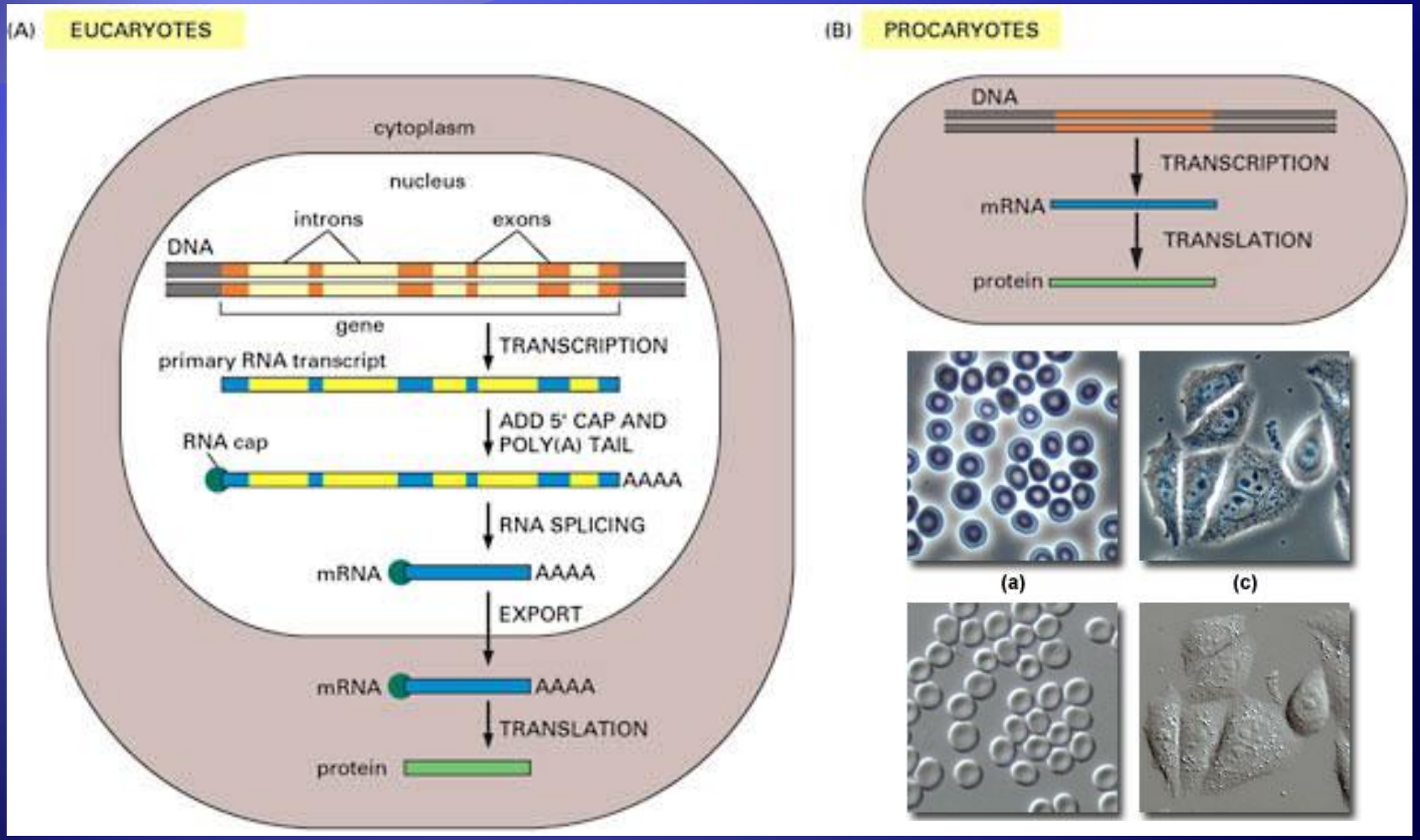
Session 3

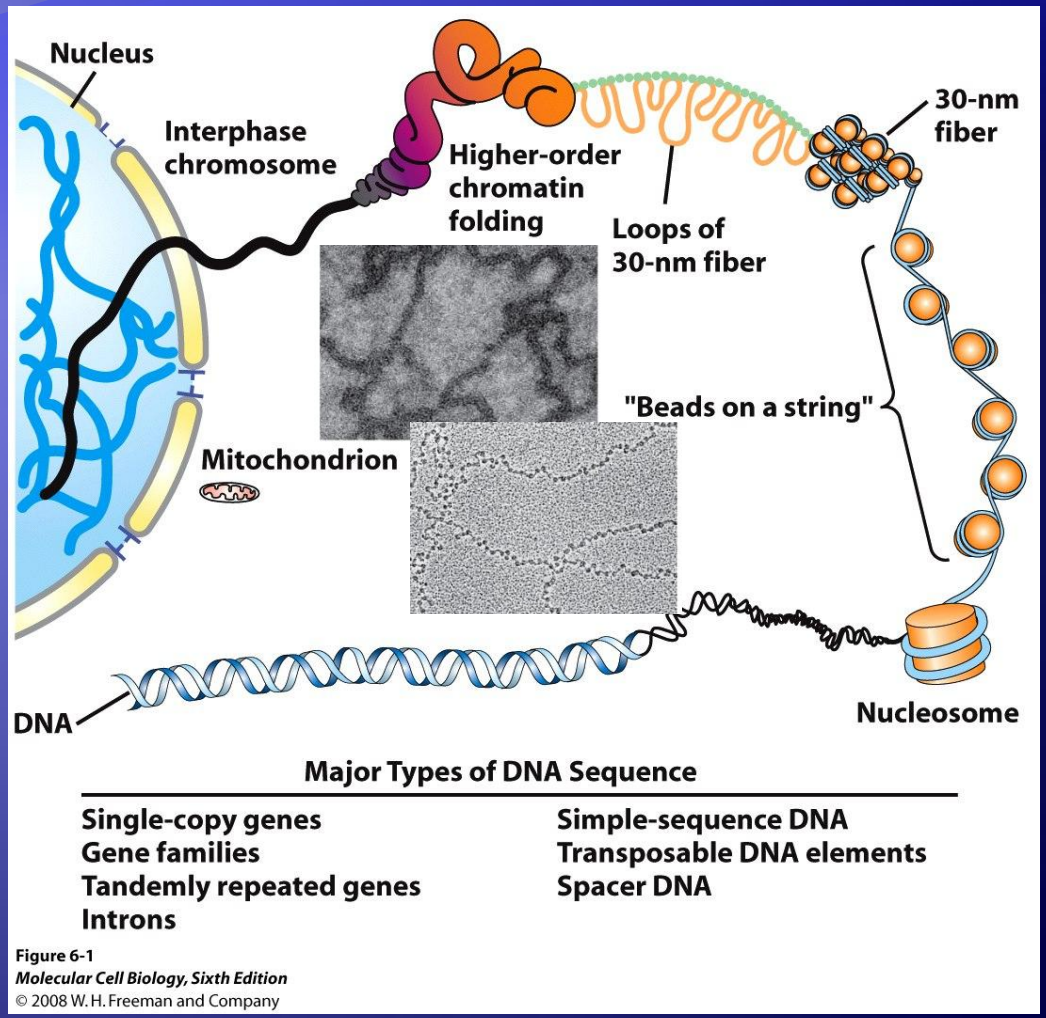
NUCLEUS

Nucleus


(A) EUCARYOTES
cytoplasm
nucleus
introns
exons
DNA
gene
TRANSCRIPTION
primary RNA transcript
ADD 5' CAP AND POLY(A) TAIL
RNA cap
AAAA
RNA SPLICING
mRNA
AAAA
EXPORT
mRNA
AAAA
TRANSLATION
protein
(B) PROCARYOTES
DNA
TRANSCRIPTION
mRNA
TRANSLATION
protein
(a)
(c)

