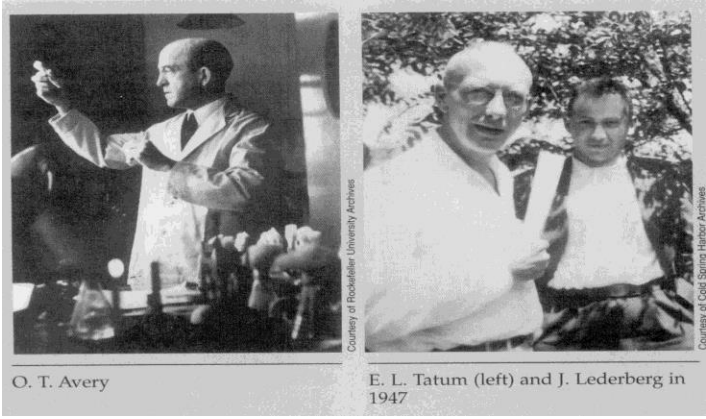
MICROBIAL GENETICS

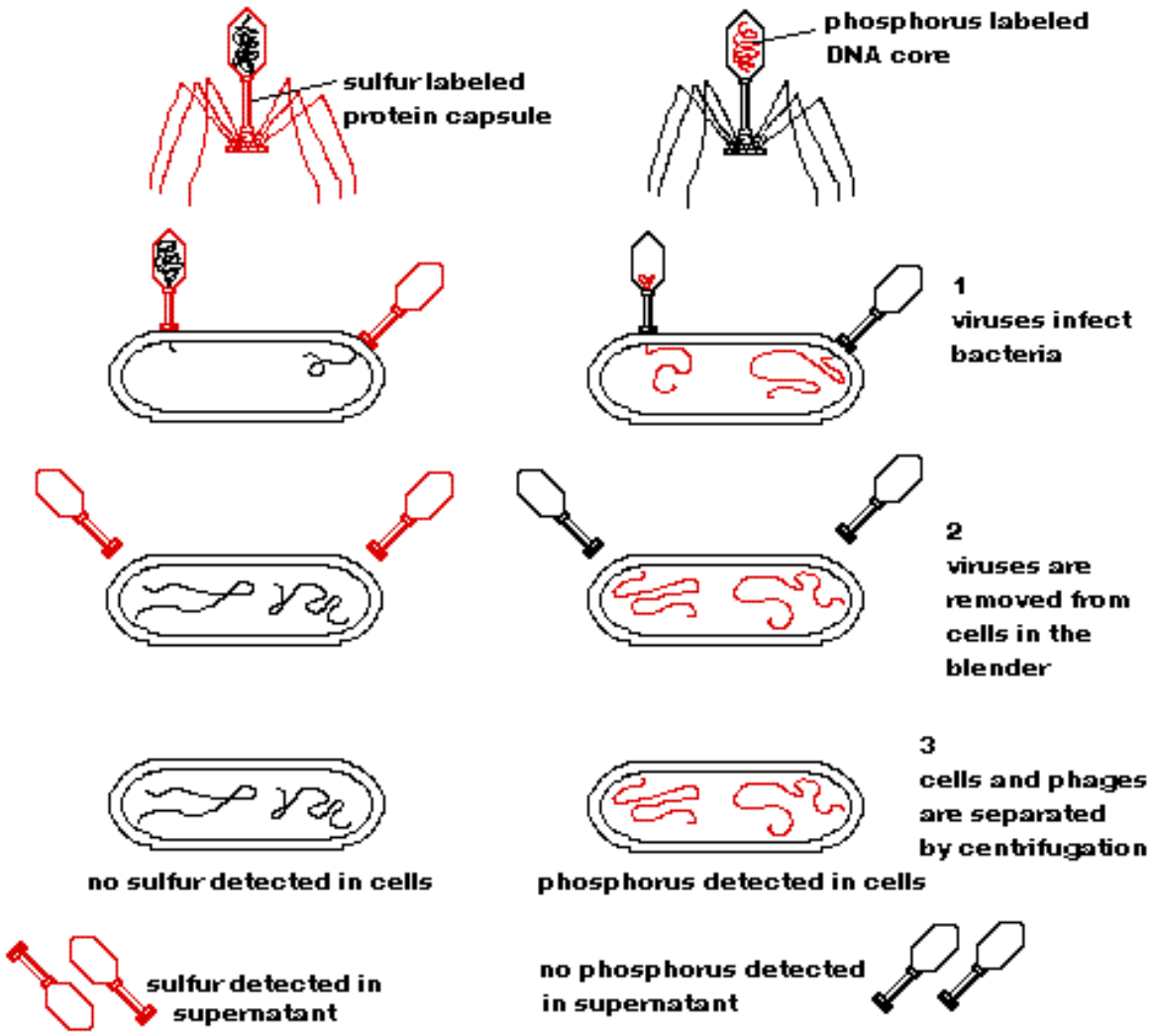
Hereditary material is DNA:

Oswald Avery's demonstration



capsule inactivated	→	Still transformed
proteins inactivated	→	Still transformed
lipids inactivated	→	Still transformed
RNA inactivated	→	Still transformed
DNA inactivated	→	Did not transform
purified DNA	→	transformed

The Hershey-Chase Blender Experiment

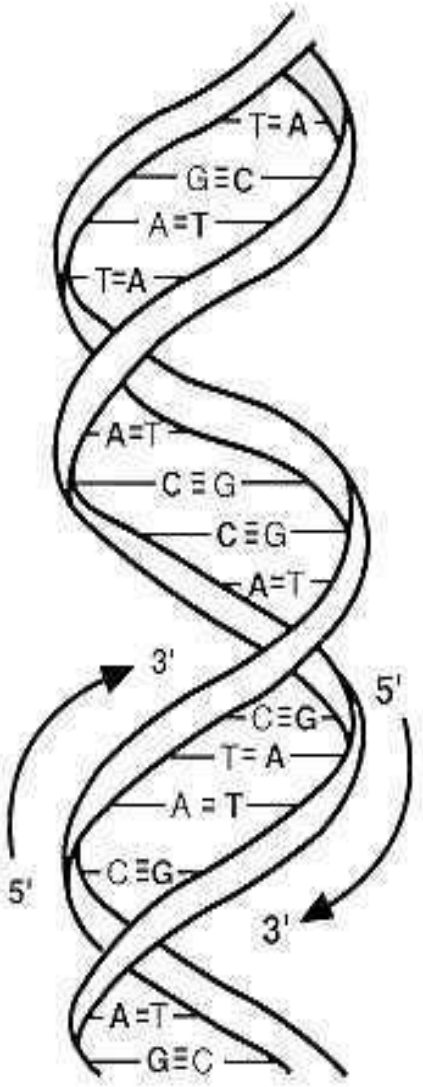


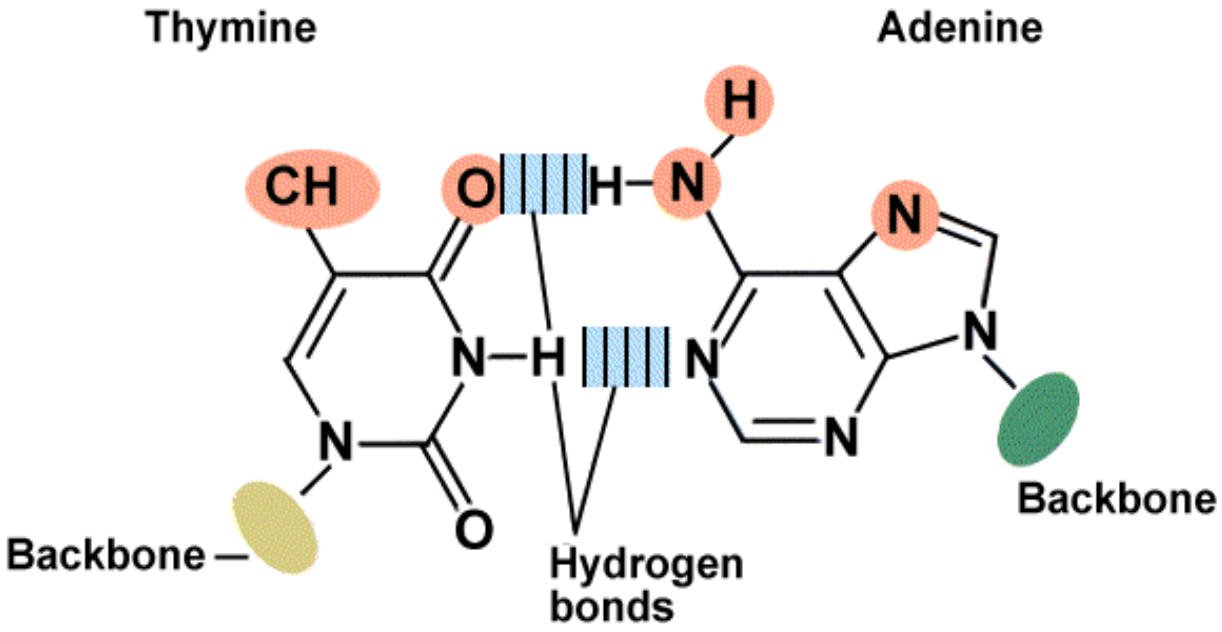
Nucleic Acid Structure

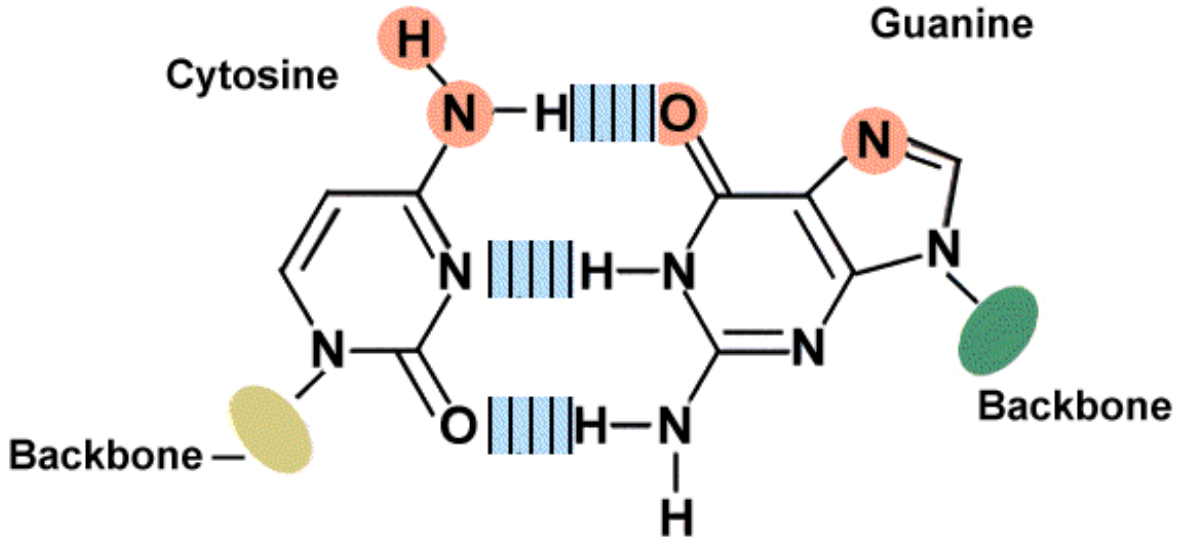
Polymer of nucleotides, each containing a sugar, a phosphate group & a purine (A, G) or pyrimidine (C, T/U)

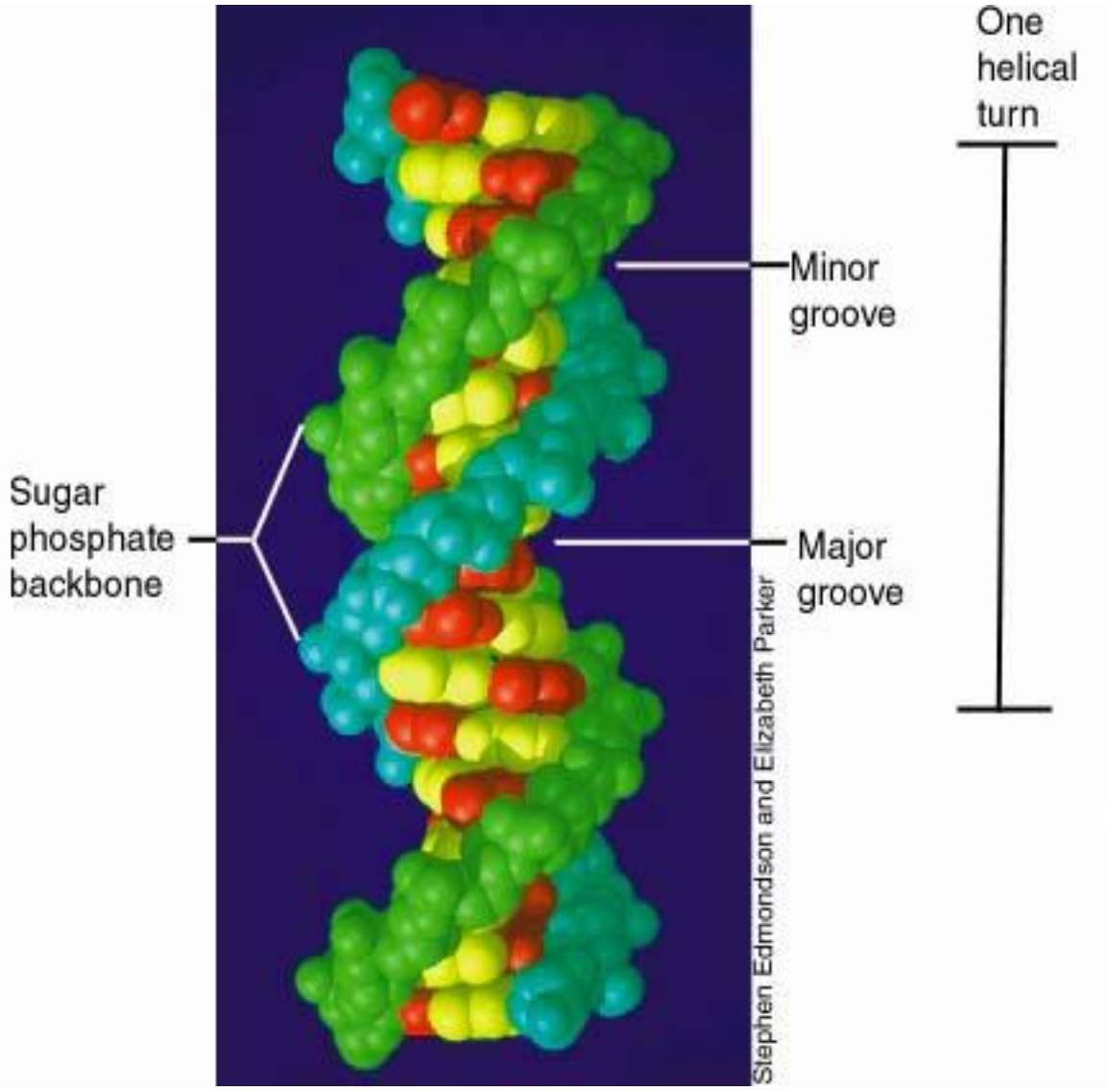
Alternating sugar (3'-OH) & phosphate residues (5'-P) form phosphodiester bond forming backbone of DNA (ribbon).

Double helix, antiparallel strands stabilized by H-bond bet. Pu & Py.

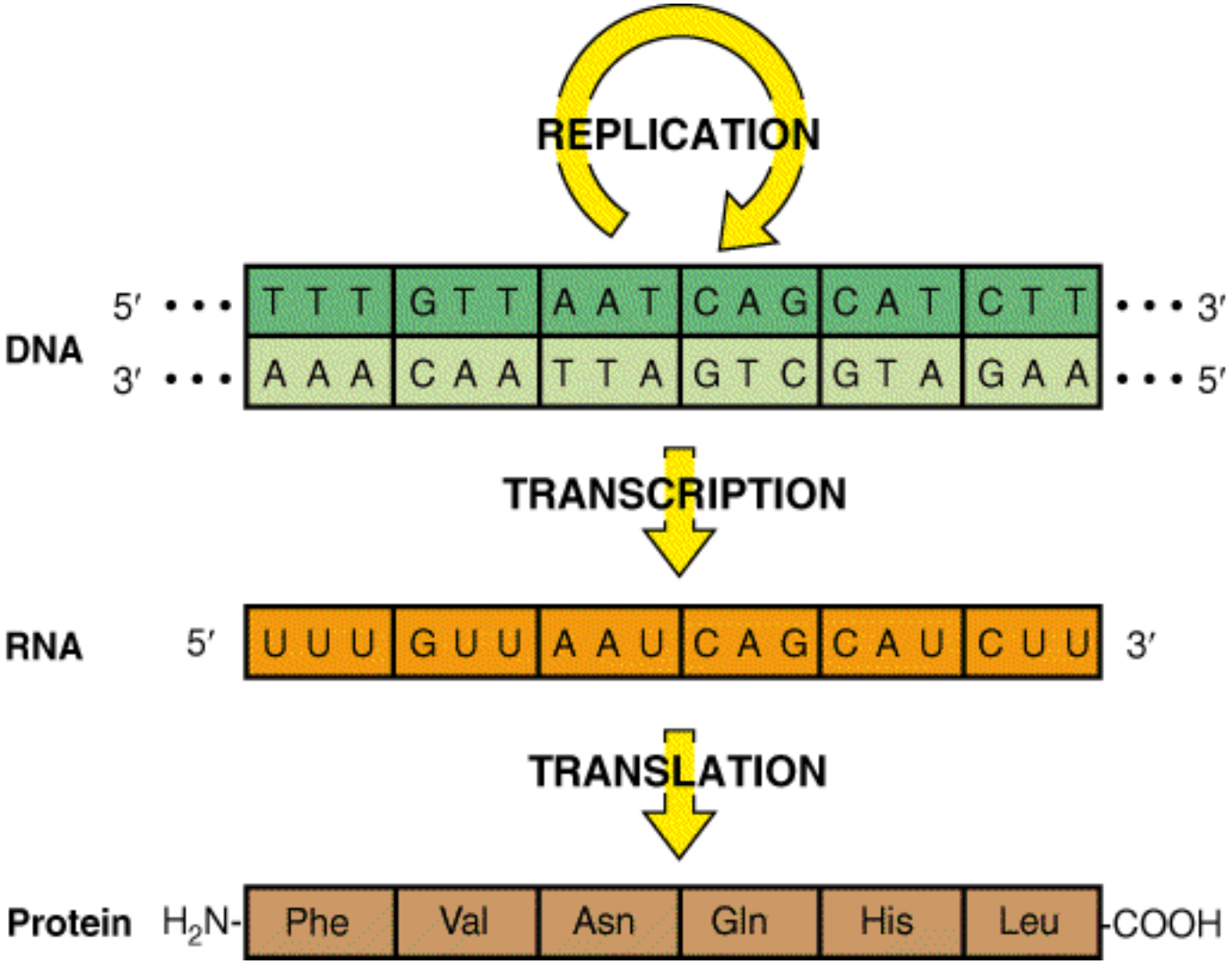



Thymine
Adenine
CH
O
H
N
H
N
N
N
N
N
N
O
Backbone
Backbone
Hydrogen bonds

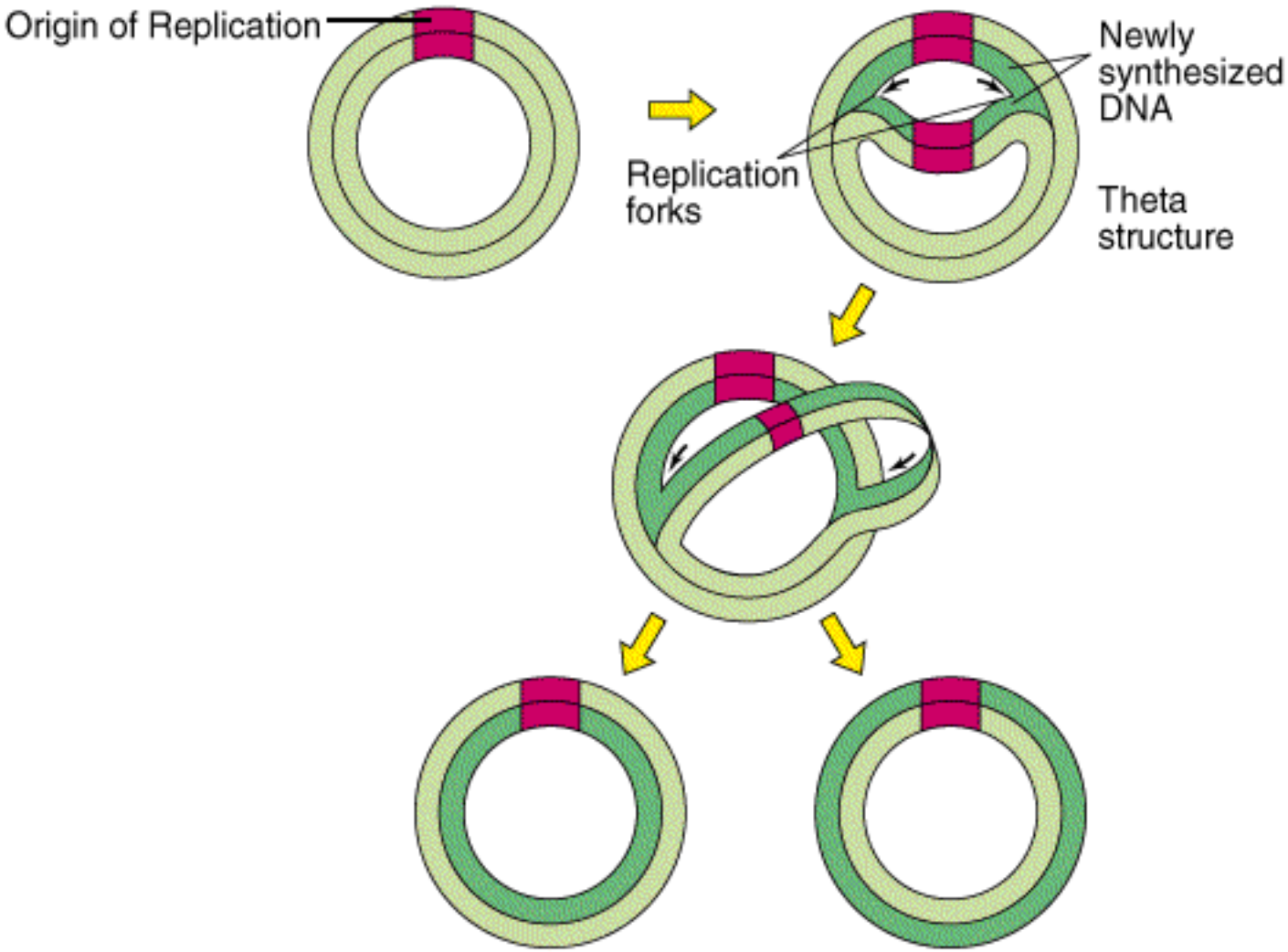

Cytosine
Guanine
H
N
H
O
N
N
H
N
N
N
N
O
H
N
H
Backbone
Backbone


One helical turn
Minor groove
Major groove
Sugar phosphate backbone
Stephen Edmondson and Elizabeth Parker

