Chapter 8:
Microbial Genetics

1. Gene Expression

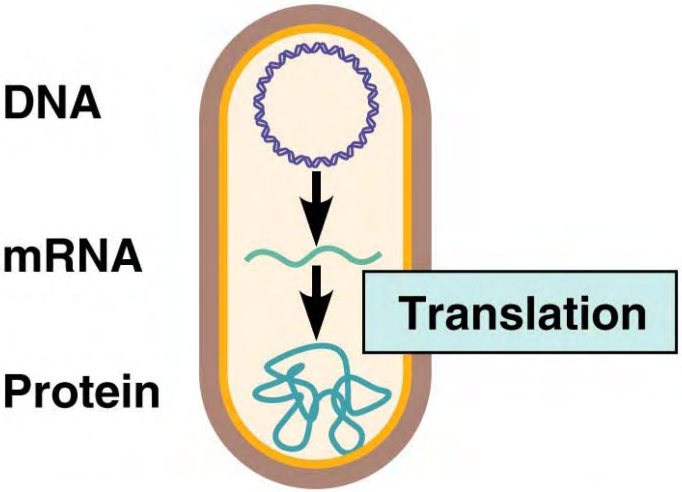
2. Gene Regulation

3. DNA Replication & Mutation

4. Mechanisms of Gene Transfer

1. <u>Gene Expression</u>

Gene Expression



The expression of a gene into a protein occurs by:

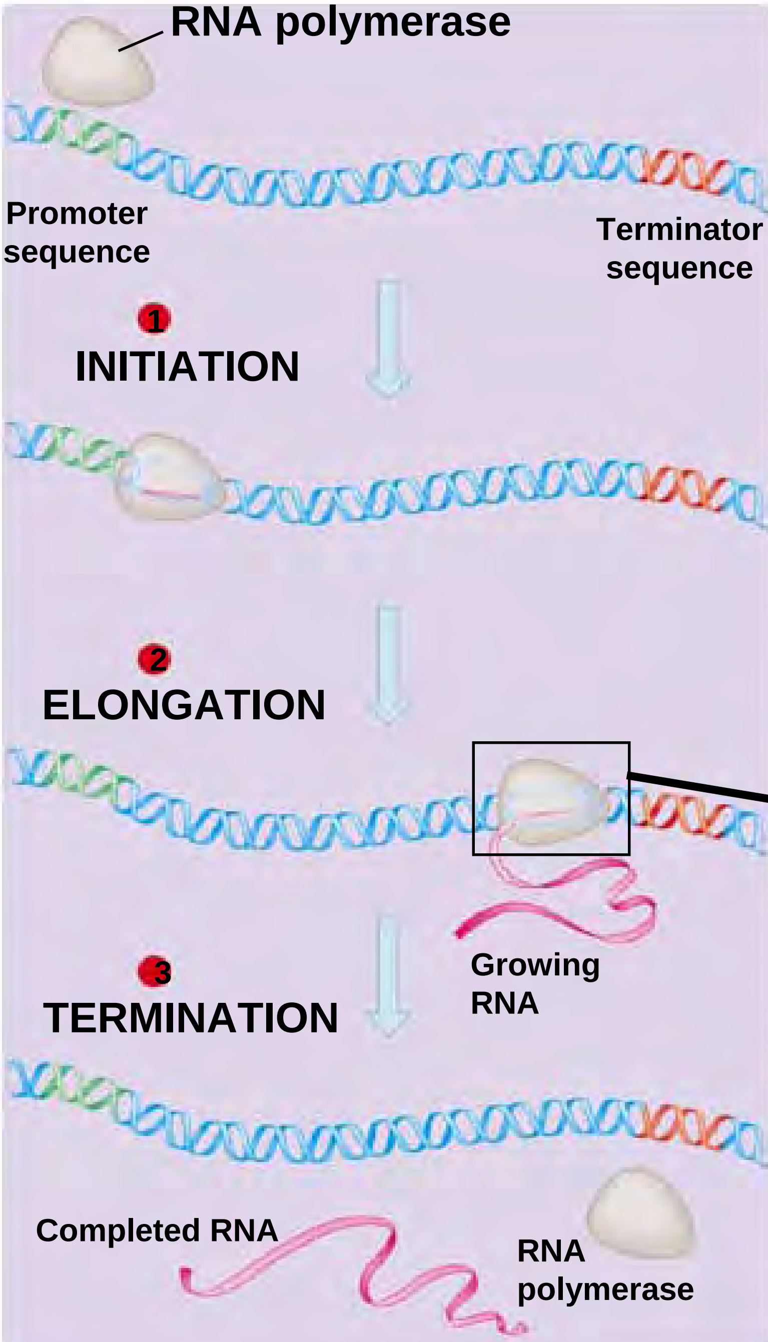
1) <u>Transcription</u> of a gene into RNA

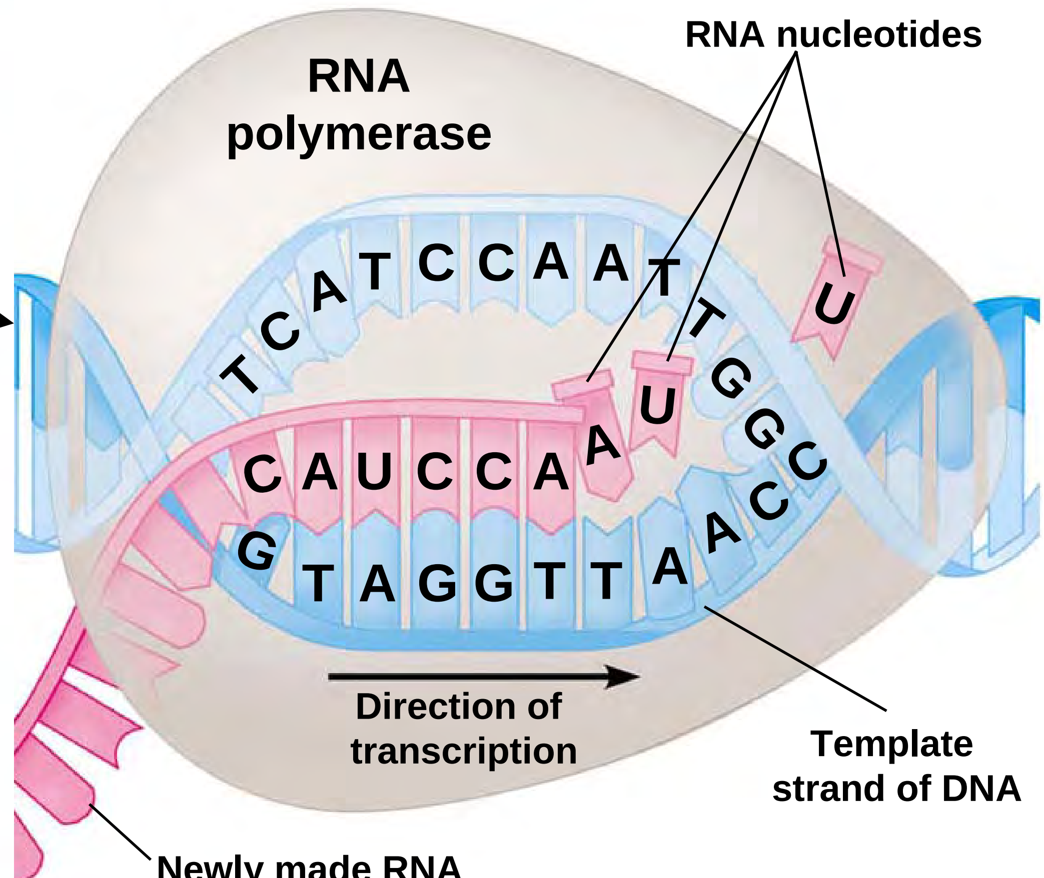
- **produces an RNA copy of the coding region of a gene**
- **the RNA transcript may be the actual gene product (rRNA, tRNA) or be translated into a polypeptide gene product (mRNA)**

2) <u>Translation</u> of mRNA transcript into polypeptide

- **accomplished by <u>ribosomes</u> with the help of tRNA**

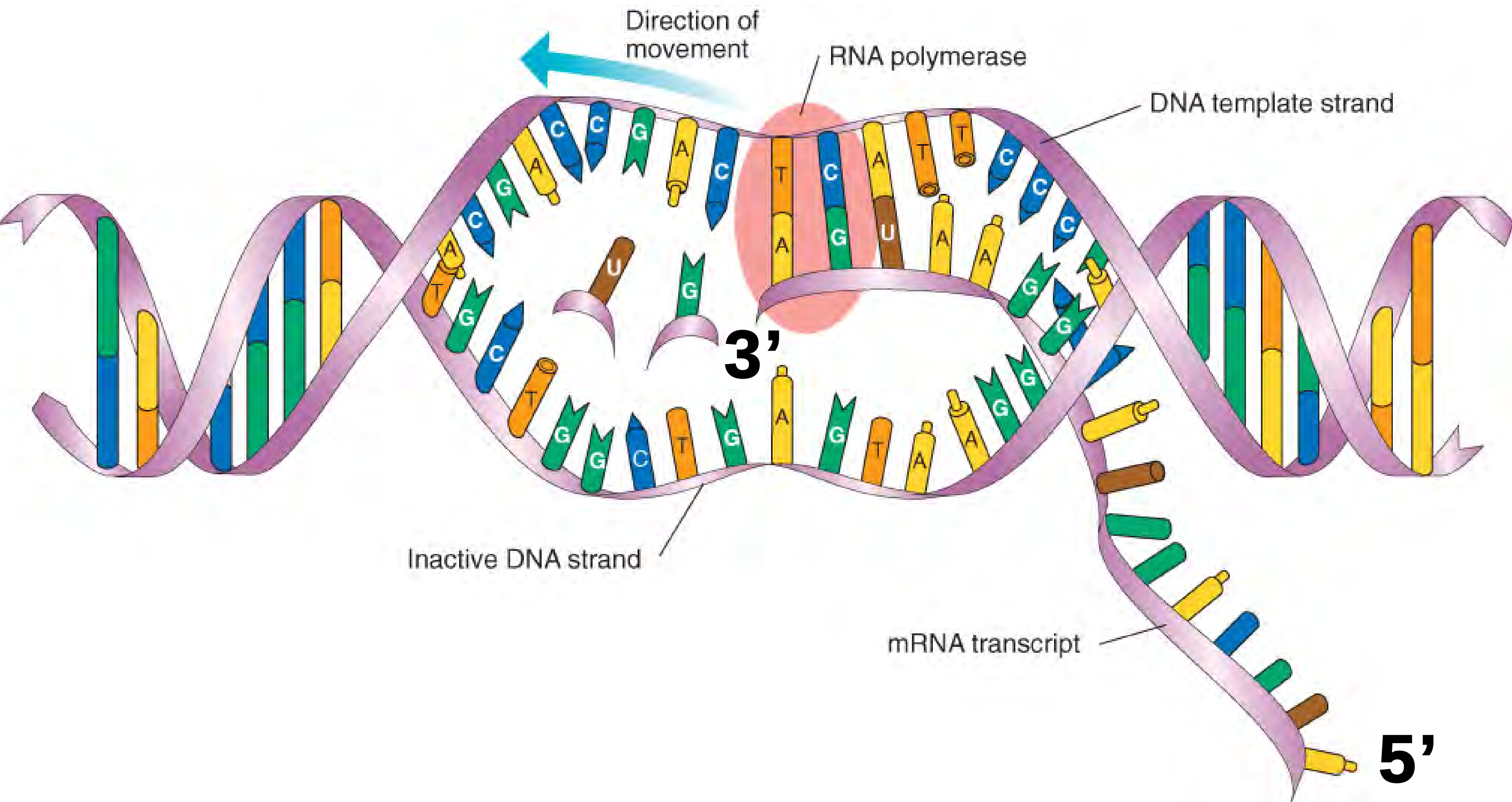
Overview of Transcription





Transcription is Uni-directional

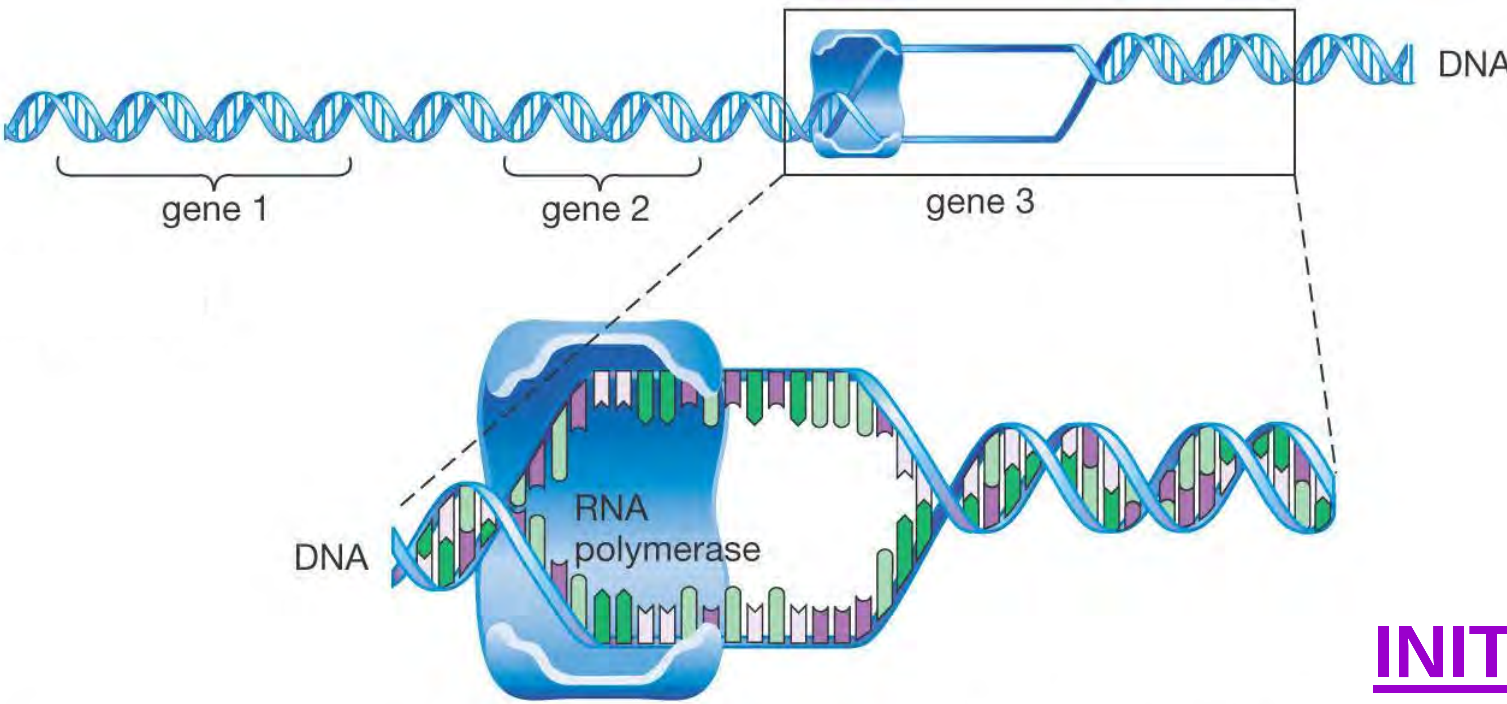
- ribo-nucleotides can only be added to the 3' end of an transcript, thus elongation is in a <u>5' → 3'</u> direction



