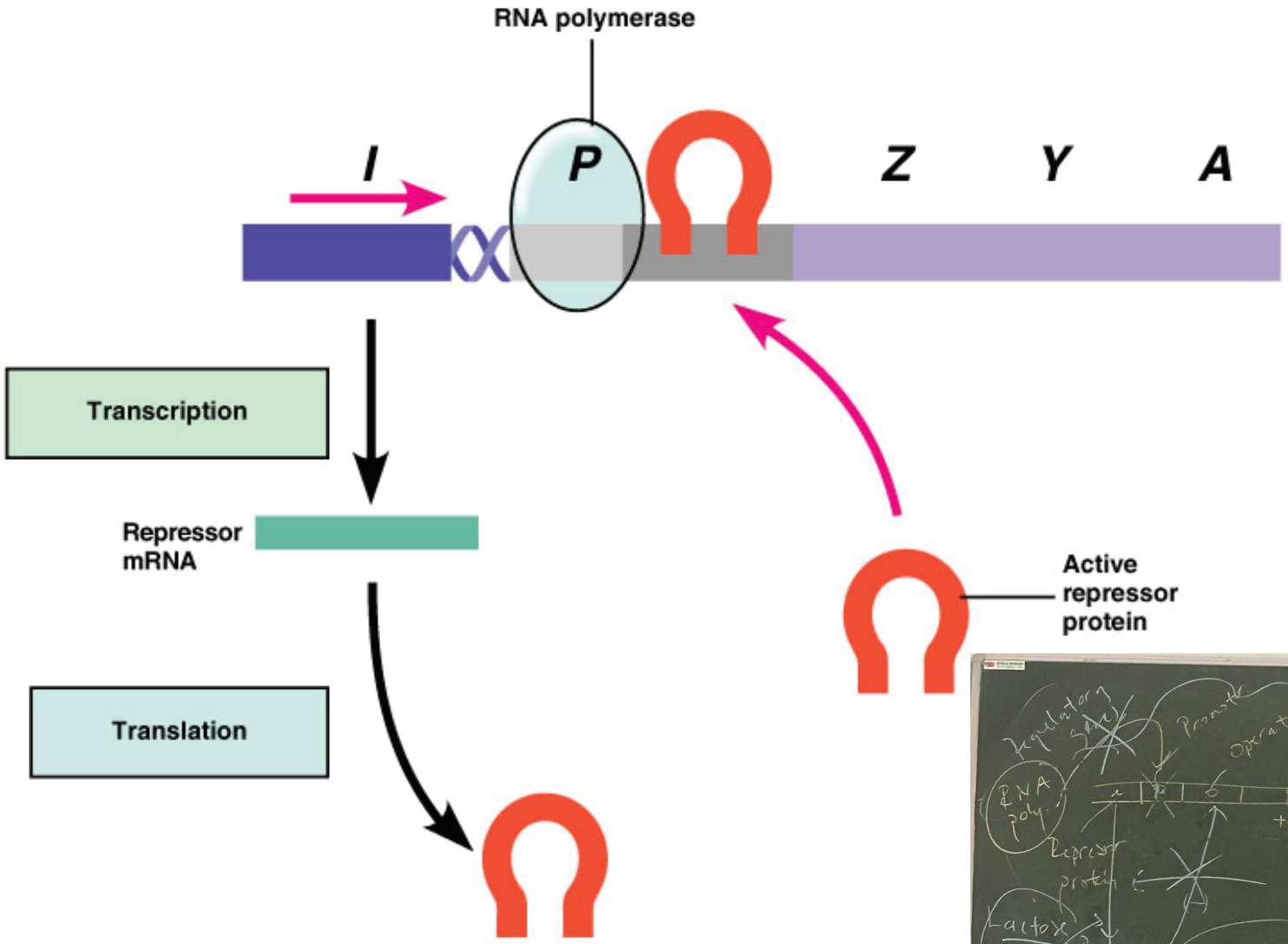
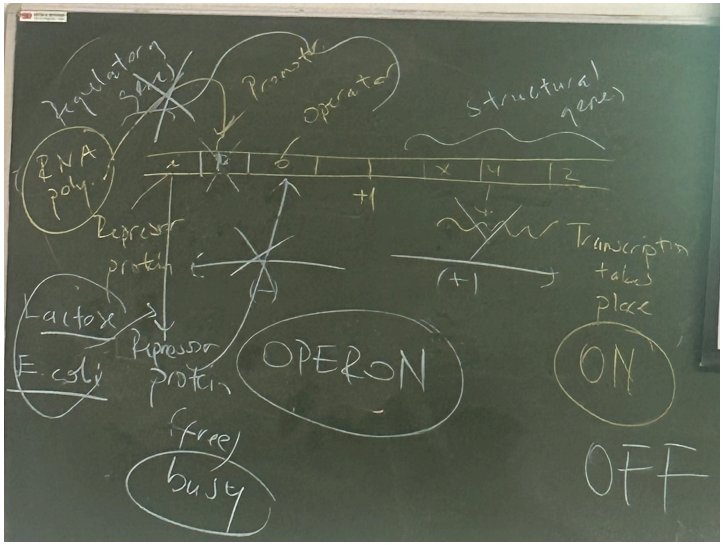
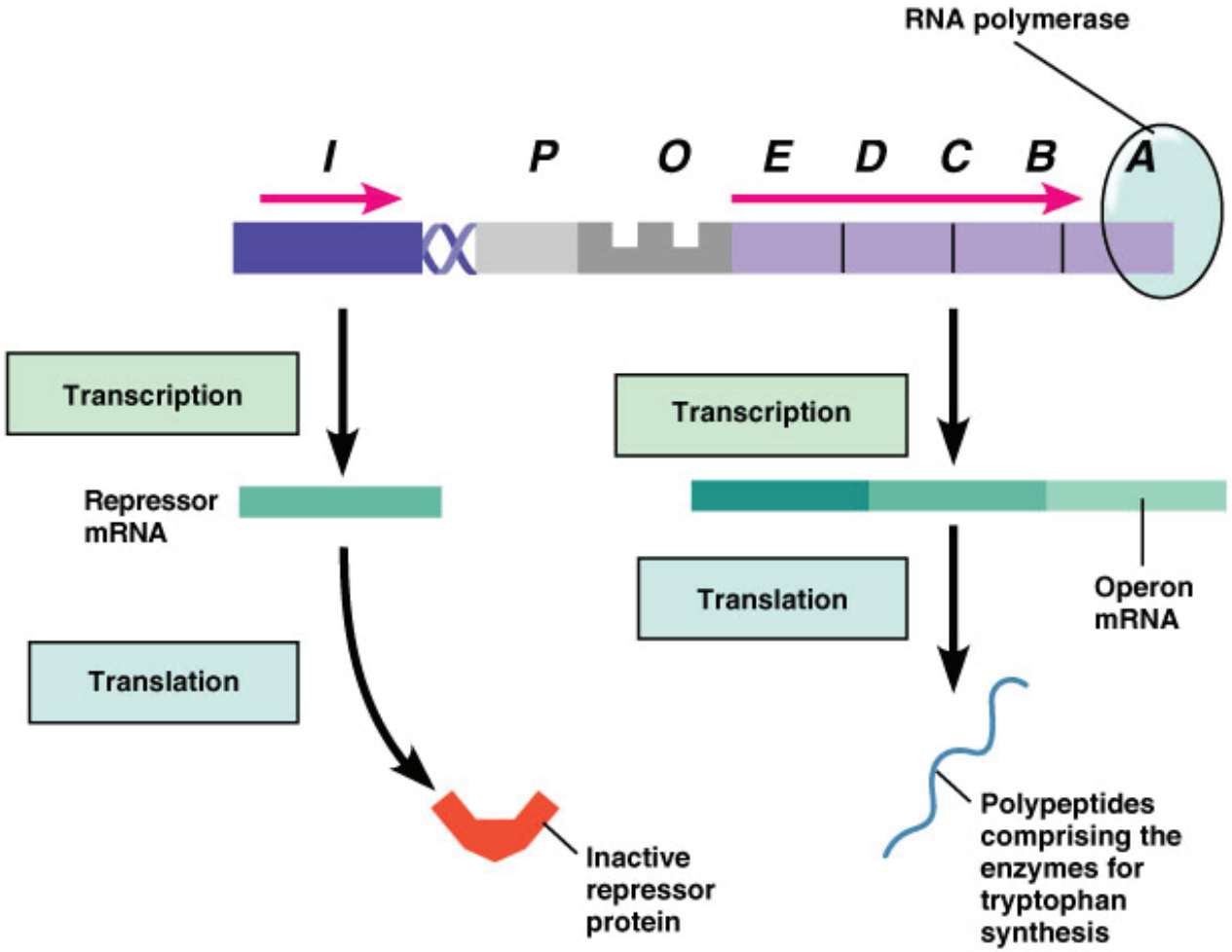
Regulation of Gene Expression



