BME 42-620 Engineering Molecular Cell Biology

Lecture 16:

DNA Packaging

Structure of Cell Nucleus

Chapter 4

Course Administration Notes

- Reading assignment 4
  - Presentation format
  - Presentation scheduled on Nov-10, 2011
    - Group 5: Jackie Chen, Jaclyn Brackett, Pitirat Pholpabu
    - Group 6: Simone Costa, Christine Bronikowski, James Rockwell

- Lecture on Nov-08 cancelled. A make-up lecture will be scheduled.

Outline

- DNA packaging and structural organization
- Overview of kinetochore structure and functions
- Overview of cell nucleus structure

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The Problem: DNA Storage and Access

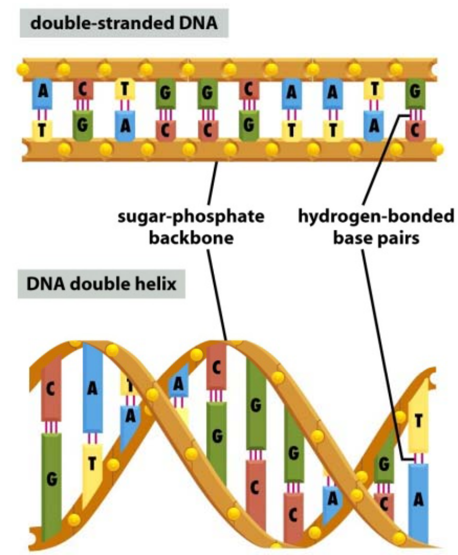
- Spacing between base pairs ≈3.4Å

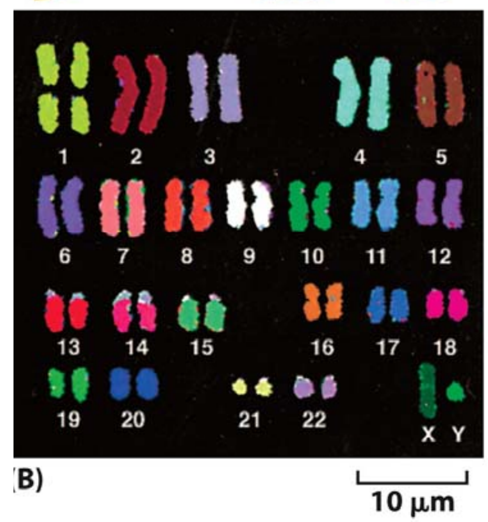
- For human genome, approximately 3.2 billion base pairs

- Total length ≈ $3.4\times10^{-10}\times3.2\times10^{9}\times2$ ≈ 2.2m

- Diameter of a nucleus: $5\sim10\times10^{-6}$m

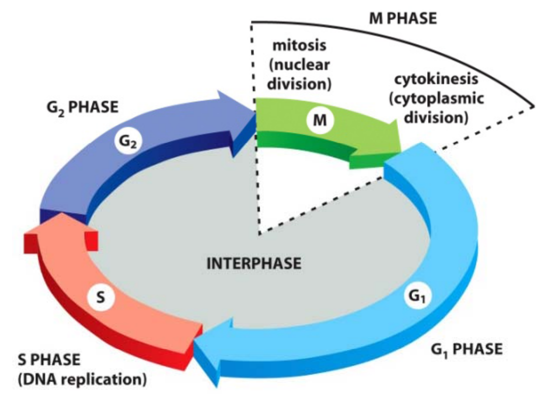
- Access to genetic information must be provided and regulated.

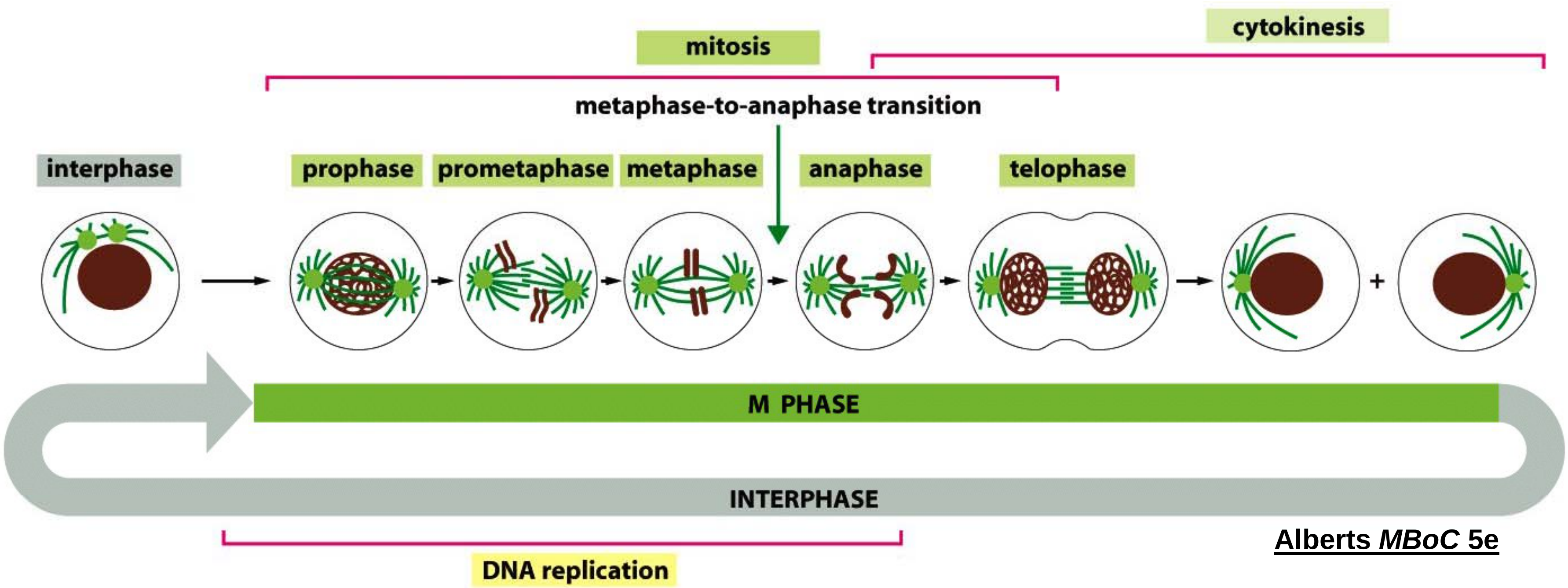




Chromosome Organization in the Cell Cycle

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





Alberts *MBoC* 5e

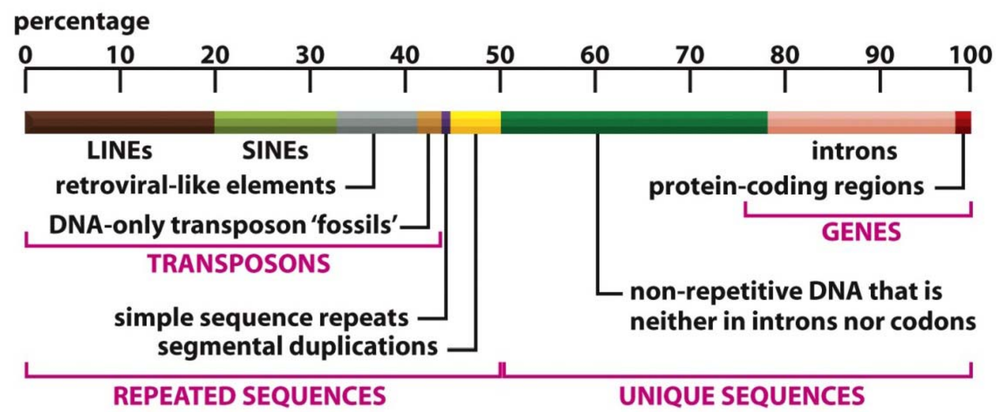
Organization of Human Chromosomes (III)

