Molecular Cell Biology A

"From nucleic acids to chromosomes to genomes"

BIOX24ZL

Tuesdays 9-10:30

Ray LC

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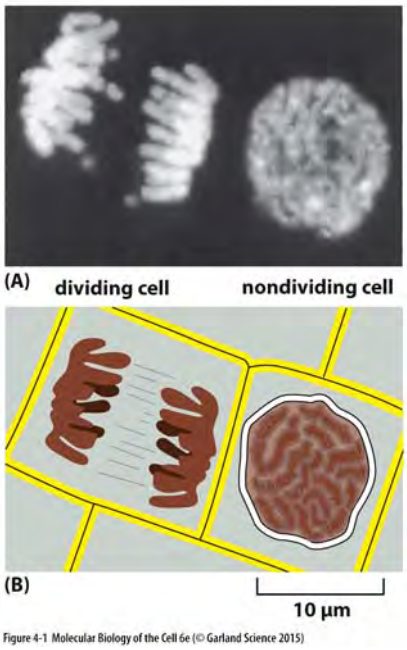


Figure 4-1 Molecular Biology of the Cell 6e (© Garland Science 2015)

Genetic info stored in an organism's DNA comprises its genome.

- DNA first found to be genetic 'stuff' in bacteria
- H-bonds between bases – 2 strands together
- Sugar-phosphate backbone 5'phos -> 3'OH
- Inside: 2-ring purine with 1-ring pyrimidine
- Efficient packing: double helix 1 turn every 10b
- Antiparallel strands; key to make replication simple is complementary base pairing
- One strand as template for replicating another
- Nuclear envelope supported by nuclear lamina, inner nucleus continuous with lumen of ER

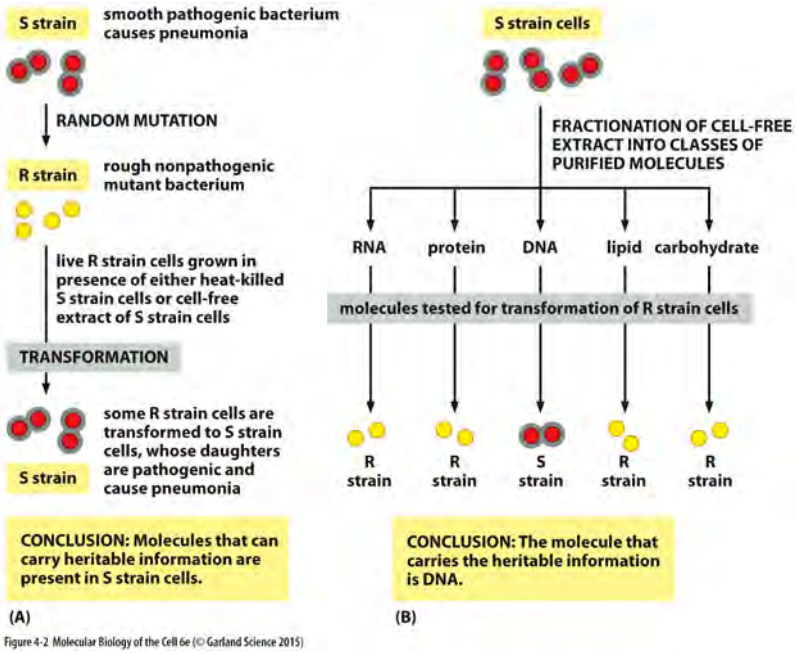


Figure 4-2 Molecular Biology of the Cell 6e (© Garland Science 2015)

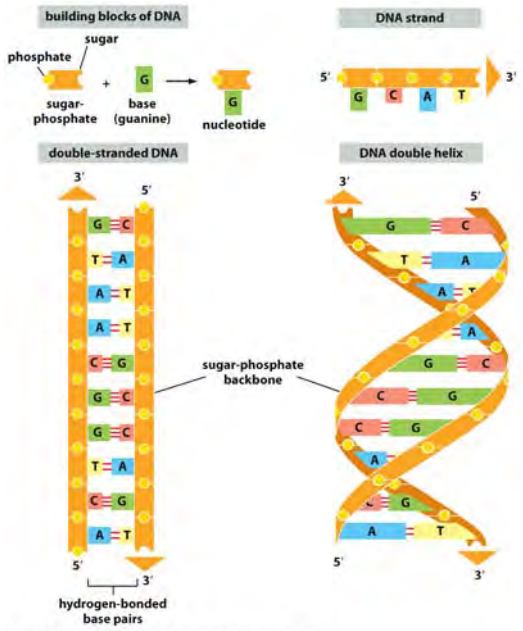


Figure 4-3 Molecular Biology of the Cell 6e (© Garland Science 2015)

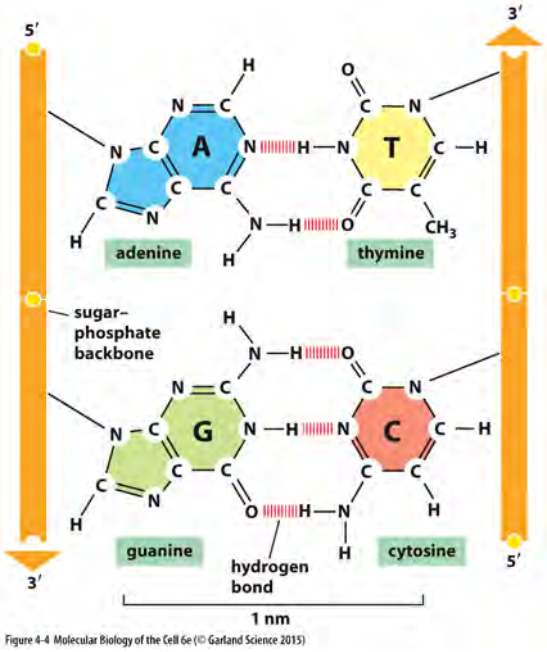


Figure 4-4 Molecular Biology of the Cell 6e (© Garland Science 2015)

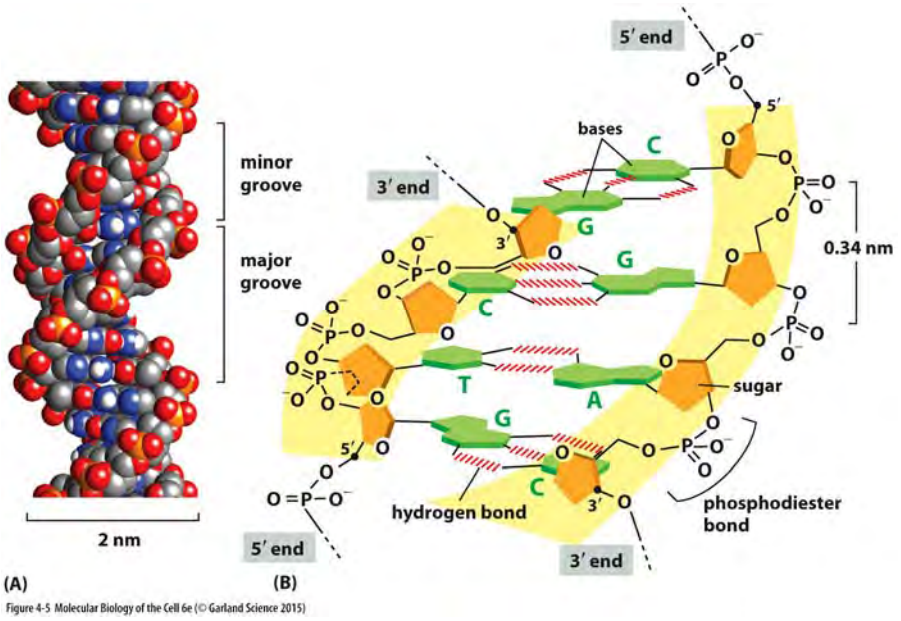


Figure 4-5 Molecular Biology of the Cell 6e (© Garland Science 2015)

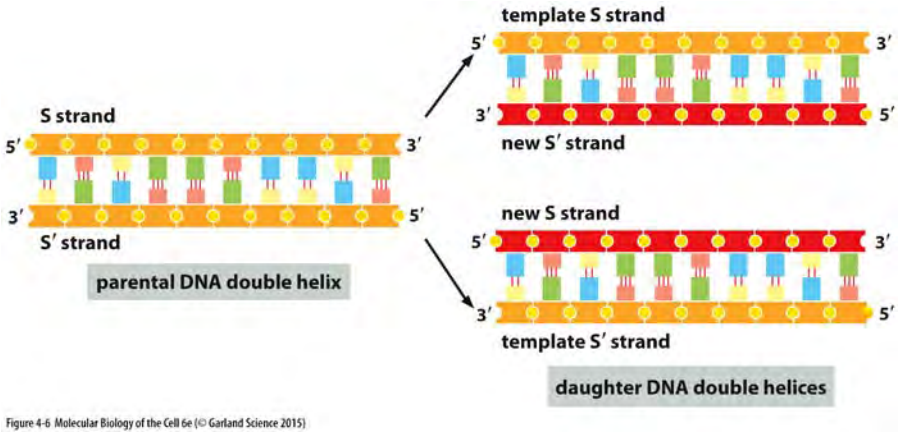


Figure 4-6 Molecular Biology of the Cell 6e (© Garland Science 2015)

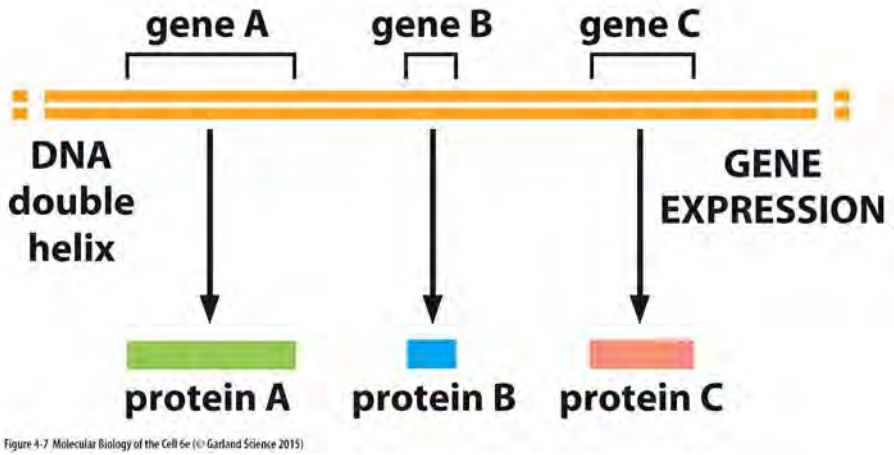


Figure 4-7 Molecular Biology of the Cell 6e (© Garland Science 2015)

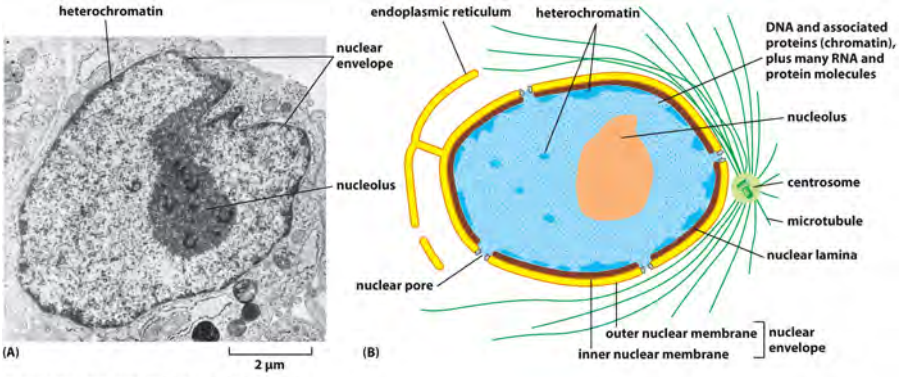


Figure 4-9 Molecular Biology of the Cell 6e (© Garland Science 2015)

DNA and accessory proteins are organized into chromosomes.

