Microbial Genetics

Lecture 13

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Microbial Genetics

A. DNA Replication

B. Gene Expression

C. Gene Regulation

D. Mechanisms of Gene Transfer

E. Mutation

*

* Factors affecting bacterial genes

Plasmid

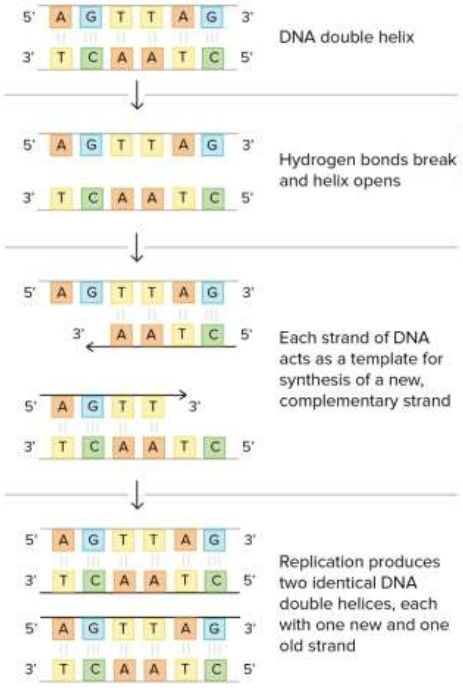
Plasmids are found in both Gram-positive and Gram-negative bacteria, but there are some differences:

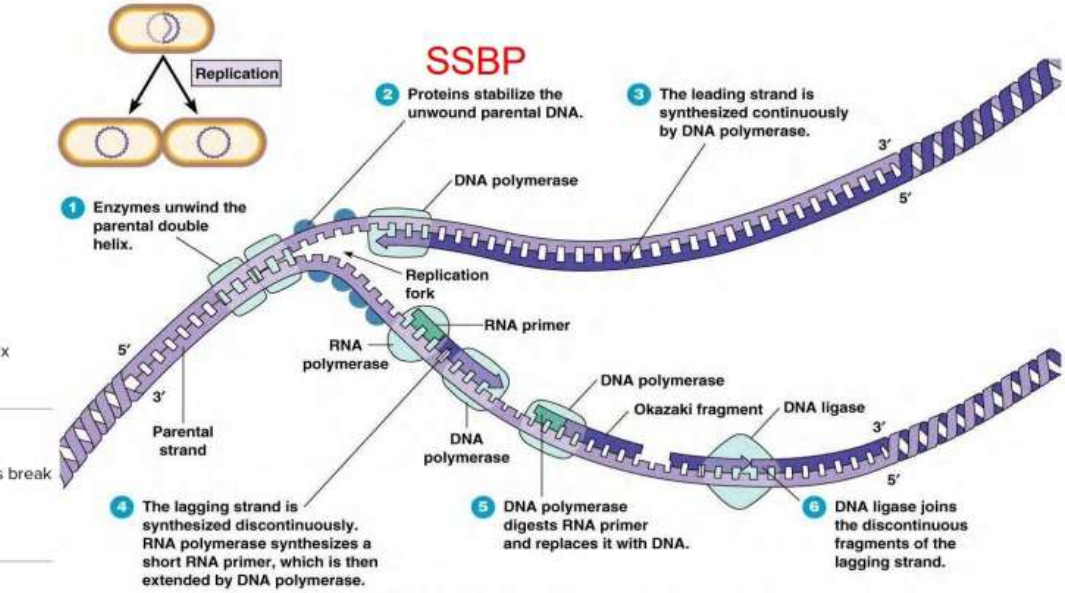
- Gram-negative bacteria (e.g., *Escherichia coli*), plasmids are very common and often carry genes for antibiotic resistance, conjugation, and virulence factors.
- Gram-positive bacteria (e.g., *Staphylococcus aureus* or *Bacillus subtilis*), may differ in structure and replication mechanisms compared to those in Gram-negative bacteria.

•Some of these genes encode traits such as:

- Antibiotic resistance
- Resistance to heavy metals
- Virulence factors that help bacteria colonize a host and evade its defenses
- Metabolic functions that enable bacteria to utilize specific nutrients or degrade toxic compounds

A. DNA Replication





Features of DNA Replication

Both strands serve as a template:

- synthesis is always 5'-3'
- *leading* strand synthesis is continuous,
  lagging strand synthesis is discontinuous

Each new DNA fragment requires an RNA primer:

- DNA synthesis cannot begin without a primer to add to

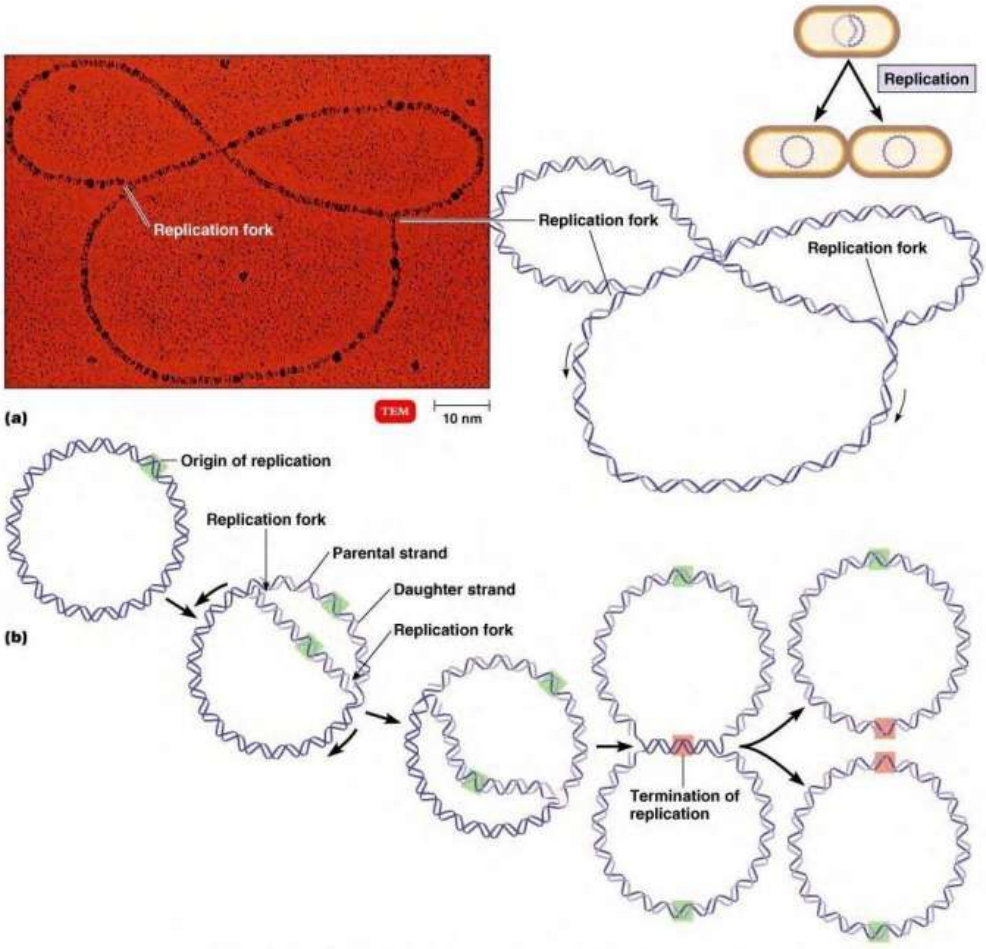
Some important enzymes:

DNA Polymerase III (synthesizes new DNA)

DNA Primase (makes RNA primers)

DNA Ligase ("seals" fragments together)

DNA Replication in Prokaryotes

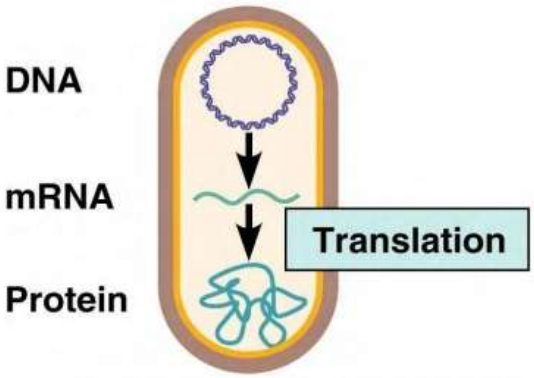


- **begins at the origin of replication (OriC)**
- **Its binding site for DnaA and DnaB**
- **can only be completed if DNA is circular**

https://www.youtube.com/watch?v=jmWuju1S9_E

B. Gene Expression

The expression of a gene into a protein occurs by:



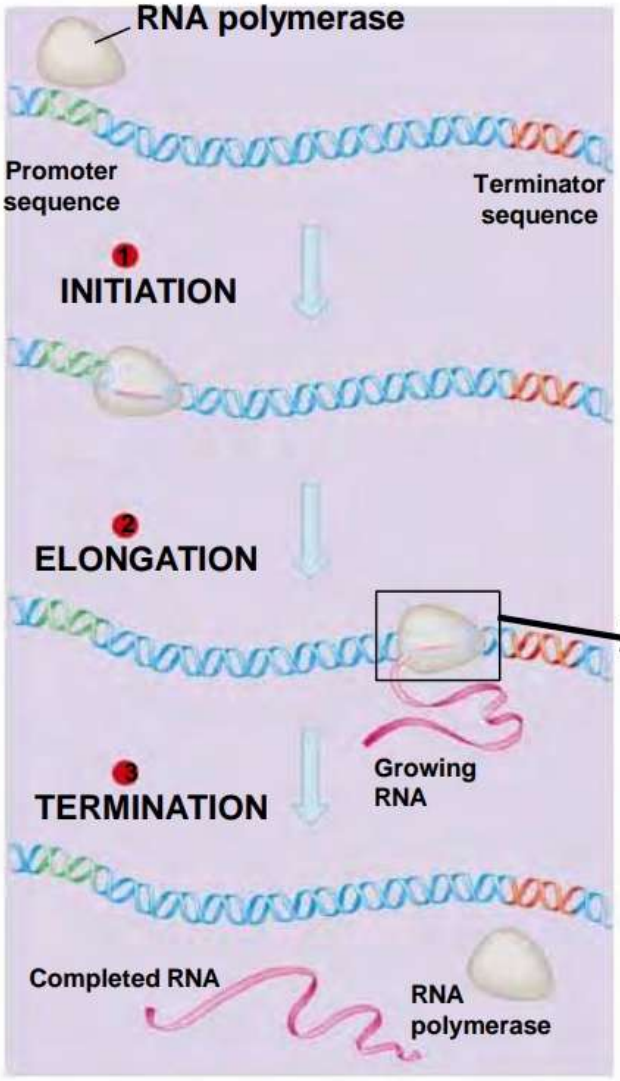
1) Transcription of a gene into RNA

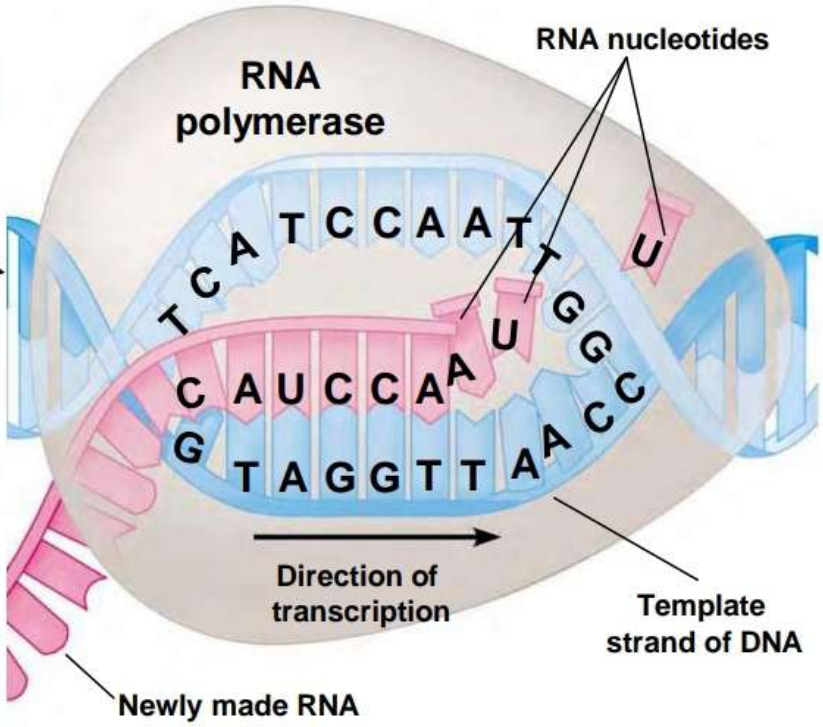
- produces an RNA copy of the coding region of a gene
- the RNA transcript may be the actual gene product (rRNA, tRNA) or be translated into a polypeptide gene product (mRNA)

