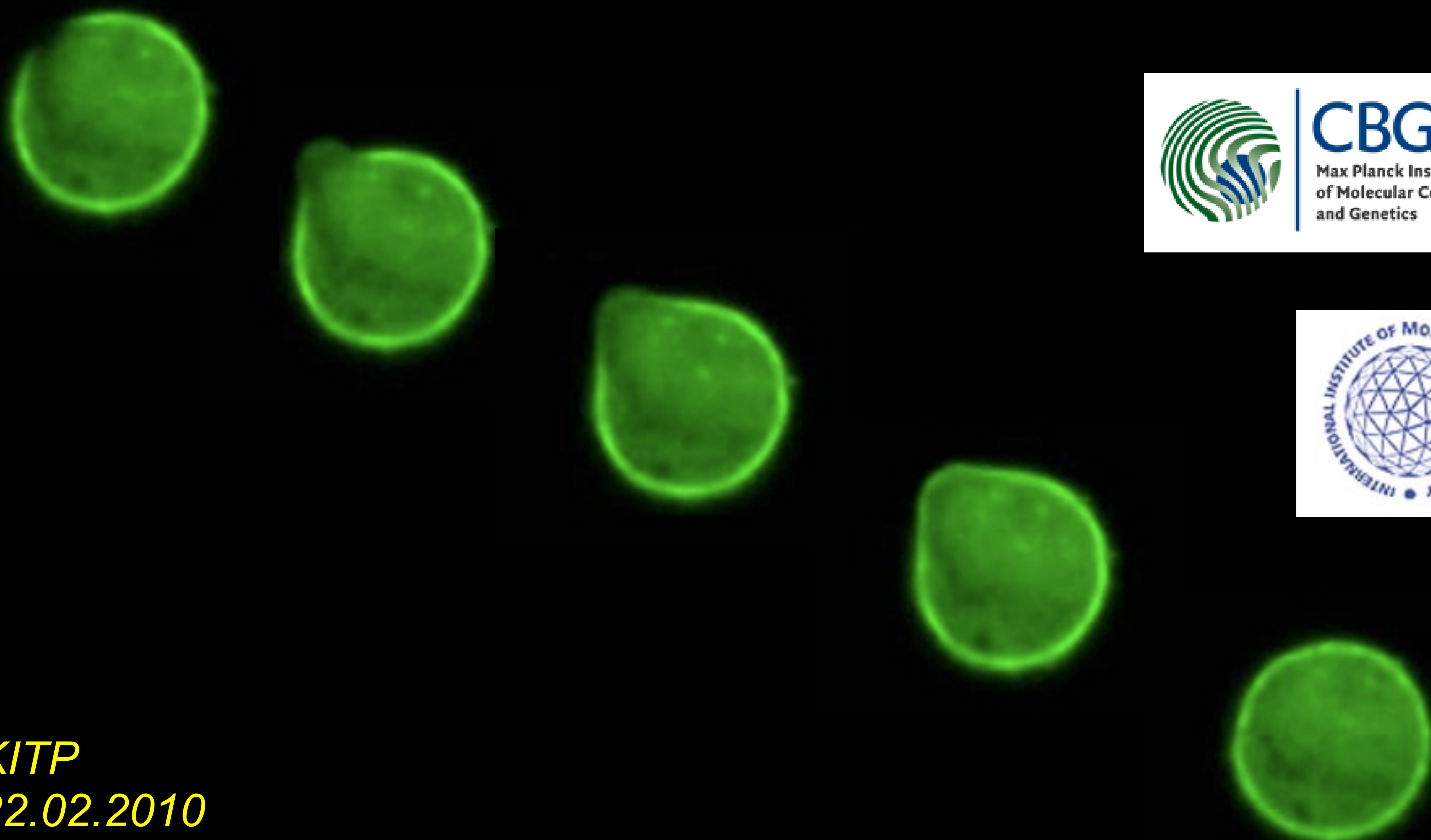
Blebs, actin cortex mechanics and the control of cell shape

Ewa Paluch



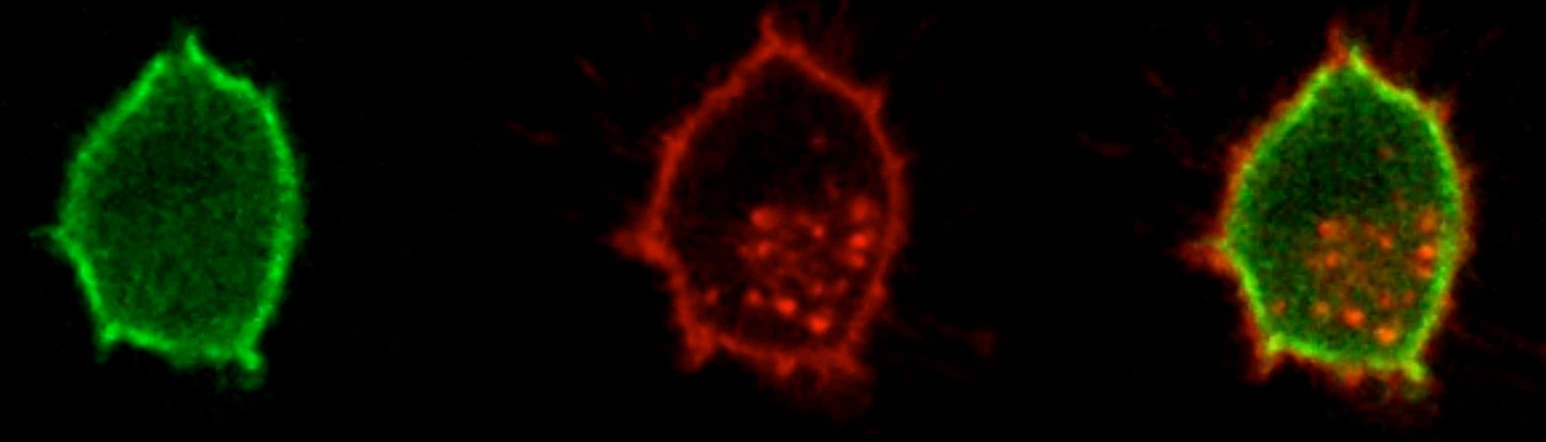
Max Planck Institute of Molecular Cell Biology and Genetics

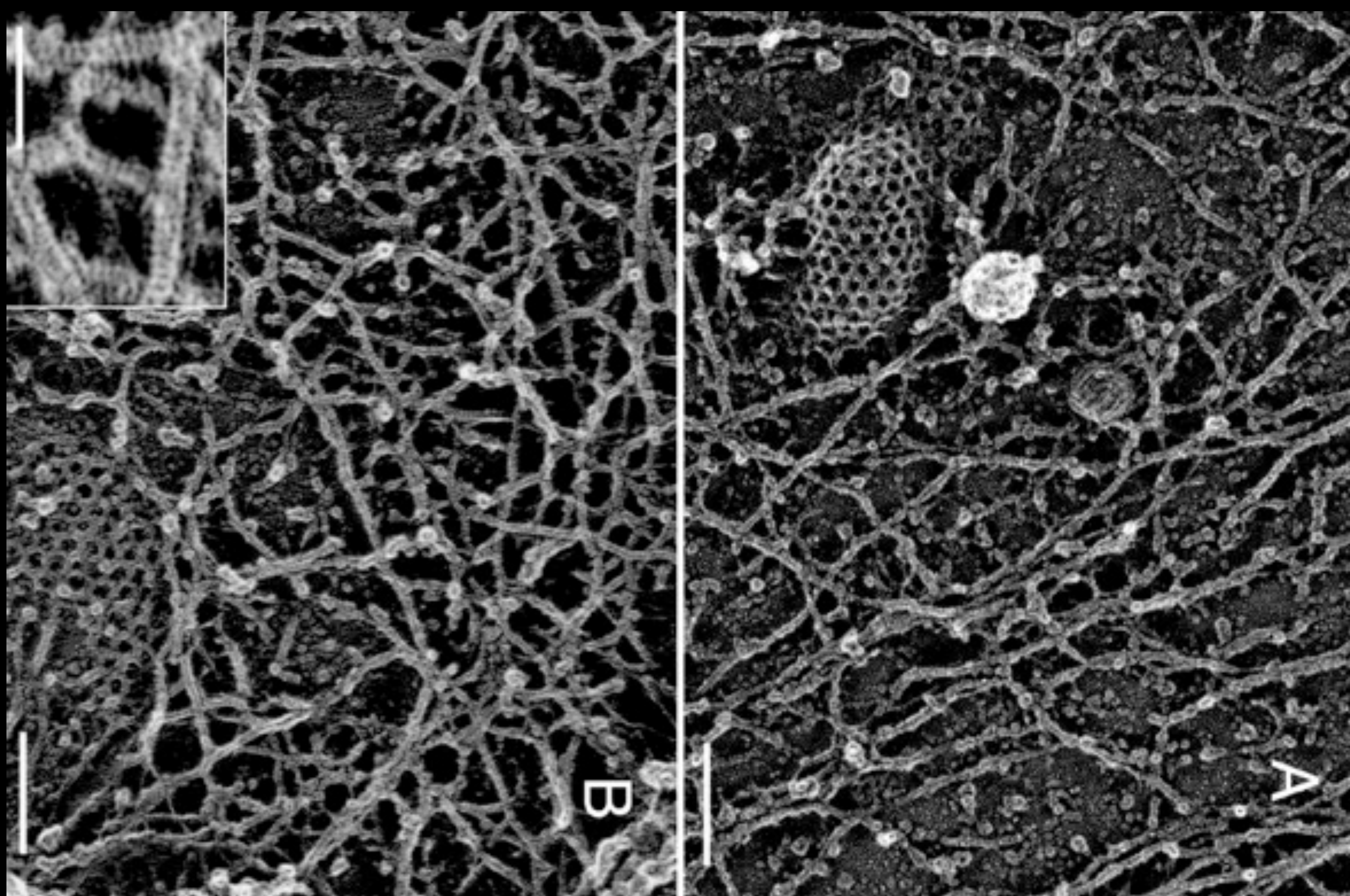
International Institute of Molecular and Cell Biology

KITP
22.02.2010

The actomyosin cortex

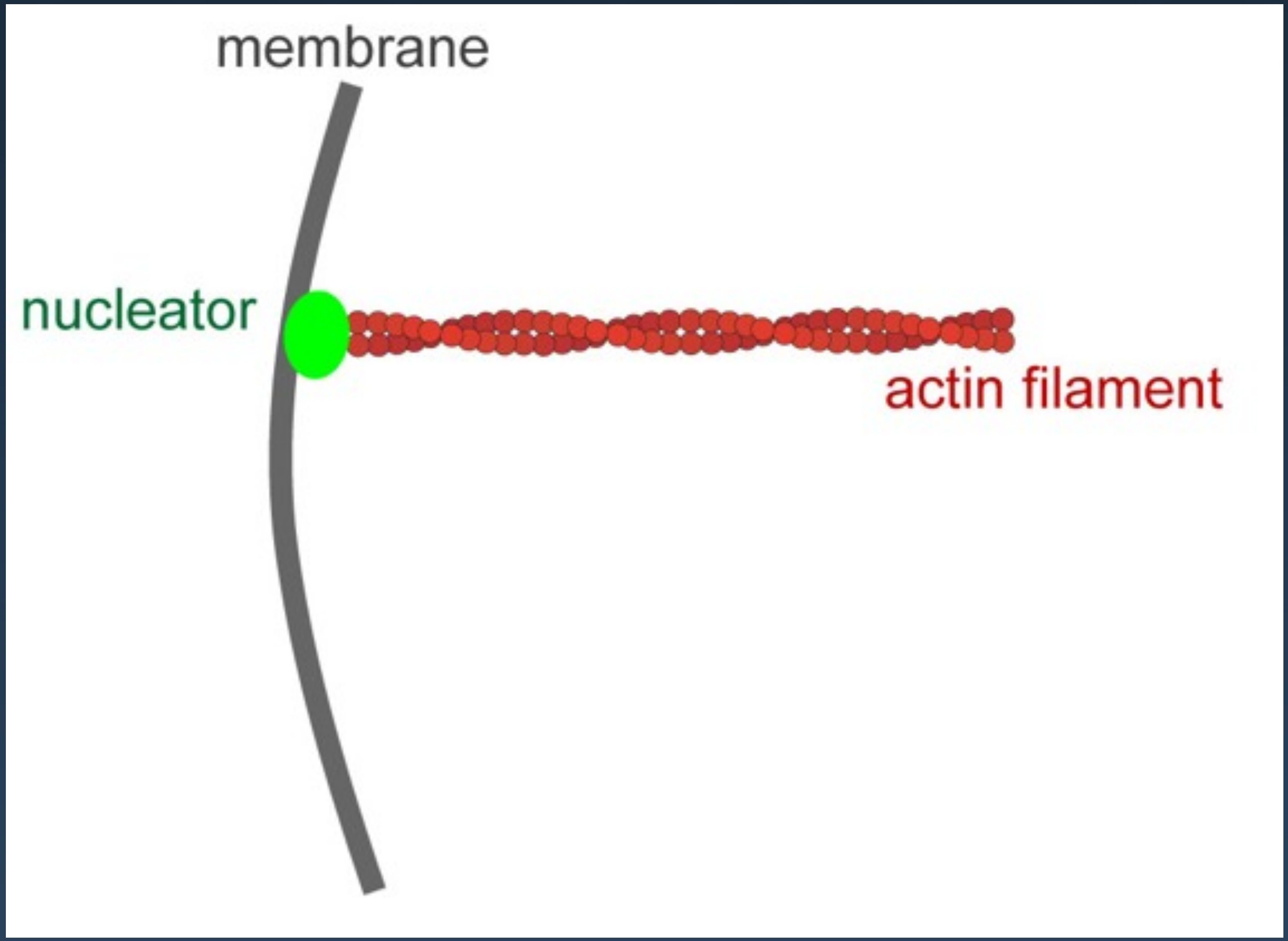
L929
(mouse fibroblasts)





NRK cells
[Morone et al., J Cell Biol 2006]

