

Microbial genetics

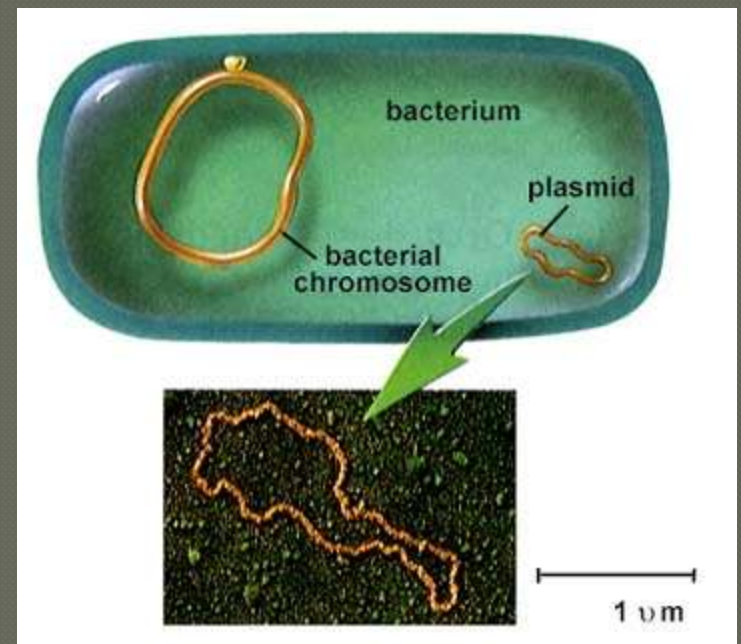
- ◉ Genetic elements
- ◉ The bacterial genome
- ◉ Gene Flow
 - Replication
 - Transcription
 - Translation
- ◉ Regulating of Gene Expression
- ◉ Genetic Variation
 - Mutation
 - Genetic Recombination
 - Transposition

Review

Prokaryotic vs. Eukaryotic

- ◉ Circular vs. linear
- ◉ Location of transcription and translation
- ◉ Single origin and termination of replication
- ◉ No introns/exons in prokaryotic

CHROMOSOME OF E. COLI



PLASMIDS

Conjugative plasmid

Carries genes for sex pili and transfer of the plasmid

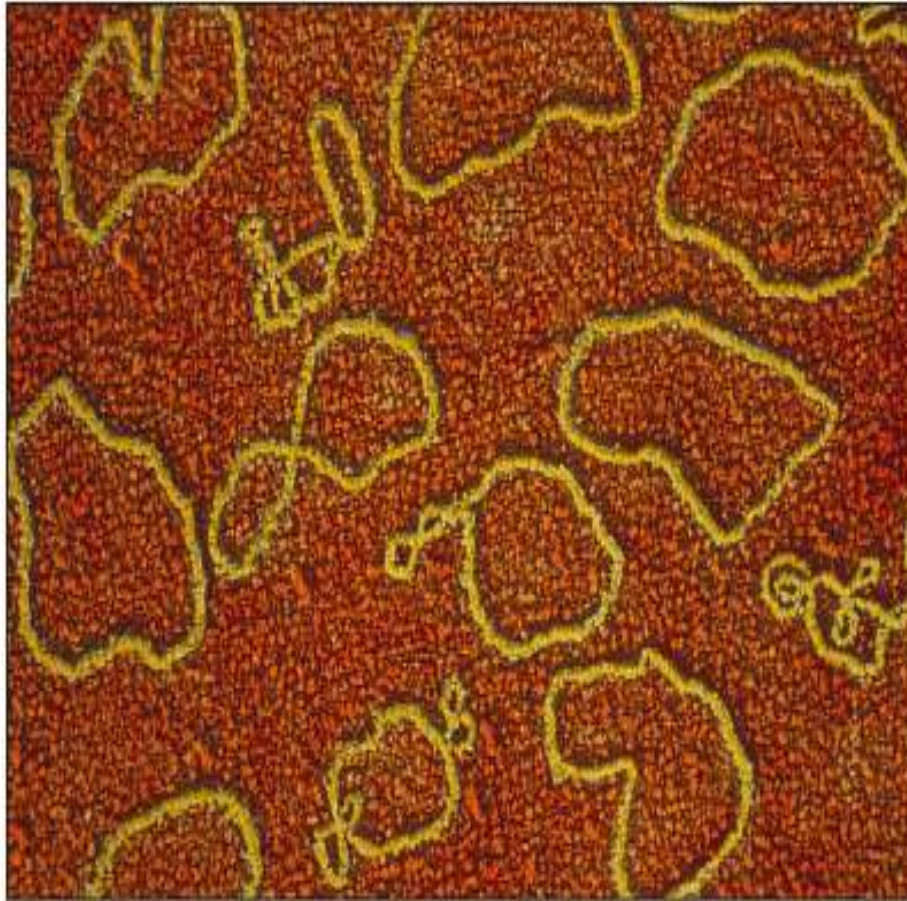
Dissimilation plasmids

Encode enzymes for catabolism of unusual compounds

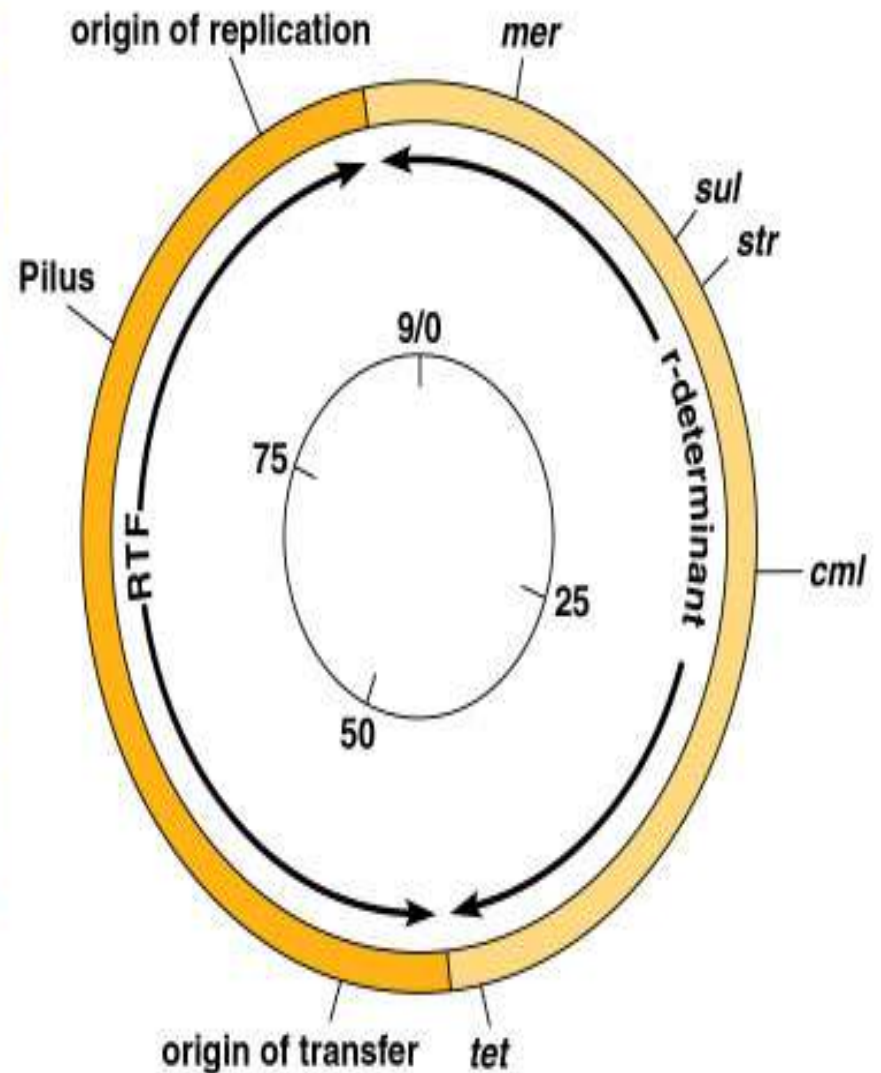
R factors

Encode antibiotic resistance

PLASMIDS

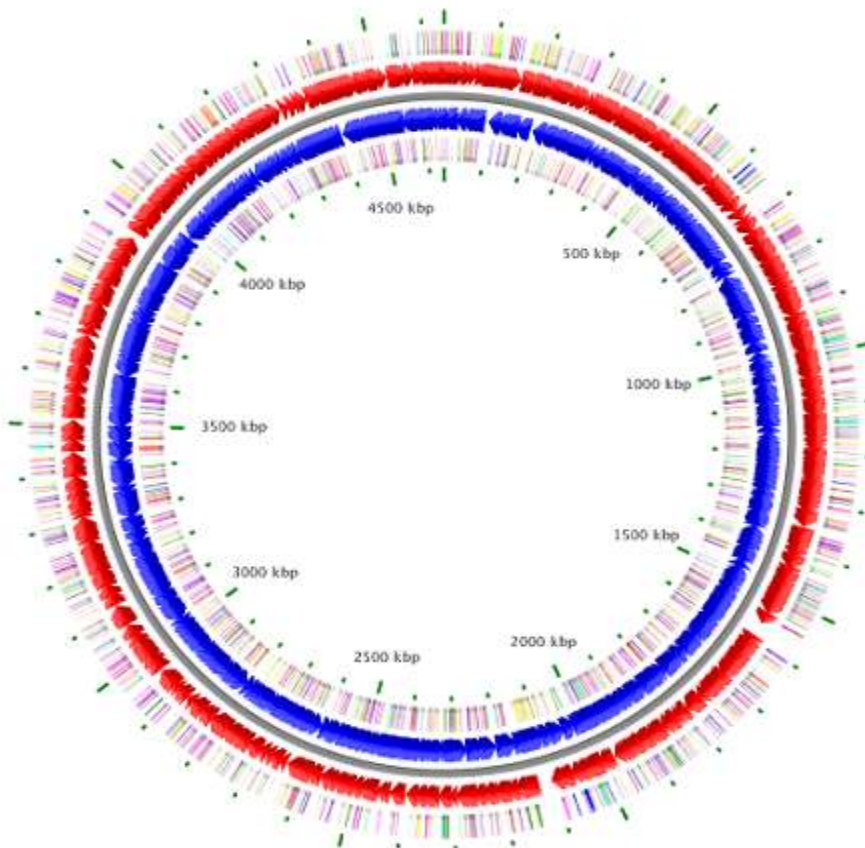


(a)



(b)

GENETIC MAP OF E. COLI



Genes encoding proteins

- Forward strand
- Reverse strand

Genes encoding functional RNA

- Forward strand
- Reverse strand

COG functional categories

Information storage and processing

- Translation, ribosomal structure and biogenesis
- Transcription
- DNA replication, recombination and repair

