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Cytoskeleton

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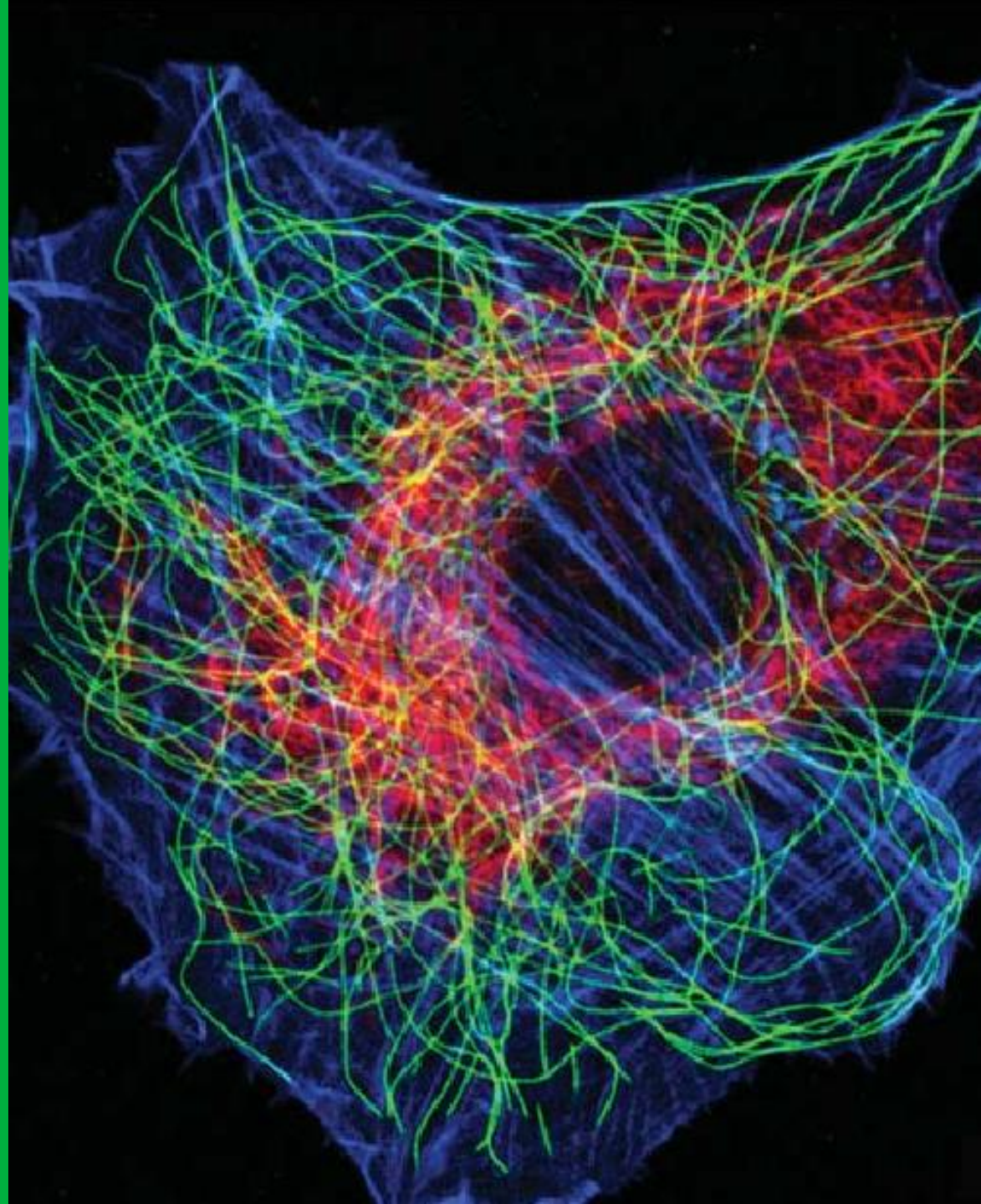
Department of Experimental Biology



Memory system / All types of cells

– System of nucleic acids and proteins (storage and expression of genetic information)

Membrane system / All types of cells – quantitative differences

– System of biomembranes (flow of matter, energy and information)

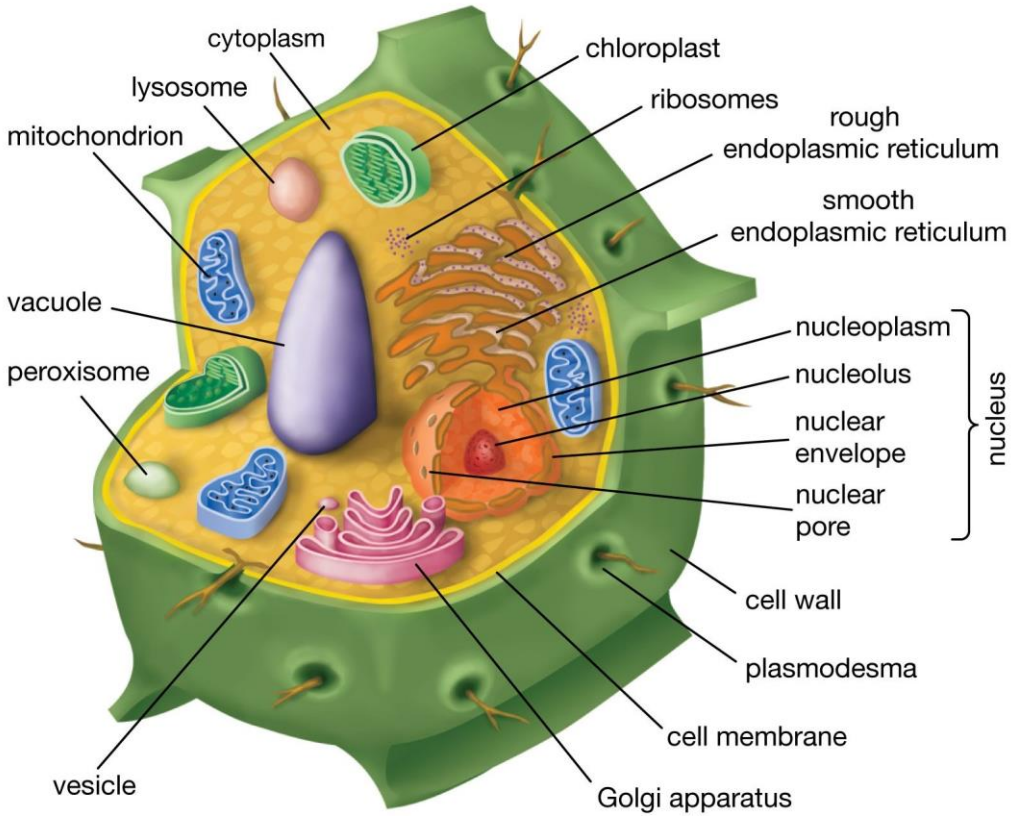
Cytoskeletal system / Eukaryotes, analogy in prokaryotes

– System of filamentous protein structures (motility, spatial organization, communication)

Outline

- Cytoskeleton components and main functions
- Visualization of the cytoskeleton
- Microtubules
- Actin filaments (microfilaments)
- Intermediary filaments
- Nuclear cytoskeleton and cell cortex
- Cytoskeleton in prokaryotes

Cytoskeleton components and main functions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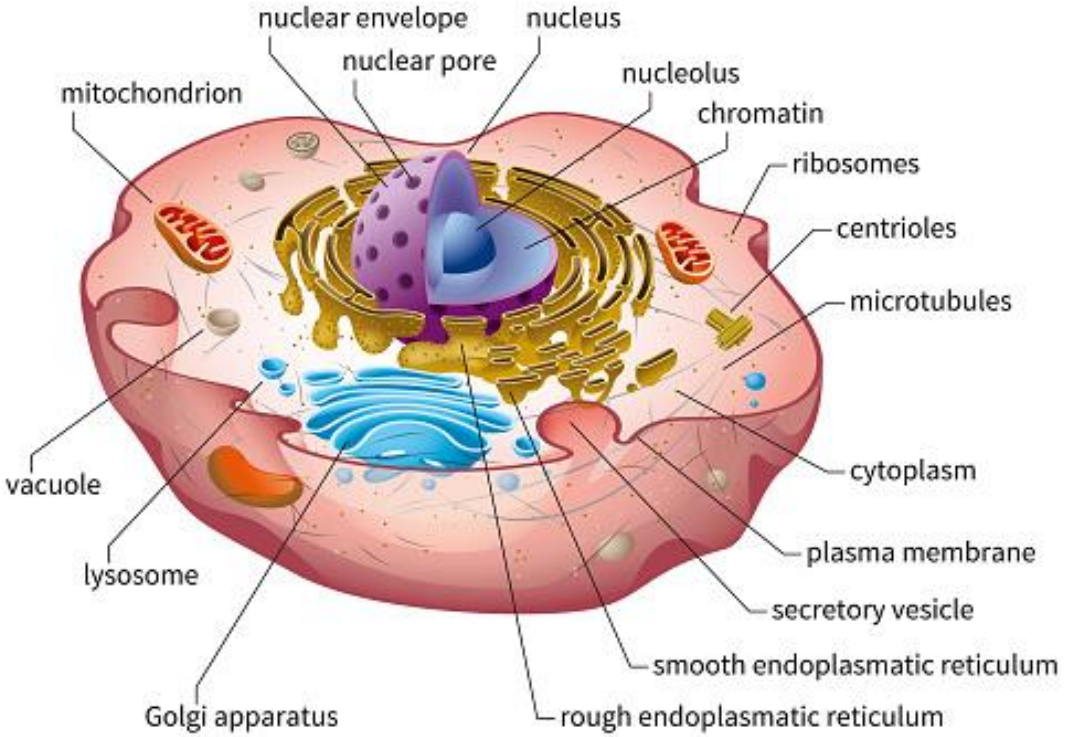
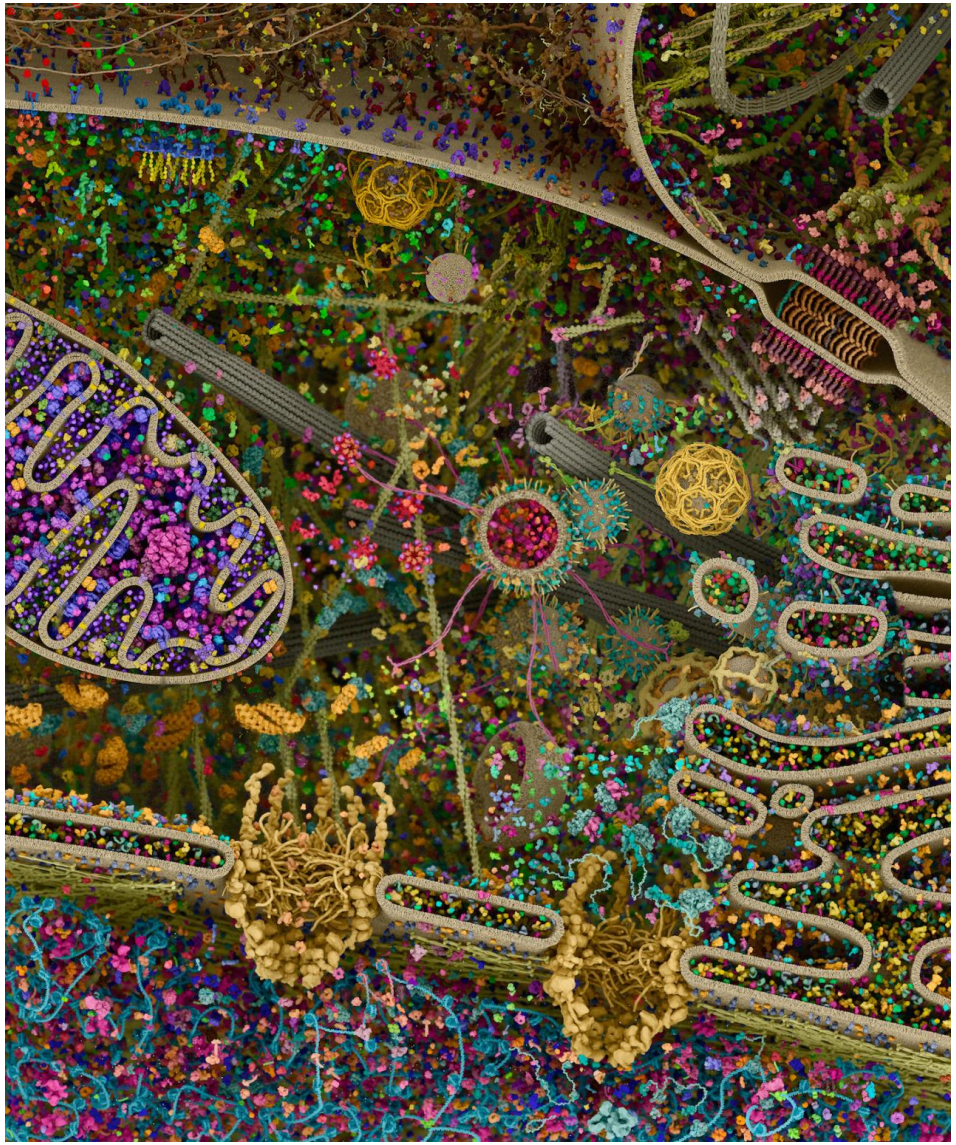
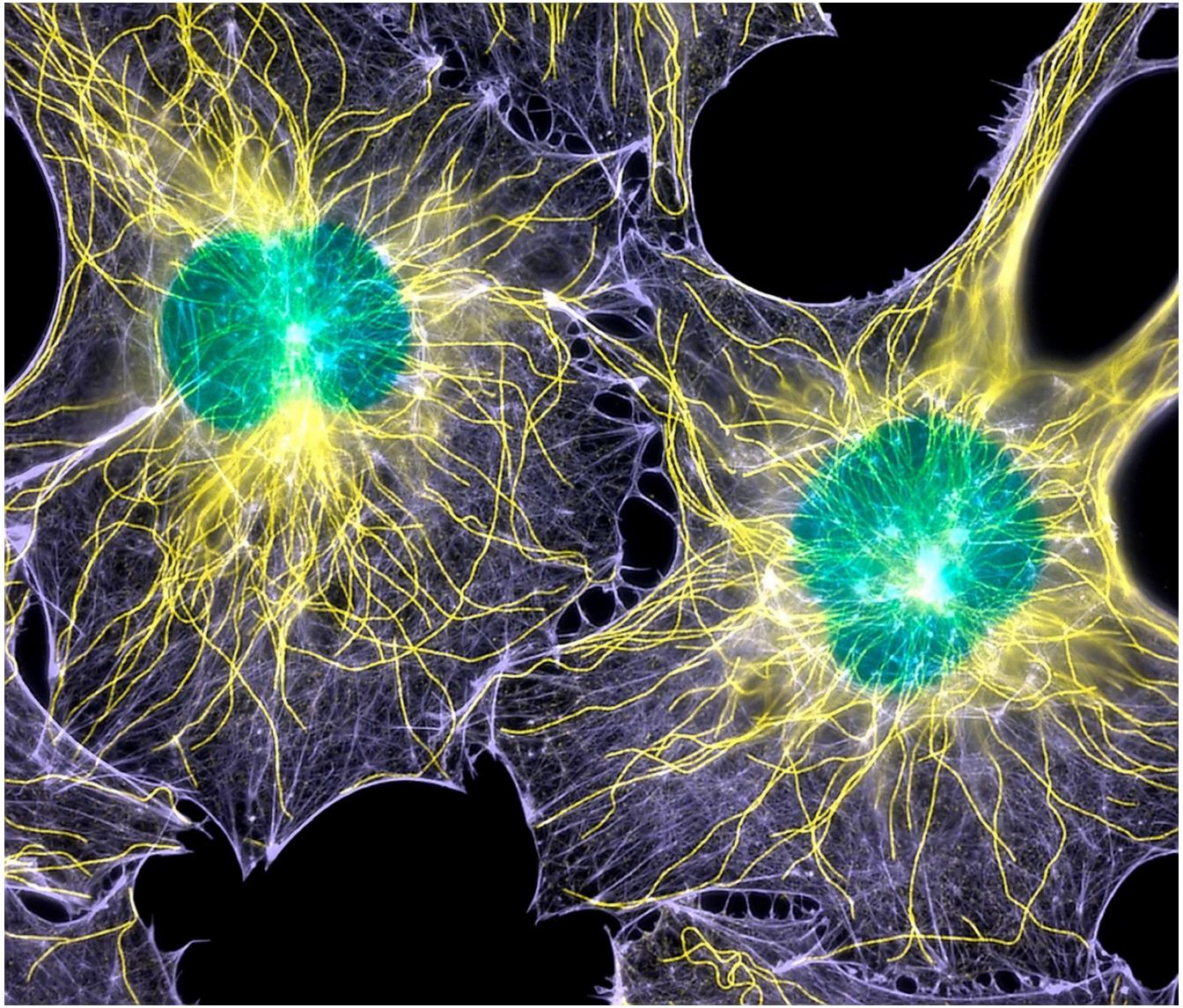
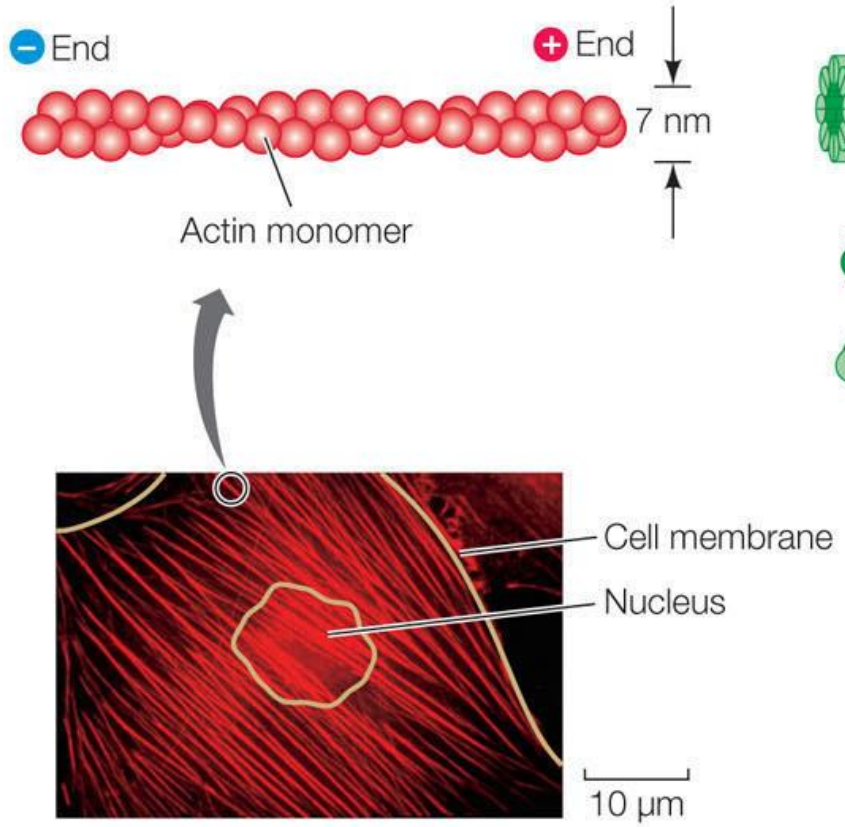
chloroplast
lysosome
ribosomes
mitochondrion
rough endoplasmic reticulum
smooth endoplasmic reticulum
vacuole
nucleoplasm
peroxisome
nucleolus
nuclear envelope
nucleus
nuclear pore
cell wall
plasmodesma
cell membrane
vesicle
Golgi apparatus


nuclear envelope
nucleus
nuclear pore
nucleolus
mitochondrion
chromatin
ribosomes
centrioles
microtubules
vacuole
cytoplasm
plasma membrane
lysosome
secretory vesicle
smooth endoplasmatic reticulum
Golgi apparatus
rough endoplasmatic reticulum

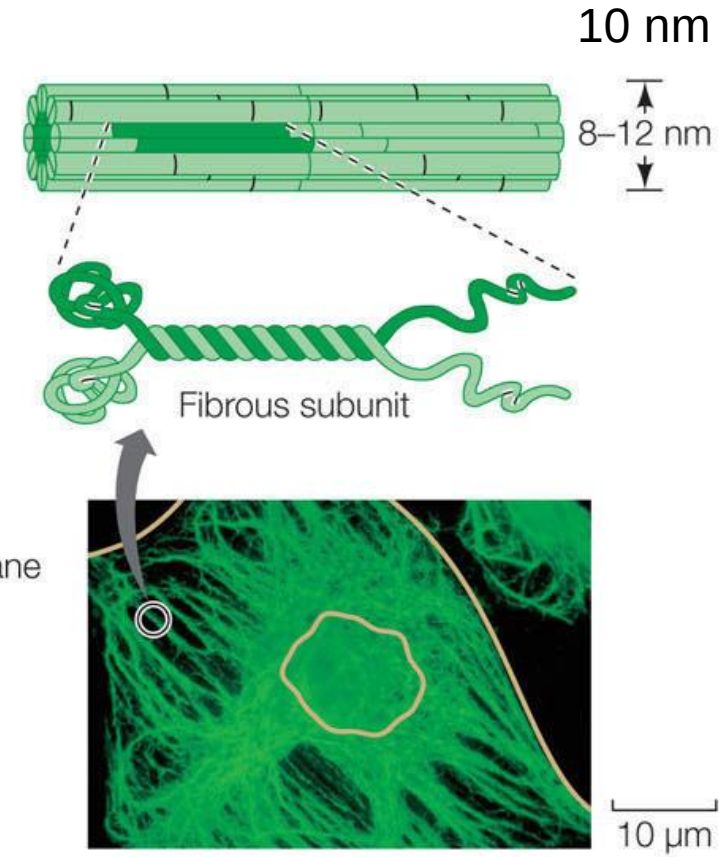




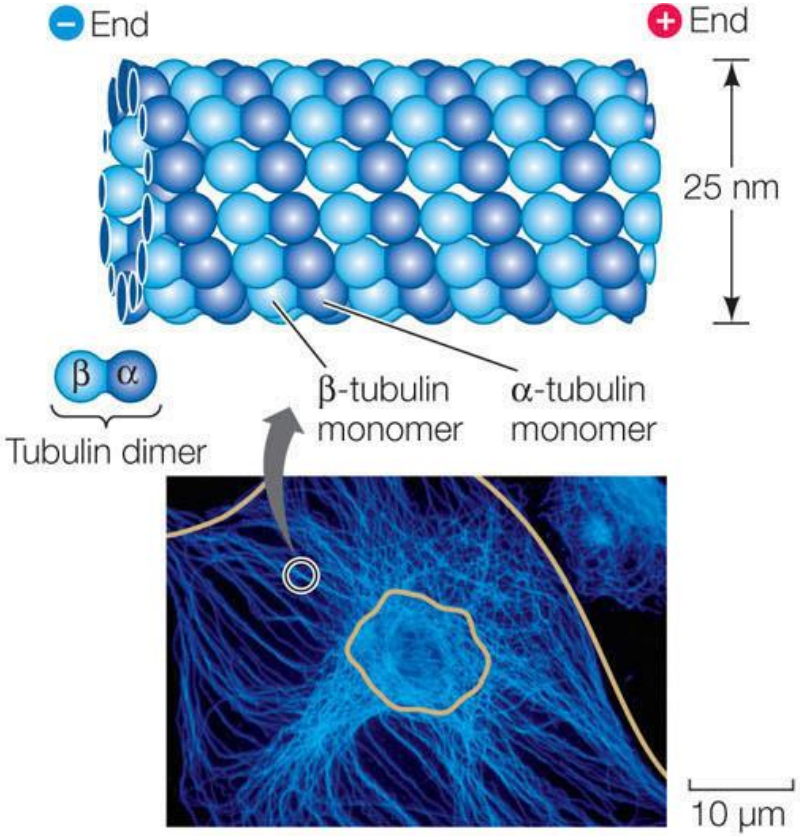
Actin filaments (microfilaments)



Intermediary filaments



Microtubules



Main functions of the cytoskeleton

Morphology and structural organization

- Animal cell morphology including cytoplasmatic protrusions
- Internal organization of the cell
- Mechanical strength

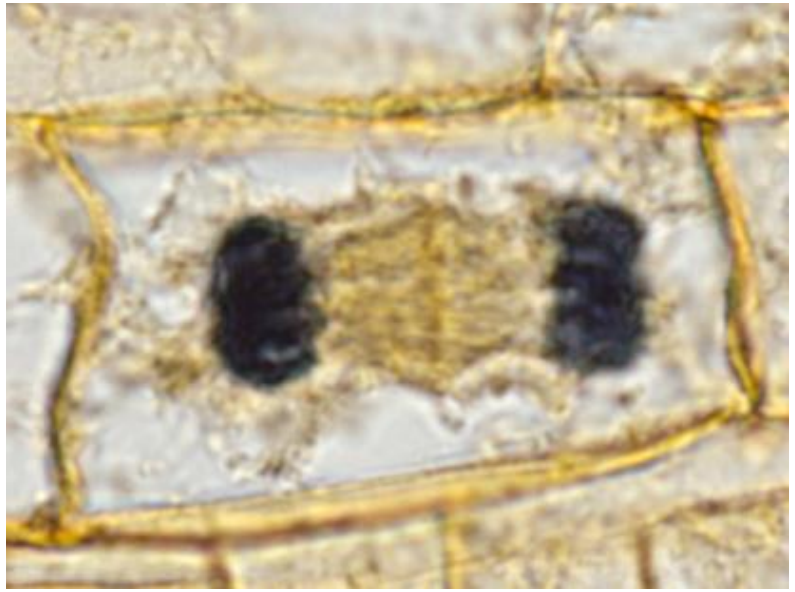
Movement and locomotion

- Intracellular transport
- Cytoplasmic streaming
- Movement of chromosomes during anaphase
- Cell migration
- Ciliary and flagellar movement
- Muscle contraction

Visualization of the cytoskeleton

Conventional light microscopy

- Insufficient contrast
- Only microtubules partially observable in some cases, e.g., anaphase/telophase



Fluorescence microscopy

- Widefield fluorescence microscopy
- **Confocal microscopy**
- **Super-resolution microscopy**

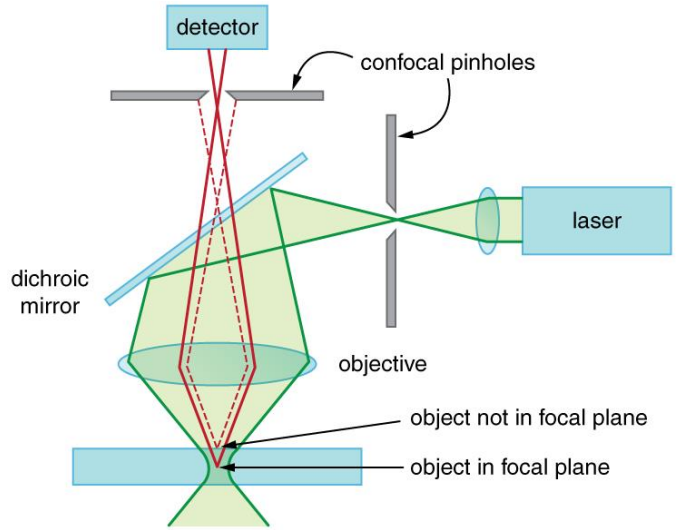
Methods of visualization

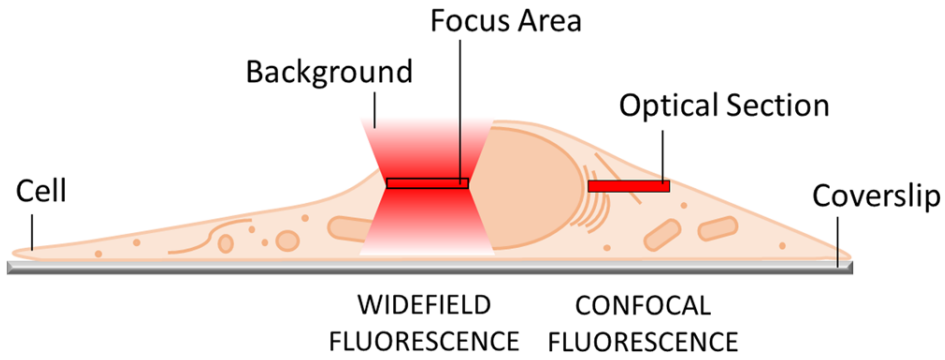
- **Fluorochrome-conjugated antibodies** (direct and indirect immunofluorescence)
- **Fluorochrome-conjugated cytoskeletal drugs** (poisons)
- **Tagging proteins with GFP and its derivatives** or **with mFruit proteins** (genetic manipulation)

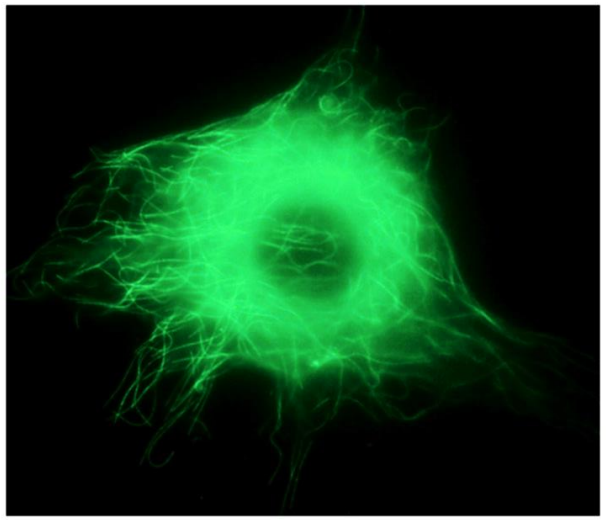
Fluorescence microscopy

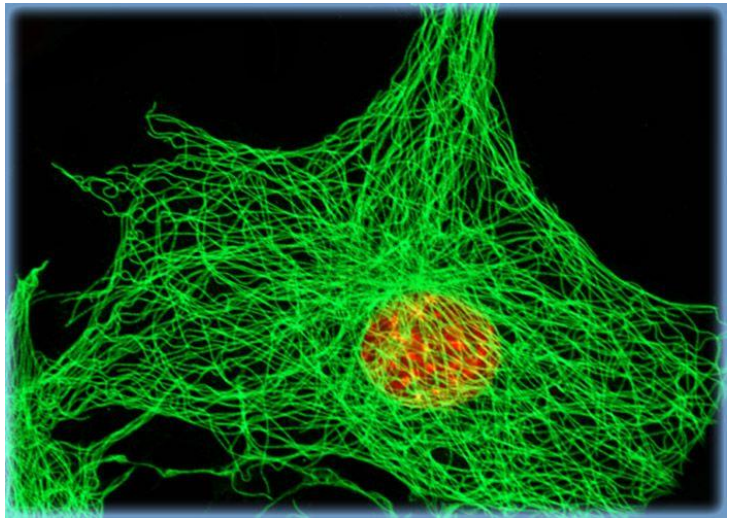
Confocal vs. widefield

- Pinholes: **block out-of-focus signal**
- Allows **optical sectioning**: Z-stack, 3D projection

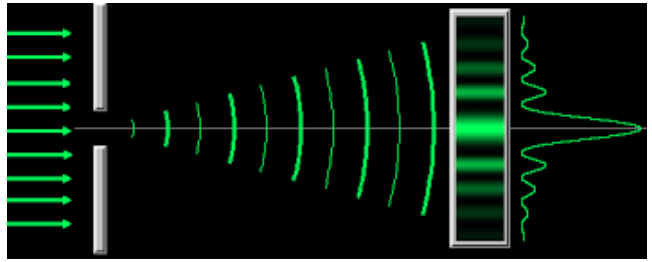






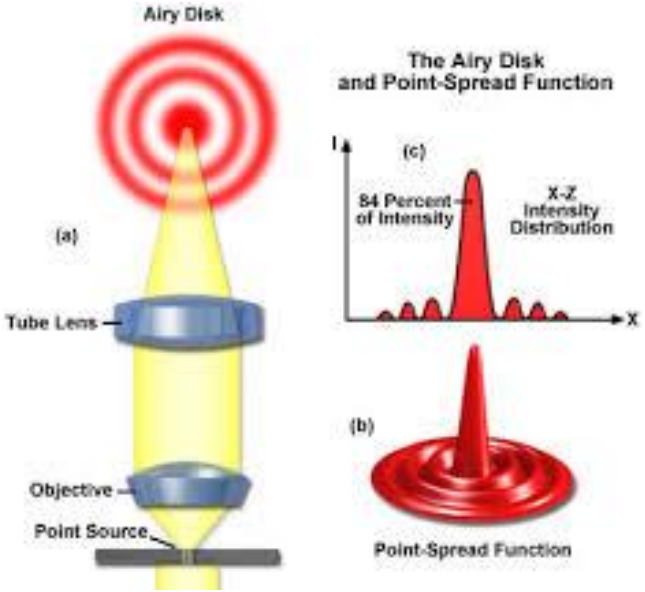


Fluorescence microscopy

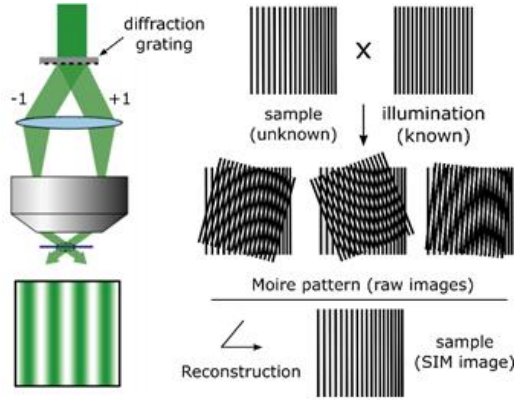


Super-resolution vs. confocal

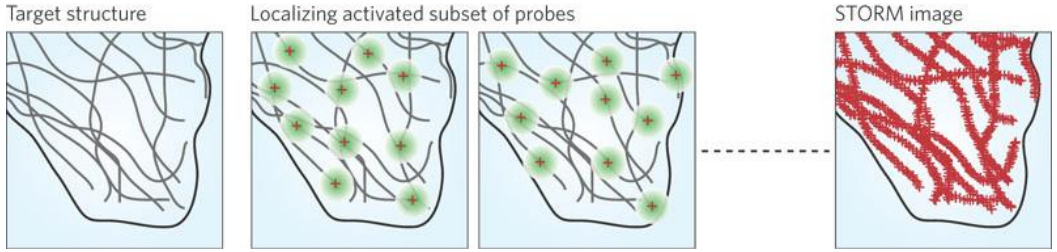
- Different approaches to overcome diffraction limit (200 nm)
- Often faster imaging (widefield microscopy) / Demanding on image processing time



Structured Illumination Microscopy (SIM):