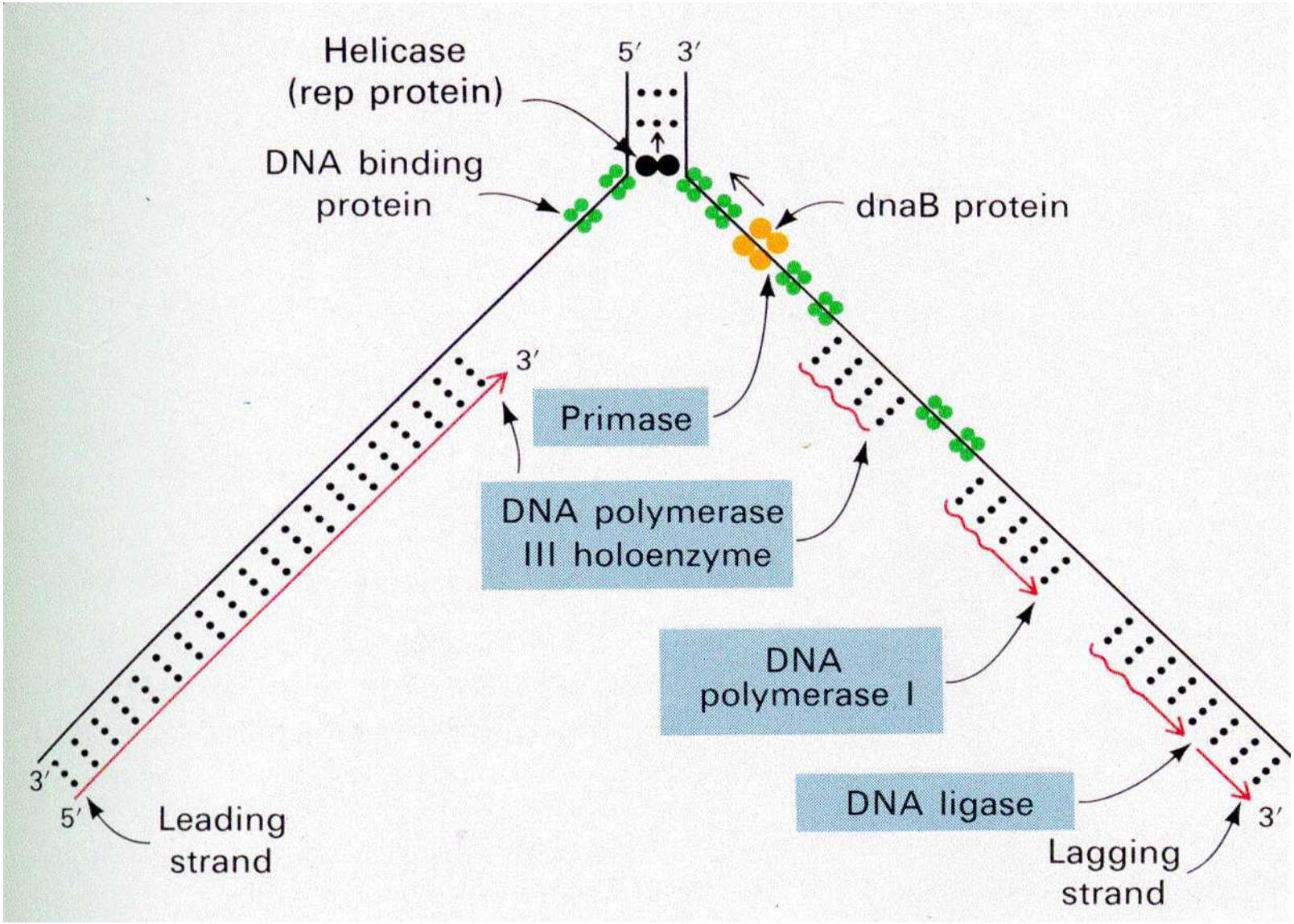
Central Dogma



Replication of the Bacterial Chromosome



DNA Replication: Semiconservative



BACTERIAL GENOMES

AND

GENETIC ELEMENTS

Genomes: Sizes and Numbers of Genes

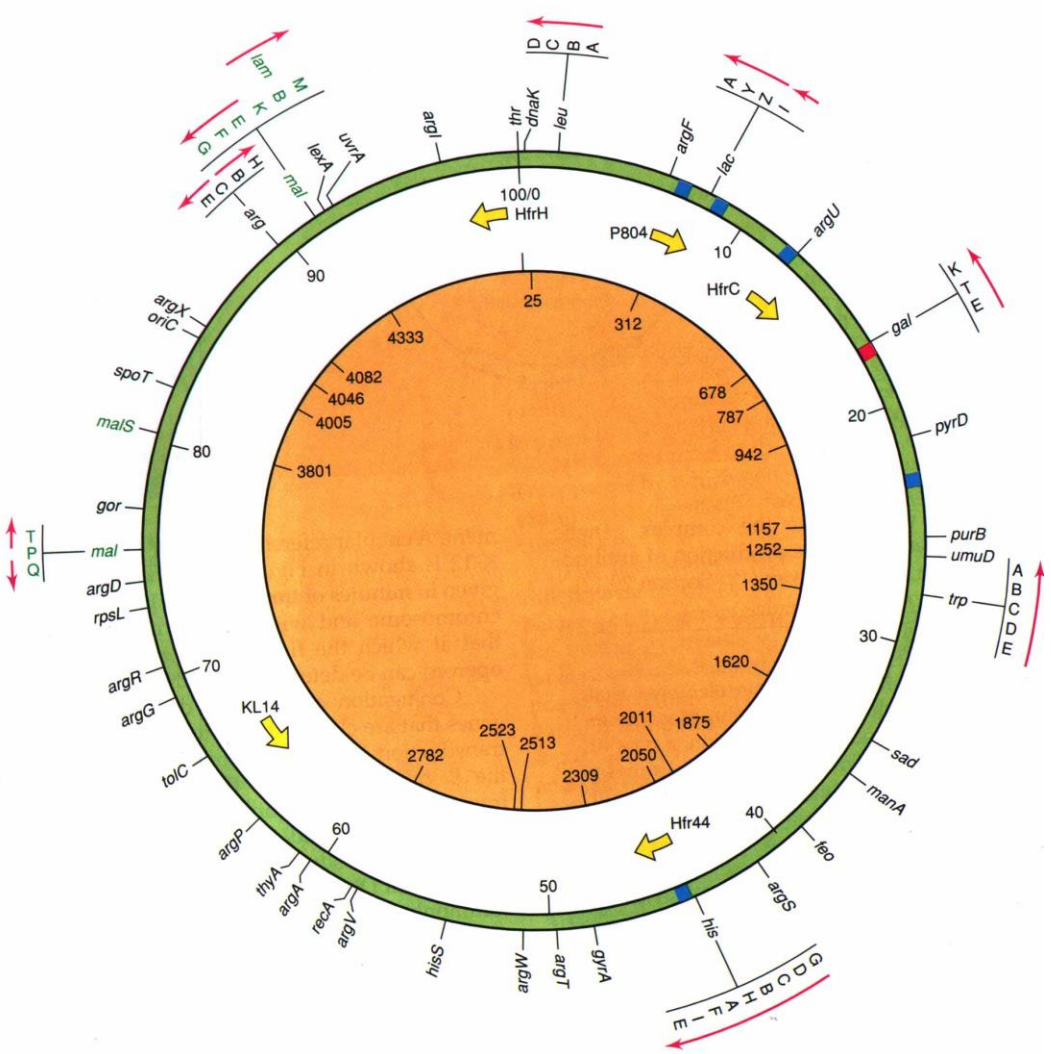
Genome	Group	Size (kb)	No. of genes
Eukaryotic nucleus			
Saccharomyces cerevisiae	Yeast	13,500 (L)	6,000
Homo sapiens	Human	3,000,000 (L)	30,000
Prokaryote			
Escherichia coli	Bacterium	4,700 (C)	4,000
Hemophilus influenzae	Bacterium	1,830 (C)	1,703
Methanococcus jannaschii	Bacterium	1,660 (C)	1,738
Viruses			
T4	Bacterial virus	172 (L/C)	300
HCMV (herpes group)	Human virus	229 (L)	200
F plasmid	In *E. coli*	100 (C)	29
kalilo	In *Neurospora,* a fungus	9 (L)	2

The genome of bacteria

- DNA is arranged in a dense clump called a nucleoid
- single chromosome
- closed, circular double helix of DNA

  Exceptions: *Borrelia* & *Streptomyces* (G+): Linear chromosome, *Agrobacterium tumefaciens*: 1 linear, 1 circular
- Bidirectional replication
- Binary fission
- genes arranged close together with little intergenic space
- functionally related genes are grouped (operon)

Structure of the *E.coli* chromosome



GENETIC ELEMENTS

Plasmids

- Small circular extrachromosomal DNA molecules distinct from the chromosome (some linear e.g. *Borrelia, Streptomyces*)
- Autonomous replication
- Range in size from 1.5 - 400 kb (so codes for a few to as many as a few hundred genes)
- Variable number of copies and size
- Non essential for the basic operation of the cell
- Code for functions for their replication and partition
- Can be transferred between bacteria and may be important in pathogenesis by carrying virulence genes or antibiotic resistance genes

Plasmids

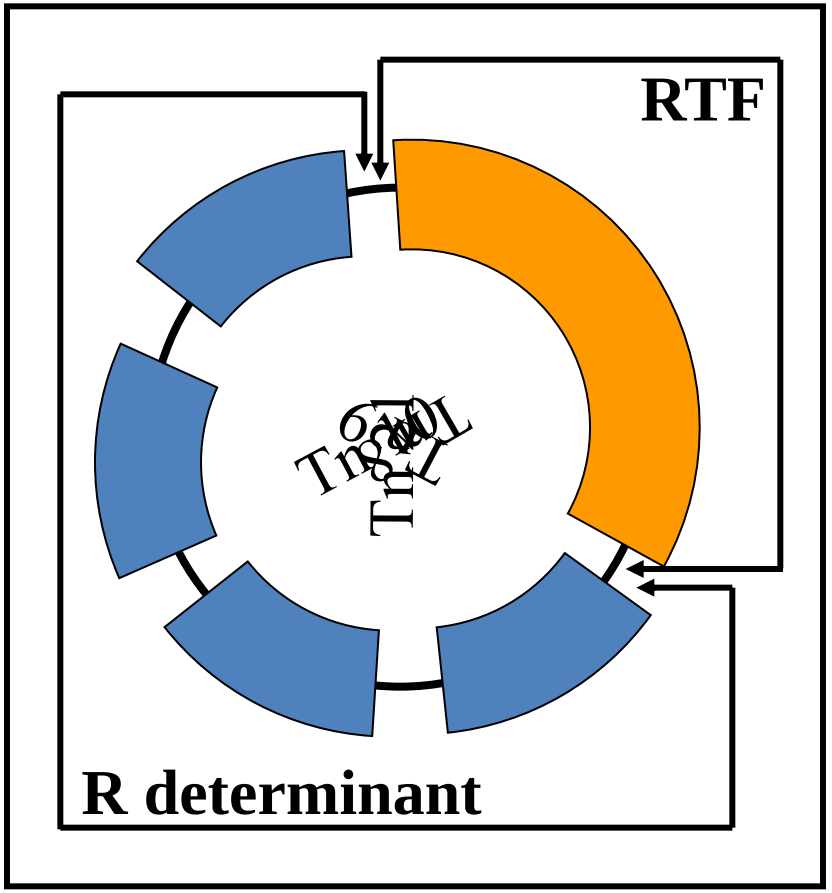
- Definition: Extrachromosomal genetic elements that are capable of autonomous replication (replicon)
- Episome - a plasmid that can integrate into the chromosome

Classification of Plasmids

- Transfer properties
  - Conjugative
  - Nonconjugative
- Phenotypic effects
  - Fertility
  - Bacteriocinogenic plasmid
  - Resistance plasmid (R factors)

Structure of R Factors

- RTF
  - Conjugative plasmid
  - Transfer genes
- R determinant
  - Resistance genes
  - Transposons



Plasmid Phenotypes

I. <u>Virulence factors</u>: toxins, adhesins, iron acquistion systems, etc.

II. <u>Antibiotic resistance</u>: R (resistance) plasmids can encode several different antibiotic resistance determinants

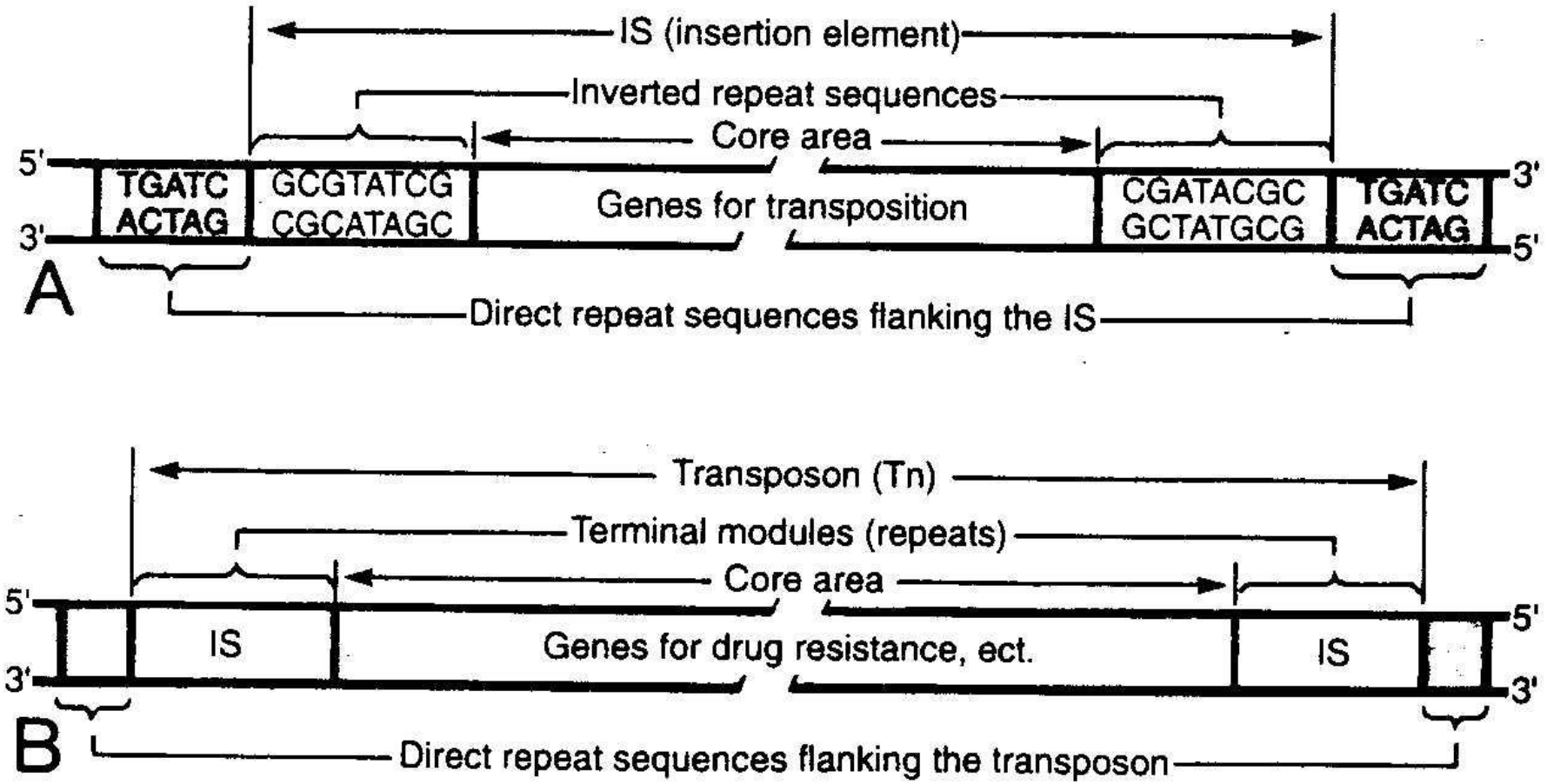
III. Conjugation plasmids- F factor

Plasmids

- Copy number – fixed or relaxed
  -- low or high
- Compatible or noncompatible
- May be lost- ‘curing’
- Can be mobilized

Insertion sequences

- Pieces of DNA that can move in the chromosome
- Insertion can cause mutations of a gene or a whole operon
- Vehicles are often plasmids and phages
- Flanked by inverted repeats & inner fragment codes for a transposase, necessary for its transposition.
- Not self replicating, must integrate to other replicons to be maintained stably in bacterial genome.


IS (insertion element)
Inverted repeat sequences
Core area
5'
3'
TGATC
ACTAG
GCGTATCG
CGCATAGC
Genes for transposition
CGATACGC
GCTATGCG
TGATC
ACTAG
3'
5'
A
Direct repeat sequences flanking the IS
Transposon (Tn)
Terminal modules (repeats)
Core area
5'
3'
IS
Genes for drug resistance, ect.
IS
5'
3'
B
Direct repeat sequences flanking the transposon

Transposons

- IS that carry additional pieces of DNA
- flanked by IR
- previously known as R factors, confer

resistance to antibiotics

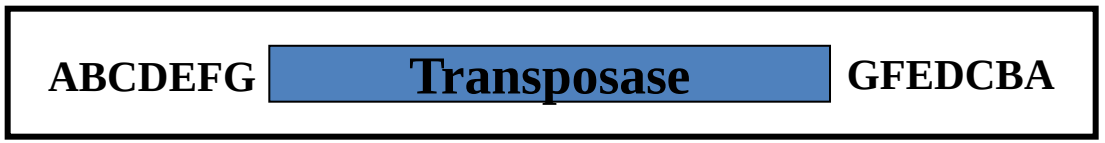
- they can also code for virulence factors e.g. toxins, adherence

Transposable Genetic Elements

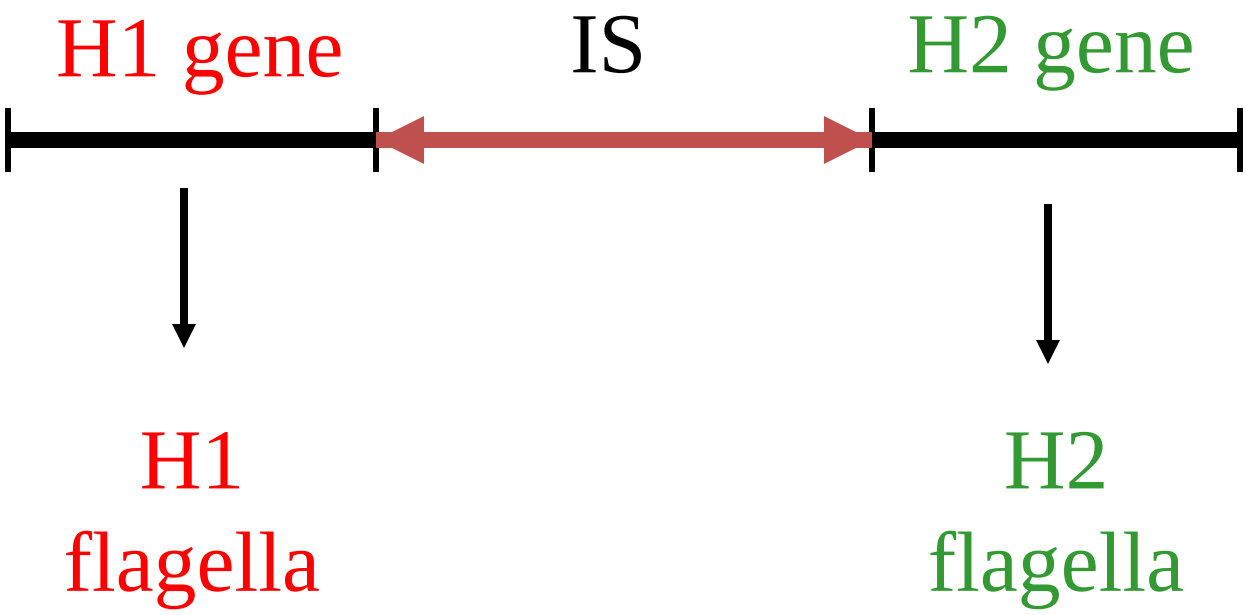
- Definition: Segments of DNA that are able to move from one location to another
- Properties
  - "Random" movement
  - Not capable of self replication
  - Transposition mediated by site-specific recombination
    - Transposase
  - Transposition may be accompanied by duplication

