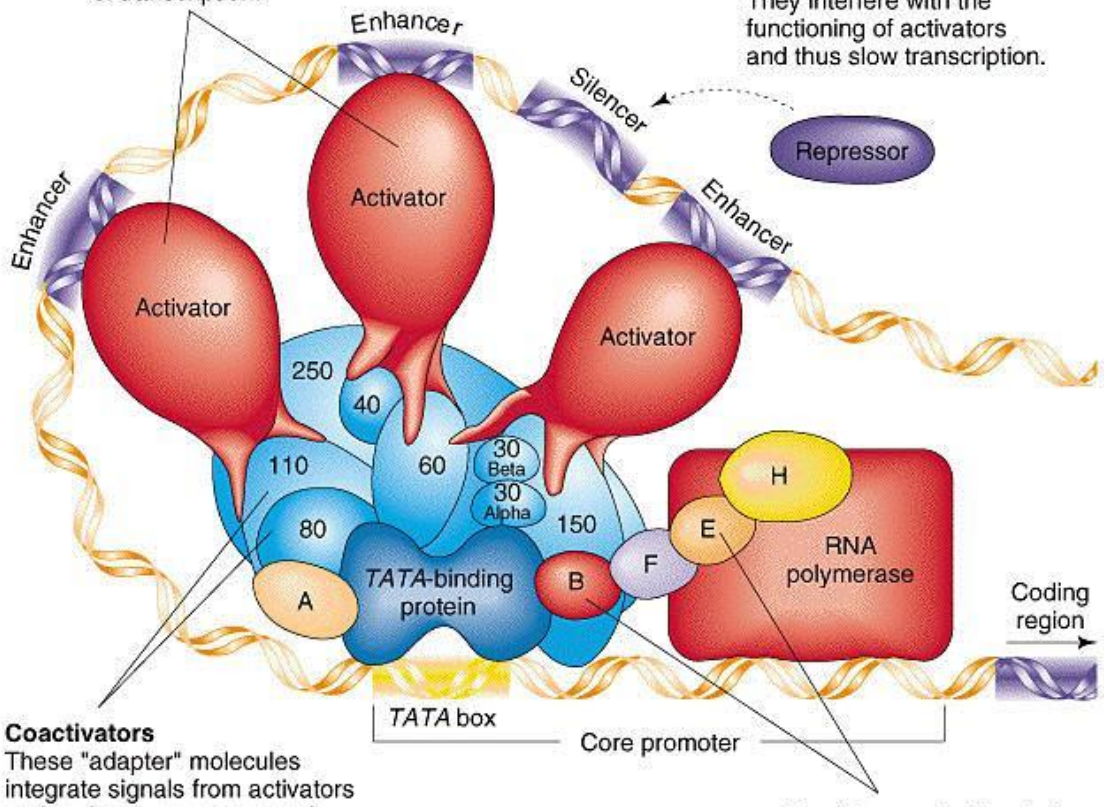
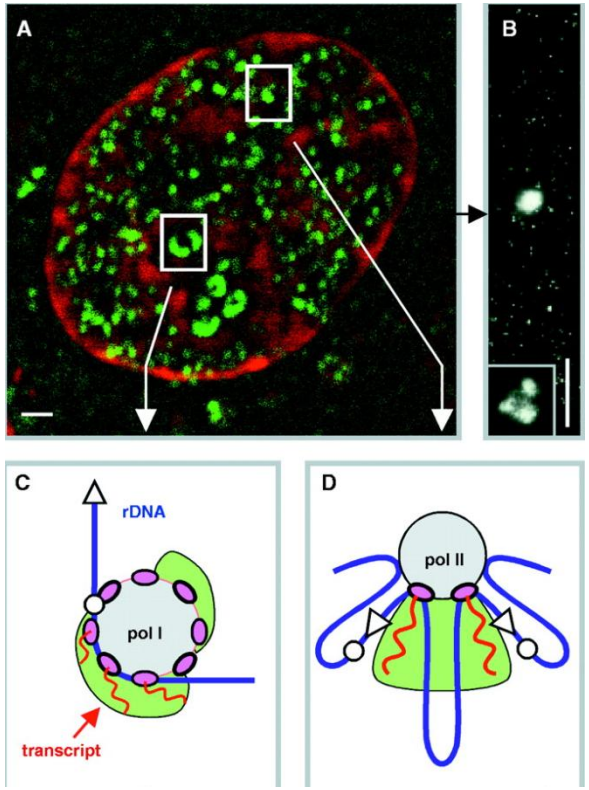
Activators
These proteins bind to genes at sites known as *enhancers.* Activators help determine which genes will be switched on, and they speed the rate of transcription.

Repressors
These proteins bind to selected sets of genes at sites known as *silencers.* They interfere with the functioning of activators and thus slow transcription.

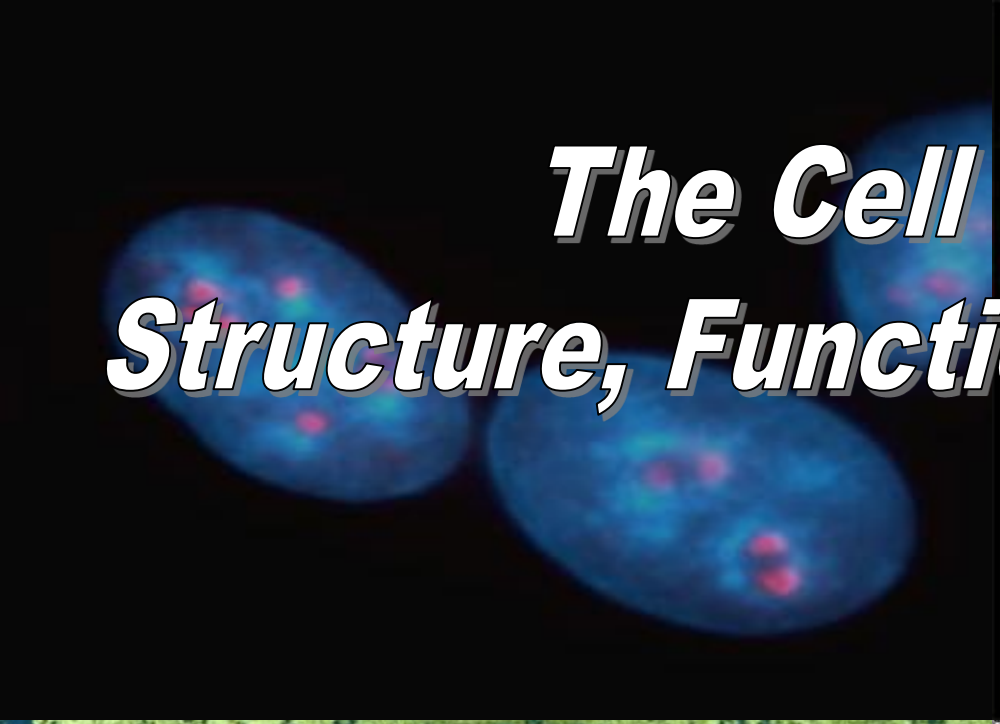


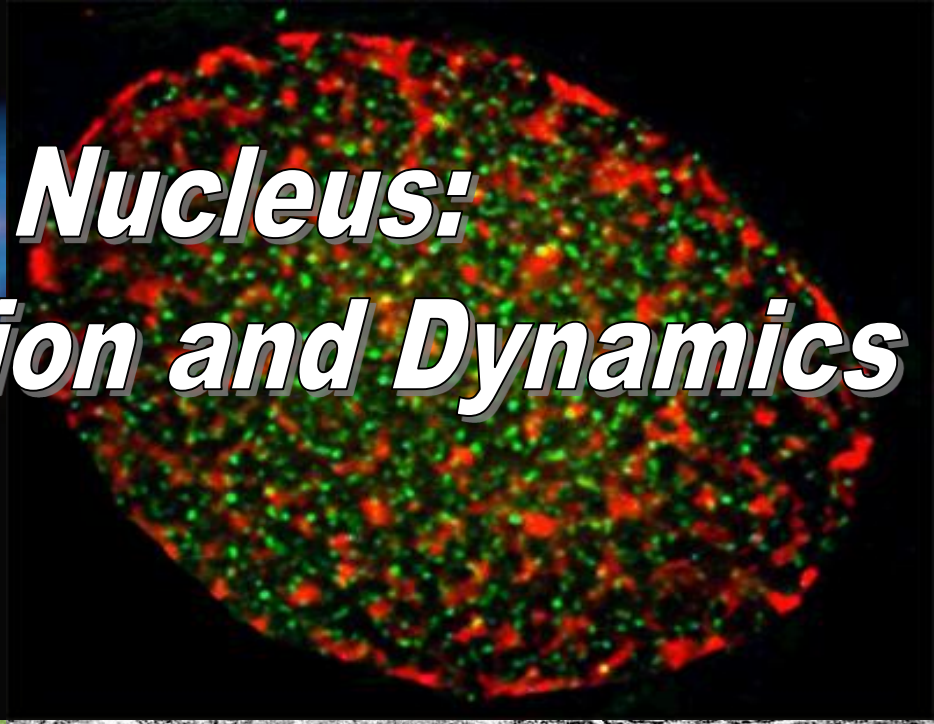
Coactivators
These "adapter" molecules integrate signals from activators and perhaps repressors and relay the results to basal factors.

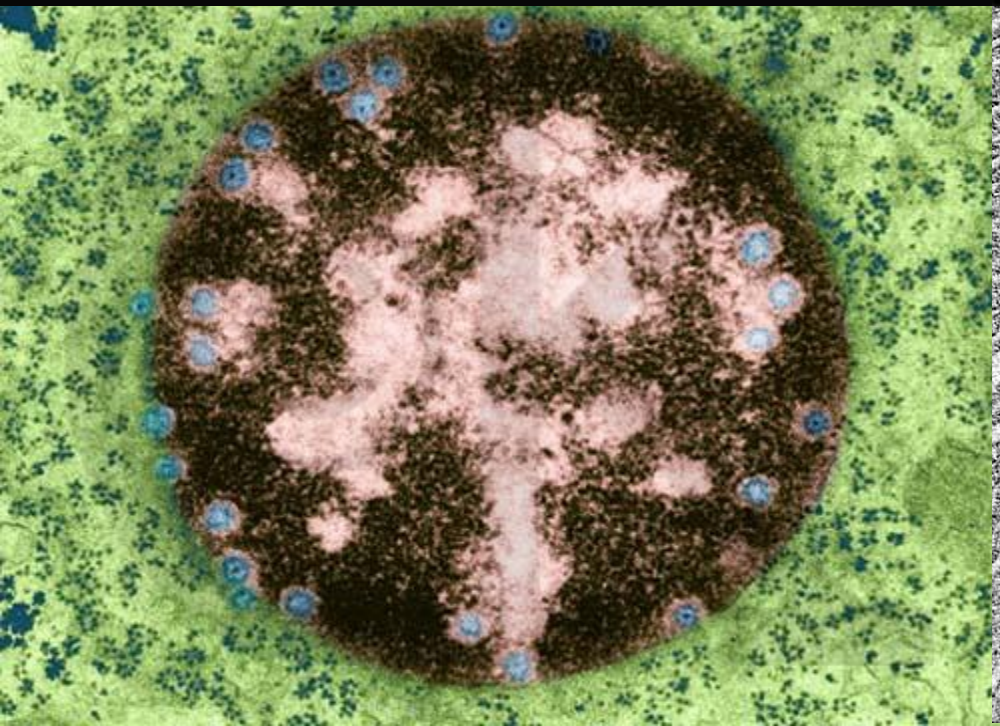
Basal transcription factors
In response to injunctions from activators, these factors position RNA polymerase at the start of the protein-coding region of a gene and send the enzyme on its way.

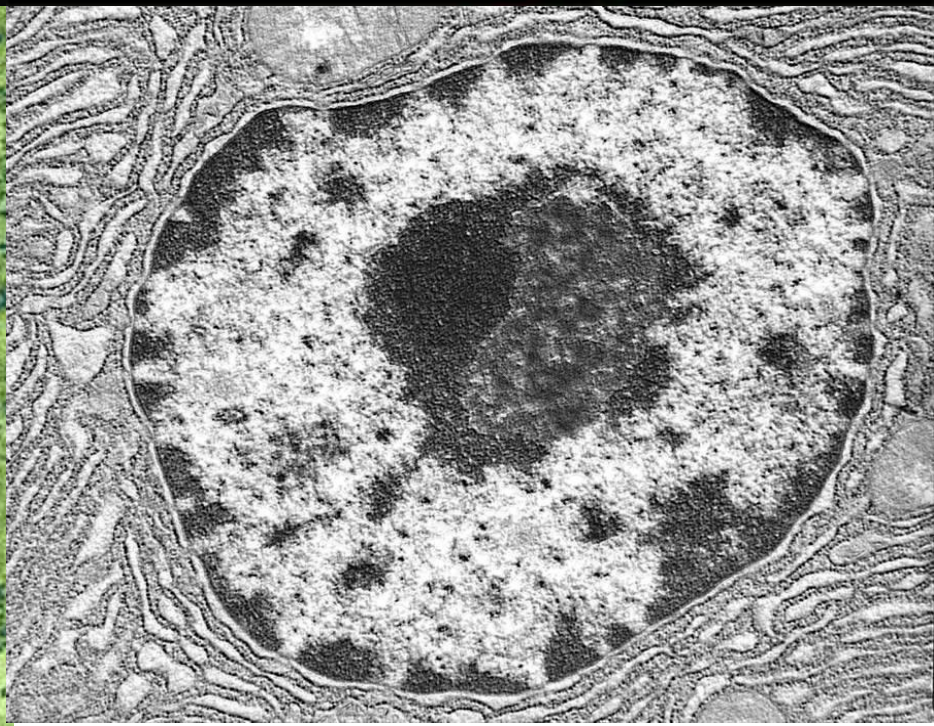


The Cell Nucleus: Structure, Function and Dynamics

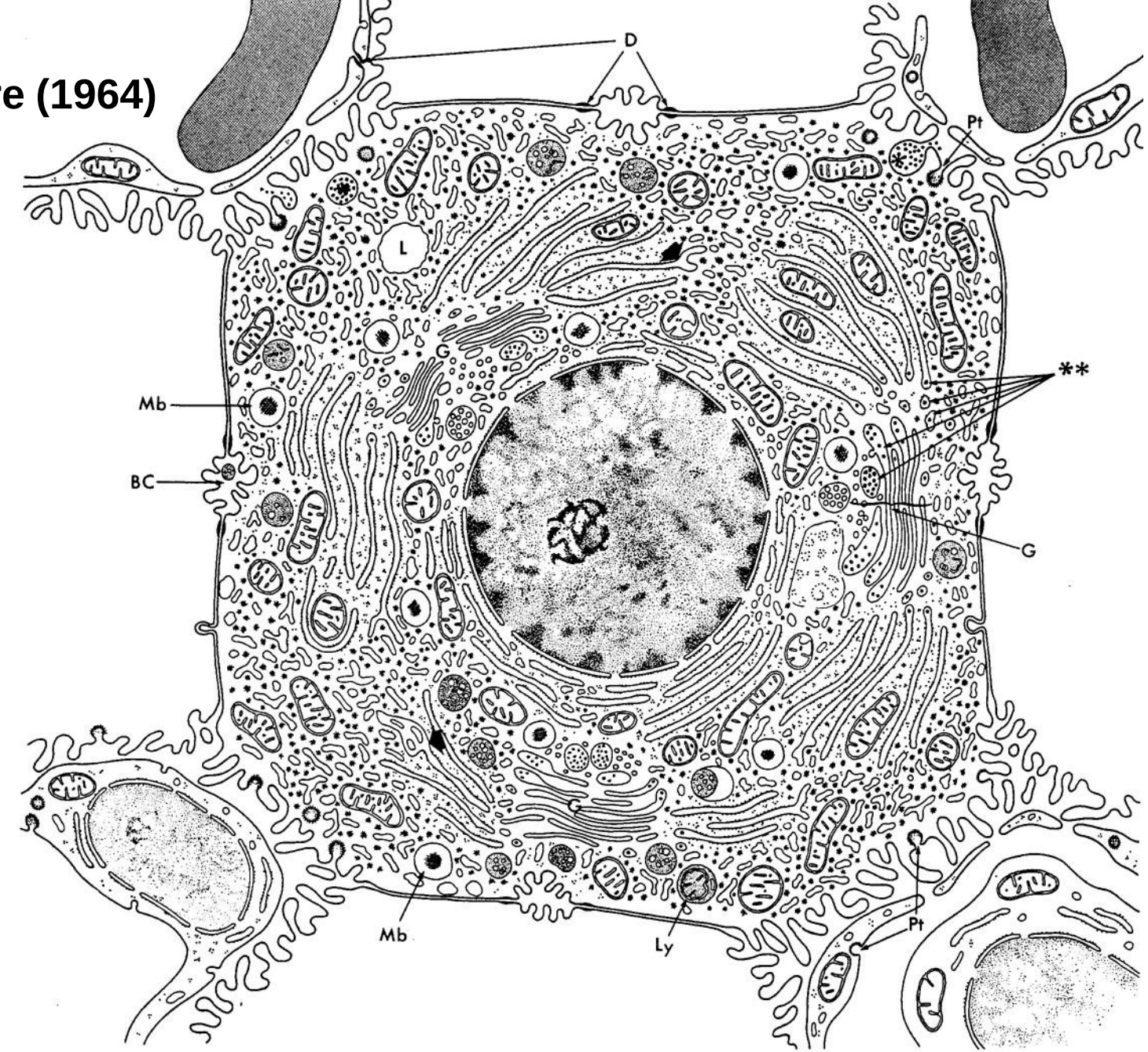






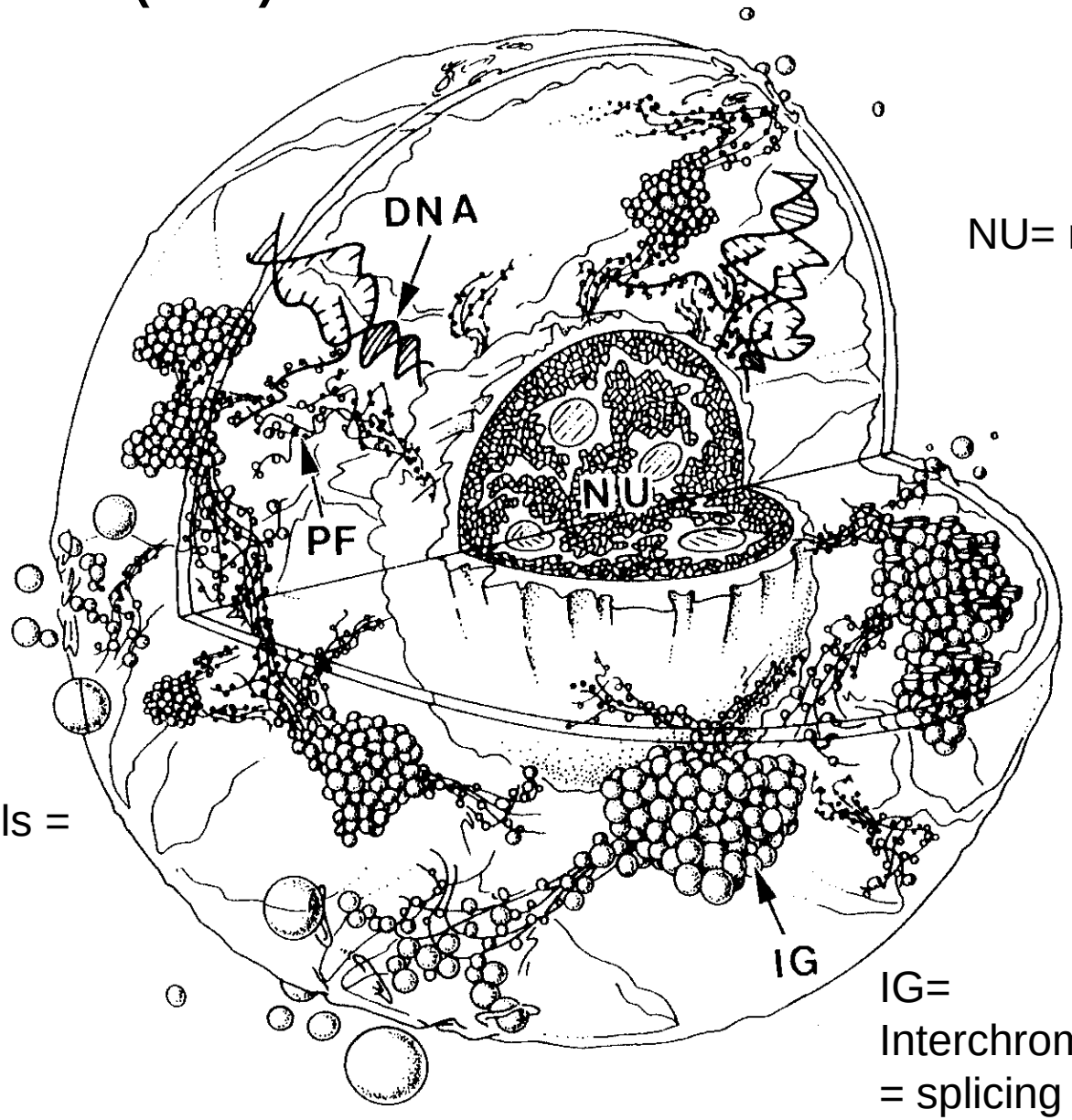


Nuclear Structure (1964)



Porter & Bonneville: An Introduction to the Fine Structure of Cells and Tissues (1964)

Nuclear Structure (1993)



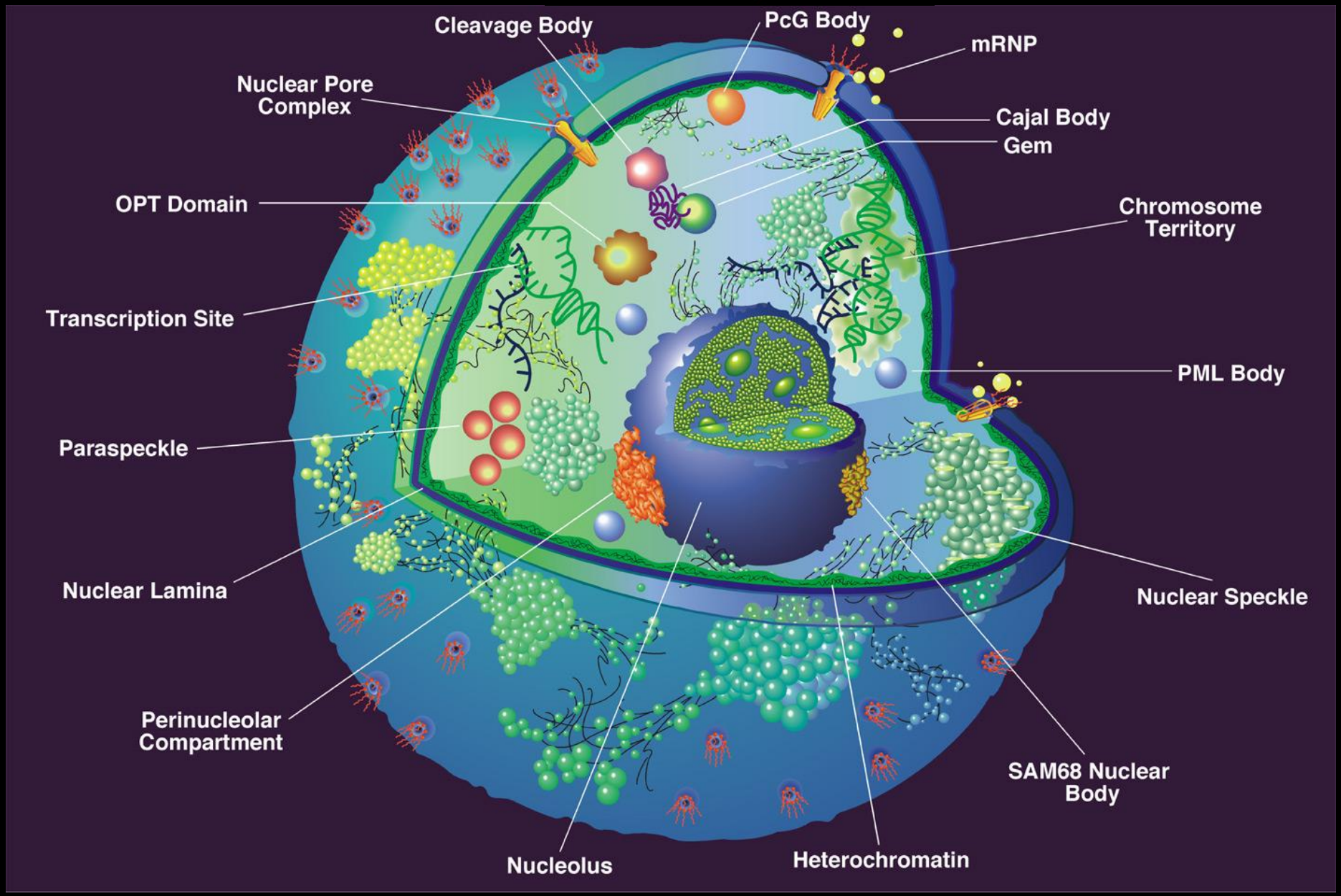
NU= nucleolus

PF=
perichromatin fibrils =
RNA

IG=
Interchromatin granules
= splicing factors

Spector DL. Annu Rev Cell Biol. 1993;9:265-315.

