

Key Enzymes and their Roles

Enzyme	Function
Helicase	Unwinds the DNA helix
SSBs	Prevents reannealing of single strands
Topoisomerase	Relieves supercoiling tension
Primase	Lays down RNA primers
DNA Polymerase III	Synthesizes the new DNA strand
DNA Polymerase I	Replaces RNA primers with DNA
Ligase	Seals Okazaki fragments

DNA Replication is Terminated at Distinct Sites in *E. coli*

- The *E. coli* chromosome contains specific termination (**Ter**) sites.
- **Tus** (termination utilization substance) binds these Ter sites to facilitate replication termination.
- **Ter-Tus** complexes are not symmetrical.
- This asymmetry is crucial for controlling the replication fork's progress.

Directional Block

- Approaching the Ter-Tus complex from one direction allows the replication fork to pass; if from the opposite direction → blocks the fork, terminating replication.

Efficient Termination

- The positions and orientations of Ter sites ensure that replication stops after one full cycle around the circular chromosome.
- This prevents replication complexes from indefinitely circling the DNA.

DNA Replication

Step 1: INITIATION

- Origin of replication (OriC) recognized
- Helicase unwinds DNA
- SSBs stabilize single strands
- Topoisomerase prevents supercoiling

Step 2: ELONGATION

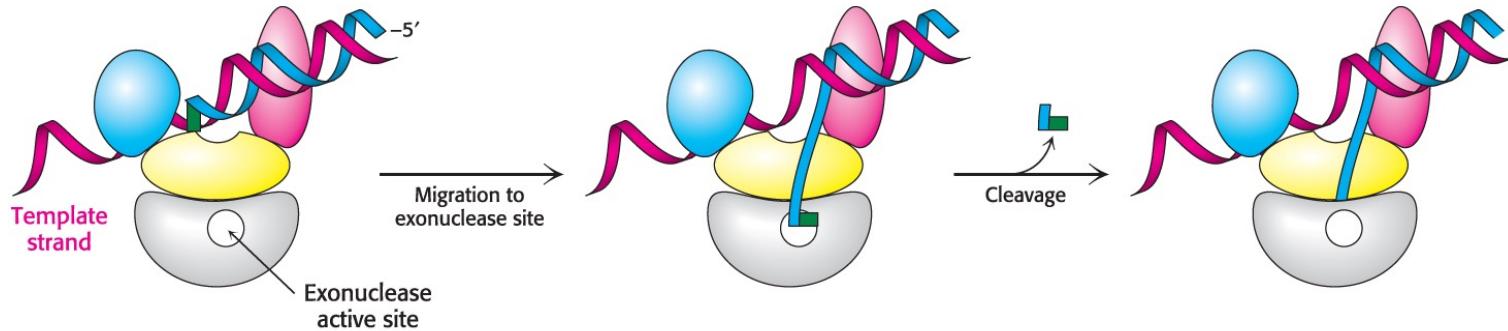
- Primase lays RNA primers
- DNA Polymerase III extends strands
- Leading strand: continuous synthesis ($5' \rightarrow 3'$)
- Lagging strand: Okazaki fragments joined by Ligase

Step 3: TERMINATION

- Replication forks meet and process stops
- DNA Polymerase I replaces primers
- Ligase seals final nicks
- Proofreading ensures accuracy

Step 4: PROOFREADING

Proofreading



If base-pairing is incorrect, the growing DNA strand shifts from the polymerase site to the exonuclease site, where erroneous nucleotides are removed.

High Fidelity Through Proofreading

- Many polymerases enhance DNA replication accuracy via proofreading mechanisms.
- DNA Polymerase I (*E. coli*) has both polymerase activity and a 3'→5' exonuclease activity.
- The exonuclease removes mismatched nucleotides from the 3' end by hydrolysis.

Mechanism of Mismatch Removal

- When an incorrect nucleotide is inserted, it forms weaker hydrogen bonds.
- The mismatched nucleotide “flops” away from the polymerase active site (due to Brownian motion) and into the exonuclease site.
- The incorrect nucleotide is then excised.

How the enzyme detects incorrect bases?

- An incorrect base will not properly pair with the template strand, making it less likely to be added to the new strand.
- Even if an incorrect base is inserted, the enzyme stalls due to the structural disruption of a non-Watson-Crick base pair.
 - The pause allows the DNA strand to move into the exonuclease site, where the mismatch is removed.

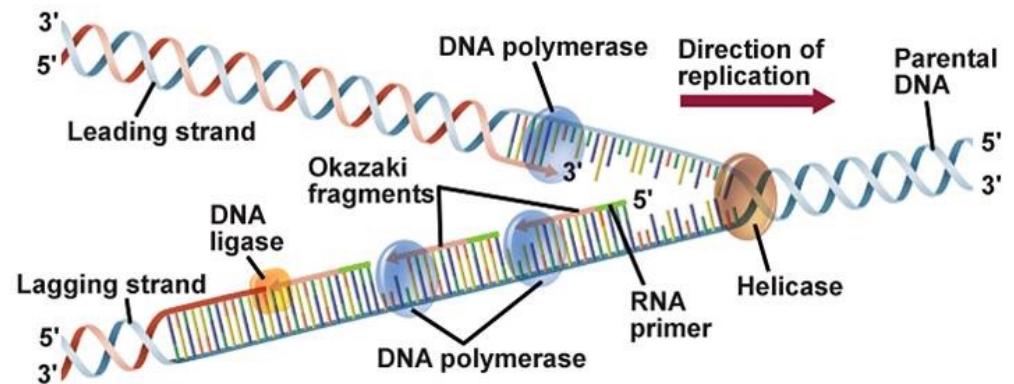
Mechanism of Stalling

- After a new nucleotide is added, the DNA is pulled by one base pair into the enzyme.
- An incorrect base causes a structural disruption, slowing the polymerase and enhancing the chance of exonuclease action.
- DNA polymerase I occasionally removes a correct nucleotide (1 in every 20).
- Despite this slight energetic cost, proofreading increases replication accuracy by about 1000-fold.

DNA Replication

Key characteristics of DNA synthesis are:

1. Four deoxynucleoside triphosphates and Mg^{2+} are required.
2. A template strand is used to direct DNA synthesis.
3. A primer from which the new strand grows must be present.
4. Many DNA polymerases have nuclease activity that allows for the removal of mismatched bases.



Quick Quiz 4

Which of the following is released during the addition of deoxyribonucleotides to the nascent DNA chain?

- A. Okazaki fragments
- B. pyrophosphate (PPi)
- C. AMP
- D. ATP
- E. dNTP

Quick Quiz 5

DNA polymerases can add nucleotides only to a free hydroxyl group. As a result, a primer made up of _____ is added by the enzyme _____.