2 Repressor active, operon off. The repressor protein binds with the operator, preventing transcription from the operon.

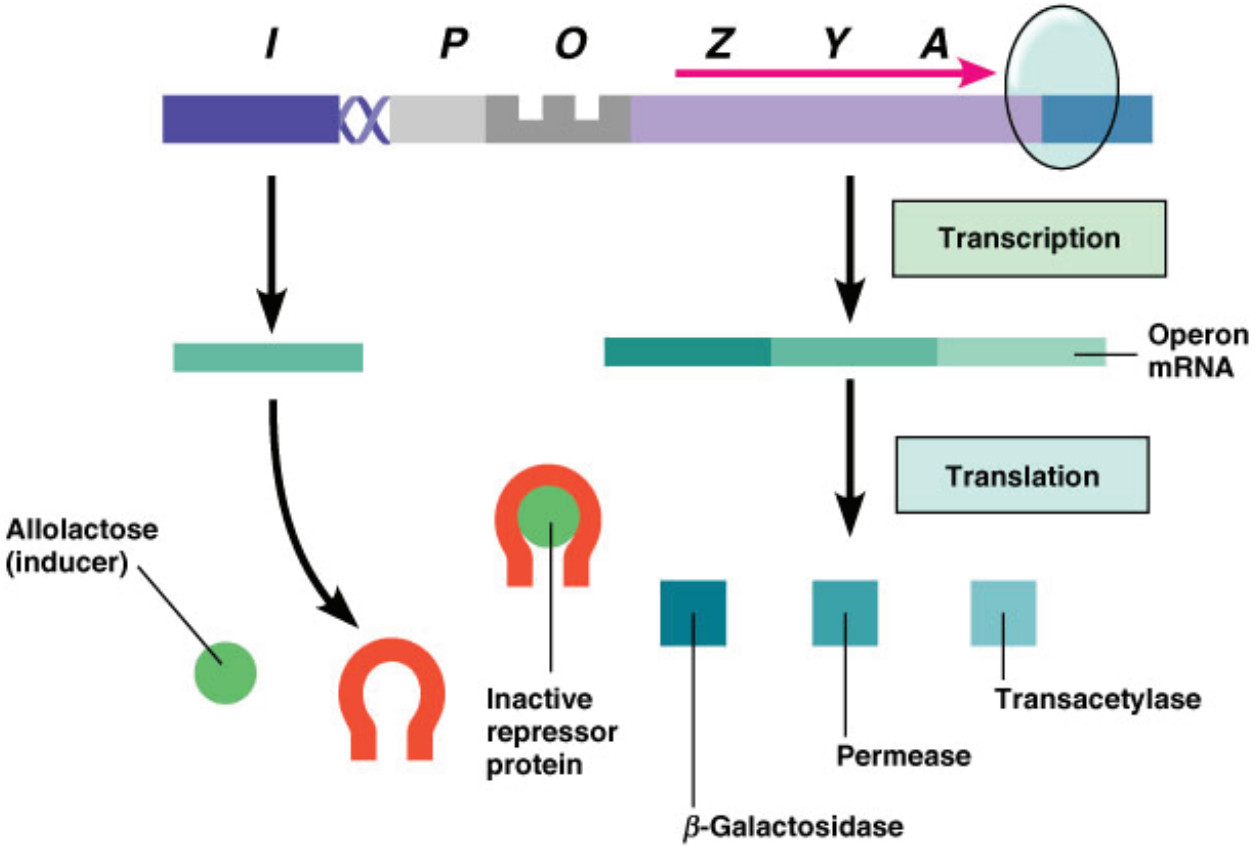


Regulation of Gene Expression



2 **Repressor inactive, operon on.** The repressor is inactive and transcription and translation proceed leading to the synthesis of tryptophan.

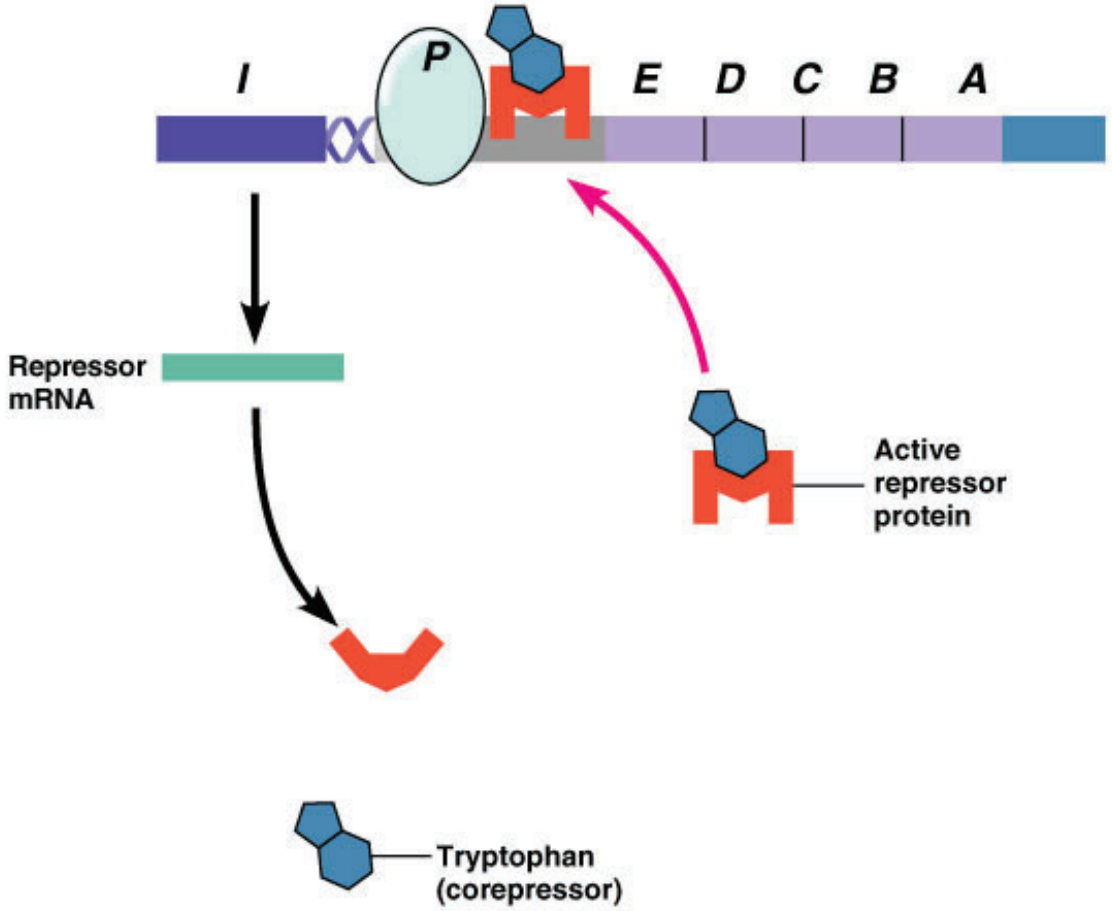
Regulation of Gene Expression



3 **Repressor inactive, operon on.** When the inducer allolactose binds to the repressor protein, the inactivated repressor can no longer block transcription. The structural genes are transcribed, ultimately resulting in the production of the enzymes needed for lactose catabolism.

(a) An inducible operon

Regulation of Gene Expression



3 **Repressor active, operon off.** When the corepressor tryptophan binds to the repressor protein, the activated repressor binds with the operator, preventing transcription from the operon.

(b) A repressible operon

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 → why ?

