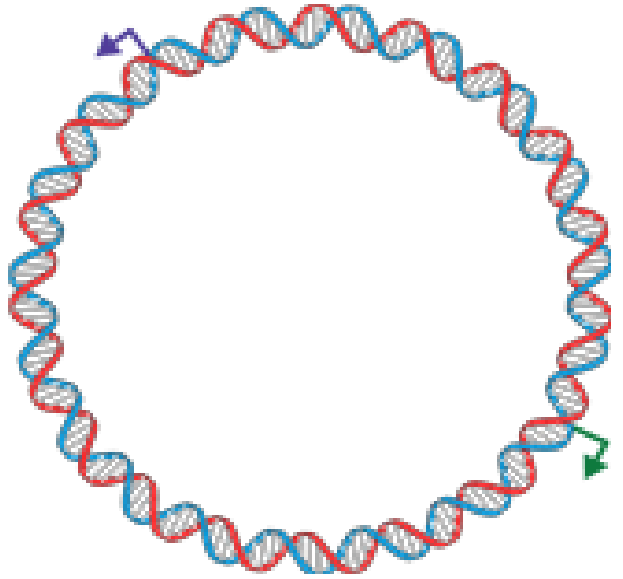
MICROBIAL GENETICS

NM
01/06/2021

CONTENT

- **Terminology in Genetics**
- **Basic structure of DNA and RNA**
- **DNA replication**
  - ✓ Replication fork, DNA synthesis & enzymes
- **Protein synthesis**
  - ✓ Transcription & translation
- **Regulation of gene expression**
  - ✓ Lactose operon & tryptophan operon
- **Mutations**
  - ✓ Silent, nonsense, missense, frameshift mutations
- **Exchange of genetic materials**
  - ✓ Conjugation, transformation, transduction



GENETICS

The science of **heredity**, including the study of **genes**, how information is **carried**, and how this information is **replicated** and **passed** on to subsequent generations

TERMINOLOGY

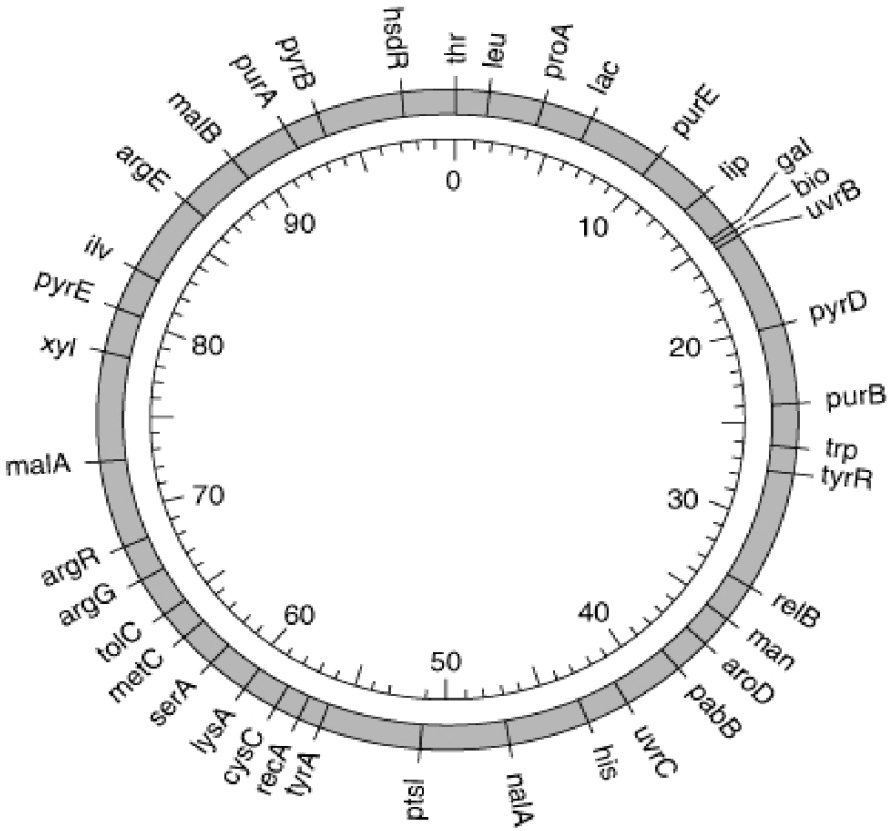
Genotype	The genetic makeup of an organism. The **entire set of genes** in an organism. The genes which encode particular characteristics of the organism (collection of genes). Determined by actual DNA sequence (gene) written eg: *flaA.*
Phenotype	The **expression of the genotype** creates observable traits. The actual, expressed properties (observed) of the gene. The result of phenotype is a **protein** (or collection of proteins) written eg: FlaA (note use of capitalization when referring to protein). One phenotype can possibly be the result of different genotypes.

TERMINOLOGY

Genome	The genetic information in a cell. It includes cell's chromosomes and plasmid.
Chromosome	Structures containing DNA that carry hereditary information in the cell. It is subdivided into genes
Genes	segments of DNA which encode for functional products (protein)
Plasmid	Small DNA independent of chromosomal DNA

The Bacterial DNA

- Bacteria generally have a **single, circular chromosome**.
- e.g. *E. coli* chromosome consists of 4 million base pairs (4 x 10^6 bp).
- This is a medium sized bacterial chromosome.
- Takes up only about 10% of the cell's volume because the DNA is **supercoiled**
- Attached to plasma membrane.



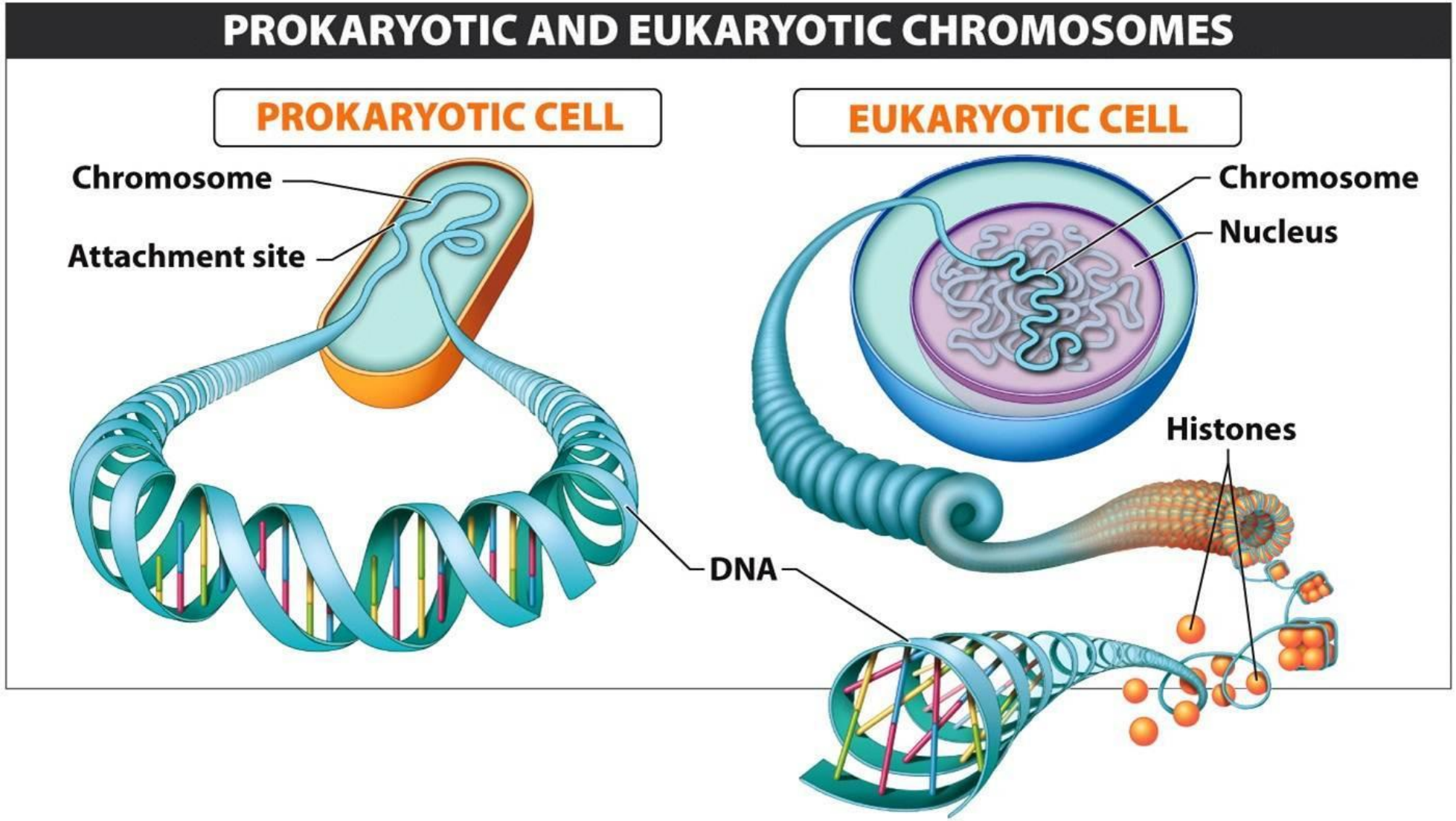
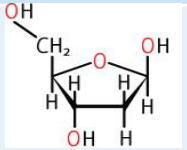
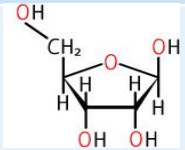


Figure 6-3
What Is Life? A Guide To Biology

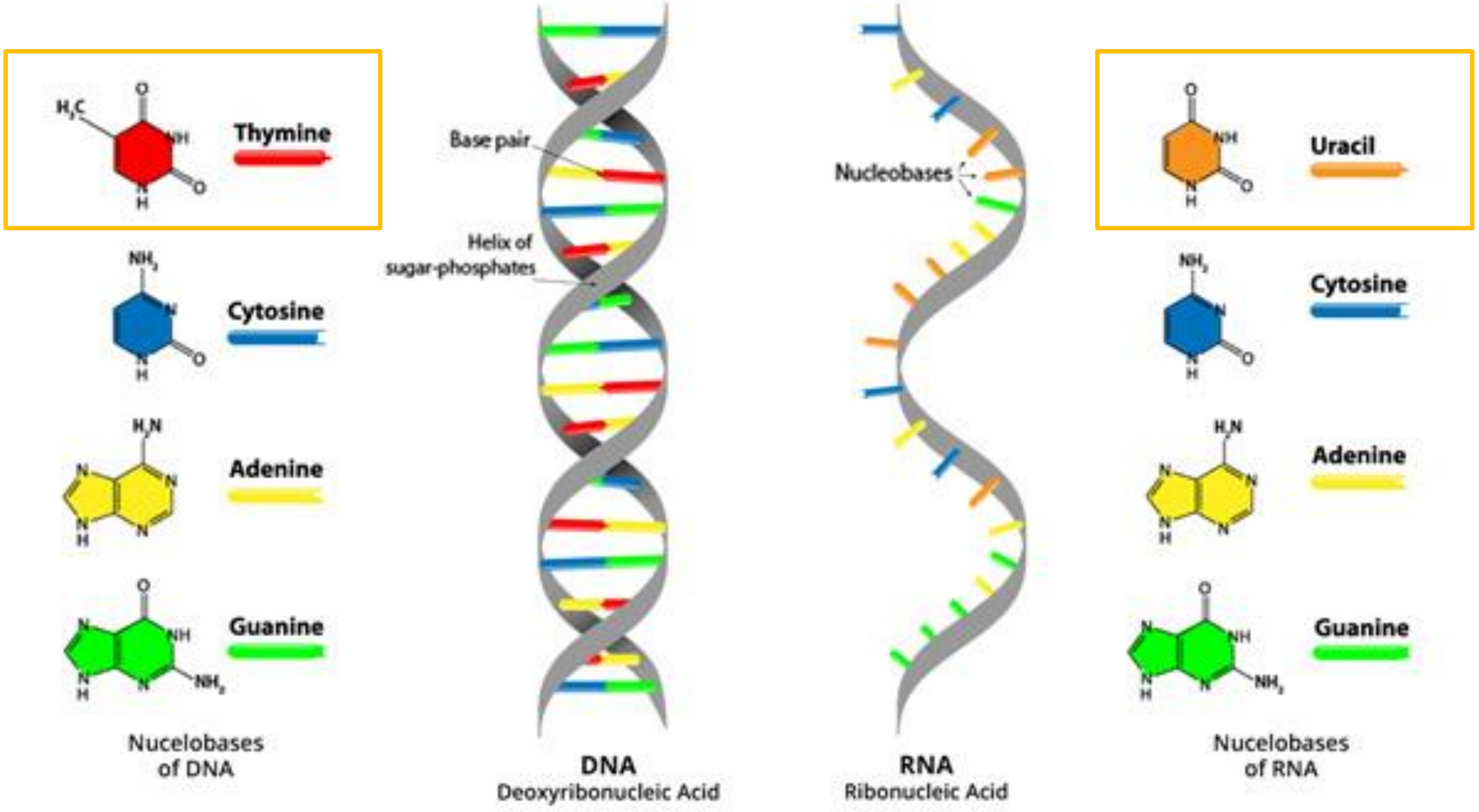
BASIC STRUCTURE OF DNA

- Deoxyribonucleic acid (DNA)
  - Contain bases **adenine, guanine, cytosine, and thymine**
  - Monomer: **deoxyribonucleotide** (Ribose + bases)
    - ✓ the sugar is deoxyribose
    - ✓ Deoxyribose is linked to bases by **glycosidic bond**
  - The monomers are linked by **phosphodiester bond** to form a polymer (polynucleotide)
    - ✓ A phosphate forms a bridge between the 3'-hydroxyl of one sugar and the 5'-hydroxyl of an adjacent sugar.
  - Large molecules & usually composed of two polynucleotide chains (DNA strands) coiled together to form a **double helix**
  - The bases from each strands interact with those of the other strand, forming **base-pairs.**
    - ✓ **Complementary** to each other : A paired with T, C paired with G
  - The sequence of bases encode genetic information.
  - DNA is a helical (coil)
    - ✓ The coiling of a coil is **supercoiling** – helps to compact DNA, 'loosen' up the DNA for separation in replication process.

What is the difference between DNA and RNA

	DNA	RNA
Function	Genetic information blueprint Stores genetic information Replicates genetic information	Converts the genetic information contained within DNA to a format that can be used to build proteins Moves it to ribosomal protein factories.
Sugar	Deoxyribose 	Ribose 
Structure	Double Stranded	Single Stranded
Nitrogenous bases	AGTC	AGUC
Base pairs	(A-T) (C-G)	(A-U) (C-G)
Length	Millions of nucleotides DNA > longer than RNA in terms of length	Hundreds to a few thousand nucleotides Variable and much shorter in length compared to DNA
Location	Mostly nucleus (in eukaryotes)	Formed in the nucleolus and moves to the cytosol and ribosomes (in eukaryotes)
Reactivity	DNA is more stable in comparison to RNA Important in keeping genetic information safe	RNA more reactive (less stable) Easily subject to attack by enzymes

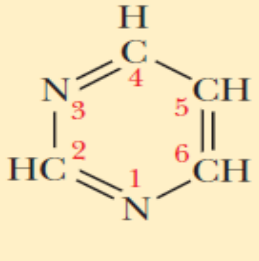
What is the difference between DNA and RNA



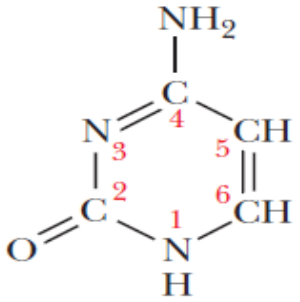
Source: https://www.technologynetworks.com/genomics/lists/what-are-the-key-differences-between-dna-and-rna-296719

Bases

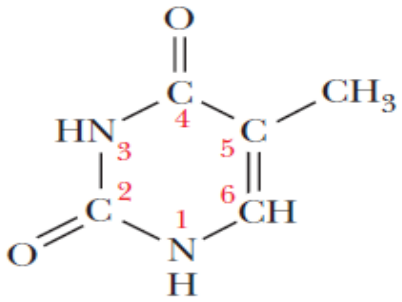
- **Two pyrimidine bases**:
  - Cytosine (C)
  - Thymine (T)
  - Uracil (U)
- **Two purine bases**:
  - Adenine (A)
  - Guanine (G)



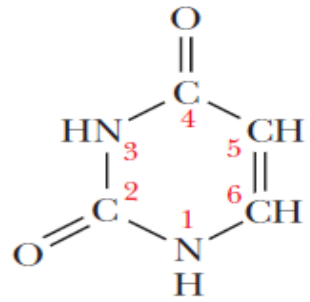
Pyrimidine



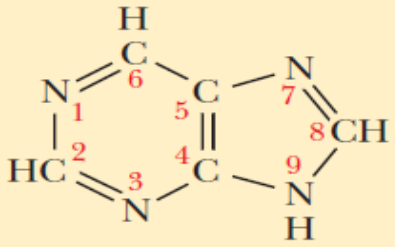
Cytosine
(in DNA & RNA)



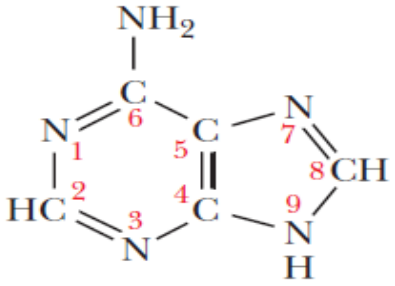
Thymine
(in DNA & some RNA)