- 2m of DNA 6um diam nucleus, tight accessible
- Human 46 chroms, chromatin = DNA + protein
- Mom and dad homolog copies, X + Y + 22 pairs
- Hybridization of probe at mitosis for 'painting'
- Karyote: full chrom set at mitosis for diagnosis, each of pair stain the same way
- Junk DNA and number of chroms vary
- Mostly inserted transposons and noncoding segments in genes introns; highly disordered

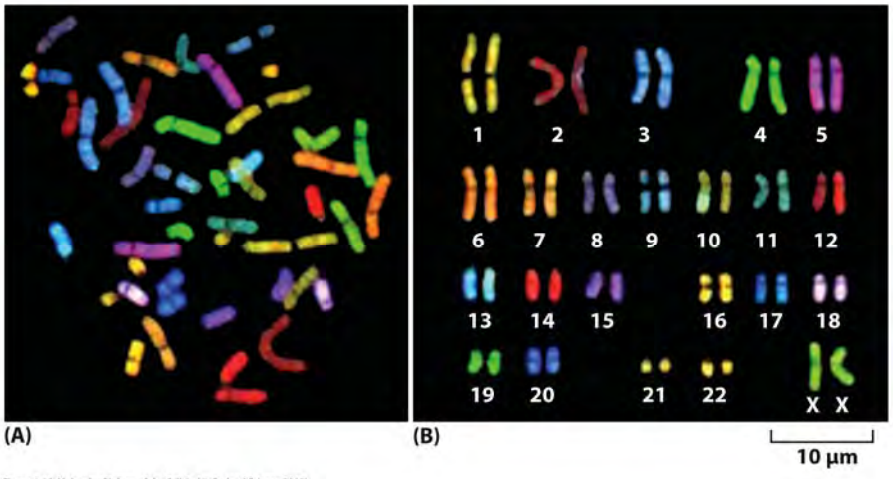
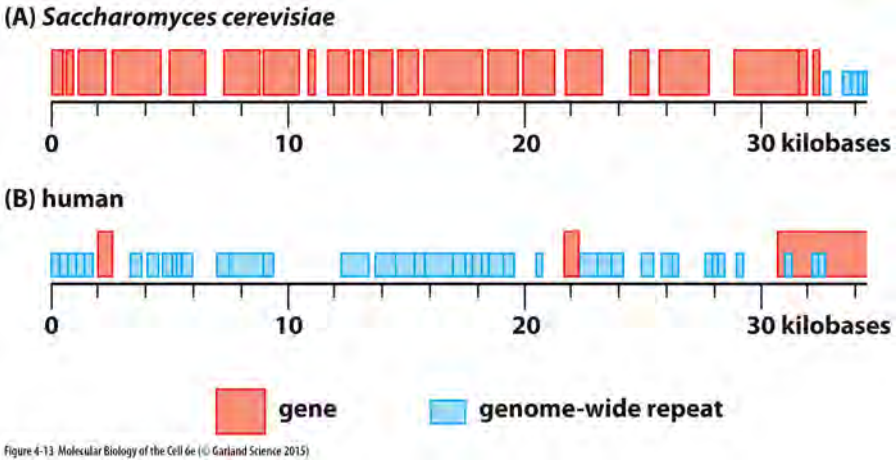


Figure 4-10 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 4-11 Molecular Biology of the Cell 6e (© Garland Science 2015)



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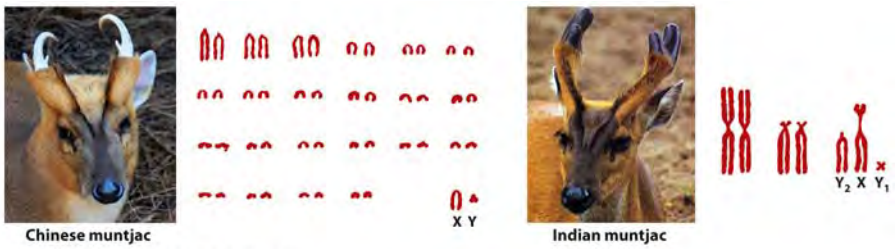


Figure 4-14 Molecular Biology of the Cell 6e (© Garland Science 2015)

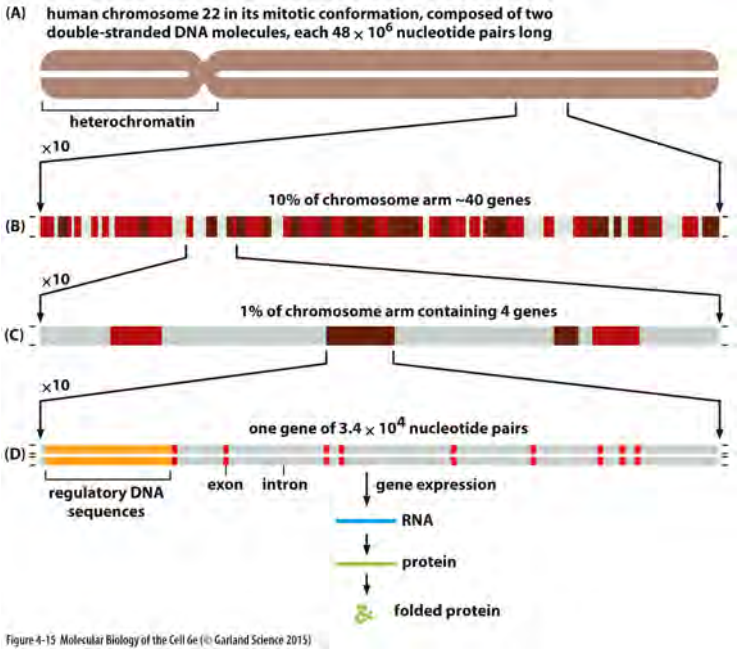


Figure 4-15 Molecular Biology of the Cell 6e (© Garland Science 2015)

Characteristic sequences of DNA are found in eukaryotes.

- Look at conserved regions for critical function
- Complexity correlates with num of genes, not size
- 1/3 of conserved code proteins, also binding sites and coding for untranslated RNA
- Species with genes in same order: synteny
- Interphase: replication, mitosis: condensed, most of time no divide thin threads expression
- Centromere: protein kinetochore attaches each chromosome to spindles -> pull apart
- Telomeres: repeat ends protect from DNA repair
- Origins of replication: at many sites in linear DNA

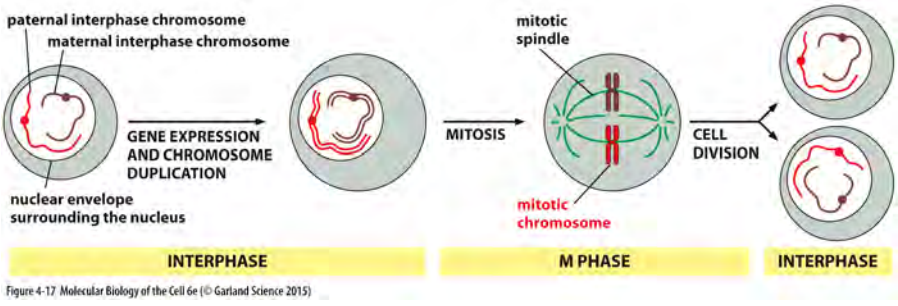


Figure 4-17 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 4-18 Molecular Biology of the Cell 6e (© Garland Science 2015)

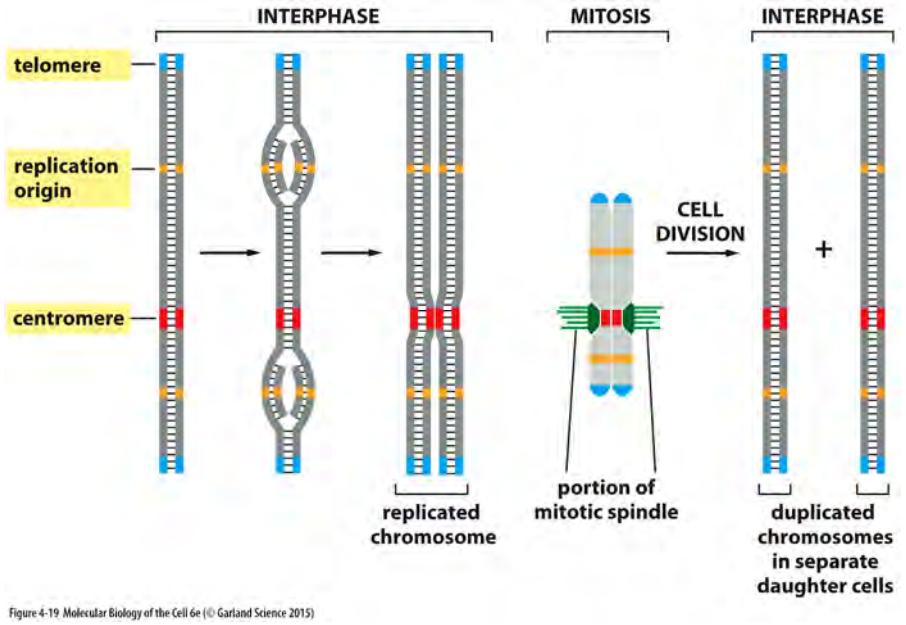
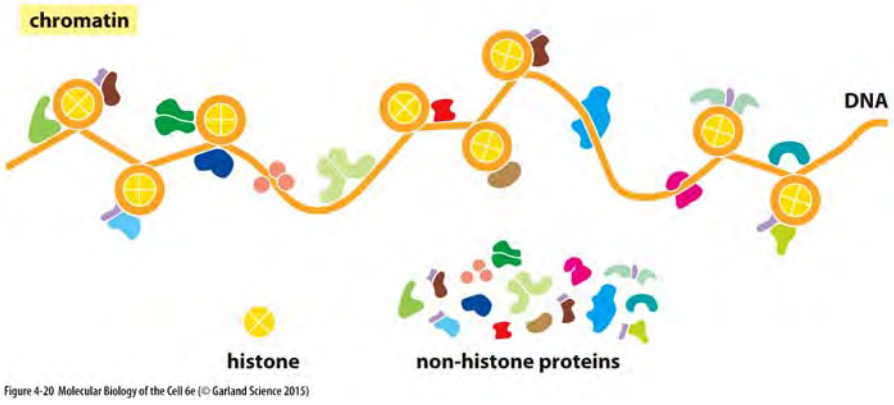


Figure 4-19 Molecular Biology of the Cell 6e (© Garland Science 2015)

Nucleosome is the basic unit of DNA organization around histone cores.

- Regional decondensation during interphase access for expression repair replicate
- Nucleosome: DNA strings on core particle beads 2x of H2A H2B H3 H4, degrade linker DNA to find repeat every 200 nucleotides
- Disc shaped core left handed coil of DNA using 2 loops 3 a-helices, H-bond amino of histones with phosphodiester of DNA, (+) charged arginine and lysine on histone with (-) charged DNA



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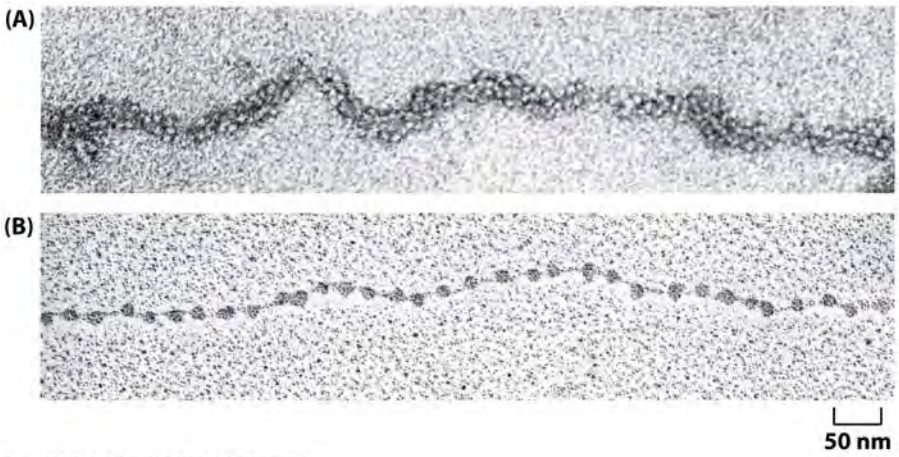

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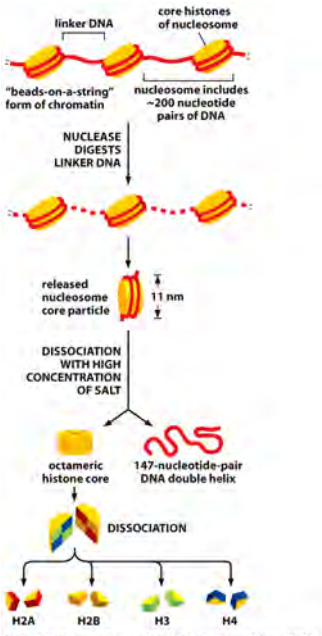

