MICROBIAL GENETICS

CHAPTER 7

MICROBIAL GENETICS

Genetics – the study of the structure of genes, their function and how genes and their cumulative effects are passed between organisms (often referred to as heredity)

Microbial Genetics

- All information for life is stored in an organism's genetic material, DNA, or for many viruses, RNA
- Heredity – the transmission of this information from an organism to its progeny (offspring)
- Chromosomes – a circular (in prokaryotes) or linear (in eukaryotes) threadlike molecule of DNA and its associated proteins

Microbial Genetics

- **Plasmids** – an extra-chromosomal circular strand of DNA that contains genetic information which supplements information in the chromosome.
  - it is self-sufficient in that it can replicate and transcribe mRNAs on its own.
  - plasmids may be gained or lost without harm to the cell
  - under certain conditions, however, plasmids are an advantage to cells.
  - plasmids may carry genes for such activities as antibiotic resistance, tolerance to toxic metals, the production of toxins and the synthesis of enzymes.

- **Genome** – the total sum of an organisms genetic information

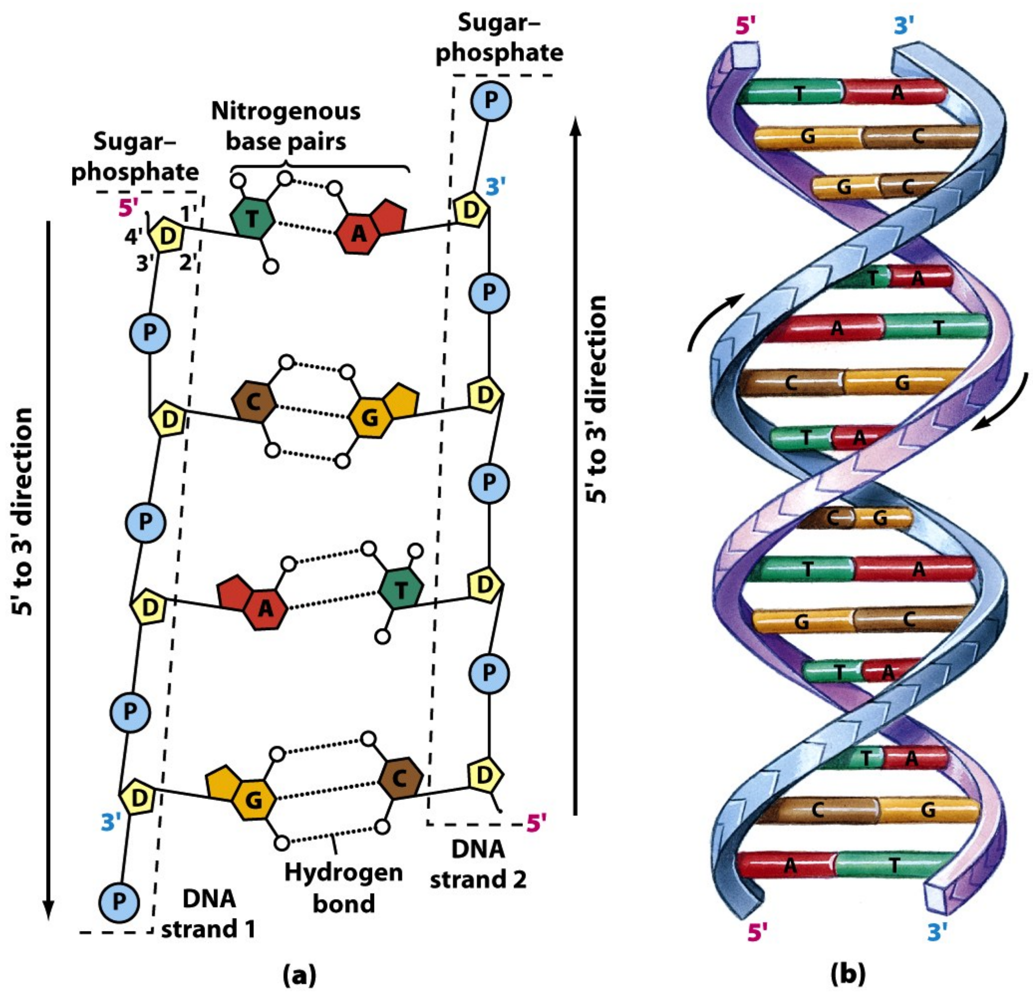
DNA STRUCTURE

- **DNA** - double chain of nucleotides

- Nucleotide - made up of sugar (5C sugar deoxyribose), a phosphate, and a base (adenine, guanine, cytosine or thymine)

- Arrangement – nucleotides arranged in helix, with the nucleotide base pairs held together by hydrogen bonds

DNA STRUCTURE



Microbial Genetics

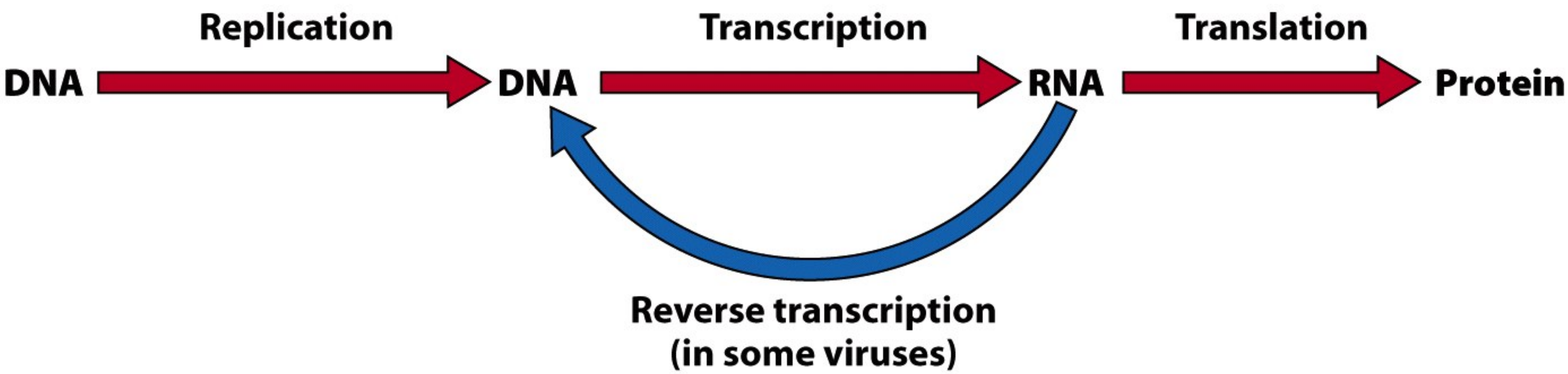
- **Gene**
  – a linear sequence of nucleotides
  - encoded in it are the information for the structure and function of an organism
- **Allele**
  - different molecular forms of the same gene
- **Mutation**

  - is a permanent alteration in DNA

  - mutations usually change the sequence of nucleotides in DNA and thereby change the information in the DNA

  - when the mutated DNA is passed on to the daughter cell, the daughter cell can be different from the parent cell in one or more characteristics

- **Genetic information transfer**



DNA Replication (semi-conservative)

- Structurally, dsDNA is referred to as antiparallel, whereby the 2 strands are parallel but oriented in opposite directions.

  - DNA strands have direction, or orientation.

  5' to 3' direction refers to carbon 5 and carbon 3 of the deoxyribose molecule

  - the 2 strands are held together by H-bonds between the bases adenine and thymine **(A-T),** and cytosine and guanine **(C-T).**

DNA Replication (semi-conservative)

- The process of **replication** always starts at a particular site in the DNA referred to as the origin of replication site, or OriC