3 Steps of Transcription

1) Initiation

- <u>RNA polymerase</u> binds to the <u>promoter</u> of a gene

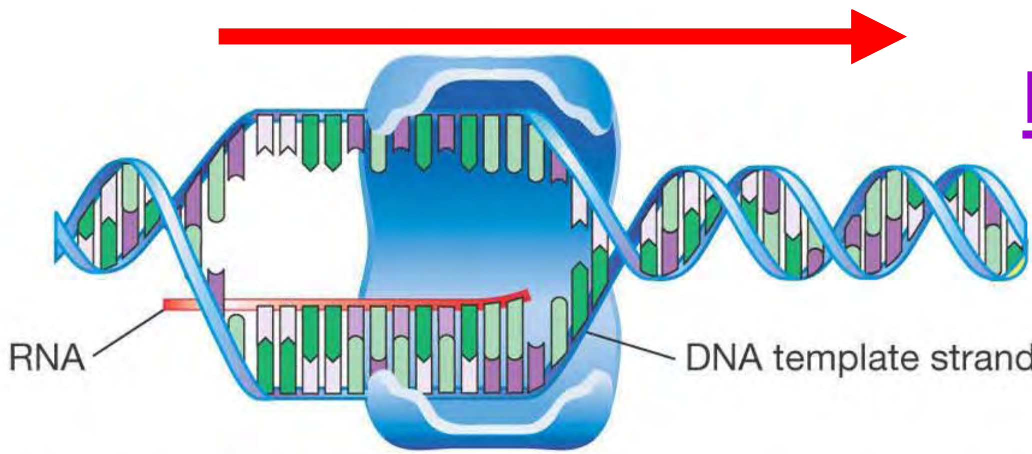


<u>INITIATION</u>

- 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**

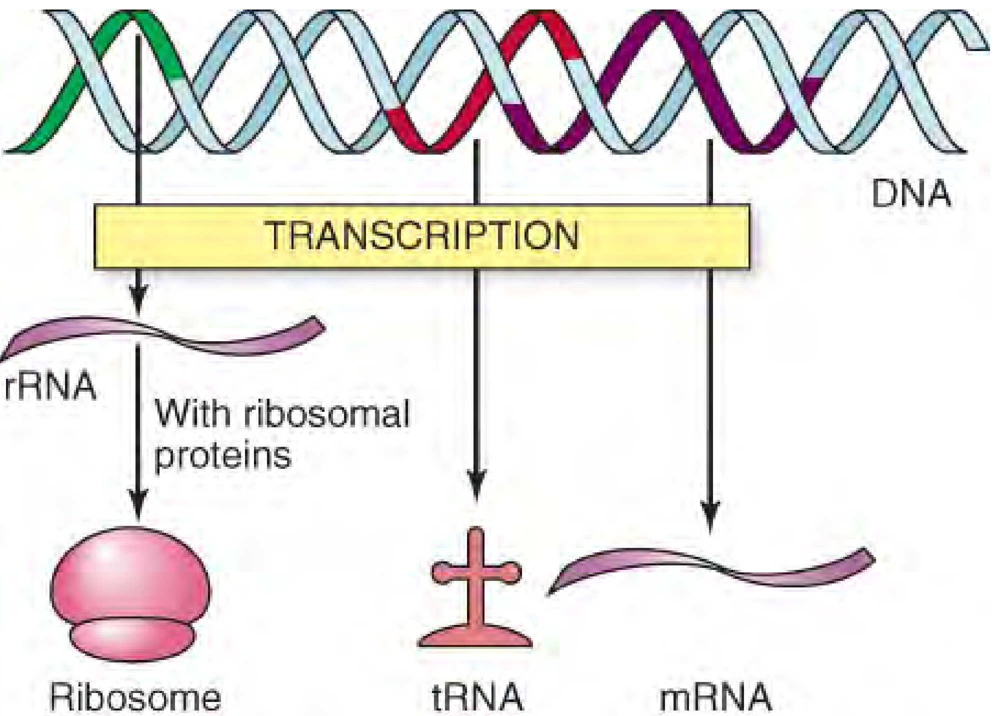
Various Roles of RNA Transcripts

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

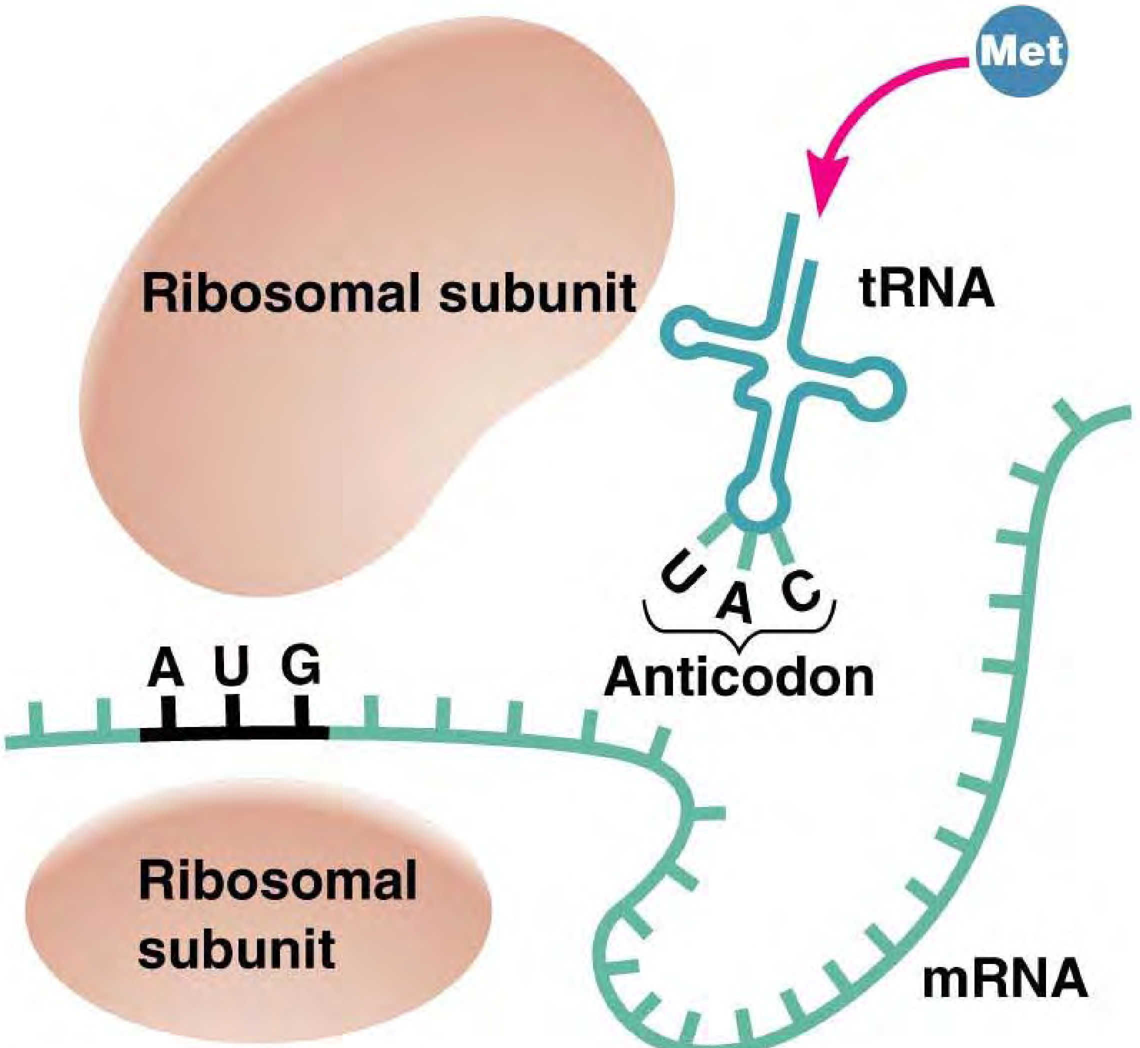
The building of a polypeptide, 1 amino acid at a time, by ribosomes using info in mRNA:

- **ribosomes bind directly to mRNA, “read” codon by codon**
  - **ribosomes always start at AUG (methionine)**

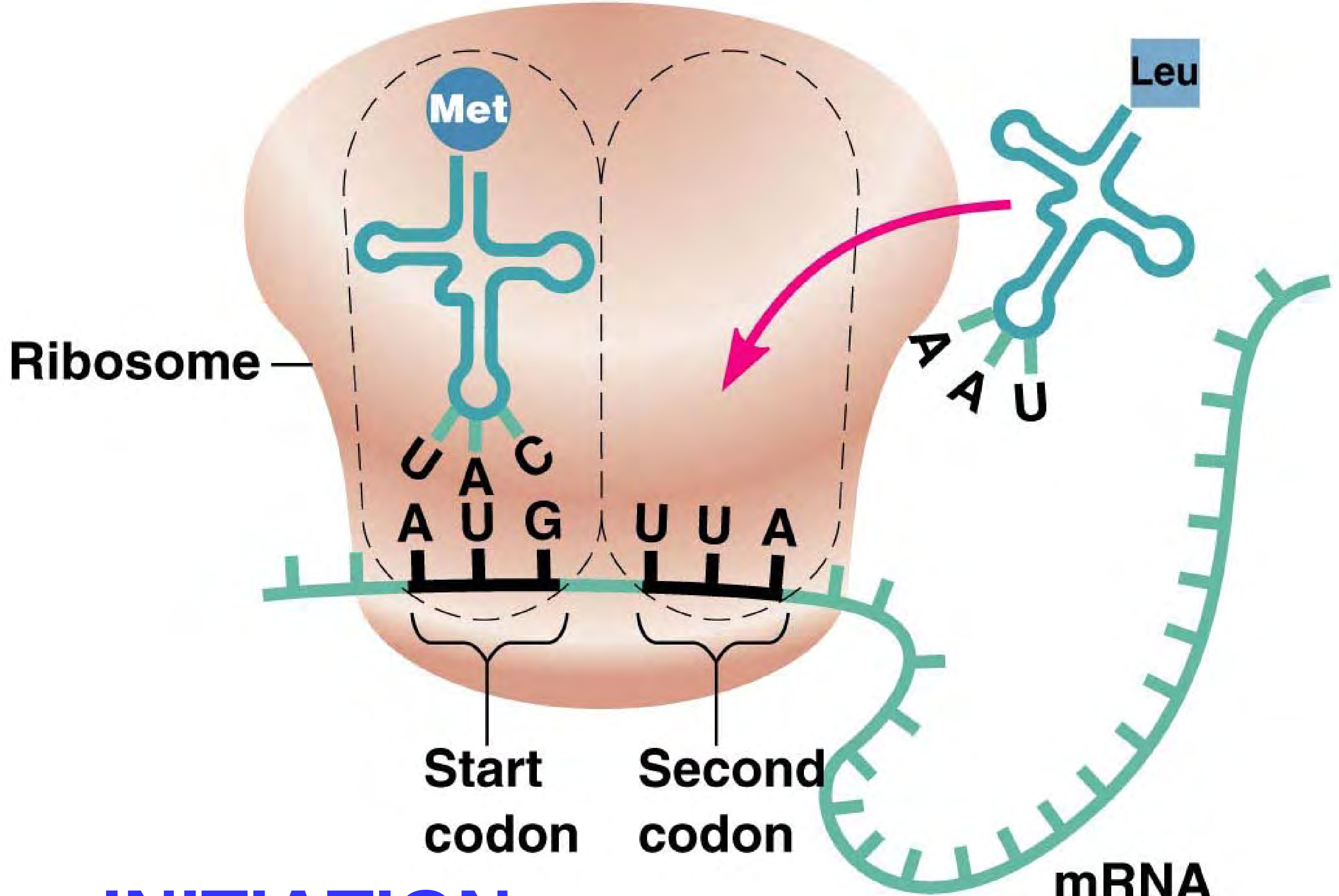
- **translation also involves tRNA, each of which is attached to 1 of the 20 amino acids (AAs)**
  - **ribosomes match the right tRNA (via anticodon) with the right codon in the mRNA, then add its AA to the growing protein**

