Animal Virus vectors

KSR

Introduction

- **Viral vectors** are a tool commonly used by molecular biologists to deliver genetic material into cells.
- This process can be performed inside a living organism (*in vivo*) or in cell culture (*in vitro*).
- Viruses have evolved specialized molecular mechanisms to efficiently transport their genomes inside the cells they infect.

Conti…

- Delivery of genes by a virus is termed transduction and the infected cells are described as transduced.
- Molecular biologists first harnessed this machinery in the 1970s.
- Paul Berg used a modified SV40 virus containing DNA from the bacteriophage lambda to infect monkey kidney cells maintained in culture

Viruses: Bacterial and Animal Types

Animal Viruses

- •DNA vs RNA animal viruses
  - •Differences in replicating viral genome
  - •both ss and ds DNA & RNA forms
- •DNA Viruses (E.g. smallpox, warts, tumors, herpes, colds)
  - •Life Cycle
    - •Attachment, penetration, (uncoating)
    - •Replication of DNA in nucleus of host
    - •Assembly in nucleus
    - •Lysis from nucleus and plasma membrane
- **•RNA Viruses (E.g. Polio, rabies, hepatitis, influenza, AIDS)**
  - •Life Cycle of RNA->RNA viruses
    - •Attachment, penetration, (uncoating)
    - •Replication of RNA to RNA in cytoplasm
    - •Assembly in cytoplasm, then lysis
  - •Retroviruses

Types of Infections: Latent (herpes, shingles), slow (encephalitis), prions (kuru, CJD)

Oncogenesis

- • 15% of cancers are virally causes (e.g. EBV (mononucleosis), HIV, papovaviruses-warts)
- • Carcinogenic agents: mutagens, radiation, viruses
- • Cancer cell characteristics (contact inhibition loss, chromosome number)

Figure 13.1

Single/Double-stranded DNA, nonenveloped viruses

Parvoviridae

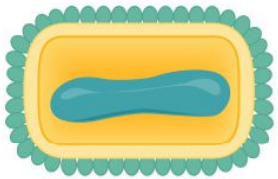
- *Parvovirus*
- **Fifths Disease**

Adenoviridae

Papovaviridae

- **Tumors**
- **Cancers**
- **Warts**

Poxviridae



- **Smallpox**
- Cowpox

Herpesviridae

- **Herpes simplex cold sores**
- **Genital herpes**
- **Kaposi's sarcoma**

Hepadnaviridae

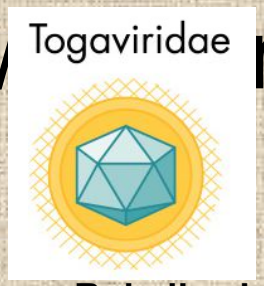
- Hepatitis C

Know the names of some DNA viruses

SS or DS RNA, +/- strand, en[illegible]d/[illegible]nv[illegible]c

- **Polio virus**
- ***Rhinovirus (colds)***
- **Hepatitis A virus** (vaccine)

- **Hepatitis E** virus
- *Norwalk gastroenteritis*



- **Rubella virus**
- **Measles**

- **Yellow fever, dengue, and West Nile viruses**
- **Hepatitis C virus**

- Mumps

- Respiratory infections

Rabies virus

Ebola & Marburg viruses

- **Hepatitis D** virus, virulent

- **Influenza**

Know the names of some RNA viruses

Hanta virus

Lassa fever

Hepatitis B virus (esp. virulent, cirrhosis, cancer); used reverse transcription – (vaccine)

Colorado tick fever

Some Viruses are Oncogenic

TABLE 14.8 Viruses Associated with Cancers in Humans

Virus	Type of Nucleic Acid	Kind of Tumor
Human papillomaviruses (HPV)	DNA	Different kinds of tumors, including squamous cell and genital carcinomas, caused by different HPV types
Hepatitis B	DNA	Hepatocellular carcinoma
Epstein-Barr	DNA	Burkitt's lymphoma; nasopharyngeal carcinoma; B-cell lymphoma
Hepatitis C	RNA	Hepatocellular carcinoma
Human herpes, virus 8	DNA	Kaposi's sarcoma
HTLV-1	RNA (retrovirus)	Adult T-cell leukemia (rare)

Key properties of a viral vector

1. *Safety*: Although viral vectors are occasionally created from pathogenic viruses, they are modified in such a way as to minimize the risk of handling them.

- This usually involves the deletion of a part of the viral genome critical for viral replication.
- Such a virus can efficiently infect cells but, once the infection has taken place, requires a helper virus to provide the missing proteins for production of new virions

Cont…

2. *Low toxicity*: The viral vector should have a minimal effect on the physiology of the cell it infects.
3. *Stability*: Some viruses are genetically unstable and can rapidly rearrange their genomes. This is detrimental to predictability and reproducibility of the work conducted using a viral vector and is avoided in their design.