Nuclear Structure (2001)



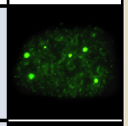
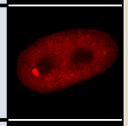
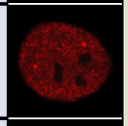
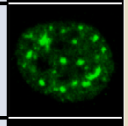
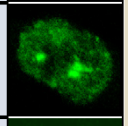
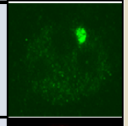
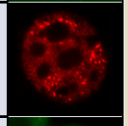
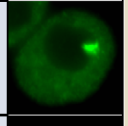
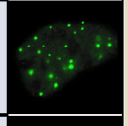
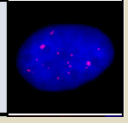
Spector DL. J Cell Sci. 2001;114:2891-3 / Ann Rev Biochem 2003

Nuclear Structure (2006)

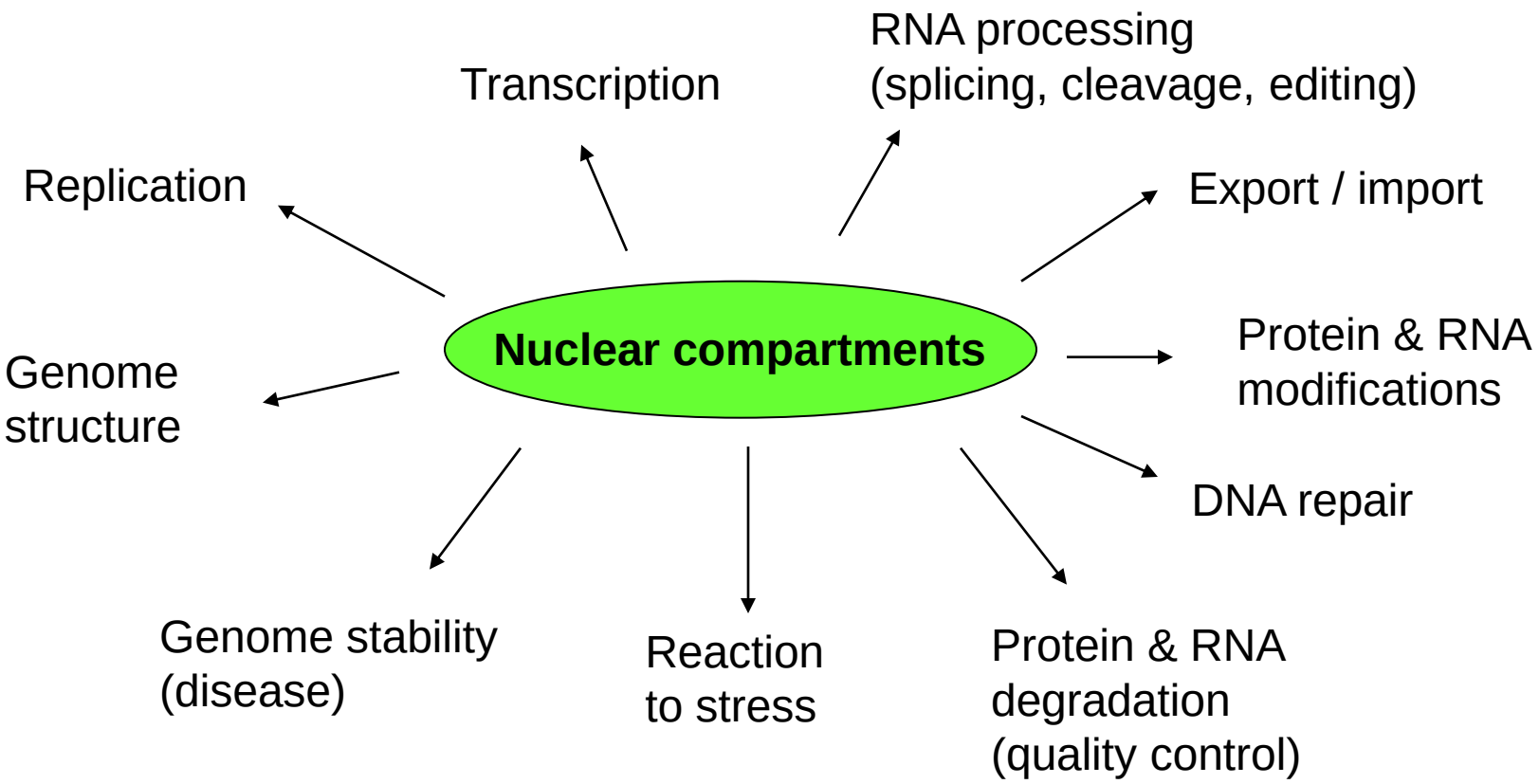
SnapShot: Cellular Bodies

David L. Spector
Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA

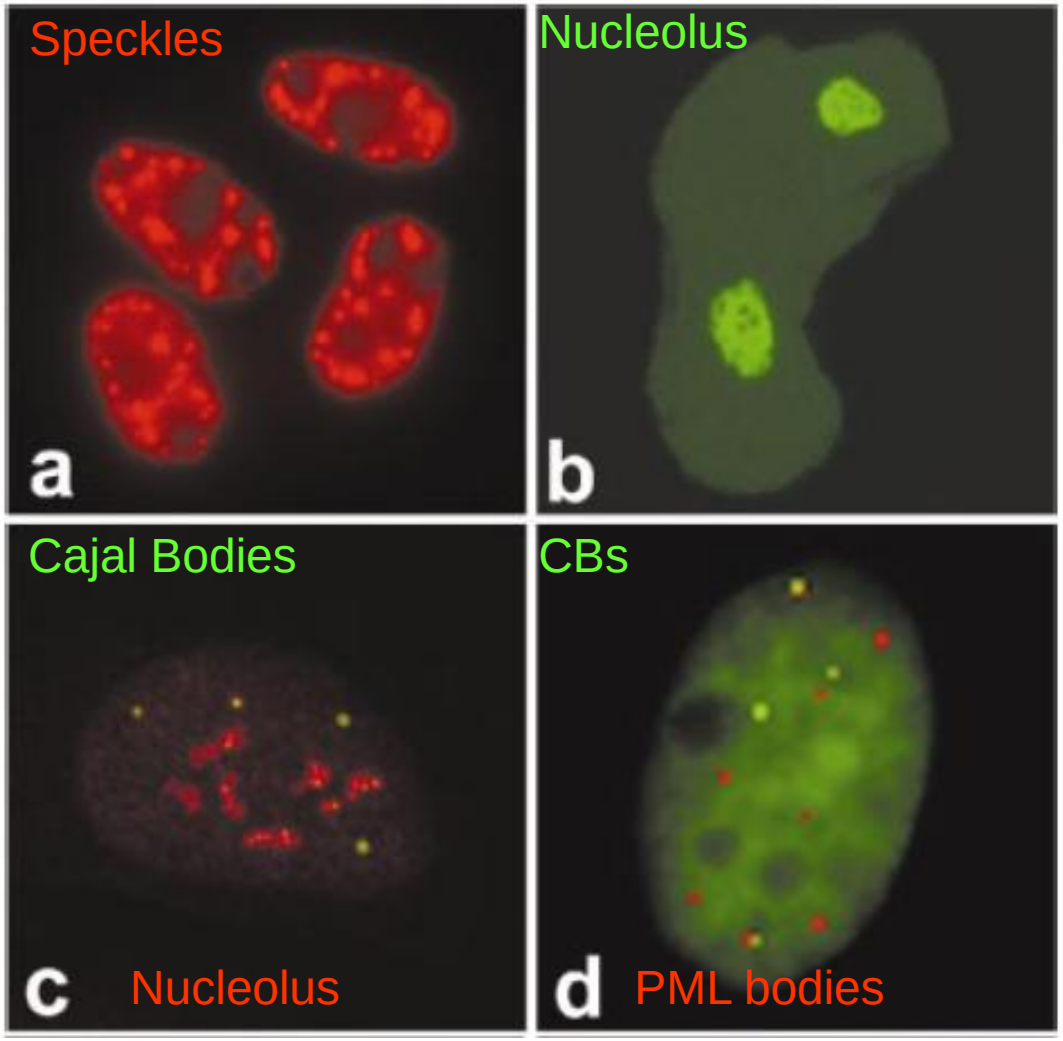
Cell

	Body Name	Number/ Cell	Typical Size and Shape	Marker Protein	Description	Image
Nuclear Bodies	**Cajal Body**	0–6	0.1–2.0 μm; round	Coilin	Involved in snRNP and snoRNP biogenesis and posttranscriptional modification of newly assembled spliceosomal snRNAs.	
	Clastosome	0–3	0.2–1.2 μm; irregular	20S core catalytic component of proteasome	Contains ubiquitin conjugates, the proteolytically active 20S core and 19S regulatory complexes of the 26S proteasome, and protein substrates of the proteasome.	
	Cleavage Body	1–4	0.2–1.0 μm; round	CstF 64 kDa	Contains several factors involved in 3′ cleavage of mRNAs. ~20% contain newly synthesized RNA. Some cleavage bodies localize adjacent to Cajal and PML bodies.	
	Nuclear Speckle or Interchromatin Granule Cluster	25–50	0.8–1.8 μm; irregular	SC35, SF2/ASF	Contains proteins for pre-mRNA processing. Involved in the storage, assembly, and/or modification of pre-mRNA splicing factors.	
	Nuclear Stress Body	2–10	0.3–3.0 μm; irregular	HSF1	Induced by heat shock response. Associates with satellite III repeats on human chromosome 9q12 and other pericentromeric regions; recruits various RNA-binding proteins.	
	OPT Domain	1–3	1.0–1.5 μm; round	PTF	Contains several transcription factors (Oct1/PTF) and RNA transcripts; predominant in late G1 cells. Often localizes close to nucleolus.	
	Paraspeckle	10–20	0.5 μm; round	p54^{nrb}, PSP1	Contains several RNA-binding proteins and nuclear-retained CTN-RNA.	
	Perinucleolar Compartment	1–4	0.3–1.0 μm; cap	hnRNPI (PTB)	Cap on surface of nucleolus; found mainly in transformed cells. Contains RNA pol III transcripts and several RNA-binding proteins.	
	PML Body	10–30	0.3–1.0 μm; round	PML	Suggested to play a role in aspects of transcriptional regulation and/or nuclear protein sequestration.	
	Polycomb Body	12–16	0.3–1.0 μm; round/ irregular	Bmi1, Pc2	Contains silencing proteins associated with Polycomb repressive complex 1; associates with heterochromatin.	

Nuclear architecture



Nuclear bodies

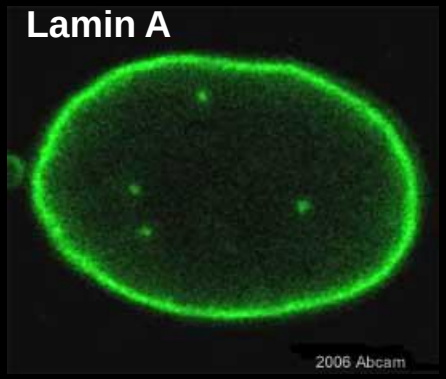


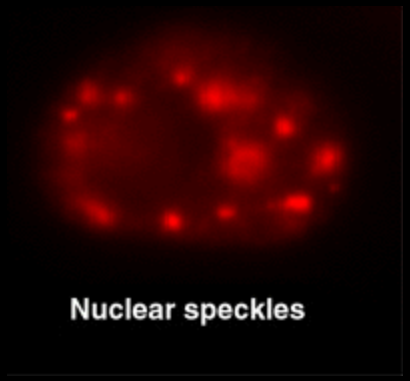
* No membrane

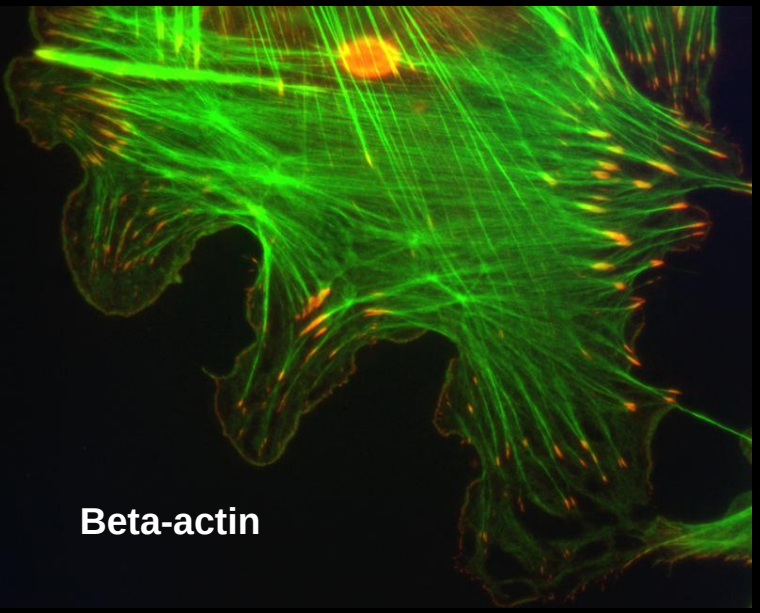
* Have a distinct set of proteins

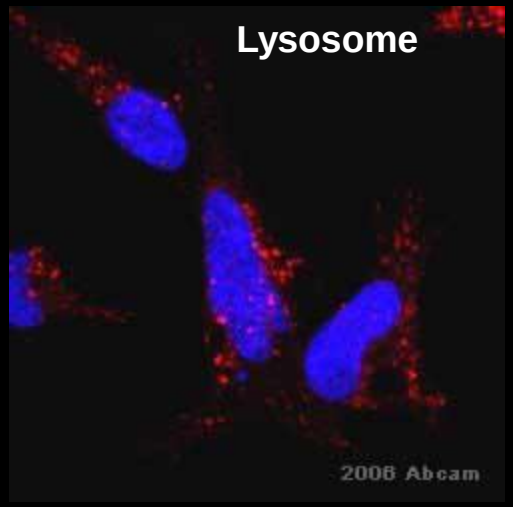
Misteli, Biochem J 2001

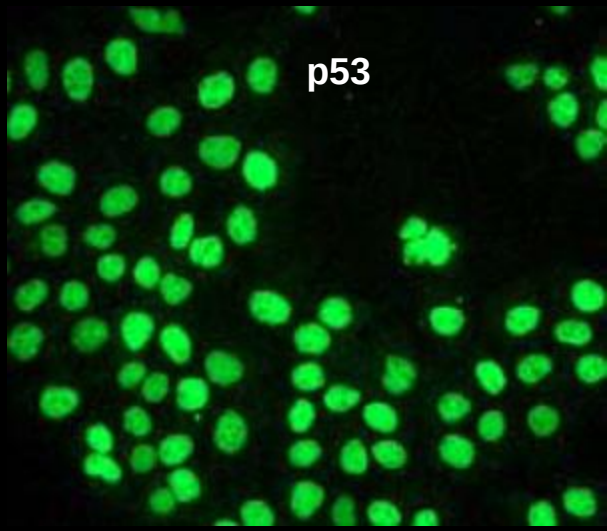
Immunofluorescence of cellular structures





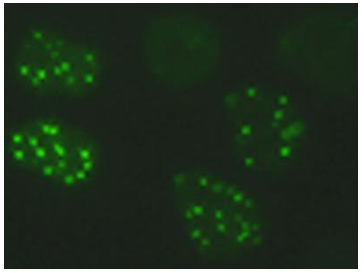




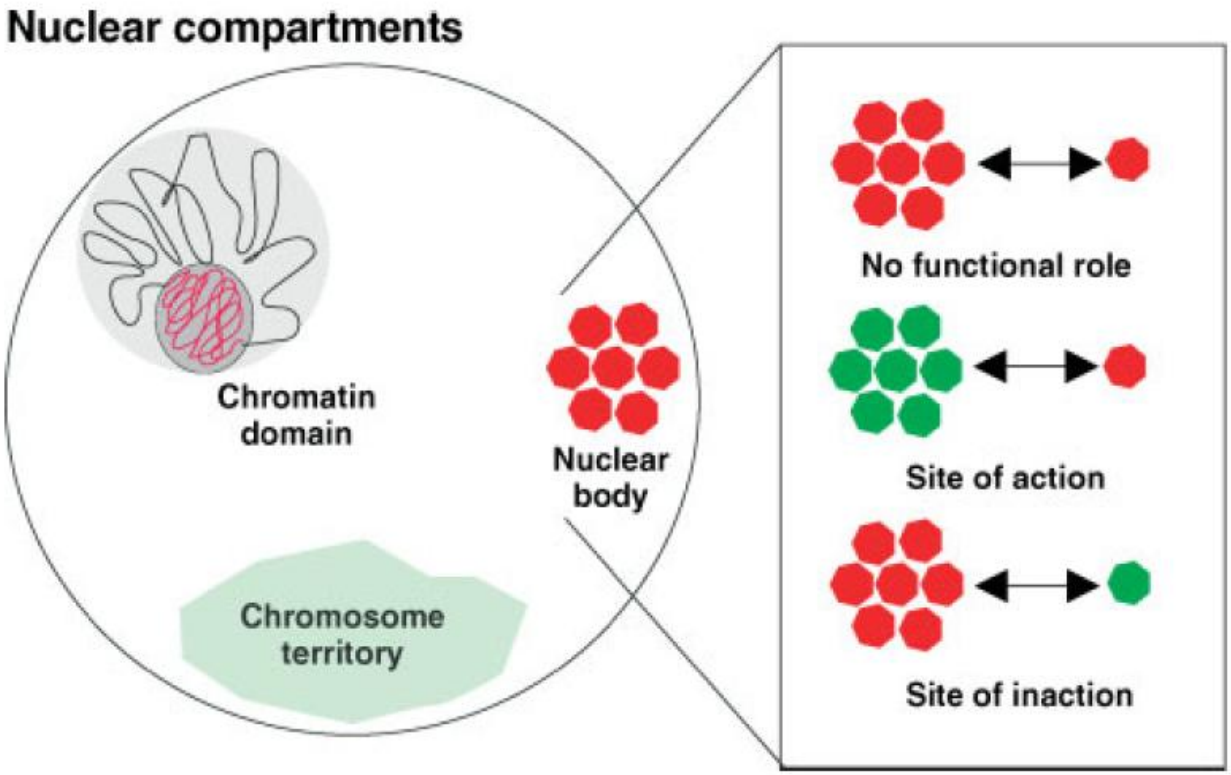


Nuclear bodies

GFP-X overexpression



Functions:

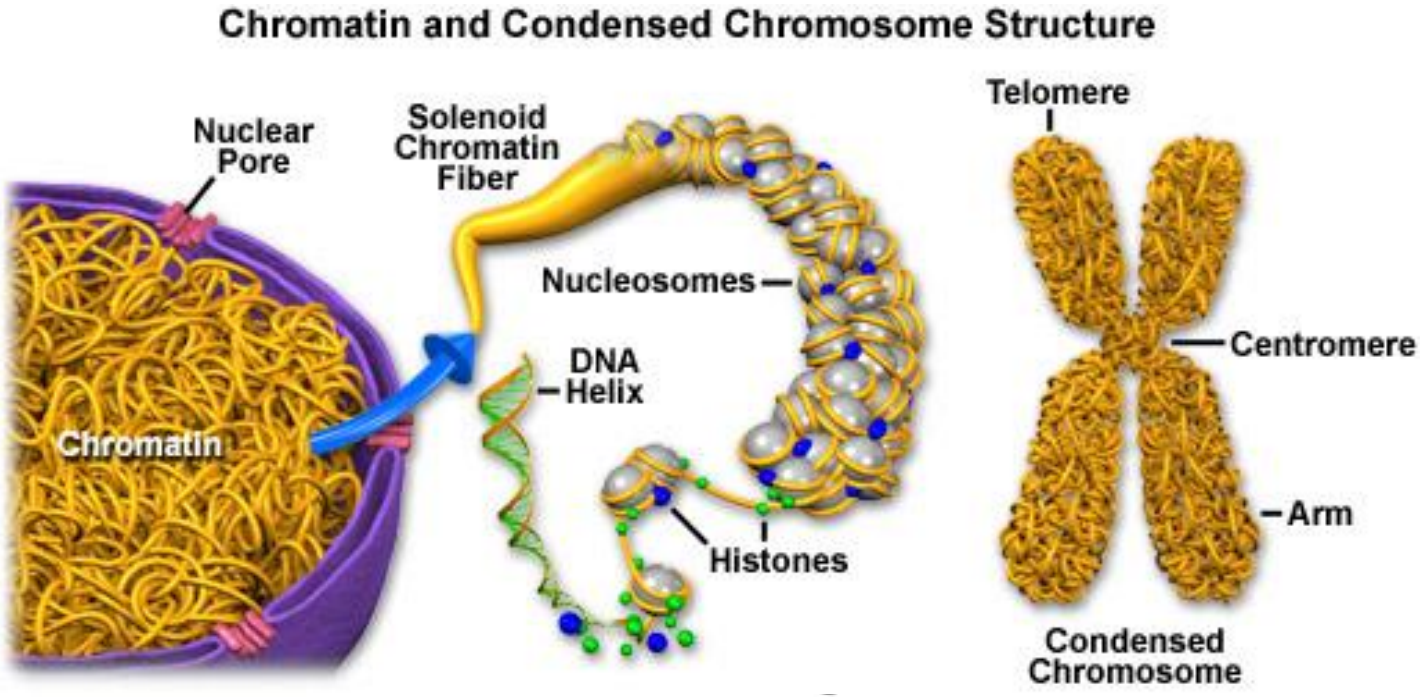


Aggregation of excess proteins.

