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Migration and invasiveness in cancer, cell movement, Epithelial-mesenchymal transition

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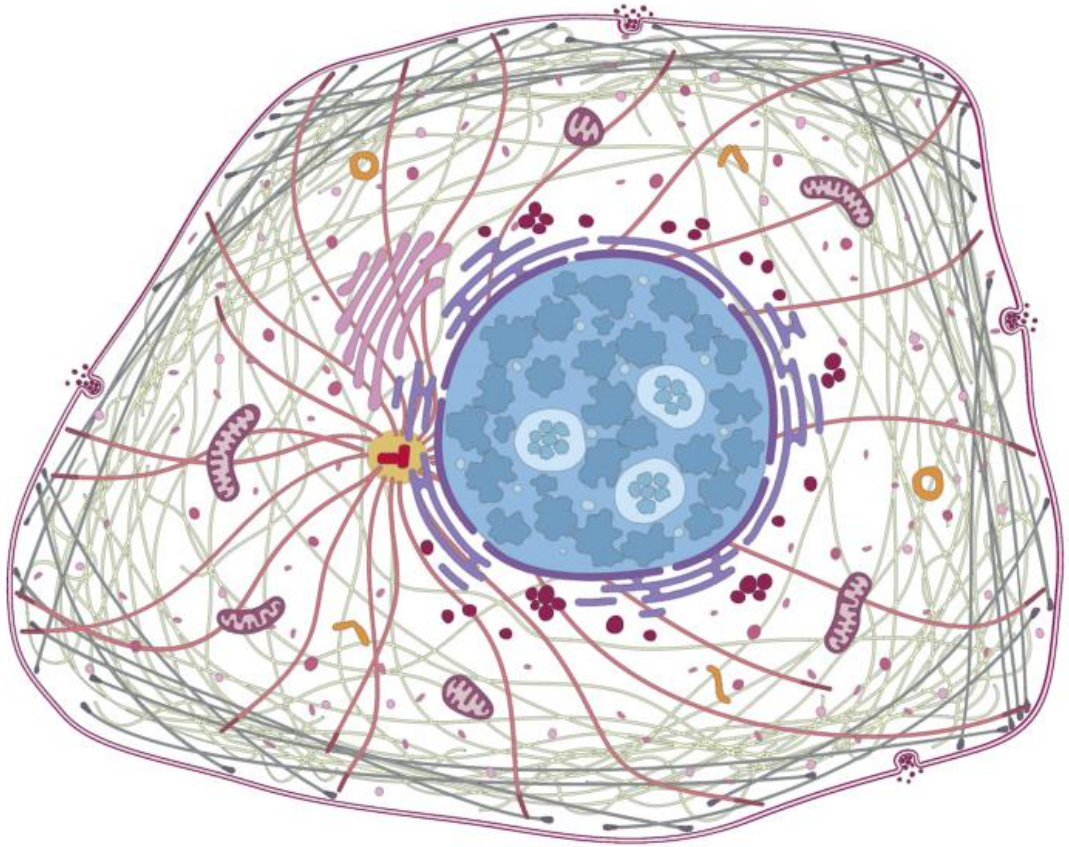
@jarogumulec

Cytoskeleton

- protein fibers that are 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



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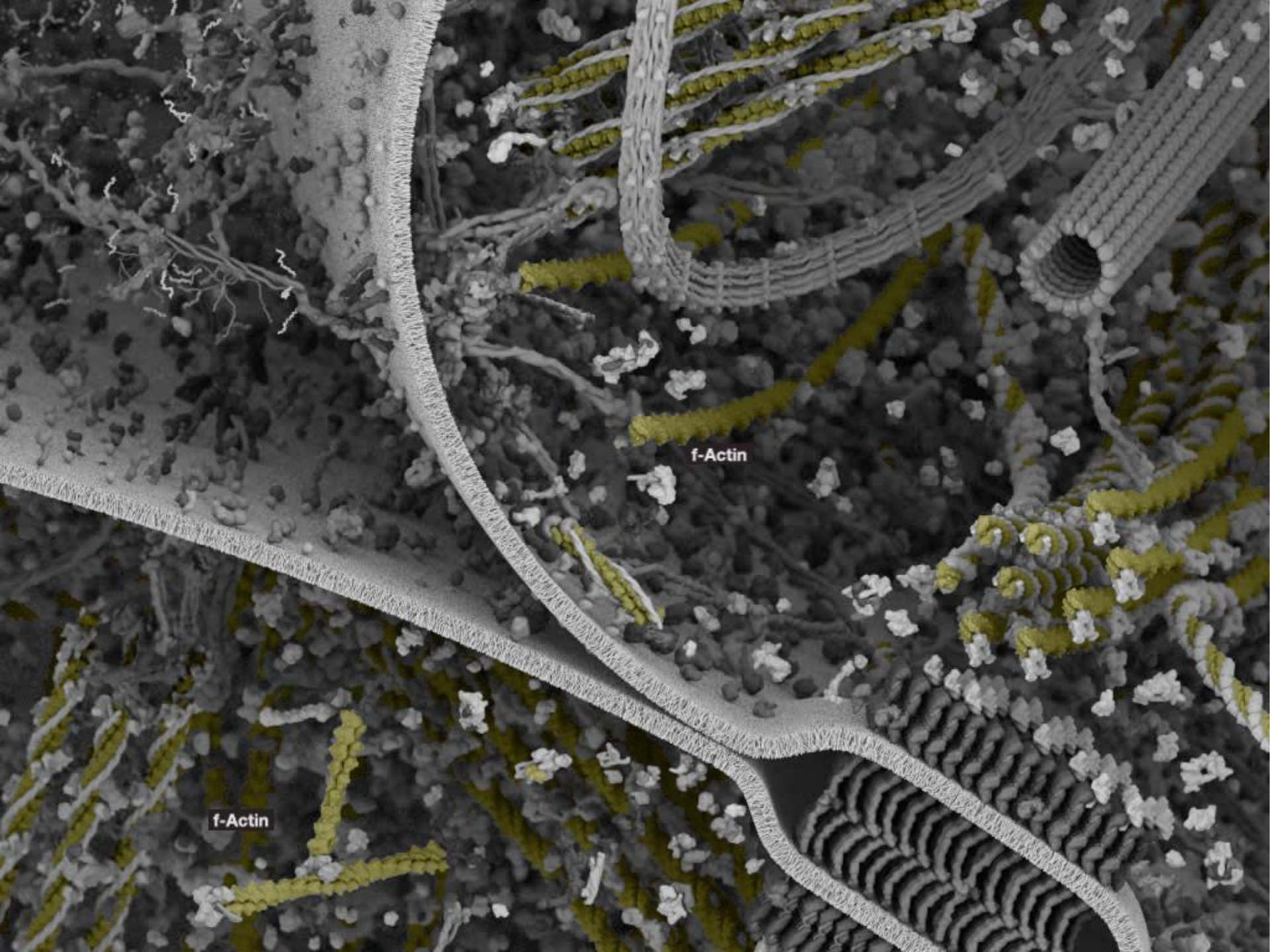

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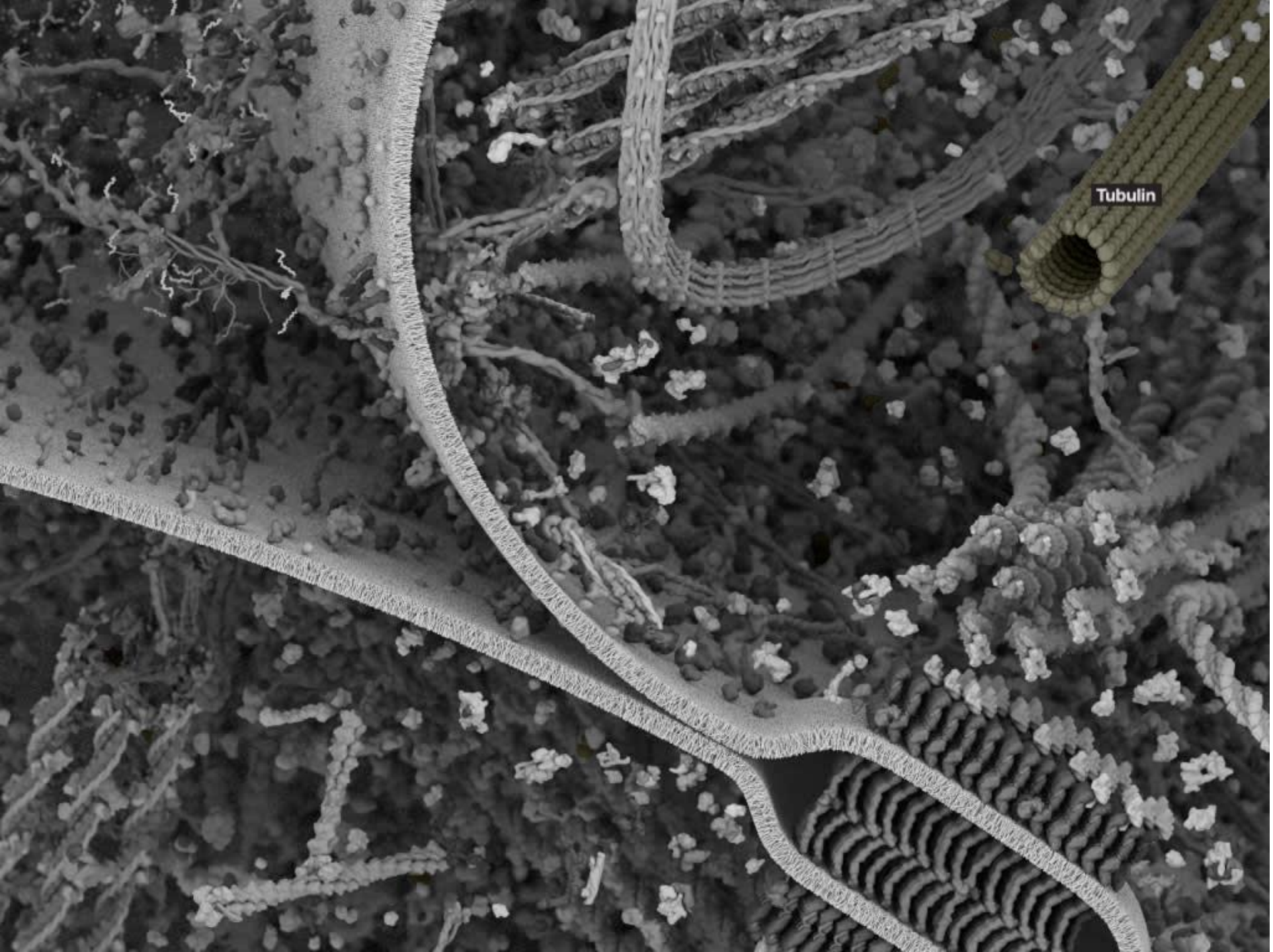

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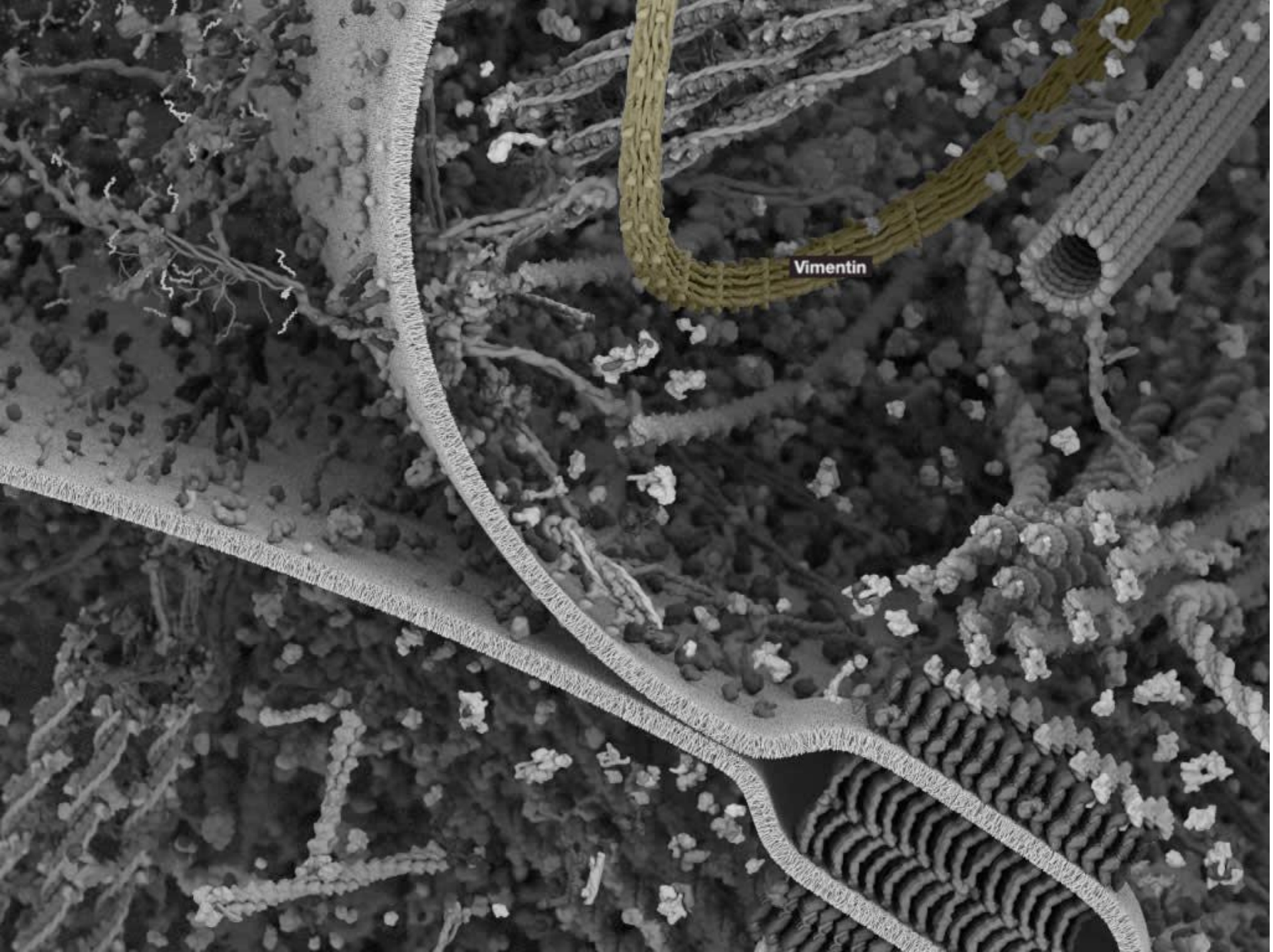

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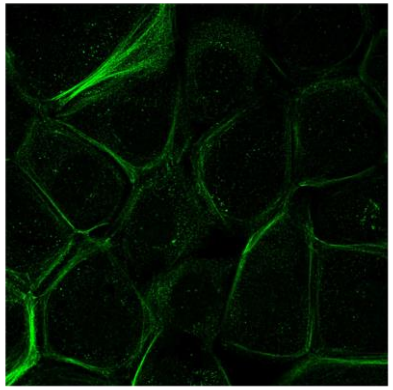
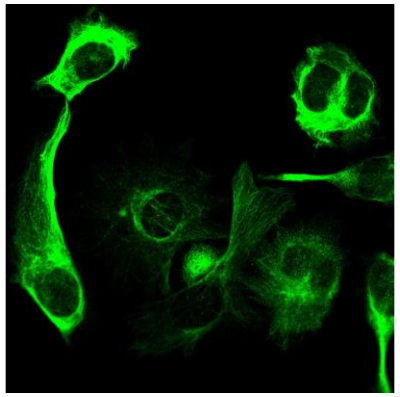
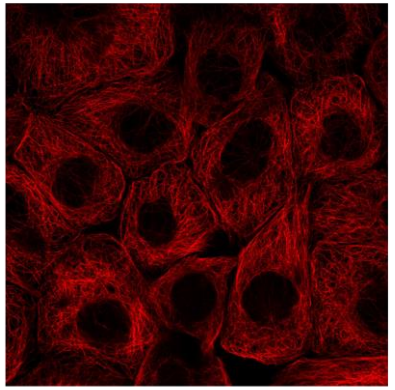
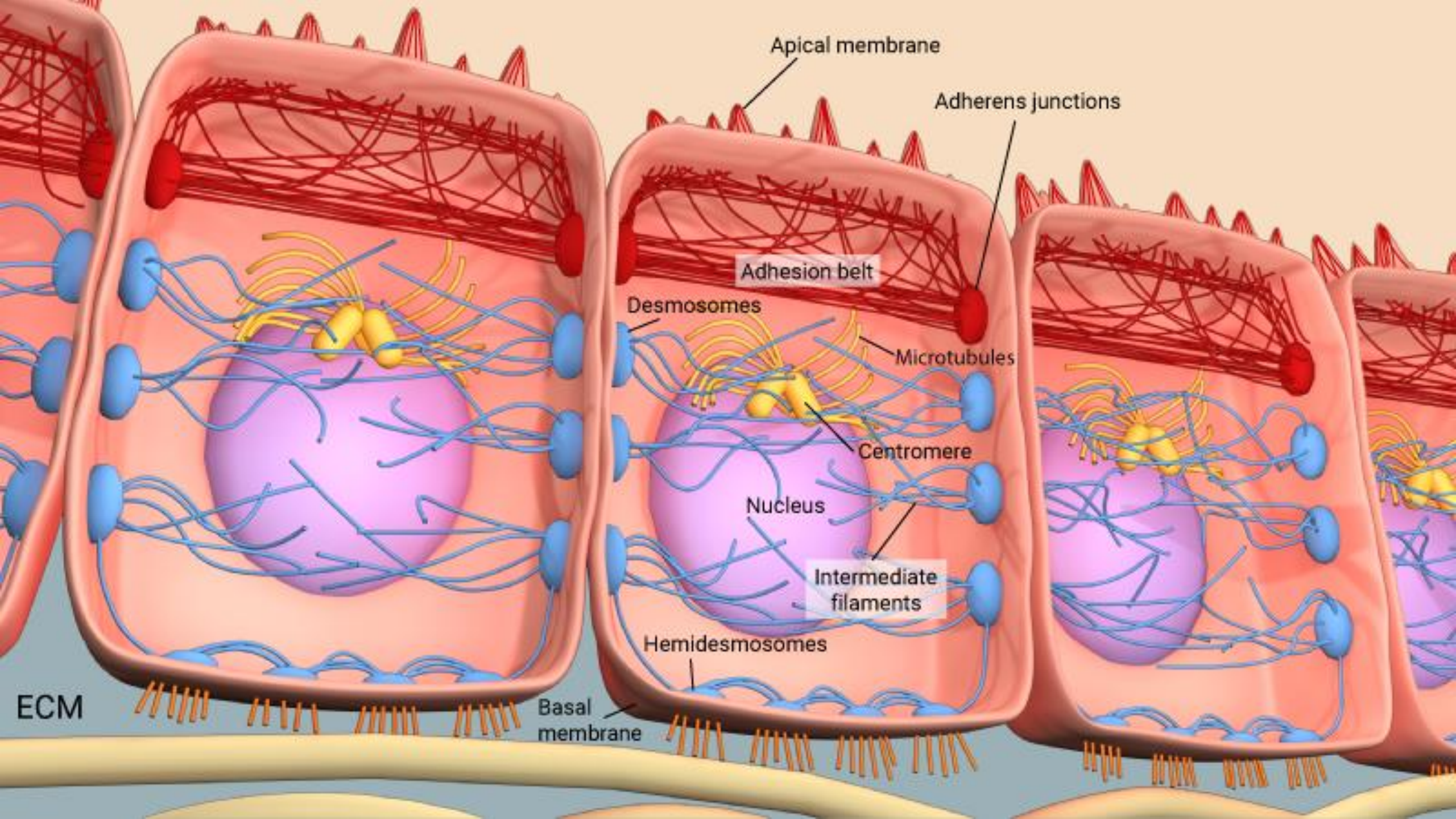
Cytoskeleton in eukaryotic cells

	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
function	structure stabilisation, muscle contraction, cytokinesis, cell movement	mechanical stability, cell-specific	intracel. transport, mitotic spindle

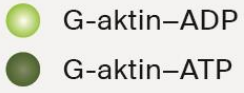


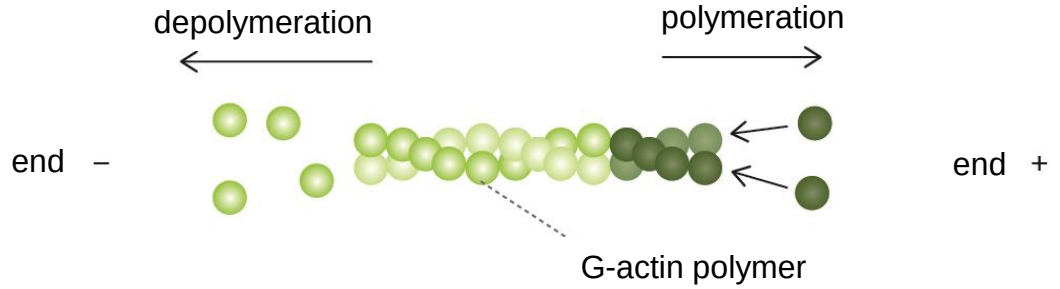




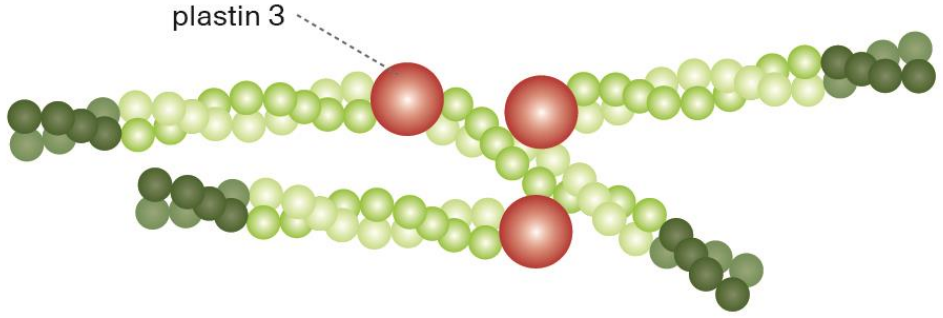

Apical membrane
Adherens junctions
Adhesion belt
Desmosomes
Microtubules
Centromere
Nucleus
Intermediate filaments
Hemidesmosomes
ECM
Basal membrane

Actin filament formation



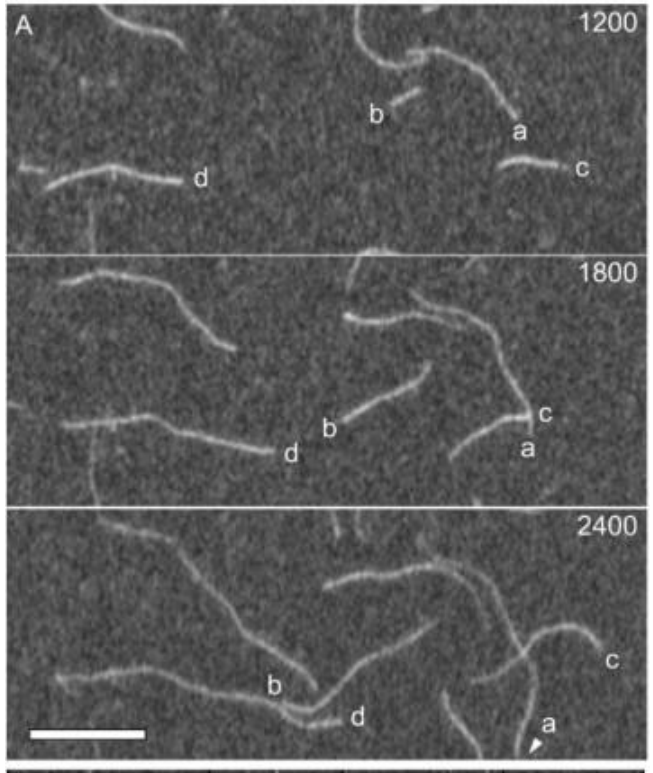


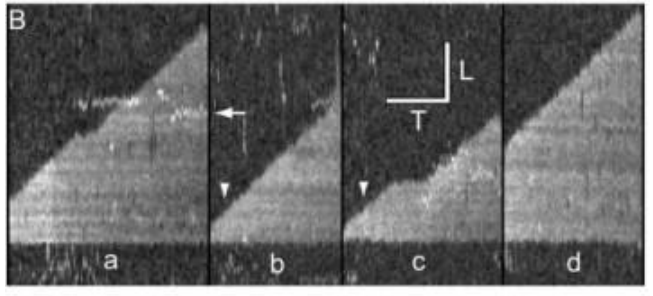
F-Actin networking



Formation and depolymerization of actin filaments.
Like microtubules, actin filaments are dynamically instable

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

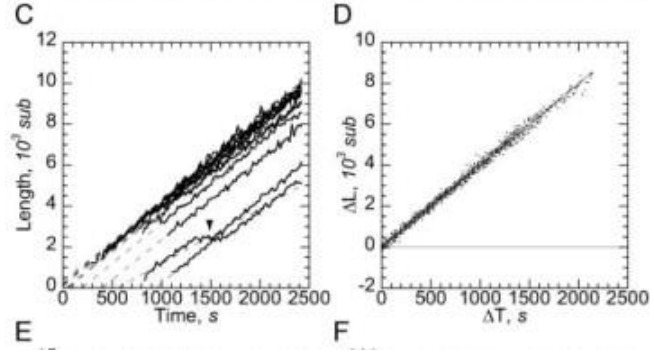




Imaging the time course of the polymerization of ATP-actin.

