
*It is only with its nucleus that a cell can see rightly*

# Components and Mechanisms of Nuclear Mechanotransduction

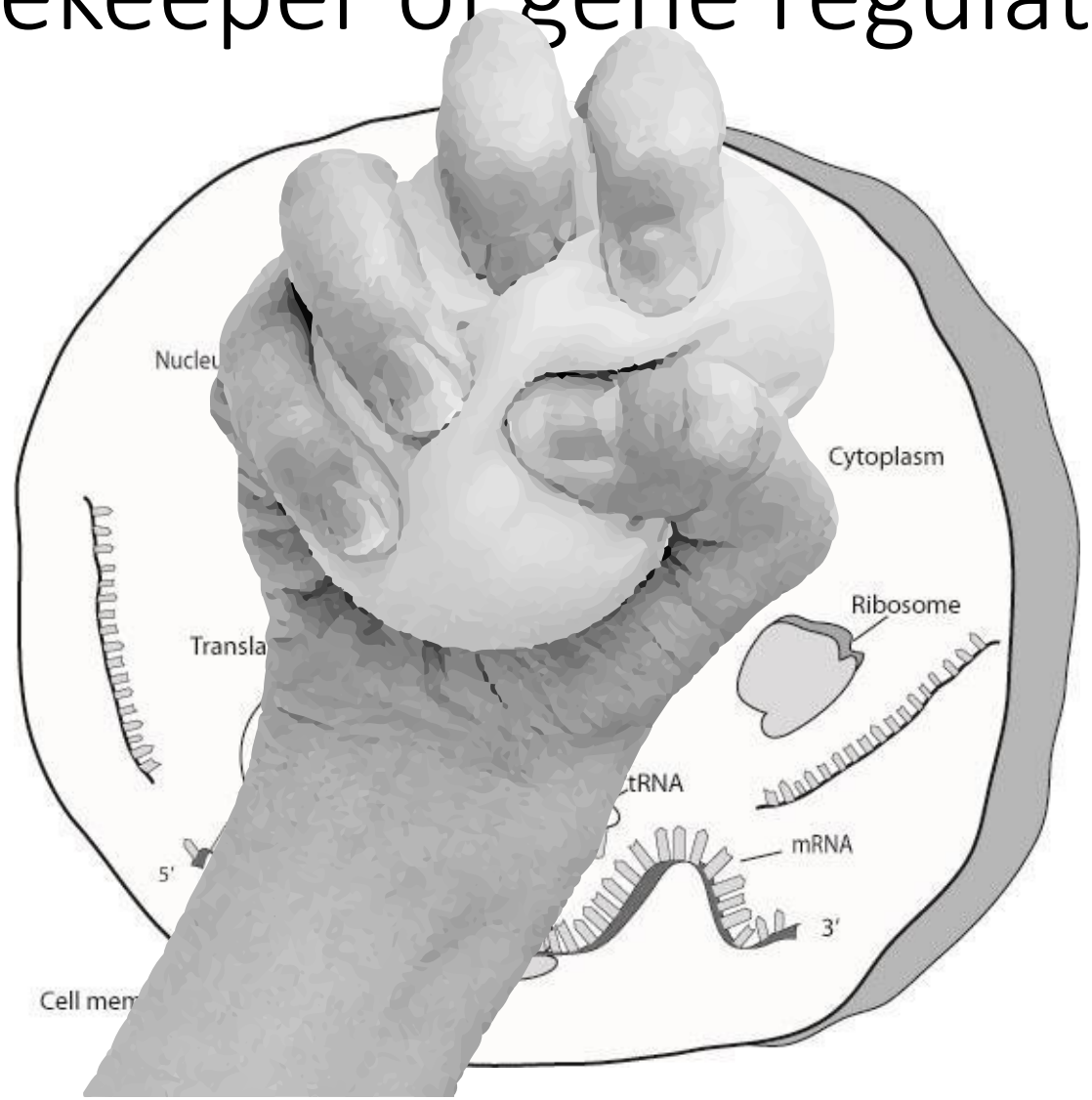
GSK Core course, Philipp Niethammer, Cell Biology Program, SKI

# Mechanotransduction

Physical signal  Chemical signal

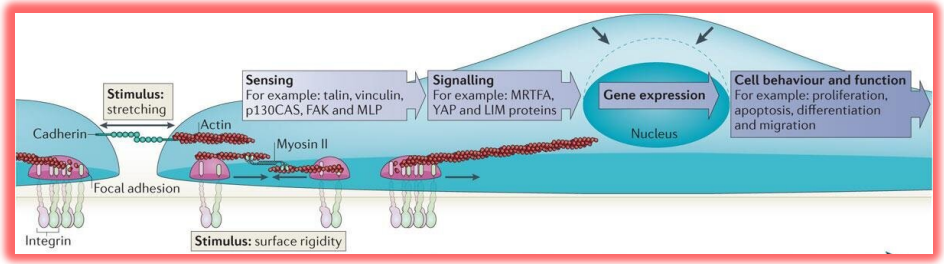
What does the nucleus have to do with this?

# Classic notion: The nucleus as gatekeeper of gene regulation



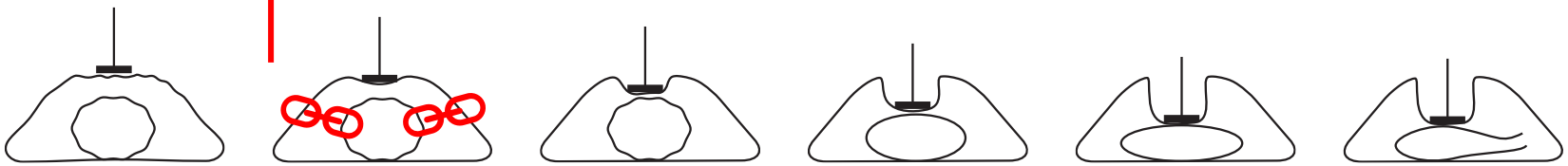

Gene transcription, from *The Mermaid's Tale*, Weiss & Buchanan, 2009

# New notion: The nucleus as sensor of critical cell body deformation




Surface deformation → Cell surface mechanotransduction

Intermediate nuclear mechanotransduction



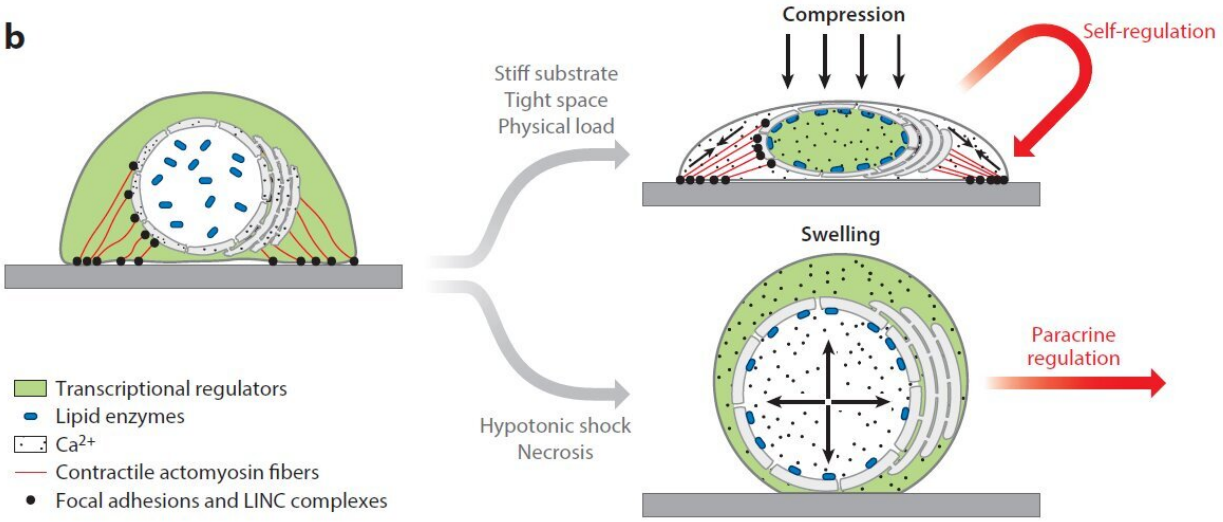
Critical deformation → Immediate nuclear mechanotransduction

# Lecture overview

1) Common types of nuclear deformation
2) Physiological consequences of nuclear deformation
3) How do the individual components of the nucleus respond to load?
    a) The nuclear membrane under load
    b) Chromatin under load
    c) The nuclear lamina under load
4) Studied mechanisms of nuclear mechanotransduction

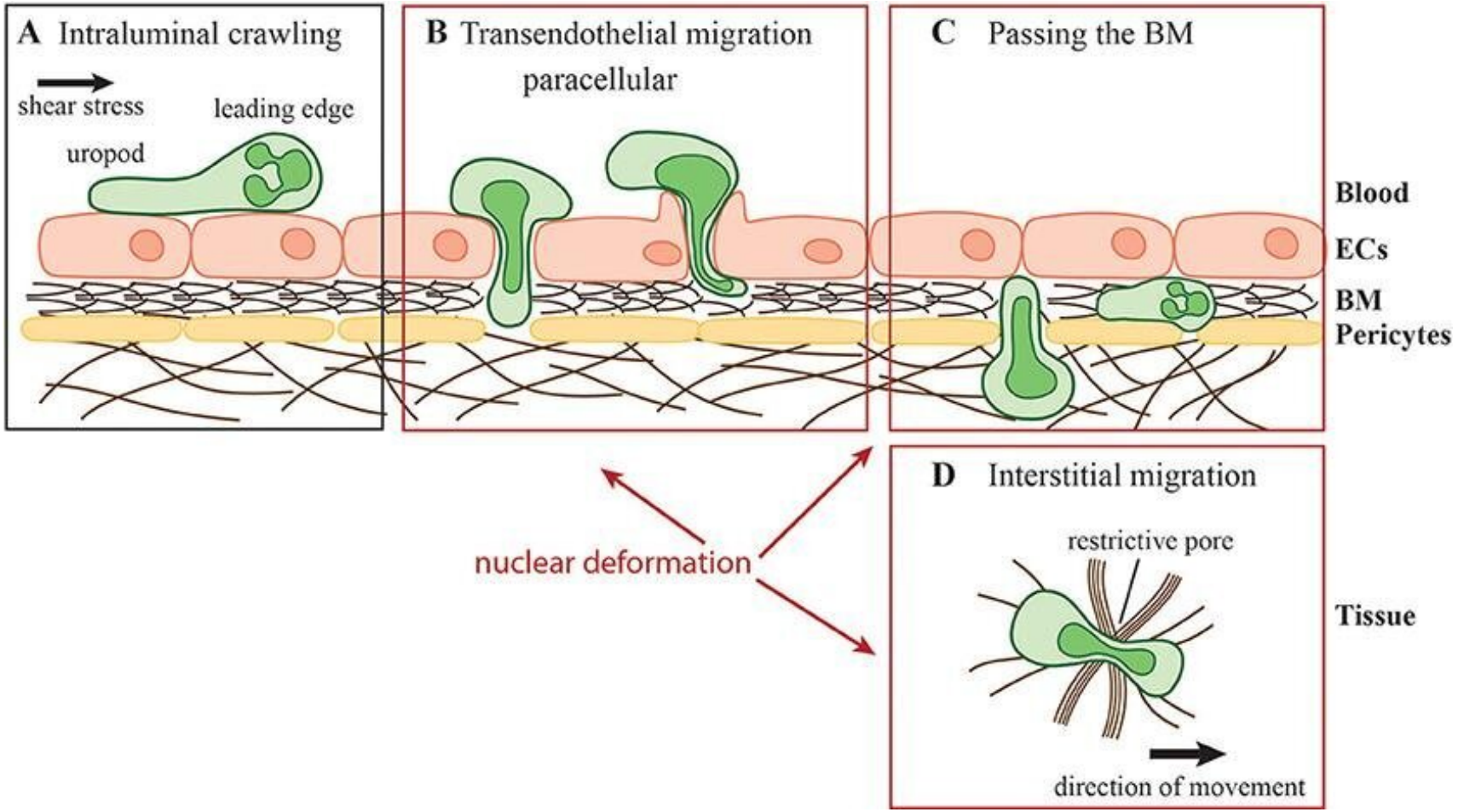
# 1.
# Common Types of Nuclear Deformation

# Two principal types of nuclear deformation




Niethammer, *Ann Rev Cell Dev Biol*,2021

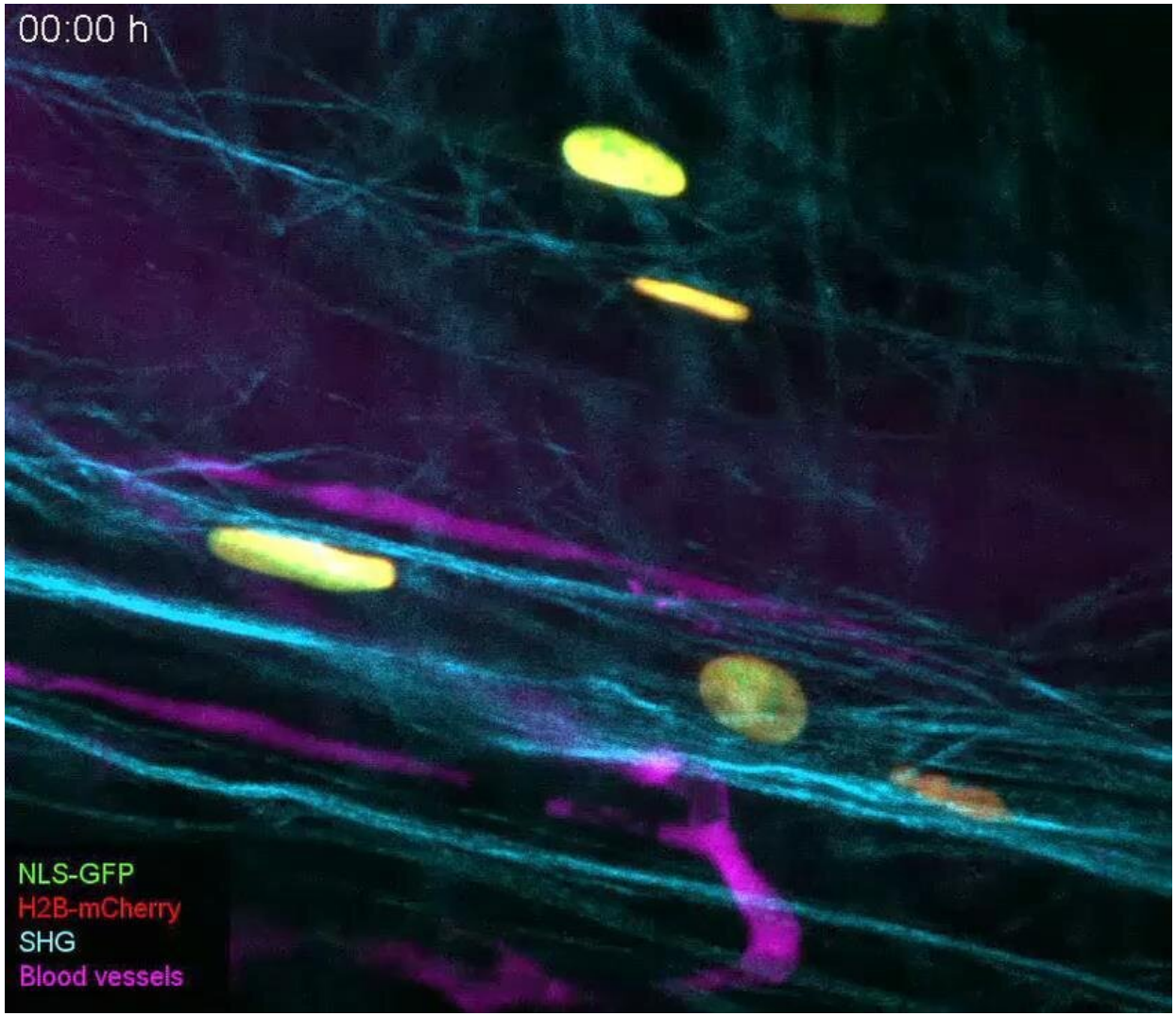
# Examples for nuclear compression




Salvermoser et al., *Front Immunol*, 2018

# Nuclear compression during cell migration

HT1080 fibrosarcoma cells transplanted to mouse dermis



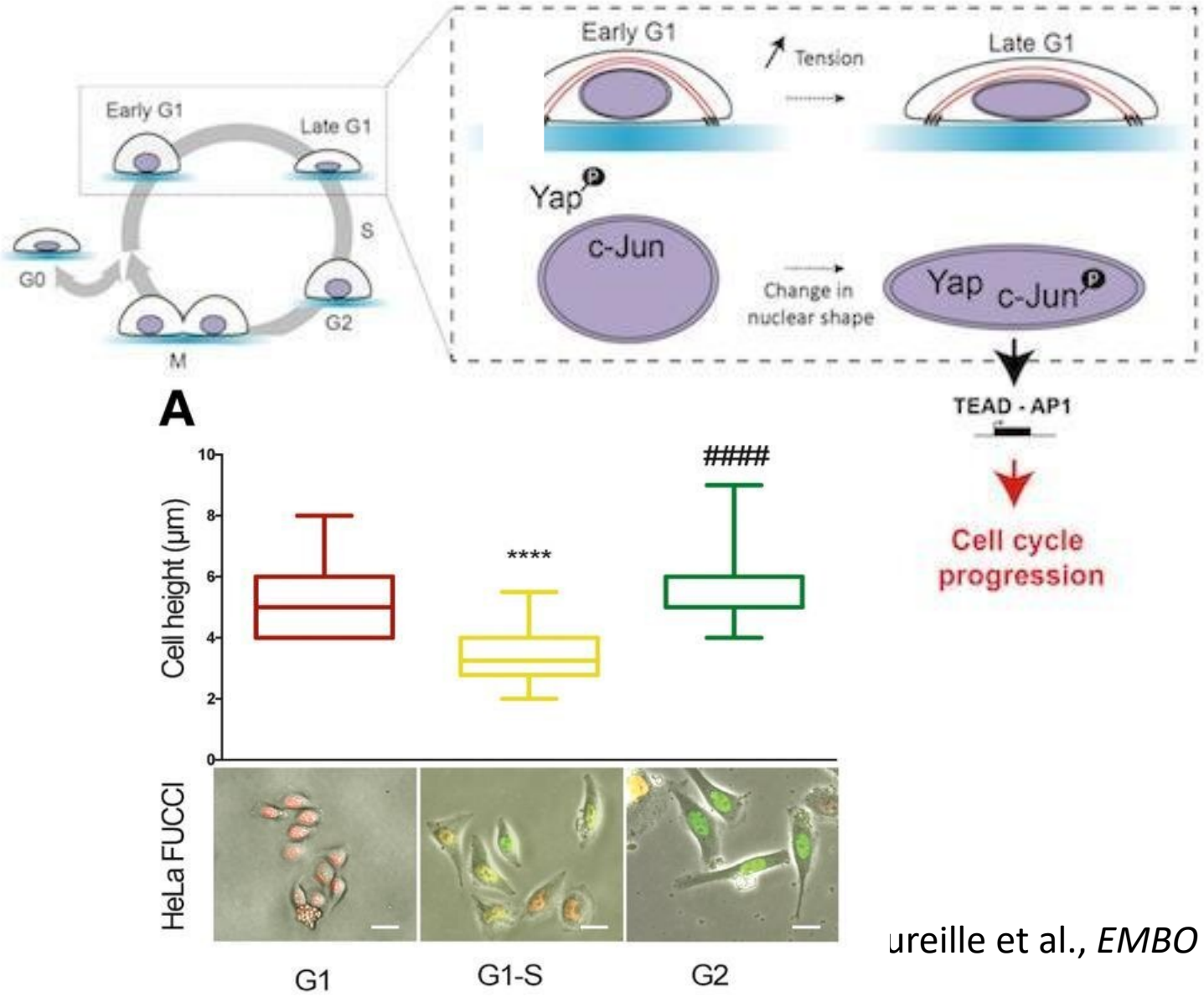

Denais et al., *Science*, 2016

# Nuclear compression during cell migration

MDA-MB-231 breast cancer cell migrating through 2 × 5 $\mu m^2$ constrictions in microfluidic device.


Denais et al., *Science*, 2016

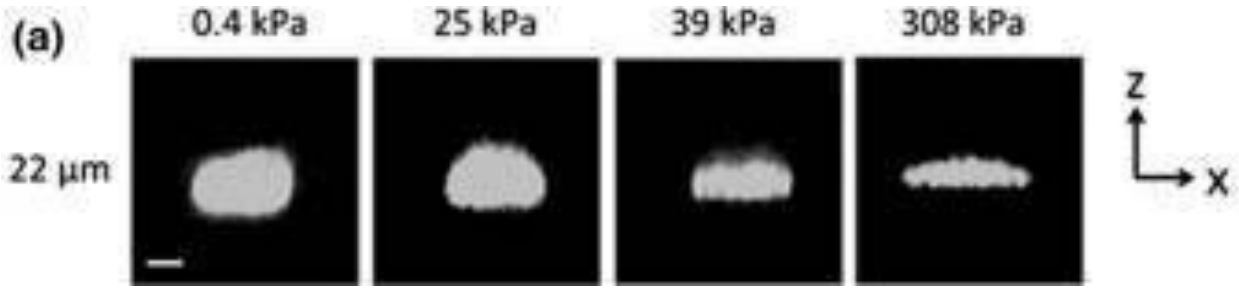
# Nuclear compression during the cell cycle

