PHARMACEUTICAL MICROBIOLOGY and IMMUNOLOGY

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OBJECTIVES

- Nucleic acids
- DNA replication
- Protein synthesis

Nucleic Acids

- Living organisms are complex systems. Hundreds of thousands of proteins exist inside each one of us to help carry out our daily functions
- These proteins are produced locally, assembled piece-by-piece to exact specifications
- An enormous amount of information is required to manage this complex system correctly
- This information, detailing the specific structure of the proteins inside of our bodies, is stored in a set of molecules called **nucleic acids**

Nucleic Acids

- **Nucleic acids** are biopolymers, or large biomolecules, essential to all known forms of life

- They are composed of monomers, which are **nucleotides** made of three components:

  - **a 5-carbon sugar**

  - **a phosphate group**

  - **a nitrogenous base**

Nucleic Acids

- A **nucleoside** consists of a **nitrogenous base** covalently attached to a **sugar** (ribose or deoxyribose) but **without the phosphate group**

- A **nucleotide** consists of a **nitrogenous base**, a **sugar** (ribose or deoxyribose) and a **phosphate group**

Nucleic Acids

- The two main classes of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)

- If the sugar is a simple **ribose**, the polymer is **RNA**; if the sugar is derived from ribose as **deoxyribose**, the polymer is **DNA**

Nucleic Acids

- Nucleotides are linked together to form polynucleotide chains
- Nucleotides are joined to one another by covalent bonds between the phosphate of one and the sugar of another
- These linkages are called **phosphodiester linkages**
- Phosphodiester linkages form the sugar-phosphate backbone of both DNA and RNA

Nucleic Acids

- Nucleotides are linked by a **phosphodiester bond**: a covalent bond is formed between the **5’ phosphate group of one nucleotide and the 3’-OH group of another**

- Nucleobases found in the two nucleic acid types are different: adenine, cytosine, and guanine are found in both RNA and DNA, while thymine occurs in DNA and uracil occurs in RNA

Nucleic Acids

	DNA	RNA
Purine Bases:	A-G	A-G
Pyrimidine Bases:	T-C	U-C
Sugar:	deoxyribose	ribose
Strands:	double-stranded	single-stranded

Nucleic Acids

DNA (Deoxyribonucleic acid)

- DNA is a nucleic acid containing the genetic instructions used in the development and functioning of all known living organisms

- The DNA segments carrying this genetic information are called **genes**. (DNA sequence forms genes, which in the language of the cell, tell cells how to make proteins)

- Likewise, other DNA sequences have structural purposes, or are involved in regulating the use of this genetic information

Nucleic Acids

DNA (Deoxyribonucleic acid)

- DNA consists of two long polymers of simple units called nucleotides, with backbones made of sugars and phosphate groups joined by ester bonds

- These two strands run in opposite directions to each other and are, therefore, anti-parallel

- DNA is normally found as a double stranded molecule

Nucleic Acids

DNA (Deoxyribonucleic acid)

- Not only is DNA double stranded, but the two seperate strands are wound around each other in a **helical arrangement (double-helix)**

- The fact that the two DNA strands that form the double helix are anti-parallel helps to twist the molecule as well

- Nucleotides are joined by linking the phosphate on the 5’ end of the deoxyribose of one to the 3’ position of the next (5’→3’ direction)

Nucleic Acids

DNA (Deoxyribonucleic acid)

- Two strands are held together by **hydrogen bonds** between the base pairs

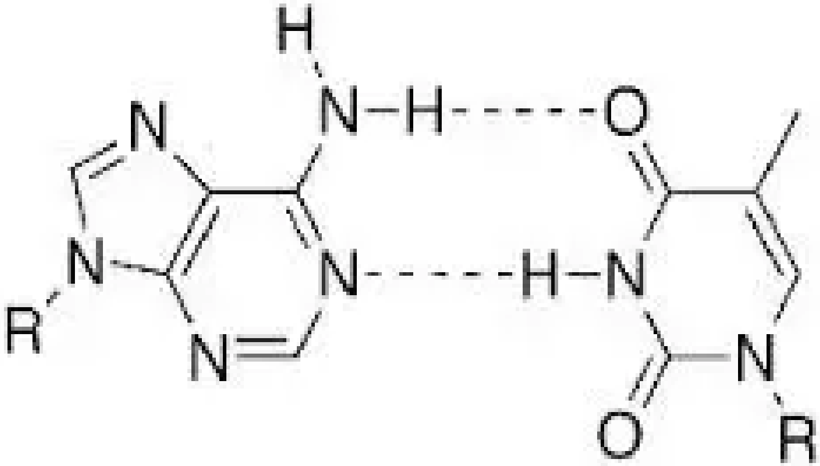
- **Thymine (T)** always pairs with **adenine (A)** with **two hydrogen bonds**

