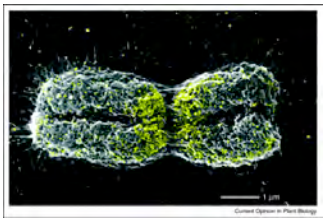
DNA packaging summary

1. Problem is packaging
2. Levels of chromatin structure (nucleosomes, 30-nm fiber, loops, bands)
3. Histone code marks active and inactive sequences
4. DNA elements for chromosome structure include (ARS), TEL and CEN.
5. CEN promotes the assembly of the kinetochore, a giant protein complex that attaches the chromosome to the spindle at division.



High resolution scanning immunogold electron micrograph.
Yellow = phosphorylated H3 in the pericentromeric region.

A severe problem of packaging

1. Largest human chromosome: ~3 x 10^8 bp

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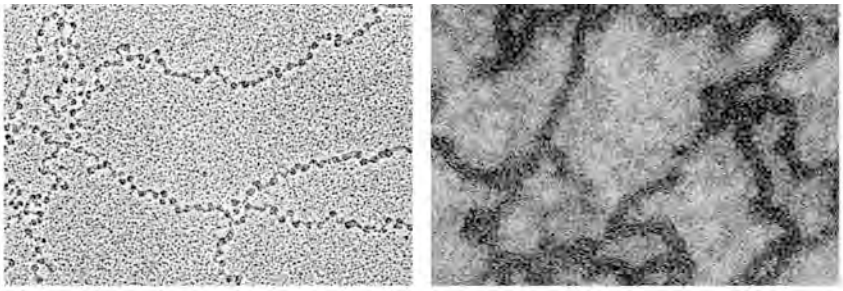
2. A typical cell = 10 μm = 10×10^{-6} m

3. Therefore the DNA must be compacted ~10^4-fold

   This is like fitting an 11-mile-long string into a 6-foot box

Visualizing chromatin breakdown products

Electron micrographs of "chromatin" preparations



1. Beads-on-a-string (low salt) 2. 30-nm fiber (0.15 M KCl)

Beads contained "histones": H1, H2A, H2B, H3, and H4

Packaging proteins discovered by DNase I digests of nuclei

1. Isolate nuclei
2. Partial digest with DNase I (nonspecific endonuclease)
3. Run agarose gel.
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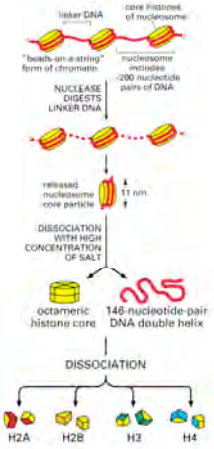

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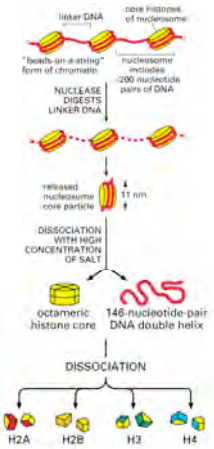
5. Treat with more DNase I.
6. Run acrylamide gel.
7. Result: Ladder of multiples of ~10 bp.



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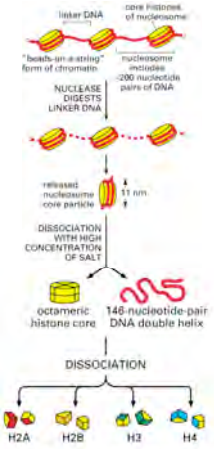
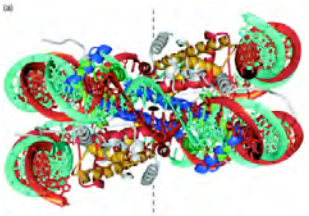
Conclusion: DNA is wrapped around the outside of the nucleosome.