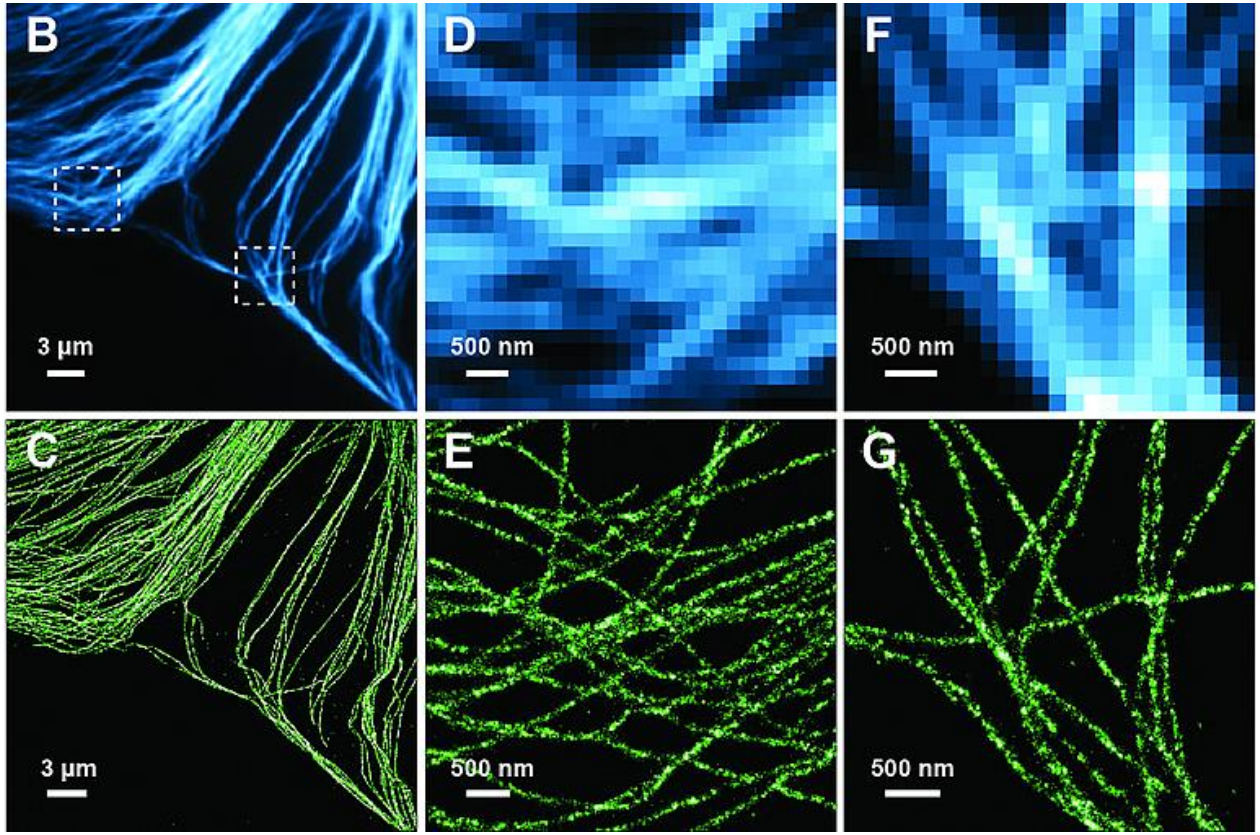


Single-molecule approaches (e.g., STORM):

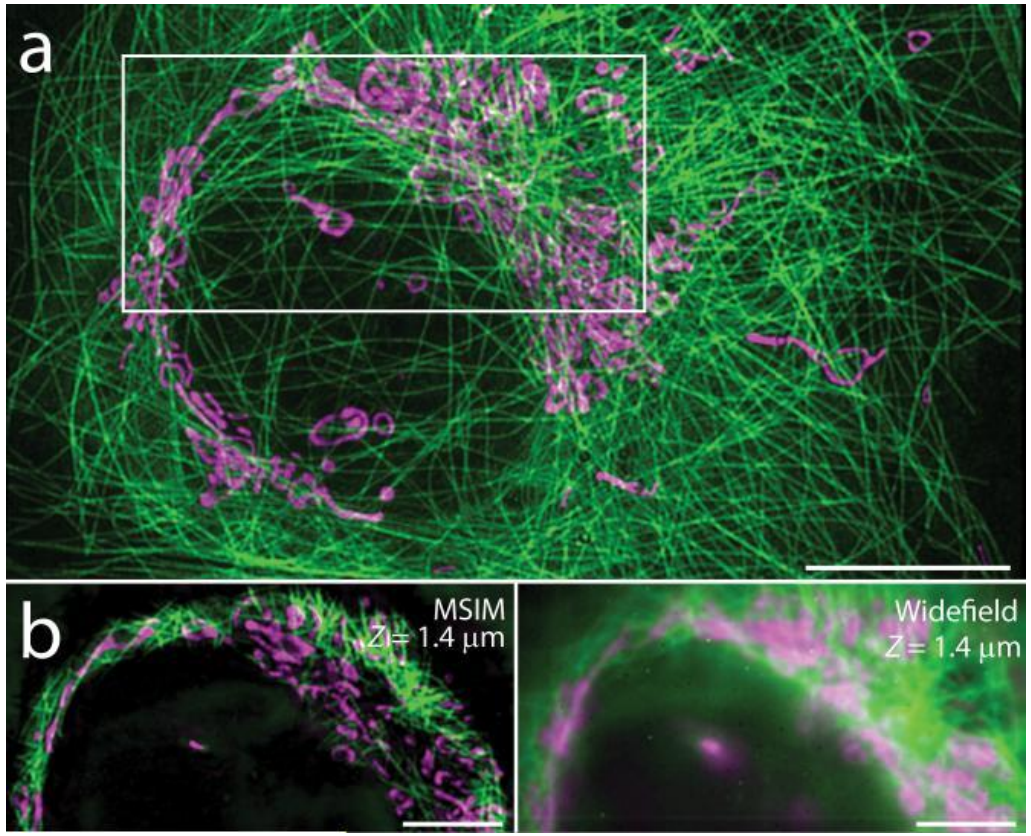


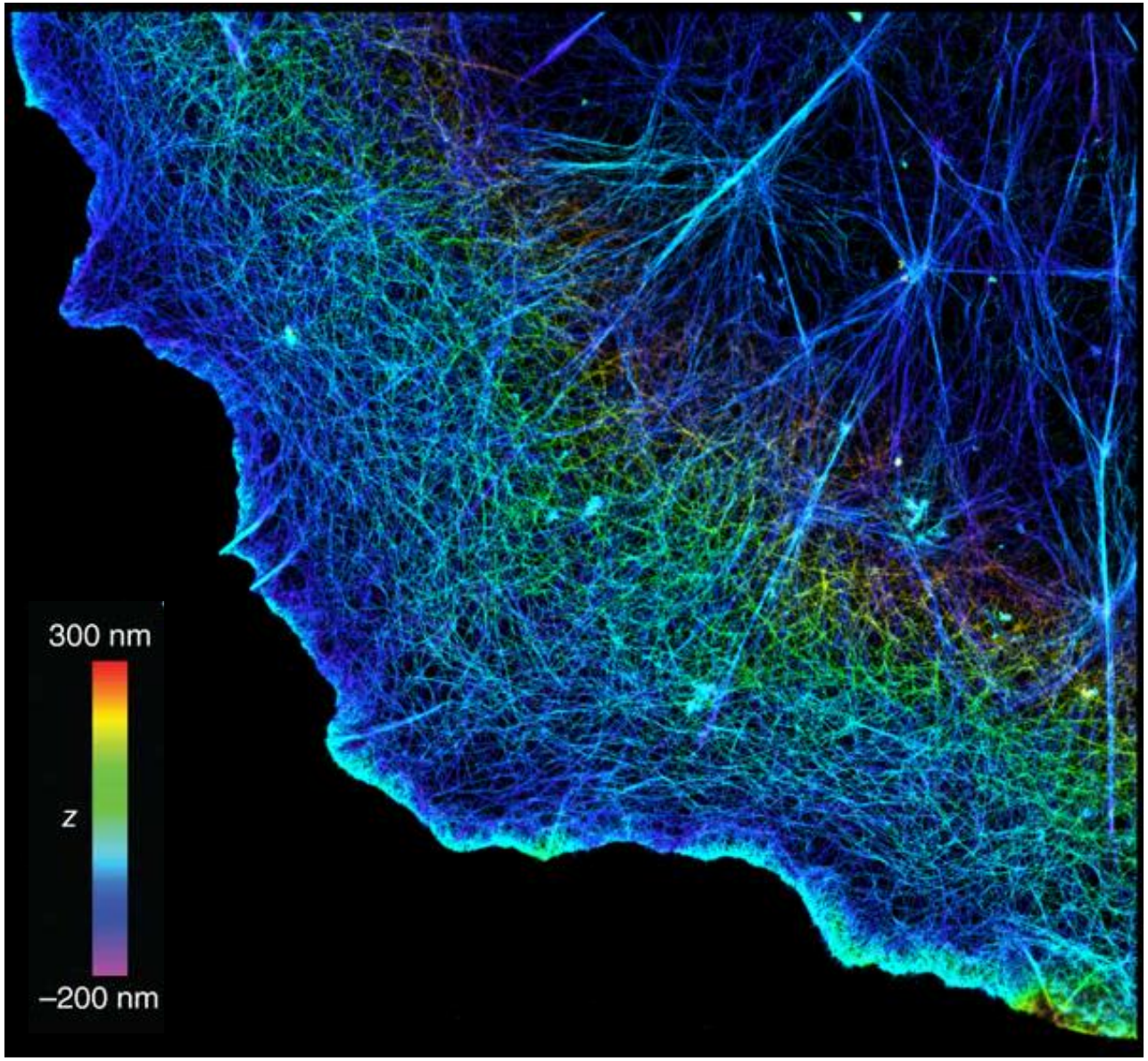
Super-resolution fluorescence microscopy

Tubulin (confocal, *top* vs. STORM, *bottom*)



Tubulin, mitochondria (SIM vs widefield)



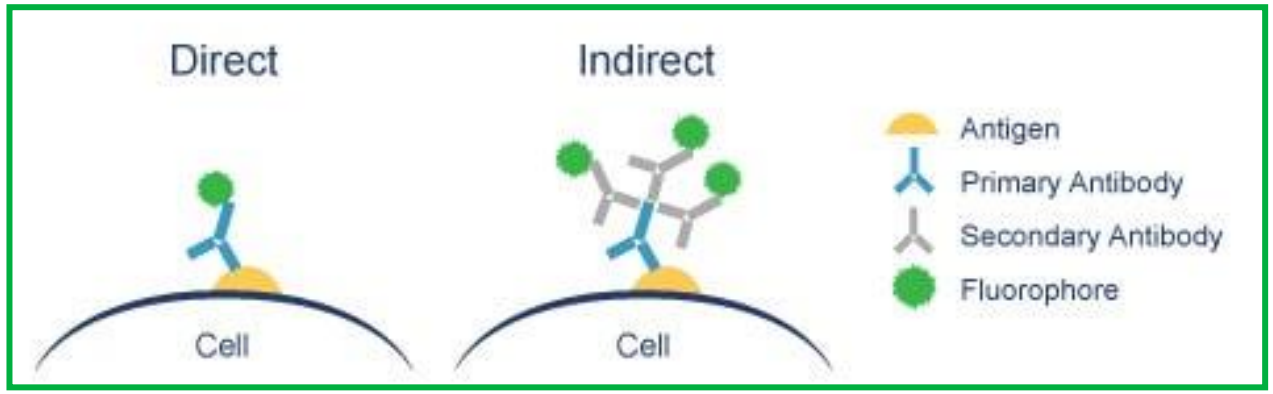


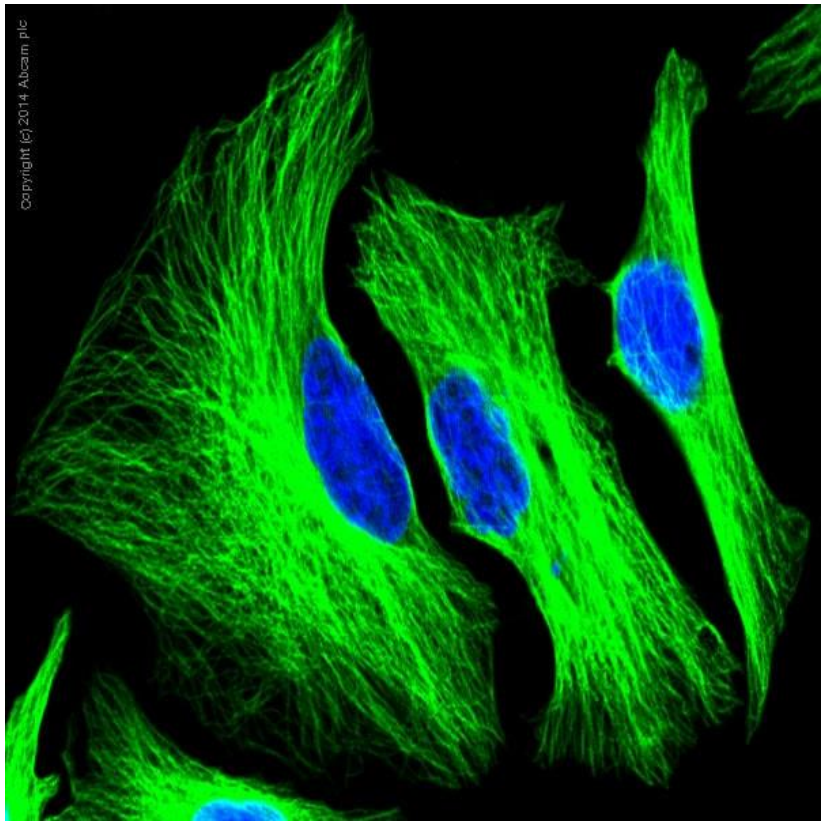
Super-resolution fluorescence microscopy

- Superior optical sectioning: 3D reconstruction and projection

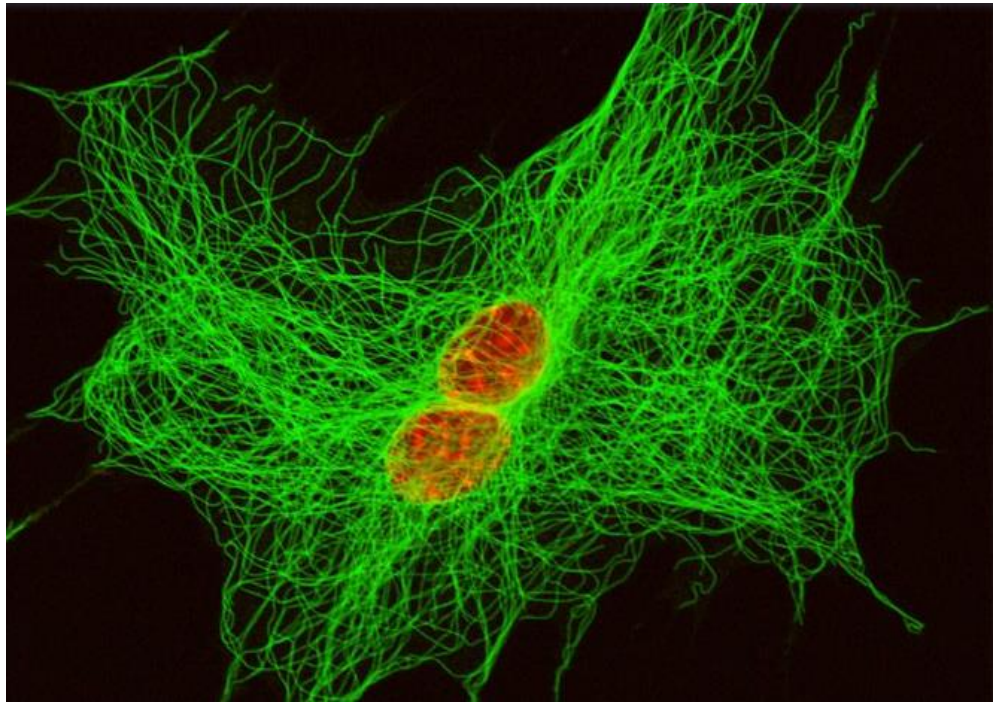
Actin
(Z-positons color-coded; 3D STORM)

Immunofluorescence





Tubulin / DNA
Alexa Fluor® 488 Anti-tubulin antibody



Tubulin
DNA

Primary anti-tubulin antibody
& Alexa Fluor® 488 secondary antibody

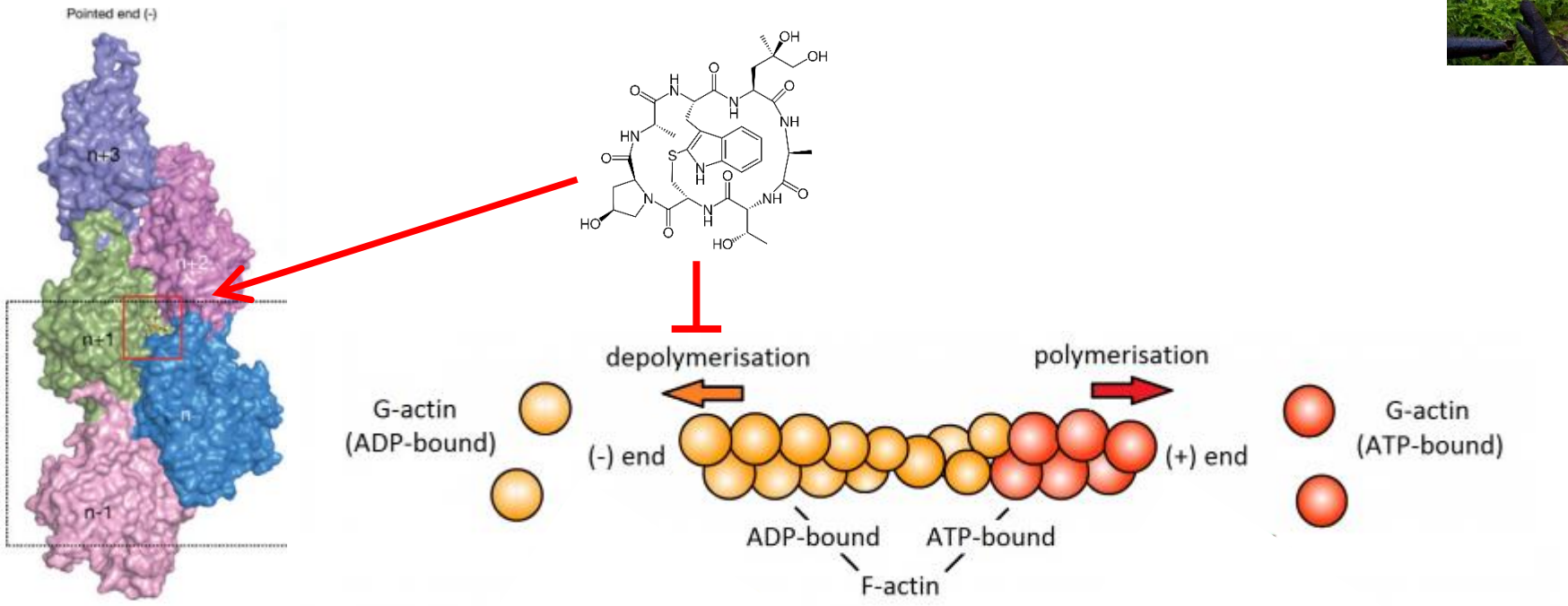
Fluorochrome-conjugated cytoskeletal drugs

- **Prevent depolymerization** / polymerization (not useful)
- Naturally occurring fatal poisons – importance of cytoskeleton dynamics

- **Phalloidin: Actin filaments**
- **Paclitaxel and derivates: Microtubules**

Phalloidin

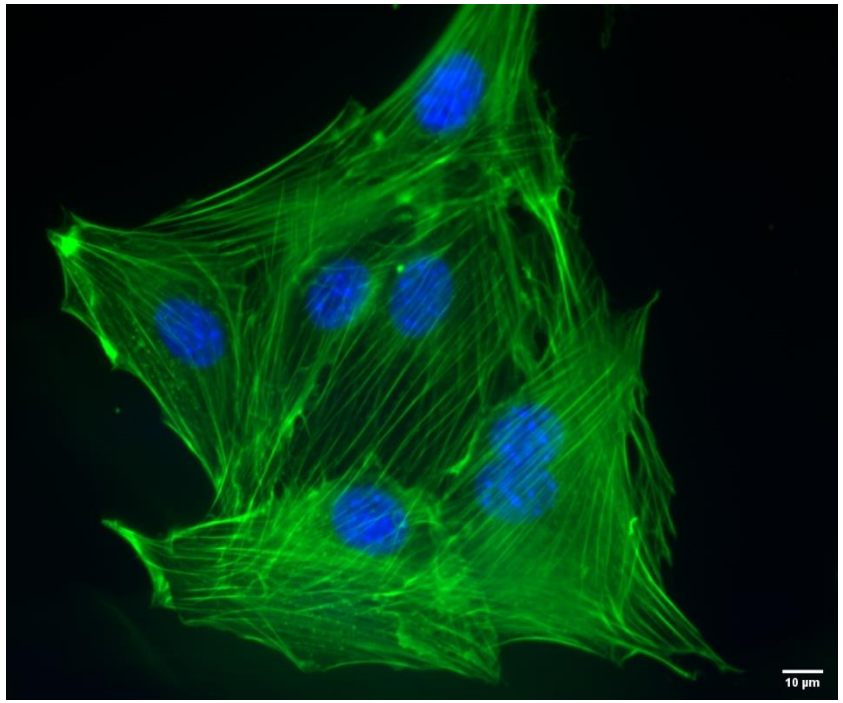
- Toxin found in *Amanita phalloides* (death cap mushroom)
- Binds F-actin and stabilizes actin filaments



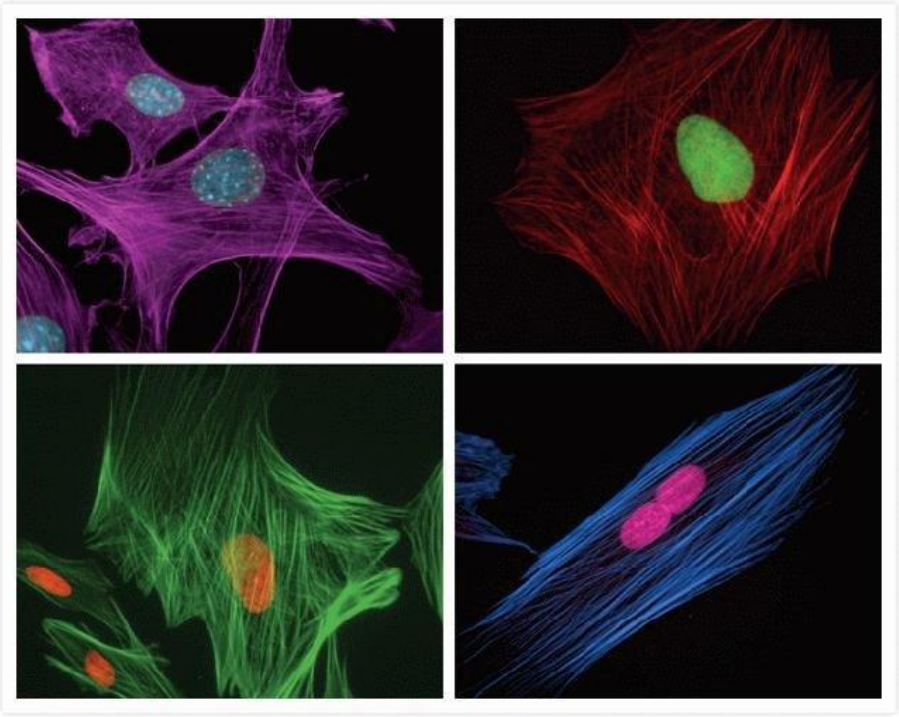
Phalloidin – visualization of actin filaments

- Conjugated with modern photostable fluorochromes
- Straightforward (direct) use as a **fluorescent probe**

CoraLite®488-Phalloidin, DNA



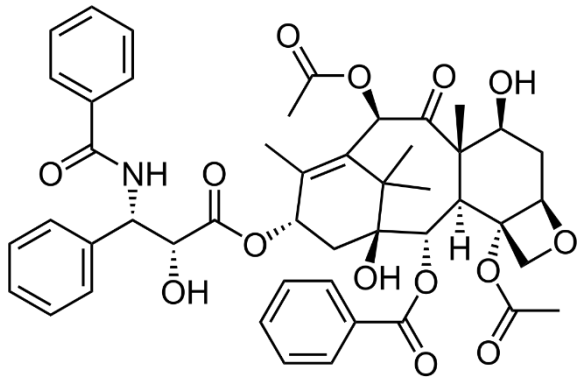
Different Alexa Fluor®-phalloidin conjugates

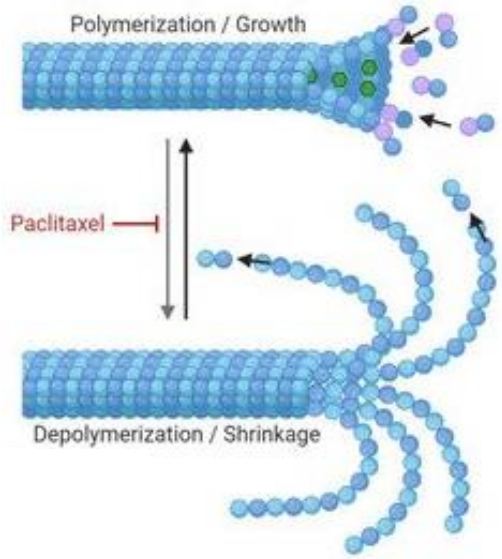


Alexa Fluor® 680
Alexa Fluor® 568
Alexa Fluor® 488
Alexa Fluor® 350

Paclitaxel (brand name: Taxol)

- Taxane alkaloid found in Taxus brevifolia (the pacific yew tree)

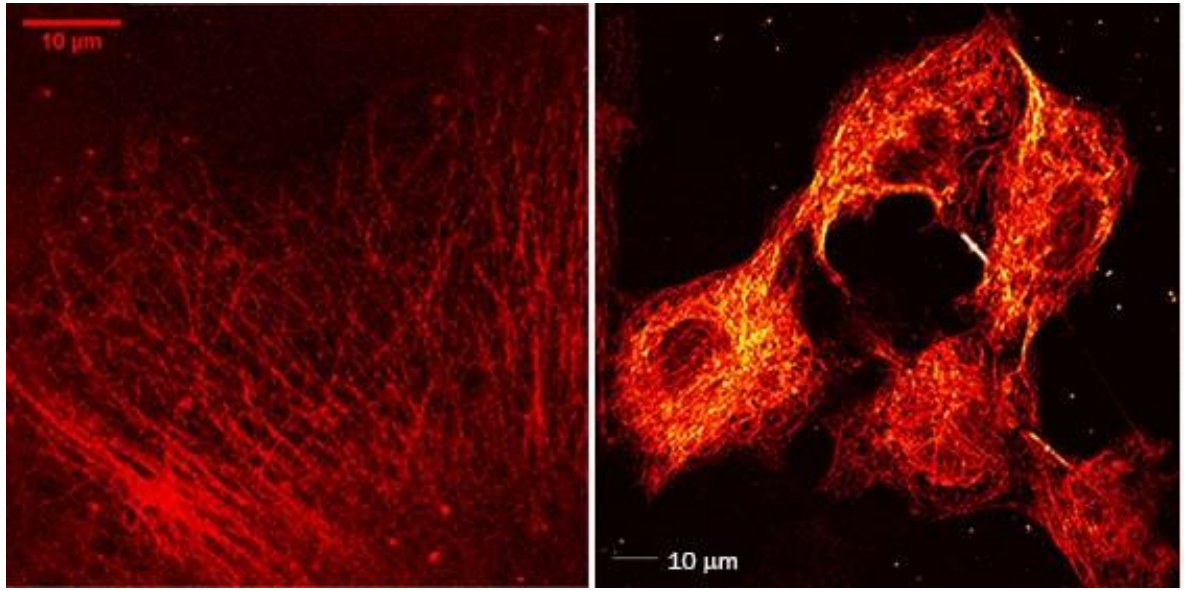




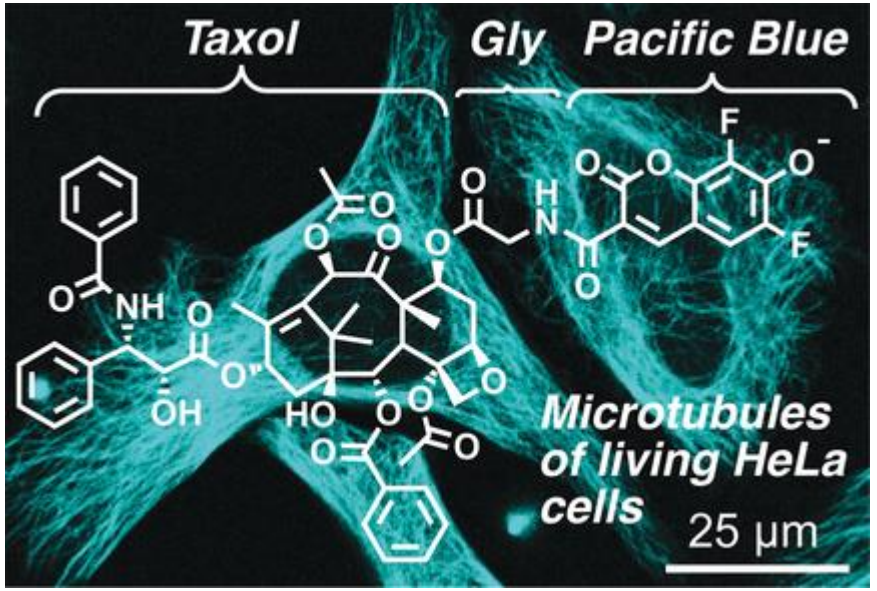
- Induces mitotic arrest / apoptosis (prolonged)

Paclitaxel-based fluorescent probes

- Useful for live cell imaging



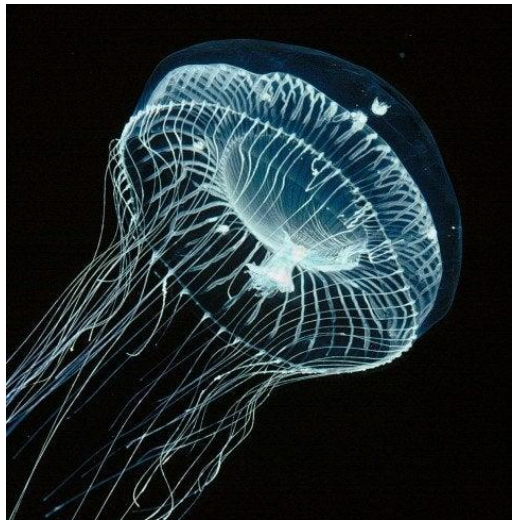
Taxol Janelia Fluor® 646



Lee et al. Angew Chem Int Ed Engl. 2017;56(24):6927-6931.