Types of Transposable Genetic Elements

- Insertion sequences (IS)
  - Definition: Elements that carry no other genes except those involved in transposition
  - Nomenclature - IS1
  - Structure
  - Importance
    - Mutation
    - Plasmid insertion
    - Phase variation

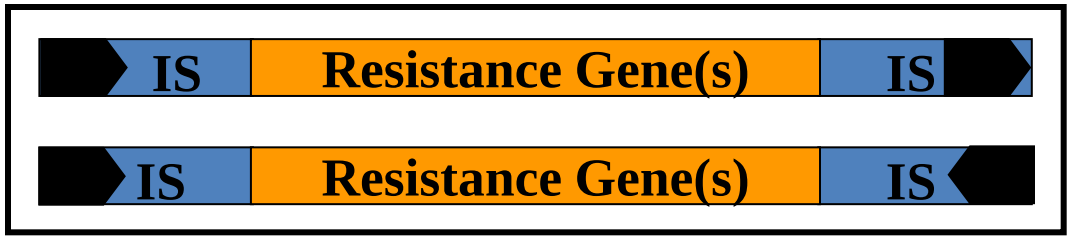


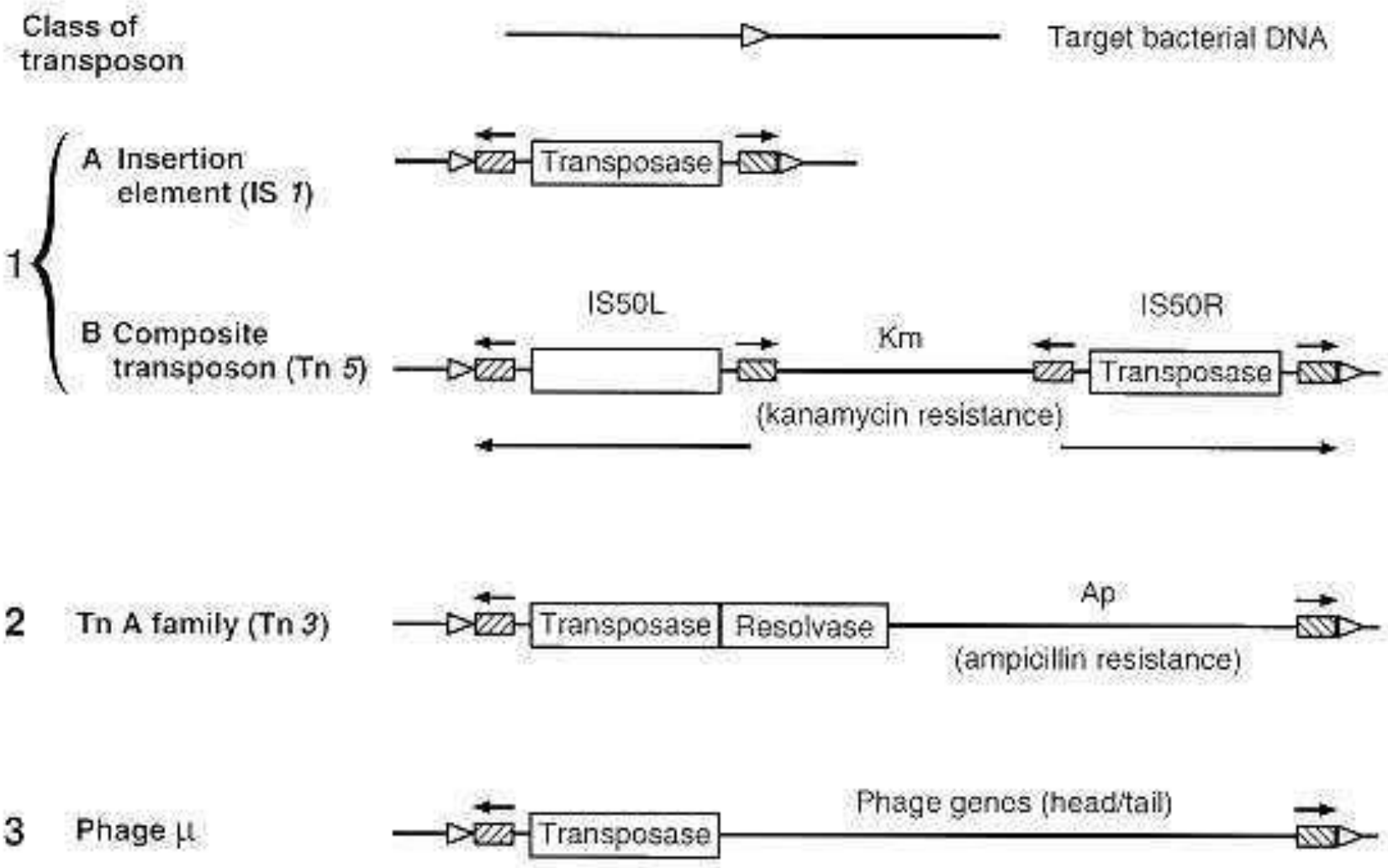
Phase Variation in *Salmonella* H Antigens



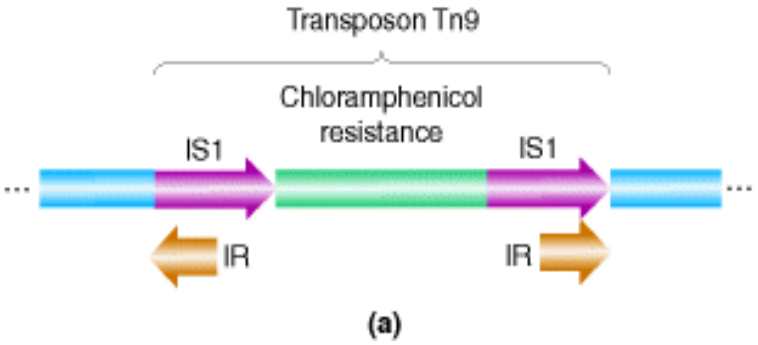
Types of Transposable Genetic Elements

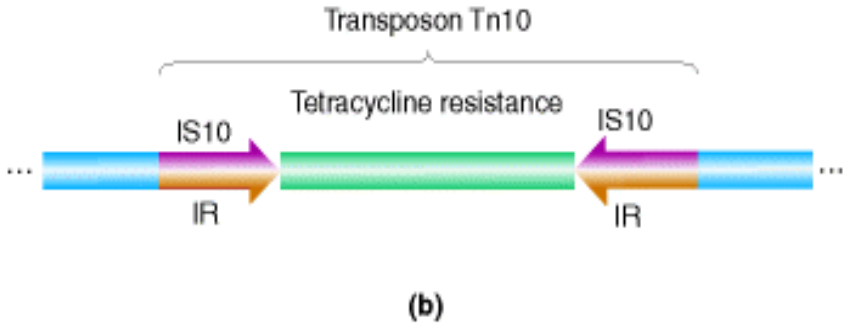
- Transposons (Tn)
  - Definition: Elements that carry other genes except those involved in transposition
  - Nomenclature - Tn10
  - Structure
    - Composite Tns
  - Importance
    - Antibiotic resistance



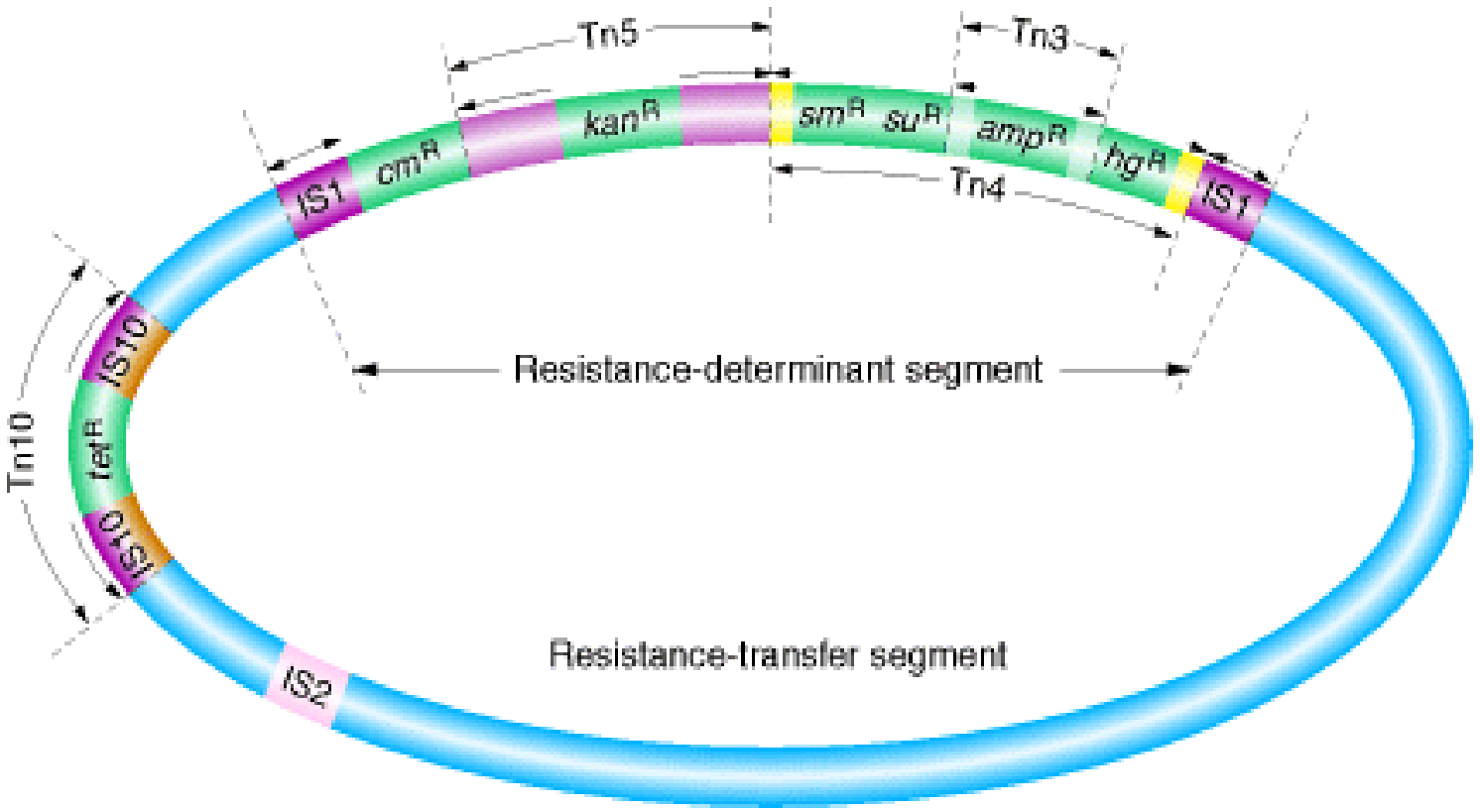

Class of transposon
Target bacterial DNA
1
A Insertion element (IS 1)
Transposase
B Composite transposon (Tn 5)
IS50L
IS50R
Km
Transposase
(kanamycin resistance)
2
Tn A family (Tn 3)
Transposase
Resolvase
Ap
(ampicillin resistance)
3
Phage μ
Transposase
Phage genes (head/tail)

Two different transposons having different inverted repeat (IR) regions and carrying different drug-resistance genes.





Plasmid carrying many antibiotic-resistance genes



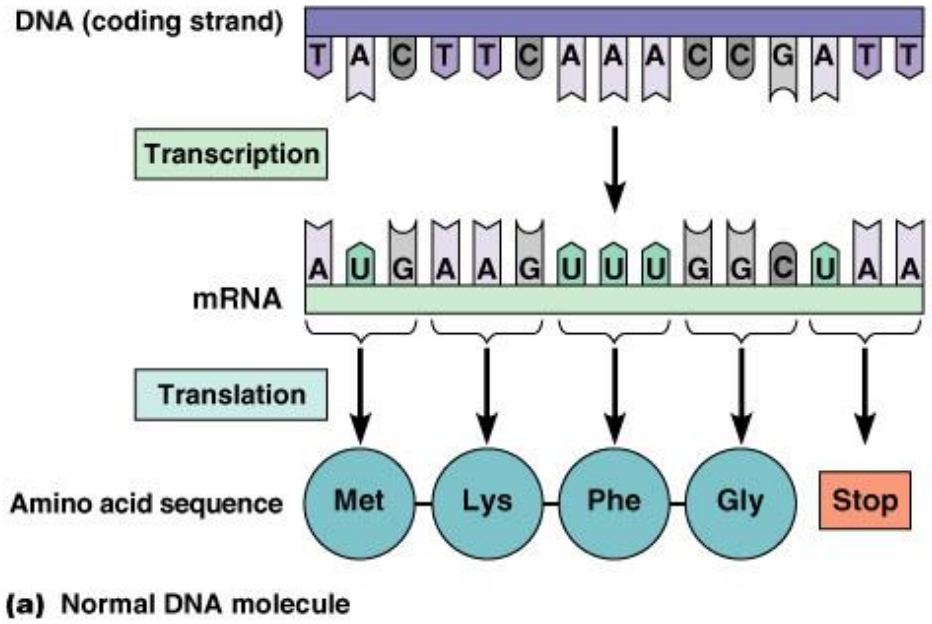
•From S. N. Cohen and J. A Shapiro, “Transposable Genetic Elements.” Copyright © 1980 by Scientific American, Inc.

Mutation

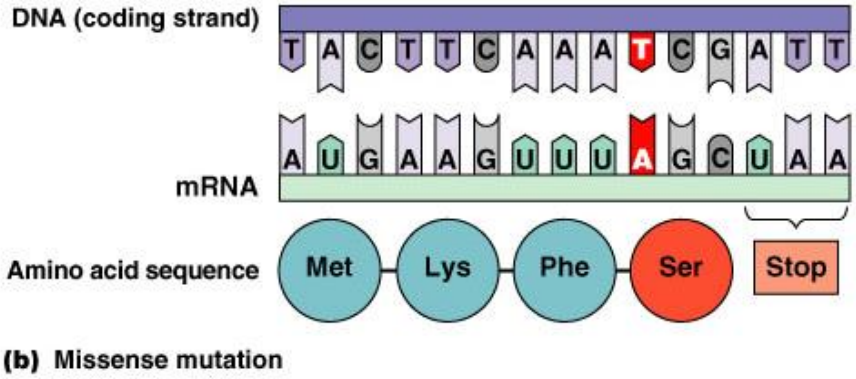
- Change in the genetic material
- Mutations may be neutral, beneficial, or harmful
- Mutagen: Agent that causes mutations
- Spontaneous mutations: Occur in the absence of a mutagen
- Spontaneous mutation rate = 1 in 10^9 replicated base pairs or 1 in 10^6 replicated genes
- Mutagens increase to 10^{-5} or 10^{-3} per replicated gene

Mutation

- Base substitution (point mutation)
  - Change in one base
- Missense mutation
  - Result in change in amino acid