The cell cortex



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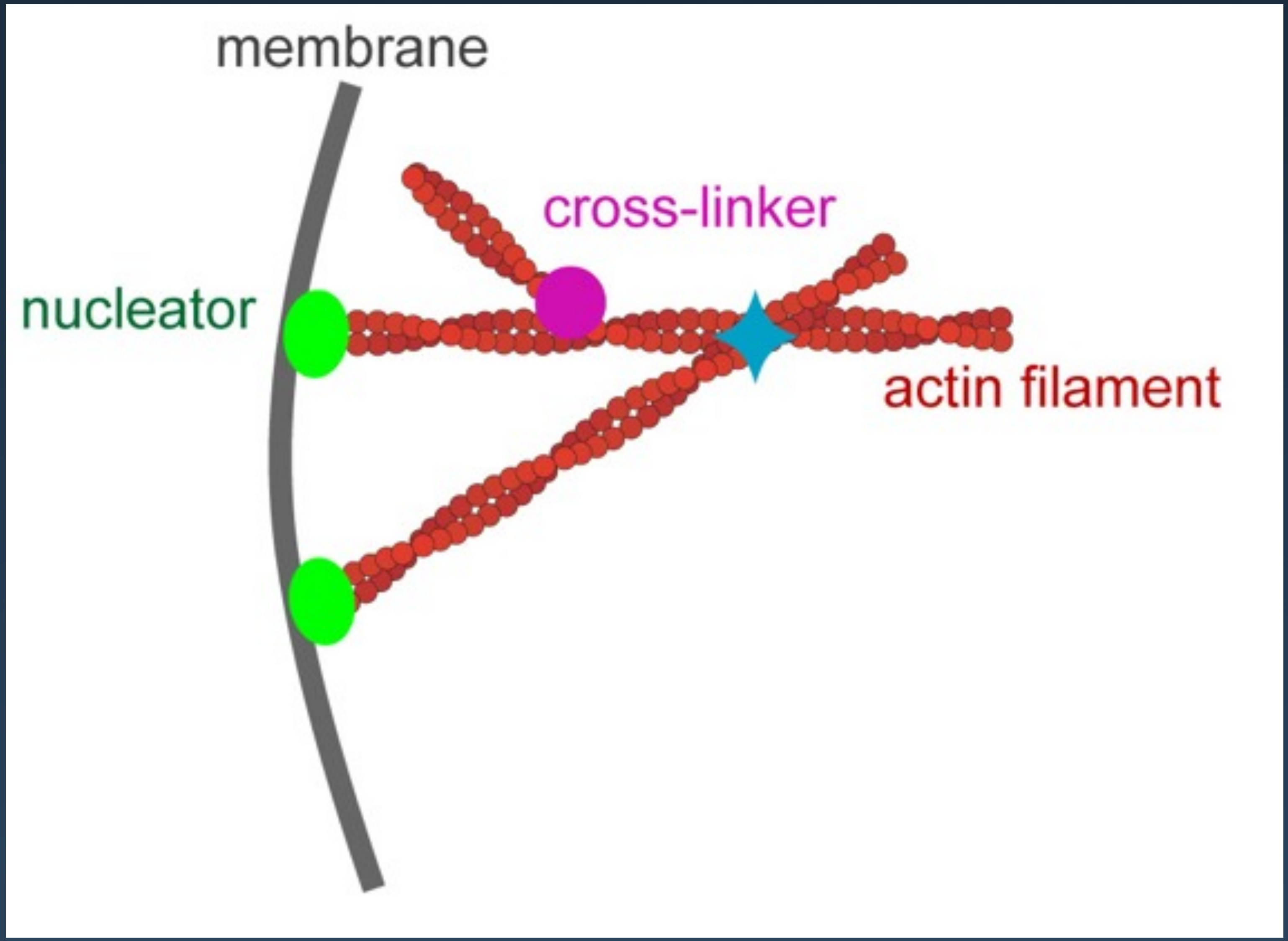
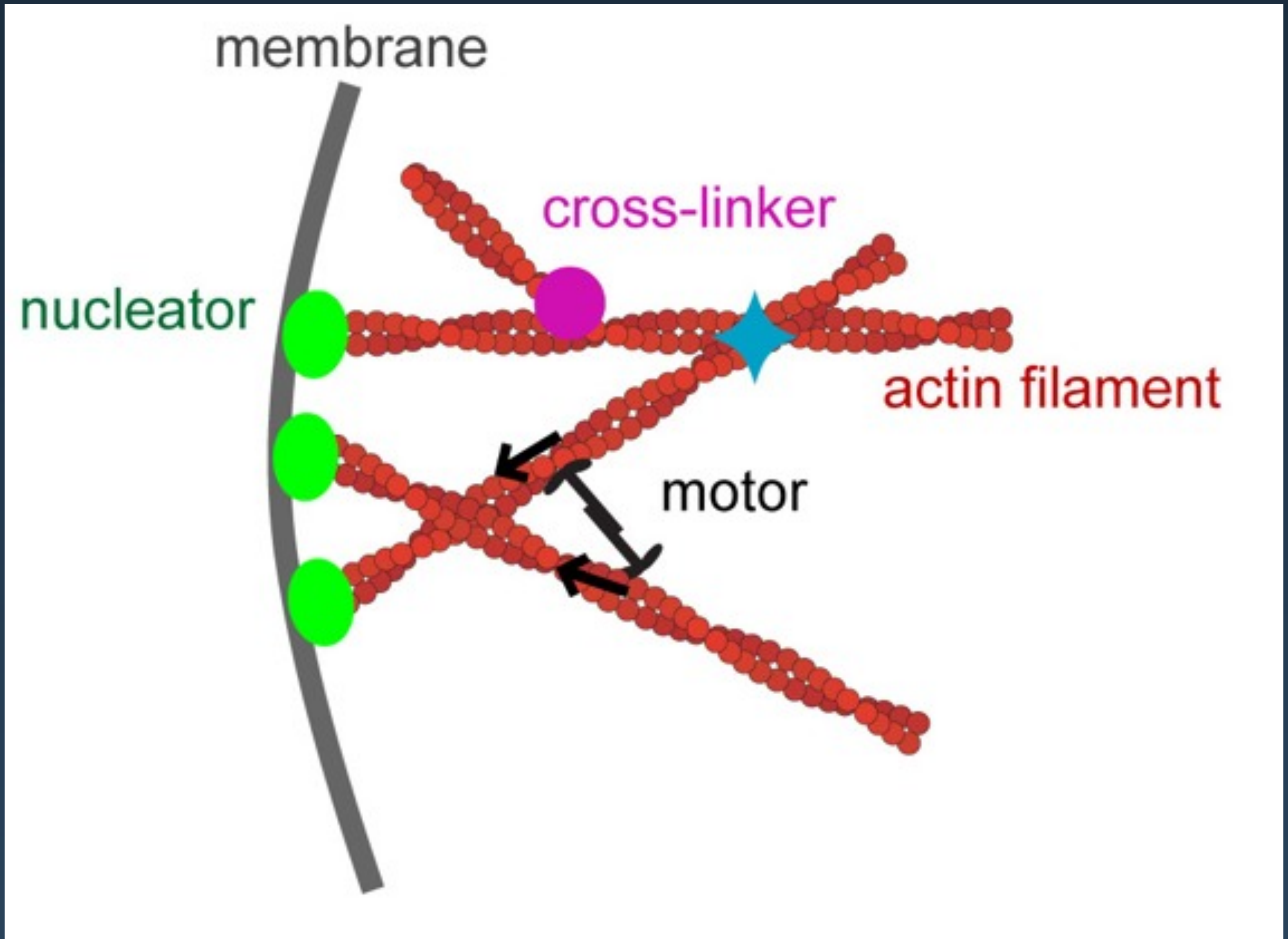
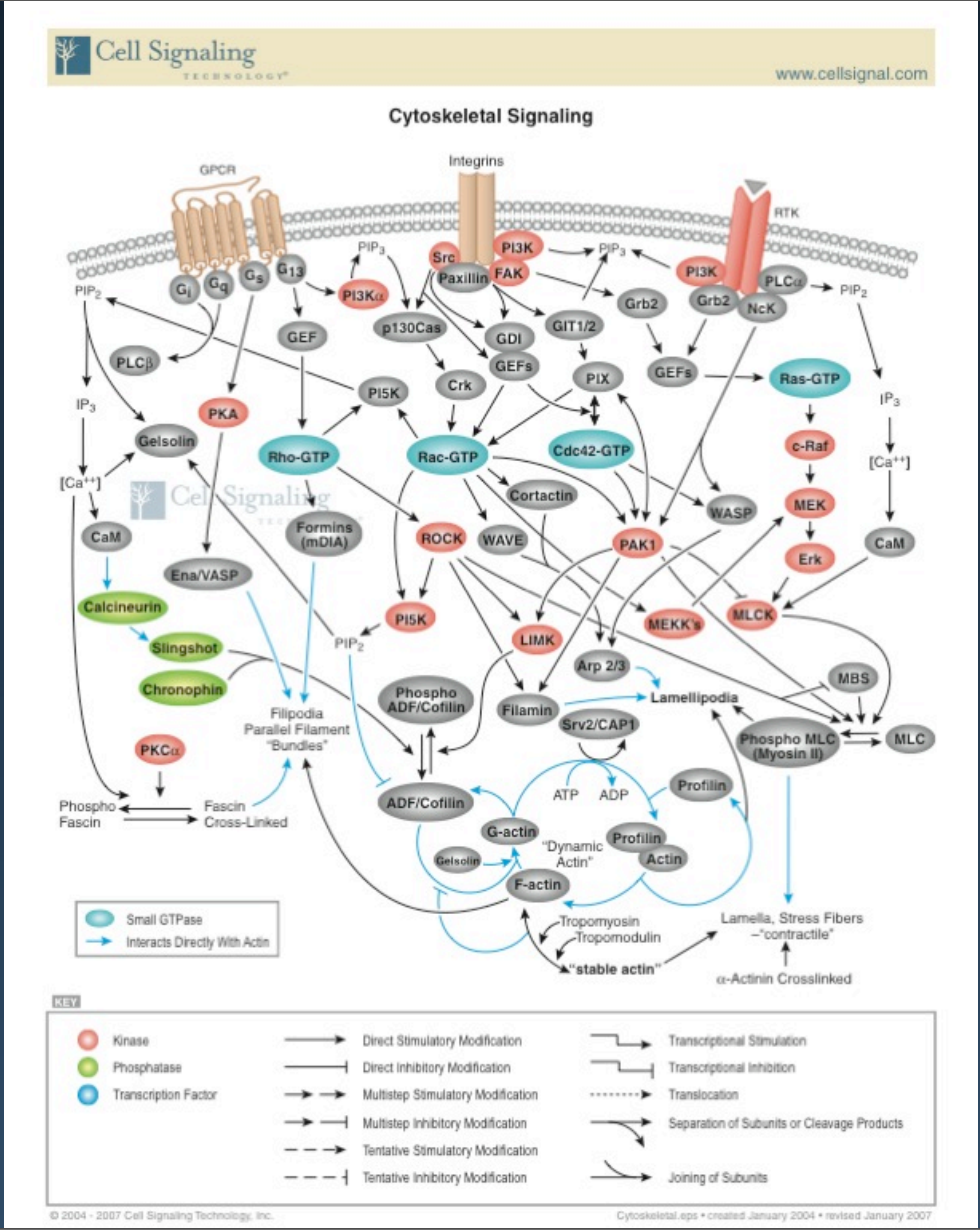


The cell cortex

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PLCβ
GEF
p130Cas
GDI
GEFs
GIT1/2
Grb2
NcK
PLCα
IP3
PKA
PI5K
Crk
PIX
Ras-GTP
[Ca++]
Gelsolin
Rho-GTP
Rac-GTP
Cdc42-GTP
c-Raf
Cortactin
WASP
MEK
CaM
Formins (mDIA)
ROCK
WAVE
PAK1
Erk
Ena/VASP
Calcineurin
Slingshot
Chronophin
LIMK
MEKK's
MLCK
Arp 2/3
MBS
Phospho ADF/Cofilin
Filamin
Lamellipodia
Srv2/CAP1
Phospho MLC (Myosin II)
MLC
PKCα
Filipodia Parallel Filament "Bundles"
Phospho Fascin
Fascin Cross-Linked
ADF/Cofilin
ATP
ADP
Profilin
G-actin
"Dynamic Actin"
Actin
F-actin
Tropomyosin
Tropomodulin
"stable actin"
Lamella, Stress Fibers –"contractile"
α-Actinin Crosslinked
Small GTPase
Interacts Directly With Actin
KEY
Kinase
Phosphatase
Transcription Factor
Direct Stimulatory Modification
Direct Inhibitory Modification
Multistep Stimulatory Modification
Multistep Inhibitory Modification
Tentative Stimulatory Modification
Tentative Inhibitory Modification
Transcriptional Stimulation
Transcriptional Inhibition
Translocation
Separation of Subunits or Cleavage Products
Joining of Subunits
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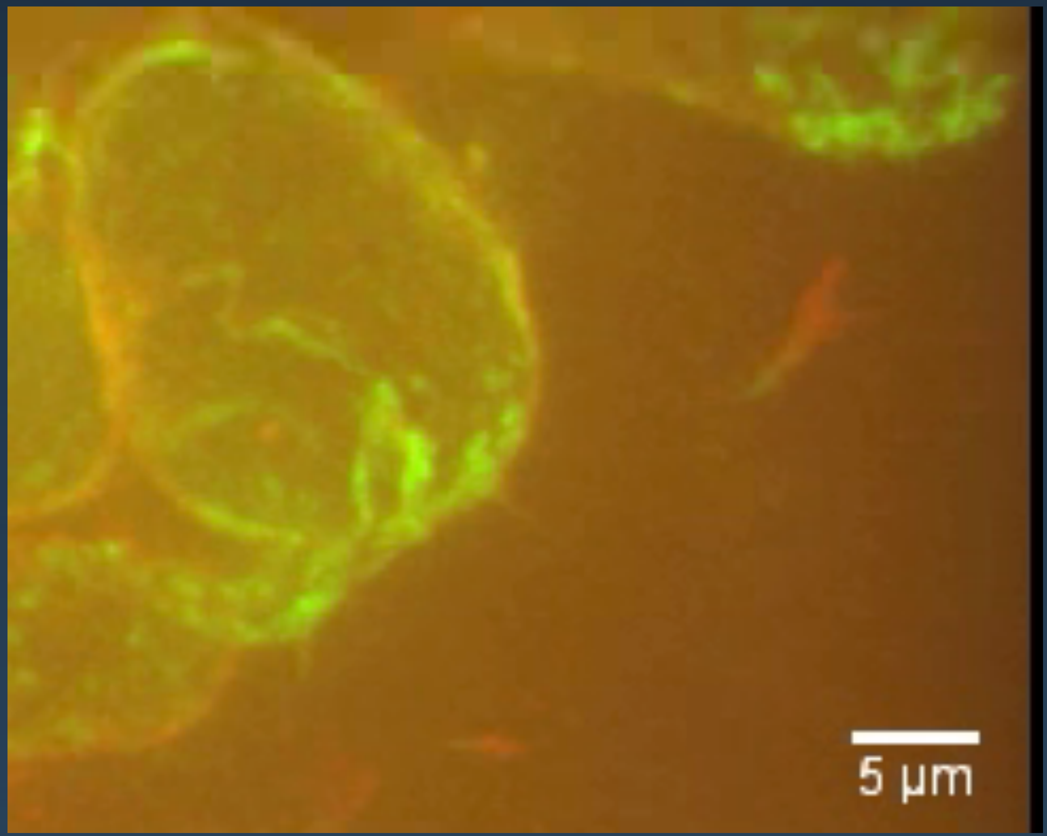
Cortical contractions drive cell deformations

L929 fibroblast dividing

Zebrafish prechordal plate progenitor cell



Lifeact-GFP (F-actin marker)

GPI anchored RFP (membrane)
Lifeact-GFP (F-actin)

Cortical contractions drive cell deformations

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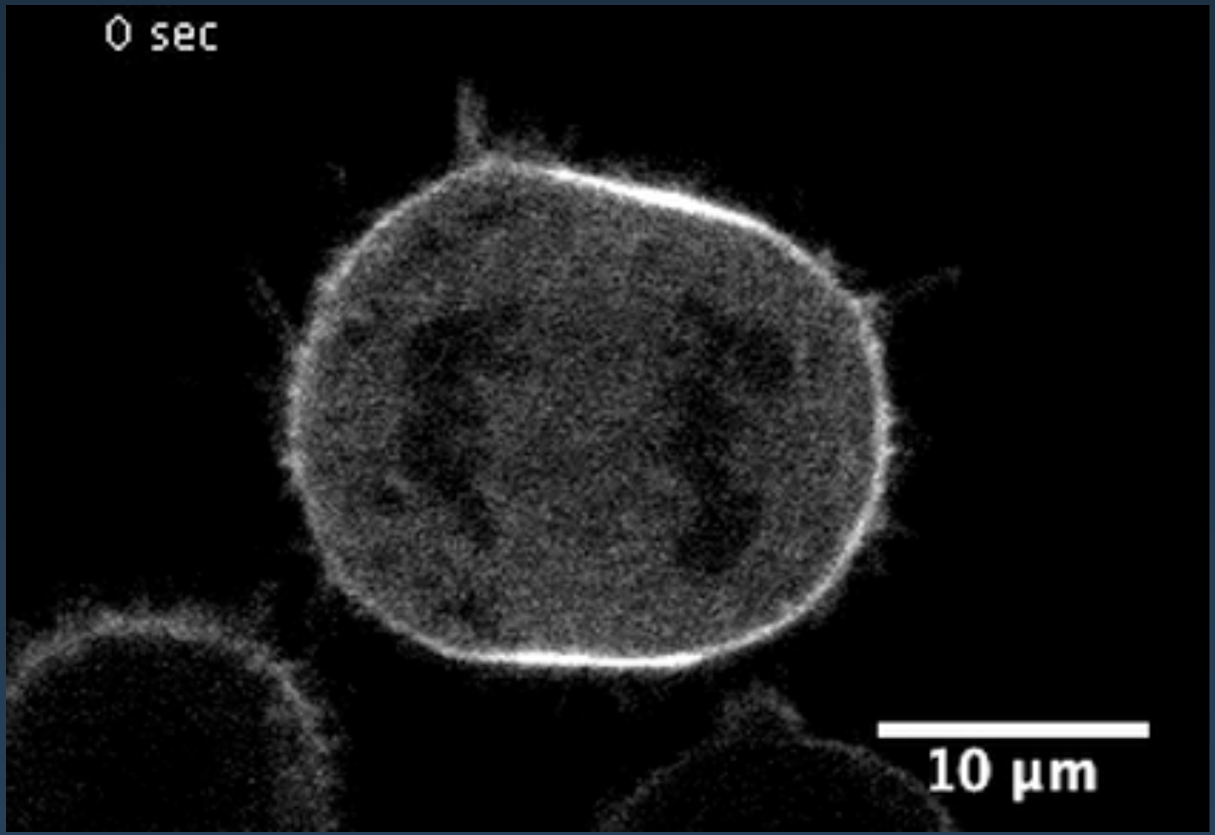

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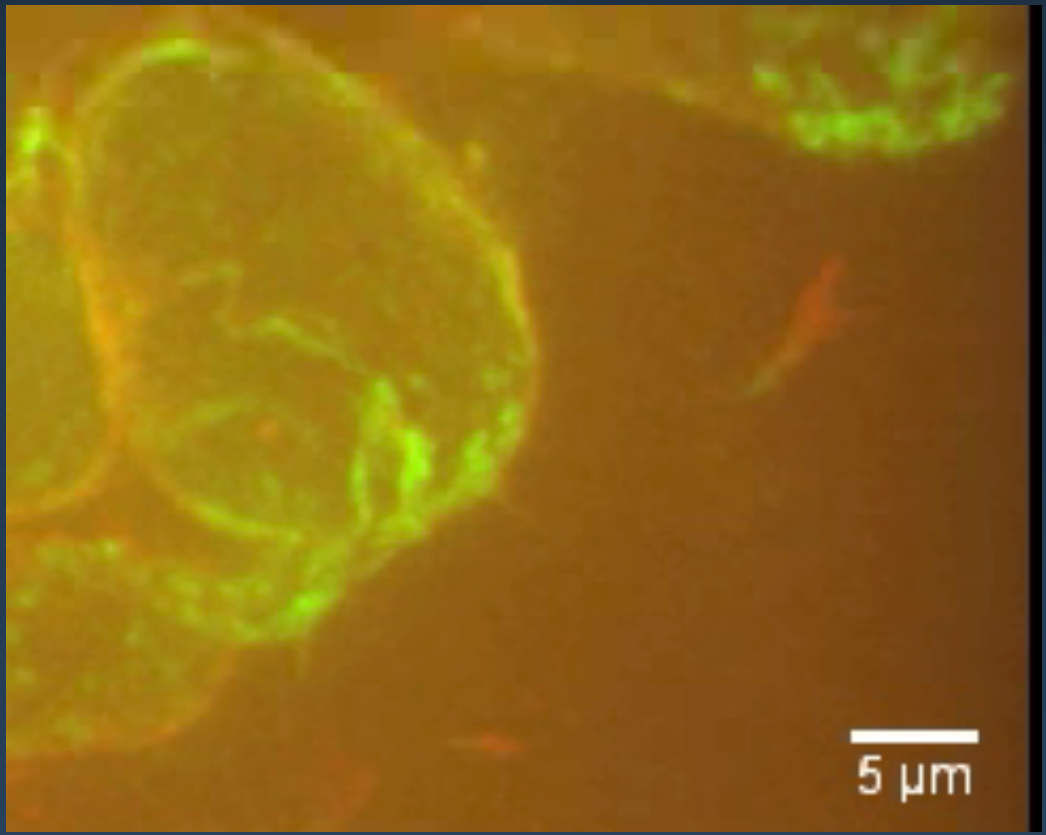
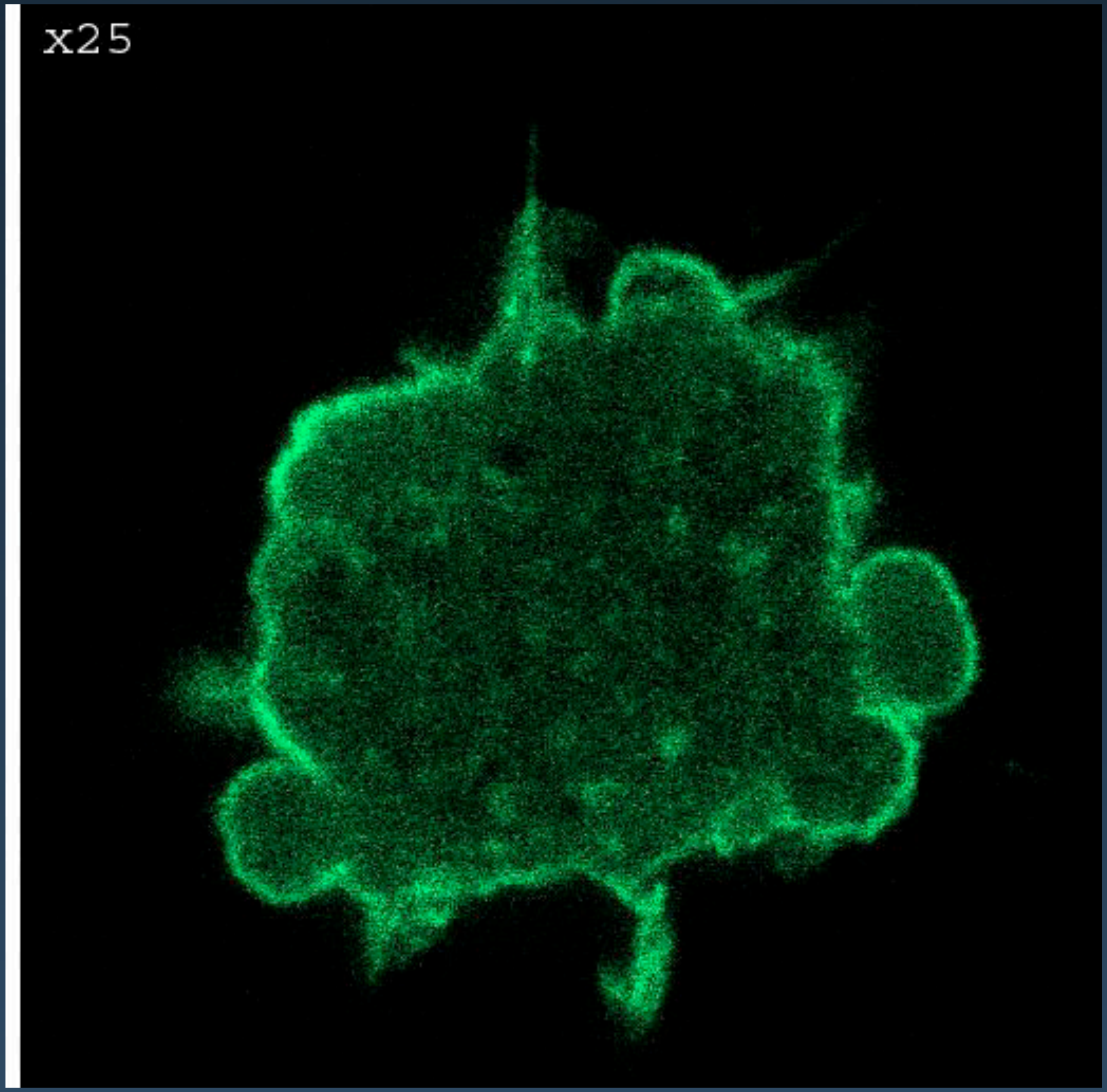


