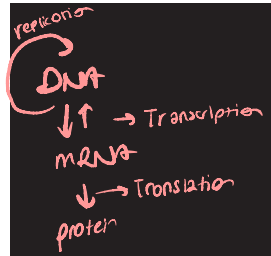


Environmental Microbiology

Microbial Genetics

prepared by Prof. Bulent Içgen



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 Genes of an organism
- Phenotype Expression of the genes

DNA (deoxychar acid) } nucleic acid
 RNA (ribonucleic acid) }

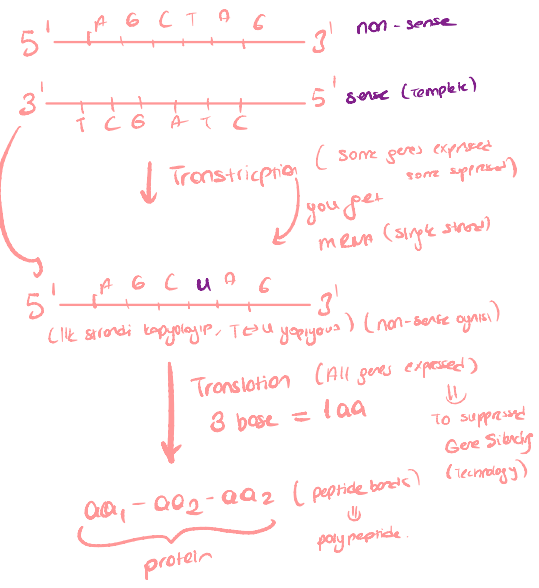
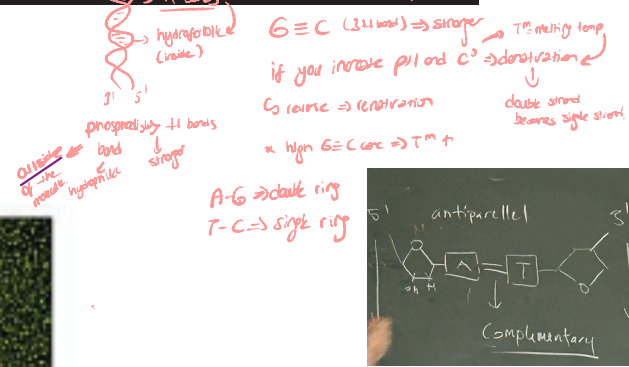
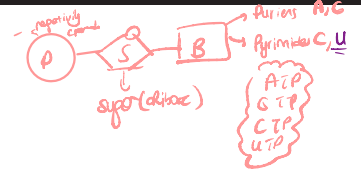
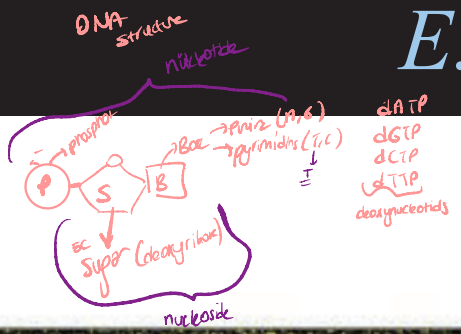
Both RNA and DNA ⇒ negatively charged due to phosphate

E. coli

RNA structure
 SS (single stranded)

DNA
 one strand direction ⇒ 5' → 3' } antiparallel
 other " " ⇒ 3' → 5' }
 A = T (2 H bonds)
 G = C (3 H bonds) ⇒ stronger
 if you increase pH and C^{3'} ⇒ deprotonation
 C^{3'} reinit ⇒ renaturation
 ⇒ high GC cont ⇒ T^m ↑
 double strand becomes single strand

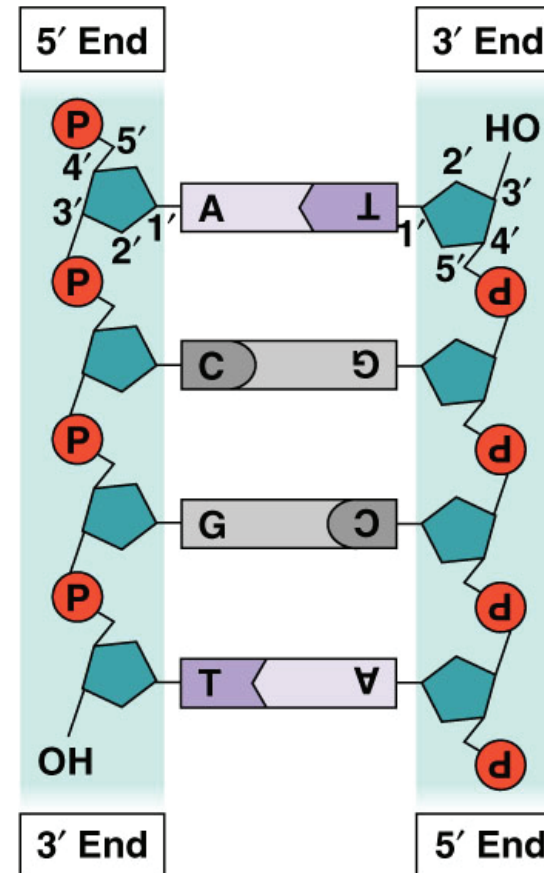
Chromosomal DNA
 ⇒ ds-ss
 ⇒ closed circular CC



housekeeping genes ⇒ all microorganism need for transcription, translation, replication
 catabolic genes ⇒ catabolic enzyme

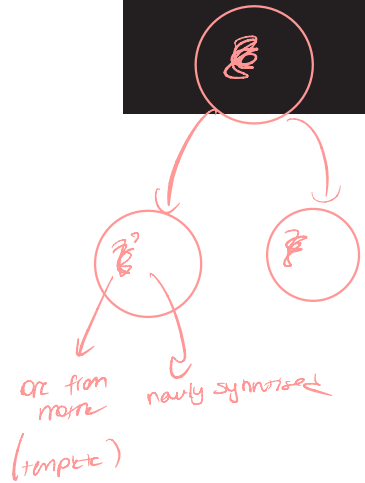
DNA

- 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



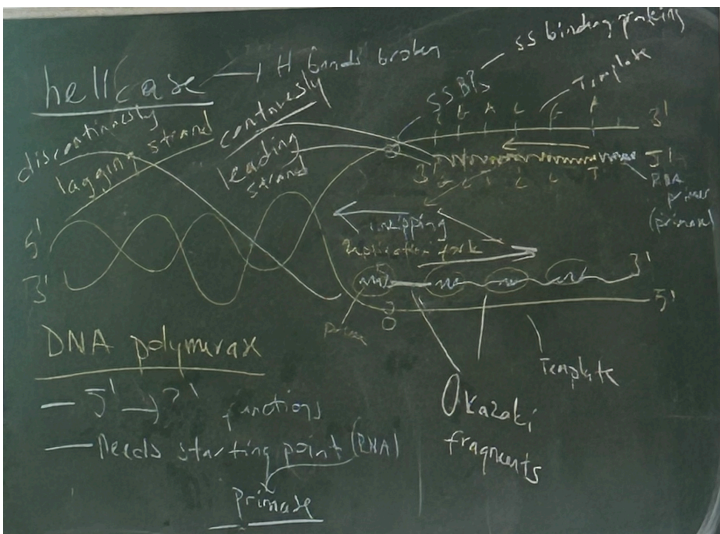
DNA

in RNA viruses they have enzyme "reverse transcriptase"
 by this enzyme you can reverse transcription meaning
 from RNA to DNA (from mRNA)



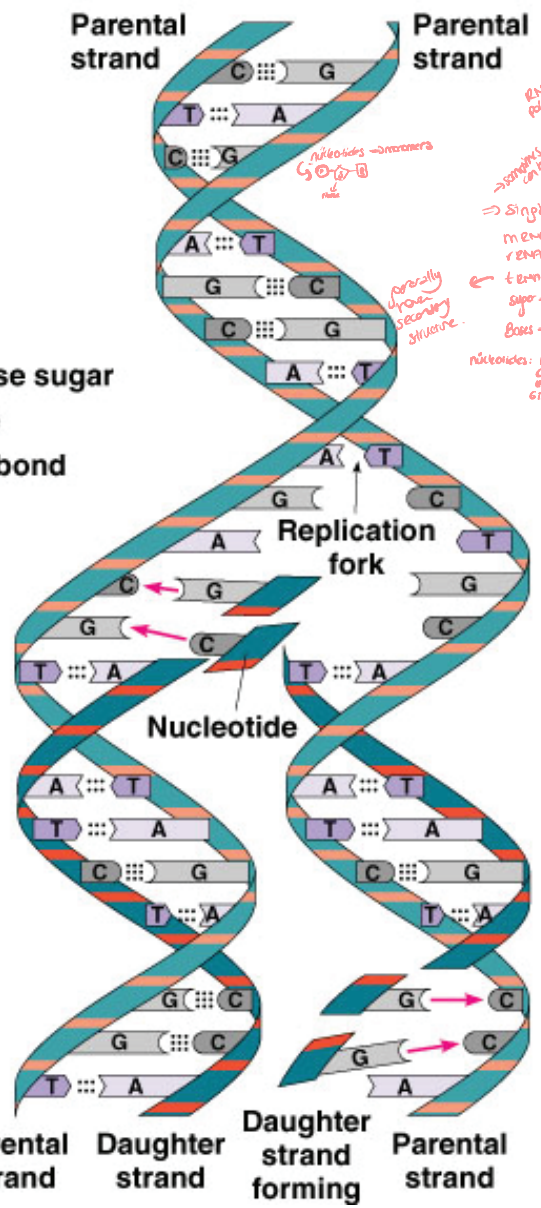
→ nick is created then strand becomes unwound (unzipped)
 ↳ hydrogen bonds broken in these. ↳ 'bidirectional' unwinding
 increase the enzyme that breaks hydrogen bonds.