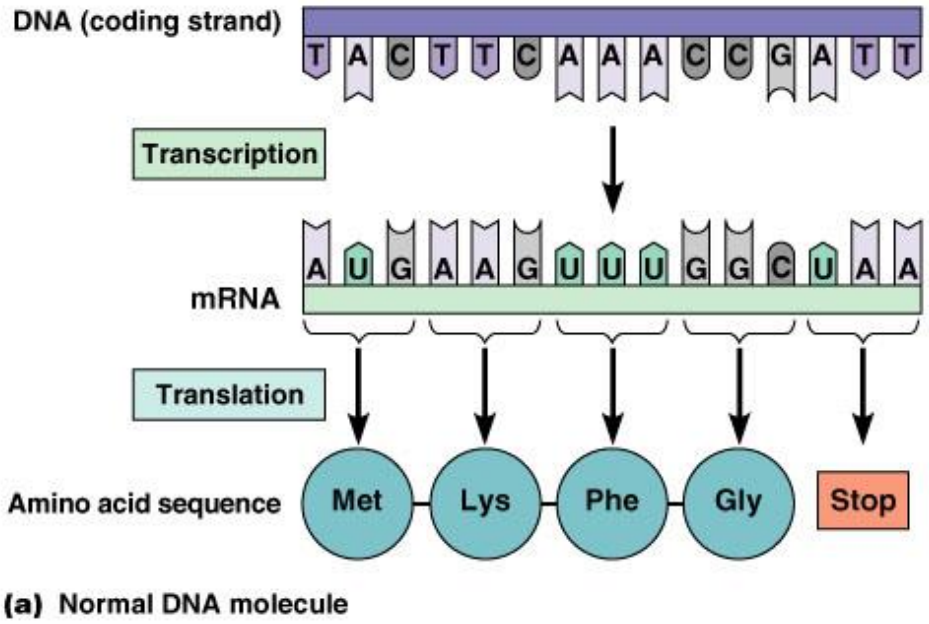
(a) Normal DNA molecule



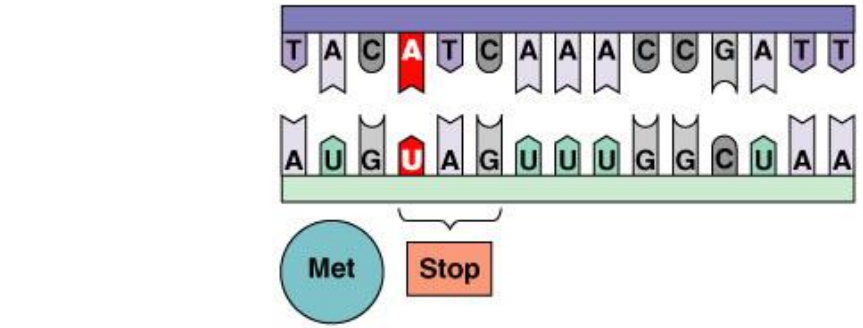
(b) Missense mutation

Mutation

- Nonsense mutation
- Results in a nonsense codon



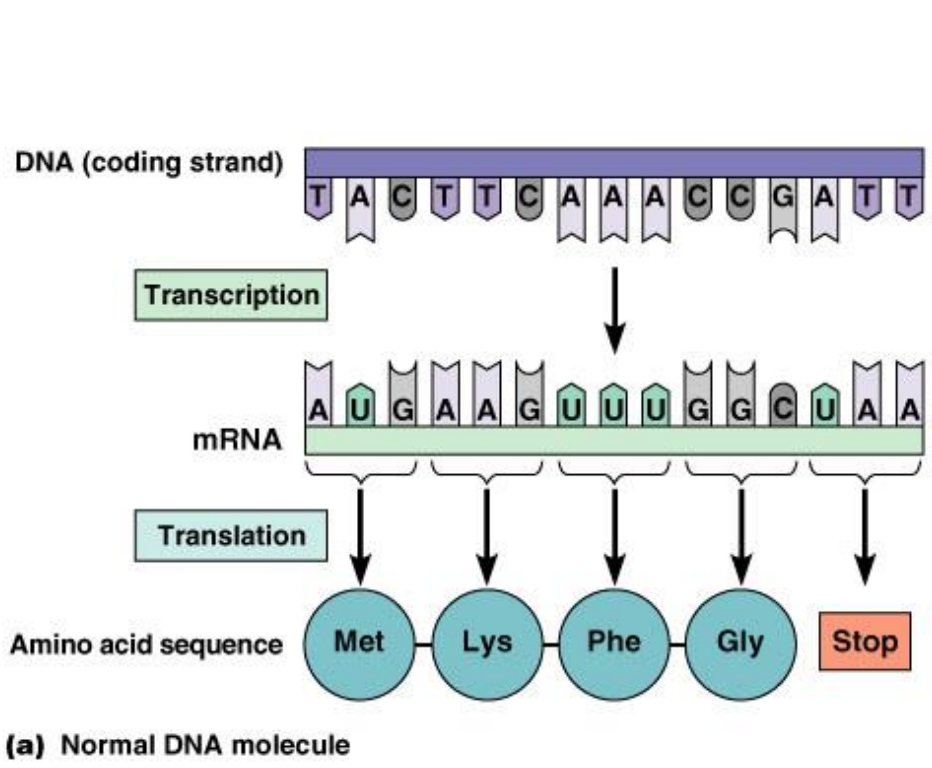
(a) Normal DNA molecule



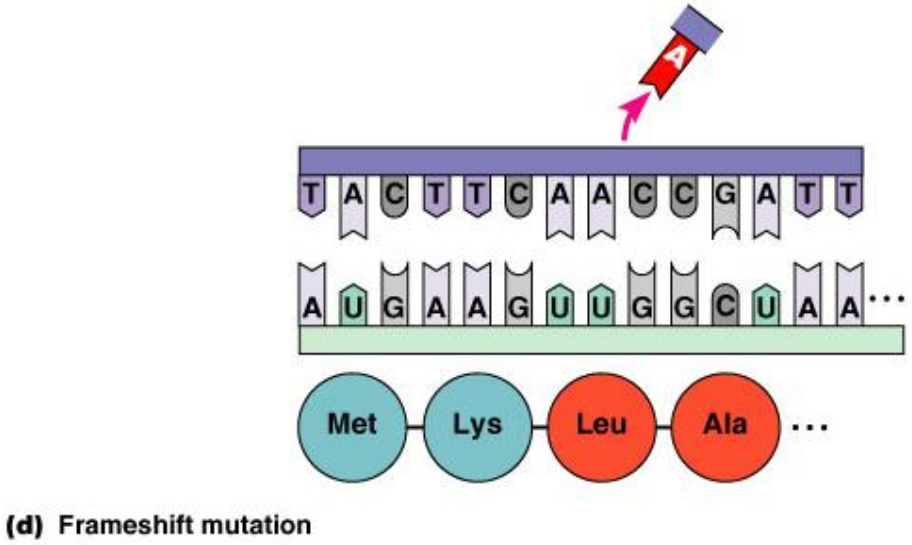
(c) Nonsense mutation

Mutation

- Frameshift mutation
- Insertion or deletion of one or more nucleotide pairs



(a) Normal DNA molecule



(d) Frameshift mutation

Recombination in bacteria

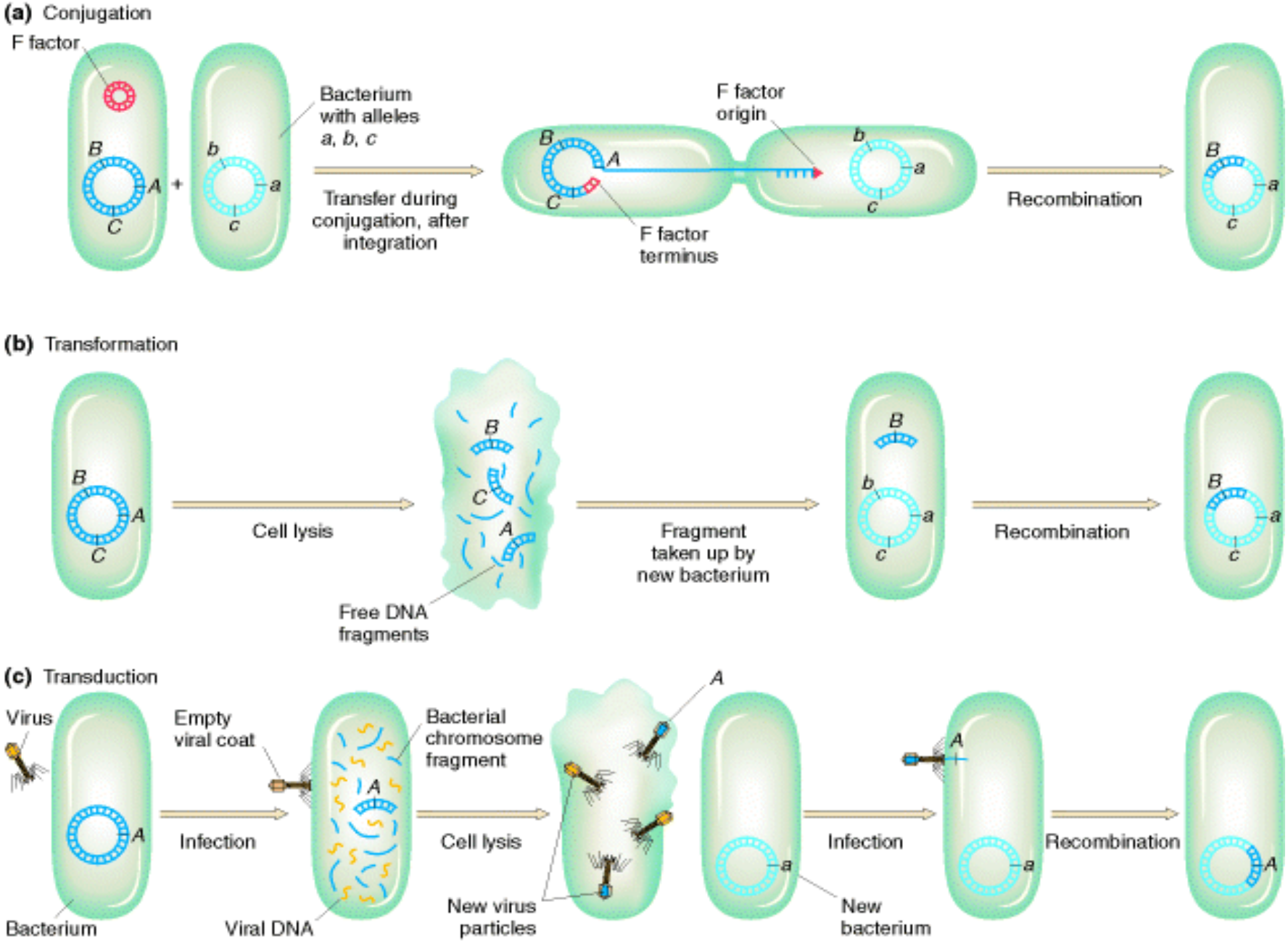
- Reassortment of genetic material
- Generalized: *rec*A dependent
- Specialized
- Replicative
- nonreplicative

Mechanisms of gene transfer

- Conjugation
- Transformation
- Transduction

General Features of Gene Transfer in Bacteria

- Unidirectional
  - Donor to recipient
- Donor does not give an entire chromosome
  - Merozygotes
- Gene transfer can occur between species

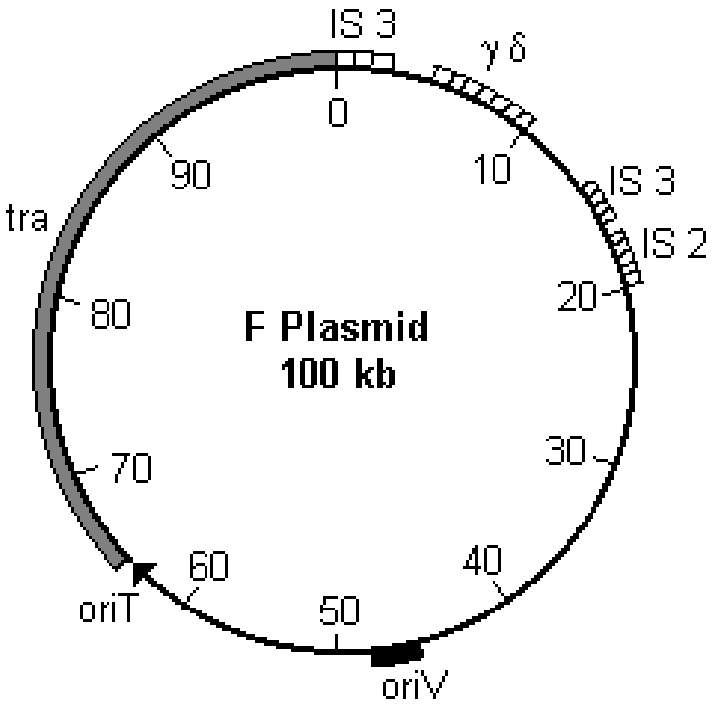

(a) Conjugation
F factor
B
A
C
b
a
c
Bacterium with alleles a, b, c
Transfer during conjugation, after integration
F factor origin
F factor terminus
Recombination
(b) Transformation
Cell lysis
Free DNA fragments
Fragment taken up by new bacterium
Recombination
(c) Transduction
Virus
Bacterium
Infection
Empty viral coat
Viral DNA
Bacterial chromosome fragment
Cell lysis
New virus particles
Infection
New bacterium
Recombination

Conjugation

- Discovery of the **fertility factor** (F) in 1953 by William Hayes
- genetic transfer occurred in one direction, not reciprocal.
- One cell acts as donor ‘male’, and the other as the recipient ‘female’
- the ability to **transfer** imposed by a **fertility factor (F).**
- **F+ and F- strains**

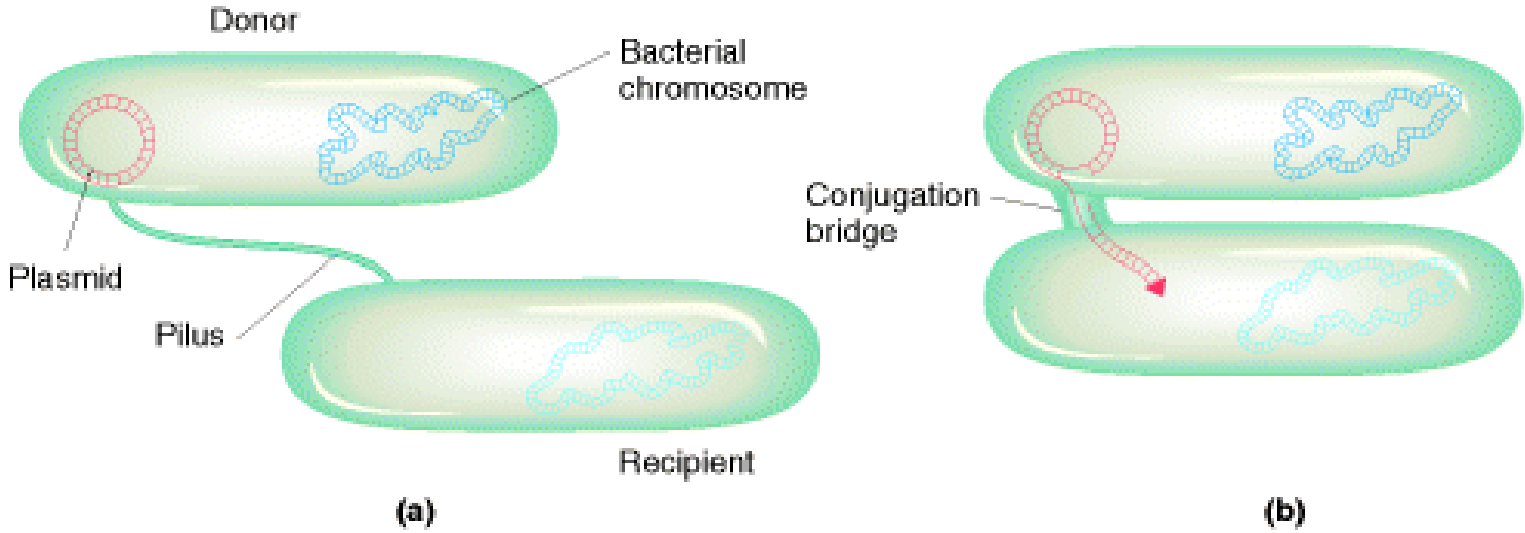
The F plasmid

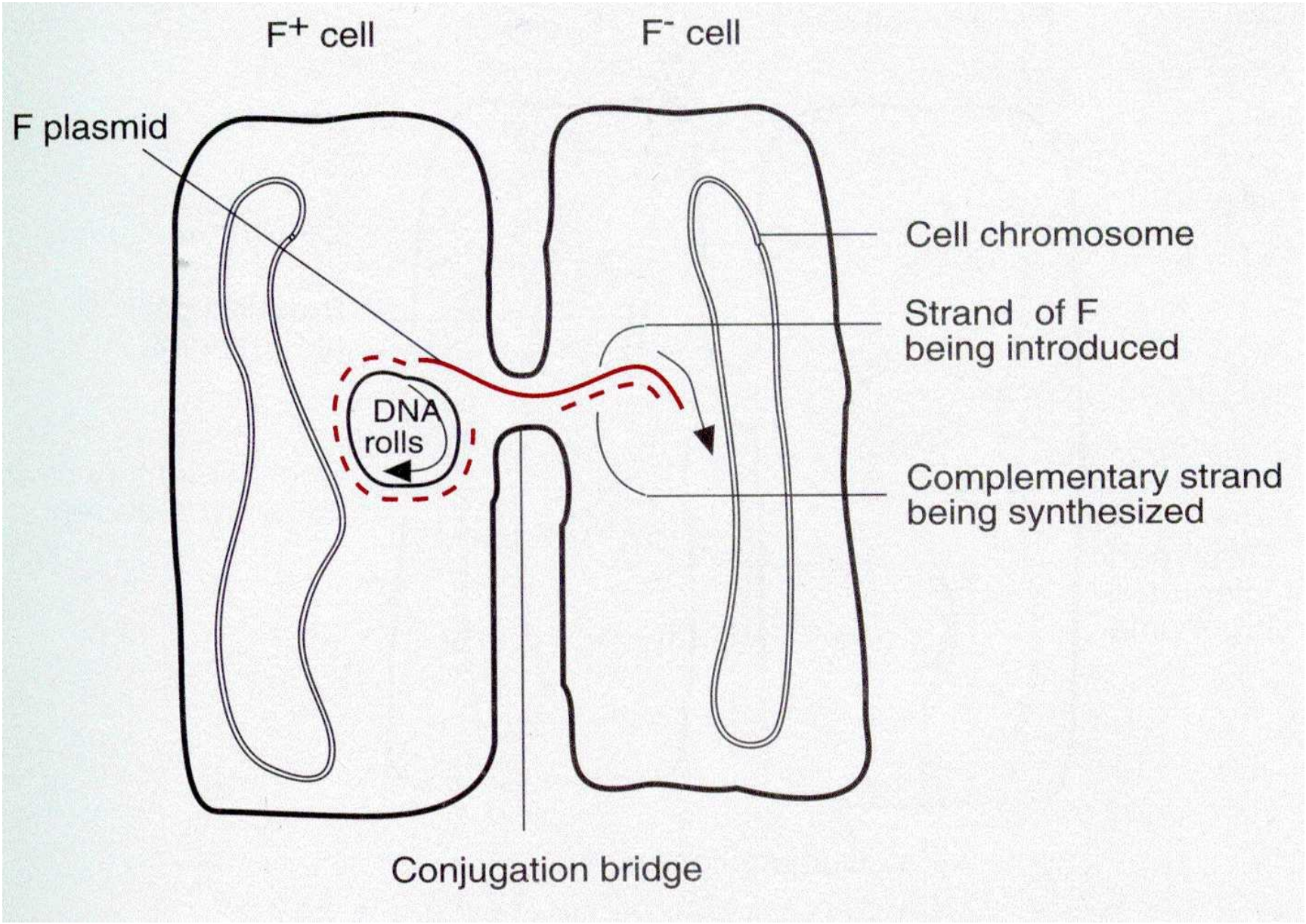
F- plasmid has about 25 tra genes for expression of F pili, synthesis & transfer of DNA during mating, interference to serve as receipient, etc.



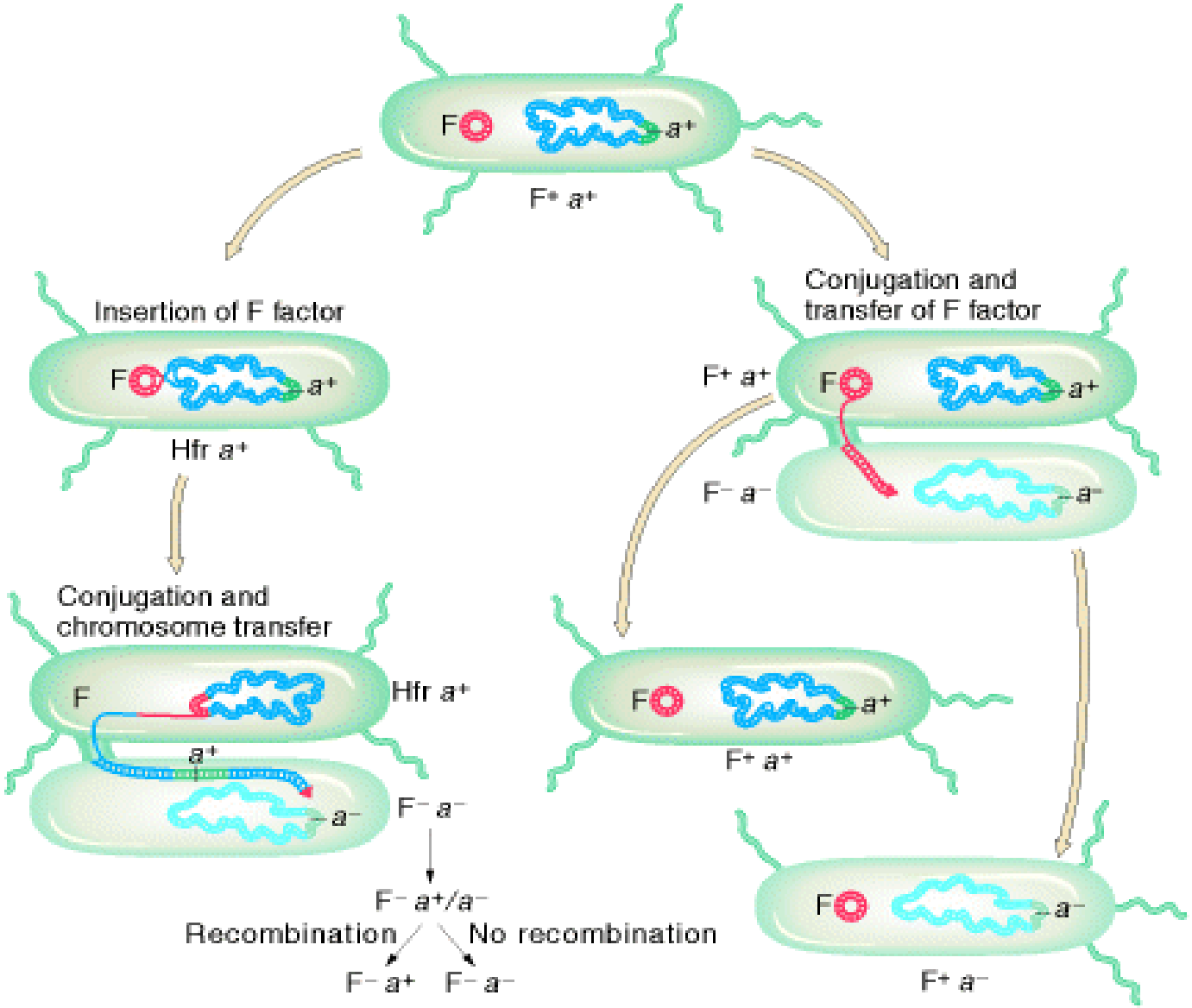
IS 3 & IS 2 = insertion sequences
γδ = transposon Tn1000
oriV = origin of replication
oriT = origin of conjugal transfer
tra = tra functions

Transfer of F plasmid by conjugation

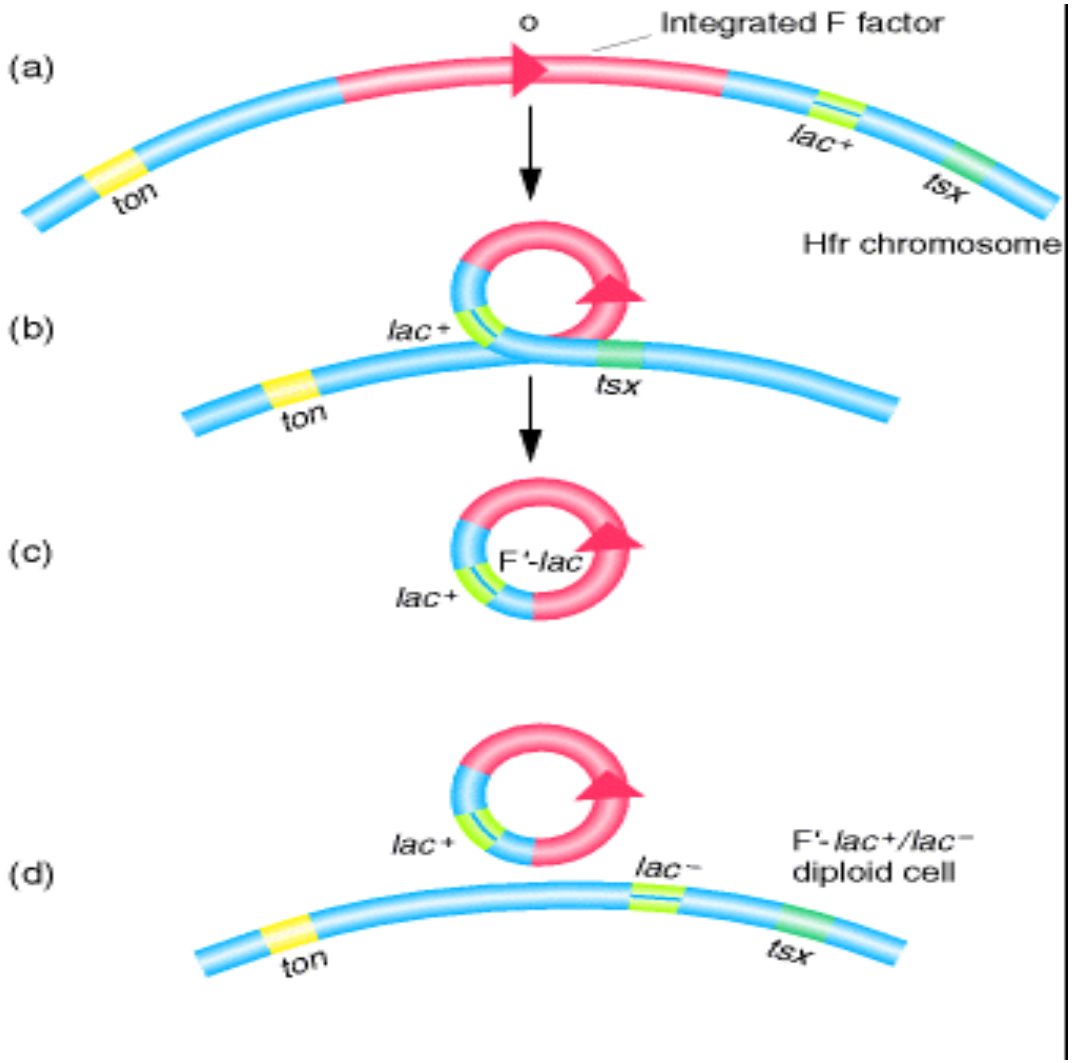



F+ cell
F- cell
F plasmid
DNA rolls
Cell chromosome
Strand of F being introduced
Complementary strand being synthesized
Conjugation bridge