Translation: step by step…

INITIATION…

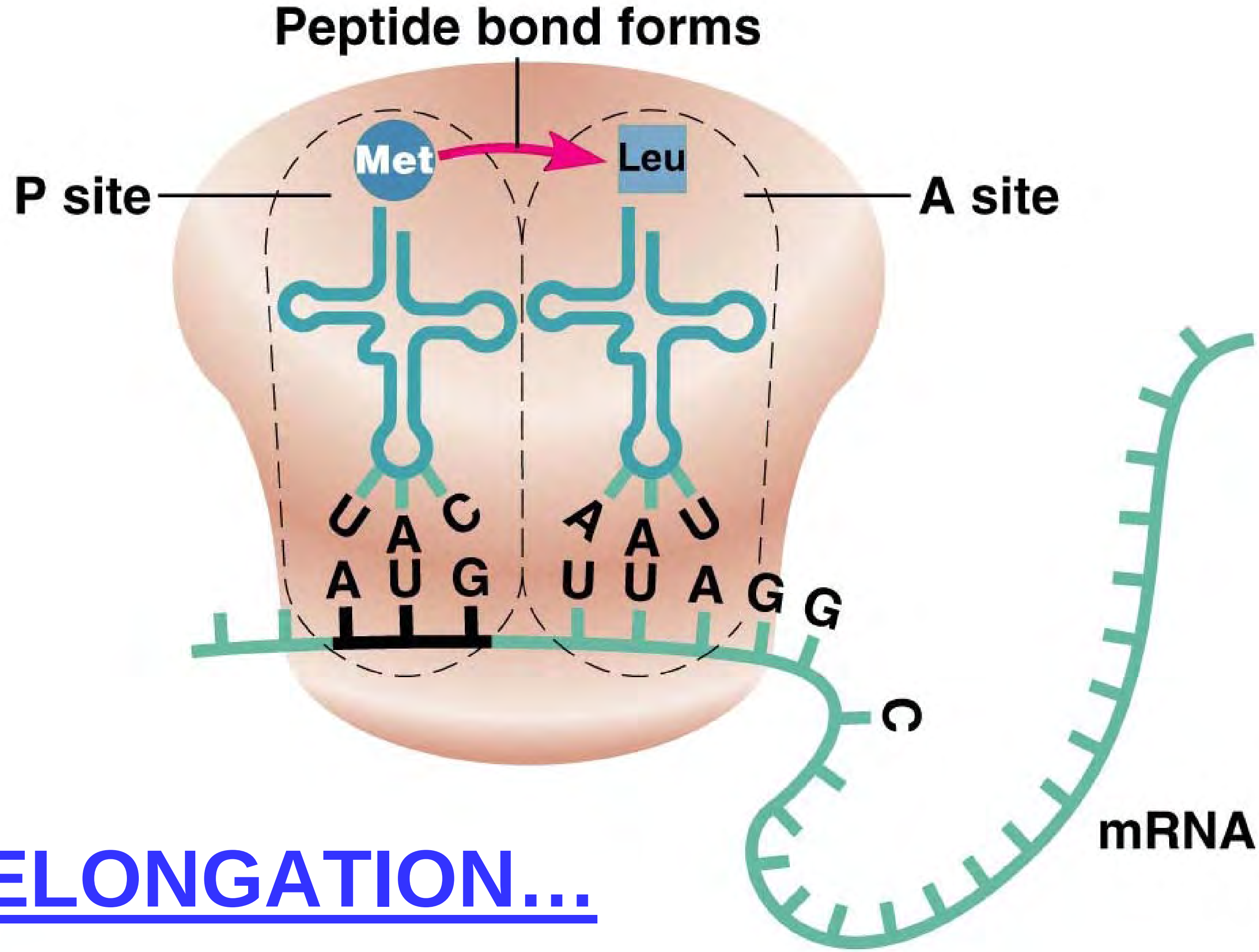


1 Components needed to begin translation come together.



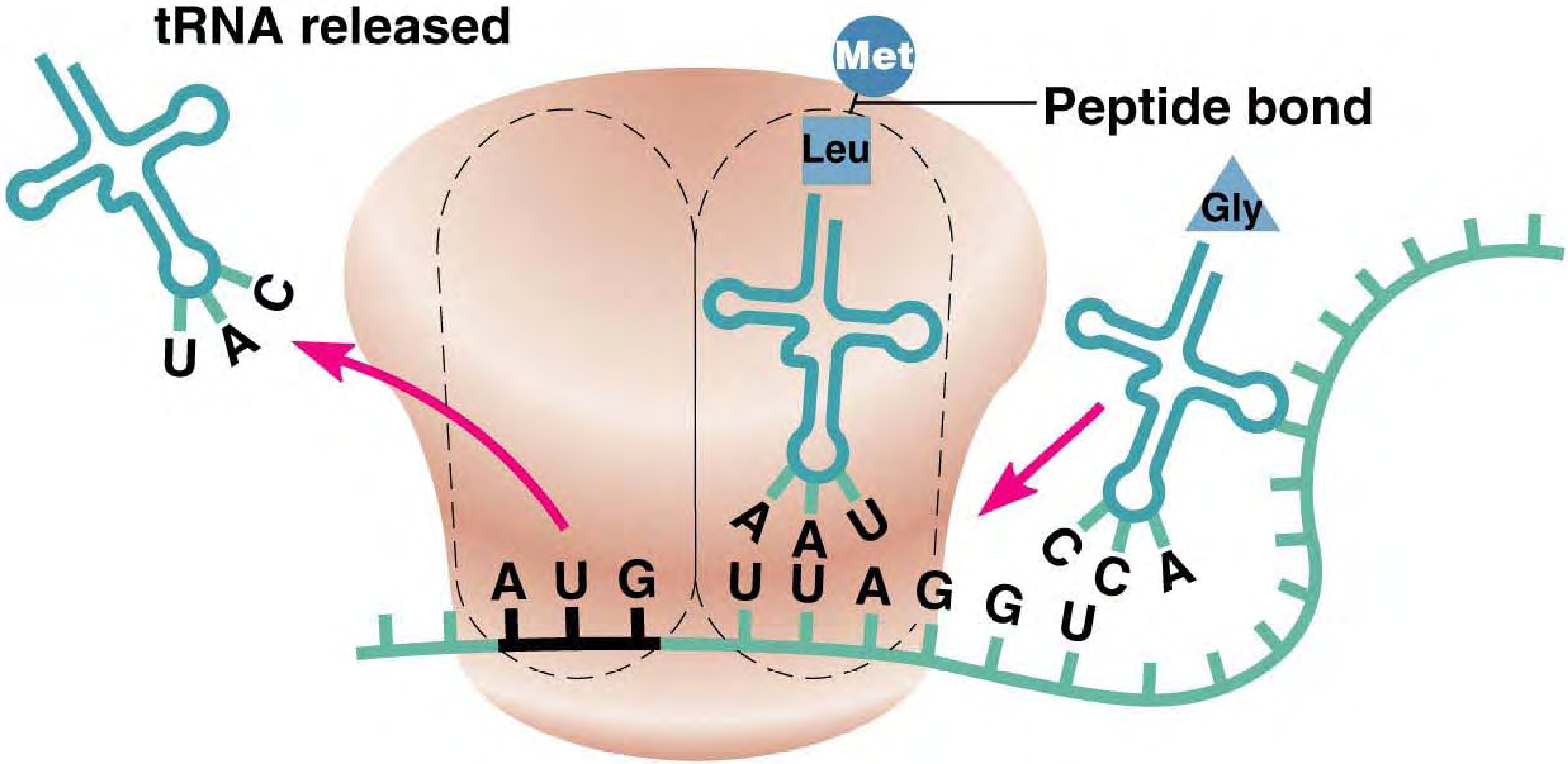
...INITIATION

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.



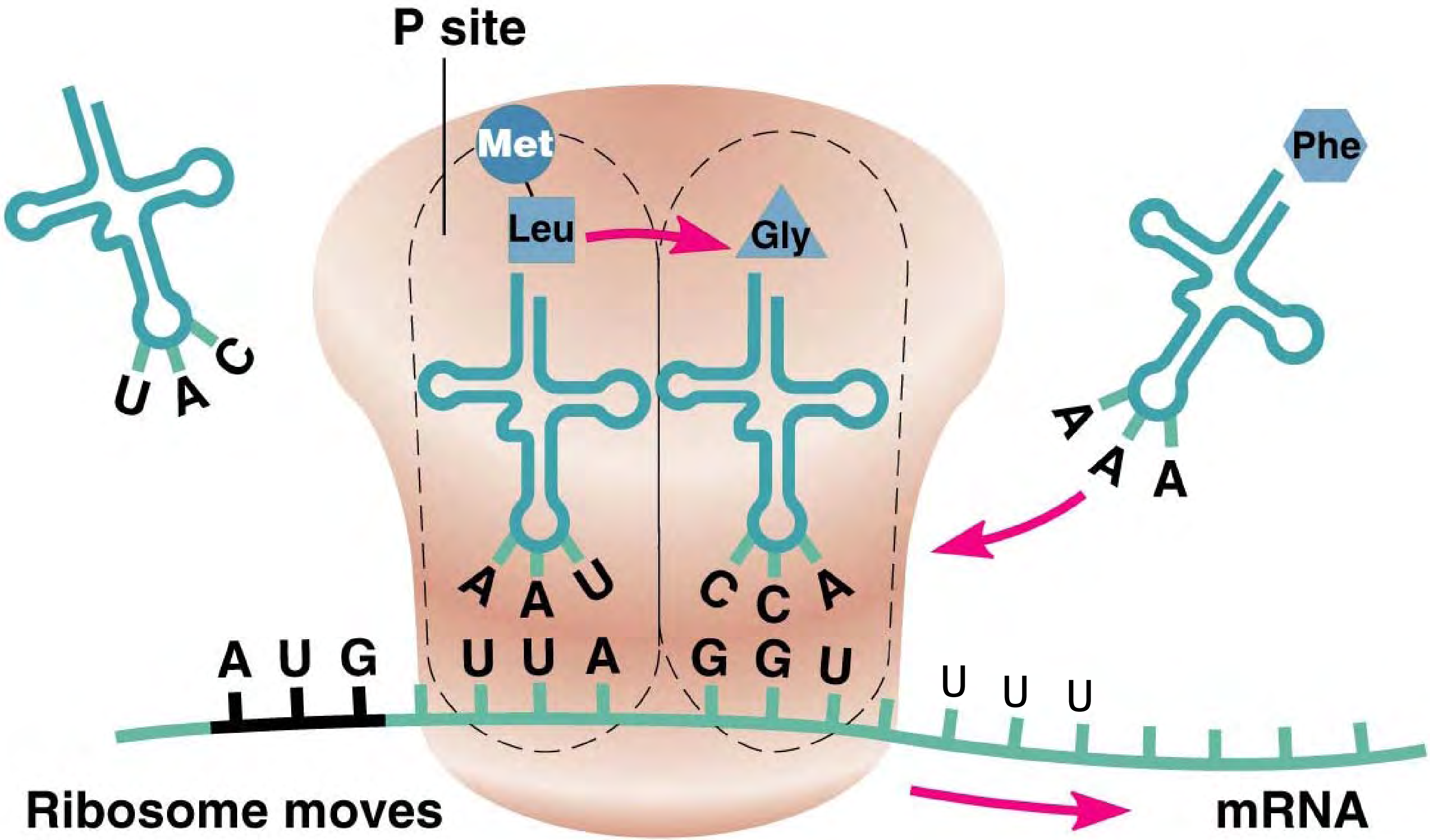
ELONGATION…

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.



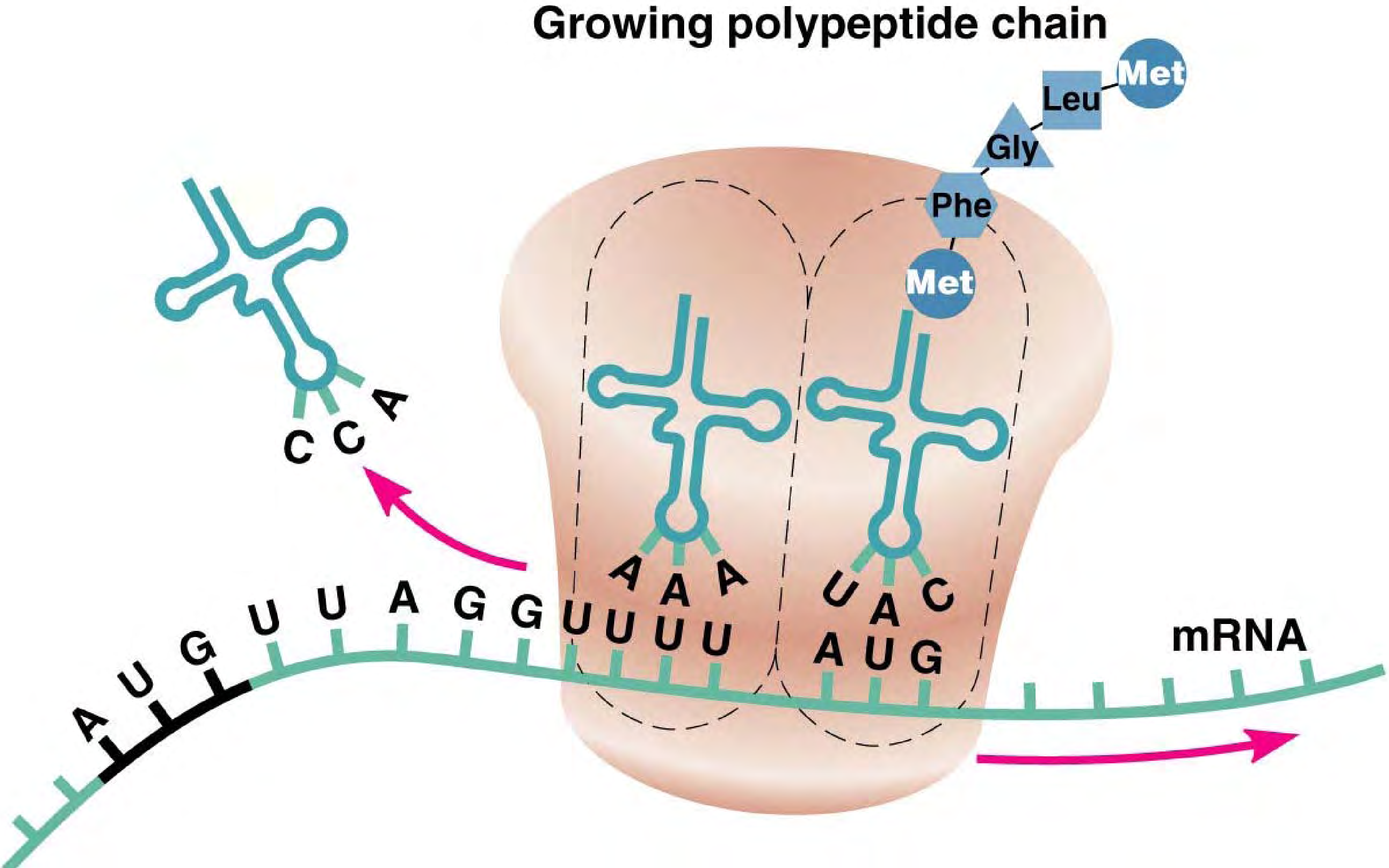
...ELONGATION...

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



…ELONGATION…

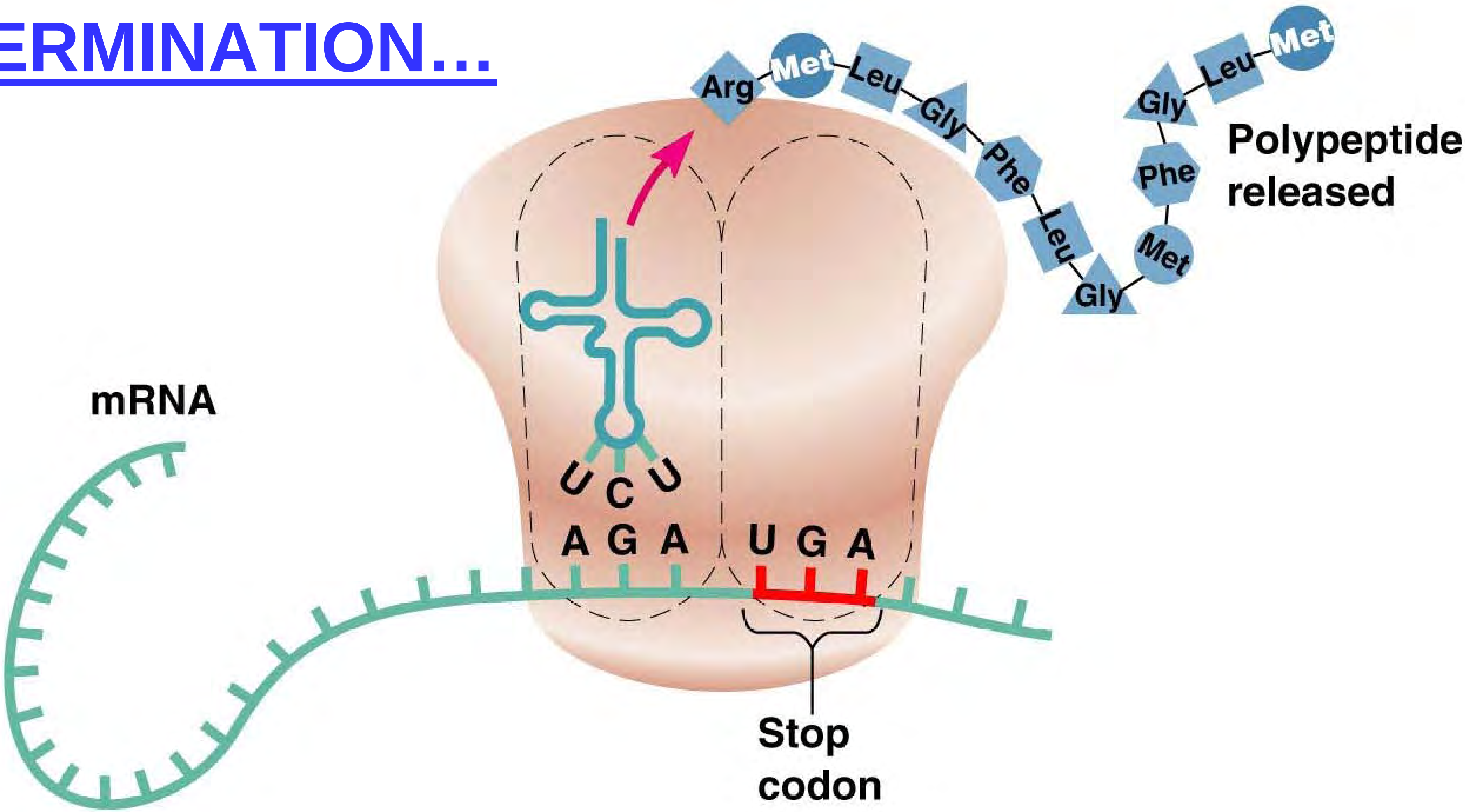
5 The ribosome moves along the mRNA until the second tRNA is in the P site, and the process continues.