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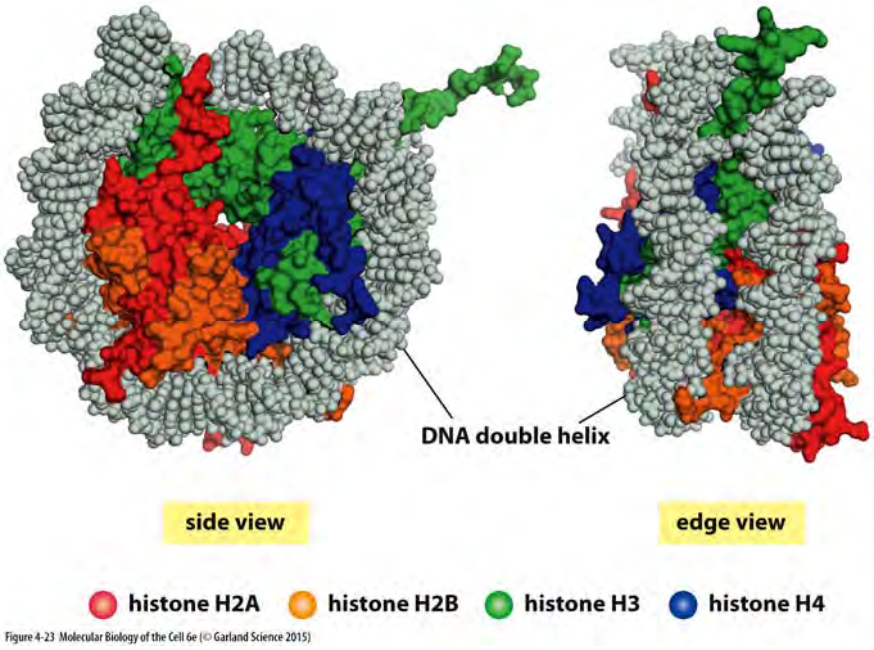

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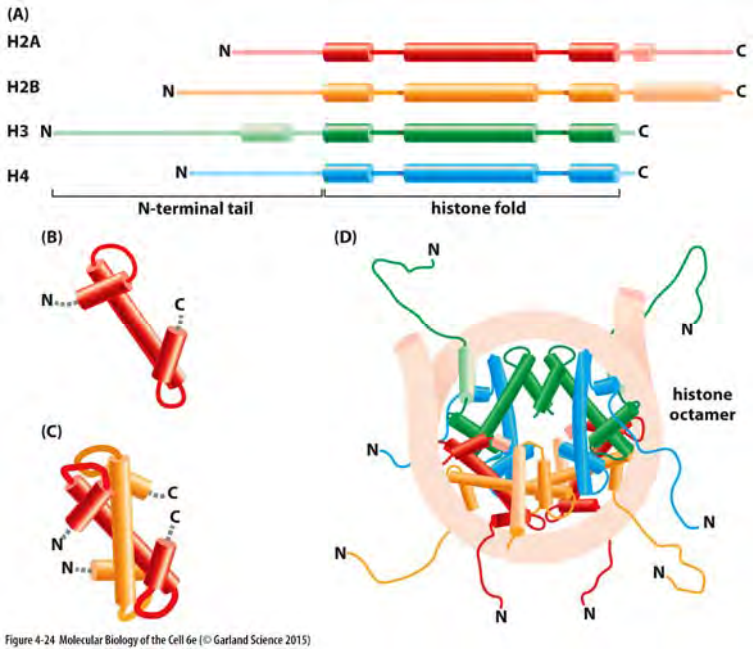
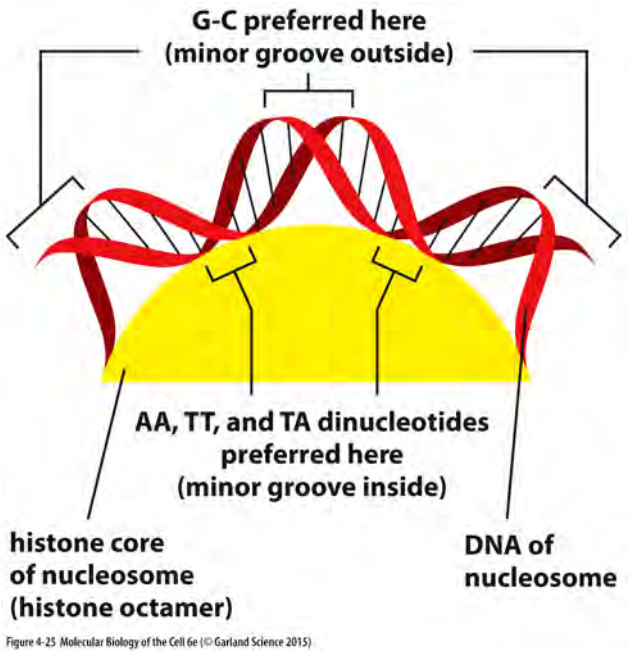


Figure 4-24 Molecular Biology of the Cell 6e (© Garland Science 2015)

Chromatin remodeling complexes bind to core, exchange histone subunits.

- Bent minor groove of DNA 1.7 turns octomer
- Covalent modification of outward protruding N-terminal tail of core histones
- Highly conserved - lethal, dedicated variants
- Chromatin remodeling complex use ATP to move DNA off the core, nucleosome sliding, work with neg chaperones to remove histones
- Nucleosome position depends on other proteins tightly bound, dynamic to cells



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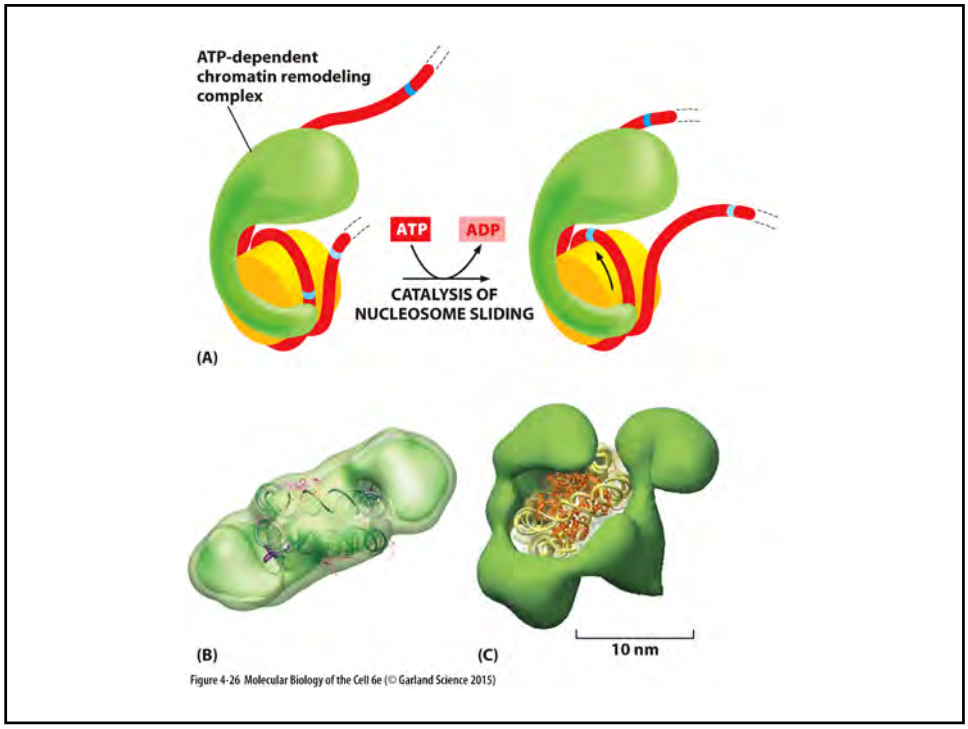

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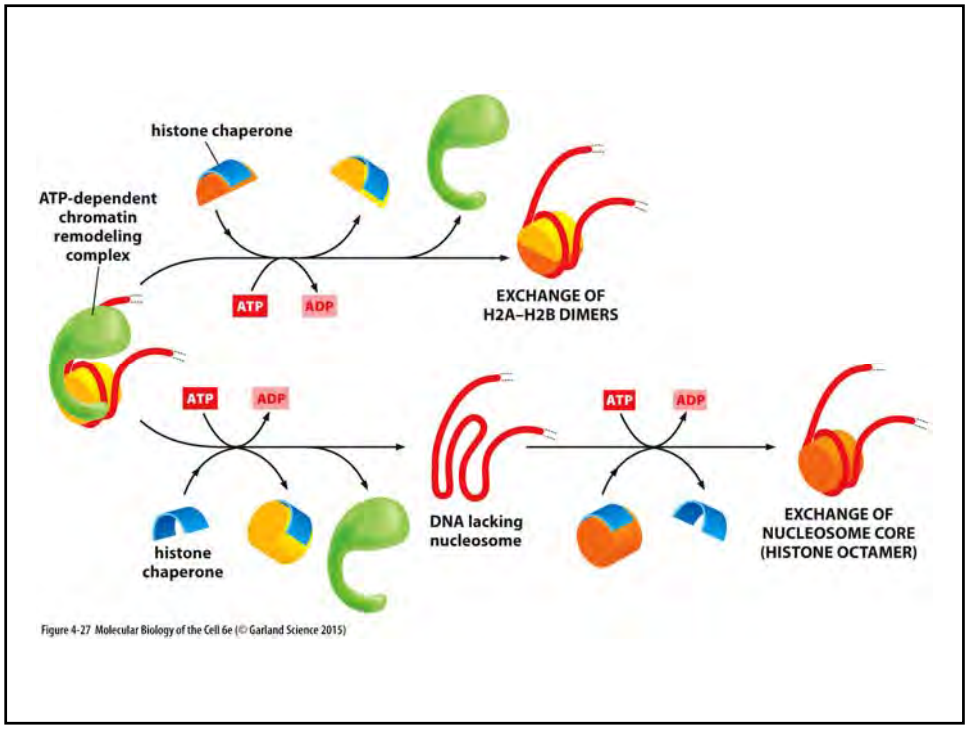
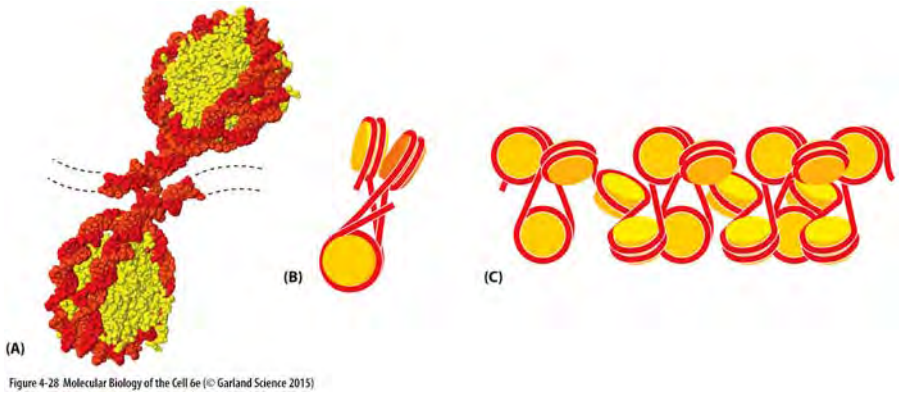


Figure 4-27 Molecular Biology of the Cell 6e (© Garland Science 2015)

Nucleosomes are packed into chromatin fiber beyond beads-strings.

- Nucleosomes packed on top of each other thicker fiber of 30nm, zig zag model of stack
- Cryoelectron microscopy supports different solenoidal intercalated nucleosome model
- Nucleosome to nucleosome H4 tail linker
- 1 core to 1 linker H1 histone less conserved, changes path of DNA as it exits nucleosome
- Variation in linker DNA length



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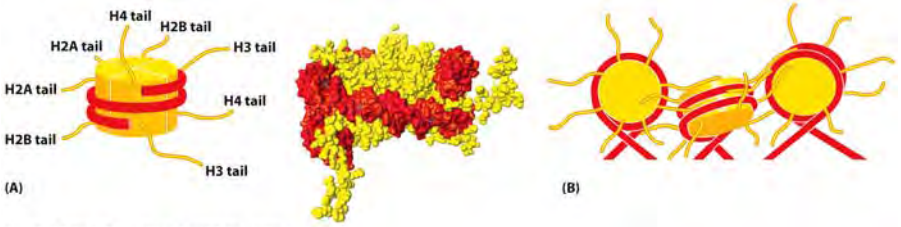

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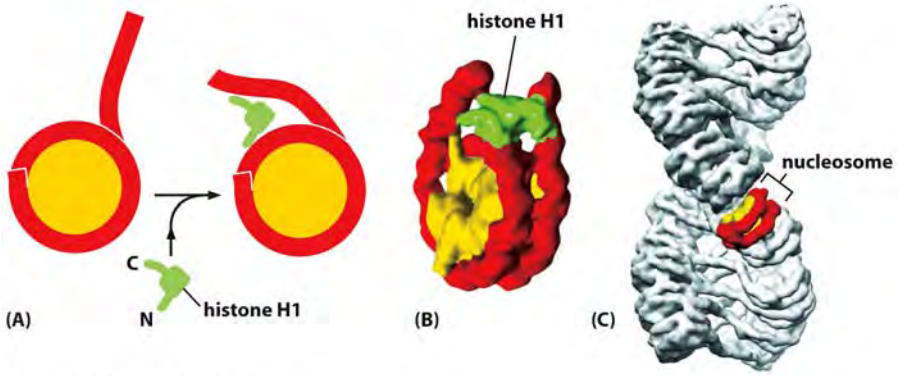
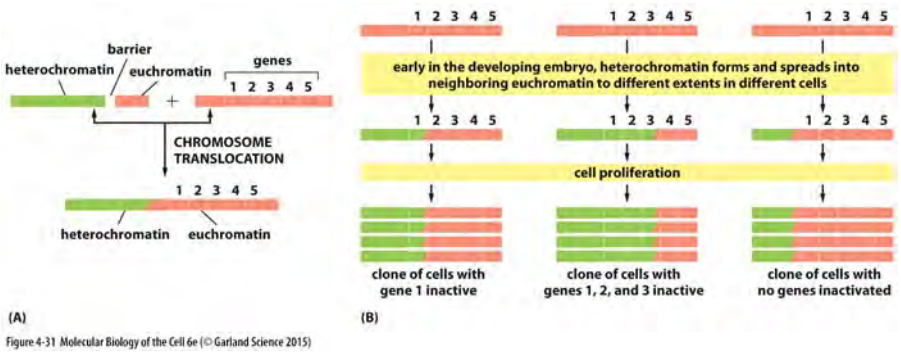


Figure 4-30 Molecular Biology of the Cell 6e (© Garland Science 2015)

Heterochromatin (condensed) is resistant to gene expression.

