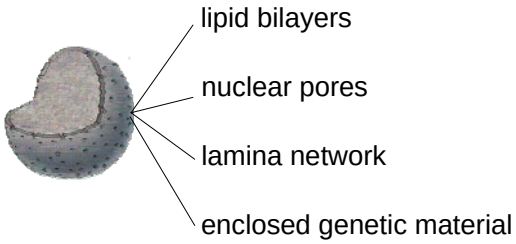
The Nucleus

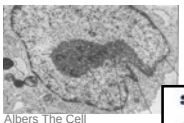
Structure and Engineering

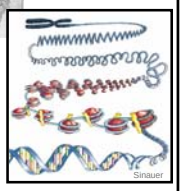
Structures of the Nucleus

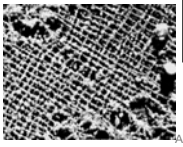


The Biological Nucleus

- The Whole Nucleus: nuclear domains and epigenetic modifications regulate gene expression
- DNA: DNA and higher order chromatin structures contain and regulate genetic information
- Lamina: the nuclear lamina organizes genes and regulates nuclear function


Albers The Cell


Sinauer

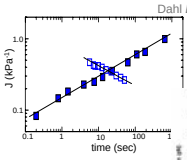

Aebi *Nature* 1986

The lipid membranes

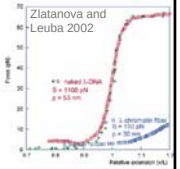
The Biophysical Nucleus

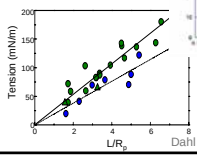
- The Whole Nucleus: complex viscoelasticity suggests shear thinning under stress
- DNA: chromatin is more deformable than DNA which exhibits a 3-state deformation
- Lamina: the lamina appears to be purely elastic, highly extendible and reversible to protect interior chromatin from shear damage

Dahl *Biophys. J.* 2005



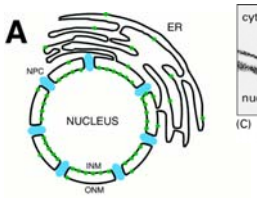
Zlatanova and Leuba 2002

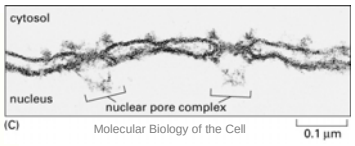




Dahl *J. Cell Sci.* 2004

Nuclear membranes

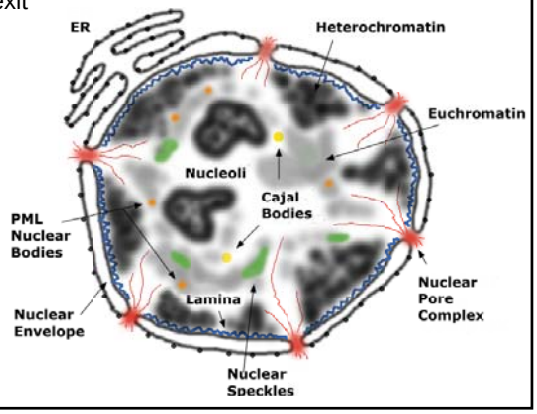




- Double membrane system
- Contiguous with endoplasmic reticulum
  - Allows membrane proteins to transport directly from the ER

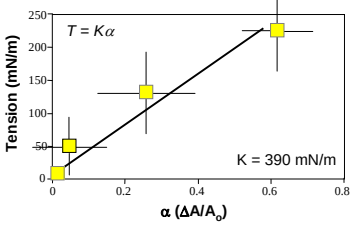
Ellenberg *J. Cell Biol.* 1997

Nuclear "Envelope" encloses, organizes and regulates entry/exit



Tension and α to Determine K

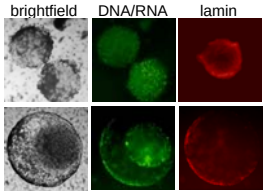
K is the dilation modulus of the lipid bilayer membranes



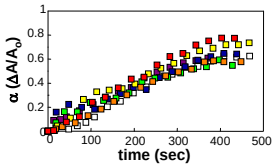
	nucleus	red cell	bilayer	2 bilayers
K (mN/m)	390	450	150-250	200-400
α-lysis	>60%	2-4%	2-3%	

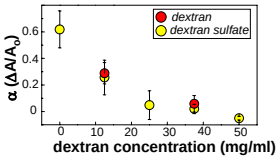
Rawicz 2000, Kwok and Evans 1981, Evans 1976, Evans and Waugh 1977, Katnik and Waugh 1990 all *Biophys J*

Xenopus oocyte nuclei swell when isolated



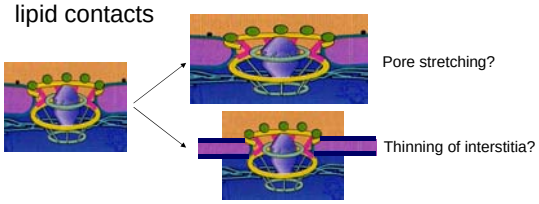
→ Used to determine membrane properties





Unique stretch properties

- Similar membrane dilation modulus
- Very high rupture modulus (>60% versus 3%)
  - Rupture is therefore not determined by lipid-lipid contacts



