Paper Code:- 402 Cytology and Molecular Biology

By:- Dr. Shubhi Avasthi (Botany Department)

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DNA Packaging (Unit - III)

* DNA is the only component of chromosome which acts as a heredity material.
* Chromatin is made up of DNA and protein (mainly histone and some non-histone acidic protein)
* The most important property of DNA is super coiling.
* Chromosomal DNA exist in the form of very long molecules, which must be tightly packed to fit into the small confines of a cell.
* So, packaging here refers to the condensing of DNA to fit easily into the nucleus.
* Process of packaging differs in prokaryotes and Eukaryotes
* In prokaryotes, the chromosome are present freely in the cytoplasm hence packaging is not required
* In Eukaryotes DNA is packaged as follows

1) Folded fiber Model
2) Nucleosome Concept
3) Solenoid Model

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Folded fiber Model

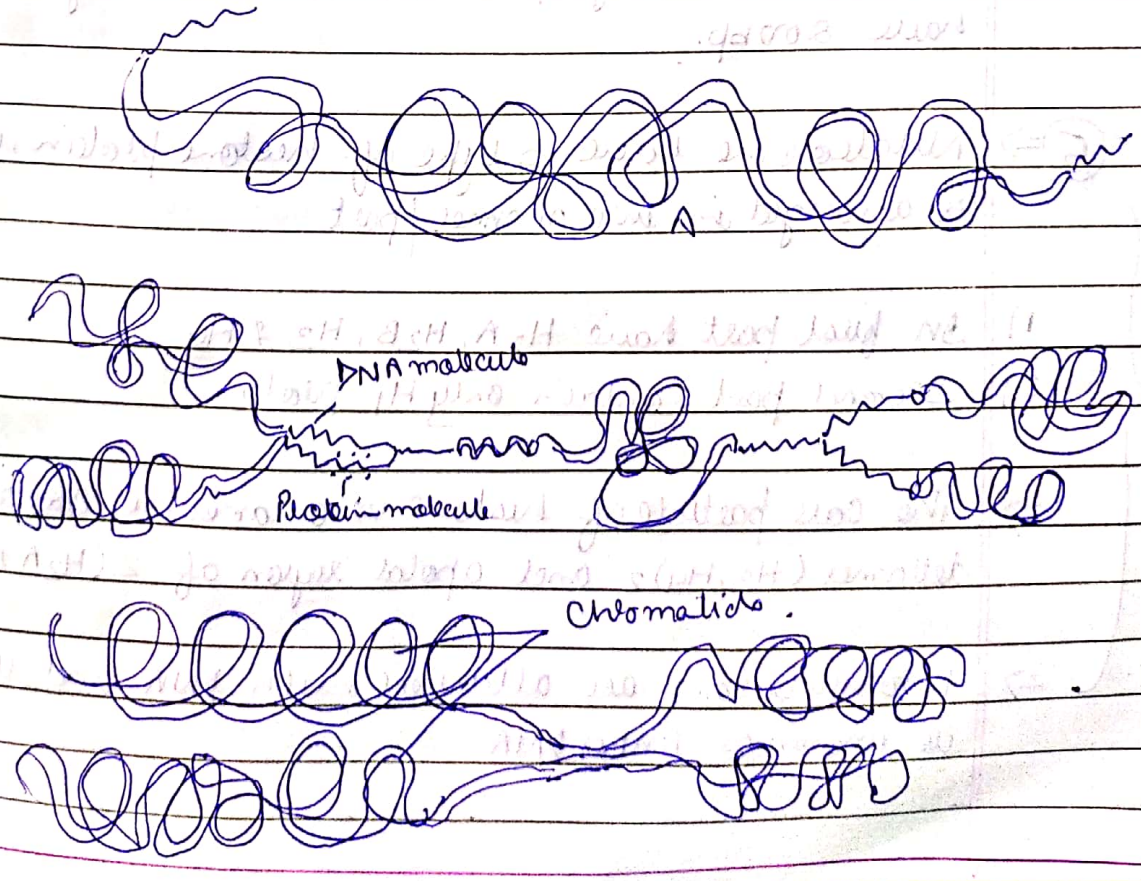
=> A single chromatid has a single long DNA molecule and this is the DNA which should be present in folded manner

=> Folded fiber model was proposed by E.J Dupraw in 1965.

=> In this model, the bulk of chromosome is visualized to be composed of a tightly folded fiber which has a diameter of 200 to 500 Å and folding is both longitudinally & transversely

=> Folded fiber model supposed to contain DNA histone helix (30Å) in a supercoiled condition

=> Folded fiber model proposed that histone need bound on the outside of the DNA (histone shell around DNA).



