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Mechanobiology: where biology and biophysics meet

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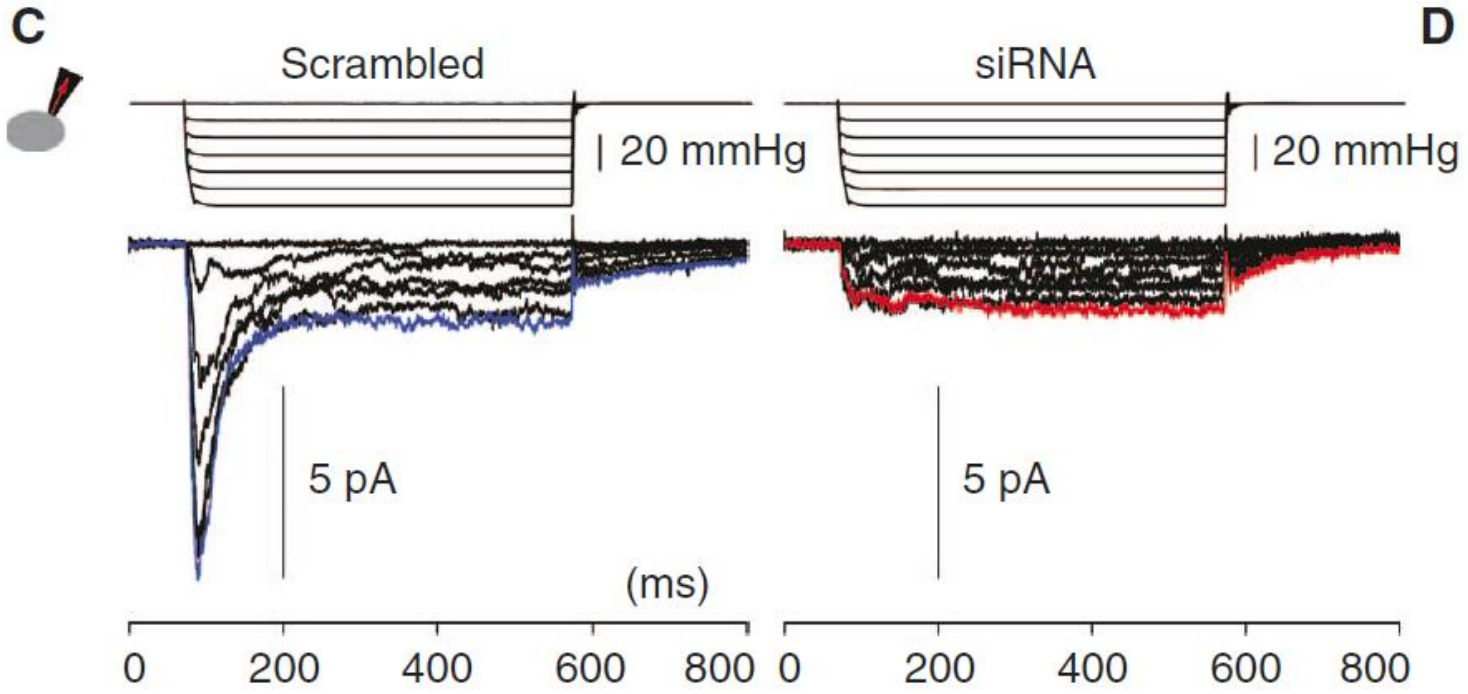
Mechanotransduction, the conversion of mechanical force into biological signals, has crucial roles in physiology. In mammals, embryonic development, touch, pain, proprioception, hearing, adjustment of vascular tone and blood flow, flow sensing in kidney, lung growth and injury, bone and muscle homeostasis, as well as metastasis are all regulated by means of mechanotransduction (*1*, *2*).

2010 Patapoutian 10.1126/science.1193270



Mechanically activated cationt channel for touch sensation

Suppression of mechanically activated currents by means of Piezo1 siRNA. representative currents (averaged traces) induced by means of negative pipette pressure in a N2A cell transfected with (left) scrambled siRNA or (right) Piezo1 siRNA.

 2010 Patapoutian 10.1126/science.1193270

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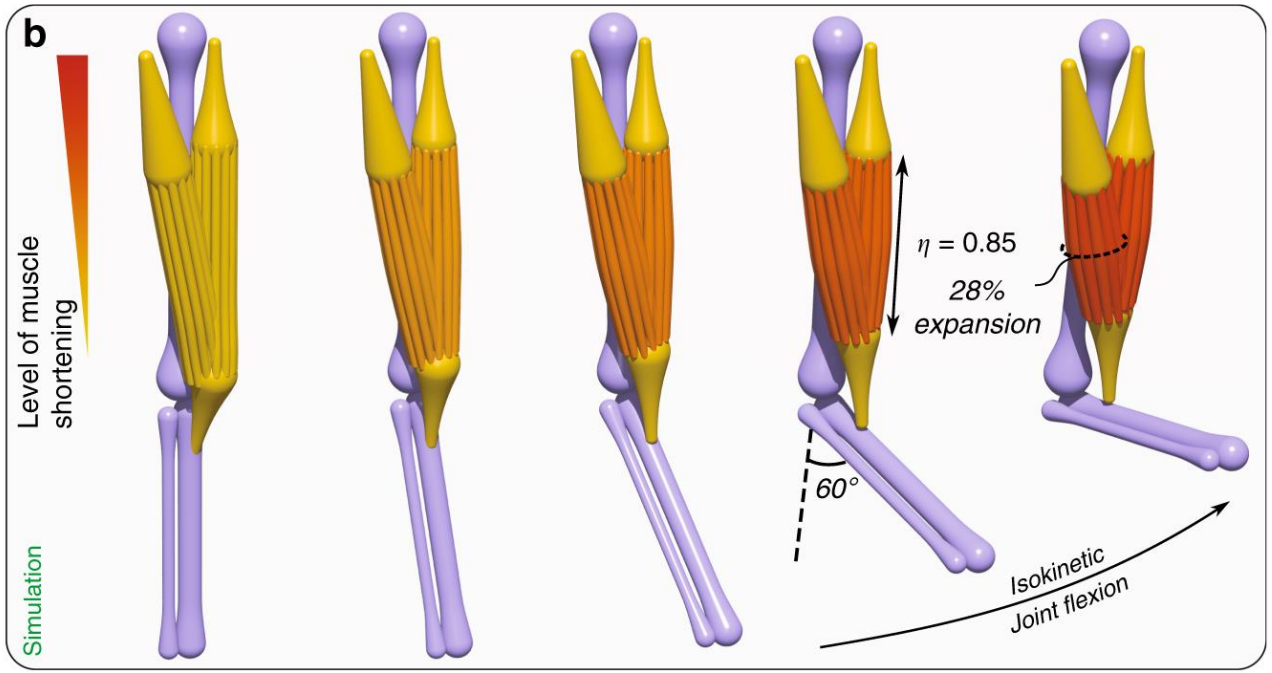

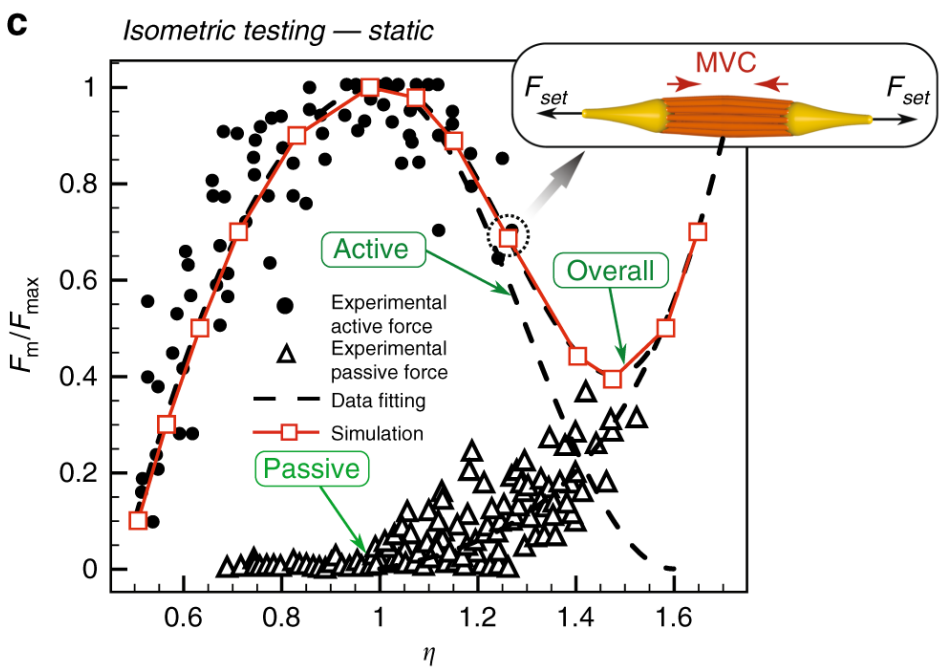

Mechanical forces in human tissues

Human elbow actuation. Simulation of an elbow composed of three bones performing a complete flexion. Simulations for active and passive force normalized with peak force (Fm/Fmax) during the isometric exercise

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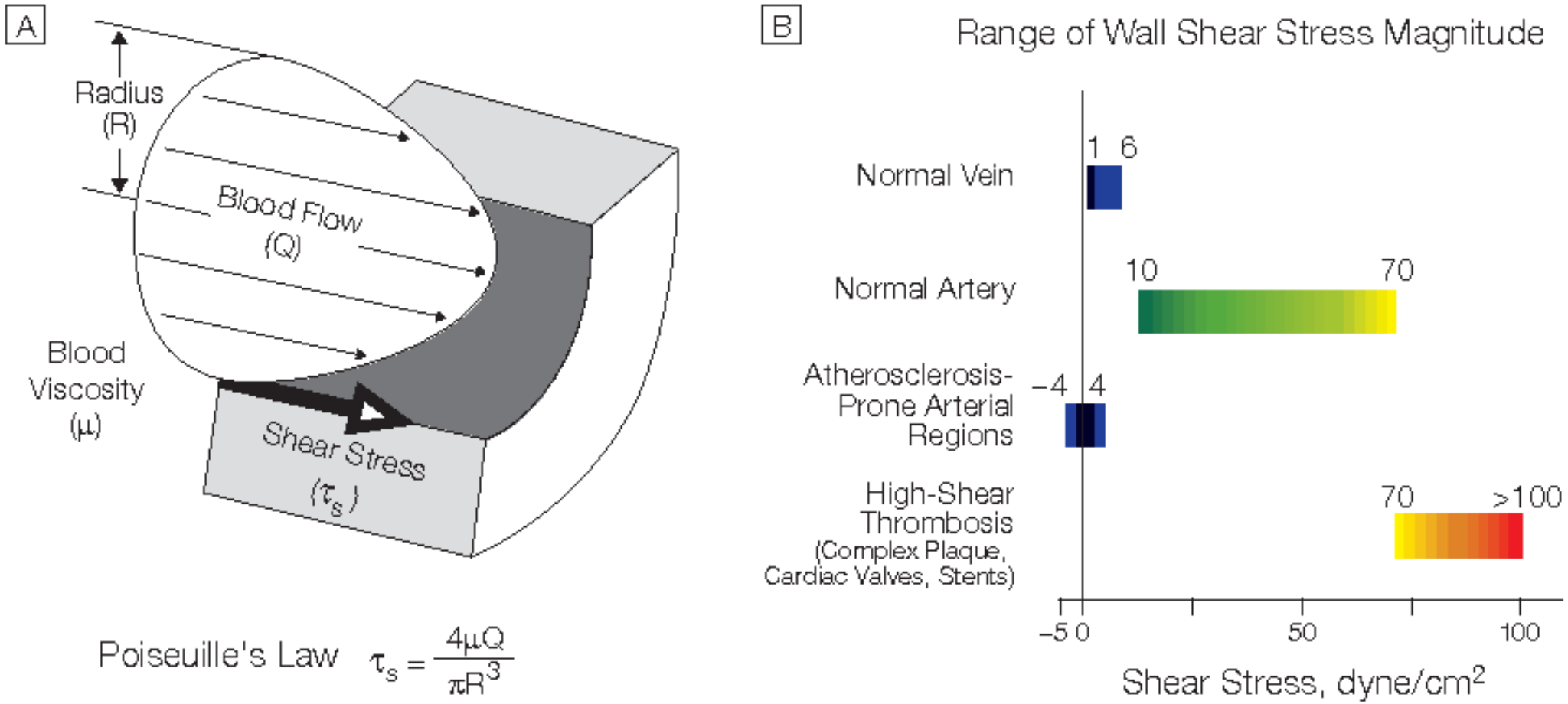

Mechanical forces in human tissues

Hemodynamic shear stress. Cross-section of blood vessels illustrating shear stress, τ_s, the frictional force per unit area acting on **inner vessel wall and endothelium as result of flow of viscous blood,** diagram of shear magnitues in vessels.

1 dyne/cm² = 0.1 Pa = 0.1 N/m²

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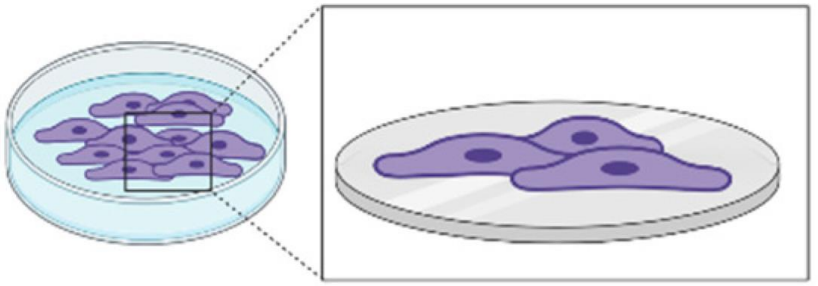
Monolayer Cell Culture



Cell mechanics

sub-field of biophysics that focuses on the mechanical properties and behavior of living cells and how it relates to cell function

PC-3 cells, 10x, quantitative phase imaging

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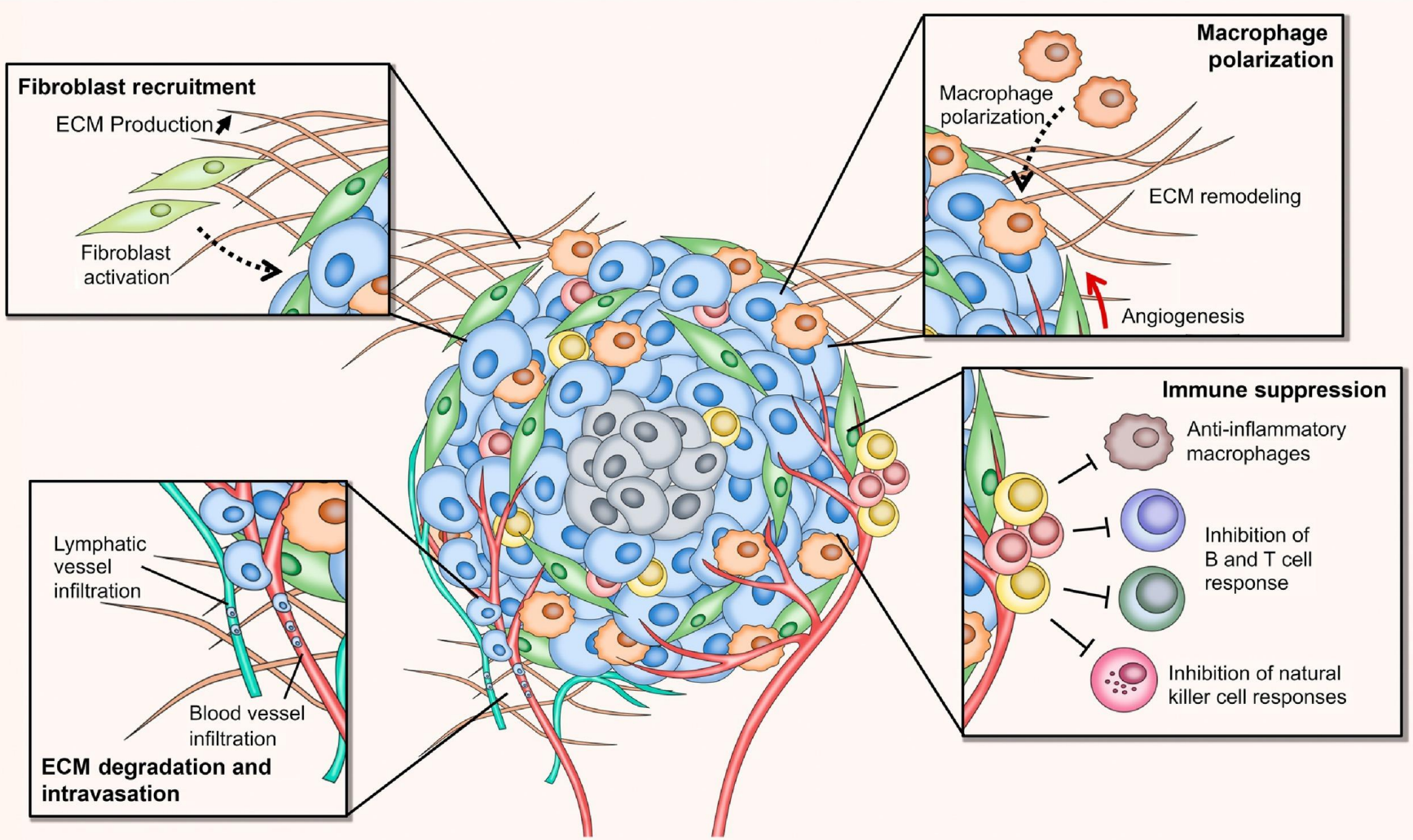

Trends in Cancer

Cells in environment, the Tumor Microenvironment

Rodrigues 2020

https://doi.org/10.1016/j.trecan.2020.10.009

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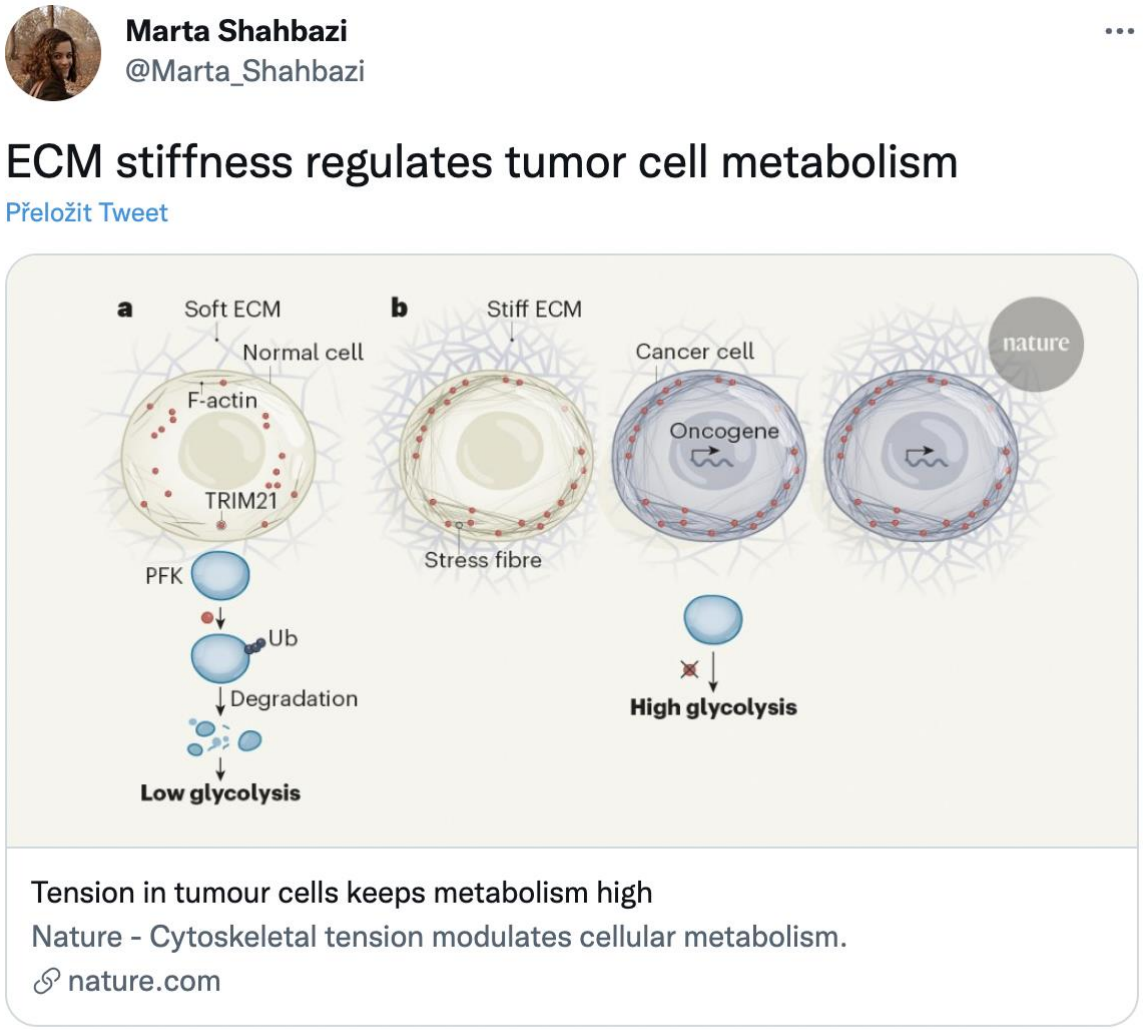
ECM stiffness regulates tumor metabolism

mechanics of the cellular microenvironment continuously modulates cell functions such as growth, survival, apoptosis, differentiation and morphogenesis via cytoskeletal remodelling and actomyosin contractility

Transfer of human bronchial epithelial cells **from stiff to soft substrates causes a downregulation of glycolysis** via proteasomal degradation of the rate-limiting metabolic enzyme phosphofructokinase

cancer cells maintain high glycolytic rates regardless of environmental mechanics

Park et al https://www.nature.com/articles/s41586-020-1998-1
https://twitter.com/Marta_Shahbazi/status/1234532810154823685