- 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



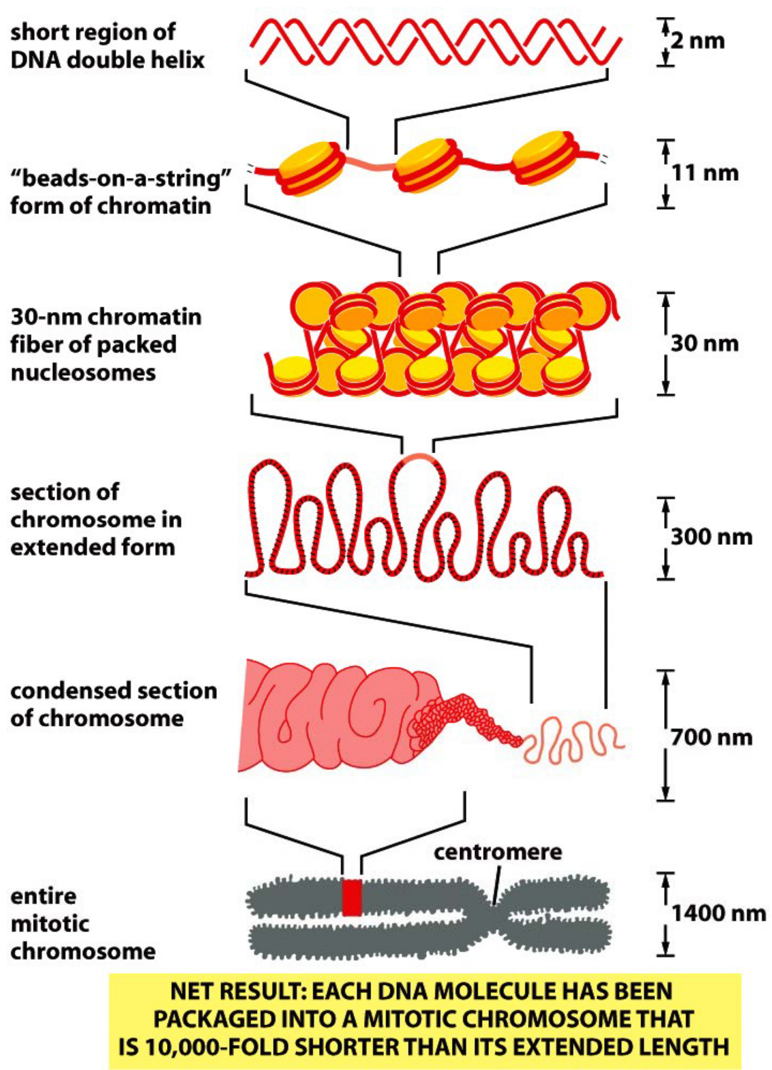
The Problem: DNA Storage

- 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

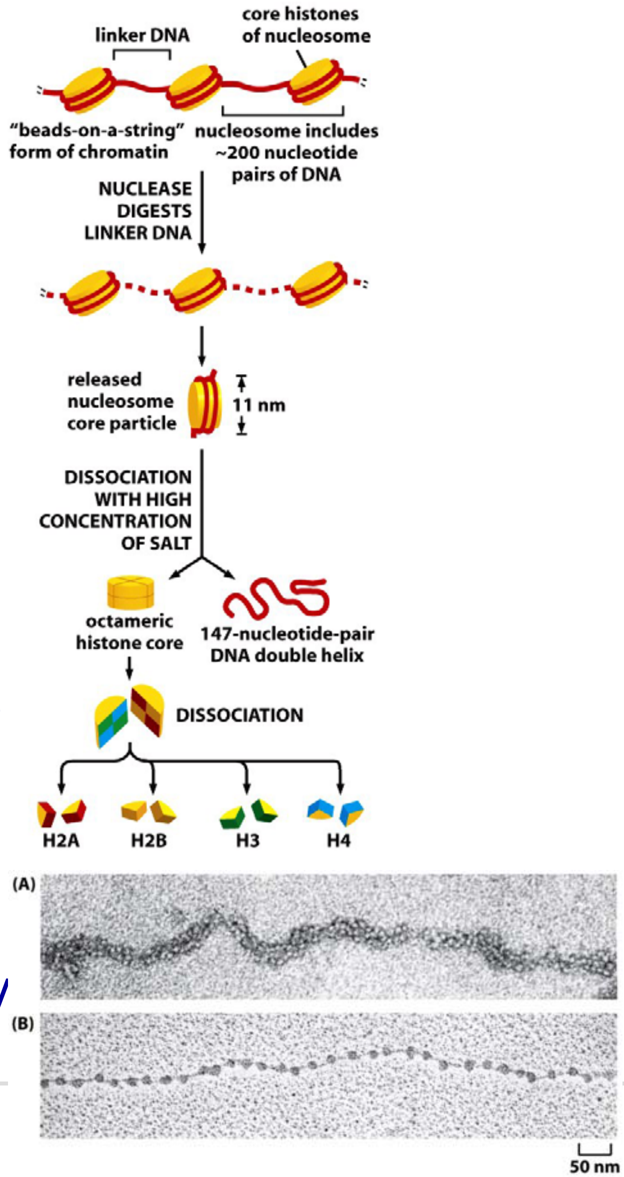
- Packaged DNA must provide controlled access to regions required for gene expression.

The Solution: Multiple Levels of DNA Packaging



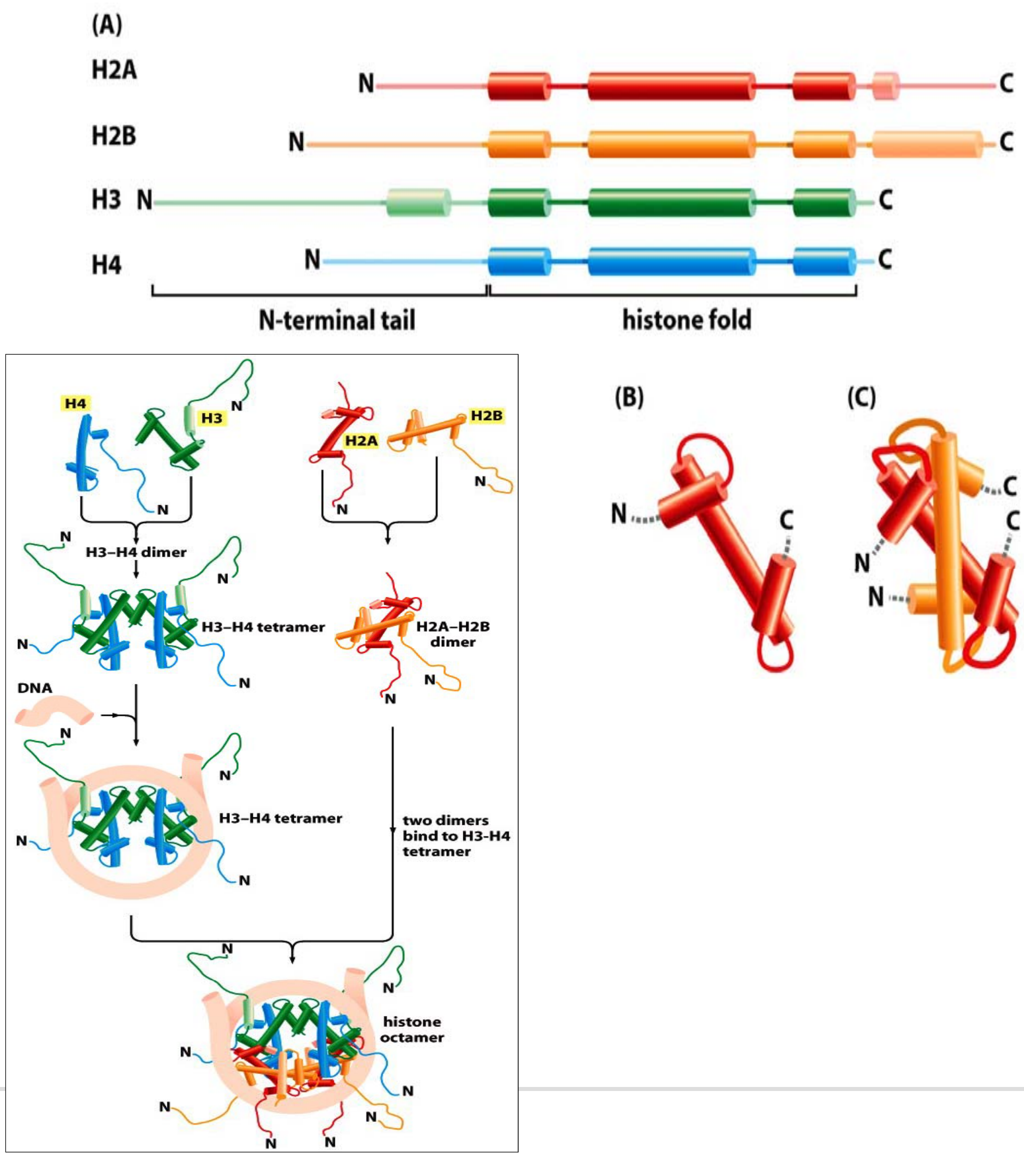
Packaging of DNA into Nucleosomes

- 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.



