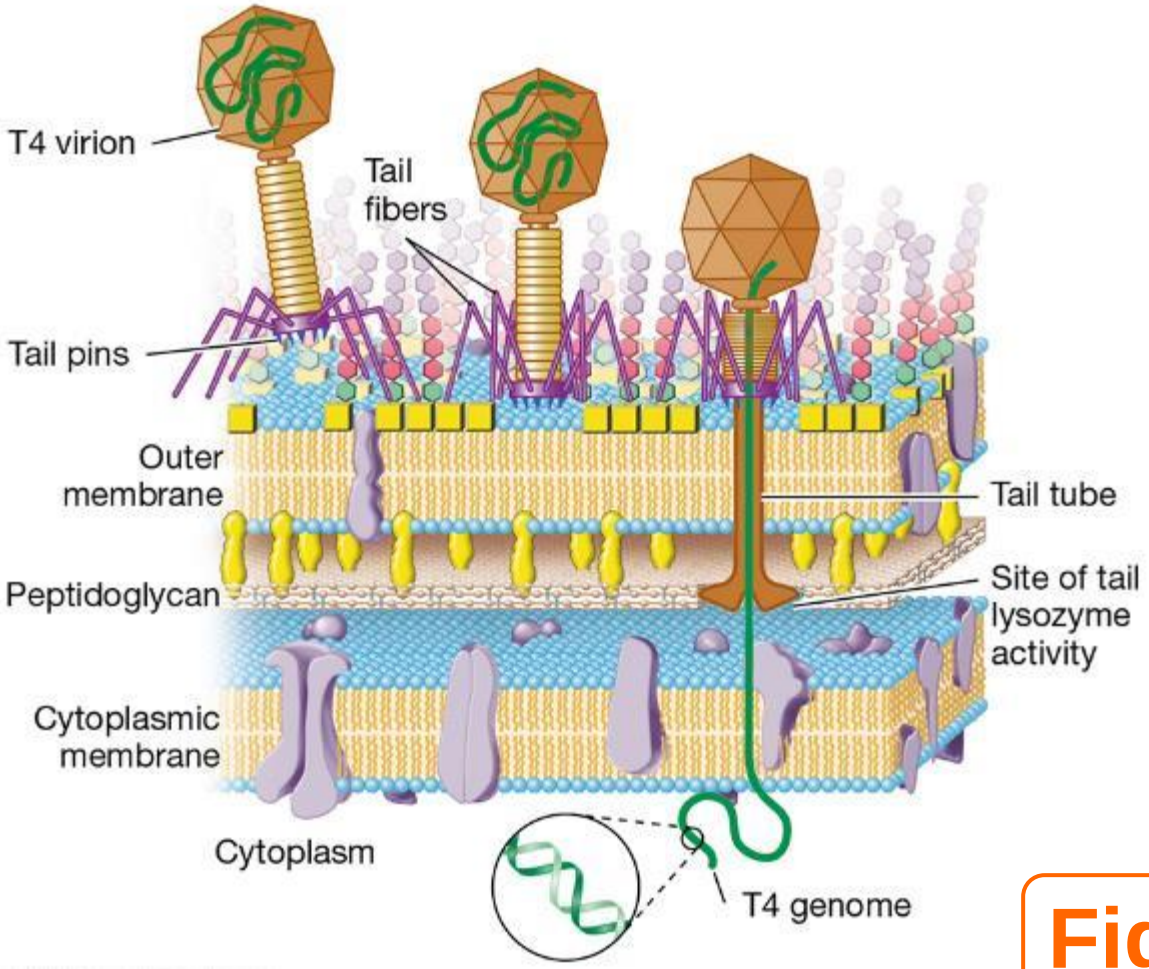
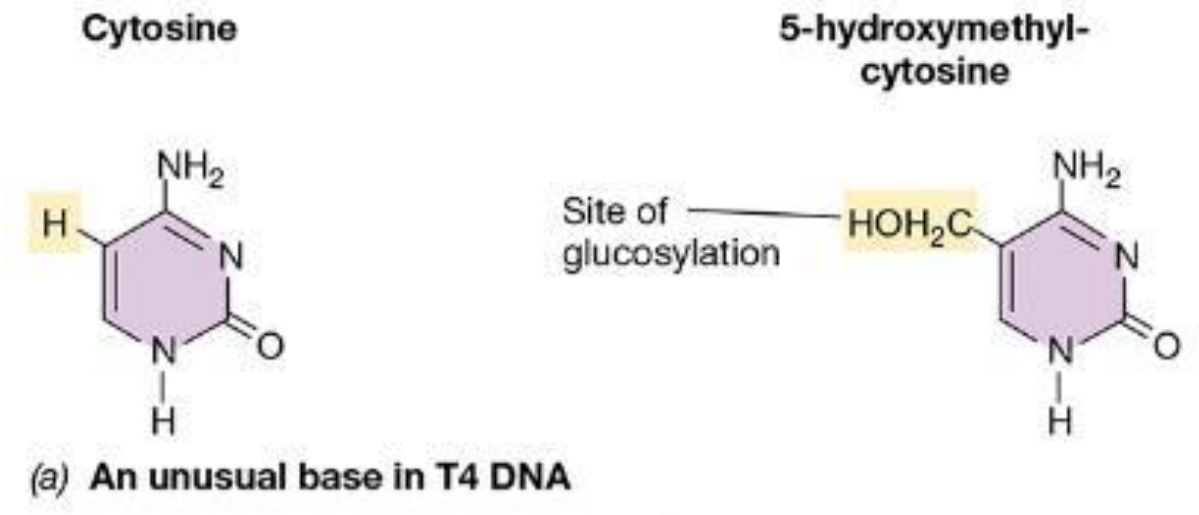


Figure 5.15

5.5 Bacteriophage T4: A Model Lytic Virus

- Prokaryotic defense against phage infections.
  - toxin-antitoxin molecules
  - CRISPR
  - *restriction endonucleases*
    - specific for dsDNA; ssDNA and RNA unaffected
    - Phage protection includes base substitution to resist restriction enzyme.
  - DNA methylation prevents cleavage of host's own DNA.

- Glycosilation of phage DNA protects against digestion.



(a) An unusual base in T4 DNA

11.4 DS DNA Phages: T4

- Genome replication and circular permutation
  - *circular permutation:* feature of many virus genomes where same genes arranged in different orders
    - *terminally redundant:* some DNA sequences duplicated on both ends
  - T4 first replicated as a unit, then forms concatemer (several genomic units recombined)

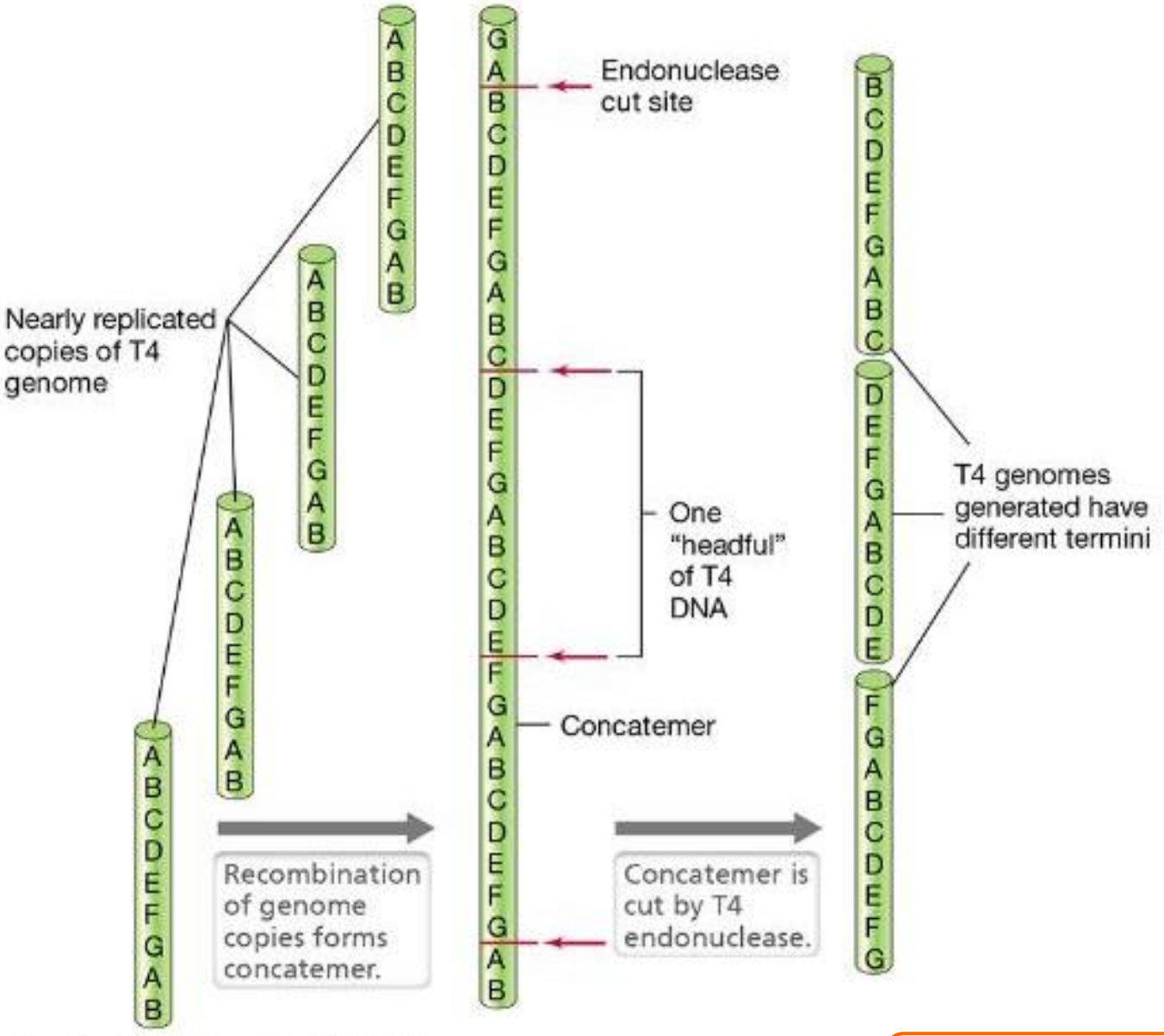


Figure 11.9

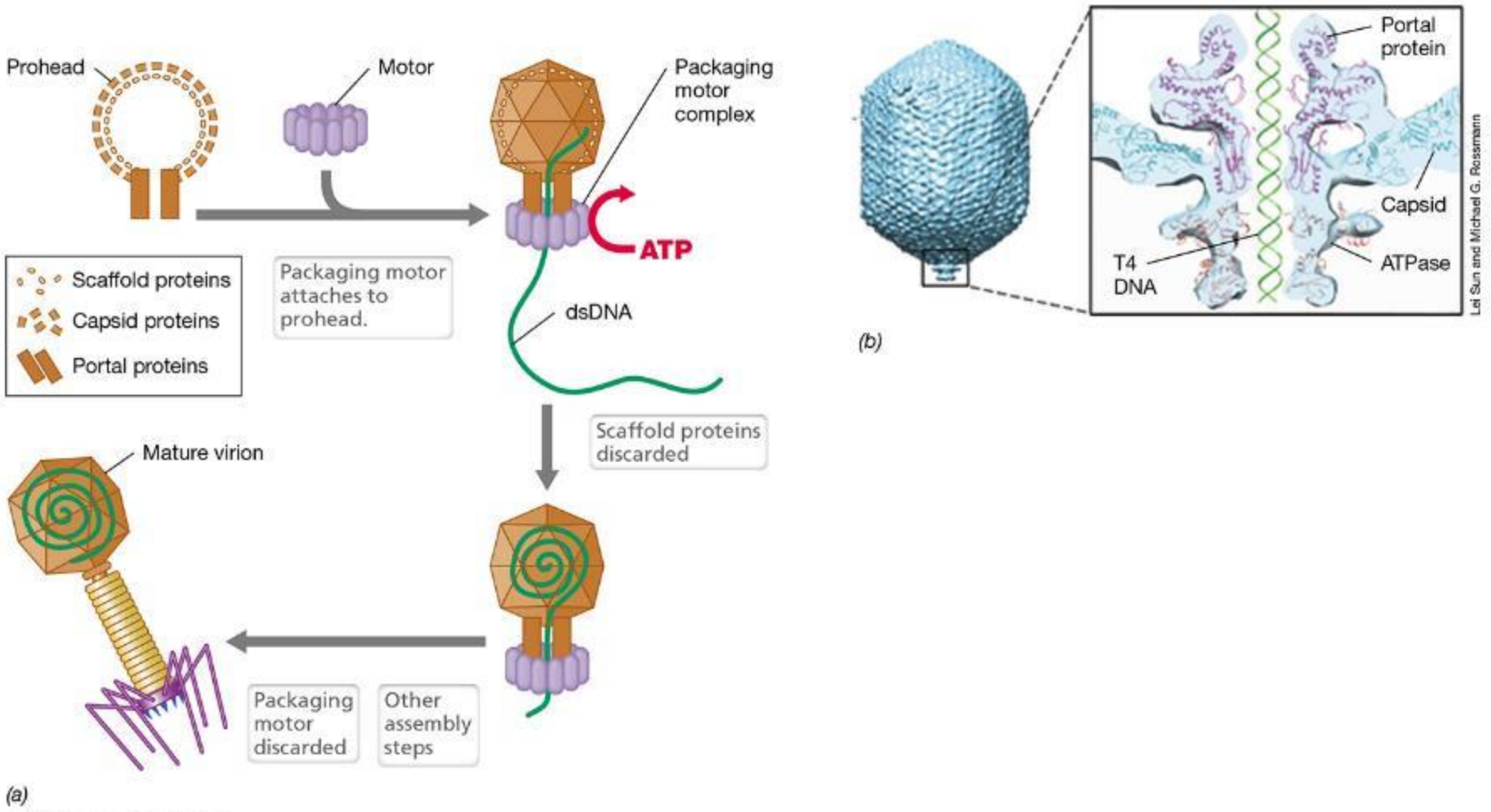


Figure 5.17

5.5 Bacteriophage T4: A Model Lytic Virus

- Transcription and translation
  - Virion synthesis takes ~30 minutes and ends in release of new virions from lysed cell. (Figure 5.16)
  - T4 genome can be divided into three parts: early, middle, and late proteins.
    - early proteins: enzymes needed for DNA replication and transcription
      - enzyme for the synthesis and glucosylation of the T4 base hydroxymethylcytosine
      - enzymes that function in T4 replisome
      - proteins that modify host RNA polymerase
    - Middle and late proteins: head and tail proteins and enzymes required to liberate mature phage particles
      - additional RNA polymerase-modifying proteins
      - viral head and tail proteins
      - enzymes for liberating new virions from cell