Osmotic Swelling to Determine T

Assumptions:

- Continuous membrane
- Covered with rigid pores
- Pores modeled as cylinders
  - *n* pores
  - radius *a*
  - length *l*
- Poiseuille flow through pores

filtration coefficient from data on pores

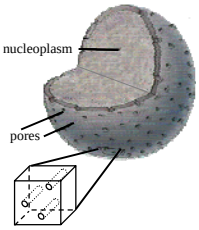
$$\Phi = \frac{n \pi a^4}{8 \eta l}$$

membrane pressure from swelling data

$$\Delta P = \frac{dV/dt\ _{initial}}{A\ \Phi}$$

membrane tension from Law of Laplace

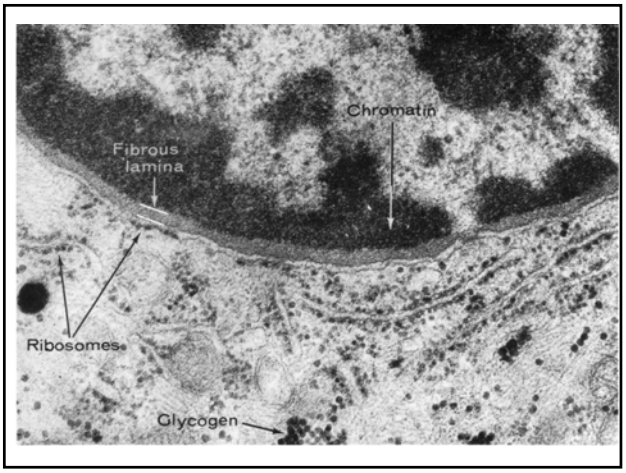
$$T = \frac{1}{2} \Delta P r$$



a = radius of pore
A = nucleus surface area
l = length of pore
n = pore density
P = pressure
r = radius of nucleus
T = tension
V = volume of nucleus
h = viscosity
α= surface area expansion
F = filtration coefficient

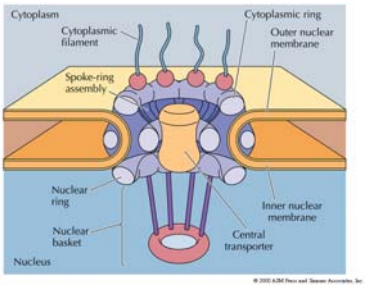
Nuclear Pore Complexes (NPCs)
and
Nucleo-cytoplasmic transport

(How stuff gets in and out of the nucleus)



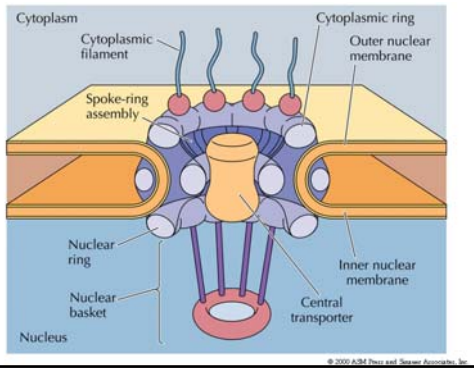
~3,000 NPCs per mammalian nucleus

Mediate ALL traffic into/out of nucleus

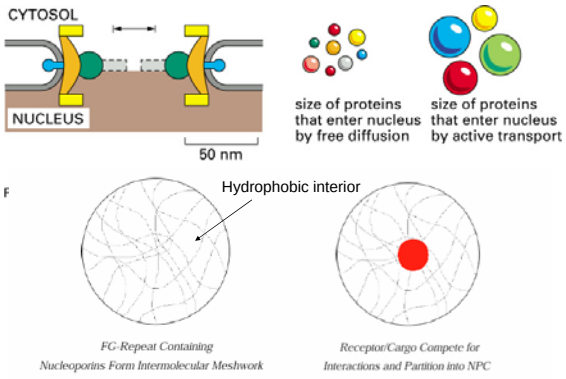


Allow passive diffusion of ions and small proteins (<40 kD)

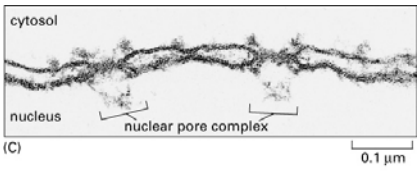
Nuclear Pore Complexes (NPCs) occupy 'pores' formed by the inner and outer membranes.
Each NPC = 8-32 copies (each) of 30 different proteins called *nucleoporins* ('Nups')

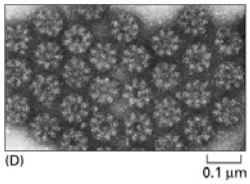


Translocation through the Nuclear Pore Complex



Nuclear Pore Complexes viewed by transmission electron microscopy (TEM)





NPCs are HUGE: 150 x 80 nm
~100,000 kDa

8-fold radial symmetry

Disassemble and reassemble with each mitosis

Figure 12–10 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Molecules over ~40 kD require a peptide **signal** to cross the NPC*

Import: Nuclear Localization Signal ('NLS')

Export: Nuclear Export Signal ('NES')

*The nuclear envelope is a 'border zone', with 'walls', import and export regulations, and smuggling activity (eg, viruses)