Lengthy DNA

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



Gene Control

- Sequence need to be readily accessible
  - Heterochromatin (closed)
  - Euchromatin (open)
- Binding regions
  - Transcription regions

    (Transcription factors)
  - Promoter sequences

    (RNA polymerase)

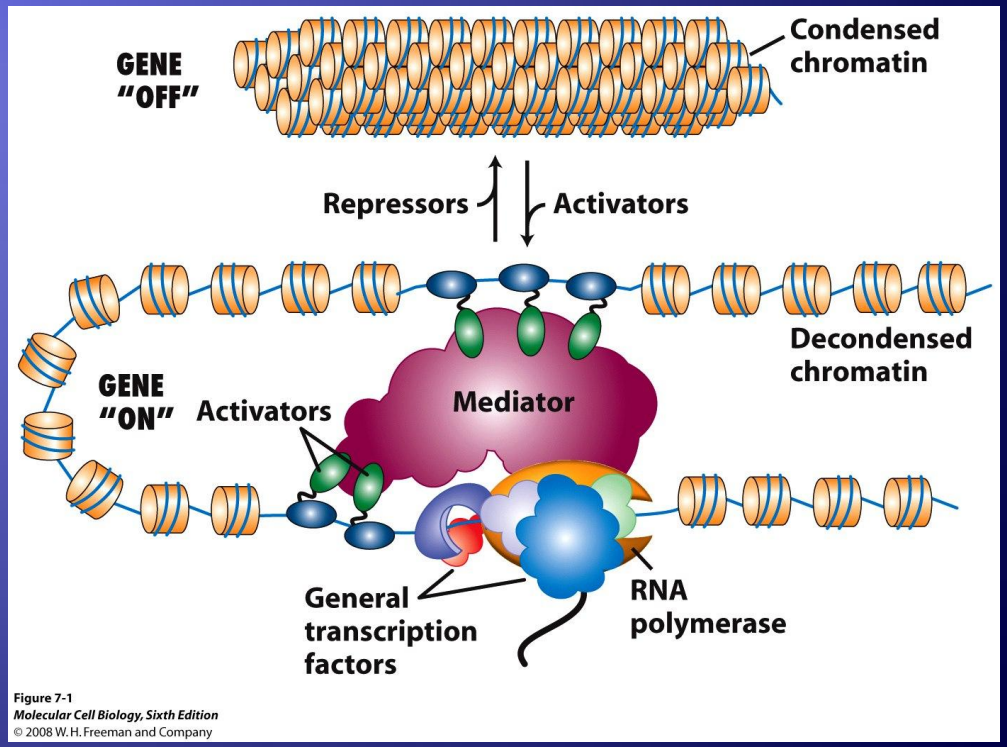
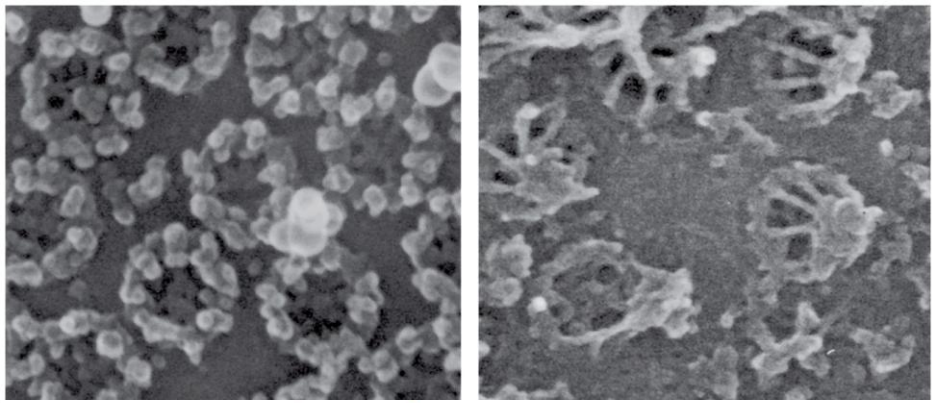