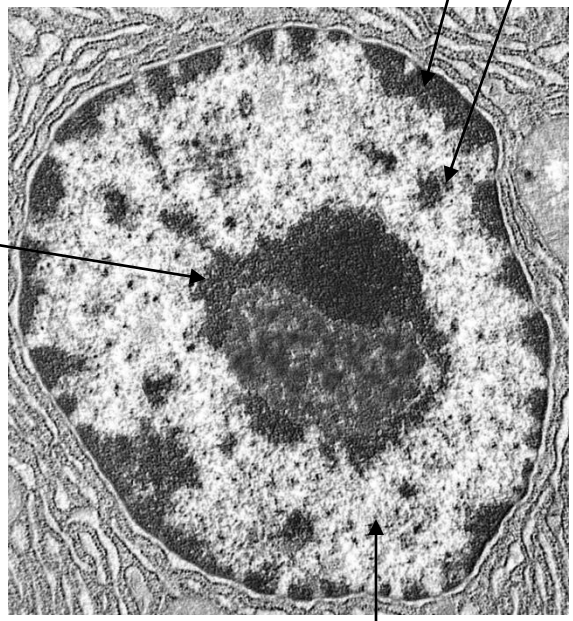
Nucleolus

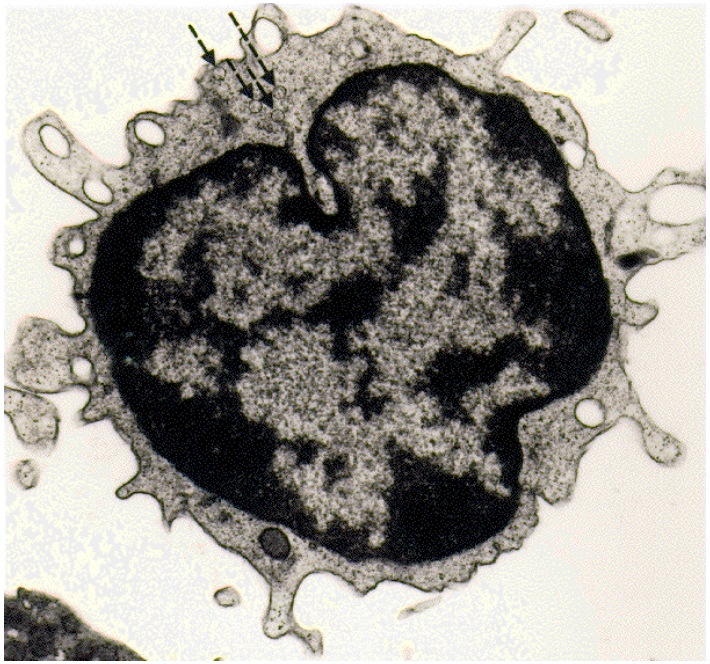
Provides components to its surroundings (speckles)
→ might regulate the concentration of factors at their sites of action

Chromatin


Chromatin and Condensed Chromosome Structure
Nuclear Pore
Solenoid Chromatin Fiber
Nucleosomes
DNA Helix
Chromatin
Histones
Telomere
Centromere
Arm
Condensed Chromosome

Chromatin domains | **Euchromatin & heterochromatin** | 1928 - Heitz



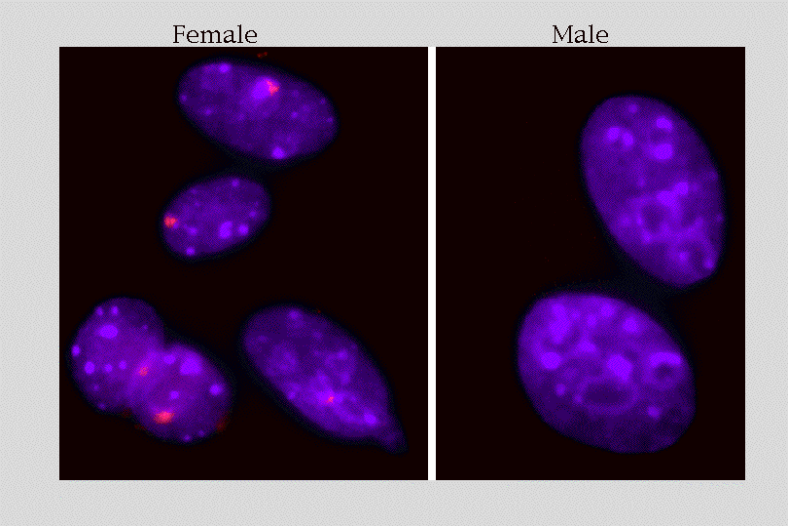


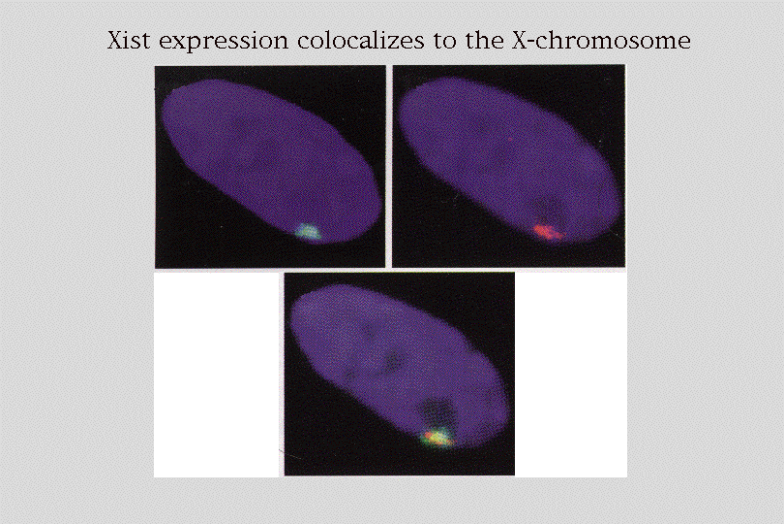
Lymphocyte
Heterochromatin clumps

Euchromatin & heterochromatin

Facultative heterochromatin is euchromatin that will adopt heterochromatic properties in a developmentally controlled manner.

Classic example: inactive X chromosomes of female mammals.

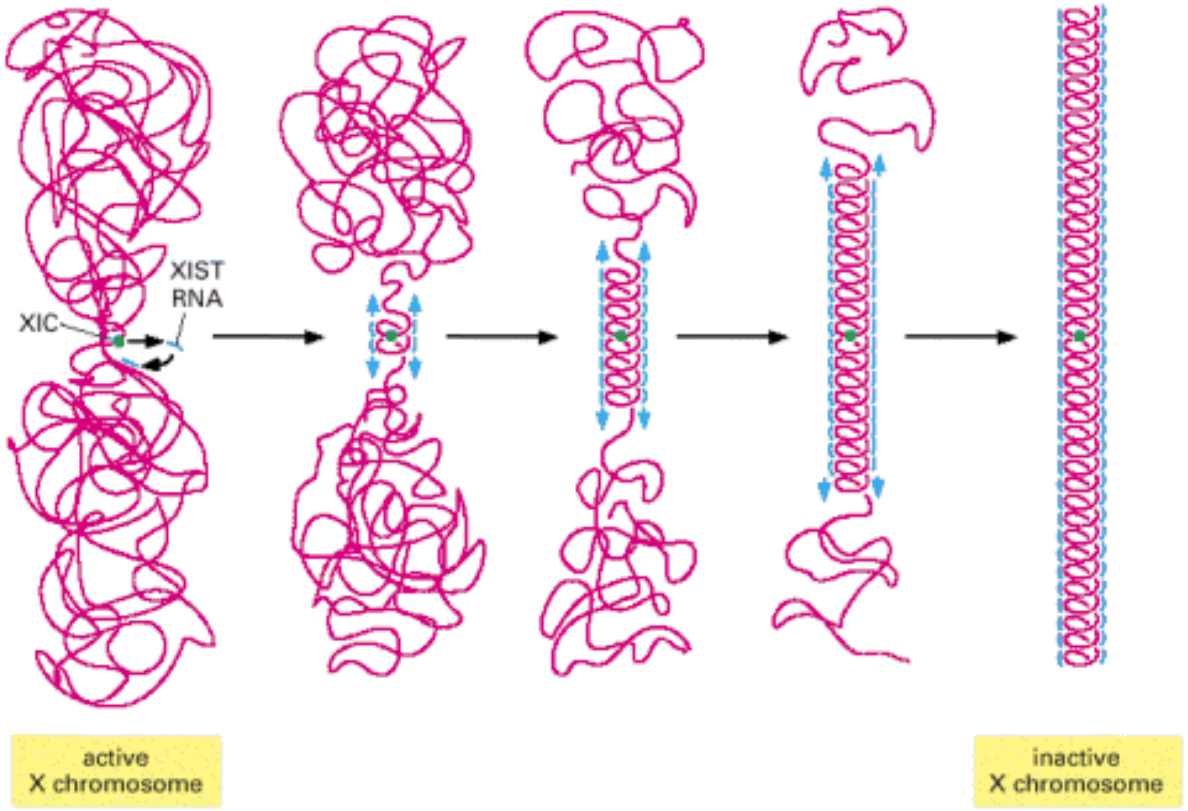


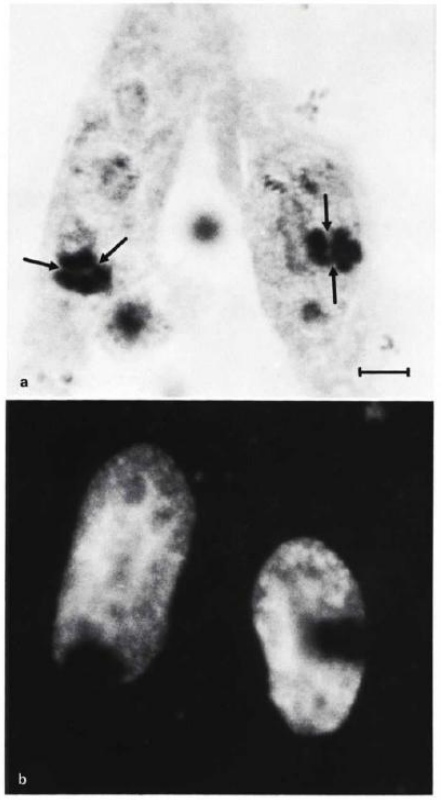


X-inactivation ensures that all cells in both males and females synthesize equivalent amounts of X-linked gene products. This “dosage compensation” results in silencing of all or almost all genes on the female’s two X chromosomes.

Euchromatin & heterochromatin

The XIST RNA coats the inactive X chromosome.



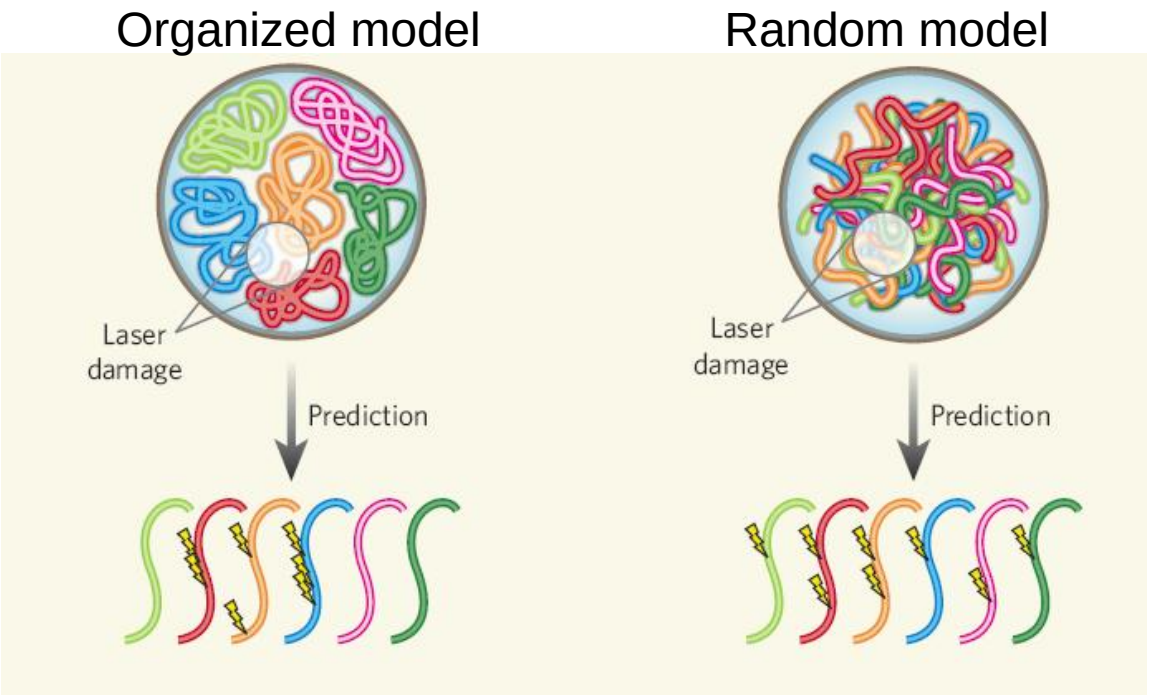


XIC (X-inactivation center) locus

Chromatin domains 1982 – Thomas Cremer

How are chromosomes organized in the nucleus?

Used a microlaser to induce local genome damage, and predicted that inflicting DNA damage within a small volume of the nucleus would yield different results depending on how chromosomes were arranged.



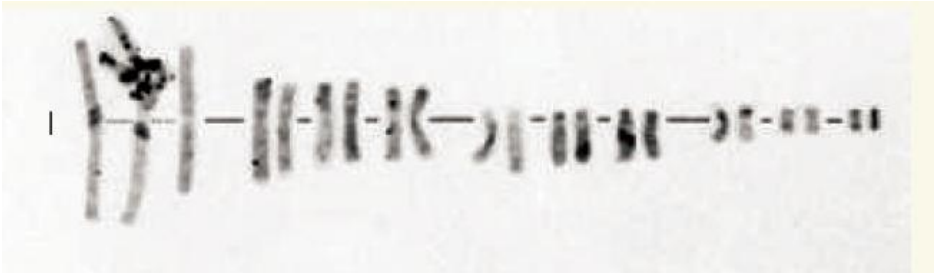
Chromatin domains 1982 – Thomas Cremer

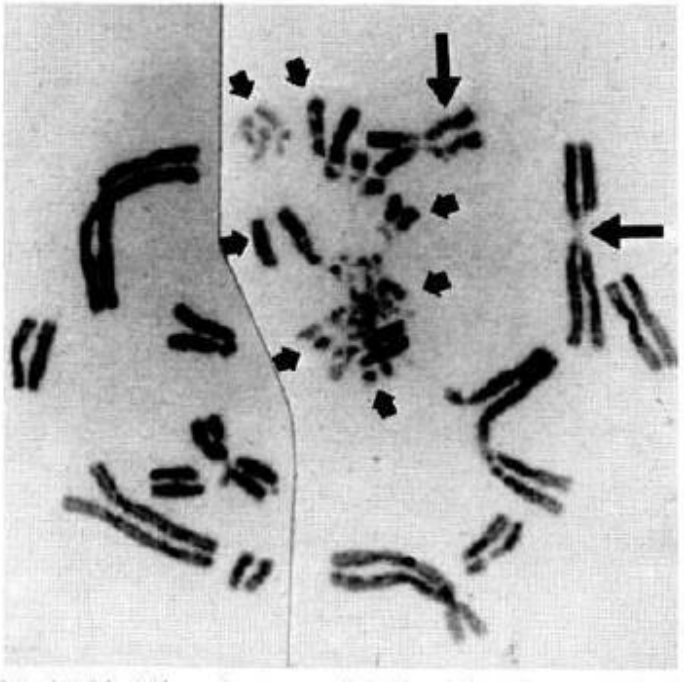
How are chromosomes organized in the nucleus?

→ Chromosome territories

Results:
* DNA repair occurred in one or a few chromosomes
* This was random

Conclusions:
* Each chromosome has its own territory
* There are no fixed positions in the nucleus

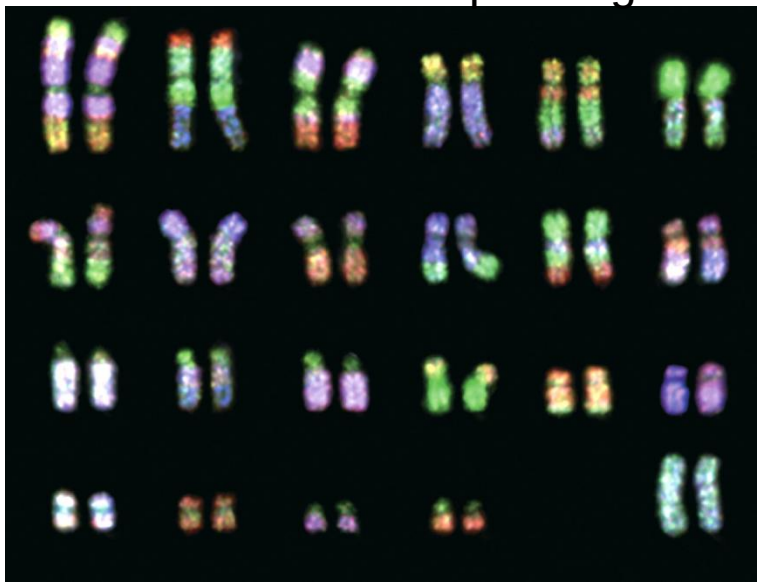




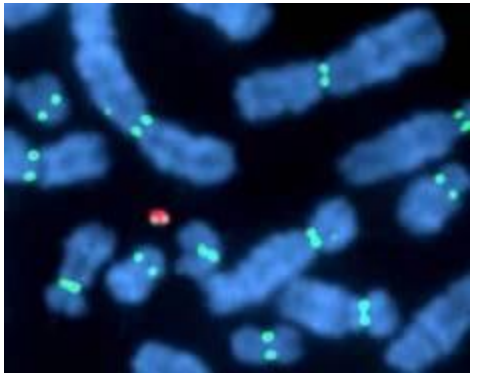
Cremer T, Hum. Genet 1982

DNA FISH – fluorescence in situ hybridization

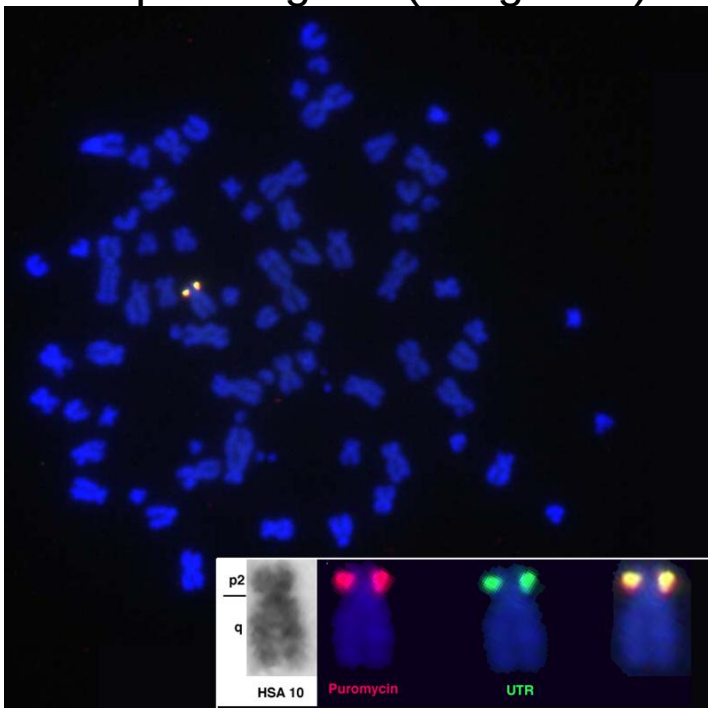
Chromosome painting



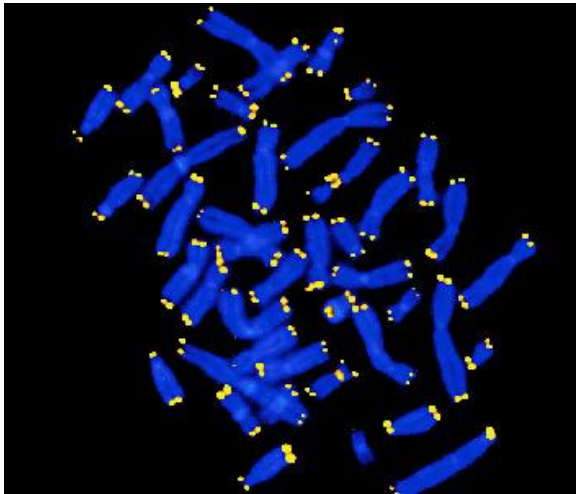
Centromeres



Specific gene (integrated)



Telomeres



Chromosomes territories

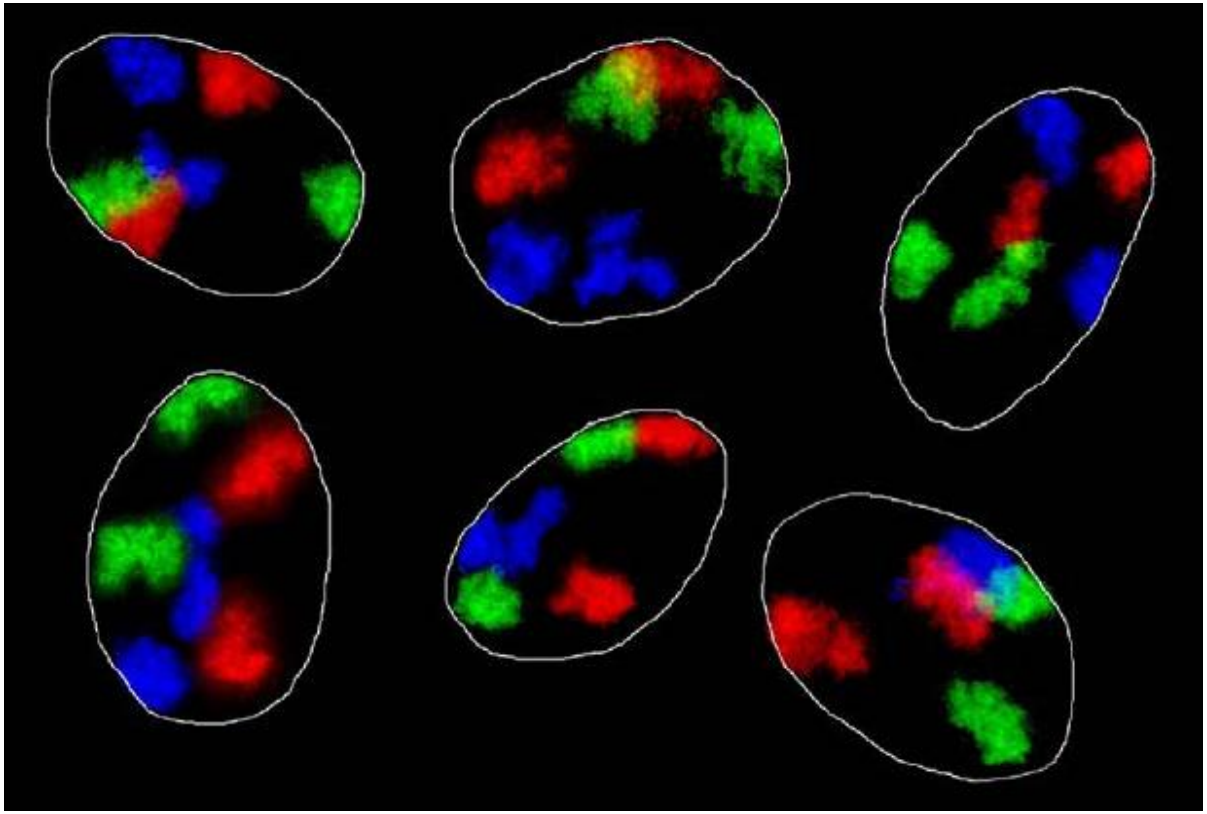
This organization is preserved throughout evolution.

(Tanabe et al., 2002)

Chromosomes territories

Genetic material is not widely distributed but occupies a spatially defined sub-volume.