- A. RNA; DNA polymerase II
- B. DNA; DNA polymerase I
- C. DNA; primase
- D. Okazaki fragments; primase
- E. RNA; primase

Bacteria vs *Homo Sapiens*

BACTERIA

- 5×10^6 base pairs
- Circular DNA packed in polyamines
- Division time: ~20 mins
- Replication Speed: 1000 bp/sec

H. sapiens

- 3.2×10^9 base pairs
- Linear DNA packed in histones
- Division time: > 8 hrs
- Replication Speed: ~50 bp/sec

Sheer Size

- *E. coli*: ~4.6 million base pairs vs *Human diploid cell*: ~6 billion base pairs

Multiple Chromosomes

- *E. coli*: 1 circular chromosome vs Humans: 23 pairs of linear chromosomes

Linear Chromosomes and Shortening

- Circular vs. linear DNA
- The lagging strand poses a problem that can lead to chromosome shortening if not addressed

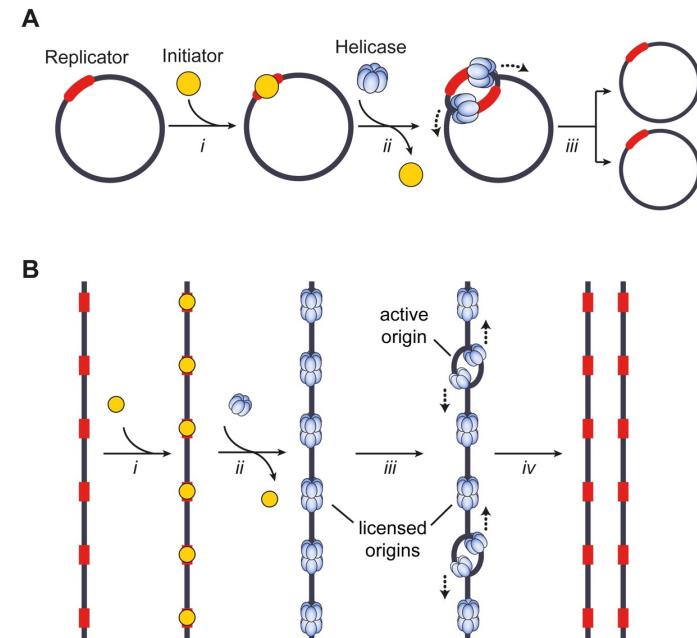
Multiple Origins and Replicon Control in Eukaryotes

Multiple Origins of Replication

- Eukaryotic genomes have many origins spaced ~30-300 kbp apart.
- Humans require ~30,000 origins for the entire genome, with each chromosome containing several hundred.
- Each origin defines a replicon, or replication unit.

Ensuring One Replication per Cell Division

- Licensing Factors bind to origins to form the initiation complex.
- Each replicon is activated once per round of DNA synthesis.
- Licensing factors are destroyed after replication begins, preventing re-initiation.

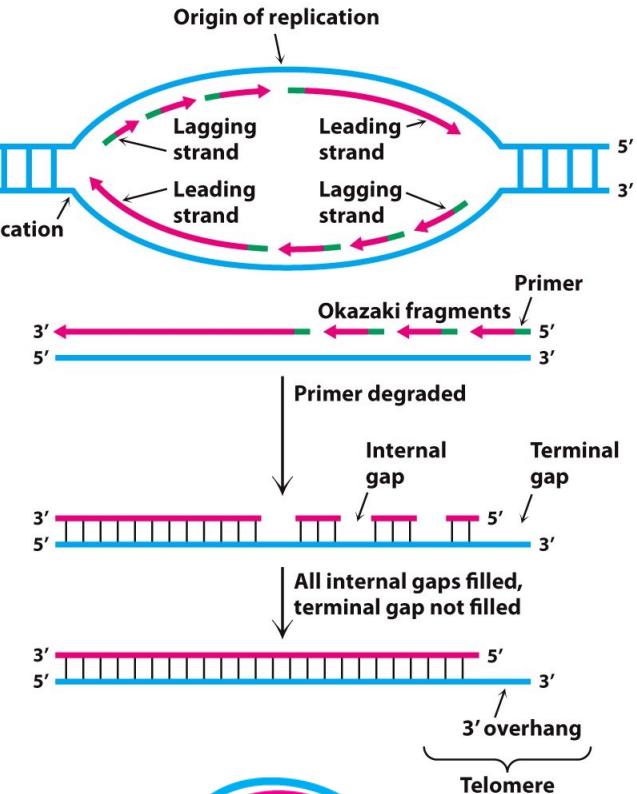


(A) In bacteria, initiator proteins recruit helicases, then replication proceeds bidirectionally.

(B) In eukaryotes, multiple origins are licensed, helicases are loaded, and replication continues bidirectionally until adjacent forks converge.

The Problem with Linear Chromosomes

- Susceptibility to exonucleases: the unprotected DNA termini are vulnerable to digestion.
- Incomplete replication:
 - DNA polymerases operate only $5' \rightarrow 3'$, making the lagging strand prone to shortening once the RNA primer is removed.
 - each replication cycle could further shorten chromosomes
- Telomeres: one strand is G-rich (3' end) and slightly longer; in humans, the repeat is AGGGTT.
- simple repeats form large duplex loops, with the single-stranded 3' end looping back to displace part of the original duplex; telomere-binding proteins help stabilize this loop structure.
- This loop (T-loop) shields chromosome ends from degradation and other damage.



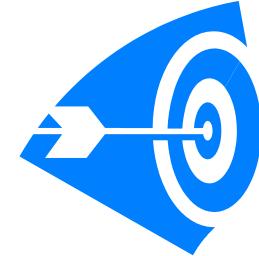
Polymerase Chain Reaction

- A method used to amplify a specific segment of DNA, creating millions of copies from a small initial amount.
- Core Components
 - **Template DNA:** sample containing the target sequence.
 - **Primers:** short DNA sequences that flank the region of interest.
 - **Taq DNA Polymerase:** Thermostable enzyme that synthesizes new DNA strands.
 - **dNTPs:** building blocks (A, C, G, and T) to assemble new DNA.
- Basic Steps
 - Denaturation (95°C): Heat separates double-stranded DNA into single strands.
 - Annealing (50–65°C): Primers bind (anneal) to complementary sequences on the single-stranded DNA.
 - Extension (72°C): Taq polymerase synthesizes new DNA by extending from the primers.
- PCRs have been used in research, forensics, and diagnostics.