Anything, including gold nanoparticle up to ~25 nm diameter, bearing an NLS (or NES) peptide can be imported (exported) through NPCs; Ribosome subunits (10-20 nm are continually exported from the nucleus

Import:

- NLSs include PKKKRKV, KR[PAATKKAGQA]KKKK, etc.
- NLS is recognized by receptors called importins
- Complex then binds NPC and enters the nucleus
- In the nucleus, Ran-GTP dissociates the imported complex

Export:

- NES are hydrophobic
- NES is recognized by a receptors called exportins and require Ran-GTP
- Complex then binds NPC, translocates through and

A gradient of RanGTP drives nuclear transport, and determines DIRECTION of movement

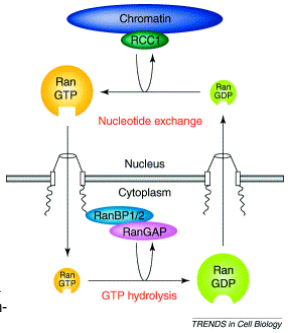
How do molecules know IN from OUT??

Ran-GTP is abundant inside nucleus due to the action of RCC1, an abundant chromatin protein that removes GDP and puts GTP onto Ran.

Ran-GTP binds EXPORTINS, promotes exportin binding to NES-cargo, and exits with them.

Ran-GDP is abundant in cytoplasm due to cytoplasmic Ran-GAP (GTPase Activating Protein) that simulates Ran to hydrolyze GTP.

Ran-GDP is shuttled by a protein named NTF1 back into nucleus, where it is recharged to Ran-GTP and promotes DISSOCIATION of newly-imported Importin/NLS-cargo complexes.



IMPORT requires a Nuclear Localization Signal (NLS)

Positive charges, but no strict consensus.
*Signal must be **exposed** on protein surface!!*

Classic NLS: P**KKKRK**V

'Two-part' NLS:
KRxxxxxxxxxx**K**x**KK**

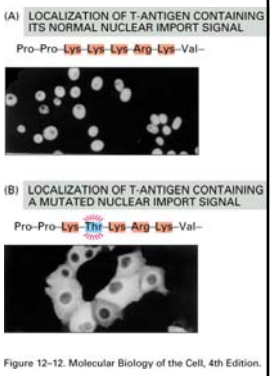
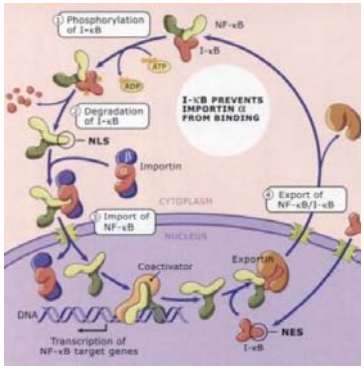


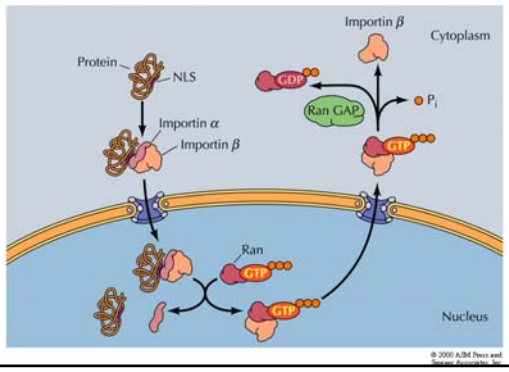
Figure 12–12. Molecular Biology of the Cell, 4th Edition.

Nuclear entry (or exit) can be regulated by hiding the NLS (or NES)

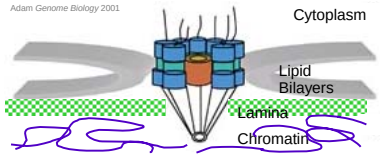


Cells by Lewin 2007

Cargo traverses NPC via importins. Cargo is released inside nucleus by Ran-GTP

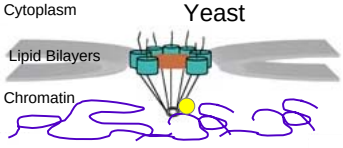


Nuclear Pore Complexes (NPC)

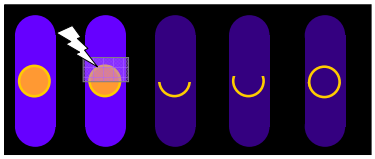


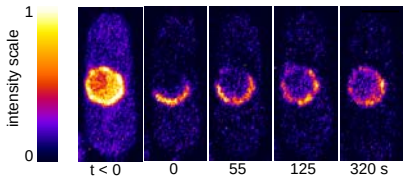
Metazoan
• Lamina anchored

Yeast
• No lamina
• DNA tethered to NPC



FRAP of yNup85 in the Nuclear Envelope

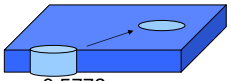




Phong T. Tran

Continuum Model of Pores Moving Through a Thick Membrane

$$D_{trans} = \frac{k_B T}{4\pi\eta h}\left(\ln\left(\frac{\eta h}{\eta_1 R}\right) - \gamma\right)$$