Human chromosomes: 3 (green), 5 (blue), 11 (red)



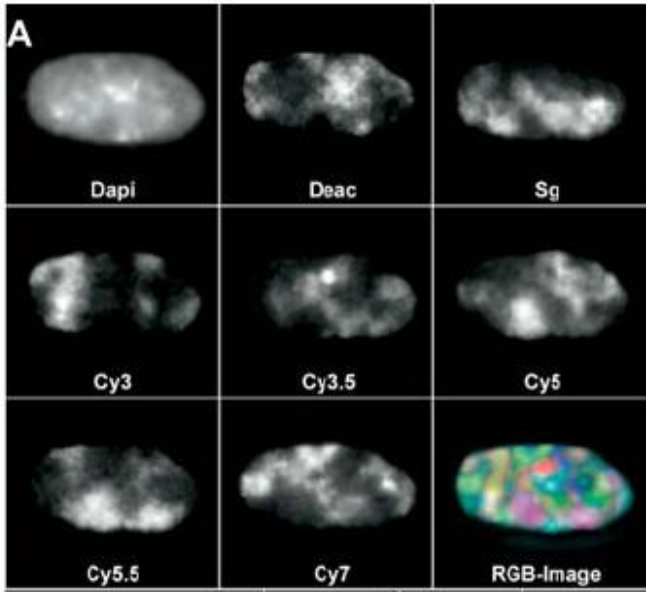
(Bridger JM, Chromosoma 2005)

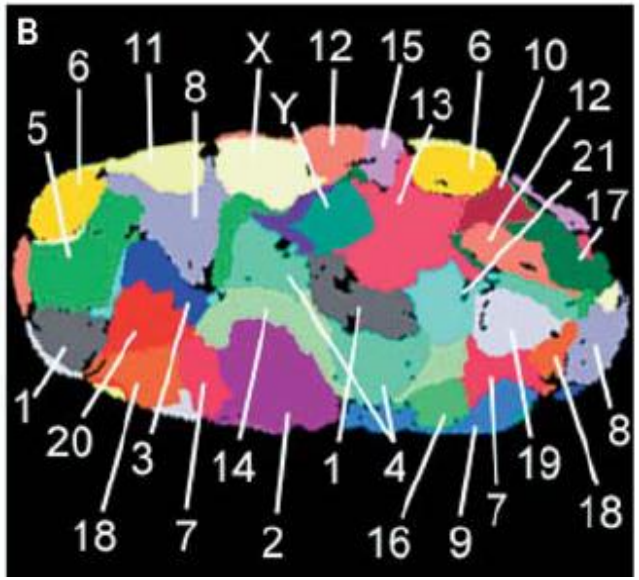
Non-random nuclear organization

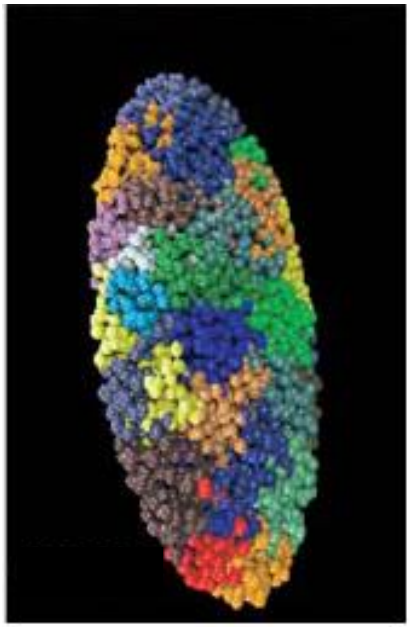
Mapping the 3D distribution of all chromosomes in normal human fibroblasts (46 XY)

3D M-FISH (multiplex) for all chromosomes, under conditions that preserve 3D structure

Differential staining - probes were differentially labeled using a combinatorial labeling scheme with seven different haptens/fluorochromes

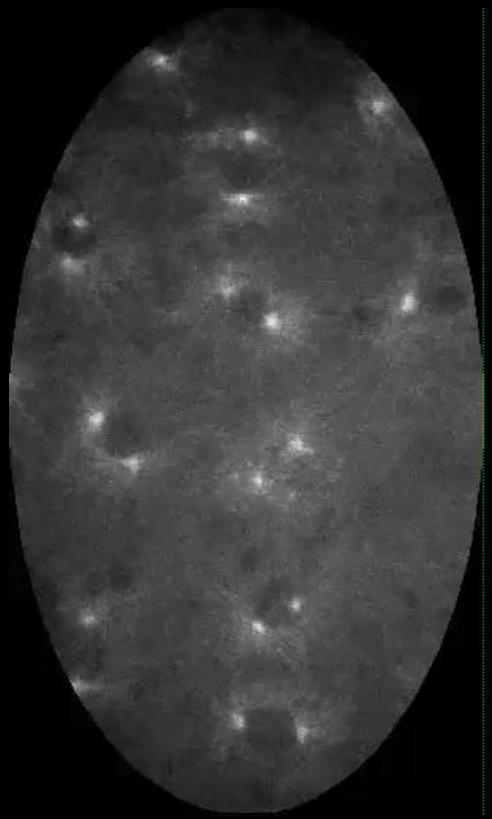






(Bolzer A. et al., Cremer PLoS Biol 2005)

חלוקות תא בדרוזופילה



חלוקת תא –
טובולין – ירוק
היסטון - אדום

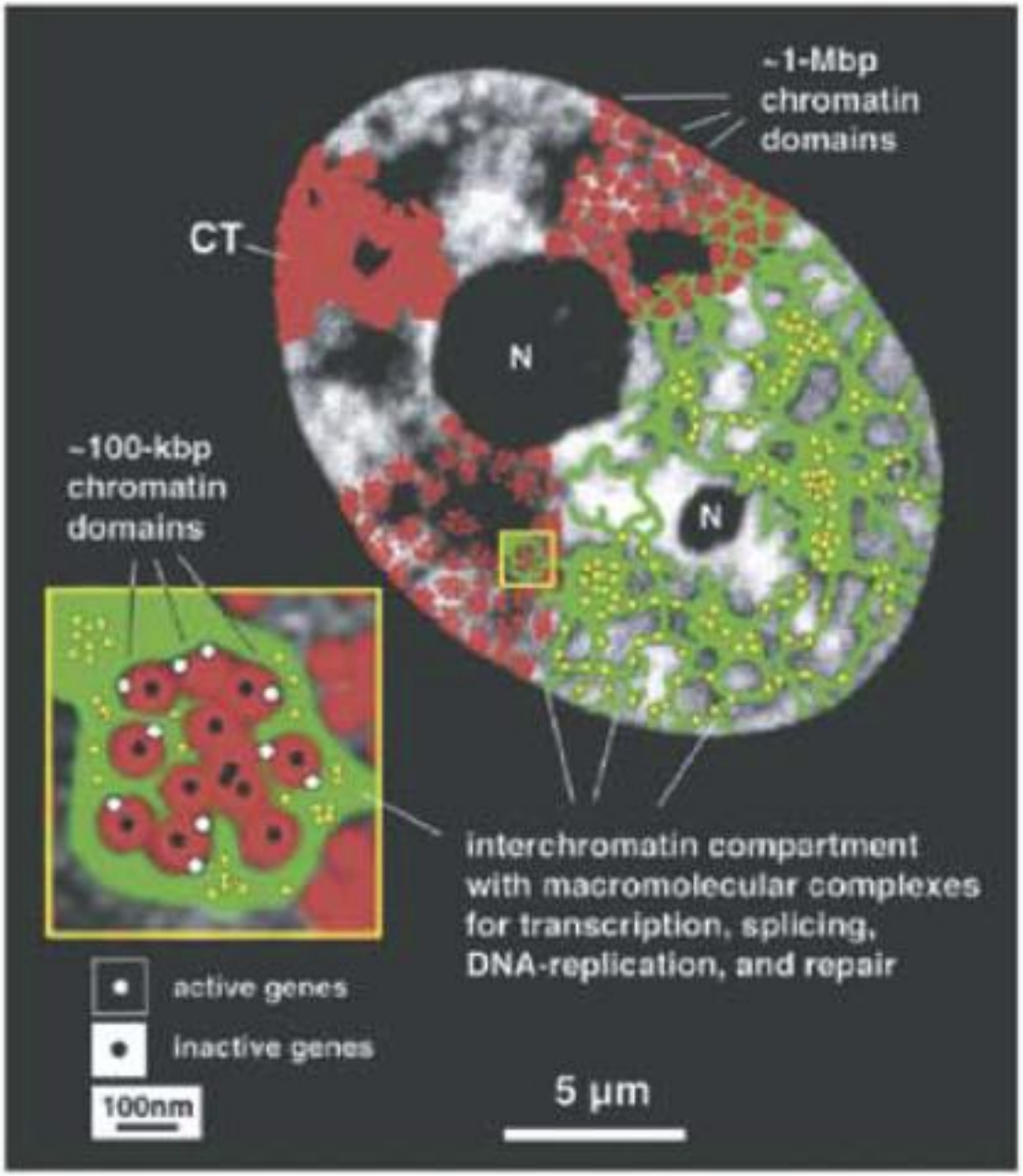
חלוקה - תנועת קינטוכורים
סימון צנטרומרים

Inter-chromatin

CT-IC model

Chromosome territory – interchromatin compartment

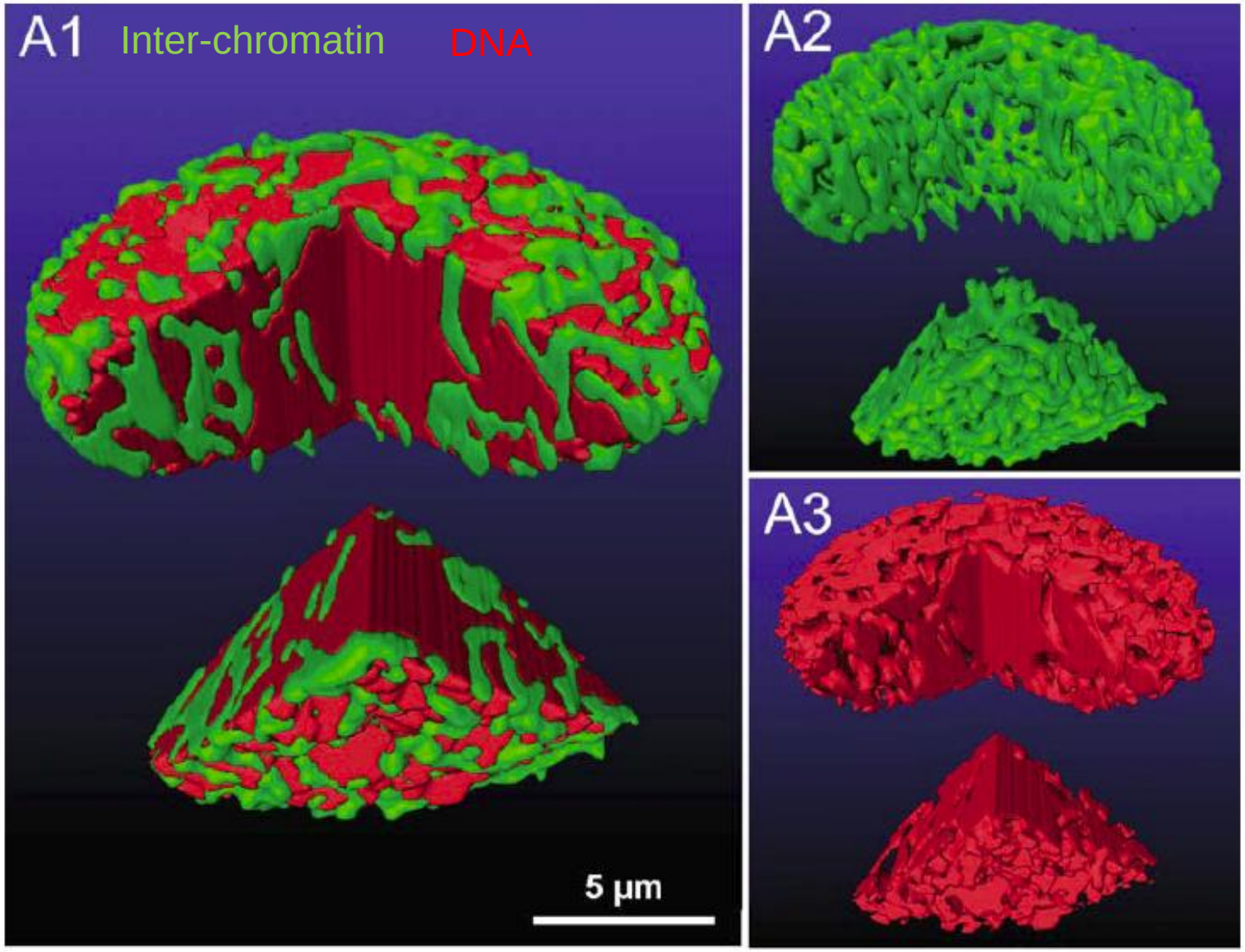
CT architecture resembles a sponge built up from condensed
chromatin domains, with a contiguous network of DNA-free channels expanding in between.



(Cremer et al. Curr Opin cell Biol 2006)

CT-IC

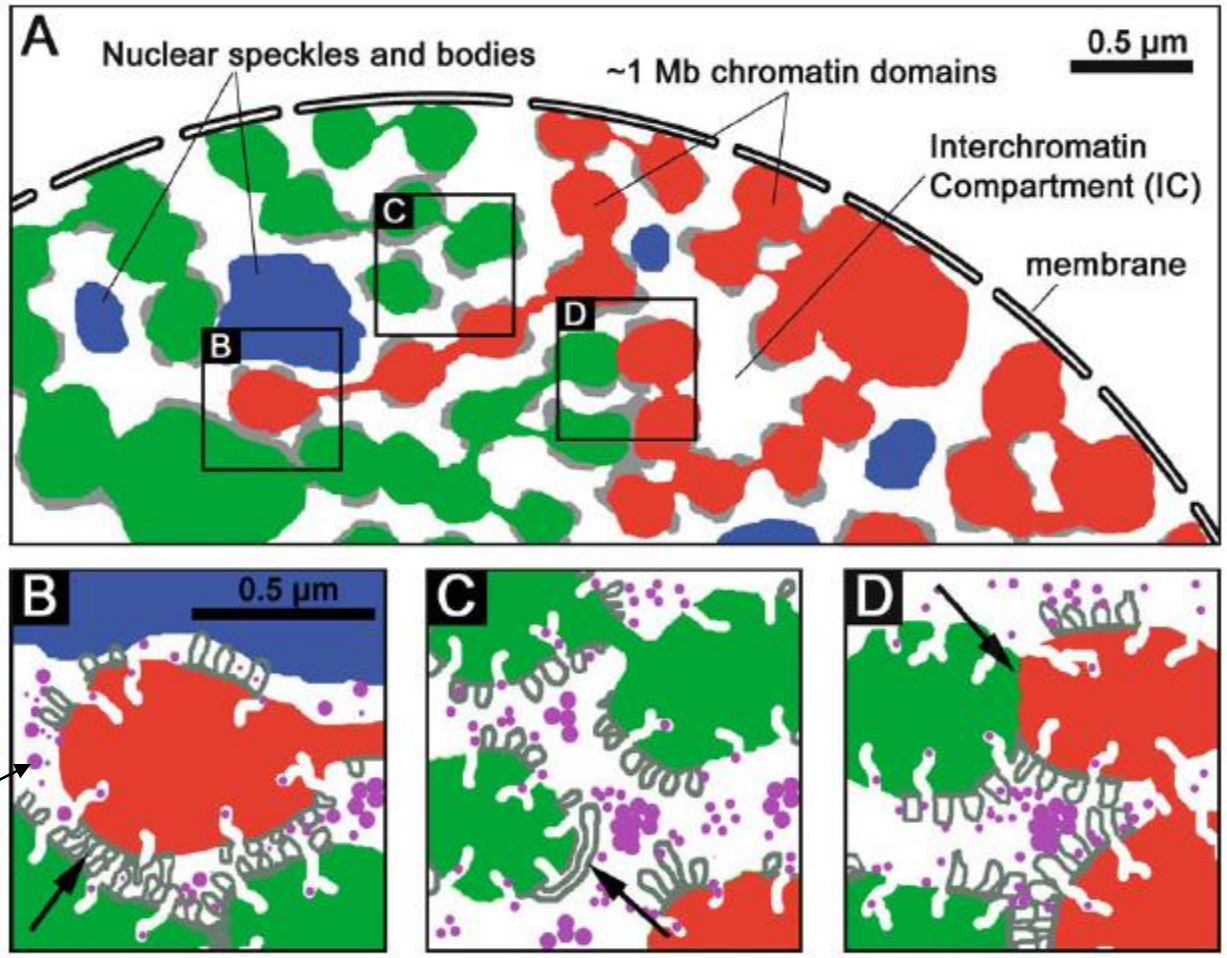
3D networks of chromatin bundles and interchromatin channels.



(Albeiz H, Cremer, Chrom Res 2006)

CT-IC

Chromosomes are not solid structures but contain nucleoplasmic channels of many sizes. Chromosome have loops of different sizes and these can cause intermingling between chromosomes.



(Albeiz H, Cremer, Chrom Res 2006)

Nuclear bodies

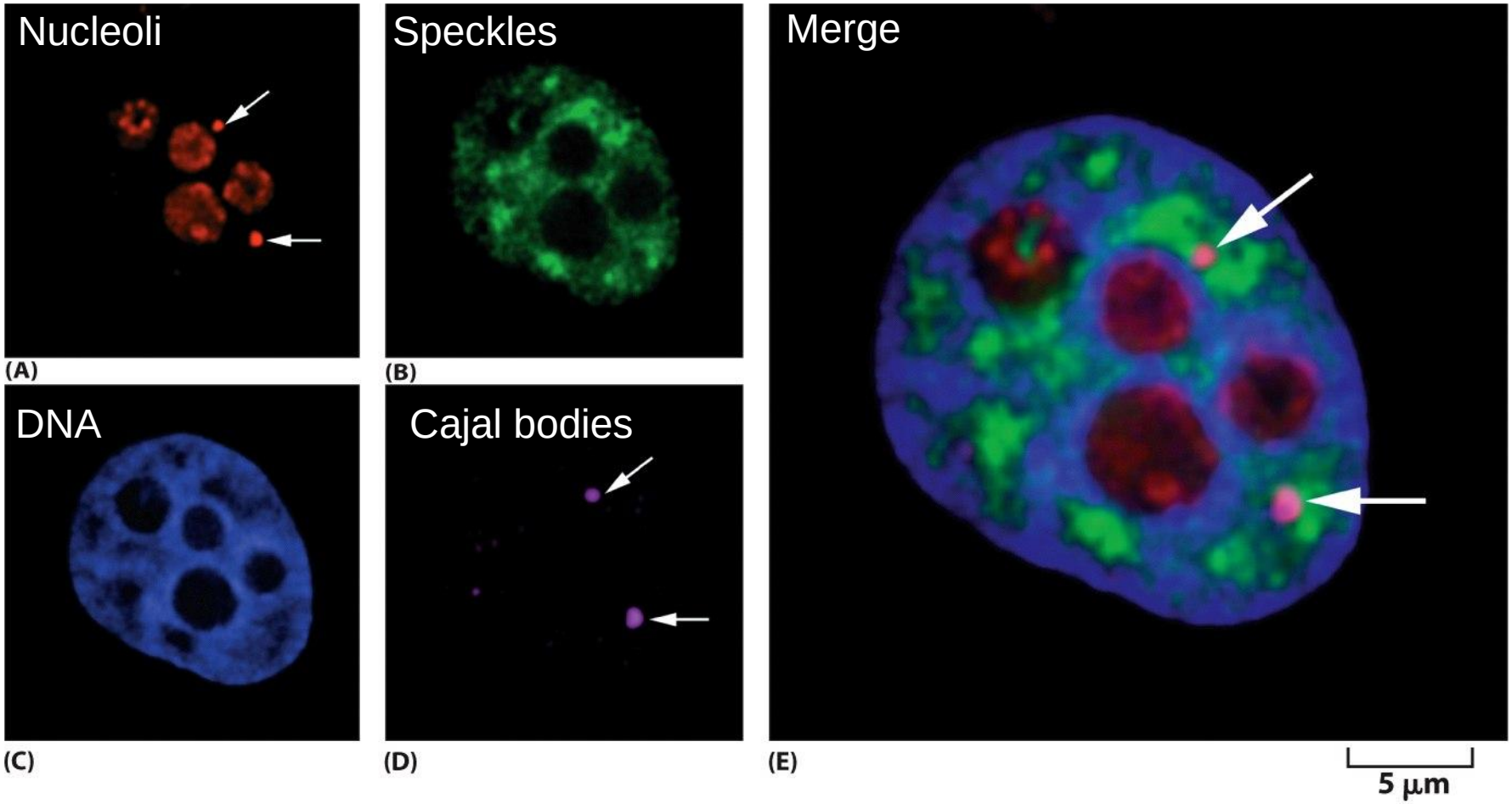


Figure 6-48 *Molecular Biology of the Cell* (© Garland Science 2008)

Nucleolus

Usually 2-3 per nucleus

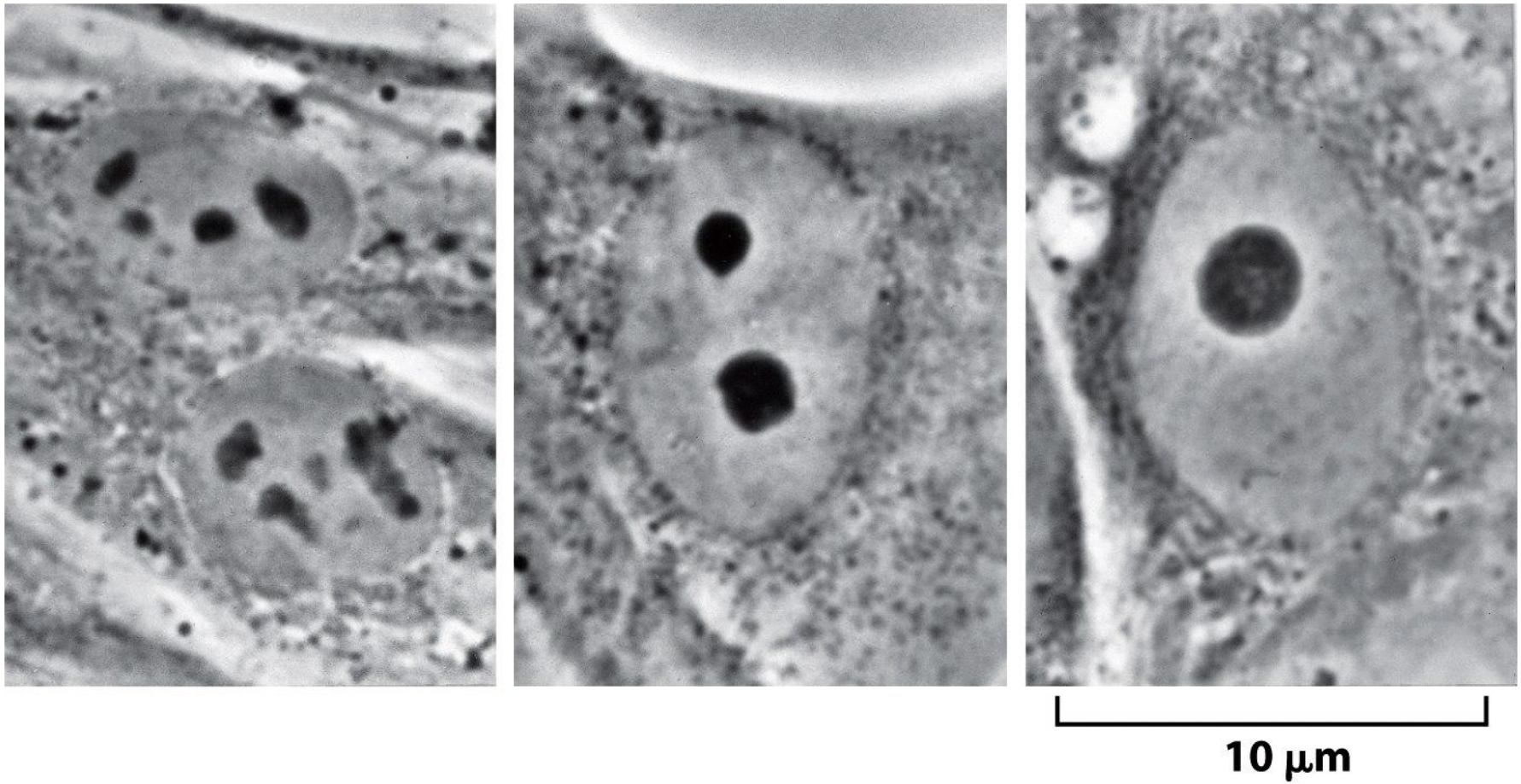
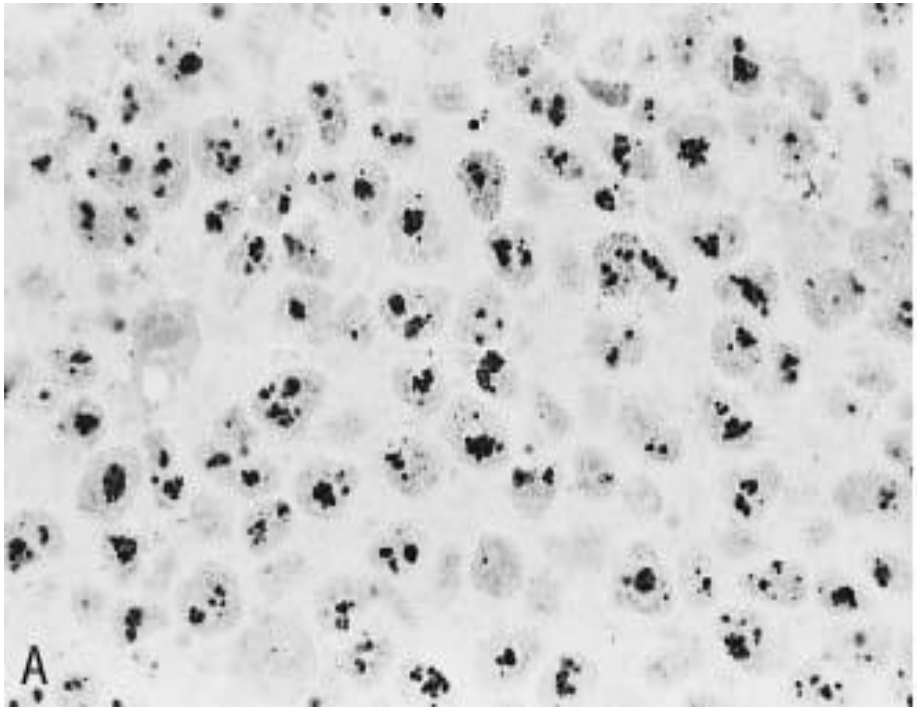