2) Translation of mRNA transcript into polypeptide

- accomplished by ribosomes with the help of tRNA

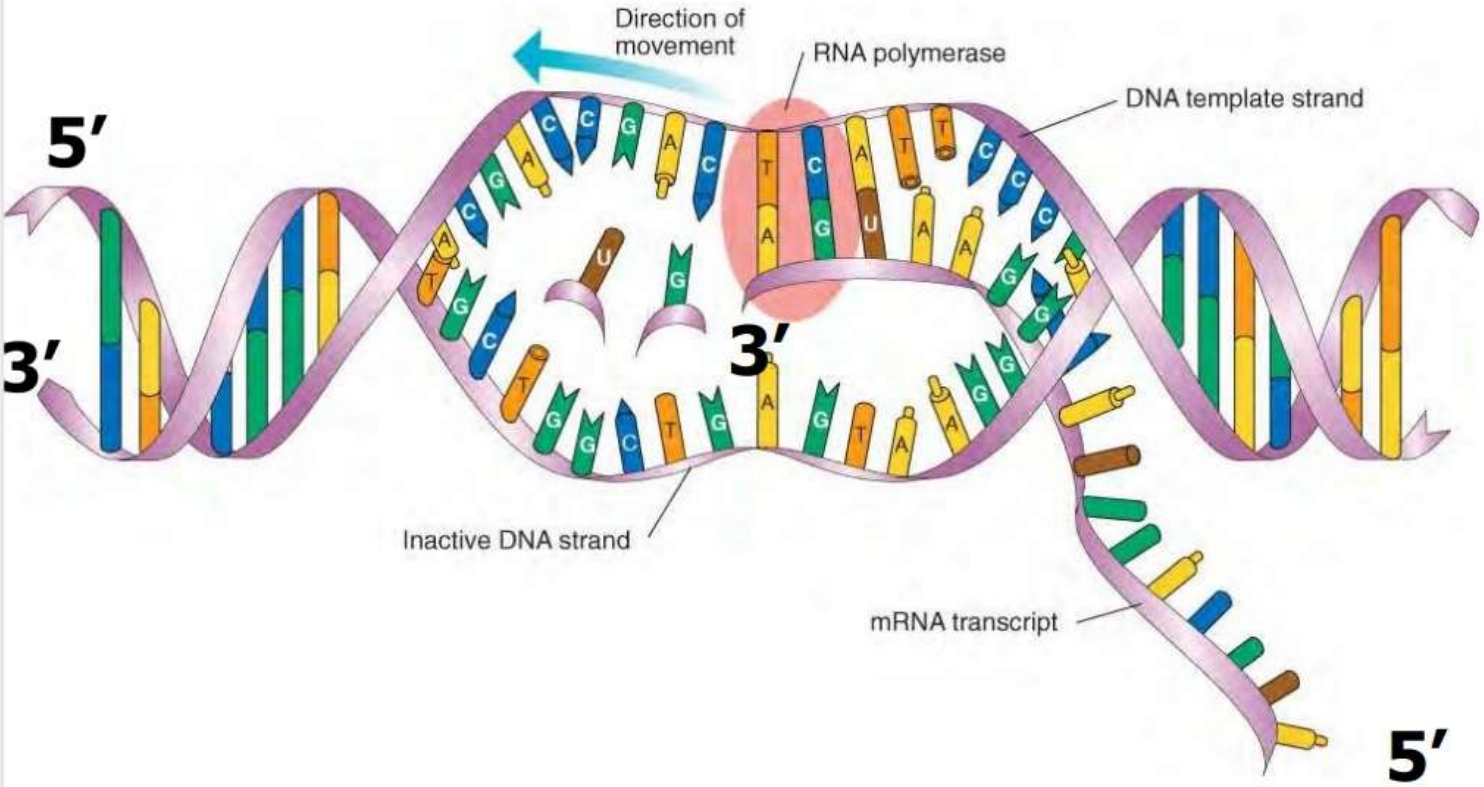
Overview of Transcription





Transcription is Uni-directional

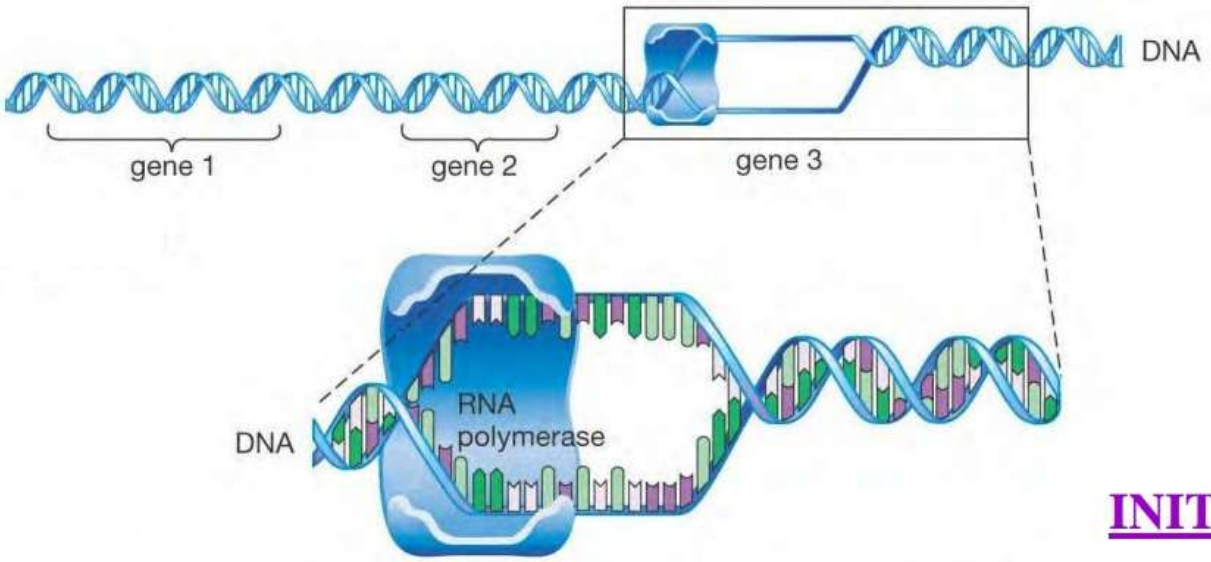
- **ribo-nucleotides can only be added to the 3′ end of an transcript, thus elongation is in a 5′ → 3′ direction**



3 Steps of Transcription

1) Initiation

- **RNA polymerase binds to the promoter of a gene**

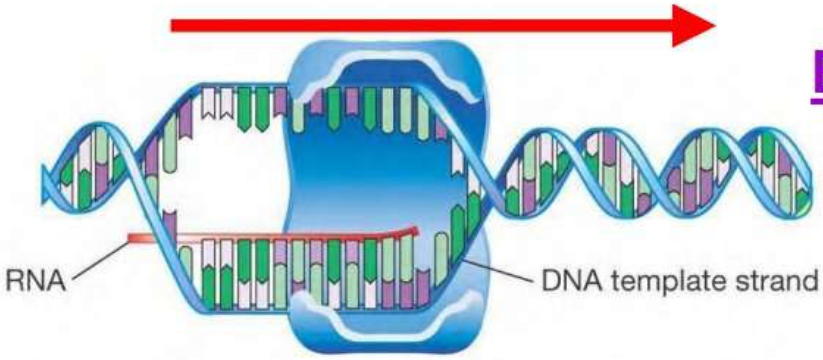


INITIATION

- **promoter serves to target and orient RNA polymerase**
- **once "docked" at promoter, RNA polymerase unzips DNA**

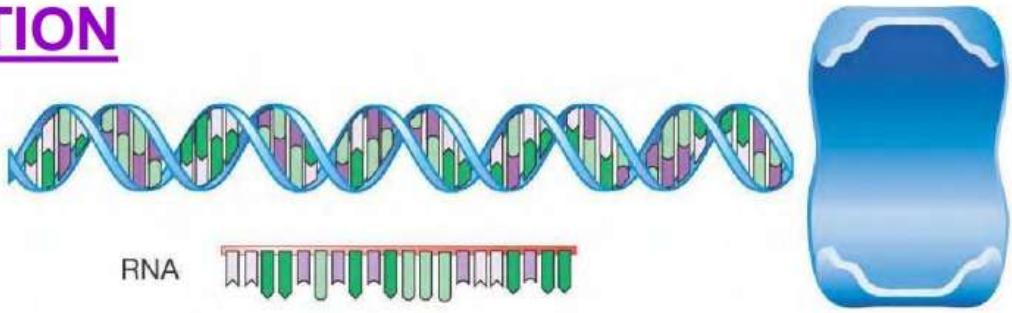
2) Elongation

- only 1 DNA strand is used as a template



ELONGATION

TERMINATION



3) Termination

- triggered by specific DNA sequences in the gene

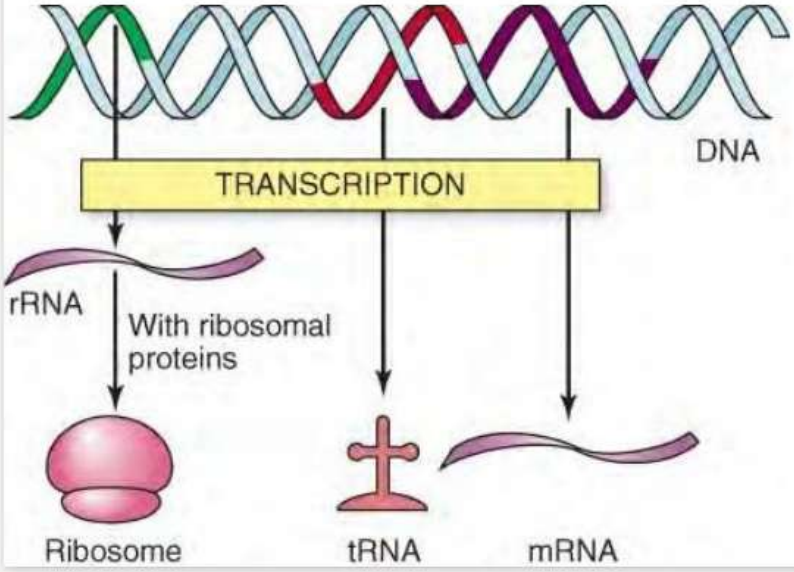
Various Roles of RNA Transcripts

1) messenger RNA (mRNA)

- RNA copy of a gene that encodes a polypeptide

2) ribosomal RNA (rRNA)

- RNA that is a structural component of ribosomes



3) transfer RNA (tRNA)

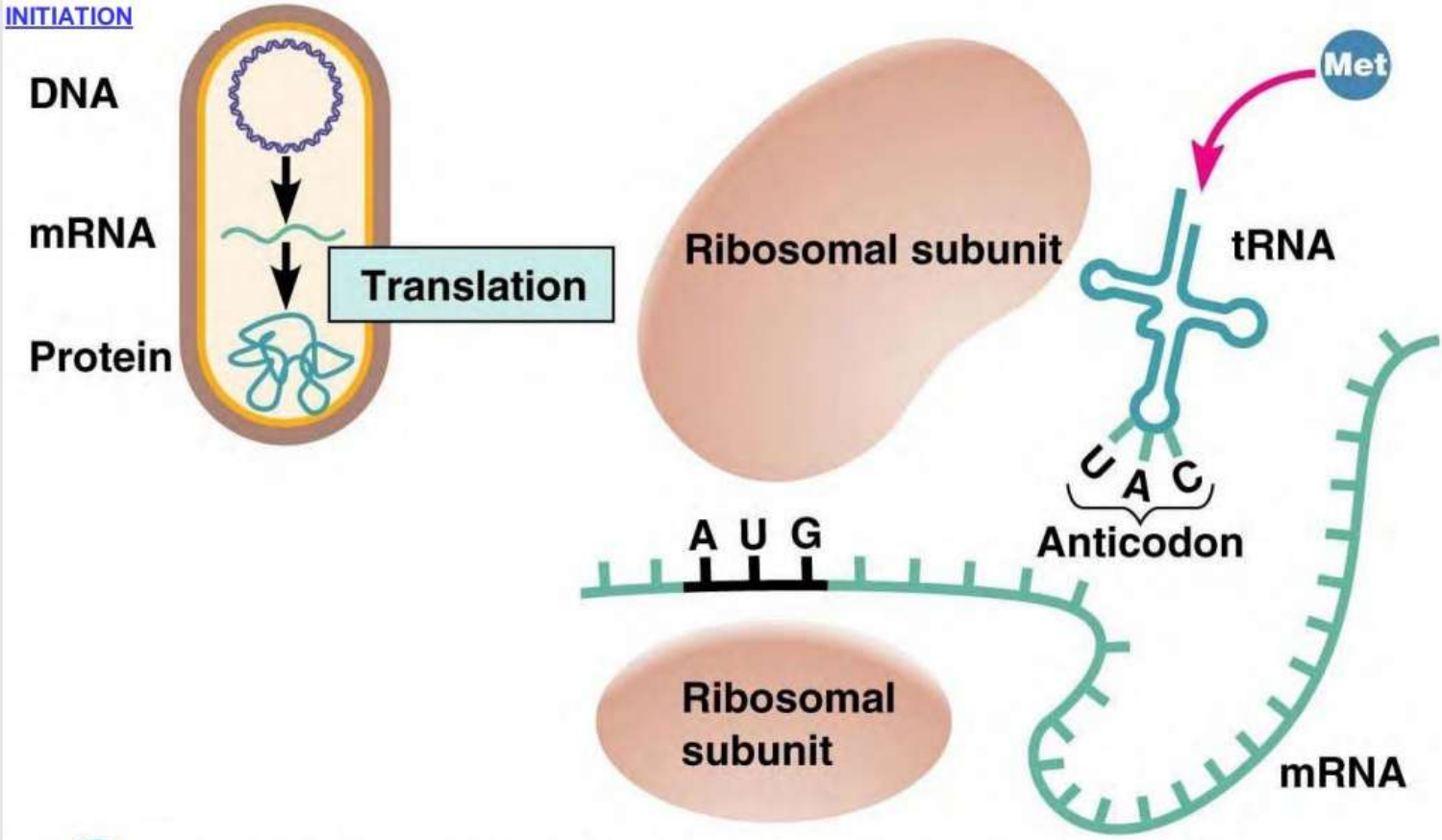
- delivery of "correct" amino acids to ribosomes during translation

