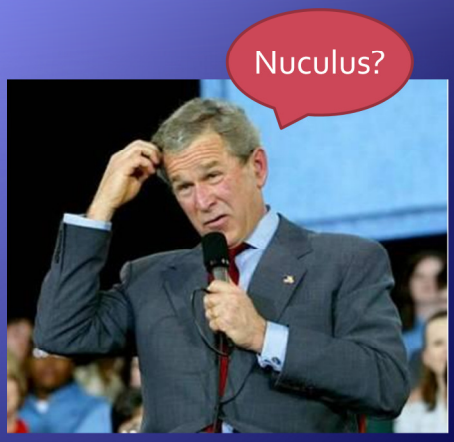
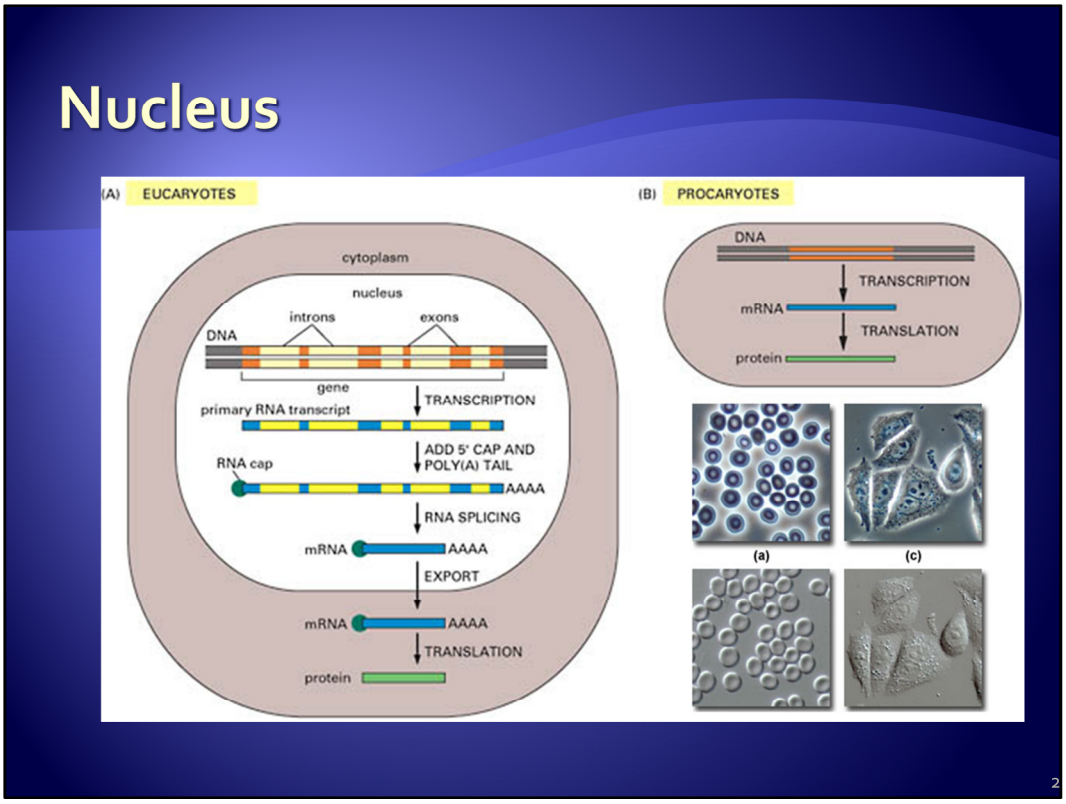
Session 3

NUCLEUS





Eucaryotic cells have a nucleus, which is the epicenter for gene expression. Prokaryotic cells like bacteria do not have a nucleus

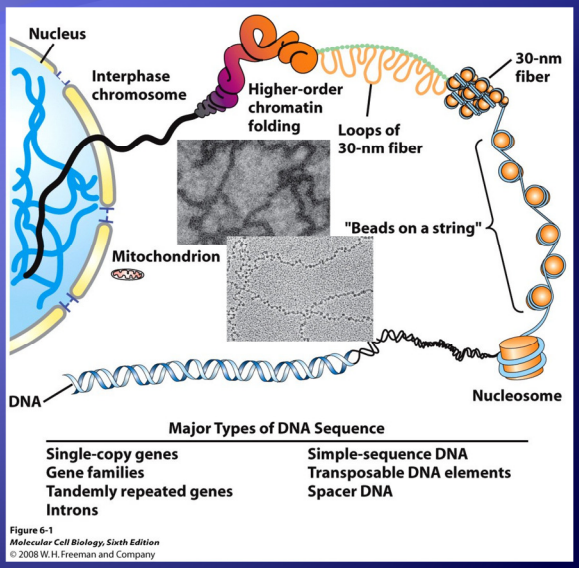
In the nucleus is where a gene is transcribed into RNA. At this point it contains exon and intron segments. Exons are the protein encoding regions. Intron are intervening regions and are non-protein coding.

