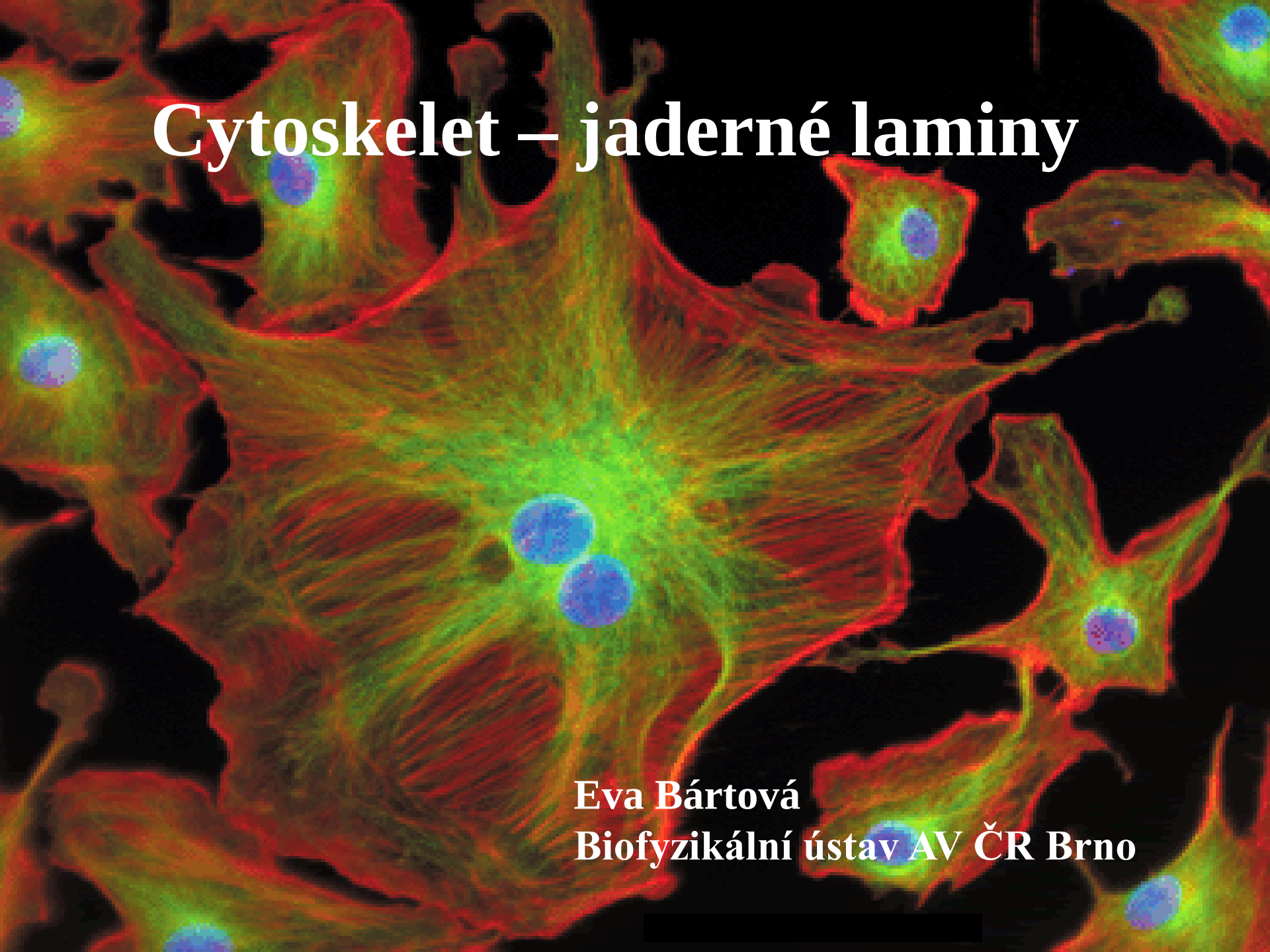
Cytoskelet – jaderné laminy



Eva Bártová
Biofyzikální ústav AV ČR Brno

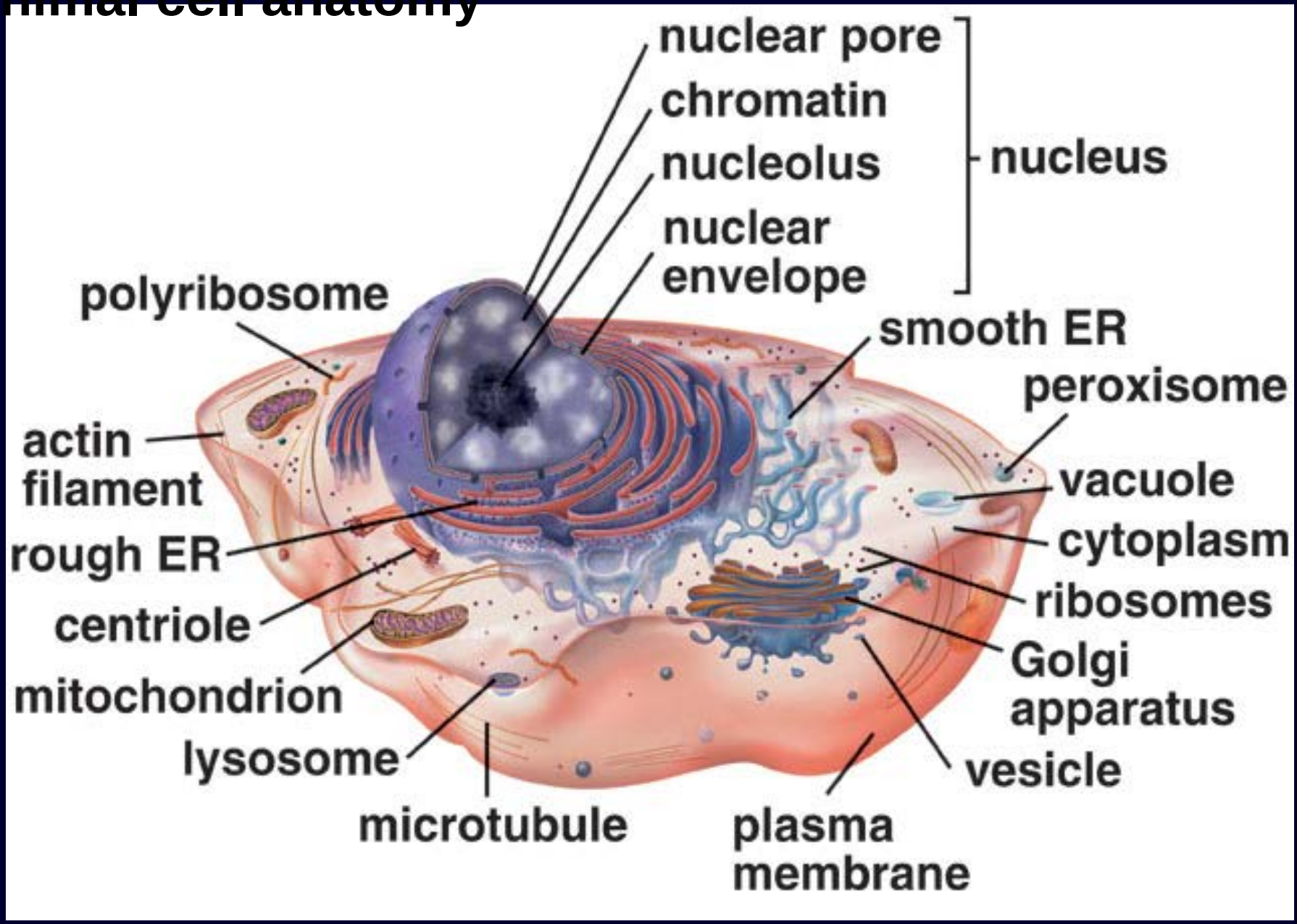
Eukaryotic Cells

All cells are surrounded by a *plasma membrane* made of phospholipids and proteins.

Eukaryotic cells have membrane bound intracellular organelles.

The most prominent is the *nucleus* that controls the workings of the cell.

Animal cell anatomy



Eukaryotické buňky jsou charakteristické velkou morfologickou variabilitou. Tvar buněk je závislý na komplexu sítí proteinových filament, které se rozpínají cytoplasmou. Tato síť filament se nazývá CYTOSKELET. Protože je cytoskelet zodpovědný za buněčný pohyb, mohl by se rovněž nazývat cytomusculaturou.

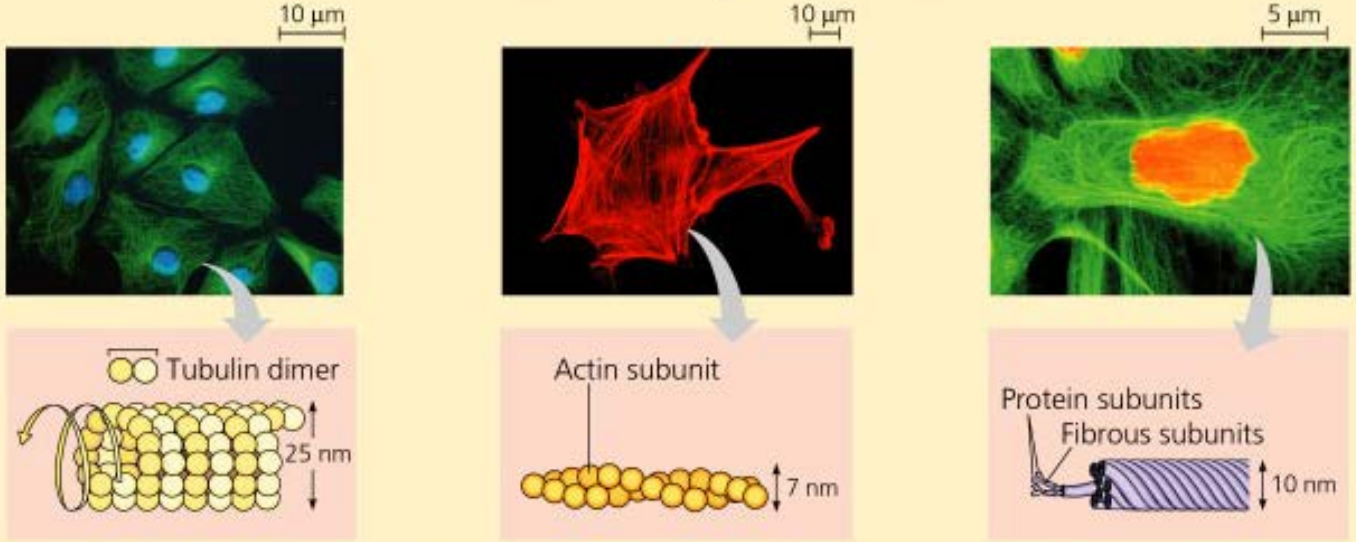
CYTOSKELET SE JEVÍ JAKO ZÁSADNÍ BUNĚČNÝ FAKTOR, KTERÝ HRAJE DULEŽITOU ÚLOHU V EVOLUCI BUNĚK

ODLIŠNÉ AKTIVITY CYTOSKELETU JSOU ZÁVISLÉ NA TŘECH TYPECH PROTEINOVÝCH FILAMENT:

A) AKTINOVÁ FILAMENTA (aktin)
B) MIKROTUBULY (tubulin)
C) INTERMEDIALNÍ FILAMENTA (vimentin, lamin)

Table 7.2 The Structure and Function of the Cytoskeleton

Property	Microtubules	Microfilaments (Actin Filaments)	Intermediate Filaments
Structure	Hollow tubes; wall consists of 13 columns of tubulin molecules	Two intertwined strands of actin	Fibrous proteins supercoiled into thicker cables
Diameter	25 nm with 15-nm lumen	7 nm	8–12 nm
Protein subunits	Tubulin, consisting of α-tubulin and β-tubulin	Actin	One of several different proteins of the keratin family, depending on cell type
Main functions	Maintenance of cell shape (compression-resisting "girders") Cell motility (as in cilia or flagella) Chromosome movements in cell division Organelle movements	Maintenance of cell shape (tension-bearing elements) Changes in cell shape Muscle contraction Cytoplasmic streaming Cell motility (as in pseudopodia) Cell division (cleavage furrow formation)	Maintenance of cell shape (tension-bearing elements) Anchorage of nucleus and certain other organelles Formation of nuclear lamina



SOURCE: Adapted from W. M. Becker, L. J. Kleinsmith, and J. Hardin, *The World of the Cell*, 4th ed. (San Francisco, CA: Benjamin Cummings, 2000), p. 753.

Eukaryotické buňky jsou tvořeny biliony proteinových molekul, které tvoří přibližně 60% buněčné masy. Existuje 10 tisíc typů proteinů, které vykazují svoji specifickou funkci.

A1) AKTINOVÁ FILAMENTA (mikrofilamenta)

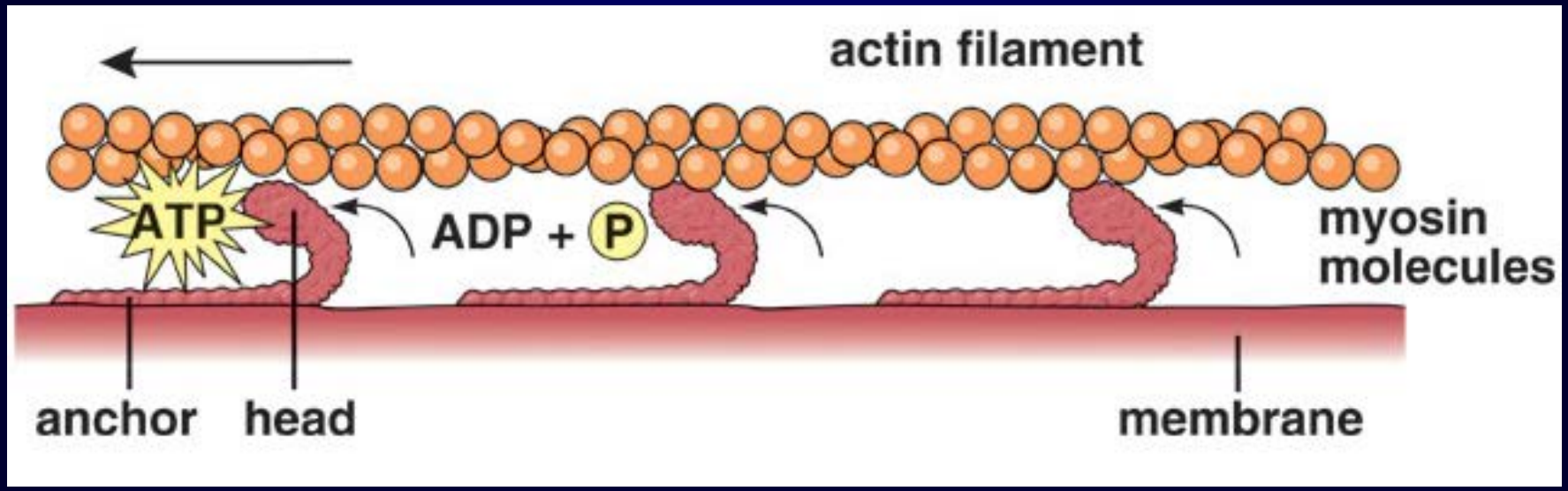
jsou dvoustranné helikální polymery tvořené proteinem aktinem. Jeví se jako flexibilní struktury s průměrem 5-9mm. Jsou organizovány do dvoudimenzionálních sítí. Jsou roztaženy po celé buňce, ale nejvíce jsou roztaženy v kortexu, těsně pod plasmatickou membránou.

B2) MIKROTUBULY

Jsou tvořeny tubulinem, který je uspořádán do dutých cylindrů. Mají průměr 25nm. Mají schopnost rychle se smršťovat a natahovat. Jsou rigidnější než aktinová vlákna. Jedna část mikrotubulů se dotýká tzv. centra organizujícího mikrotubuly (MTOC), které se nazývá CENTROZOM. Centrozomy jsou polární struktury s + a – koncem, jsou schopné rychlého růstu. Několik stovek mikrotubulů vybíhá z centrosomu tak že mohou dosahovat mnoho mikronů. + konec mikrotubulů se tak dotýká kraje buněk.

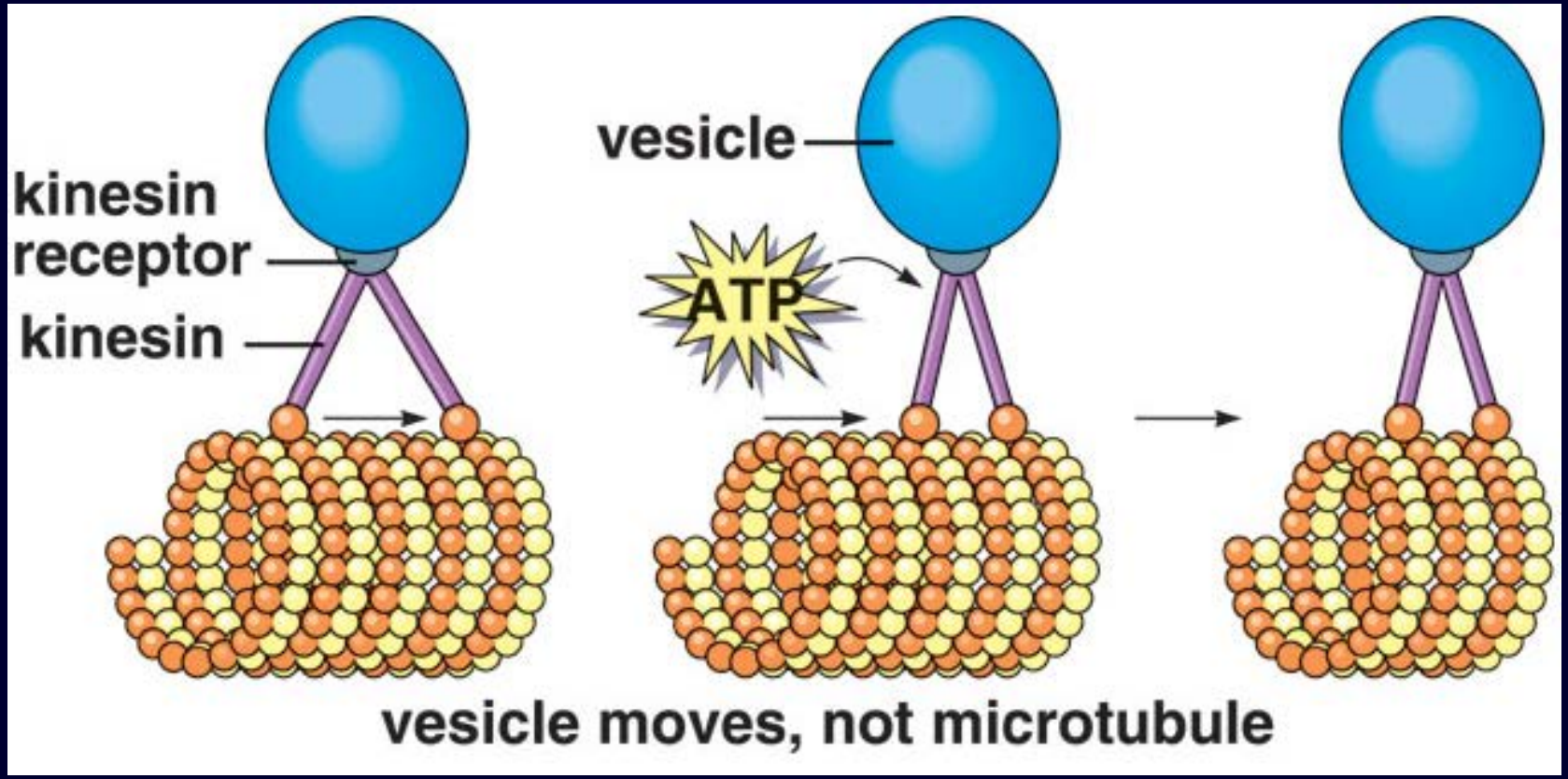
Actin filaments occur in bundles or mesh-like networks.

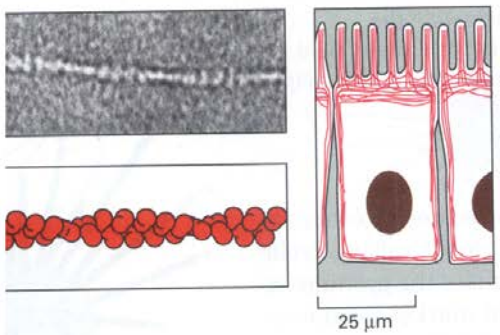
Actin filaments play a structural role in intestinal microvilli and also interact with motor molecules, such as myosin.

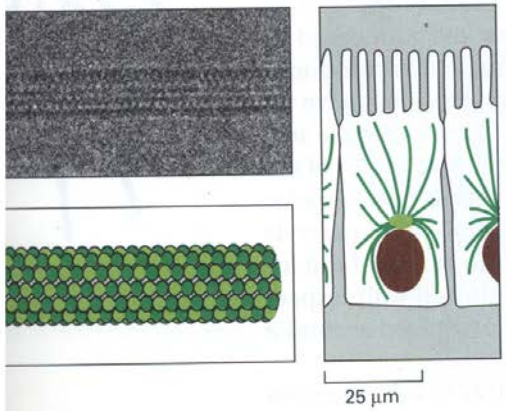


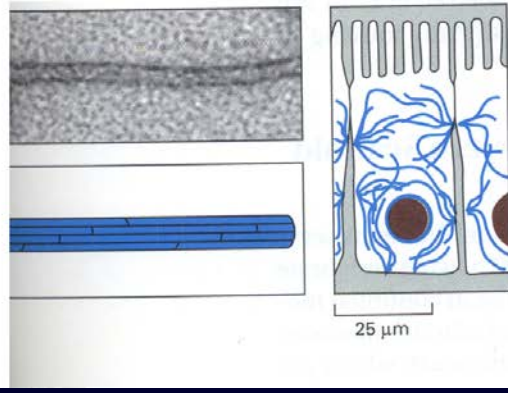
Microtubles are small hollow cylinders made of the globular protein *tubulin*.

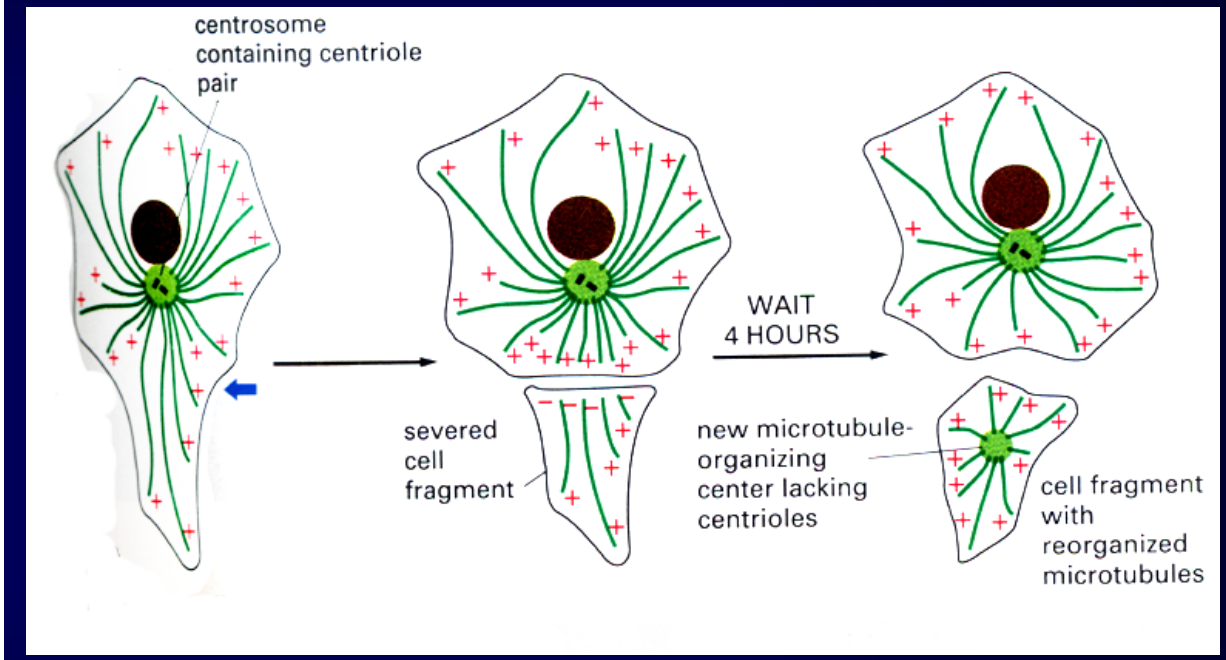
Microtubules help maintain the shape of the cell and act as tracks along which organelles can move.