Crystal structure of the nucleosome

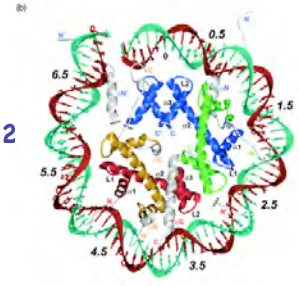
Ribbon drawings

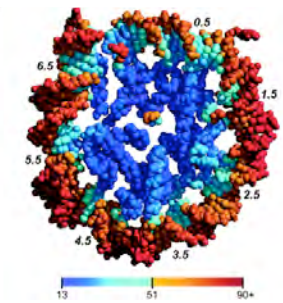
"Top" view



147 bp of DNA wrapped almost twice around 8 core histones: $(H2A, H2B, H3, H4)_2$

"Side" view of 1/2 nucleosome

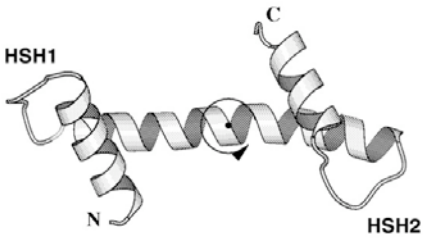




Space-filling drawing

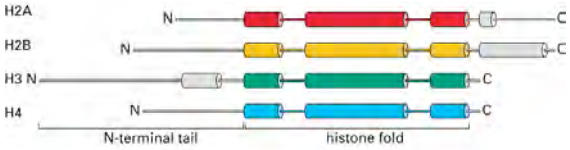
Carolyn Luger and Tim Richmond

The histone fold



Ribbon drawing

Simple.
Conserved.
Adopted by all 4 "core" histones (H2A, H2B, H3 and H4).



