

MICROBIAL GENETICS

BY

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Terminology

Genetics

Study of what genes are, how they carry information, how information is expressed, and how genes are replicated

Gene

Segment of DNA that encodes a functional product, usually a protein

Terminology

Genome

- ▣ All of the genetic material in a cell

Genomics

- ▣ Molecular study of genomes

Genotype

- ▣ Genetic make up of an organism (potential properties)
- ▣ Collection of genes

Phenotype

- ▣ Expression of the genes
- ▣ Collection of proteins

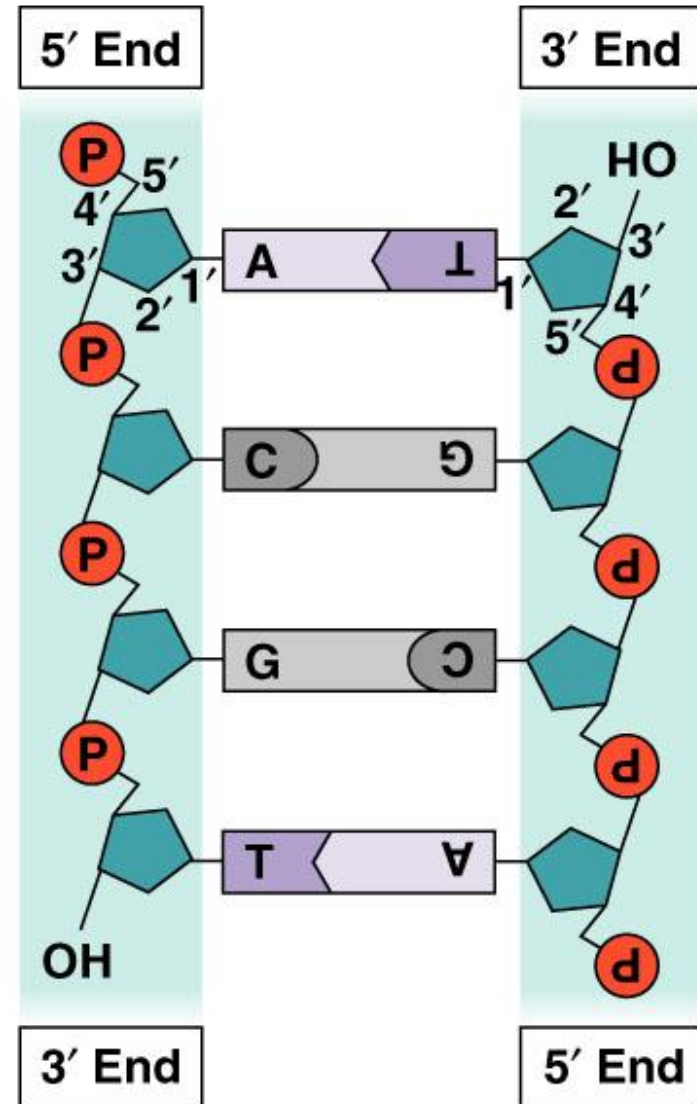
Chromosome (*E. coli*)

- 4.6 million base pairs
- 1 meter long
- 1000 times longer than bacteria



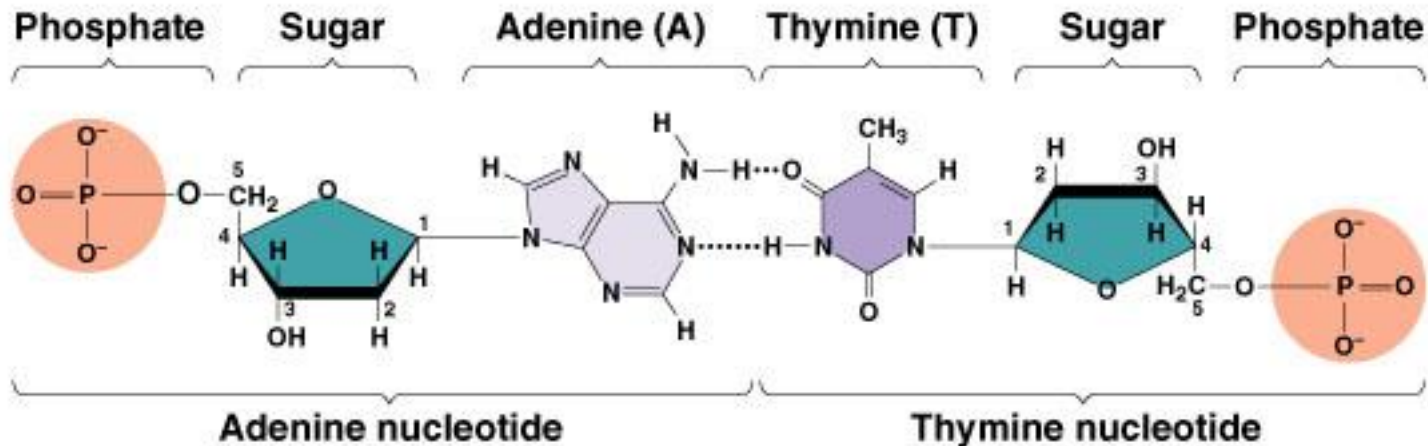
DNA

- Polymer of nucleotides: adenine, thymine, cytosine, guanine
- Double helix associated with proteins
- "Backbone" is deoxyribose-phosphate
- Strands held together by hydrogen bonds between AT and CG
- Strands are antiparallel

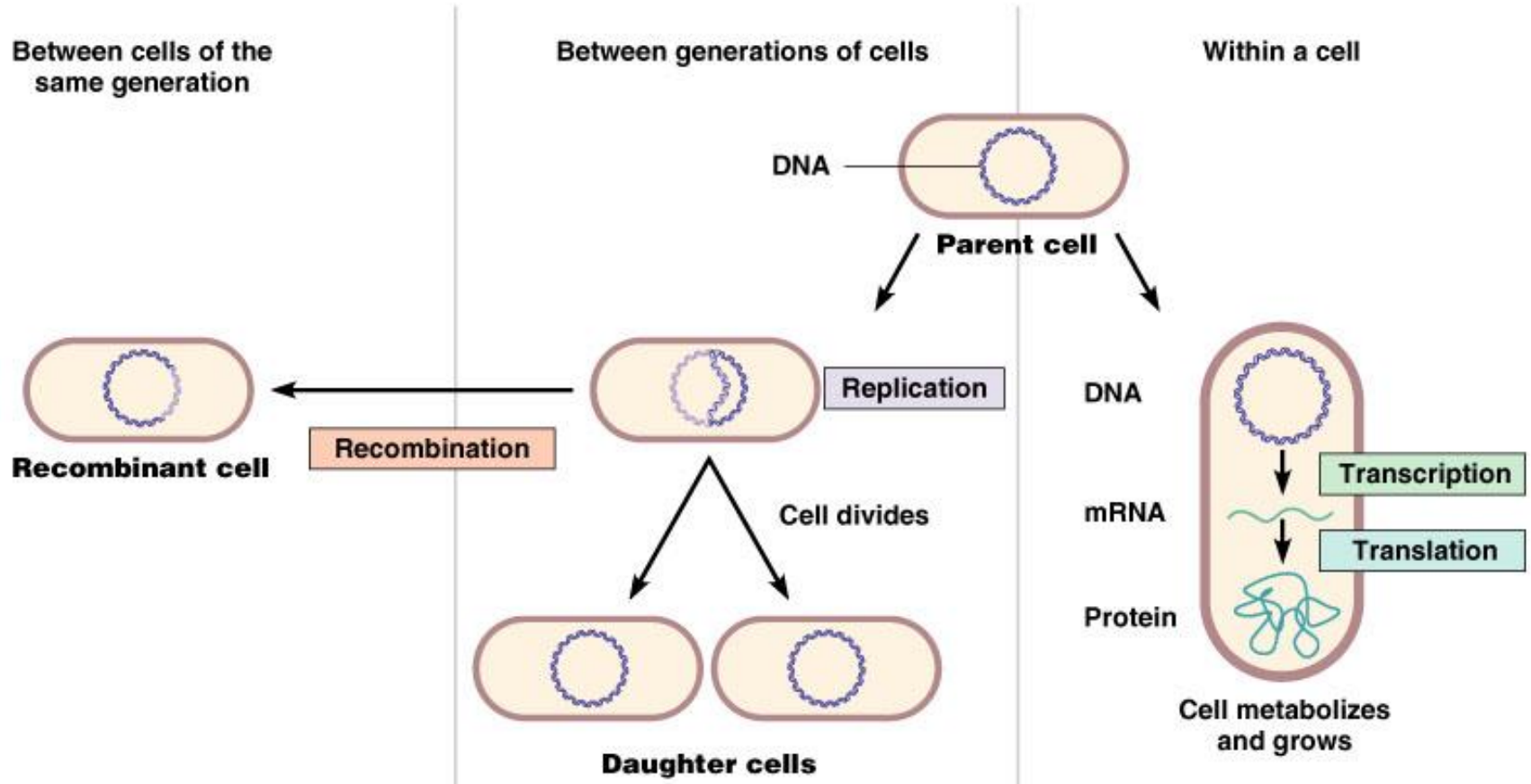


Nucleic Acids

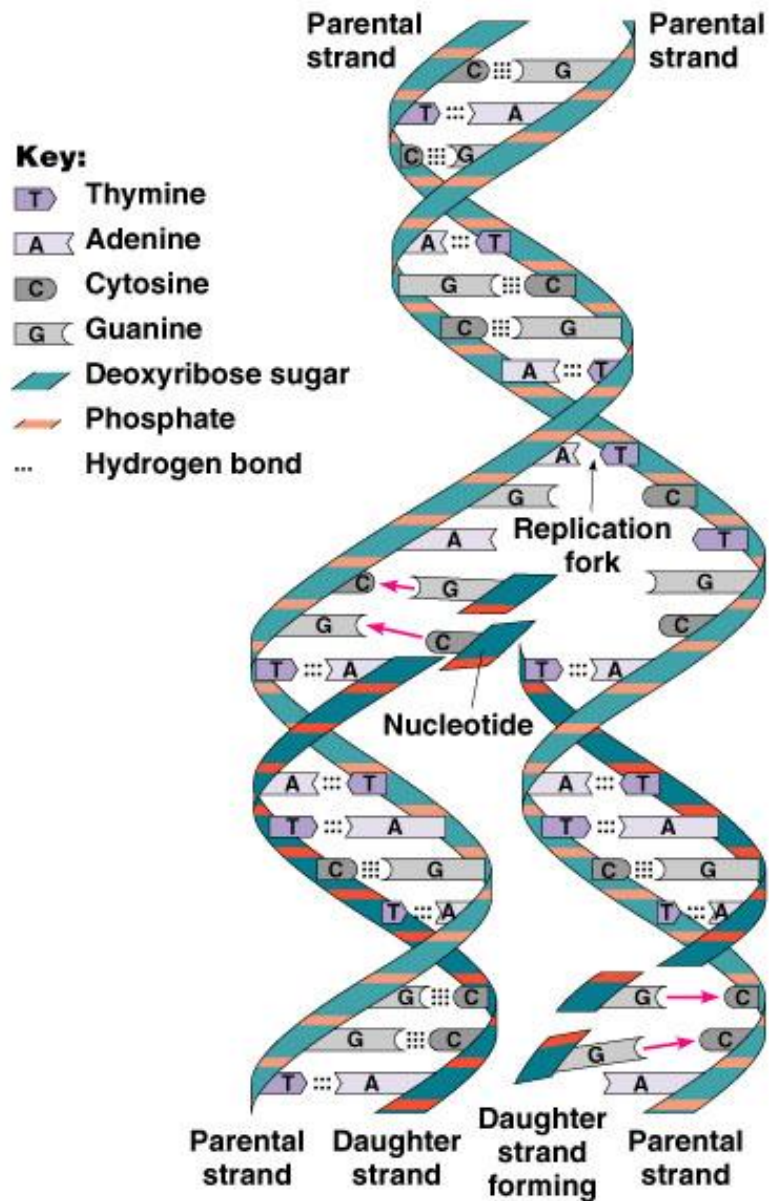
- Consist of nucleotides.
- Nucleotides consist of a:
 - Pentose
 - Phosphate group
 - Nitrogen-containing (purine or pyrimidine) base



Flow of Genetic Information



DNA replication



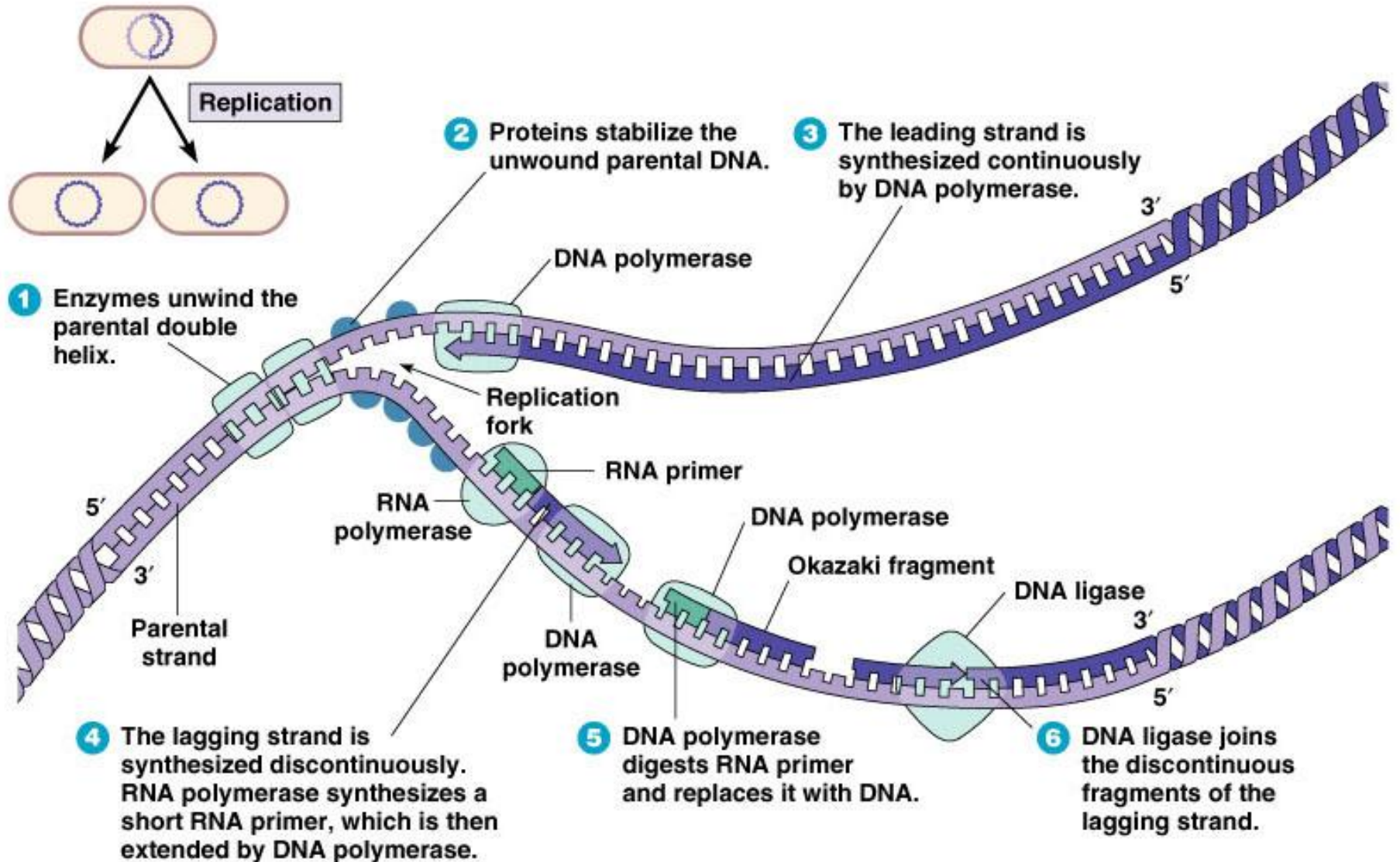
DNA replication

- Two parent strands unwound
- DNA polymerase
- **Replication fork**
- Semiconservative replication
 - ▣ Each parent strand make complementary strand

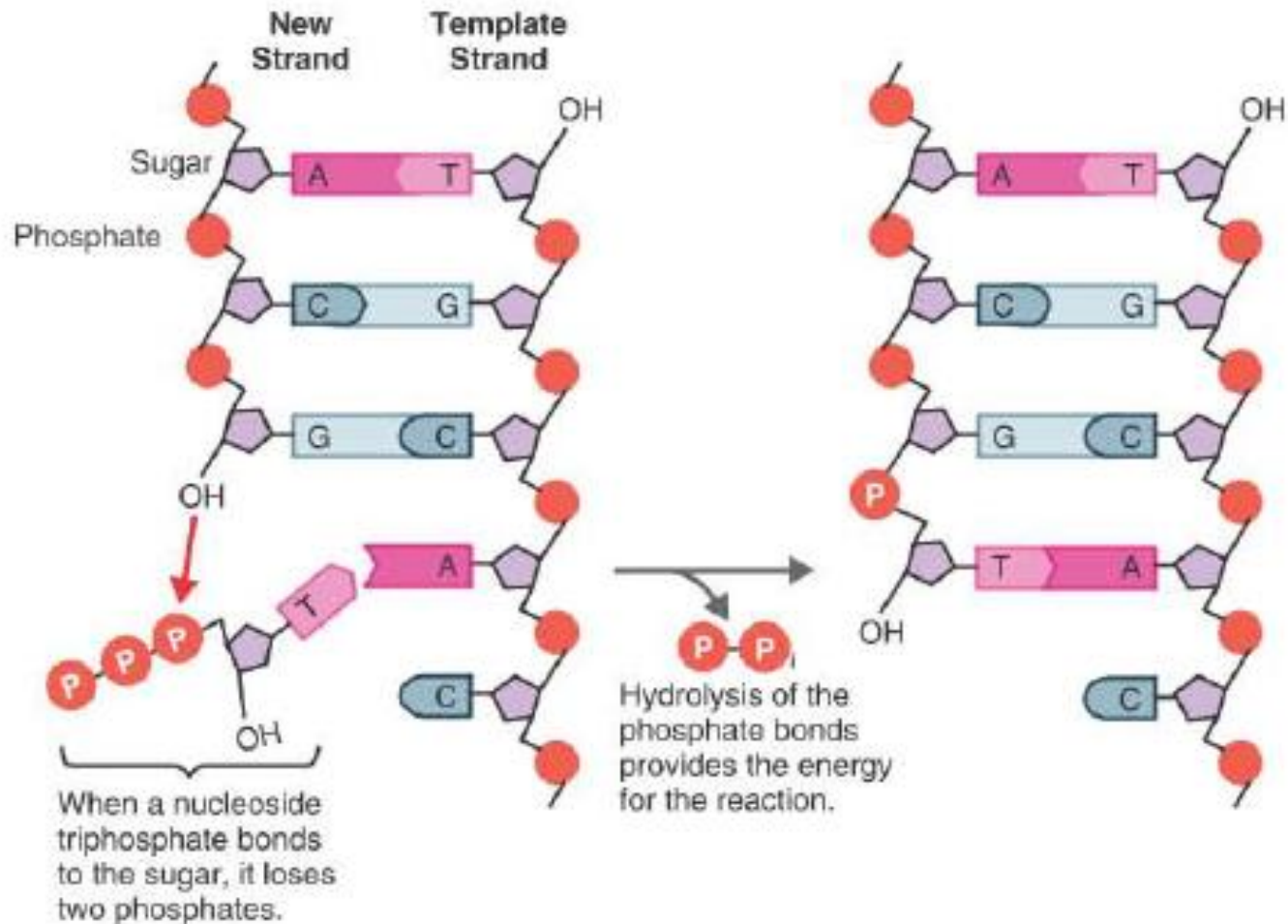
DNA replication

- DNA is copied by DNA polymerase
 - ▣ In the 5' → 3' direction
 - ▣ Initiated by an RNA primer
 - ▣ Leading strand synthesized continuously
 - ▣ Lagging strand synthesized discontinuously
 - ▣ Okazaki fragments
 - ▣ RNA primers are removed and Okazaki fragments joined by a DNA polymerase and DNA ligase

DNA replication

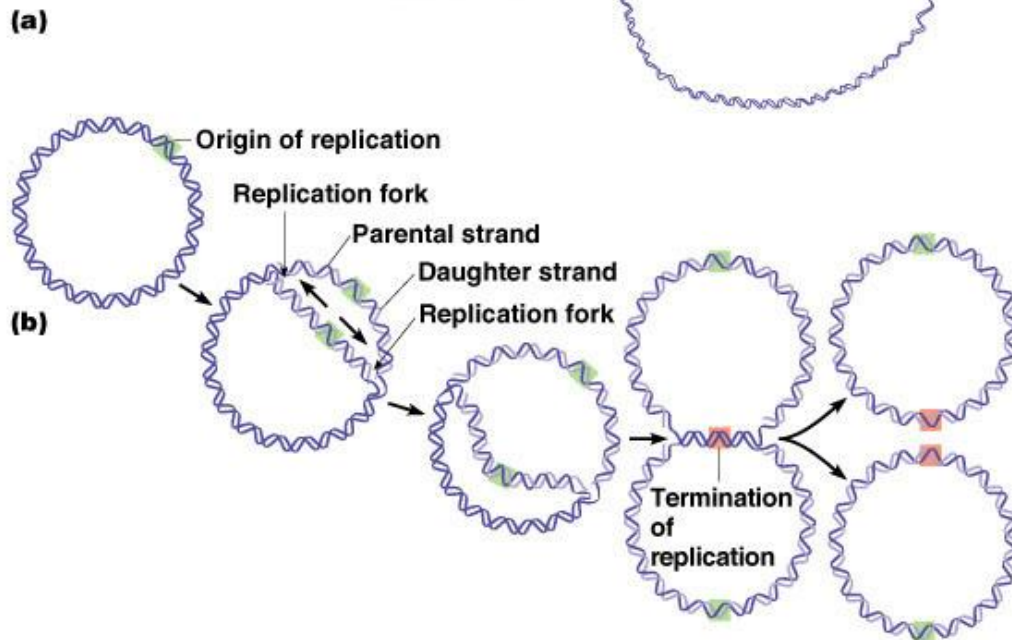
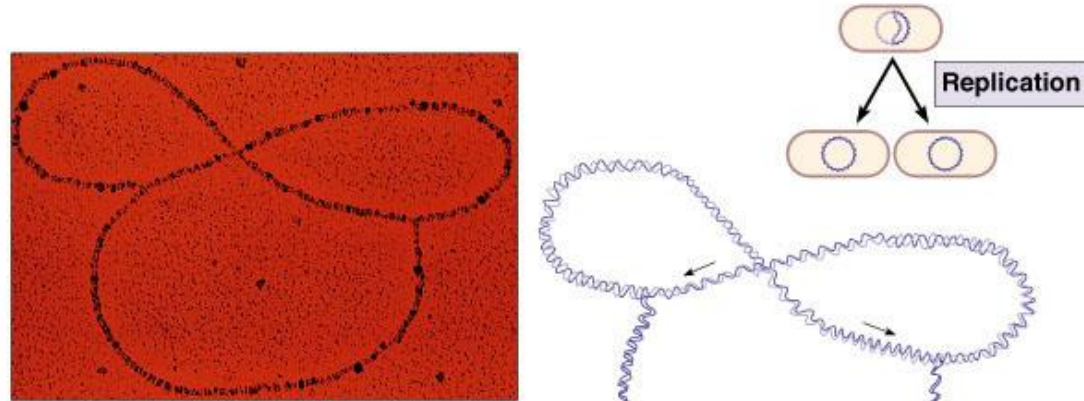


