BME 42-620 Engineering Molecular Cell Biology

Lecture 15:

Chromosome Organization & DNA Packaging

Chapter 4

Review: Reading Assignments 1 & 2

- Reading assignment 1
  - Protein folding: Mean = 17.3 STD = 2.1 MAX = 20 MIN = 12
  - Fluorescence microscopy: Mean = 17.2 STD = 2.1 MAX = 20 MIN = 12

- Reading assignment 2
  - Cell motility: Mean = 17.5 STD = 1.3 MAX = 20 MIN = 15
  - Molecular motors: Mean = 16.9 STD = 1.2 MAX = 20 MIN = 15

- Comments
  - Design a clear/logical structure for your presentation. Avoid simple clustering of technical details.
  - Make efforts to ensure accuracy and rigor in your presentation.

Outline

- Structural and functional organization of chromosomes
- DNA packaging and structural organization
- Overview of kinetochore structure and functions

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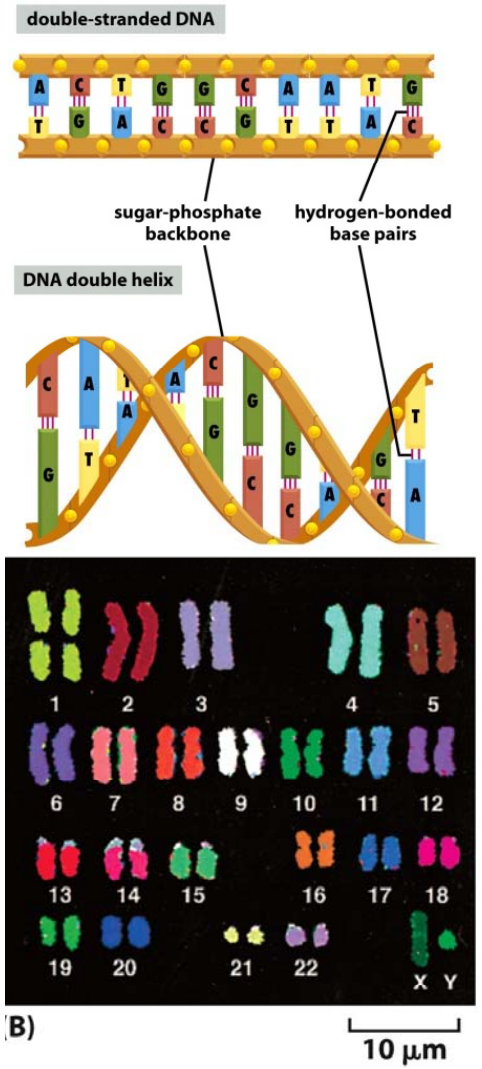
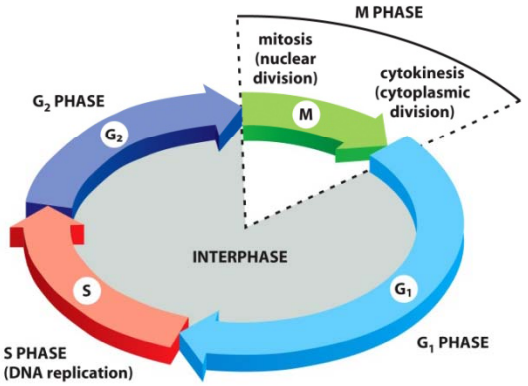
The Problem

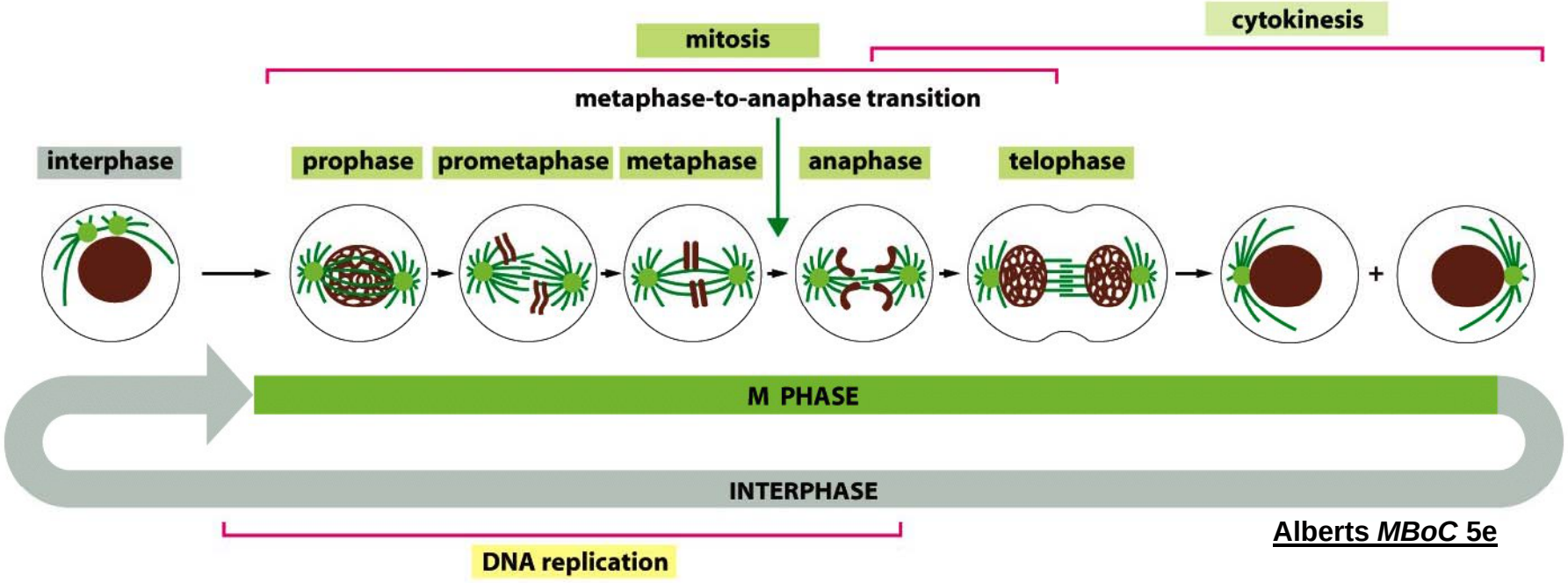
- Spacing between base pairs ≈3.4Å
- For human genome, approximately 3.2 billion base pairs
- Total length ≈ $3.4\times10^{-10}\times3.2\times10^{9}\times2 \approx 2.2$m
- Diameter of a nucleus: $5\sim10\times10^{-6}$m
- Access to genetic information must be regulated.



Chromosome Organization in the Cell Cycle

- DNA are packaged with associated proteins into chromosomes.
- Chromosomes undergo dramatic reorganization when cells divide.





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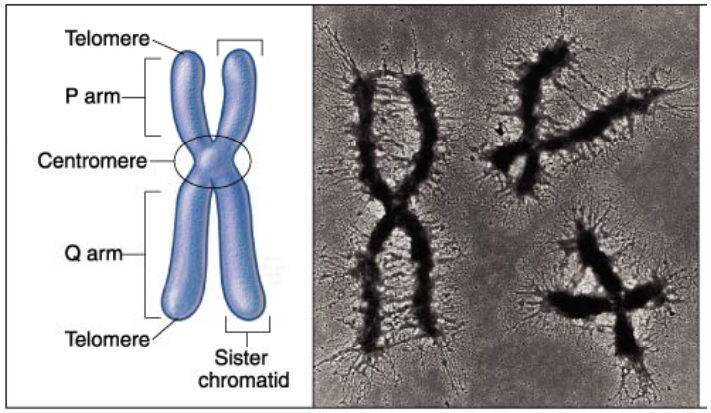
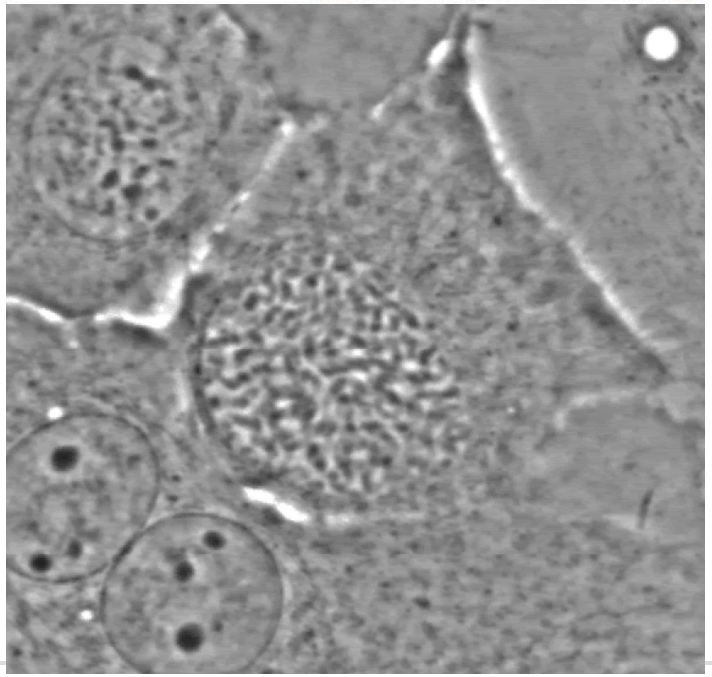
Chromosome Related Nomenclature (I)

- Chromatin: the complex of DNA, histones, and nonhistone proteins within the nucleus of a eukaryotic cell (DNA persistence length ~50nm). The material of which chromosomes are made.