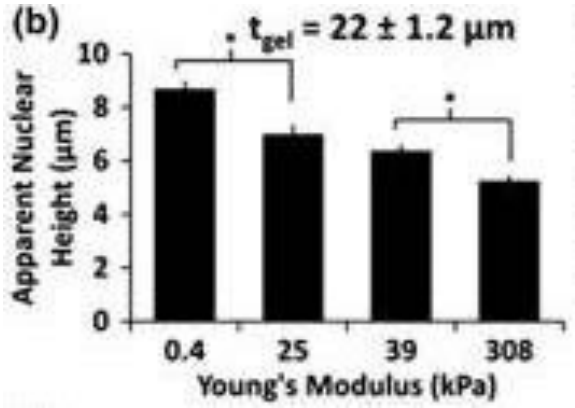



ureille et al., *EMBO Rep,* 2019

# Nuclear compression on stiff substrates

NIH-3T3 cells on polyacrylamide gels of different stiffness

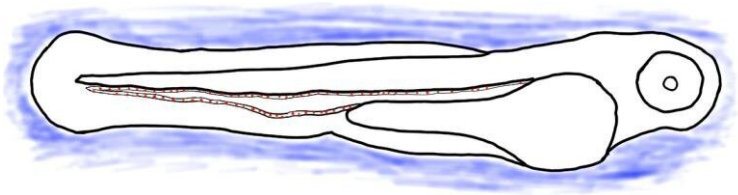


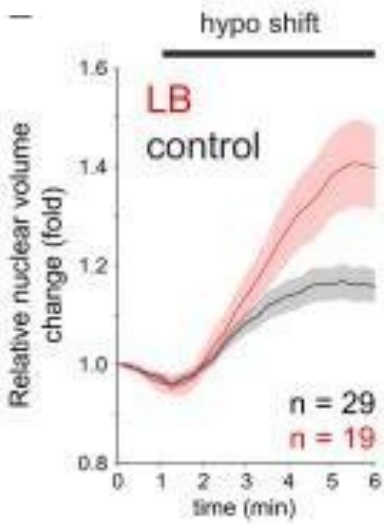

Lovett et al., *Cell Mol Bioeng,* 2013

# Nuclear swelling after hypotonic shock

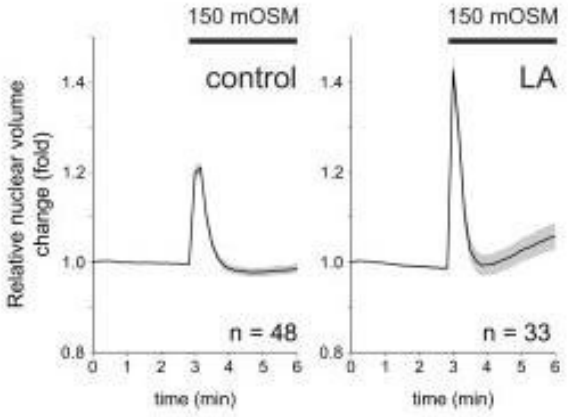
Hypotonic fresh water



Zebrafish wound



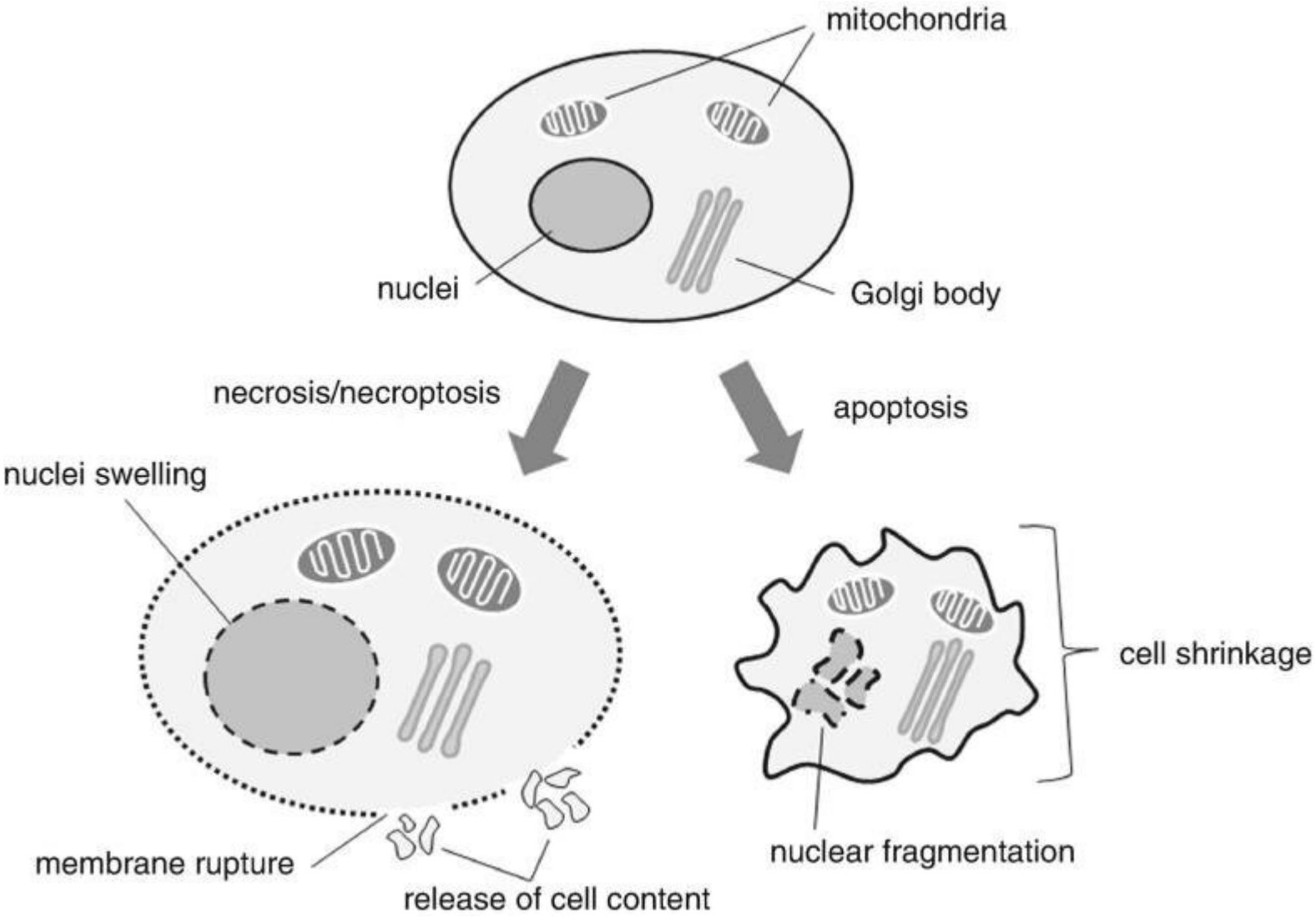

HeLa cells




Enyedi et al., *Cell,* 2016

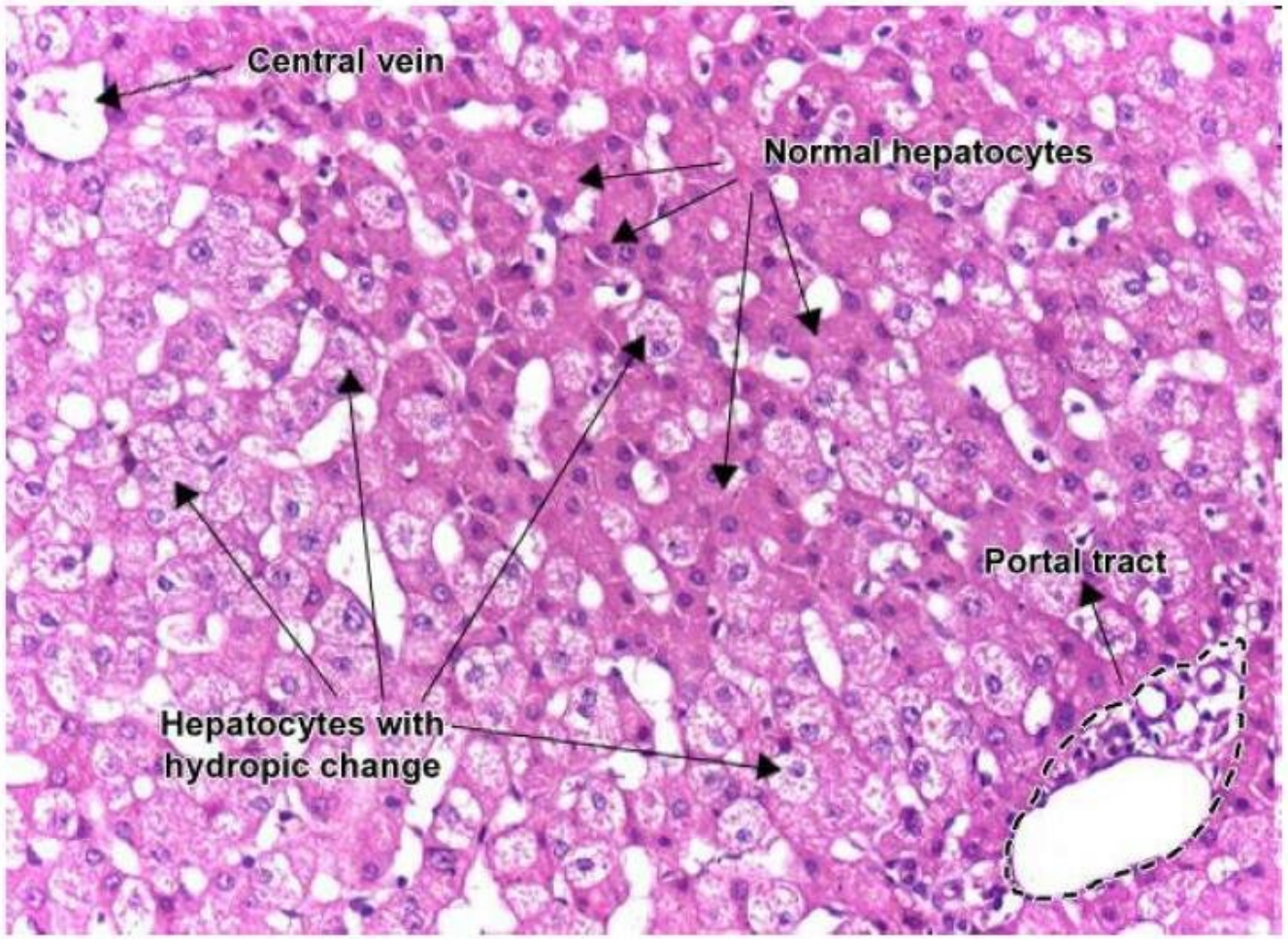
(fresh water ≅ saliva)

# Nuclear swelling during cell death



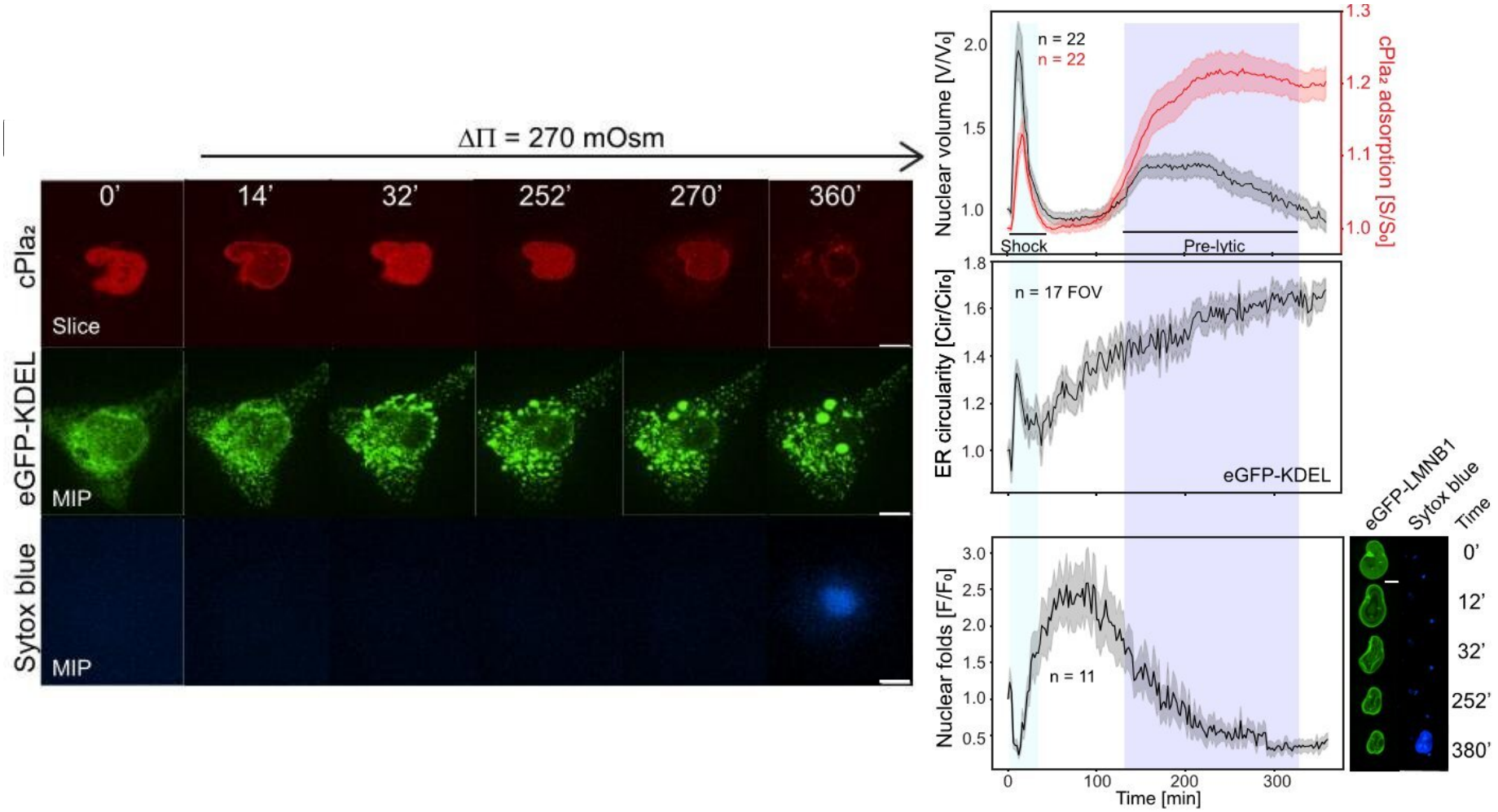

Yamashita & Abe, 2020

# Liver damage




https://www.life.illinois.edu/mcb/458/private/lectures/ppt_pdf/Path_ggf_3_2020.pdf

# Necrosis after osmotic shock



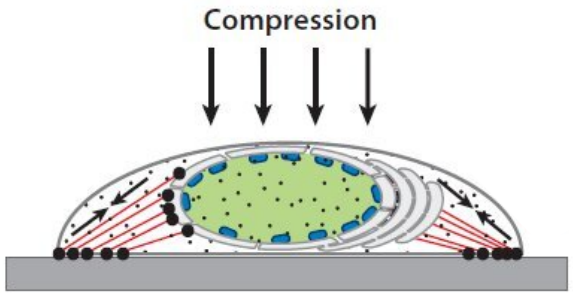

PMID: 39711539
NCB, *in press*

# Morphological/molecular proxies of nuclear membrane stretch

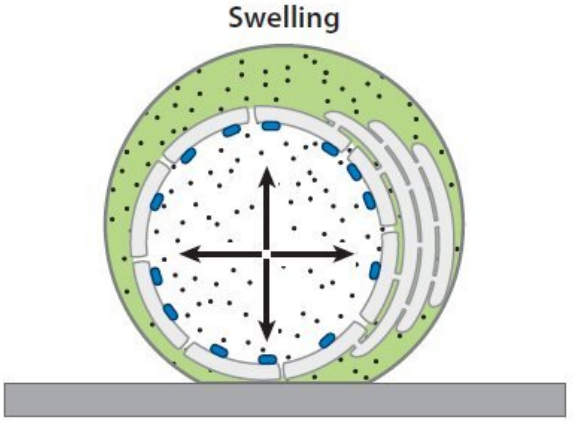
- Disappearance of nuclear membrane invaginations (smooth surface)
- Increase of nuclear pore distance
- Insertion of hydrophobic protein residues

# 2.
# Consequences of Nuclear Deformation

# Consequences of nuclear deformation

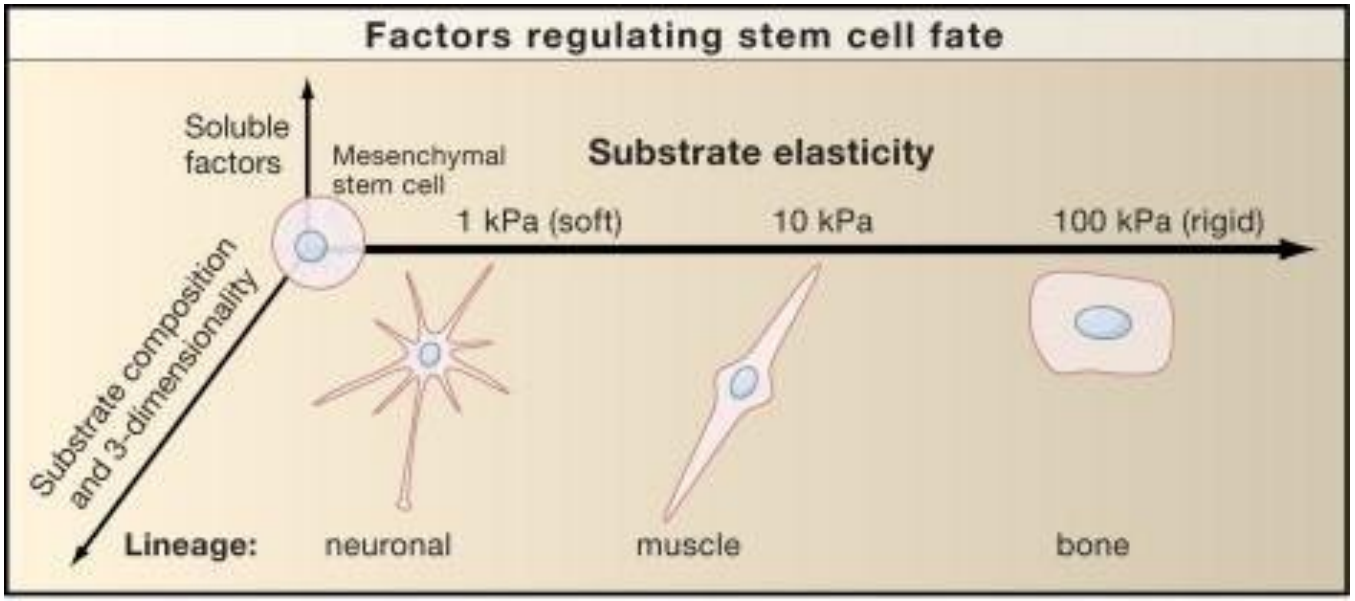



- Cell differentiation
- DNA damage/mutations
- Nuclear rupture/cytosolic DNA sensing
- Increase of cortical contractility/switch to ameboid motility



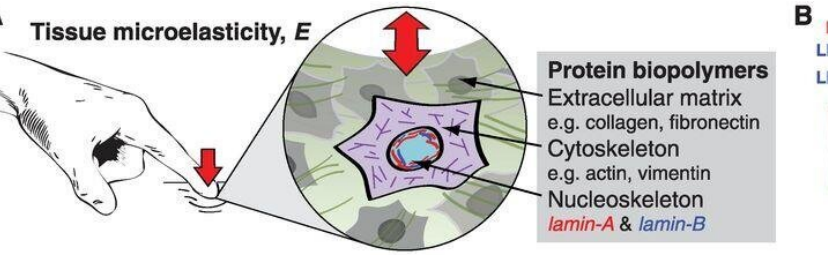

- Inflammatory signaling
- Extracellular chromatin release (NETosis)

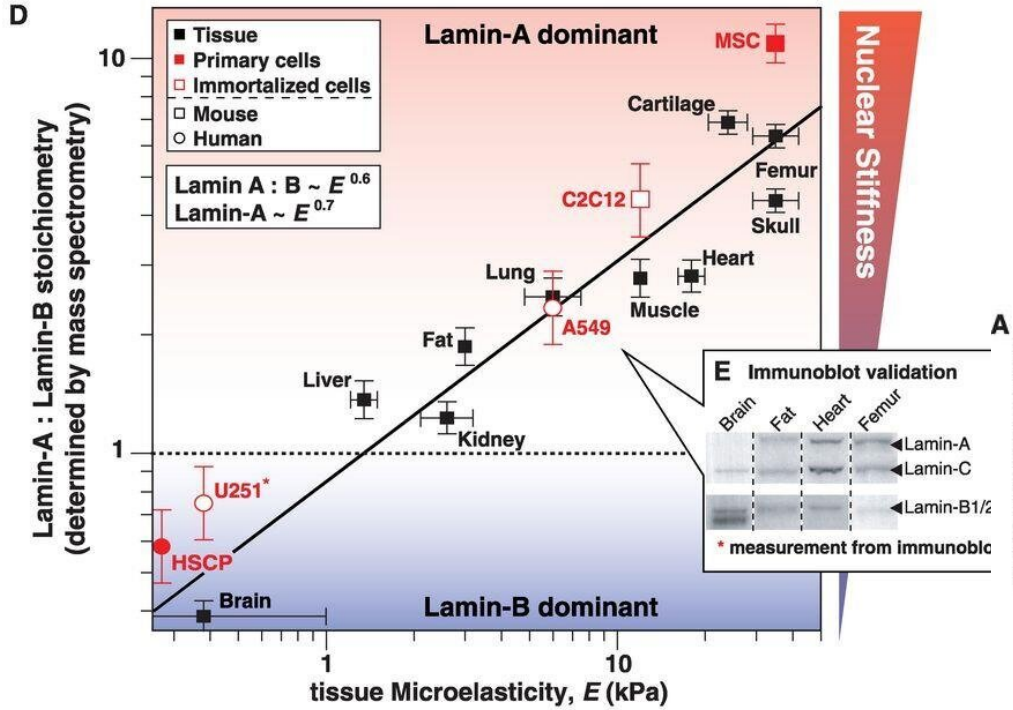
# Consequences of nuclear deformation: **Cell differentiation**

