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COURSE: MBLG1001

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Lecture 8

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MBLG Lecture 8

- Stability of DNA
- UV absorbance
- Negative charge
- Detection by fluorescence
- Manipulation of the weak forces experimentally
- Proteins that interact with DNA

The Physical and chemical stability of DNA (pages 112 – 115).

Information stored in DNA must be passed on from one generation to the next over millions of years. To do this DNA molecules must be very stable. They have evolved over time to be just that. Initially it is thought that life started as RNA. After all RNA can store and transfer information like DNA and it can catalyse some reactions like proteins. DNA and proteins then evolved becoming macromolecules with more specialized functions.

Properties which make DNA ideal as a store of genetic information:

- The sugar phosphate backbone is very stable to chemical attack.
- The absence of the –OH at position 2 in the sugar makes DNA very resistant to alkali (unlike RNA). The –OH at position 2 in ribose of RNA makes the backbone vulnerable to base attack. It is in the right position for the phosphate nearby to form a cyclic compound when the proton is removed (at high pH). This cyclic compound rapidly breaks the phosphodiester bond resulting in a 2’ or 3’ Phosphorylated nucleotide.
• The N-glycosidic bond to the bases is very stable as the bases are hydrophobic (if they were more hydrophilic the bond would not be so stable).

• The tightly stacked bases exclude almost all water. This protects the information (in the more polar section in the center of the double strand) from water soluble compounds which may react with the charged groups.

• The two strands, which gives a double copy of the information and a template for repair. The information (in the form of charged and polar groups on the bases) is found, “buried” at the very center of the double stranded helix, protected by a hydrophilic sugar phosphate backbone and then the hydrophobic bases. These two layers with distinctly different properties make it difficult for possible mutagenic compounds to reach the information. With single stranded RNA the polar groups on the bases are exposed to all the chemical compounds in the cell (there are a lot).

• Uracil is replaced by thymine. Cytosine is deaminated to uracil spontaneously by a process called oxidative deamination. In a given day about 100 cytosines are deaminated to uracils in each cell. These uracils are a corruption of the code and need to be removed and replaced. But if uracil was a normal constituent of DNA there would be no way of knowing whether the
uracils were supposed to be there or not. By converting uracils to thymines (by a methylation) before incorporation into DNA, any uracils found in the DNA must have come from cytosine and can thus be excised and replaced.

![Reaction](image)

**UV Absorption**

- This is the most commonly used technique to measure the concentration of DNA and RNA
- It is not sequence or source dependent
- An absorbance of 1 at 260 nm gives 50 mg/mL DNA.
- An absorbance of 1 at 260 nm gives 40 mg/mL RNA.

See the lab manual section 9: DNA Isolation.

**The Negative Charge**

See Section 10 of the lab manual.

**Detecting DNA by Fluorescence**

- DNA can be detected by ethidium bromide.
- This dye intercalates with the double stranded DNA.
- It fluoresces under UV light.
- It is very sensitive, detecting as little as 10 ng of DNA in one band.

**Manipulation of the weak forces experimentally:**

Many of the techniques used in molecular biology involve creating an environment that promotes or disrupts hydrogen bonding. The manipulation of this environment is done in one or more of the following ways:

- **Increased temperature** will disrupt the H bonds
- **Increased pH** will remove the ring N proton from Thymine and Guanine, thus preventing the H-bonding
• **The addition of formamide.** Formamide offers an alternative molecule for H bonding.

• **The addition of formaldehyde.** Formaldehyde reacts with the amine groups of adenine, guanine and cytosine forming a Schiff’s base and preventing H bonding.

• **Ethanol Precipitation.** The ethanol is able to disrupt the base stacking and the bases slide off each other (into a mess!!).

**Disrupting the DNA double helix.**
Formamide, formaldehyde, high pH and high temperature will all disrupt the double helix. High pH achieves this by removing the proton from the ring N. No protons → no H bonding. The high temperature is referred to as “melting” DNA.

**Melting DNA (pages 40 – 42).** If double stranded DNA is heated from room temperature, it melts (becomes single stranded) over a short temperature range. This melting can be monitored by absorbance at 260 nm. DNA absorbs at 260 nm due to the ring structure of the bases. The same solution of DNA will have a higher absorbance at 260 nm when it is single stranded, than in its double stranded form. This is known as the **hyperchromic** effect. The reason for this is the ordered base stacking in double stranded DNA which hinders photon access. Single stranded DNA has 1.4 * higher absorbance at 260 nm. The equivalent concentration of free nucleotides have even higher absorbance again. A $T_m$ can be determined for the DNA solution (the midpoint of the transition) and this is dependent on the GC content of the DNA sample. The higher the GC content the higher is the $T_m$ or melting temperature. The dependence of $T_m$ on GC content is related to the 3 H bonds in the GC pair (as opposed to 2 in the AT pair) although the base stacking may have a larger effect than the number of H bonds. Base stacking interactions with GC pairs is thought to be stronger than AT pairs.