Deoxynucleotide (dNTP) Solution Set

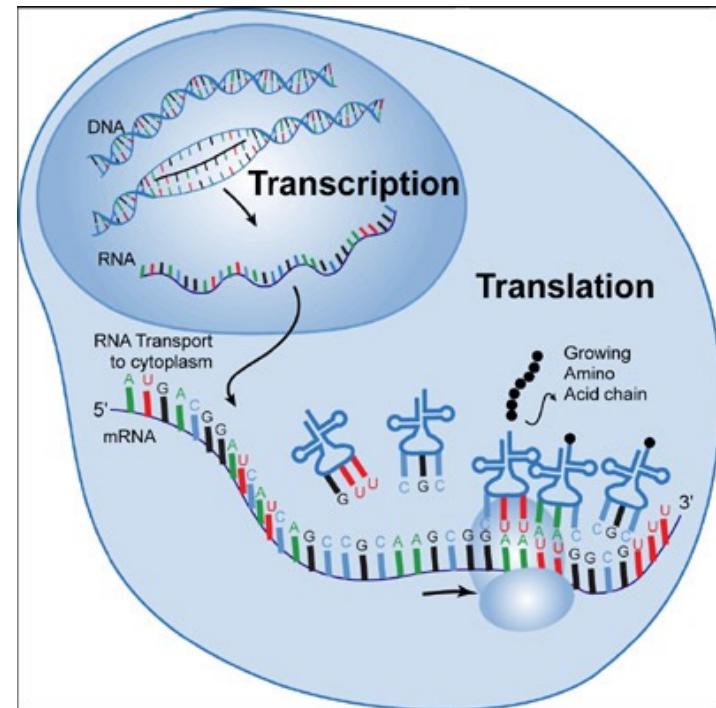
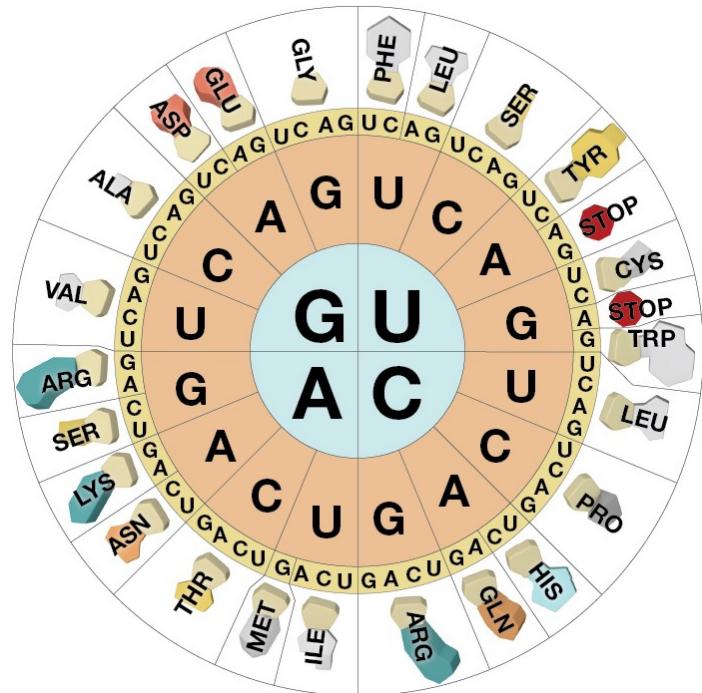
Assigned Problems



Chapter	Tymochko, Berg, Stryer, Biochemistry, 2 nd Edition,	Chapter	Tymochko, Berg, Stryer, Biochemistry, 2 nd Edition,
33	1, 3, 4, 5, 7, 10, 12, 16, 17, 19, 20, 21.	34	5, 6, 8, 9, 11, 12, 14, 17.
Chapter	Tymochko, Berg, Stryer, Biochemistry, 3 rd Edition, 4 th Edition (bottom line)	Chapter	Tymochko, Berg, Stryer, Biochemistry, 3 rd Edition, 4 th Edition (bottom line)
33	1, 3, 4, 5, 7, 11, 13, 17, 18, 20, 21, 22. 1, 3, 4, 5, 7, 11, 13, 17, 18, 20, 21, 22.	34	5, 6, 8, 9, 11, 12, 14, 17. 5, 6, 8, 9, 11, 12, 14, 17.

Lecture 8

RNA Synthesis and Regulations. The Genetic Code.





**first step in gene expression is transcription
(instructions are in the DNA template)**

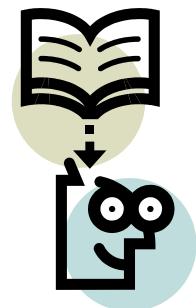
RNA Synthesis and Regulations. The Genetic Code

Lecture Outline:

- Cellular RNA Synthesis
- Three Stages Of RNA Synthesis
- The *lac* Operon Illustrates the Control of Bacterial Gene Expression
- The Genetic Code
- Amino Acid Activation Process
- A Ribosome Is a Ribonucleoprotein Particle Made of Two Subunits

Readings:

Tymochko, Berg, Stryer,
Biochemistry, 2nd Edition,
Ch. 36, 39, pp. 629 – 643; 675 – 688
3rd Edition,
Ch. 36, 39; pp.657-675; 705-721
4th Edition,
Ch. 36, 39; pp.733-744; 787-796

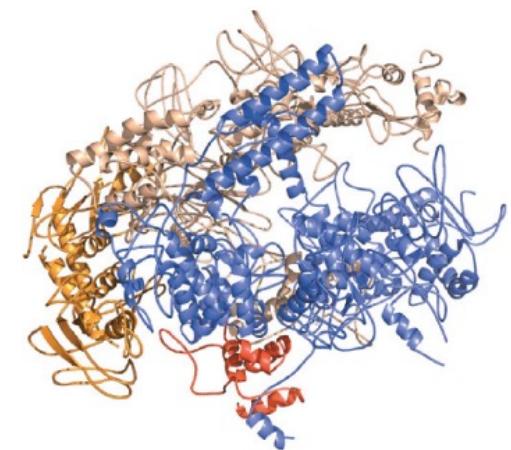


RNA Synthesis

- The synthesis of RNA from a DNA template is called **transcription**, a process catalyzed by **RNA polymerase**.
$$(\text{RNA})_n \text{ residues} + \text{ribonucleoside triphosphate} \rightleftharpoons (\text{RNA})_{n+1} \text{ residues} + \text{PPi}$$
- RNA polymerase has the following requirements:

1. A template. The sequence of the newly synthesized RNA is complementary to the DNA template. The DNA strand that has the same sequence as the RNA product (with T instead of U) is called the coding strand.
2. Activated precursors in the form of the four ribonucleoside triphosphates.
3. Divalent metal ions (serve as cofactor), usually Mg^{2+} or Mn^{2+} .