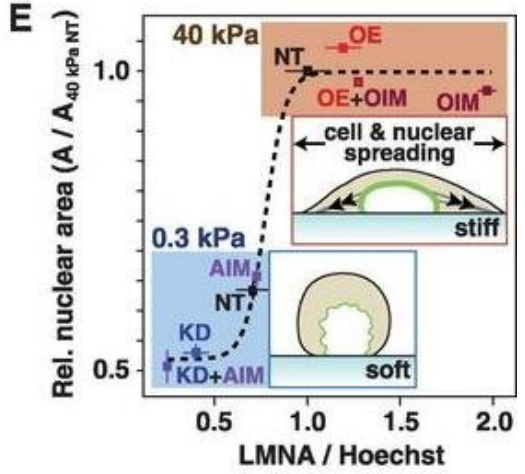


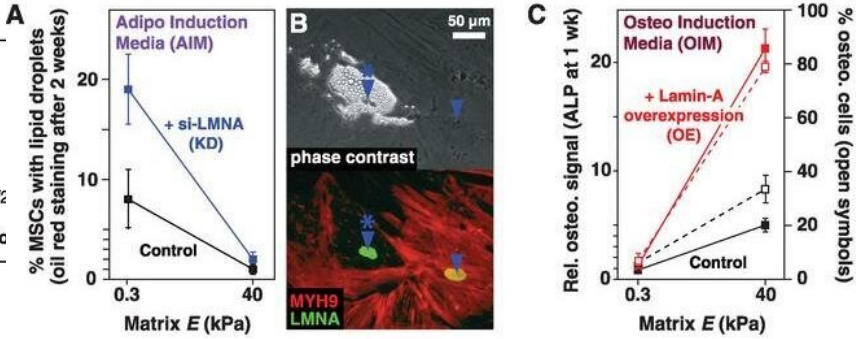

Even-Ram et al., *Cell,* 2006

# Consequences of nuclear deformation: **Cell differentiation**



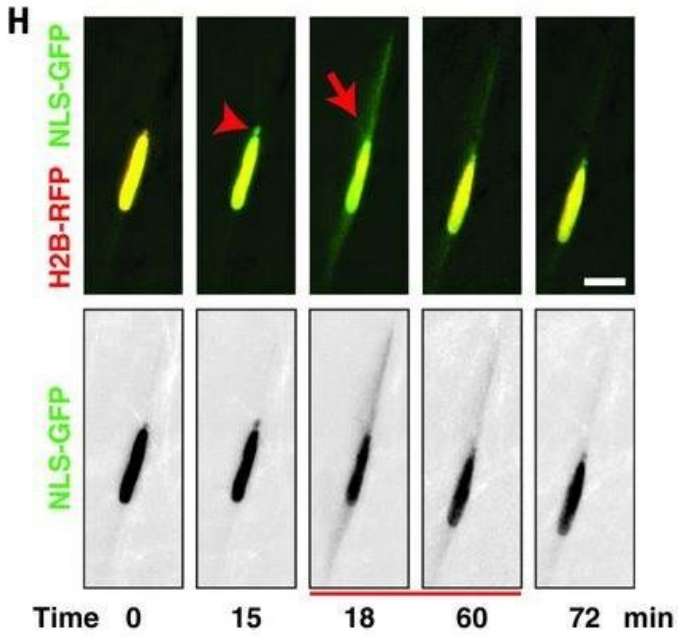



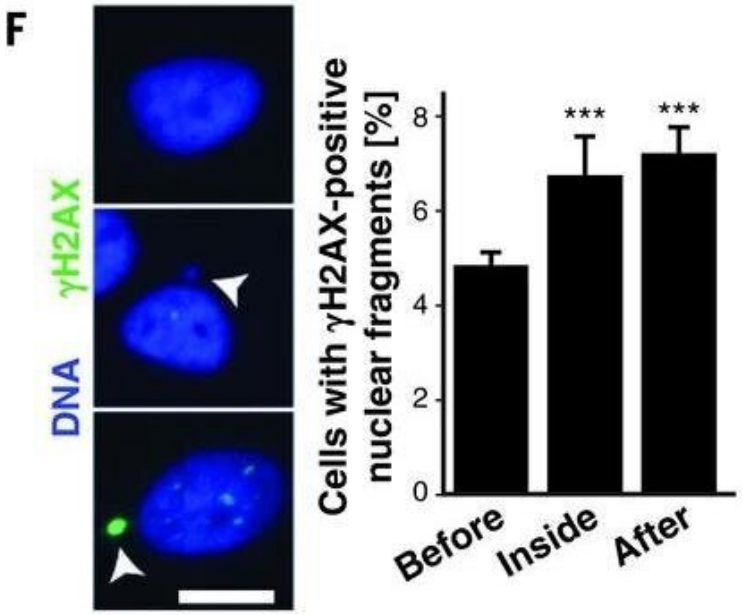




Swift et al., *Science,* 2013

# Consequences of nuclear deformation: **DNA damage**

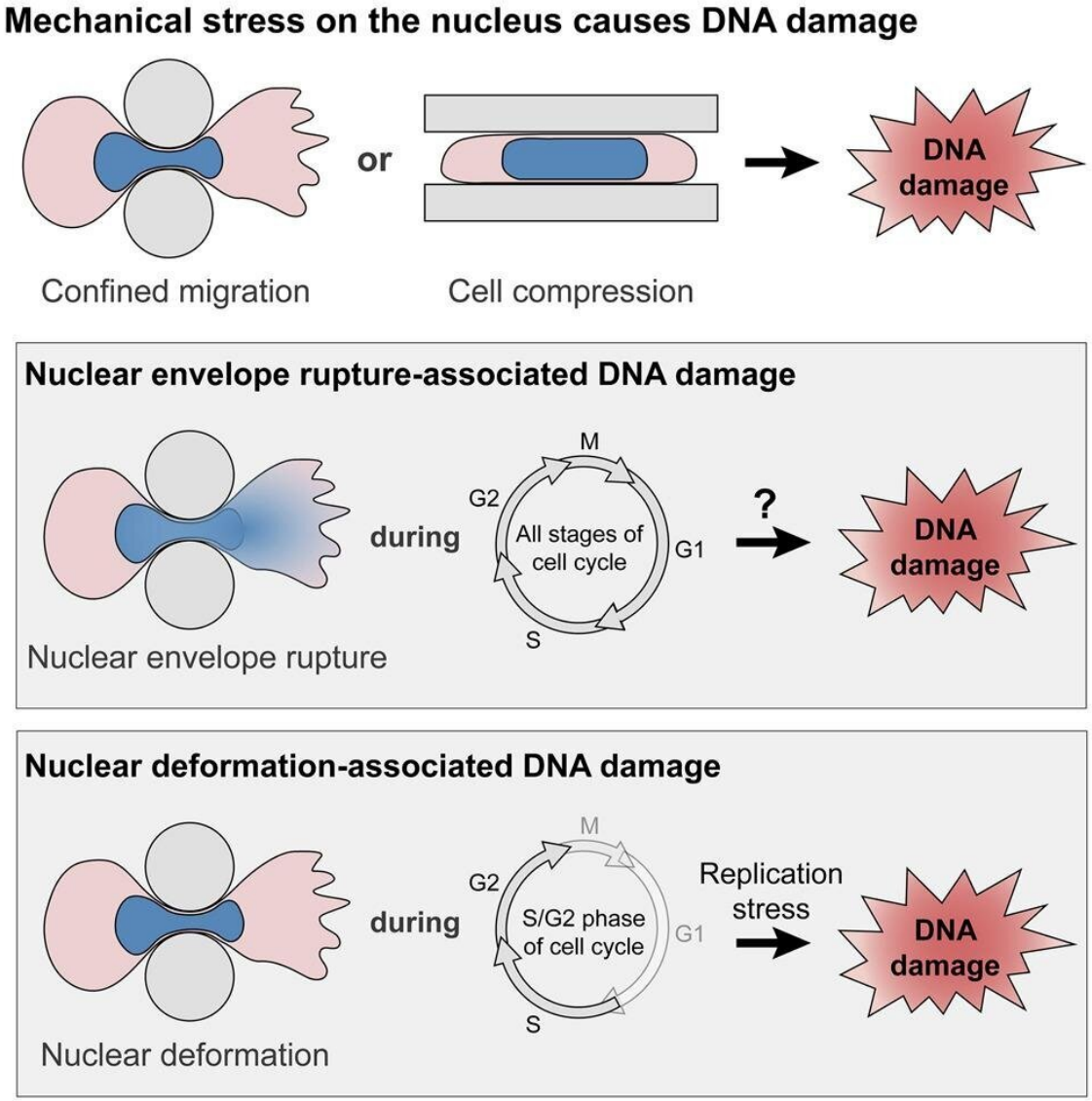
Nuclear membrane rupture

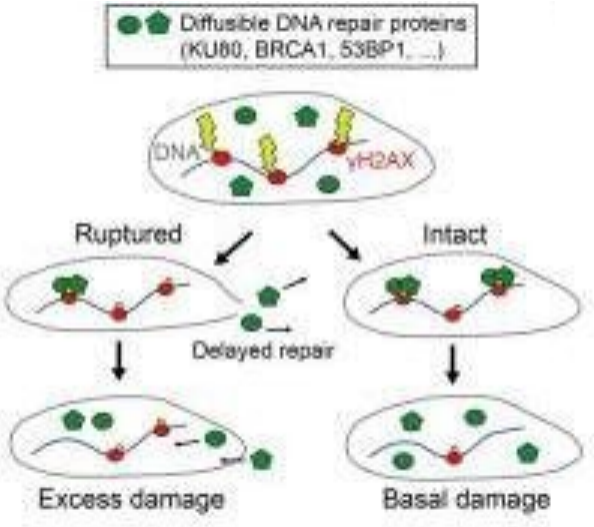



Denais et al., *Science,* 2016

# Consequences of nuclear deformation: **DNA damage**



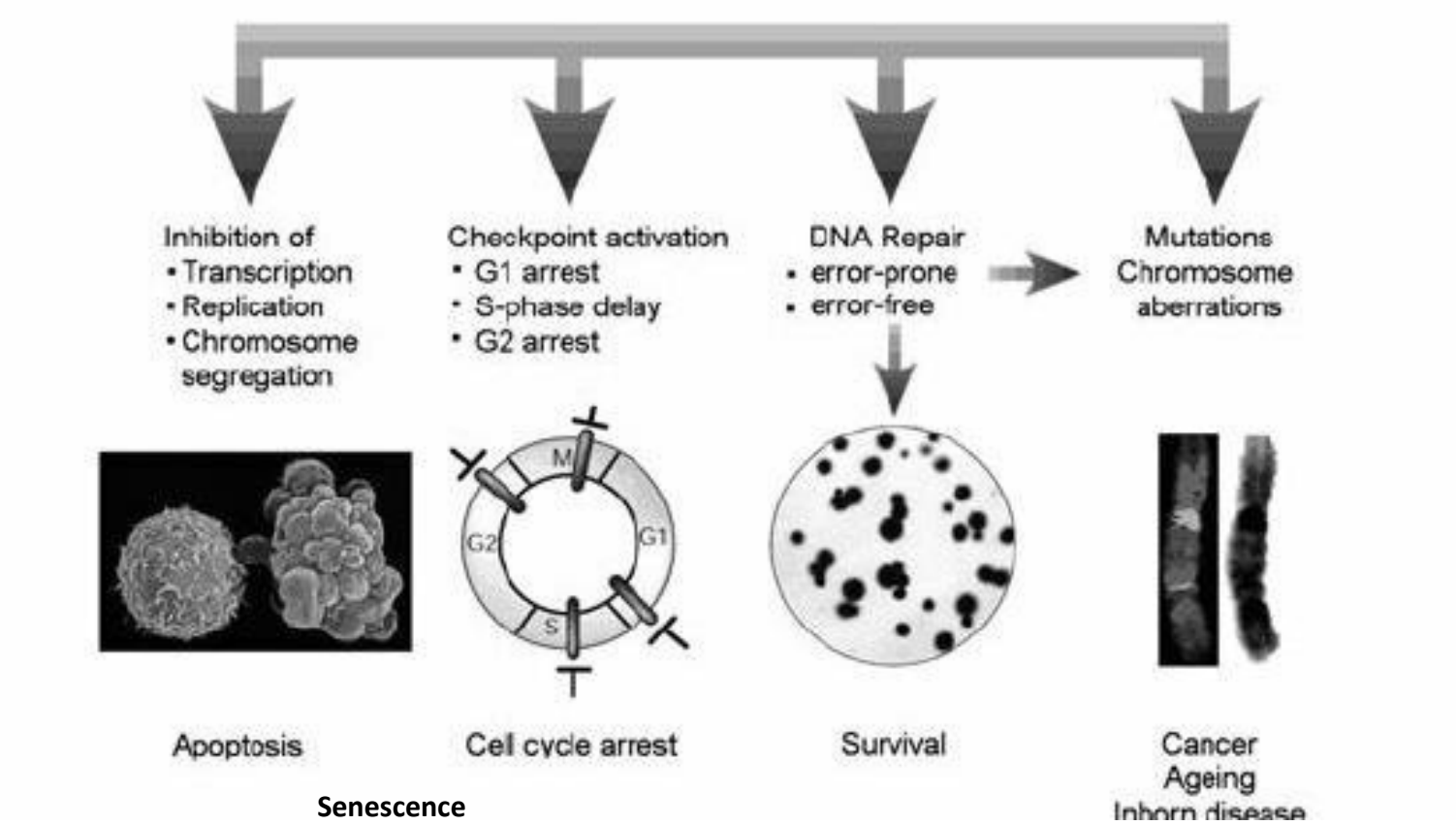




Contesting hypothesis: Deformation causes DNA damage and replication stress independent from each other

Sha et al., *Curr Biol,* 2021

Pfeifer et al., *MBoC,* 2018

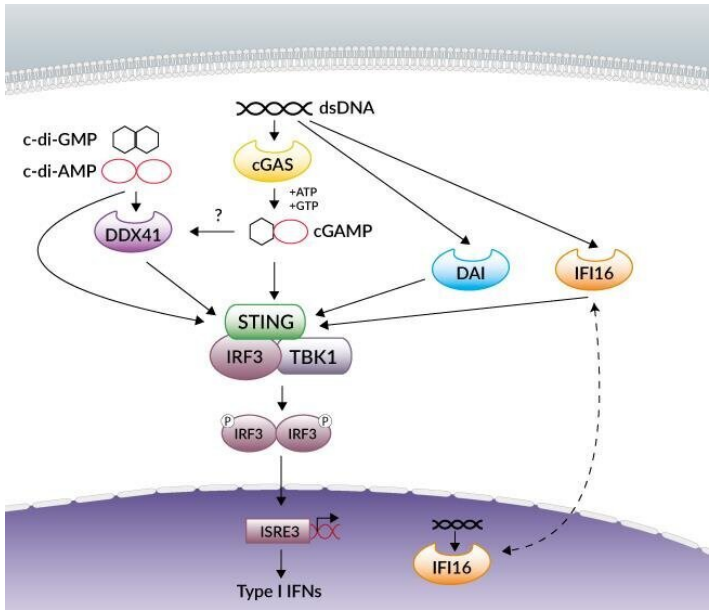
# Consequences of DNA damage



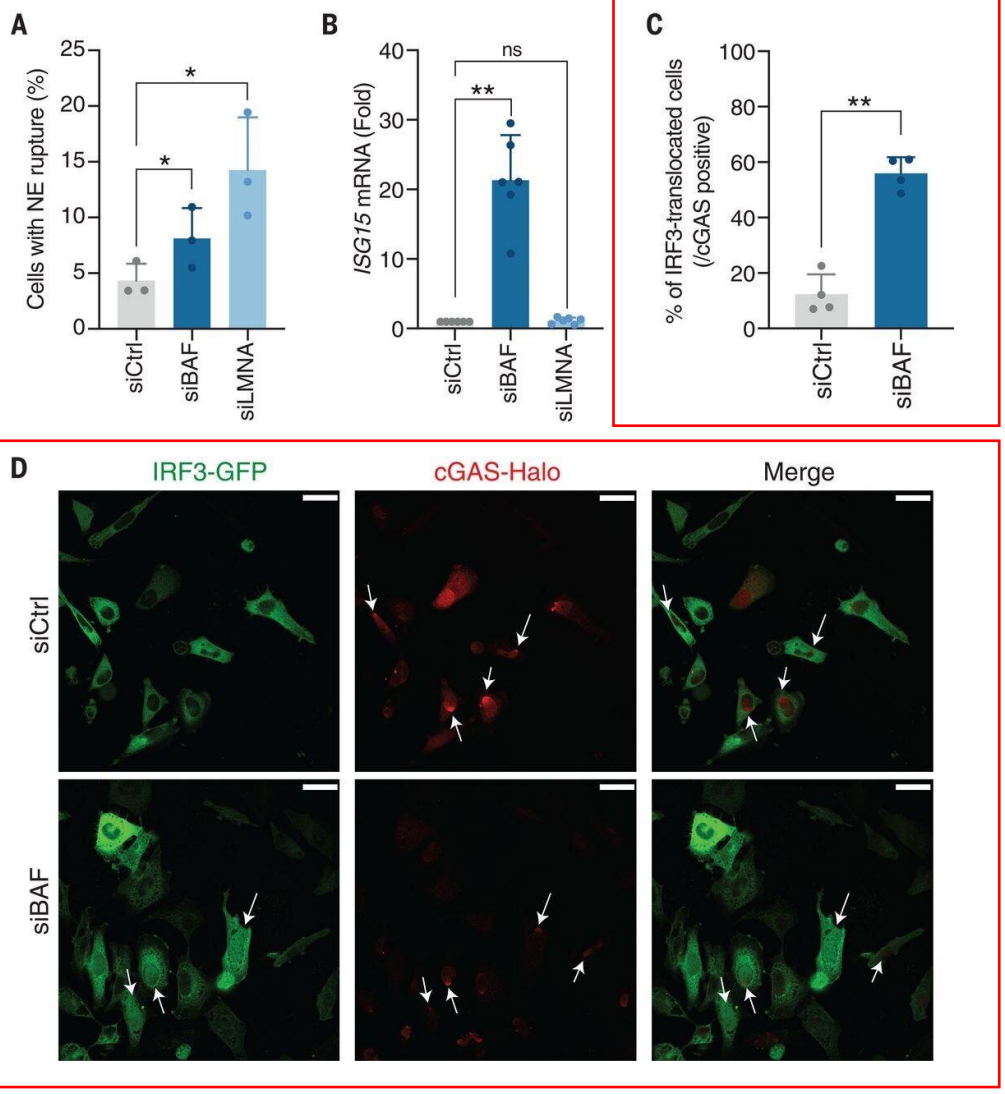

Baumstark-Khan et al., *Adv Biochem Engin Biotechnol,* 2010

# Consequences of nuclear rupture: Cytosolic DNA-sensing

cGAS/STING pathway




https://www.invivogen.com/review-cgamp



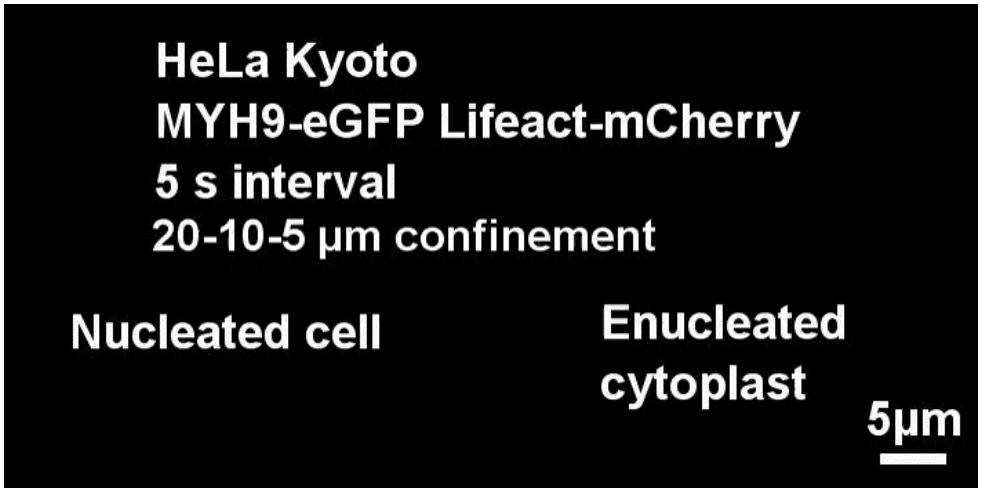

3 µm confinement

Guey et al., *Science*, 2020

# Consequences of nuclear deformation: Switch in cortical contractility


Lomakin et al., *Science,* 2020

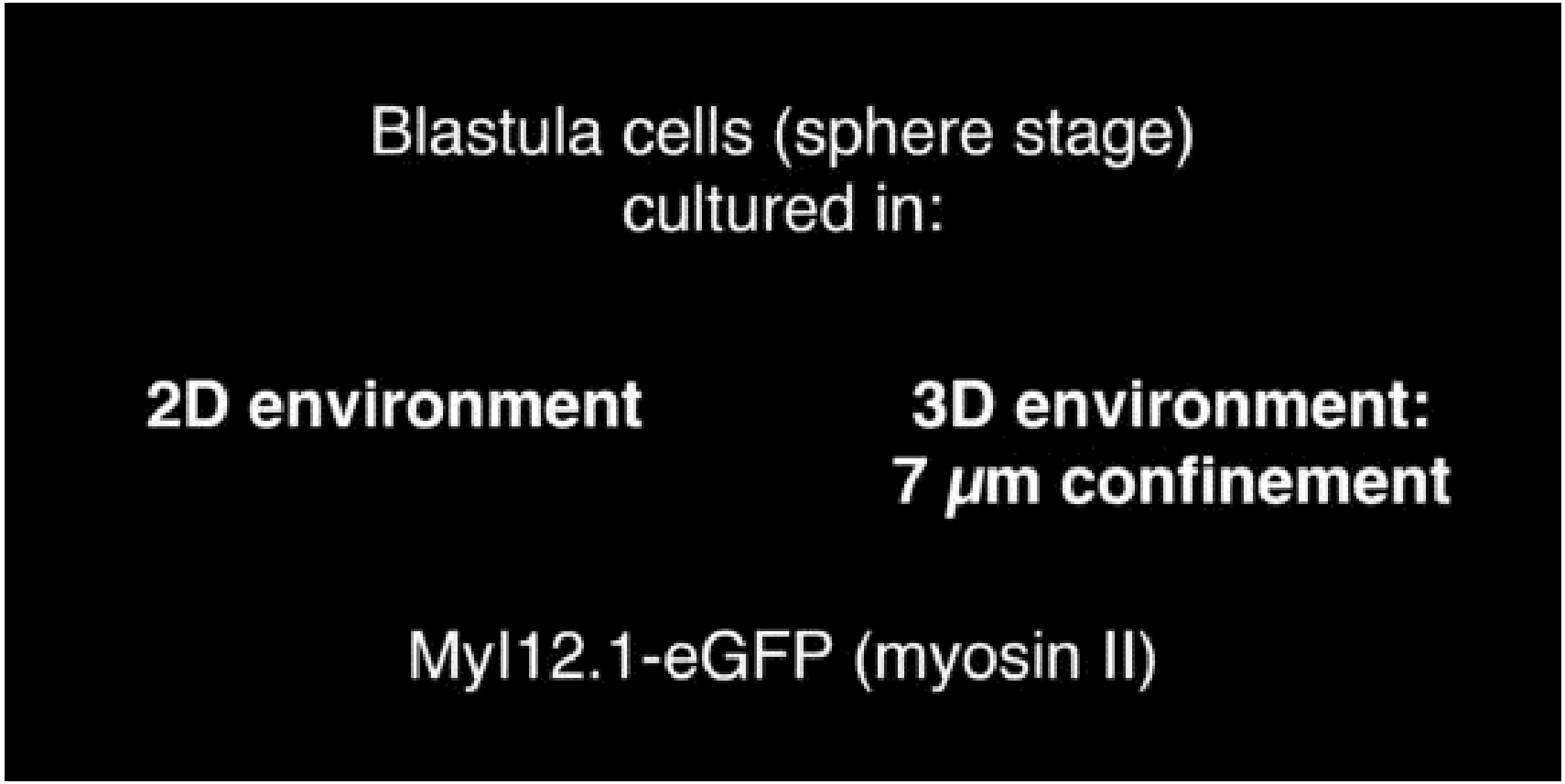
# Consequences of nuclear deformation: Switch in cortical contractility





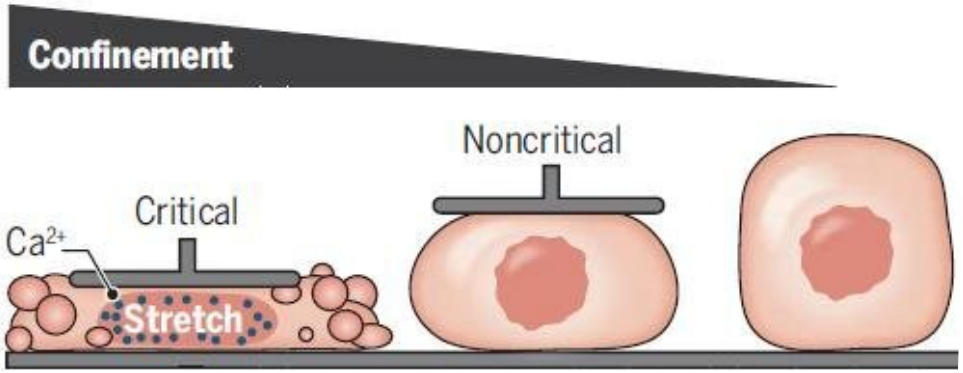
Lomakin et al., *Science,* 2020

# Consequences of nuclear deformation: Switch in motility



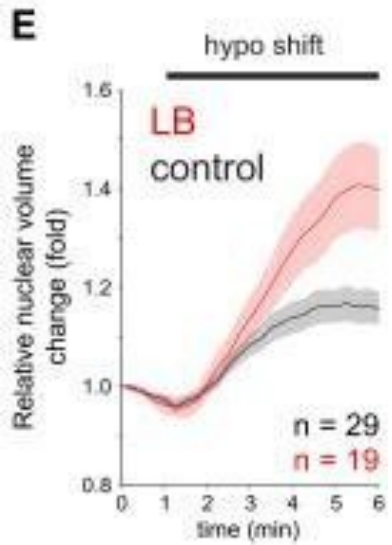

Venturini et al., *Science,* 2020

# Consequences of nuclear deformation: A cellular sense of space and pressure

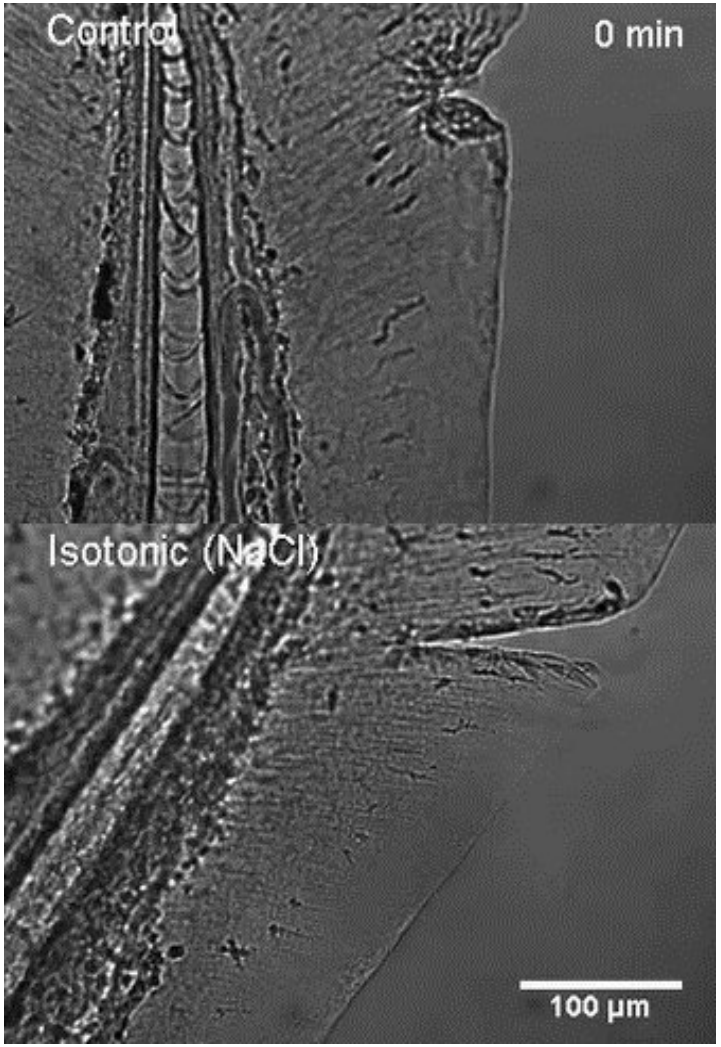



Shen & Niethammer et al., *Science,* 2020

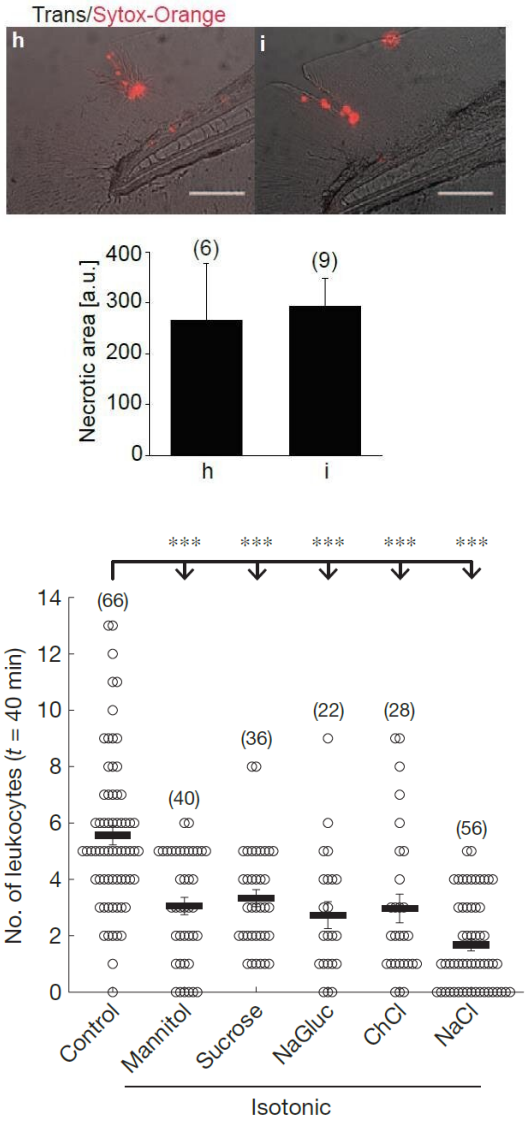
# Consequences of nuclear deformation: inflammation/osmotic surveillance



