Chromatin Structure

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ICPH E250P

DNA Packaging Is a Formidable Challenge

- Single DNA molecule in human chromosome ca. 5 cm long
- Diploid genome contains ca. 2 meters of DNA
- Nucleus of human cell ca. 5 μm in diameter
- Human metaphase chromosome ca. 2.5 μm in length
- 10,000 to 20,000 packaging ratio required

Overview of DNA Packaging

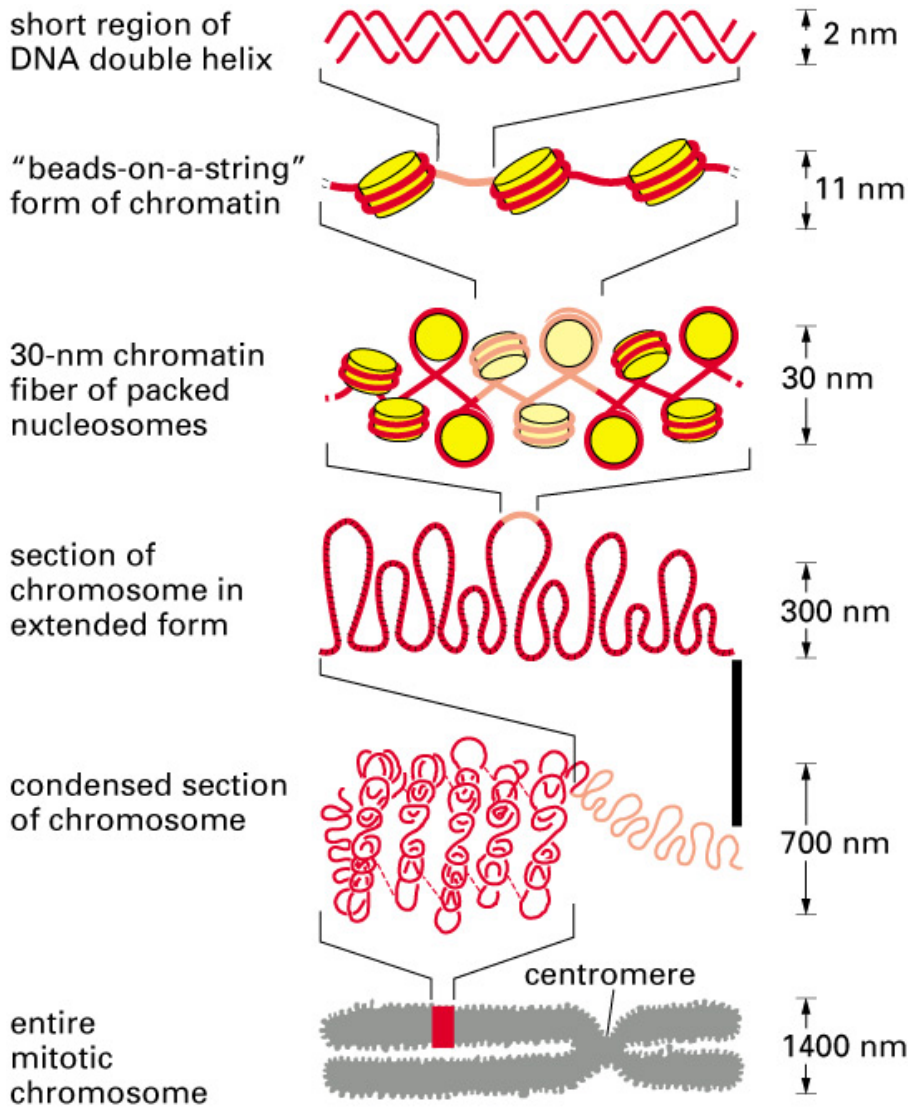
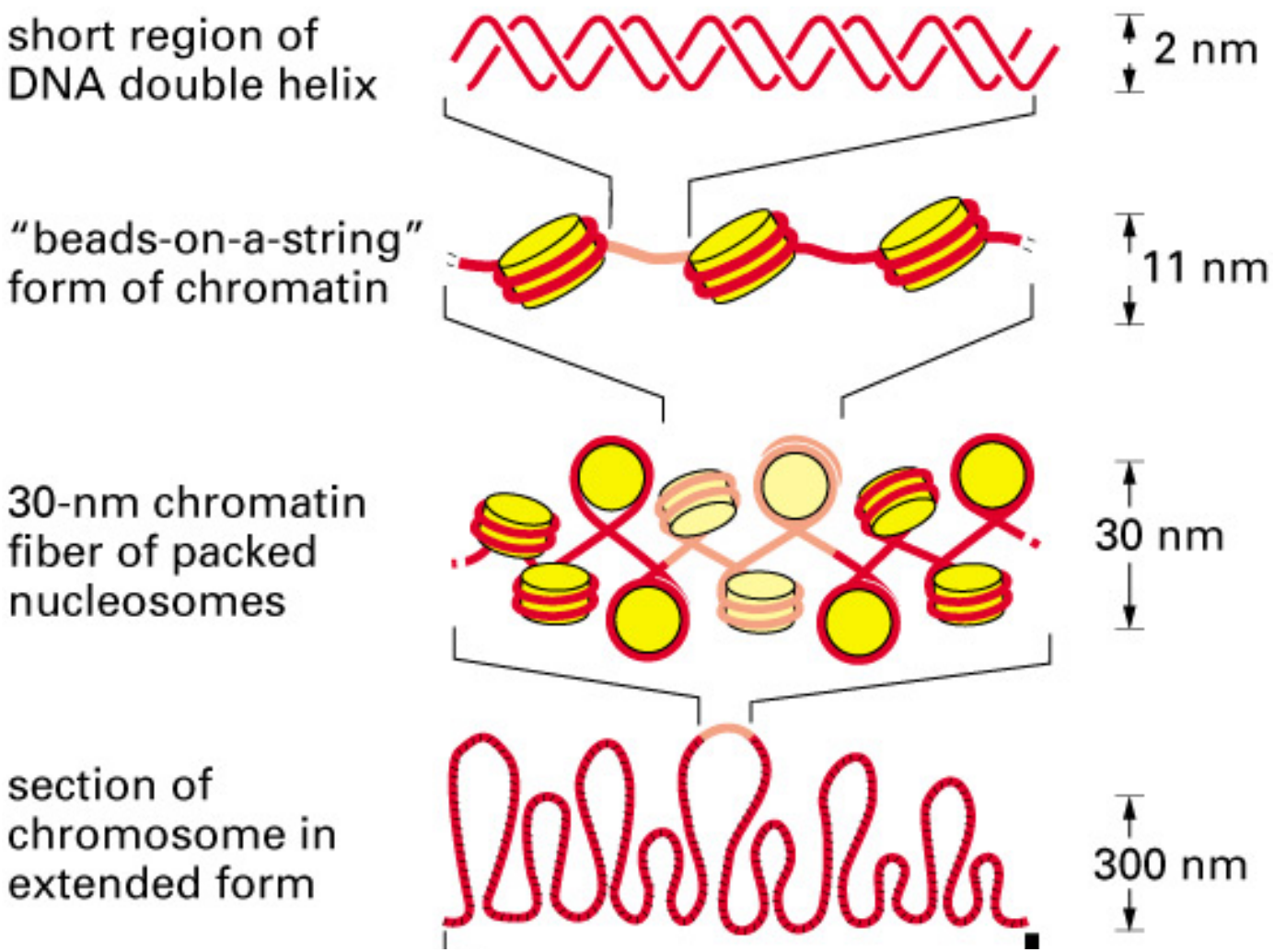


Figure 4–55. Molecular Biology of the Cell, 4th Edition.

Packaging in Interphase Nucleus

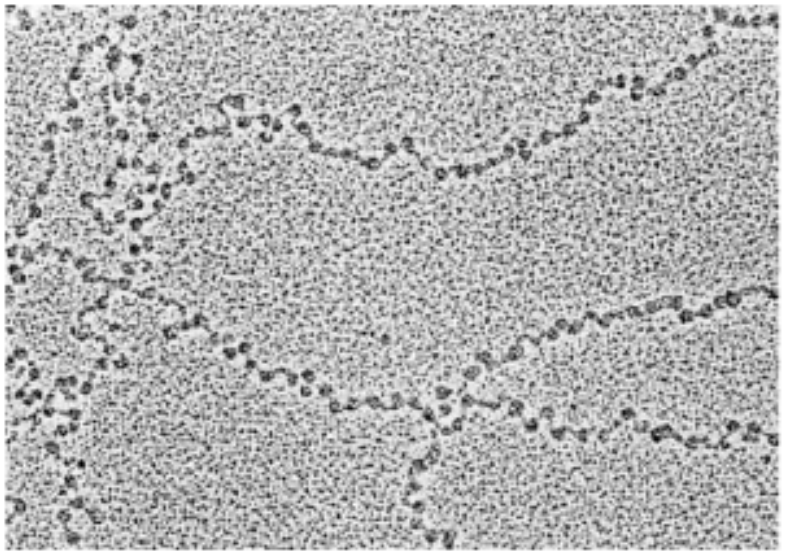


Chromatin Composition

- Complex of DNA and histones in 1:1 mass ratio
- Histones are small basic proteins
  - highly conserved during evolution
  - abundance of positively charged aa's (lysine and arginine) bind negatively charged DNA
- Four core histones: H2A, H2B, H3, H4 in 1:1:1:1 ratio
- Linker histone: H1 in variable ratio

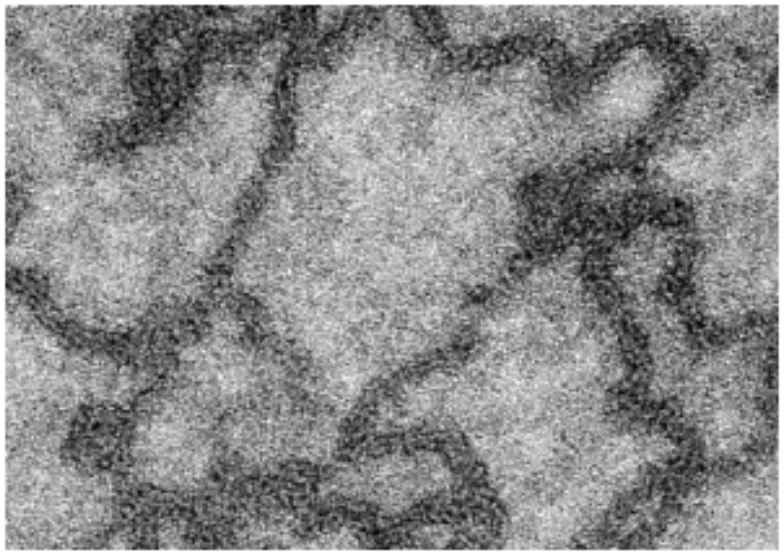
Chromatin Fibers

(a) 11-nm fiber



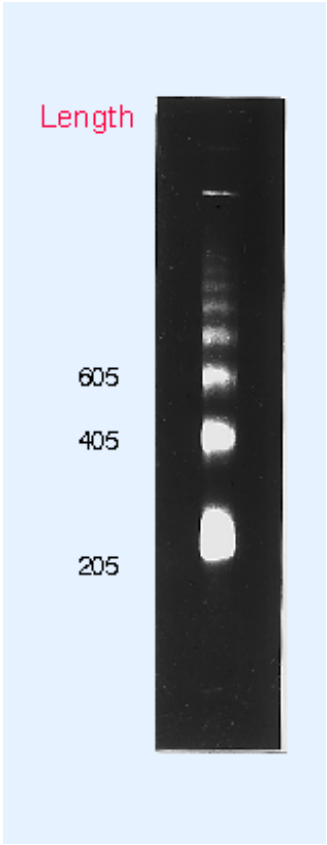
- beads = nucleosomes
- compaction = 2.5X
- low ionic strength buffer
- H1 not required