- Chromatid: one of the two copies of a replicated chromosome that is joined at the centromere to the other copy. The two identical chromatids are called sister chromatids.

- Centromere: the chromosomal region that holds sister chromatids together and where the kinetochore forms.

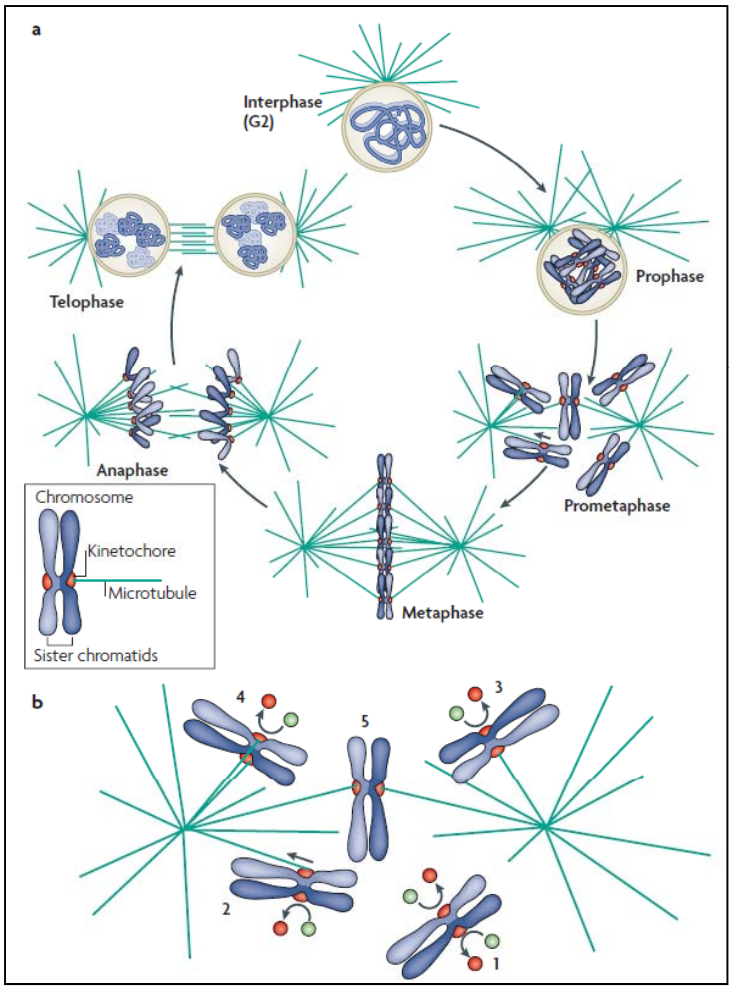




Chromosome Related Nomenclature (II)

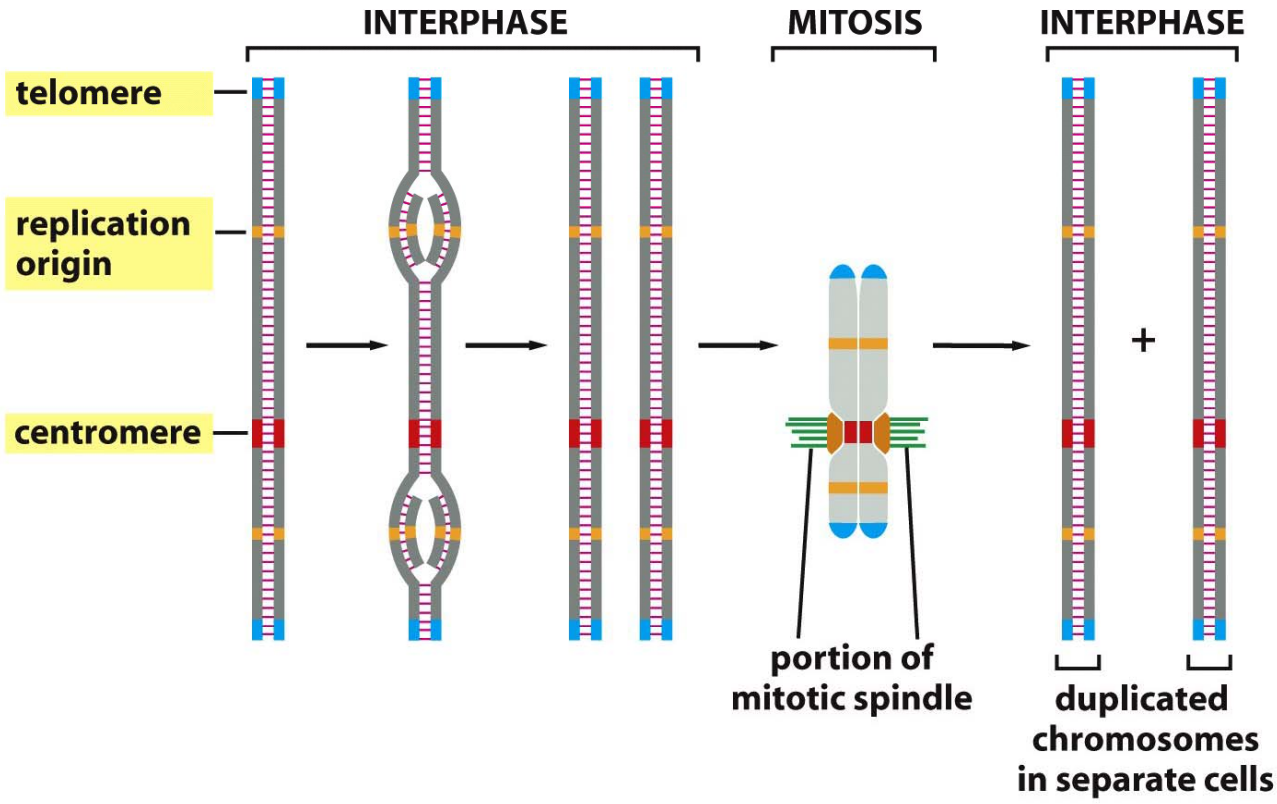
- Kinetochore: the centromeric substructure that binds microtubules and directs chromosome movement in mitosis.

- Gene: a segment of DNA encoding a functional RNA or protein product.



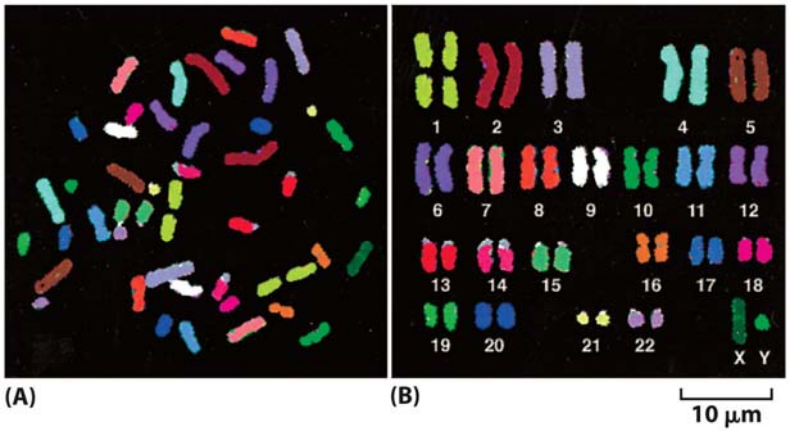
Cheeseman & Desai, *Nat. Rev. Mol. Cell Biol.* 9:33, 2008

General Organization of Chromosomes



An Overview of Human Chromosomes

- Human genome
  - 3.2×10^9 bp
  - distributed over 24 chromosomes
  - 20,000~25,000 protein coding genes