25 μm


25 μm

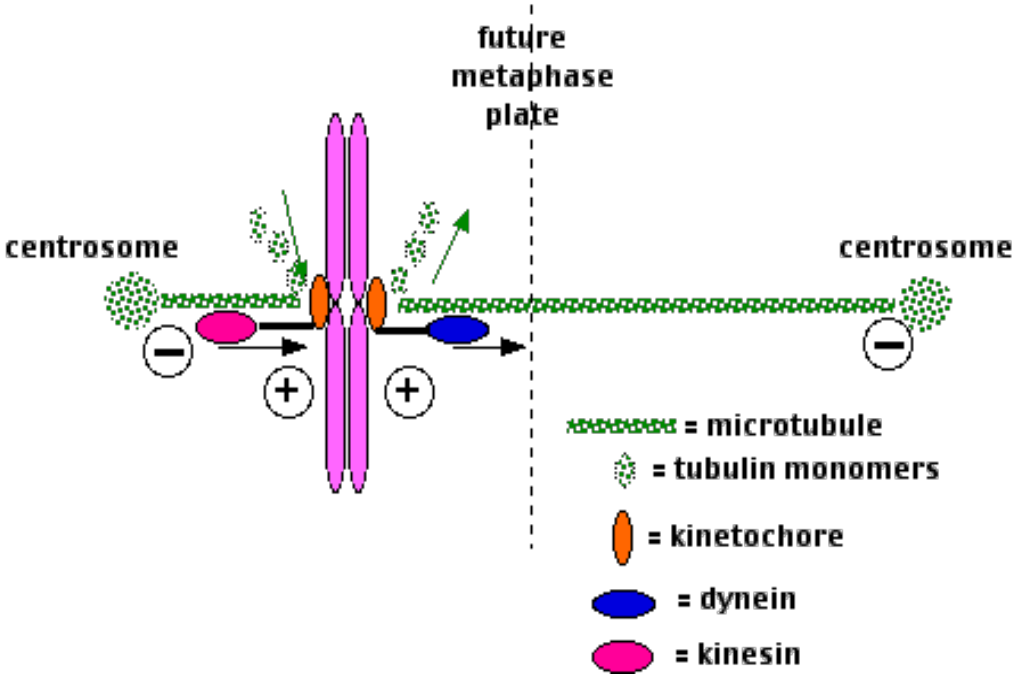

25 μm


centrosome
containing centriole
pair
WAIT
4 HOURS
severed
cell
fragment
new microtubule-
organizing
center lacking
centrioles
cell fragment
with
reorganized
microtubules

Microtubule motors

There are two major groups of microtubule motors:

- **kinesins (most of these move toward the plus end of the microtubules) and**
- **dyneins (which move toward the minus end).**
- **The rapid transport of organelles, like vesicles and mitochondria, along the axons of neurons takes place along microtubules with their plus ends pointed toward the end of the axon. The motors are kinesins.**
- **CENTROSOMES located in the cytoplasm attached to the outside of the nucleus.**
- **Just before mitosis, the centrosome duplicates.**
- **The two centrosomes move apart until they are on opposite sides of the nucleus.**
- **As mitosis proceeds, microtubules grow out from each centrosome with their plus ends growing toward the metaphase plate. These clusters of microtubules are called spindle fibers.**



Bez přítomnosti centrozómu není možné jaderné dělení. Konkrétní funkcí centrozómu je organizování mikrotubulů do prostorové sítě. Během buněčného dělení zajišťuje navázání chromozomů na mikrotubuly. Nachází se v něm asi 100 různých proteinů, které jsou většinou zcela unikátní a neexistují k nim analogické proteiny v jiných částech buňky.

Vyskytuje se v cytoplazmě těsně u jaderné membrány (karyolema), která je v jeho okolí mírně prohloubena, a před mitózou se duplikuje. Dva centrozómy tvoří póly dělícího (mitotického) vřeténka, jehož vytváření a orientaci zajišťují. V živočišných buňkách (na rozdíl od rostlinných) jsou centrozómy pozorovatelné po celou dobu mitózy.

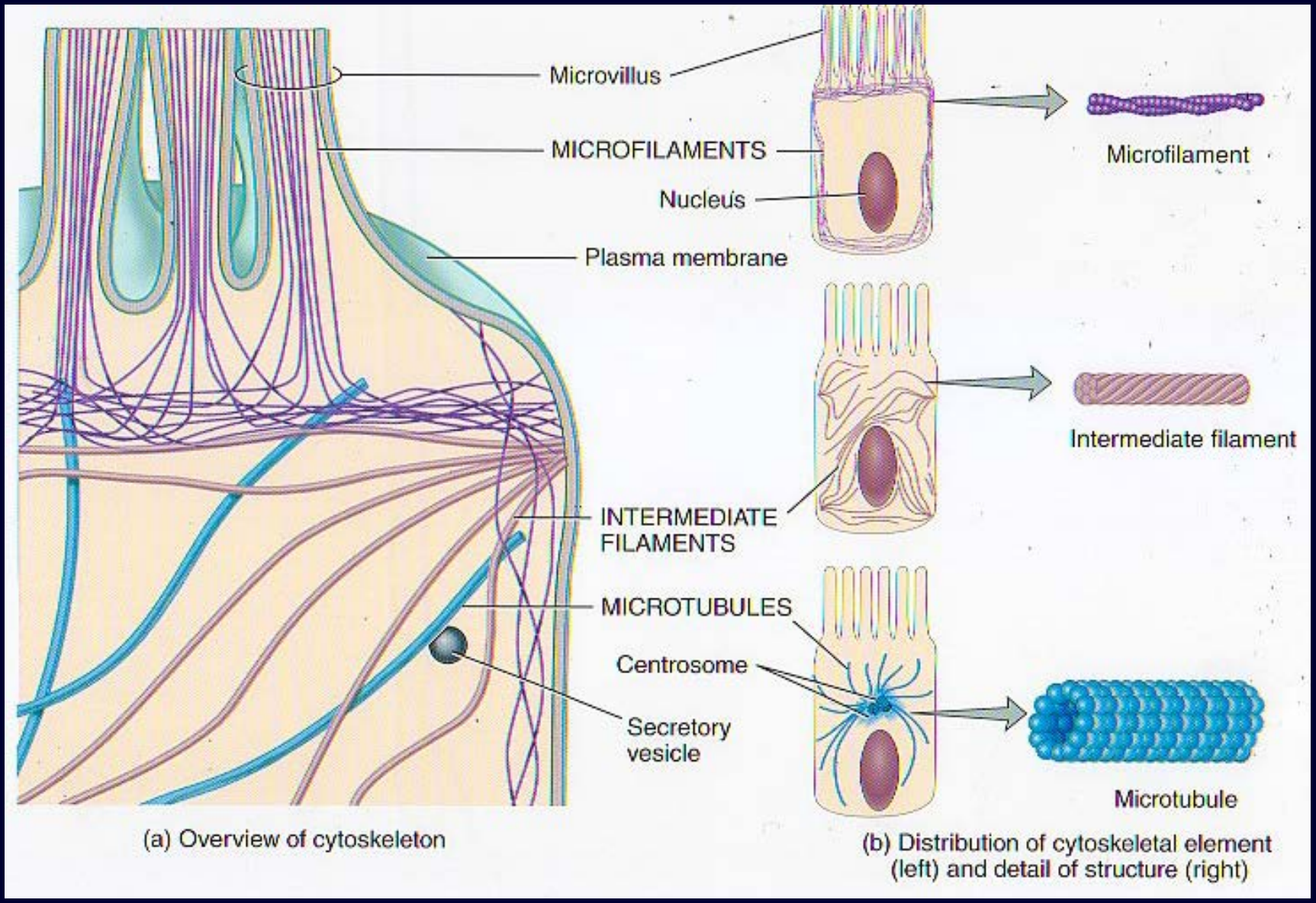
Centriola je válcovitá buněčná organela, která se nachází ve většině eukaryotických buněk vyjma vyšších rostlin a hub. Buňky obvykle obsahují dvě centrioly. Pár centriol, které jsou vzájemně kolmo orientované, vytváří centrozóm. Před mitózou vyrostou nové centrioly z obou stávajících, čímž vzniknou dva páry centriol. Dceřinné buňky získají vždy jednu mateřskou a jednu dceřinnou centriolu.

3) INTERMEDIÁLNÍ FILAMENTA

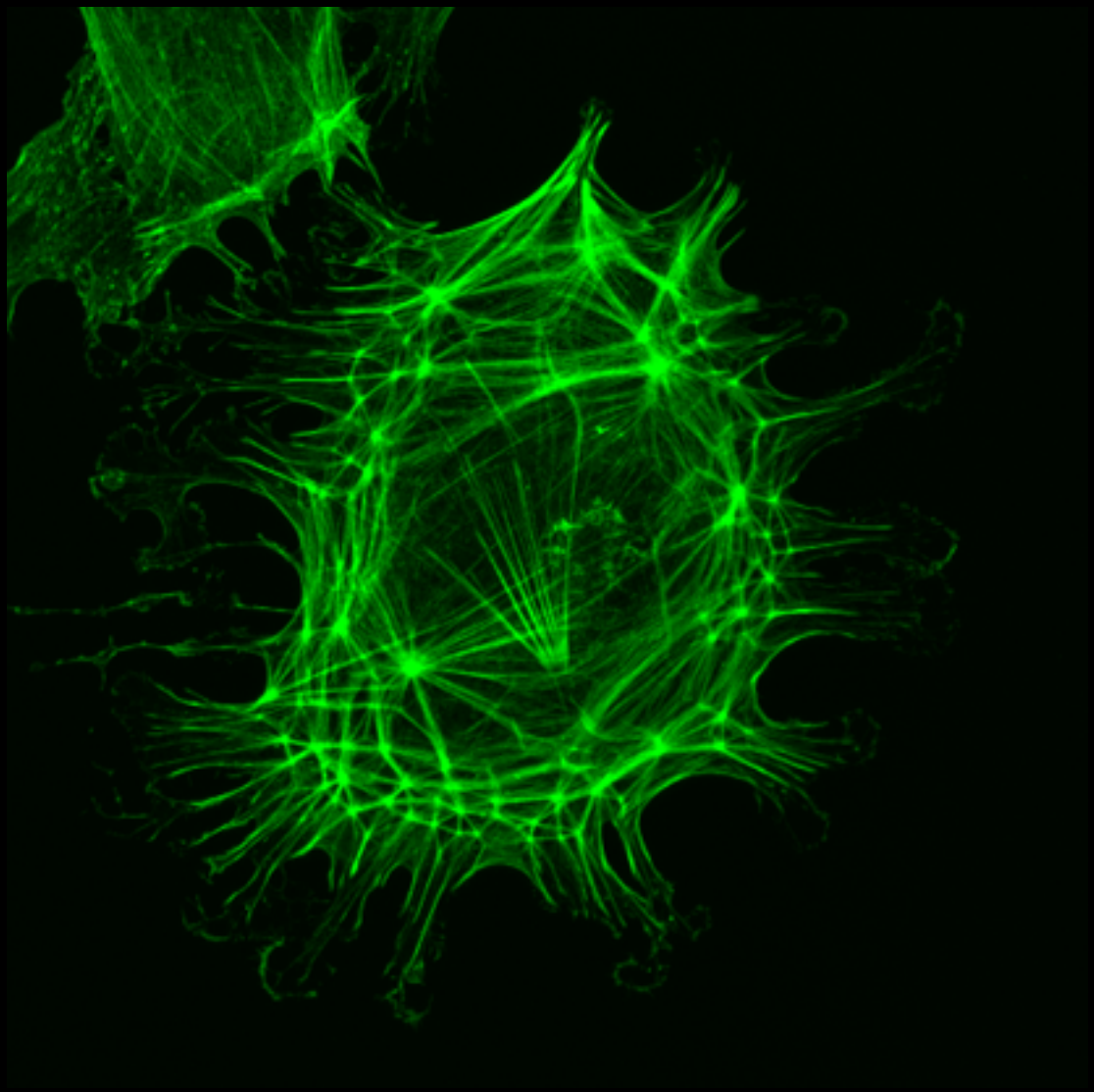
provazcovitá vlákna o průměru 10 nm, jsou tvořena intermediálními proteiny. Jedním typem je jaderná lamina, přiléhající k jaderné membráně. Jiný typ těchto filament je natažený zkrz cytoplasmu a tak poskytuje buňkám mechanistické rozpínání, to umožňuje zprostředkování mezibuněčných komunikací.

There are several types of intermediate filament, each constructed from one or more proteins characteristic of it.

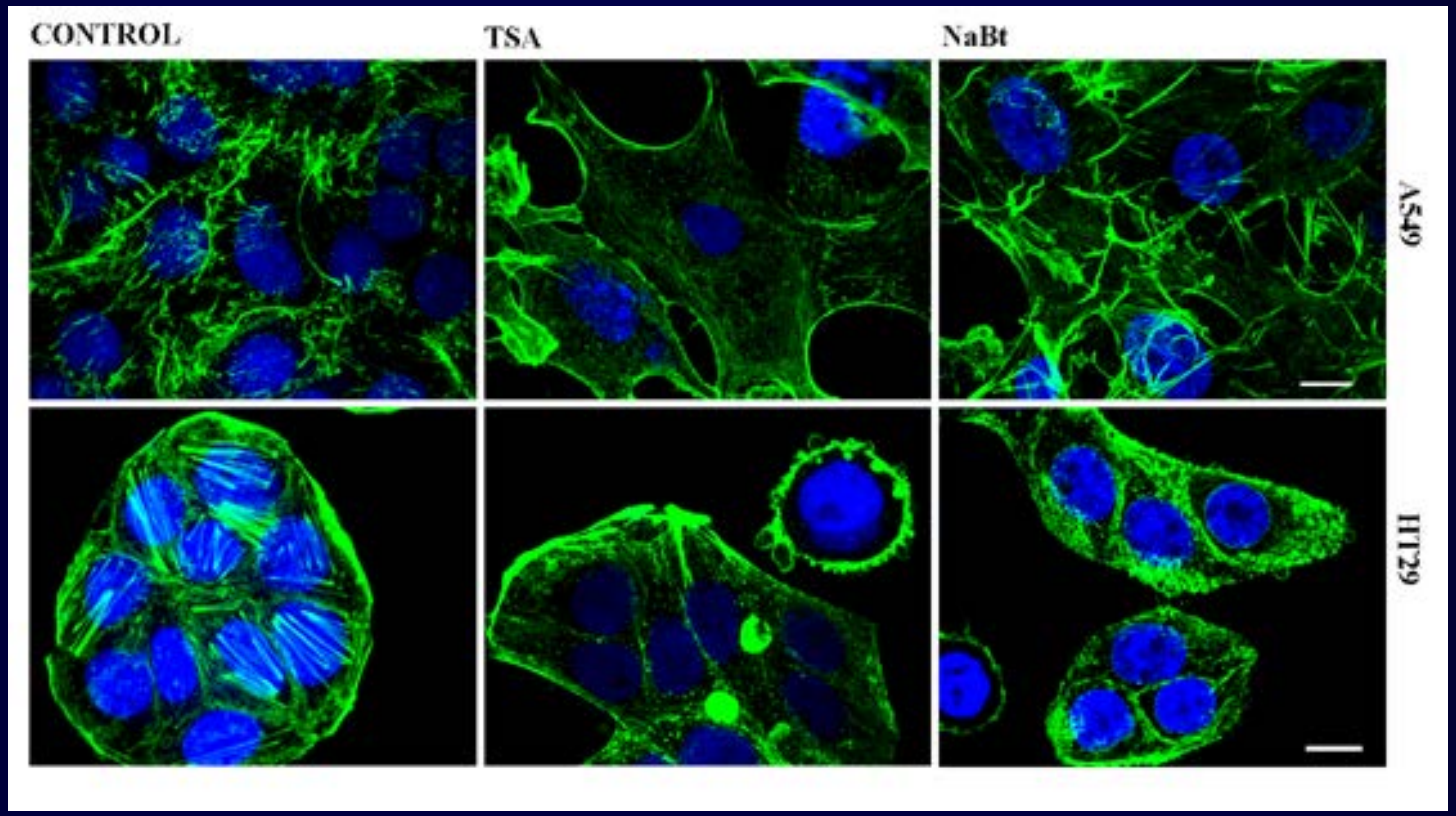
- **keratins are found in epithelial cells and also form hair and nails;**
- **nuclear lamins form a meshwork that stabilizes the inner membrane of the nuclear envelope;**
- **neurofilaments strengthen the long axons of neurons;**
- **vimentins provide mechanical strength to muscle (and other) cells.**


Microvillus
MICROFILAMENTS
Nucleus
Plasma membrane
INTERMEDIATE FILAMENTS
MICROTUBULES
Centrosome
Secretory vesicle
Microfilament
Intermediate filament
Microtubule
(a) Overview of cytoskeleton
(b) Distribution of cytoskeletal element (left) and detail of structure (right)

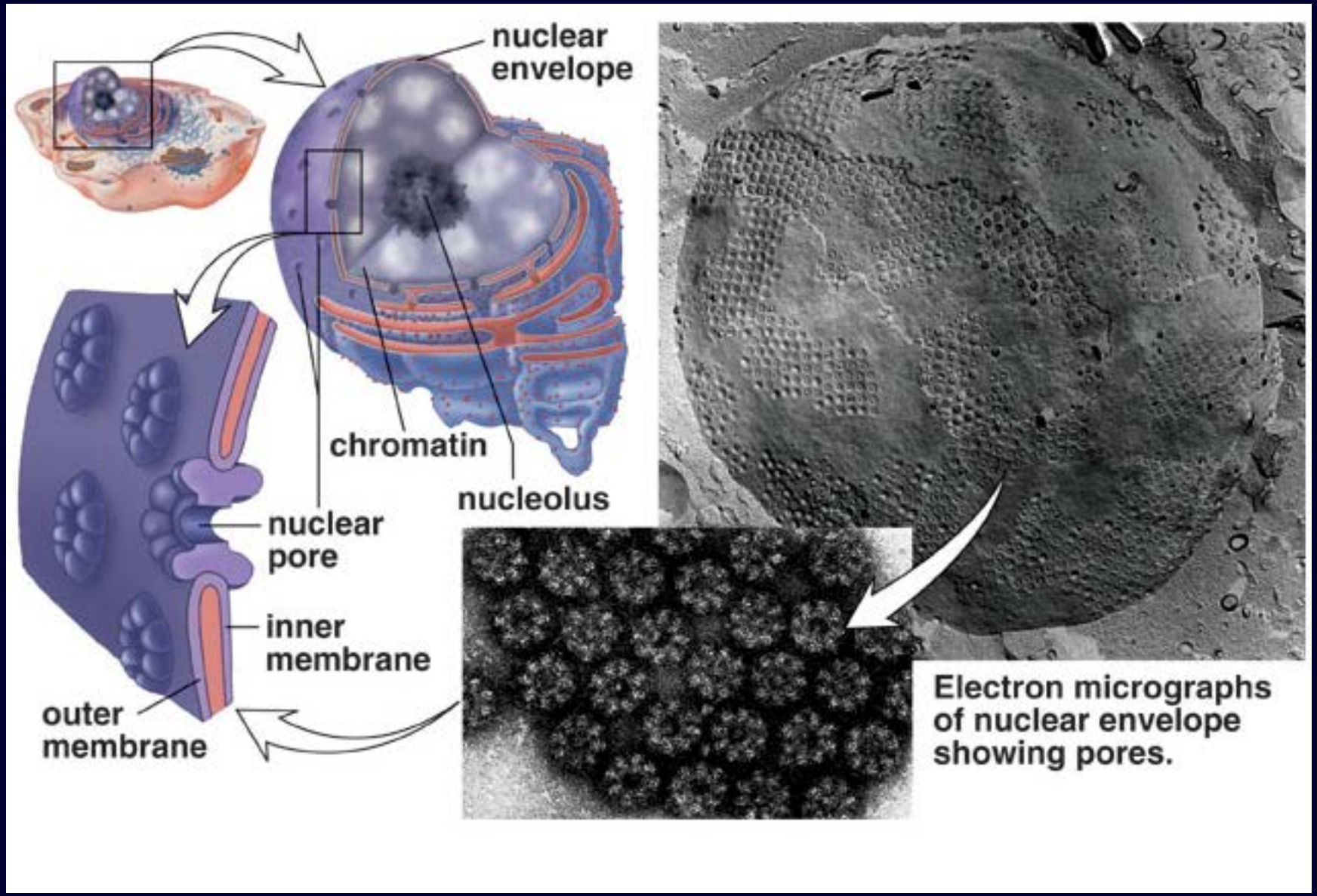
F-actin in bovine articular chondrocytes (Actin forms microfilaments)