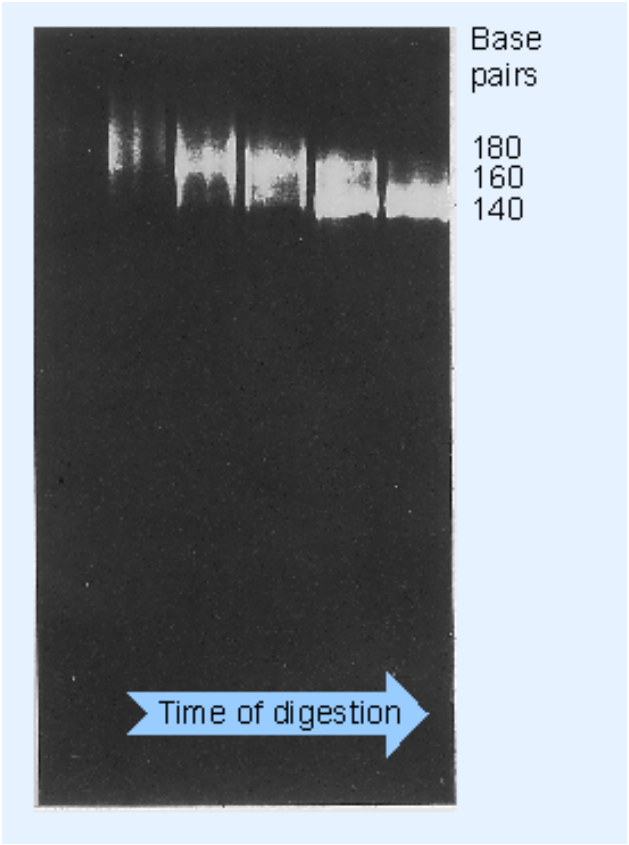
(b) 30-nm fiber

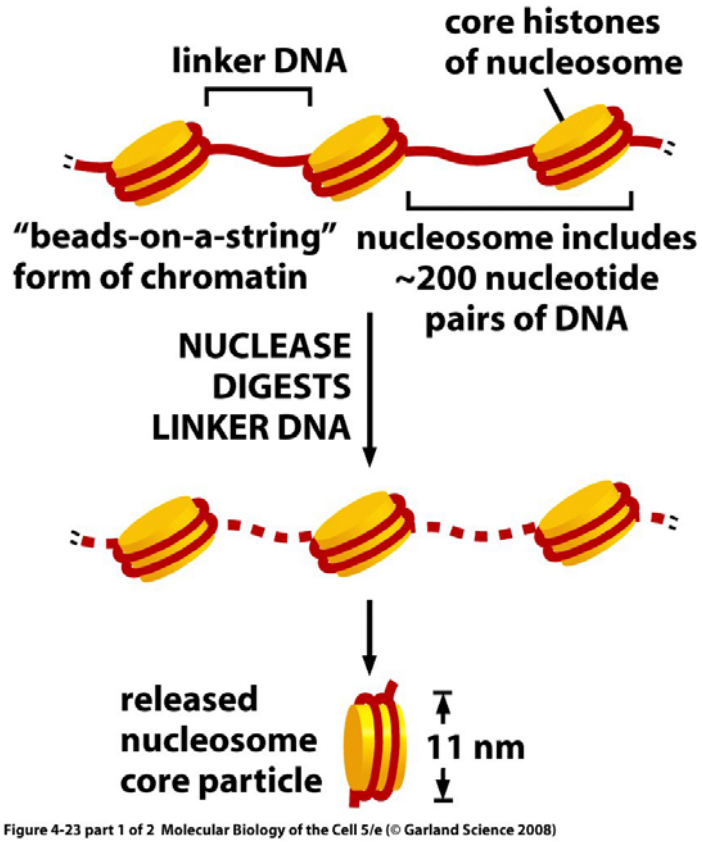


- physiological ionic strength (0.15 M KCl)
- compaction = 42X
- H1 required

Micrococcal Nuclease Digestion of Chromatin







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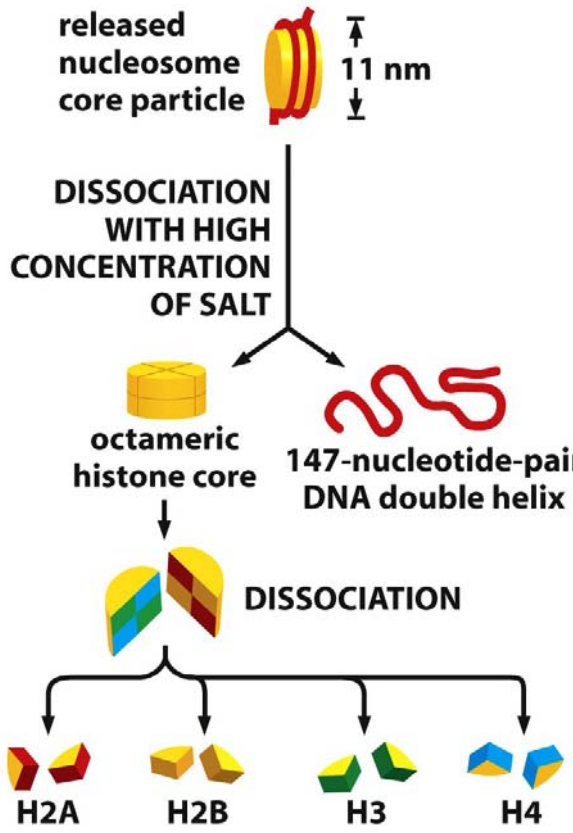
Stochiometry of Histones and DNA



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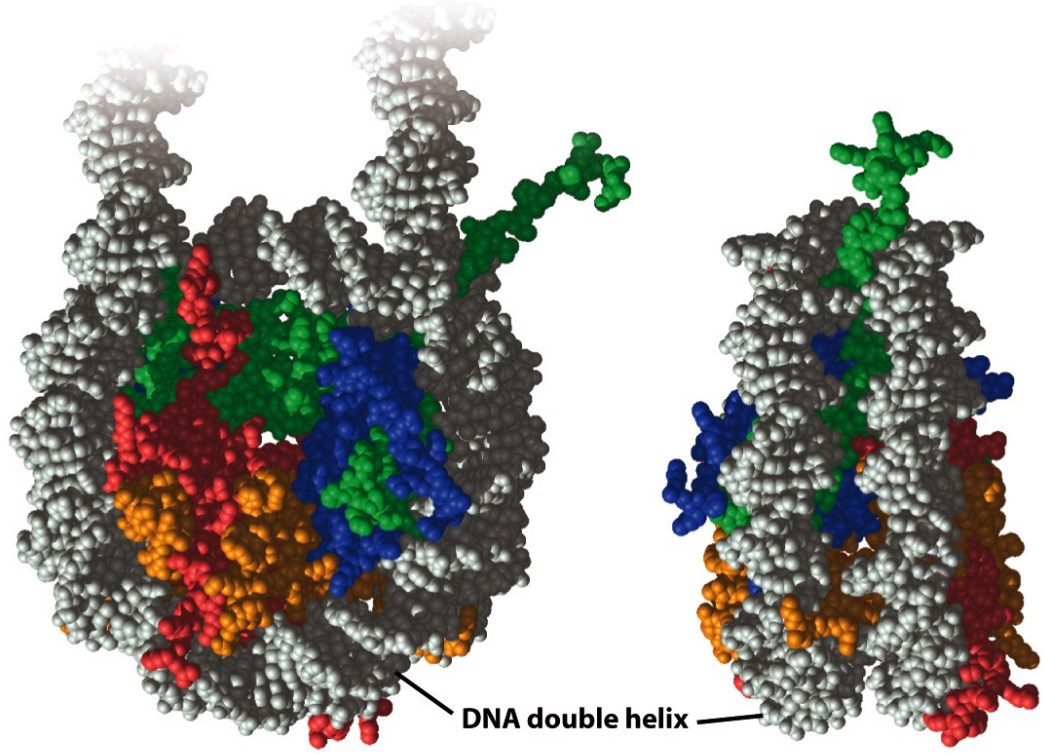
- 146 bp DNA ca. 100 kDa
- 8 histones ca 108 kDa
- mass ratio of DNA:protein 1:1

Structure of Core Nucleosome

1.65 left handed turns of DNA around histone octamer



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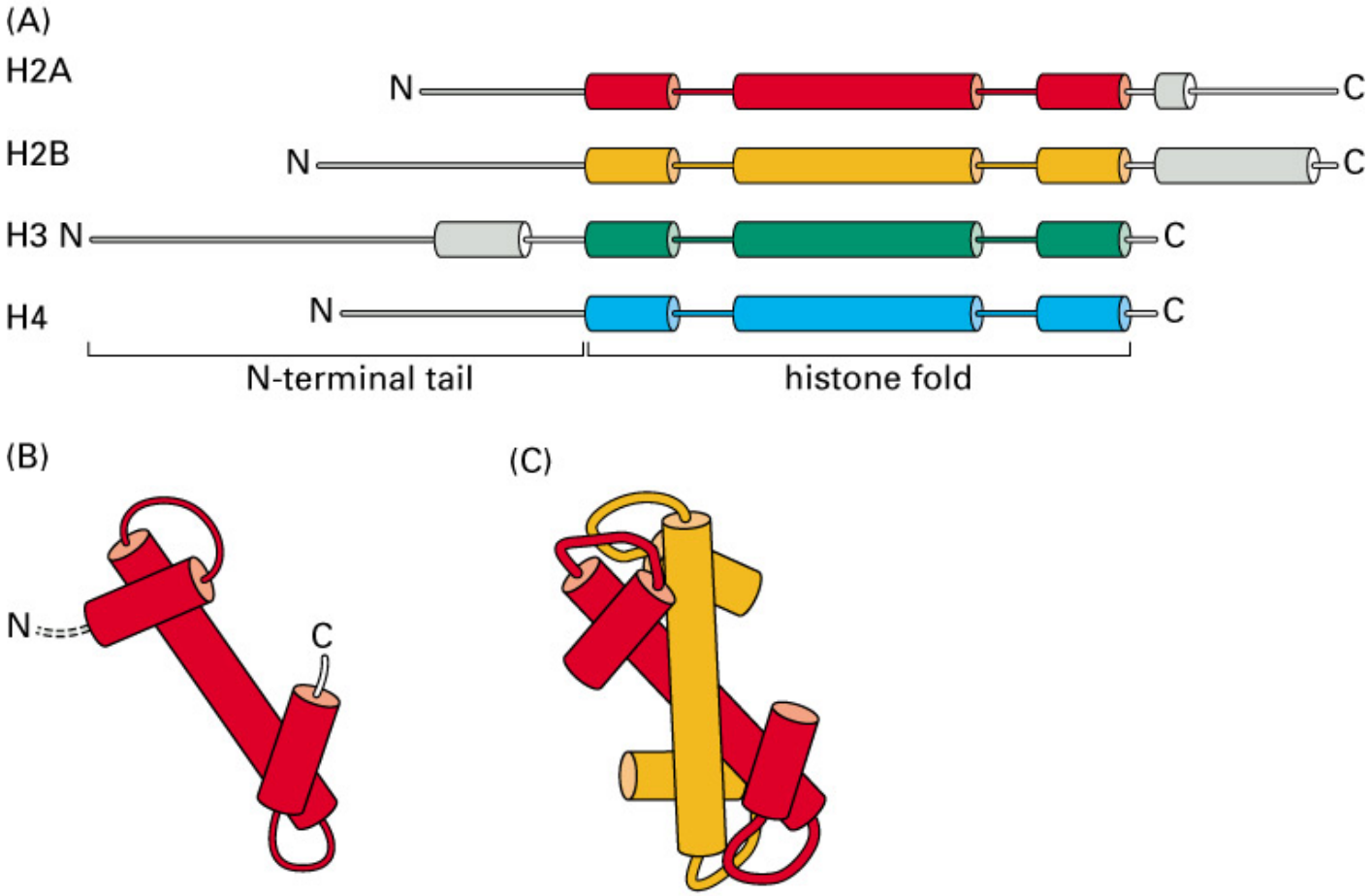
Histone Structure



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Assembly of a Histone Octamer

