CHAPTER 2

STRUCTURES OF NUCLEIC ACIDS

What is the chemical structure of a deoxyribonucleic acid (DNA) molecule? DNA is a polymer of deoxyribonucleotides. All nucleic acids consist of nucleotides as building units. A nucleotide has three components: sugar, base, and a phosphate group. (The combination of a sugar and a base is a nucleoside.) In the case of DNA, the nucleotide is known as deoxyribonucleotide, because the sugar in this case is deoxyribose. The base is either a purine (adenine or guanine) or a pyrimidine (thymine or cytosine) (Fig. 2.1). Another type of nucleic acid is ribonucleic acid (RNA), a polymer of ribonucleotides also consisting of three components - a sugar, a base and a phosphate, except that the sugar in this case is a ribose, and that the base thymine is replaced by uracil.

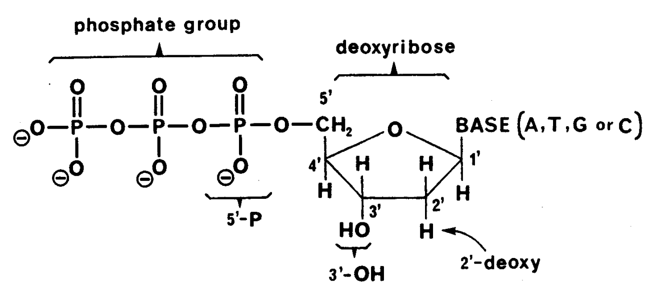

Fig. 2.1. Chemical structure of deoxyribonucleotide

2.1 3'-OH and 5'-P Ends

In DNA, the hydroxyl (OH) group is attached to the carbon at the 3' position. One of the three phosphates (P) in the phosphate group is directly attached to the carbon at the 5' position (Fig. 2.1). The OH group and the P group in a nucleotide are called the 3'-OH (3 prime hydroxyl) and 5'-P. A nucleotide is more appropriately described as 2'-deoxynucleoside 5'-triphosphate to indicate that the OH at the 2' position is deoxygenated and the phosphate group is attached to the 5' position.

A DNA molecule is formed by linking the 5'-P of one nucleotide to the 3'-OH of the neighboring nucleotide (Fig. 2.2). A DNA molecule is therefore a polynucleotide with nucleotides linked by 3'-5' phosphodiester bonds. The 5'-P end contains 3 phosphates but in the 3'-5' phosphodiester bonds, two of the phosphates have been cleaved during bond formation. An important consequence to a phosphodiester linkage is that DNA molecules are directional- one end of the chain with a free phosphate group, and the other end with a free OH group. It is particularly important in cloning to specify the two ends of a DNA molecule: 5'-P end (or simply 5' end) and 3'-OH end (or 3' end).

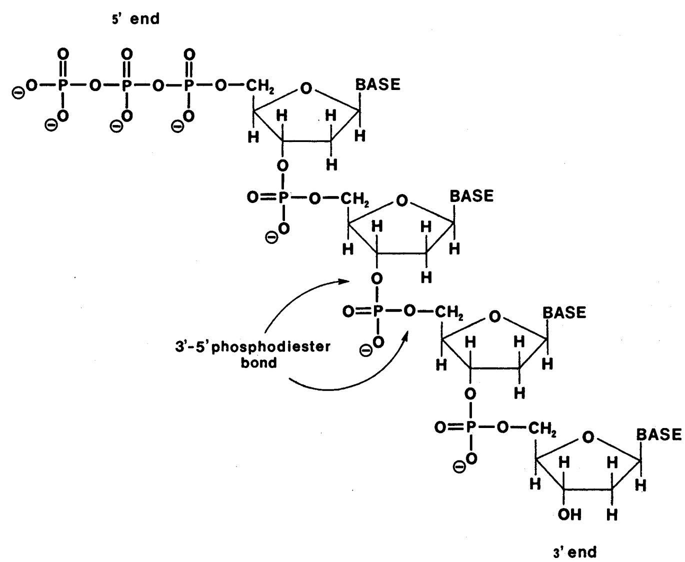

Fig. 2.2. Polynucleotide showing a 3'-5 phosphodiester bond.