Adding nucleotides to a DNA



DNA replication

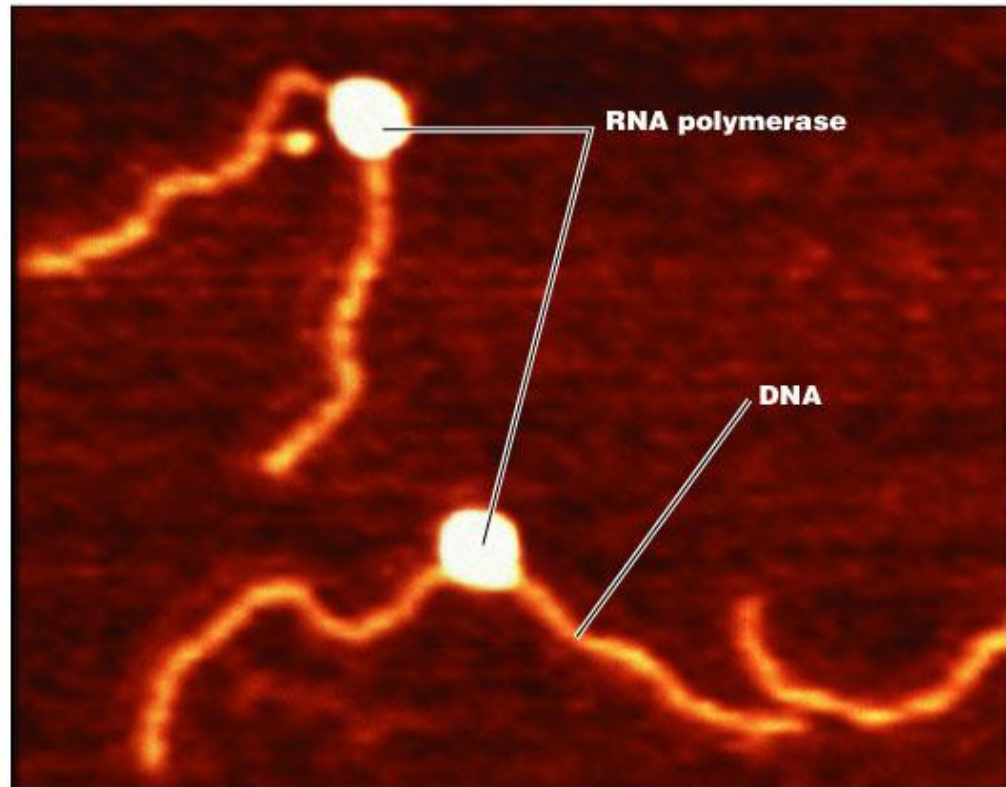
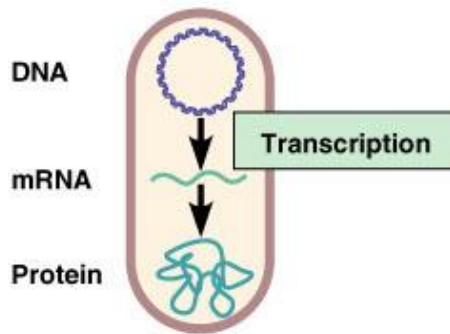
Bidirectional
Closed loop DNA



Transcription

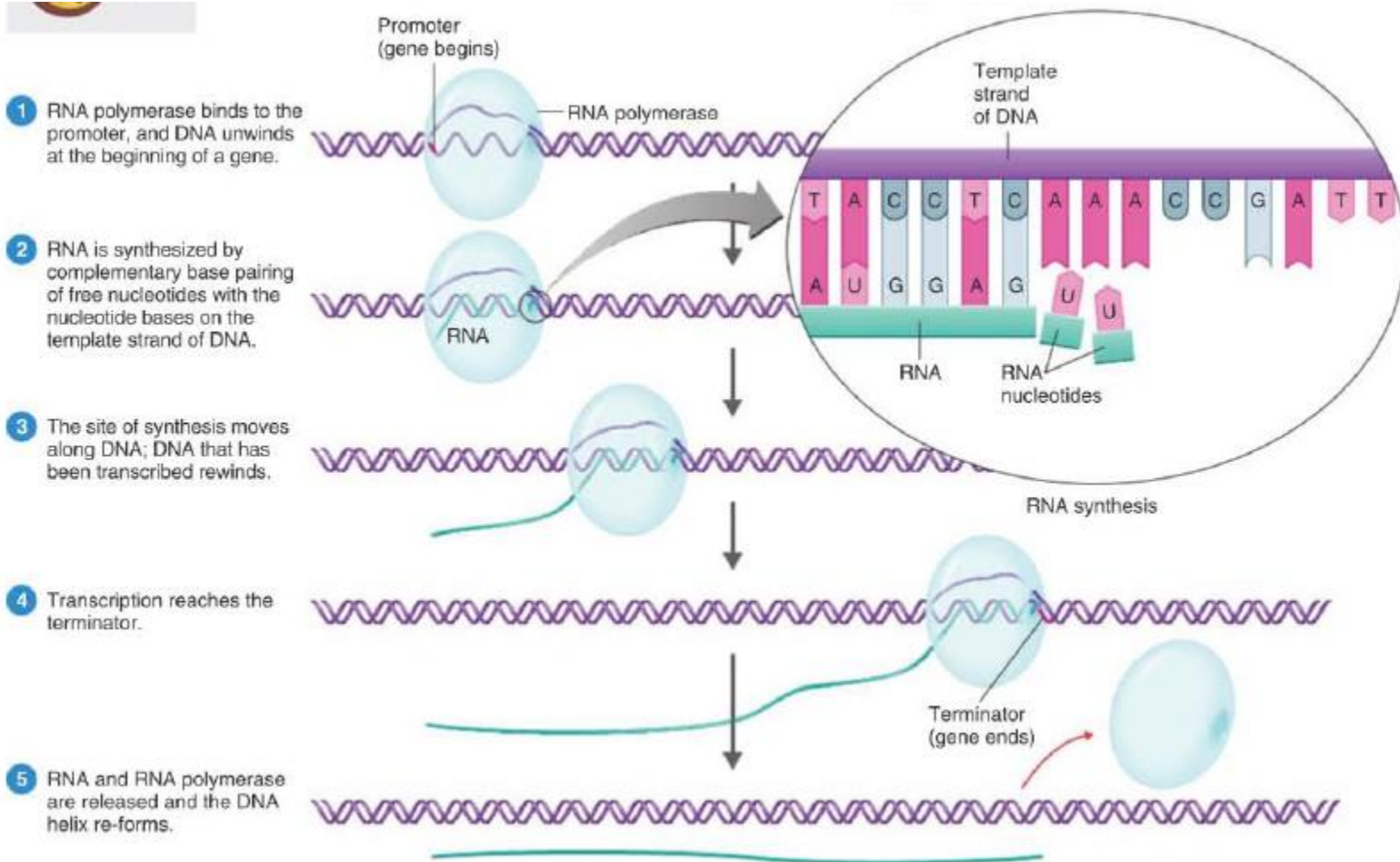
- DNA is transcribed to make RNA (mRNA, tRNA, and rRNA)
- Transcription begins when RNA polymerase binds to the promoter sequence
- Transcription proceeds in the 5' → 3' direction
- Transcription stops when it reaches the terminator sequence

Transcription

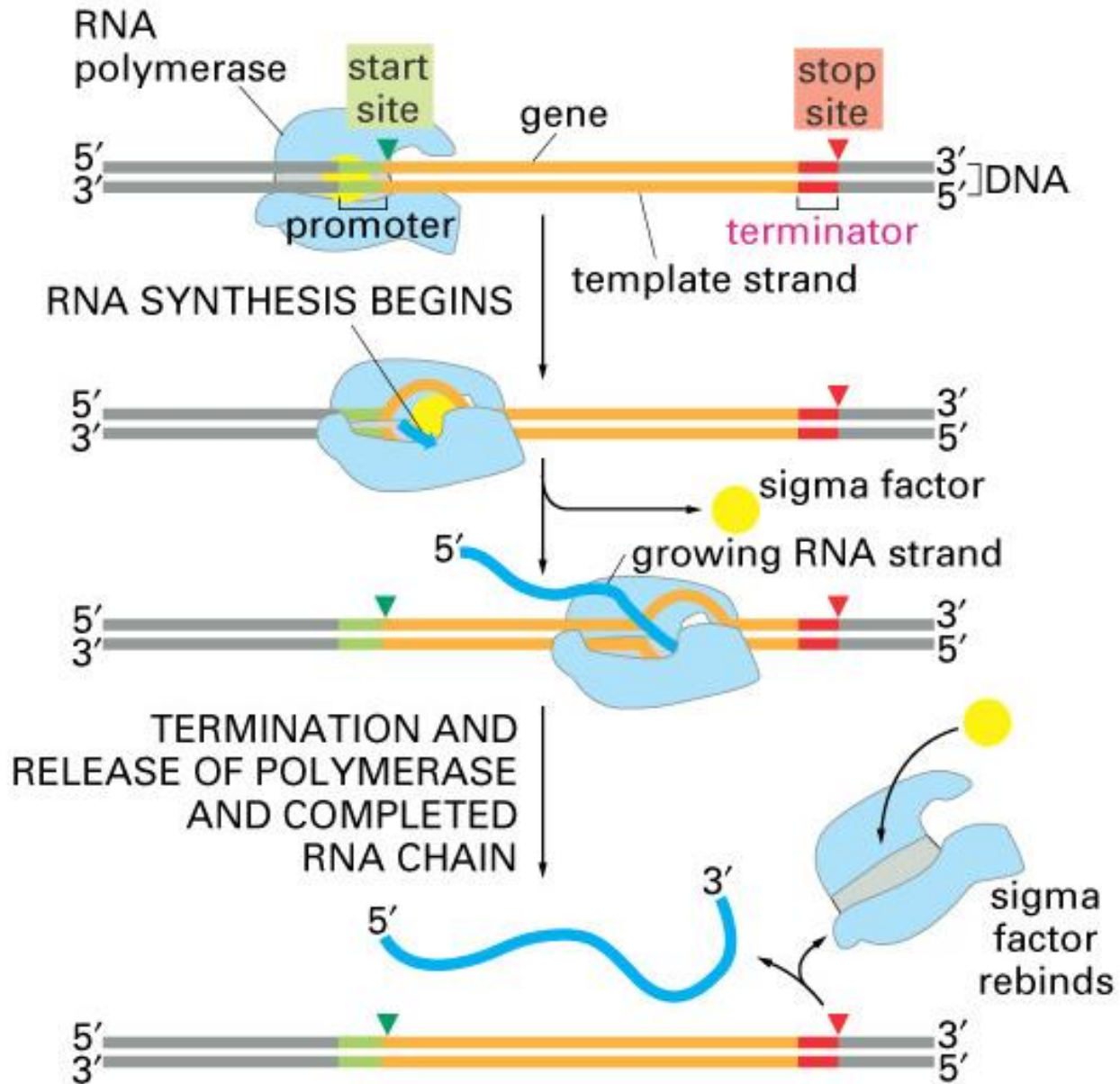


RNA Polymerase bound to DNA

Transcription

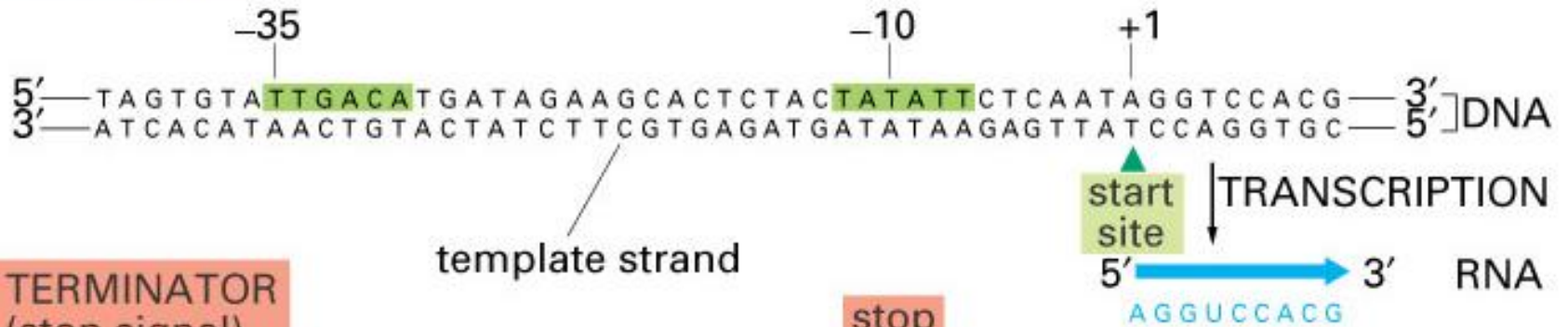


Transcription



Transcription

PROMOTER
(start signal)



TERMINATOR
(stop signal)

stop site



Translation

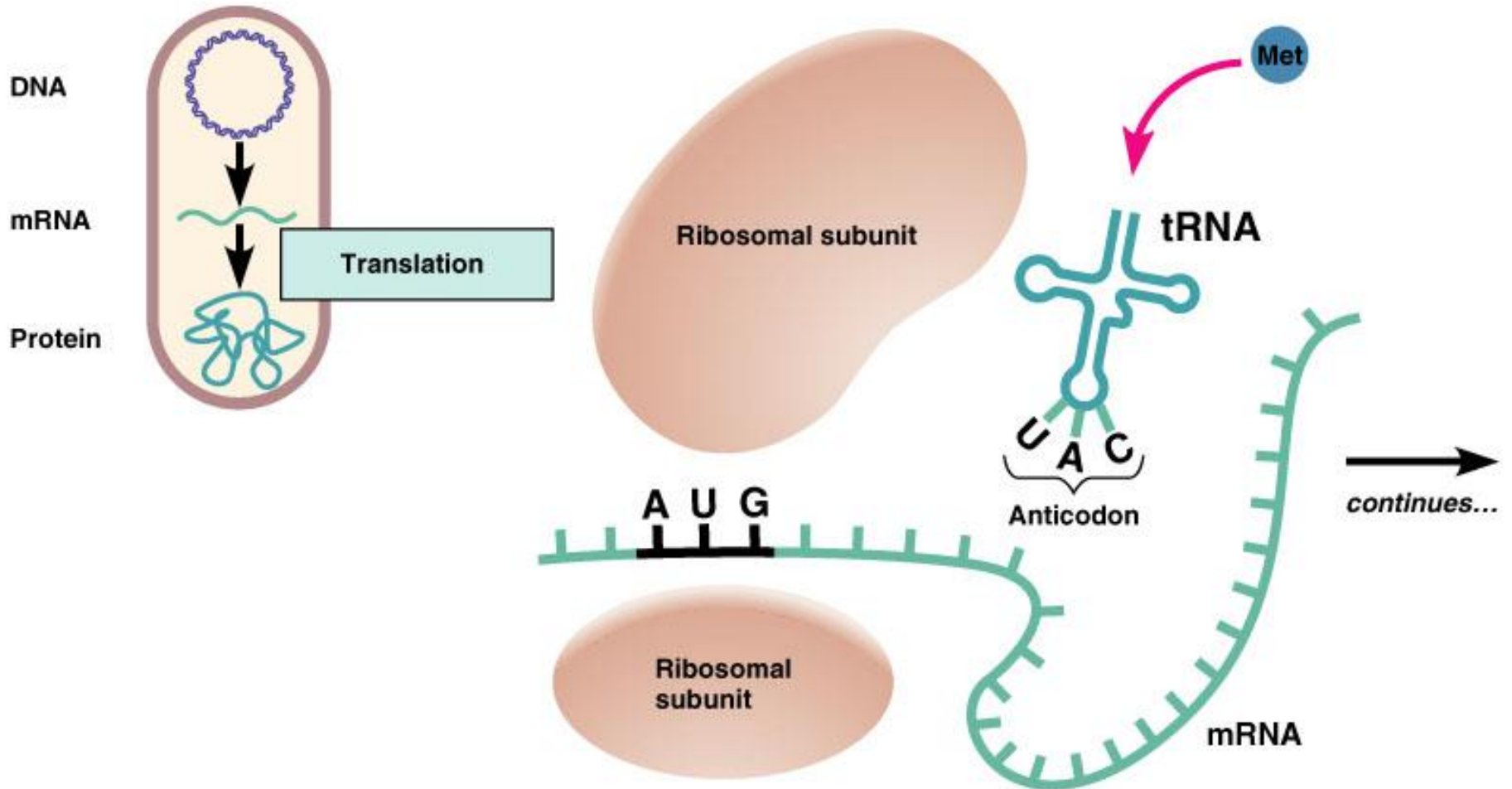
- Process of protein synthesis from mRNA strand
- mRNA is translated in **codons** (3 nucleotides)
- Translation of mRNA begins at the **start codon**: AUG
- Translation ends at a **STOP codon**: UAA, UAG, UGA
- **Sense codons** (61)
- **Nonsense codons** (3) stop codons
 - ▣ UAA, UAG, UGA

Translation

Degeneracy of code?

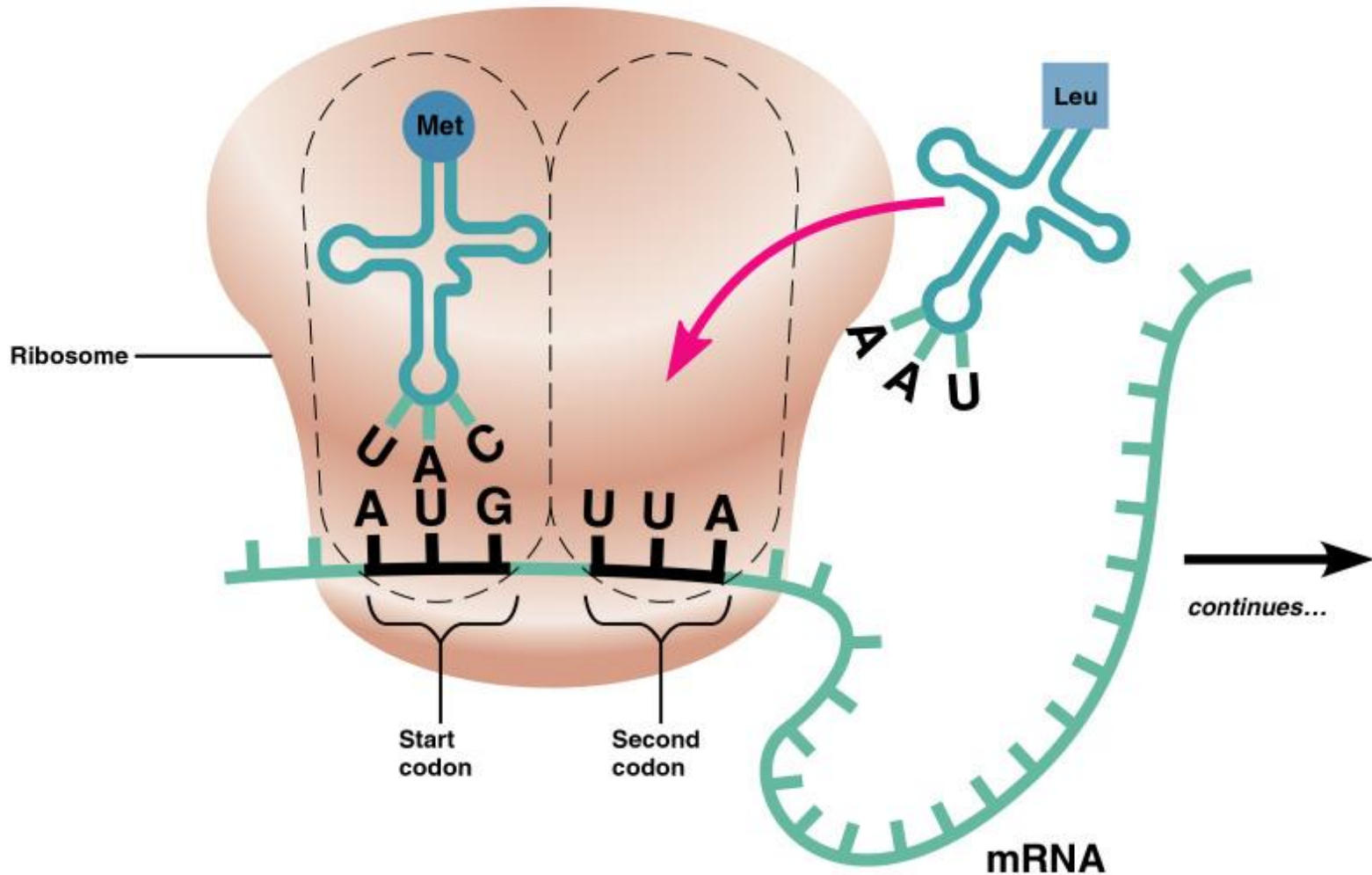
		Second position				
		U	C	A	G	
First position	U	UUU } Phe	UCU } Ser	UAU } Tyr	UGU } Cys	U
		UUC } Phe	UCC } Ser	UAC } Tyr	UGC } Cys	C
		UUA } Leu	UCA } Ser	UAA Stop	UGA Stop	A
		UUG } Leu	UCG } Ser	UAG Stop	UGG Trp	G
	C	CUU } Leu	CCU } Pro	CAU } His	CGU } Arg	U
		CUC } Leu	CCC } Pro	CAC } His	CGC } Arg	C
		CUA } Leu	CCA } Pro	CAA } Gln	CGA } Arg	A
		CUG } Leu	CCG } Pro	CAG } Gln	CGG } Arg	G
	A	AUU } Ile	ACU } Thr	AAU } Asn	AGU } Ser	U
		AUC } Ile	ACC } Thr	AAC } Asn	AGC } Ser	C
		AUA } Ile	ACA } Thr	AAA } Lys	AGA } Arg	A
		AUG Met/start	ACG } Thr	AAG } Lys	AGG } Arg	G
	G	GUU } Val	GCU } Ala	GAU } Asp	GGU } Gly	U
		GUC } Val	GCC } Ala	GAC } Asp	GGC } Gly	C
		GUA } Val	GCA } Ala	GAA } Glu	GGA } Gly	A
		GUG } Val	GCG } Ala	GAG } Glu	GGG } Gly	G