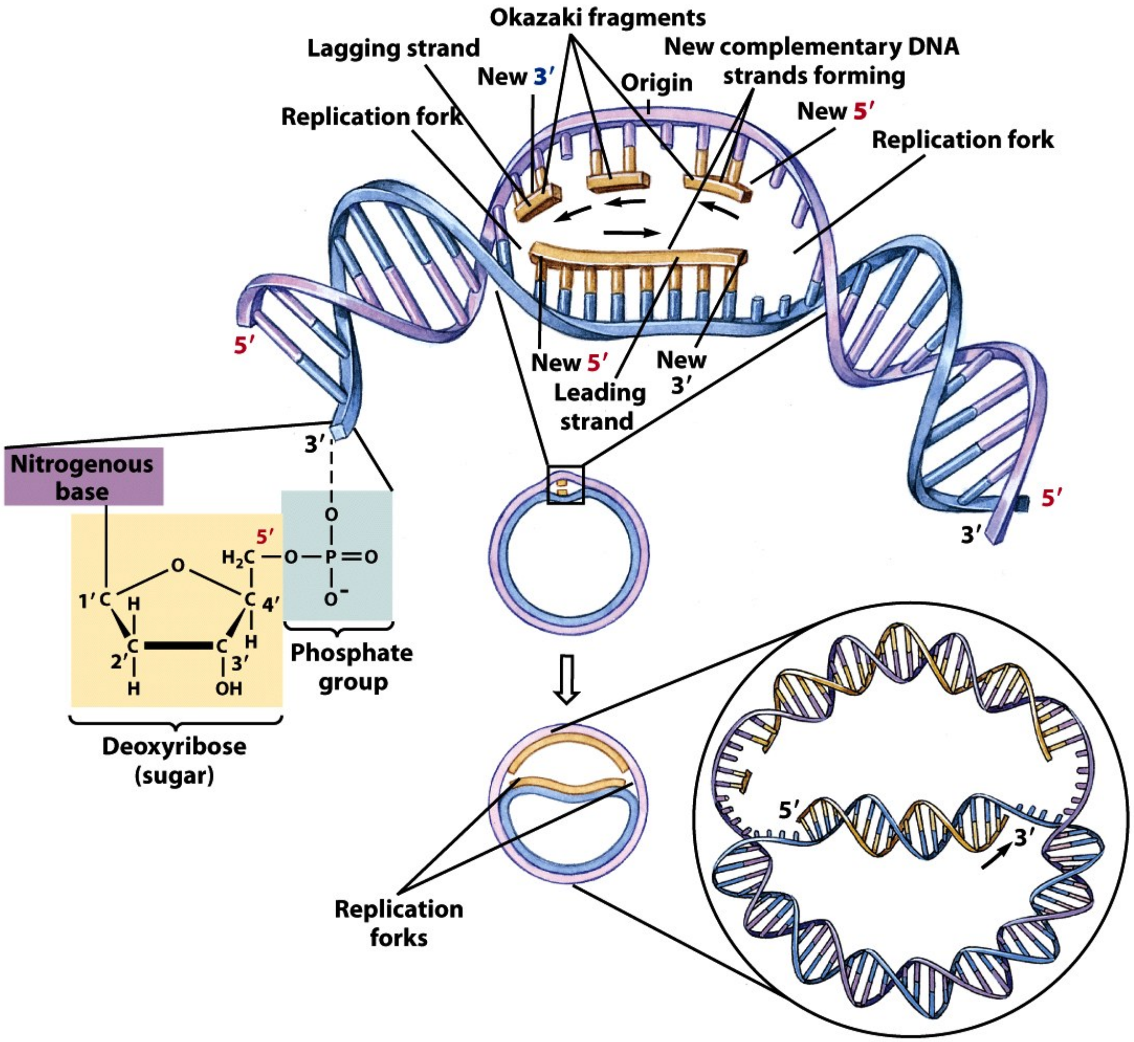
  - Helicase splits the dsDNA (by breaking the H-bonds) into 2 strands, creating 2 replication forks traveling in opposite direction

  -DNA polymerase then binds each strand and synthesizes a compliment strand in 5' to 3' direction. DNA polymerase can synthesize one continuous complimentary strand, leading strand

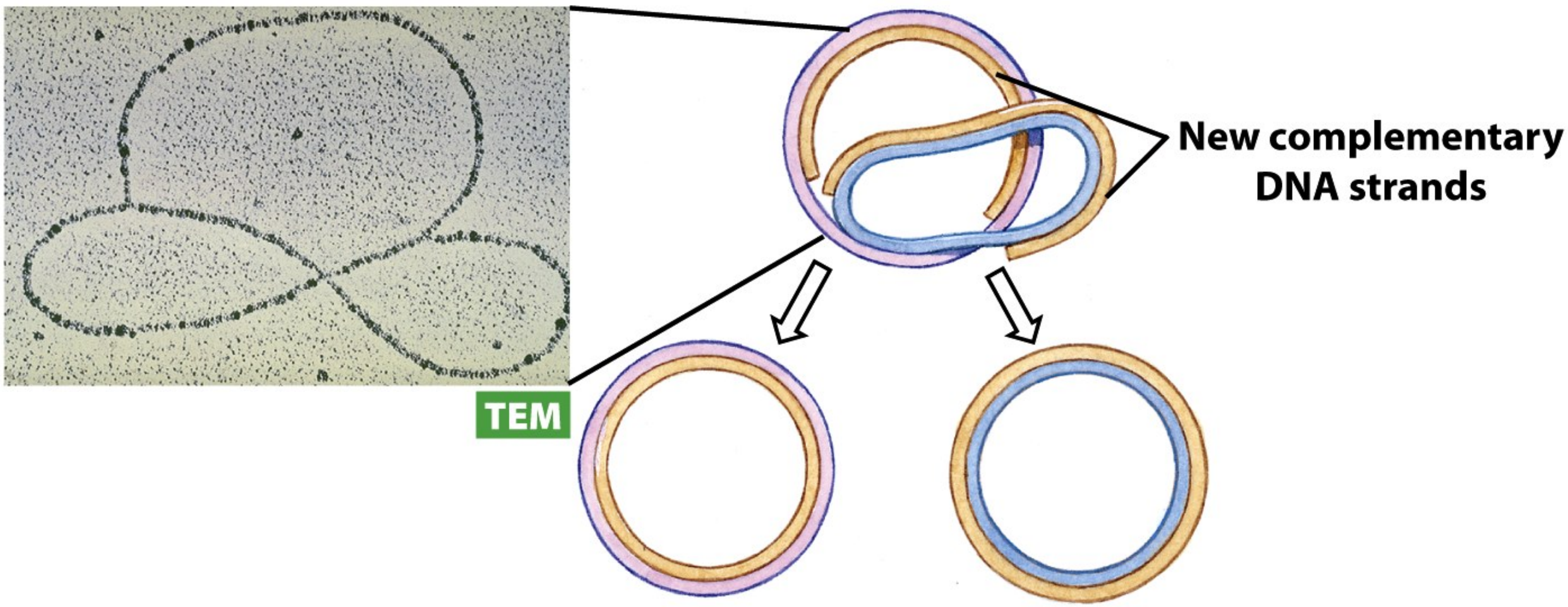
  -The lagging strand is synthesized discontinuously as short fragments (Okazaki fragments) and then extended by DNA polymerase

  -Ligase then links the Okazaki fragments making one continuous strand

DNA Replication (semi-conservative)



DNA Replication (semi-conservative)

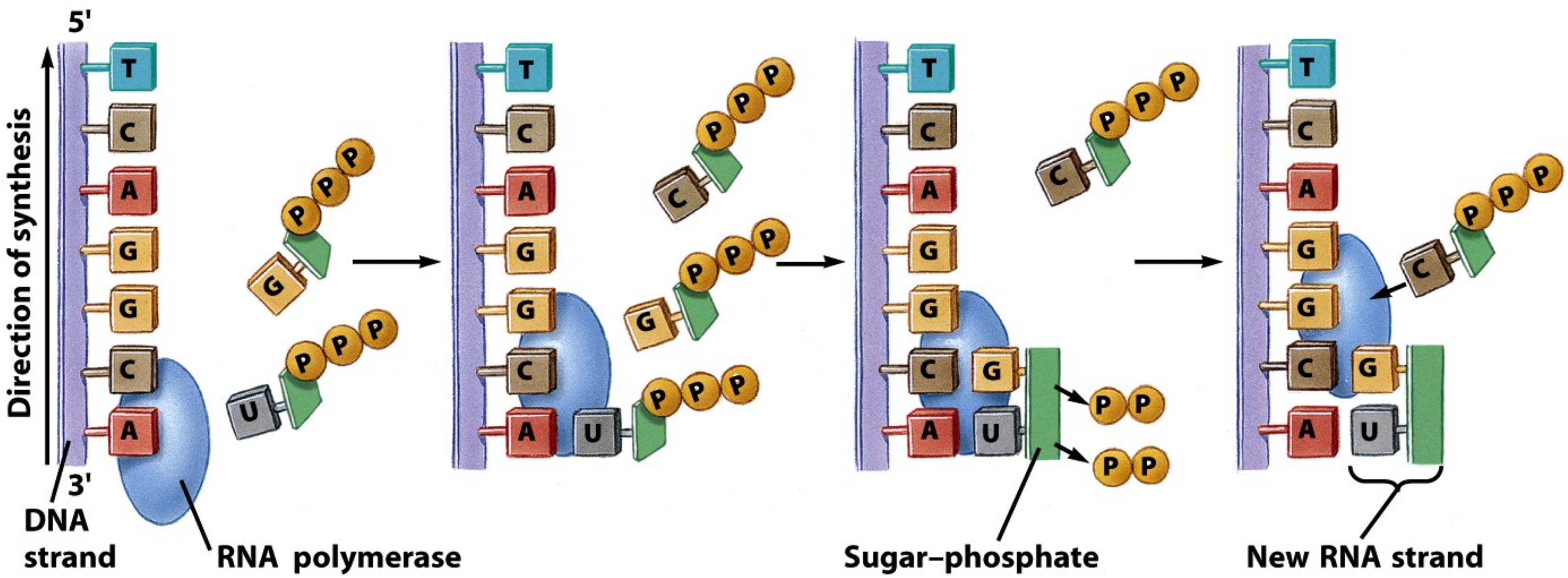


Transcription

Transcription is the synthesis of the complimentary strand of RNA from the DNA template

