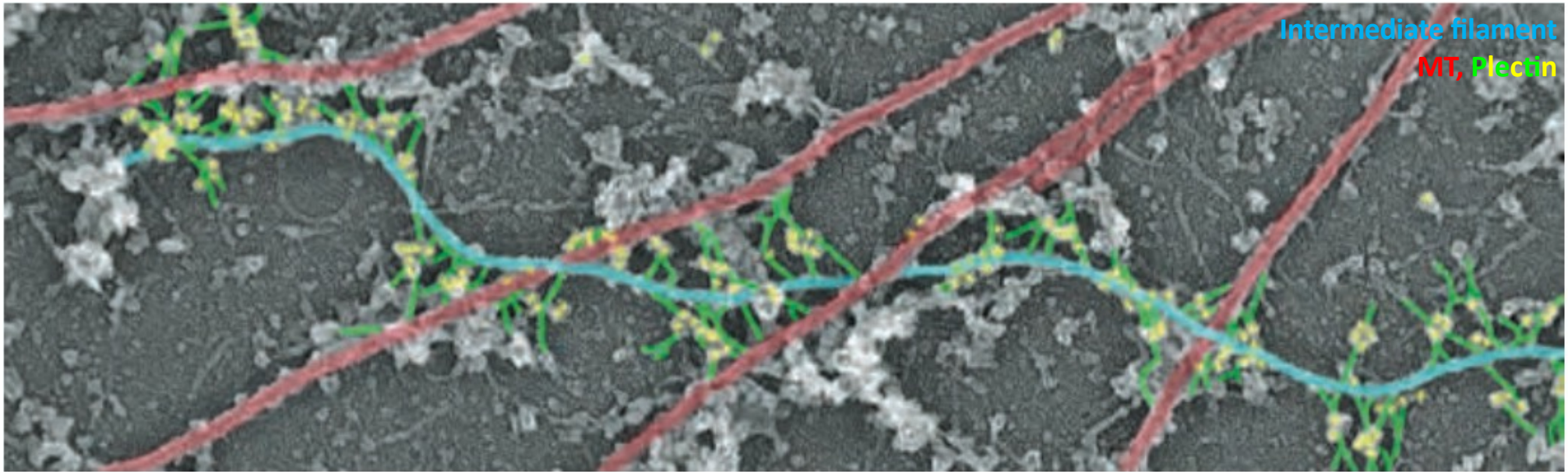
Cytoskeleton



M1 International, Cancer Cell Biology, TU n°05

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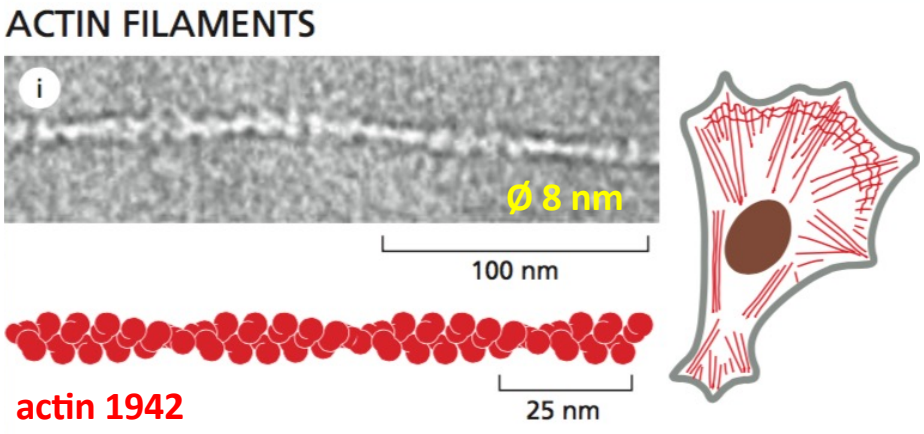
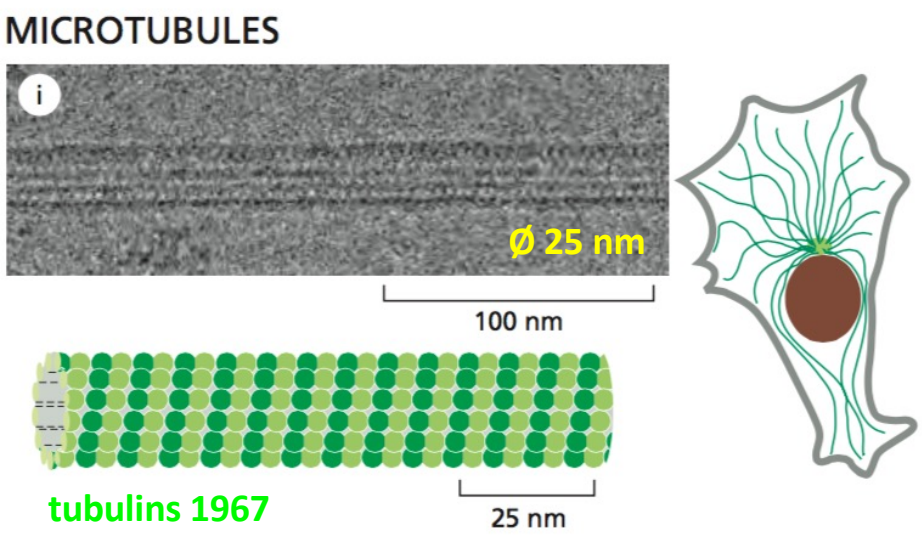
GRADUATE SCHOOL
Health and Drug Sciences

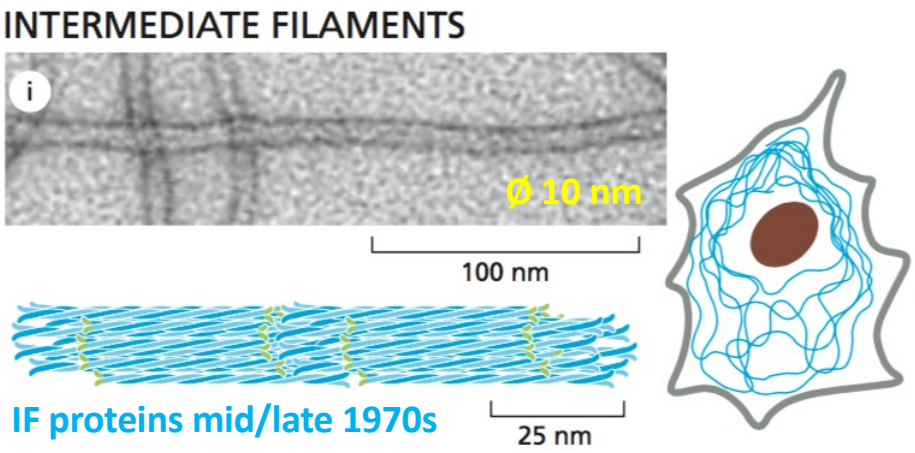
d2hp

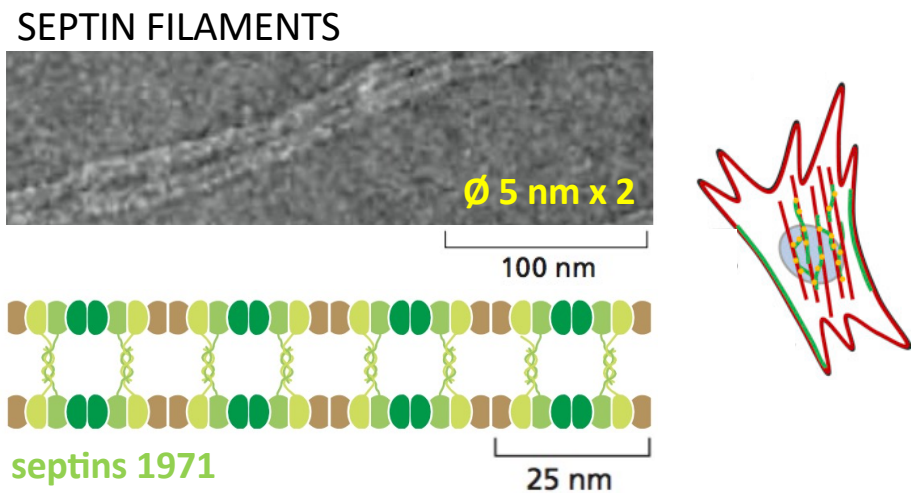
université PARIS-SACLAY
FACULTÉ DE PHARMACIE