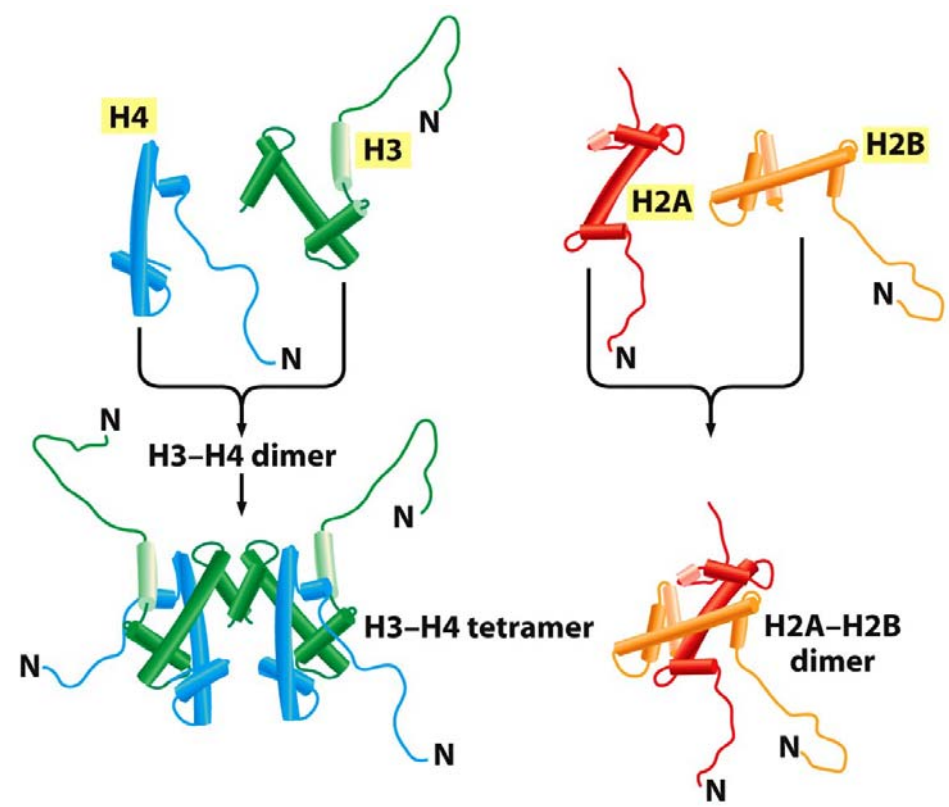

Figure 4-26 part 1 of 2 Molecular Biology of the Cell 5/e (© Garland Science 2008)

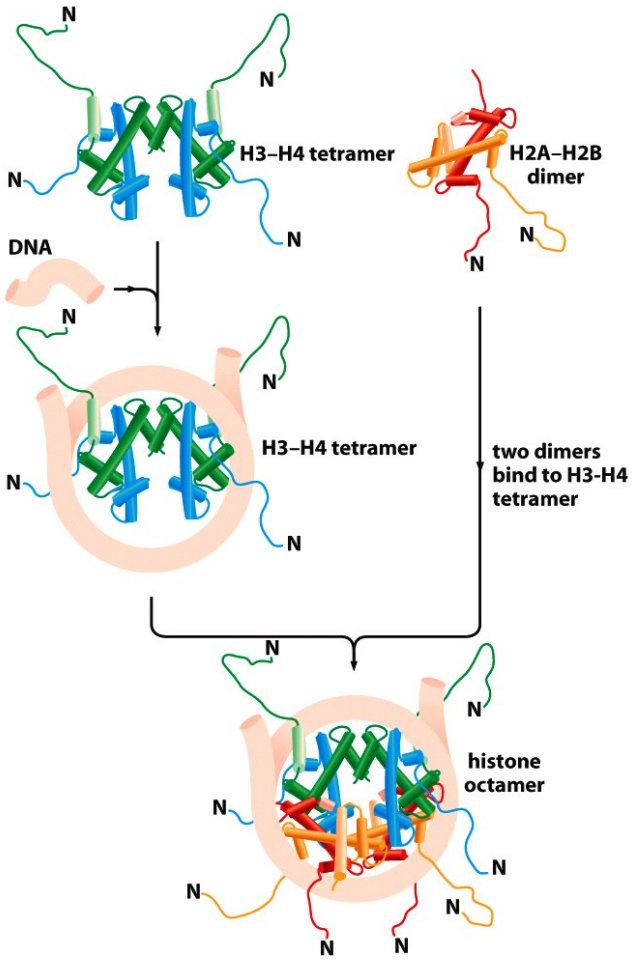

Figure 4-26 part 2 of 2 Molecular Biology of the Cell 5/e (© Garland Science 200

Nucleosomes Are Dynamic

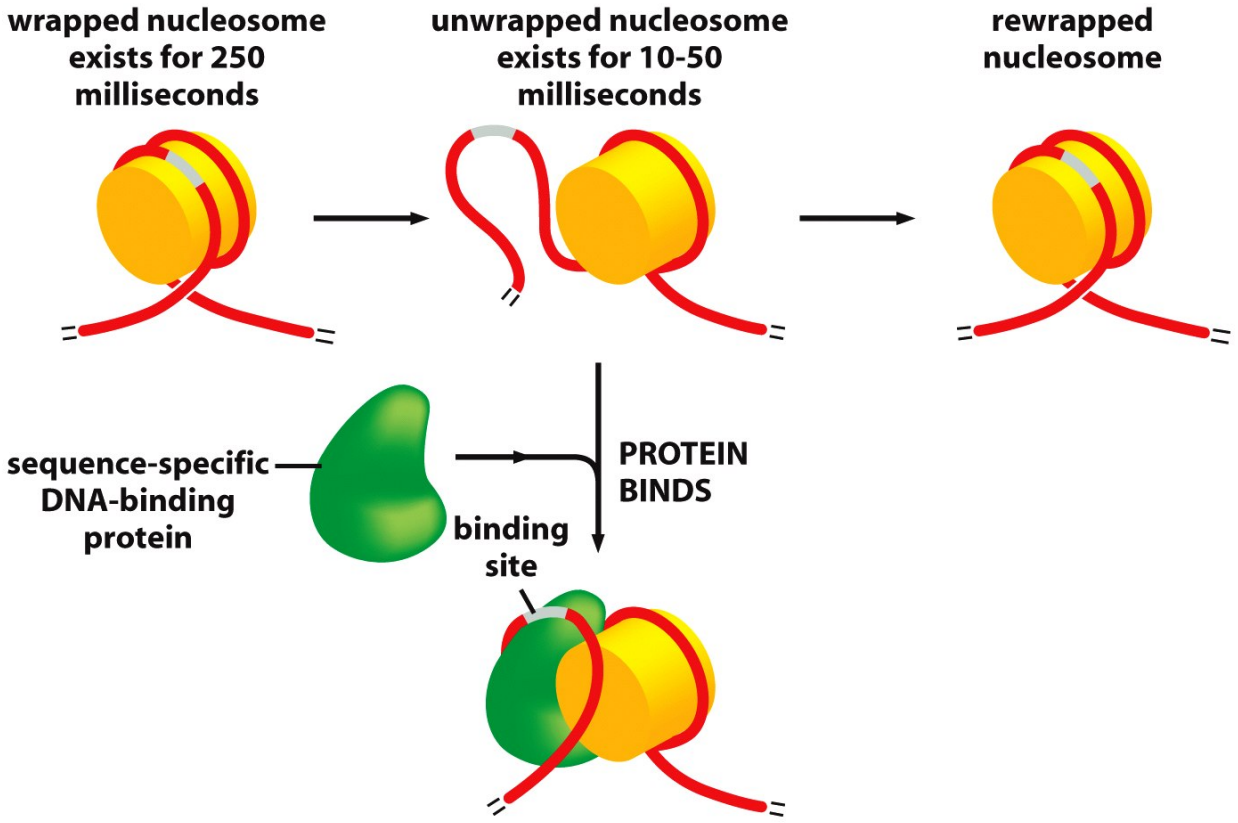

Figure 4-28 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Chromatin Remodeling

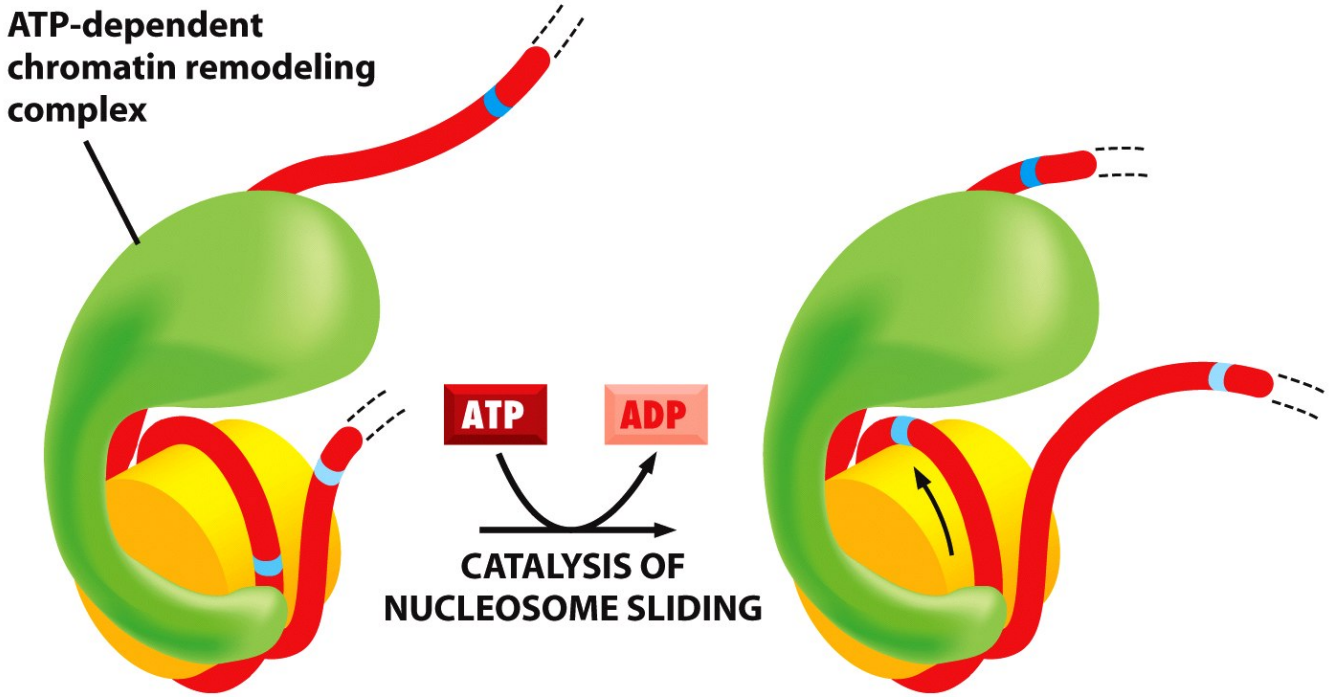

Figure 4-29 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Large complexes of ≥ 10 proteins

Use energy of ATP hydrolysis to partially disrupt histone-DNA contacts

Catalyze nucleosome sliding or nucleosome removal

30-nm Chromatin Fiber Structure

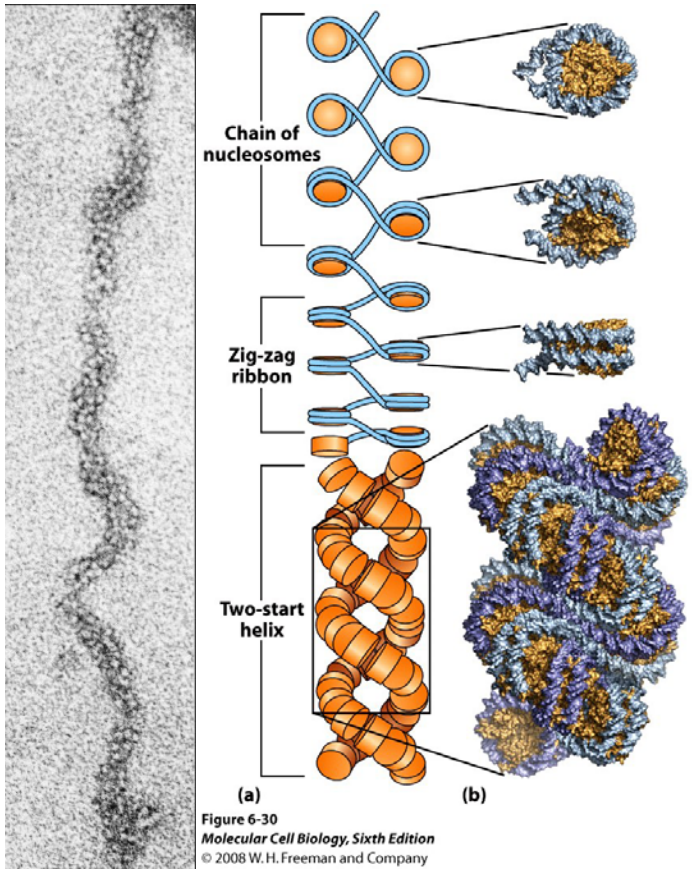

Figure 6-30
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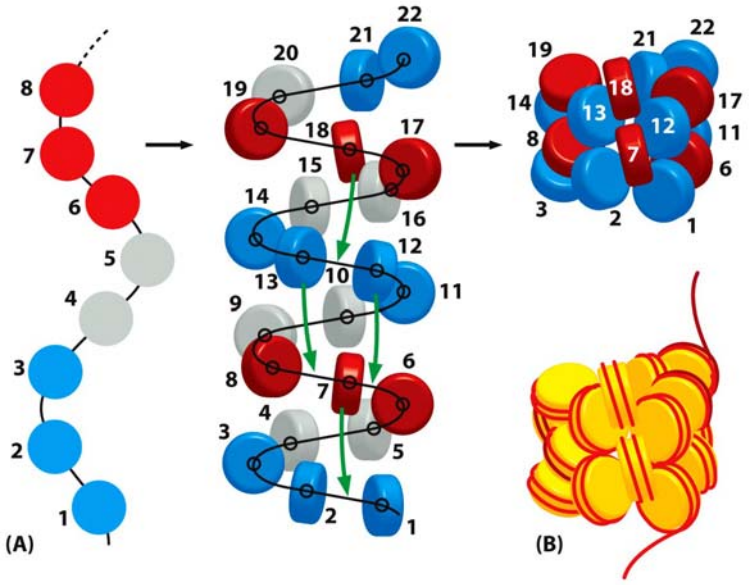