Cellular processes

- Cell division and chromosome partitioning
- Posttranslational modification, protein turnover, chaperones
- Cell envelope biogenesis, outer membrane
- Cell motility and secretion
- Inorganic ion transport and metabolism
- Signal transduction mechanisms

Metabolism

- Energy production and conversion
- Carbohydrate transport and metabolism
- Amino acid transport and metabolism
- Nucleotide transport and metabolism
- Coenzyme metabolism
- Lipid metabolism
- Secondary metabolites biosynthesis, transport and catabolism

Poorly characterized

- General function prediction only
- Function unknown

Microbial genetics

Genetic variation: MUTATION

MUTATION

- Is any heritable change in the genetic material
- Mutations may be neutral, beneficial, or harmful
- Mutant- organism or strain whose genome carries a mutation
- Wild type- the usual (native) form of the organism
- Occurrence of mutations:
 - Spontaneous - Occur in the absence of a mutagen
 - Induced - Occur in the presence of a mutagen
- Mutagen: Agent that causes or accelerates rate of mutations

FREQUENCY OF MUTATION

- Spontaneous mutation rate = 1 in 10^9 replicated base pairs
 - 1 in 10^6 replicated genes
- Mutagens increase to 10^{-5} or 10^{-3} per replicated gene

Mutagens

- Mutagens are chemical, physical or biological agents that increase the mutation rate i.e. induce mutations
- Can classify mutagens according to mode of action:
 - Incorporation of base analogues
 - Direct reaction with DNA
 - intercalation

Base analogues

- Incorporated into polypeptide chain during replication like normal bases
 - They have different base pairing properties and in subsequent replication events may form a stable mutation
-
- eg. 5-bromouracil: incorporate into the growing DNA as if it were thymine

Mutagens acting directly on DNA

- These mutagens change the structure of a base and alter the base pairing characteristics
 - e.g. methyl nitrosoguanidine (NTG), adds methyl groups to guanine, causing it to base pair with thymine

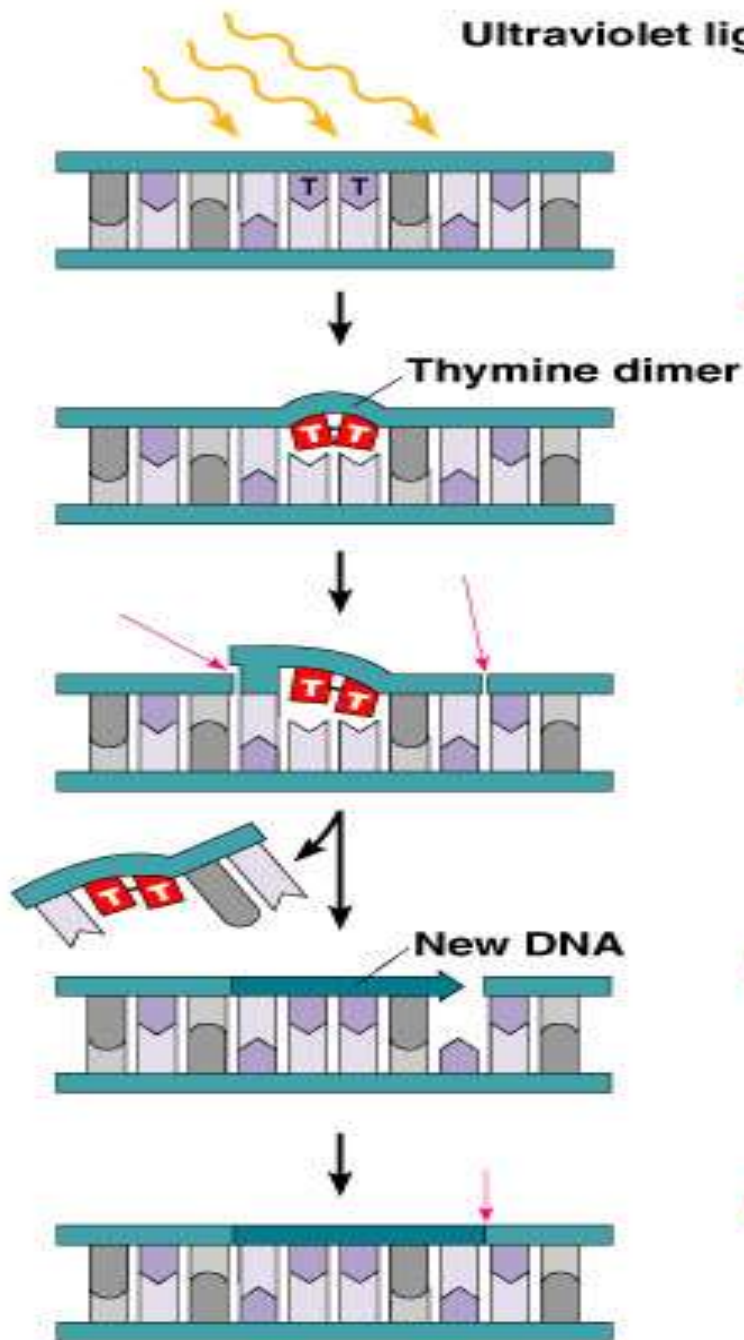
Intercalating Agents

- These mutagens insert themselves between adjacent base pairs and push them apart
- During subsequent replication this abnormal structure leads to microinsertions/deletions and frameshifts
 - e.g. acridine orange, ethidium bromide

Physical mutagens

- Ionizing radiation (X rays and gamma rays) causes the formation of ions that can react with nucleotides and the deoxyribose-phosphate backbone
 - Nucleotide excision repairs mutations
- Non ionizing radiation
 - UV radiation causes thymine dimers
 - Light-repair separates thymine dimers

Ultraviolet light

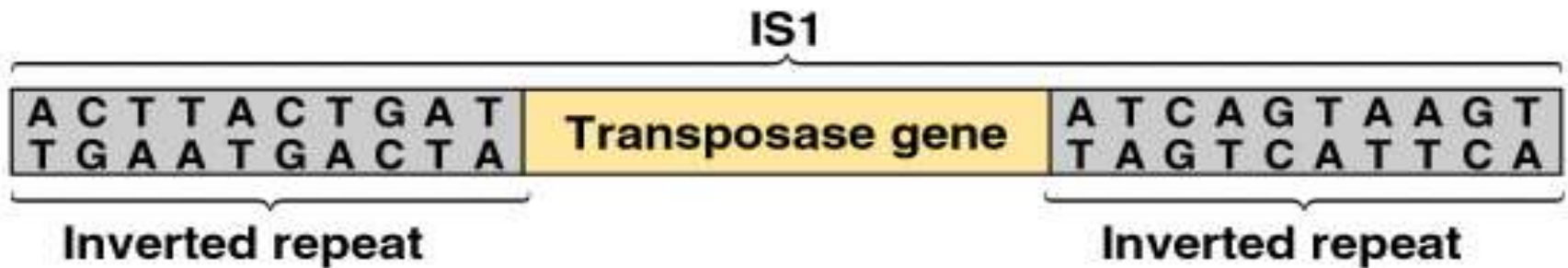


- 1** Exposure to ultraviolet light causes adjacent thymines to become cross-linked, forming a thymine dimer and disrupting their normal base pairing.
- 2** An enzyme cuts out and removes the damaged DNA.
- 3** DNA polymerase fills the gap by synthesizing new DNA, using the intact strand as a template.
- 4** DNA ligase seals the remaining gap by joining the old and new DNA.

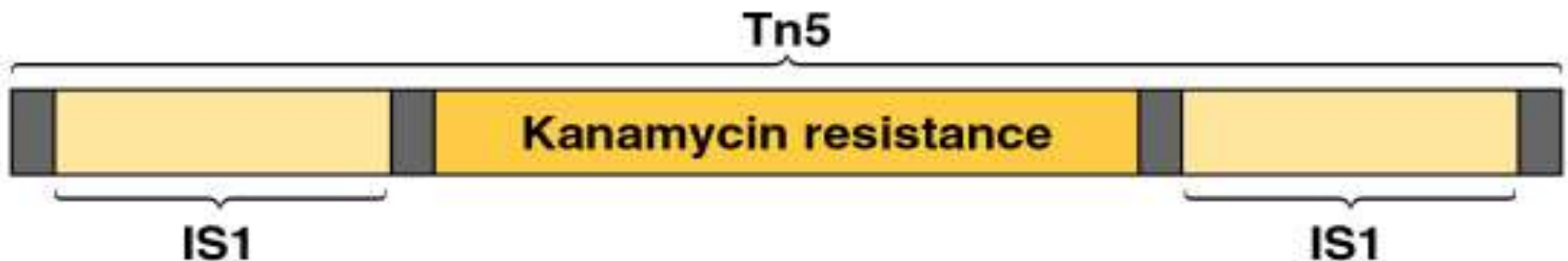
TRANSPOSONS

- ◉ Biological mutagen
- ◉ Segments of DNA that can move from one region of DNA to another
- ◉ Contain insertion sequences for cutting and resealing DNA (transposase)
- ◉ Complex transposons carry other genes

TRANSPOSONS



(a) Insertion sequence "IS1"

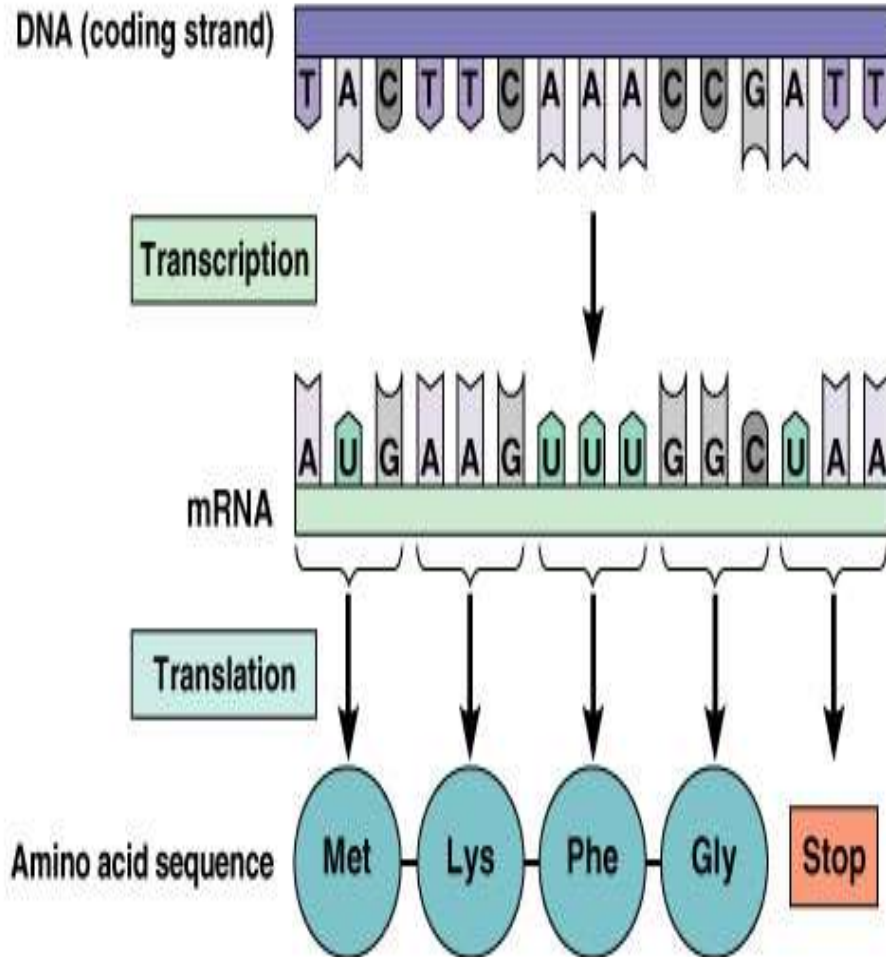


(b) Complex transposon "Tn5"