5'—GCGGCGACGCGCAGUUAAUCCCACAGCCGCCAGUUCGCUGGCGGCAU—3'
3'—CGCCGCTGCGCGTCAATTAGGGTGTGGCGGTCAAGGCGACCGCCGTA—5'
5'—GCGGCGACGCGCAGTTAATCCCACAGCCGCCAGTTCGCTGGCGGCAT—3'



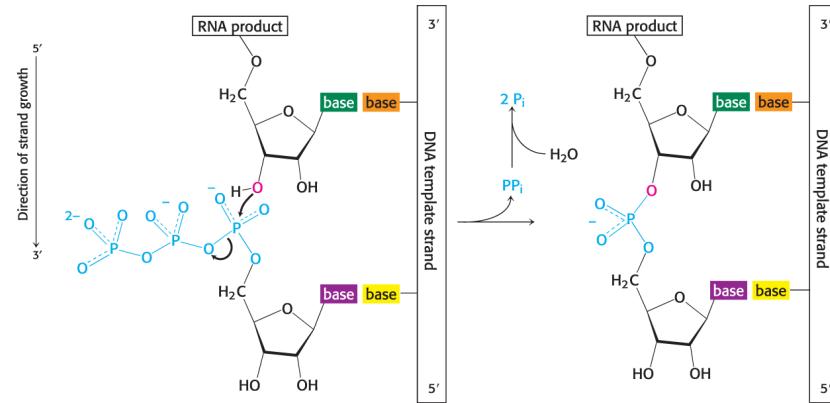
RNA polymerase from
Thermus aquaticus.

mRNA

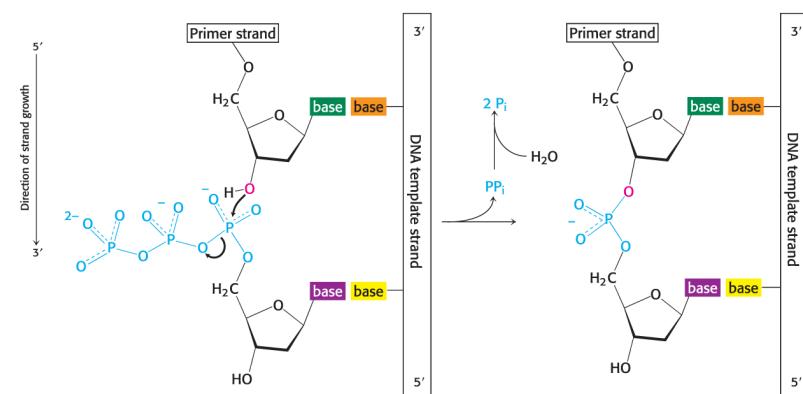
Template (antisense) strand of DNA
Coding (sense) strand of DNA

RNA Synthesis

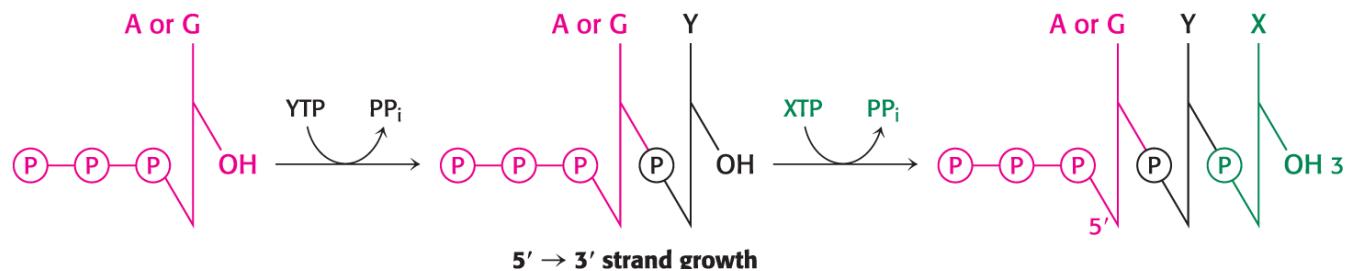
- RNA polymerase initiates and elongates the RNA product, with the chain growing in the 5' to 3' direction. The 3'OH of the growing chain attacks the inner most phosphoryl (α) group of the incoming ribonucleoside triphosphate.



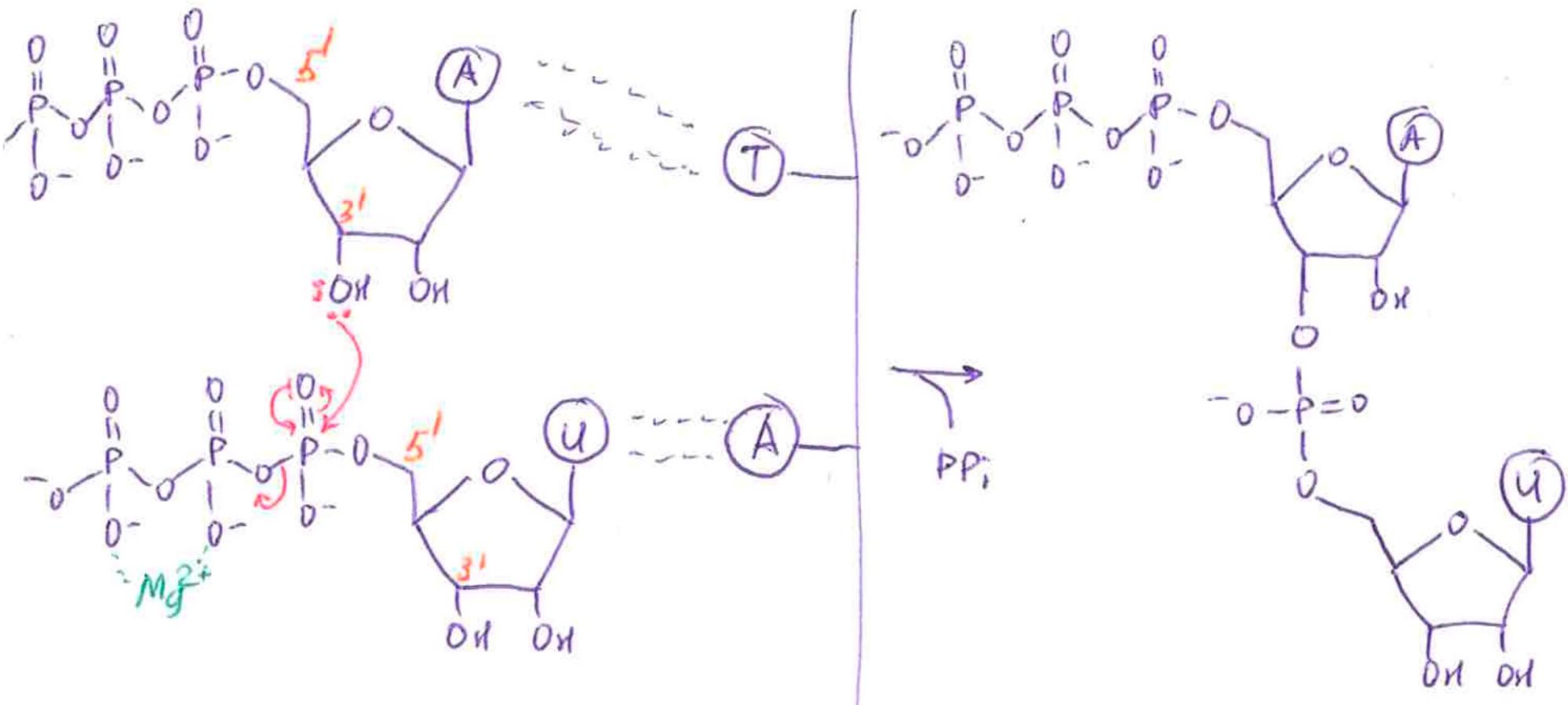
RNA strand-elongation reaction.



