Molecular Biology

Lecture 2

NUCLEIC ACID STRUCTURE

Nucleoside

A nucleoside consists of a base covalently bonded to the 1′-position of a pentose sugar molecule. In RNA the sugar is ribose and the compounds are ribonucleosides, or just nucleosides, whereas in DNA it is 2′-deoxyribose, and the nucleosides are named 2′-deoxyribonucleosides, or just deoxynucleosides.Base + sugar = nucleoside

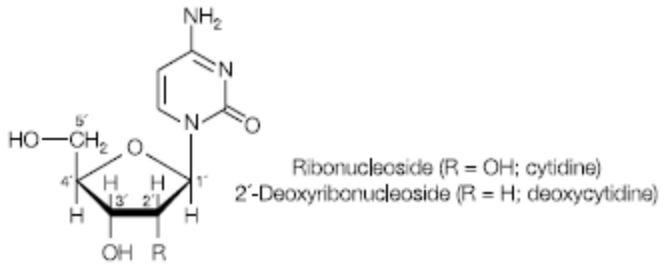

Fig. 2. Nucleosides.

Nucleotides

Nucleotides are nucleosides with phosphate groups covalently bound to the 5′ in ribonucleotides. Base + sugar + phosphate = nucleotide. The nucleoside 5′-triphosphates (NTPs or dNTPs) are respectively the building blocks of polymeric RNA and DNA.

Phosphodiester bonds

In nucleic acid polymers, the ribose or deoxyribose sugars are linked by a phosphate bound between the 5′-position of one sugar and the 3′-position of the next, forming a 3′,5′-phosphodiester bond. Nucleic acids hence consist of a directional sugar–phosphate backbone with a base attached to the sugar. The repeat unit is a nucleotide. Nucleic acids are highly charged polymers with a negative charge on each phosphate.

DNA/RNA sequence

Conventionally, the repeating monomers of DNA or RNA are represented by **sequence** their single letters A, T, G, C or U. In addition, there is a convention to write the sequences with the 5′-end at the left. Hence a stretch of DNA sequence might be written 5′-ATAAGCTC-3′, or even just ATAAGCTC. An RNA sequence might be 5′-AUAGCUUGA-3.

DNA double helix

DNA most commonly occurs in nature as the well-known **'double helix'**. The basic features of this structure were deduced by James Watson and Francis Crick in 1953. Two separate chains of DNA are wound around each other, each following a helical (coiling) path, resulting in a **right-handed** double helix (*Fig. 5a*). The negatively charged sugar–phosphate backbones of the molecules are on the outside, and the planar bases of each strand stack one above the other in the center of the helix (*Fig. 5b)*. Between the backbone strands run the **major** and **minor grooves**, which also follow a helical path. The strands are joined noncovalently by hydrogen bonding between the bases on opposite strands, to form **base pairs**. There are around 10 base pairs per turn in the DNA double helix.