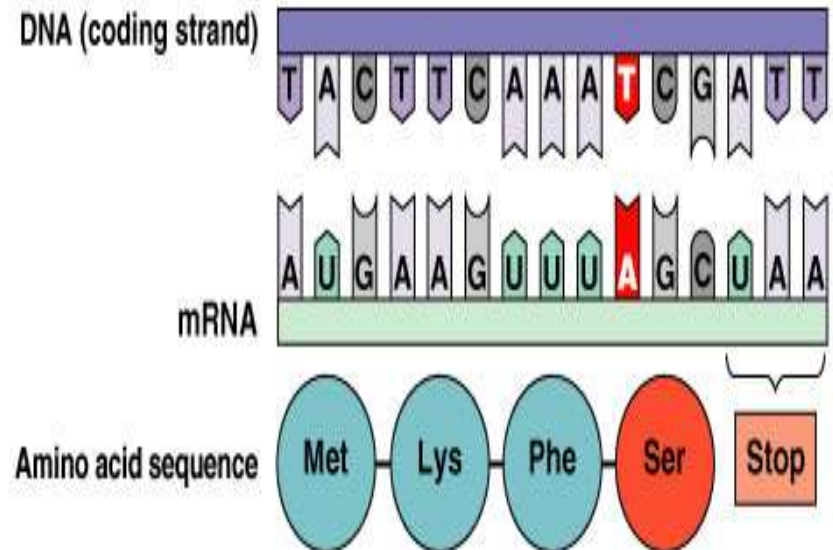
TYPES OF MUTATION

- Base substitution (point mutation)
 - Change in one base
- Missense mutation
 - Result in change in amino acid
- Nonsense mutation
 - Results in a nonsense codon
- Frameshift mutation
 - Insertion or deletion of one or more nucleotide pairs

MISSENSE

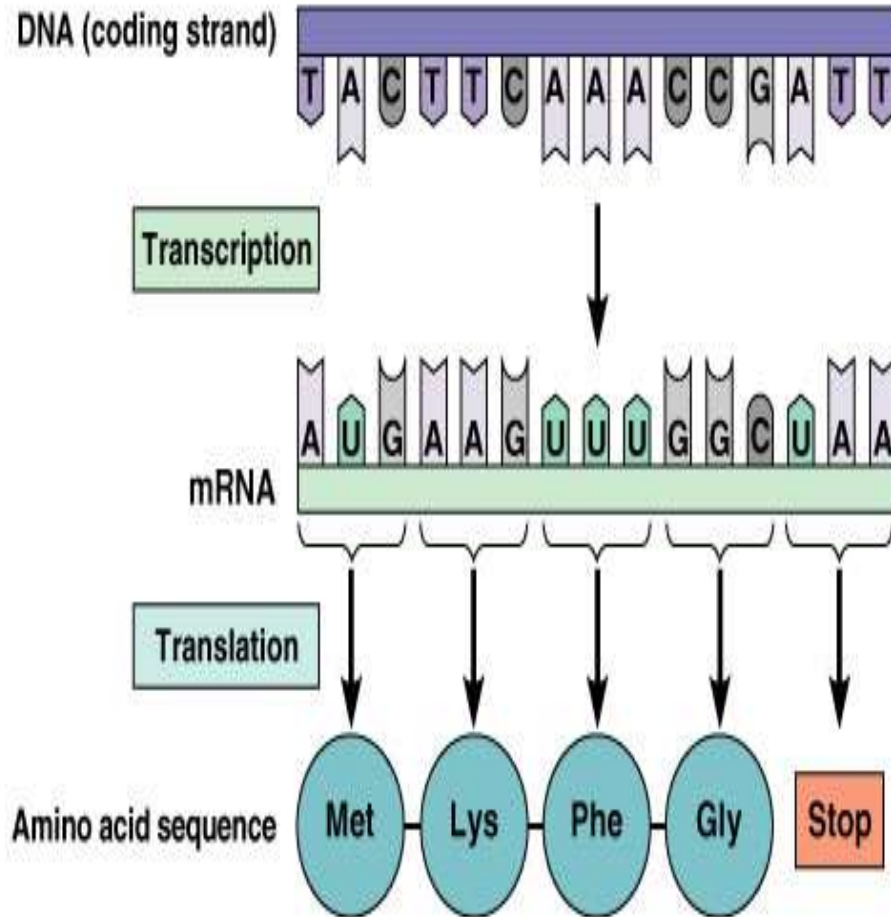


(a) Normal DNA molecule

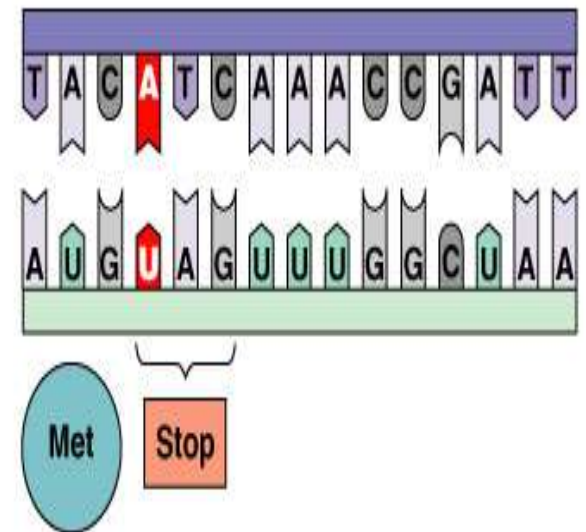


(b) Missense mutation

NONSENSE

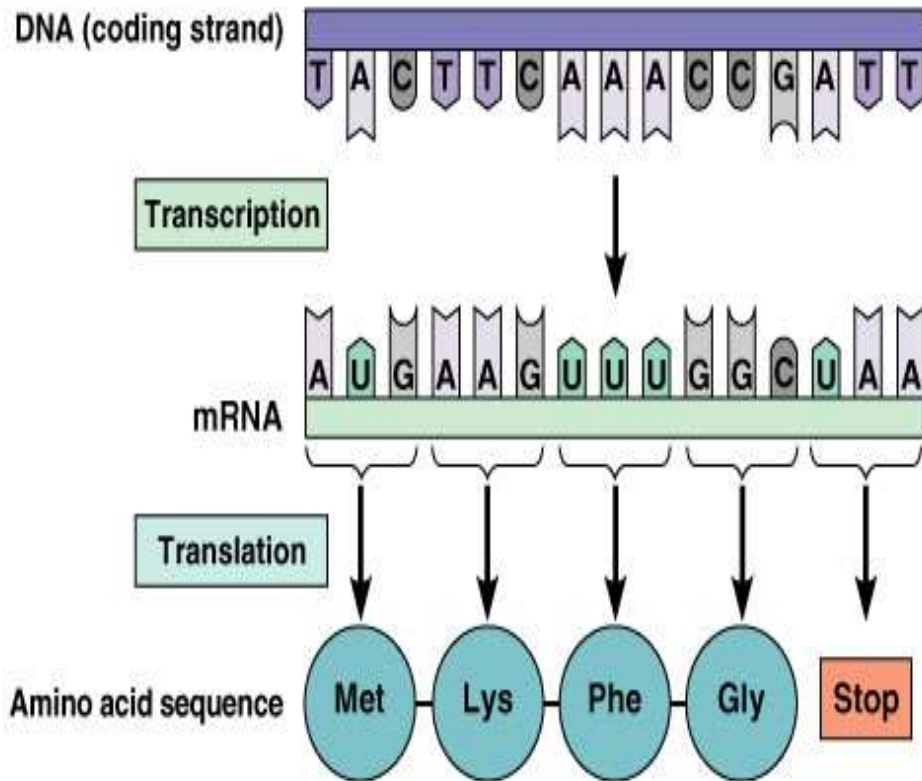


(a) Normal DNA molecule

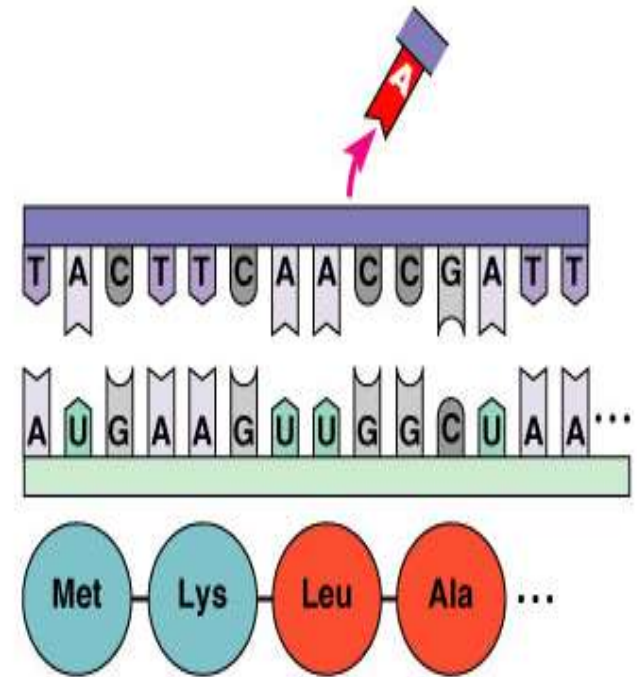


(c) Nonsense mutation

FRAMESHIFT



(a) Normal DNA molecule



(d) Frameshift mutation

Bacterial mutants

- ◉ Mutants in bacteria are mostly biochemical in nature, because we can't generally see the cell
- ◉ 3 major types:
 - Auxotroph
 - Chemoauxotroph
 - Antibiotic resistant

Other types are temperature sensitivity and viral resistance

Auxotrophy

- An auxotroph needs some nutrient that the wild type strain (prototroph) can make for itself.
- For example,
 - trp- auxotroph can't make its own tryptophan (an amino acid). To grow trp- bacteria, you need to add tryptophan to the growth medium. Prototrophs are trp+; they don't need any tryptophan supplied since they make their own.