* DNA is tightly bound to an equal mass of histone, which serve to form a repeating array of DNA-protein particle called Nucleosome.

Nucleosome Concept

=> This model was proposed by R.D Kornberg and Confirmed by P. Oudet et al (1975)

(1) => He stated that DNA alone cannot make a 10 nm repeating unit. ~~This is for the repeating organization~~ Thus, histone is nessessay for the repeating organization of Chromatin.

(3) * Chromosome structure is beaded in appearance, each bead is termed as Nucleosom

(2) => Histone play a crucial role in packing this very long DNA molecule in an orderly way, into nucleus only a few micrometers in diameter.

(4) * Nucleosome is a Complex of DNA and histone protein.

(6) => Each nucleosome is a disc shaped particle with a diameter of about 11 nm and 5.7 to 6 nm in height, on (3) which 67 nm lenght DNA is coiled or alonged, which have 200 bp.

(5) => Nucleosome have 5 type of histone protein, which is alonged in two manner / part

(5)
1) In first part have H_2A, H_2B, H_3 & H_4
2) Second part Contain only H_1 protein

=> The core particle of histone is octamer i.e. Coe of histone tetramer $(H_3, H_4)_2$ and apolar region of 2 (H_2A & H_2B).

=> Two nucleosome are attached with DNA and this DNA is known as linker DNA.

remain associated with the H1 molecule.

7 ⇒ All these evidence indicate that, histone never cover the DNA, while DNA is coiled or bound on the histone core protein.

(8) ⇒ Thus, nucleosome is a fundamental packing unit particles of the chromatin and give chromatin beads on a string.

9 ⇒ The nucleosome beads can be removed from the DNA by digestion with enzyme, that degrade DNA such as bacterial enzyme micrococal nuclease.

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10 ⇒ Electron microscopic studies revealed that core particle is neither completely ~~round~~ spherical / circular nor flat but wedge shaped about 11 nm in diameter and 6 nm in height.

⇒ ~~DNA molecule bound / coiled on the outer portion of the core & made a super coiled structure.~~

⇒ ~~DNA double helix is wound around the core particle. It is wound 1 3/4 times containing.~~ coiled

11 ⇒ DNA Double helix is coiled into a large helix or super helix by making two turns around the histone in the middle part of the particle.

12 ⇒ From the structure of DNA & diameter of core particle, it has been estimated that one superhelical turn would have 160 bp.

13 ⇒ Since, the lenght of DNA in the core is only 146 bp. therefore it makes only $1\frac{3}{4}$ turns. So the core particle is thin on one side accounting for the wedge shaped.

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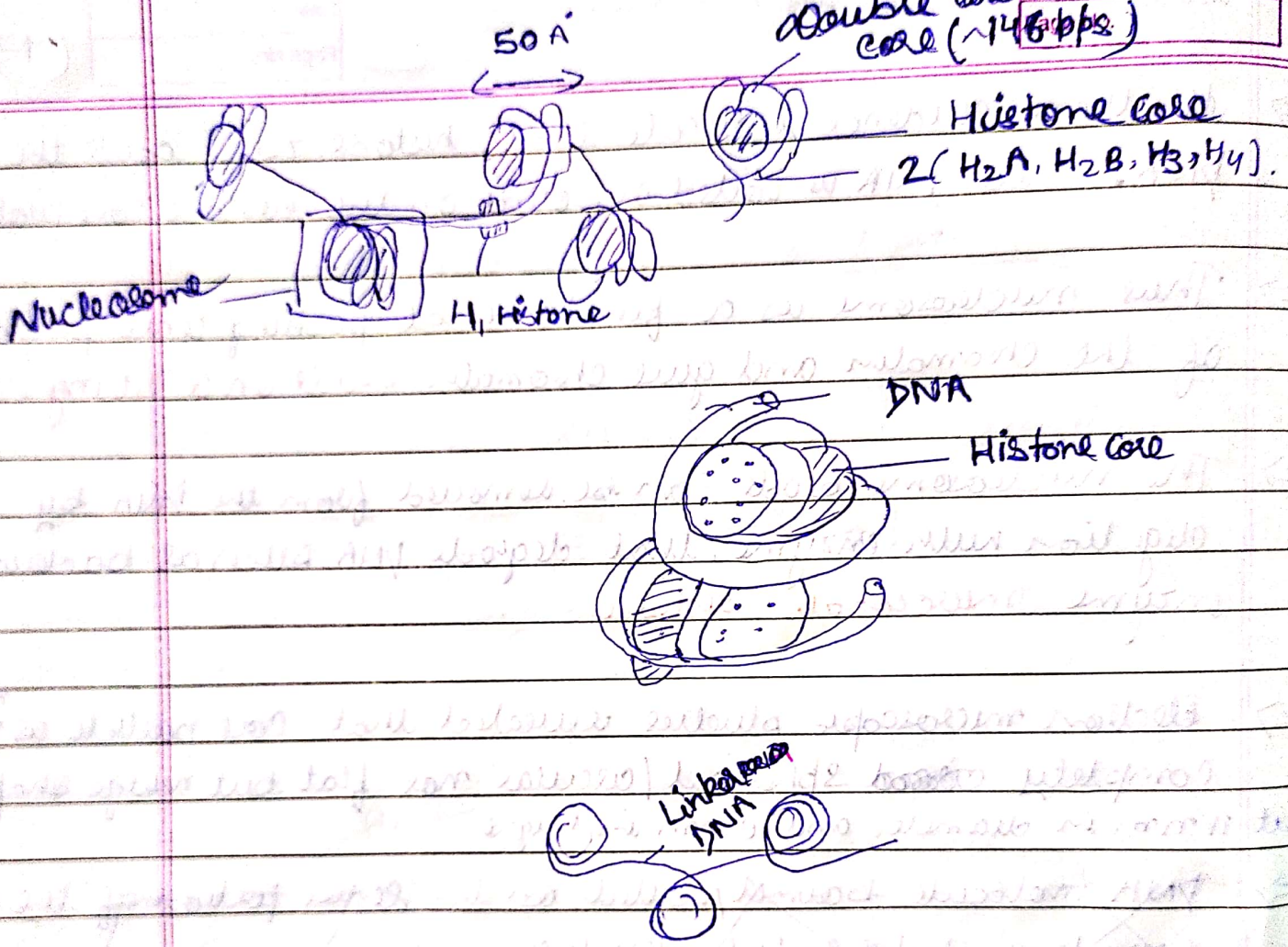