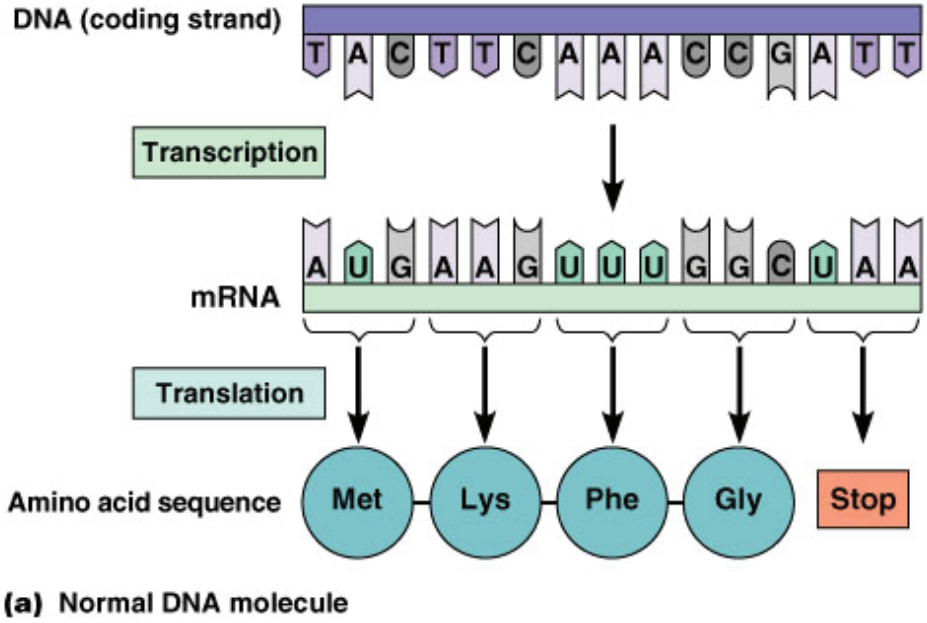
enzymes make mistake

↳ corrected by DNA polymerase proof reading → part of the process

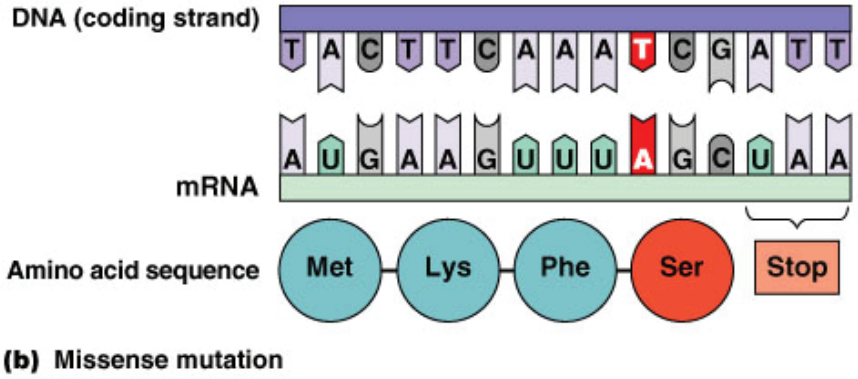
Mutation

- Base substitution (point mutation)
- Missense mutation

- Change in one base
- Result in change in amino acid



(a) Normal DNA molecule



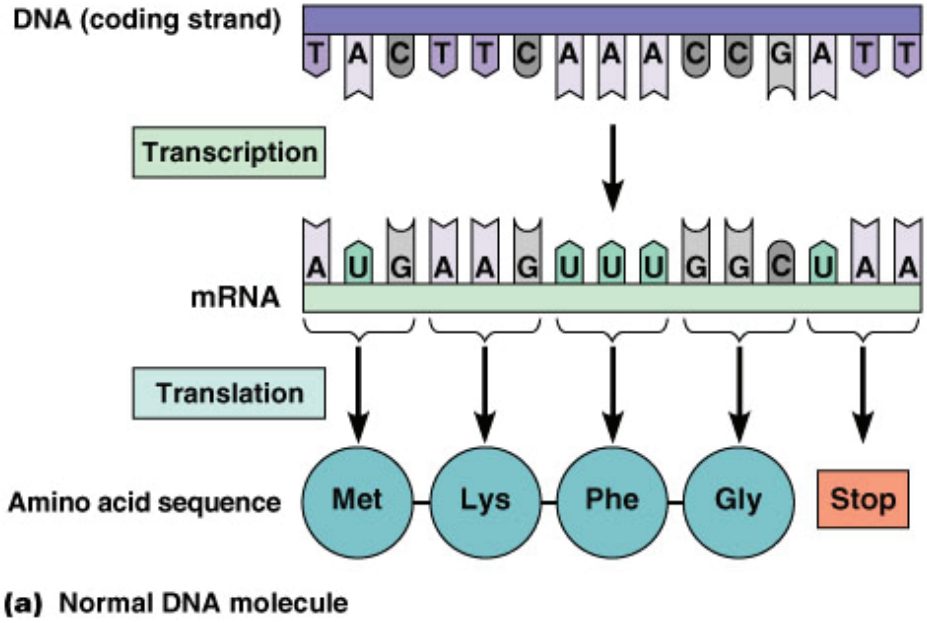
(b) Missense mutation

Mutation

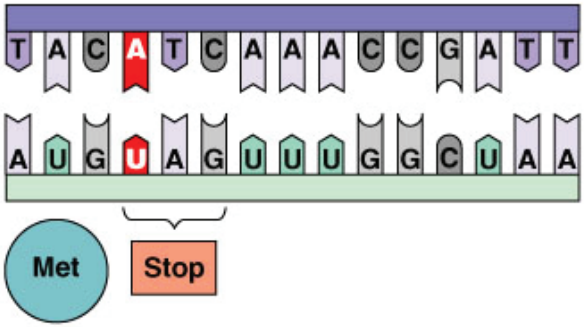
- Nonsense mutation
  - 1 codon becomes stop codon
- Results in a nonsense codon

- silent mutation => wobble base change. => amino acid not changing

All three of them are point mutation,



(a) Normal DNA molecule

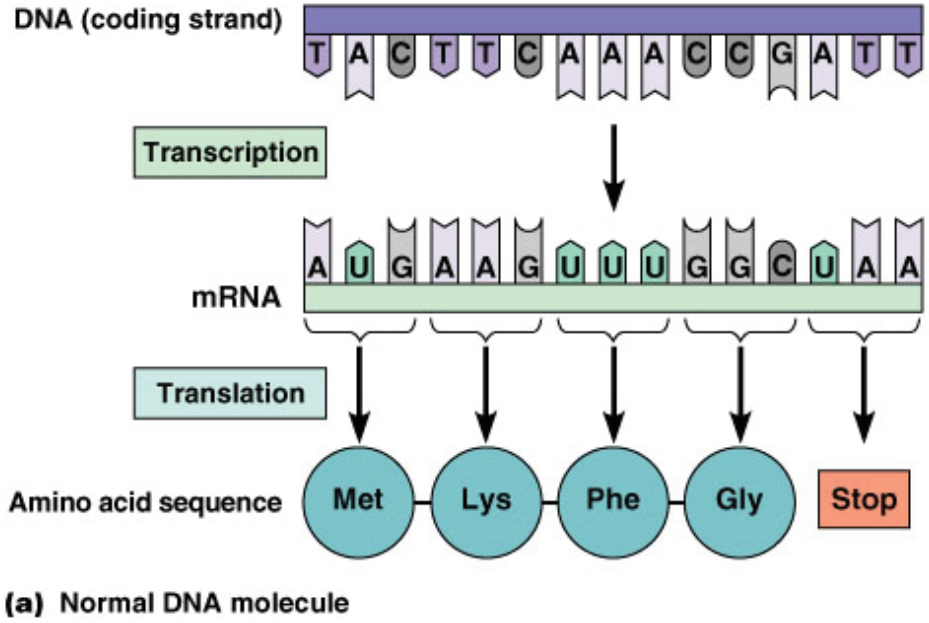


(c) Nonsense mutation

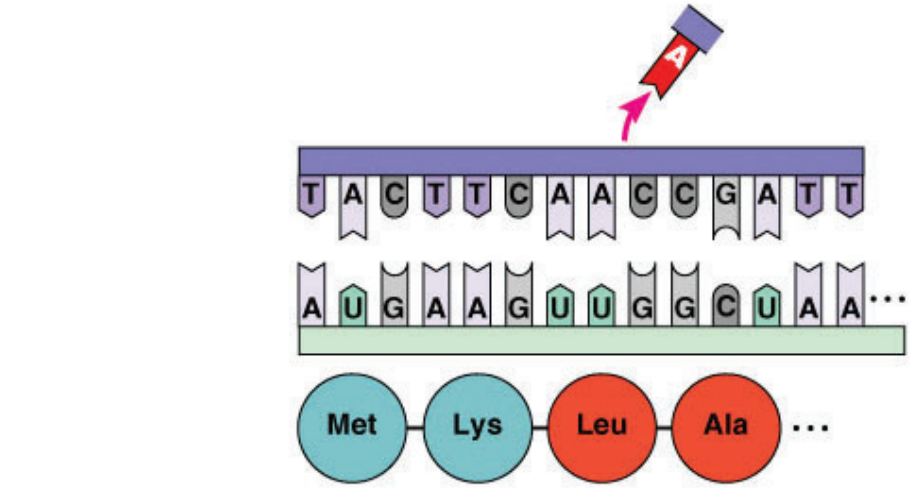
Mutation

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

one of the pairs deleted or inserted ⇒ frame is shifting. ⇒ many amino acid changing ⇒ this is the difference from point mutation