Cell cytoskeleton









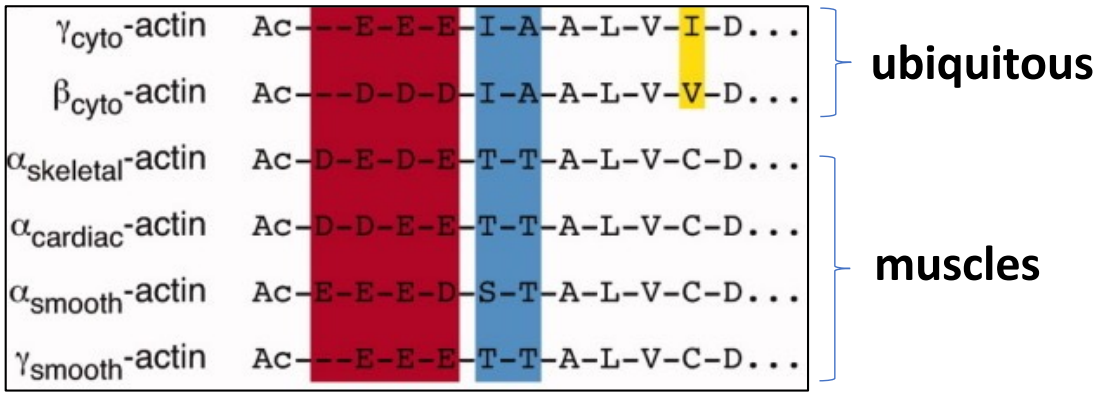
Calvo et al., Cell reports, 2015

Adapted from panel 16-1 & fig 74 , Molecular Biology of the Cell 6th

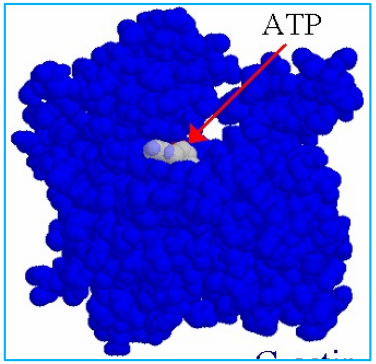
Actin microfilaments

Actin microfilament structure

Actin isotypes in humans



G-actin (globular)



- end
pointed

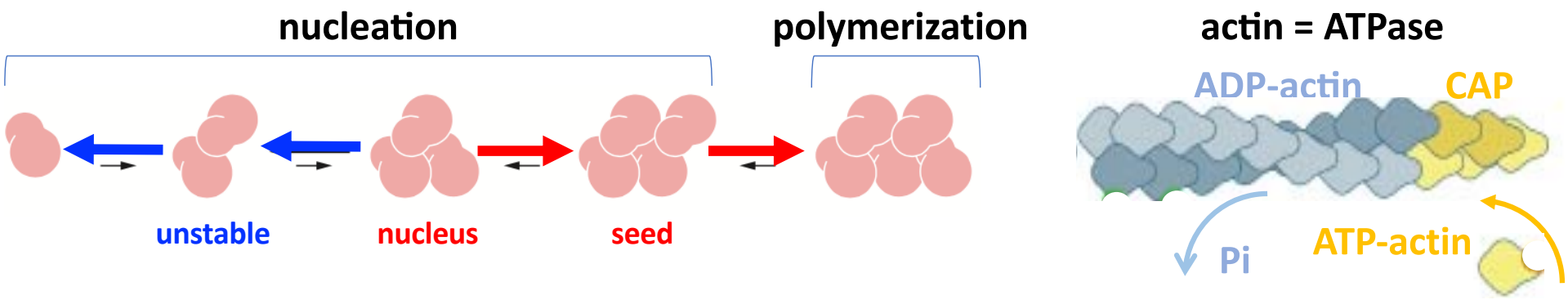
+ end
barbed

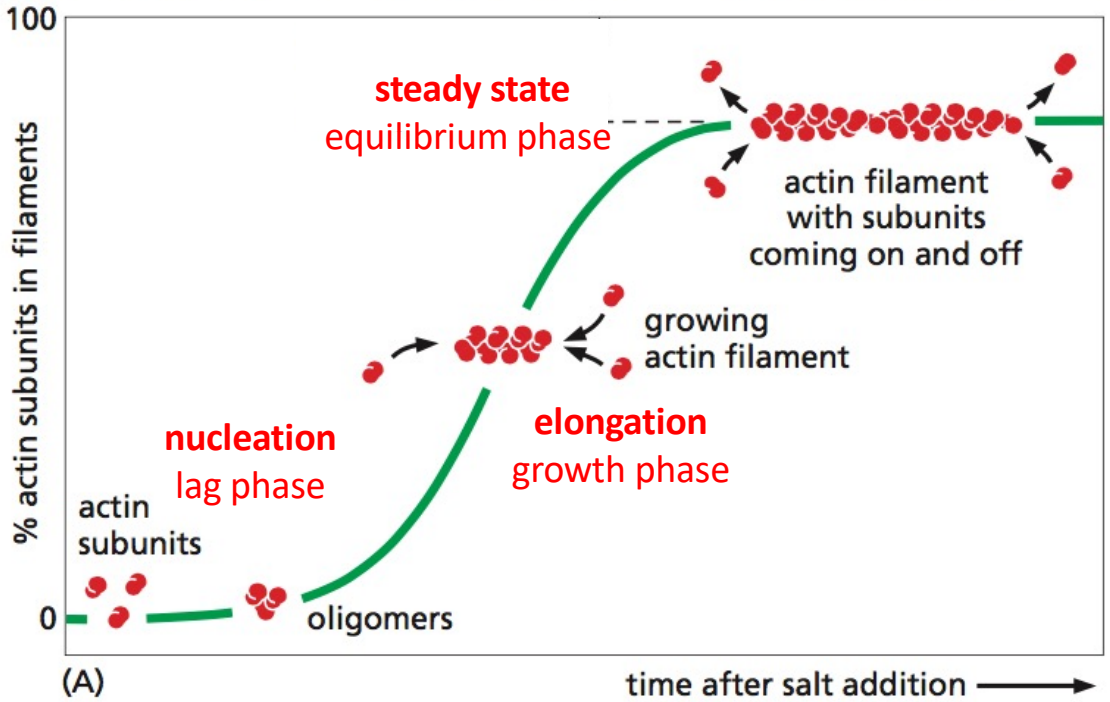
F-actin (filamentous) is a polarized double helix of ~ 8 nm wide

- end **+ end**

Perrin & Ervasti, cytoskeleton, 2010
Joyce J. Diwan, Rensselaer Polytechnic Institute
Figure 16-11 Molecular Biology of the Cell 6th