Chemoauxotrophy

- ◉ Chemoauxotrophs are mutants that can't use some nutrient (usually a sugar) that prototrophs can use as food.
- ◉ For example:
 - lac- mutants can't grow on lactose (milk sugar), but lac+ prototrophs can grow on lactose

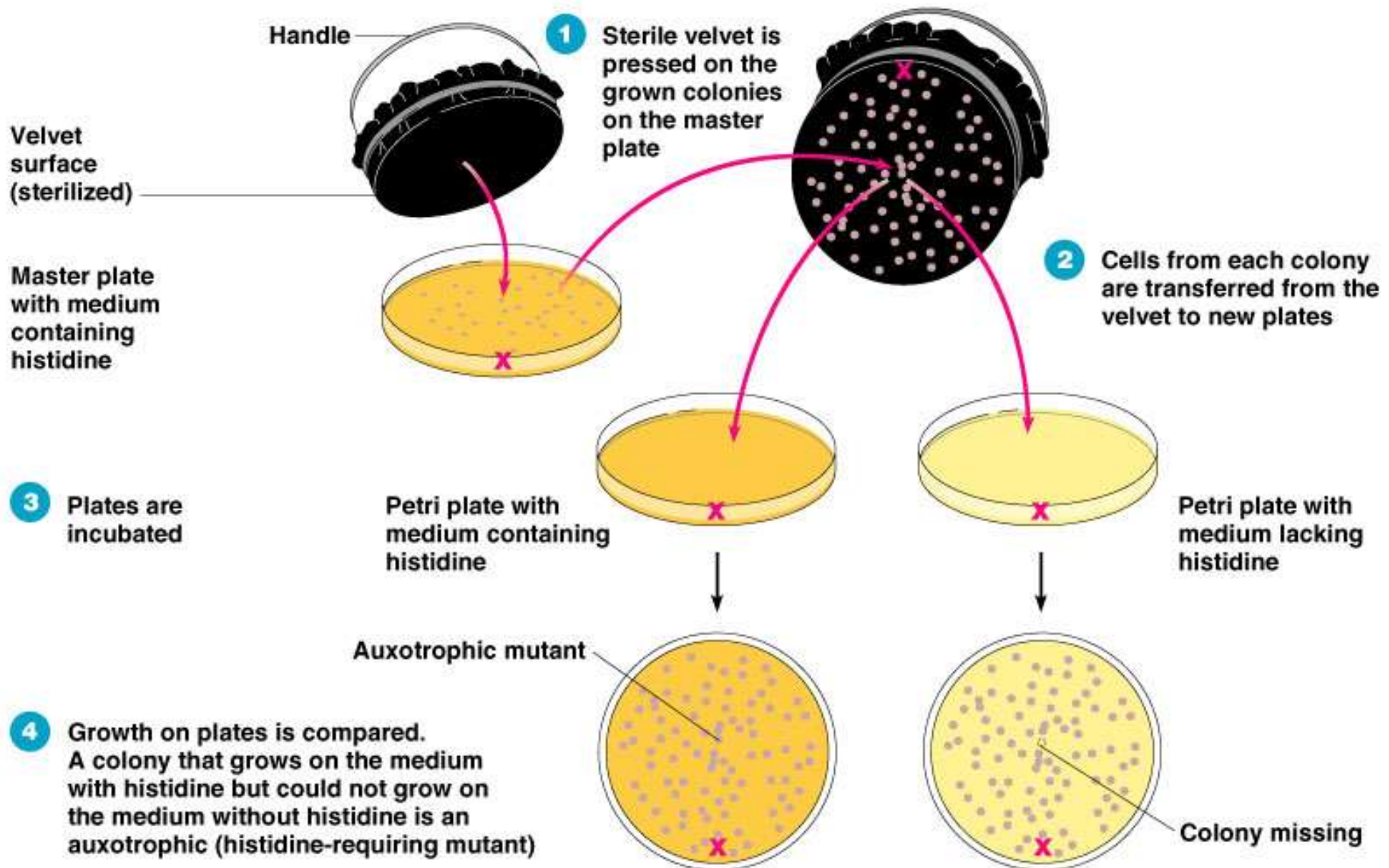
Antibiotic resistance

- confer resistance antibiotics
- Example:
 - Amp^R causes bacteria to be resistant to ampicillin, a common antibiotic related to penicillin
- Note: Auxotrophs and chemoauxotrophs are usually recessive; drug resistance mutants are usually dominant

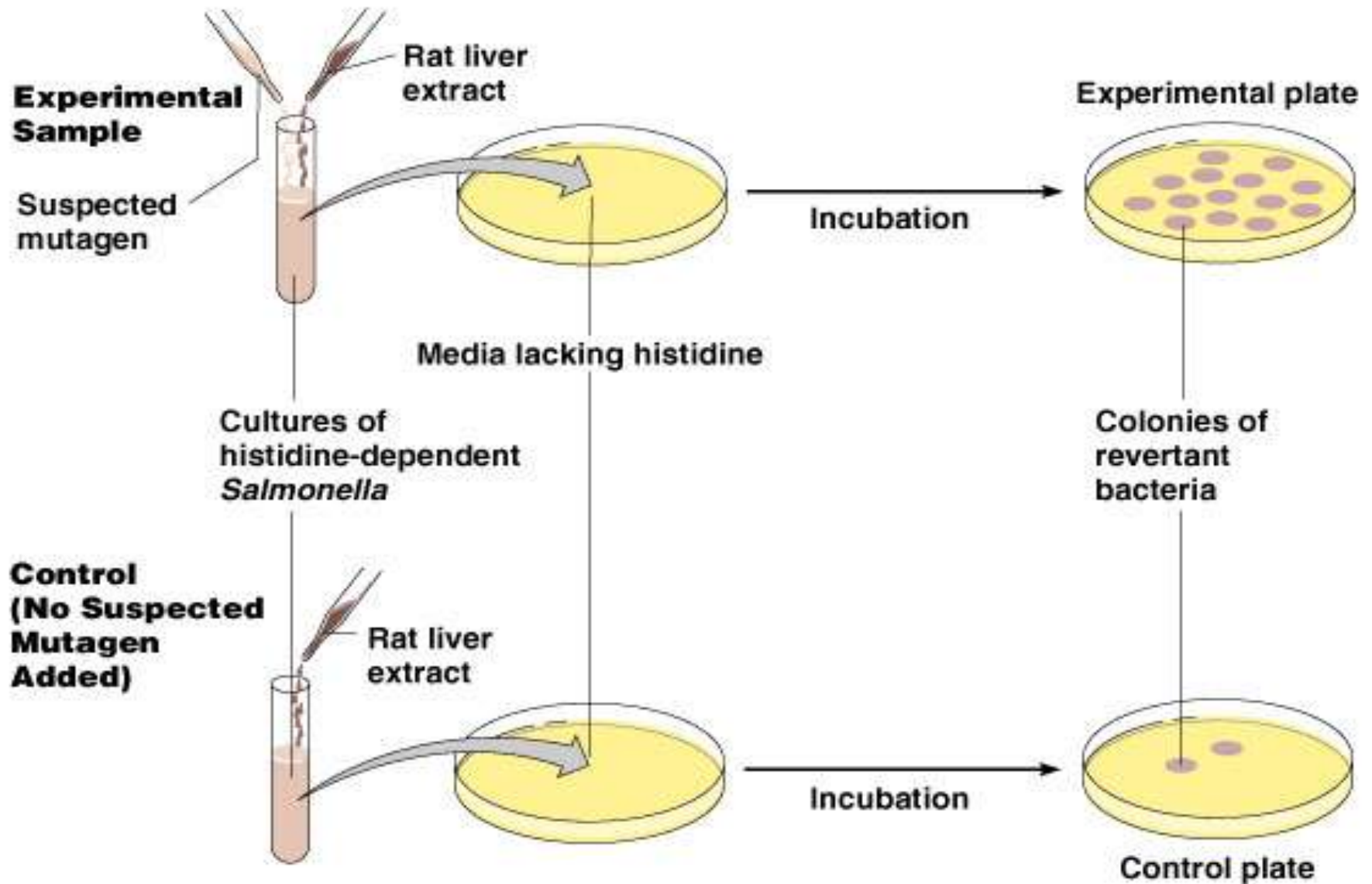
SELECTION

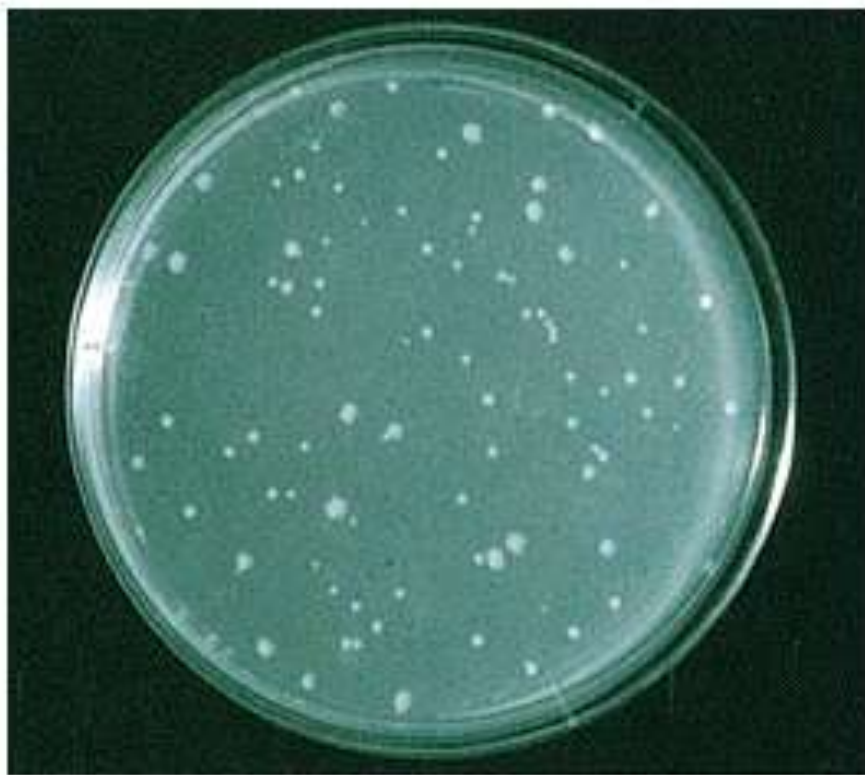
- Positive (direct) selection detects mutant cells because they grow or appear different
- Negative (indirect) selection detects mutant cells because they do not grow

THE REPLICA PLATING



AMES TEST





Microbial genetics

Recombination

Bacterial Sexual Processes

- Not regularly occurring
- Cause mixing of genes\
- The three bacterial sexual processes:
 - 1. conjugation: direct transfer of DNA from one bacterial cell to another.
 - 2. transduction: use of a bacteriophage (bacterial virus) to transfer DNA between cells.
 - 3. transformation: naked DNA is taken up from the environment by bacterial cells.