Conti…

4. *Cell type specificity*: Most viral vectors are engineered to infect as wide a range of cell types as possible. However, sometimes the opposite is preferred. The viral receptor can be modified to target the virus to a specific kind of cell. Viruses modified in this manner are said to be pseudotyped.

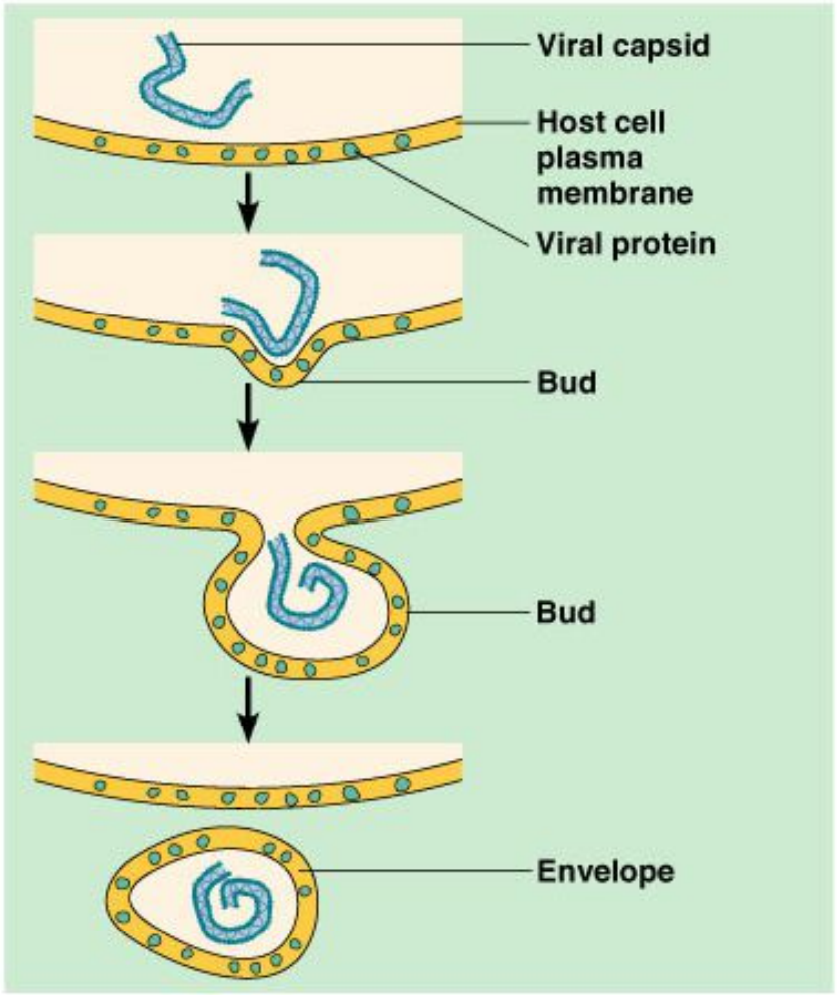
- *Identification*: Viral vectors are often given certain genes that help identify which cells took up the viral genes. These genes are called Markers, a common marker is antibiotic resistance to a certain antibiotic. The cells can then be isolated easily as those that have not taken up the viral vector genes do not have antibiotic resistance and so cannot grow in a culture with antibiotics present.

Multiplication of Animal viruses

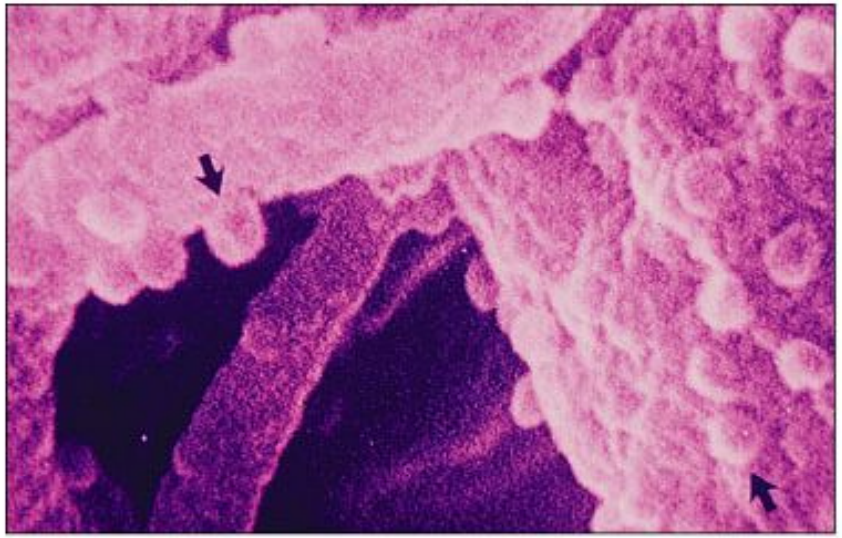
- Attachment — Viruses attaches to cell membrane
- Penetration — By endocytosis or fusion
- Uncoating — By viral or host enzymes
- Biosynthesis — Production of nucleic acid and proteins
- Maturation — Nucleic acid and capsid proteins assemble
- Release — By budding (enveloped viruses) or rupture