GPI anchored RFP (membrane)
Lifeact-GFP (F-actin)

Blebbing

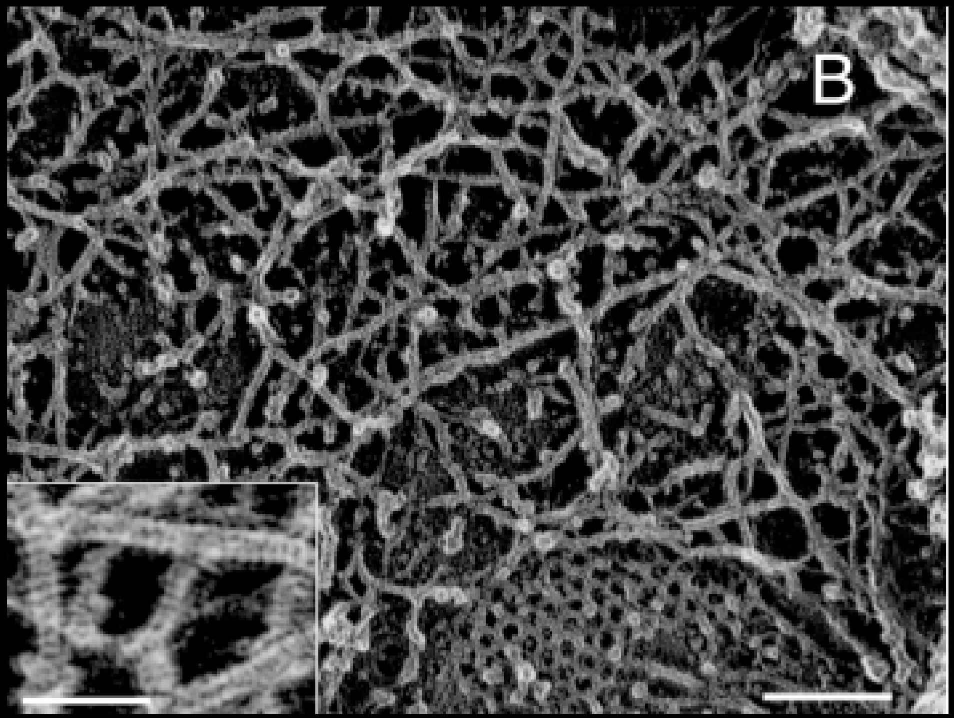
L929 fibroblast NZ-treated, actin-GFP

Blebbing



L929 fibroblast NZ-treated, actin-GFP

The actomyosin cortex



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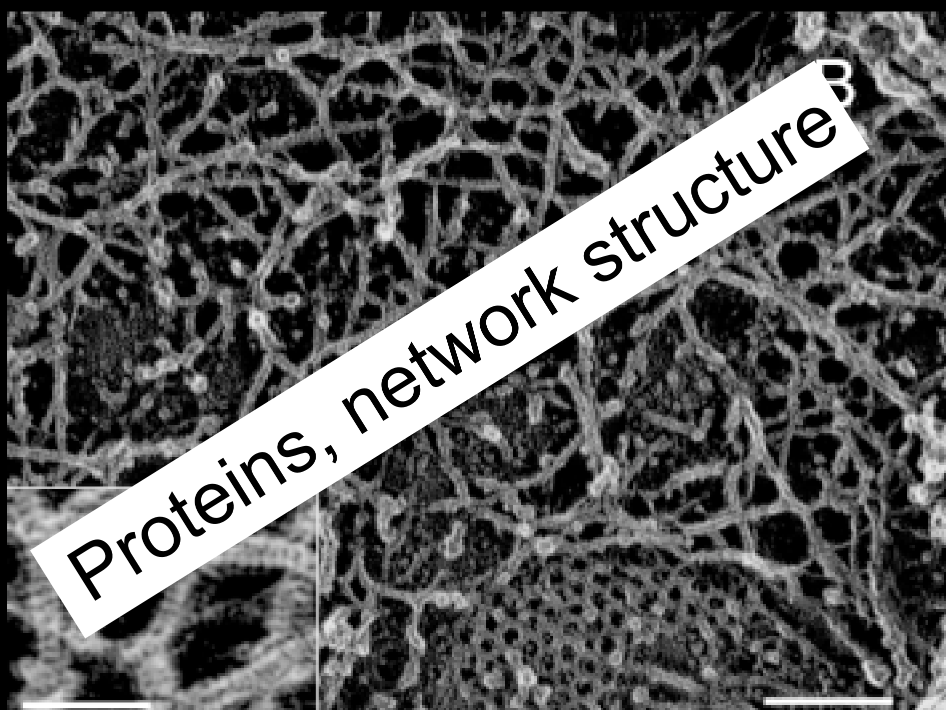

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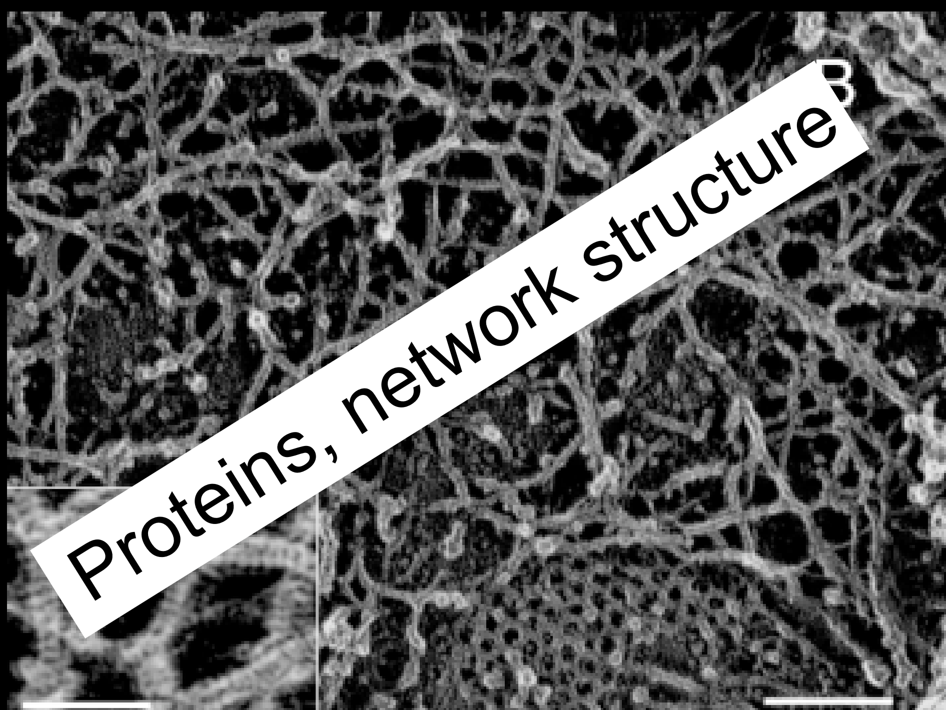

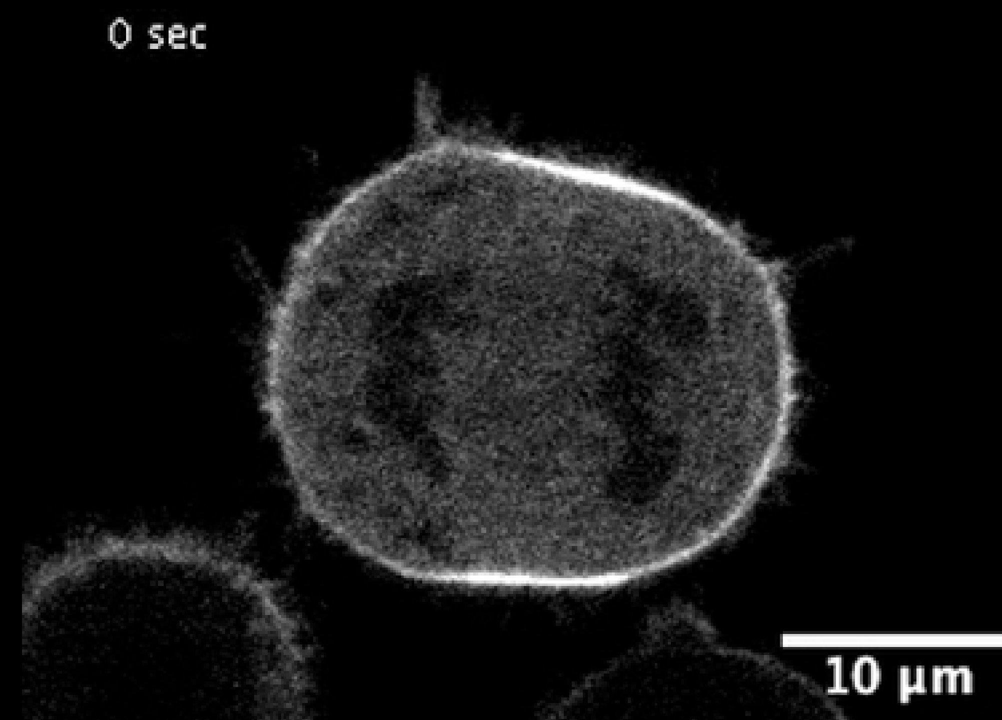

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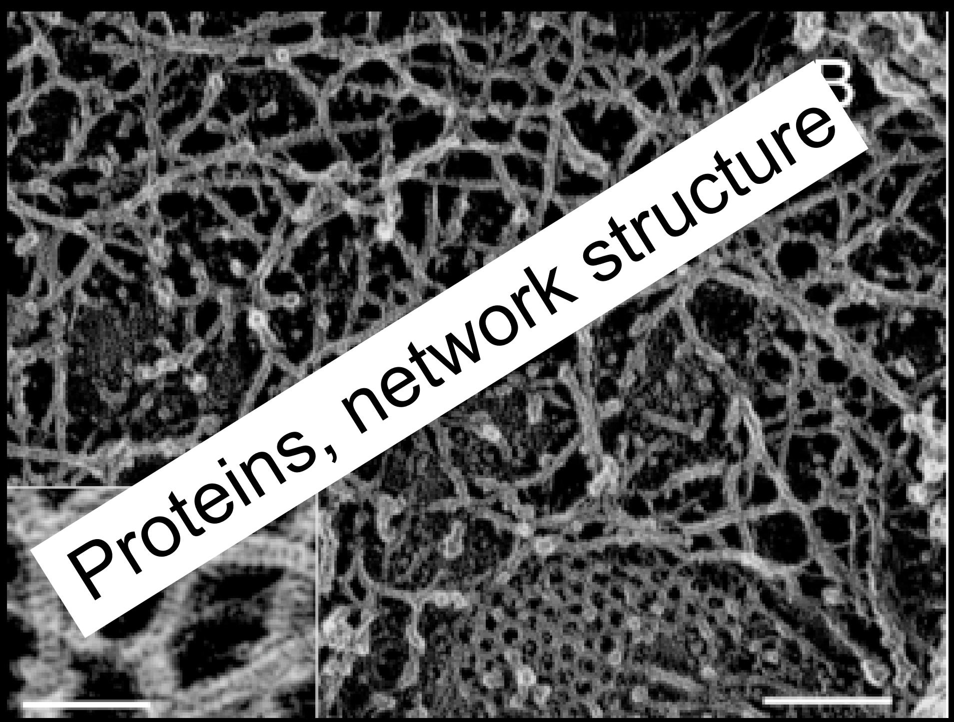

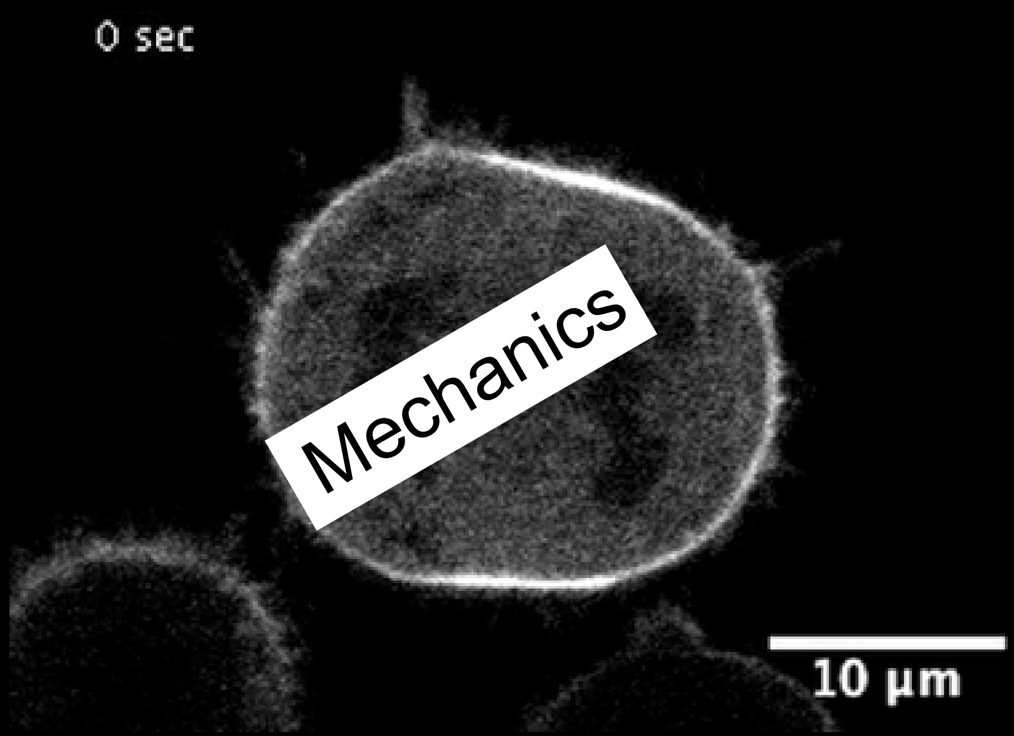

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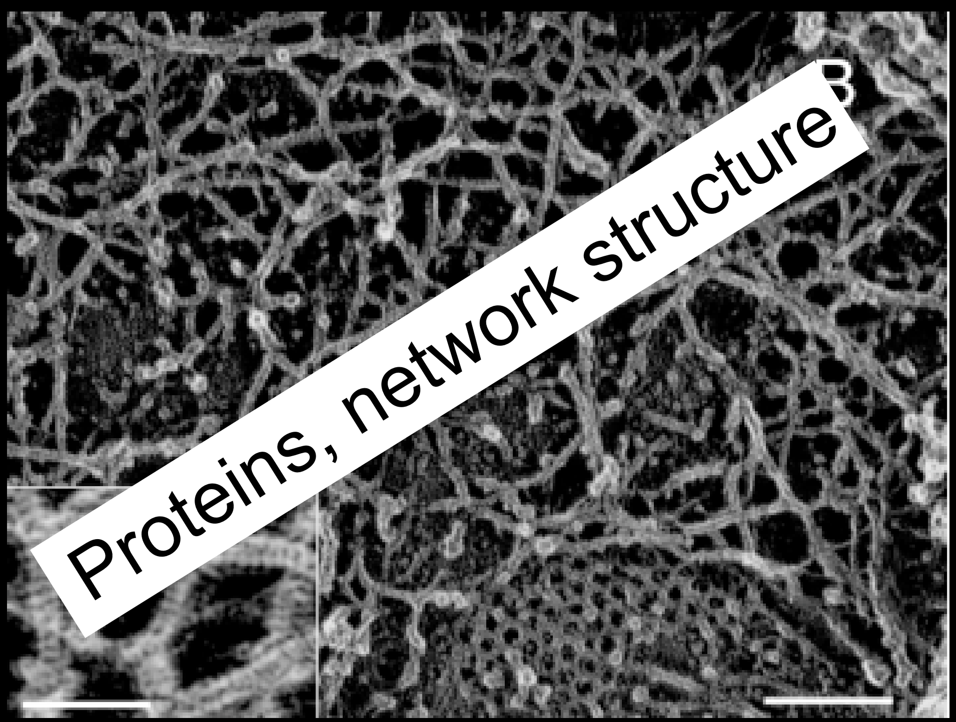