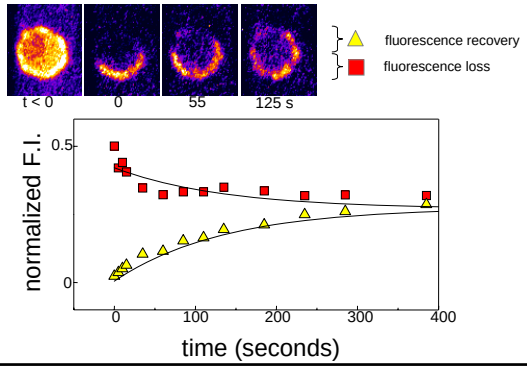
γ = Euler's constant	0.5772
η = bilayer viscosity	5P
η_1 = cytoplasm viscosity	0.01P
R = radius of the complex	(960/2)Å
h = bilayer height	300 Å

D_{trans} = 0. 11 $\mu m^2/s$ freely diffusing body

$D_{FRAP\text{-}calc}$ = 0.04 $\mu m^2/s$

Saffman and Delbruck *PNAS* 1975

FRAP Quantification



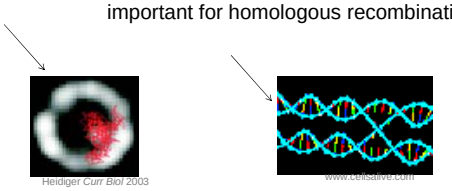
Yeast NPC Mobility

Diffusion rate comparable to cellular transmembrane proteins with slight cytoplasmic steric hindrance

D_{nuc} = 0.04 μm^2/sec $D_{band\ 3}$ = 0.004 – 0.20 μm^2/sec

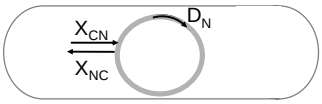
Poo and Cone *Nature* 1974, Golan and Veatch *PNAS* 1980

Allows chromatin more freedom at nuclear envelope, important for homologous recombination



Heidiger *Curr Biol* 2003

Half-times of yNup85 Movement



	D_N	X_{CN}	X_{NC}
	Nuclear Diffusion	**Cytoplasm to Nucleus**	**Nucleus to Cytoplasm**
$\tau_{1/2}$ (s)	**58 +/- 4**	**289 +/- 42**	**553 +/- 94**
n	15	10	10

compared with metazoan NPC
$t_{1/2}$ = 20 hours

Daigle *J Cell Biol* 2001

Nuclear Lamins

First Purification and Characterization of Lamins

Gerace, Blum, and Blobel (1978)

Rat Liver Nuclei

⇩ DNase

Nuclear Envelopes

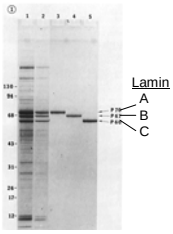
⇩ Triton X-100

⇩ Salt Wash

Nuclear Pore Complex/ Lamina Fraction

⇩ Preparative Gel Electrophoresis

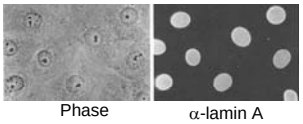
Purified Lamins (A, B, C)