single strand binding proteins → to keep them single strands (used together)
 each single strand becomes a template
DNA-dependent enzyme ⇒ only works in 5'→3' direction
 ⇒ it needs a primer (starting point) (usually RNA)
 ⇒ this enzyme joins them together by primase (enzyme)
 replication fork are first strand same direction
 in second strand are synthesized as small fragments (discontinuous where first strand continuous)
 ↳ because of opposite directions
 ↳ to second strand lagging strand (LSS) → Okazaki fragments as each requires RNA primer
 ⇒ DNA polymerase degrades RNA primers in both strands
 and filled gaps → but discontinuity made by DNA ligase



→ replication
 ↓
 semi-conservative (type of replication)
 (1 one strand from mother or is newly synthesised)

- Key:**
- T Thymine
 - A Adenine
 - C Cytosine
 - G Guanine
 - Deoxyribose sugar
 - Phosphate
 - ... Hydrogen bond



RNA polymerase → synthesizes catalytic 2-subunit 'backbone' part
 ⇒ single stranded structure
 mRNA → messenger
 rRNA → ribosome
 tRNA → transfer
 sugar → ribose
 Bases = A-G C-U
 nucleotides: ATP GTP UTP CTP
 DNA → double stranded
 ⇒ deoxyribose (sugar)
 ⇒ Bases: A-G C-T
 ⇒ nucleotides: dATP dGTP dCTP dTTP

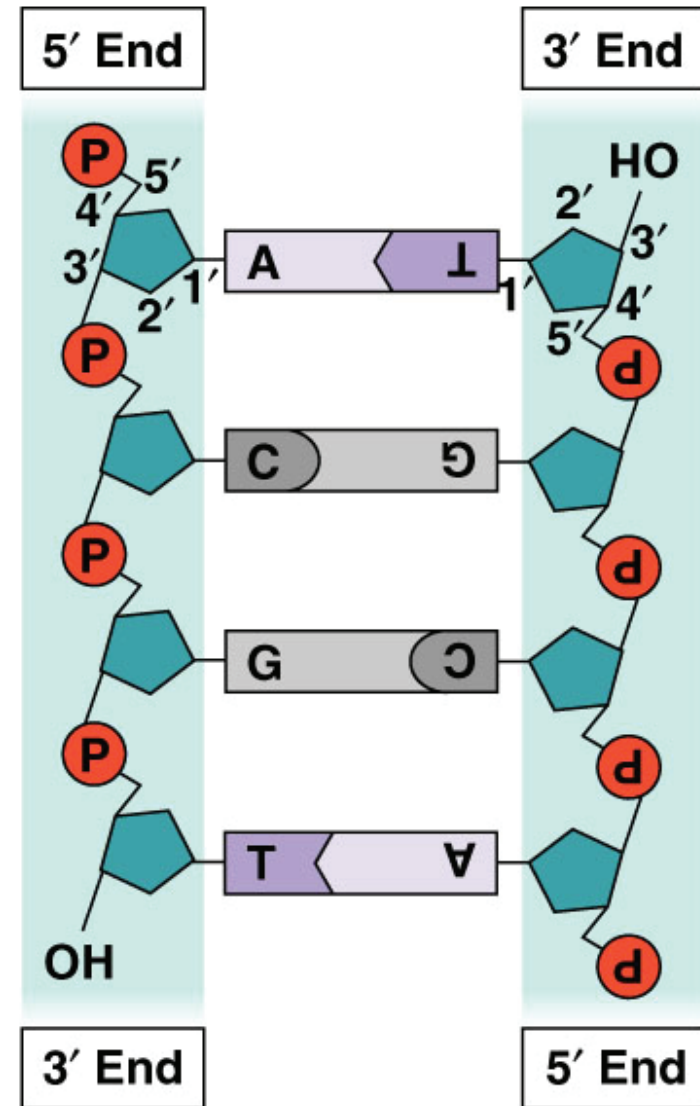
in RNA viruses they have enzyme "reverse transcriptase"
 by this enzyme you can reverse transcription meaning
 from RNA to DNA (from mRNA)
 replication → DNA polymerase
 DNA → mRNA
 mRNA → protein
 Transcription
 Translation

DNA

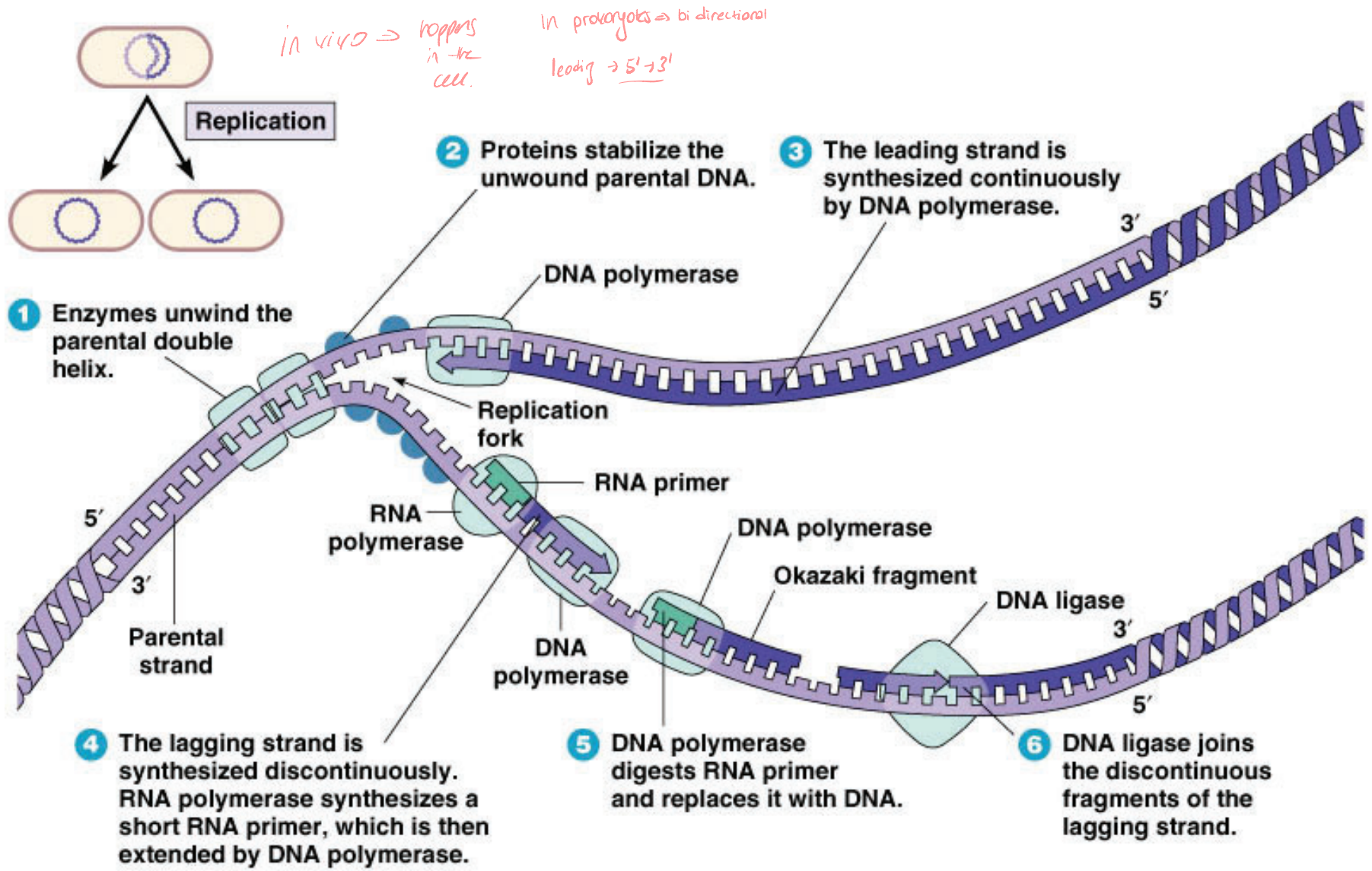
- DNA is copied by DNA polymerase
 - In the 5' → 3' direction
 - Initiated by an RNA primer
 - Leading strand synthesized continuously
 - Lagging strand synthesized discontinuously
 - Okazaki fragments
 - RNA primers are removed and Okazaki fragments joined by a DNA polymerase and DNA ligase