Tagging proteins with GFP and derivatives

- **G**reen **F**luorescent **P**rotein
- Isolated from ***Aequorea Victoria*** (crystal jelly fish)



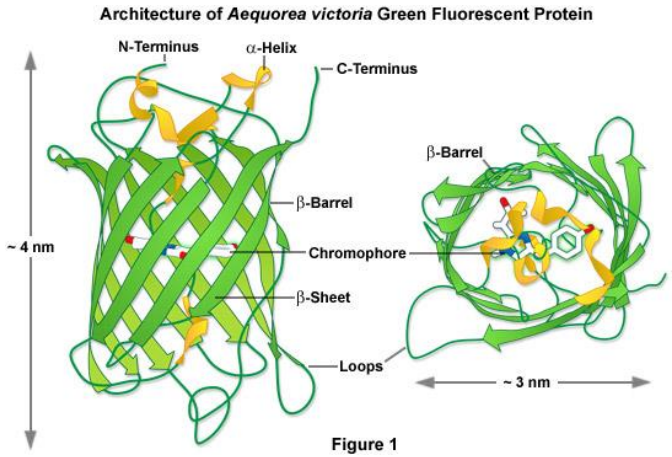


Figure 1

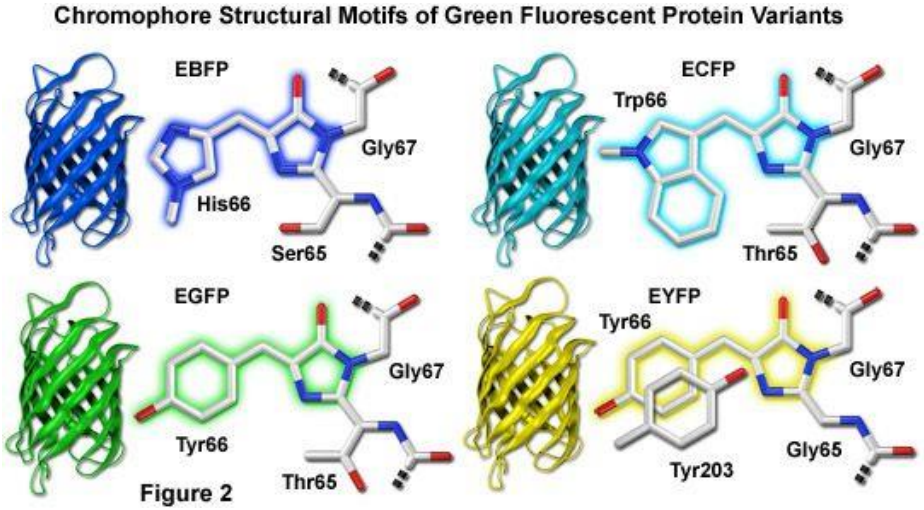


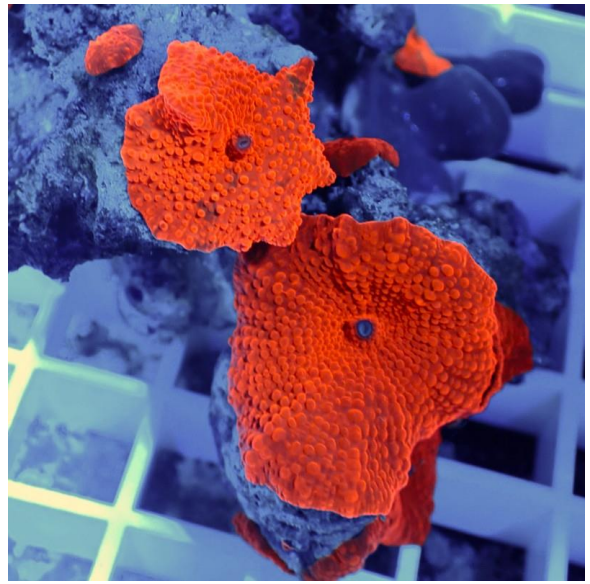
Figure 2

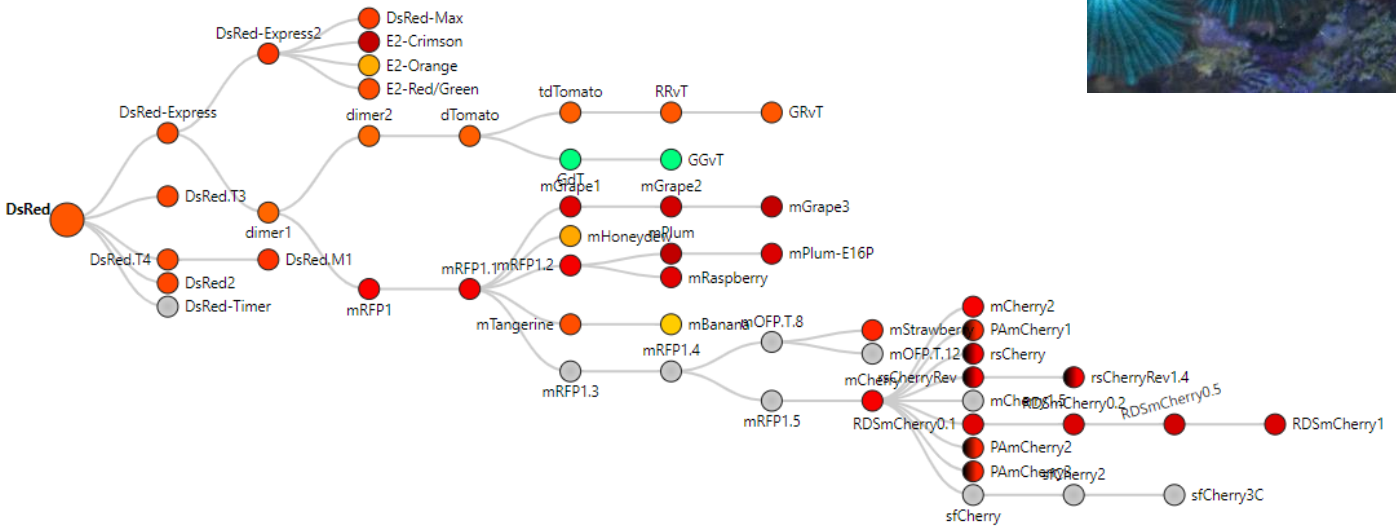
Increased photostability and brightness

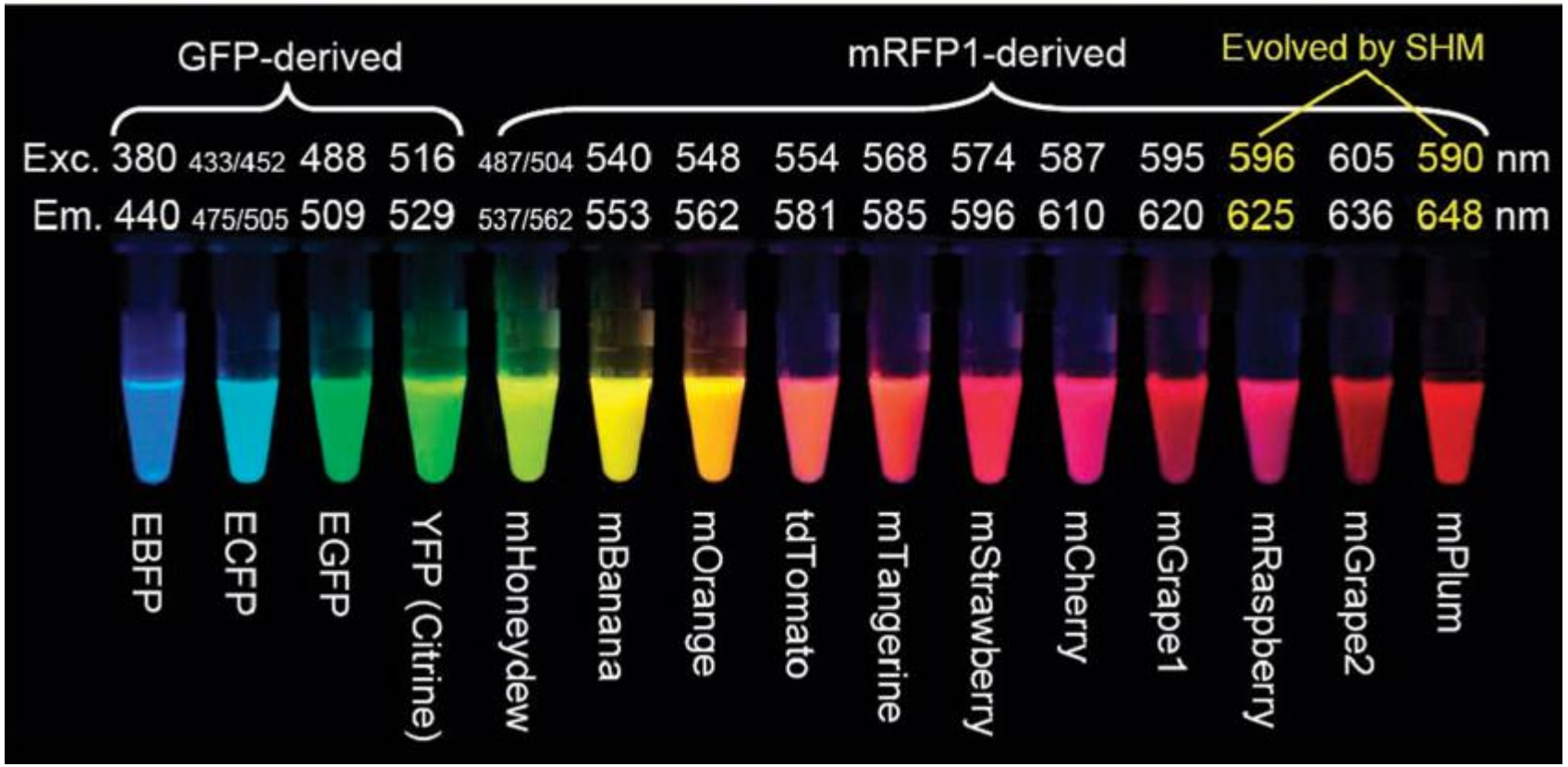
- EGFP – enhanced green FP
- EBFP – enhanced blue FP
- ECFP – enhanced cyan FP
- EYFP – enhanced yellow FP…

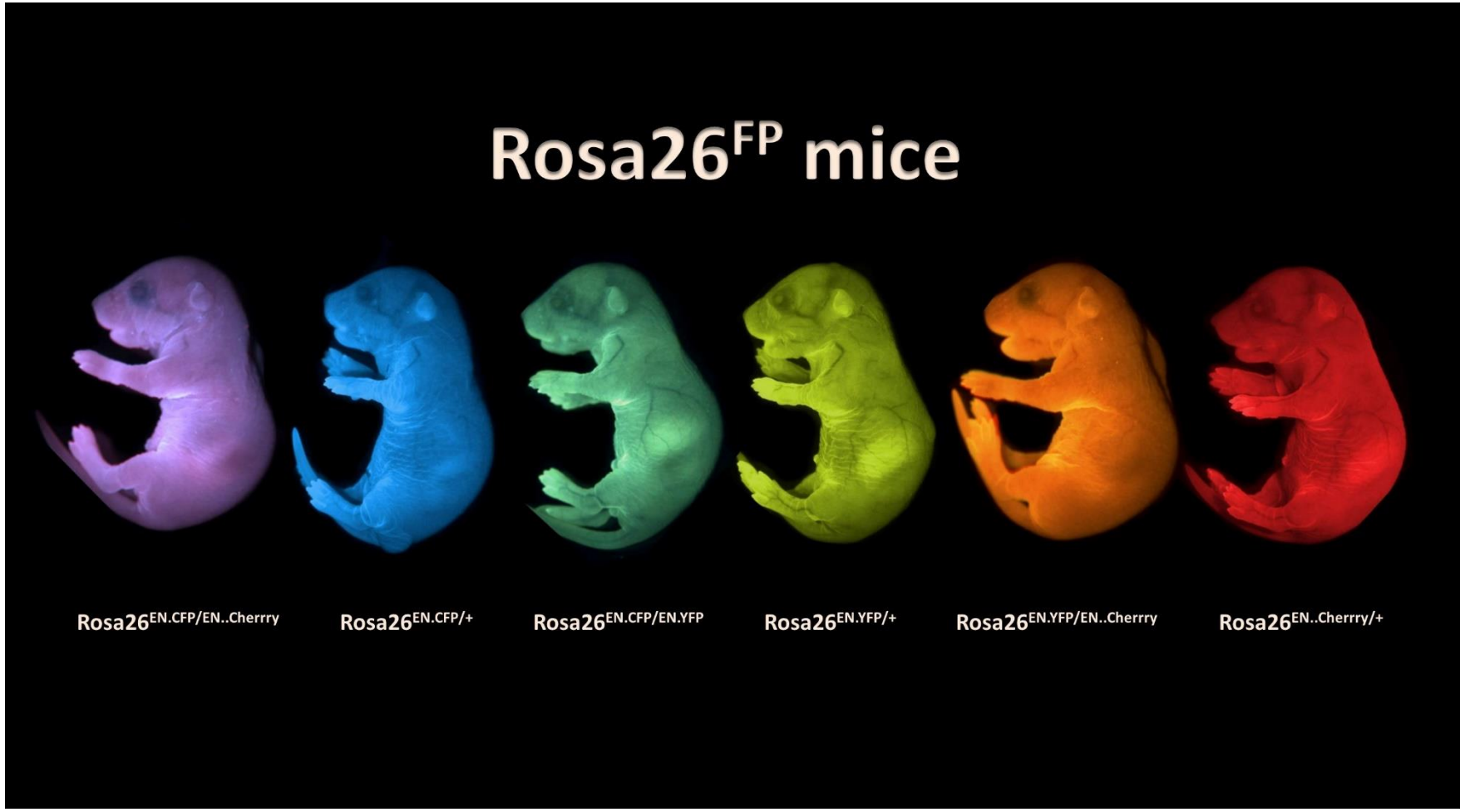
Tagging with mFruit proteins

- **Derivatives of DsRed** protein cloned from *Discosoma striata* (radiating mushroom coral)

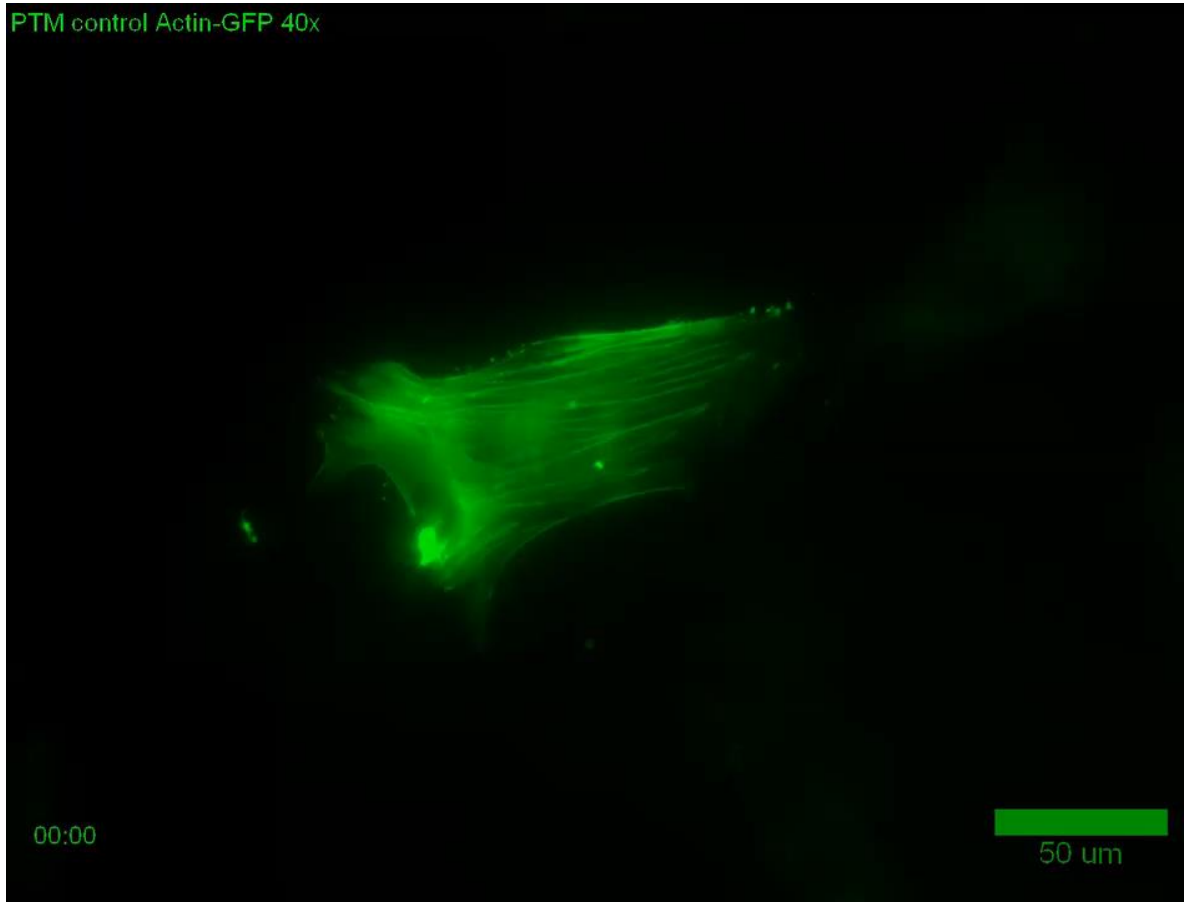




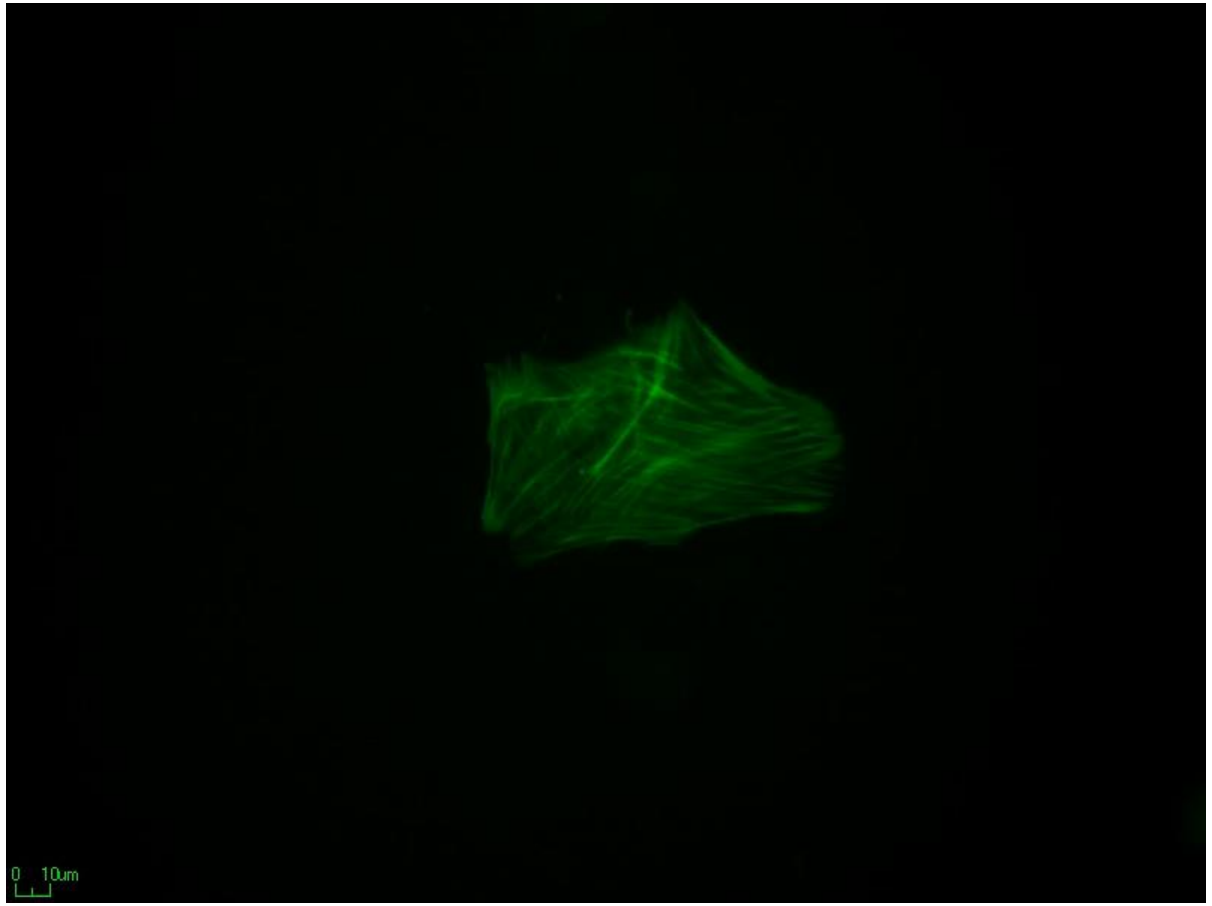

GFP-derived
mRFP1-derived
Evolved by SHM
Exc. 380 433/452 488 516 487/504 540 548 554 568 574 587 595 596 605 590 nm
Em. 440 475/505 509 529 537/562 553 562 581 585 596 610 620 625 636 648 nm
EBFP
ECFP
EGFP
YFP (Citrine)
mHoneydew
mBanana
mOrange
tdTomato
mTangerine
mStrawberry
mCherry
mGrape1
mRaspberry
mGrape2
mPlum


Rosa26FP mice
Rosa26EN.CFP/EN..Cherrry
Rosa26EN.CFP/+
Rosa26EN.CFP/EN.YFP
Rosa26EN.YFP/+
Rosa26EN.YFP/EN..Cherrry
Rosa26EN..Cherrry/+

Actin-GFP in porcine cells (30 min time-lapse)



Actin-GFP in porcine cells (72 h time-lapse)



Microtubules

Microtubular structures

- Microtubule network within the cytoplasm
- Spindle apparatus (mitotic spindle, meiotic spindle)
- Microtubule-organizing center (MTOC)
- Primary cilia, motile cilia and flagella – animal cells

& their functions

- Intracellular transport of cell structures
- Movement of chromosomes during anaphase
- Cell locomotion – ciliary and flagellar movement;
- Cell signaling (primary cilium)
- Formation of axons and axon transport

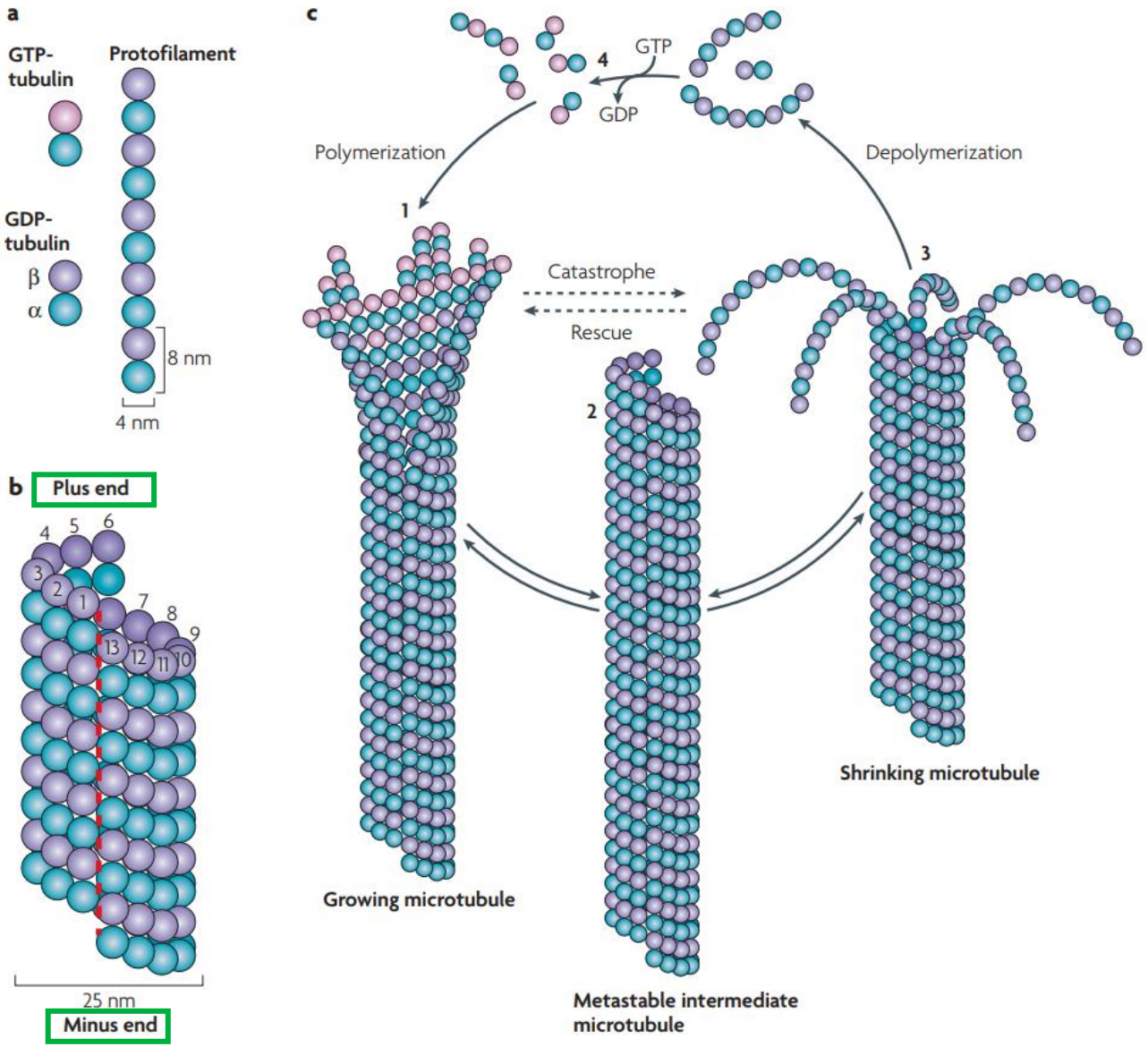
The structure of microtubules

Tubulin

- microtubule: **α-tubulin** & **β-tubulin heterodimers**
- **γ-tubulin** only in MTOCs – essential for microtubule nucleation

Microtubule-organizing center

- **Centrosome** (animal cells, some fungi)**,** only **γ-tubulin ring complex** in plant cells**; basal bodies** (cilium, flagellum)

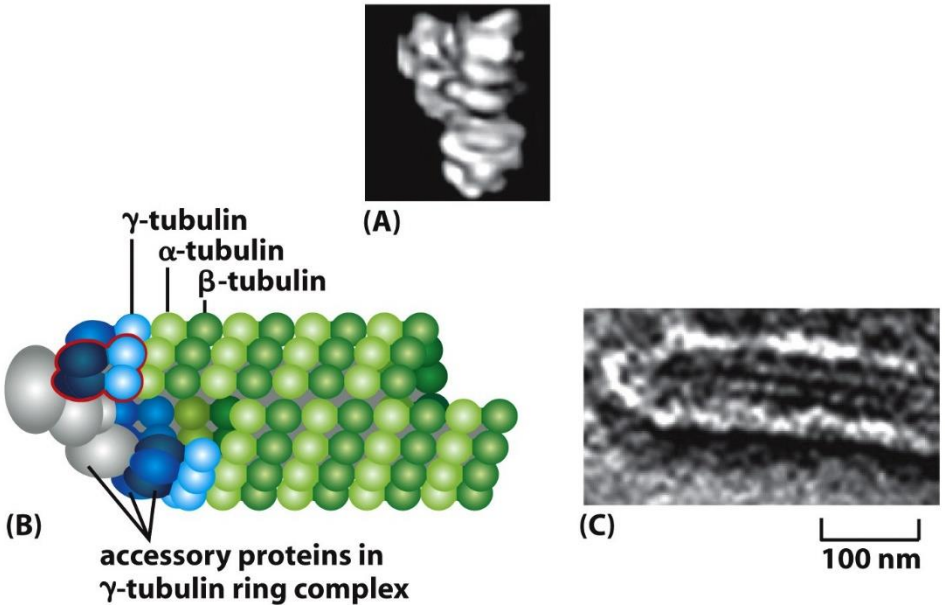


Microtubule organization & dynamics

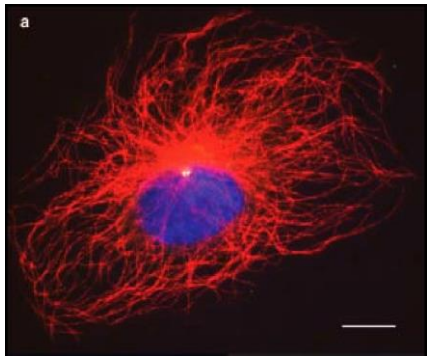
- **13 protofilaments:** polymerization of α/β-tubulin dimers
- Helical structure, **25 nm**
- **Polarization: + end, - end**
  - Constant balance of polymerization & depolymerization
  - Polymerization: hydrolysis of GTP at β-tubulin → GDP metastable state
  - **Growth/shrinkage: + end**
  - **Nucleation: - end**

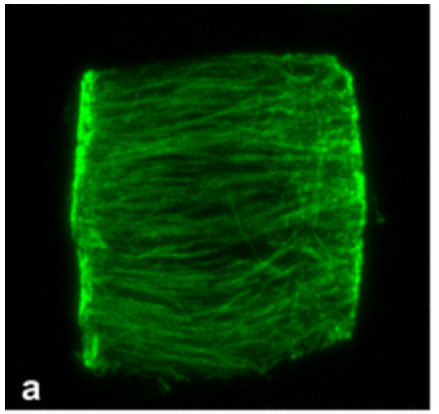
γ-tubulin ring complex: MT nucleation

- **Minus end of the microtubule**



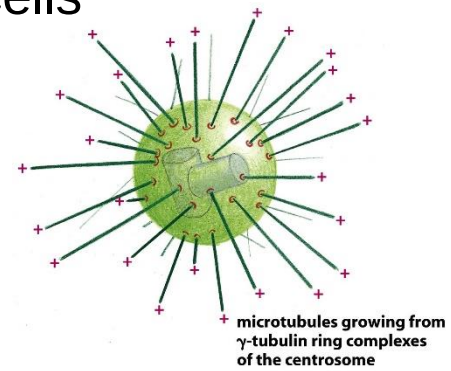
MTOCs





Centrosomal

- Animal cells
- Fungi

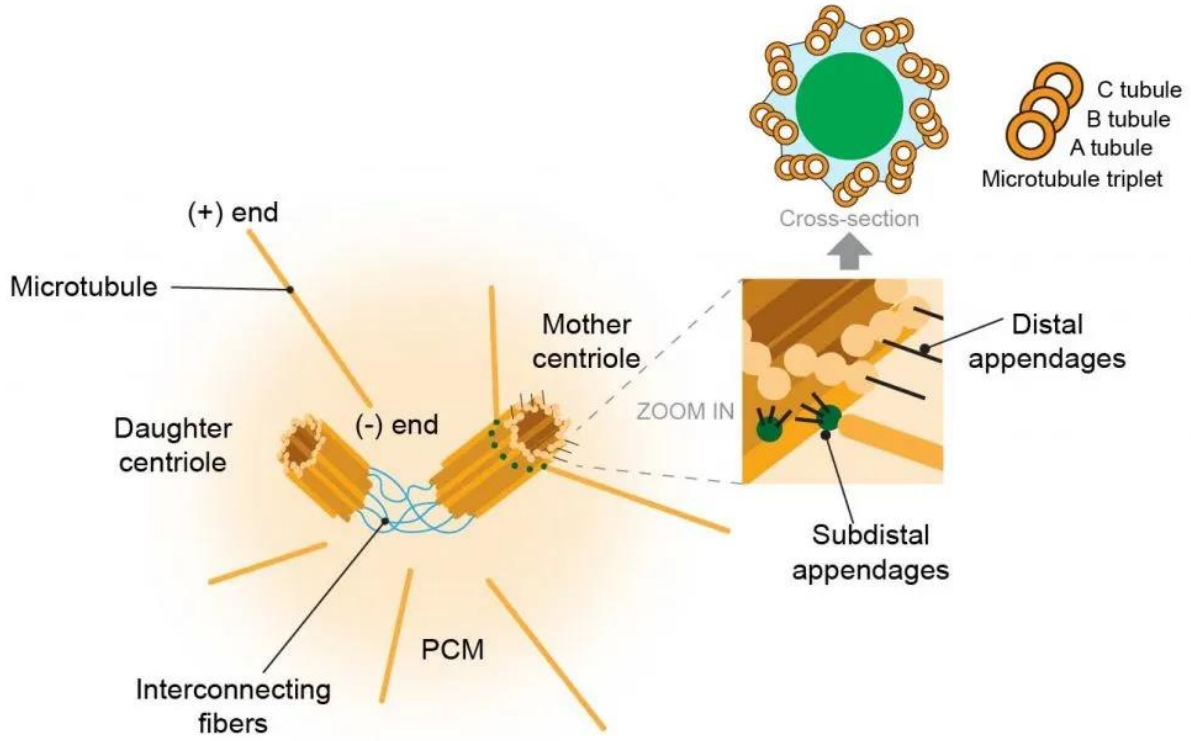


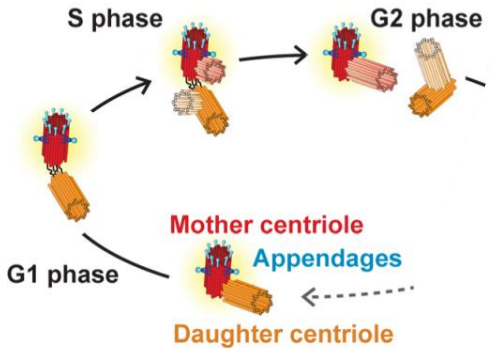
Non-centrosomal

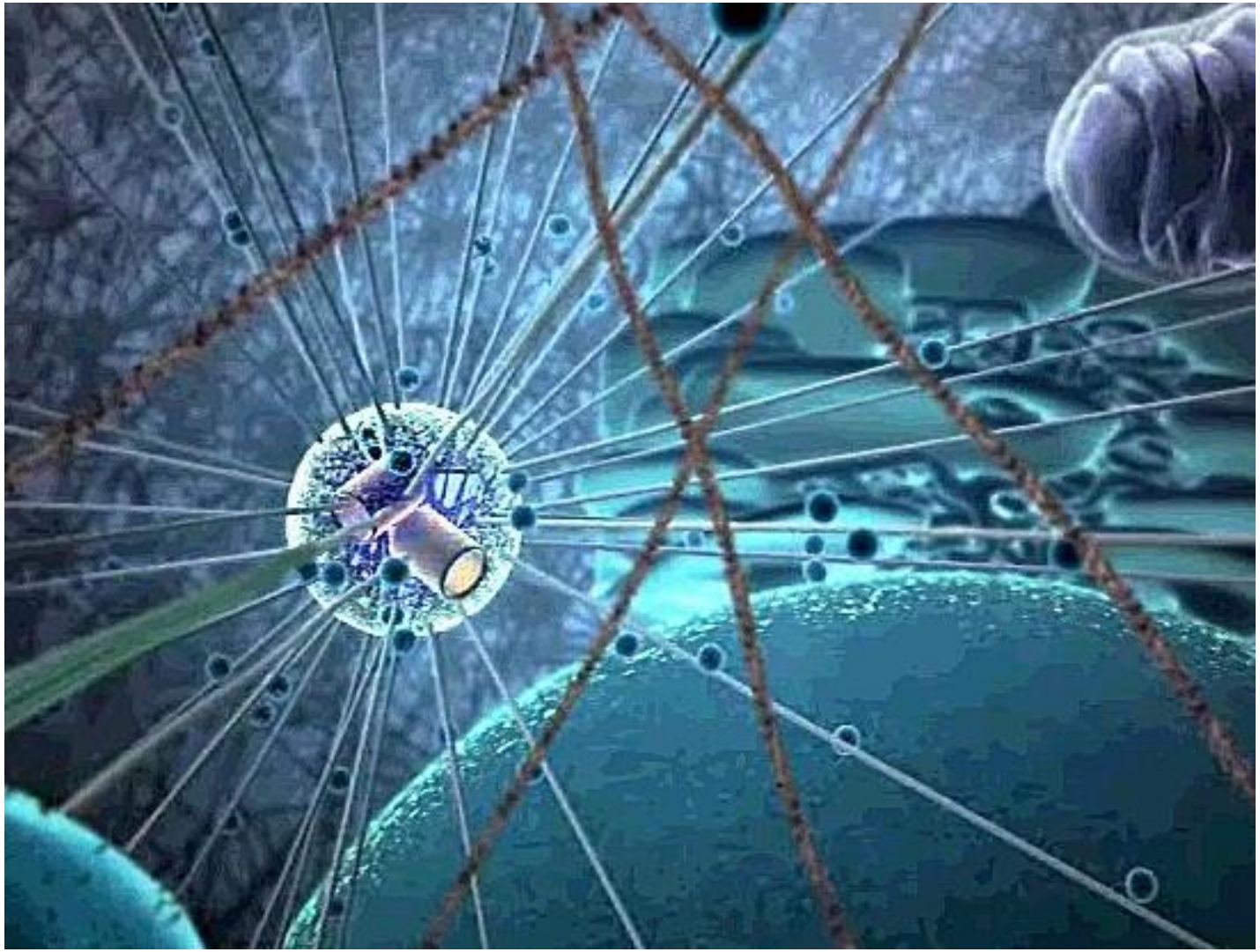
- Plant cells
- Fungi

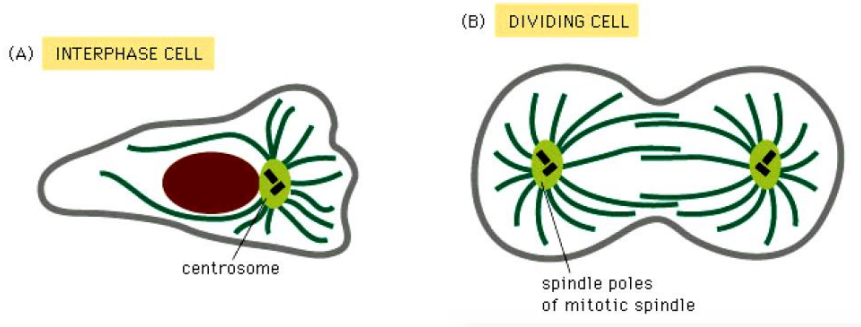
Centrosome

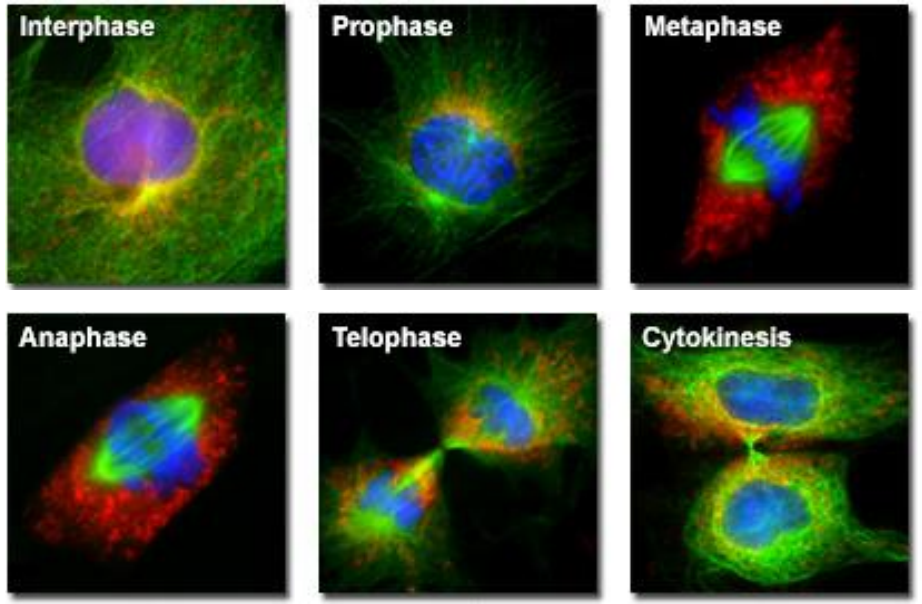
- 2 **centrioles** (**mother** & daughter) and **pericentriolar material**
- **9 triplets of microtubules** (A,B,C)
- **9+0 structure**
- Duplication during cell cycle: daughter centriole separates → mother centriole