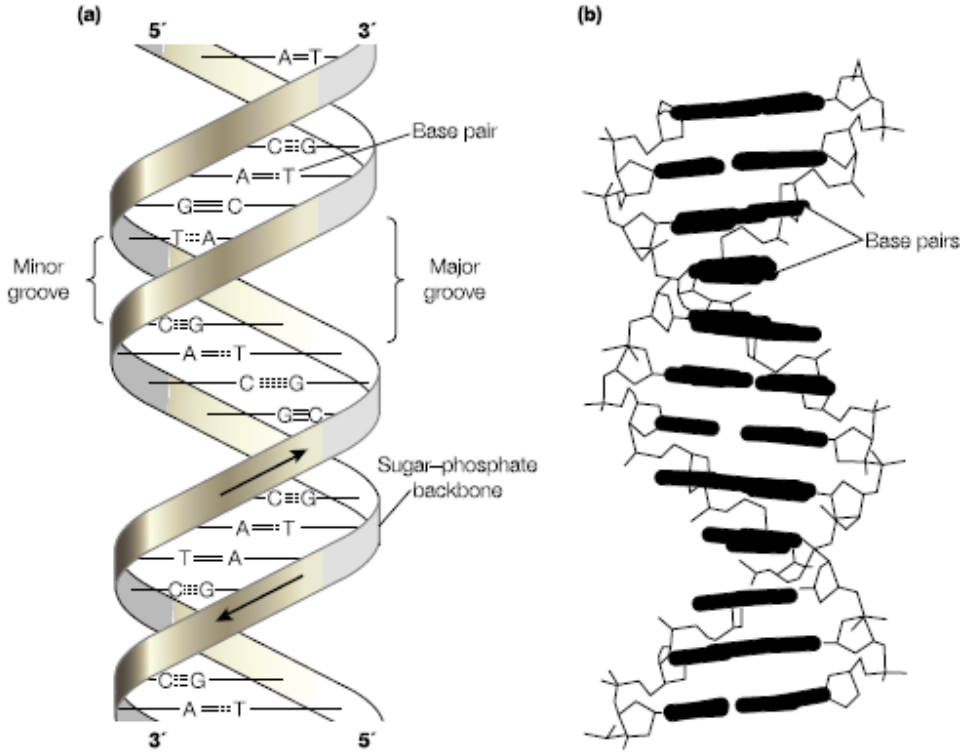

Fig. 5. The DNA double helix. (a) A schematic view of the structure; (b) a more detailed structure, highlighting the stacking of the base pairs (in bold).

RNA secondary structure

RNA normally occurs as a single-stranded molecule, and hence it does not adopt a long regular helical structure like double-stranded DNA. RNA instead forms relatively globular conformations, in which local regions of helical structure are formed by **intramolecular** hydrogen bonding and base stacking within the single nucleic acid chain.

CHEMICAL AND PHYSICAL PROPERTIES OF NUCLEIC ACIDS

Stability of nucleic acids

Although it might seem obvious that DNA double strands and RNA structures are stabilized by hydrogen bonding, this is not the case. H-bonds determine the specificity of the base pairing, but the stability of a nucleic acid helix is the result of hydrophobic and dipole–dipole interactions between the stacked base pairs.

Effect of acid

Highly acidic conditions may hydrolyze nucleic acids to their components: bases, sugar and phosphate. Moderate acid causes the hydrolysis of the purine base glycosylic bonds to yield apurinic acid. More complex chemistry has been developed to remove particular bases, and is the basis of chemical DNA sequencing.

Effect of alkali

High pH denatures DNA and RNA by altering the state of the bases and disrupting specific hydrogen bonding. RNA is also susceptible to hydrolysis at high pH, by participation of the 2′-OH in intramolecular cleavage of the phosphodiester backbone.

Chemical denaturation

Some chemicals, such as urea and formamide, can denature DNA and RNA at neutral pH by disrupting the hydrophobic forces between the stacked bases.

SPECTROSCOPIC AND THERMAL PROPERTIES OF NUCLEIC ACIDS

UV absorption

Nucleic acids absorb UV light due to the conjugated aromatic nature of the bases; the sugar–phosphate backbone does not contribute appreciably to absorption.The wavelength of maximum absorption of light by both DNA and RNA is 260 nm, which is conveniently distinct from the maximum absorption of protein (280 nm). The absorption properties of nucleic acids can be used for detection, quantitation and assessment of purity.

Purity of DNA

The approximate purity of dsDNA preparations may be estimated by determination of the ratio of absorbance at 260 and 280 nm (***A*260/*A*280**). pure dsDNA has an *A*260/*A*280 of 1.8, and pure RNA one of around 2.0. Protein, of course, with max = 280 nm has a 260/280 ratio of less than 1 (actually around 0.5). Hence, if a DNA sample has an *A*260/*A*280

greater than 1.8, this suggests RNA contamination, whereas one less than 1.8 suggests protein in the sample.

Thermal denaturation

Increased temperature can bring about the denaturation of DNA and RNA. RNA denatures gradually on heating, but double-stranded DNA 'melts' cooperatively to give single strands at a defined temperature, *T*m, which is a function of the G+C content of the DNA. Denaturation may be detected by the change in *A*260.

Renaturation

DNA renatures on cooling, but will only form fully double-stranded native DNA if the cooling is sufficiently slow to allow the complementary strands to anneal.

CHROMATIN STRUCTURE

Chromatine

Eukaryotic chromosomes each contain a long linear DNA molecule, which must be packaged into the nucleus. The name chromatin is given to the highly ordered DNA–protein complex which makes up the eukaryotic chromosomes. The chromatin structure serves to package and organize the chromosomal DNA, and is able to alter its level of packing at different stages of the cell cycle.

Histones

The major protein components of chromatin are the histones; small, basic (positively charged) proteins which bind tightly to DNA. There are four families of core histone, H2A, H2B, H3, H4, and a further family, H1, which has some different properties, and a distinct role. Individual species have a number of variants of the different histone proteins.

Nucleosomes

The nucleosome core is the basic unit of chromosome structure, consisting of a protein octamer containing two each of the core histones, with 146 bp of DNA wrapped 1.8 times in a left-handed fashion around it. The wrapping of DNA into nucleosomes accounts for virtually all of the negative supercoiling in eukaryotic DNA.