Conjugation

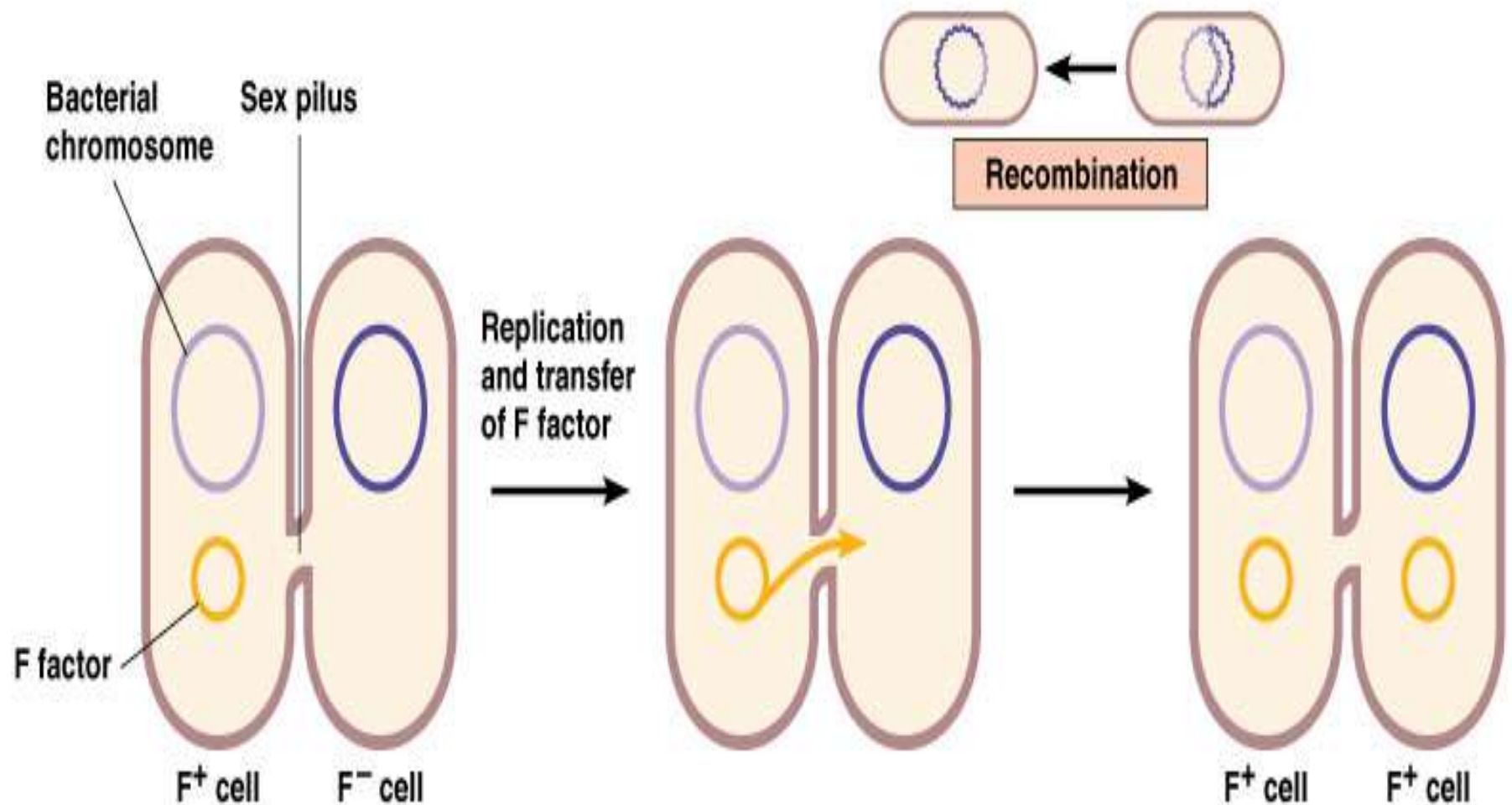
- Conjugation is the closest analogue in bacteria to eukaryotic sex.
- The ability to conjugate is conferred by the F plasmid.
 - A plasmid is a small circle of DNA that replicates independently of the chromosome.
 - Bacterial cells that contain an F plasmid are called “F+”. Bacteria that don’t have an F plasmid are called “F-”.
- F+ cells grow special tubes called “sex pilli” from their bodies. When an F+ cell bumps into an F- cell, the sex pilli hold them together, and a copy of the F plasmid is transferred from the F+ to the F-. Now both cells are F+.

Mating of F+ and F- Bacterial Strains

**Animation by
Thomas M. Terry**

Why aren't all *E. coli* F+, if it spreads like that? Because the F plasmid can be spontaneously lost.

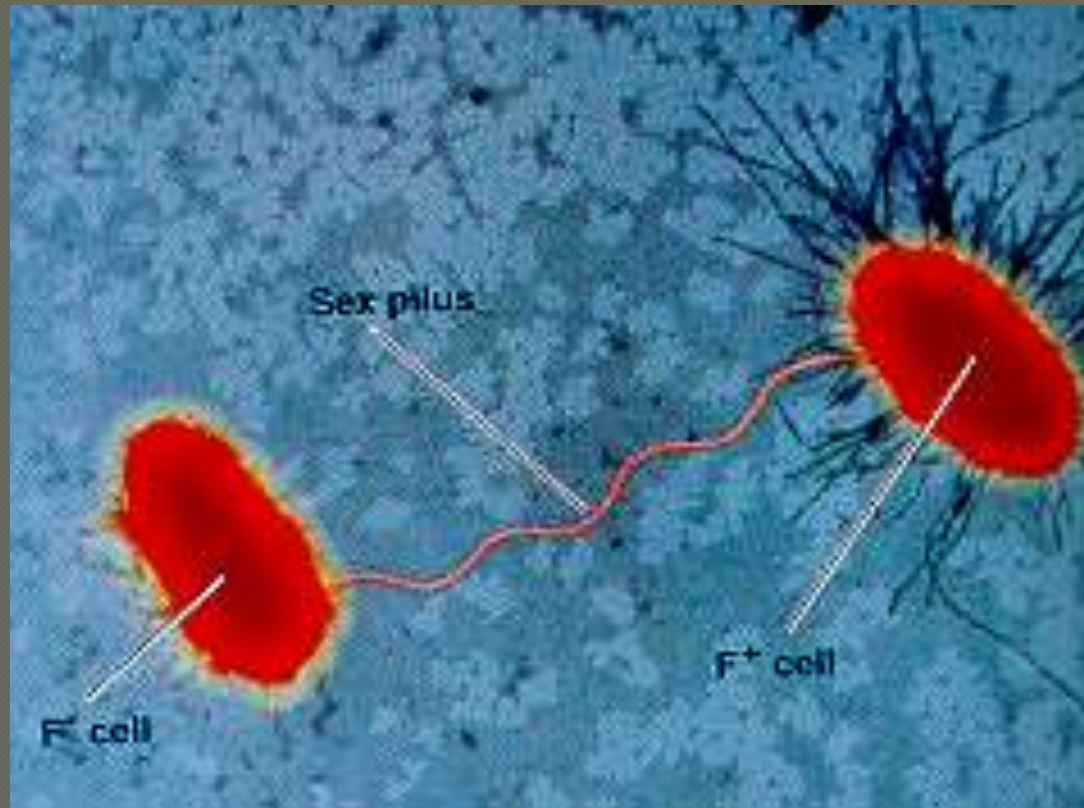
CONJUGATION



(a) When an F factor (a plasmid) is transferred from a donor (F^+) to a recipient (F^-), the F^- cell is converted into an F^+ cell.

Conjugation

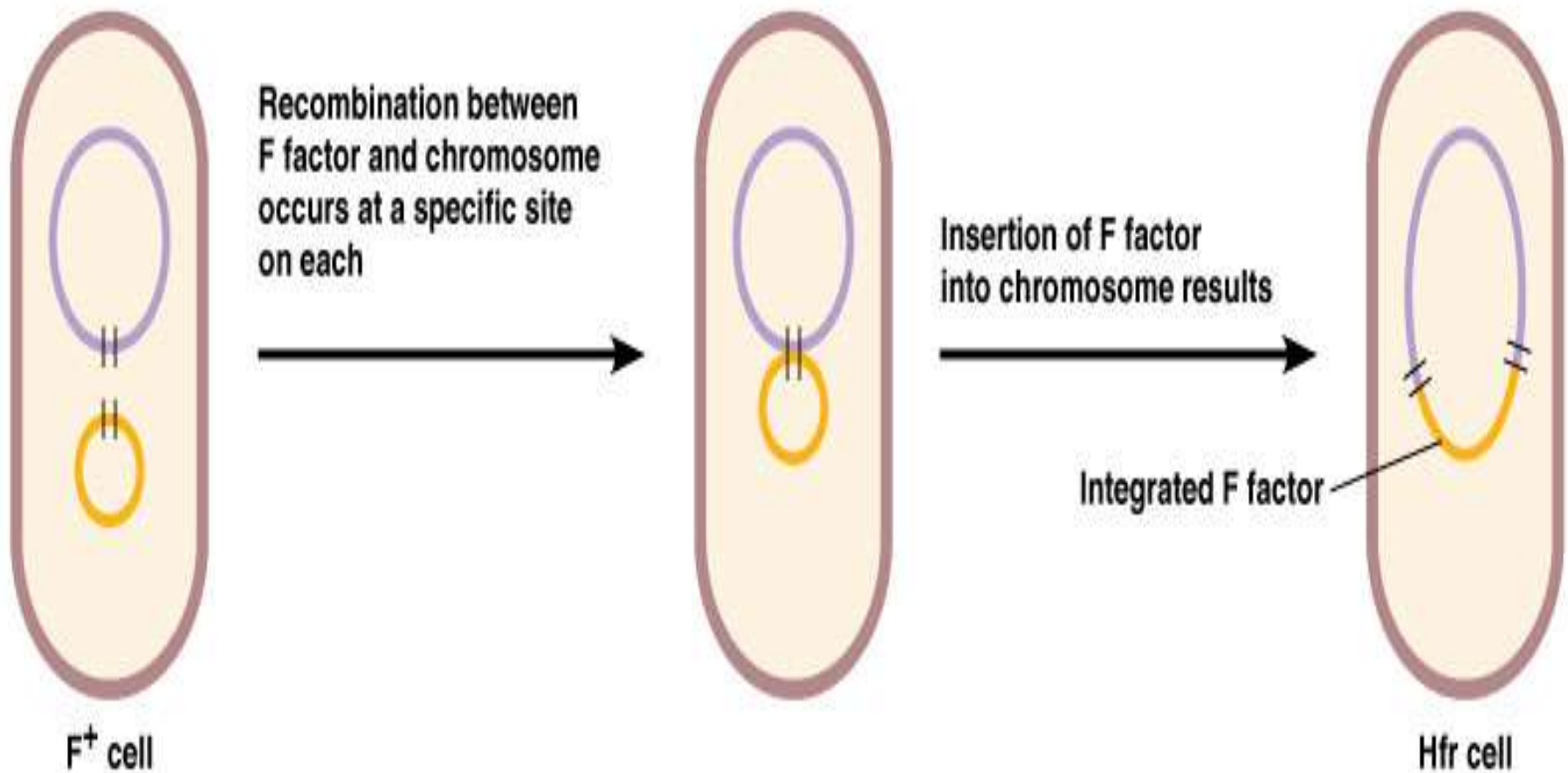
One bacterium passes some DNA (in a plasmid) to another bacterium