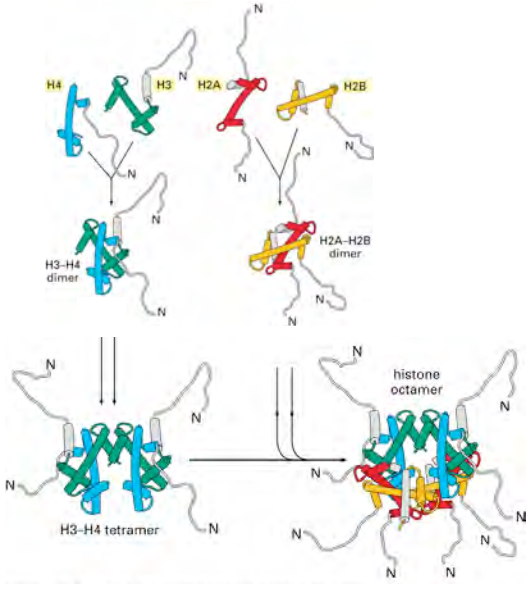
Sequence schematic

H3-H4 tetramer binds two H2A-H2B dimers to form the histone octamer

Monomers

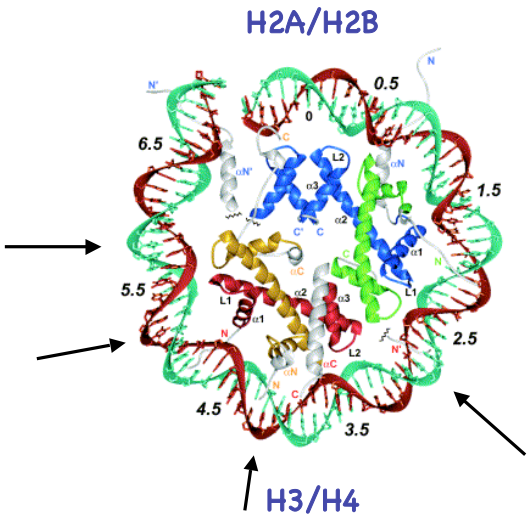
Dimers

Tetramer



Octamer

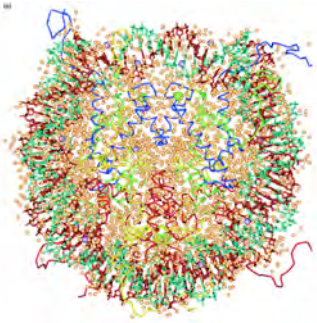
The DNA is not smoothly bent

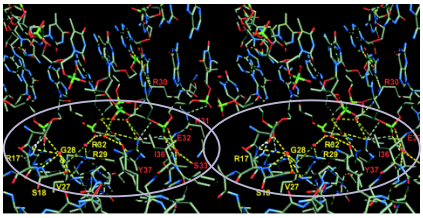


Ribbon drawing of a single gyre

Water mediates many histone:PO_4 contacts

Dots = Visible waters

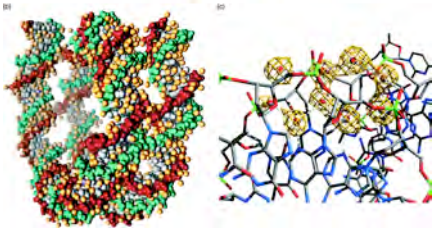




DNA

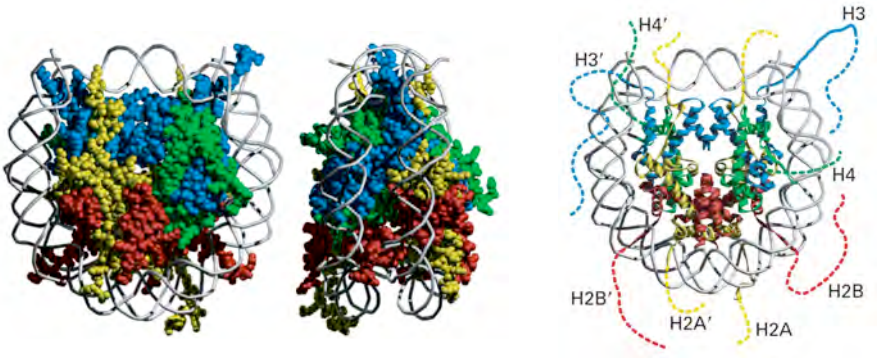
Protein

"Stick" drawing



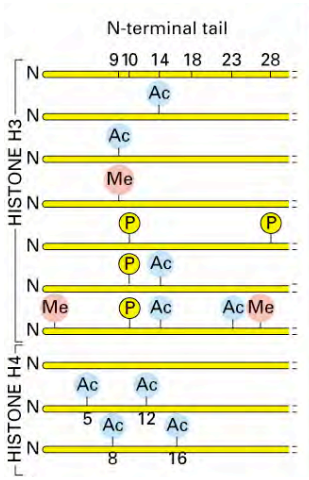
Waters on the DNA

Histone "tails" stick out between the gyres of DNA



Post-translational chemical modification of the tails controls function.
Modification patterns comprise the "histone code".

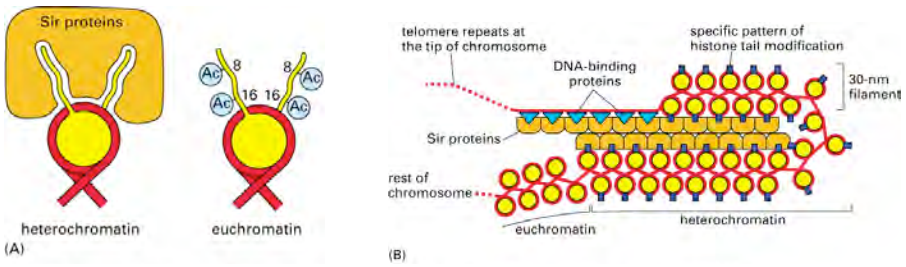
Examples of the "histone code" for H3/H4



modification state	"meaning"
unmodified	gene silencing?
acetylated	gene expression
acetylated	histone deposition
methylated	gene silencing/ heterochromatin
phosphorylated	mitosis/meiosis
phosphorylated/ acetylated	gene expression
higher-order combinations	?
unmodified	gene silencing?
acetylated	histone deposition
acetylated	gene expression

Ac - acetyl (lysine), Me - methyl (lysine), P - phosphoryl (Ser or Thr)

Hypo-acetylated histones in heterochromatin



Sir proteins (Sir 1, 3, 4) bind cooperatively to de-acetylated chromatin, tightly packaging the 30-nm filament.
Sir2 is a histone deacetylase.
The combined actions of the packaging proteins and the deacetylase spread heterochromatin (and silence packaged genes).

Replication distributes nucleosomes to the daughters

Experiment: replicate mixed chromatin and nucleosome-free templates *in vitro*

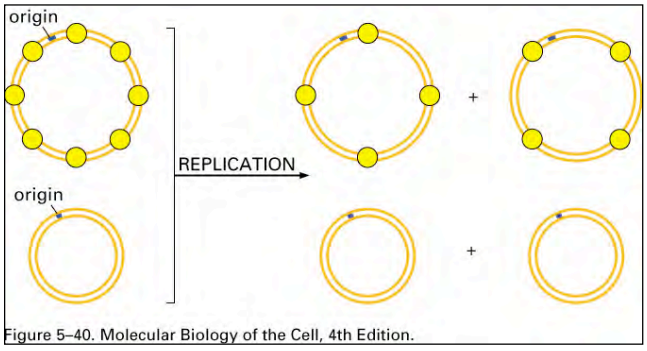
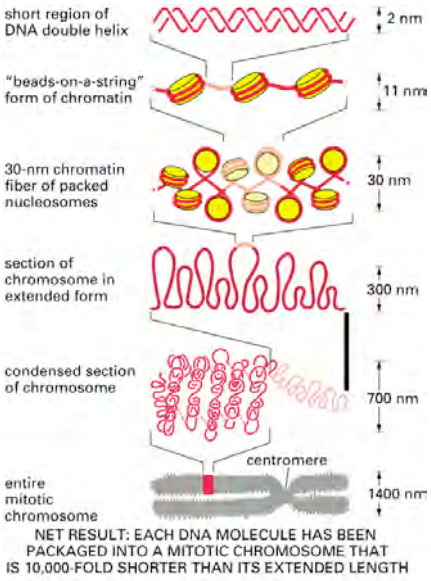