Figure 4-32ab Molecular Biology of the Cell 5/e (© Garland Science 2008)

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Models for H1 and Core Histone Tails in Formation of 30-nm Fiber

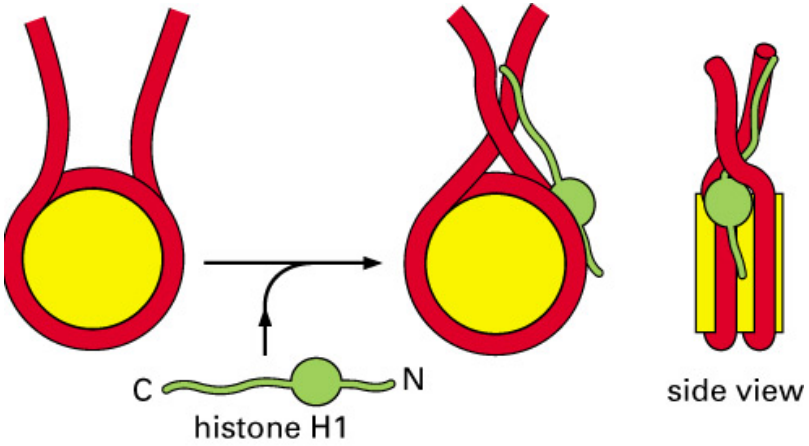

Figure 4–31. Molecular Biology of the Cell, 4th Edition.

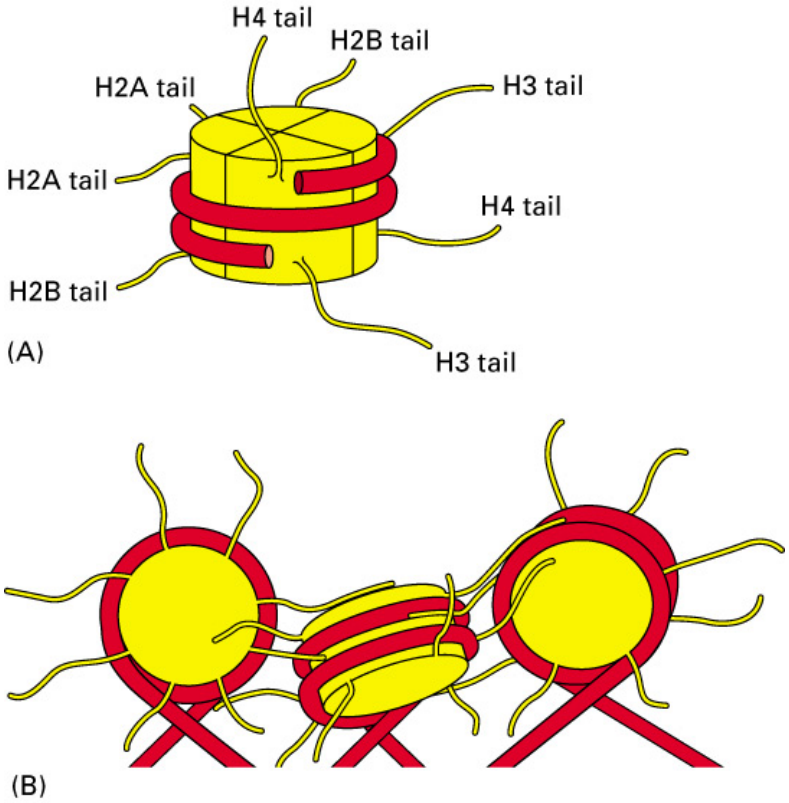

Figure 4–32. Molecular Biology of the Cell, 4th Edition.

Histone Tails

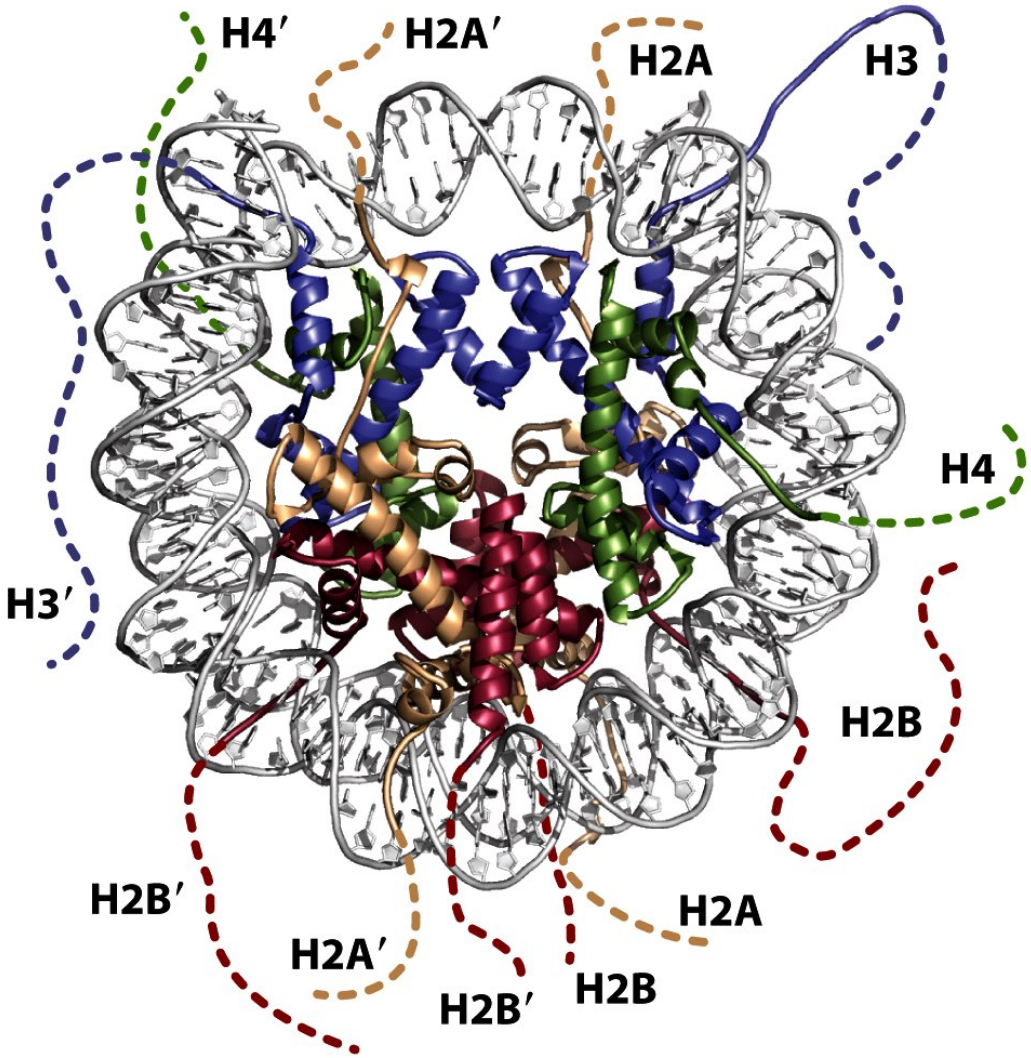

Figure 6-31a
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Covalent Modifications of Histone Tails Control Chromatin Function

Acetylation (K)

Phosphorylation (S)

Methylation (K, R)

Ubiquitinylation (K)

Sumoylation (K)

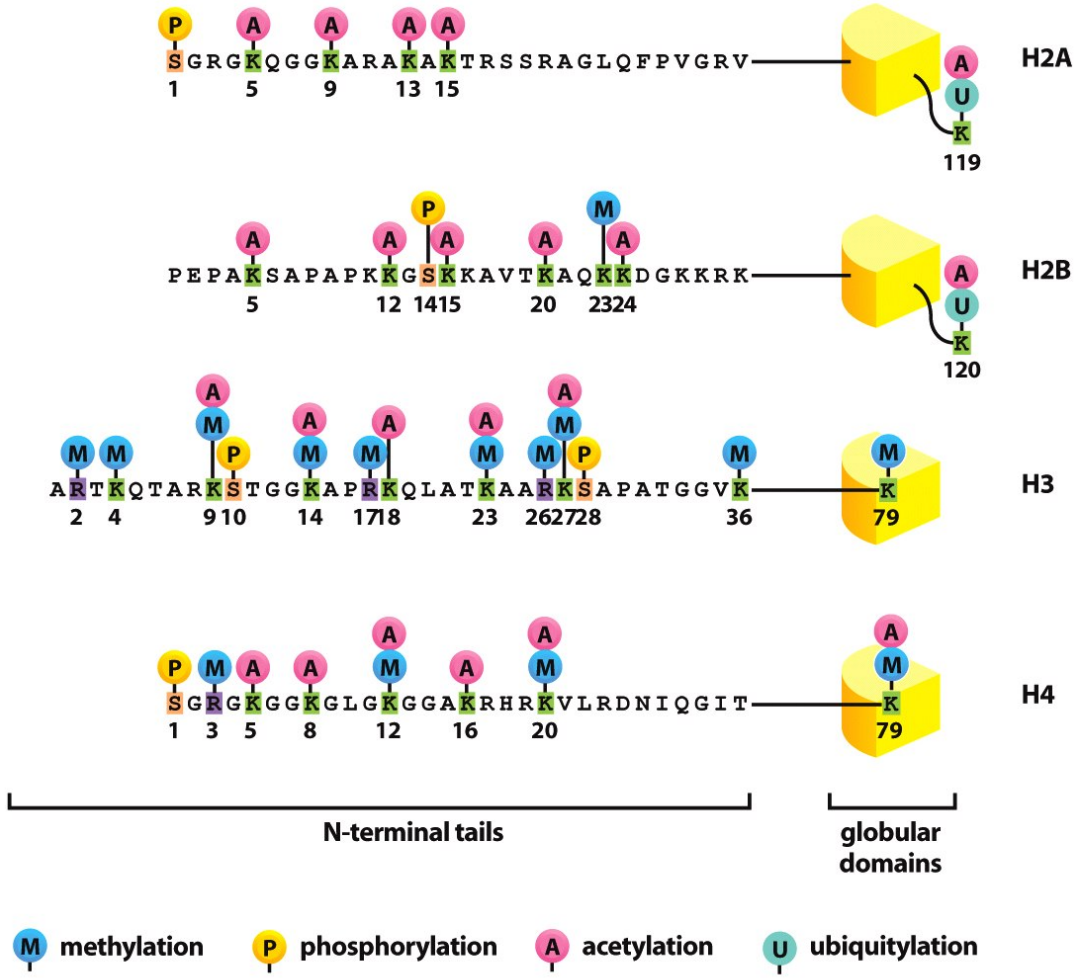


Figure 4-39b Molecular Biology of the Cell 5/e (© Garland Science 2008)