Generate Antibodies

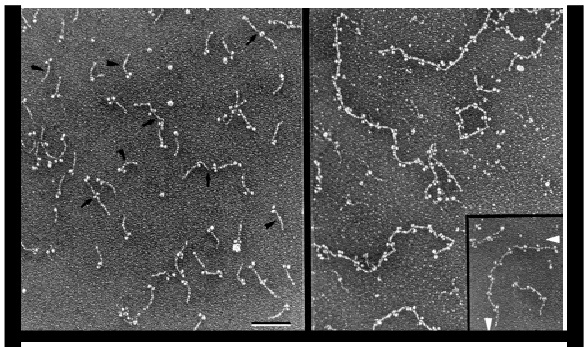
⇩

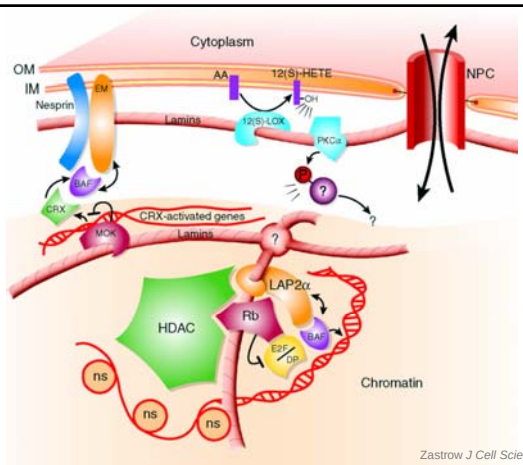
Immunofluoresence



Phase α-lamin A

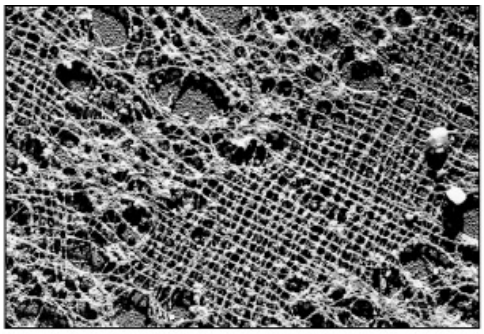
Head-to-tail association of IF dimers





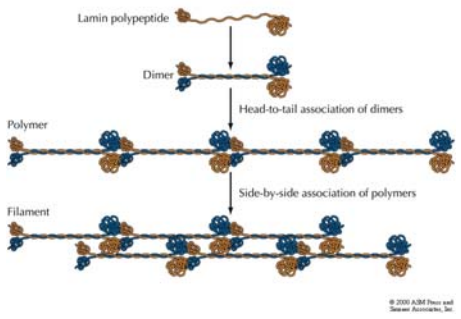
Zastrow *J Cell Science* 2004

Lamin filaments @ oocyte nuclear envelope



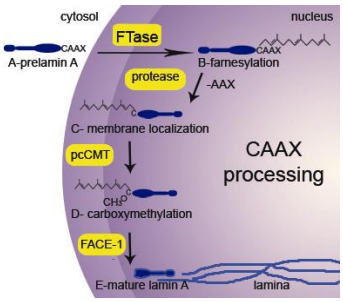
Lamins

Nuclear intermediate filament proteins



Lamin processing for localization

- Prelamin A (with CAAX box) is farnesylayed and carboxymethylated for membrane localization
- Hydrophobic residues are then cleaved to make mature lamin A



B-type lamins (~1-2 million copies / nuc)

Ancestral

Expressed in all human cells

Essential for LIFE in multicellular eukaryotes

Required for DNA replication, mRNA transcription

A-type lamins (~1 million copies / nuc)

Non-essential

Absent from stem cells

Expressed upon differentiation

Partners: Rb, actin, histones, NE proteins

"Laminopathies"

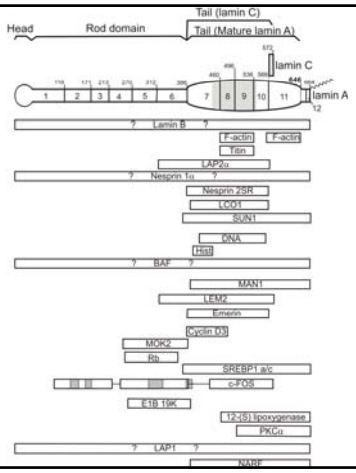
Diseases caused by mutations in *LMNA* *or lamin-binding proteins*

Striated muscle+	EDMD	***EMERIN, NESPRIN-1, NESPRIN-2***
	Limb-girdle muscular dystrophy	***EMERIN***
	Dilated cardiomyopathy	***EMERIN, LAP2***
Adipose tissue	Lipodystrophy	***Lamin B2***
Neurons?	Charcot-Marie-Tooth axonal neuropathy	
Bone+	Mandibuloacral dysplasia Heart-Hand syndrome	
Human development	Restrictive dermopathy (lethal at birth)	
Human aging	Hutchinson-Gilford Progeria Syndrome atypical Werner Syndrome	

Profound disruption of lamina network, as seen in progeria patients, disturbs epigenetic regulation

Lamin A polymers as 'scaffolds'

Many nuclear proteins bind lamin A directly



Zastrow *J Cell Sci* 2004

Other pathologies suggest widespread and subtle roles for lamins and nuclear membrane proteins in human physiology

White blood cell development/function [*LBR*]

Neutrophil migration/oxidative burst defects; Ichthyosis
Pelger-Huet anomaly of neutrophils

Bone disorders *[LBR]*

Lethal HEM (Hydrops-Ectopic calcification-Moth-eaten) skeletal dysplasia
Syndactyly
Brachydactyly [short fingers/toes]

STEM CELL dysfunction

Epidermal stem cells [*LAP2, LMNA*]
Erythroid stem cells [*LAP2, LMNA*]
Ovarian germ cells in *Drosophila* [*Otefin*]

CENTRAL NERVOUS SYSTEM

CNS demyelination (adult-onset AD Leukodystrophy) [*LMNB1 duplic'n*]
Cerebellar hypoplasia, cataracts, mental retardation [Nesprin-1/*SYNE-1*]
Lissencephaly (AR cerebellar ataxia and atrophy) [Nesprin-1/*SYNE-1*]

"LAMINOPATHIES"

>15 human diseases/conditions caused by mutations in lamins or lamin-binding proteins

E.g., *LMNA* mutations can disrupt specific tissues (muscle, heart, fat, skin, bone, neurons),
or cause ***accelerated aging***
(Hutchinson Gilford Progeria; atypical Werner syndrome)

Lamins are required for:

Nuclear SHAPE

MECHANICAL strength (molecular 'shock absorber')

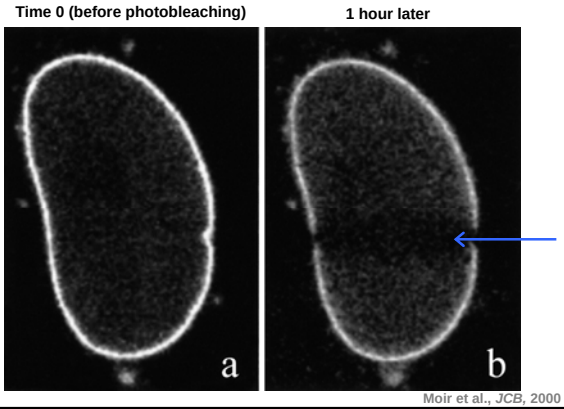
Chromatin attachment to NE

Nuclear Pore Complex anchorage/spacing

DNA replication (!!)