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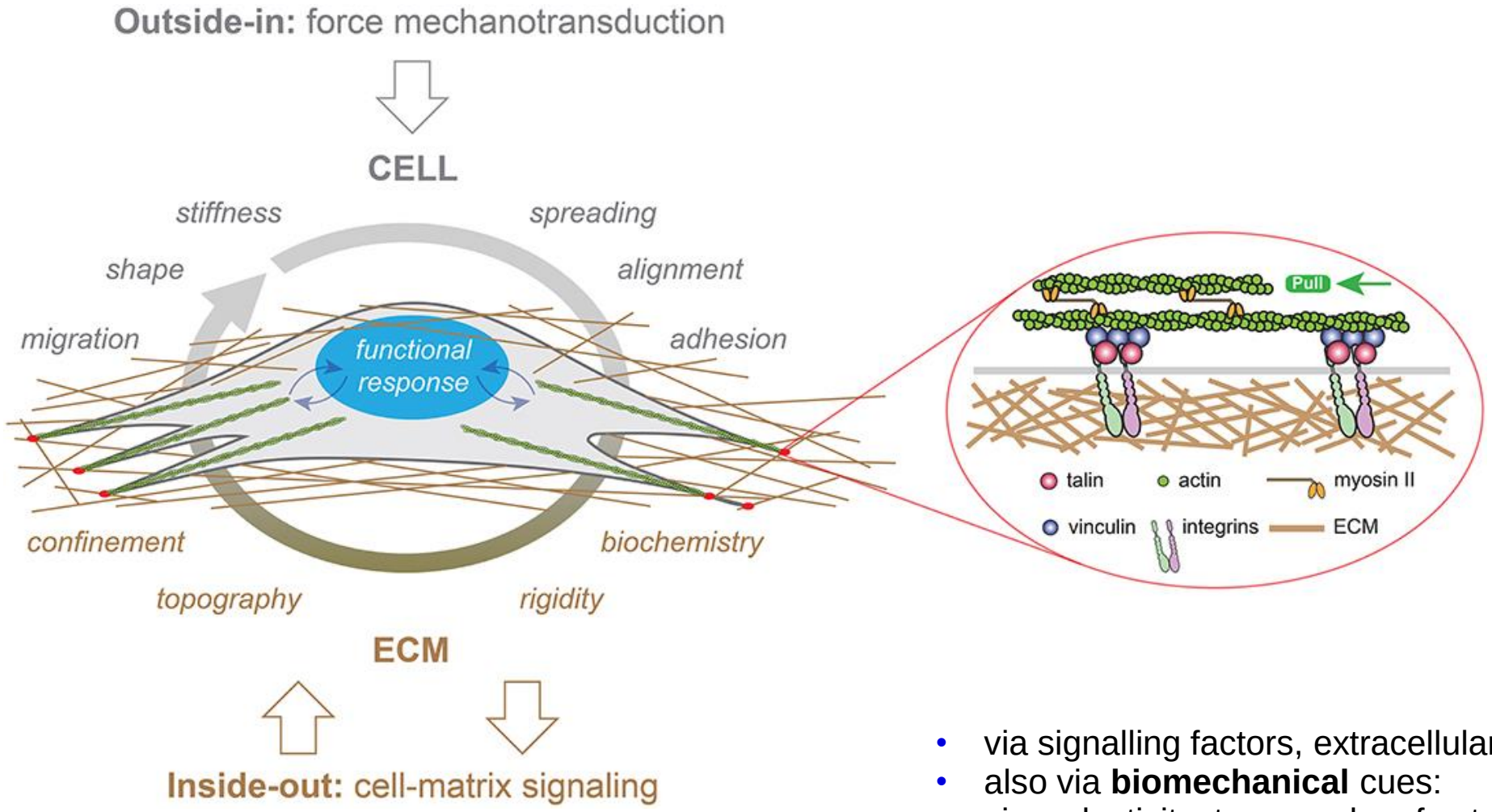
Cell functions affected by surrounding microenvironment



- via signalling factors, extracellular matrix ligands
- also via **biomechanical** cues: viscoelasticity, topography of extracellular matrix

2019 Mohamed https://doi.org/10.3389/fbioe.2019.00162

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Outline

- mechanobiology, mechanotransduction
- factors involved in mechanotransduction
- celluar consequences following mechanotransduction
- examples in physiology
- cell mechanics and pathology

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Mechanotransduction

- the molecular mechanisms by which cells sense and respond to mechanical signals

Signaling and mechanics

genetic and biochemical basis of disease

X

changes in cell mechanics, extracellular matrix, or mechanotransduction contribute to the development diseases:

atherosclerosis,
fibrosis,
asthma,
osteoporosis,
heart failure
cancer

- mechanotransduction contributors
  - stretch-activated ion channels,
  - caveolae,
  - integrins,
  - cadherins,
  - growth factor receptors,
  - myosin motors,
  - cytoskeletal filaments,
  - nuclei,
  - extracellular matrix,
  - and numerous other signaling molecules

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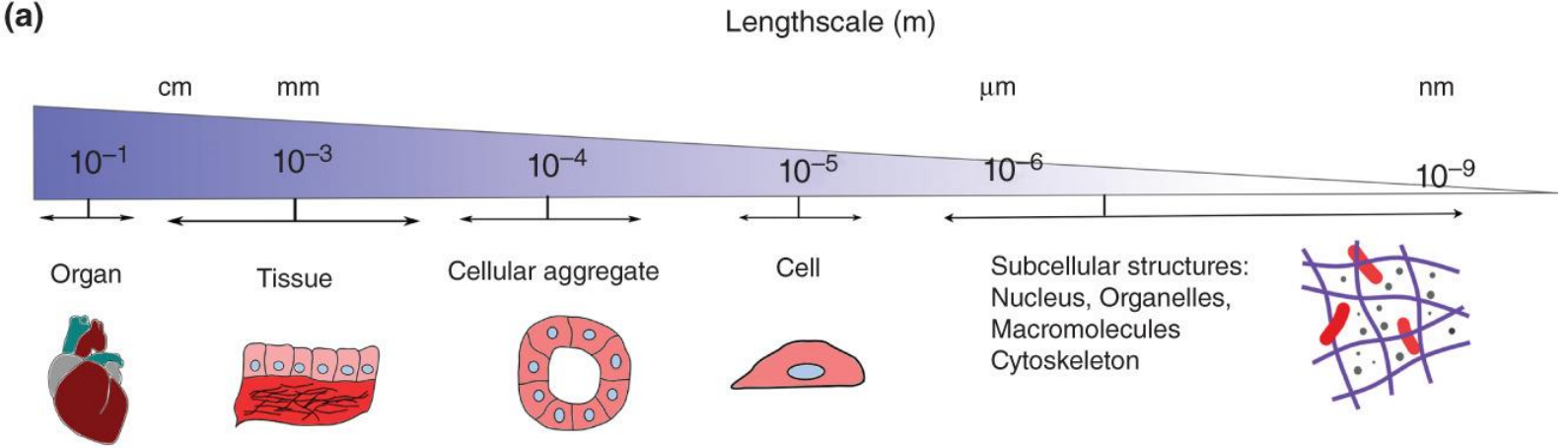

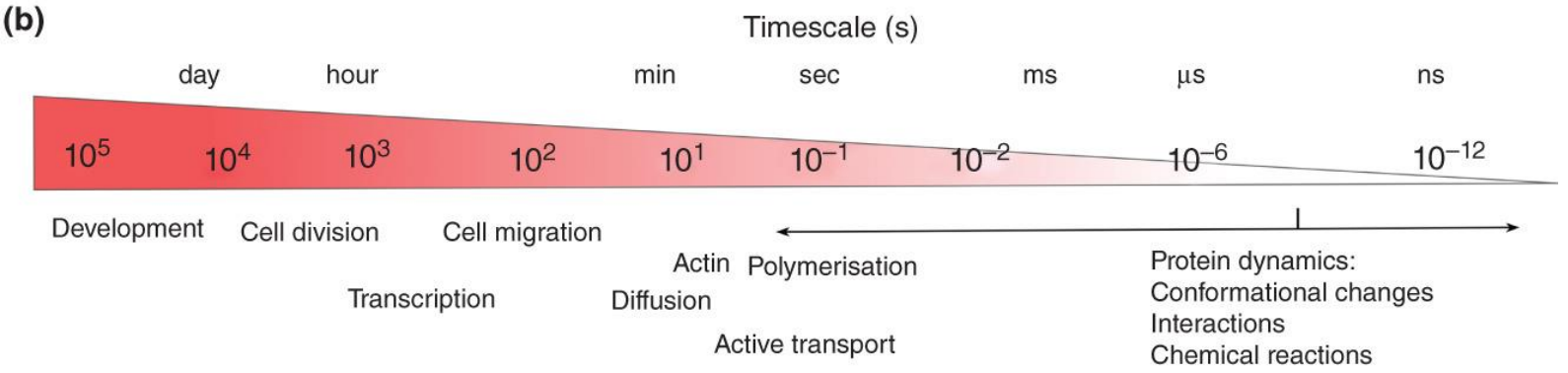

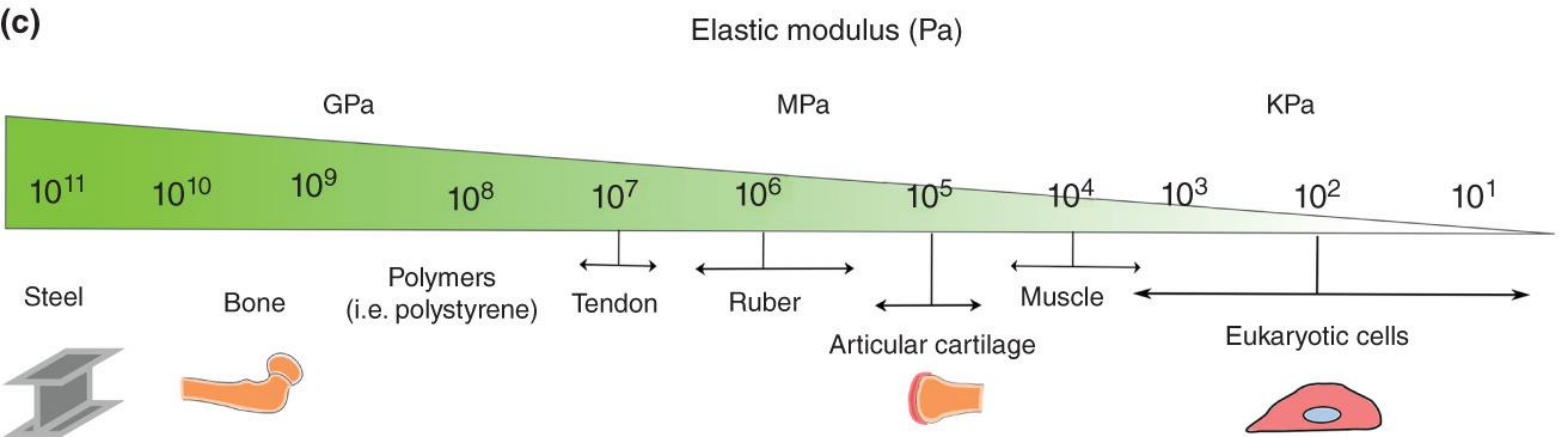

Parameters involved in choosing the mechanical measurement tool.

The choice of experimental tool requires consideration of (a) the lengthscale, (b) the timescale of the measurement and (c) the level of forces (or elasticity of the sample).

Moeendarbary https://doi.org/10.1002/wsbm.127

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- Whole-body scale:
  - bodies exposed to forces, e.g. gravity
  - body source of forces: locomotion permitted by tensile muscular forces
- Tissue scale
  - blood pressure and shear stress on vessels due flow
- Single cell scale
  - mechanical forces on cells regulate functions
  - cell movement

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Mechanotransduction

1

Load-sensitive cells: fibroblasts, chondrocytes, osteoblasts, endothelial cells, smooth muscle cells,...

2

cell components in mechanotransduction: Extracellular matrix, cytoskeleton, integrins, G-proteins, receptor tyrosine kinases, MAPK, stretch-activated protein channels

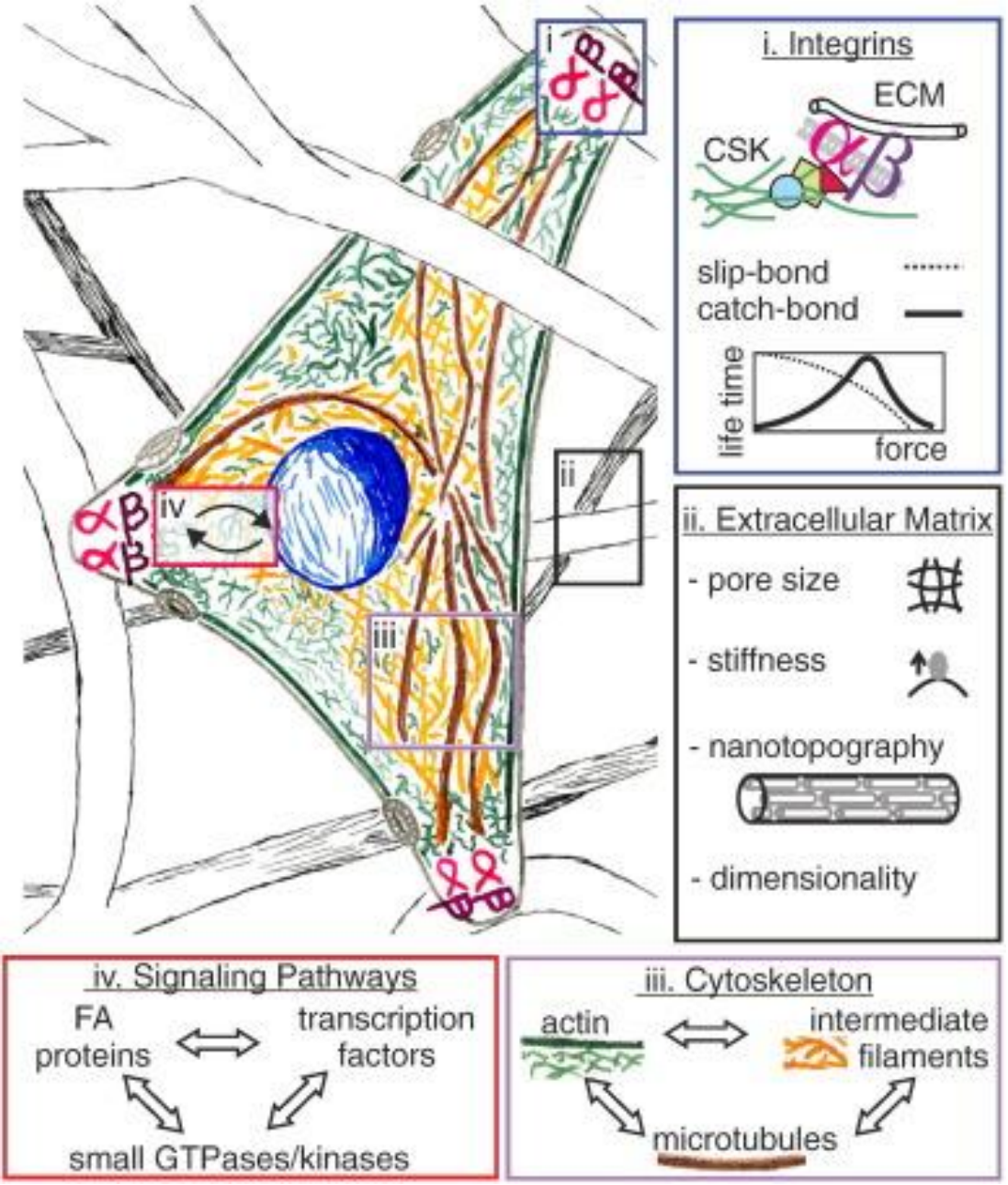
3

mechanical forces on cells **regulate functions**: gene expression, protein synthesis, cell growth, differentiation.

4

excessive/abnormal mechanical load – tilts cell equilibrium to catabolism, tissue pathophysiogy

 2005 Wang



Cell inside a three dimensional fibrous ECM network.

(i) Integrins : α and β subunit - clustered in focal adhesions (FAs) together with other FA proteins (triangle, square and circle).
- The adhesions connect the extracellular matrix (ECM) and the (actin) cytoskeleton.

(ii)ECM provides multiple cues to the cell, specifically pore size, stiffness, nanotopography and dimensionality.

(iii)Cytoskeleton: actin, intermediate filaments and microtubules

(iv)Signalling pathways.

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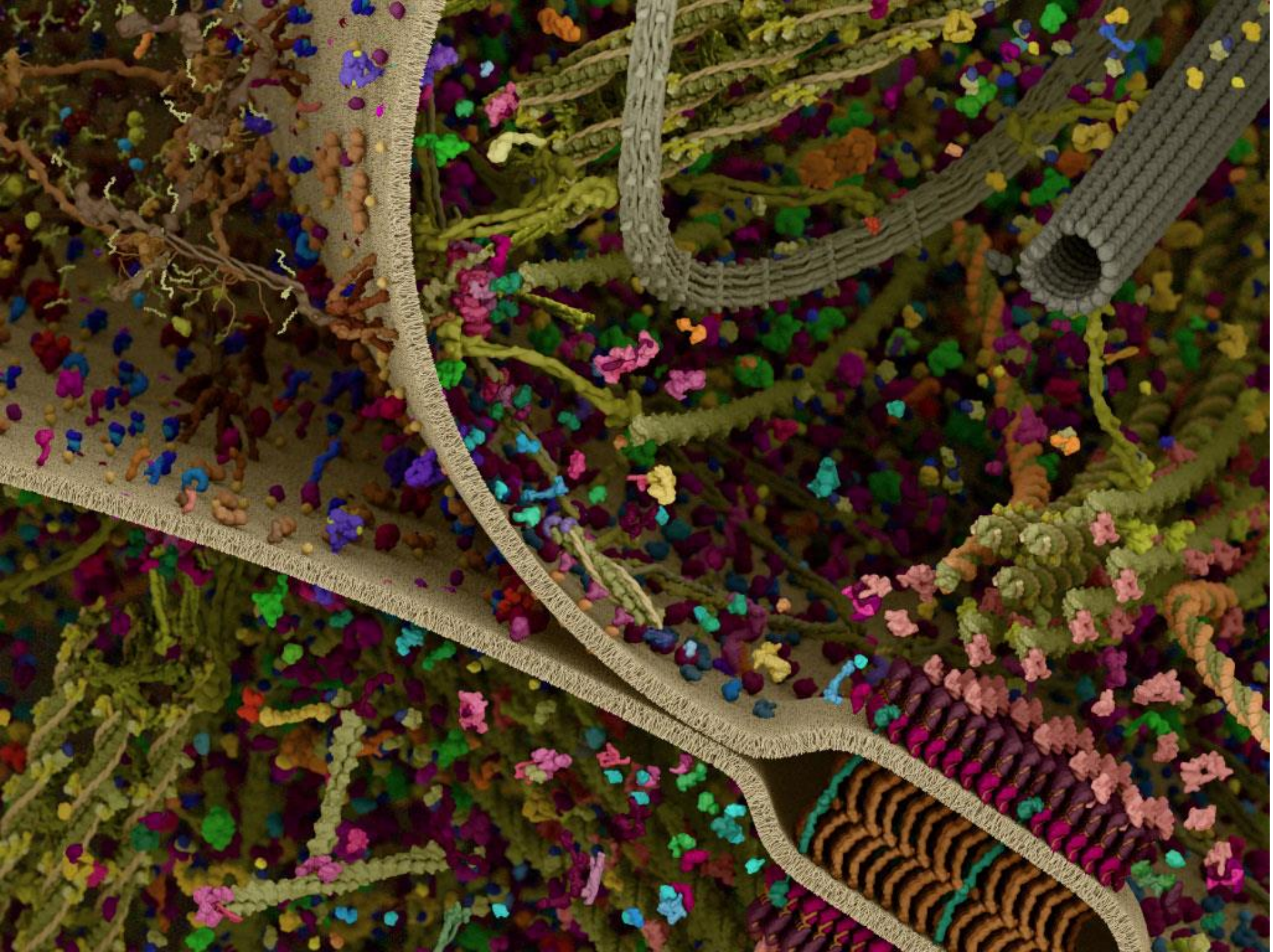
Cytoskeleton



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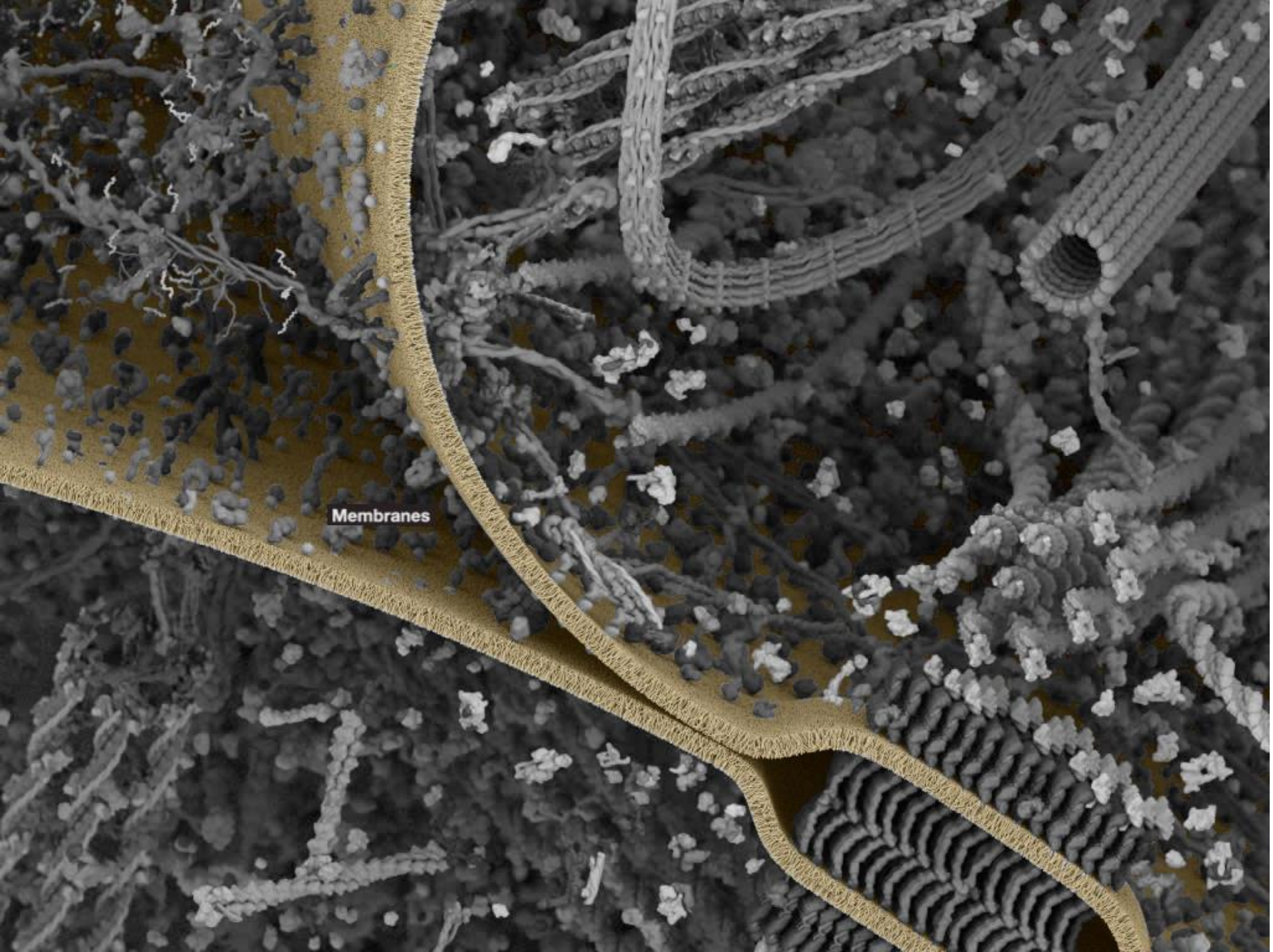