DNA

- 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

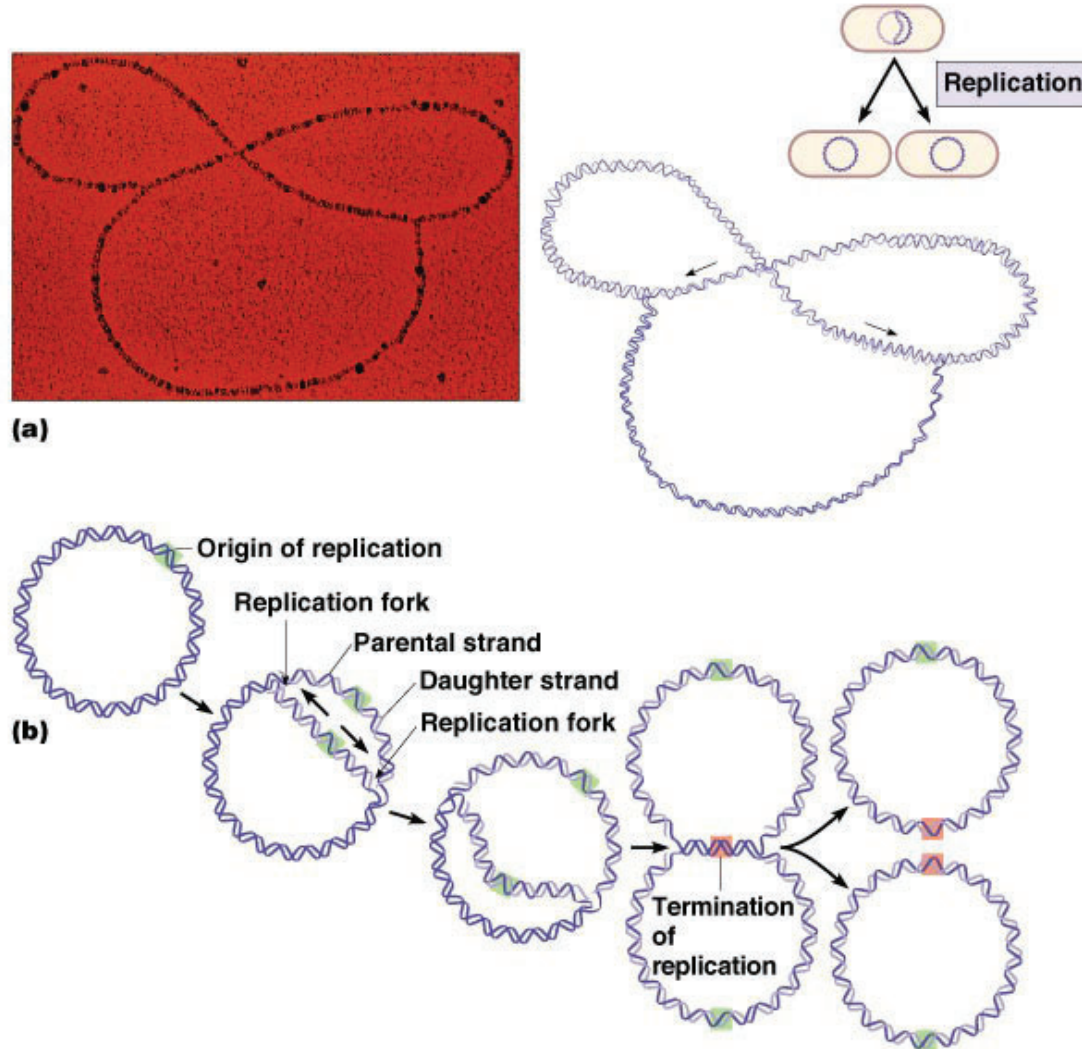


DNA

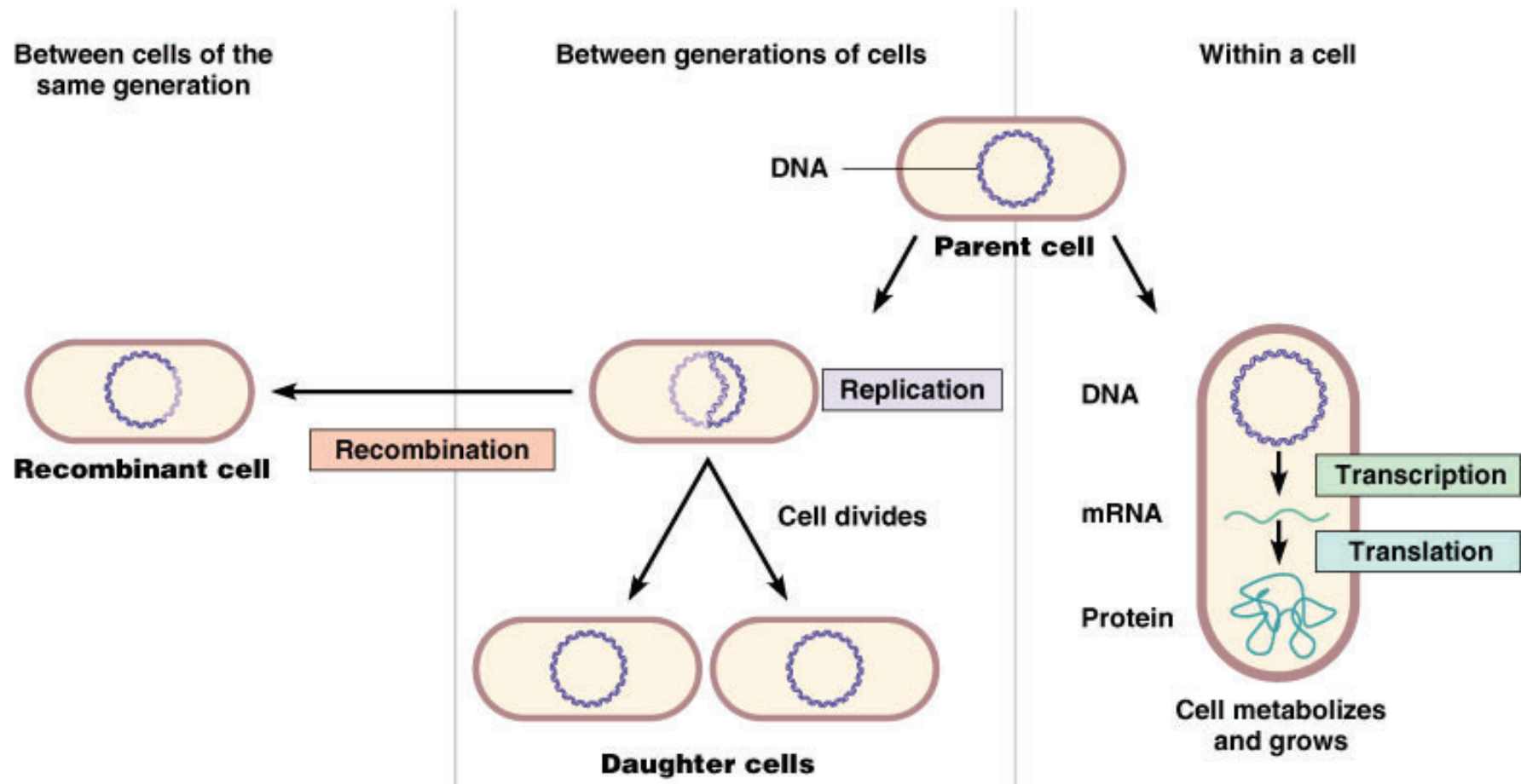


DNA

- DNA replication is semiconservative



Flow of Genetic Information



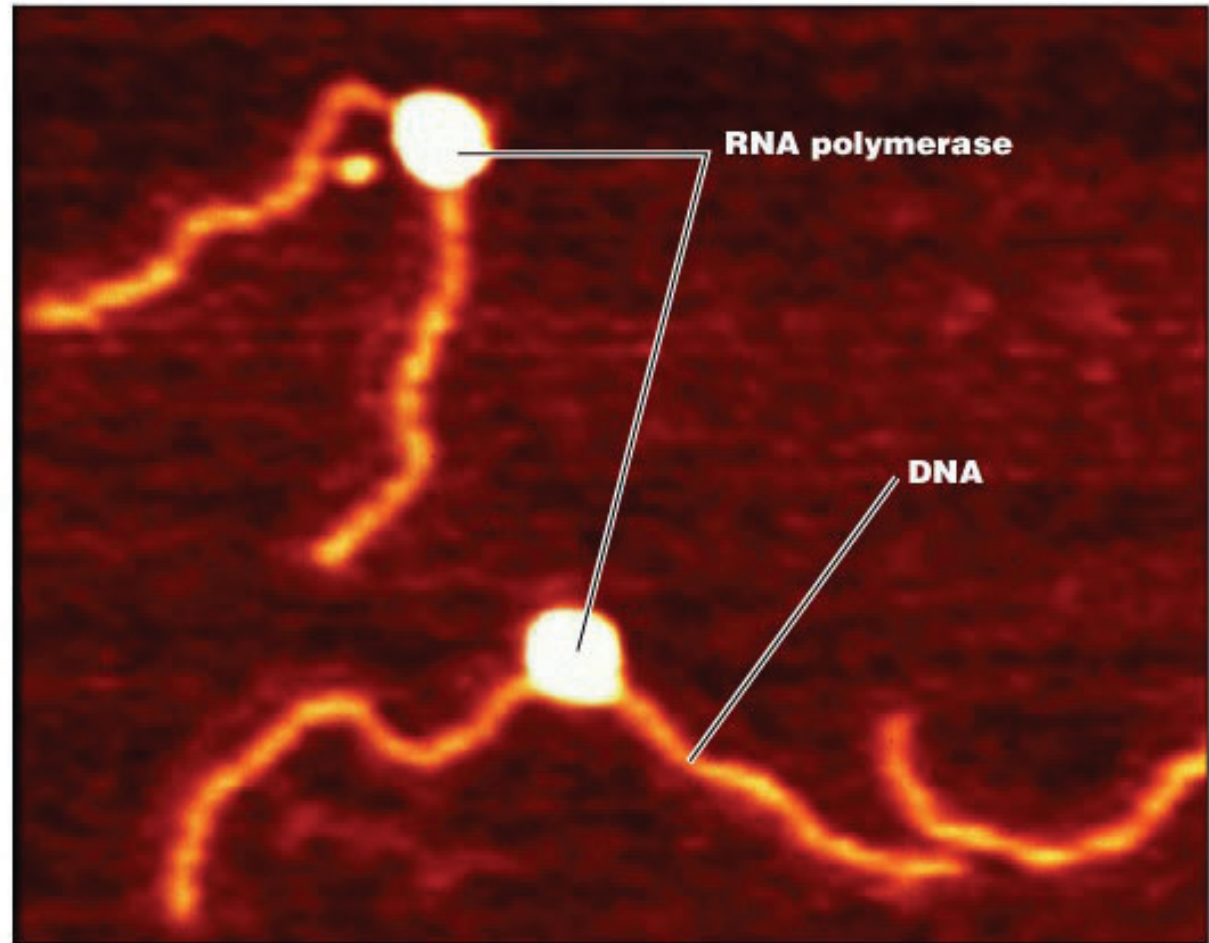
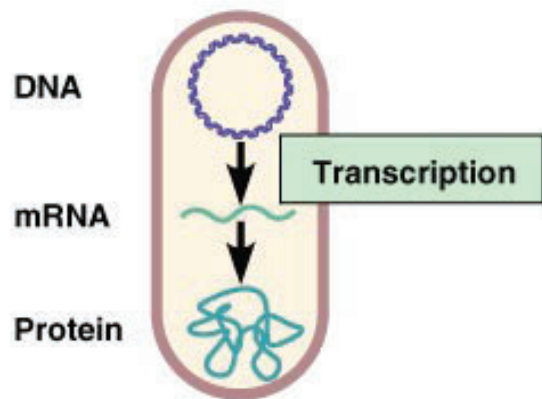
Transcription

