# Centromeric DNA

The centromere generally appears as primary constriction of mitotic chromosomes, first described by Walther Flemming in 1882.

## Centromeres are –

- heterochromatic components
- relatively gene poor
- play a vital role in kinetochore assembly
- dense, with various types of repeats like – SAT DNA, transposable elements etc.

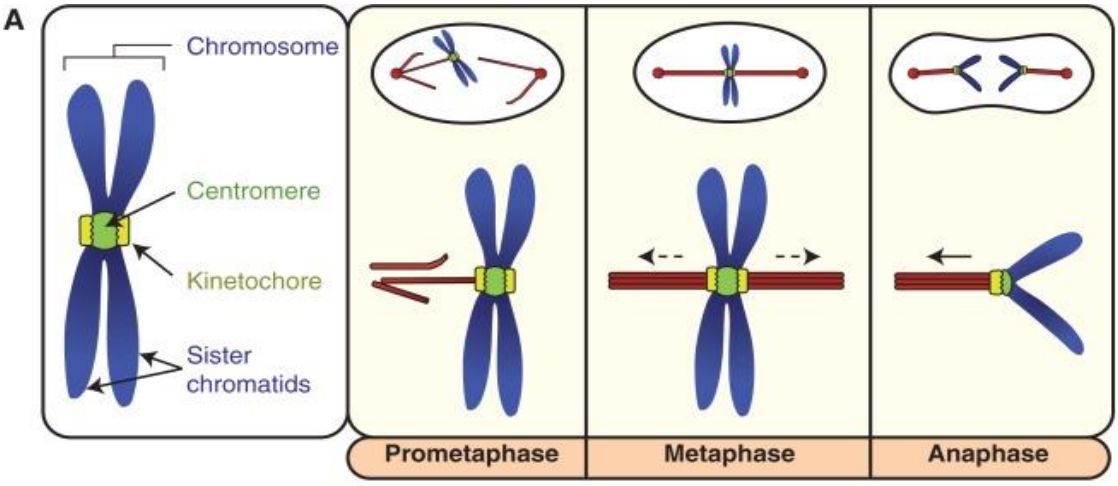



**Fig: Centromere and kinetochore**

### Structural pattern -

DNA at the centromere is often

- has no defined sequences, characterized by tandemly repeated sequence, repeats are orderly arranged, species specific and sometimes chromosome specific
- transposable elements are often found (plants, humans)
- has a **centromere core** that binds with a specialized protein – **CENP-A** a histone variant. Its nucleosome structure is altered due to a variant H3 protein which in turn, facilitates heterochromatinisation of this particular part of chromosome. These regions are involved in the formation of three dimensional structures that link to kinetochores and serve as capture devices for the mitotic microtubules.

Besides, CENP-A centromere function also requires a set of various other proteins that form the **constitutive centromere-associated network (CCAN).** CCAN components CENP-T, CENP-

W, CENP-S, and CENPX have been identified independently and are mostly involved in making supercoiled DNA of that part.
This core centromere region is generally flanked by pericentric heterochromatin, characterized by nucleosomes containing H3 methylated on lysine 9 (H3K9me) that are bound by heterochromatin proteins. During mitosis, these two domains together form a three-dimensional structure that exposes CENP-A-containing chromatin to the surface for interaction with the kinetochore and microtubules.

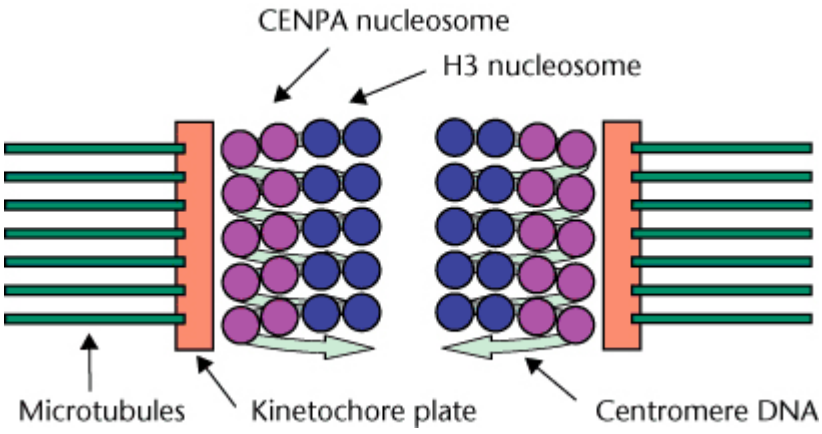



**Fig: Organization of centromeric DNA with CENP-A proteins**

## Study of centromere in different organism –

### A. In Budding yeast-

- Centromere for all its 16 chromosomes is about 125 bp in length and has 3 parts – I.II and III.
- CEN I and III are short, highly conserved, 8-26 bp in length.
- CEN III showed diad symmetry, most importently involved in microtubule attachment
- CEN II is much larger, about80-85 bp, AT rich and not conserved structure among chromosomes. Central region is base for kinetochore assembly while flanking regions are for sister chromatid attachment.