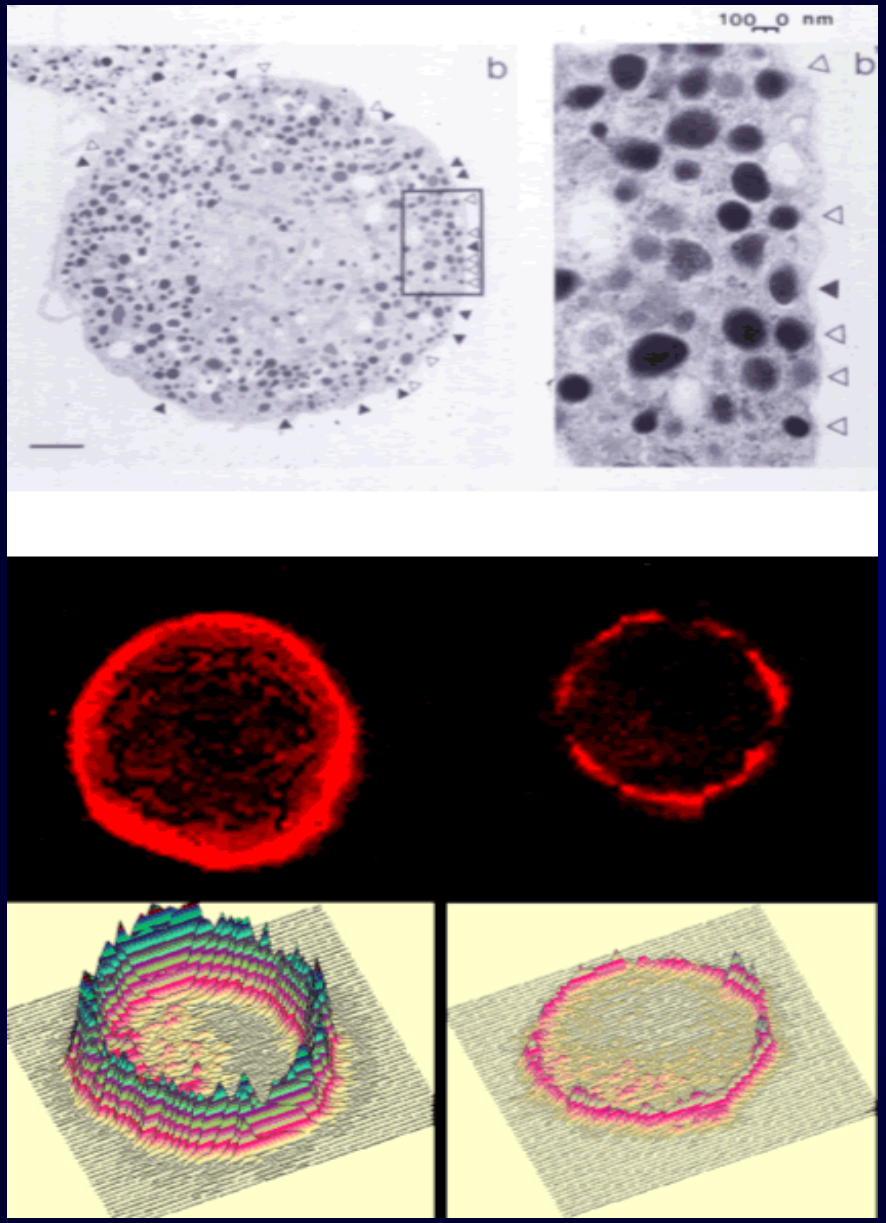


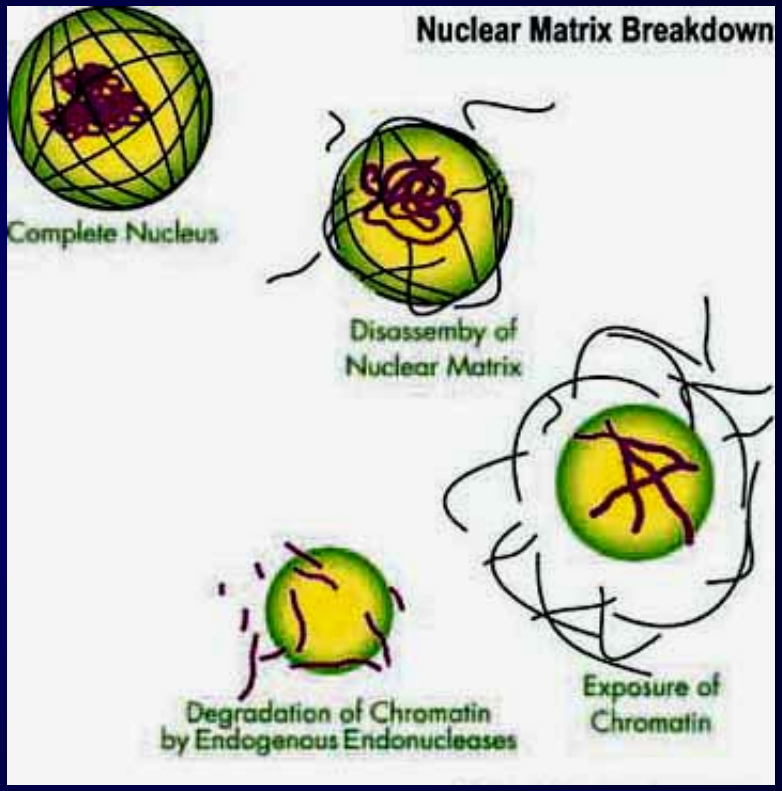
F-actin



The nucleus and the nuclear envelope




100 nm
b
b

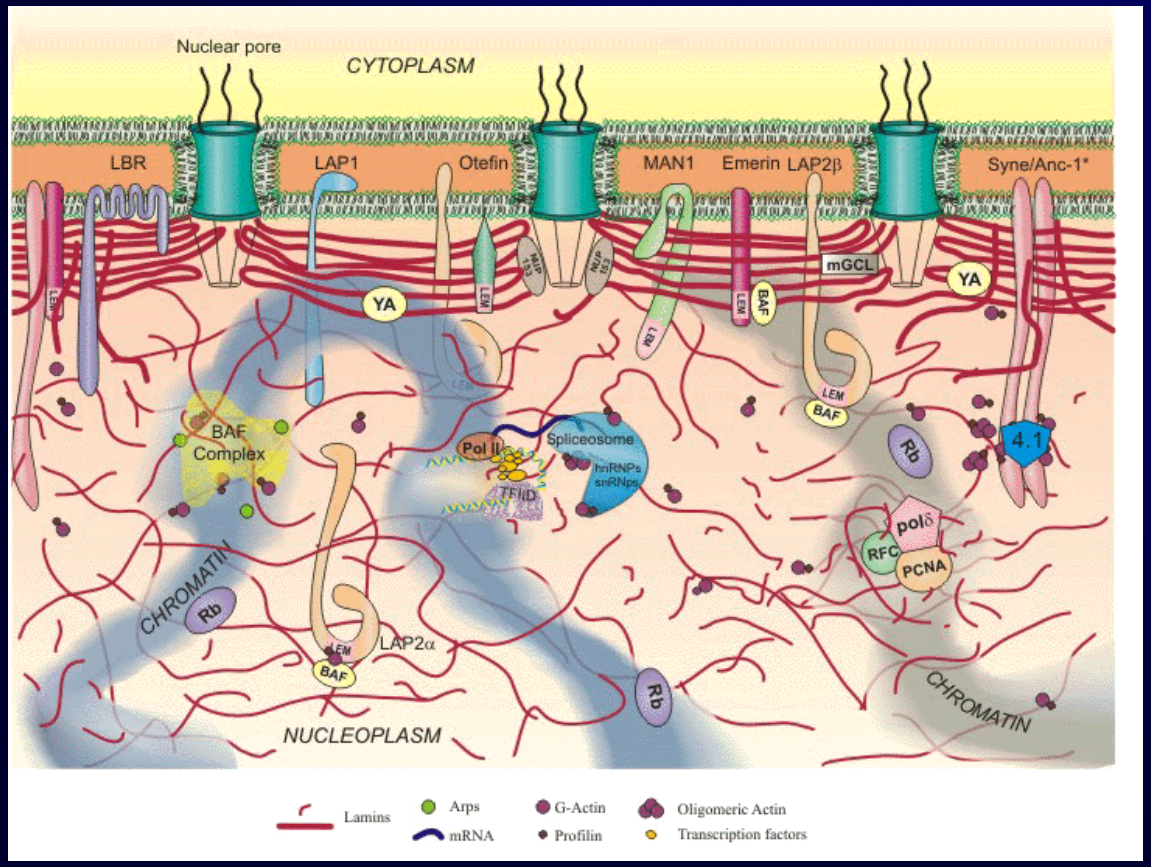

Nuclear Matrix Breakdown
Complete Nucleus
Disassemby of
Nuclear Matrix
Exposure of
Chromatin
Degradation of Chromatin
by Endogenous Endonucleases

Laminy a další proteiny jsou složky jaderné membrány. Byl podán důkaz, přítomnosti laminů i v nukleoplasmě. Proteiny typu laminů jsou zodpovědny za jadernou architekturu, z důvodů jejich kontaktu se strukturami chromatinu. Tyto vzájemné vztahy se mění během buněčného cyklu a diferenciace.

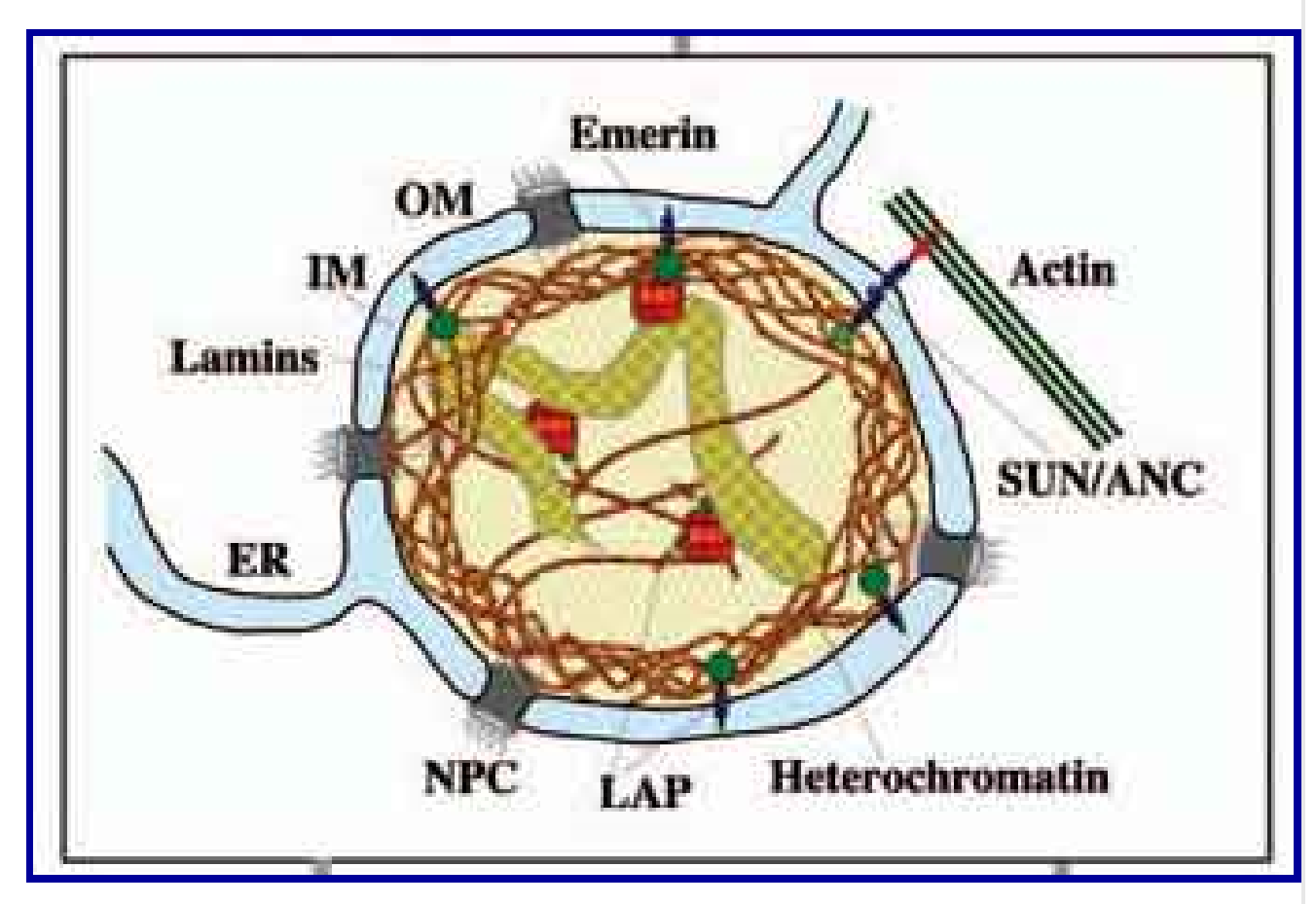
Změny v expresi laminů (A, B1, B2) korelují se změna v buněčné diferenciaci, s vývojově-závislými změnami a se změnami v expresních profilech, které probíhají během zmíněných procesů.

Laminy jsou hlavní komponety i jaderné matrix, polymerizované laminy tvoří hlavní kostru buněčného jádra. Mutace v laminech, a dalších proteinech jaderné matrix jako je emerin, nurim, způsobují další nestability chromatinu a fragilnost buněčných jader. Specifické mutace laminů A/C a emerinů jsou zodpovědné za vznik Emery-Dreifuss svalové dystrofie.

Laminy mají rovněž schopnost se vázat na proteiny asociované s chromatinem. laminy jsou považovány i za regulátory transkripce. LA a LC se vážou na pRb protein (centrantrální regulator buněčného cyklu, inhibuje geny důležité pro vstup do S-fáze, tím že aktivuje HDAC komplexy, které jsou zodpovědné za inaktivitu struktur chromatinu), který se váže na LAP2 alfa. Vyblokování LA i LC inhibuje aktivitu pol II a mění formování některých transkripčních faktorů.

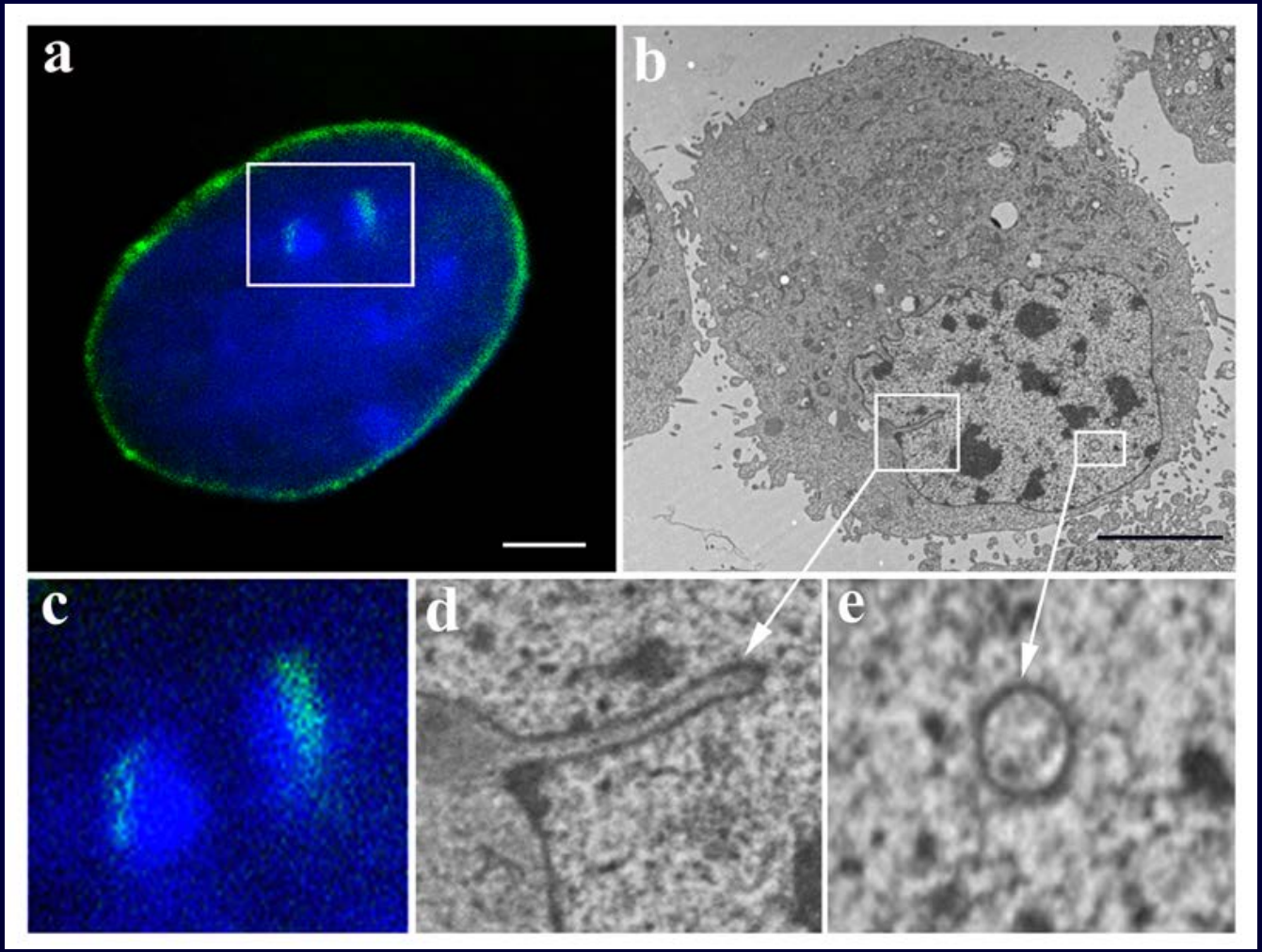


Z tohotofaktu je zřejmé, že proteiny jaderné slupky ovlivňují strukturu chromatinu.

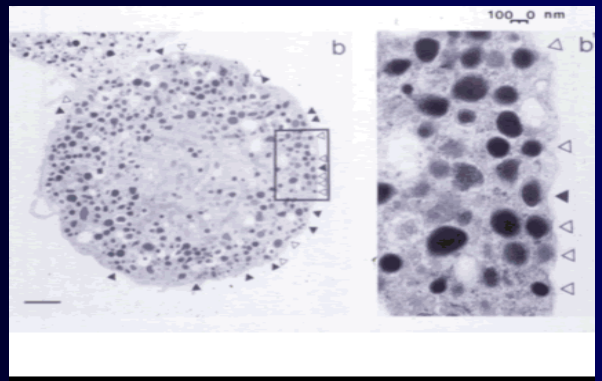


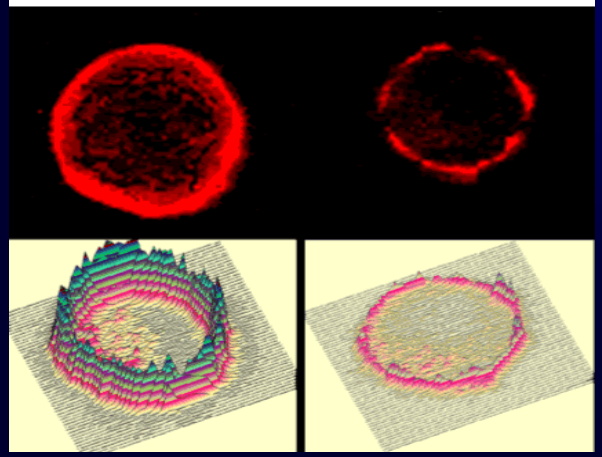
Laminy- intermediální filamenta (IF), komunikují s dalšími IF, jsou součástí tzv. nuclear lamina a tudíž i matrix, jsou zodpovědné za organizaci chromatinu.

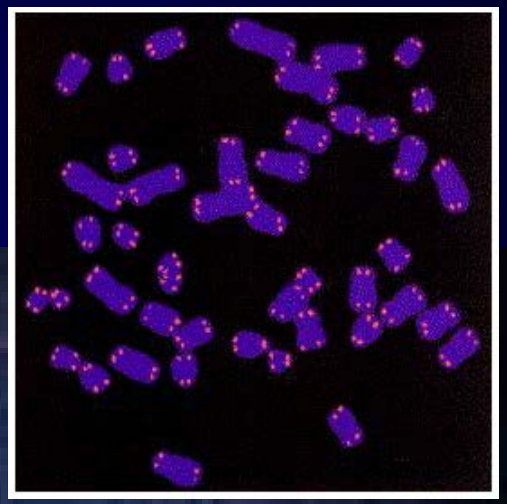
Lamin B (green) and invagination of nuclear membrane

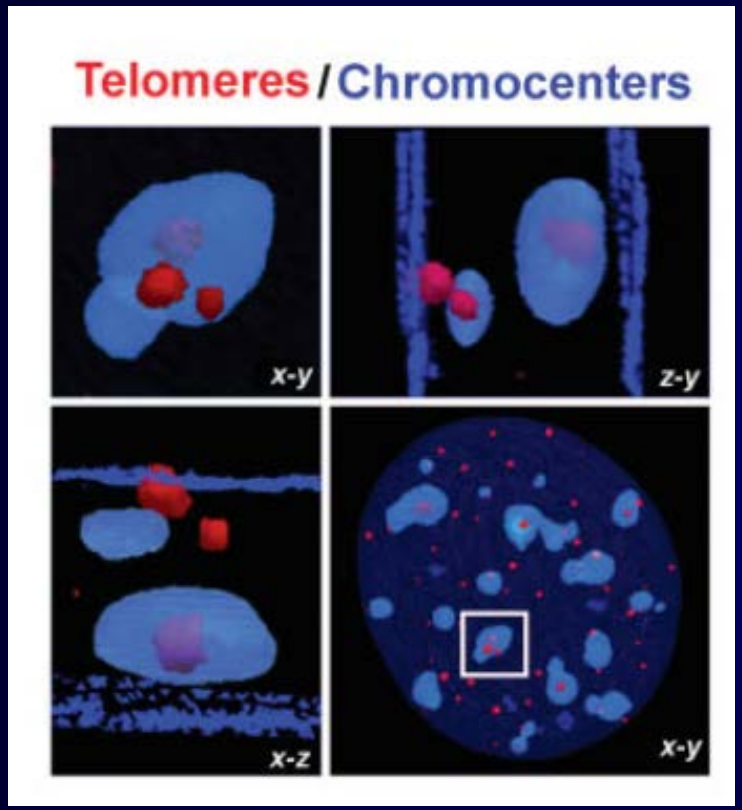


Laminy A/C se rovněž těsně dotýkají heterochromatických struktur jako jsou telomery a centromery, které jsou často umístěny v těsné blízkosti jaderné periferie