DNA strand-elongation reaction. DNA polymerase catalyze the formation of phosphodiester bridge.



RNA Synthesis



Cellular RNA is Synthesized by RNA Polymerases

- Three major classes of RNA are synthesized.
 - *messenger RNA (mRNA)* encodes the information to generate a protein.
 - *transfer RNA (tRNA)* and
 - *ribosomal RNA (rRNA)* play key roles in translating mRNA information into protein.
- RNA polymerase is composed of five subunits (~500 kDa).
- The holoenzyme, consisting of $\alpha_2\beta\beta'\omega\sigma$ subunits, initiates RNA synthesis.
- The core enzyme, composed of $\alpha_2\beta\beta'\omega$ subunits, elongates the RNA product (contains the active site).

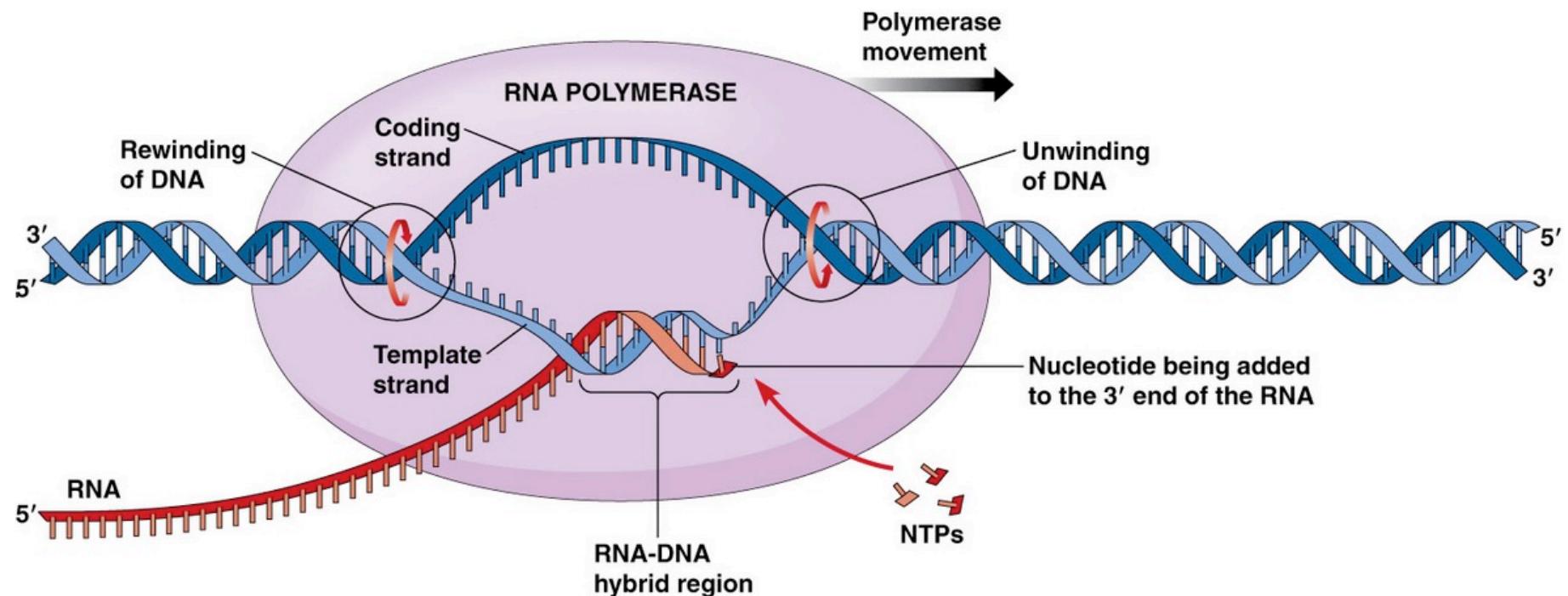
Table 36.1 Subunits of *E. coli* RNA polymerase

Subunit	Gene	Number	Mass (kd)	Function
α	<i>rpoA</i>	2	37	Required for assembly of core enzyme; interacts with regulatory factors
β	<i>rpoB</i>	1	151	Takes part in all stages of catalysis
β'	<i>rpoC</i>	1	155	Binds to DNA; takes part in catalysis
ω	<i>rpoZ</i>	1	10	Required to restore denatured polymerase to its native form
σ^{70}	<i>rpoD</i>	1	70	Takes part in promoter recognition

σ subunit looks for the site where transcription begins, participates in initiating RNA synthesis, then releases itself from the core enzyme.

Stages of RNA Synthesis

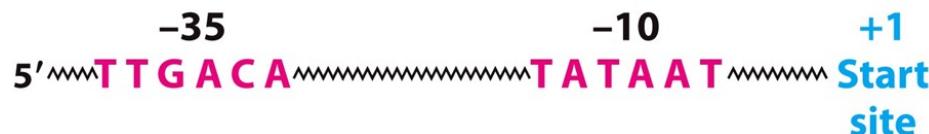
- The three stages of RNA synthesis are initiation, elongation, and termination.



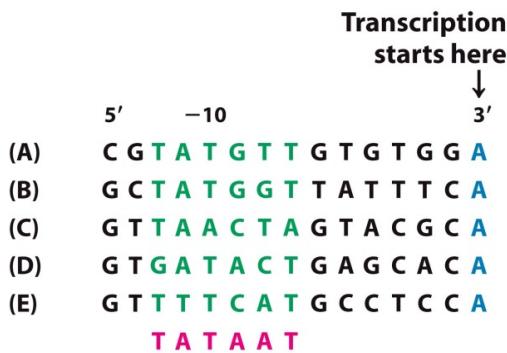
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(1) Initiation of RNA Synthesis

- Promoters are specific DNA sequences that direct RNA polymerase to the proper initiation site
- In *E. coli*, two DNA sequences that act as a promoter for many genes are the -10 sequence and the -35 sequence