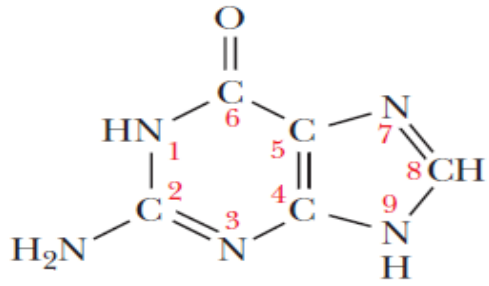
Uracil
(in RNA)



Purine



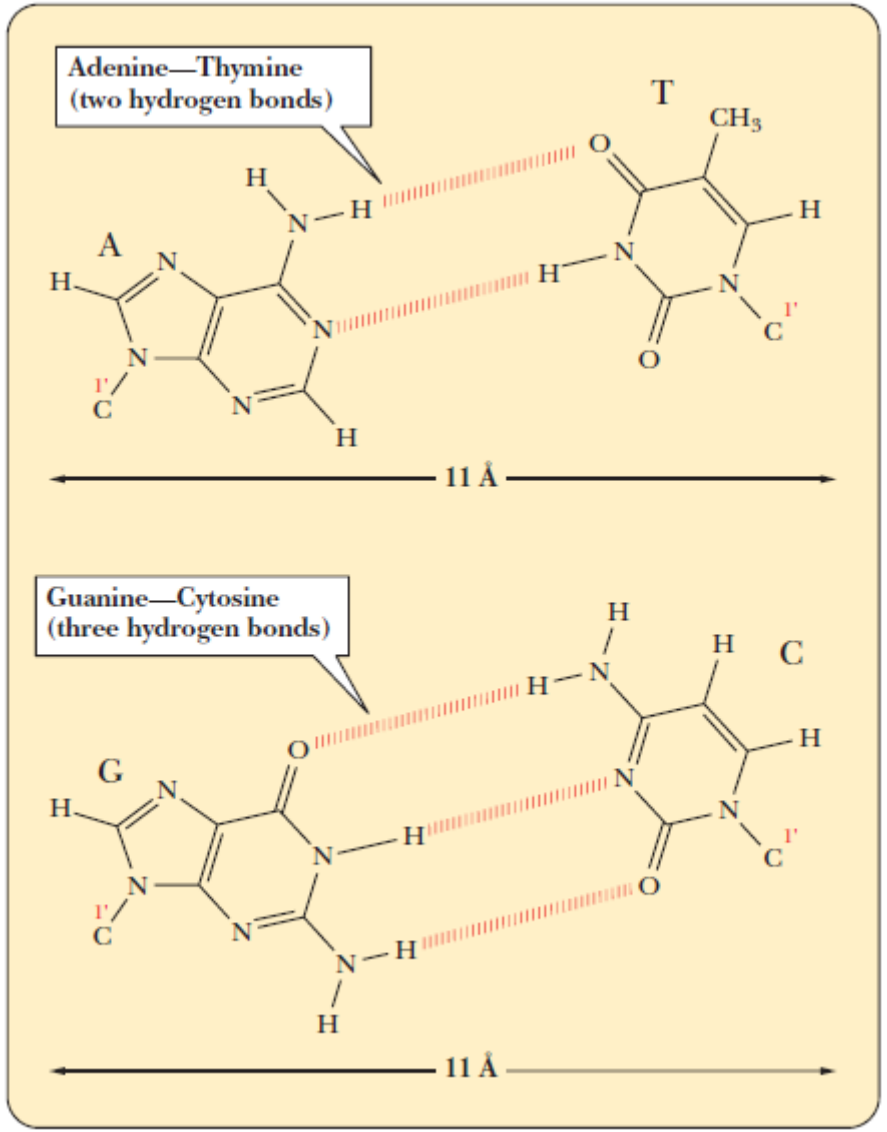
Adenine
(in DNA & RNA)



Guanine
(in DNA & RNA)

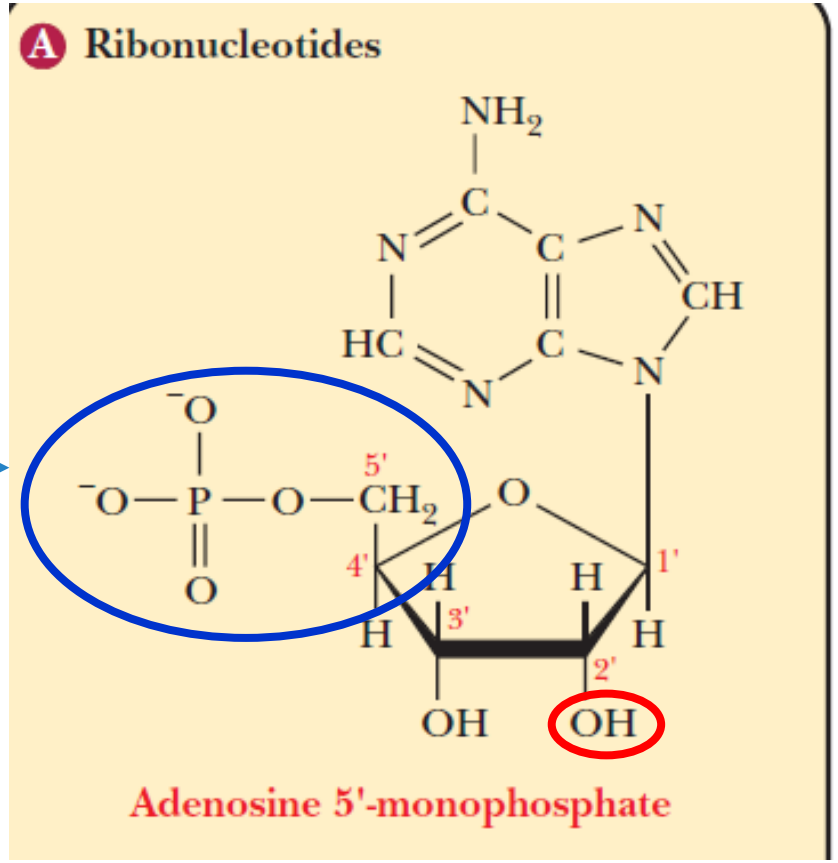
Base Pairing

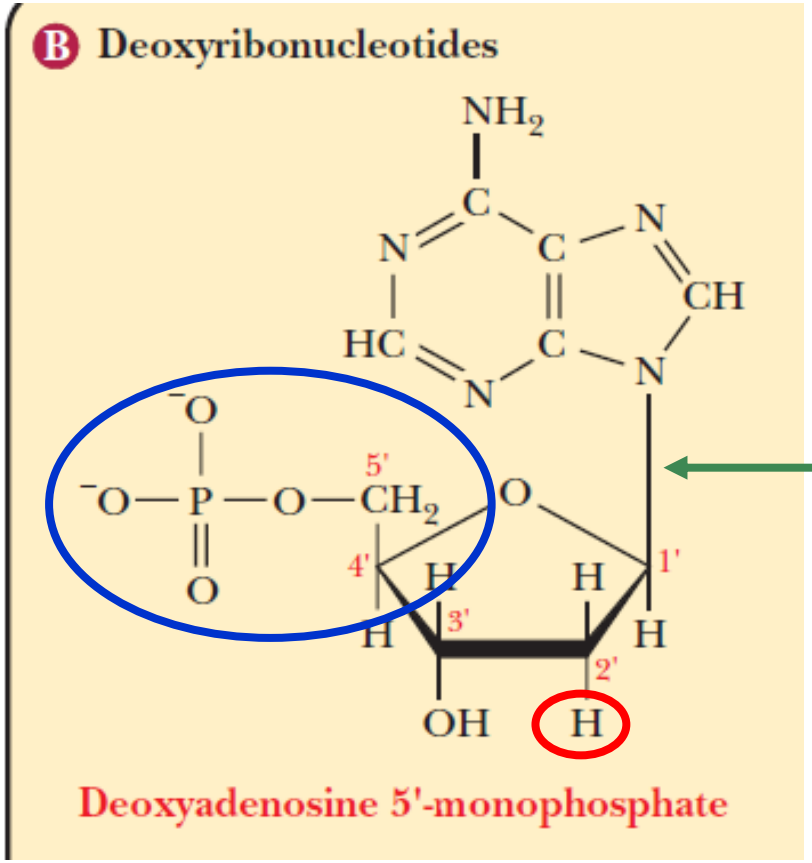
- Bases associate in DNA by complementary base pairing
- Bases are paired by hydrogen bond
- A always pairs with T (or U in RNA)
- G always base pairs with C



Nucleotides

Phosphate group





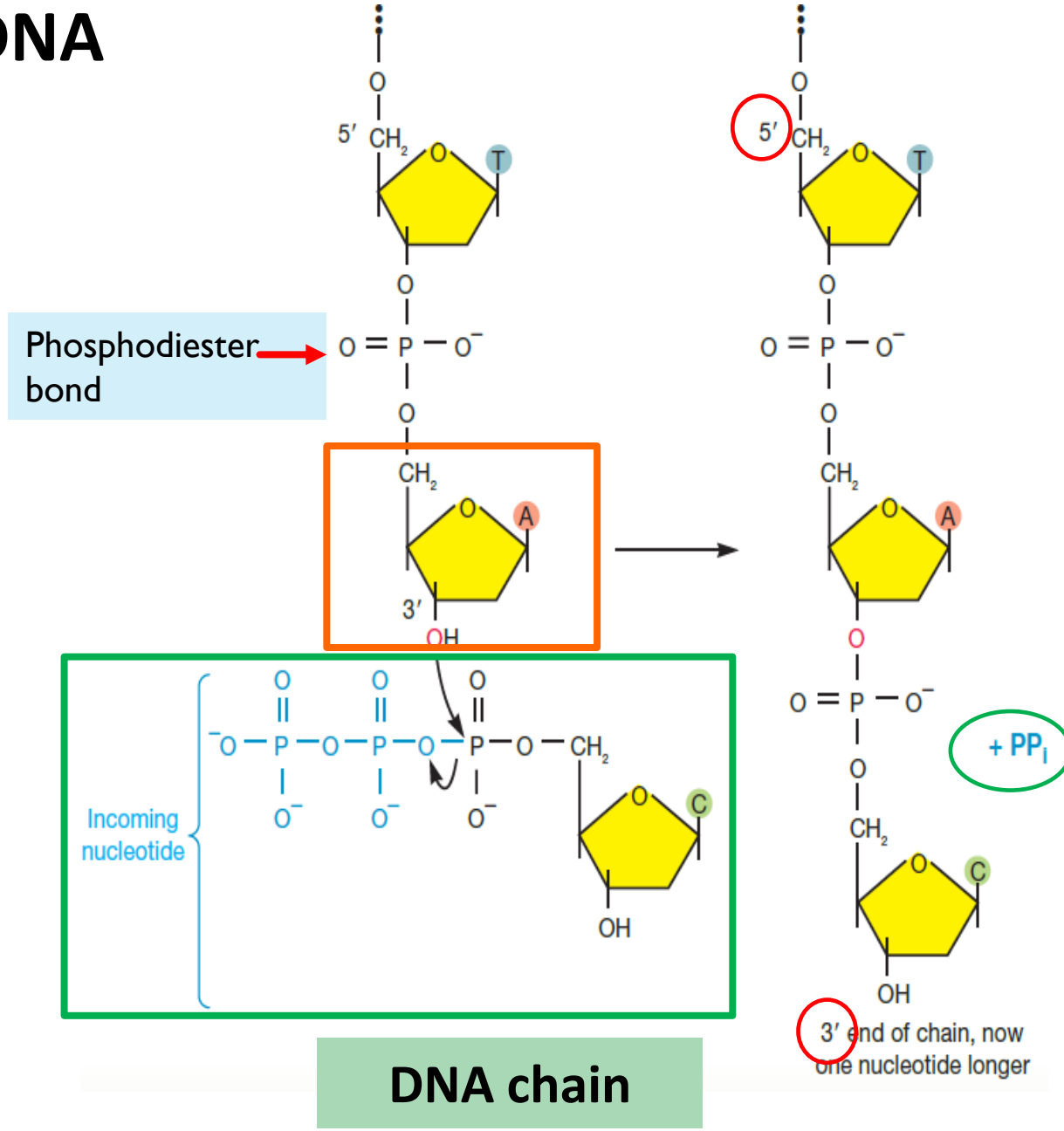
Glycosidic bond is formed between sugar (ribose) and bases

RNA

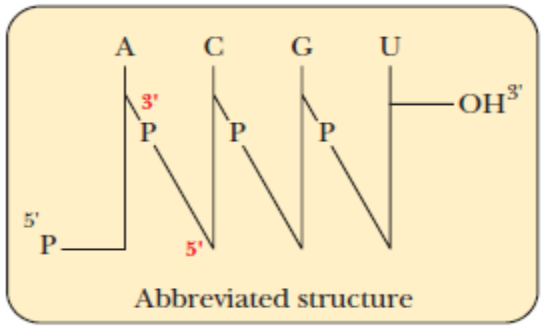
DNA

How nucleotides are added in DNA replication?

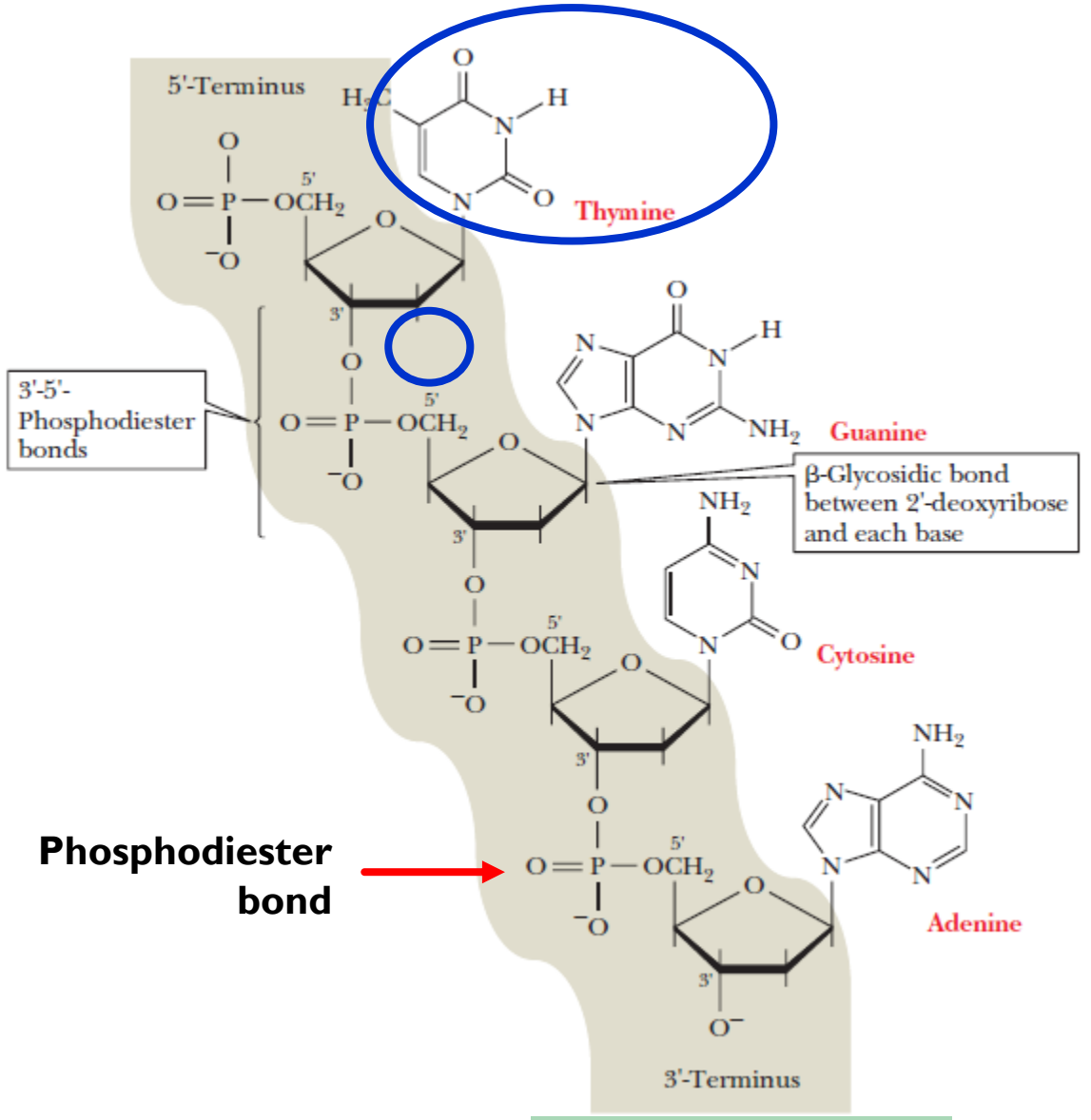
- DNA polymerase catalyze DNA synthesis in the 5’ to 3’ direction
- Nucleotide [Deoxynucleoside triphosphate (dNTP)] is added to the 3’ end.
  - ✓ Linked by phosphodiester bonds (reactions between a hydroxyl group at the 3’ end of the growing DNA strand, and the phosphate at the 5’ carbon of the incoming nucleotide.
  - ✓ The energy to form the phosphodiester bond is provided by the release of 2 phosphate group from the nucleotide that is added.



What are the differences between RNA and DNA chain?