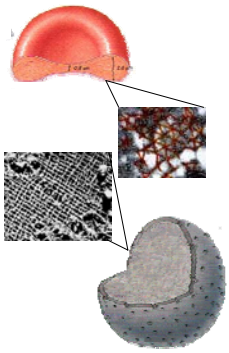
mRNA transcription (!!)

The lamin network is very stable



Moir et al., *JCB*, 2000

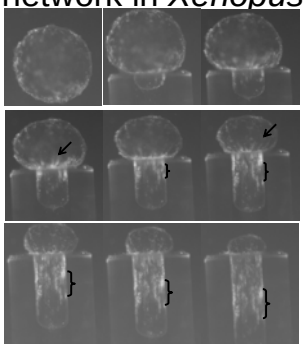
Comparison of Red Cell and Nuclear Membrane Skeletons



	nucleus	red cell
E (mN/m)	25	0.01
filament	lamin	spectrin
lp (nm)	100-500	2.5-10

Mohandas and Evans *Annu Rev Biophys Biomol Struct*.1994,Hohenadl *Biophys J* 1999, Discher 1998 *Biophys J* 1998, Lenormand *Biorheology* 2003

Micropipette aspiration of lamina network in *Xenopus* oocyte nucleus



Membrane Wrinkles
Suggests solid-like nature of the envelope

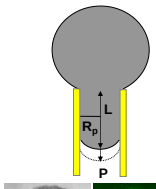
Distance Between Spots
Show stretch of membrane during aspiration

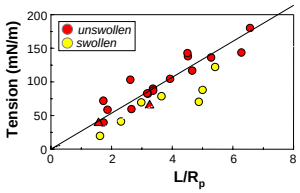
Hypothesis
Resistance to aspiration dominated by the lamin network

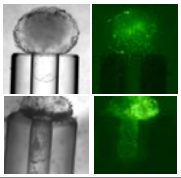
Chromatin

Tension and L/R_p to Determine E

E is the extensional modulus of the membrane skeleton



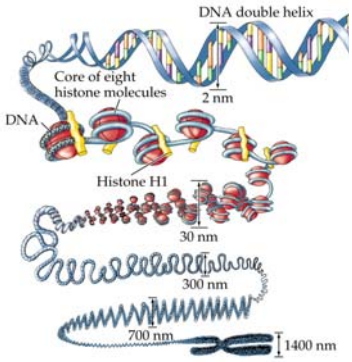




swollen
no DNA involved
E=24±9 mN/m

unswollen
DNA deformable
E=28±8 mN/m

E = 25 mN/m
measure of lamina
not nucleoplasm interior
not lipid bilayers



DNA

Nucleosome: DNA winds around a histone

Chromatin: packed nucleosomes

Chromosome: bundled and interconnected chromatin

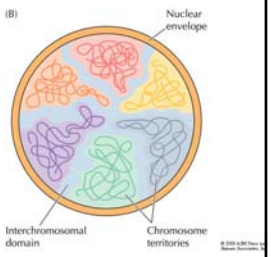
Higher-order chromatin structure is regulated and dynamic

Chromosome ‘territories’

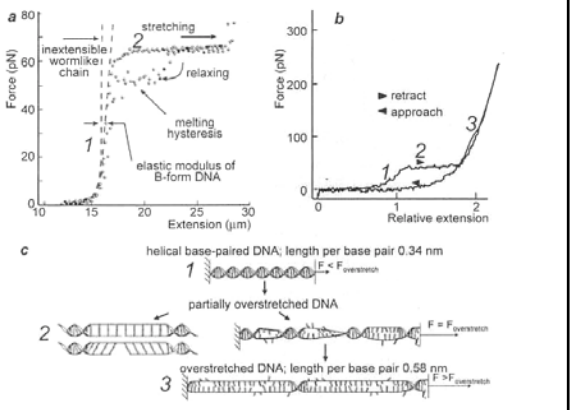
Can infiltrate neighbors.
No ‘fences’!!

Certain pairs of chromosomes are consistently found as ‘near neighbors’ in specific tissues.

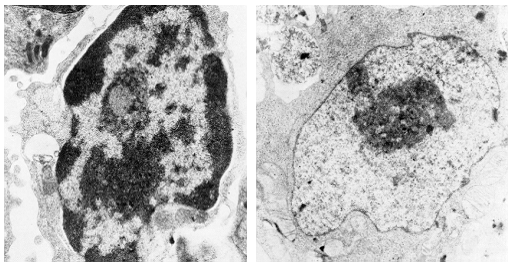
When a gene activates, its chromatin UNFOLDS to occupy a huge volume as diffusible proteins arrive to transcribe, splice and process the mRNA.



Stretched DNA

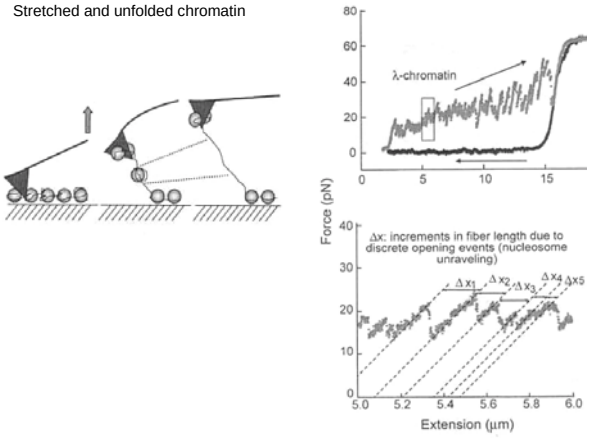


Chromatin can re-organize rapidly!