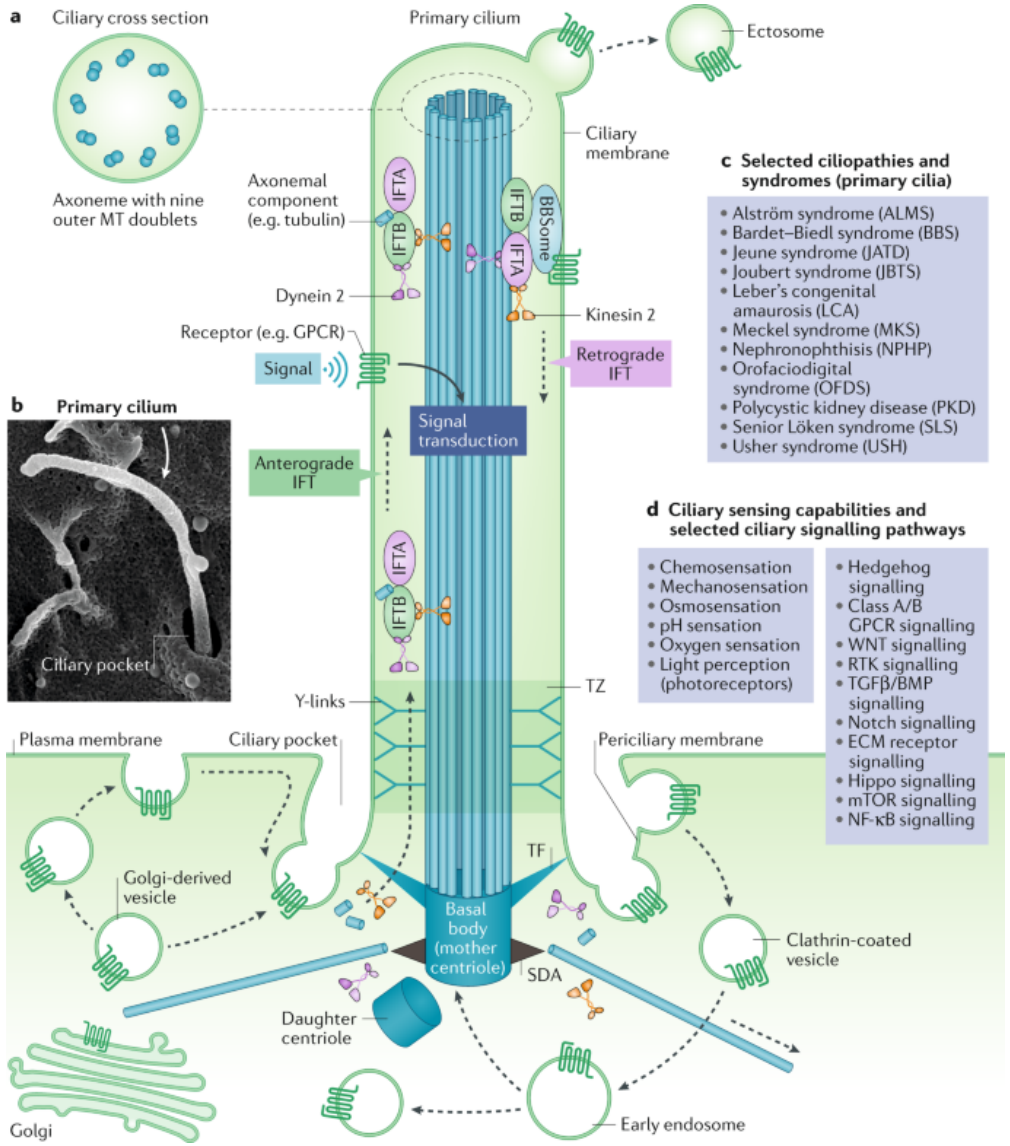



(A) INTERPHASE CELL
centrosome
(B) DIVIDING CELL
spindle poles
of mitotic spindle


Interphase
Prophase
Metaphase
Anaphase
Telophase
Cytokinesis

Primary cilium

- Non-motile cilium, **animal cells**
- **Basal body** = mother centriole, same structure (9 MT triplets, 9+0)
- Important for **cell signaling**
- Microtubules form **axoneme: 9 MT doublets, 9+0**
- Found on nearly every type of cell
- Most often only one primary cilium



Proteins interacting with microtubules

Non-motor proteins: Regulate the stability of microtubules

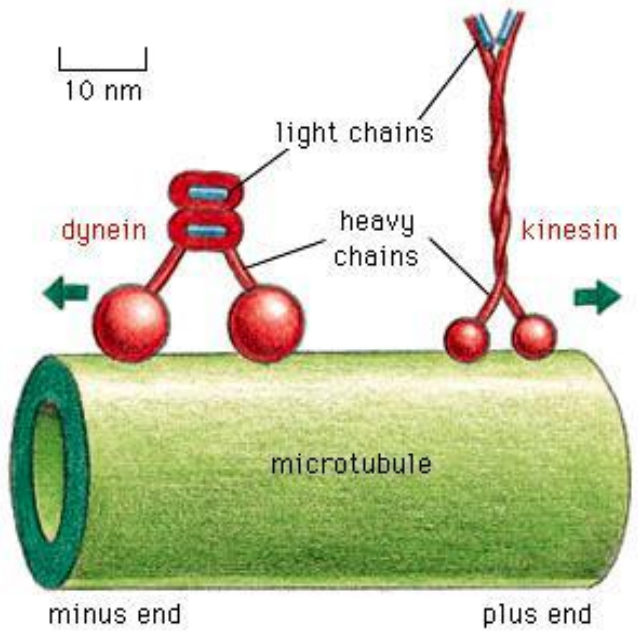
- **Microtubule-associated proteins (MAPs):** MAP1, MAP2…
- **Tau proteins** – in neurons, stability of axonal microtubules; abnormal phosphorylation → aggregation in Alzheimer's disease

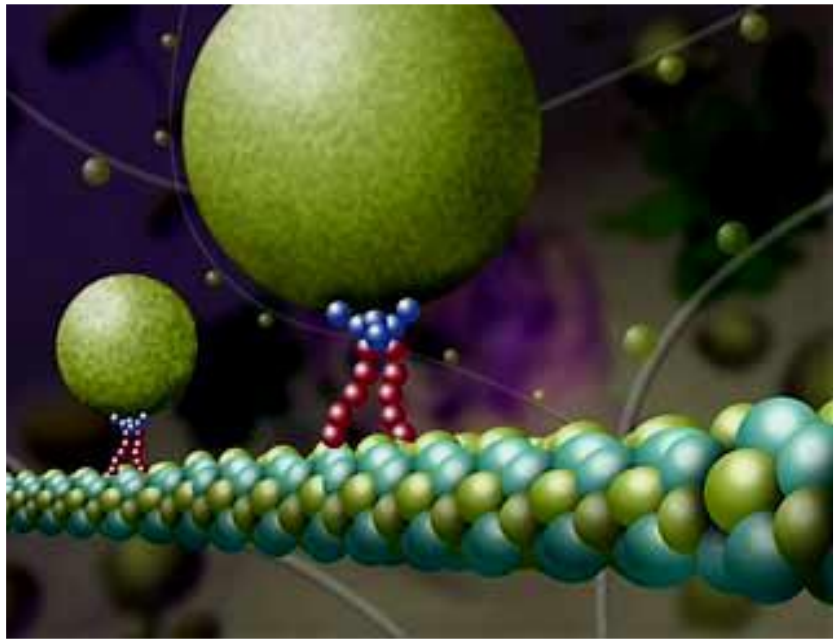
Motor proteins: ATPase activity

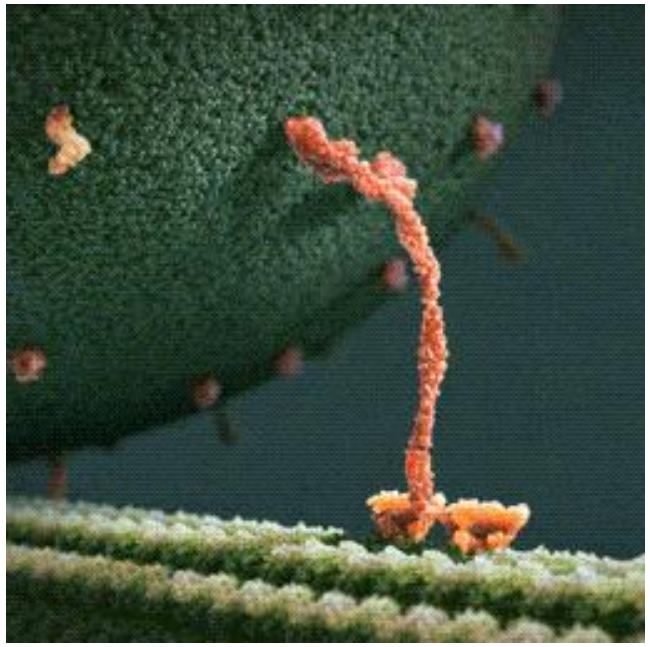
- **Dyneins** (movement **to - end**)
- **Kinesins** (movement **to + end**)

Microtubule-associated motor proteins

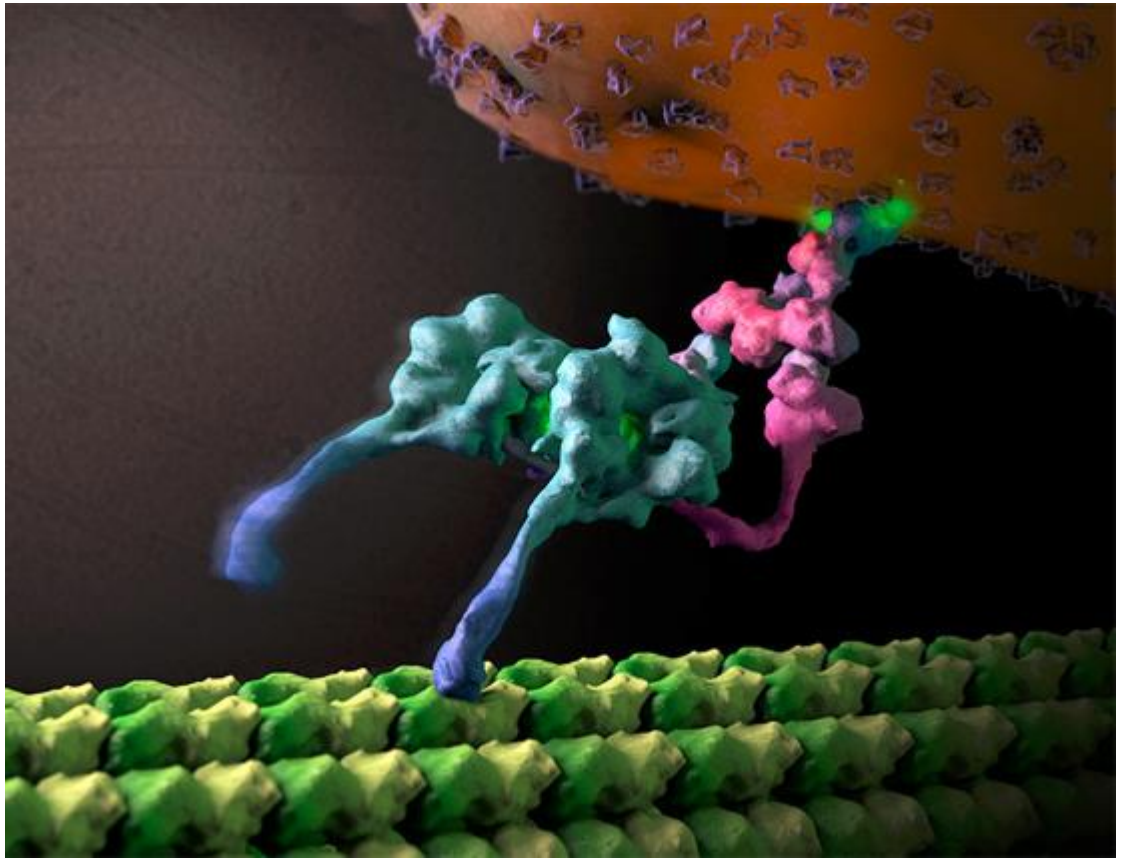
– Transport of cargo, ciliary/flagellar movement





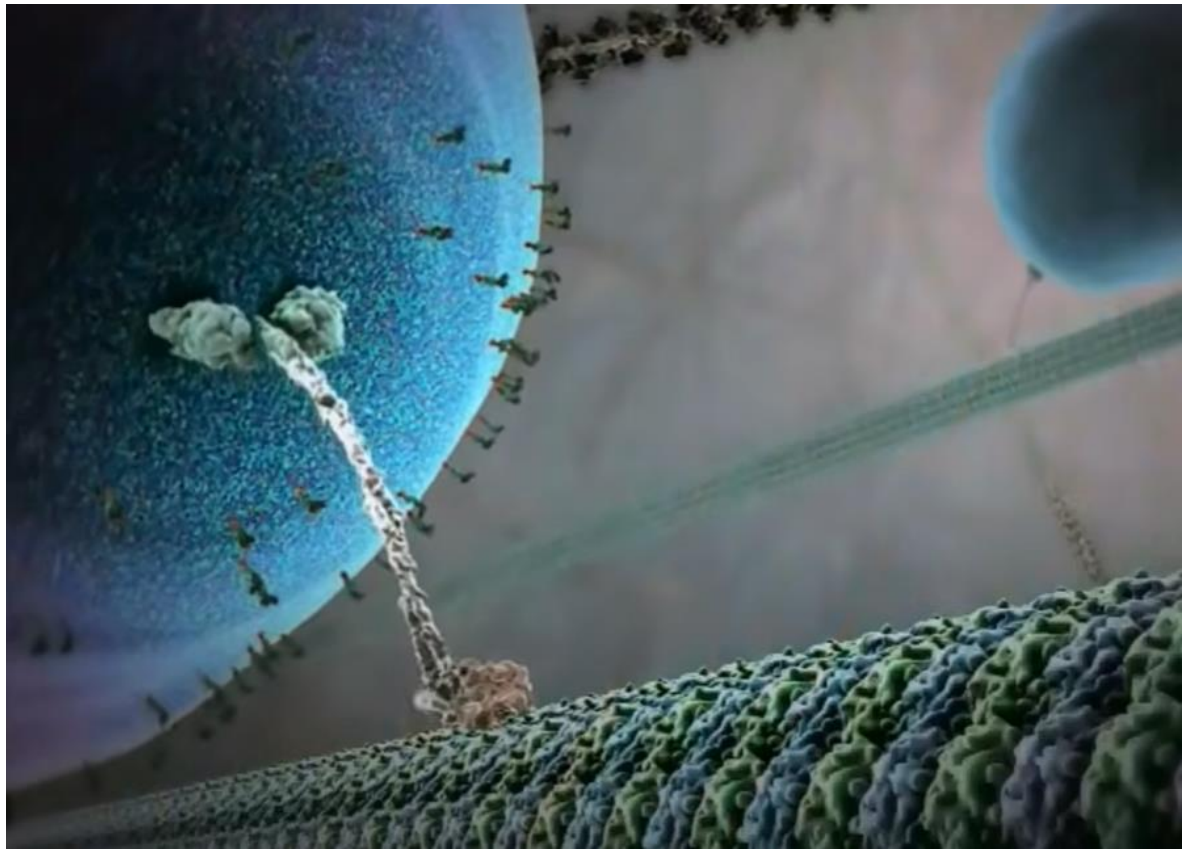


Dynein movement



https://youtu.be/-7AQVbrmzFw

Kinesin movement (walking)

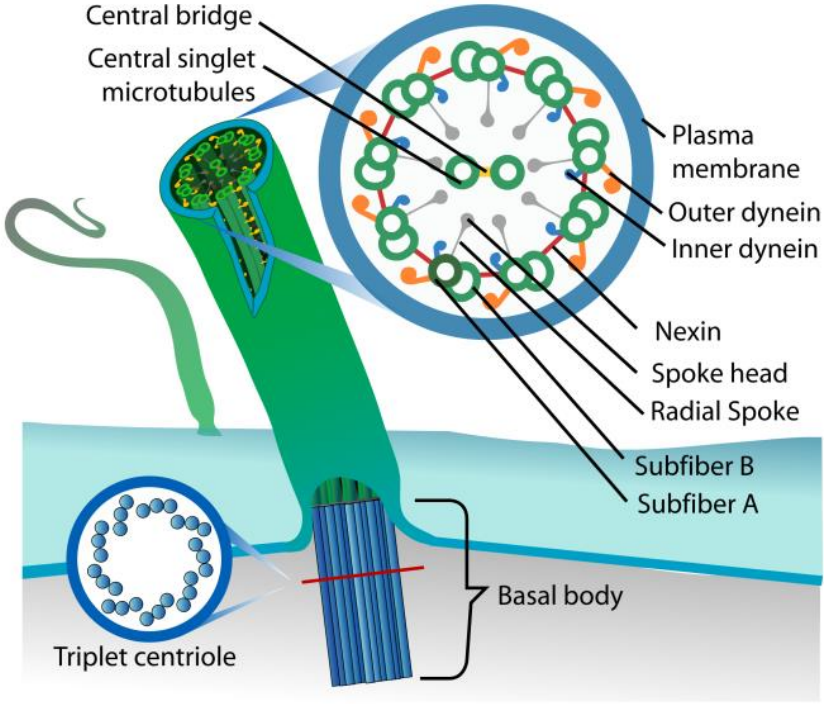


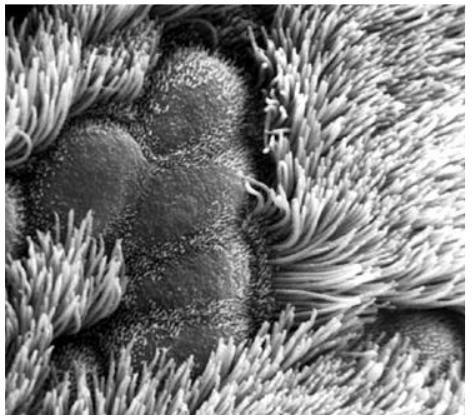
https://youtu.be/y-uuk4Pr2i8

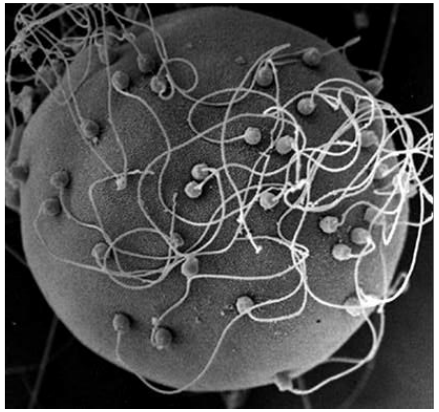
Motile cilium and flagellum

- Similar structure: **9 MT doublets + 2 central singlet MTs, 9+2**
- **Basal body** – centriole
- **Dynein** & **nexin** linkers

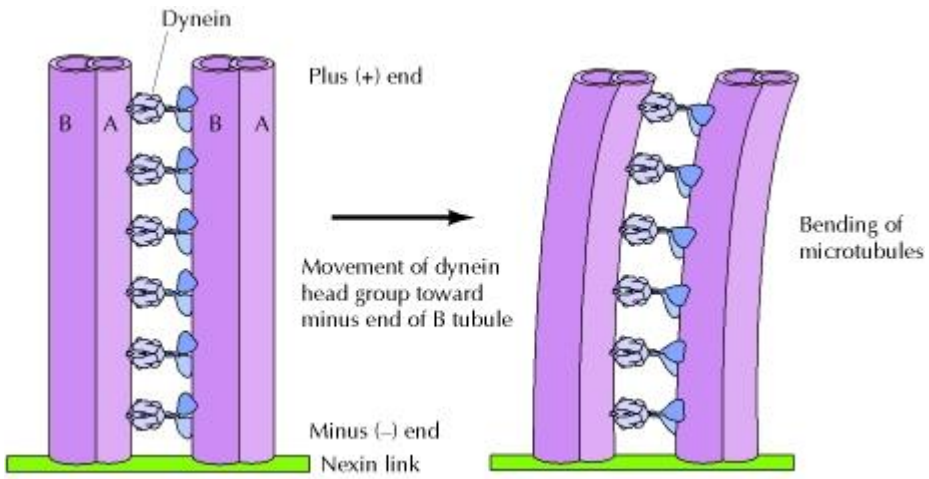
- **Motile cilia** – large numbers (= multiple centrioles), shorter
- **Flagellum** – 1-3 per cell, longer
- Protists, epithelial cells, sperm cells



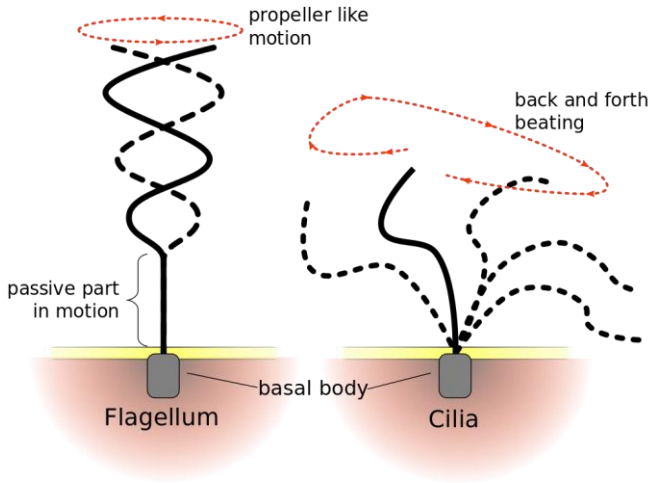


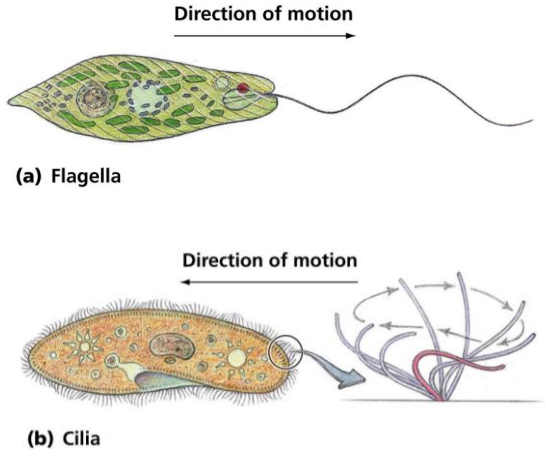


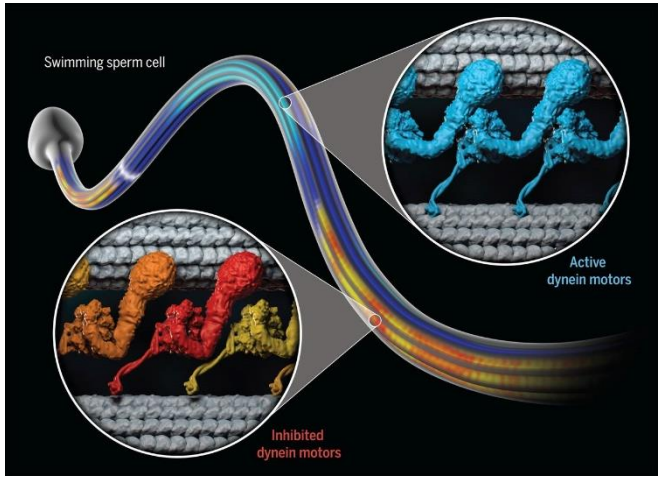
Mechanism of movement

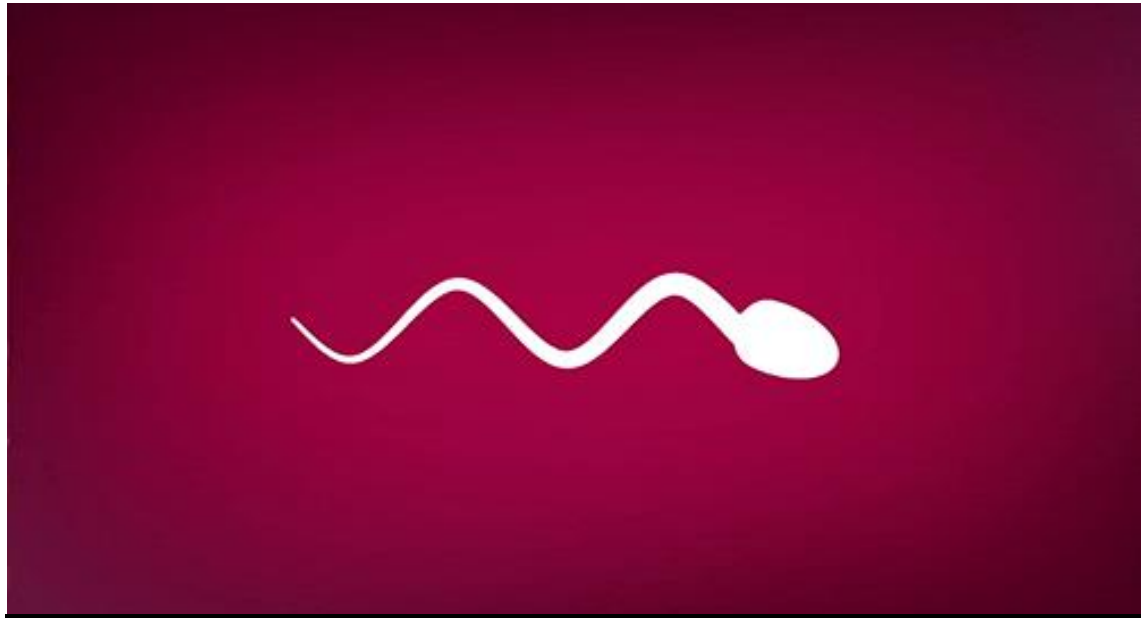


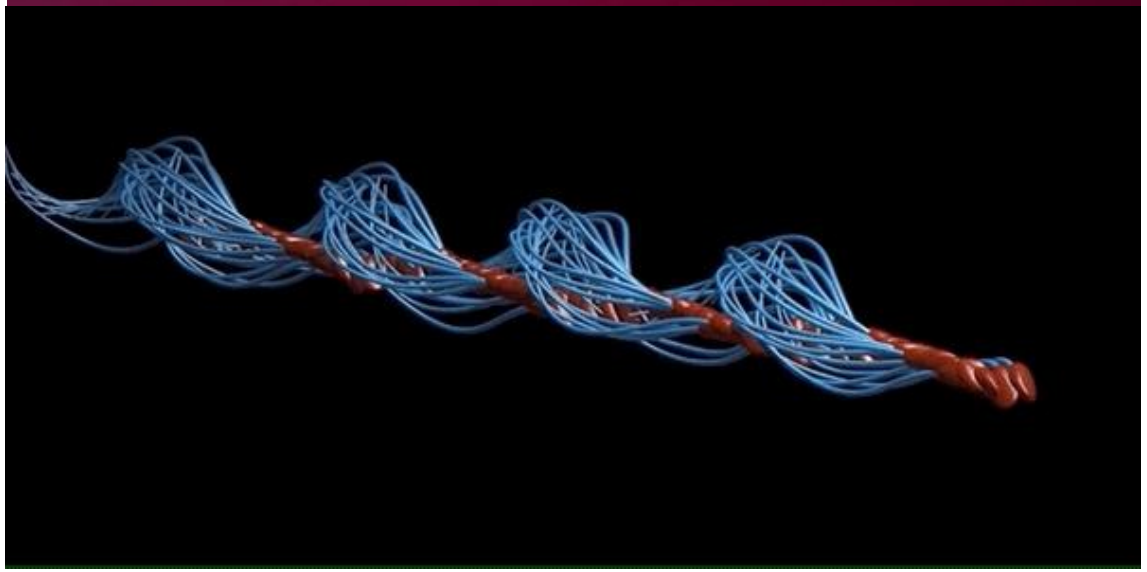
- ATP-dependent movement of dynein stalks → bending
- Flagellum vs. cilium – wave differences: **switching dynein activity**

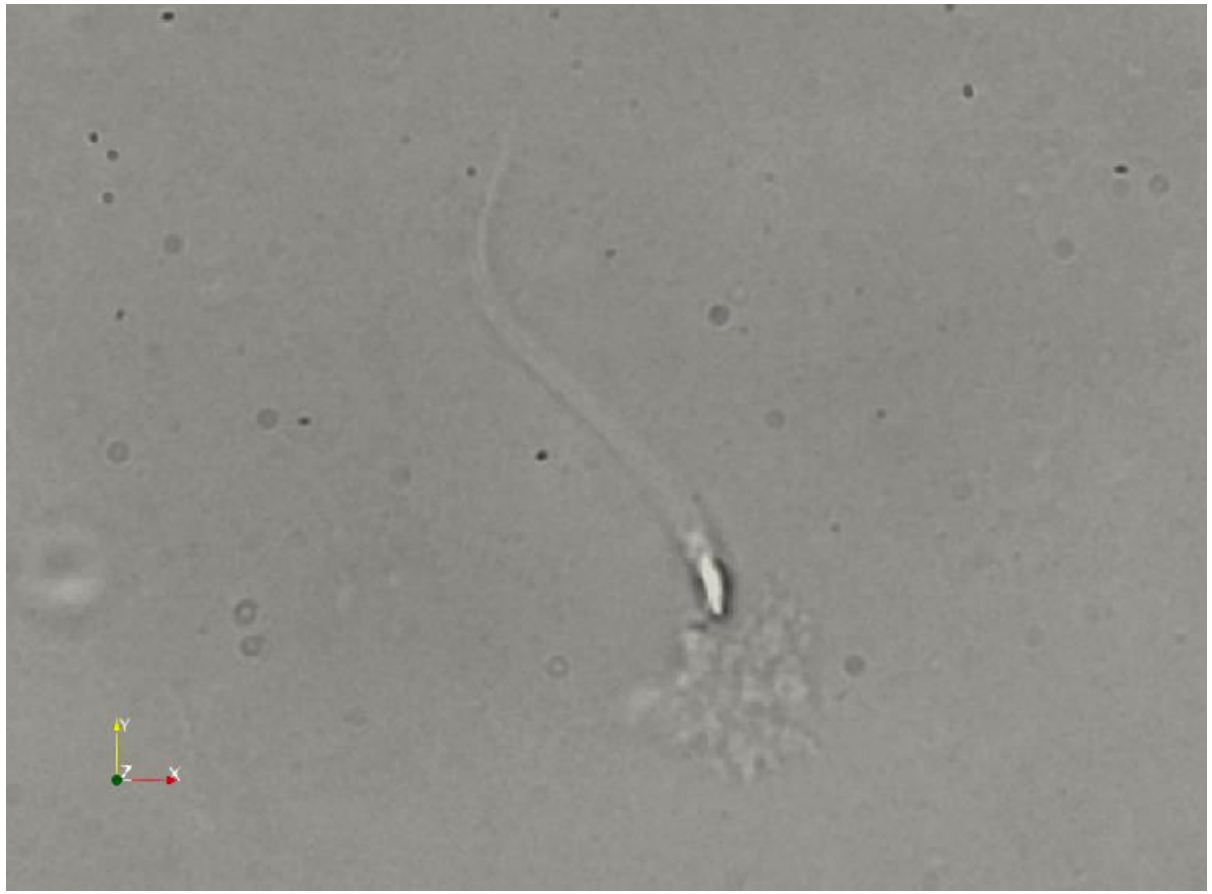












Actin filaments (microfilaments)

Actin structures

- Actin filament network within the cytoplasm
- Cell cortex (plasma membrane-associated cytoskeleton)

Animal cell-specific structures:

- Stress fibers
- Cytoplasmic protrusions: microvilli, lamellipodia, filopodia
- Contractile ring (cytokinesis)

