Lecture Series 10

The Genetics of Viruses and Prokaryotes

The Genetics of Viruses and Prokaryotes

A. Using Prokaryotes and Viruses for Genetic Experiments

B. Viruses: Reproduction and Recombination

C. Prokaryotes: Reproduction, Mutation, and Recombination

The Genetics of Viruses and Prokaryotes

D. Regulation of Gene Expression in Prokaryotes

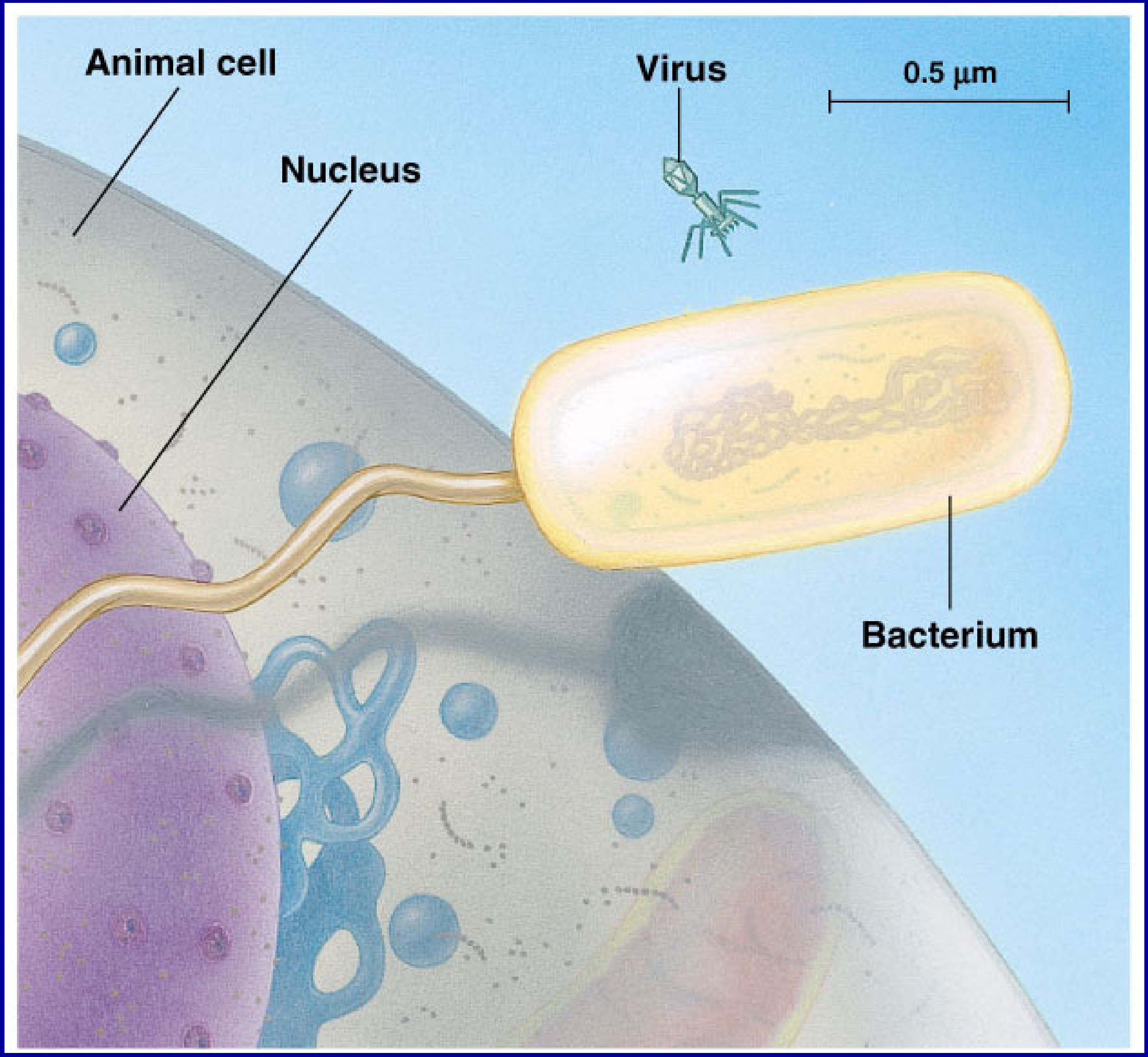
E. Control of Transcription in Viruses

F. Prokaryotic Genomes

A. Using Prokaryotes and Viruses for Genetic Experiments

- Prokaryotes and viruses are useful for the study of genetics and molecular biology because they contain less DNA than eukaryotes, grow and reproduce rapidly, and are **haploid**.

Comparing the size of a virus, a bacterium, and a eukaryotic cell



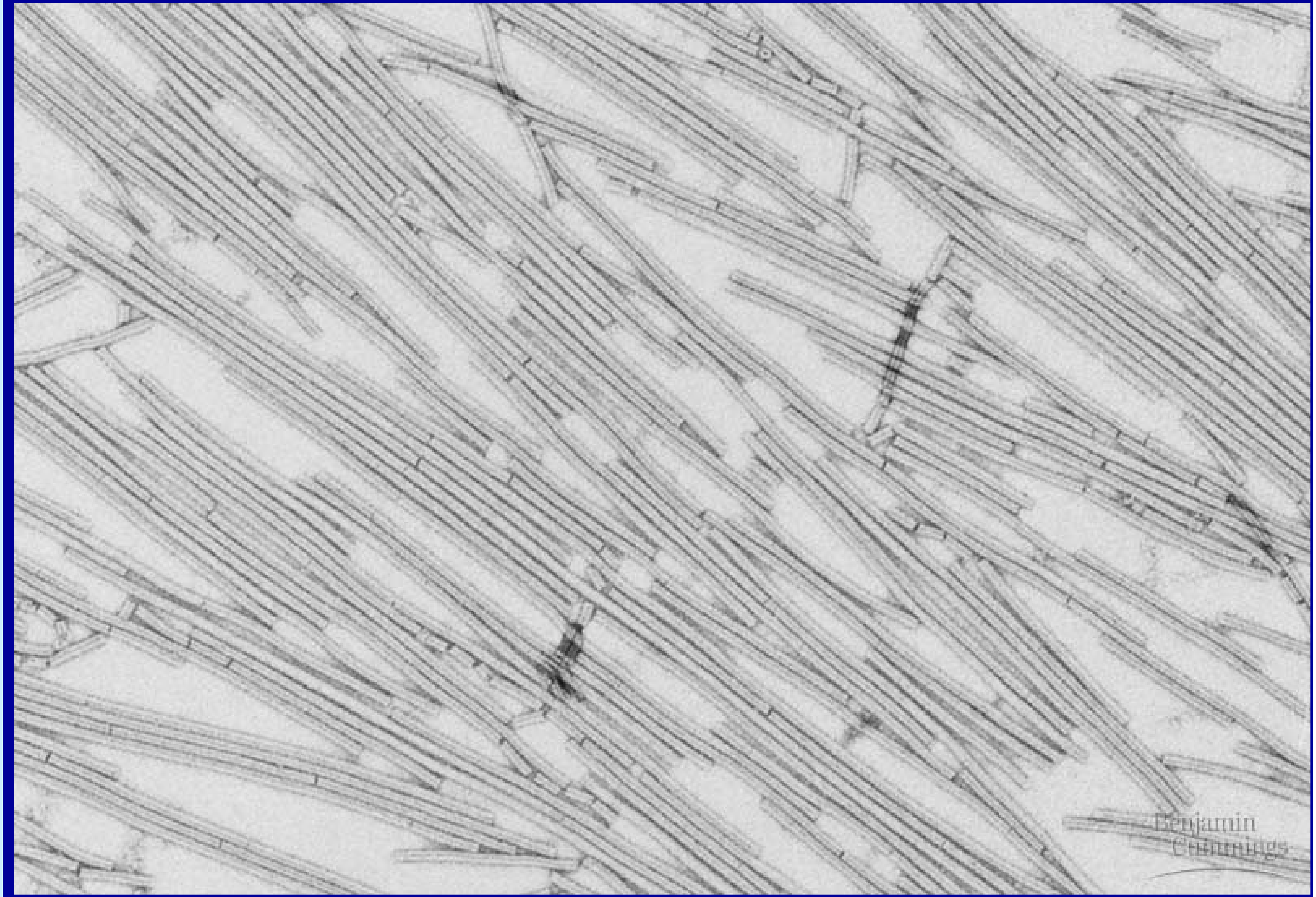
13.1 Common Sizes of Microorganisms

MICROORGANISM	TYPE	TYPICAL SIZE RANGE (μm^3)
Protists	Eukaryote	5,000–50,000
Photosynthetic bacteria	Prokaryote	5–50
Spirochetes	Prokaryote	0.1–2.0
Mycoplasmas	Prokaryote	0.01–0.1
Poxviruses	Virus	0.01
Influenza virus	Virus	0.0005
Poliovirus	Virus	0.00001

B. Viruses: Reproduction and Recombination

- Viruses were discovered as disease-causing agents small enough to pass through a filter that retains bacteria.
- The first to describe viruses was Beijerinck (1898), a Dutch microbial ecologist who showed that they were not killed by alcohol, did not grow on any media, and only reproduced inside a host.
- Scientists couldn’t see them till advent of EM.

Tobacco mosaic virus



B. Viruses: Reproduction and Recombination

- In addition to size and shape, viruses are classified by whether they are naked or enveloped, by their genetic material, and by their host range.
- Some viruses have a lipid membrane derived from host membranes, which determines if they are enveloped or naked.
- They have a nucleic acid genome, that can be DS or SS, RNA or DNA.
- The host range can be at the level of cells, tissues or even species specific.

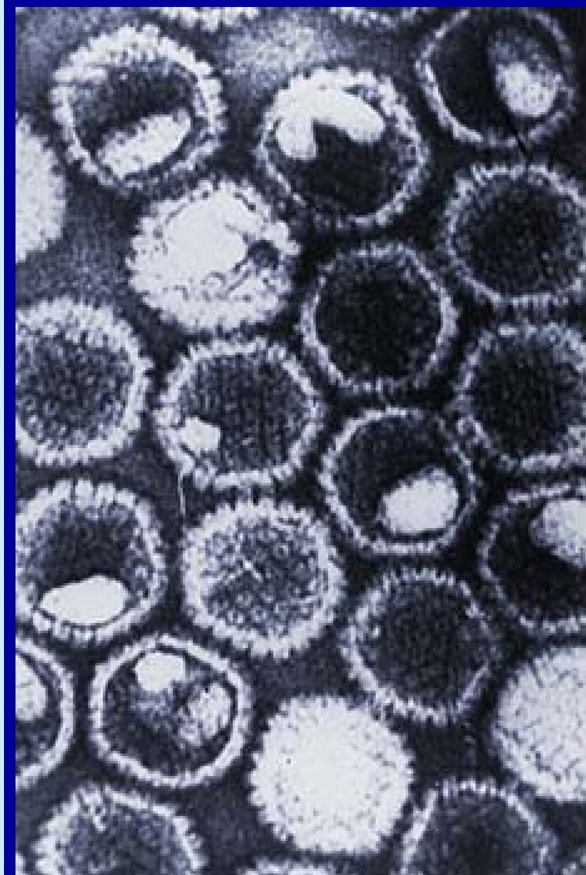
Classes of Animal Viruses, Grouped by Type of Nucleic Acid

Table 18.1 Classes of Animal Viruses, Grouped by Type of Nucleic Acid

Class*	Examples/Diseases
I. dsDNA**	
Papovavirus	Papilloma (human warts, cervical cancer); polyoma (tumors in certain animals)
Adenovirus	Respiratory diseases; some cause tumors in certain animals
Herpesvirus	Herpes simplex I (cold sores), herpes simplex II (genital sores); varicella zoster (chicken pox, shingles); Epstein-Barr virus (mononucleosis, Burkitt's lymphoma)
Poxvirus	Smallpox; vaccinia, cowpox
II. ssDNA	
Parvovirus	Roseola; most parvoviruses depend on co-infection with adenoviruses for growth
III. dsRNA	
Reovirus	Diarrhea; mild respiratory diseases
IV. ssRNA that can serve as mRNA	
Picornavirus	Poliovirus; rhinovirus (common cold); enteric (intestinal) viruses
Togavirus	Rubella virus; yellow fever virus; encephalitis viruses
V. ssRNA that is a template for mRNA	
Rhabdovirus	Rabies
Paramyxovirus	Measles; mumps
Orthomyxovirus	Influenza viruses
VI. ssRNA that is a template for DNA synthesis	
Retrovirus	RNA tumor viruses (e.g., leukemia viruses); HIV (AIDS virus)

*The subclasses within each class differ mainly in capsid structure and in the presence or absence of a membranous envelope.
**ds = double-stranded; ss = single-stranded.

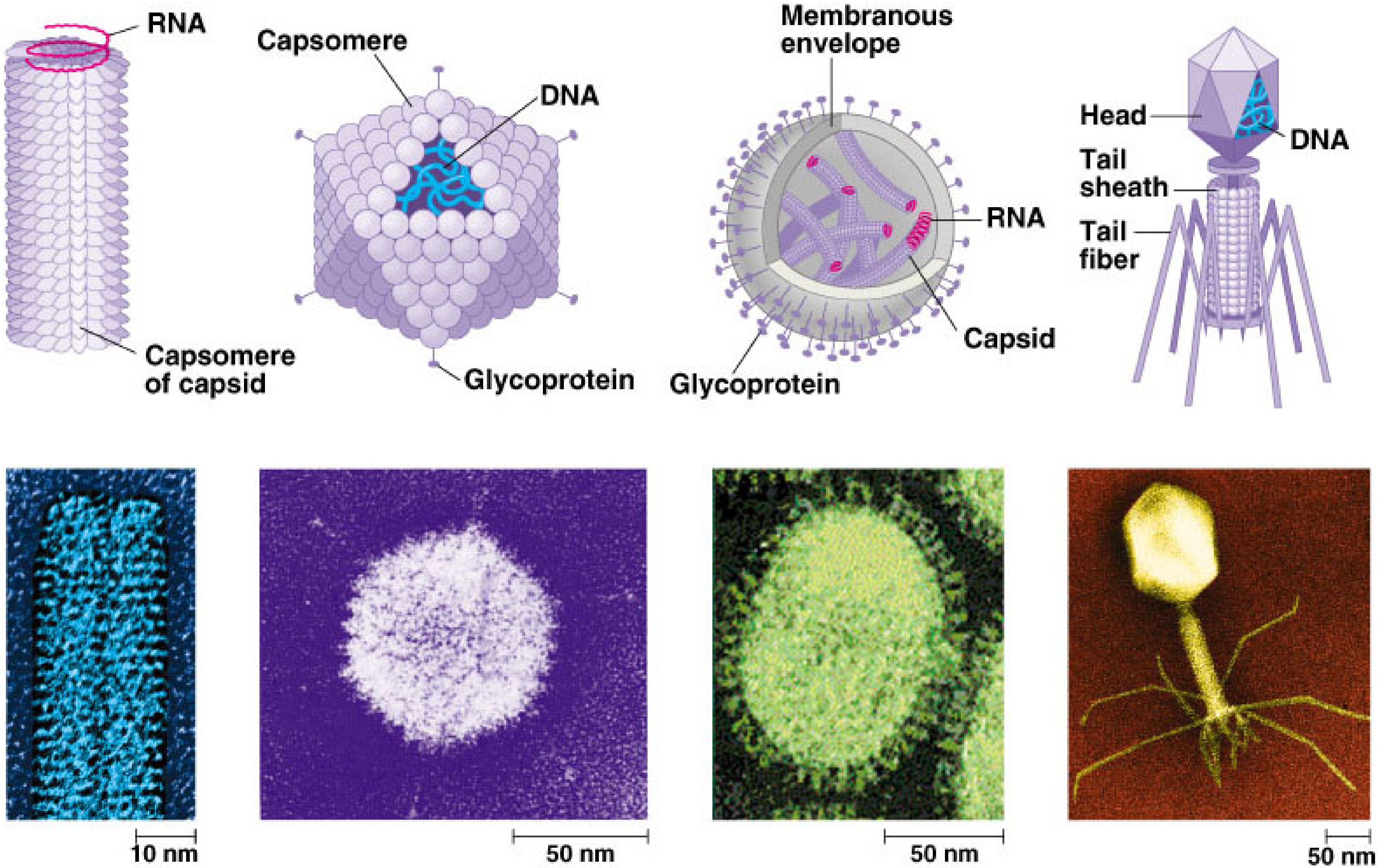
Herpes Simplex I



B. Viruses: Reproduction and Recombination

- Viruses are obligate intracellular parasites, needing the biochemical machinery of living cells to reproduce.
- Their genome is relatively small and generally codes for just a few proteins, including a protein capsid.

