

CHEM 3331
Fundamentals of Biochemistry

Chapter
11 Nucleic Acids and Protein Synthesis

Organic and Biochemistry for Today (4th ed.)
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1

Components of Nucleic Acids

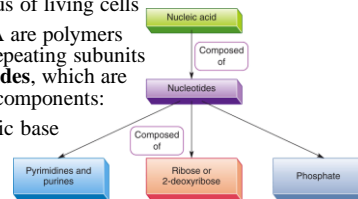
Nucleic Acids

The transfer of genetic information to new cells is accomplished through the use of biomolecules called **nucleic acids**:

- **ribonucleic acid (RNA)** — found mainly in the cytoplasm of living cells
- **deoxyribonucleic acid (DNA)** — found mainly in the nucleus of living cells

DNA and RNA are polymers consisting of repeating subunits called **nucleotides**, which are made of three components:

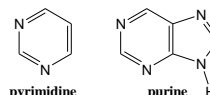
- a heterocyclic base
- a sugar
- phosphate



2

The Heterocyclic Bases

- A ring that contains elements other than carbon is called a **heterocyclic ring**.
- The bases found in RNA and DNA contain two types of heterocyclic rings: **pyrimidine** and **purine**.



– pyrimidine bases: **uracil (U)**, **thymine (T)**, **cytosine (C)**

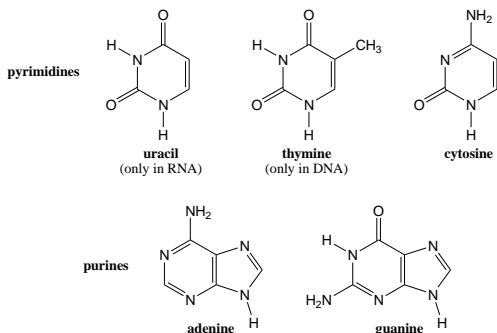
– purine bases: **adenine (A)**, **guanine (G)**

– DNA contains A, G, T, C; RNA replaces T with U

3

4

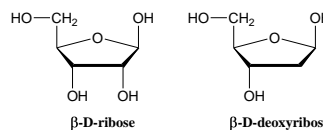
The Heterocyclic Bases



5

The Sugars, and the Phosphate Group

- In RNA, the sugar component is D-ribose, and in DNA the sugar is D-deoxyribose. (Note that both sugars are in the β -anomeric form.)



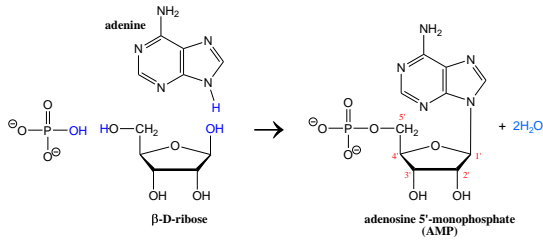
- The phosphate group in nucleotides is derived from phosphoric acid, H_3PO_4 , and at physiological pH exists in the ionic form:



6

Putting the Pieces Together: Nucleotides

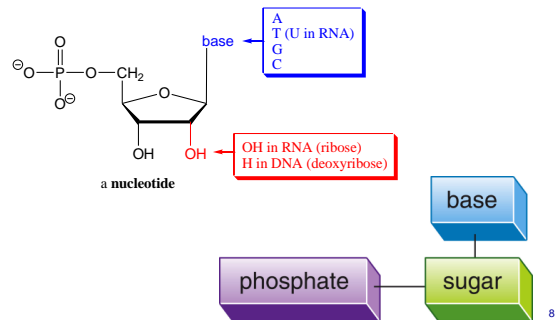
- Nucleotides** are formed from the combination of a sugar with a phosphate group at the 5' position and a heterocyclic base at the 1' position. (' is used to indicate the carbon number in the sugar to distinguish them from the atoms in the bases.)



7

General Nucleotide Structure

- The general structure of a nucleotide is illustrated below:



8

The Structure of DNA

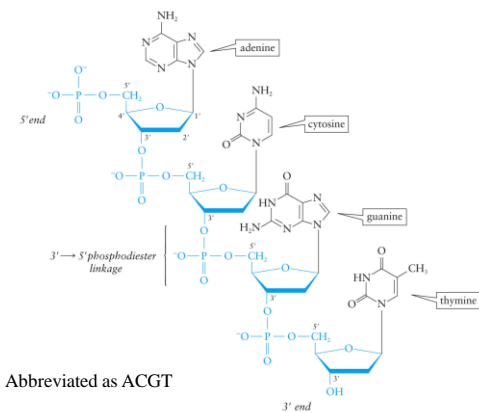
The Primary Structure of DNA

- DNA is one of the largest molecules known, containing between 1 and 100 million nucleotide units.
- The nucleotides in DNA are linked by phosphate groups that connect the 5' carbon of one nucleotide to the 3' carbon of the next.
 - Because these connections occur on two oxygen atoms of the phosphate group, they are called **phosphodiester bonds**.
- The **nucleic acid backbone** then is a sequence of sugar-phosphate groups, which differ only in the sequence of bases attached to the sugars along the backbone (the *primary structure* of DNA):