- only one strand of dsDNA can be used as the coding region for a gene since each strand reads the opposite nucleotides and therefore different codons
- enzyme RNA polymerase binds to one a sequence of DNA bases that indicates the start of the gene (promoter sequence)
- the transcript is called mRNA (messenger RNA)
- during transcription, the base **uracil** pairs with adenine and cytosine with guanine
- mRNA is synthesized in the 5’ to 3’ direction
- the newly formed transcript is released from the template DNA

Transcription



Kinds of RNA

- Three kinds of RNA participate in <u>protein synthesis</u>

  - ribosomal RNA (rRNA)

  - messenger RNA (mRNA)

  - transfer RNA (tRNA)

Kinds of RNA

- **ribosomal RNA (rRNA)**

  - binds closely to certain proteins to form two kinds (30S and 50S) of ribosome subunits. A subunit of each kind combines to form a ribosome, sites of protein synthesis

  - serve as binding sites for tRNA, and some of their proteins act enzymes that control protein synthesis

  - after the two subunits join together around the strand of mRNA, the synthesis of a peptide begins

ribosomal RNA (rRNA)

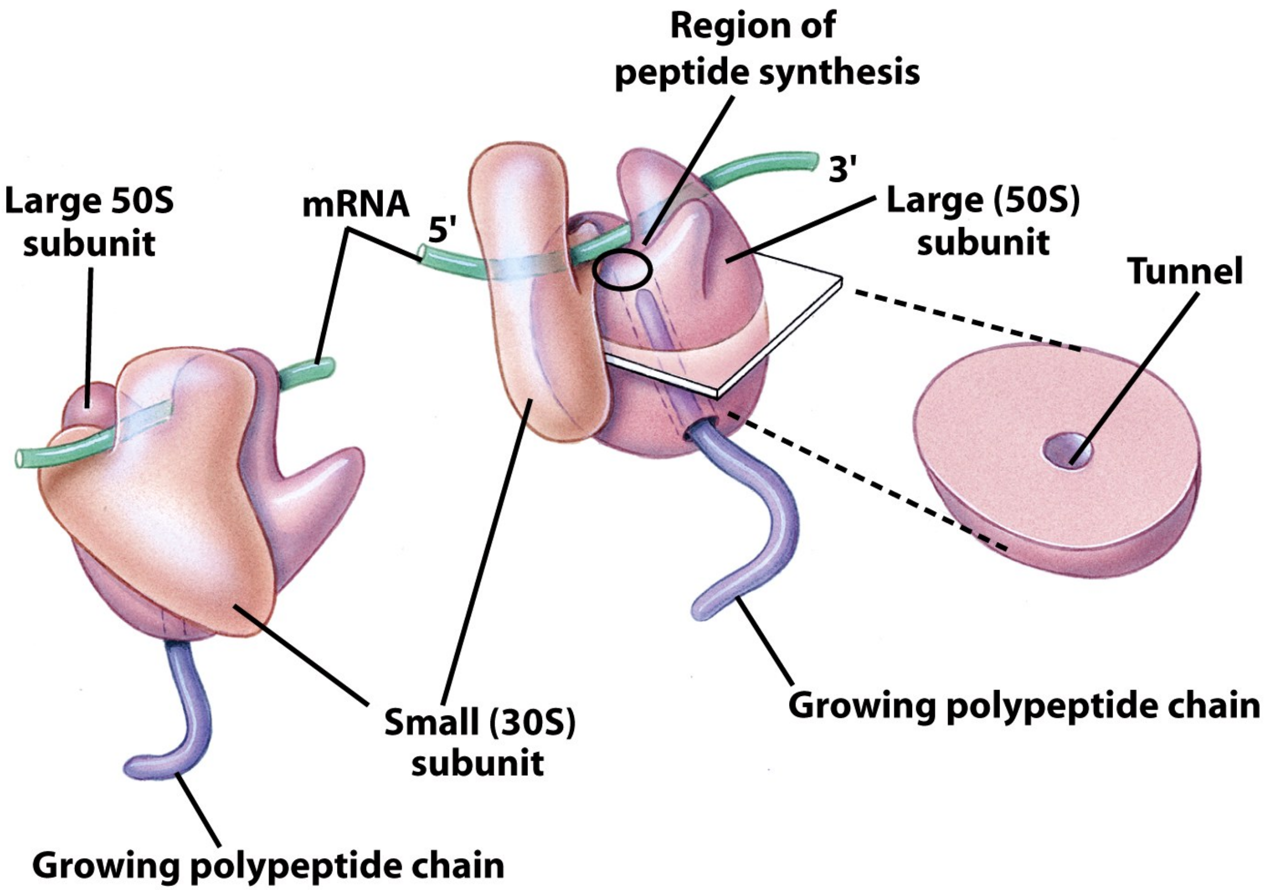


Figure 7-7 Microbiology, 7/e

Kinds of RNA

- messenger (mRNA)

  - is synthesized in units that contain sufficient information to direct the synthesis of one or more polypeptides chains

  - one mRNA transcript corresponds to one or more genes

  - the information coded in mRNA acts during translation to dictate the sequence of amino acids in the protein

  - each triplet (sequence of three bases) in mRNA constitutes a **codon.**

  - each codon specifies a particular amino acid or acts as a terminator of translation

  - the first codon **AUG** acts as a start codon and codes for amino acid methionine

  - **codon** **(UAA, UAG or UGA)** act as a terminator or stop codon

Genetic Code

First position	Second position: U		Second position: C		Second position: A		Second position: G		Third 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	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

Kinds of RNA

- **transfer RNA (tRNA)**
  - transfers amino acids from the cytoplasm to the ribosomes
  - consists of 75 to 80 nucleotides and is folded back on itself to form several loops that are stabilized by complementary base pairing
  - each tRNA has a three-base **anticodon**, that is complementary to a particular mRNA codon
  - It also has a binding site for a an amino acid –the particular amino acid specified by the mRNA codon
  - The anticodon attaches by complementary base pairing to the appropriate mRNA codon

Transfer RNA (tRNA)