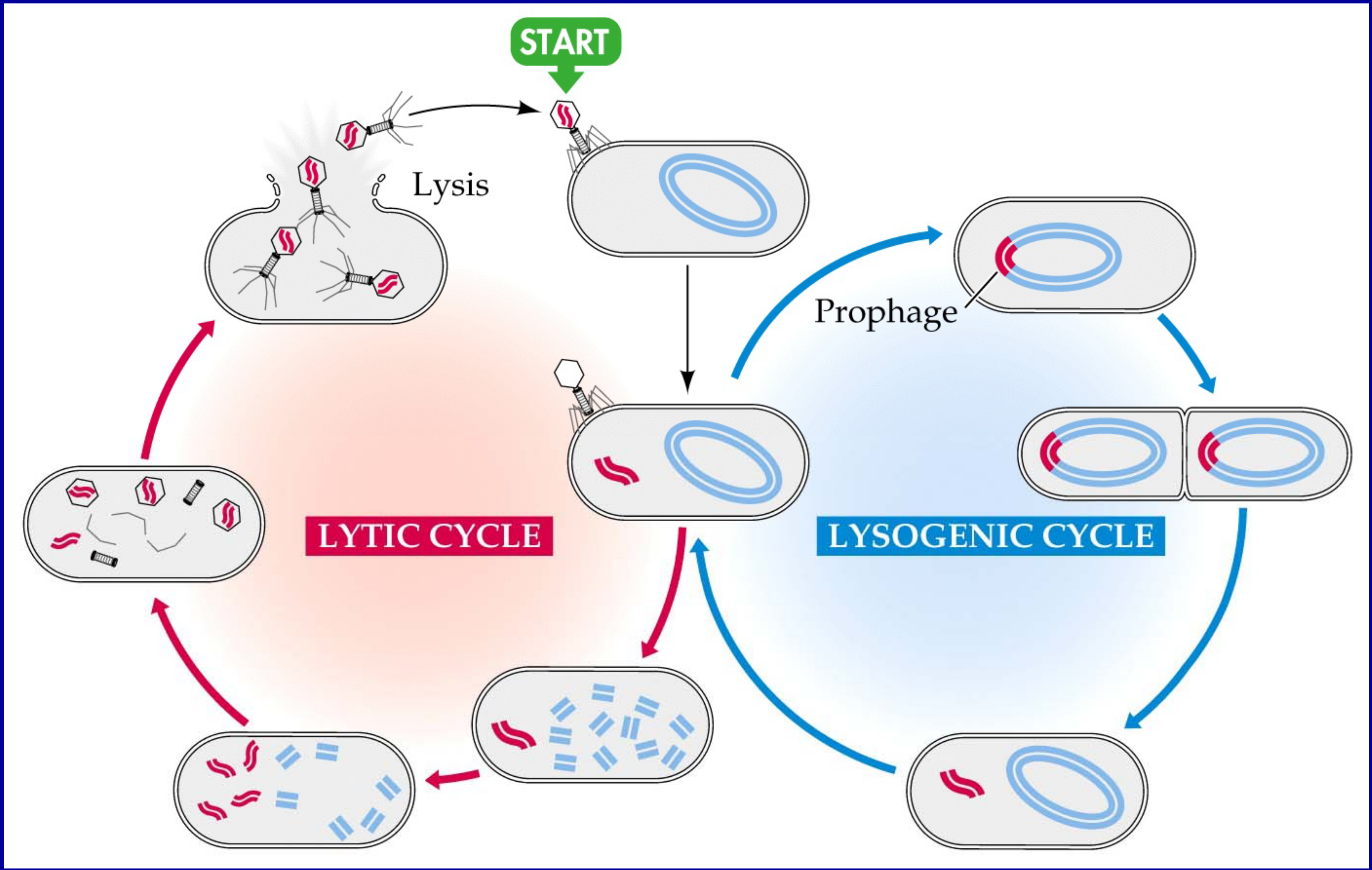
Viral structure



(a) Tobacco mosaic virus (b) Adenoviruses (c) Influenza viruses (d) Bacteriophage T4

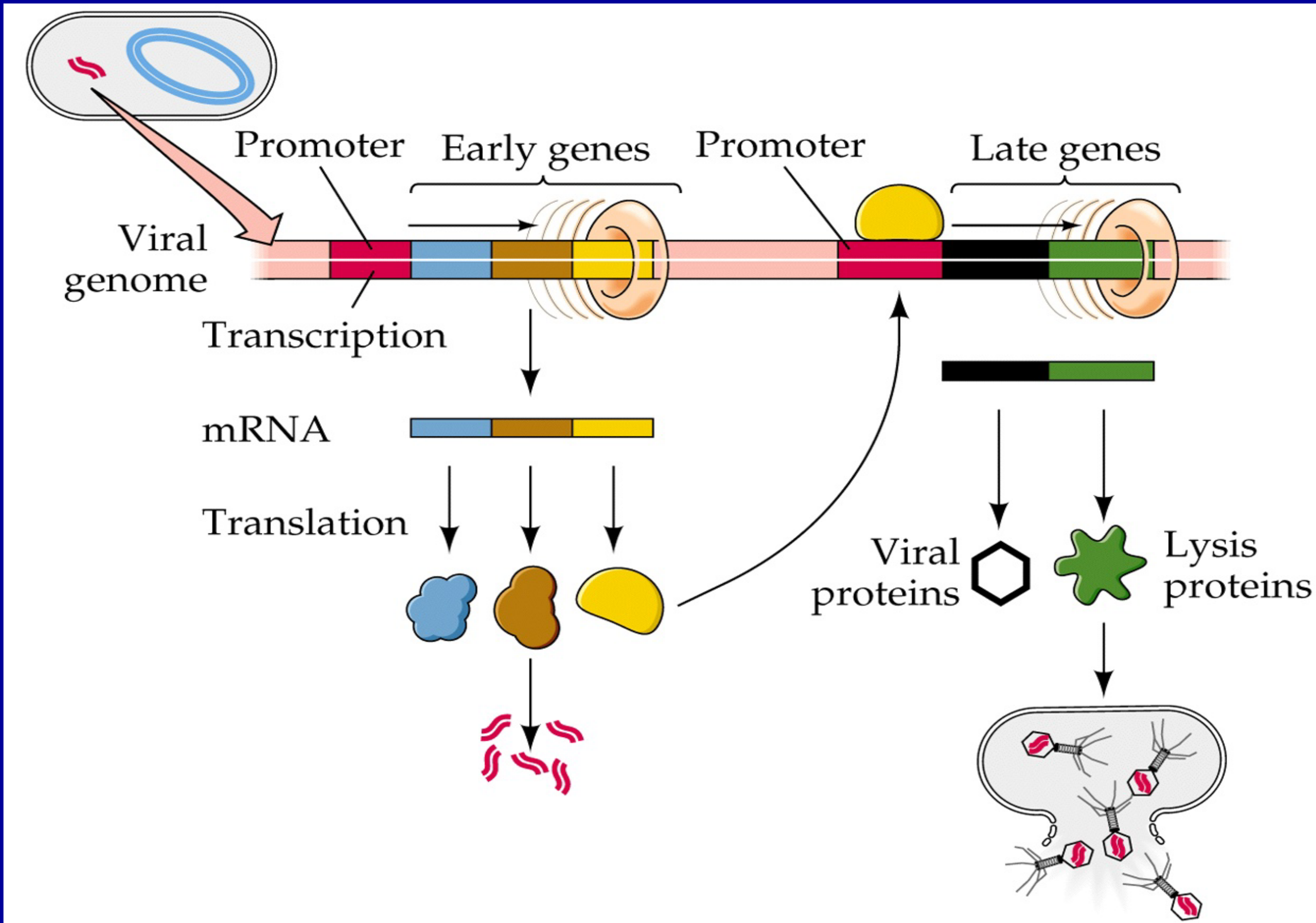
B. Viruses: Reproduction and Recombination

- Bacteriophages are viruses that infect bacteria. In the lytic cycle, the host cell breaks open, releasing many new phage particles. Some phages can also undergo a lysogenic cycle: their DNA is inserted into the host chromosome, where it replicates for generations. When conditions are appropriate, the lysogenic DNA exits the host chromosome and enters a lytic cycle.


START
Lysis
Prophage
LYTIC CYCLE
LYSOGENIC CYCLE

B. Viruses: Reproduction and Recombination

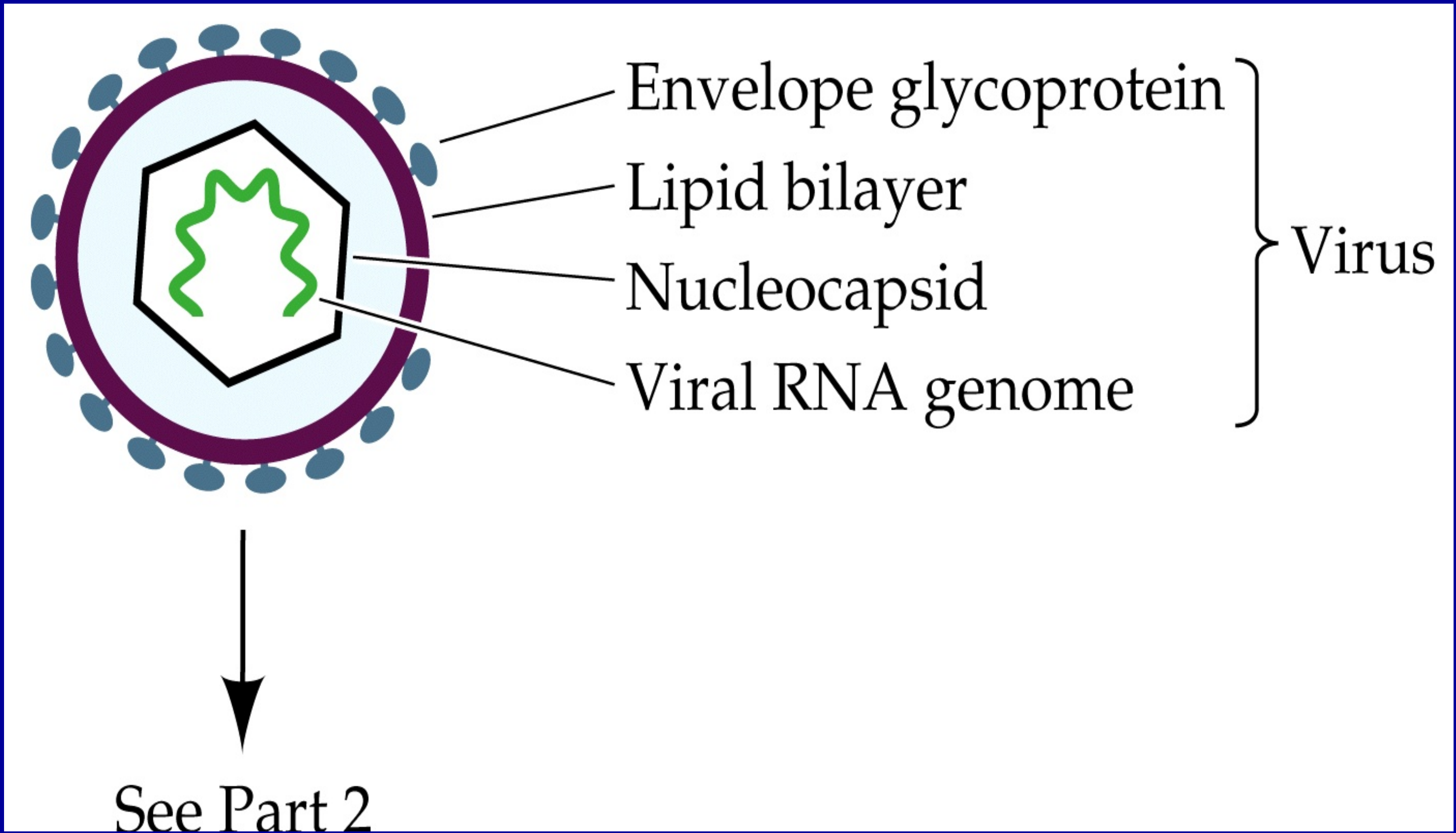
- Some viruses have promoters for host RNA polymerase, which they use to transcribe their own genes.
- They can shut down host gene transcription and stimulate viral genome reproduction.

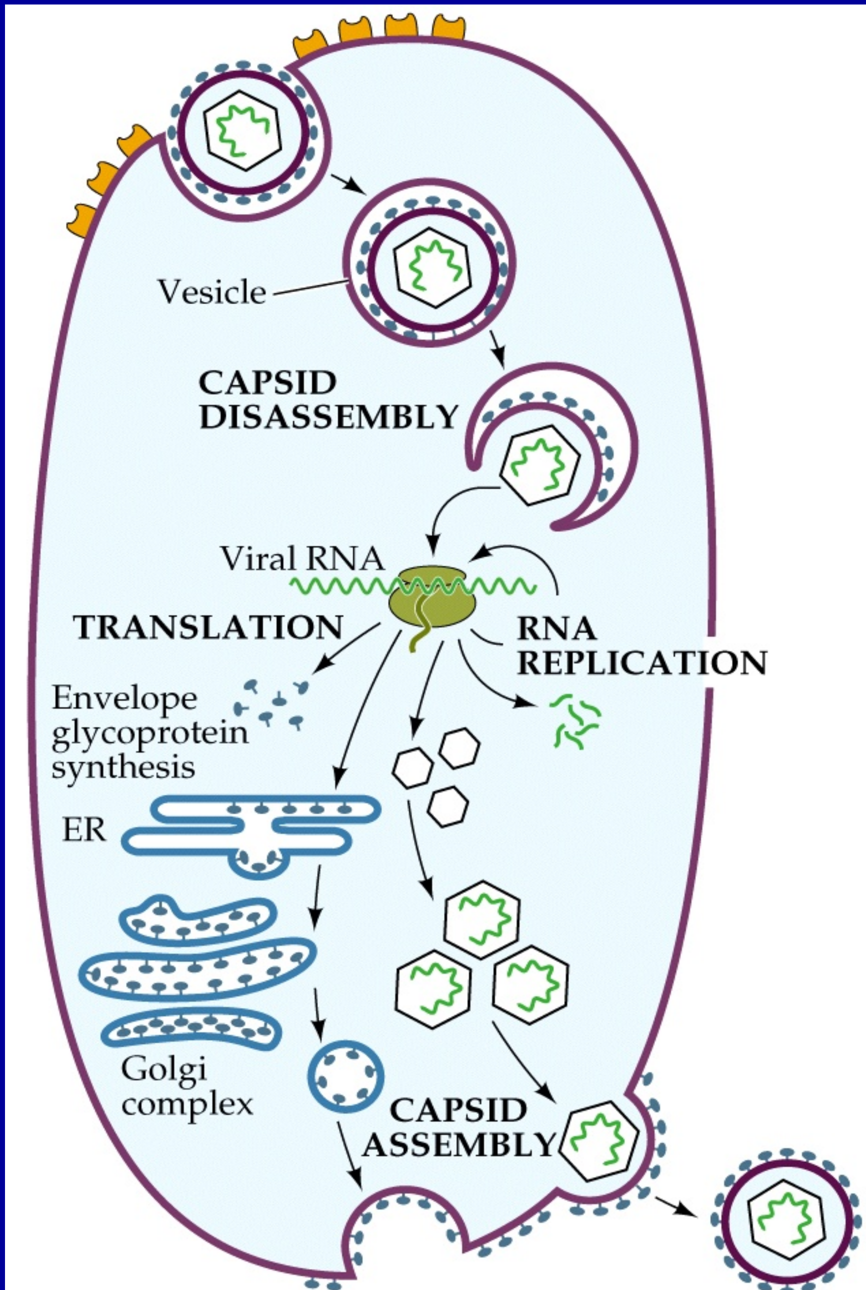

Promoter
Early genes
Promoter
Late genes
Viral genome
Transcription
mRNA
Translation
Viral proteins
Lysis proteins

B. Viruses: Reproduction and Recombination

- Most RNA and DNA viruses that infect animals cause diseases. Some animal viruses are surrounded by membranes derived from host plasma membrane.
- Retroviruses have RNA genomes that they reproduce through a DNA intermediate. Others use their RNA as mRNA directly or as template for mRNA to code for enzymes and replicate their genomes without DNA.