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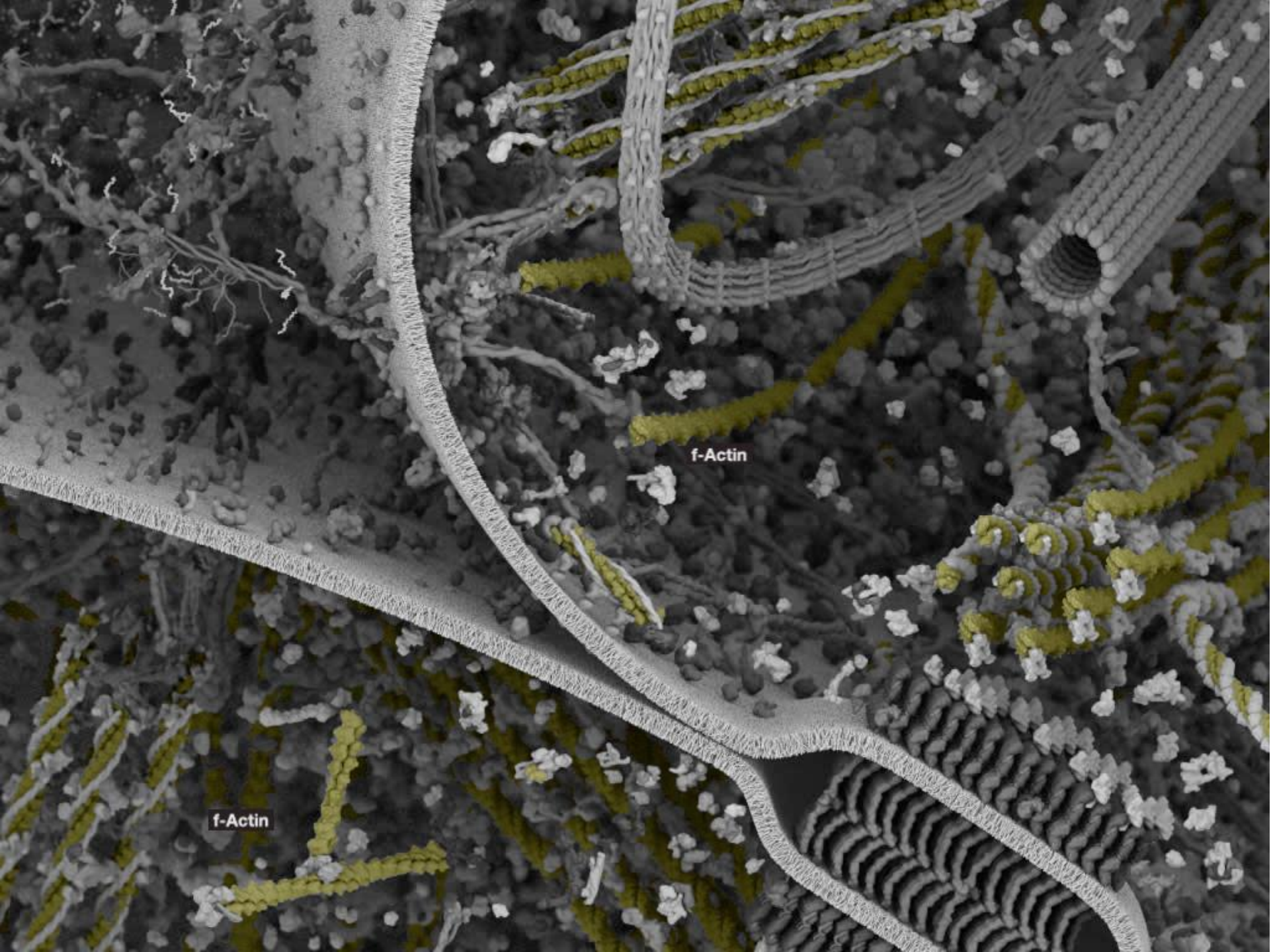

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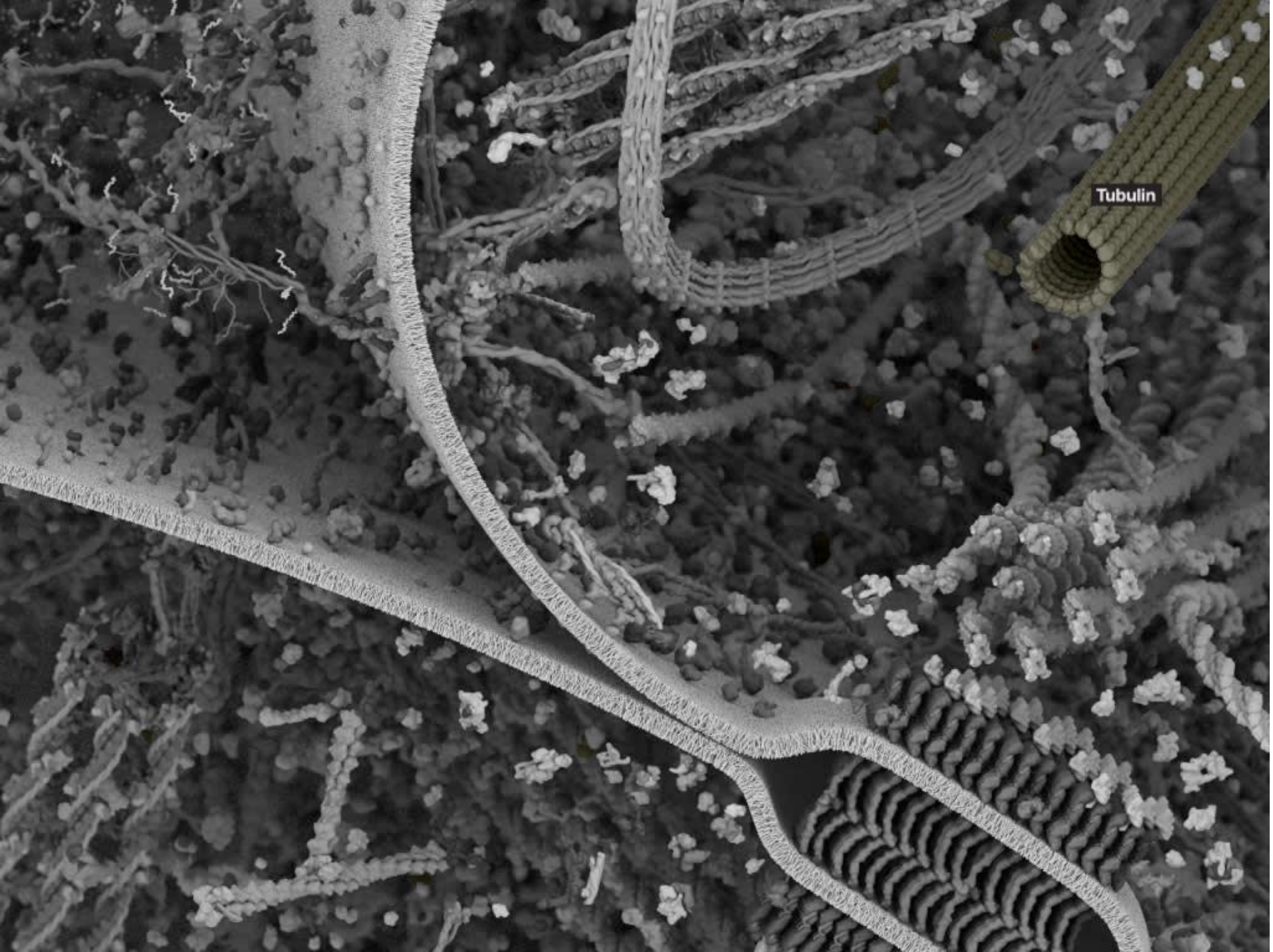

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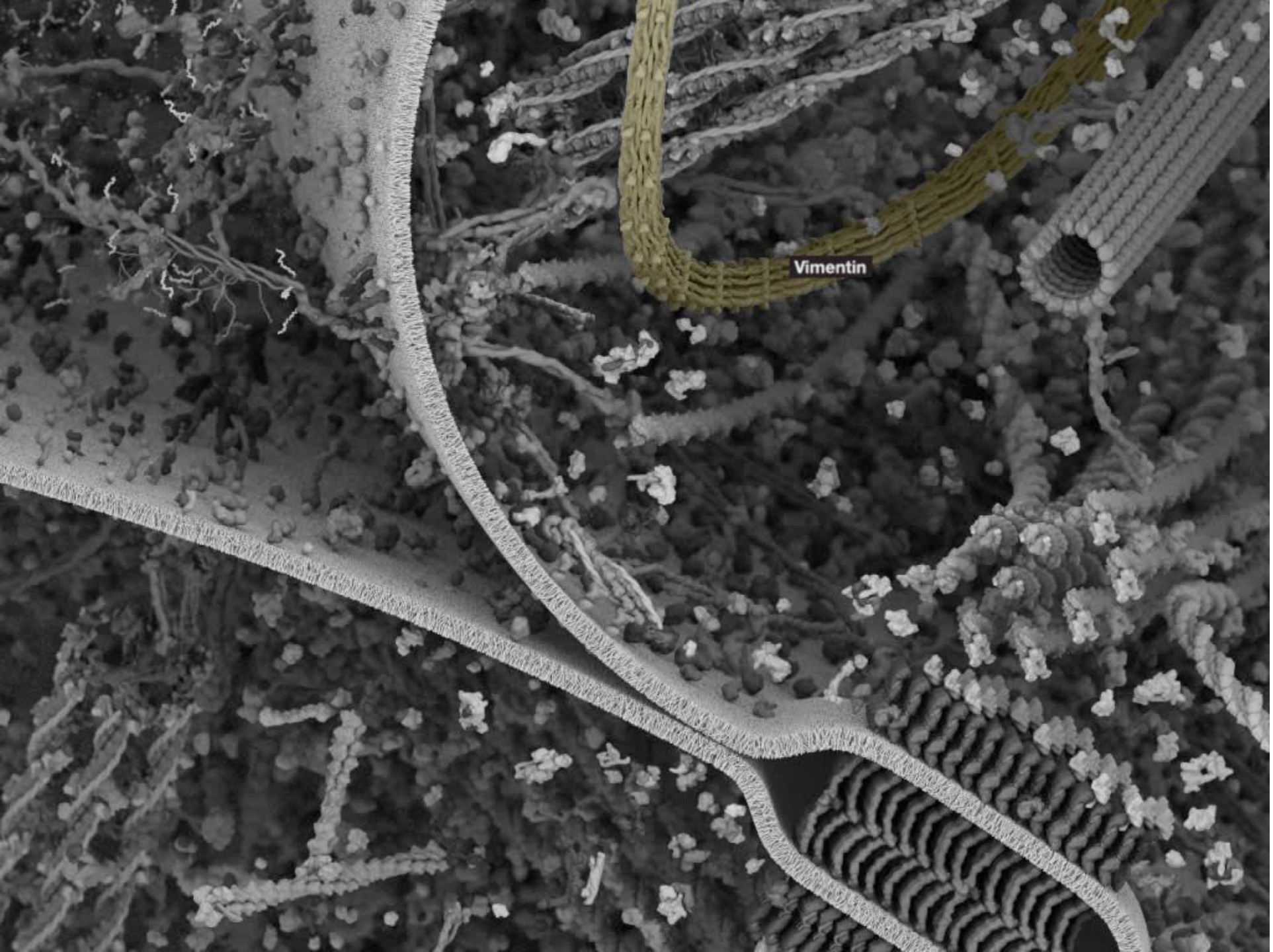

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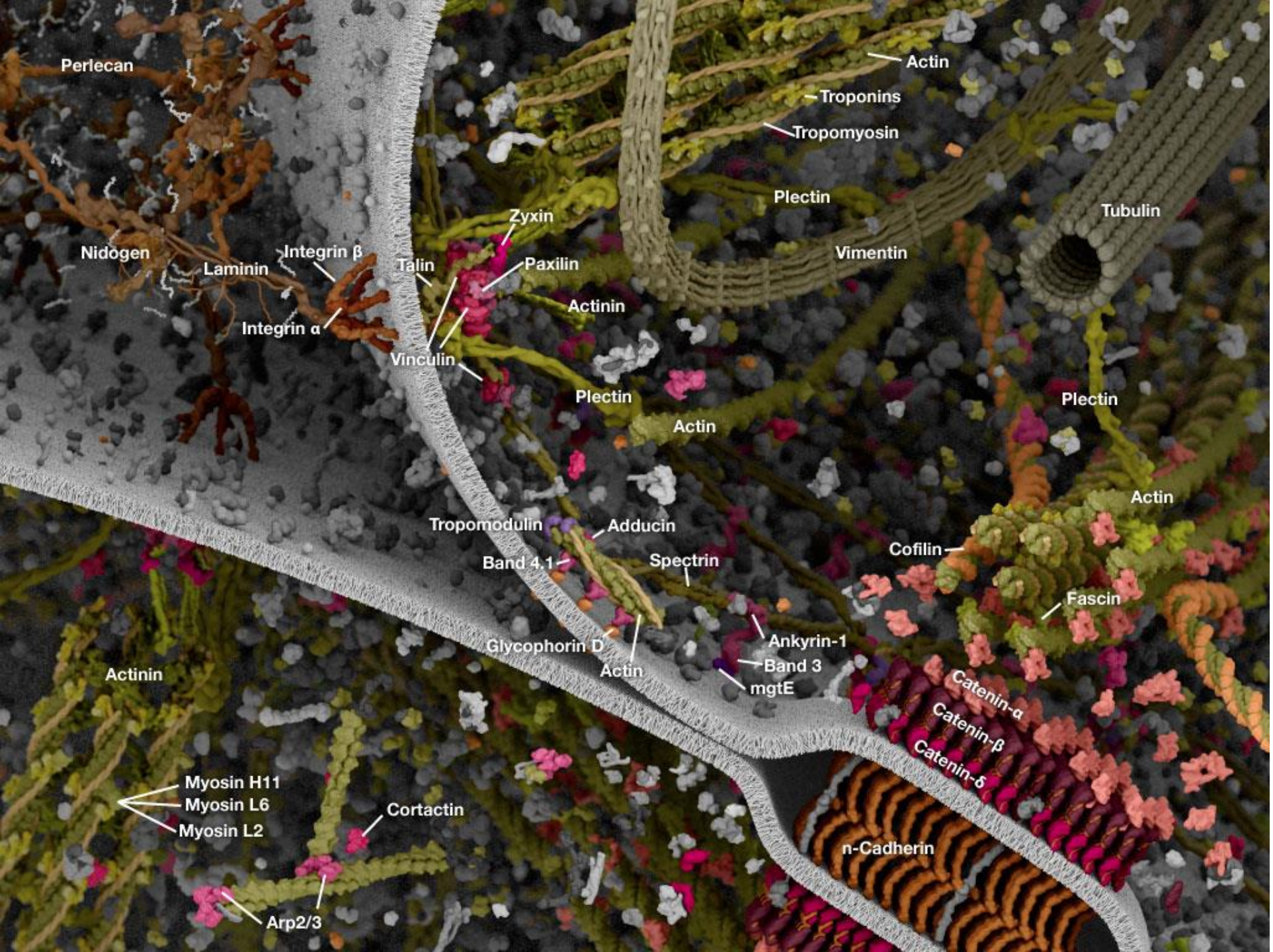

cytoskeleton involved in

- cell shape and cell mechanic properties
  (no cell wall in animal cell)
- providing mechanical strength
- cell movement
- chromosome separation
- intracellular transport of organelles
- enable cell communication

- cytoskeletal fibers + motor proteins
- dynamic instability,
- self-assembly

http://media.cellsignal.com/www/html/science/landscapes/adhesion

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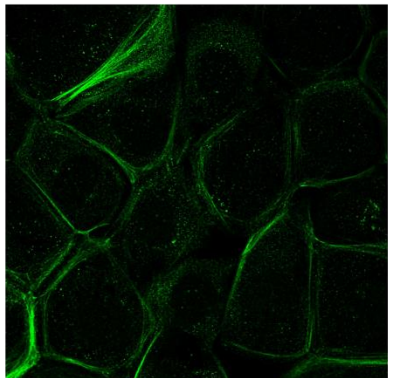
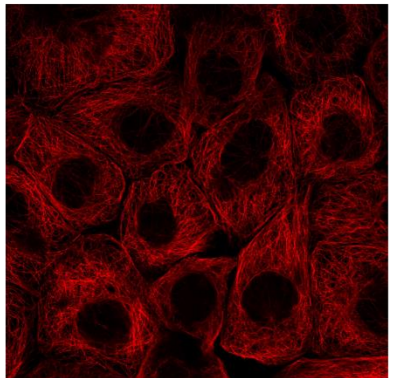
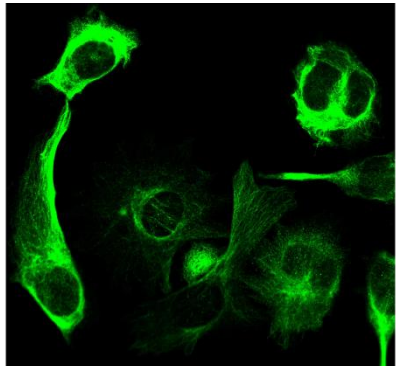
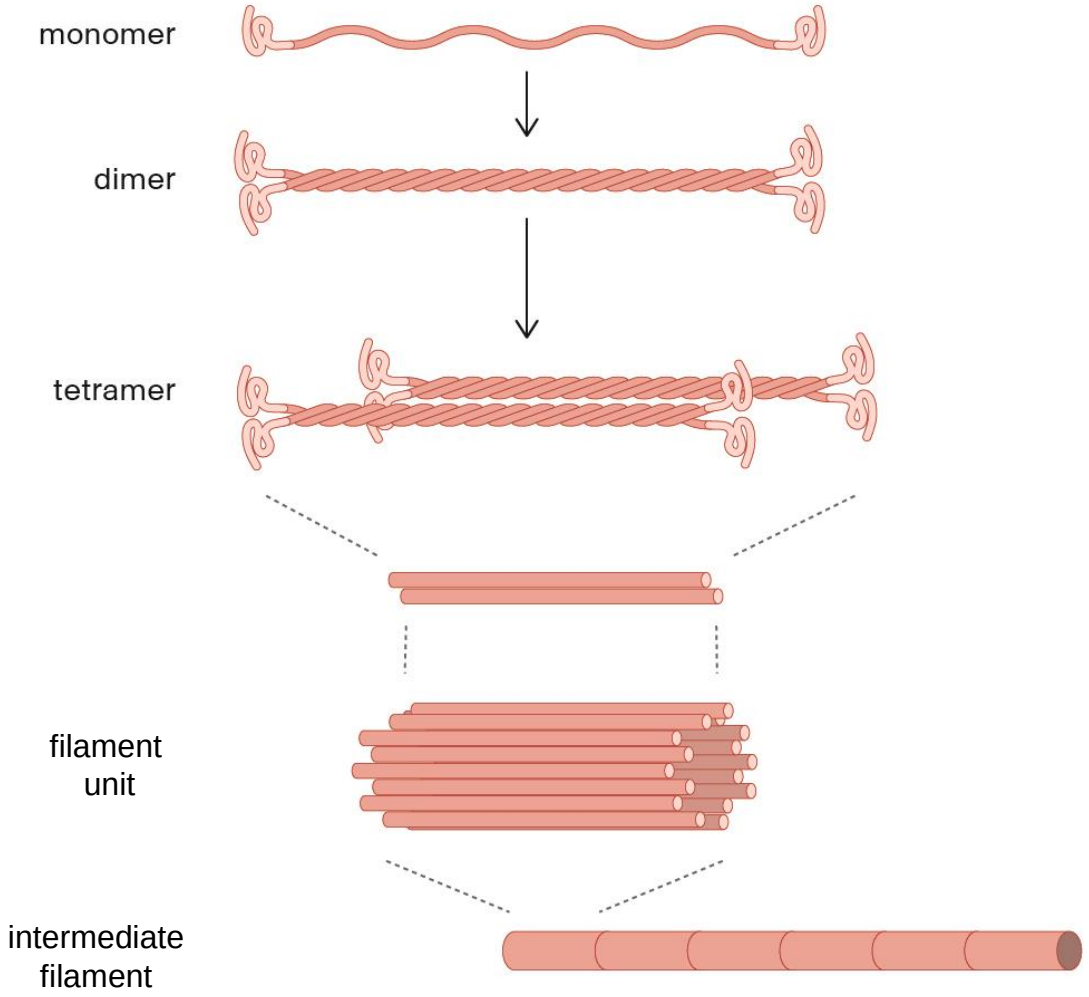
Cytoskeleton in eukaryotic cells

	Microfilaments	Intermediary filaments	Microtubules
build of	G-actin/F-actin	various	a-tubulin/ b-tubulin
diameter	7 nm	10-12 nm	25 nm
molecular motors	myosins	none	kinesin / dynein
polymeration fuel	ATP	none	GTP
properties	most flexible, fast assembly	very flexible, more permanent	stiff
function	structure stabilisation, muscle contraction, cytokinesis, cell movement	mechanical stability (bearing tension, retaining shape). structure, securing organelles . cell-specific	resist compression, intracel. transport, mitotic spindle









Structure of intermediate filaments

actin microfilaments and microtubules are formed from globular subunits

Intermediate filaments formed from fibrous protein units, globular parts localized at the ends

monomers round around themselves, forming polymers.

tetramers are basic organisation unit

 2018 Raudenská https://www.lekarskeknihy.cz/produkt/109803-vybrane-kapitoly-z-bunecne-fyziologie/

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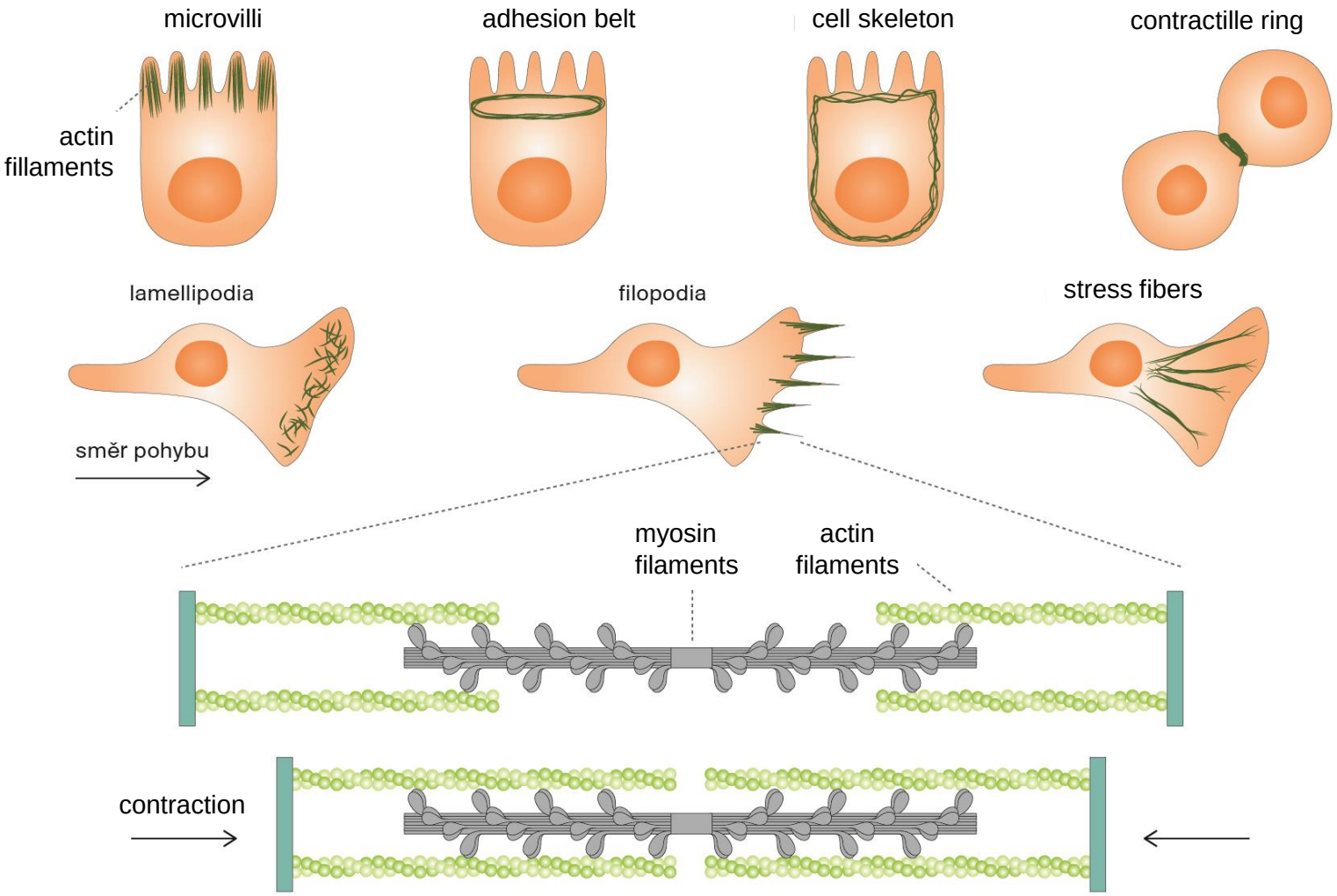