Solenoid Model

=> When the nucleosome is more tightly packed then Solenoid model is formed.

=> Due to Solenoid coiling of ~~chromosome~~ nucleosome containg fiber the following type of chromosomal structure can be observed during cell cycle.

1) The 10 nm fiber :- When nucleosomes are in close apposition they form the 10 nm filament, in which packing of DNA is about 5 to 7 folds i.e. 5-7 times more compact than free DNA.

2) The 30 nm fiber :- When nuclei are very gently lysed onto an electron microscopy grid, most of the chromatin is seen to be in the form of a fibers with a diameter of about 30 nm.

* 30 nm fibers can be observed in metaphase chromosome & in interphase nuclei

* The 30 nm fibers consist of closely packed nucleosome. It probably arise from the folding of nucleosome chain into Solenoid structure having about 6 nucleosome per turn.

* Histone H_1 molecule are found responsible for packing nucleosome into 30 nm fibers

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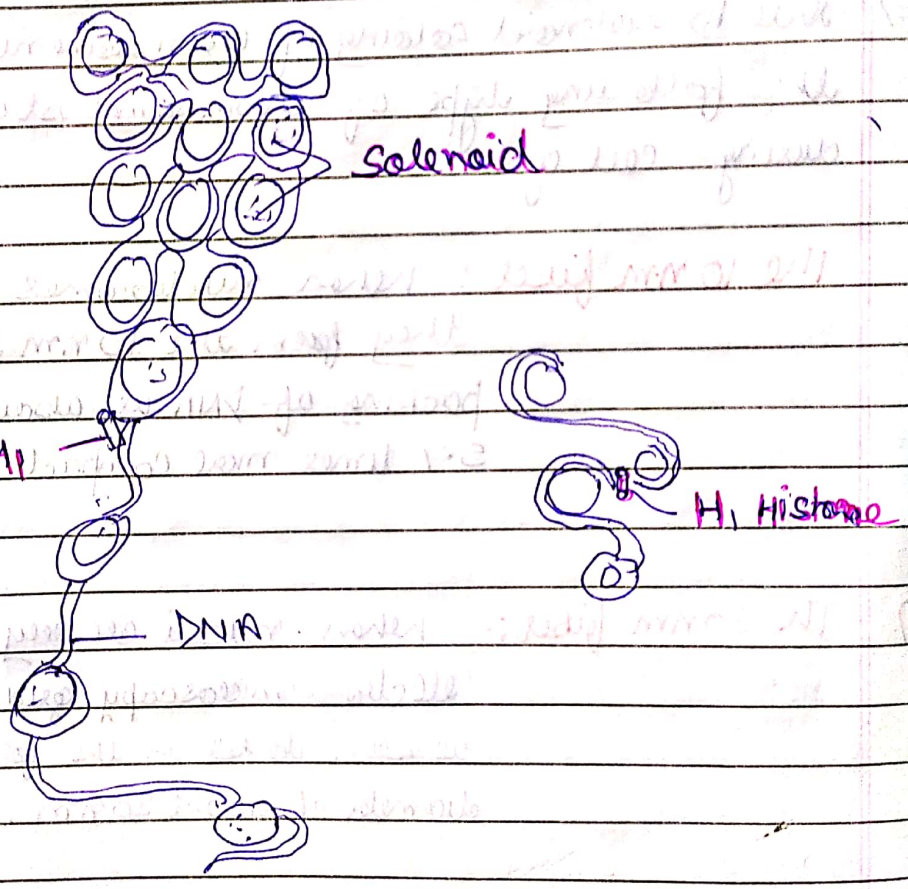
* When H_1 was removed the order folding was absent. H_1 molecules aggregate by crossing - linking to form polymers & may control the formation of solenoid