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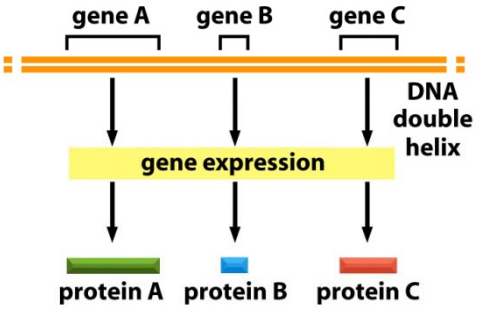
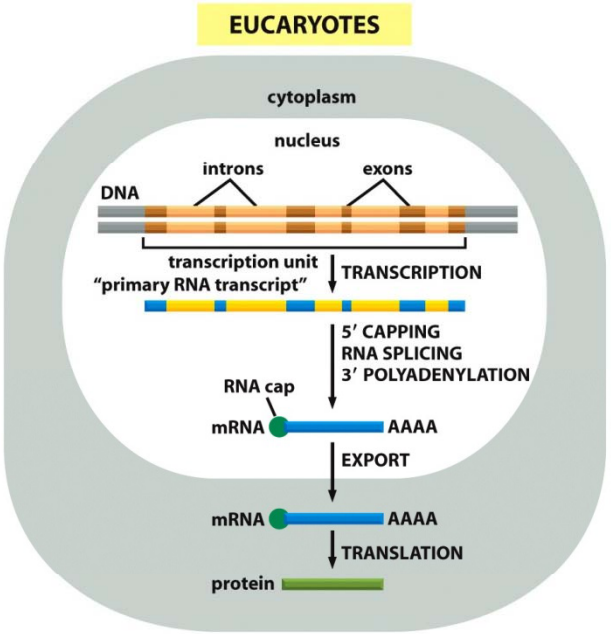


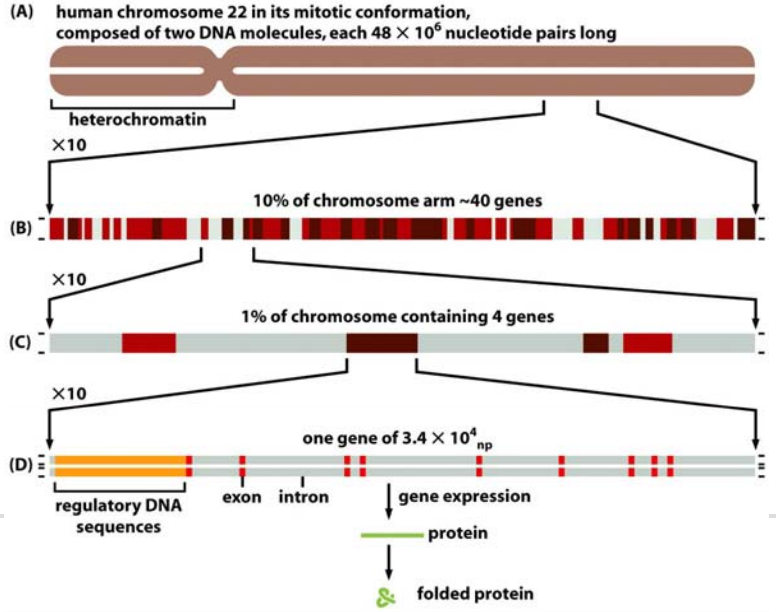


Organization of Human Chromosomes (I)

- Bacterial genomes make very efficient use of space. Coding sequences occupies 90% of the genome .
- For human genome, protein-coding genes occupies 1.5% of the genome.
- Human genes range in size from several hundred bp to one million bp, with an average of ~27,000 bp.

Table 4–1 Some Vital Statistics for the Human Genome

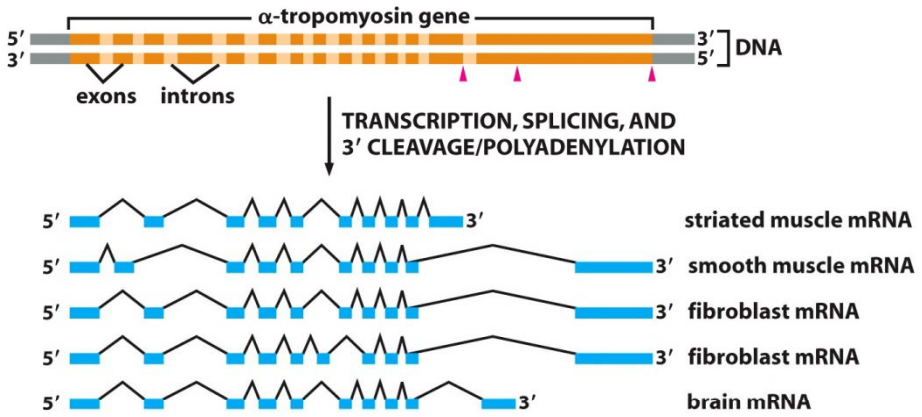
	HUMAN GENOME
DNA length	3.2×10^9 nucleotide pairs*
Number of genes	approximately 25,000
Largest gene	2.4×10^6 nucleotide pairs
Mean gene size	27,000 nucleotide pairs
Smallest number of exons per gene	1
Largest number of exons per gene	178
Mean number of exons per gene	10.4
Largest exon size	17,106 nucleotide pairs
Mean exon size	145 nucleotide pairs
Number of pseudogenes**	more than 20,000
Percentage of DNA sequence in exons (protein coding sequences)	1.5%
Percentage of DNA in other highly conserved sequences***	3.5%
Percentage of DNA in high-copy repetitive elements	approximately 50%



Organization of Human Chromosomes (II)

- Number of genes per one million bp

  - 483 in budding yeast
  - 197 in C. elegans
  - 117 in fruit fly
  - 7~9 in human



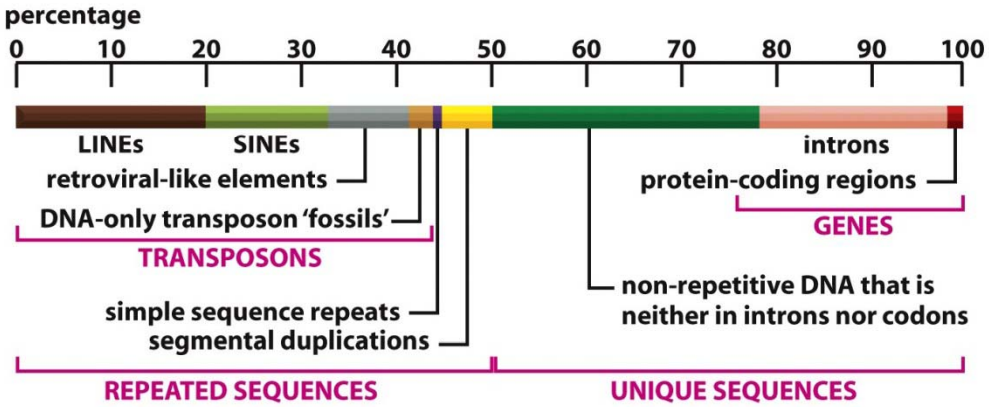
- Distributions of genes on chromosomes are highly variable.

  - On human chromosome 9, 3~22 genes per one million bp
  - One region on human chromosome 21 with 7×10^6 bp has no genes.

Organization of Human Chromosomes (III)

- Much (40~50%) of non-protein-coding DNA in the human genome is transcribed into RNA.
- Coding regions are usually unique.
- Eukaryotic genomes often contain large numbers of repetitive DNA sequences that are present in many copies.

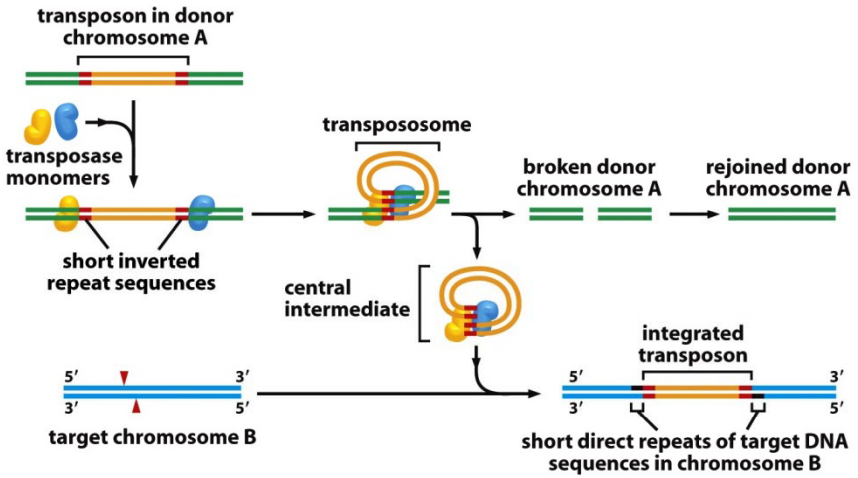
  - Transposons
  - Satellite DNAs (e.g. centromeres)



Organization of Human Chromosomes (IV)

- Pseudogenes are DNA fragments that contain too many mutations that render a n ancestral gene inactive and nonfunctional.