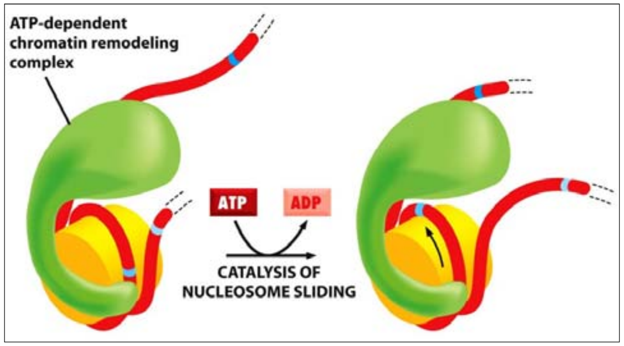
Histone Organization

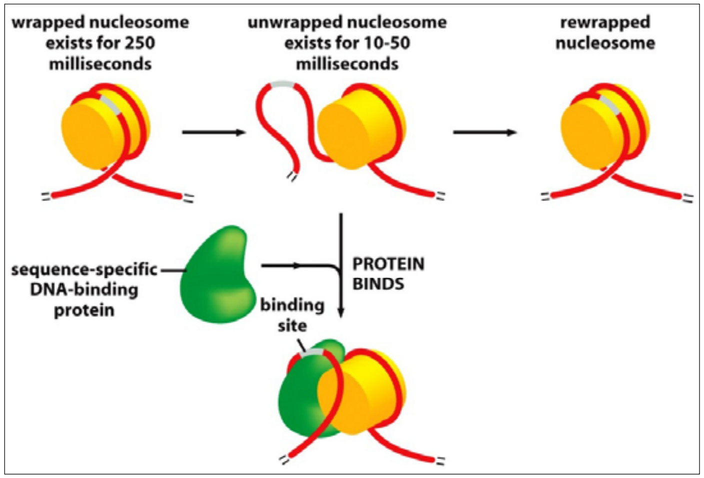
- N-terminal tail is subject to different forms of modification.
- H2A & H2B form a dimer through handshaking.
- H3 & H4 form a dimer in a similar fashion and then a tetramer.
- Extensive interactions between histones and DNA

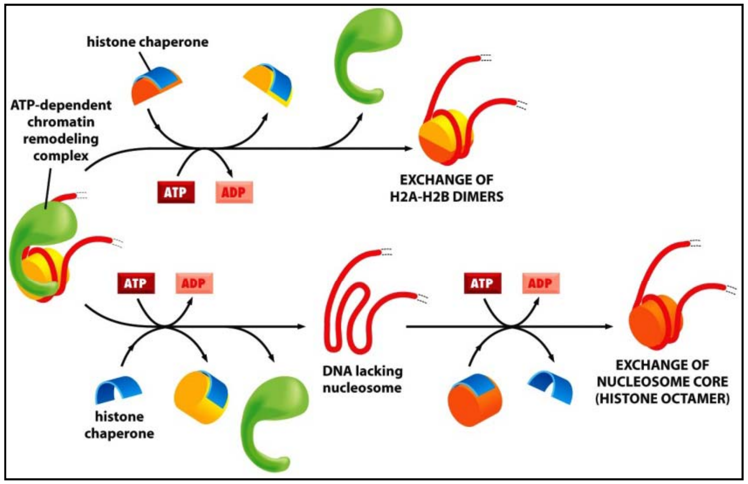


Dynamics of the Nucleosome

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

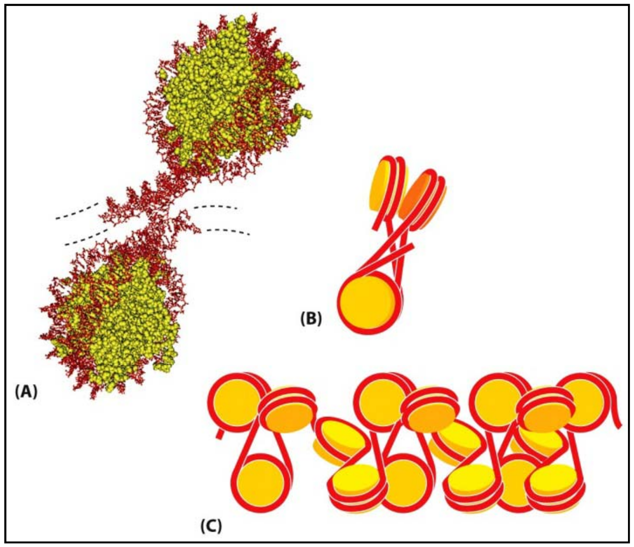


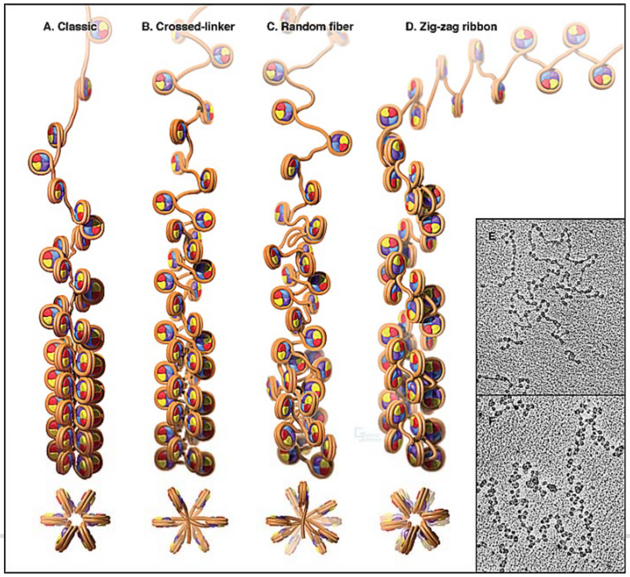




Second level of DNA Packaging: 30-nm Fibre

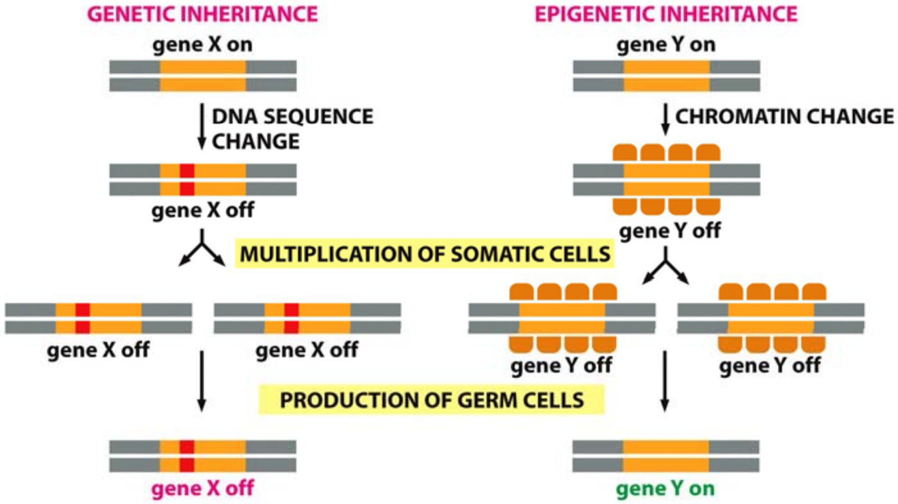
- Chromatin structure beyond nucleosomes is generally less well understood.
- Nucleosomes are further packaged into 30-nm fibers.
- The precise structure of the 30-nm fiber is not yet known.
- ~40 folds in packaging.