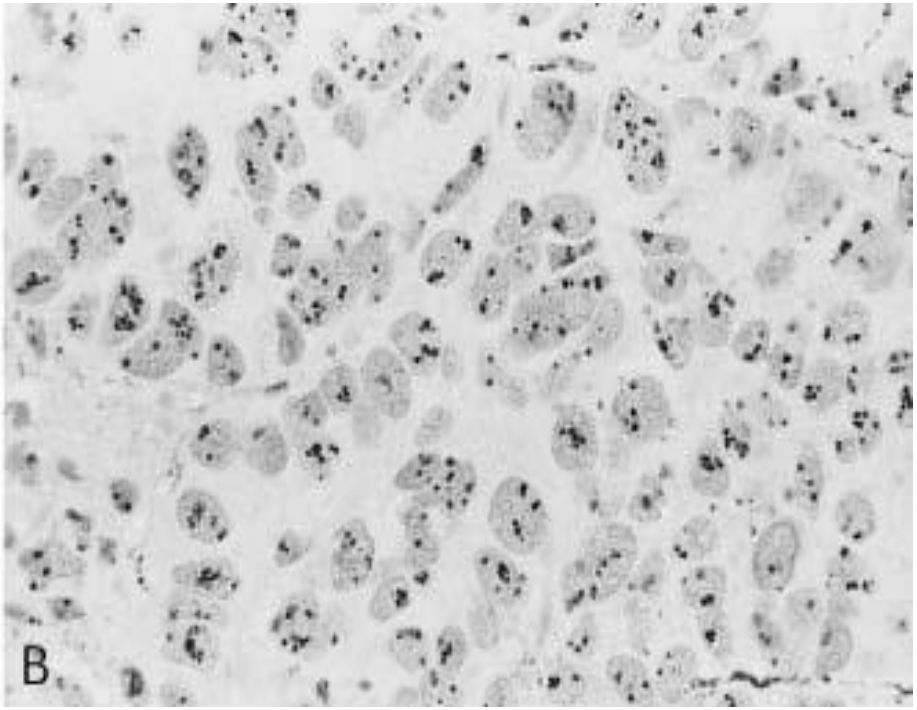
Figure 6-46 *Molecular Biology of the Cell* (© Garland Science 2008)

Nucleolus

Silver Staining of NORs in Carcinomas



NB 100 (DT = 3.15 days)

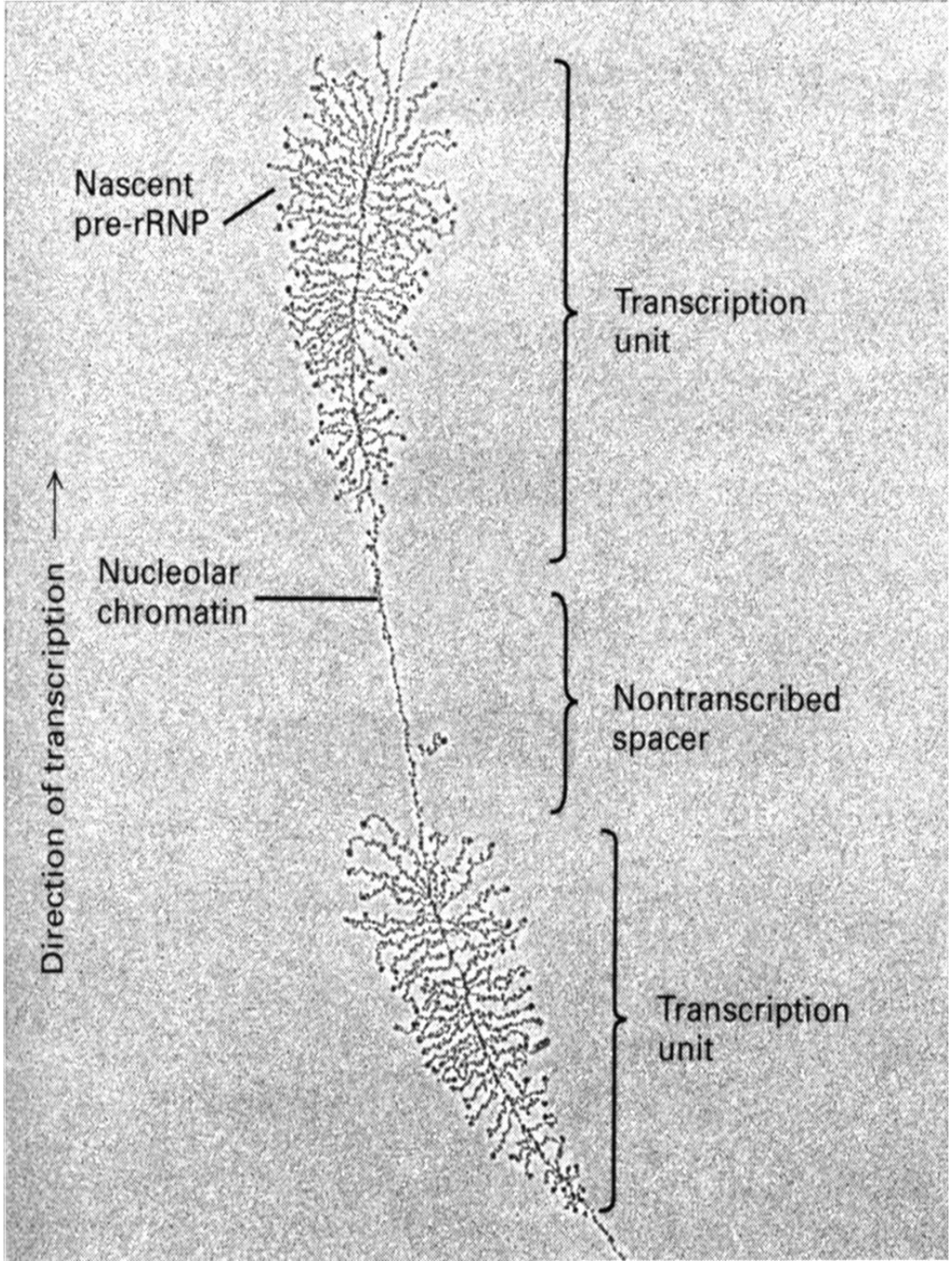


LoVo (DT = 15.23 days)

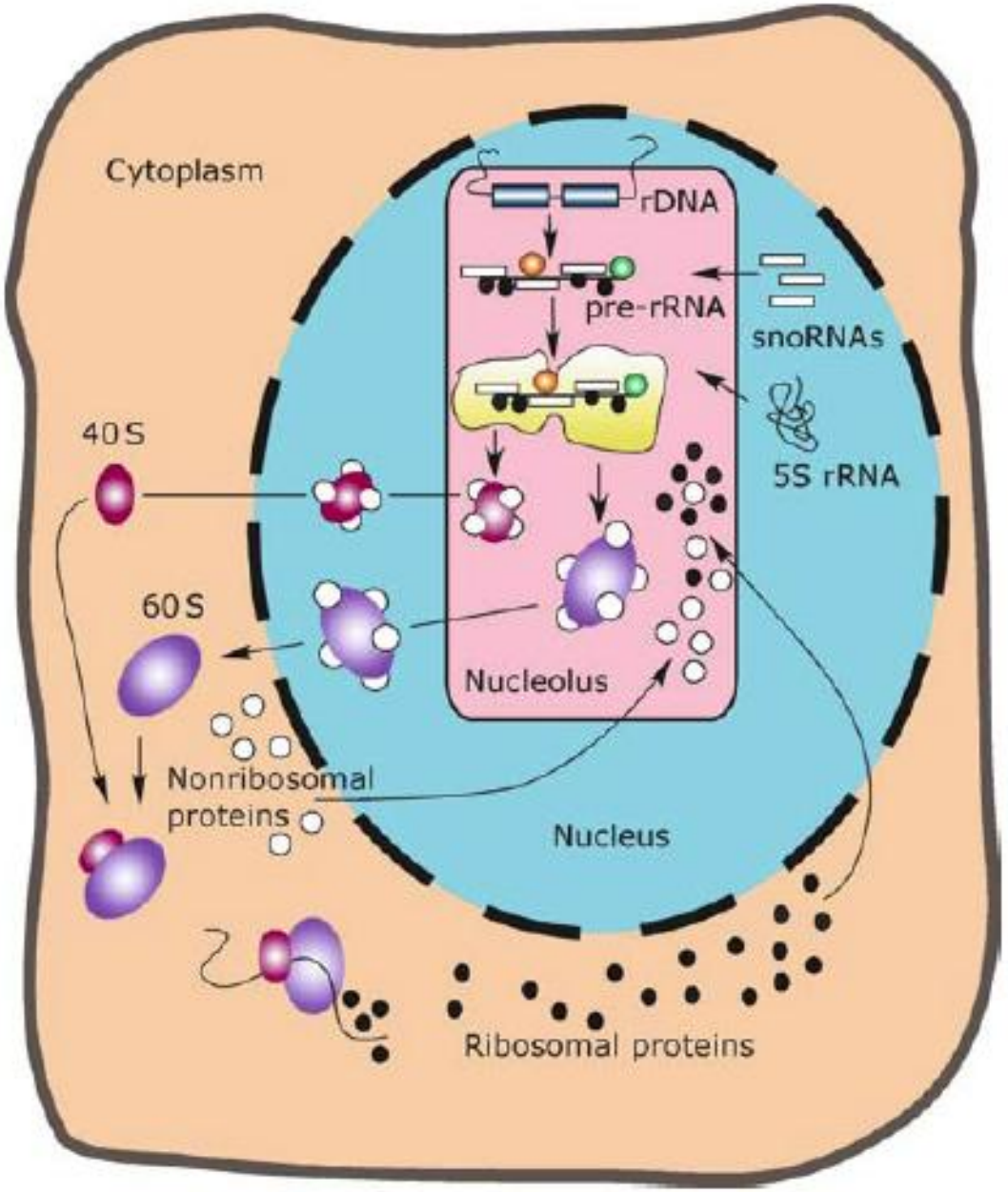
(Derenzini et al., 2000. *J. Pathol.* **191:**181)

Nucleolus

rDNA



Nucleolus



(Olson MO, Dundr, HistochemCellBiol 2005)

Nucleolus

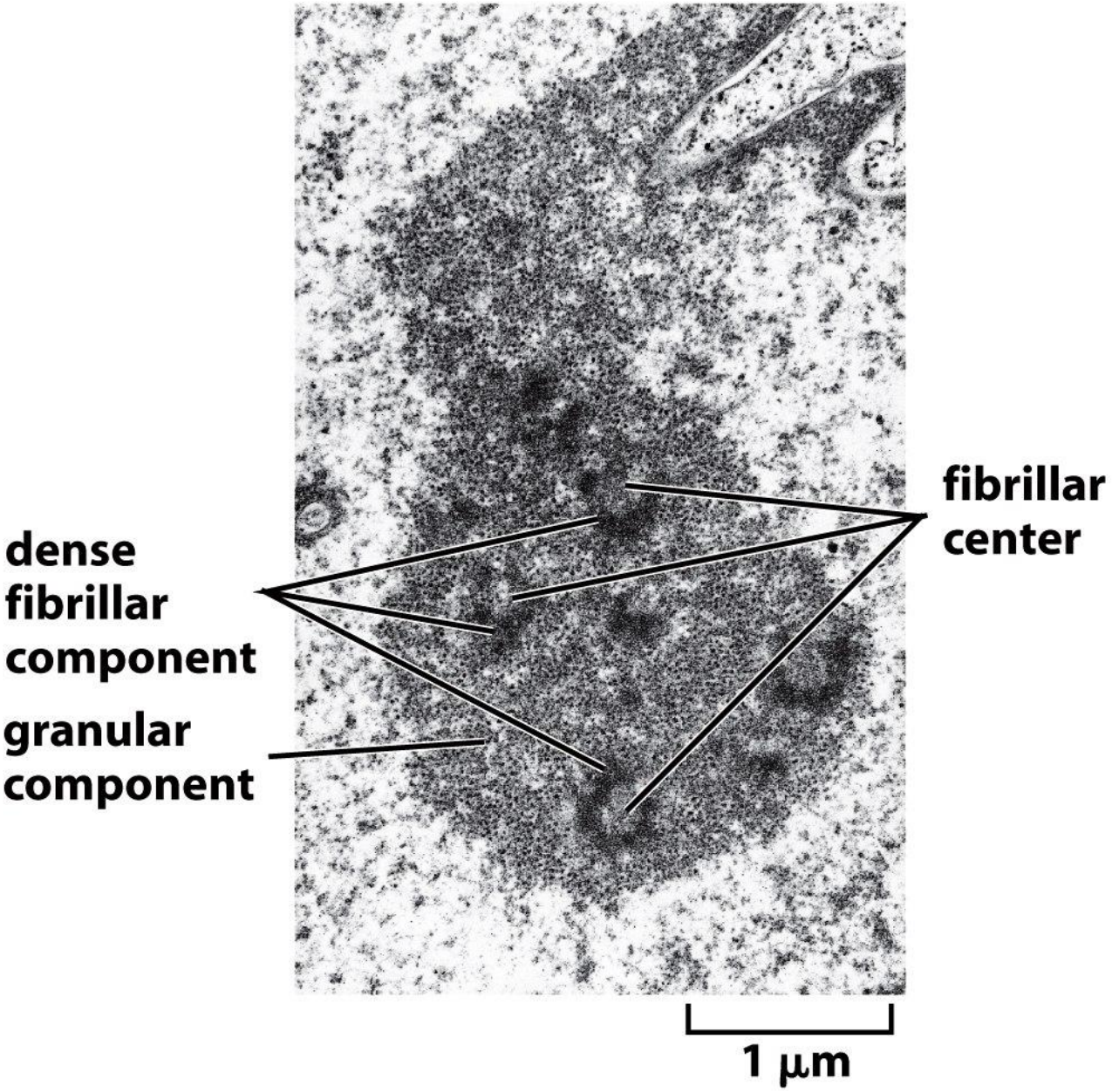
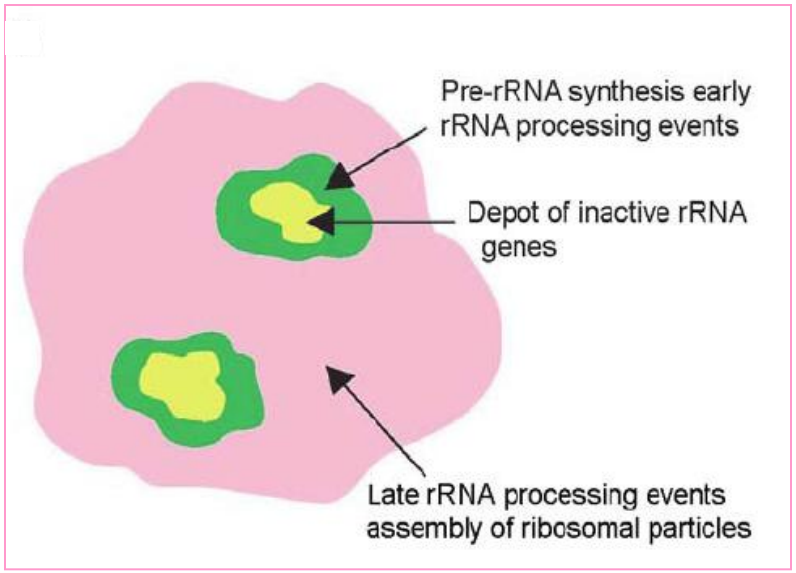
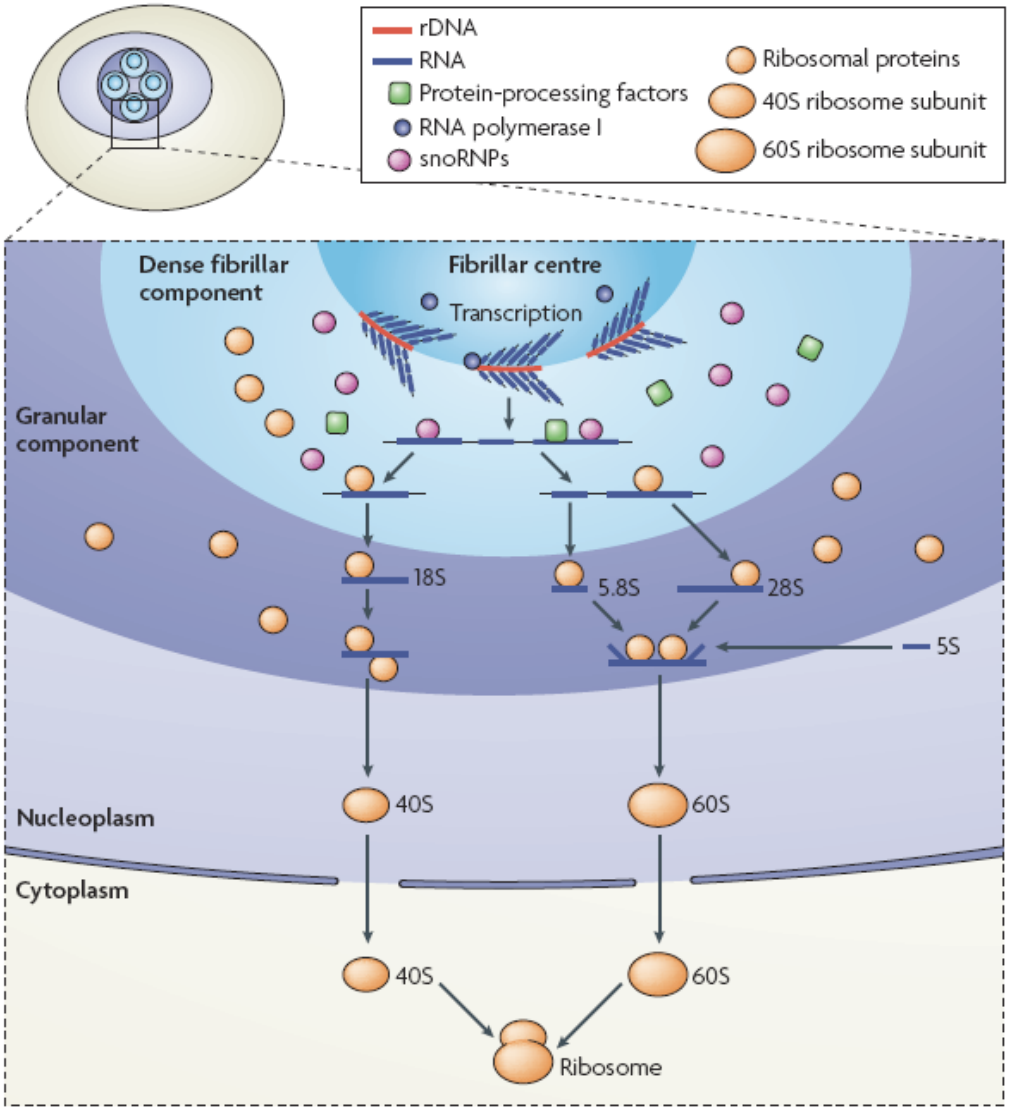


Figure 6-44b *Molecular Biology of the Cell* (© Garland Science 2008)

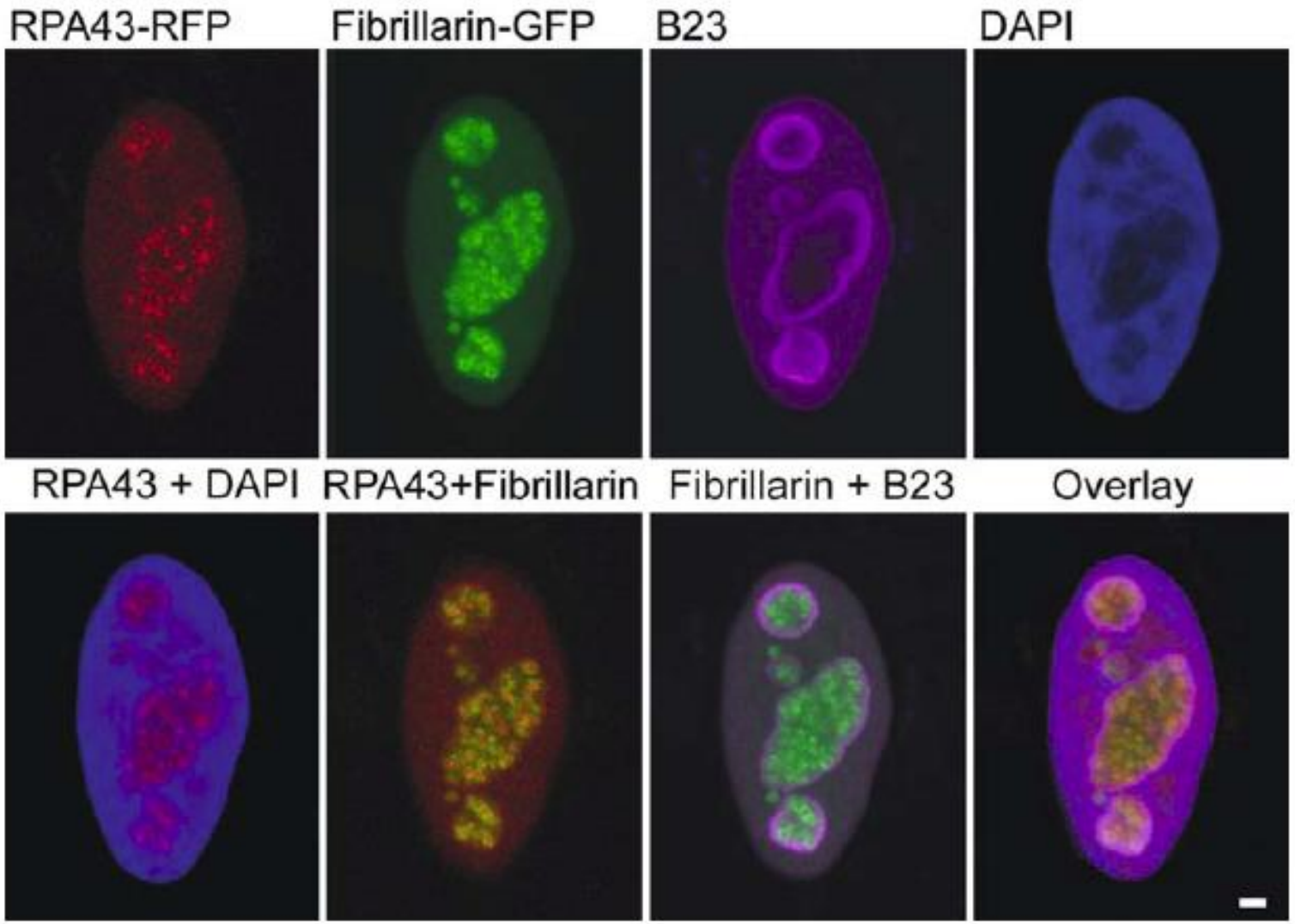
Nucleolus





(Raska I, IntRevCytol 2006)
(Lamond AI, NatRevMolCellBiol 2007)