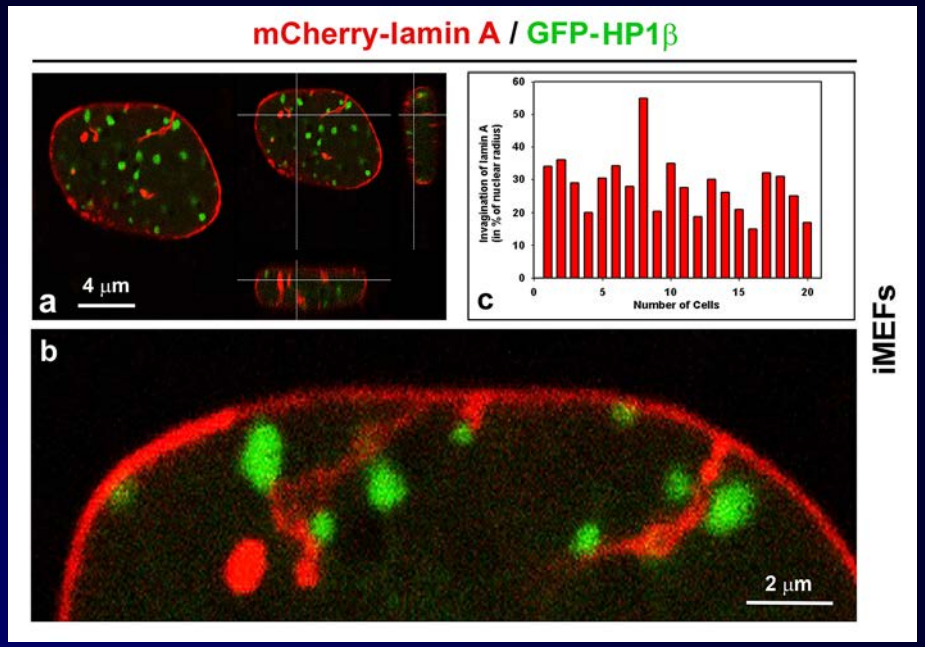


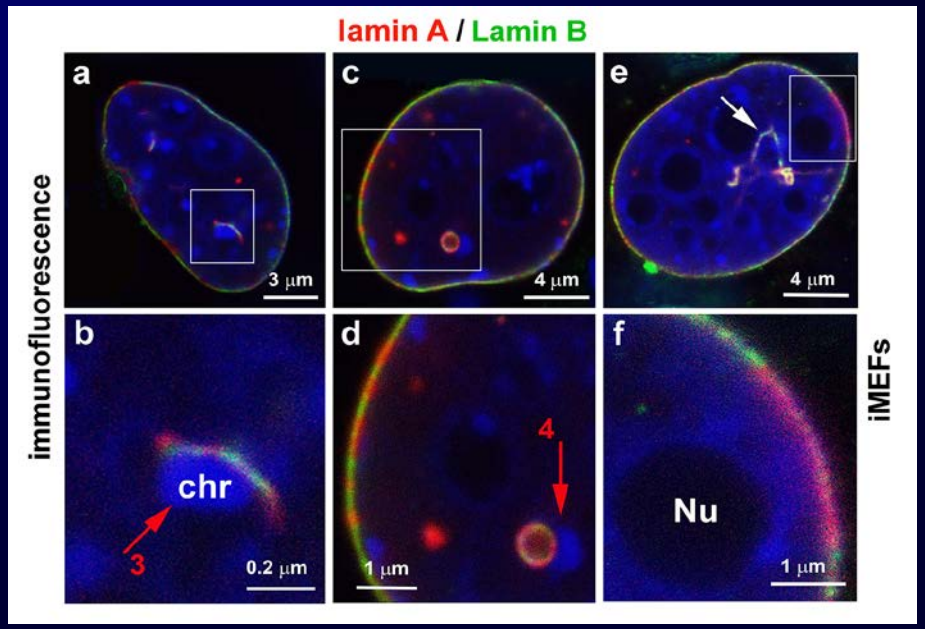




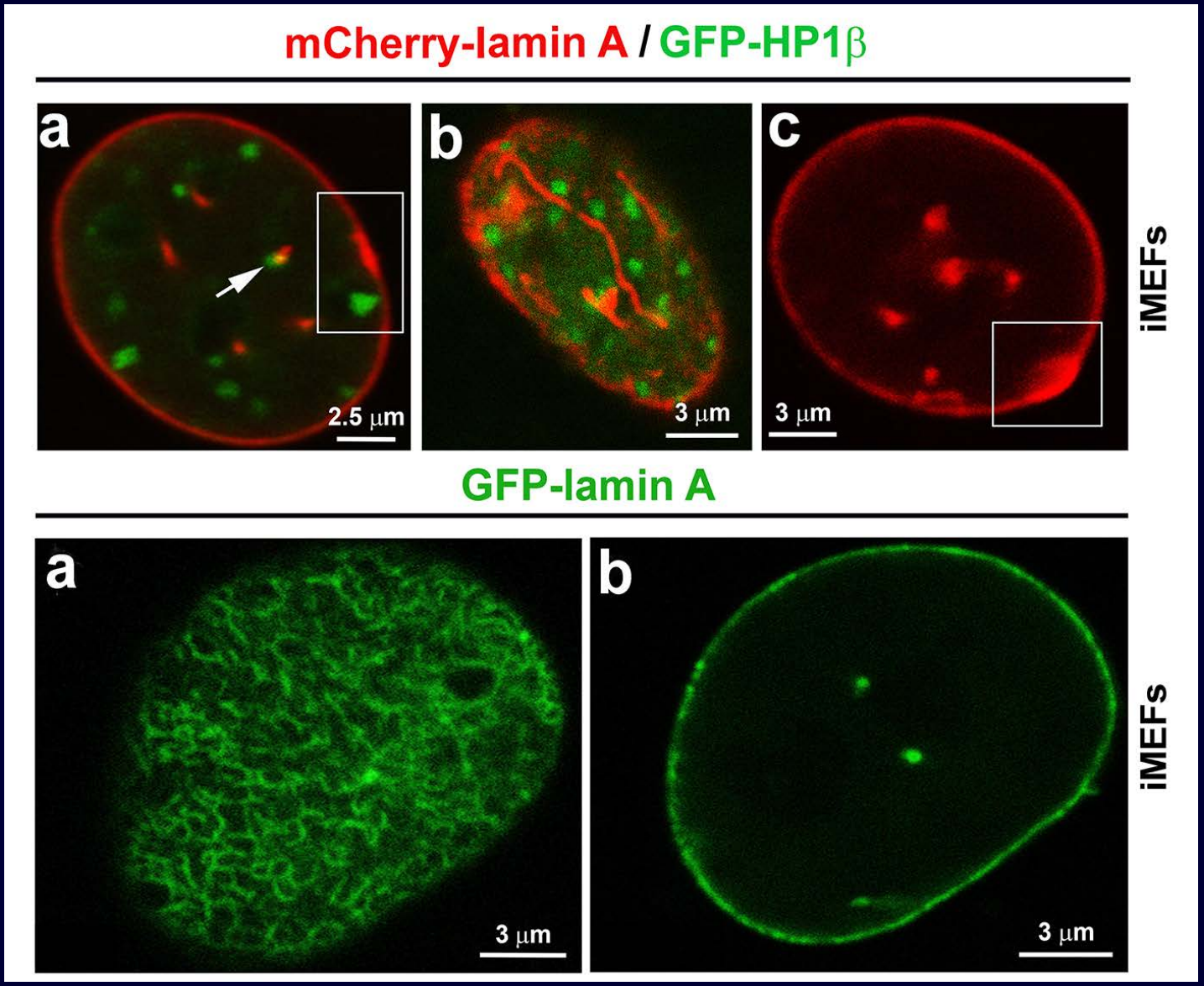


Uhlířová et al. (2010)

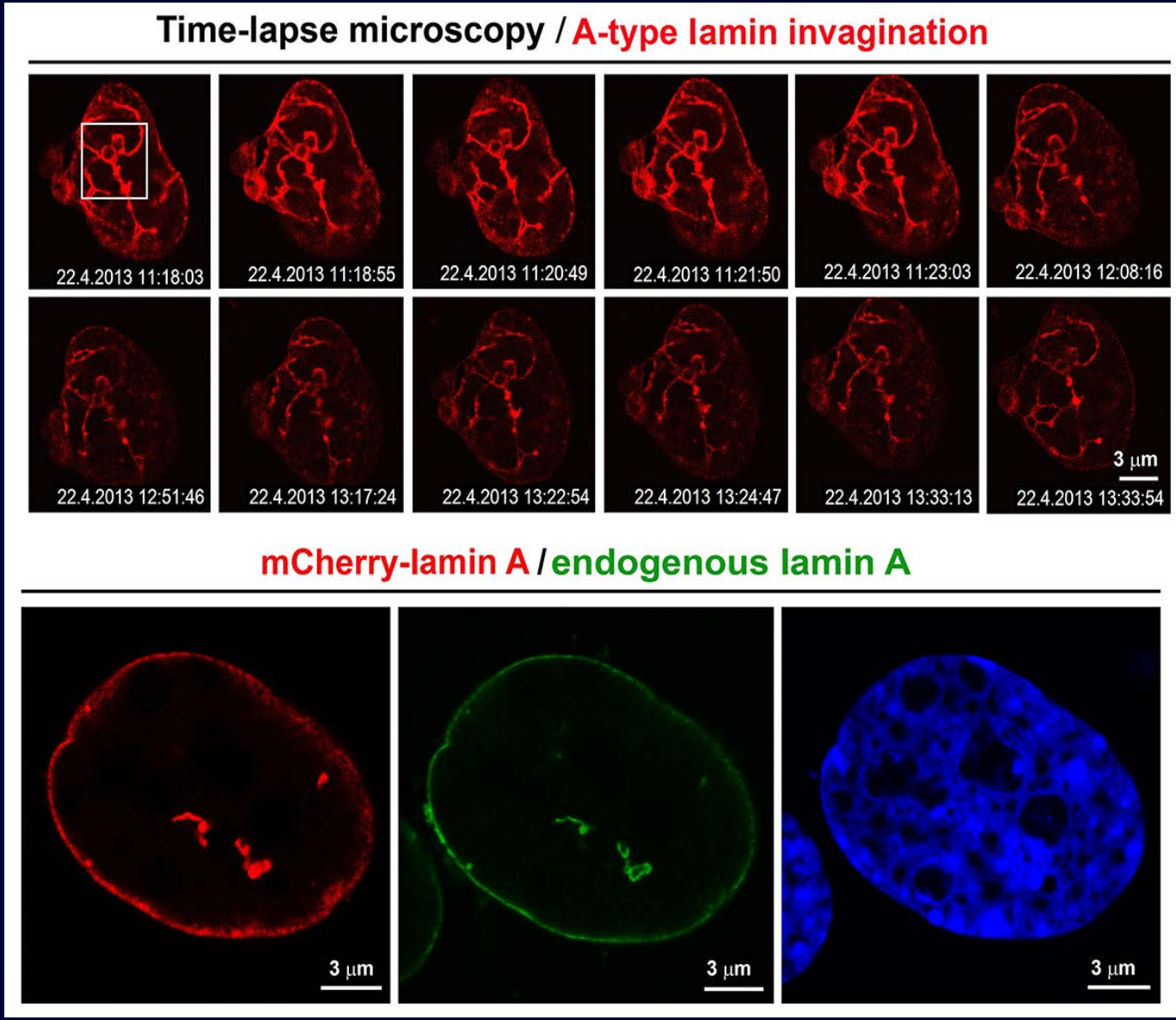




Legartová et al. (2013)



Legartová et al. (2013)



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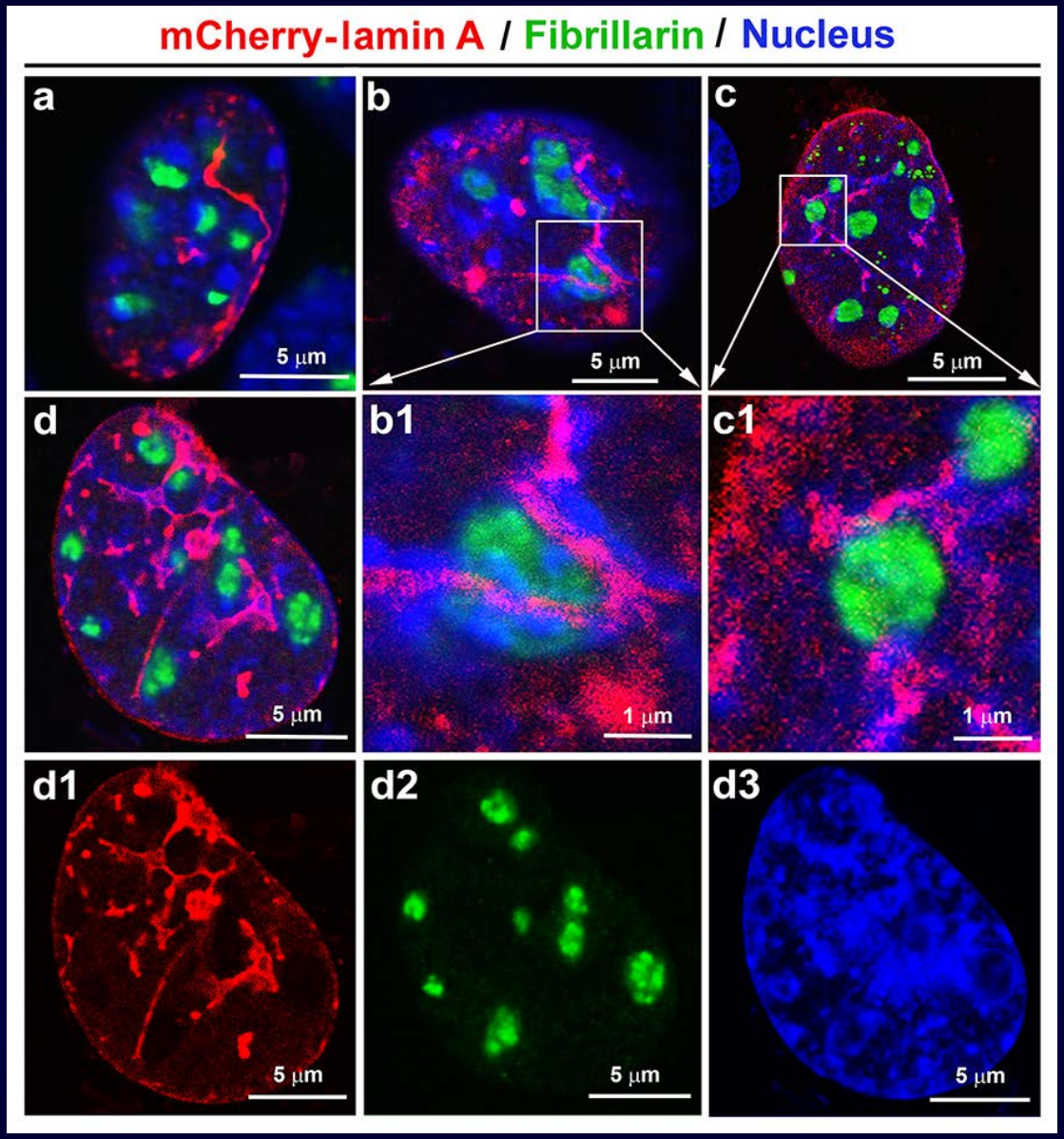
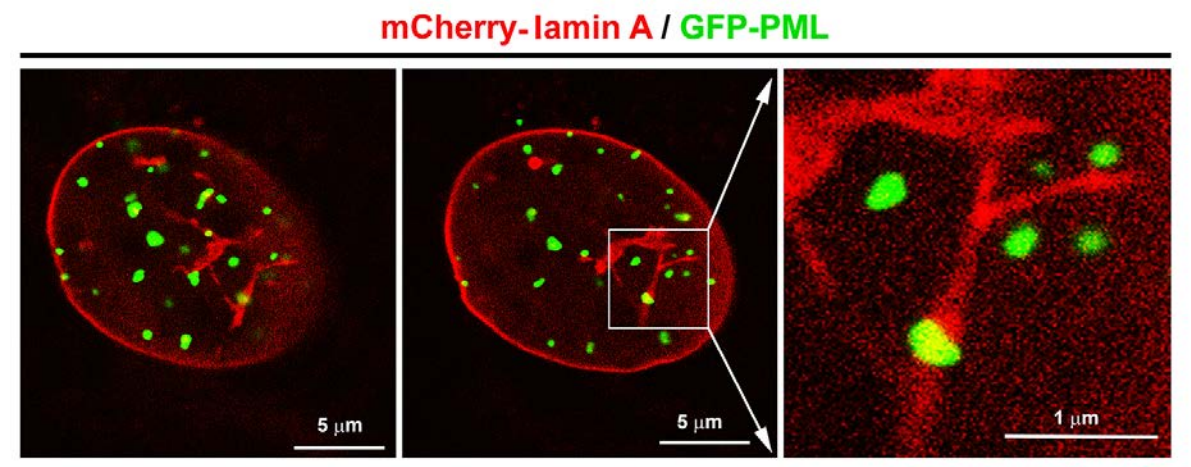
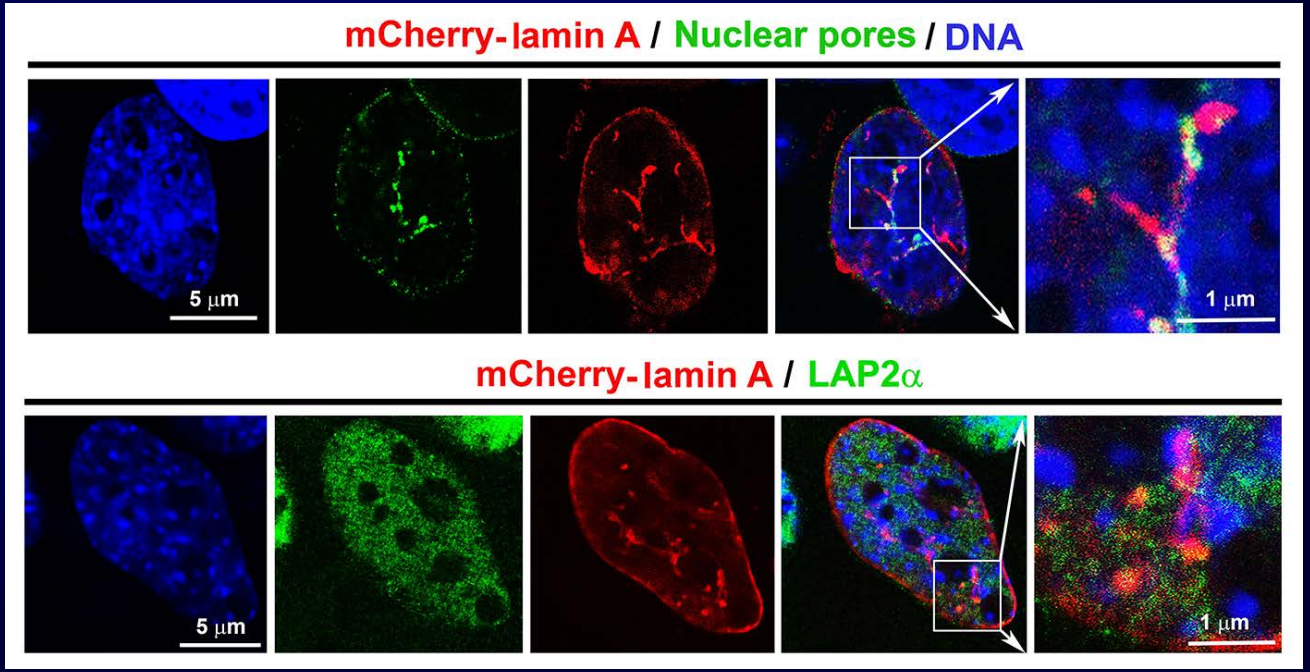
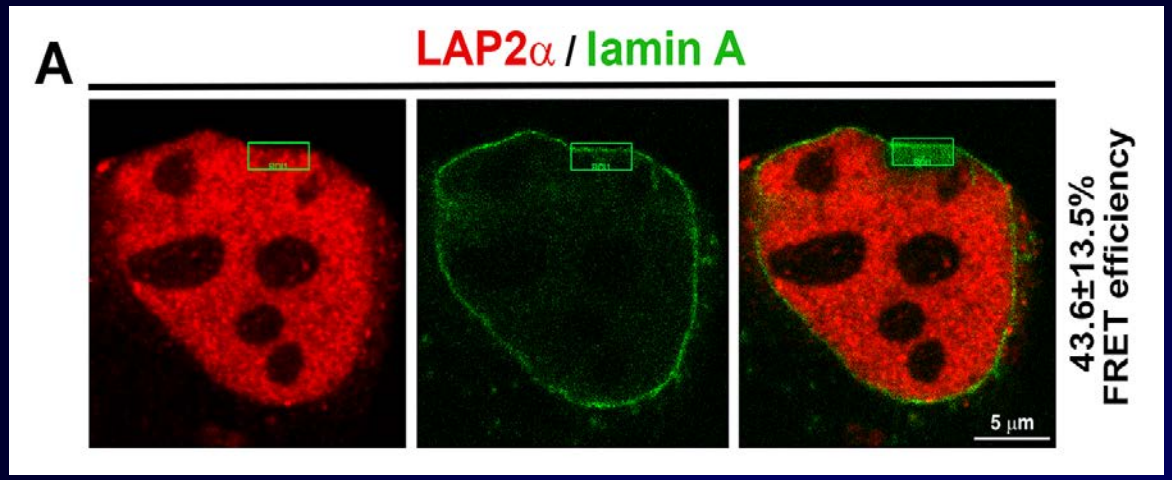


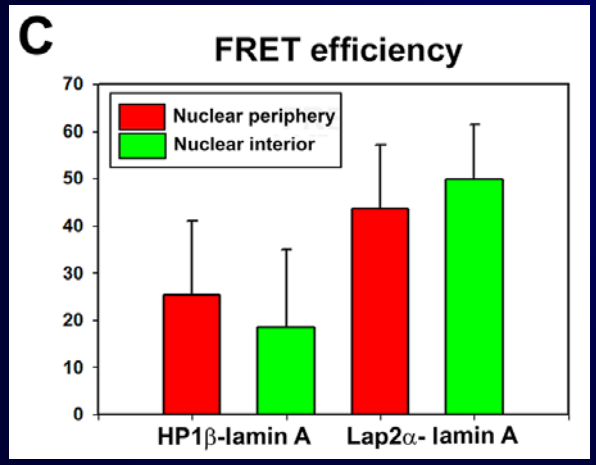
Legartová et al. (2013)

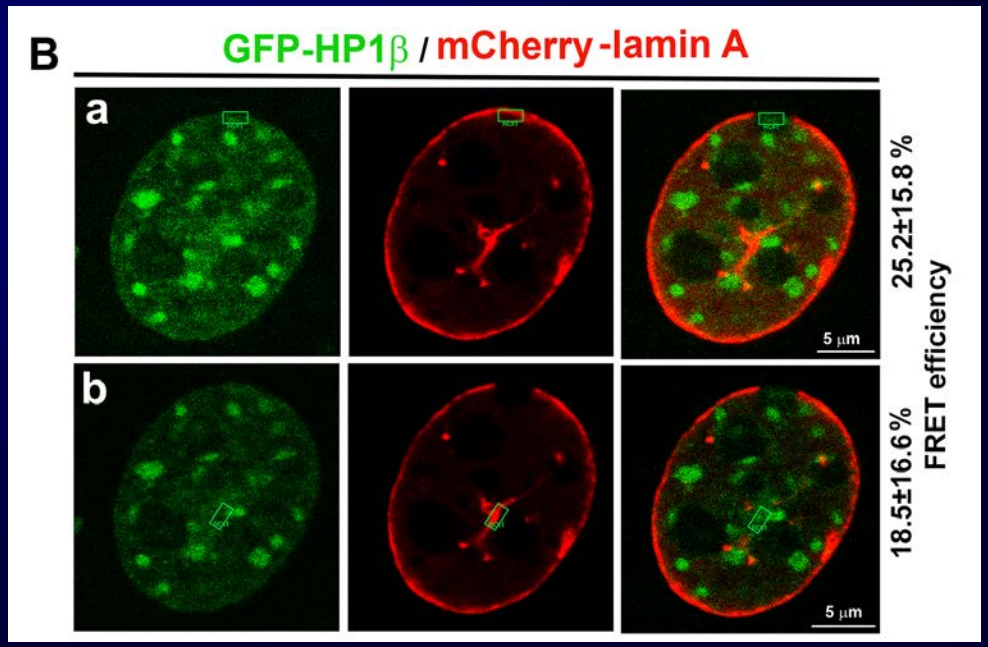




Legartová et al. (2013)