- Transposons are DNA segments that can move from one location of the genome to another location.



- There are three types of transposons
  - DNA-only transposons
  - Retroviral-like retrotransposons
  - Nonretroviral-like retrotransposons

Outline

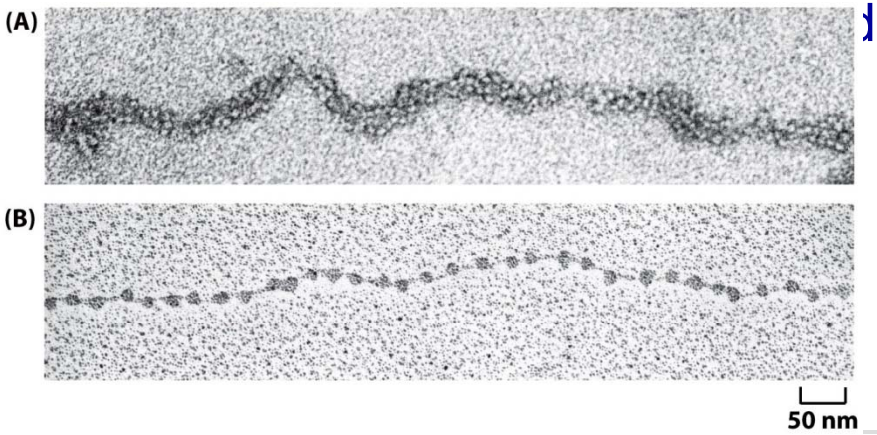
- Structural and functional organization of chromosomes
- DNA packaging and structural organization
- Overview of kinetochore structure and functions

DNA Packaging

- Long sequence of DNA must be stored within the geometry of a nucleus

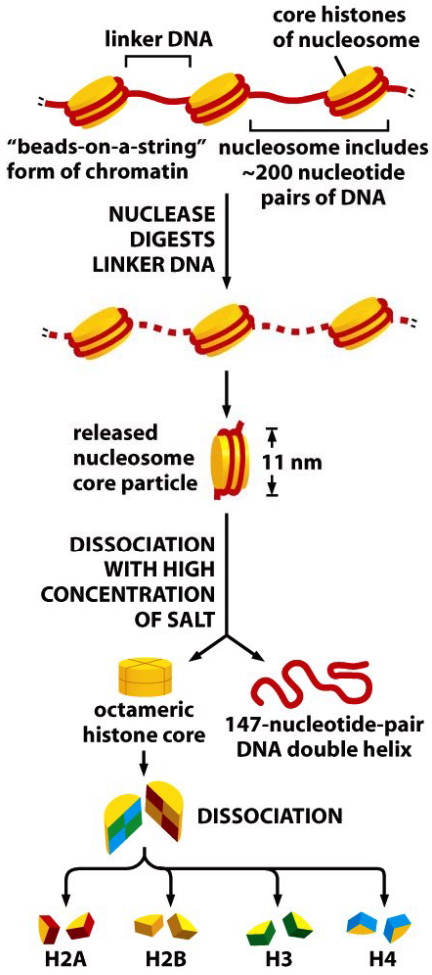
  - Example: human chromosome 22, 48 million bp
  - Extends to length of ~1.5 cm
  - Measures 2 μm in mitosis
  - Packaging ratio on the level of 10^4 in mitosis
  - Packaging ratio ~500 in interphase

- Packaged DNA must provide [illegible] for gene expression.



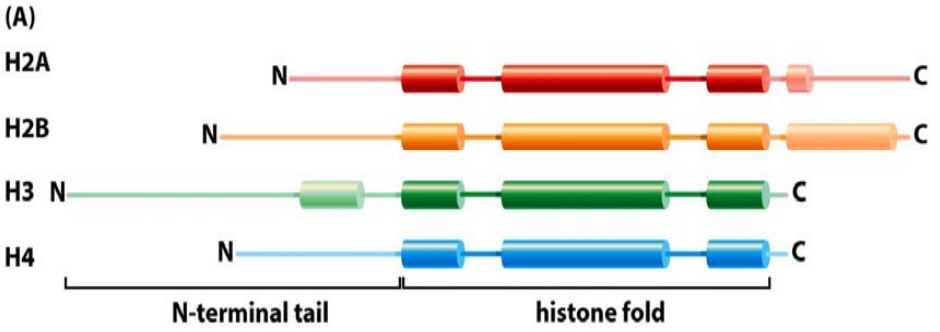
Packaging of DNA into Nucleosomes

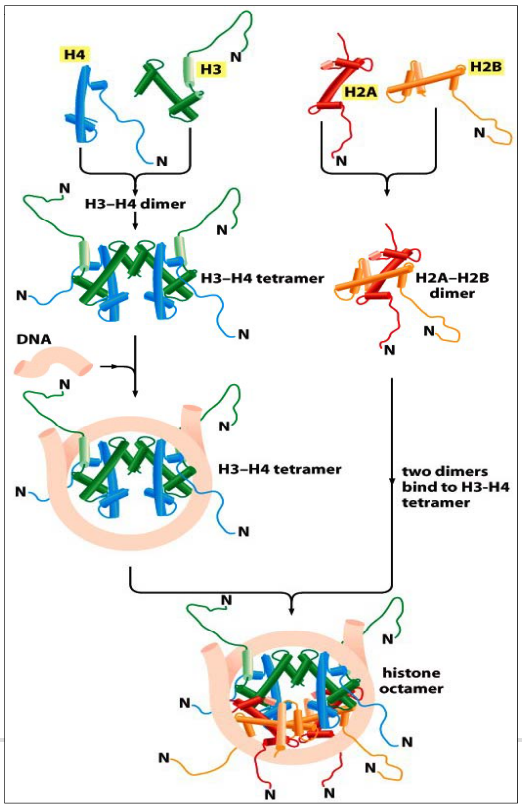
- DNA is coiled around a protein core to form nucleosomes: 142 hydrogen bonds.
- ~7 folds in packaging.
- Histone H2A, H2B, H3, H4 with 147 bp DNA.
- Nucleosomes repeat at every 200 bp. So ~30 million nucleosomes in a human cell.
- Total mass of histones approximately equal to that of DNA.

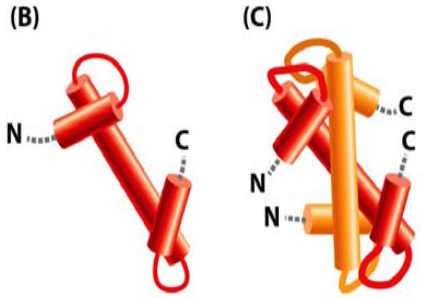


Histone Organization

- N-terminal tail is subject to different forms of modification.
- H2A & H2B form a dimer through handshaking.
- H3 & H4 form a dimer in a similar fashion.
- Extensive interactions between histones and DNA