Having an RNA genome means transcription may not be necessary but an RNA-dependent RNA polymerase must be provided for replication

Release of an enveloped virus by budding



(a) Release by budding



(b) *Alphavirus*

Figure 13.20

Multiplication of DNA Virus

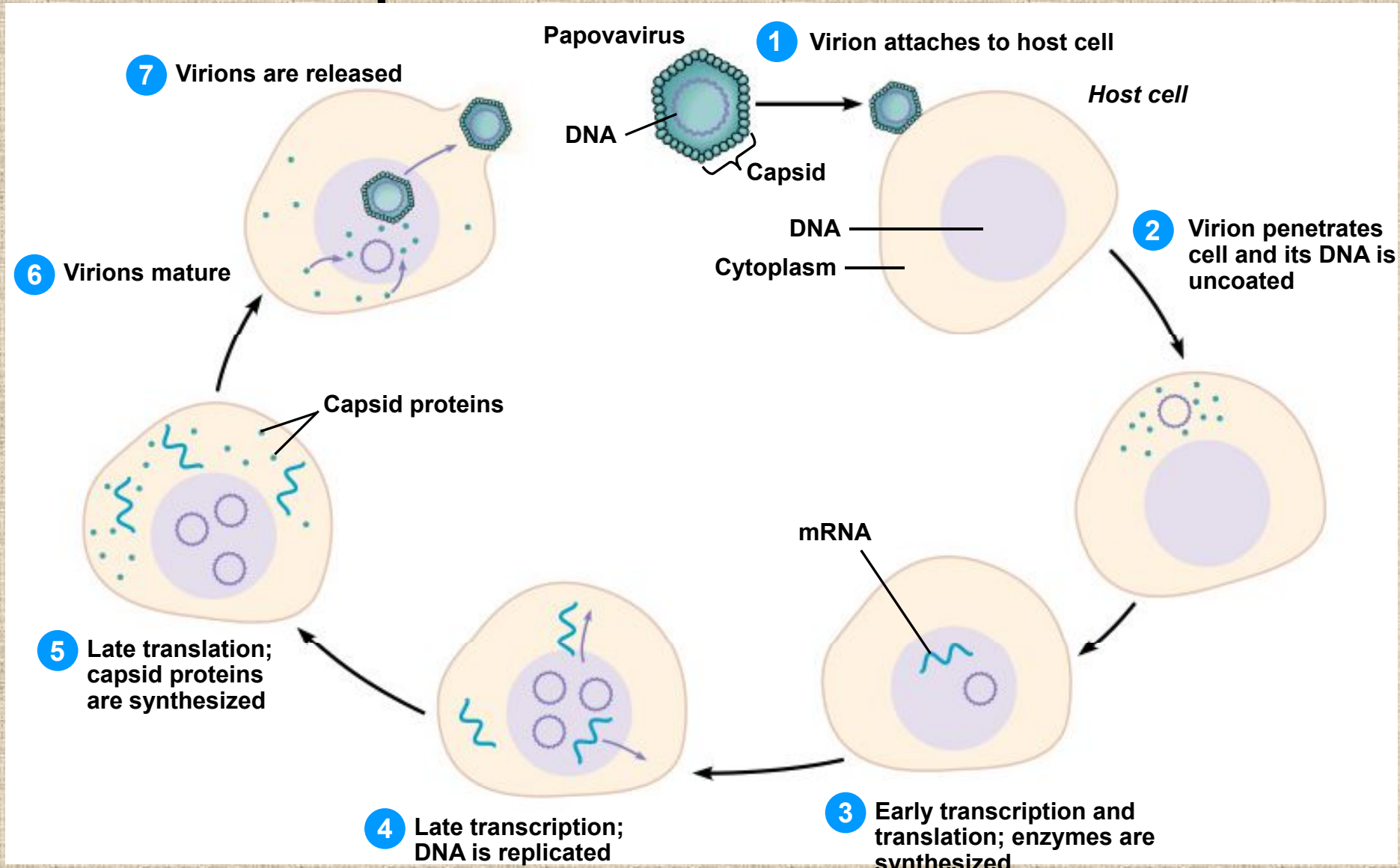


Figure 13.15

Pathways of Multiplication for RNA-

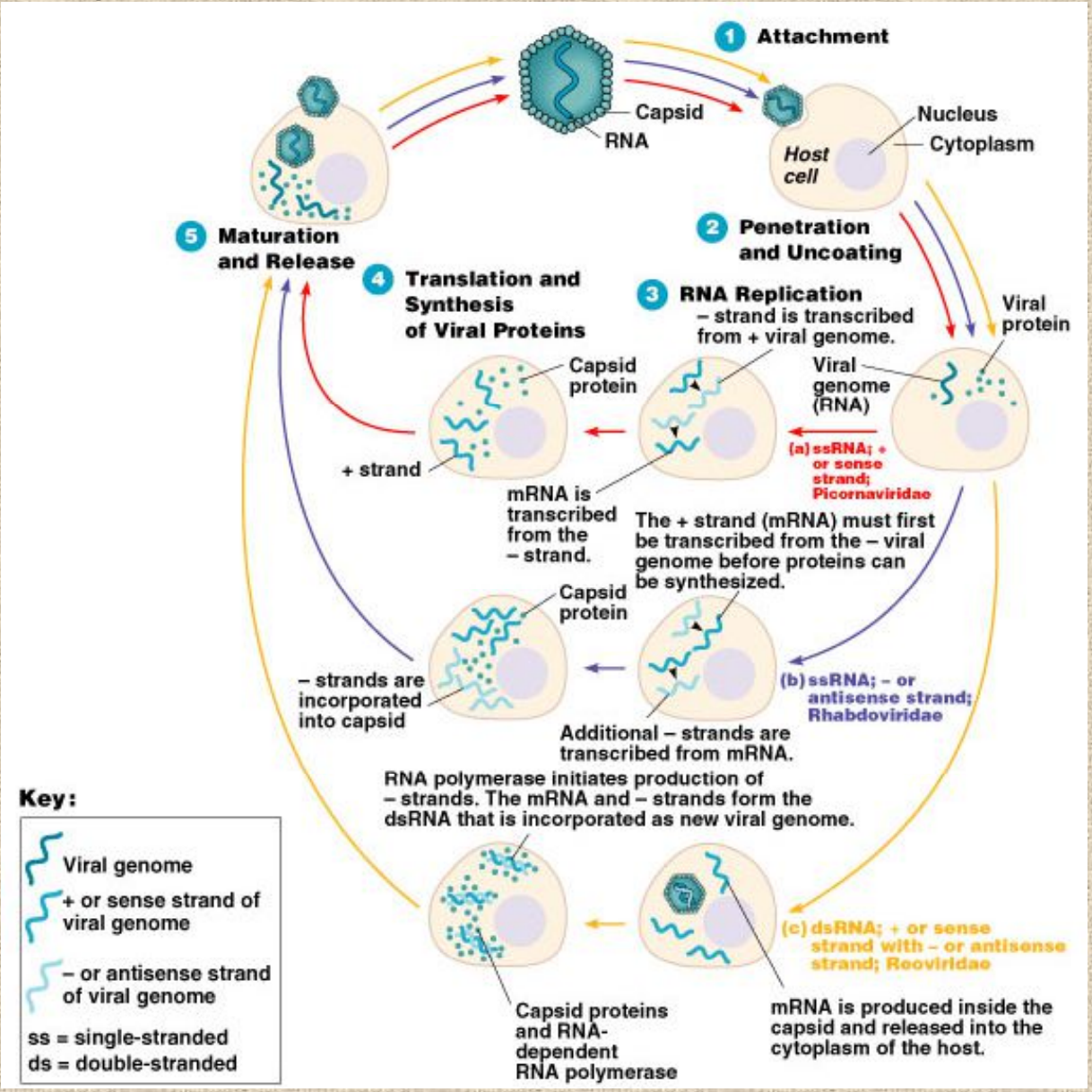
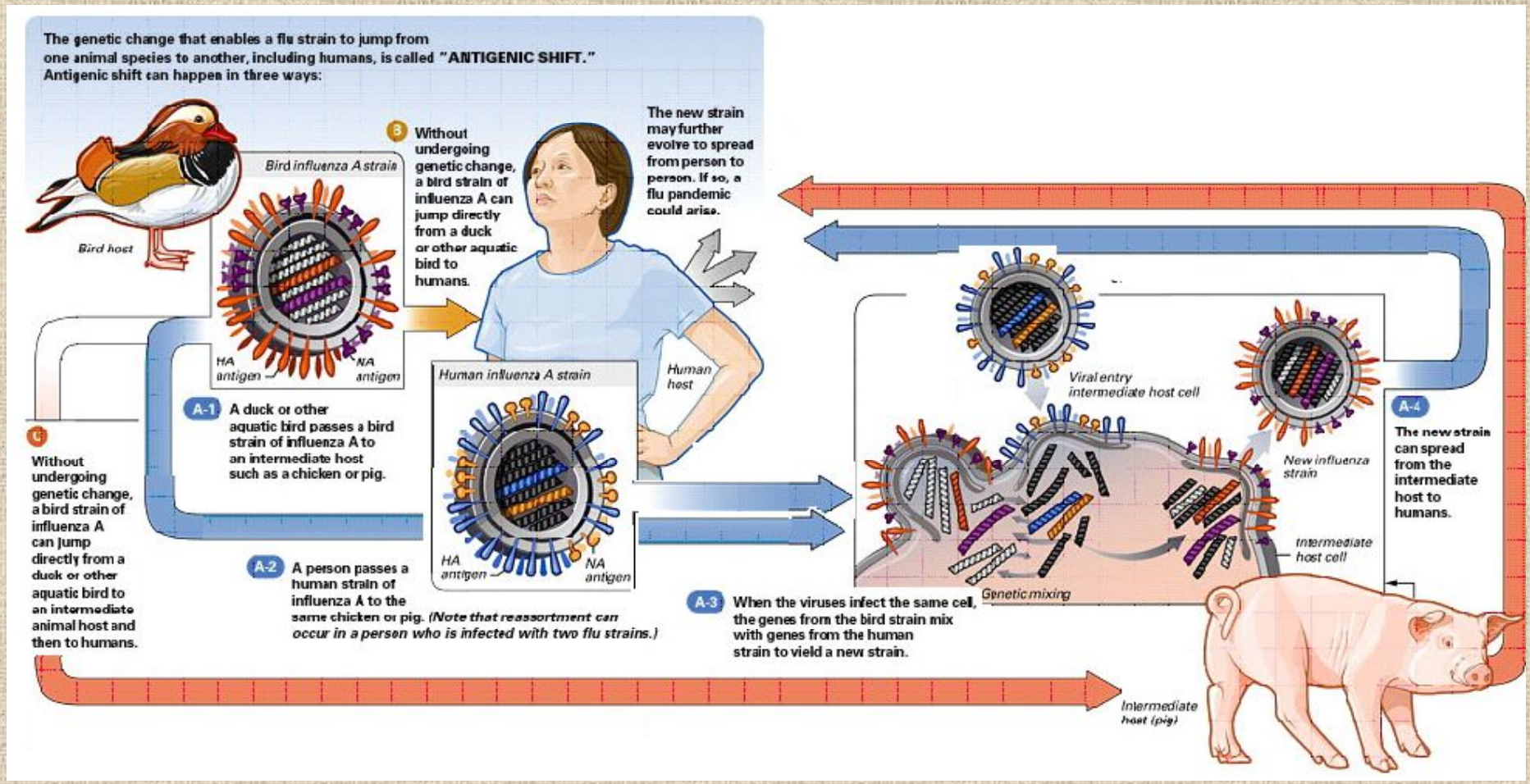