Length scale bar (*L*) is 10 *μ*m and timescale bar (*T*) is 1000 s.
(*C*) Lengths of 13 filaments as a function of time

https://www.sciencedirect.com/science/article/pii/S0006349505732057



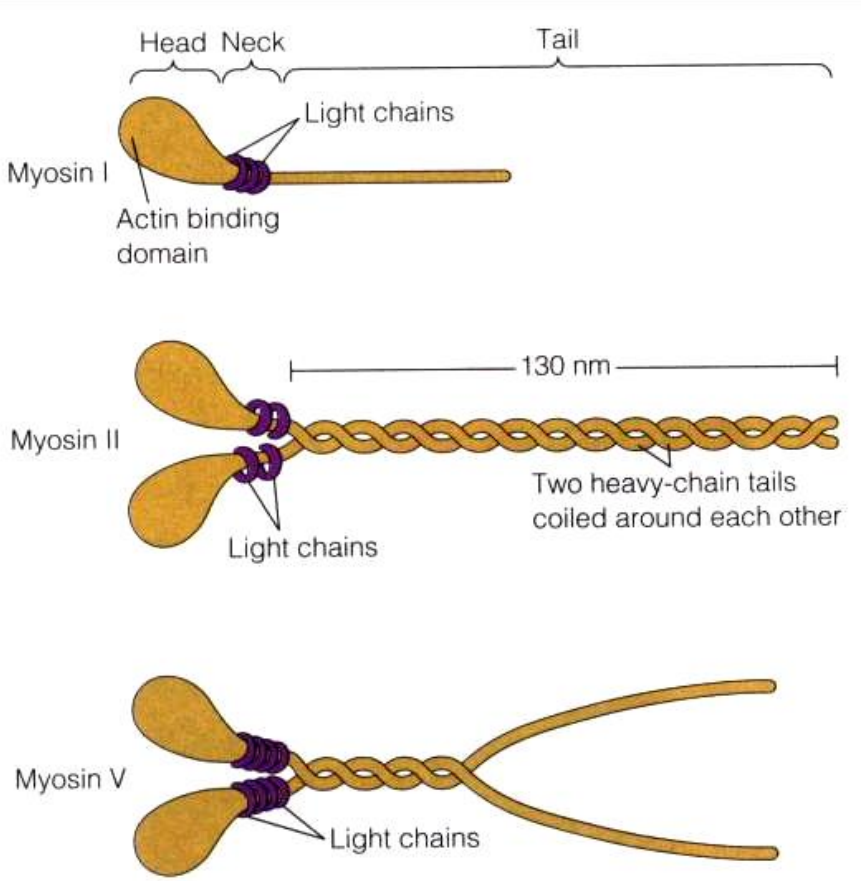
Actin cytoskeleton

- higher order structures – connecting proteins (eg plastin 3)
- polarized, grow on + end (by ATP hydrolysis)

- associated with other protein complexes
- specialized functions in various parts of cells

Myosin motor protein families

- The head is both actin-binding and ATP binding; the purple light chain has a regulatory role. Myosin II is muscle myosin.
- 18 different myosin families have been identified (I –XVIII)



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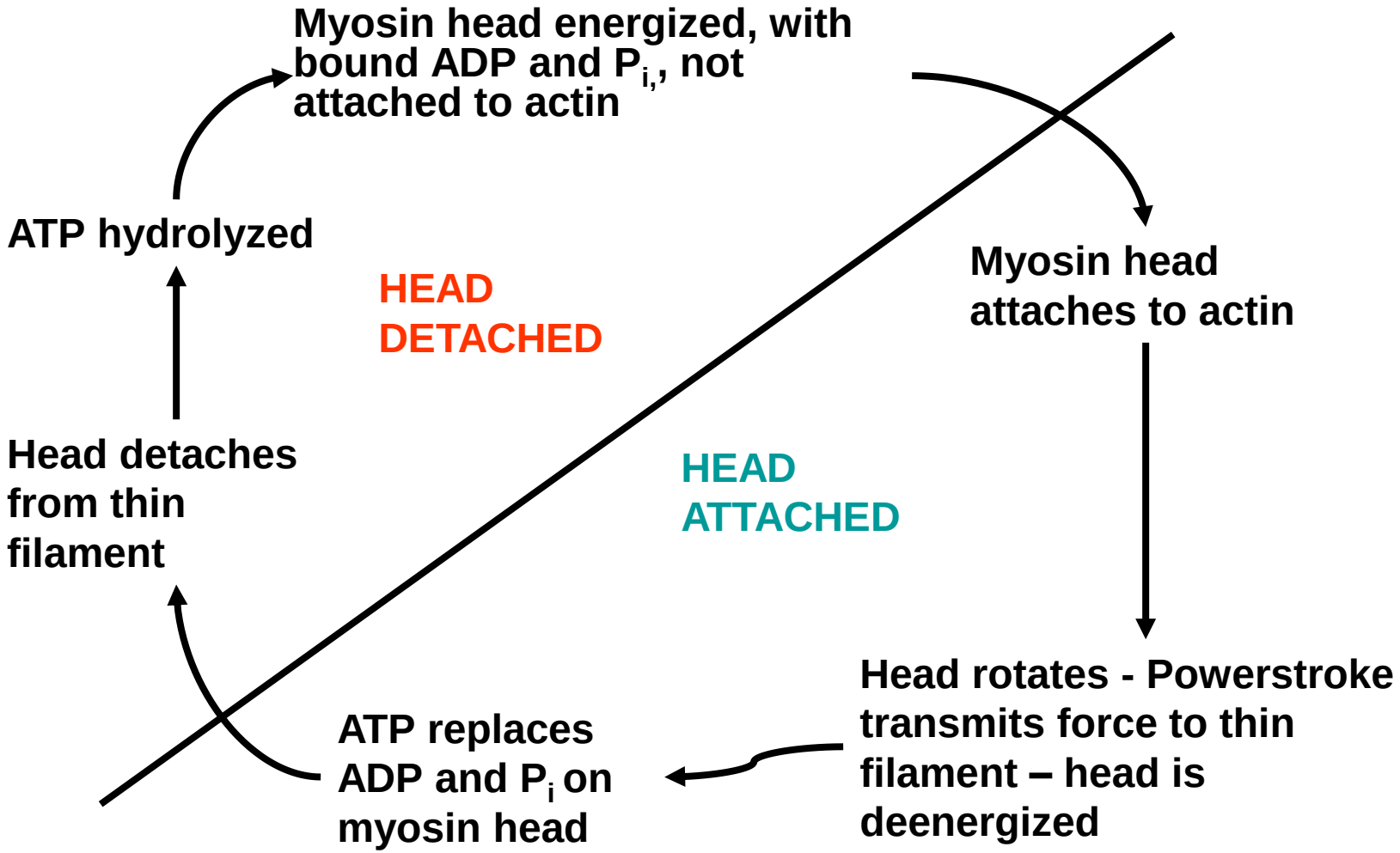
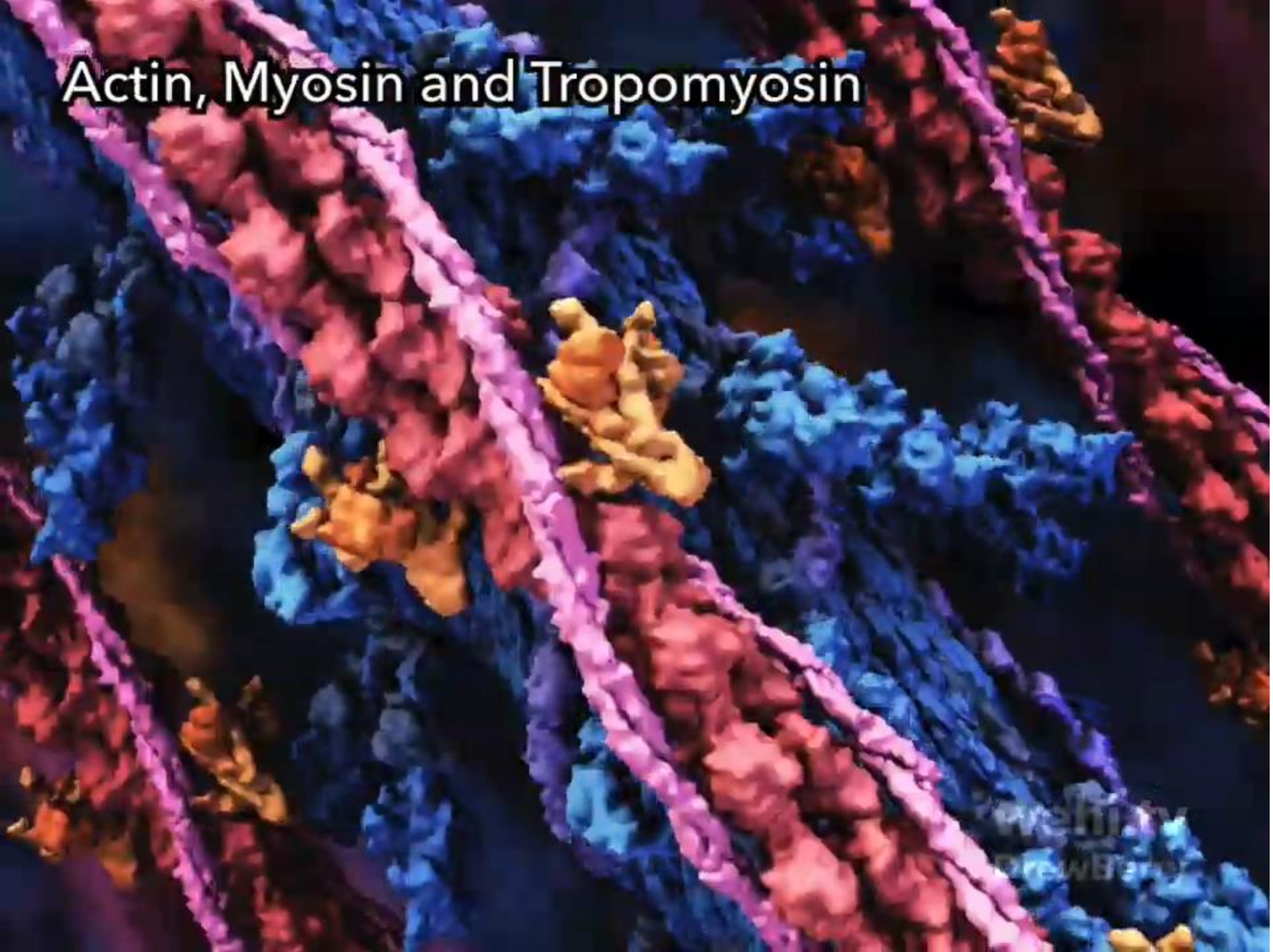

Myosin head energized, with bound ADP and P_i, not attached to actin
ATP hydrolyzed
HEAD DETACHED
Myosin head attaches to actin
Head detaches from thin filament
HEAD ATTACHED
Head rotates - Powerstroke transmits force to thin filament – head is deenergized
ATP replaces ADP and P_i on myosin head



Muscle contraction molecular mechanism.

Actin
Myosin
Tropomyosin
Troponin
Calcium ions

https://twitter.com/drewberryIV/status/1264431344689414146

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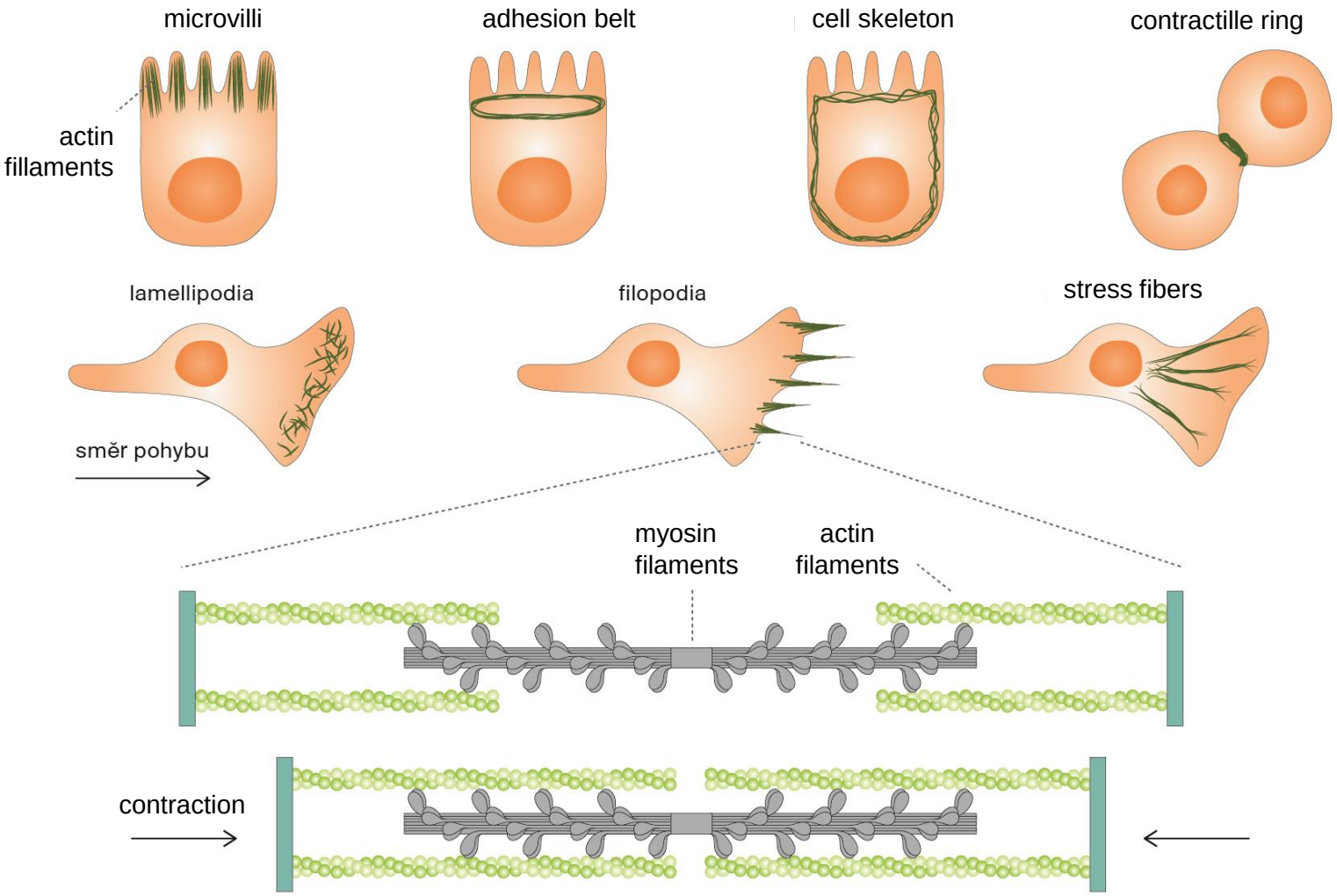

Actin filaments location in cells.

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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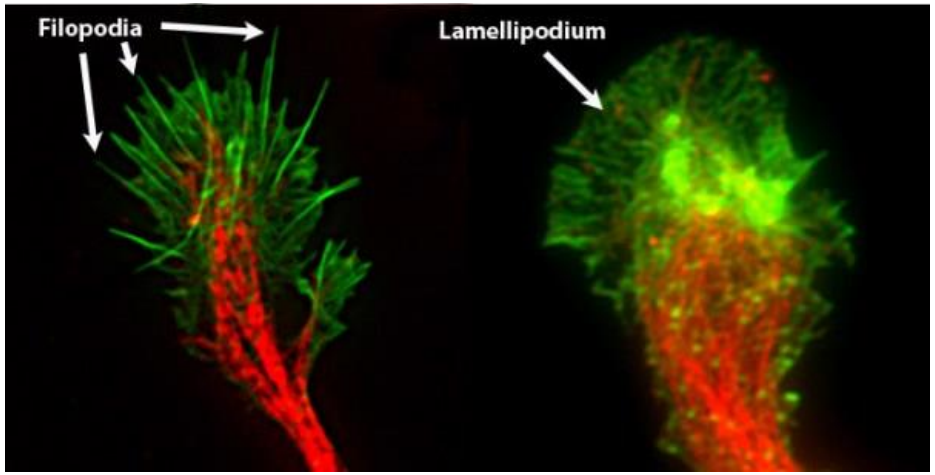
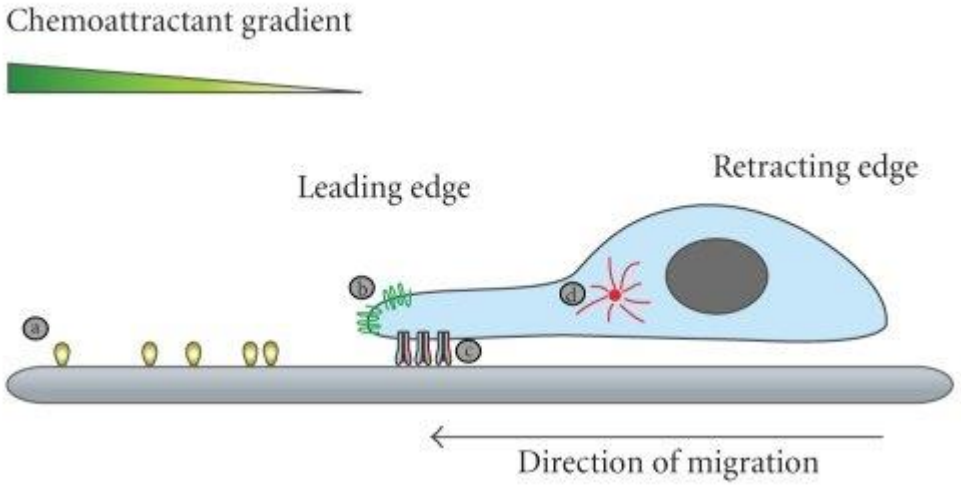
	Fillopodia	Lamellipodia	Stress fibers
morphology	thin protrusions at leading edge	veil-like cytoplasm extension	actin bundles in cells
regulation		↓ RhoA + ROCK	↑ RhoA + ROCK
function	environment probing modulation of adhesion	migration in 2D and 3D	cellular contractility, force for adhesion, migration



Leading edge in migrating cell



- Formation of polarised cells:
  - change its morphology + intracellular organization.
- and integrins forming focal adhesions, localise to the leading edge.

Ngalim 2010 10.1155/2010/363106

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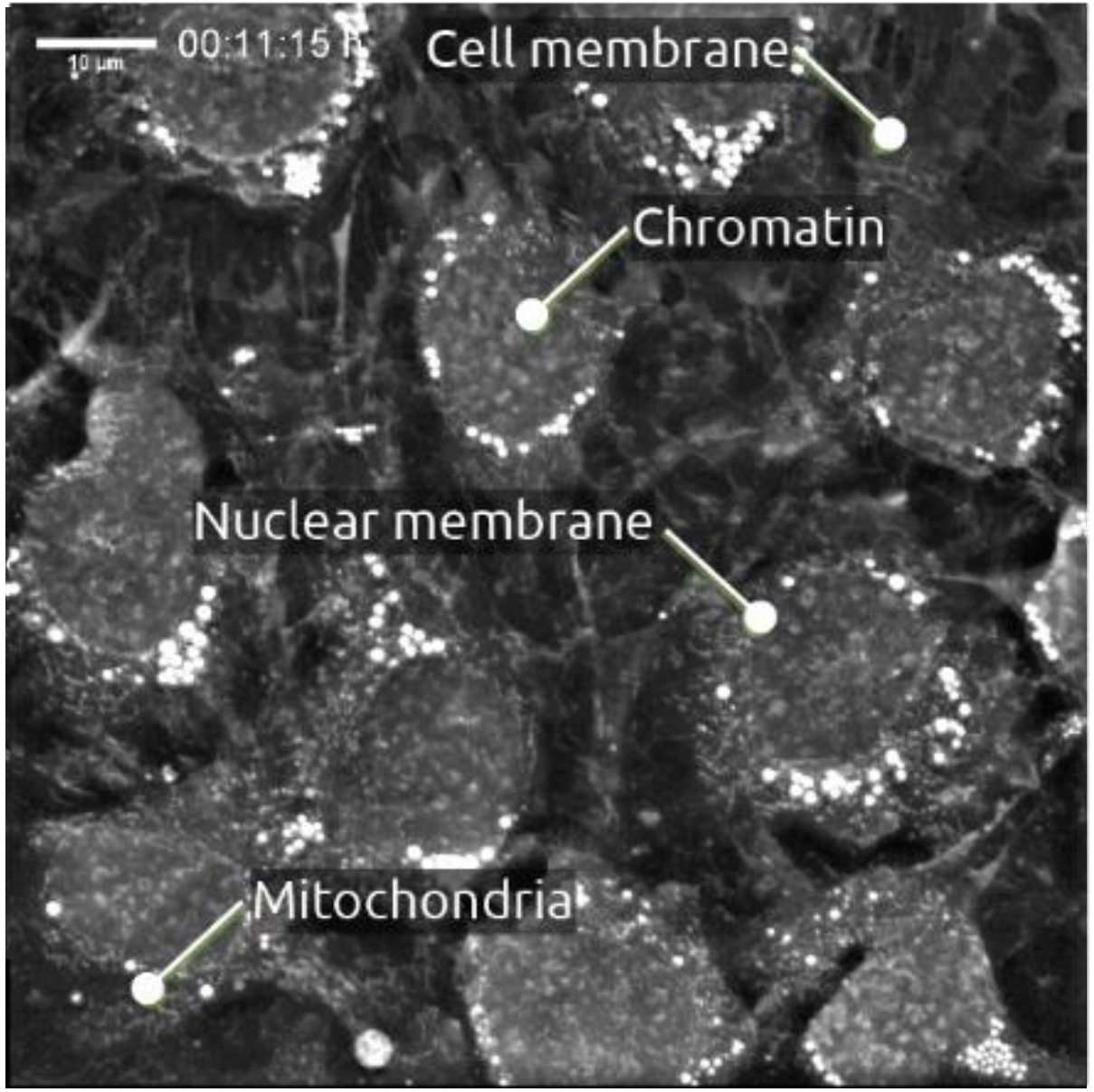
BEFORE FIXATION (0.2% PFA)



