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How DNA is packaged inside the eukaryotic cell

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Abstract
DNA packaging is a critical process that enables the lengthy eukaryotic DNA to fit inside the microscopic confines of the cell nucleus. In humans, each cell contains approximately 2 meters of DNA that must be efficiently condensed to fit within a nucleus of just 5-10 µm in diameter. This is achieved through multiple levels of folding, beginning with the formation of nucleosomes—the fundamental unit of chromatin—composed of DNA wrapped around histone proteins. Subsequent higher-order structures, such as 30 nm chromatin fibers and chromatids, contribute to the further compaction of DNA, ultimately forming chromosomes. This chapter explores the mechanisms of DNA packaging, highlighting the role of supercoiling, histone proteins, and non-histone proteins. Additionally, the models of chromatin fiber organization, including the solenoid and zigzag models, are discussed. The complexity of DNA packaging not only facilitates chromosome formation but also plays an essential role in regulating gene expression, replication, and transcription.

Keywords: DNA packaging, chromatin, protein, chromosome, nucleosome

Introduction
The haploid human genome contains approximately 3 billion base pairs of DNA packaged in 23 pairs of chromosome. Most cells in the body are diploid with 23 base pairs of chromosomes that makes a total of 6 billion base pairs of DNA per cell. Each base pair is around 0.34 nm long, each diploid cell hence contains [$6 \times 10^{9\ bp} \times 0.34$ nm/ bp = 2.04×10^{9} nm] = 2 m per cell. The diameter of nucleus is around 5-10 µm Moreover, it is estimated that the human body contains around 50 trillion cells which means that length of DNA is 100 trillion meters of DNA per human. It is folding of DNA into compact structure so it can get fit into the nucleus. The packaging of DNA is necessary so that it can get accommodated into the tiny nucleus. DNA has to condense itself so that it can get arranged in the cell.

Prerequisite for DNA packaging
Supercoiling
DNA has to supercoil itself for compacting itself. Supercoiling means bending or twisting of DNA upon itself. There are two types of supercoiling (Table 1).

- Positive supercoiling
- Negative supercoiling

Table 1: Types of supercoiling

Positive supercoiling	Negative supercoiling
There is right handed twisting (clockwise) of DNA	There is left handed twisting (anticlockwise) of DNA
Overwinding of DNA takes place	Underwinding of DNA takes place
Twisting in the direction of helical conformation	Twisting against the helical conformation
More strain on the DNA (knot formation)	Less strain on the DNA

Topoisomerase
As there is less strain on DNA in negative supercoiling so all organisms DNA is negatively supercoiled. Positive supercoiling takes place at the time when cellular activities like replication, transcription and translation takes place.

Due to positive supercoiling DNA bears more strain and there is formation of knots in the downstream area. So this knot have to be broken down as DNA polymerase cannot move across the knot. So for this there is a class of enzyme called Topoisomerase which cuts the DNA Strands and relaxes the DNA so now replication can take place (Table 2) (Forterre *et al*., 2007) [4].

Table 2: Types of topoisomerase

Topoisomerase I	Topoisomerase II
It makes single strand cut and rejoins it	It makes double strand cut on both strands
It is present in eukaryotes only	It is present in both prokaryotes and eukaryotes

DNA, Histones and Chromatin

For packaging of DNA there is a requirement of proteins called Histone proteins. It is one of the most important class of proteins. They are evolutionary conserved proteins. DNA is wrapped tightly around histone proteins and make it more compact. The 2 m long DNA condenses to 0.66 m length. This is done by histone proteins. The resulting DNA-protein complex is called chromatin.

Order of DNA packaging

There are various orders of DNA packaging:

1. Nucleosome
2. 30 nm chromatin fibre
3. Chromatid
4. Chromosome

Nucleosome – basic unit of DNA condensation

It is the fundamental repeating unit of chromatin fibre. It is composed of DNA (negatively charged) + Histone octamer (positively charged). DNA is tightly coiled around histone proteins by forming hydrogen bonds (Simpson *et al*., 2018) [9]. There are around formation of 142 hydrogen bonds. DNA is folded around 7 times till 1st order.