9

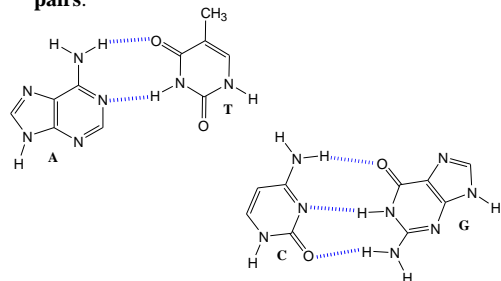
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11

The Secondary Structure of DNA

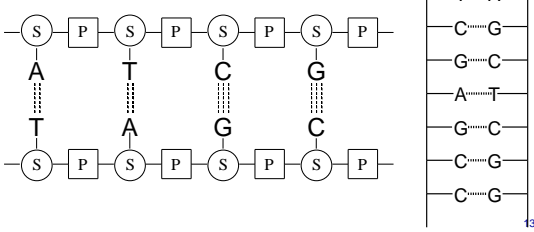
- The bases hydrogen bond to each other in a specific way: A hydrogen bonds to T, and G hydrogen bonds to C, forming a set of **complementary base pairs**:



12

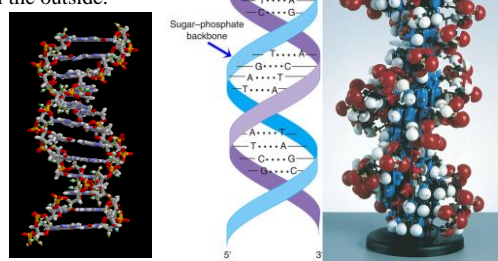
The Secondary Structure of DNA

- This allows two separate strands of sugar-phosphate backbones to run alongside each other, held together by the hydrogen bonds between the complementary base pairs:



The Double Helix

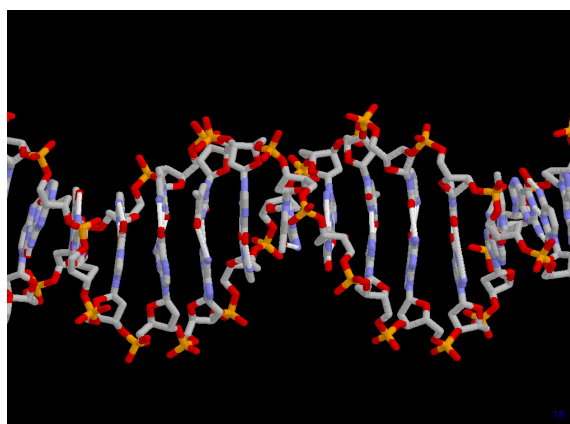
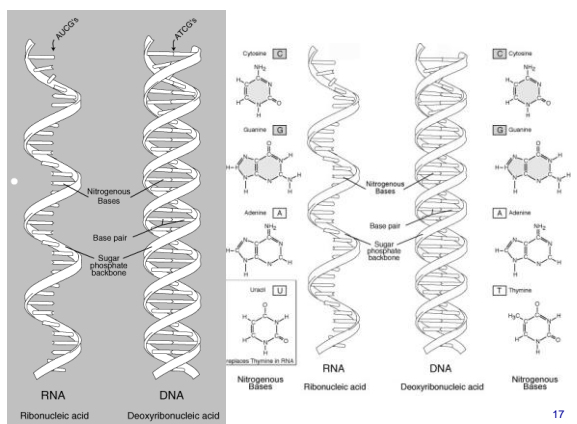
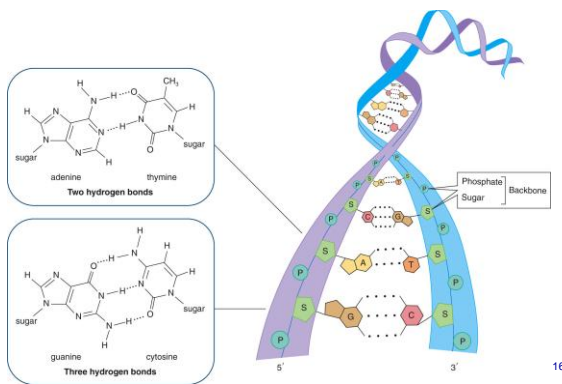
- The “ladder-like” structure folds in on itself to form a **double helix**, with the bases on the inside and the sugar-phosphate backbone on the outside:



The Double Helix

- The two intertwined polynucleotide chains run in opposite (antiparallel) directions, with the 5' end of one chain on the same side as the 3' end of the other.
 - The base sequence of a DNA strand is always written from the 5' end to the 3' end.
- The sugar-phosphate backbone runs along the outside of the helix, with the bases pointing inwards, where they form hydrogen bonds to each other.
- The two strands of DNA are **complementary** to each other, because of the specific pairing of G to C and A to T.