Translation



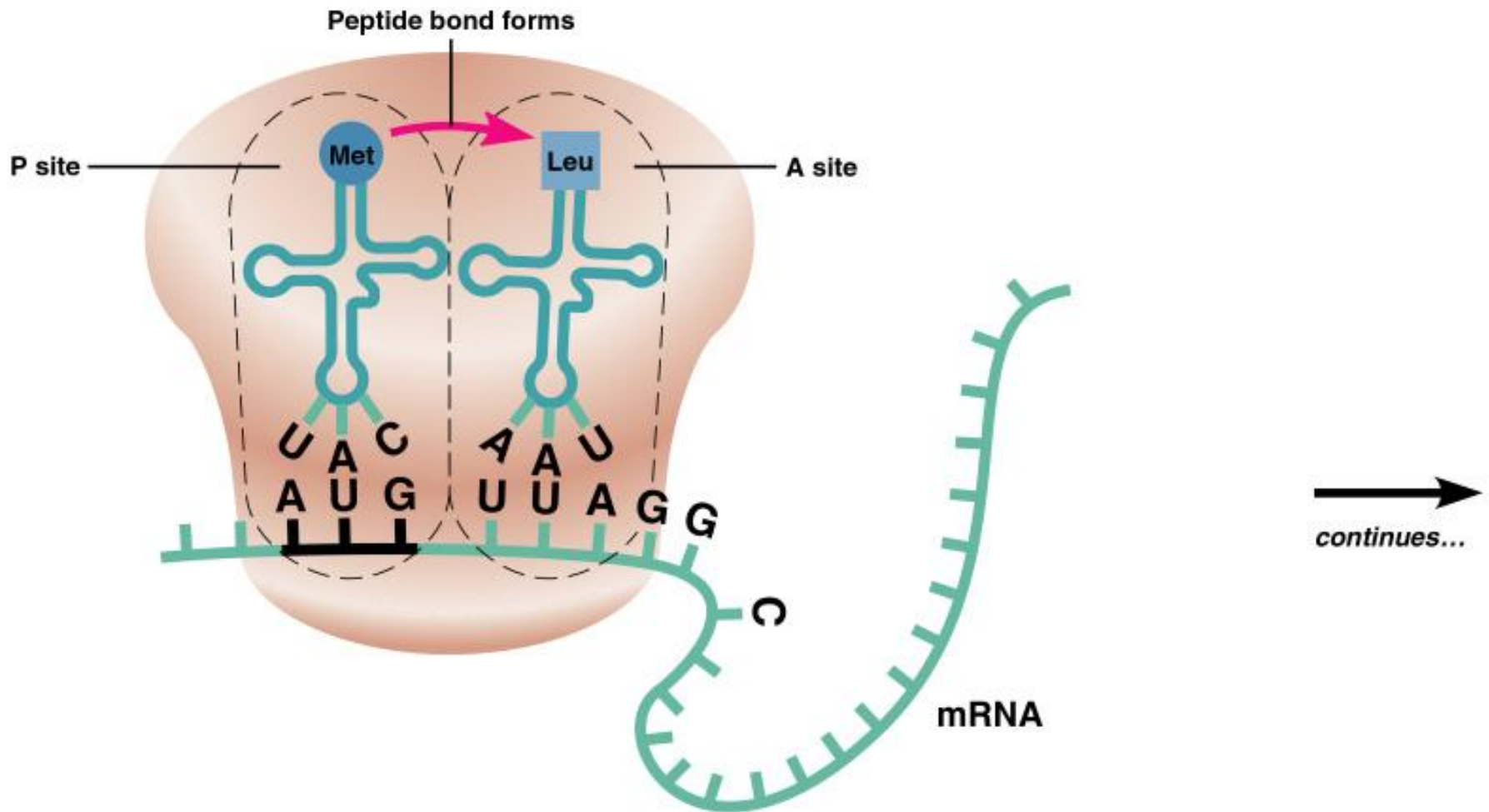
1 Components needed to begin translation come together.

Translation



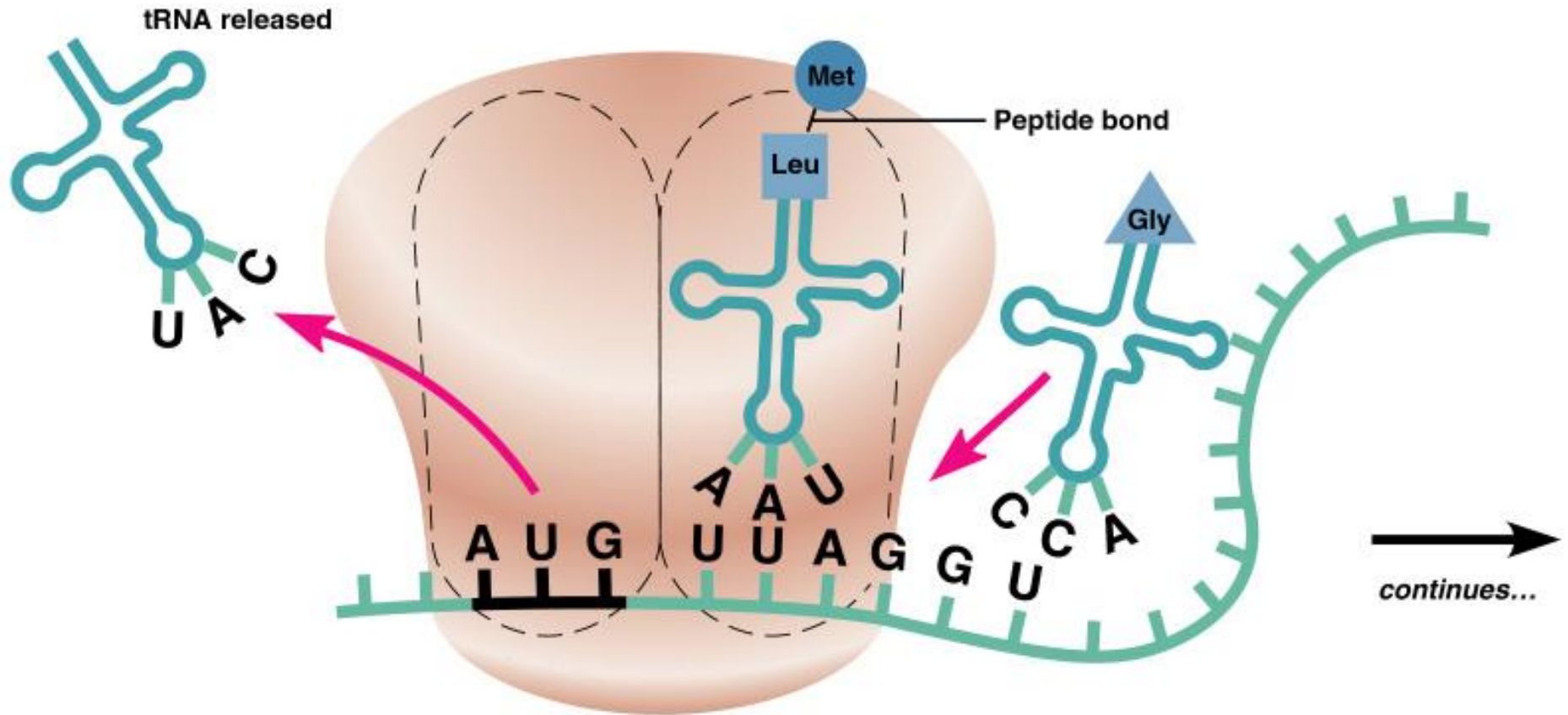
- 2 On the assembled ribosome, a tRNA carrying the first amino acid is paired with the start codon on the mRNA. A tRNA carrying the second amino acid approaches.

Translation



- 3 The place on the ribosome where the first tRNA sits is called the P site. In the A site next to it, the second codon of the mRNA pairs with a tRNA carrying the second amino acid.

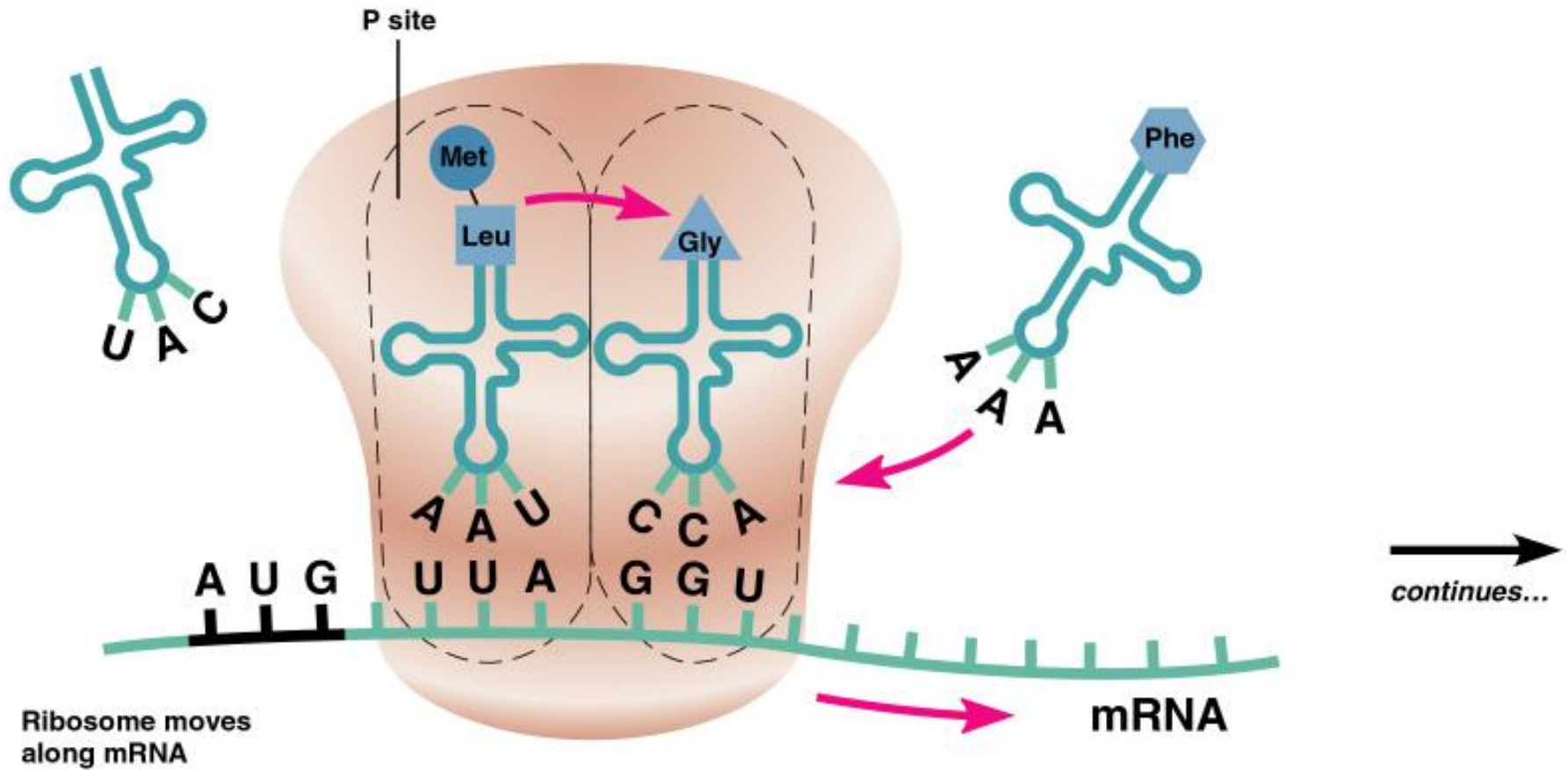
Translation



4

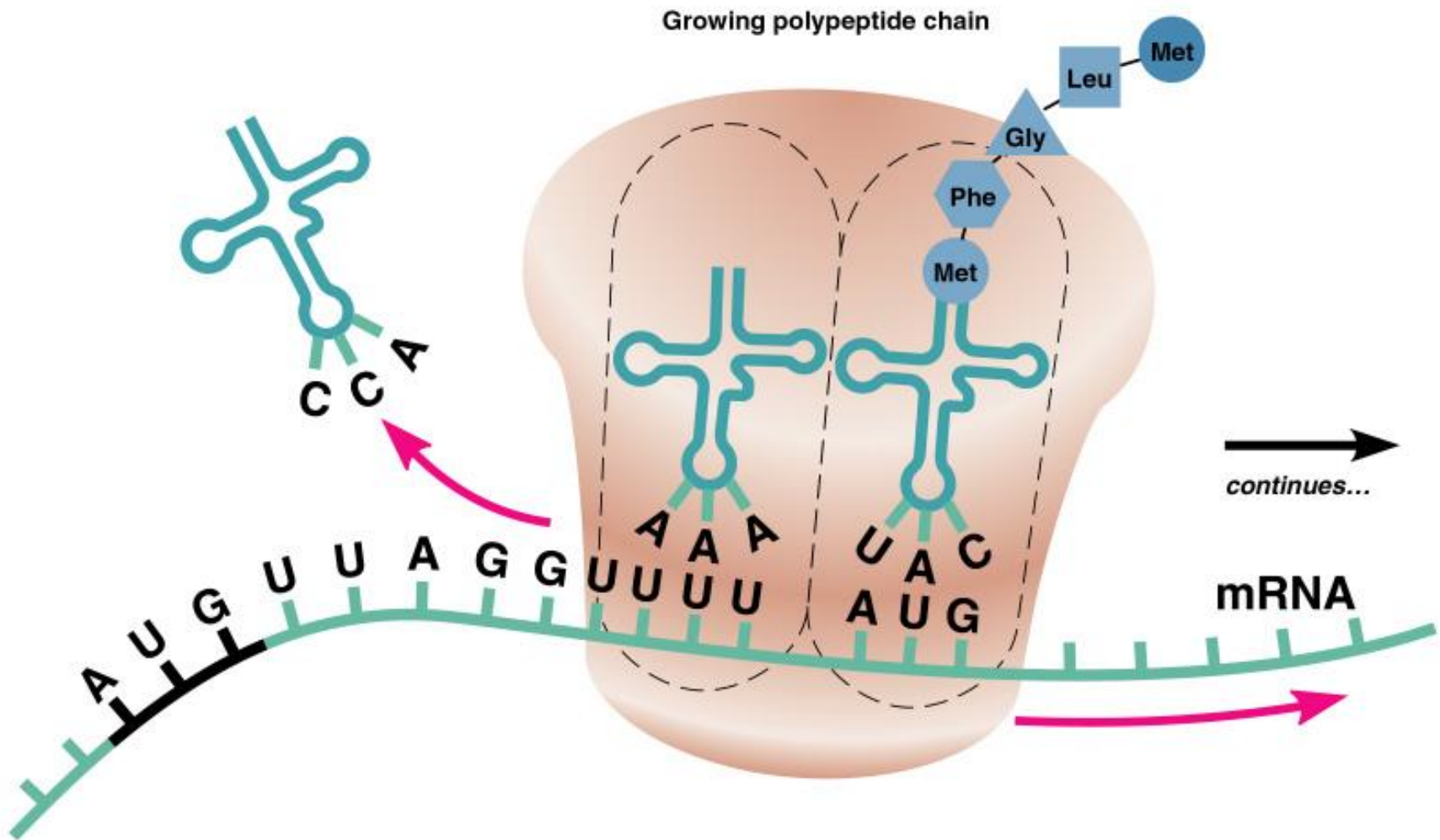
The first amino acid joins to the second by a peptide bond, and the first tRNA is released.

Translation



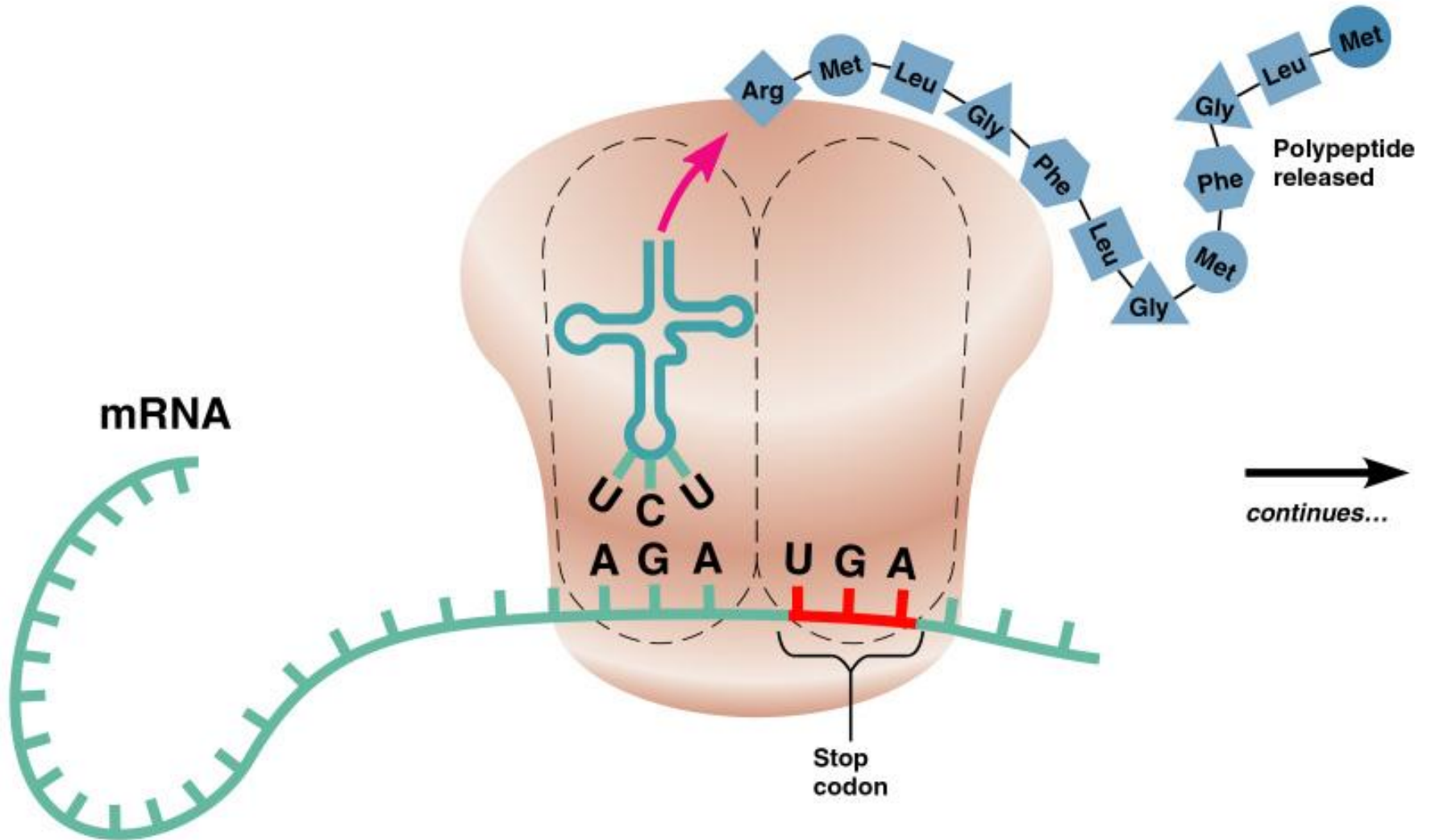
- 5 The ribosome moves along the mRNA until the second tRNA is in the P site, and the process continues.

Translation



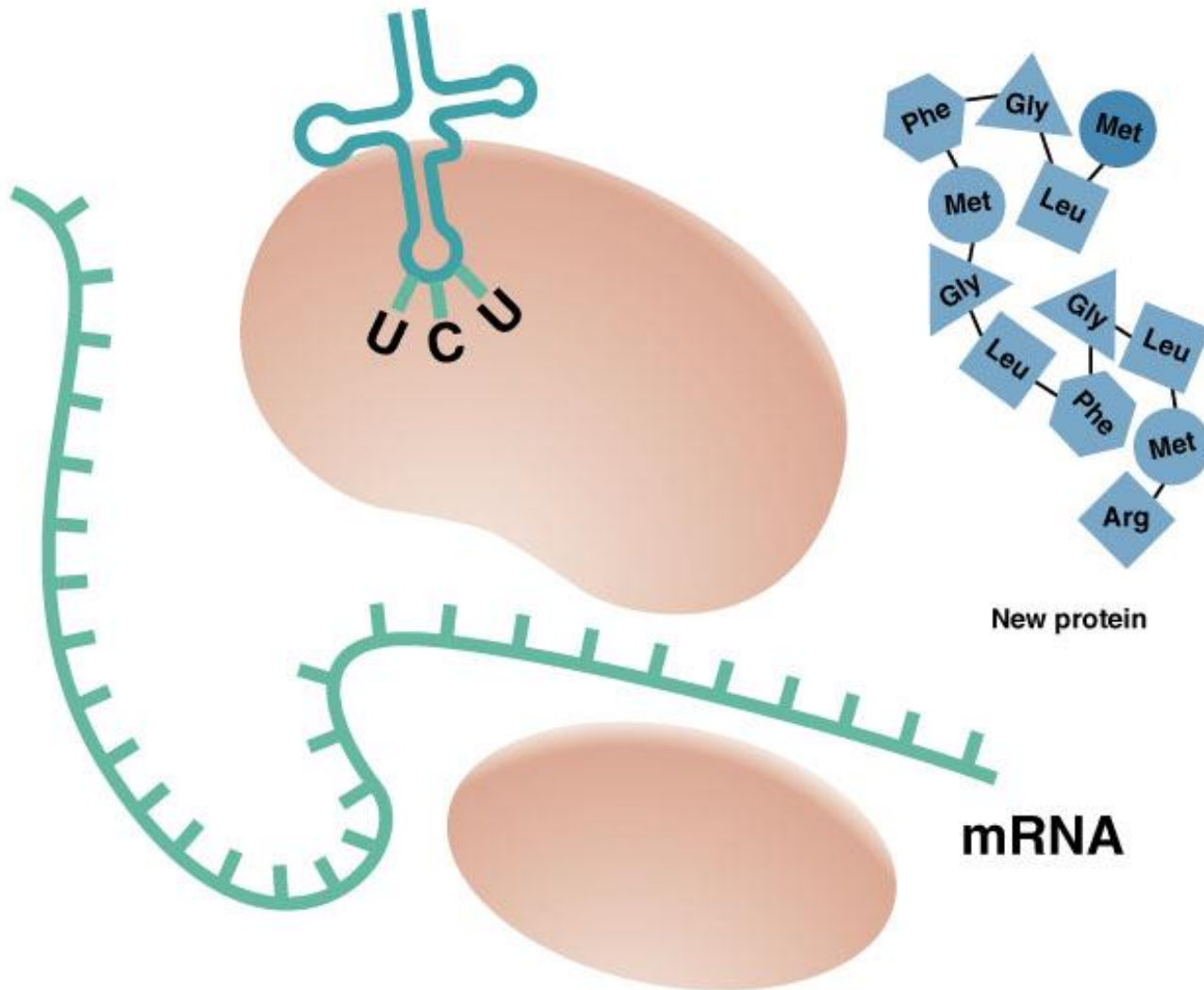
- 6 The ribosome continues to move along the mRNA, and new amino acids are added to the polypeptide.