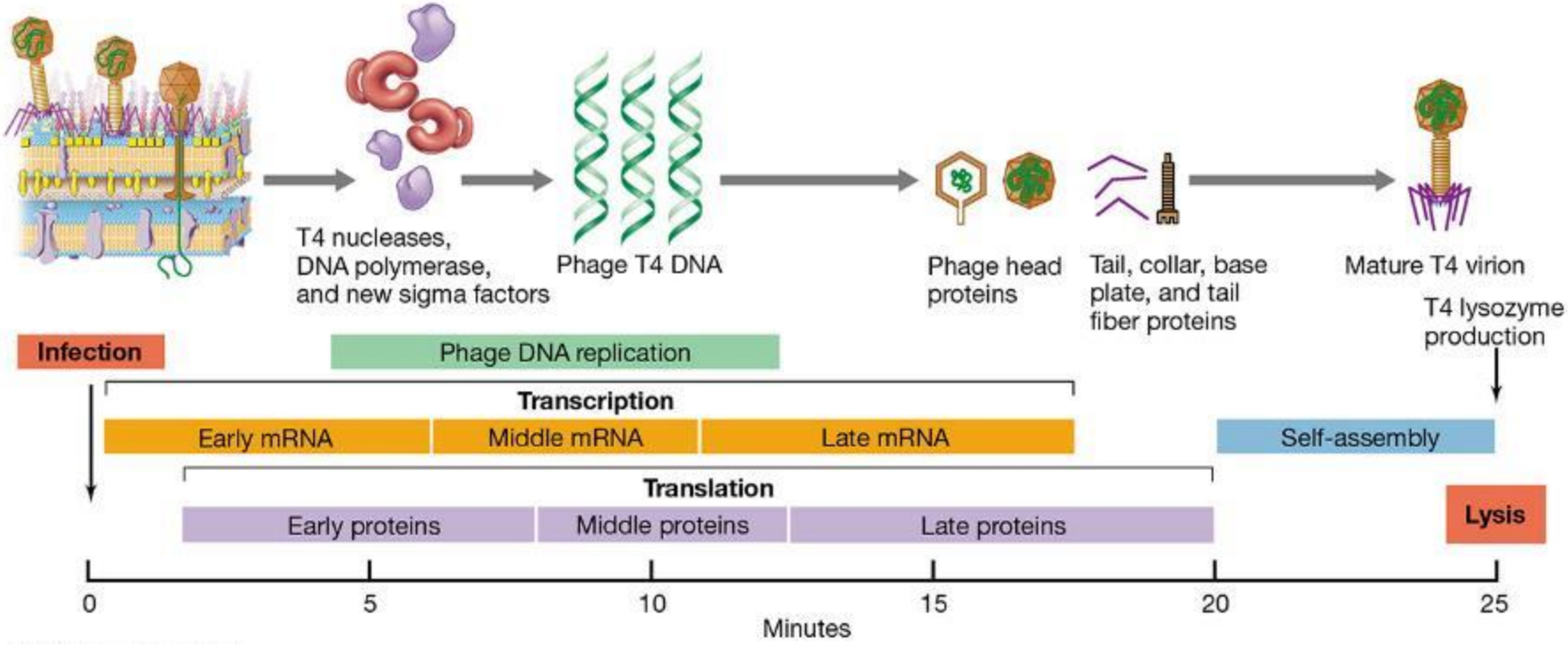


Figure 5.16

5.5 Bacteriophage T4: A Model Lytic Virus

- Transcription and translation
  - T4-specific proteins modify host RNA polymerase specificity to recognize only phage promoters.
  - host transcription shut down

5.5 Bacteriophage T4: A Model Lytic Virus

- Packaging the T4 genome and virion assembly and release
  - Genome is pumped into head under pressure using ATP.
  - packaging in three stages (Figure 5.17)
    - *proheads* (bacteriophage head precursors) assembled
    - packaging motor assembled at opening (Figure 8.15b)
    - double-stranded linear genome pumped into prohead using ATP
  - After head is filled with DNA, T4 tail, tail fibers, and other components are self-assembled.
  - late enzymes break membrane and peptidoglycan
  - lysis occurs, virions released

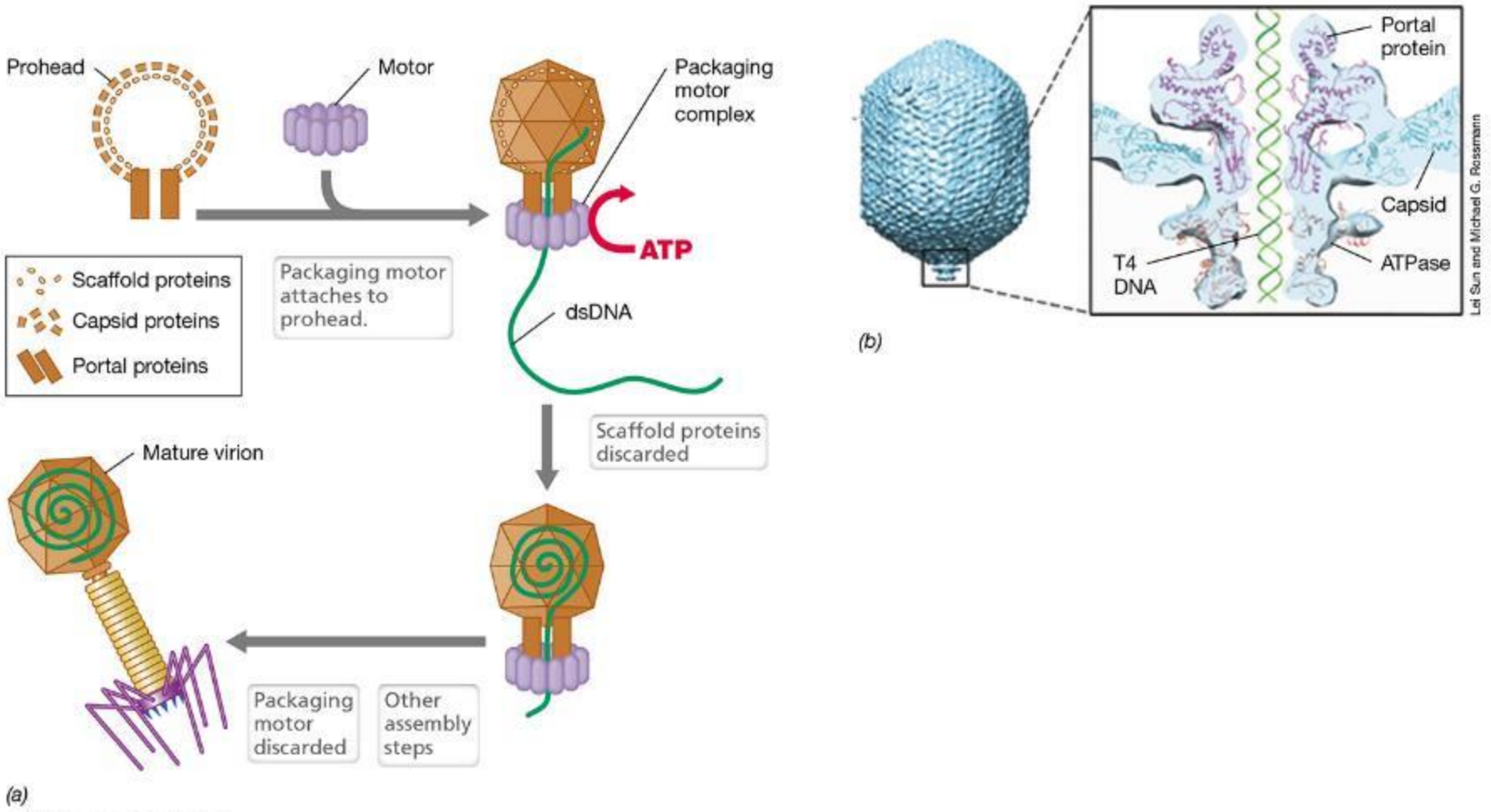
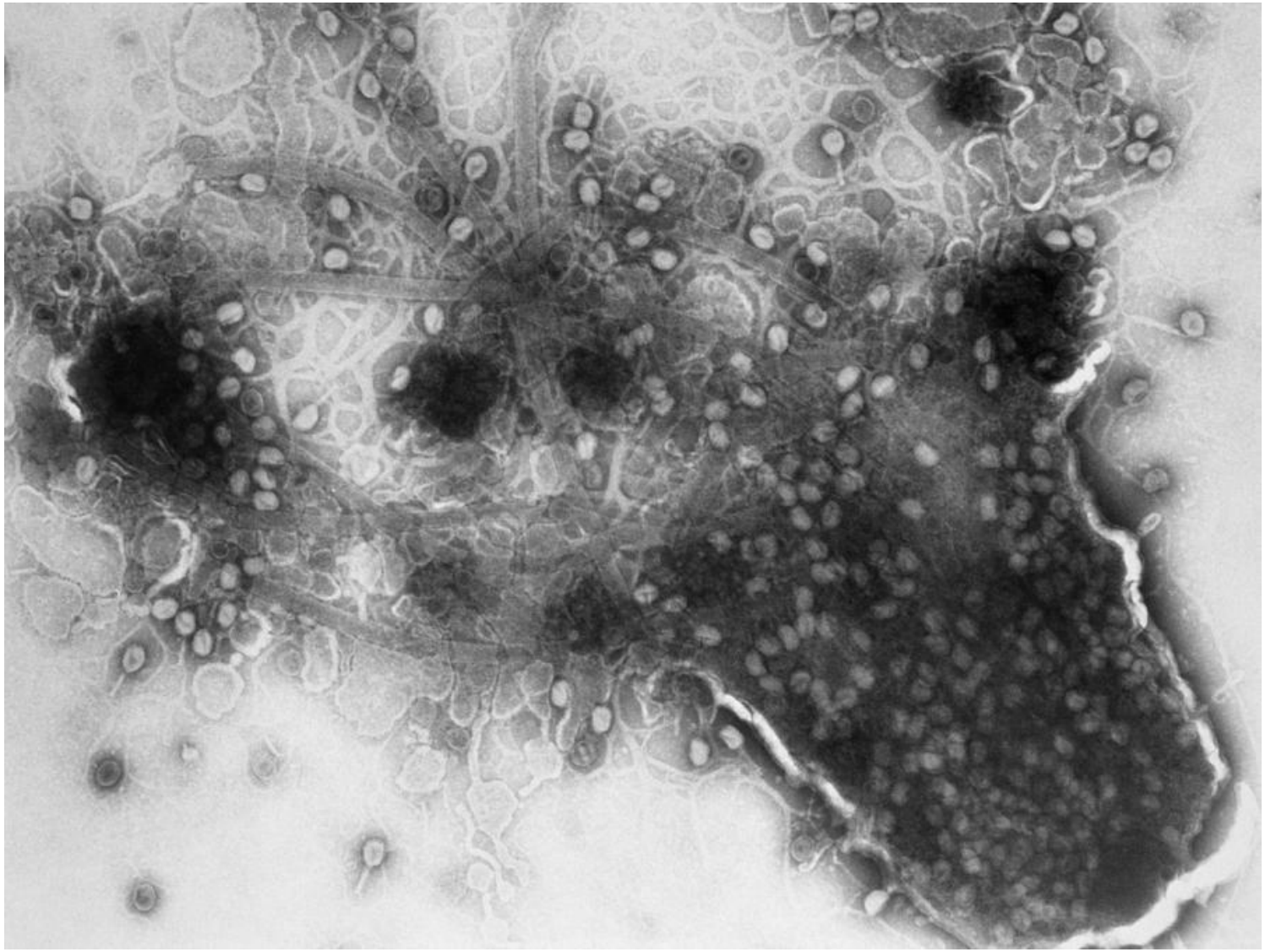