(a) Normal DNA molecule



(d) Frameshift mutation

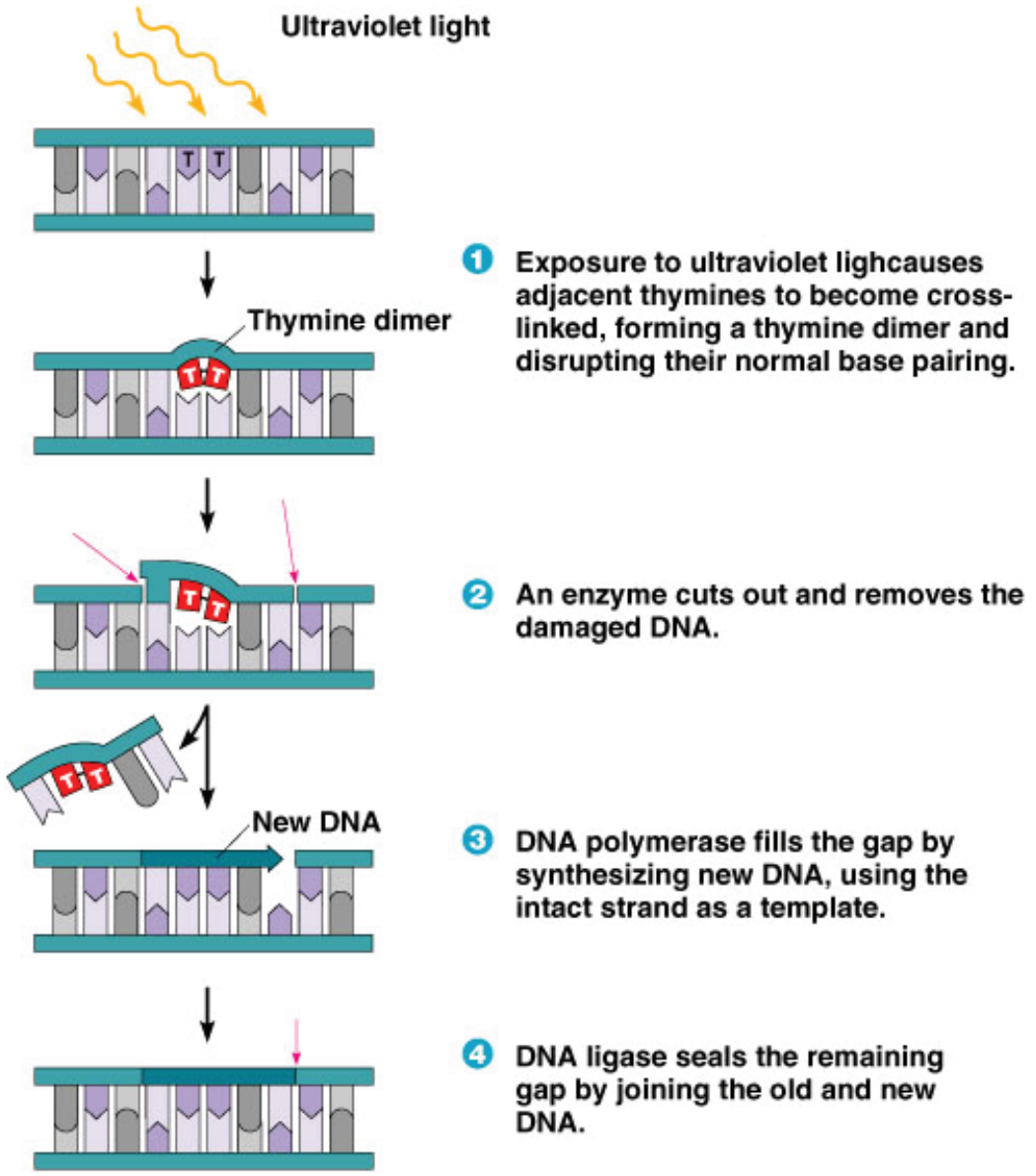
Mutation

- 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

Mutation

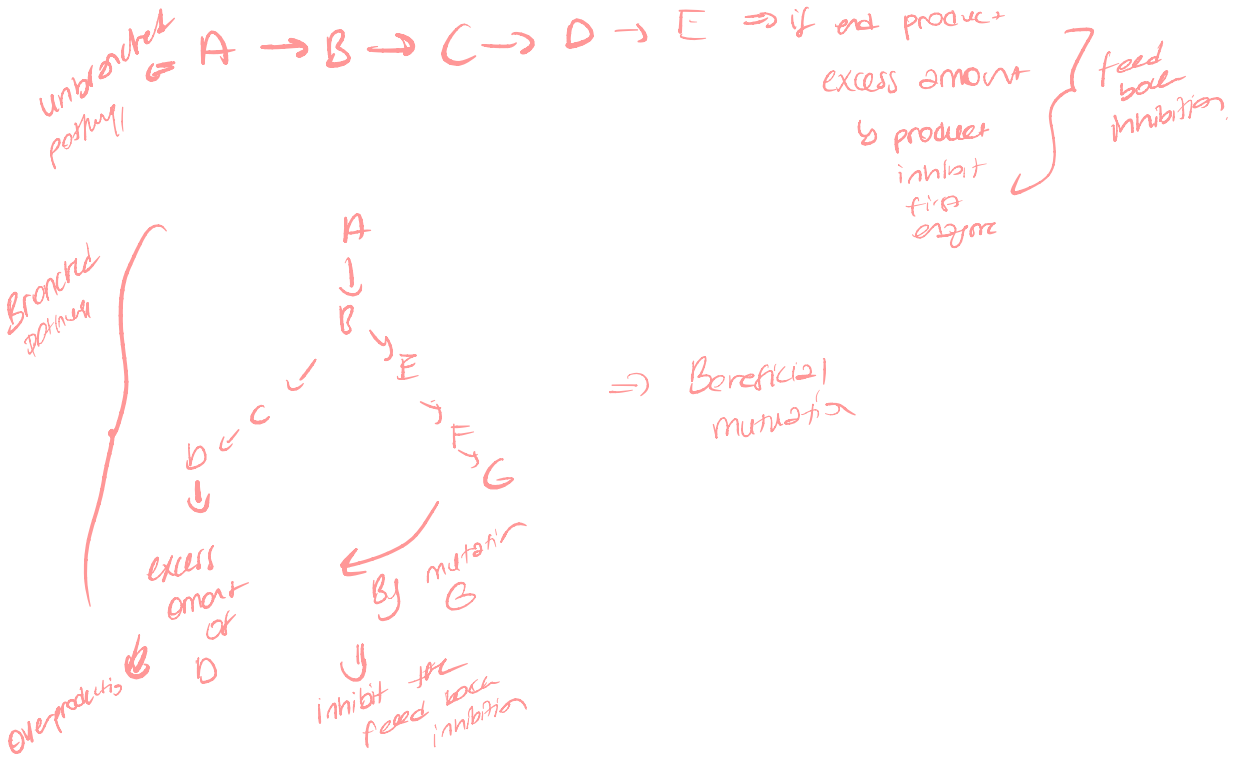
Pyrimidins (C, T) → dimers cause mutation (TT, CC)

- UV radiation causes thymine dimers
- Light-repair separates thymine dimers

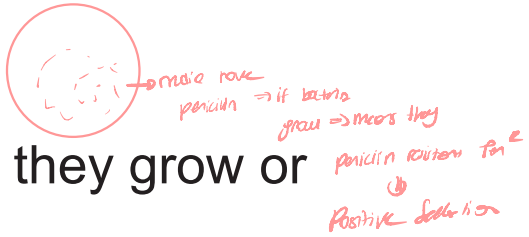


The Frequency of Mutation

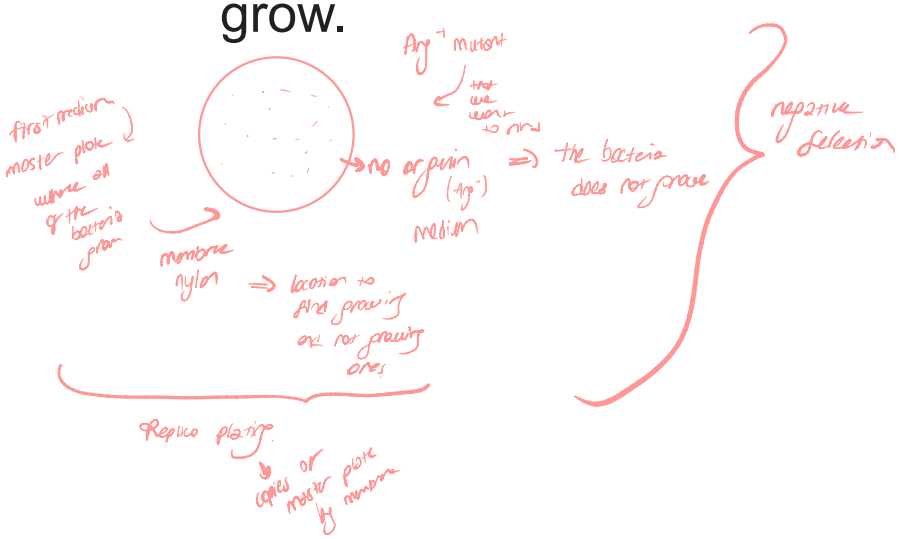
- Spontaneous mutation rate = 1 in 10^9 replicated base pairs or 1 in 10^6 replicated genes
- Mutagens increase to 10^{-5} or 10^{-3} per replicated gene



Selection



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



Genetic Transfer and Recombination

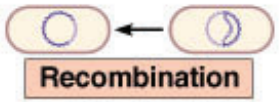
- Vertical gene transfer → replication
  - Occurs during reproduction, between generations of cells
- Horizontal gene transfer → Tarctal for transfe
  - Transfer of genes between cells of the same generation
  - Transformation
  - conjugation
  - transduction

really imp. for public health.