Overview of Translation

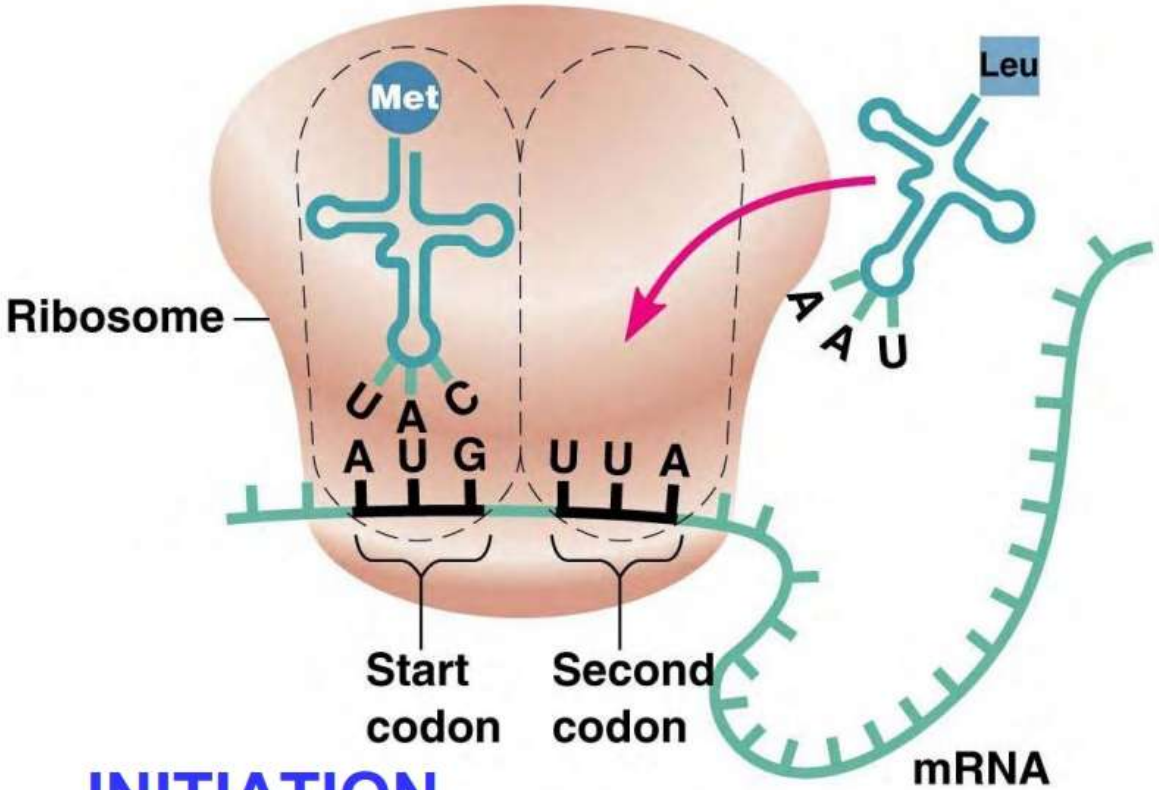
The building of a polypeptide, 1 amino acid at a time, by ribosomes using info in mRNA:

- ribosomes bind directly to mRNA, "read" codon by codon
    - ribosomes always start at AUG (methionine)
- translation also involves tRNA, each of which is attached to 1 of the 20 amino acids (AAs)
    - ribosomes match the right tRNA (via anticodon) with the right codon in the mRNA, then add its AA to the growing protein

Translation: step by step…

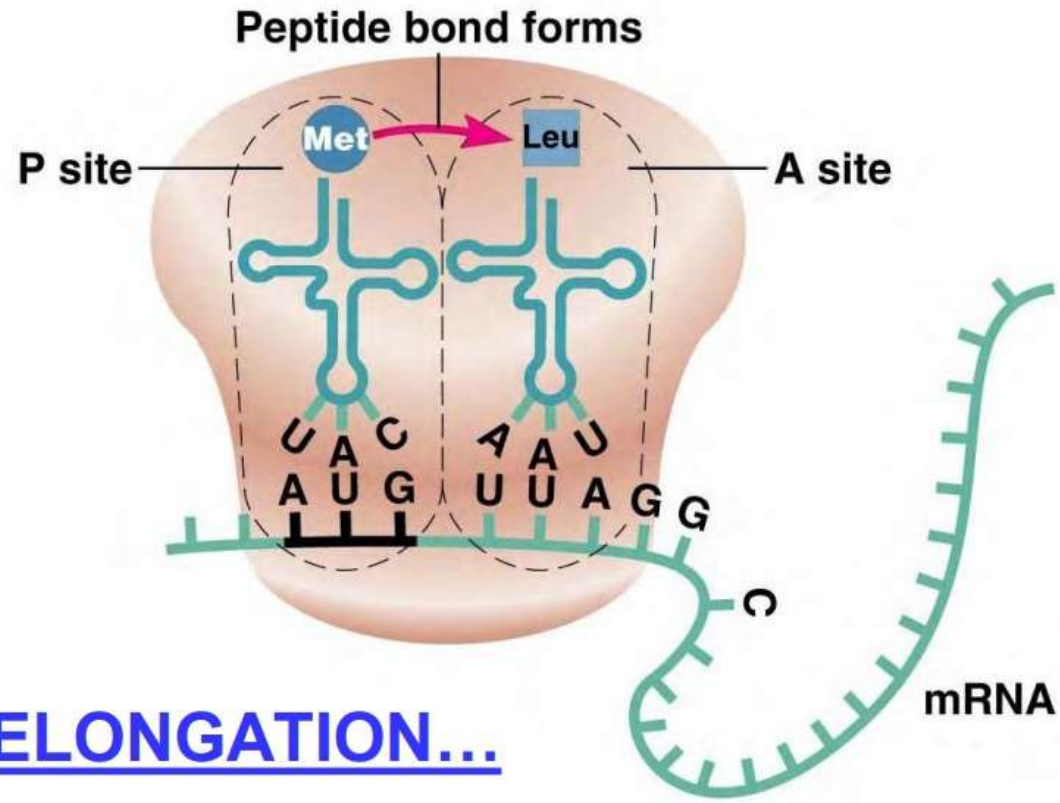


1 Components needed to begin translation come together.



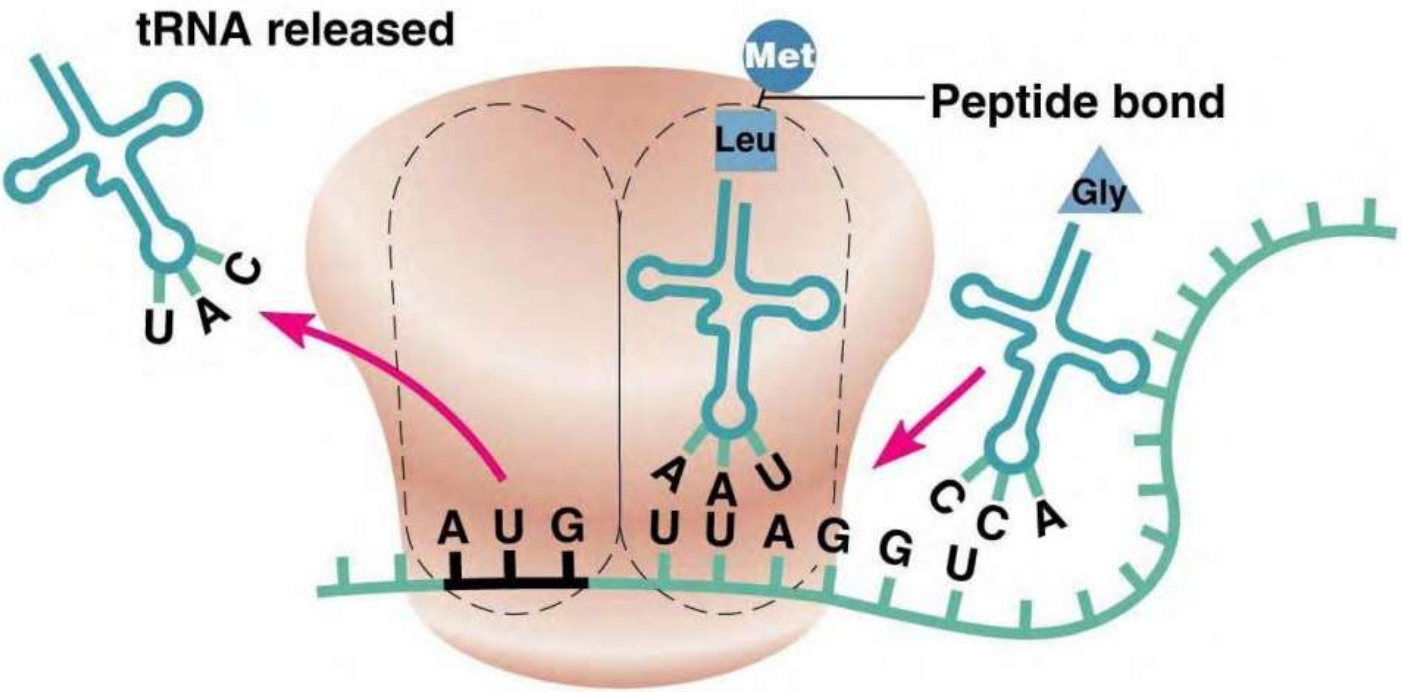
…INITIATION

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.



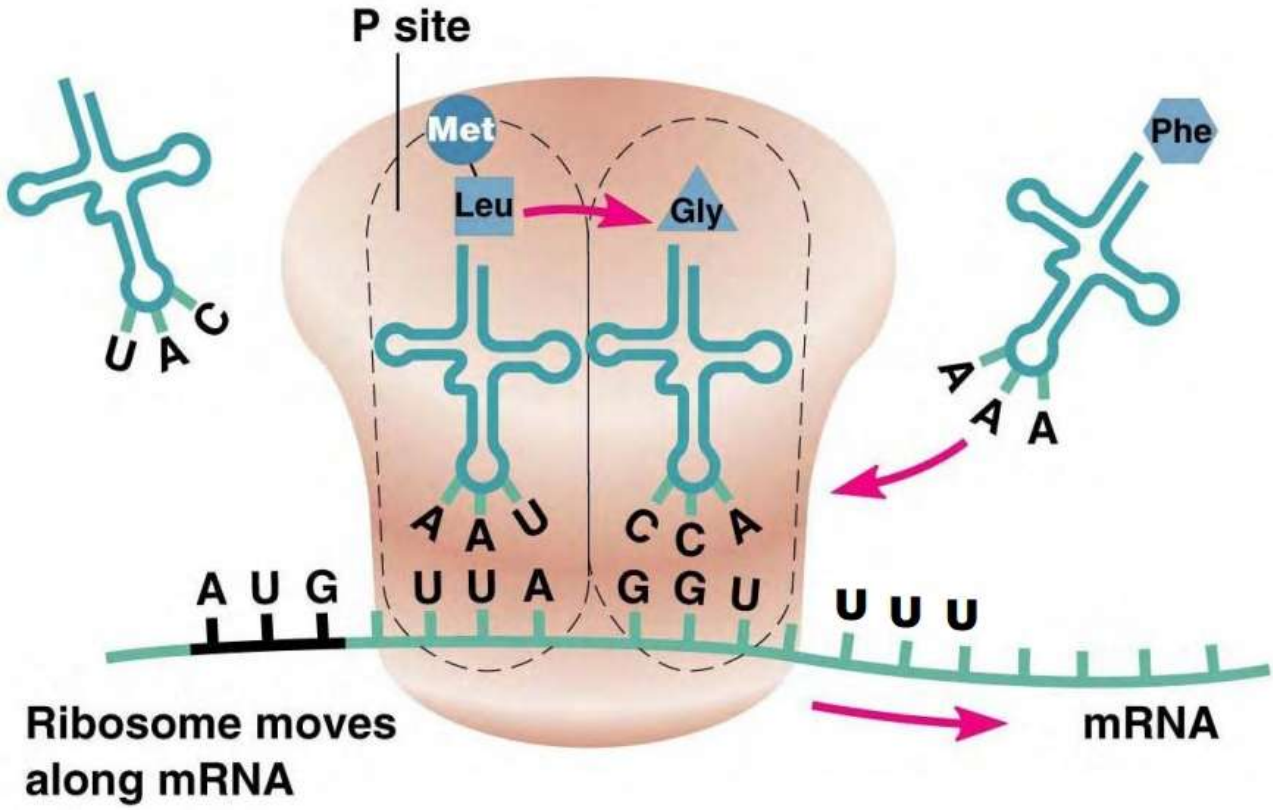
ELONGATION…

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.