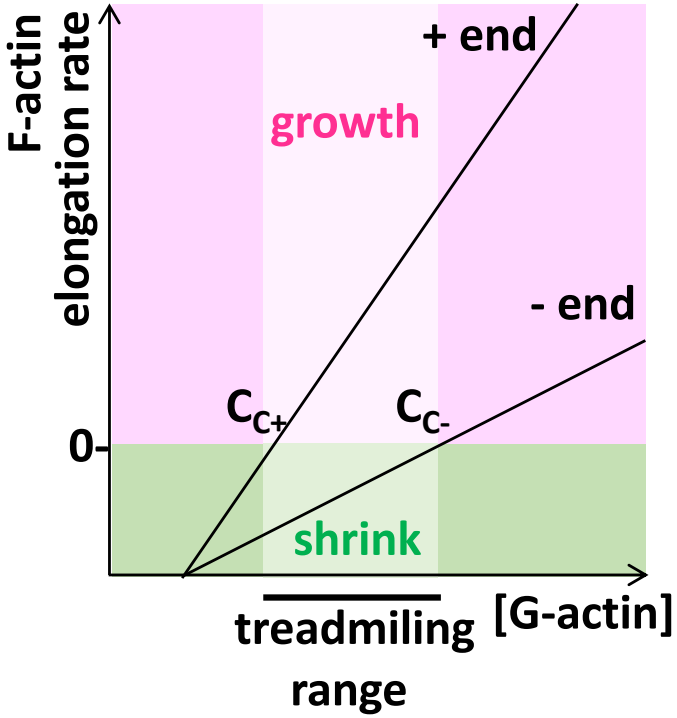
Actin microfilament dynamics



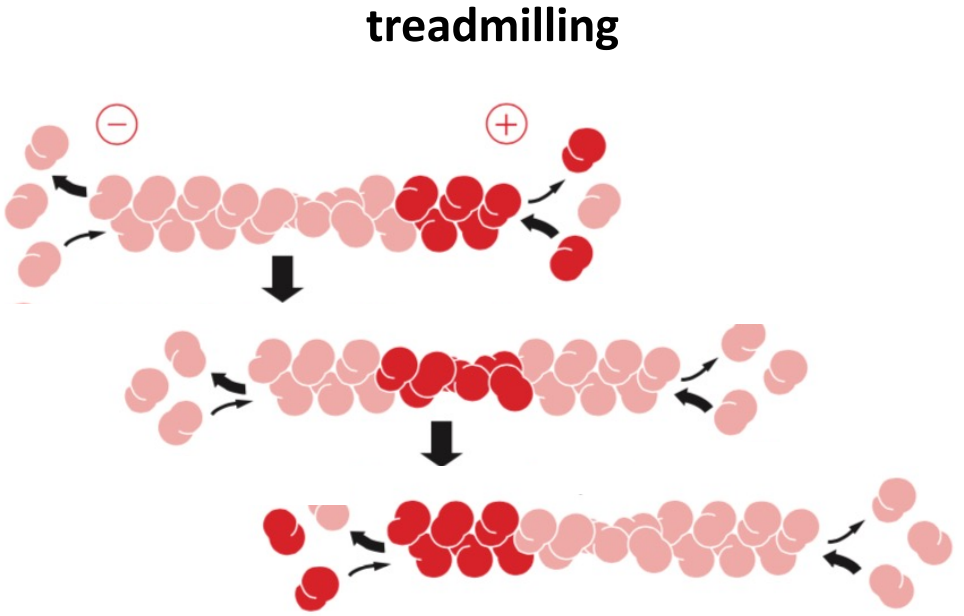


Panel 16-2, Figure 16-13
Molecular Biology of the Cell 6th

Actin microfilament dynamics



C_C : critical concentration
elongation rate = 0

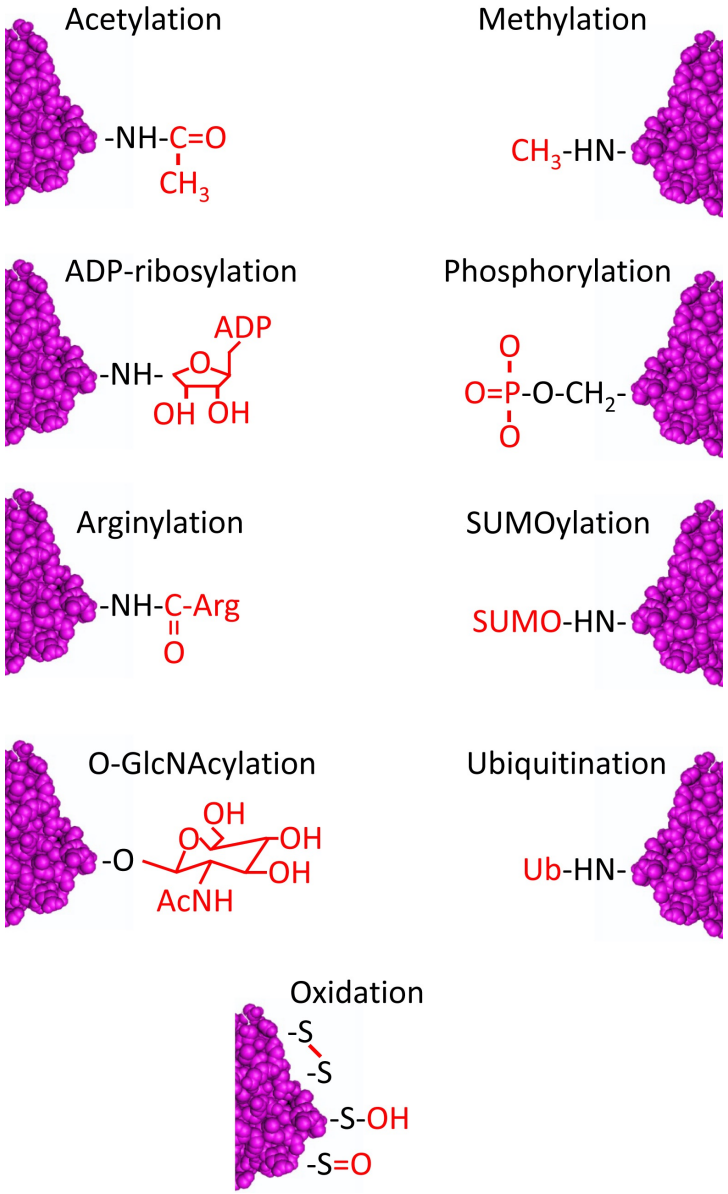


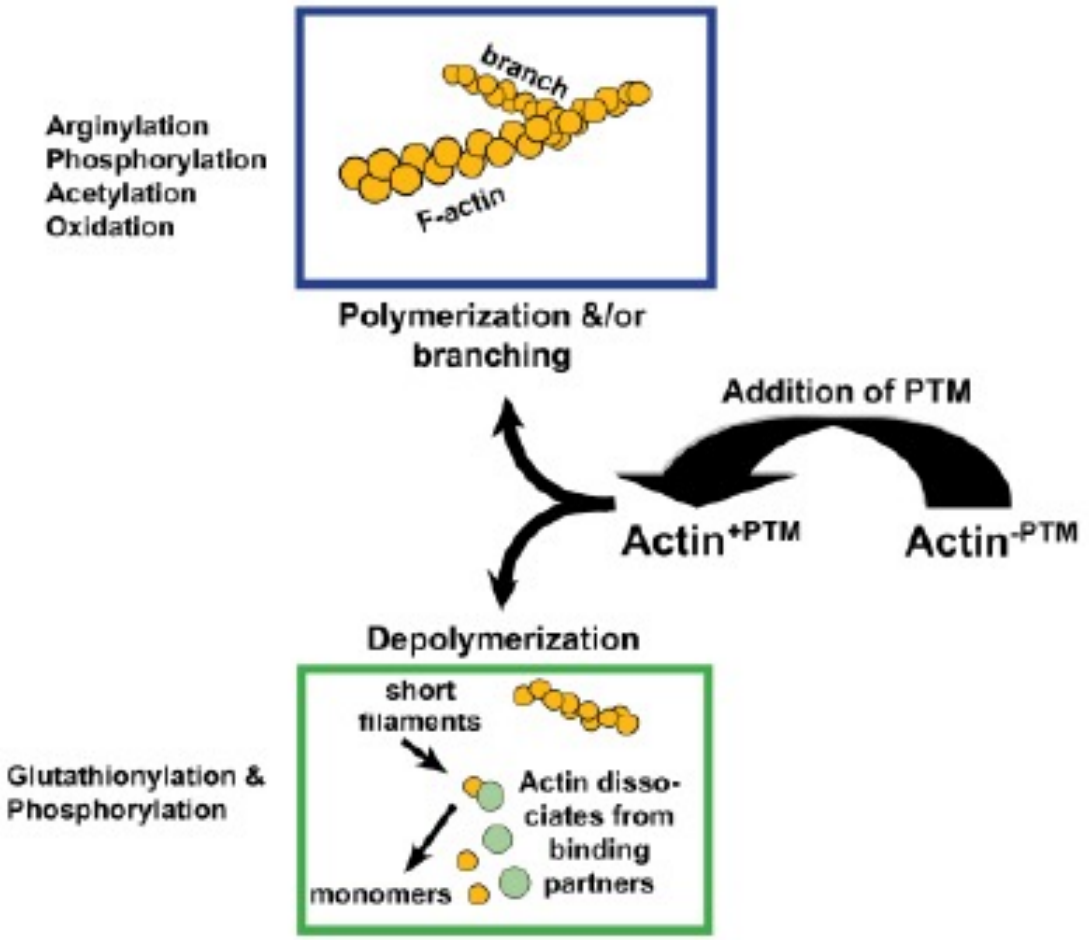
- end
depolymerization

+ end
polymerization

Adapted from Molecular Biology of the Cell 6th
Panel 16-2, Figure 16-14

Actin code : post-translational modifications (PTMs)

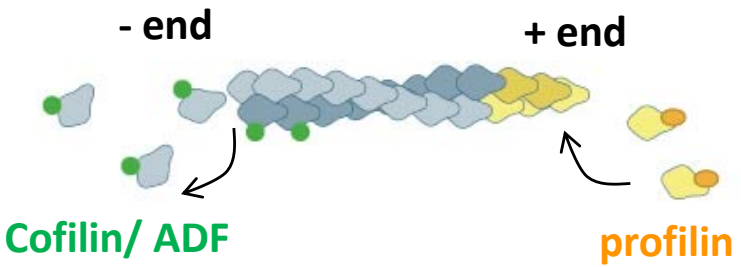


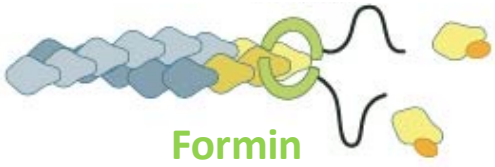


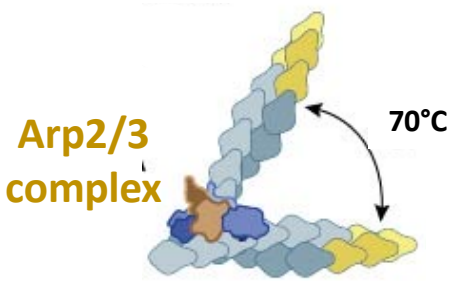
https://www.cytoskeleton.com/actin-post-translational-modifications

Actin-binding proteins (ABPs)