Actin filaments location in cells.

actin shown green

muscle contraction: motor molecule of myosin interacts with actin, resulting in contraction. by hydrolysis of ATP and resulting morphology changes

2018 Raudenská https://www.lekarskeknihy.cz/produkt/109803-vybrane-kapitoly-z-bunecne-fyziologie/

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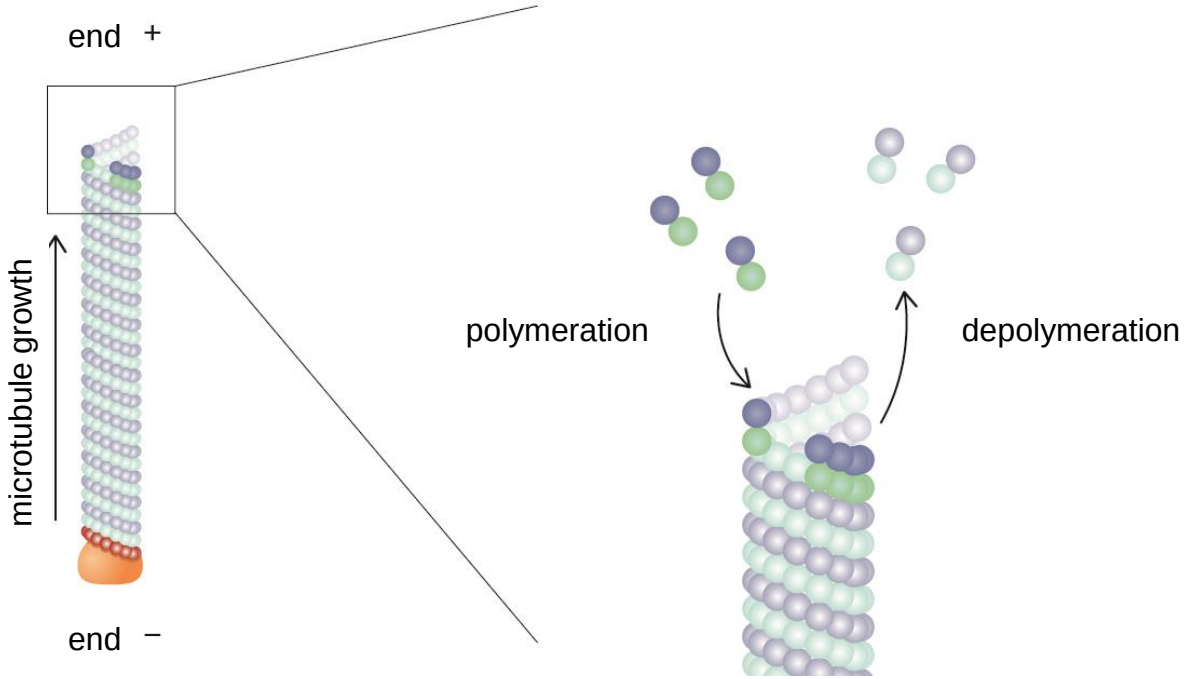

α-tubulin

β-tubulin

γ-tubulin

GTP-binding tubulin dimer

GDP-binding tubulin dimer

Microtubule structure

tubulin dimer composed of two subunits: alpha-tubulin and beta-tubulin

dynamic structure always changing:
dynamic instability:
polymeration – tubulin elongation,
depolymeration tubulin shortening

GTP as energy source

 2018 Raudenská https://www.lekarskeknihy.cz/produkt/109803-vybrane-kapitoly-z-bunecne-fyziologie/

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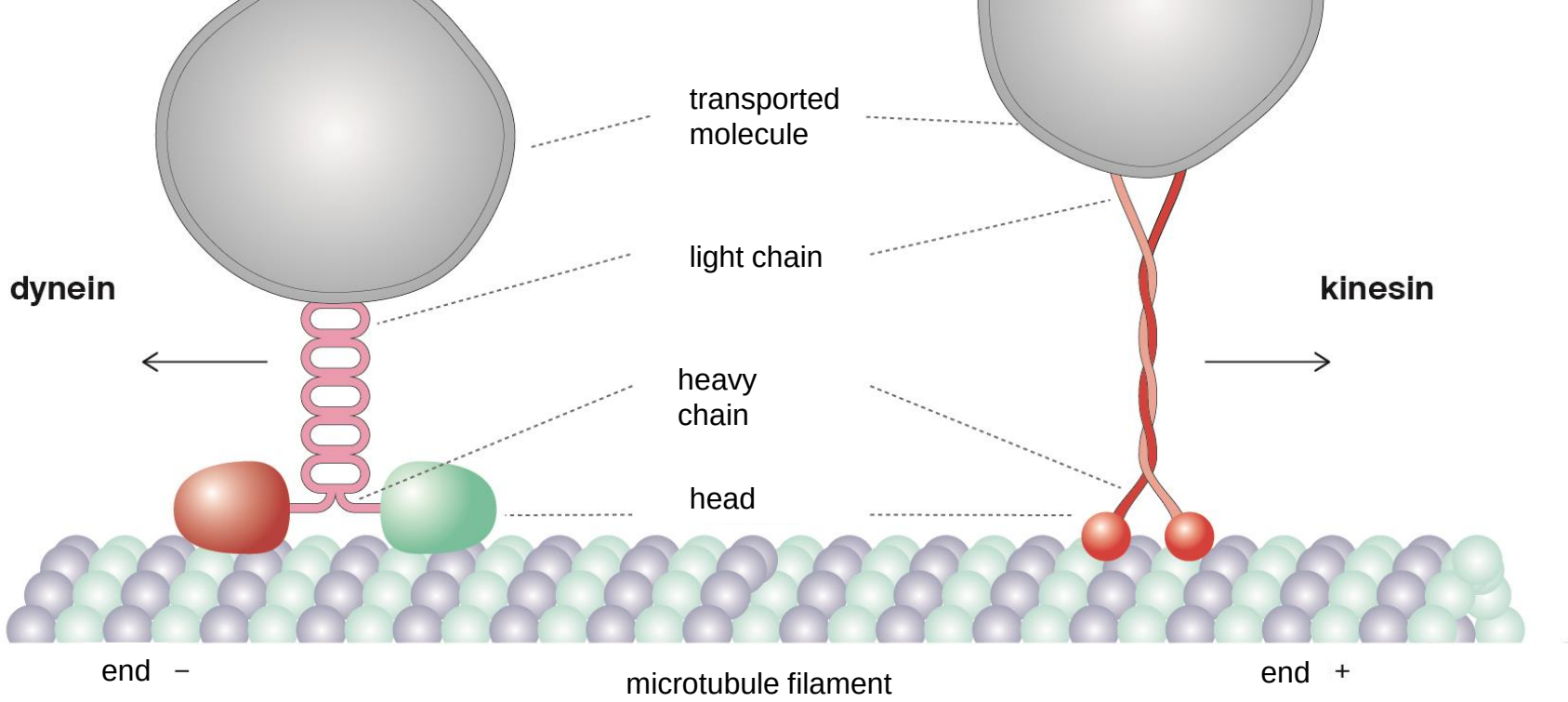
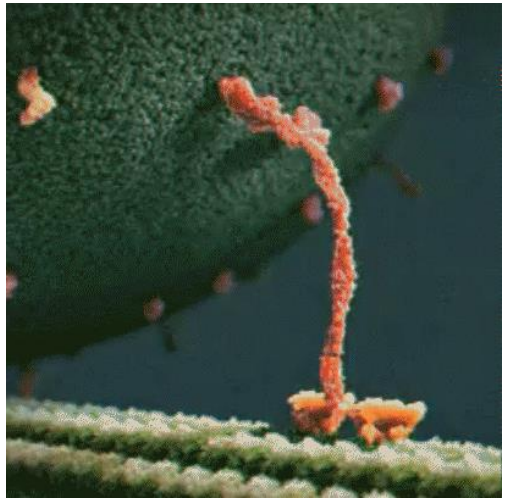


Molecular motors

transport of membrane vesicles and organelles along microtubules driven by molecular motors: kinesins and dyneins:
Kinesin to + end (to cell periphery)
Dynein to - end (to cell centre)

ATP as energy source



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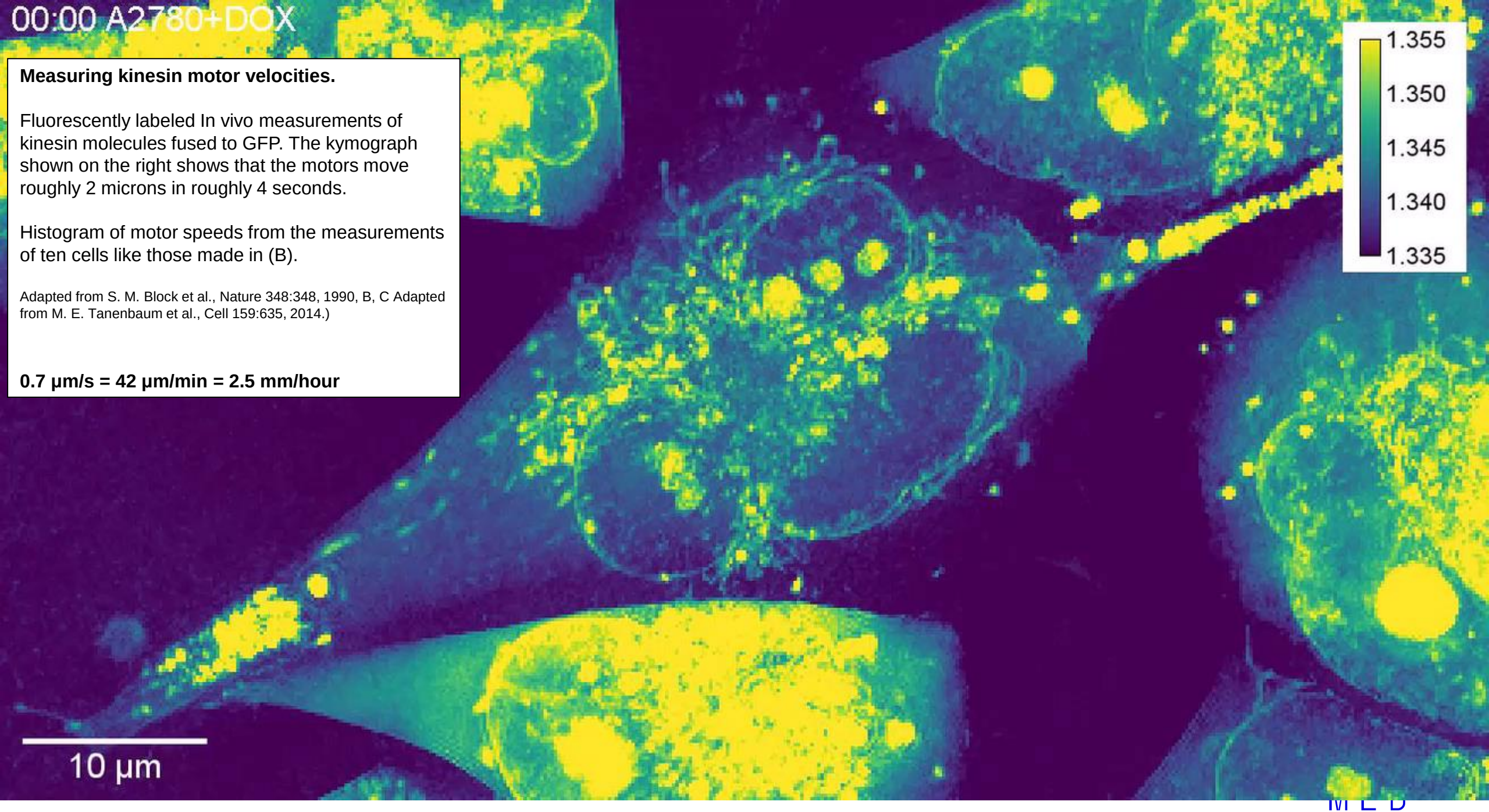


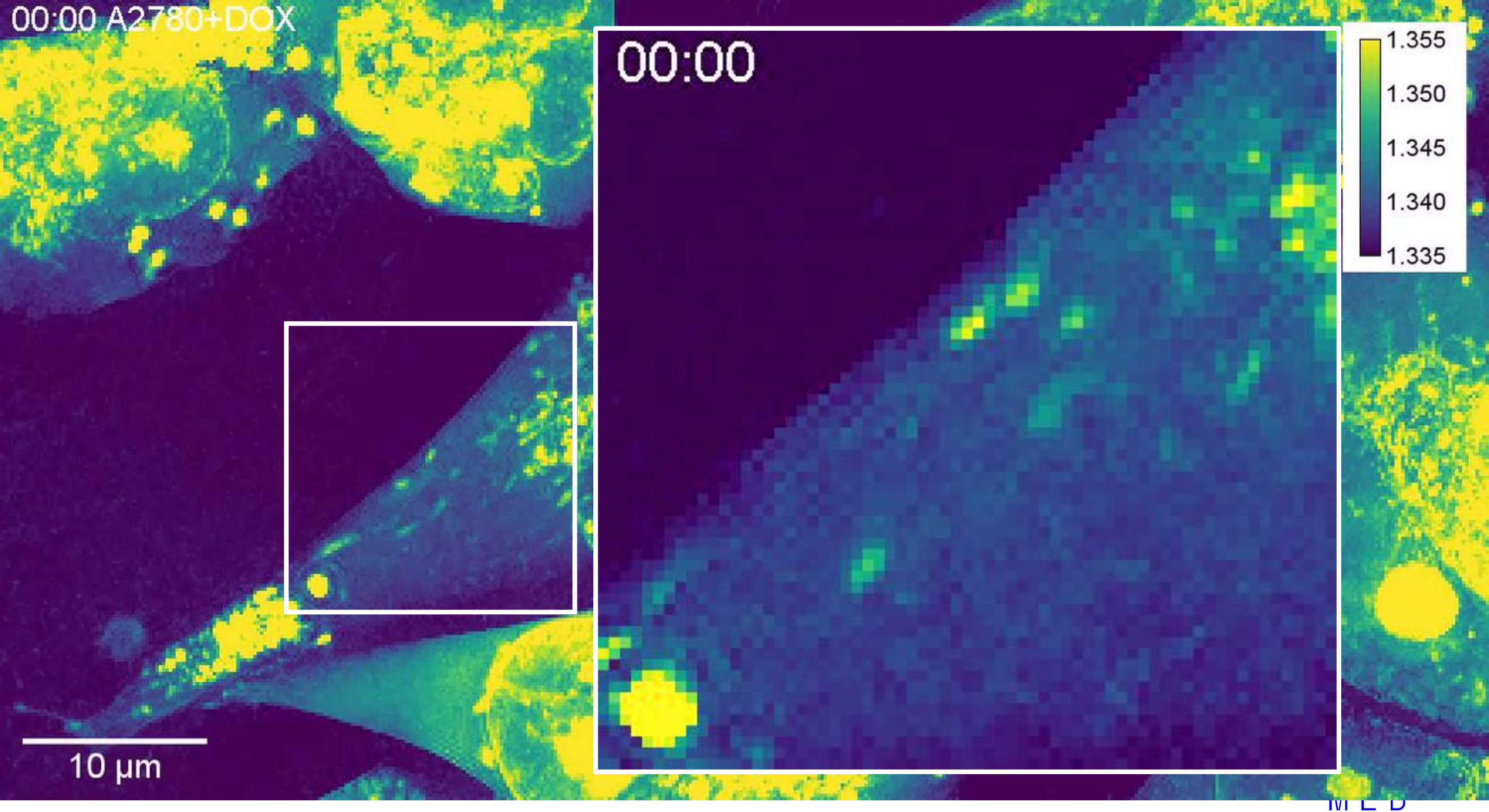
Measuring kinesin motor velocities.

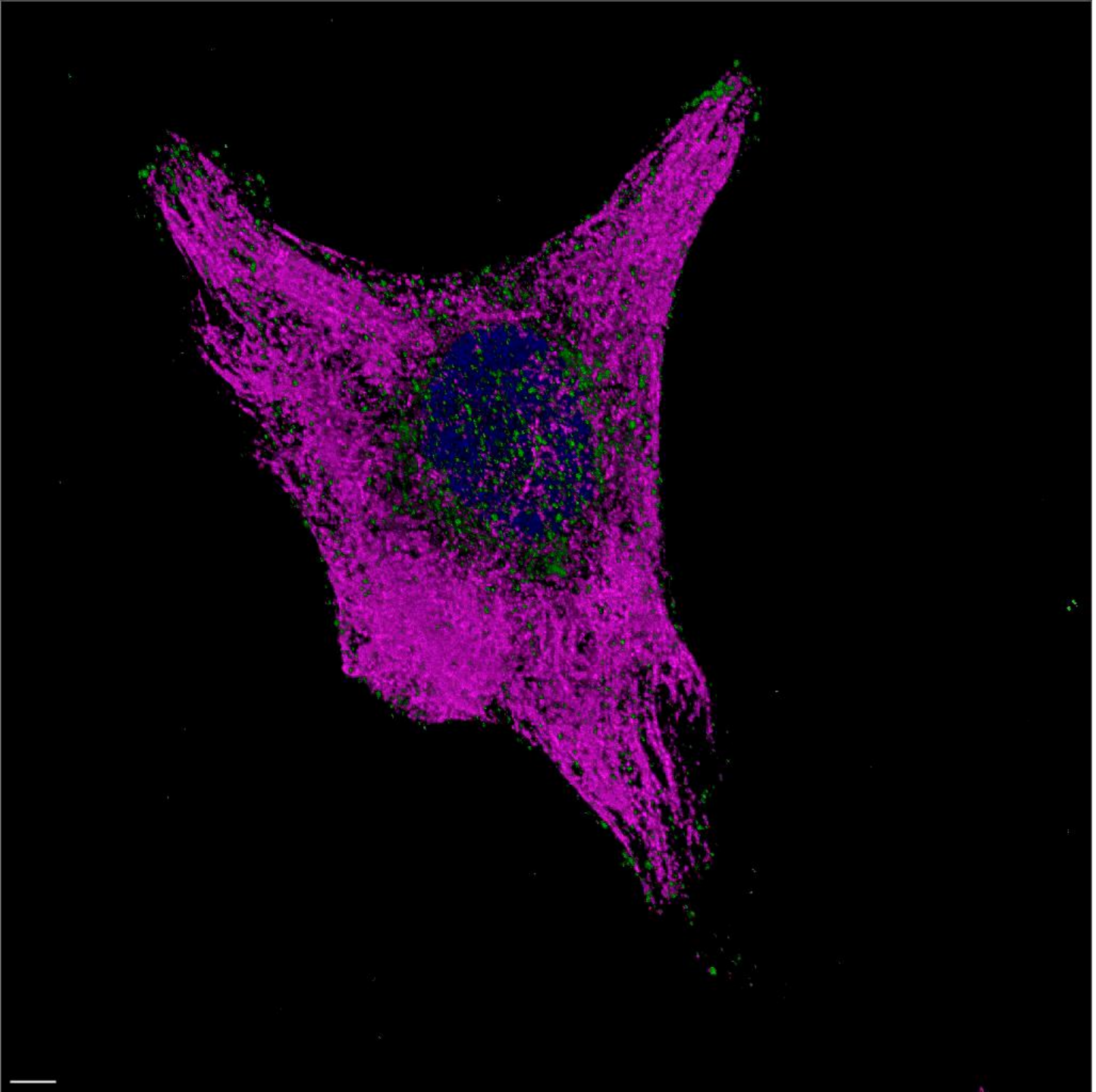
Fluorescently labeled In vivo measurements of kinesin molecules fused to GFP. The kymograph shown on the right shows that the motors move roughly 2 microns in roughly 4 seconds.

Histogram of motor speeds from the measurements of ten cells like those made in (B).

Adapted from S. M. Block et al., Nature 348:348, 1990, B, C Adapted from M. E. Tanenbaum et al., Cell 159:635, 2014.)

0.7 µm/s = 42 µm/min = 2.5 mm/hour


00:00 A2780+DOX
00:00
1.355
1.350
1.345
1.340
1.335
10 µm



Caveolin-1 is ubiquitously distributed throuhhout cells, although originally described as mebrane protein.

FaDu primary oropharyngeal cancer, untreated control, confocal microcopy, 64x. magenta goes for tubulin.