Figure 5.17

TEM of bacterial lysis due to T4 phage infection



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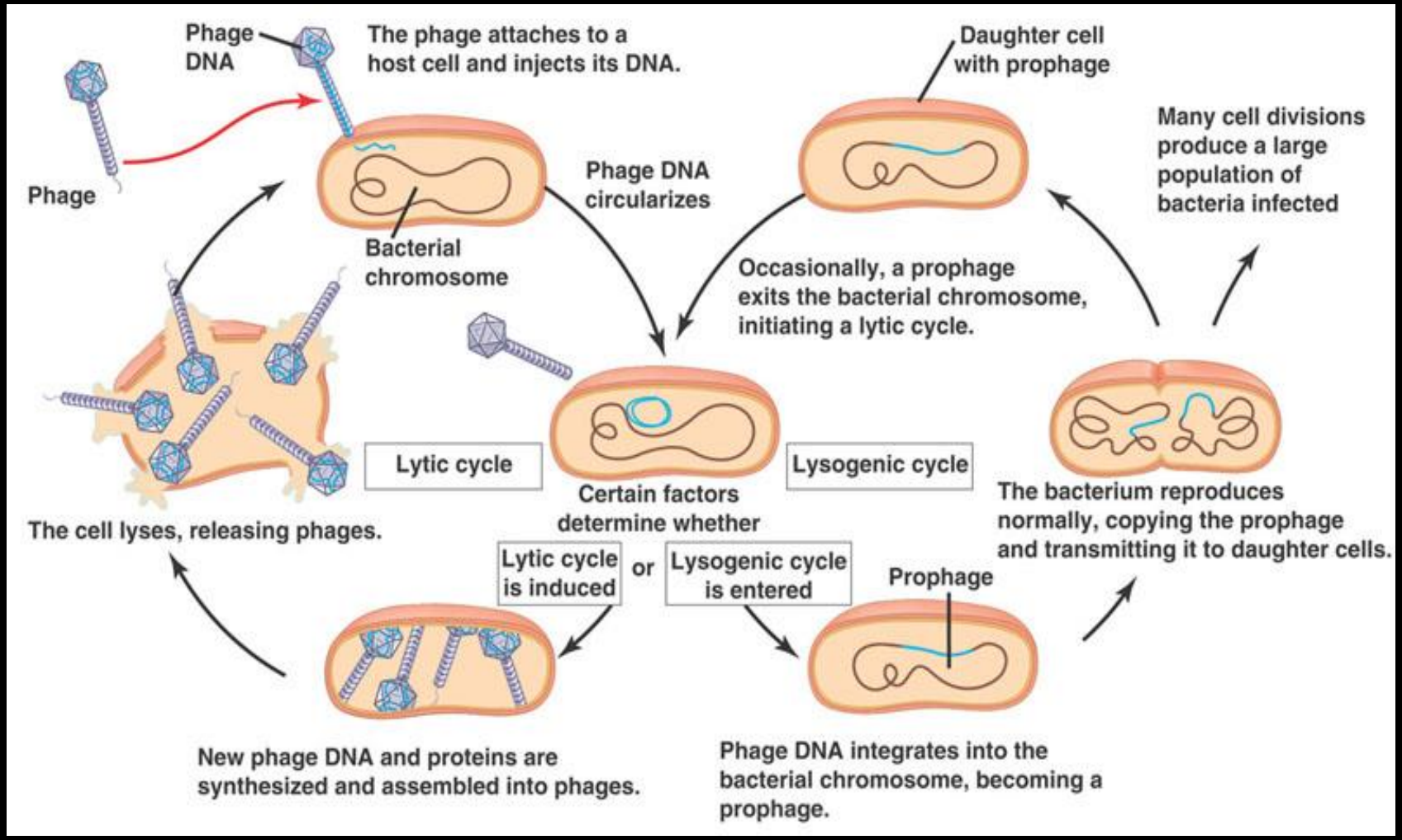
Microbiologie 2: Les 6

II. The Viral Replication Cycle



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6	5 en 11	5.7, 5.8, 11.1, 11.2
7	11	11.6, 11.7, 11.8 (MS2 niet)
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I. The Nature of Viruses

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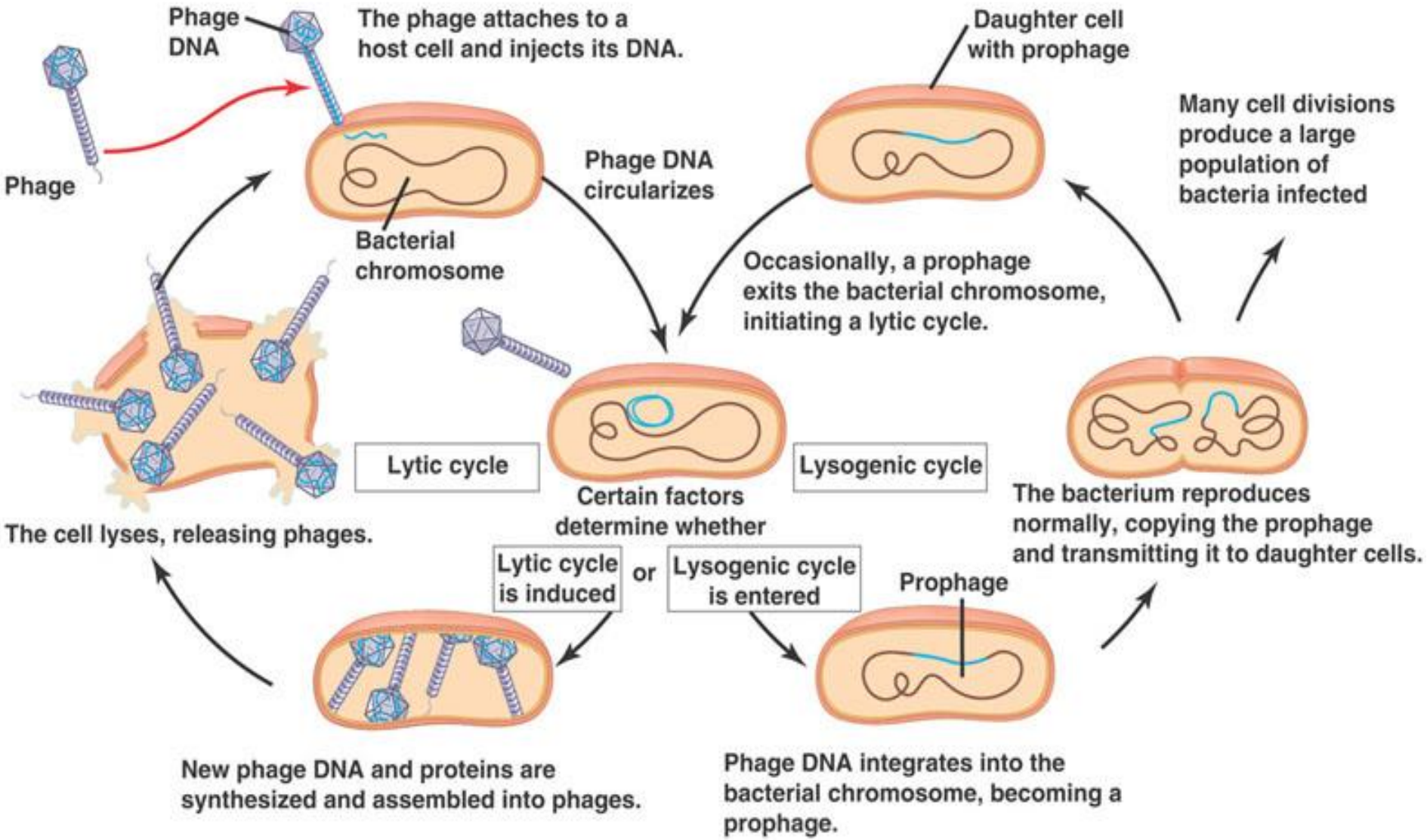
Lytisch vs lysogeen

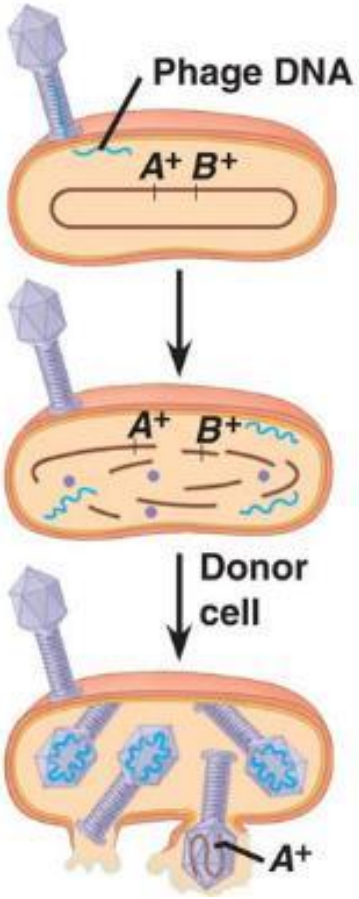


5.6 Temperate Bacteriophages and Lysogeny

- Viral life cycles
  - Virulent: Viruses always lyse and kill host after infection.
  - Temperate: Can establish long-term, stable relationship without killing host.
    - can enter lysogeny: most viral genes are not transcribed, viral genome is replicated with host chromosome
    - lysogen: host cell that harbors temperate virus
    - can result in *lysogenic conversion* with new properties (*e.g.,* virulence in pathogens)

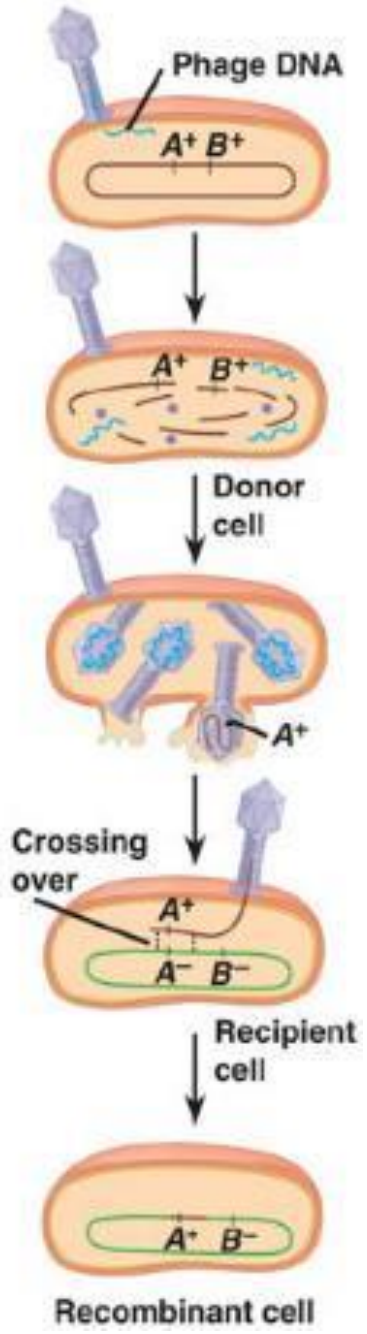
lysogenic conversion / transductie

1. Phage infects a bacterial cell that has alleles A^+ and B^+.
2. Host DNA (brown) is fragmented, and phage DNA and proteins are made. This is the donor cell.
3. A bacterial DNA fragment (in this case a fragment with the A^+ allele) may be packaged in a phage capsid.



lysogenic conversion / transductie

1. Phage infects a bacterial cell that has alleles A^+ and B^+.
2. Host DNA (brown) is fragmented, and phage DNA and proteins are made. This is the donor cell.
3. A bacterial DNA fragment (in this case a fragment with the A^+ allele) may be packaged in a phage capsid.
4. Phage with the A^+ allele from the donor cell infects a recipient A^-B^- cell, and crossing over (recombination) between donor DNA (brown) and recipient DNA (green) occurs at two places (dotted lines).
5. The genotype of the resulting recombinant cell (A^+B^-) differs from the genotypes of both the donor (A^+B^+) and the recipient (A^-B^-).



11.4 DS DNA Phage: Lambda

- Replication cycle of a temperate phage (Figure 5.18)
  - examples: lambda and P1
  - In lysogeny, genome is either integrated into bacterial chromosome (lambda) or exists as a plasmid (P1).
  - prophage: viral DNA
  - lysogeny maintained by phage-encoded *repressor protein*
  - Inactivation of repressor induces lytic stage.
  - Cell stress (*e.g.,* DNA damage) induces lytic pathway.