Translation



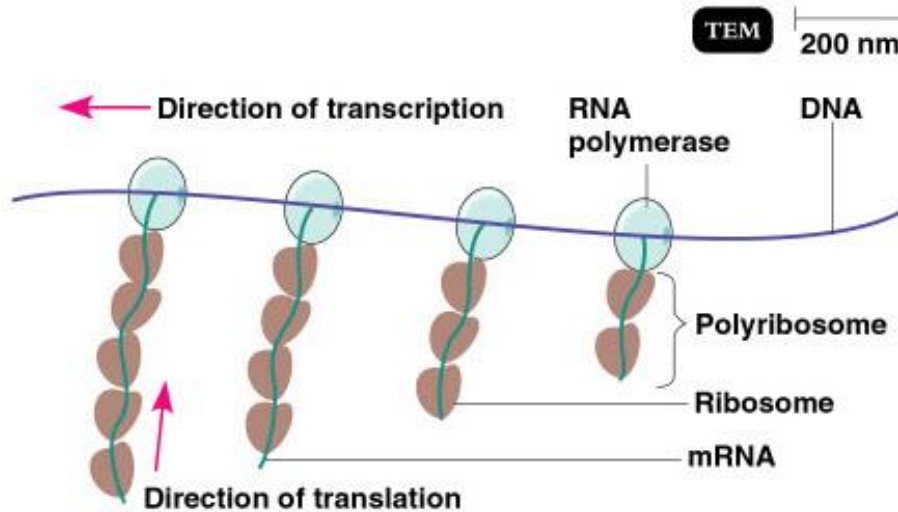
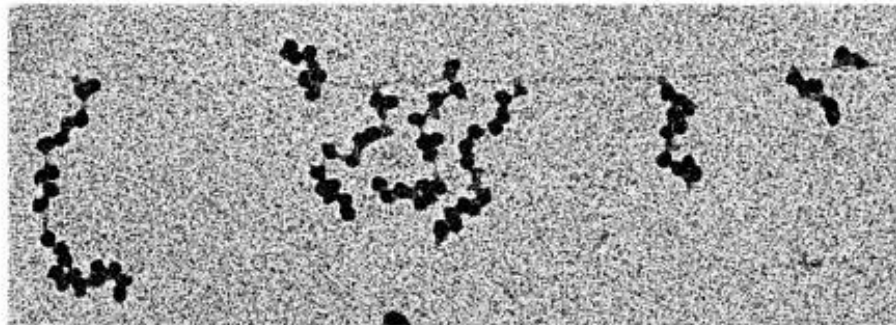
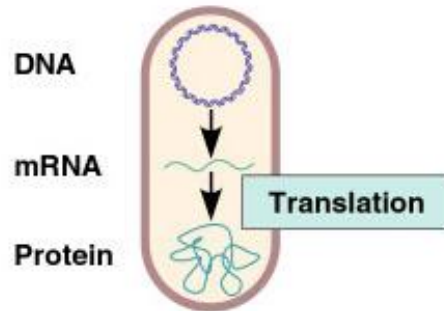
- 7 When the ribosome reaches a stop codon, the polypeptide is released.

Translation

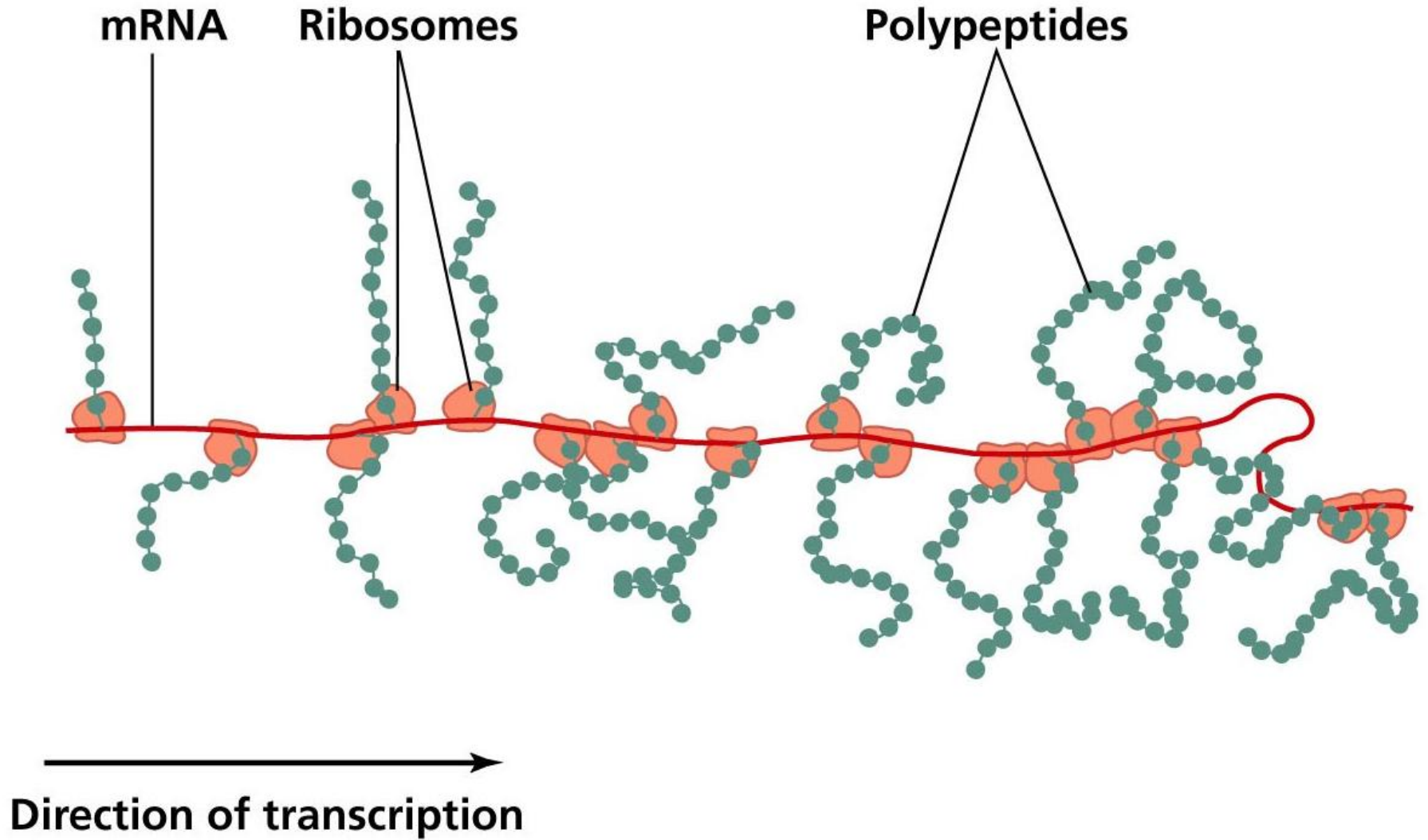


- 8** Finally, the last tRNA is released, and the ribosome comes apart. The released polypeptide forms a new protein.

Translation

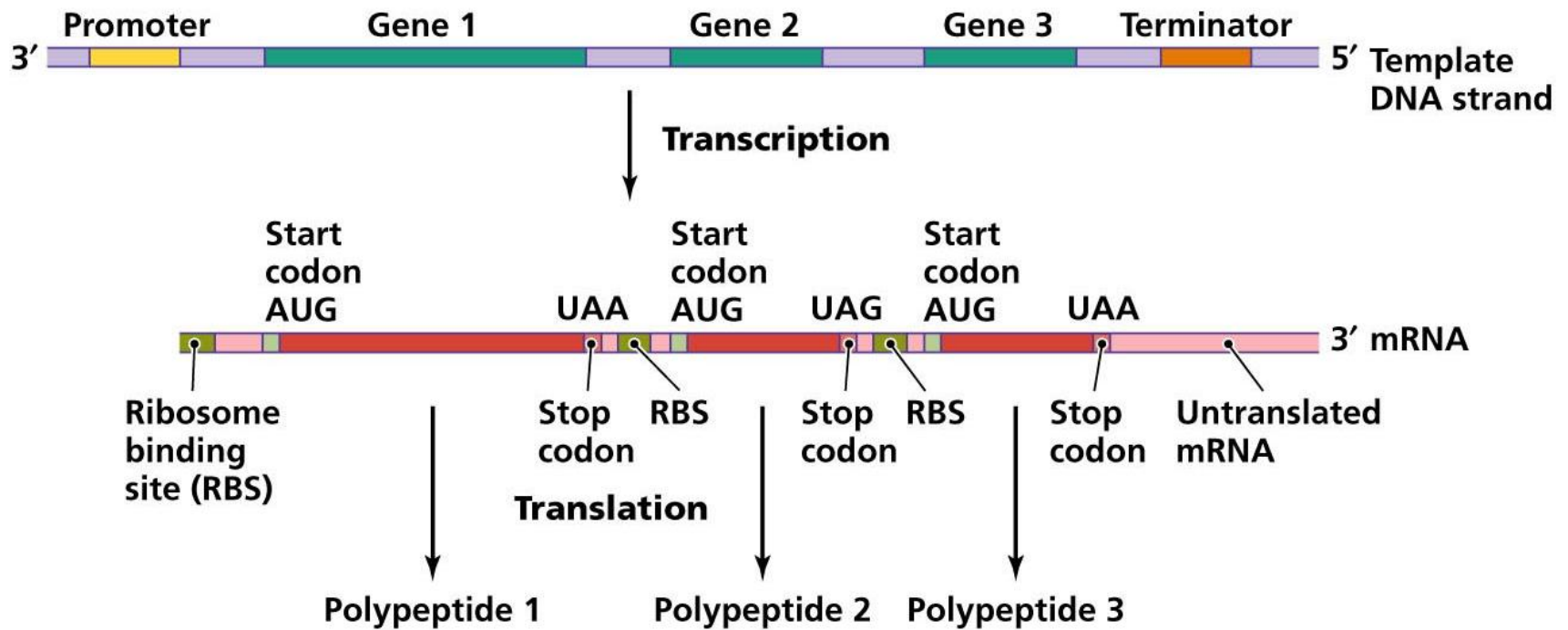


Polyribosome

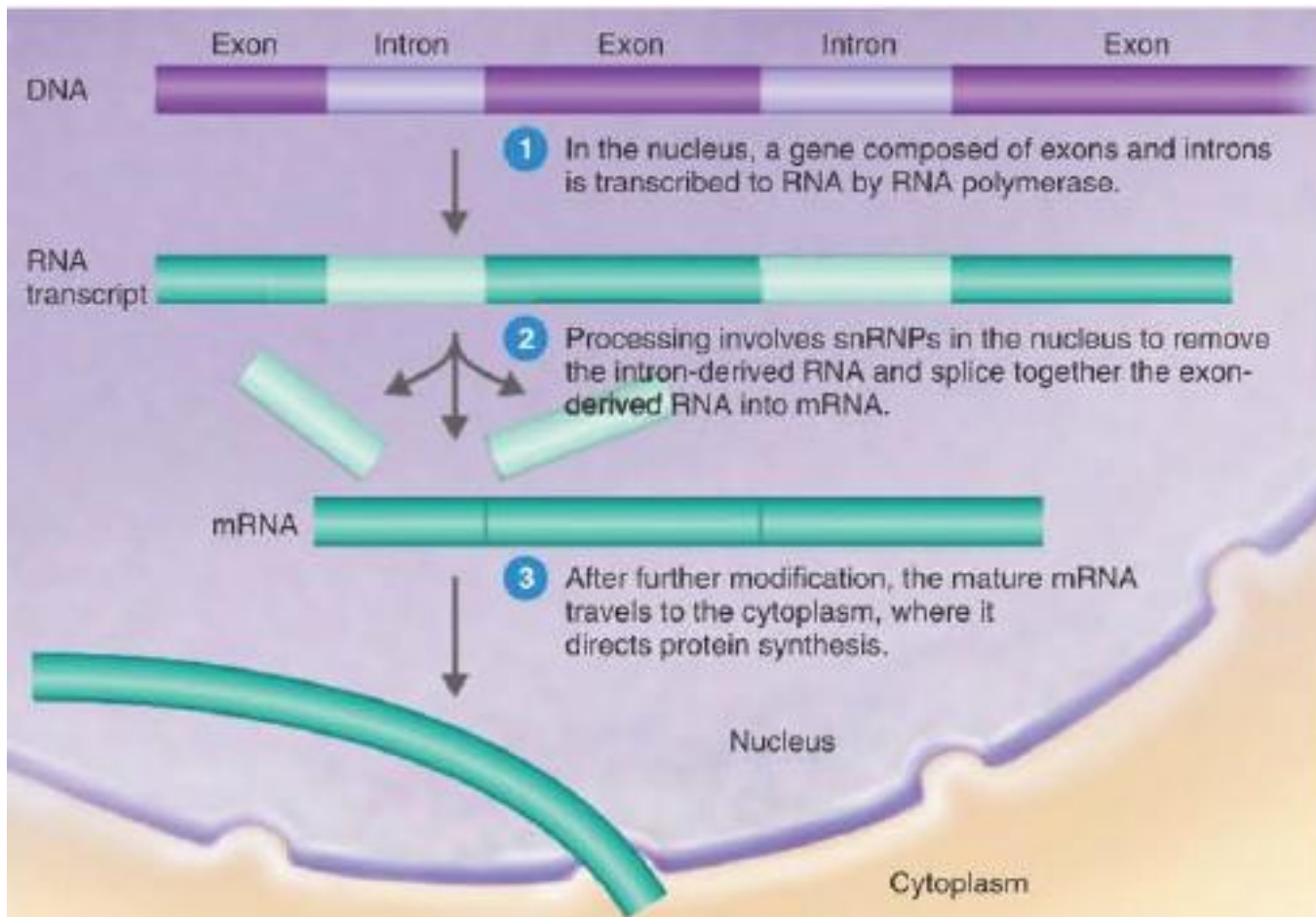


(a)

RNA processing in prokaryotes



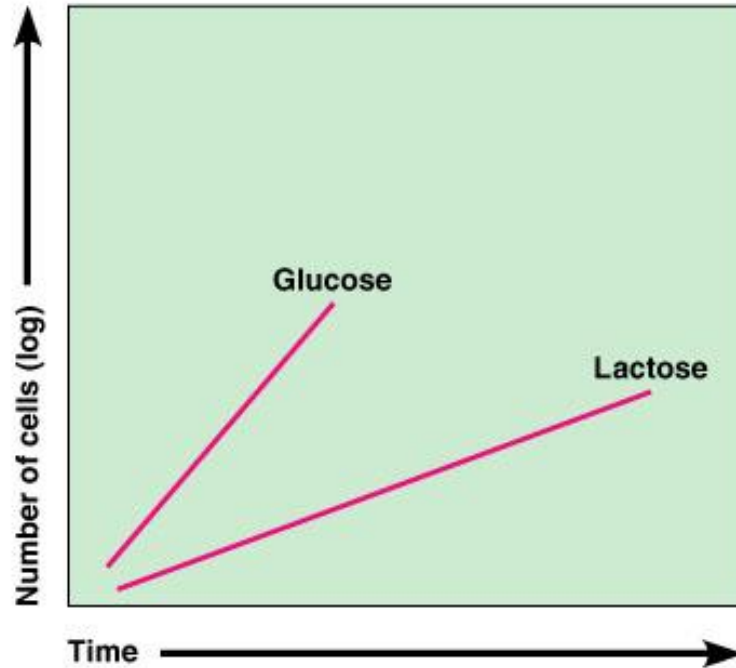
RNA processing in eukaryotes



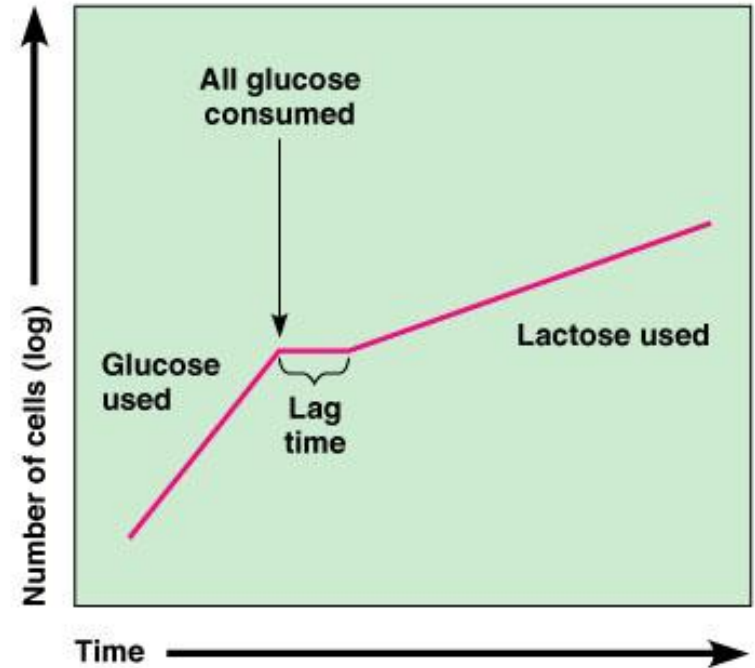
Regulation of Bacterial Gene Expression

- Constitutive enzymes are expressed at a fixed rate
- Other enzymes are expressed only as needed
 - ▣ Repressible enzymes
 - ▣ Inducible enzymes
 - Beta galactosidase in *E.coli*
 - Insulin

Regulation of Gene Expression

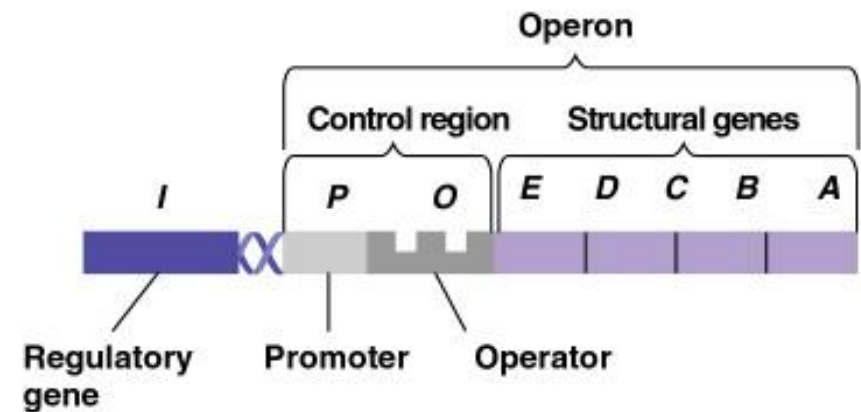
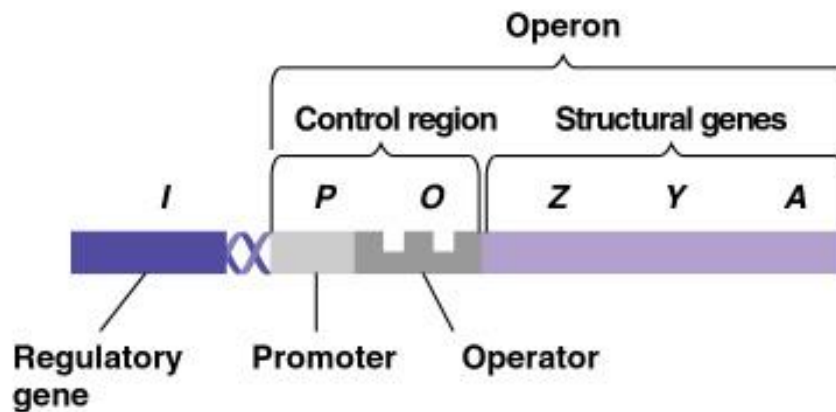


(a) Growth on glucose or lactose alone



(b) Growth on glucose and lactose combined

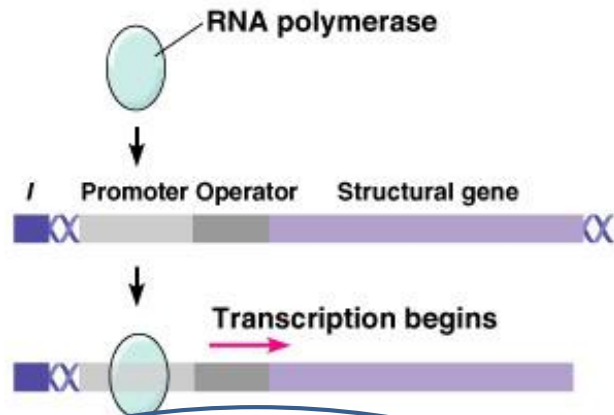
Operon



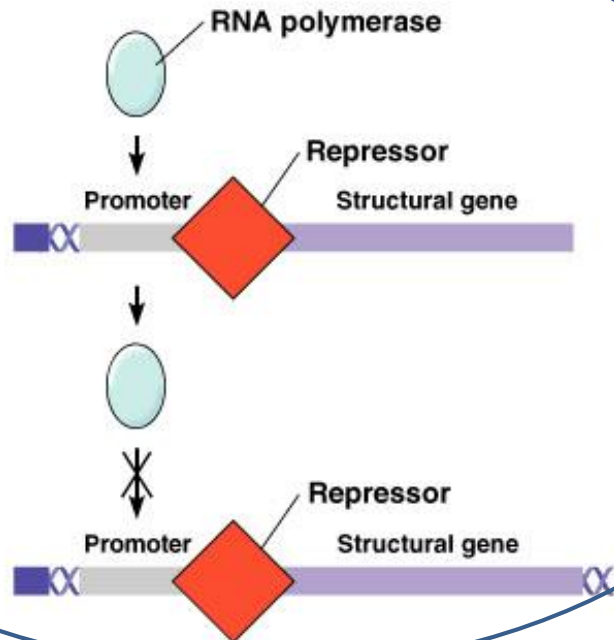
- 1 Structure of the operon.** The operon consists of the promoter (*P*), and operator (*O*) sites, and structural genes which code for the protein. The operon is regulated by the product of the regulatory gene (*I*).