2.2 Purine and Pyrimidine Bases

The deoxyriboses and phosphate groups forming the backbone of a DNA molecule are unchanged throughout the polynucleotide chain. However, the bases in the nucleotides vary because there are 4 bases - adenine, thymine, guanine and cytosine, abbreviated as A, T, G and C, respectively (Fig. 2.3). A and G are purines (with double-ring structures); T and C are pyrimidines (with single-ring structures). Consequently, there are four different nucleotides.

Base	DNA Nucleotide (deoxynucleoside triphosphates)	dNTP
Adenine (A)	2'-deoxyadenosine 5'-triphosphate	dATP
Thymine (T)	2'-deoxythymidine 5'-triphosphate	dTTP
Guanine (G)	2'-deoxyguanosine 5'-triphosphate	dGTP
Cytosine (C)	2'-deoxycytidine 5'-triphosphate	dCTP

A DNA molecule with n number of nucleotides would have 4^n possible different arrangement of the 4 nucleotides. For example, a 100 nucleotide long DNA has 4^{100} different possible arrangements. The particular arrangement of the nucleotides (as determined by the bases) of a DNA molecule is known as the nucleotide (or DNA) sequence.

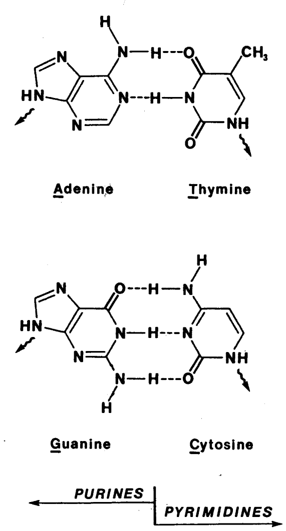

Fig. 2.3. Chemical structures of purine and pyrimidine bases.

2.3 Complementary Base Pairing

The unique structures of the four bases result in base pairing between A and T, and between G and C, by the formation of hydrogen bonds (electrostatic attraction between hydrogen atom and two electronegative atoms, such as nitrogen and oxygen) (Fig. 2.3). It is important to note that there are 3 hydrogen bonds in a GC pair whereas only 2 hydrogen bonds are formed in an AT pair. Therefore, AT pairs are less tightly bound (hence, less stable) than GC pairs.

Base pairing provides a major force for two polynucleotides to interact. A DNA molecule in its native (natural) state exists as a double-stranded molecule, with the nucleotides of one strand base pairing with the nucleotides of the other strand. The two strands in a DNA molecule are therefore complementary to one another. If the bases in one strand are known, the alignment of the bases in the complementary strand can be deduced.

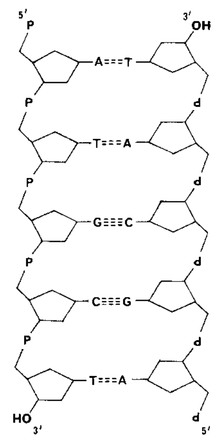

Fig. 2.4. Base-pairing in double-stranded DNA.

In addition to complementary base pairing, the two strands of a DNA molecule assume a double helical structure because of energetic factors of the bonds, a subject beyond the scope of this book. The two strands in a DNA molecule are antiparallel. One strand goes from 5' to 3' in one direction, while the other strand goes in the opposite direction (Fig. 2.4).

2.4 Writing a DNA Molecule

Taking all the information described thus far, a DNA molecule can be represented by a simple scheme. Since the deoxyribose and phosphate backbones are the same for every nucleotide, a DNA molecule can simply be represented by the bases, with indication of the 5' end of the DNA strand. The four bases A, T, G, and C are used synonymously with their respective nucleotides, with the understanding that it is a convenient way to simplify a complicated structure. For example, a DNA sequence is represented: 5'-ATGTCGGTTGA. Also note that a DNA sequence is always read in a 5' to 3' direction. In writing a DNA sequence, always starts with the 5' end. Conventionally, only the sequence of one strand is presented because the complementary strand can readily be deduced. The question then is: Which strand of a DNA molecule do we choose to present? The answer to this is related to the process of transcription and translation, and will be described in Sections 4.5 and 5.2.