- Inherited chromatin structure is epigenetic i.e. beyond DNA inheritance, via covalent mod of conserved histones -> site for protein binding
- Condensed heterochromatin esp in centromere telemere very few genes express
- Position effects: expression depends on proximity to heterochromatin (silenced there)
- Position effect variegation: e.g. Female X chrom, zone of inactivation stably inherited by progeny



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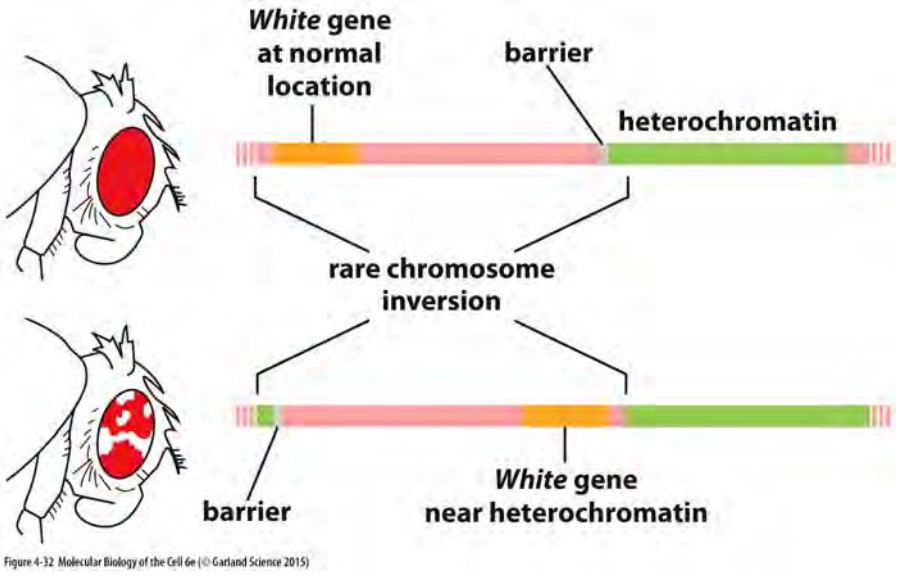
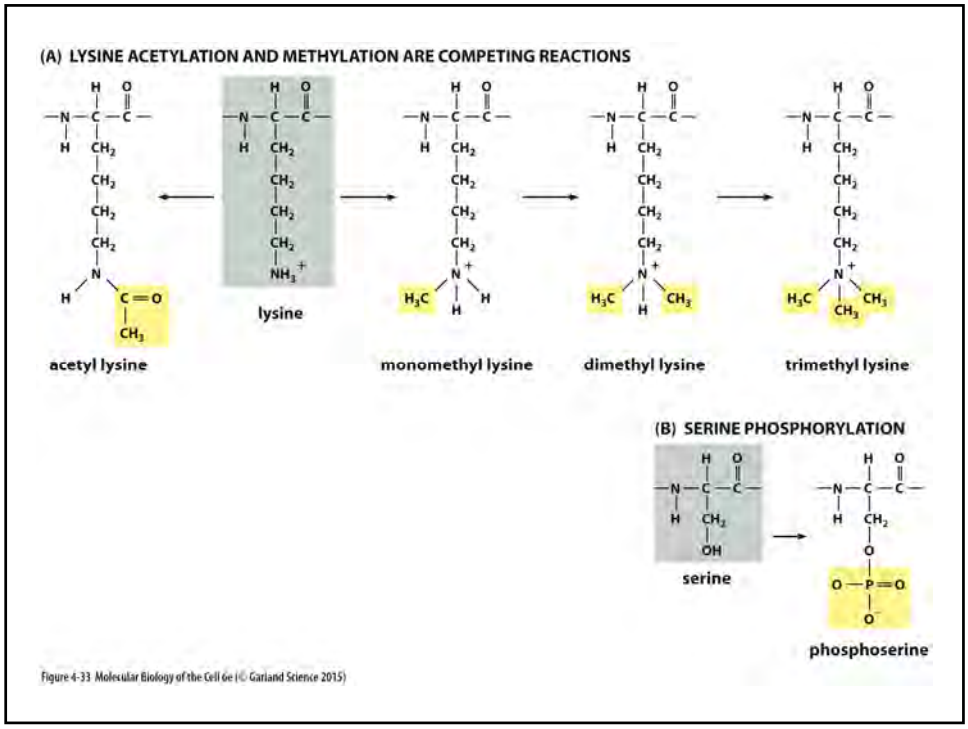


Figure 4-32 Molecular Biology of the Cell 6e (© Garland Science 2015)

Core histones and N-terminam tails can be covalently modified.

- 4 histone side chains & 8 N-term histone tails: acetylate lysine, methyl lysine, phosph serine
- Histone acetyl transferase (HATS) add acetyl to lysines, removal - deacetylase complex (HDAC)
- Reversible, recruited to chromatin at specific stage of cell by gene regulatory proteins, but modification persists after cell cont develop
- Acetylate lysine on N-term tail removes positive charge losens chromatin structure via recruited proteins that recognize the acetylation.



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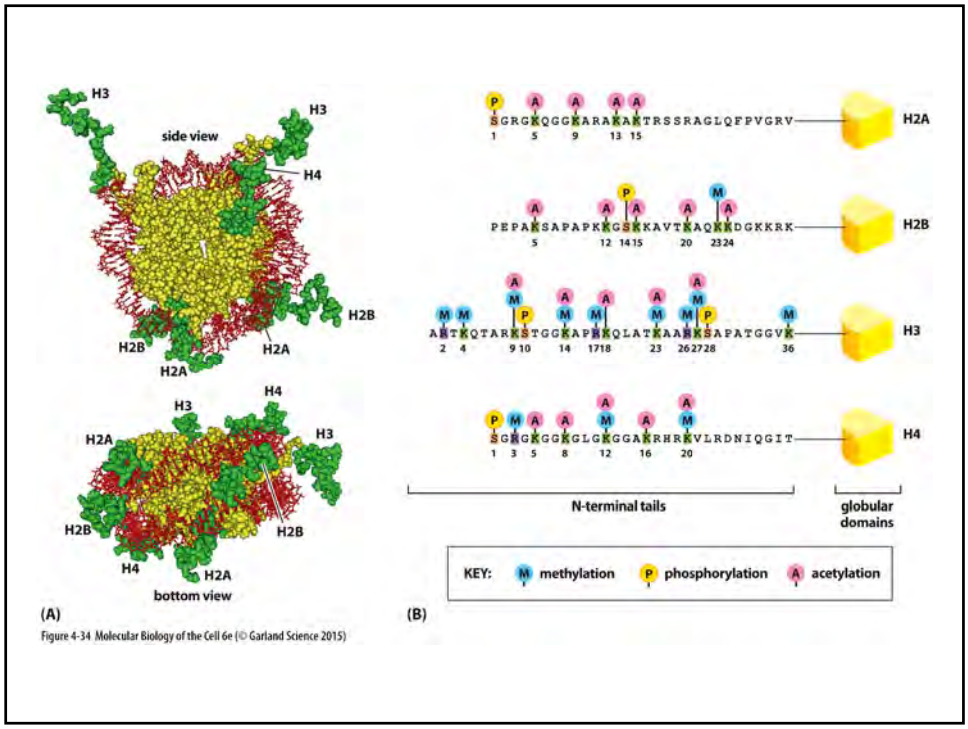
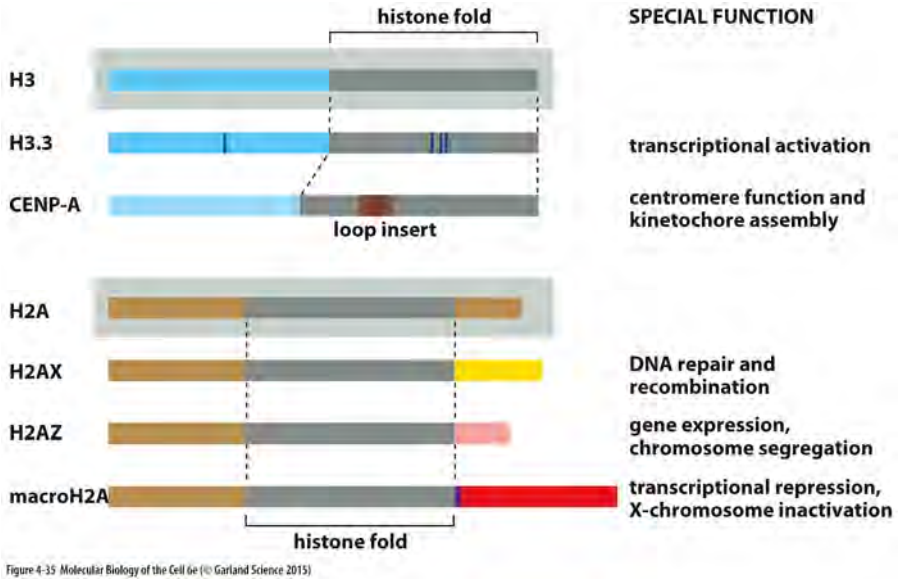


Figure 4-34 Molecular Biology of the Cell 6e (© Garland Science 2015)

Histone code marking on chromatin, spread of mods over long distance.

- Histone variants made in interphase (not like major histones in S phase), inserted via remodeling complexes into specific sites
- Histone code: marker signals like cov mods and variants recruit proteins for bio function
- Writer mark -> reader reads mark -> attached writer writes on adjacent nucleosome -> read again, ATP-dep chromatin remodeling attached to decondense stretch of chromatin



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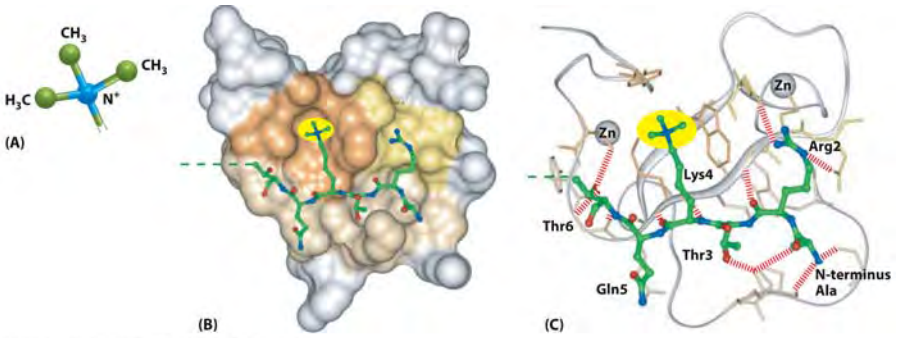

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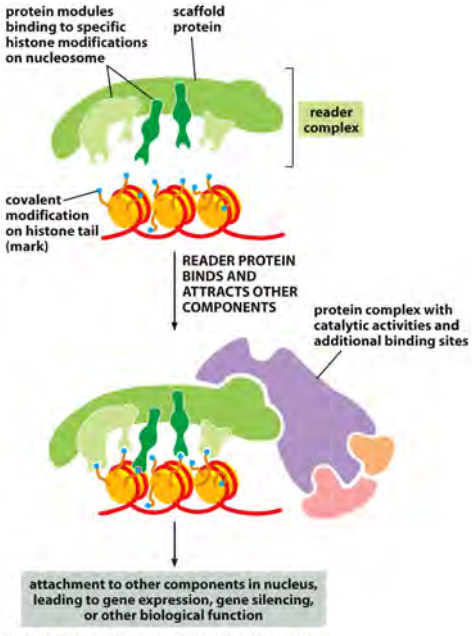