### B. In Fission Yeast –

- Several kb in length
- Has a central core flanked by inverted repeats
- Several tandem repeats are present flanking the inverted repeats.

### C. In Drosophila –

- Centromere contains highly repeated sequences of DNA with islands of unique sequences.
- Mostly AT rich repeats along with transposable elements

### D. In Human –

- Centromeric region ranges from 0.3-5 Mb in size
- Contain repeats called α- Sat DNA, 171 bp in length and repeated about 18,000 times (average).
- This171 bp α- Sat DNA repeats are the binding sites for altered H3 the CEN-A protein and are called CEN box.

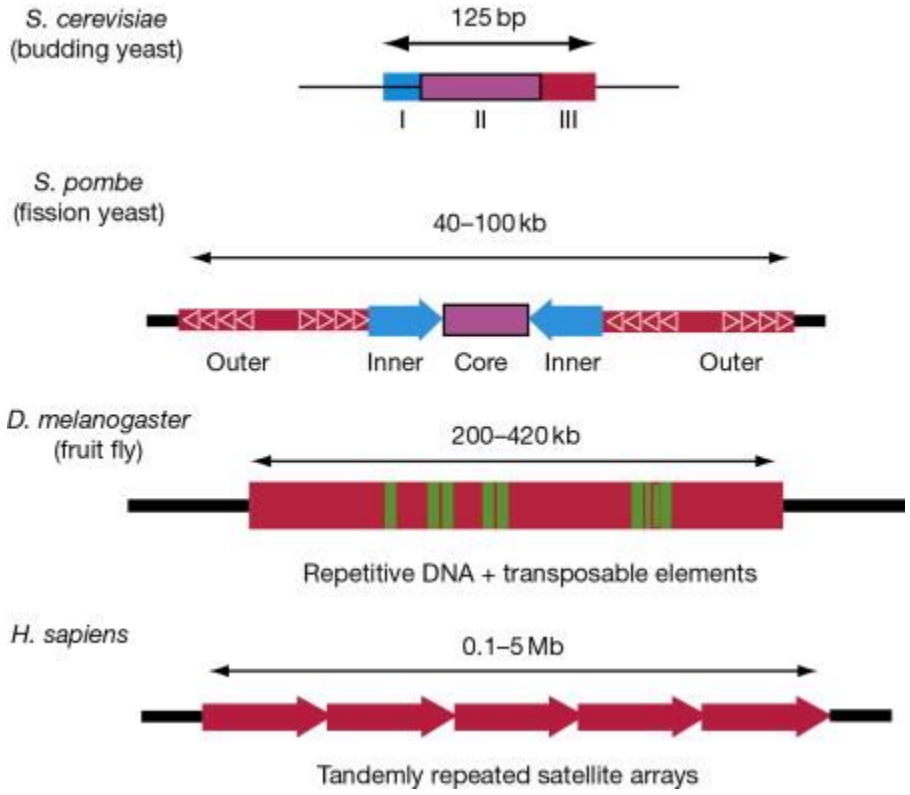



**Figure: Centromeric structure in various eukaryotes.**

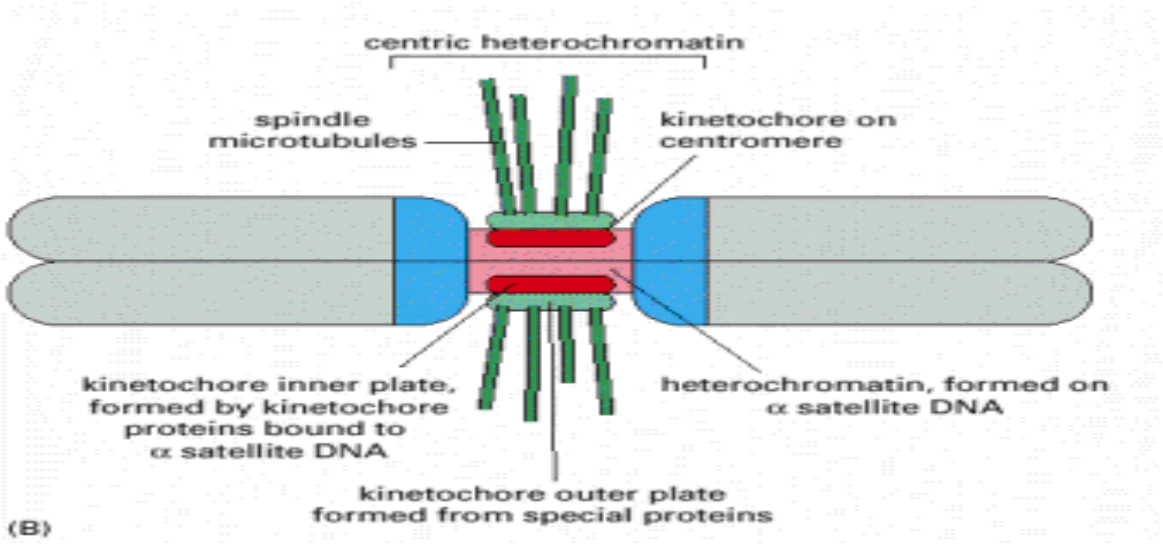
**Budding yeast (*S. cerevisiae*)** centromeres are 125 bp and are composed of three distinct elements, two of which are conserved (I and III).
***S. pombe* (fission yeast)** centromeres contain a unique central core flanked by inverted inner and outer repeats.
***Drosophila*** centromeres extend for 200–420 kb and contain repetitive DNA (red boxes) that is interspersed with transposable elements (green lines).
**Human** centromeres consist of tandemly repeated alpha-satellite DNA arranged into higher-order repeats that extend over several megabases.

## Functions –

- The centromere is essential for the equal segregation of chromosomes during mitosis and meiosis.