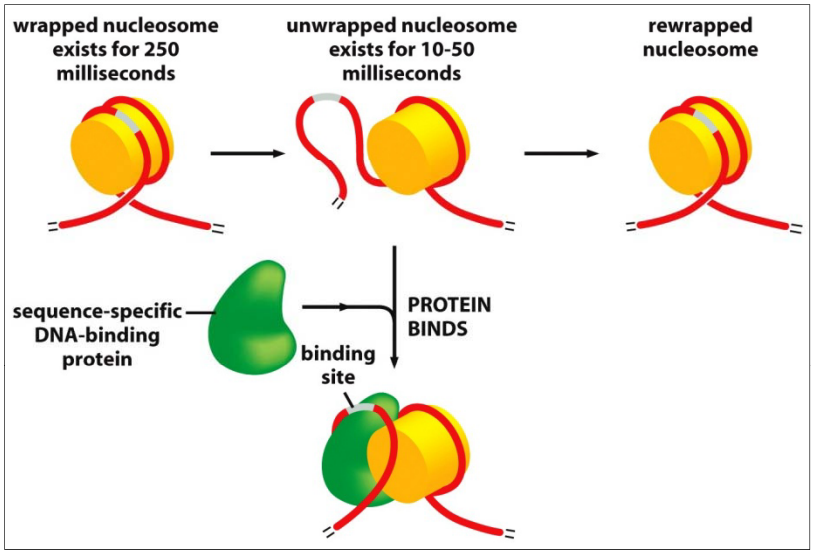
Formation of the Histone Octomer

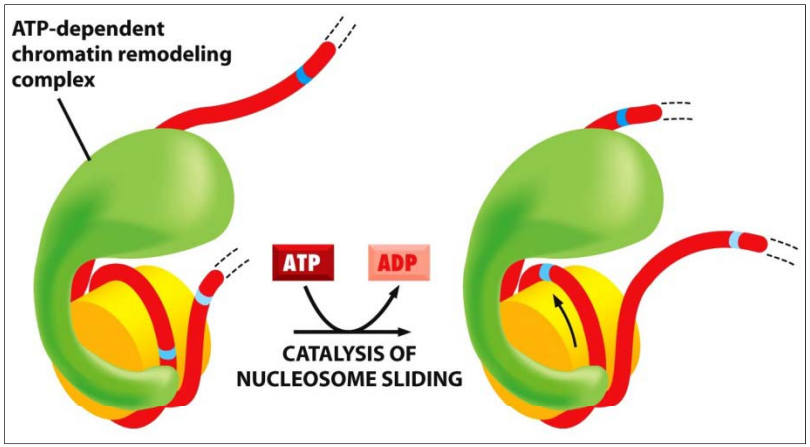
- Histone code hypothesis:

  Histone modifications determine whether particular regions of chromatins are transcribed or held in an inactive state.

- Nucleosomes are dynamic.

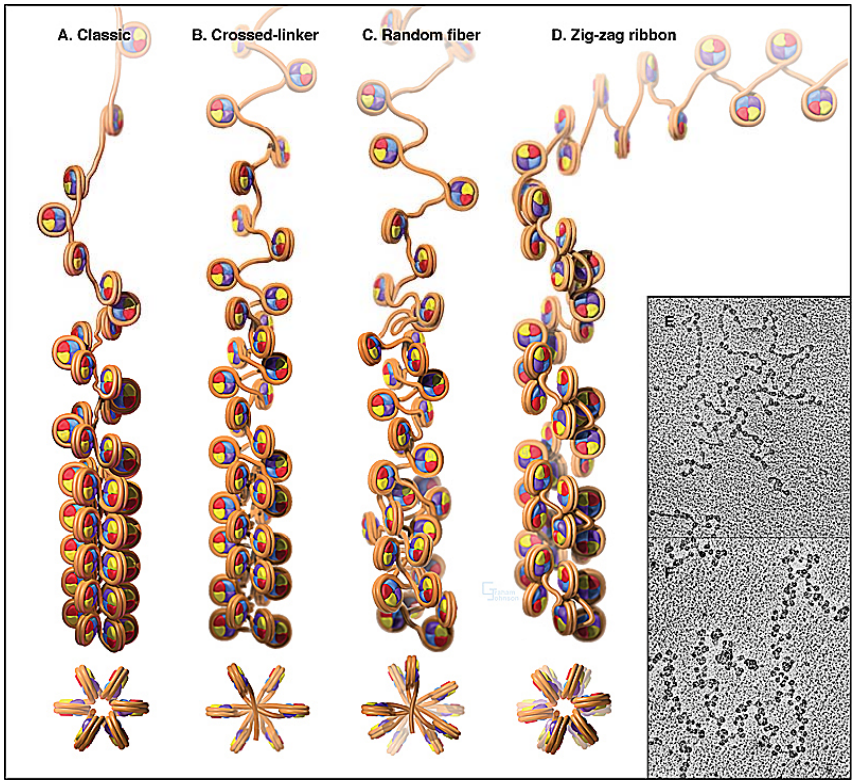
- Eukaryotic cells have a large variety of ATP-dependent chromatin remodeling complexes.





Second level of DNA Packaging: 30-nm Fibre

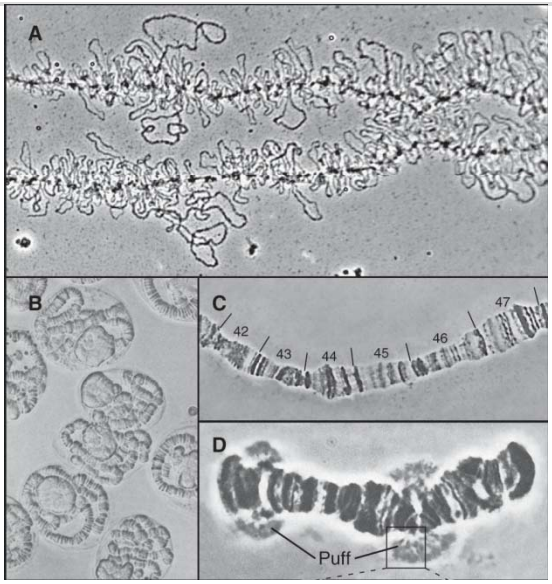
- Nucleosomes are further packaged into 30-nm fibers.
- The precise structure of the 30-nm fiber is not yet known.
- Chromatin structure beyond nucleosomes is poorly understood.
- ~40 folds in packaging.



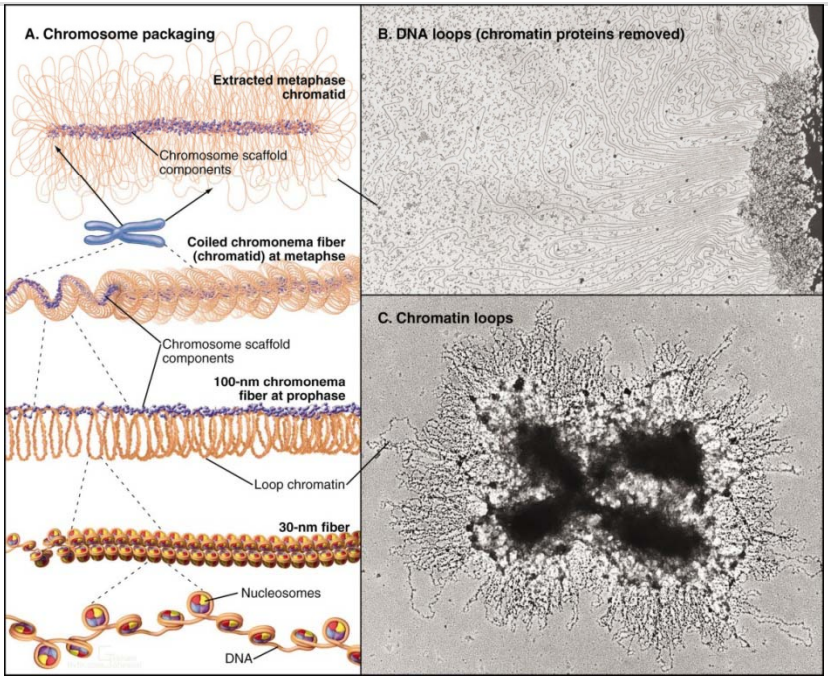
Different models of the 30-nm fiber

Higher Order Structure of Chromosomes (I)

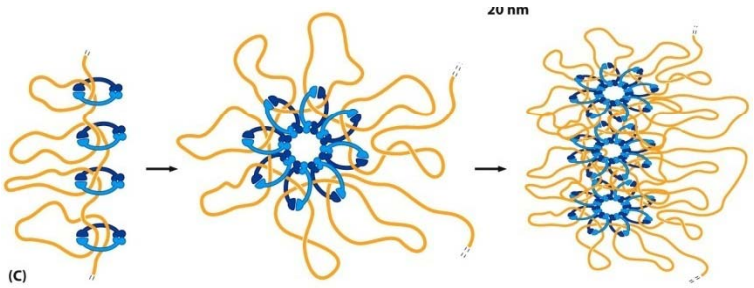
- Two different cases
  - Interphase chromosomes
  - Mitotic chromosomes
- Interphase chromosomes
  - Formation of loops
  - Formation of bands
- Higher order structures are also actively regulated.