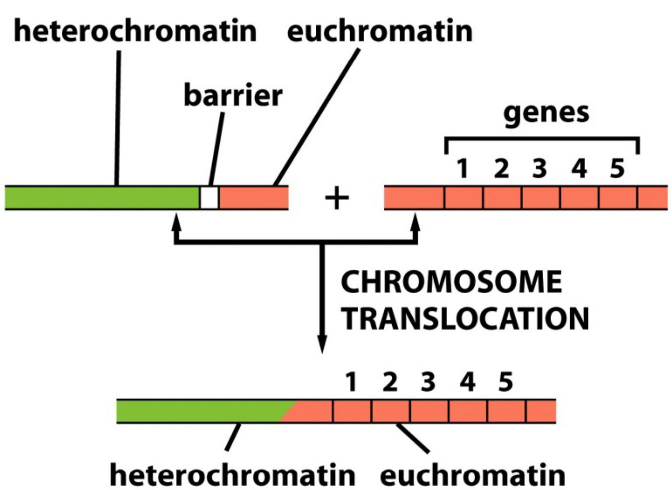
Regulation of Chromatin Structure (I)

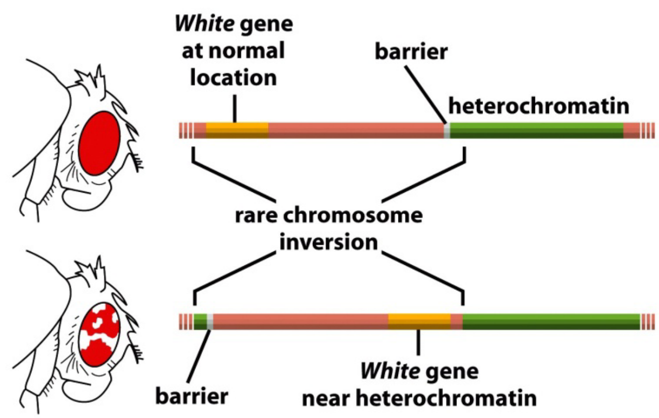
- Certain types of chromatin structures can be inherited.
- This is one form of epigenetic inheritance.
- Epigenetic information is usually but not always erased during the formation of eggs and sperms.



Regulation of Chromatin Structure (II)

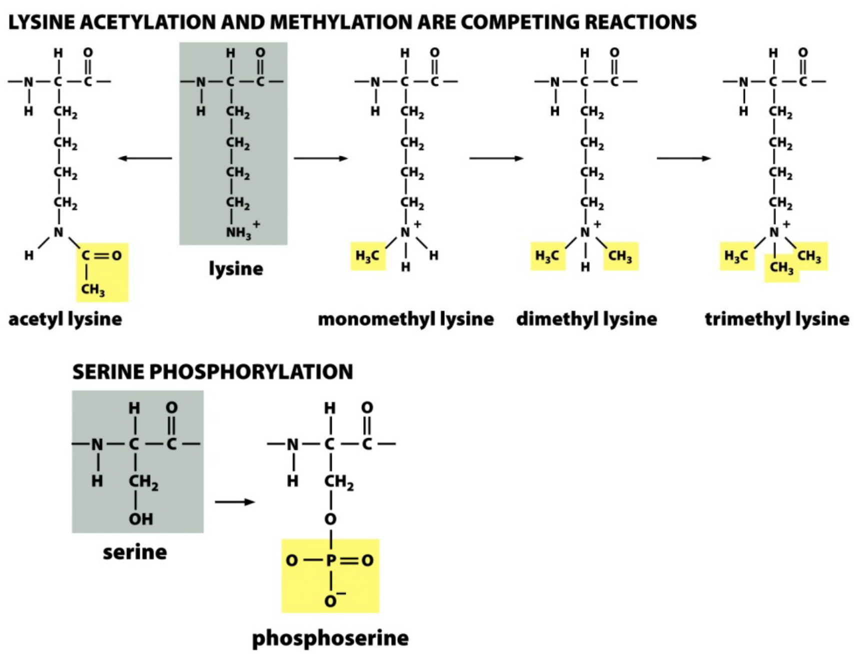
- Heterochromatin vs euchromatin
  - Heterochromatin refers to a highly condensed region of a chromosome that is generally inactivated for transcription.
  - Euchromatin refers to a uncondense region of a chromosome that is active for transcription.

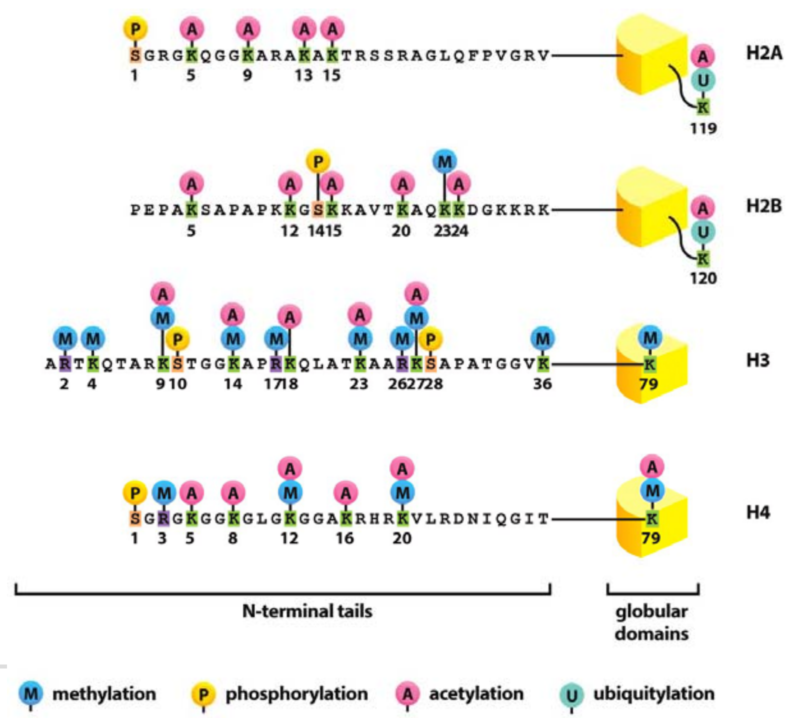




Regulation of Chromatin Structure (III)

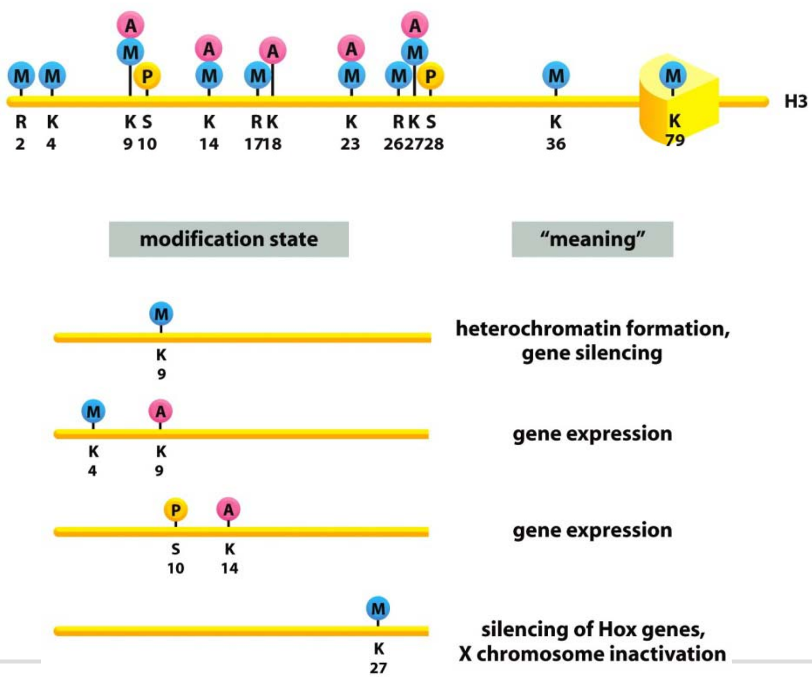
- Core histones can be covalently modified at different sites.
- All these modifications are reversible.
- Such modifications enable the recruitment of specific regulatory proteins.

