Section B and C

Volume-04

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1. STRUCTURE OF CHROMATIN AND CHROMATIN

The word *Chromosome* was coined by Waldeyer in 1888 (Gr. Chroma = colour and soma = body) which literally means 'coloured body'. The name is derived from the fact that the chromosomes stain darkly when treated during mitosis. Chromosomes are always present in the nucleus, although they are generally not visible under the light microscope during interphase. During this phase of the cell cycle, the DNA of the chromosome is extended and is complexed with proteins to form chromatin. During mitotic and meiotic cell division the chromatin becomes visible, usually in the form of filamentous bodies, the chromosomes. Most of the chromosomes in a cell are the autosomes, and the genes localized in them show autosomal inheritance. In addition, eukaryotes with separate sexes have a chromosome or a group of chromosomes called the sex chromosomes (*heterosomes* and *allosomes*) which are represented differently in males and females. Genes localized in these chromosomes show sex-linked inheritance.

Chromosomes are discrete units (linkage groups) carrying a specific linear sequence of genes which in turn carry the genetic information. Chromosomes have two main activities:

(i) Trails formation of genetic information from generation to generation and from cell to cell (during cell division),

(ii) Gene expression or the conversion of genetic information for cellular function and development.

Chromosomes are auto-duplicating structures, which divide longitudinally and are normally distributed equally to the two daughter cells. This ensures that all the cells in the organism have identical genetic complements (in the absence of mutations or recombinations). The number, organization and morphology of chromosomes show species-specific characteristics.

Unlike the circular DNA molecules of prokaryotes, nuclear DNA in eukaryotes consists of linear double-stranded DNA molecules that are packaged with proteins into individual chromosomes. As large as eukaryotic genomes are, it is essential that the DNA be packaged in a highly organized way to prevent it from becoming hopelessly tangled. DNA packaging must also be highly organized in order for the DNA to replicate and its genes to be expressed.

When packaged DNA is at its highest level of condensation, a tremendous amount of DNA is held tightly in a small space. A condensed chromosome has been treated with detergents to disrupt the proteins that hold the DNA in condensed form. Once the proteins are removed, the tightly packed DNA spills out of the chromosome into its surroundings. All of the

fiber like material surrounding the residual protein is double-stranded DNA that was packaged into a chromosome.

DNA packaging follows a hierarchy with several stages of coiling. At each stage of coiling, specific proteins hold the DNA in its coiled state. The DNA and its associated proteins are collectively called chromatin.

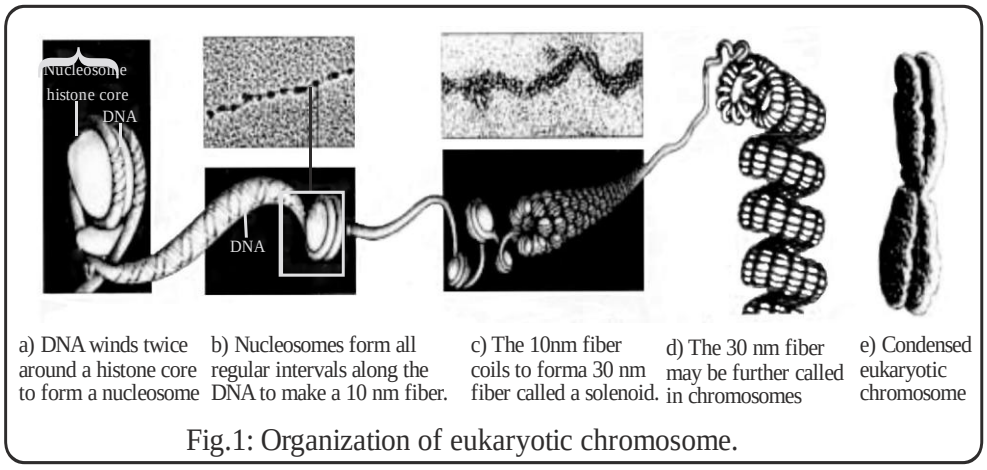


Fig.1: Organization of eukaryotic chromosome.

The hierarchical packaging follows a set pattern, diagrammed in figure 1, first DNA winds around individual cores composed of proteins called histones. The histone cores act as spools that hold the coiled DNA around them. DNA winds around a histone core to form a beadlike structure called a nucleosome. When visualized with electron microscopy, the nucleosomes look like a set of beads along the DNA to make a string. In the cell nucleosomes form at regular intervals along the DNA to make a string that is 10 nm in diameter and is called the 10 nm fiber.

In the next level of packaging the 10 nm fiber coils into a helix called a *solenoid*. Each coil of the solenoid contains six nucleosomes. The solenoid is 30 nm in diameter and is called the 30 nm fiber. Chromatin may be condensed even more, particularly when chromosomes condense in preparation for cell division.