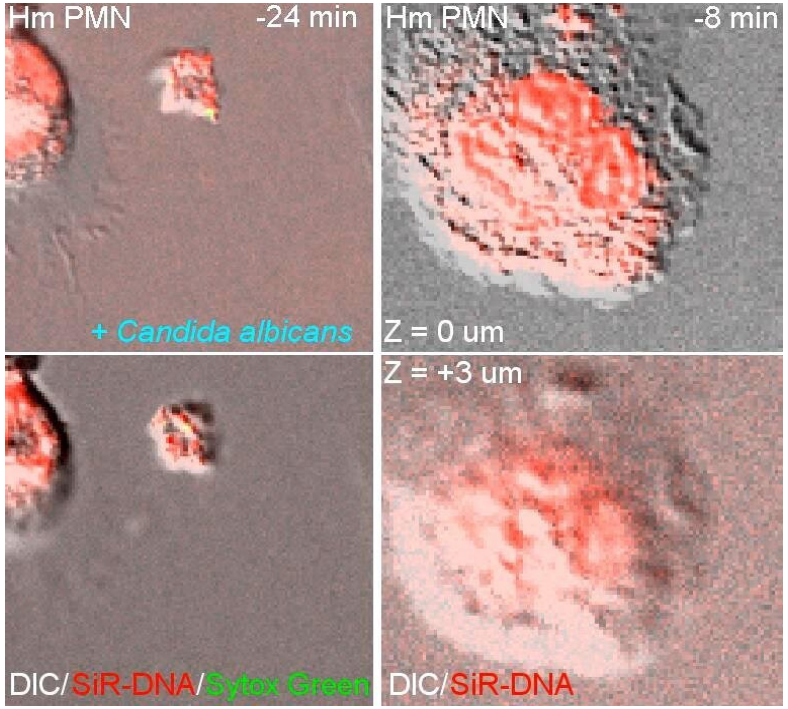

Enyedi et al., *Cell*, 2016



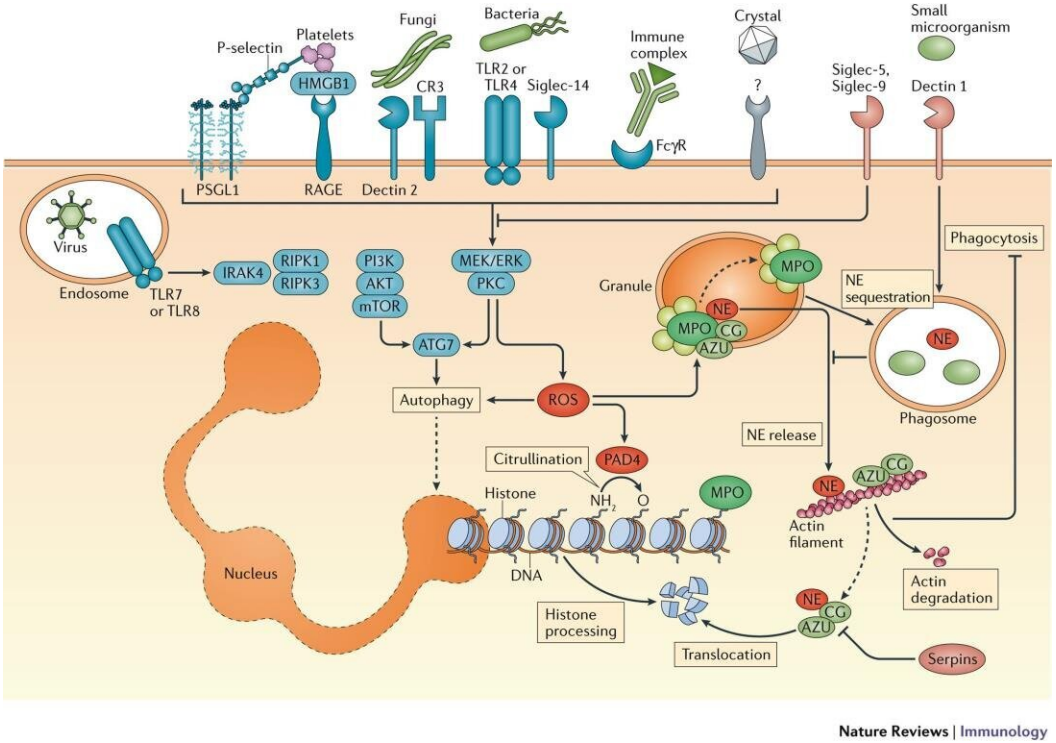

Enyedi et al., *Nat Cell Biol*, 2013



# Consequences of nuclear deformation: NETosis (chromatin puking)



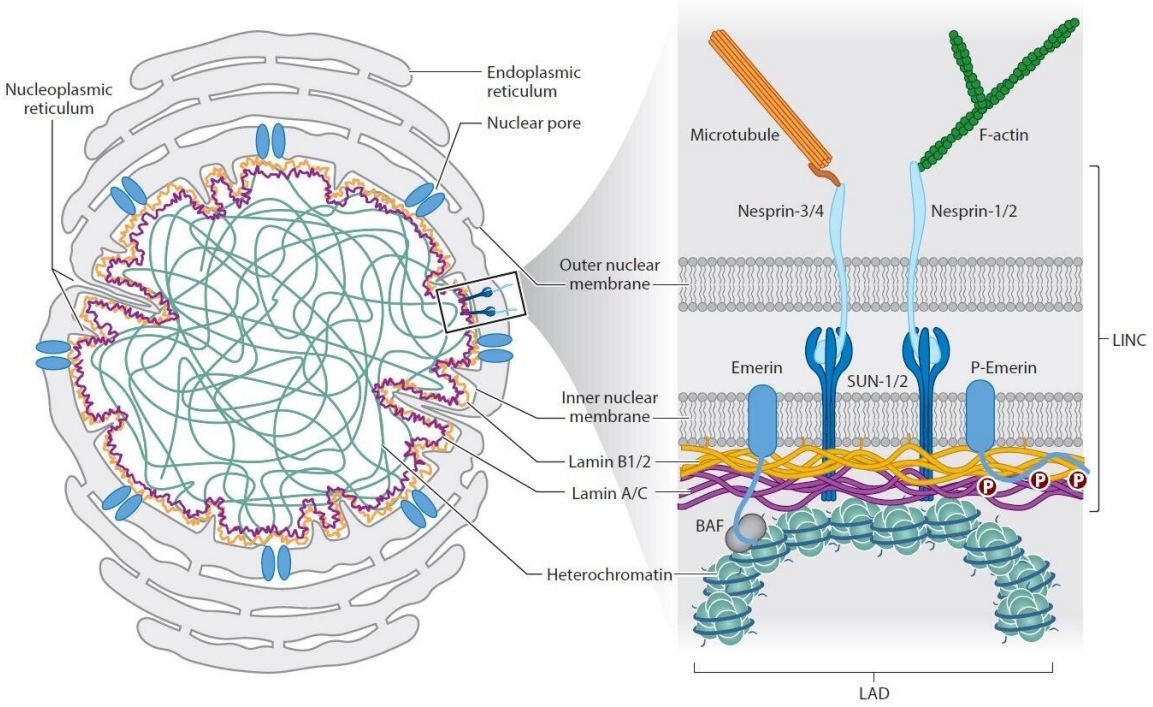

Thiam et al., *PNAS*, 2020




Papayannopoulos, *Nat Rev Immun,* 2017

# 3.
# How do Nuclear Components Respond to Load

# The components of nuclear mechanotransduction



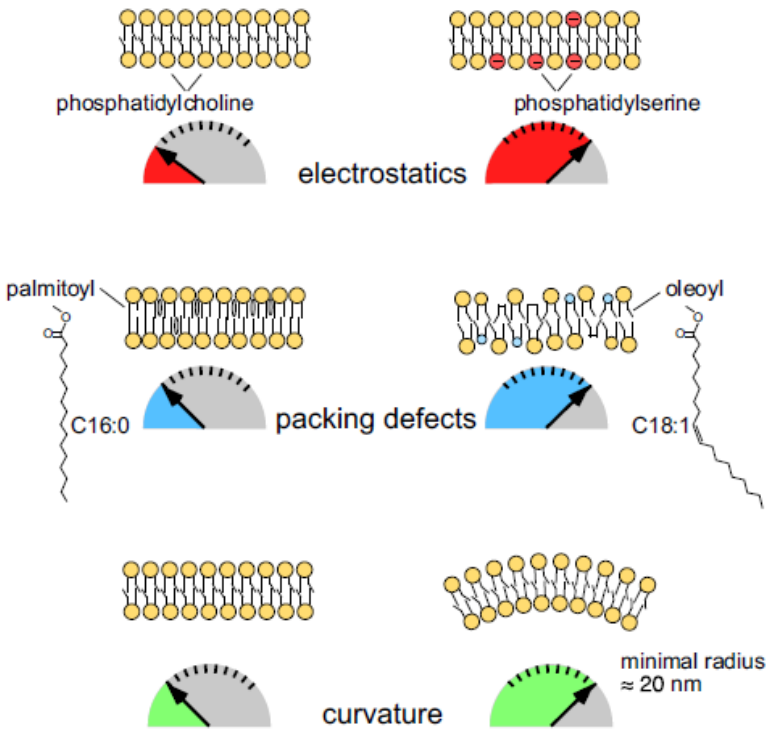

a) Nuclear membrane (NM)
b) Chromatin
c) Nuclear lamina

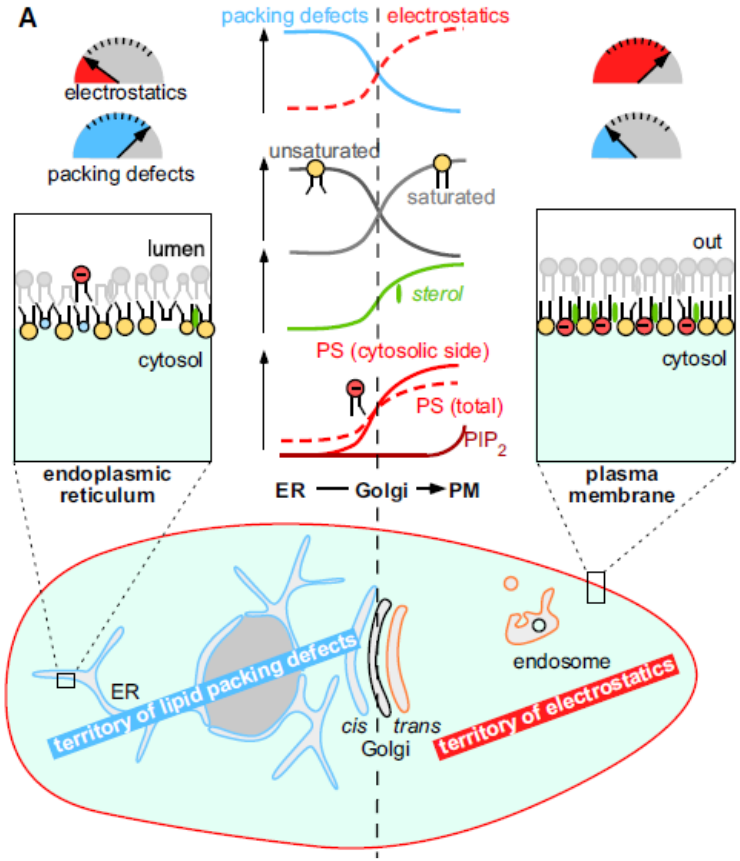
Form composite structure that responds to mechanical stress as unit (=complex mechanics)

Niethammer, *Ann Rev Cell Dev Biol*,2021

# 3a.
# The nuclear membrane under load

# Nuclear vs. plasma membrane lipid composition



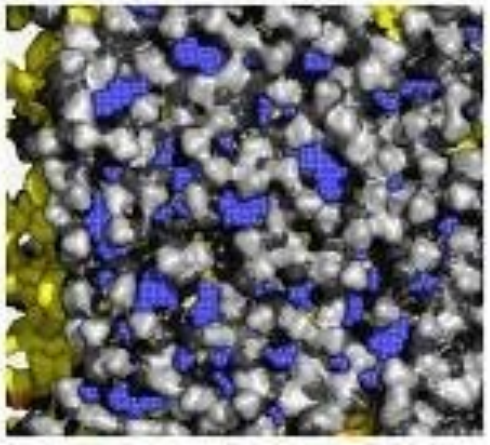




Bigay & Antonny, *Dev Cell*,2012

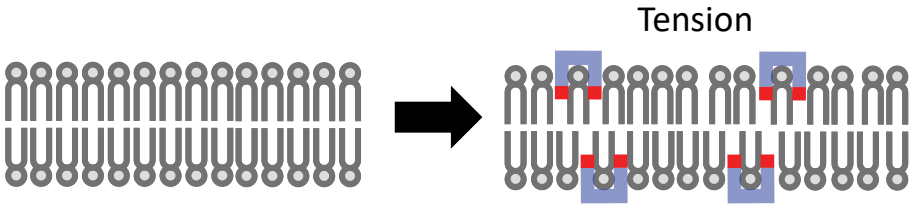
# Nuclear membrane protein composition

- >250 NM-associated proteins
- PL:Protein (w/w)~ 0.2-0.5
  (PM~0.3-0.8)
- NPC-proteins
- LEM family proteins (INM, integral)
- SUN-proteins (INM, integral)
- KASH proteins (ONM, integral)
- INM vs. ONM localization by diffusion-retention
- Lamin B associated via farnesyl anchor

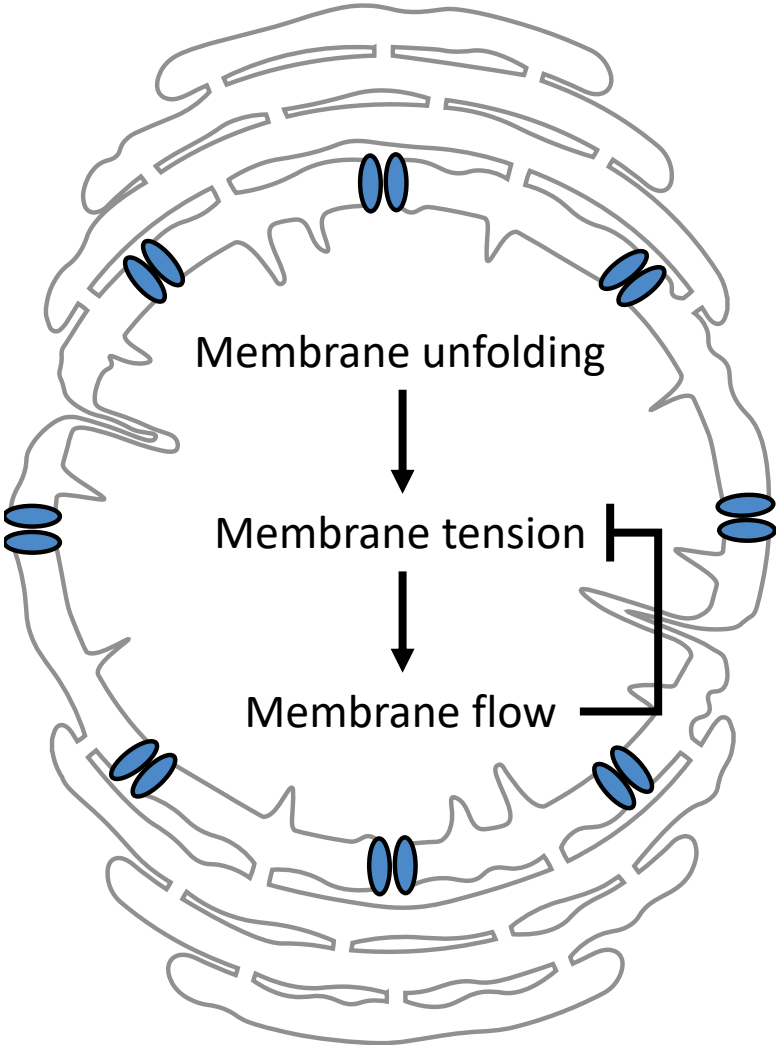
# The nuclear membrane under load

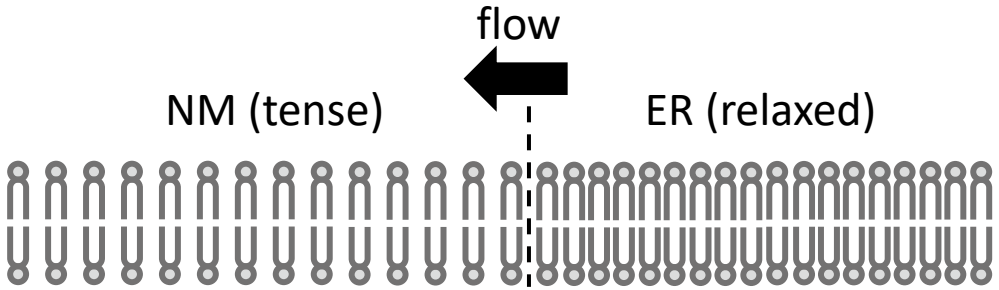


Vanni et al., *Nat Comms*, 2014



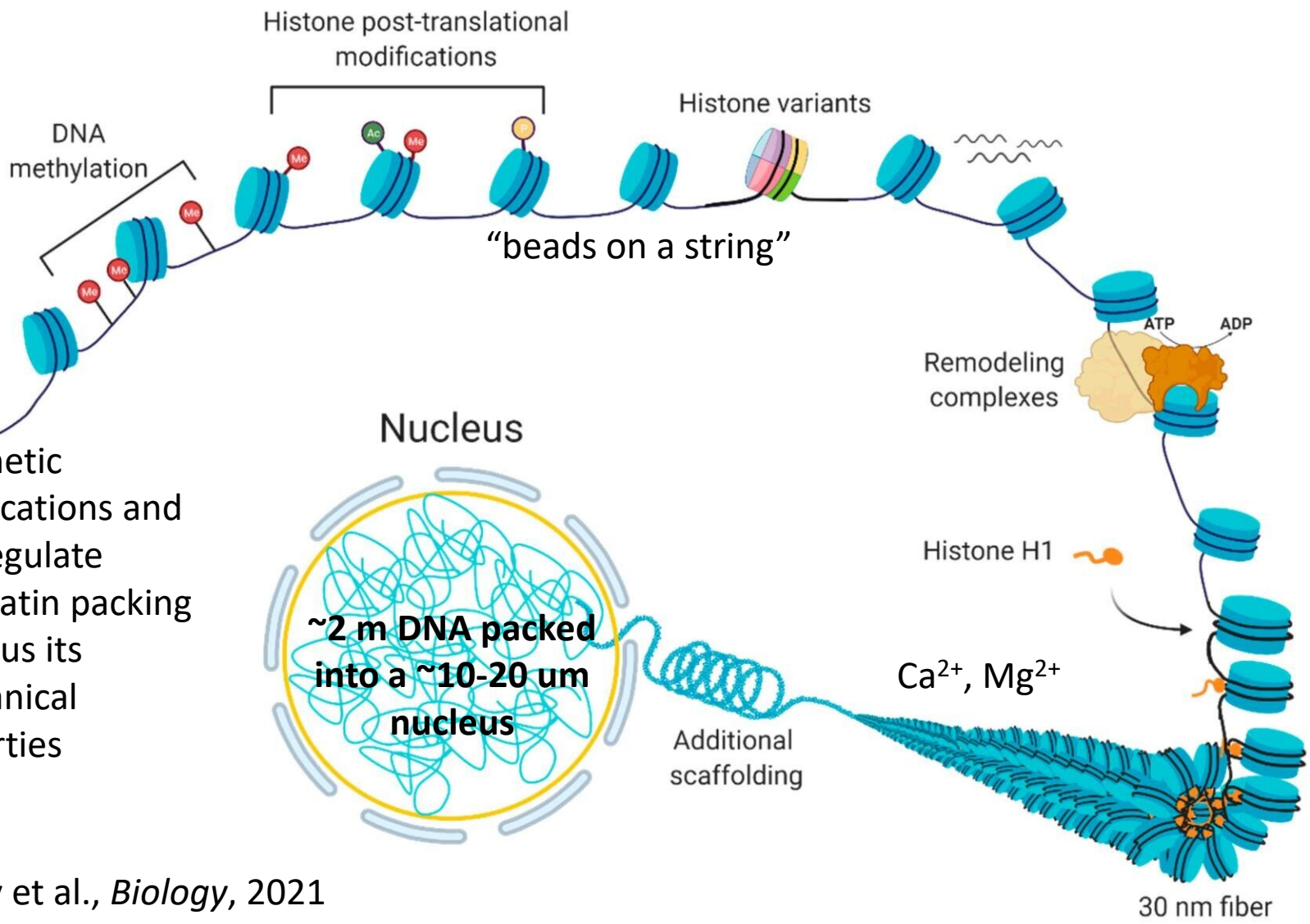
# The nuclear membrane under load



# 3b.
# Chromatin under load

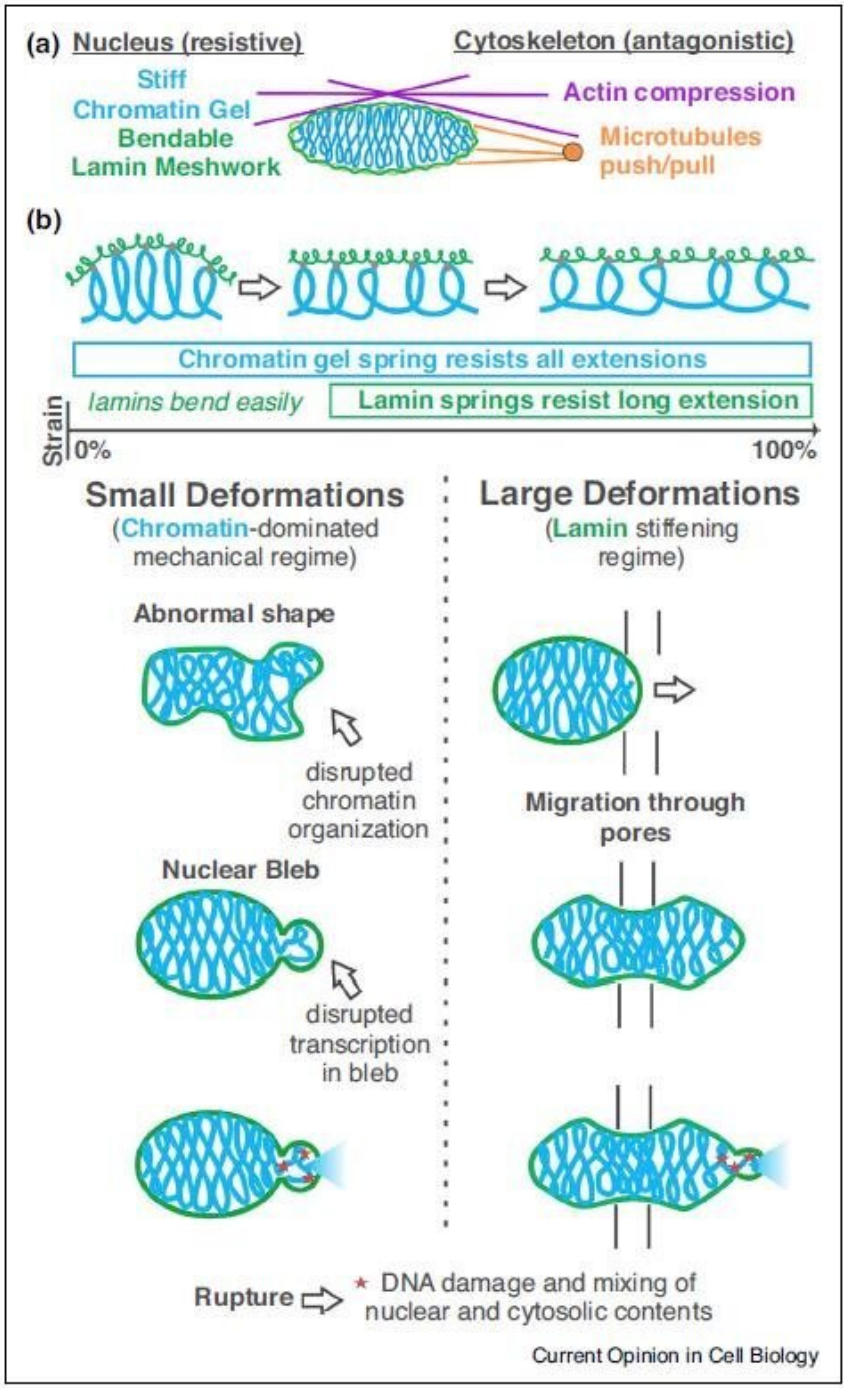
# Chromatin structure




Epigenetic modifications and ions regulate chromatin packing and thus its mechanical properties