Sirovdo učunů de sarucokmis

Transformation → Naked DNA inserted in the cell.

Transformation frequency very low (probability low)
→ to increase $CaCl_2$ → in the lab
→ Put the cell called transformant



(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

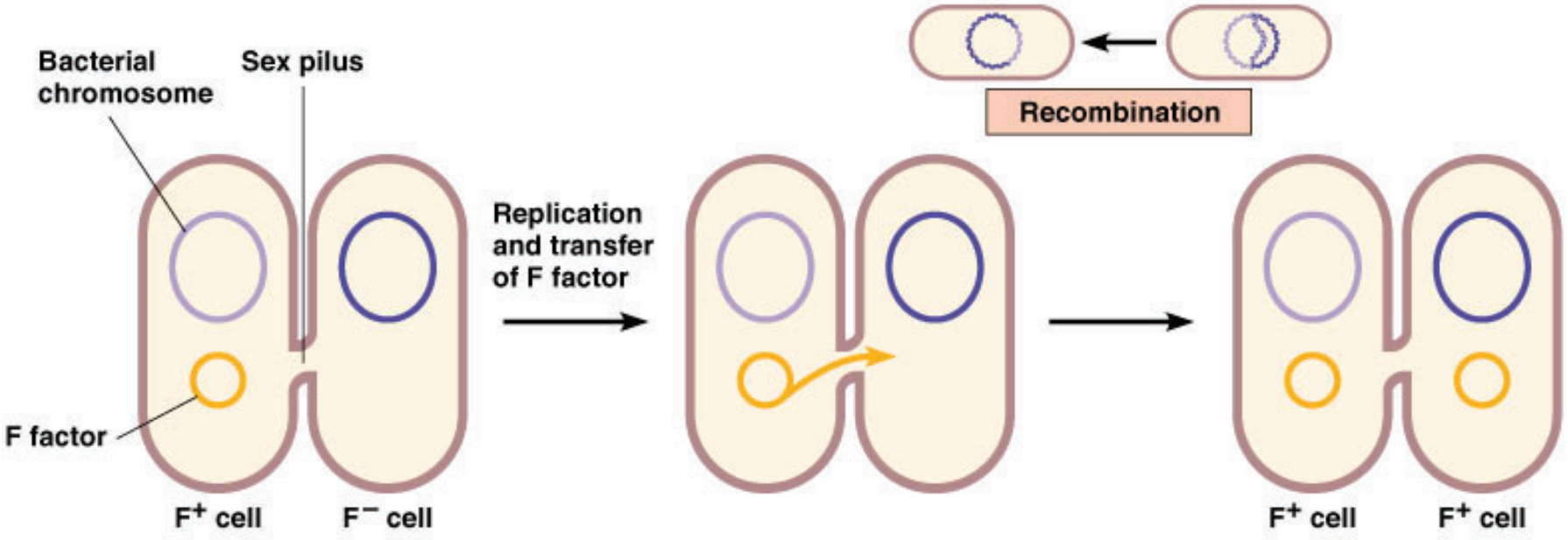
(c)

1. Heat-killed encapsulated bacteria injected into mouse
2. Mouse remained healthy
3. No colonies were isolated from mouse

(d)

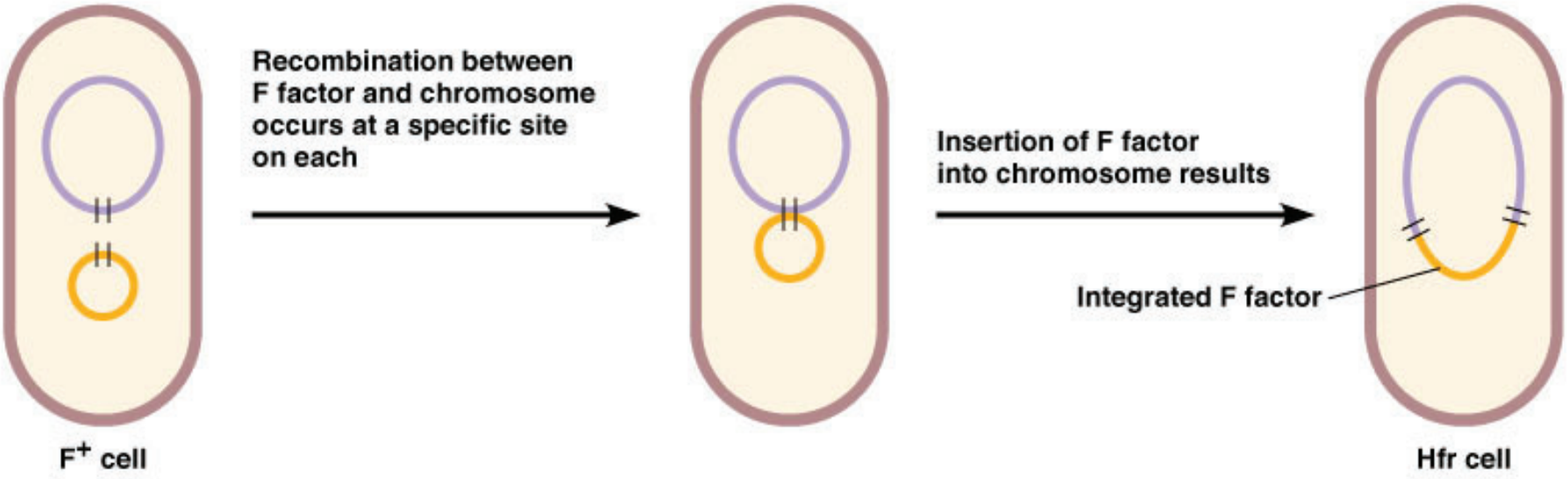
1. Living nonencapsulated and heat-killed encapsulated bacteria injected into mouse
2. Mouse died
3. Colonies of encapsulated bacteria were isolated fromdead mouse

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.