Figure 13.17

Viruses Can Recombine Within Host Cells: E.g. Flu Virus



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  - **•Retroviruses (HIV)**

Types of Infections: Latent (herpes, shingles), slow (encephalitis), prions (kuru, CJD)

Oncogenesis

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Figure 13.1

Multiplication of a Retrovirus

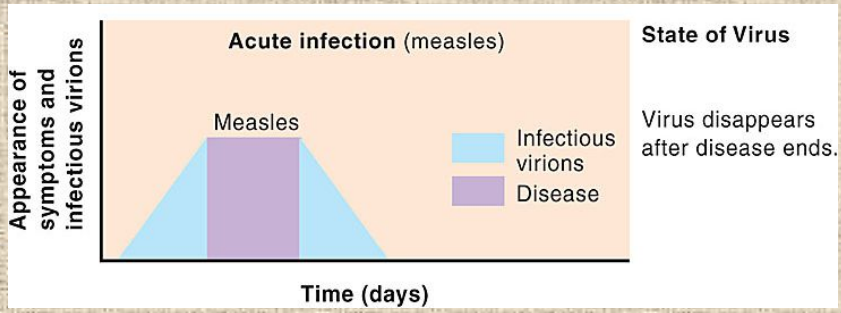
RNA tumor viruses
HIV-AIDS

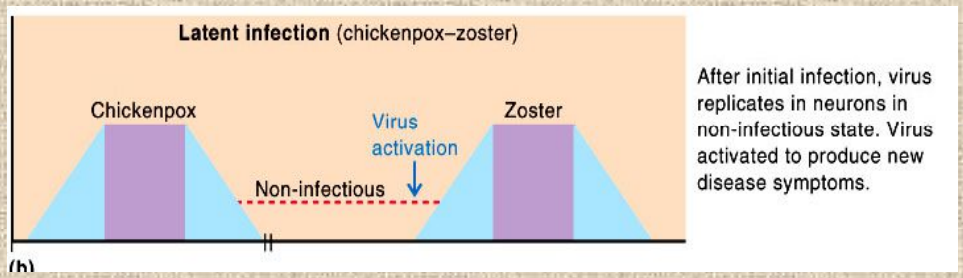
(Hepatitis B also uses reverse transcriptase)