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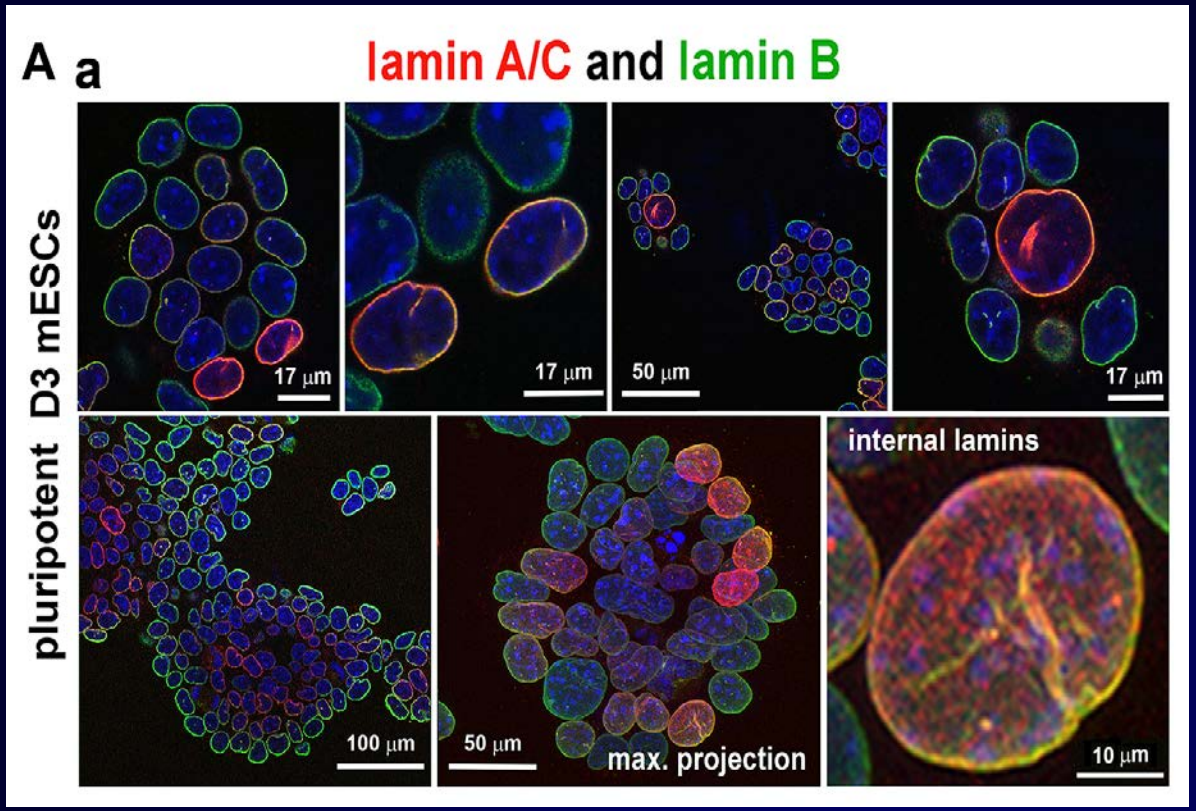

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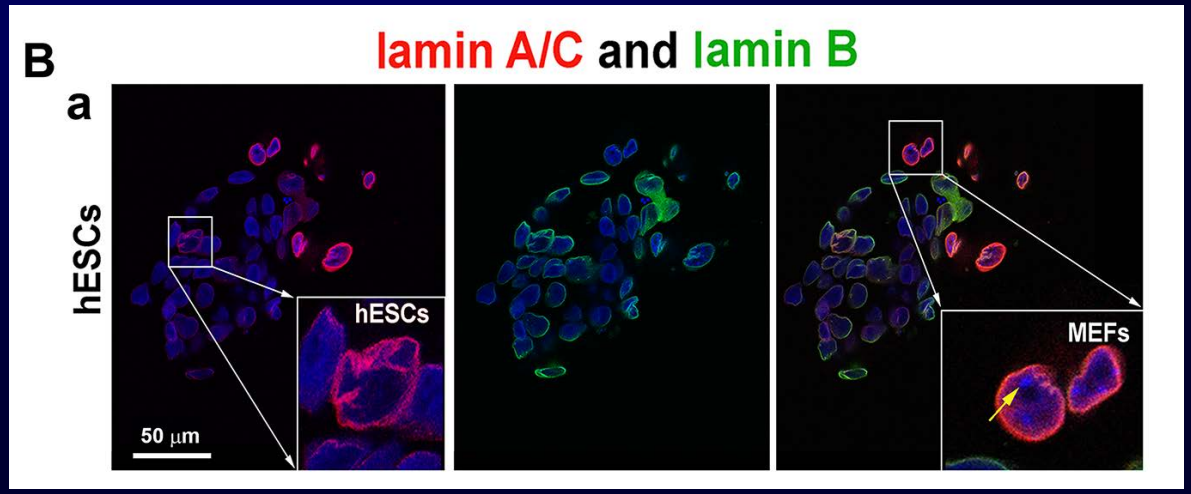
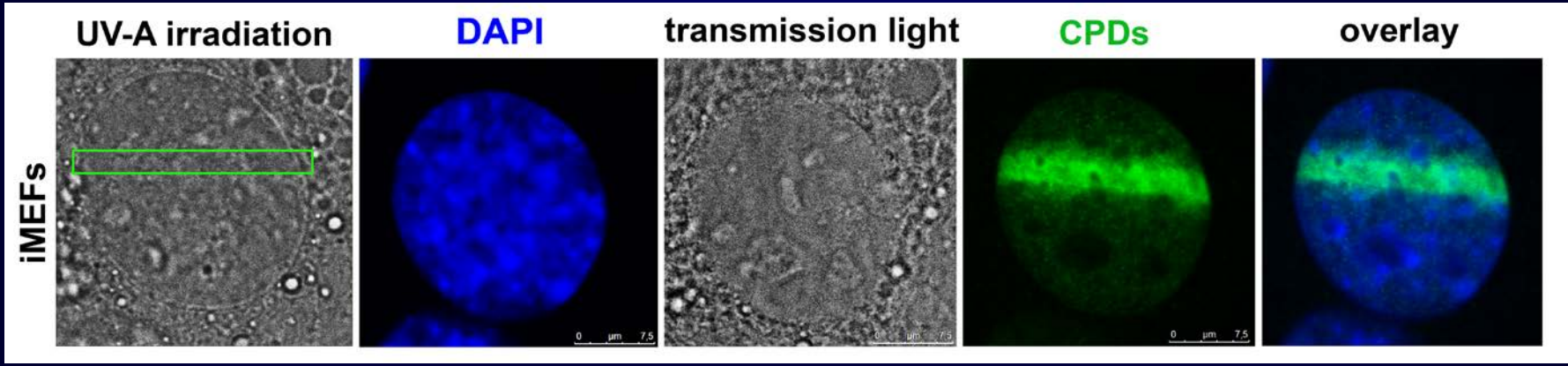
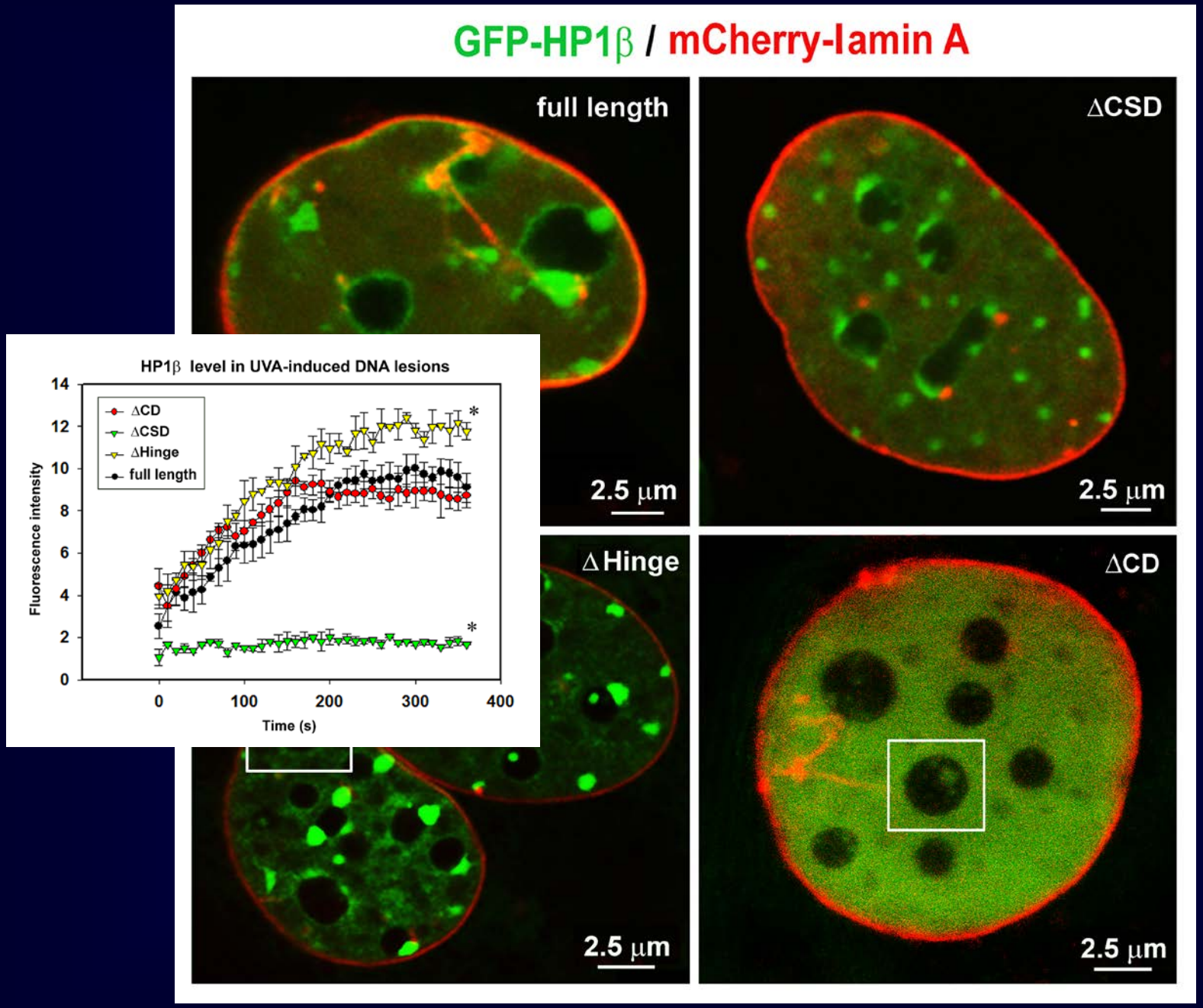


DNA repair studies

Eva Bártová, Soňa Legartová, Petra Sehnalová, Lenka Stixová, Gabriela Šustáčková, Stanislav Kozubek

Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i. Brno

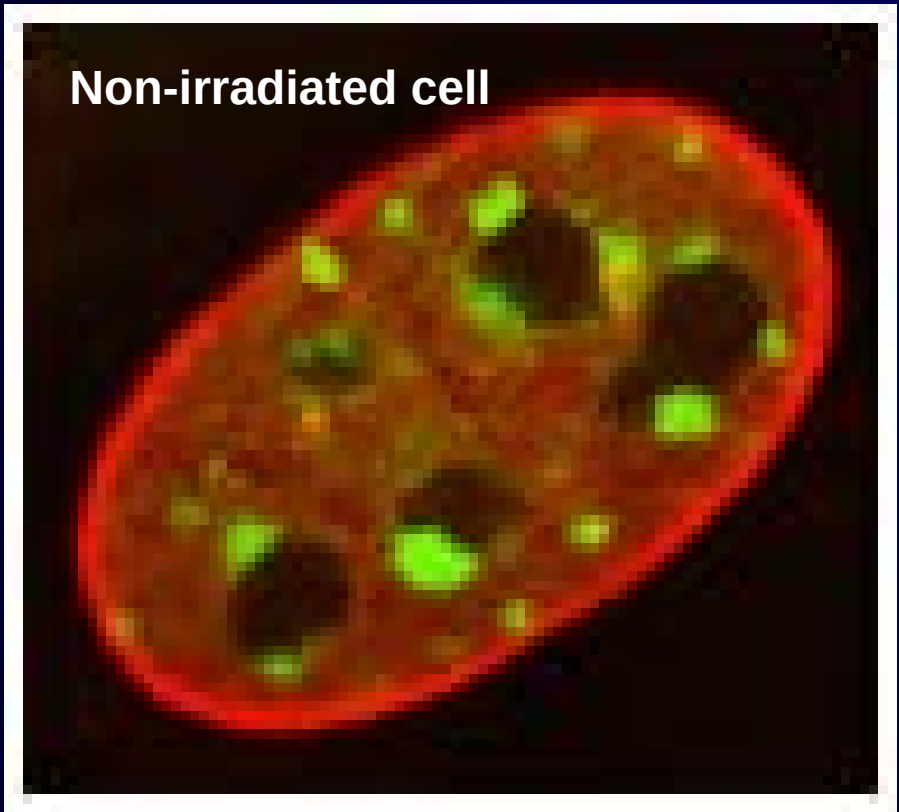


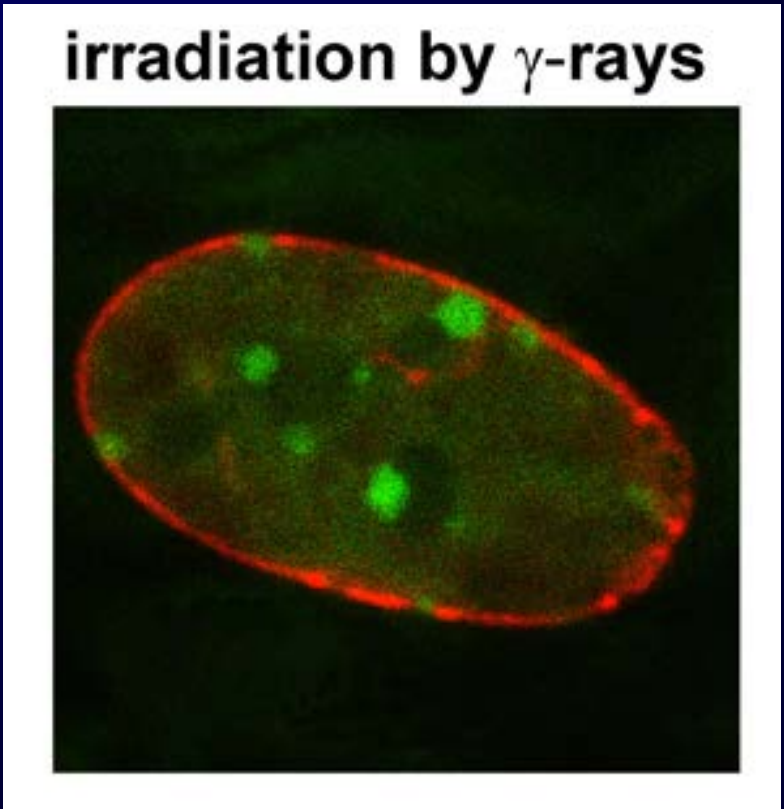


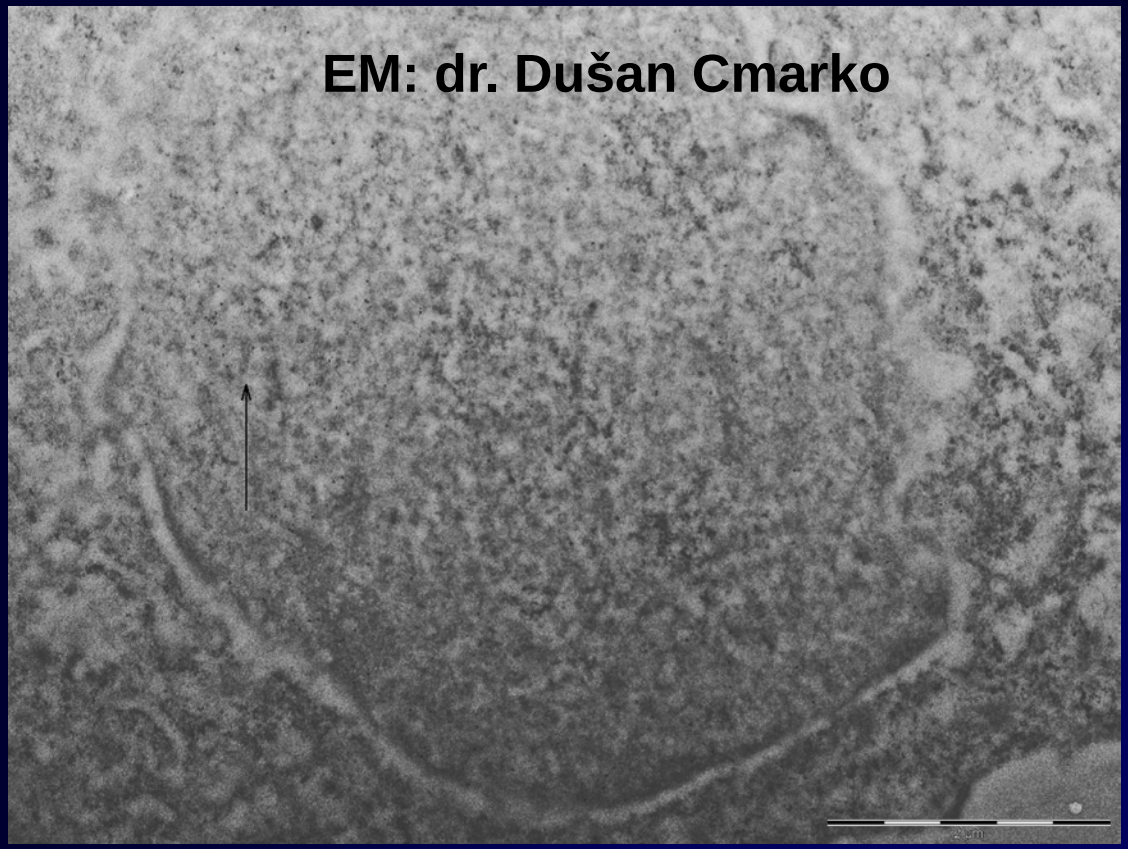
Experiments of Petra Sehnalová

HP1β after γ-irradiation

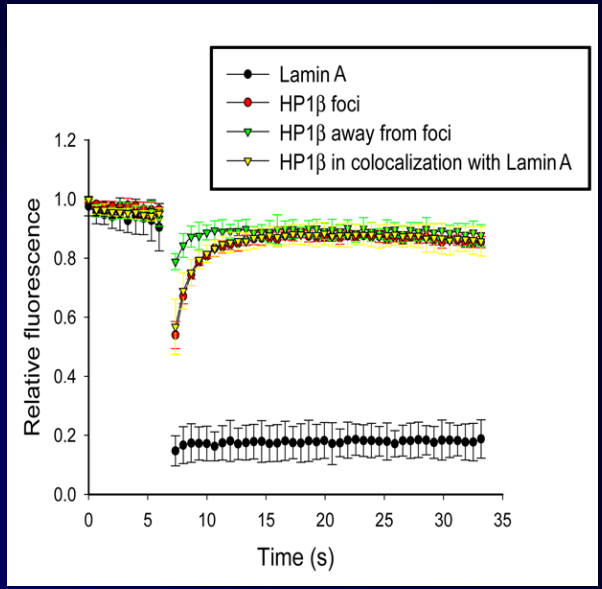
HP1β and Lamin A



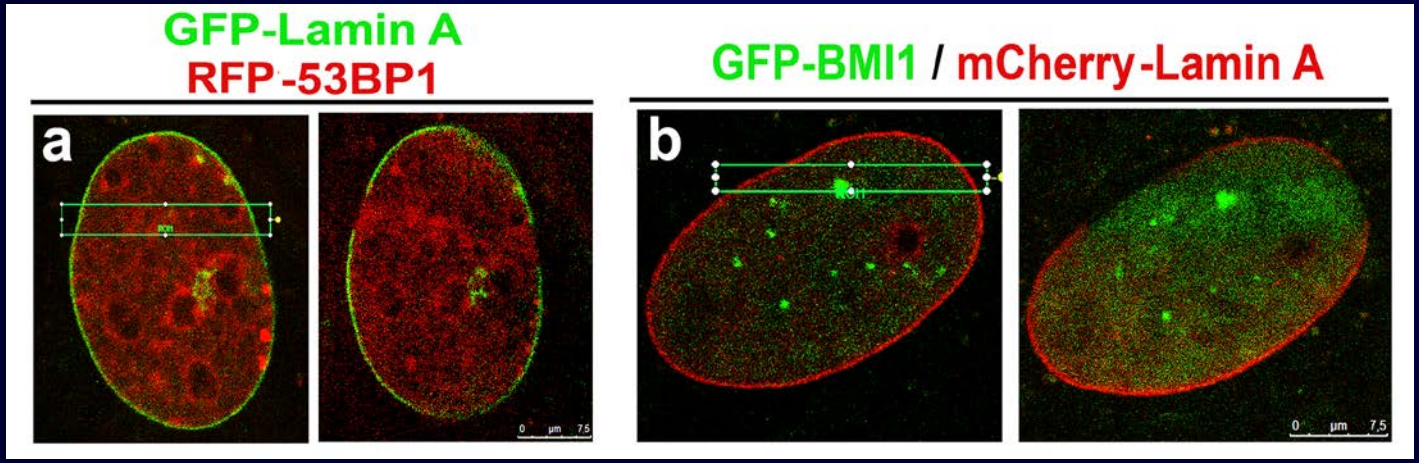


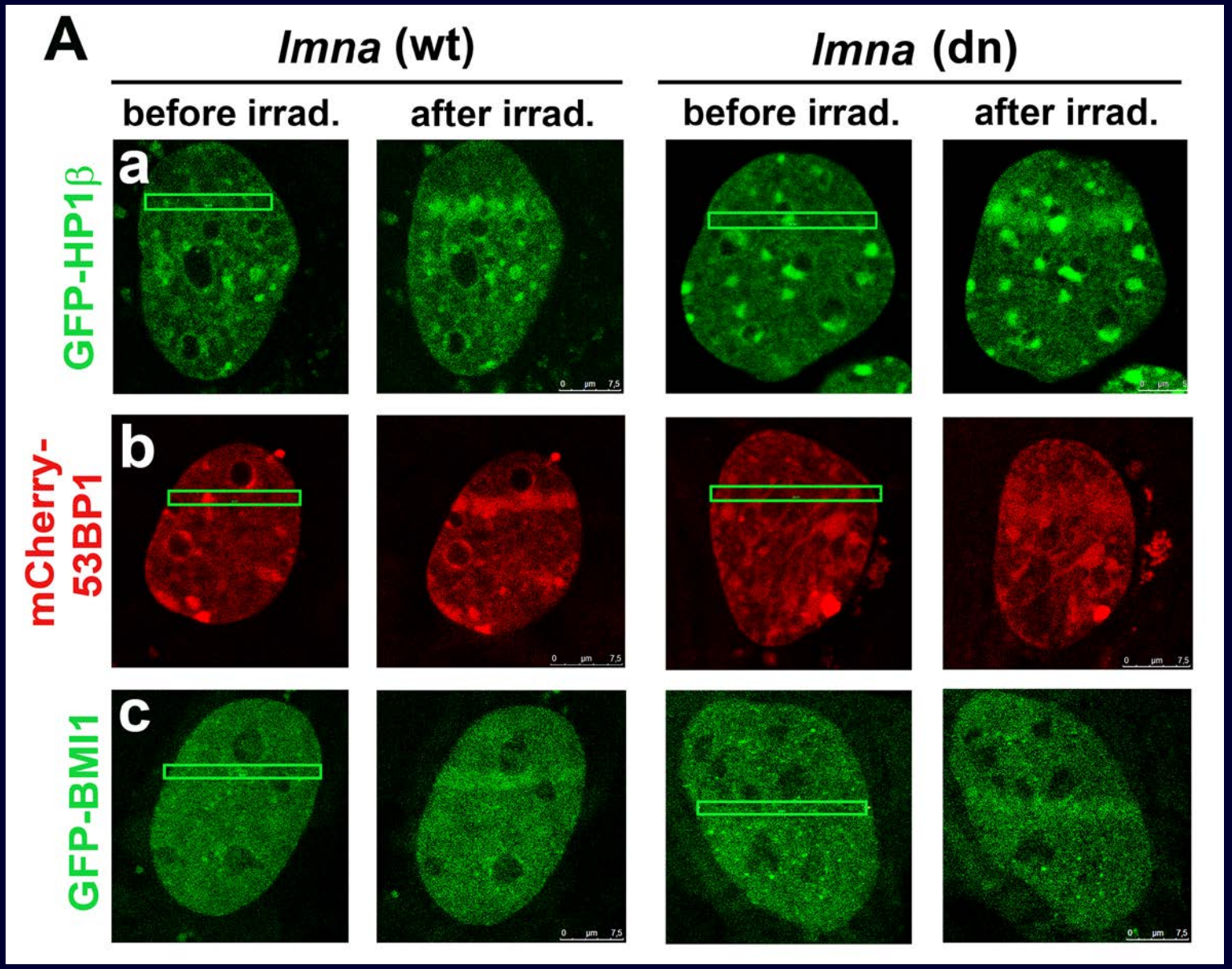


FRAP data

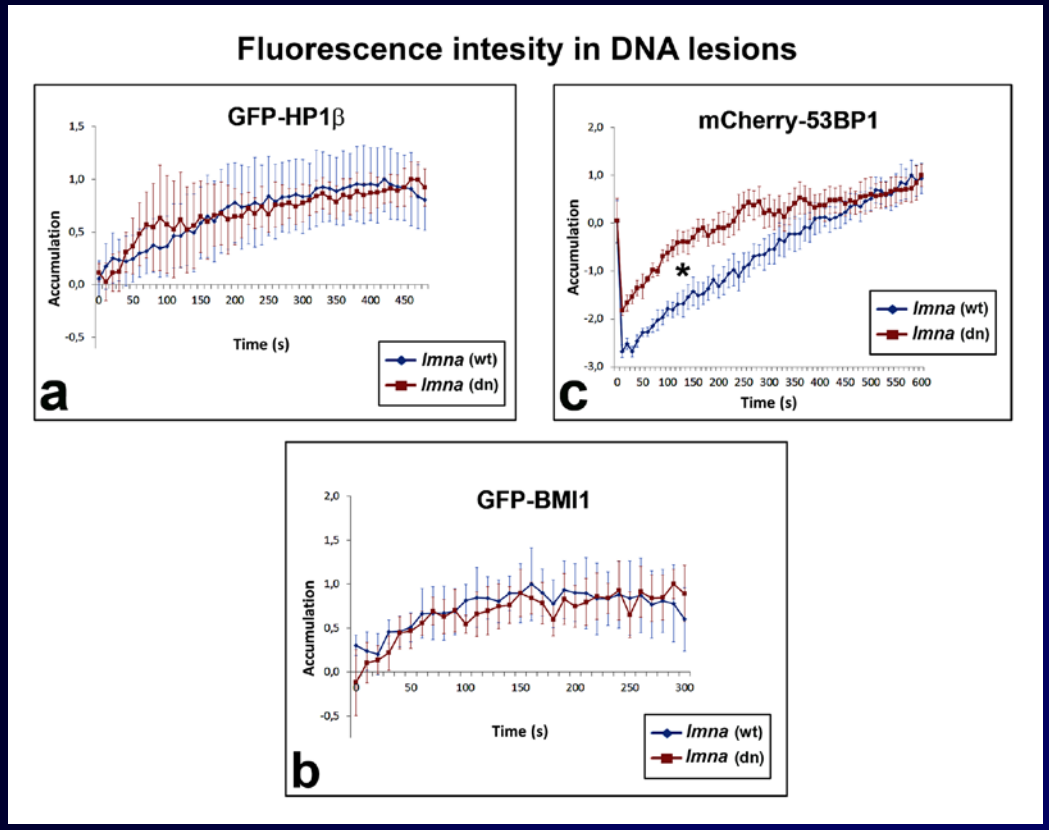


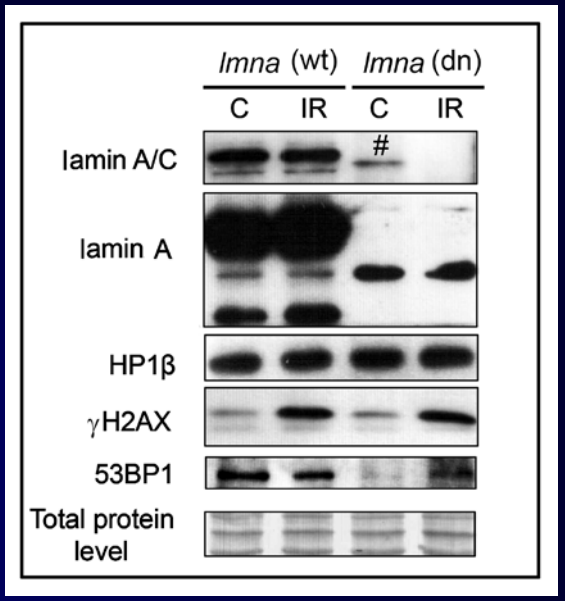
Experiments: Petra Sehnalová

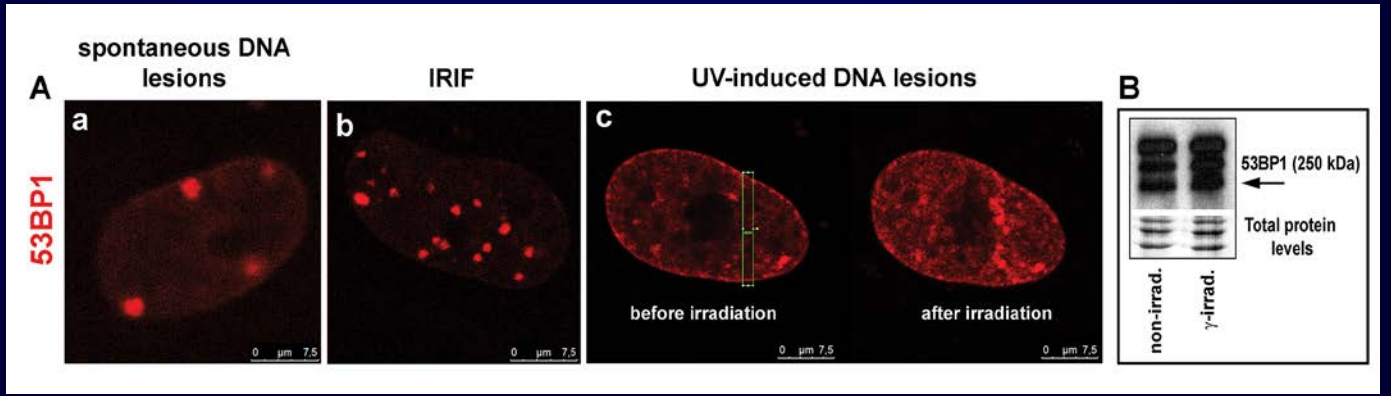




Experiments: Petra Sehnalová

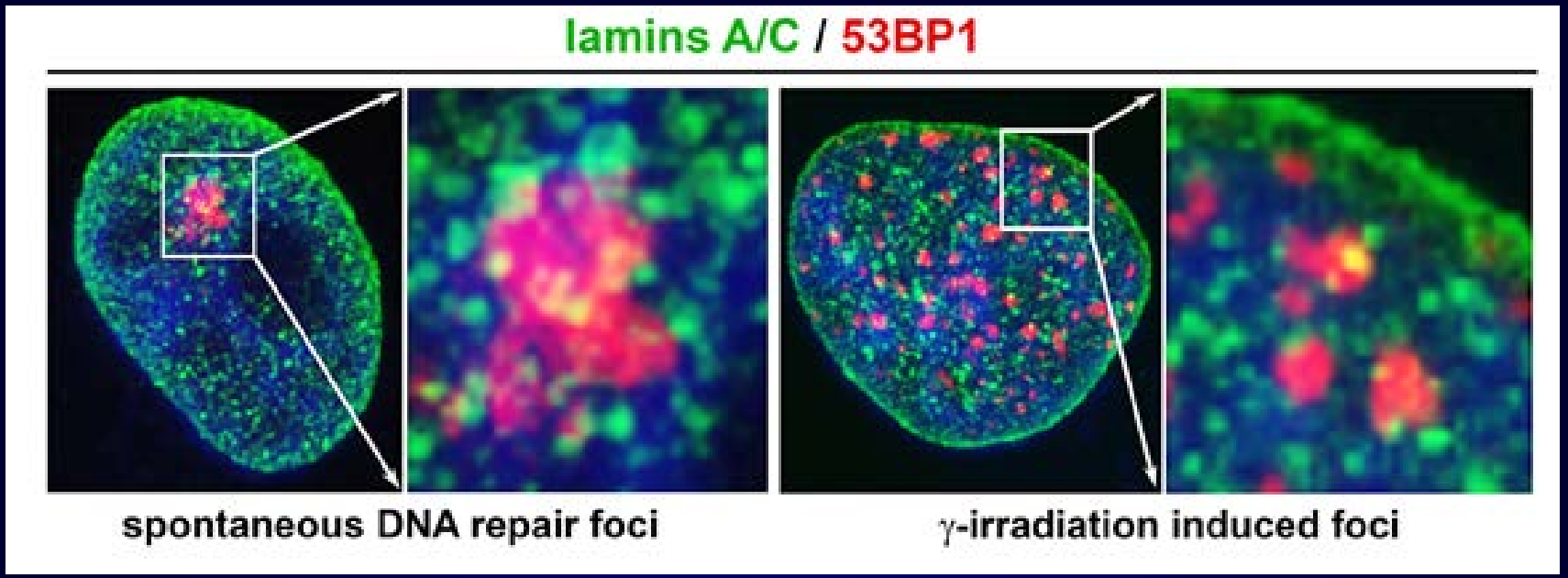




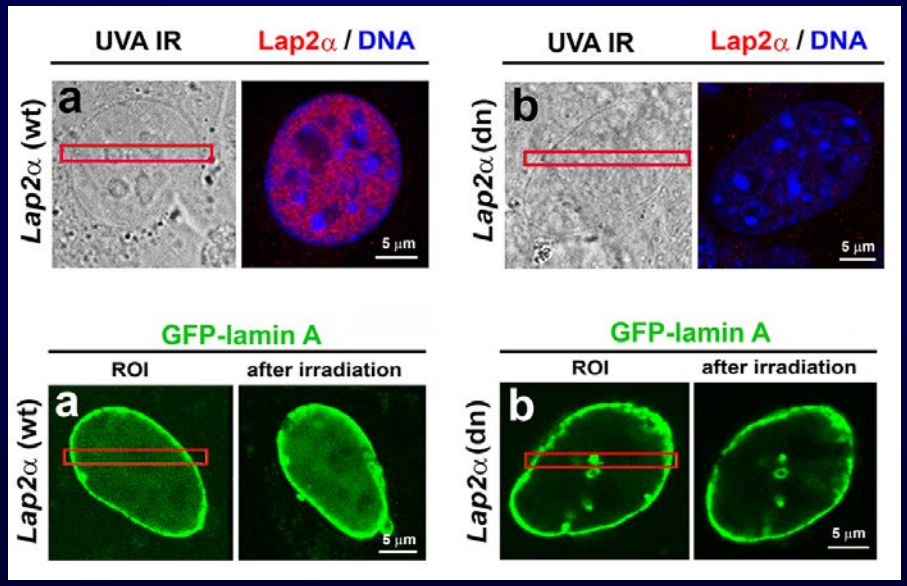


Experiments: Petra Sehnalová and Veronika Foltánková

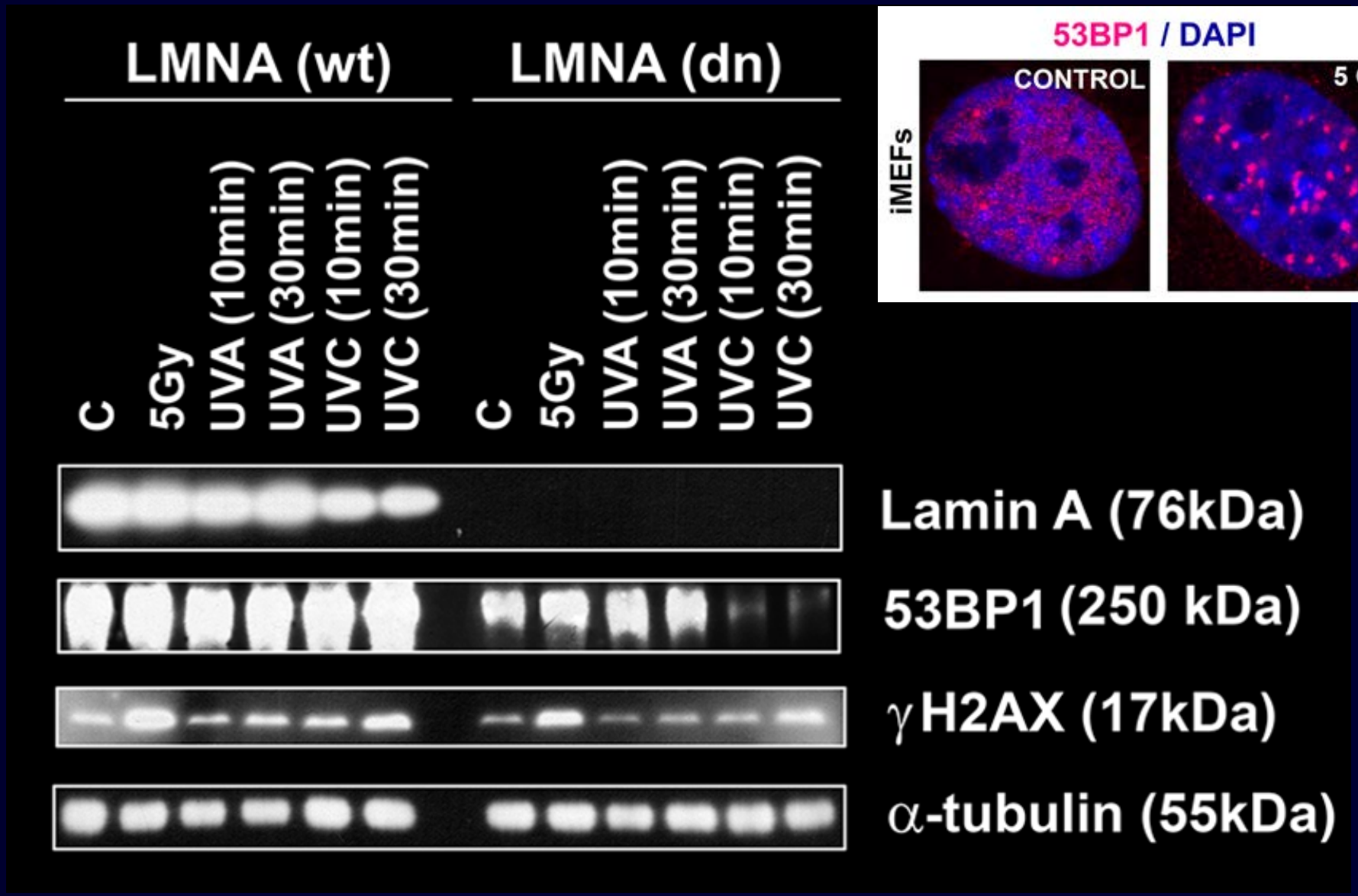
Study 2: A-type lamins and DNA repair



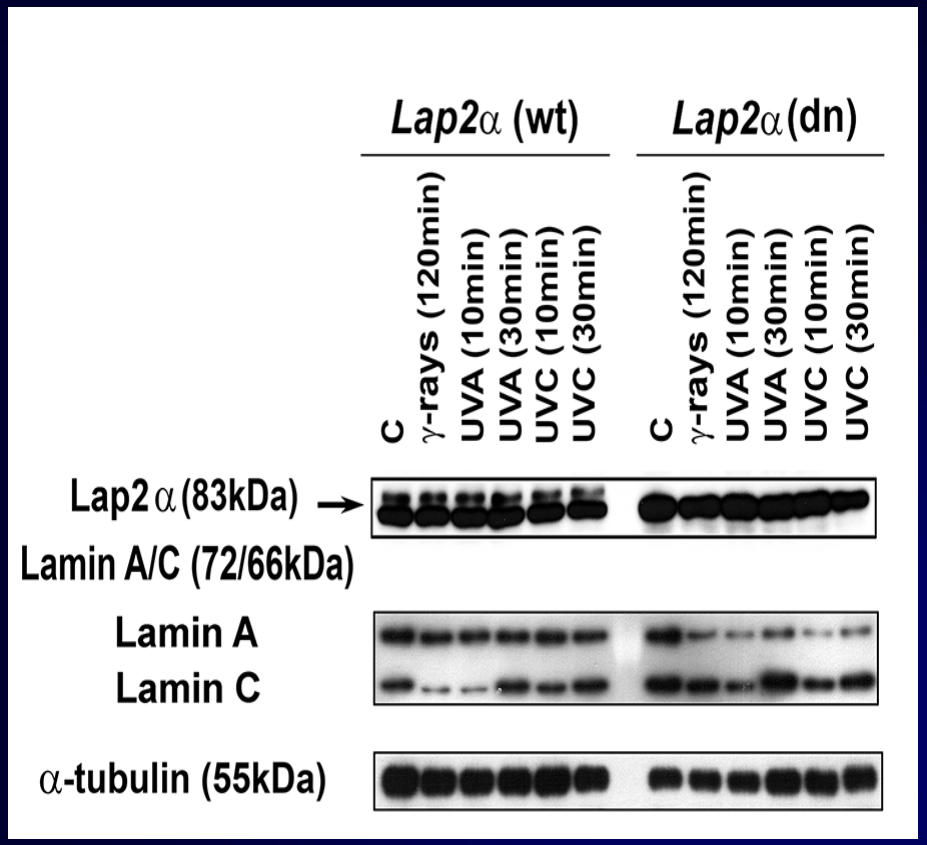
Experiments: Petra Sehnalová

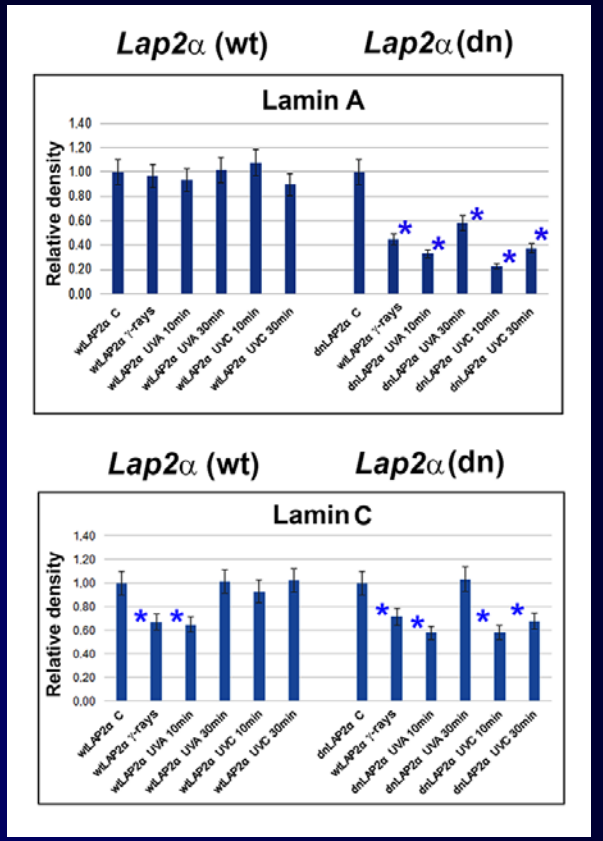


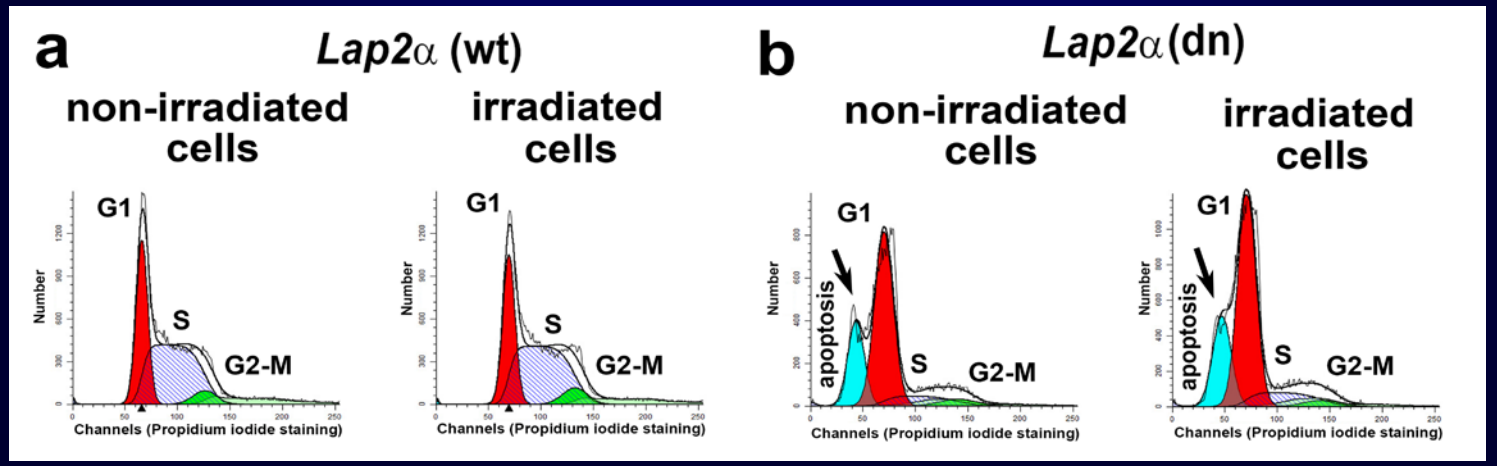
Bártová et al., J. Cell. Biochem. (2018)