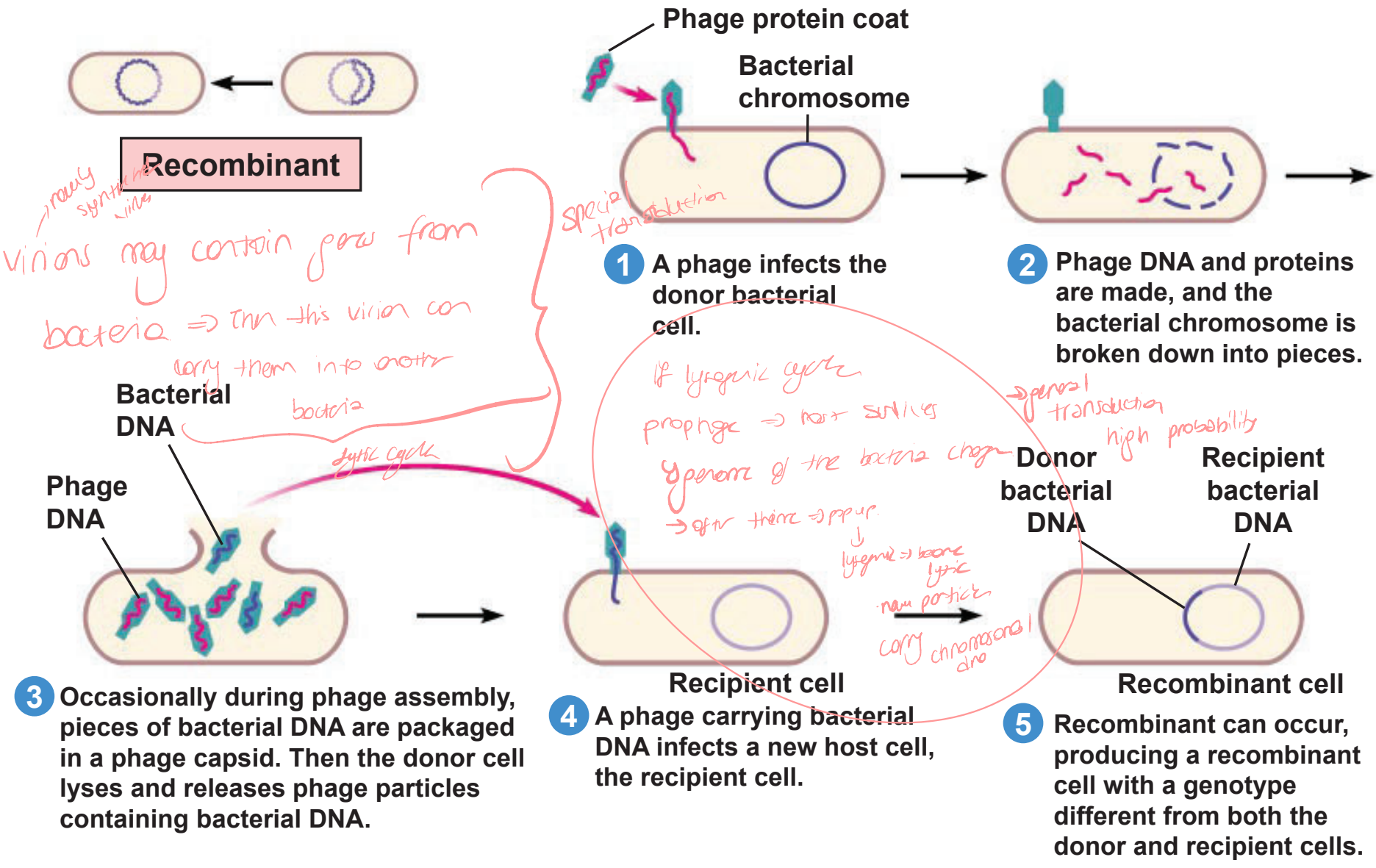
Conjugation



(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.

Hfr $\xrightarrow[\text{conjugation}]{}$ F^- $\Rightarrow$ F' (cord cell)

Transduction



Plasmids → Extra chromosomal DNA

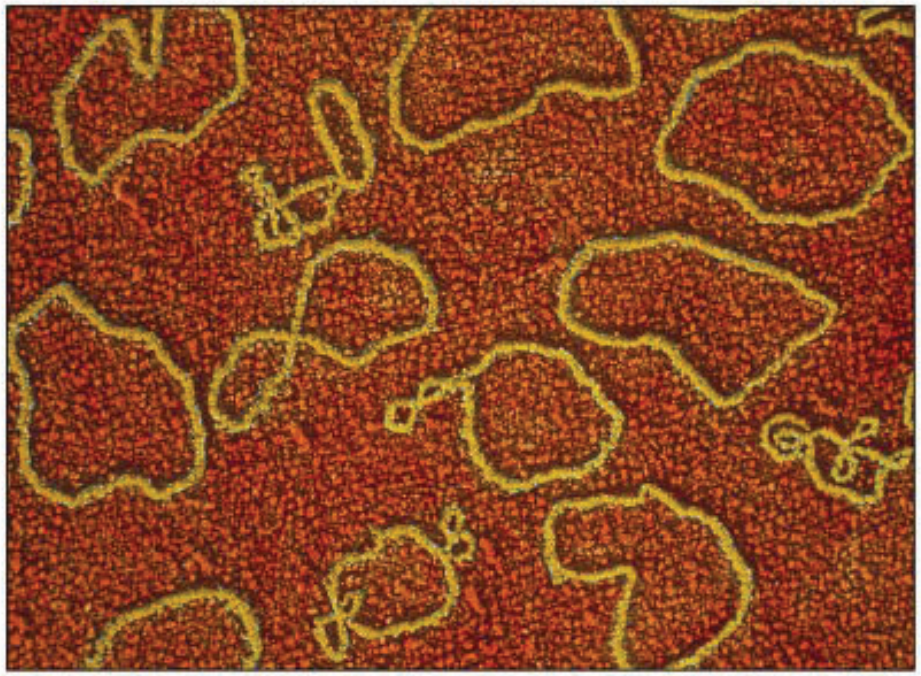
not all microbes have them

carry genes.

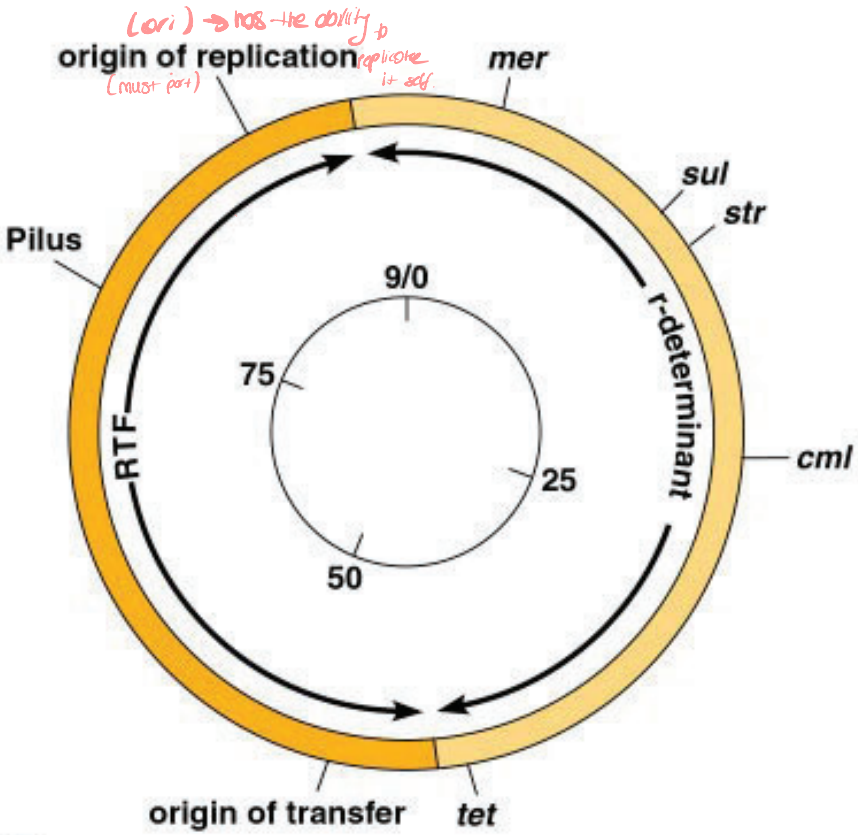
Plasmid → cc, ds dna molecule
cc → closed circular
ds → double stranded

• Conjugative plasmid ⇒ carry genes for pili (Fertility factor) F	Carries genes for sex pili and transfer of the plasmid
• Dissimilation plasmids ⇒ for enzymes (catabolic genes)	Encode enzymes for catabolism of unusual compounds
• R factors → antibiotic resistant genes	Encode antibiotic resistance

Plasmids



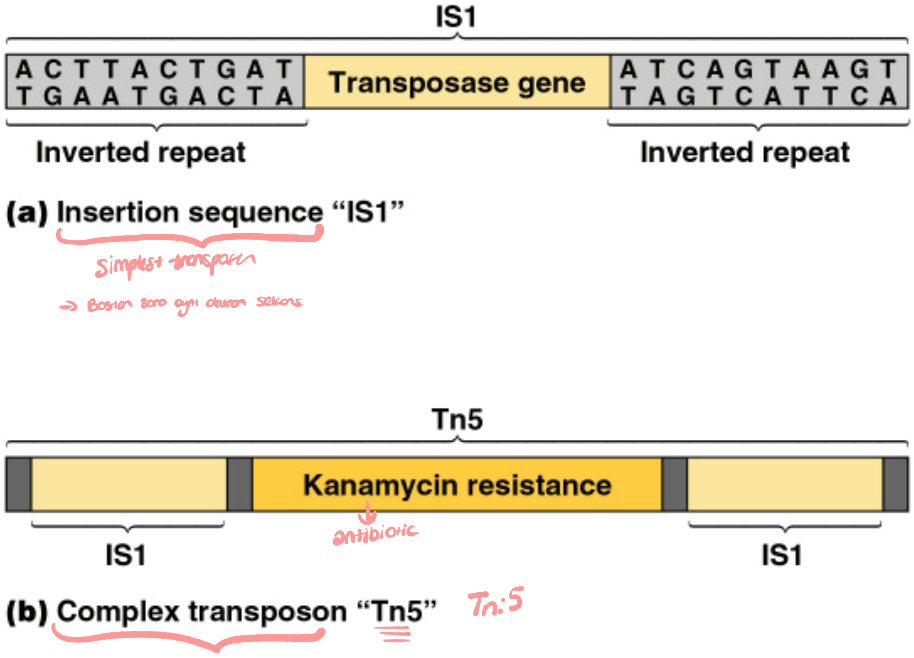
(a)


origin of replication
mer
sul
str
Pilus
9/0
75
25
50
r-determinant
RTF
cml
origin of transfer
tet

(b)