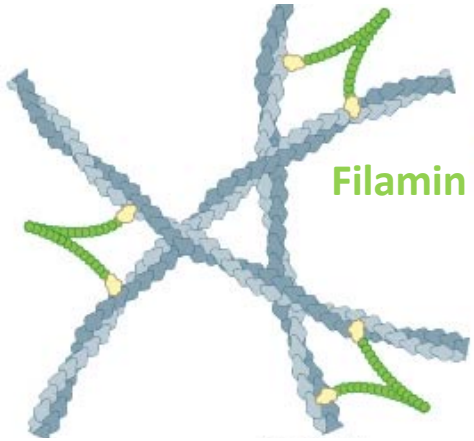
Nucleation/(de)polymerization

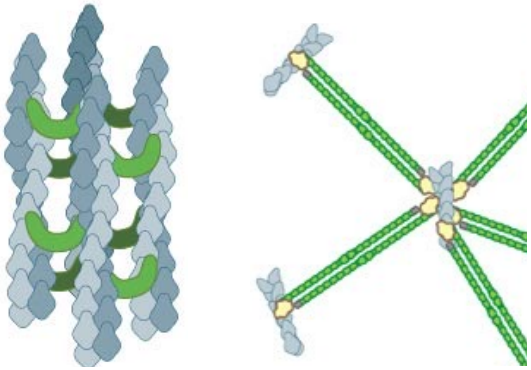




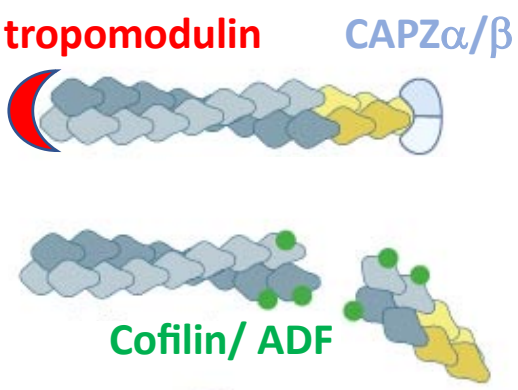


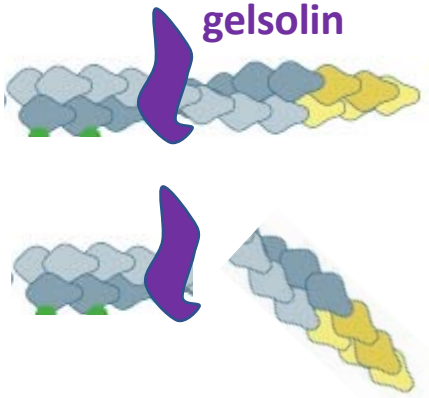
Crosslinking/bundling





Capping / severing

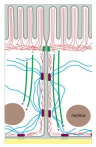




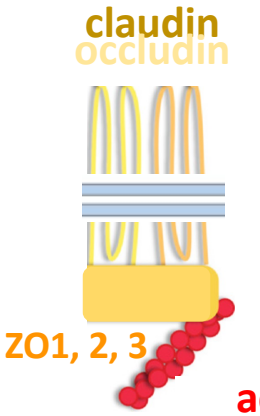
Adapted from https://www.mechanobio.info/cytoskeleton-dynamics/

Actin-binding proteins (ABPs)

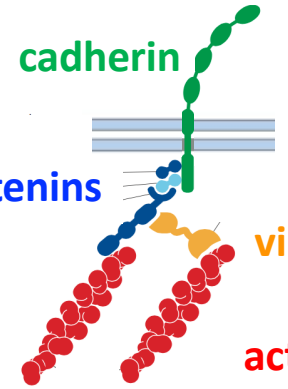
Membrane attachment



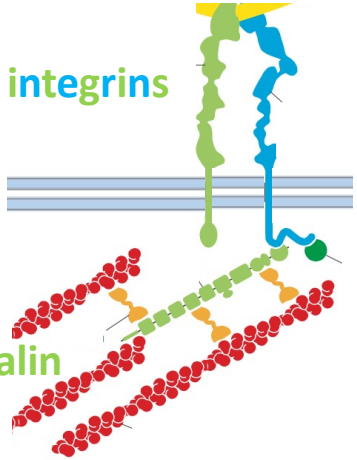
tight junction



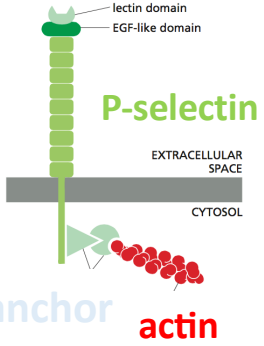
adherens junction



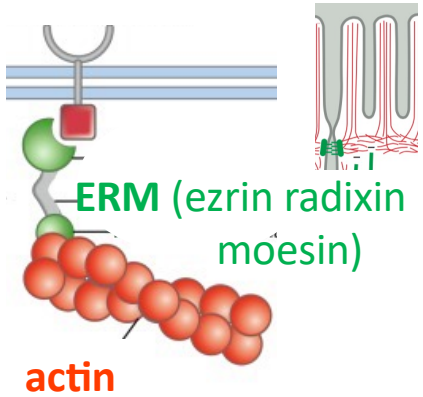
focal adhesion (FA)



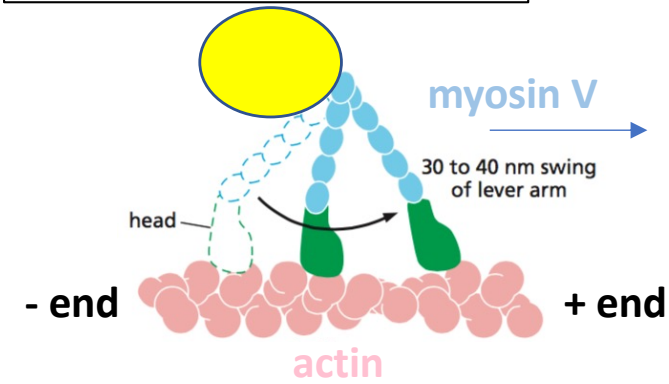
transient junction



filopodia, microvilli

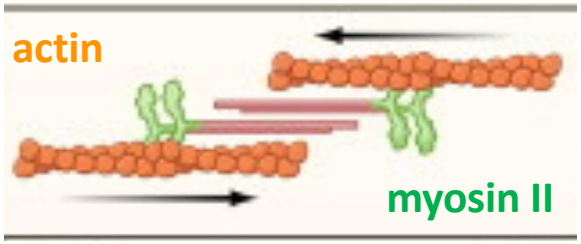


Motor for cargo transport



ATP hydrolysis

Motor for contraction



Contractile actomyosin in stress fibers, rings (cytokinesis), sarcomeres (muscle)

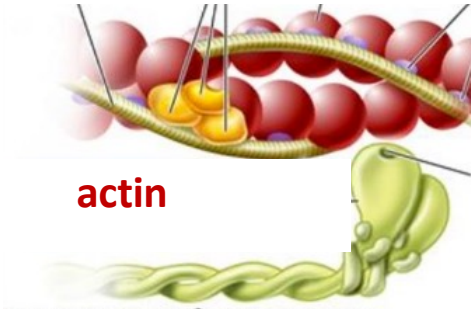
Figure 16-2, 41, 19-10, 55 Molecular Biology of the Cell 6th
Fig 3-28 Goodman's Medical Cell Biology 4th 2021
Wühr et al., Cell, 2019; https://www.solidbio.com/