- 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

→ instead T → U
⇒ 5' → 3' direction
↳ sense strand
⇒ template 3' → 5'
↳ non-sense strand

enzyme
↓
should attach

→ signal for RNA polymerase which takes the enzyme to the right promoter



RNA Polymerase bound to DNA

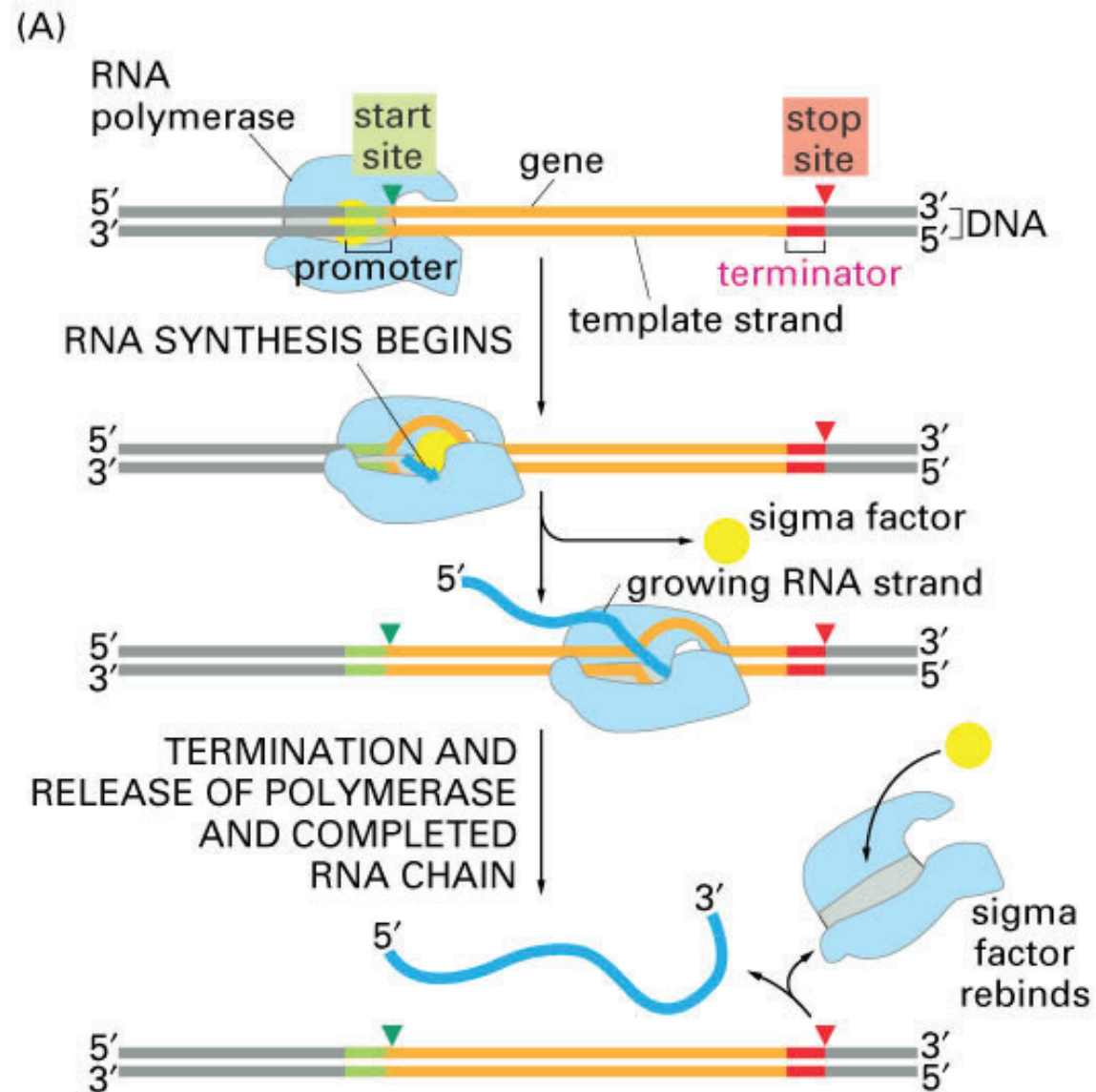
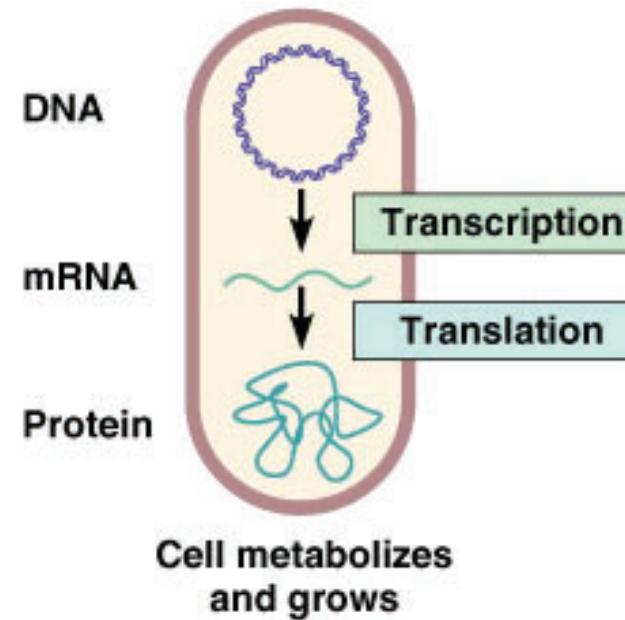


Figure 7-9 part 1 of 2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Translation

- mRNA is translated in codons (3 nucleotides)
- Translation of mRNA begins at the start codon: AUG *→ methionine*
- Translation ends at a STOP codon: UAA, UAG, UGA *→ process starts with methionine and if necessary remove it after*