Higher Order Structure of Chromosomes (II)

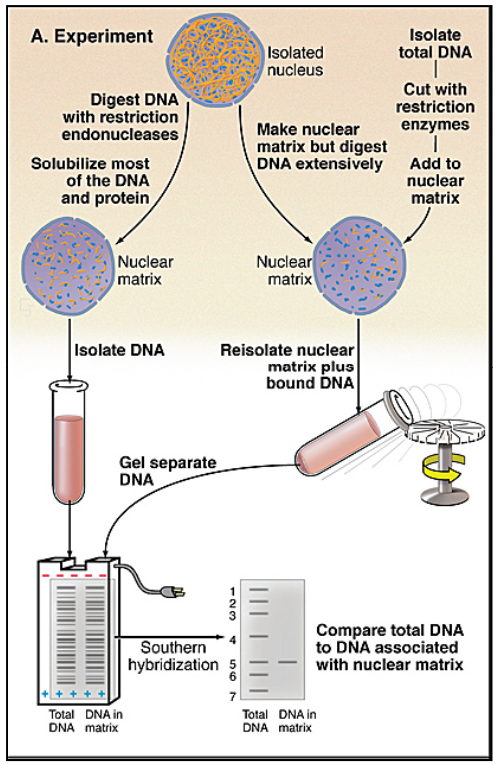


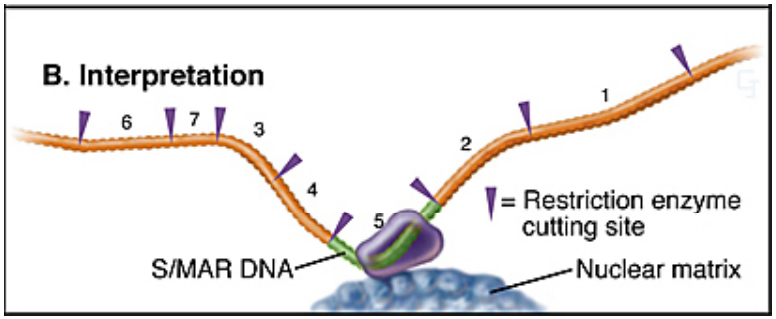
Model of mitotic chromosomes



The Nuclear Matrix

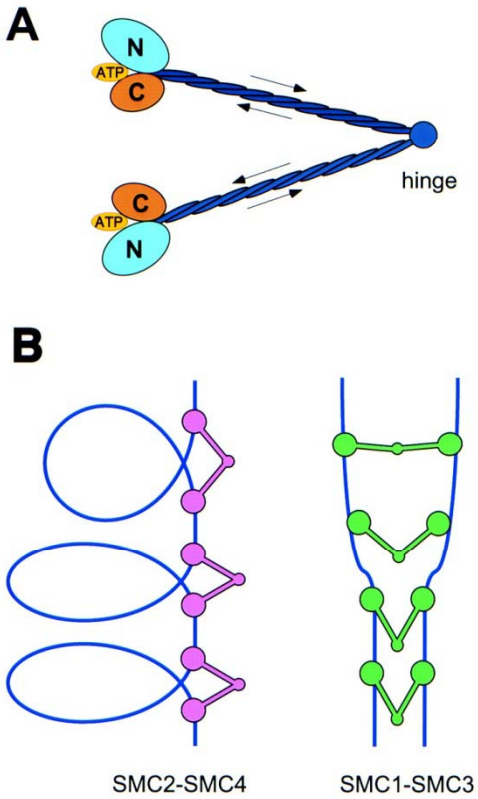
- The identification of nuclear matrix is controversial.
- The proposed function of nuclear matrix is to organize chromosomes compartments and chromatin loops.





Mitotic Chromosome Scaffold Protein: SMC

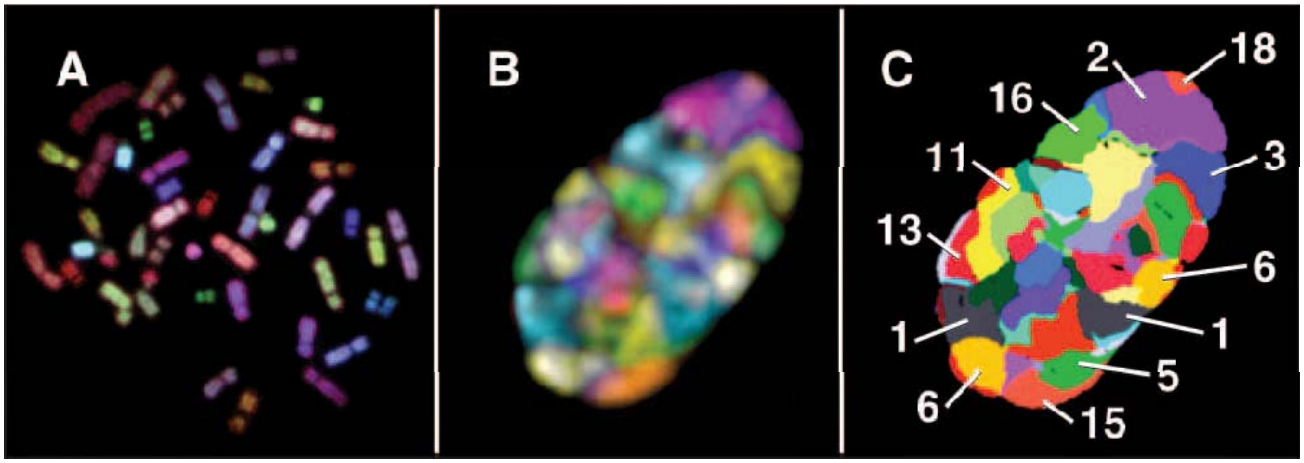
- SMC protein: structural maintenance of chromosome.
- Condensin: mediates condensation
- Cohesin: holds sister chromatids together; cleaved by separase.
- Hundreds of other chromosome scaffold proteins with unknown functions.



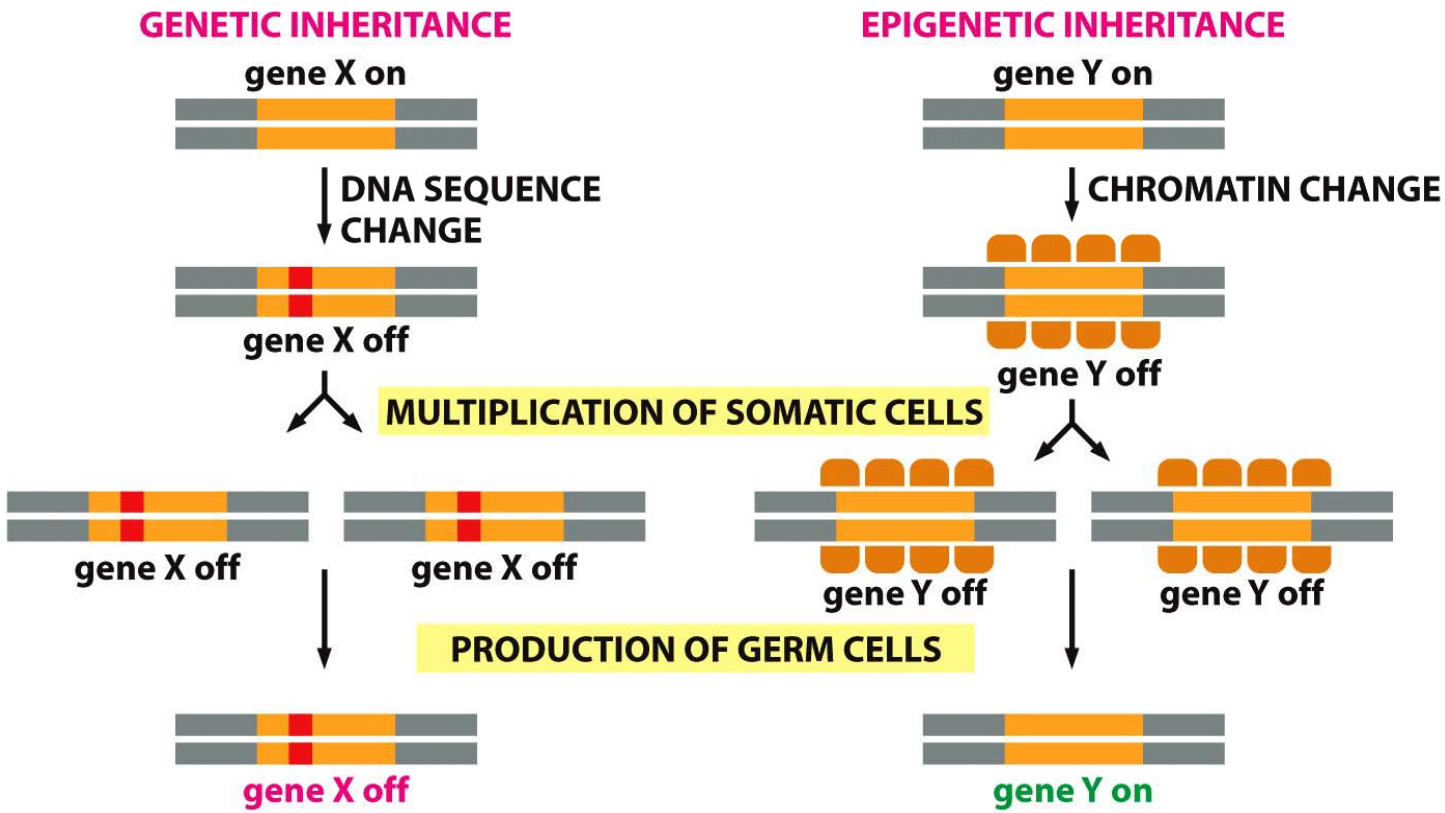
Hirano T, Genes Dev. 13:11-19, 1999

Structural Compartmentation of the Nucleus

- Individual chromosomes tend to concentrate within discrete territories with limited intermingling.
- Chromosomes active in transcription (euchromatin) tend to concentrate to the middle of the nucleus.
- Chromosomes inactive (heterochromatin) in transcription tend to concentrate at the periphery.