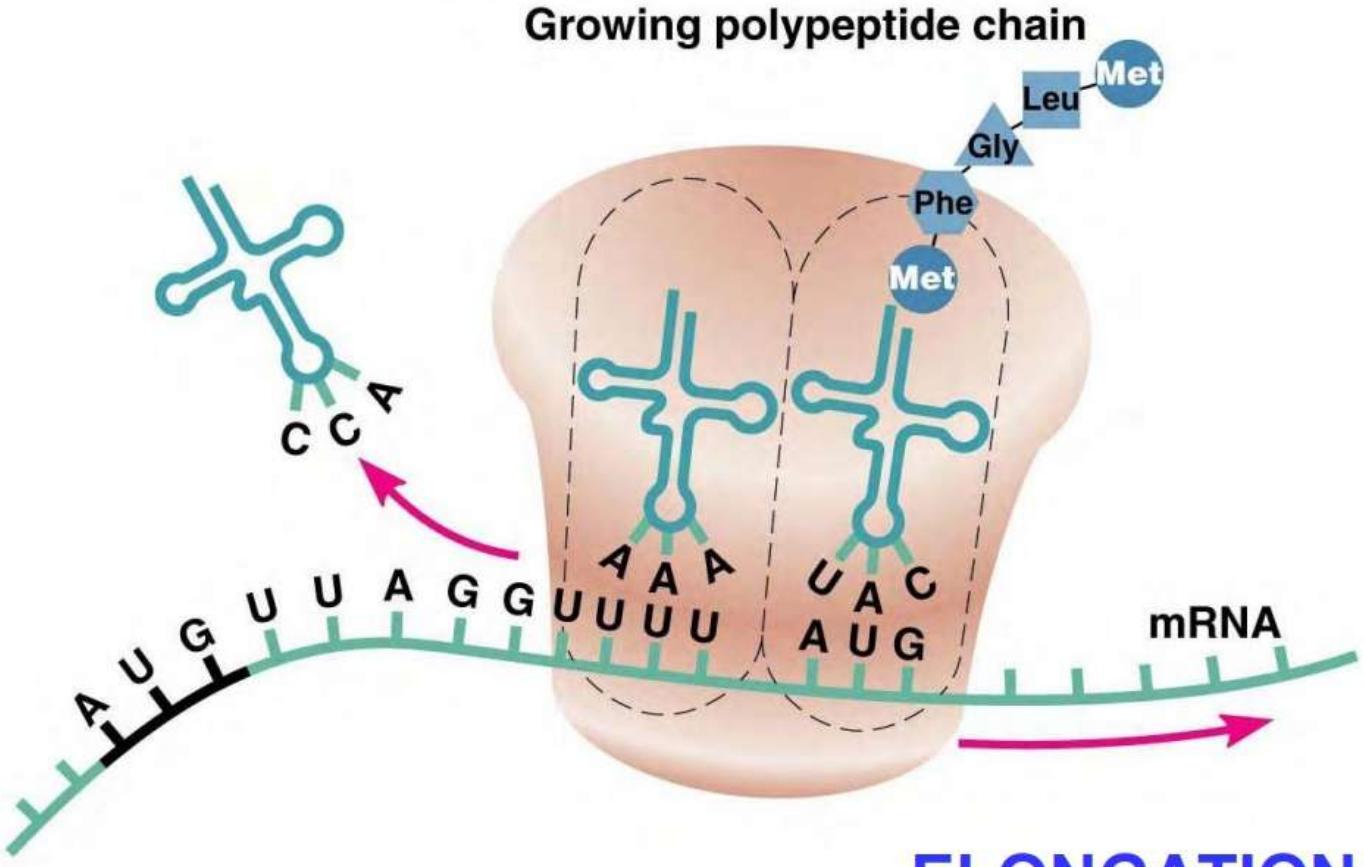
…ELONGATION…

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



…ELONGATION…

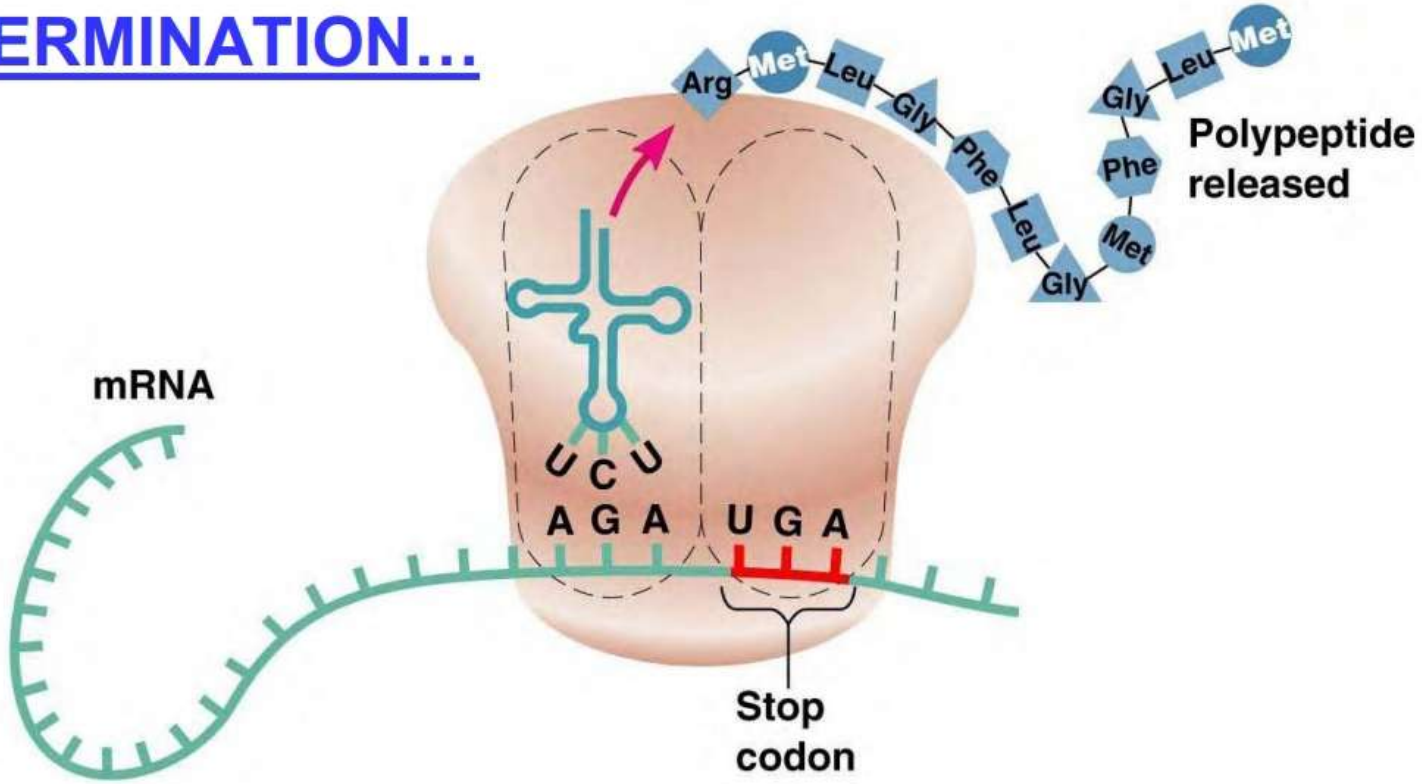
5 **The ribosome moves along the mRNA until the second tRNA is in the P site, and the process continues.**



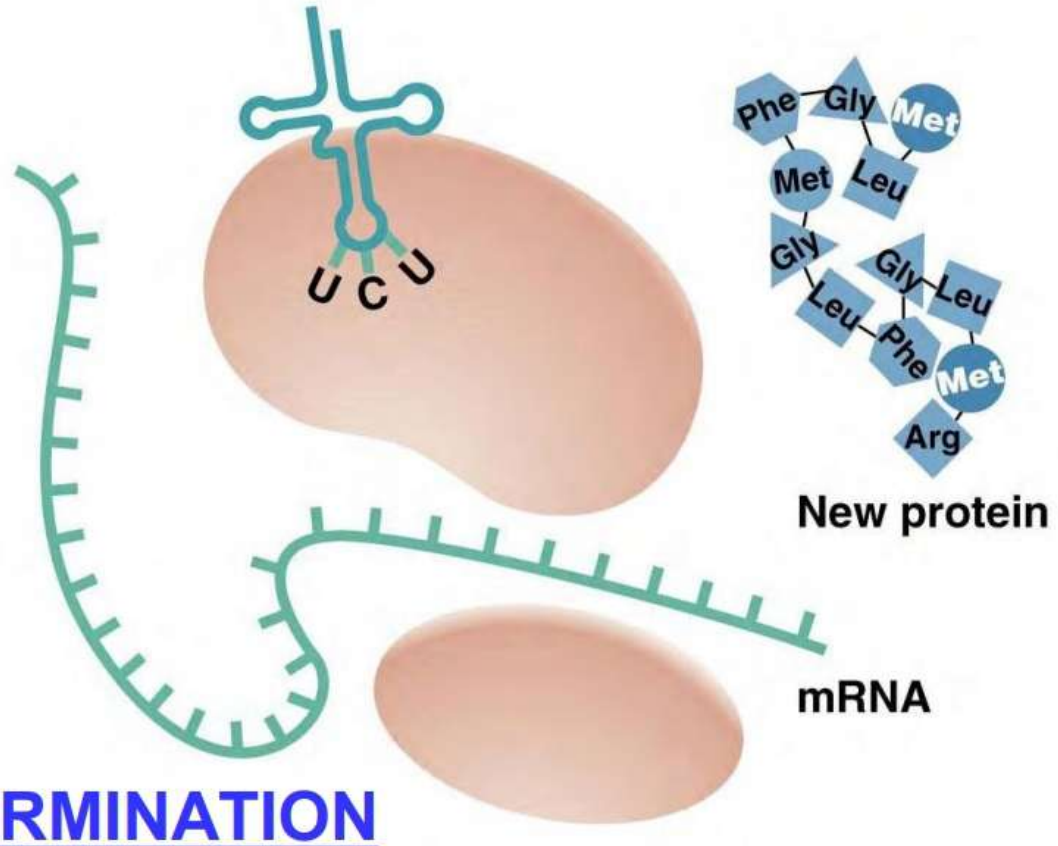
...ELONGATION

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

TERMINATION…



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



…TERMINATION

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

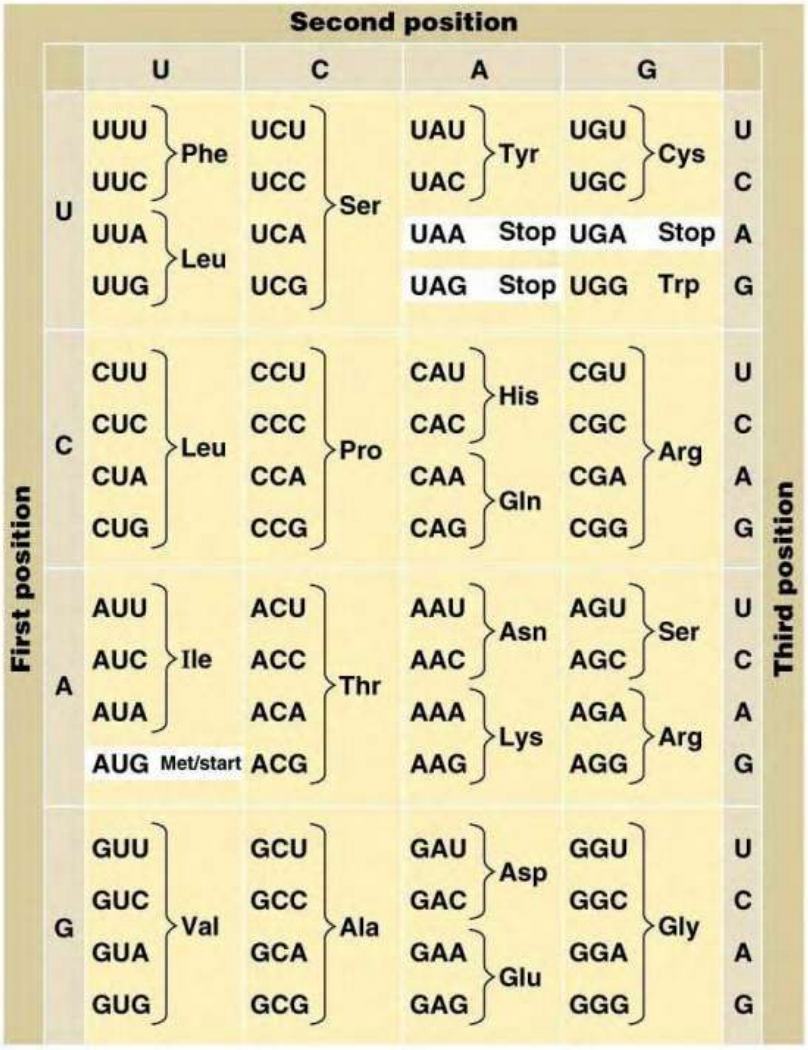


Table of the Genetic Code

If the DNA sequence is:
5′CATGCCTGGGCAATAG 3′

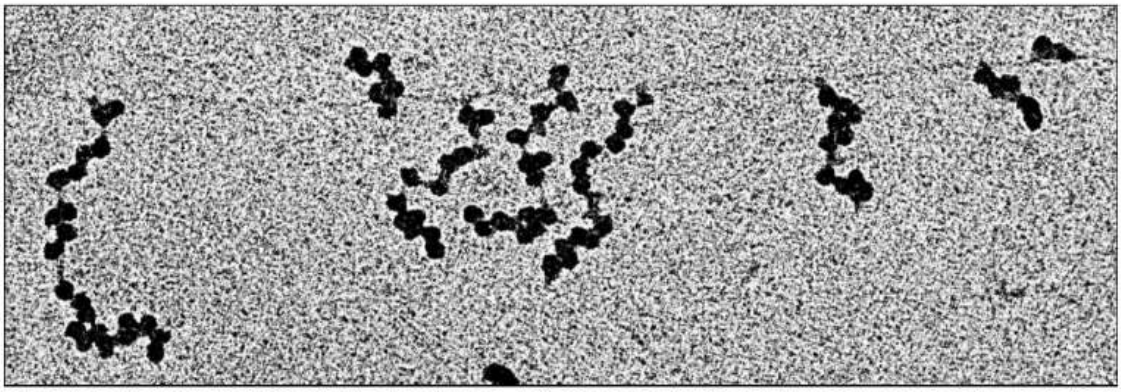
(transcription)

The mRNA copy is:
5′ CAUGCCUGGGCAAUAG 3′

(translation)