Components of Nucleosome (Fig 1)

1. **Histone core:** It contains the histone octamer which is formed by 2 pairs of histone proteins that are H2A, H2B, H3, and H4. These are the core histones that form the octamer.
2. **Nucleosome:** It is comprised of negatively charged DNA wrapped around positively charged histone protein.
3. **Chromatosome:** It is comprised of nucleosome (146 bp) and H1 histone (linker DNA).

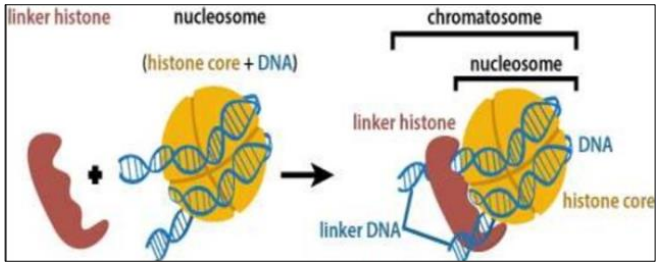

Fig 1: Components of Nucleosome

146 bp of DNA takes around 1 ¾ turns around histone proteins. H1 histone provides additional 20 bp so two complete turns of DNA takes place. The H1 histone act as clamp and attaches to the nucleosome and gives more compactness to DNA. The 146 bp of DNA gets locked now it will not going to move (McGhee and Felsenfeld, 1980) [8]

30nm chromatin fibre

It is the 2nd order of DNA packaging and the semi condensed form of DNA (Fig 2). The DNA during the non-dividing phase that is interphase remains in the form of chromatin fibre. It is formed by the long chain of repeating units of nucleosomes. DNA is folded around 40 times till this level. Chromatin structure beyond this is generally less understood (Luger *et al*, 1997) [7]

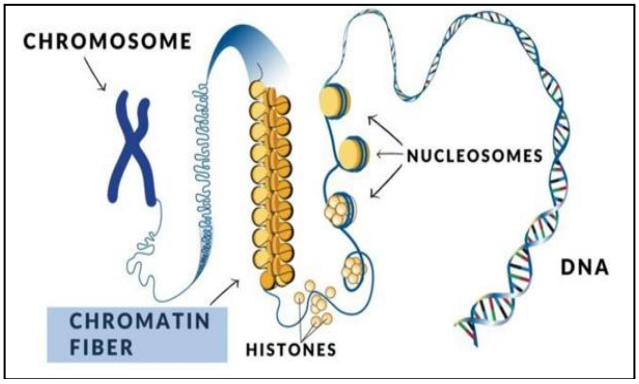

Fig 2: Chromatin Fibre

It is thread like structure formed by the association of DNA and proteins. It was discovered by 'Walther Flemming' in 1878.In chromatin fibre DNA content is around 30-40% and protein content is 50-65%.

Types of chromatin fibre

1. Euchromatin
2. Heterochromatin

Models of Chromatin fibre formation

Folded Fibre Model

This model was proposed by Dupraw in 1966. He published this model on his studies on human chromosomes using electron microscope.

Important features of this model are:

- Each chromosome contains a single but long and coiled chromatin fibre.
- It is more or less uniform in thickness.
- He suggested the thickness of this fibre around 200 to 250 Å.
- The 20 Å thick DNA double helix is packed spirally to form a fibre.
- This fibre is then coiled to form a 10 to 100 Å type A fibre.
- Packing ratio is 6:1.
- Type a fibre is further coiled to form 200 to 250 Å thick type B fibre with folding ratio of 10:1.
- The 200-250 Å type B fibre is further folded to form the chromatid.
- This fibre is supposed to contain DNA histone helix.
- The histone protein bound on outer side of DNA and forms a shell around the DNA.
- Dupraw called this histone as 'histone shell'.
- In the S phase of cell cycle, the chromatid undergo a round of replication to form two sister chromatids.

- The sister chromatids are held by an unreplicated region of the chromatin fibre.

Demerits of Folded fibre model of chromosome

- It is not accepted by scientific community.
- We know that histone are not forming a shell around DNA.
- Histones are imbedded in between the DNA fold.

Nuclease digestion technique (Fig 3)

- Hewish and Burgoyne (1973) have developed this technique.
- They have isolated the enzyme DNA nuclease from rat liver cell and used it to digest DNA.
- They got results that support the idea of DNA packaging.