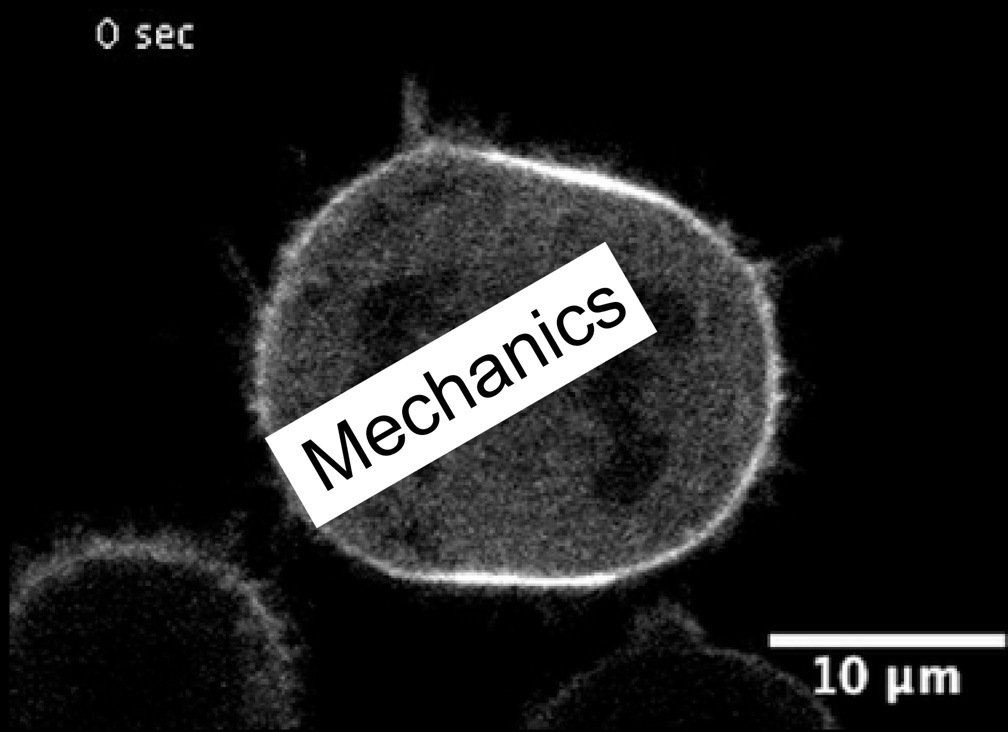

NRK cells
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L929 fibroblast dividing, Lifeact-GFP

- Cortex mechanics and blebs
- Blebs and cleavage furrow stability
- Blebs in migration

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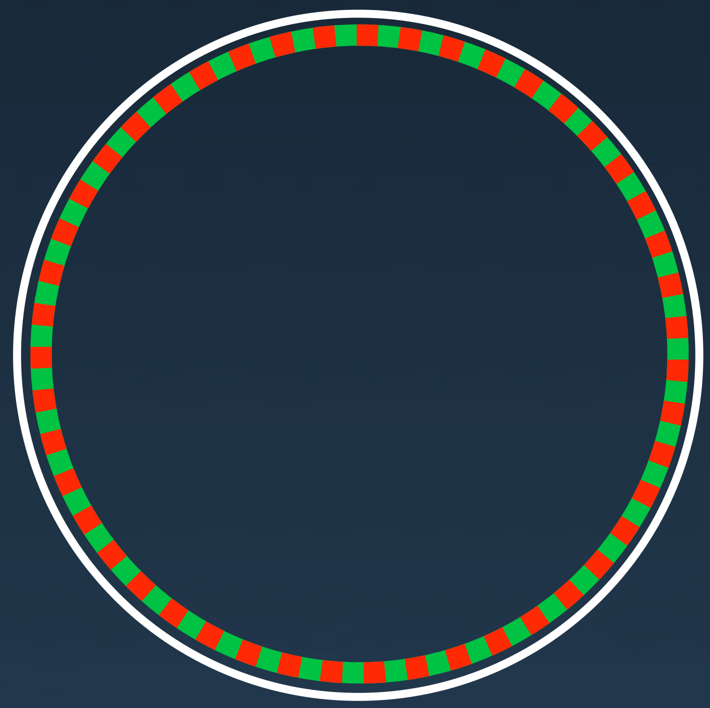
- Blebs in migration

Cortical tension



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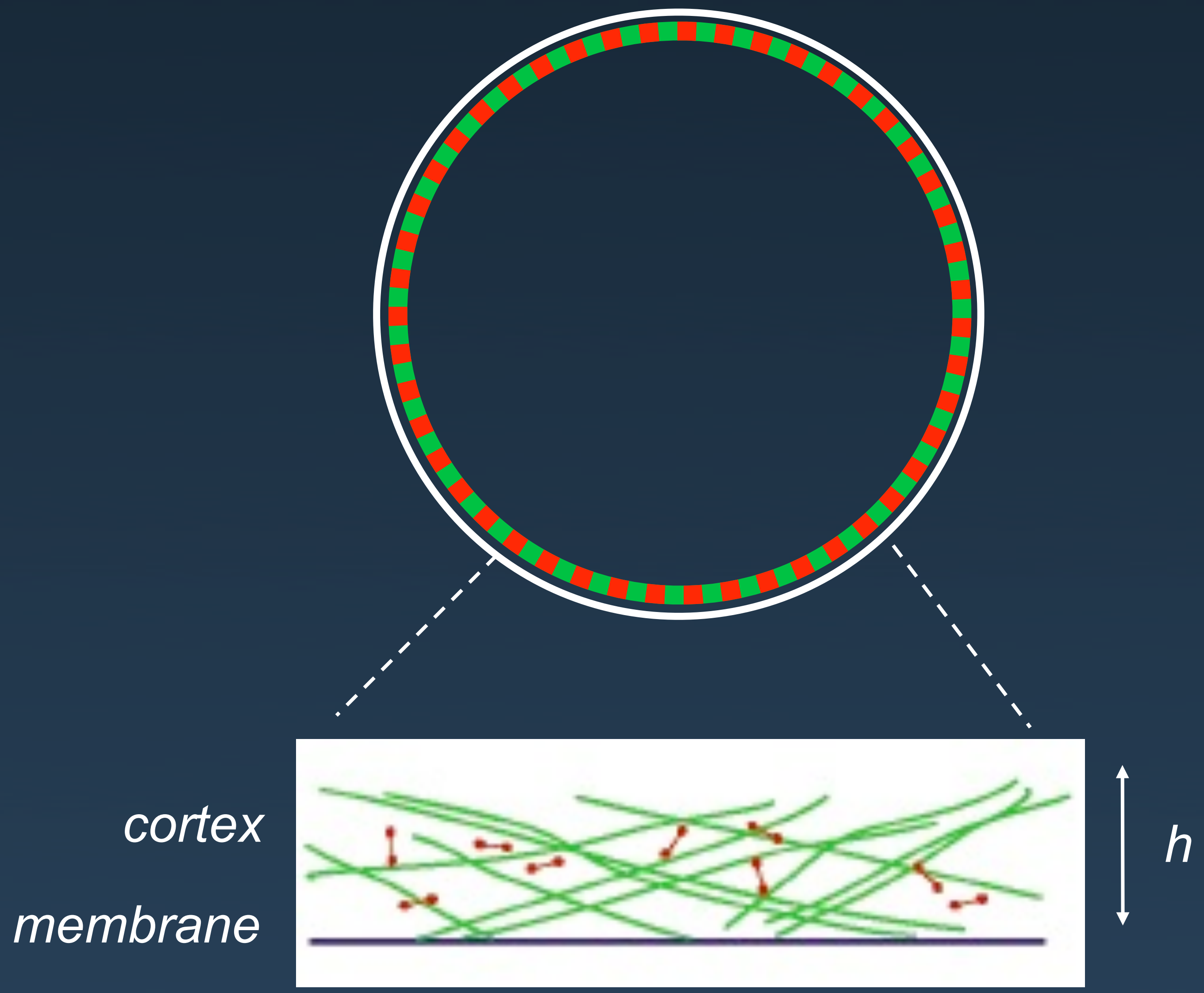

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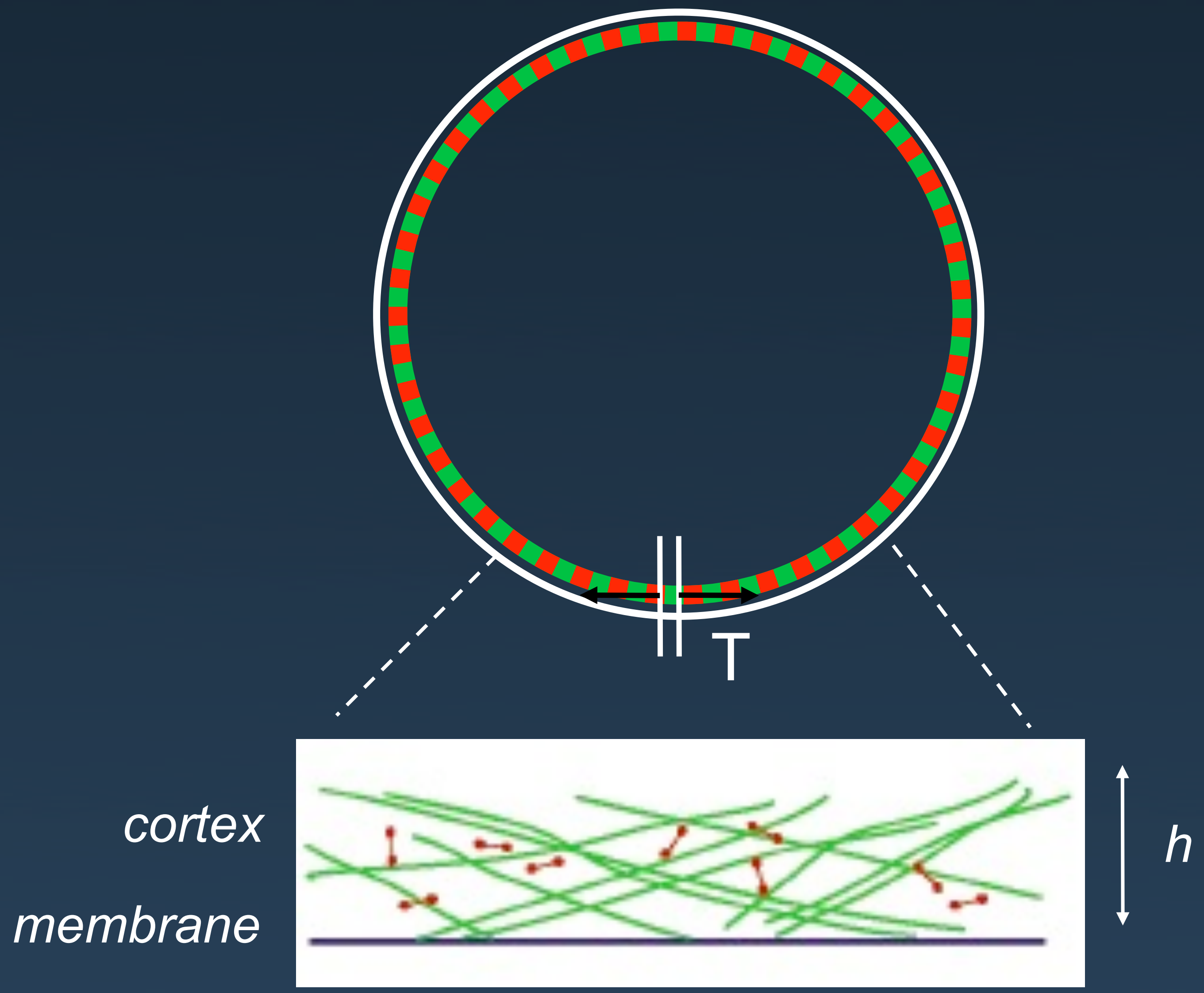
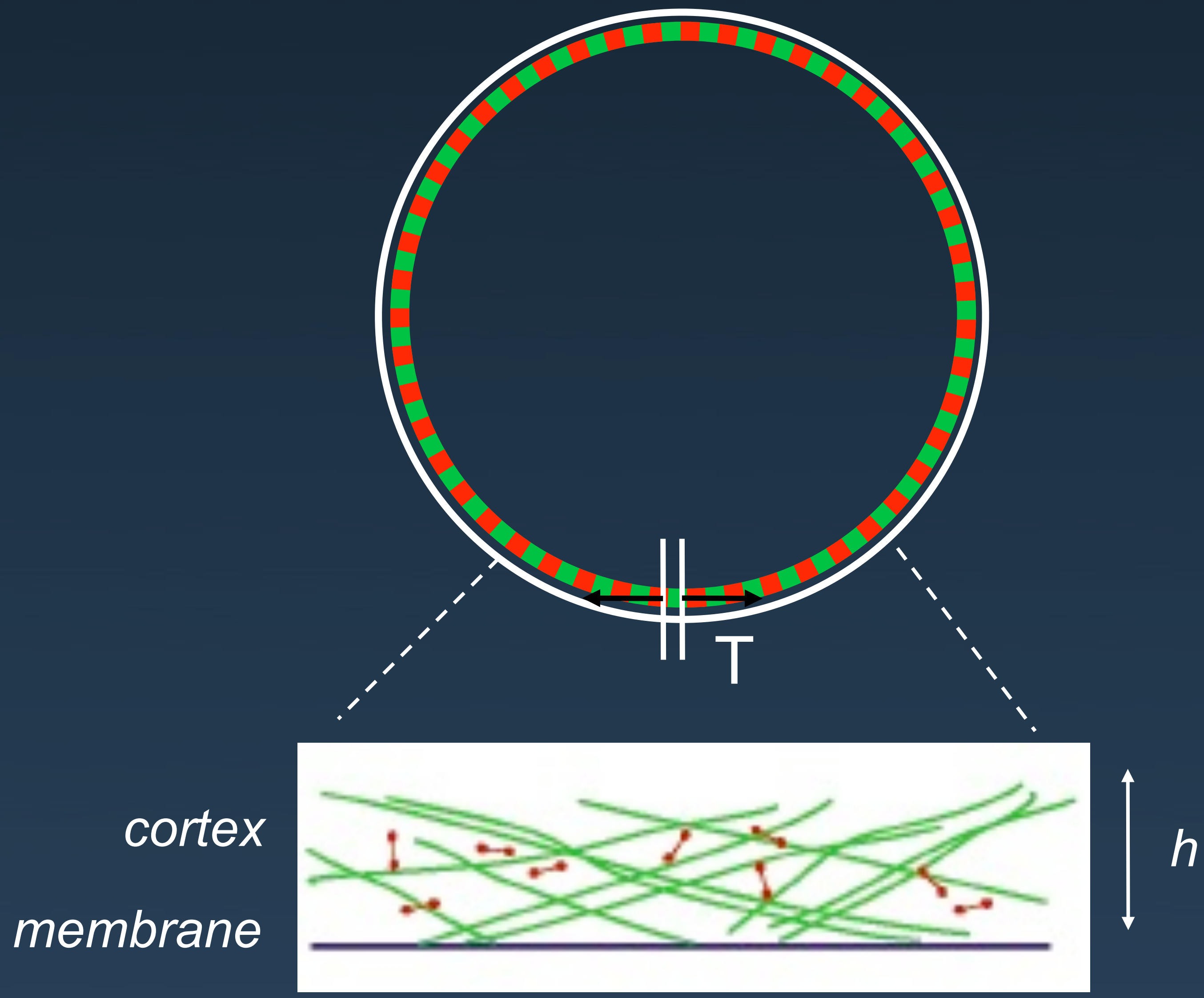
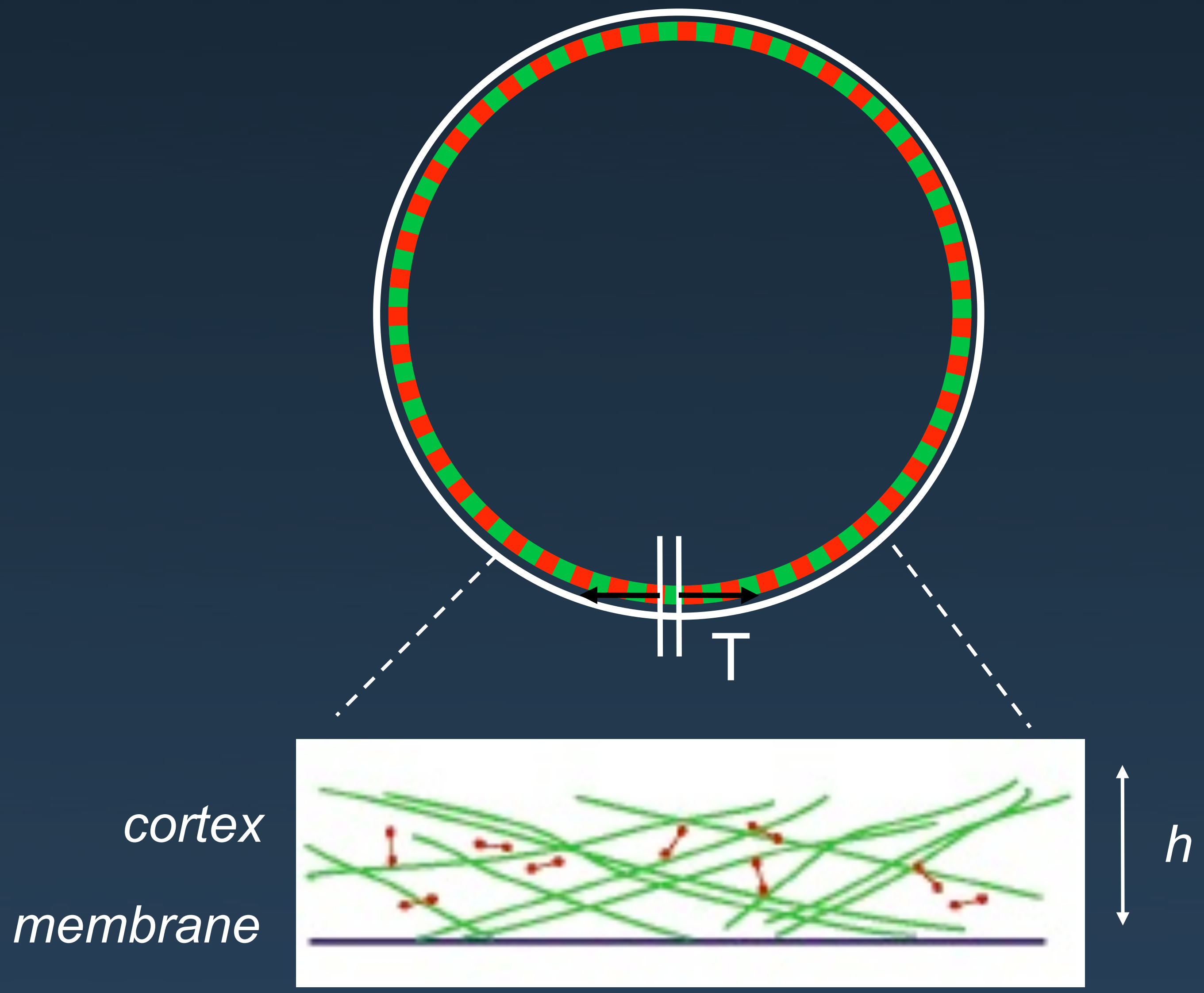