The Double Helix



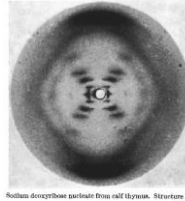
The Discovery of the DNA Structure

- DNA was discovered in 1869 by the Swiss physician Friedrich Miescher in the pus of discarded surgical bandages; he named it “nuclein” because it was located in the nucleus of the cell.
- In 1878, Albrecht Kossel isolated the pure nucleic acid, and later isolated the five nitrogenous bases.
- Many scientists believed that nucleic acids were far too simple to be the agent that carried genetic information from one generation to the next, and that the genetic material would turn out to be a protein.
- In 1943, Oswald Avery, Colin MacLeod, and Maclyn McCarty identified DNA as the carrier of genetic information.
- The race was on to determine the structure of DNA, and how it was able to transmit genetic information.

19

The Discovery of the DNA Structure

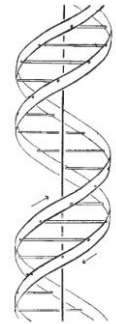
- In 1952, Rosalind Franklin obtained an X-ray crystal structure (“Photo 51”) of a sample of DNA which contained structural features which lead James D. Watson and Francis H. C. Crick to deduce the double helix structure of DNA (Nobel Prize in Medicine, 1962).



Model of deoxyribose nucleic acid (DNA). Structure #

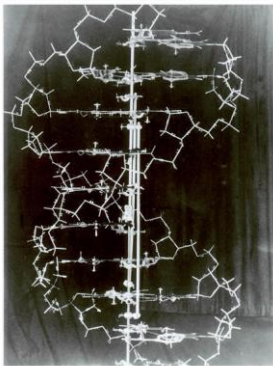
Molecular Configuration in Sodium Thymonucleate
R. Franklin, and R. G. Gosling,
Nature 171, 740-741 (1953)

A Structure for Deoxyribose Nucleic Acid
J. D. Watson and F. H. C. Crick,
Nature 171, 737-738 (1953)



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate-sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis.

The Discovery of the DNA Structure



Courtesy of Cold Spring Harbor Archives. Noncommercial, educational use only.

We wish to suggest a structure for the salt of deoxyribose nucleic acid (DNA). This structure has novel features which are of considerable biological interest....

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

“Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid,” *Nature* (April 25, 1953)

21

Examples: The Structure of DNA

- One strand of a DNA molecule has the base sequence CCATTG. What is the base sequence for the complementary strand?

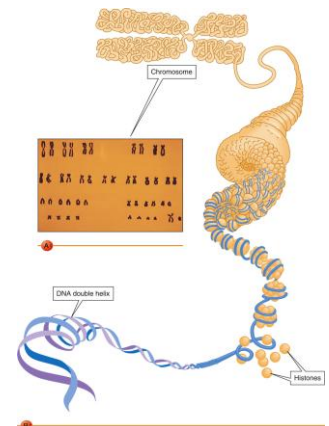
22

DNA Replication

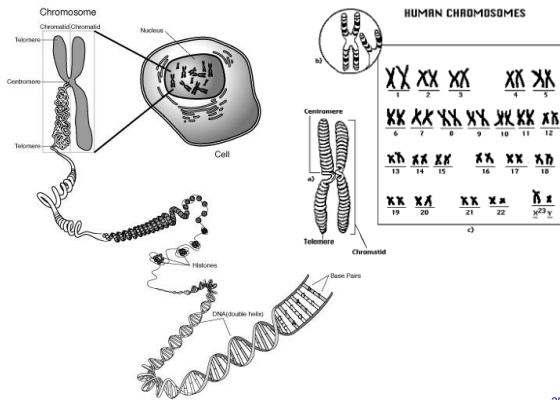
23

Chromosomes

- A normal human cell contains 46 **chromosomes**, each of which contains a molecule of DNA coiled tightly around a group of small basic proteins called **histones**.



24



25

Genes

- Individual sections of DNA molecules make up the **genes**, which are the fundamental units of heredity that direct the synthesis of proteins.
 - Viruses contain a few to several hundred genes.
 - Escherichia coli* (*E. coli*) contains ~1000 genes.
 - Humans cells contain ~25,000 genes.

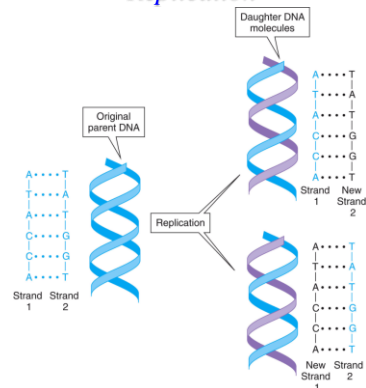
26

Replication

- Replication** is the process by which an exact copy of DNA is produced.
 - Two strands of DNA separate, and each one serves as the template for the construction of its own complement, generating new DNA strands that are exact replicas of the original molecule.
 - The two daughter DNA molecules have exactly the same base sequences of the parent DNA.
 - Each daughter contains one strand of the parent and one new strand that is complementary to the parent strand. This type of replication is called **semiconservative replication**.