Transposons

- 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

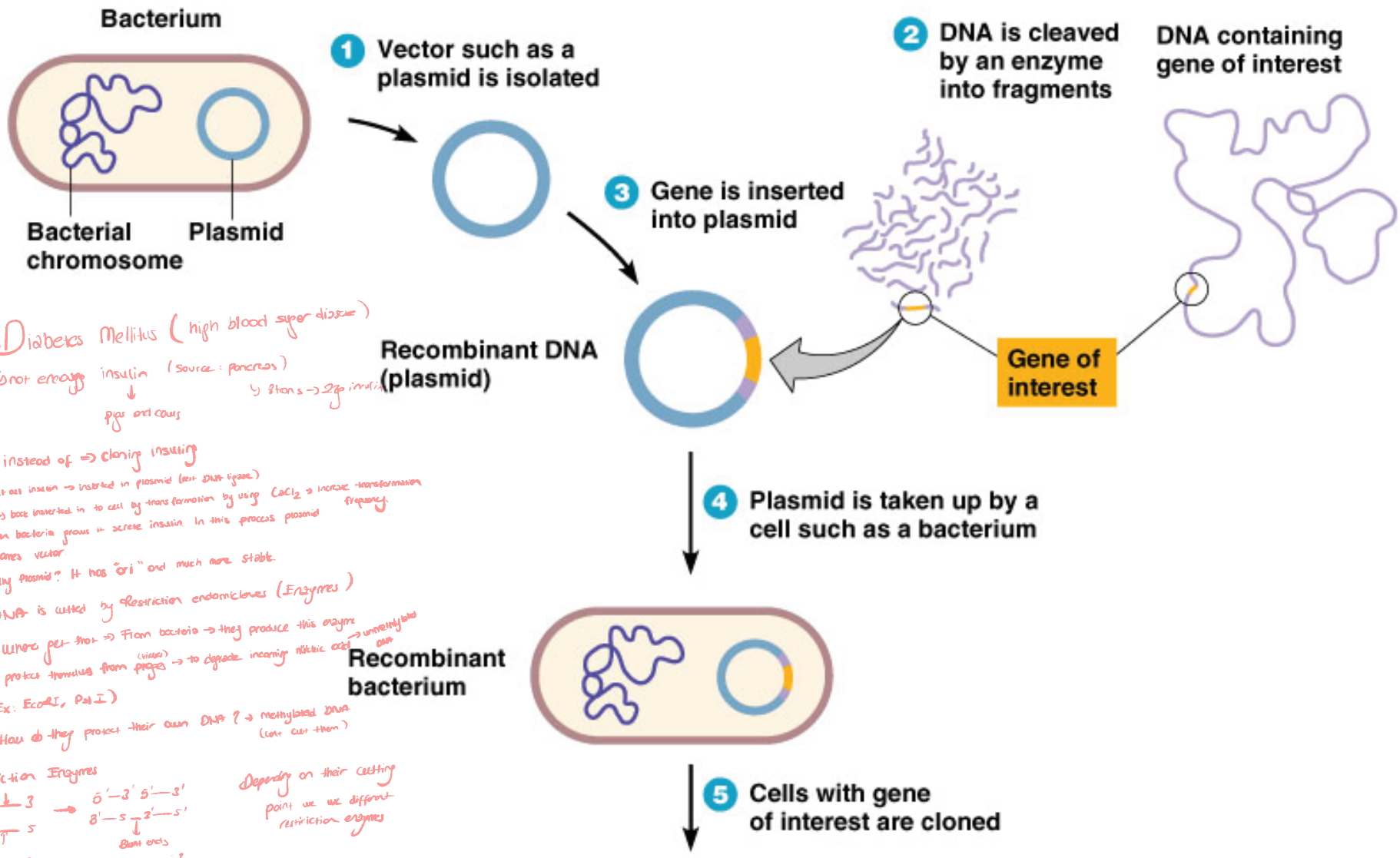


Biotechnology and Recombinant DNA

- Biotechnology:
  - The use of microorganisms, cells, or cell components to make a product
  - Foods, antibiotics, vitamins, enzymes
- Recombinant DNA Technology:
  - Insertion or modification of genes to produce desired proteins

vector → we → plasmid then as a tool to transfer the genes into the bacteria in the lab.

cloning



Diabetes Mellitus (high blood sugar disease)
Not enough insulin (source: pancreas)
↓
pigs and cows
1 gram → 2g insulin

instead of ⇒ cloning insulin

cut out insulin → inserted in plasmid (ext. DNA ligase)
↳ back inserted in to cell by transformation by using $CaCl_2$ → increase transformation frequency.
when bacteria grows it secrete insulin. In this process plasmid becomes vector.

Why Plasmid? It has "ori" and much more stable.

DNA is cutted by Restriction endonucleases (Enzymes)
↳ where per from ⇒ From bacteria → they produce this enzyme
to protect themselves from phages (viruses) → to degrade incoming nitrilic acid → unmethylated own
(Ex: EcoRI, PstI)
↳ How do they protect their own DNA? → methylated DNA (can't cut them)

Restriction Enzymes

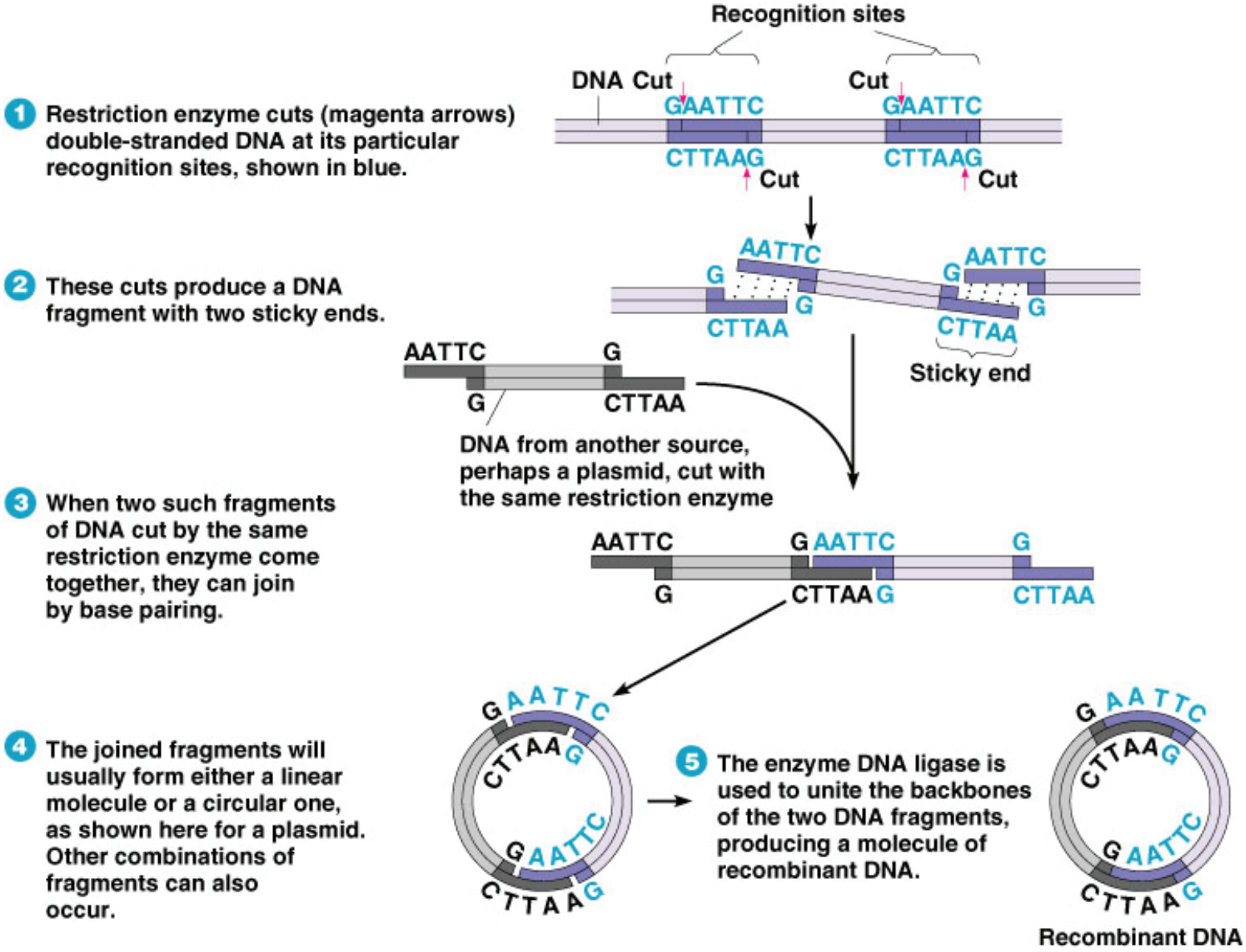
5 ↓ 3 → 5' — 3' 5' — 3'
3 ↑ 5 → 3' — 5' 3' — 5'
↓
Blunt ends