Hfr Conjugation

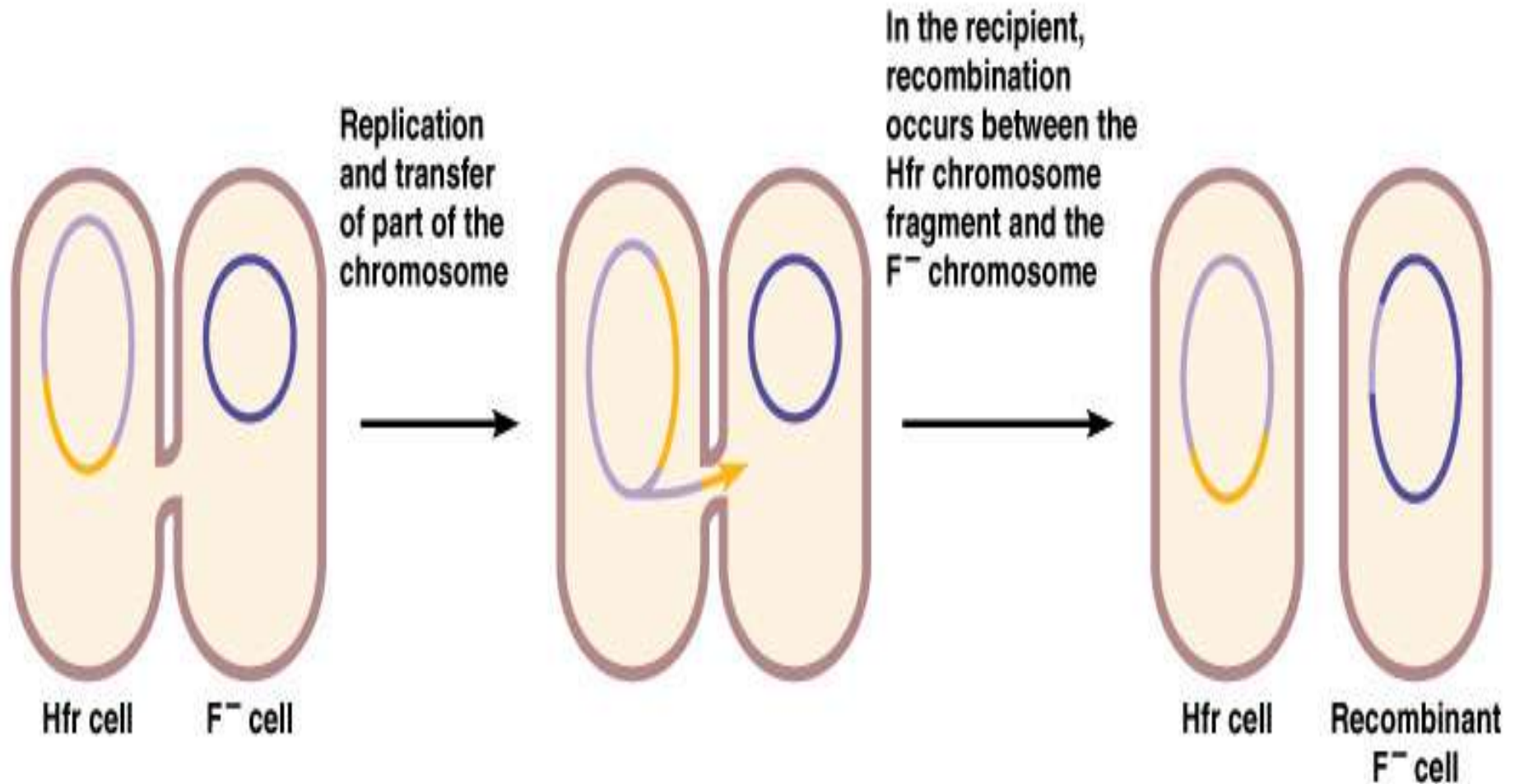
- ◉ sometimes the F plasmid can become incorporated into the bacterial chromosome, by a crossover between the F plasmid and the chromosome.
- ◉ The resulting bacterial cell is called an “Hfr”, which stands for “High frequency of recombination”.
- ◉ Hfr bacteria conjugate just like F+ do, but they drag a copy of the entire chromosome into the F- cell.

CONJUGATION



(b) When an F factor becomes integrated into the chromosome of an F^+ cell, it makes the cell a high frequency of recombination (Hfr) cell.

CONJUGATION



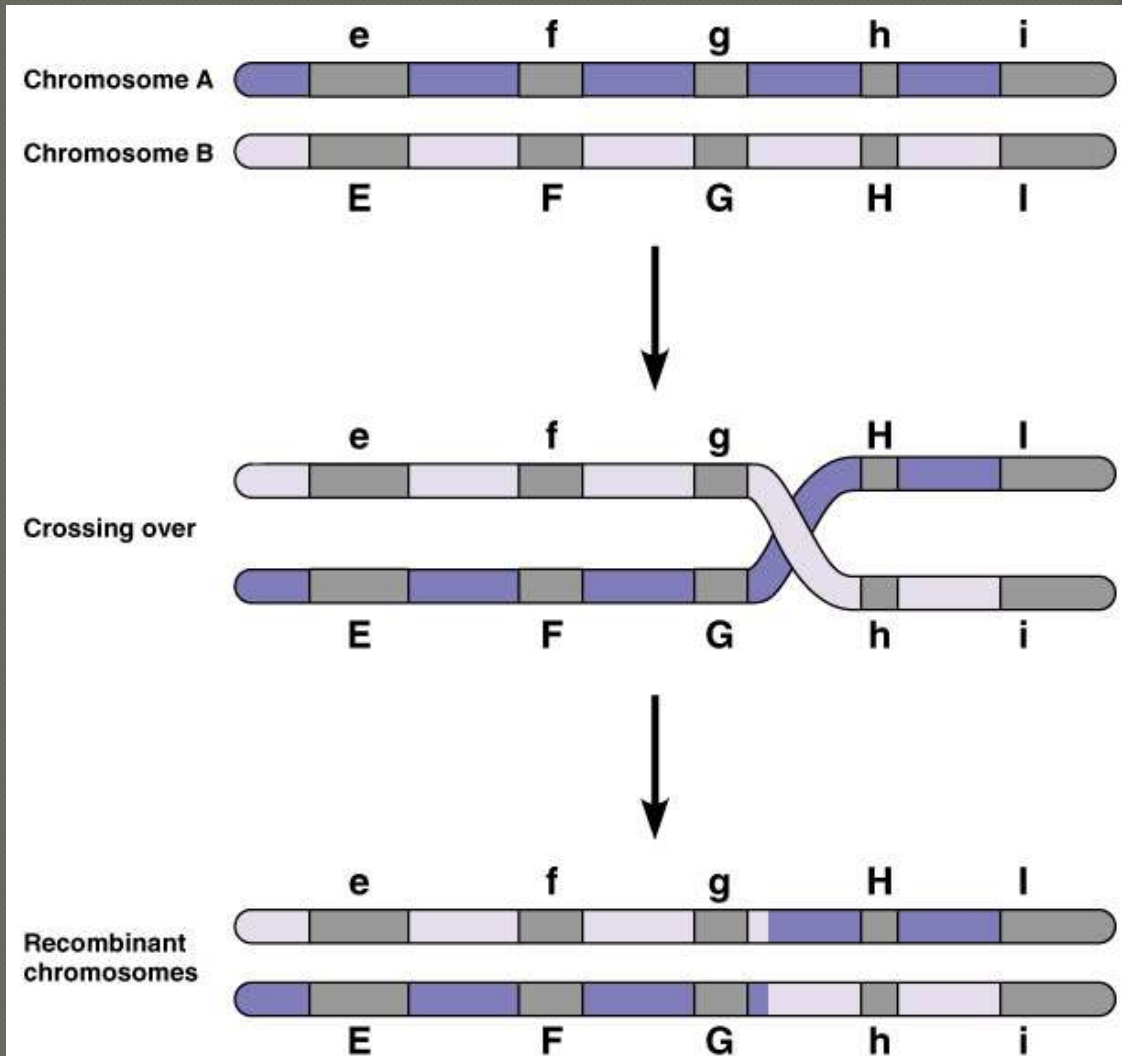
(c) When an Hfr donor passes a portion of its chromosome into an F⁻ recipient, a recombinant F⁻ cell results.

F-prime (F')

- The process of making an Hfr from an F⁺ involves a crossover between the F plasmid and the chromosome. This process is reversible: an Hfr can revert to being F⁺ when the F plasmid DNA incorporated into the Hfr chromosome has a crossover and loops out of the chromosome forming an F plasmid once again.
- Sometimes the looping-out and crossing-over process doesn't happen at the proper place. When this happens, a piece of the bacterial chromosome can become incorporated into the F plasmid. This is called an F' (F-prime) plasmid.

GENETIC RECOMBINATION

- Exchange of genes between two DNA molecules
 - Crossing over occurs when two chromosomes break and rejoin



Transduction

- Transduction is the process of moving bacterial DNA from one cell to another using a bacteriophage.
- Bacteriophage or just “phage” are bacterial viruses.
- Two forms of transduction:
 - 1. generalized: any piece of the bacterial genome can be transferred
 - 2. specialized: only specific pieces of the chromosome can be transferred.