Translation

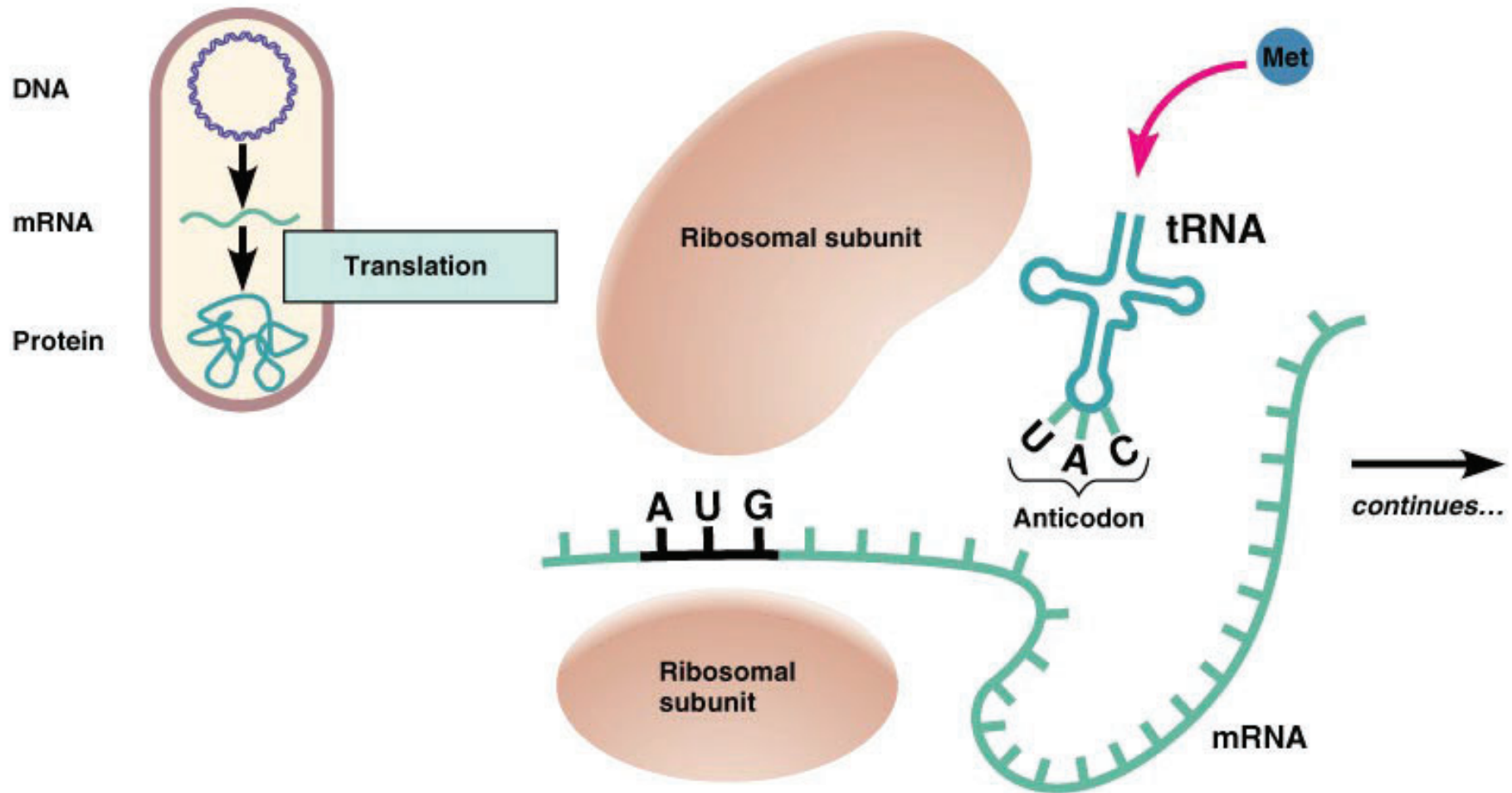
First 2 base highly specif for aa. → first 2 identifies aa.

→ but 3rd not that specific ⇒ wobble base

↓
more of the 3rd
codon dependency

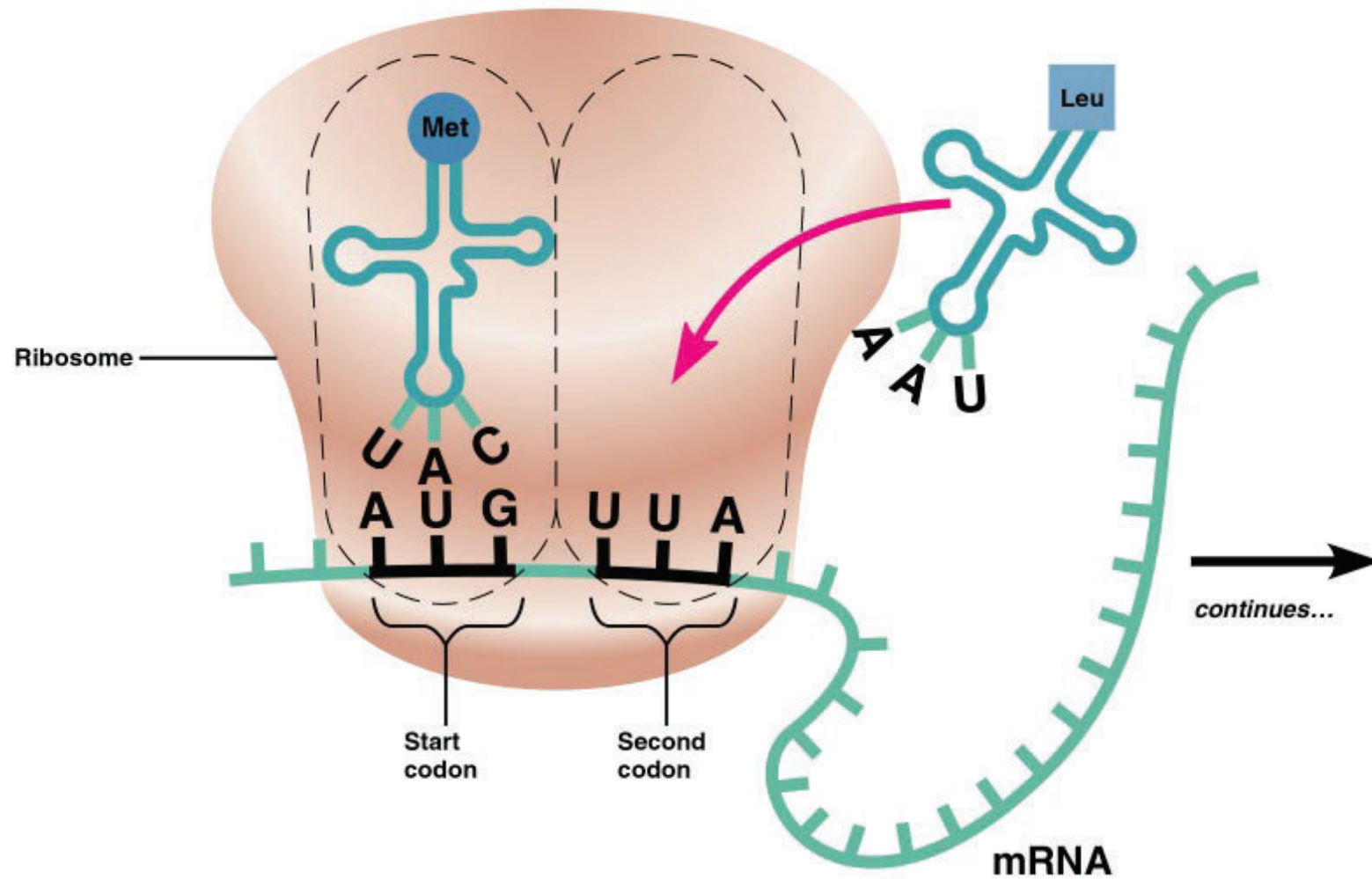
		Second position				
		U	C	A	G	
First position	U	UUU } Phe	UCU } Ser	UAU } Tyr	UGU } Cys	U
		UUC } Phe	UCC } Ser	UAC } Tyr	UGC } Cys	C
		UUA } Leu	UCA } Ser	UAA Stop	UGA Stop	A
		UUG } Leu	UCG } Ser	UAG Stop	UGG Trp	G
	C	CUU } Leu	CCU } Pro	CAU } His	CGU } Arg	U
		CUC } Leu	CCC } Pro	CAC } His	CGC } Arg	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



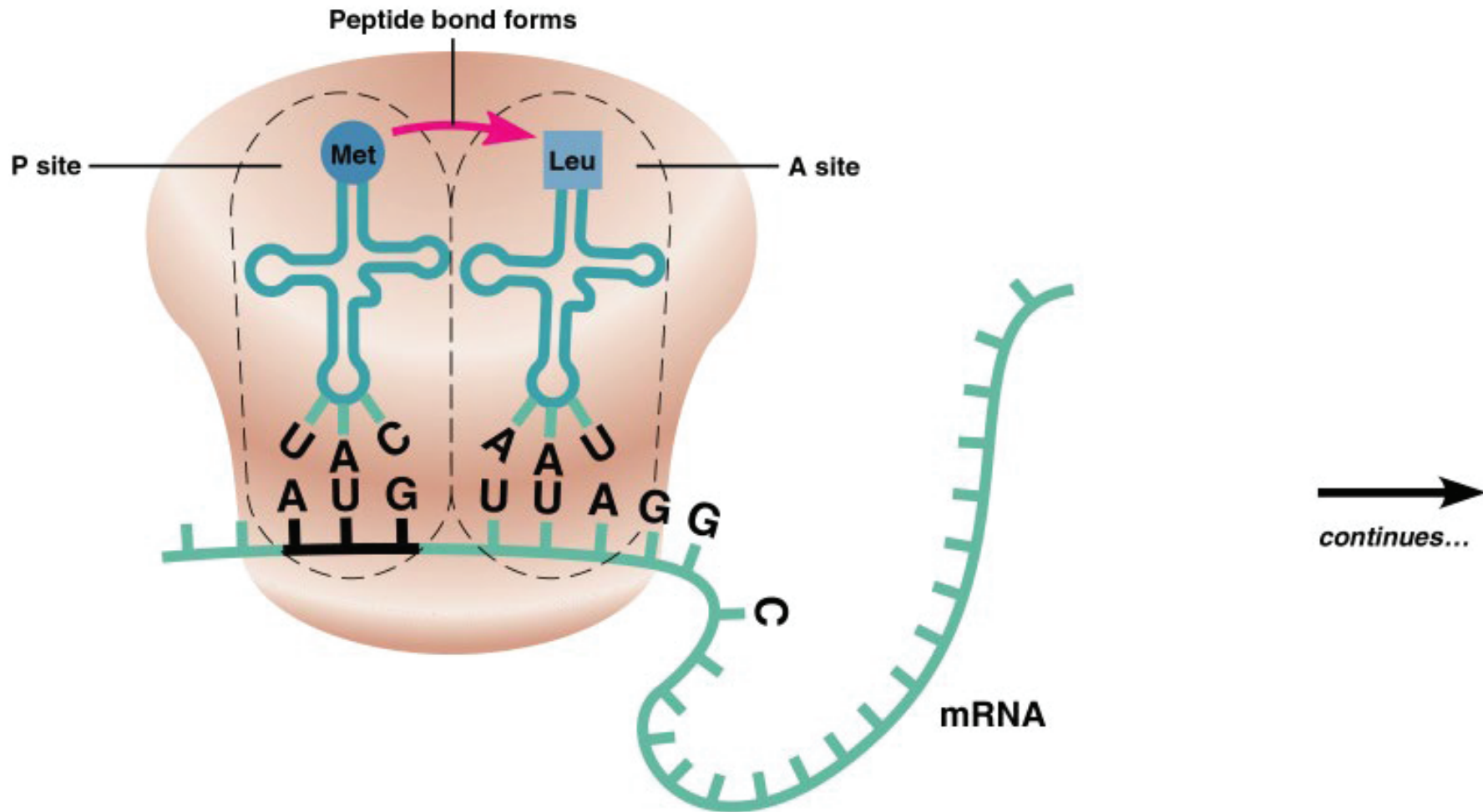
- 1 Components needed to begin translation come together.