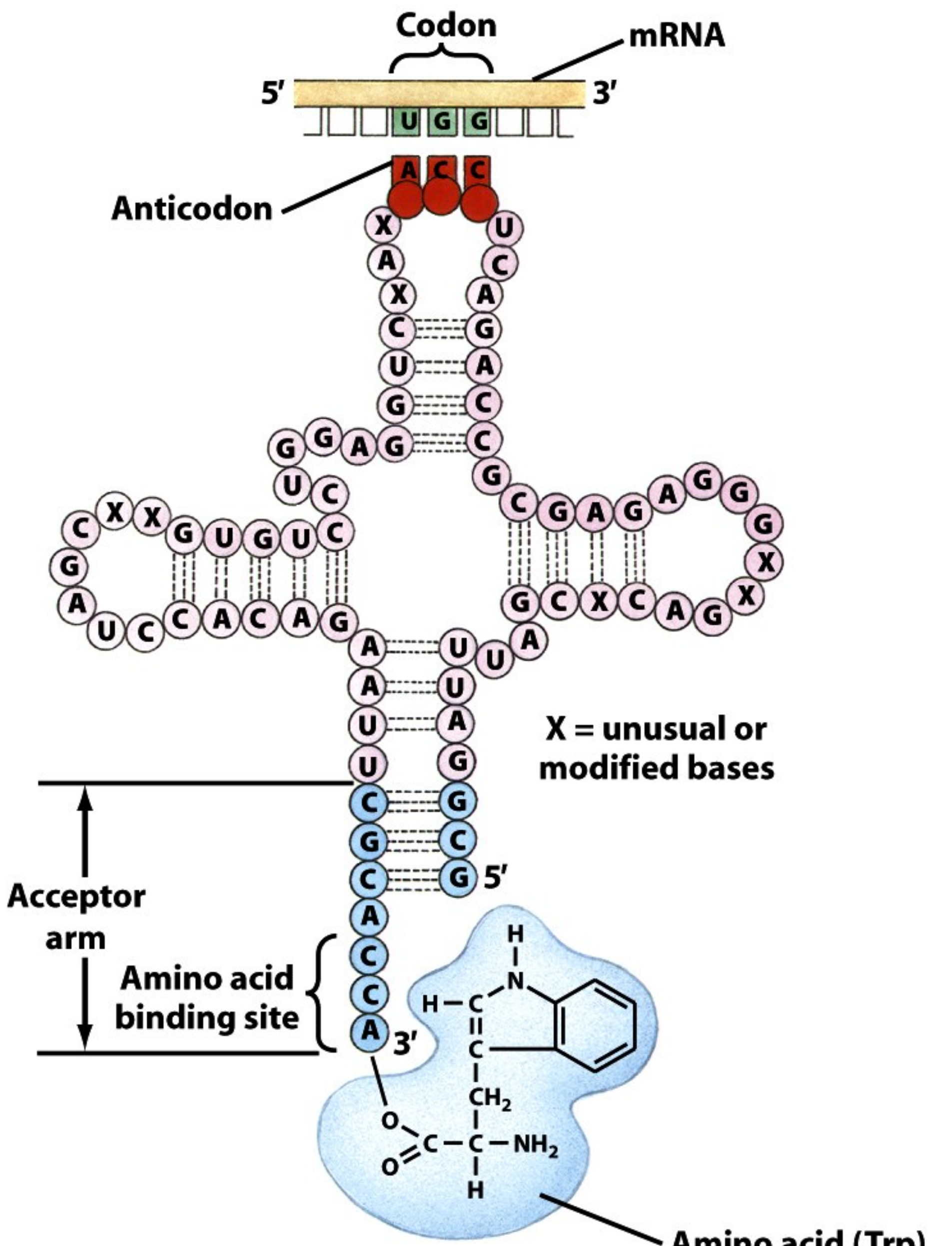


Figure 7-9a Microbiology, 7/e

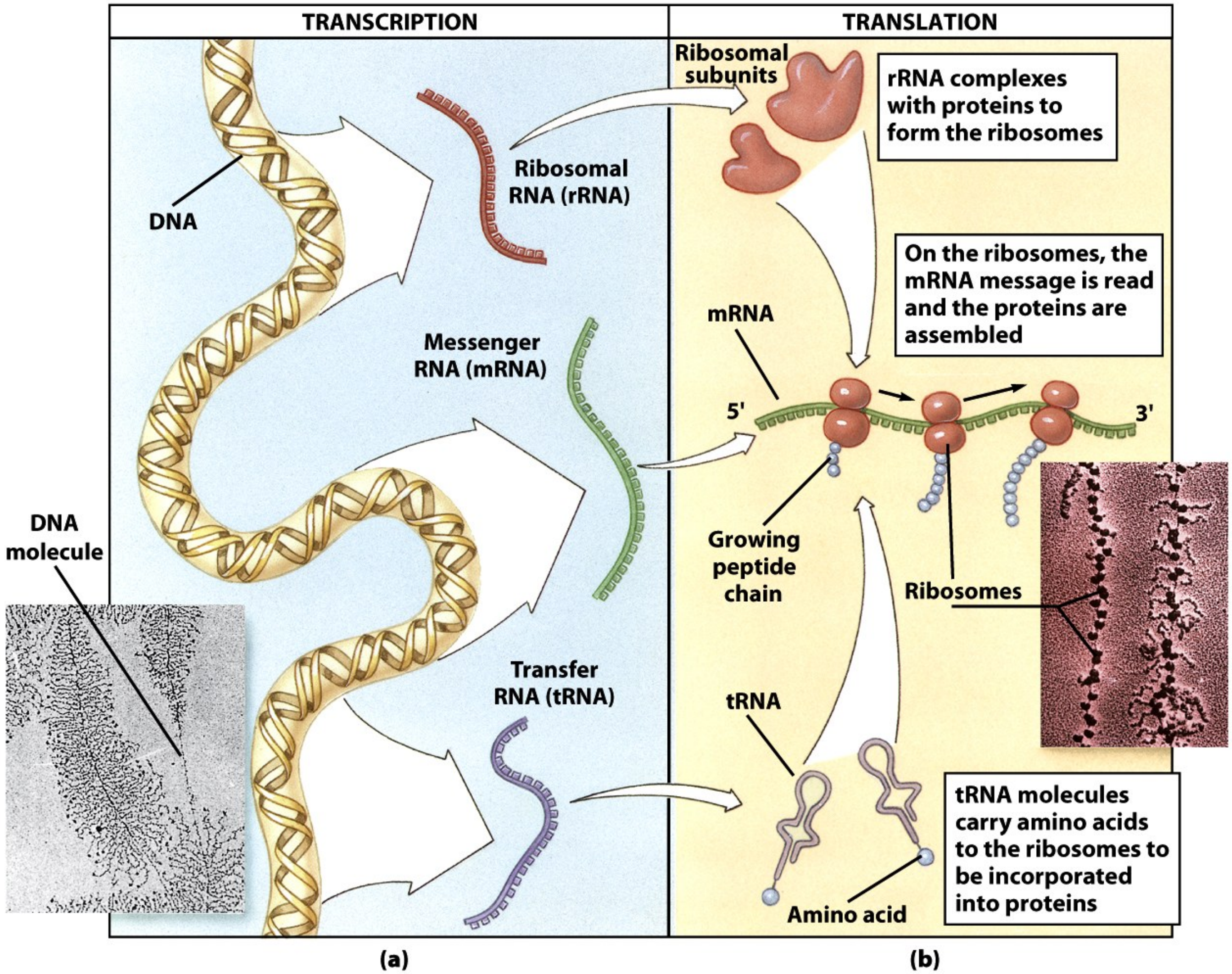


Figure 7-10 Microbiology, 7/e

(a) Fron O. L. Miller, Jr., and B. R. Beatty, *Journal of Cellular Physiology* 74 (1969); (b) E. Kiselva & D. Fawcett/Visuals Unlimited