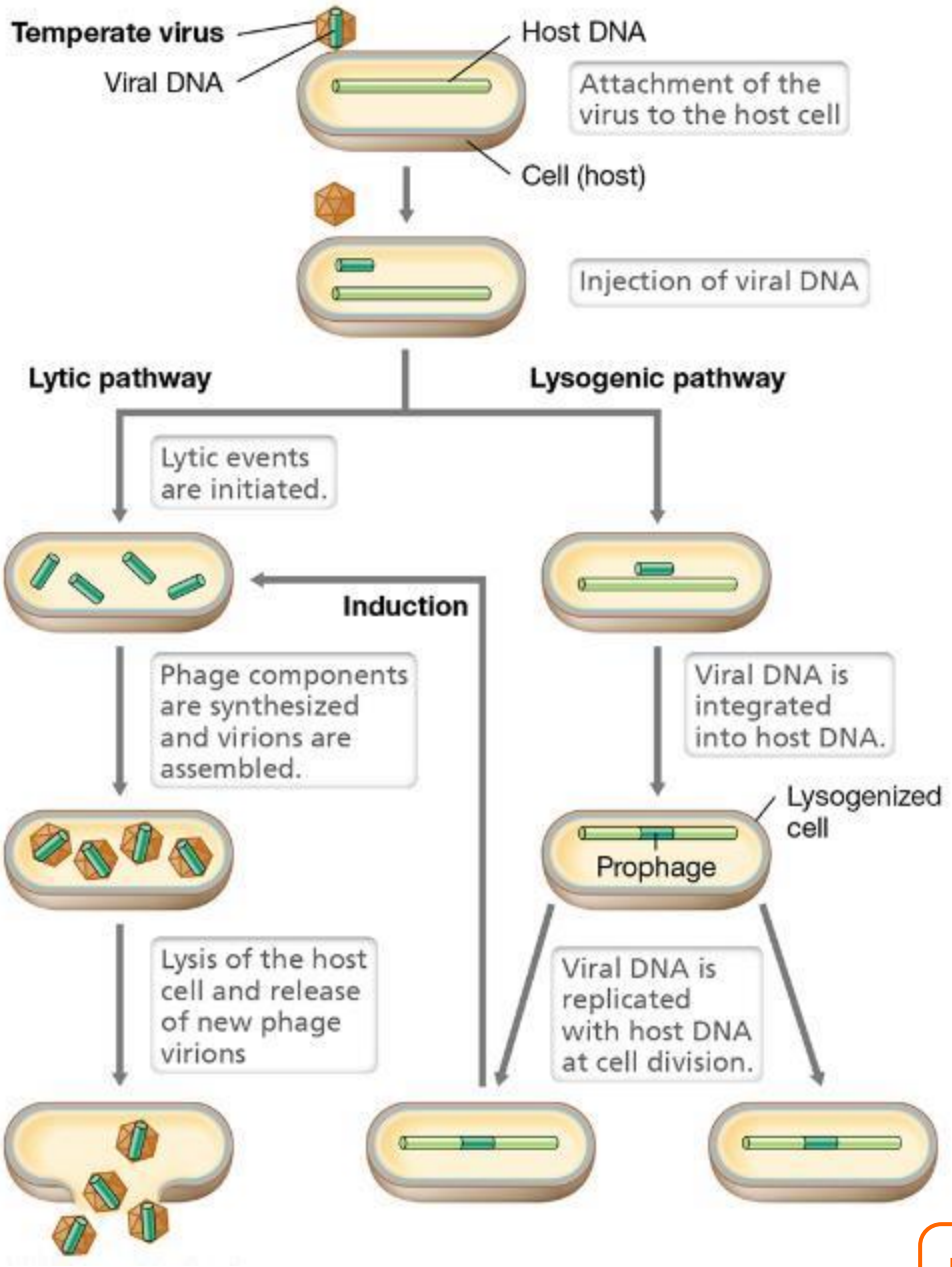
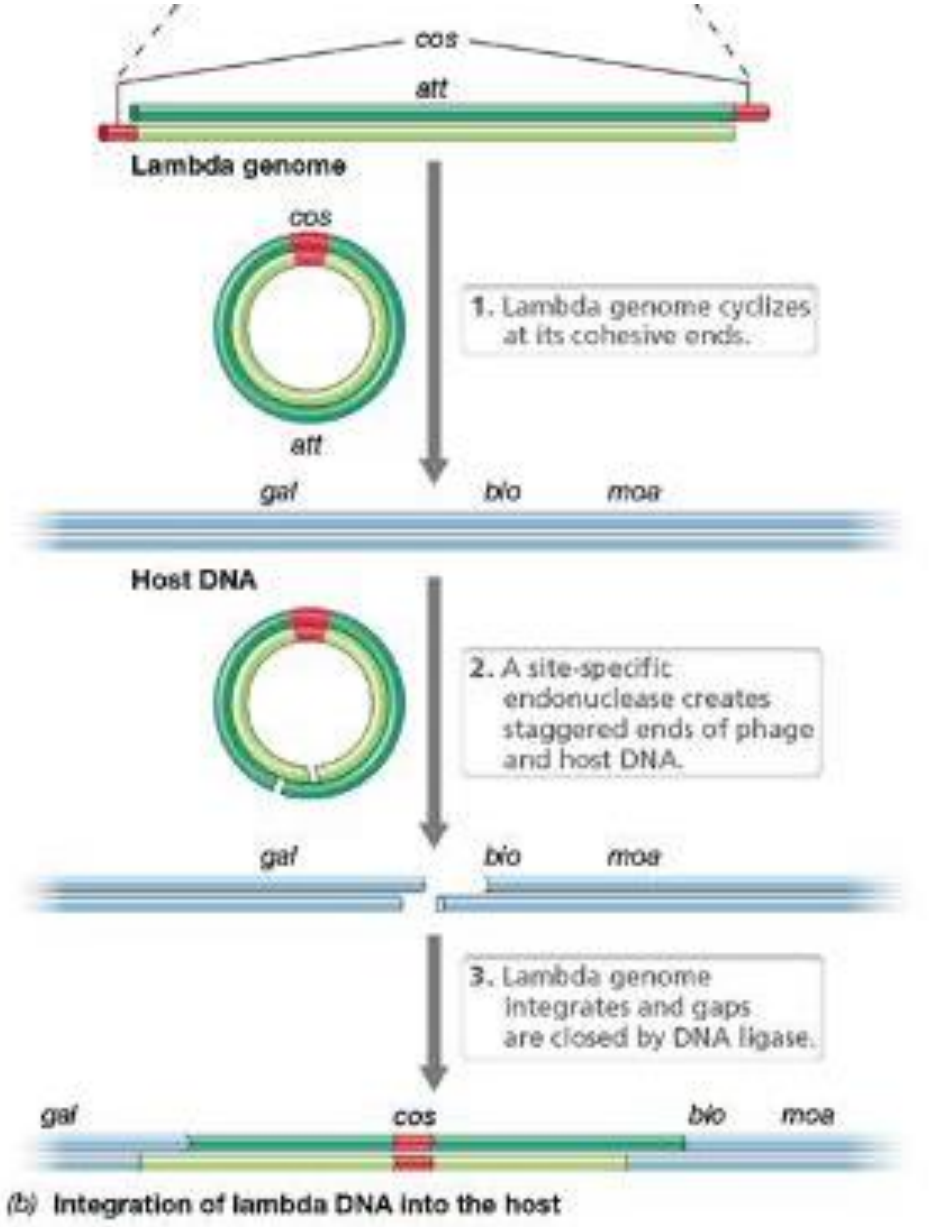


Figure 5.18

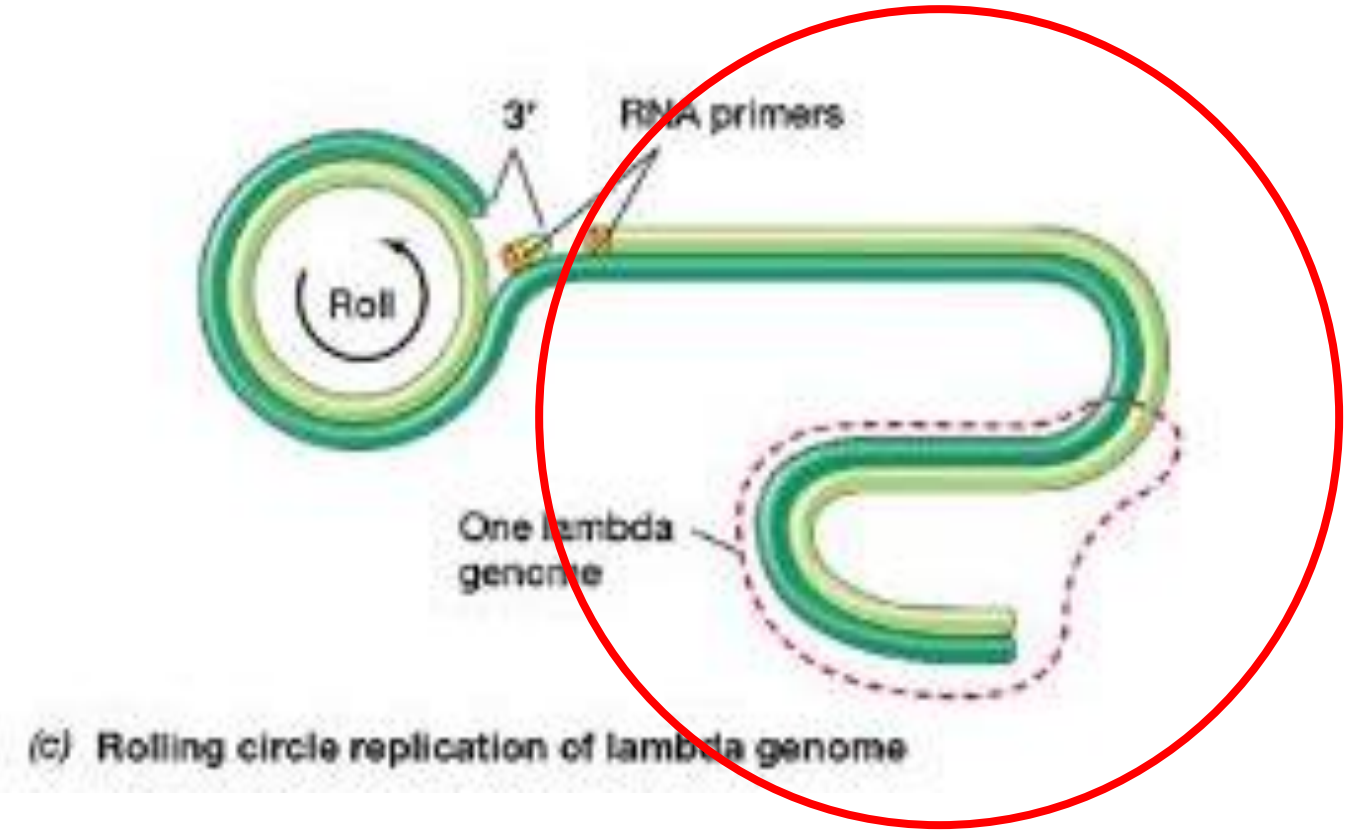
11.4 DS DNA Phage: Lambda

- Bacteriophage lambda
  - linear, dsDNA virus with head and tail
  - complementary, single-stranded “cohesive” regions 12 nucleotides long at the 5′ terminus of each strand
  - Upon penetration, DNA ends base pair, forming the *cos* site, and the DNA ligates and forms double-stranded circle.