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.

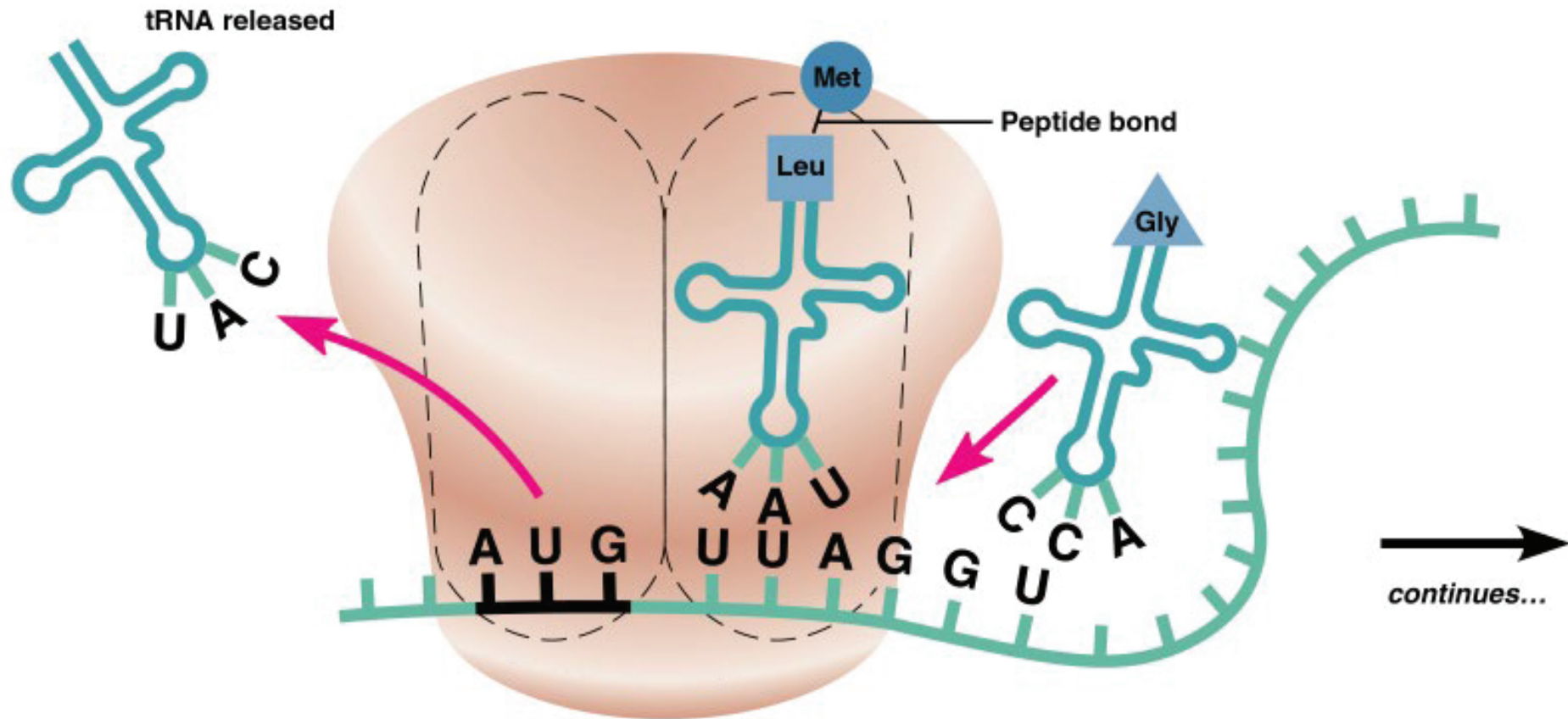
Translation



3

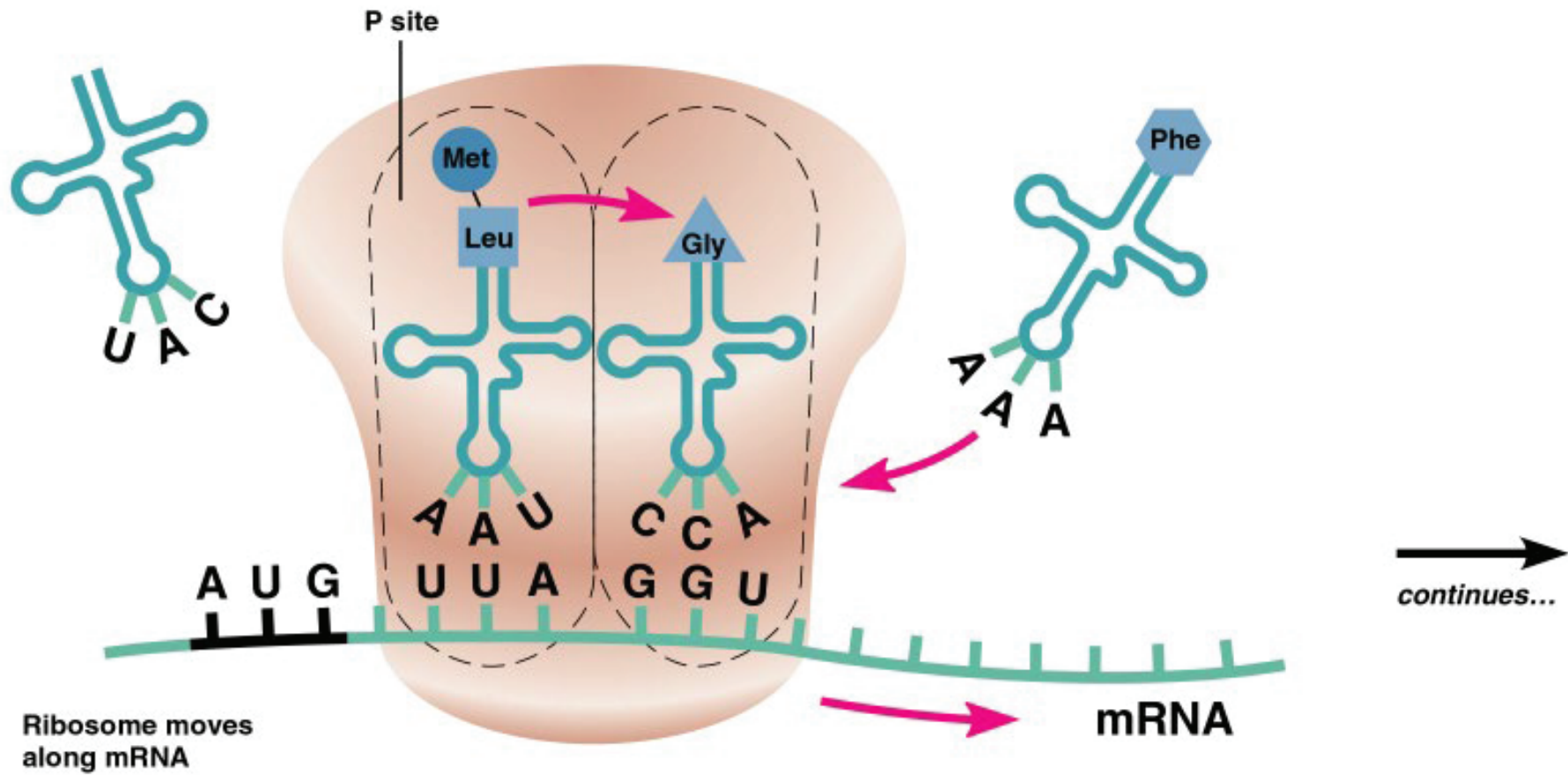
The place on the ribosome where the first tRNA sits is called the P site. In the A site next to it, the second codon of the mRNA pairs with a tRNA carrying the second amino acid.