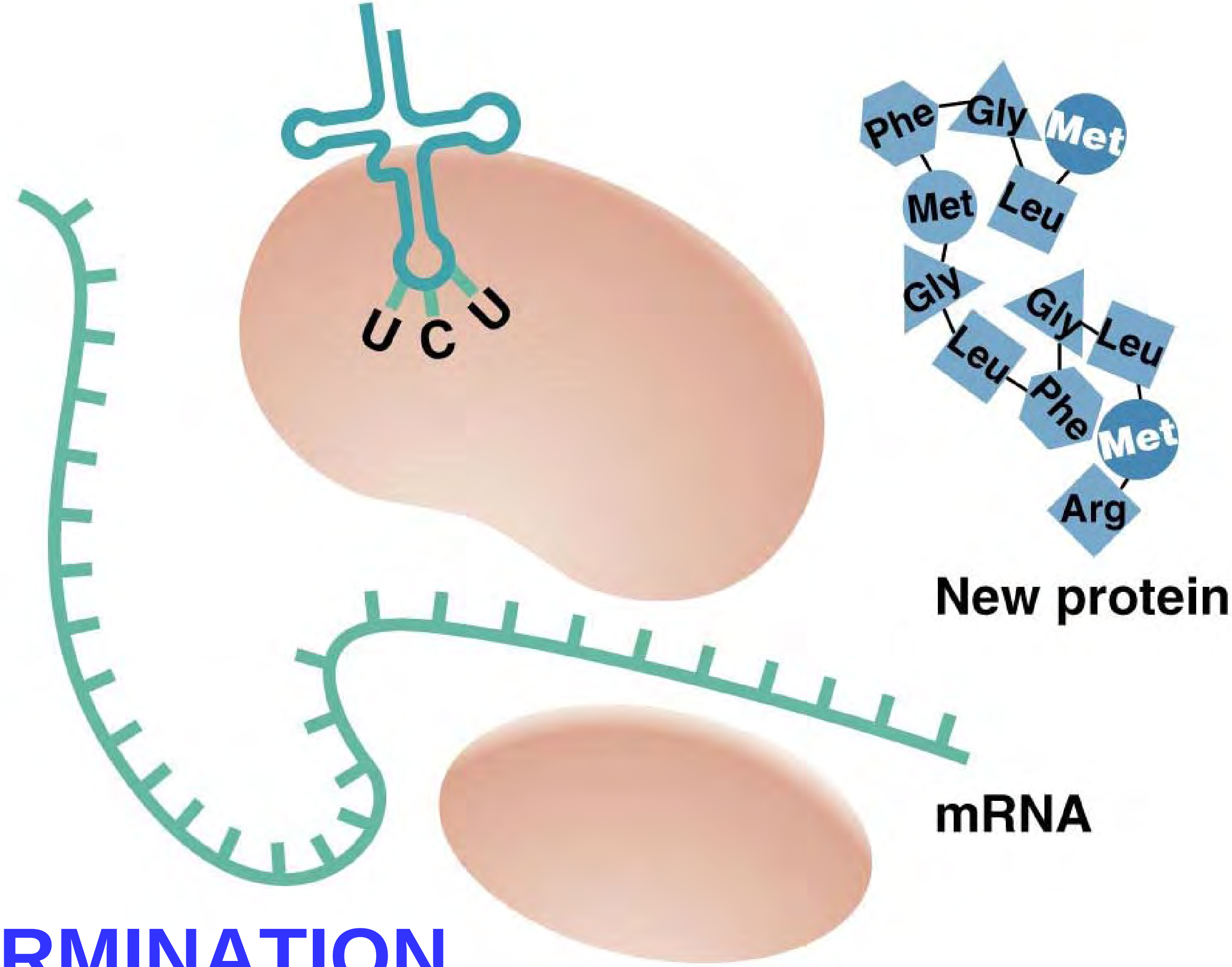
...ELONGATION

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.**

Summary of Translation

INITIATION

- ribosome assembles at specific AUG of mRNA
- ribosome binds 2 tRNA-AAs, 2 codons at a time
  - matching complementary anti-codons with mRNA codons

ELONGATION

- ribosome catalyzes peptide bond formation between amino acids attached to each tRNA
- ribosome shifts 3 nucleotides (1 codon) on mRNA and repeats the process

TERMINATION

- “stop” codon causes translation to end

Table of the Genetic Code

Second position

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

If the DNA sequence is:
CATGCCTGGGCAATAG

(transcription)

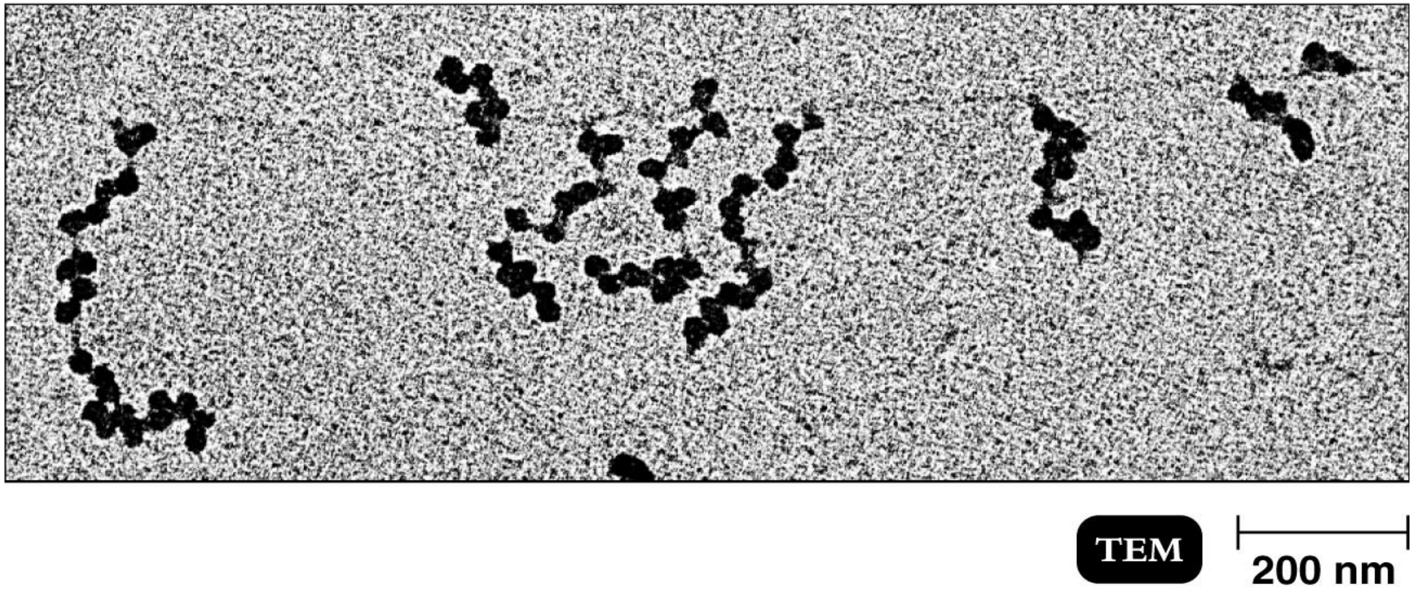
The mRNA copy is:
CAUGCCUGGGCAAUAG

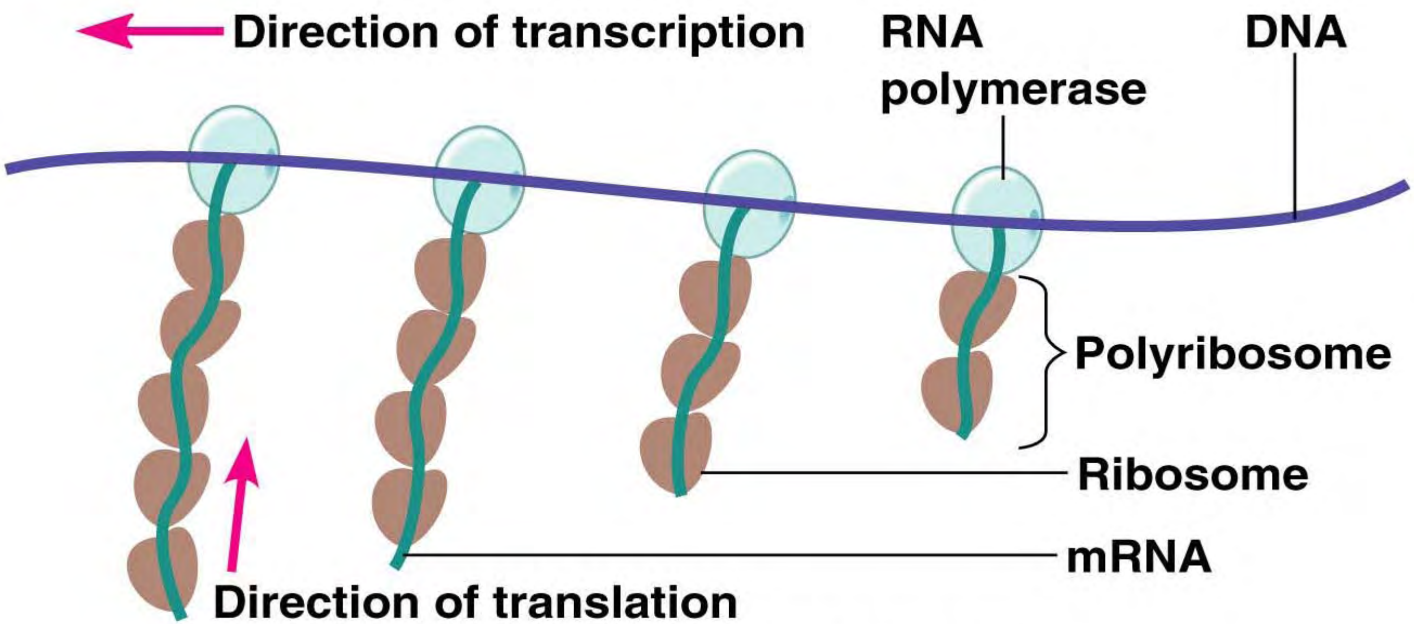
(translation)

The polypeptide is:
***Met-Pro-Gly-Gln-(stop)**

***all proteins begin w/Met**

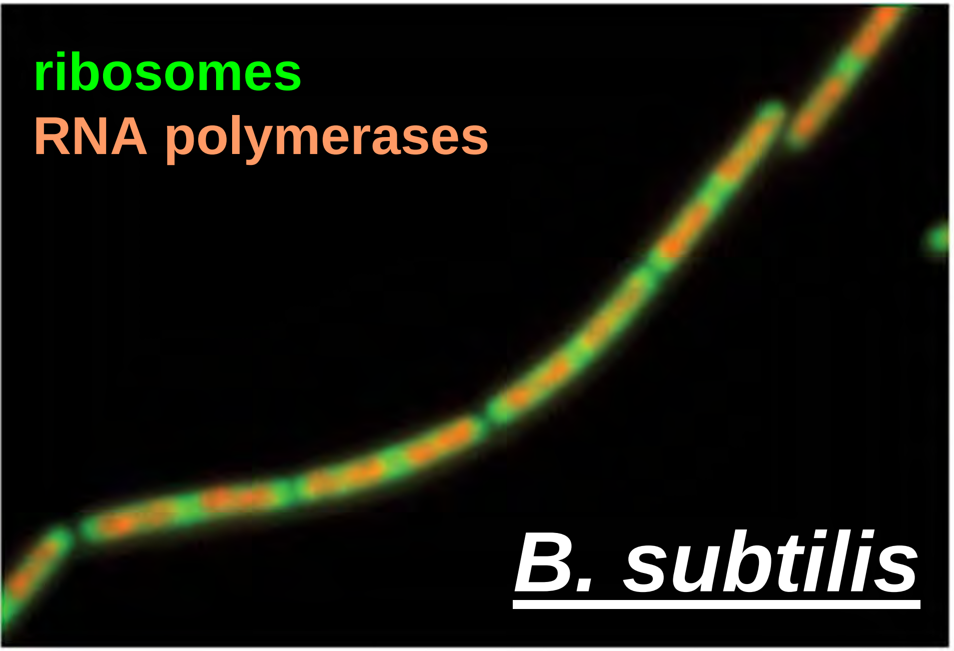
Gene Expression in Prokaryotes

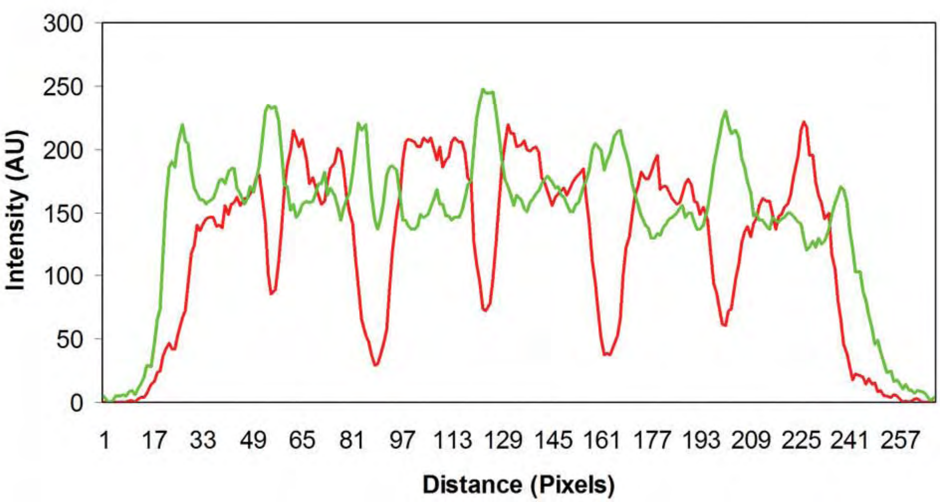




- gene expression is not necessarily "segregated"
- transcription & translation can occur simultaneously

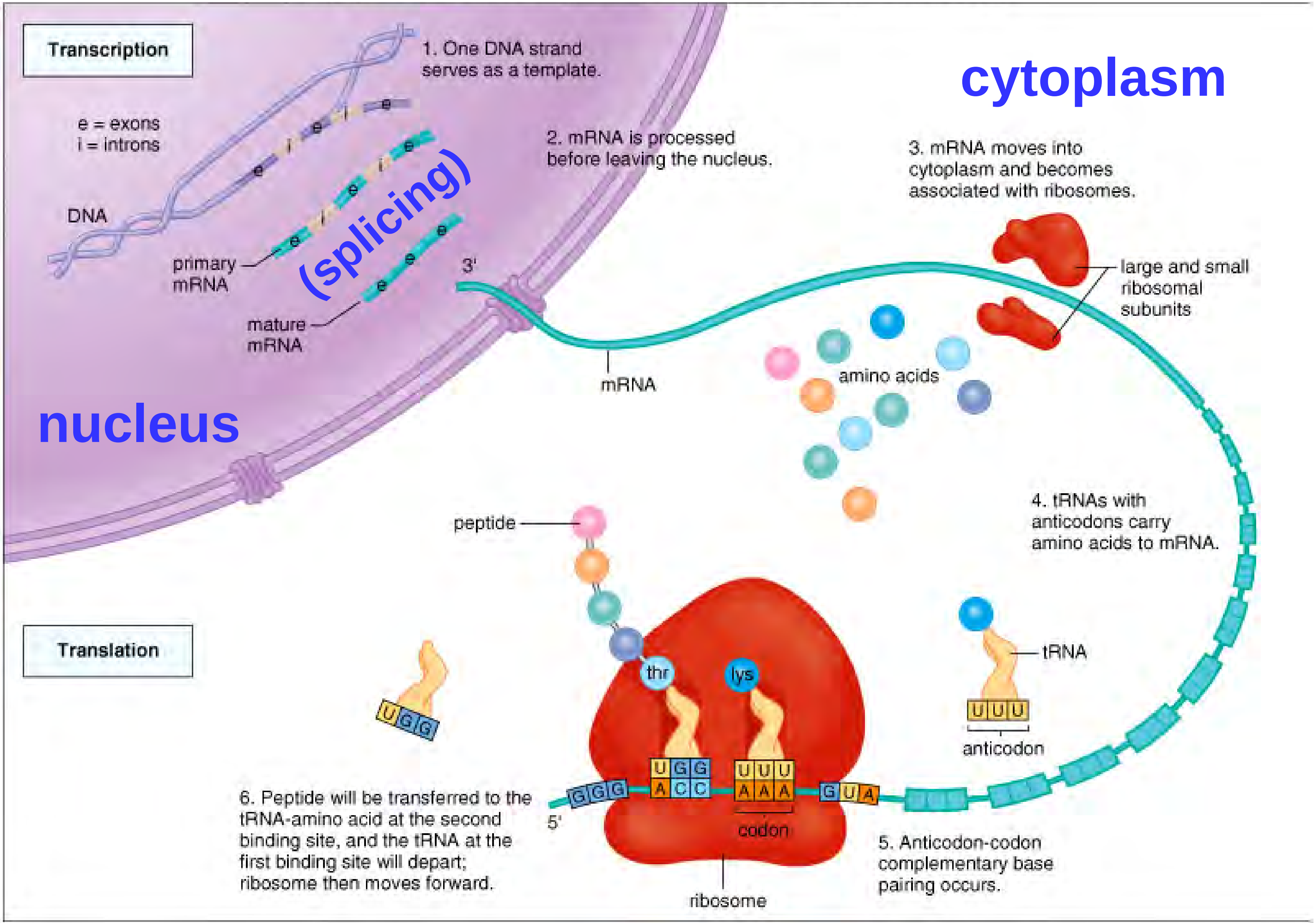
Compartmentalization of Gene Expression in Prokaryotes