Repression

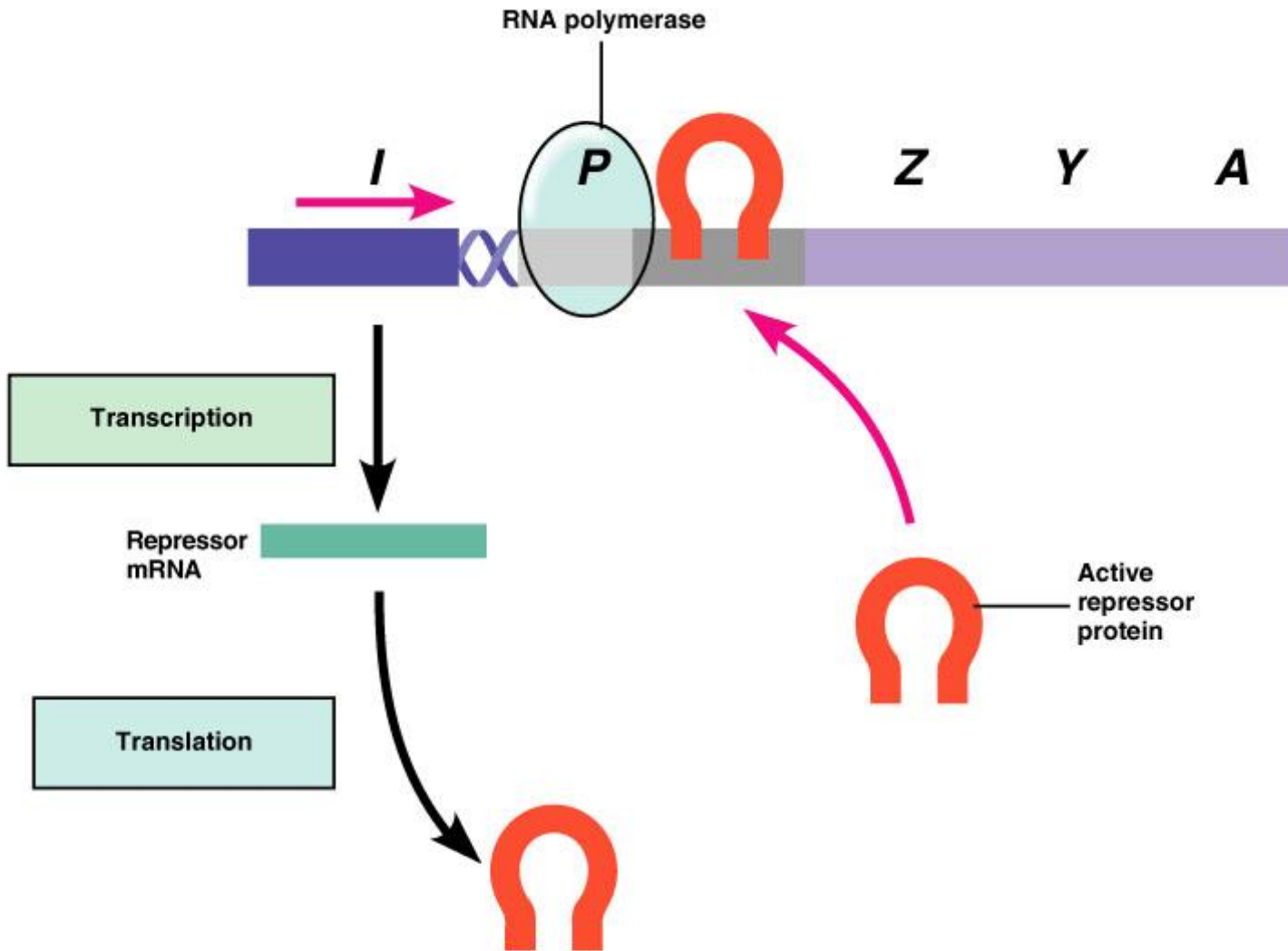
Without repressor:



With repressor:

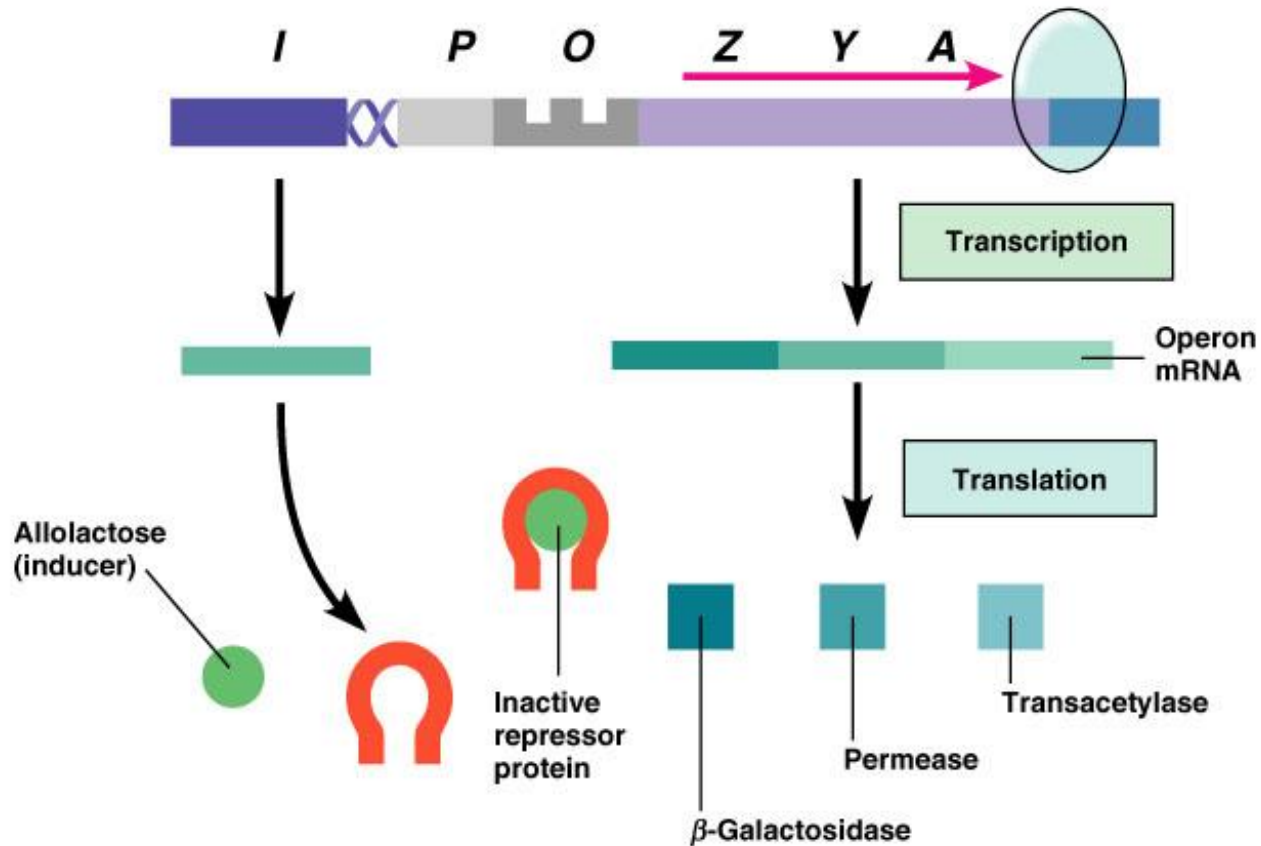


Regulation of Gene Expression



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

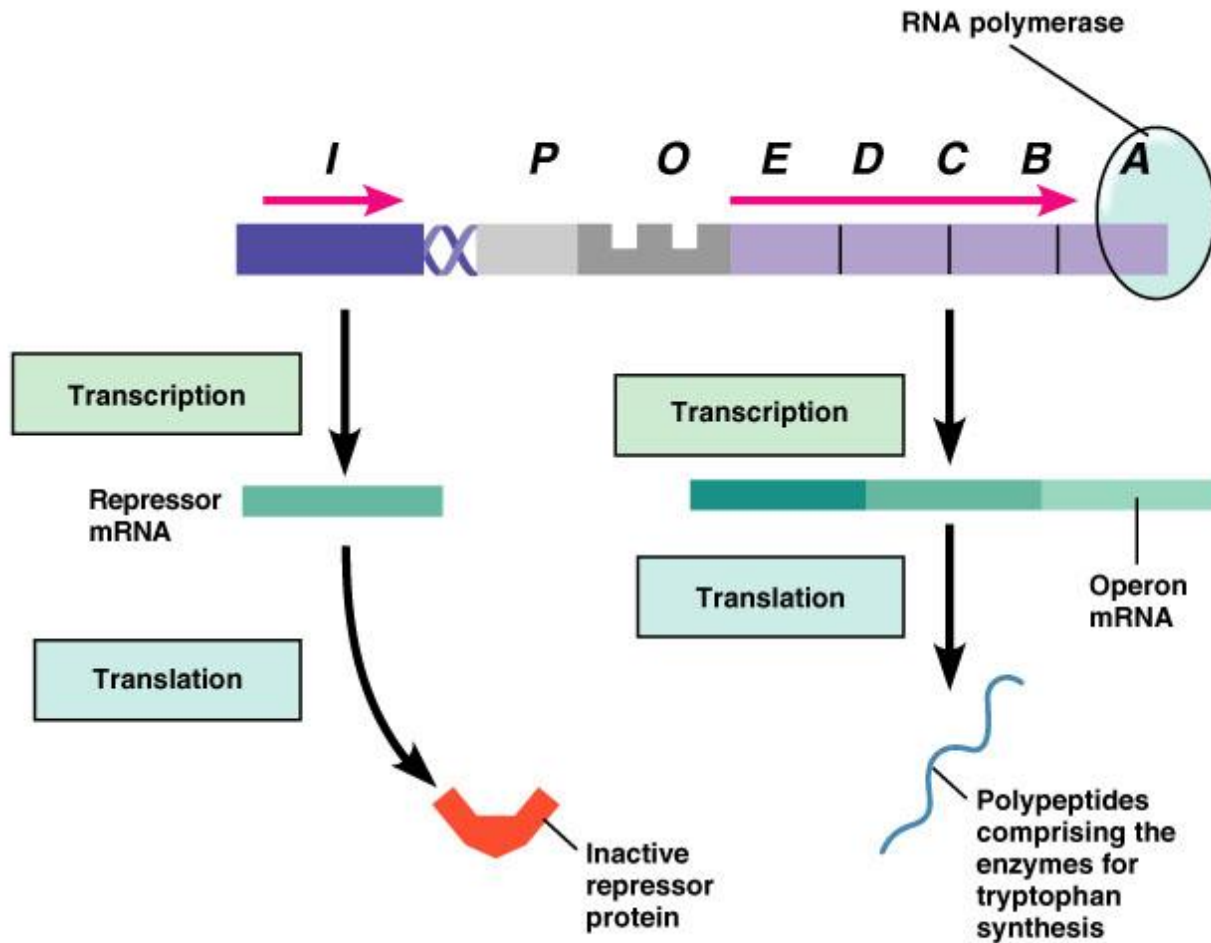
Regulation of Gene Expression



- 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.

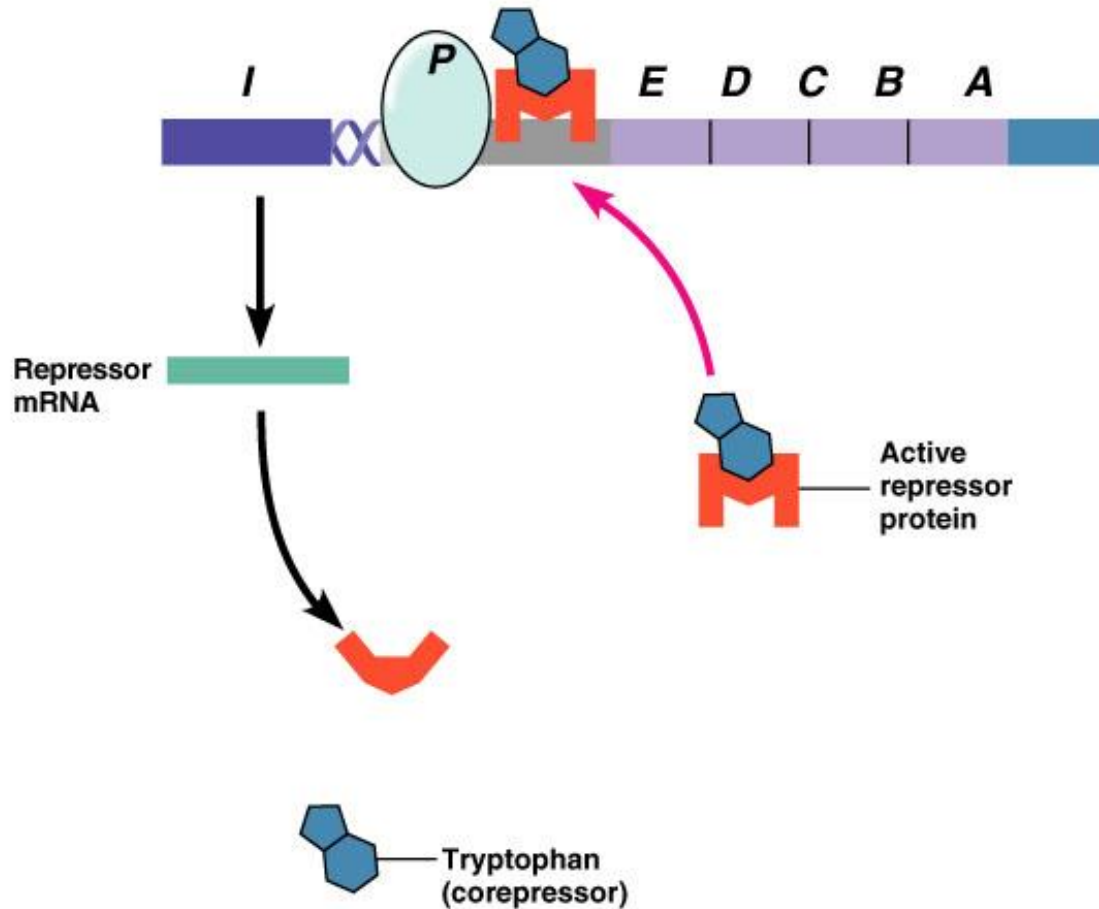
(a) An inducible operon

Regulation of Gene Expression



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

Regulation of Gene Expression



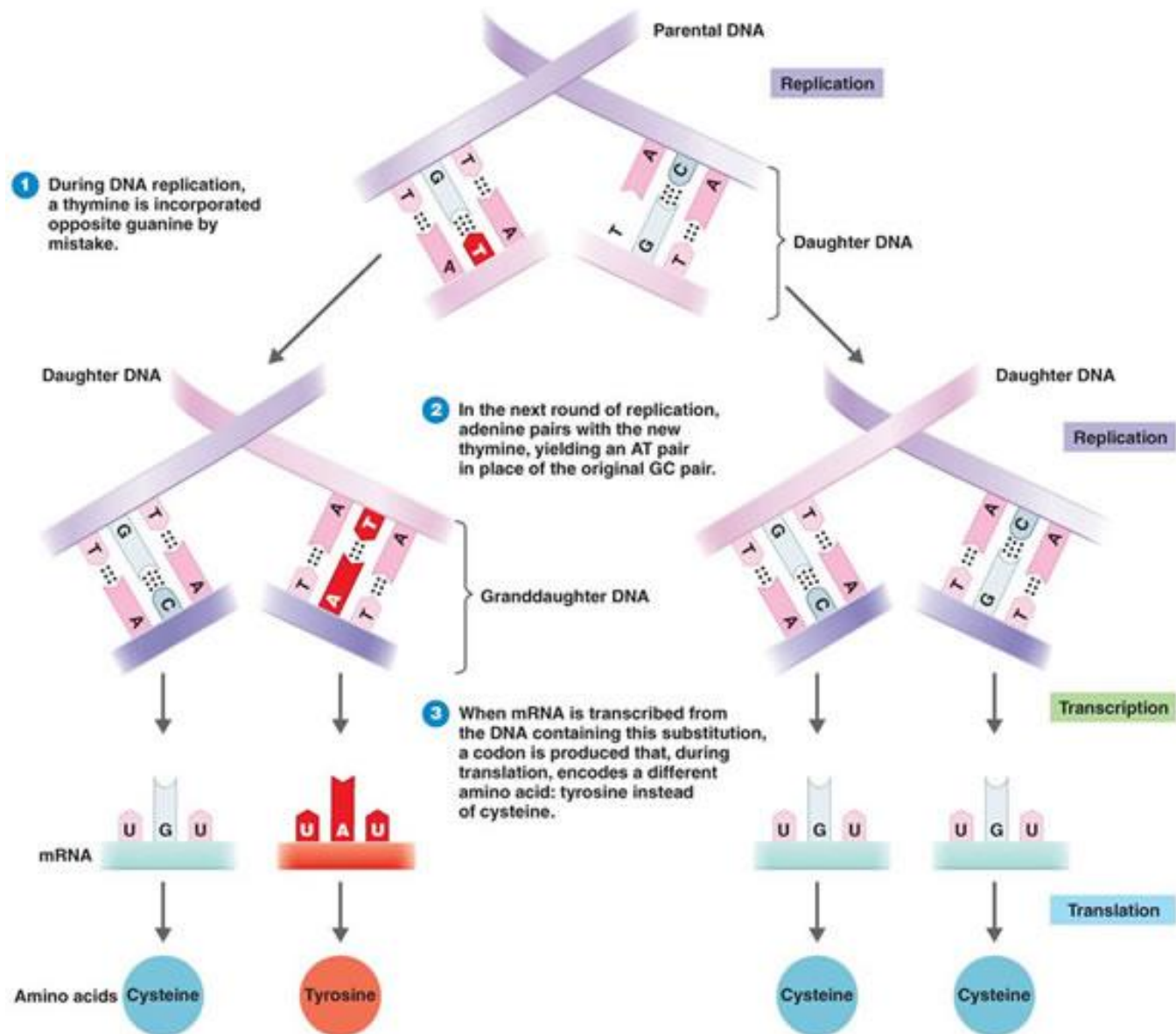
- 3** **Repressor active, operon off.** When the corepressor tryptophan binds to the repressor protein, the activated repressor binds with the operator, preventing transcription from the operon.

(b) A repressible operon

Mutation

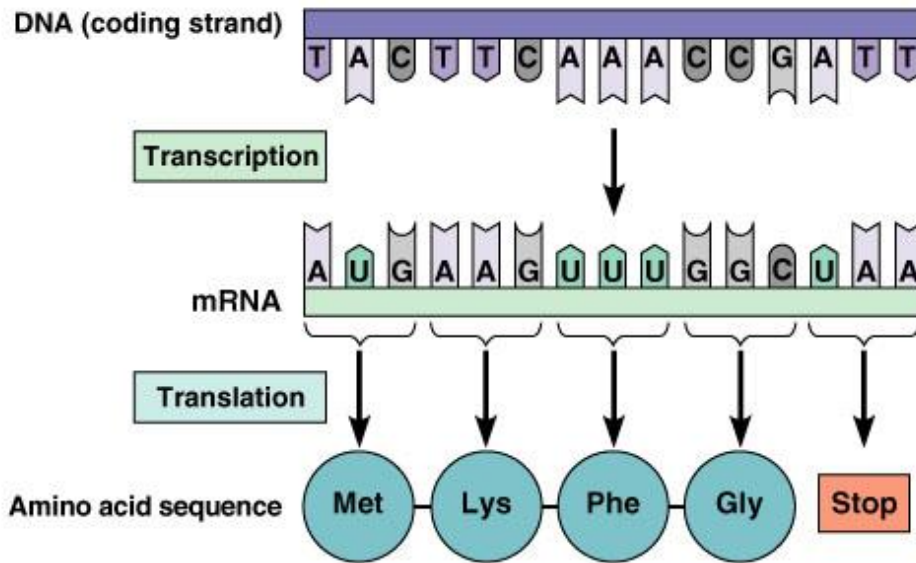
- Change in the genetic material
- Mutations may be neutral, beneficial, or harmful
- **Mutagen:** Agent that causes mutations
- Spontaneous mutations: Occur in the absence of a mutagen