Translation



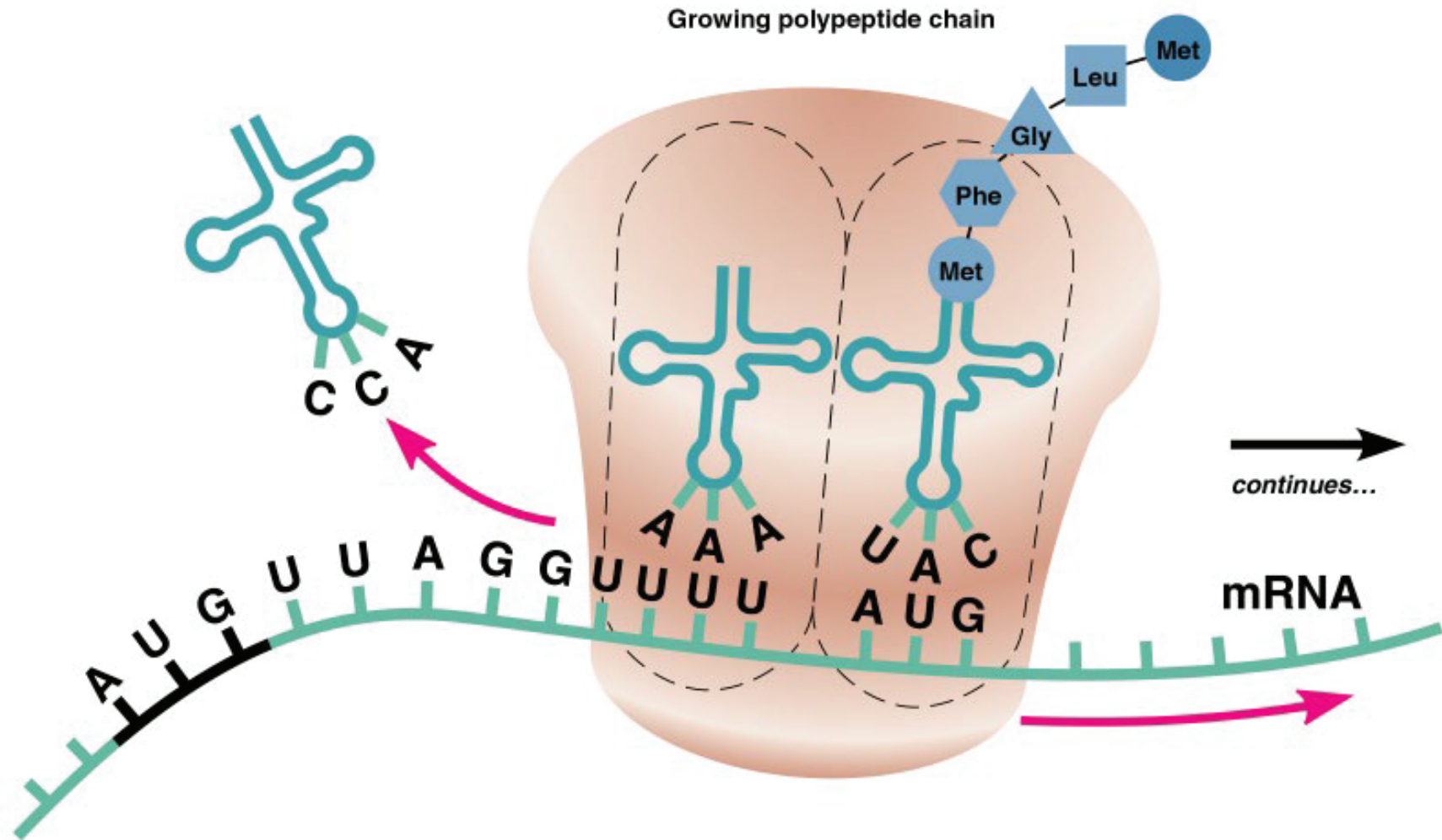
- 4 The first amino acid joins to the second by a peptide bond, and the first tRNA is released.

Translation



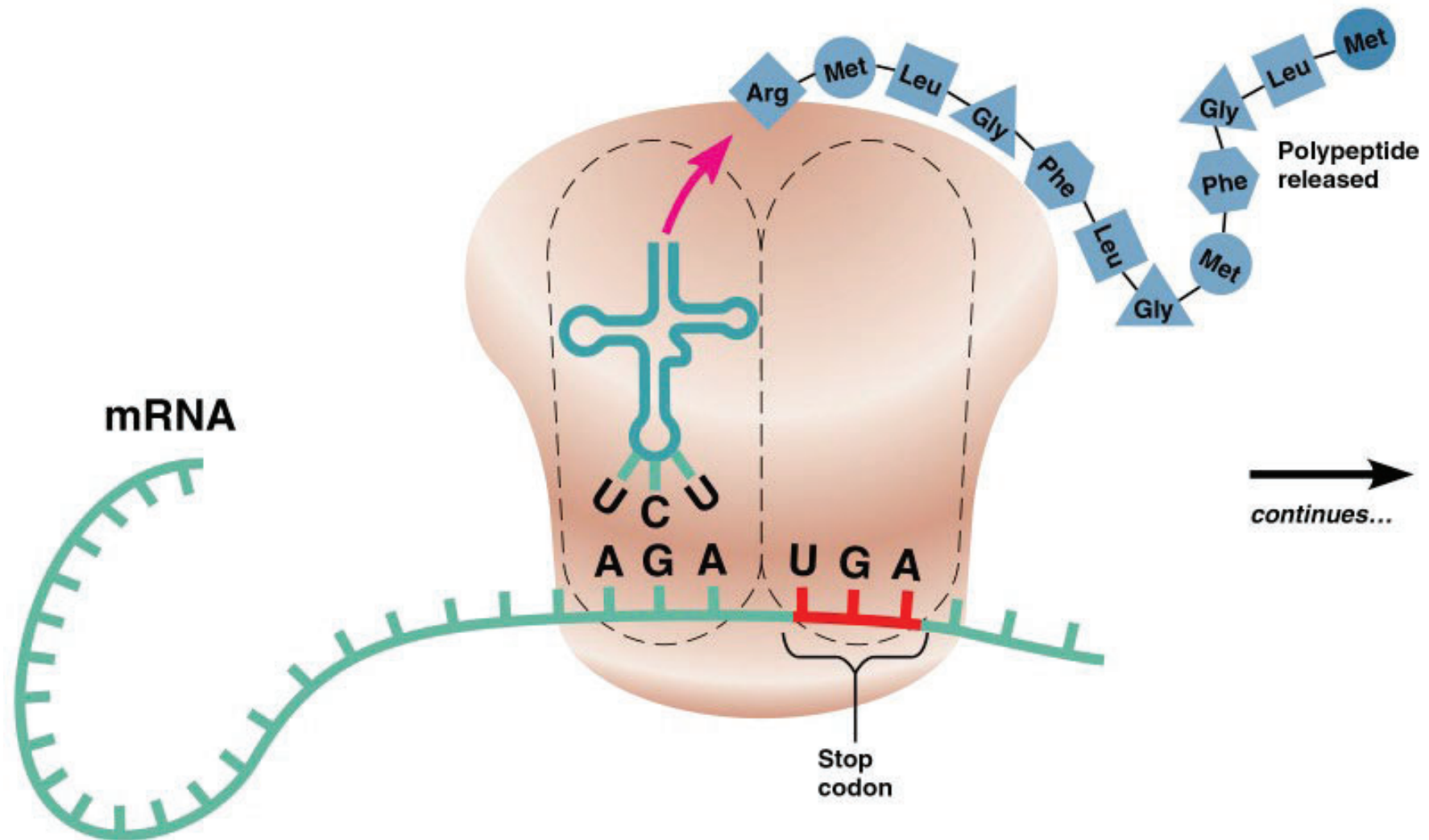
- 5 The ribosome moves along the mRNA until the second tRNA is in the P site, and the process continues.