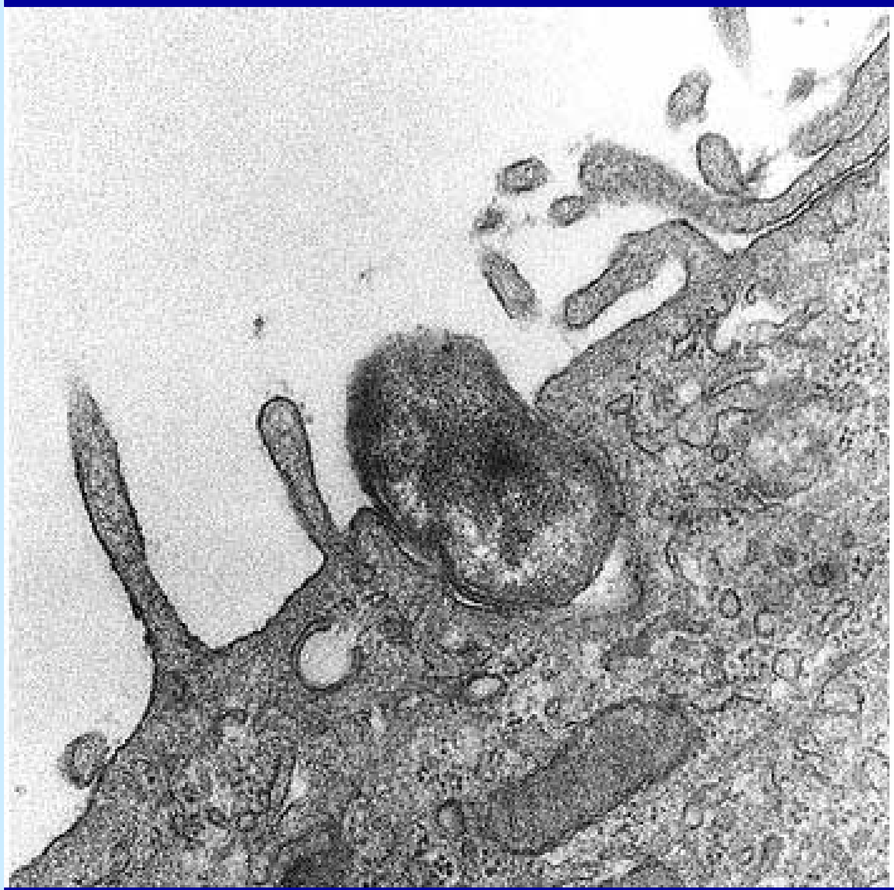
The Reproductive Cycle of the Influenza Virus

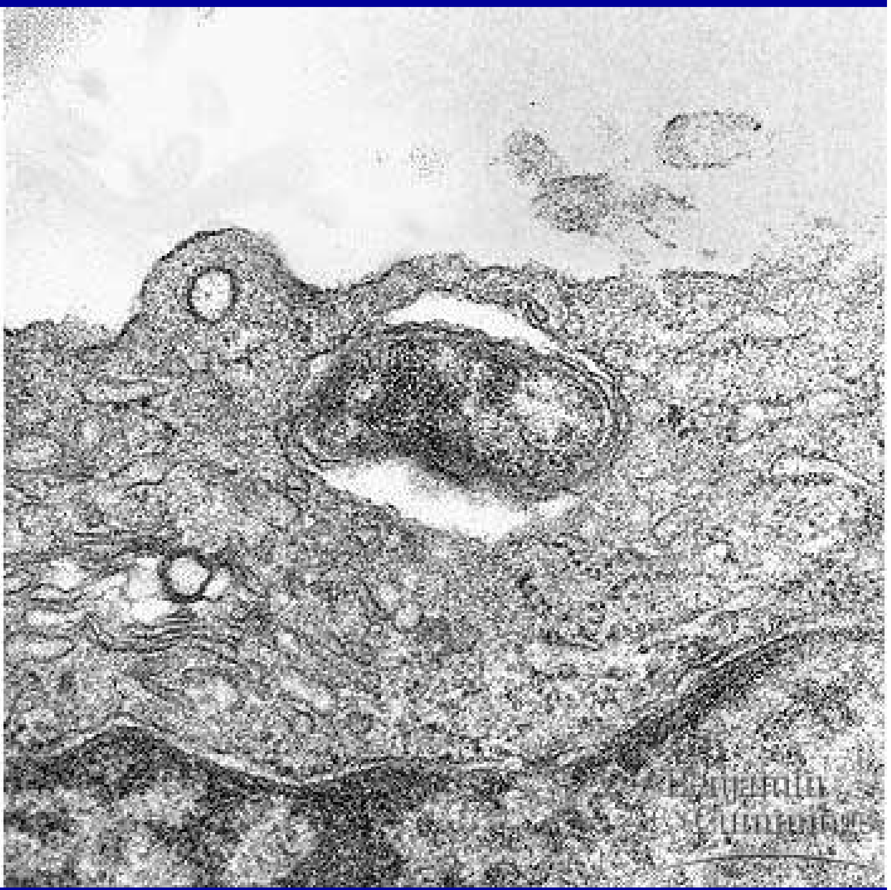




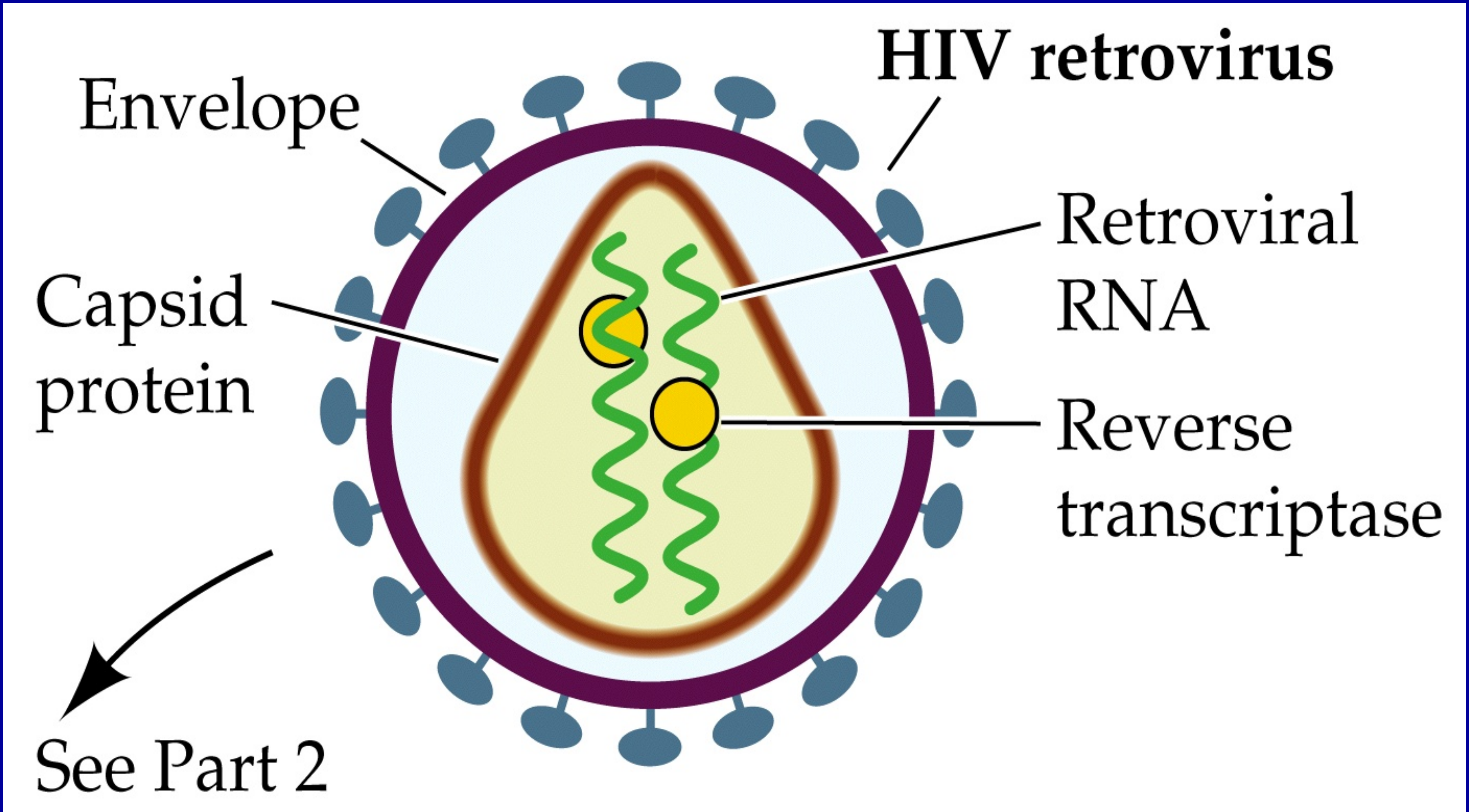
Vesicle
CAPSID
DISASSEMBLY
Viral RNA
TRANSLATION
RNA
REPLICATION
Envelope
glycoprotein
synthesis
ER
Golgi
complex
CAPSID
ASSEMBLY

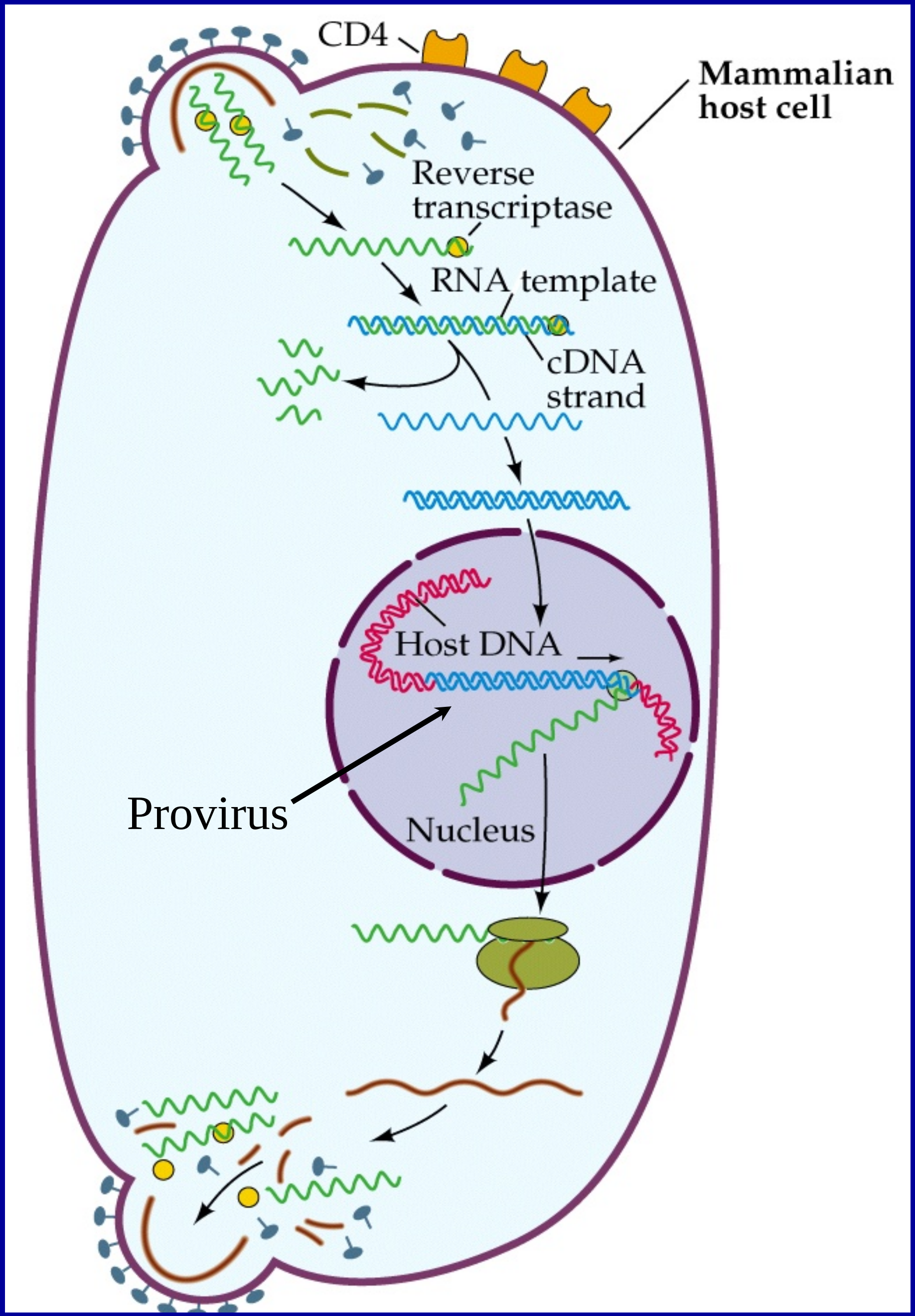
HIV infection





The Reproductive Cycle of the HIV Virus

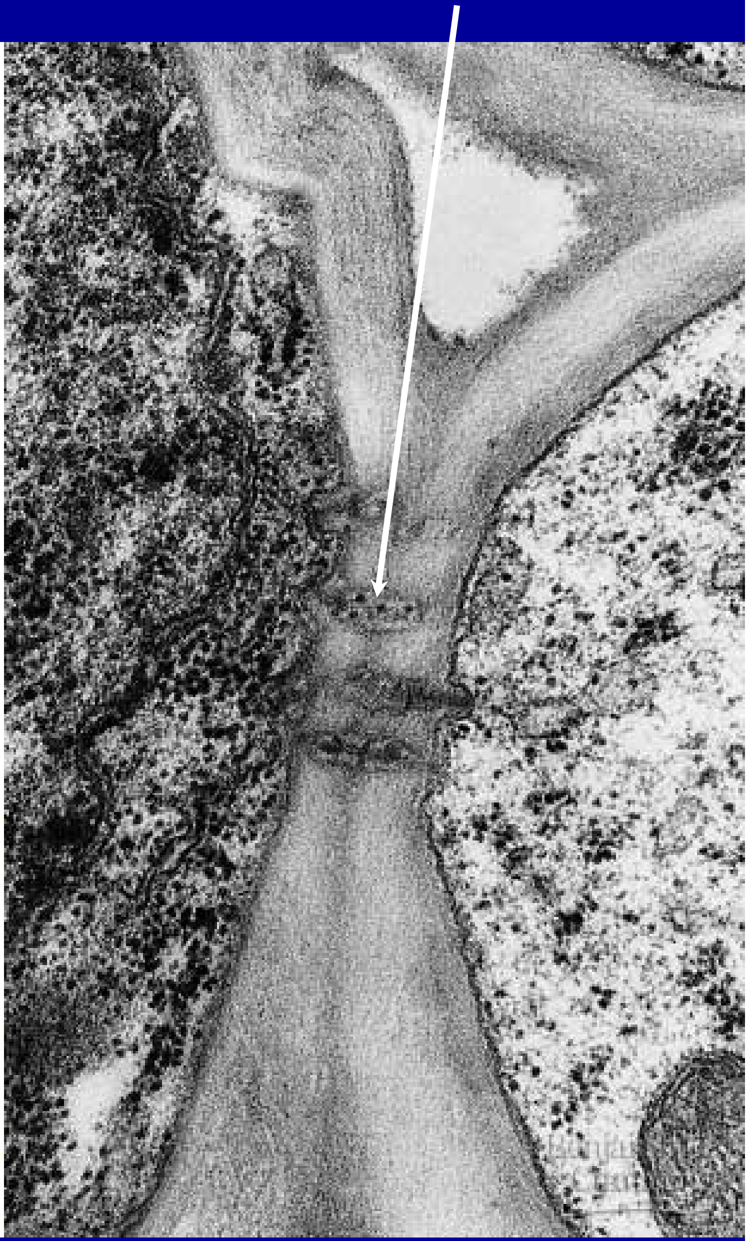



CD4
Mammalian host cell
Reverse transcriptase
RNA template
cDNA strand
Host DNA
Provirus
Nucleus

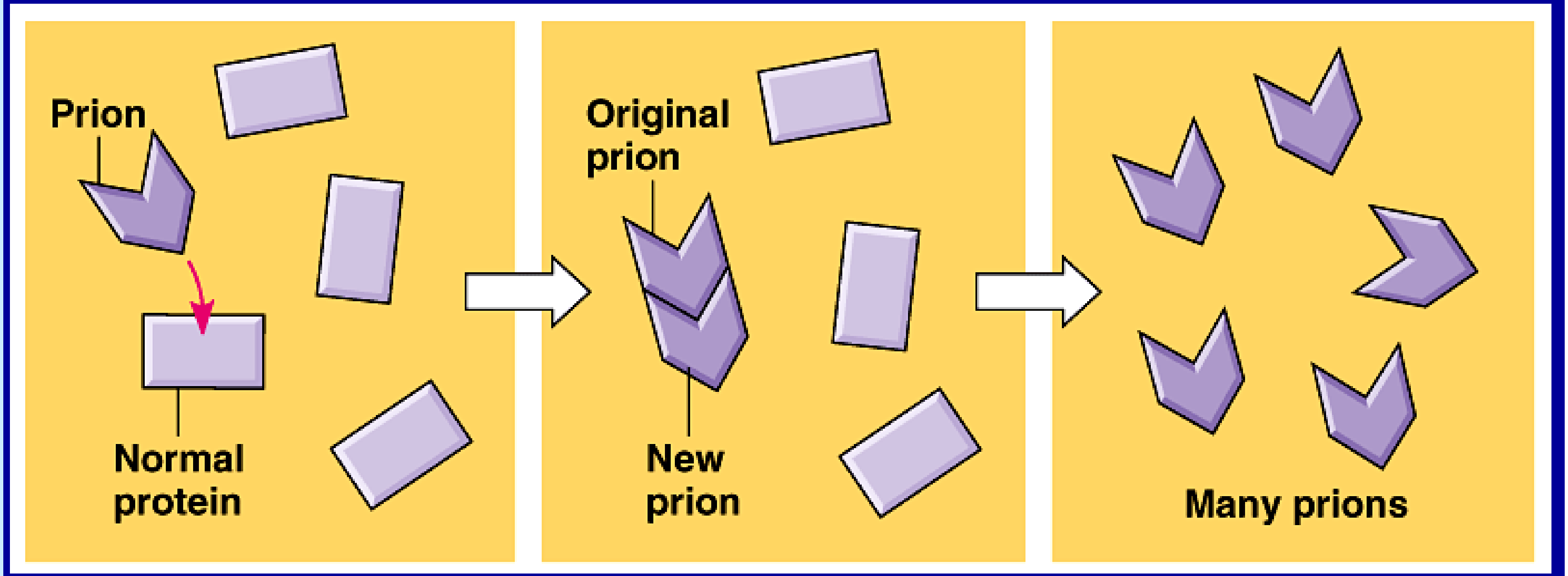
B. Viruses: Reproduction and Recombination

- Many plant viruses are spread by other organisms, such as insects.
- Viroids are made only of RNA molecules and infect plants. They are replicated by the plant’s enzymes.
- Prions are infectious chaperones that cause degenerative brain diseases.