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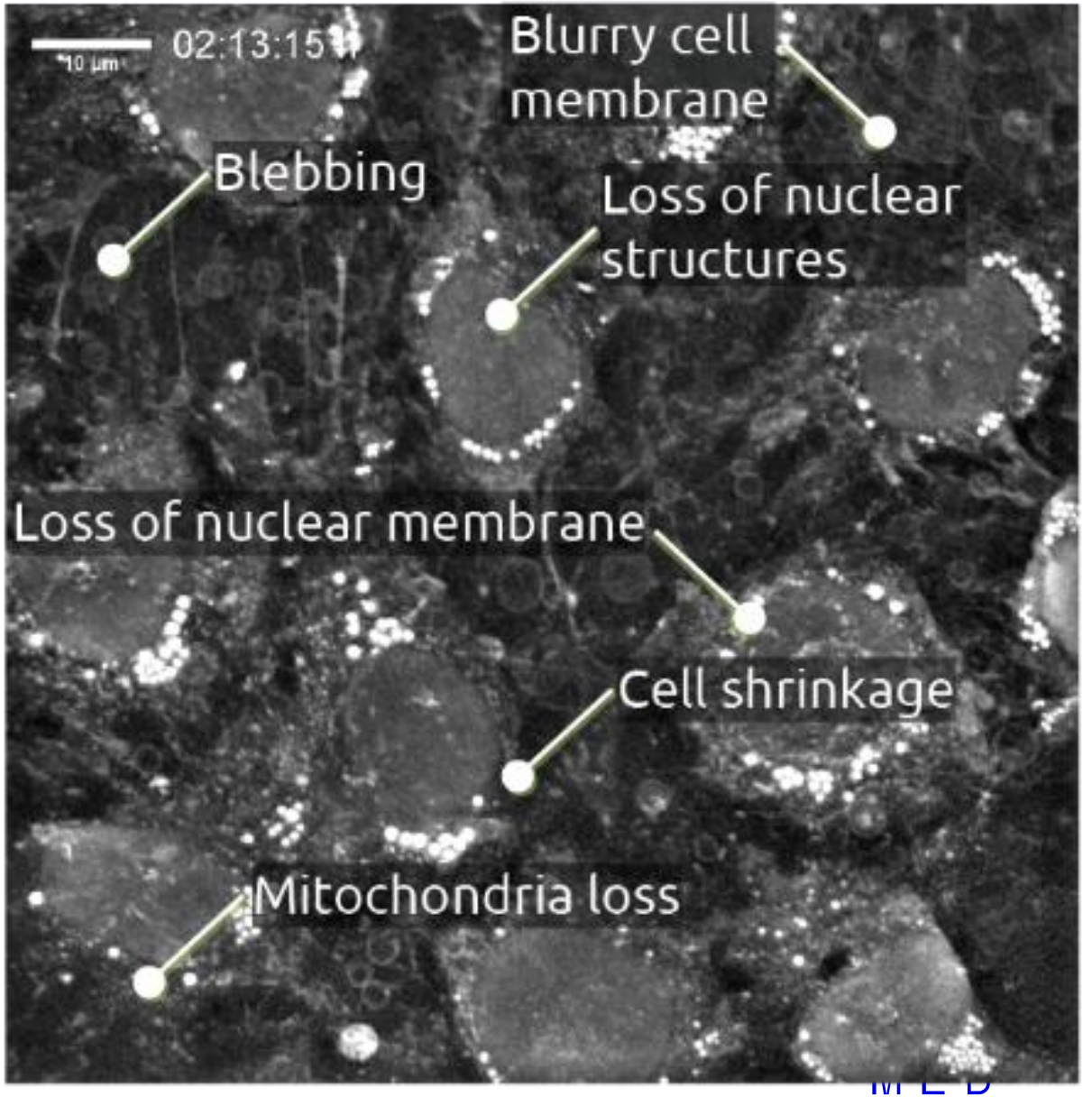

Cytoskeleton is a highly dynamic structure

Am I the only one who likes to watch the #LeadingEdge all day long? The orange bundles make #RetrogradeFlow easy to follow. #Actin assembly
@VUCellImaging
@VUBasicSciences
#VandyCytoskeleton

https://twitter.com/i/status/1251982170421428224

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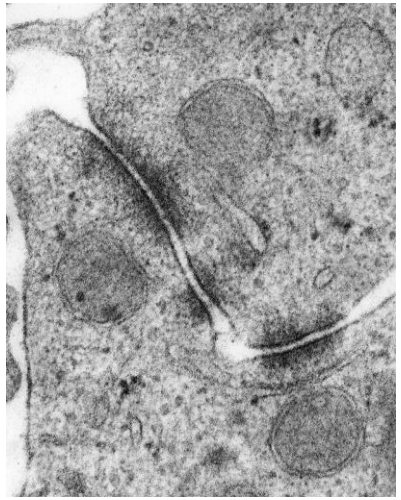
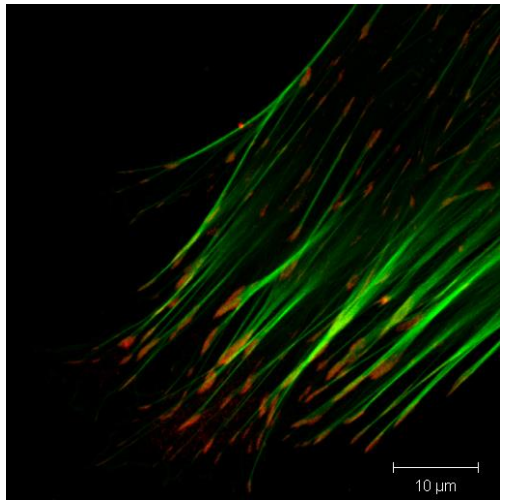
F-actin associated structures

	Adherens junctions	focal adhesions
associated protein	cadherins (α- / β-catenin) + others	integrins + 200 others
function	cell-cell adhesion	cell-ECM adhesion, mechanotransduction, migration





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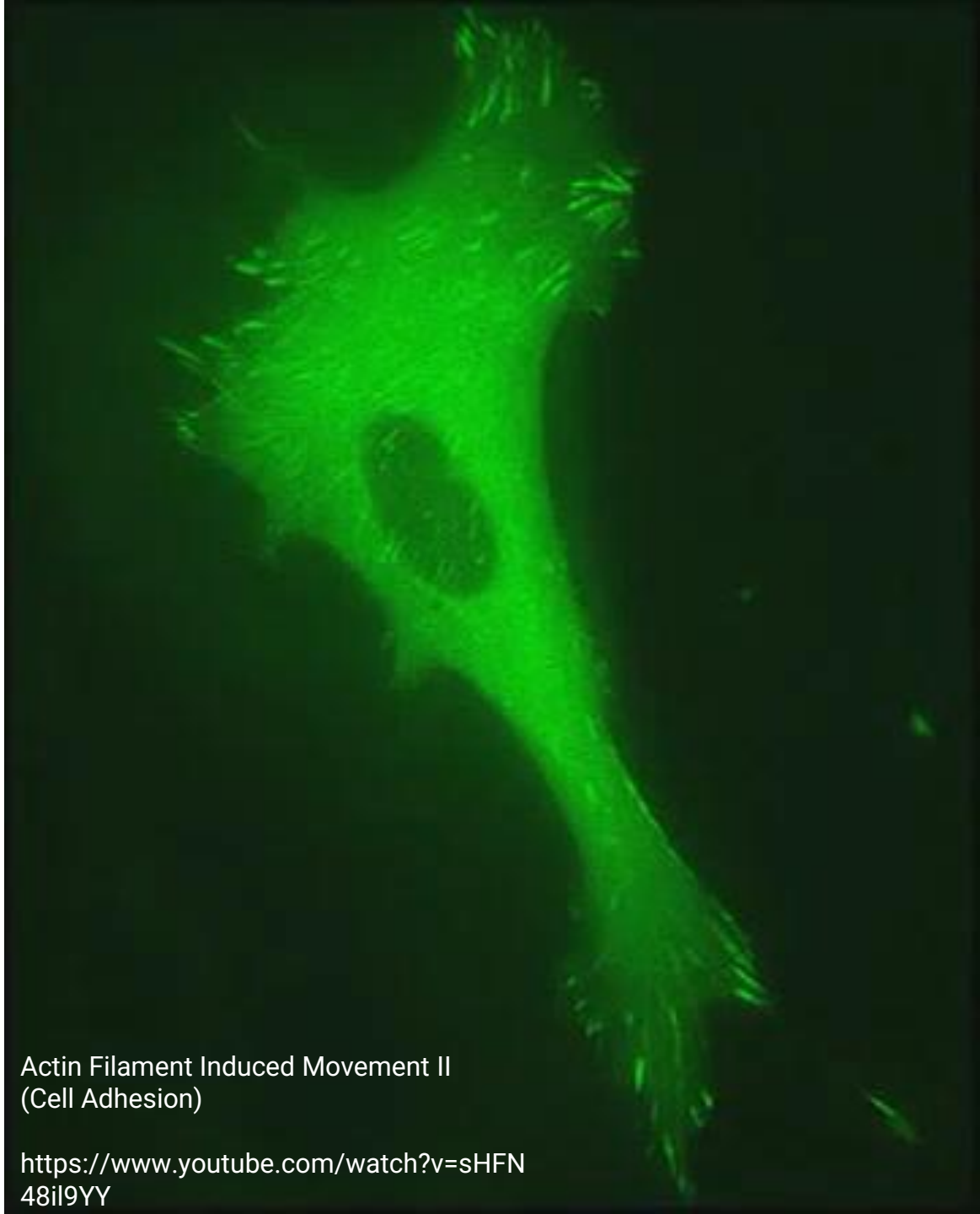
Focal adhesions

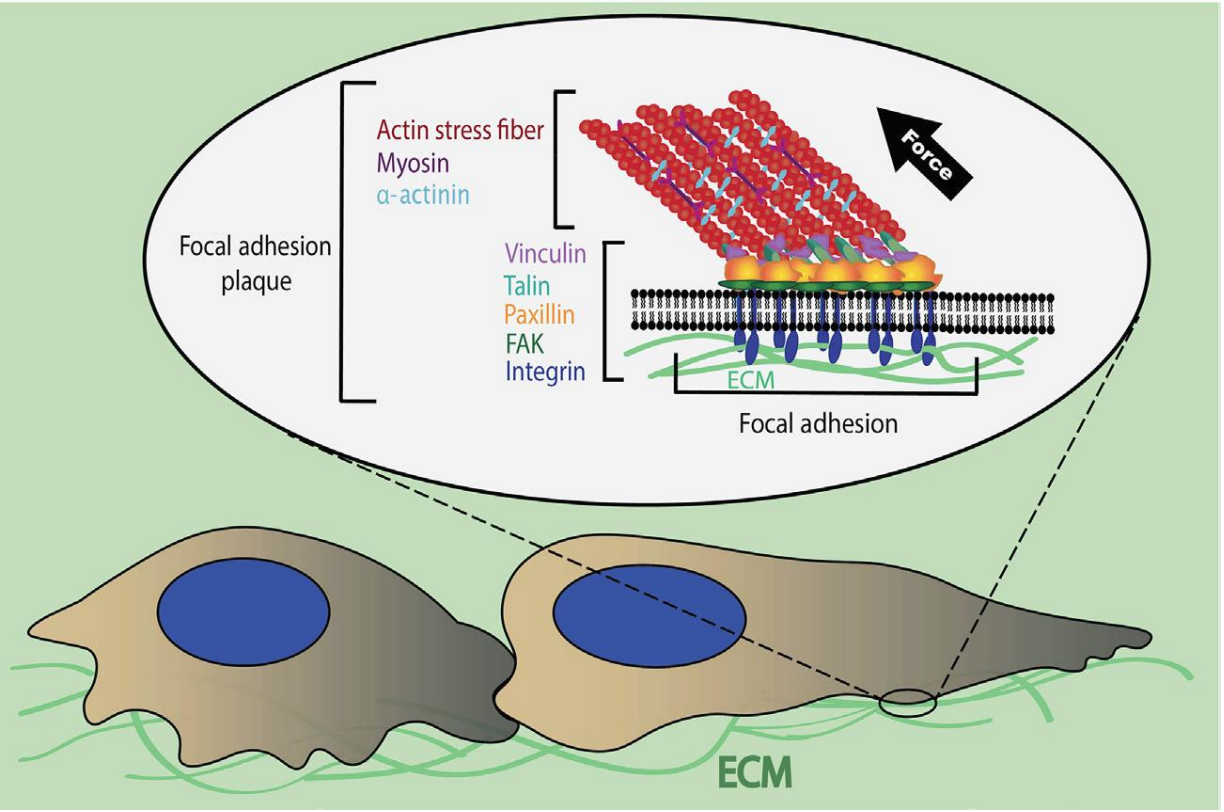
- connection between a cell's cytoskeleton and ECM.
- sub-cellular structures that mediate the regulatory effects of a cell in response to **ECM adhesion**
- in a state of **constant flux**: proteins associate and disassociate with it continually as signals are transmitted to other parts of the cell, relating to anything from cell motility to cell cycle
- **contact with ECM via integrins**: integrins bind to extra-cellular proteins via short amino acid sequences
- white blood cells migrate along the connective endothelium



Actin Filament Induced Movement II (Cell Adhesion)

https://www.youtube.com/watch?v=sHFN48il9YY

Four layers of Focal adhesions



- **integrin extracellular layer**,
  - responsible for binding to the ECM.
  - extracellular domain **"outside-in**" signaling: integrin-ECM binding changes in intracellular signaling.
  - cytoplasmic domain "**outside-in**" signalling: intracellular signaling affect extracell integrin binding.
- **integrin signaling layer**
- **force transduction layer**

  FA stabilization and mechanosensitive signaling via focal adhesion kinase (FAK), paxillin, talin, vinculin.
- **actin regulatory layer:** the regulation of actin assembly, disassembly, and actomyosin contractility

 2017 Collins http://dx.doi.org/10.1016/B978-0-12-803394-4.00001-X

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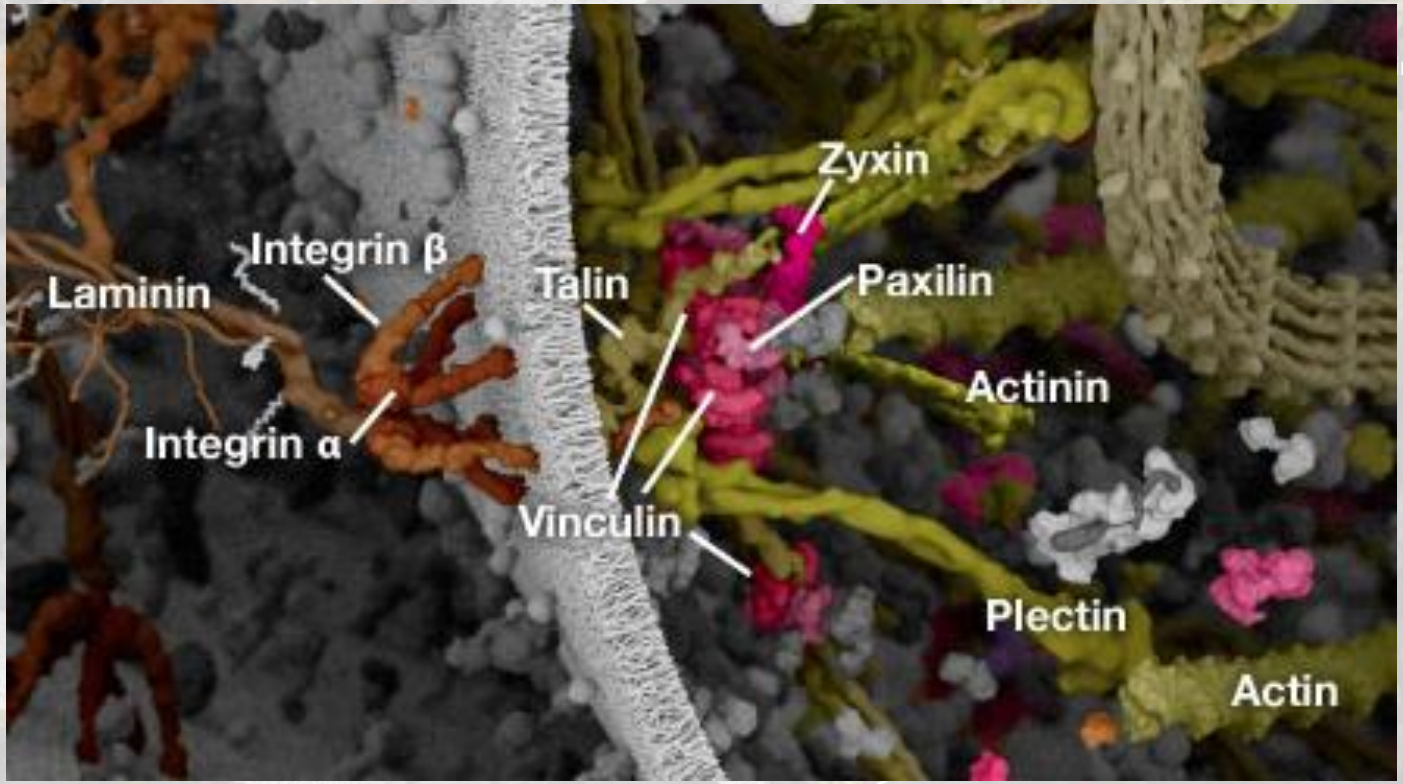

integrin extracellular layer,

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integrin signaling layer

force transduction layer

FA stabilization and mechanosensitive signaling via focal adhesion kinase (FAK), paxillin, talin, vinculin.

actin regulatory layer: the regulation of actin assembly, disassembly, and actomyosin contractility

Plectin
Actin
Cofilin
Band 4.1
Spectrin
Fascin
Glycophorin D
Actin
Ankyrin-1
Band 3
mgtE
Actinin
Catenin-α
Catenin-β
Catenin-δ
Myosin H11
Myosin L6
Myosin L2
Cortactin
n-Cadherin
Arp2/3

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

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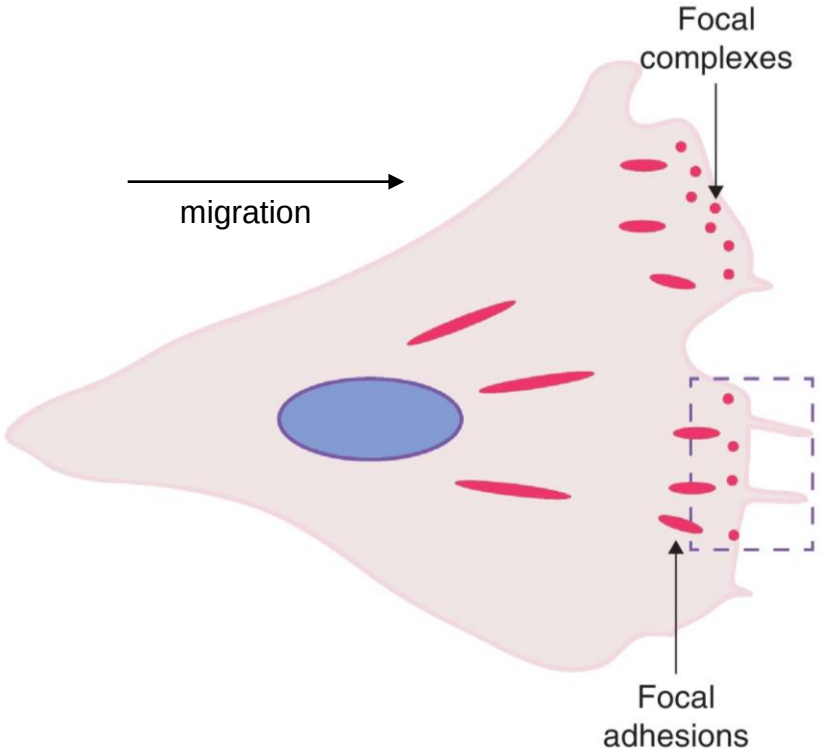

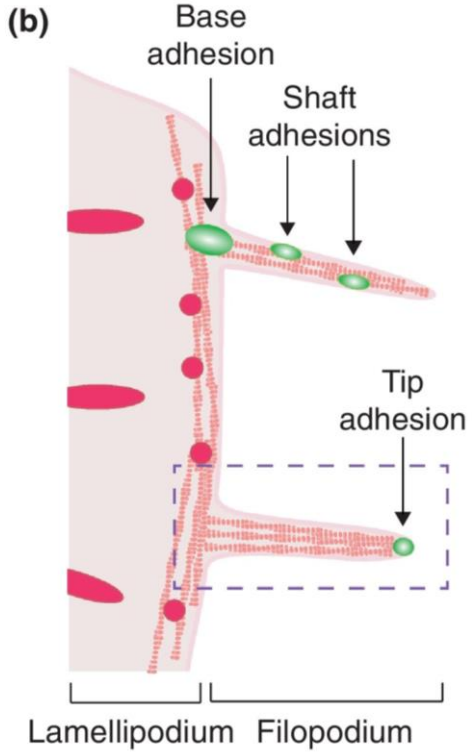

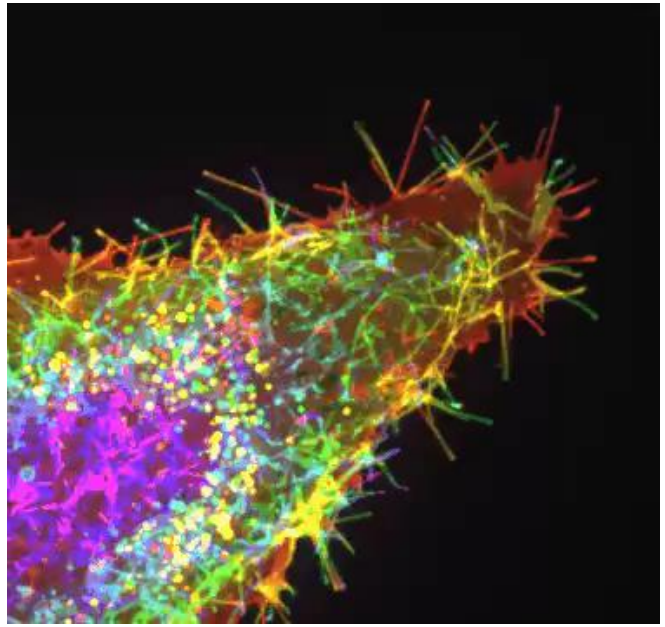

Filopodia during cell migration as "mechanical antennae“

- probing ECM stiffness
- Fillopodia function as signaling platforms:
  - probing ECM topography
  - probing ECM stiffness
  - at the leading edge regulates Arp2/3-mediated actin remodeling and polymerization to drive lamellipodia membrane protrusions and forward cellular movement.

Filopodia probe the ECM by assembling specialized adhesion complexes at specific sub-filopodial locations

Fillopodia

A spinning disk is a device that enables high signal:noise fluorescence microscopy by eliminating out of focus light.

https://twitter.com/TyskaLabActual/status/1277592591882768385

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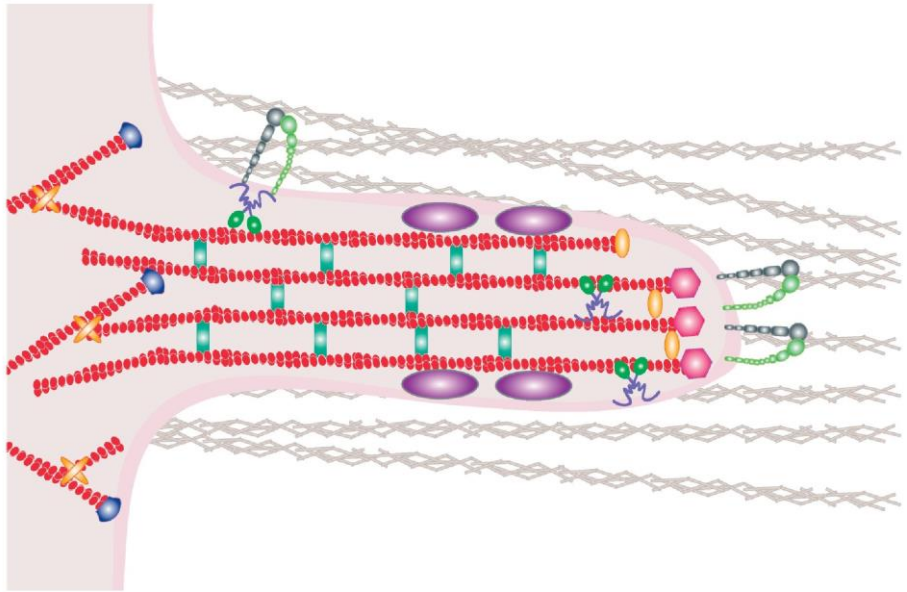
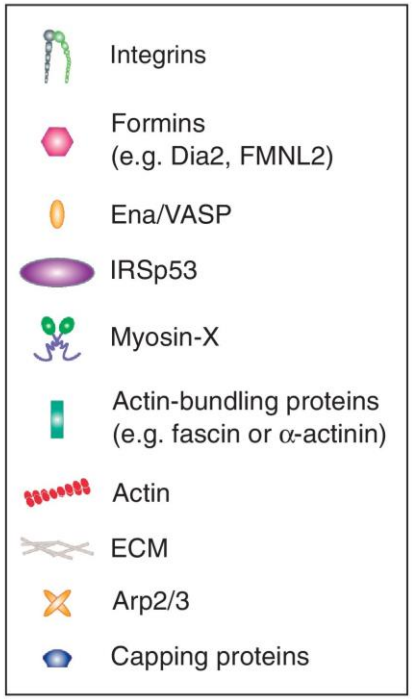
Fillopodia





Filopodia during cell migration

actin polymeration = strenght generated for fillopodia and lamellipodia growth

fillopodia formation preceeds lamellipodia formation

formation facilitated by

- insulin-receptor substrate p53 (IRSp53) and others: **deform and/or tubulate the plasma membrane**, and
- by motor activity **of myosin-X -** actin fiber convergence at the cell periphery.