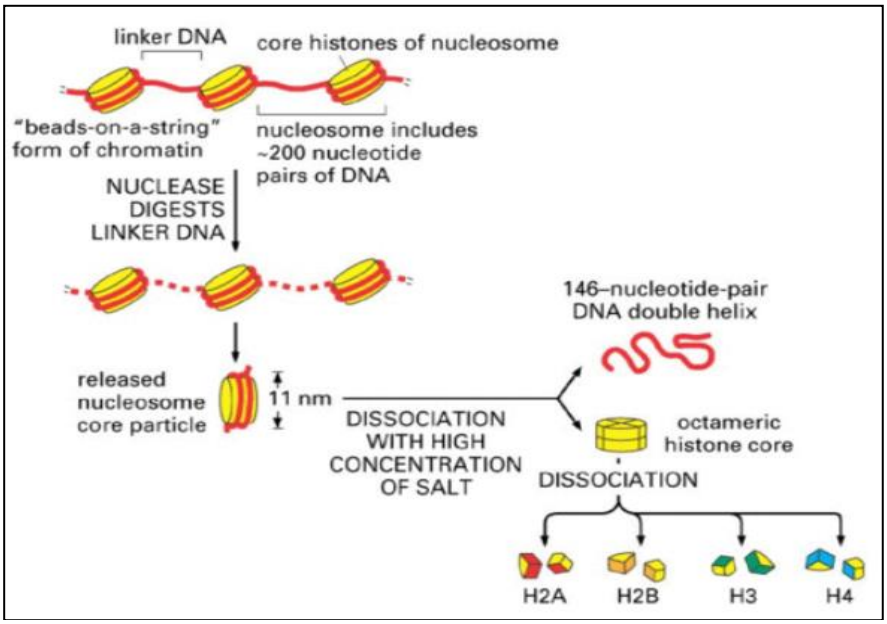

Fig 3: Nuclease Digestion Technique

Nucleosome Solenoid Model

It was proposed by R.D. Kornberg in 1974 [6]. It is the best accepted model for DNA packaging. It says that DNA is tightly bound to histone proteins which serve to form a repeating array of DNA-protein complex called Nucleosomes and further condenses to fit into the small chromosome structure (Kornberg, 1974) [6]

Important Features are:

- Appears as 'Beads on string' on chromatin fibre under electron microscope.
- DNA takes (1¾) turns around Histone octamer.
- Diameter of Nucleosome is 11 nm.
- Adjacent Nucleosome attached via linker DNA.
- Nucleosome coils itself to form solenoid fibre.

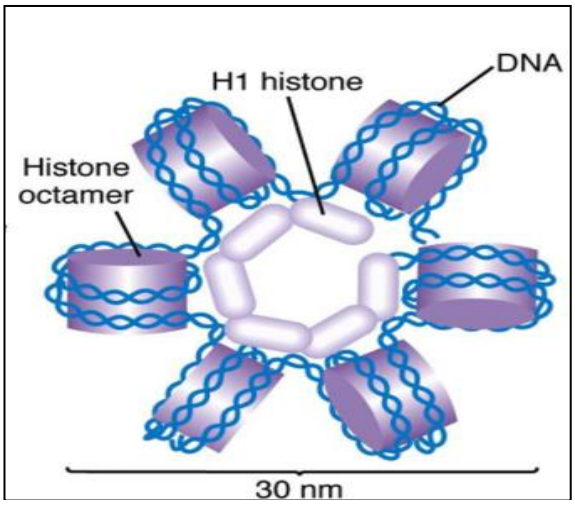

Fig 4: Solenoid Model

Chromatin fibre – nucleosomal arrays arrangement

There are two models proposed for the arrangement of nucleosomal arrays

Solenoid model

In this model there is formation of helical loop in which nucleosomes assemble over one another. There is formation of 6 nucleosomes per turn (Simpson *et al*., 2018) [9]. In this configuration there is consecutive interaction between nucleosomes. The linker DNA remains outside the central channel which is present at the centre. H1 histone arranges itself in the centre (Thoma *et al*., 1979) [4] (Fig 4).