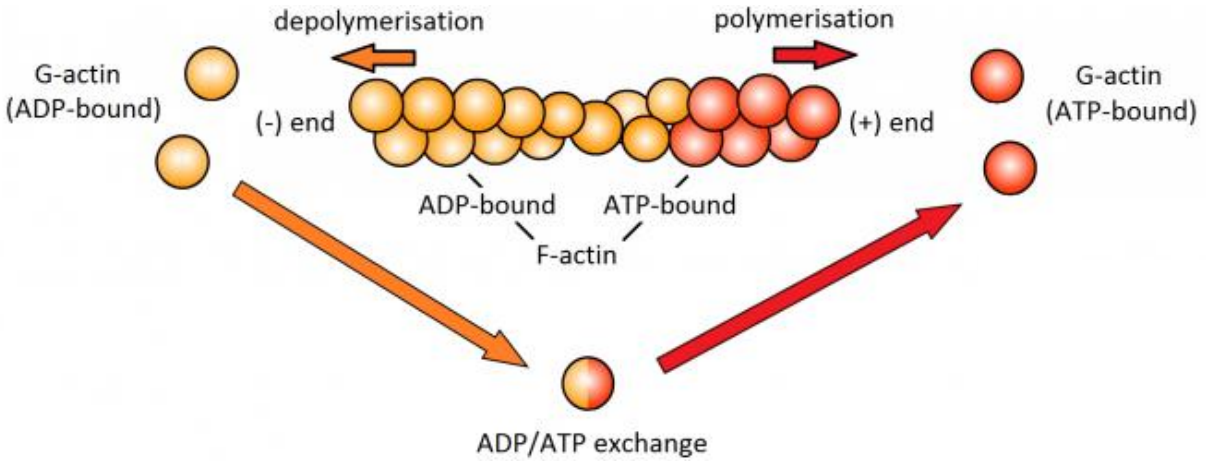
The structure of actin filaments

Actin

- About 5% of cell proteins; different isoforms (major: α,β,γ)
- **G-actin** (globular): monomeric pool
- **F-actin** (filamentous/fibrillar): polymerized, helical structure – 2 strands wound around each other

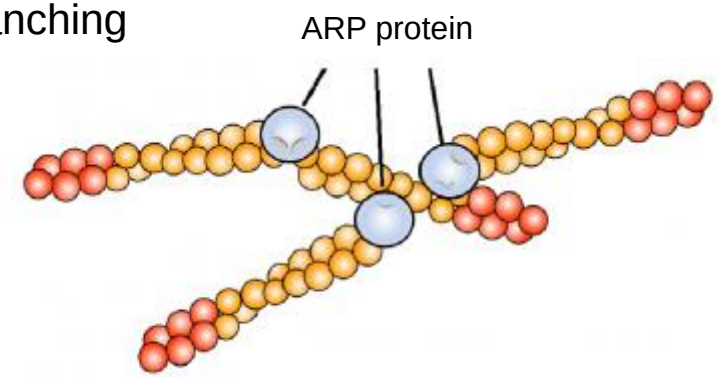
- Actin filament (AF) – **thinner, shorter, and more flexible than MT**
  - **! overall length of actin filaments ~ 30× greater than MTs**

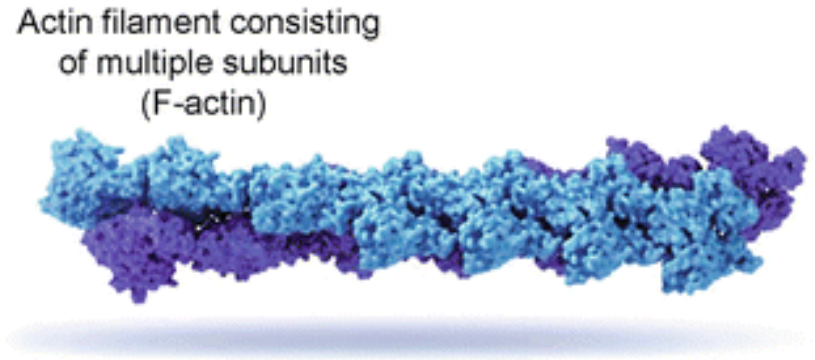
AF organization, dynamics & branching

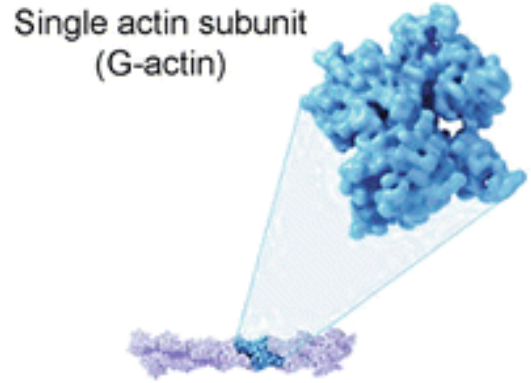


- ATP-dependent polymerization
- Helical structure, **7 nm**
- **ARP proteins**: nucleation & branching

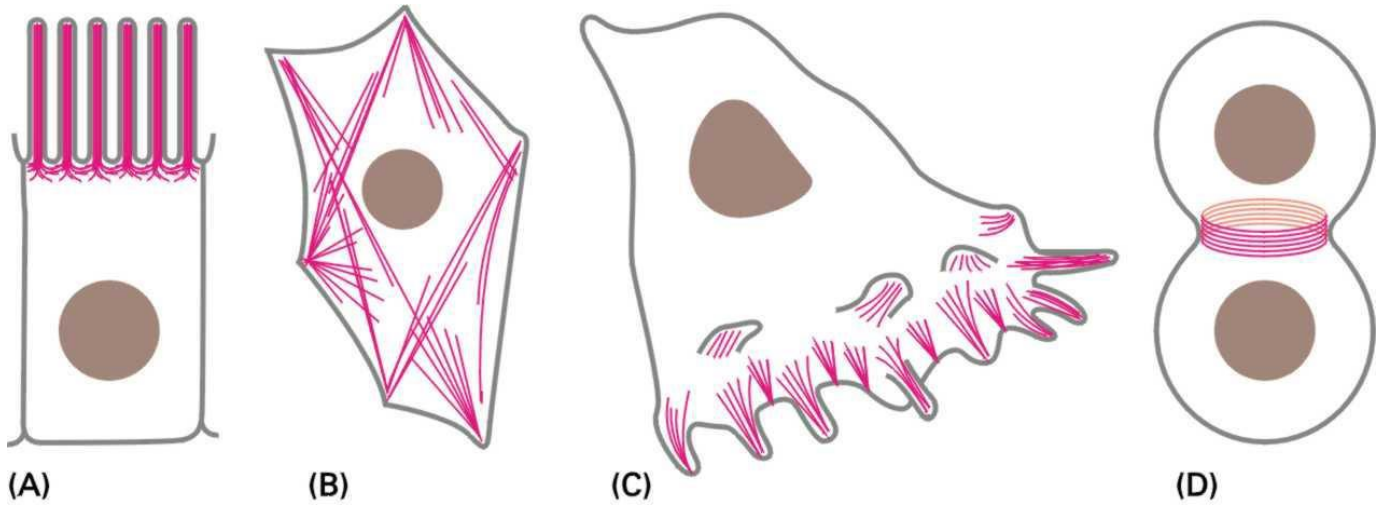
Branching







Examples of actin structures in animal cells

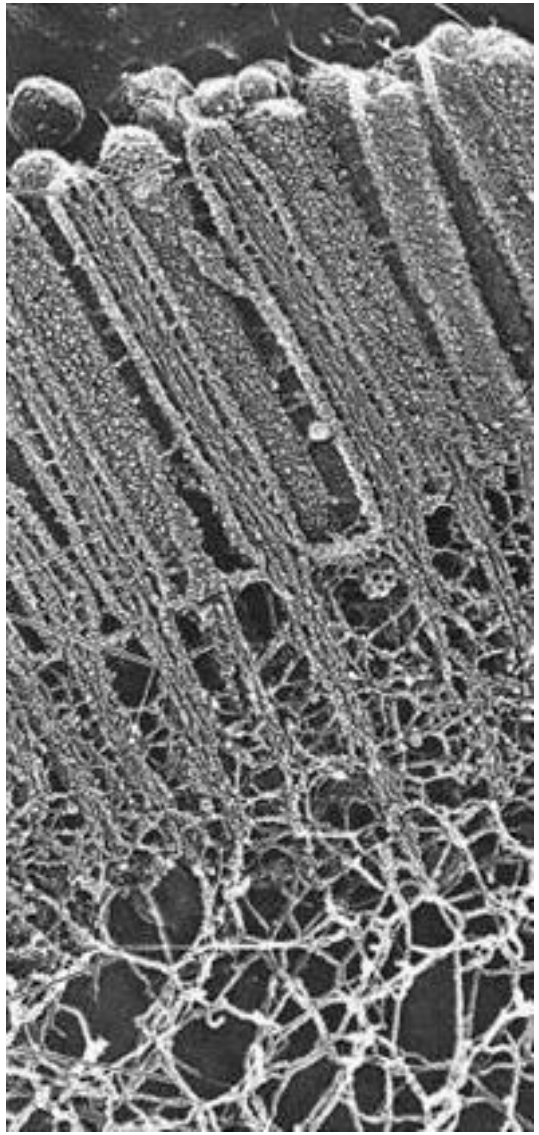


(A) **Microvilli** (e.g., intestinal enterocytes)

(B) **Stress fibers**: cell adhesion, morphogenesis and mechanotransduction

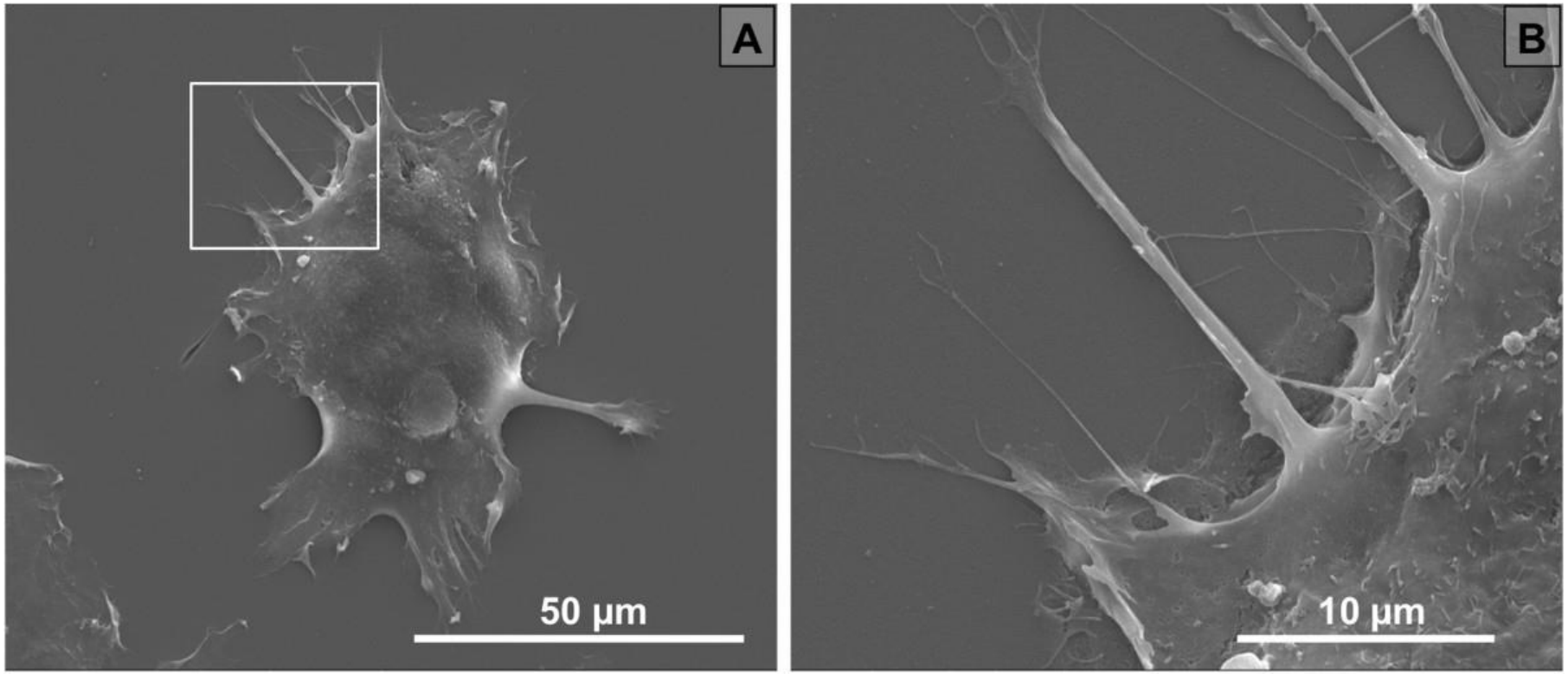
(C) **Lamellipodia, filopodia**: cell migration

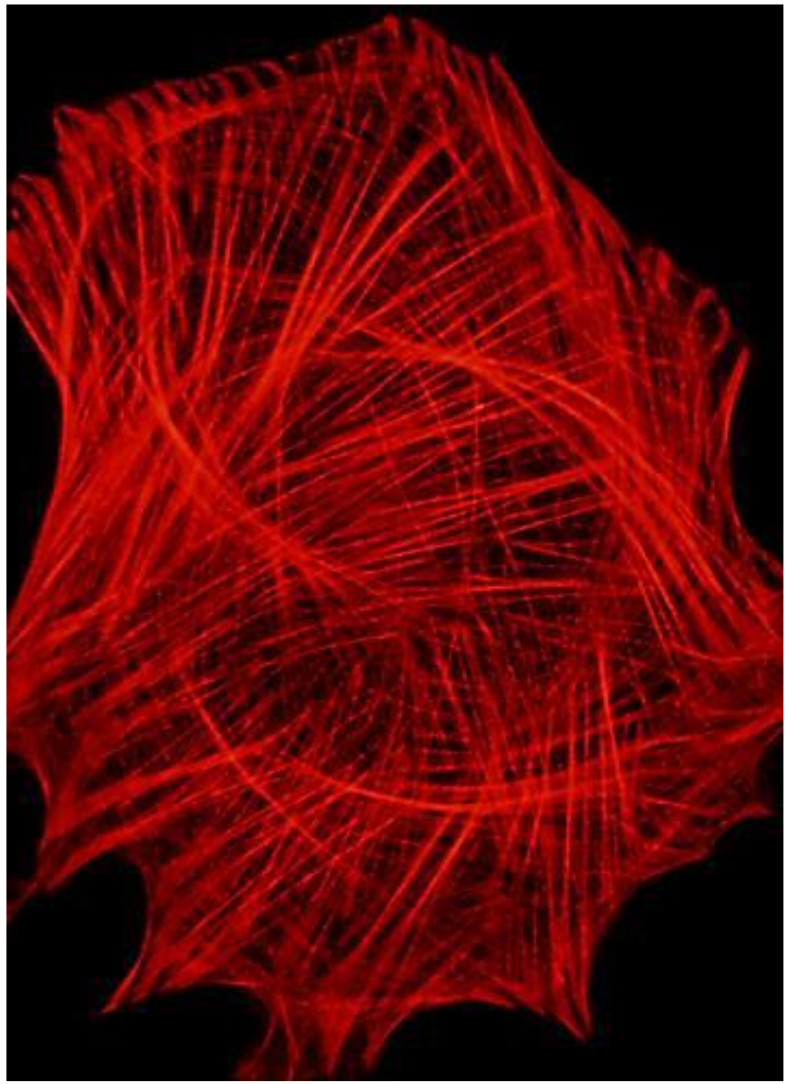
(D) **Contractile ring**: cytokinesis



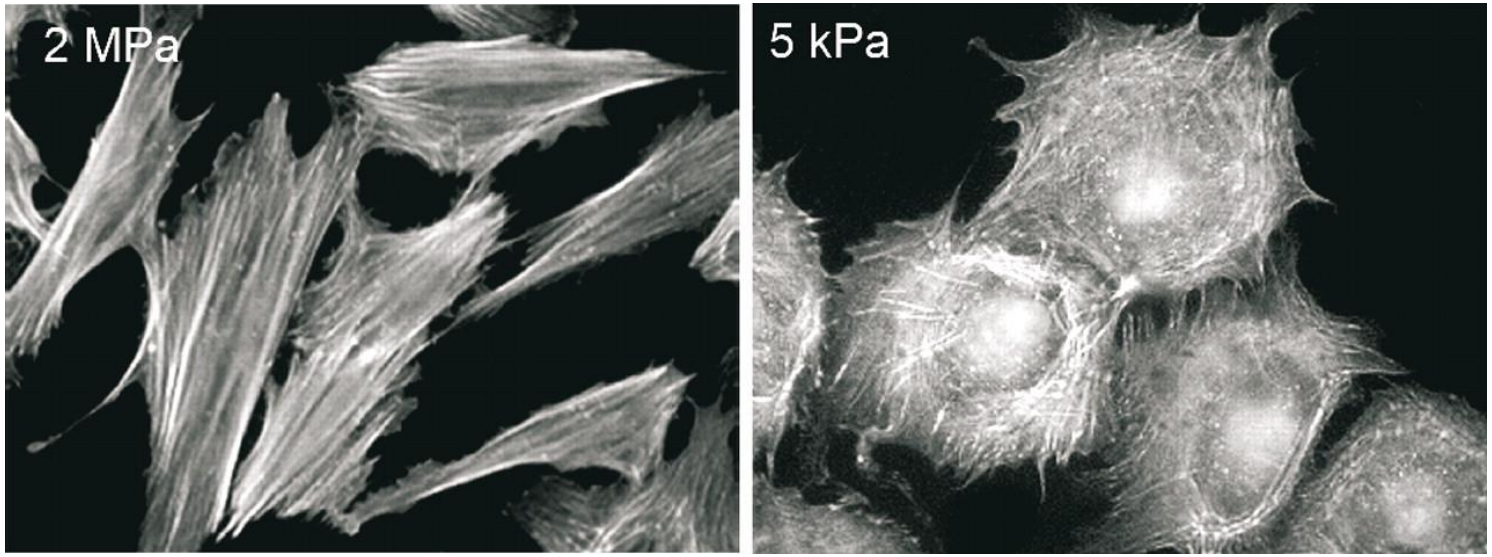
Microvilli

Lamellipodia and filopodia

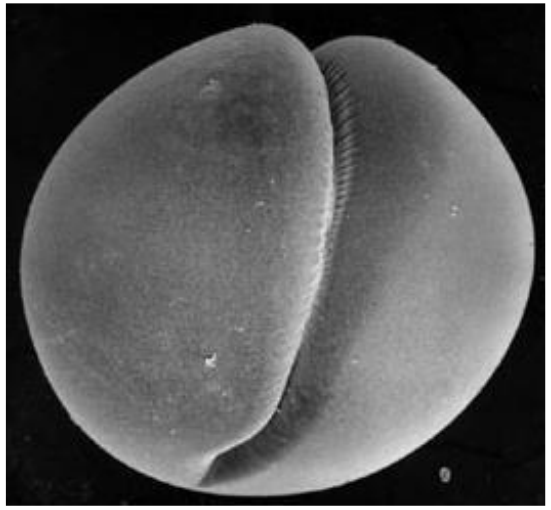


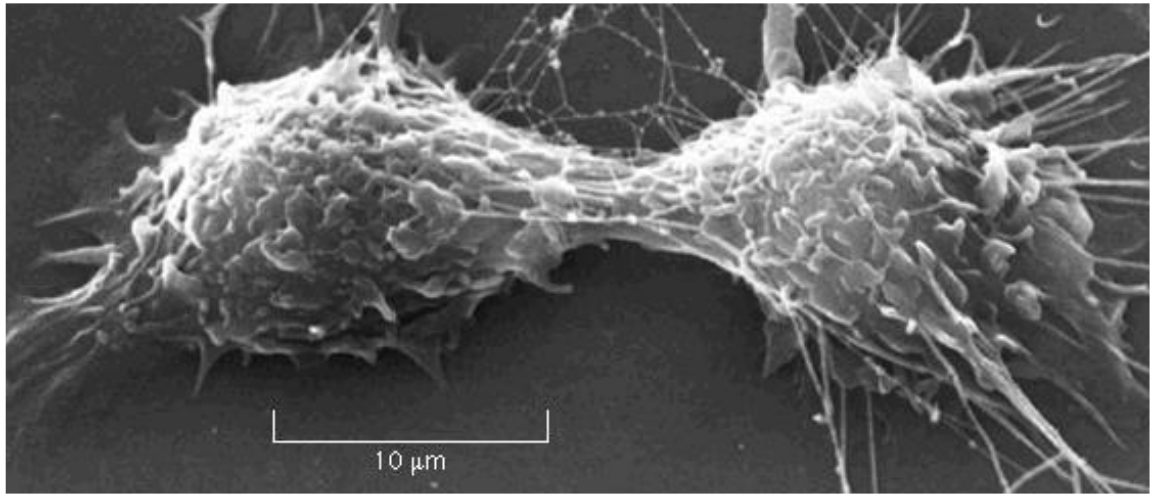


Stress fibers

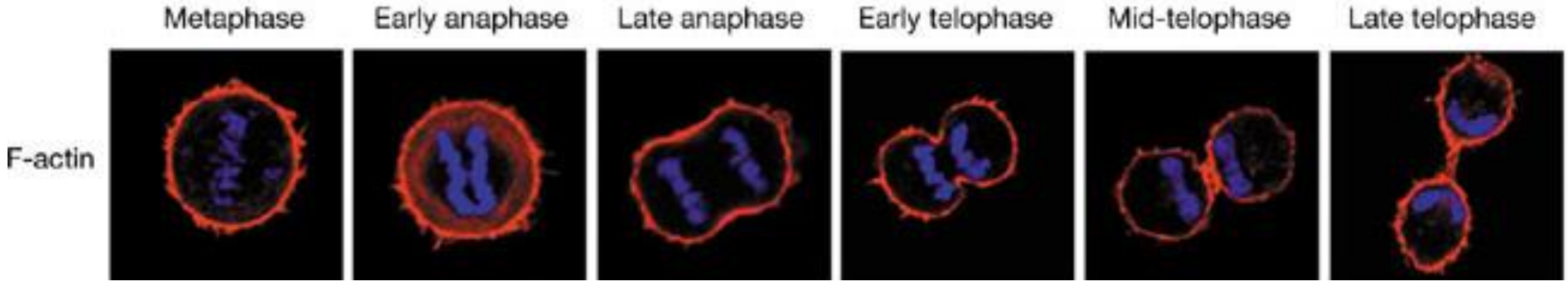


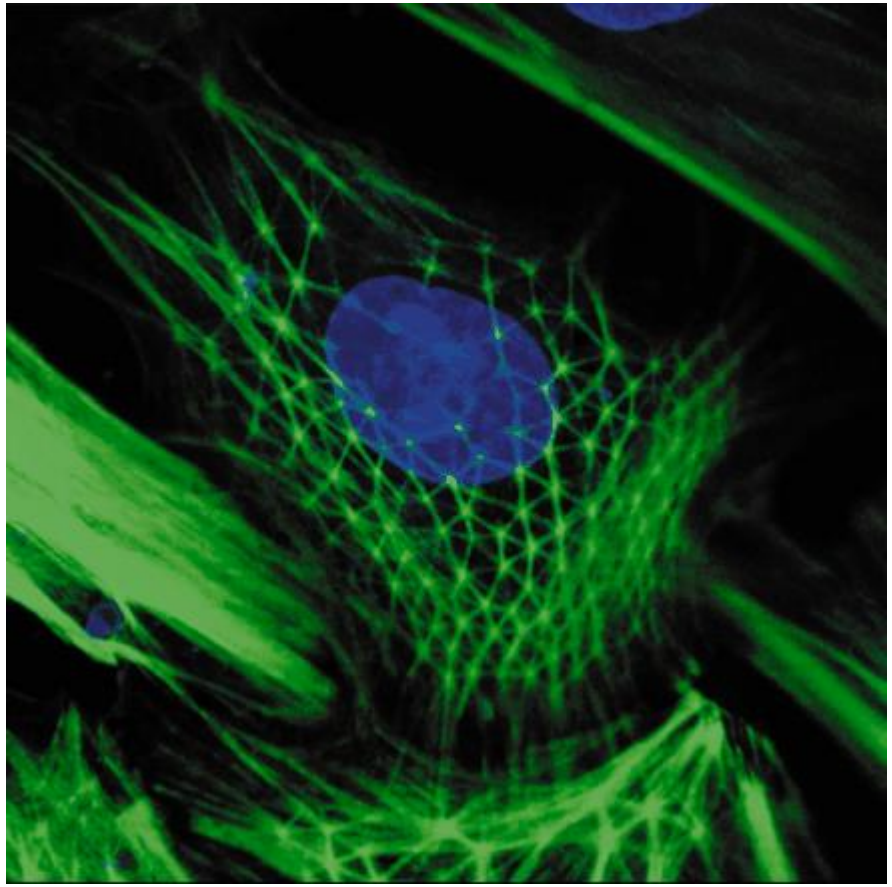
Substrate stiffness mechanosensing: Stress fibers are more prominent in cells that are grown on rigid than on soft substrates.





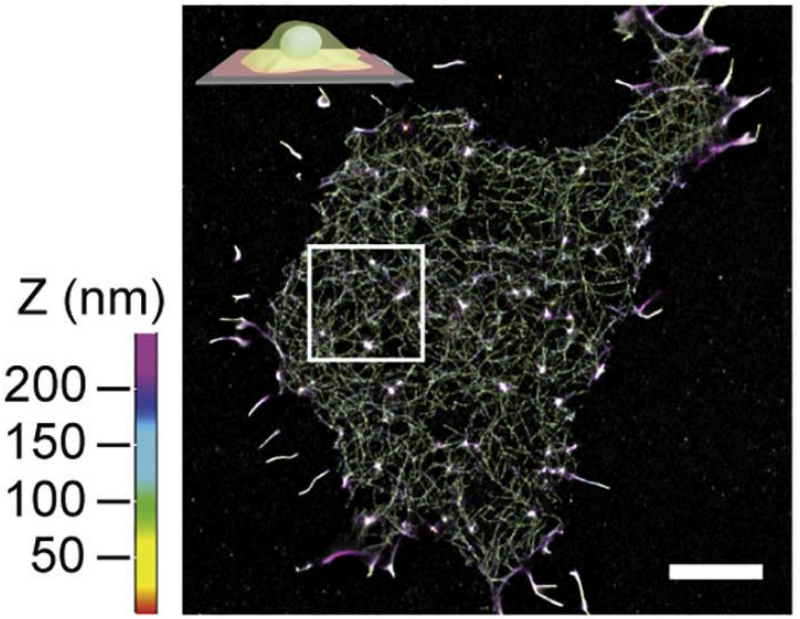
Contractile ring (cleavage furrow)

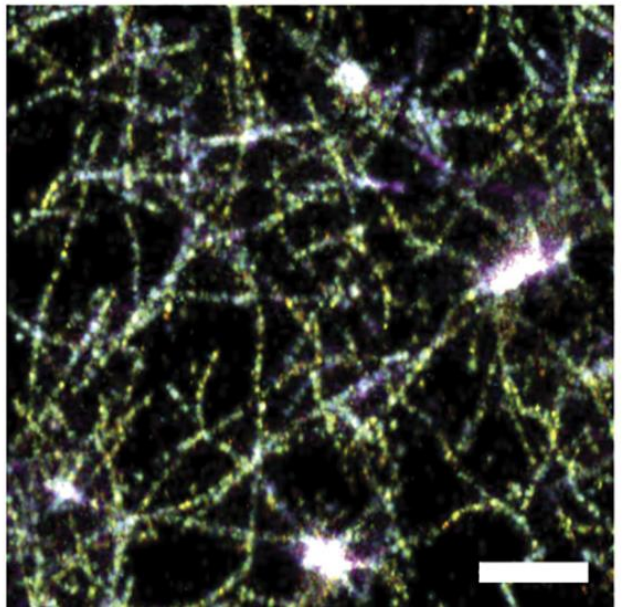


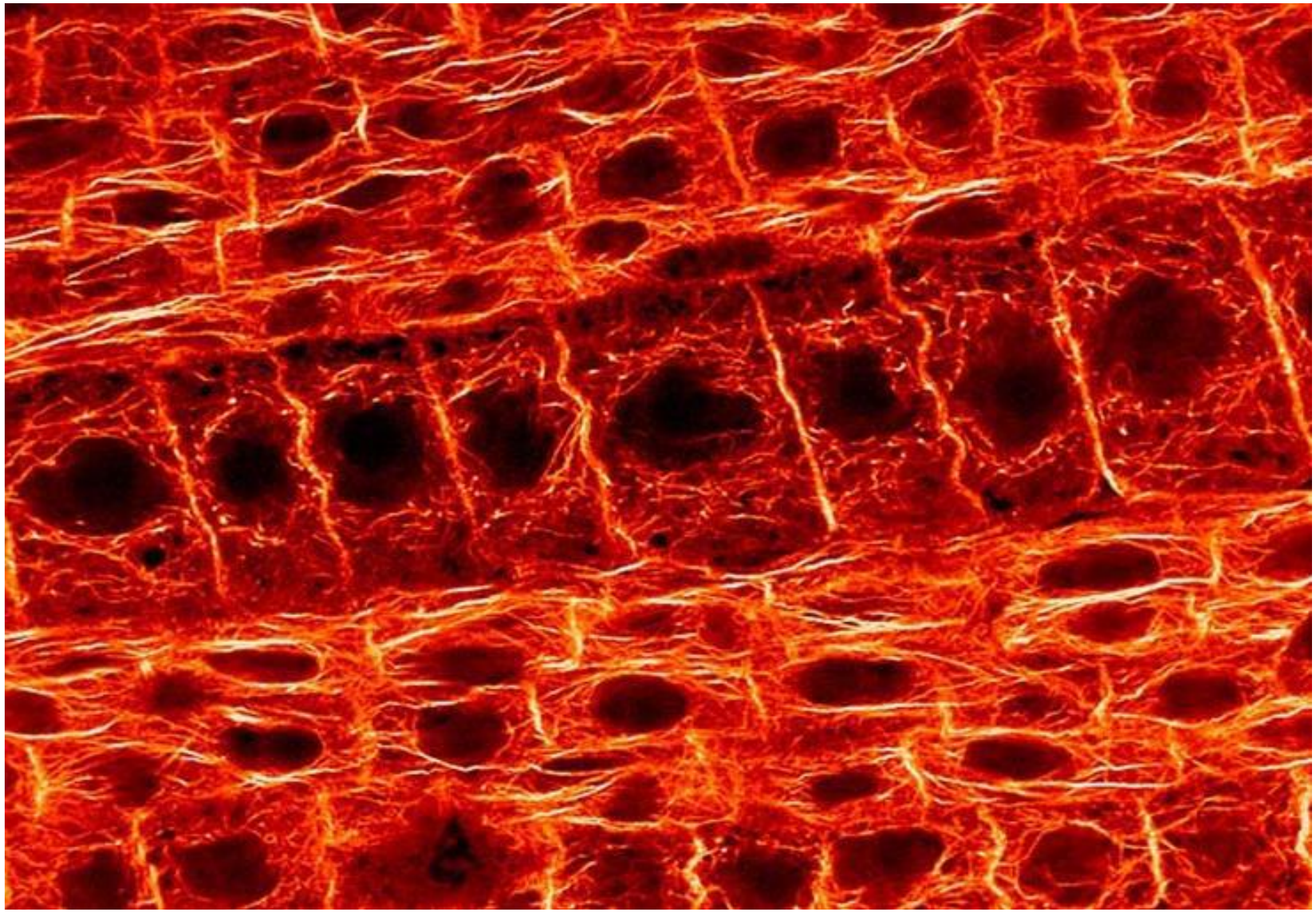


Actin cortex
in neonatal fibroblasts

Actin cortex in mESCs

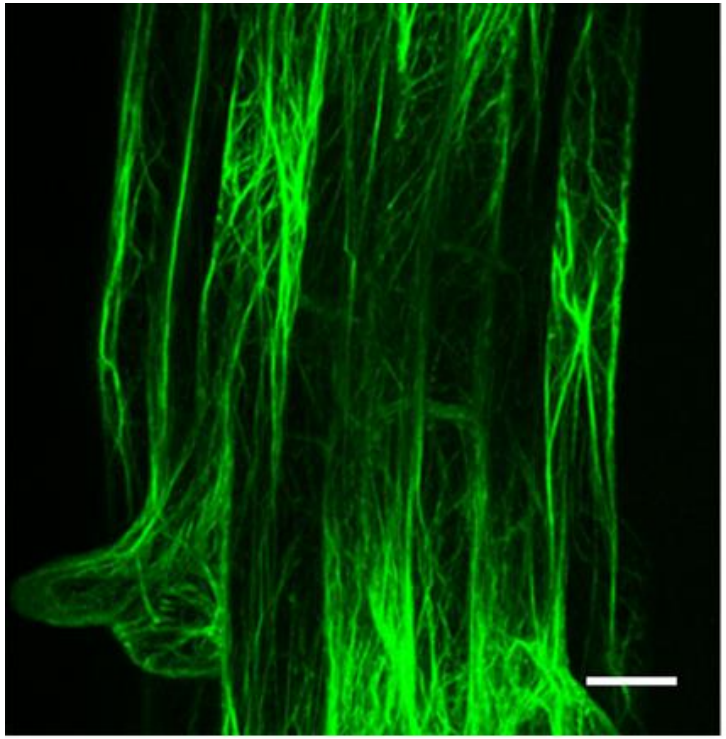






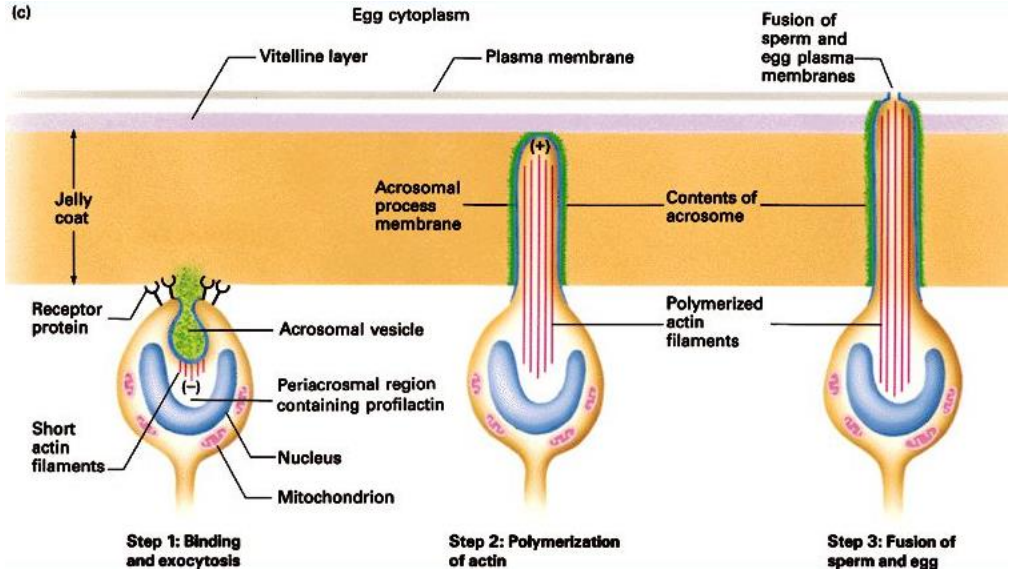
Maize root tip meristem

***Arabidopsis* root tip mature zone**



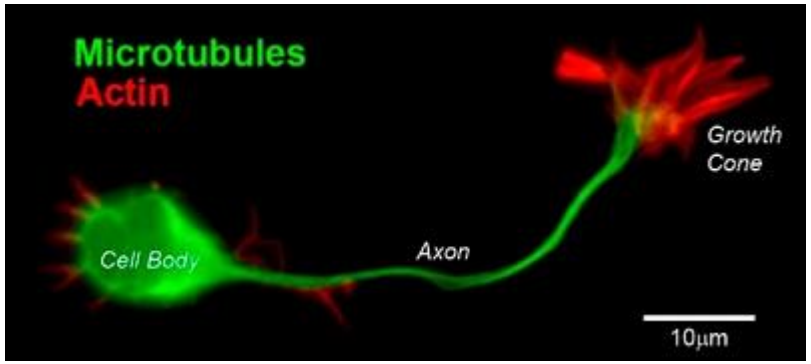
Functions of actin filaments

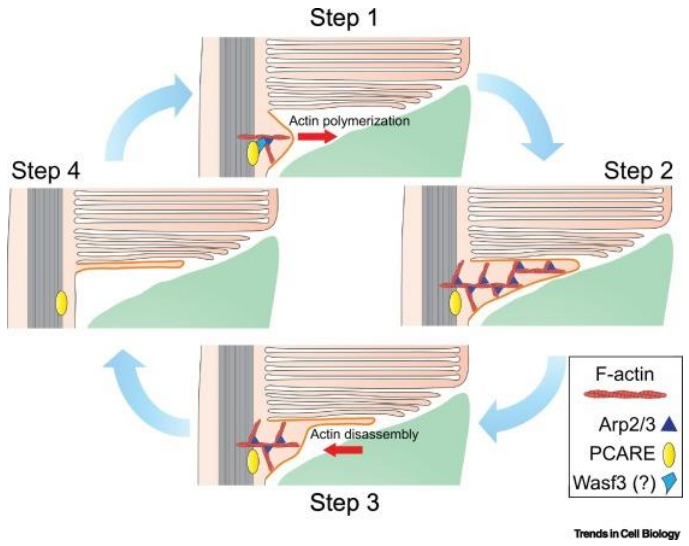
- **Cytokinesis** (contractile ring)
- **Phagocytosis**
- **Cell migration** & **morphology changes** (lamellipodia, filopodia, stress fibers)
- Substrate interactions – **focal adhesions** (stress fibers)
- **Oocyte fertilization** – acrosomal process formation in sperm cells
- **Muscle contraction**