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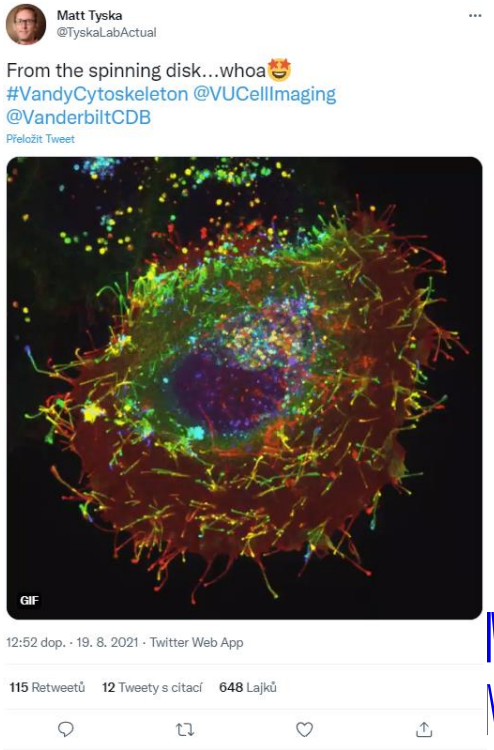
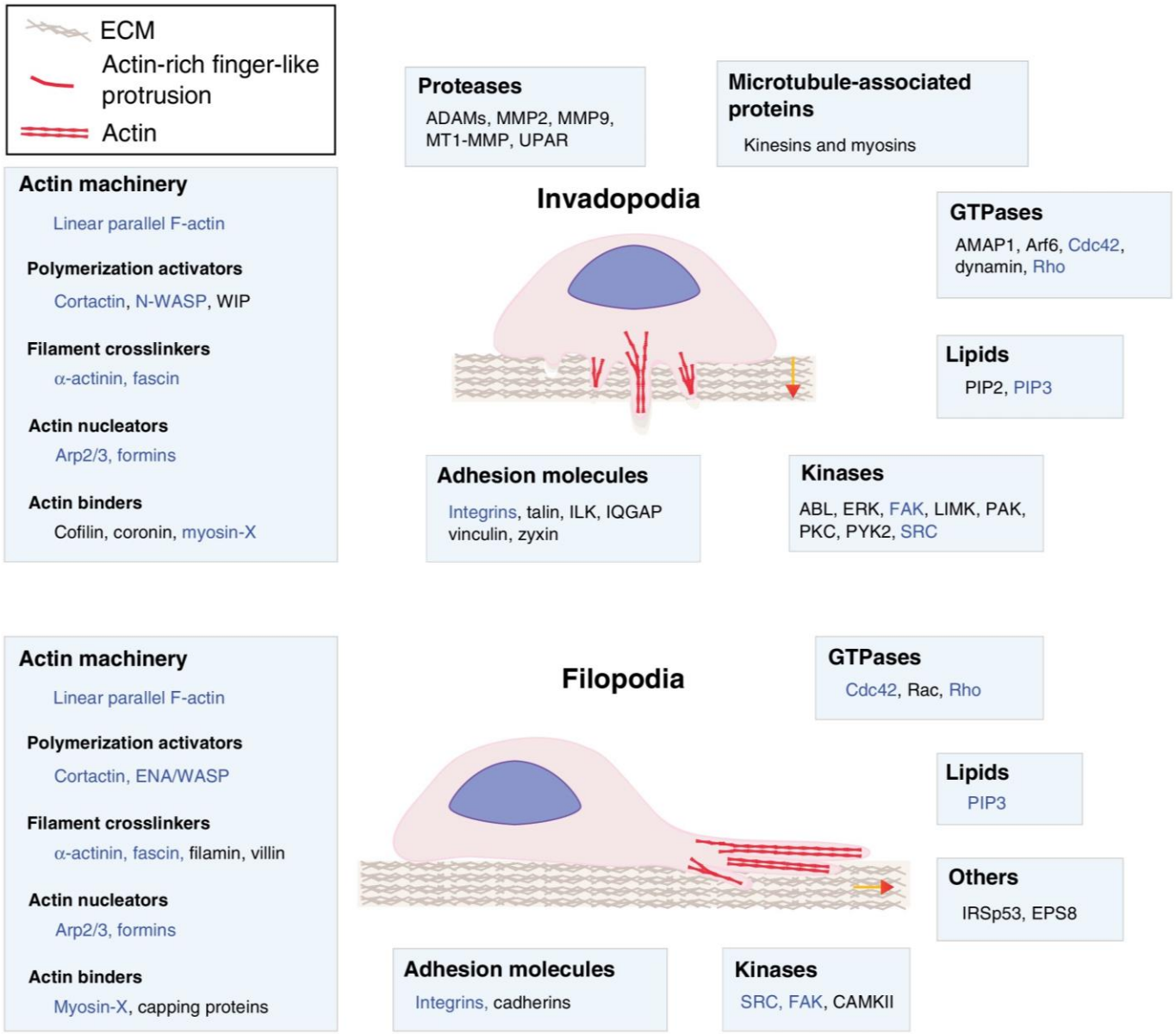
Fillopodia

GFP-stained transmembrane protein, colors code for depth, spining disk miscoscopy

https://twitter.com/TyskaLabActual/status/1428127612497317892





Filopodia, invadopodia and filopodia-like structures.

In 2D, cells form well-defined finger-like, actin-rich structures including filopodia and invadopodia.

Filopodia
transient and extend out from the advancing lamellipodium
associated with myosin-X and fascin,

Invadopodia
actin-rich, more stable, localize **beneath** the cell body
possess **substrate degradation** properties.
assoc: cortactin and the ECM-degrading protease MT1-MMP
spaces used for consequent **migration**

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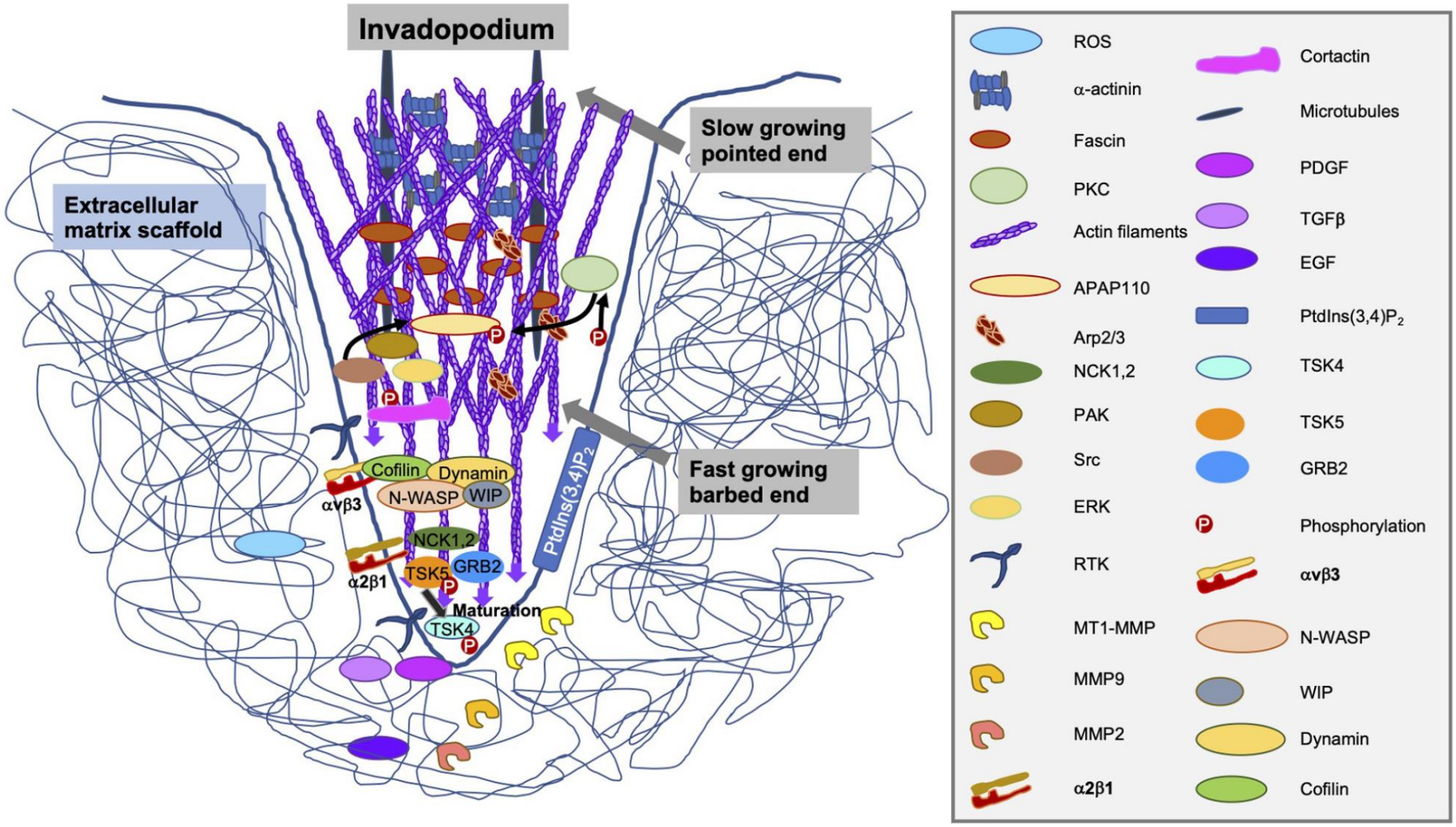

Structure, components and secreted enzymes of an invadopodium

Mierke, 2020, https://doi.org/10.3389/fcell.2020.583226

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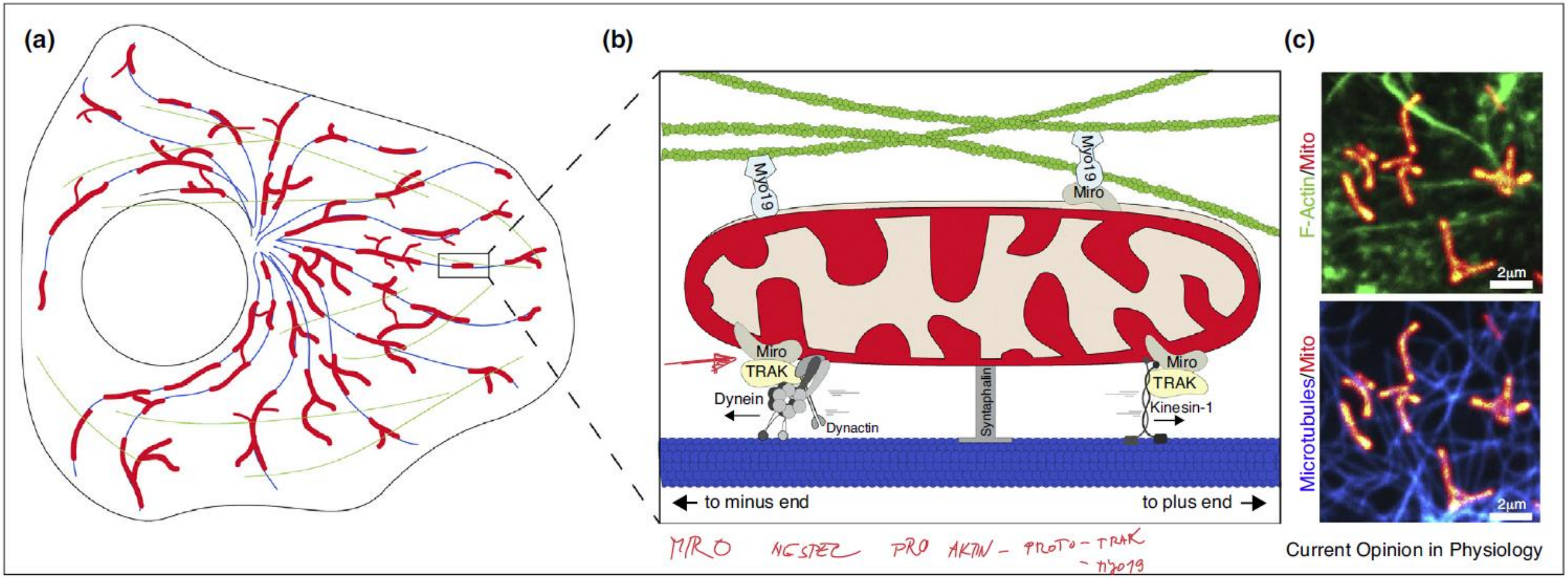
Tubulin, actin and mitochondria anchoring



Schematic of mitochondria (red), microtubules (blue), and f-actin (green) distribution in an undifferentiated cell. (b) Mitochondria associate with microtubules (blue, bottom) and with actin (green, top) via motor/adaptor complexes. Dynein/dynactin associate with mitochondria via TRAK and Miro to drive retrograde mitochondrial motility. In contrast, Kinesin-1 coordinates anterograde motility toward the cell periphery. Myo19 can associate with the mitochondria outer membrane either directly or through Miro. Syntaphilin anchors mitochondria to microtubules.
https://doi.org/10.1016/j.cophys.2018.03.003

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Extracellular matrix, 2D vs 3D

- in 2D use actin polymerisation to extend leading edge
- in 3D this is just one of the movement strategies.
- migration modes (in 3D) dependent on physical properties of ECM
- ECM properties distinguished by **leading edge** cells
- single cell can switch between leading edge structures

 Petrie 2012 10.1242/jcs.093732

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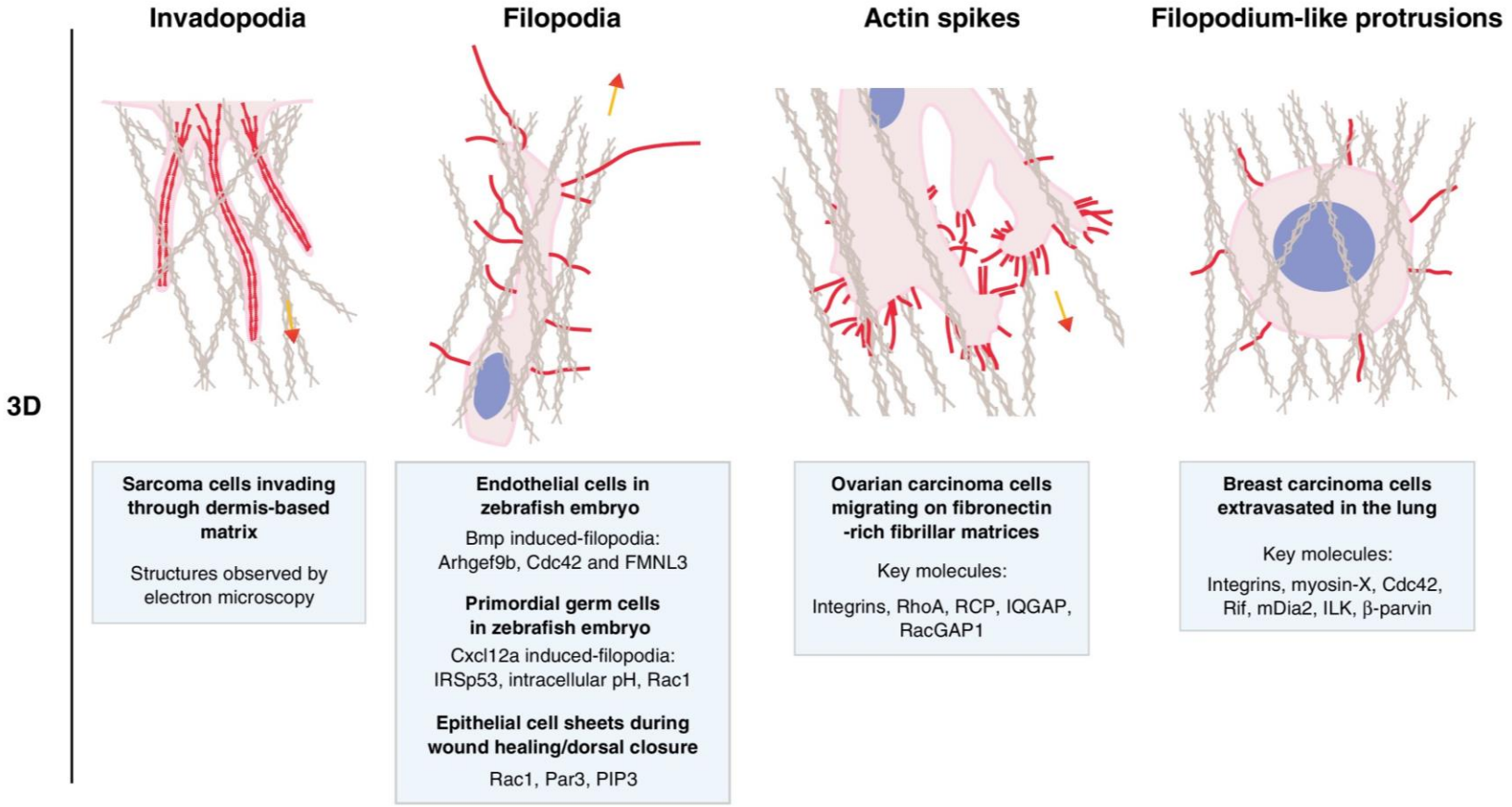

Filopodia, invadopodia and filopodia-like structures.

In 3D and *in vivo*,
cells form **filopodia-like protrusions**, (lack of clear classification criteria)

filopodia, invadopodia, filopodium-like protrusions and actin spikes.

molecular machinery associated is poorly understood

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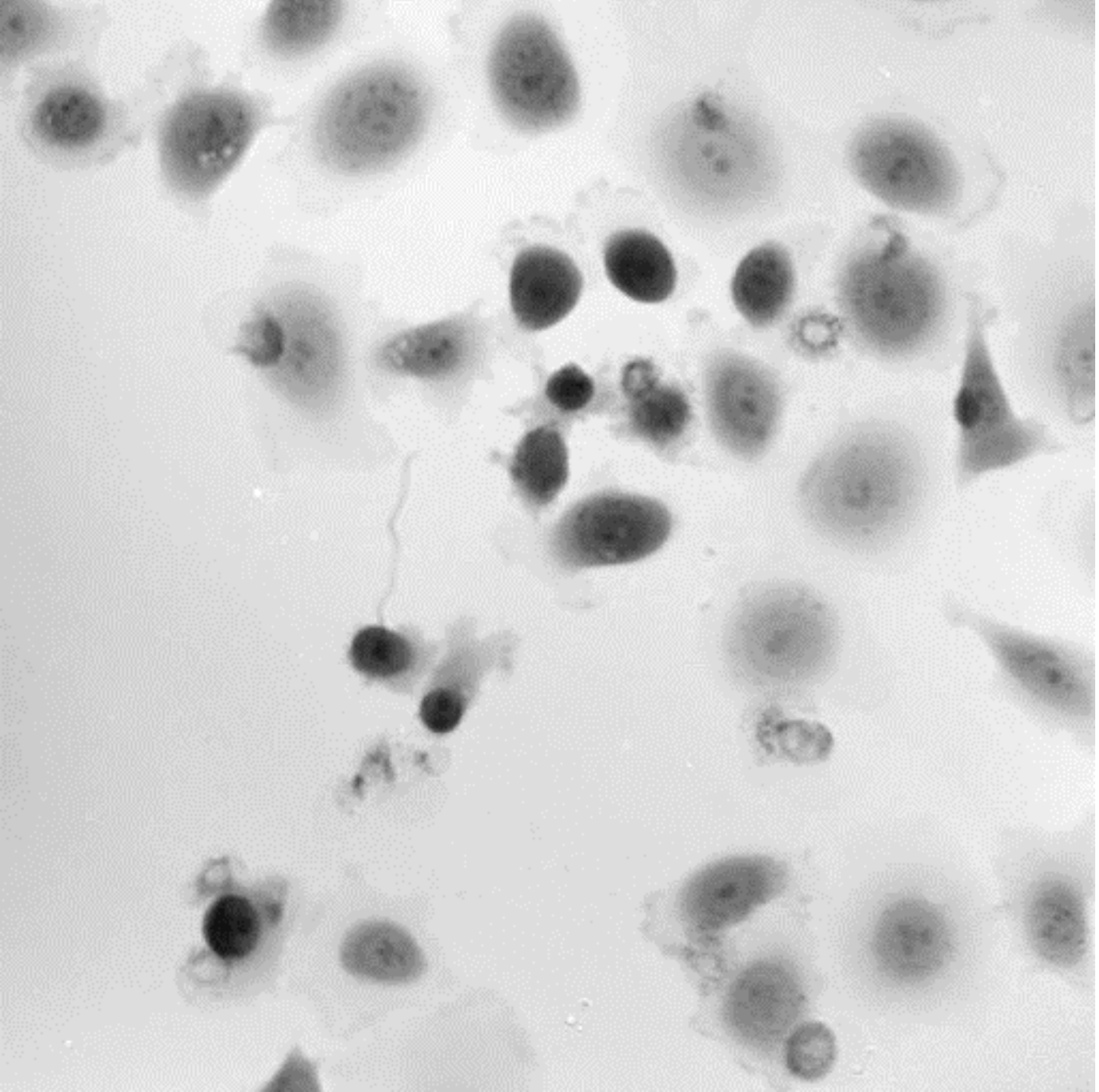

metastatic prostate cancer cell line PC3

Migration

- Single cell migration
  - ameboid
  - mesenchymal
  - lobopodial
  - pseudopodial
- Collective migration
  - Cell migration in which **groups of cells migrate while in physical contact and in the same net direction**. This is in contrast to single cell migration in which cells move individually and are not in physical contact with other

Types of movements

- mesenchymal x pseudopodial migraiton?