Nucleolus

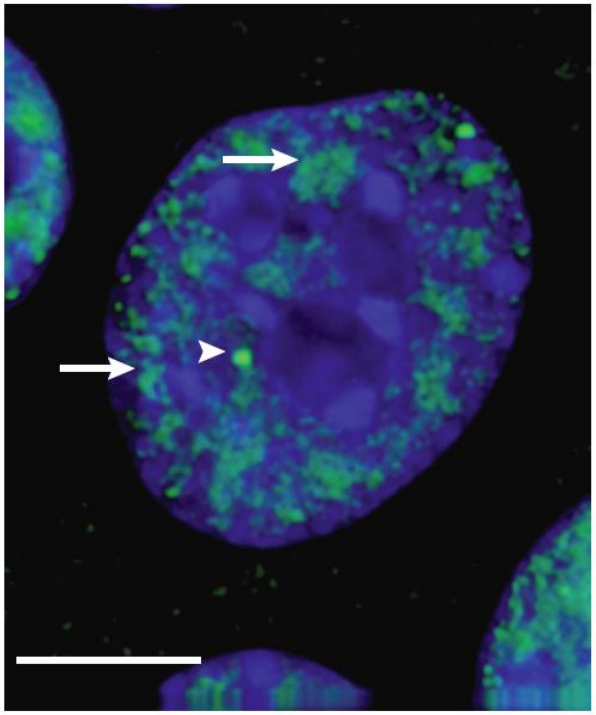


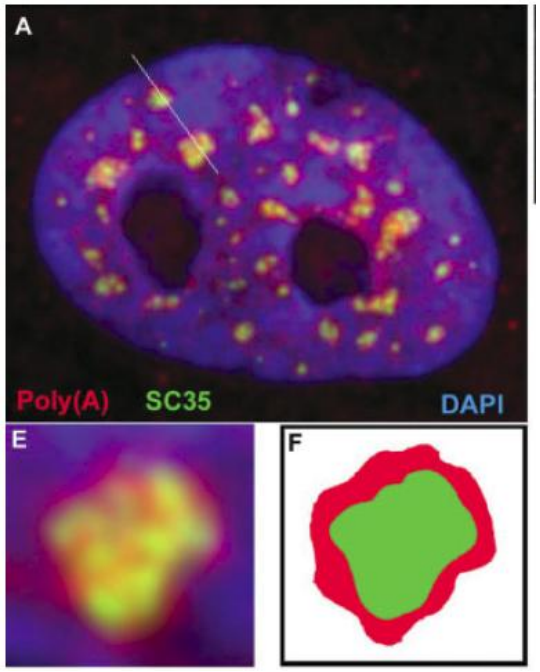
(Olson MO, Dundr, HistochemCellBiol 2005)

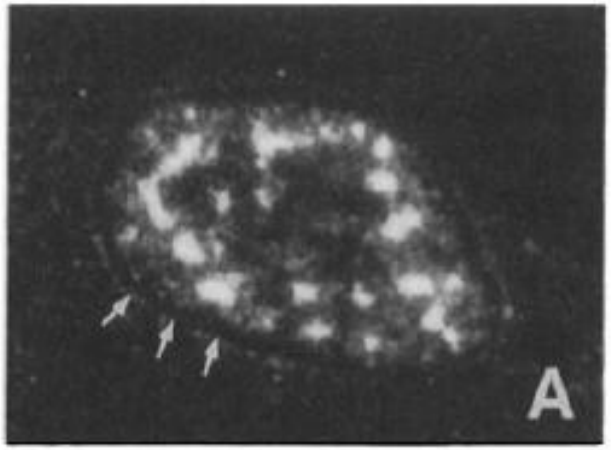
Inter-chromatin granules / “Speckles”

Subnuclear structures enriched in pre-mRNA splicing factors, located in the inter-chromatin regions.

Variable size (1 to several microns), irregular shape. Composed of 20-25nm granules.





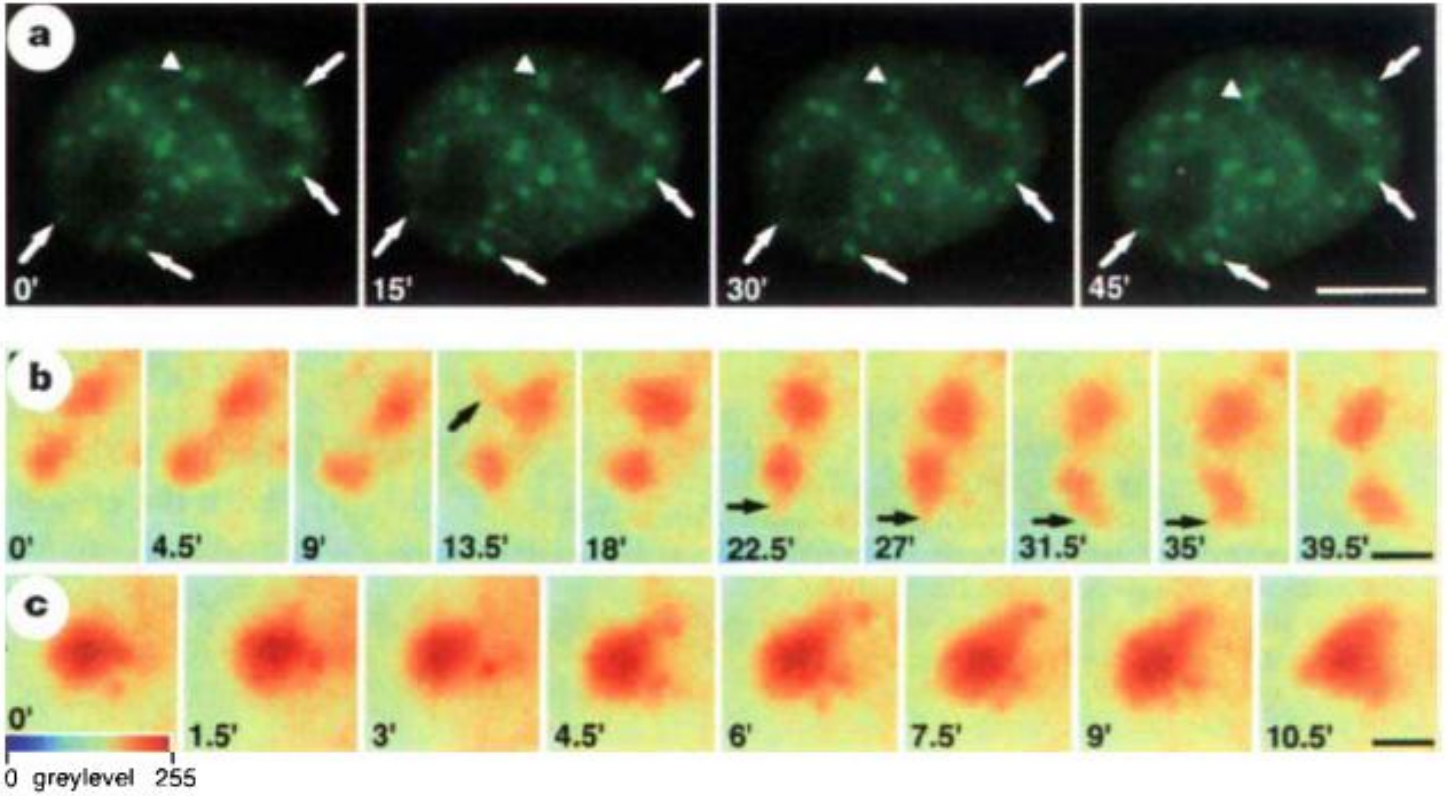


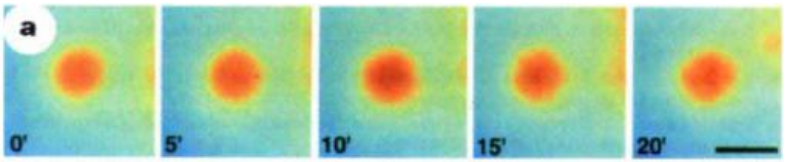
(Hall, Lawrence JB, The Anantomical Record ptA 2006)

(Lamond AI, Spector DL, NatRevMolCellBiol 2003)

Inter-chromatin granules / “Speckles”

Live-cell studies show that splicing factors are recruited from speckles to sites of transcription

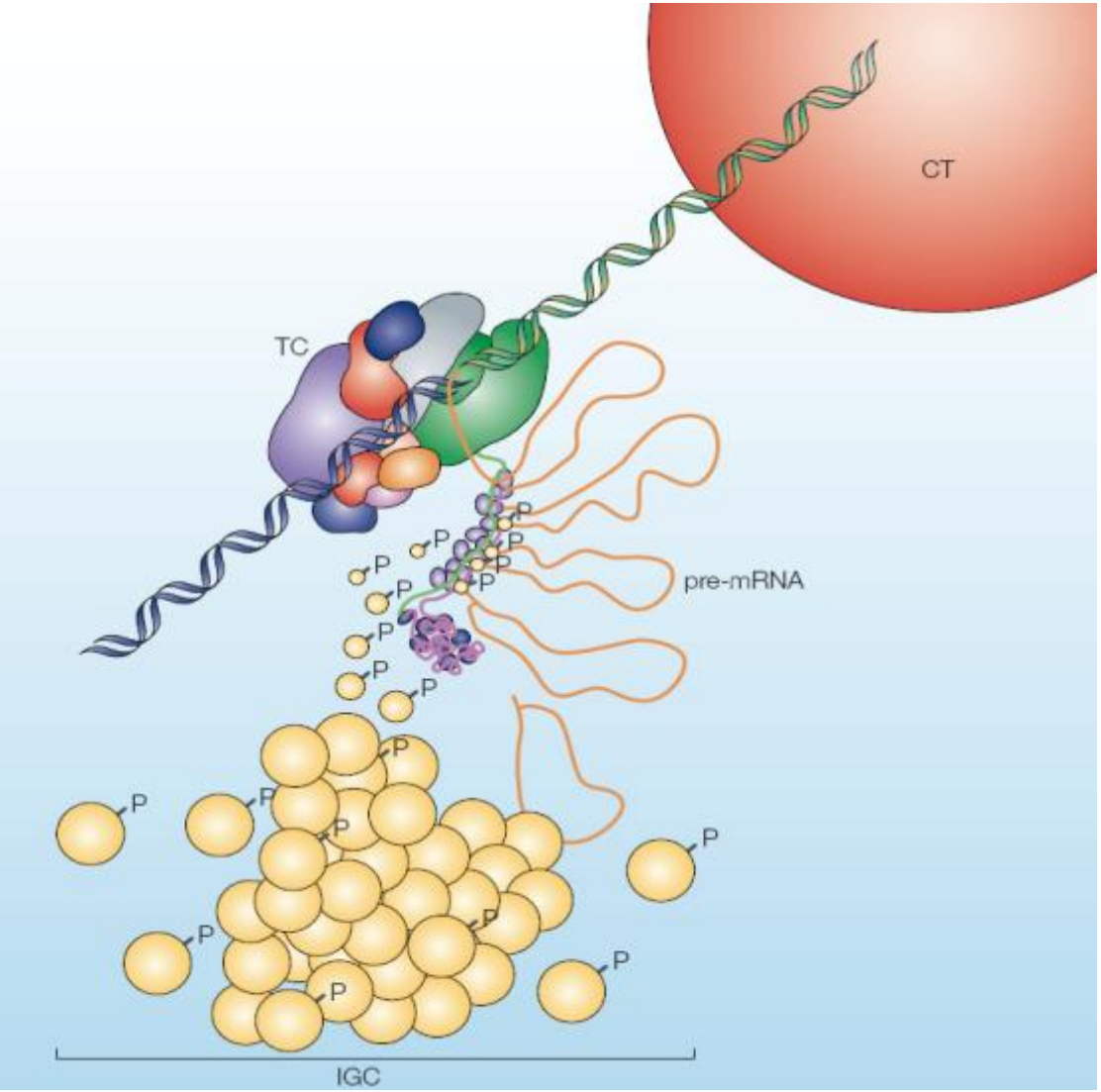




Speckles round-up when Pol II txn is inhibited

(Misteli T, Spector DL, Nature 2000)

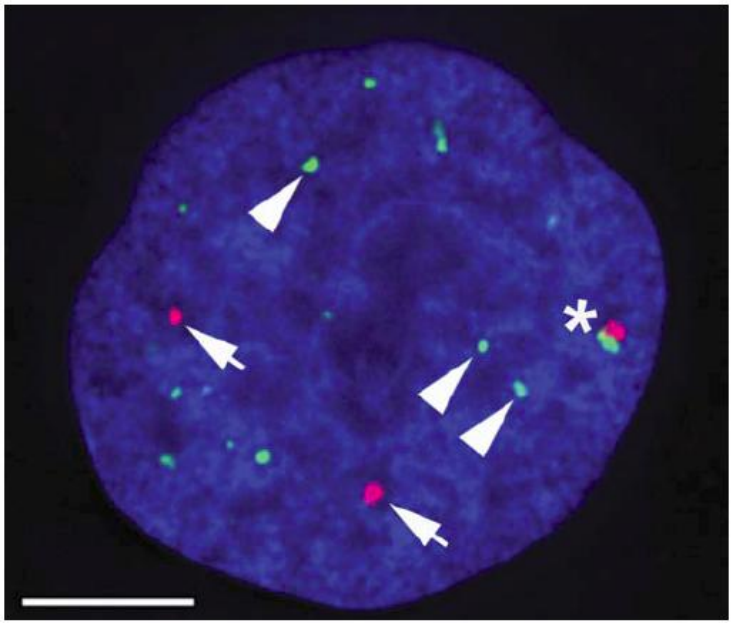
Inter-chromatin granules / “Speckles”



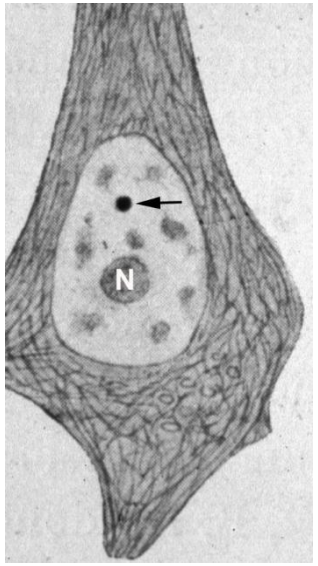
(Lamond AI, Spector DL, NatRevMolCellBiol 2003)

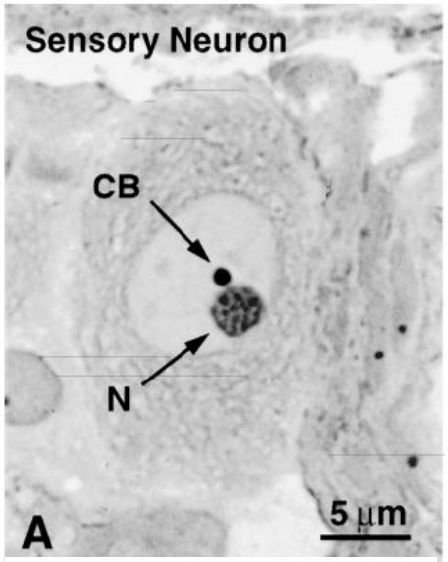
Cajal bodies

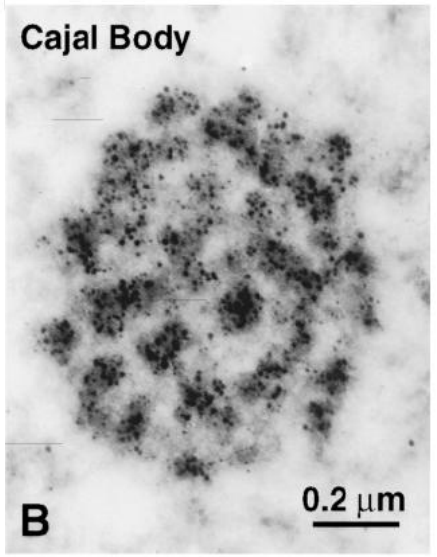
CBs are subnuclear organelles found in both plant and animal cells. They can vary in size from less than 0.2 *μ*m up to 2 *μ*m or even larger, depending on cell type and species.



Cajal bodies – red, anti-coilin
PML bodies - green







(Gall JG, AnnRevCellDevBiol 2000)

Cajal bodies

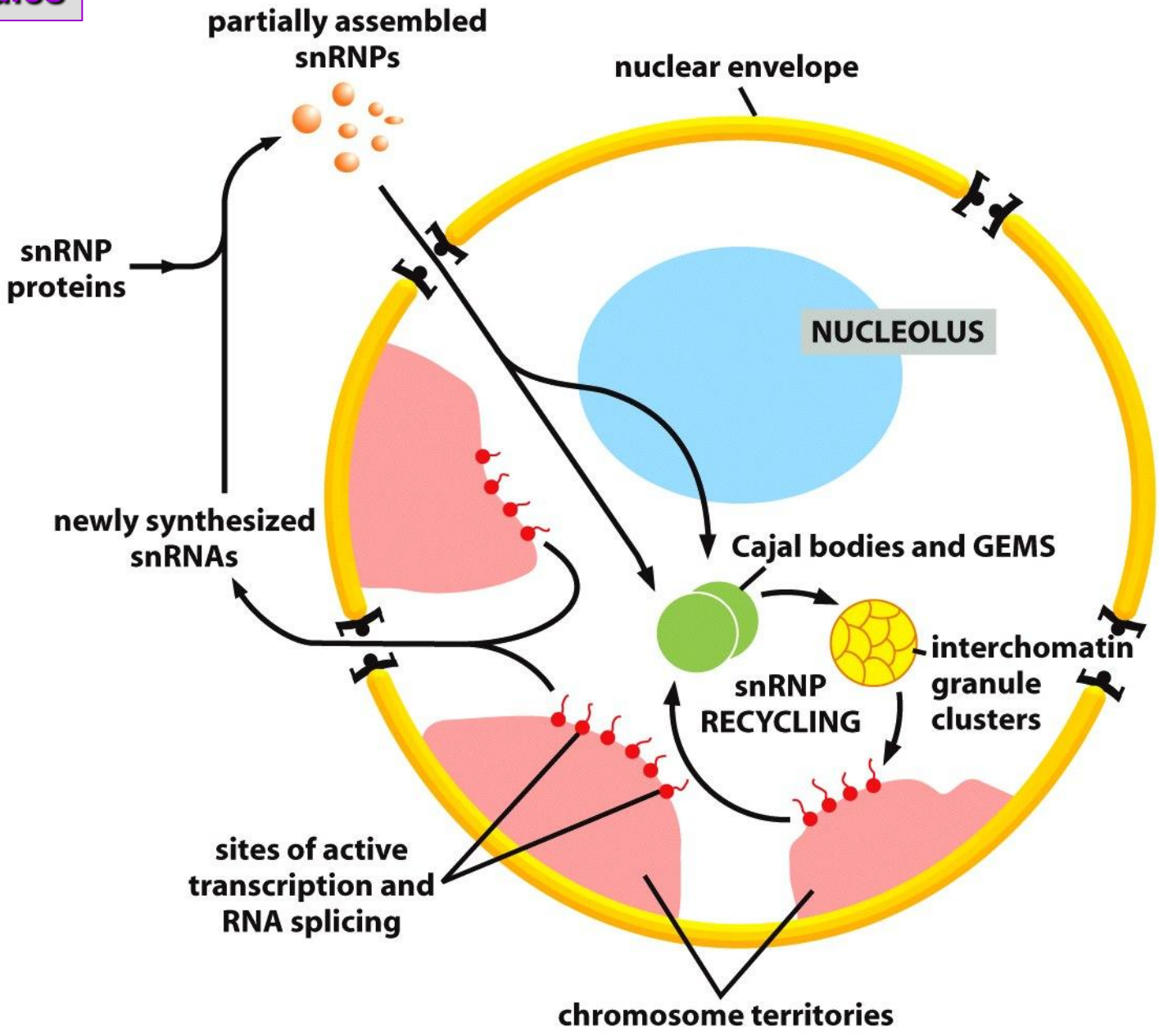
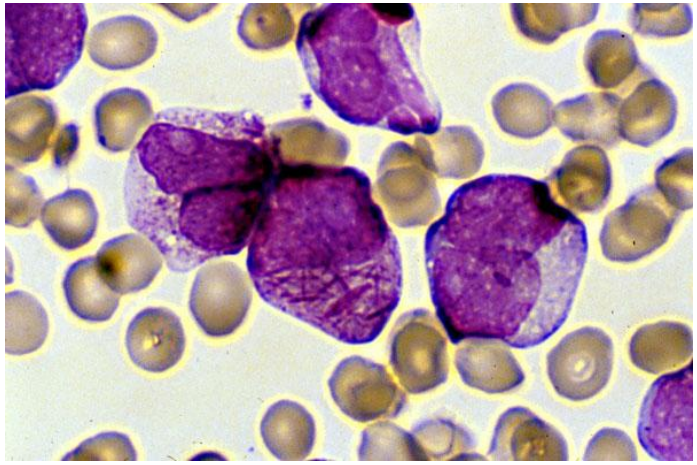
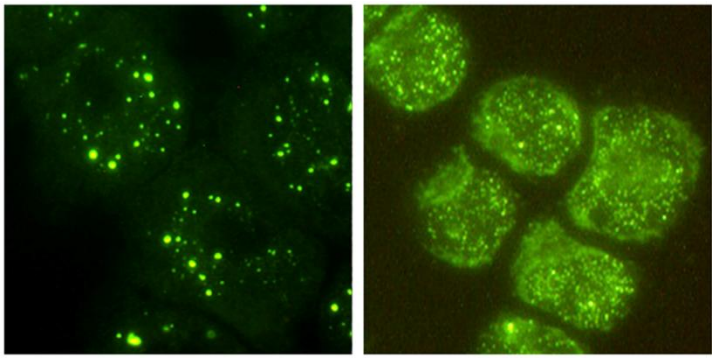


Figure 6-49 *Molecular Biology of the Cell* (© Garland Science 2008)