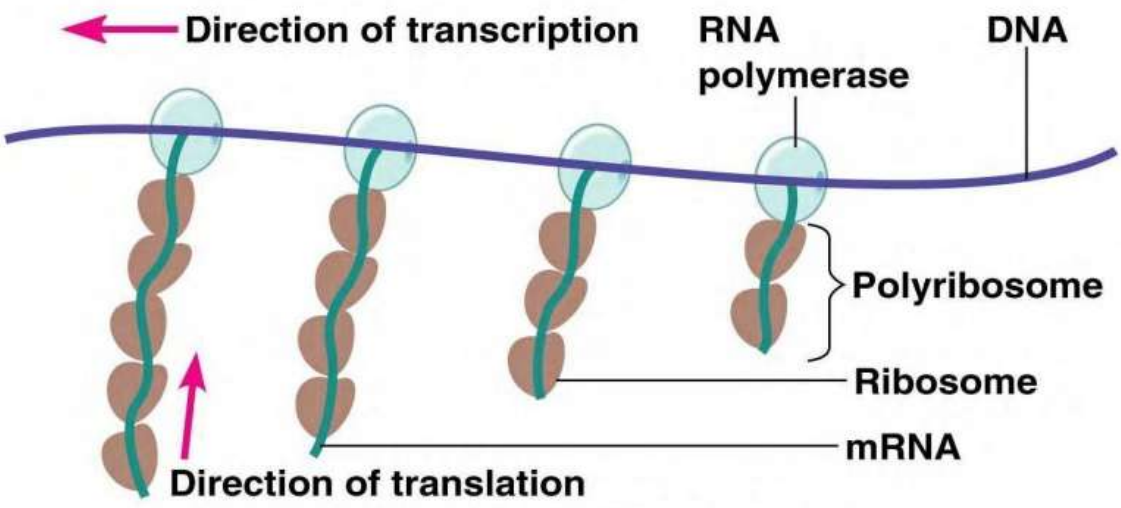
The polypeptide is:
***Met-Pro-Gly-Gln-(stop)**
***all proteins begin w/Met**

Gene Expression in Prokaryotes



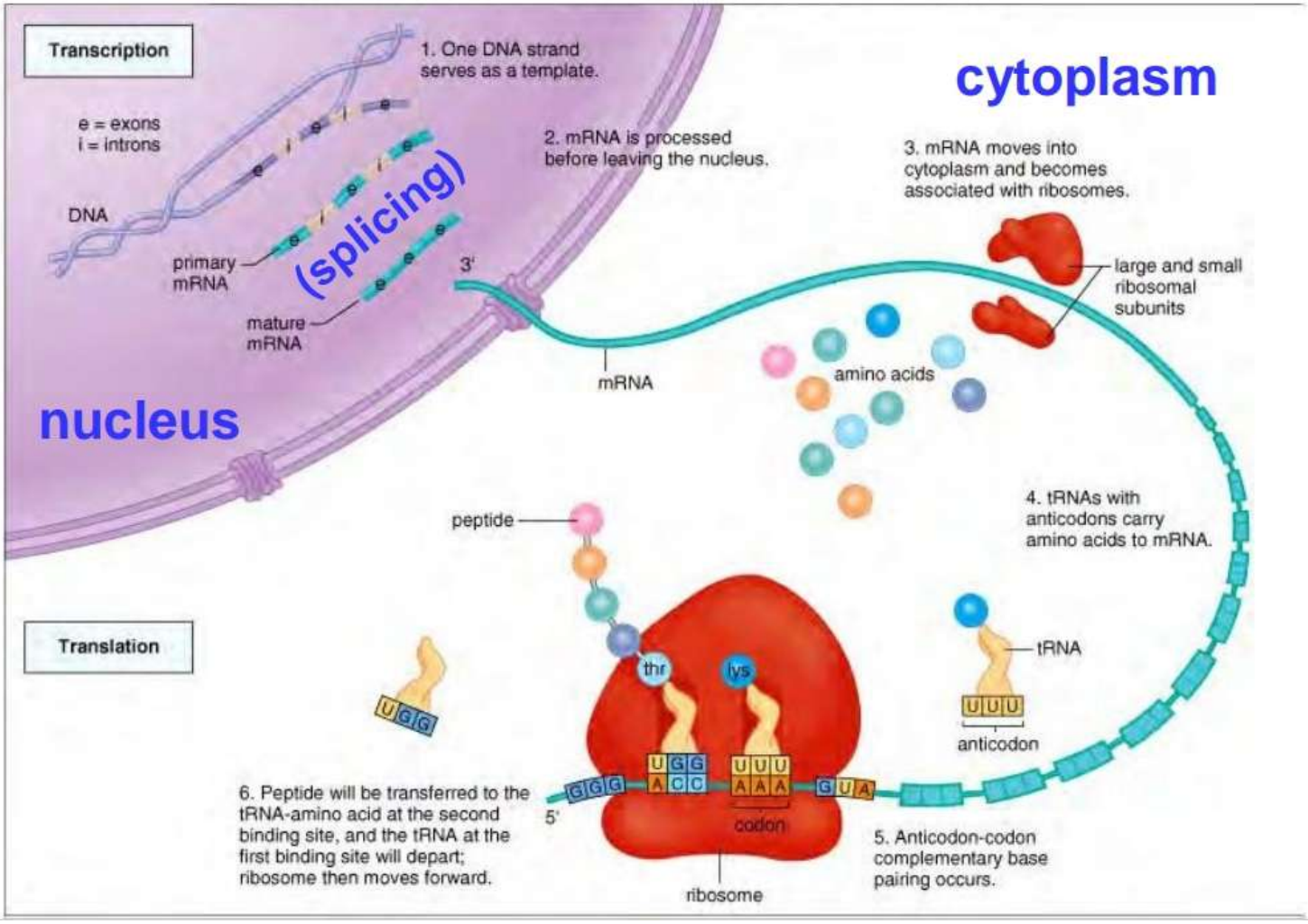
TEM

200 nm

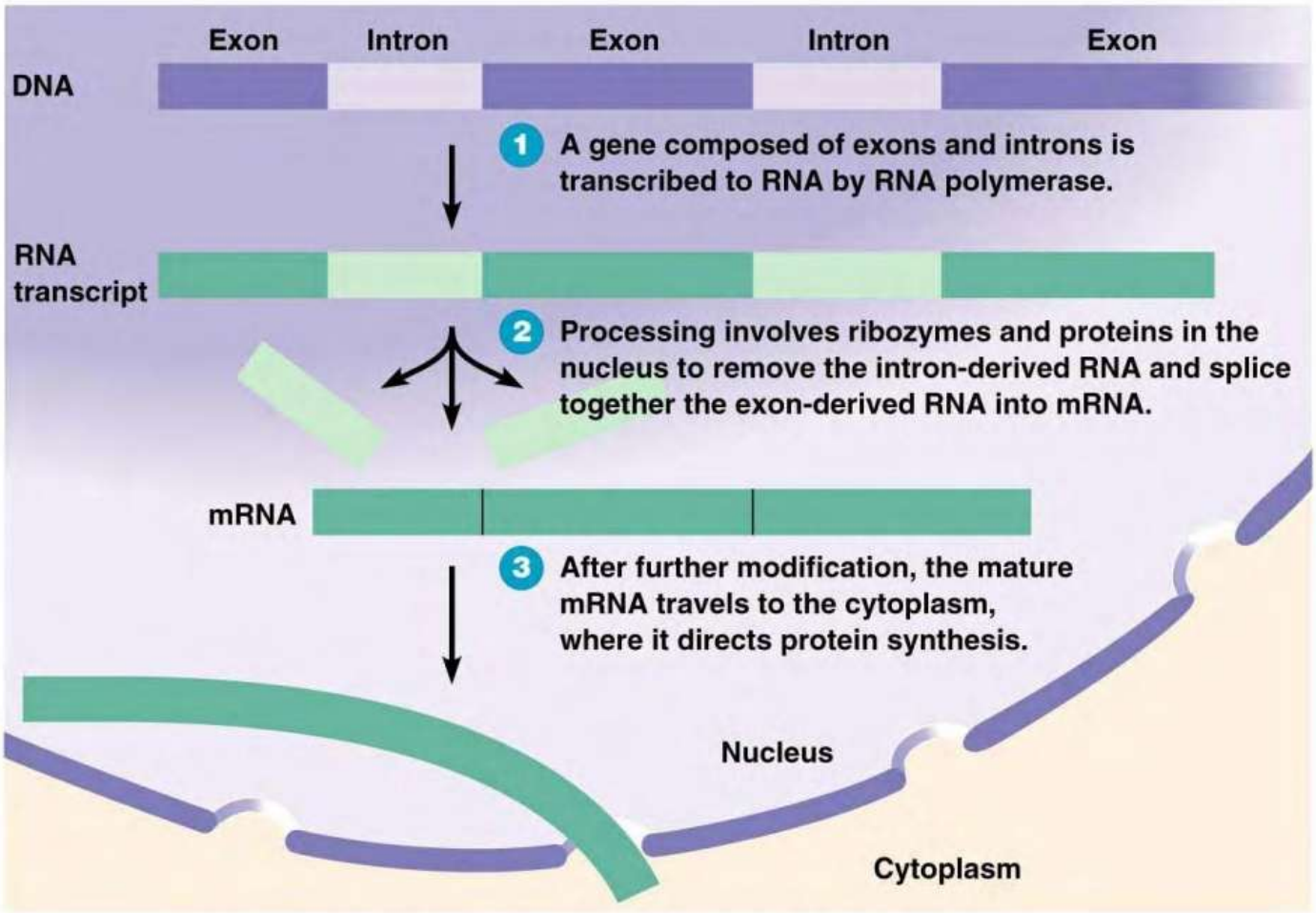


- gene expression is not necessarily "segregated"
- transcription & translation can occur simultaneously

Gene Expression in Eukaryotes



Splicing of Eukaryotic Transcripts



C. Regulation of Transcription

The focal point is whether or not RNA polymerase binds the promoter of a gene and initiates transcription which depends on:

1) Affinity of RNA polymerase for a given promoter

- some promoters are "strong" and bind RNA polymerase with high affinity
- some promoters are "weak" and bind RNA polymerase with low affinity, requiring help from special proteins called transcription factors
- the strength of a promoter depends on its sequence

2) Influence of proteins collectively referred to as transcription factors

- proteins that help RNA polymerase bind a promoter (referred to as "activators")

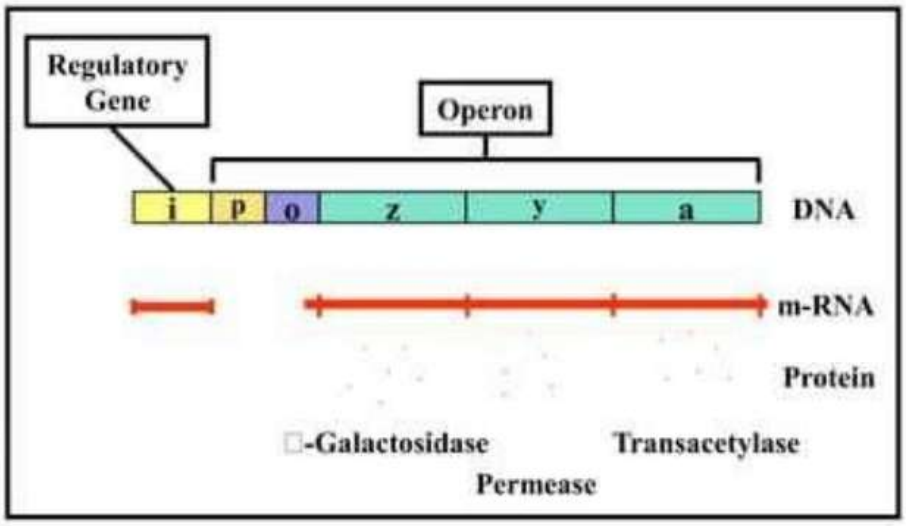
- proteins that inhibit or prevent RNA polymerase from binding a promoter (referred to as "repressors" or "inhibitors")

- the levels of various repressors & activators of transcription depend on the cellular environment, which thus determines which genes are ON or OFF!

Let's see how this works in genes involved with lactose metabolism in *E. coli*…

Lactose Operon

- **Structural genes**
  - *lac z, lac y, & lac a*
    - ❖ Polycistronic mRNA
- **Regulatory gene**
  - Repressor
- **Operator**
- **Promoter**
- **Inducer – (lactose)**



Difference between Polycistronic and Monocistronic mRNA ?

Structure of the lac Operon

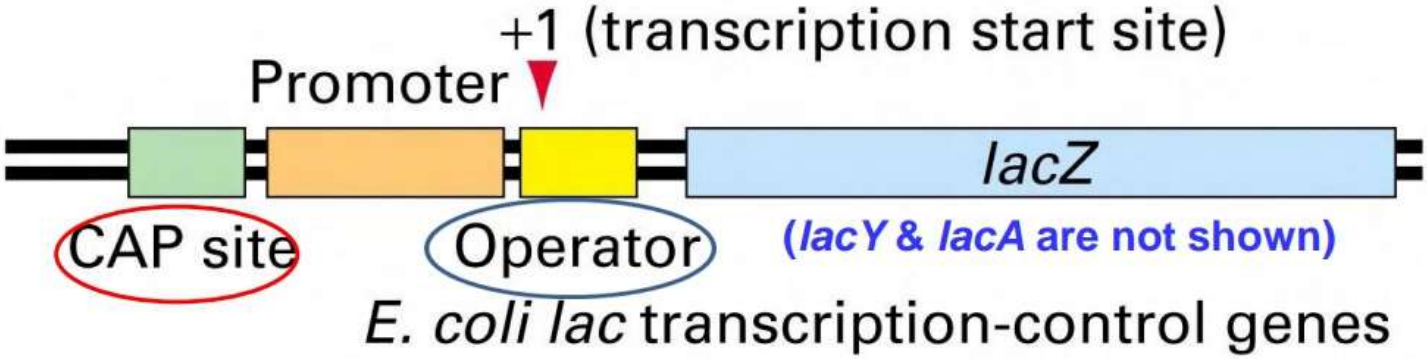
- The lac operon consists of 3 protein-coding genes plus associated control regions.
- The 3 genes are called z, y, and a.
- lacZ codes for the enzyme beta-galactosidase, which splits lactose into glucose plus galactose.
- lacY codes for a "permease" protein that allows lactose to enter the cell
- lacA codes for an enzyme that acetylates lactose.

Together these three genes are called the "structural genes".