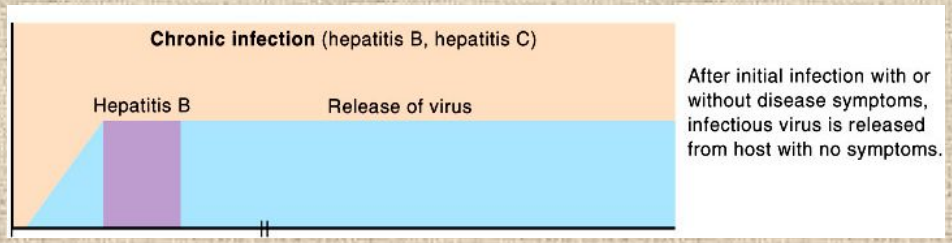
Figure 13.19

Special Types of Viral or Viral-Like Infections

- **Acute infections**
- **Peristent Viral Infections**
  - Latent: Virus remains in asymptomatic host cell for long periods
    - Cold sores, shingles
  - Chronic: Infectious virus can be detected at all times
    - Hep B and C
- **Slow Viral Infections**
  - Disease processes occurs over a long period, generally fatal
    - Subacute sclerosing panencephalitis (measles virus)
    - Retrovirus disease(HIV)
- Prions (Protein infectious particles) – CJD, kuru, scrapie

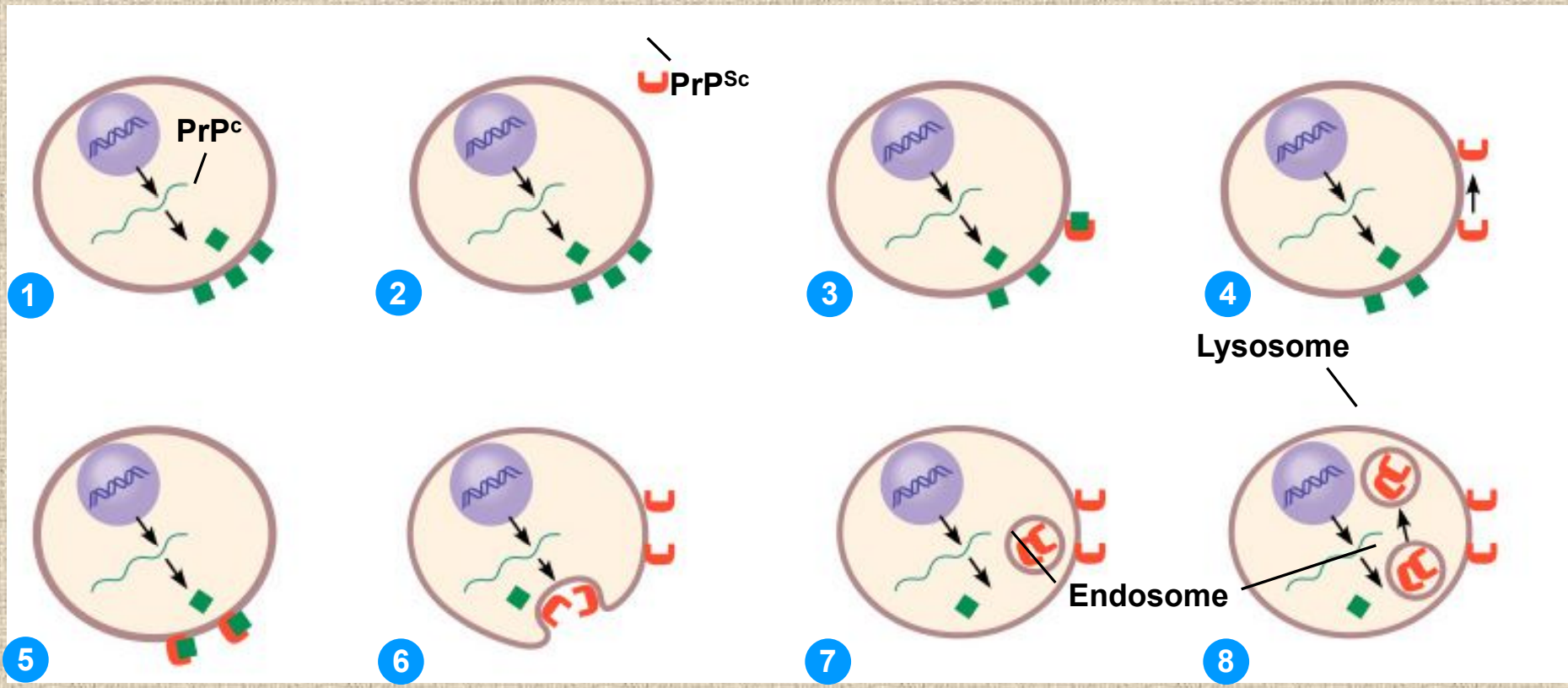






Prions (Proteinaceous Infectious Agents)