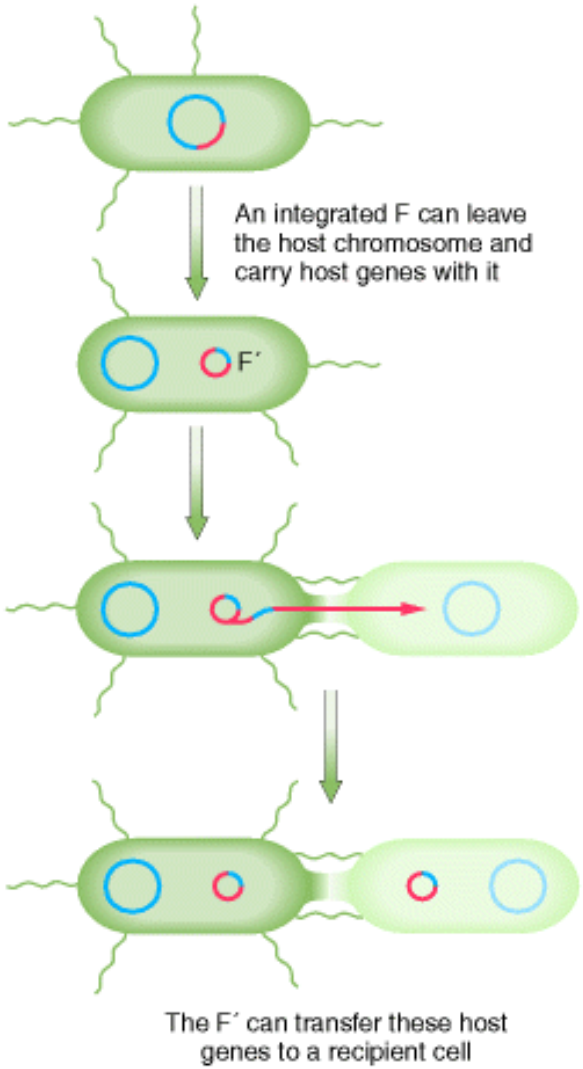
Events that take place in the conjugational cycle of *E. coli.*



F’ plasmid

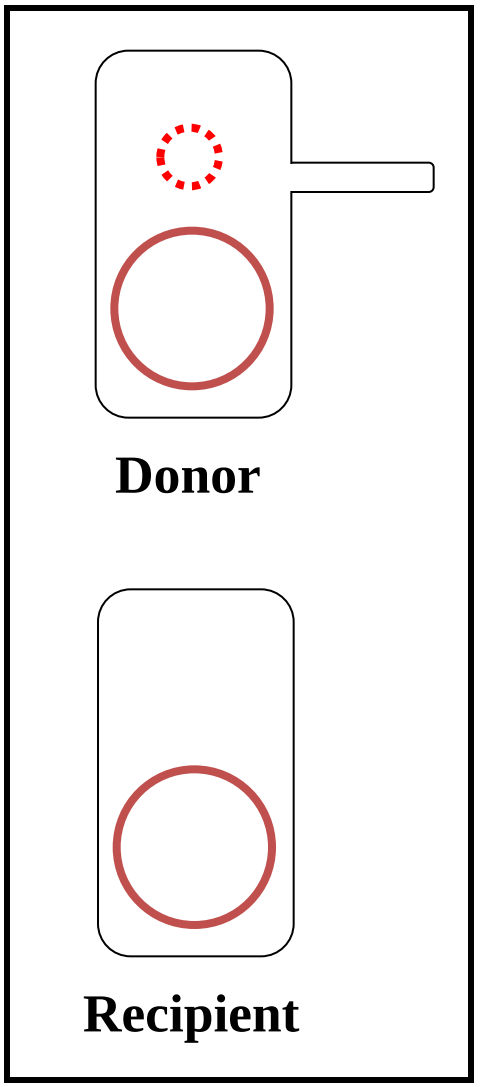

(a)
o
Integrated F factor
lac+
tsx
ton
Hfr chromosome
(b)
lac+
tsx
ton
(c)
F'-lac
lac+
(d)
lac+
lac−
F'-lac+/lac−
diploid cell
ton
tsx

Conjugation with a F’ plasmid



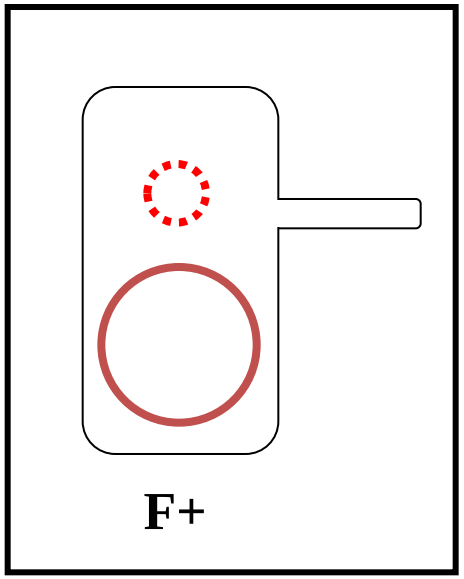
Conjugation

- Definition: Gene transfer from a donor to a recipient by direct physical contact between cells
- Mating types in bacteria
  - Donor
    - F factor (Fertility factor)
      - **F (sex) pilus**
  - Recipient
    - Lacks an F factor



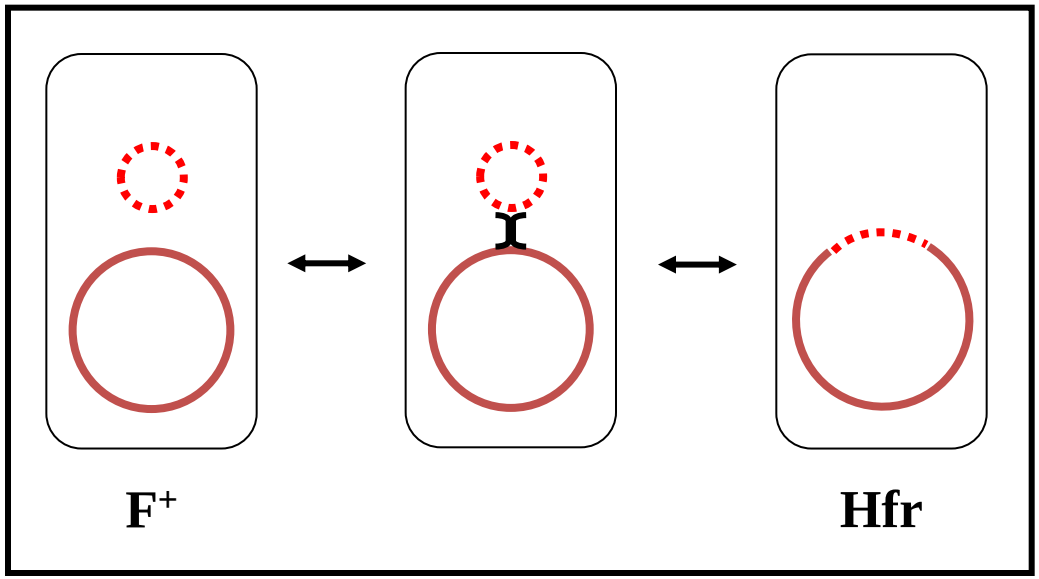
Physiological States of F Factor

- Autonomous (F^+)
  - Characteristics of F^+ x F^- crosses
    - F^- becomes F^+ while F^+ remains F^+
    - Low transfer of donor chromosomal genes



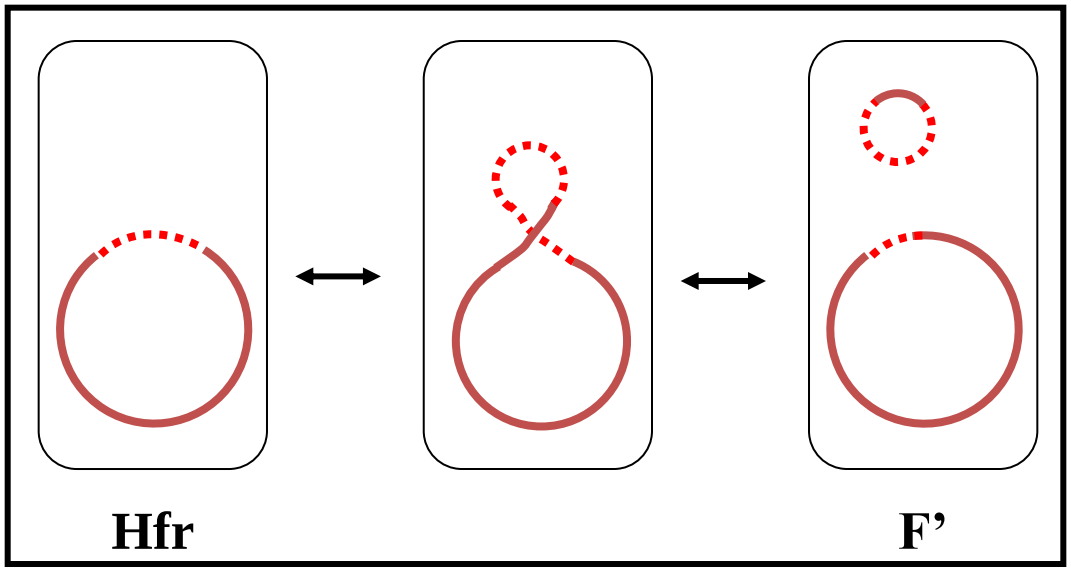
Physiological States of F Factor

- Integrated (Hfr)
  - Characteristics of Hfr x F^- crosses
    - F^- rarely becomes Hfr while Hfr remains Hfr
    - High transfer of certain donor chromosomal genes



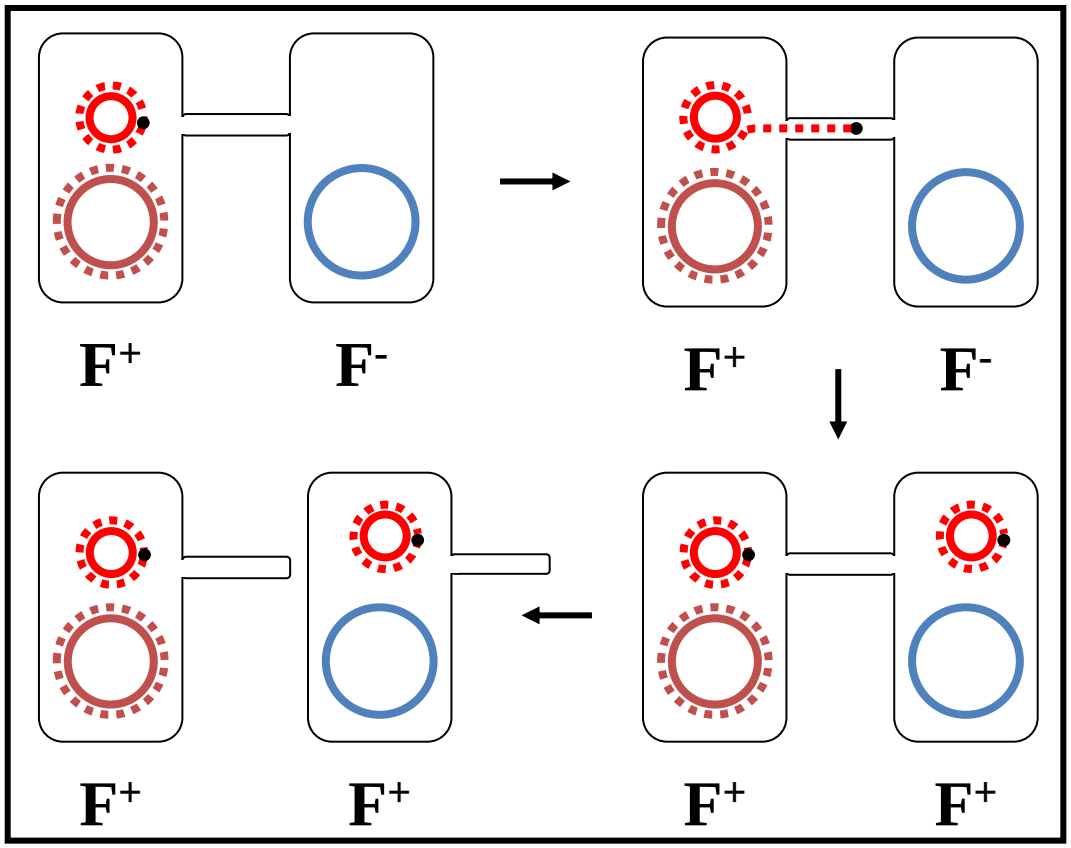
Physiological States of F Factor

- Autonomous with donor genes (F')
  - Characteristics of F' x F^- crosses
    - F^- becomes F' while F' remains F'
    - High transfer of donor genes on F' and low transfer of other donor chromosomal genes



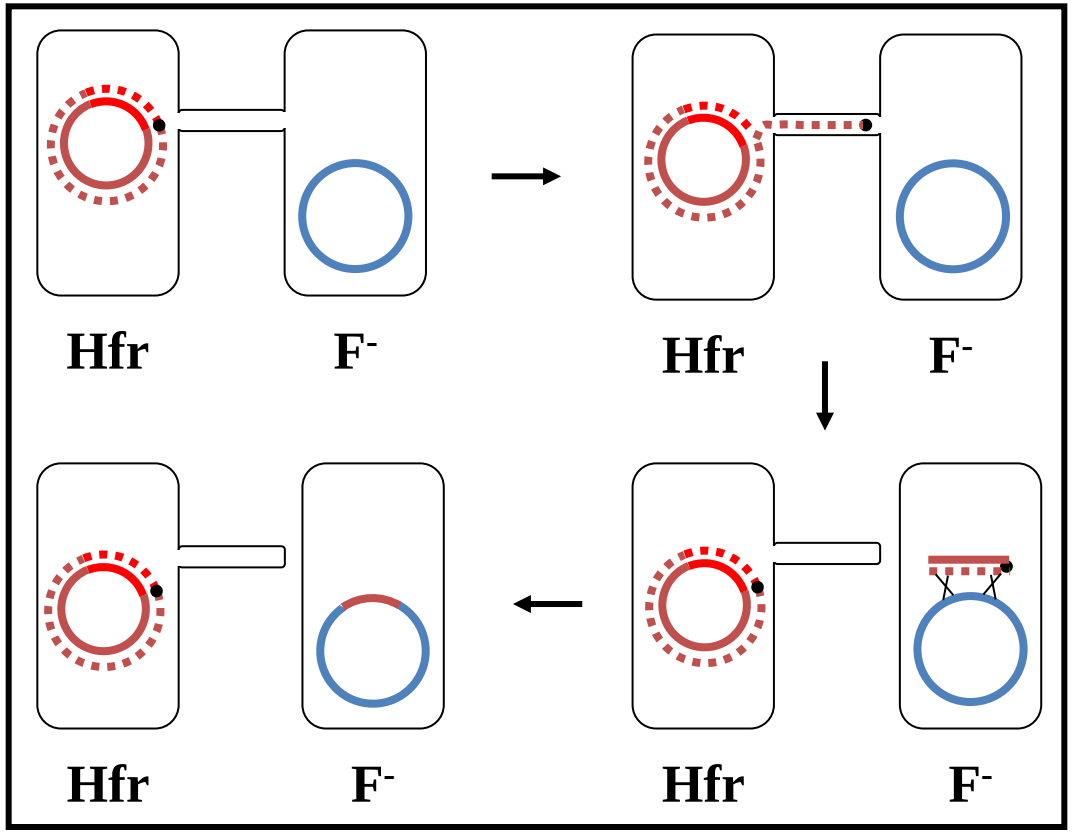
Mechanism of F^+ x F^- Crosses

- Pair formation
  - Conjugation bridge
- DNA transfer
  - Origin of transfer
  - Rolling circle replication



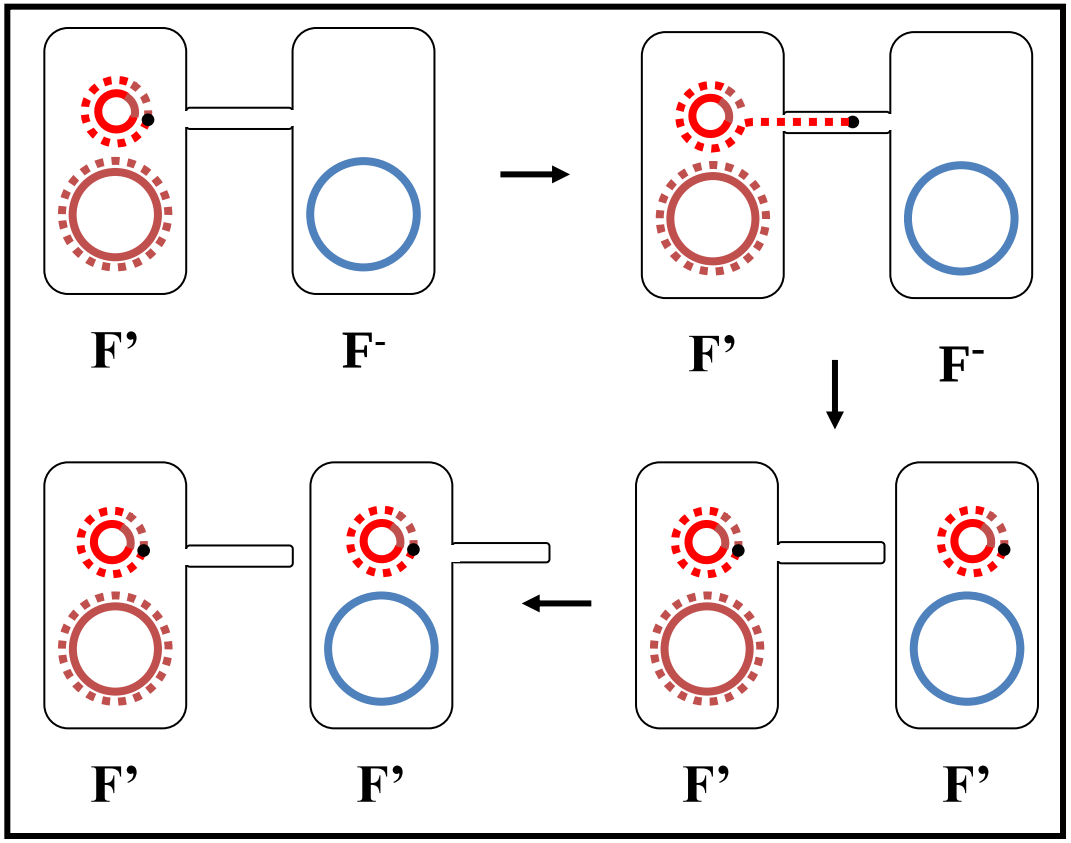
Mechanism of Hfr x F$^-$ Crosses

- Pair formation
  - Conjugation bridge
- DNA transfer
  - Origin of transfer
  - Rolling circle replication
- Homologous recombination



Mechanism of F’ x F$^-$ Crosses

- Pair formation
  - Conjugation bridge
- DNA transfer
  - Origin of transfer
  - Rolling circle replication

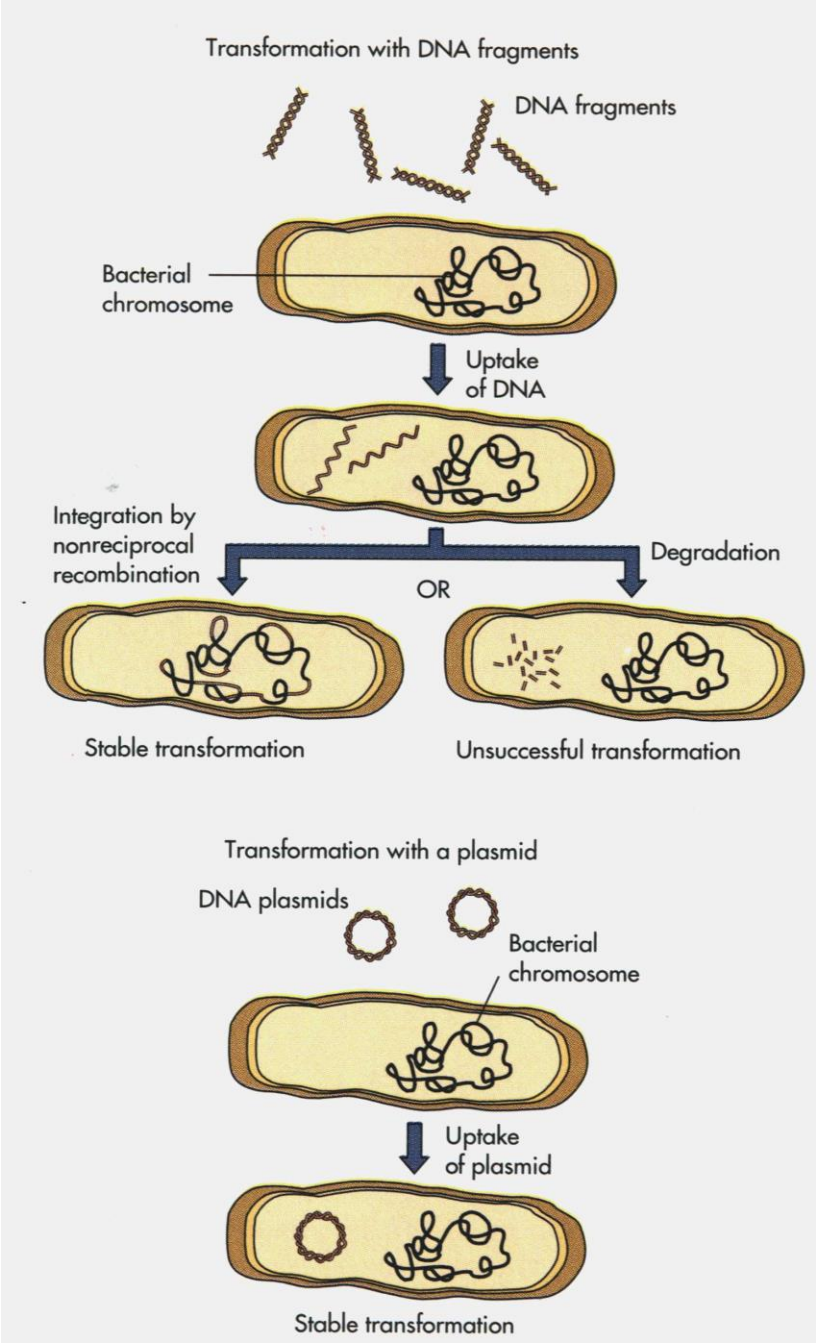


Conjugation

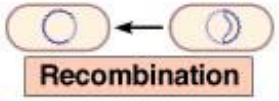
- Significance
  - Gram - bacteria
    - Antibiotic resistance
    - Rapid spread
  - Gram + bacteria
    - Production of adhesive material by donor cells

Transformation

- Pieces of DNA directly taken up by recipient bacteria e.g. *Strept. Pneumoniae*, *Haemophillus, Bacillus.*
- Ability to get transformed is called **Competence**.
- Restriction-modification system in recipient cells.