Melting DNA is not only done in the test-tube. Whenever we want access to the sequence of the DNA, as in replication and transcription, we need to melt localized regions of the double helix. This is achieved in various ways, usually with enzymes. Because the forces holding the two strands together are only weak forces this process is not as energy demanding as it would be if the strands were held together by covalent bonds (we would never get past the first mitosis!!).

**Promoting base pairing (42 – 45); renaturation, hybridisation.**
Increasing ionic strength promotes base pairing as the cations (positive ions eg Na$^+$, Mg$^{2+}$) shield the phosphates and thus reduce the repulsive effect of the strong negative charges. Mg$^{2+}$ ions while giving the best shielding are rarely used as these are necessary for reactions which hydrolyse the phosphodiester bond, thus breaking the sugar phosphate backbone (and you want to avoid this at all cost!). This type of reaction needs no encouragement so chelator e.g. EDTA are often added to buffers to protect against hydrolysis.

We create an environment which promotes base pairing when you hybridise one strand of nucleic acid to another e.g. gene chips. The hybridization technique refers to the base pairing of nucleic acid strands from different sources.
Proteins which interact with DNA (pages 85 – 87).

Key Concept: **Proteins are composed of amino acids and so cannot “base pair” to the DNA.**
Amino acid side chains interact with the nucleic acid with the same non-covalent weak forces used to maintain the double helix i.e. H bonds, ionic interactions, van der Waals and hydrophobic interactions.

Nucleases: enzymes responsible for the hydrolysis of nucleic acids

Exonucleases “chew” from the ends of the nucleic acid.

**Endonucleases** eg. Restriction enzymes, cleave internal phosphodiester bonds and are usually sequence specific. There are 3 types of restriction enzymes; types I, II and III. These enzymes are produced by certain species of bacteria to restrict bacteriophage DNA. Bacteriophage are viruses that infect bacteria. The bacteria produce the enzymes to prevent the infection. They protect their own DNA by methylation. The most relevant restriction enzymes for molecular biology are type II, which cut a specific sequence. **The cleavage site is within the recognition sequence.** Type I enzymes cleave DNA some 1 000 bp from the recognition site while type III enzymes cut the DNA 24 to 26 bps from the recognition site. Restriction enzymes are named after the genus and species they were first isolated from e.g. *EcoR*I was first isolated from *Escherichia coli* subspecies R and the I means it was the first enzyme isolated. *EcoRI* cuts at G\(\text{AATTTC}\). This sequence if found in the host DNA is methylated at the second A: GA\(\text{me}\)ATTTC. Type II restriction enzymes cut at palindromic sequences (two fold rotational symmetry). The enzymes themselves are homodimers with each subunit recognizing the sequence on one of the strands. If the cleavage site is staggered then the digest will produce sticky ends. If the cleavage is symmetrical then blunt ends will result. This distinction becomes important when trying to insert DNA into the cleavage site. Most restriction enzymes recognize a specific 4 base sequence (termed 4 base cutters) or a 6 base pair sequence (6 base cutters).

**Calculating the probability:** The probability of finding the exact recognition site for a 4 base cutter is 1 in \(4^4\) and a 6 base cutter 1 in \(4^6\). If you have a piece of DNA 1 200 base pairs long you are, in all probability, going to have 4.69 sites or between 4 and 5 sites for a 4 base cutter.

**Ribozymes** are RNA molecules capable of cleaving and reforming phosphodiester bonds.

**Synthesis:** enzymes responsible for synthesizing new strands of nucleic acid.

**DNA polymerases, I and III,** make a new deoxyribonucleic acid strand in the 5’ → 3’ direction from a DNA template. They require a primer and use dNTPs as substrate.

**RNA polymerases** make an RNA copy from a DNA template in the 5’ → 3’ direction, using NTPs as substrate. They do not need a primer.

**Reverse transcriptase** makes a complementary DNA copy (cDNA) from an RNA template in the 5’ → 3’ direction using dNTPs as substrate. It needs a primer.

**Transcription Regulation:** proteins responsible for the initiation of transcription at specific sites on the genome. Refer to the notes in lecture 4; leucine zippers, helix-turn-helix and zinc finger motifs.

**Packaging:** proteins responsible for the packaging of the genome eg histones.