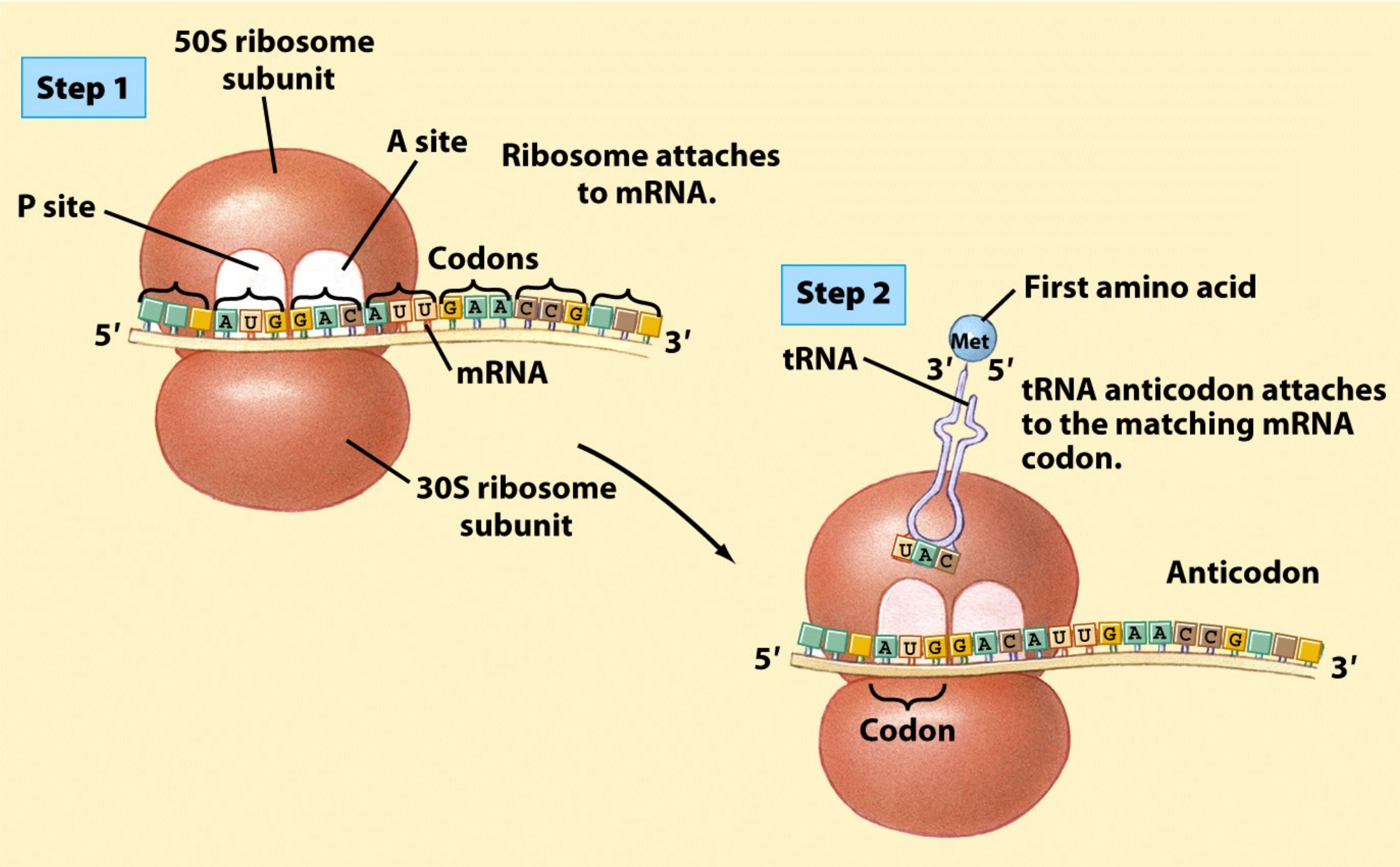


Figure 7-12 part 2 Microbiology, 7/e

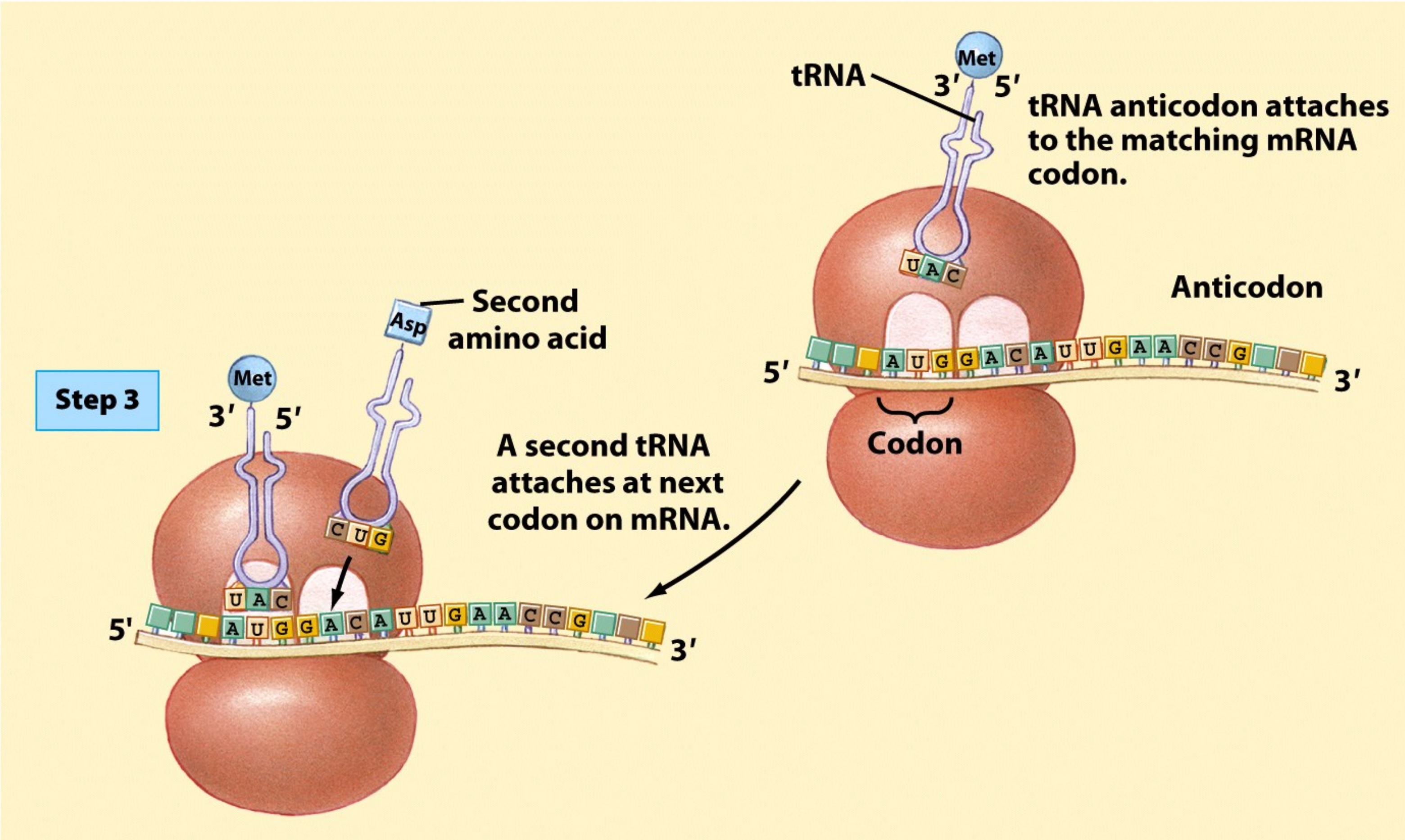


Figure 7-12 part 3 Microbiology, 7/e

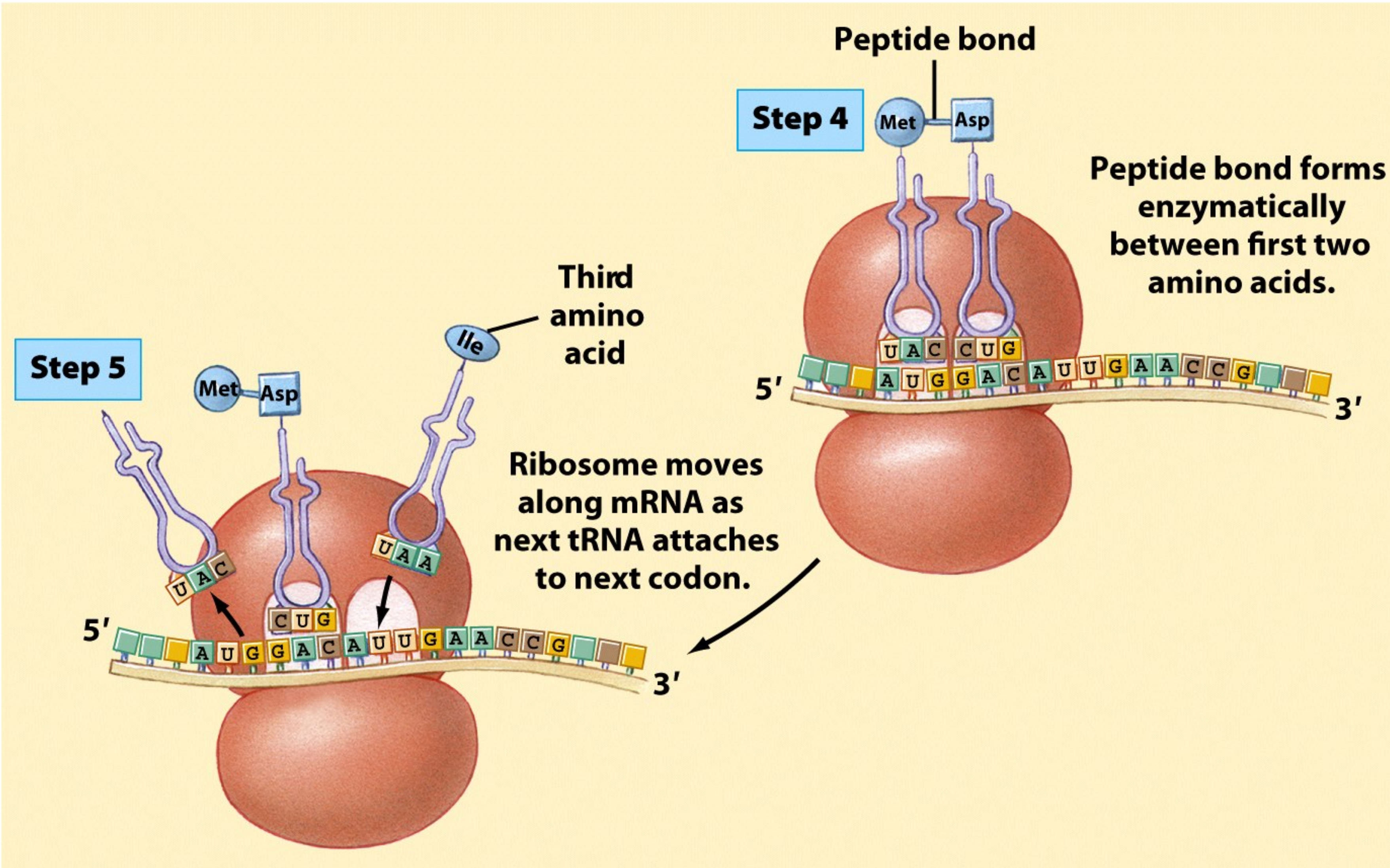


Figure 7-12 part 5 Microbiology, 7/e

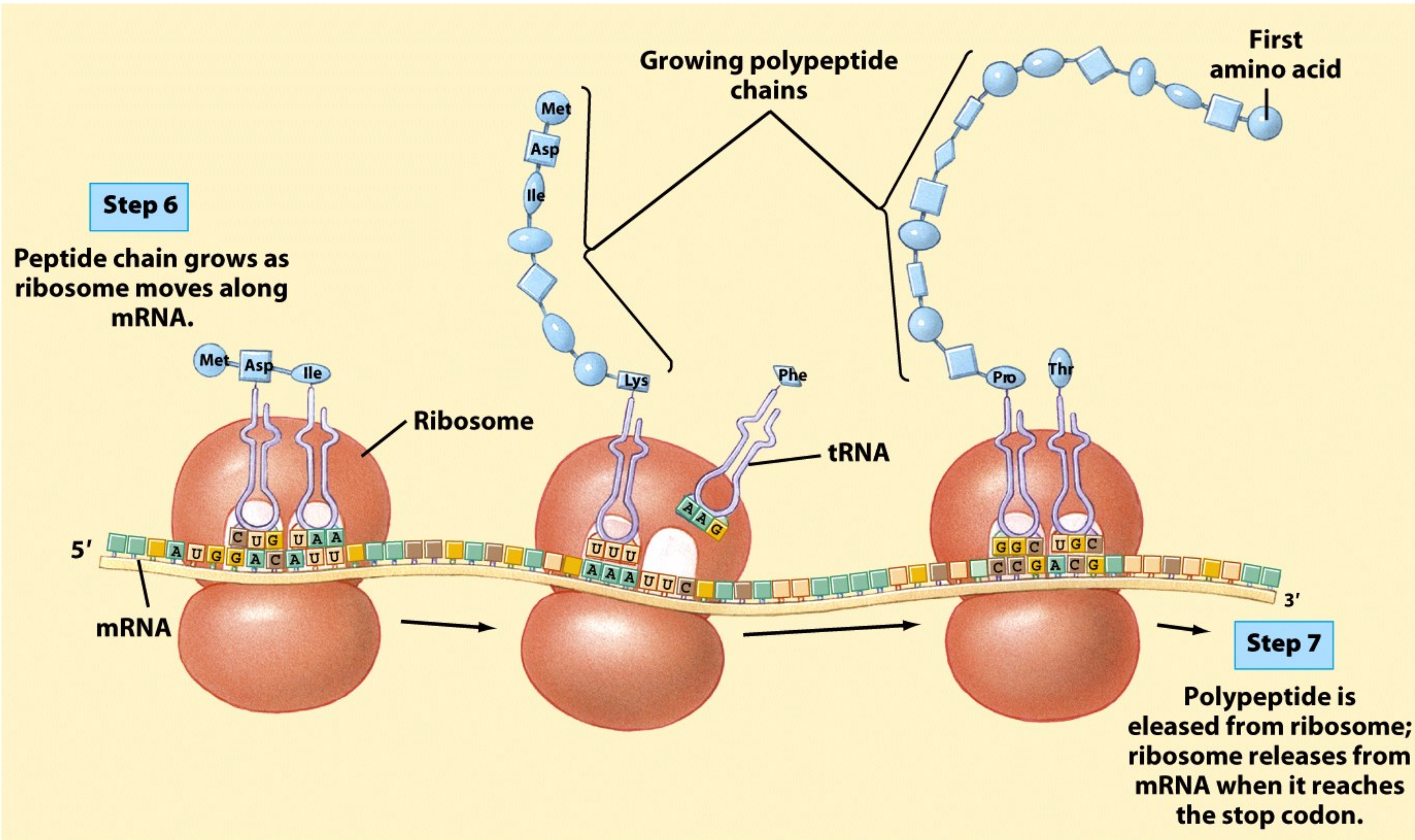


Figure 7-12 part 6 Microbiology, 7/e

Regulation of Metabolism

- **Categories of regulatory mechanisms**

  - The mechanisms that control metabolism either regulate enzyme activity directly or regulate the synthesis of enzymes by turning on or off genes that code for particular enzymes. The three well study mechanisms are:

    - feedback inhibition
    - enzyme induction
    - enzyme repression