27

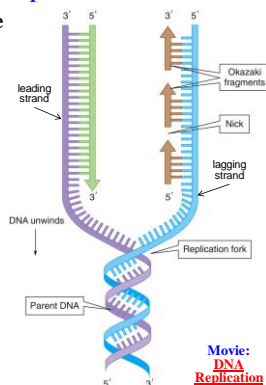
Replication



28

Steps in DNA Replication

- Step 1: Unwinding of the double helix.**
 - The enzyme *helicase* catalyzes the separation and unwinding of the nucleic acid strands at a specific point called a **replication fork**.
 - The hydrogen bonds between the base pairs are broken, and the bases are exposed.
 - An RNA primer attaches to the DNA at the point where replication begins.



Movie:
DNA
Replication

Steps in DNA Replication

- Step 2: Synthesis of DNA segments.**
 - DNA replication takes place from the 3' end towards the 5' end of the exposed strands (the template).
 - Because the strands are antiparallel, the synthesis of new nucleic acid strands proceeds:
 - toward* the replication fork on one strand (the **leading strand**)
 - away* from the replication fork on the other strand (the **lagging strand**).
 - Nucleotides complementary to the ones on the exposed strands are attached to the growing chain, and are linked together by the enzyme DNA polymerase to form a new *daughter* strand.

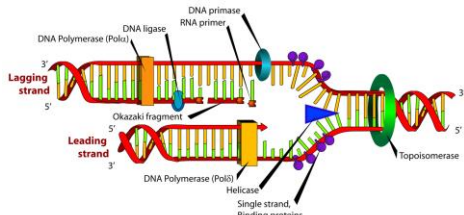
30

Steps in DNA Replication

- **Step 2: Synthesis of DNA segments.**
 - As the replication fork moves down the DNA backbone, the leading strand grows smoothly towards the 5' end.
 - Since the lagging strand was growing away from the first fork, new segments grow from the new location of the replication fork, until they meet the areas where the RNA primers are located.
 - This daughter strand is thus synthesized as a series of fragments that are bound together in Step 3. The gaps or breaks between segments in this daughter strand are called **nicks**, and the DNA fragments separated by the nicks are called **Okazaki fragments** (after Reiji Okazaki).

31

Steps in DNA Replication — Summary



- **Step 1:** The DNA is unwound by **helicase** and a **replication fork** forms.
- **Step 2:** With the help of the enzyme **DNA polymerase**, DNA is replicated smoothly along the **leading strand** which grows *towards* the replication fork. DNA segments (**Okazaki fragments**) are synthesized by DNA polymerase along the **lagging strand** as the replication fork moves.
- **Step 3:** The Okazaki fragments are joined by DNA ligase, resulting in two new DNA molecules.

33

Polymerase Chain Reaction (PCR)

- An important laboratory technique called the **polymerase chain reaction (PCR)** mimics the natural process of replication. [Kary Mullins and Michael Smith, Nobel Prize, 1993]
 - A small quantity of target DNA, a buffered solution of DNA polymerase, the cofactor $MgCl_2$, the four nucleotide building blocks, and primers are added to a test tube.
 - The primers are short polynucleotides that bind to the DNA strands and serve as starting points for new chain growth.
 - The mixture goes through several three-step replication cycles:
 - Heat ($94-96^\circ C$) is used for one to several minutes to unravel DNA into single strands.

35

Steps in DNA Replication

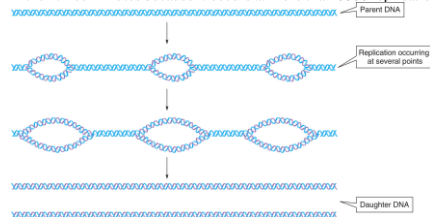
- **Step 3: Closing the nicks.**
 - The daughter strand along the leading strand is synthesized smoothly, without any nicks.
 - The Okazaki fragments along the lagging strand are joined by an enzyme called DNA ligase, which removes the RNA primer and replaces it with the correct nucleotides.
 - The result is two DNA double-helix molecules of DNA that are identical to the original DNA molecule, each of which contains one old strand from the parent DNA and one new daughter strand (*semiconservative* replication).

32

DNA Replication in More Than One Place

- In eukaryotes, DNA replication occurs simultaneously at many replication forks along the original DNA molecule. The zones where replication occur eventually combine to form complete strands. This allows long molecules to be replicated quickly.

- The largest chromosome of the fruit fly (*Drosophila*) would take more than 16 days to replicate in one segment. The actual process takes less than three minutes because it occurs at more than **6000** replication forks.



34

Polymerase Chain Reaction (PCR)

- The tube is cooled to $50-65^\circ C$ for one to several minutes to allow primers to hydrogen-bond to the separated strands of target DNA.
- The tube is heated to $72^\circ C$ for one to several minutes while DNA polymerase synthesizes new strands.
- Each cycle doubles the amount of DNA; following 30 cycles, a theoretical amplification factor of 1 billion is attained.