Figure 5–40. Molecular Biology of the Cell, 4th Edition.

Results:
Replisome copied through nucleosomes!
Nucleosomes were found only on the daughters of the chromatin template.
No nucleosomes moved to the daughters of the nucleosome-free template.

Levels of chromatin structure



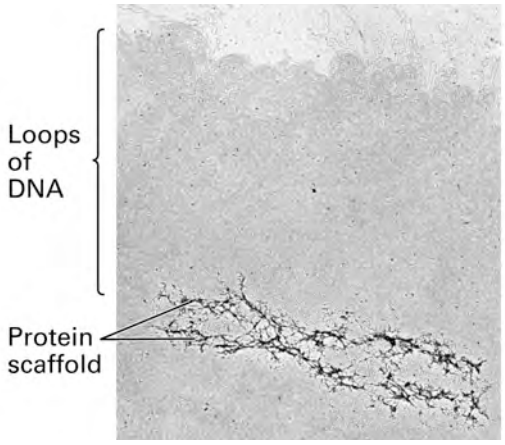
Larger DNA structures mediate more compaction.

Euchromatin: transcribed and less condensed

"Loops" of 30-nm fibers seen at interphase

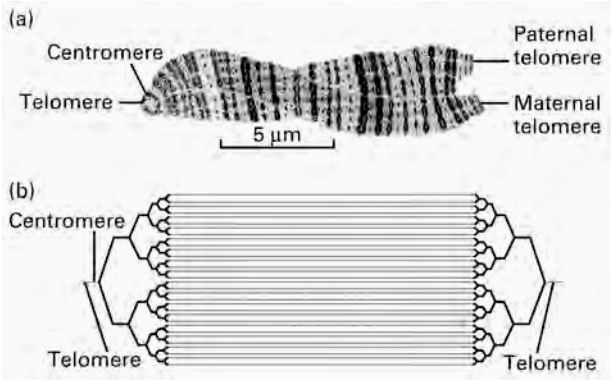
Heterochromatin: more condensed, genes silenced, replicated later in S phase.

Loops and scaffold

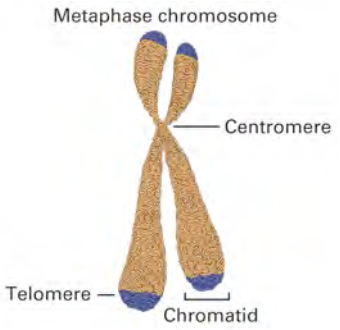


Electron micrograph of "histone-depleted" chromosome

DNA amplification produces visible "bands" in staining pattern of fly "polytene" chromosomes



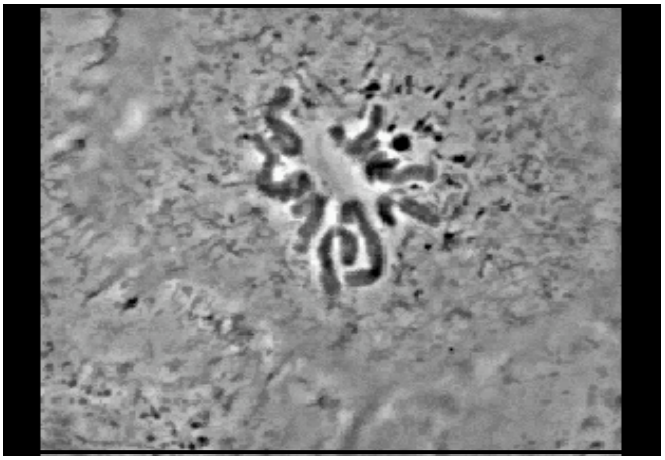
Functional DNA sites -- Telomere & Centromere



Schematic and electron micrograph of X chromosome.