- **Cytosine (C)** always pairs with **guanine (G)** with **three hydrogen bonds**

- Two strands are coiled around each other, each running in the opposite direction

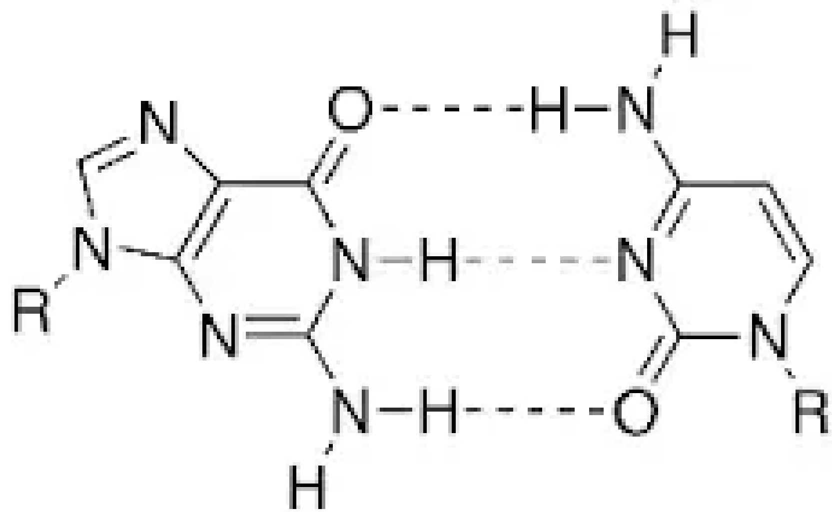
- The bases are on the inside of the resulting helix

Nucleic Acids



Adenine Thymine

There are two hydrogen bonds between A=T



Guanine Cytosine

There are three hydrogen bonds between G≡C

Nucleic Acids

DNA (Deoxyribonucleic acid)

- In addition, the ratios of **Adenine/Thymine** and **Guanine/Cytosine** are always **one**, that is, the number of molecules is the same for Adenine and Thymine (A=T) and for Guanine and Cytosine (G=C)

A/T=1 G/C=1

Nucleic Acids

DNA (Deoxyribonucleic acid)

- The molar content of **purine** bases is always **equal** to that of **pyrimidine** bases, or in other words, the sum of **adenine plus guanine** molecules always **equals** that of **cytosine plus thymine**

  (A+G=T+C)

- The proportion of bases in the DNA is characteristic for each species

  (A+T)/(C+G)

Nucleic Acids

DNA (Deoxyribonucleic acid)

- The structure of DNA does not only exist as secondary structures such as double helices, but it can fold up on itself to form tertiary structures by **supercoiling**

- Supercoiling allows for the compact packing of circular DNA

- DNA has two important functions:
  - It is self-replicating, it can make an identical copy of itself
  - the production of proteins

Nucleic Acids

RNA (Ribonucleic acid)

The chemical structure of RNA is very similar to that of DNA, but differs in three main ways:

- Unlike double-stranded DNA, **RNA is a single-stranded molecule**
- While DNA contains deoxyribose, **RNA contains ribose**
- The **complementary base to adenine** in DNA is thymine, whereas **in RNA, it is uracil**

Nucleic Acids

RNA (Ribonucleic acid)

There are three main types of RNA:

- **messenger RNA (mRNA):**is the RNA that carries information from DNA to the ribosome, the sites of protein synthesis in the cell

- **transfer RNA (tRNA):** is the RNA that carries the specific amino acid to a growing polypeptide chain at the ribosomal site of protein synthesis during translation

- **ribosomal RNA (rRNA):**is a type of stable RNA that is a major constituent of ribosomes

Nucleic Acids

Messenger RNA (mRNA)

- Messenger RNA (mRNA) is a linear molecule transcribed from one strand of DNA
- It carries the base sequence complementary to DNA template strand
- The base sequence of mRNA is in the form of consecutive triplet codons
- **A codon is a sequence of three nucleotides, coding for one aminoacid.** The synthesized amino acids combine to form the protein

Nucleic Acids

Messenger RNA (mRNA)

- Ribosomes translate these triplet codons into amino acid sequence of polypeptide chain
- **Transcription** is the first step of gene expression, in which a particular segment of **DNA is copied into mRNA** by the enzyme **RNA polymerase (mRNA is created in the 5' → 3' direction)**
- Length of mRNA depends upon the length of polypeptide chain it codes for. Polypeptide length varies from a chain of a few amino acids to thousands of amino acids

Nucleic Acids

Messenger RNA (mRNA)

- A triplet encodes the same aminoacid, both in bacteria and human

- Some amino acids are encoded by only one codon whereas some of them are encoded by more than one codon

AAU-AAC→Asparagin   AUG→Methionine

- There are **twenty major amino acids** which make up proteins

Nucleic Acids

Messenger RNA (mRNA)