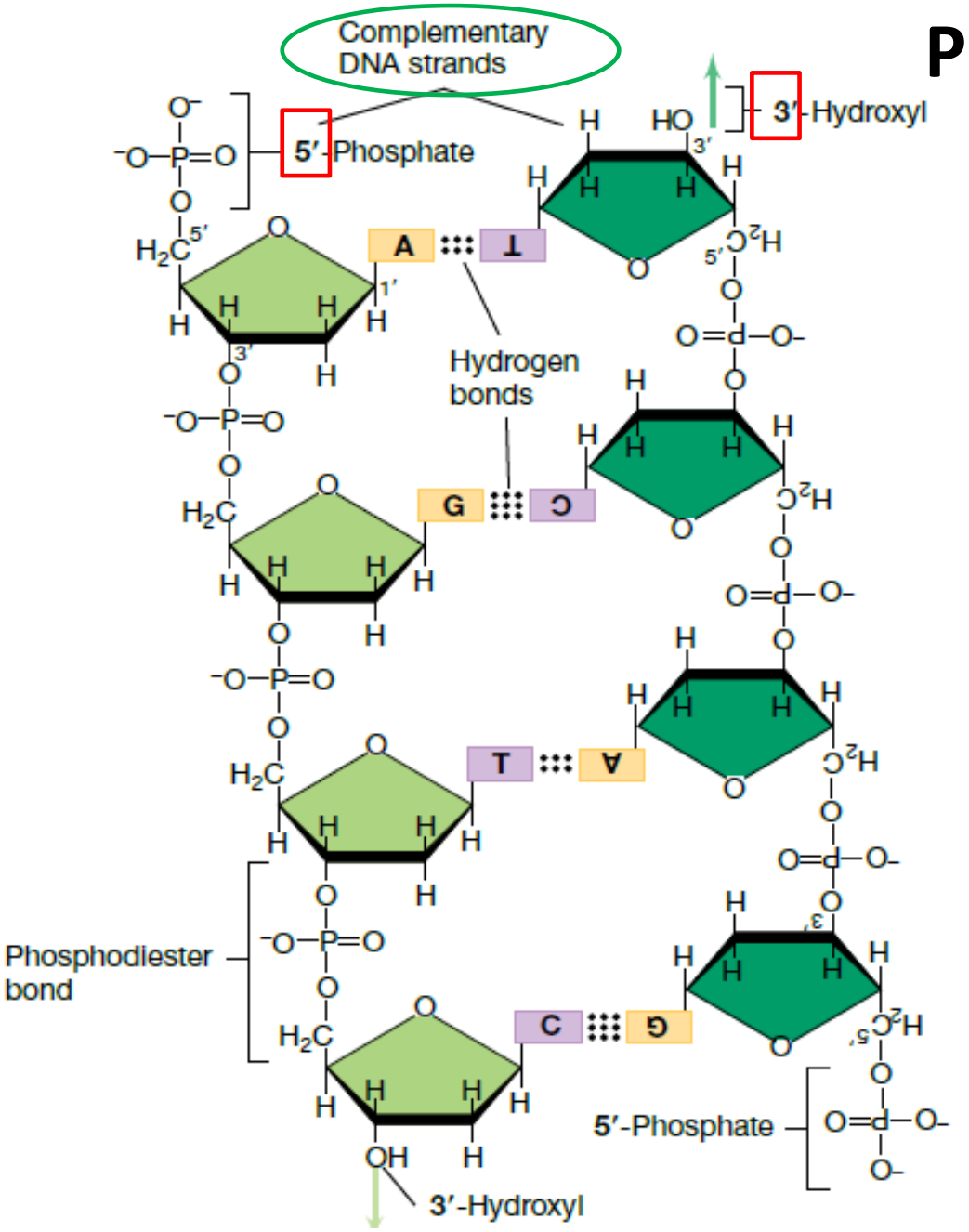


RNA chain

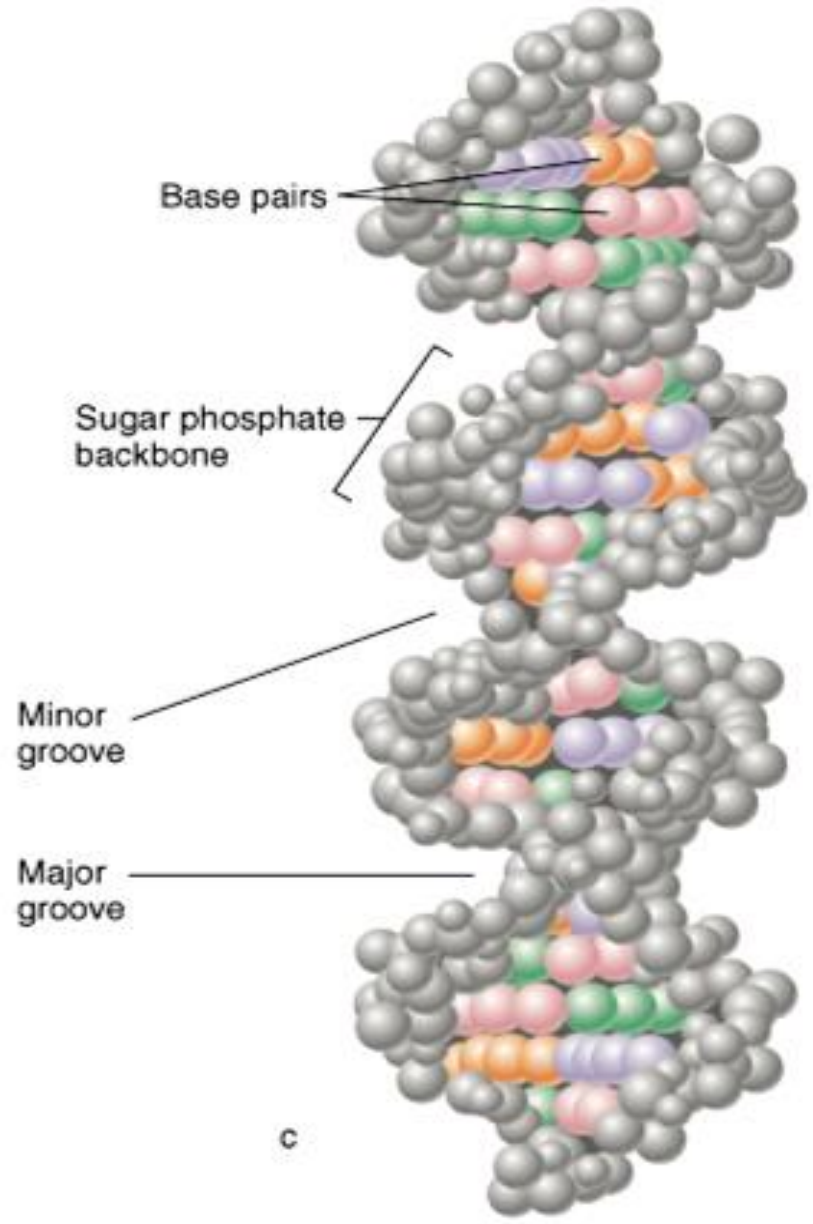


DNA chain

Physical Structure of DNA



Schematic, nonhelical model, anti-parallel



Helical & anti-parallel arrangement

DNA REPLICATION

- The DNA of most bacteria is contained in a single **circular molecule**, called the bacterial chromosome
- DNA is **double-stranded** which is **anti-parallel** (opposite direction)
- Replication is copying of each strand.
- Since base pairing is specific, each strand can serve as the **template** for the opposite.
- One parent molecule of double-stranded DNA gives two daughter molecules.
- Each daughter molecule contains one "original" strand and one newly synthesized strand.
- This is called **semi-conservative** replication

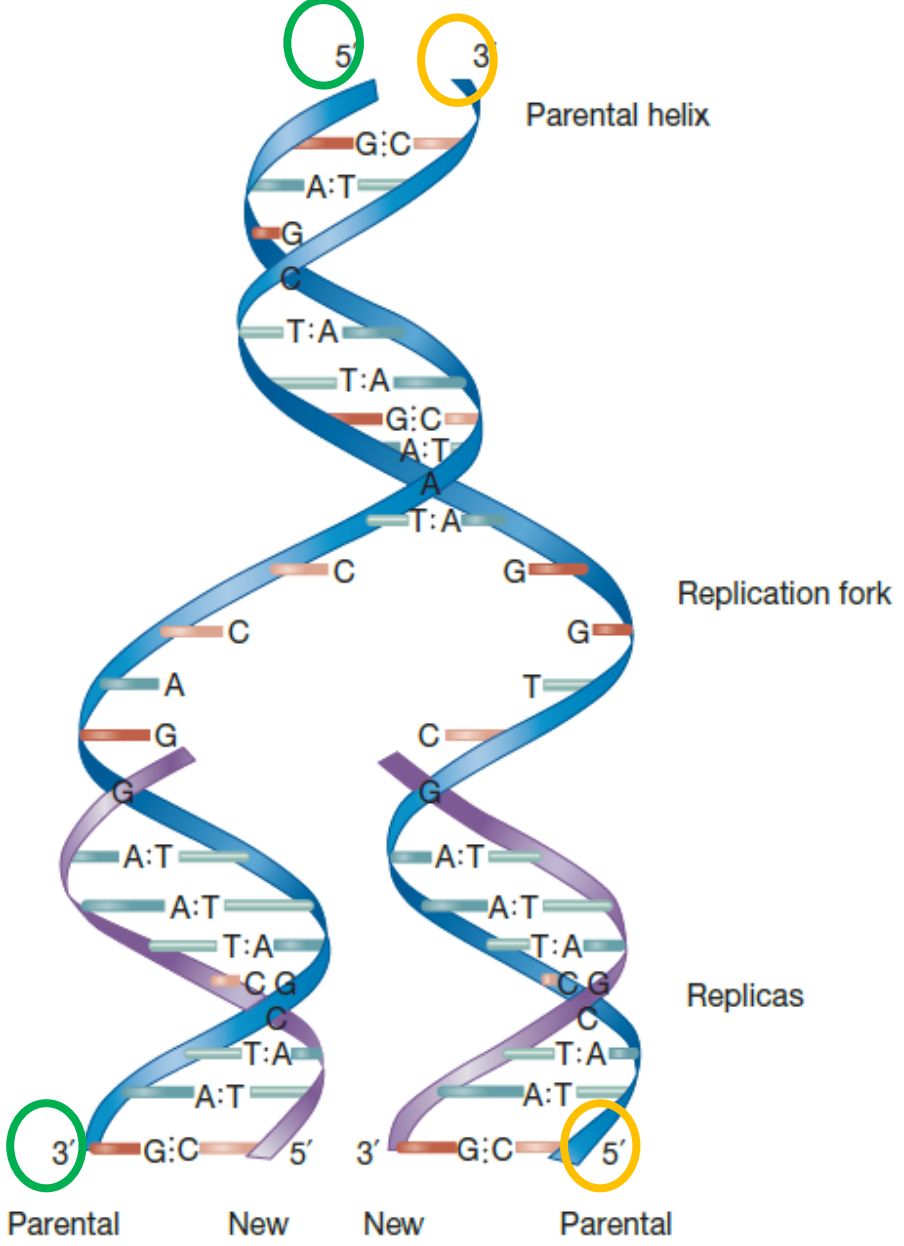
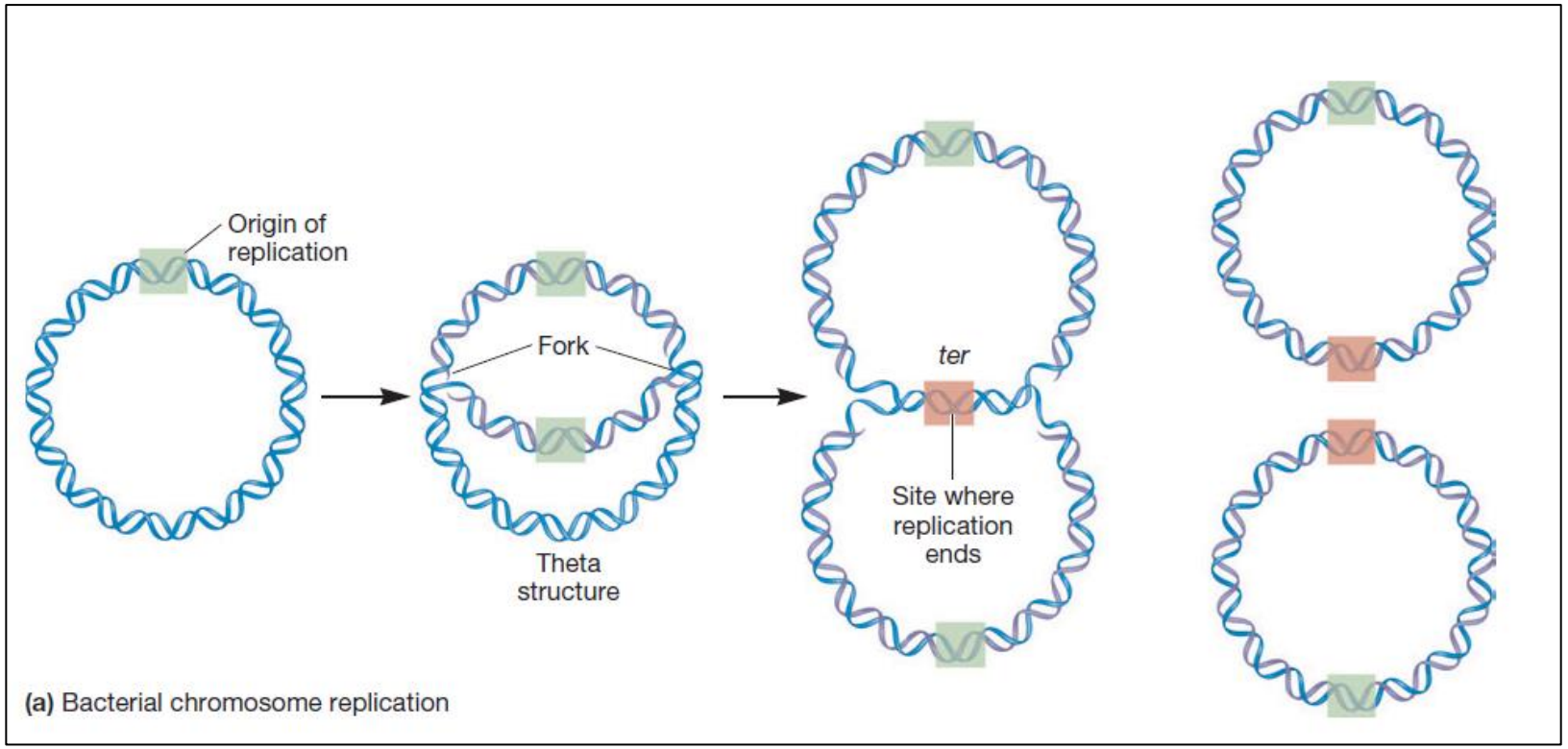
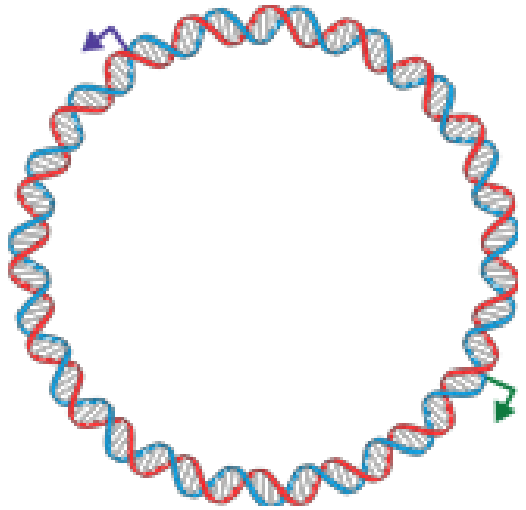


Figure 13.8 Semiconservative DNA Replication. The replication fork of DNA showing the synthesis of two progeny strands. Newly synthesized strands are purple. Each copy contains one new and one old strand.