Control Regions

- Near the lac operon is another gene, called lacI, or just "i". It codes for the lac repressor protein,
- Plays an essential role in lac operon control.
- The lac repressor gene is expressed "constitutively", meaning that it is always on (but at a low level).
- It is a completely separate gene, producing a different mRNA than the lac operon.

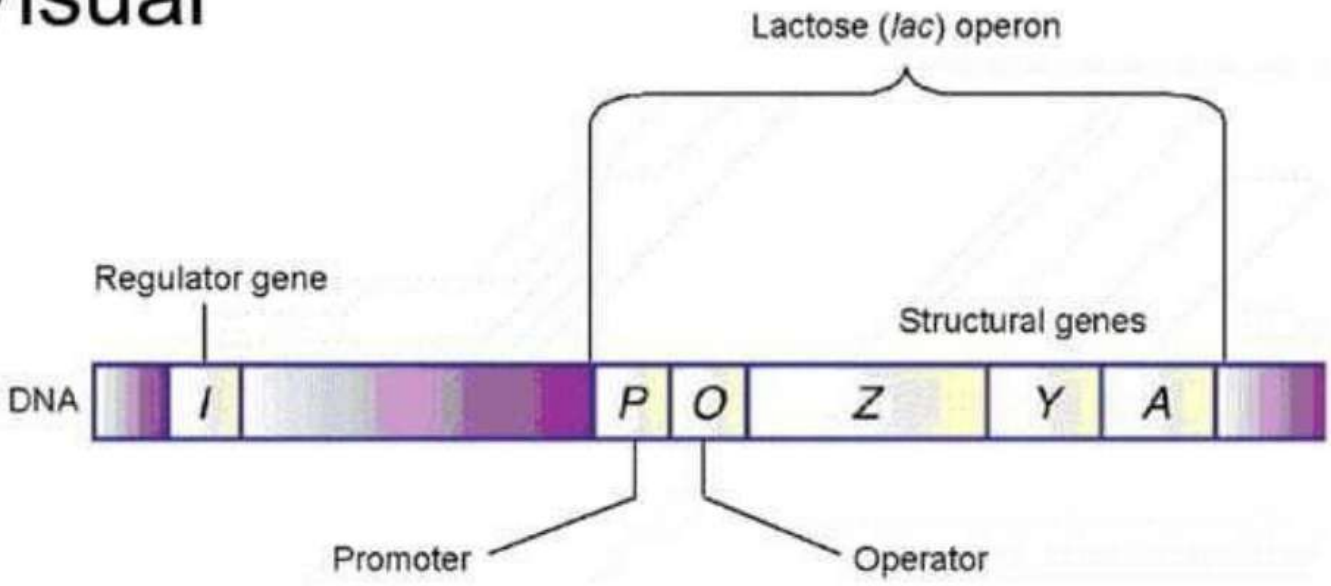
The *lac* operon of *E. coli*



On either side of the promoter are 2 special sequences

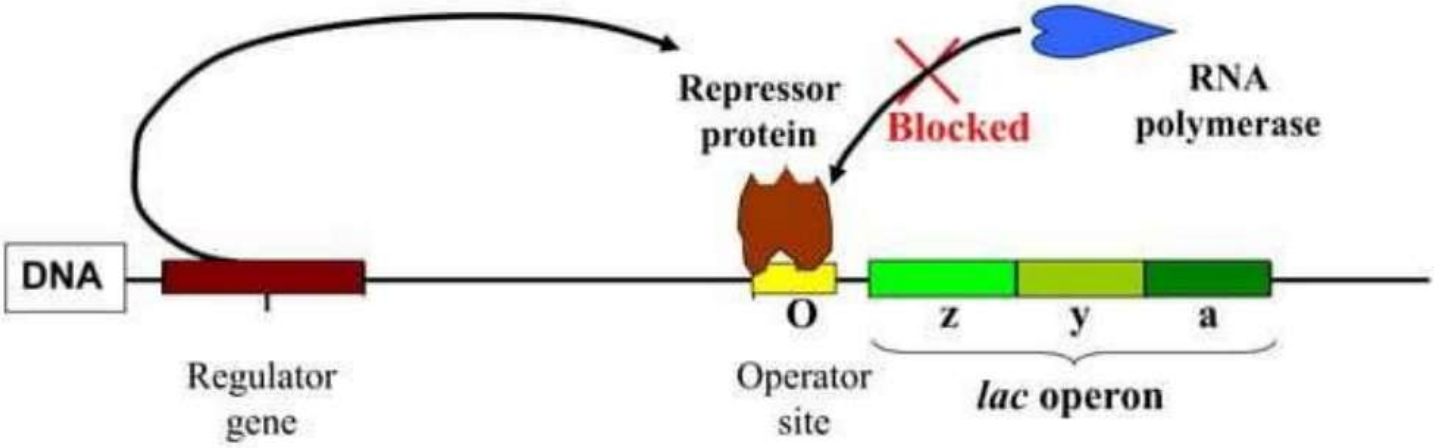
- **Catabolite Activator Protein (CAP site) which binds the activator CAP (cAMP)**
- **Operator which binds the *lac* repressor.**

Visual


Lactose (lac) operon
Regulator gene
Structural genes
DNA
I
P
O
Z
Y
A
Promoter
Operator

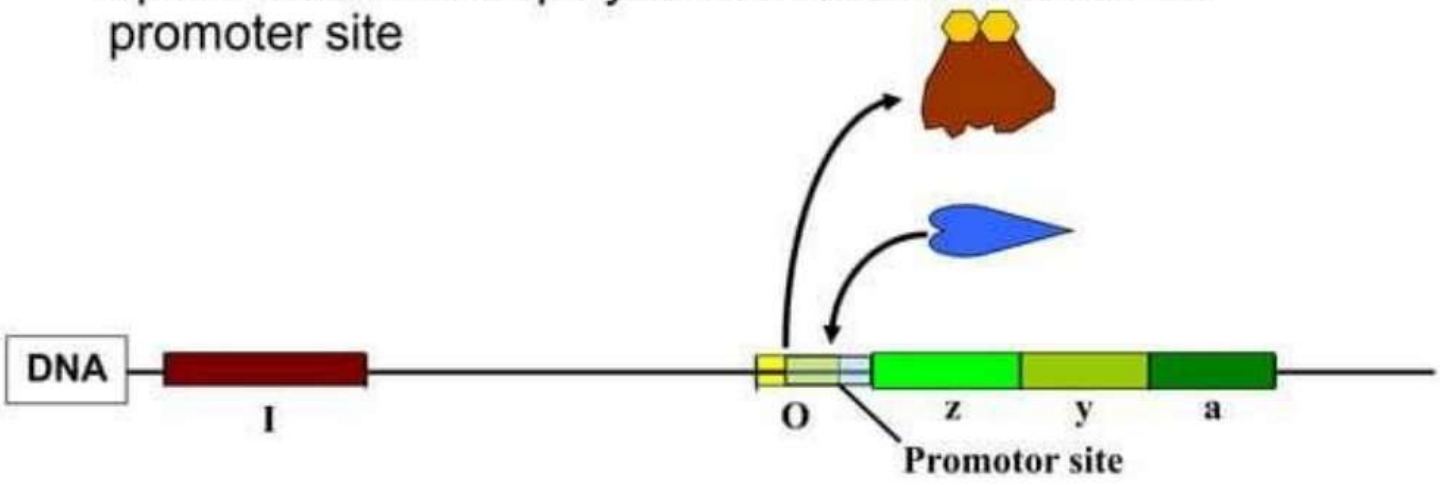
1. When lactose is absent

- A repressor protein is continuously synthesised. It sits on a sequence of DNA just in front of the *lac* operon, the **Operator site**
- The **repressor protein** blocks the **Promoter site** where the RNA polymerase settles before it starts transcribing



2. When lactose is present

- A small amount of a sugar allolactose is formed within the bacterial cell. This fits onto the repressor protein at another active site (**allosteric site**)
- This causes the repressor protein to change its shape (a **conformational change**). It can no longer sit on the operator site. RNA polymerase can now reach its promoter site

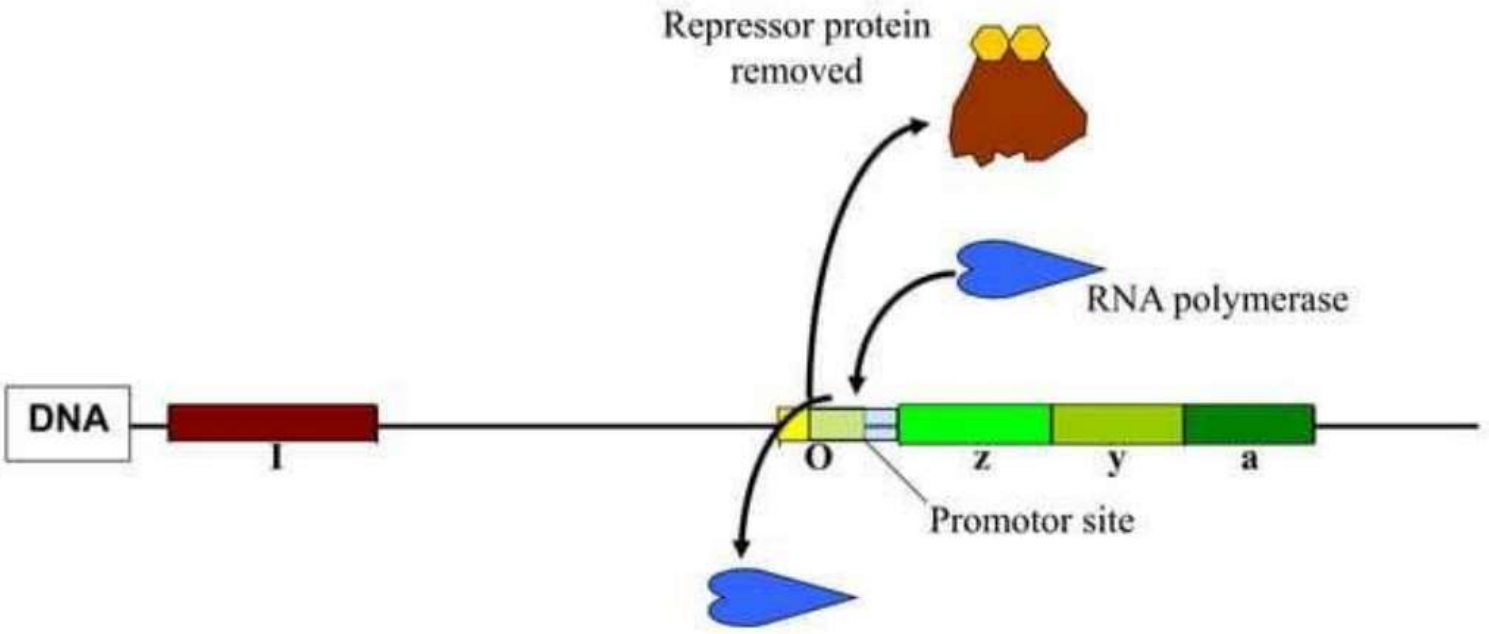


3. When both glucose and lactose are present

- This explains how the *lac* operon is transcribed only when lactose is present.

- BUT….. this does not explain why the operon is not transcribed when both glucose and lactose are present.

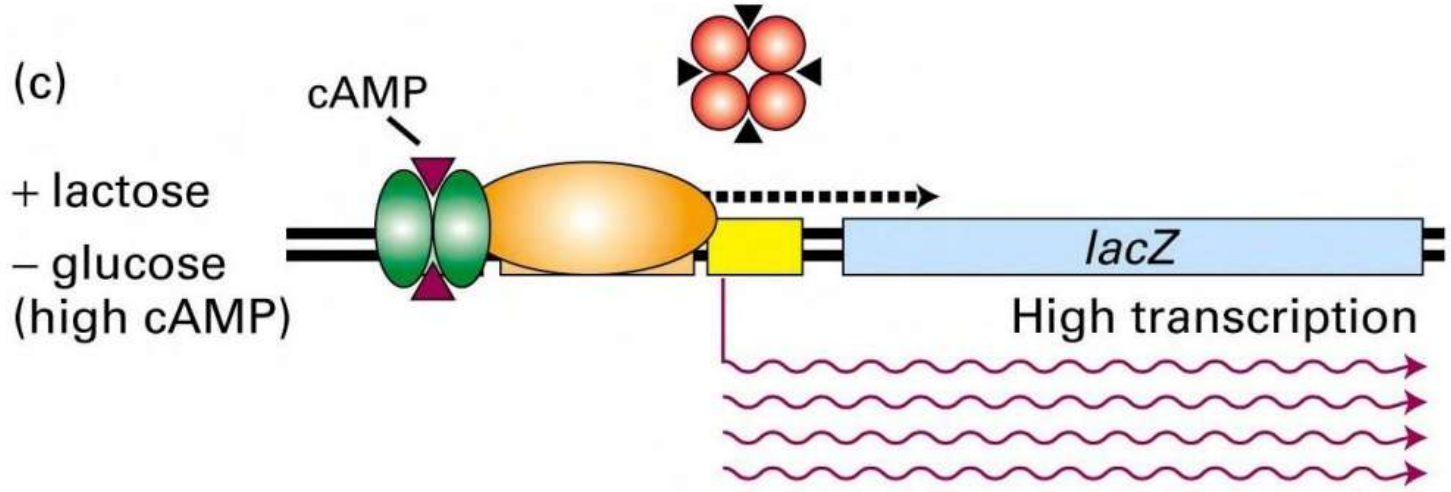
- When glucose and lactose are present RNA polymerase can sit on the promoter site but it is unstable and it keeps falling off



4. When lactose is present w/o glucose:

The *lac* repressor is bound by lactose and inactive, and the low glucose levels activate CAP, a transcriptional activator, which binds the CAP site & enhances binding of RNA polymerase to the promoter.