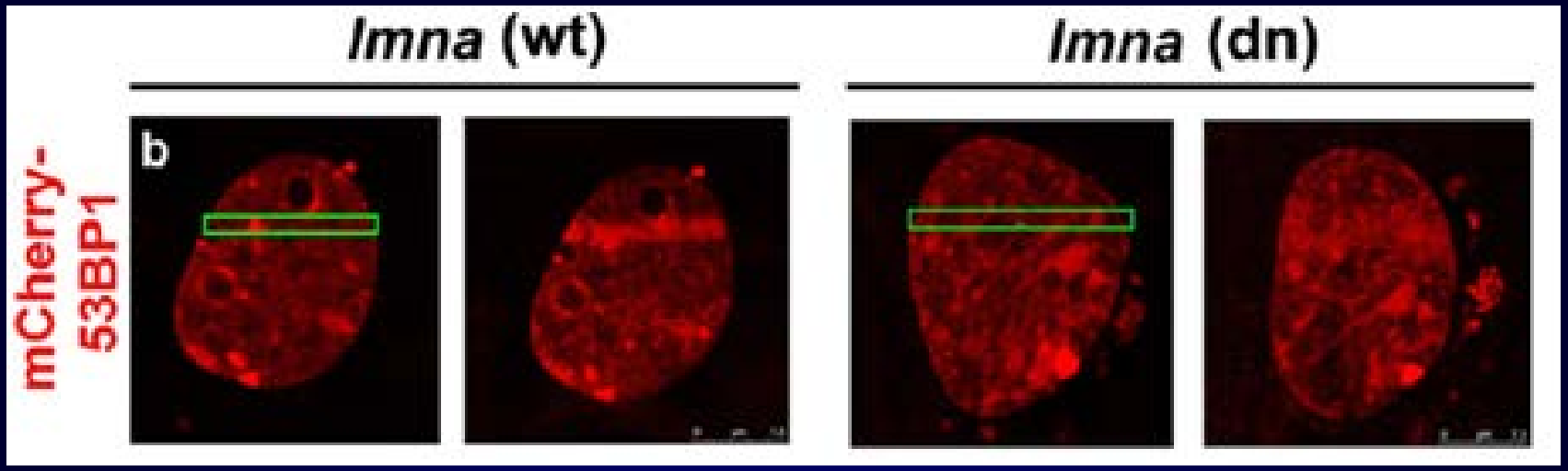


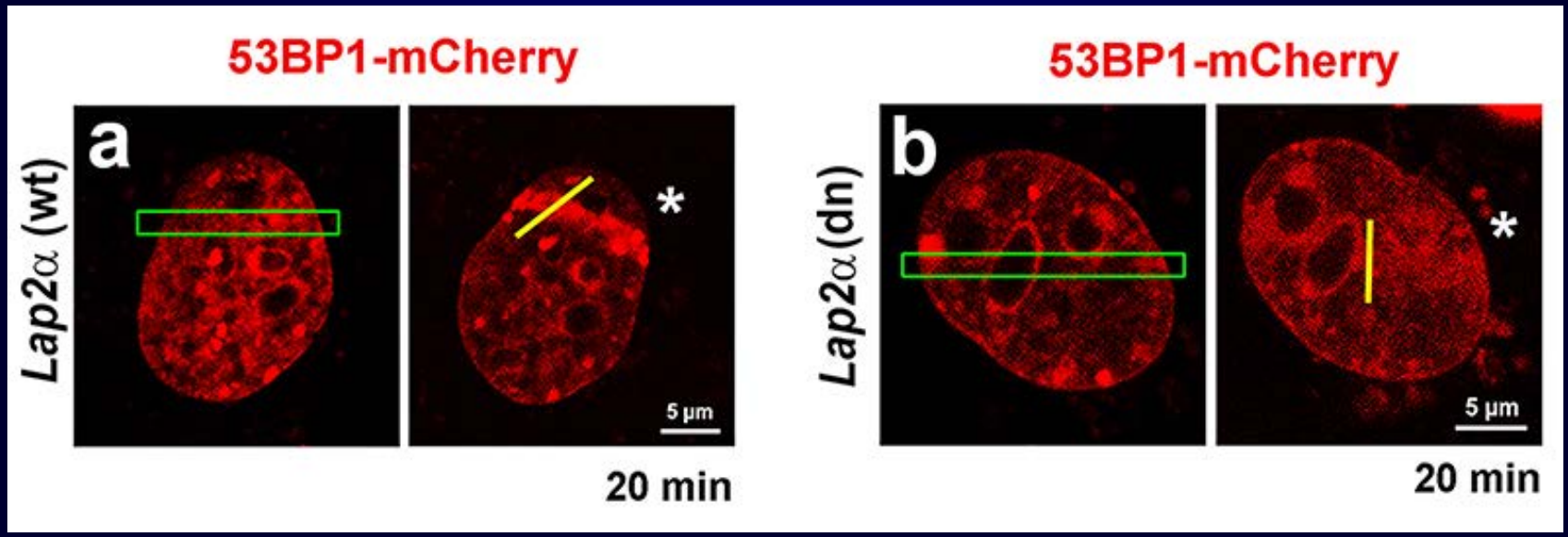
Experiments: Soňa Legartová

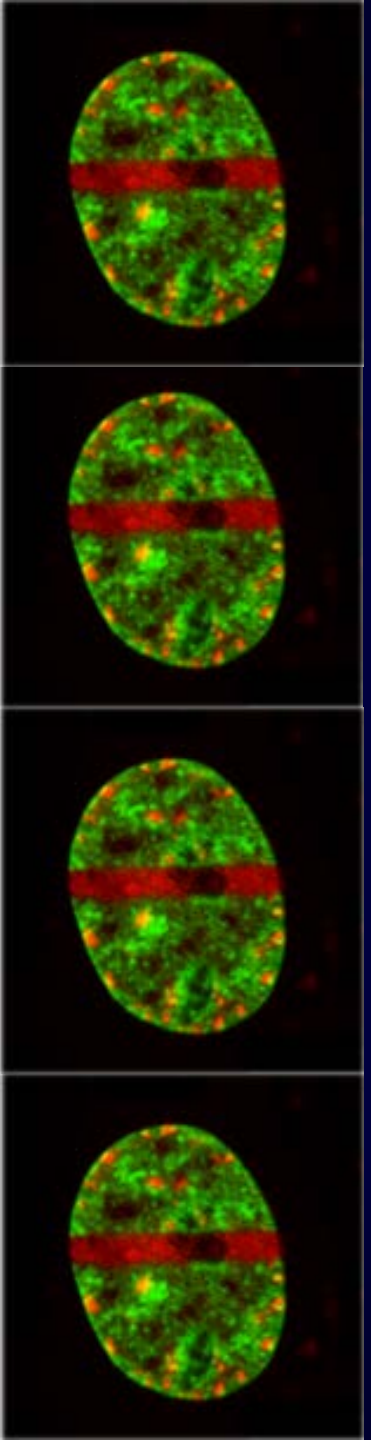

Lap2α (wt)
Lap2α(dn)
C
γ-rays (120min)
UVA (10min)
UVA (30min)
UVC (10min)
UVC (30min)
Lap2α(83kDa)
Lamin A/C (72/66kDa)
Lamin A
Lamin C
α-tubulin (55kDa)


Lap2α (wt)
Lap2α(dn)
Lamin A
Relative density
Lamin C

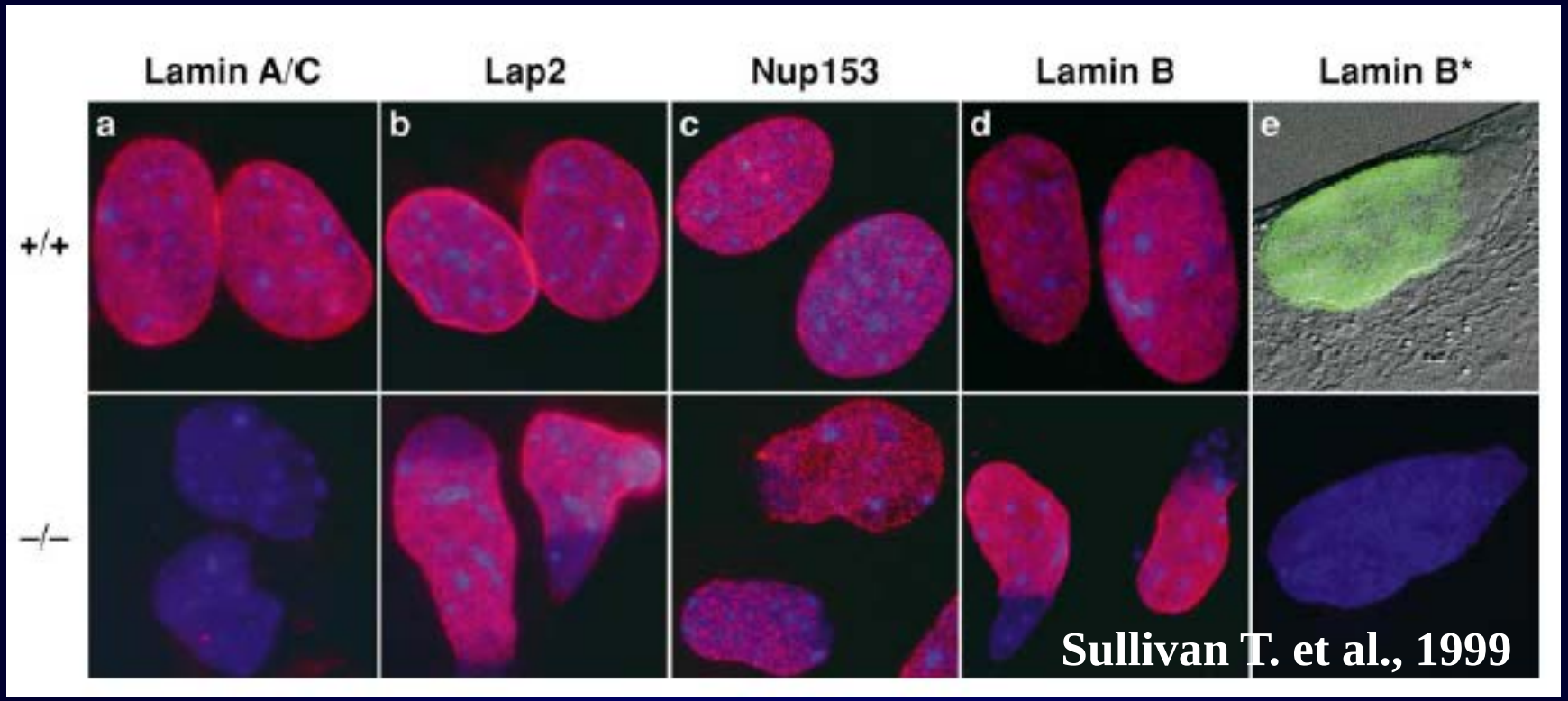

a
Lap2α (wt)
non-irradiated cells
irradiated cells
b
Lap2α(dn)
non-irradiated cells
irradiated cells
G1
S
G2-M
apoptosis
Number
Channels (Propidium iodide staining)


Imna (wt)
Imna (dn)
mCherry-53BP1
b

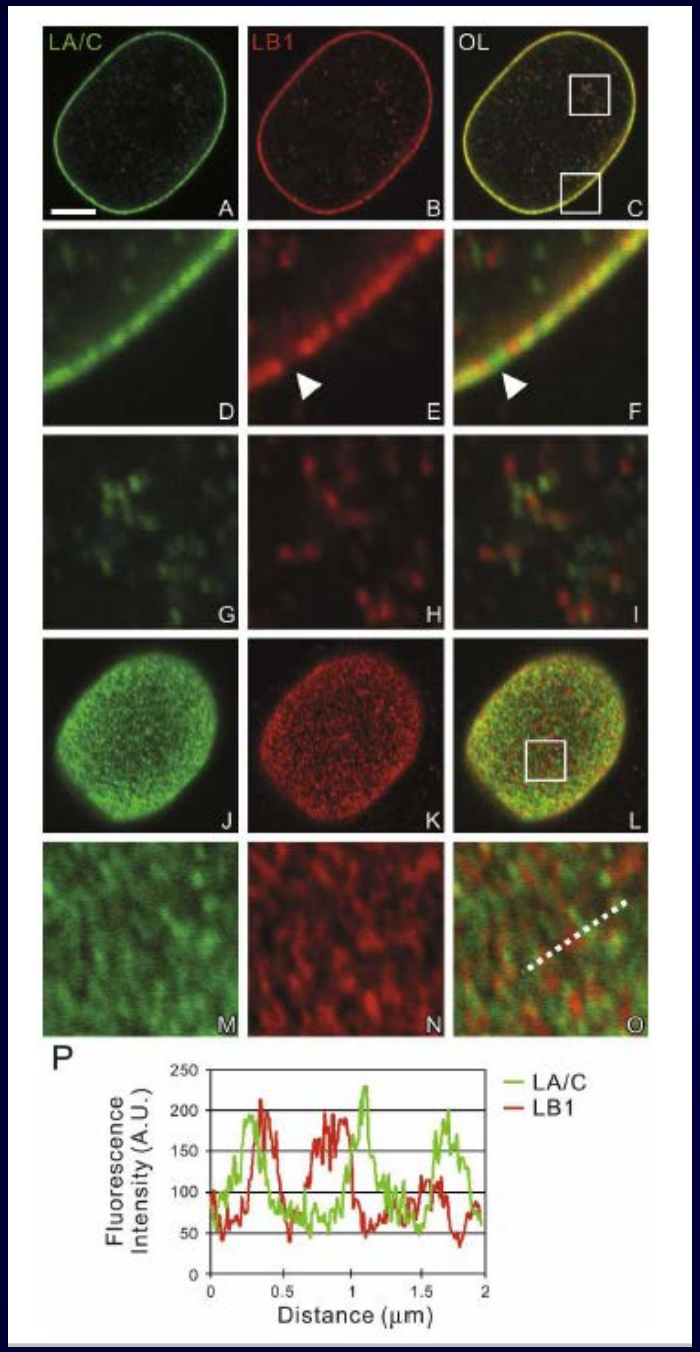

53BP1-mCherry
53BP1-mCherry
Lap2α (wt)
Lap2α(dn)
a
b
*
*
5 μm
5 μm
20 min
20 min

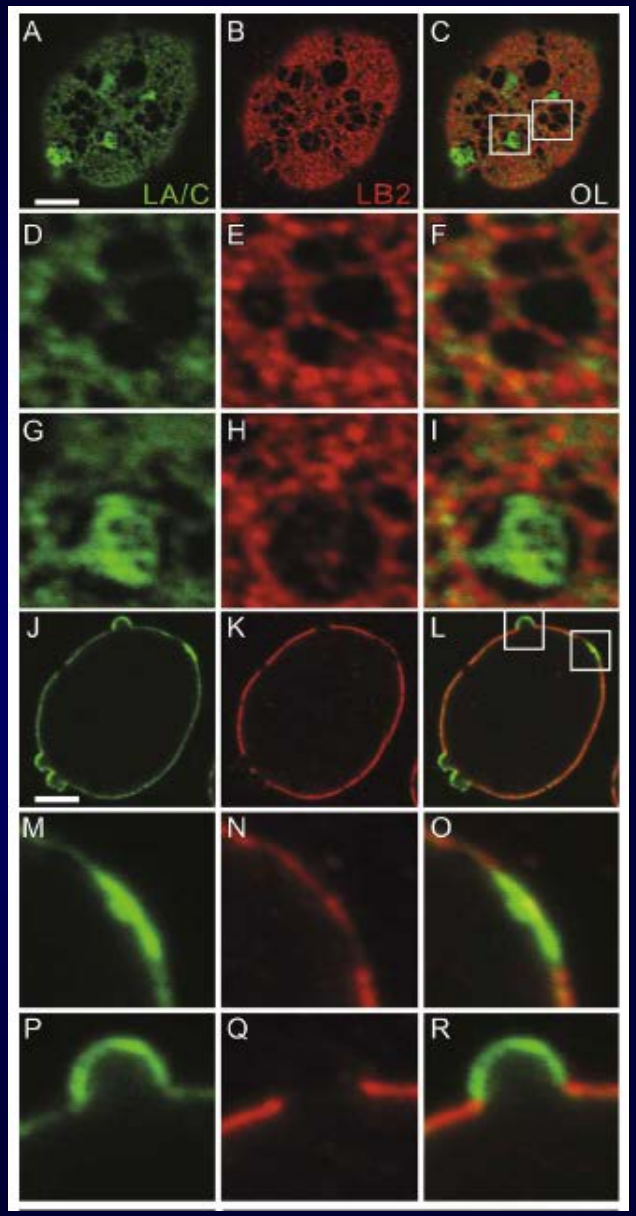


Conclusion: A-type lamins and Lap2α protein were not recruited to local DNA lesions. However, A-type lamin depletion and Lap2α deficiency affected recruitment kinetics of 53BP1 to DNA lesions. The levels of A-type lamins were reduced in irradiated Lap2α deficient cells.



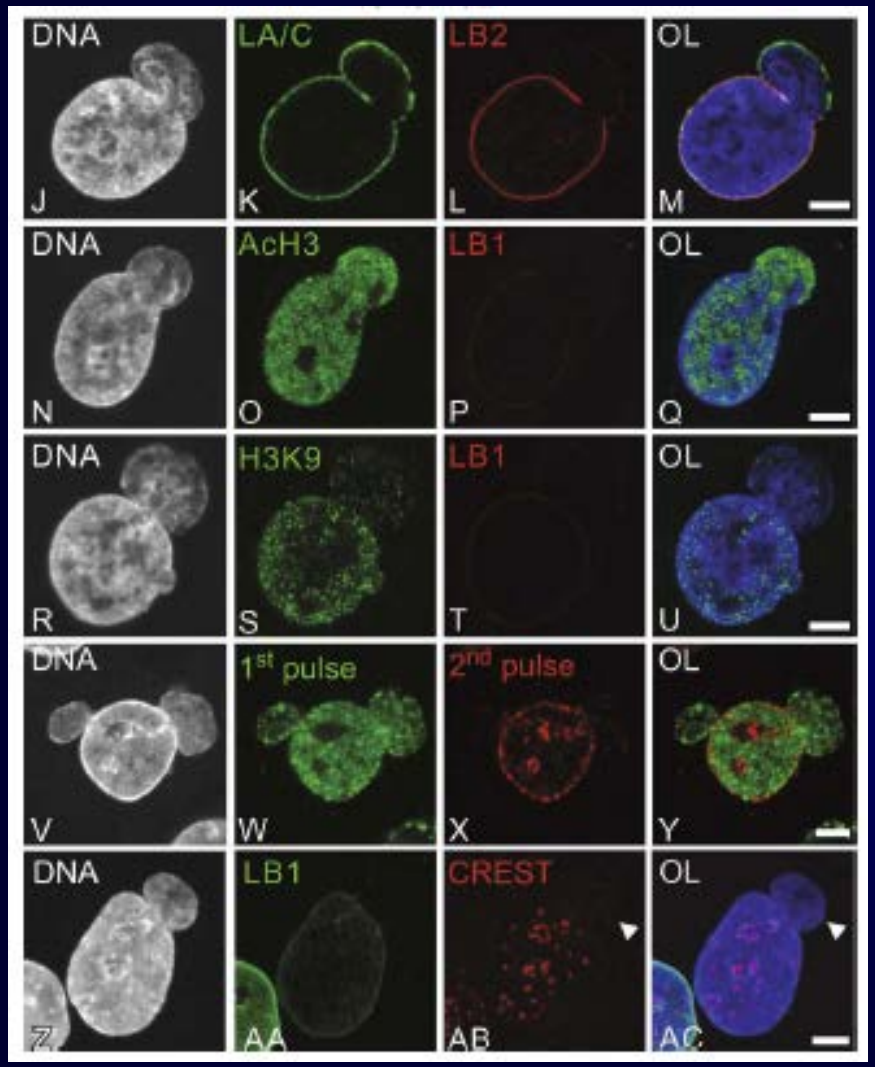
MEF buňky izolované z LA/C knock out myší – vhodný model ke studiu laminů. Ztráta LA/C není pro buňky fatální, ale RNAi vyblokovaný LB – apoptóza (Večeřová J.). Ztráta LA/C neovlivňuje distribuci a morfologii „nuclear specles“ a ani formování faktorů sestřihu (Večeřová et al., 2005). Z experimentů plyne, že dynamicky organizované proteiny nehistonové povahy asociované s chromatinem vykazují schopnost vlastní organizace, která není závislá na proteinech nukleoskeletálních intermediálních filament typu laminů.

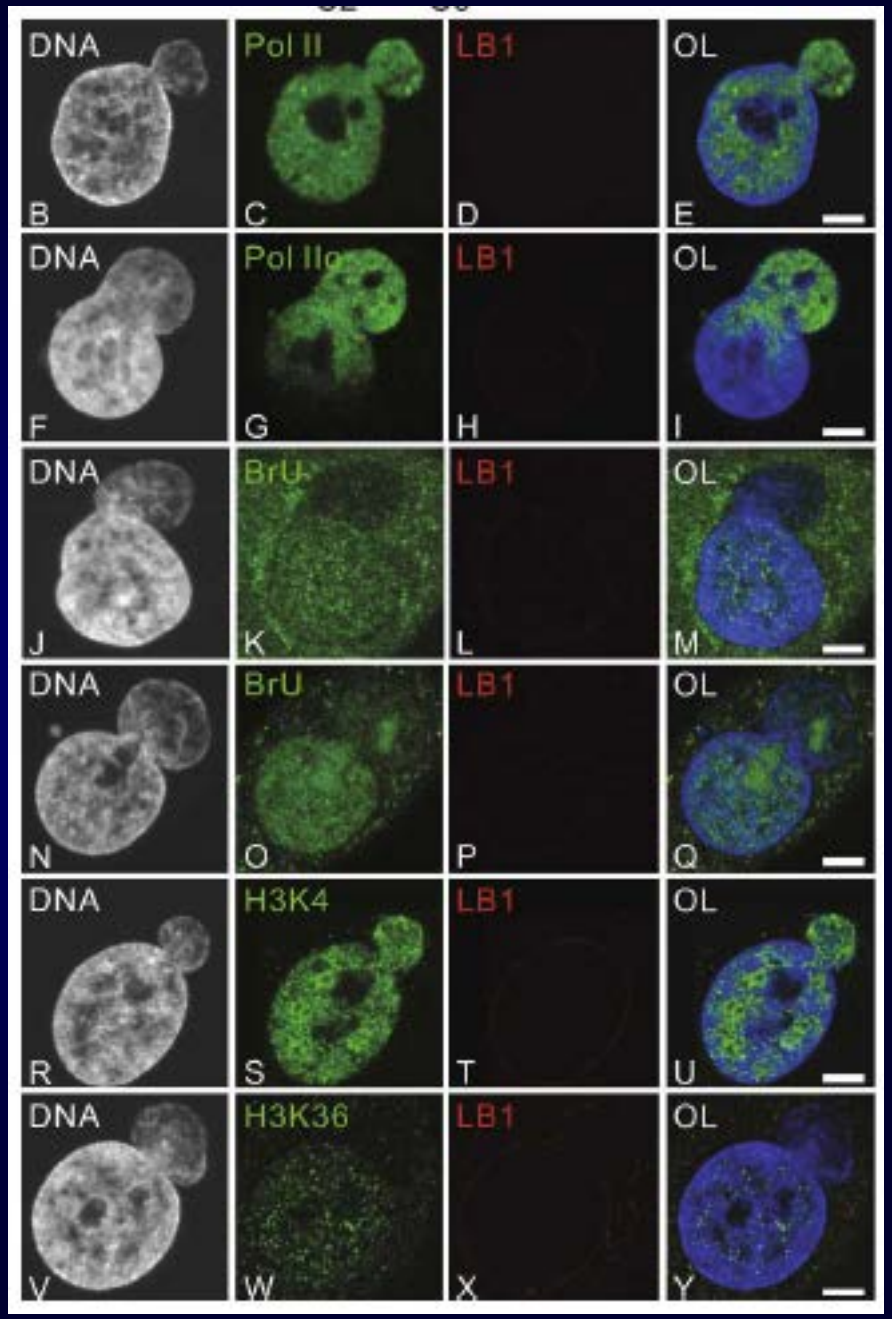




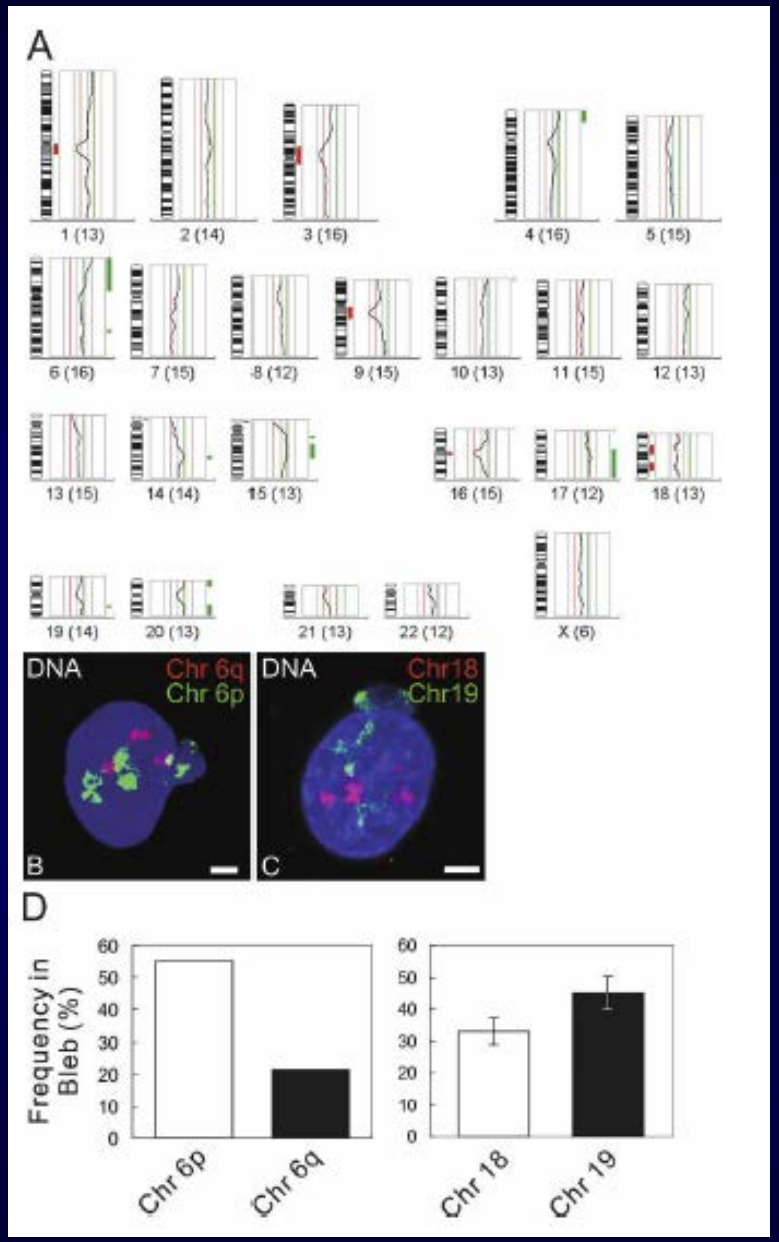
Shimi et al. Genes Dev (2008)