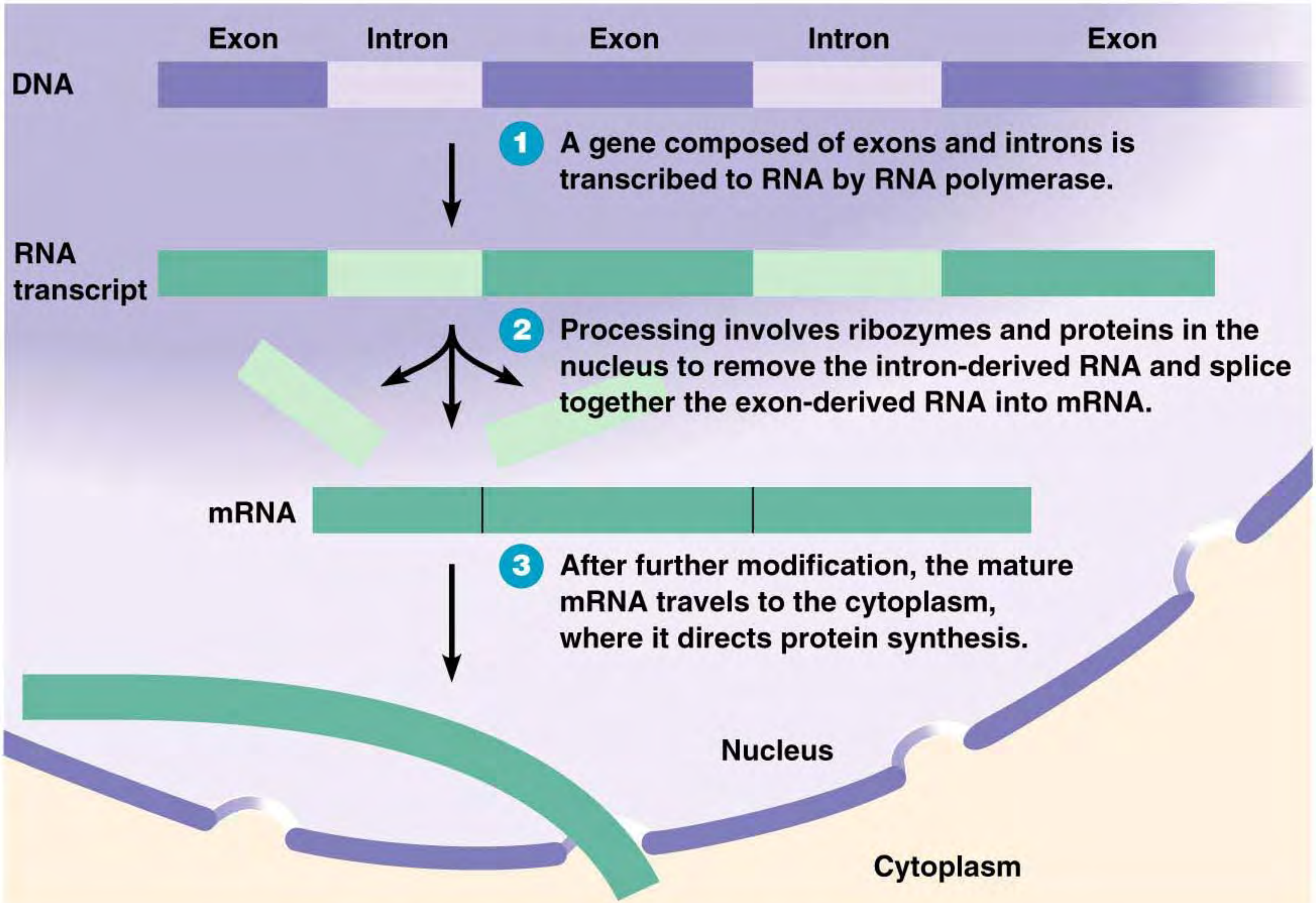


- as shown above, there is evidence of the segregation of transcription & translation in some prokaryotes

Gene Expression in Eukaryotes



Splicing of Eukaryotic Transcripts



2. Gene Regulation

Levels of Gene Regulation

The expression of a gene into functional proteins can be regulated at multiple levels:

TRANSCRIPTION*
(regulation of rate at which gene is transcribed)

mRNA transcript stability
(“half-life” of transcripts)

***key level of regulation**

TRANSLATION
(regulation of translation of mRNA)

post-translational modifications
(e.g., cleavage of polypeptides, addition of chemical groups)

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 <u>transcription</u> <u>factors</u>

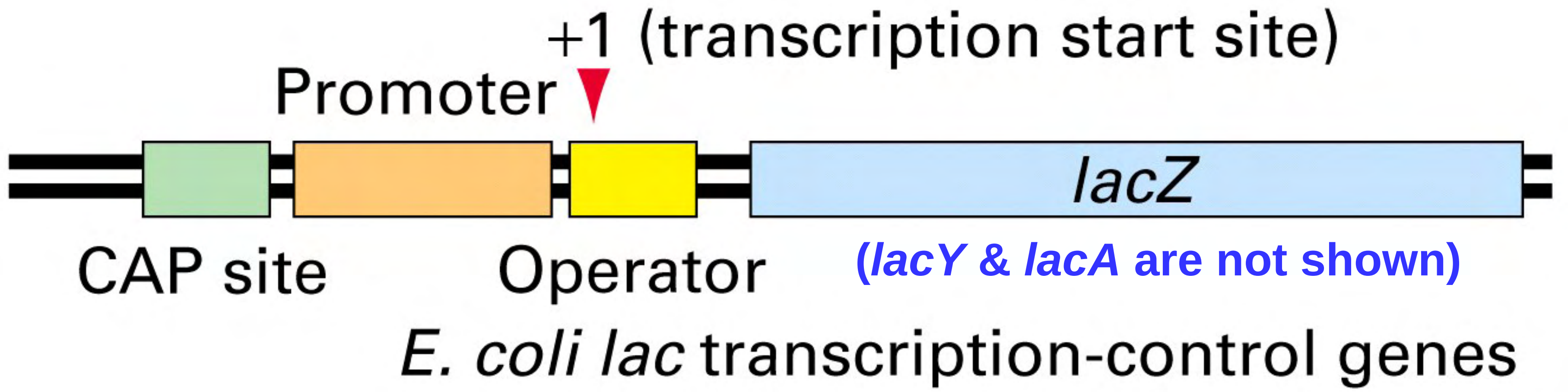
- **proteins that help RNA polymerase bind a promoter (referred to as "<u>activators</u>")**

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

- **the levels of various <u>repressors</u> & <u>activators</u> 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*…

The *lac* operon of *E. coli*



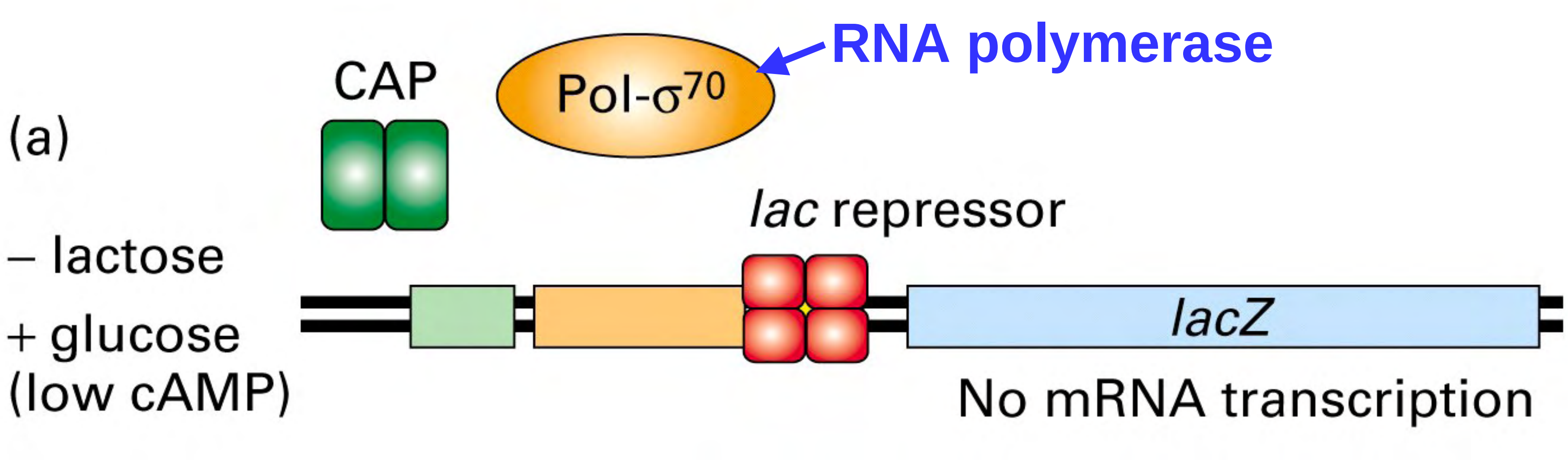
The *lac* operon is a module of 3 genes involved in lactose metabolism, *lacZ*, *lacY* & *lacA*, that are transcribed in a single mRNA from a single promoter.

On either side of the promoter are 2 special sequences, the CAP site which binds the activator CAP, and the Operator which binds the *lac* repressor...

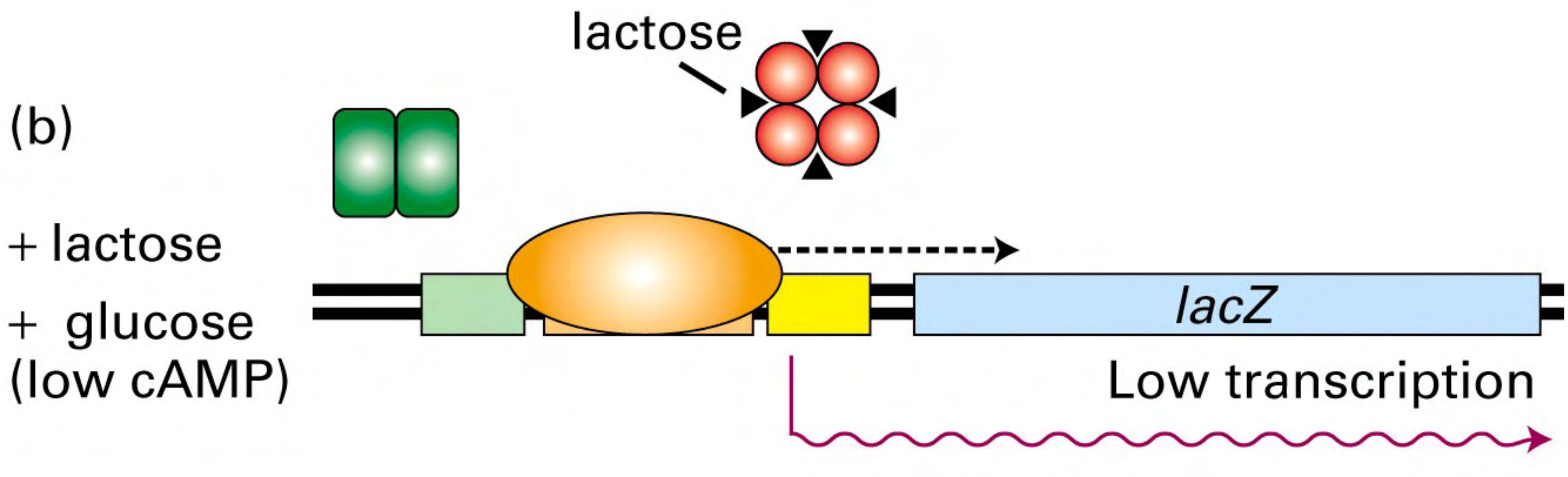
When lactose is absent:

The *lac* repressor protein by default is bound to the operator sequence, thus blocking part of the promoter and preventing RNA polymerase from binding and initiating transcription of the *lacZ*, *lacY* & *lacA* genes.

- **the *lac* operon is OFF since there's no need for these gene products in the absence of lactose**



When lactose is present w/glucose:



Lactose binds to the *lac* <u>repressor</u>, inducing a change in shape that prevents its binding the Operator sequence.