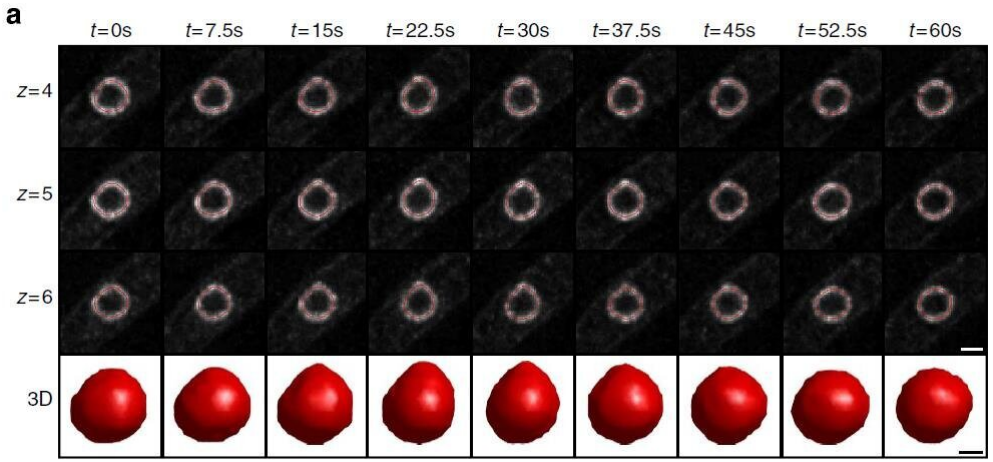
Alpsoy et al., *Biology*, 2021

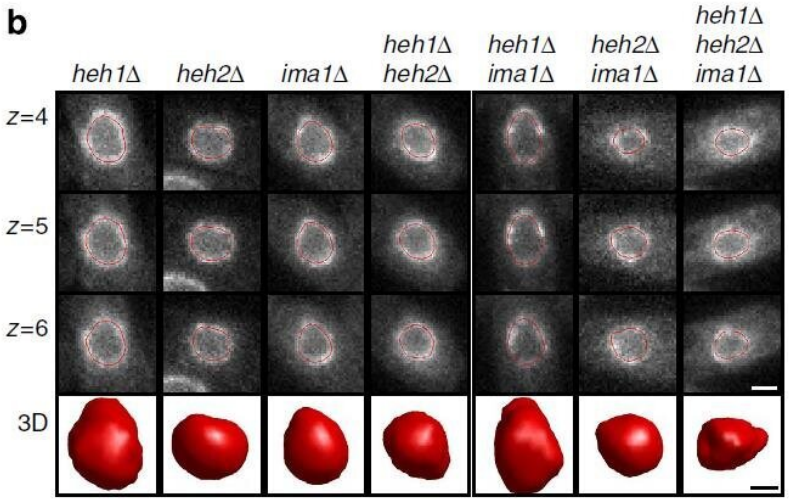
# Chromatin resists small deformations

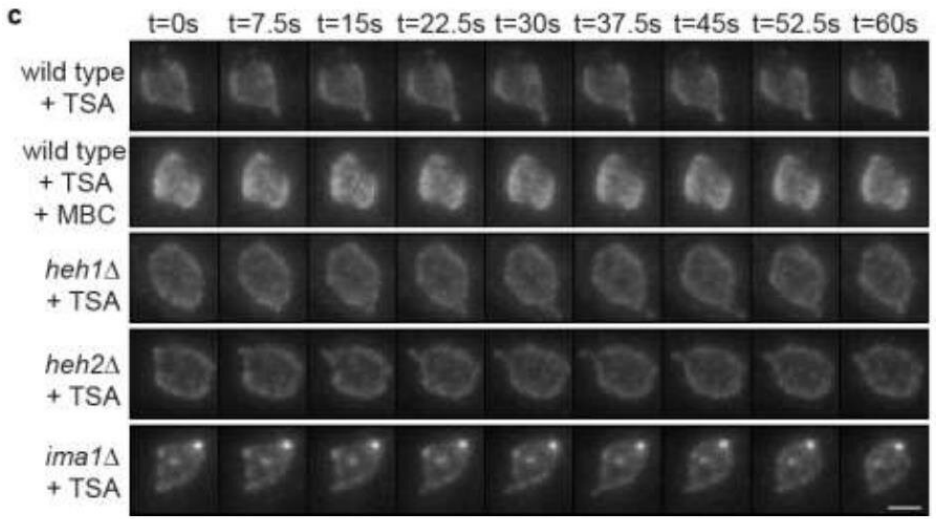


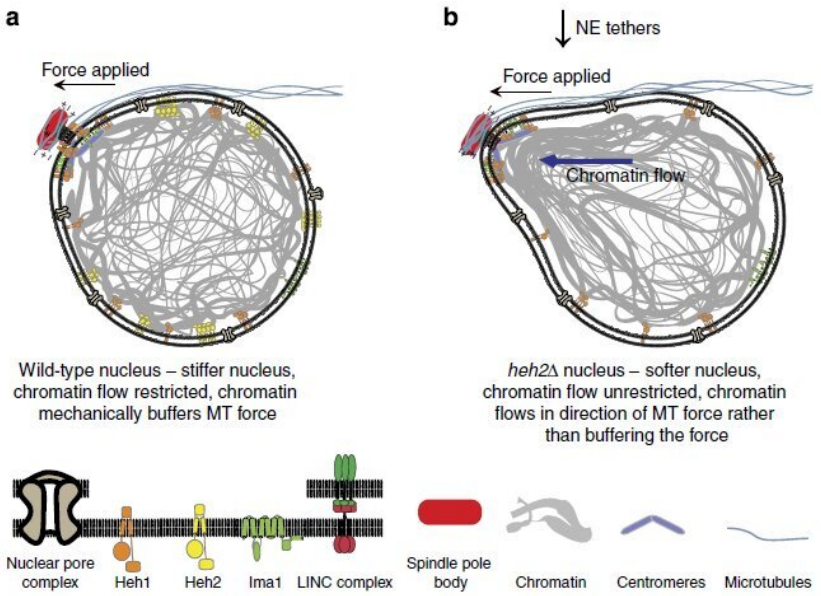

Stephens et al., *Curr Opin Cell Biol*, 2019

# Chromatin-tethering to the NM restricts small deformations in yeast



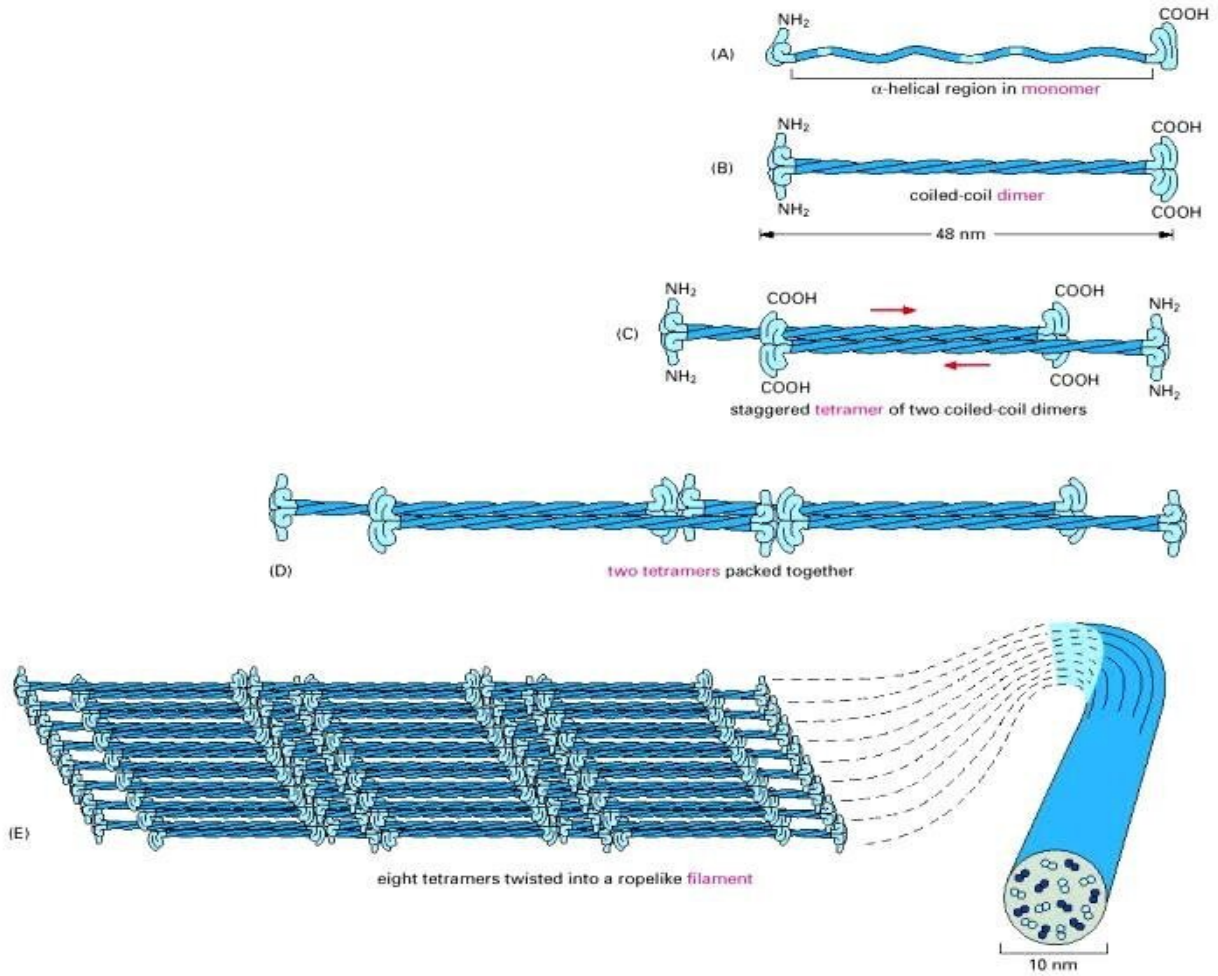




Schreiner et al., *Nat Comm*, 2015

# 3c.
# The lamina under load

# Intermediate Filaments are rope-like and lack intrinsic polarity




Monomer

Parallel Dimer

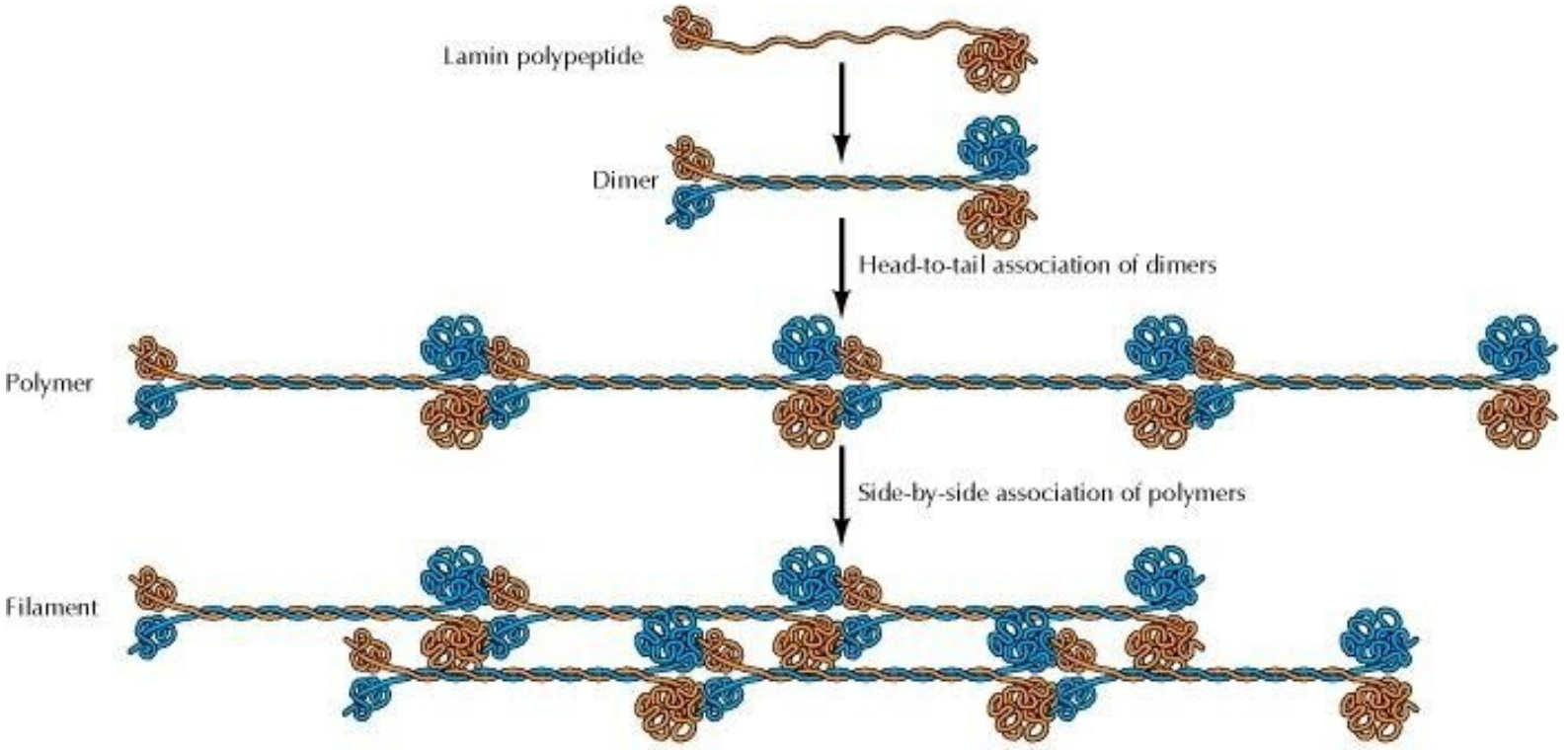
Antiparallel
Staggered
Tetramer

Protofilament

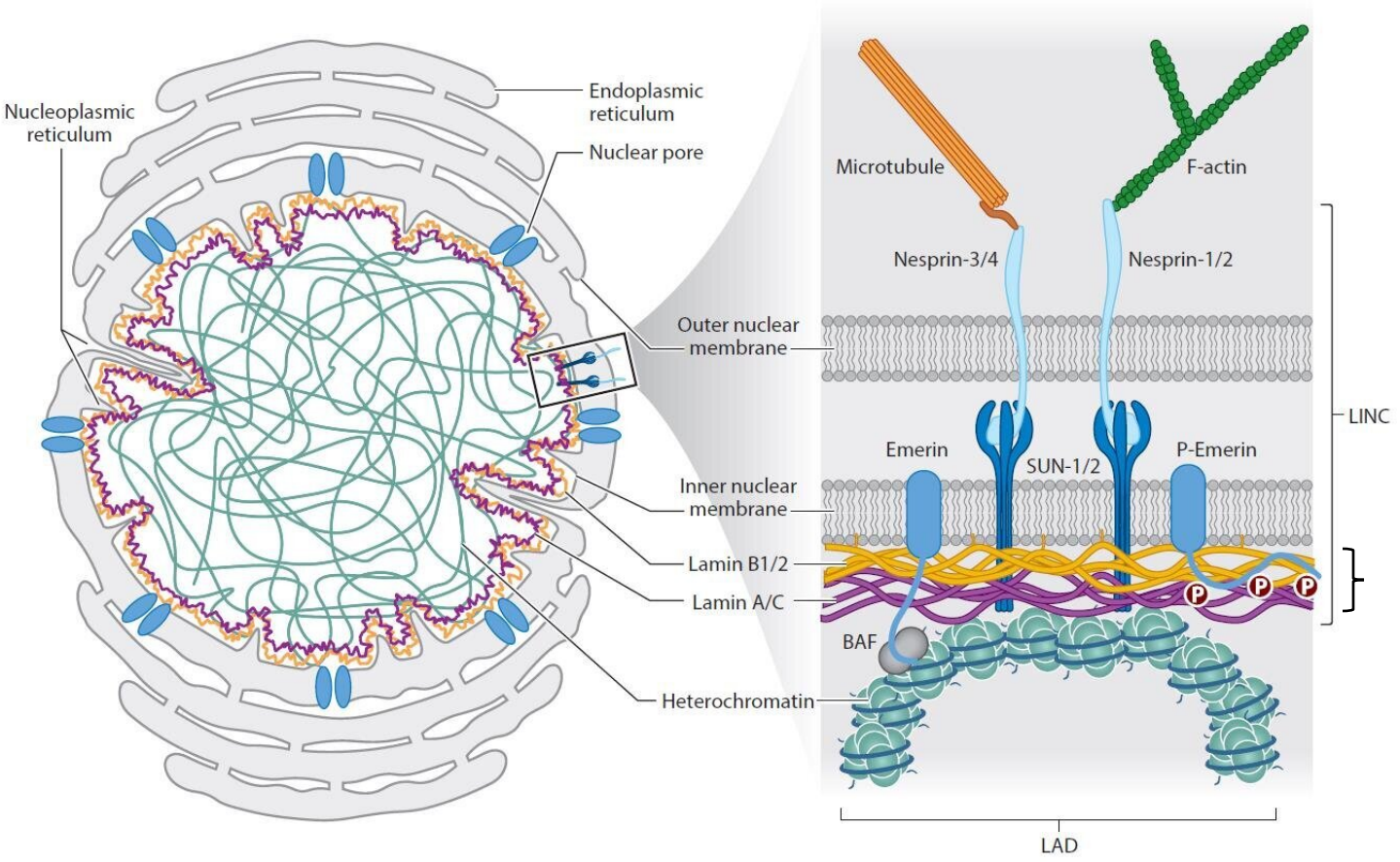
Filament
(16 coiled-coils)

# Lamins: Class V intermediate filaments

B-type lamins: Every animal cell. Not in yeast. Plant functional homolog: CROWDED NUCLEI (CRWN)
A-type lamins (Lamin A/C): Only expressed after gastrulation. Low in stem cells. Form homodimers.



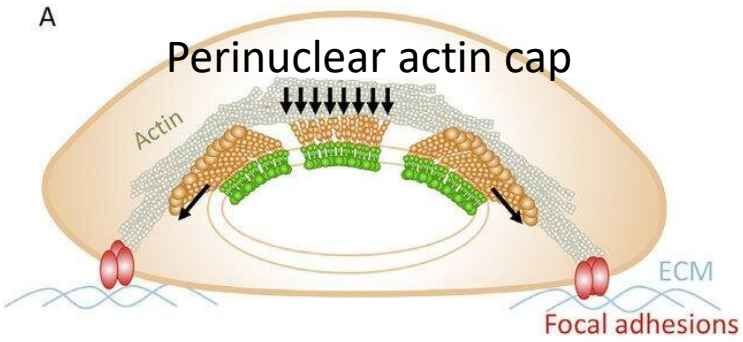
# Nuclear lamina structure & connectivity



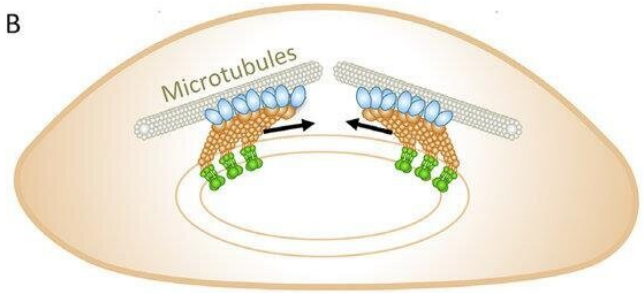

~10-200 nm thickness

Niethammer, *Ann Rev Cell Dev Biol*,2021

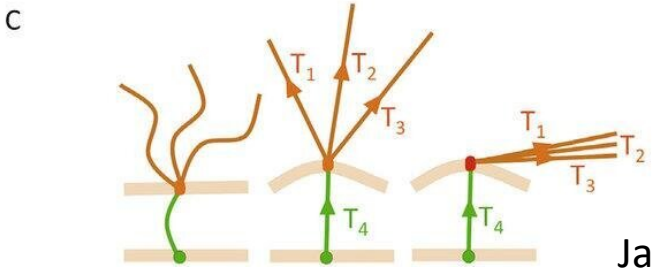
# Lamina-cytoskeleton mechanically couple the nucleus to the extracellular space




Direct mechanical regulation of gene expression
(

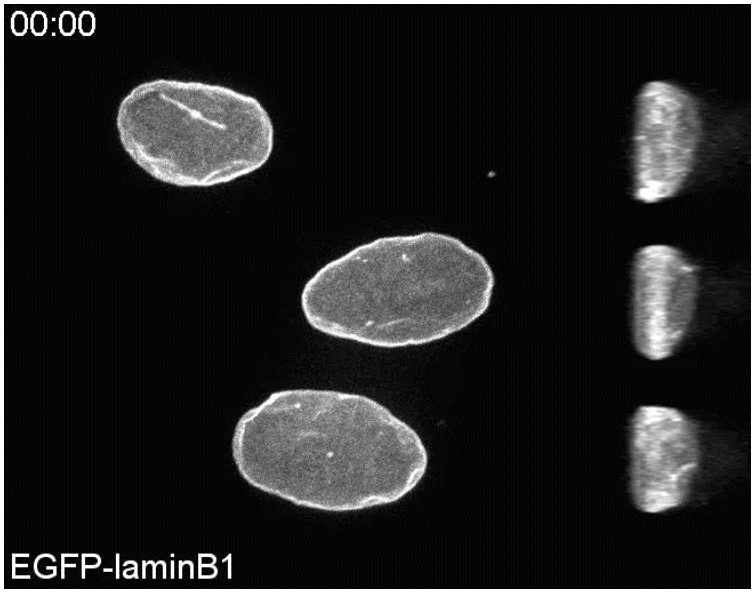



Stiffening of the nucleus (Guilluy et al., *Nat Cell Bio*, 2014)

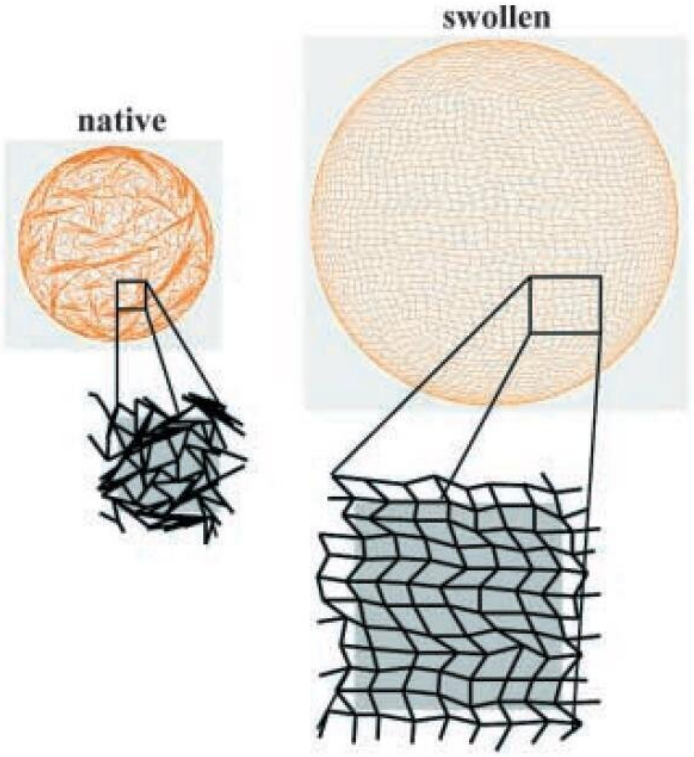



Jahed & Mofrad, 2018

# The nuclear lamina under load: a “molecular shock absorber”

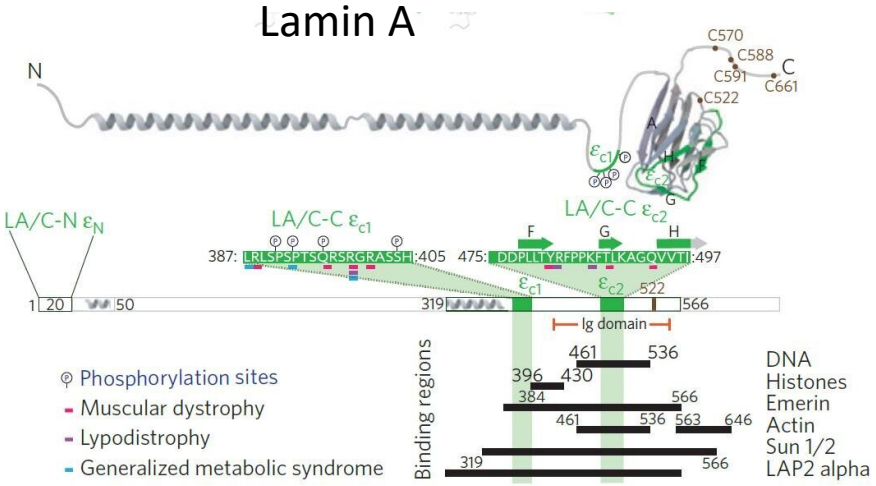


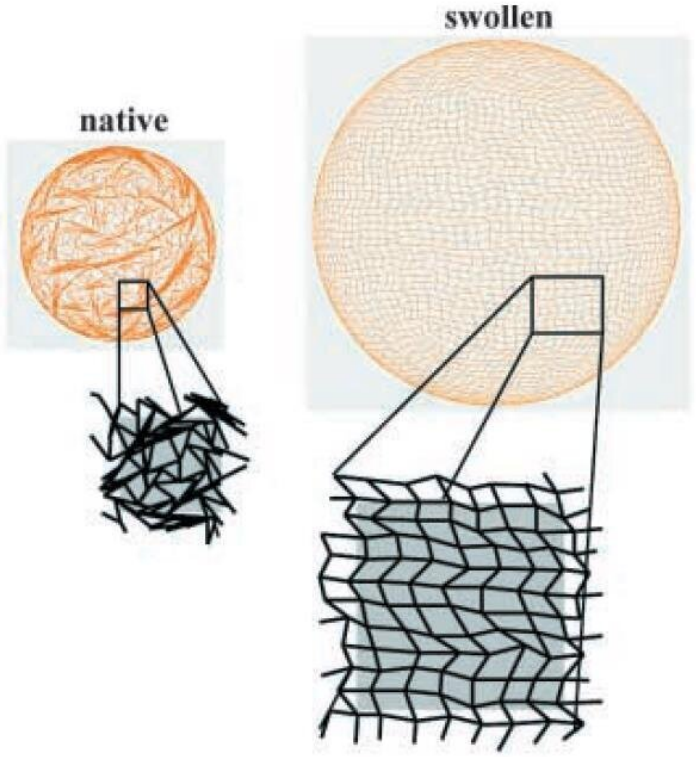

Enyedi et al., *Cell,* 2016




Dahl et al., *JCS,* 2004

# The nuclear lamina under load: a “molecular shock sensor”



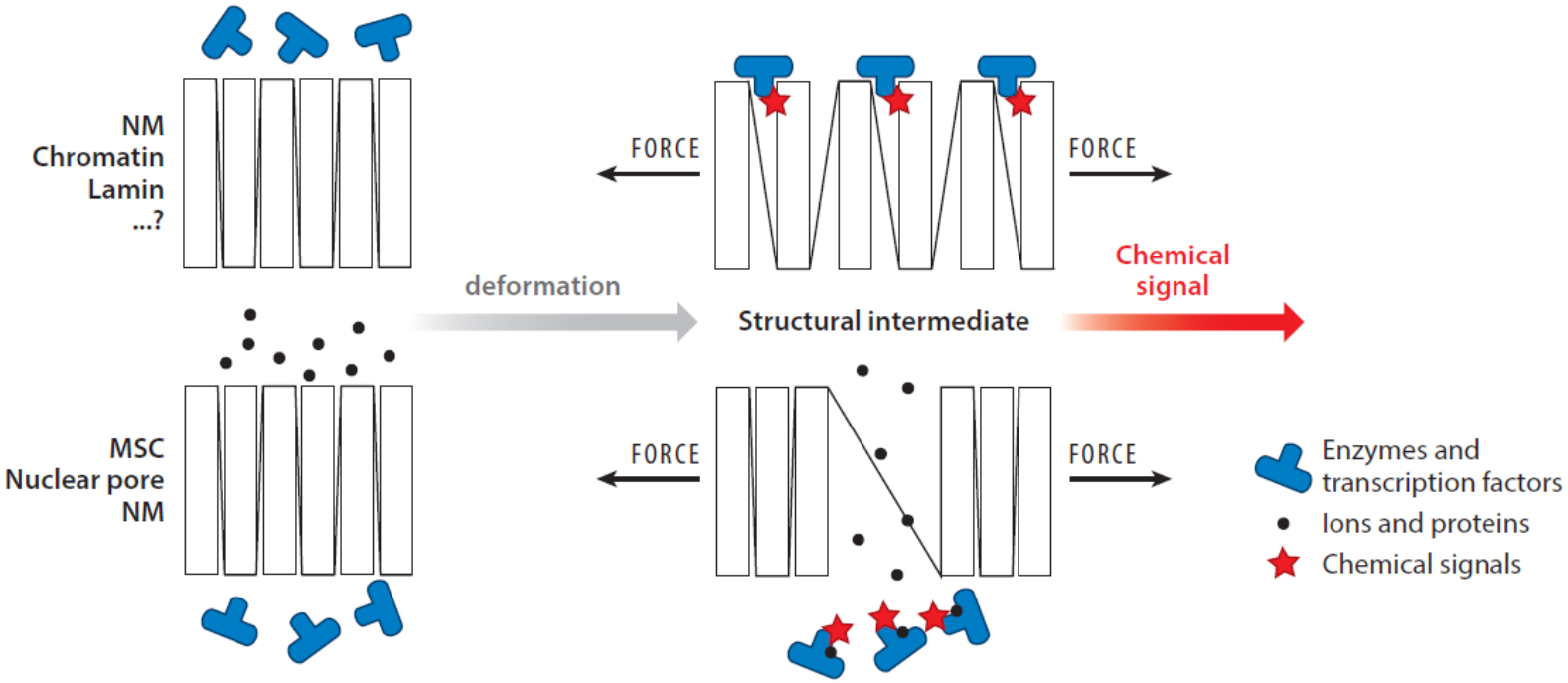




Dahl et al., *JCS,* 2004

Ihalainen et al., *Nat Mat,* 2015

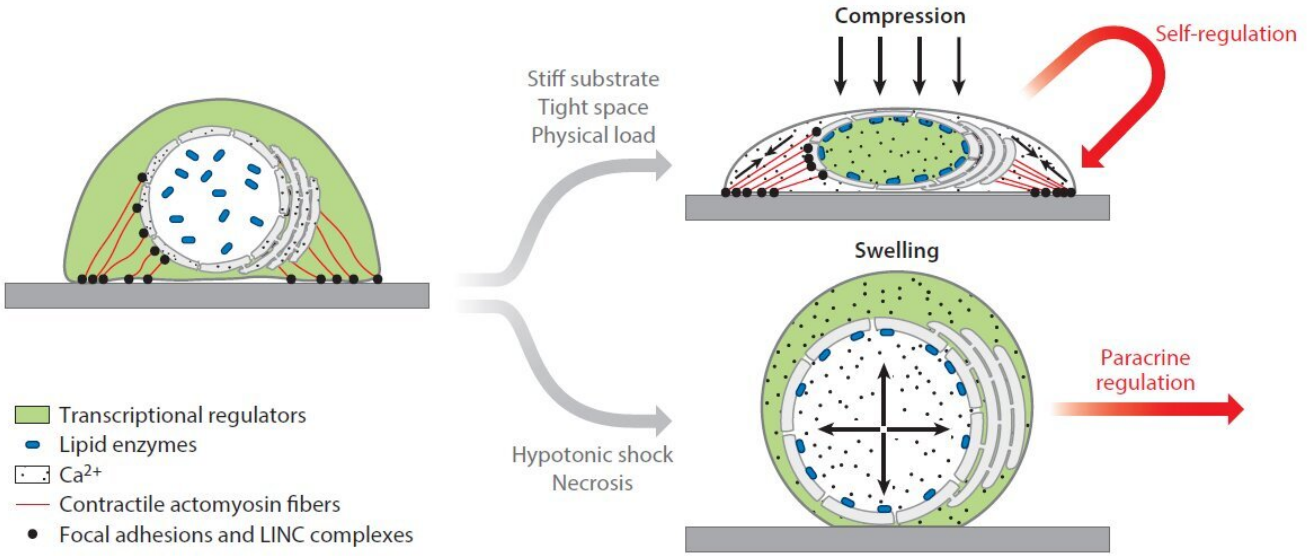
# 4.
# Mechanisms of nuclear mechanotransduction