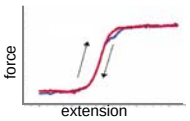
Inactive Lymphocyte Activated Lymphocyte

Stretched and unfolded chromatin



DNA, chromatin, chromosomes

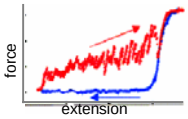
λ-DNA



Bennink et al. *Nature Struct. Biol.* 2001

DNA: very little hysteresis

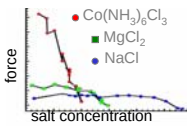
chromatin



Bennink et al. *Nature Struct. Biol.* 2001

chromatin: hysteresis
stepwise unfolding
from histones

chromosomes



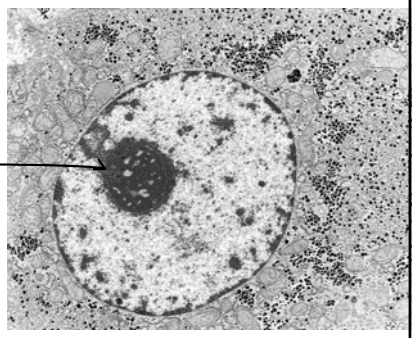
Poirer et al. *J. Cell Biochem.* 2002

Other Nuclear Bodies

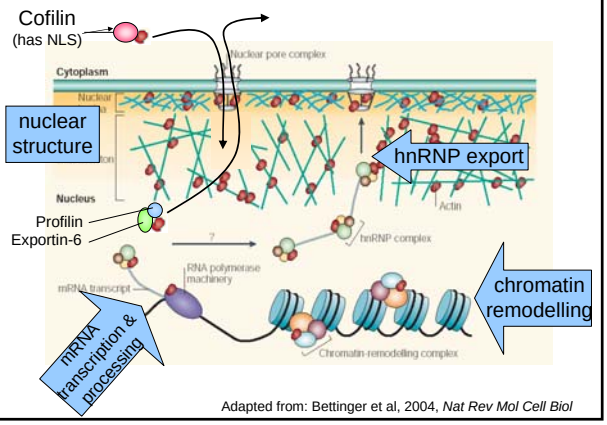
Subnuclear organelles have specific roles, e.g.:

The nucleolus

(factory for making ribosomes)



New concept: Roles for ACTIN in the nucleus



Adapted from: Bettinger et al, 2004, *Nat Rev Mol Cell Biol*

Inside the cell nucleus

David Spector

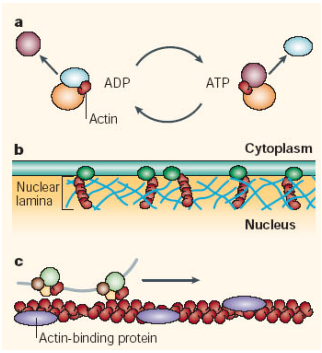
Cajal Bodies: ribosome biogenesis and transcription and are involved in small nuclear and nucleolar RNA metabolism, snRNP biogenesis
Gems: coincident or adjacent to Cajal bodies; assembly of snRNP and snRNP maturation
Nucleolus: ribosome synthesis and assembly
Heterochromatin: inactive chromatin
PcG bodies: contain polycomb group proteins (silencing proteins) such as RING1, BMI1 and hPc2
Nuclear Speckles: contains groups of pre-mRNA splicing factors
IGC: interchromatin granule clusters assembly, modification of pre-mRNA splicing factors
Transcription Sites: diffuse in nucleus and on periphery of IGC, several thousand foci
OPT Domains: (Oct1/PTF/transcription) appear in G1 but disappear in S phase; contain transcription factors but not RNA processing factors
Cleavage Bodies: cleavage and polyadenylation of pre-mRNA processing
PNC (perinucleolar compartment) SAM68 nuclear body: contain RNA and RNA binding proteins found mainly in cancer cells
PML bodies: transcriptional regulation affected by viral infection

Possible Forms of Nuclear Actin

Monomeric G-actin
Conformation changes through ATPase activity might regulate the composition or function of multiprotein complexes

Short filaments
Proposed structural roles at the nuclear envelope

Novel oligomeric forms
Structural roles?



