Nucleic acids

BIOS 10016

30 Jun 2025

Objectives

- Know all definitions associated with nucleic acids.
- Draw and name the nucleosides, nucleotides and Watson-Crick base pairs associated with DNA and RNA.
  - Nucleotides: adenine, guanine, thymine, cytosine, uracil
  - Nucleosides: adenosine, guanosine, thymidine, cytidine, uridine
  - Adenine → thymine/uracil (2 H-bonds), cytosine → guanine (3 H-bonds)
- Know the numbering schemes for the components of nucleotides.
  - Pyrimidines: start with sugar nitrogen, number around the ring in the direction of the closest nitrogen
  - Purines: start with nitrogen diagonally across sugar nitrogen, number around the ring in the direction of the closest nitrogen, jump to closest nitrogen on other ring, repeat
- Describe and explain the absorbance properties of nitrogenous bases. (absorb UV light at 260 nm)
- Describe the chemical and structural similarities and differences between DNA and RNA.
  - DNA:
    - Double-stranded
    - Deoxyribose (no OH) sugar
    - Thymine as a nitrogenous base
    - Stable, less reactive
    - Stores genetic information
  - RNA:

    * Single-stranded (mostly)
    * Ribose (OH) sugar
    * Uracil as a nitrogenous base
    * Less stable, more reactive (enzymatic activity)
    * Involved in protein synthesis and regulation
- Use Chargaff's rules to predict the composition of DNA molecules. (% nucleotide = % complementary nucleotide)
- Describe the different double helical structures that DNA and RNA can adopt. (B-DNA, A-DNA, Z-DNA, mRNA, tRNA, rRNA)
- Write out the complementary strand based upon Watson-Crick base pairing.
- Predict the stability of double stranded DNA sequences. (C to G is more stable, higher percentage of C-G bonds increases stability)
- Identify major and minor grooves in B-DNA.
- Describe how proteins interact with DNA, and why the major groove is often bound. (major groove is wider and deeper, allowing for more interactions with amino acid side chains. Positive amino side chains interact with the negatively charged phosphate backbone)
- Describe the features/forces that stabilize nucleic acid structure. (LDFs stabilize overall structure, hydrogen bonds hold strands together)
- Describe the phenomenon and role of supercoils, and the supercoil forms that exist.
  - Positive supercoiling: overwinding, left-handed supercoil
  - Negative supercoiling: underwinding, right-handed supercoil
- Describe the higher order structures that DNA adopts in cells.
- Describe the roles of messenger, transfer, ribosomal and other non-coding RNAs.
- Describe the features and exceptions to the central Dogma. (DNA $\rightarrow$ RNA $\rightarrow$ protein through transcription and subsequently translation, but also RNA $\rightarrow$ DNA in retroviruses, and RNA can have catalytic roles)
- Describe the concepts behind DNA replication:
  - what is the meaning of semi-conservative? (one original strand, one daughter strand)
  - the stages involved in the process
    * Initiation: helicase unwinds the protein, single-stranded binding proteins stabilize the unwound DNA, primase lays down RNA primer

    * Elongation: DNA polymerase synthesizes new DNA strand, topoisomerases relieve supercoiling
    * Termination: ligase seals gaps, telomerase extends telomeres, Tus protein terminates replication in prokaryotes
- main protein players in prokaryotic and eukaryotic systems and the roles that they play in the process
- mechanism by which nucleotides are added to growing strand (5' continuously, 3' added in Okasaki fragments)

Nucleic acids, RNA, DNA

The building blocks of nucleic acids

Nucleotides

Nitrogenous bases with pentose sugar (β-D-ribofuranose or β-D-2'[1]-deoxyribofuranose) with one or more phosphate groups (α, β, γ). Nitrogenous bases can be **purines** with two rings, 4 nitrogens (adenine, guanine) or **pyrimidines** with one ring (cytosine, uracil, thymine). Nitrogenous bases are heterocycles, aromatic, and flat and absorb UV light (260 nm).

Nucleosides

Nitrogenous bases with pentose sugar (ribose or deoxyribose) with no phosphate groups.

Numbering positions on nitrogenous bases

Pyrimidines: start with the nitrogen where the sugar is attached, count in a circle toward the next nitrogen.

Purines: start with the position opposite the sugar nitrogen, then jump to the next closest nitrogen.

Nitrogenous base pairings

adenine → thymine (in DNA), 2 hydrogen bonds
guanine → cytosine, 3 hydrogen bonds
adenine → uracil (in RNA), 2 hydrogen bonds

Nucleotide nomenclature

Nitrogenous base	Ribose nucleoside	Within a nucleic acid
Adenine	Adenosine	Adenylate
Guanine	Guanosine	Guanylate
Cytosine	Cytidine	Cytidylate
Uracil	Uridine	Uridylate
Thymine	Thymidine	Thymidylate

Structure of DNA

Nucleic acid orientation

- Linear or circular, depending on organism
- DNA - deoxyribonucleotide polymer: dAMP, dCMP, dGMP and TMP

[1]The ' is added to differentiate between the sugar carbon numbering and the nucleotide numbering.