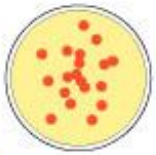
Transformation



(a)

1. Living encapsulated bacteria injected into mouse

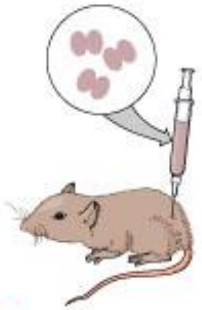
2. Mouse died



3. Colonies of encapsulated bacteria were isolated from dead mouse

(b)

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

(c)

1. Heat-killed encapsulated bacteria injected into mouse

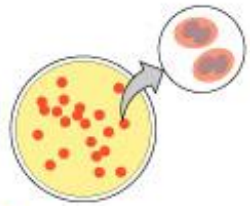
2. Mouse remained healthy

3. No colonies were isolated from mouse

(d)

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

2. Mouse died



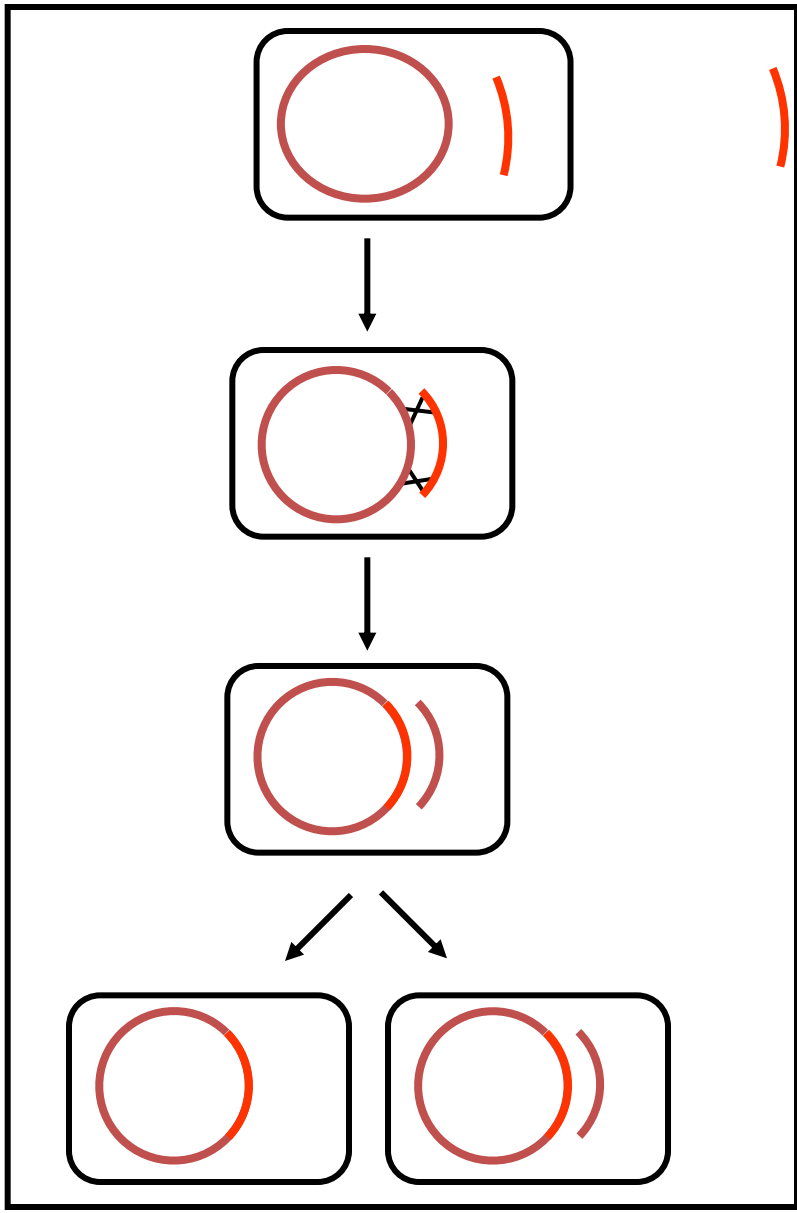
3. Colonies of encapsulated bacteria were isolated fromdead mouse

Transformation

- Definition: Gene transfer resulting from the uptake of DNA from a donor.
- Factors affecting transformation
  - DNA size and state
    - Sensitive to nucleases
  - Competence of the recipient (*Bacillus, Haemophilus, Neisseria, Streptococcus*)
    - Competence factor
    - Induced competence

Transformation

- Steps
  - Uptake of DNA
    - Gram +
    - Gram -
  - Recombination
    - Legitimate, homologous or general
    - recA, recB and recC genes
- Significance
  - Phase variation in *Neiseseria*
  - Recombinant DNA technology



Transformation

- Cells need to be competent to take up DNA from the external milieu. In gram positive bacteria this requires the presence of a DNA-binding protein on the surface of the cell. The presence of this protein is correlated with nutritional shift-down -- i.e. when the cells start to run out of nutrients

Transformation

- **In *Bacillus subtilis* and *Streptococcus pneumoniae*,a complex of 3 - 5 proteins including**
- **a labile competence factor**

  a specific endonuclease

  the DNA-binding polypeptides

  an autolysin to increase cell permeability

- **are required for transformation. The competence complex is exposed by autolysin and can then bind to double-stranded DNA fragments. Only dsDNA can be used to transform cells. If ssDNA is used, no tranformants are observed**

Mechanism

- Bound fragments are digested by an endonuclease into fragments of size ~15 Kbp and then an exonuclease degrades one of the two strands as the other enters the cell (it is protected by ssDNA binding proteins from further degradation). The endonucleolytic reaction may be required to position the end of the DNA fragment correctly so that it can be imported into the cell.
- The resulting ssDNA is recombined into the host chromosome by some sort of strand displacement mechanism.

- DNA uptake is associated with the formation of small membraneous structures, called transformasomes, which protrude outside the cell. The transforming DNA is taken into these vesicles where it is then internalized into the cell. One of the two strands is degraded while the remaining strand may recombine with the host chromosome.

- Unlike gram positive bacteria, DNA uptake in gram negative bacteria appears to require or involve the recognition of specific sequences. The sequences or some bacteria are as follows:

- ***H. influenza*** **:** **AAGTGCGGTCA**
- ***N. gonorrhoeae:*** **GCCGTCTCAA**

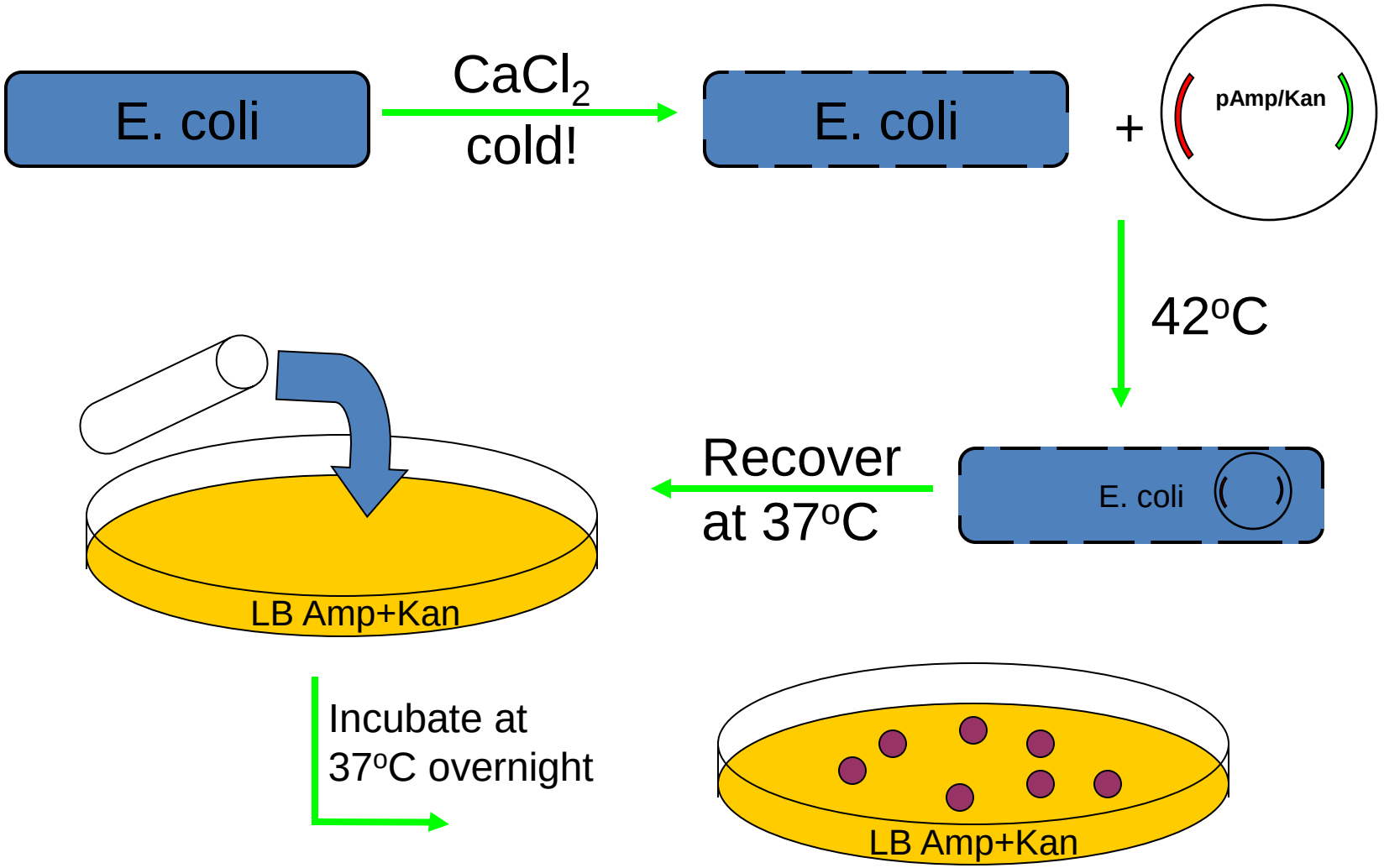
Bacterial transformation

- Transformation is the act of putting foreign DNA into a bacterial cell
- Occurs in nature, but VERY RARELY!
- We've perfected an artificial method to force bacteria to take up DNA

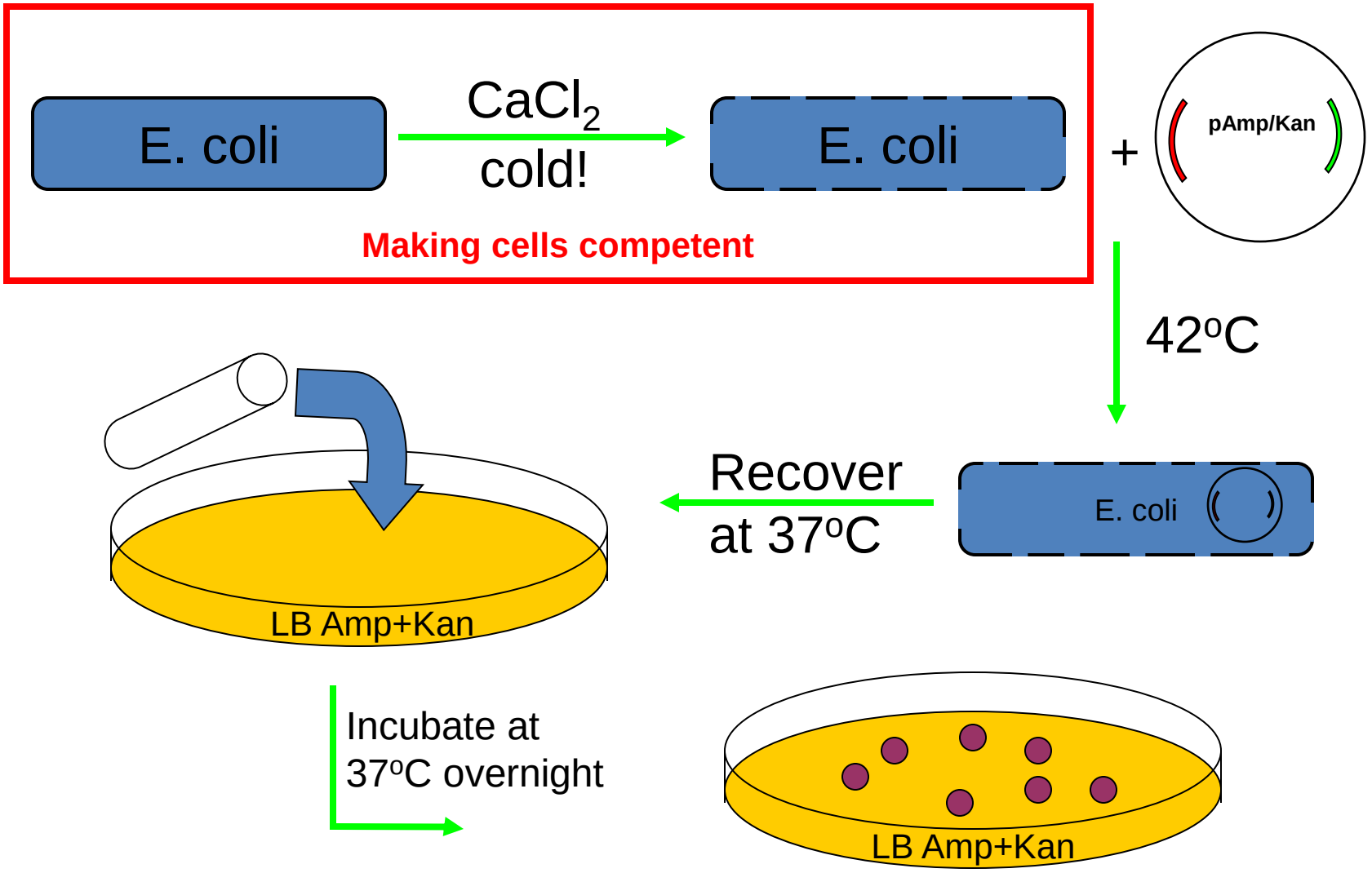
Transformation procedure—probable mechanism

- Cells in mid-logarithmic phase are suspended in cold calcium chloride which makes them permeable to DNA
- They are mixed with the DNA to be inserted
- They are “heat shocked” which drives the DNA into the cell
- They are allowed to recover at 37^{o}C
- They are spread on a plate containing a selection drug(s)

Transformation procedure



Transformation procedure



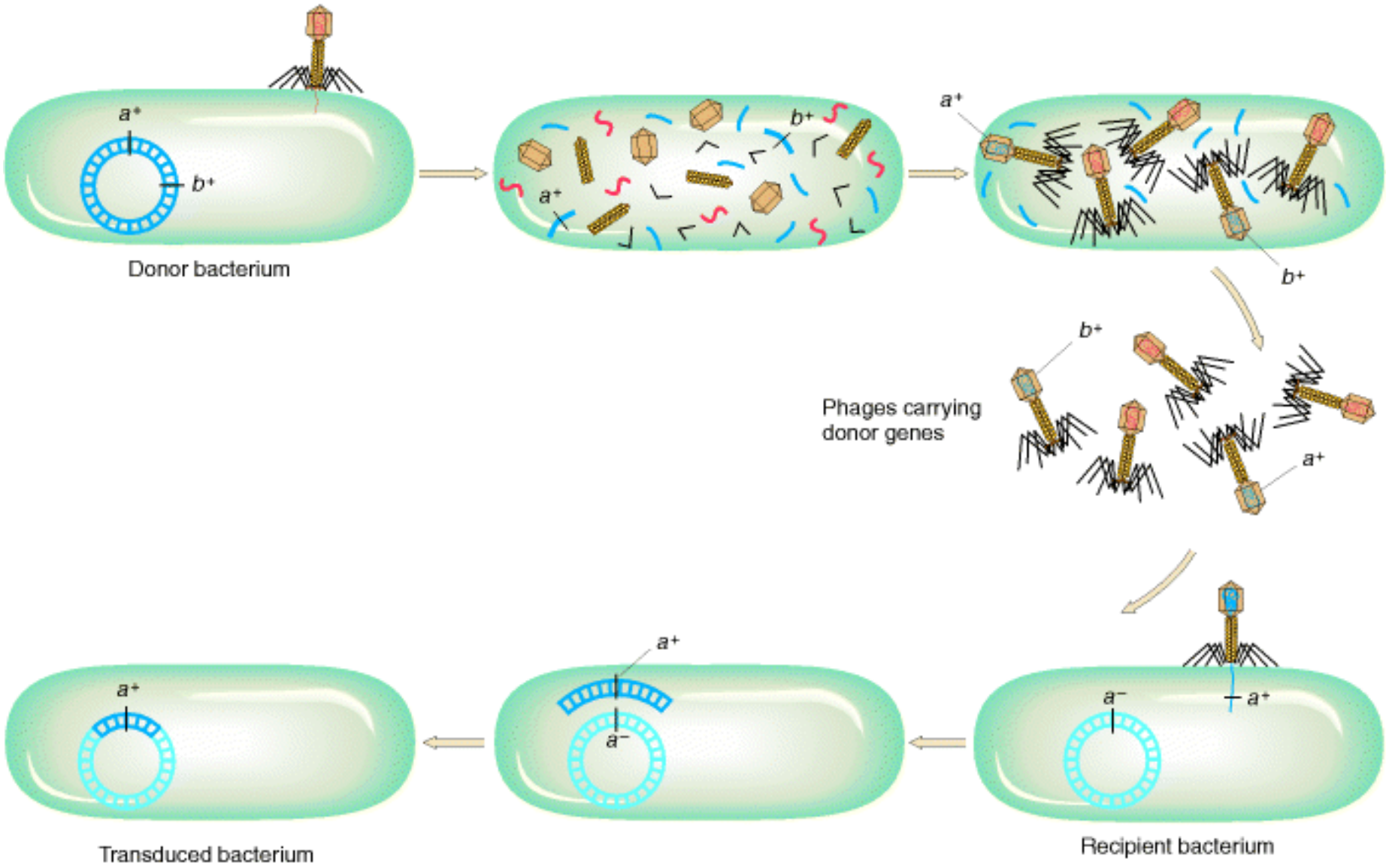
Important considerations

- Cells must be in log phase when making competent!
- Cells must be kept cold!
- Cells must recover after heat shock!