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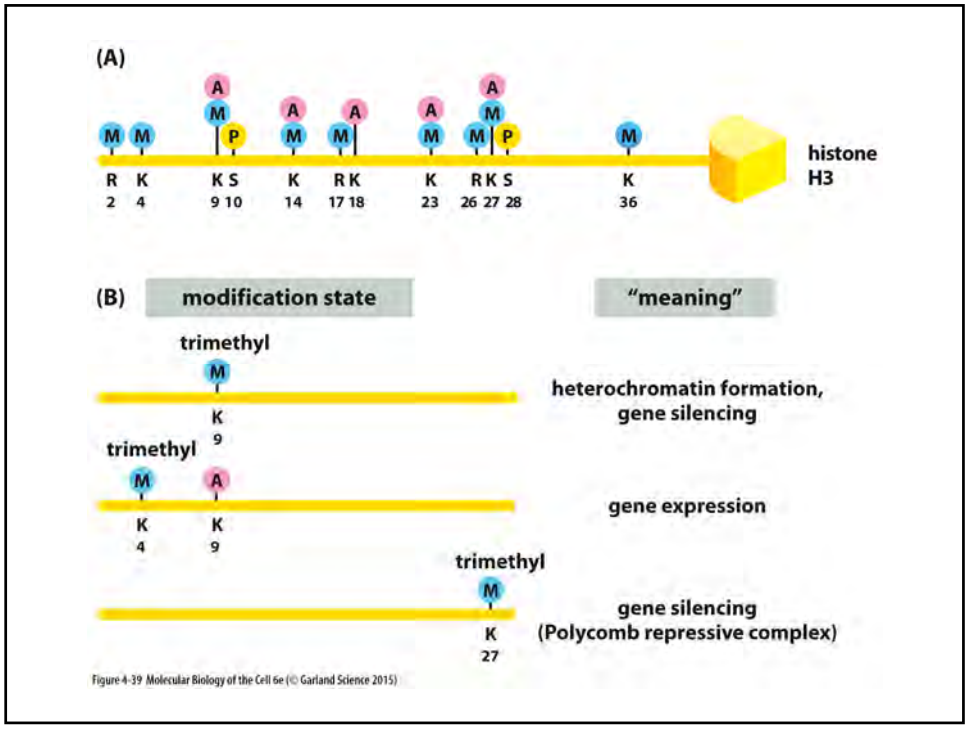

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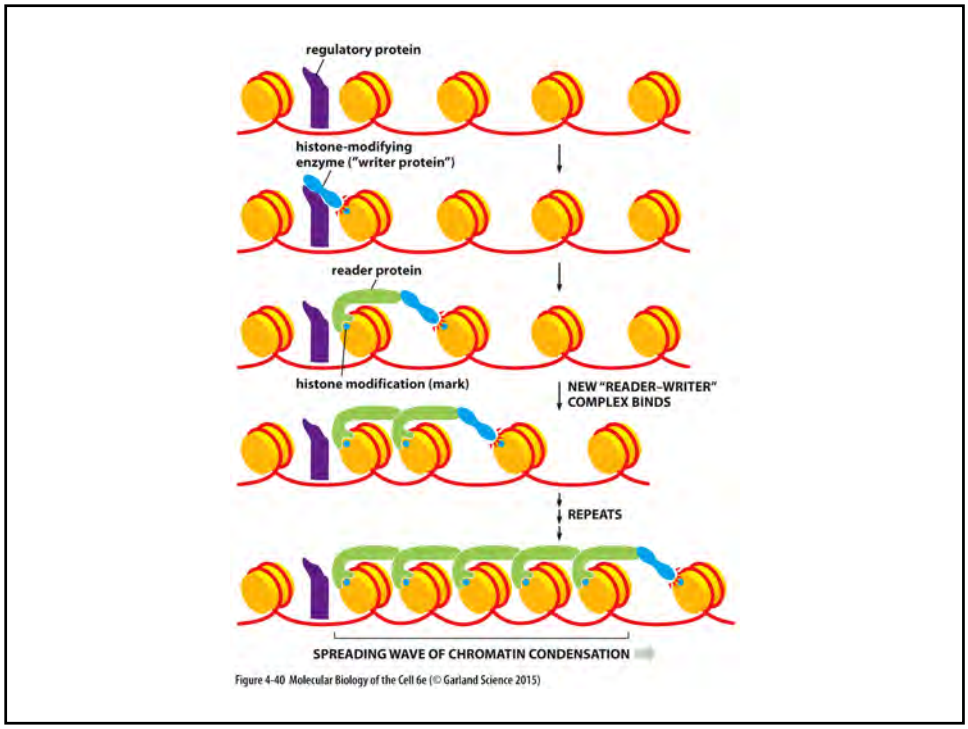
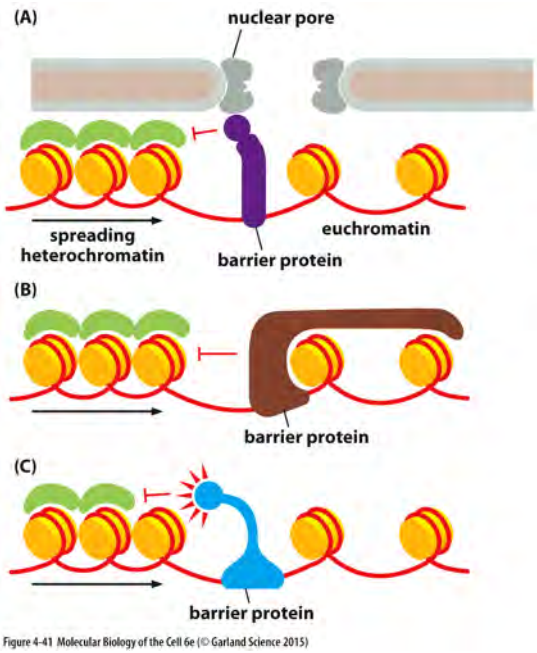


Figure 4-40 Molecular Biology of the Cell 6e (© Garland Science 2015)

Centromere protein arrangement allows kinetechore plate binding.

- Barrier sequences like HS4 separate target gene from adjacent condensed chromatin, if deleted spreading inactivation lead to gene silencing, HS4 sites for histone acetylation
- Interphase centric heterochromatin in centromeres has CENP-A histone that packs nucleosomes densely into kinetechore for spindle attachment
- Alpha satellite DNA in centromeres in humans packaged into alt blocks of chromatin CENP-A enhances seeding centromeres but not necessary, neocentromeres in fragments (no satellite DNA)



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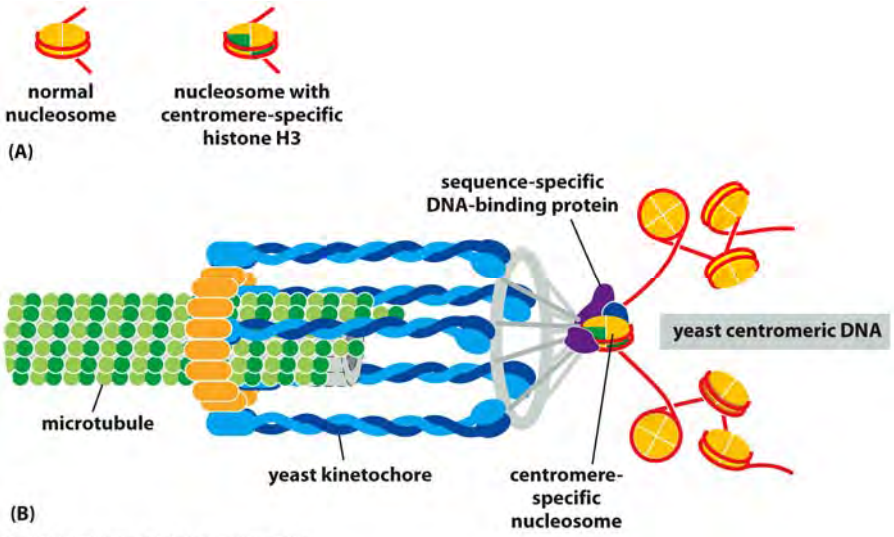

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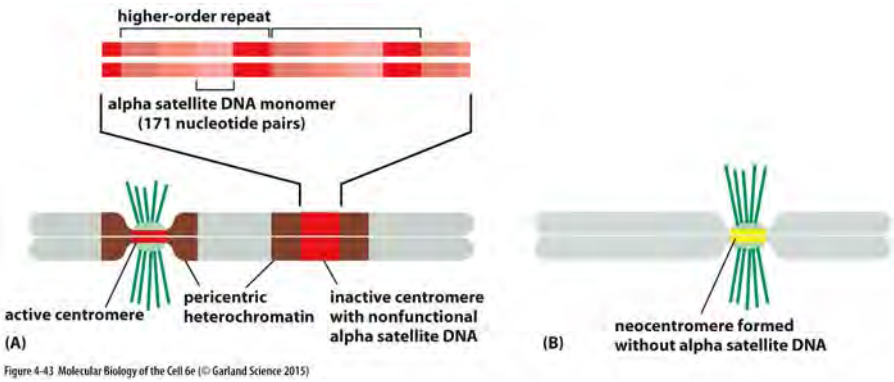


Figure 4-43 Molecular Biology of the Cell 6e (© Garland Science 2015)

Team work.

The chromatin remodeling complexes play an important role in chromatin regulation in the nucleus. They …

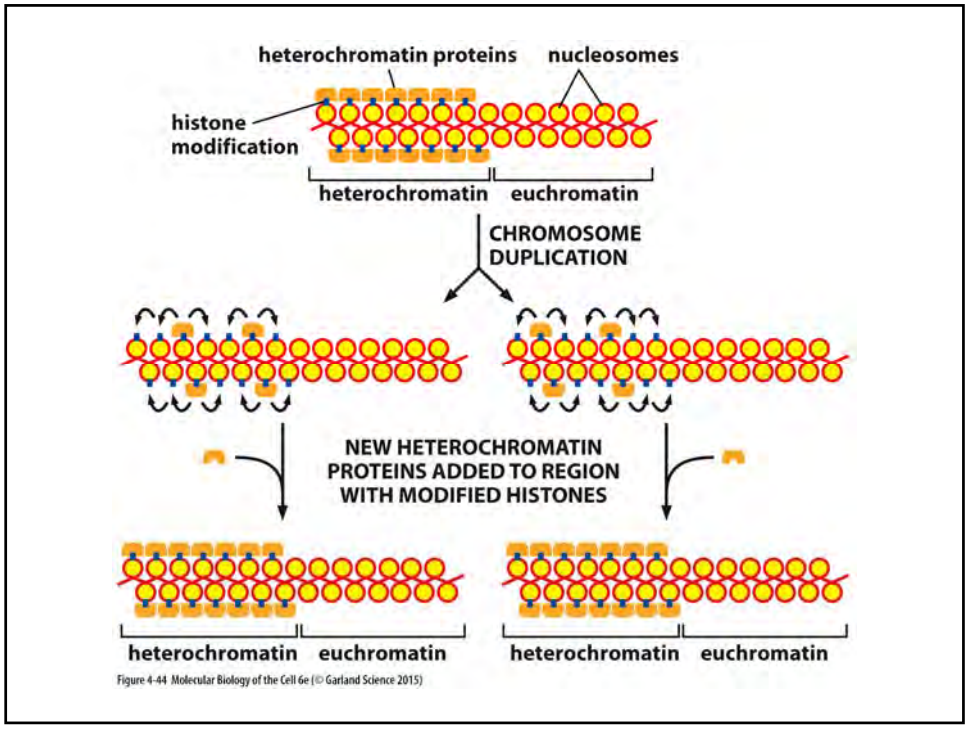
- A. can slide nucleosomes on DNA.
- B. have ATPase activity.
- C. interact with histone chaperones.
- D. can remove or exchange core histone subunits.
- E. All of the above.