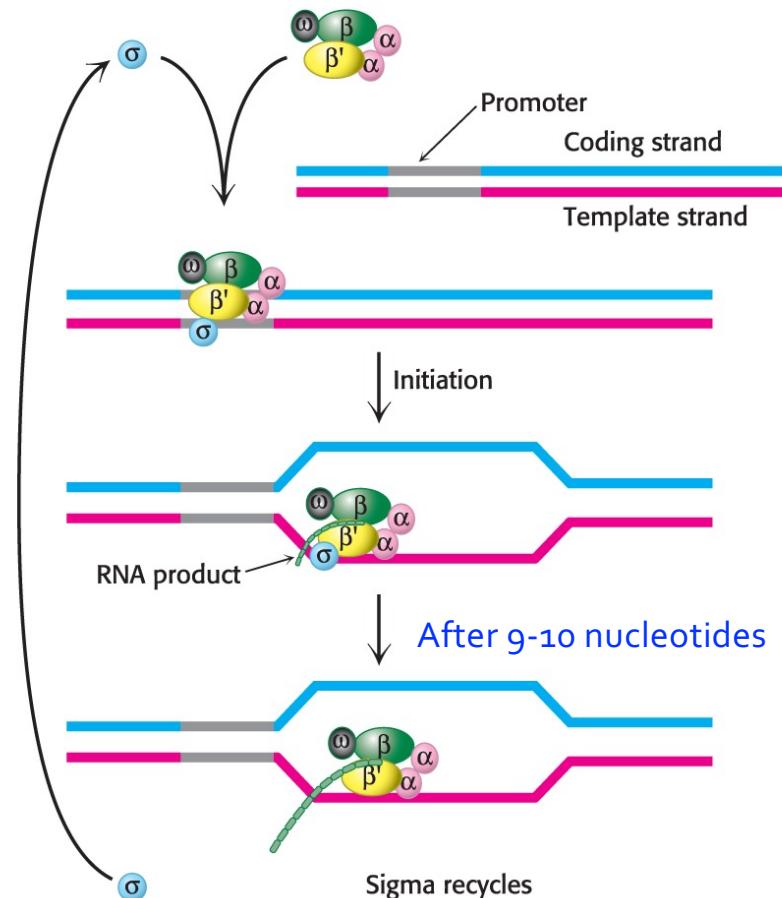
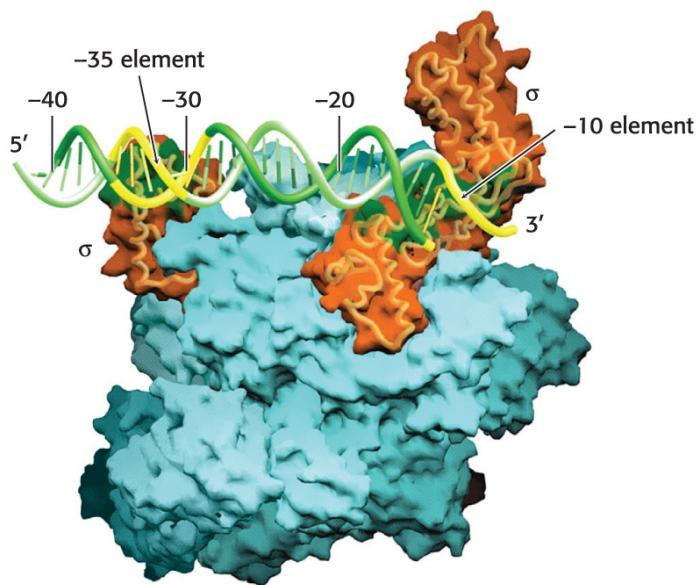
- There are variations in the sequence of the promoter for different genes. The average or consensus sequences are



- Other sequences upstream of the promoter can enhance promoter effectiveness (creates an additional binding site for the enzyme, hence, increases transcription efficiency).

Sigma subunits recognize promoter sites

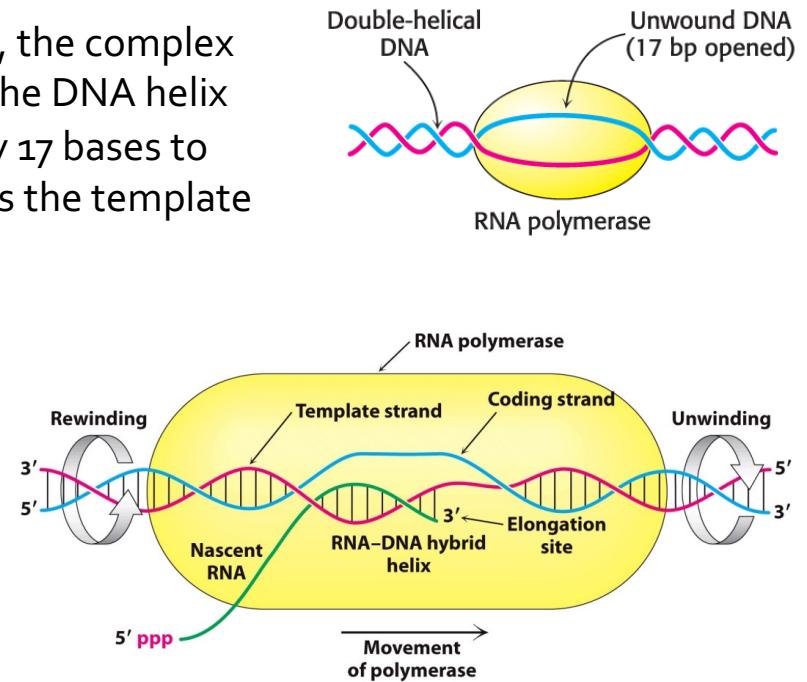
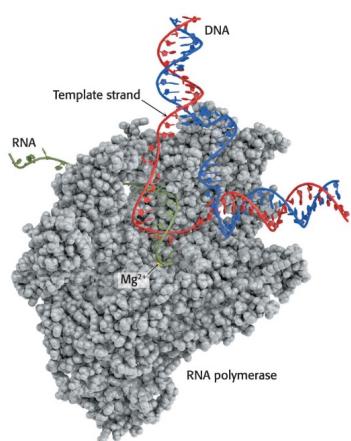
- Sigma subunits of RNA polymerase recognize promoter sites.



1. Decreases the enzyme's affinity for general regions of DNA (by 10^4).
2. The sigma subunit enables the enzyme to identify promoter sites.