Cortical tension



Tension: $T = \zeta \Delta\mu \times h$

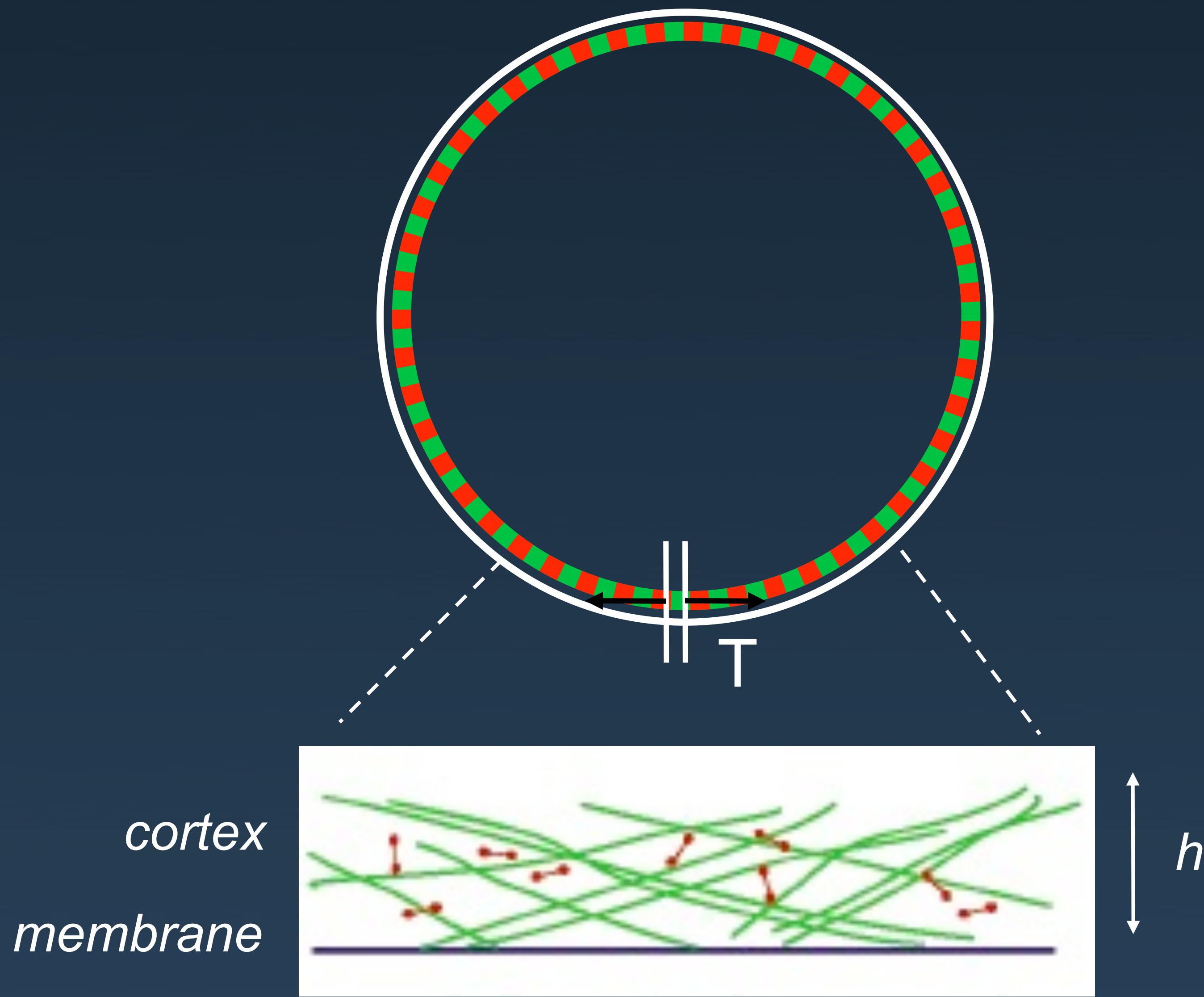
Cortical tension



Tension: $T = \zeta\Delta\mu \times h$

Active stress in the network
Proportional to myosin
concentration

Cortical tension



Tension: $T = \zeta \Delta\mu \times h$

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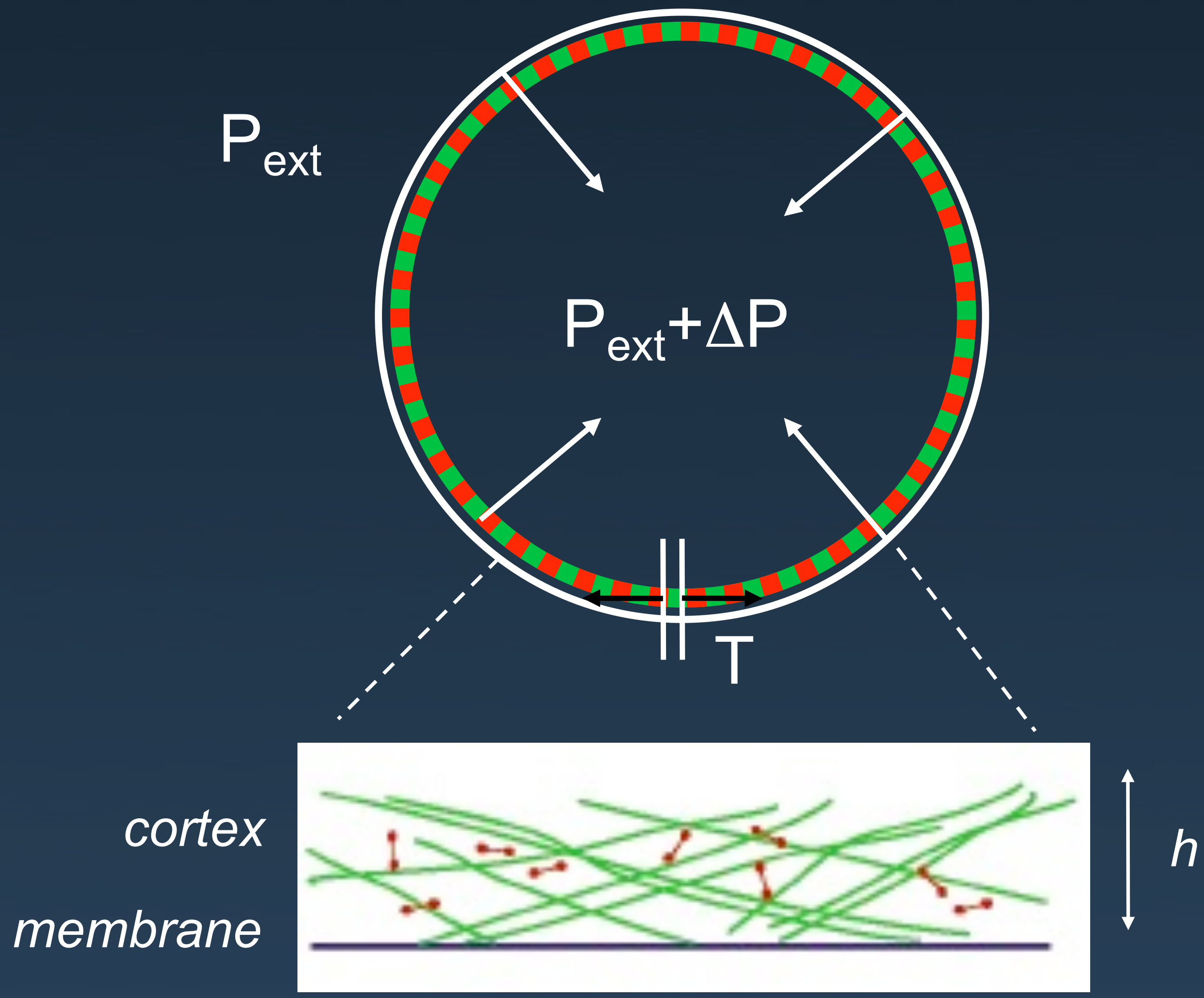
Cortex thickness

Cortical tension



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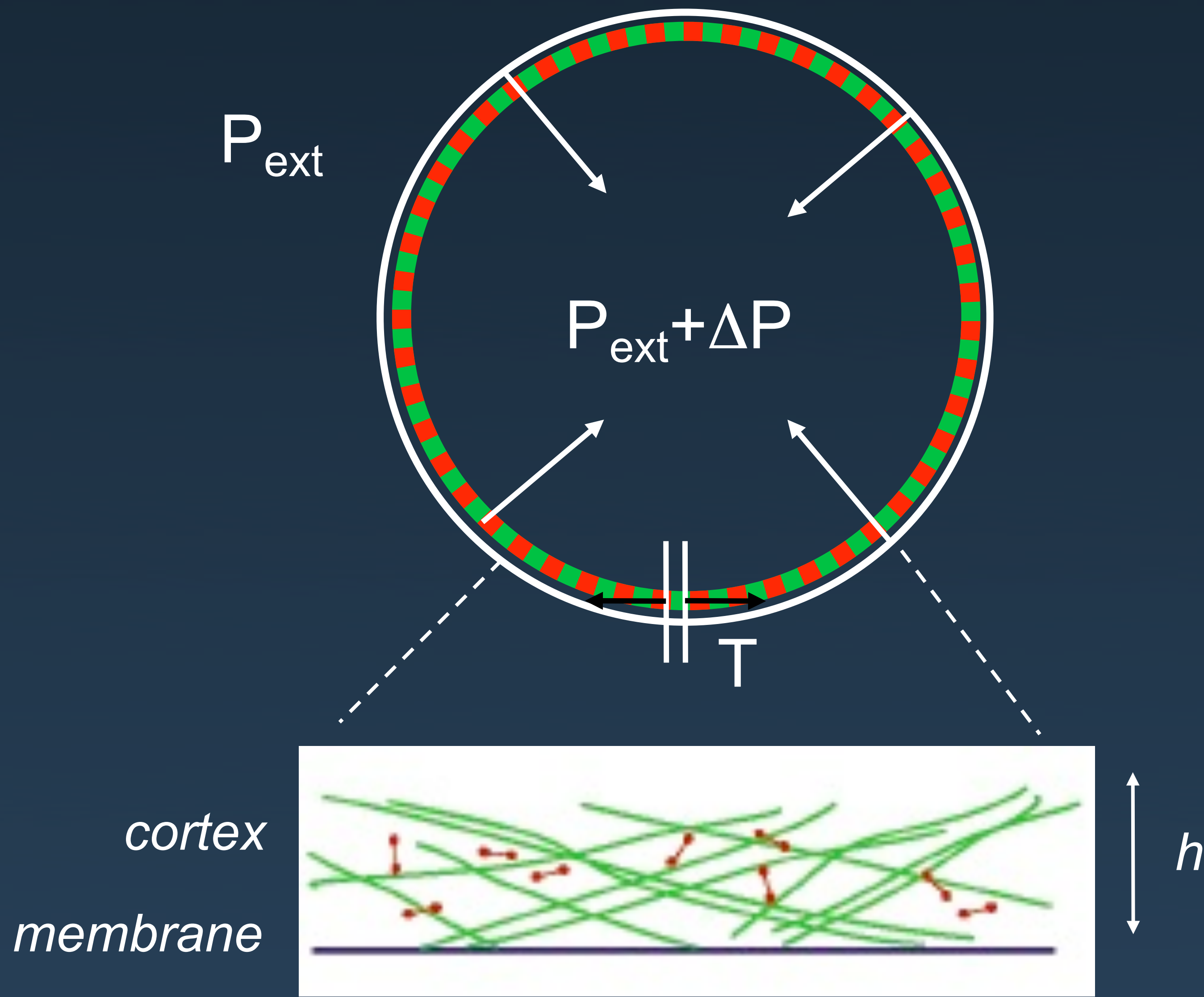


Tension: $T = \zeta\Delta\mu \times h$

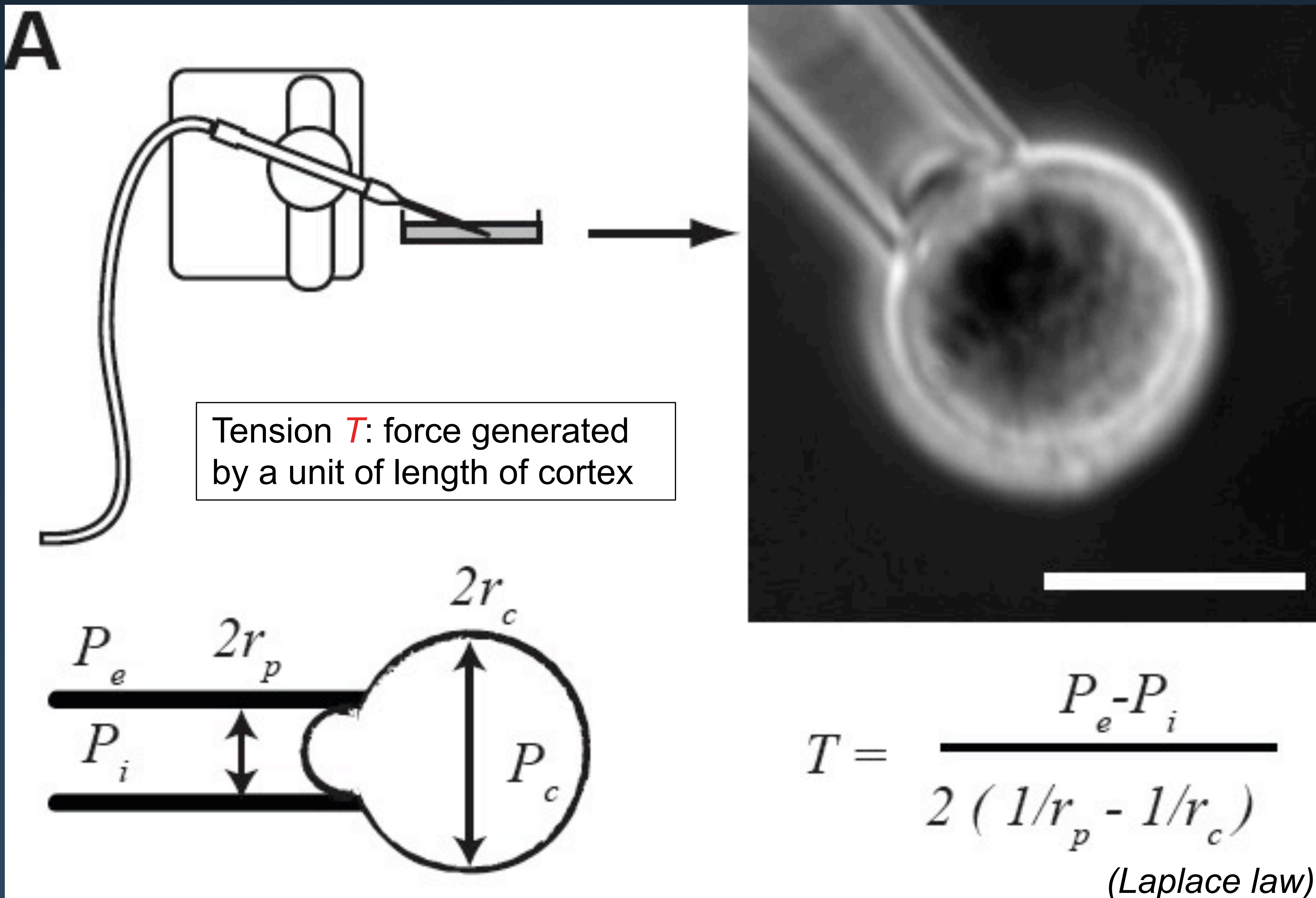
Active stress in the network
Proportional to myosin
concentration

Cortex
thickness

Laplace law gives intracellular pressure:

$$\Delta P = \frac{2T}{R}$$

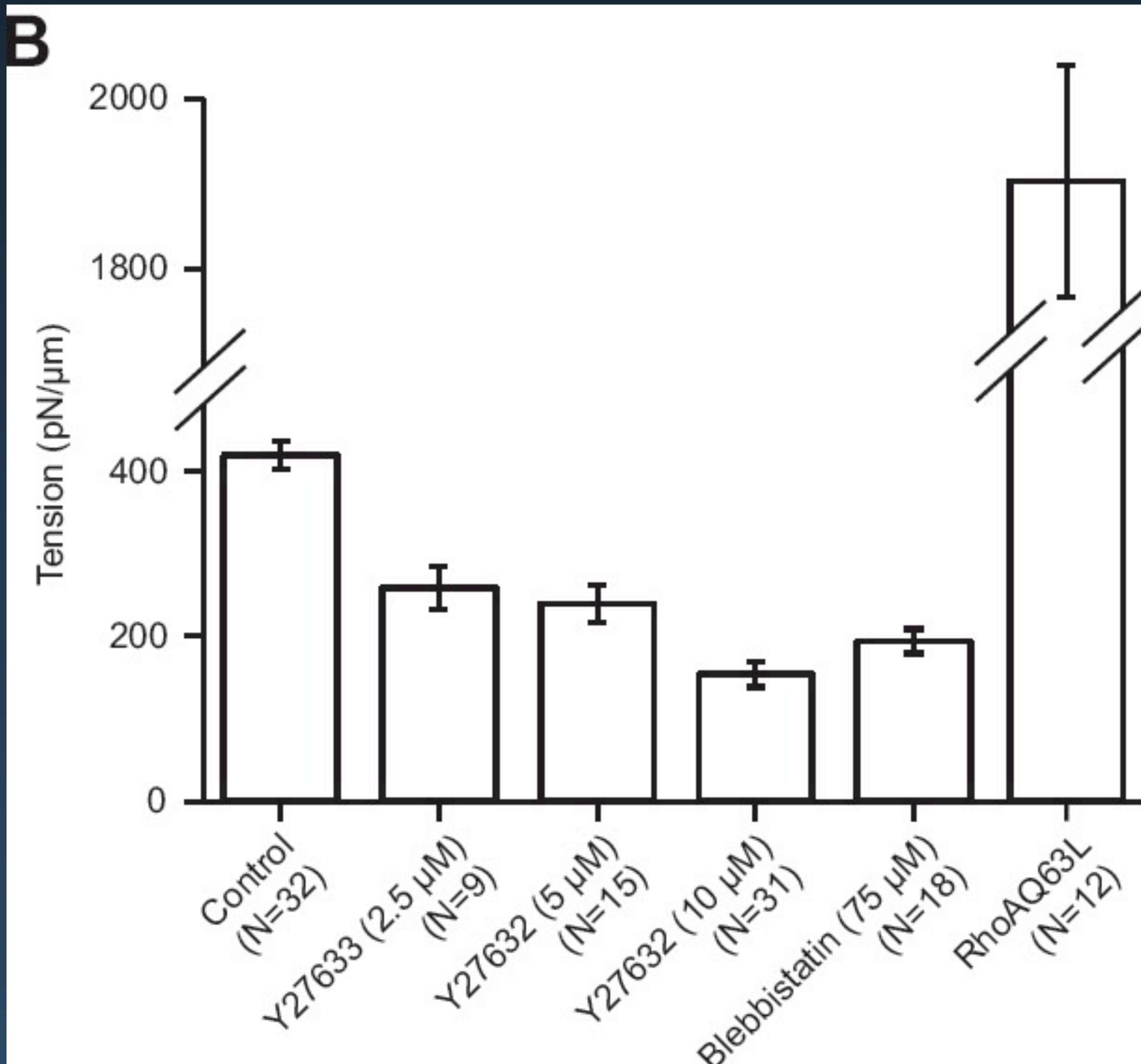
Cortex tension measurement



$$T = \frac{P_e - P_i}{2\,(1/r_p - 1/r_c)}$$

(Laplace law)