Figure 7-1
Molecular Cell Biology, Sixth Edition

Nuclear Mechanics



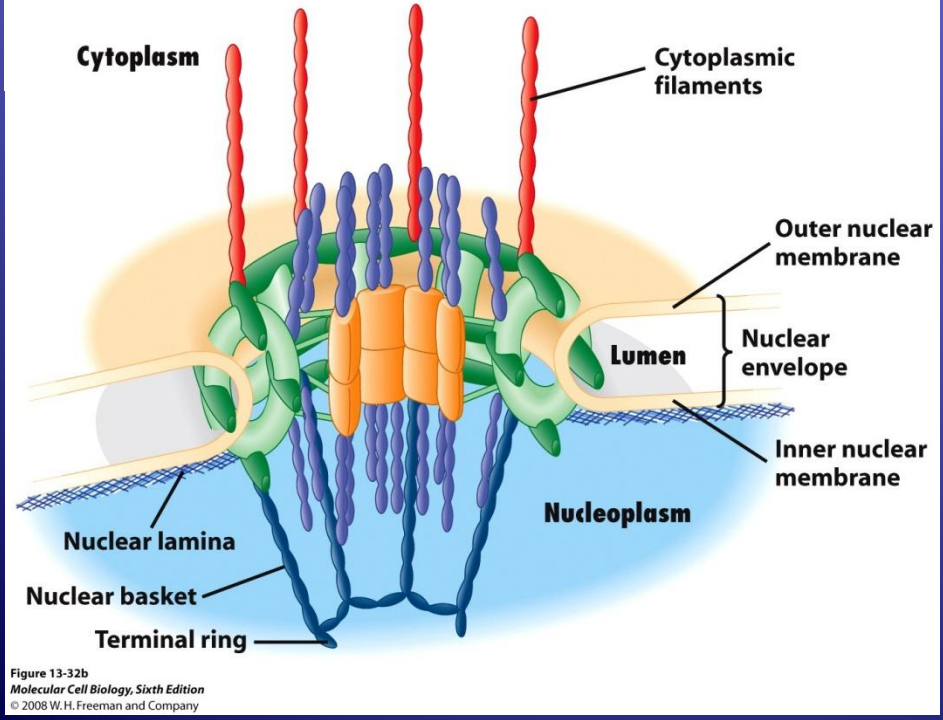


Figure 13-32b
Molecular Cell Biology, Sixth Edition

- Stress transition to nuclear elements
  - Opening of pores
  - Chromatin stretching