Lamin B1 deficiency



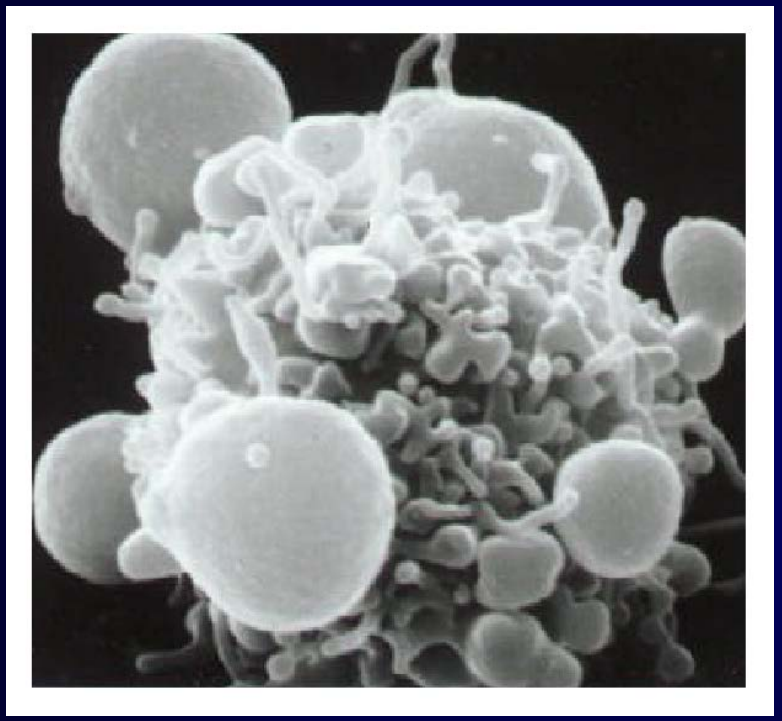


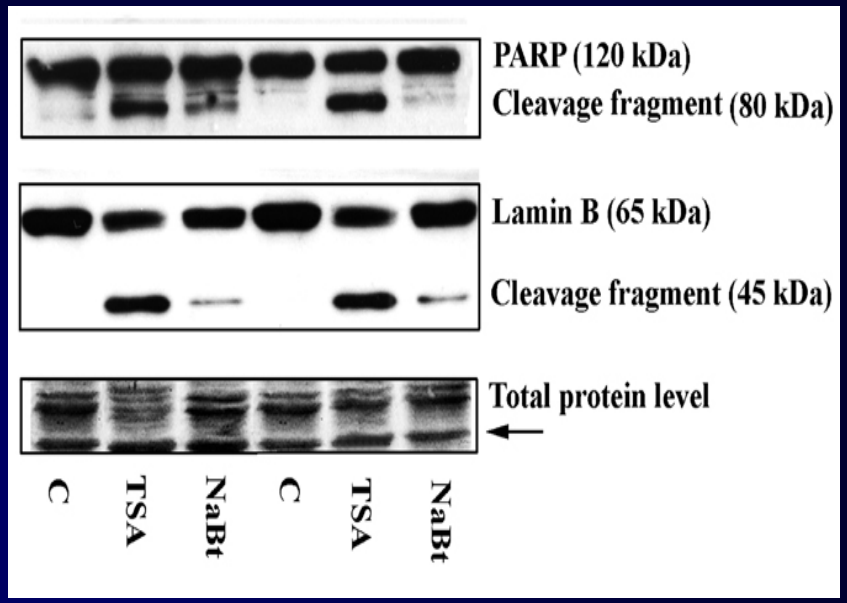
Shimi et al. Genes Dev (2008)

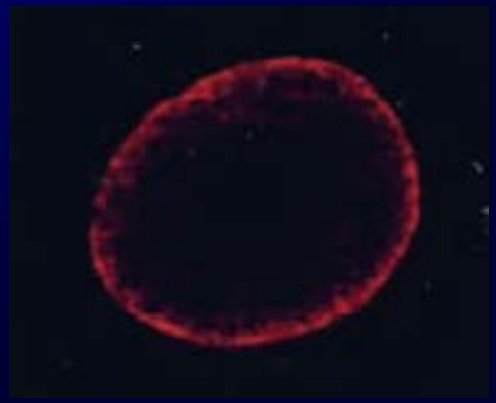


Shimi et al. Genes Dev (2008)

Apoptotic lamin B cleavage

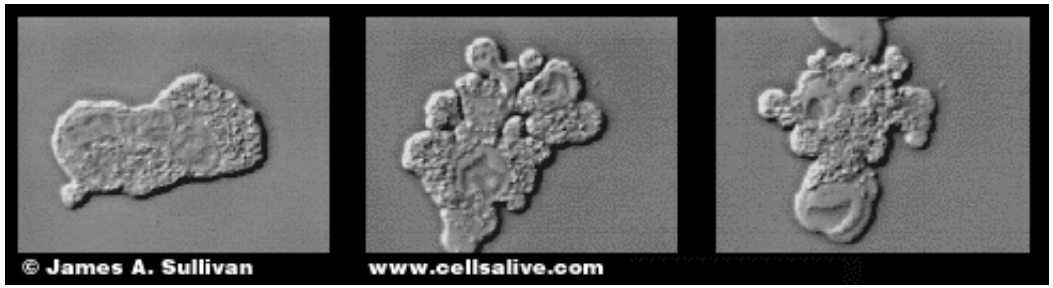


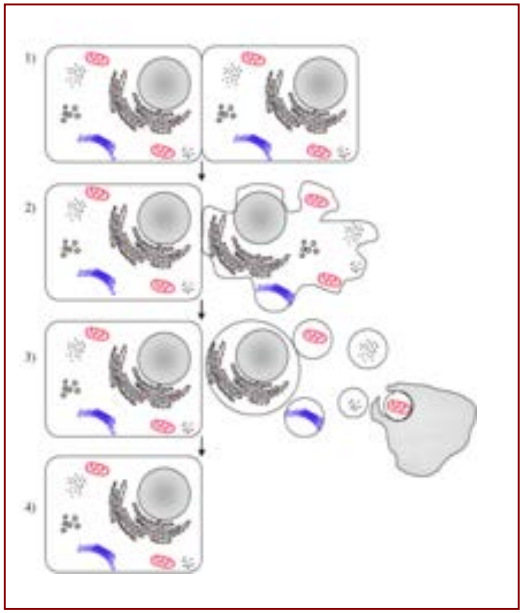




Laminy a apoptóza

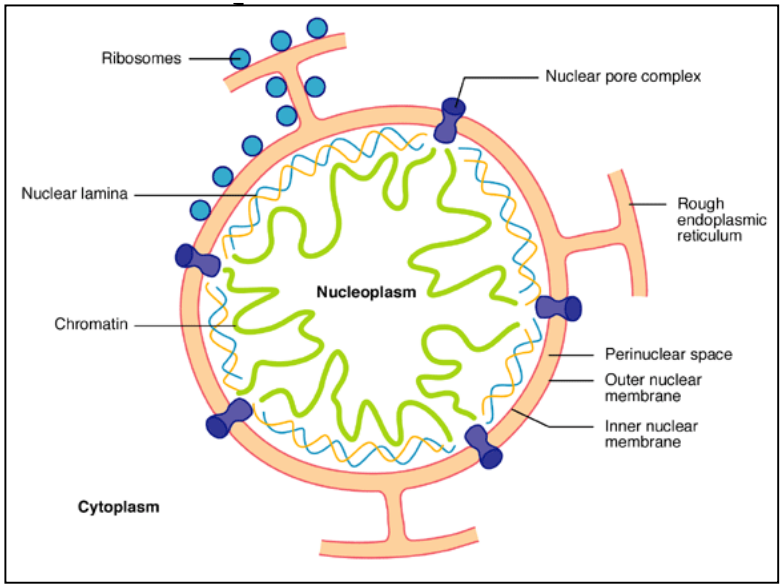
- apoptóza je charakterizována zmenšením jádra a kondenzací chromatinu (fragmentace na „bodies“)
- během apoptózy jsou laminy štěpeny kaspázami v místě kyseliny aspartové na centrální doméně laminu A (jedna z nejvíce konzervovaných oblastí v proteinech IF)
- degradace laminů hraje důležitou roli úspěšném zakončení apoptózy





Nuclear lamina and lamins

- connection between cytoplasm and genome
- mechanical stability for the NE and organizing of chromatin structure at the nuclear periphery
- essential role in chromatin and NPCs architecture and organization
- NE breakdown and reassembly mitosis
- DNA replication
- RNA polymerase II-dependent expression
- transcriptional repression
- laminopathies



(Ellis and Maidment, 2002)

Nuclear lamina and lamins

- A-type lamins – *LMNA* gene (A, AΔ10, C, C2)

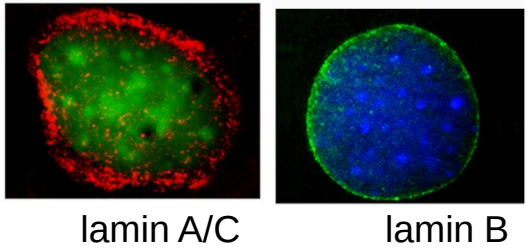
(in differentiated cells, tissue homeostasis)

- B-type lamins – *LMNB1*, *LMNB2* genes (B1, B2, B3)

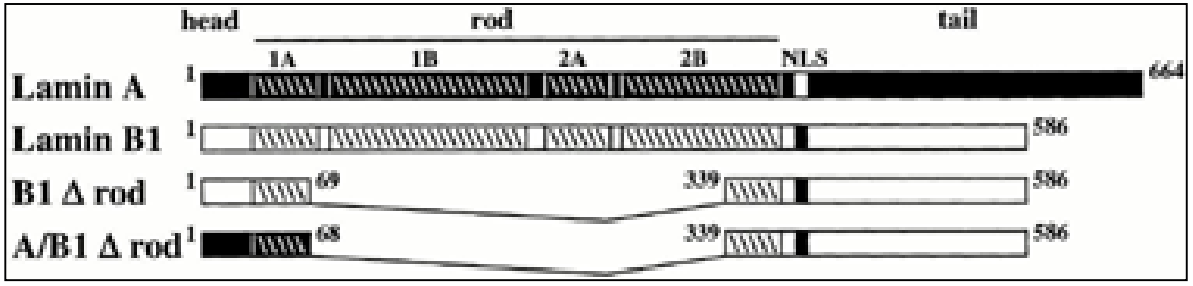
(in somatic cells, essential for cell viability, development)

Lamin structure

- short N-terminal „head“ domain (NH_2)
- long α-helical coiled-coil „rod“ domain
- globular „tail“ domain (COOH)



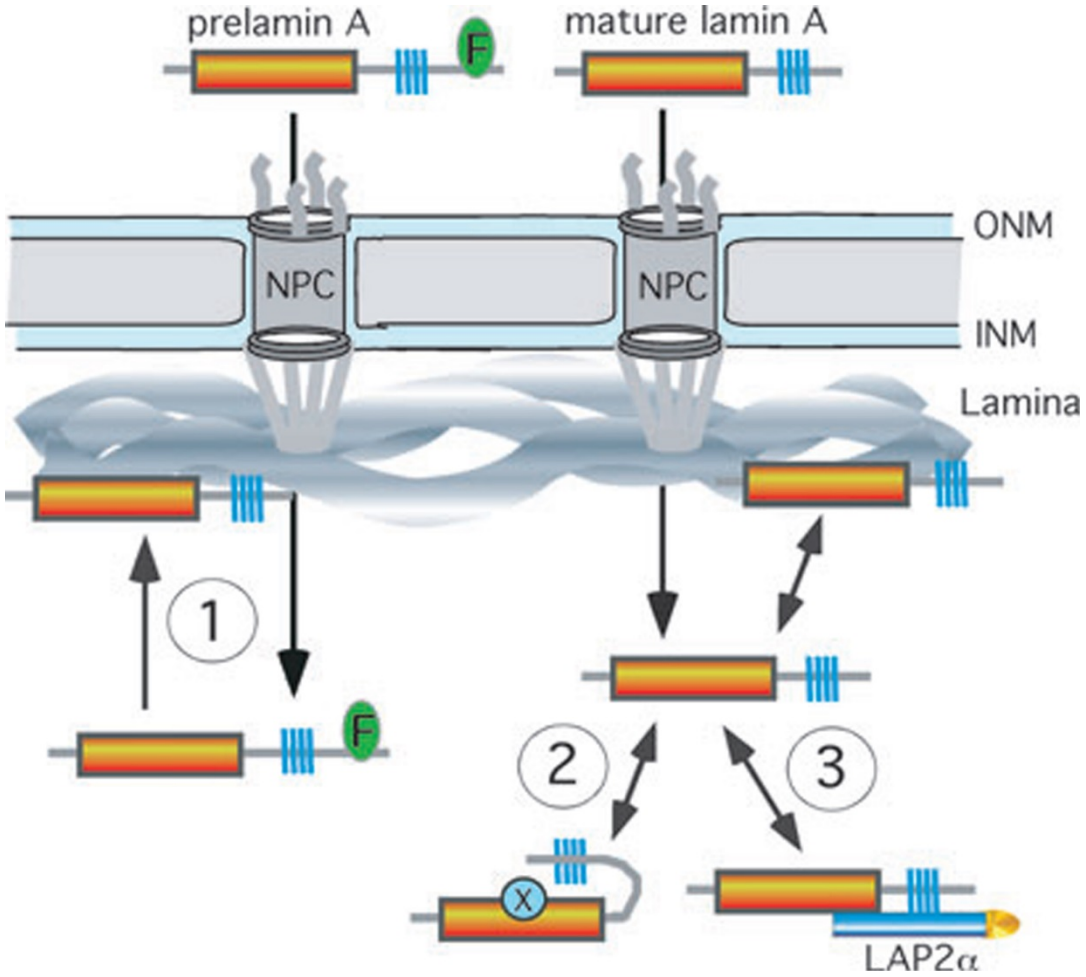
lamin A/C lamin B



(E.C. Schirmer et al., 2001)

• nucleoplasmic pool of the lamin A

Three possible mechanisms of targeting lamins A/C to the nucleoplasm



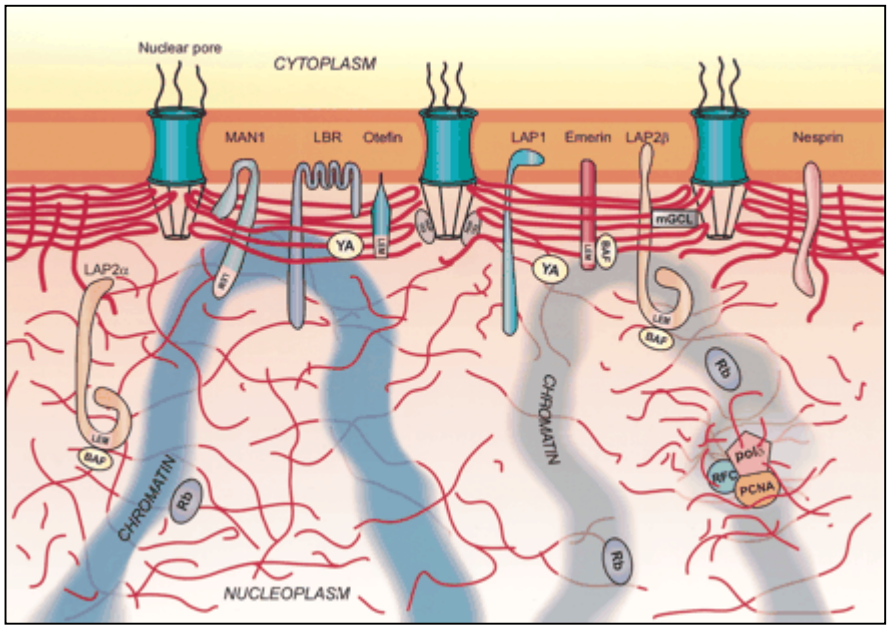
(Dorner et al., 2006)

Prelamin A is farnesylated and carboxymethylated on the cysteine residue of a carboxyl-terminal CaaX motif. This post-translationally modified cysteine residue is removed from prelamin A when it is endoproteolytically processed into mature lamin A. The protein encoded by this gene binds to the prenylated prelamin A carboxyl-terminal tail domain. It may be a component of a prelamin A endoprotease complex. The encoded protein is located in the nucleus, where it partially colocalizes with the nuclear lamina. It shares limited sequence similarity with iron-only bacterial hydrogenases. Alternatively spliced transcript variants encoding different isoforms have been identified for this gene, including one with a novel exon that is generated by RNA editing.

Lamin associated proteins

INM proteins

- LBR (lamin B receptor)
- LAP1 (lamin associated polypeptide)
- LAP2α, β (lamin associated polypeptide α, β)
- emerin
- MAN1
- Nesprin-1α

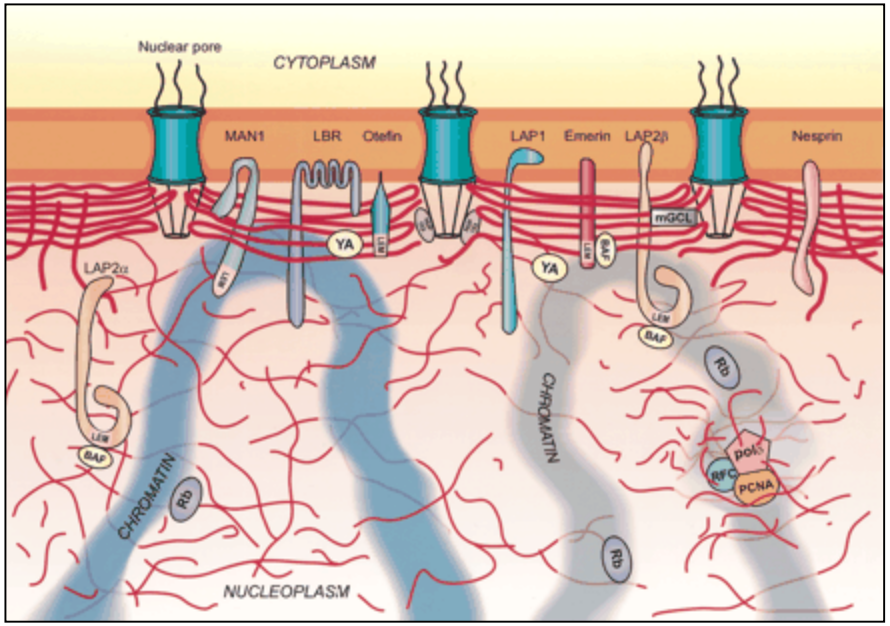


Goldman et al., 2002

Lamin associated proteins

Nucleoplasmic soluble proteins

- BAF (barrier-to-autointegration factor)
- components of RNA polymerase II transcription complexes
- DNA replication complexes
- H2A, H2B dimmers
- actin
- RB protein



Goldman et al., 2002

Heterochromatin

- dissociation of the Oct-1 from lamin B and the nuclear periphery correlate with reduced inhibitor activity
- Nup2p was shown to tether chromatin to the nuclear pore complex blocking propagation of chromatin
- loss of heterochromatin or its altered organization at the nuclear periphery in lamin A knockout mice

Laminopathies
(nuclear envelopathies)

- premature ageing syndroms, myopathies, neuropathies, lipodystrophies, dermopathies
- lamin A/C (EDMD, HGPS), emerin (EDMD), MAN1, LBR, LAP2α (cardiomyopathy)
- **mechanical stress model** - mutated nuclei are mechanically more fragile, defects in NE structure (cardiac- and skeletal-muscle pathologies)
- **perturbed gene expression** - mutations in lamina proteins could promote diseases by compromising various gene regulatory pathways in different tissues (pRB + lamin A and LAP2α, important for skeletal muscle and adipose tissue differentiation)

Laminopatie

- Emery Dreifuss Muscular Dystrophy (EDMD) – mutace v *LMNA* (autozomálně dominantní), v emerinu (recesivní)
- jiné příznaky než u myší až po 4-5 letech
- Dilated cardiomyopathy (DCM)
- Familiar partial lipodystrophy (FPLD)
- Limb girdle muscular dystrophy (LGMD)
- asi 50 mutací v *LMNA* (bodové mutace, delece, nonsence mutace,…)

Laminopathies

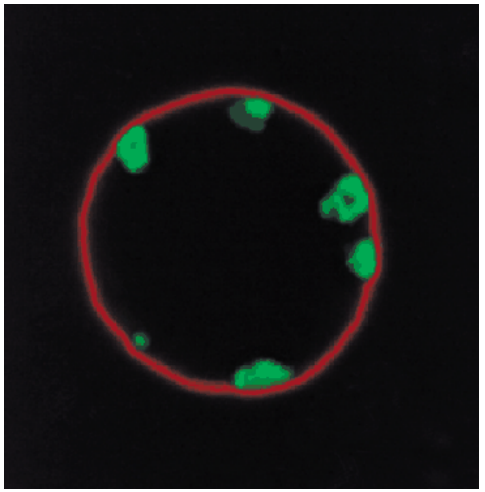
- loss of heterochromatin in HPGS patients (epigenetic marks, Goldman et al.)
- nuclei from old people have similar defects as HGPS patients (Scaffidi and Misteli) – including changes in histone modifications and increased DNA damage
- direct link between heterochromatinization defects leading to HGPS, and ageing

Lamin A and nuclear lamina-dependent epigenetic alterations are involved no only in nuclear envelopathies but also in the physiological processes of ageing.

Laminy a chromatin

- interakce laminů a LAPs s chromatinem
- vazba laminů na sekvence DNA v místech matrix attachment regions (MARs) a scaffold attachment regions (SARs)
- stabilní navázání LAPs vyžaduje LEM doménu (oblast 43 aminokyselin)
- ovlivnění organizace chromatinu

  a tím i aktivity genů



R.D. Goldman et al., 2002