Actin microfilament networks / functions

Muscle contraction

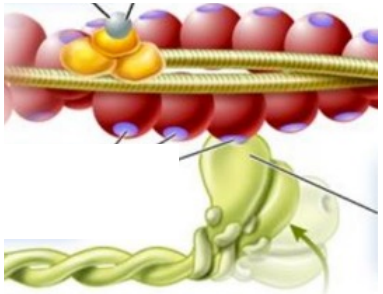
Low calcium : relaxed

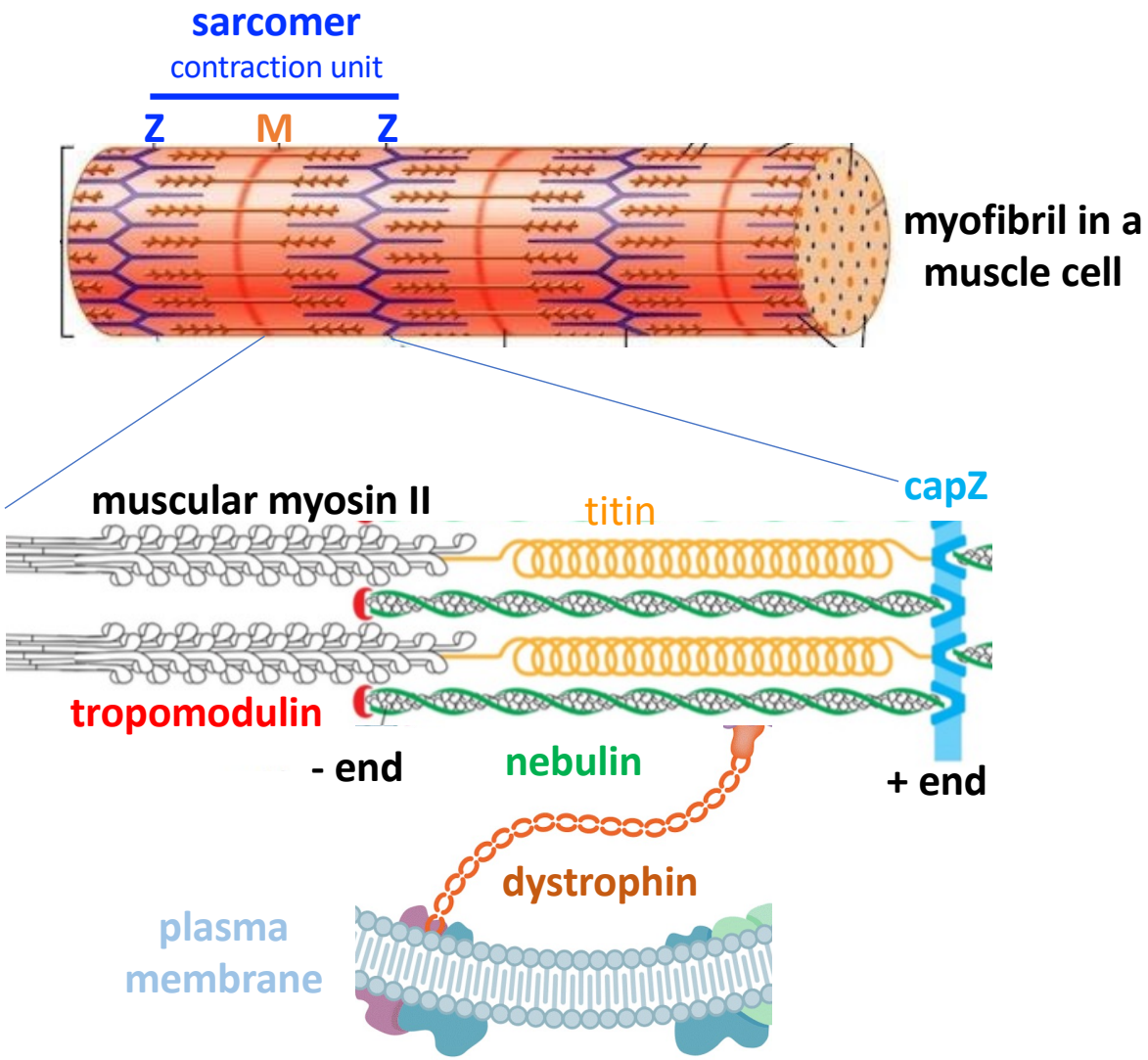
tropomyosin troponin



High calcium : contraction

Ca2+ myosin-binding sites





https://www.orthobullets.com/basic-science/9021/muscle-biology-and-physiology

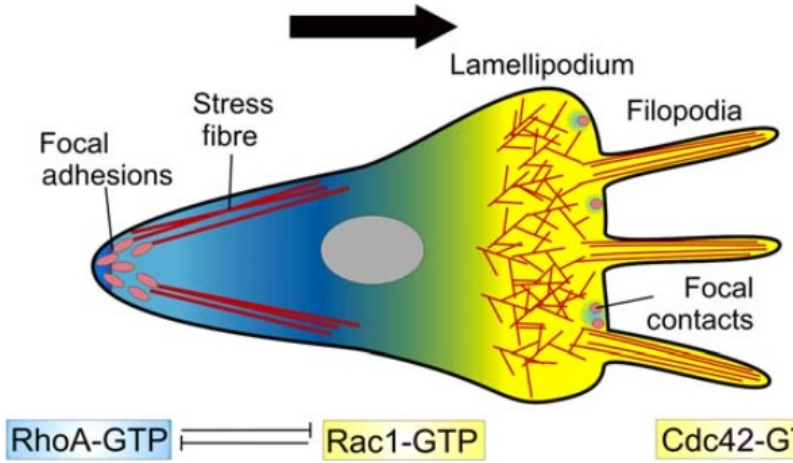
https://www.pinterest.fr/pin/627055948095820460/

Figure 16-34 Molecular Biology of the Cell 6th

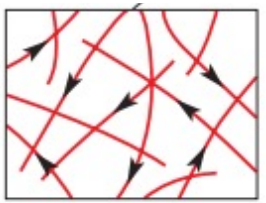
Actin microfilament networks / functions

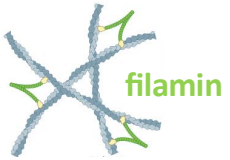
Mesenchymal migration

RhoA, Rac1, Cdc42 = small GTPases, G proteins
Active if GTP linked
= Regulators of actin



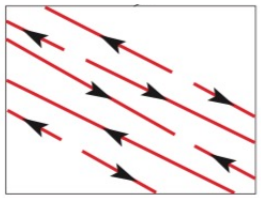
cell cortex



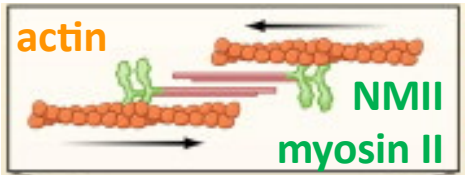


meshwork : gel-like

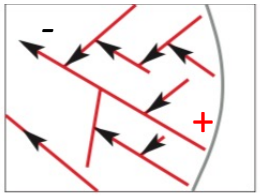
stress fiber (+ FA)



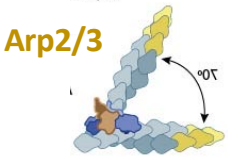
contractile bundle



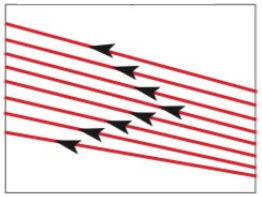
lamellipodium



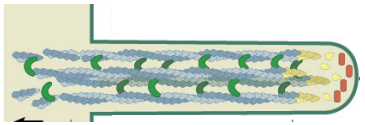
dendritic network



filopodia



tight parallel



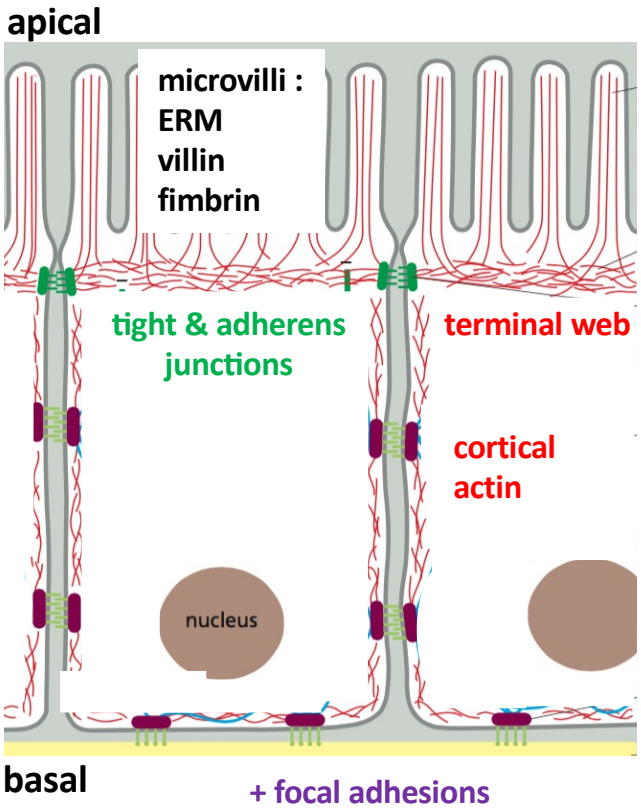
fascin

actin microfilaments

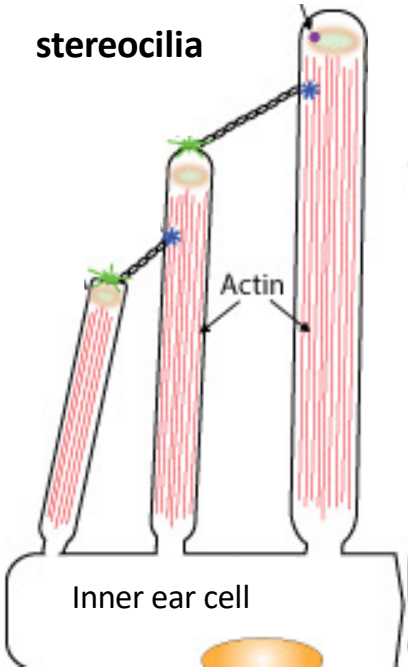
Mayor & Carmona-Fontaine, Trends Cell Biol , 2010
Adapted from figure 16-21, Molecular Biology of the Cell 6th
Wühr et al., Cell, 2019, www.mechanobio.info/cytoskeleton-dynamics/