(b) Integration of lambda DNA into the host

Figure 11.11



(c) Rolling circle replication of lambda genome

concatemeer

Figure 8.17

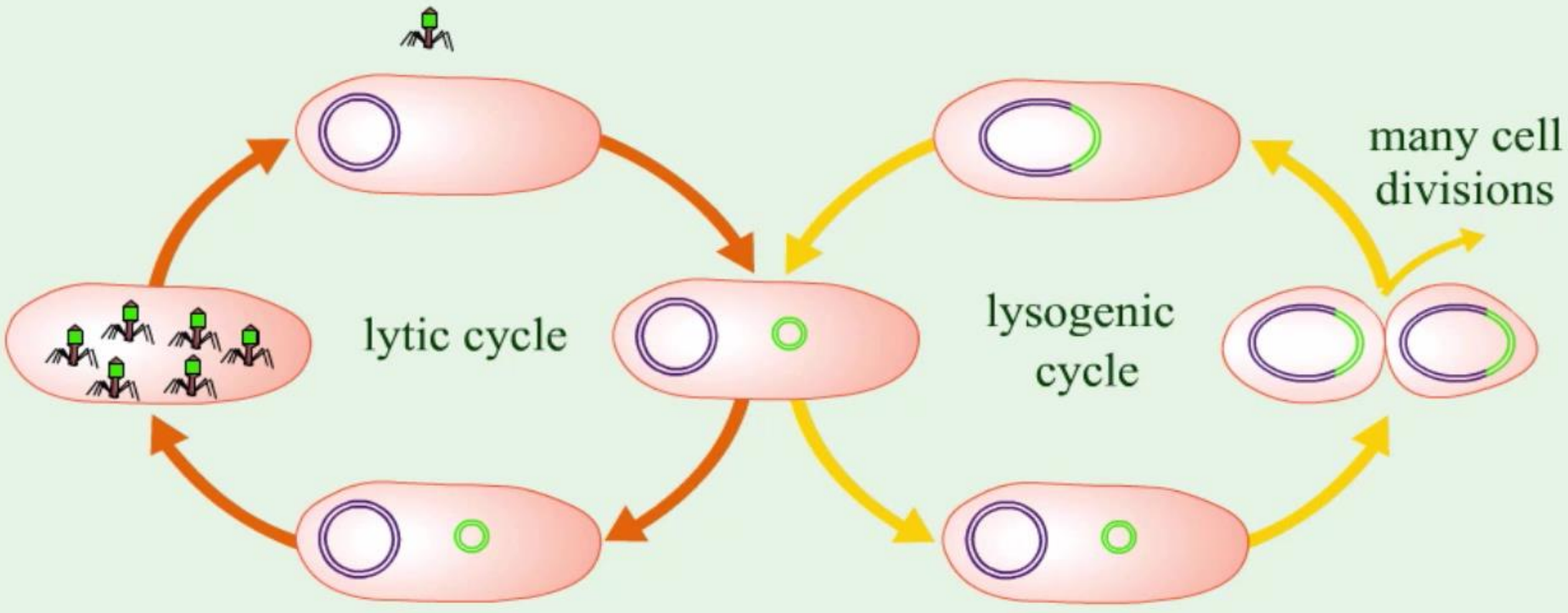
11.4 DS DNA Phage: Lambda

- Bacteriophage lambda
  - When it enters lytic pathway, lambda synthesizes long, linear concatemers of DNA by rolling circle replication. (Figure 11.11)
  - genome-sized lengths cut at *cos* sites; genomes packaged into phage heads
  - after tails added, lysis occurs
  - *Transduction* (packaging of host chromosomal genes and transfer to new host) can also occur.
  - When lambda is lysogenic, its DNA integrates into *E. coli* chromosome at the lambda attachment site (*attλ*) using *lambda integrase.*

11.4 DS DNA Phage: Lambda

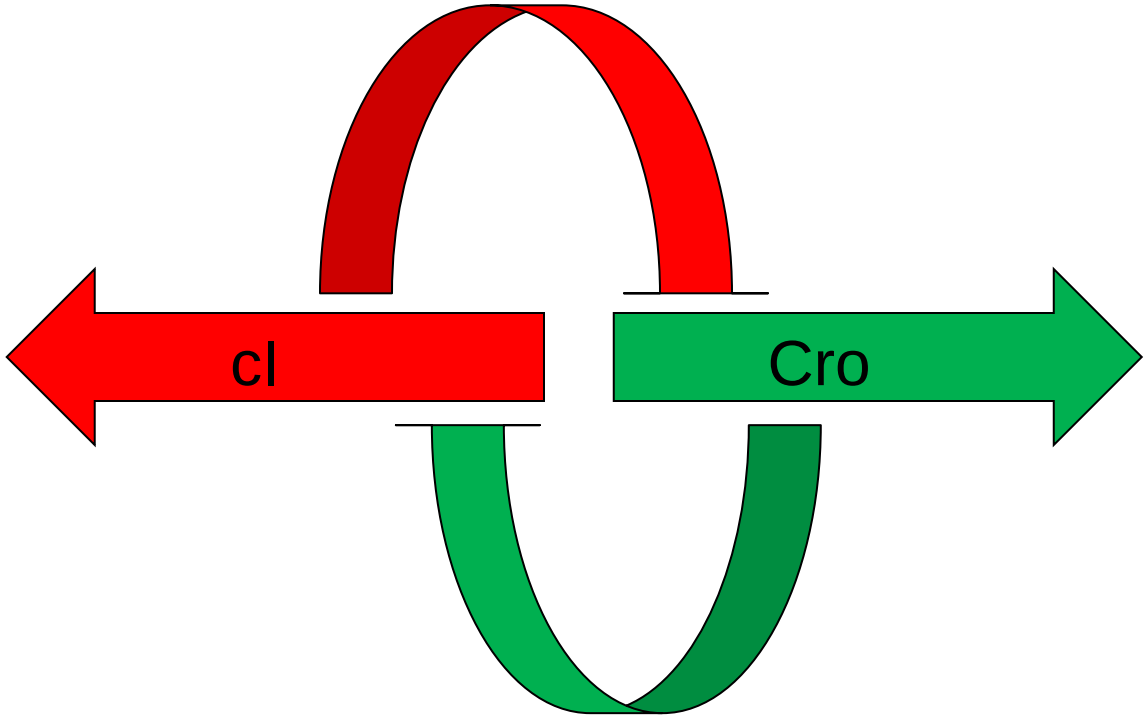
- Lysis or lysogeny: regulation of the lambda lifestyle
  - Key elements are two repressor proteins.
    - *cI protein* (the *lambda repressor*): causes repression of lambda lytic events
    - *cro* repressor: controls activation of lytic events
  - First repressor to accumulate controls infection outcome. (Figure 11.12)

Viral Replication: Temperate Bacteriophages



Temperate bacteriophages carry out two types of life cycle:the lytic cycle and the lysogenic cycle.
The lytic cycle for temperate bacteriophages is similar to the lytic cycle for virulent phages.

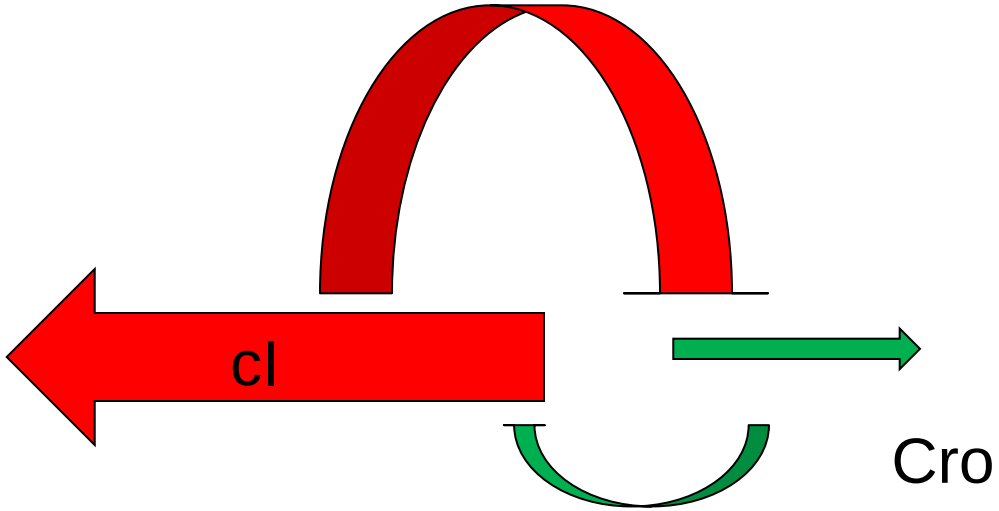
Sterk vereenvoudigd: bistabiele switch.



Netto effect: cI > Cro => lysogeny

cI < Cro => lytic

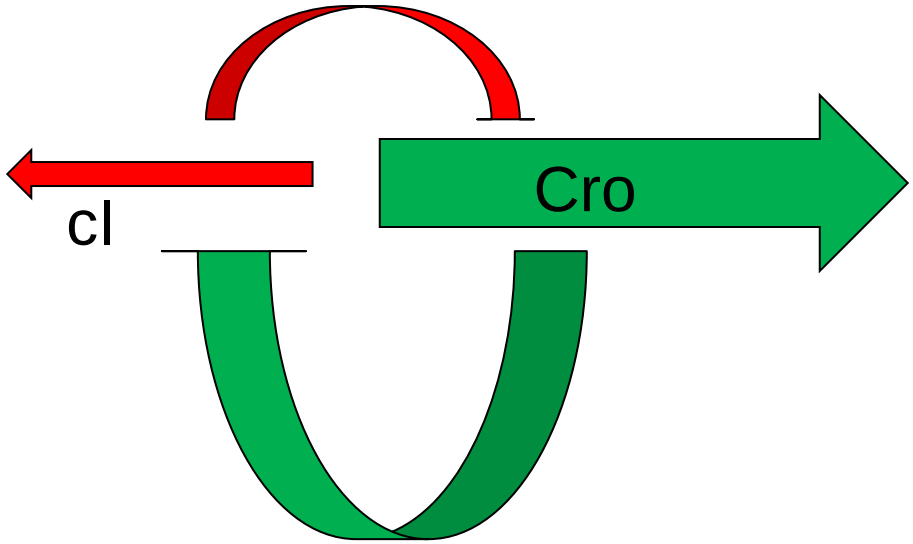
Sterk vereenvoudigd: bistabiele switch.



Netto effect: **<u>cI > Cro => lysogeny</u>**

cI < Cro => lytic

Sterk vereenvoudigd: bistabiele switch.