Bacterial DNA replication





- Synthesis of bacterial DNA occur at the replication fork
- DNA helix is unwound & individual strand is replicated
- Two replication folk move outward from the origin and copied the whole genome

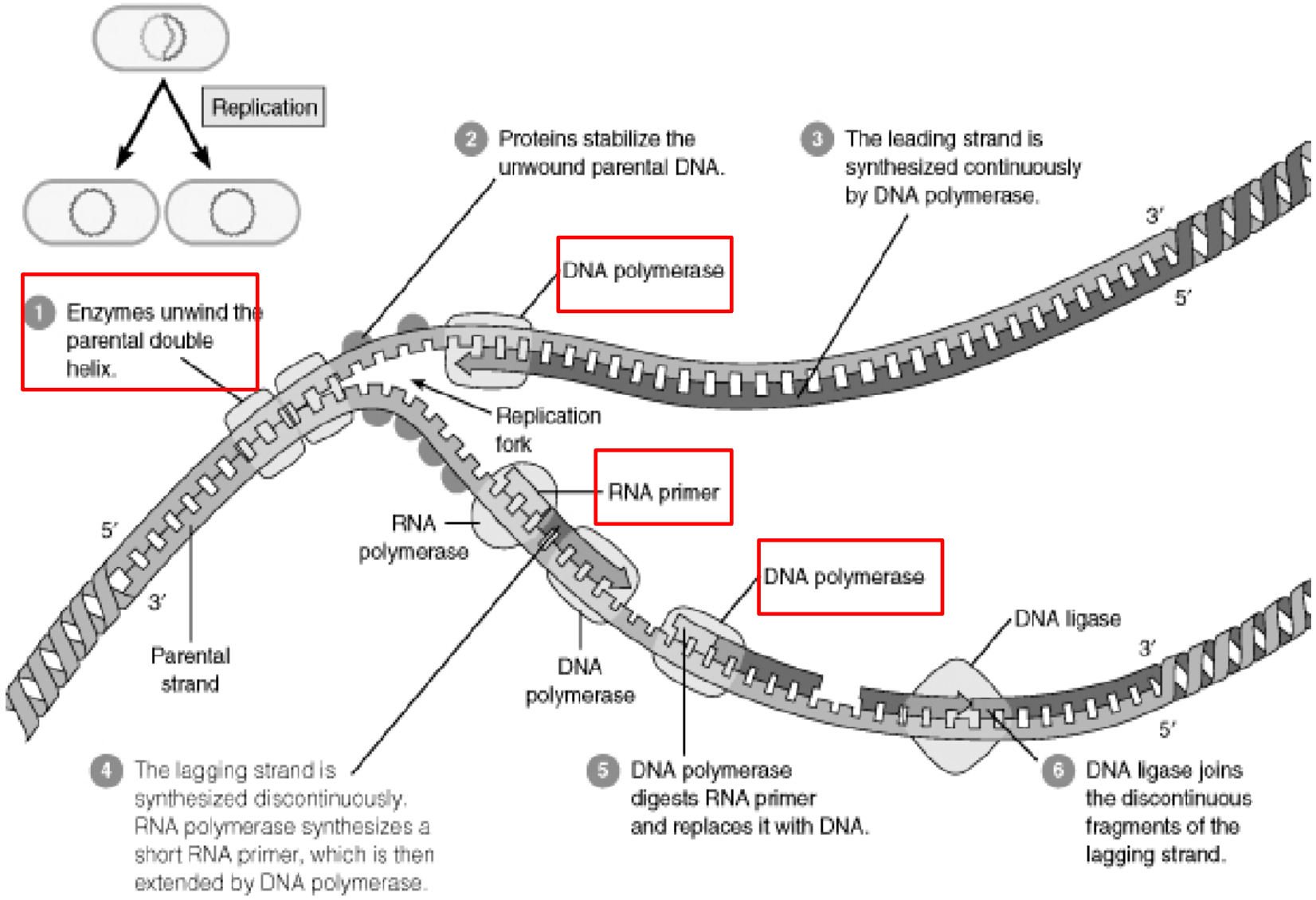
Replication Machinery

Table 13.1 Components of the *E. coli* Replication Machinery

Protein	Function
DnaA (initiator protein)	Initiation of replication; binds origin of replication (*oriC*)
DnaB	Helicase (5′→3′); breaks hydrogen bonds holding two strands of double helix together; promotes DNA primase activity; involved in primosome assembly
DNA gyrase	Relieves supercoiling of DNA produced as DNA strands are separated by helicases; separates daughter molecules in final stages of replication
SSB proteins	Bind single-stranded DNA after strands are separated by helicases
DnaC	Helicase loader; working with DnaA, directs DnaB (helicase) to DNA template
DNA primase	Synthesis of RNA primer; component of primosome
DNA polymerase III holoenzyme	Catalyzes most of the DNA synthesis that occurs during DNA replication; has 3′→5′ exonuclease (proofreading) activity
DNA polymerase I	Removes RNA primers; fills gaps in DNA formed by removal of RNA primer
Ribonuclease H	Removes RNA primers
DNA ligase	Seals nicked DNA, joining DNA fragments together
Tus	Termination of replication
Topoisomerase IV	Separation of chromosomes upon completion of DNA replication

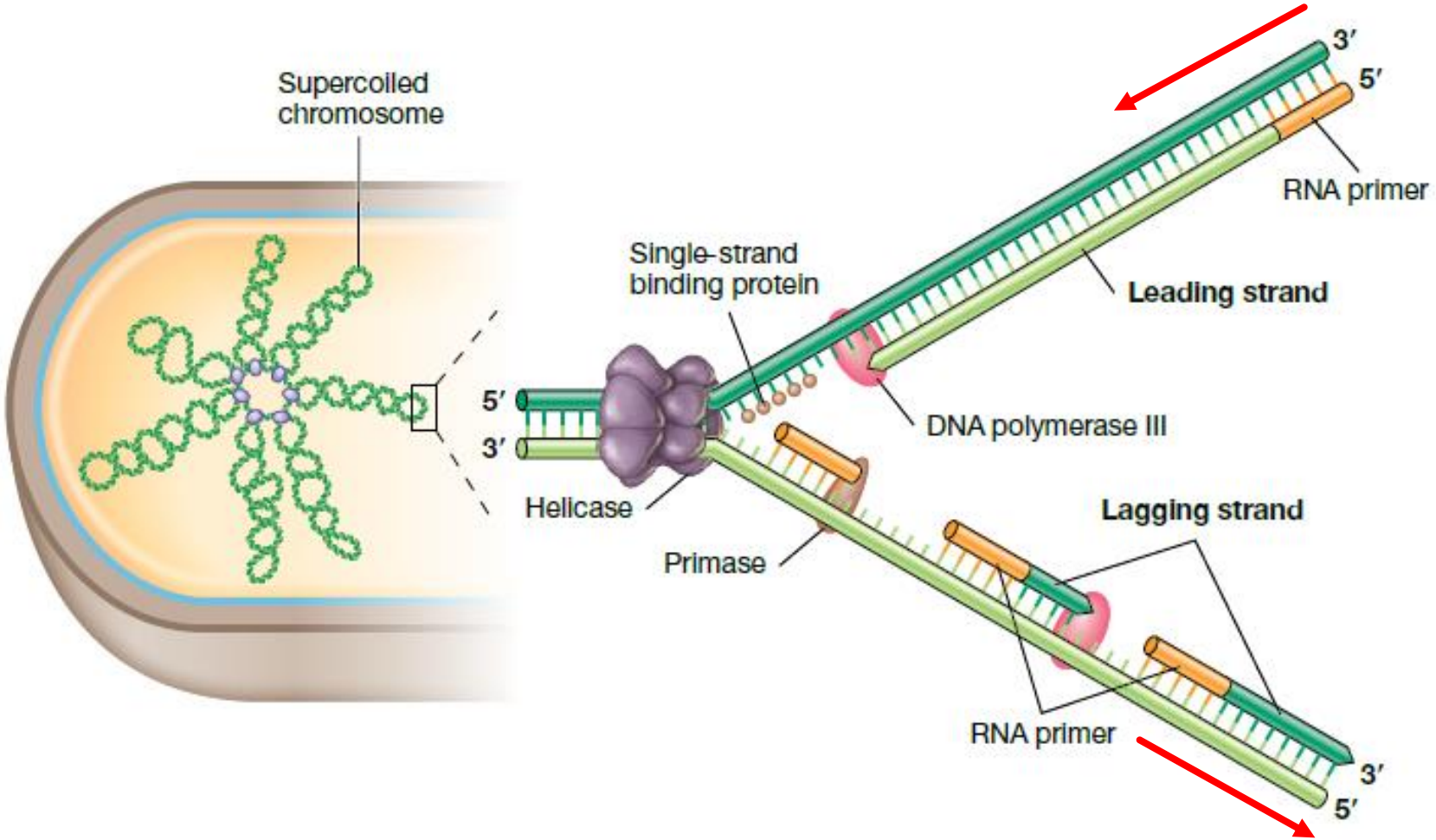
Synthesis of DNA

- DNA synthesis required:
  - ✓ DNA template (read from 3’ – 5’)
  - ✓ Primer (to provide free 3’-OH group to which nucleotides can be added
  - ✓ A set of dNTPs
  - ✓ DNA polymerase (I – V)
- Begins at an origin of replication
- **Helicase** unwinds and unzips the DNA double helix at the replication forks
- **An RNA primer** is synthesized at the origin of replication
- **DNA polymerase III** adds nucleotides in a 5’ to 3’ direction
  - **Leading strand** – synthesized continuously in 5’ to 3’ direction
  - **Lagging strand** (okazaki fragments) – synthesized 5’ to 3’ in short segments



Event at the DNA Replication Fork

Figure 4.14 Events at the DNA replication fork on the nucleoid. Note the polarity and antiparallel nature of the DNA strands. Helicase unwinds the DNA while primase adds the RNA primer.



Replication occurs at a replication fork. In most bacteria, there are two forks which are moving in opposite directions (bidirectional replication).

Synthesis of DNA

- **DNA polymerase I** removes the RNA primers and replaces them with DNA (exonuclease activity).
- Synthesis occurs in a specific direction on the DNA.
- New DNA synthesis ALWAYS occurs from 5’⟶3’ (DNA template is 3’⟶5’)
- One strand is the **leading strand**. This strand runs into the unzipping fork in the 5’-3’ direction, so open space is always in-front of the newly forming strand.
- The other is called the **lagging strand** because it cannot synthesize continuously due to direction of DNA
- The short pieces on the lagging strand are called **Okazaki fragments.**

Synthesis of DNA

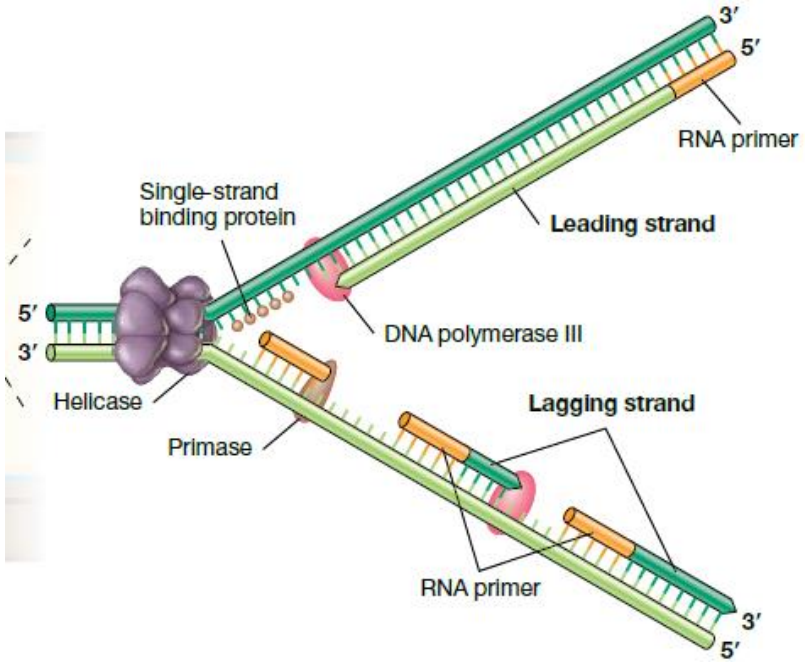
- Lagging strand synthesis requires **RNA primers** to begin each segment. DNA Polymerase requires a free end to start from. It can't start at an empty space.

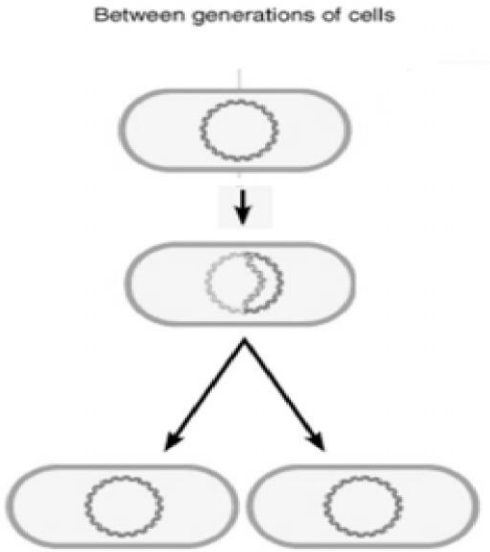
- DNA Polymerase can't fit against ends of earlier segments so it leaves a small gap. These gaps are closed by **DNA Ligase**

- In *E. coli*, DNA replication stop when the replisome reaches a termination site (*ter* of the DNA). Protein Tus bind to *ter* sites & stop the progression of the forks.

- When replication forks meet, **ligases** link the DNA fragments along the lagging strand to complete the synthesis

- Separation of the daughter molecules is complete



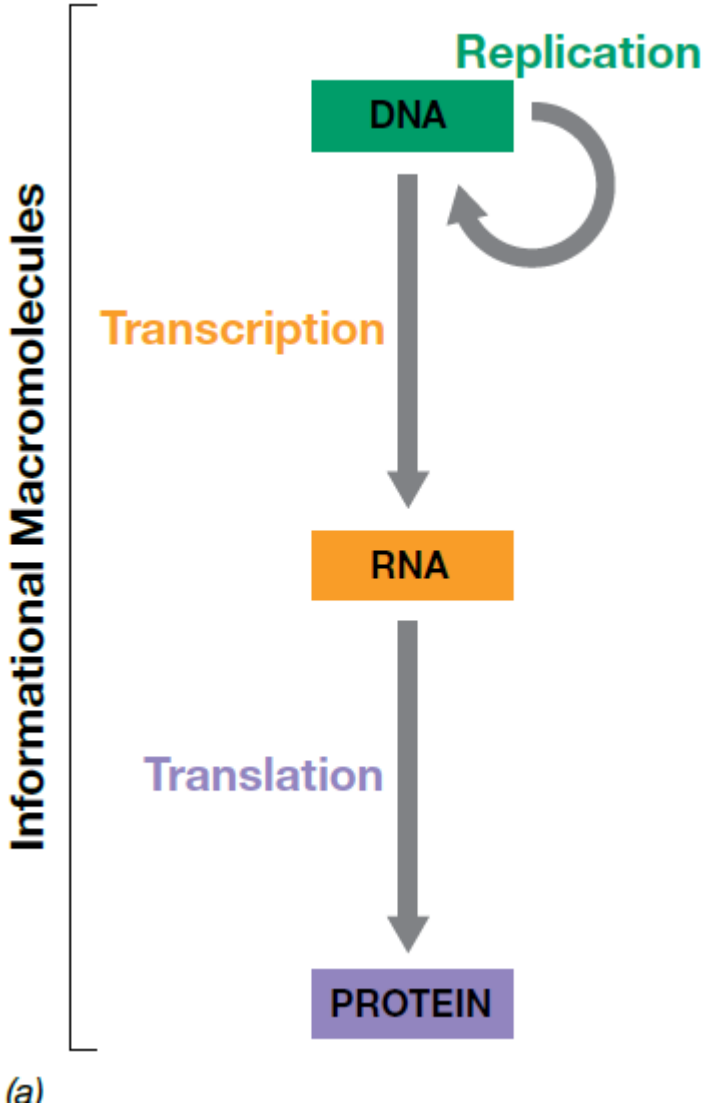


Summary of steps in bacterial DNA Replication

1. Enzymes unwind the parental double helix.
2. Proteins stabilizes the unwound parental DNA.
3. The leading strand is synthesized continuously by DNA polymerase.
4. The lagging strand is synthesized discontinuously, RNA polymerase synthesizes a short RNA primer, which is then extended by DNA polymerase.
5. DNA polymerase digest the primer and replaces it with DNA.
6. DNA ligases joins the discontinuous fragments of the lagging strand.

PROTEIN SYNTHESIS

- The DNA (gene) stores the hereditary information.
- Information stored on the DNA molecule is conveyed to RNA molecules through the process of **transcription**
- The information contained in the RNA molecule is then used to produce proteins in the process of **translation**