36

Polymerase Chain Reaction (PCR)

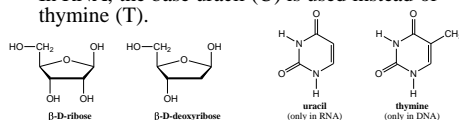
- PCR is a standard research technique that:
 - detects all manner of mutations associated with genetic disease.
 - is used to detect presence of unwanted DNA (bacterial or viral infection).
 - is a fast and simple to lengthy procedures involving sample cultures that can take weeks.
 - can be used on degraded DNA samples:
 - forensic analysis, DNA fingerprinting.
 - Recovery of DNA from extinct mammals, Egyptian mummies, and ancient insects trapped in amber to be amplified and analyzed.

37

Ribonucleic Acid (RNA)

RNA

- RNA is a long unbranched polymer consisting of nucleotides joined by 3' to 5' phosphodiester bonds.
- RNA strands consist of from 73 to many thousands of nucleotides.
- Whereas DNA is only found in the nucleus, RNA is found throughout cells: in the nucleus, in the cytoplasm, and in the mitochondria.
- Differences in RNA and DNA primary structures:
 - In RNA the sugar is ribose instead of deoxyribose.
 - In RNA, the base uracil (U) is used instead of thymine (T).

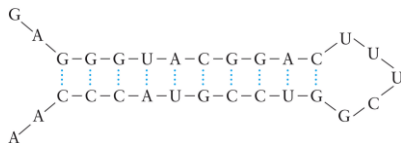


39

40

Secondary Structure of RNA

- Most RNA molecules are single-stranded, although many contain regions of double-helical structure where they form loops. (A::U, G::C)



41

Kinds of RNA — Messenger RNA (mRNA)

- There are three kinds of RNA: **messenger RNA (mRNA)**, **ribosomal RNA (rRNA)**, and **transfer RNA (tRNA)**.
- **Messenger RNA (mRNA)** — functions as a carrier of genetic information from the DNA in the cell nucleus to the site of protein synthesis in the cytoplasm.
 - The bases of mRNA are in a complementary sequence to the base sequence of one of the strands of nuclear DNA.
 - mRNA has a short lifetime (usually less than one hour); it is synthesized as it is needed, then rapidly degraded to the constituent nucleotides.

42

Kinds of RNA — Ribosomal RNA (rRNA)

- **Ribosomal RNA (rRNA)** — the main component of ribosomes that are the site of protein synthesis.
 - rRNA accounts for 80-85% of the total RNA of the cell.
 - rRNA accounts for 65% of a ribosome’s structure (the remaining 35% is protein).

Kinds of RNA — Transfer RNA (tRNA)

- **Transfer RNA (tRNA)** — delivers individual amino acids to the site of protein synthesis.
 - tRNA is specific to one type of amino acid; cells contain at least one specific type of tRNA for each of the 20 common amino acids.
 - tRNA is the smallest of the nucleic acids, with 73-93 nucleotides per chain.

Table 21.1 Different Forms of RNA Molecules in Escherichia coli (E. coli)

Class of RNA	% in Cells	Number of RNA Subtypes	Number of Nucleotides
Ribosomal RNA (rRNA)	80	3	120
			1700
			3700
Transfer RNA (tRNA)	15	46	73-93
Messenger RNA (mRNA)	5	Many	75-3000

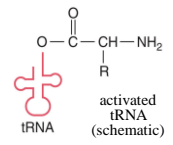
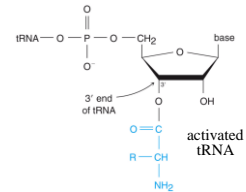
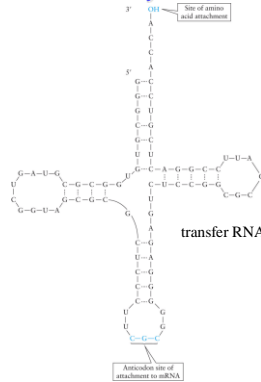
43

44

Kinds of RNA — Transfer RNA (tRNA)

- **Transfer RNA (tRNA)** — cont.
 - tRNA has regions of hydrogen bonding between complementary base pairs, separated by loops where there is no hydrogen bonding.
 - Two regions of tRNA have important functions:
 - the **anticodon** is a three-base sequence which allows tRNA to bind to mRNA during protein synthesis. (It is complementary to one of the codons in mRNA.)
 - the 3' end of the molecule binds to an amino acid with an ester bond and transports it to the site of protein synthesis. An enzyme matches the tRNA molecule to the correct amino acid, “activating” it for protein synthesis.

Kinds of RNA — Transfer RNA (tRNA)



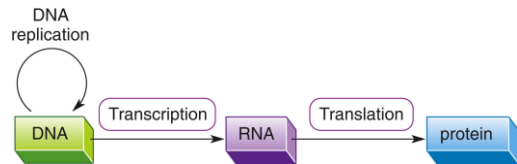
45

46

The Flow of Genetic Information

The Central Dogma of Molecular Biology

- The **central dogma of molecular biology** states that genetic information contained in the DNA is transferred to RNA molecules and then expressed in the structure of synthesized proteins.
- **Genes** are segments of DNA that contain the information needed for the synthesis of proteins.
- Each protein in the body corresponds to a DNA gene.