- It serves as attachment site for microtubules via kinetochore to mediate chromosome segregation.
- Specialized structural features of the CENP-A nucleosomes allow assembly of the kinetochore proteins on centromere which in turn help in micro-tubular attachment.

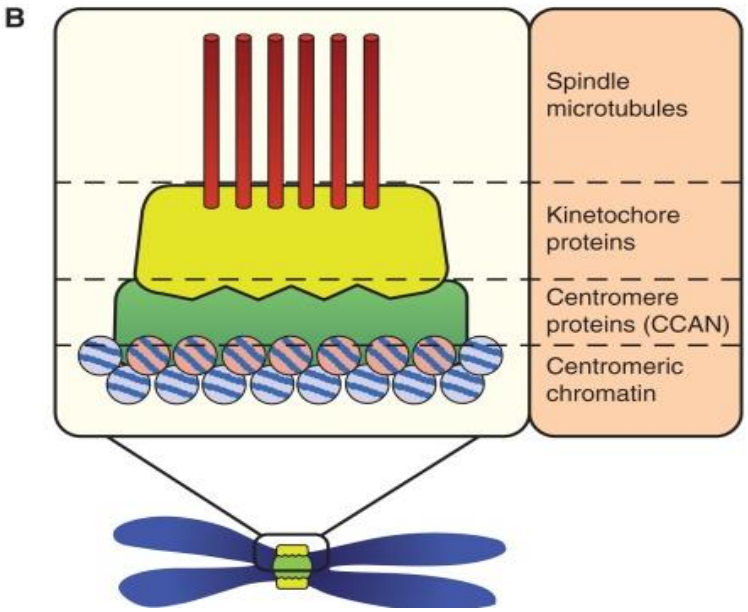
## Kinetochore DNA

It is a complex structure that specifies the attachment between chromosome and microtubules of spindle and aids chromosome segregation. The centromere is the chromosomal locus where a kinetochore is built.




**Structure** –

consists of 2 ‘’cups’’ on either side of the centromere and appears to have a trilaminar morphology in cross section, with an electron-dense outer plate, lightly staining middle layer, and a darker inner area immediately adjacent to the underlying centromeric heterochromatin. Spindle microtubules are specifically attached to the outer plate



## Fig: Trilamilar structure of kinetochore

3 layers are –

- **Inner layer-** matrix like, immediately adjacent to the centromeric heterochromatin, 40-60 nm in thickness, contain CEN-A, maintains throughout the cell cycle.

In vertebrates, it is electron dense plate of proteins anchors the kinetochore on centromeric heterochromatin and recruits all other kinetochore components.