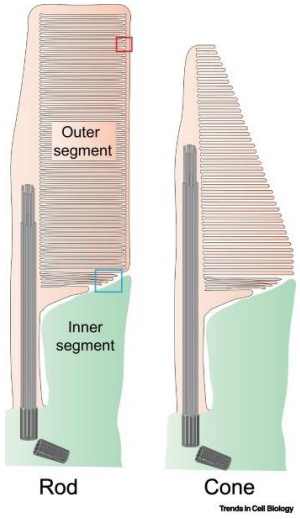


Functions of actin filaments

- **Axon growth** (growth cone): nerve synapse formation
- Expansion of cell surface
  - **Microvilli**
  - **Formation of photoreceptor discs** in rods & cones



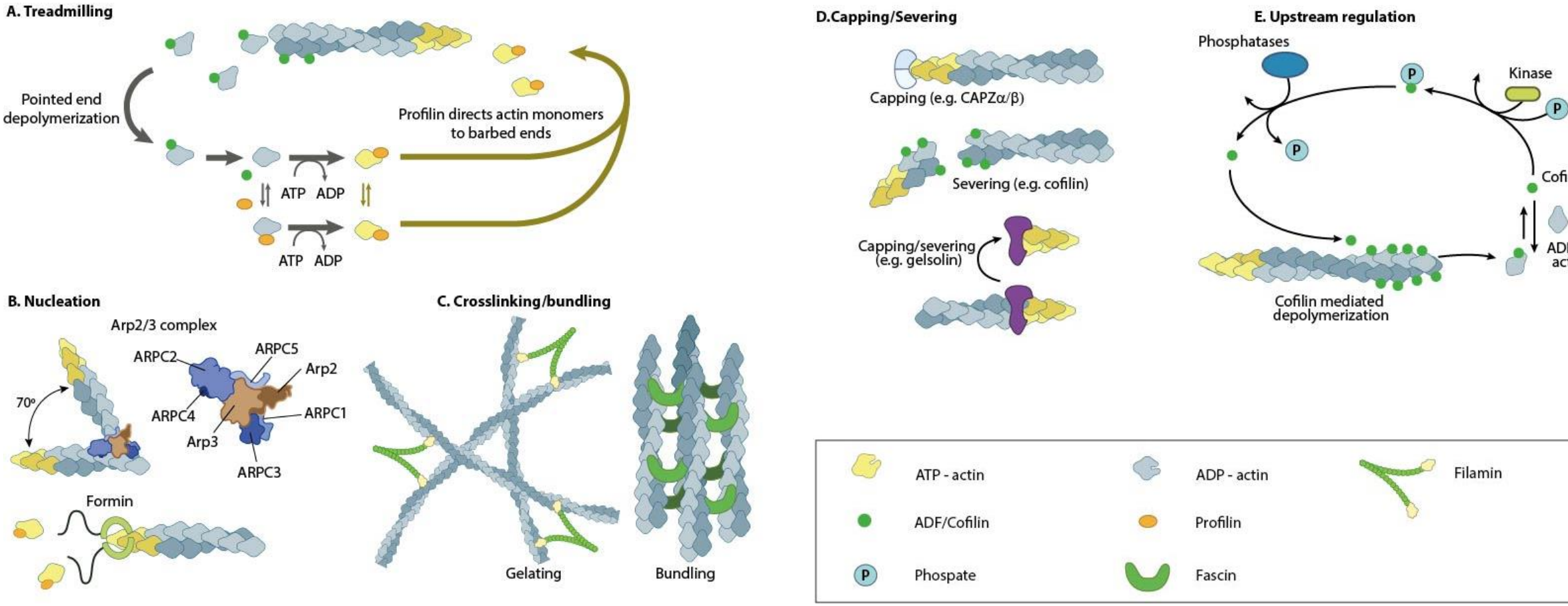




Non-motor proteins associated with AF

- **Regulate polymerization and spatial organization**
- Nucleation factors: actin related proteins (**ARPs**)
- Maintaining the pool of (monomeric) G-actin: **thymosins**
- Capping proteins – bind to +end, terminate AF elongation, prevents addition/loss of subunits: **β-actinin, CapZ or Cap32/34**
- Proteins stimulating polymerization: **profilin**
- Proteins depolymerizing AF: **ADF, cofilin, depactin**
- Crosslinking protein**: filamin, ABP, gelactin, villin, fimbrin, fascin, spectrin, α-actinin**
- Proteins cutting AF (actin-severing):**gelsolin, brevin**

Non-motor proteins associated with AF



Actin motor proteins

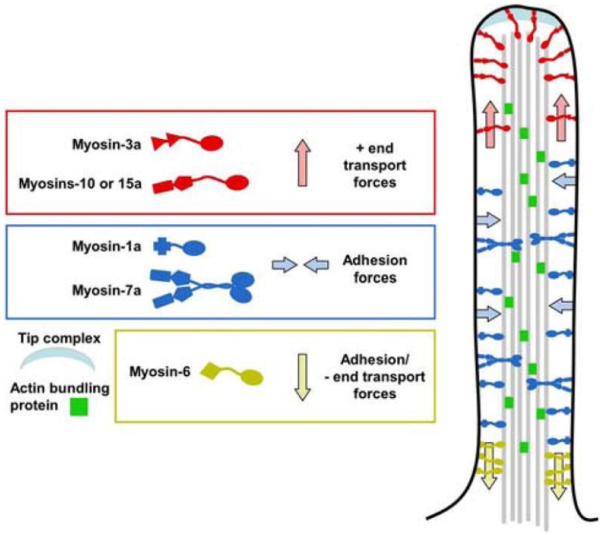
Myosins

Conventional myosin (type II)

- Muscle contraction – regulated by **tropomyosin** and **troponin**
- Cytokinesis, stress fibers

Non-conventional myosins (type I, III-XVIII)

- Vesicular transport (to + or - end)
- Maintaining membrane protrusions
- Movement of organelles in plant cells

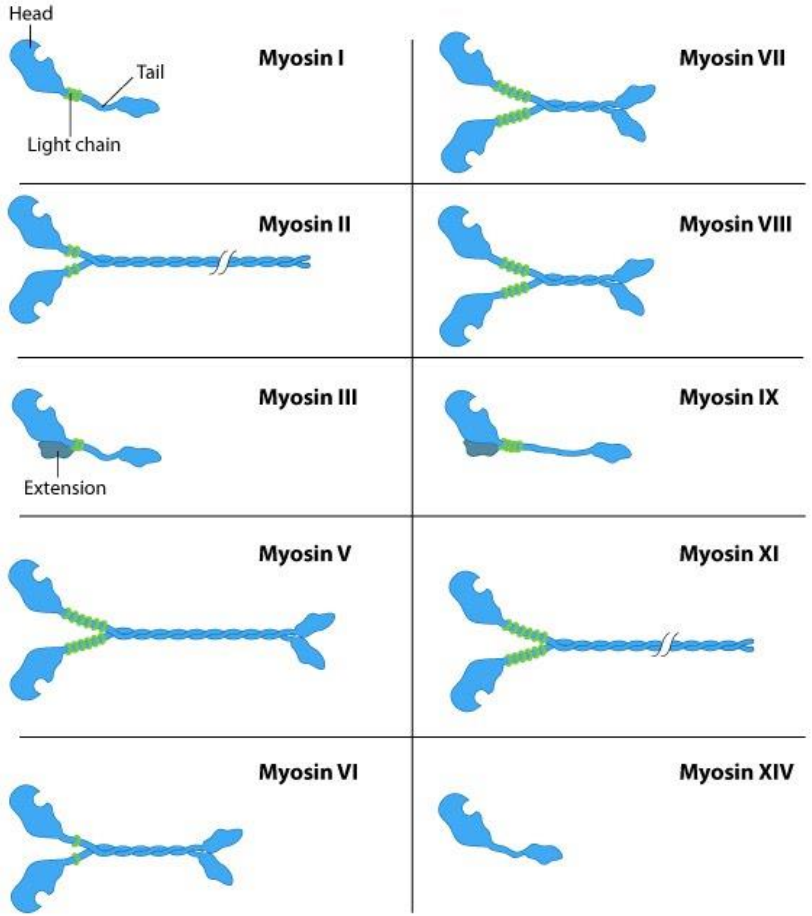


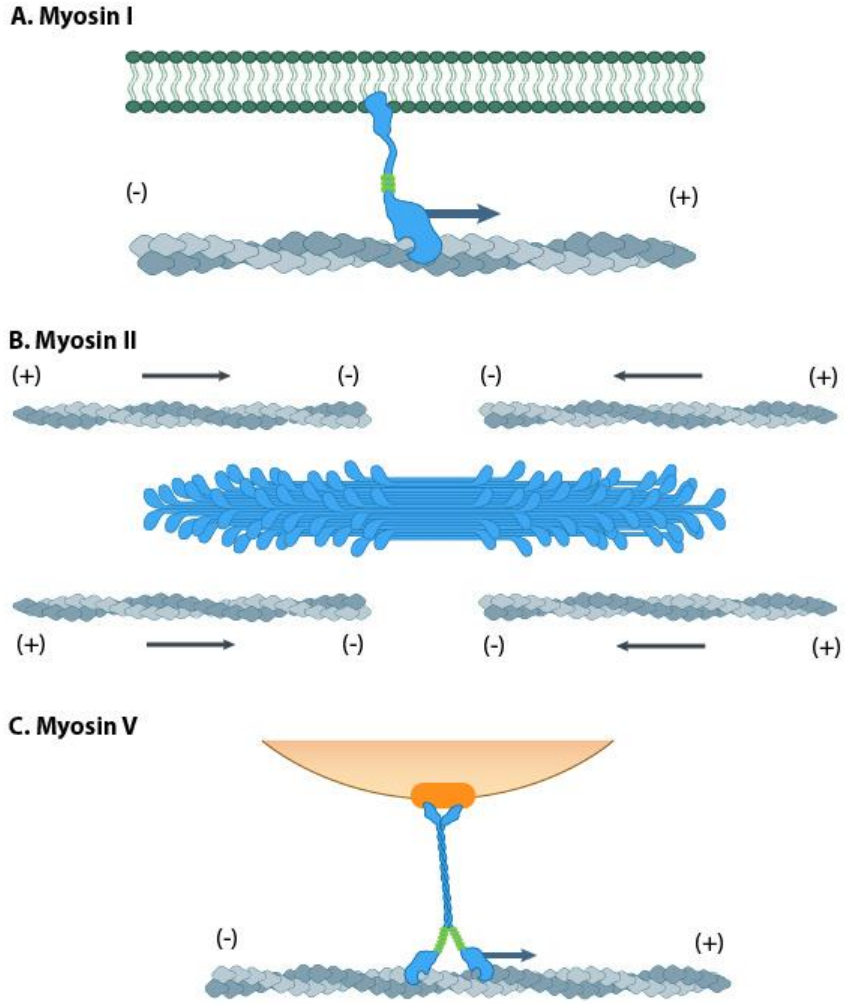
Diversity of myosins

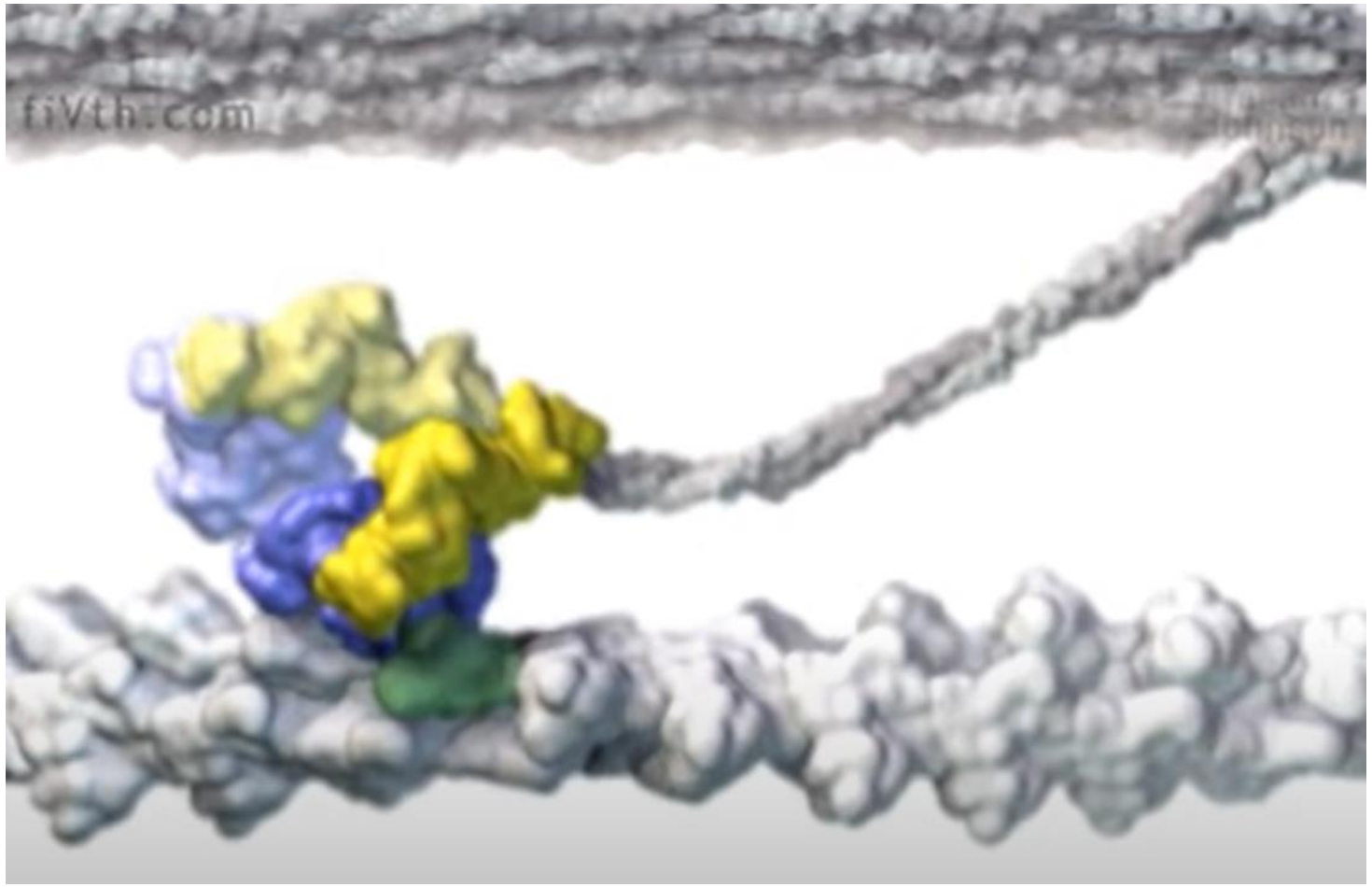
– Monomeric or dimeric

Movement

ATP-dependent detachment of myosin head

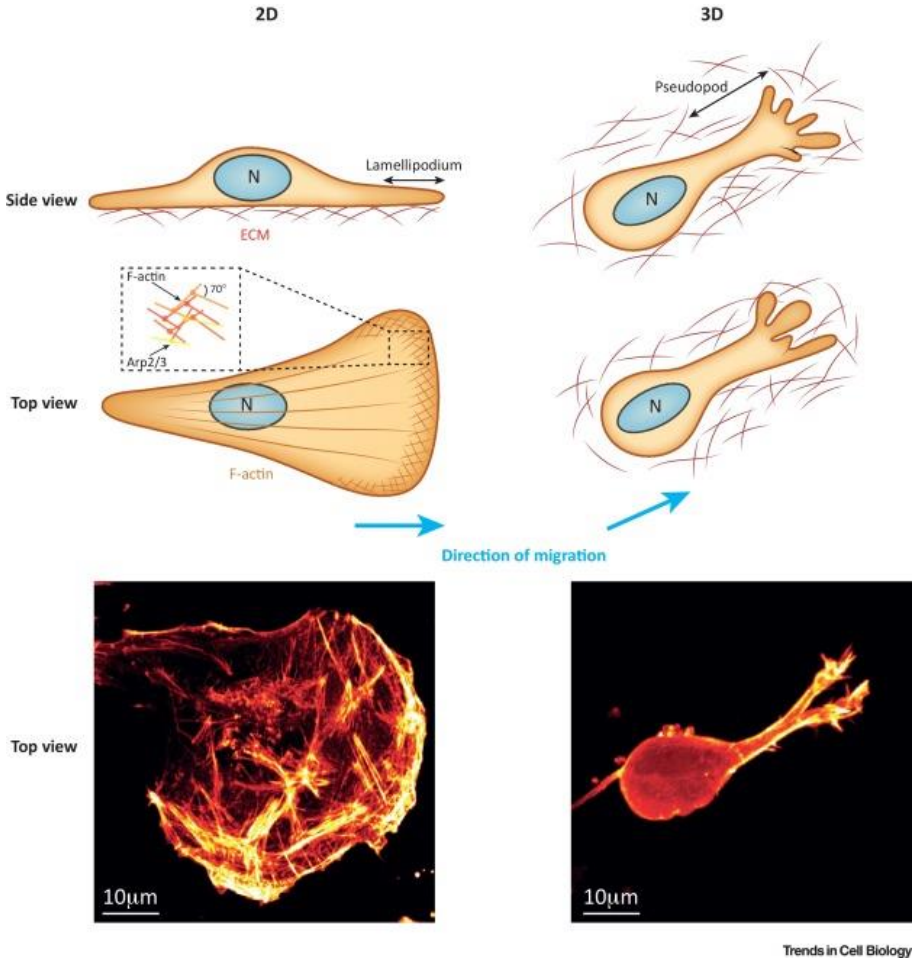




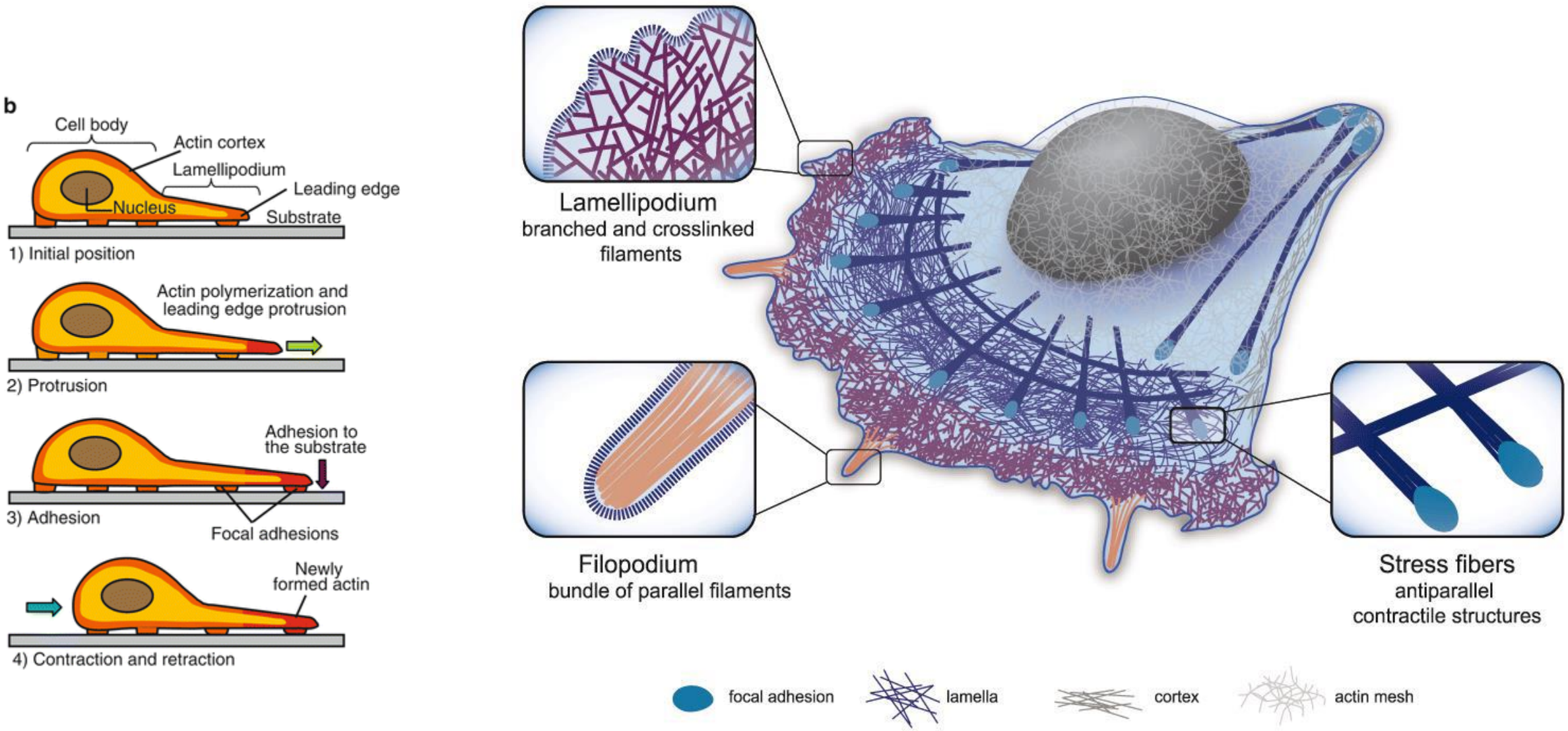


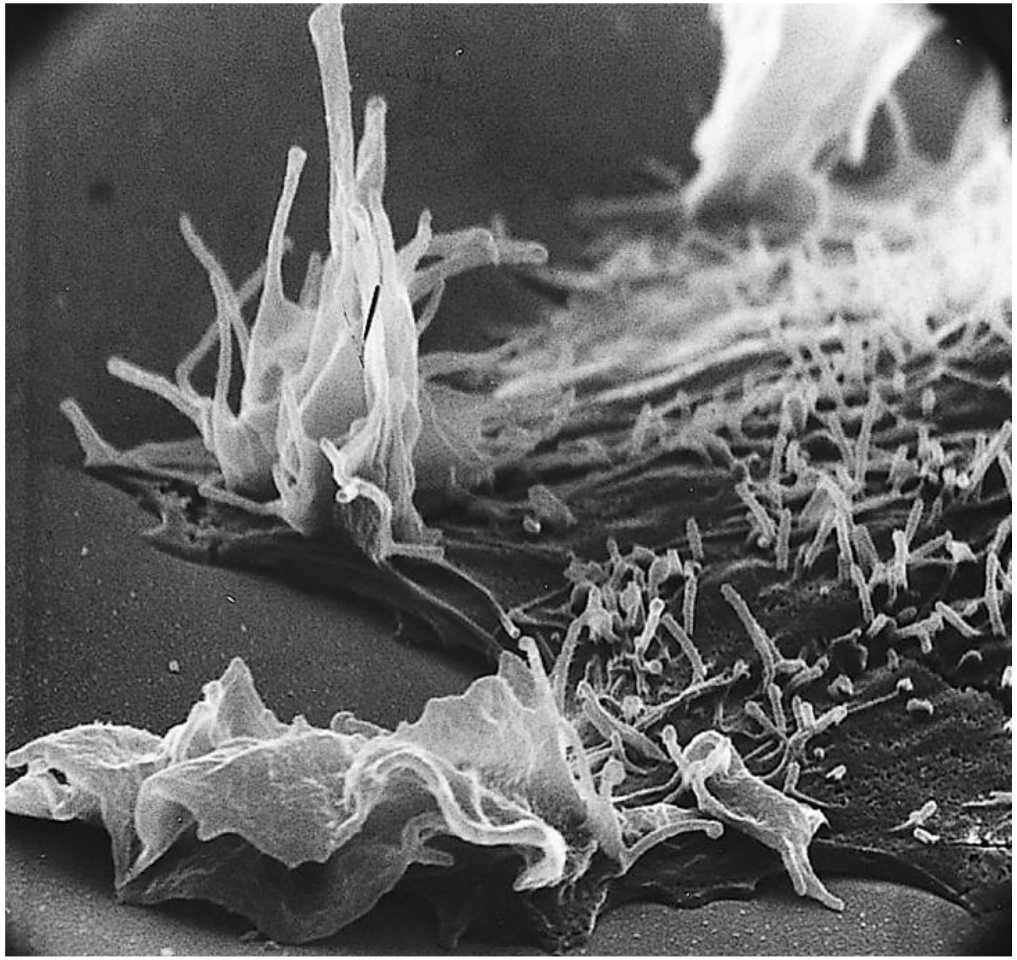
https://youtu.be/oHDRIwRZRVI

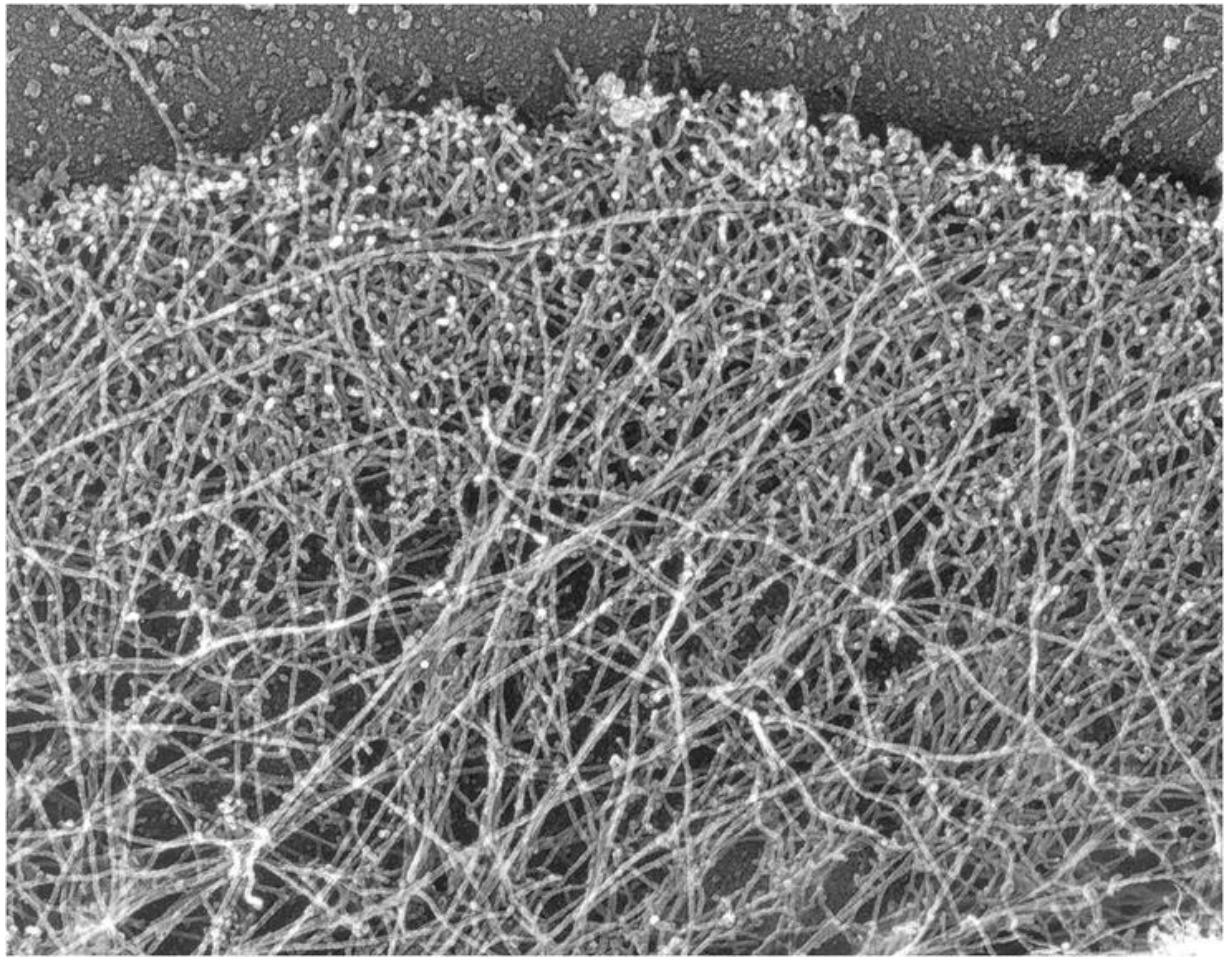
Actin-based movement: cell migration

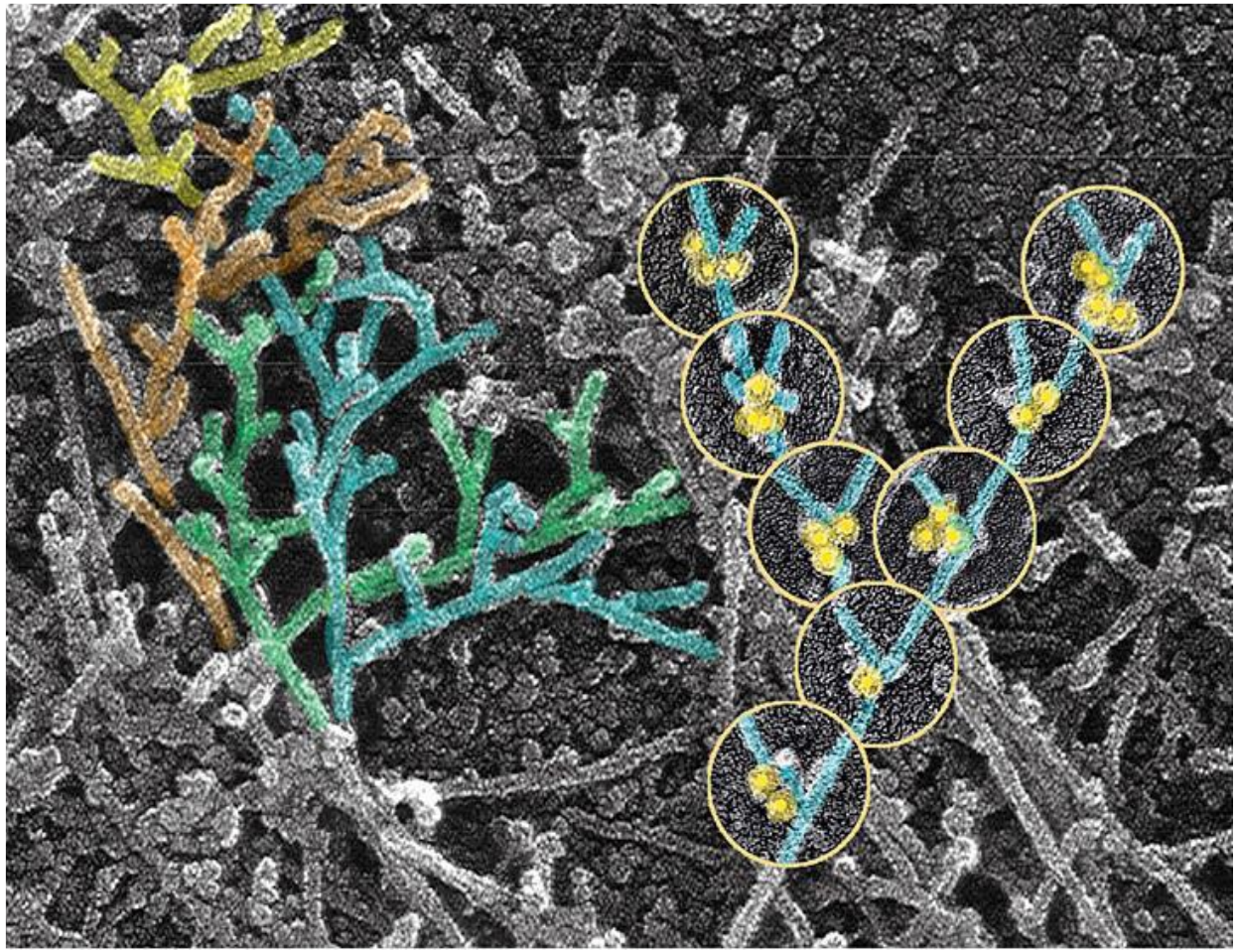


- **Mesenchymal migration** on 2D surfaces
  - Lamellipodium with filopodia
- **Ameboid migration** in 3D
  - Pseudopod with lamellipodia and filopodia