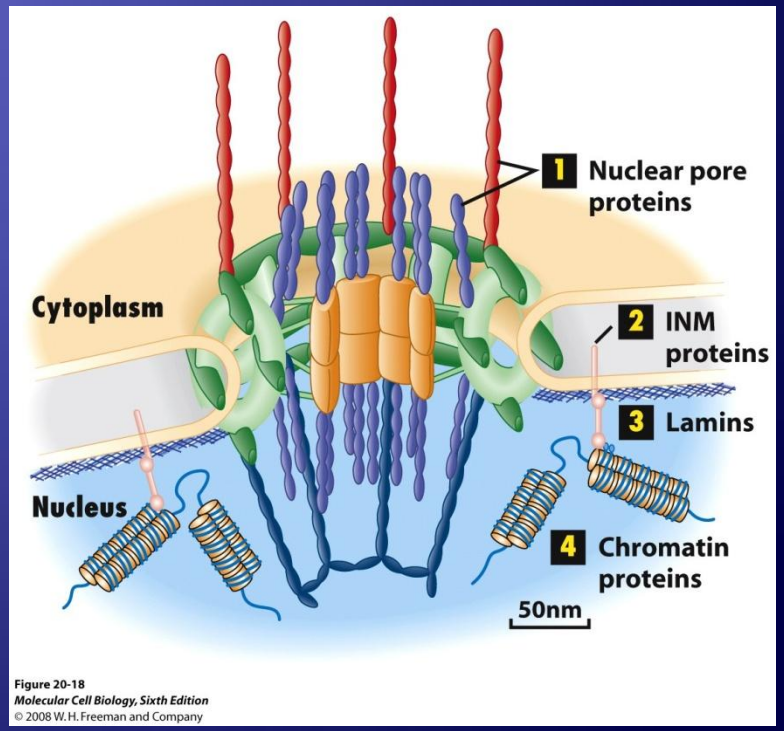
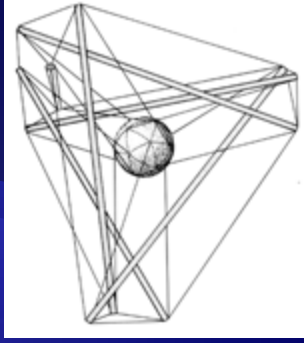


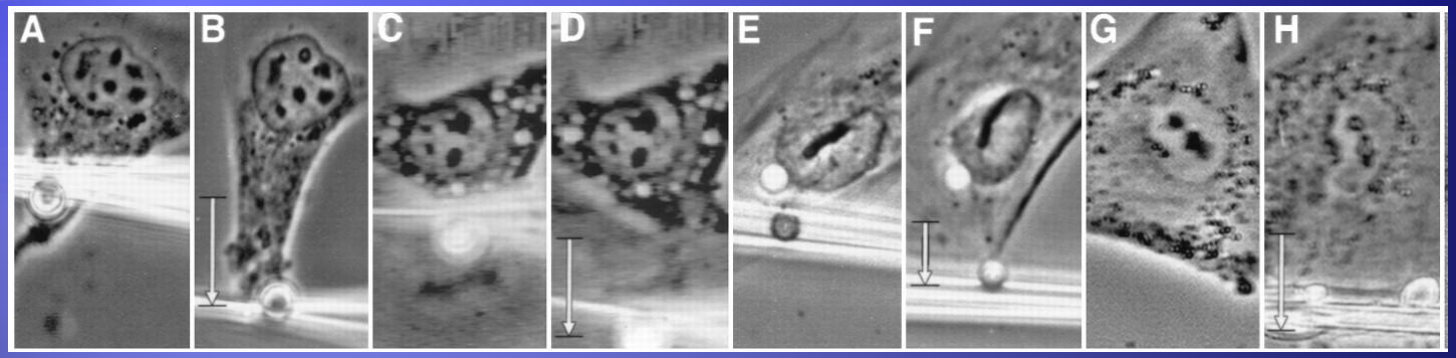
Figure 20-18
Molecular Cell Biology, Sixth Edition

Tensegrity



Mechanical Connections

- 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.