Prion diseases: Creutzfeld-Jacob Disease (CJD), kuru, scrapie, bovine spongiform encephalopathy (BSE)

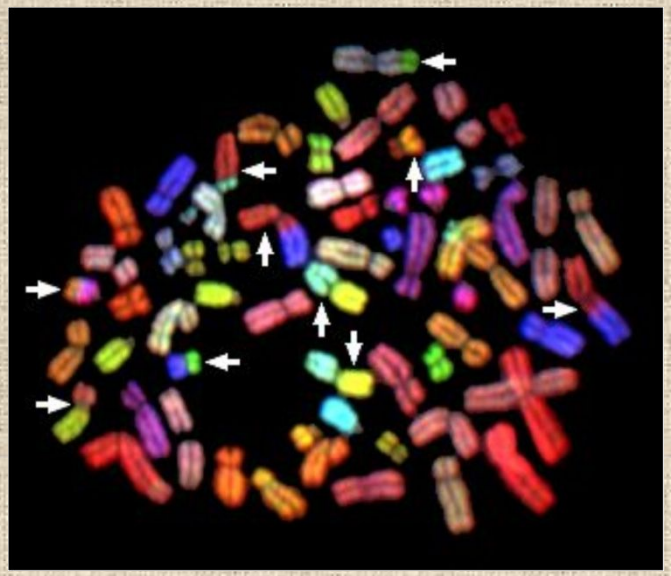


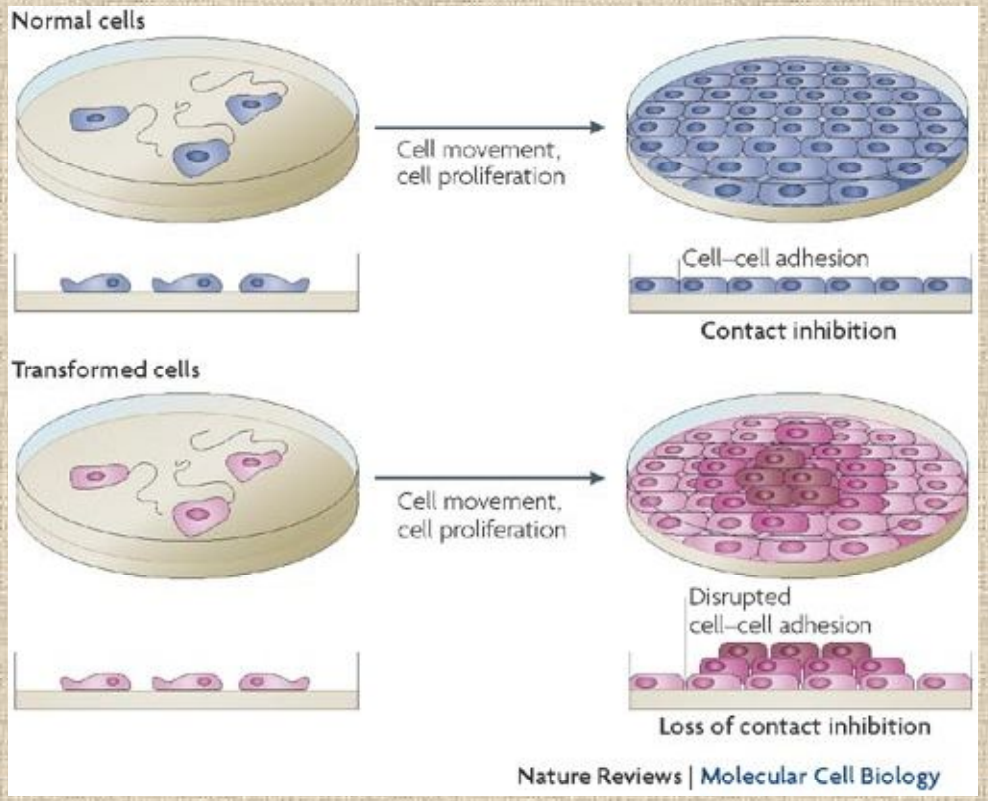
Abnormally folded protein "recruits" normal ones to fold abnormally, leading to accumulations of abnormal proteins and neuron death. Abnormal protein is resistant to UV light, and proteases