Mosaic Viral infection of plants



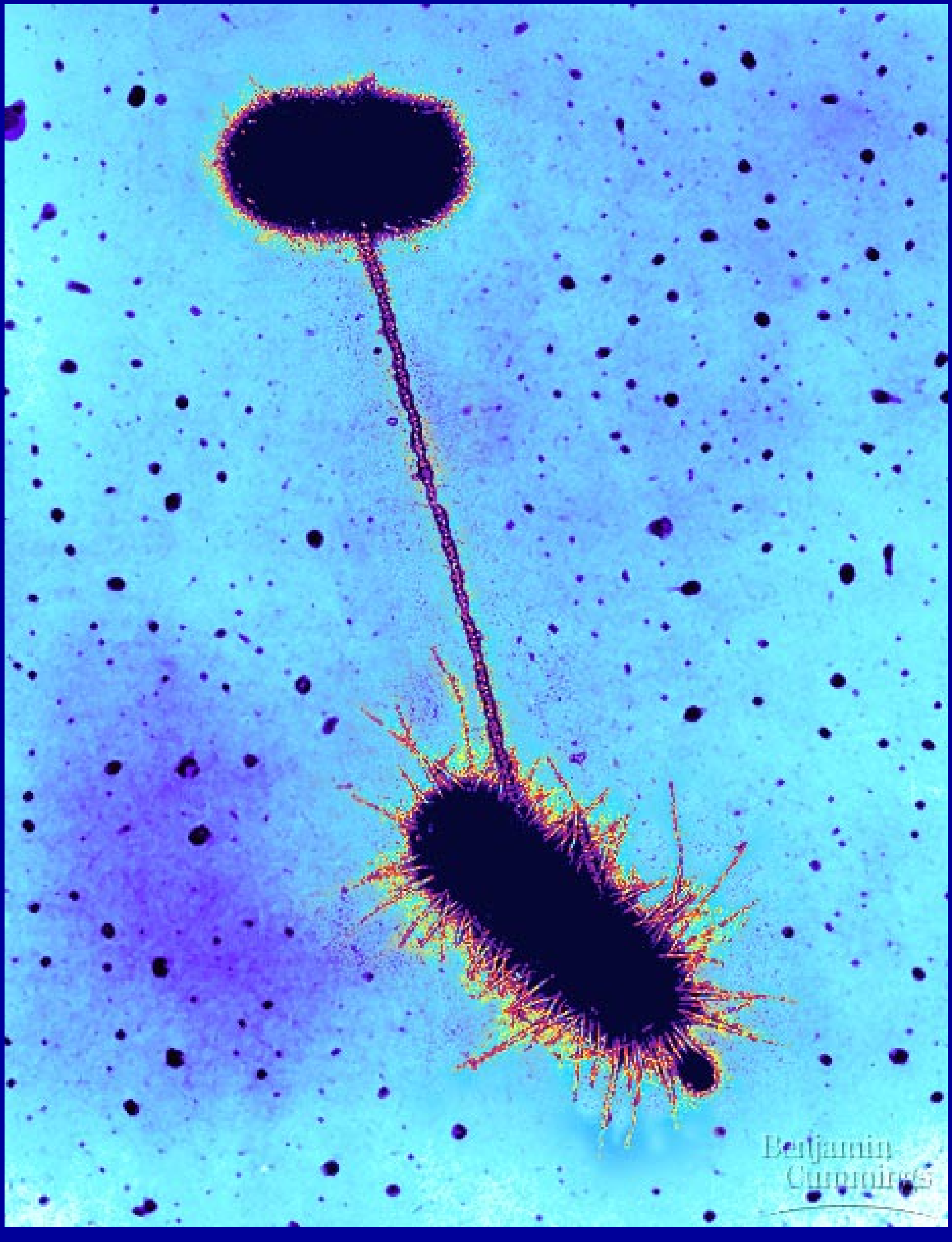
A hypothesis to explain how prions propagate



C. Prokaryotes: Reproduction, Mutation, and Recombination

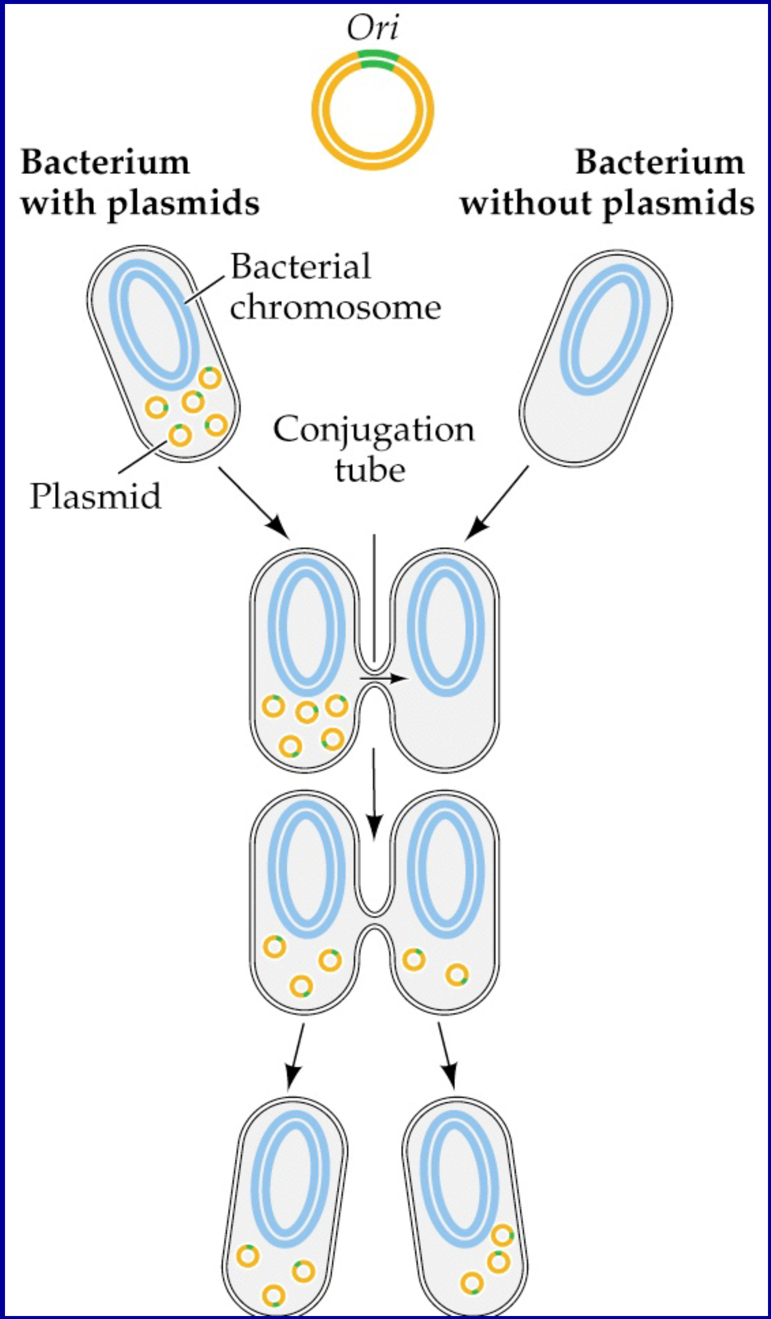
- A bacterium can transfer its genes to another bacterium by conjugation, transformation, or transduction.
- Unlike sexual reproduction, these processes are **unidirectional** and transfer only a few genes via recombination events.
- In conjugation, a bacterium attaches to another bacterium and passes a partial copy of its DNA to the adjacent cell via a plasmid.

Bacterial Conjugation

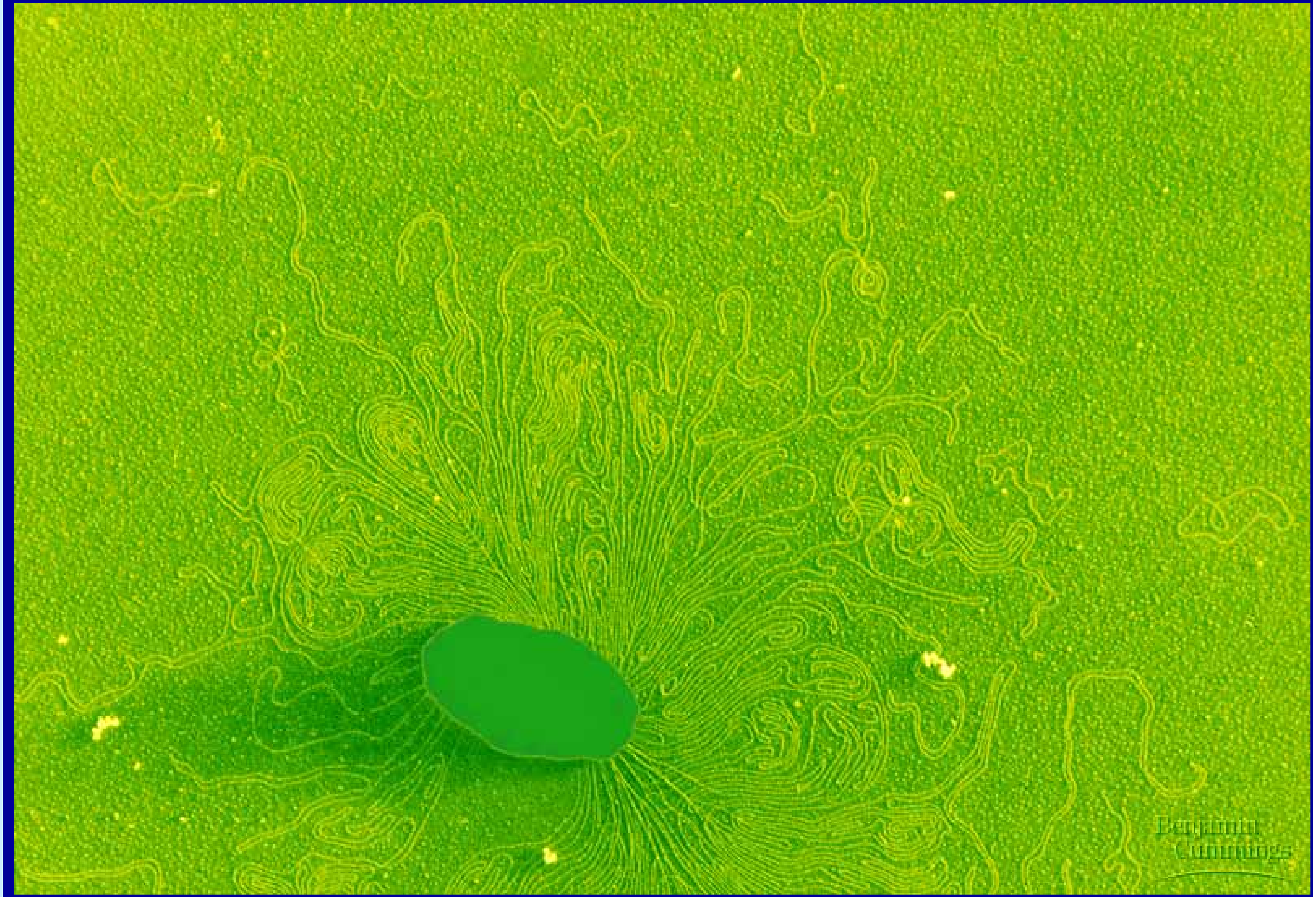


C. Prokaryotes: Reproduction, Mutation, and Recombination

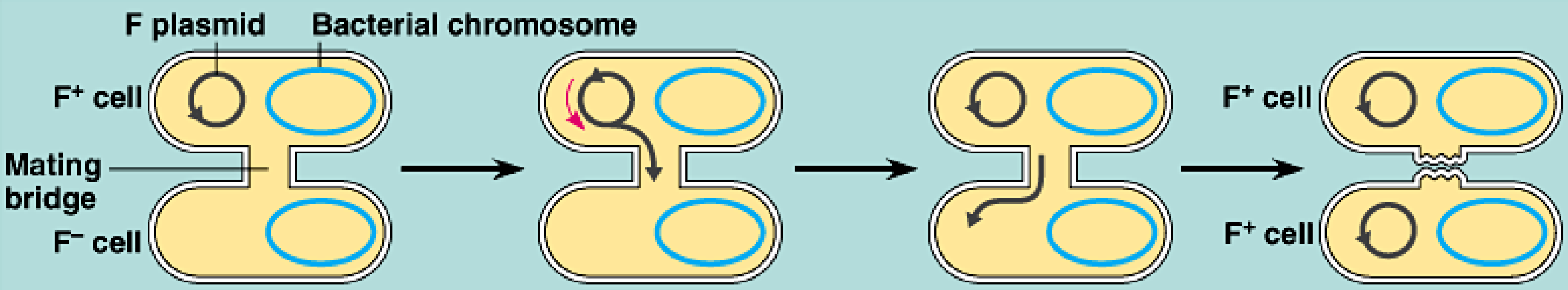
- Plasmids are small bacterial chromosomes independent of the main chromosome.
- F plasmids carry genes allowing for conjugation, F is for fertility.
- R plasmids carry genes for antibiotic resistance, are a serious public health threat, R is for resistance.