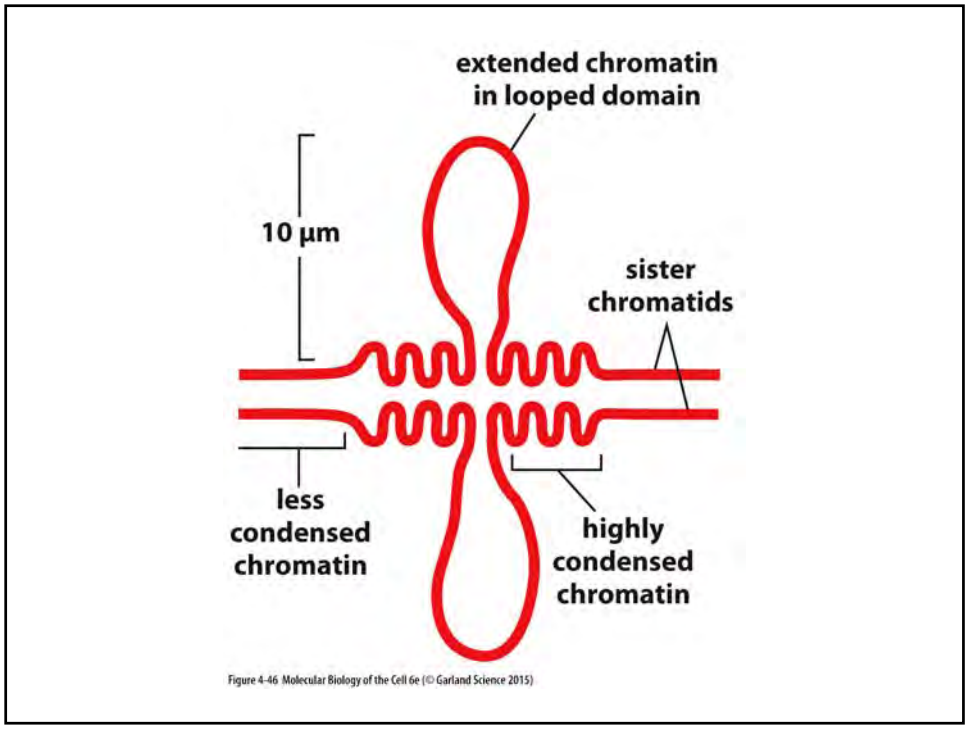
The acetylation of lysines on the histone tails …

- A. is sufficient for the formation of an open chromatin structure.
- B. loosens the chromatin structure because it adds positive charges to the histone.
- C. recruits the heterochromatin protein HP1, resulting in the establishment of heterochromatin.
- D. can be performed on methylated lysines only after they are first demethylated.
- E. is a covalent modification and is thus irreversible.

From chromatin structure to chromosome structure.

- Seeding event on alpha satellite DNA -> CENP-A H3 histone -> centromere made & inherited
- Memory of genes and local chromatin structure, inherited centric heterochromatin
- Further folding of 30nm fiber into loops coils
- Most DNA found in nonexpress chromomeres on axis, loops of 50000 to 200000 nucleotides
- Determine loop position by chromosome conformation capture 3C to find link location


heterochromatin proteins
nucleosomes
histone modification
heterochromatin
euchromatin
CHROMOSOME DUPLICATION
NEW HETEROCHROMATIN PROTEINS ADDED TO REGION WITH MODIFIED HISTONES
heterochromatin
euchromatin
heterochromatin
euchromatin
Figure 4-44 Molecular Biology of the Cell 6e (© Garland Science 2015)


extended chromatin in looped domain
10 µm
sister chromatids
less condensed chromatin
highly condensed chromatin
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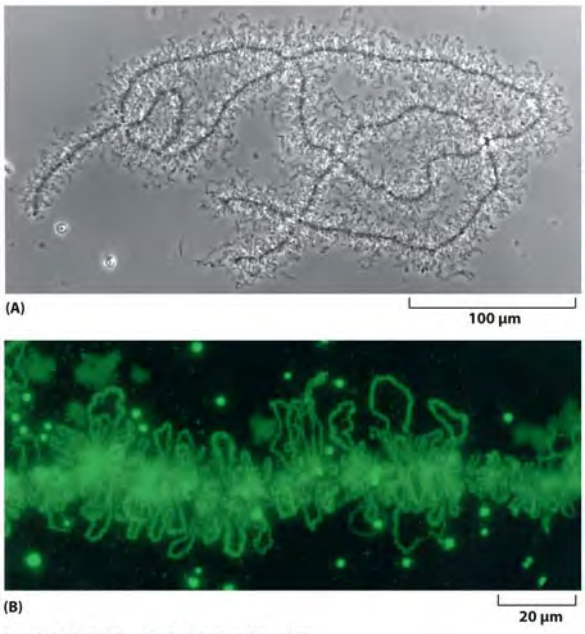

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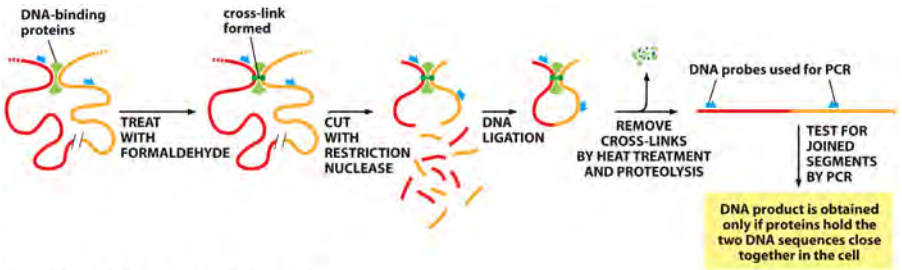

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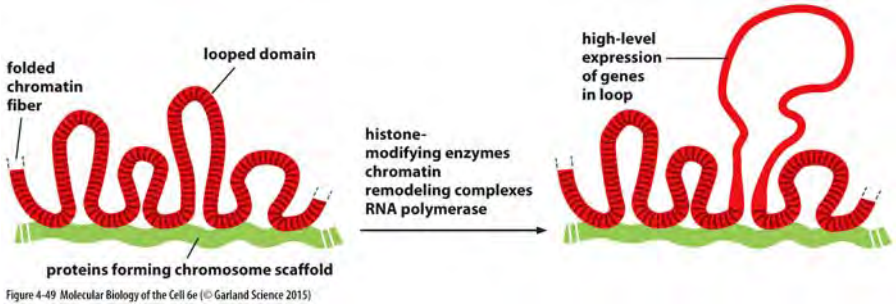


Figure 4-49 Molecular Biology of the Cell 6e (© Garland Science 2015)

Chromosomes move around nucleus when their genes are expressed.

- Polytene chromosomes contain many copies, easy to study due to distinctive banding
- Most DNA in bands condensed, antibody label
- Multiple forms of heterochromatin: polycomb form proteins in nonoverlapping PcG proteins
- Loops decondense - puffs for gene expression
- Each chromosome its own area attached to lamina, assembly extends when transcribed
- Nuclear regions mark by inositol phopholipids

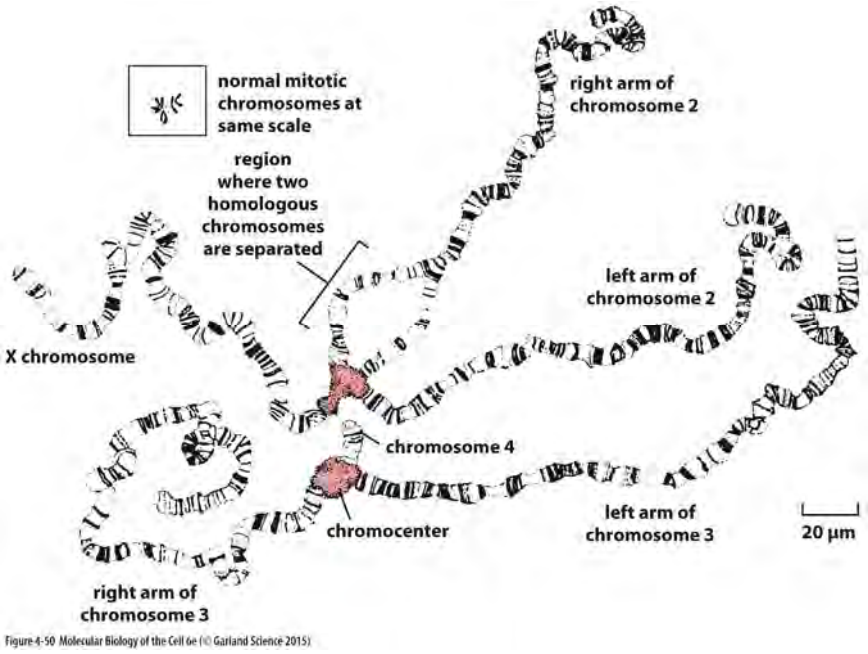


Figure 4-50 Molecular Biology of the Cell 6e (© Garland Science 2015)

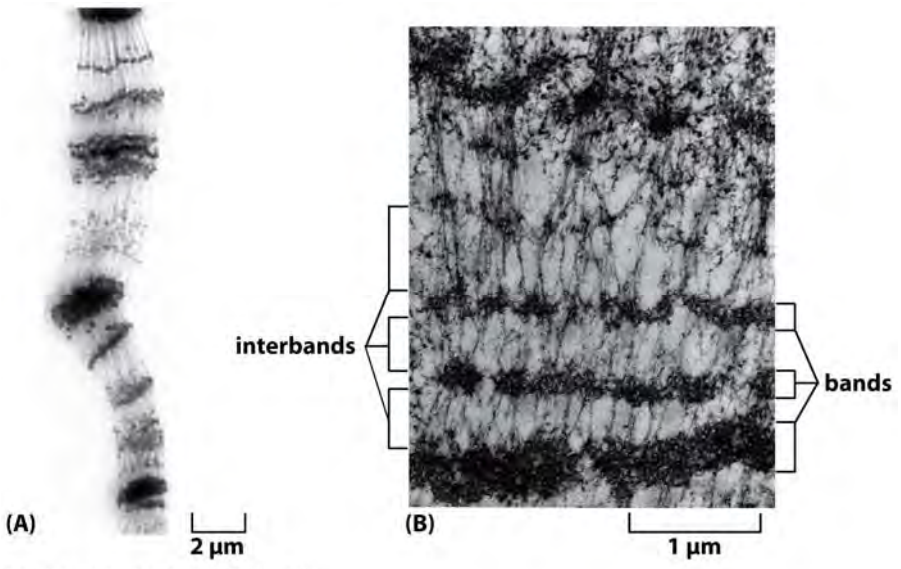
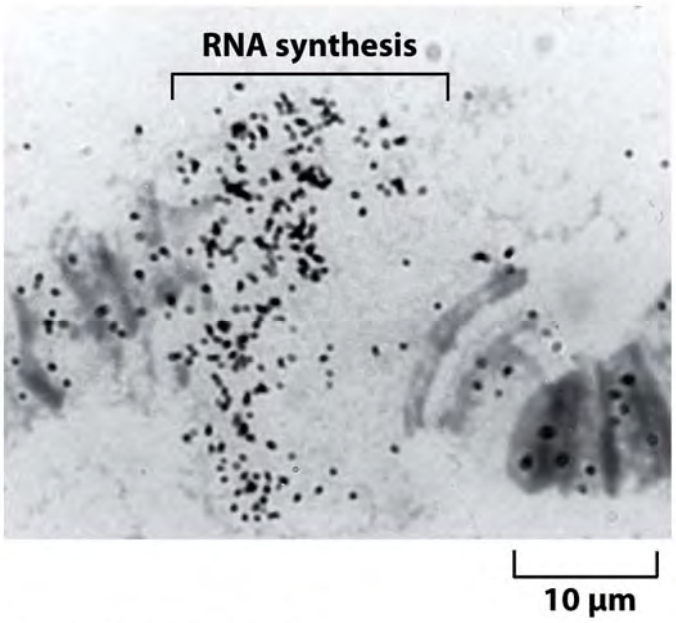


Figure 4-51 Molecular Biology of the Cell 6e (© Garland Science 2015)



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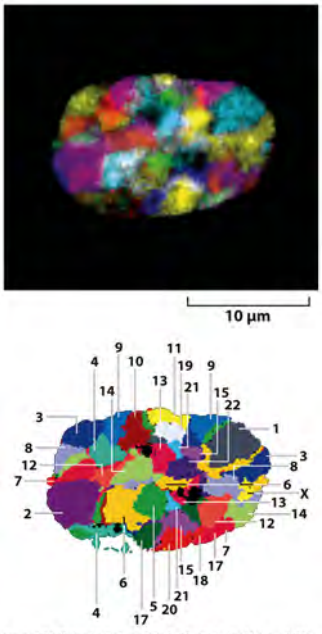