Figure 13.21

Cancer Cell Characteristics

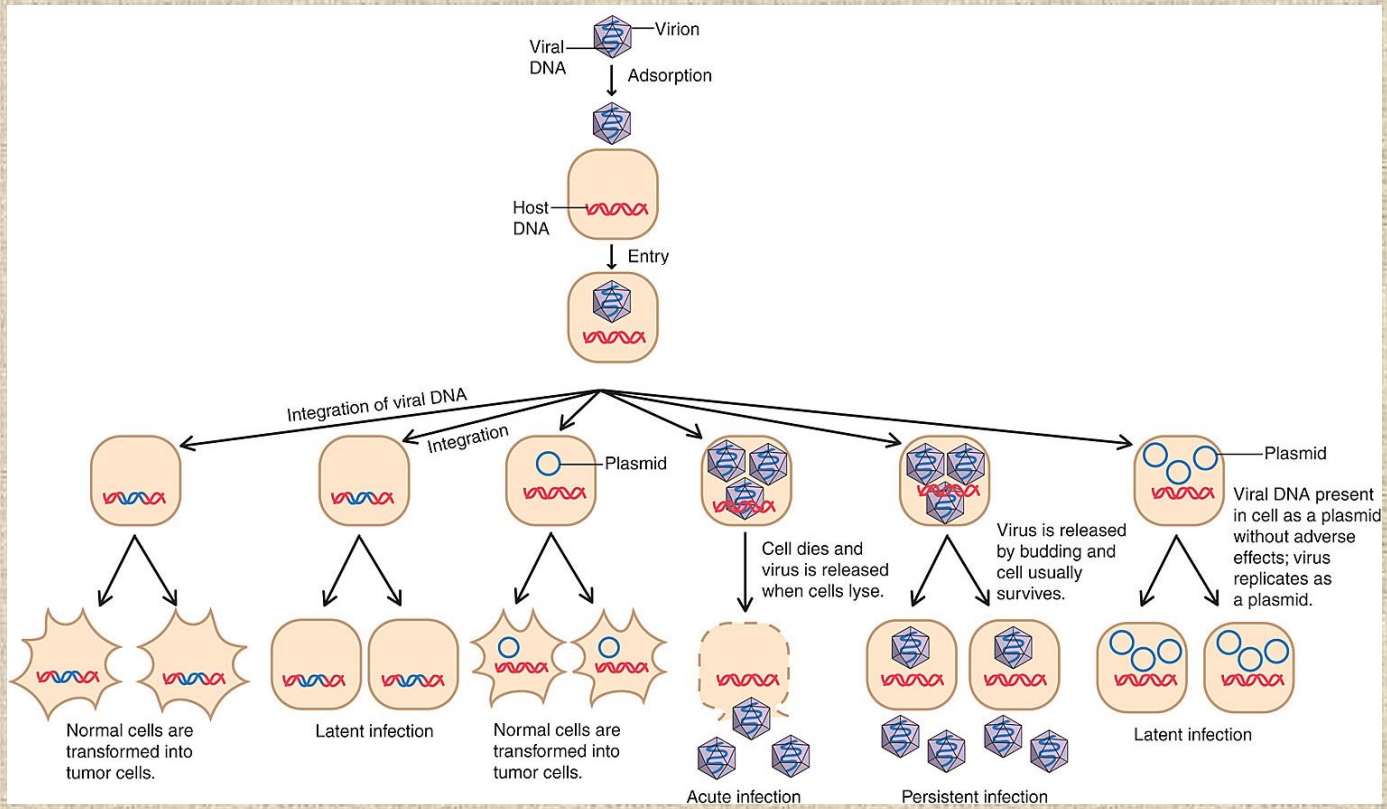
Cancer cell characteristics: loss of contact inhibition, chromosome abnormalities, cell fusion (polykaryocytes)





Viral Cancer Results Mostly From Activation of Oncogenes

- Proto-oncogenes: Normal genes in human cells that control cellular division (mitosis)
  - Activated proto-oncogenes (oncogenes) *transform* normal cells into cancerous cells. Requires **at least "2 hits"**
  - Double-stranded DNA viruses cause most virus-induced tumors
  - Virus can go through productive cycle and lyse cell OR
  - Virus can transform cell without killing it by integrating into the middle of an oncogene



Applications

- Gene therapy is a technique for correcting defective genes responsible for disease development.
- In the future, gene therapy may provide a way to cure genetic disorders, such as severe combined immunodeficiency, cystic fibrosis or even Haemophilia A.

Applications

- **Vaccines:** Viruses expressing pathogen proteins are currently being developed as vaccines against these pathogens, based on the same rationale as DNA vaccines.
- T-lymphocytes recognize cells infected with intracellular parasites based on the foreign proteins produced within the cell.
- T cell immunity is crucial for protection against viral infections and such diseases as malaria.