- The genetic code includes **64 possible combinations**, of three-letter nucleotide sequences that can be made from the **four nucleotides**
- Of the 64 codons, 61 represent amino acids, and three are stop signals
- A stop codon (Termination codon) is one of three triplets (**UAG, UAA, UGA**) that causes protein synthesis to terminate

Nucleic Acids

Messenger RNA (mRNA)

- An **operon** is a functioning unit of genomic DNA containing a cluster of genes under the control of a single promoter. The genes are transcribed together into a mRNA strand and either translated together in the cytoplasm

- The genes contained in the operon are either expressed together or not at all. Several genes must be co-transcribed to define an operon

- A mRNA is constituted for each operon

Nucleic Acids

Ribosomal RNA (rRNA)

- Most of the RNA of the cell is in the form of ribosomal RNA which constitutes about 85% of the total RNA

- Ribosomes consist of many types of **rRNA.** The **70S ribosome** of prokaryotes, in its smaller subunit of **30S has 16S rRNA**. The **50S** larger subunit consists of **23S** and **5S rRNA**

- **Polyribosome** is a cluster of ribosomes linked together by a molecule of messenger RNA and forming the site of protein synthesis

Nucleic Acids

Transfer RNA (tRNA)

- Each nucleotide triplet codon on mRNA represents an amino acid. The tRNA plays the role of an adaptor and matches each codon to its particular amino acid in the cytoplasmic pool
- Transfer RNA carries the correct amino acid to the site of protein synthesis in the ribosome
- It is the base pairing between the tRNA and mRNA that allows for the correct amino acid to be inserted in the polypeptide chain being synthesized

Nucleic Acids

Transfer RNA (tRNA)

- Any mutations in the tRNA or rRNA can result in global problems for the cell because both are necessary for proper protein synthesis

- The tRNA has two properties:

- It represents a single amino acid to which it binds covalently
- It has two sites. One is a trinucleotide sequence called **anticodon**, which is complementary to the codon of mRNA. The codon and anticodon form base pairs with each other. The other is **amino acid binding site**

Nucleic Acids

Transfer RNA (tRNA)

- There are many different kinds of tRNA molecules in a cell
- Each tRNA is named after the amino acid it carries. For example if tRNA carries amino acid tyrosine it is written as $tRNA^{Tyr}$
- Sometimes there are more than one tRNA for an amino acid, then it is denoted as $tRNA_1^{Try}$ and $tRNA_2^{Try}$
- The tRNA charged with an amino acid is called amino acyl tRNA

Nucleic Acids

Transfer RNA (tRNA)

- The primary structure of all tRNA molecules is small, linear, single stranded nucleic acid ranging in size from 73 to 93 nucleotides

- The tRNA due to its property of having stretches of complementary base pairs forms secondary structure, which is in the form of a **cloverleaf**

Nucleic Acids

Transfer RNA (tRNA)

- Several regions of the single stranded molecule form double stranded stems or arms and single stranded loops due to folding of various regions of the molecule. These double stranded stems have complementary base pairs

- The various regions of the clover leaf model of tRNA are as follows:

- amino acid arm - D-arm
- anticodon arm -an extra arm - T- arm

Movie time

DNA Replication

- DNA replication employs a large number of proteins and enzymes, each of which plays a critical role during the process

- Enzymes required for DNA replication are located in mesosome

- **DNA gyrase** is the enzyme that unwinds **supercoiled DNA**

- **DNA helicase** is the enzyme that unwinds **double helical DNA.** It separates the DNA to form a replication fork at the **origin of replication** where DNA replication begins

DNA Replication

- DNA polymerase adds nucleotides one by one to the growing DNA chain that are complementary to the template strand

- Three main types of polymerases are known: DNA polymerase III is the enzyme required for DNA synthesis; DNA polymerase I and DNA polymerase II are primarily required for repair

- Bacterial DNA is replicated by **DNA polymerase III** in the **5′ to 3′ direction** at a rate of 1000 nucleotides per second

DNA Replication

- There are specific nucleotide sequences called **origins of replication** where replication begins
- The origin of replication is recognized by certain proteins that bind to this site
- **DNA helicase** unwinds the DNA **by breaking the hydrogen bonds** between the nitrogenous base pairs
- ATP hydrolysis is required for this process. As the DNA opens up, Y-shaped structures called **replication forks** are formed

DNA Replication

- Two replication forks at the origin of replication are extended bi-directionally as replication proceeds
- **Single-strand binding proteins** coat the strands of DNA near the replication fork to prevent the single-stranded DNA from winding back into a double helix
- **DNA polymerase** is able to add nucleotides only in the **5′ to 3′ direction** (a new DNA strand can be extended only in this direction)