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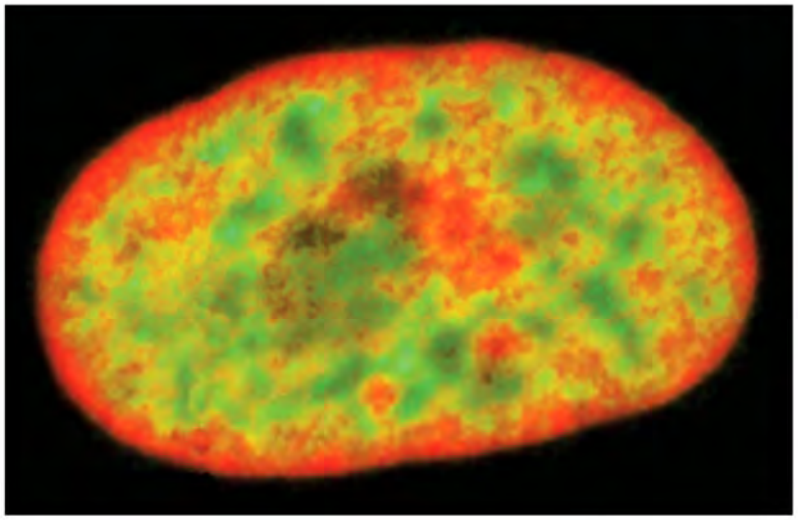

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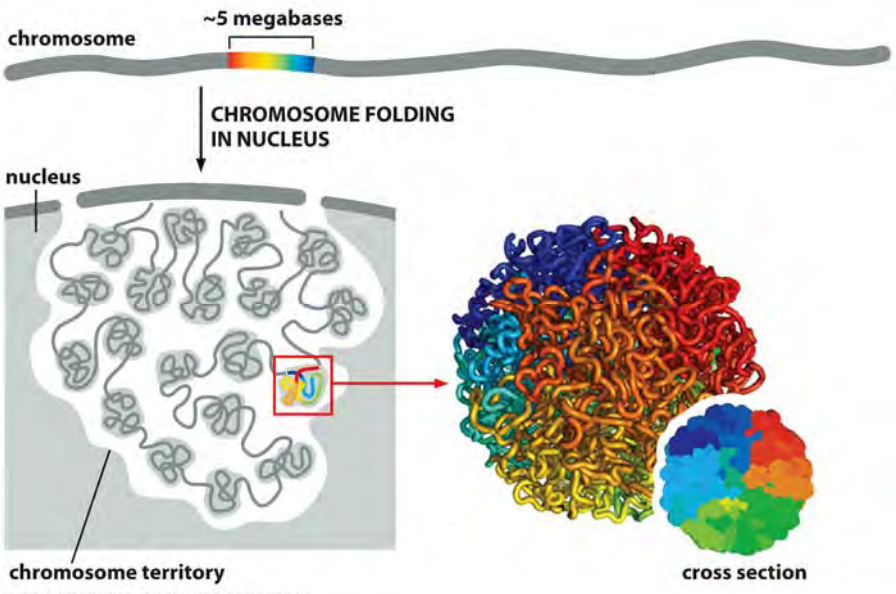

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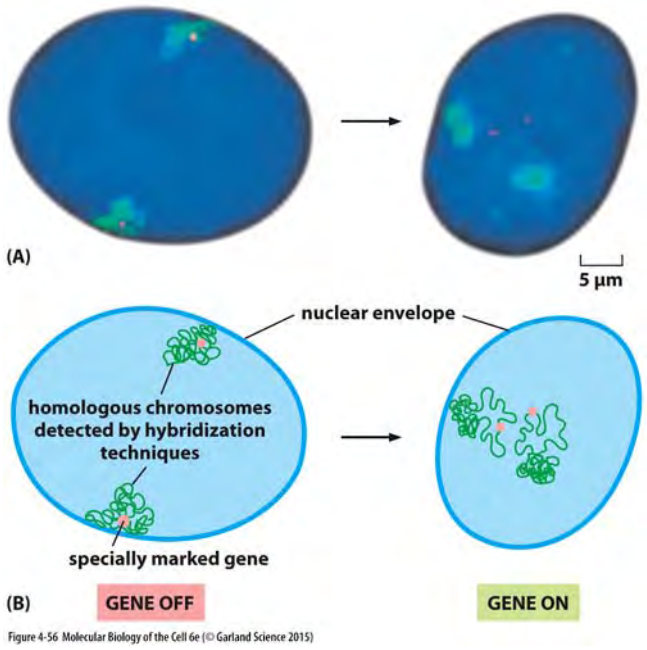
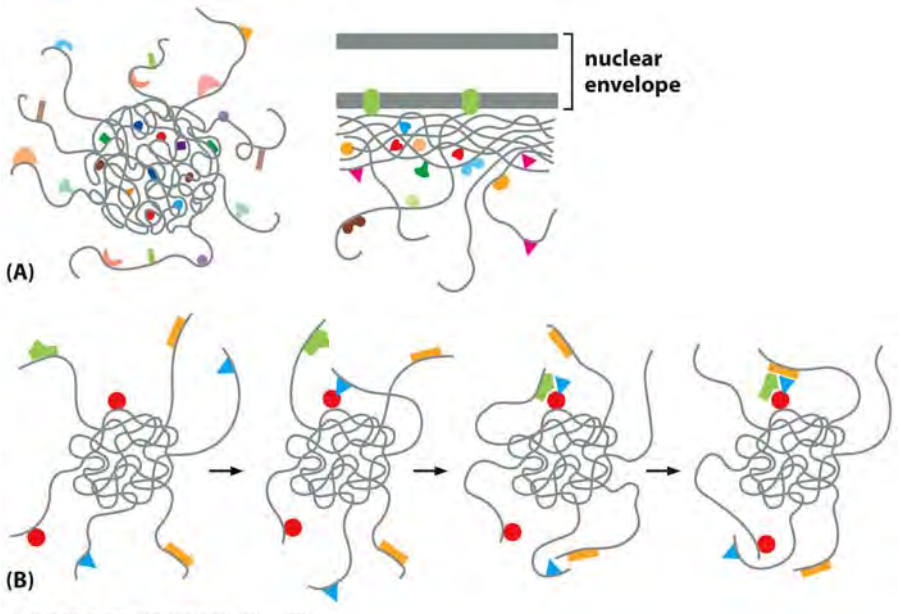


Figure 4-56 Molecular Biology of the Cell 6e (© Garland Science 2015)

Nucleus subcompartmentalization is selective and organized in 'gel'.

- Nucleolus: ribosomal RNA assembly RNAs
- Subcompartment forms only when needed
- Cajal bodies, interchromatin granule clusters
- Concentrate macromolecules RNA envelopes pores to local efficient reaction compartment
- Polypeptide tethering of proteins and RNA for increased efficiency; nuclear scaffold matrix
- Mitosis sister chromatids lined up, condensins (SMC dimers) use ATP to coil DNA into right handed loops, protect fragile DNA

Figure 4-57 Molecular Biology of the Cell 6e (© Garland Science 2015)



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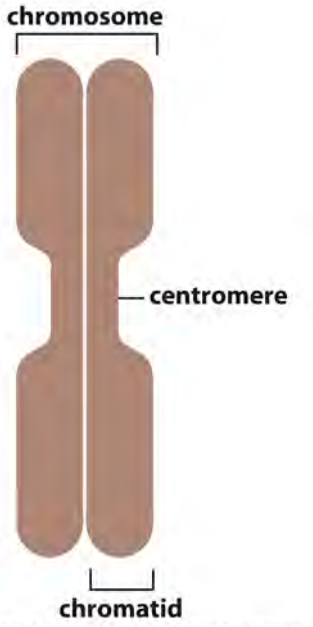

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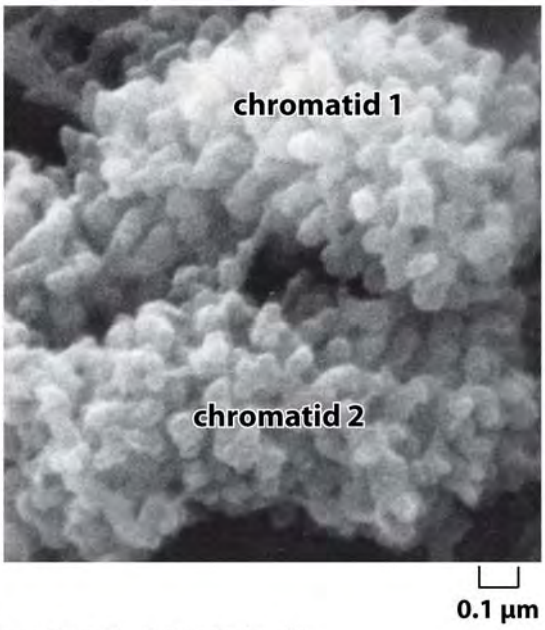

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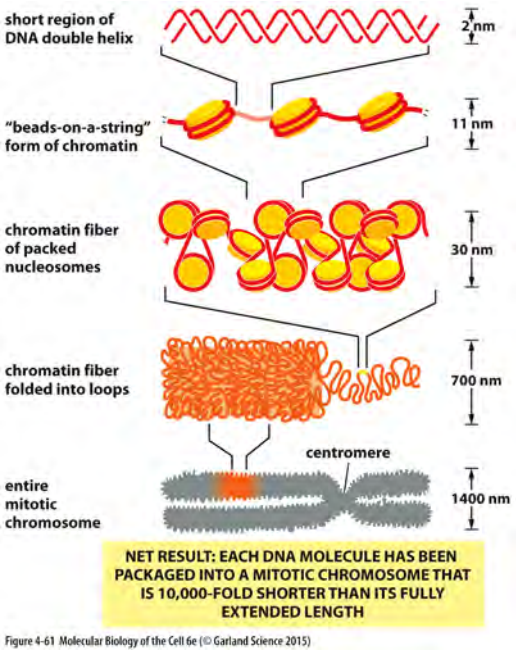


Figure 4-61 Molecular Biology of the Cell 6e (© Garland Science 2015)

Mutations in stable and unstable DNA sequences reveal phylogenicity.

- Homologues to study human based on others
- Genome alterations from errors in replication, recombination, repair, or transposition (half?)
- Phylogenetic tree of species divergence
- Purifying selection eliminates indiv with mutations that interfere with critical functions
- Molecular clock fast on introns pseudogenes and slow on constrained genes; rapid change means positive selection; time resolution
- Genome size increase due mostly to transposons

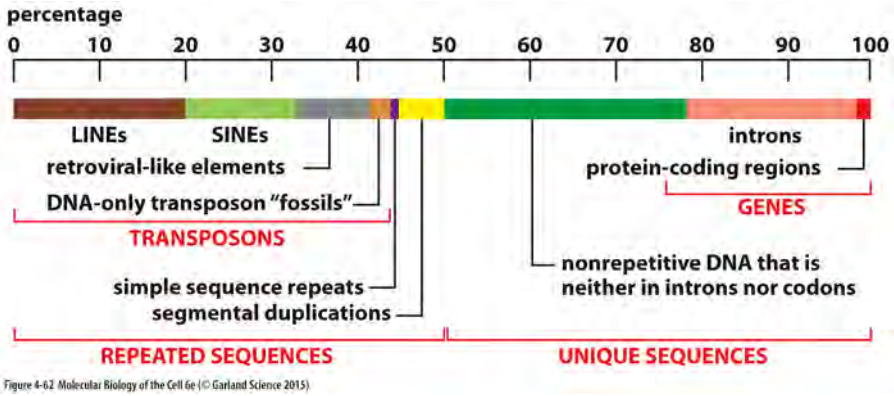
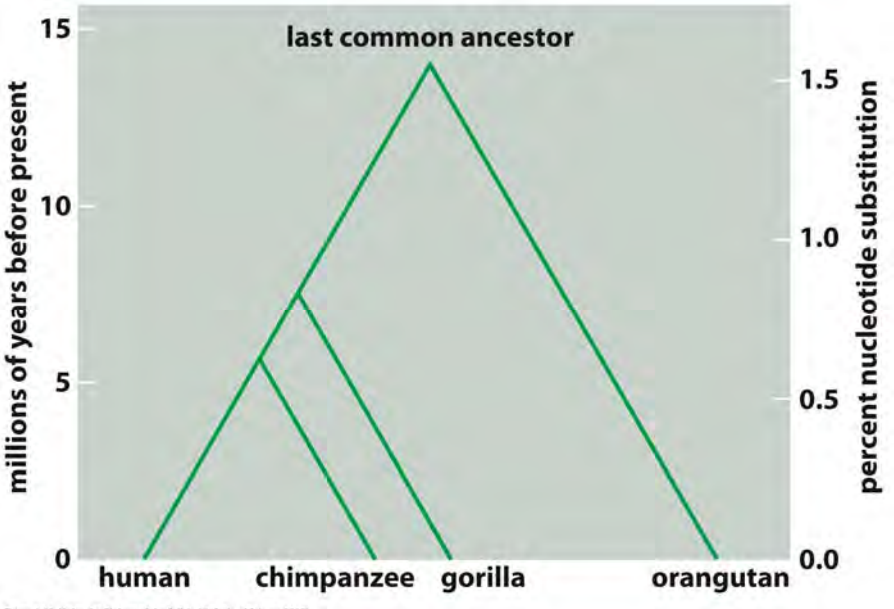