Regulation of Metabolism

- **Feedback inhibition**
  - also called end-product inhibition
  - the end-product of a biosynthetic pathway directly inhibits the first enzyme in the pathway
  - because feedback inhibition acts quickly and directly on a metabolic process, it allows the cell to conserve energy
  - Requires less energy than the more complex processes that regulate gene expression

Regulation of Metabolism

- **Feedback inhibition**

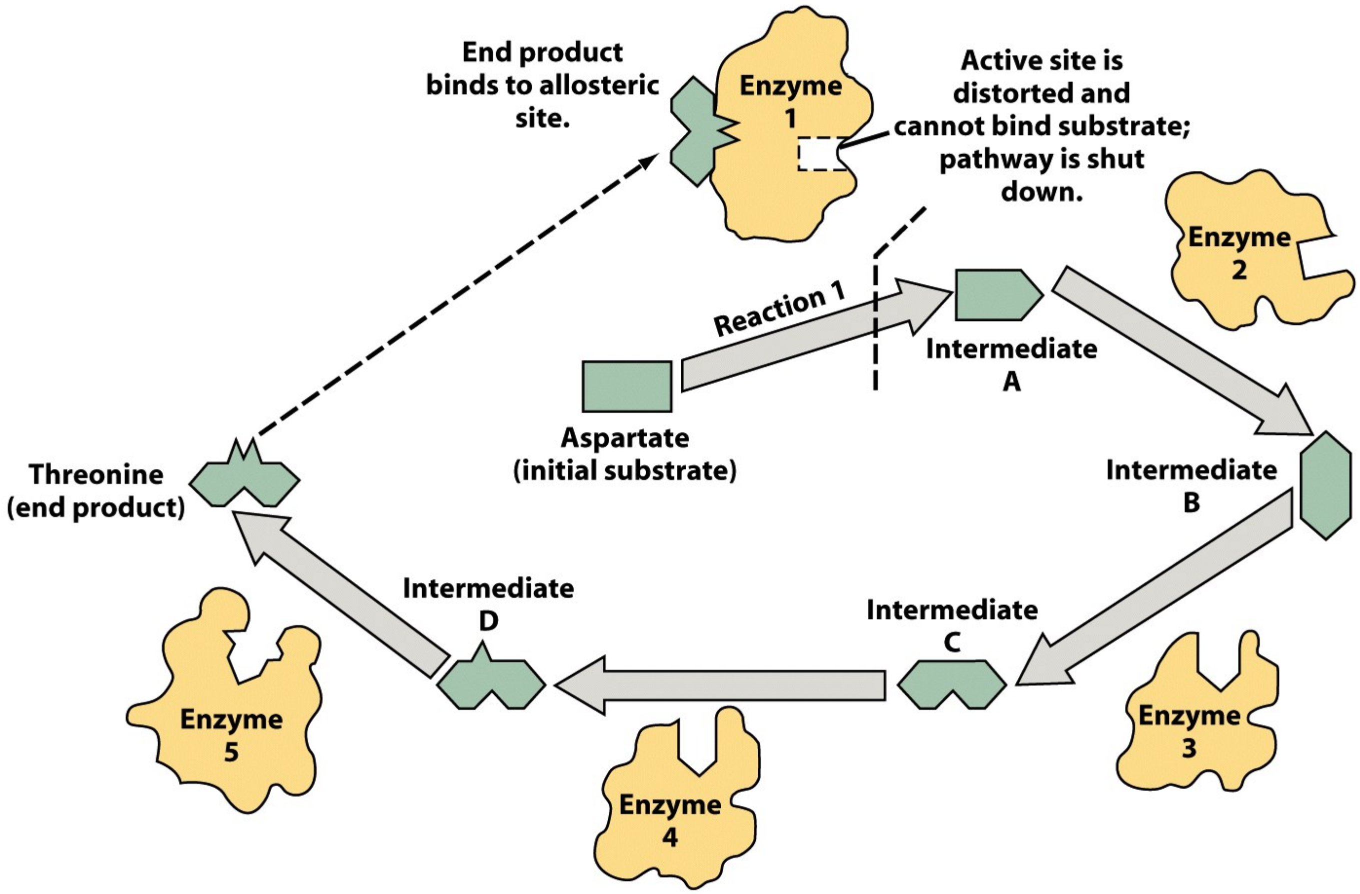


Figure 7-13 Microbiology, 7/e

- **Constitutive enzymes**
  - enzymes that are synthesized continuously regardless of the nutrients available to the organisms
  - the genes that make these enzymes are always active