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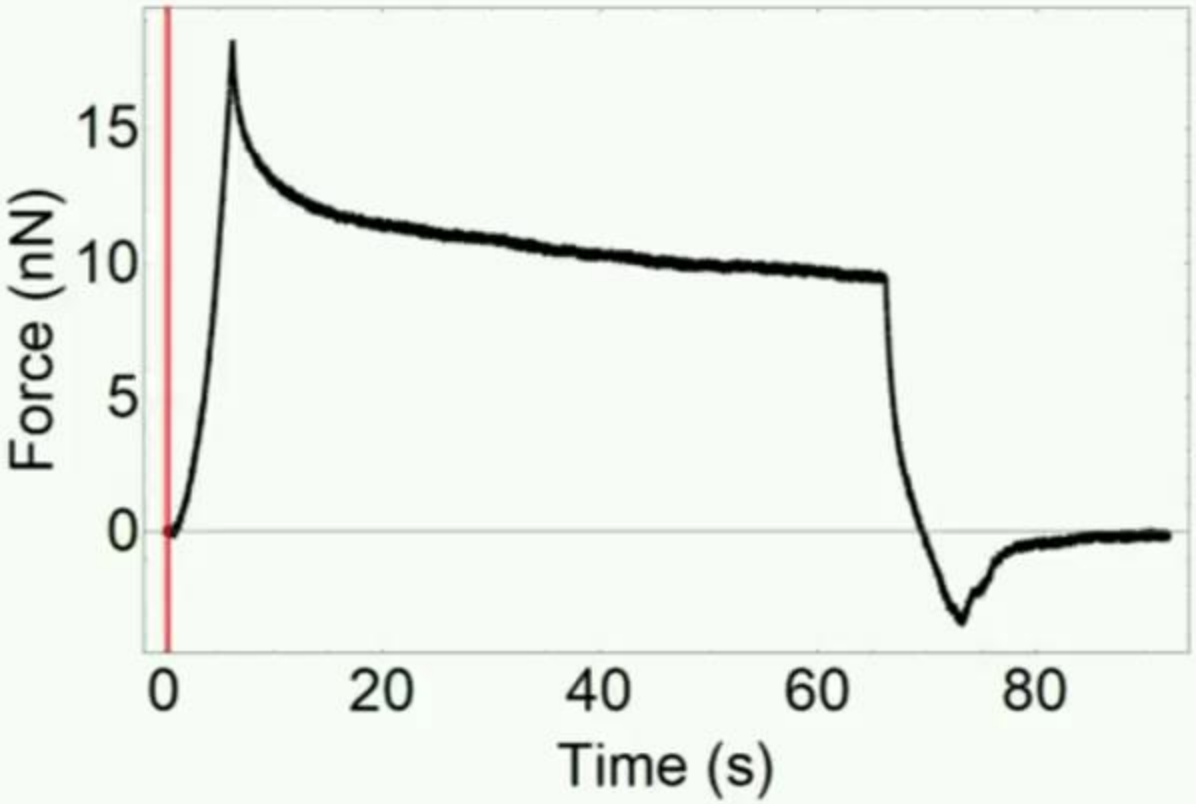
Cytoskeleton and mechanical role

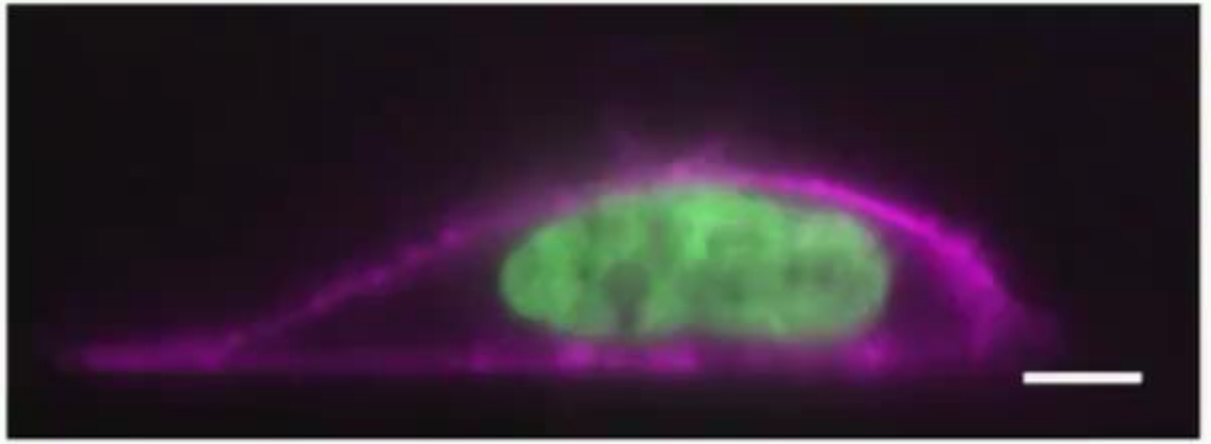
- **actin and intermediate filaments**: main source of cell stiffness
- **microtubules**: resistence to compression force

Physical limits for migration

- Nuclear size and stiffness control confined migration
- as confinment increases, deformation and squeezing is challenging
  - knockdown of lamin A, (component of the nuclear lamina) decreases nuclear stiffness and enhances the transmigration
  - progerin (a mutant form of lamin A) increases nuclear stiffness and suppresses confined cell migration

 Paul, 2017, https://www.nature.com/articles/nrc.2016.123





Nucleus stiffness. lamin A/C as limiting factor in migration

We use combined AFM and side-view SPIM to study how forces correlate with nuclear shape change under compression in live cells. https://twitter.com/C_M_Hobson/status/1227278696798539777
Hobson 2020 https://doi.org/10.1091/mbc.E20-01-0073

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Extracellular matrix

Extracellular matrix

- precisely organised structural and functional

  to biomechanical function of tissues
  - proteins (collagen, glycoprotien, elastin),
  - proteoglycans
  - adhesive glycoproteins (fibronectin, laminin,...)
- ECM provides
  - structural support,
  - mechanical strength,
  - attachement sites for cell receptors = ligand for integrins
  - reservoir for signalling molecules

classess of ECM

Basement membrane

- thin
- 2D substrate for adhesion of epithelial and endothelial cells
- laminin, collagen IV, nidogen, heparan sulphate

connective tissue

- fibrous 3D scaffold
- fibrillar colagens (type I, II mixed with III and V), proteoglycans, glycosaminoglycans

Extracellular matrix

- ECM component synthesis regulated

  by various factors and cytokines

- ECM degradated by matrix metalloproteases (MMPs)
  - selectively digest ECM components collagen, fibronectin
  - functional regulation of non ECM molecules (growth factors, cytokines,...)

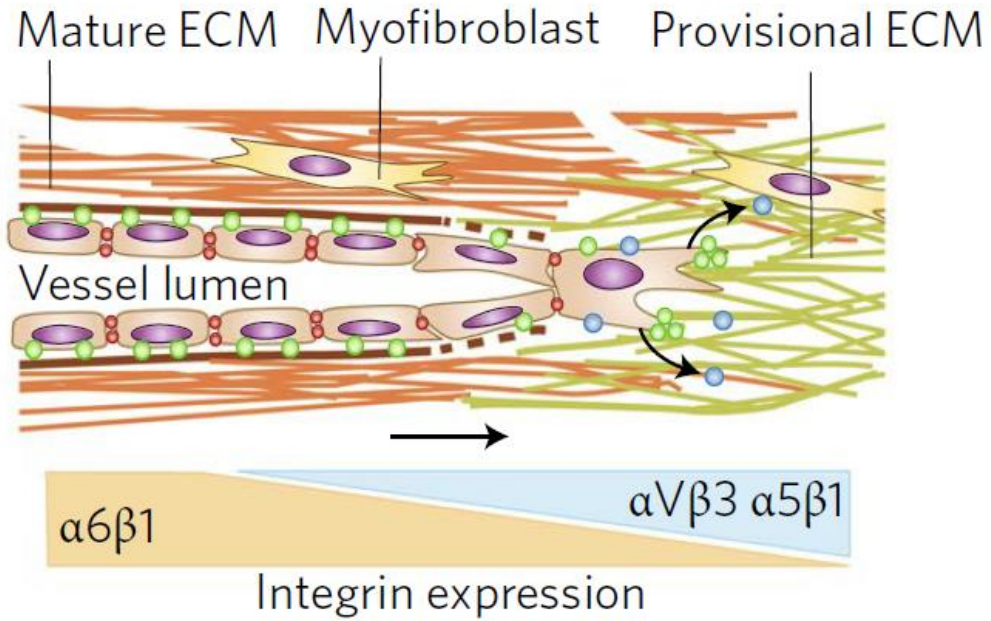
Dynamic ECM remodelling by cells - mechanoreciprocity

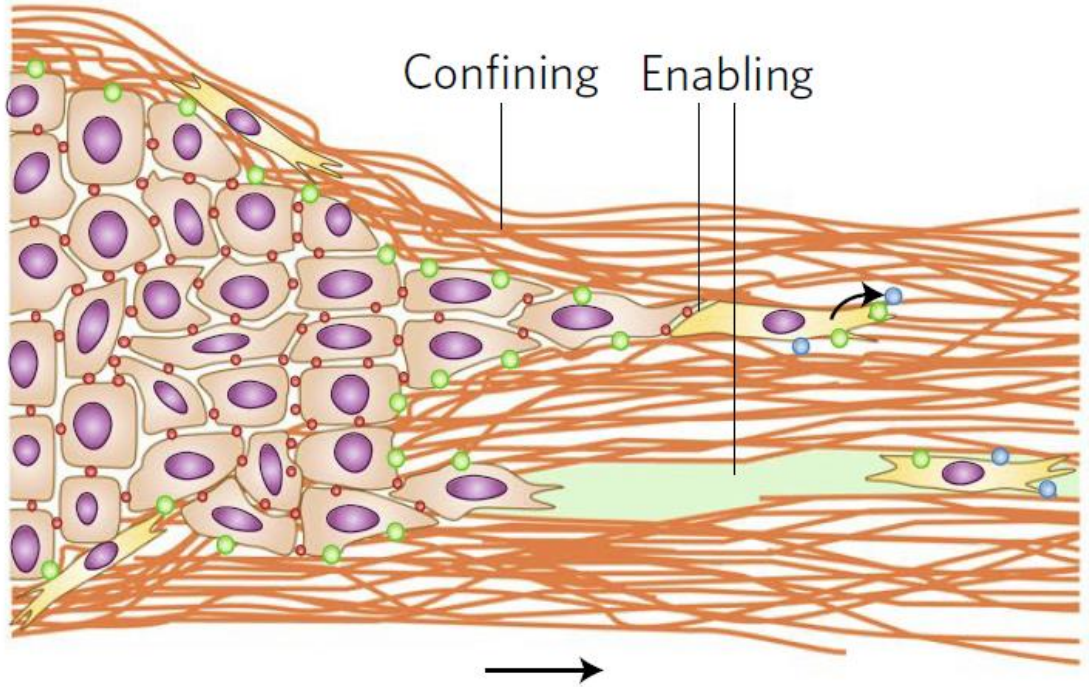
- Moving cells sense and respond to tissue mechanics and induce

transient or permanent tissue modifications:

- extracellular matrix stiffening,
- compression and deformation,
- protein unfolding,
- proteolytic remodelling
- jamming transitions.

 van Helvert https://doi.org/10.1038/s41556-017-0012-0





Integrins Matrix degradation Cell–cell adhesion Basement membrane

migration of fibroblasts and endothelial cells into wound

- realign and degrade provisional ECM
- synthesize collagen and basement membrane proteins
- undergo a transition of engaged integrin systems.
- **Outcome:** tissue alignment, density and stiffness are reciprocally linked to fibroblast function

Mechanoreciprocity in cancer invasion. Dual function of ECM deposition and stiffening by myofibroblasts in subregions, leading to tumour encapsulation or invasion along collagen interfaces.

 van Helvert https://doi.org/10.1038/s41556-017-0012-0

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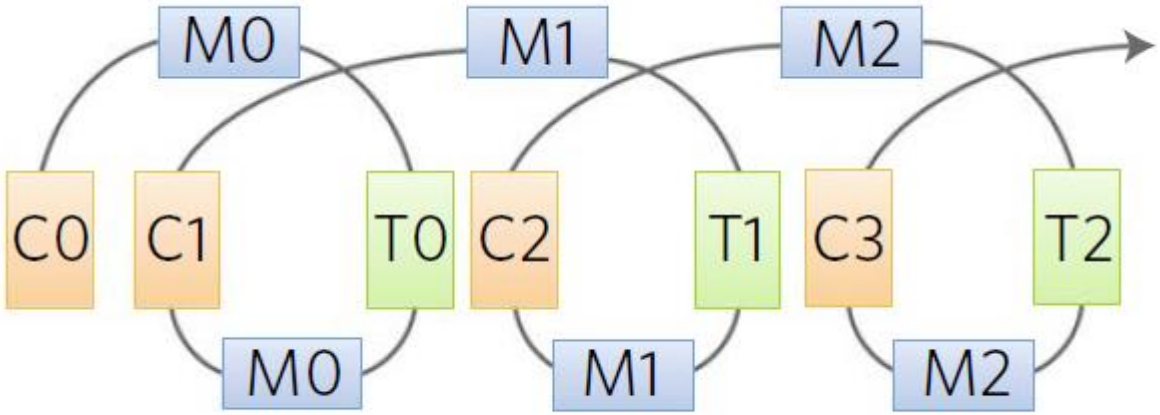

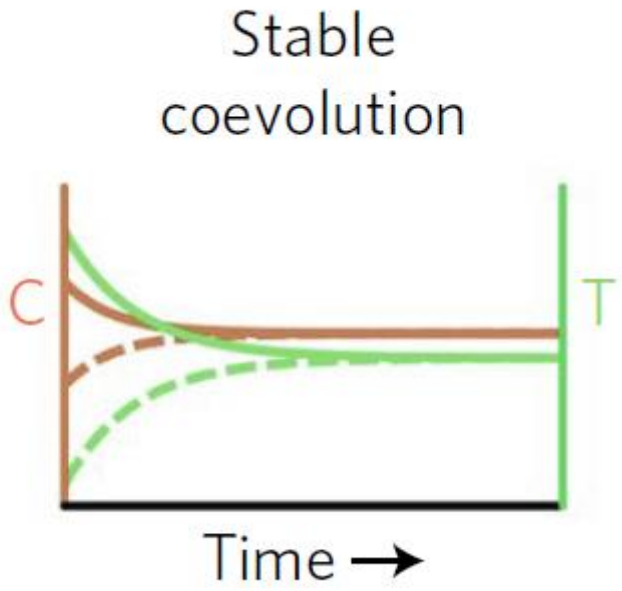

C Cell state M Mediator T Tissue state

Mechanoreciprocity: Spiral concept of mechanical cell–tissue interactions

Cells impose ‘mediators’ (pulling, pushing, ECM deposition and ECM degradation) and thereby alter tissue modules. Through iterative reinforcement and positive or negative feedback (indicated by the spiral), both cell and tissue modules undergo coevolution towards altered morphology and function.

 van Helvert https://doi.org/10.1038/s41556-017-0012-0

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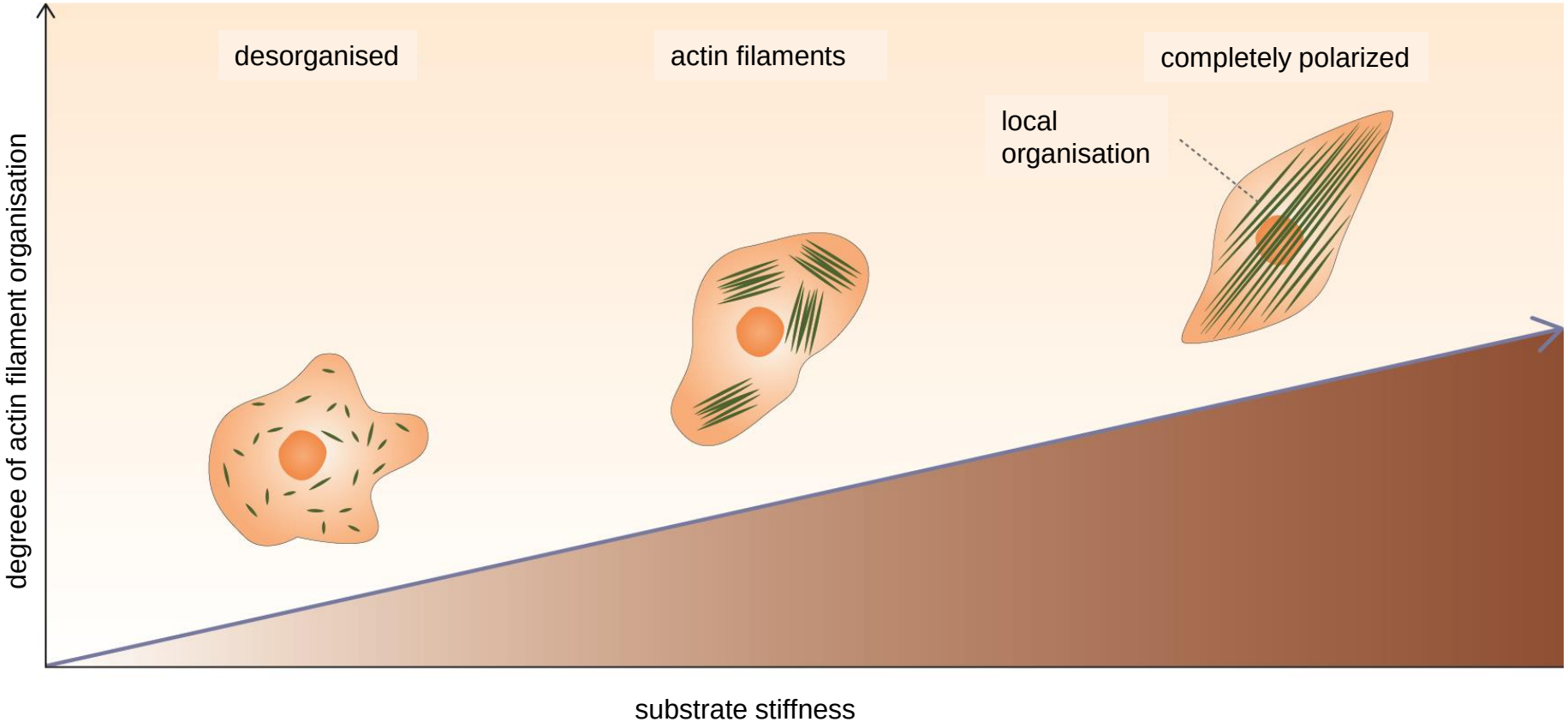

Strain-stiffening: Cell reaction to substrate stiffness

Cells adapt their structure to substrate they grown on by cytoskeletal remodelling

actin and intermediate fillaments increase stiffness under influence of force:

cells strain-stiffen on harder substrates

2018 Raudenská https://www.lekarskeknihy.cz/produkt/109803-vybrane-kapitoly-z-bunecne-fyziologie/
2015 Janssen

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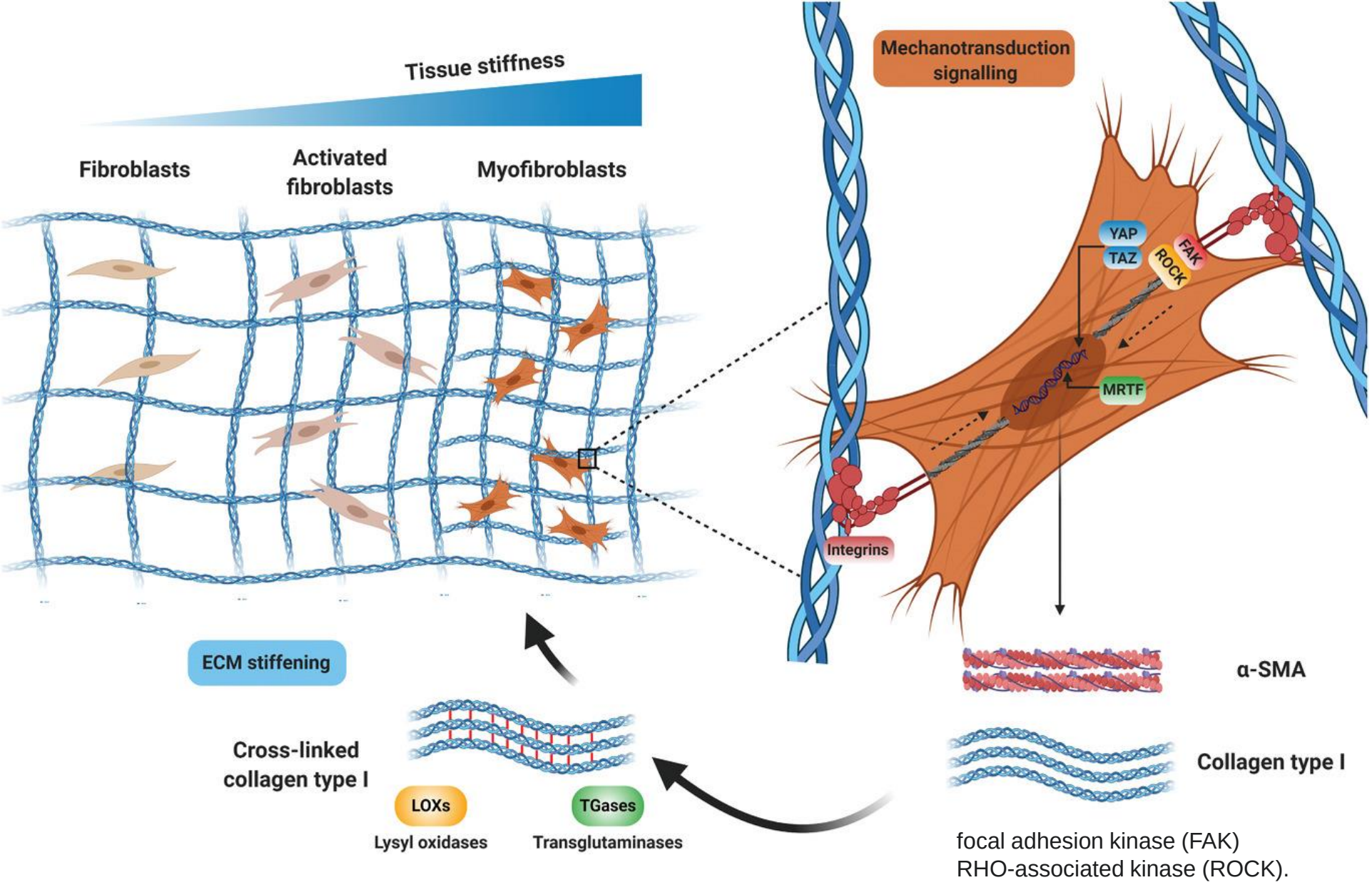

focal adhesion kinase (FAK)
RHO-associated kinase (ROCK).

Profibrotic matrix stiffness and mechanotransduction feedback loop

Mechanotransduction pathways mediate matrix stiffness-induced myofibroblast activation. Stiffness-mediated traction forces are transmitted across **integrins**, which **induce actomyosin cell contractility**

signals activate the effectors YAP (Yes-associated protein), TAZ (transcriptional coactivator with PDZ-binding motif), which increase the expression of **profibrotic α-SMA and collagen type I**.