5 ↓ 3 → 5' — 3 5 — 3'
3 — ↑ 5' → 3' — 5 3' — 5'
↓
Sticky ends → more common

Depending on their cutting point we use different restriction enzymes

Restriction Enzymes

- Cut specific sequences of DNA
- Destroy bacteriophage DNA in bacterial cells (not all bacteria have them)
- Cannot digest (host) DNA with methylated cytosines


Recognition sites
DNA Cut
Cut
GAATTC
CTTAAG
Cut
Cut
1 Restriction enzyme cuts (magenta arrows) double-stranded DNA at its particular recognition sites, shown in blue.
AATTC
G
G
CTTAA
Sticky end
2 These cuts produce a DNA fragment with two sticky ends.
DNA from another source, perhaps a plasmid, cut with the same restriction enzyme
3 When two such fragments of DNA cut by the same restriction enzyme come together, they can join by base pairing.
4 The joined fragments will usually form either a linear molecule or a circular one, as shown here for a plasmid. Other combinations of fragments can also occur.
5 The enzyme DNA ligase is used to unite the backbones of the two DNA fragments, producing a molecule of recombinant DNA.
Recombinant DNA

Vectors

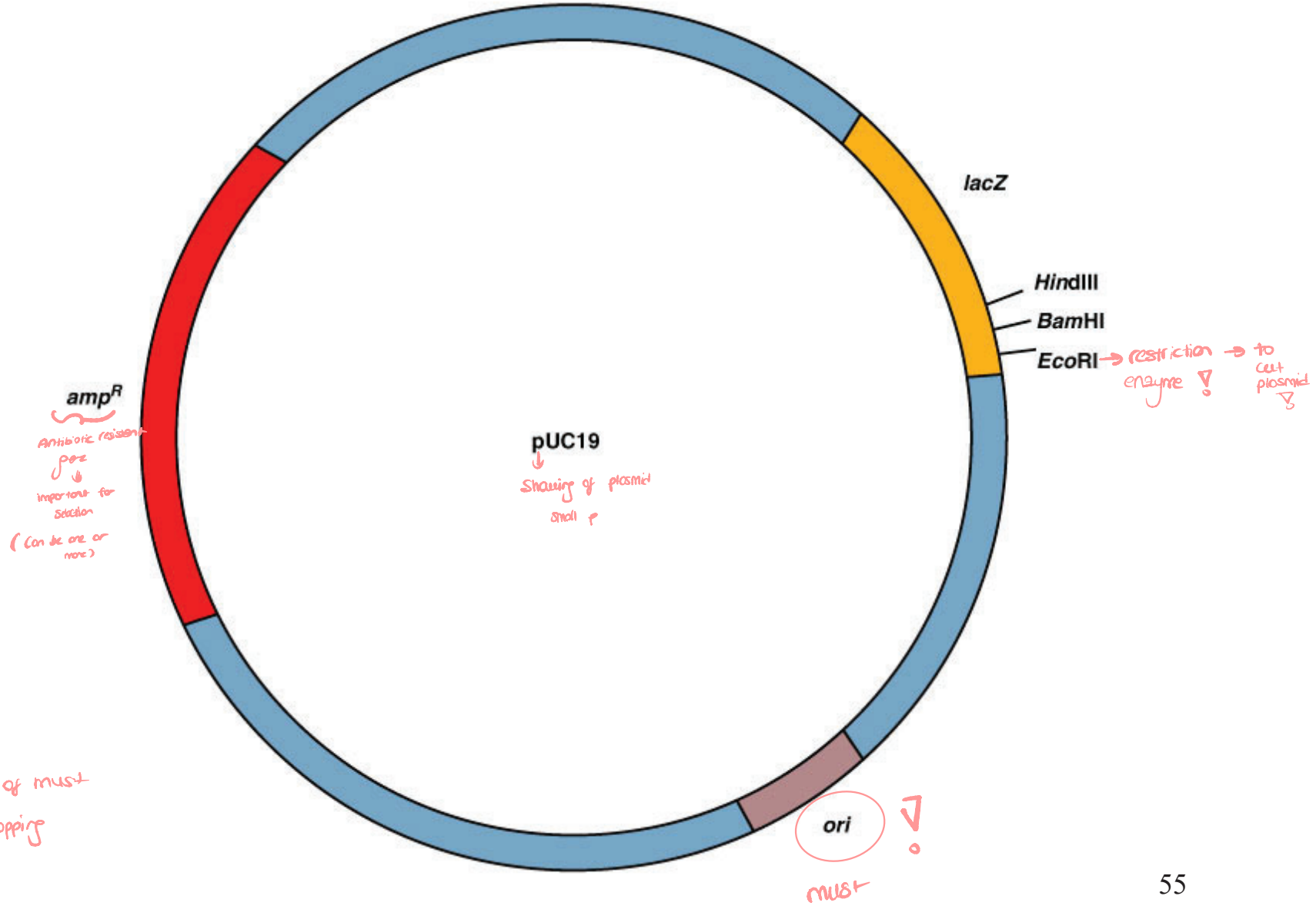
Bacteria (Prokaryotic) Gene (GOI Gene of Interest)

↳ If coming GOI from bacteria (prokaryotic) → no problem (which we will insert in prokaryotic)

↳ If coming GOI from eukaryotic ⇒ This vector (plasmid) should contain promotors for both prokaryotic and eukaryotic cells ⇒ Named "Shuttle Vectors"

- Carry new DNA to desired cell
- Shuttle vectors can exist in several different species
- Plasmids and viruses can be used as vectors

Vectors



55

Figure 9.3

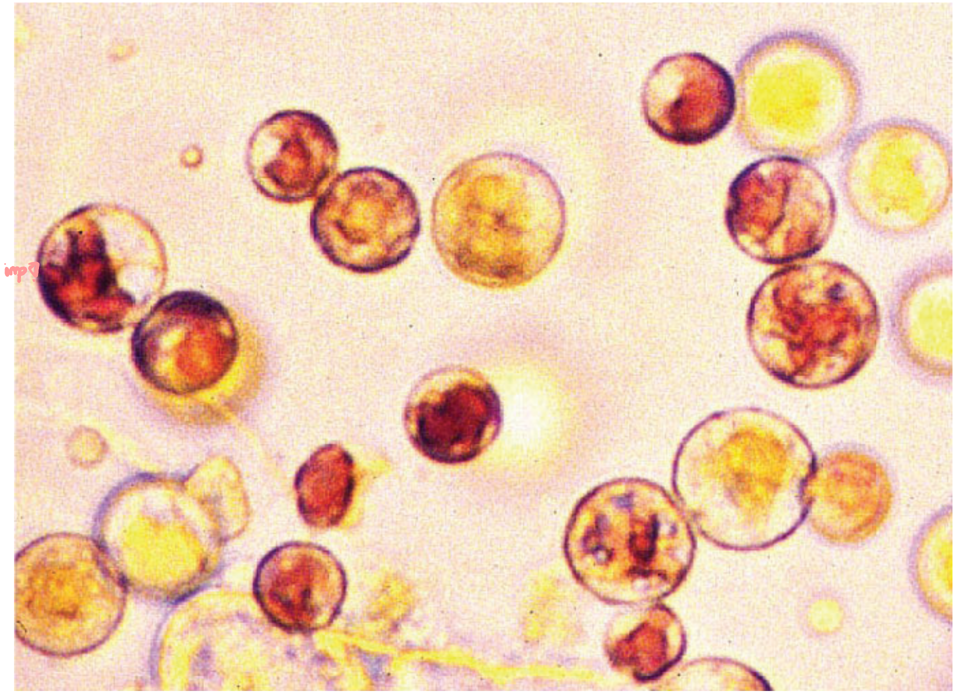
DNA can be inserted into a cell by:

(In vitro (in lab)) methods

- Transformation → $CaCl_2$ (Easier)
- Electroporation → pore of the cells wide → put DNA in the cell; container filled with buffer; power supply; → Optimization is very imp: voltage and Amper
- Protoplast fusion

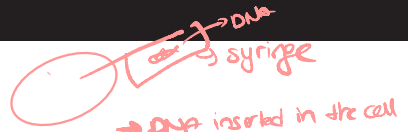
protoplast (removed cell wall and some remaining part) — usually for plant cells

PEG (Polyethylene Glycol) → fused hybrid protoplast



DNA can be inserted into a cell by:

- Microinjection (In vitro fertilization IVF)
  - → in the lab
  - → IVF Babies
- Gene gun (Biolistics / microprojectile Bombardment)
  - ↳ Usually used for plant cell.
  - genes → nano particles (golds) → DNA attached to gold particle
  - ↳ this gold shotted into the plant → transgenic plant



→ DNA inserted in the cell under microscope !