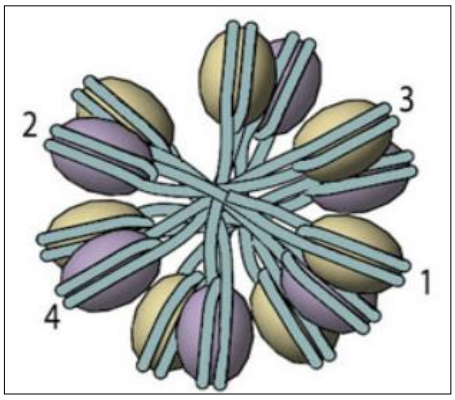

Fig 5: Zig Zag model

Zig zag model: Nucleosomes are arranged in a zig zag ribbon that twists or supercoils. There is alternate interaction among nucleosomes. No central channel is formed in the centre. Linker DNA passes through central pore. The packaging is more condensed (Rydberg, 1998) [9] (Fig 5).

Higher order packaging of DNA: There is a formation of loops of 300nm fibre length. Each loop encompasses some 20,000 to 1,00,000 bp of DNA . 300 nm loops are further packed and folded to produce 250 nm wide fibre. Tight helical coiling of 250 nm fibre produces 700 nm width chromatids during metaphase stage. DNA is in a very condensed at this stage so there is electrostatic repulsion between there is electrostatic repulsion between the adjacent DNA which is counter balanced by polycationic ions like Mn^{+2} and $Mg^{+2.}$ (Fig 6).

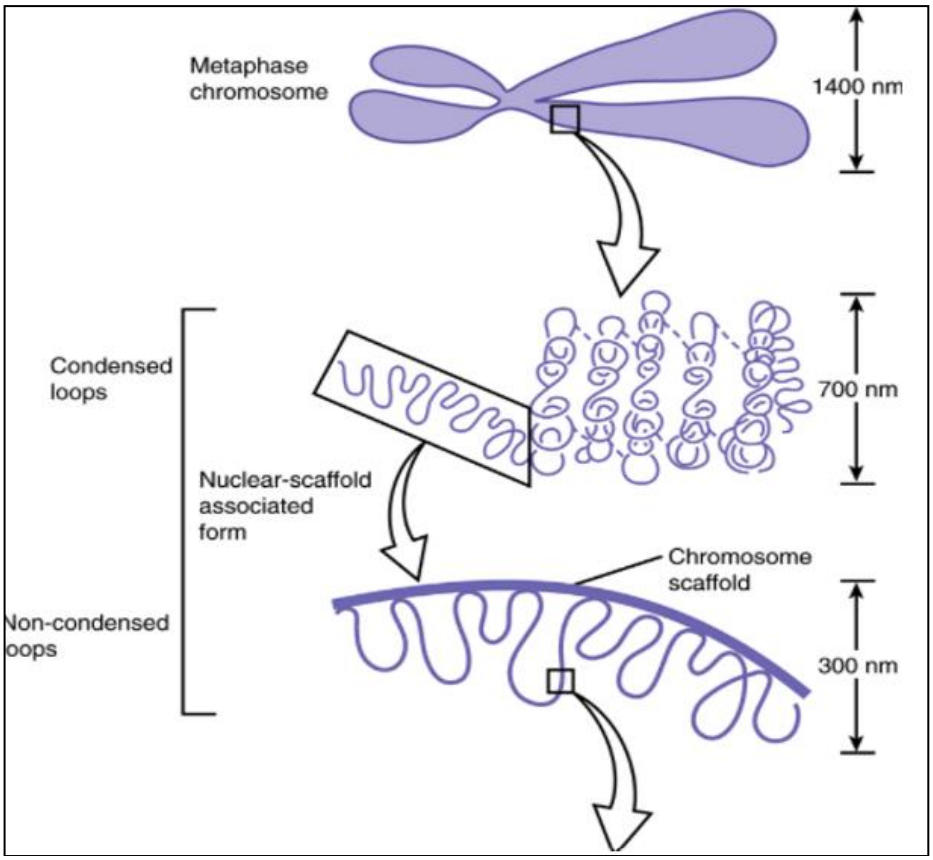

Fig 6: Higher order packaging of DNA

Level of folding	Consists of	Base pair/turn
DNA double helix	Nucleotides	10
Nucleosomes	200 bp each	100
30 nm fibre	6 Nucleosomes /turn	1200
Loops	50 Solenoids/loop	60,000
Mini band	18 loops	1,080,000
Chromatid	1,000,000 minibands	