Increased collagen deposition and crosslinking further increases ECM stiffening,

 2021 De Pieri https://doi.org/10.1002/adbi.202000168

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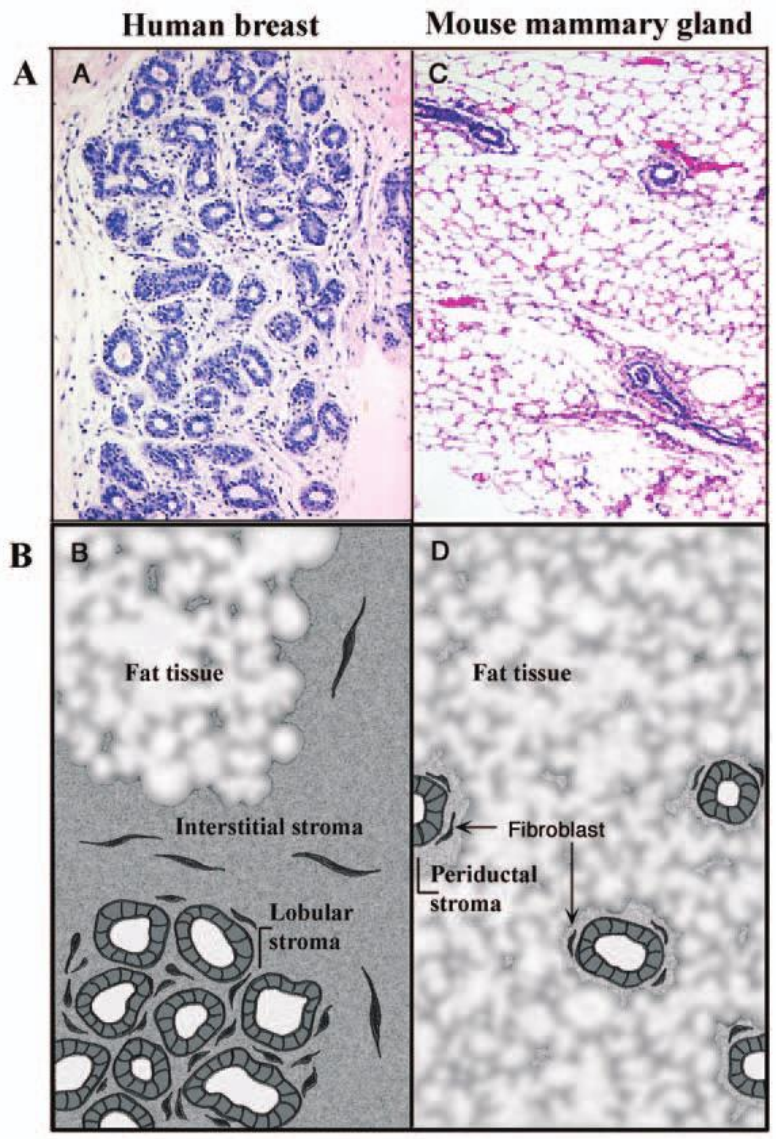
Unclear: cells sense environment by:

- by applying constant stress (force) and reading out strain (deformation)
- OR vice versa

- ability of cancer cells to invade via
  - MMP-independent **amoeboidal mode versus**
  - **an MMP-dependent** mesenchymal mode
  - may not solely be attributed to **cell-intrinsic** properties
  - but also to the **3D architecture of the local microenvironment.**

  - mouse mammary gland:significantly less fibrous tissue than the corresponding human



Comparison of human and mouse mammary glands. (A) Hematoxylin & eosin (H&E) stained section of human breast tissue showing a terminal ductal lobular unit comprised of ducts and acini embedded in a fibrous connective tissue stroma. (B) Schematic representation of a human terminal ductal lobular unit, emphasizing the **intimate association of epithelial structures with interstitial fibrous connective tissue stroma and the more distant adipose tissue.** (C) H&E stained section of the mouse mammary gland, showing ducts imbedded in a stroma composed of adipose tissue. (D) Schematic representation of the mouse mammary gland, **displaying ducts in intimate contact with fibroblasts and adipocyte**

Parmar et al 2004
10.1677/erc.1.00659

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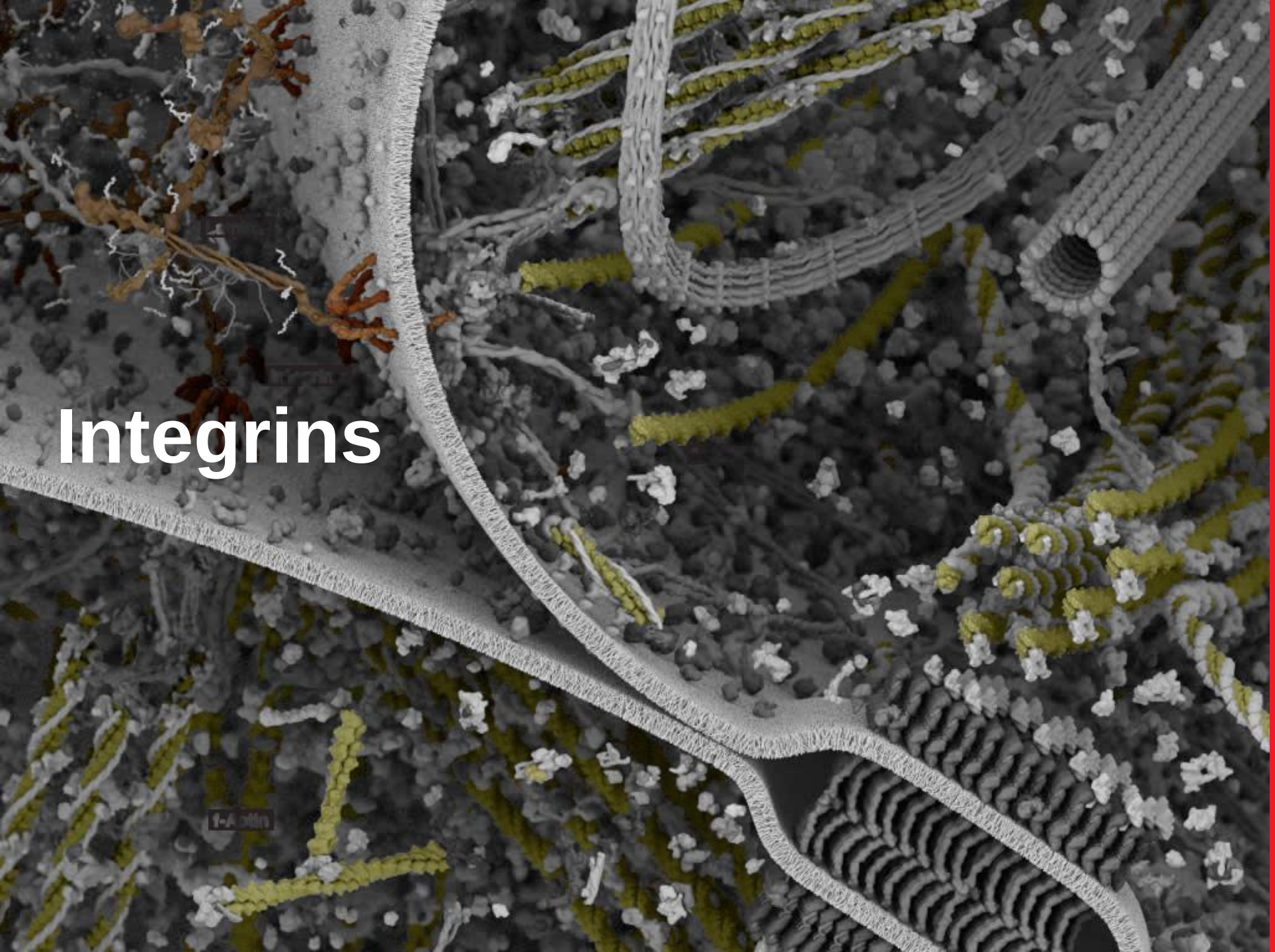
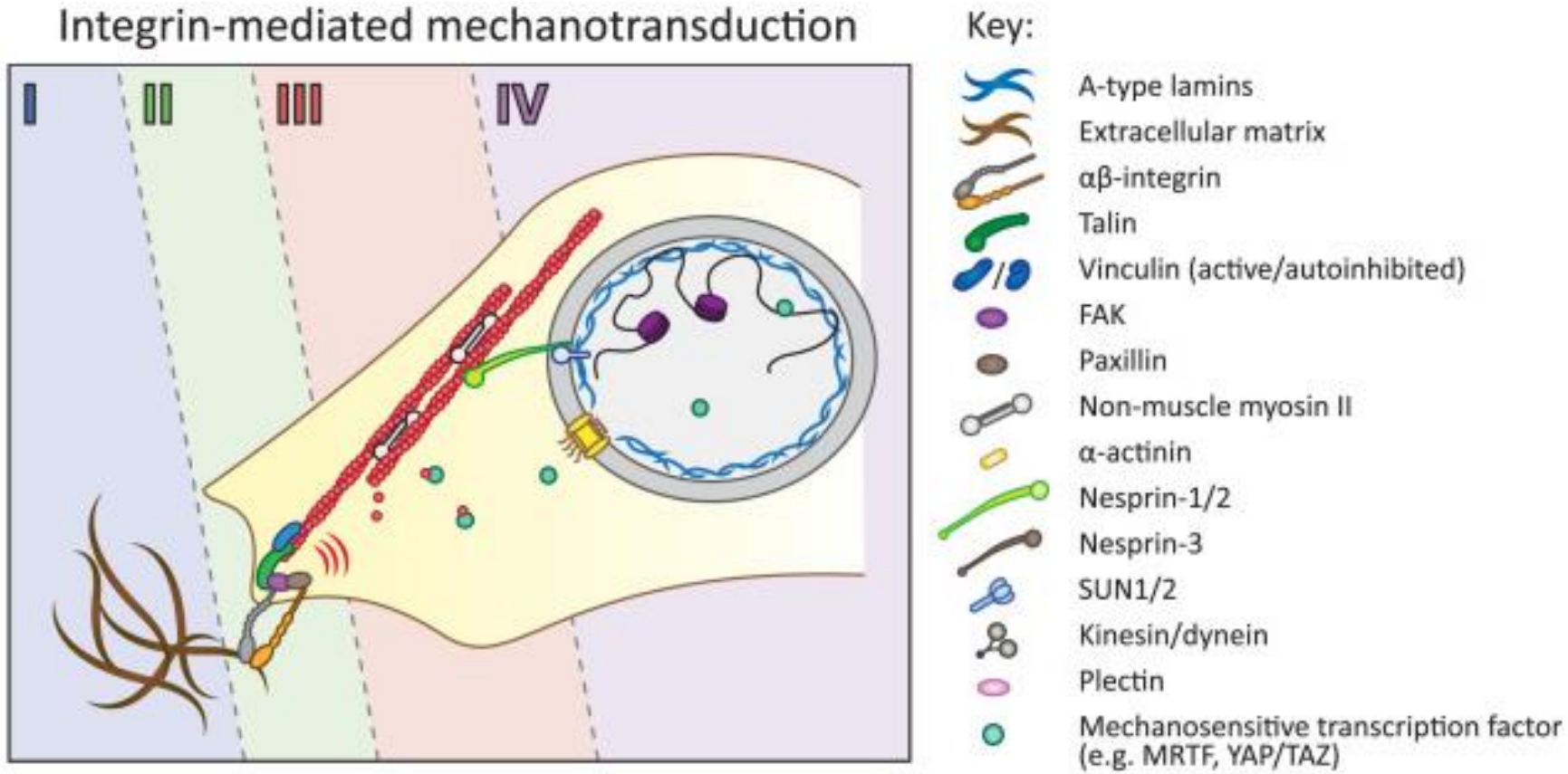


Integrins



- heterodimeric transmembrane proteins connecting ECM to cytoskeleton
- act as bi-directional signalling receptor
  - outside-in signalling: ligand binding > conformational changes modulating signalling cascades
  - inside-out signalling: intracell. proteins > increase integrin affinity for extracel. ligands

 2019 Isomursu, 2015 Jansen

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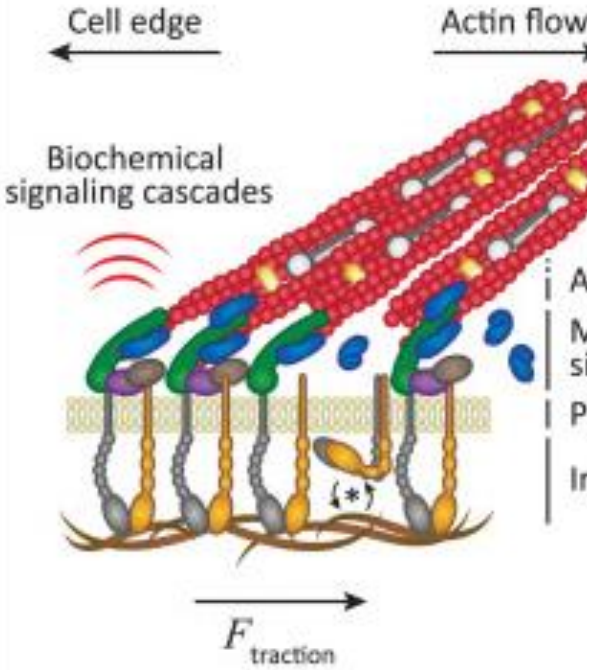
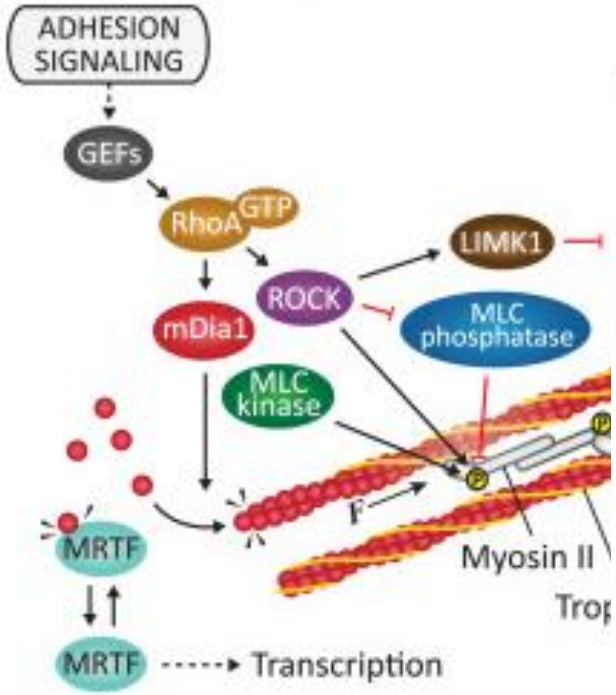
I Biomechanical properties of the extracellular environment

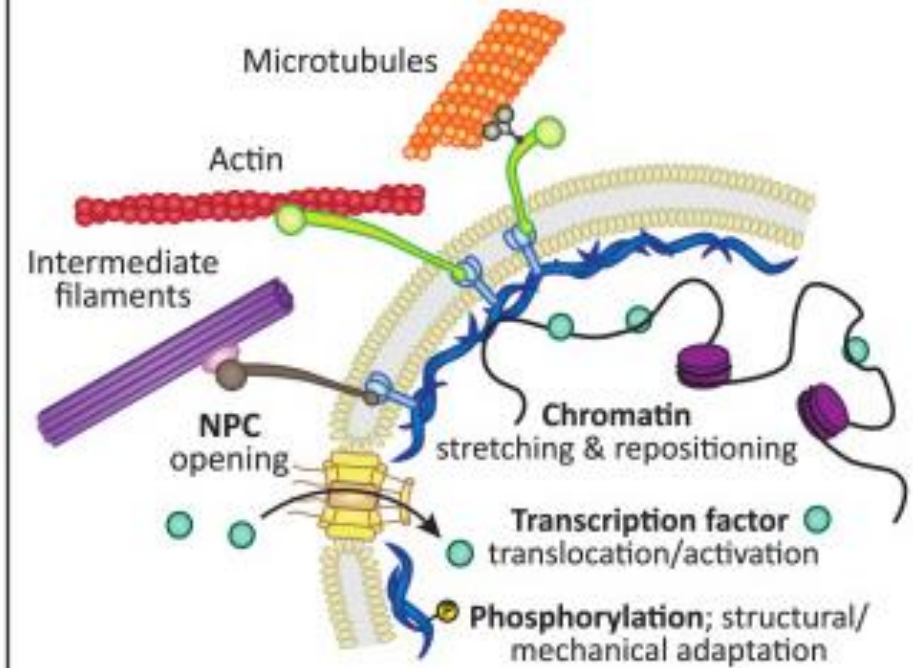
II Integrin-mediated adhesions

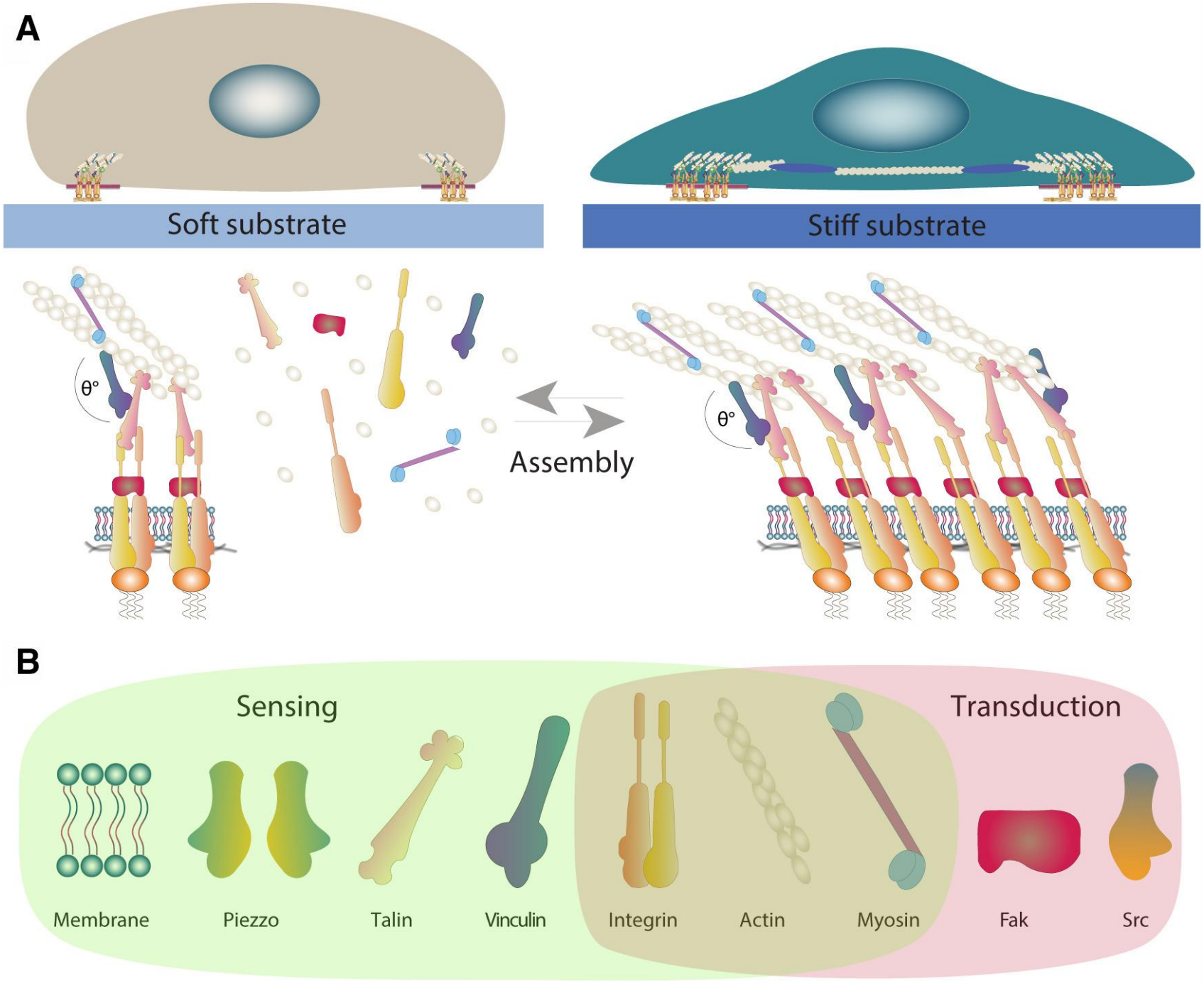


III Actin cytoskeleton and actomyosin contractility



IV Nuclear and transcriptional mechanoresponses





Mechanosensing in soft and stiff substrates

1. substrate rigidity can modulate the composition and dimensions of the FAs.
2. FAs promote cytoskeleton reorganization
3. thereby mediates tension (FA angle)

cells on **soft substrates** have small FA complexes, with low degree of assembly, which experience **low levels of tension**,

cells plated on **stiff substrates** present a higher degree of FA assembly and experience **greater levels of tension**.

the **most common elements of the mechanical response to substrate rigidity** including sensing and transduction modules. (integrin or some cytoskeleton elements, can exhibit both functions).

Espina 2021
https://doi.org/10.1111/febs.15862

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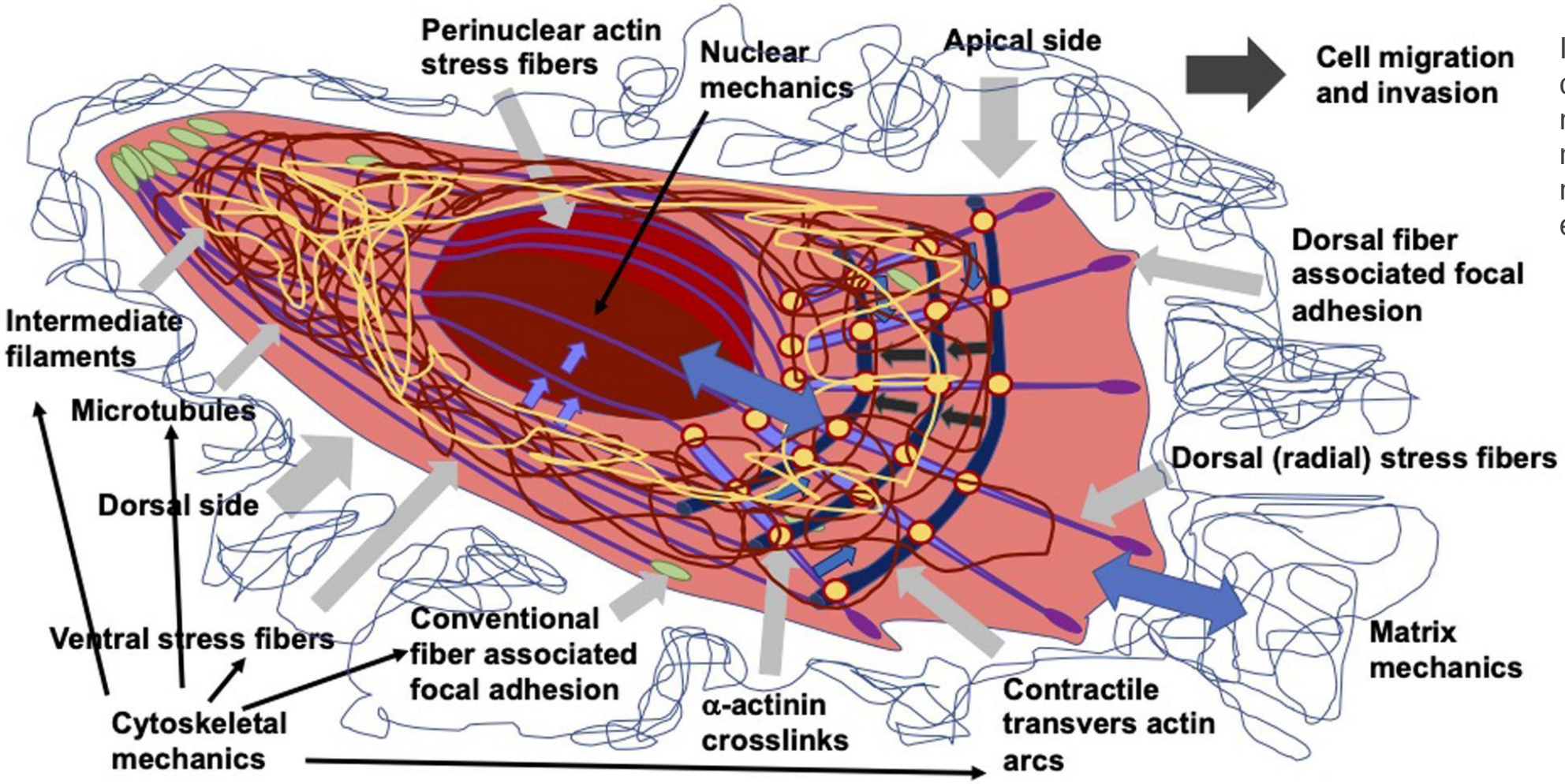

Interaction between the cell's cytoskeletal mechanics, nuclear mechanics and the matrix mechanics impacts cell migration and invasion through environmental confinements