- **since lactose is a much more important source of energy in the absence of glucose, the *lac* operon is ON "high"**



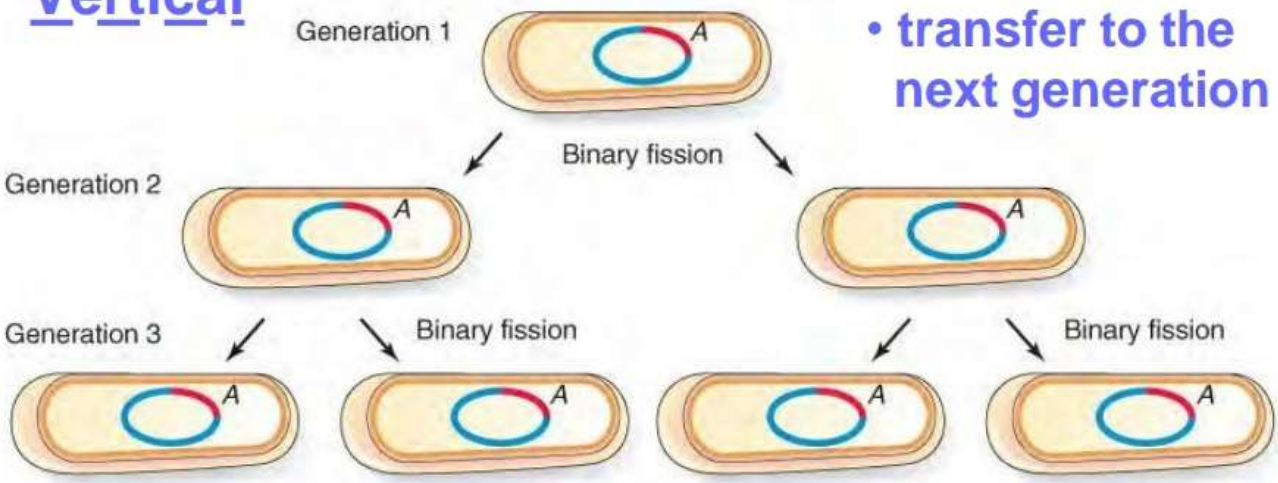
Summary

Carbohydrates	Activator protein	Repressor protein	RNA polymerase	*lac* Operon
+ GLUCOSE + LACTOSE	Not bound to DNA	**Lifted off operator site**	Keeps falling off promoter site	**Basal level transcription**
+ GLUCOSE - LACTOSE	Not bound to DNA	Bound to operator site	Blocked by the repressor	**No transcription**
- GLUCOSE - LACTOSE	**Bound to DNA**	Bound to operator site	Blocked by the repressor	**No transcription**
- GLUCOSE + LACTOSE	**Bound to DNA**	**Lifted off operator site**	**Sits on the promoter site**	**Activated level Transcription**

D. Mechanisms of Gene Transfer

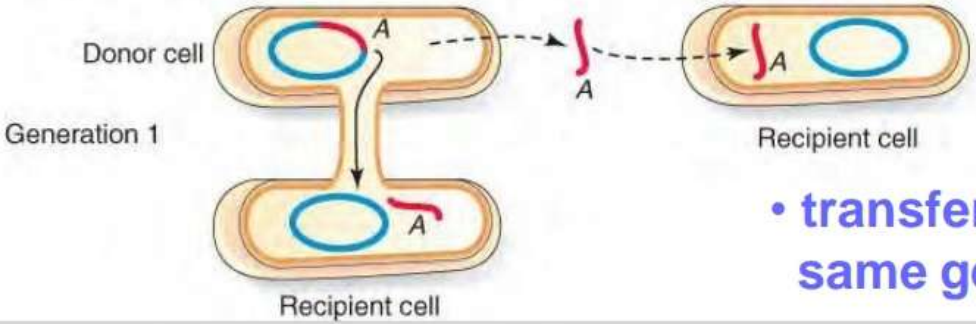
Horizontal vs Vertical Gene Transfer

Vertical



- transfer to the next generation

Horizontal (or lateral)



- transfer within the same generation

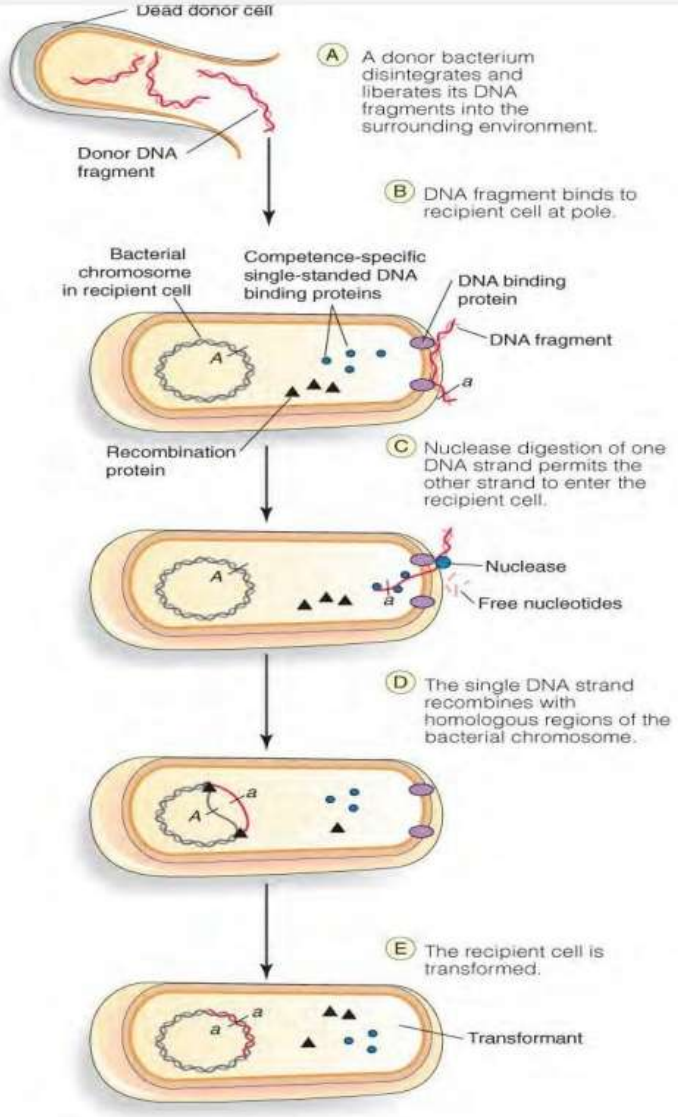
Methods of Gene Transfer

Bacteria can acquire DNA (i.e., new genes) in 3 basic ways:

1) **Transformation**
   - uptake and retention of external DNA molecules

2) **Conjugation**
   - direct transfer of DNA from one bacterium to another

3) **Transduction**
   - the transfer of DNA between bacteria by a virus



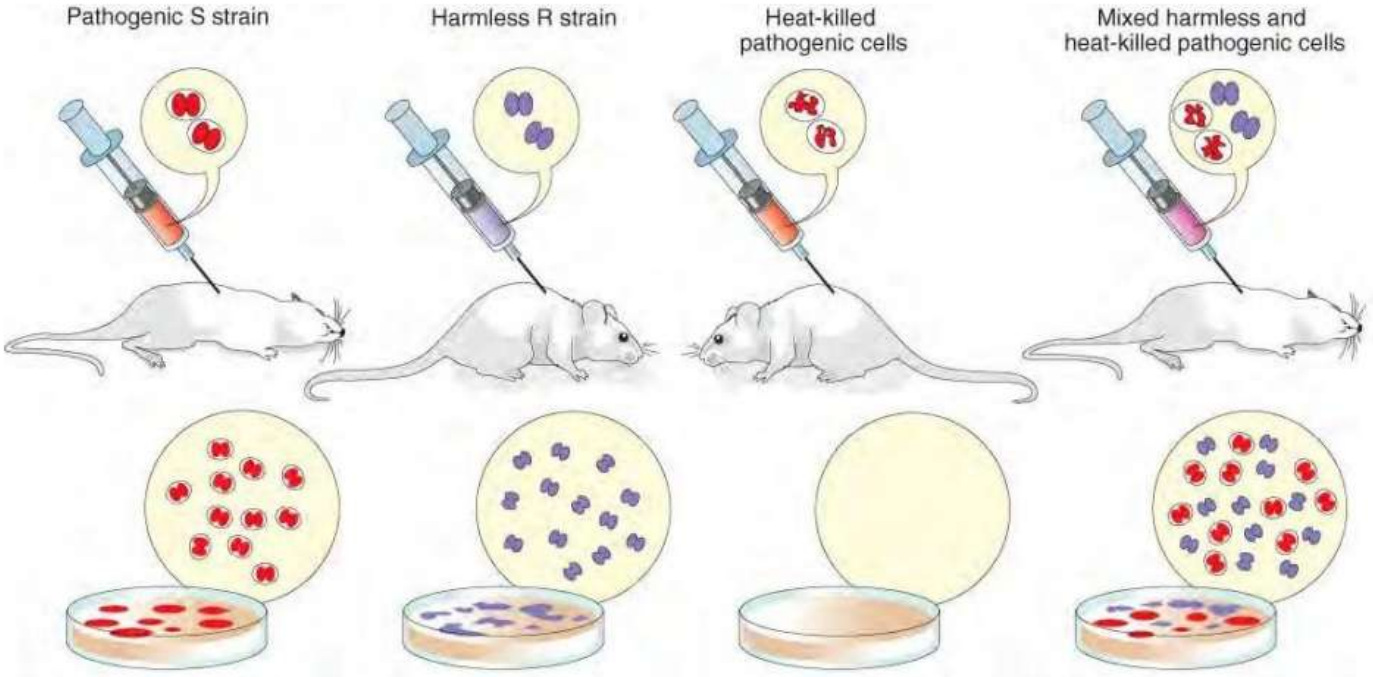
Transformation

Under the right conditions, bacteria can "take in" external DNA fragments (or plasmids) by <u>transformation</u>.

- **DNA binding proteins transfer external DNA across cell envelope**
- **homologous recombination can then occur**
- **bacterial cells capable of transformation are referred to as <u>competent</u>**

Griffith's Transformation Experiment

Streptococcus pneumonia



(A) When Griffith injected S strain (encapsulated, pathogenic) cells into the mouse, it developed pneumonia and died.

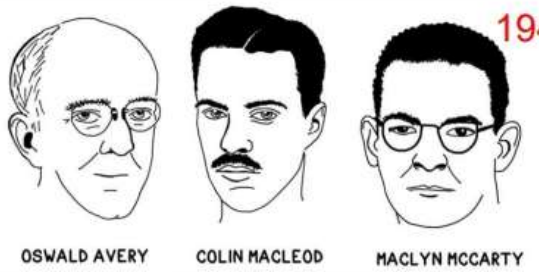
1928!

(B) An injection of R strain (unencapsulated,harmless) cells did no harm to the mouse.

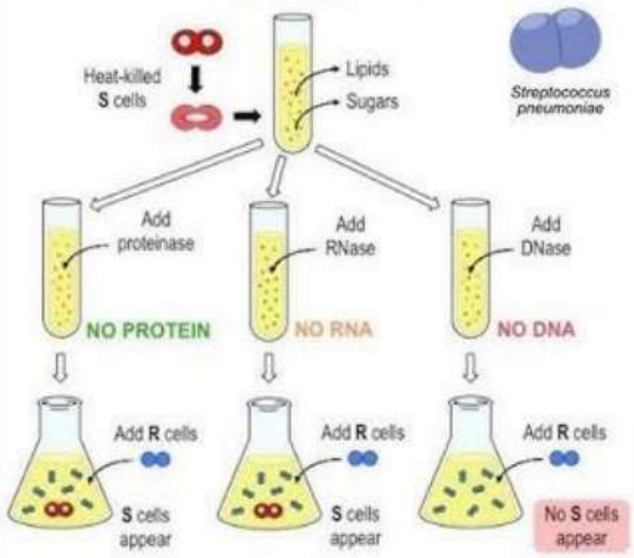
(C) Furthermore, an injection of heat-killed S strain cells did no harm because the cells were dead.

(D) But when Griffith injected a mixture of live R strain and heat-killed S strain cells into the mouse, it died. When Griffith cultivated the organism from the blood, he found live S strain cells.