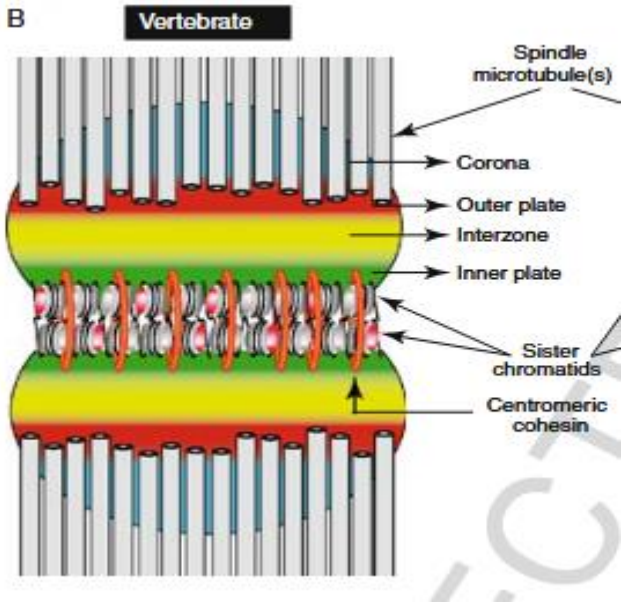
- **Middle electron lucent layer –** 25-30 nm thick, protein components are not fully studied.

In vertebrates, it is an electron-lucent interzone and act in tension sensing and spindle checkpoint signaling.

- **Outer layer –** 30-40 nm thick, electron opaque, plate like. Contains majority of microtubule interacting proteins, CENP-F, cell cycle signaling proteins etc. This structure assembled on chromosome after nuclear membrane dissolution.

In vertebrates, an electron-dense outer plate harbors components that make end-on contact with up to 20–30 micro-tubules. It consists of microtubule-associated proteins (MAPs), kinesins and structural units involved in the regulation of spindle dynamics and checkpoint signaling.

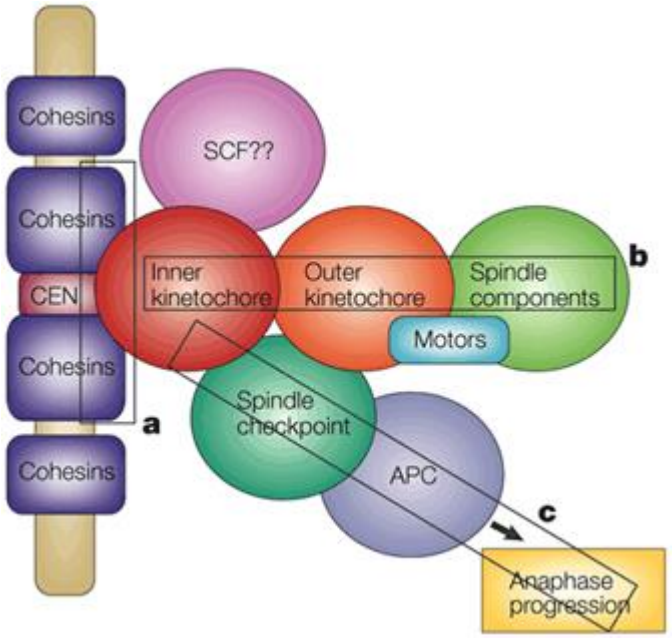
Besides, a fibrillar network called **fibrous corona** is also present when microtubules are not attached. It radiates about100 nm. This part contains various motor proteins. As the kinetochore plate attaches to microtubule ends, corona becomes less distinct and plate diameter decreases.
The corona contains resident and transient kinetochore components that are involved in recruiting spindle checkpoint proteins, establishing kinetochore–microtubule attachment, and regulating microtubule dynamics and chromosome segregation.



## Three layers of kinetochore (vertebrates)

The kinetochore is composed of more than 100 different proteins with multiple copies in vertebrate cells, (about 7o different types in Yeast) and is grouped into three main categories:

- Inner kinetochore proteins that are required to form the connection with the centromere DNA and provide a platform to assemble the kinetochore,
- Outer kinetochore proteins that form connections with microtubules, and
- Regulatory proteins that monitor or control the activities of the kinetochore.