Jianmei Zhu, S-1

Bettinger BT et al, Nat Rev Mol Cell Biol, 2004

Other Nuclear Structures

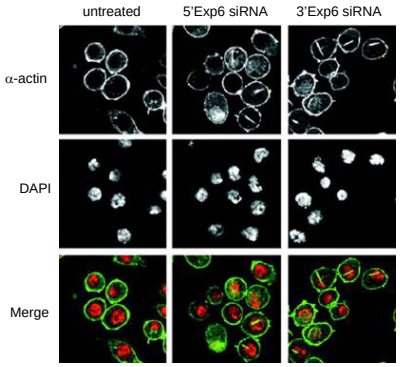
Actin
Titin
Spectrin
LINC
INM proteins and signaling

Evidence for nuclear actin polymers

- FRAP experiments suggest ~20% of nuclear actin is in a polymeric state (McDonald et al. 2006)
- Inhibition of N-WASP-dependent actin polymerization reduces RNA Polymerase II-dependent transcription (Wu et al. 2006)
- Polymerizable actin and Nuclear Myosin 1c (nMyo1c) are required for RNA Polymerase I-dependent transcription (Ye et al. 2007)
- Movement of active genes from heterochromatic to euchromatic regions requires polymerizable actin and nMyo1c (Chuang et al. 2006; Dundr et al. 2007)
- Emerin, an inner nuclear membrane protein, caps F-actin *in vitro* (Holaska et al. 2004)

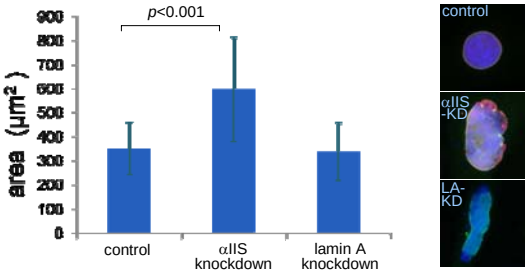
Too much actin in the nucleus is BAD

When its nuclear export is blocked, actin forms intra-nuclear spikes in Drosophila Schneider cells



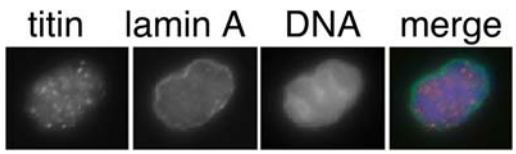
Stuven et al. (2003)

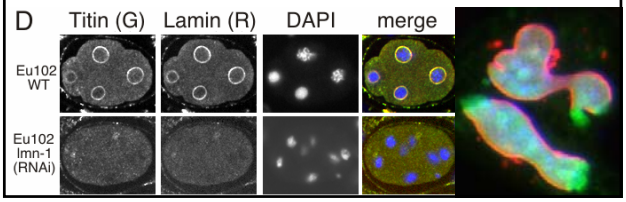
Increase in Nuclear Area from αIIspectrin reduction



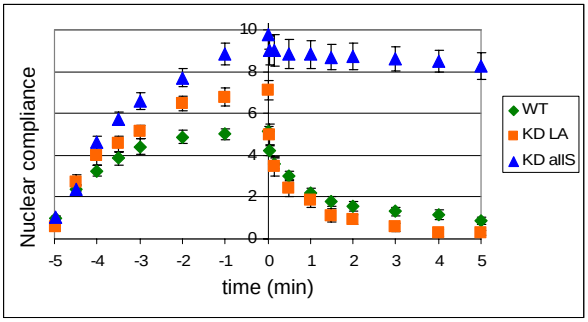
n>100

Nuclear Titin



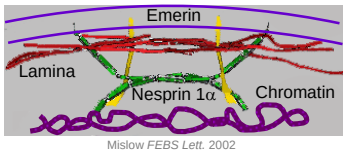


αIIS Helps Nuclei Recover From Deformation



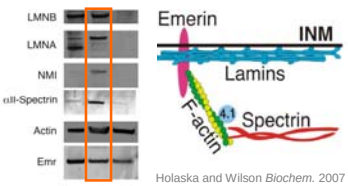
Lamin-Associated Spectrin Proteins

- nesprin: spectrin-homolog nesprin-1α binds lamin A and emerin



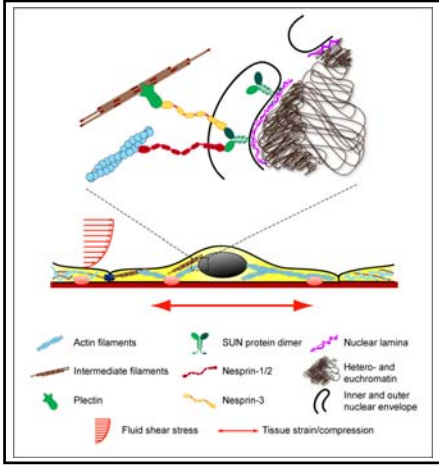
Mislow *FEBS Lett.* 2002

- spectrin: pull down assays show αII-spectrin in emerin complex with lamins and actin



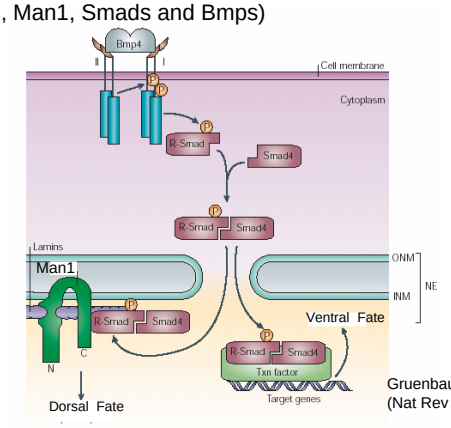
Holaska and Wilson *Biochem.* 2007

LINC Complex



Dahl *Circ Res* 2008

New concept: NE proteins regulate signal transduction
(e.g., Man1, Smads and Bmps)



Gruenbaum et al., 2005
(Nat Rev MCB)