# Structural intermediates of nuclear mechanotransduction




Niethammer, *Ann Rev Cell Dev Biol*,2021

# Mechanisms of Nuclear Mechanotransduction




a) Mechanosensitive protein redistribution
- to the nuclear membrane
- between cytoplasm and nucleus
- within the nuclear membrane

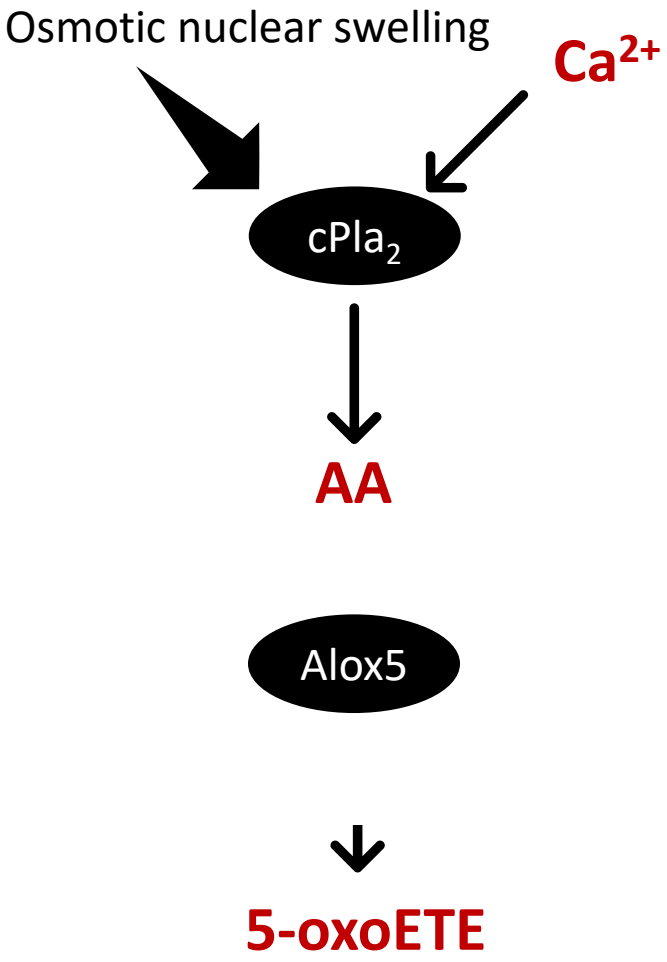
b) Mechanosensitive $Ca^{2+}$ release

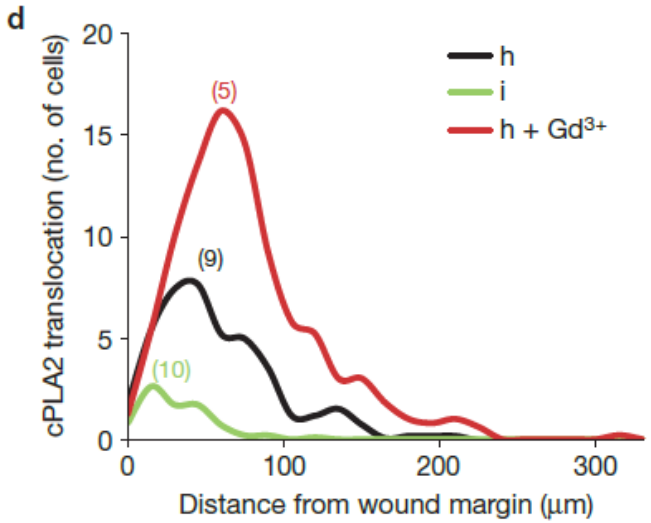
c) Direct mechanical control of gene transcription

Niethammer, *Annu Rev Cell Dev Biol*,2021

# Mechanosensitive protein redistribution to the nuclear membrane

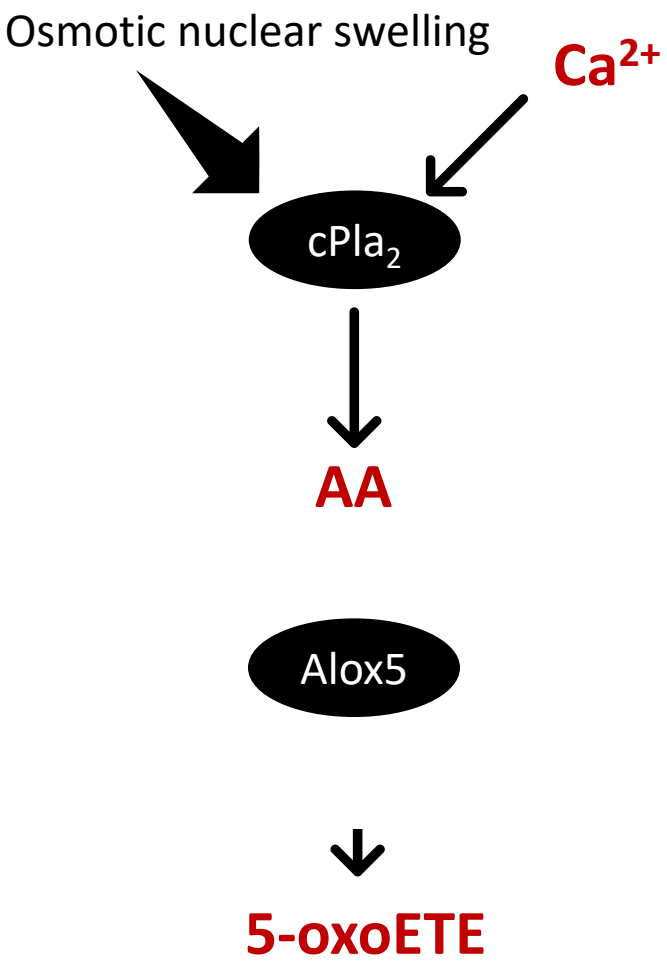
# $cPLA_2$ directly senses nuclear swelling and converts it into an inflammatory signal



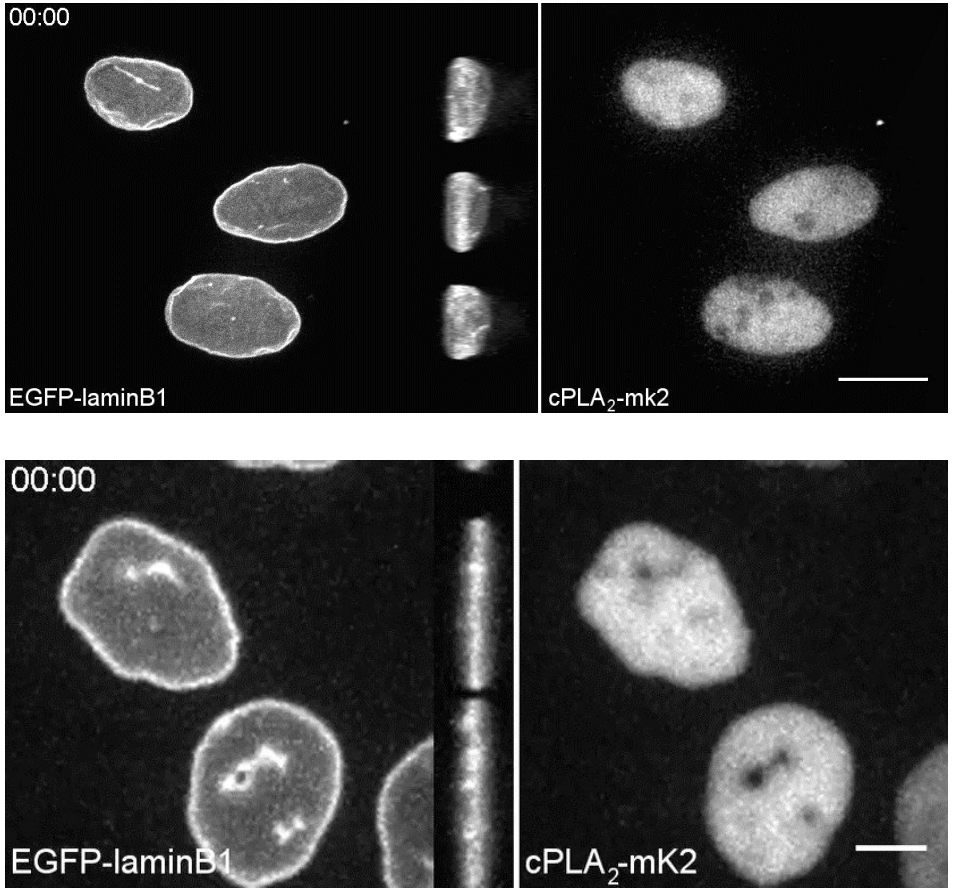




Enyedi et al., *Nat Cell Biol*, 2013

# $cPLA_2$ directly senses nuclear swelling and converts it into an inflammatory signal

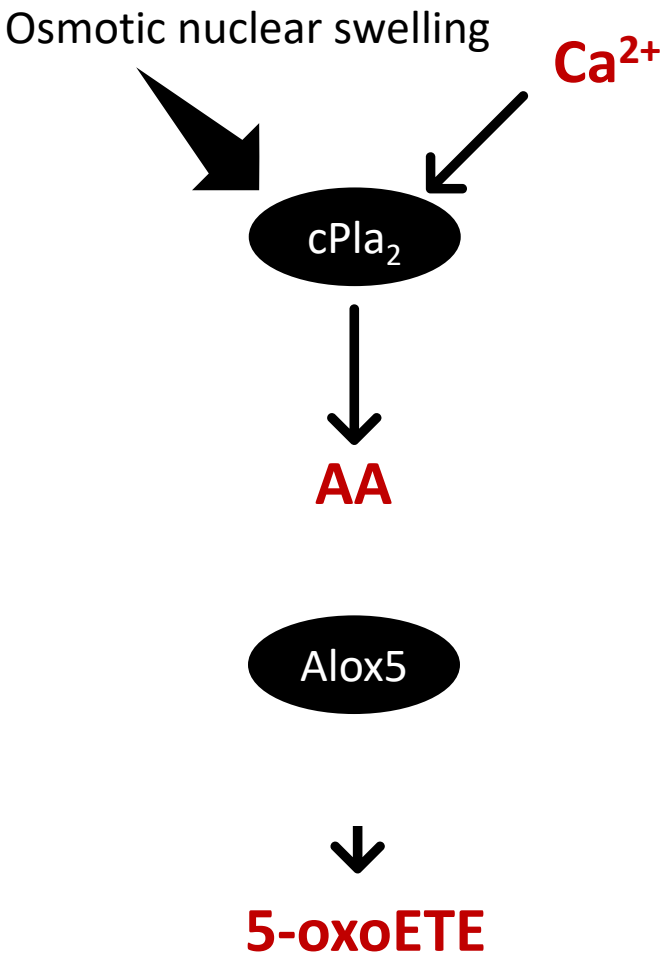



**Exp:** $cPla_2$ translocation in permeabilized HeLa cells at constant $[Ca^{2+}]$

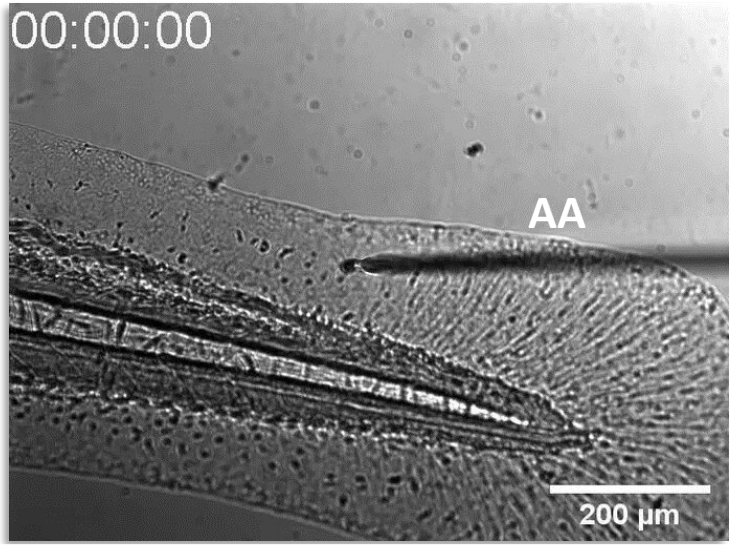



Enyedi et al., *Cell*, 2016

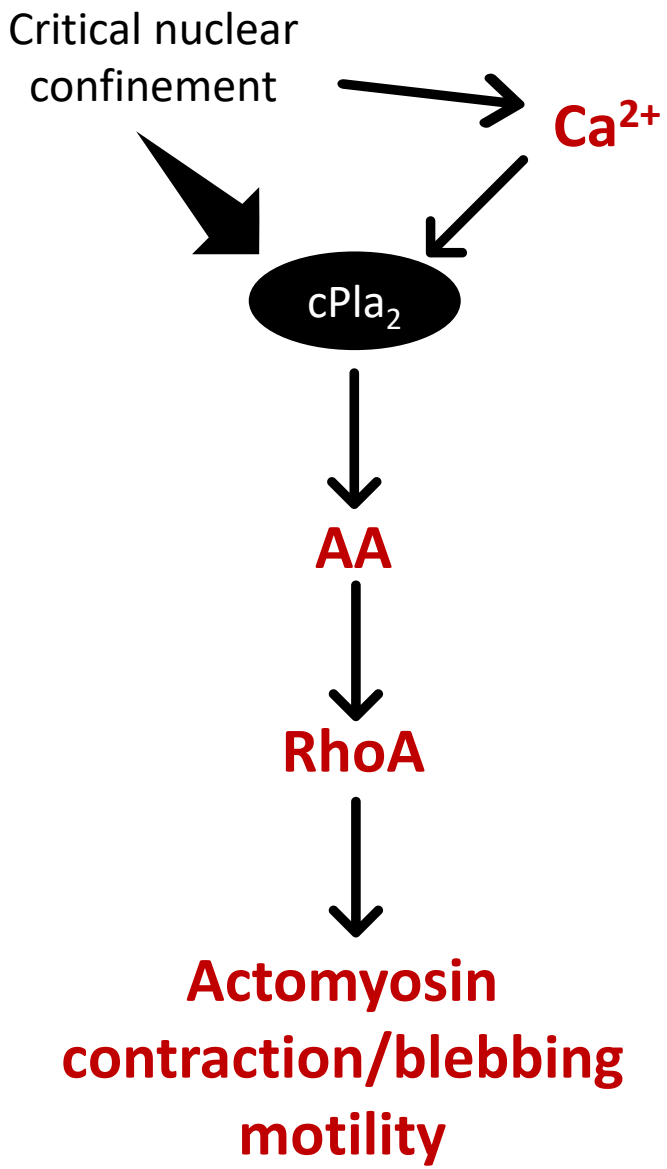
# $cPLA_2$ directly senses nuclear swelling and converts it into an inflammatory signal




Katikaneni et al., *Nat Cell Biol*, 2020



# $cPLA_2$ directly senses nuclear compression and converts into activation of actomyosin contractility




Progenitor cells under mechanical confinement:

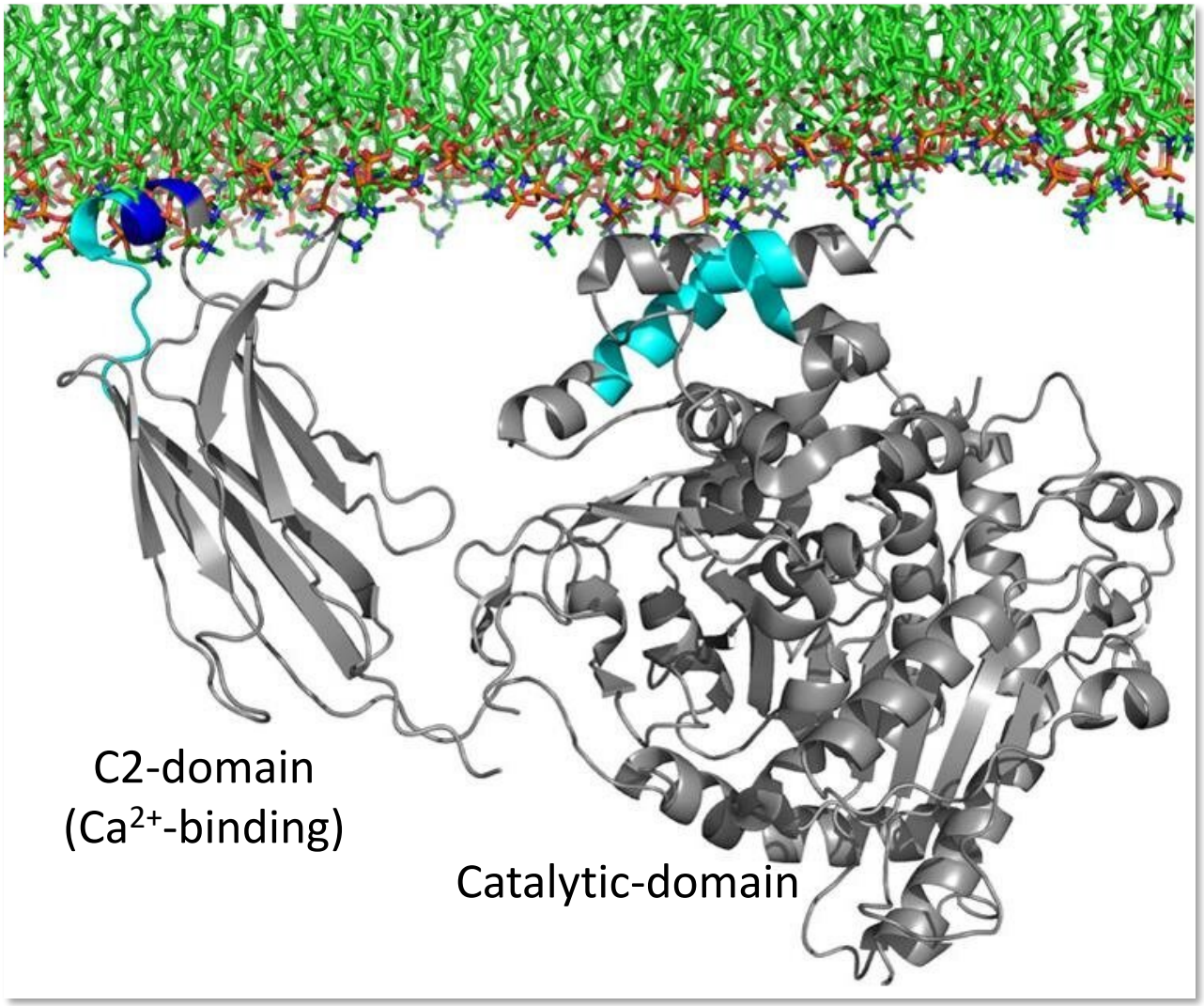
(i) confinement release

Myl12.1-eGFP (myosin II)

Progenitor cells expressing Myl12.1-eGFP (myosin II)

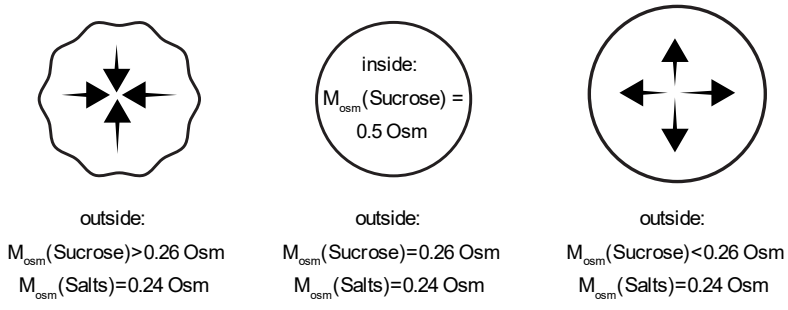
Venturini et al., *Science,* 2020

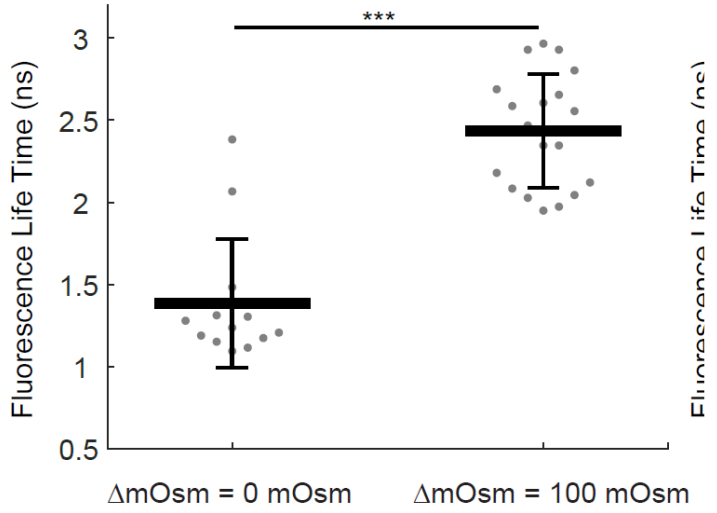
# How does $cPLA_2$ sense membrane deformation?



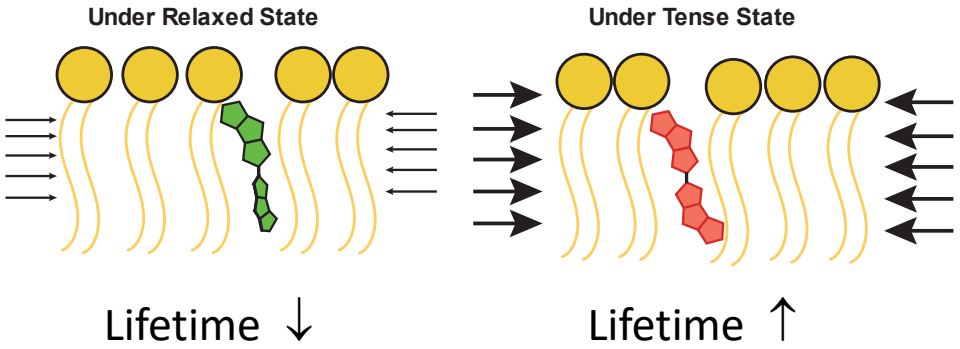

Cao et al., 2013

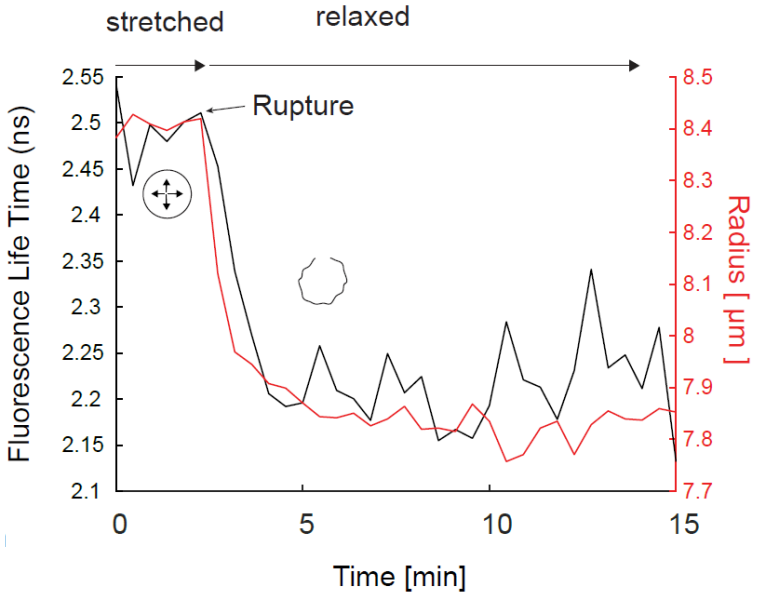
# Can the mechanism be reconstituted in vitro on artificial membranes?