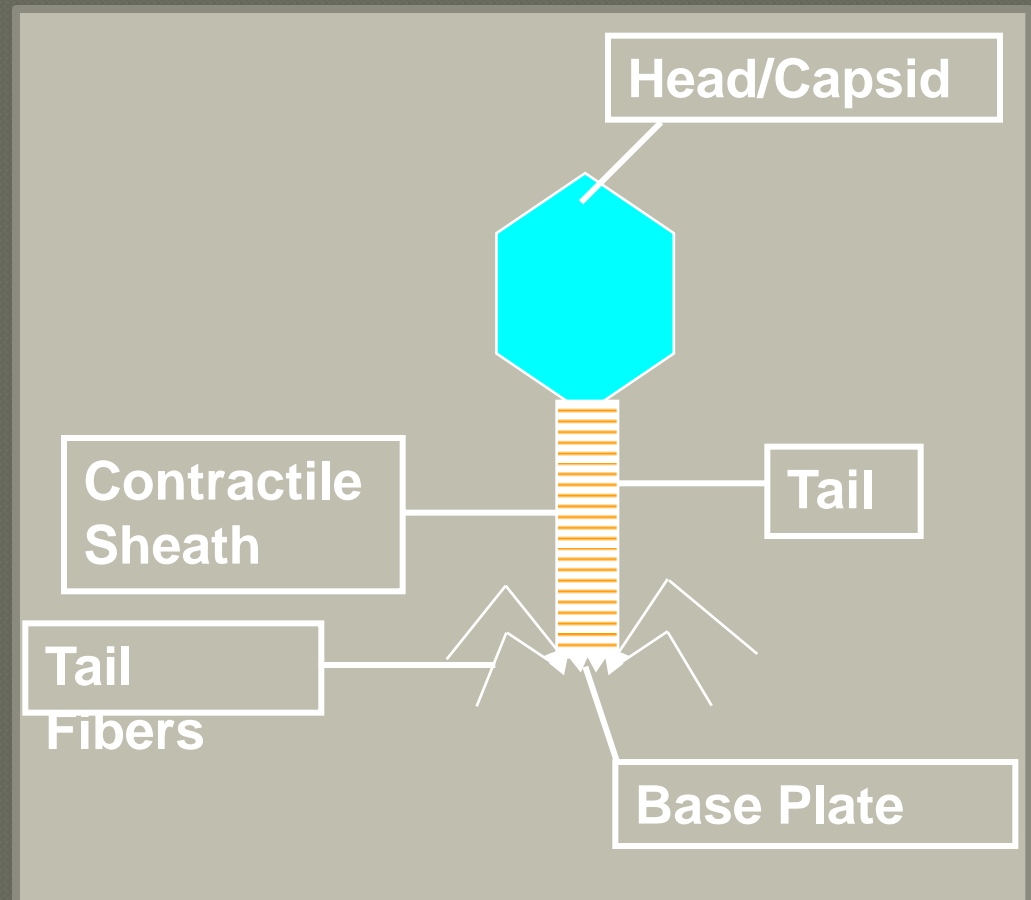
Bacteriophage

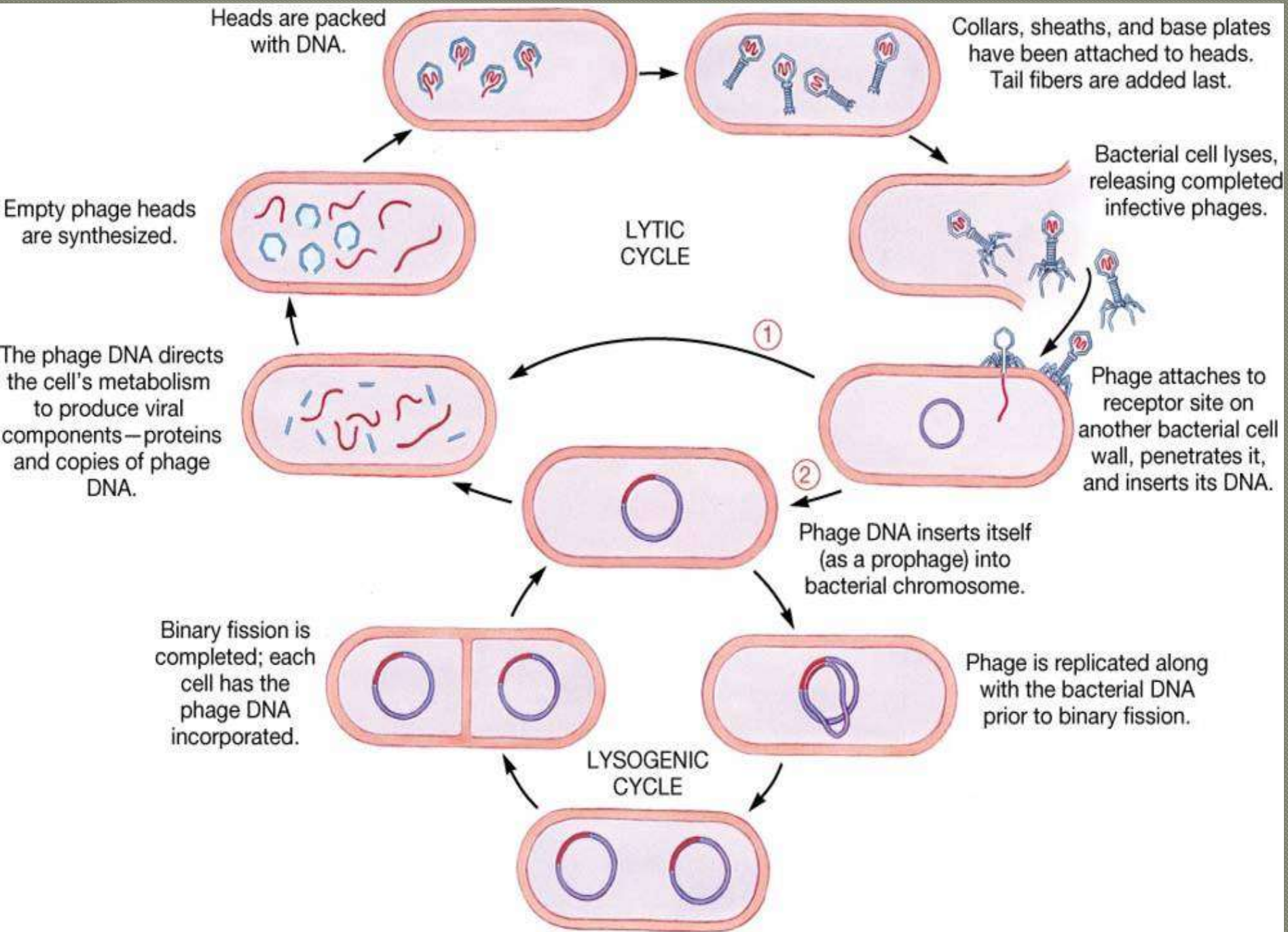
- Definition - Obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery

Composition and Structure

Composition

- Nucleic acid
 - Genome size
 - Modified bases
- Protein
 - Protection
 - Infection
- Structure (T_4)
 - Size
 - Head or capsid
 - Tail





Generalized Transduction

- Some phages, such as phage P1, break up the bacterial chromosome into small pieces, and then package it into some phage particles instead of their own DNA.
- A phage containing bacterial DNA can infect a fresh host
- After infection by such a phage, the cell contains an exogenote (linear DNA injected by the phage) and an endogenote (circular DNA that is the host's chromosome).
- A double crossover event puts the exogenote's genes onto the chromosome, allowing them to be propagated.