(2) Elongation of RNA Synthesis

- When the promoter is initially located by the polymerase, the complex formed is called the ***closed promoter complex*** because the DNA helix is not unwound; RNA polymerase unwinds approximately 17 bases to form an open promoter complex in which the DNA acts as the template
- Once the DNA is unwound, elongation can take place
- The region containing the RNA polymerase, DNA, and the RNA product is called the ***transcription bubble***
- The transcription bubble moves along the DNA as DNA is unwound and then rewound, while the RNA product is extruded from the complex
- A DNA-RNA hybrid helix of approximately 8 nucleotides is an intermediate in RNA synthesis

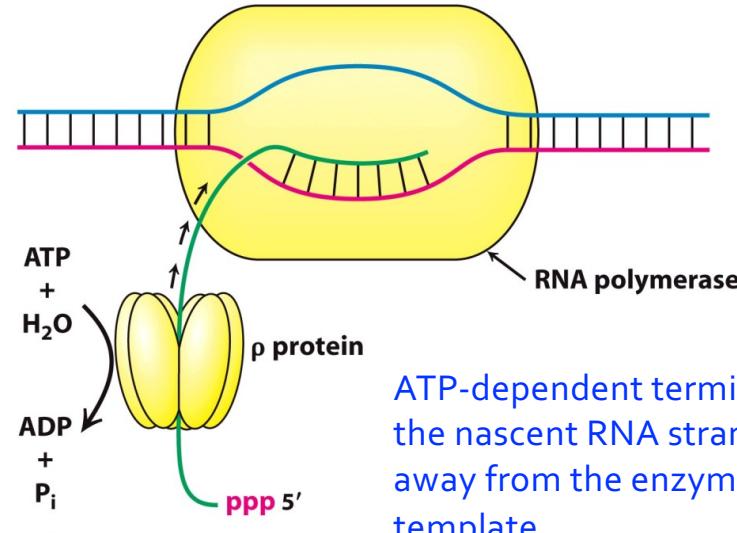
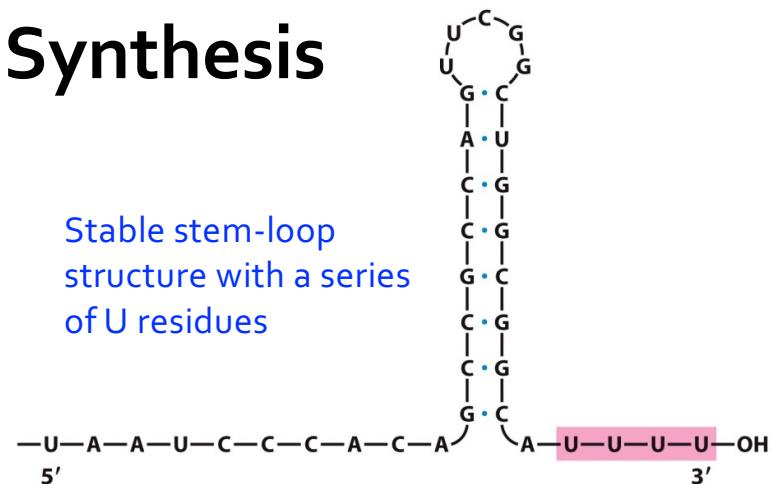


RNA rate of elongation: 50 nt/s

DNA rate of elongation: 1000 nt/s

(3) Termination of RNA Synthesis

- Elongation continues until a termination signal is detected
 - The simplest stop signal is the transcribed product of a segment of palindromic DNA
 - Another type of termination signal requires the protein *rho* (*ρ*)
 - Rho binds to a particular sequence on the RNA product and uses the energy of ATP hydrolysis to chase down the polymerase in the transcription bubble
 - Contact with rho causes the transcription bubble to dissociate



ATP-dependent termination: binds the nascent RNA strand and pulls it away from the enzyme and DNA template.

Quick Quiz 1

The template DNA strand for transcription by RNA polymerase

- A. has a sequence that is complementary to the transcribed RNA
- B. has the same sequence as the transcribed RNA
- C. has thymine (T) replaced by Uracil (U)
- D. has Uracil (U) replaced by thymine (T)
- E. is also referred to as the sense strand

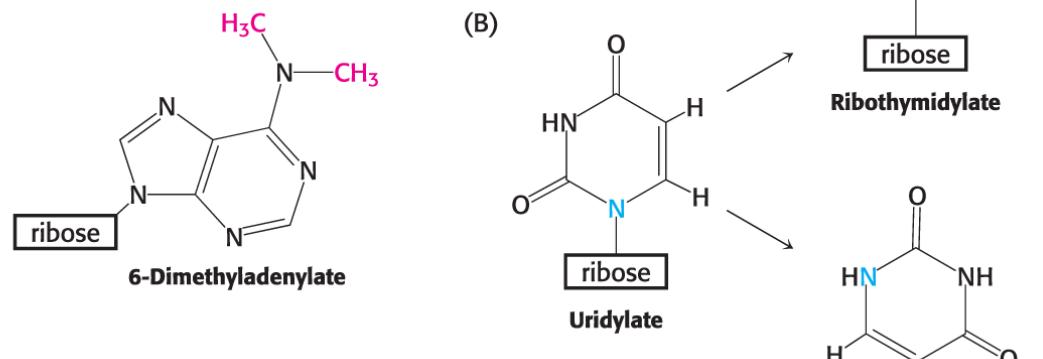
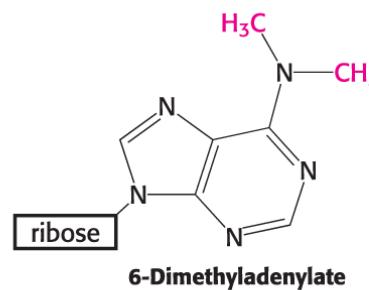
Quick Quiz 2

Which of the following affect the strength or the efficiency of the promoter?

- A. The sequence of the -10 and -35 sites.
- B. The distance between the -10 and -35 sites.
- C. Transcription factors that bind to the promoter region.
- D. All of the above.
- E. None of the above

Cleavage and Chemical Modification in tRNA and rRNA Maturation

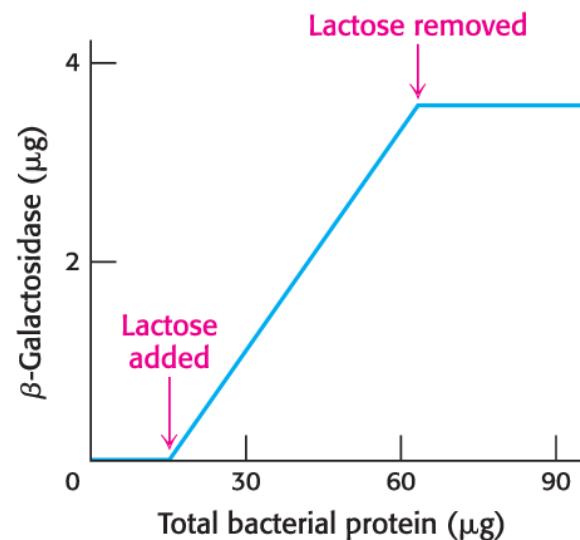
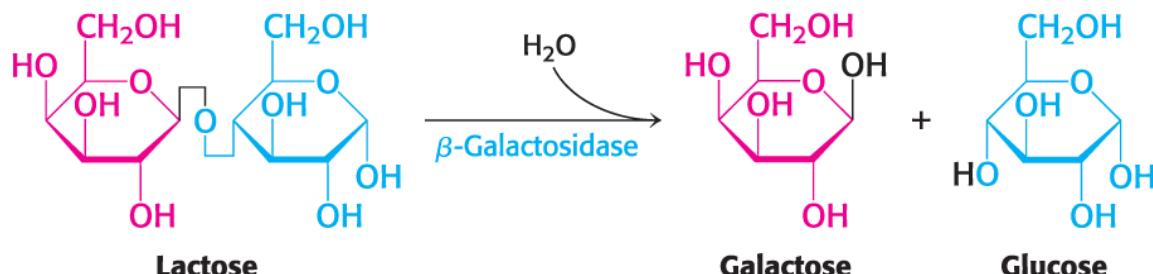
- Precursors of transfer and ribosomal RNA are cleaved and chemically modified after transcription.
- Although mRNA undergoes little or no modification after synthesis in bacteria, the same is not true for **rRNA** or **tRNA**.
- **Ribosomal** and **transfer RNA** are modified as follows:
 - The final mature RNA is cleaved from a larger precursor molecule.
 - Many tRNA transcripts lack CCA sequence at the 3' end of the strand. These nucleotides are added post-transcriptionally.
 - The bases and riboses of tRNA and rRNA are modified, for instance, by the attachment of methyl groups.