Transduction

- DNA transfer mediated by viruses
  - Generalised transduction
    - Fragment of DNA from infected cell is packaged in a phage particle
    - Defective phage particle introduces bacterial DNA into another bacterial cell

Generalized transduction



Transduction

- Definition: Gene transfer from a donor to a recipient by way of a bacteriophage

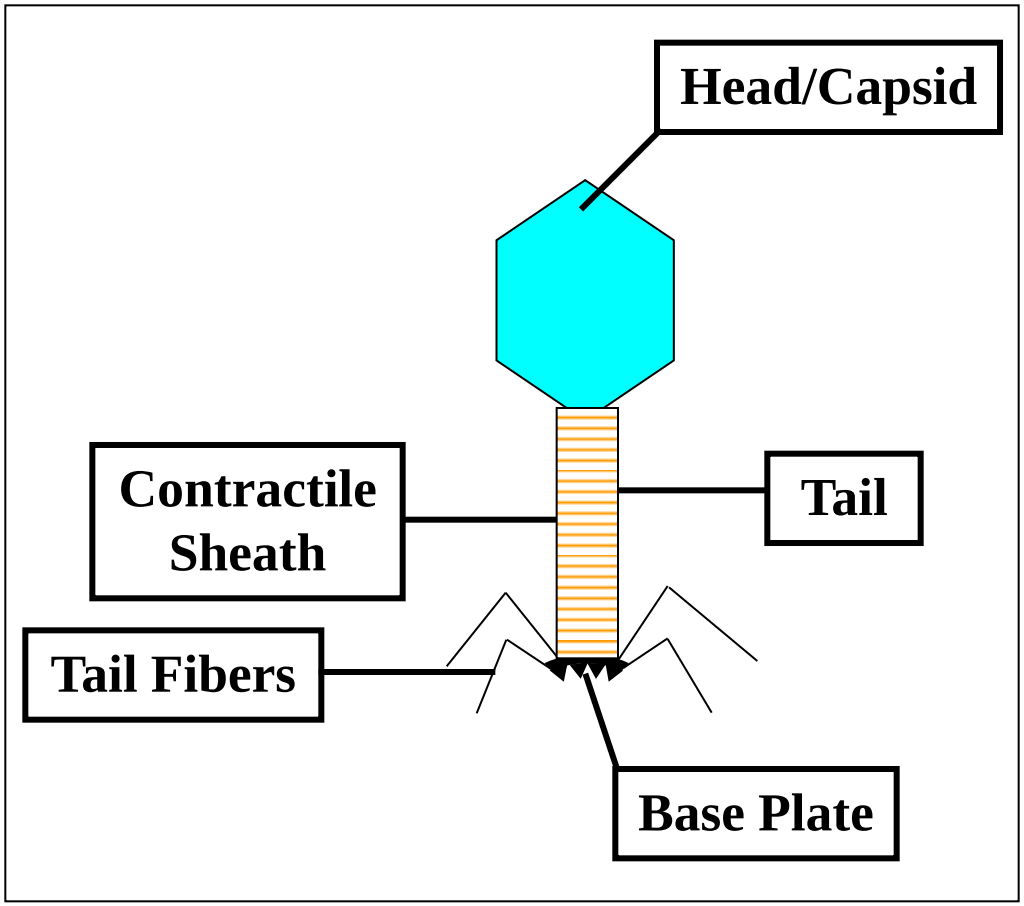
Transduction

- Definition
- Types of transduction
- Significance
  - Common in Gram+ bacteria
  - Lysogenic (phage) conversion

Phage Composition and Structure

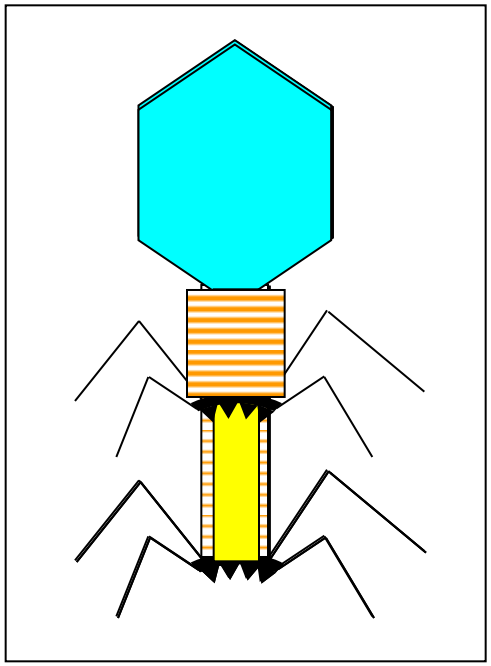
- Composition
  - Nucleic acid
    - Genome size
    - Modified bases
  - Protein
    - Protection
    - Infection

- Structure (T_4)
  - Size
  - Head or capsid
  - Tail



Infection of Host Cells by Phages

- Adsorption
  - LPS for T4
- Irreversible attachment
- Sheath Contraction
- Nucleic acid injection
- DNA uptake



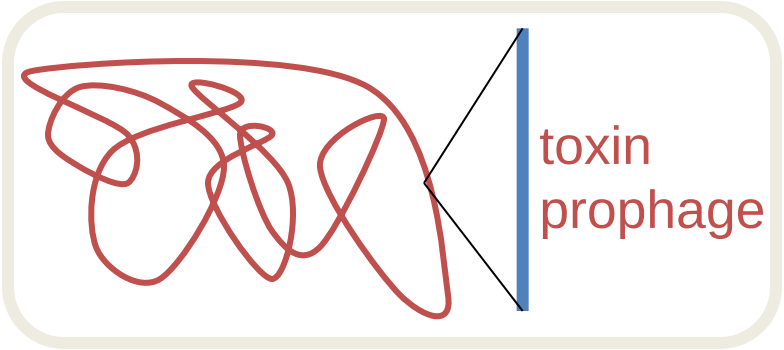
Types of Bacteriophage

- Lytic or virulent – Phage that multiply within the host cell, lyse the cell and release progeny phage (*e.g.* T4)
- Lysogenic or temperate phage: Phage that can either multiply via the lytic cycle or enter a quiescent state in the bacterial cell. (*e.g.,* )
  - Expression of most phage genes repressed
  - Prophage
  - Lysogen

Phage conversion

Dormant prophage – integrated bacteriophage – carries genes that alter the phenotype of the microbe

- best examples are pathogens and toxin production



Corynebacterium diptheriaea

Phage produces diptheria toxin

This is what makes people sick

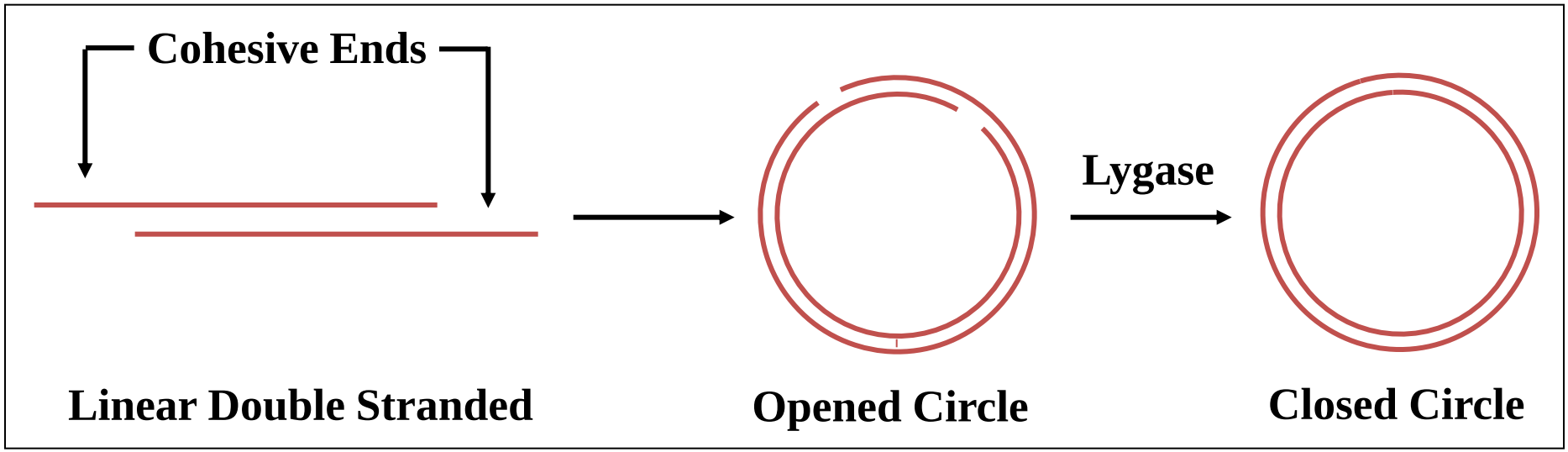
C.diptheriaea

without phage strain produces no toxin

Does not cause diptheria

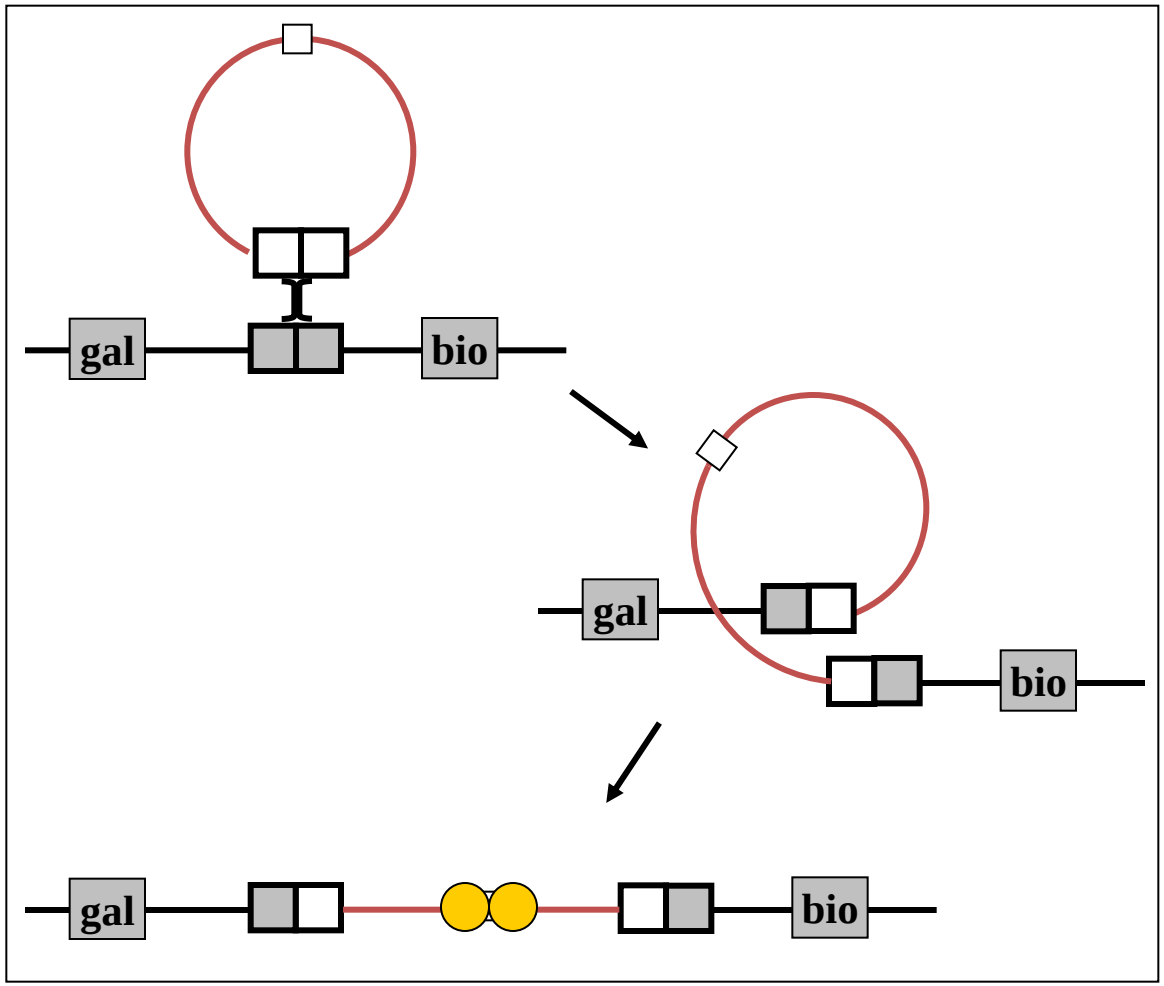
Events Leading to Lysogeny

- Circularization of the phage chromosome
  - Cohesive ends



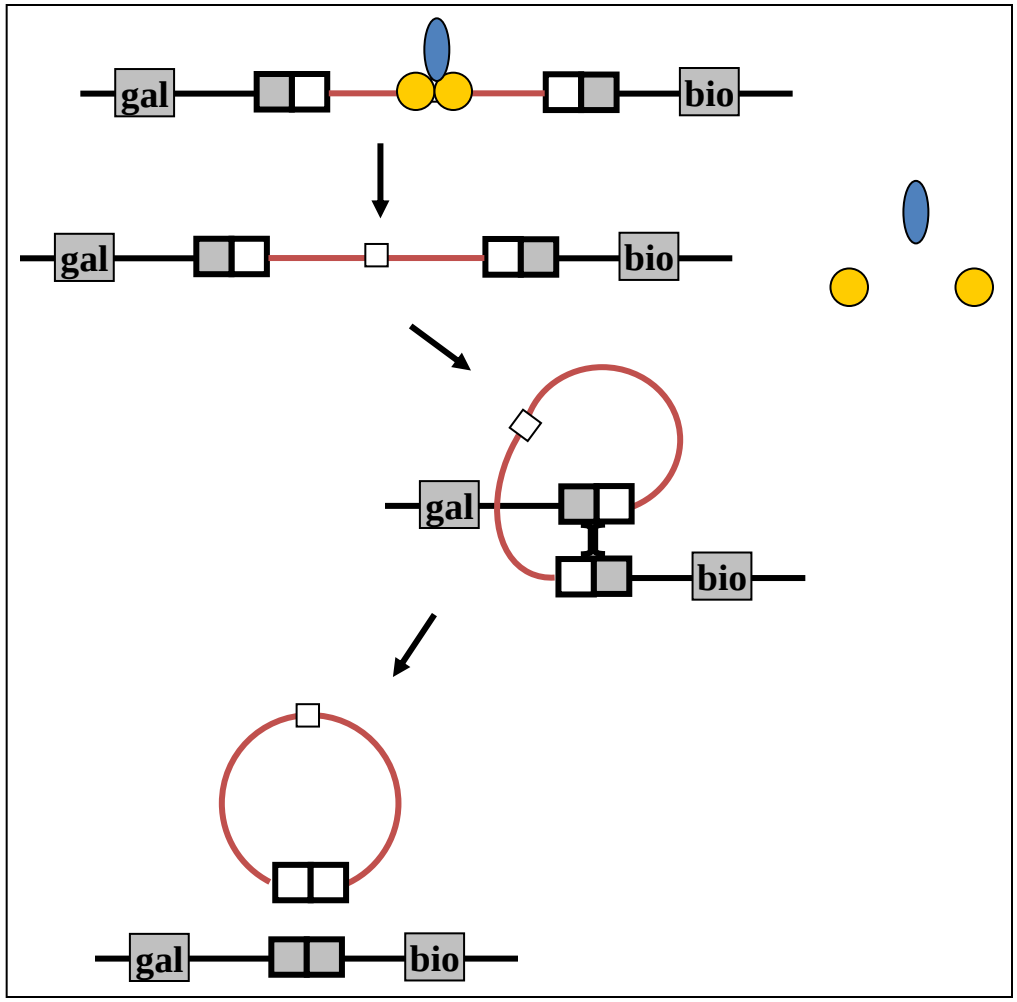
Events Leading to Lysogeny

- Site-specific recombination
  - Phage coded enzyme
- Repression of the phage genome
  - Repressor protein
  - Specific
  - Immunity to superinfection



Termination of Lysogeny

- Induction
  - Adverse conditions
- Role of proteases
  - recA protein
  - Destruction of repressor
- Gene expression
- Excision
- Lytic growth



Transduction

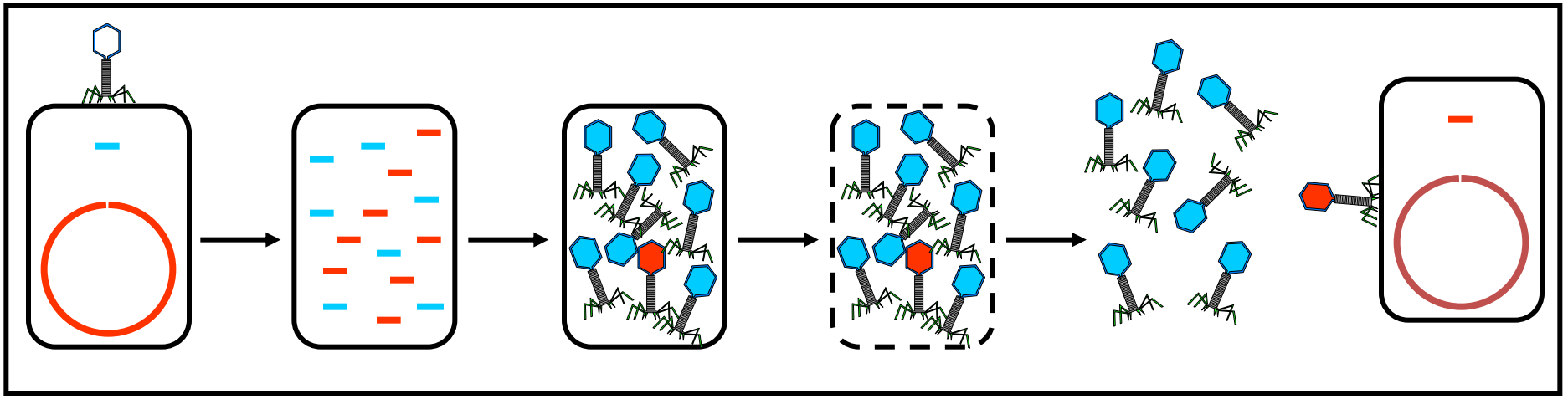
- Definition: Gene transfer from a donor to a recipient by way of a bacteriophage
- Resistant to environmental nucleases

Transduction

- Types of transduction
  - Generalized - Transduction in which potentially any donor bacterial gene can be transferred

Generalized Transduction

- Infection of Donor
- Phage replication and degradation of host DNA
- Assembly of phages particles
- Release of phage
- Infection of recipient
- Legitimate recombination



An example – P22 phage transduction of *Salmonella typhimurium*

P22 HT is a efficient generalized transducer

- its sloppy – 50% of the viral particles contain host cell DNA (ie are transducing particles or TPs)

Each transducing particle (TP) carries 44 kb of DNA – the *Salmonella* genome is app. 4400 kb in size

Therefore, if the process is random 100 different tranducing particles should represent the entire genome.

10^{11} P22 HT viruses are produced per ml – therefore 5 x 10^{10} of these particles carry host DNA→ 1 ml carries app. 5 x 10^{8} copies of the *Salmonella genome*

(0.5)(10^{11} viruses/ml)/(100 TP [1 genome]) = 10^{8} copies of the genome/ml of lysate

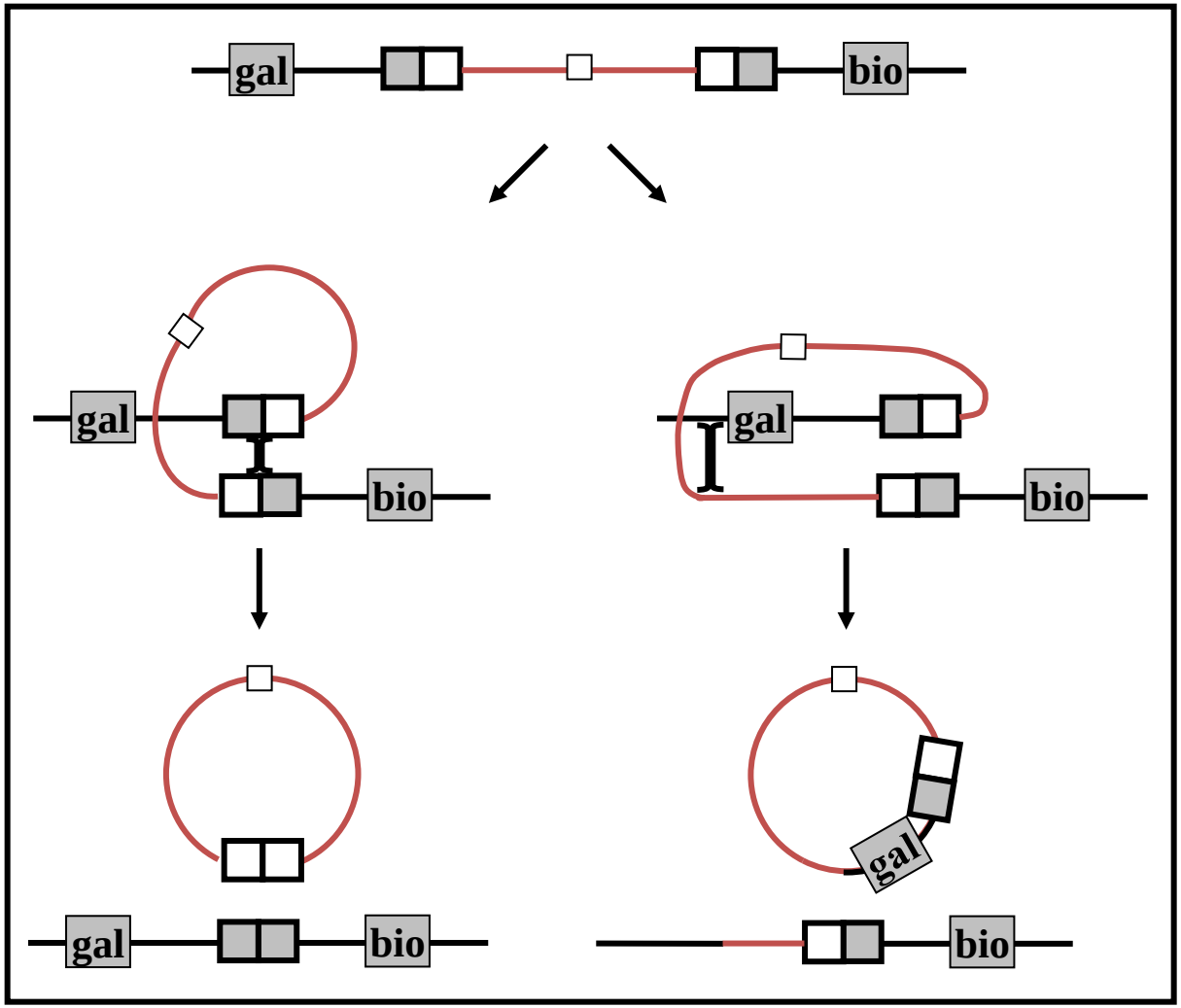
Transduction

- Types of transduction
  - Generalized - Transduction in which potentially any dornor bacterial gene can be transferred.
  - Specialized - Transduction in which only certain donor genes can be transferred

