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Programme: M.Sc., Biomedical Science

Course Code: BM35C5

Course Title: Molecular Biology

Unit-I

Structure and Properties of Nucleic Acids

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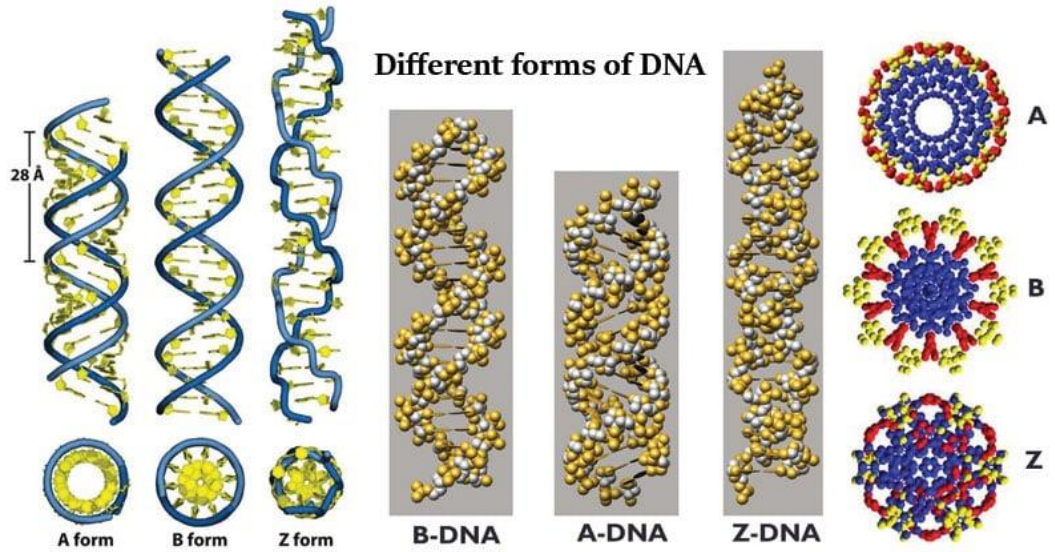
Unit I:

Structure and Properties of Nucleic acids : Nucleic acid as the genetic material (Griffith's experiment, Avery, MacLeod and McCarty‟s experiment, Hershey-Chase experiment)- Components of DNA and RNA -Watson and Crick model of DNA structure, Various forms of DNA- Physical properties of nucleic acids; Denaturation and renaturation, Cot curves – Structure and function of mRNA, rRNA, tRNA- Chromatin structure-Euchromatin, Heterochromatin- Central Dogma of Molecular Biology.

PRESENTATION: 2

Different forms of DNA

- The right-handed double-helical **Watson – Crick Model** for B-form DNA is the most commonly known DNA structure.
- In addition to this classic structure, several other forms of DNA have been observed.
- The helical structure of DNA is thus variable and depends on the sequence as well as the environment.



B-form DNA

- B-DNA is the Watson–Crick form of the double helix that most are **familiar** with.
- They proposed two strands of DNA — each in a right-hand helix — wound around the same axis. The two strands are held together by H-bonding between the bases (in anti-conformation).
- The two strands of the duplex are antiparallel and plectonemically coiled. The nucleotides arrayed in a 5′ to 3′ orientation on one strand align with complementary nucleotides in the 3′ to 5′ orientation of the opposite strand.
- Bases fit in the double helical model if pyrimidine on one strand is always paired with purine on the other. From Chargaff's rules, the two strands will pair A with T and G with C. Two H-bonds can form between A and T, and three can form between G and C.
- These are the complementary base pairs. The base-pairing scheme immediately suggests a way to replicate and copy the genetic information.
- 34 nm between bp, 3.4 nm per turn, about 10 bp per turn
- About 2.0 nm or 20 Angstroms in diameter.

A-form DNA

- The major difference between A-form and B-form nucleic acid is in the confirmation of the deoxyribose sugar ring. It is in the C2′ endoconformation for B-form, whereas it is in the C3′ endoconformation in A-form.
- A second major difference between A-form and B-form nucleic acid is the placement of base-pairs within the duplex.
- In B-form, the base-pairs are almost centered over the helical axis but in A-form, they are displaced away from the central axis and closer to the major groove. The result is a ribbon-like helix with a more open cylindrical core in A-form.
- Right-handed helix
- 11 bp per turn

Z-form DNA

- Z-DNA is a radically different duplex structure, with the two strands coiling in left-handed helices and a pronounced zig-zag (hence the name) pattern in the phosphodiester backbone.
- Z-DNA can form when the DNA is in an alternating purine-pyrimidine sequence such as GCGCGC, and indeed the G and C nucleotides are in different conformations, leading to the zig-zag pattern.
- The big difference is at the G nucleotide.
- It has the sugar in the C3′ endo conformation and the guanine base is in the syn conformation.
- Discovered by Rich, Nordheim &Wang in 1984.
- It has antiparallel strands as B-DNA.
- It is long and thin as compared to B-DNA.
- 12 bp per turn; 1.8 nm helix diameter