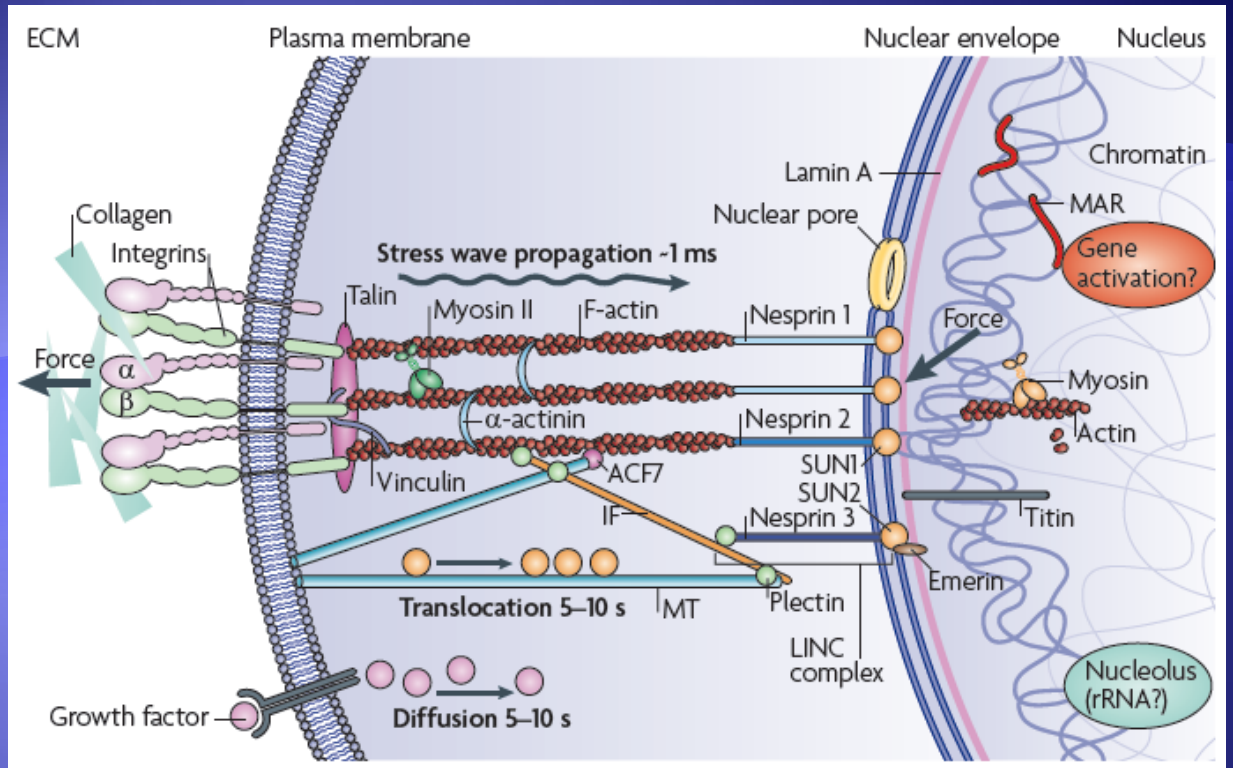


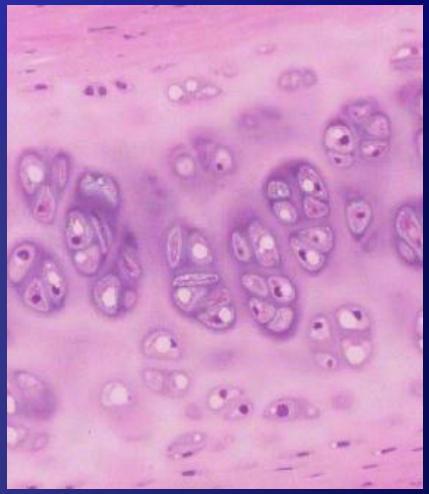
Figure 3 | **Molecular connectivity from the ECM to the nucleus.** A local force applied to integrins through the extracellular matrix (ECM) is concentrated at focal adhesions and channelled to filamentous (F)-actin, which is bundled by α-actinin and made tense by myosin II, which generates prestress. F-actins are connected to microtubules (MTs) through actin-crosslinking factor 7 (ACF7), and to intermediate filaments (IFs) through plectin 1. Plectin 1 also connects IFs with MTs and IFs with nesprin 3 on the outer nuclear membrane. Nesprin 1 and nesprin 2 connect F-actin to the inner nuclear membrane protein SUN1; nesprin 3 connects plectin 1 to SUN1 and SUN2. Owing to cytoplasmic viscoelasticity, force propagation from the ECM to the nucleus might take up to ~1 ms. The sun proteins connect to the lamins that form the lamina and nuclear scaffold, which attaches to chromatin and DNA (for example, through matrix attachment regions (MARs)). Nuclear actin and myosin[102] (and nuclear titin) might help to form the nuclear scaffold, control gene positioning and regulate nuclear prestress. The force channelled into the nuclear scaffold might directly affect gene activation within milliseconds of surface deformation. By contrast, it takes seconds for growth factors to alter nuclear functions by eliciting chemical cascades of signalling, which are mediated by motor-based translocation or chemical diffusion. LINC, linker of nucleoskeleton and cytoskeleton; rRNA, ribosomal RNA.

Wang, Tytell, Ingber, Nat Rev Mol Cell Biol, 2009, 10:75-82

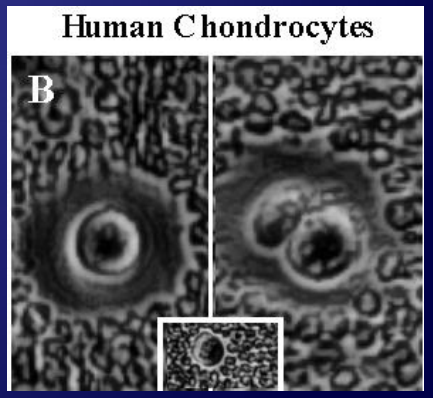
Case study: Chondrocytes

- 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 cartilage phenotype and transform into flattened fibroblast-like cells.