Actin microfilament networks / functions

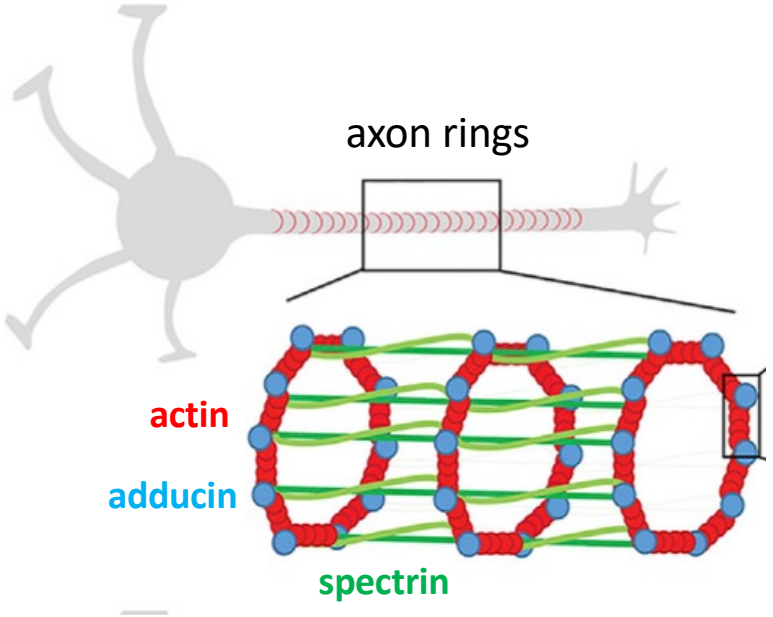
Epithelial cells



Axon in neurons



Also in brain cells



Mitosis : cytokinesis

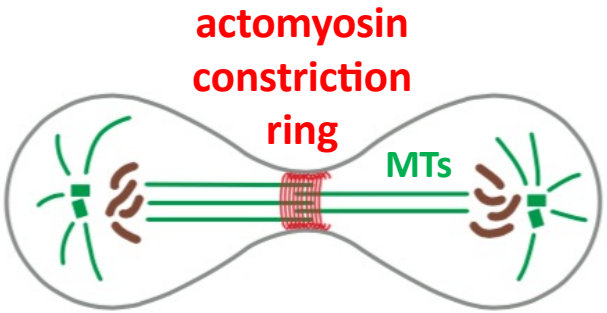
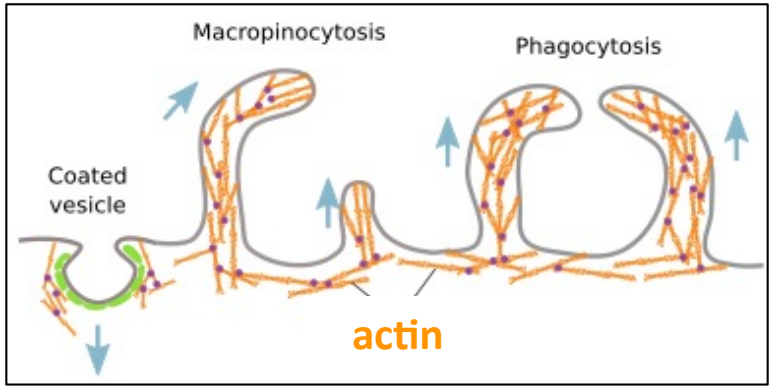
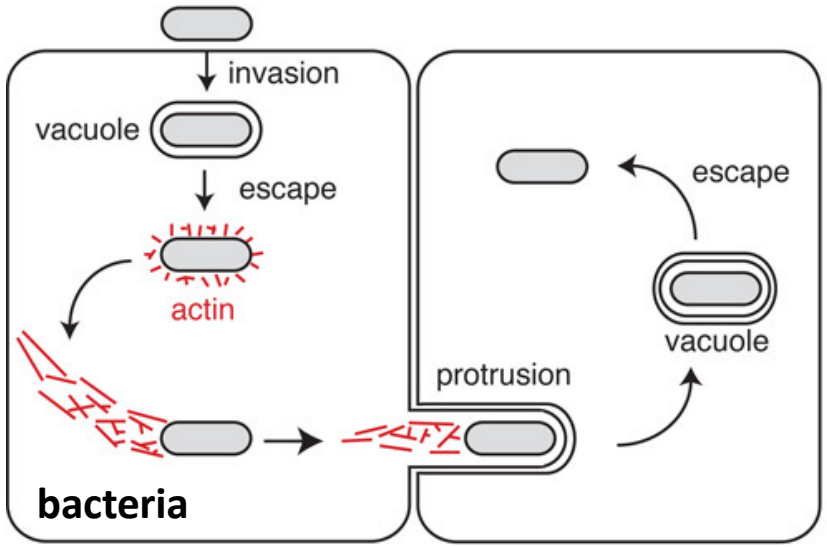


Figure 16-4 Molecular Cell Biology 8th 2016
Cytoskeleton Inc, Leite et al., Cell Reports, 2016

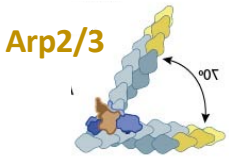
Actin microfilament networks / functions