The level of DNA packaging is not constant; it changes over time during different stages of the cell's life. When the cell is not preparing to divide, the chromosomes are decondensed and cannot be distinguished from one another microscopically, even at magnifications possible with an electron microscope. Even so, much of the DNA is still packaged as 30 nm fiber loops connected to a nuclear protein matrix. However, after DNA replicates and the cell prepares for division, the chromosomes condense into compact, easily distinguished masses within the cell. Not only does the level of DNA packaging vary over time, it also varies within each chromosome. Nearly all eukaryotic DNA in the cell nucleus is

condensed to at least the level of 10 nm fibers, and in most parts of the chromosome the DNA is condensed to 30 nm fibers, which may be coiled even further.

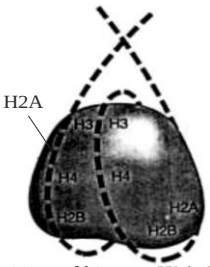
Nucleosomes

A nucleosome forms when 140-180 nucleotide pairs of DNA wind two times around the histone core. The DNA within the nucleosome is called core DNA. Between each nucleosome is a segment of DNA called linker DNA that connects each nucleosome to the next, giving the DNA the *beads-on-a-string* appearance. The number of nucleotide pairs in each segment of linker DNA averages about 50 but may vary from as few as 8 to over 100. Histones contain an unusually large proportion of basic amino acids, which confer a net positive charge to histones. The phosphate groups in DNA are negatively charged and bind to the positively charged histones. There are eight histone molecules in a nucleosome core, two molecules each of histones H2A, H2B, H3, and H4.

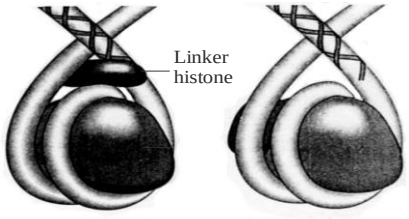
Table:1 Characteristic of some Histone Proteins

Histone	Molecular wt.	Number of Amino acids
H1	17,000-28,000	200-265
H2A	13,900	129-156
H2B	13,800	121-148
H3	15,300	135
H4	11,300	102

These subunits are arranged as illustrated in figure 2, with DNA wound around them. Most nucleosomes contain an additional histone called a linker histone. Linker histones have two binding sites for DNA, where they link the DNA coil in the nucleosome. Linker histones include H1 or H5 or H°.



The positions of histones H2A, H2B, H3 and H4.



a. The original model places the linker histone outside of the nucleosomal DNA

b. A more recent model places the linker histone within the nucleosome surrounded by the core DNA.

Fig.2: Two models of linker histone placement in the nucleosome.

Most models of nucleosome structure predict that a single linker histone resides on the outer surface of the nucleosome, where it links the DNA coil in the nucleosome, as diagrammed in figure 2.

However, the results of some experiments have challenged this model and suggest that the linker histone may reside within the nucleosome, as diagrammed in figure 2. In fact, the two models need not be mutually exclusive. In some instances the linker histone may resides outside of the nucleosome and in others it may resides inside of the nucleosome.

Linker histones play an important role in gene regulation. Experiments in which a mutant H1 gene fails to encode functional H1 show that expression of many genes is then altered. Some genes are transcribed more often in the absence of H1, while others are transcribed less. These results suggest that H1 may have a positive or a negative effect on transcription, depending on the interaction of H1 with other transcription factors.

Some histones are among the most highly conserved proteins in nature, meaning that the histones differ very little in amino acid sequence from one species to another. For example, there is no difference in the amino acid sequence of histones H3 and H4 from several plants and animals, suggesting that these proteins play a vital and identical role among a wide variety of organisms. Linker histones, on the other hand, are much more varied among species, and are even absent in certain species.

DNA Packaging and Regulation of Transcription

In a specialized cell, DNA packaging averts transcription of most genes. RNA polymerase cannot physically access the genes in highly condensed DNA. In order for a eukaryotic gene to be transcribed, its DNA must be decondensed. How far decondensation proceeds depends on how intensively the gene is transcribed! For instance, rRNA genes are often saturated with RNA polymerase-I molecules transcribing the genes. These intensively transcribed rRNA genes, as well as the spacer DNA between them, are free of nucleosomes.

Most genes that encode mRNAs are not transcribed intensively, and the histones remain attached to DNA during transcription, although the 30 nm fiber must be unraveled. RNA polymerase molecules are about the same size as nucleosomes, making RNA polymerases very large to transcribe DNA when it is bound tightly to histones. Nucleosome structure is therefore temporarily altered during transcription making the DNA more accessible to RNA polymerase. Once RNA polymerase has passed the nucleosome resumes its normal conformation.

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