- **Inducible enzymes**
  - enzymes that are synthesized by genes that are sometimes active and sometimes inactive, depending on the presence or absence of substrates

- **Repression**
  - is a regulatory mechanism that inhibits gene expression and decreases the synthesis of enzymes
  - is usually a response to the overabundance of end product of a metabolic pathway
  - mediated by regulatory proteins called **repressors**, which block the ability of RNA polymerase to initiate transcription from the repressed genes
- **Induction**
  - Process that turns on the transcription of gene or genes
  - Substances that induce gene transcription are called **inducers** and the enzymes that are synthesized in the presence of inducers are called inducible enzymes

Regulation of Metabolism

- **Operon model of gene expression**
  - A model that explains the regulation of some protein synthesis in bacteria
  - An operon includes one or more **structural genes**, which carry information for the synthesis of specific proteins such as enzyme molecules, and the **operator** and **promoter**
  - The **operator** region is a short sequence that repressor-proteins and inducer-proteins bind to in order to prevent or enhance transcription
  - The **promoter** region is a common sequence that RNA polymerase recognizes, binds and begins transcription from
  - The **structural** region of the gene is the actual coding-sequence, which will represent the transcribed mRNA sequence

Regulation of Metabolism

- **Enzyme induction (*lac* operon)**

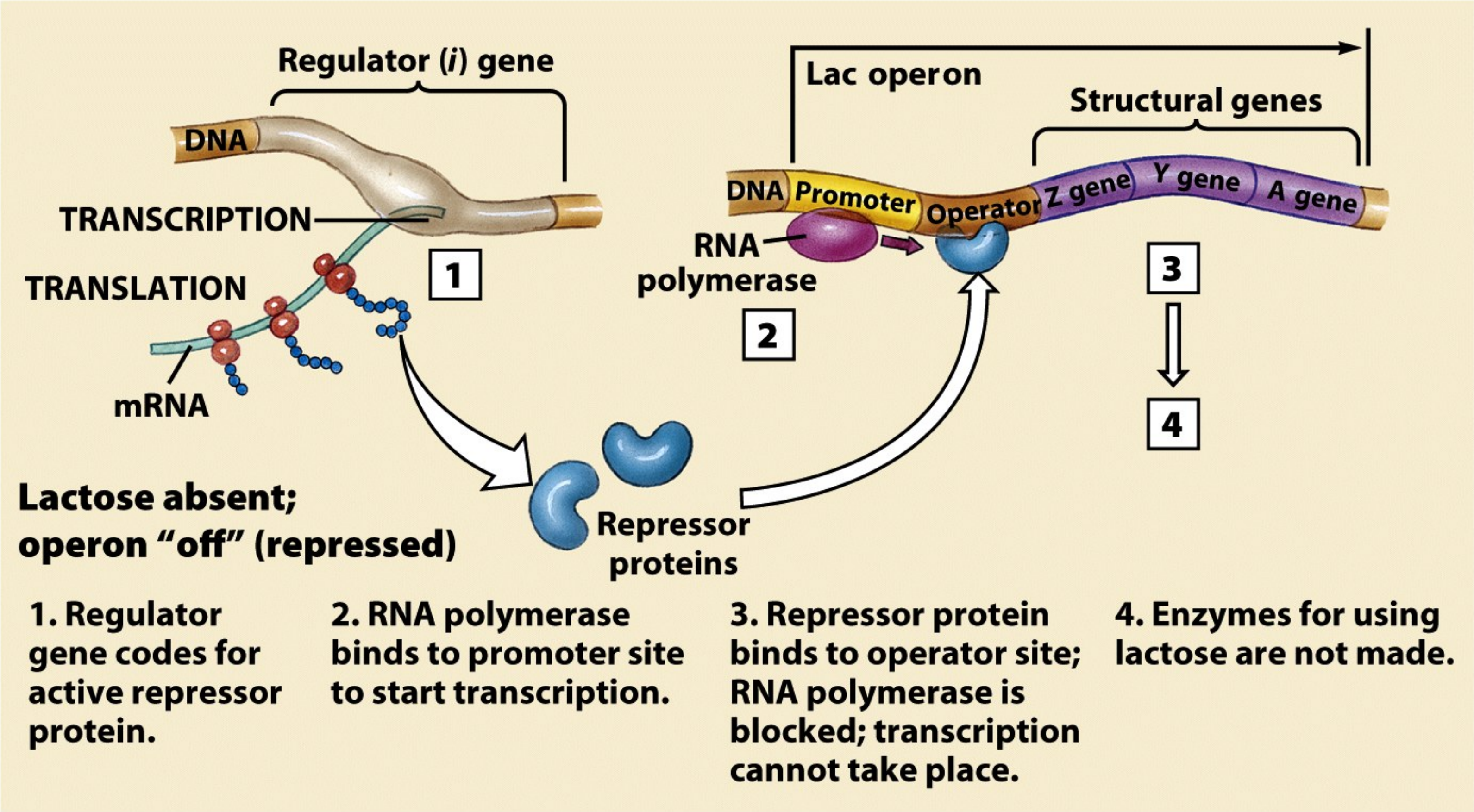


Figure 7-14a Microbiology, 7/e

Regulation of Metabolism

- **Enzyme induction (*lac* operon)**

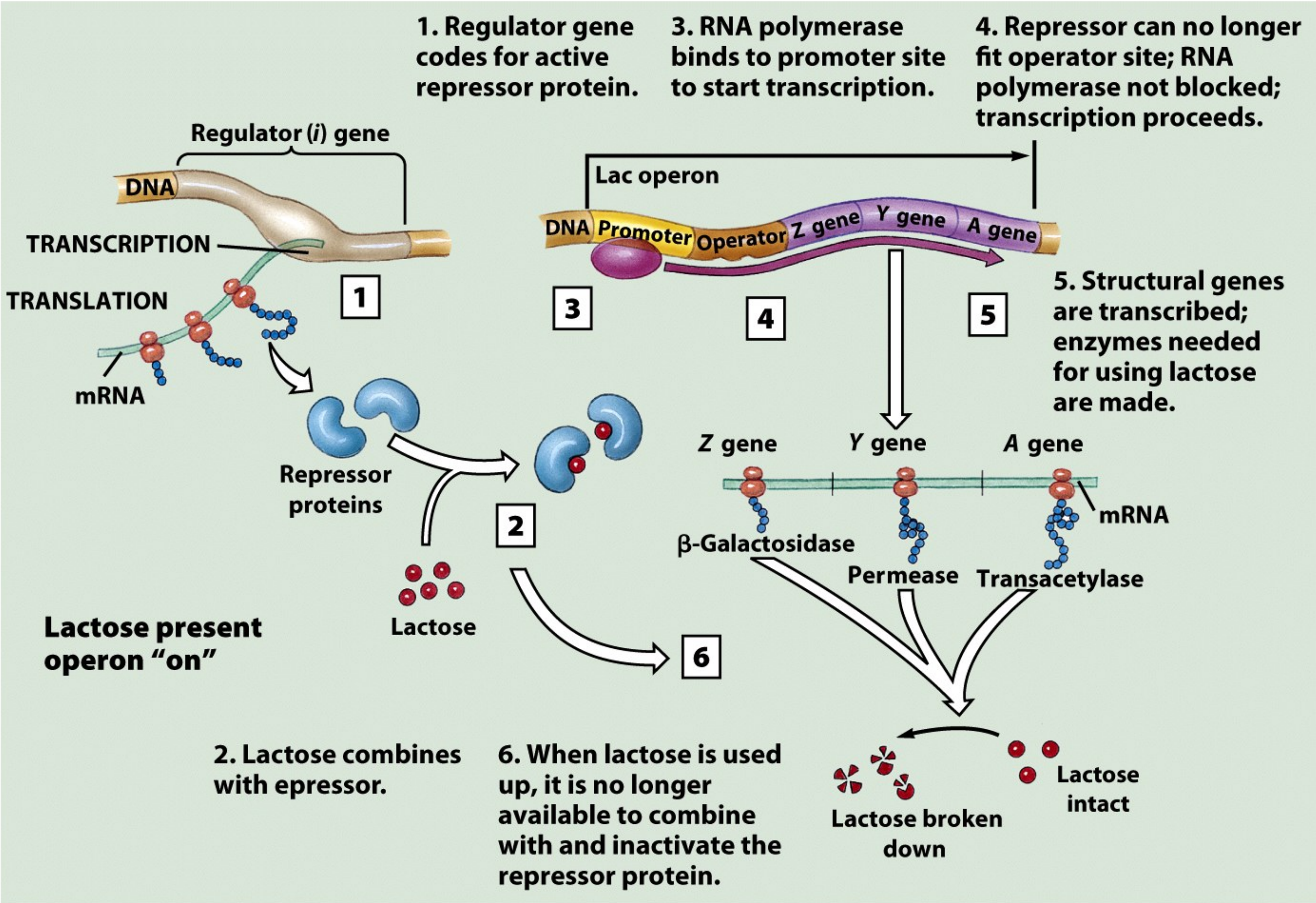


Figure 7-14b Microbiology, 7/e

Regulation of Metabolism

- **Enzyme repression** (*trp* operon)
  - the structural genes are transcribed until they are turned off, or ***repressed****.*
  - genes for enzymes involved in the synthesis of tryptophan are regulated by this mechanism
  - When excess tryptophan is present, the tryptophan acts as a **corepressor** binding to the **repressor** protein
  - The repressor protein then binds to the operator and stops further tryptophan synthesis