Enzymes that Modify Histones

- Histone acetyltransferases (HATs)
- Histone deacetylases (HDACs)
- Histone methyl transferases (HMTs)
- Histone kinases

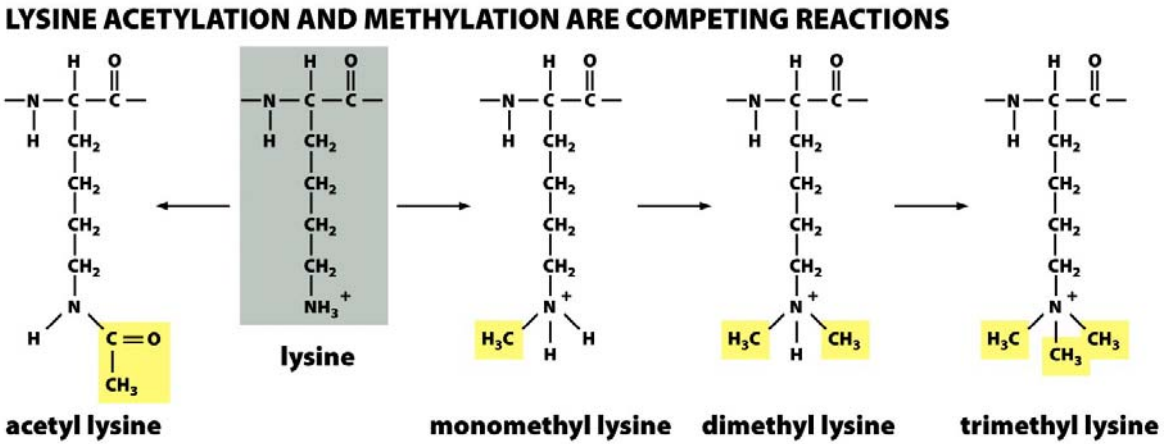
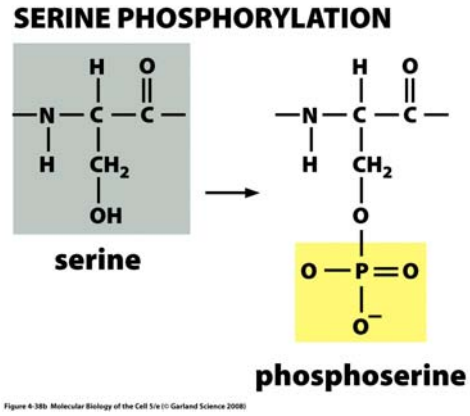


Figure 4-38a Molecular Biology of the Cell 5/e (© Garland Science 2008)



Meanings of Histone ‘Code’

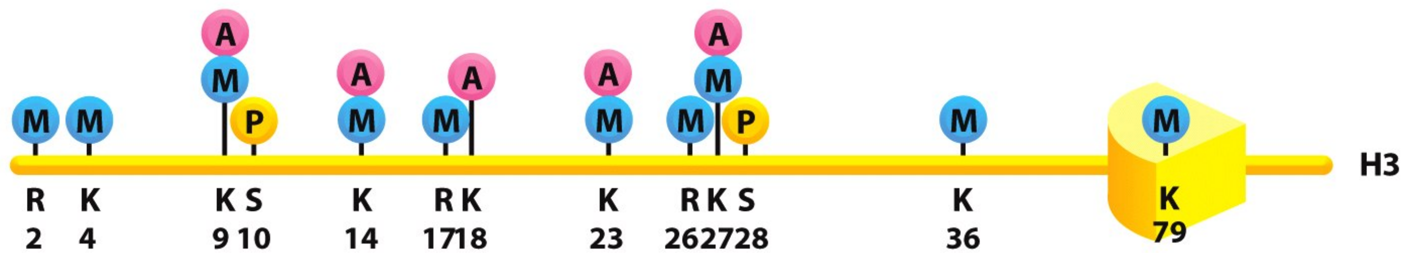


Figure 4-44a Molecular Biology of the Cell 5/e (© Garland Science 2008)

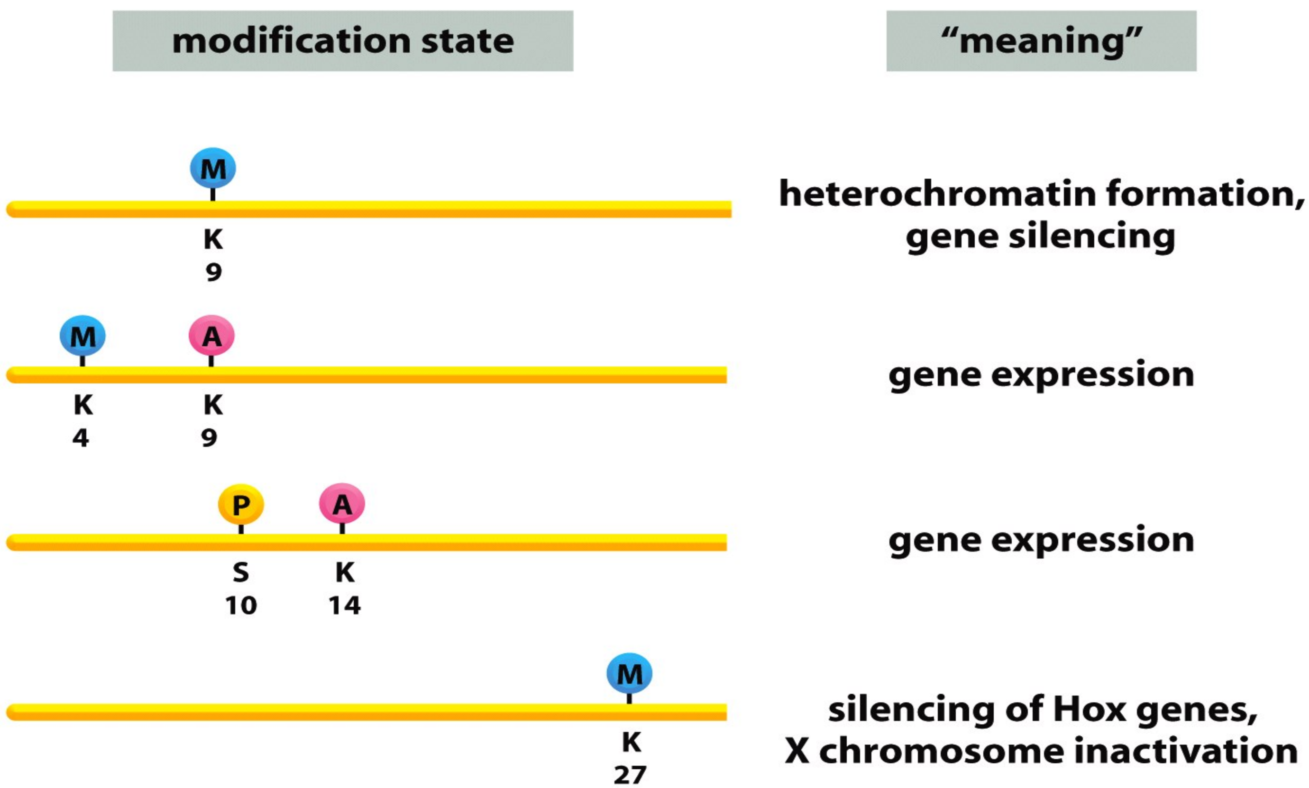


Figure 4-44b Molecular Biology of the Cell 5/e (© Garland Science 2008)