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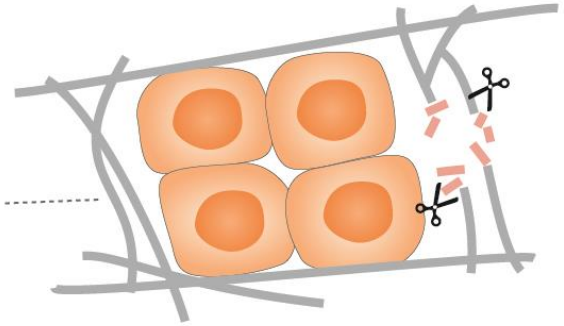
collective strategy

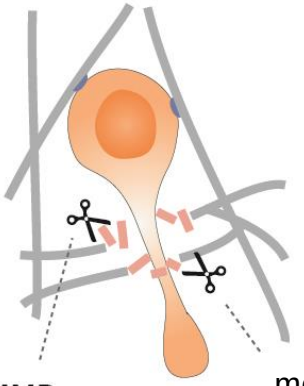
extracellular matrix (ECM)



individual strategy

mesenchymal
↓ cell-cell adhesion

ameboid
↓ cell-cell adhesion
↓ cell-extracellular matrix adhesion

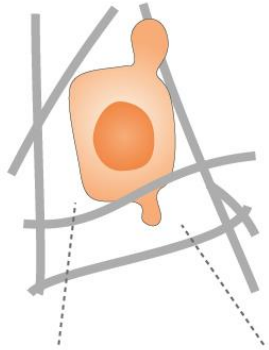


MMP

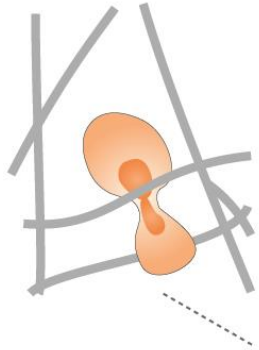
movement through ECM due cleavage of ECM by matrix metalloproteases

↑ Rho ↓ Rac

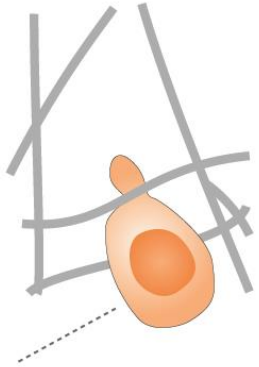
↓ Rho ↑ Rac



loss of cell polarity



membrane blebbing



push through spaces in ECM

Main types of migration in cancer cells

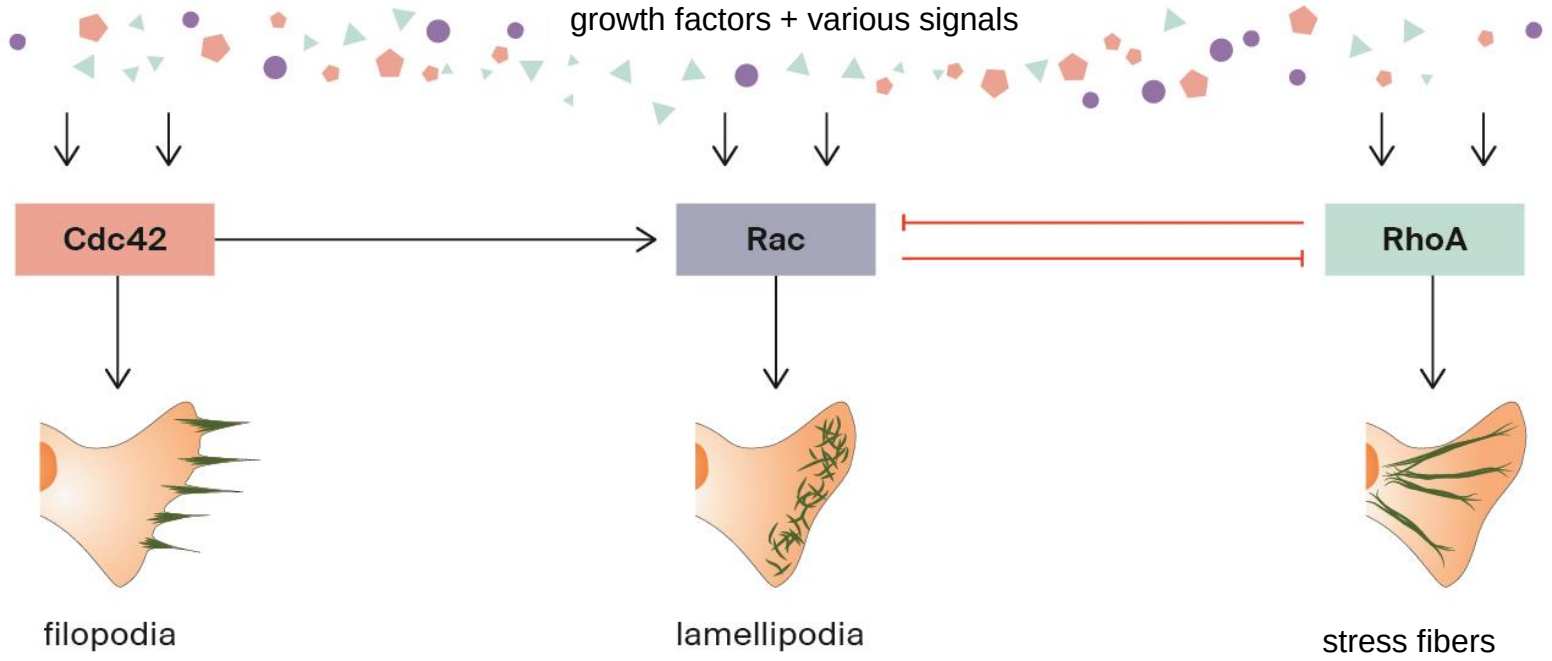
cancer cells employ variety of invasivenes modes

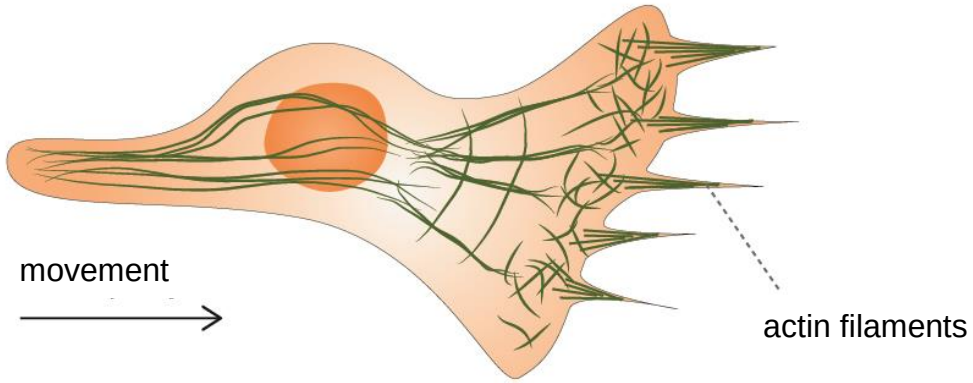
migration strategies can be switched

Key switches: Rho, Rac moleculles

Rac1 = Ras-related C3 botulinum toxin substrate
RhoA = Transforming protein RhoA).

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





Location of movement structuresin the <u>mesenchymal</u> 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/

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

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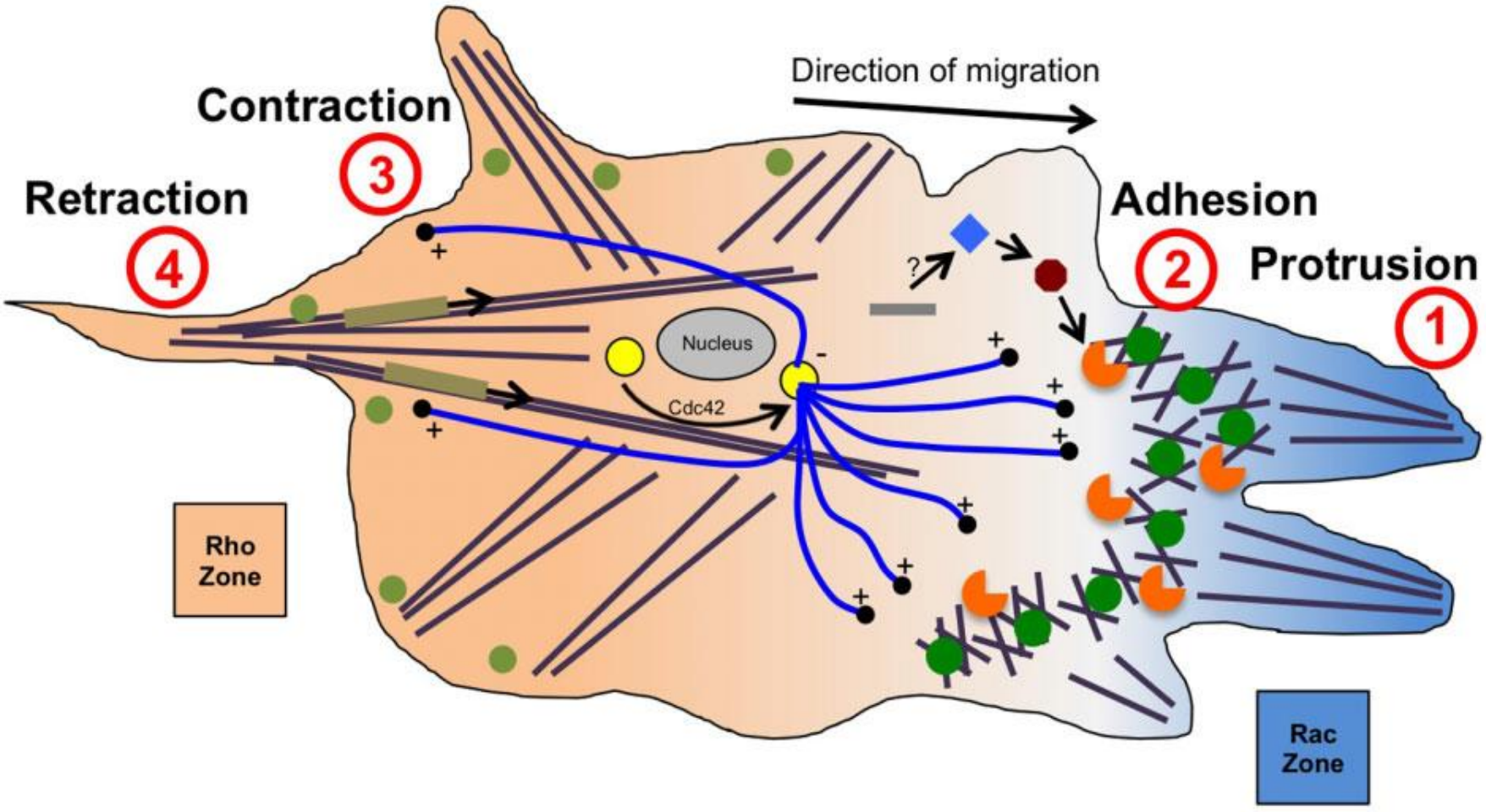
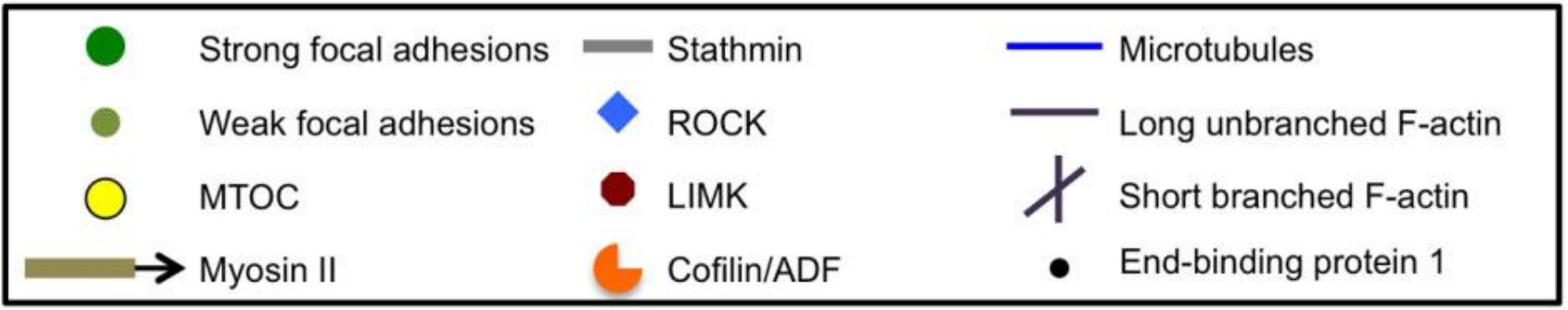




4 steps of migration: protrusion, adhesion, contraction, retraction.

F-actin

- short, branched F-actin at the leading edge
- long, unbranched F-actin stress fibres at the rear

Microtubules with ends emanating from the MTOC

Strong and weak focal adhesions

The gradients of active Rho and Rac

Cytoskeleton is a highly dynamic structure

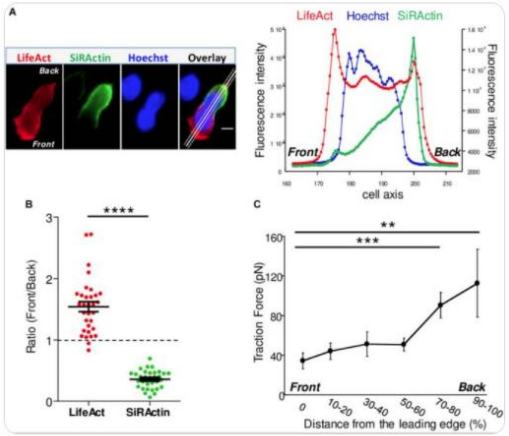
visualization of static (arteficially stabilised) and dynamic actin filaments by co-imaging SiR-actin (green) and LifeAct (magenta).

https://twitter.com/joachimgoedhart/status/1402234543646679042

Joachim Goedhart
@joachimgoedhart

Here's a cool trick that I hadn't seen before: visualization of static and dynamic actin filaments by co-imaging SiR-actin (green) and LifeAct (red). Thoughts?
Figure from this paper: frontiersin.org/articles/10.33...

Přeložit Tweet



2:02 odp. · 8. 6. 2021 · Twitter Web App

10 Retweetů 1 Citovat Tweet 47 Lajků

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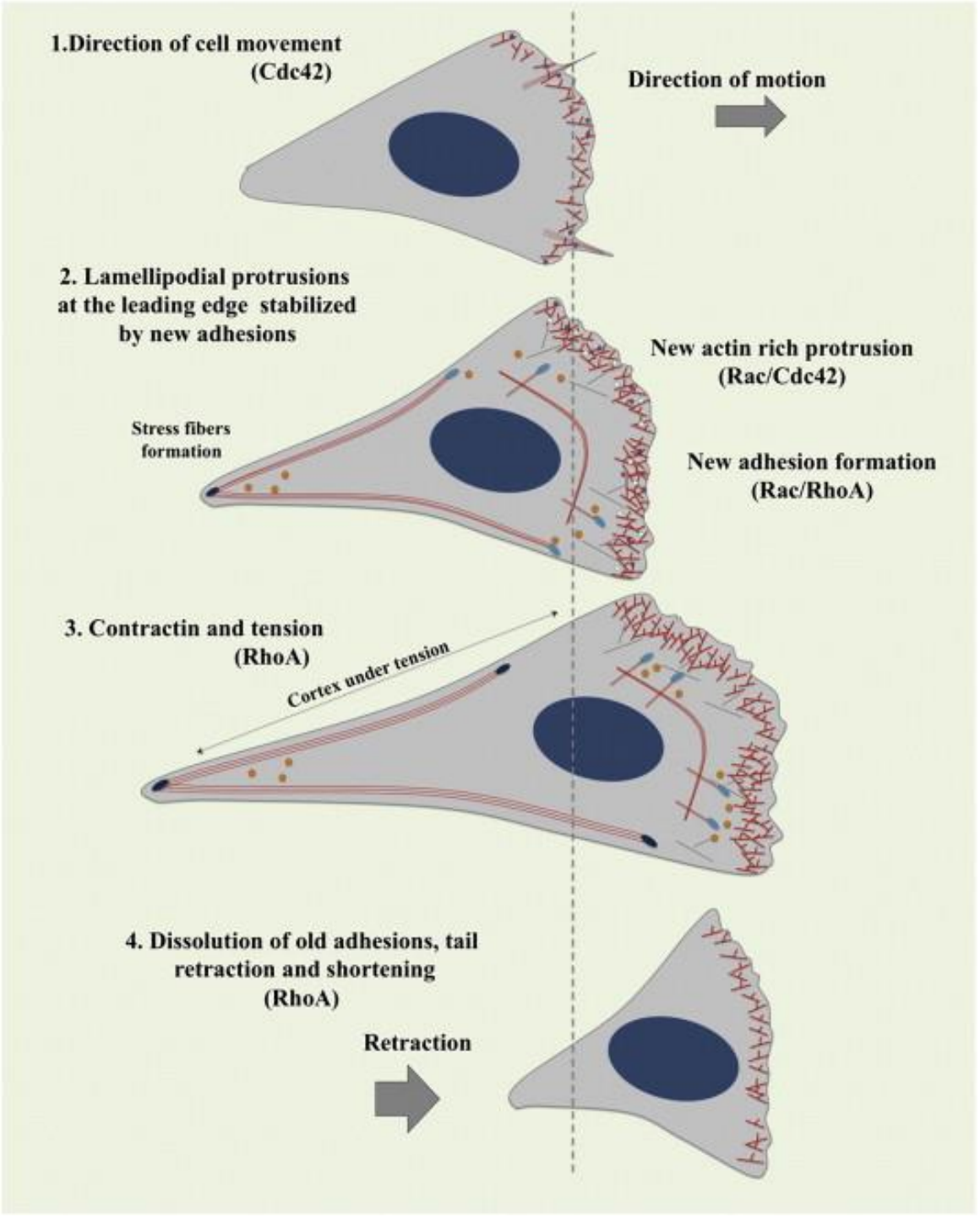
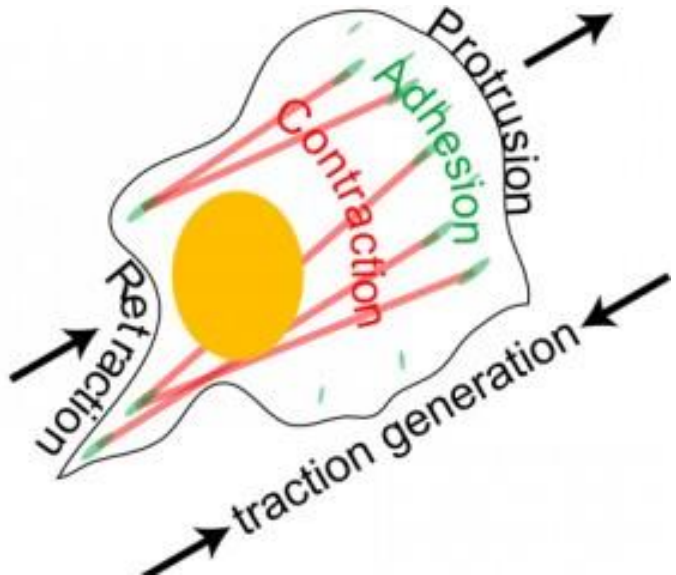


Rho GTPases in the cell motility cycle.

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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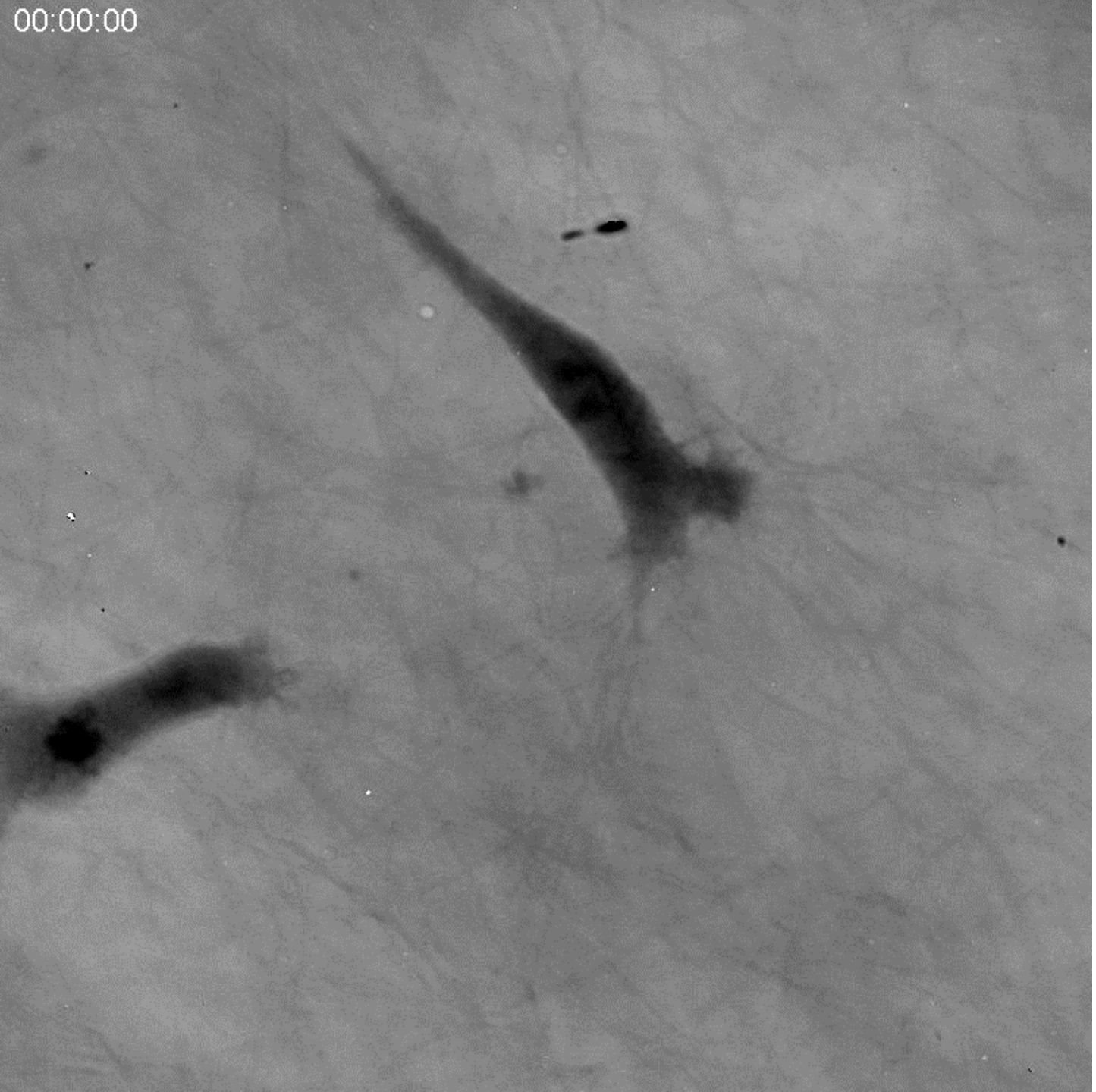

A migration of mesenchymal cell within collagen matrix. Cells were embedded within bovine collagen gel (1mg/ml) and observed using CCHM.

Tolde 2018 https://doi.org/10.1038/s41598-018-30408-7

Mesenchymal characteristics

- elongated morphology
- pseudopodial protrusions
- adhesion to substrate

generates coordinated action of MMPs and actomyosin machinery „paving the way“

Pseudopodial migration

- **filopodial**, **lamellipodial** or other migration relying on protrusions driven by **actin polymerisation**

follower cells are MMP-independent

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

Paul 2017

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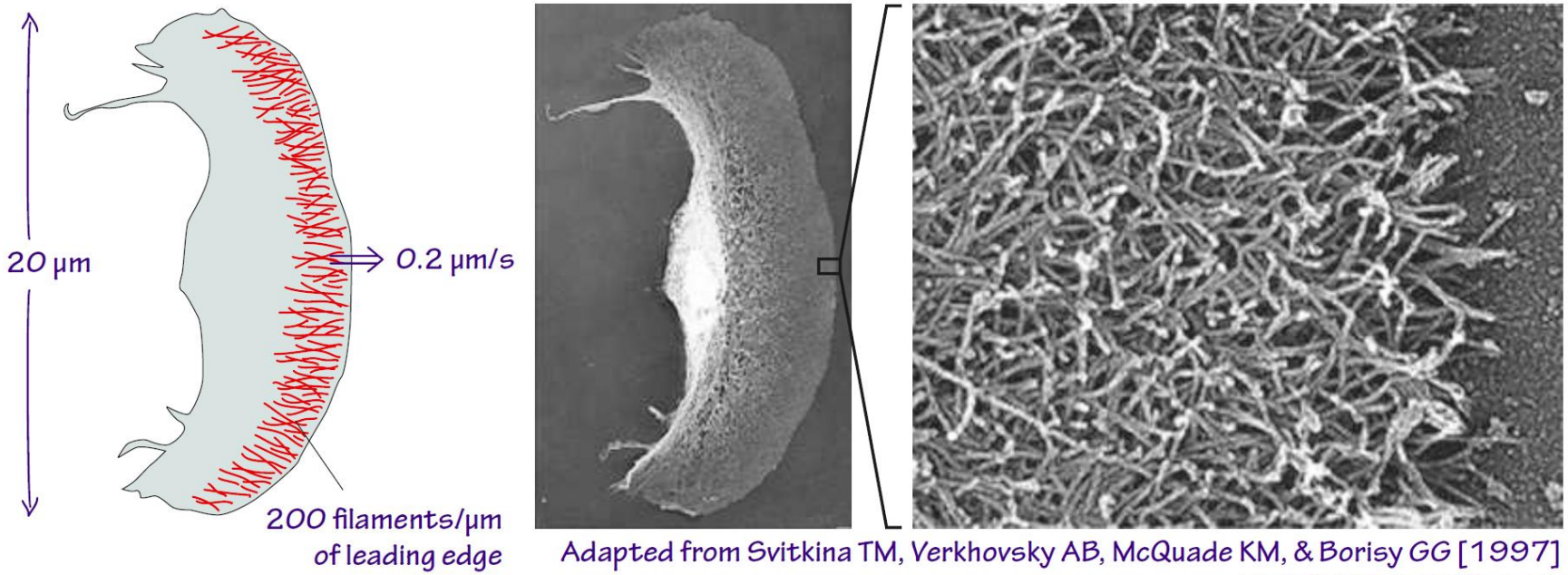
How much ATP is required for actin-driven motility?