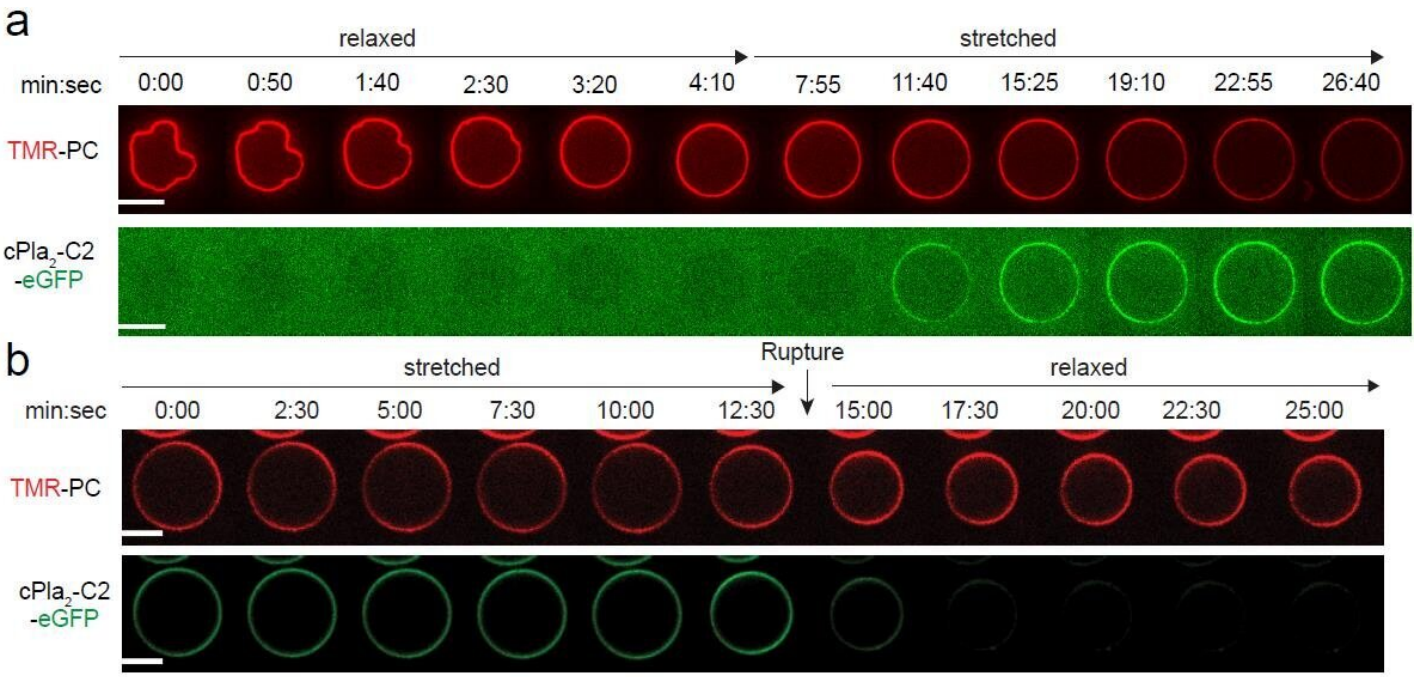

Flipper-TR
(fluorescence lifetime probe for membrane tension)

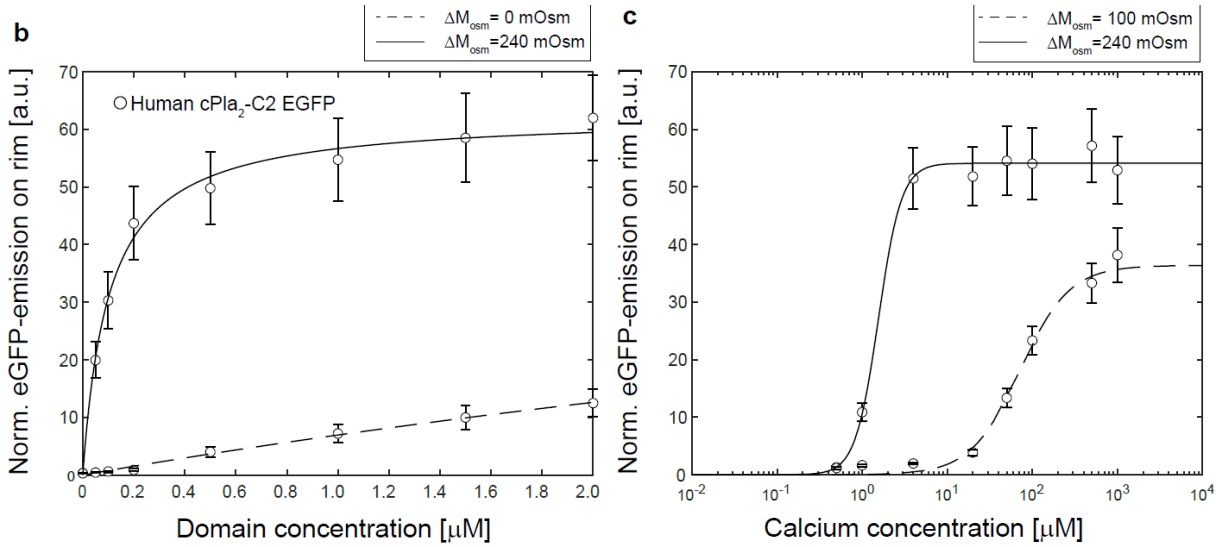



Shen et al., *PNAS, 2022*

# $cPLA_2$ directly senses membrane deformation with its C2-domain

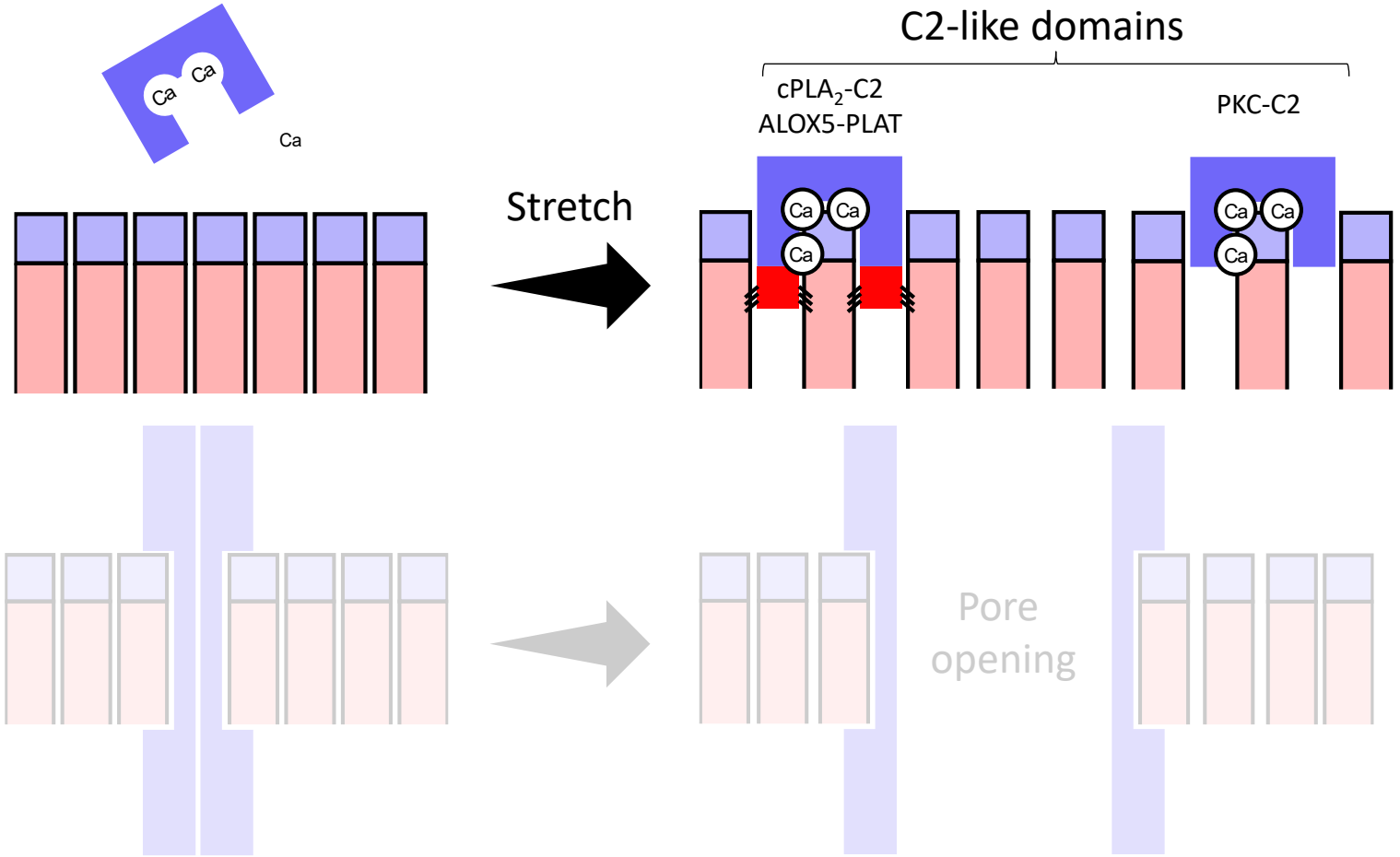


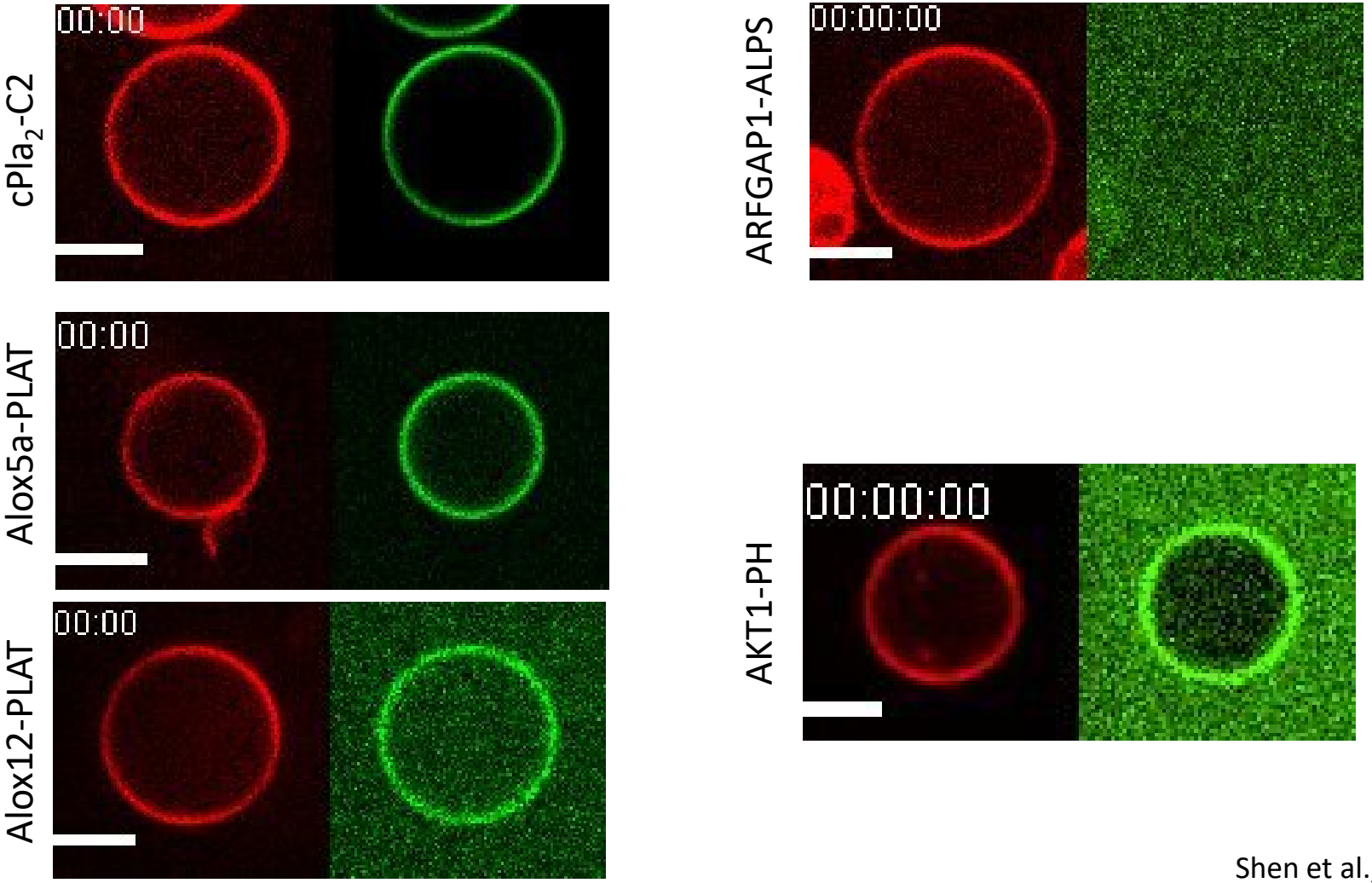

Constant $[Ca^{2+}]$ in all conditions

Shen et al., *PNAS, 2022*

# Sensing internal membrane stresses by protein insertion



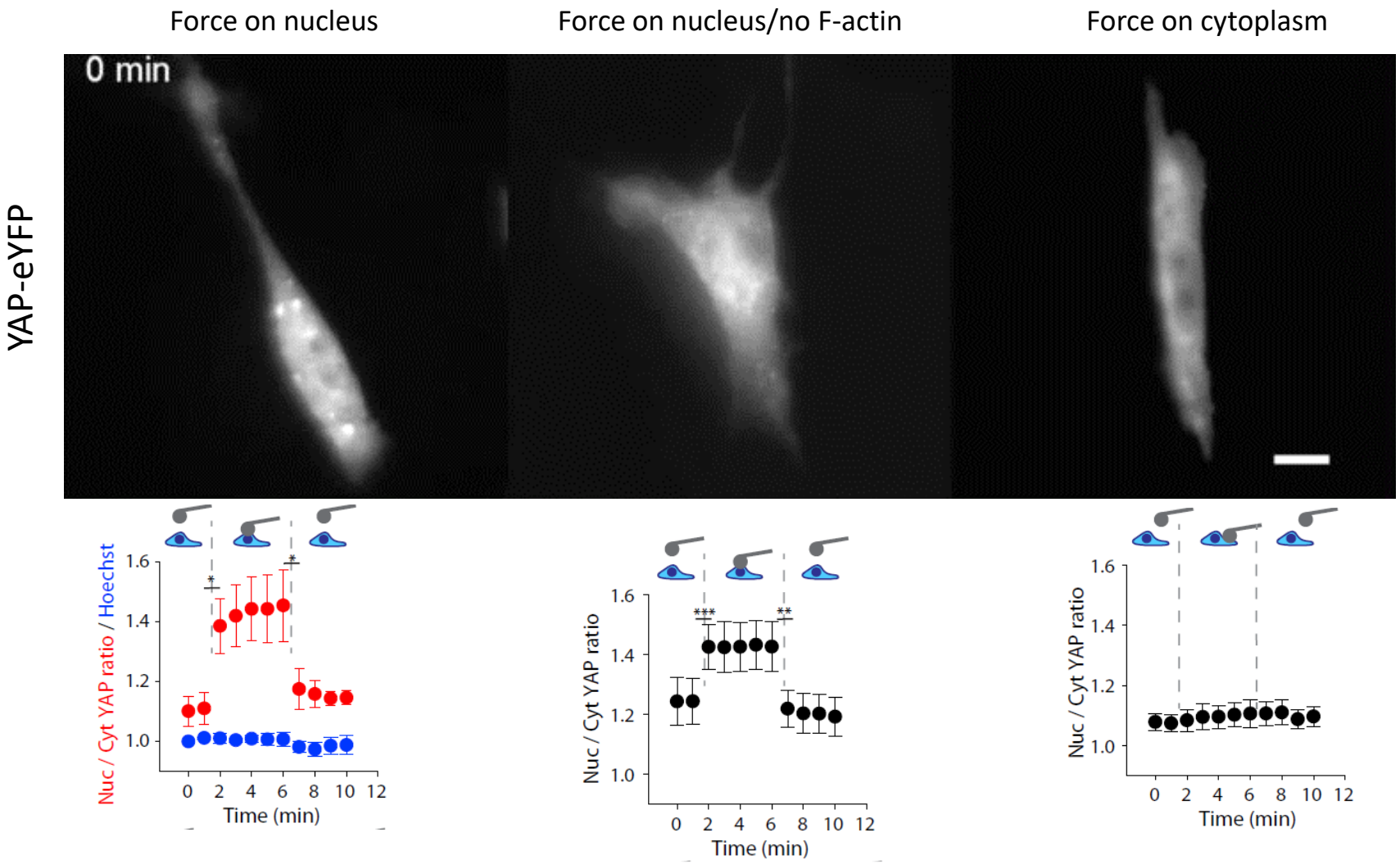
# Differential tension sensing among membrane interaction domains



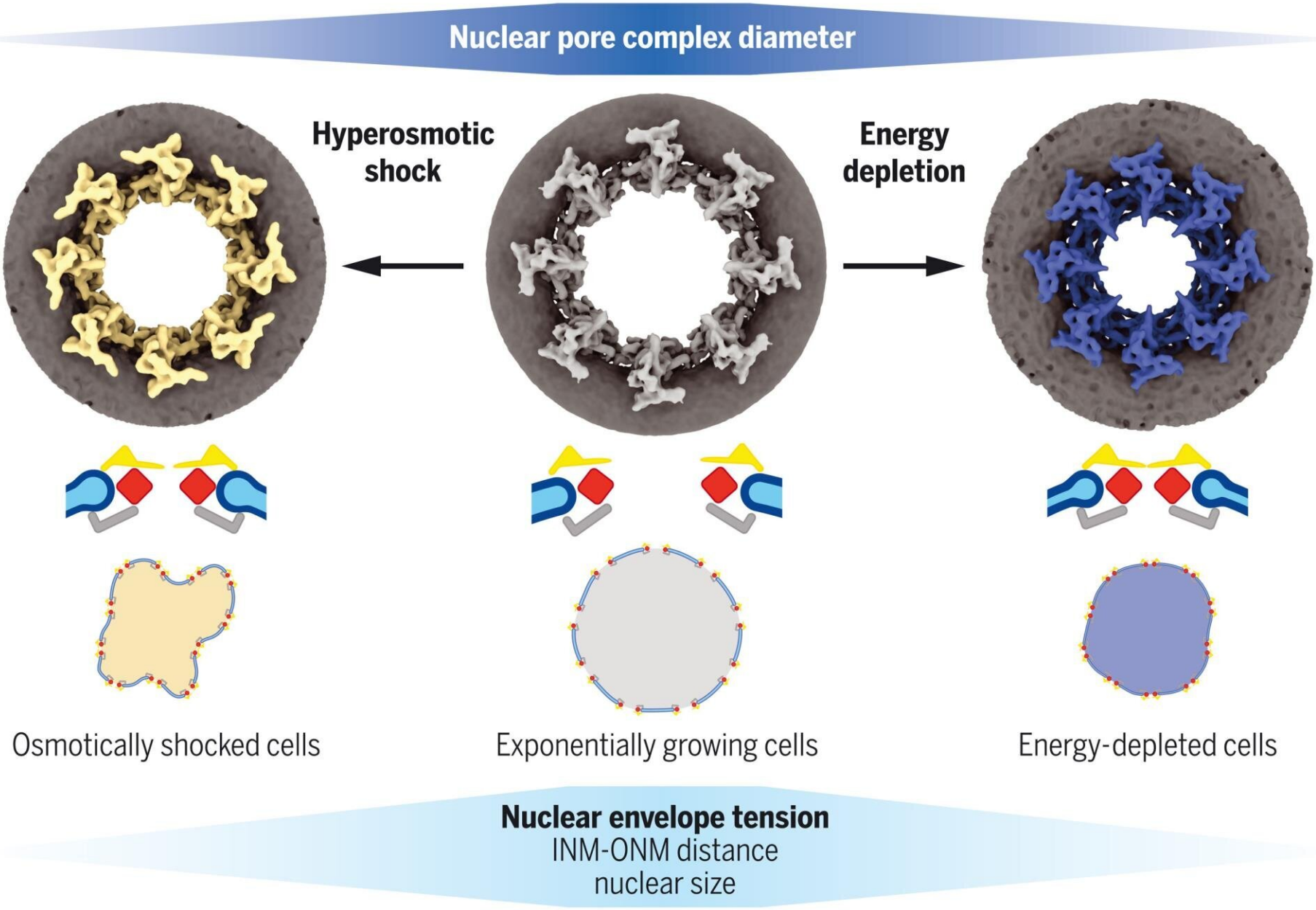

Shen et al., *PNAS, 2022*

# Mechanosensitive protein redistribution between the nucleus and cytoplasm

# Mechanosensitive nuclear translocation of YAP



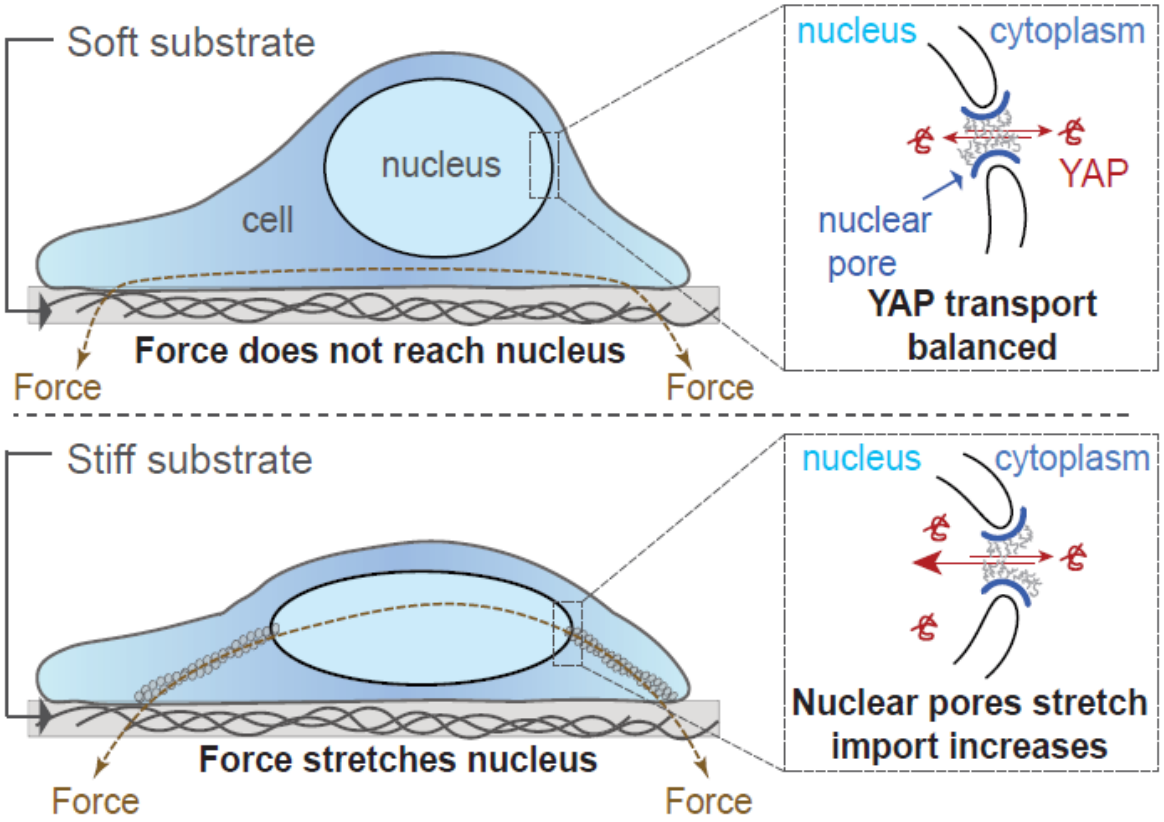

Elosegui-Artola et al., *Cell,* 2017




Zimmerli et al., *Science,* 2021

# Mechanosensitive nuclear translocation of YAP

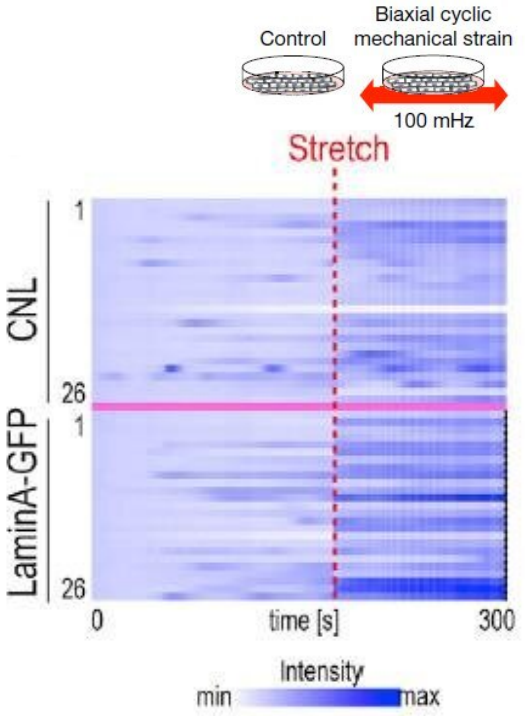
## The nuclear pore as a valve?