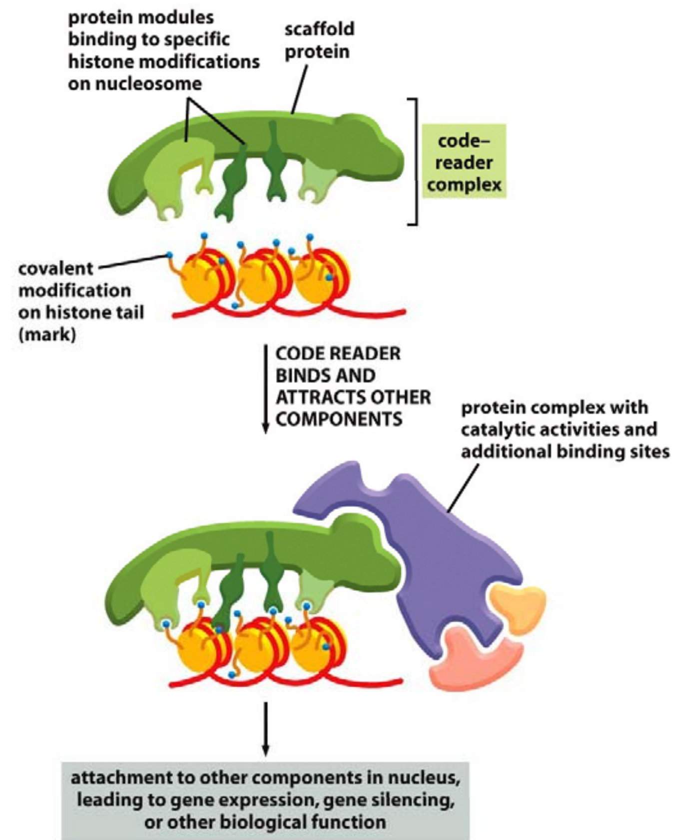




The Histone Code Hypothesis

- The hypothesis: Covalent modifications and histone variants of histones encode specific controls of gene expression.



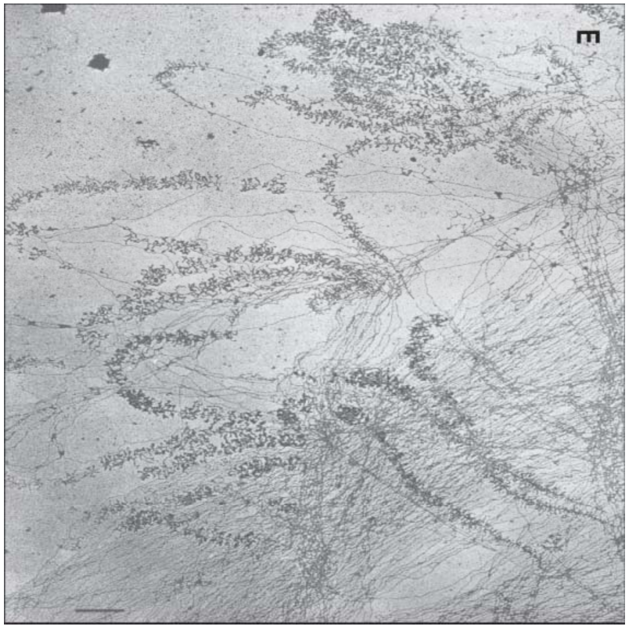


Higher Order Structure of Chromosomes (I)

- Two different cases:
  - interphase chromosomes
  - and mitotic chromosomes.

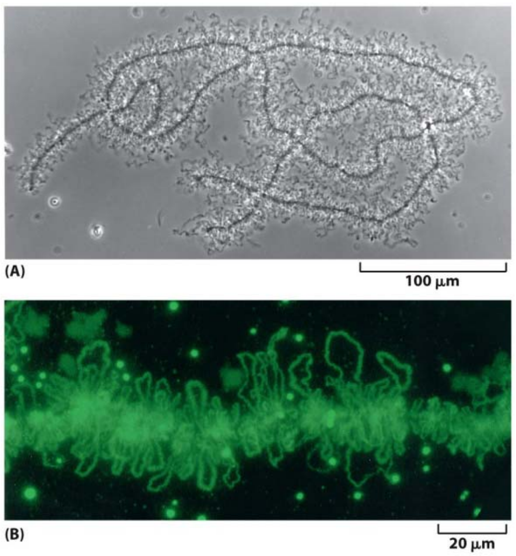
- Different organization configurations:
  - loops and band
  - Condensation

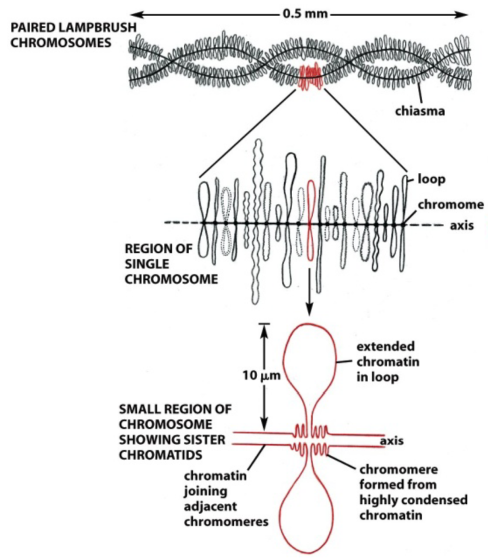
- Higher order structures are also actively regulated for control of gene expression.

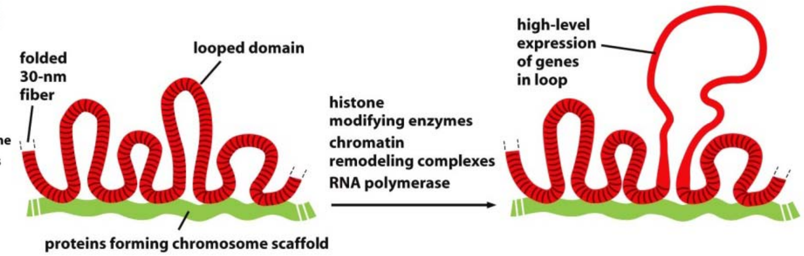


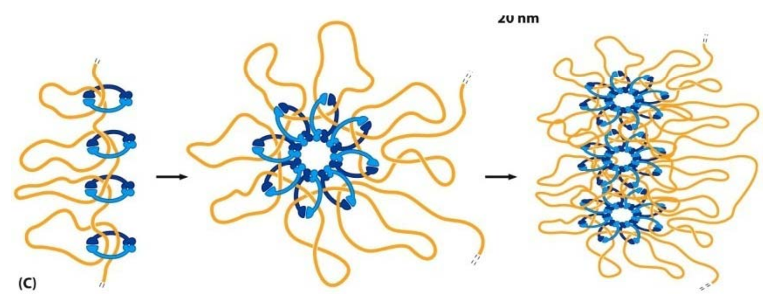
Higher Order Structure of Chromosomes (II)

- Organization of mitotic chromosome involves condensation. .



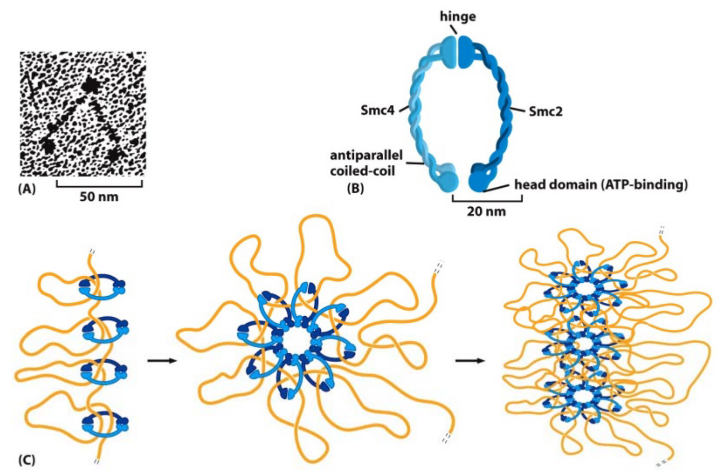






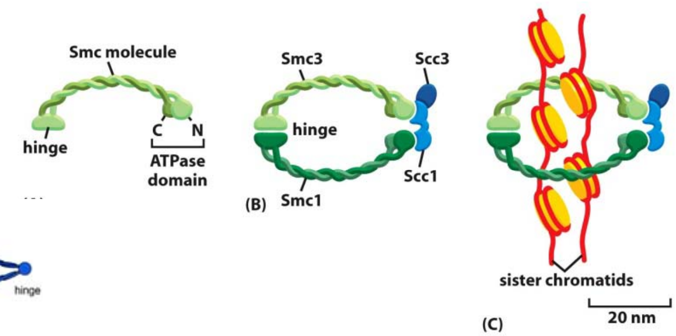
Mitotic Chromosome Scaffold Protein: SMC

- Condensation of mitotic chromosomes is mediated by condensin, a large protein complexes built from SMC dimers.