2.5 Describing DNA Sizes

The size of a DNA molecule is measured by the number of nucleotides (or simply the number of bases). The common unit for double-stranded DNA (dsDNA) is the base pair (bp). A thousand bp is a kilobase (kb). Likewise, a million bp is known as megabase pair (Mb). One kb of dsDNA has a molecular weight of 6.6 x 10^5 daltons (330 gram per mole).

2.6 Denaturation and Renaturation

The two strands of a DNA molecule are held by hydrogen bonds that can be broken down by heating or increasing pH of the DNA solution.

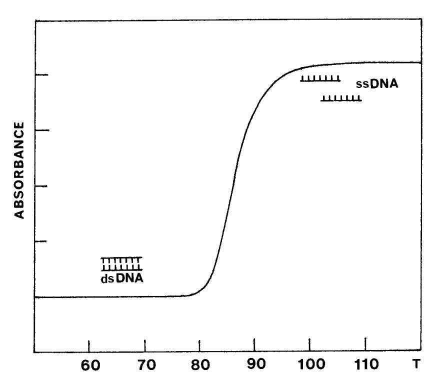

Fig. 2.5. Denaturation and renaturation curve.

In a process known as denaturation, the two strands separate into single-stranded DNA (ssDNA) at a sharp melting temperature ~90°C. Upon cooling of the DNA solution, the two strands reassociate into a dsDNA molecule, a process known as renaturation (Fig. 2.5). The process of thermal denaturation and renaturation is utilized in cloning for creating ssDNA strands, for the annealing of DNA primers in DNA sequencing, and in the polymerase chain reaction (see Sections 8.8 and 8.9).

2.7 Ribonucleic Acid

A second type of nucleic acid is ribonucleic acid (RNA). Like DNA, RNA is also a polynucleotide, but with the following differences (Fig. 2.6): (1) In RNA, the sugar is ribose, not deoxyribose (The nucleotide in RNA is therefore known as ribonucleotide.); (2) The bases in RNA are A, U (uracil), G, C, instead of A, T, G, C in DNA; (3) The OH group at he 2' position is not deoxygenated; (4) RNA is single-stranded. However, it can form base pairs with a DNA strand. For example:

5' - ATGCATG ---- 3'   ssDNA
3' - UACGUAC ---- 5'   RNA

Base	RNA nucleotides(Nucleoside triphospates)	NTP
Adenine (A)	Adenosine 5'-triphosphate	ATP
Uracil (U)	Uridine 5'-triphosphate	UTP
Guanine (G)	Guanosine 5'-triphosphate	GTP
Cytosine (C)	Cytidine 5'-triphosphate	CTP

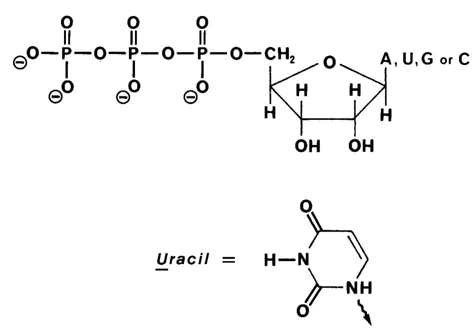

Fig. 2.6. Chemical structure of ribonucleotide.

Review

1. A DNA molecule is formed by linking the _______ of one nucleotide to the ________ of the neighboring nucleotide. The bond formed by linking two nucleotides is a _______________ bond.
2. Deoxyribonucleic acid (DNA) is double stranded. The two strands are _________________ to one another, with A (adenine) pairing with ________ and G (guanine) pairing with _________. DNA strands are directional, with _______ and ______ ends. The two strands are _________ to one another.
3. List the differences in the components between deoxyribonucleotide and ribonucleotide.

Nucleotide	Sugar	Base	Phosphate
Deoxyribonucleotide			
Ribonucleotide			

4. Given the following DNA strand: 5'-TCTAATGGAGCT, write down the complementary strand, _____________________. Indicate the directions by properly labeling the 5' end.
5. What are the conventional rules for writing a DNA sequence?
6. What is the size of the following DNA fragment?

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5'-AATGGCTAGT GGCAAATGCT AGGCTGCAAG
   CCTTTCCAAT GGTGTGTCAA ACAAAAAACG
   TGCCCGTCAG CAAGTTGTG
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7. Suppose the DNA fragment in problem 7 is RNA. What will be the sequence like?

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