1944



Avery Macleod and Mccarty Experiment (Transformation)



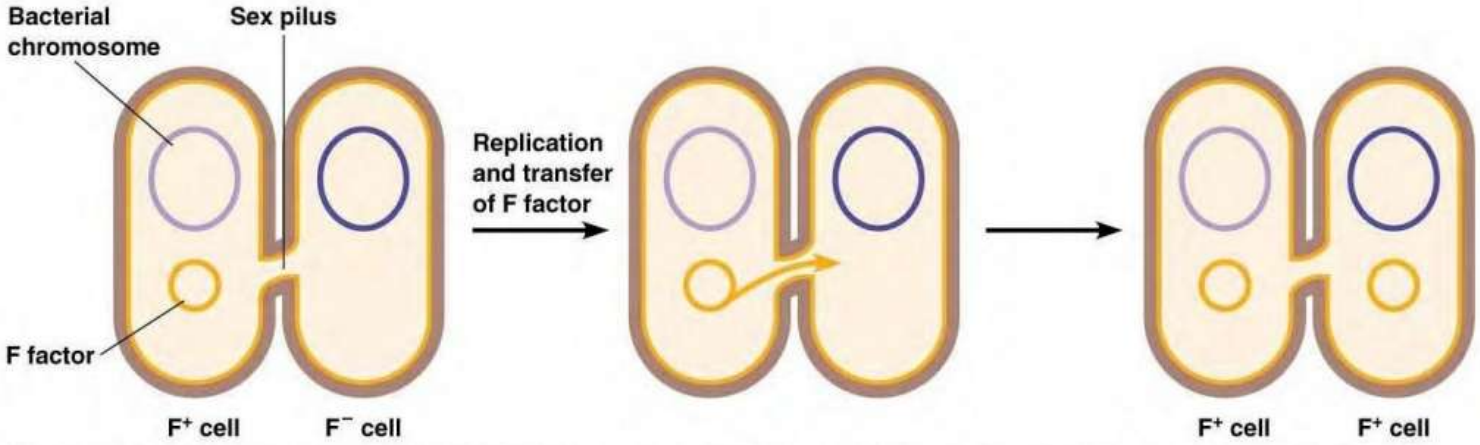
Remove lipids and sugars from a solution of heat-killed **S** cells. Proteins, RNA and DNA remain

Treat solutions with enzymes to destroy protein, RNA or DNA

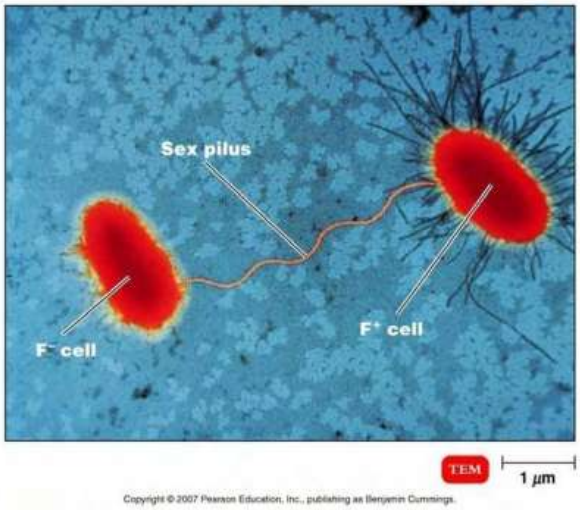
Add to culture containing living **R** cells. Observe for transformation by testing for the presence of virulent **S** cells

Result: Hereditary materials (DNA) was the transforming factor

Bacterial 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.



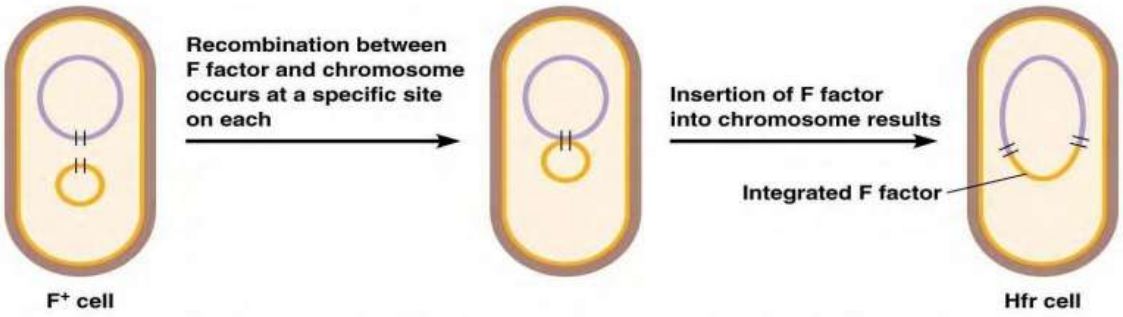
Requires an F factor plasmid

- **has all "conjugation genes"**
- **directs formation of a sex pilus**
- **single DNA strand produced by DNA replication is transferred to F- cell through the sex pilus, recipient produces 2nd**

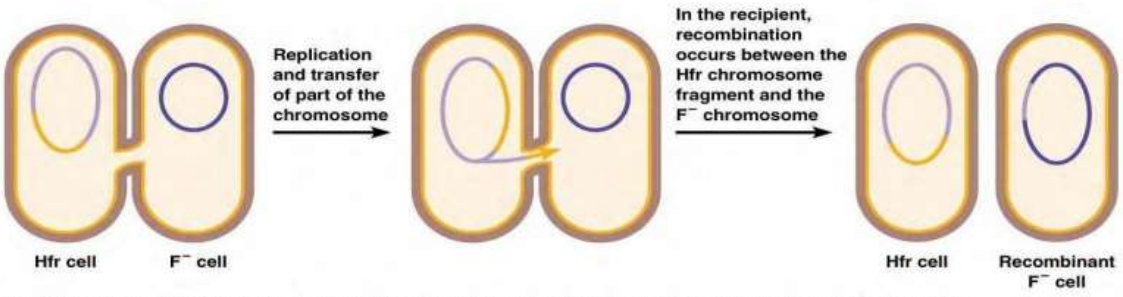
https://www.youtube.com/watch?v=hm8SZaFmlWg

Hfr Conjugation

If F factor plasmid is inserted into host chromosome (Hfr cell), this will result in the transfer of the entire DNA complex.



(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.



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

Hfr = "High frequency of recombination

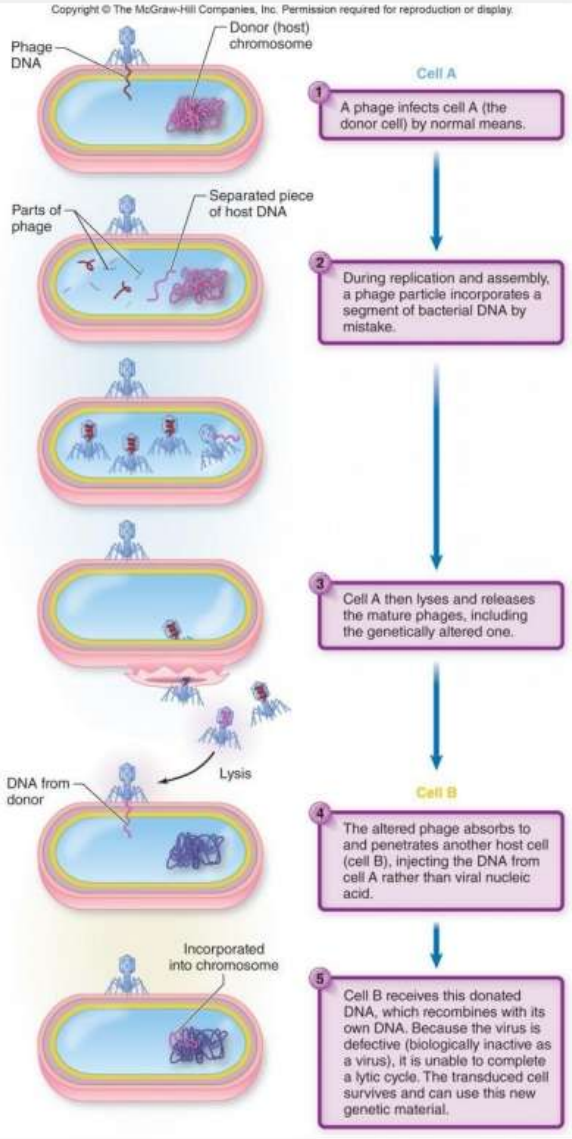
https://www.youtube.com/watch?v=3rFpkmdOM_Y

Transduction

Foreign genetic material introduced via Virus called Bacteriophage

- **Bacteriophage (phage):** A virus that infects bacteria

- **Types of transduction**
  - Generalized
  - Specialized



1. Generalized transduction:

- The bacteriophage picks up any part of bacterial DNA during the lytic cycle and transfers it into a new bacteria after infecting it.
- Virtually any genetic marker can be transferred from donor to recipient.
- It occurs at a low frequency.

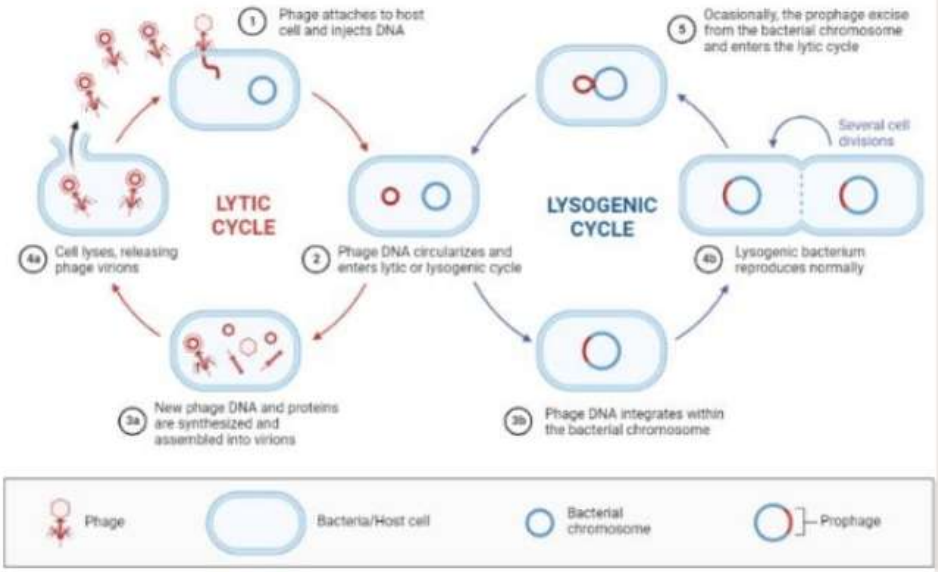
Mechanism of Generalized Transduction

1. Attachment/adsorption of bacteriophage to the bacteria
2. Penetration of phage DNA
3. Replication of phage DNA/RNA
4. Synthesis of nucleic acid and proteins
5. **Assembly of phage protein and nucleic acid**
6. Release of mature bacteriophage

2. Specialized transduction

Examples of Transduction in Bacterial Population

1.Escherichia coli
2.Pseudomonas spp
3.Salmonella spp
4.Staphylococcus spp



- Process where the phage carries only a specific part of the host's (bacteria) DNA as a part of the viral genome.
- It only occurs during the lysogenic cycle of bacteriophage.
- But specialized transduction is a highly efficient gene transfer mechanism.

E. Mutations

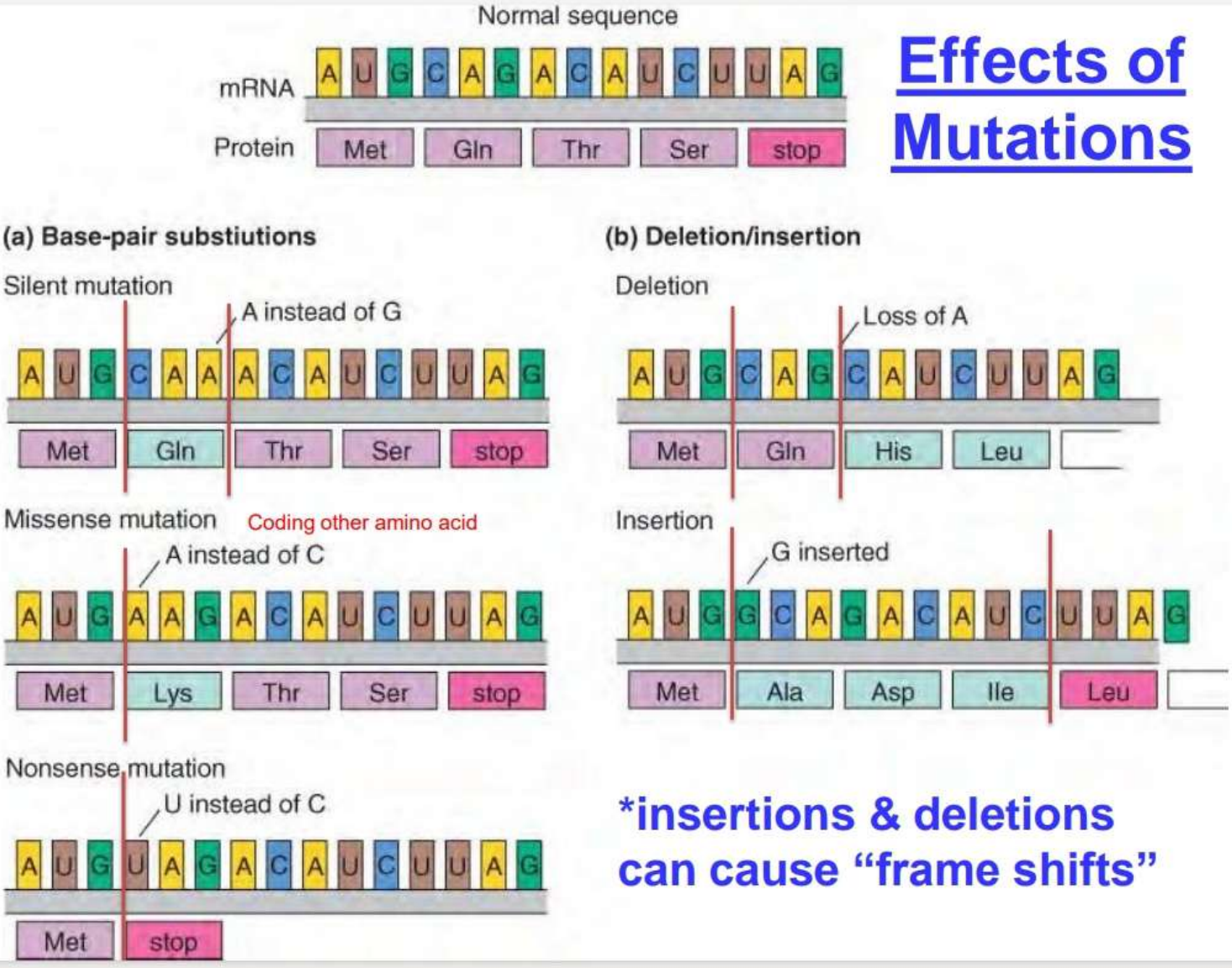
A mutation is *any* change in DNA sequence:

- change of one nucleotide to another
- insertion or deletion of nucleotides or DNA fragments
- inversion or recombination of DNA fragments

What causes mutations?

- errors in DNA replication, DNA repair
- chemical mutagenesis
- high energy electromagnetic radiation
    - UV light, X-rays, gamma rays

Effects of Mutations



***insertions & deletions can cause "frame shifts"**