Point mutation

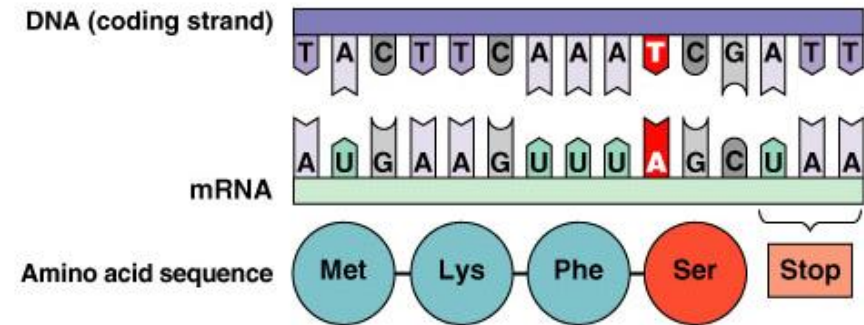


Mutation

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



(a) Normal DNA molecule

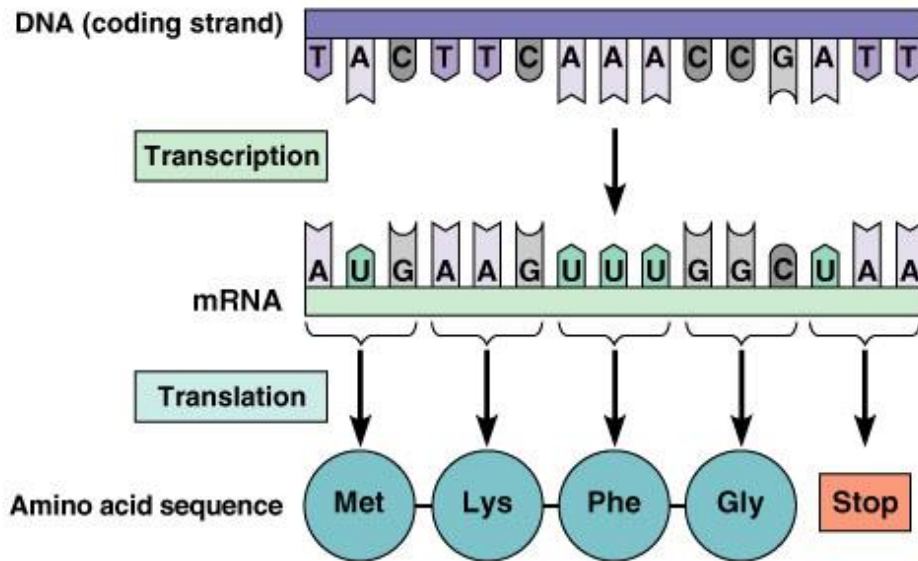


(b) Missense mutation

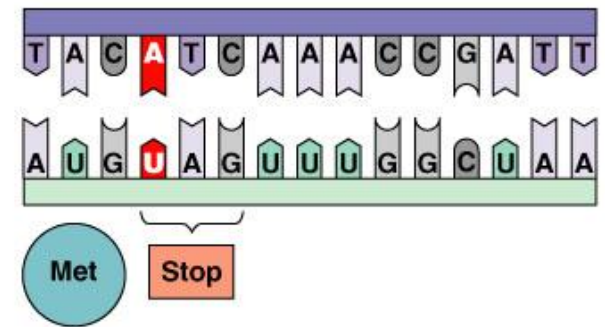
Mutation

□ Nonsense mutation

- Results in a nonsense codon



(a) Normal DNA molecule

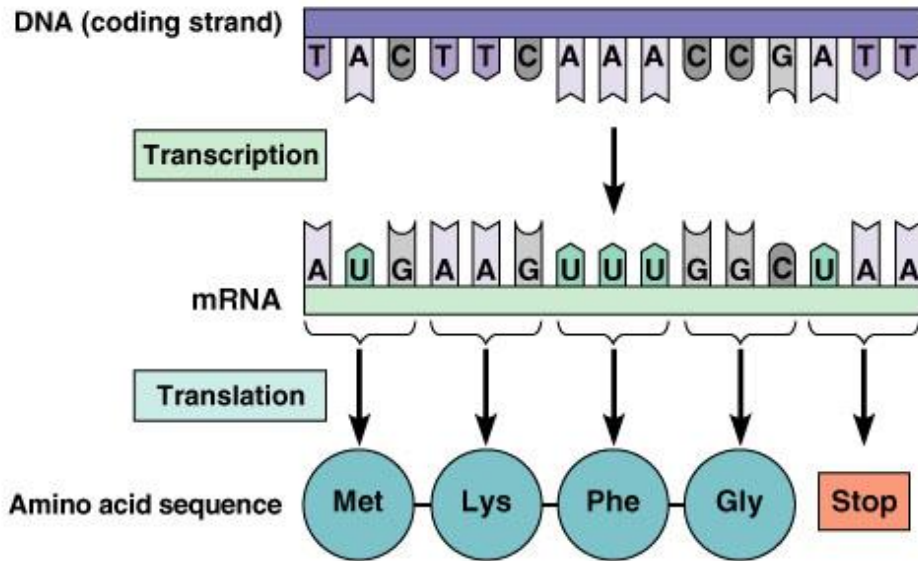


(c) Nonsense mutation

Mutation

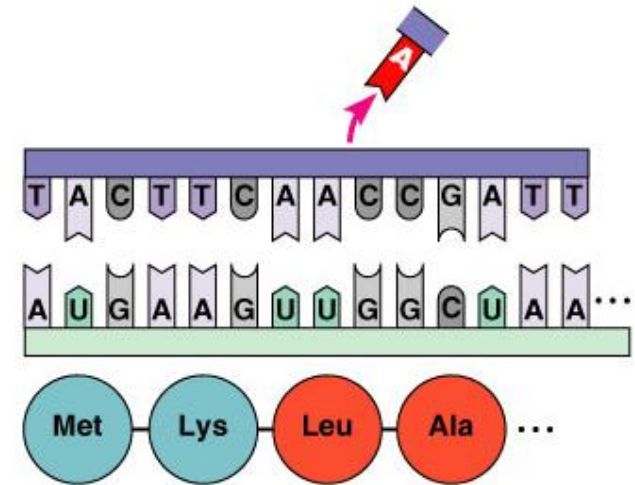
□ Frameshift mutation

• Insertion or deletion of one or more nucleotide pairs



(a) Normal DNA molecule

(d) Frameshift mutation



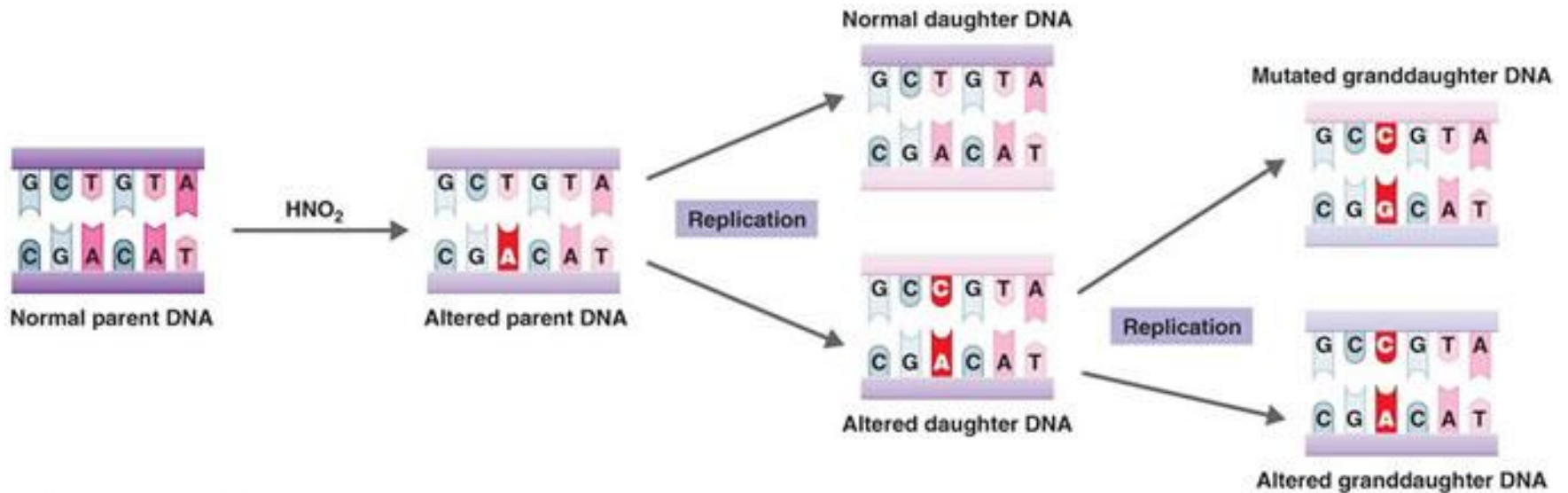
Mutation

Mutagens

- Chemical mutagens
 - ▣ A number of household and lab chemicals sodium azide
- Physical mutagens
 - ▣ Radiation, UV light

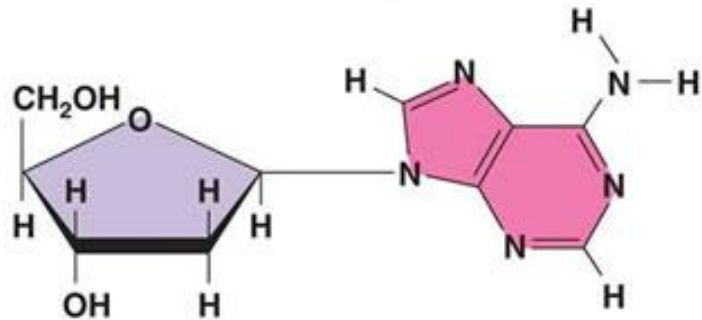
Chemical mutagens

Nitrous acid as a mutagen



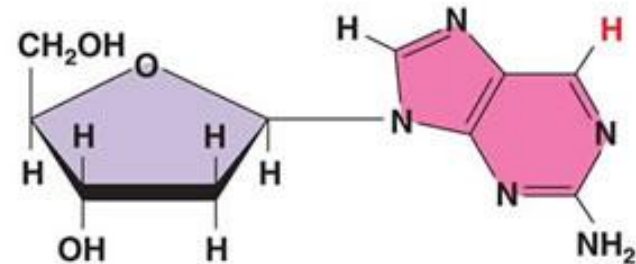
Nucleoside analogs

Normal nitrogenous base



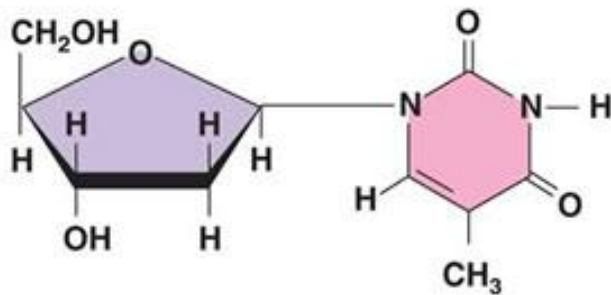
Adenine nucleoside

Analog

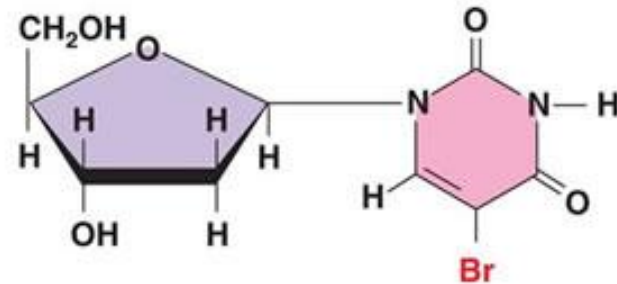


2-Aminopurine nucleoside

(a) The 2-aminopurine is incorporated into DNA in place of adenine but can pair with cytosine, so an AT pair becomes a CG pair.



Thymine nucleoside



5-Bromouracil nucleoside

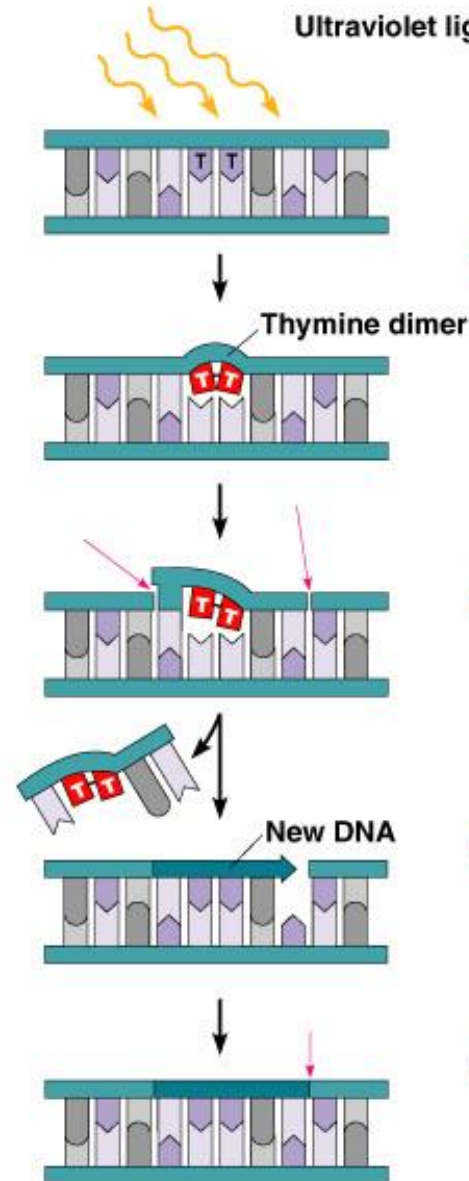
(b) The 5-bromouracil is used as an anticancer drug because it is mistaken for thymine by cellular enzymes but pairs with cytosine. In the next DNA replication, an AT pair becomes a GC pair.

Radiations

- ❑ Ionizing radiation (X rays and gamma rays) causes the formation of ions that can react with nucleotides and the deoxyribose-phosphate backbone.
- ❑ Breakage of covalent bonds in phosphate sugar backbone of DNA
- ❑ Covalent bonds between adjoining nitrogenous bases caused by UV light (thymin dimer)
- ❑ Nucleotide excision repairs mutations

Mutation

- UV radiation causes thymine dimers
- Light-repair separates thymine dimers
- Skin cancer by accumulation of TDs in the skin cells



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.

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

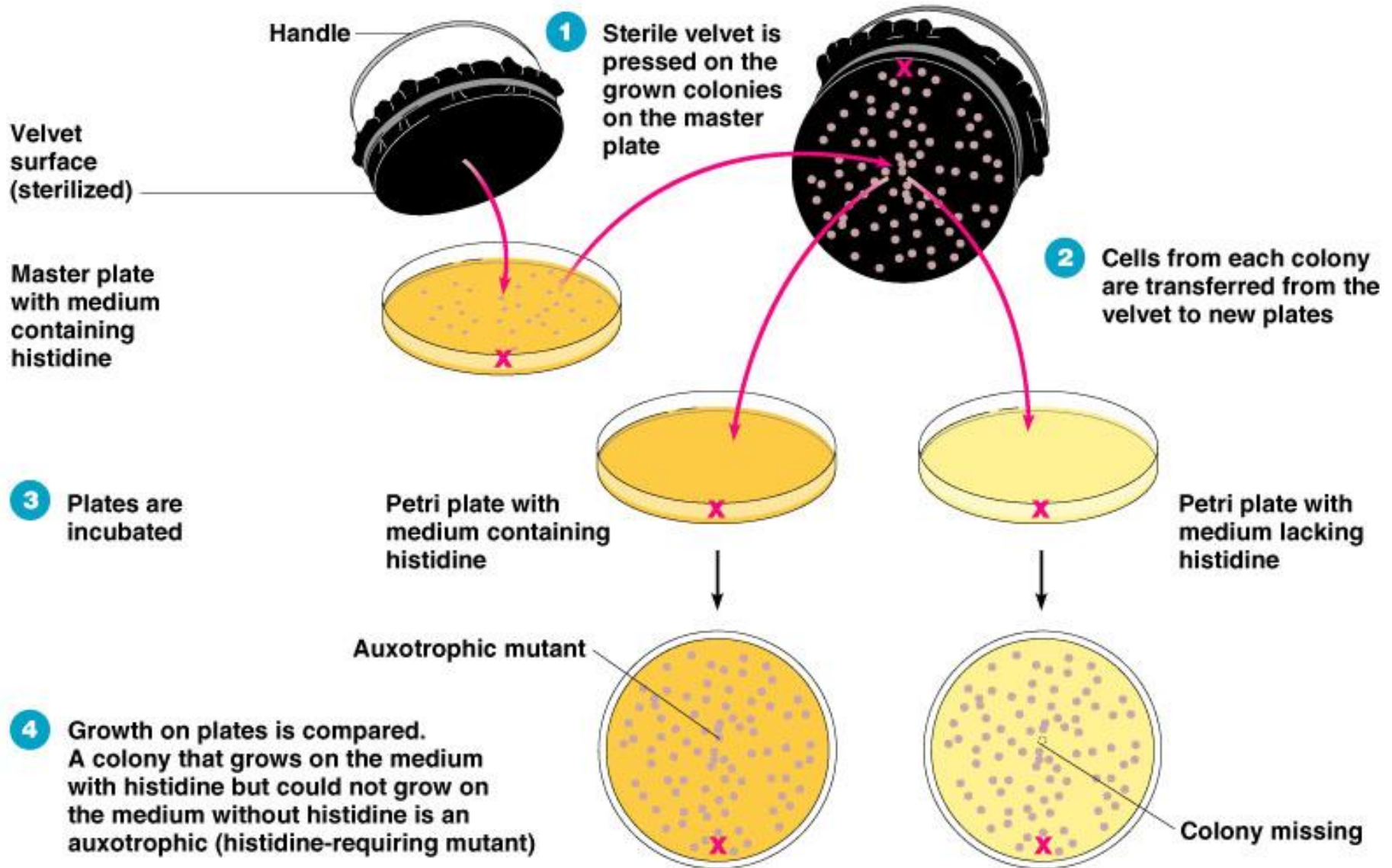
Identifying mutants

- Bacteria have one copy of each gene so identifying mutation is relatively easy

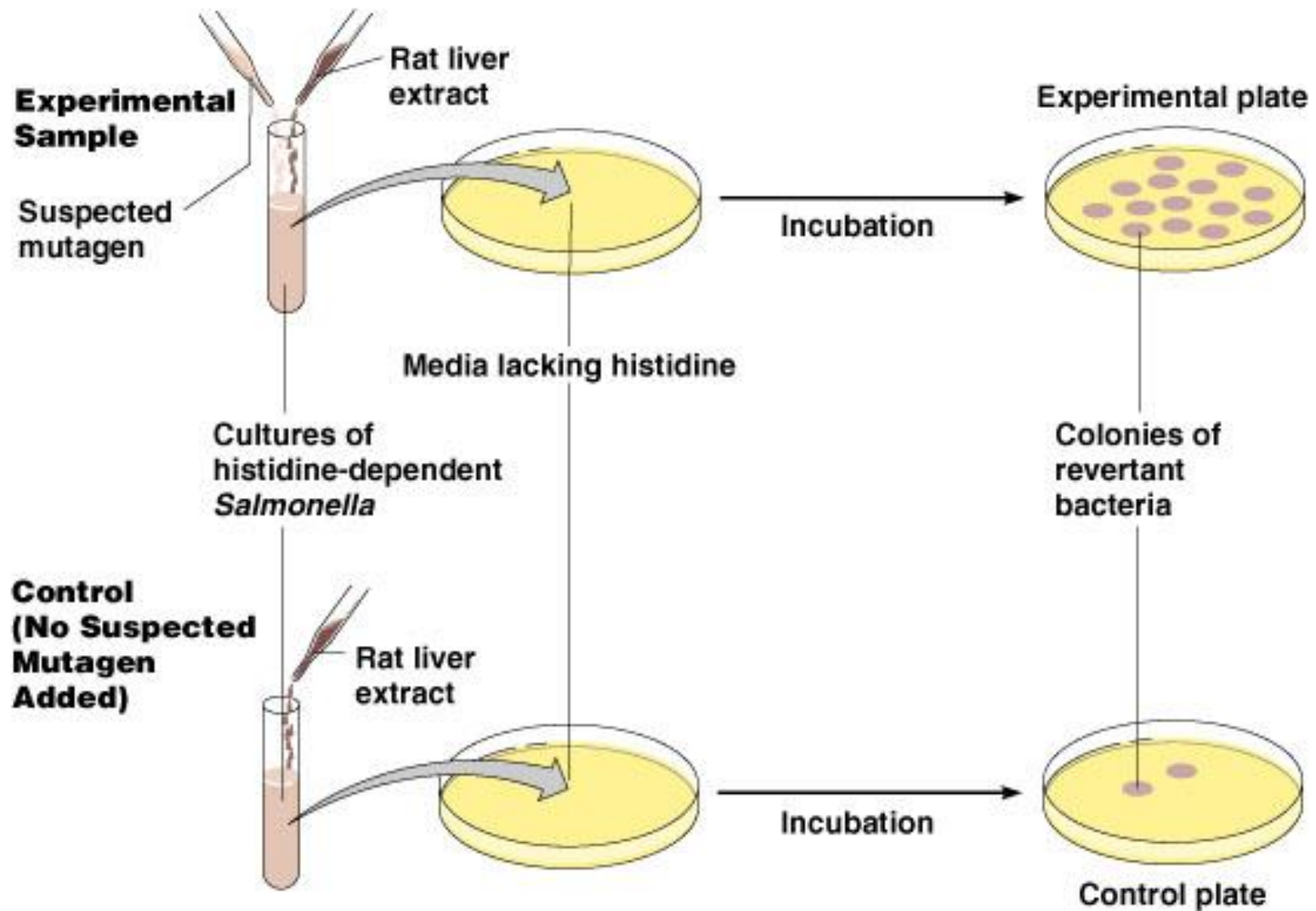
Selection:

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

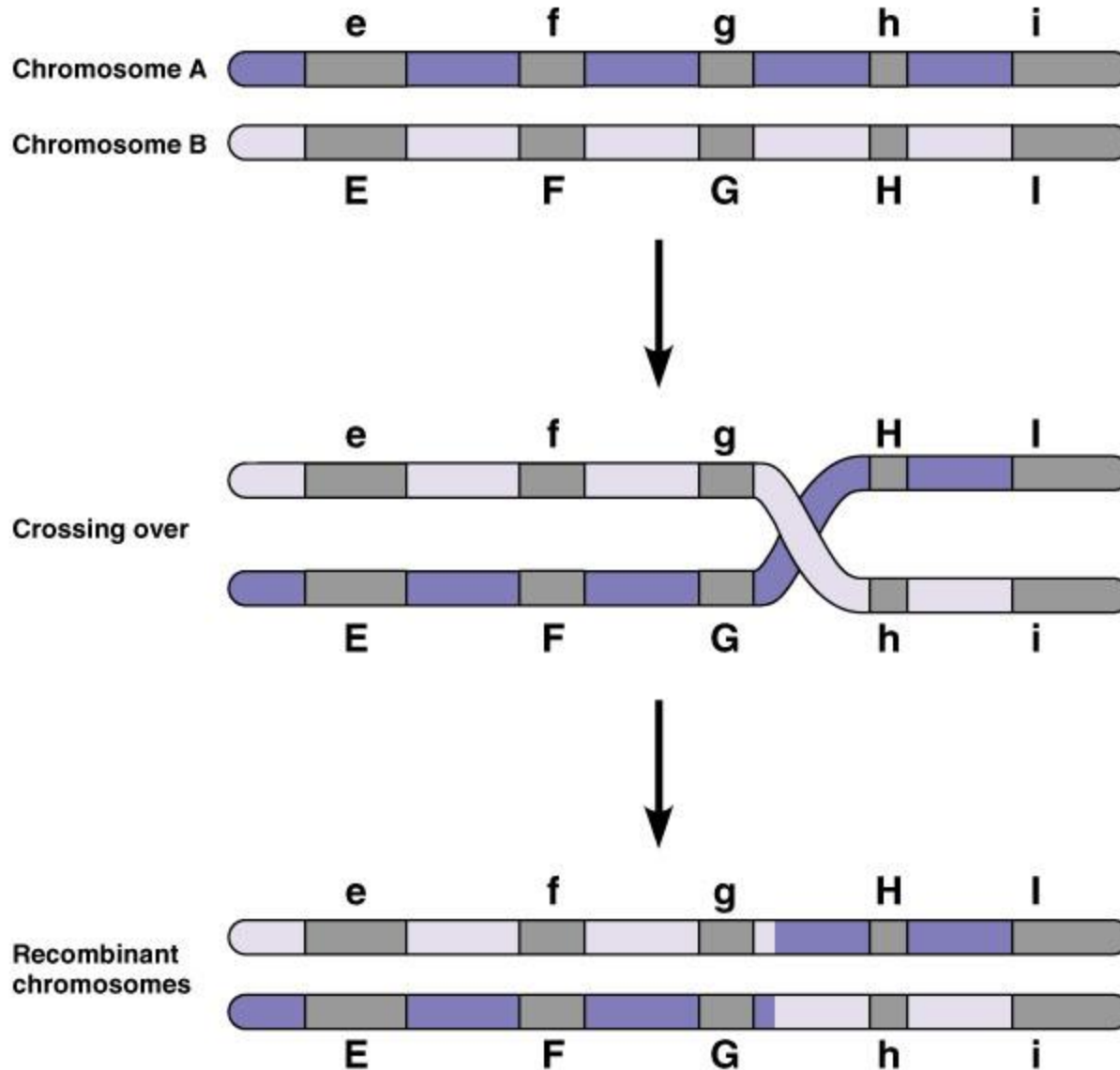
Replica Plating



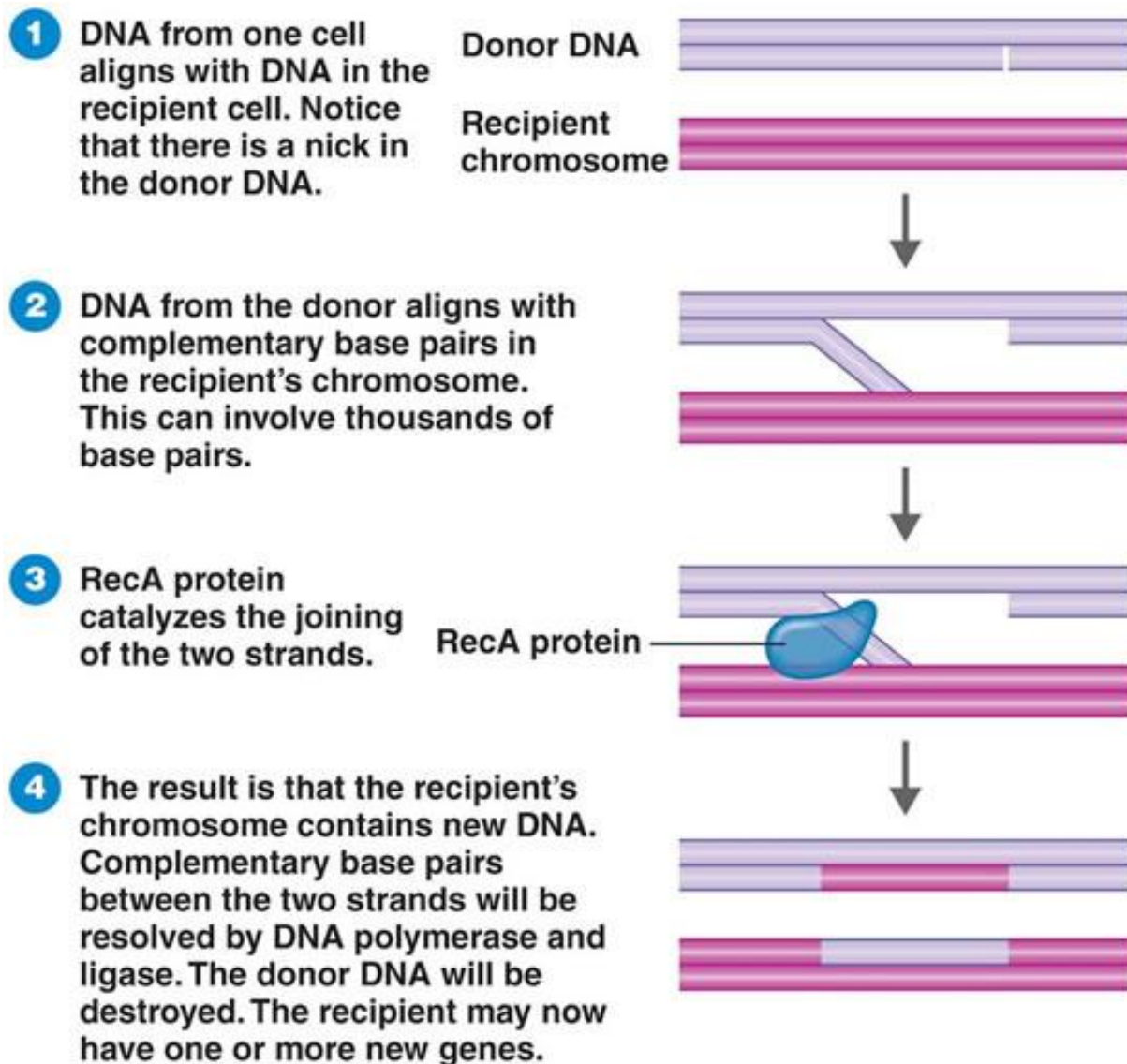
The Ames Test for Chemical Carcinogens



Genetic transfer and Recombination



Genetic transfer and Recombination



Genetic Transfer and Recombination

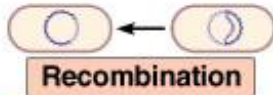
□ Vertical gene transfer

Occurs during reproduction, between generations of cells

□ Horizontal gene transfer

Transfer of genes between cells of the same generation

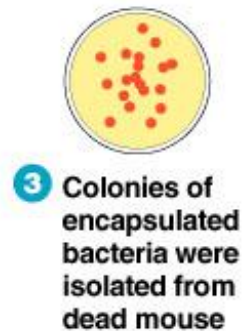
Transformation



- 1** Living encapsulated bacteria injected into mouse



- 2** Mouse died



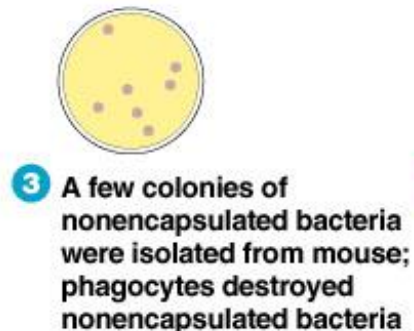
- 3** Colonies of encapsulated bacteria were isolated from dead mouse

(a)

- 1** Living nonencapsulated bacteria injected into mouse



- 2** Mouse remained healthy



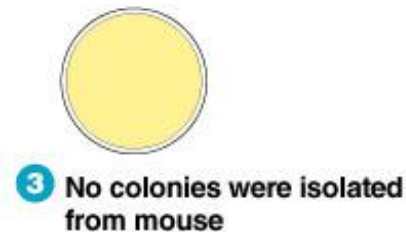
- 3** A few colonies of nonencapsulated bacteria were isolated from mouse; phagocytes destroyed nonencapsulated bacteria

(b)

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



- 2** Mouse remained healthy



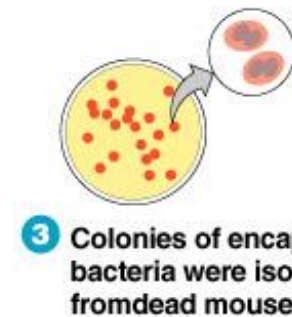
- 3** No colonies were isolated from mouse

(c)

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



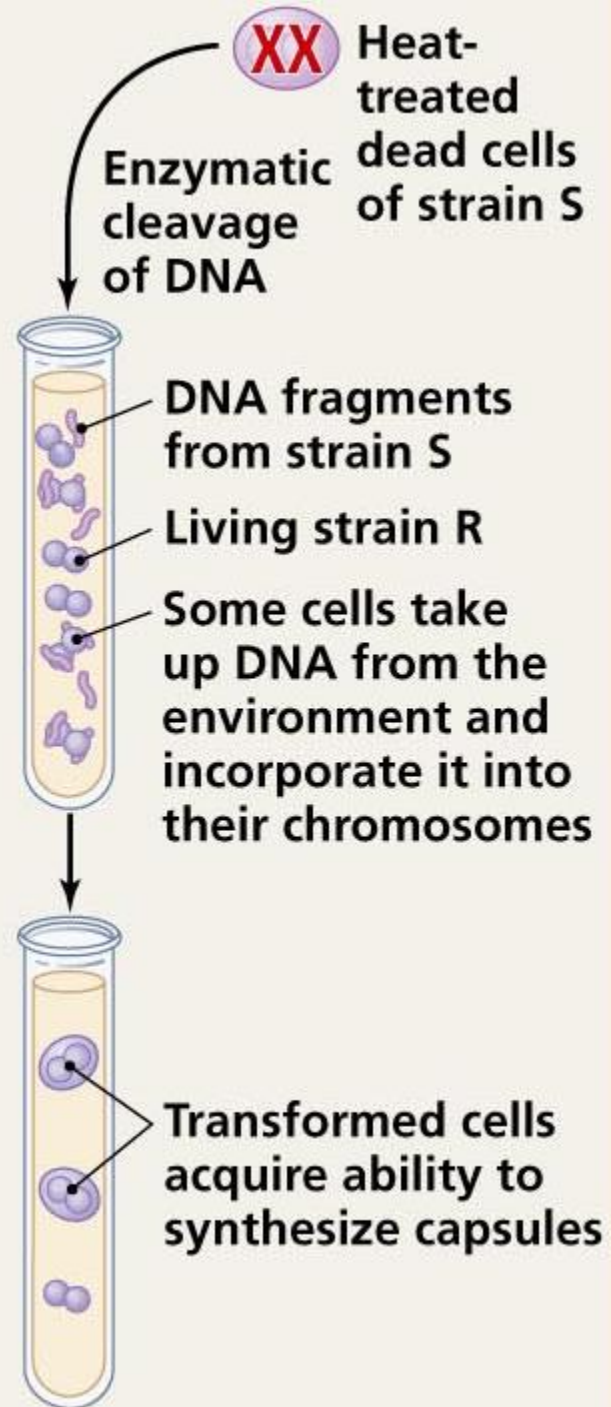
- 2** Mouse died



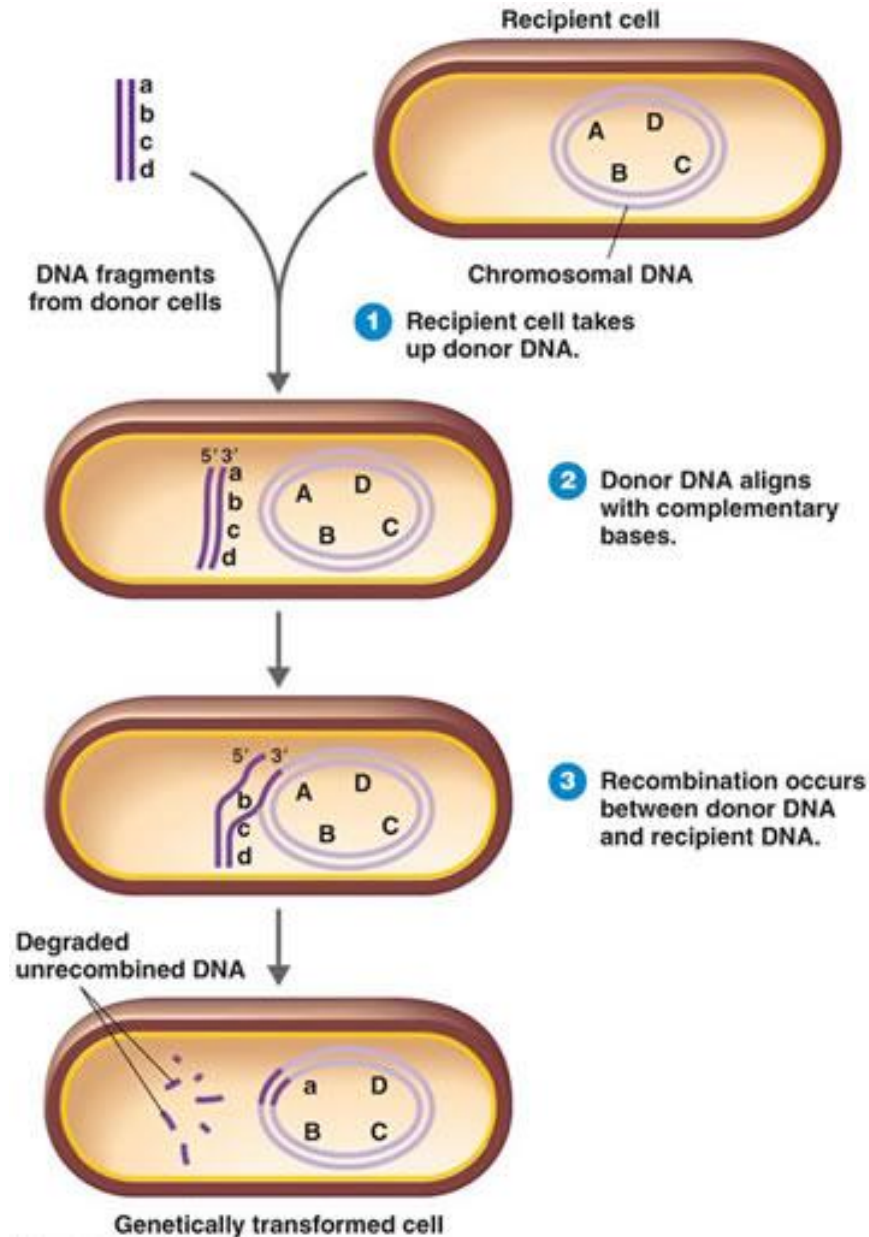
- 3** Colonies of encapsulated bacteria were isolated from dead mouse

(d)