The role of H1 histone

A single molecule of H1 stabilizes the DNA at the point at which it enters and leaves the nucleosome core, and organizes the DNA between nucleosomes. A nucleosome core plus

H1 is known as a chromatosome. In some cases, H1 is replaced by a variant, H5, which binds more tightly, and is associated with DNA which is inactive in transcription.

The linker DNA

The linker DNA between the nucleosome cores varies between less than 10 and more than 100 bp, but is normally around 55 bp. The nucleosomal repeat unit is hence around 200 bp.

The 30 nm fiber

Chromatin is organized into a larger structure, known as the 30 nm fiber or solenoid, thought to consist of a left-handed helix of nucleosomes with approximately six nucleosomes per helical turn. Most chromatin exists in this form.

Higher order structure

On the largest scale, chromosomal DNA is organized into loops of up to 100 kb in the form of the 30 nm fiber, constrained by a protein scaffold, the nuclear matrix. The overall structure somewhat resembles that of the organizational domains of prokaryotic DNA.

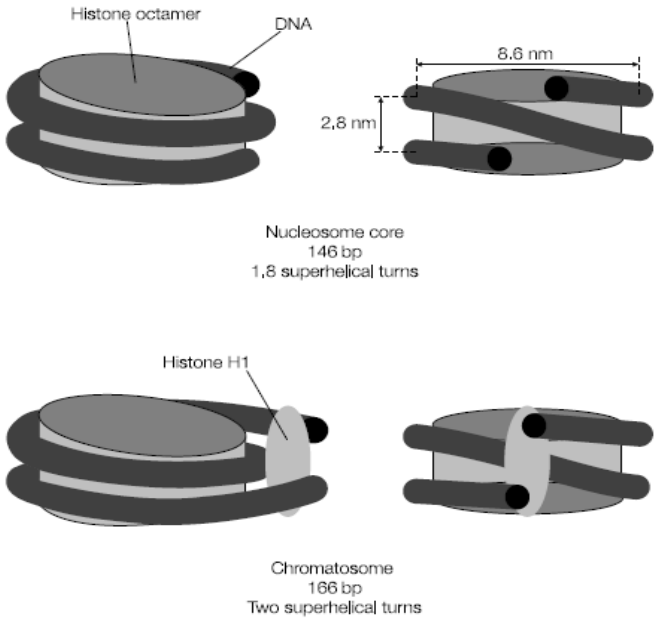

Fig. 1. A schematic view of the structure of a nucleosome core and a chromatosome.

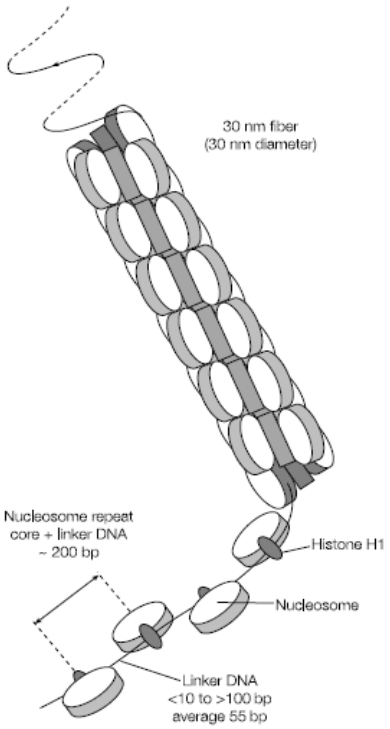

Fig. 2. An array of nucleosomes separated by linker DNA, and the 30 nm fiber.

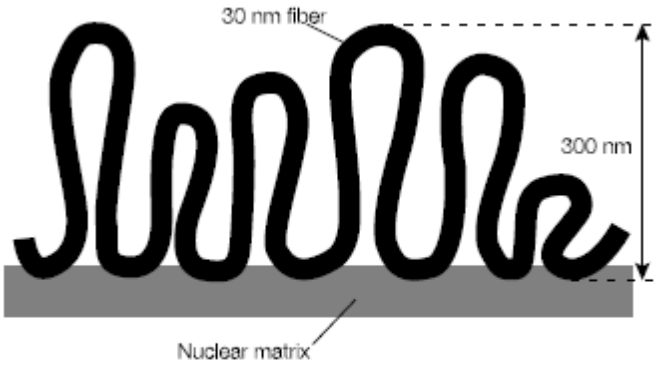

Fig. 3. The organization of 30 nm fiber into chromosomal loops.