Transcriptional Regulators Modify Histone Acetylation

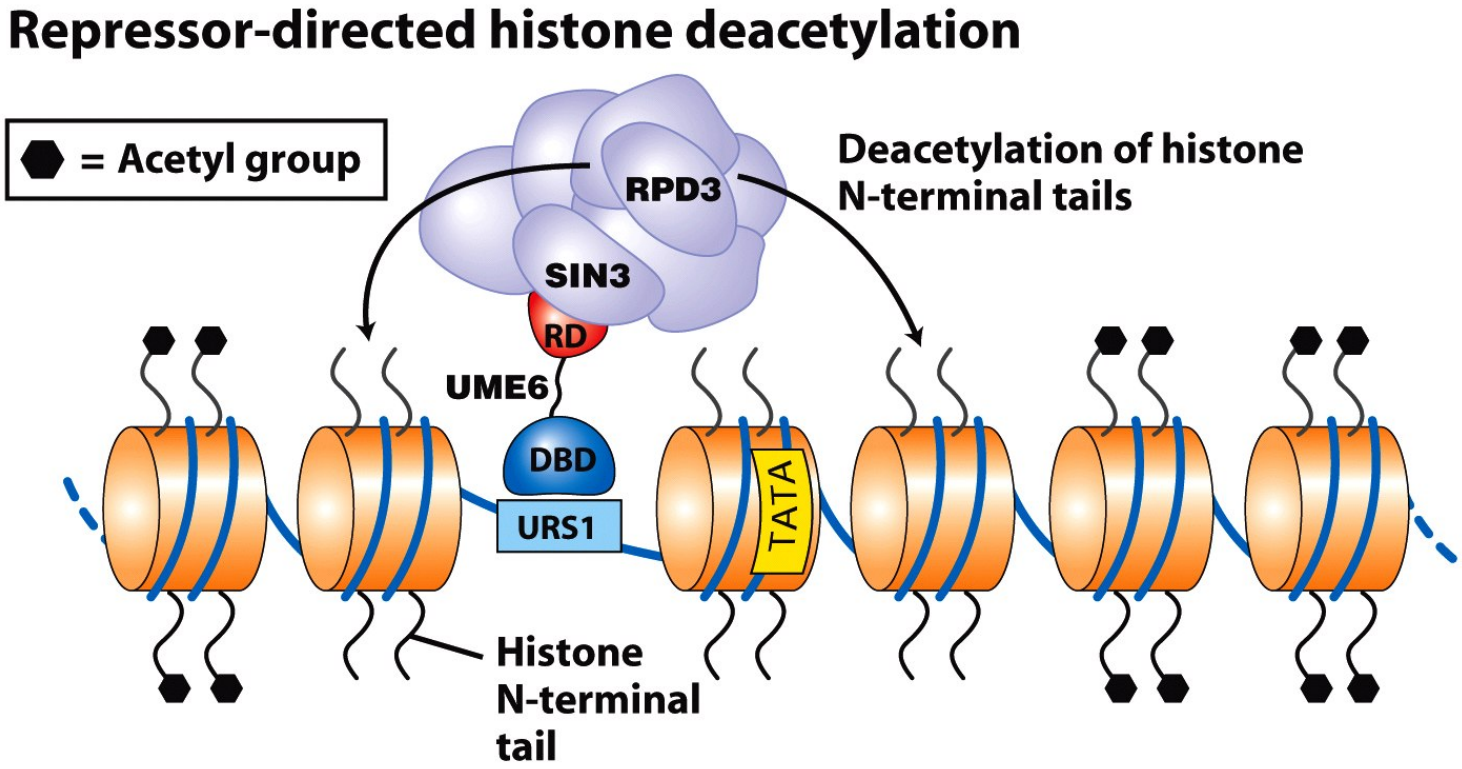


Figure 7-38a
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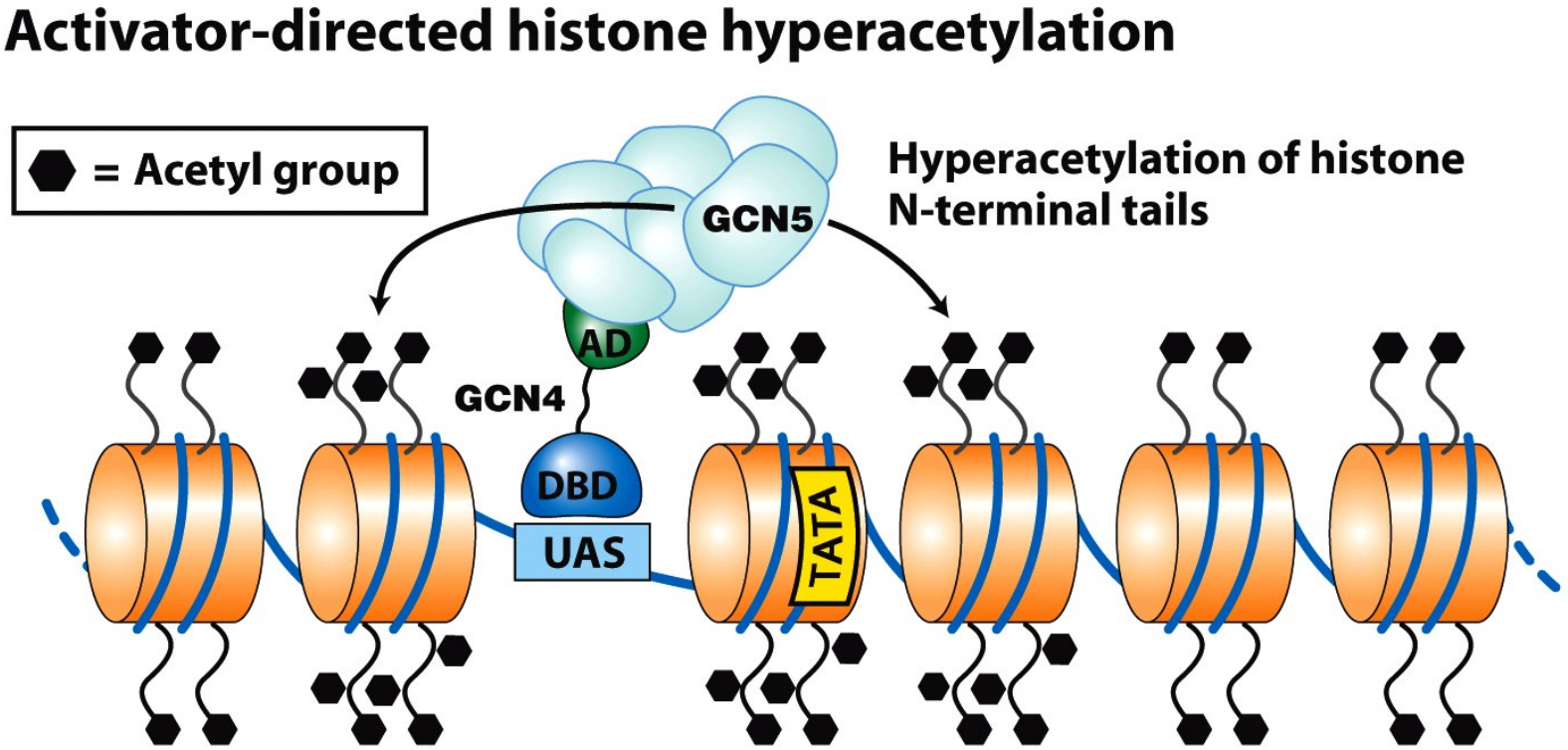
Transcriptional Regulators Modify Histone Acetylation



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Histone Code Readers

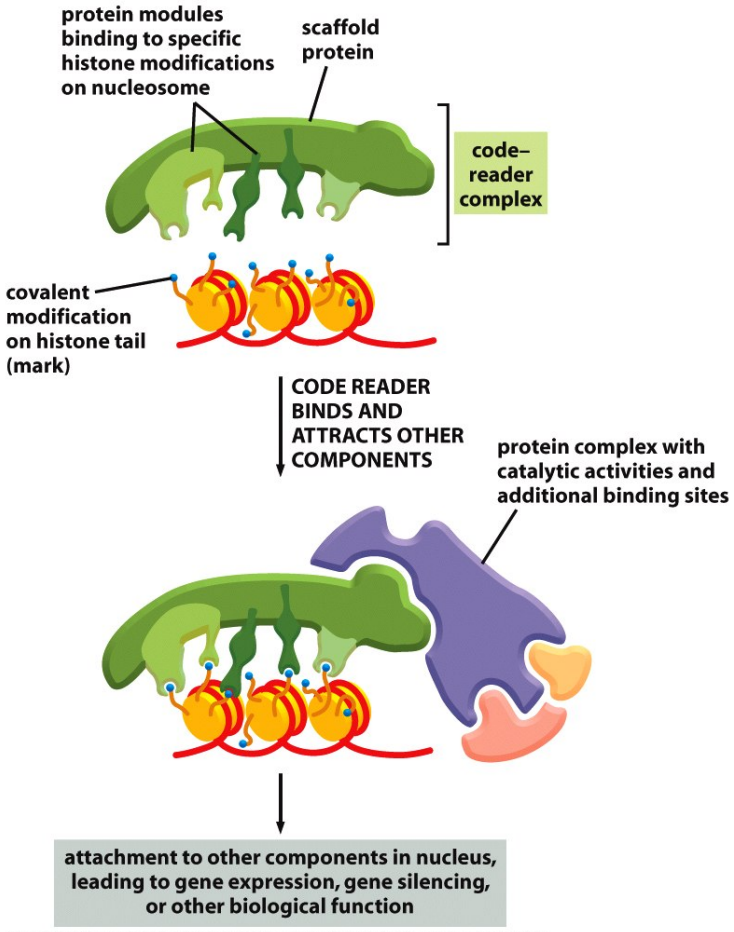
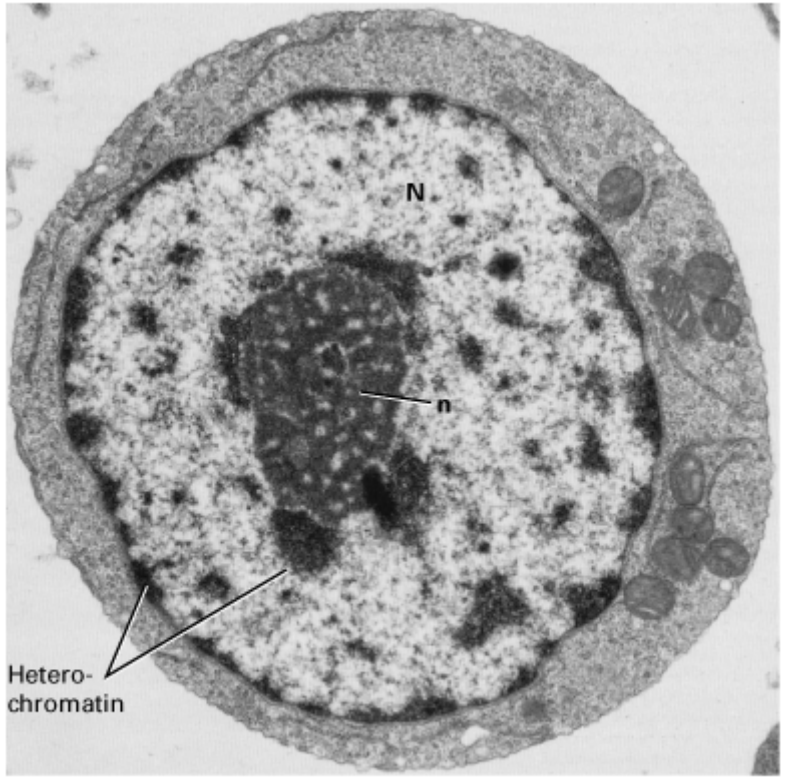


Figure 4-43 Molecular Biology of the Cell 5/e (© Garland Science 2008)

- Code reader complexes recognize particular marks on chromatin
- Attract additional protein complexes that execute biological function

Formation of Heterochromatin Silences Gene Expression

- Heterochromatin–regions of darkly staining chromatin in eukaryotic nuclei
- Transcriptionally silent DNA
- Centromeres, telomeres are heterochromatic
- Genes near heterochromatin show metastable expression patterns
  - position effect variegation in flies
  - telomere position effects in yeast



Formation of Heterochromatin in Mammalian Cells

- Requires specific modification: Histone H3 lysine 9 trimethylation ($H3K9Me_3$) by H3K9 HMT
- Heterochromatin protein 1 (HP1)

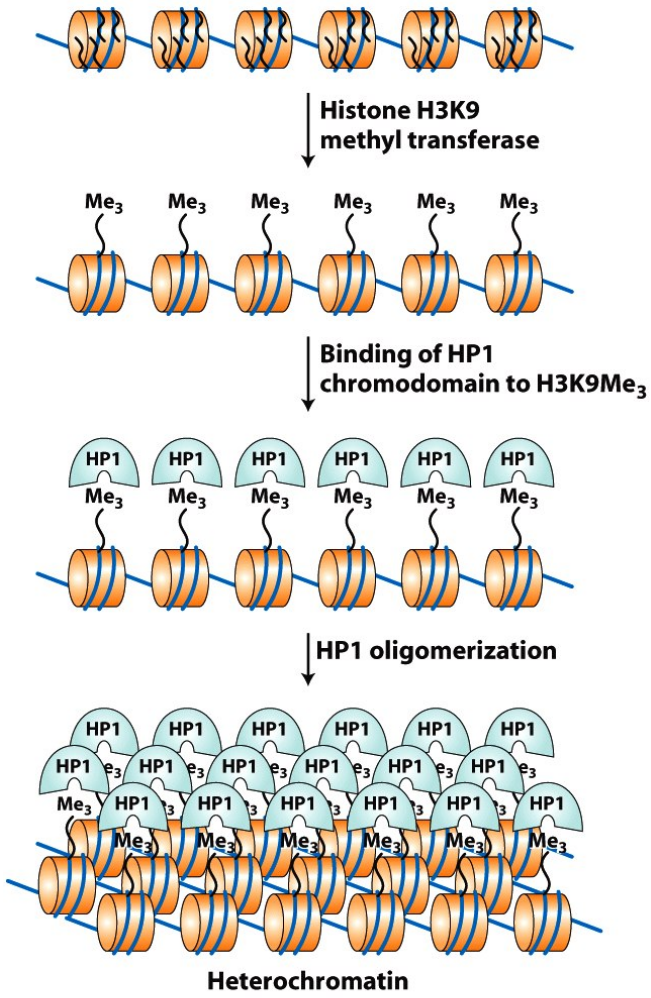


Figure 6-34a
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Boundary Elements Prevent Spread of Heterochromatin