Adapted from Svitkina TM, Verkhovsky AB, McQuade KM, & Borisy GG [1997] *J Cell Biol* 139: 397–415.

$$\text{actin polymerization rate} = 0.2\ \mu m/s \times \frac{1000\ nm}{1\ \mu m} \times \frac{2\ \text{monomers}}{5\ nm} \approx \frac{100\ \text{monomers}}{(s \times \text{filament})}$$

$$\text{ATP requirement} = 20\ \mu m \times \frac{200\ \text{filaments}}{1\ \mu m} \times \frac{100\ \text{monomers}}{(s \times \text{filament})} \times \frac{1\ \text{ATP}}{\text{monomer}} \approx 4 \times 10^5\ \text{ATP/s}$$

- actin filaments in moving goldfish epithelial keratocytes polymerize at the same rate that the cell moves—about 0.2 µm/s
- each filament must grow by about 100 monomers/s to support motility, which costs ≈100 ATP per polymerizing filament per second
- Lamellipodium is about 20 µm long and contains roughly 200 actin filaments per micron
- this value turns out to be a **very minor ATP requirement** (cells produce 10^9 ATP/second)

Milo et al., Cell biology by Numbers, 2016, p.202

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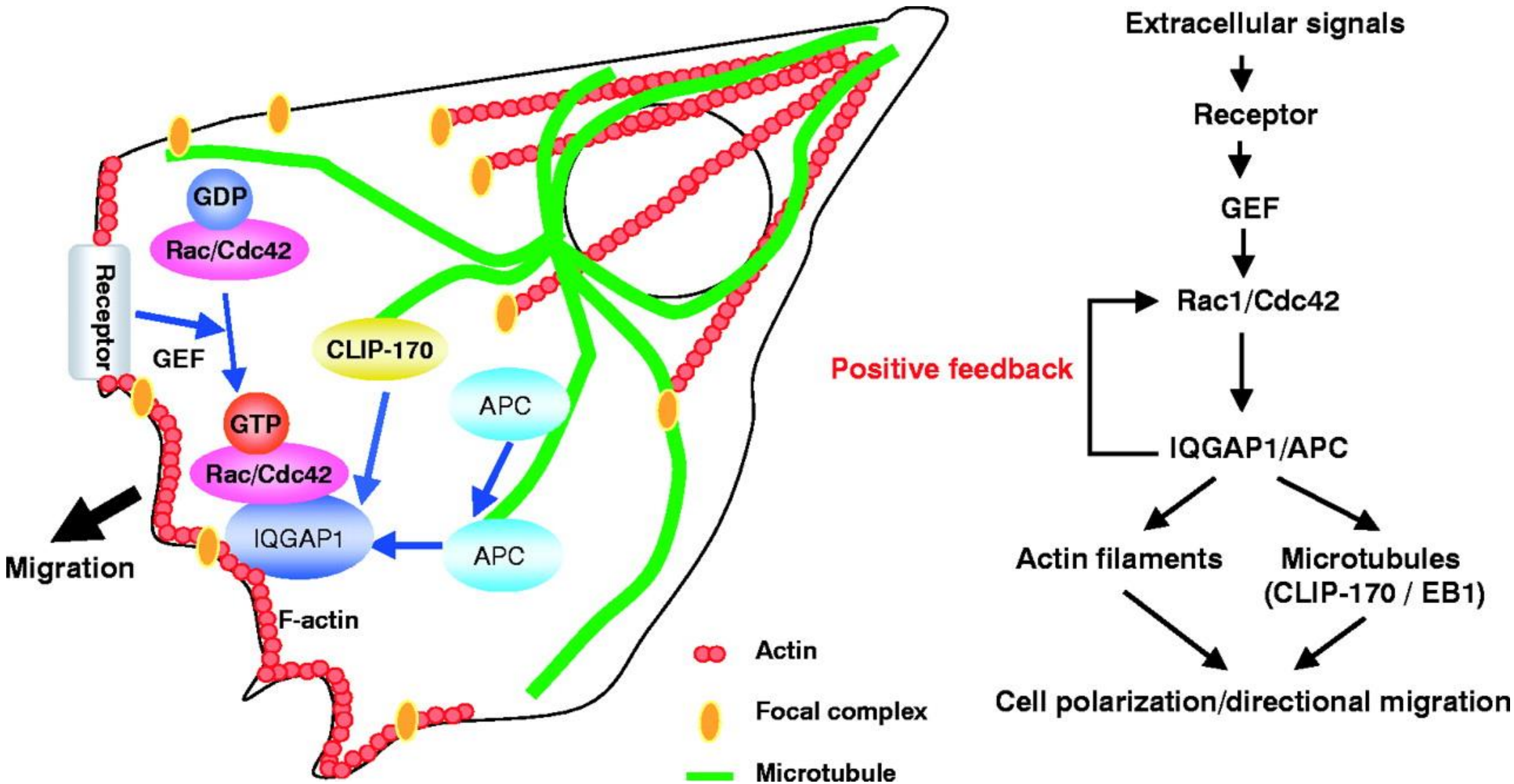
GDP
Rac/Cdc42
Receptor
GEF
CLIP-170
APC
GTP
Rac/Cdc42
IQGAP1
APC
Migration
F-actin
Actin
Focal complex
Microtubule
Extracellular signals
Receptor
GEF
Rac1/Cdc42
Positive feedback
IQGAP1/APC
Actin filaments
Microtubules
(CLIP-170 / EB1)
Cell polarization/directional migration

Translocation of an **amoeboid cell** through a narrow pore. An amoeboid cell embedded in rat-tail collagen (1 mg/ml) was observed using CCHM. This video demonstrates the dynamic cell body deformation during invasion through a narrow pore

Tolde 2018 https://doi.org/10.1038/s41598-018-30408-7

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Disorders associated with impaired cytoskeleton

- Tumor diseases
  - Metastasis is responsible for the greatest number of cancer deaths

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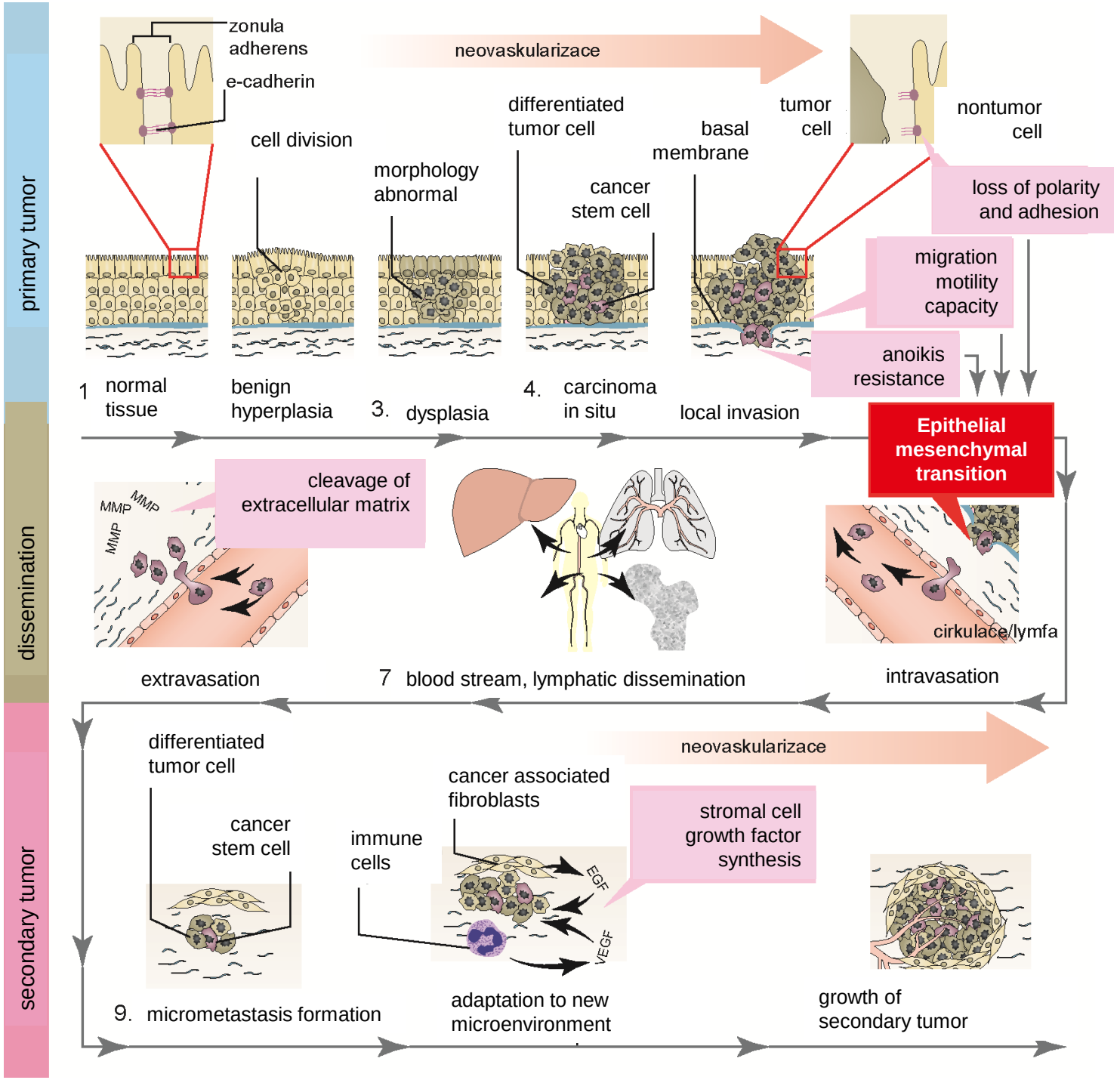

- Metastatic disease, or the movement of cancer cells from one site to another, is a **complex process requiring dramatic remodelling of the cell cytoskeleton**.
- For cancer cells to metastasize, they must successfully complete all of the steps of the **metastatic cascade.**

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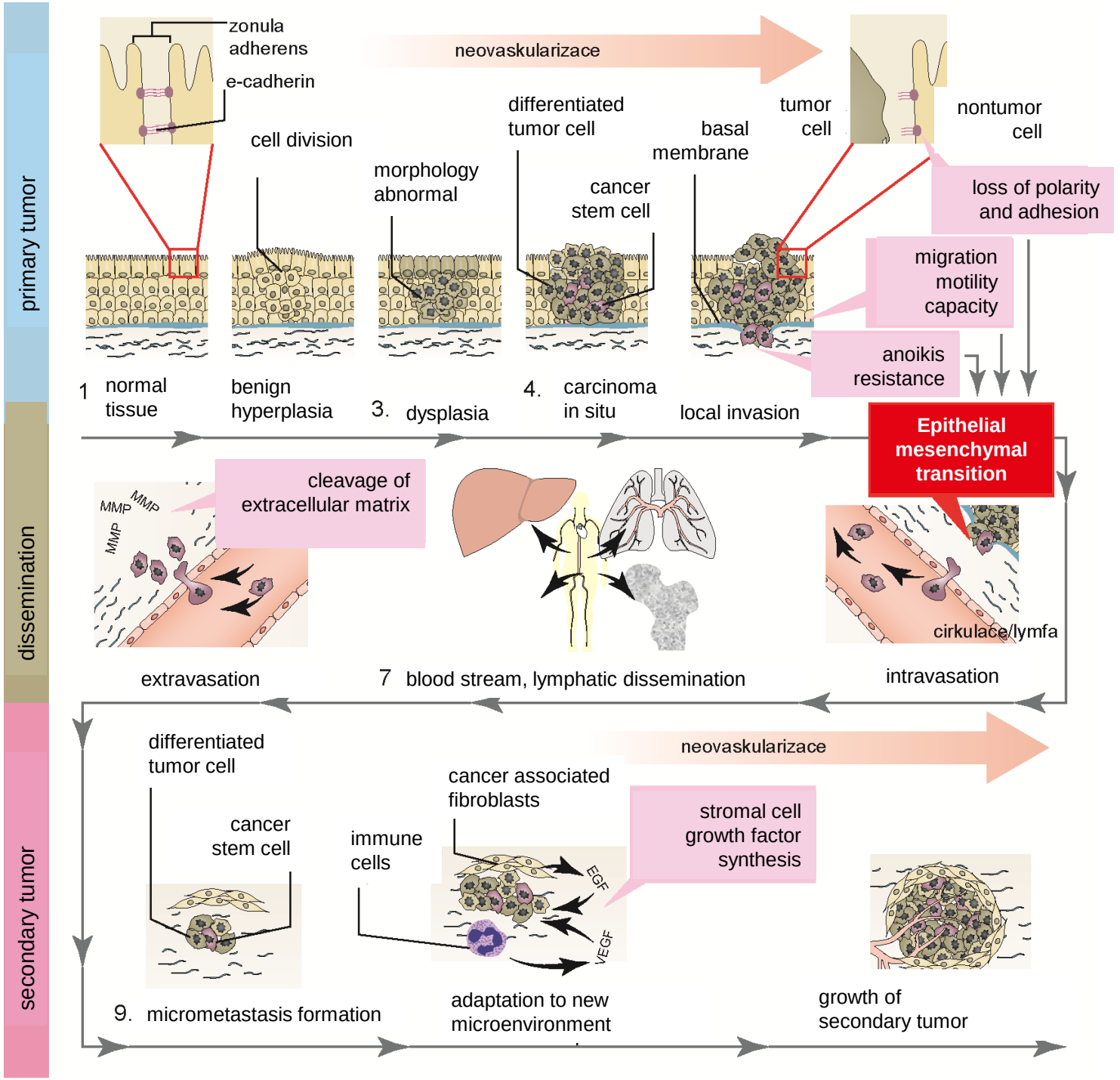

- Cancer cells in **the primary tumour acquire the ability to detach** from the primary tumour and migrate through the surrounding ECM and stroma.
- Degradation of the vascular basement membrane and travel across the endothelium, termed **intravasatio**n.
- Tumour cells transport through the vasculature, arrest in a capillary bed and cross the vasculature (termed extravasation).
- Disseminated cells **grow and interact** with the extracellular environment to form metastatic tumours.

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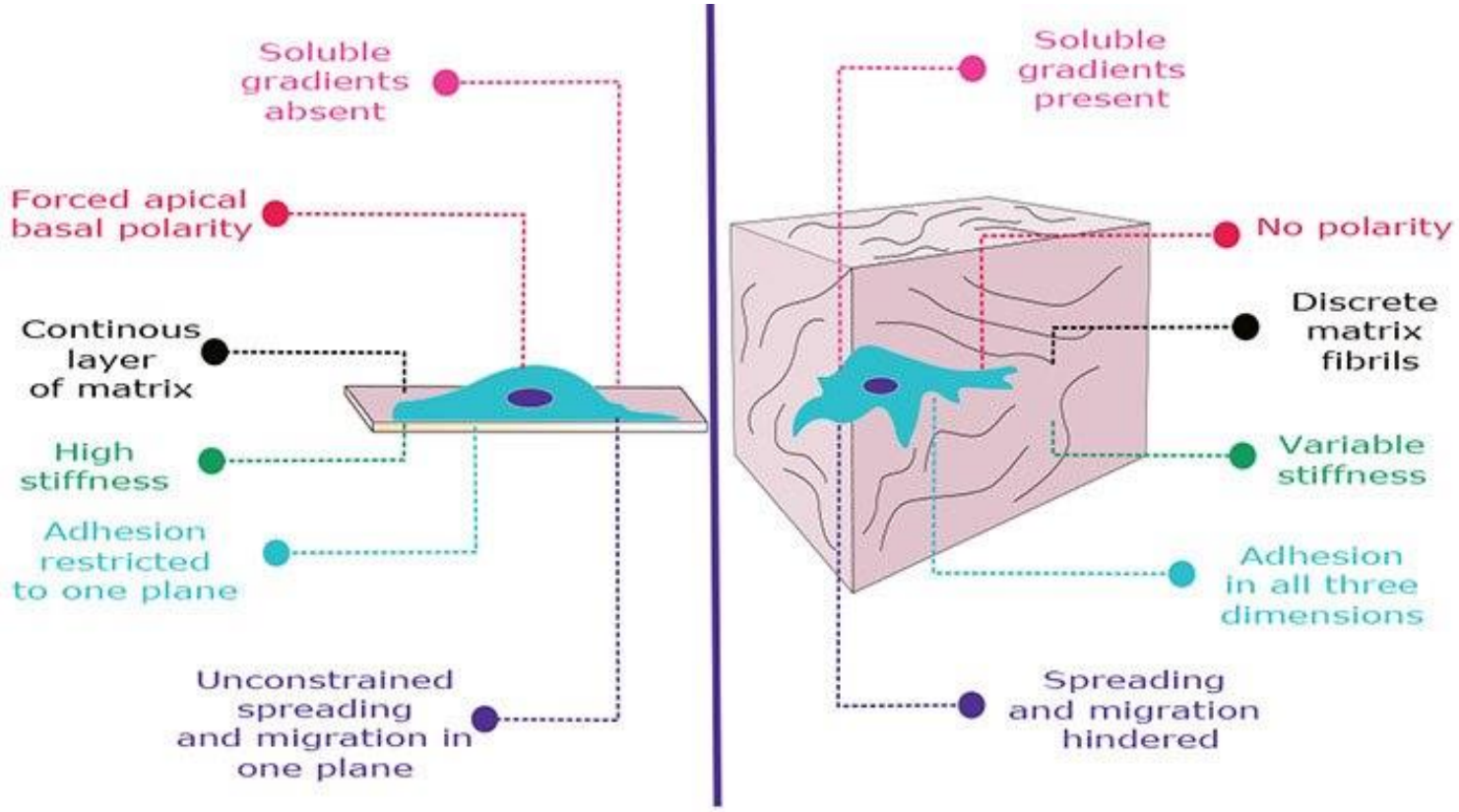
2D vs 3D

- Migration in unconfined 2D surfaces X in confined spaces

- migration in confining microenvironments not predicted by 2D assays



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Migration and invasion of cells

- Metastasis regulated by biochemical and mechanical cues of **microenvironment**
- Decreased cell adhesion → enhanced invasive capacity
- Migration subject to fluctiations due alteration of
  - cytoskeleton,
  - matrix mechanics,
  - organelle mechanics

 Mierke, 2020, https://doi.org/10.3389/fcell.2020.583226

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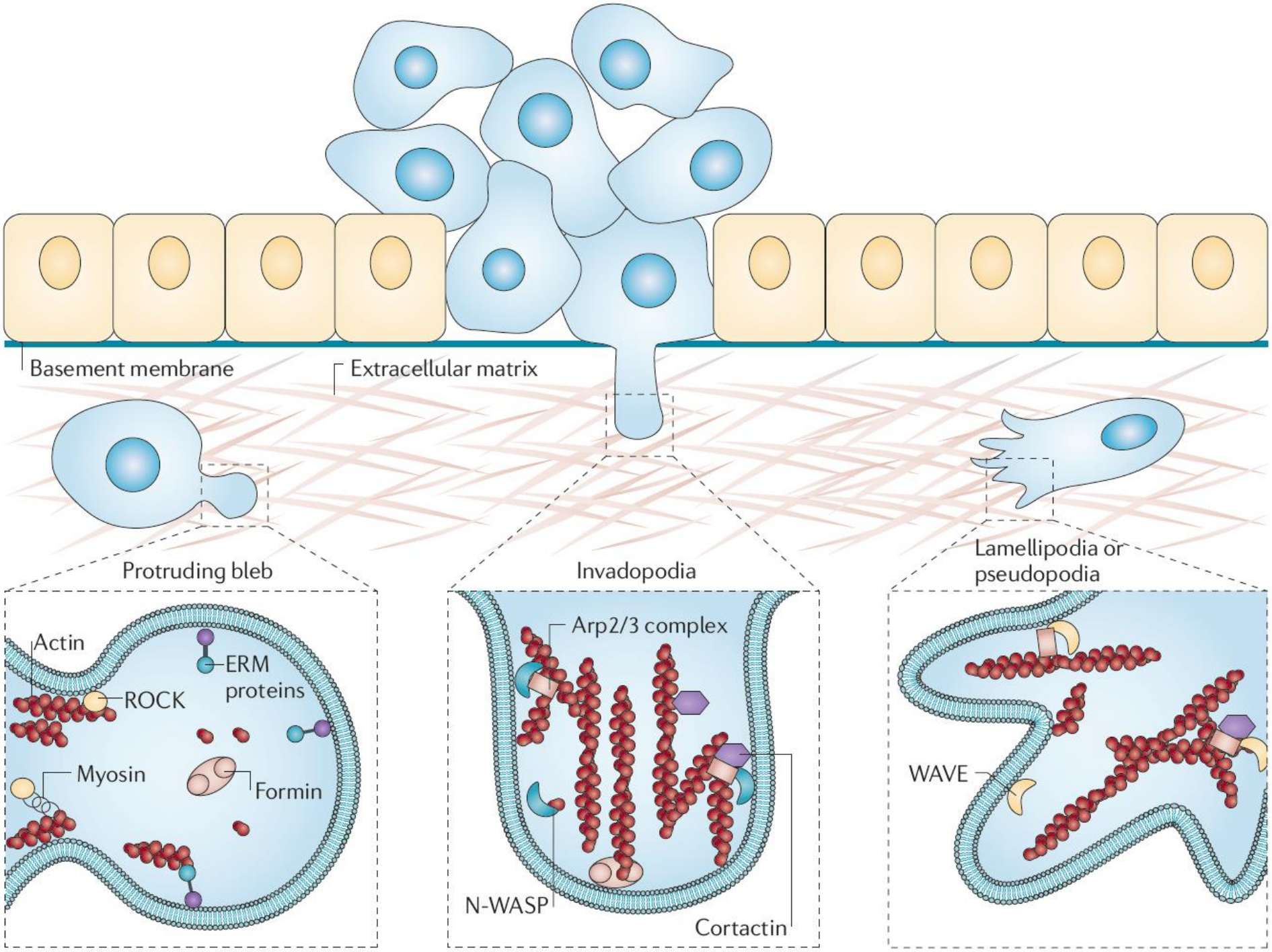

Typical protrusive structures in invasive cancer cells

- Cancer formation of structures:
- **plasma membrane blebs, invadopodia or pseudopodia**
- **actin-dependent**
- Nonapoptotic blebs are highly dynamic protrusions in which the plasma membrane bulks out owing to increased hydrostatic pressure on regions of **weak** cortical actin.
- :10.1038/nrc3003

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Migration through confining tracks

- endogeneous: features of tissues
- made by tumors/tumour associated cells

- types of migration
- 2dx3d

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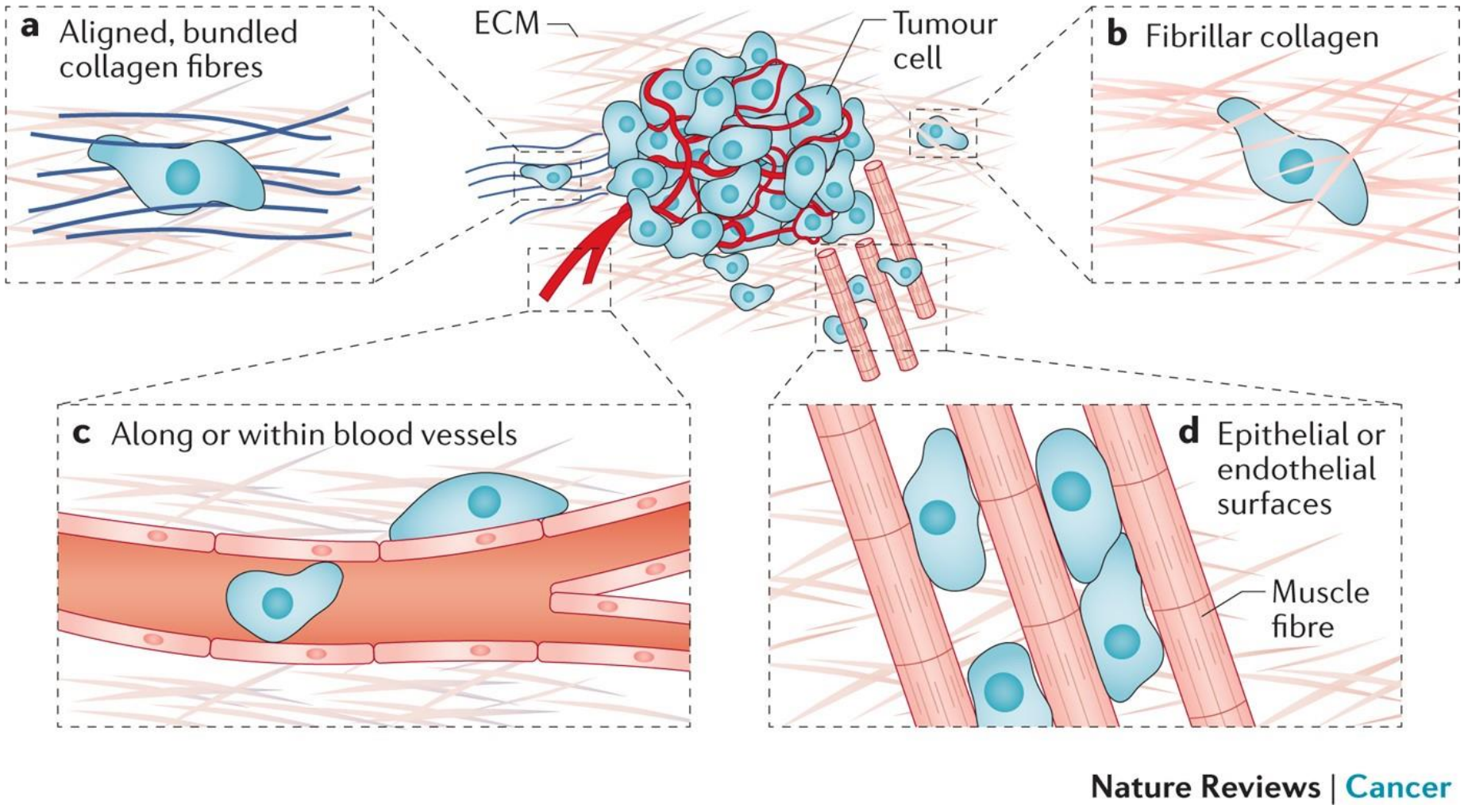

Cancer cell migration occur in pre-defined paths.