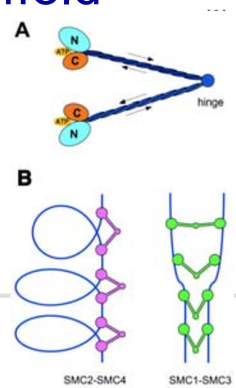


- SMC protein: structural maintenance of chromosome.

- 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

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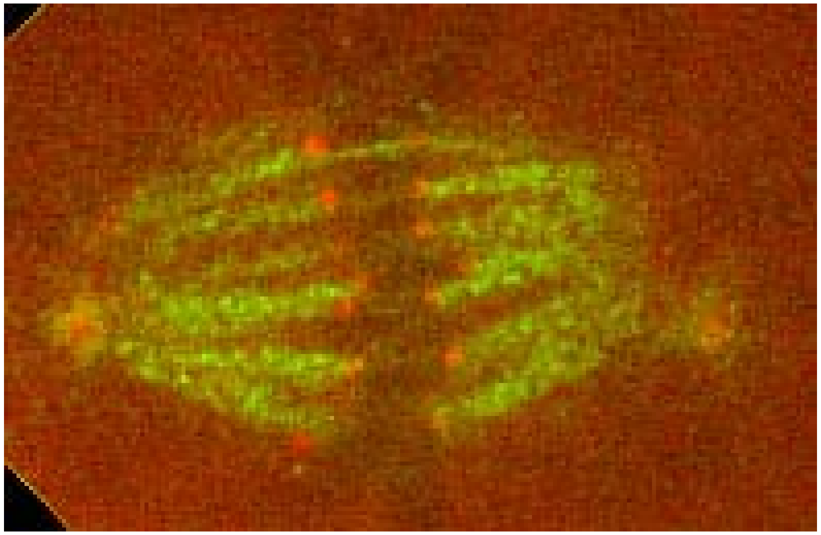
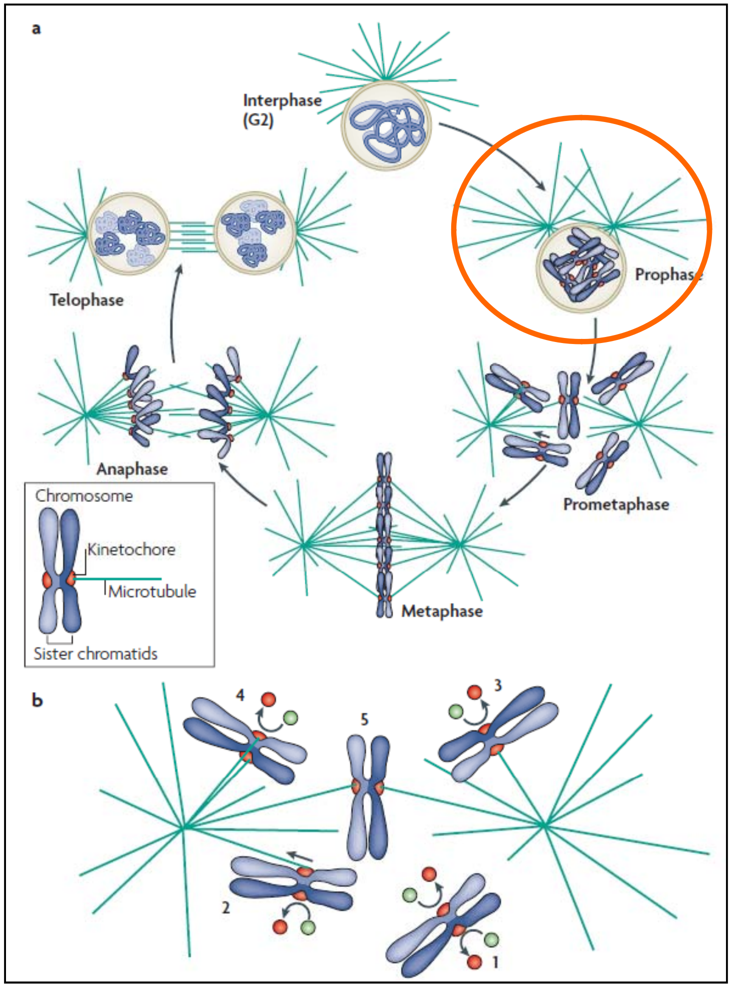
Kinetochore (I)

- Kinetochore becomes visible in mitosis.

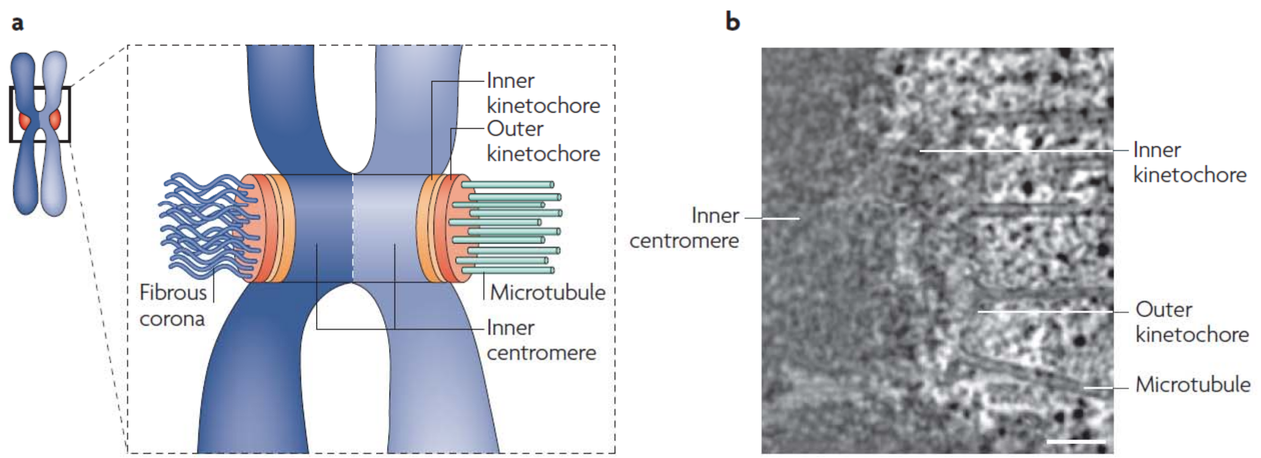




Cheeseman & Desai, *Nat. Rev. Mol. Cell Biol.* 9:33, 2008

Kinetochore (II)

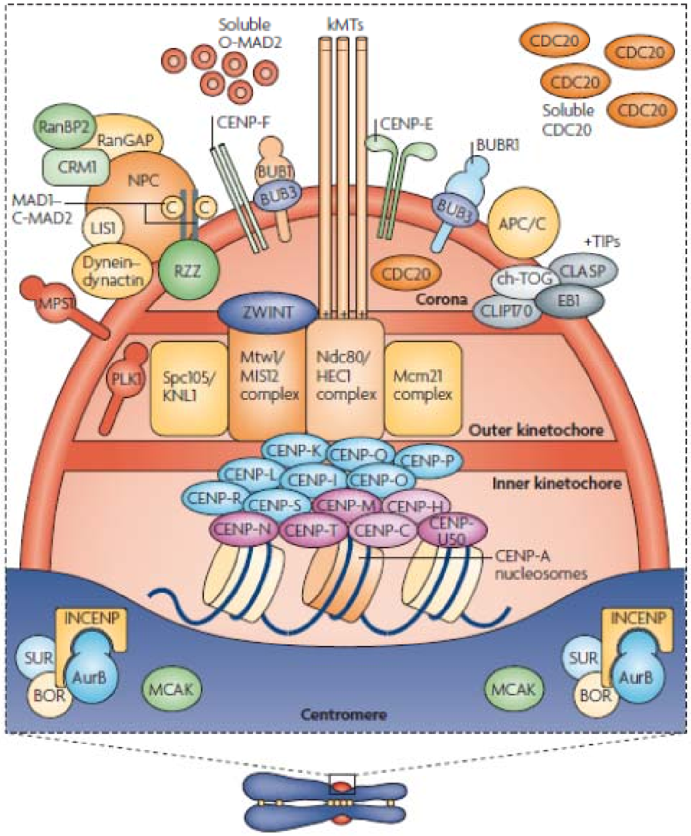
- Embedded in the surface of centromere.
- Kinetochores assemble in prophase; dissemble after mitosis.
- Primary functions
    - Directs chromosome movement
    - Regulating microtubule dynamics
    - Form signaling pathways to regulate cell cycle
- Fibrous corona is detected on unattached kinetochores.