In vivo



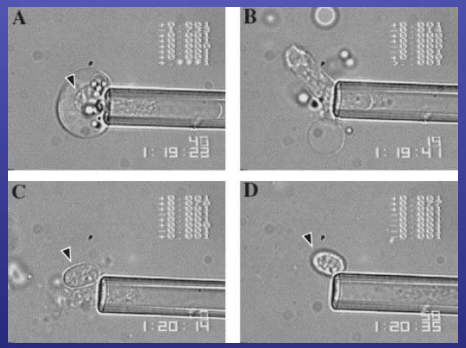
In vitro

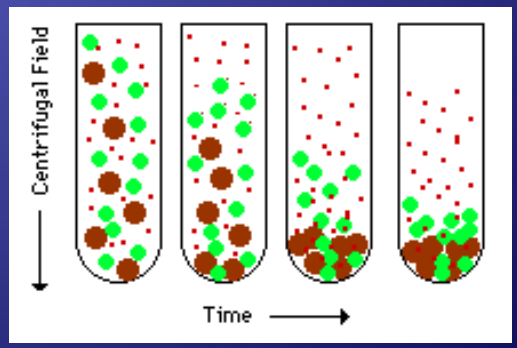


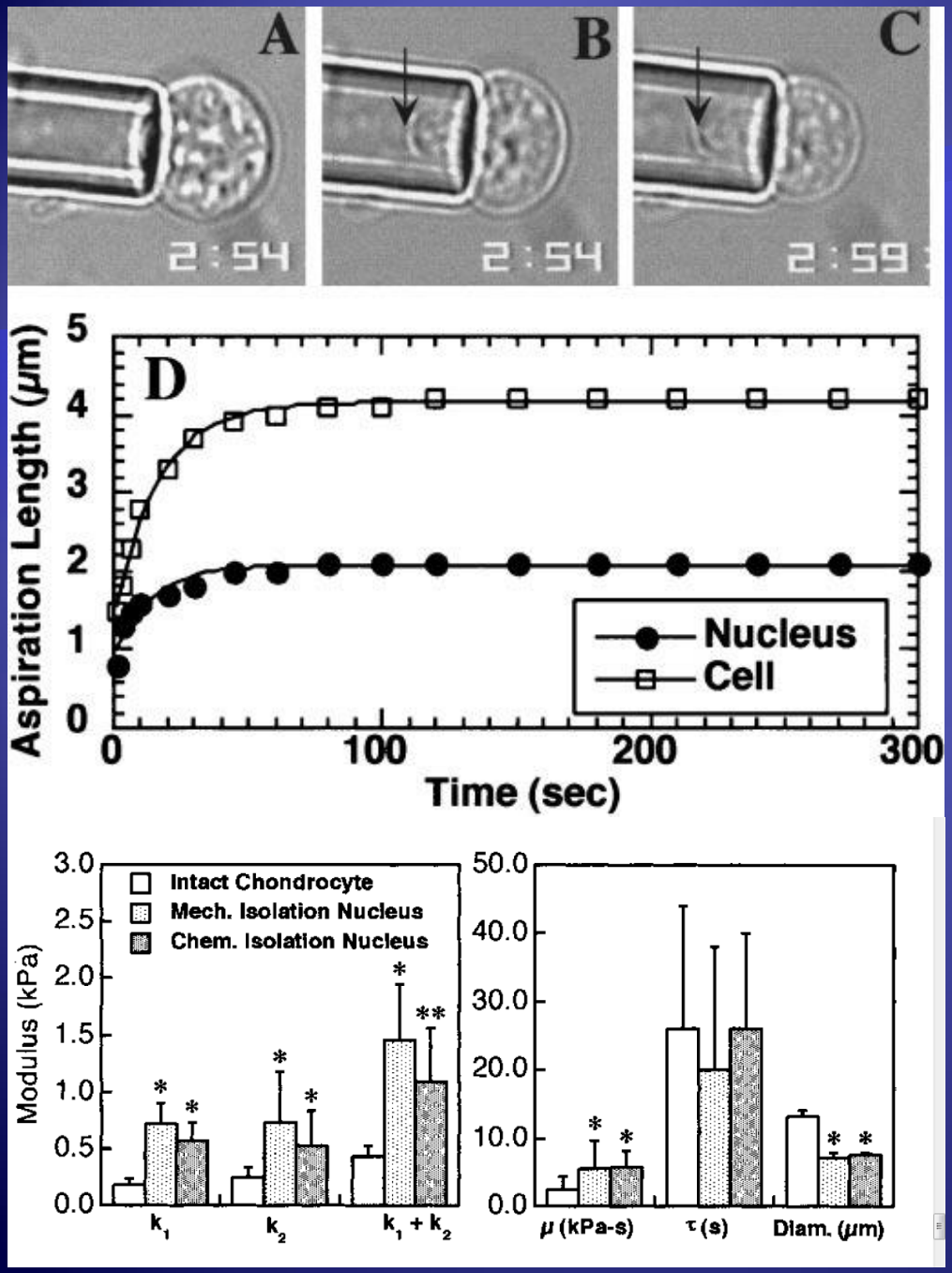
Technique

- Micropipette system on inverted microscope

- Mechanical and chemical isolation of nuclei