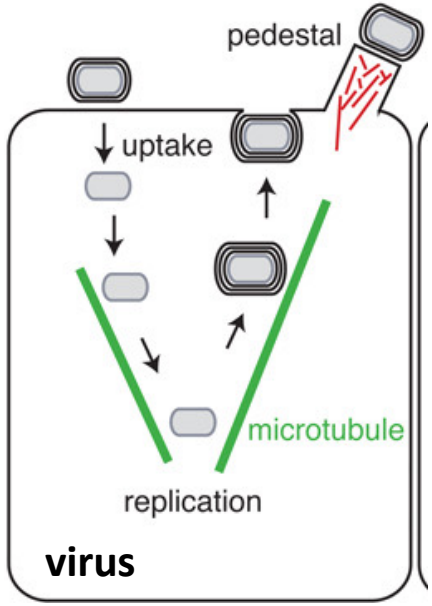
Endocytosis / engulfment





Actin comet tail induced by bacteria / virus infection



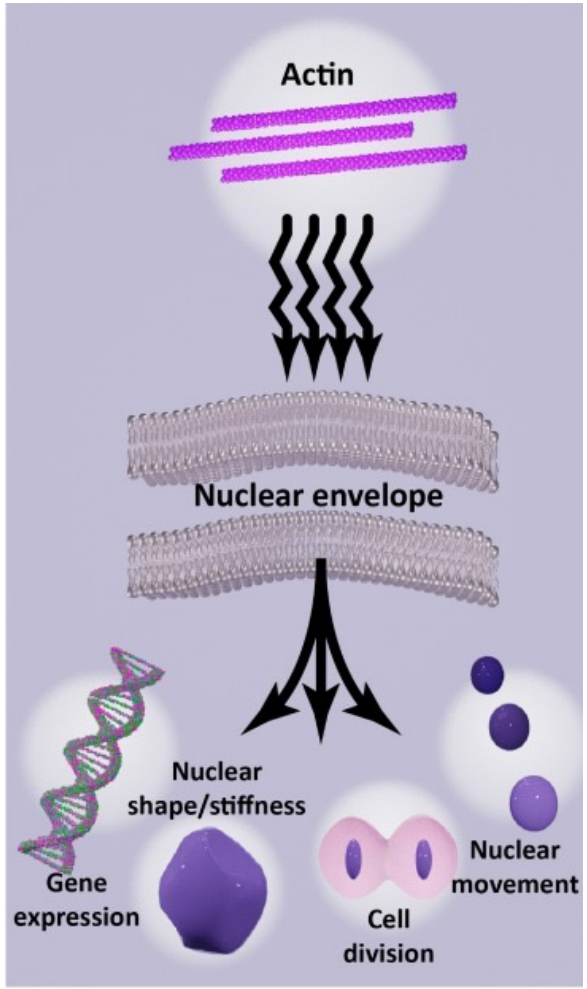


https://mmegias.webs.uvigo.es/02-english/5-celulas/7-actina.php

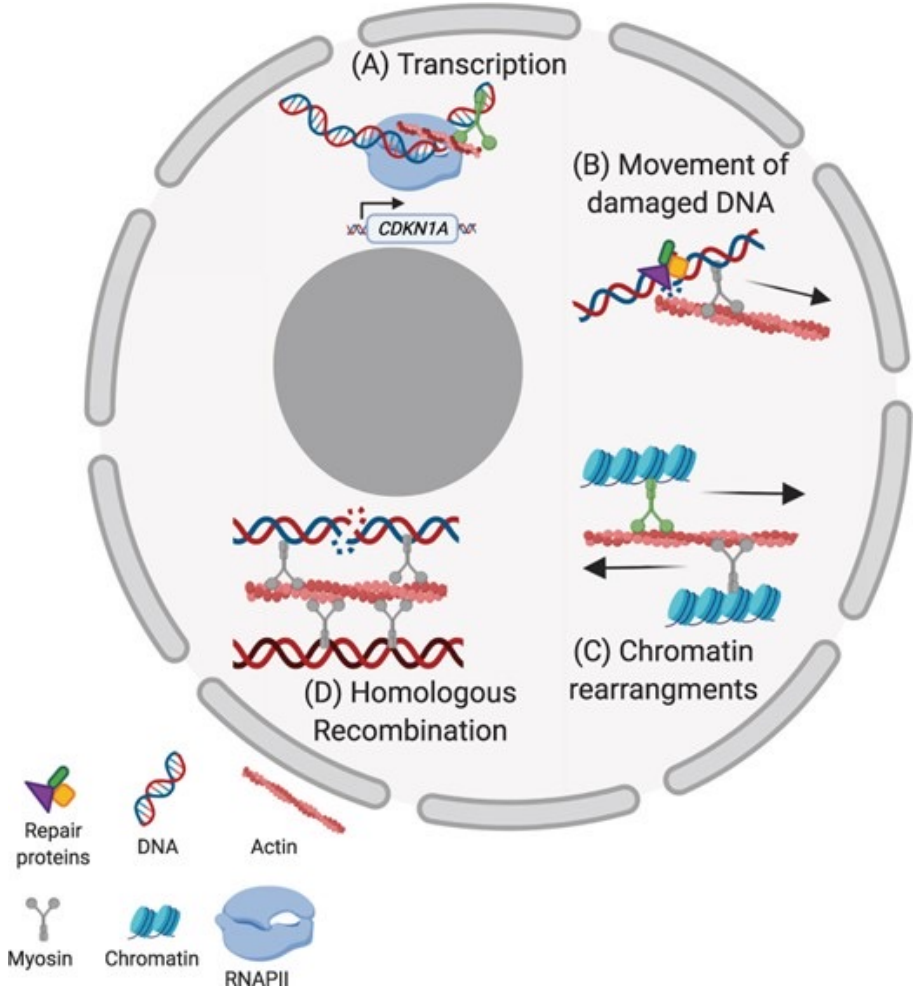
Welch & Way, Cell Host Mirobe, 2014

Actin microfilaments and nucleus

Perinuclear actin



Nuclear actin



Davidson & Cadot, Trends Cell Biol., 2021
Cook & Toseland, J. Biochem. 2021

Actin microfilaments and diseases

Actin mutations

- Nemaline myopathy (respiratory muscle weakness)
- Cardiomyopathy (heart muscle)
- Megacystis microcolon-Intestinal hypoperistalsis syndrome (enteric muscle)
- Baraitser-Winter syndrome (brain, heart, kidney)
- Familial thoracic aortic aneurysms (vascular smooth muscle cells)
- Bleeding disorders
- Deafness (inner ear, stereocilia)

Abnormal ABPs

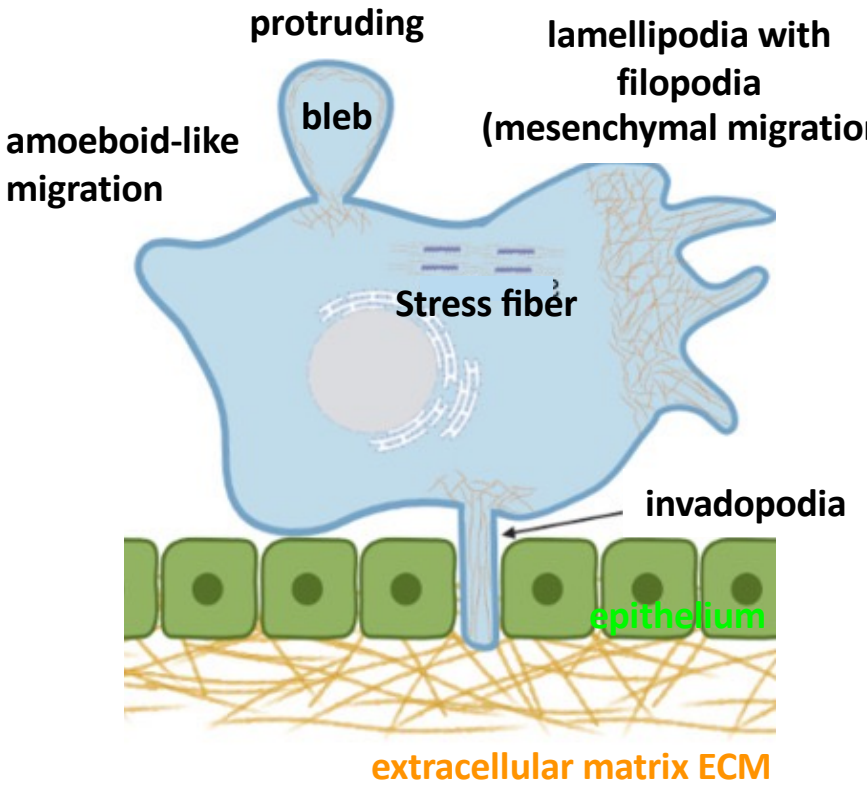
- Myopathies
- Neurodegeneration
- Wiskott–Aldrich syndrome (immune dysregulation)

…

Sprenkeler et al., J. Innate Immun., 2021

Actin microfilaments and cancer

Metastasis facilitation (migration, invasion)



Angiogenesis

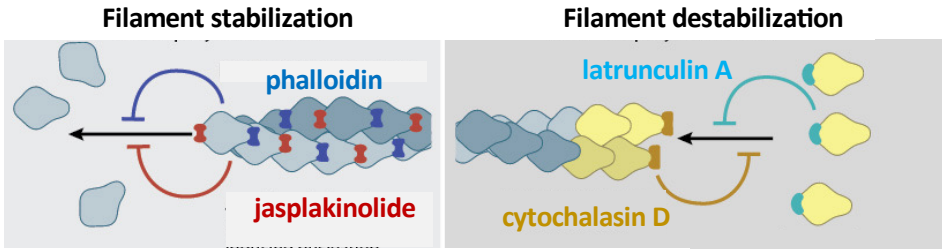
Mitosis defects

Actin / ABPs misregulation

- Actin isoforms (often up)
- Formin (up)
- N-WASp = Arp2/3 activator (up or down)
- Tropomyosin isoforms

Targeting actin in cancer therapy ?

Too severe off-target effects (toxicity)



Targeting actomyosin in cancer therapy ?

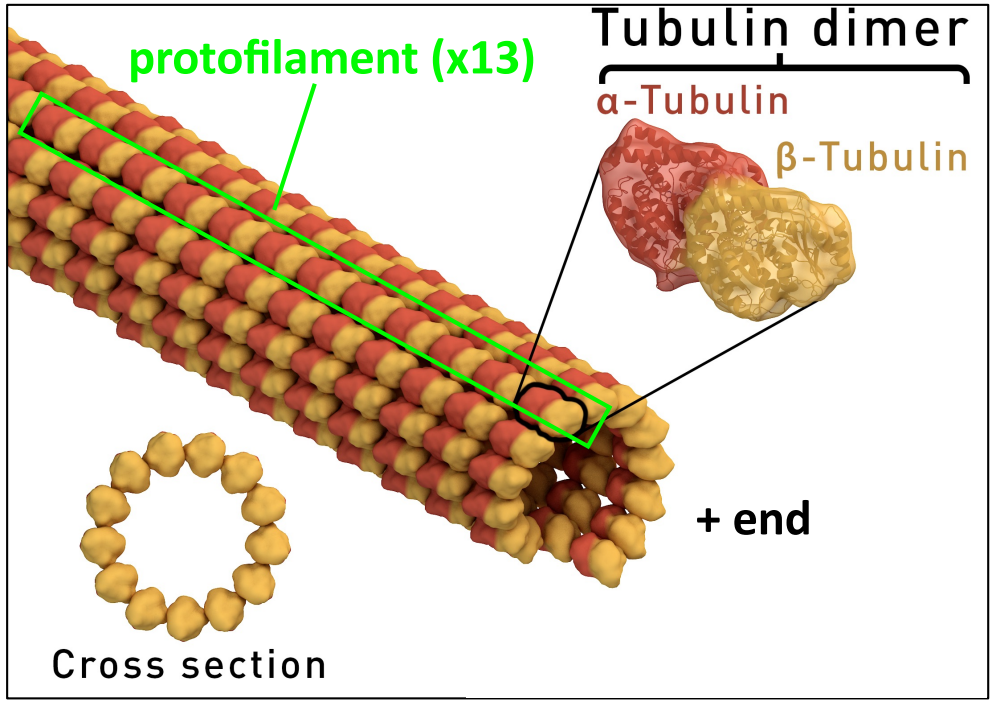
Tropomyosin (TR100, ATM3507) ?

Biber et al., Int. Rev. Cell Mol. Biol., 2020
https://www.mechanobio.info/cytoskeleton-dynamics/

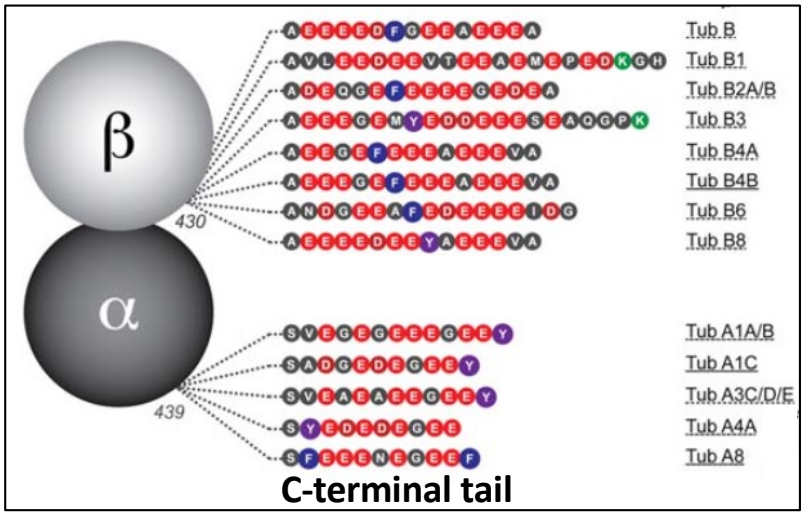
Microtubules

Microtubule structure : tubulin

Hollow polarized tube with a diameter of 25 nm



Tubulin isotypes in humans

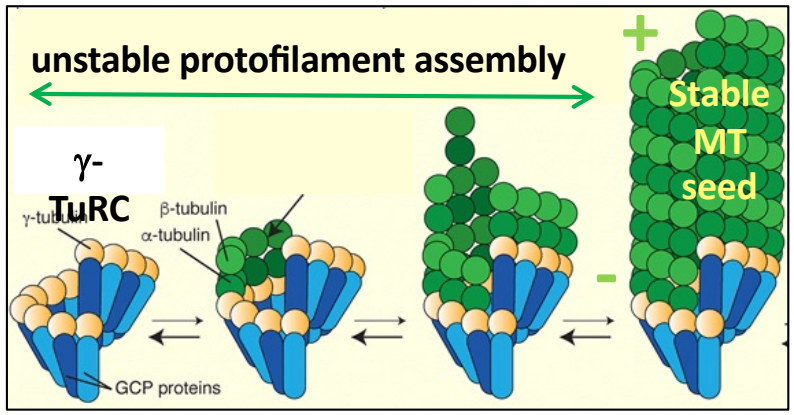


Part of the tubulin code (+ PTMs)