PML bodies

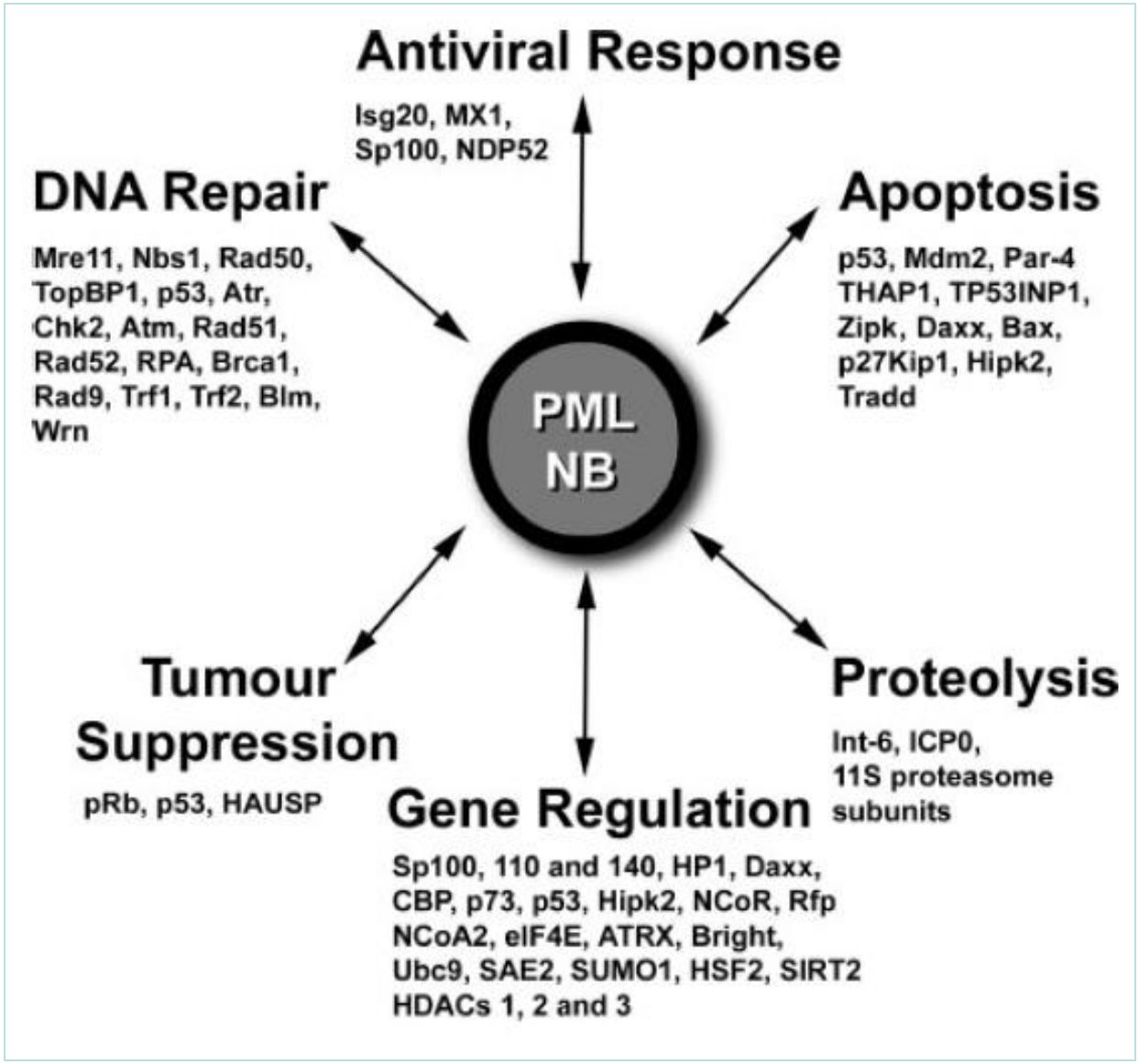
The gene encoding the PML protein was isolated at the break point of a common translocation found in patients suffering from acute promyelocytic leukaemia (APL), which results in the expression of an oncogenic fusion protein consisting of the PML protein fused to the retinoic acid receptor-α (RAR-α)





This leukemia is treatable to complete clinical remission with retinoic acid, the physiological ligand of RAR-α, and also induces their reorganization.

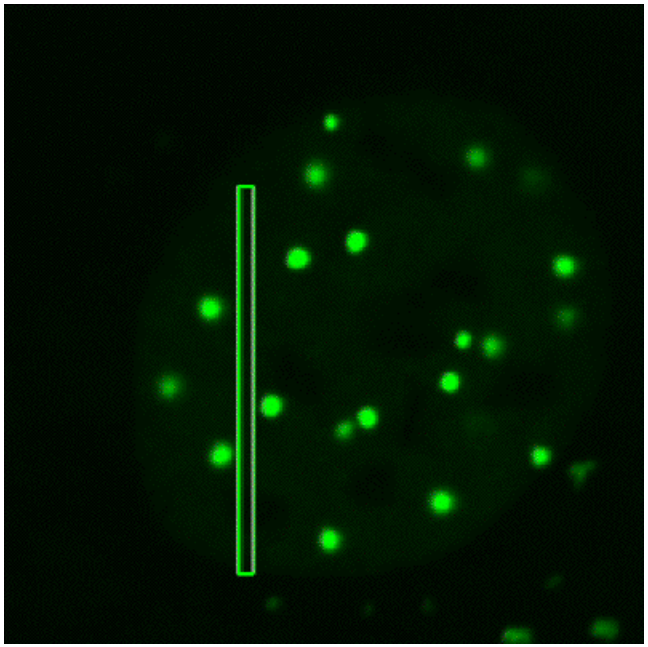
PML bodies

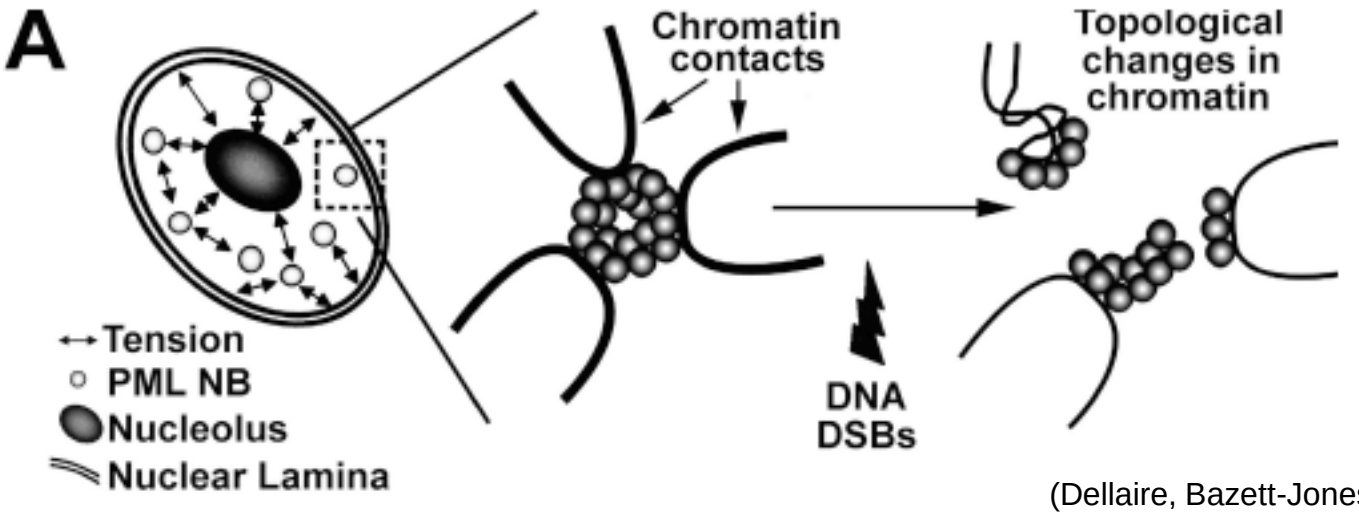


(Dellaire G, Bazett-Jones DP, Bioessays 2004)

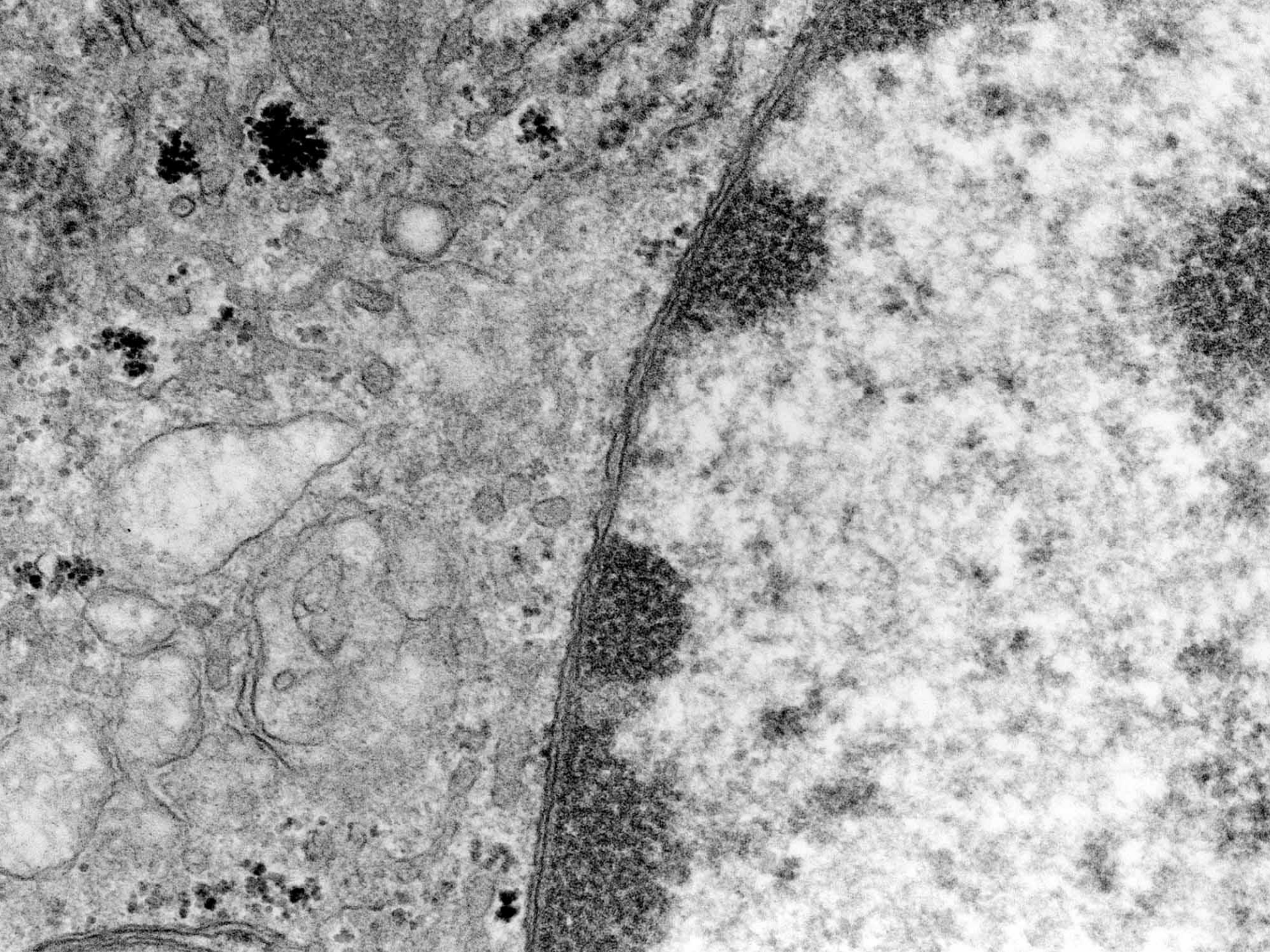
PML bodies

Site-specific DSBs induced by UV laser irradiation.





(Dellaire, Bazett-Jones DP, JCB 2006)



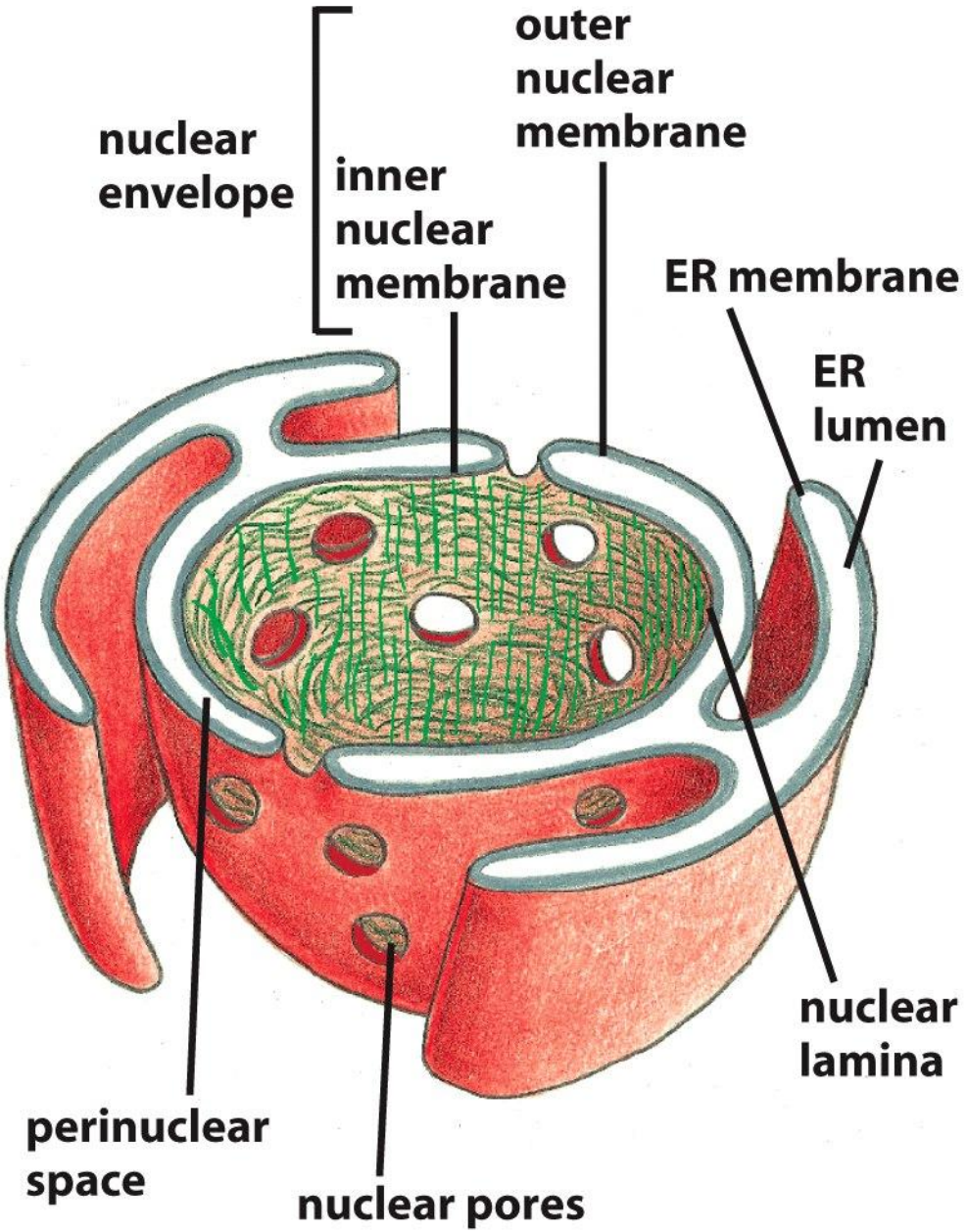
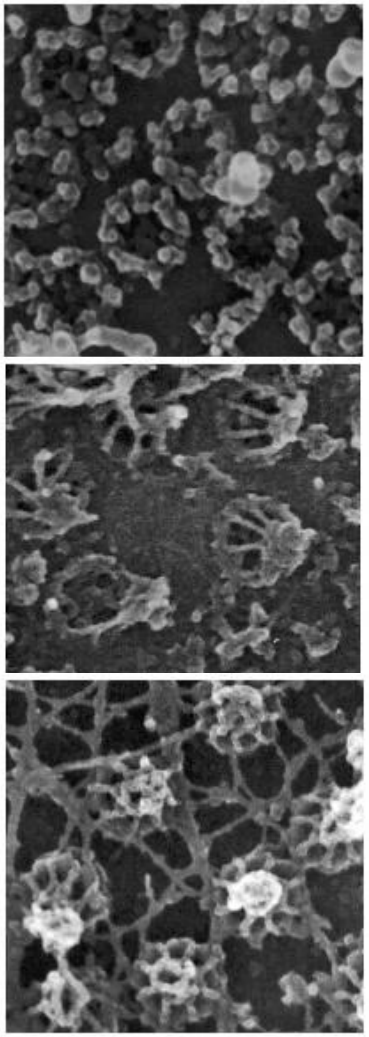
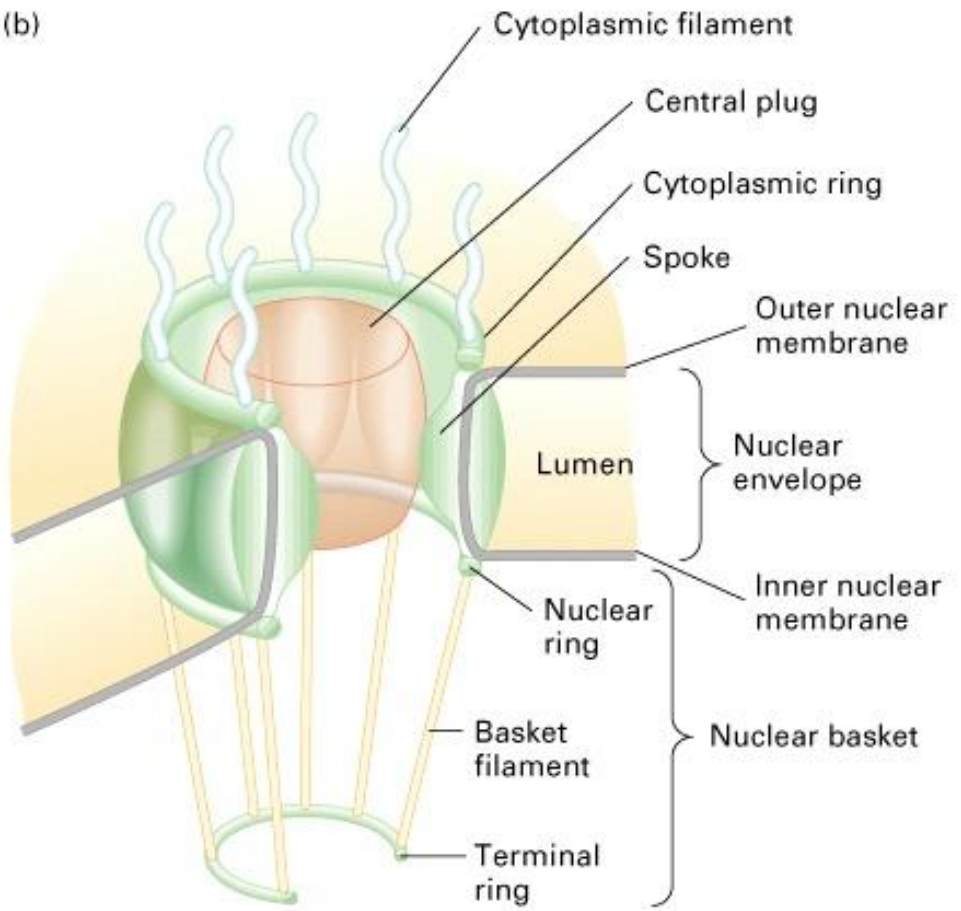


Figure 12-8 *Molecular Biology of the Cell* (© Garland Science 2008)

The nuclear pore complex





Nuclear Transport

Imported

- Polymerases
- Histones
- Transcription, replication, modification and repair factors
- Ribosomal proteins

Exported

- tRNAs, small RNAs
- mRNPs
- Ribosomal subunits

HeLa cells: 10 million ribosomes, divide each day
10^7 ribosomes every day = 10^7 per 1440 minutes = 7000 ribosomes/minute

Each ribosome has 80 proteins, made into two subunits

→ 560,000 ribosomal proteins imported/min
14,000 ribo subs exported/min

3,000-4,000 pores/cell →
Per pore per minute: 100 proteins in, 3 subunits out
Also 100 histones/min/pore etc.

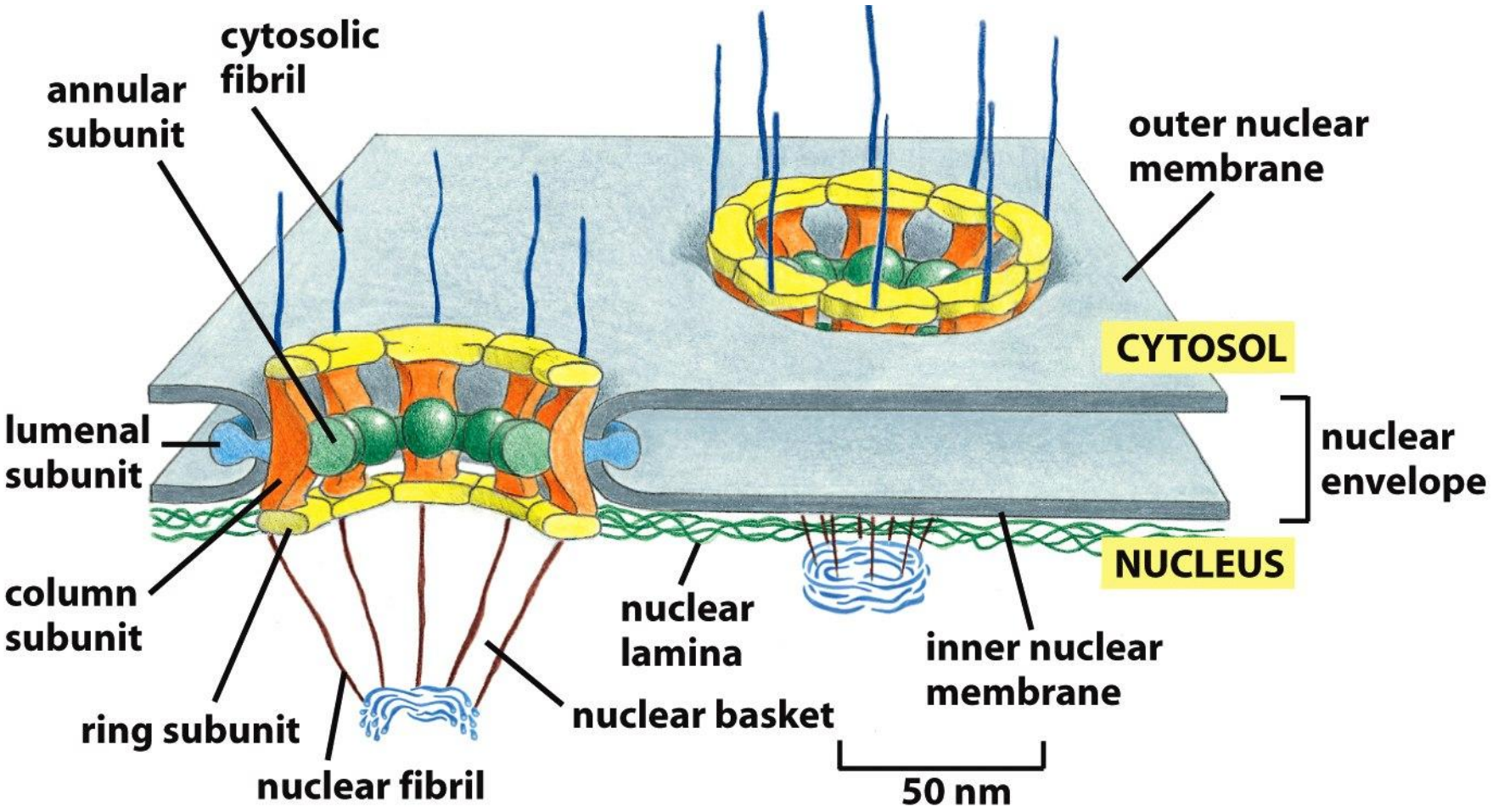


Figure 12-9a *Molecular Biology of the Cell* (© Garland Science 2008)

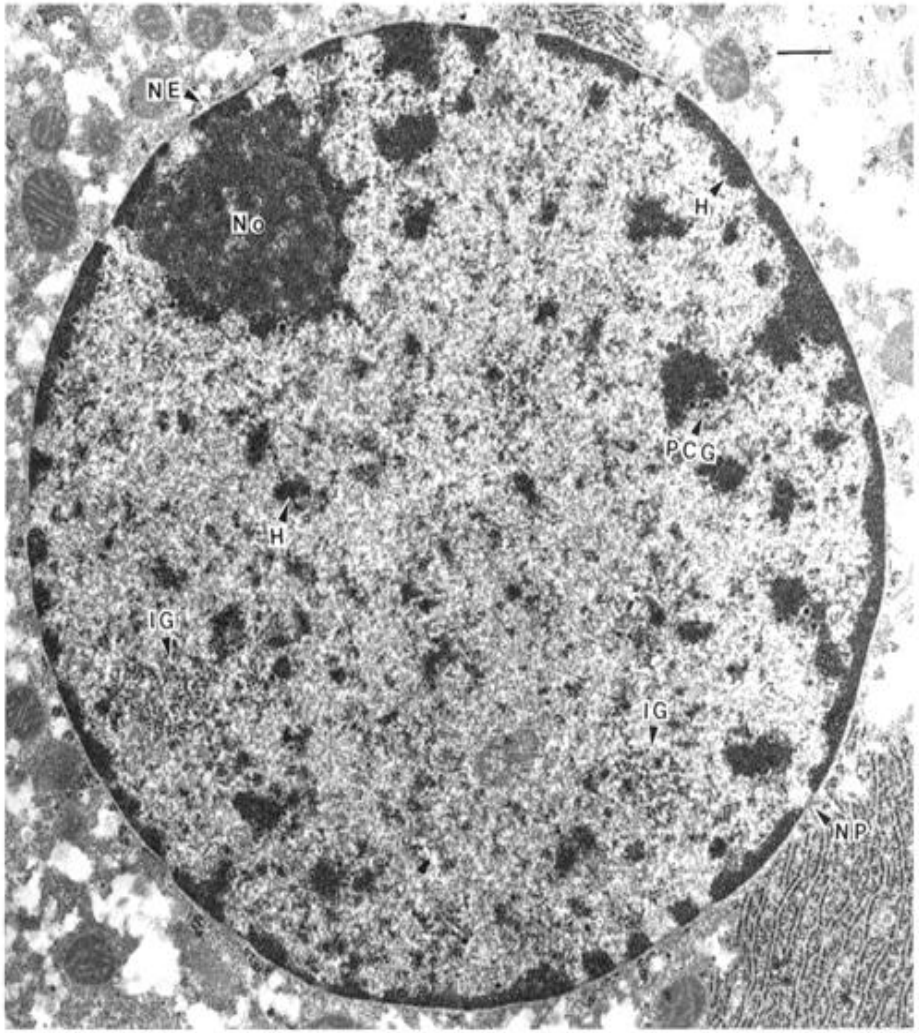


FIGURE 4.2
Thin-section transmission electron micrograph of a rat liver cell nucleus. *Bar*, 0.5 μm.