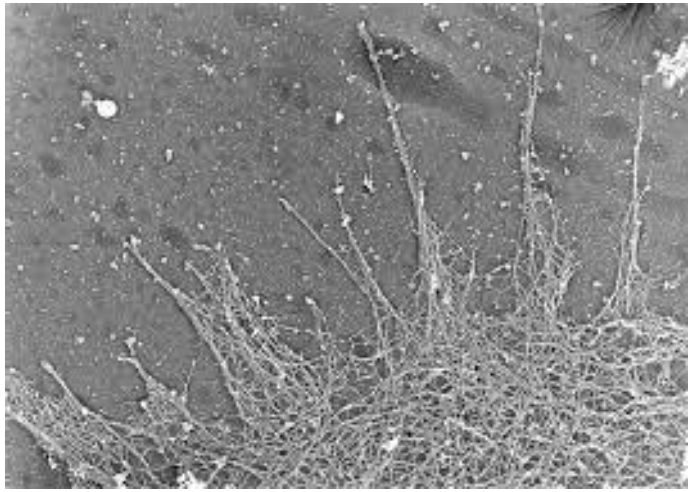

b
Cell body
Actin cortex
Lamellipodium
Leading edge
Nucleus
Substrate
1) Initial position
Actin polymerization and
leading edge protrusion
2) Protrusion
Adhesion to
the substrate
3) Adhesion
Focal adhesions
Newly
formed actin
4) Contraction and retraction
Lamellipodium
branched and crosslinked
filaments
Filopodium
bundle of parallel filaments
Stress fibers
antiparallel
contractile structures
focal adhesion
lamella
cortex
actin mesh

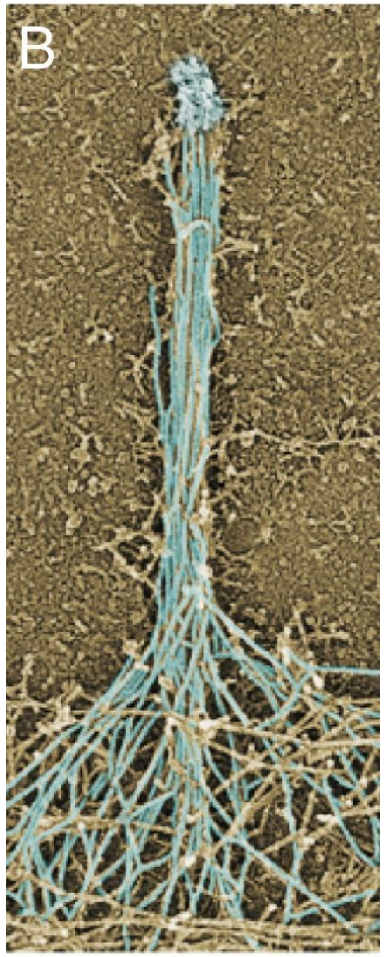






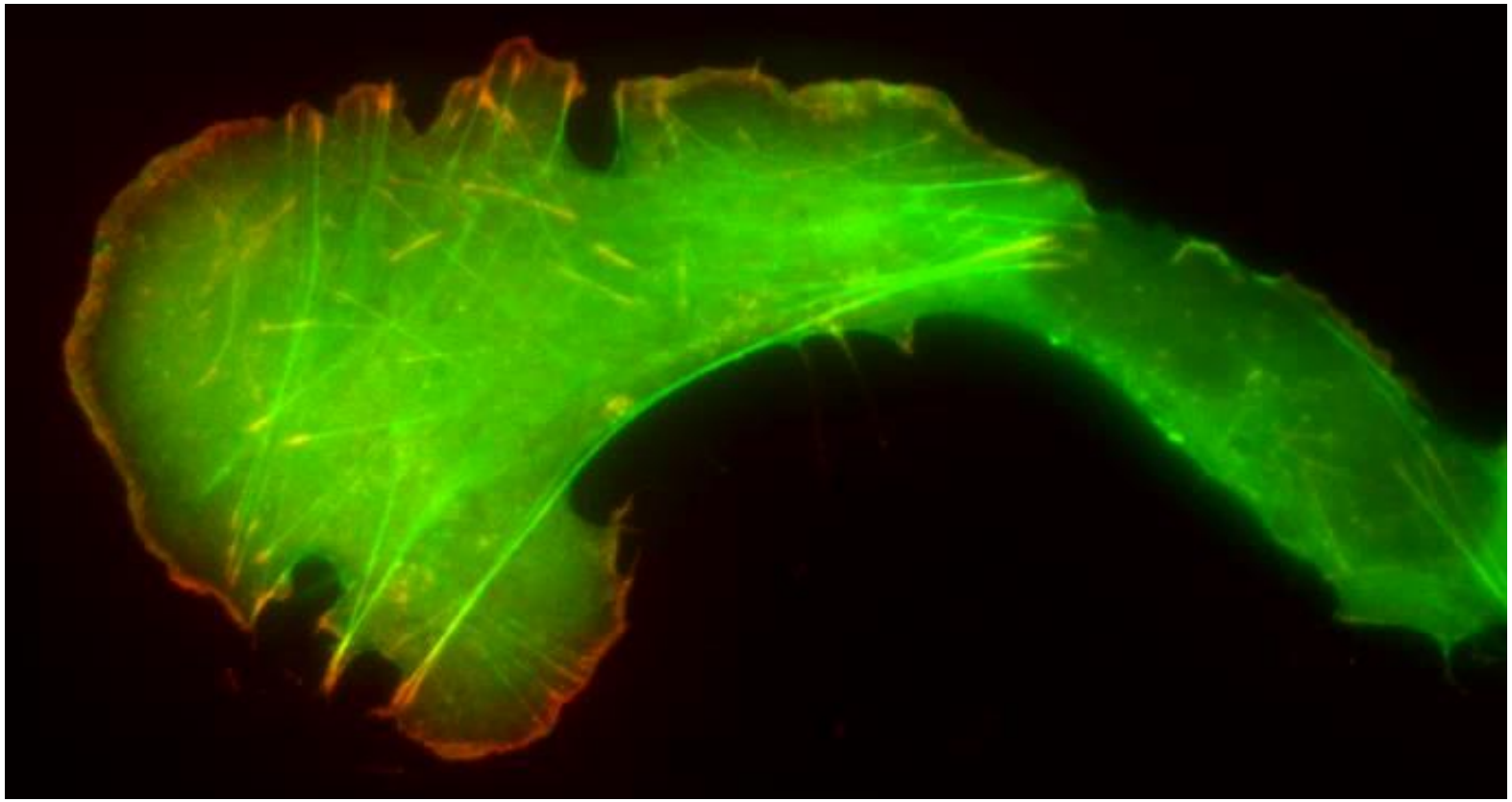
Lamellipodium with filopodia



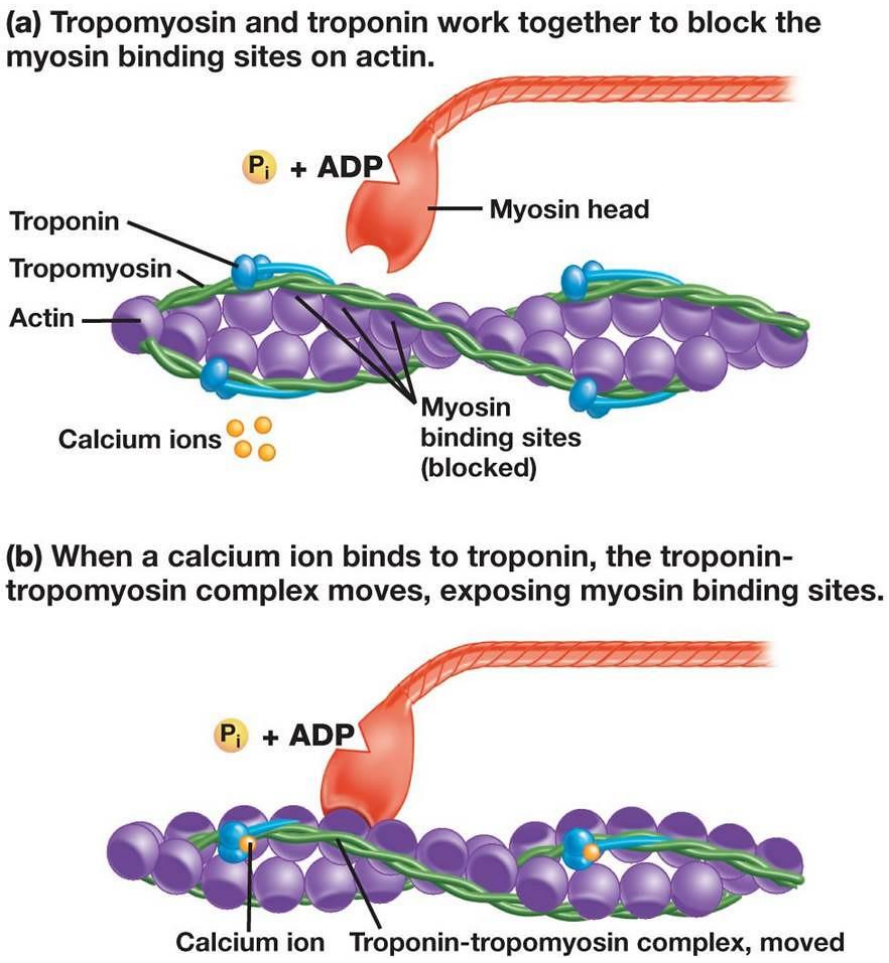


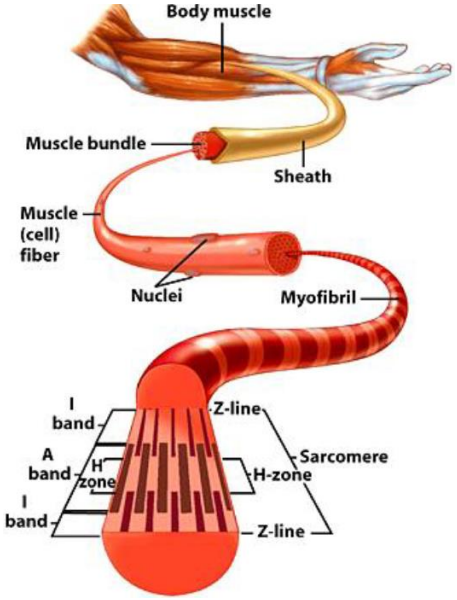
Actin branching
in lamellipodium

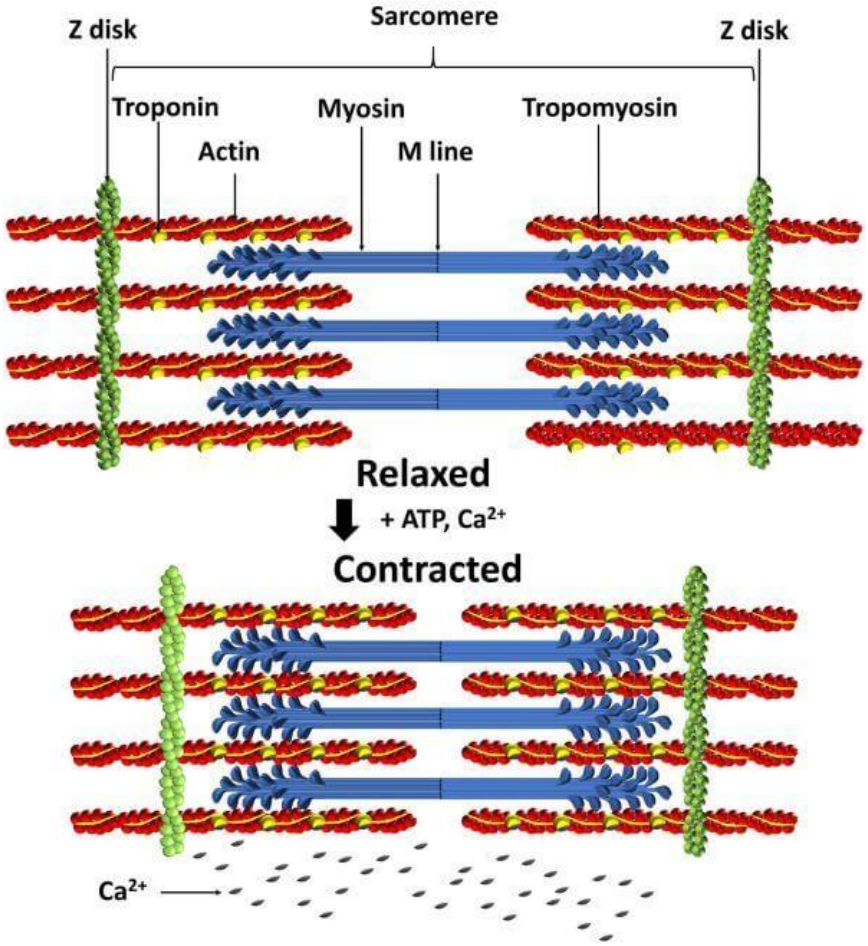
Actin TIRF & widefield microscopy

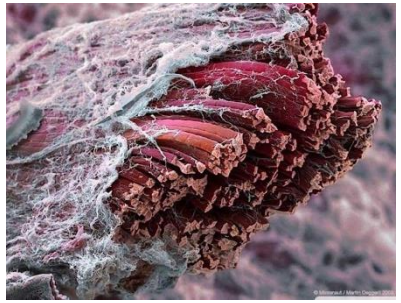


Actin-based movement: muscle contraction

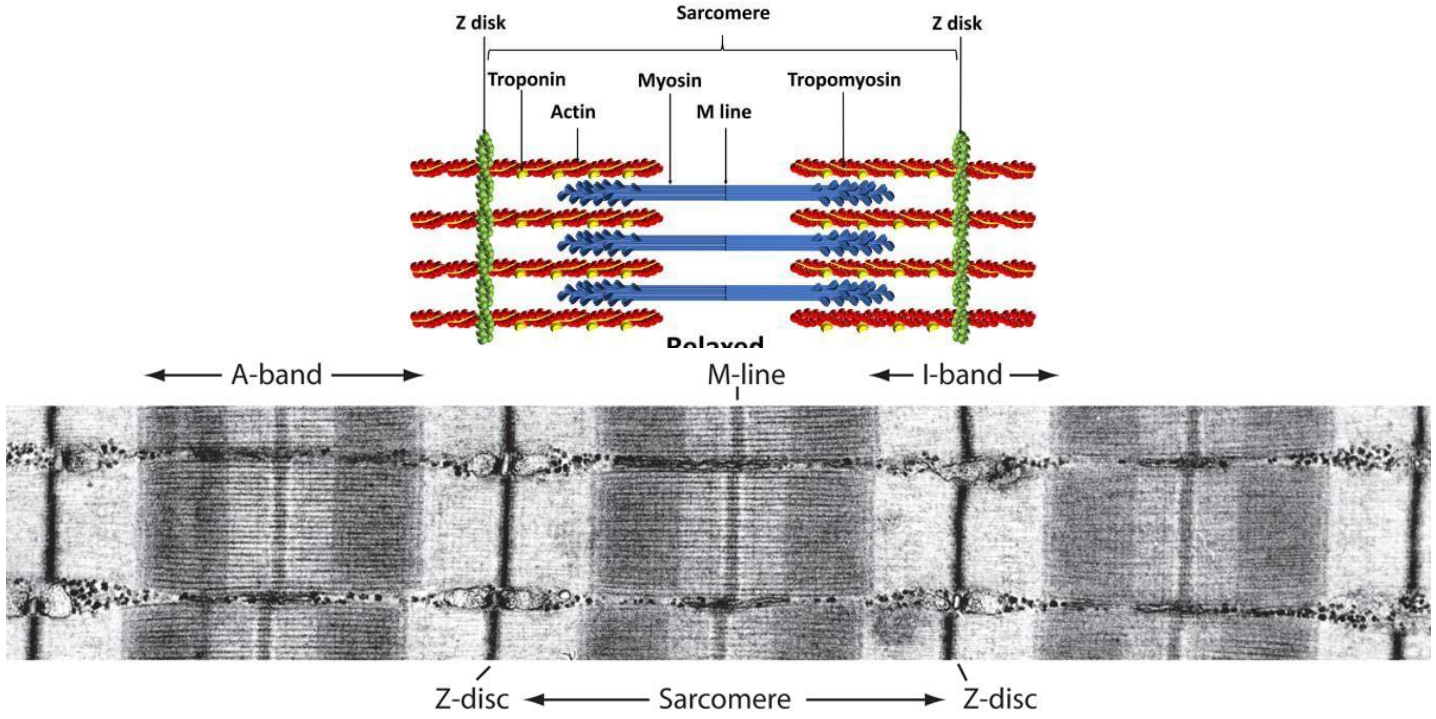


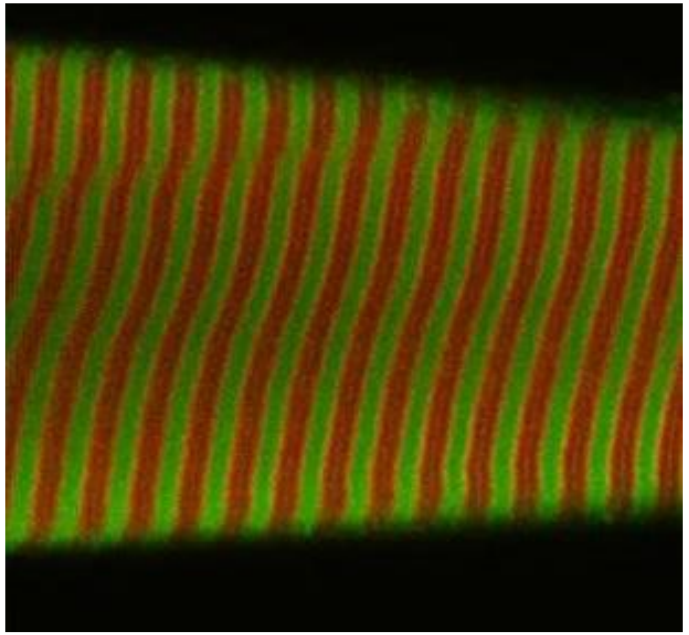






Myofibrils

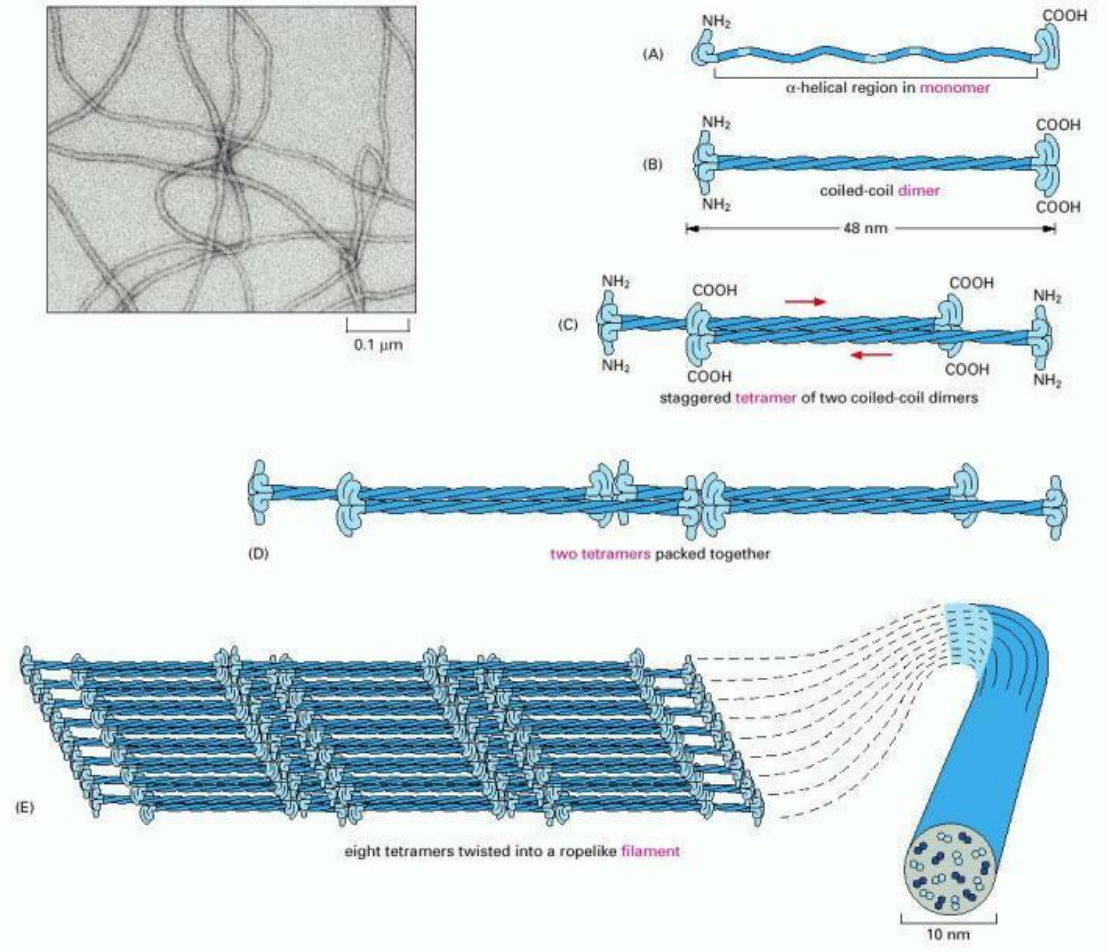




F-actin, myosin

Intermediary filaments

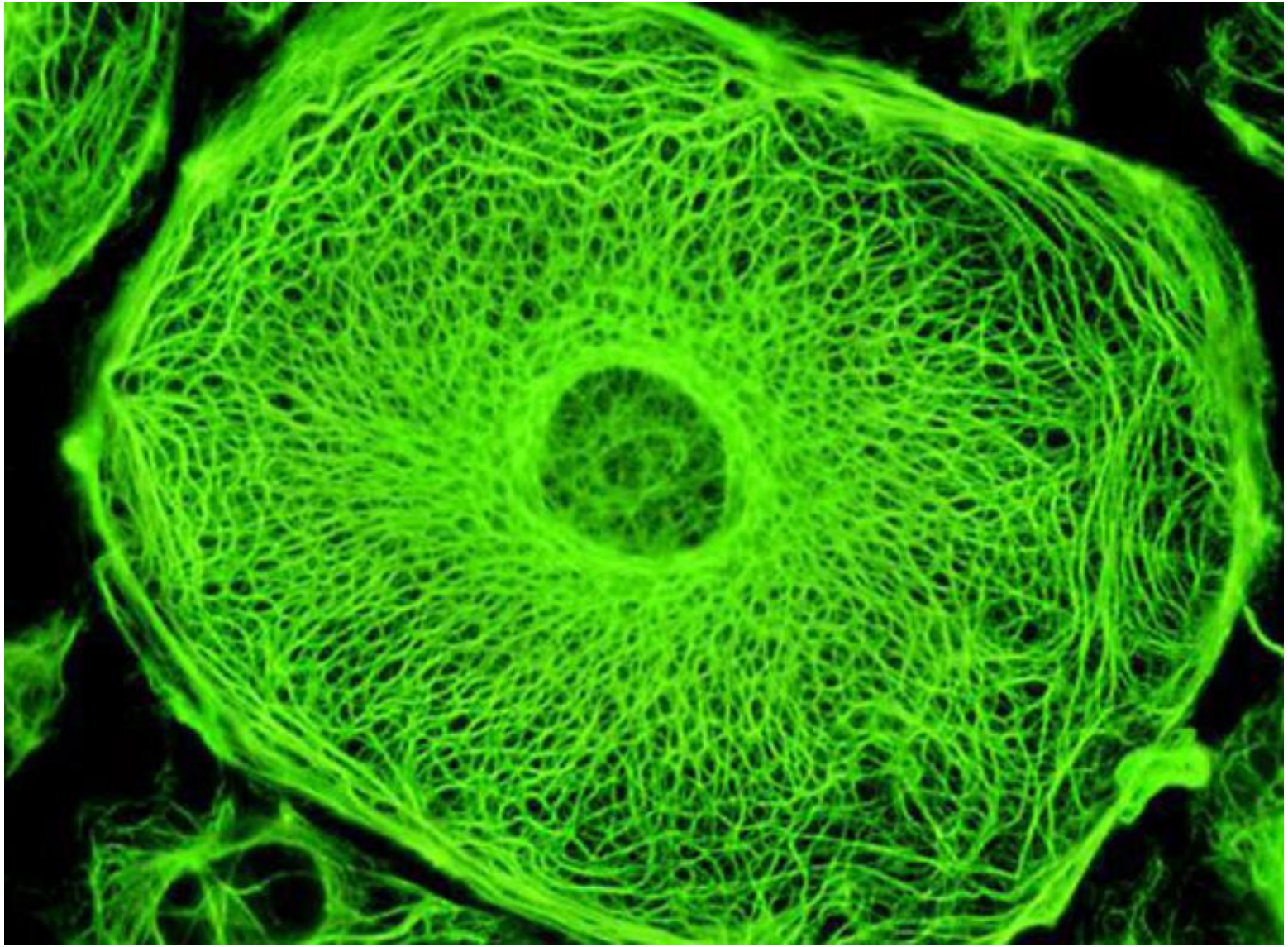
The structure of intermediary filaments



- **Present only in animal cells**
- **Many different tissue- and cell type-specific proteins**
- Polymerization:
  - Fibrillar monomeric peptide (central rod domain)
  - Homodimers/heterodimers (coiled-coil structure)
  - Tetramer (dimers staggered antiparallel)
  - Protofilament – two packed tetramers
  - **Filament 10 nm** – 8 protofilaments; **rope-like structure**

Classification of intermediary filaments

- **Type I and II**: **cytokeratins**
  - Epithelial cells
  - Associated proteins: **filaggrin, plectin, dermoplakin**
- **Type III**: **vimentin, desmin, GFAP, peripherin**
  - Mesenchymal tissue, muscle cells, neuroglia
  - Associated proteins: **plectin, filensin, epinemin, paranemin**
- **Type IV**: **neurofilaments, synemin**
  - Neurons; Associated proteins: **plectin**
- **Type V**: **lamins** (nuclear lamina, not cytoskeleton)
- **Type VI**: **nestin**
  - Neural stem cells, cancer cells, endothelial cells

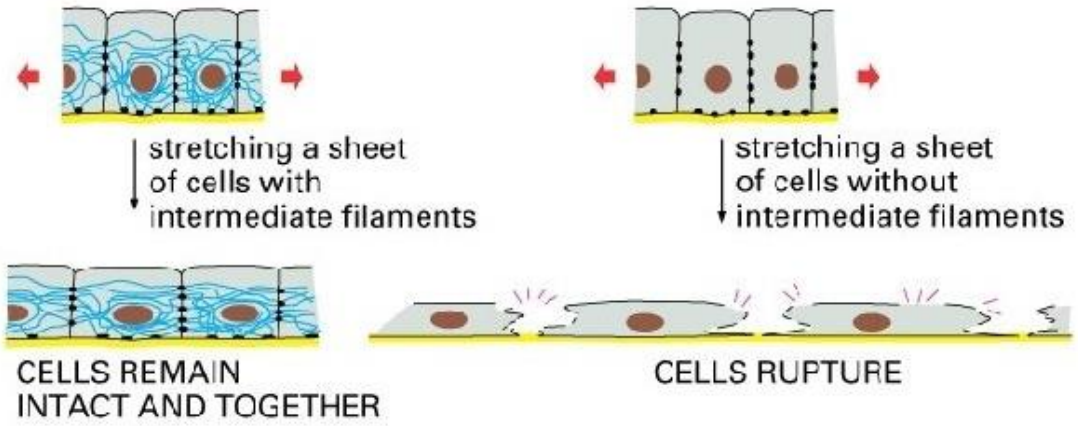


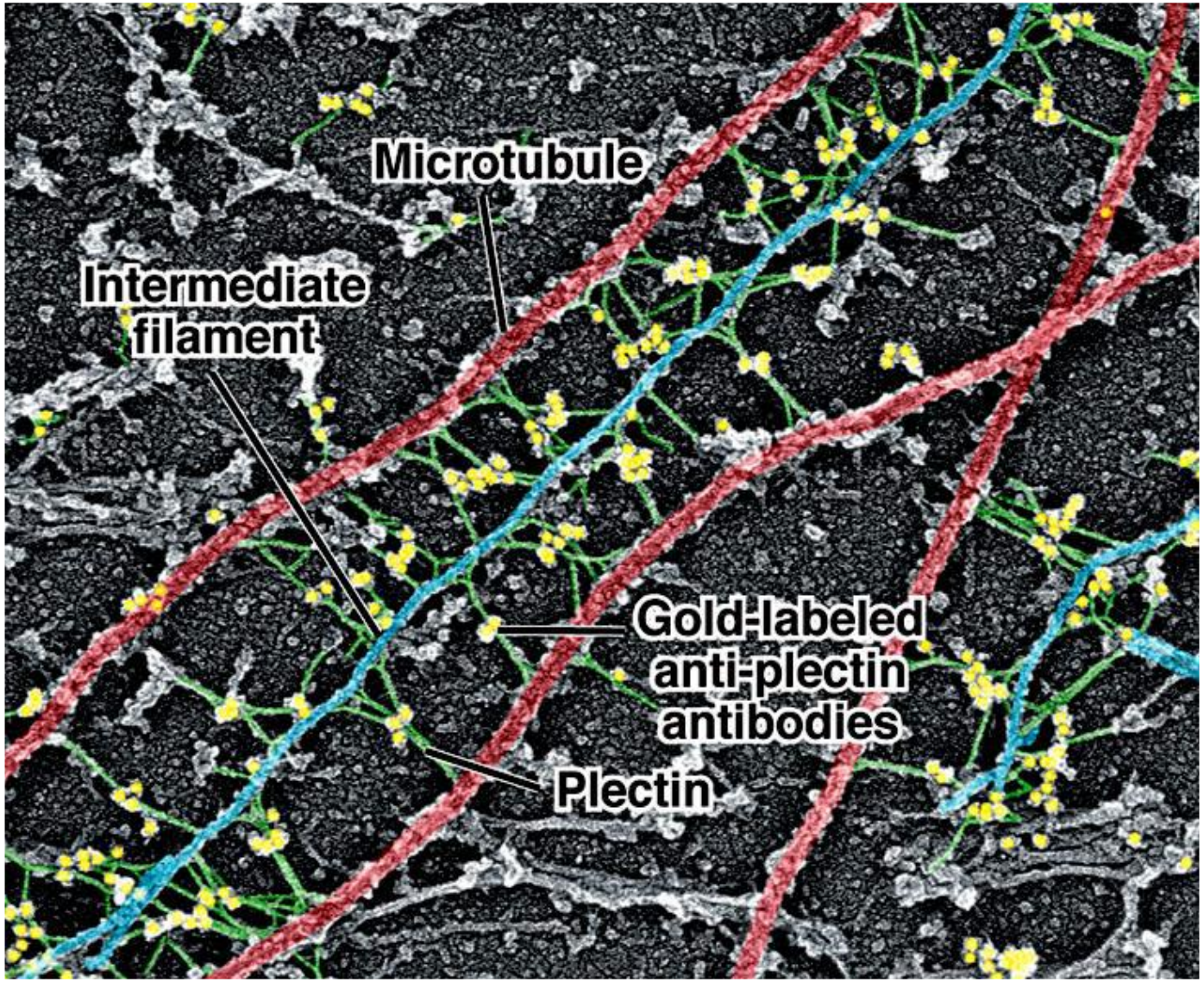
Kidney cell line Ptk2

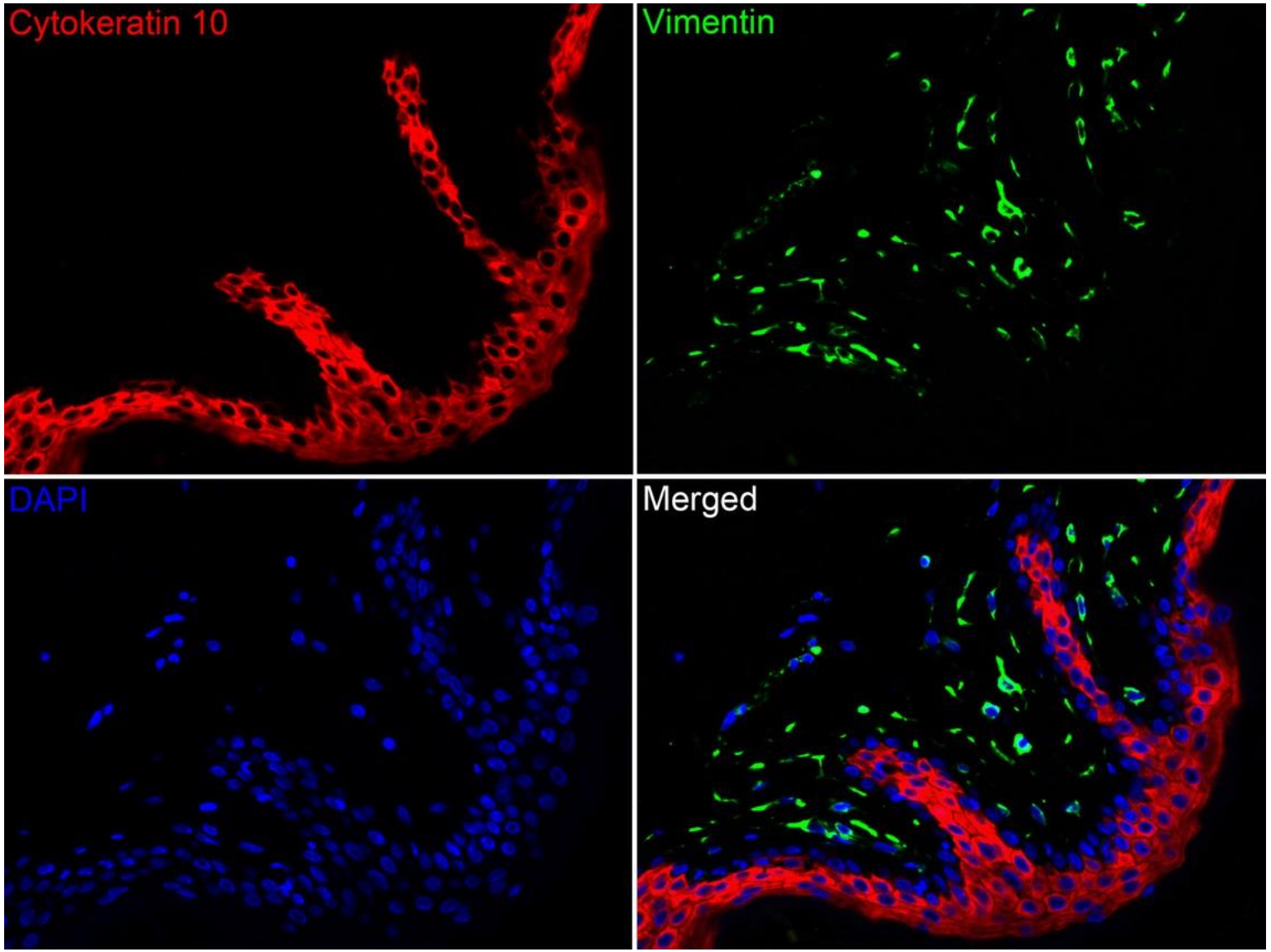
Cytokeratin

Functions of intermediary filaments

- Increase **mechanical strength**
- **Highly stretchable: cell survival under extreme deformations**
- Shaping cell morphology, localization of organelles
- Integrating other components of the cytoskeleton