The intron segments will be cut out by the aptly named splicosome and reformed into a single strand that exits out of the nuclear membrane through nuclear pores. In the cytoplasm, it will be transported to ribosomes where it translated into protein. Interestingly, the activity of splicosome is tissue-specific and invalidates the "one-gene-one-protein" idea. In drosophila, Exon 5 for myosin light chain 1 is excluded for indirect flight muscles (thorax) while all six exons are included for all others.

Here are some phase contrast (top row) and differential interference contrast (bottom) micrographs of RBCs (left) and HeLa cells (right). RBCs and platelets are cells in our bodies that do not have nuclei and do not contain genetic DNA. They have mitochondrian DNA.

Lengthy DNA

- A cell's DNA is 2 meters long!
- Compaction scheme
  - DNA (-)
  - Histones (+)
  - Chromatin



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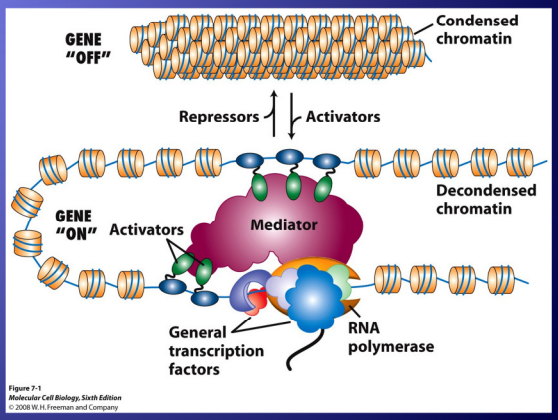
Cells can be small as 10 microns so DNA is 200,000X longer.

DNA (acidic, neg charge) is compacted and organized by Histones (rich in basic amino acids, pos charge). The complex of DNA, Histones, and other proteins constitute chromatin (50% DNA, 50% protein). Chromatin is normally dispersed throughout the nucleus during interphase but become more folded and compacted during mitosis (the readily visible chromosomes of metaphase).

The beads on a wire arrangement is seen as low Mg salt concentrations. At physiological salt levels, a 30nm fiber is formed.

Gene Control

- Sequence need to be readily accessible
  - Heterochromatin (closed)
  - Euchromatin (open)
- Binding regions
  - Transcription regions
    (Transcription factors)
  - Promoter sequences
    (RNA polymerase)



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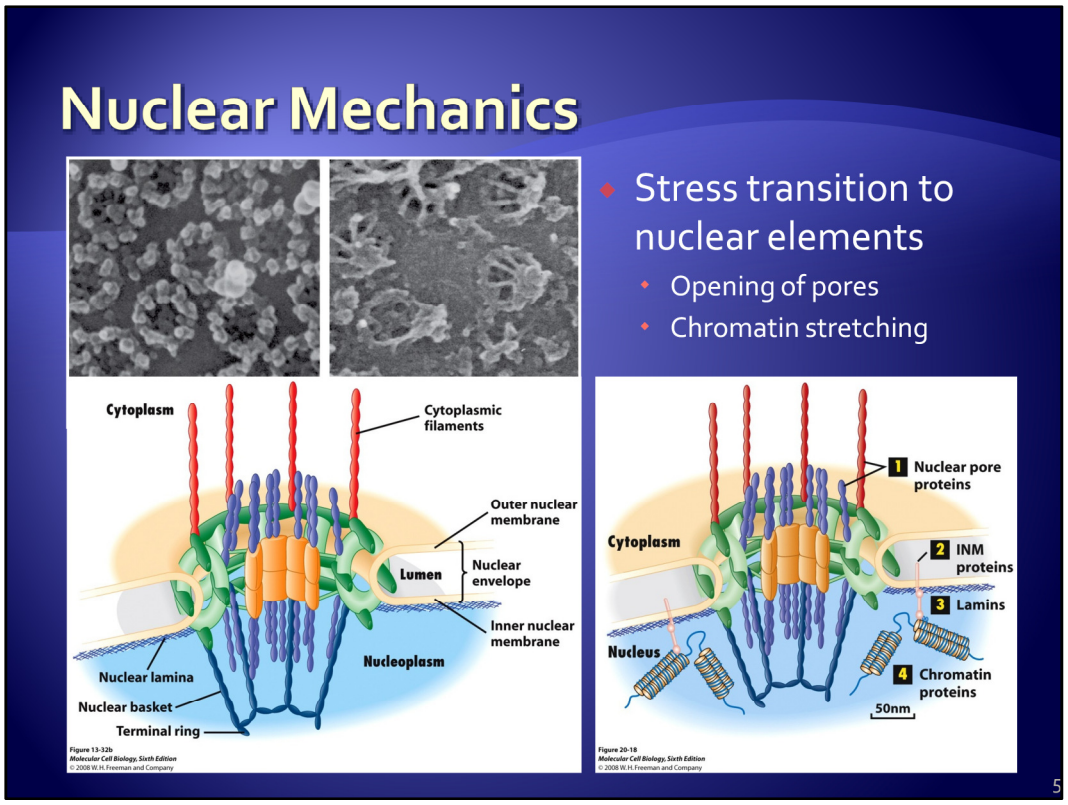
For transcription (gene activation), post-translational covalent modification of the amino acids in the tails of histones controls the compaction of the chromatin and its ability to be transcribed, replicated, and repaired. Heterochromatin are highly dense chromatin. Euchromatin is less condensed from acetylation of the lysine group that neutralizes their neg charge and unwinds the chromatin. Methylation of lysine prevents acetylation and keeps it wound tight.