DNA Replication

- It also requires a free 3′-OH group to which it can add nucleotides by forming a phosphodiester bond between the 3′-OH end and the 5′ phosphate of the next nucleotide. This means that it cannot add nucleotides if a free 3′-OH group is not available

- Another enzyme, **RNA primase**, synthesizes an RNA primer that is about five to ten nucleotides long and complementary to the DNA, priming DNA synthesis

DNA Replication

- **A primer provides the free 3′-OH end to start replication.** DNA polymerase then extends this RNA primer, adding nucleotides one by one that are complementary to the template strand

- **DNA polymerase can only extend in the 5′ to 3′ direction**, which poses a slight problem at the replication fork

- As we know, the DNA double helix is anti-parallel; that is, **one strand is in the 5′ to 3′ direction** and **the other is oriented in the 3′ to 5′ direction**

DNA Replication

- One strand (the leading strand), complementary to the 3′ to 5′ parental DNA strand, is synthesized continuously towards the replication fork because the polymerase can add nucleotides in this direction

- The other strand (the lagging strand), complementary to the 5′ to 3′ parental DNA, is extended away from the replication fork in small fragments known as **Okazaki fragments**, each requiring a primer to start the synthesis

DNA Replication

- The leading strand can be extended by one primer alone, whereas the lagging strand needs a new primer for each of the short **Okazaki fragments**
- The overall direction of the lagging strand will be 3′ to 5′, while that of the leading strand will be 5′ to 3′
- The sliding clamp (a ring-shaped protein that binds to the DNA) holds the DNA polymerase in place as it continues to add nucleotides
- **DNA gyrase (Type II topoisomerase)** prevents the over-winding of the DNA double helix ahead of the replication fork as the DNA is opening up

DNA Replication

- As synthesis proceeds, the RNA primers are replaced by DNA
- The primers are removed by the exonuclease activity of **DNA polymerase I**, while the gaps are filled in by deoxyribonucleotides
- The nicks that remain between the newly-synthesized DNA (that replaced the RNA primer) and the previously-synthesized DNA are sealed by the enzyme **DNA ligase** that catalyzes the formation of phosphodiester linkage between the 3′-OH end of one nucleotide and the 5′ phosphate end of the other fragment

Movie time

DNA Replication

- **Replicon:** a DNA molecule or a region of DNA that replicates as an individual unit

- A replicon may be, for instance, a chromosome, a plasmid or a phage

Protein Synthesis

- Protein synthesis is accomplished through a process called **translation**

- After **DNA is transcribed into a mRNA** molecule during **transcription**, the mRNA must be **translated** to produce a protein

- In translation, **mRNA** along with **tRNA** and **ribosomes** work together to produce proteins

Protein Synthesis

- **tRNA** plays a huge role in **protein synthesis** and **translation**. Its job is to translate the message within the nucleotide sequence of mRNA to a specific amino acid sequence. These sequences are joined together to form a protein

- tRNA contains an **amino acid attachment site** on one end and a special section in the middle loop called the **anticodon site**. The anticodon recognizes a specific area on a mRNA called a **codon**

Protein Synthesis

- For each tRNA there is a specific enzyme that recognize both the tRNA and the corresponding aminoacid. The enzyme, known as **amino-acyl tRNA synthetases**, attach the aminoacid to the tRNA

- The bases of mRNA are read in groups of three, **starting at the 5’ end**. Protein synthesis always begin with the **start codon (AUG)**

Protein Synthesis

The three major steps during protein synthesis by the ribosome are:

- Initiation
- Elongation
- Termination

- The first codon is always **AUG**, which stands for the amino acid **methionine**

Protein Synthesis

- A special tRNA, the initiator tRNA will be charged with chemically tagged methionine (formyl-methionine or fMet) and will bind to the start codon

- So all polypeptide chains begin with methionine

- Before protein synthesis starts, the two subunits of the ribosome are floating around separately

Protein Synthesis

- The ribosome has two sites for tRNA

  - A (acceptor) site - P (peptide) site

- Protein synthesis start with the fMet initiator tRNA in the P-site

- Another tRNA, carrying the next amino acid, arrives and enters the A-site

- The fMet is cut loose from its tRNA and bonded to amino acid No. 2 instead

Protein Synthesis

- Next another charged tRNA arrives carrying the third amino acid
- As the peptide chain continues to grow, it is constantly cut off from the tRNA holding it and joined instead to the newest amino acid to be brought by its tRNA into the A site
- Eventually protein synthesis reach the end of the message. This is marked by a **stop codon**. There are three of these, **UGA, UAG, and UAA**

Protein Synthesis

- There are no tRNA molecules with anticodons for STOP codons
- However, protein **release factors** recognize these codons when they arrive at the A site
- Binding of these proteins releases the polypeptide from the ribosome
- The ribosome splits into its subunits, which can later be reassembled for another round of protein synthesis

Movie time