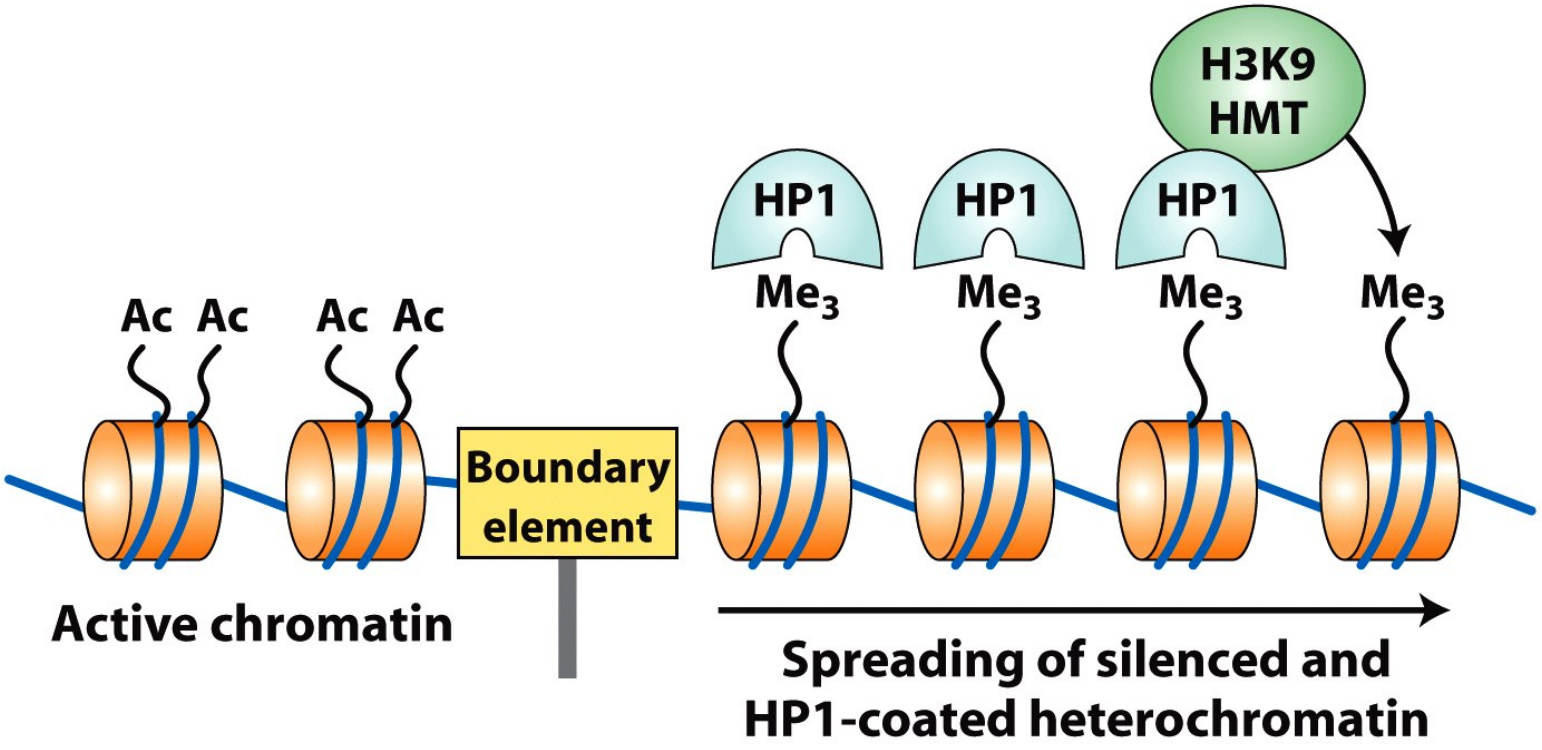

Figure 6-34b
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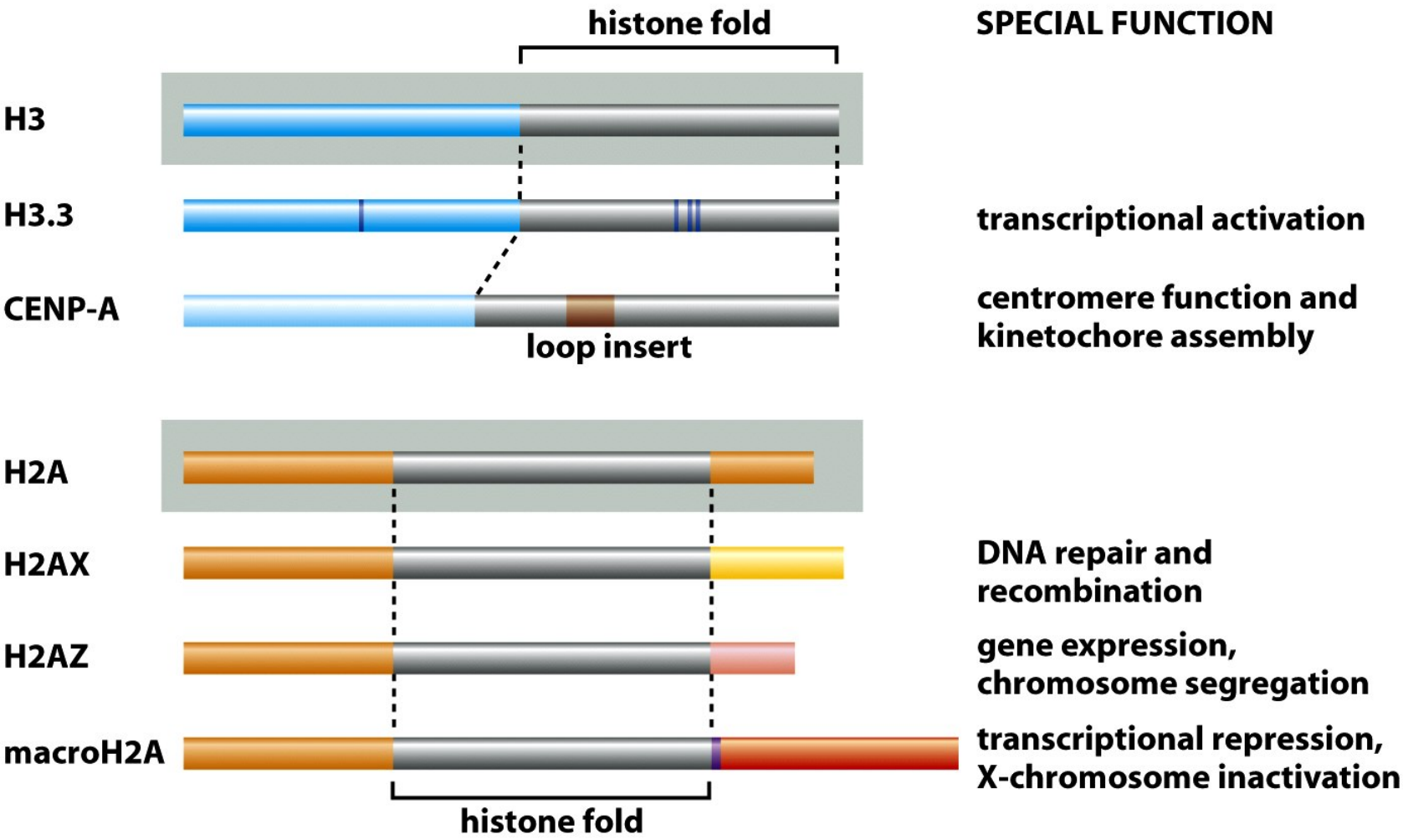
Histone Variants Have Special Functions



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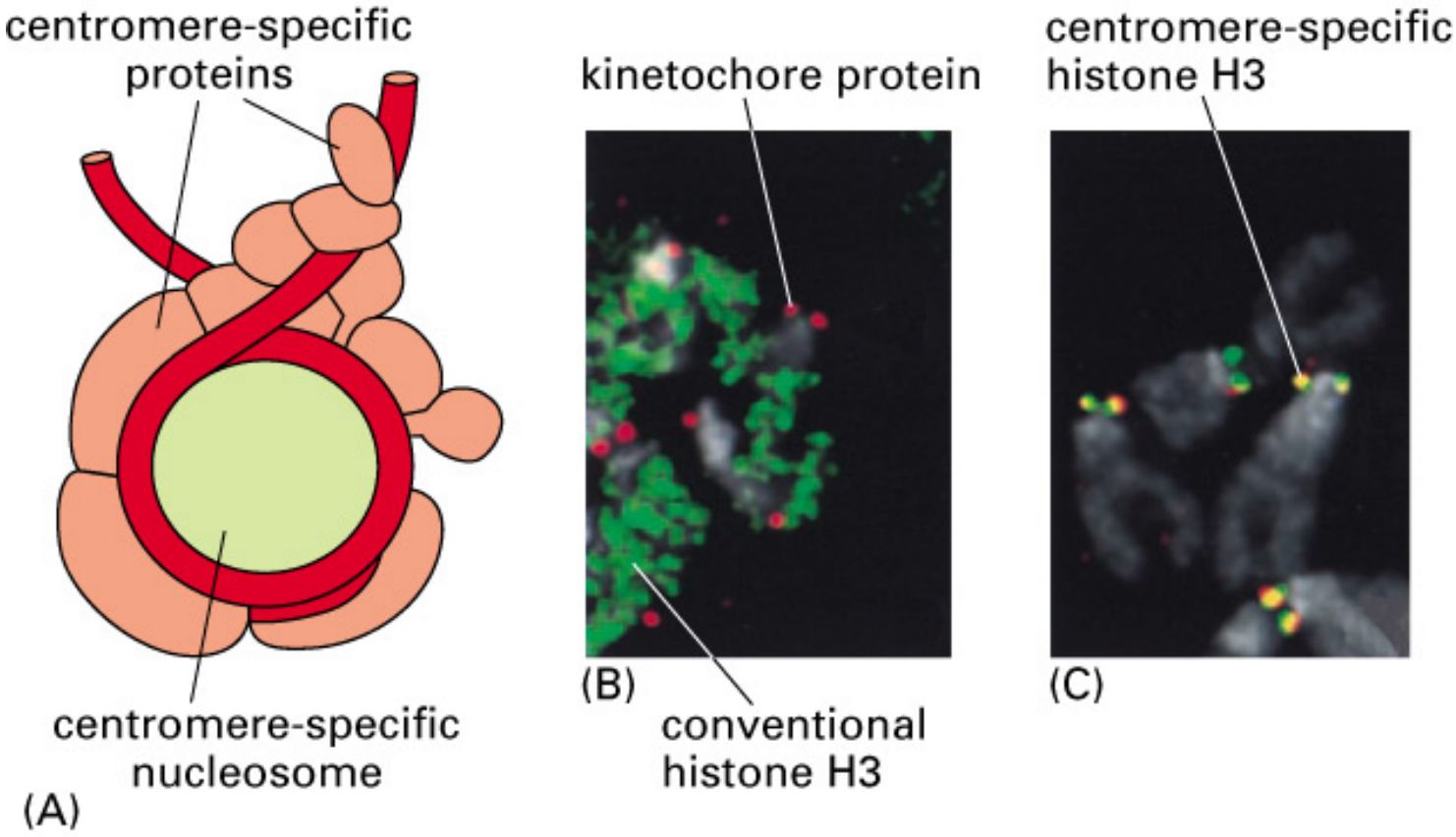
Centromeres Are Heterochromatic and Contain Specialized Nucleosome