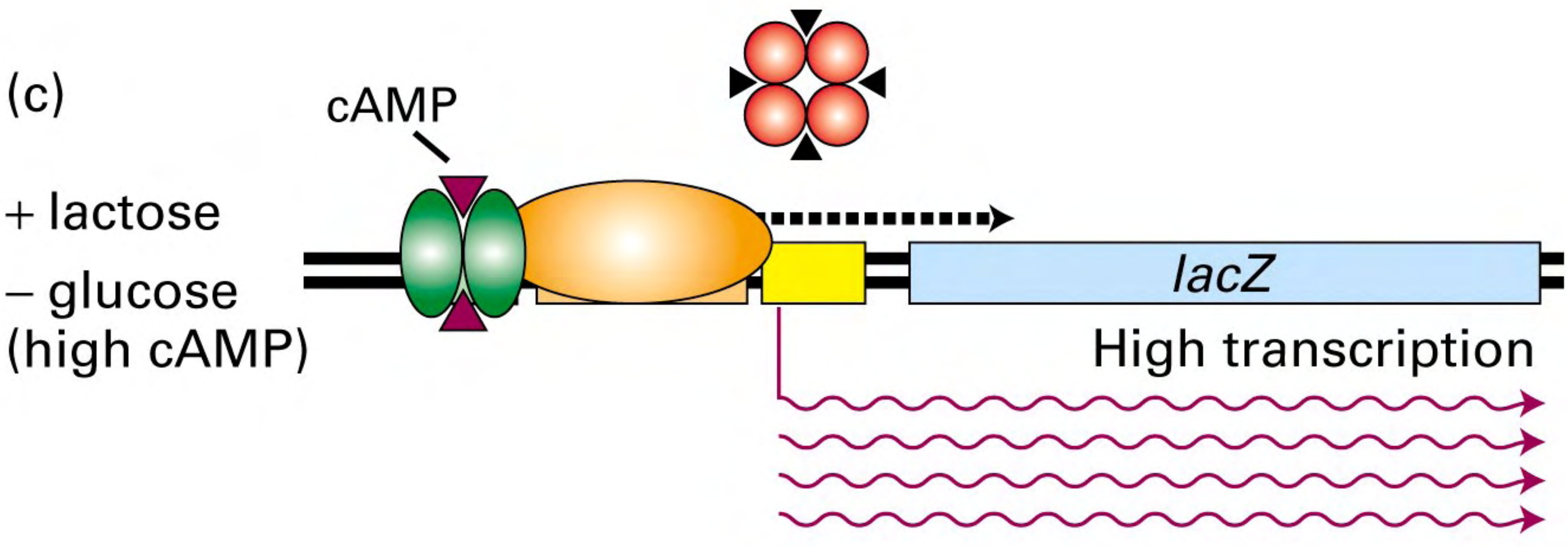
- **with the operator no longer occupied, RNA polymerase can bind promoter & initiate a low level of transcription**

- **since glucose (a preferred energy source) is present, the *lac* operon is ON "low"**

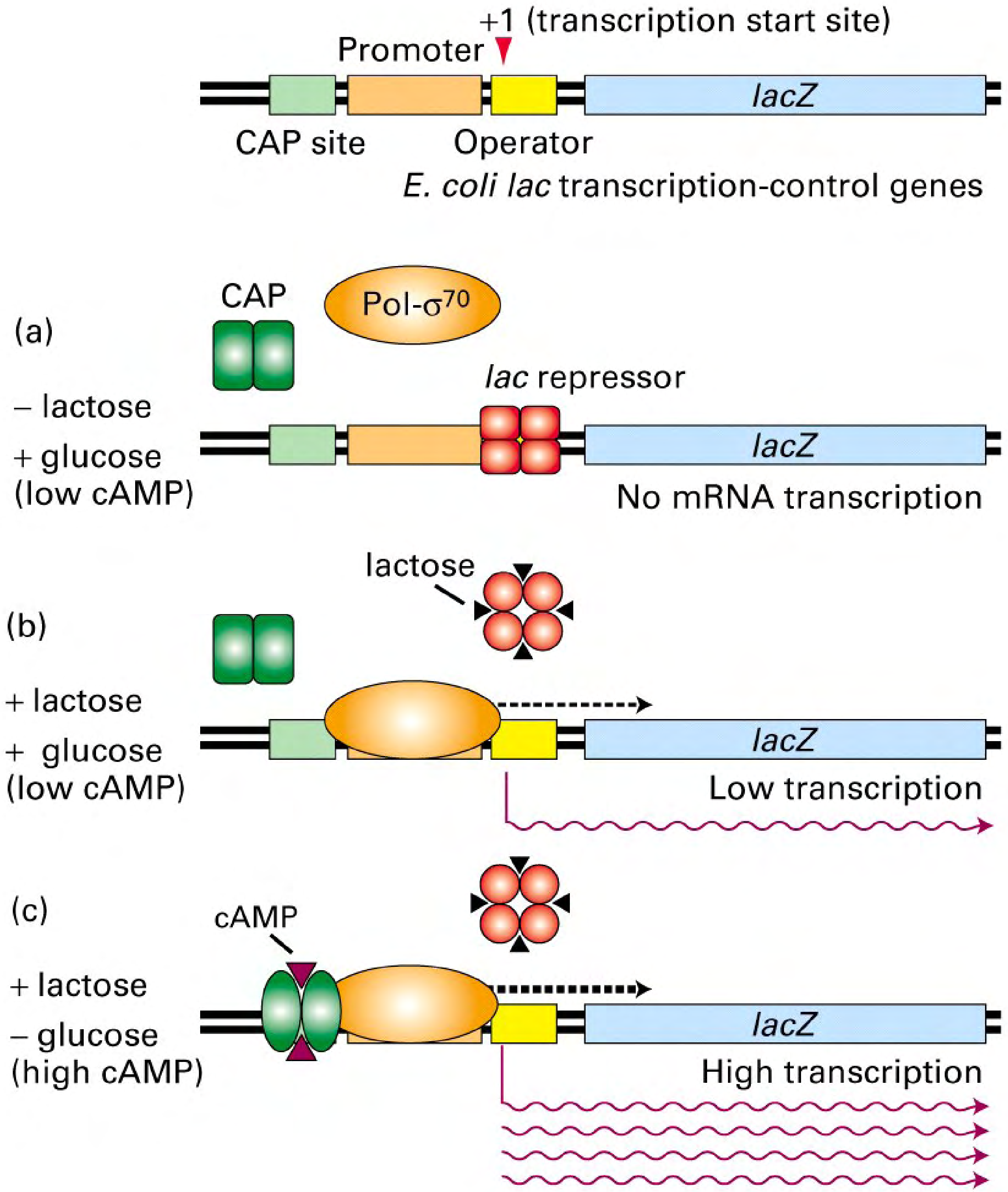
When lactose is present w/o glucose:

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"**

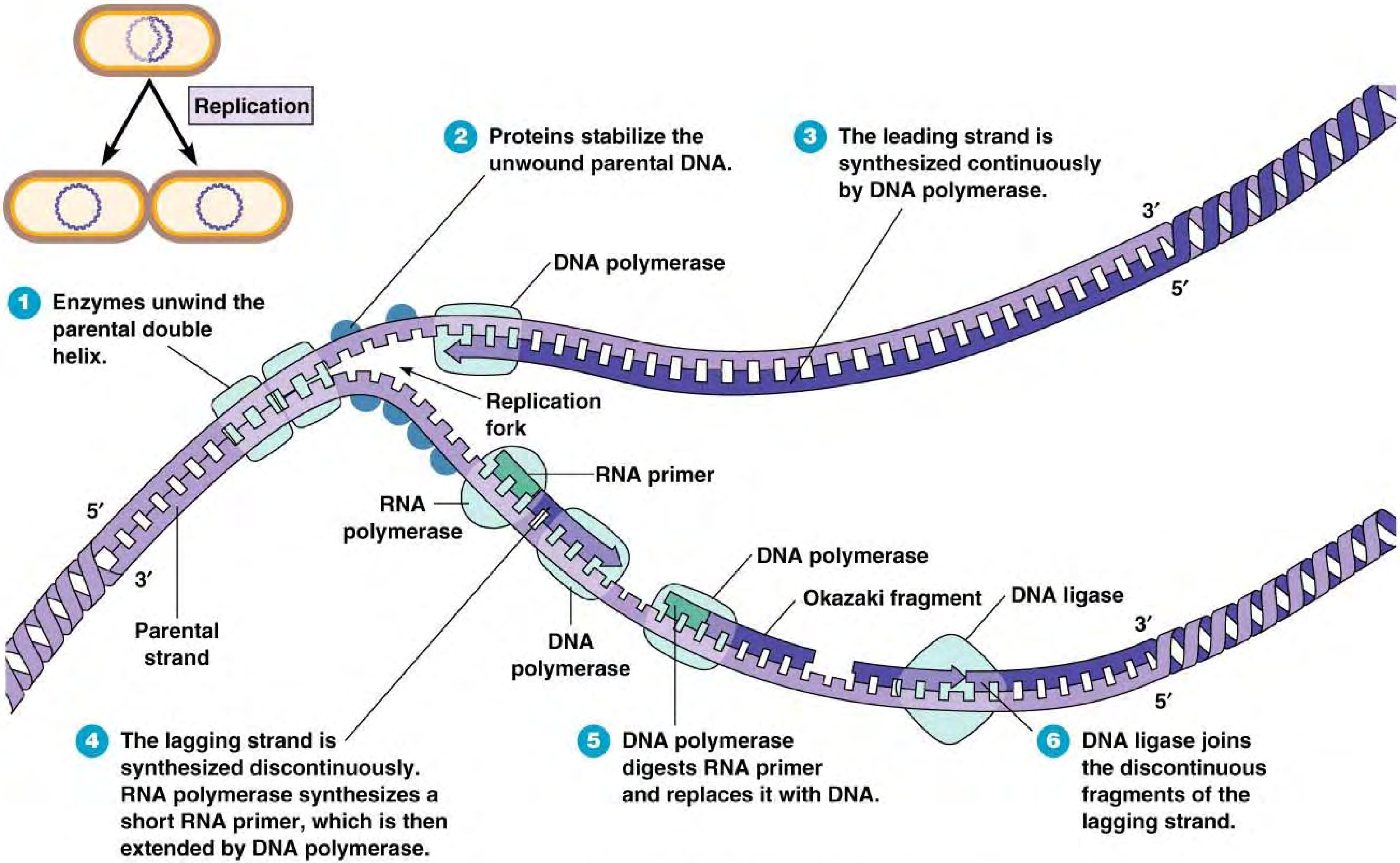


Summary of the *lac* operon



3. DNA Replication & Mutation

DNA Replication



Features of DNA Replication

Both strands serve as a template:

- **synthesis is always 5'-3'**
- ***leading* strand synthesis is <u>continuous</u>,**
  ***lagging* strand synthesis is <u>discontinuous</u>**

Each new DNA fragment requires an RNA <u>primer</u>:

- **DNA synthesis cannot begin without a primer to add to**

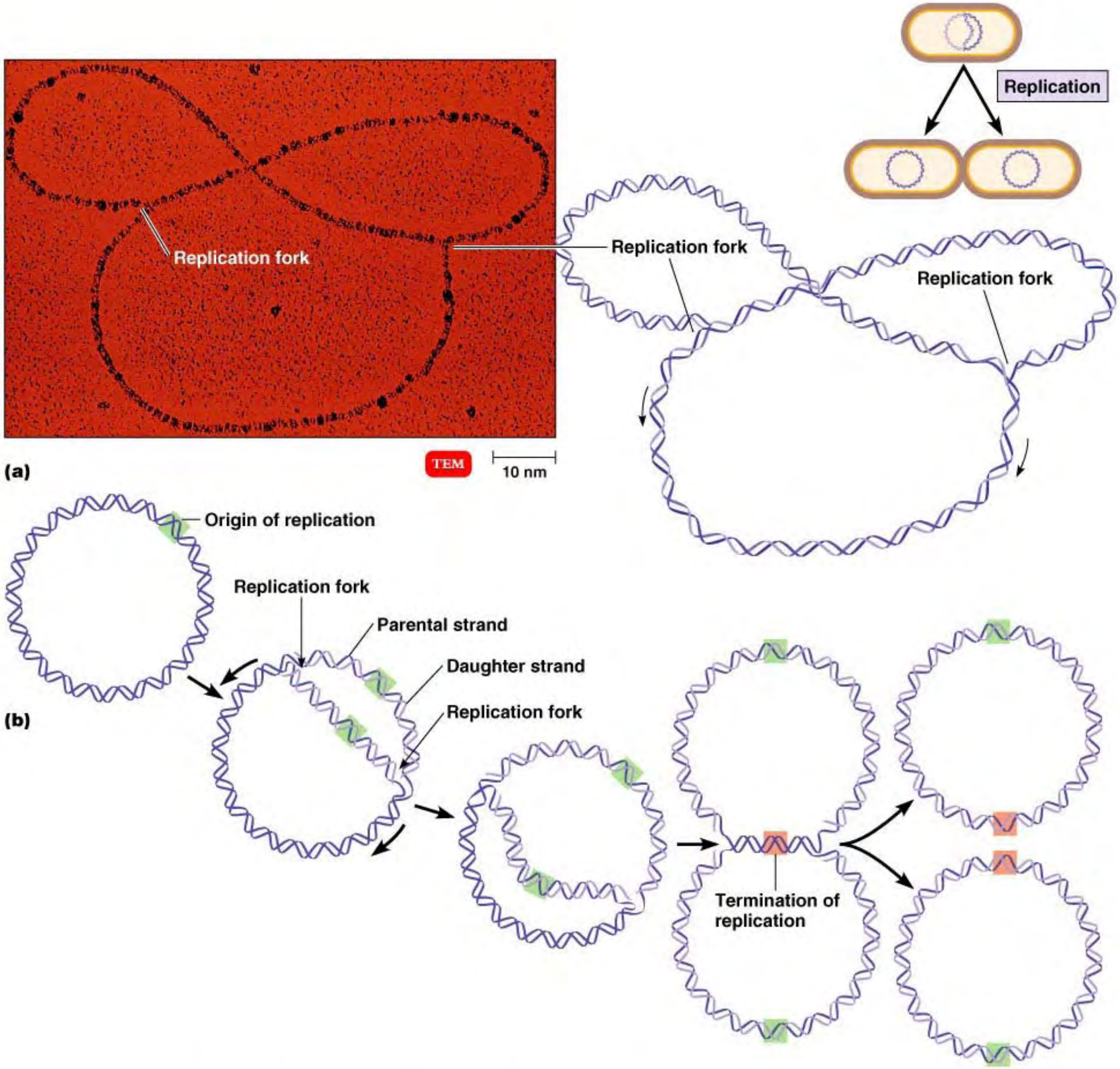
Some important enzymes:

<u>DNA Polymerase</u> (synthesizes new DNA)

<u>Primase</u> (makes RNA primers)

<u>DNA Ligase</u> ("stitches" fragments together)

DNA Replication in Prokaryotes



- **begins at the origin of replication (OriC)**
- **can only be completed if DNA is circular**

Mutations

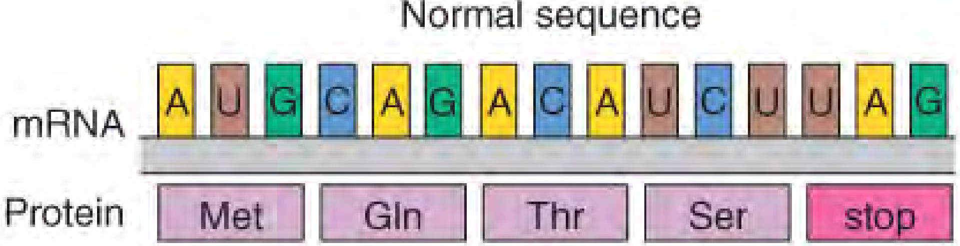
A mutation is *any* change in DNA sequence:

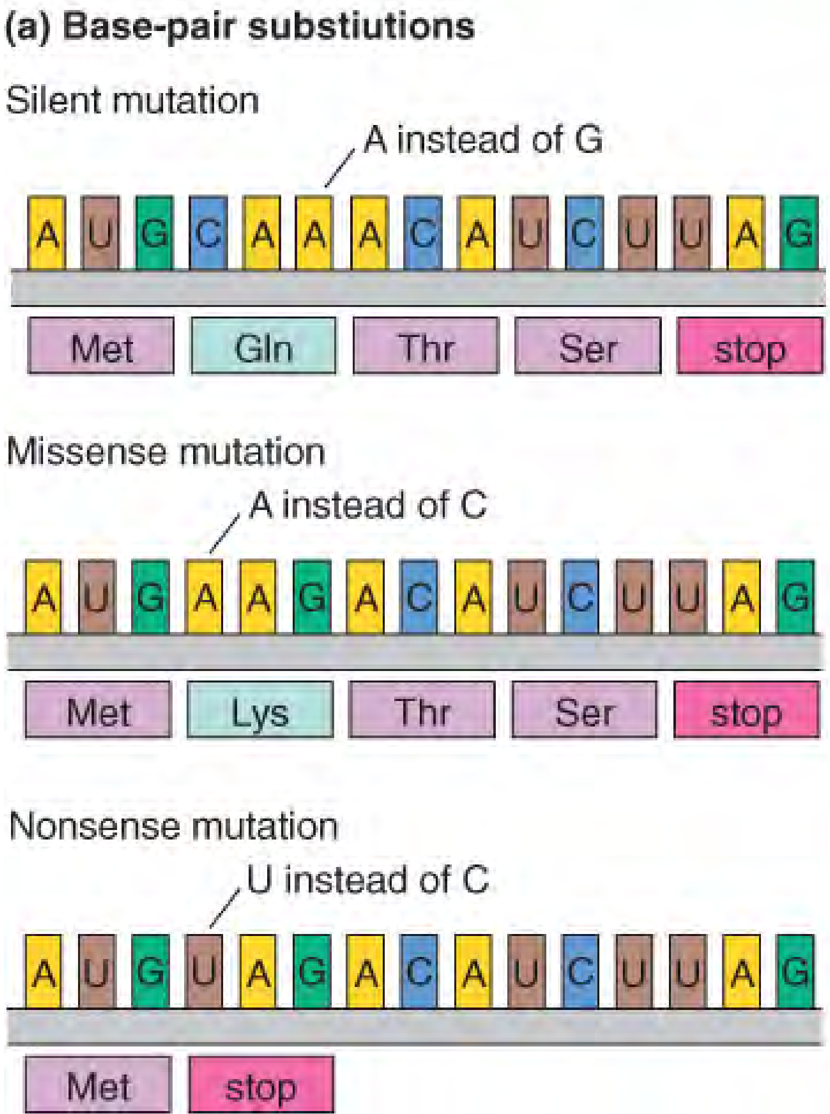
- change of one nucleotide to another
- insertion or deletion of nucleotides or DNA fragments
- inversion or recombination of DNA fragments