Translation



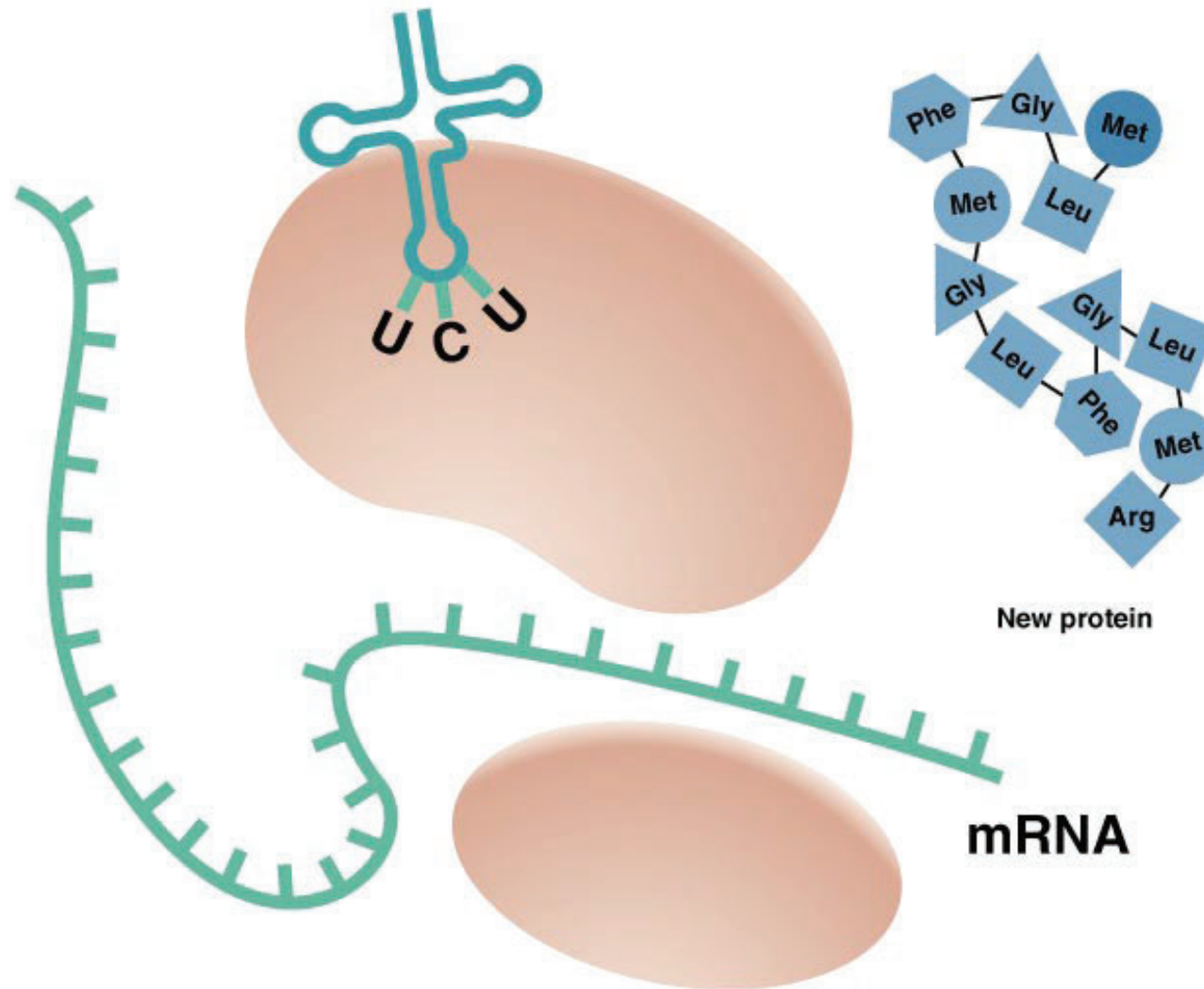
- 6 The ribosome continues to move along the mRNA, and new amino acids are added to the polypeptide.

Translation



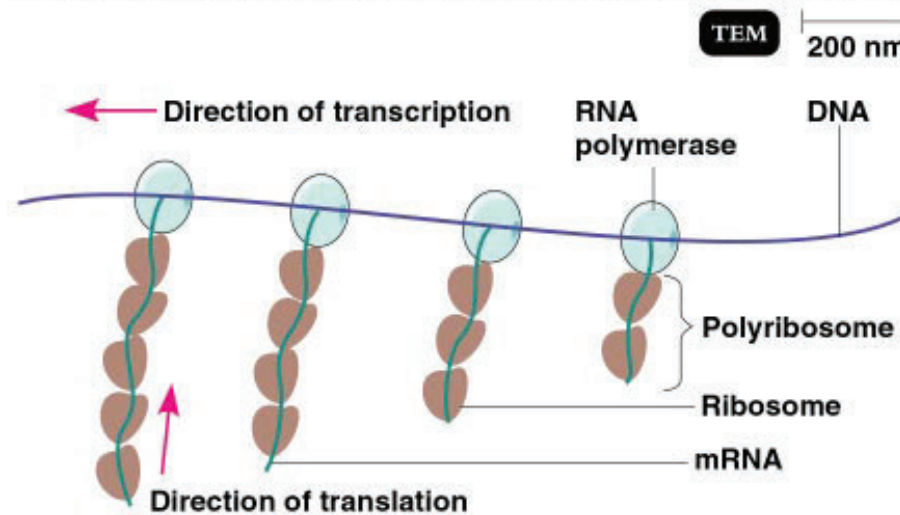
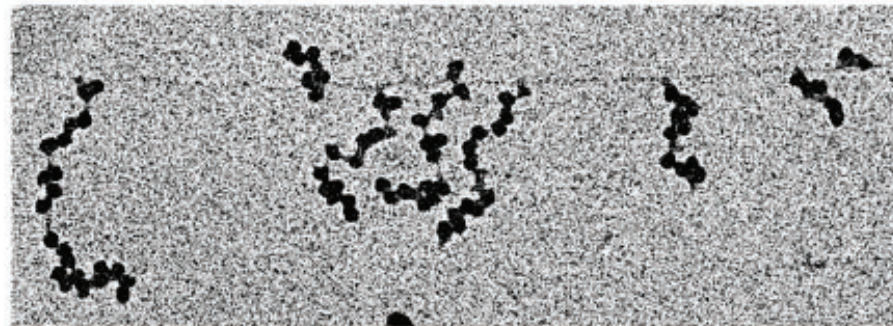
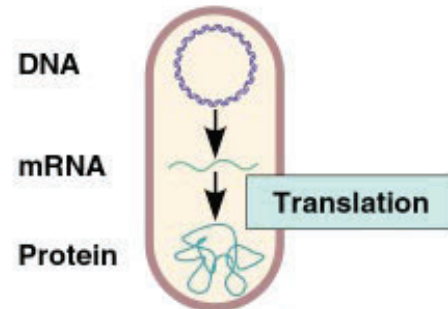
- 7 When the ribosome reaches a stop codon, the polypeptide is released.

Translation



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

Translation

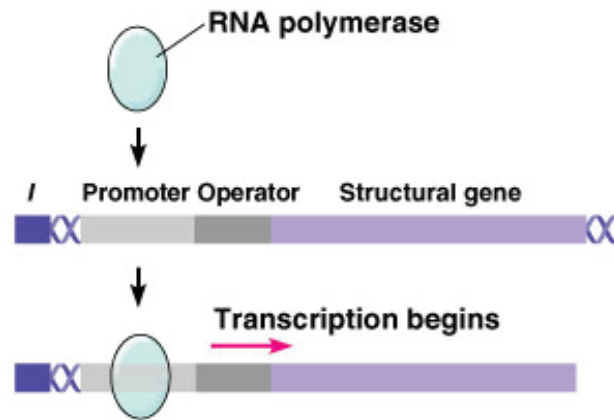


Regulation of Bacterial Gene Expression

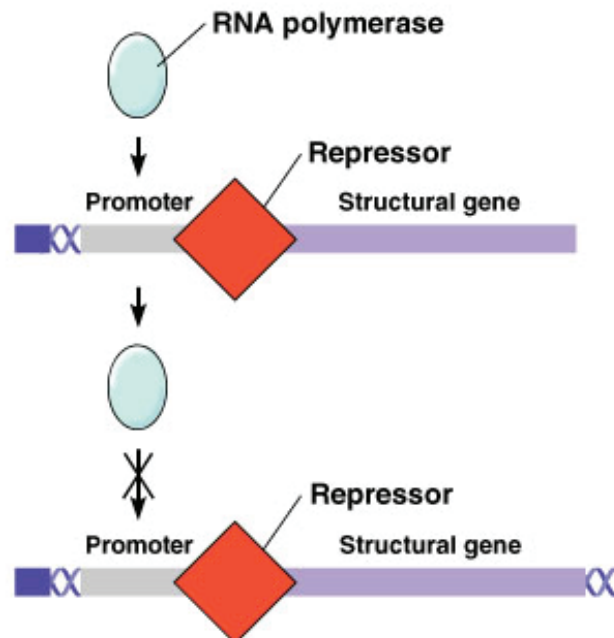
- *→ housekeeping gene*
Constitutive enzymes are expressed at a fixed rate
- Other enzymes are expressed only as needed
 - Repressible enzymes
 - Inducible enzymes

Repression

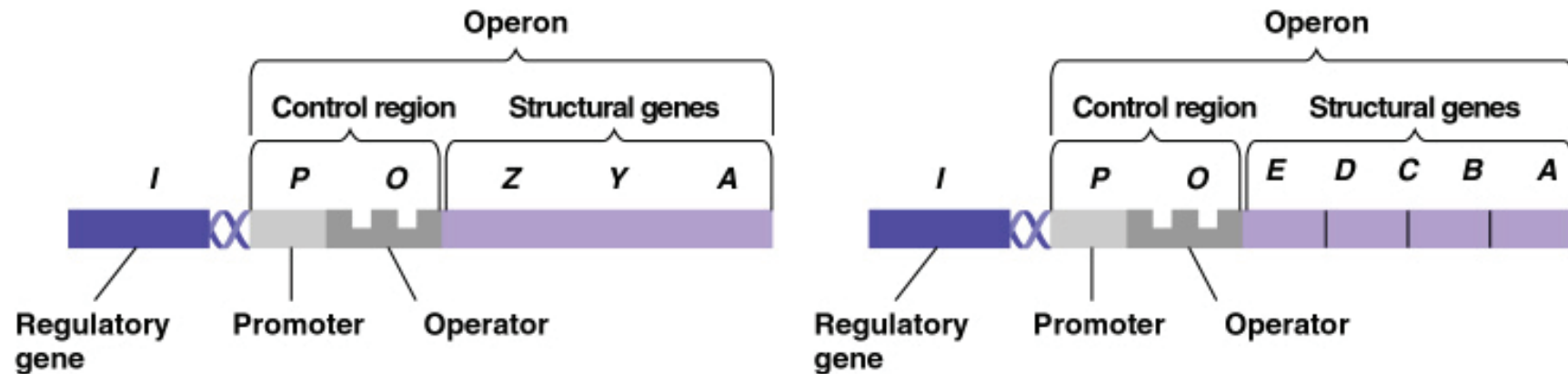
Without repressor:



With repressor:



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*).