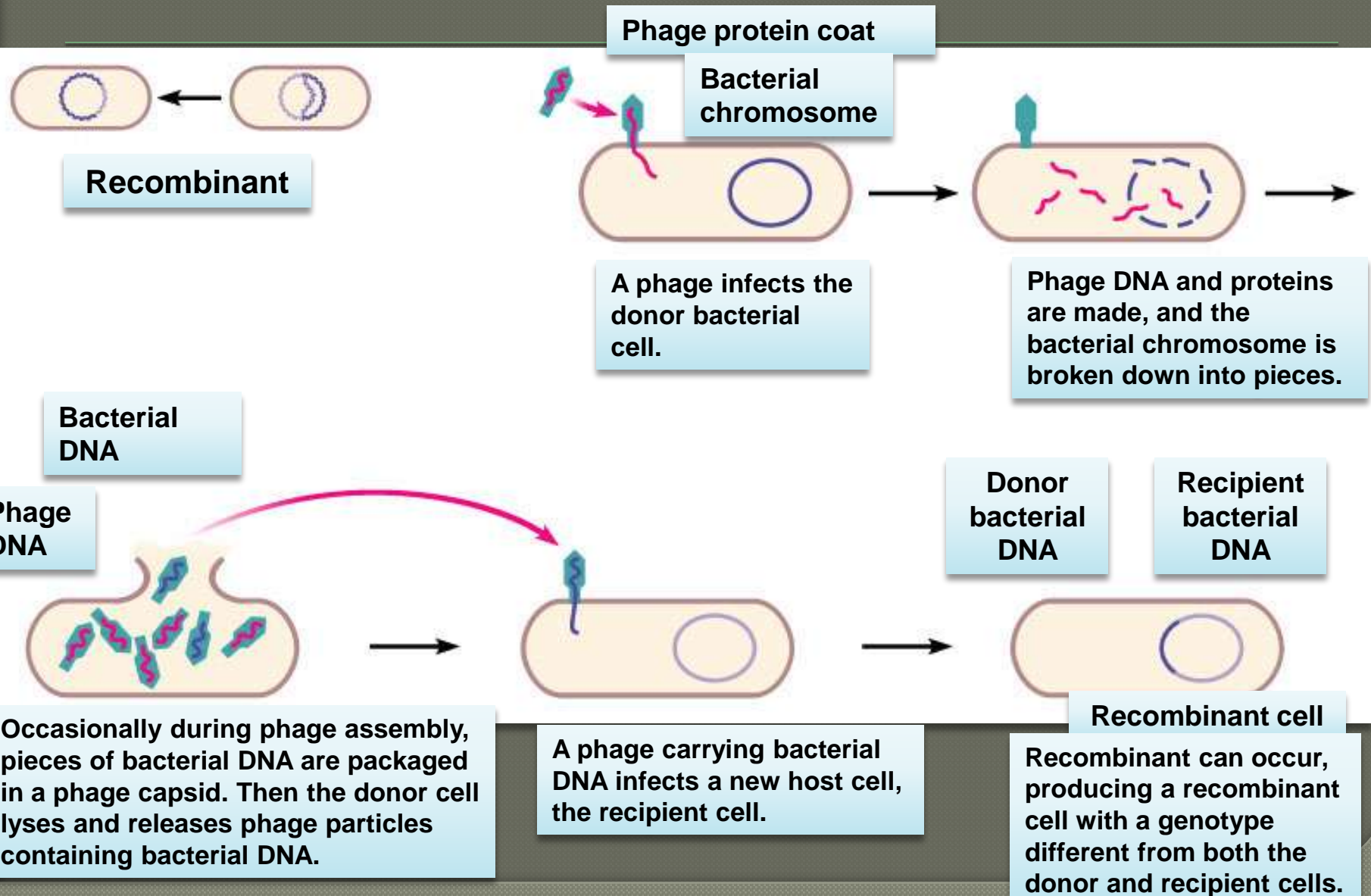
Specialized Transduction

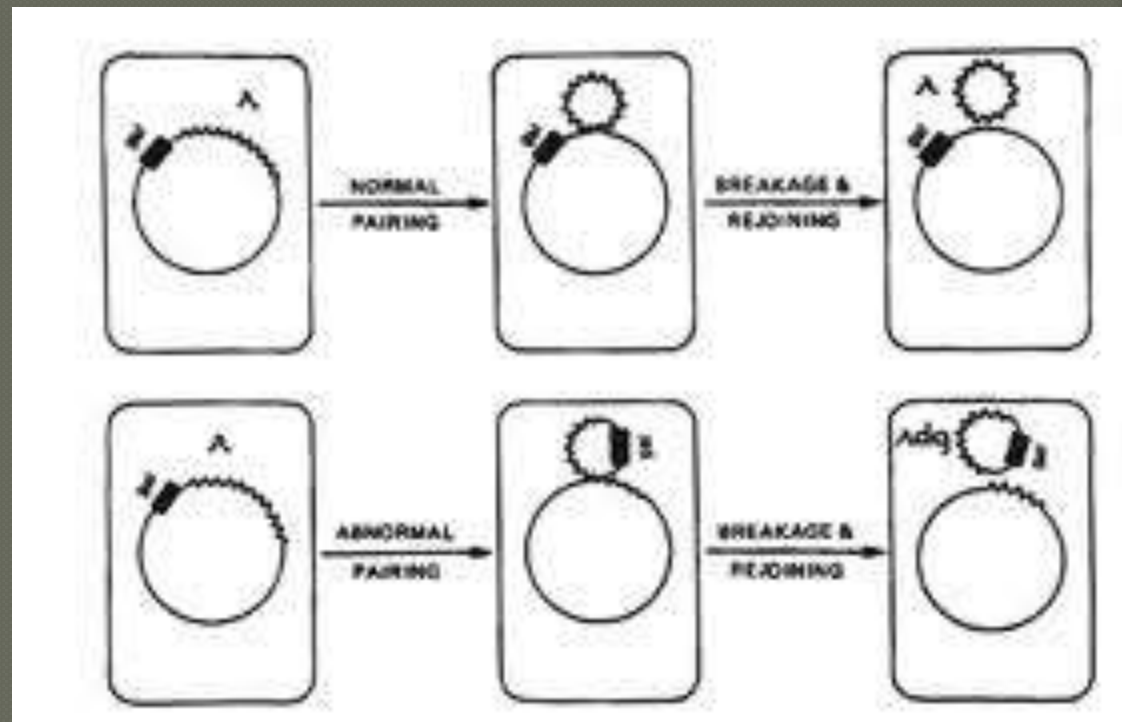
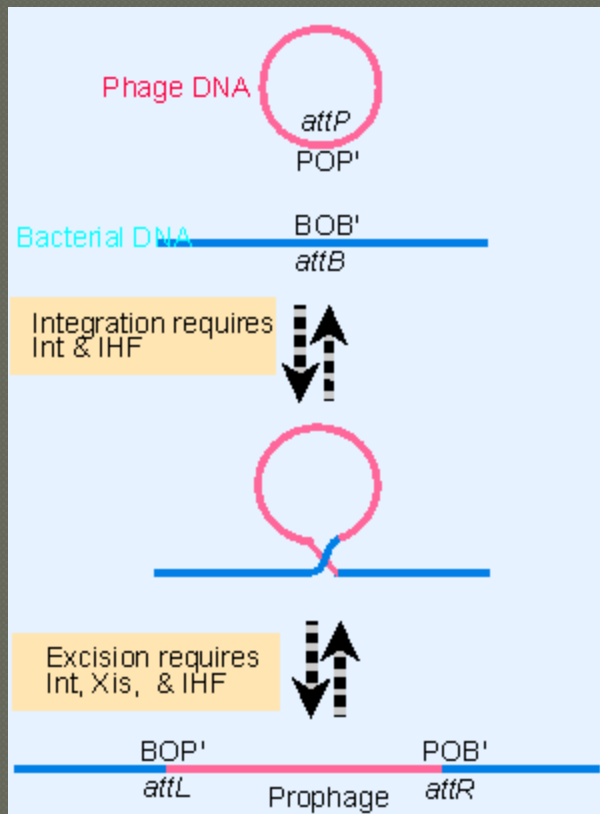
- Some phages can transfer only particular genes to other bacteria.
- Phage lambda (λ) has this property.
- lambda has 2 distinct phases of its life cycle. The “lytic” phase is the same as we saw with the general phage life cycle: the phage infects the cell, makes more copies of itself, then lyses the cell to release the new phage.
- Then it also has lysogenic phase

Lysogenic Phase

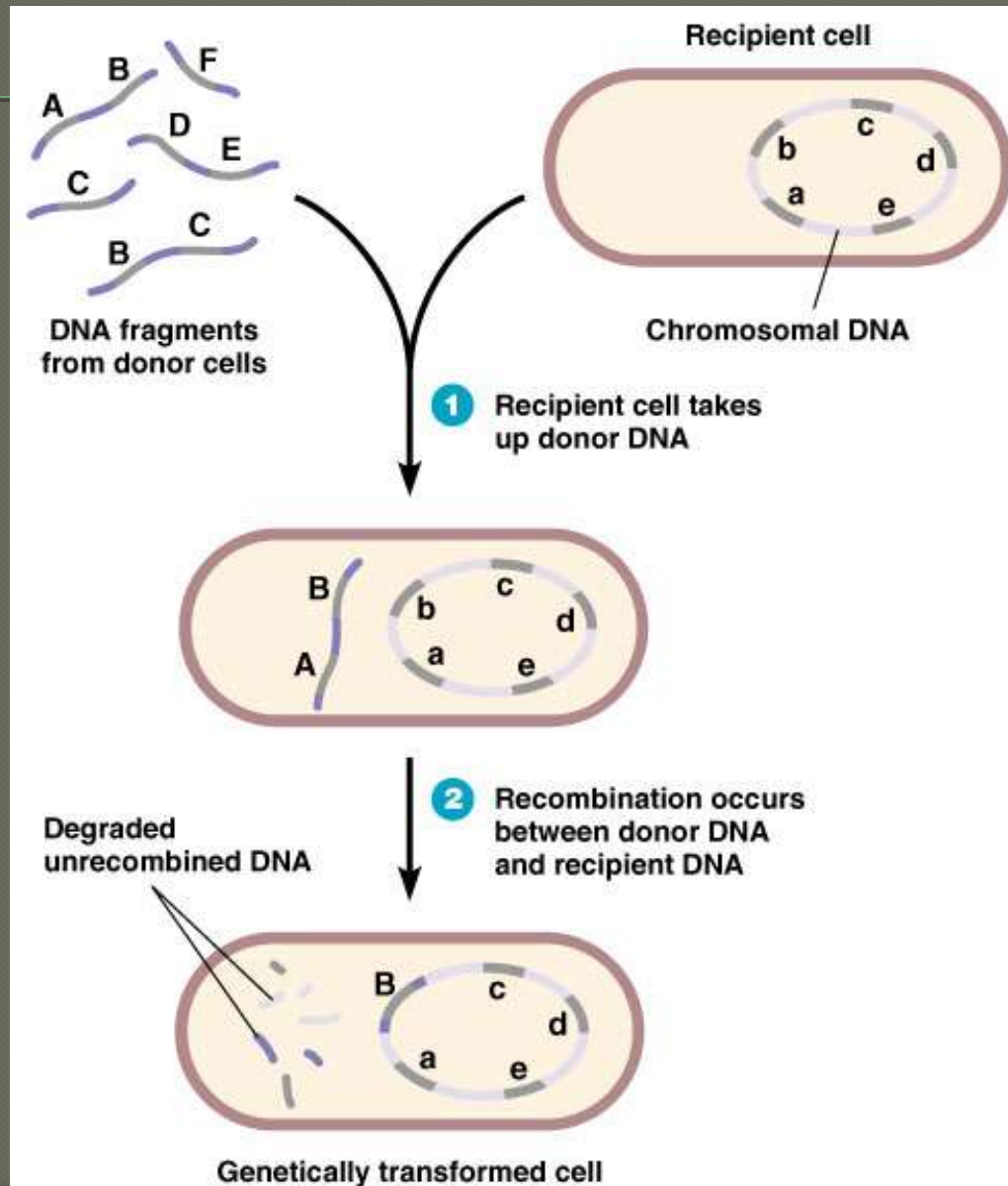
- Once inside the cell, the lambda DNA circularizes, then incorporates into the bacterial chromosome by a crossover
- Once incorporated into the chromosome, the lambda DNA becomes quiescent: its genes are not expressed and it remains a passive element on the chromosome, being replicated along with the rest of the chromosome. The lambda DNA in this condition is called the “prophage”.
- After many generations of the cell, conditions might get harsh. For lambda, bad conditions are signaled when DNA damage occurs.
- When the lambda prophage receives the DNA damage signal, it loops out and has a crossover, removing itself from the chromosome. Then the lambda genes become active and it goes into the lytic phase, reproducing itself, then lysing the cell.

TRANSDUCTION

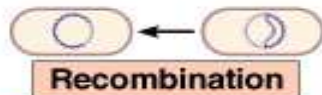




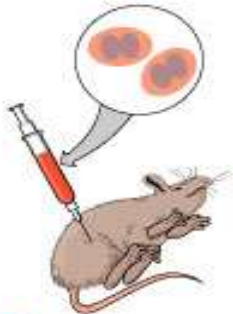
Transformation



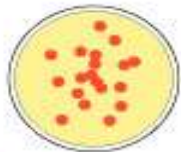
RECALL: TRANSFORMATION



- 1** Living encapsulated bacteria injected into mouse



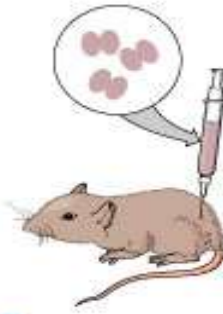
- 2** Mouse died



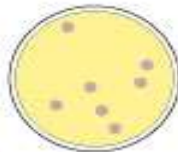
- 3** Colonies of encapsulated bacteria were isolated from dead mouse

(a)

- 1** Living nonencapsulated bacteria injected into mouse



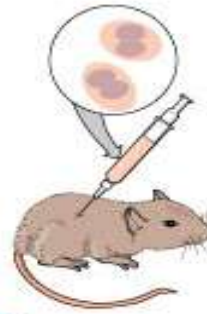
- 2** Mouse remained healthy



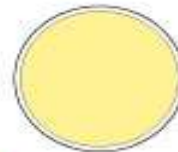
- 3** A few colonies of nonencapsulated bacteria were isolated from mouse; phagocytes destroyed nonencapsulated bacteria

(b)

- 1** Heat-killed encapsulated bacteria injected into mouse



- 2** Mouse remained healthy



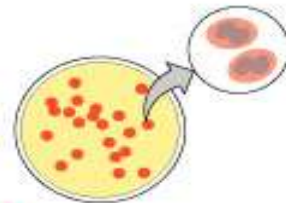
- 3** No colonies were isolated from mouse

(c)

- 1** Living nonencapsulated and heat-killed encapsulated bacteria injected into mouse



- 2** Mouse died



- 3** Colonies of encapsulated bacteria were isolated from dead mouse

(d)