Ori
Bacterium with plasmids
Bacterium without plasmids
Bacterial chromosome
Conjugation tube
Plasmid

Bacterium releasing DNA with plasmids

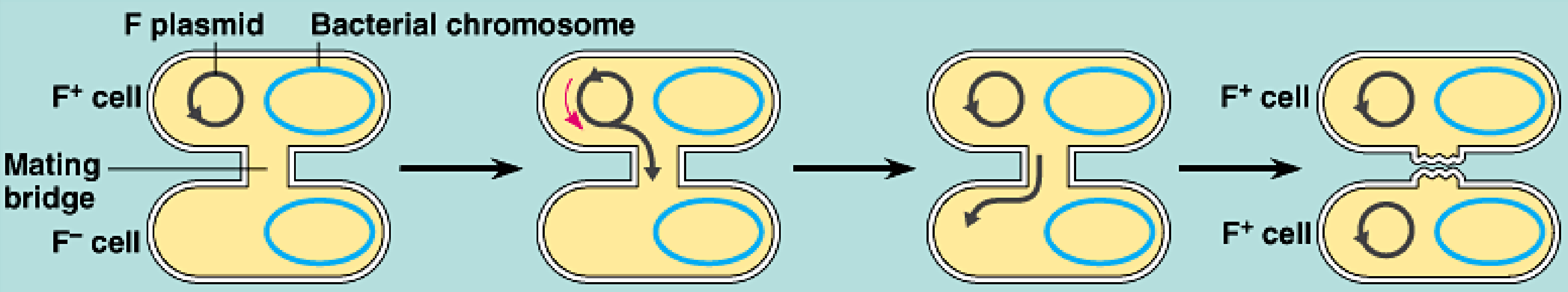


Conjugation and recombination in *E. coli* (Layer 1)

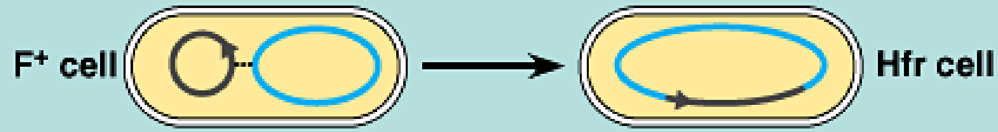


(a) Conjugation between an F⁺ (male) and an F⁻ (female) bacterium

Conjugation and recombination in *E. coli* (Layer 2)

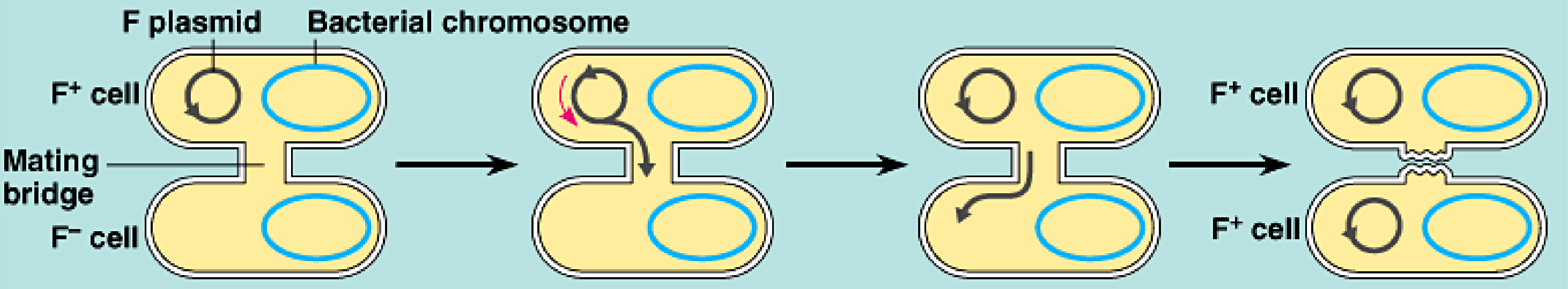


(a) Conjugation between an F⁺ (male) and an F⁻ (female) bacterium

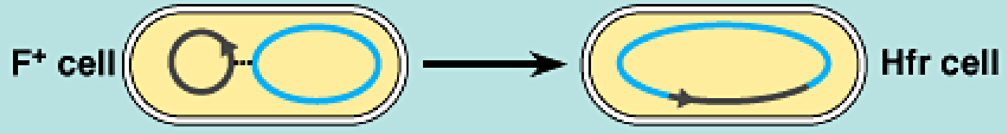


(b) Conversion of an F⁺ male into an Hfr male by integration of the F plasmid into the chromosome

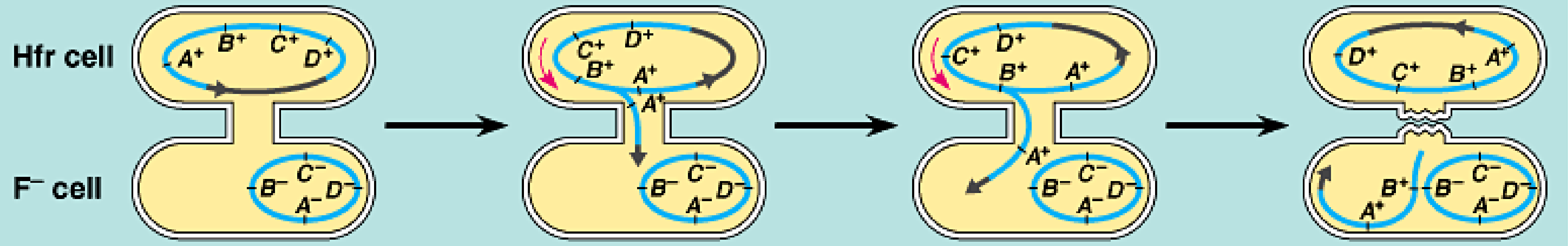
Conjugation and recombination in *E. coli* (Layer 3)



(a) Conjugation between an F⁺ (male) and an F⁻ (female) bacterium

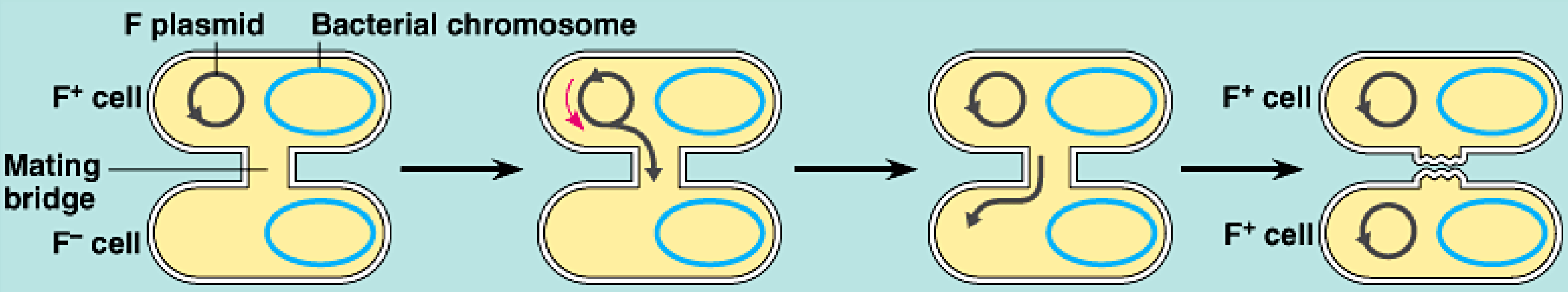


(b) Conversion of an F⁺ male into an Hfr male by integration of the F plasmid into the chromosome

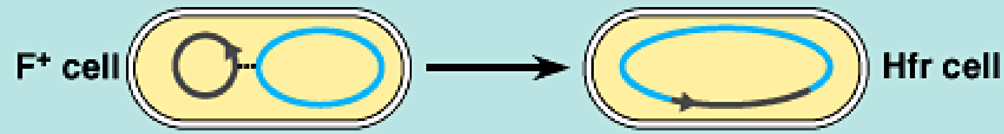


(c) Conjugation between an Hfr and an F⁻ bacterium

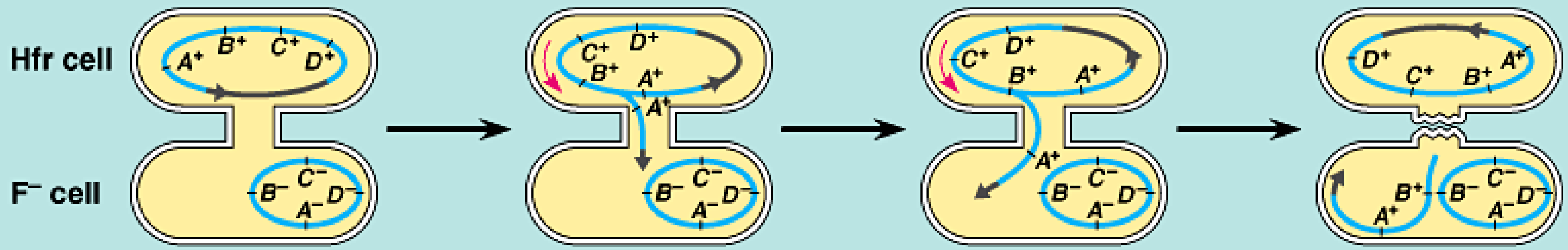
Conjugation and recombination in *E. coli* (Layer 4)



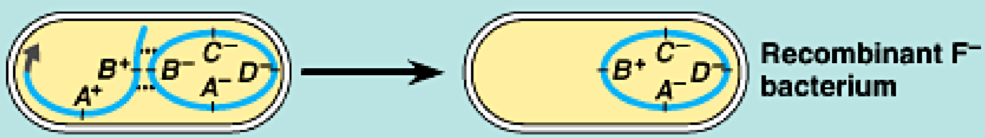
(a) Conjugation between an F+ (male) and an F− (female) bacterium



(b) Conversion of an F+ male into an Hfr male by integration of the F plasmid into the chromosome



(c) Conjugation between an Hfr and an F− bacterium

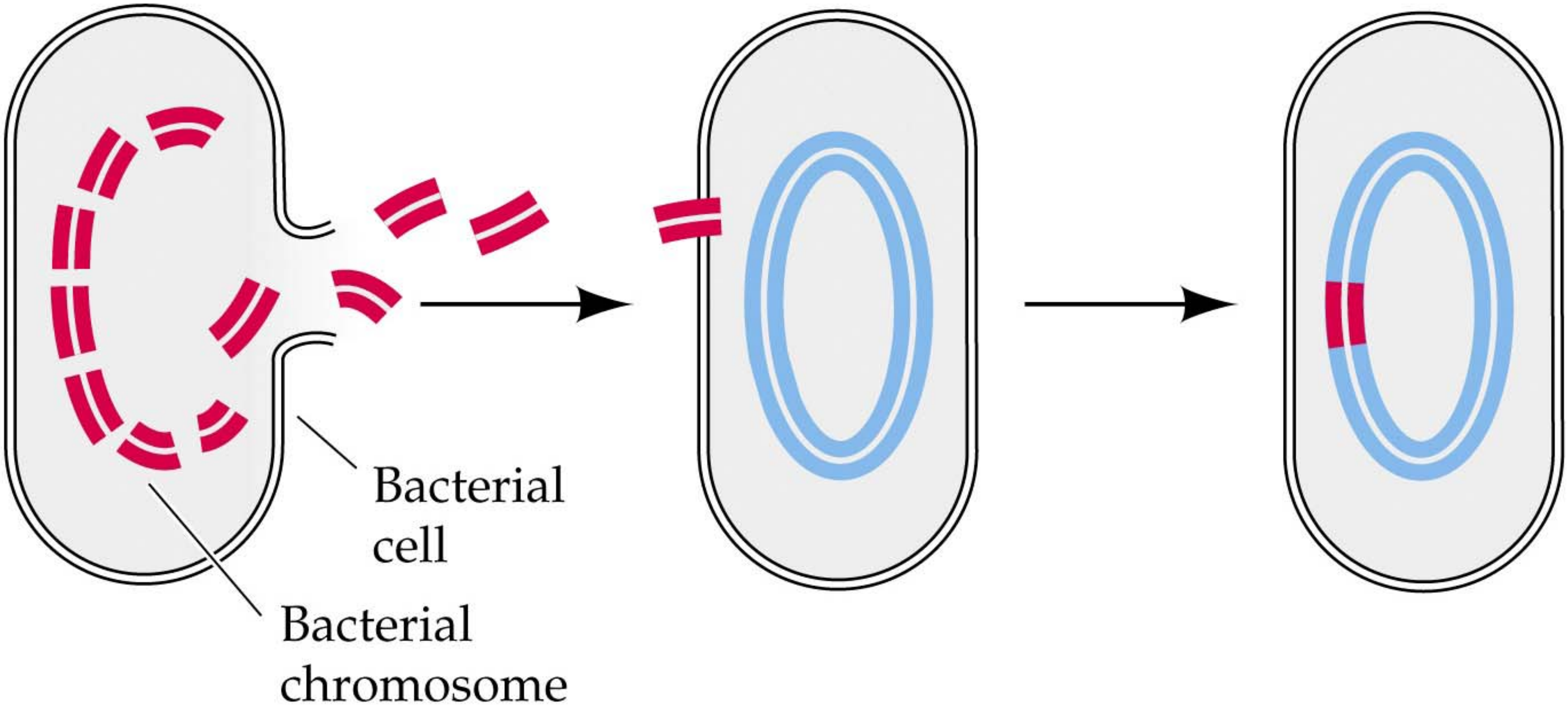


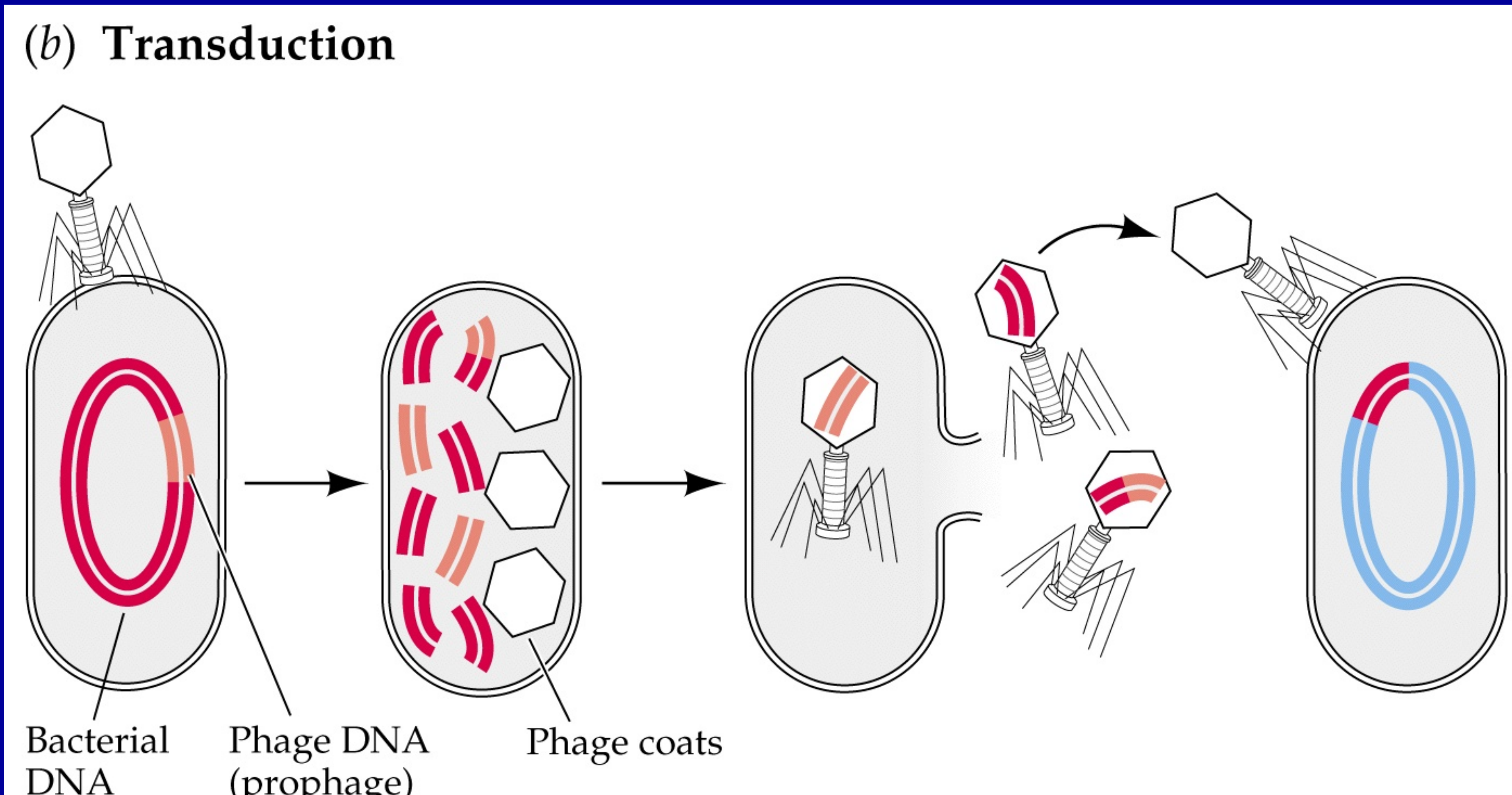
(d) Recombination between the Hfr chromosome fragment and the F− chromosome

C. Prokaryotes: Reproduction, Mutation, and Recombination

- In transformation, genes are transferred between cells when fragments of bacterial DNA are taken up by a cell from the medium.
- In transduction, phage capsids carry bacterial DNA from one bacterium to another.
- These fragments may recombine with the host chromosome, permanently adding new genes.