- **Genotype**
  - refers to the genetic information contained in the DNA of the organism

- **Phenotype**
  - refers to the specific characteristics displayed by the organisms

  - **Mutations** always change the genotype
  - such a change may or may not be expressed in the phenotype, depending on the nature of the mutation

Mutations

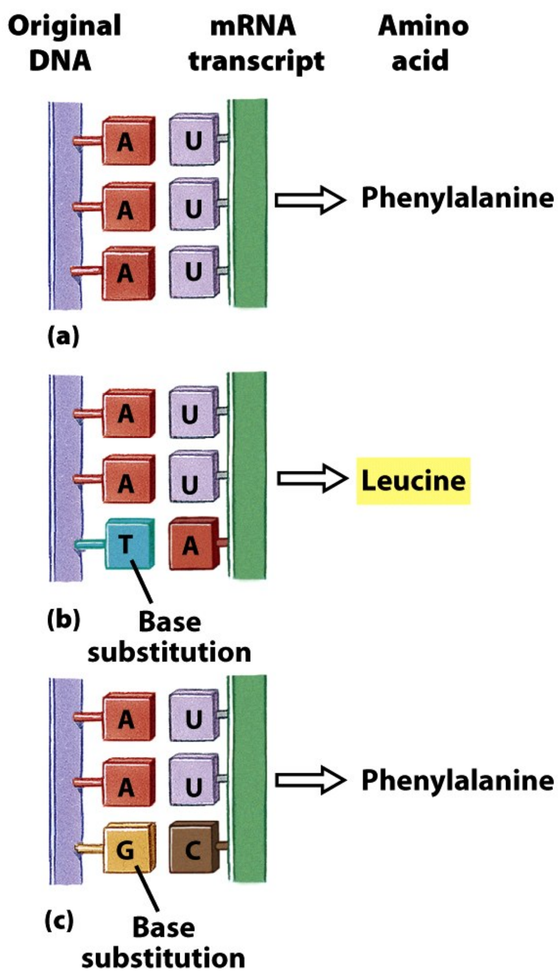


Figure 7-16 Microbiology, 7/e

Types of Mutations and Their Effects on Organisms

Types of Mutations	Effects on Organisms
Point Mutation	
Single base change in DNA with no change in the amino acid specified by the mRNA codon.	No effect on protein; a "silent" mutation.
Change in DNA with change in the amino acid sequence specified by the mRNA codon.	Change in protein by substitution of one amino acid for another; can significantly alter function of protein.
Change in DNA that creates a terminator codon in mRNA.	Produces polypeptide of no use to organism and prevents synthesis of normal protein.
Frameshift Mutation	
Deletion or insertion of one or more bases in DNA.	Changes entire sequence of codons and greatly alters amino acid sequence; can introduce terminator codon and produce useless polypeptides instead of normal proteins.

Table 7-3 Microbiology, 7/e

Mutations

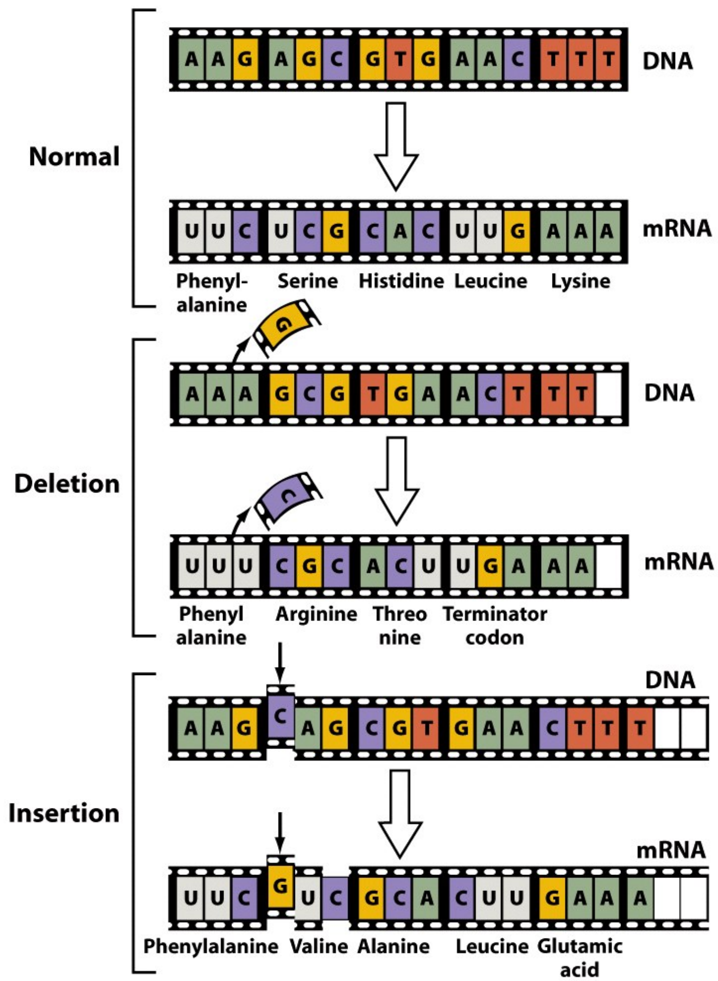


Figure 7-17 Microbiology, 7/e

Types of Mutations and Their Effects on Organisms

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Deletion or insertion of one or more bases in DNA.	Changes entire sequence of codons and greatly alters amino acid sequence; can introduce terminator codon and produce useless polypeptides instead of normal proteins.

Table 7-3 Microbiology, 7/e

Mutations

Inversions – when a segment of DNA is excised and reinserted upside down

Transposition – when a segment of DNA is excised and reinserted somewhere else

- **Phenotypic variations in mutated bacteria**
  - include alterations in colony morphology, colony color, or nutritional requirements
  - impaired synthesis of certain cell surface substances
  - organisms that typically form capsules may no longer form the external structures
  - mutations that alter nutritional requirements generally increase the nutritional needs of organisms, usually by impairing the organism's ability to synthesize one or more enzymes. As a result the organism may require certain amino acids or vitamins in its medium
  - still another variation is temperature sensitivity

- Not all phenotypic variations are the result of mutations
- Some phenotypic variations may be caused by environmental factors aswell

- **Spontaneous mutations**
  - occur in the absence of any agents known to cause changes in the DNA sequence
  - they arise during replication of DNA and appear to be errors in base pairing of nucleotides in the old and new strands of DNA
- **Induced mutations**
  - are produced by agents called mutagens
  - increases rate of the mutation compared to spontaneous mutation rate
  - mutagens include chemical agents and radiation

Some Mutagens and Their Effects

Mutagen	Effects
Chemical Agents	
Base analog *Examples:* caffeine, 5-bromouracil	Substitutes "look-alike" molecule for the normal nitrogenous base during DNA replication → point mutation.
Alkylating agent *Example:* nitrosoguanidine	Adds an alkyl group, such as methyl group (—CH_3), to nitrogenous base, resulting in incorrect pairing → point mutation.
Deaminating agent *Example:* nitrous acid, nitrates, nitrites	Removes an amino group (—NH_2) from a nitrogenous base → point mutation.
Acridine derivative *Example:* acridine dyes, quinacrine	Inserts into DNA ladder between backbones to form a new rung, distorting the helix → frameshift mutation.
Radiation	
Ultraviolet	Links adjacent pyrimidines to each other, as in thymine dimer formation, and thereby impairs replication.
X-ray and gamma ray	Ionize and break molecules in cells to form free radicals, which in turn break DNA.

Table 7-4 Microbiology, 7/e

Repair of DNA Damage

- Many bacteria can repair certain kinds of damage to DNA
  - ***light repair*** or photoreactivation, occurs in the presence of visible light in bacteria previously exposed to UV light
  - mutations that might have been passed along to daughter cells are corrected and the DNA is returned to its normal state
  - ***Dark repair***, which occurs in some bacteria in the presence or absence of light, requires several enzyme controlled reactions

THE END