ECM alignment provides migration cues

collagen alignment and bundling at tumour periphery provide cues for directed migration.

unbundled ECM (fibrillar collagen), which present pore-like migration spaces

Microtracks intravascularly and perivascularly

between epithelial or endothelial surfaces (eg between muscle and nerve fibres).

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

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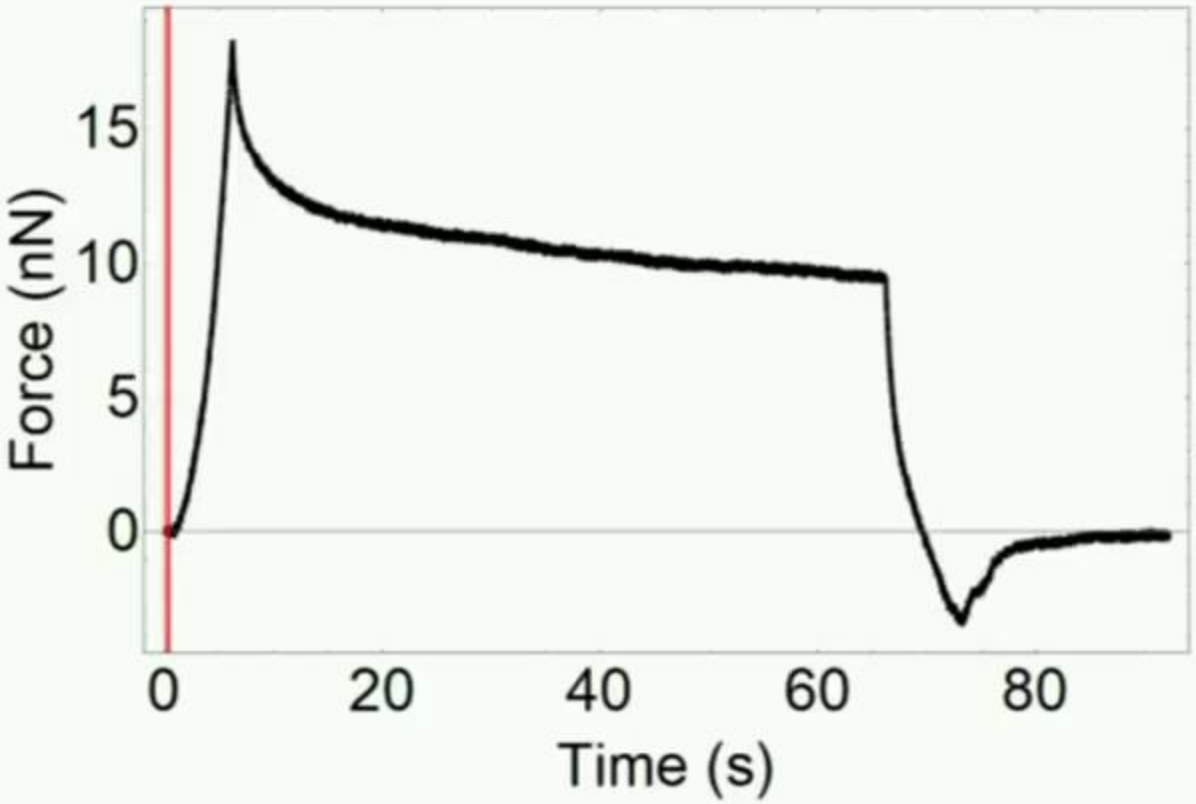
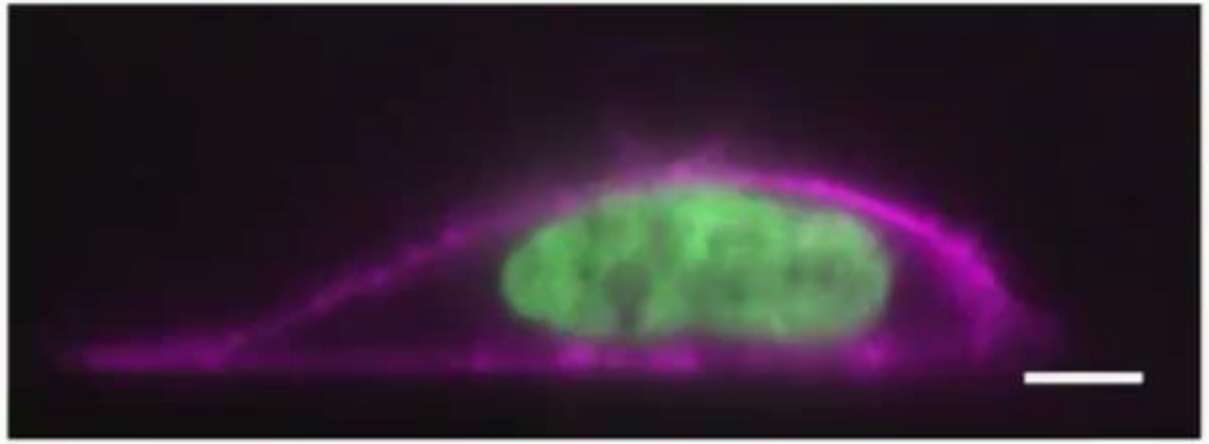
Physical limits for migration

- 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 limitin 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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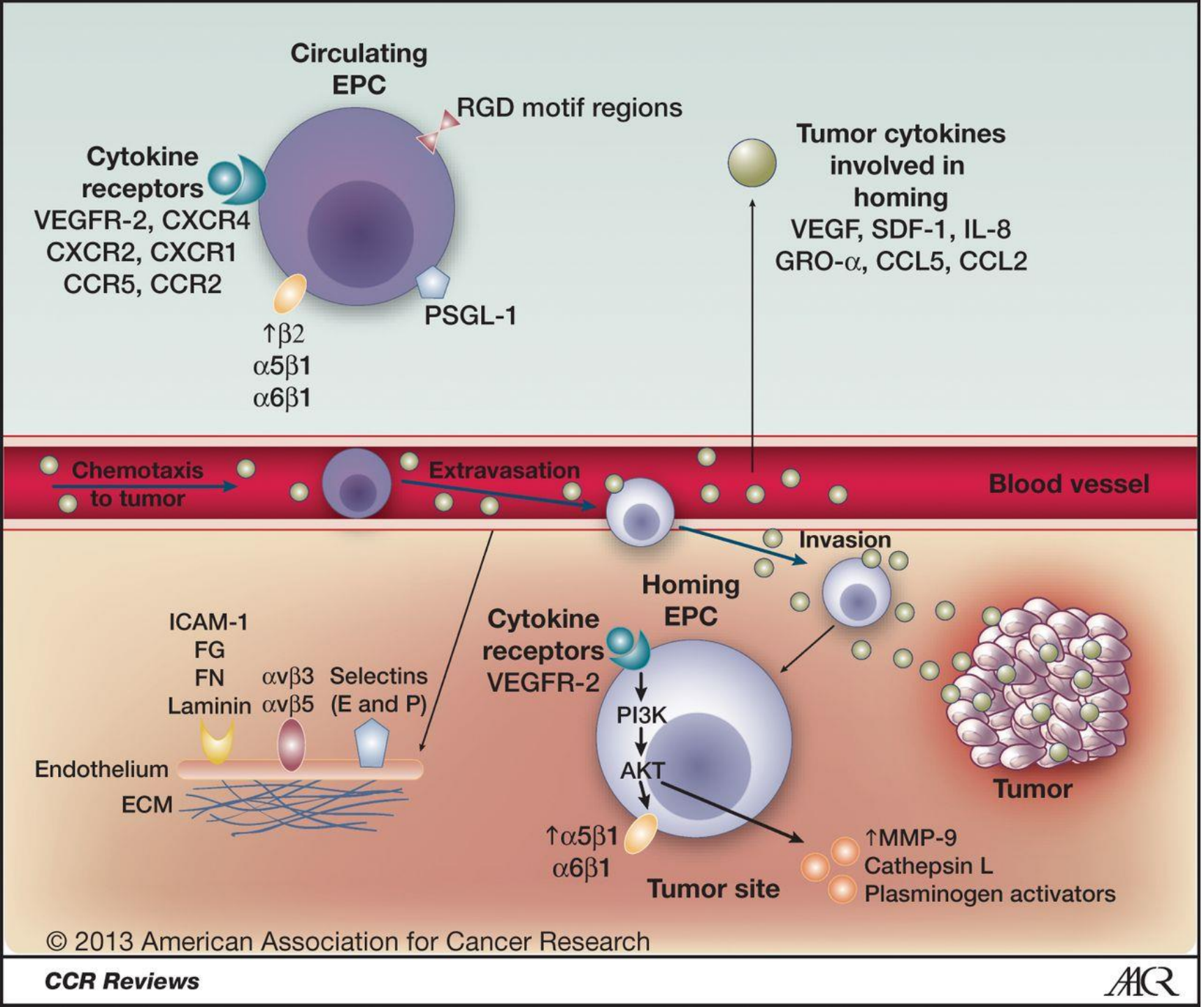

Homing of Endothelial Progenitor Cells to tumor

Cells migrate to tumor in **a chemotaxis response** to tumor-secreted cytokines (VEGF, IL-8, CCL5, and others) which interact with their respektive receptors

de la Puente 2013 10.1158/1078-0432.CCR-13-0462

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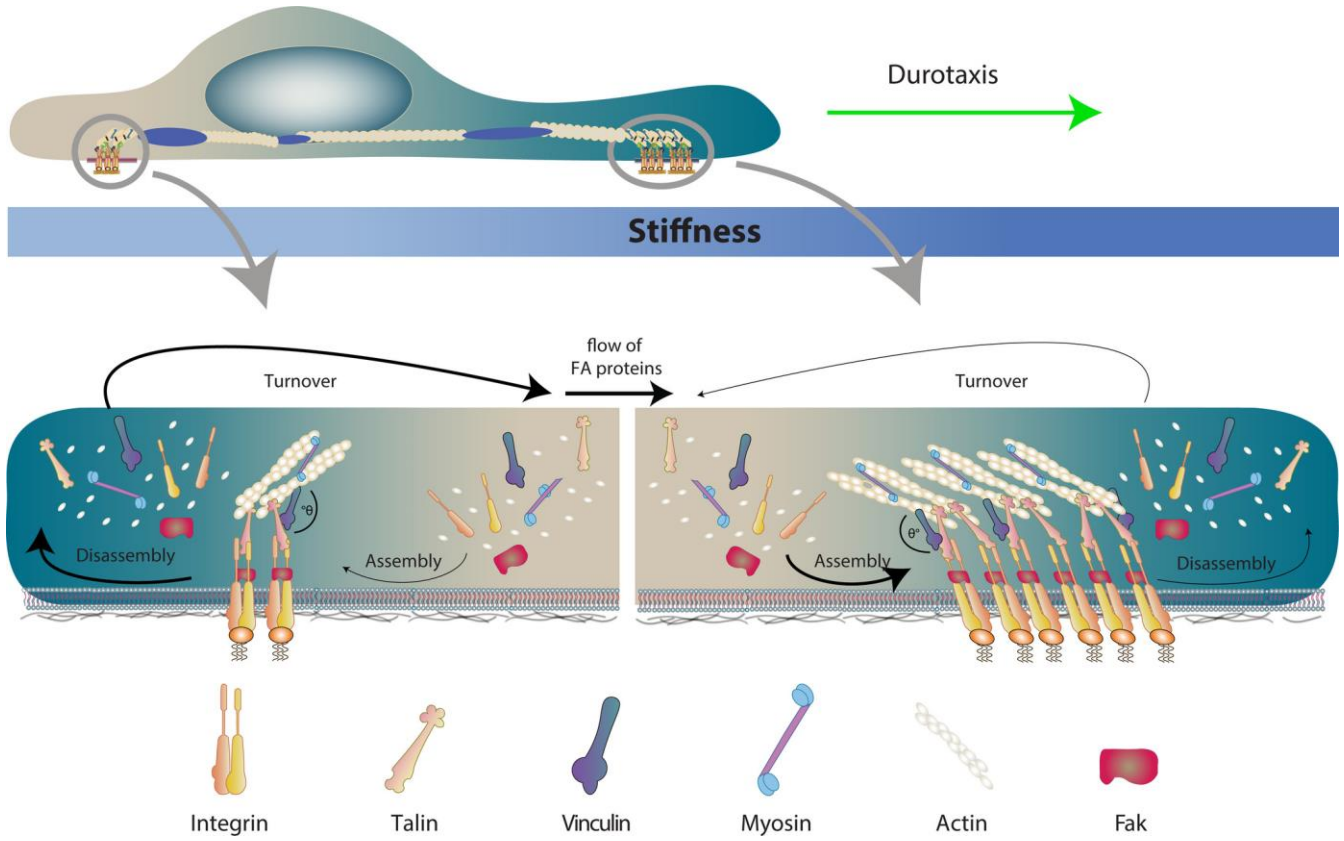
Durotaxis: mechanosensing and transduction in action



directed cell motility in response to gradiens in substrate rigidity

- Fillopodia - affinity for stiffer ECM
  - ECM stiffness favor migration and attract movement to stiffer parts of ECM)
  - explain migration to vasculature: **tumor-associated vessels stiffer than nontumor**

Gradient cause asymetry in cells

- front: higher rigidity → ↑ FA asembly → larger FA
- rear: the softer substrate → FA **dis**assembly.
- net flow of FA proteins to the leading edge → mature FAs in front promote **protrusion extension and establish the direction of migration** to the stiffer regions of the substrate.

 2015 Jansen, 2021 Raudenska, 2021 Espina

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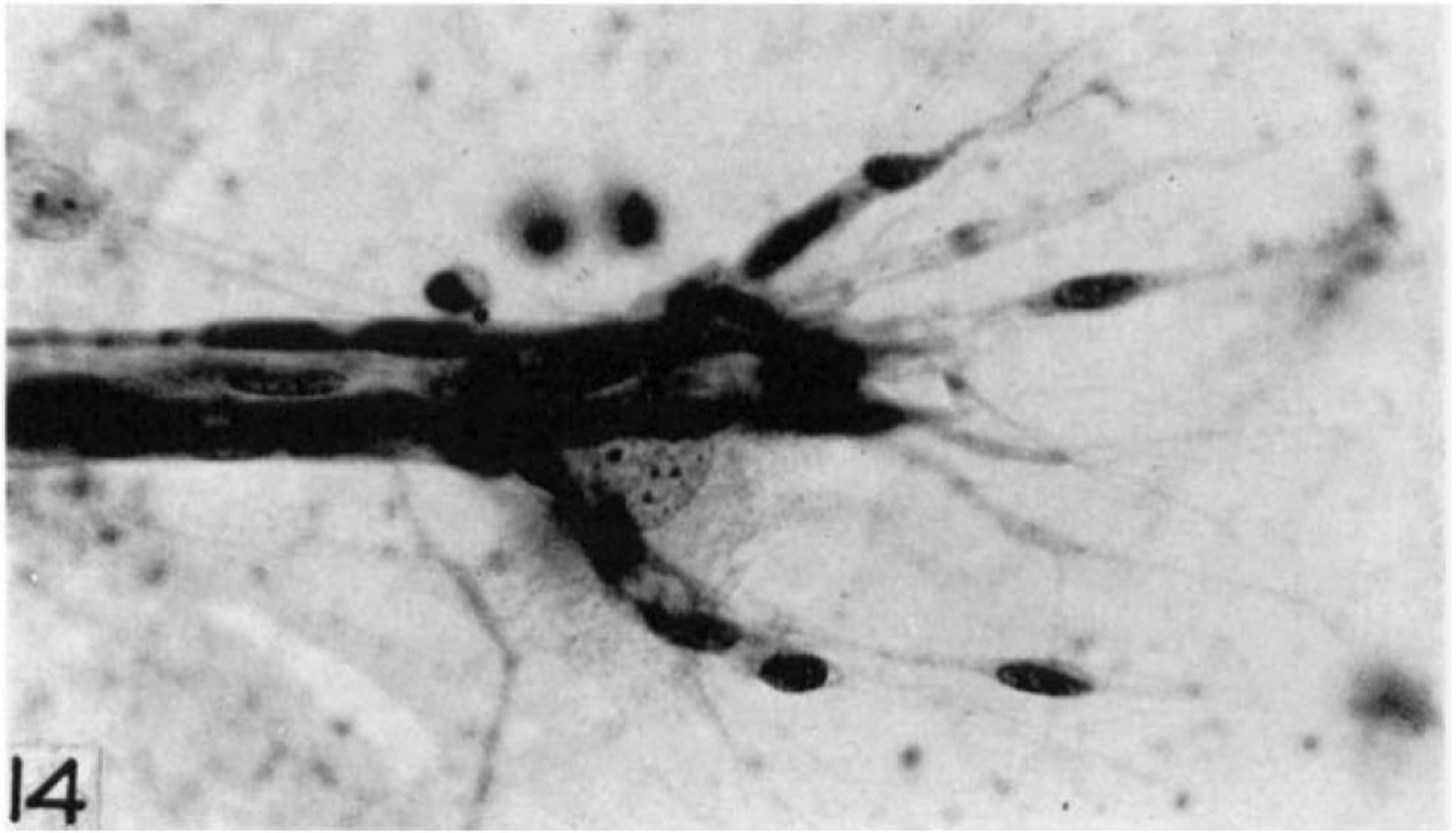
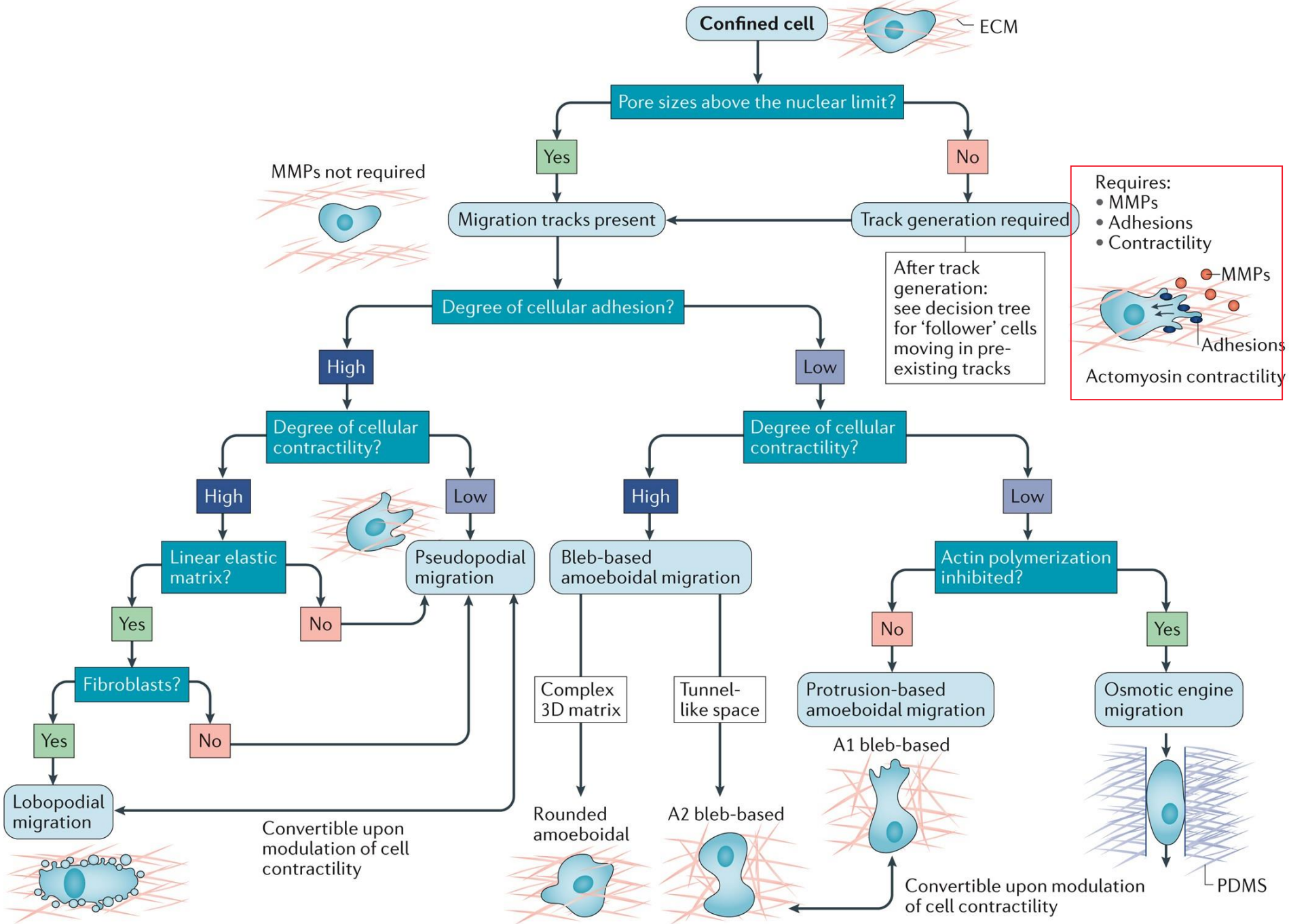


Fig. 14 Passage of cells from glass fiber to plasma clot. Explant: rat nerve, 14 days predegenerated. Medium (clot): as in figure 9. Period of cultivation: 3 days. × 530. Note numerous filopodial extensions of terminal cells into plasma clot; in a few instances, the main nucleated parts of the cell bodies have followed.

Contact guidance

- orientation of cells and stress fibers influenced by geometrical patterns of stroma
- directed cell migration/orientation based on microenvironment alignment

1945 Weiss https://doi.org/10.1002/jez.1401000305



Nature Reviews | Cancer

intrinsic and extrinsic cues influence the migration

migrate proteolytically through the secretion of matrix metalloproteinases (MMPs), which create microtracks for migration. **Follower cells** moving through these tracks and cells moving through microenvironments with pre-existing migration tracks use diverse migration mechanisms that depend on the levels of adhesion and cell contractility, and are thus dependent on both the cell and the microenvironment.

- **when cell adhesions** to the substrate are present, tumour cells migrate **using a pseudopodial-based mechanism that is dependent on protrusions.**
- when high contractility - fibroblasts move using a **lobopodial migration** mode.
- **low cellular adhesion** migrate using a bleb-based mode of **amoeboidal migration** dependent on high cortical contractility.
- contractility is inhibited, tumour cells may use a protrusion-based amoeboidal migration mode (A1 bleb-based migration) dependent on actin at the leading edge.
- absence of actin polymerization, cell movement is achievable through **front-to-rear flow of water through the cell** (which is termed osmotic engine migration).