- Linked by phosphodiester bonds between the 5' phosphate of one nucleotide and the 3' hydroxyl of the next (3' $\rightarrow$ 5' linkage)
- RNA - ribonucleotide polymer: AMP, CMP, GMP and UMP
- Conventionally, named and drawn in the 5' to 3' direction (example: 5' ATGC 3')
- Double helix structure of B-DNA was published in 1953 by James Watson and Francis Crick (but it was all because of Rosalind Franklin's X-ray diffraction data!)
- Nobel prize in 1962 along with Maurice Wilkins
- **Anti-parallel, right-handed helix**
- Two grooves: major (wider, deeper) and minor (shallower, smaller)
- 10.5 base pairs per turn
- Watson-Crick base pairing (cytosine to guanine, thymine or uracil to adenine)

Chargaff's rule for double-stranded DNA

%C = %G and %A = %T. G to C is more stable because of 3 hydrogen bonds

Base pair stacking

- Base pairs stack on each other like books on a shelf
- **Stabilized by London interactions between pi orbitals** (π-π interaction)
- Edges of bases define grooves

DNA conformations

B-DNA ist he most common form adopted, but other forms exist:

- A-DNA (dehydrated B-DNA)
  - Right-handed helix
  - Observed in RNA, dehydrated DNA, and DNA-RNA hybrid structures
  - 11 base pairs per turn
  - Pitch: 25.3 Å
- Z-DNA
  - Zigzag conformation
  - Left-handed helix
  - Common for alternating purine/pyrimidine sequences
  - Arises from **torsional strain** imposed by supercoiling

  - 12 base pairs per turn
  - Pitch: 45.6 Å

Amino acid interactions

- Positively charged amino acids (R, H, K) form salt bridges with PO_4^-, elevating protein pI
- Amino acid residues "read" the DNA sequence by recognising H-bond acceptors (A) and donors (D) in the major groove
  - Adenine: $-NH_2$, N_7
  - Thymine: O_4
  - Guanine: O_6, N_7
  - Cytosine: $-NH_2$

Structure stability

- Hydrogen bonds within base pairs
- Hydration (phosphate group and ribose OH are hydrophilic)
- Ionic (phosphate backbone is rich in negative charges, high energy state that can be stabilized by Mg)
- Hydrophobic interactions and base stacking (bases are aromatic - hydrophobic, London force interactions between π-π orbitals result in stacking)

Supercoiling of DNA

- Positive and negative supercoiling
- Imposed strain causes supercoiling
- Results from attempted unwinding of DNA
- Energy is stored in the supercoil as torque
- A right-handed coil
- The energy helps in the separation of the strands for transcription and replication of DNA

Positive supercoiling

Results from overwinding, a left-handed supercoil

Negative supercoiling

Results from underwinding, a right-handed supercoil

Higher order structures of DNA

- **Nucleosomes**: a complex between DNA and histone proteins
- "Beads on a string" structure
- Positively charged amino acids needed on histone proteins

RNA - Ribonucleic acids

- A versatile molecule:
  - Protein synthesis
  - Structural roles
  - Catalytic roles
- Differs from DNA:
  - Ribose sugar instead of deoxyribose
  - Uracil nitrogenous base replaces thymine
  - Predominantly exists in single-stranded form
  - Adjacent bases adopt 2° structures: hairpins, A-form helices, internal loops, stem loops
  - Adopts 3° and 4° structures, very protein-like
  - Structures are also stabilized by unique base pairs/triplets
- Modification of bases is common

Types of RNA

messenger RNA, mRNA carries genetic information from DNA through to protein synthesis

transfer RNA, tRNA molecules transport amino acids to the ribosomes for polypeptide assembly (15% of cellular RNA)

ribosomal RNA, rRNA the functional component oand the framework for the ribosome

ribozyme, catalytic RNA formation of peptide bonds, associated proteins only play structural roles

Processes involving DNA

Central Dogma of genetic information transfer

DNA transcripted into RNA translated into proteins (and DNA can replicate itself)

- The traditional central dogma is overly simplified since information also flows in the other direction
  - Proteins are involved in regulation
  - RNA molecules involved at all steps
  - Viruses have RNA genomes
  - **RNA world**: the hypothesis that RNA was the first self-replicating molecule
  - Like proteins, RNA molecules can perform many functional roles

DNA replication

- Process by which DNA is duplicated
- Similar in all organisms
- **Semi-conservative**: new DNA molecules contain one parent and one daughter strand (original strand separates into 2, old strand + new strand)

Steps:

1. Initiation:
   - Helicase (important to rip strands apart)
   - Single-stranded DNA binding proteins
   - Primase (allows recognition by polymerase, laying the foundation)
2. Elongation
   - Polymerases
   - Topoisomerases (release supercoiling so processes continue)
3. Termination
   - Ligase
   - Telomerase
   - Tus protein

DNA replication sites

- In prokaryotes: 1 site: oriC
- In eukaryotes: multiple origin sites
- Recognised by helicases that destabilise the double-stranded DNA duplex, requires ATP (energy)
- Very energy-intensive process
- Leads to the formation of **replication bubbles** from origins of replication
- **Single-stranded DNA binding (SSB) proteins** stabilize single stranded DNA state
- **Primase** "primes" the open DNA for replication
- Recognises the exposed bases in the replication fork
- Synthesises a short primer sequence on the DNA template (RNA in prokaryotes, RNA-DNA in eukaryotes)

DNA polymerase

- Main polymerases: DNA polymerase III (prokayreotes) and DNA polymerases δ and ε (eukaryotes)
- Large multi-enzyme complex
- Recognises the RNA-DNA duplex in the replication bubble
- Highly processive, not released until completed replication of DNA
- β-sliding clamp (encircles DNA and enhances processivity)
- Requires $Mg_2{}^+$, DNA template, primers with a free 3' OH group, and deoxyNTPs (dATP, dCTP, dGTP and TTP)

5' to 3' synthesis of DNA

- DNA template read in the 3' $\rightarrow$ 5' direction
- Parental DNA opens
- 5' is the leading strand (continuous), 3' is the lagging strand (discontinuous, happens in **Okazaki fragments**)
- DNA made in the 5' $\rightarrow$ 3' direction