Netto effect: cI > Cro => lysogeny

cI < Cro => lytic

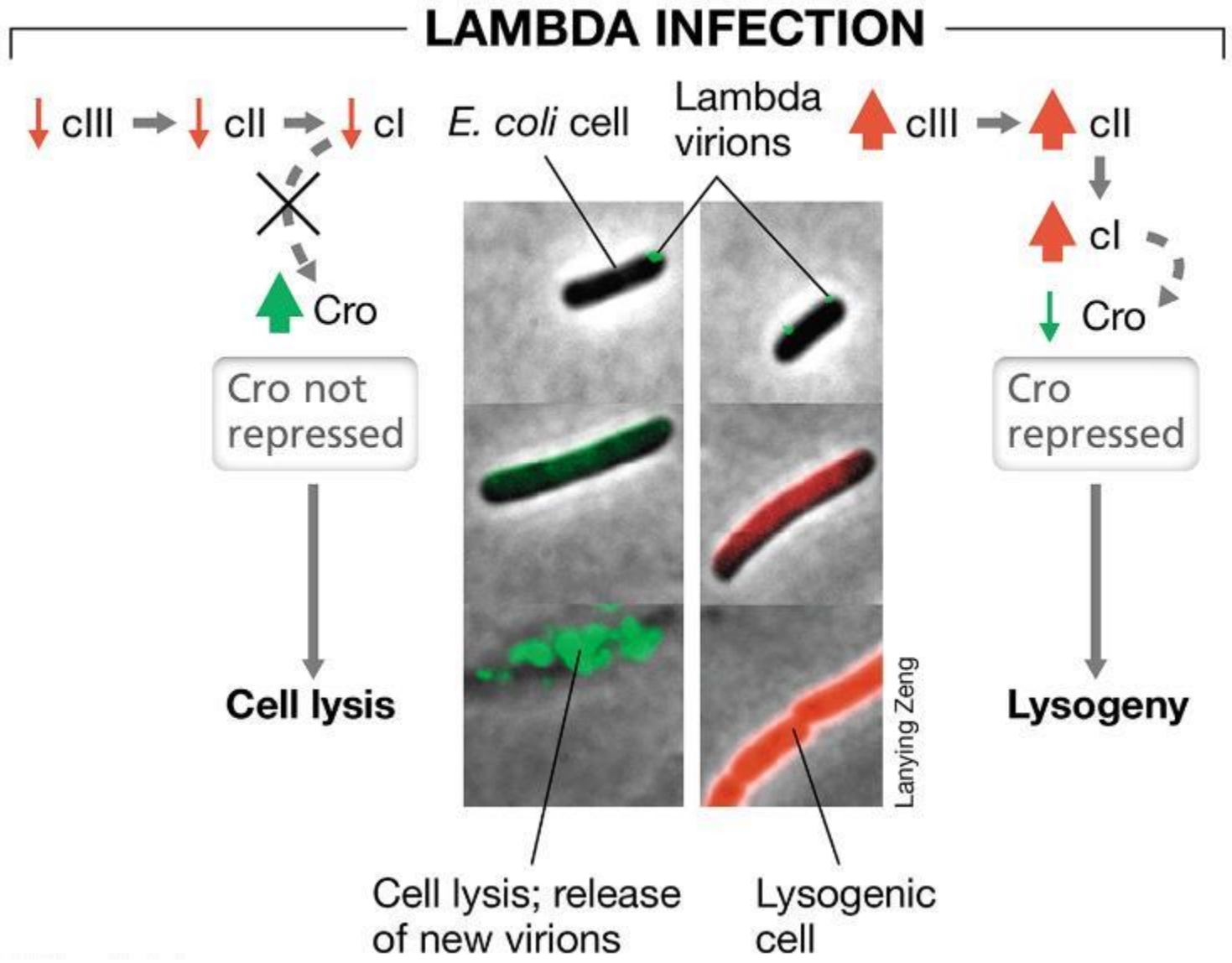


Figure 11.12

Microbiologie 2: Les 6

5.7 An Overview of Viruses of Eukaryotes

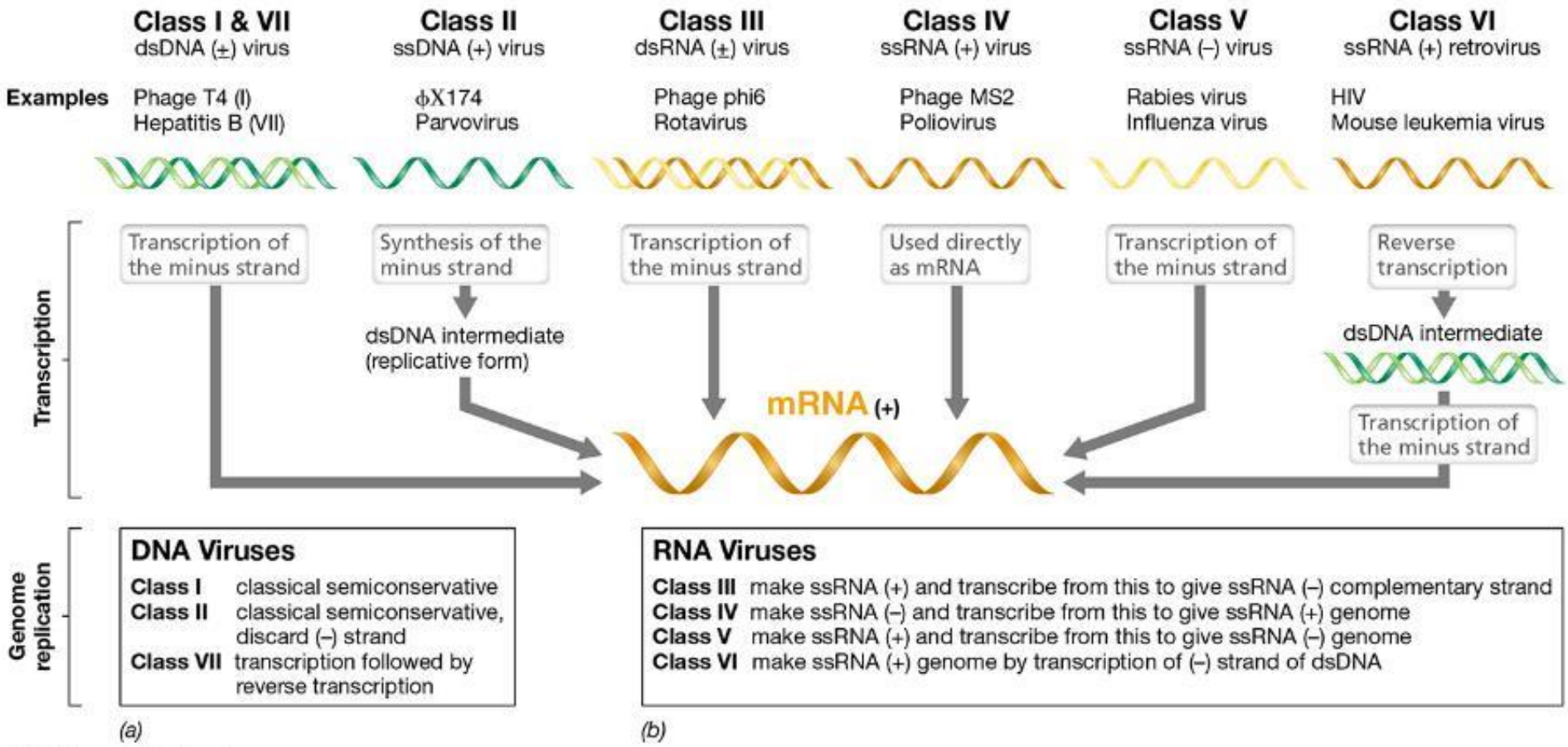
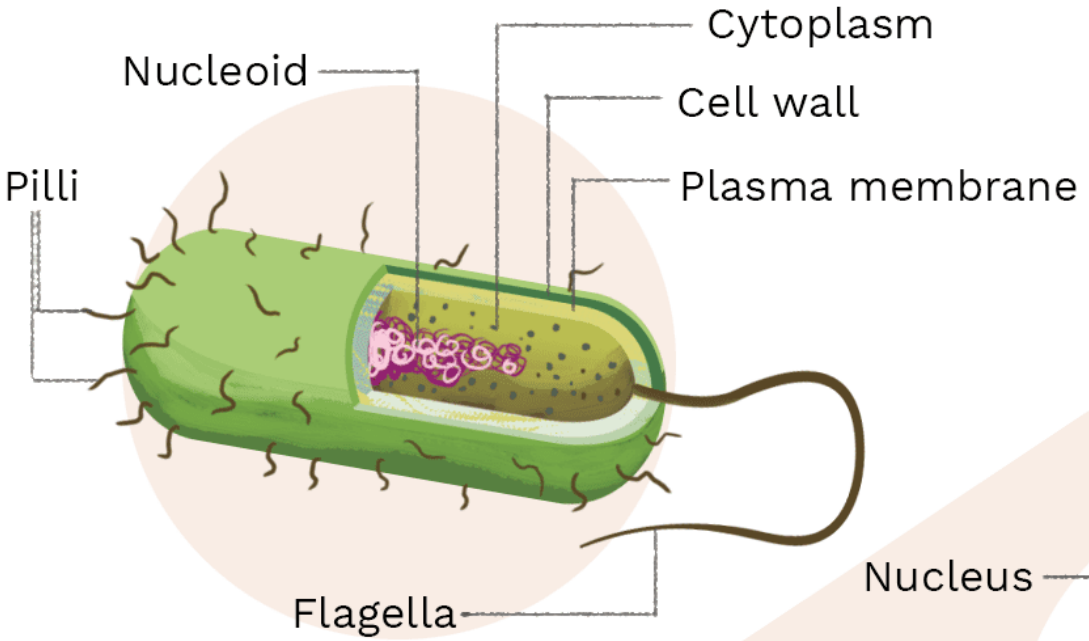
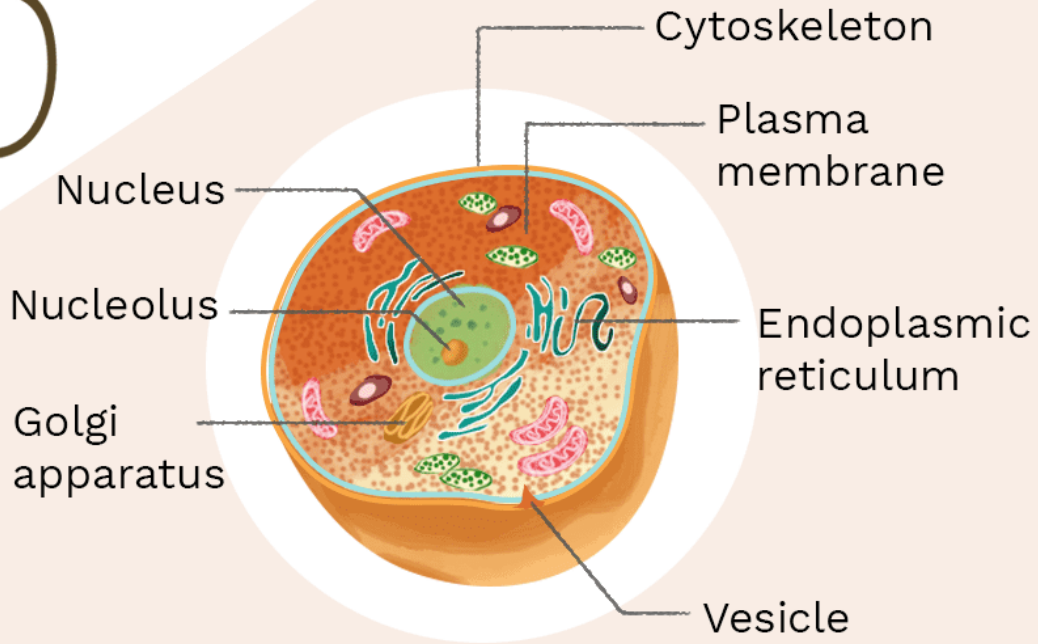


Figure 11.2

PROKARYOTIC CELL





EUKARYOTIC CELL

ThoughtCo.

5.7 An Overview of Viruses of Eukaryotes

- Major tenets (capsid and DNA/RNA genome, infection and takeover of host, assembly and release) universal
- Classified by genomes
- Most human viral diseases are caused by RNA viruses. (Table 5.1)
- Two key differences compared to phages
  - Entire virion enters the animal cell.
  - Eukaryotic cells contain a nucleus, the site of replication for many animal viruses.

5.2 Structure of the Virion

Komen we nu op terug!

- Enveloped viruses (Figure 5.2)
  - lipoprotein membrane surrounding nucleocapsid
  - RNA or DNA genomes
  - Envelope proteins attach to and infect animal host cell.
  - relatively few enveloped plant or bacterial viruses because of cell walls surrounding cell membrane
  - Entire virion enters animal cell during infection.
  - Enveloped viruses exit more easily.