Figure 4-62 Molecular Biology of the Cell 6e (© Garland Science 2015)



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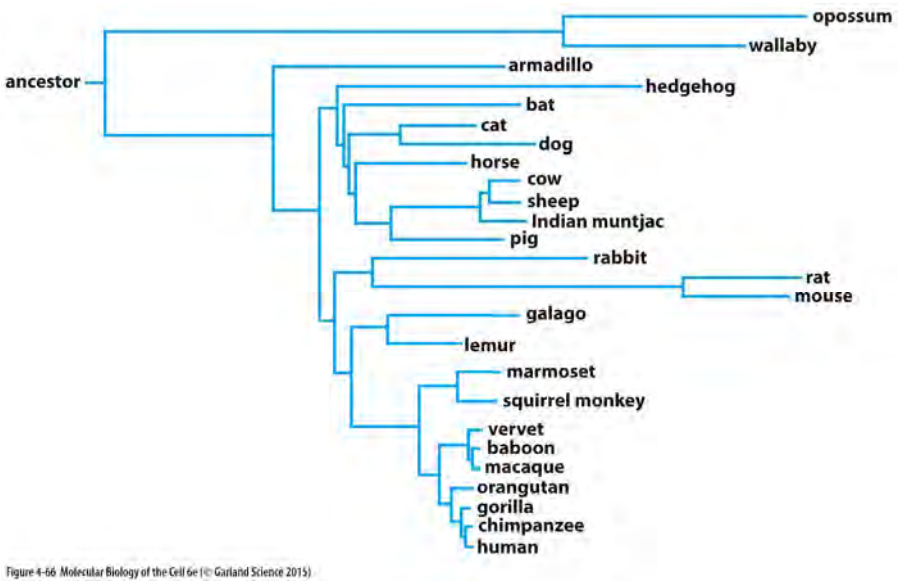
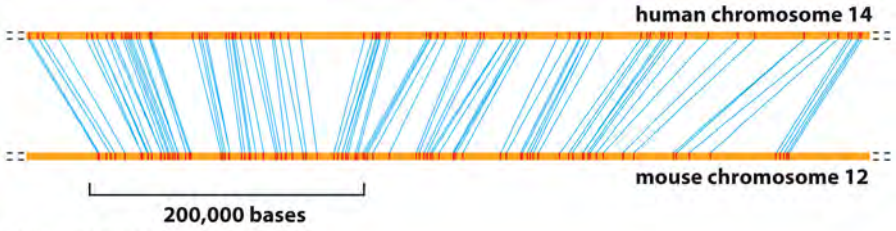


Figure 4-66 Molecular Biology of the Cell 6e (© Garland Science 2015)

Speed of evolution of genes provides clues to evolutionary lineage.

- Mouse genome evolved faster, break-and-join to 20 chromosomes, synteny conserved order
- Rapid deletion and insertion, small amt coding
- DNA addition slow -> cleansing of *fugu* genome -> small introns but positions same
- Can infer ancient genomes of extinct species
- Multispecies conserved non-protein sequences are short, for untranslated RNA and regulation
- Human accelerated regions (HARs) fast changing seq that mark specifically human, neural develop



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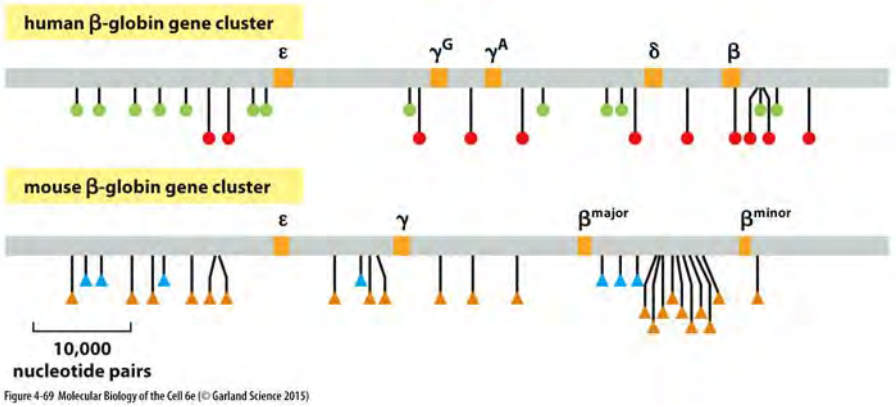
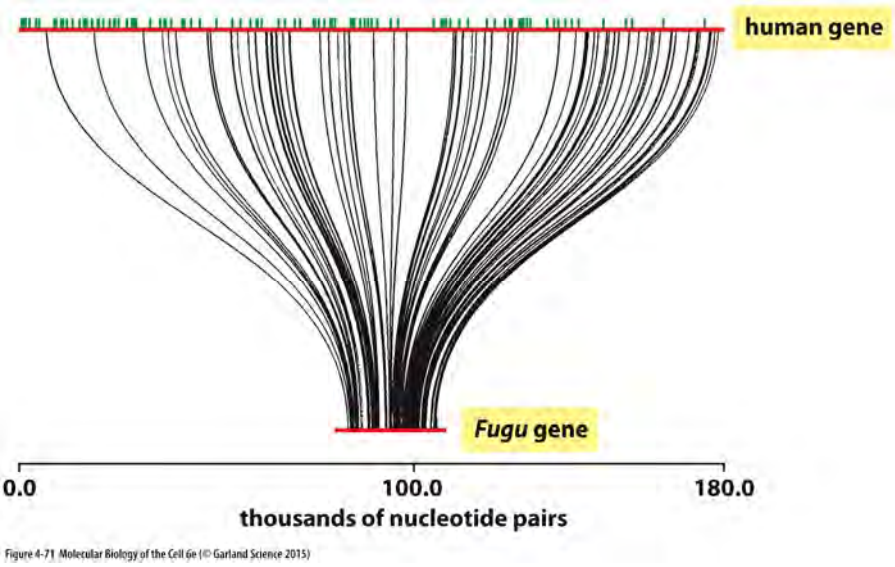


Figure 4-69 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 4-70 Molecular Biology of the Cell 6e (© Garland Science 2015)

Net DNA LOSS over time -> cleansing of only nonessential seq



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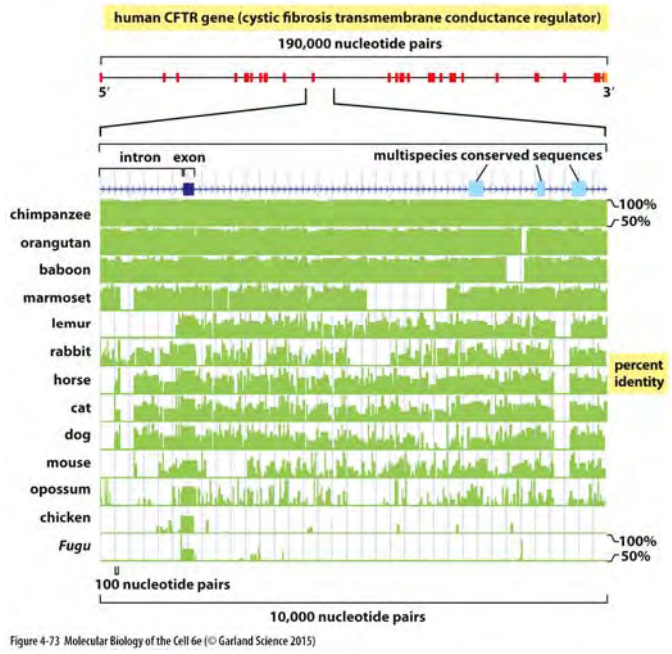
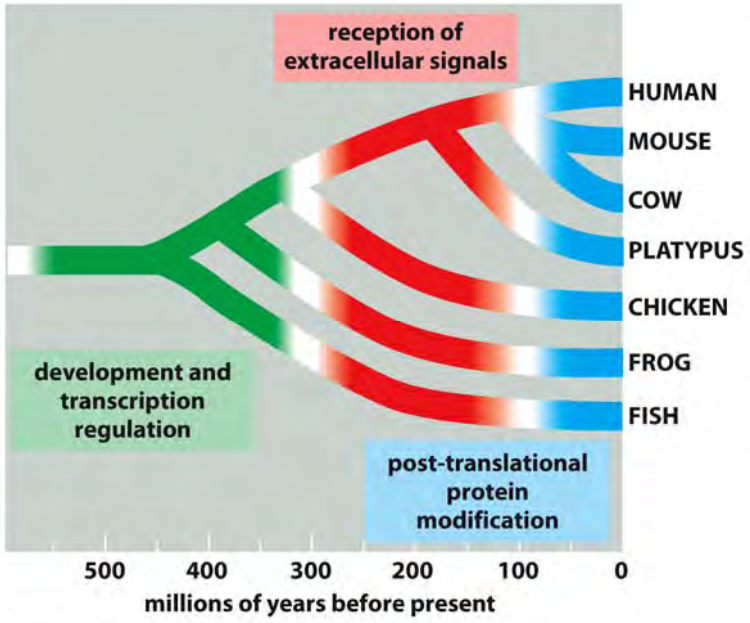


Figure 4-73 Molecular Biology of the Cell 6e (© Garland Science 2015)

Divergence by duplication and specialization in human genes.

- Gene duplication -> each take on diff function, lead to DNA addition biggest contribute to diff
- Duplicate and mutate one copy to noncoding pseudogene or diverge in expression pattern
- Whole genome duplication -> specialization (loss in diff type of tissue for each duplicate)
- Single chain to alpha and beta forms in globin gene -> more efficient, beta duplicate to form more tight gamma in infants, mutate again to eta and gamma forms for even earlier development, also changes in regulation, alpha on diff chrom



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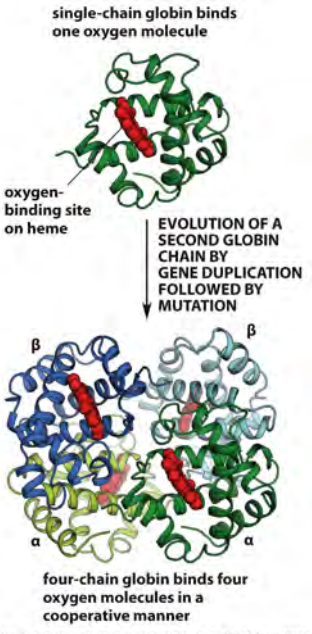


Figure 4-75 Molecular Biology of the Cell 6e (© Garland Science 2015)

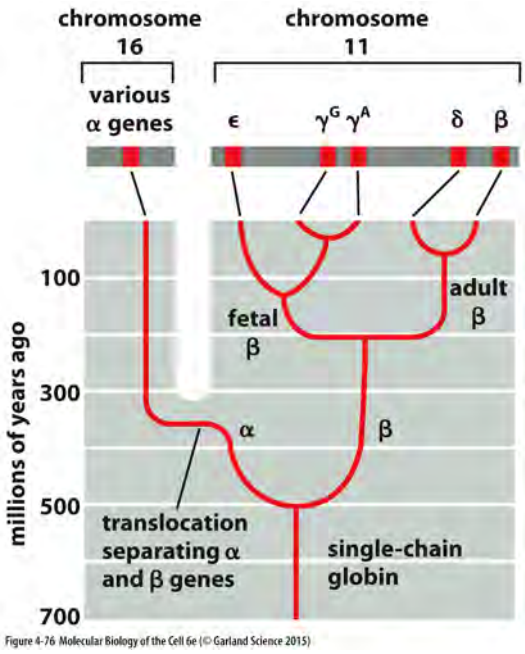


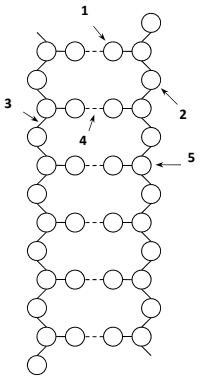
Figure 4-76 Molecular Biology of the Cell 6e (© Garland Science 2015)

Variations in DNA repeats contribute to diversity of indiv genomes.

- Recombination of exons break at intron ends
- Neutral mutations can spread slowly and be fixed modeled assuming constant population size and random mating
- Single nucleotide polymorphisms (SNPs) are points in human population where some have one others two nucleotides, polymorphic
- (CA)n low fidelity repeats maintain variability

Team work.

- What is indicated by (5) in the schematic drawing below of the DNA double helix?



- A. Phosphate group
- B. Covalent linkage
- C. Hydrogen-bonding
- D. Nitrogen-containing base
- E. Deoxyribose sugar