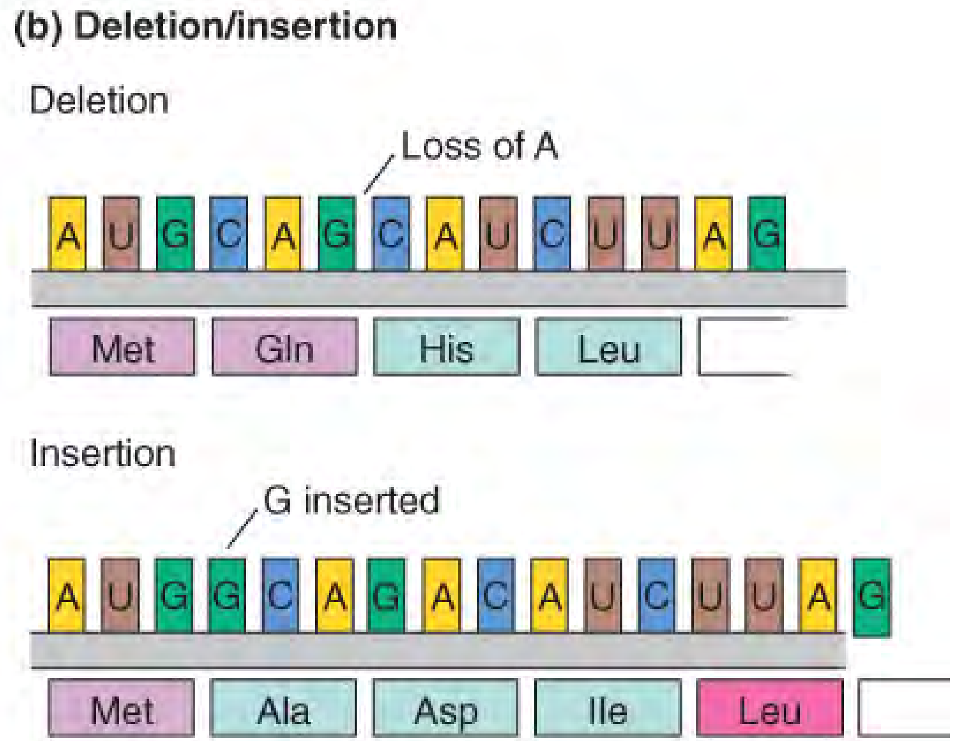
What causes mutations?

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

Effects of Mutations





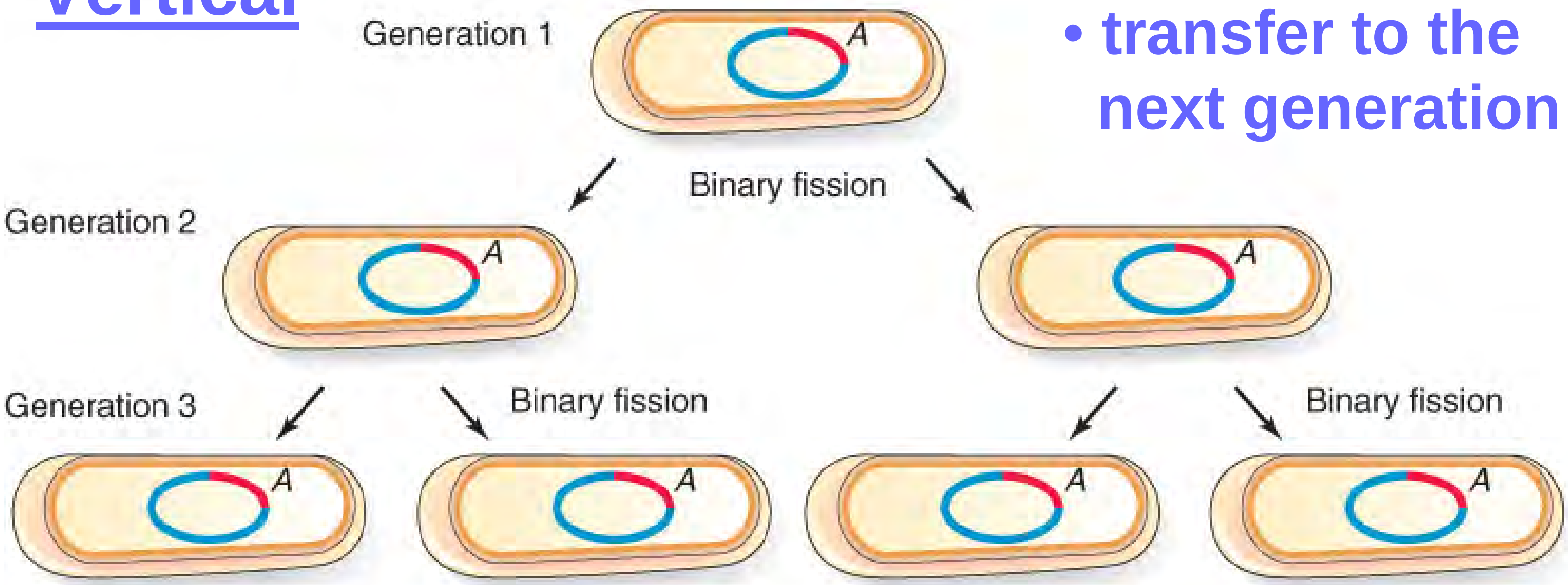


***insertions & deletions can cause "frame shifts"**

4. Mechanisms of Gene Transfer

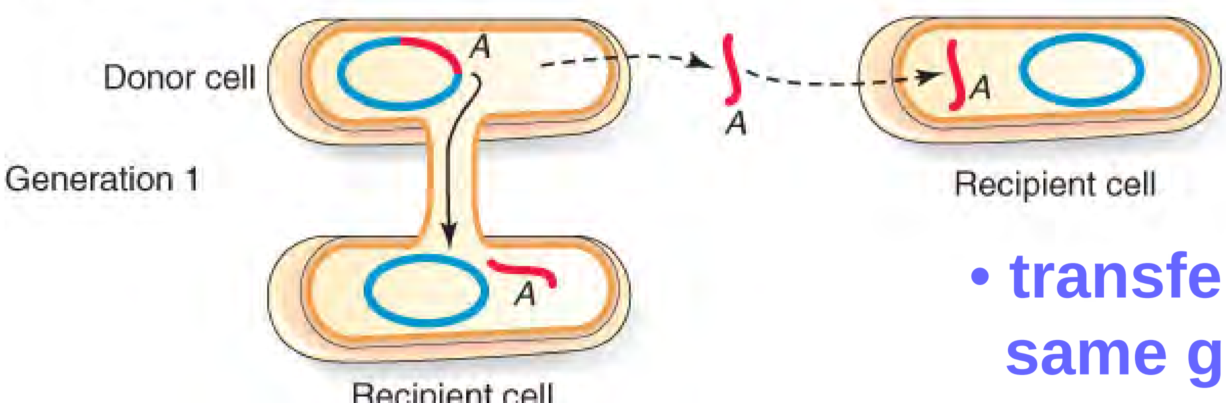
Horizontal vs Vertical Gene Transfer

Vertical



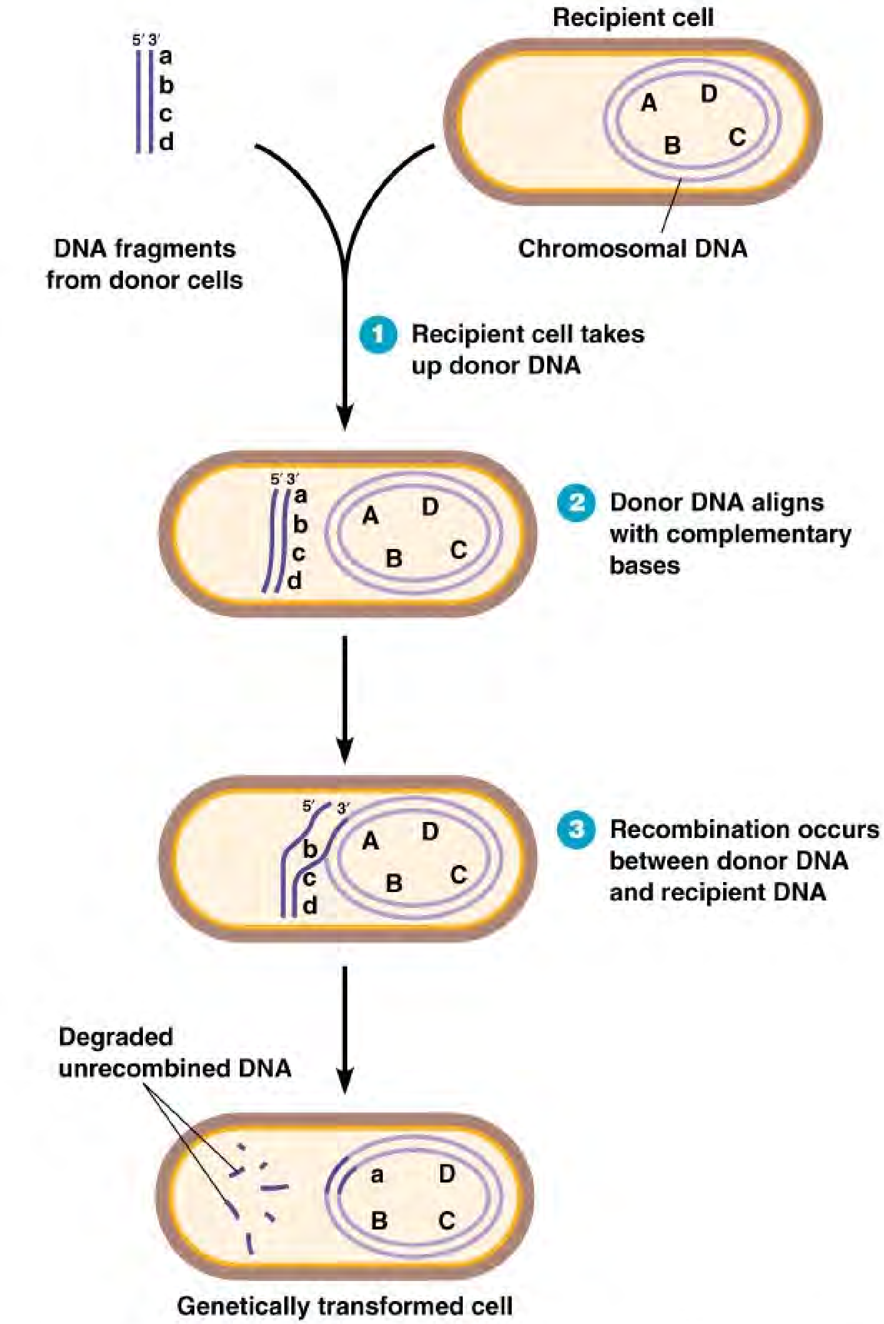
- transfer to the next generation

Horizontal (or lateral)



- transfer within the same generation

Homologous Recombination



Unless transferred DNA is circular w/Ori (plasmid), it must recombine with host DNA to be retained

Recombination can occur between *homologous* (similar) DNA sequences:

- **DNA with "same" genes**
- **facilitated by special proteins**
- **original DNA is lost**