Once the DNA structure is unwound, it its open for binding of the proteins like transcription factors and RNA polymerase that transcribe the DNA sequence into RNA.

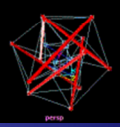


After transcription, the RNA needs to leave the nucleus and does so through nuclear pores in the nuclear envelope (a lipid bilayer). These structures are connect to the cytoskeleton and it has been suggested that tension can strength open the pores. The pores are a two-way street because transcription factors in the cytoplasm need to enter into the nucleus.

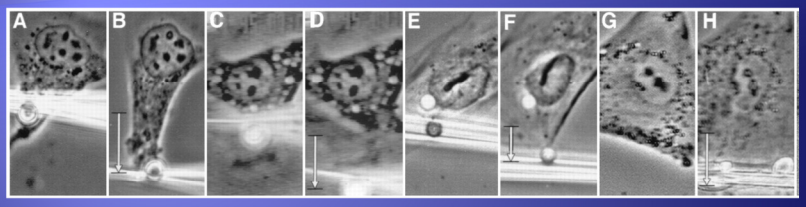
The nuclear membrane is held up by intermediate filament proteins that form the nuclear lamina. Another interesting concept to consider is that the lamins connect to chromatin structure. It has been suggest that mechanical distortion of the nuclear membrane can transmit strain to the chromatins and expand their structure to expose new genes to be transcribe that were previously hidden to transcription factors

Mechanical Connections

Tensegrity



- Maniotis, Chen, Ingber, PNAS, 1997, 94:849-54



- Phase-contrast (*A–H*) views of endothelial cells before (*A*, *C*, *E*, *G*) and after (*B*, *D*, *F*, *H*) mechanical stresses were applied to cell surface receptors. (*A* and *B*) Pulling on a single RGD-coated microbead (4.5-μm diameter) 15 min after binding to integrins using an uncoated glass micropipette; only 2 sec passed between *A* and *B*. (*C* and *D*) Similar displacement of a surface-bound bead coated with acetylated low density lipoprotein (AcLDL), a ligand for transmembrane metabolic receptors, microbead. (*E* and *F*) Mechanical displacement of RGD-coated (Arg–Gly–Asp) beads bound to the surface of a cell permeabilized with 0.5% Triton X-100 prior to force application. (*G* and *H*) A spread cell before (*G*) and after (*H*) a fibronectin-coated micropipette was bound to cell surface integrins for 5 min and pulled laterally (downward in this view). The movement of the pipette is downward, and vertical black arrows indicate the extent of pipette displacement in all views.

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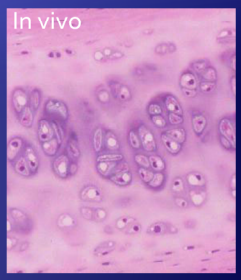
This study shows that there is a mechanical connection from the integrin receptors to the nuclear envelope. This connection indicates that strains in the extracellular matrix of a tissue can be transmitted to the nucleus through a unique mechanical pathway that involves only integrins and not other transmembrane proteins (AcLDL).