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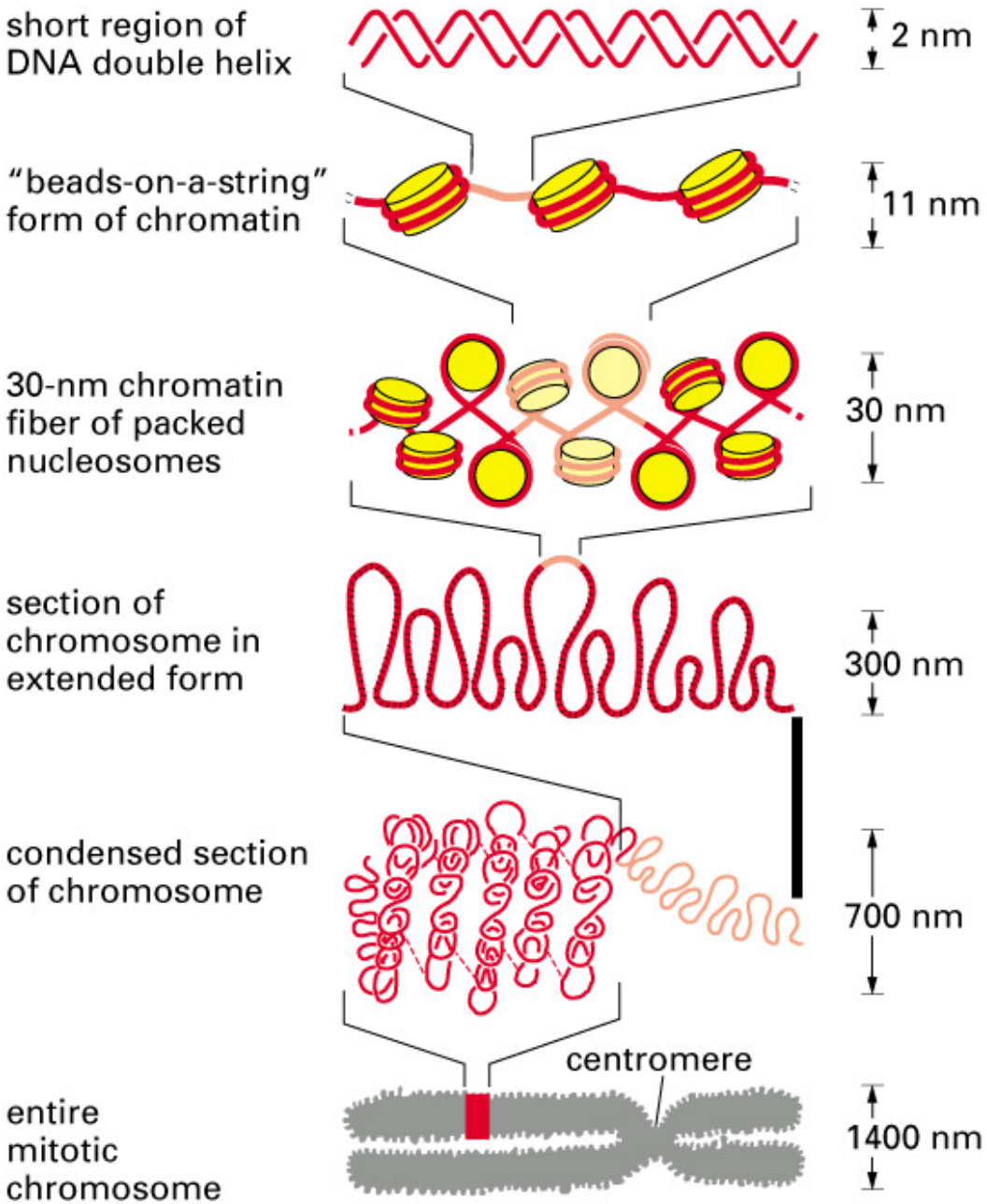
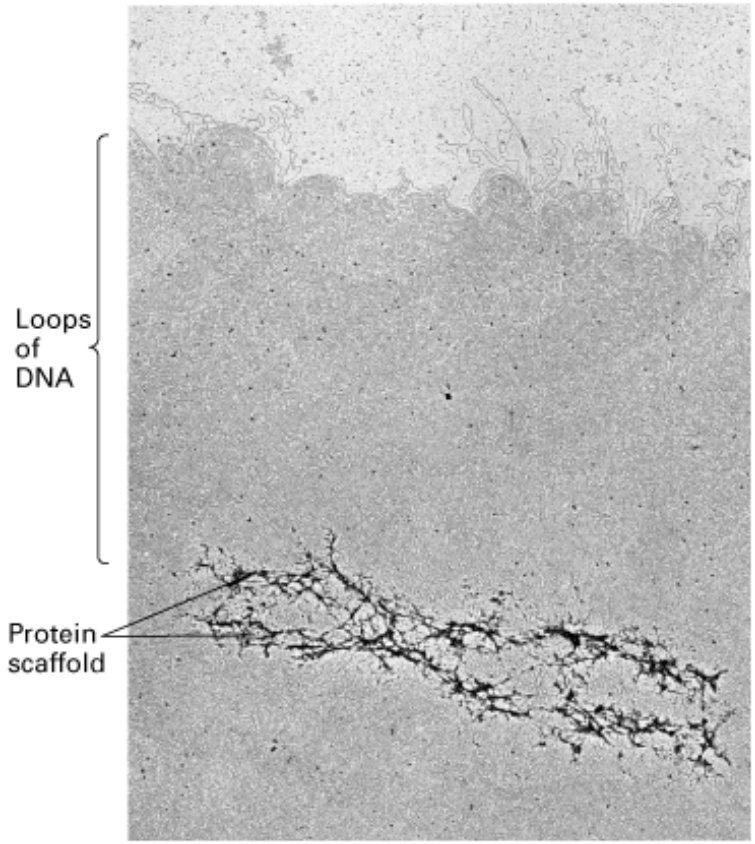
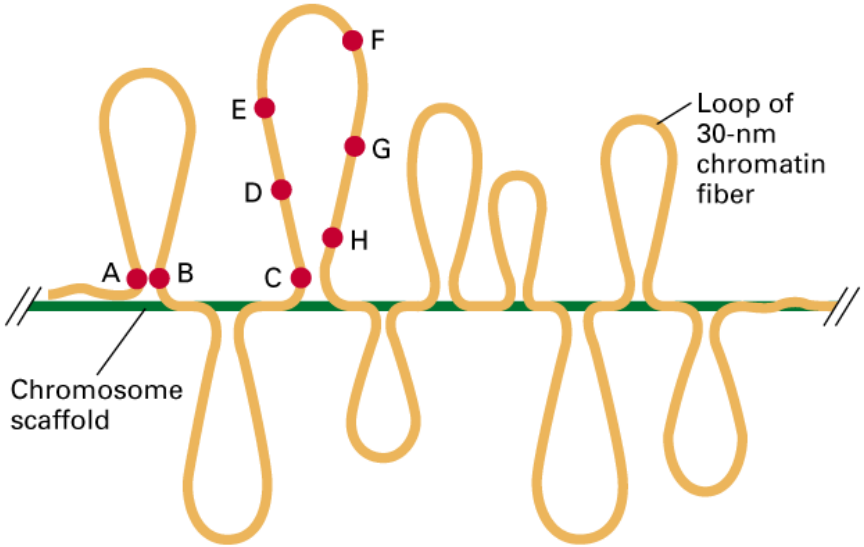
Higher Order Packaging



Higher Order Packaging (300-nm fiber)

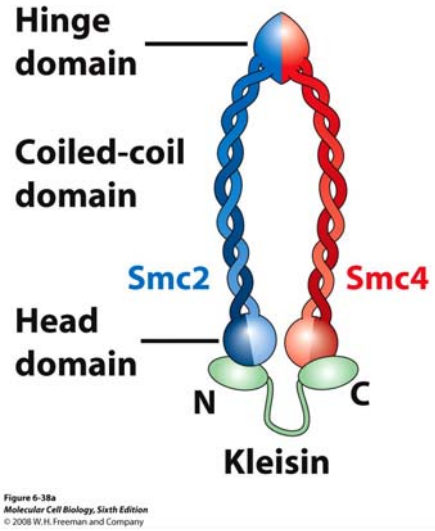




Mechanism of looping unknown

Mitotic Chromosome Condensation

- Depends on SMC (structural maintenance of chromosomes) proteins, which are conserved from bacteria to man
  - Condensins (SMC2 & SMC4)
  - Cohesins (SMC1 & SMC3)
  - large proteins with coiled-coiled domains and ATPase domains



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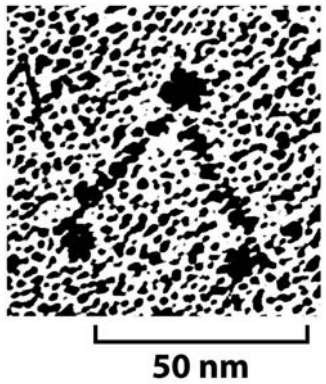

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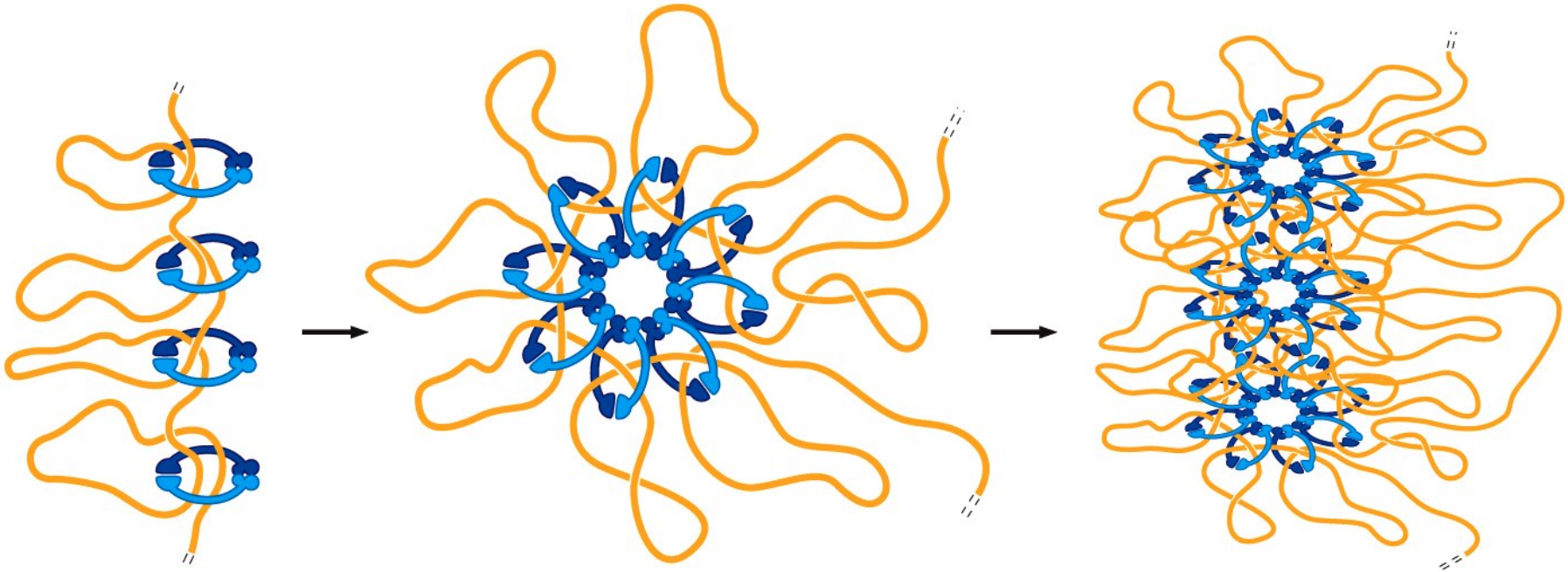
Model of Cohesin in Mitotic Chromosomes



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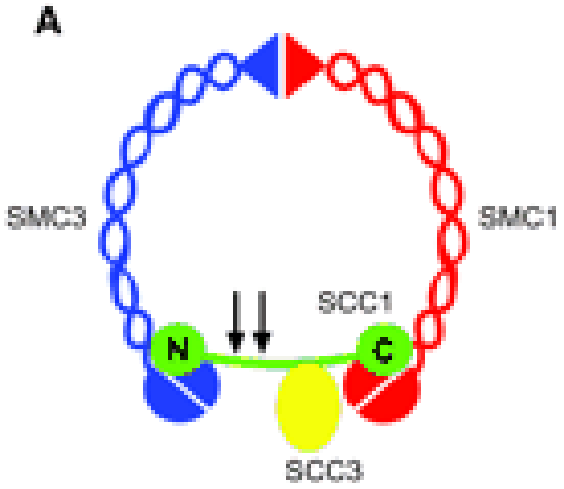
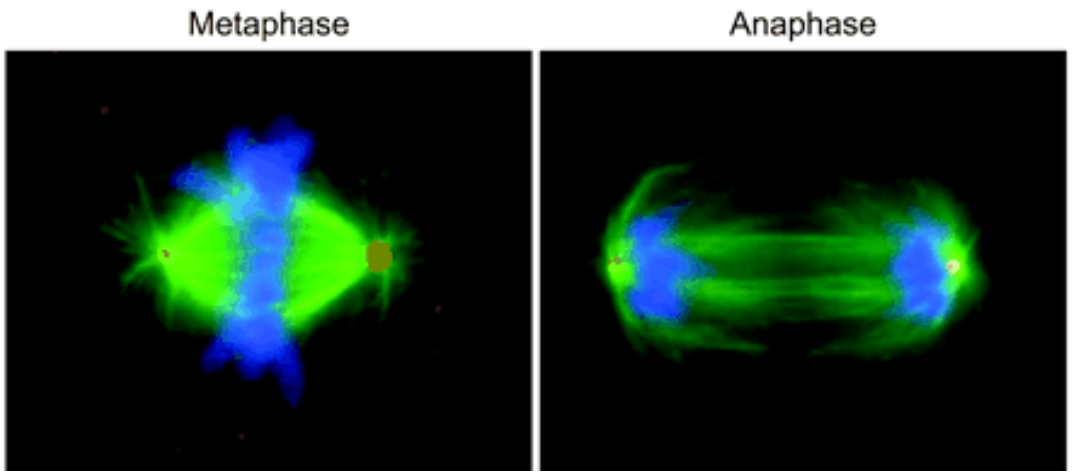
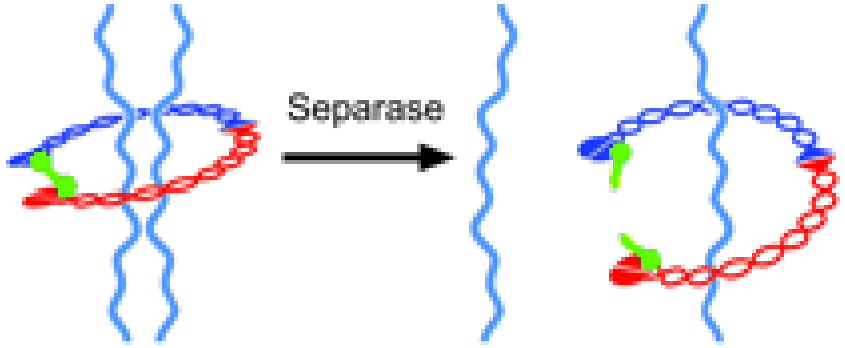
Molecular Basis of Cohesion





Cohesin



Nasmyth 2002 Science 297:559