Fundamental process in protein synthesis

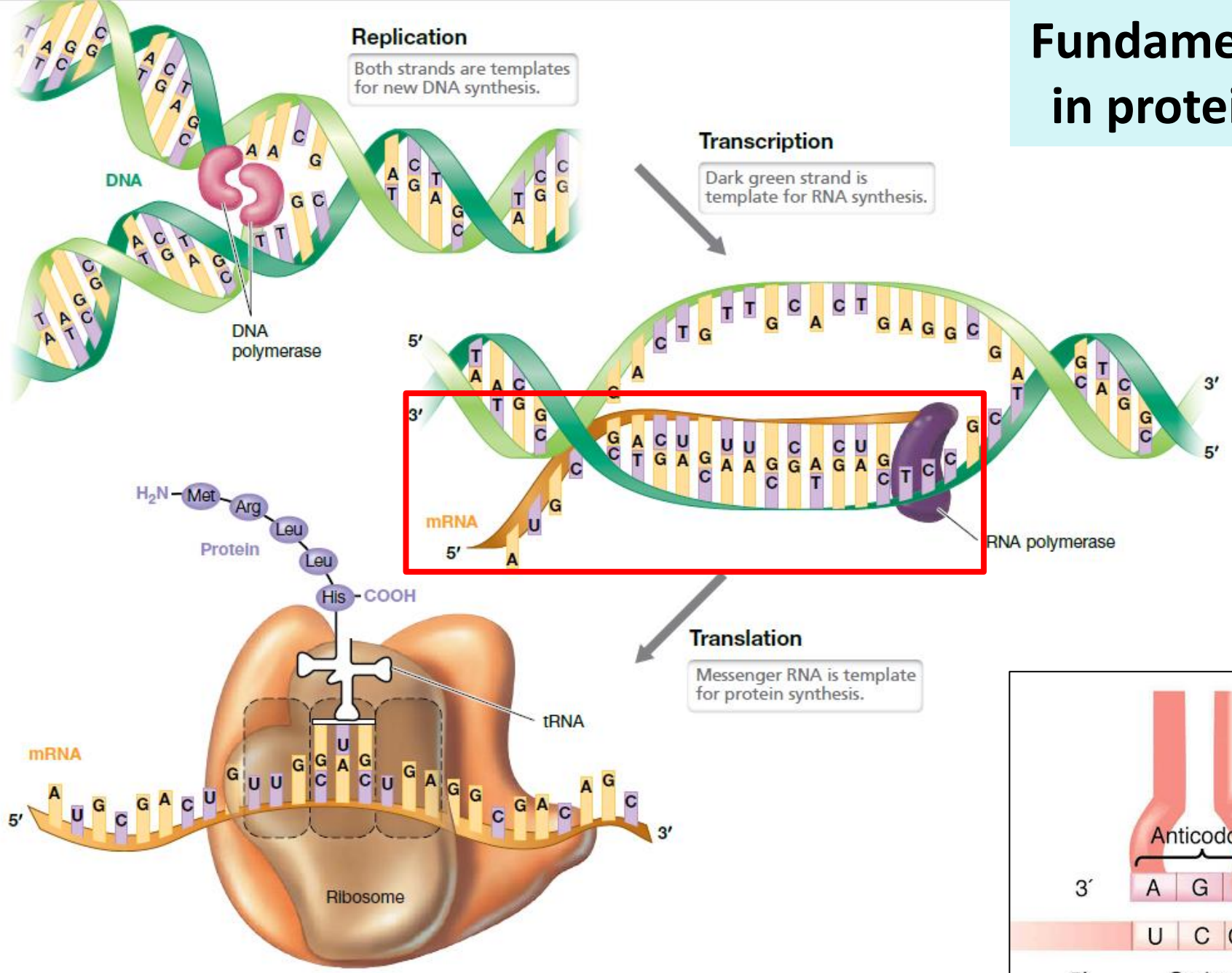
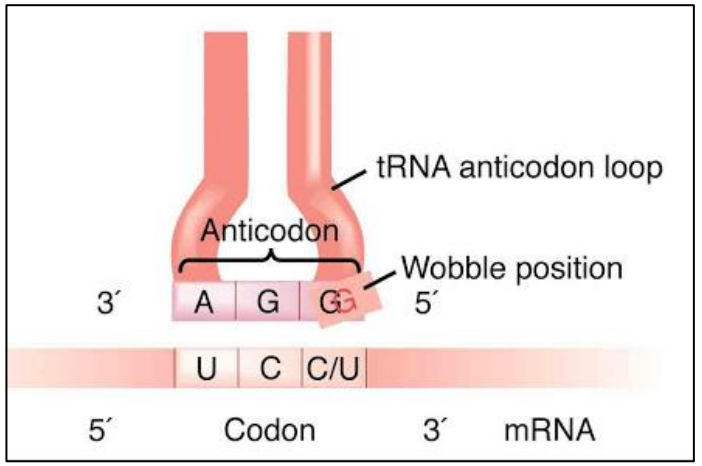
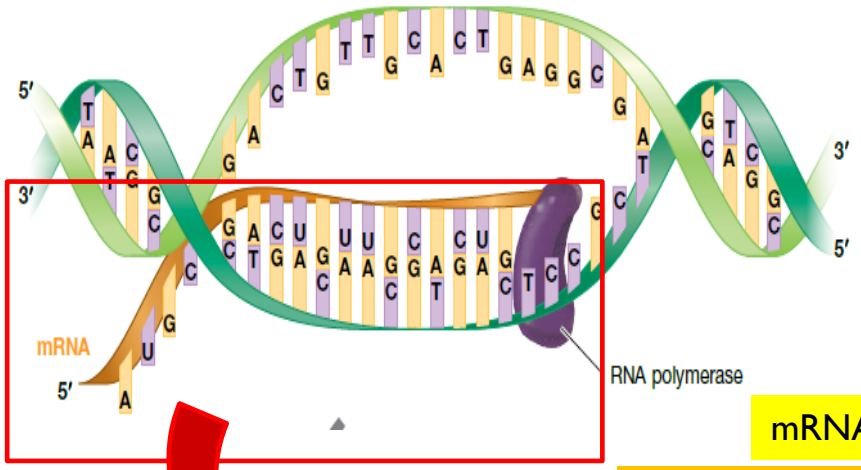
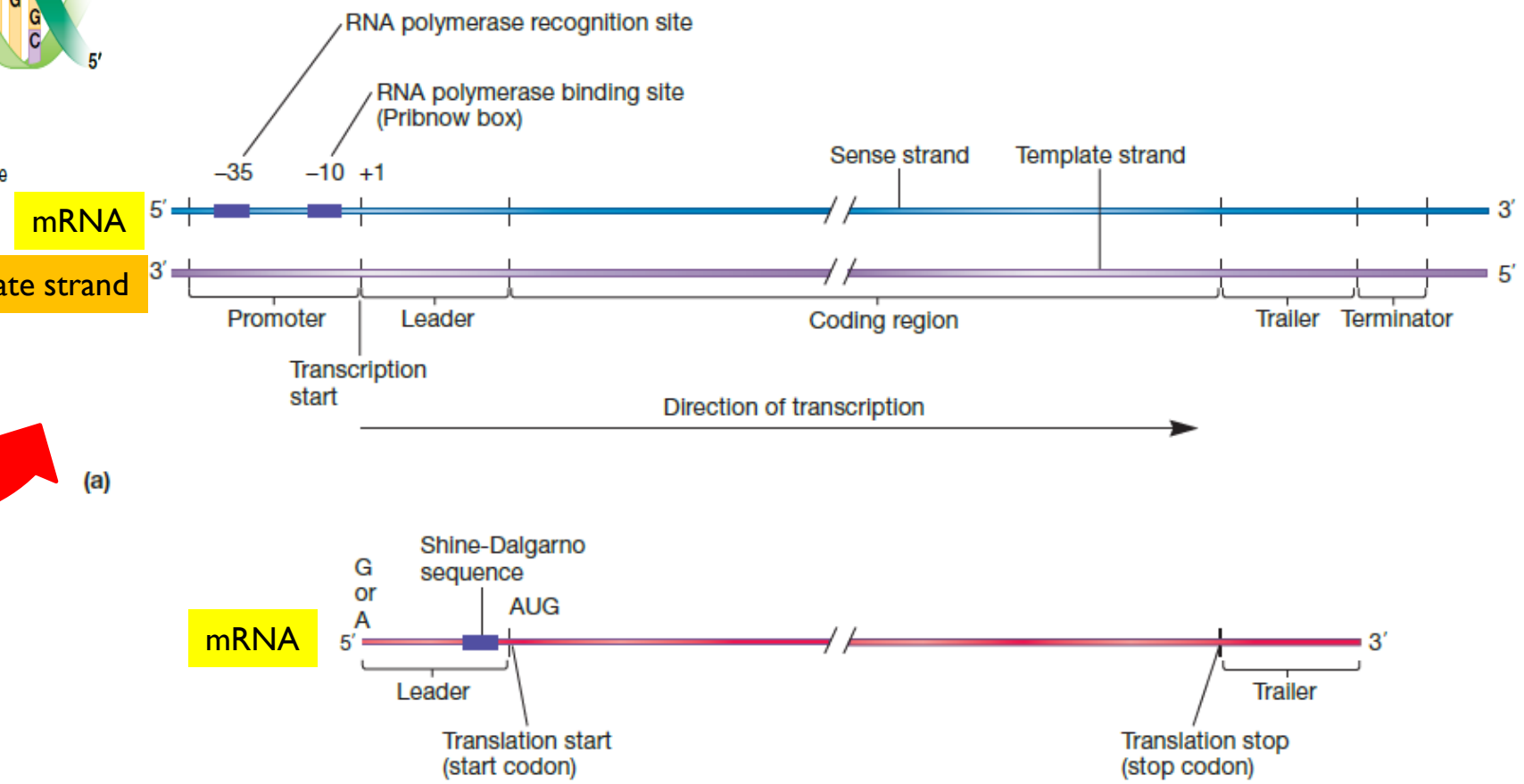


Figure 4.6 Synthesis of the three types of informational macromolecules. Note that for any particular gene only one of the two strands of the DNA double helix is transcribed.



Transcription





Promoter : Binding site for RNA polymerase
: Transcription start at +1, but do not code for amino acid

Leader : Involve in regulation

Coding region: start codon AUG
: end with a stop codon UAA, UAG or UGA

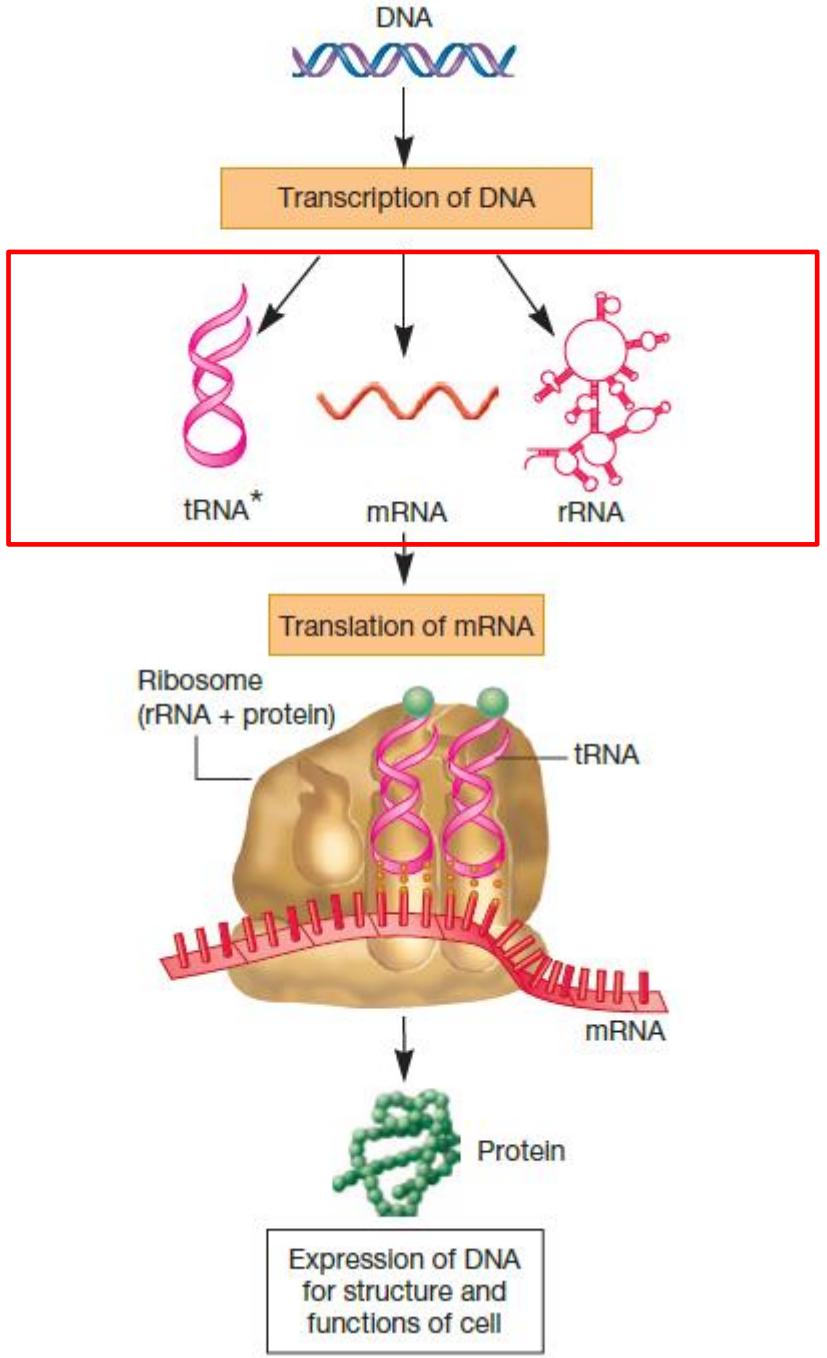
Trailer : transcribed but not translated,
: prepare for the release of RNA pol.

Terminator : sequence that signals the RNA pol. To stop transcription.

Figure 13.20 A Bacterial Structural Gene and Its mRNA Product. (a) The organization of a typical structural gene in a bacterial cell. Some genes lack leaders or trailers or both. Transcription begins at the +1 position in DNA and proceeds to the right, as shown. The numbering of nucleotides to the left of this spot is in a negative direction, while the numbering to the right is in a positive direction. For example, the nucleotide that is immediately to the left of the +1 nucleotide is numbered −1, and the nucleotide to the right of the +1 nucleotide is numbered +2. There is no zero nucleotide in this numbering system. In many bacterial promoters, sequence elements at the −35 and −10 regions play a key role in promoting transcription. During transcription, the template is read in the 3′ to 5′ direction. Regulatory sites are not shown but are usually upstream of the coding region and may overlap with the promoter. (b) Messenger RNA product of the gene shown in part a. The first nucleotide incorporated into mRNA is usually GMP or AMP. Translation of the mRNA begins with the AUG start codon.

Types of RNA

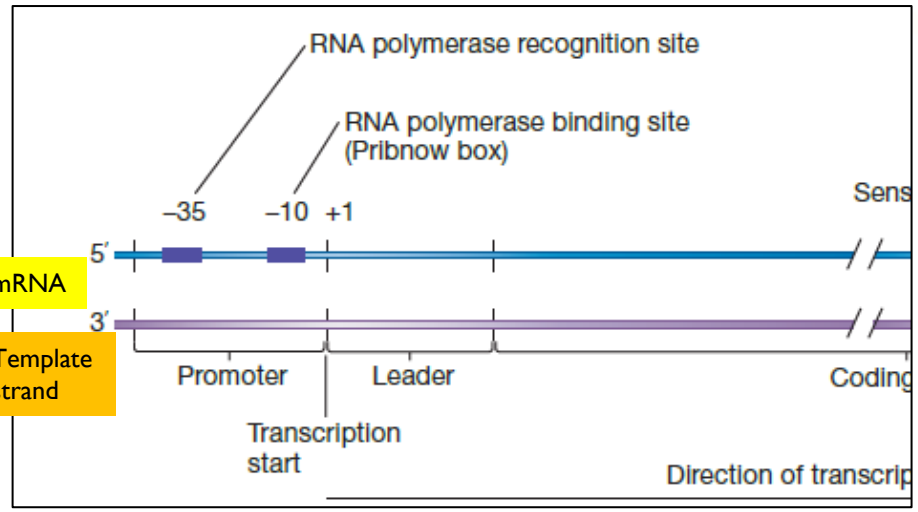
- Three types of RNA are made in the cell, **all are involved in protein synthesis**
  - **Ribosomal (rRNA)** is a required as part of ribosomes – the machinery of protein synthesis
  - **Transfer (tRNA)** brings the amino acids to the ribosomes in a specific manner
  - **Messenger (mRNA)** carries the coded information from DNA for the synthesis of specific proteins
- All RNA is transcribed from DNA by RNA polymerase



RNA Synthesis - Transcription

- RNA polymerase transcribes mRNA using the DNA template (the "coding" strand of the double-stranded DNA)
- **3 stages in transcription:**
  - **Initiation** - RNA polymerase binds to a promoter (special start site on DNA), Sigma factor helps to position the RNA polymerase at the promoter
  - **Elongation** - then polymerizes the new chain using complementary bases.
  - **Termination** - polymerization stops upon reaching a terminator (*stop* site) where it releases from the DNA
- The new RNA strand has ***ribonucleotides*** instead of *deoxyribonucleotides* & **uracil (U)** is used in place of thymine (T) to base pair with adenine (A)
- The reaction catalyzed by RNA polymerase is quite similar to that catalyzed by DNA polymerase.

The Bacterial Transcription Cycle



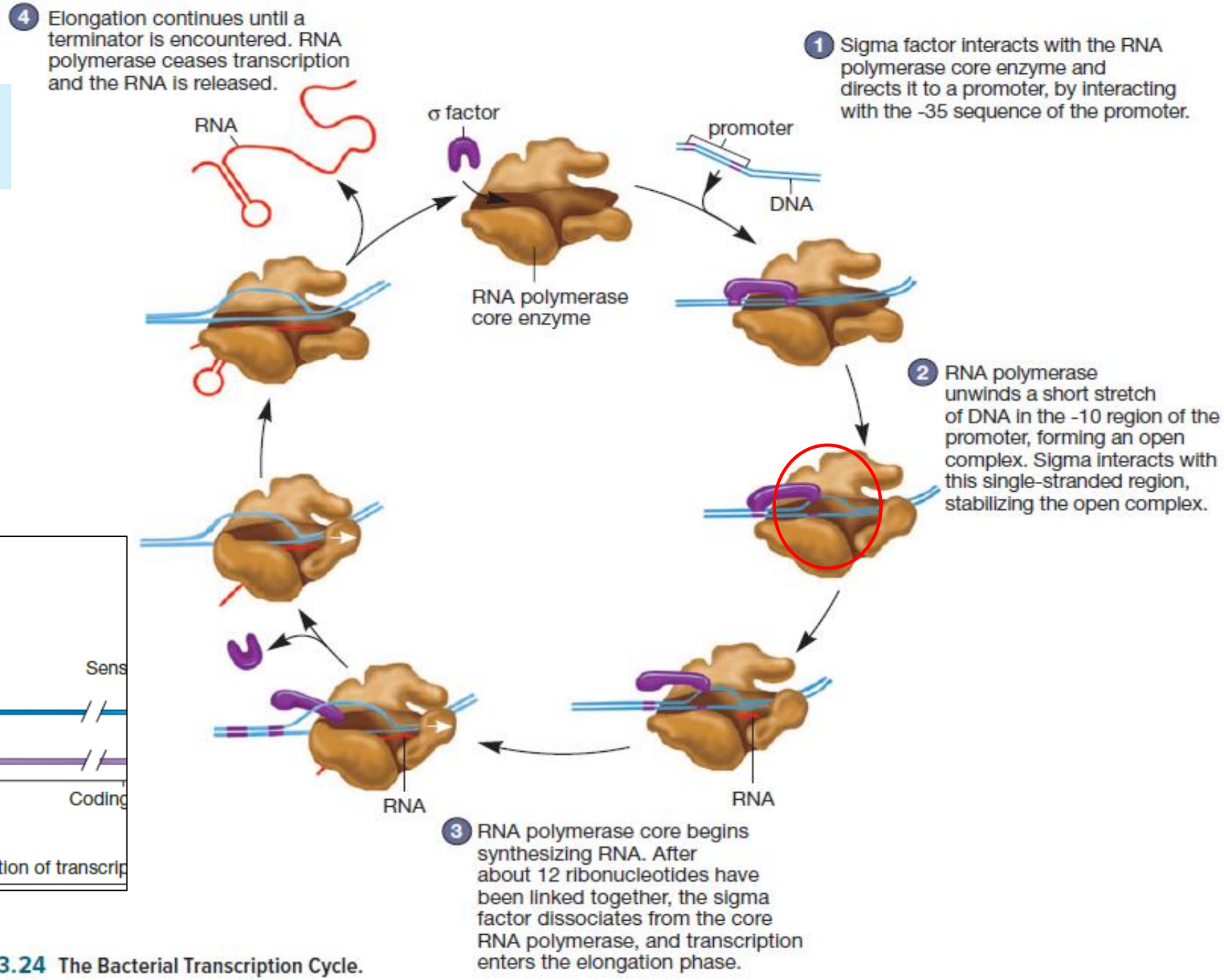


Figure 13.24 The Bacterial Transcription Cycle.