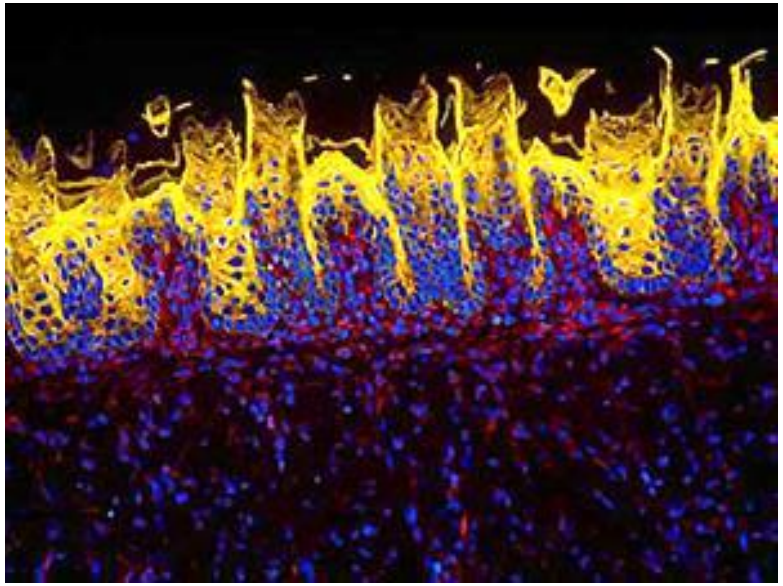



Microtubule
Intermediate filament
Gold-labeled anti-plectin antibodies
Plectin

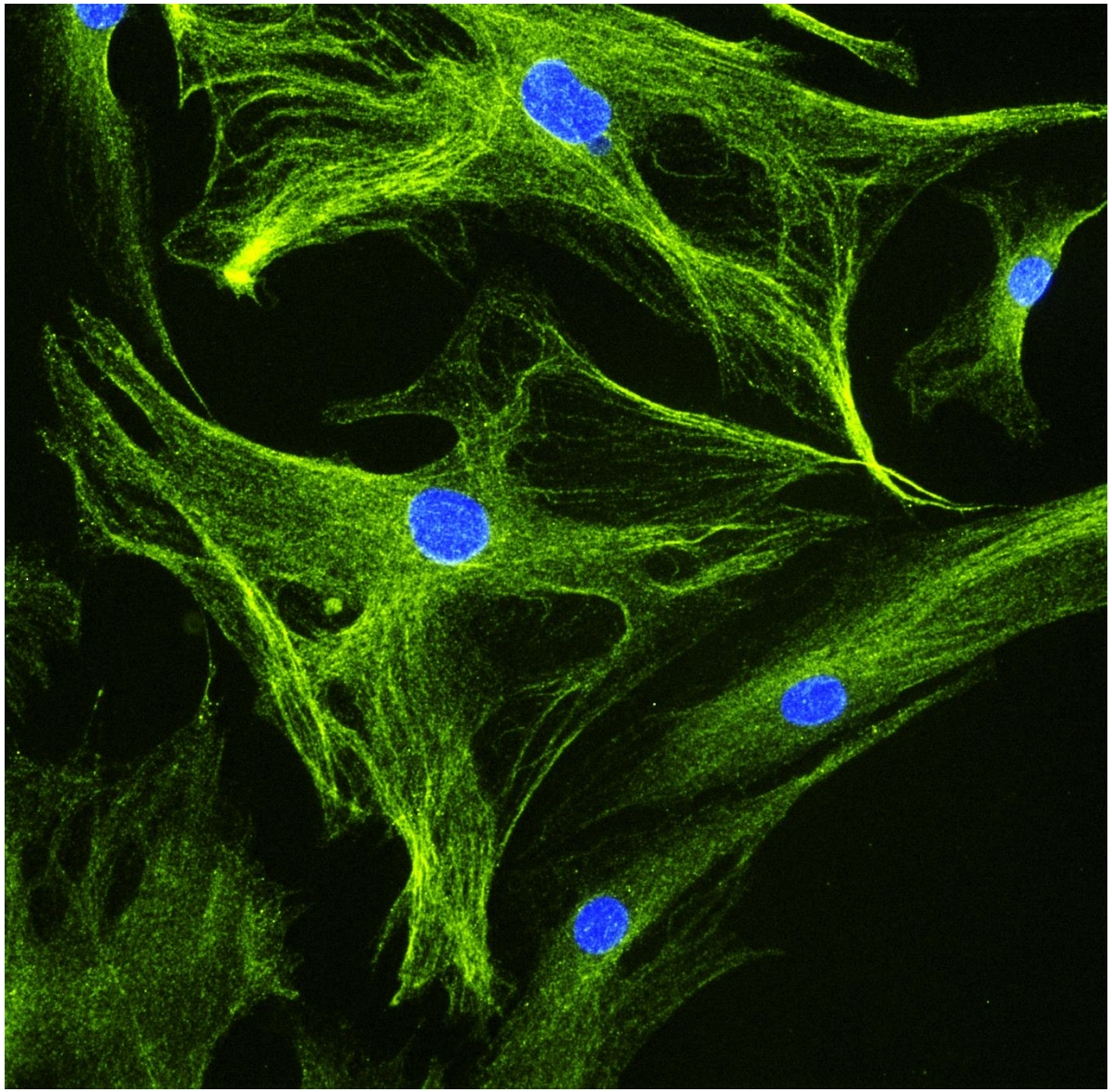


Human skin tissue

Section of rat tongue



Cytokeratin, vimentin, DNA



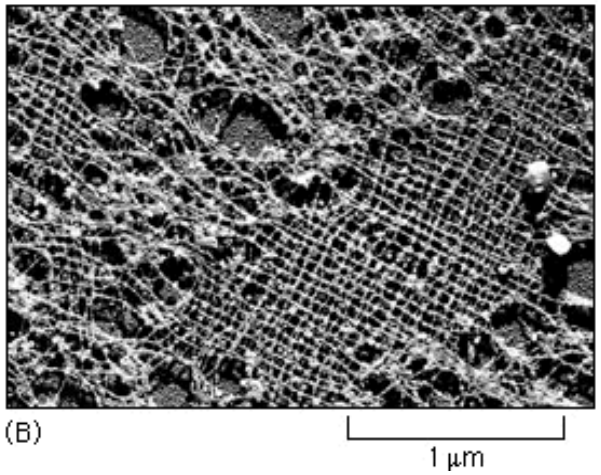
Human Ewing’s sarcoma cell line

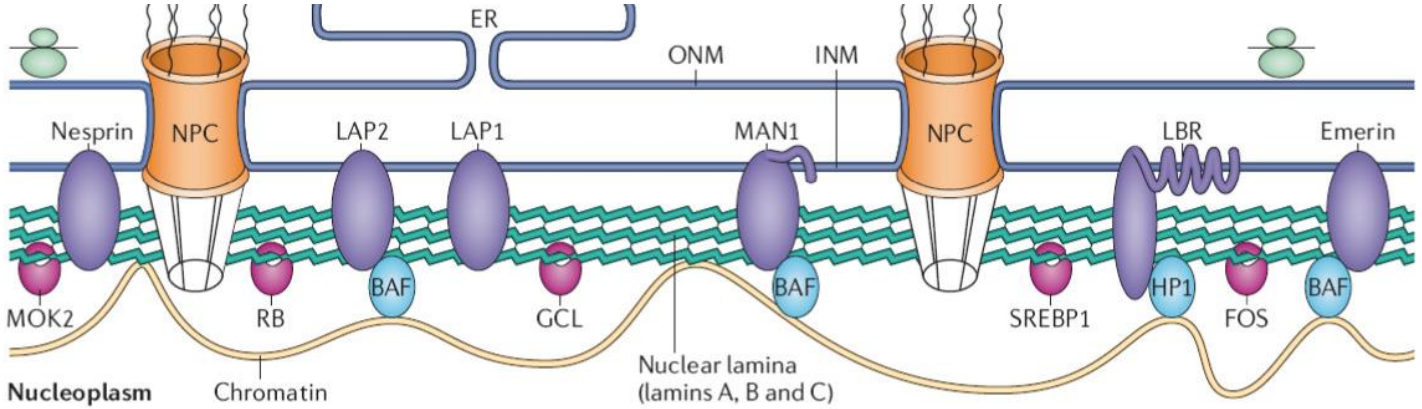
Nestin, DNA

Nuclear cytoskeleton & cell cortex

Nucleoskeleton = nuclear lamina

- **Attached to nuclear envelope**: lamina associated polypeptides (LAP1, LAP2), emerin, lamin B-receptor (LBR), MAN1
- **Lamin A** (alternative splicing: **lamin C**) & **Lamin B**
- Create **fibrous layer**; involved in **chromatin organization**



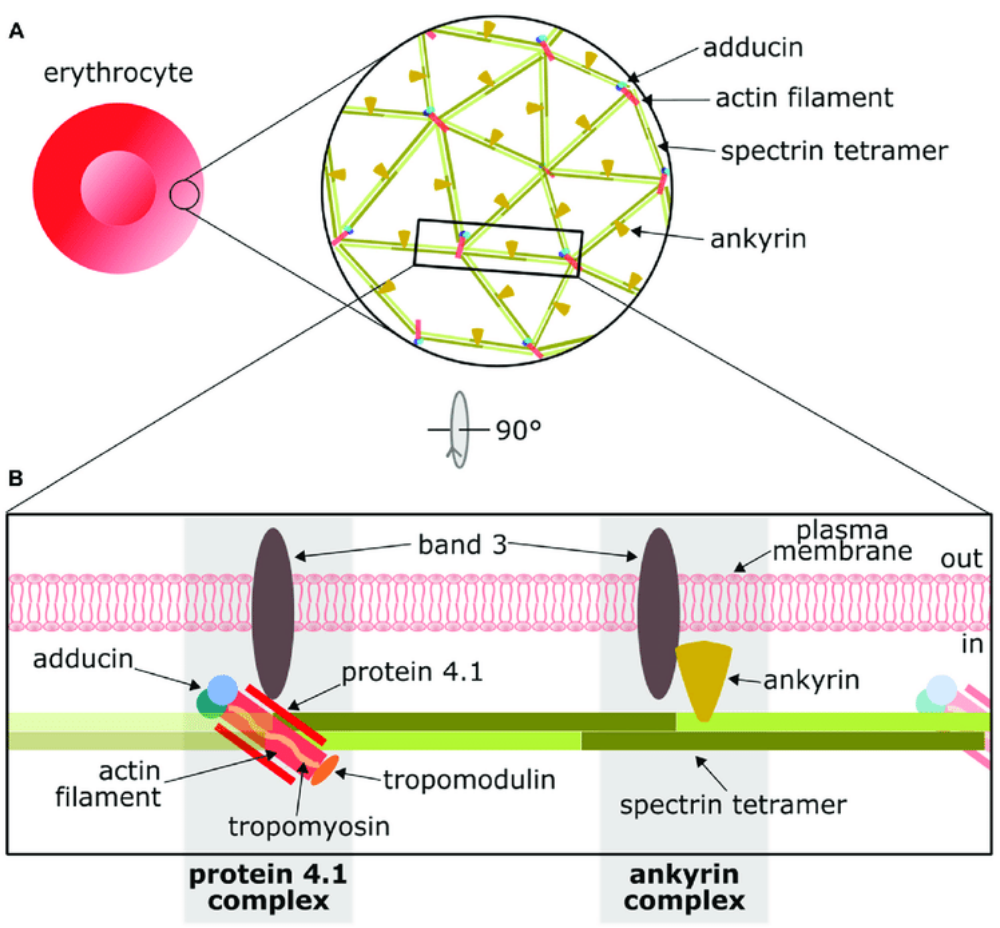


BAF & HP1 bind to histones

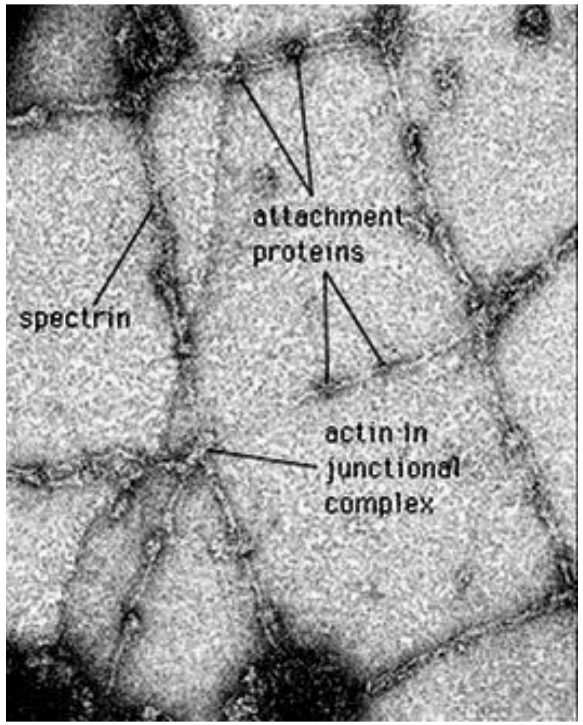
Cell cortex = actin cortex

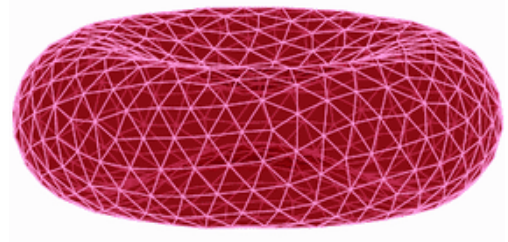
- Cross-linked actin network at the inner face of the plasma membrane
- **Spectrin** (erythrocytes), **Spectrin Alpha, Non-Erythrocytic 1** (aka fodrin; other cell types)
- Associated proteins:
  ankyrin – binding to transmembrane proteins
  protein 4.1 – links spectrin with actin
- **Morphology** maintenance (e.g., erythrocytes) and changes,
  cell migration

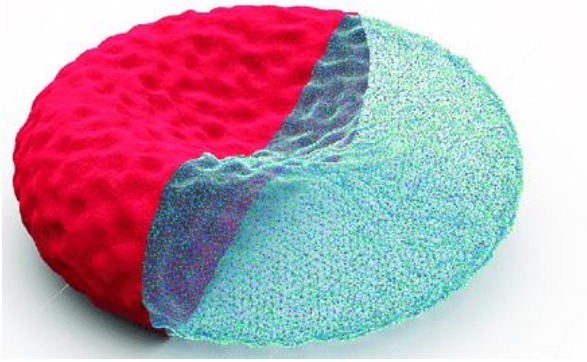
Cell cortex = actin cortex



– Supports otherwise fragile plasma membrane



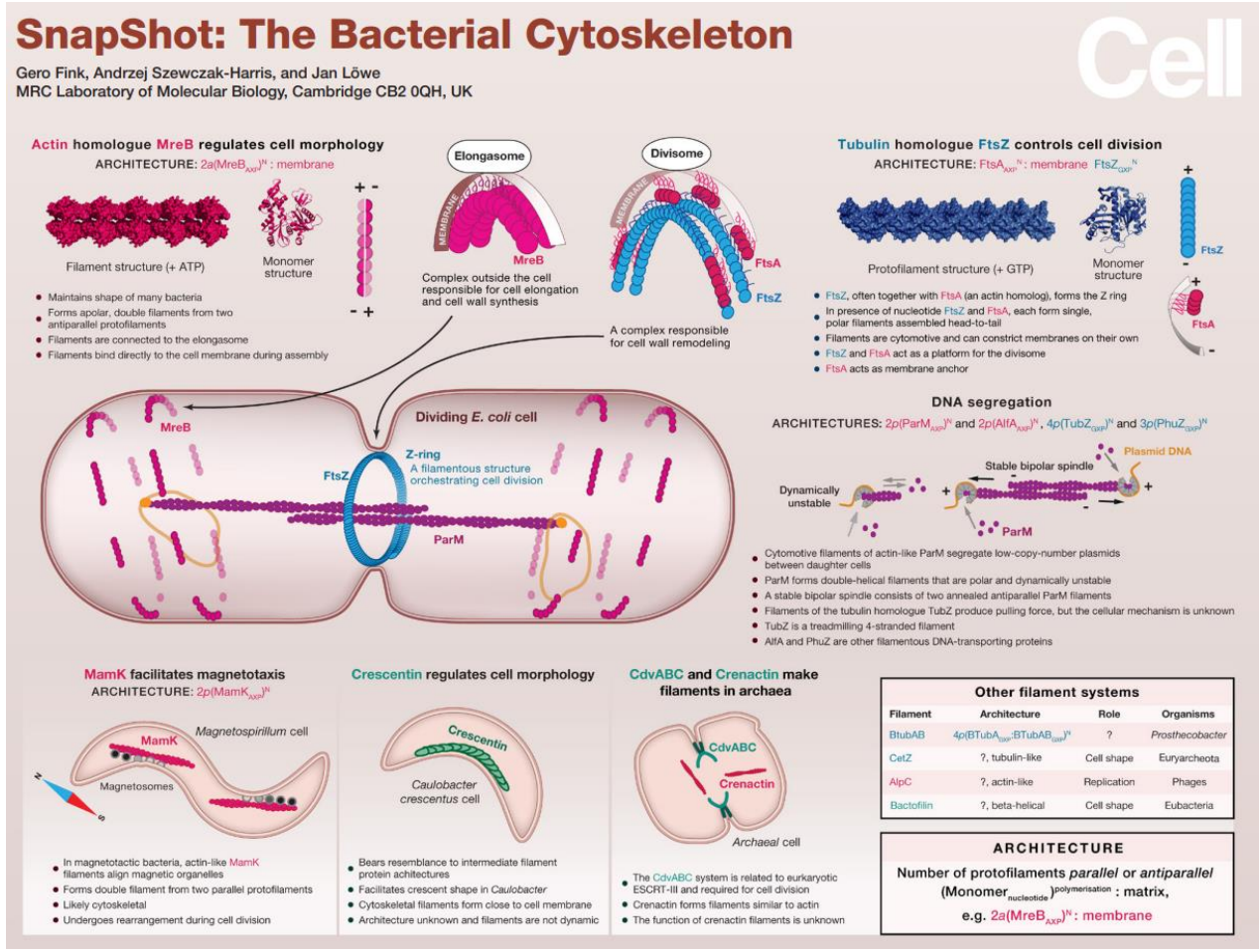




Cytoskeleton in prokaryotes

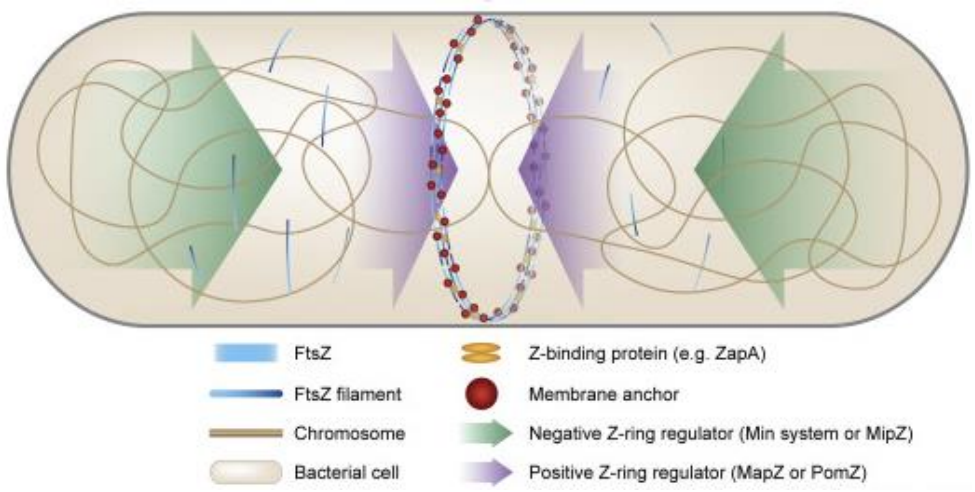
Discovered ~20 years ago

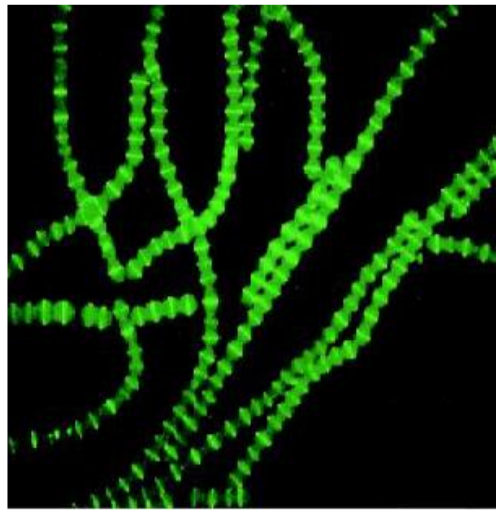
- Previously thought bacteria lacked cytoskeleton
- 1991 – **FtsZ protein**
  - tubulin homolog
- 2001 – **MreB** and **Mbl**
  - actin homolog
- …

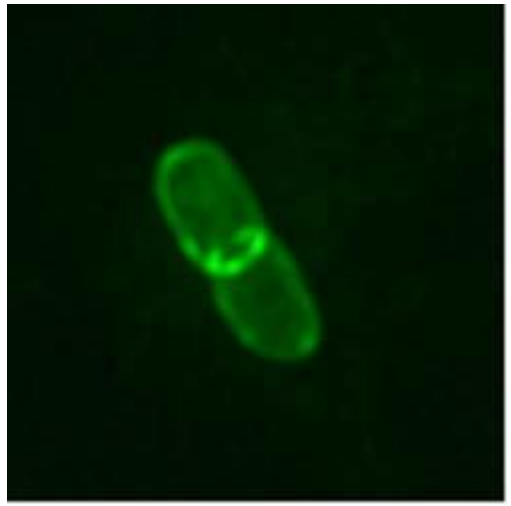


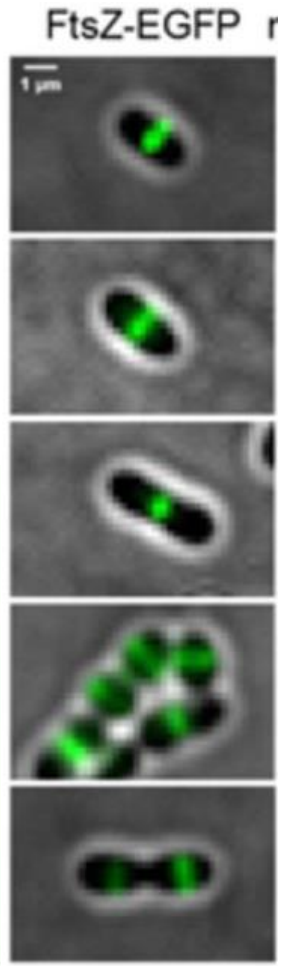
FtsZ

- Prokaryotic **tubulin homolog**
- Creates **Z-ring** structures in the central region of the cell
- Mediating **cell division – cooperation with FtsA (actin homolog)**



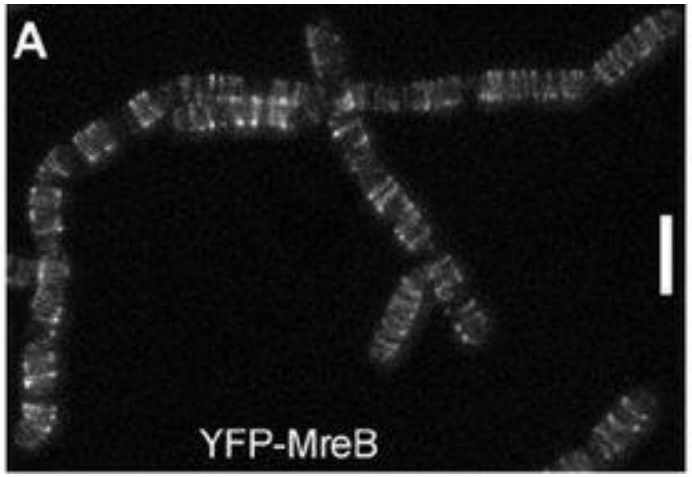


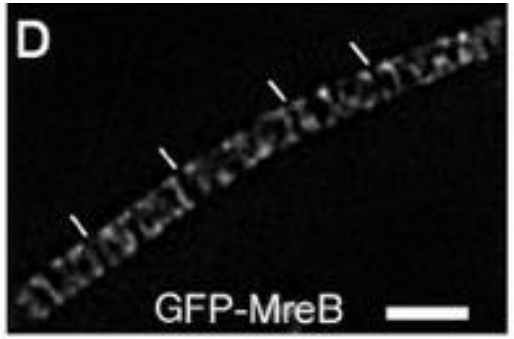




MreB, MbI, MreBH

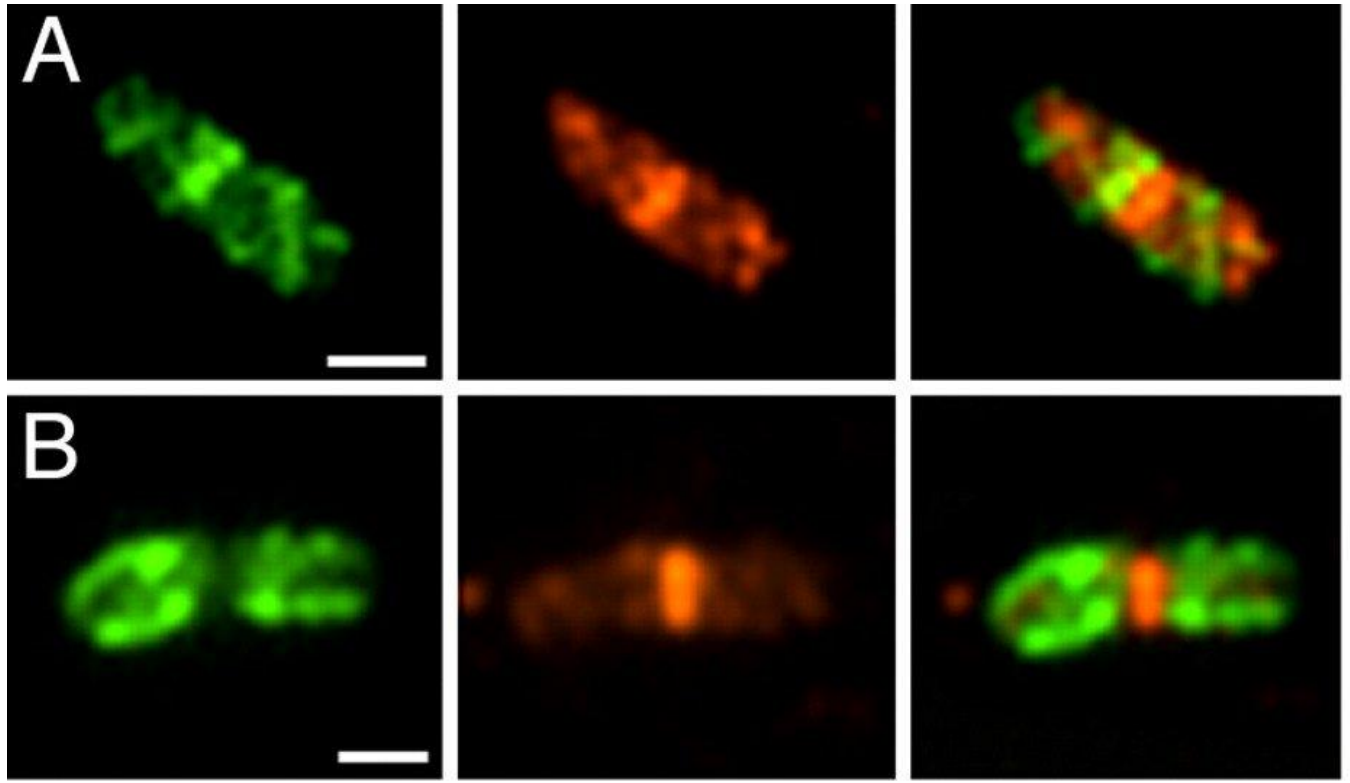
- Prokaryotic **actin homologs**
- **Cell morphology and elongation**
- Double filaments (antiparallel protofilaments) in proximity of the plasma membrane





ParM

- Actin homolog
- Segregation of low-copy-number plasmids before cytokinesis

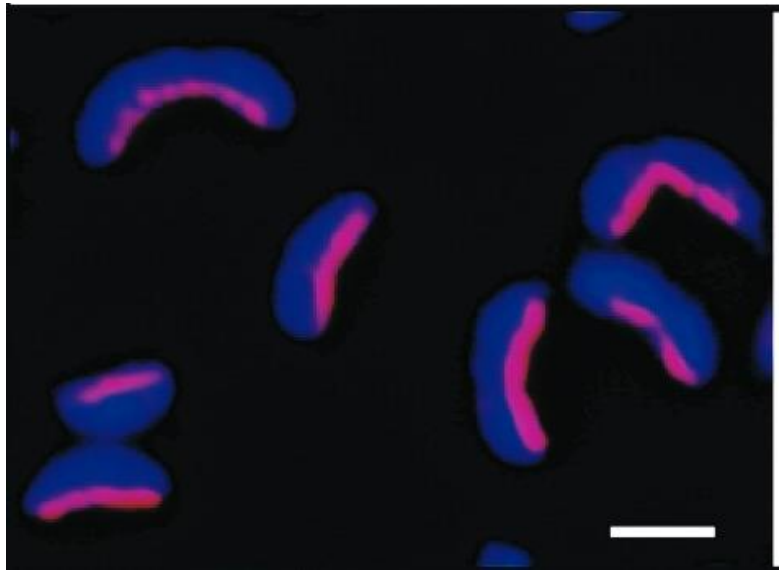


E. coli

MreB, FtsZ

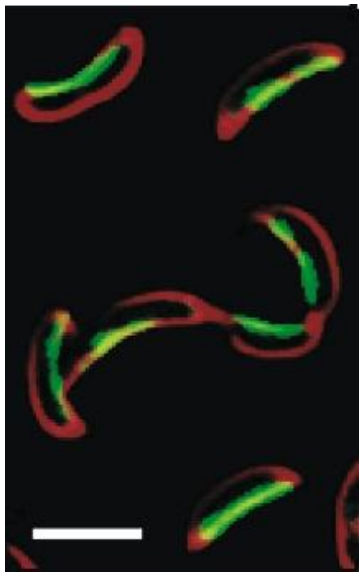
Crescentin (CreS)

- Prokaryotic **intermediary filament homolog**
- Cytoskeletal filaments close to plasma membrane
- **Crescent (bend) shape:** *Caulobacter crescentus, Helicobacter pylori*



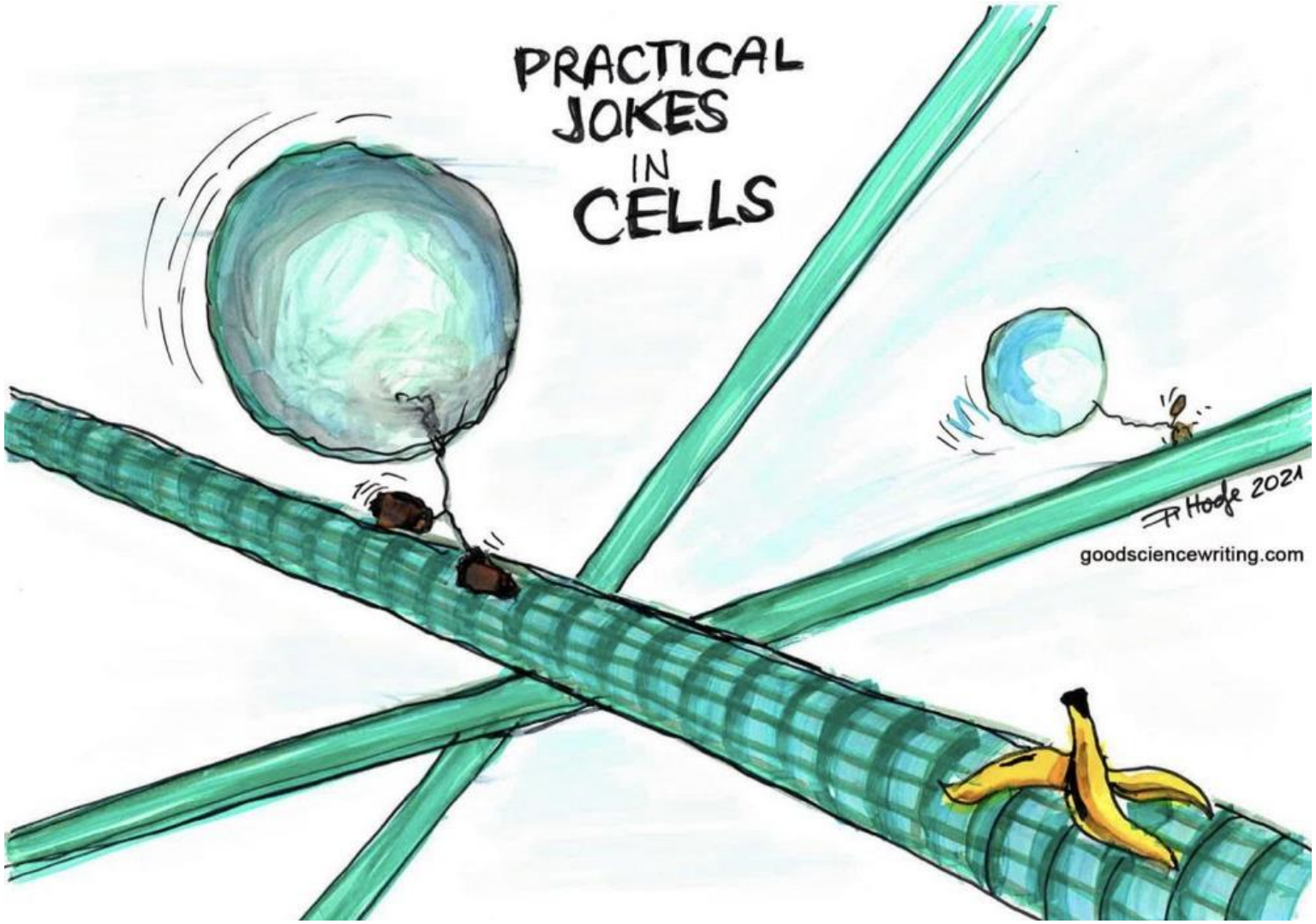
C. crescentus

Crescentin
DNA



C. crescentus

Crescentin
Plasma membrane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