(*a*) **Transformation**



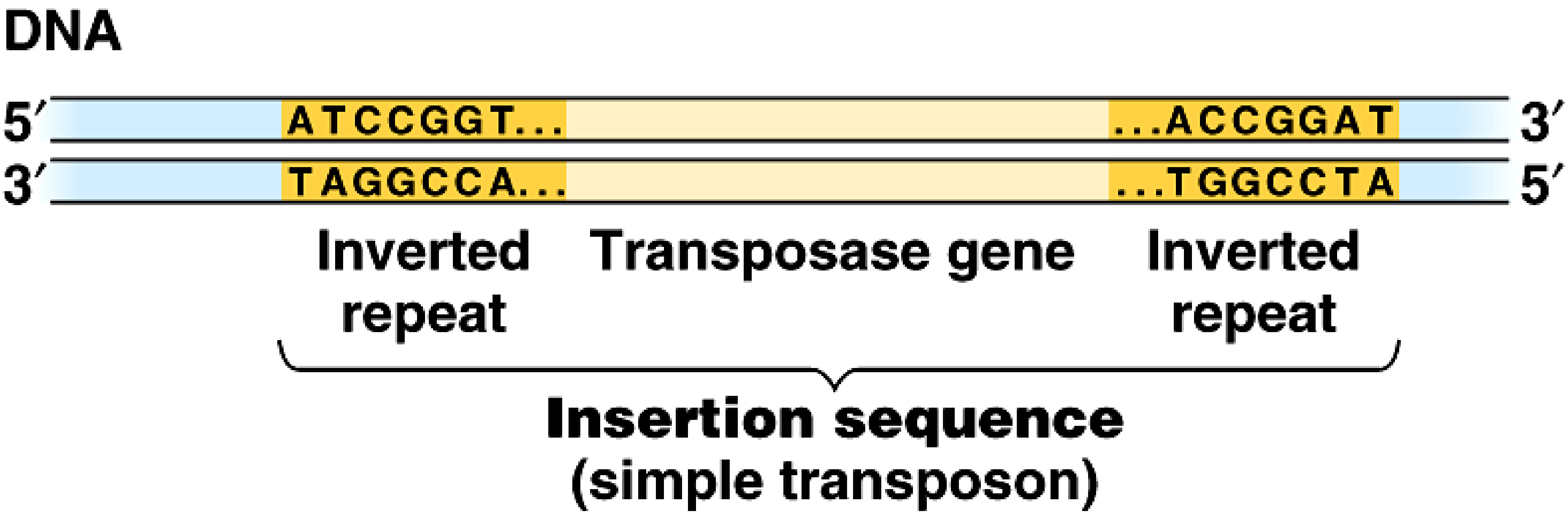


(b) Transduction
Bacterial DNA
Phage DNA (prophage)
Phage coats

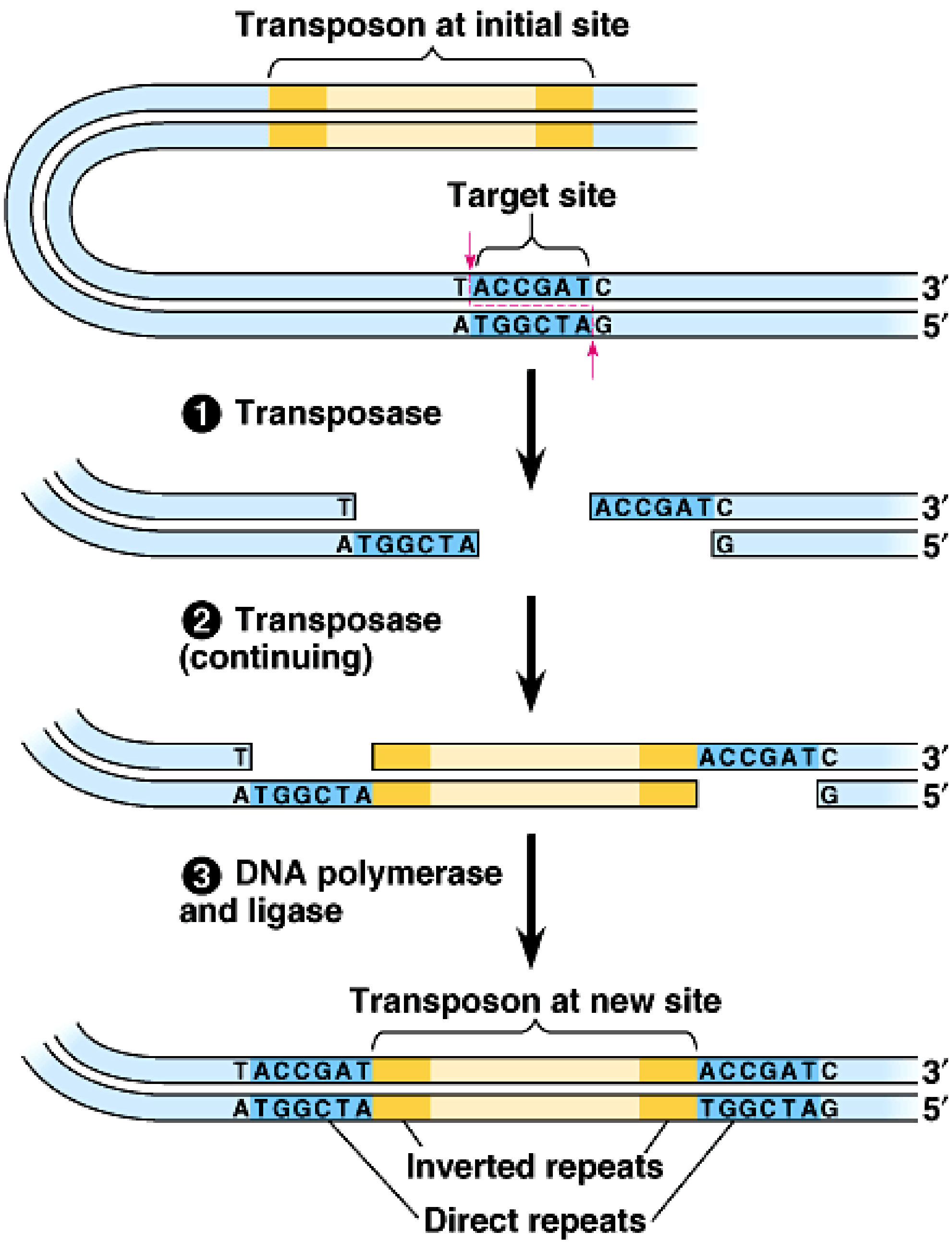
C. Prokaryotes: Reproduction, Mutation, and Recombination

- Transposable elements are movable stretches of DNA that can jump from place to place on the bacterial chromosome by actually moving or by making a new copy, inserted at a new location.

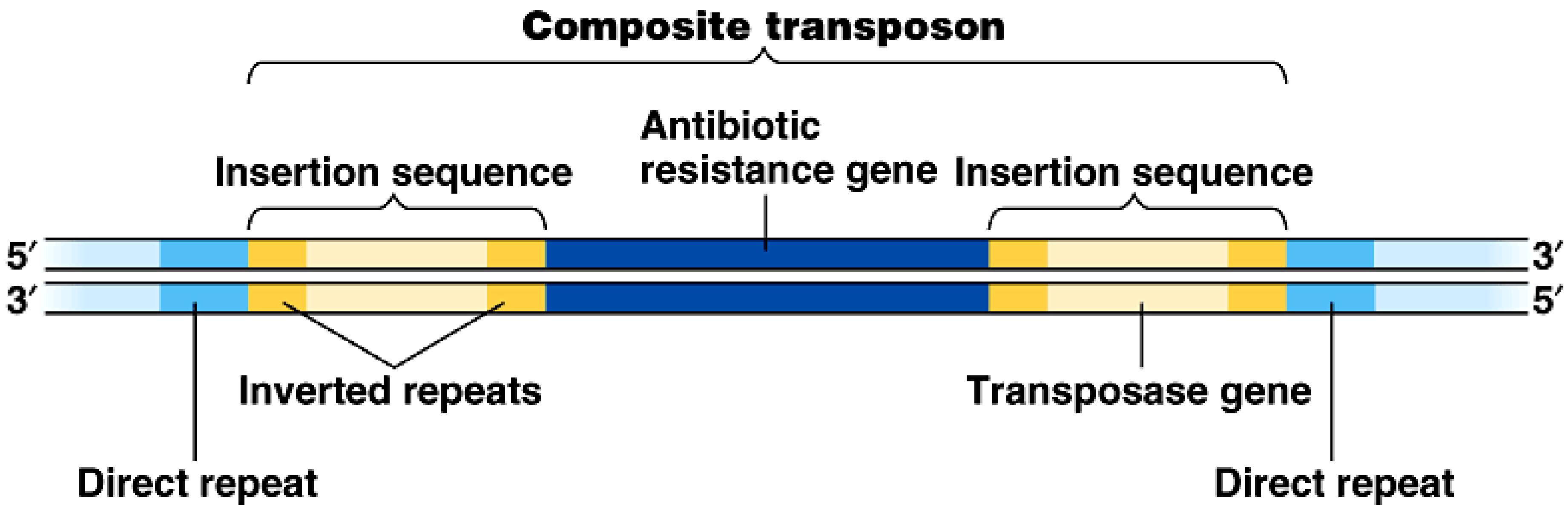
Insertion sequences, the simplest transposons



Insertion of a transposon and creation of direct repeats



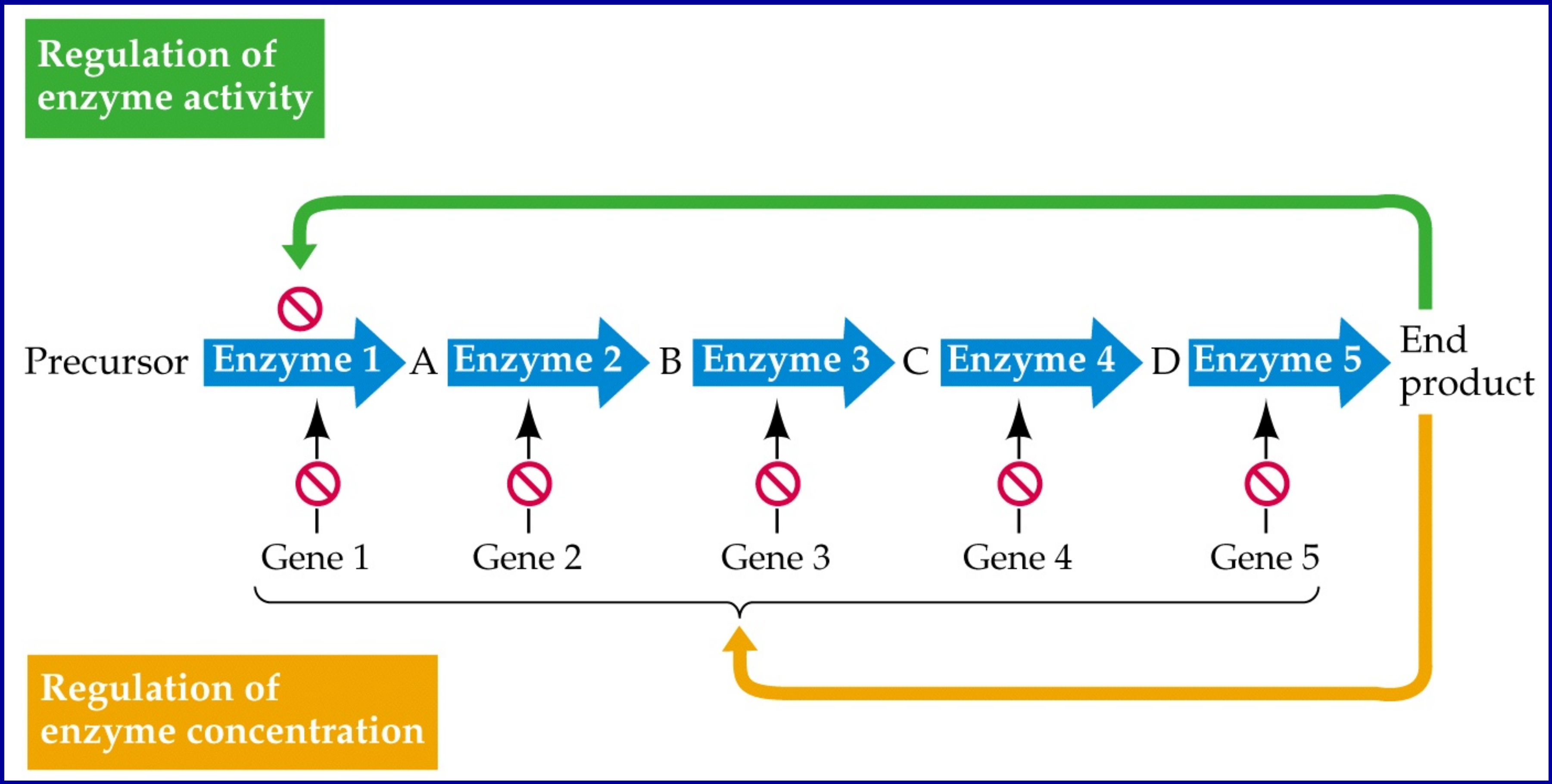
Anatomy of a composite transposon



D. Regulation of Gene Expression in Prokaryotes

- In prokaryotes, the expression of some genes is regulated to save energy; their products are made only as needed.
- Other genes, constitutive genes, whose products are essential at all times, are constantly expressed.
- A compound that stimulates the synthesis of an enzyme needed to process it is called an inducer.

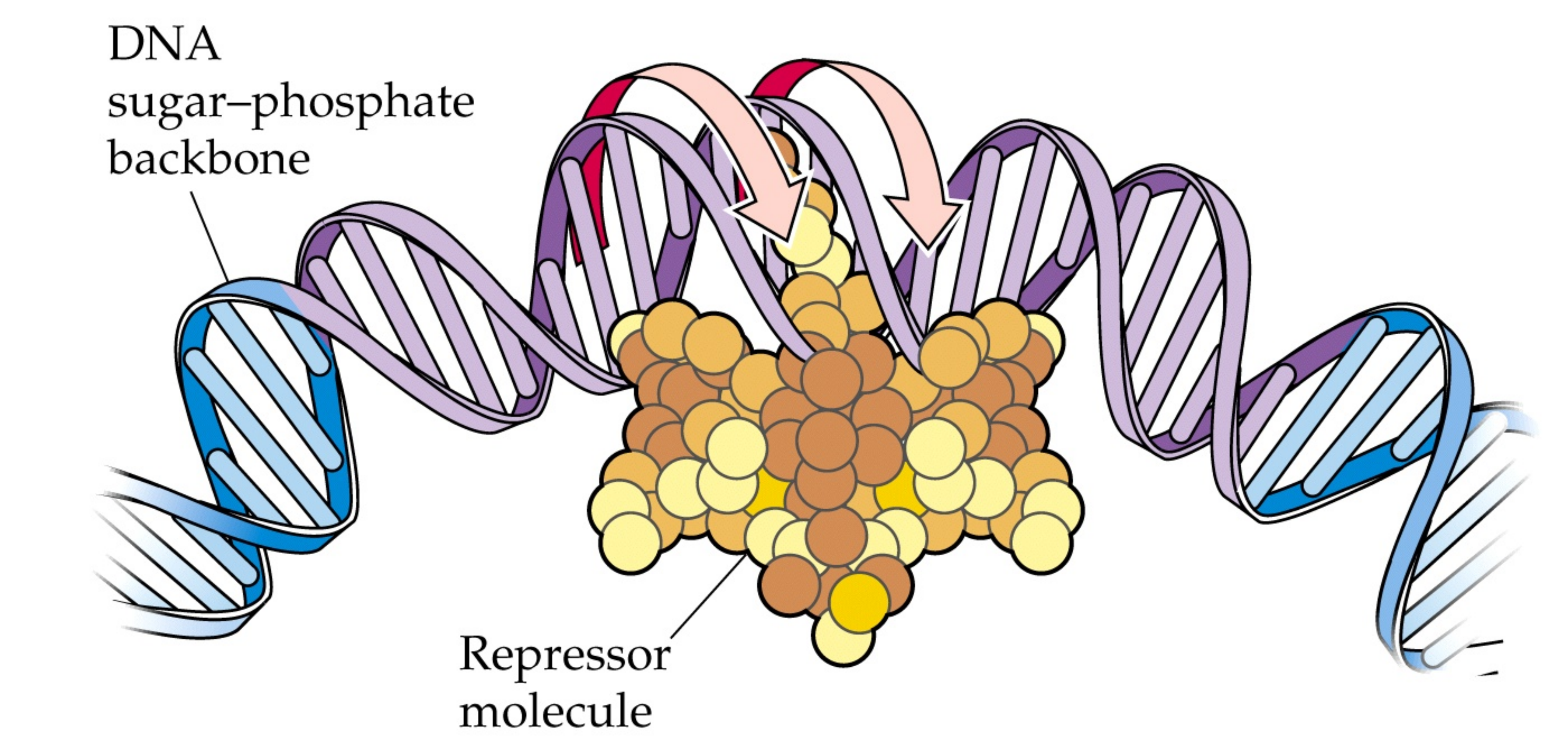
Two Ways to Regulate a Metabolic Pathway



D. Regulation of Gene Expression in Prokaryotes

- An operon consists of a promoter, an operator, and structural genes. Promoters and operators do not code for proteins, but serve as binding sites for regulatory proteins.
- When a repressor protein binds to the operator, transcription of the structural genes is inhibited.