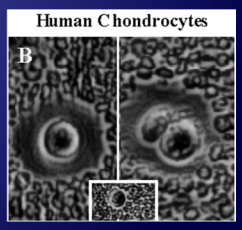
Chondrocytes

- Cells of cartilage responsible for synthesizing and maintaining the tissue.
- Mechanoresponsive to compression
  - Static 15% strain inhibits synthesis of cartilage matrix proteins and proteoglycans
  - Cyclic compression can stimulate matrix production
    - Low frequency (<1 mHz, 5% strain) has little effect
    - Hi frequency (10 mHz – 1 Hz, 5%) increases matrix changes
  - Strain rate is important
    - Low rate (0.01 s^{-1}, 50%) has no effect on chondrocyte activity
    - Hi rate (0.1-1 s^{-1},50%) decreases matrix production and kills cells
- In vitro, grown in a 3D bed of alginate beads to prevent dedifferentiation.
  - In culture, they loose their cartilage phenotype and transform into flattened fibroblast-like cells.
  - Specific markers are downregulated.

In vivo



In vitro



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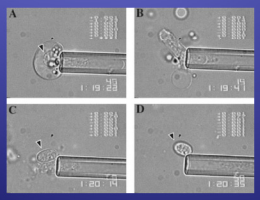
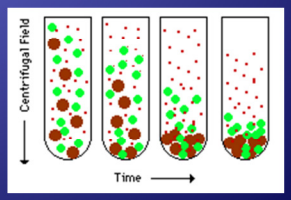
Technique

- Micropipette system on inverted microscope

- Mechanical and chemical isolation of nuclei





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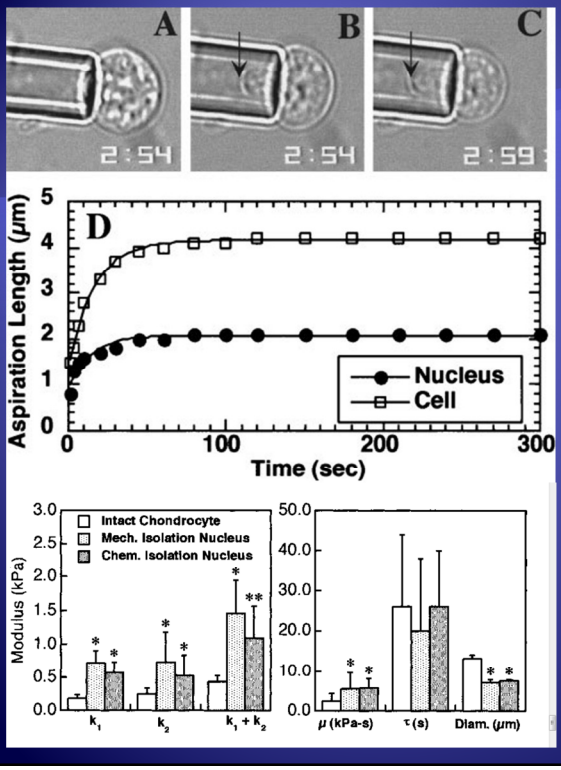

Results

- Nuclei are stiffer than intact cells
- Nuclei are more viscous
- Viscous fluid drop model with elastic shell not appropriate
- Nuclei exhibit more solid characteristics than viscous fluid

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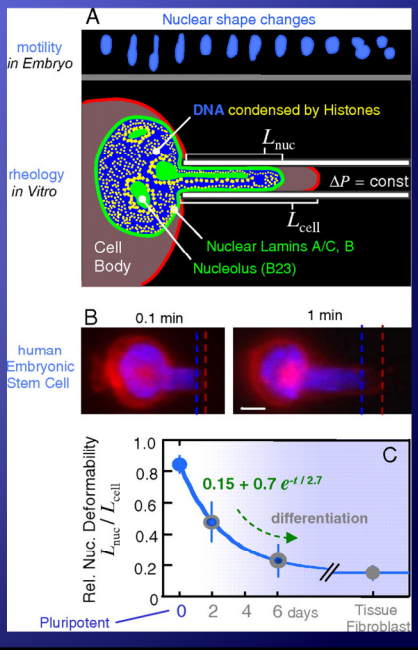
Stem Cell Plasticity?

- Pajerowski, Dahl, Zhong, Sammak, Discher, PNAS, 2007, 104:15619-24
- What about splicosomes?



Questions?