Example: Genetic vs Epigenetic Inheritance



Outline

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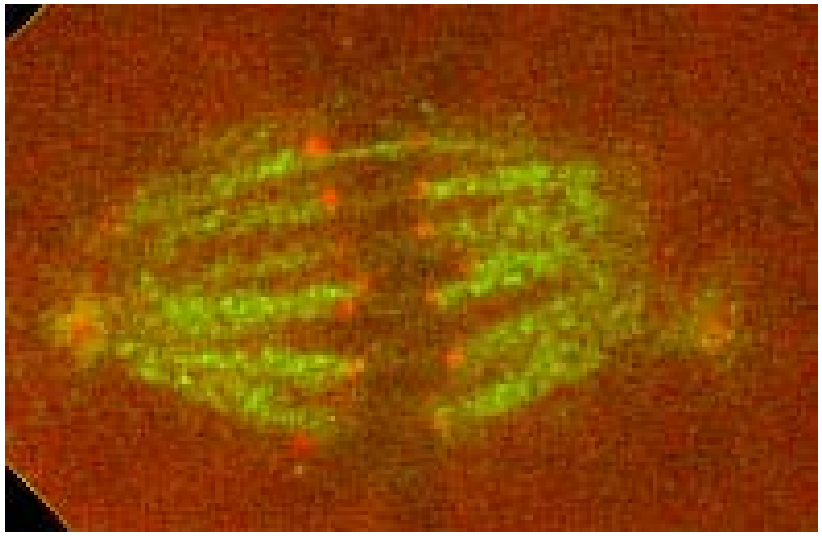
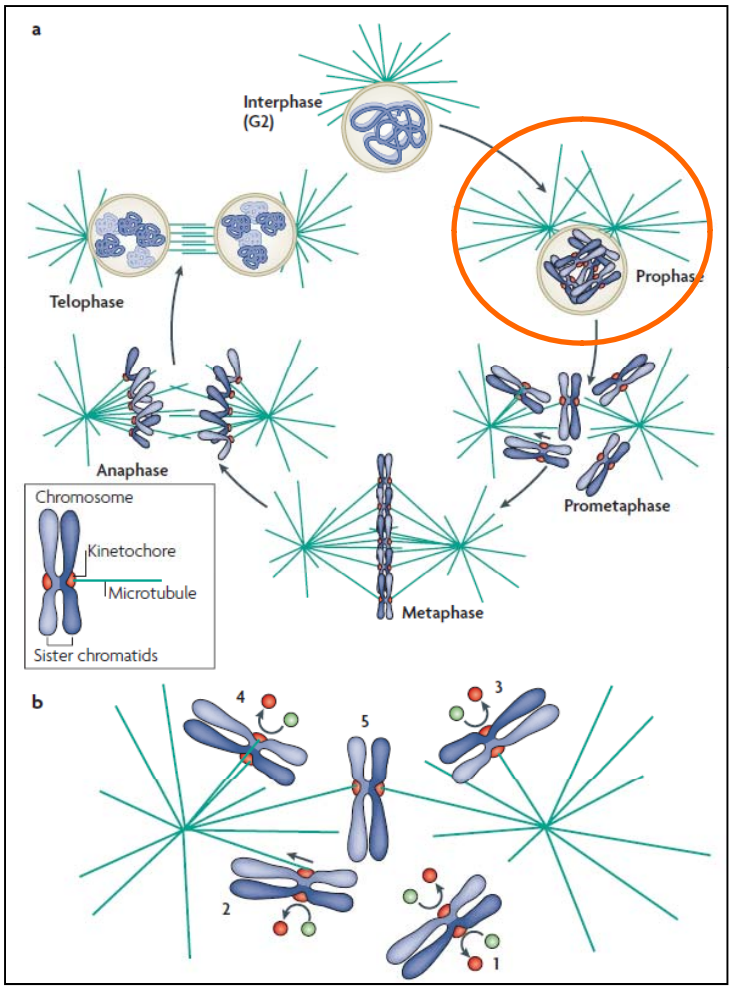
Kinetochore (I)

- Kinetochore becomes visible in mitosis.

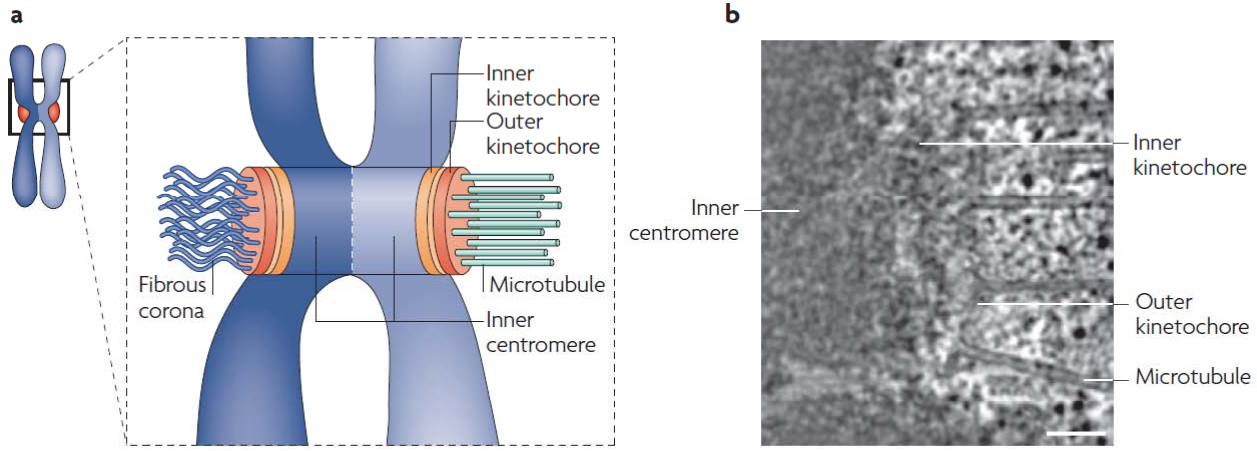




Cheeseman & Desai, *Nat. Rev. Mol. Cell Biol.* 9:33, 2008

Kinetochore (II)

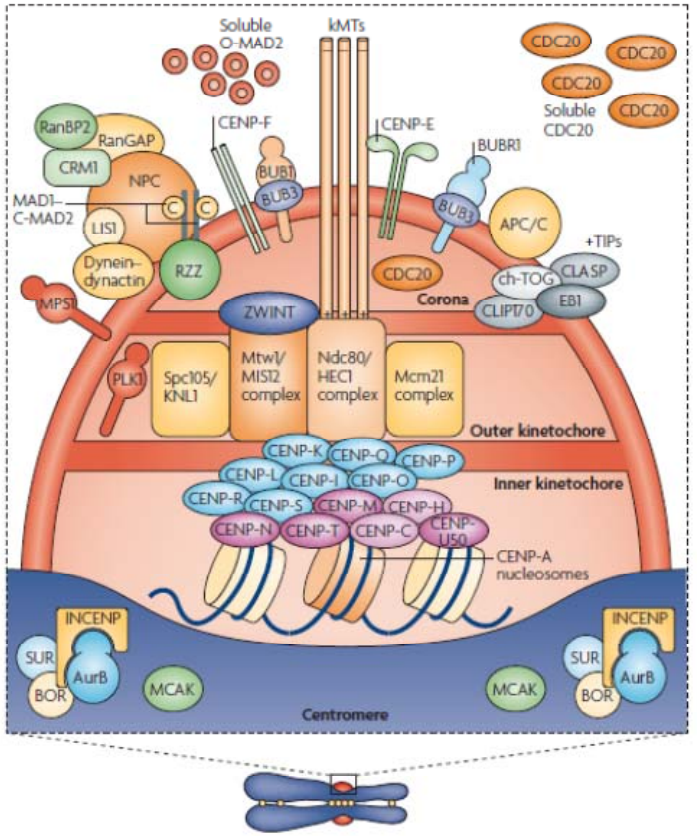
- Embedded in the surface of centromere.
- Kinetochores assemble in prophase; dissemble after mitosis.
- Primary functions
  - Directs chromosome movement
  - Regulating microtubule dynamics
  - Form signaling pathways to regulate cell cycle
- Fibrous corona is detected on unattached kinetochores.