Translation of Proteins

- Translation is a decoding process of mRNA for protein synthesis
- Series of three (triplets) bases in mRNA form ***codons***
- Each codon corresponds to a specific amino acid
- Reading frame: The sequence of codon that is 'read'

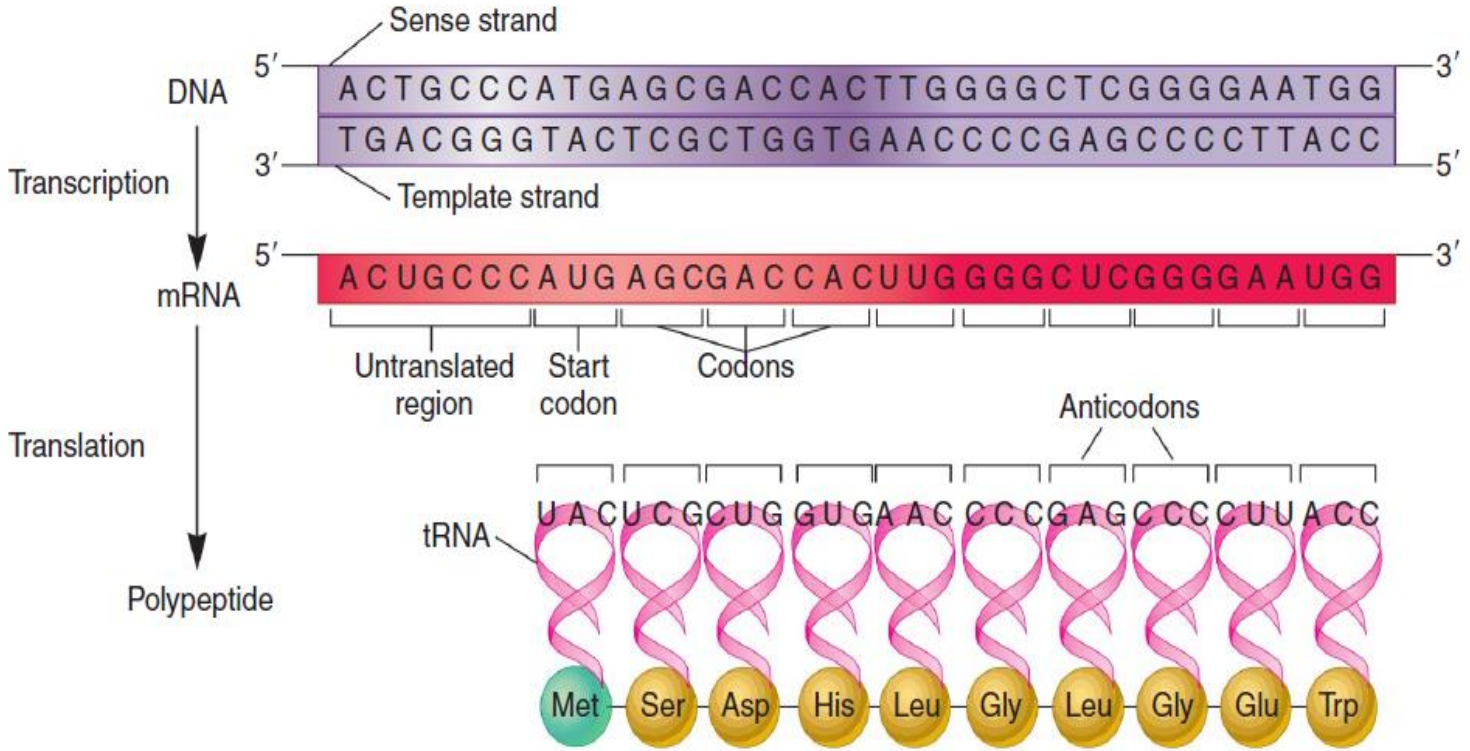


Figure 13.30 **Reading Frame.** During transcription, an mRNA complementary to the template strand of DNA is synthesized. The nucleotides in the mRNA are organized into groups of three, each group being a codon. The first codon translated into protein is the start codon. It establishes the reading frame and therefore the sequence of amino acids in the polypeptide chain that is made from the mRNA.

Genetic Code

- 61 different codons (mRNA)
- code for **20 different amino acids**
- ONE codon **AUG** signals START and occurs at the beginning of every mRNA. It codes for the amino acid methionine, so every proteins starts with this amino acid.
- Three codons signals STOP and one of these will be found at the end of each mRNA **UAA**, **UAG** or **UGA**

Table 13.3 The Genetic Code[1]

First Position (5′ End)[2]	SECOND POSITION U	SECOND POSITION C	SECOND POSITION A	SECOND POSITION G	Third Position (3′ End)
U	UUU Phe F	UCU Ser S	UAU Tyr Y	UGU Cys C	U
	UUC Phe F	UCC Ser S	UAC Tyr Y	UGC Cys C	C
	UUA Leu L	UCA Ser S	UAA STOP	UGA STOP	A
	UUG Leu L	UCG Ser S	UAG STOP	UGG Trp W	G
C	CUU Leu L	CCU Pro[3] P	CAU His H	CGU Arg R	U
	CUC Leu L	CCC Pro[3] P	CAC His H	CGC Arg R	C
	CUA Leu L	CCA Pro[3] P	CAA Gln Q	CGA Arg R	A
	CUG Leu L	CCG Pro[3] P	CAG Gln Q	CGG Arg R	G
A	AUU Ile I	ACU Thr T	AAU Asn N	AGU Ser S	U
	AUC Ile I	ACC Thr T	AAC Asn N	AGC Ser S	C
	AUA Ile I	ACA Thr T	AAA Lys K	AGA Arg R	A
	AUG Met M	ACG Thr T	AAG Lys K	AGG Arg R	G
G	GUU Val V	GCU Ala A	GAU Asp D	GGU Gly G	U
	GUC Val V	GCC Ala A	GAC Asp D	GGC Gly G	C
	GUA Val V	GCA Ala A	GAA Glu E	GGA Gly G	A
	GUG Val V	GCG Ala A	GAG Glu E	GGG Gly G	G

1 Next to each codon or set of codons is the R group characteristic of that amino acid.
2 The code is presented in RNA form. Codons run in the 5′ to 3′ direction. The 3- and 1-letter abbreviations for the amino acids designated by the codon are provided, as is the structure of the R chain of that amino acid.
3 Proline is an imino acid, rather than an amino acid.

Let's Translate A Protein

- AAA AGU **AUG** CGU UGG UGU GGU GGC GAU GCA GUA UGU UAC UCA **UAA** CCU AAA AAA GUA UGC GUU GGU GUG GUG GCG AUG CAG UAU GUU ACU CAU AAC CUA

- If our mRNA reads like this…

Find the START codon (AUG) and break the sequence into codons (3 base sections) from that point… continue until you reach a STOP codon (UAA, UAG or UGA)

- Then read the codons to determine the appropriate amino acid to use

- Met- Arg- Trp- Cys- Gly- Gly- Asp- Ala- Val- Cys- Tyr- Ser- STOP

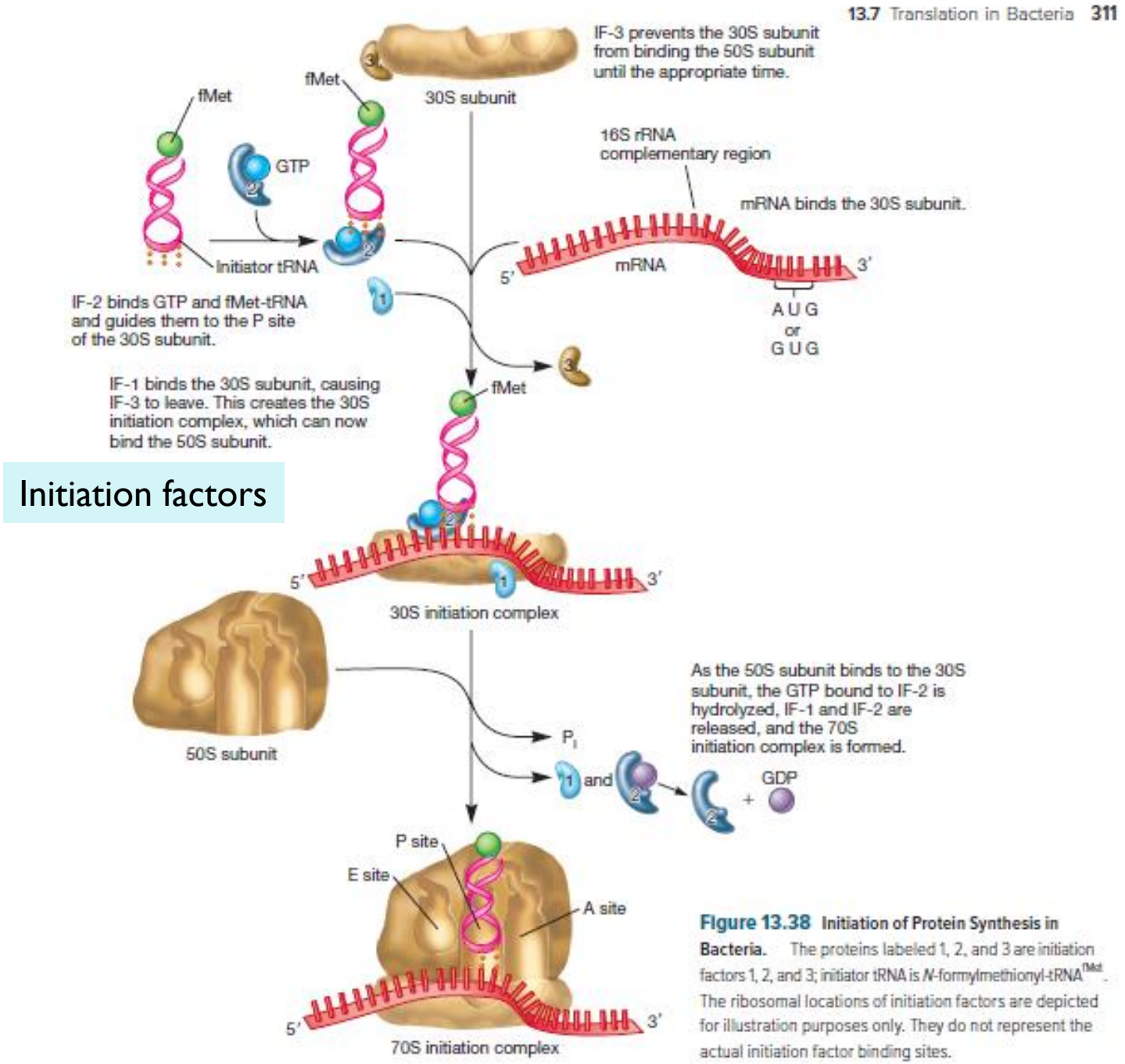
Translation proses

- **Initiation**

  - Ribosome has 3 sites
    - Peptidyl / donor site – P site
    - Aminoacyl / acceptor site – A site
    - Exit site – E site

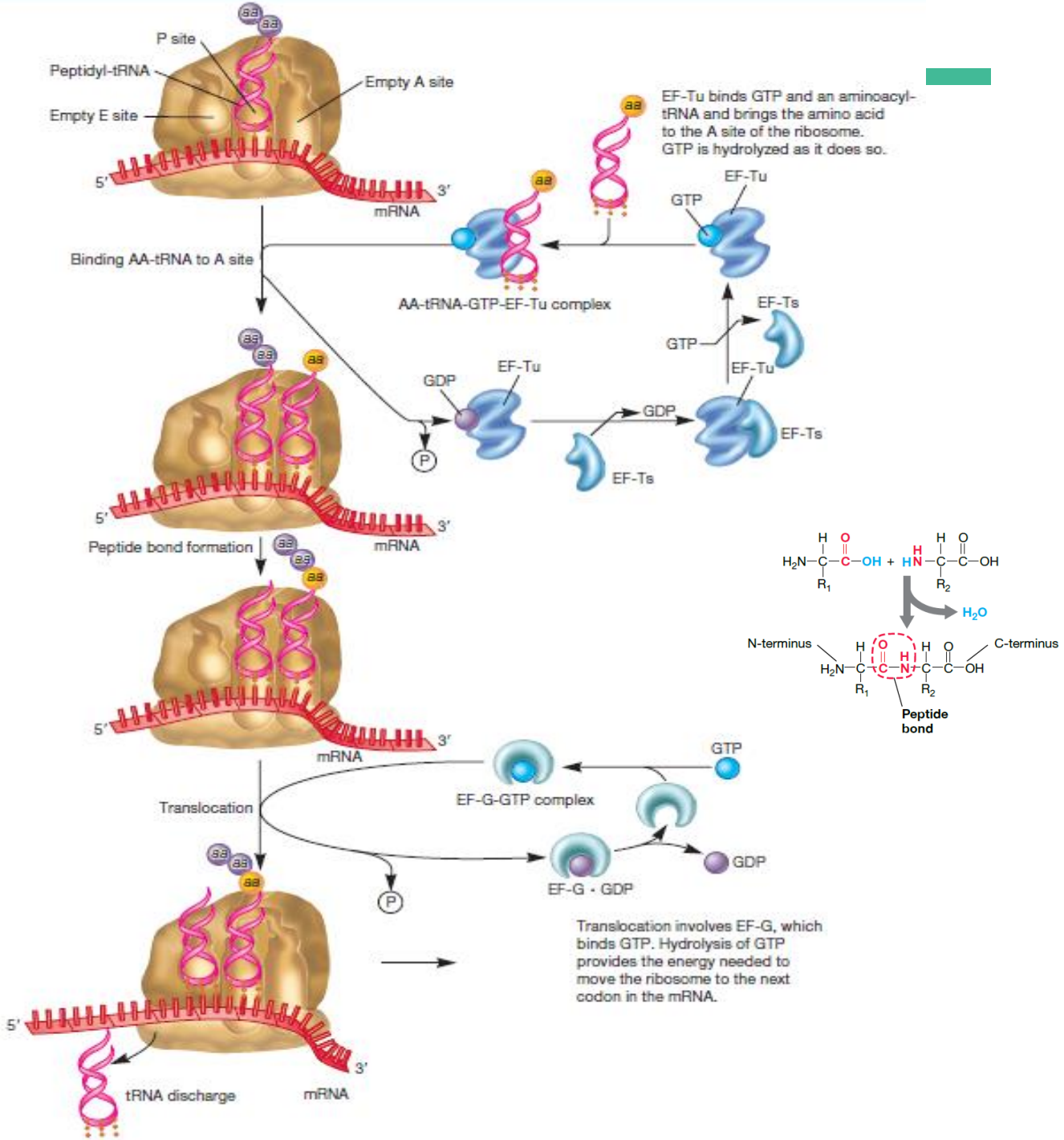
  - First tRNA, carrying an amino acid (Met), binds to the start codon AUG of mRNA at the P site.

  - Then, the two subunits of a ribosome attach to mRNA and form an active ribosomes-mRNA complex with the first tRNA carrying Met, is positioned at the P-site.