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

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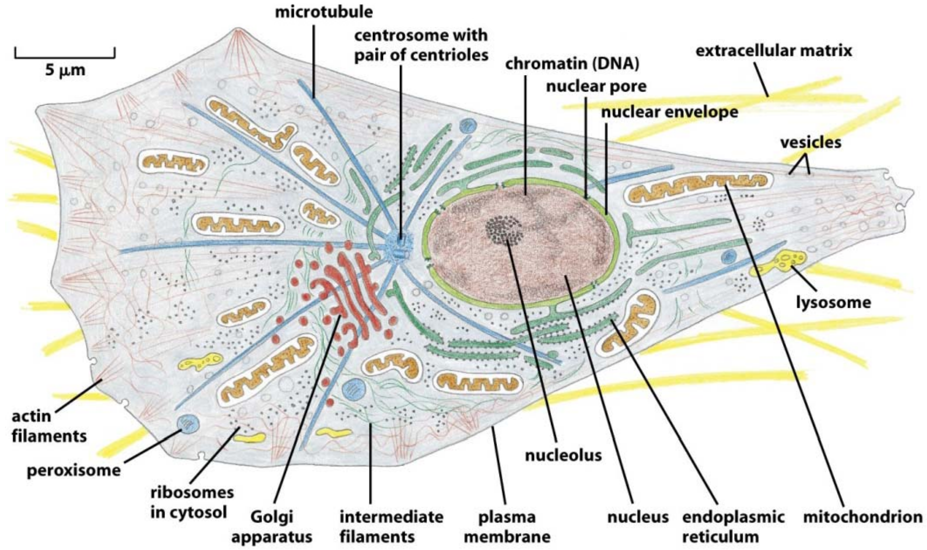
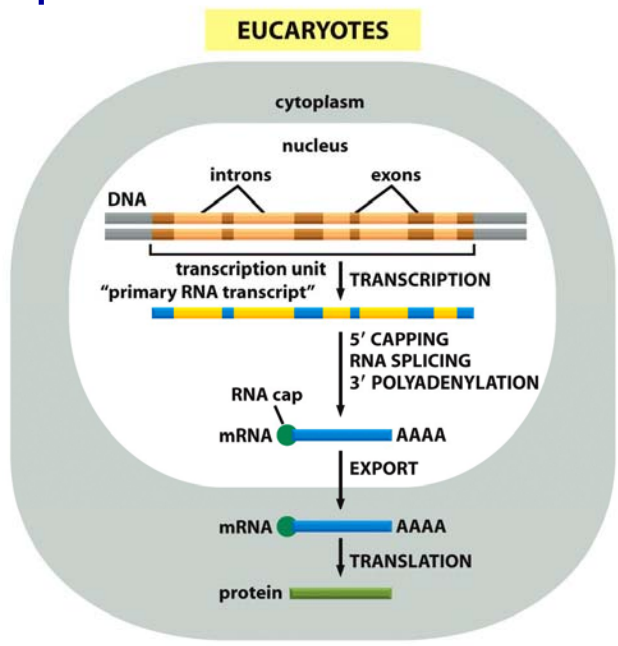
The Cell Nucleus

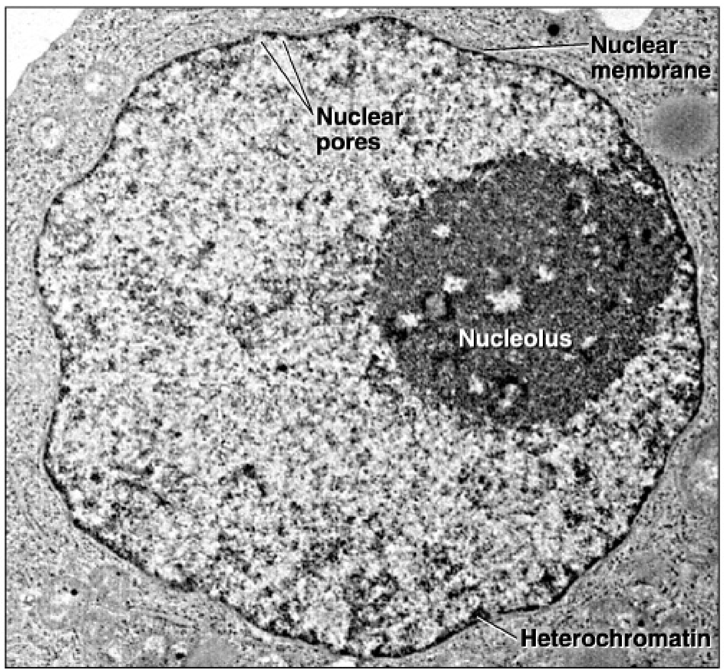
- The cell nucleus is the largest organelle in a eukaryotic cell.
- This compartmentation serves many purposes.

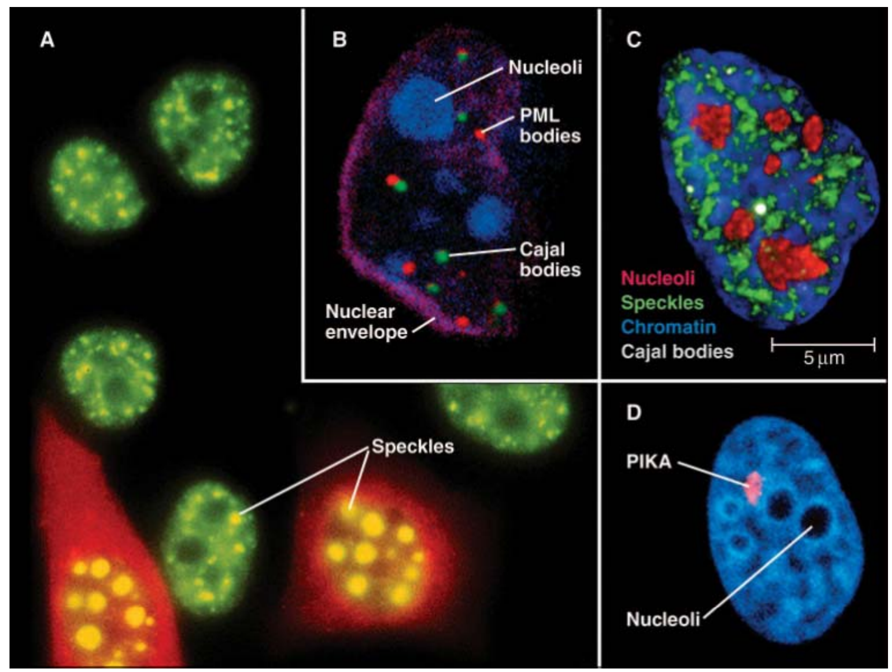




Structure of the Cell Nucleus

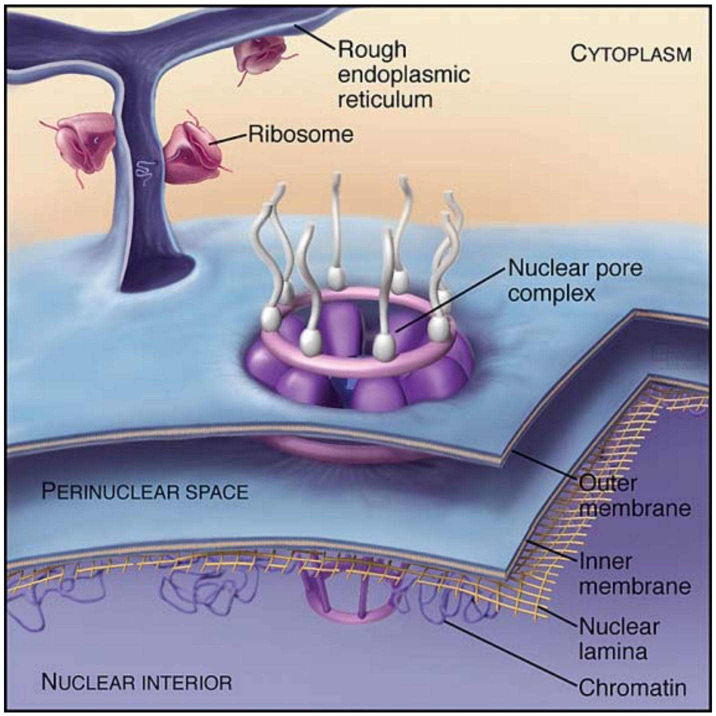
- The cell nucleus is organized into multiple subdomains:
  - nuclear membrane
  - subnuclear organelles