Cortical tension depends on myosin activity



CA Rho: myosin overactivated

Myosin activity reduced

(Ulrike Schulze)

Tinevez et al. PNAS 2009

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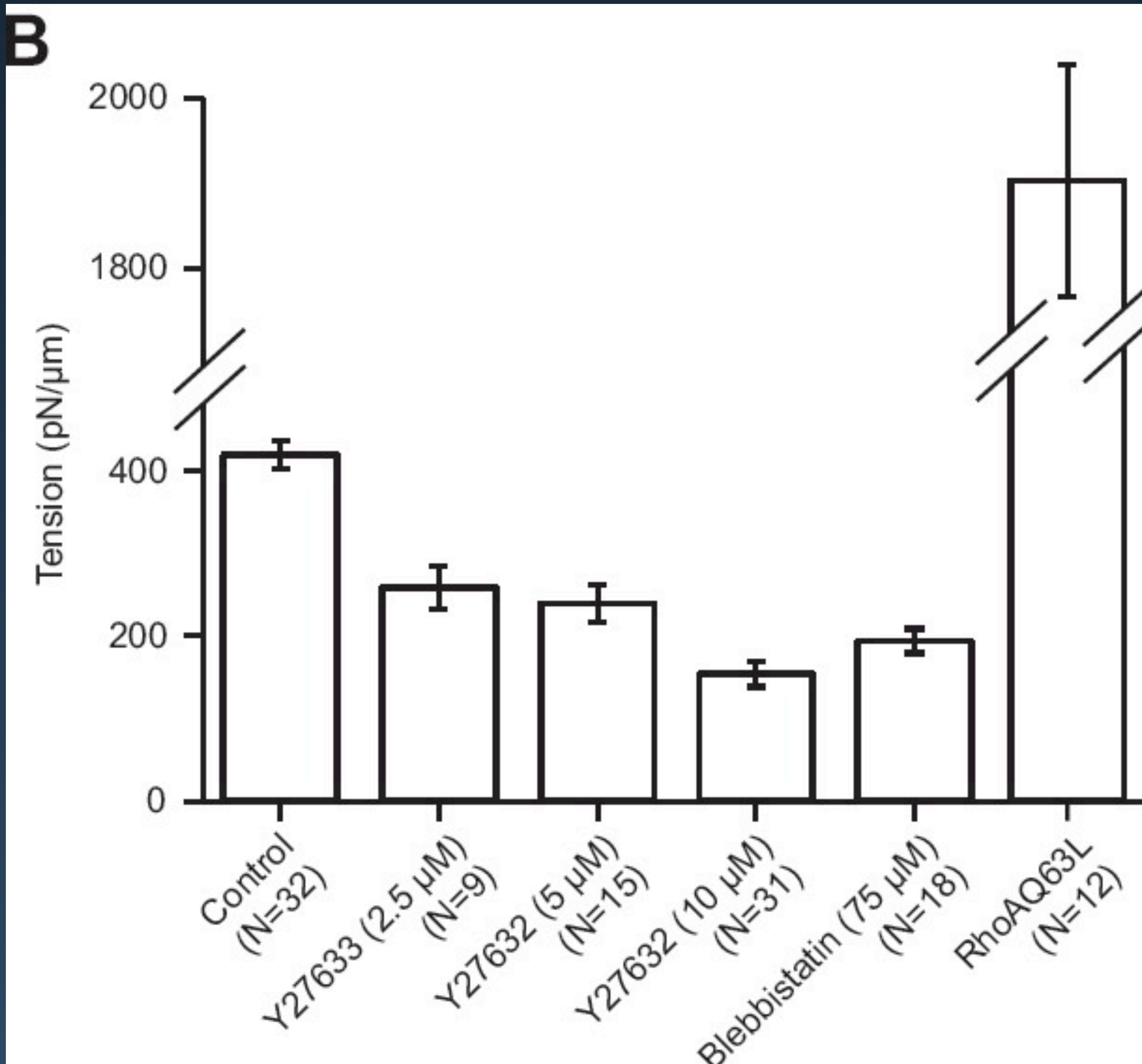


P_{in}-P_{out} ≈100 Pa

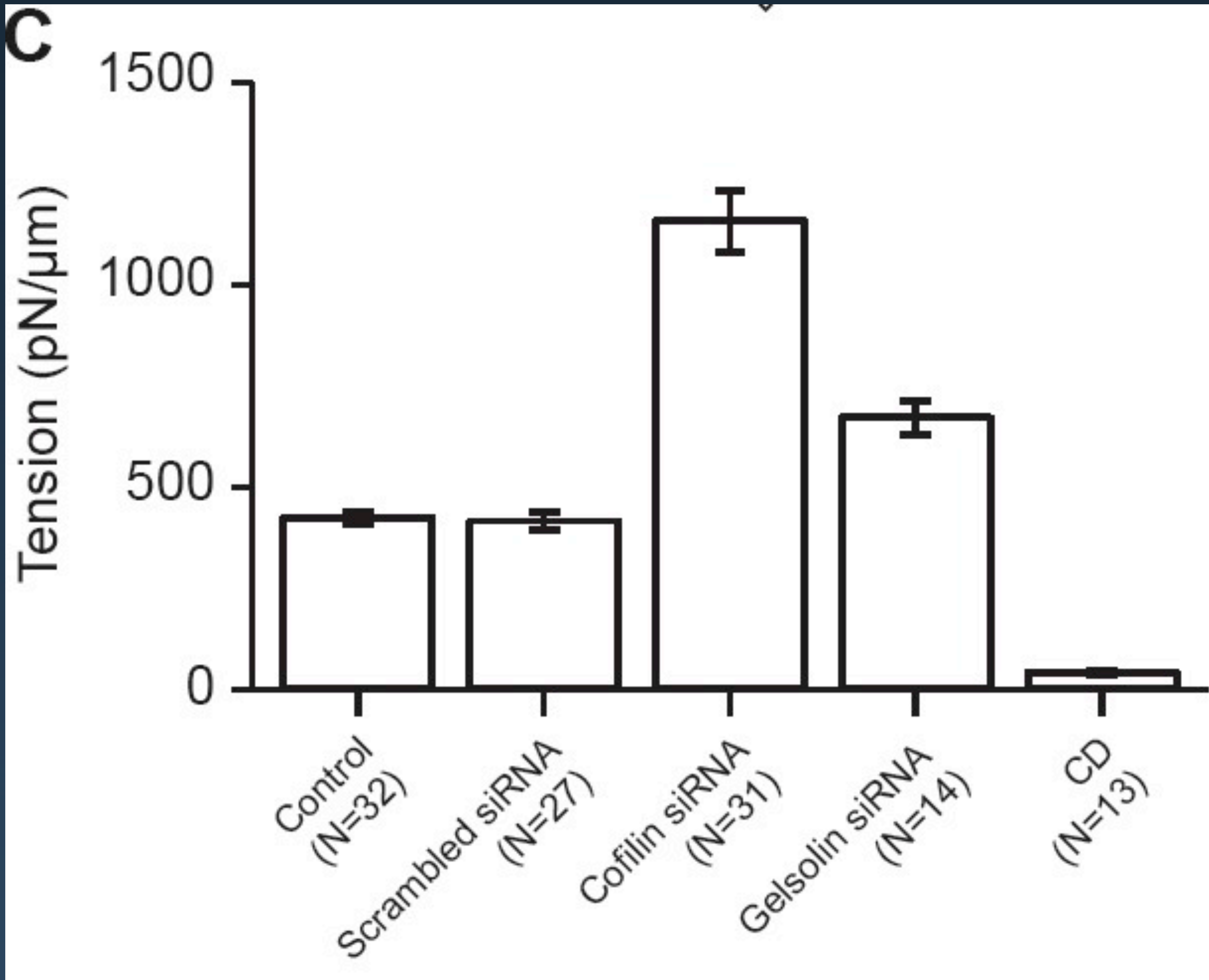
CA Rho: myosin overactivated

Myosin activity reduced

(Ulrike Schulze)

Tinevez et al. PNAS 2009

Cortical tension depends on actin turnover



Cofilin: actin-depolymerizing factor
Gelsolin: actin capping protein

CD: cytochalasin D,
depolymerizes actin F

Bleb formation is thought to result from contractions of the actomyosin cortex

L929 fibroblast NZ-treated, actin-GFP

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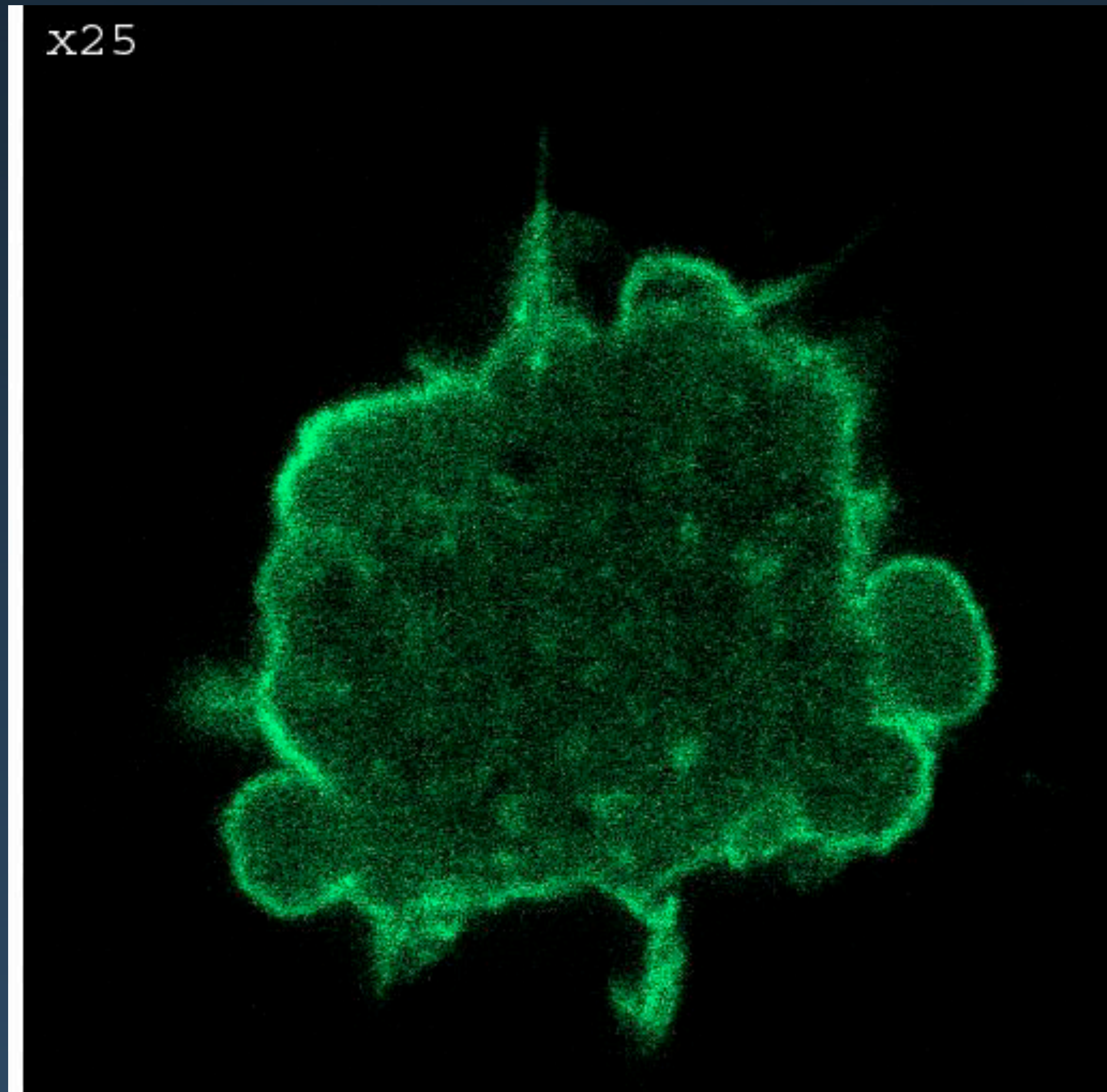
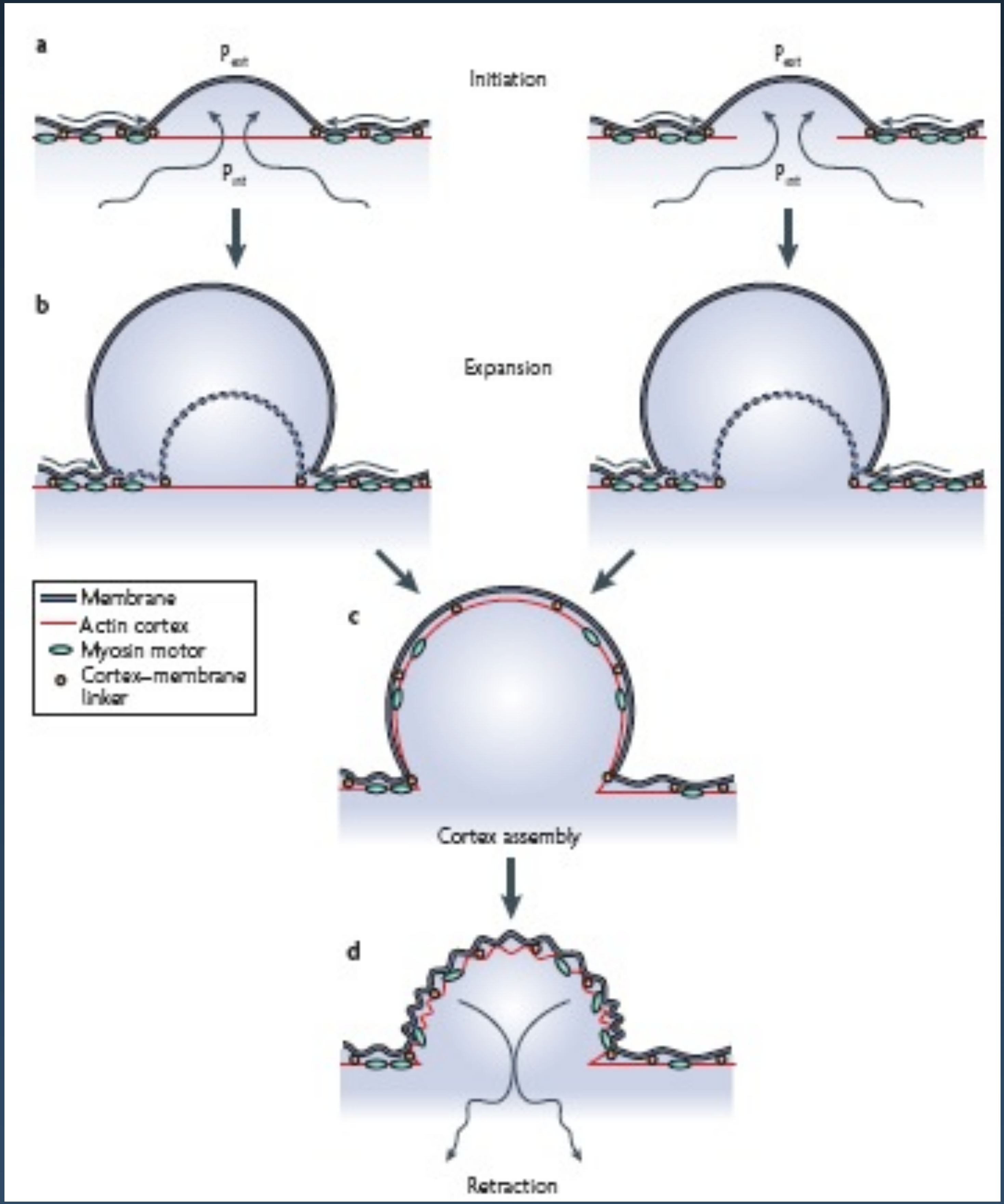