Translation proses

- **Elongation**
  - The next codon position (A site) is filled by the appropriate charged tRNA
  - The first amino acid (Met) is transferred to the amino acid on the next tRNA –attached by peptide bond
  - After forming the peptide bond, the ribosome releases the first tRNA (now empty) at the E site.
  - A new charged tRNA binds in the open position (A site)
  - The ribosome moves along the mRNA and the growing polypeptide is added to the new amino acid by forming a peptide bond



Translation proses

- **Termination**

  - The process repeats so that one amino acid is added at a time to the growing polypeptide
  - Polypeptide is always anchored to a tRNA bound within the ribosome
  - The polypeptide continues to grow until the ribosome reaches a stop codon (UAA, UAG or UGA)
  - At the stop codon, the polypeptide chain is released from the last tRNA and is complete
  - The two subunits of the ribosome detach from each other and the mRNA

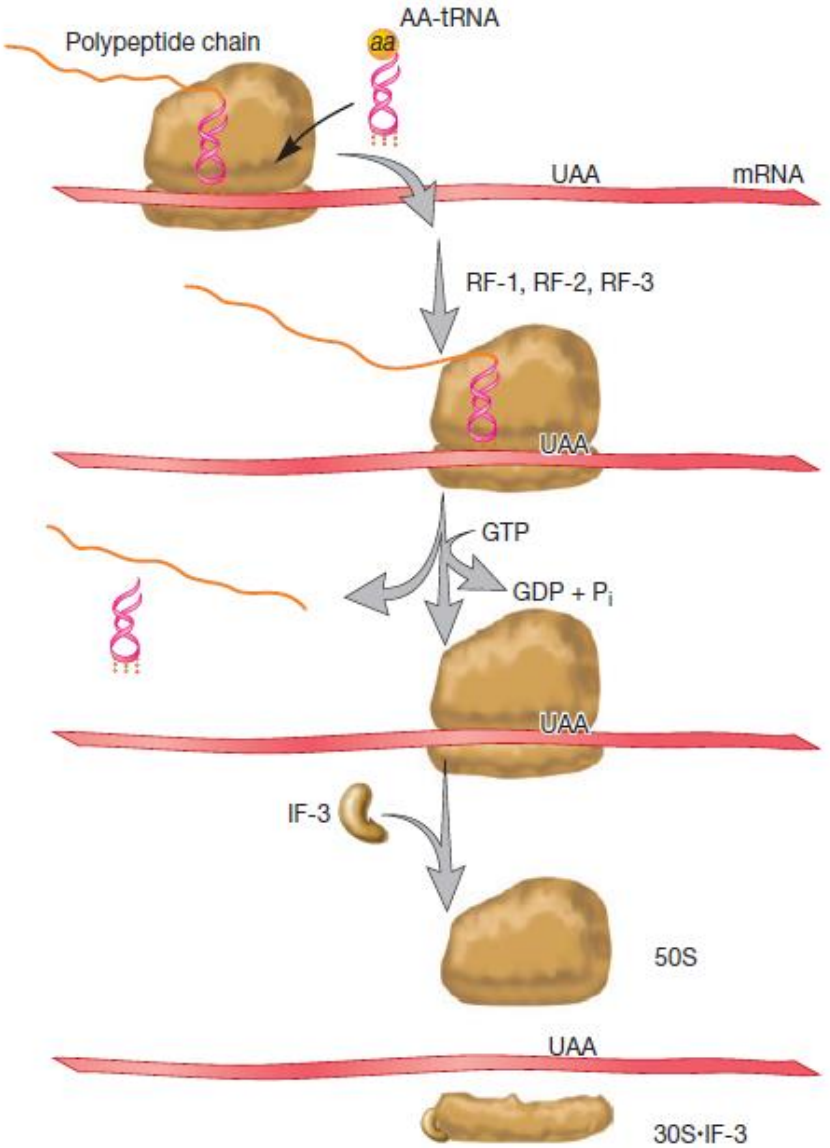


Figure 13.41 Termination of Protein Synthesis in Bacteria. Although three different nonsense codons can terminate chain elongation, UAA is most often used. Three release factors (RF) assist the ribosome in recognizing nonsense codons and terminating translation and GTP is hydrolyzed. Binding of IF-3 to the 30S subunit prepares it for the next round of translation initiation. Transfer RNAs are in pink.

REGULATION OF GENE EXPRESSION

- The process of transcription and translation can be regulated

- A gene may be **constitutive**: gene which is always turned on
  - E.g. enzyme of the central metabolic pathway
  - Continuously expressed the genes and protein is synthesized

- If not ALWAYS ON, a gene must be **regulated** in some fashion…
  - E.g. β-galactosidase enzyme (lactose --> glucose + galactose)
  - enzymes are only needed at certain time/environment
  - The genes are expressed only when needed

- 2 phenomena of regulation
  - Induction of enzyme synthesis – e.g. synthesis of enzymes in **catabolic** pathway (β-galactosidase enzyme), **lac operon**
  - Repression of enzyme synthesis – e.g. synthesis of enzymes involve in **biosynthetic p**athway (tryptophan biosynthesis), **tryptophan operon**

- Three basic categories of genes
  - Genes that code for proteins – **structural genes**
  - Genes that control gene expression – **regulatory genes**
  - Genes that code for RNA

- Group of genes in regulation
  - **Operons**: a group of genes located together in the DNA and which are regulated together. When an operon is activated all genes in the region tend to be made
  - **Promoter**: Region of DNA that initiates transcription of particular gene.
  - **Operator**: A segment of DNA to which a transcription factor binds. A segment between promoter and genes of the operon

Gene regulation - *lac* Operon

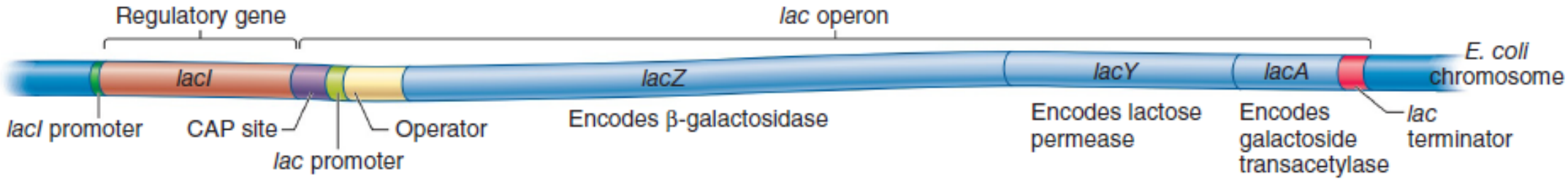
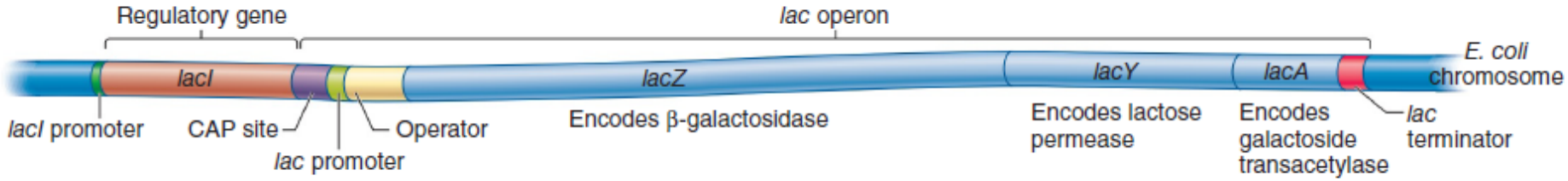


Figure 14.5 The *lac* Operon. The *lac* operon consists of three structural genes—*lacZ, lacY,* and *lacA*—that are transcribed as a single unit from the *lac* promoter. The operon is regulated both negatively and positively. Negative control is brought about by the *lac* repressor, which is the product of the *lacI* gene. The operator is the site of *lac* repressor binding. Positive control results from the action of CAP. CAP binds the CAP site located just upstream from the *lac* promoter. CAP is partly responsible for a phenomenon called catabolite repression, an example of a global control network, in which numerous operons are controlled by a single protein (section 14.5). For simplicity, the operator is represented as a single region. In reality, the *lac* operator consists of three distinct sites, as shown in figure 14.6.

Lac Operon is activated when lactose is presence
The enzymes are used to catabolize lactose to glucose and galactose

Lactose Operon: Inducible Operon

- Made of 3 segments:
  1. **Regulator** – gene that codes for repressor
  2. **Control locus** – composed of promoter and operator
  3. **Structural locus** – made of 3 structural genes, each coding for an enzyme needed to catabolize lactose (*lac Z, lac Y, lac A*)
     - ✓ β-galactosidase – hydrolyzes lactose
     - ✓ lactose permease – brings lactose across cell membrane
     - ✓ galactosidase transacetylase – uncertain function



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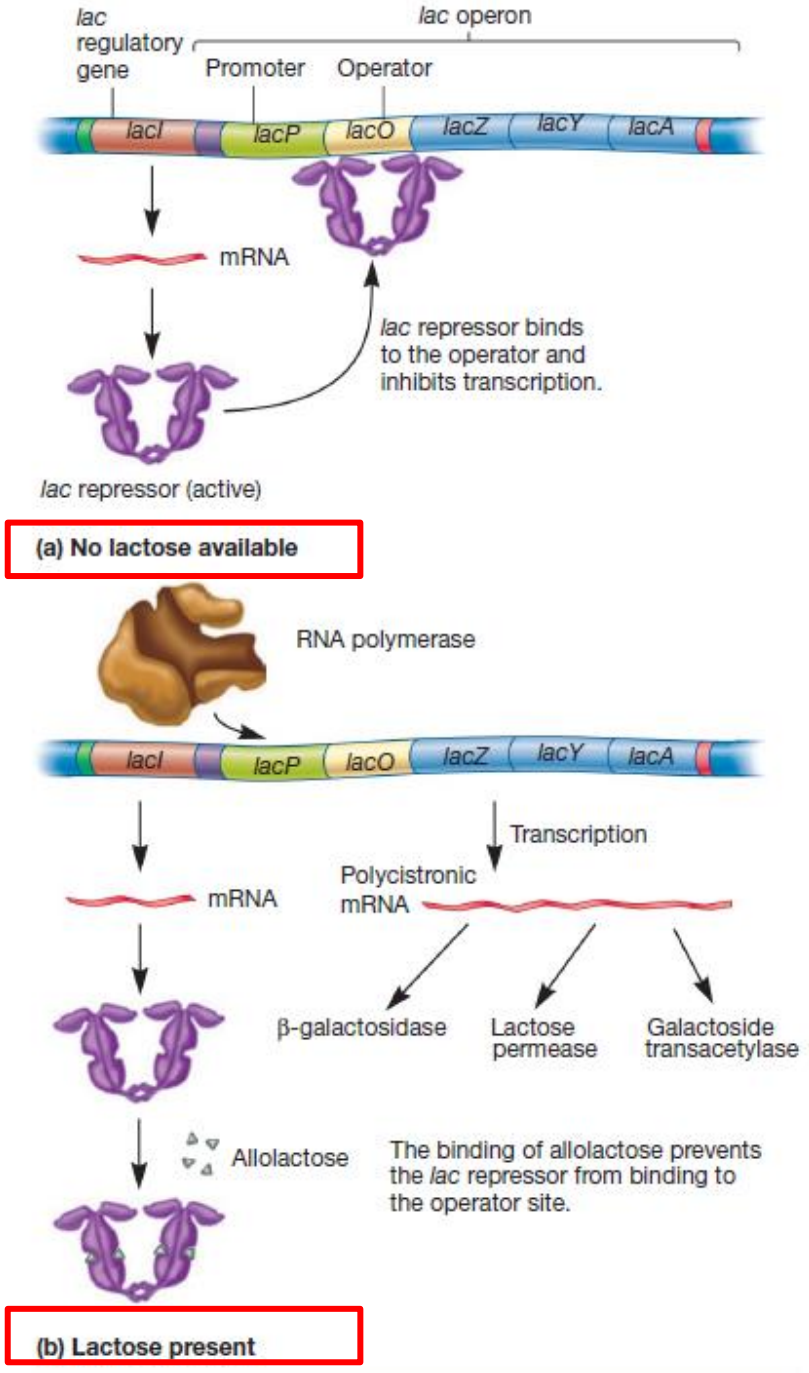
Regulation of the *lac* Operon (Inducible Operon)

- The **expression** of lactose Operon (*lac* Operon) is **high when lactose is available** & glucose (preferred carbon & energy source) is absent.

- **NO LACTOSE**
  - The lac repressor bind to operator site & inhibits transcription when there is no lactose.
  - The RNA polymerase is blocked from moving into the coding region

- **LACTOSE AVAILABLE**
  - β-galactosidase convert lactose to allolactose (operon inducer)
  - Allolactose interact with lac repressor and change it shape --> unable to bind the operator site
  - Transcription of the operon occurs

- Second regulatory protein – catabolite activator protein (CAP)
  - Allow microorganism to utilize glucose preferentially (catabolite repression mechanism)

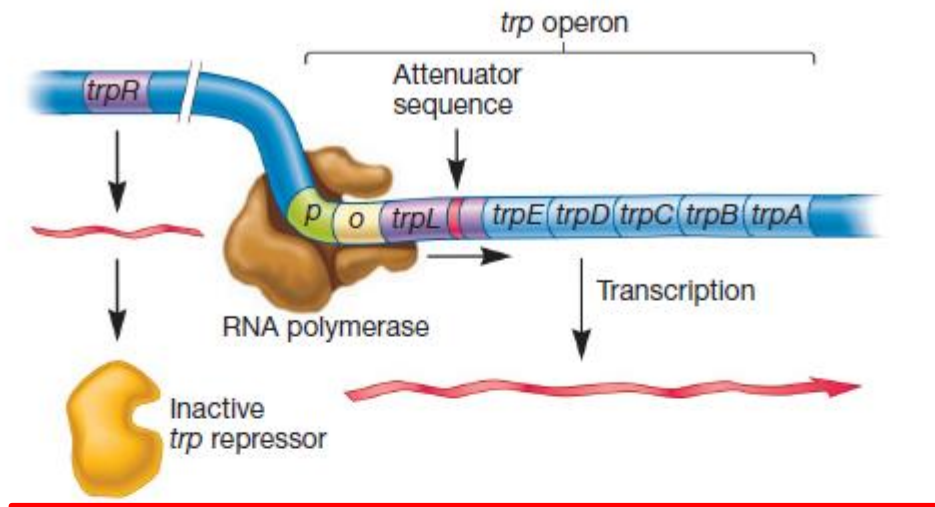


Regulation of the *trp* Operon (Repressible Operon)

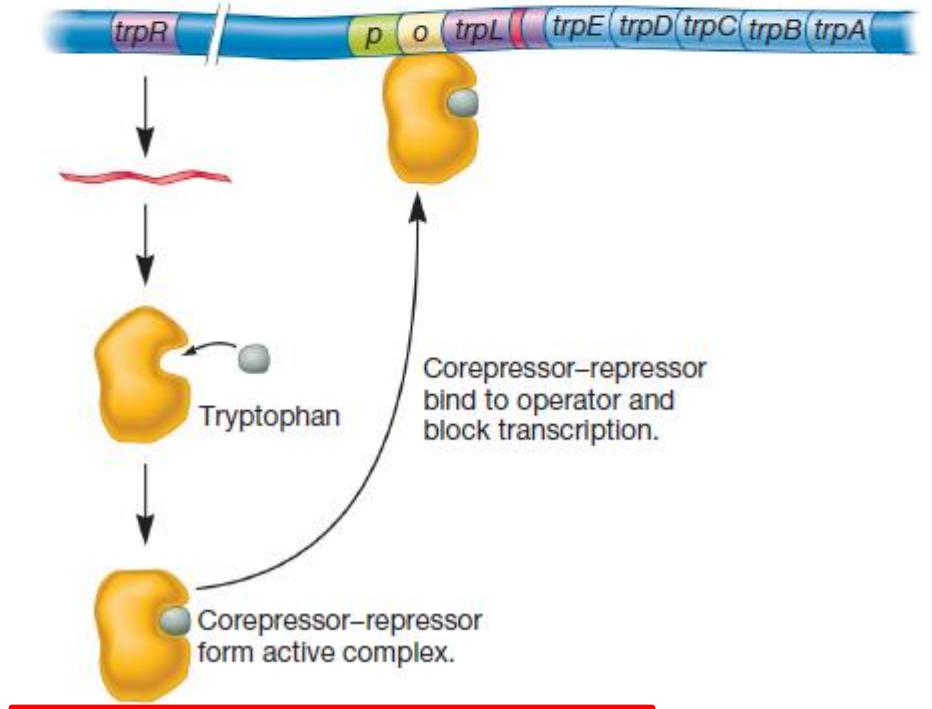
- Tryptophan operon (*trp* Operon) encode enzymes for synthesis of amino acid tryptophan
- Function in biosynthetic pathway – usually occurs in anabolism
- Regulated by *trp* repressor

- **Tryptophan level low**
  - Repressor is in inactive form – cannot bind the operator
  - Transcription of the operon occurs

- **Tryptophan level high**
  - Tryptophan act as corepressor – binds to repressor & activate it
  - The complex attaches to operator, blocking transcription of the operon



(a) Low tryptophan levels, transcription of the entire *trp* operon occurs



(b) High tryptophan levels, repression occurs

MUTATIONS

- Genetic diversity in bacteria can be created by mutation and other genetic exchange/transfer mechanism.
- Mutation: a **change in the base sequence** of DNA
- **Point mutation**: Single base substitution
  - Substituting a single base pair is the most common form of mutation.
  - Usually takes place in DNA replication.
- **Source of mutations**
  - can be spontaneous (natural errors of replication)
  - can be induced by a mutagen (any agent that causes mutation)
  - chemical
  - radiation (UV or ionizing)

Types of Point Mutations in Protein-coding genes

- **Silent mutation**
  - Change the nucleotide sequence of codon but do not change the amino acid
- **Nonsense Mutation**
  - It causes a stop codon
  - Cause early termination of translation. Result in shorten polypeptides
- **Missense Mutation**
  - Change a codon for amino acid (e.g. Lys to Arg)
  - Alter protein structure - loss activity
- **Frameshift mutation**
  - adding or deleting bases – reading frame is shifted
  - a premature stop codon
  - Synthesis of non-functional protein
  - Can yield mutant phenotype