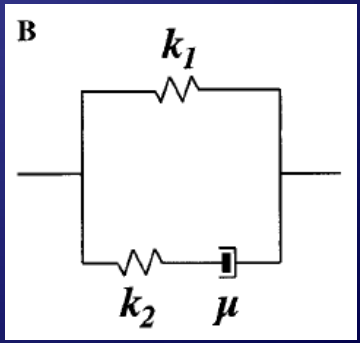




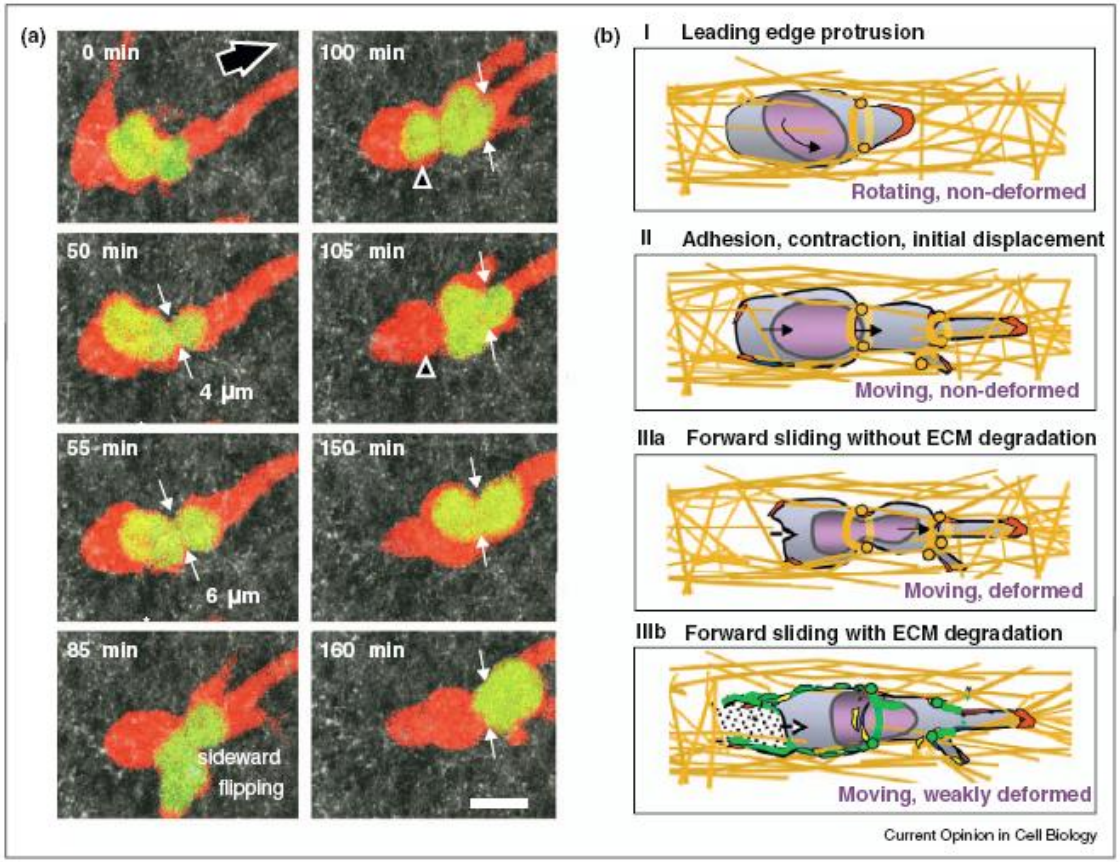


Results

- Nuclei stiffer and more viscous than whole cells
- Viscous fluid drop model with elastic shell not appropriate
- Nuclei exhibit more viscoelasticity characteristics than viscous fluid