Physical Properties of Nucleic Acids

1. Chemical Composition

- **DNA**: Composed of deoxyribonucleotides, which include a deoxyribose sugar, a phosphate group, and one of four nitrogenous bases (adenine, thymine, cytosine, guanine).
- **RNA**: Composed of ribonucleotides, which include a ribose sugar, a phosphate group, and one of four nitrogenous bases (adenine, uracil, cytosine, guanine).

2. Structure

Primary Structure:

- The sequence of nucleotides in a linear chain.

Secondary Structure:

- Involves **interactions between bases within the same molecule.**
- For DNA, this typically forms a double helix with complementary base pairing (A with T, and C with G).
- RNA can form various secondary structures like hairpins, loops, and bulges due to intramolecular base pairing.

Tertiary Structure:

- The **three-dimensional shape** formed by the entire nucleic acid chain.
- For DNA, this includes the supercoiled structure seen in chromosomes. RNA molecules can form complex 3D shapes crucial for their function.

3. Stability

- **DNA**: Generally more stable than RNA due to the **absence of the 2'-hydroxyl group on the sugar**, making it less susceptible to hydrolysis. The double-stranded nature of DNA also contributes to its stability.
- **RNA**: Less stable due to the **presence of the 2'-hydroxyl group**, which makes it more prone to hydrolysis. Single-stranded RNA is also more susceptible to degradation.

4. Solubility

- Both DNA and RNA are soluble in water and in aqueous solutions of low ionic strength. Their solubility is influenced by the presence of the negatively charged phosphate backbone.

5. Viscosity

- Solutions of high molecular weight DNA are highly viscous due to the long, intertwined nature of the DNA strands.
- RNA solutions are generally less viscous than DNA solutions of similar molecular weight.

6. Absorbance

- Nucleic acids absorb ultraviolet (UV) light, with a peak absorbance around 260 nm due to the aromatic rings in the nitrogenous bases. This property is used to quantify nucleic acid concentration using spectrophotometry.

7. Denaturation and Renaturation

- **Denaturation**: The process where double-stranded DNA unwinds and separates into single strands due to the breaking of hydrogen bonds between bases. This can be induced by heat, high pH, or chemical denaturants.
- **Renaturation (Annealing)**: The process where complementary single strands of DNA recombine to form double-stranded DNA upon returning to favorable conditions (lower temperature, neutral pH).

8. Electrophoretic Mobility

- Nucleic acids are negatively charged due to their phosphate backbone and move towards the positive electrode during electrophoresis. Smaller fragments migrate faster through the gel matrix than larger ones.

9. Hydration

- Nucleic acids are highly hydrated molecules, with water molecules interacting with the phosphate groups and the grooves of the double helix in DNA. This hydration is crucial for maintaining their structure and stability.

DENATURATION AND RENATURATION

Denaturation

- **Denaturation** refers to the process by which nucleic acids, such as DNA or RNA, lose their native secondary and tertiary structures. This involves the separation of double-stranded DNA (dsDNA) or double-stranded RNA (dsRNA) into single strands. Denaturation can be induced by various factors:

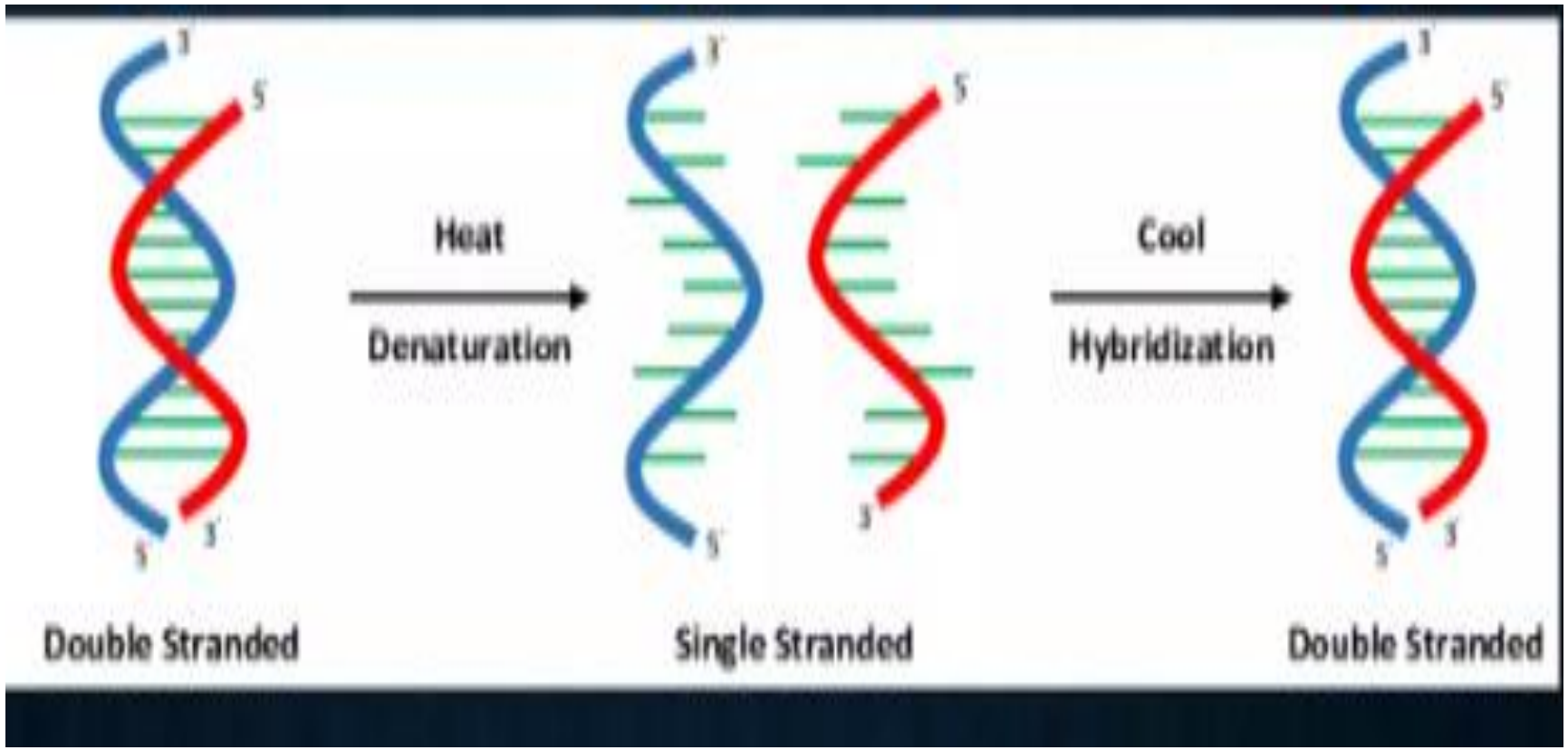
1. **Heat:** High temperatures can disrupt the hydrogen bonds between complementary base pairs, causing the strands to separate.
2. **pH:** Extreme pH levels can ionize the bases, disrupting hydrogen bonds and destabilizing the helical structure.
3. **Chemical agents:** Chemicals like **urea or formamide** can disrupt hydrogen bonds and destabilize the double helix.

- During denaturation, the nucleic acid transitions from a structured, double-stranded form to a more flexible, single-stranded form. This process is usually reversible under the right conditions.

Renaturation

- **Renaturation** (or annealing) is the process by which single-stranded nucleic acids reform their double-stranded structure by re-establishing hydrogen bonds between complementary bases. This process is critical for the accurate function of nucleic acids in biological systems and can be influenced by several factors:

1. **Temperature:** Lowering the temperature gradually after denaturation allows the complementary strands to find each other and re-form hydrogen bonds. This is often done slowly to ensure proper base pairing.
2. **Concentration:** Higher concentrations of nucleic acids increase the likelihood of complementary strands encountering each other.
3. **Ionic conditions:** The presence of certain ions, such as Mg^{2+} or Na^{+}, can stabilize the double-stranded structure by shielding the negative charges on the phosphate backbone, facilitating hydrogen bond formation between bases.


3'
5'
5'
3'
Heat
Denaturation
3'
5'
5'
3'
Cool
Hybridization
3'
5'
5'
3'
Double Stranded
Single Stranded
Double Stranded

Cot curves

- Cot curves, or Cot analysis, is a method used in molecular biology to study the complexity and **reassociation kinetics of DNA**. The term "Cot" stands for the product of the DNA **co**ncentration (C) and the reassociation **t**ime (t).