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

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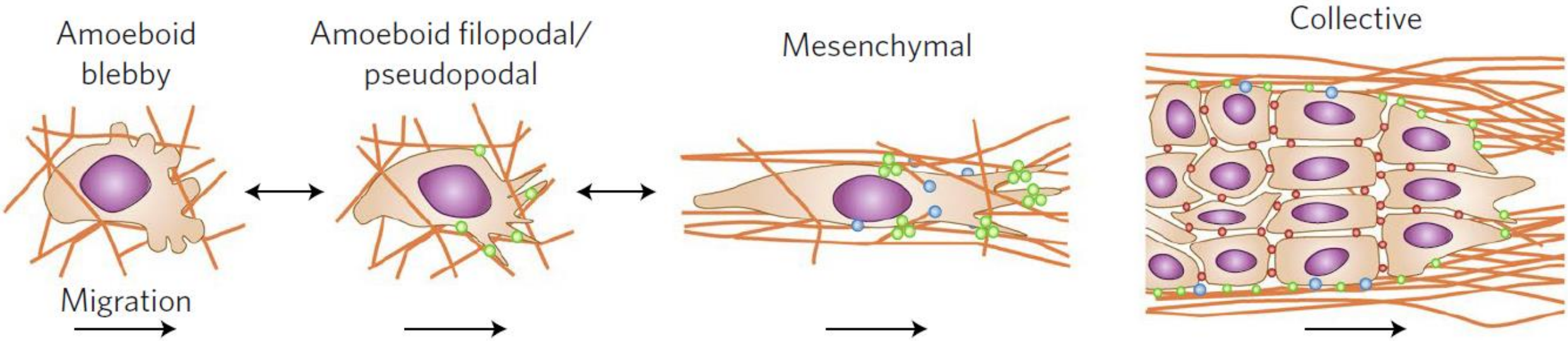
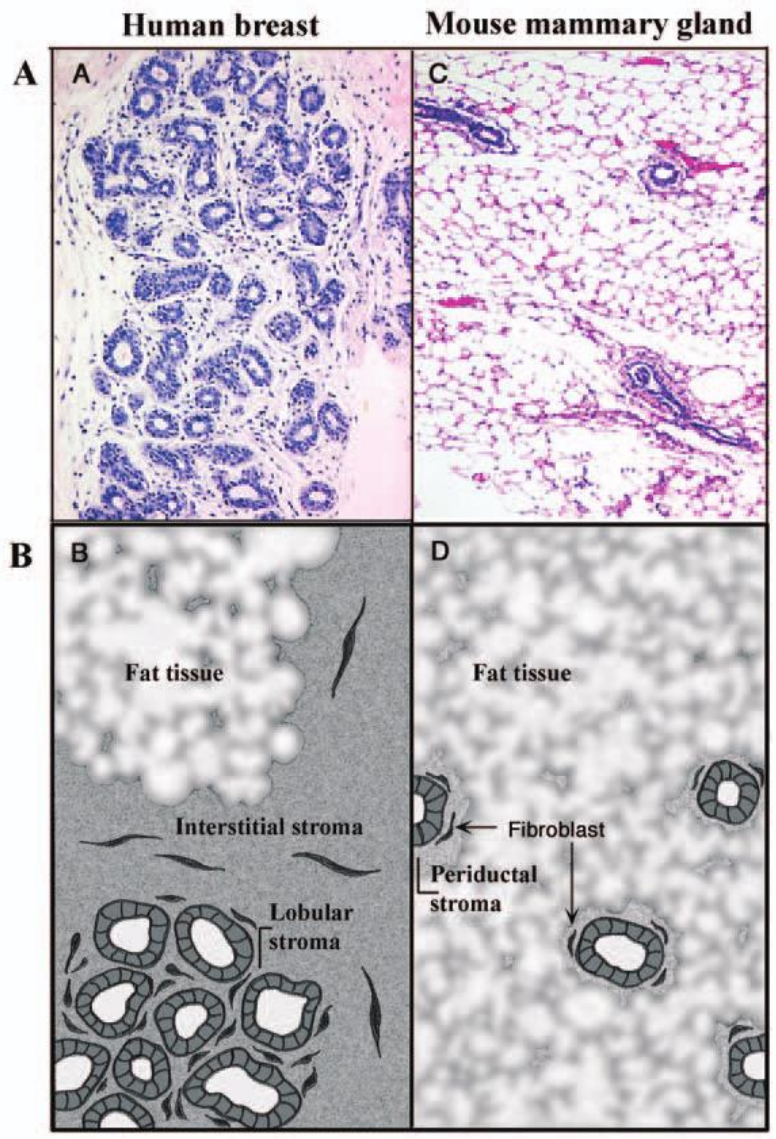


Cell migration modes in 3D environments, including single-cell and collective migration.

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

- 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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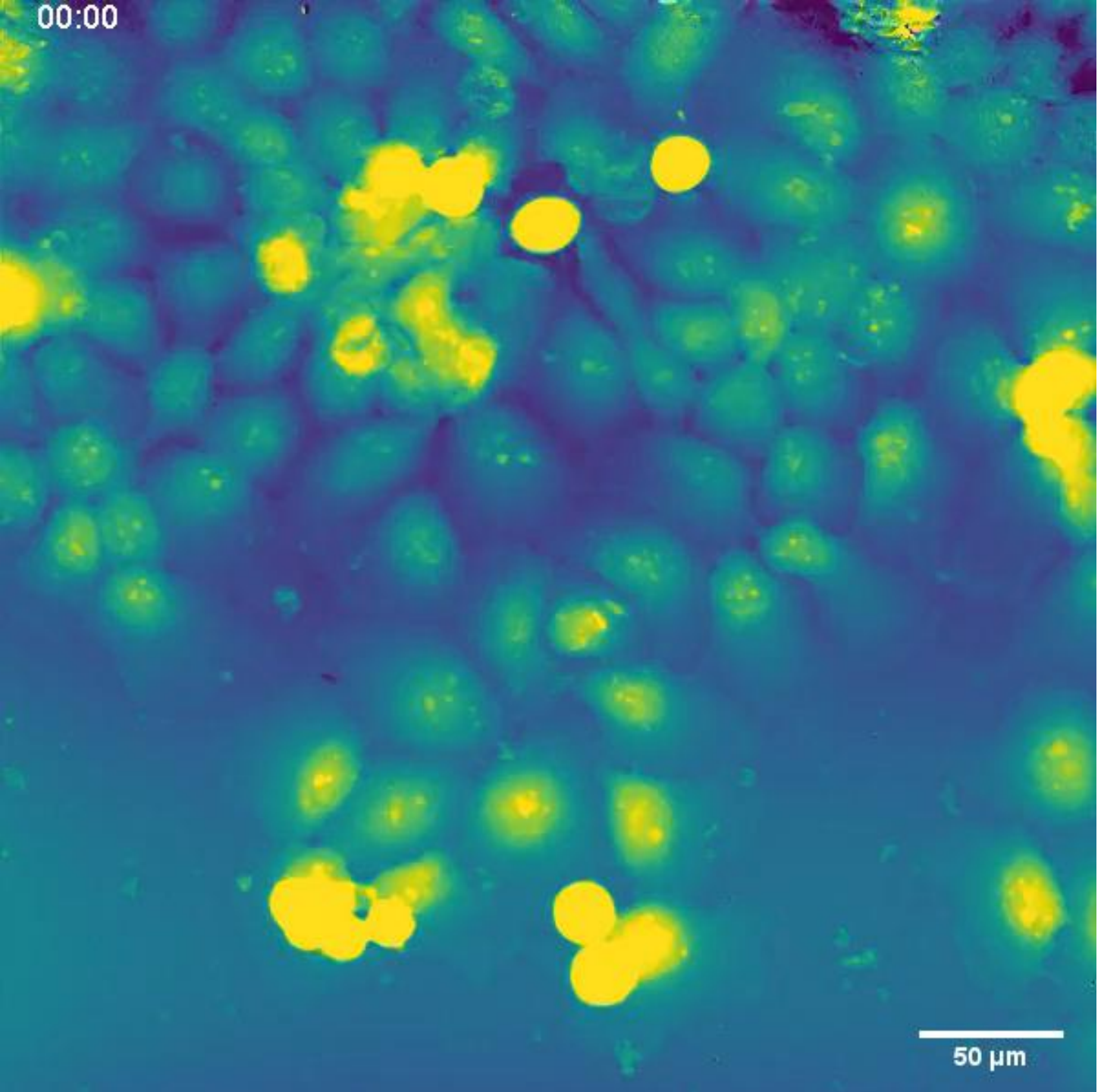
Collective migration

- movements of group of cells and the emergence of collective behavior from cell-environment interactions and cell-cell communication.
- essential process for **embryonic development, wound healing and cancer spreading**



FaDu head and neck cancer cells, collective migration and division, QPI, 10X

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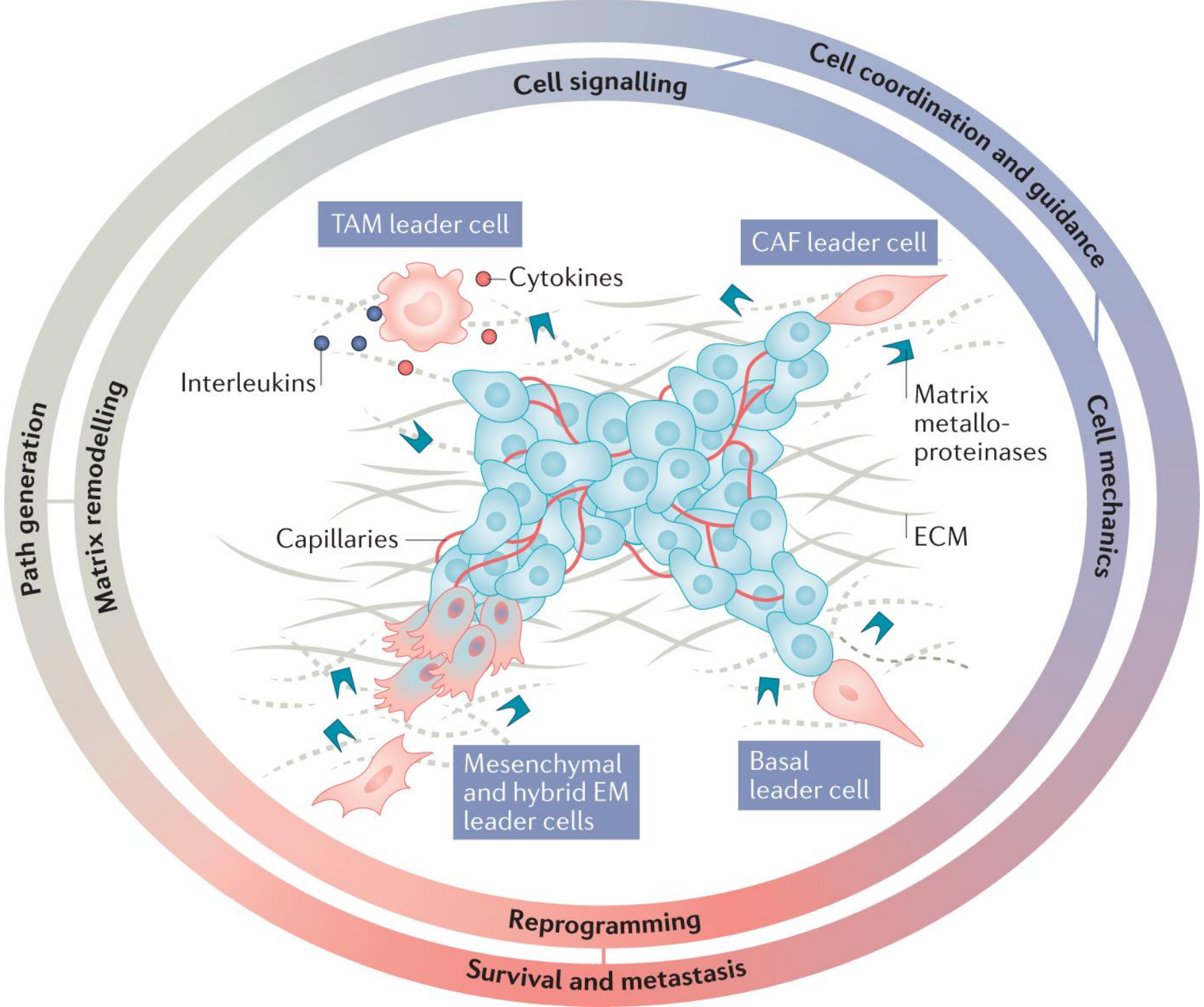

Mesenchymal and hybrid epithelial–mesenchymal (EM), basal, cancer-associated fibroblast (CAF) and tumour-associated macrophage (TAM) represent **four major categories of leader cell** that drive collective cancer invasion. Multiple leader cell types may arise in a tumour, though not necessarily all together. Key functions of leader **cells include generating a migration path**, **coordinating** with nearby cells to enable collective movement and enhancing the survival and metastatic capabilities of the tumour. Leader cells perform these functions using several **molecular programmes** such as

- matrix remodelling,
- cell mechanics and cell signalling,
- cell reprogramming.

ECM, extracellular matrix.

Mercedes 2021 https://www.nature.com/articles/s41568-021-00376-8

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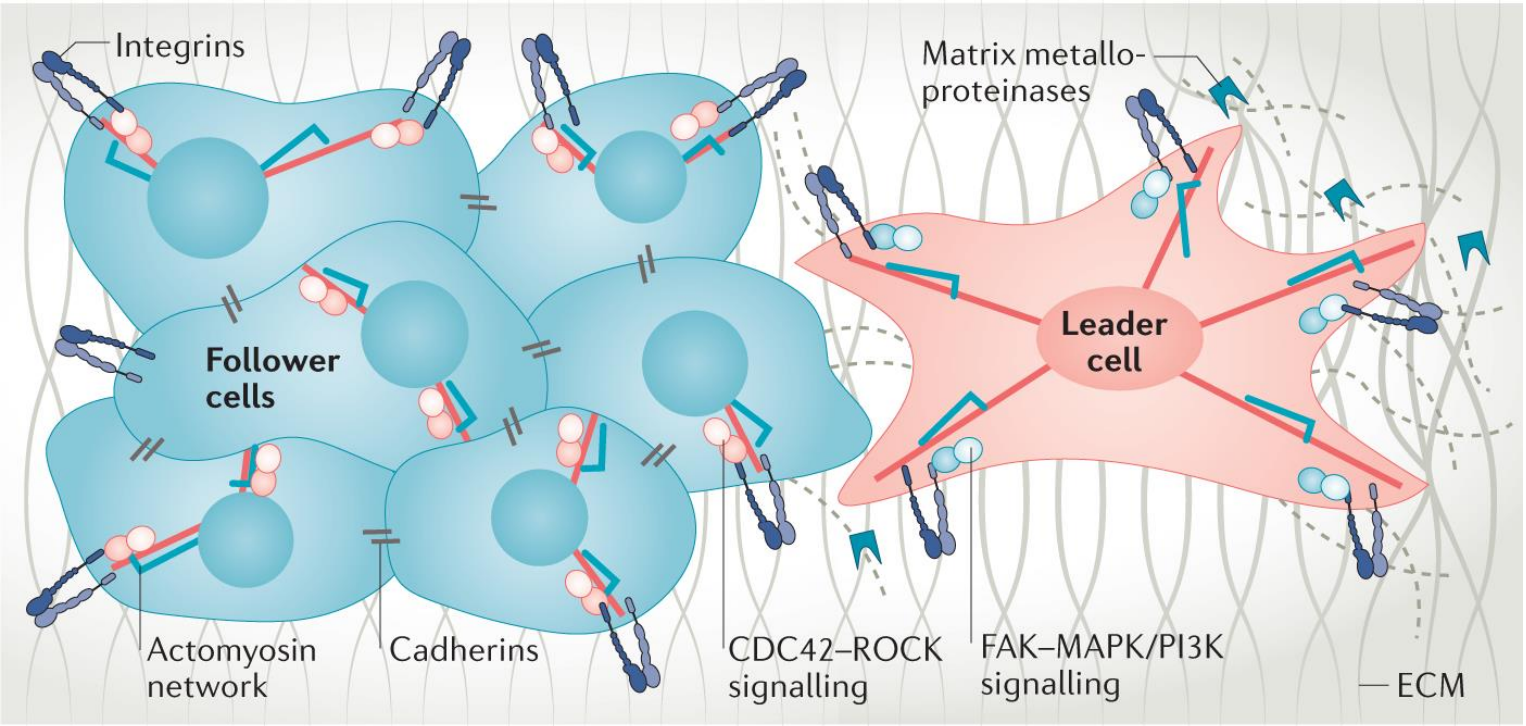

Leader cells communicate with other cells and their environments mechanically. This illustration highlights the key components of the cell mechanics cascade for cell–cell and cell–environment coordination. Leader and follower cells modulate RHO signalling according to their cell type and matrix density; this can in turn activate other pathways such as mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K) and transforming growth factor-β (TGFβ) pathways in leader cells.

Mercedes 2021 https://www.nature.com/articles/s41568-021-00376-8

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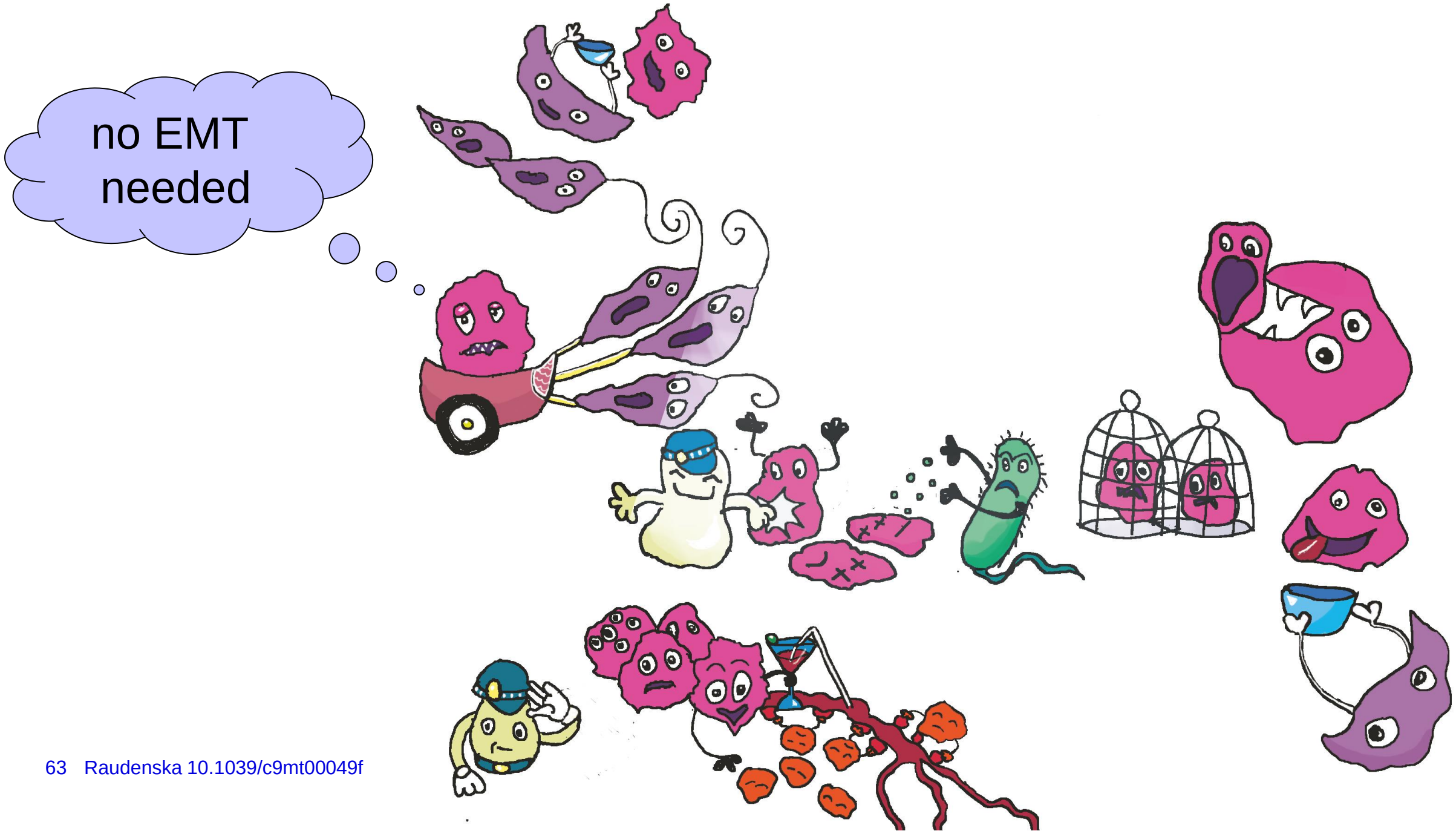
no EMT
needed

Cytoskeleton therapeutic target

- Actin targetable by mycotoxins:
  - block polymeration: cytochalasins
  - block depolymeration: phalloidin

Death cap (*Amanita phalloides*)

amantadin + phalloidin phallotoxins are highly toxic to liver cells, they have since been found to add little to the death cap's toxicity, as they are not absorbed through the gut

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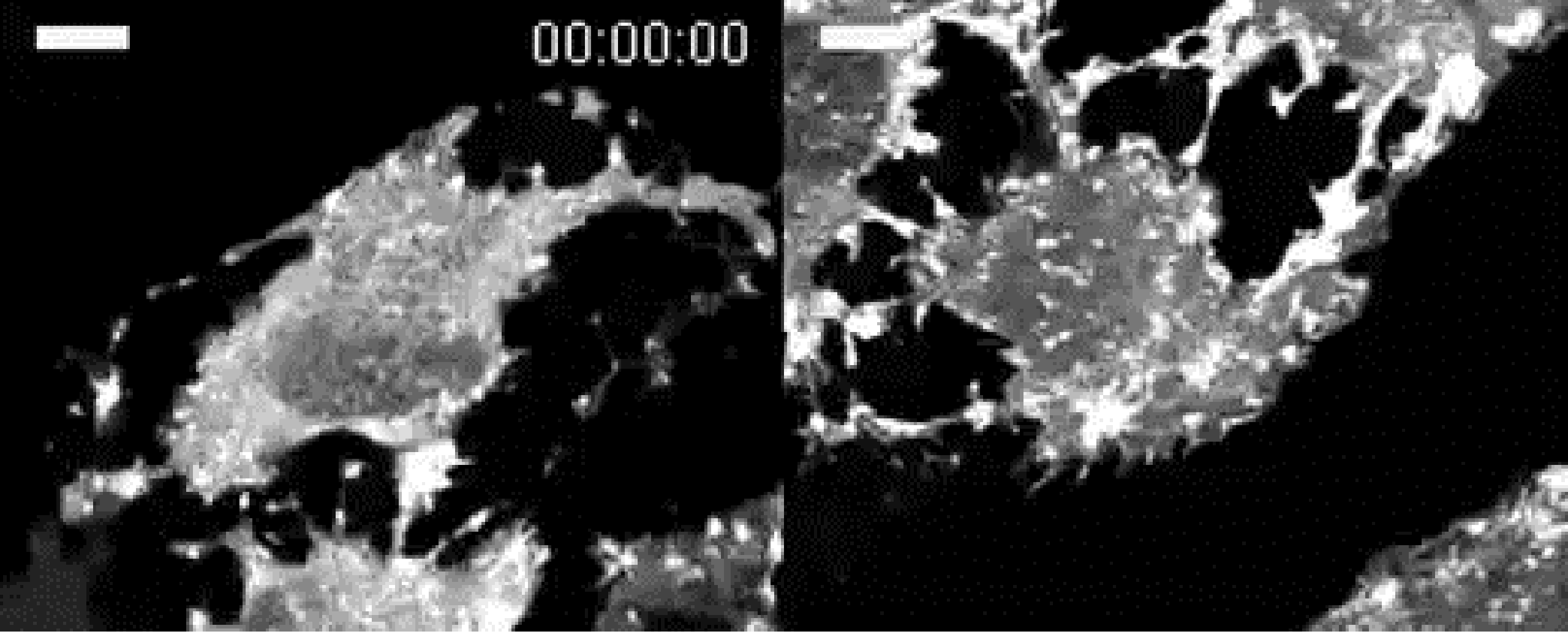

Stress fiber recovery after Cytochalasin D washout is enhanced by activated FHOD1
U2OS cells expressing mCherry-Actin together with EGFP-FHOD1 constructs (not shown) were subjected to Cytochalasin D washout to stimulate stress fiber formation. Left: FHOD1 WT; right: FHOD1 V228E. Images were acquired by time-lapse confocal microscopy

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