## Kinetochore (KT) Proteins

### Kinetochore becomes assemble in prophase and destroy after mitosis/meiosis

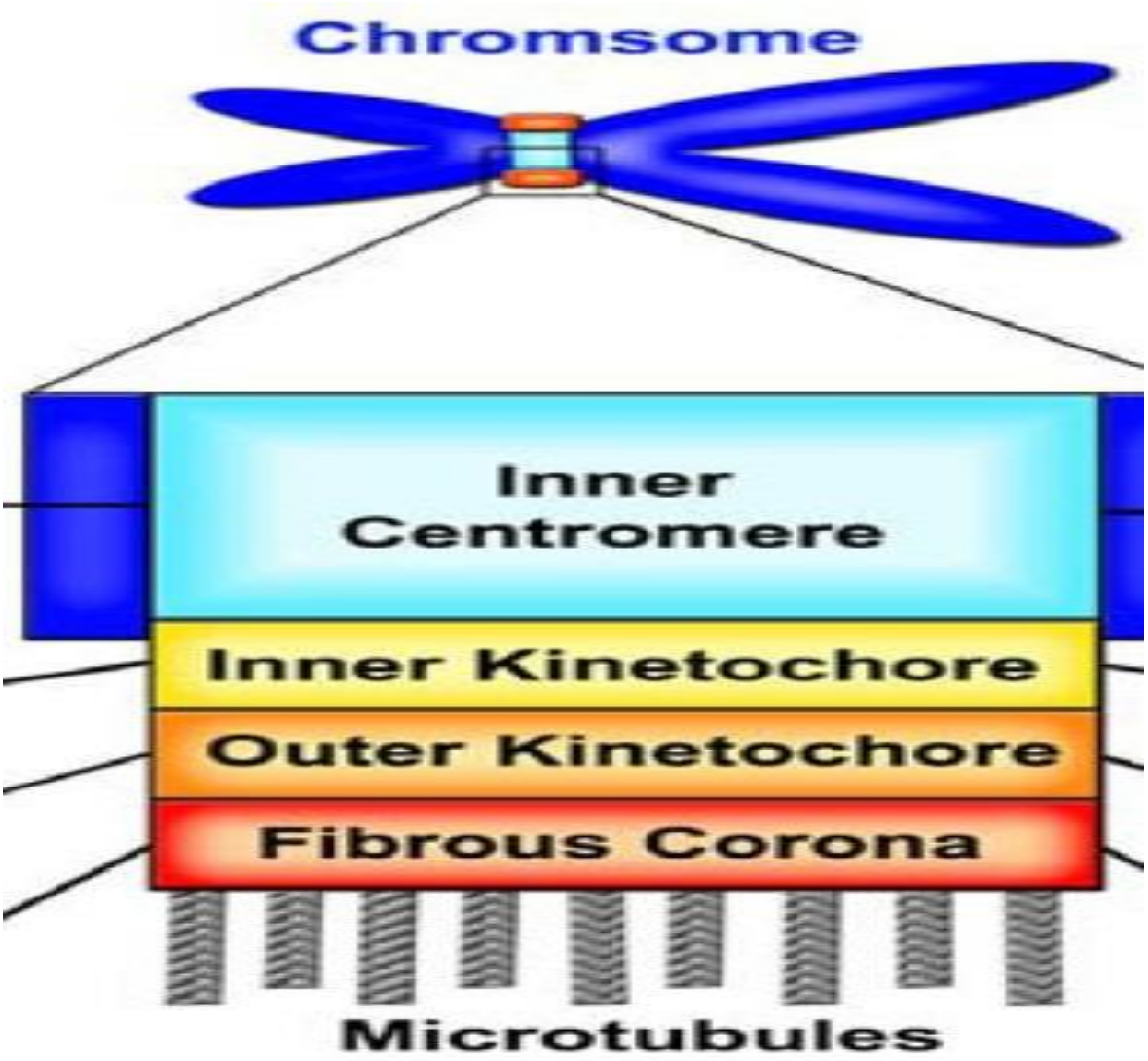
**Kinetochore protein assembly –**

Studies with **HeLa cells** showed that assembly of KT proteins occurs in a temporal order. Some proteins appear at late G2 and prophase while others after nuclear membrane breakdown. Most of these proteins are DNA binding proteins or microtubule binding proteins. Eg. –CENP-A; Motorproteins- dynein, kinesin; cell cycle signaling proteins.

## The primary functions of the kinetochore:

- Anchoring of the microtubules to the centromeres,
- Subsequent force and tension generation for the accurate segregation of the sister chromatids during cell division, and

- Activation of the spindle assembly checkpoint (SAC) to detect improper kinetochore attachments preventing cell cycle defects leading to aneuploidy.




**For 2nd Sem Hons. (CC-4)**

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