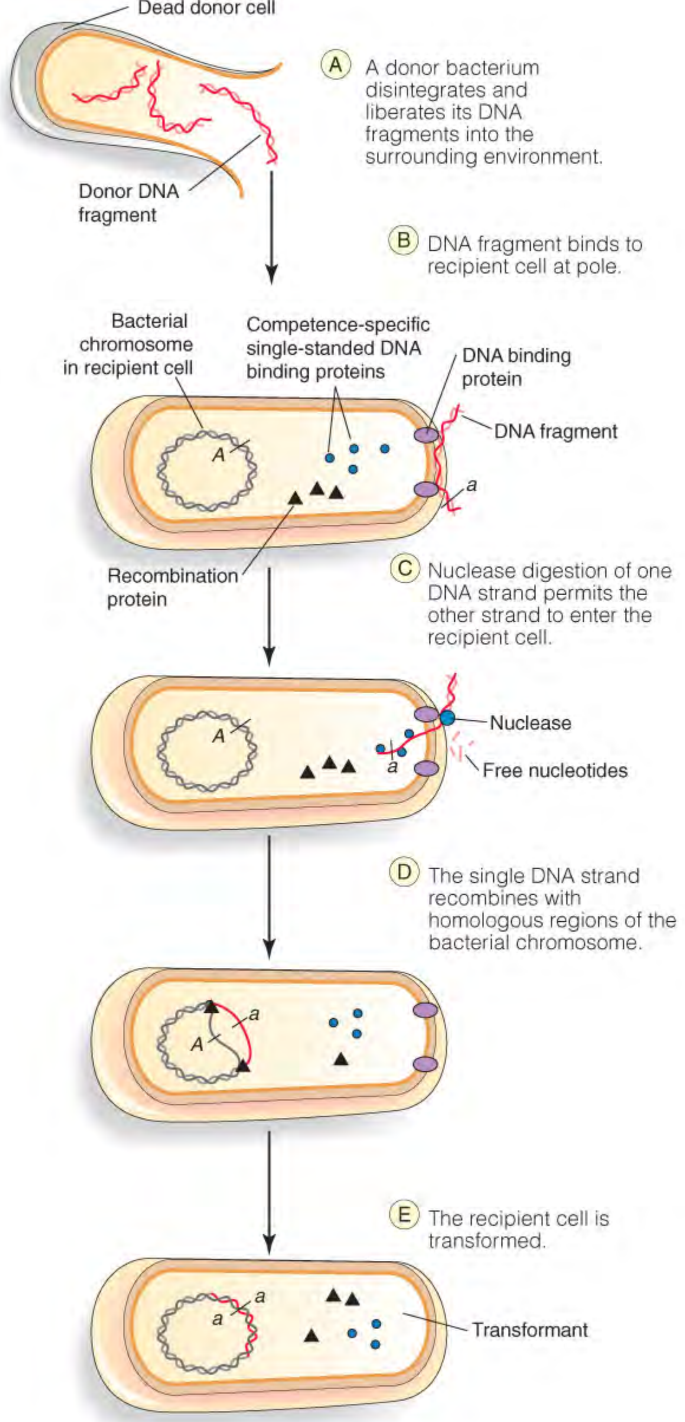
Methods of Gene Transfer

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

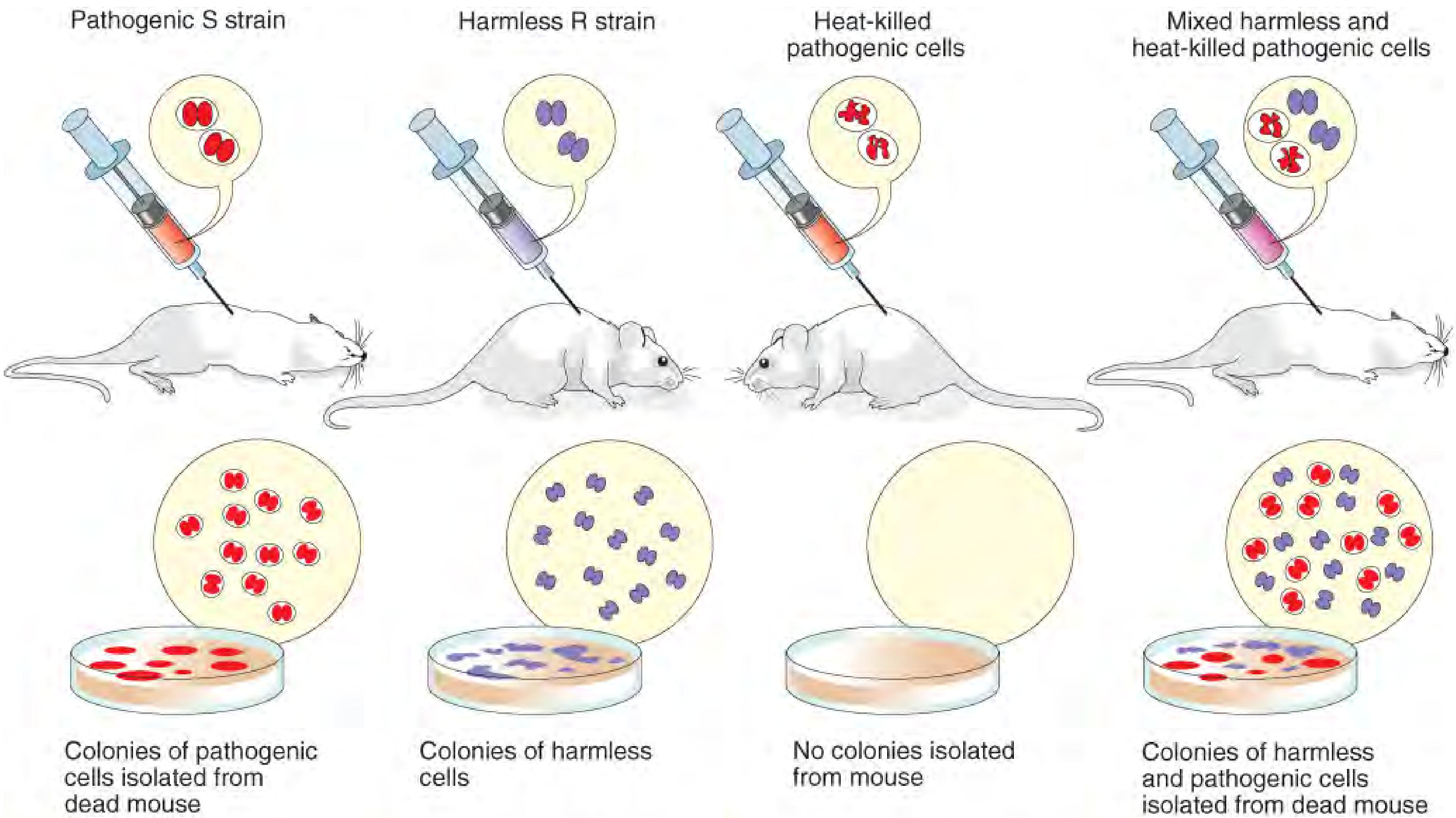


Transformation

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



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

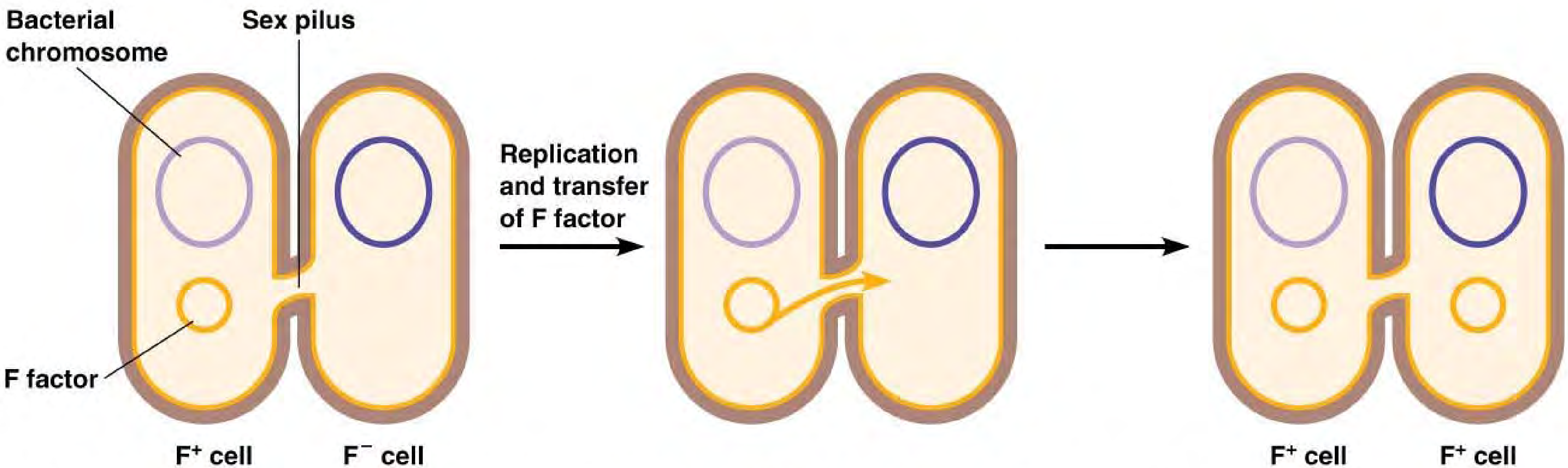
(B) An injection of R strain (unencapsulated,harmless) cells did no harm to the mouse.

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

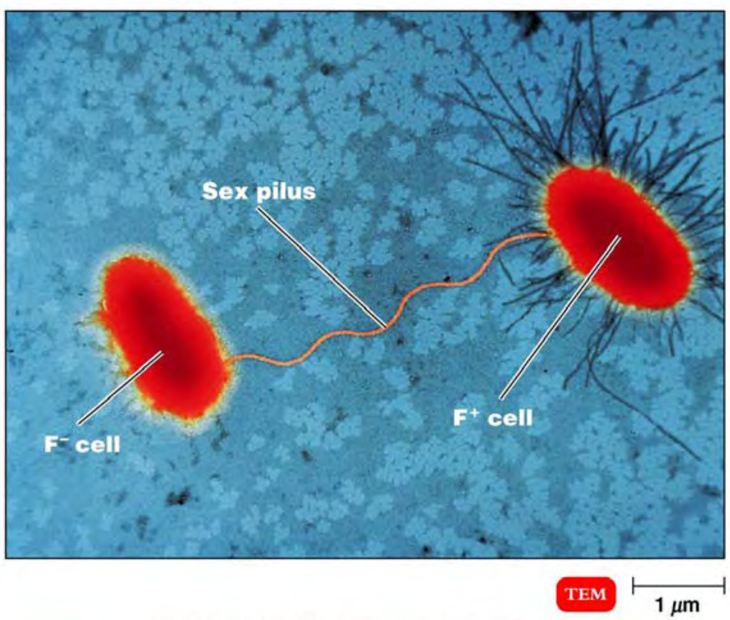
(D) But when Griffith injected a mixture of live R strain and heat-killed S strain cells into the mouse, it died. When Griffith cultivated the organism from the blood, he found live S strain cells.

1928!

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.

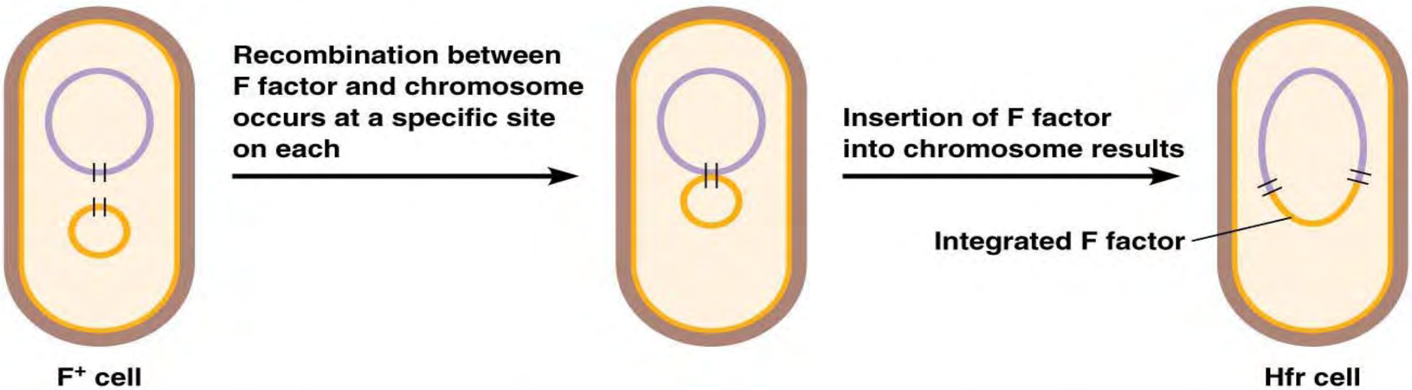


Requires an F factor plasmid

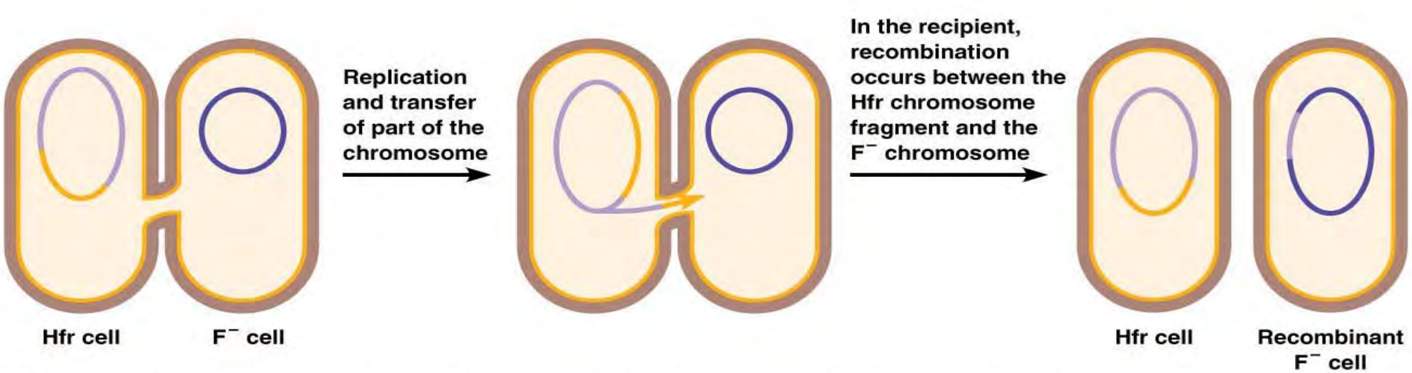
- has all "conjugation genes"
- directs formation of a sex pilus
- single DNA strand produced by DNA replication is transferred to F- cell through the sex pilus, recipient produces 2nd strand

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.

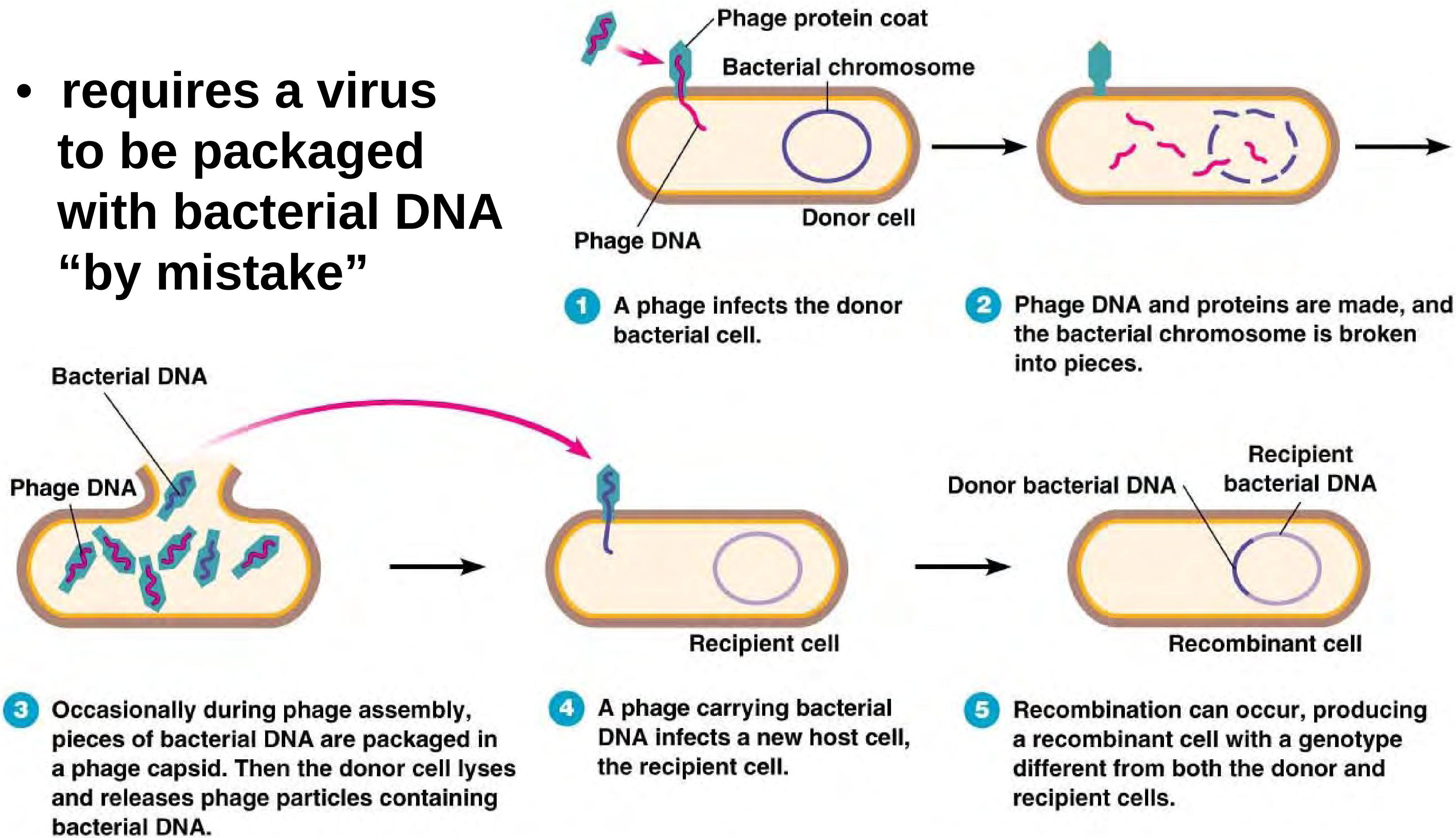
- **recipient can incorporate donor cell genes by recombination**
- **also useful for mapping bacterial genes based on the rate of transfer**

****Hfr = "High frequency of recombination****

Transduction

A virus (phage) particle can transfer DNA fragments from one host cell to another followed by recombination

- requires a virus to be packaged with bacterial DNA “by mistake”



Key Terms for Chapter 8

- **transcription factor, activator, repressor**
- **lac operon, lac repressor, operator, CAP**
- **leading strand, lagging strand, primase, DNA ligase**
- **missense, nonsense, silent mutations, frame shift**
- **horizontal vs vertical gene transfer**
- **homologous recombination**
- **transformation, transduction, conjugation, Hfr**

Relevant Chapter Questions

rvw: 1-4, 8, 9, 11, 13   MC: 1, 2, 4, 5, 7-10