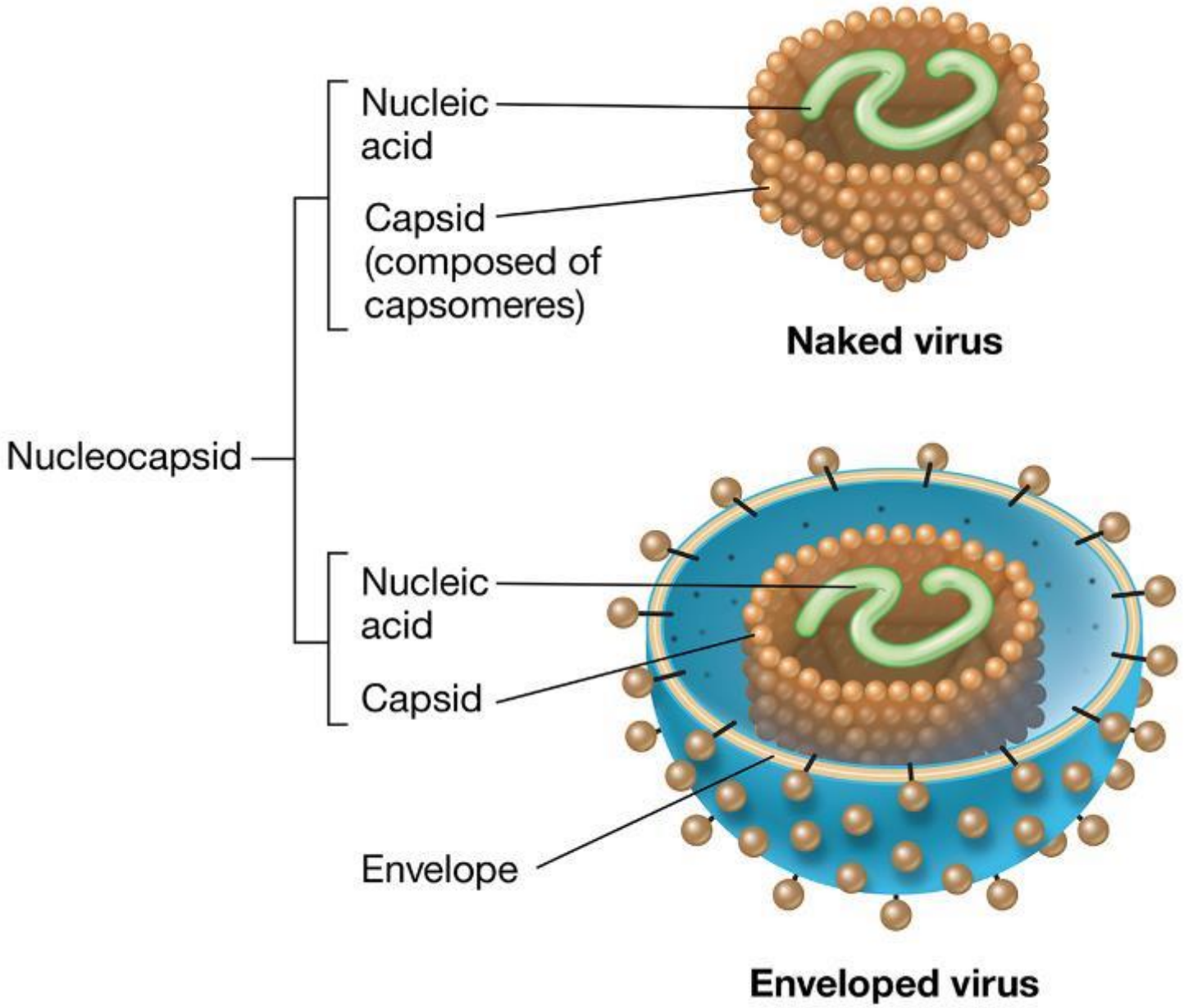


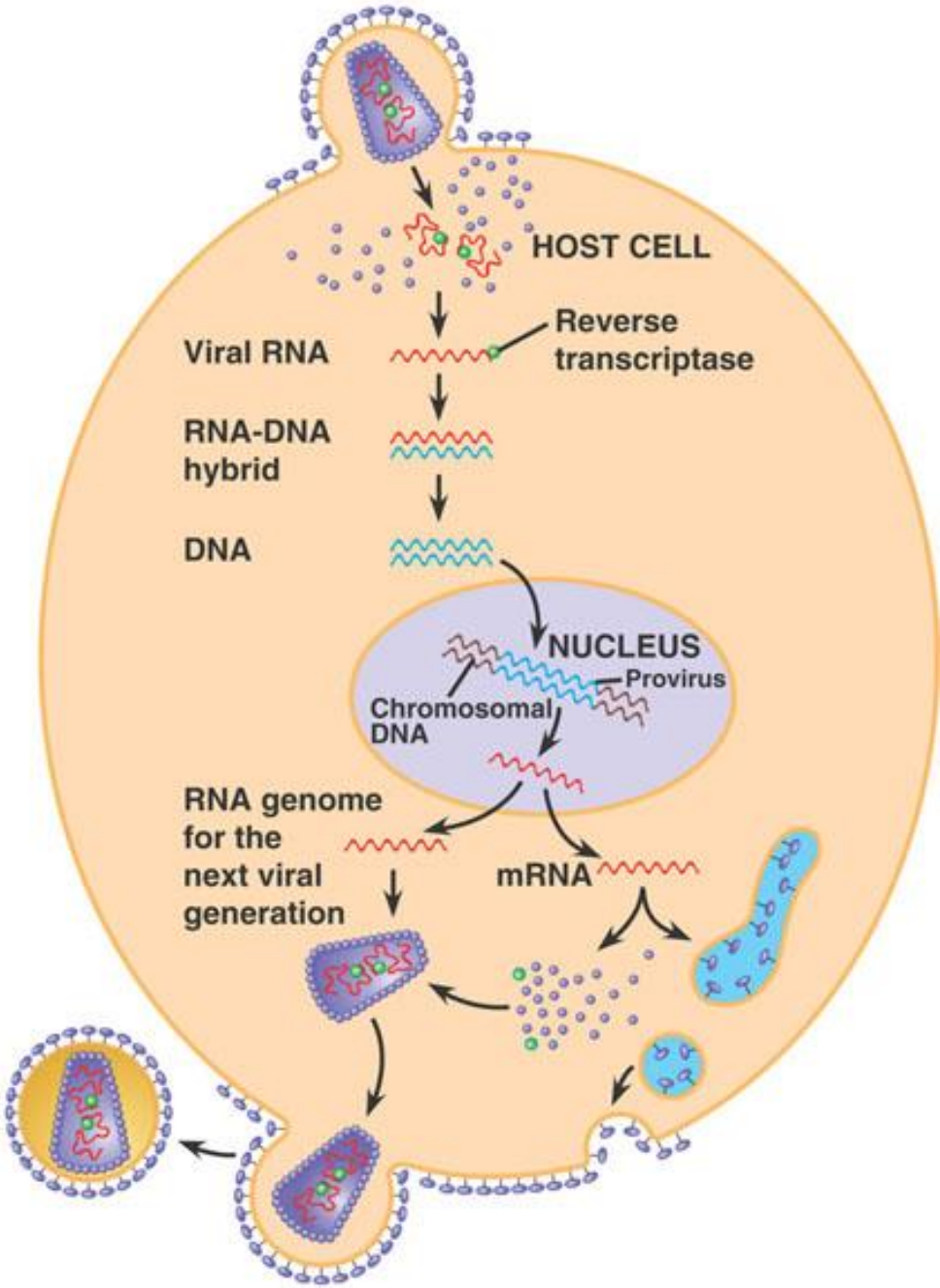
Figure 5.2

Envelop? Vaak cel-membraan

Cel kan zo intact blijven! (Itt lytic cycle)

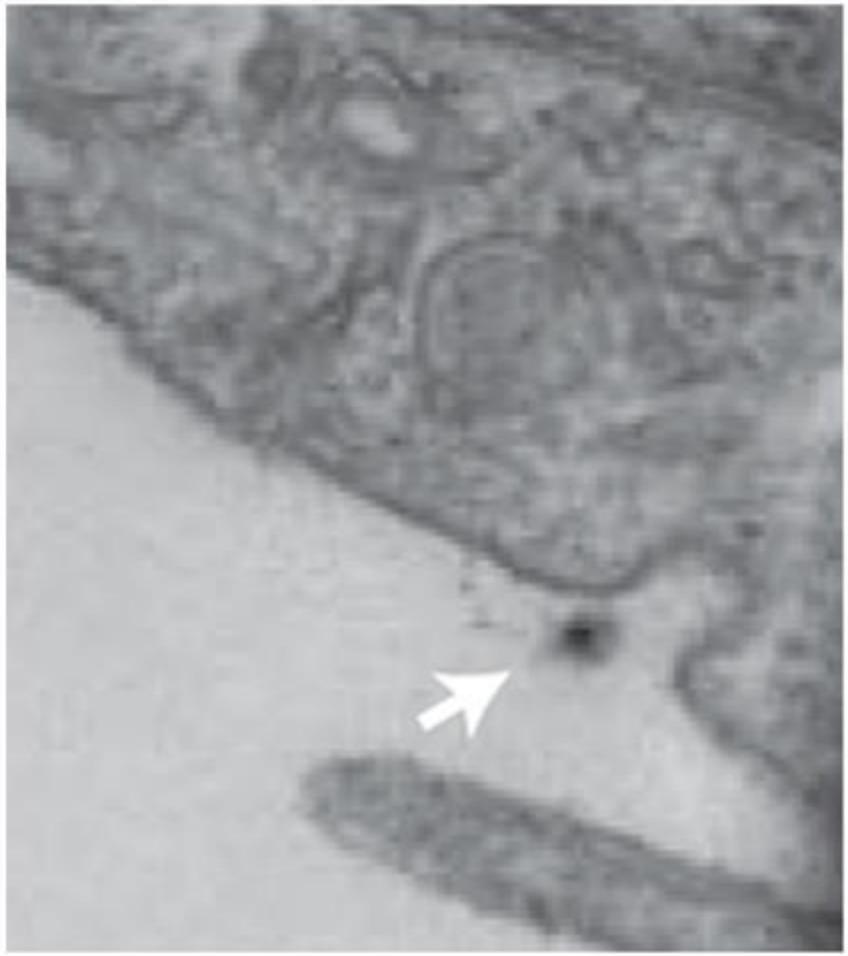
HIV entering a cell


New HIV leaving a cell

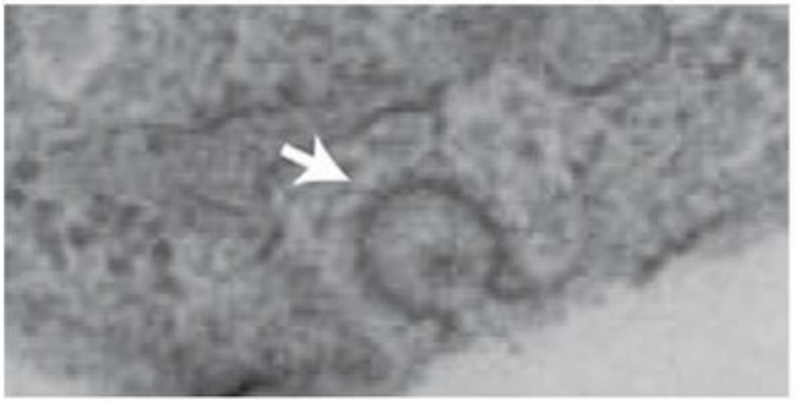


5.7 An Overview of Viruses of Eukaryotes

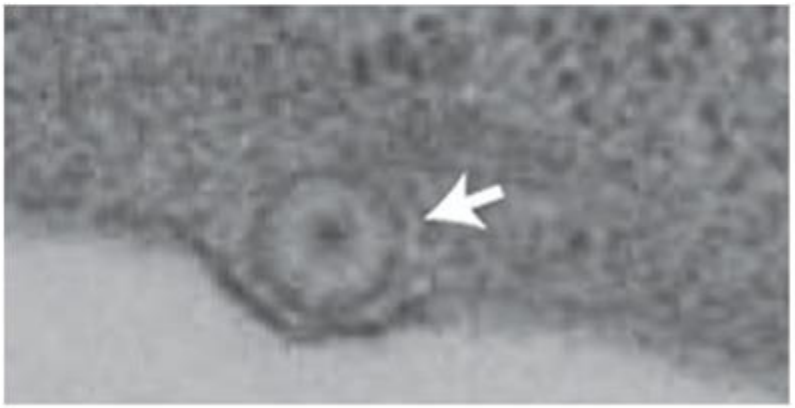
- Viral infection of animal cells
  - bind specific host cell receptors, typically used for cell-cell contact or immune function
  - Different tissues and organs express different cell surface proteins.
    - Often viruses only infect certain tissues.
  - Entry usually occurs by fusion with cytoplasmic membrane or endocytosis. (Figure 5.20)



(a)



(b)



(c)

Stephen C. Harrison

Rotavirus (no envelop)

Figure 5.20

5.7 An Overview of Viruses of Eukaryotes

- Viral infection of animal cells
  - Uncoating occurs at cytoplasmic membrane or cytoplasm.
  - Viral DNA genomes enter nucleus, most viral RNA is converted to DNA within nucleocapsid.
  - bind specific host cell receptors, typically used for cell-cell contact or immune function

5.7 An Overview of Viruses of Eukaryotes

- Virion assembly and infection outcomes (Figure 5.22)
  - *Virulent infection:* lysis of host cell, most common
  - *Latent infection:* Viral DNA exists in host genome and virions are not produced; host cell is unharmed unless/until virulence is triggered.
  - *Persistent infections:* Release of virions from host cell by budding does not result in cell lysis.
    - Infected cell remains alive and continues to produce virus
  - *transformation:* conversion of normal cell into tumor cell

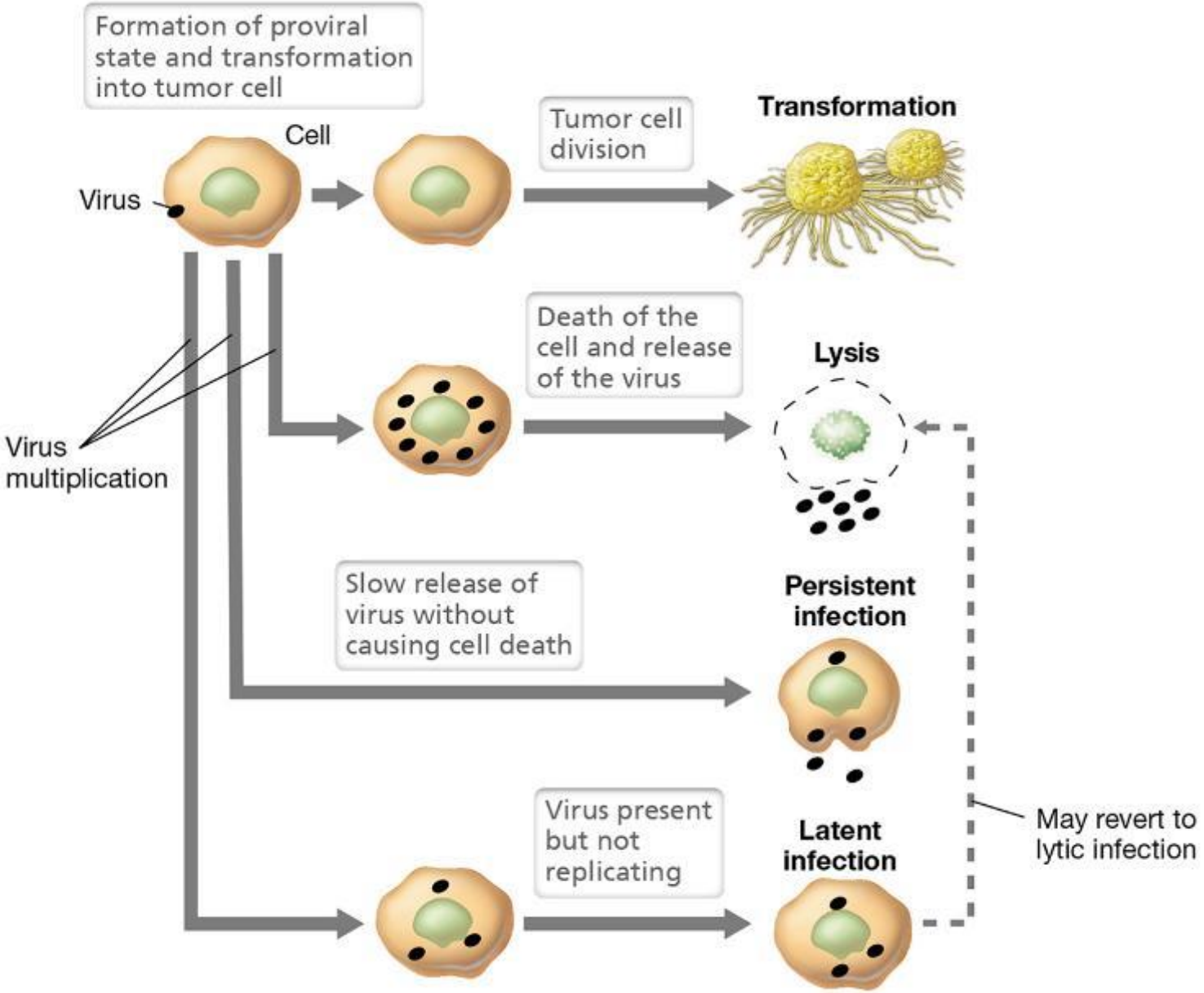


Figure 8.20

11.11 Virusses That Use Reverse Transcriptase

- Retroviruses and reverse transcriptase
  - retroviruses: RNA viruses that replicate through a DNA intermediate (*e.g.*, human immunodeficiency virus [HIV])
  - contain a reverse transcriptase (copies information from RNA to DNA), integrase, and protease
  - enveloped (Figure 11.26a)