Histone assembly

Histones are family of basic proteins which associate with DNA in the nucleus condensing it into chromatin fibre. It has positive charge due to presence of Lysine and Arginine amino acid. It helps to package DNA into nucleosome. It contains 80% histone proteins. They are present in the molar ratios of 1:H1, 2:H2, 2:H2B, 2:H3, 2:H4 (Fig 7).

- 3 Domains are present which are alpha helices
- 2 loops (L1 and L2) are there in between
- Histone Octamer formation steps –
- Two alpha helices cross over one another i.e. Domain I and Domain III fold on Domain II
- Dimer formation between (H3 – H4) and (H2A – H2B)
- Octamer formation between (H3-H4) tetramer and (H2A – H2B) heterodimer
- N tails are not the part of the folding domain
- H3 and H4 form dimer through handshaking (head and tail association)
- H2A and H2B form dimer in same manner

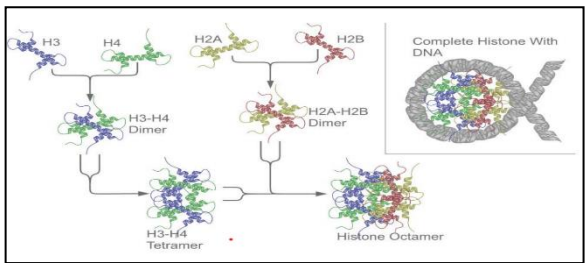

Fig 7: Histone octamer formation

N terminal tail

It stabilizes the 30 nm fibre by interacting with adjacent Nucleosomes. Hydrogen bond formation takes place between n terminal tail and DNA. It undergoes various structural modifications like methylation, acetylation and phosphorylation and regulates the gene expression in this manner (Fig 8).

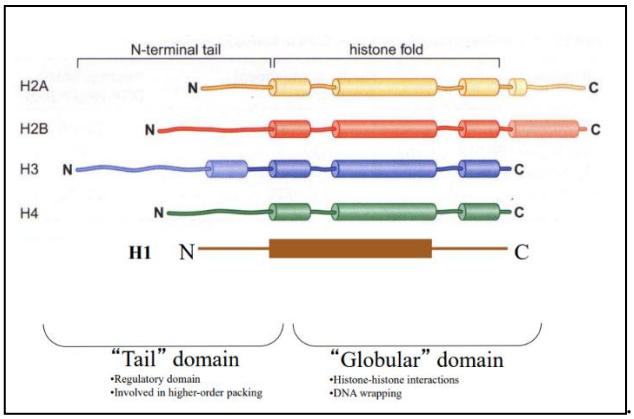

Fig 8: N terminal tail

Non-Histone proteins

The Histones have the role till DNA packaging after that role of non-histone proteins starts. It plays role in functions related to DNA folding, nucleosome remodelling, replication and transcription. The DNA contains 20% amount of non-histone protein. Example of non-histone proteins are scaffolding proteins and cohesin proteins.

Scaffolding proteins

The Scaffold means to provide or support with framework. It plays role in folding and packaging of chromatin fibre (Fogacci *et al*., 2024) [3]

Conclusion

DNA packaging is a highly regulated and intricate process essential for organizing the human genome within the limited space of the cell nucleus. Through interactions with histone and non-histone proteins, DNA forms progressively condensed structures that enable the formation of chromosomes. The nucleosome, consisting of DNA wrapped around histone proteins, serves as the fundamental unit of chromatin, with further levels of organization leading to the formation of chromatin fibers, chromatids, and ultimately chromosomes. The packaging of DNA ensures that genetic material remains accessible for cellular functions such as transcription and replication while maintaining the structural integrity of the genome. Understanding the detailed mechanisms of DNA packaging not only elucidates the complexities of chromosomal organization but also provides insights into epigenetic regulation and cellular function.

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