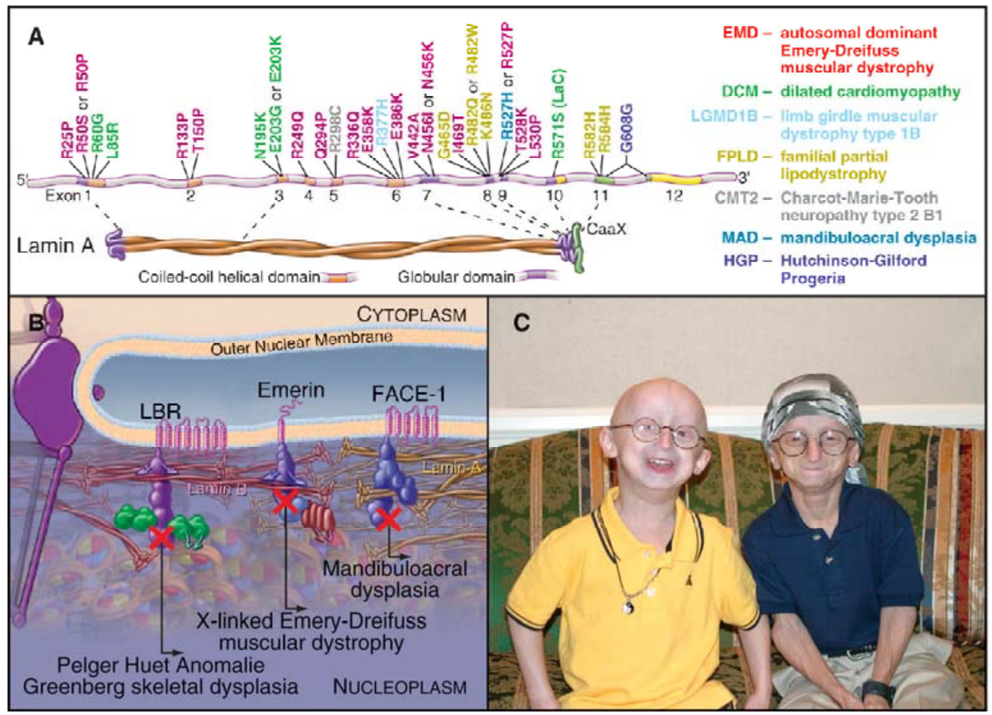




Nuclear Envelop & Defect Related Disease

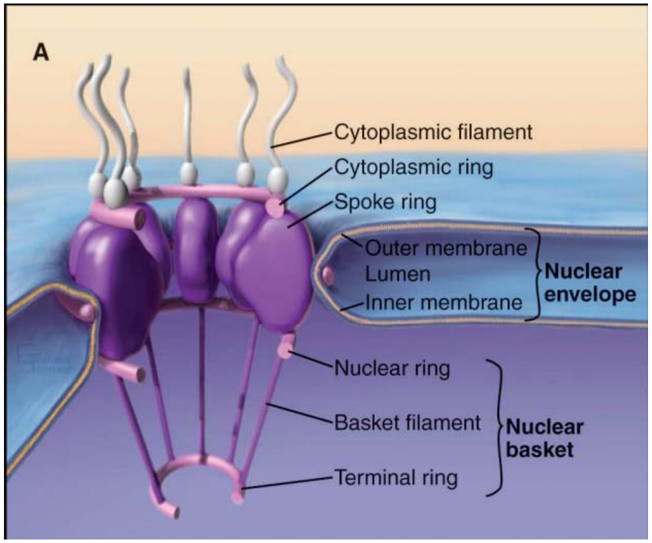
- The nuclear envelope breaks down during mitosis and reassembles upon exit.

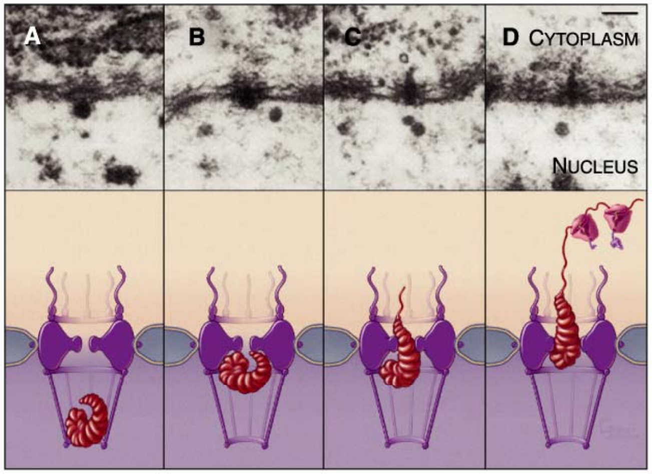




Traffic Between Nucleus and Cytoplasm

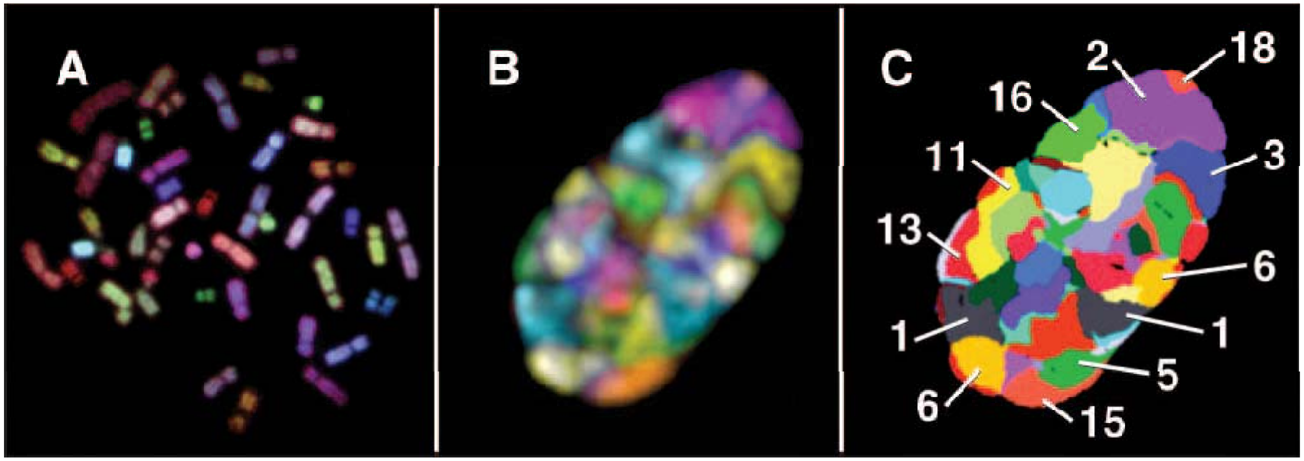
- Traffic between nucleus and cytoplasm goes through the nuclear pore complex and is controlled by different signal sequences.
- Proteins imported into the nucleus bears a nuclear localization sequence (NLS: PKKKRKV).
- Proteins exported from the nucleus bears a nuclear export sequence (NES: LQLPPLERLTL)
- Immature RNAs bear a nuclear retention signal (RNS).





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.



Questions ?