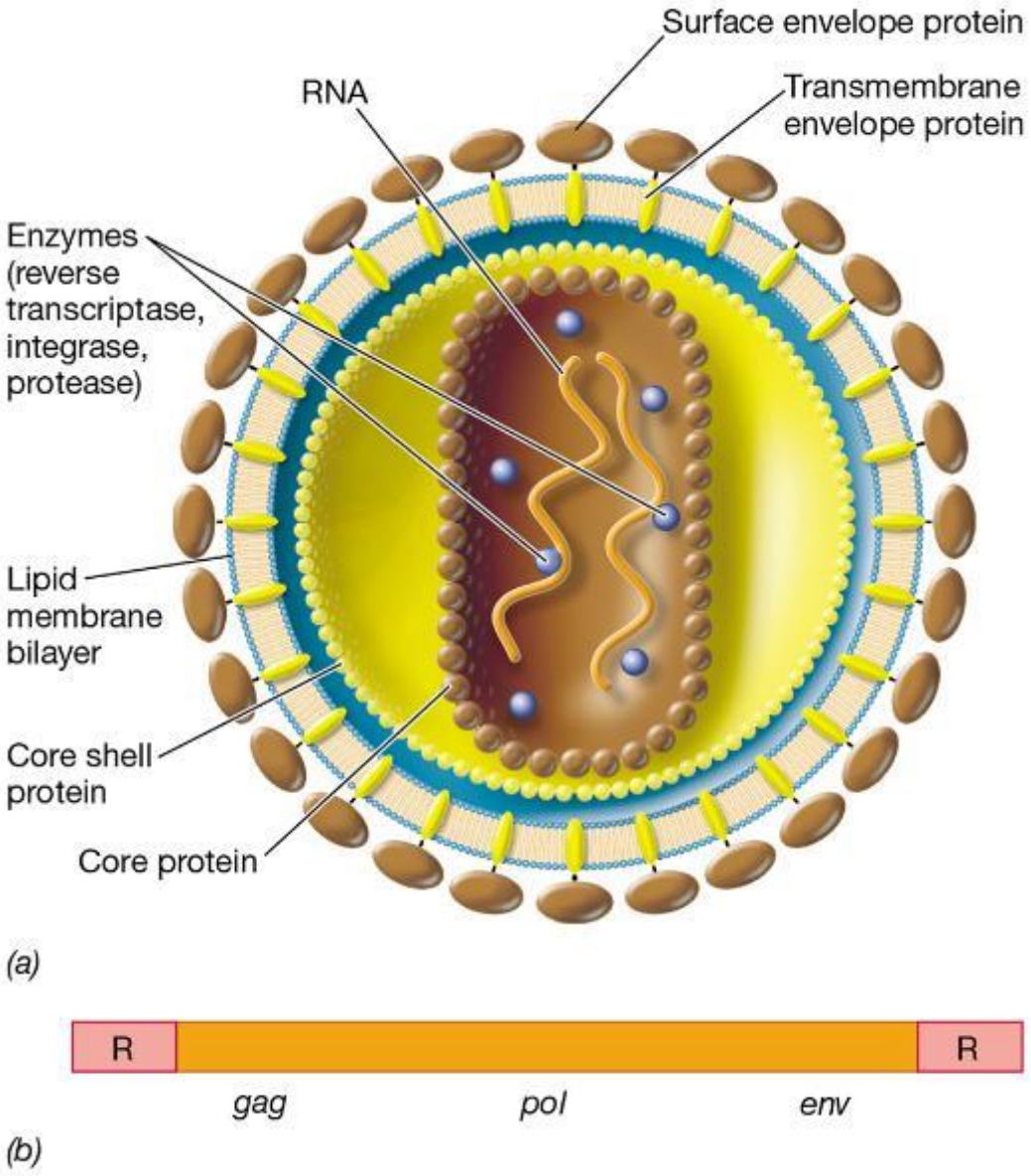


Figure 11.26a

8.8 An Overview of Animal Virus Infection

- Retroviruses have a unique genome.
  - two identical ss(+)RNA molecules
  - contains specific genes
    - *gag:* encode structural proteins
    - *pol:* encode reverse transcriptase and integrase
    - *env:* encode envelope proteins

8.8 An Overview of Animal Virus Infection

- Process of retroviral replication (Figure 11.26)
  - entrance into the cell with removal of envelope at the membrane
  - reverse transcription of one RNA genome begins in nucleocapsid
  - single DNA strand produced
  - Reverse transcriptase uses this to make a complementary strand, forming dsDNA product.
  - dsDNA enters nuclease with integrase, which incorporates retroviral DNA into host genome to form provirus, which remains indefinitely
  - transcription of retroviral DNA
  - assembly and packaging of genomic RNA
  - budding of enveloped virions and release from cell

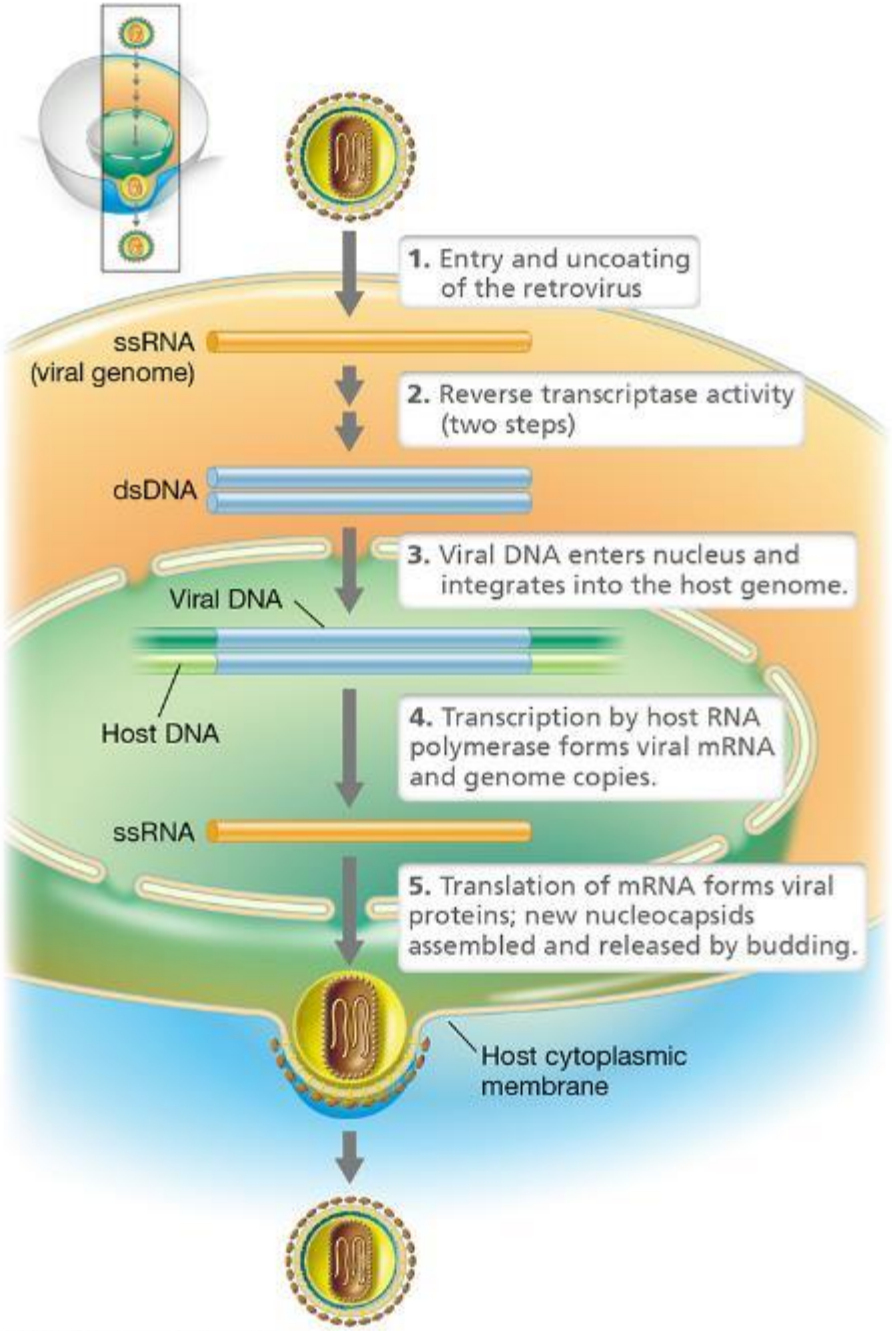


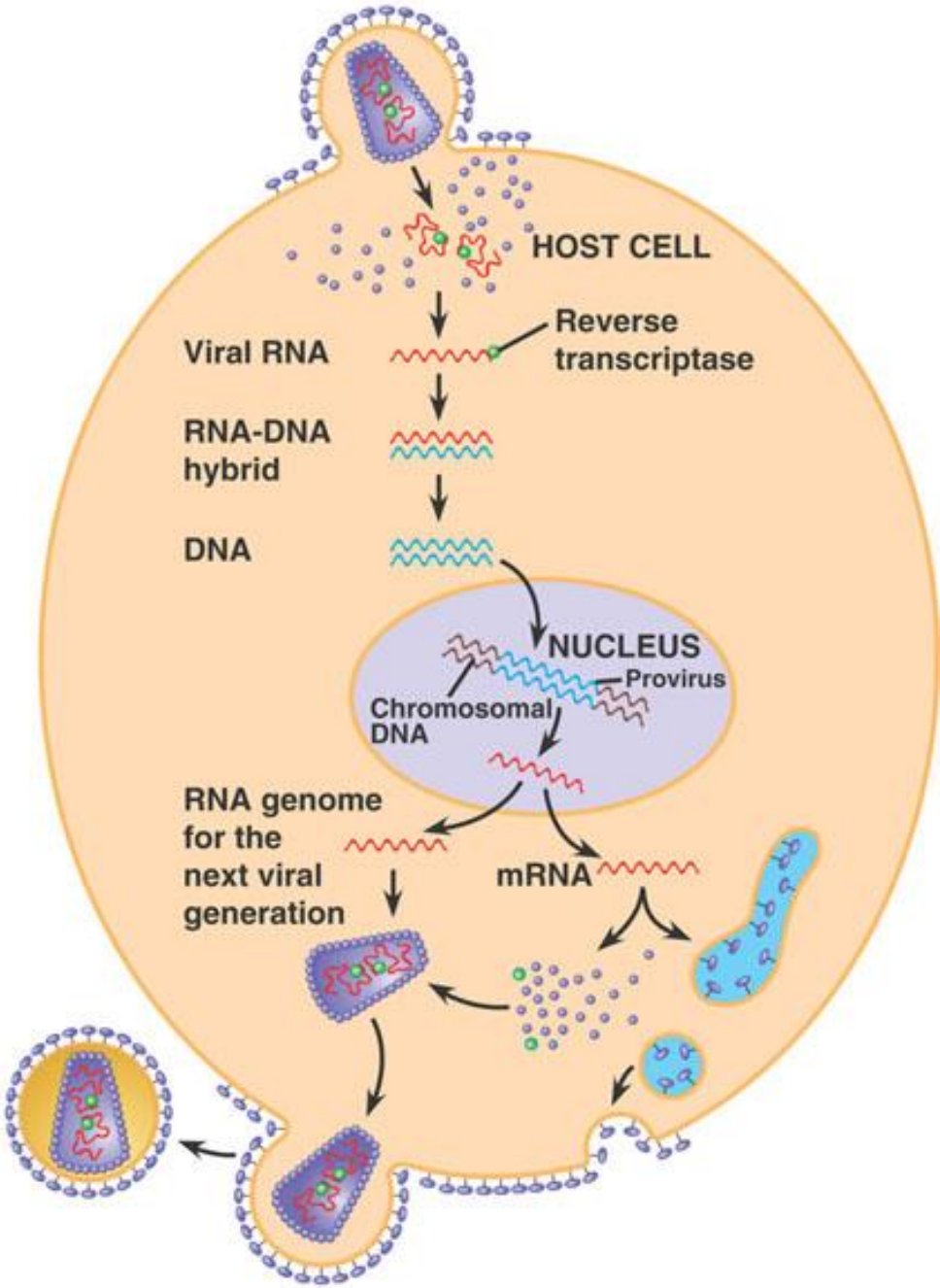
Figure 11.26

Envelop? Vaak cel-membraan

Cel kan zo intact blijven! (Itt lytic cycle)

HIV entering a cell


New HIV leaving a cell



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