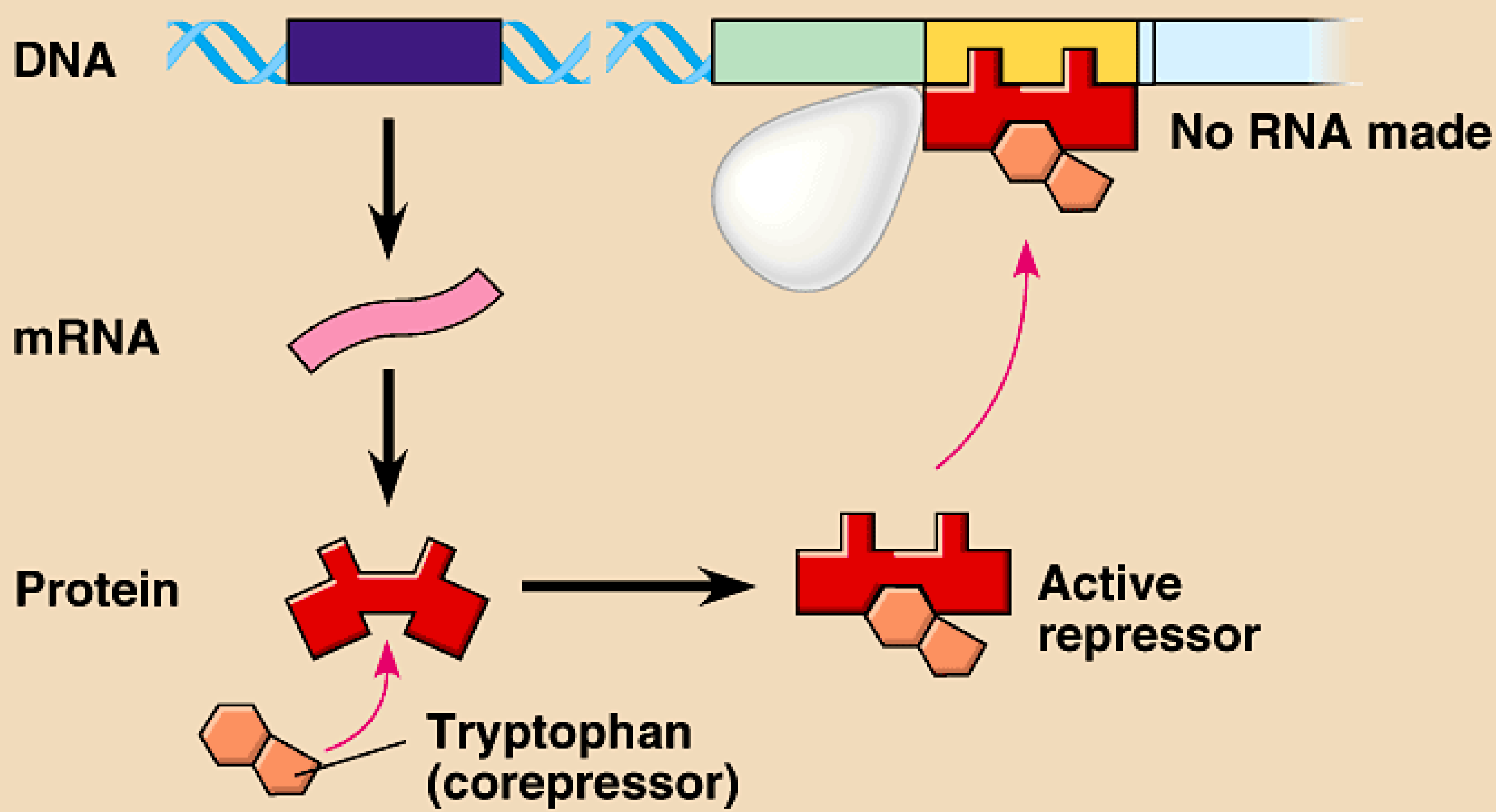
Repressor Bound to an Operator Blocks Transcription



D. Regulation of Gene Expression in Prokaryotes

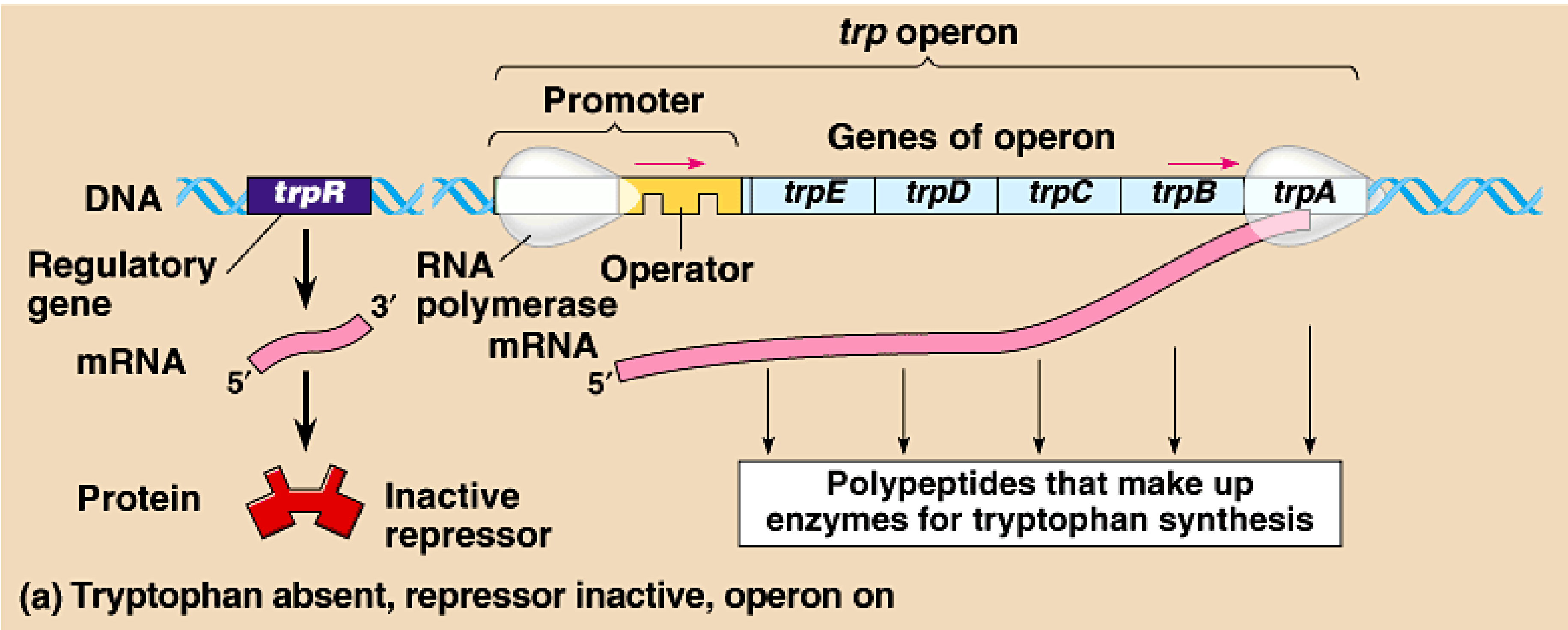
- The expression of prokaryotic genes is regulated by: inducible operator–repressor systems, repressible operator–repressor systems (e.g., both negative control), and systems that increase the efficiency of a promoter (e.g., positive control).
- Repressor proteins are coded by constitutive regulatory genes.