Cheeseman & Desai, *Nat. Rev. Mol. Cell Biol.* 9:33, 2008

Kinetochore (III)

- Kinetochore is a very large protein assembly.
- At least 70 kinetochore associated proteins have been identified in budding yeast.
- Its structure is likely to be fully solved in the near future.

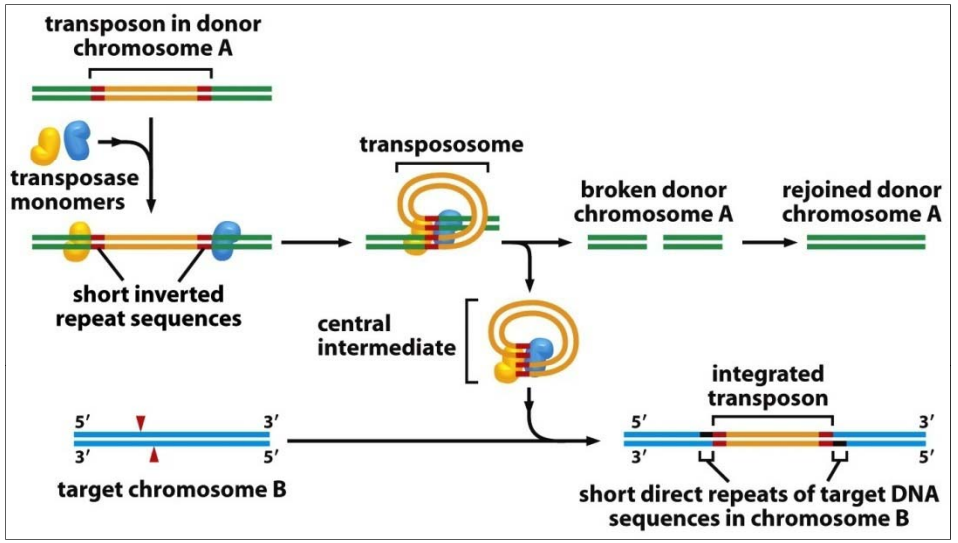


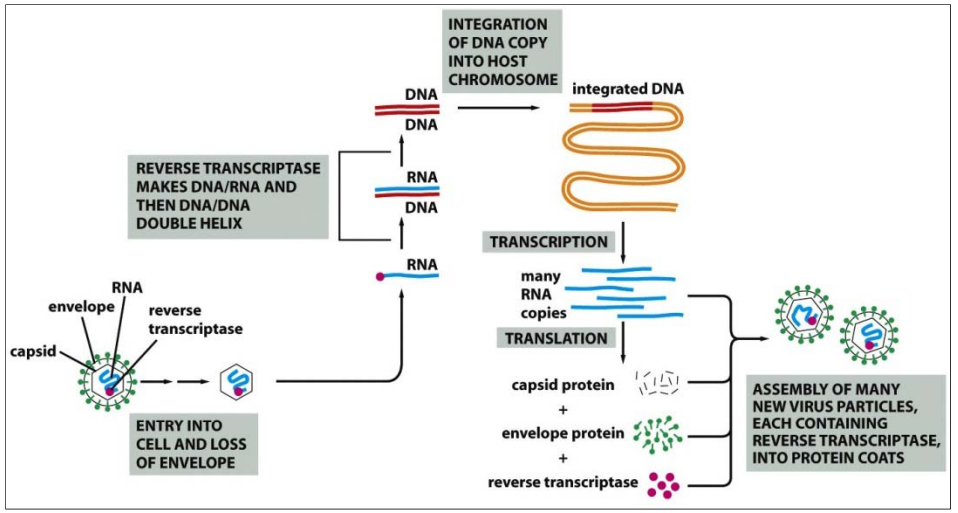
Massachio & Salmon, *Nat. Rev. Mol. Cell Biol.* 8:379, 2007

Chromosome Organization & DNA Packaging

Transposons and Retrotransposons

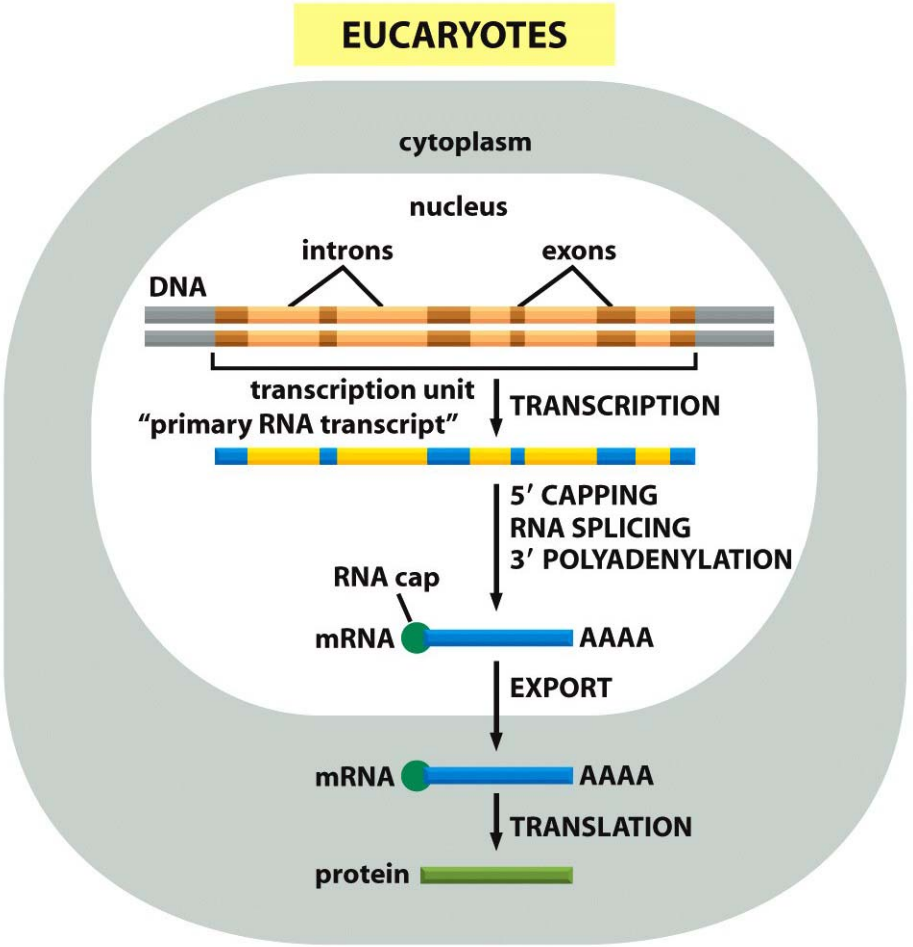
- Transposons make much of human genome.
- Transposons are small discrete DNA elements that are capable of moving along DNA. Most, if not all, of them are inactive in human genome.
- Transposons move in a cut-and-paste fashion.
- Retrotransposons move via RNA intermediates.
- Retrotransposons move in a copy-and-paste fashion.





Pseudogenes

- Pseudogenes are non-functional derivatives of genes.
- Processed pseudogenes are created by reverse transcription of mature mRNA.
- Unprocessed pseudogenes are created by reverse transcription of pre-mRNA.
- Human genome contains many pseudogenes, perhaps more than functional genes.



Background Information: Epigenetics & ISH

- Epigenetics refer to changes in phenotype or gene expression caused by factors other than DNA sequence differences.
- One example: a fertilized egg cell can differentiate into many different cell types.
- In situ hybridization: the technique of using a labeled complementary DNA/RNA to specifically match with a specific DNA/RNA sequence in cells or tissues.

Background Information: Budding Yeast and Fission Yeast

- No nuclear envelope breakdown during yeast cell division.