1. Reassociation Kinetics:

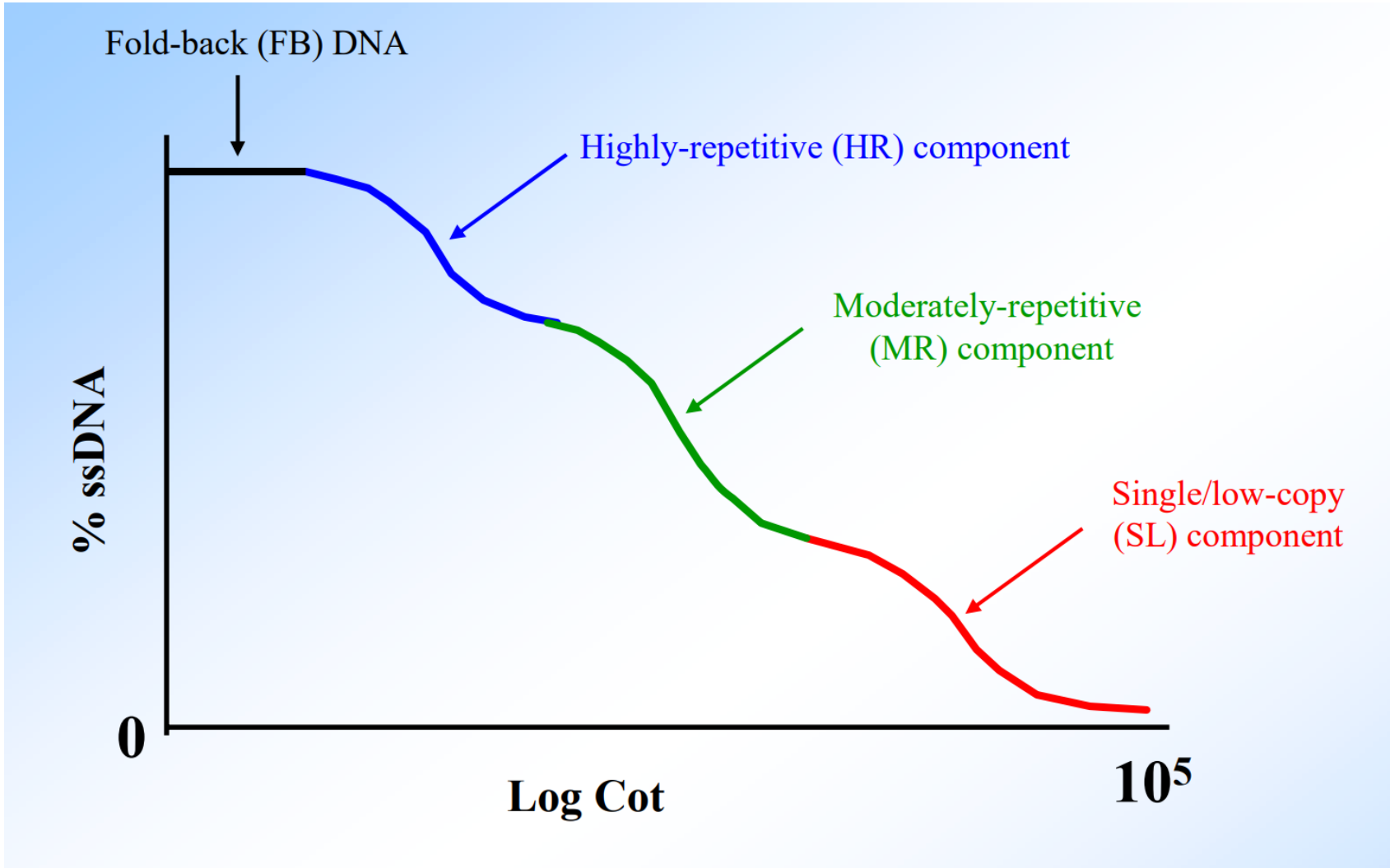
1. When double-stranded DNA is denatured by heat or chemical means, it separates into single strands.
2. If the temperature is slowly lowered or the chemical denaturant is removed, the single strands can reassociate or "reanneal" to form double-stranded DNA again.
3. The rate at which this reassociation occurs depends on the complexity of the DNA sequence.

2. Cot Value:

1. Cot is a measure of DNA concentration (C) multiplied by the time (t) it takes for reassociation.
2. It is usually expressed in molar-seconds (M·s).

3. Reassociation Curve:

1. A plot of the fraction of single-stranded DNA remaining (y-axis) versus the logarithm of the Cot value (x-axis) generates a Cot curve.
2. The shape of the curve provides information about the complexity and repetition of sequences within the DNA.


Fold-back (FB) DNA
Highly-repetitive (HR) component
Moderately-repetitive
(MR) component
Single/low-copy
(SL) component
% ssDNA
0
Log Cot
10^5

Applications

1. Estimating Genome Complexity:

1. By analyzing the Cot curve, scientists can estimate the amount of repetitive versus unique DNA in a genome.
2. The complexity of the genome correlates with the length of time required for reassociation.

2. Characterizing Repetitive DNA:

1. Cot analysis can be used to identify the proportion of repetitive sequences in different organisms and understand their evolutionary significance.

3. DNA Renaturation Kinetics:

1. The method helps in understanding the kinetics of DNA renaturation, which is important in various molecular biology applications, such as hybridization techniques.