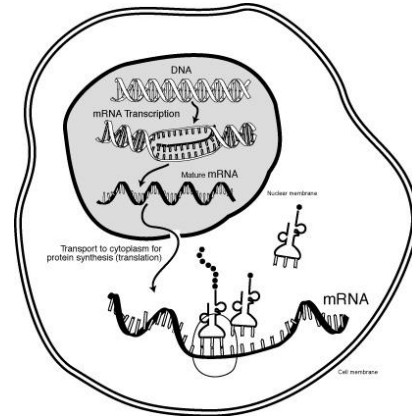


47

48

Transcription, Translation, and Information Flow

- There are two steps in the flow of genetic information:
 - **transcription** — in eukaryotes, the DNA containing the stored information is in the nucleus of the cell, and protein synthesis occurs in the cytoplasm. The information stored in the DNA must be carried out of the nucleus by mRNA.
 - **translation** — mRNA serves as a template on which amino acids are assembled in the sequence necessary to produce the correct protein. The code carried by mRNA is translated into an amino acid sequence by tRNA.
- The communicative relationship between mRNA nucleotides and amino acids in a protein is called the **genetic code**.



49

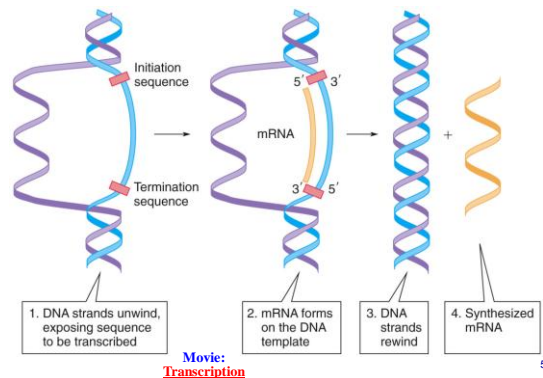
50

Transcription: RNA Synthesis

- Under the influence of the enzyme **RNA polymerase**, the DNA double helix unwinds at a point near the gene that is being transcribed (the **initiation sequence**). Only one strand of the DNA is transcribed.
- Ribonucleotides are linked along the DNA strand in a sequence determined by the base pairing of the DNA and ribonucleotide bases (A::U, G::C).
- mRNA synthesis occurs in the 3' to 5' direction along the DNA strand (in the 5' to 3' direction along the RNA strand) until the **termination sequence** is reached.
- The newly-synthesized mRNA strand moves away from the DNA, which rewinds into the double helix.
- Synthesis of tRNA and rRNA is similar to this.

51

Transcription: RNA Synthesis



Movie: **Transcription**

52

Examples: The Synthesis of RNA

- Write the sequence for the mRNA that could be synthesized using the following DNA base sequence as a template:

5' G-C-A-A-C-T-T-G 3'

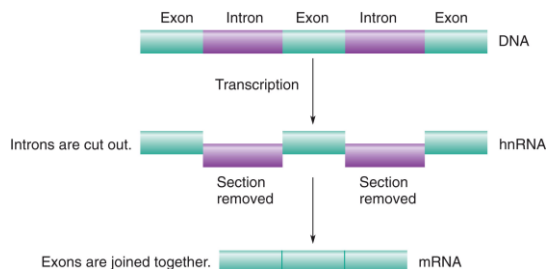
53

Introns and Exons

- In prokaryotes, each gene is a continuous segment along a DNA molecule. Transcription of the gene produces mRNA that is translated into a protein almost immediately, because there is no nuclear membrane separating the DNA from the cytoplasm.
- In eukaryotes, the gene segments of DNA that code for proteins (**exons**) are interrupted by segments that do not carry an amino acid code (**introns**).
 - Both exon and intron segments are transcribed, producing **heterogenous nuclear RNA (hnRNA)**.
 - A series of enzymes cut out the intron segments and splice the exon segments together to produce mRNA.

54

Introns and Exons



55

The Genetic Code

- Once the 3D structure of DNA was known, it was clear that the sequence of the bases along the backbone in some way directed the order in which amino acids were stacked to make proteins.
- In 1961, Marshall Nirenberg and his coworkers began to unravel the connection between the base sequence in DNA and the amino acid sequence in proteins.
- The **genetic code** uses a sequence of three bases (a triplet code) to specify each amino acid. (A triplet code gives $4^3=64$ possible combinations, which is more than enough to specify the 20 amino acids.)
- Each base triplet sequence that represents a code word on mRNA molecules is called a **codon**.