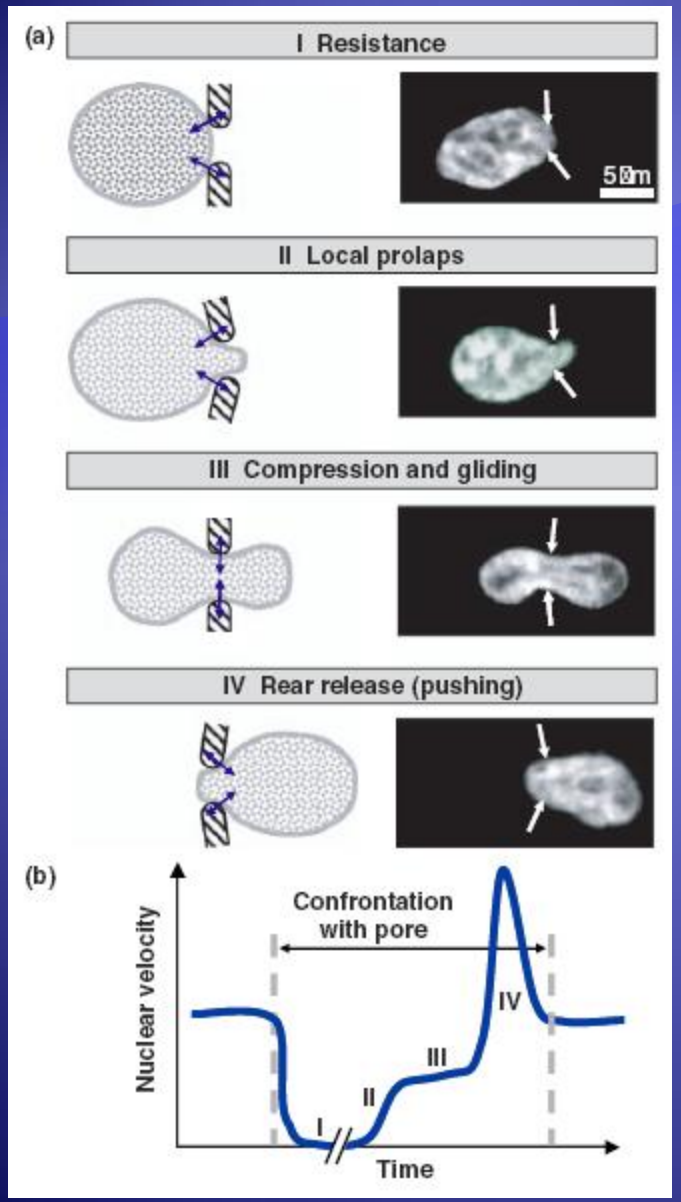


Cell Migration in 3D



Nuclear dynamics and deformation during cell migration. **(a)** HT-1080 cells expressing DsRed2 in the cytoplasm and H2B/eGFP in the nucleus migrating in 3D collagen lattice. Confocal time-lapse sequence in mid-density (3.3 mg/ml) collagen shows phases of shape change. Bar, 10 µm. **(b)** Initial cell polarization leads to rotational dynamics (I) followed by translocation of the nucleus (II). When physical barriers are encountered, the nucleus deforms during forward sliding (III). In the absence of ECM degradation capability, the nuclear deformation is more pronounced (IIIa). Alternatively, the cell degrades ECM structures proteolytically (green color) and generates a small trail-like matrix defect (IIIb, asterisk), thereby minimizing nuclear deformation. Red label, cortical actin; yellow label, surface-localized MMP.

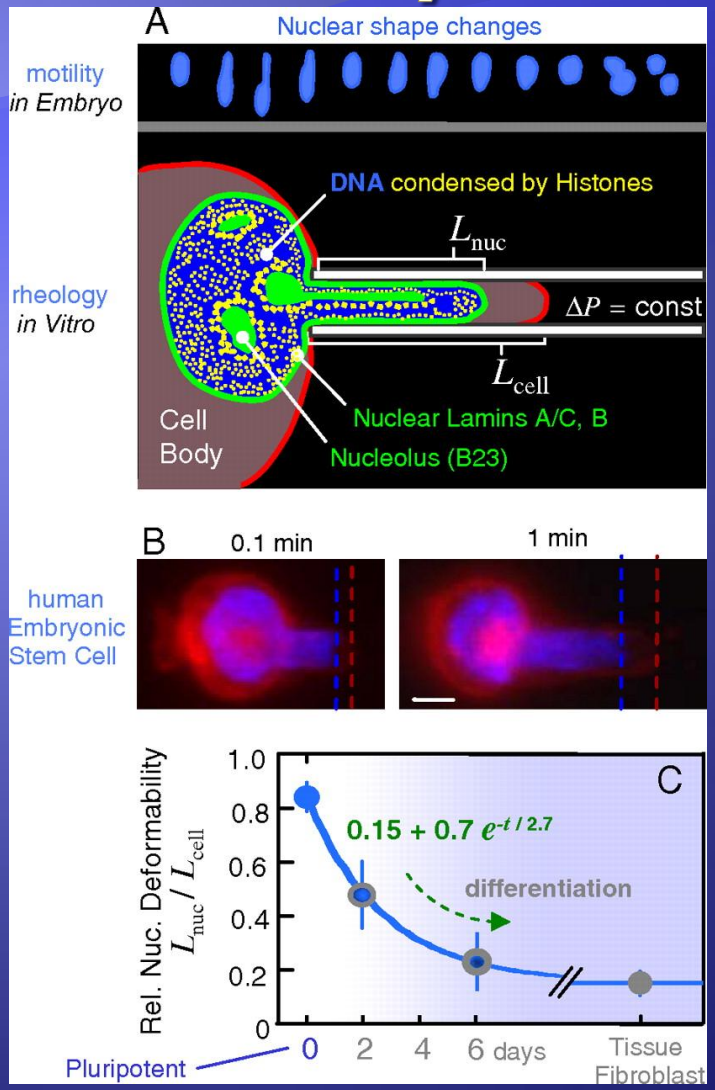
Friedl, Wolf, Lammerding, *Curr Op Cell Biol* 2011, 23:55-64



- White Blood Cells
  - Neutrophils shut down DNA replication
  - Activated T-cells have upregulation of lamins
- Cancer
  - Lamin deregulation in leukemia, GI, and lung cancer
  - Lamin upregulation in ovarian, colon, and prostate cancer
- Effect on migration and/or chromatin configuration?

Friedl, Wolf, Lammerding, *Curr Op Cell Biol* 2011, 23:55-64

Stem Cell Plasticity?



Pajerowski, Dahl, Zhong, Sammak, Discher, PNAS, 2007, 104:15619-24

Questions?