Modifications generate diversity → more structural and functional diversity

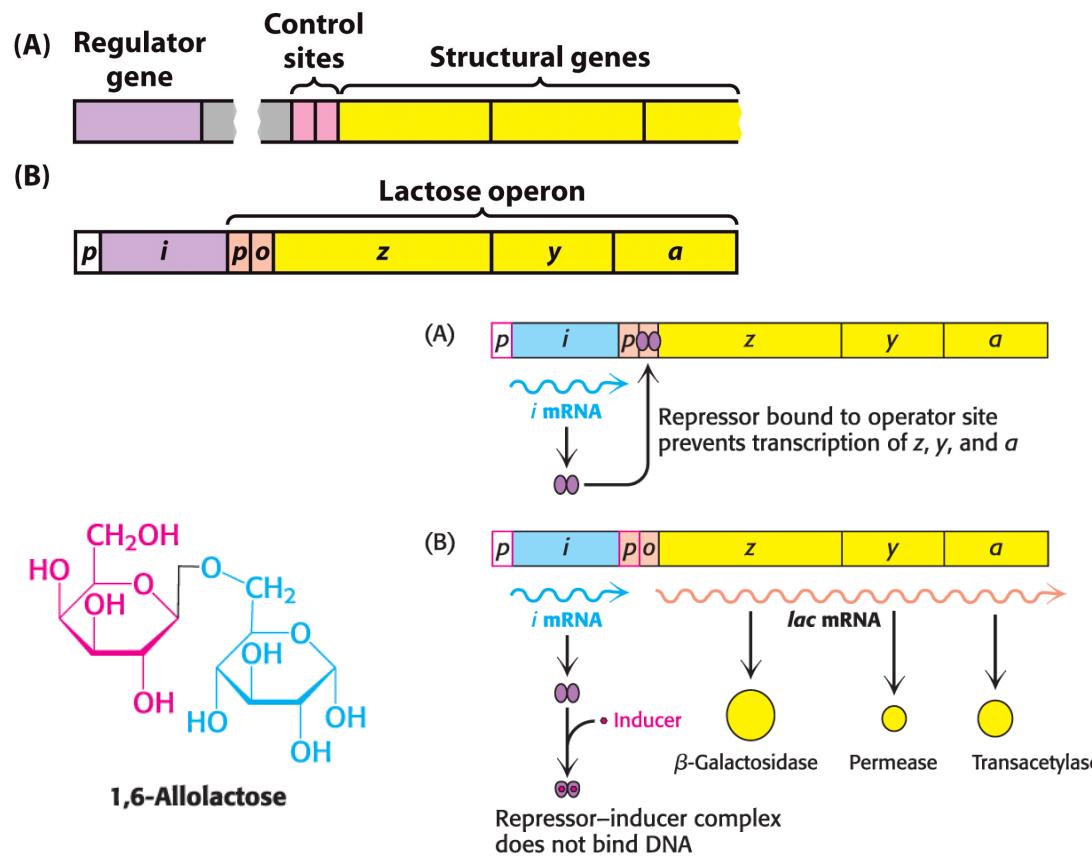
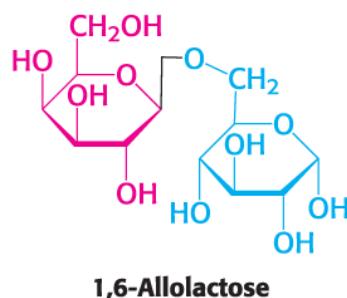
The *lac* Operon Illustrates the Control of Bacterial Gene Expression

- Transcription is a regulated process. For instance, the gene for β -galactosidase, which metabolizes lactose, is minimally transcribed unless lactose is present
- In the presence of lactose, the genes for β -galactosidase as well as two other enzymes—a permease and thiogalactoside transacetylase—are expressed
- Such a coordinated unit is called an operon, and in the case of lactose metabolizing enzymes, the unit is called the *lac operon*.



The *lac* Operon Illustrates the Control of Bacterial Gene Expression

- The DNA components of an operon consist of a *regulator gene*, an *operator*, a *promoter*, and structural genes.
 - In the lac operon, the regulatory gene encodes a protein called the lac repressor that binds to the operator site in the absence of lactose and prevents transcription of the structural genes.



Quick Quiz 3

RNA polymerase forms phosphodiester bonds with the concomitant release of pyrophosphate. The subsequent conversion of pyrophosphate to this molecule drives the reaction in the direction of RNA synthesis.

- A. Orthophosphate
- B. Ribonucleotide
- C. Deoxyribonucleotide
- D. Water
- E. Diphosphate

The Genetic Code Links Nucleic Acid and Protein Information

- Protein synthesis is a process of translation. Nucleic acid sequence information is translated into amino acid sequence information. The genetic code links these two types of information.
- Characteristics of the genetic code are:
 1. *Three nucleotides, called a codon, encode an amino acid*
 2. *The code is not overlapping*
 3. *The code has no punctuation*
 4. *The code is read in the 5' to 3' direction*
 5. *The code is degenerate in that some amino acids are encoded by more than one codon.*

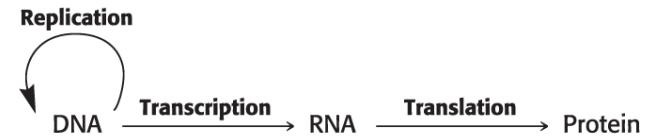


Table 39.1 The genetic code

First position (5' end)	Second position				Third position (3' end)
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Note: This table identifies the amino acid encoded by each triplet. For example, the codon 5'-AUG-3' on mRNA specifies methionine, whereas CAU specifies histidine. UAA, UAG, and UGA are termination signals. AUG is part of the initiation signal, in addition to coding for internal methionine residues.