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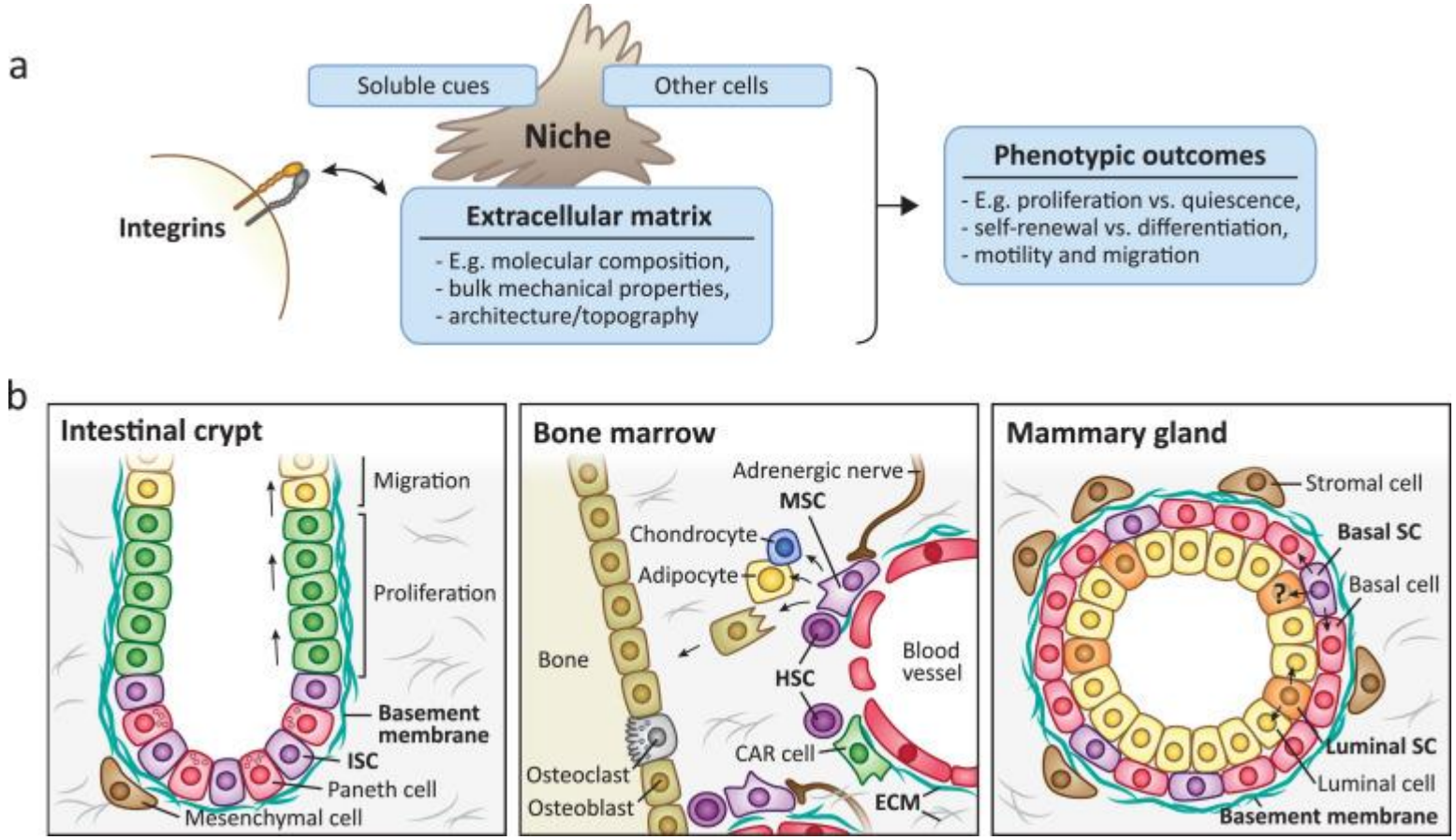
a
Soluble cues
Other cells
Niche
Integrins
Extracellular matrix
- E.g. molecular composition,
- bulk mechanical properties,
- architecture/topography
Phenotypic outcomes
- E.g. proliferation vs. quiescence,
- self-renewal vs. differentiation,
- motility and migration
b
Intestinal crypt
Migration
Proliferation
Basement membrane
ISC
Paneth cell
Mesenchymal cell
Bone marrow
Adrenergic nerve
MSC
Chondrocyte
Adipocyte
Bone
HSC
Blood vessel
CAR cell
Osteoclast
Osteoblast
ECM
Mammary gland
Stromal cell
Basal SC
Basal cell
?
Luminal SC
Luminal cell
Basement membrane

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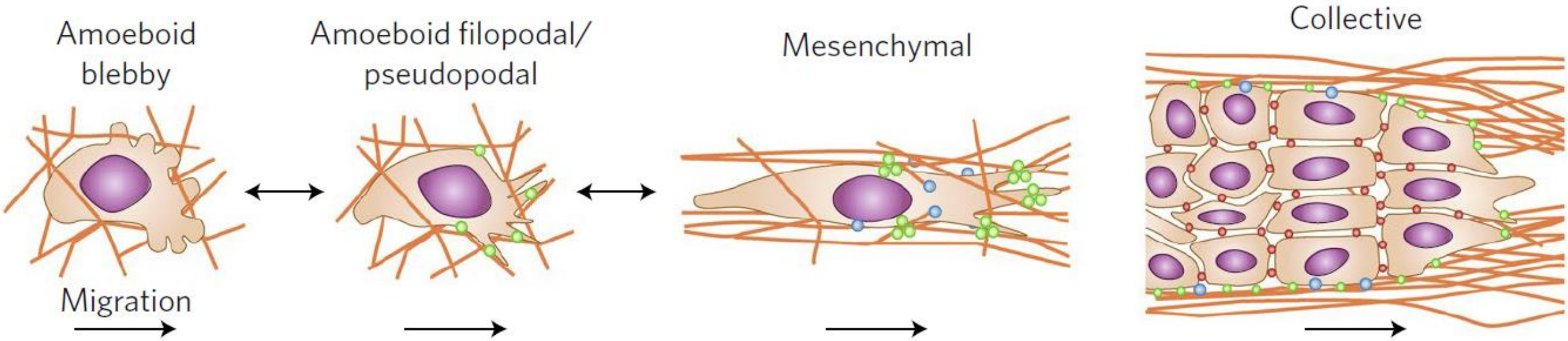

Cell migration modes in 3D environments, including single-cell and collective migration.

 van Helvert https://doi.org/10.1038/s41556-017-0012-0

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Mechanics in pathology

Effect of mechanical force

- Physiologically cells adapted to force:
  - blood elements x compression and shear stress
  - muscle cells X tensile and compression forces

- bone change shape, density and stiffness after altered mechanical loads
- blood vessels remodel in response to altered blood pressure
- **cells responsible for tissue remodeling**

ECM stiffness in disease

- excessive ECM fibrotisation:
- remodelation via release of proteolytic enzymes + demosition of components
  - collagen elongation and crosslinking
  - lysyloxidase activity (lysine – aldehydes – forming crosslinks in ECM proteins
- Stiff ECM increase metastasing probability:

stromal stiffening modulation as therapy target?

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Cell response to forces related to pathology

- abnormal mechanical loading
  - alter cell function
  - alter extracellular matrix
- lead to pathologies
  - osteoporosis, osteoarthritis, tendinopathy, fibrosis, ...

ECM stiffness in cancerogenesis

ECM affected by

- desmoplastic reaction in tumors
- compression forces – tumor expansion
- tensile forces of rigid ECM

Cancer cell actively response

to ECM stiffness via mechanotransduction

- YAP/TAZ signalling
- FAK signaling

„mechanosensitive“ cancer cell pro-survival

adhesion, migration, gene expression, cell-cell interactions, stem cell differentiation

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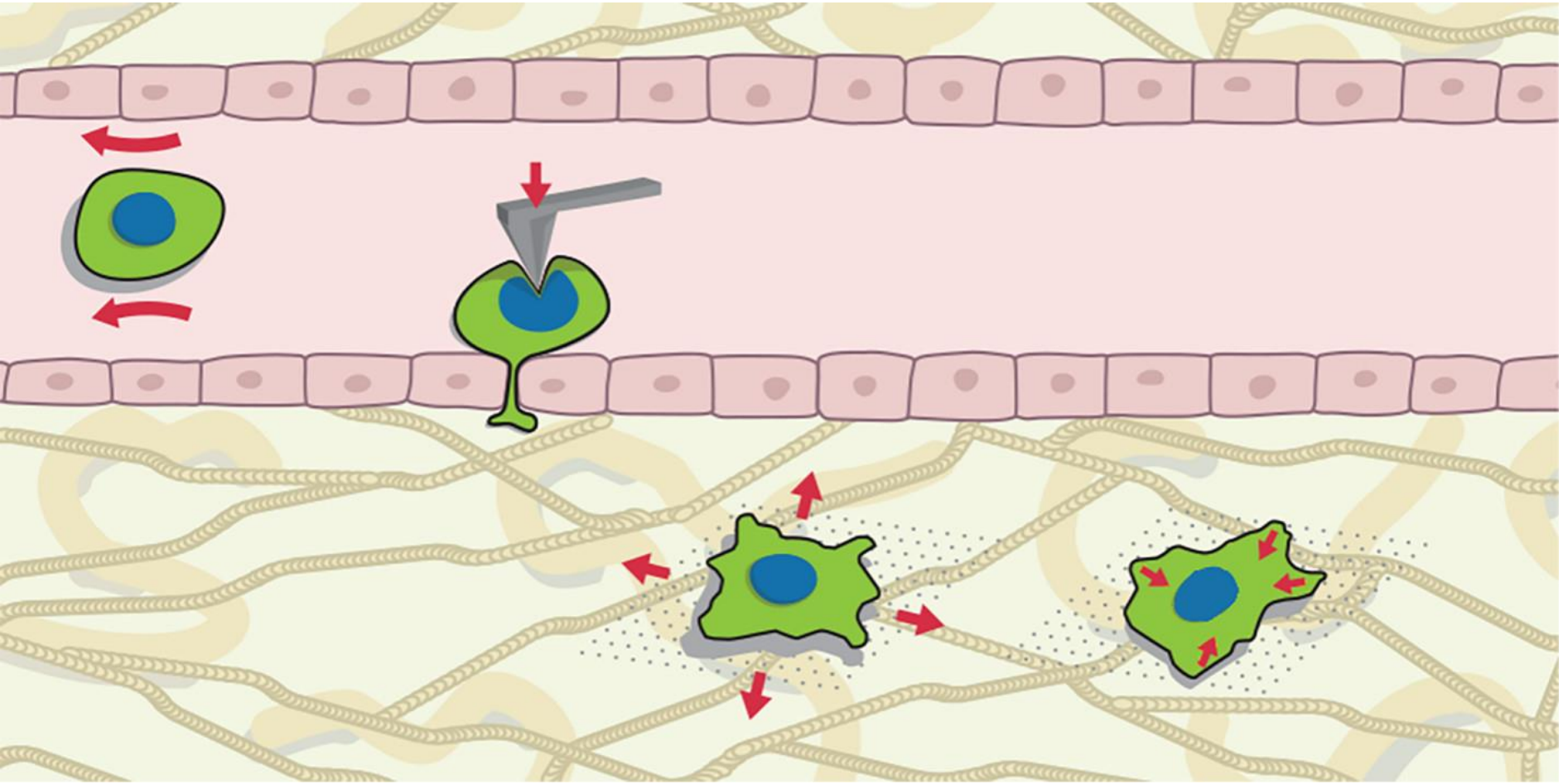
- cells exposed to mechanical stimuli: compression, tensile forces between cells and ECM, compression of interstitial fluid, shear stress
- tumor ECM change during progression
- tumor ECM may support cancer cell aggresiveness

Tumor primary stroma variable X selection during metastasis stereotypic



Late stages of the metastatic cascade and biomechanical interrogation.

During their metastatic journey, cancer cells are exposed to a number of biophysical challenges. Their adaptation to overcome these threats can be explored using different tools. Each one of the phenotyping techniques relies on the application of a force of known magnitude and tracking of the resulting cell deformation.

10.1091/mbc.E18-08-0545

blood circulation

shear forces (red arrows around the cell)
collision
65

extravasation

Nuclear size and **compressive stiffness** (as measured using AFM, red arrow in cell sitting on the endothelium)

target organ

tensile stresses caused by tissue deformations

target organ

adhering cells exert **contractile forces**
- parallel to the surface
- out-of-plane ones

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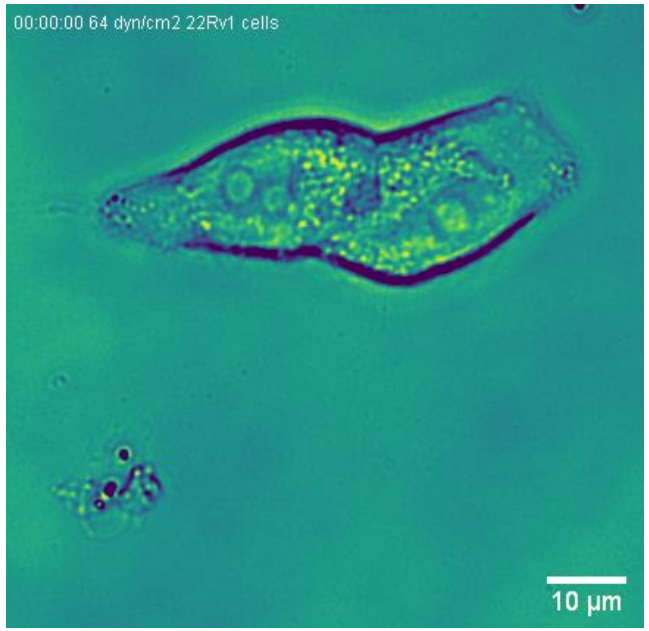
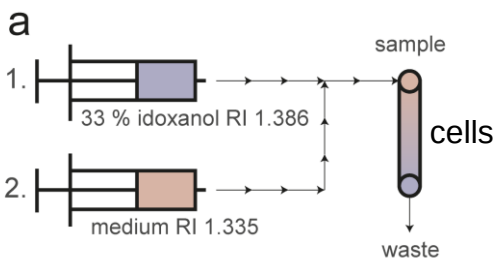
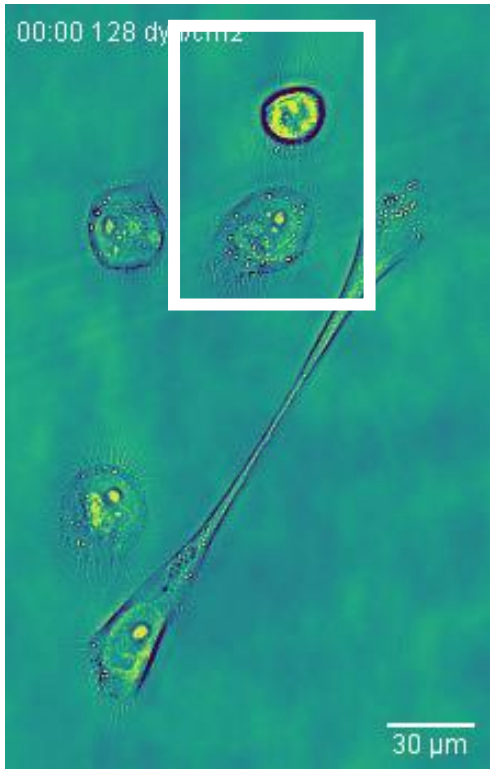
Shear stress

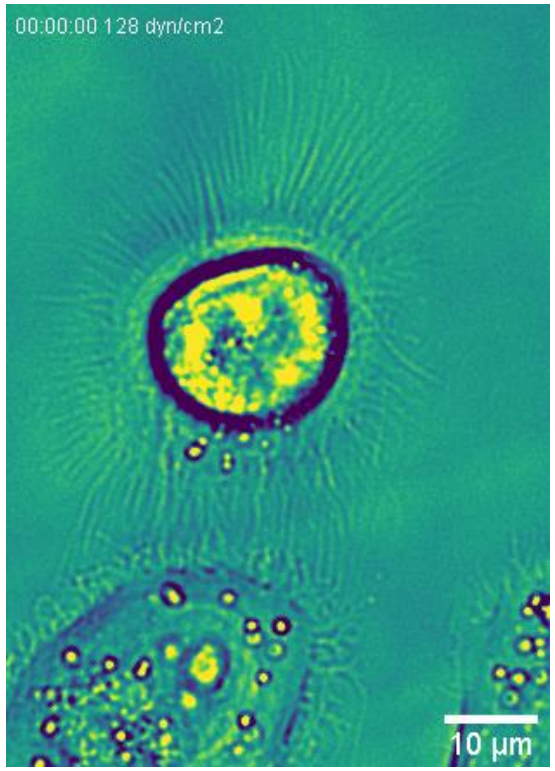
22Rv1 cells on fibronectin-coated plastic, 6.4 Pa





PC-3 cells on fibronectin-coated plastic, 12,8 Pa





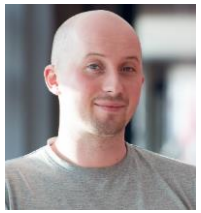
12x time compression

QPI provides shear modulus

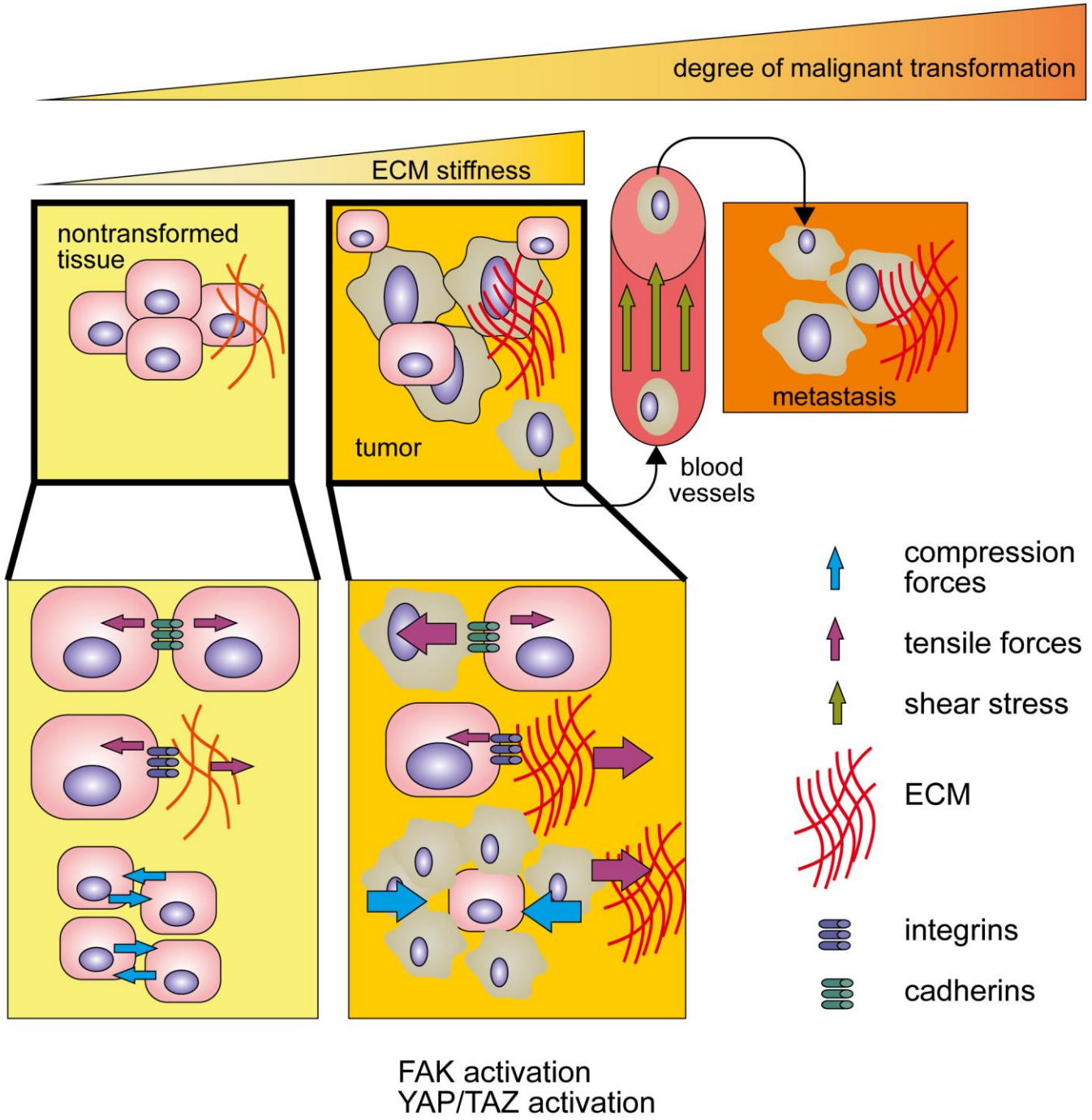
We present, to our knowledge, a new method based on QPI for measuring cellular shear moduli. Compared to typical CP-AFM measurements, our method does not involve cell contact and is well suited for high-throughput assays. The basis of our approach is to analyze the plane through which the COM moves in response to an applied shear flow. Consider a box of deformable material with height h subjected to shear flow with shear stress τ_s. A transverse displacement, dX, on the top of the box causes a shear strain γ. Once shear stress and shear strain are known, the shear modulus, G, can be determined:

$$G = \frac{\tau_s}{\tan(\gamma)} \approx \frac{\tau_s}{dX/h}. \quad (4)$$

Eldridge et al. 2019, Biophysical Journal



Tomas Vicar
BUT



Mechanical stimuli on tumor cells

- non-tumor tissue ECM x cell forces in **equilibrium**
- in response to **tensile force** (increased ECM stiffness in tumor stroma) → mechanosensitive signalling activation
  - YAP/TAZ: proliferation, dedifferentiation, invasion, resistence
  - focal adhesion kinase (FAK): increased migration (FA maturation, actomyosin contractility in stress fibers),
- tumor cell expansion limited by stroma: ↑ interstitial pressure
- increased ECM stiffness → ↑ mechanical stress → gradient supporting metastatic spread → migration along collagen fibers
- vessels: **shear stress**

Raudenska 2021 10.48095/ccko2021202.