L929 fibroblast NZ-treated, actin-GFP

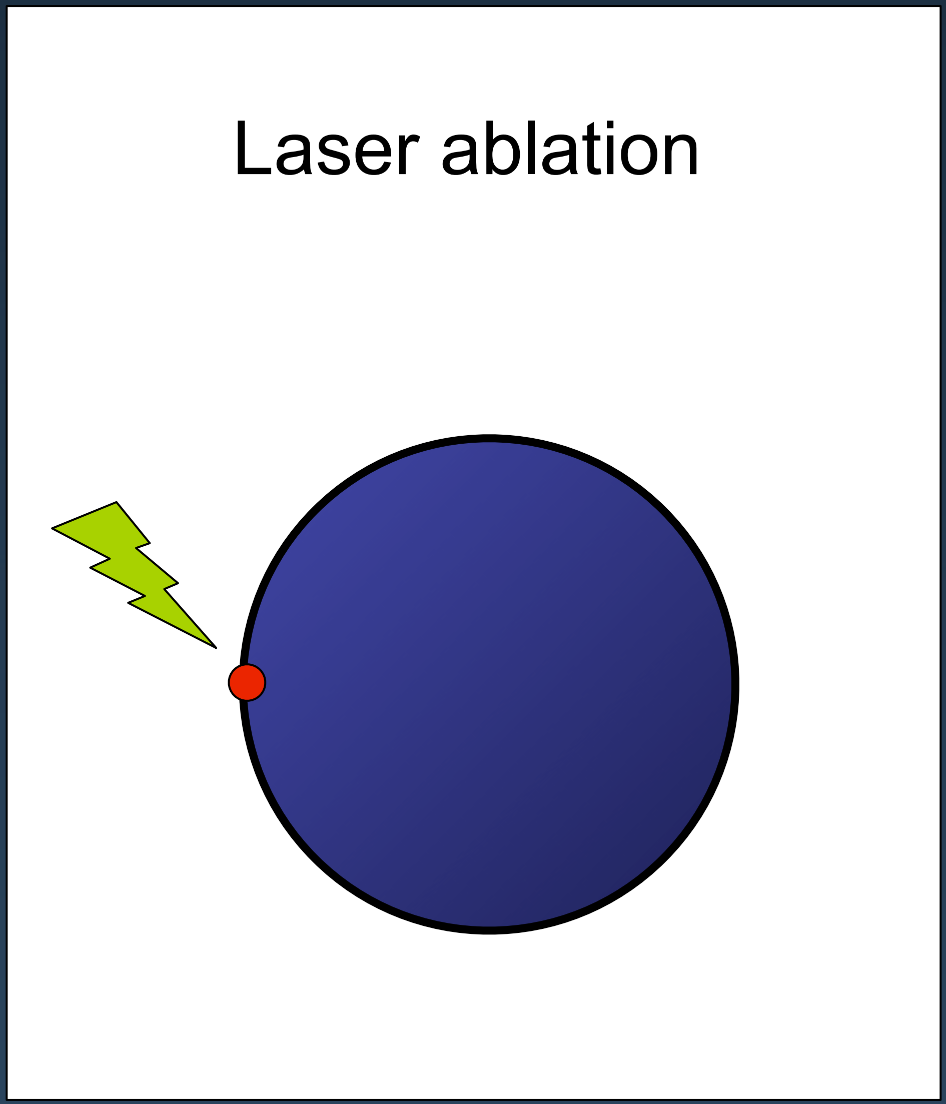
The life-cycle of a bleb



[G. Charras & E. Paluch, *Nat Rev Mol Cell Biol,* 2008]

Bleb growth can be triggered by laser ablation

Laser ablation of the cortex:



L929 cell
Picosecond pulsed laser
(405 nm, 3 mW)

(Jean-Yves Tinevez)

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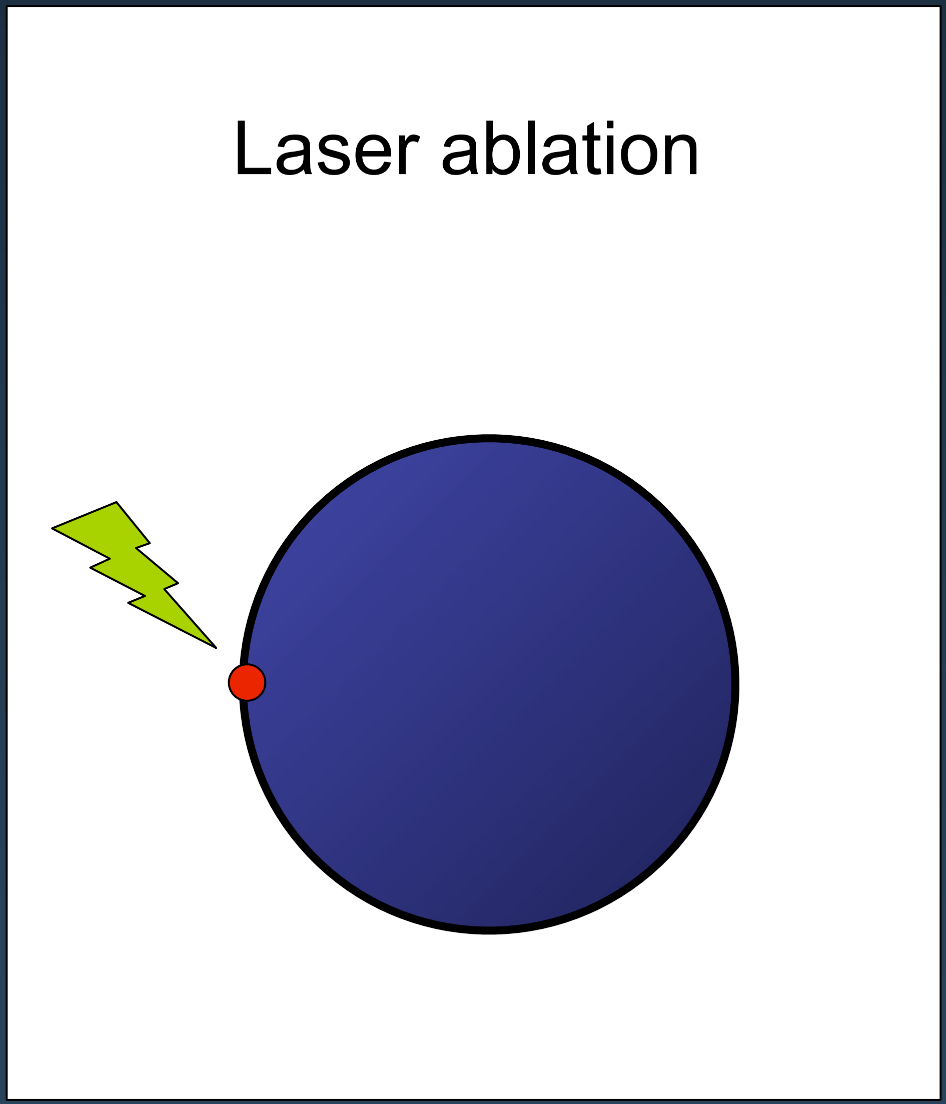

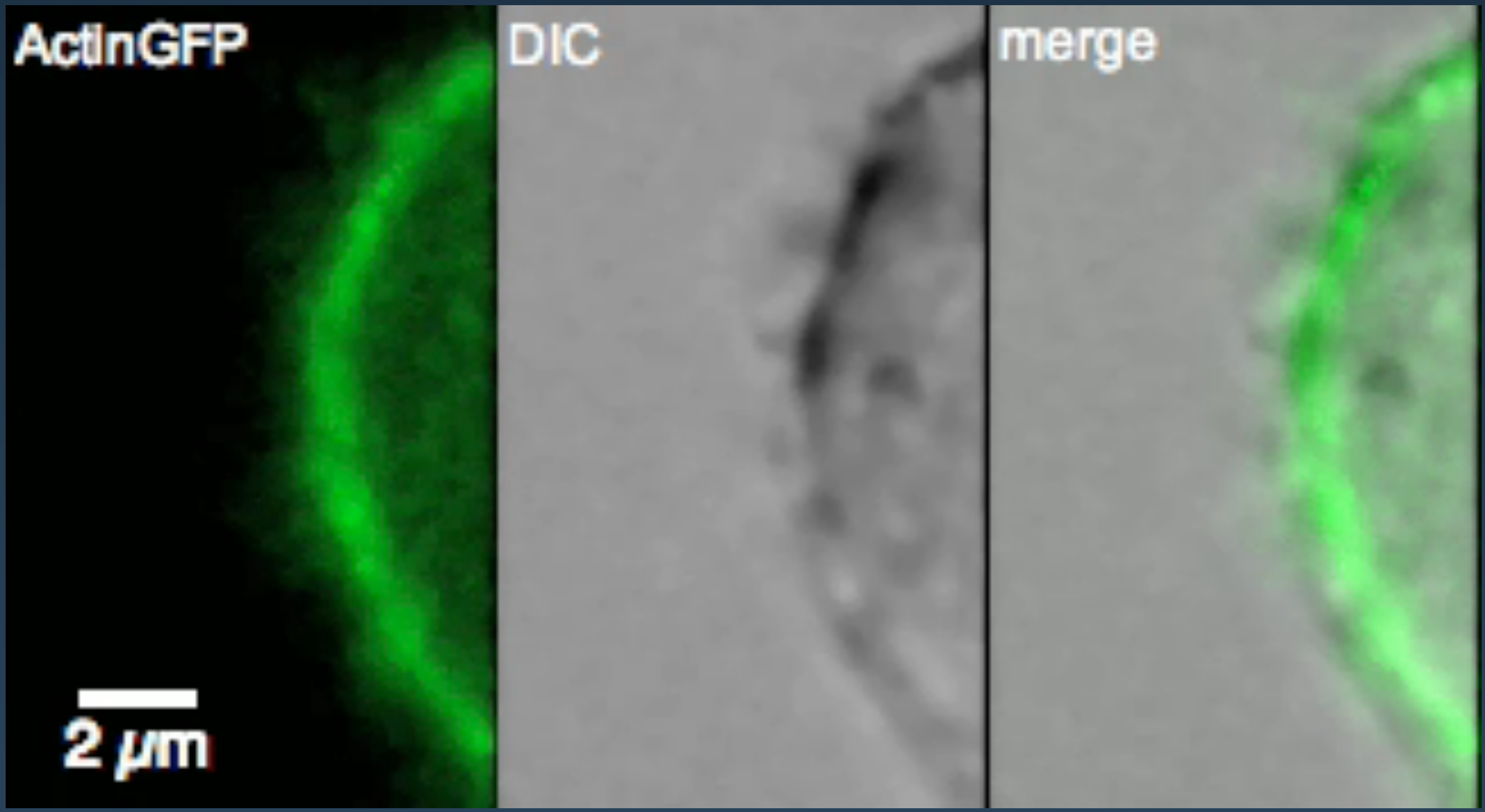

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Picosecond pulsed laser
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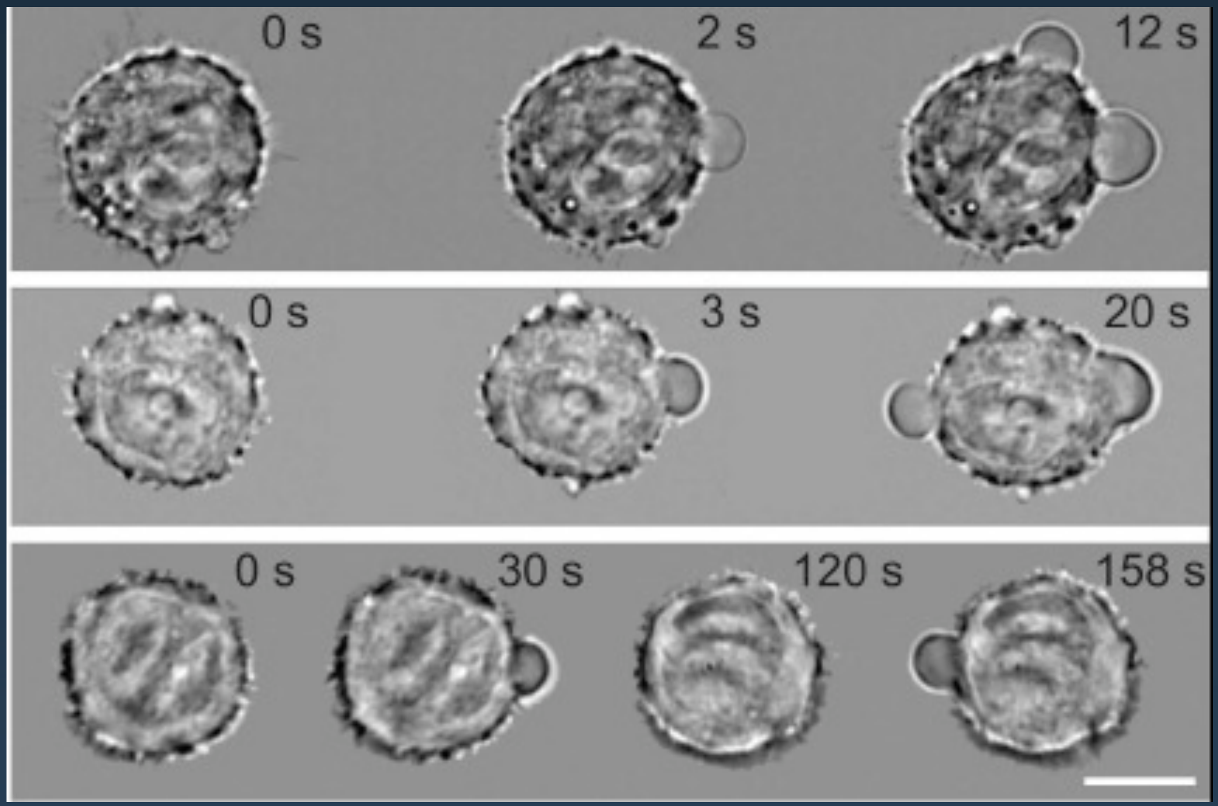
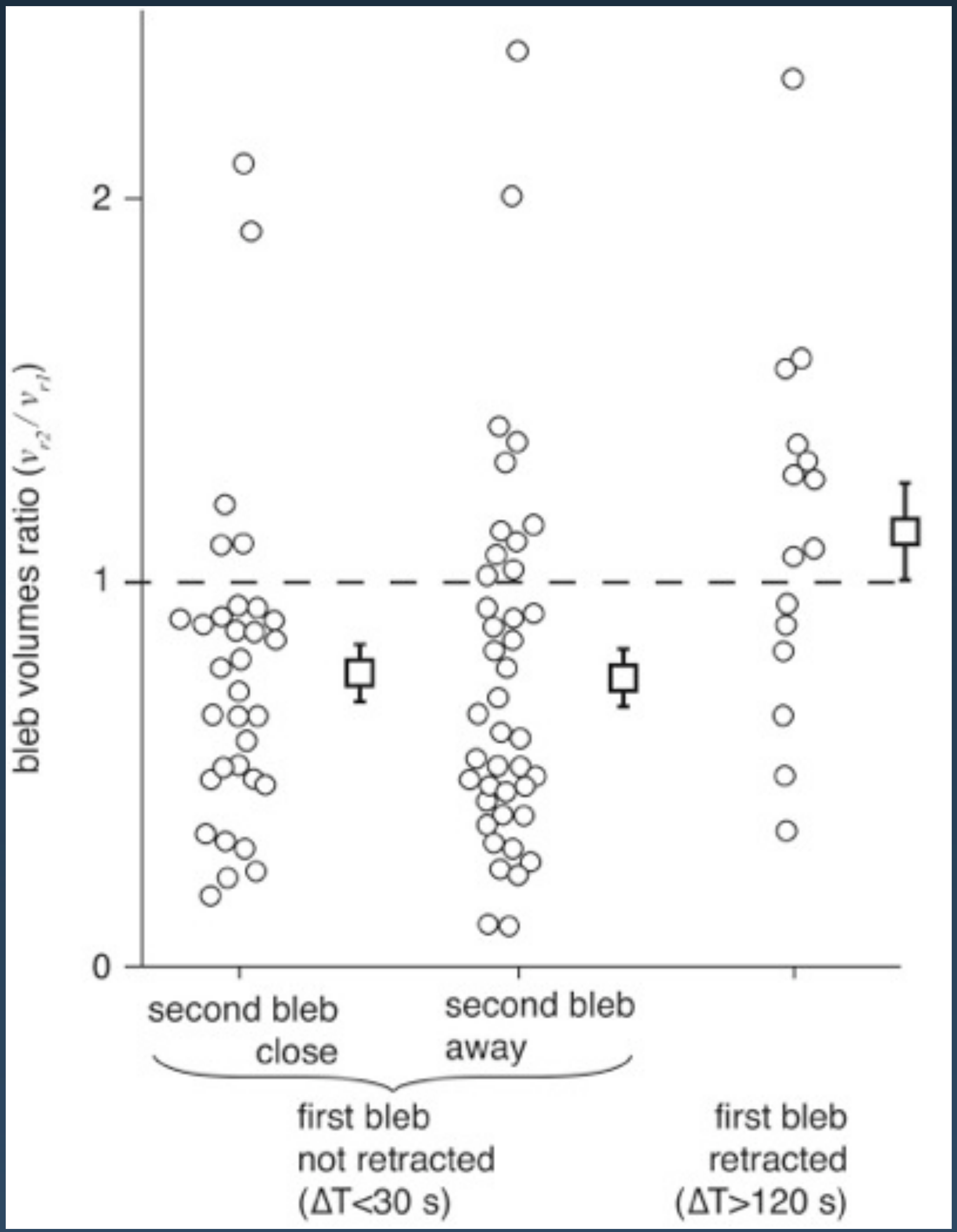
(Jean-Yves Tinevez)