Loops, Domain & Scaffold :-

+ The 30 nm solenoid fiber is then organized into looped domain structure.

+ Each loop has approximately 50 turns of solenoide. Each loop is is with 85Kb of DNA and a lenght of 10-30 µm.

+ These loops surrounds a central core of scaffold of matrix, made up of non-histone fibrous network of chromosome protein. The scaffold is involved in condensing solenoid fiber into lightly packed metaphase chromosome.

+ The scaffold protein include two abundant protein - the larger DNA topoisomerase II and the smaller matrix protein.

+ Both initiation & continued replication of DNA occurin association with matrix protein & topoisomerase II binding sites all found on matrix associated DNA.

+ The binding sites for topoisomerase II are called Scaffold Attachment regions and Matrix protein bind matrix attachment region

1) The first level of condensation involves packaking DNA as a supercoil into nucleosome. This produce 10 nm diameter interphase fiber

2 Second level involves an additional folding and or supercoiling of 10 nm nucleosome to produce the 30 nm solenoid

3. Finally solenoid fiber of 30 nm is organised into loops around a central scaffold to make the lightly packed metaphase chromosome

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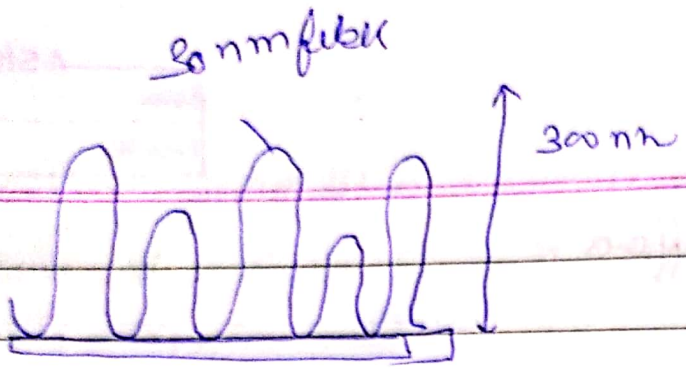

Organisation of 30 nm fiber into chromosomal loop.

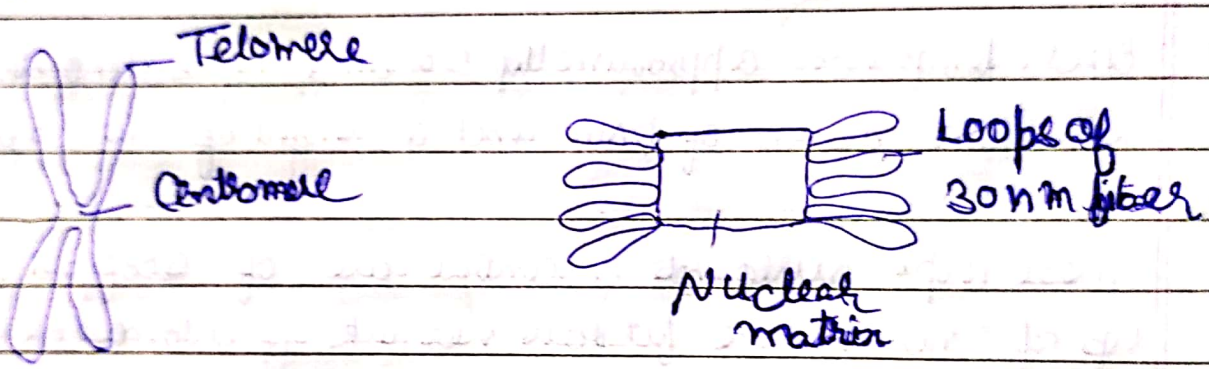

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Diagram of some of many orders of chromatin packing ~~which may give highly condensed metaphase chromosome~~