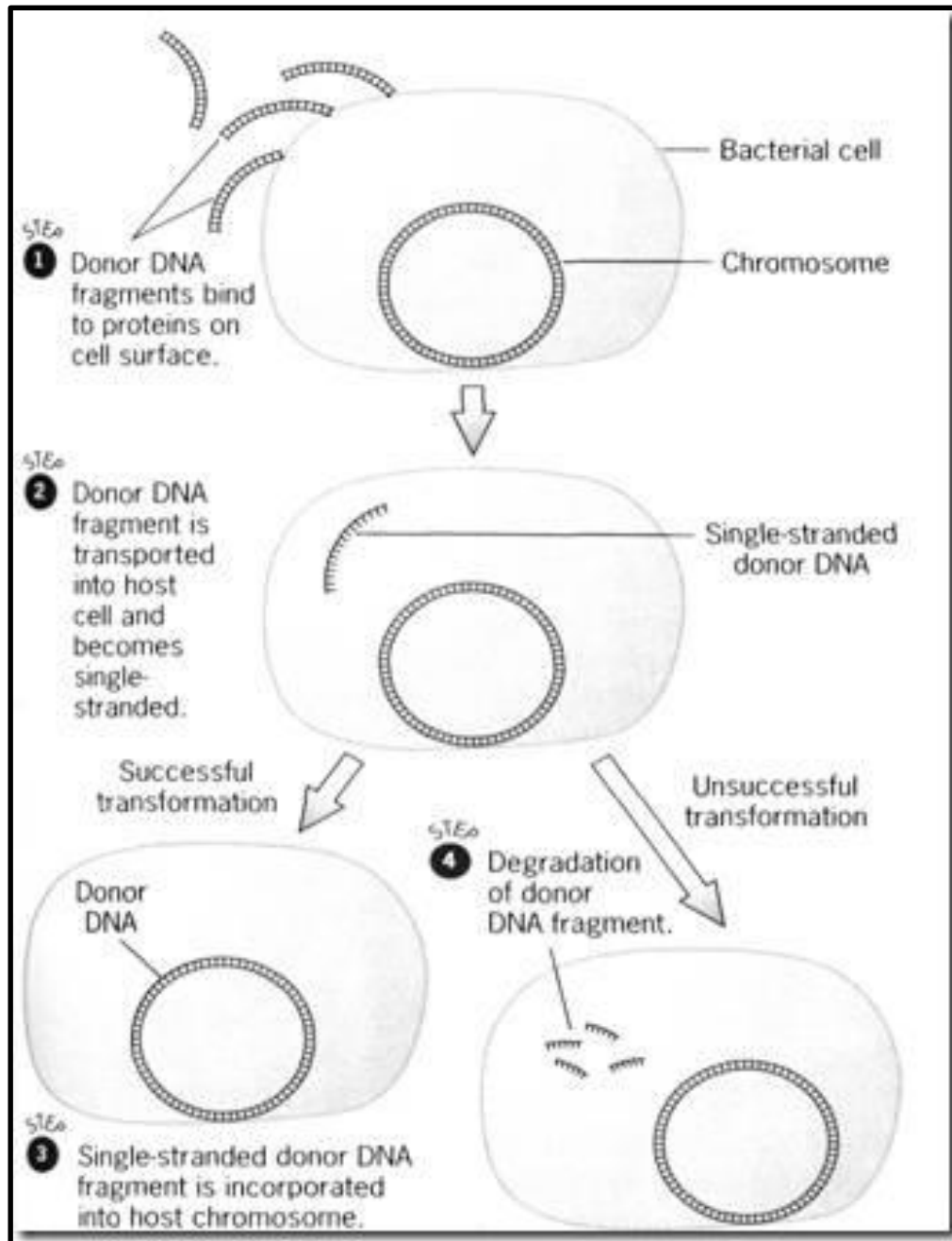
In vitro transformation



Mechanism of transformation



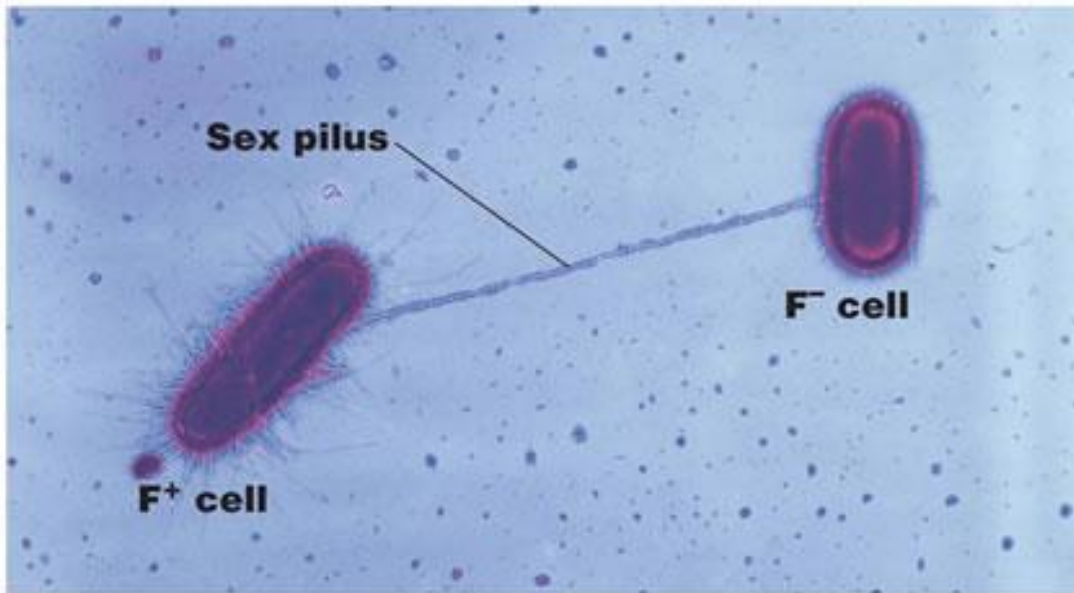
Mechanism of transformation



Transformation

- Competant cells
 - ▣ Naturally occurring
 - ▣ Induced
 - Calcium chloride

Conjugation



(a) Sex pilus

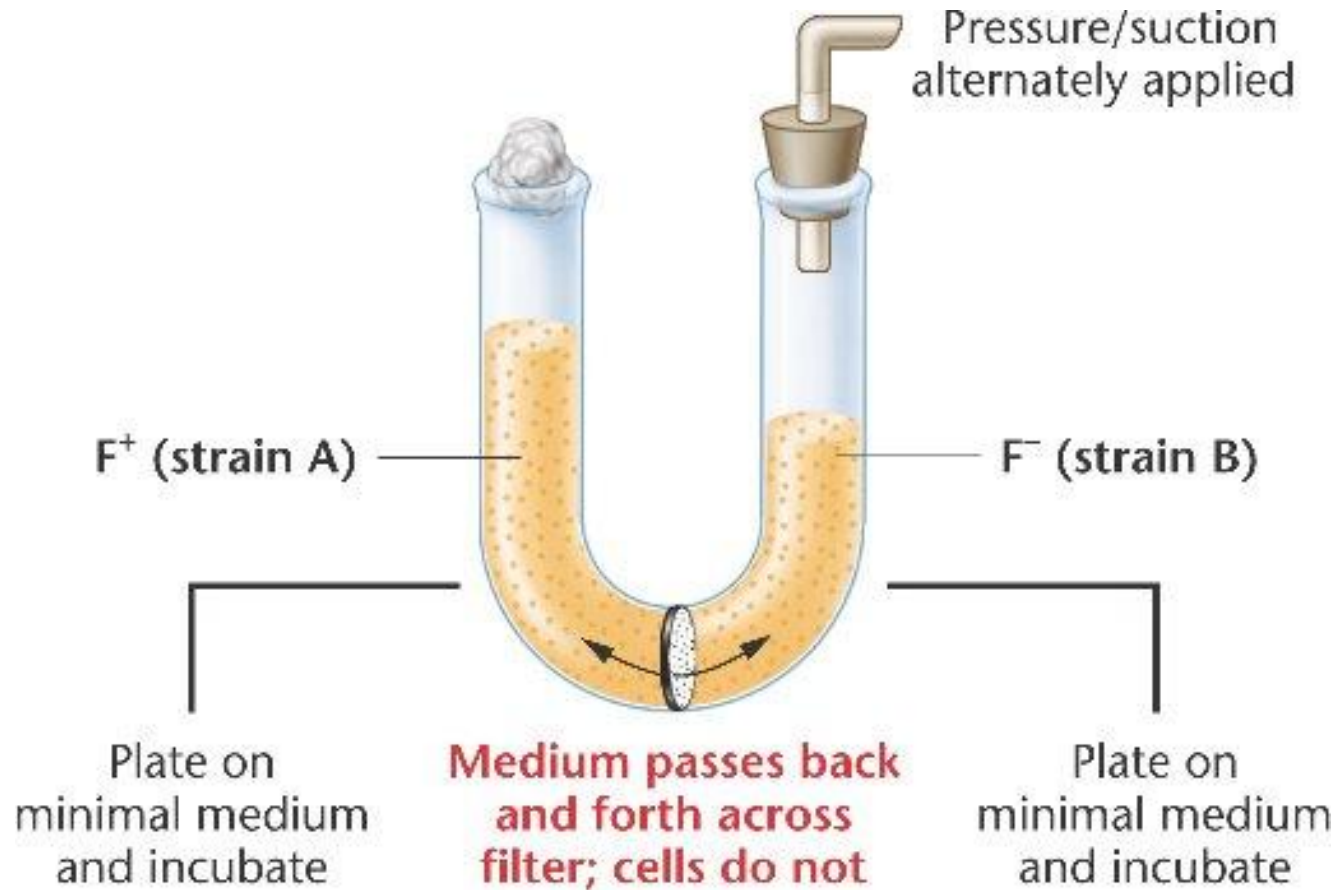
TEM 1 μ m



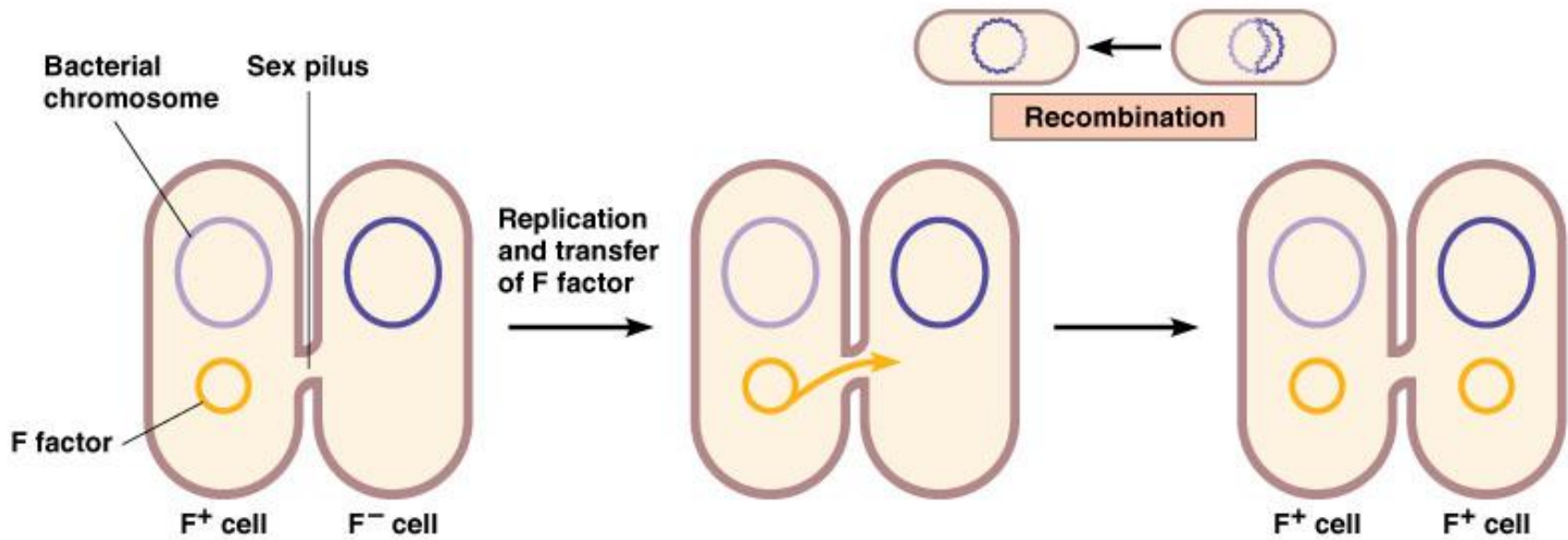
(b) Mating bridge

TEM 0.3 μ m

Conjugation

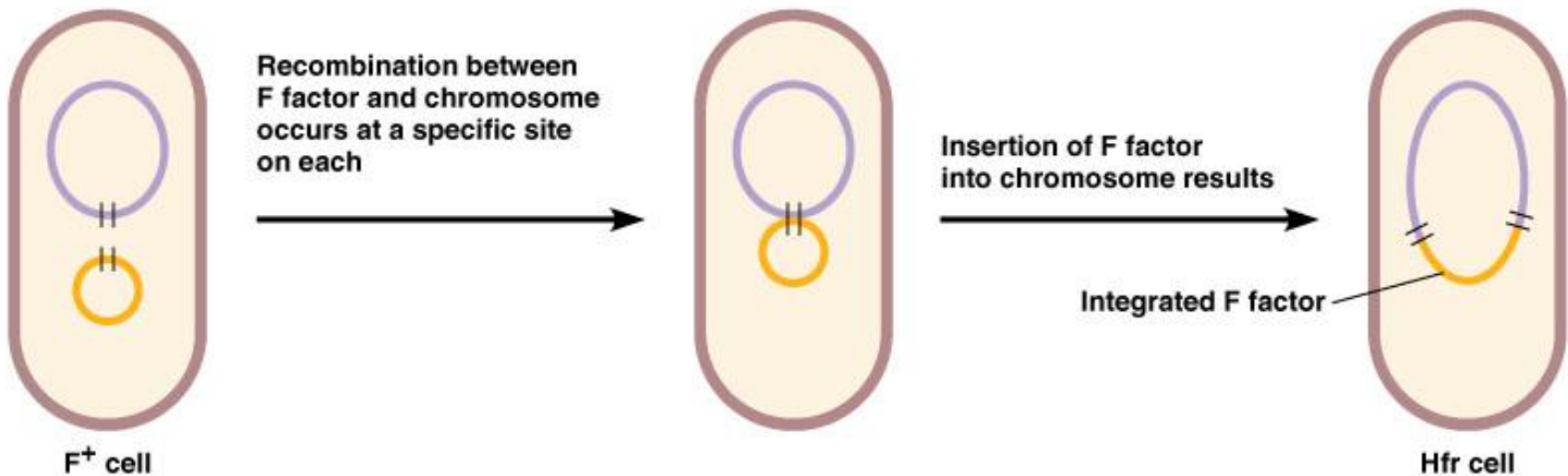


Conjugation



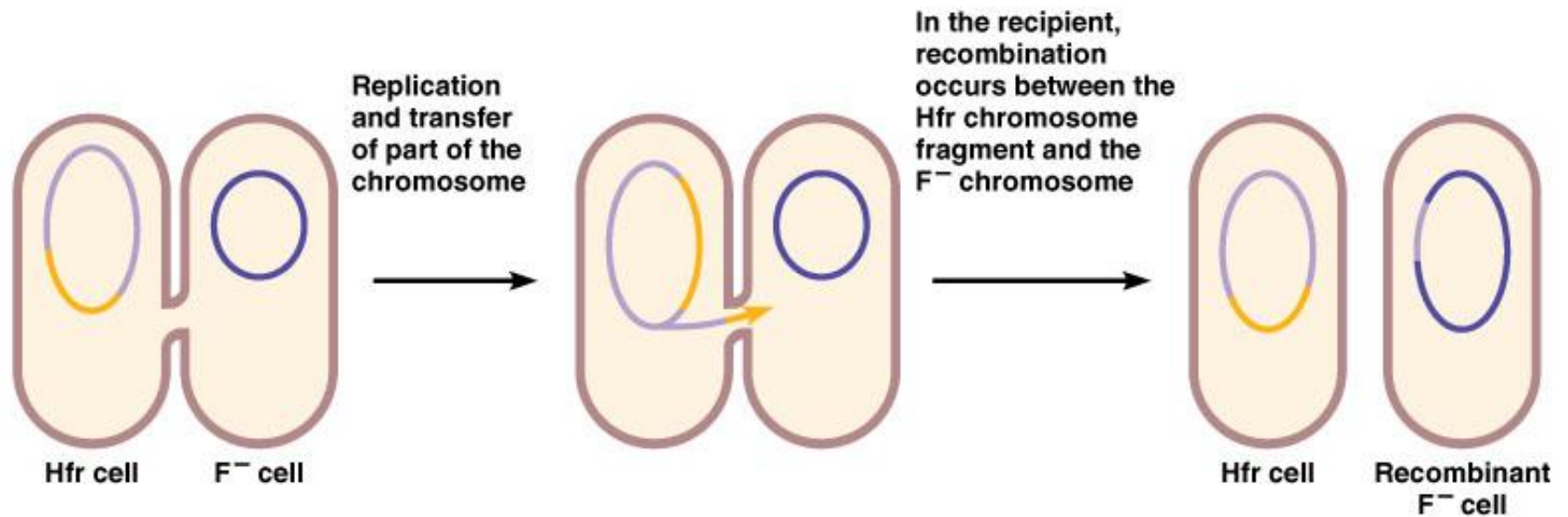
(a) When an F factor (a plasmid) is transferred from a donor (F^+) to a recipient (F^-), the F^- cell is converted into an F^+ cell.

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.

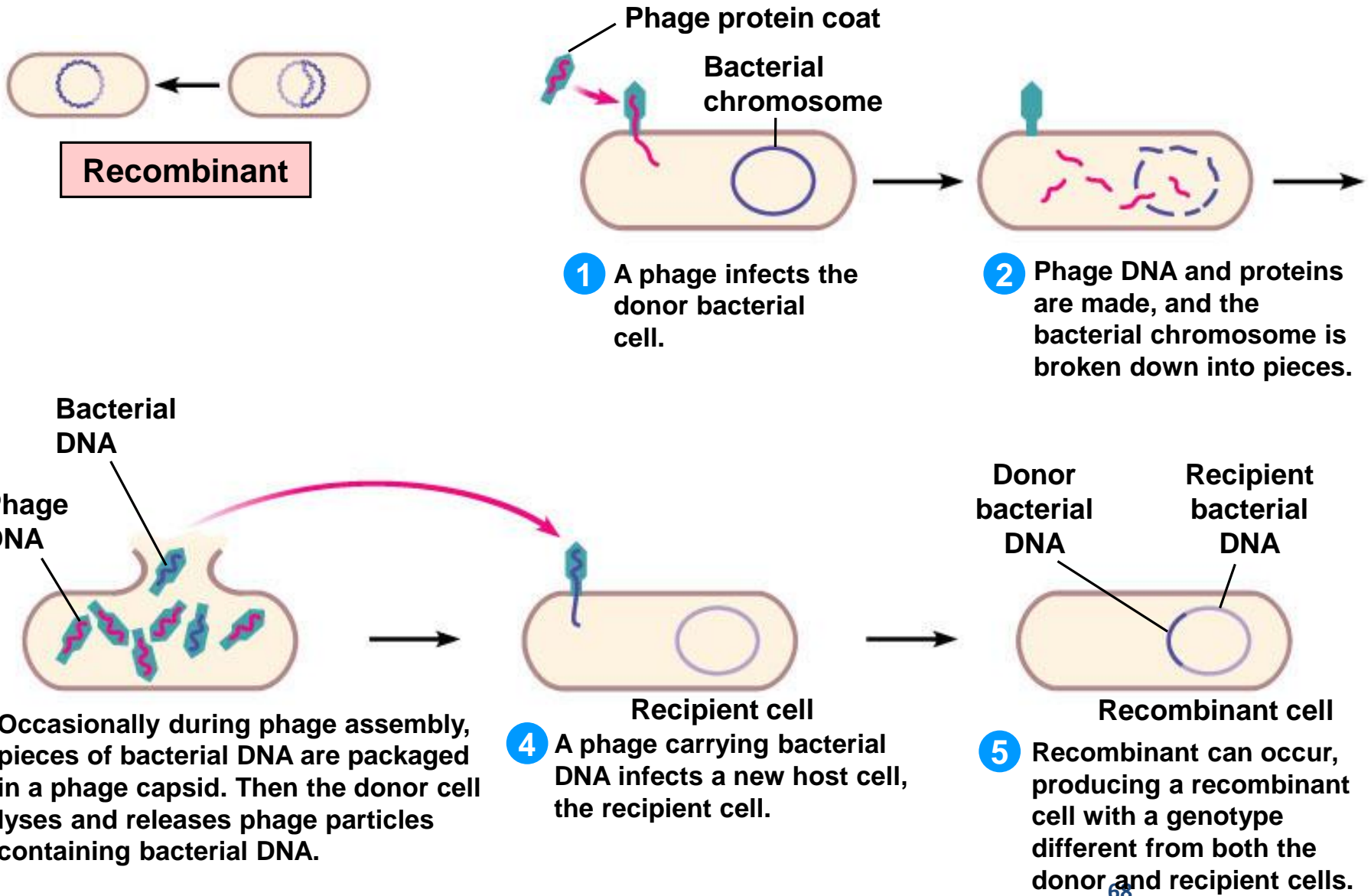
Conjugation



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

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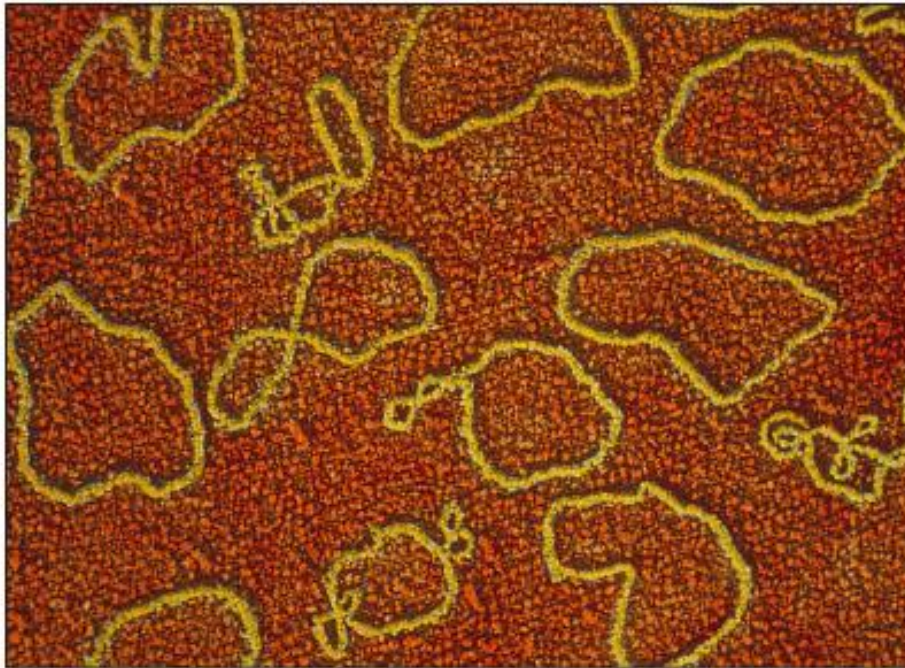
Transduction



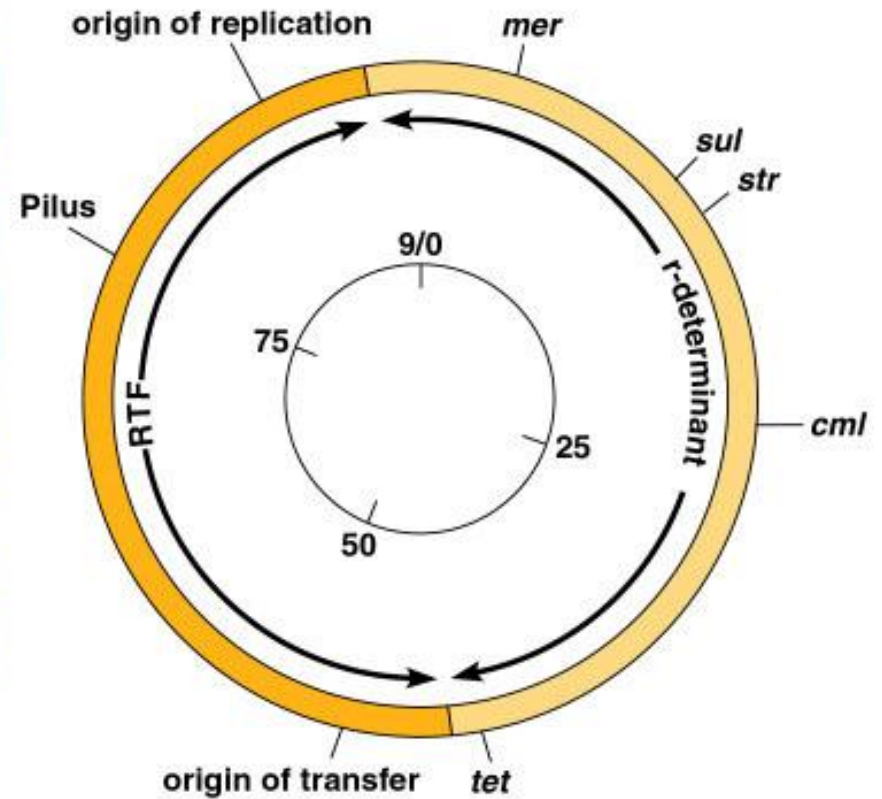
Plasmids

- 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)

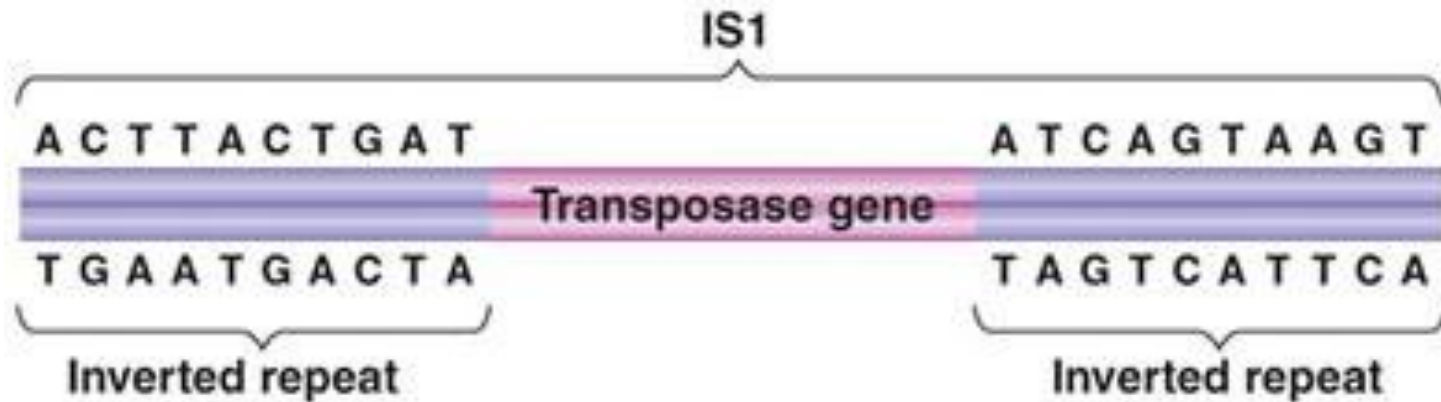


(b)

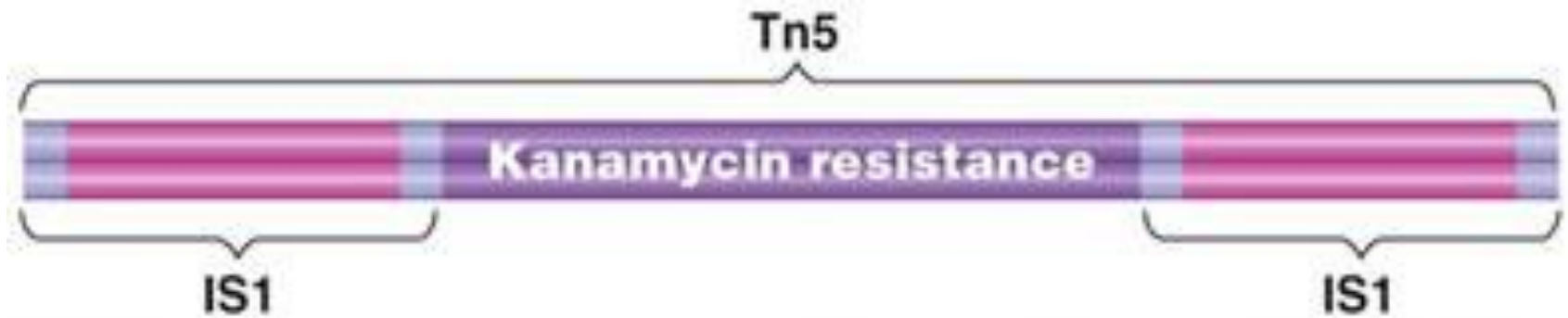
Transposons

- 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

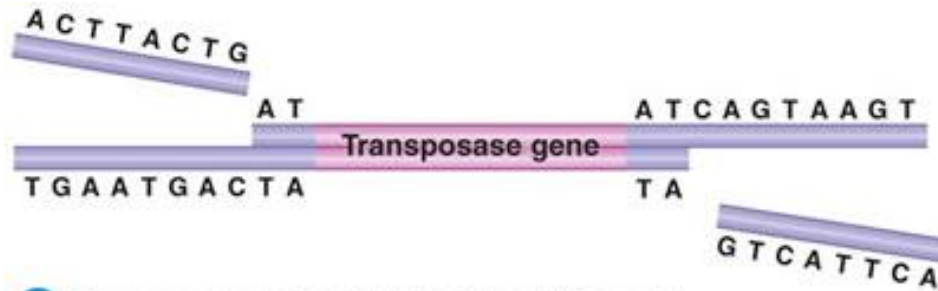
Insertion sequence



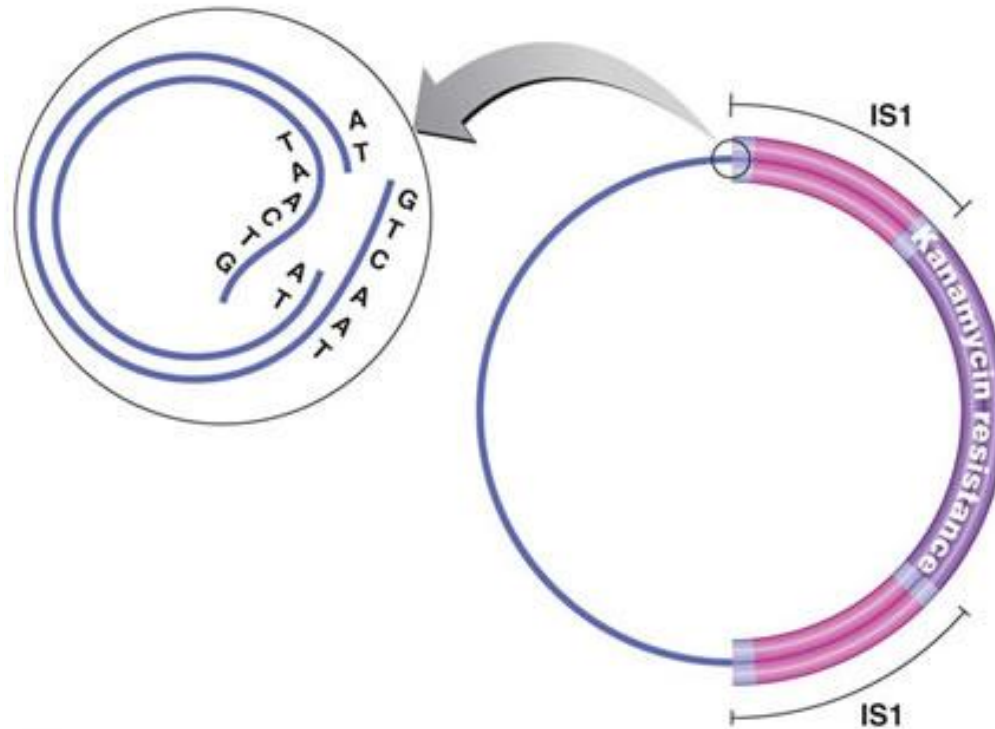
Complex transposons



Insertion of transposons into plasmids



1 Transposase cuts DNA, leaving sticky ends.



2 Sticky ends of transposon and target DNA anneal.

(c) Insertion of the transposon Tn5 into R100 plasmid.

THANK YOU