Double-bleb experiments: bleb growth releases intracellular pressure





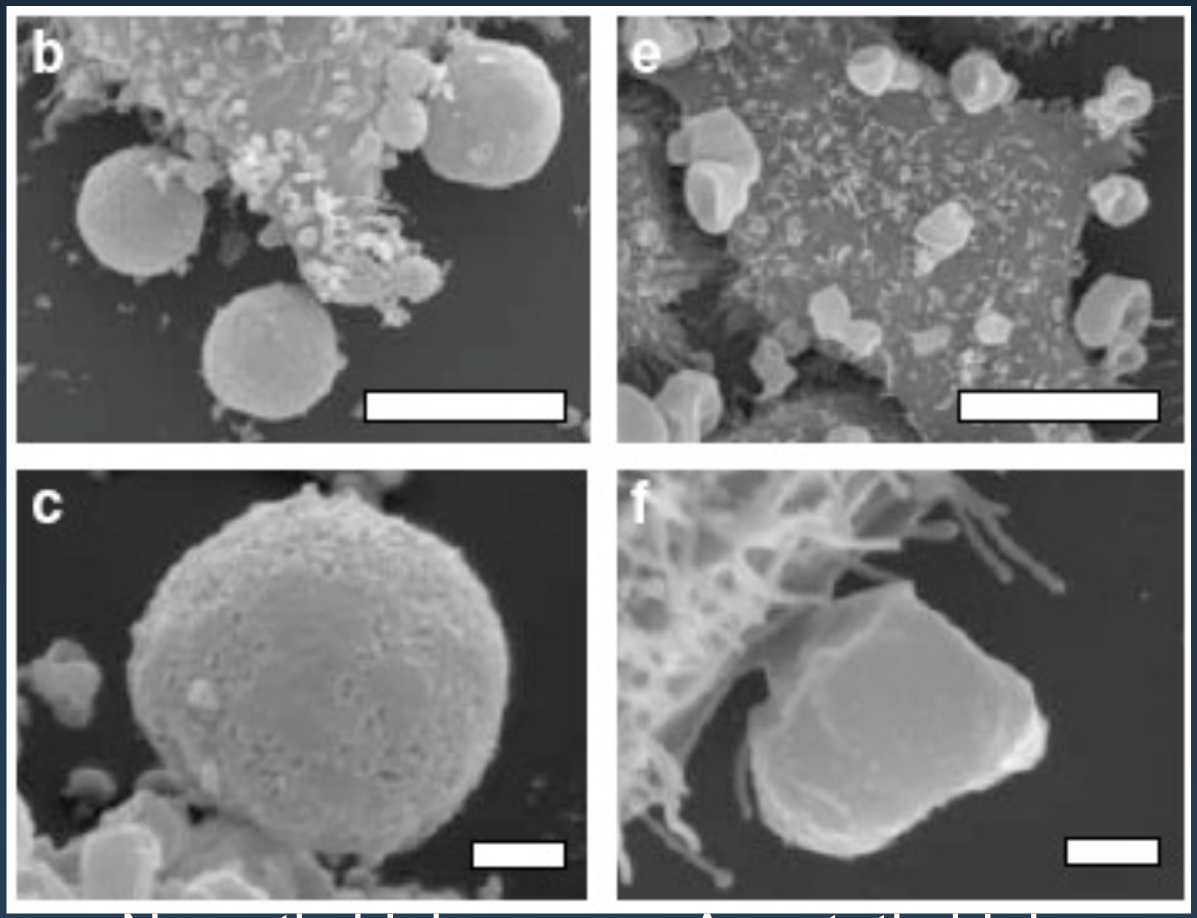
<u>Successive blebs:</u>
2nd bleb triggered immediately after 1st bleb is <u>smaller</u>

(Jean-Yves Tinevez, Julia Roensch)

Tinevez et al. PNAS 2009

Blebs in apoptosis

One of the most dramatic features of the execution phase of apoptosis:



Necrotic blebs Apoptotic blebs

[L. F. Barros et al., *Cell Death and Differentiation* 2003]

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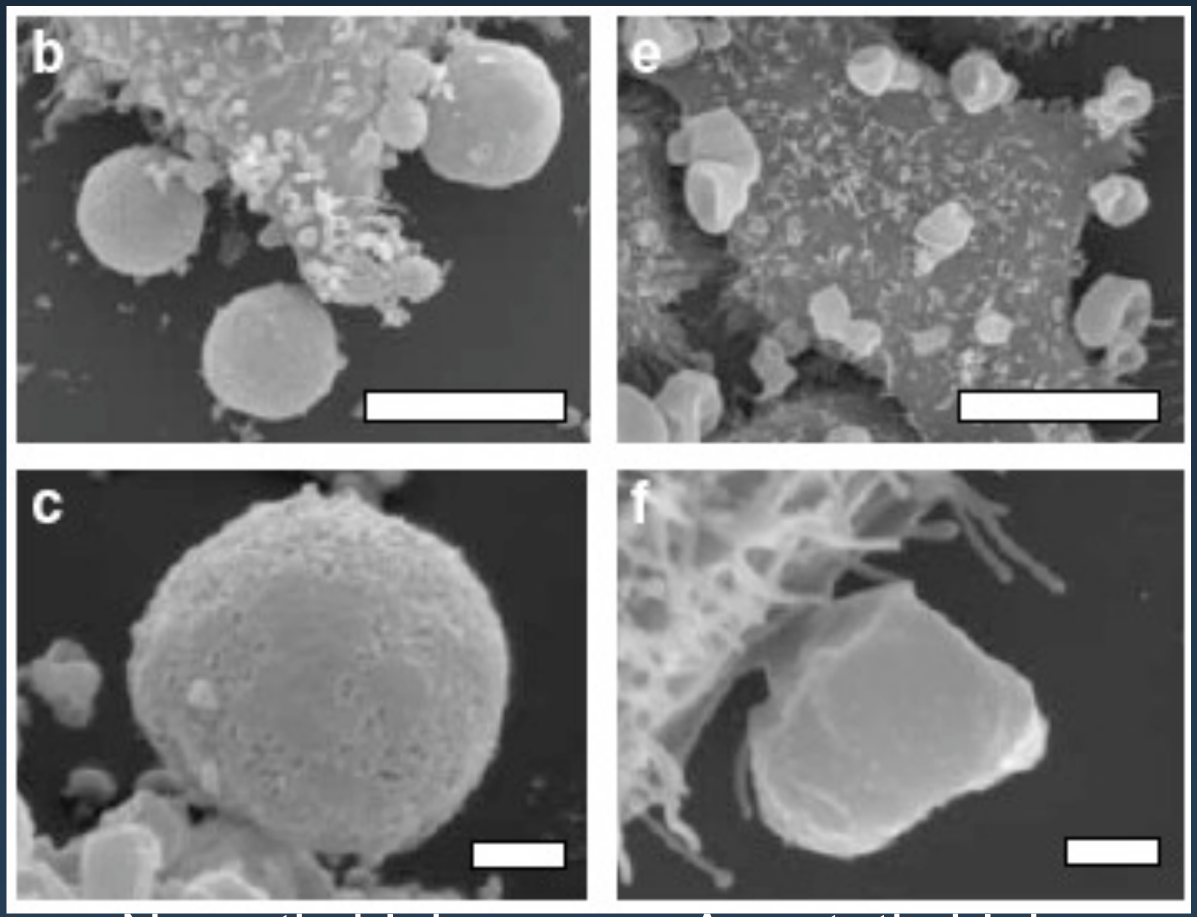

Necrotic blebs Apoptotic blebs

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Depends on myosin activation by:
- MLCK
- ROCK1 (caspase-cleaved)
- or ROCK2 (granzymeB-cleaved in caspase-independent apoptosis)

May facilitate dispersion of fragmented DNA into apoptotic bodies. Not lethal by themselves.

Blebs in cytokinesis

Division of a L929 fibroblast:

May release extra membrane for cell spreading (Trinkaus, ‘70s)
May help stabilize the cleavage furrow.

Blebs in cytokinesis

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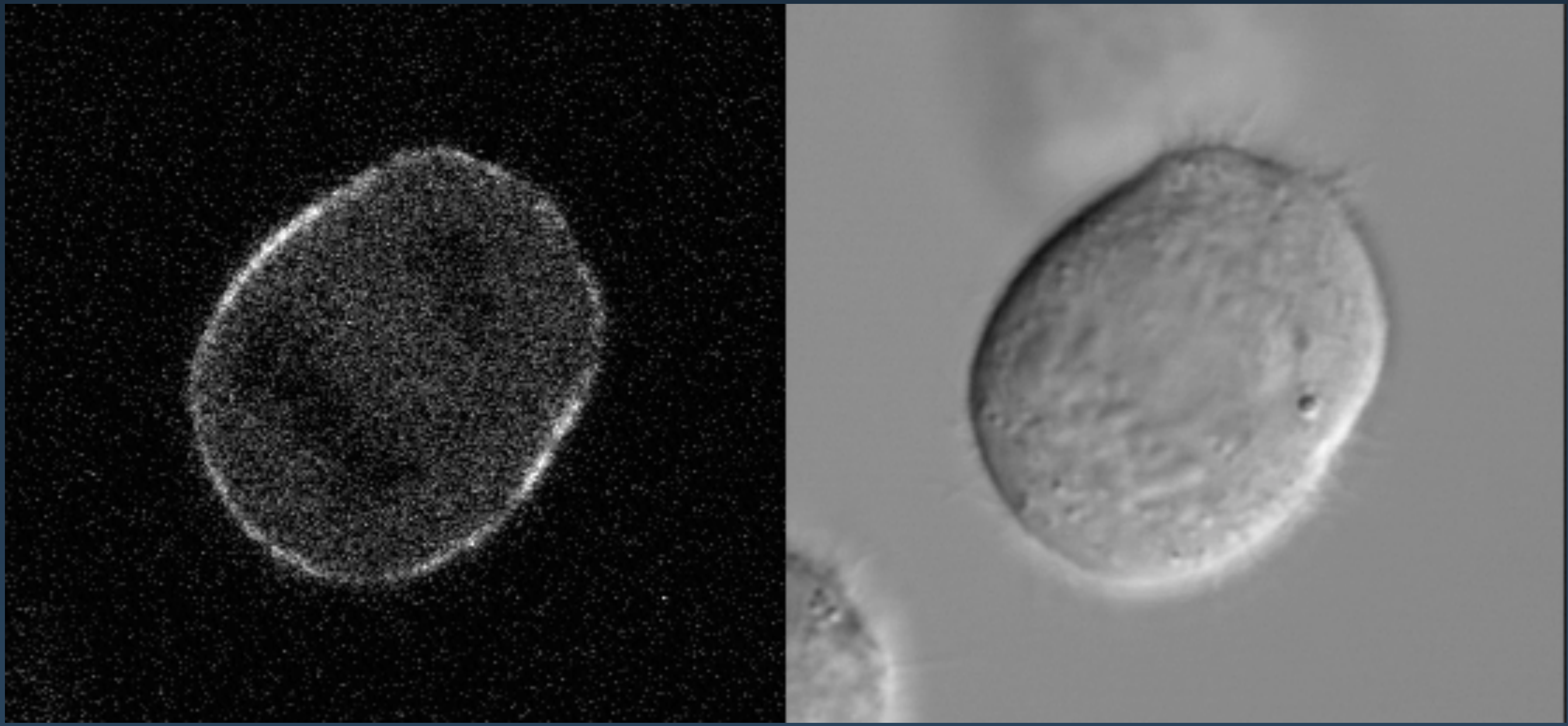

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Blebs in cytokinesis

Already observed a long time ago:

At this stage most interesting changes at the outline of the cell develop. Small balloons of cytoplasm project from the surface of the cell (figs. 6, 7, 8, 9, 10), these remain for a few seconds and then collapse. The granules in the cytoplasm can be seen (figs. 6 to 10), flowing in when the balloons are formed and streaming out when they collapse. This movement continues for about 6 minutes, new balloons being formed as the others collapse. This balloon formation is unlike amœboid movement, and appears due to local changes of surface tension. During this stage the cell begins to divide and

Observations on the Changes seen in Living Cells during Growth and Division.

[T. Strangeways, *Proc. Roy. Soc. London Series B,* 1922]

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Blebs during migration

Fundulus Killfish embryo deep cell:

Zebrafish progenitor cells:

http://www.mtholyoke.edu/courses/rfink/Videopages/index.htm

(Alba Diz Muñoz)

Blebs during migration

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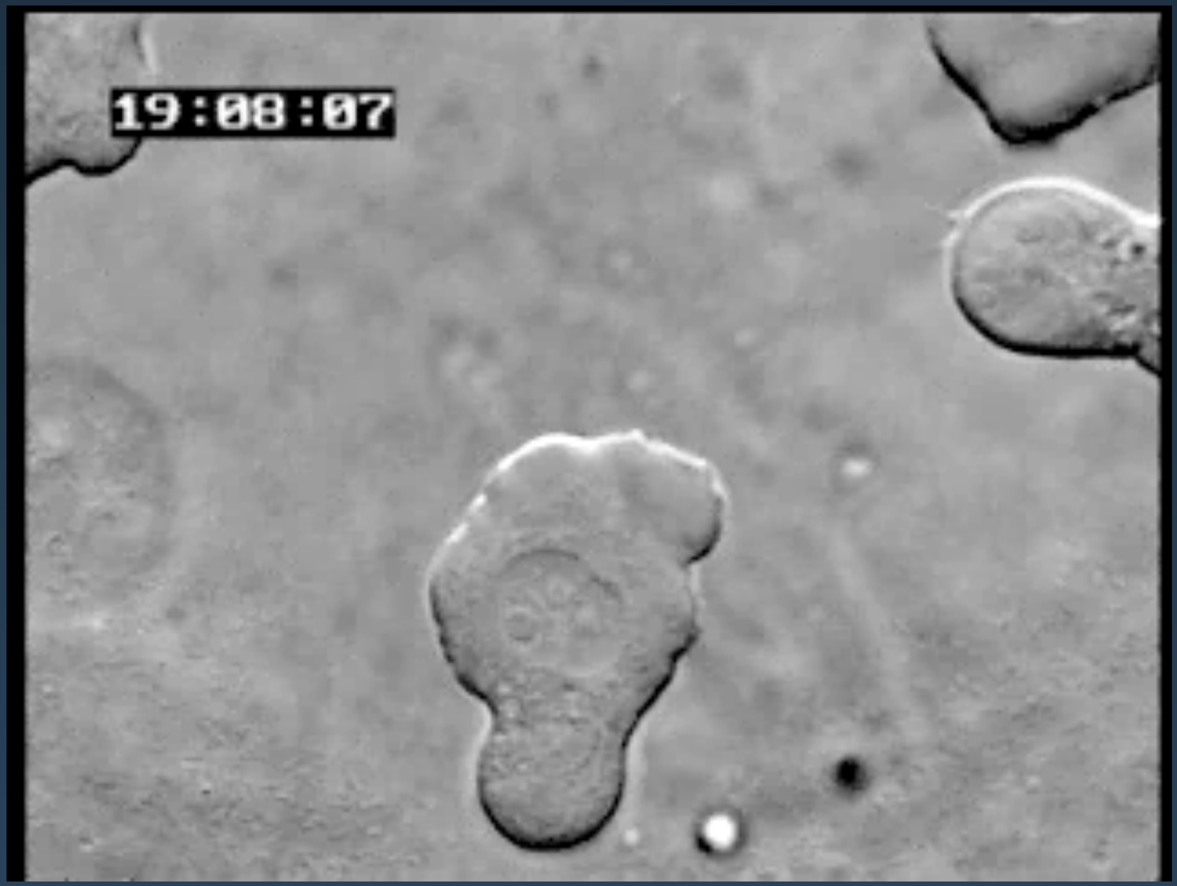

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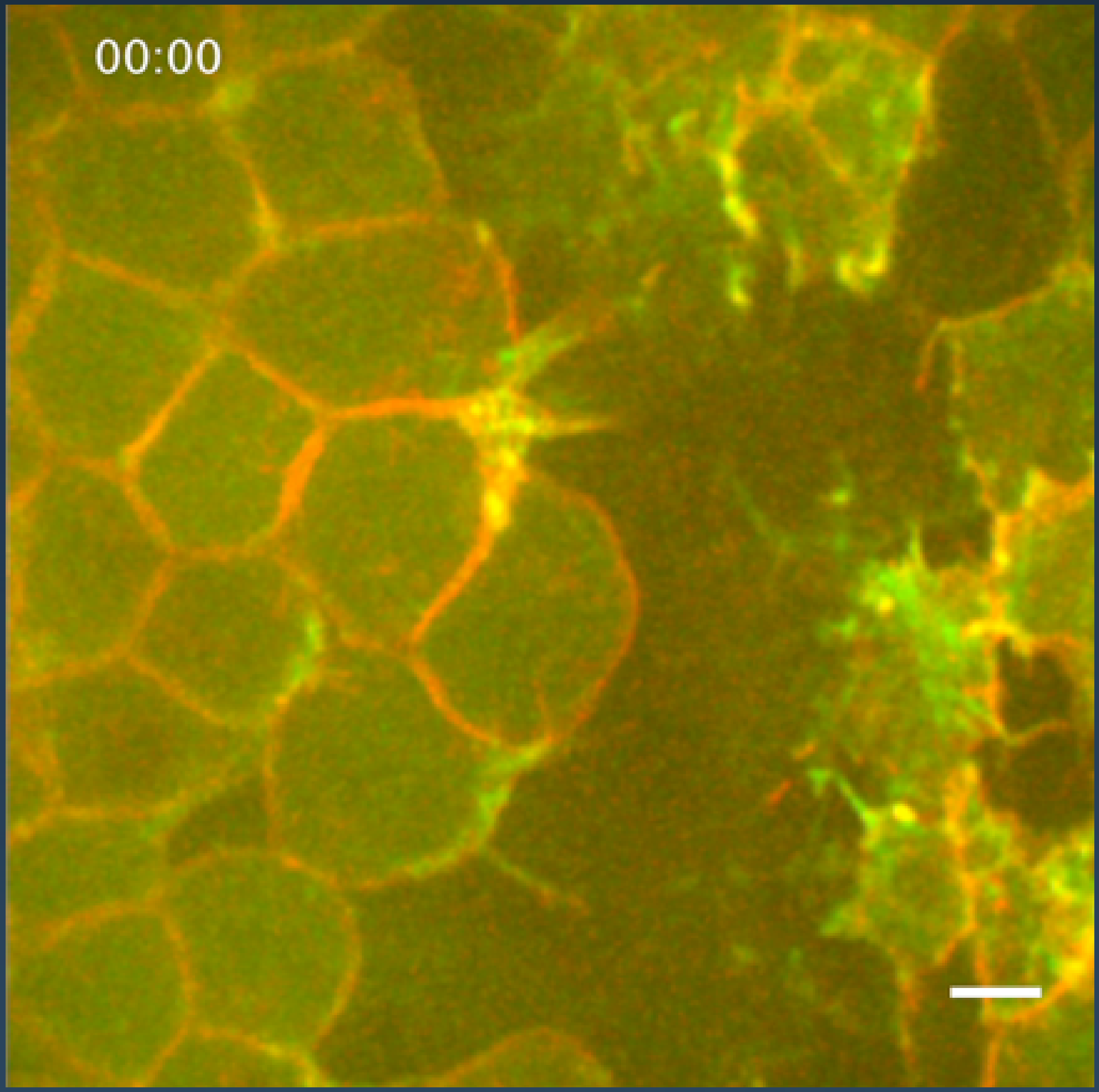
Zebrafish progenitor cells:



(Alba Diz Muñoz)

Blebs overview

- **Apoptosis** - may help forming apoptotic bodies?

- **Cell spreading** - may allow searching for an adhering surface more efficiently?

- **Cell division** - may provide additional membrane for cell spreading?

- **Virus uptake** - may help viral infection?

- **Cell migration** - protrusion at the cell leading edge, alternative to lamellipodia

Reviews:
- O. Fackler and R. Grosse, "Cell motility through plasma membrane blebbing", J Cell Biol, 2008
- E. Paluch and G. Charras, "Blebs lead the way: how to migrate without lamellipodia", Nature Rev Mol Cell Biol, 2008