- Telomeres protect the ends.
- Centromere is at the primary constriction. It mediates chromosome cohesion, spindle attachment and chromosome segregation.

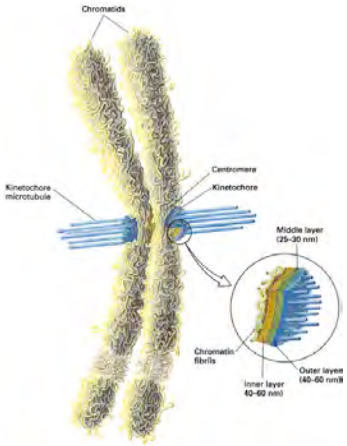
Eukaryotic Cell Division



Ted Salmon

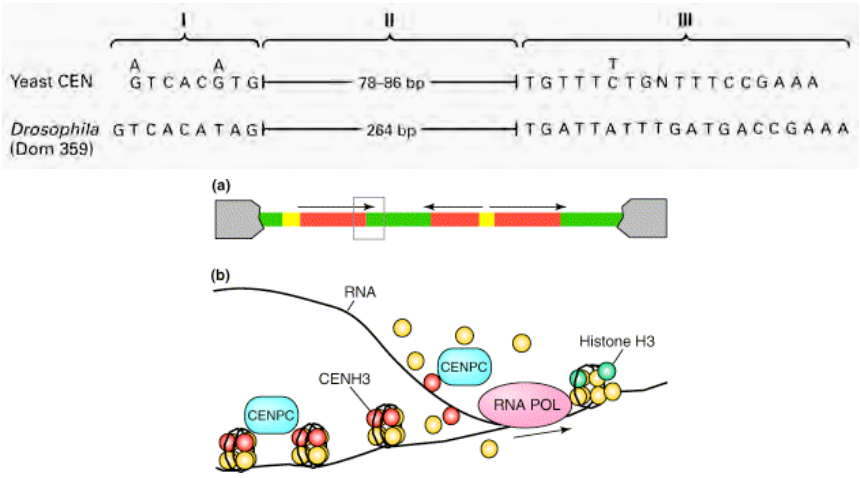
Kinetochores mediate chromosome-microtubule attachments

Kinetochore mediates attachment to the spindle



Schematic drawing.
Centromere (DNA segment) is at the primary constriction. The kinetochore is a huge, complicated protein complex with several layers. The outer layer provides attachment sites for microtubules.

Centromeres contain special DNA sequences that assemble kinetochores

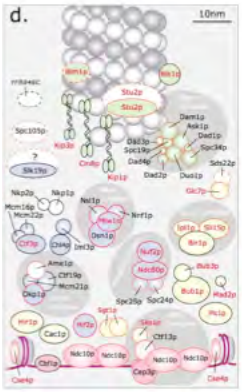


Does transcription promote replacement of centromeric histone H3?