The *trp* operon: regulated synthesis of repressible enzymes

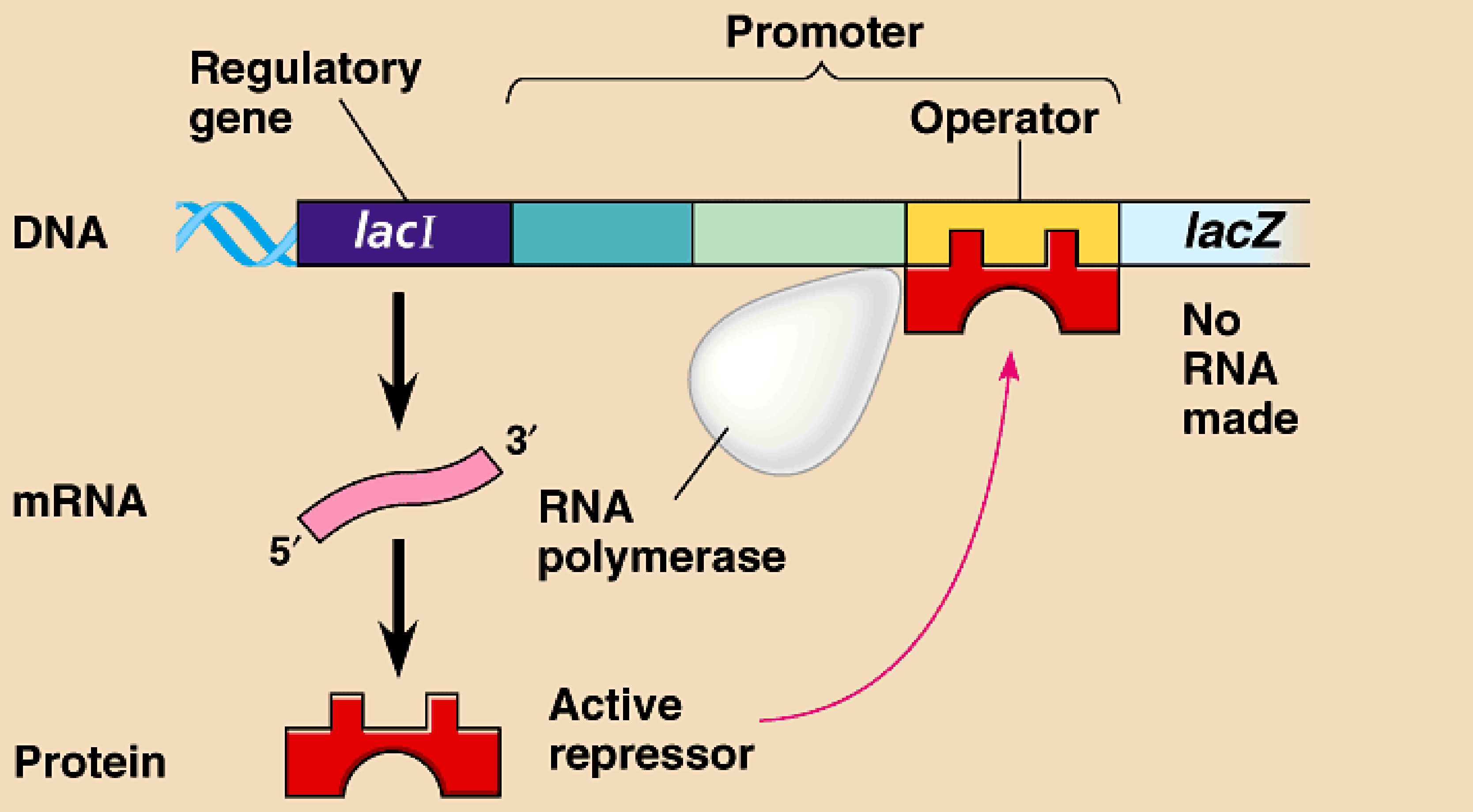


(b) Tryptophan present, repressor active, operon off

The *trp* operon: regulated synthesis of repressible enzymes

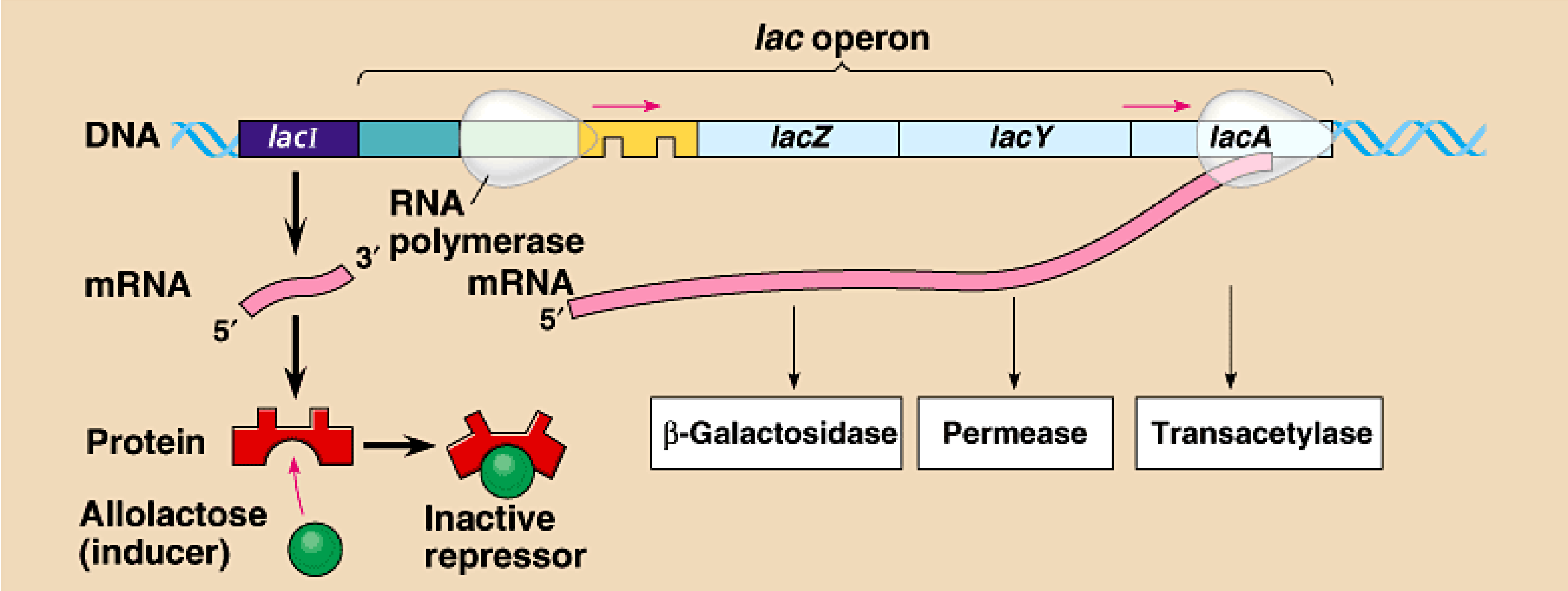


The *lac* operon: regulated synthesis of inducible enzymes



(a) Lactose absent, repressor active, operon off

The *lac* operon: regulated synthesis of inducible enzymes

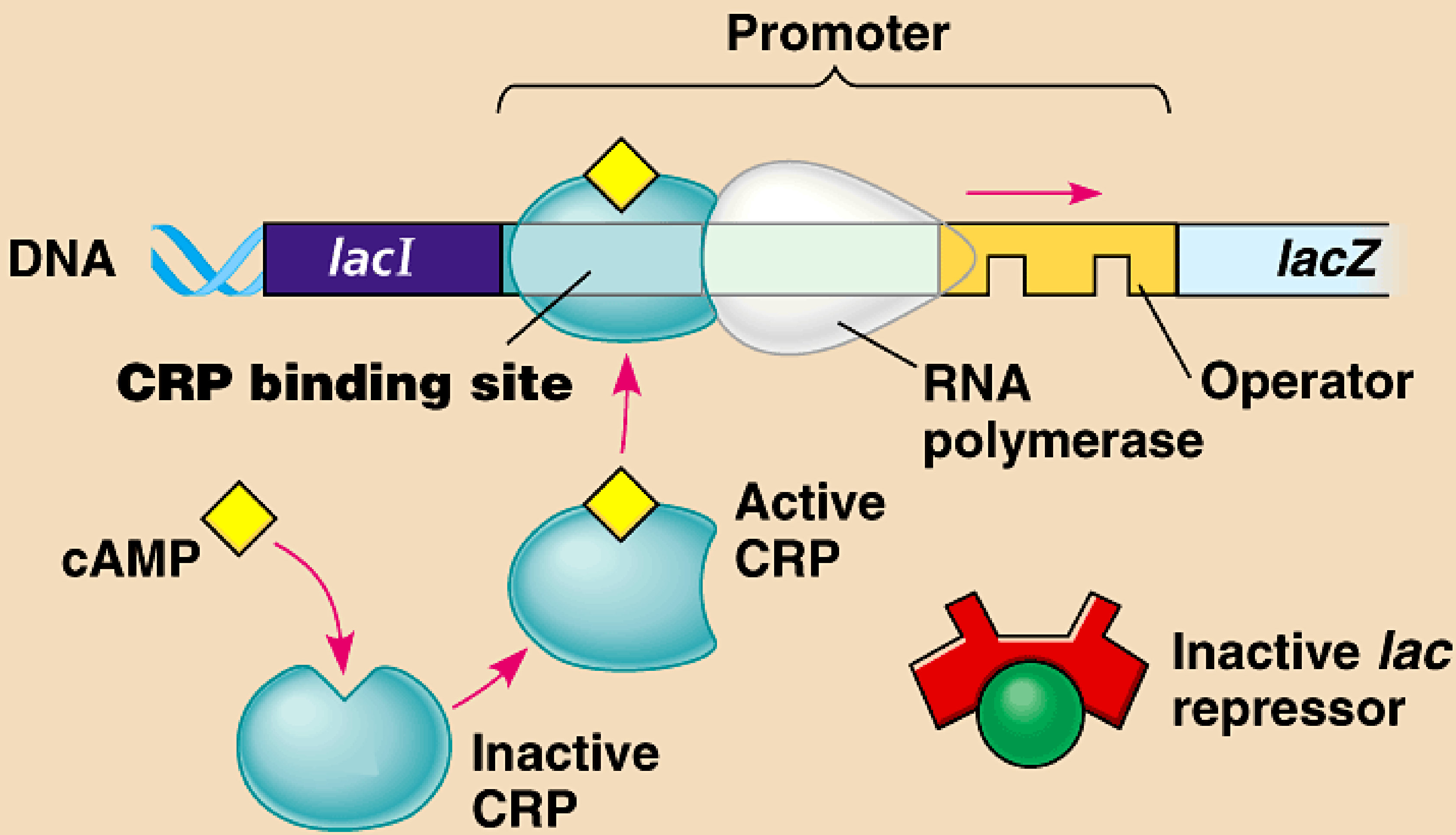


(b) Lactose present, repressor inactive, operon on

D. Regulation of Gene Expression in Prokaryotes

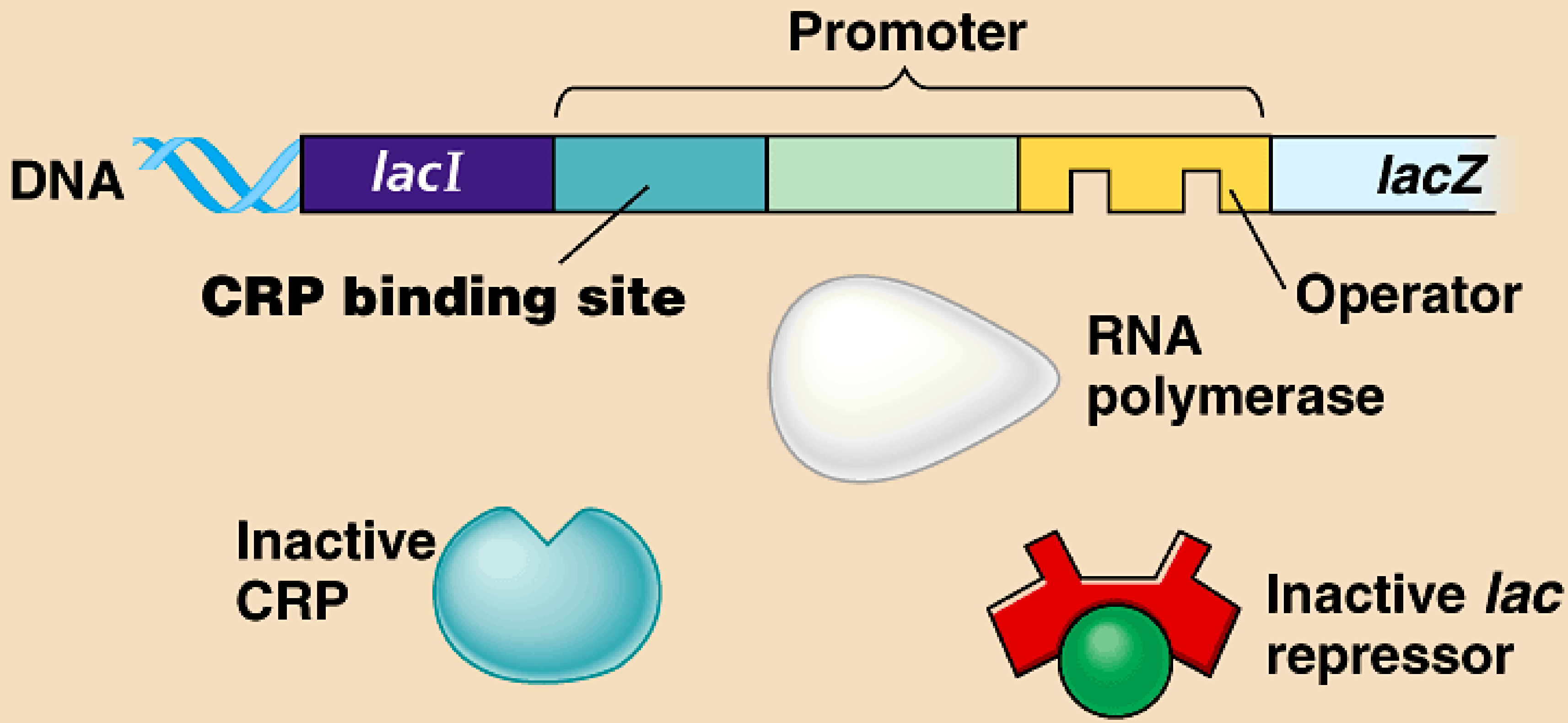
- The efficiency of RNA polymerase can be increased by regulation of the level of cyclic AMP, which binds to CRP (cAMP receptor protein).
- The CRP–cAMP complex then binds to a site near the promoter of a target gene, enhancing the binding of RNA polymerase and hence transcription.

Positive control: cAMP receptor protein



(a) Lactose present, glucose scarce (cAMP level high): abundant *lac* mRNA synthesized

Positive control: cAMP receptor protein



(b) Lactose present, glucose present (cAMP level low): little *lac* mRNA synthesized

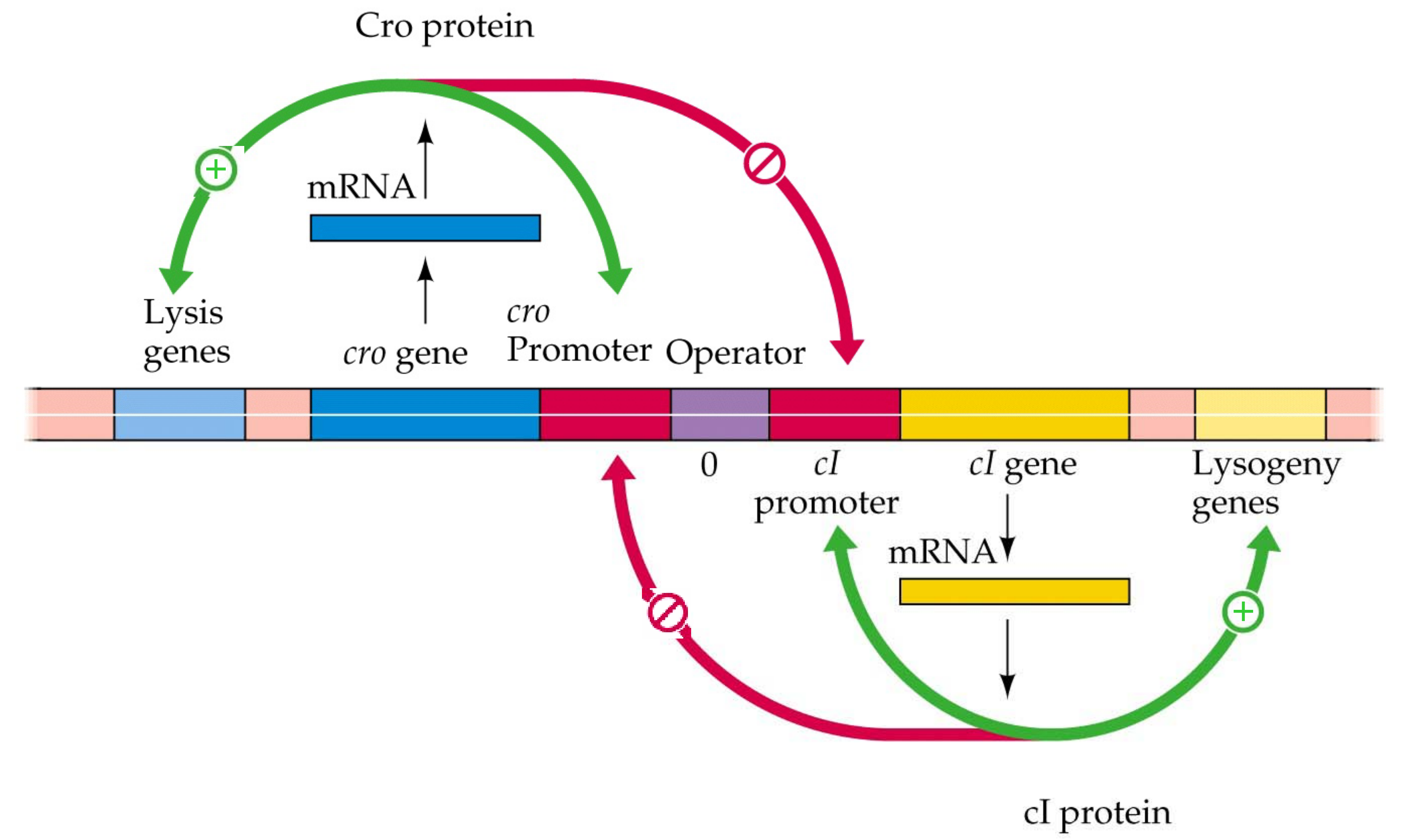
13.2 *The Relationships Between Positive and Negative Control in the* lac *Operon*

GLUCOSE	cAMP LEVELS	RNA POLYMERASE BINDING TO PROMOTER	LACTOSE	*LAC* REPRESSOR	TRANSCRIPTION OF *LAC* GENES?	LACTOSE USED BY CELLS?
Present	Low	Absent	Absent	Active and bound to operator	No	No
Present	Low	Absent	Present	Inactive and not bound to operator	No	No
Absent	High	Present	Present	Inactive and not bound to operator	Yes	Yes
Absent	High	Absent	Absent	Active and bound to operator	No	No

E. Control of Transcription in Viruses

- In bacteriophages that can undergo a lytic or a lysogenic cycle, the decision as to which pathway to take is made by operator–regulatory protein interactions.
- Two regulatory proteins, Cro and cI compete for these operators & promotors.

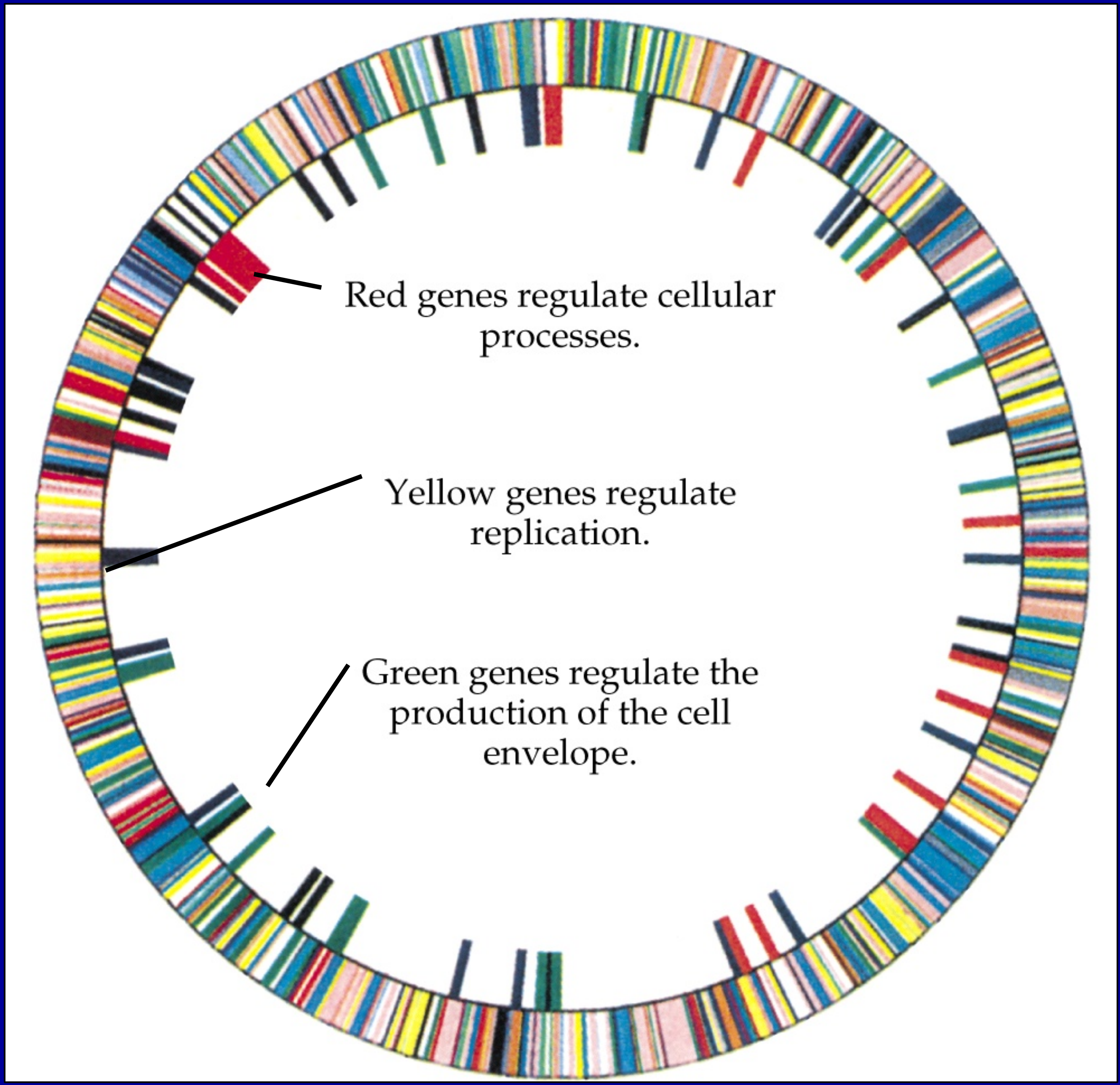
Control of Lambda Phage Lysis and Lysogeny



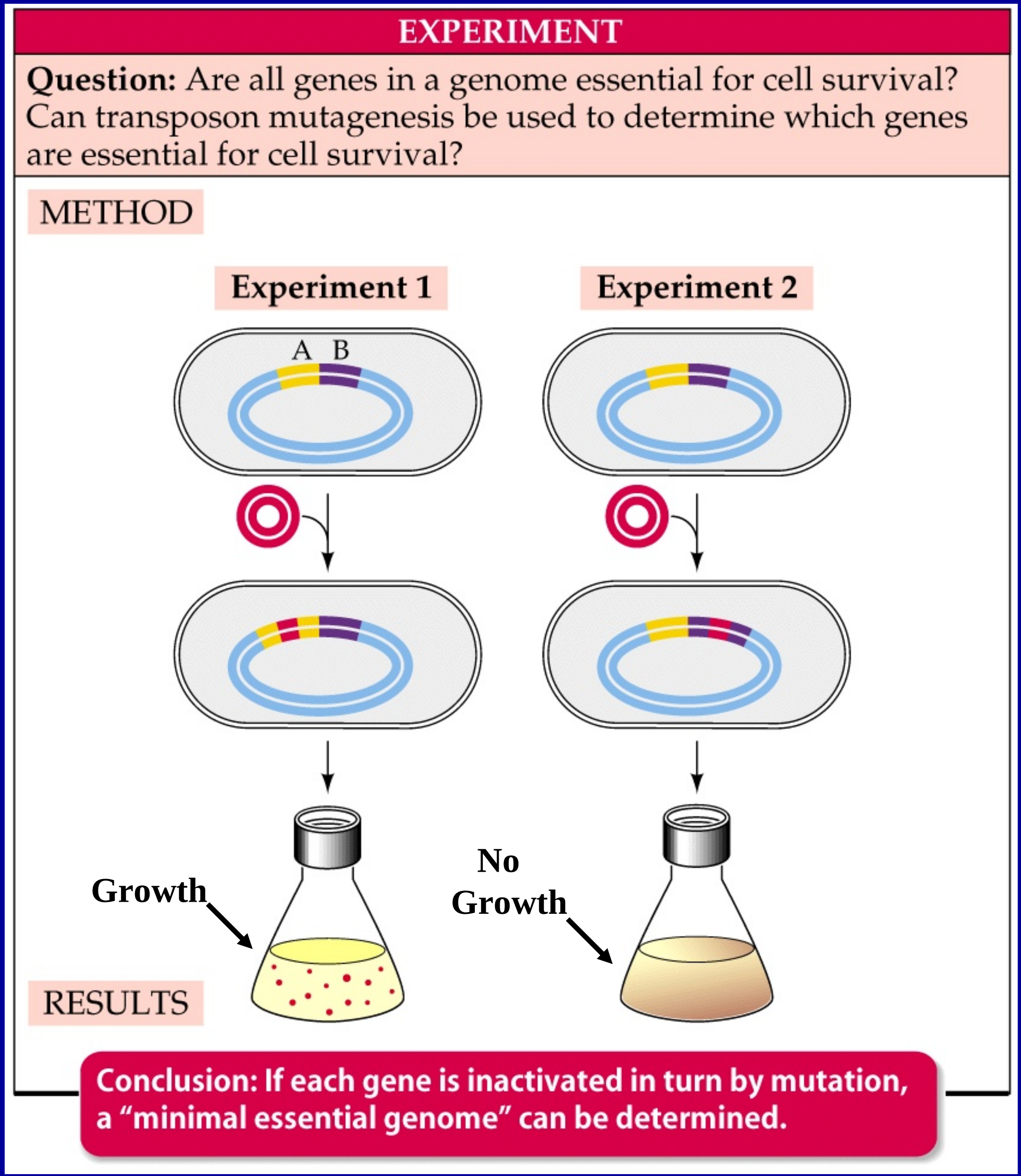
F. Prokaryotic Genomes

- Functional genomics relates gene sequences to functions.
- By mutating individual genes in a small genome, scientists can determine the minimal genome required for a prokaryote.

Functional Organization of the Genome of *H. influenzae*



Transposon Mutagenesis to Estimate the Minimal Genome



Using Mycobacterium with only 470 genes to start and Found it requires only 337 to grow in the lab.