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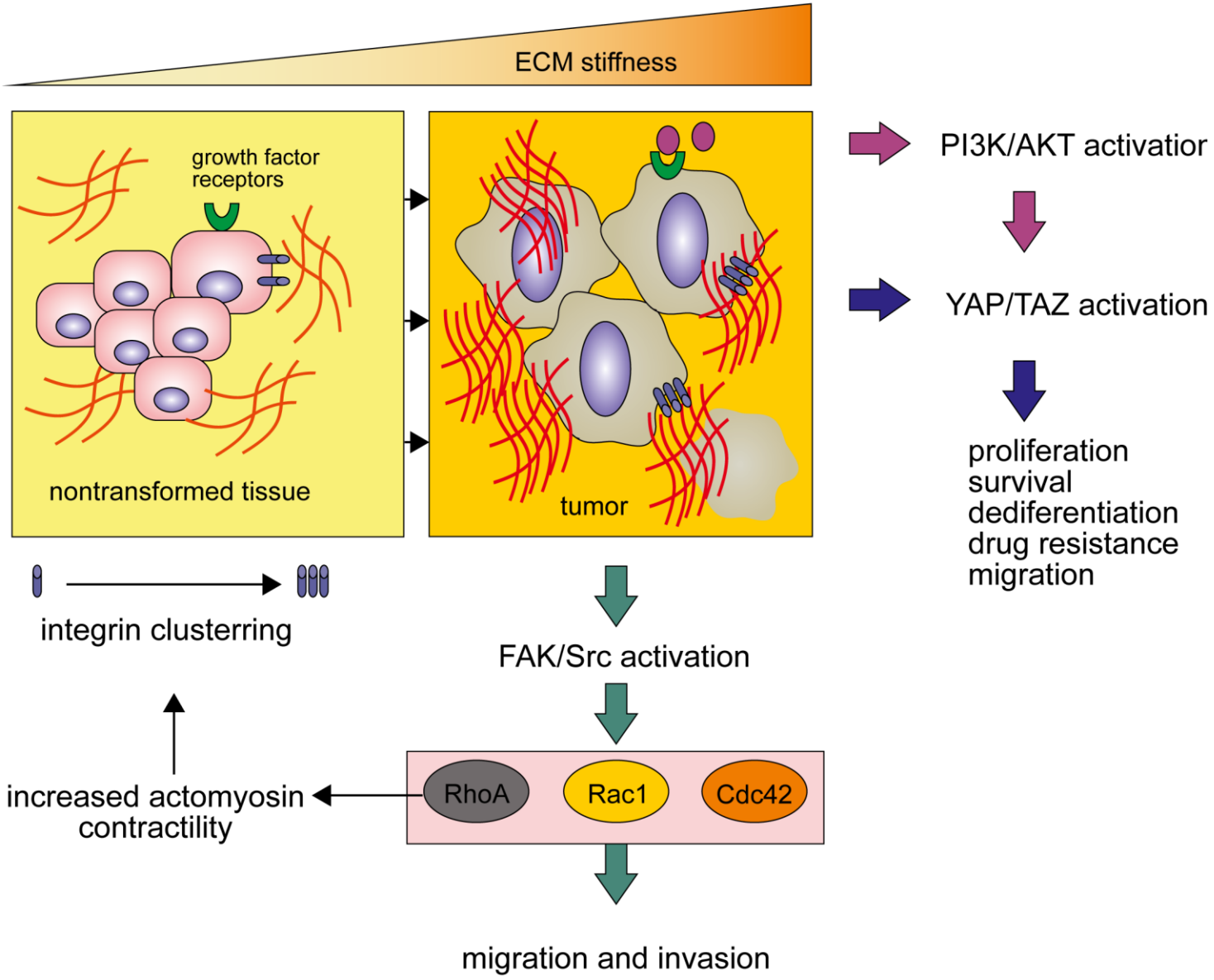

Mechanotransduction affects cancer cells

- stiff ECM activates mechanotransduction driving proliferation, invasion, migration
- increased deposition and crosslinking of collagen stiffens ECM → ↑ integrin clustering → FAK activation → Rac1/Cdc42 GTPases activation → migration
  - RhoA > Rac1/Cdc42 → stress fiber formation + FA maturation
- **stiff ECM** → ↑ YAP/TAZ sctivation via PI3K

Raudenska 2021 10.48095/ccko2021202.

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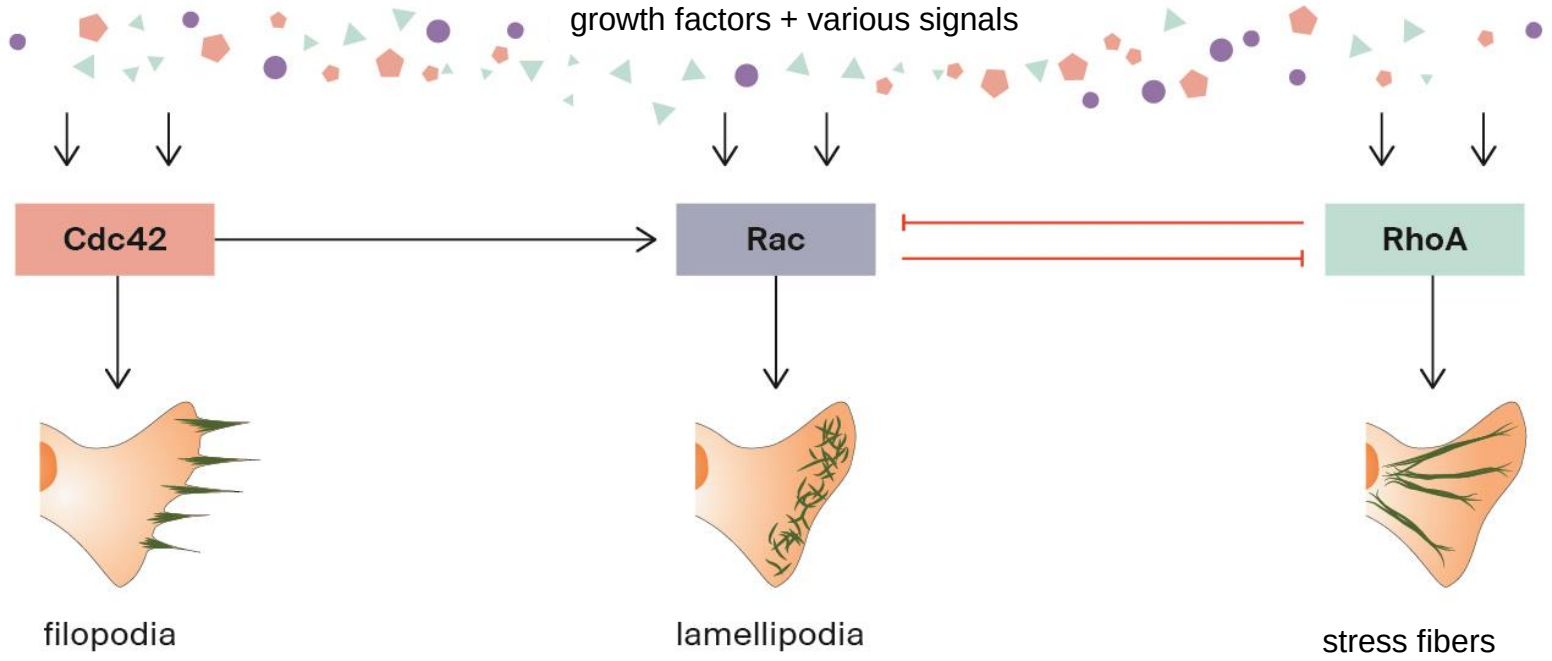
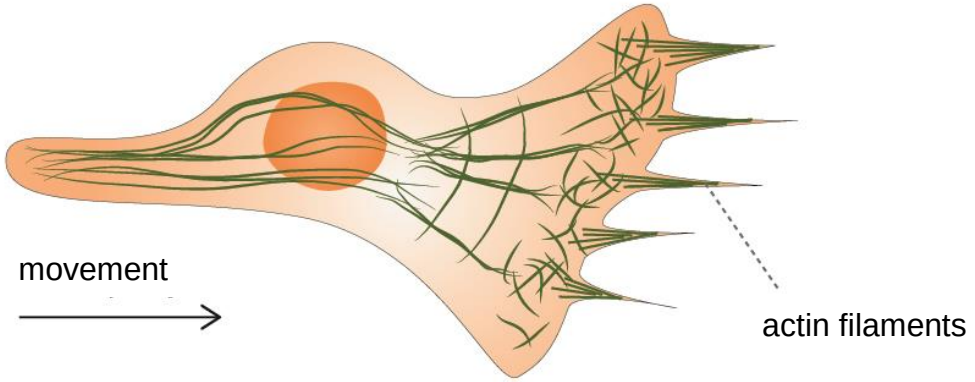
Location of movement structures in the mesenchymal type of movement.

Formation of structures enabling cell movementis significantly regulated by the activity of **small GTPase** from the Rho family

- Rac1 = Ras-related C3 botulinum toxin substrate 1,
- Cdc42 = Celldivision control protein 42 homolog
- RhoA= Transforming protein RhoA

2018 Raudenská https://www.lekarskeknihy.cz/produkt/109803-vybrane-kapitoly-z-bunecne-fyziologie/





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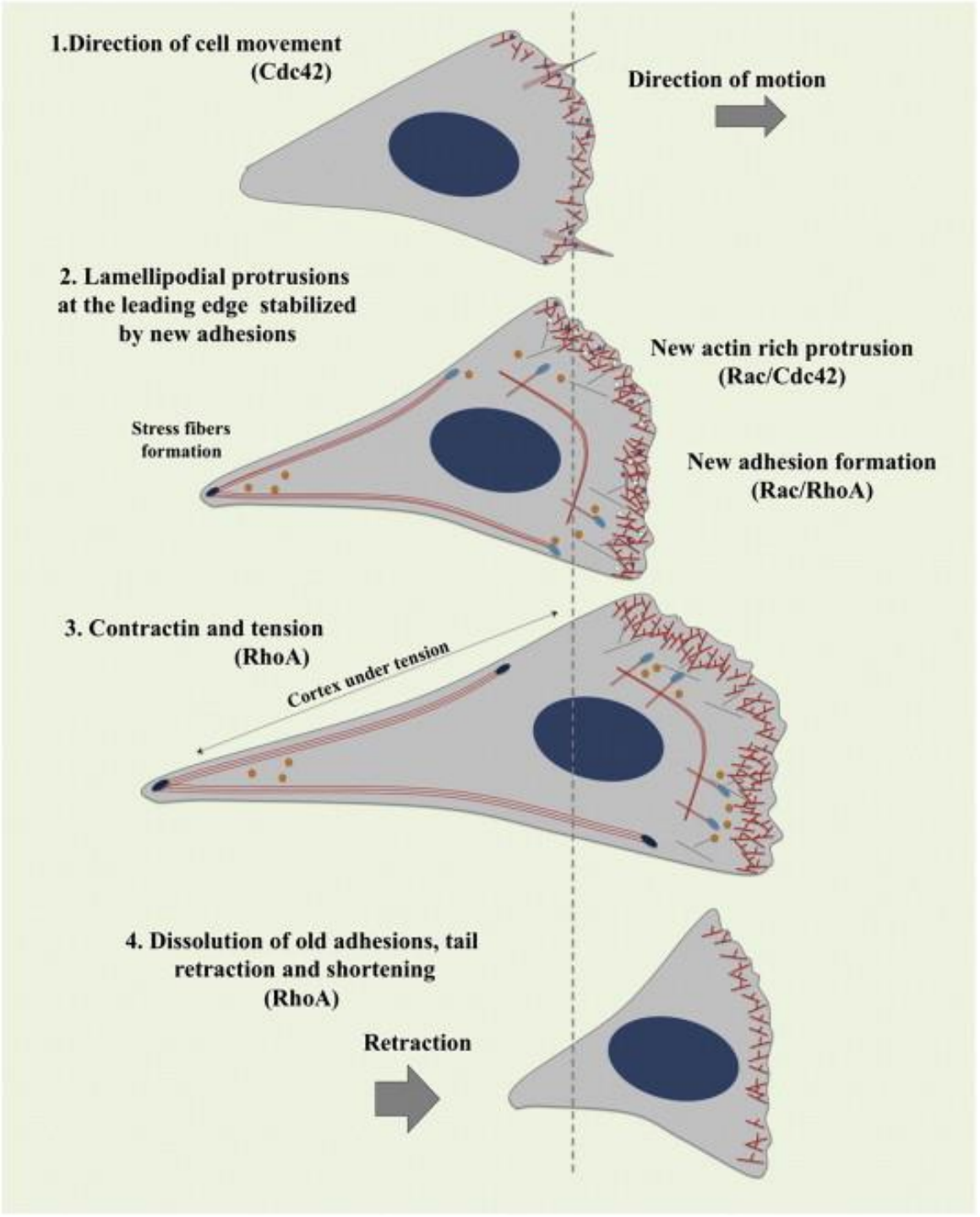
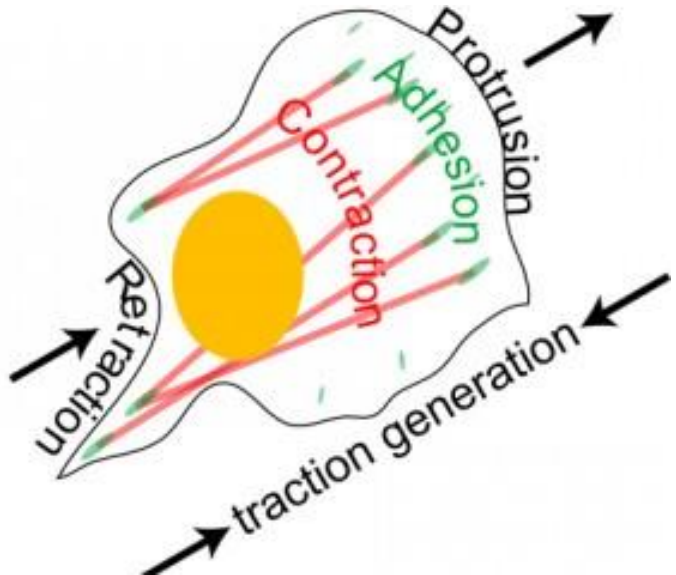


Rho GTPases in the cell motility cycle.

1. A migratory cell enters the cell motility cycle in response to a chemoattractant signal.
2. **Cdc42** determines the direction of motion.
3. **Rac** induces the formation of actin-rich lamellopodial protrusion at the leading edge.
4. New protrusion is stabilized by the formation of new adhesions to the underlying substratum, a process controlled mainly by Rac and RhoA .
5. **Rho** acts at the rear end leading to the formation of **stress fibers** and actin–myosin contractility providing tension for the cell to retract its tail and move forward.

2013 Hanna http://dx.doi.org/10.1016/j.cellsig.2013.04.009



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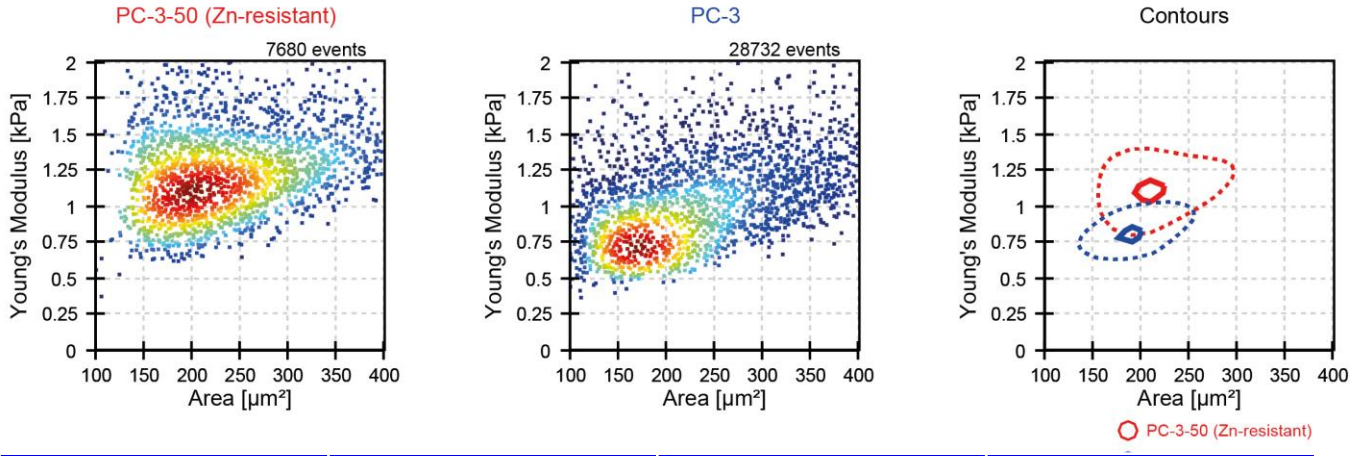
Cell deformability

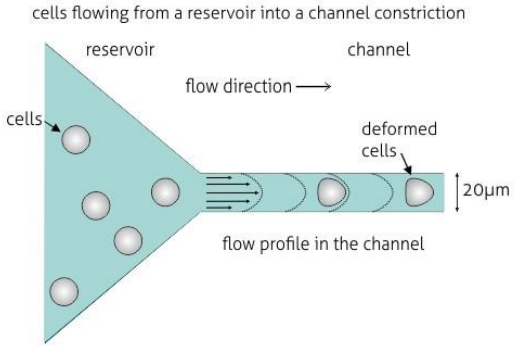
scales with Young modulus (AFM)

… and with zinc resistance

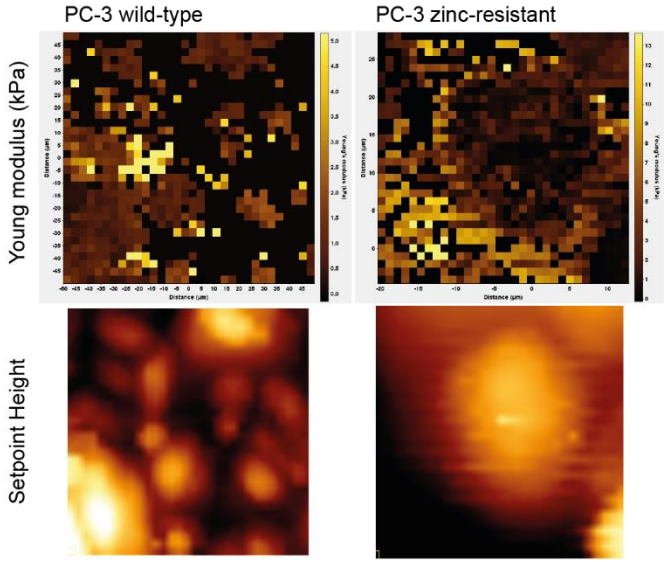
PC-3 cells, wild-type vs zinc-resistant



Cells	AFM, sharp tip Young modulus (kPa)	AFM, 5 um sphere Young modulus (kPa)	RTDC, 30um Young modulus (kPa)
PC-3, wild type	1.20	0.99	0.89
PC-3, Zn-resist.	1.70	2.00	1.23

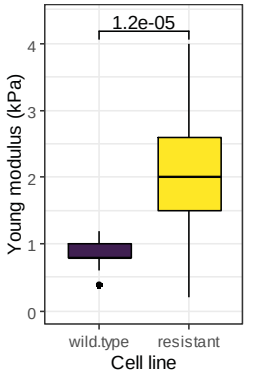


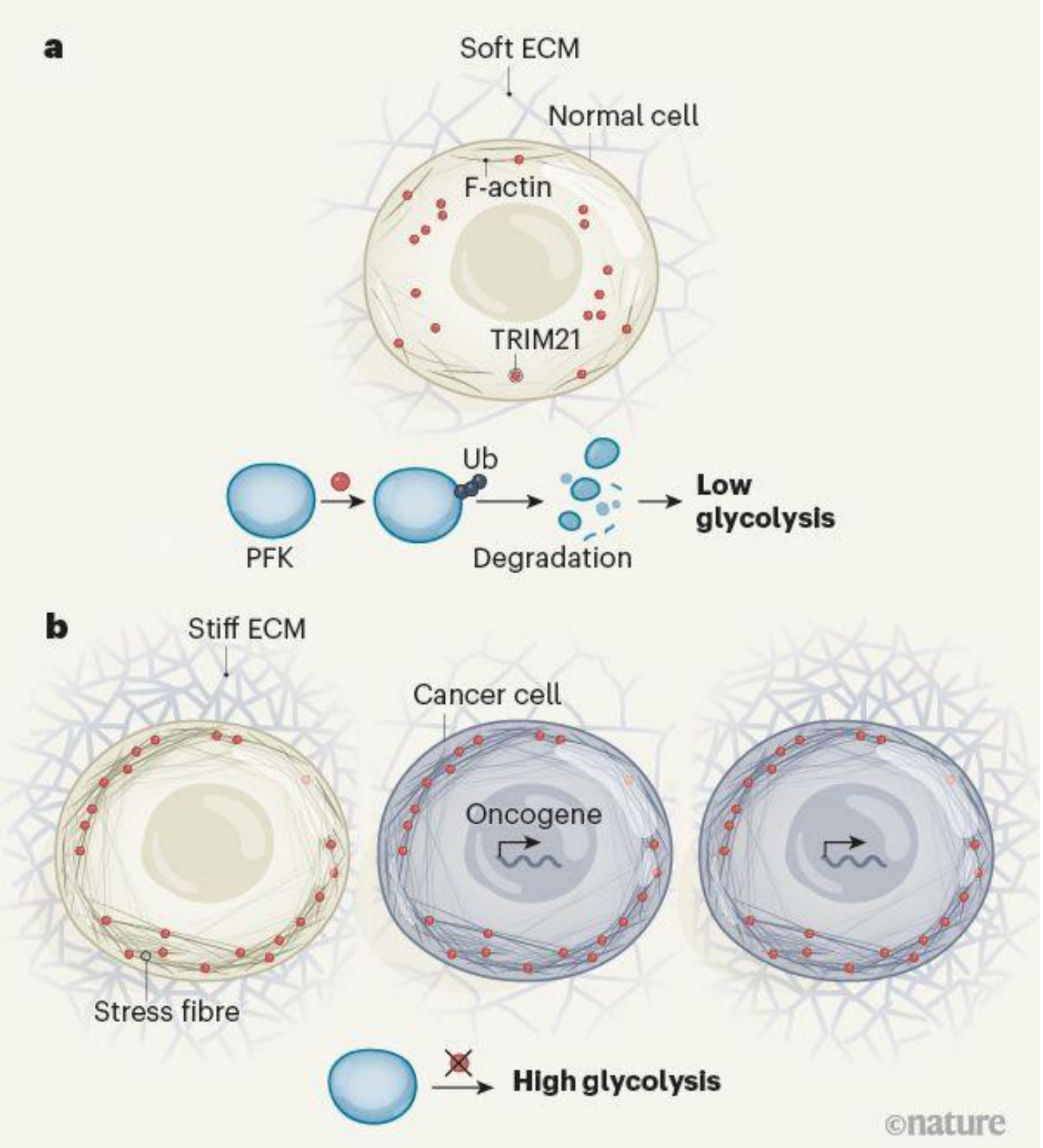
prof. Jochen Guck
MPL Erlangen



AFM, qp-SCONT (0,01 N/m) 5 µm silica sphere, AtomicJ (DMT model)
N=18

Kyoohun Kim
MPL Erlangen



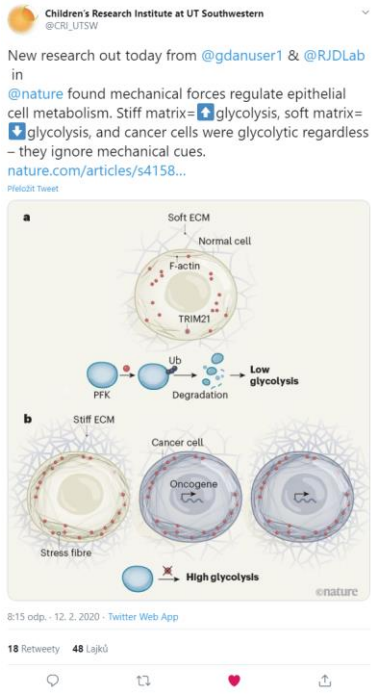


Stiff matrix increase glycolysis, cancer cells regardless stifness

New research out today from @gdanuser1 & @RJDLab in @nature found mechanical forces regulate epithelial cell metabolism. **Stiff matrix ↑glycolysis, soft matrix ↓glycolysis**, and cancer cells were glycolytic regardless – they ignore mechanical cues.
https://nature.com/articles/s41586-020-1998-1nn

https://twitter.com/CRI_UTSW/status/1227672569777664005?s=20

(Park study)



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