A model for the yeast kinetochore

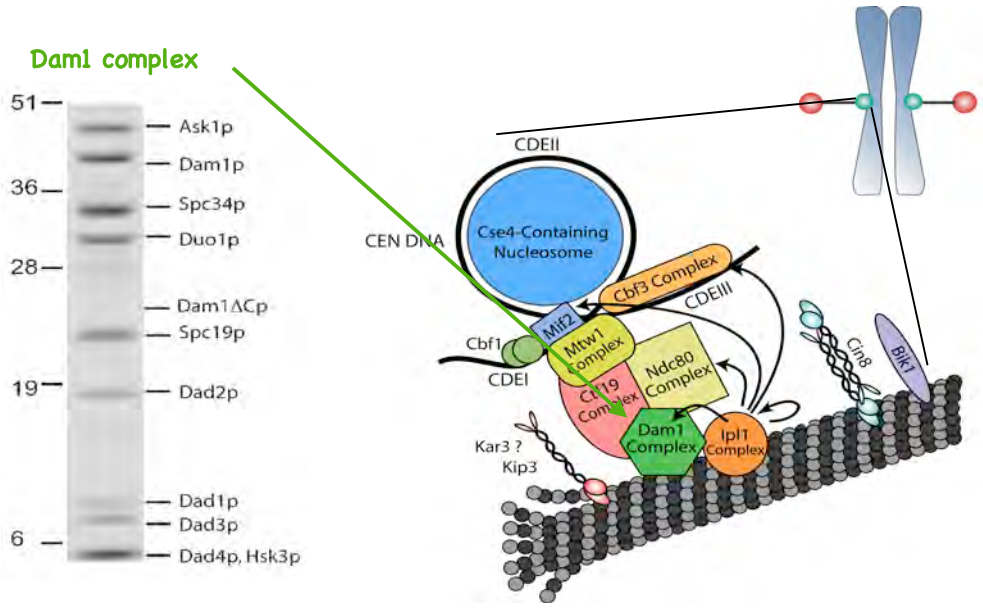
Microtubule

DNA



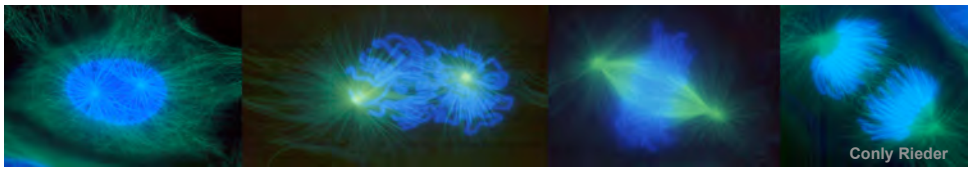
Yeast kinetochore assembly and architecture. Speculative model for the organization of known components. Protein complexes are drawn approximately to scale.

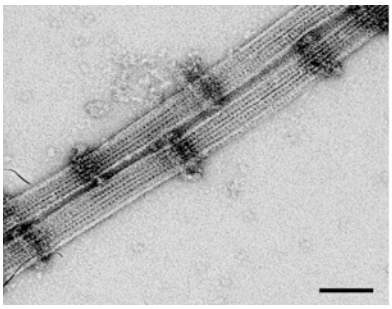
Kinetochore complexity & structural challenge

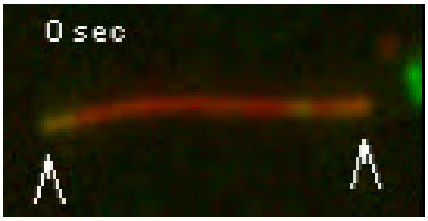


The Kinetochore Puzzle

The Yeast Complex Dam1 Solution

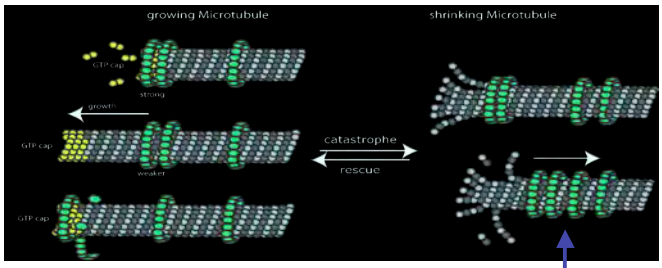






Westermann et al. (2005) Mol. Cell
Westermann et al. (2006) Nature

Dam1: Mechanism and structure



Model: Dam1 rings slide along shrinking microtubules!

YACS = yeast artificial chromosomes

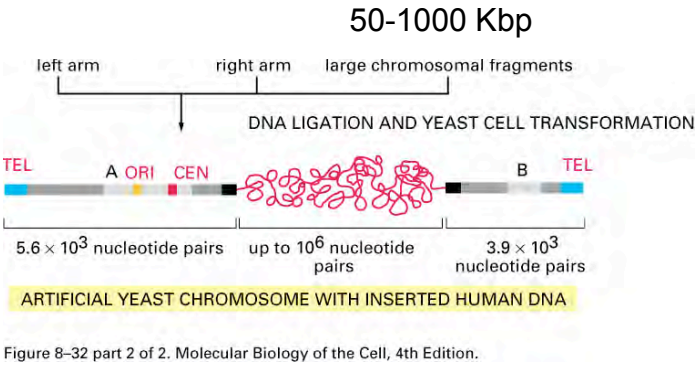


Figure 8–32 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Needed to sequence the human genome

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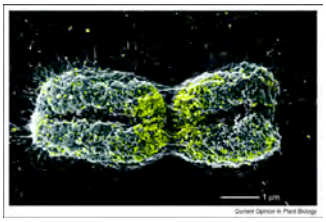
5. CEN promotes the assembly of the kinetochore, a giant protein complex that attaches the chromosome to the spindle at division. YACs = CEN, TEL, ARS + >50 Kb.



High resolution scanning immunogold electron micrograph.
Yellow = phosphorylated H3 in the pericentromeric region.