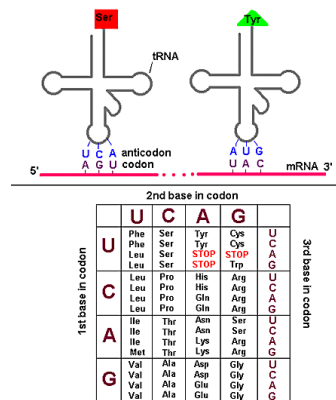
56

The Genetic Code

Table 21.2 The Genetic Code: mRNA Codons for Each of the 20 Amino Acids

Amino Acid	Codons	Number of Codons
alanine	GCA, GCC, GCG, GCU	4
arginine	AGA, AGG, CGA, CGC, CGG, CGU	6
asparagine	AAC, AAU	2
aspartic acid	GAC, GAU	2
cysteine	UGC, UGU	2
glutamic acid	GAA, GAG	2
glutamine	CAA, CAG	2
glycine	GGA, GGC, GGG, GGU	4
histidine	CAC, CAU	2
isoleucine	AUA, AUC, AUU	3
leucine	CUA, CUC, CUG, CUU, UUA, UUG	6
lysine	AAA, AAG	2
methionine, initiation	AUG	1
phenylalanine	UUC, UUU	2
proline	CCA, CCC, CCG, CCU	4
serine	UCA, UCC, UCG, UCU, AGC, AGU	6
threonine	ACA, ACC, ACG, ACU	4
tryptophan	UGG	1
tyrosine	UAC, UAU	2
valine	GUA, GUC, GUG, GUU	4
Stop signals	UAG, UAA, UGA	3
Total number of codons		64

57



The Genetic Code

58

Characteristics of the Genetic Code

- The genetic code applies almost universally: with minor exceptions, the same amino acid is represented by the same codon(s) in all species.
- Most amino acids are represented by more than one codon (a feature known as *degeneracy*).
 - Only methionine and tryptophan are represented by a single codon.
 - Leucine, serine, and arginine are represented by six codons.
 - No codon codes for more than one amino acid.
- Only 61 of the 64 possible triplets represent amino acids. The other three are used as signals for chain termination (a “stop” signal).
- The AUG codon (which also codes for methionine) functions as a “start” signal, but only when it occurs as the first codon in a sequence.

59

The Genetic Code

Table 21.3 General Characteristics of the Genetic Code

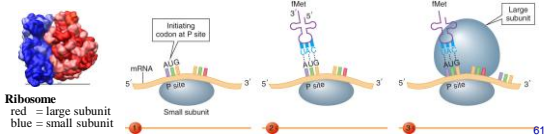
Characteristic	Example
Codons are three-letter words	GCA = alanine
The code is degenerate	GCA, GCC, GCG, GCU all represent alanine
The code is precise	GCC represents only alanine
Chain initiation is coded	AUG
Chain termination is coded	UAA, UAG, and UGA
The code is almost universal	GCA = alanine, perhaps, in all organisms

60

Translation and Protein Synthesis

Step 1: Initiation of the polypeptide chain.

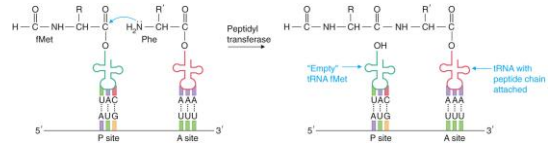
- mRNA and a small ribosomal subunit join; the initiating codon (AUG) is aligned with P (peptidyl) site of the subunit.
- tRNA brings in methionine (eukaryotes) or N-formylmethionine (prokaryotes).
- The resulting complex binds to the large ribosomal subunit to form a unit called the *initiation complex*.



Translation and Protein Synthesis

Step 2: Elongation of the chain.

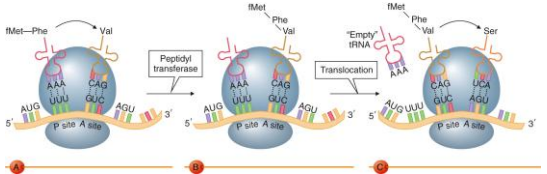
- The next incoming tRNA with an anticodon that is complementary to the mRNA codon bonds at the A (aminoacyl) site on the mRNA.
- A peptide bond is formed between the amino acid segments, (catalyzed by peptidyl transferase), which releases the amino acid chain from the P site.



Translation and Protein Synthesis

Step 2: Elongation of the chain, cont.

- The “empty” tRNA released, and the whole ribosome moves one codon along the mRNA towards the 3’ end (*translocation*).
- Another tRNA attaches to the A site, and the elongation process is repeated.

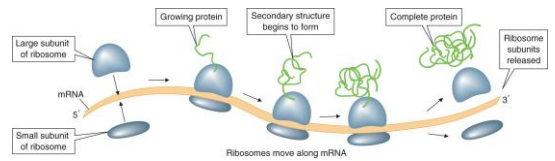


Movie: Translation

Translation and Protein Synthesis

Step 3: Termination of polypeptide synthesis.

- Elongation continues until the ribosome complex reaches a stop codon (UAA, UAG, or UGA).
- A *termination factor* protein binds to the stop codon, and separates the protein from the final tRNA.
- The ribosome can then synthesize another protein molecule.