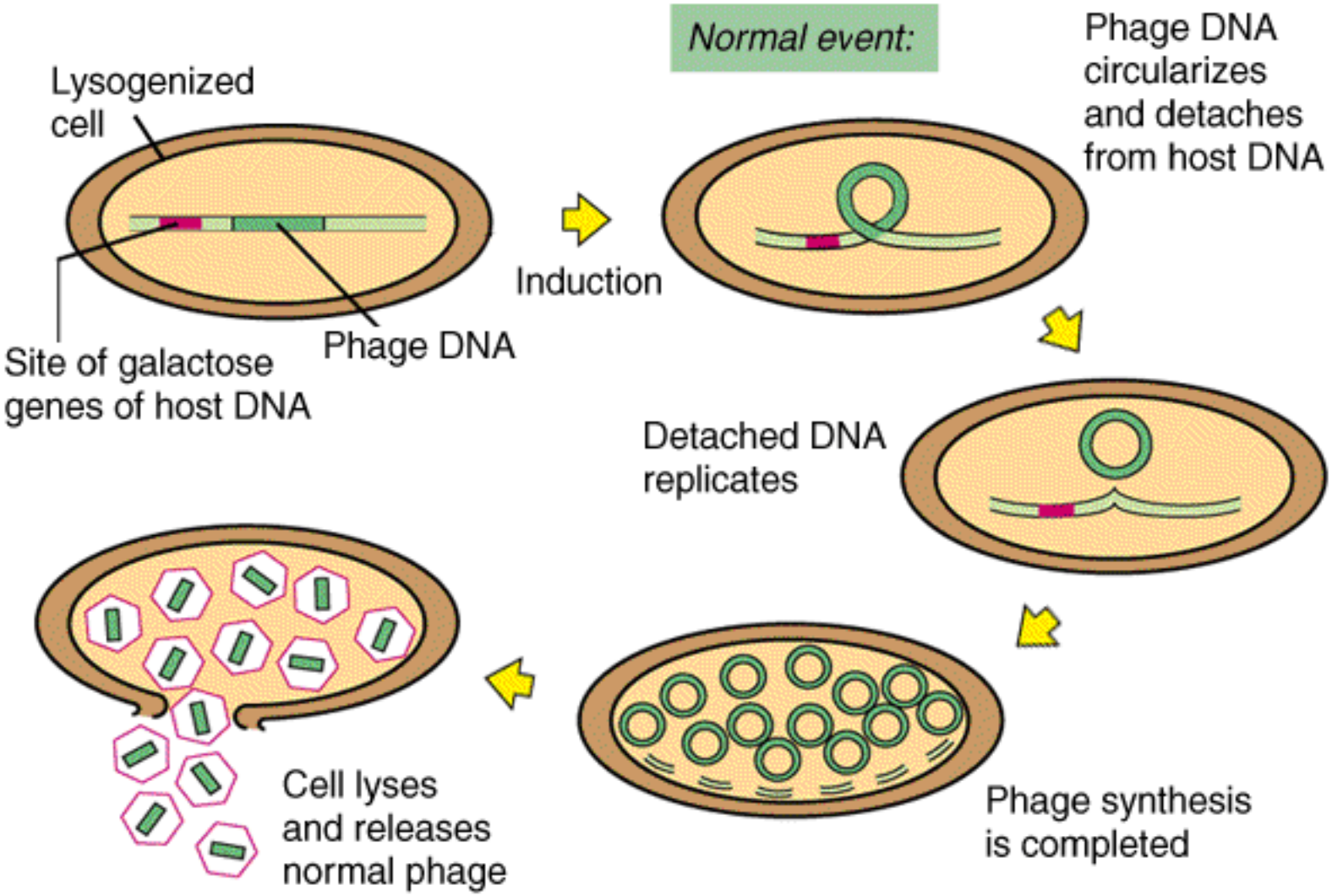
Specialized Transduction

Lysogenic Phage

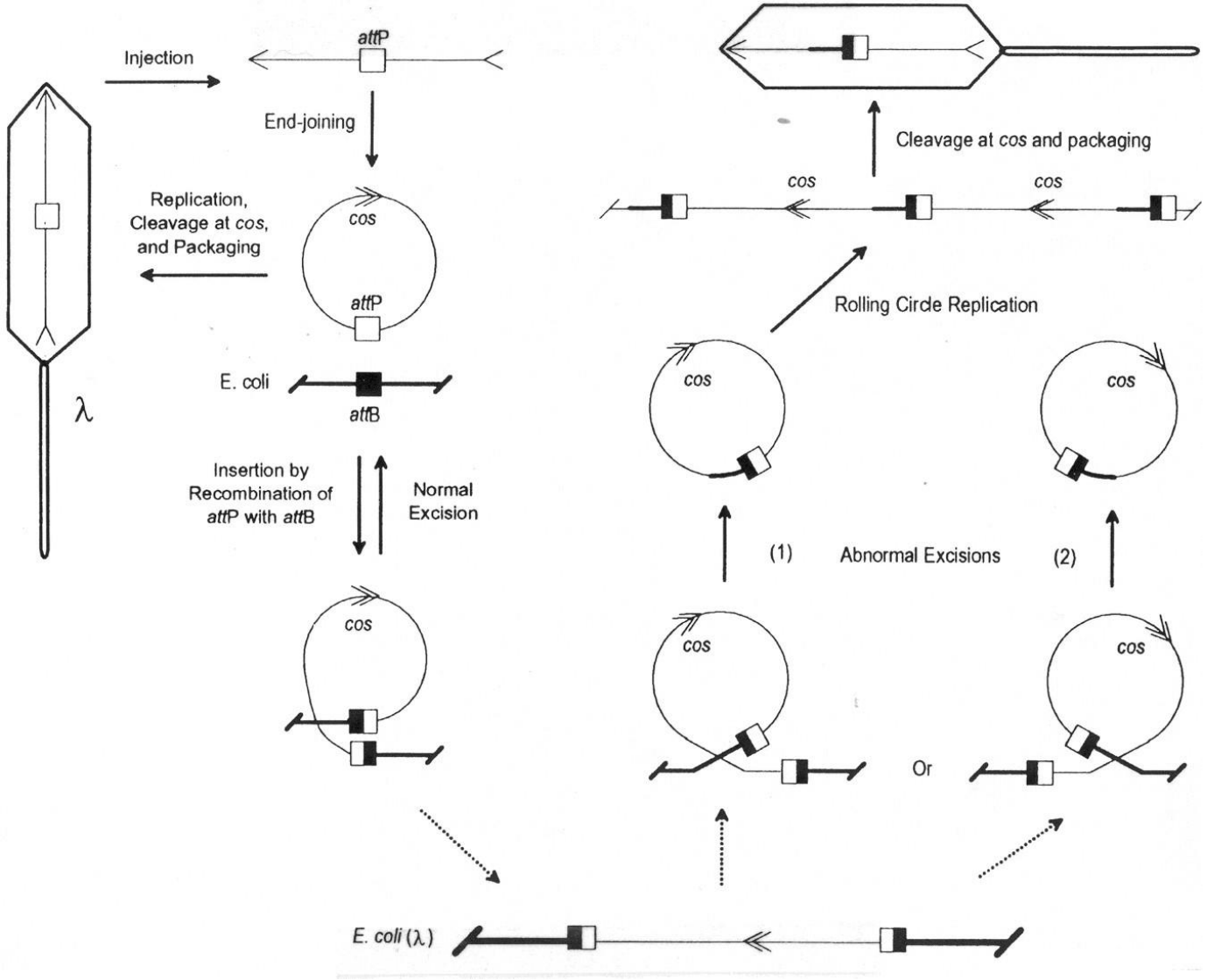
- Excision of the prophage
- Replication and release of phage
- Infection of the recipient
- Lysogenization of the recipient
  - Legitimate recombination also possible



Simple mechanism for specialized transduction

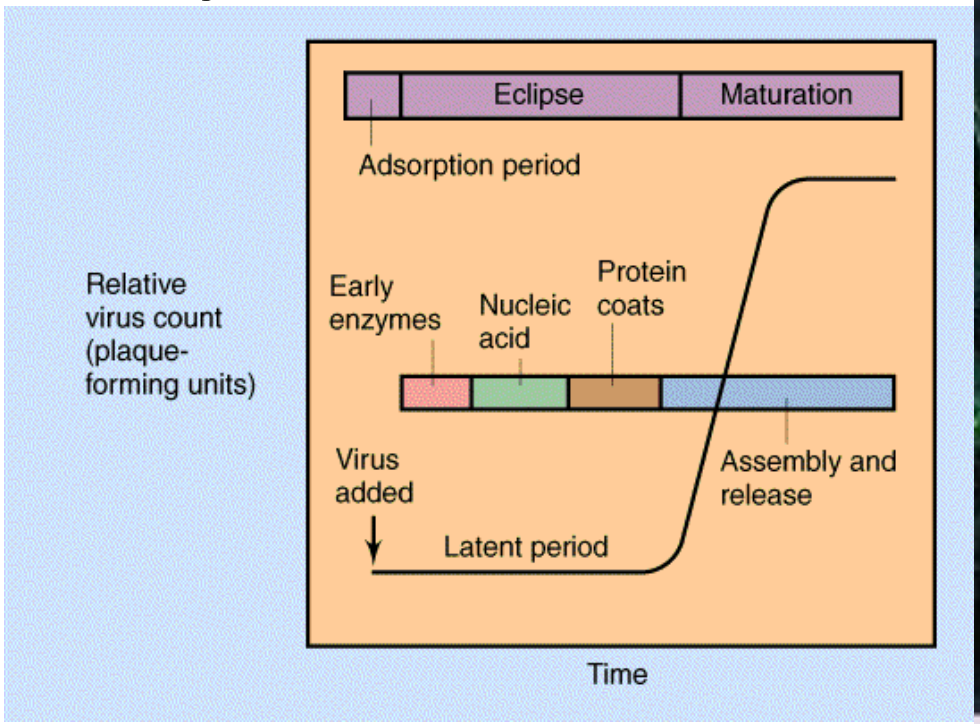


Mechanism of specialized transduction

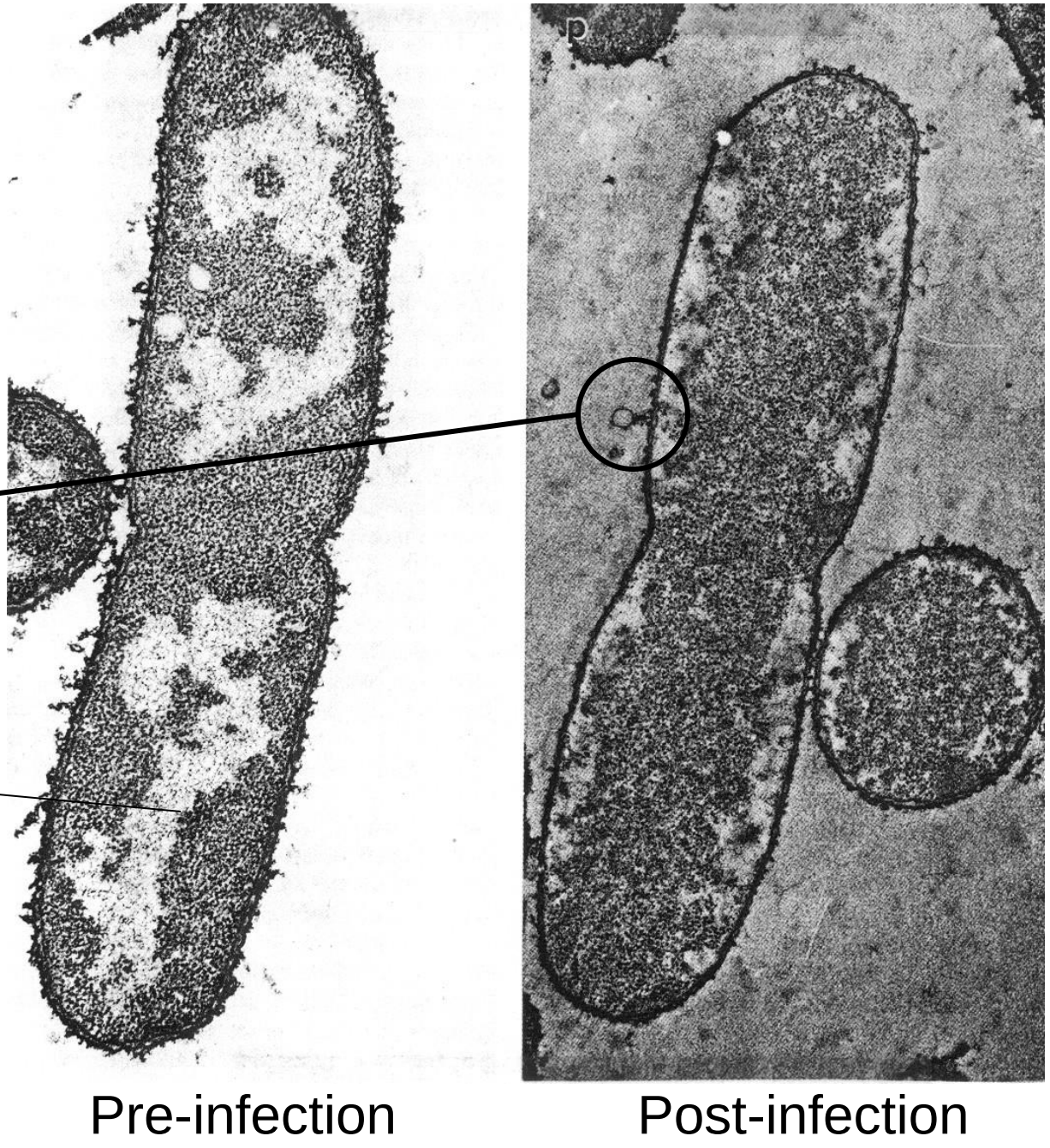


Lytic viruses go through a one step growth curve

- during the latent period, the viral genome is replicated and the host genome is generally destroyed

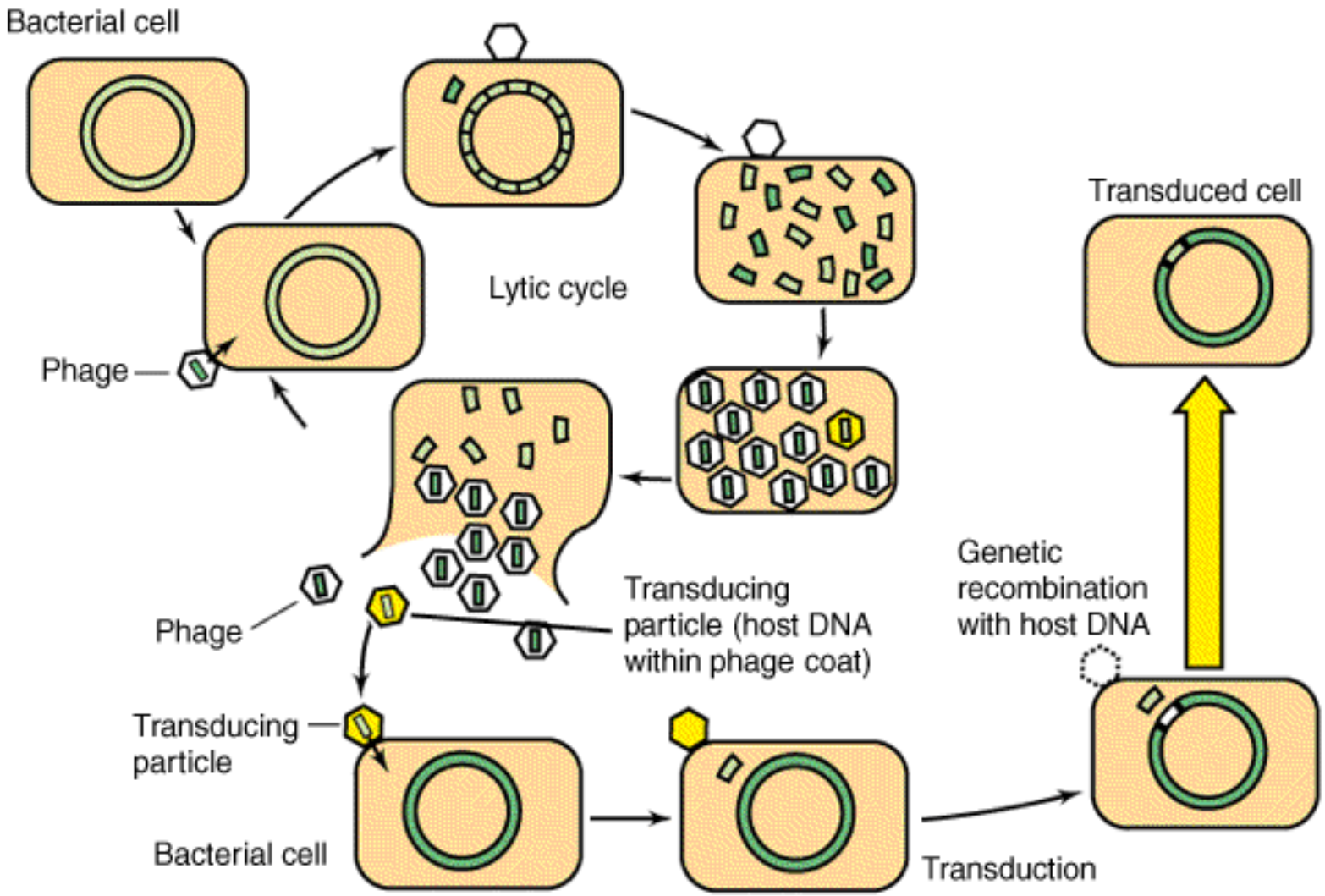


Visible effects on DNA during viral infection



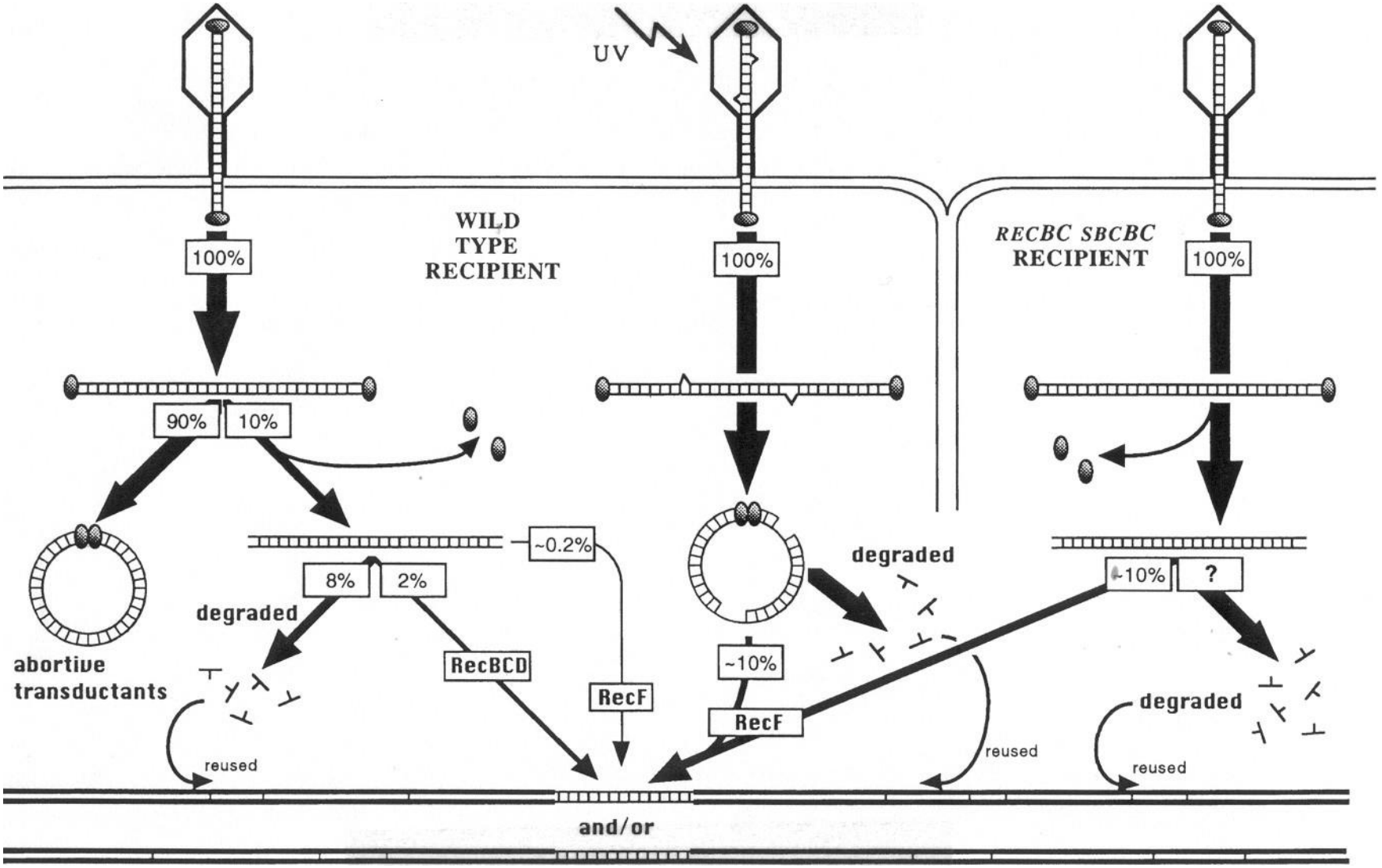
Pre-infection

Post-infection


Bacterial cell
Lytic cycle
Phage
Transduced cell
Genetic
recombination
with host DNA
Phage
Transducing
particle (host DNA
within phage coat)
Transducing
particle
Bacterial cell
Transduction

B

What happens in the recipient cell?



Generalized transduction is a useful way to exchange genes between bacteria

Also extremely useful for mapping of genetic markers relative to each other