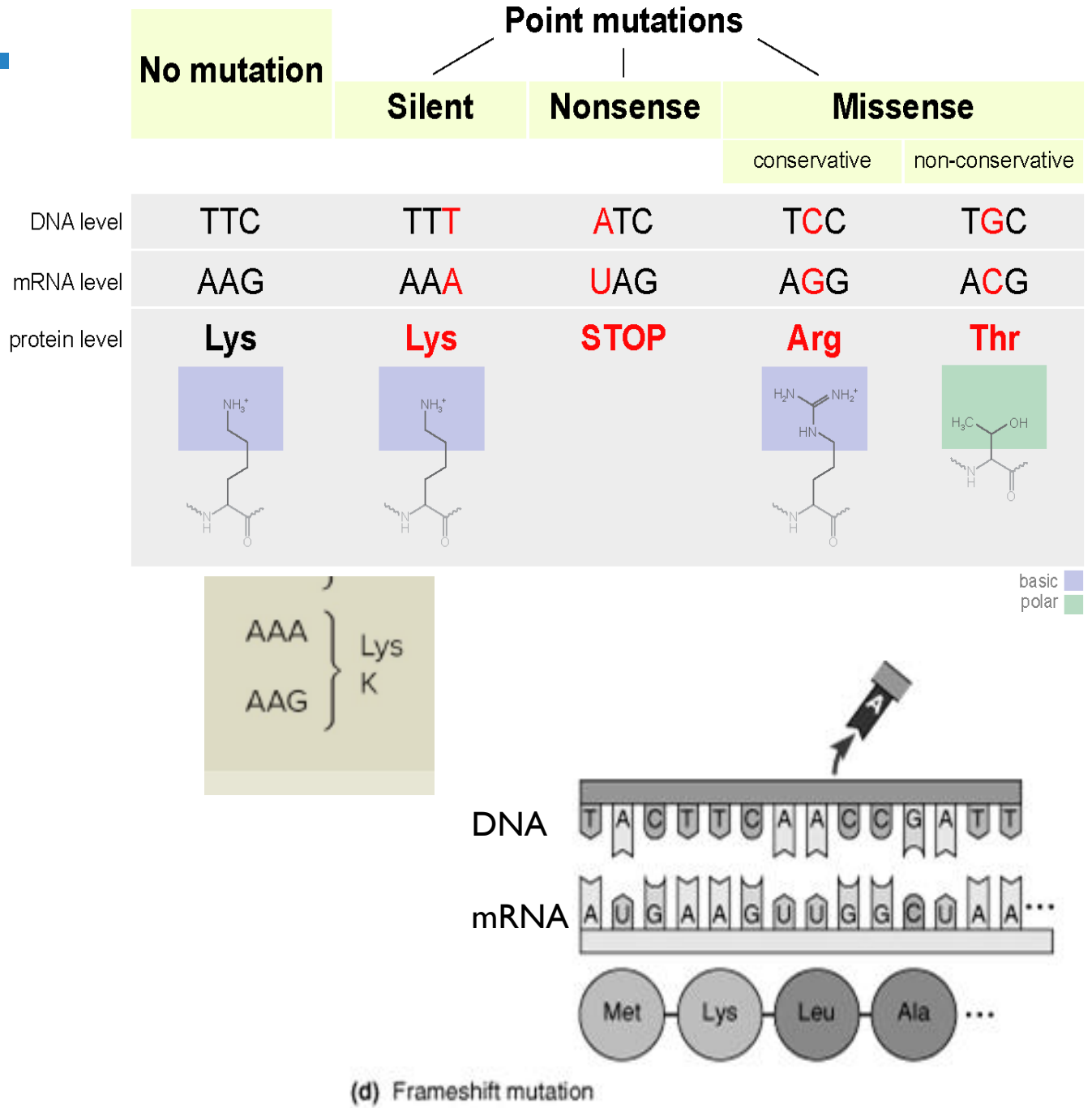


Table 16.2 Types of Point Mutations

Type of Mutation	Change in DNA	Example
Forward Mutations		
None	None	5′-A-T-G-A-C-C-T-C-C-C-C-G-A-A-A-G-G-G-3′ Met - Thr - Ser - Pro - Lys - Gly
Silent	Base substitution	5′-A-T-G-A-C-A-T-C-C-C-C-G-A-A-A-G-G-G-3′ Met - Thr - Ser - Pro - Lys - Gly
Missense	Base substitution	5′-A-T-G-A-C-C-T-G-C-C-C-G-A-A-A-G-G-G-3′ Met - Thr - Cys - Pro - Lys - Gly
Nonsense		5′-A-T-G-A-C-C-T-C-C-C-C-G-T-A-A-G-G-G-3′ Met - Thr - Ser - Pro - STOP
Frameshift	Insertion/deletion	5′-A-T-G-A-C-C-T-C-C-G-C-C-G-A-A-A-G-G-G-3′ Met - Thr - Ser - Ala - Glu - Arg

ACU
ACC
ACA
ACG
} Thr
T

DNA repair

- Mutations can have detrimental effects
- Microorganism need to repair the changes
- Repair mechanism in bacteria:
  - **Proofreading**: the first line of defense during DNA replication
    - ✓ DNA polymerase can detect any mistake that has been made, it backs up and removing the incorrect mucleotide (3’ – 5’ exonuclease activity)
  - **Mismatch repair**
    - ✓ Useful when DNA polymerase fails
    - ✓ Enzyme MutS scan the newly synthesized DNA for mismatched pairs and MutH removed a stretch of newly synthesized DNA around the mismatch.
    - ✓ The excised nucleotides are replaced by the DNA polymerase and the nick is sealed by DNA ligase
  - **Excision repair**
    - ✓ Correct damage that cause distortion in the double helix
    - ✓ Damaged portion of DNA is removed
    - ✓ The intact complementary strand serves as the template for new DNA synthesis

EXCHANGE/TRANSFER OF GENETIC MATERIAL

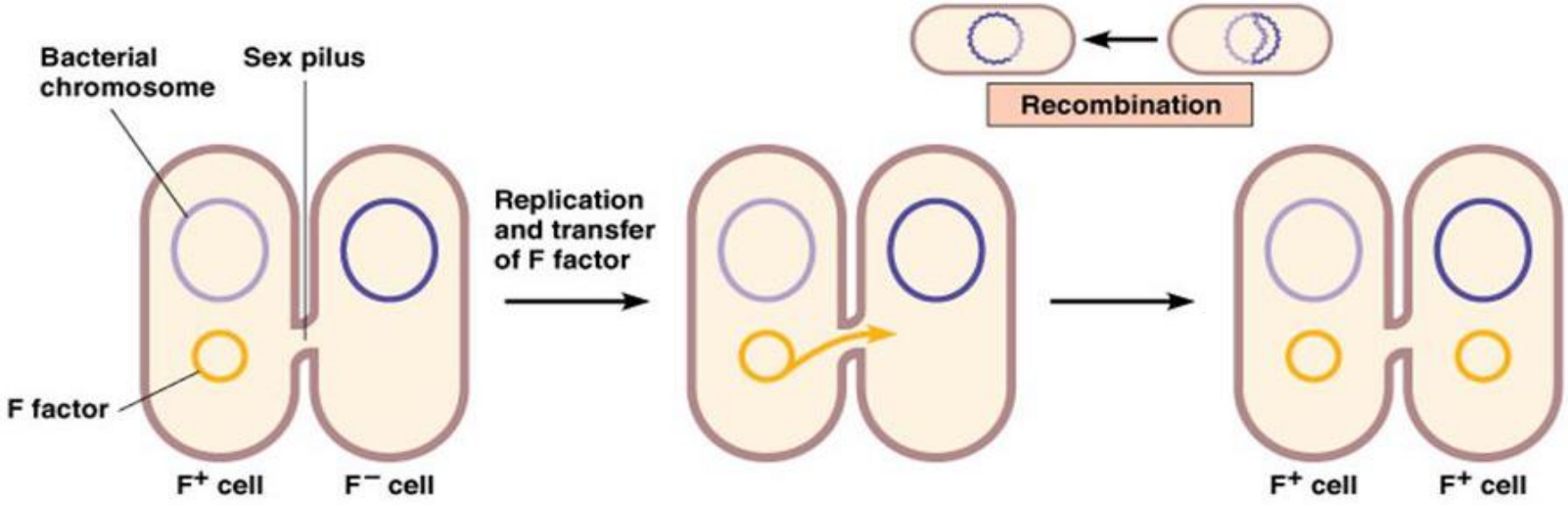
3 mechanisms for the exchange of genetic material

- ✓ Conjugation
- ✓ Transformation
- ✓ Transduction

- **Conjugation**
  - ➢ Transmission of genetic material (new DNA) via cell-cell contact using a sex pilus
  - ➢ Depend on the presence of conjugative plasmid , e.g. F factor
  - ➢ Plasmid: small, double stranded DNA that can exist independently of host chromosomes
  - ➢ F-factor = fertility factor; genes encode proteins for building the sex pilus

Bacterial Conjugation

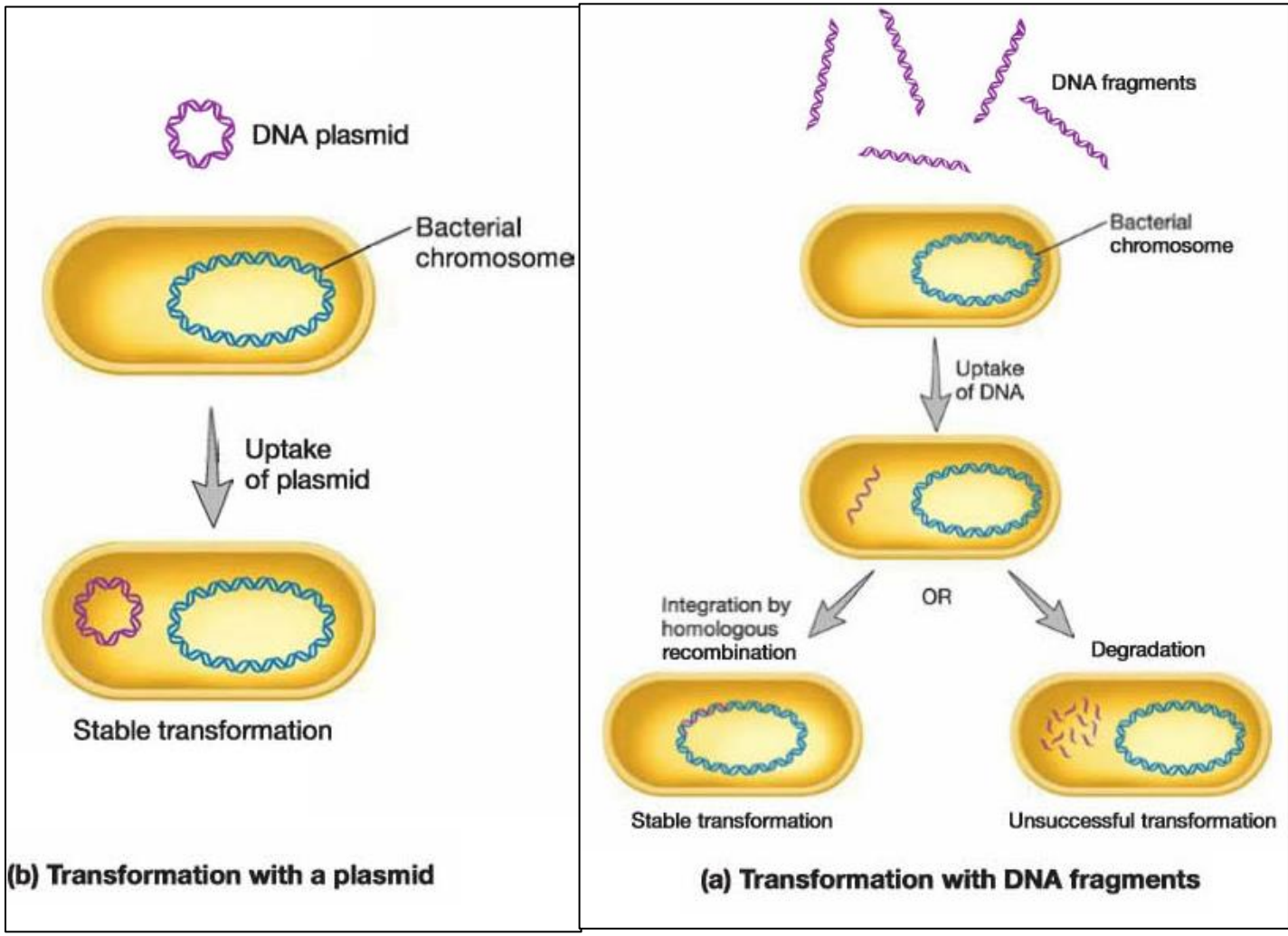
- **Conjugation steps**
  - ✓ Donor cell produces pilus.
  - ✓ Pilus attaches to recipient cell and brings the two cells together.
  - ✓ The mobile plasmid (F factor) is nicked and a single strand of DNA is then transferred to the recipient cell.
  - ✓ Both cells synthesize a complementary strand to produce a double stranded circular plasmid and also reproduce pili; both cells are now viable donors.



(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.

Bacterial Transformation

- **Transformation**
  - The uptake and incorporation of genes (naked DNA, e.g. plasmid or DNA fragment) from the surrounding.
    - ✓ DNA is released into the surrounding when bacteria lyse
    - ✓ It can be taken up by a cell and integrate into their DNA



Bacterial Transduction

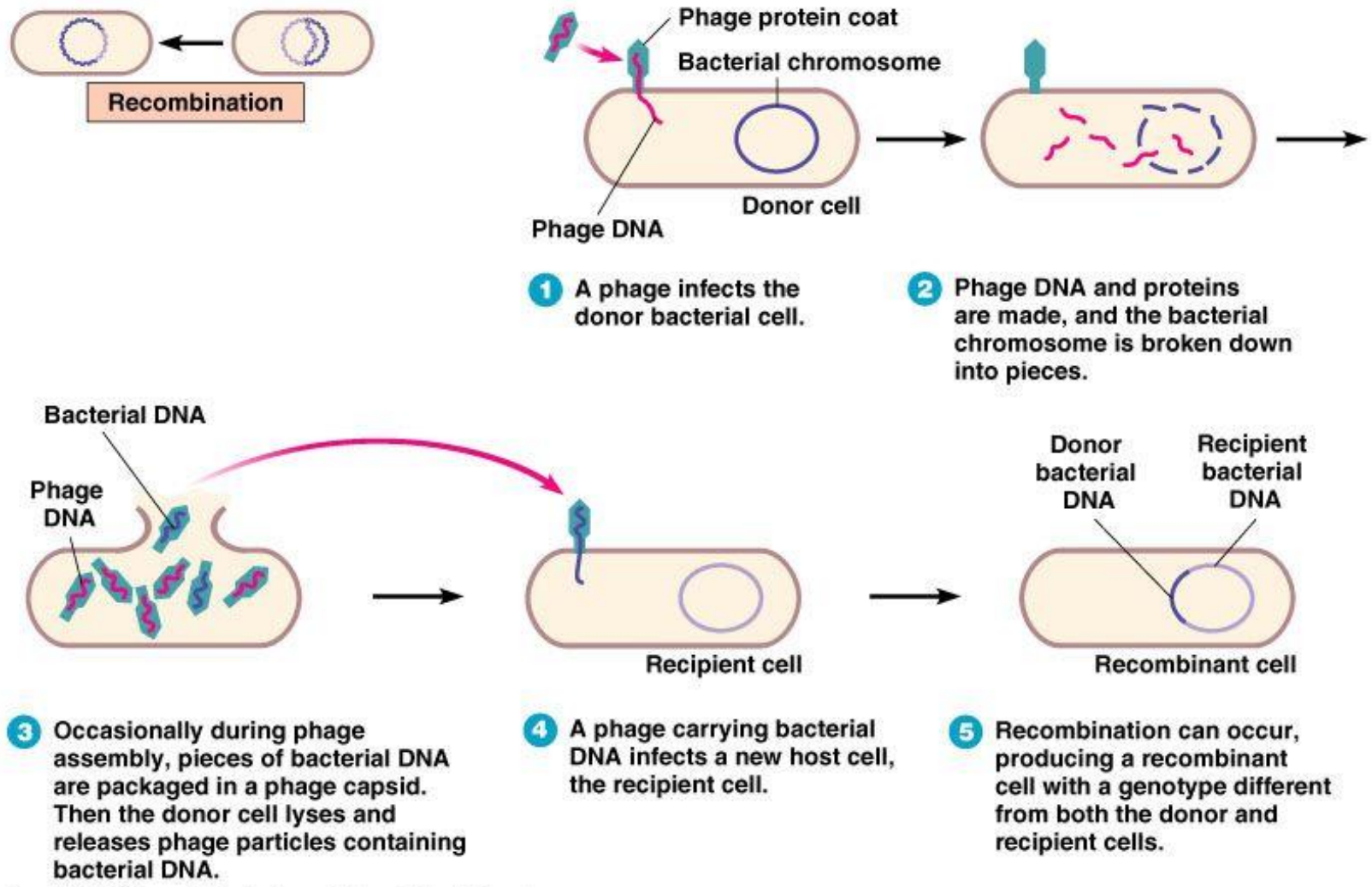
- **Transduction**

➢ Viral mediated transfer of DNA

➢ Two kinds:

✓ generalized (any gene)

✓ specialized (always a specific gene)



Summary

- Basic structure of DNA and RNA
- DNA replication
- Protein synthesis – transcription & translation
- Regulation of gene expression – inducible & respresible operon.
- Mutations – Point, Missense, Nonsense, frameshift mutation
- Exchange of genetic material – Transformation, Conjugation & Transduction

THANK YOU