Elosegui-Artola et al., *Cell,* 2017

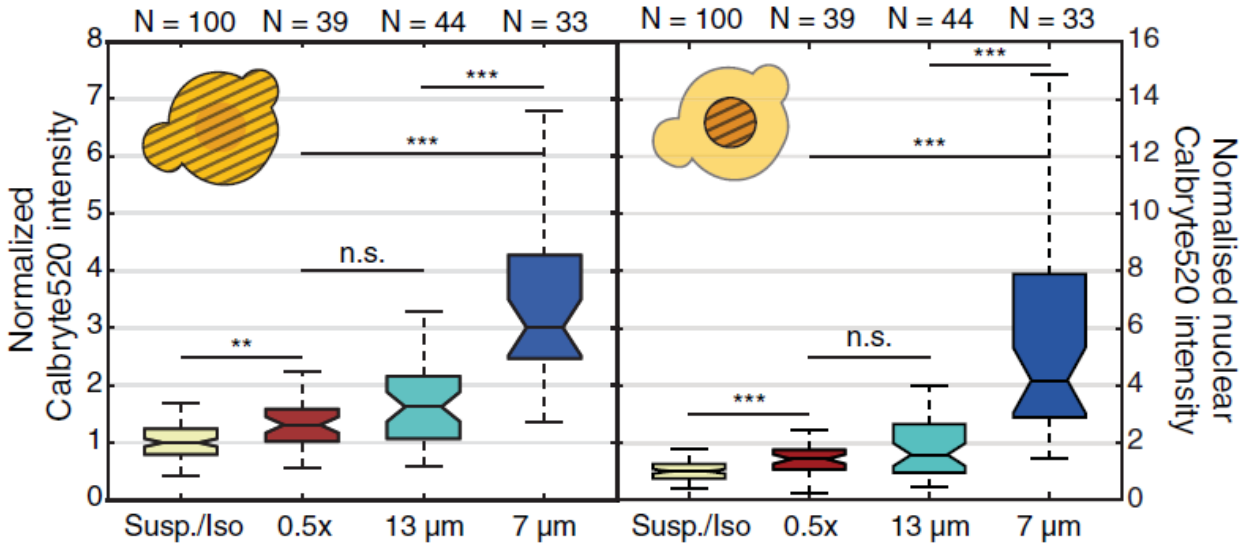
# Mechanosensitive $Ca^{2+}$ release

# Nuclear deformation regulates intracellular $Ca^{2+}$ release




Nava et al., *Cell,* 2020

Cell confinement:

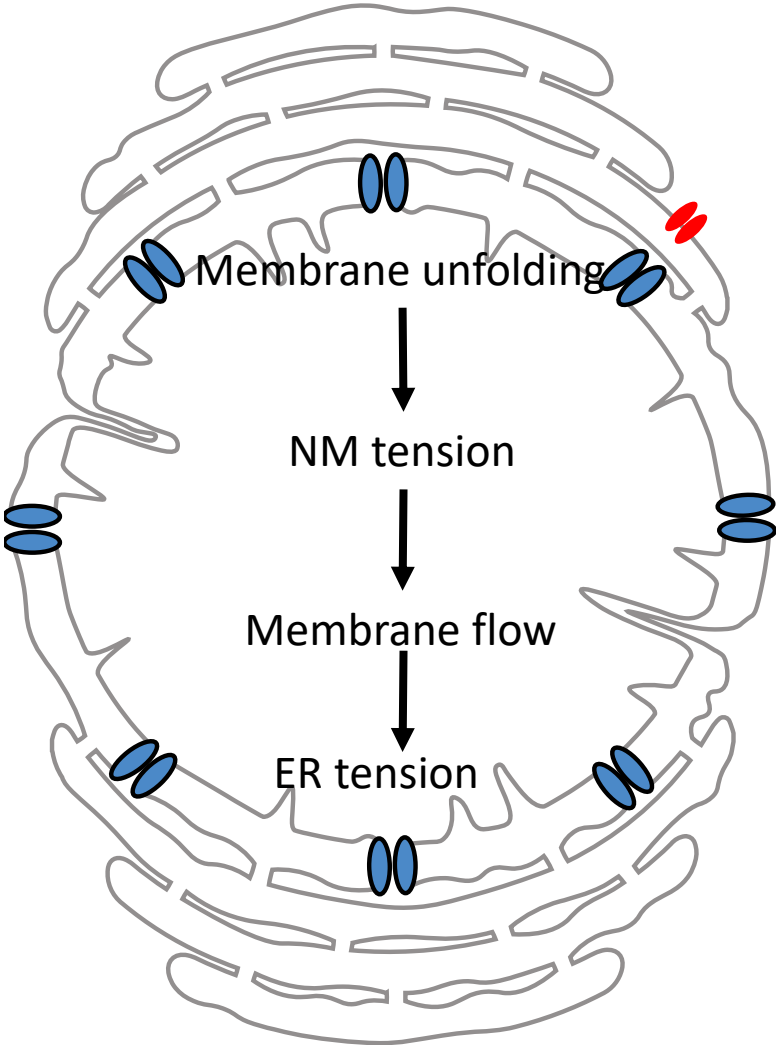


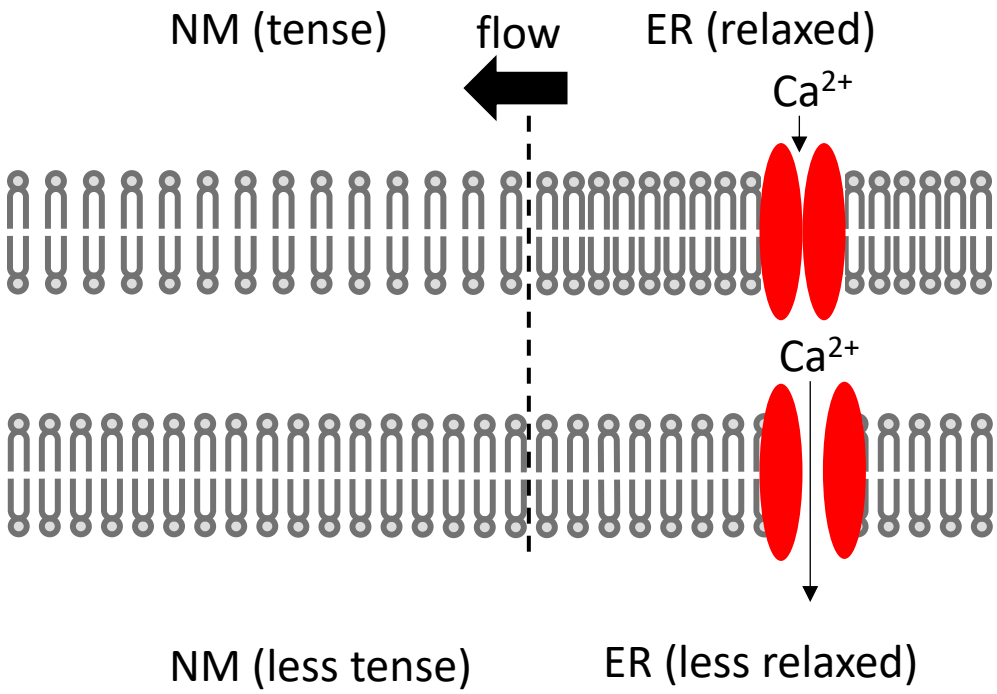

Venturini et al., *Science,* 2020

Nuclear deformation → $Ca^{2+}$ → $cPLA_2$, myosin II contraction, etc.,

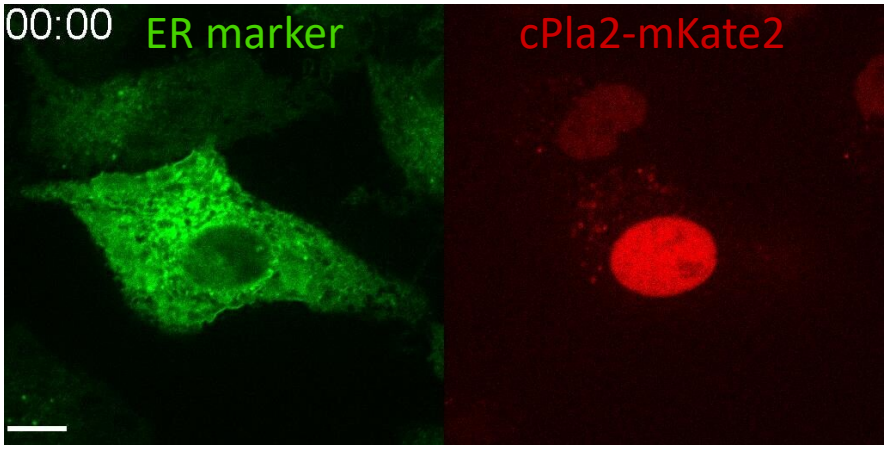
$Ca^{2+}$ → Chromatin rheology (fluidization to prevent damage)

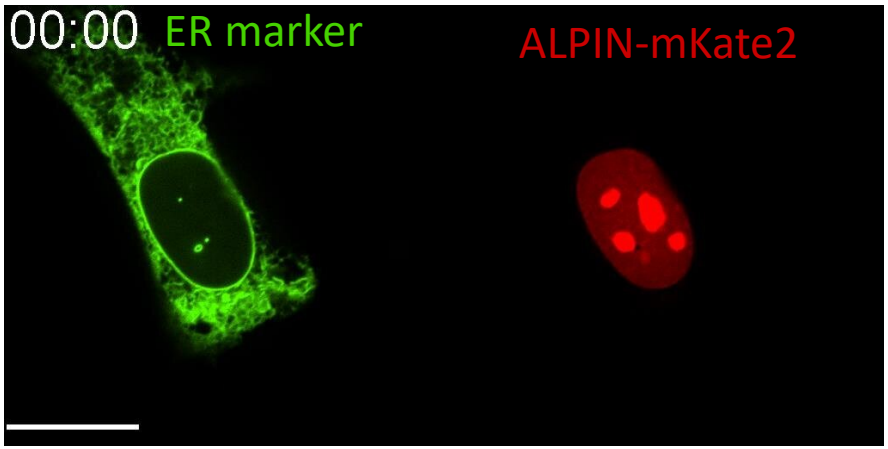
# Nuclear deformation regulates intracellular $Ca^{2+}$ release



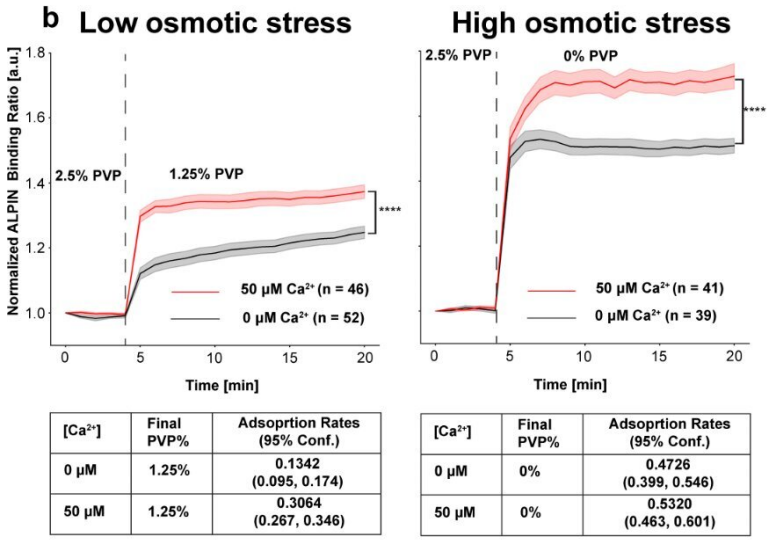



# The ER buffers nuclear membrane tension




(Shen et al., *in review*)



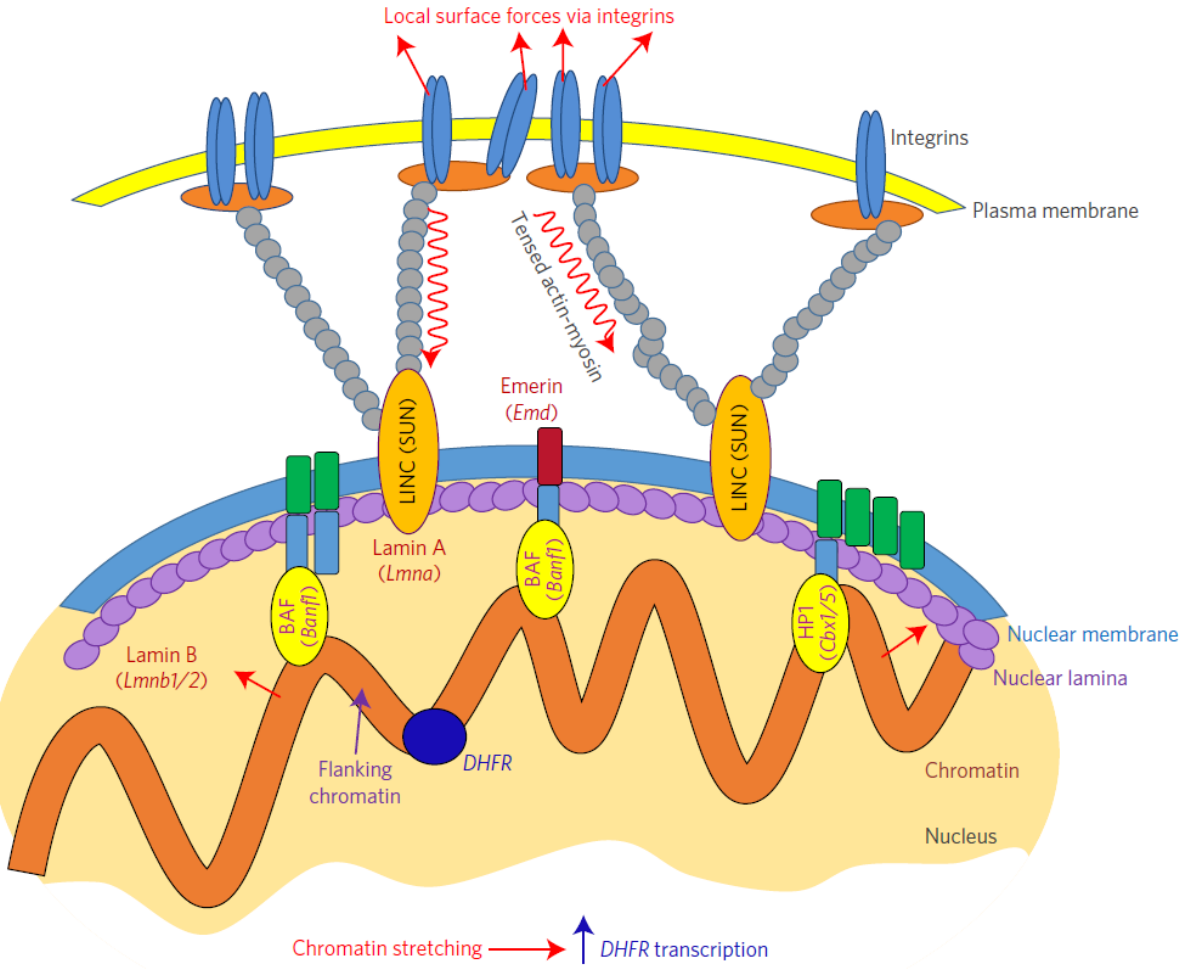

| [Ca²⁺] | Final PVP% | Adsoprtion Rates (95% Conf.) |
|---|---|---|
| 0 µM | 1.25% | 0.1342 (0.095, 0.174) |
| 50 µM | 1.25% | 0.3064 (0.267, 0.346) |

| [Ca²⁺] | Final PVP% | Adsoprtion Rates (95% Conf.) |
|---|---|---|
| 0 µM | 0% | 0.4726 (0.399, 0.546) |
| 50 µM | 0% | 0.5320 (0.463, 0.601) |

# Direct mechanical regulation of gene regulation

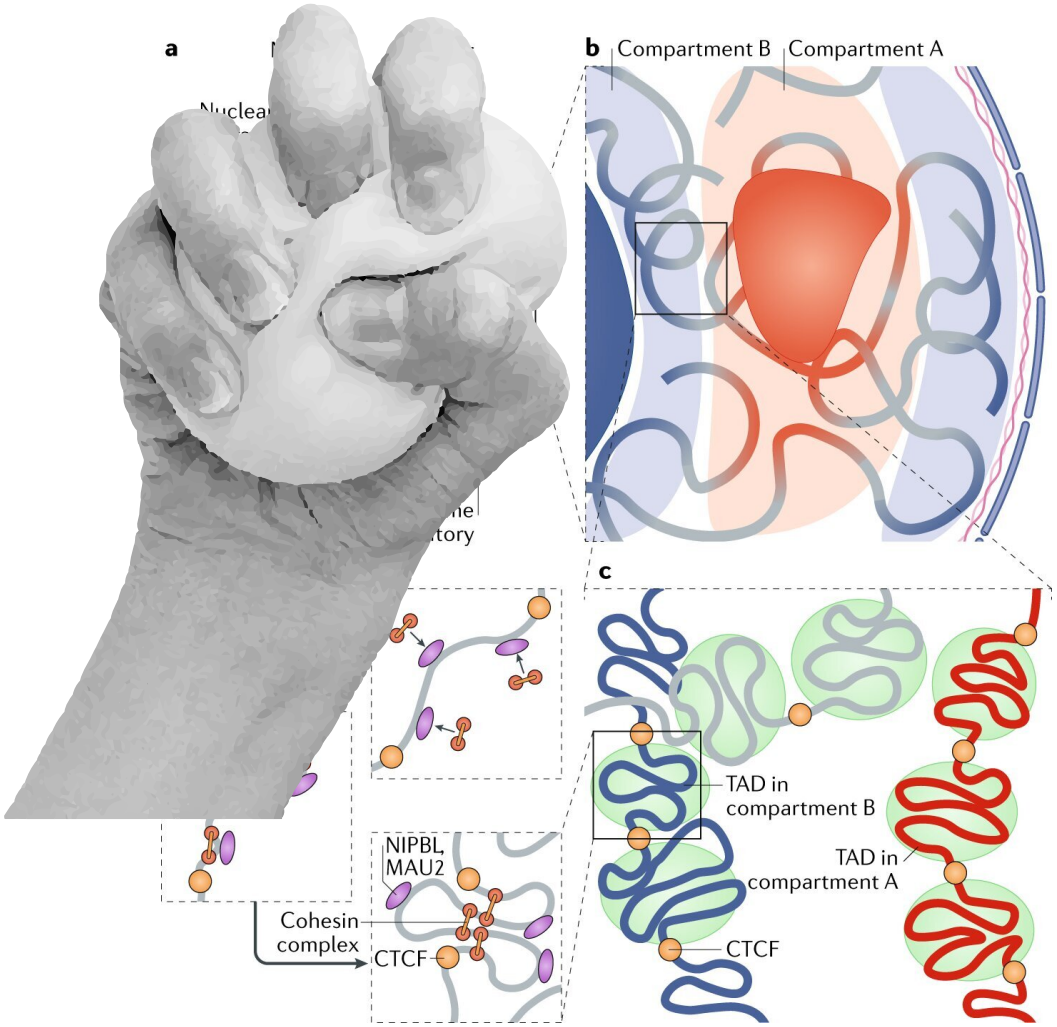
(from all outlined mechanisms to date the most hypothetical)

# Forces on integrin may be transduced to the nucleus to directly alter gene transcription

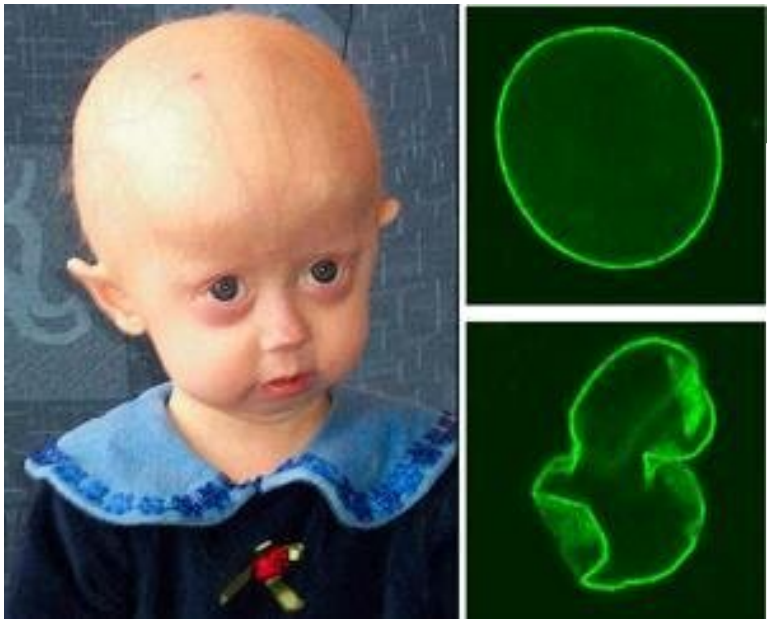



Tajik et al., *Nat Mat*, 2016

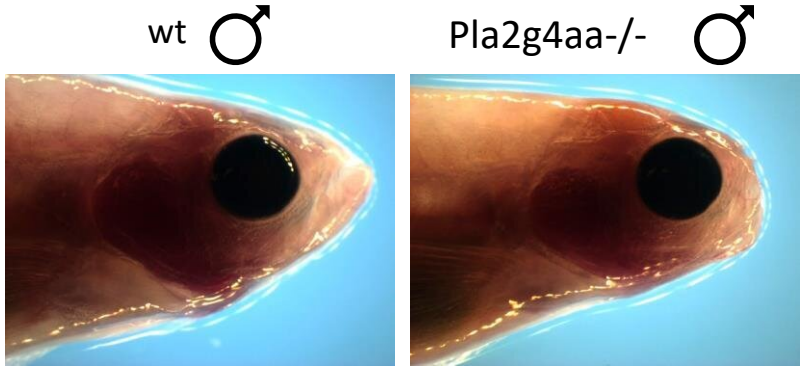
# Nuclear deformation may cause intermingling of chromosome territories and associated changes in transcription

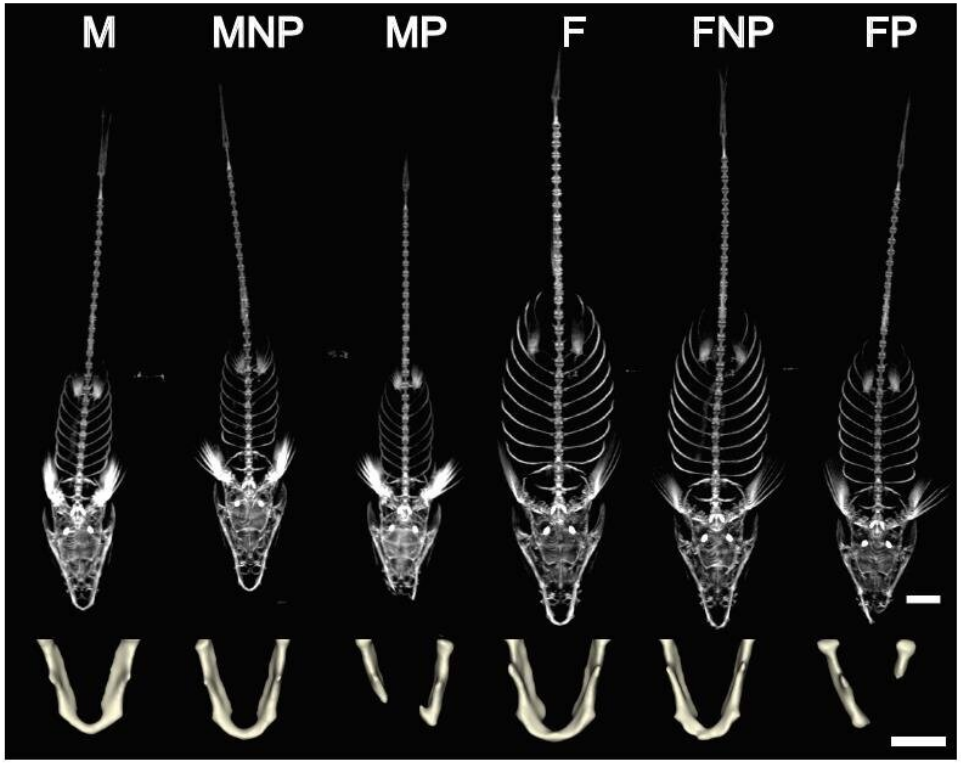


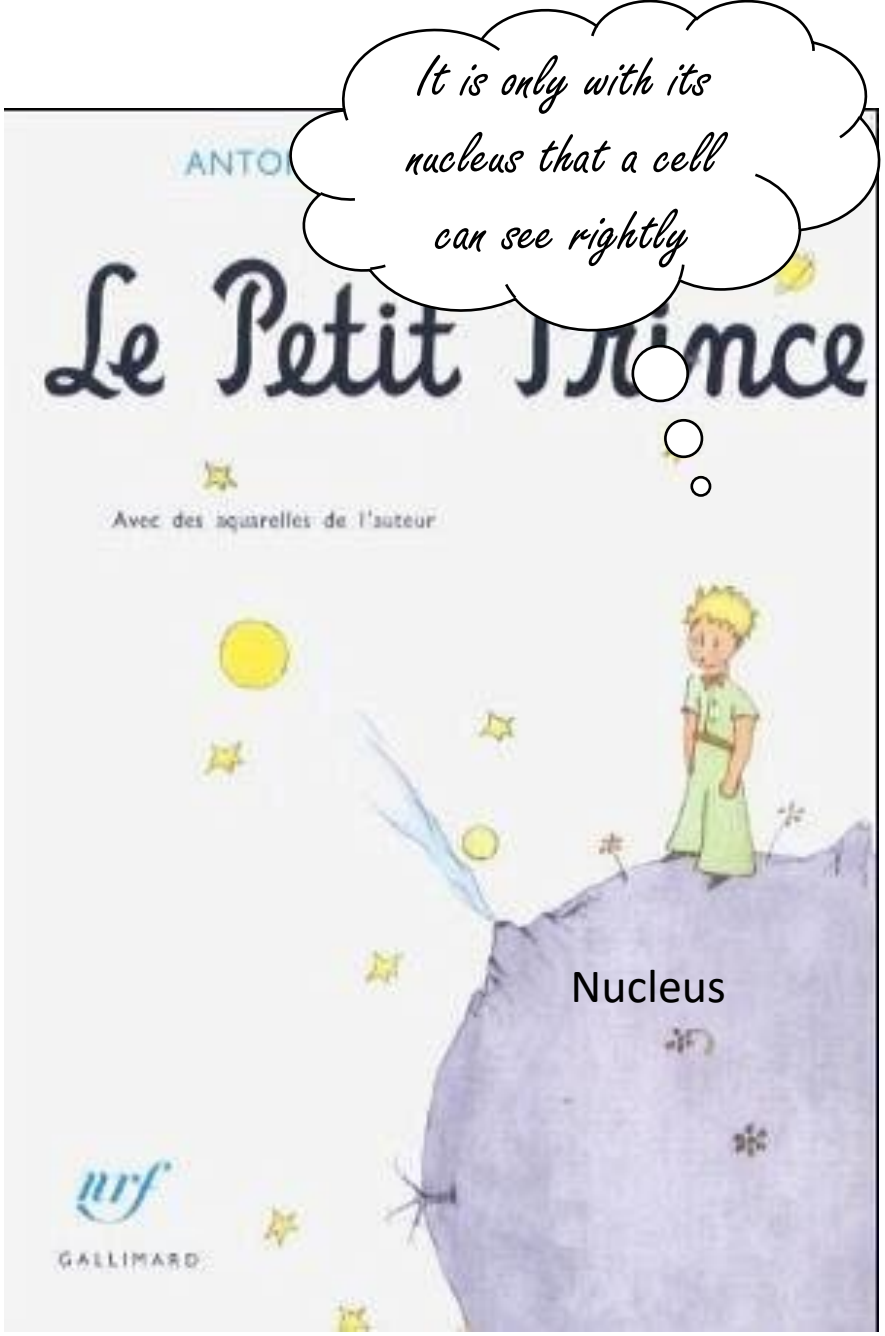
# Frontier: How does nuclear mechanotransduction contribute to physiology and pathophysiology?



Lamin A/C mutation -> progeria






## Bottom Line

- For decades, the plasma membrane as the one and only mechanosensory surface of a cell
- The nuclear envelope now emerges as second sensory surface
- Nuclear mechanotransduction can function downstream of cell surface mechanotransduction (“intermediary role”)
- Or it can detect nuclear deformation independently of the cell surface (“immediate role”)

## Future challenges

- Non-invasive measurement of intracellular forces
- Delineation of nuclear and cell surface mechanotransduction mechanisms
- Delineation of mechanotransducive and structural roles of nuclear components

For further details:

*Annual Review of Cell and Developmental Biology*

# Components and Mechanisms of Nuclear Mechanotransduction

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email: niethamp@mskcc.org