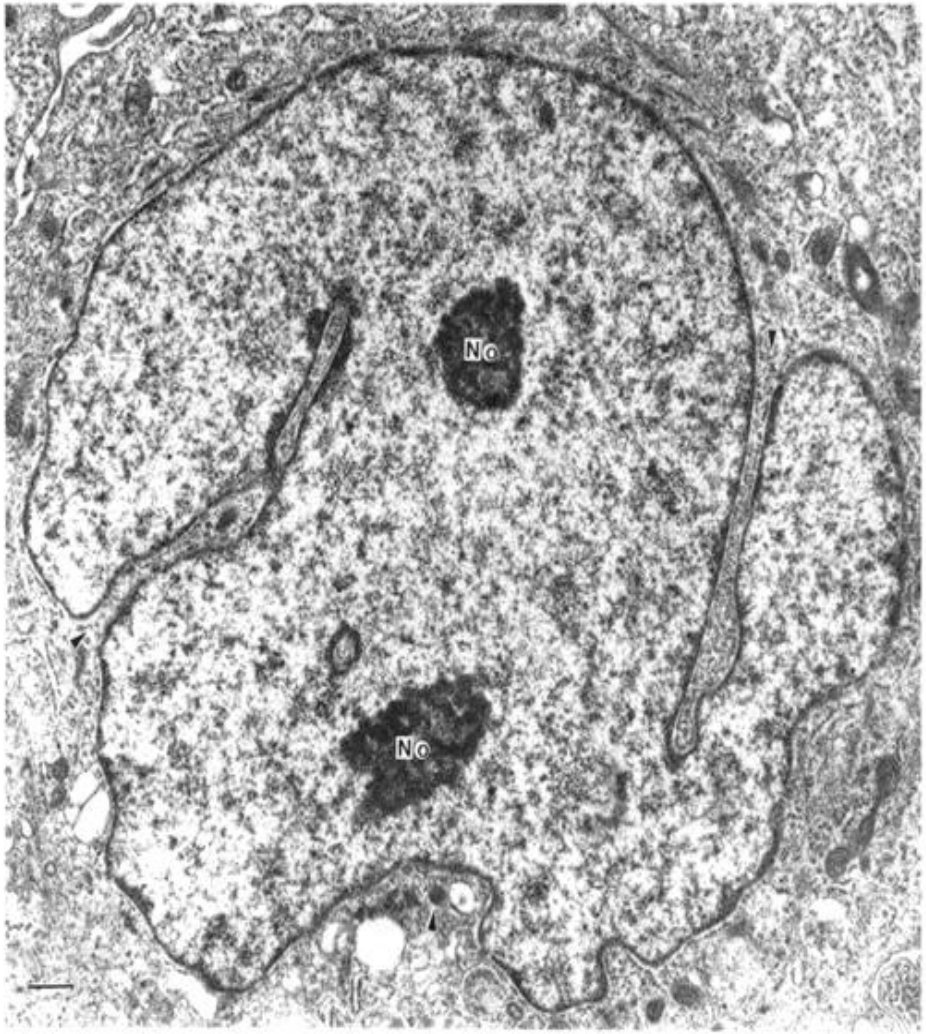
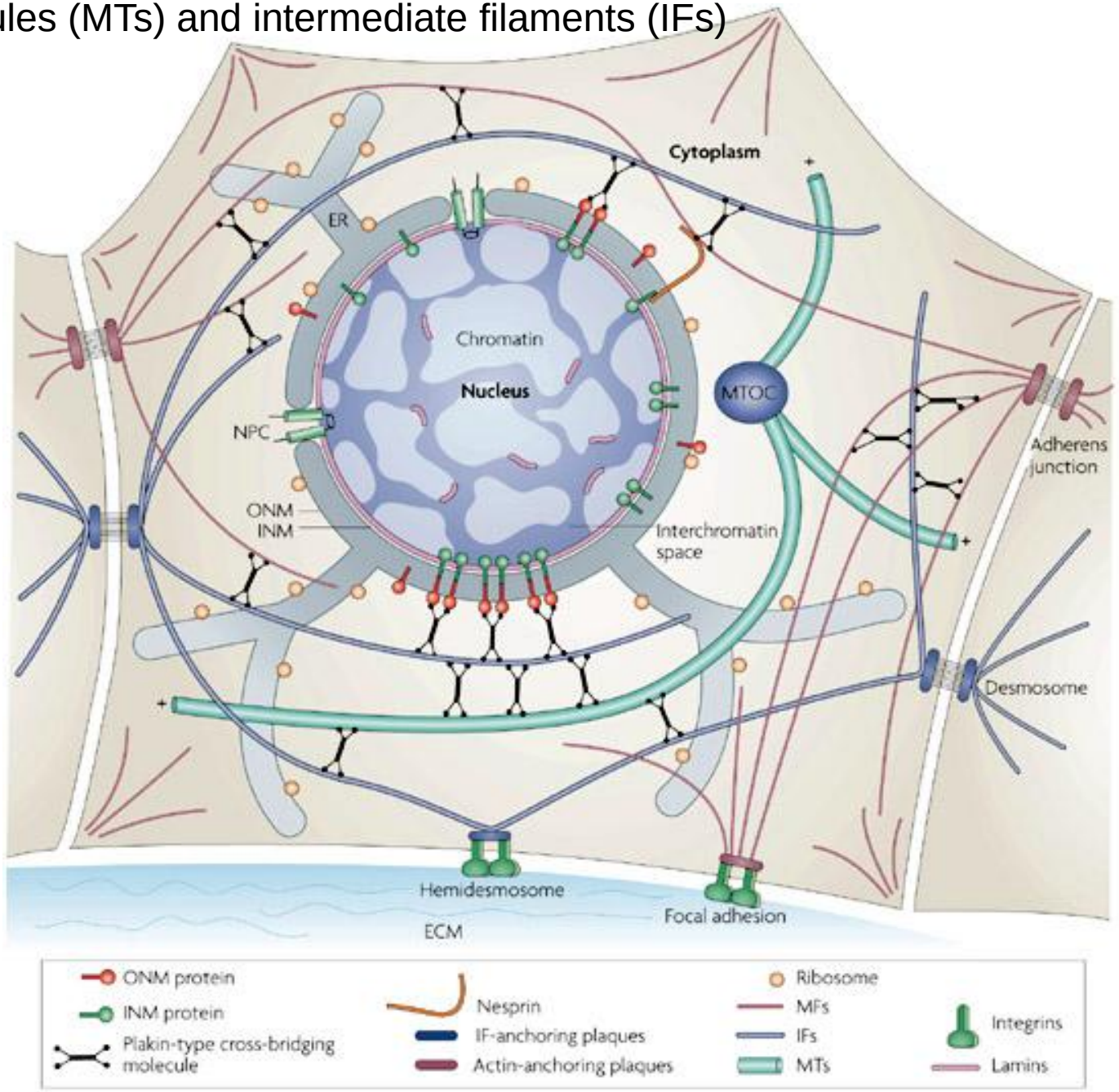


FIGURE 4.3
Thin-section transmission electron micrograph of a human hepatoma cell nucleus showing numerous infoldings (*arrowheads*) of the nuclear envelope. The nucleoplasm is nearly devoid of heterchromatin. *Bar*, 0.5 μm.

The three key filament systems of the cytoskeleton, microfilaments (MFs), microtubules (MTs) and intermediate filaments (IFs)



(Aebi U, NatRevMolCellBiol 2007)

Lamins

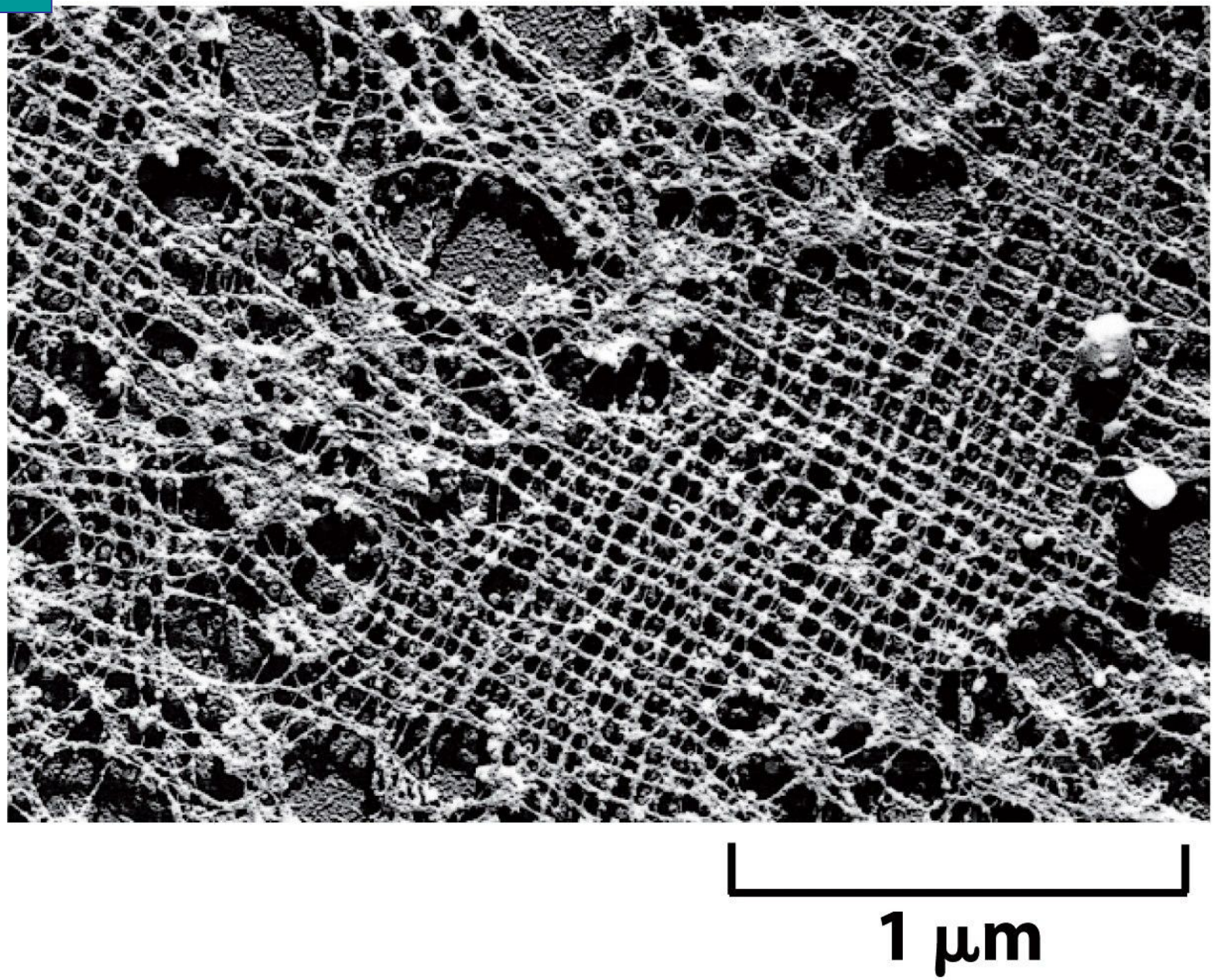
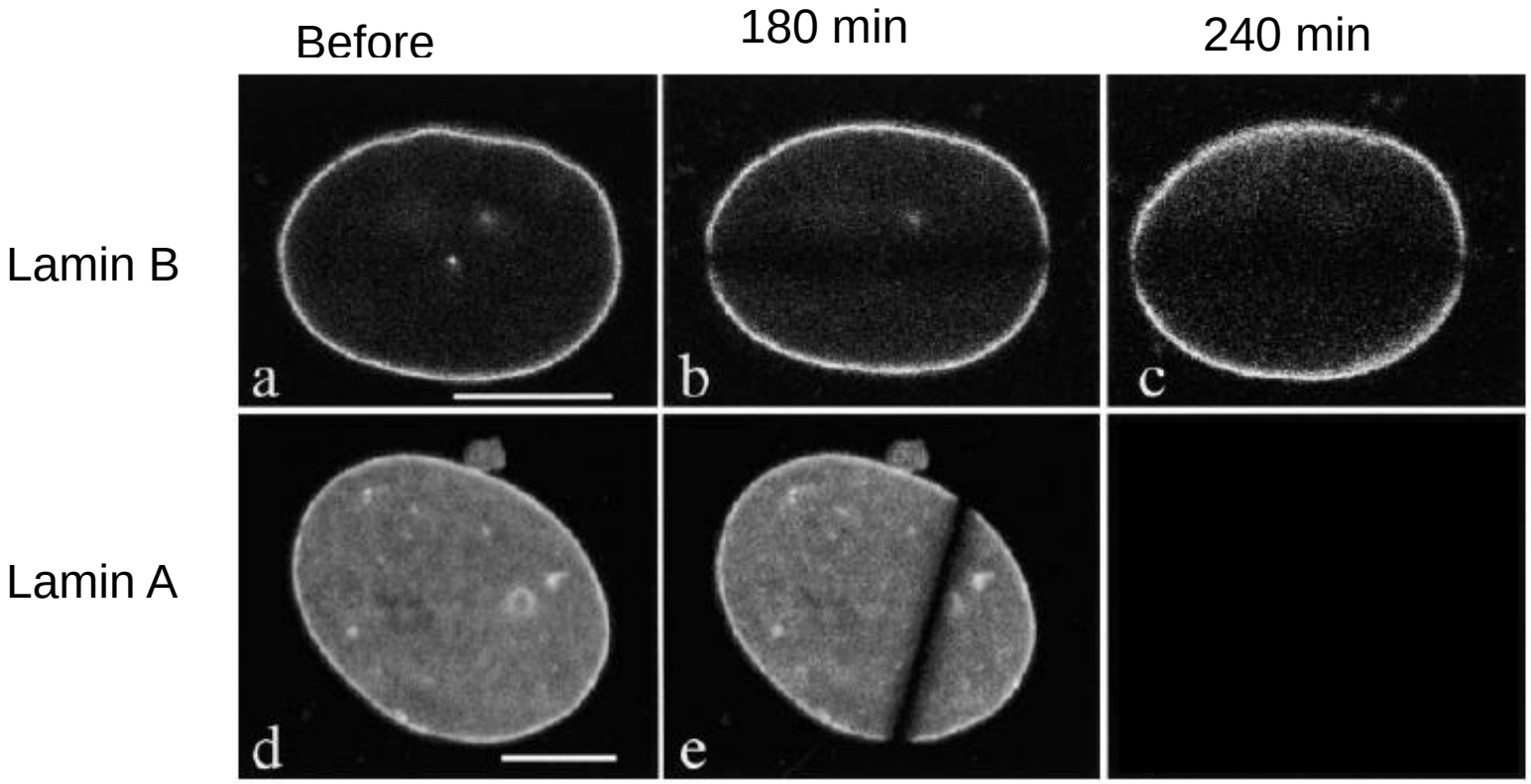


Figure 12-19 *Molecular Biology of the Cell* (© Garland Science 2008)

Lamins

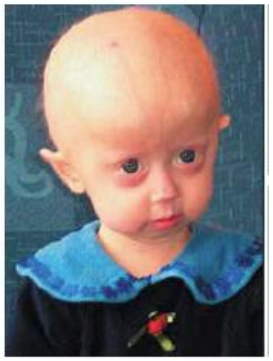
In interphase, lamin polymers are nearly immobile, with FRAP halftimes of about 3 hrs



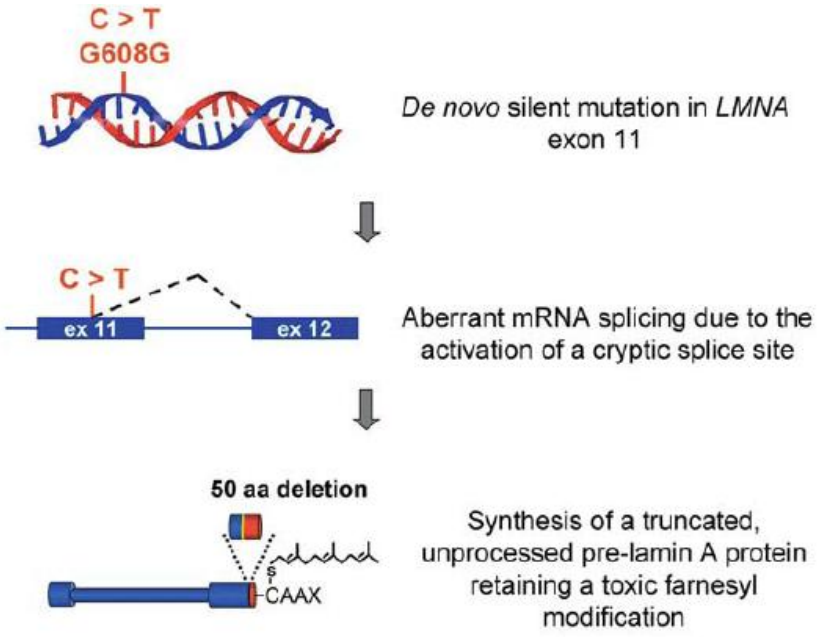
(Moir, Goldman RD, JCB 2000)

Laminopathies

Progeria – accelerated aging

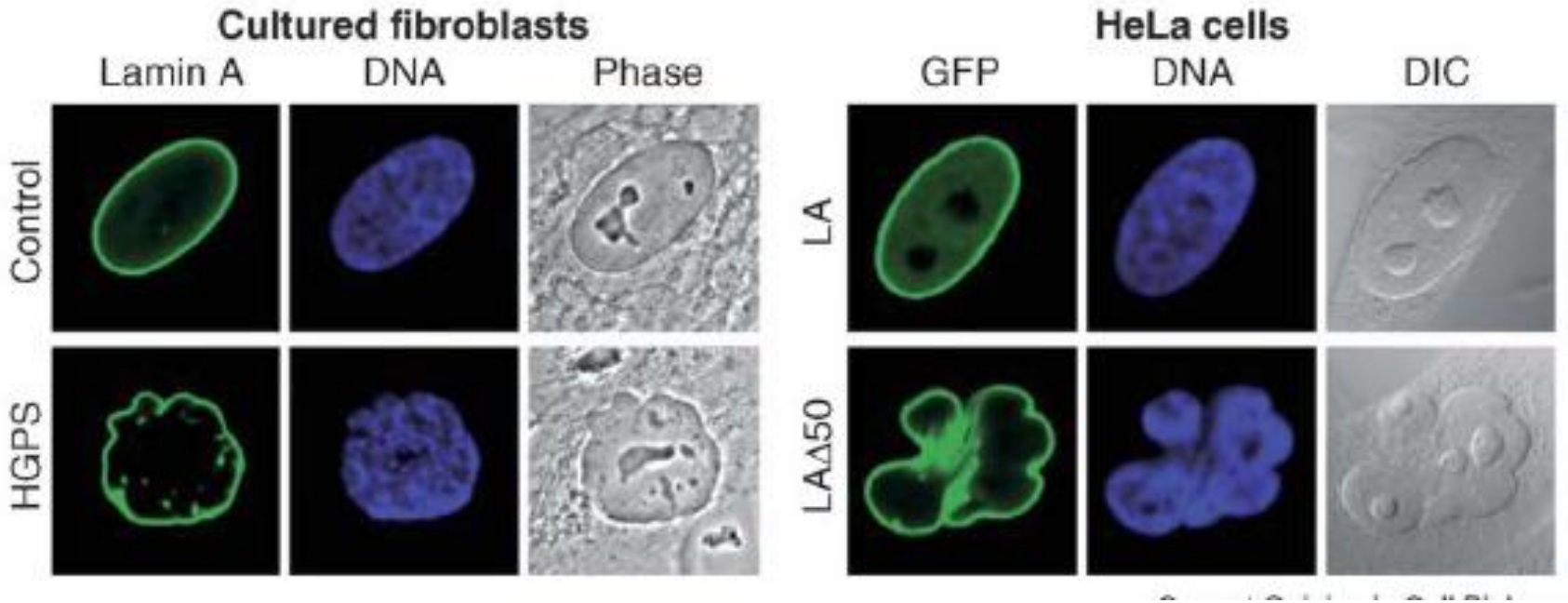


HGPS (Hutchinson-Gilford progeria syndrome) – children
Becomes apparent in 1-2 year olds – old skin, hair loss, bone deformations, growth retardation, loss of fat
Die at 13-20 yrs



(Scaffidi, Misteli T, PLOS Biol 2005)

Laminopathies



(Mattout A, Gruenbaum Y, CurrOpinCellBiol 2006)