Translation and Protein Synthesis

- Several ribosomes can move along a single strand of mRNA, producing several identical proteins simultaneously. These complexes are called *polyribosomes* or *polysomes*.
- The growing polypeptide chain emerging from the end of the ribosome spontaneously folds into the characteristic 3D shape of that protein.

Mutations, Recombinant DNA, and Genetic Engineering

Mutations

- **Mutations** are any changes resulting in an incorrect base sequence on DNA.
- Even though the base-pairing mechanism provides a nearly perfect way of copying DNA, on average one out of every 10^{10} bases are copied incorrectly.
 - This leads to a change in the amino acid sequence in a protein, or causes the protein not to be made at all.
- Mutations occur naturally during replication. They can also be induced by environmental factors:
 - *ionizing radiation* (X-rays, UV, gamma rays).
 - *mutagens*, which are chemical agents.

67

68

Mutations

- Mutations may be beneficial to an organism by making it more capable of surviving in its environment, ultimately (over millions of years of accumulating changes) leading to the evolution of new species.
- Since much of an organism's DNA does not code for anything, mutations in these regions are neutral.
- Other mutations can be harmful, either producing genetic diseases or other debilitating conditions.

69

Recombinant DNA

- **Recombinant DNA** is produced when segments of DNA from one organism are introduced into the genetic material of another organism.
- “Genetic engineering” of *E. coli* to include the gene for the production of human insulin enables large quantities of insulin to be made available for the treatment of diabetes.

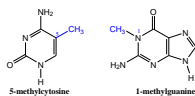
Table 21.4 Some Substances Produced by Genetic Engineering

Substance	Use
Human insulin	Treatment of diabetes mellitus
Human growth hormone	Treatment of dwarfism
Interferon	Fights viral infections
Hepatitis B vaccine	Protection against hepatitis
Malaria vaccine	Protection against malaria
Tissue plasminogen activator	Promotes the dissolving of blood clots

70

Restriction Enzymes

- **Restriction enzymes**, found in a wide variety of bacterial cells, catalyze the cleaving of DNA molecules. These enzymes are normally part of a mechanism that protects certain bacteria from invasion by foreign DNA such as that in viruses. In these bacteria, some of the bases in their DNA have methyl groups attached:

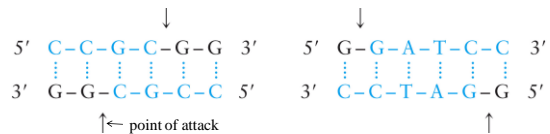


- The methylated DNA of these bacteria is left untouched by the restriction enzymes, for foreign DNA that lacks these bases undergoes rapid cleavage, and is rendered nonfunctional.

71

Restriction Enzymes

- Restriction enzymes act at sites on DNA called *palindromes*, where two strands have the same sequence but run in opposite directions:

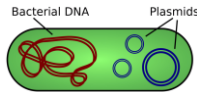


- Restriction enzymes are used to break DNA up into fragments of known size and nucleotide sequence, which can then be spliced together with DNA ligases.

72

Plasmids

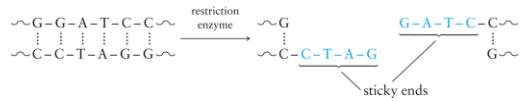
- The introduction of a new DNA segment (gene) into a bacterial cell requires a DNA carrier called a **vector**, which is often a circular piece of double-stranded DNA called a **plasmid**.
 - Plasmids range from 2000 to several hundred thousand nucleotides, and are found in the cytoplasm of bacterial cells.
 - Plasmids function as accessories to chromosomes by carrying genes for the inactivation of antibiotics and the production of toxins. They are also able to replicate independently of chromosomal DNA.



73

The Formation of Recombinant DNA

- A plasmid is isolated from a bacterium, and a restriction enzyme is added, which cleaves it at a specific site:



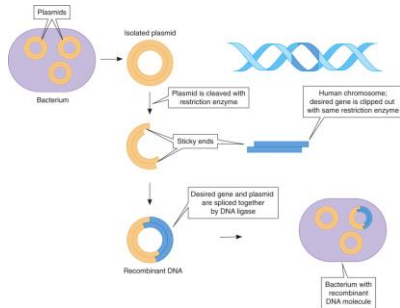
- When the circular DNA is cut, two “sticky ends” are produced, which have unpaired bases.
- The “sticky ends” are provided with complementary sections for pairing from a human chromosome to which the same restriction enzyme has been used:



74

The Formation of Recombinant DNA

- The breaks in the strands are joined using DNA ligase, and the plasmid becomes a circular piece of double-stranded, recombinant DNA.



75

The Formation of Recombinant DNA

- When the bacteria reproduce, they replicate all of the genes, including the new recombinant DNA plasmids.
- Because bacteria multiply quickly, there are soon a large number of bacteria containing the modified plasmid, which are capable of manufacturing the desired protein.

76

The End

77