Adapted from Thomas Splettstoesser (www.scistyle.com)
https://commons.wikimedia.org/w/index.php?curid=41014850
Chakraborti, cytoskeleton, 2016

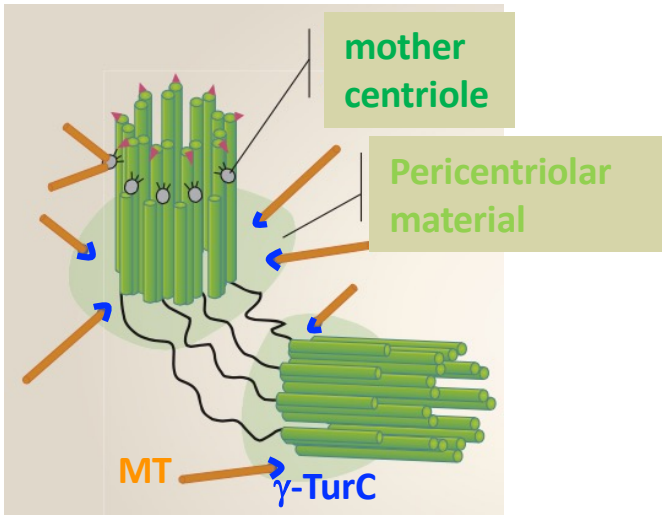
Microtubule dynamics

Nucleation : γ-tubulin ring complex



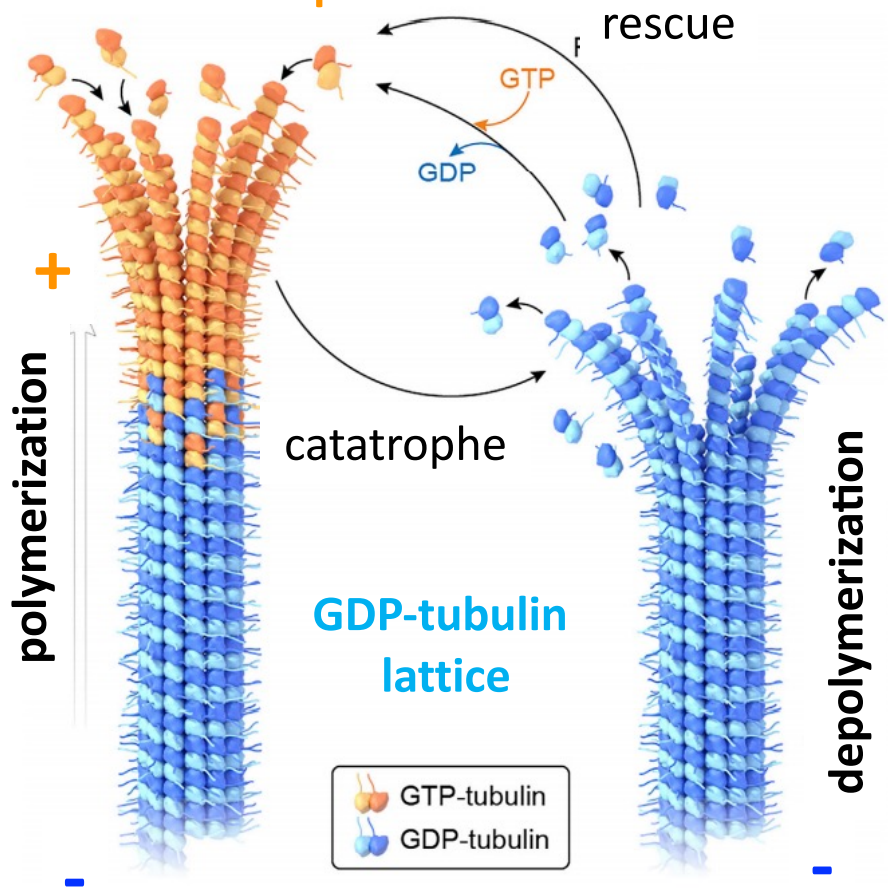
Centrosome : MTOC

Microtubule organizing center



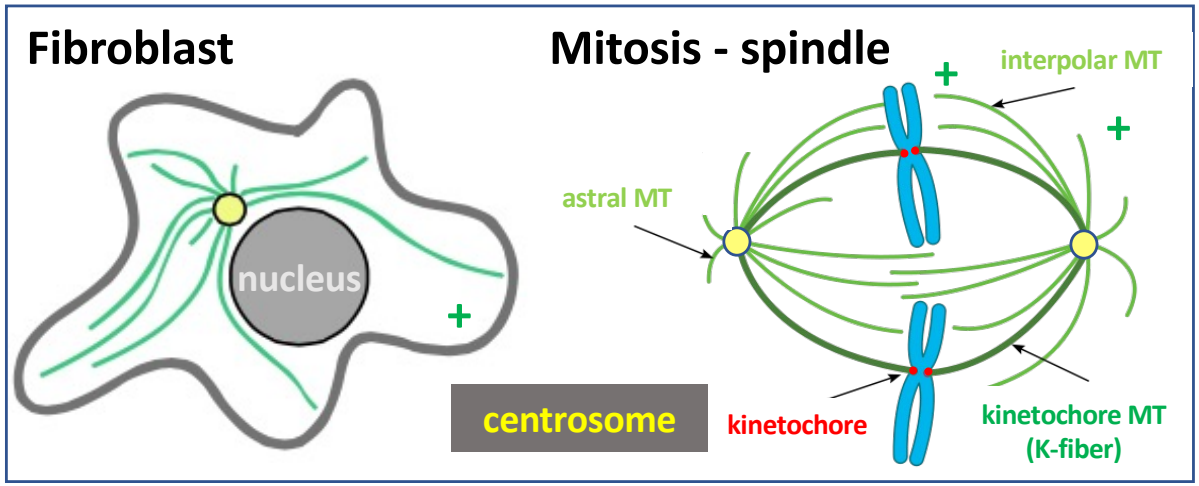
Dynamic instability

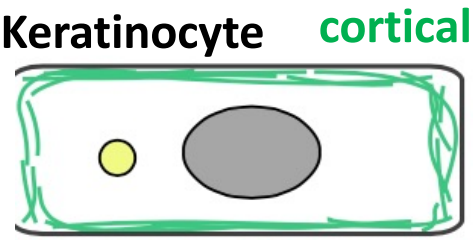
β-tubulin is a GTPase

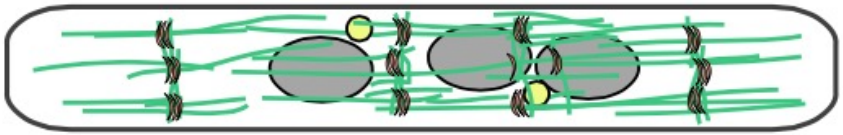


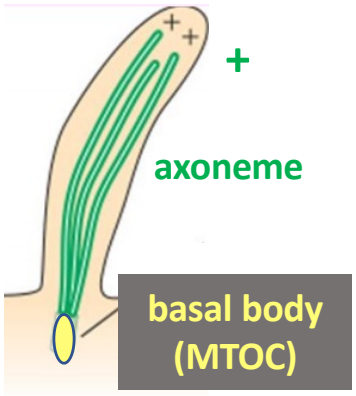
Tovey & Conduit, Essays in Biochem., 2018
Paz & Lüders, Trends Cell Biol 2018
Roll-Mecak, Dev. Cell, 2020

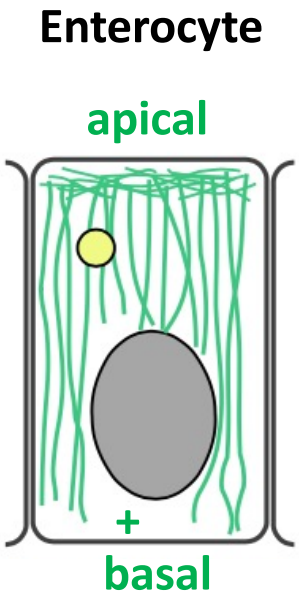
Microtubule networks

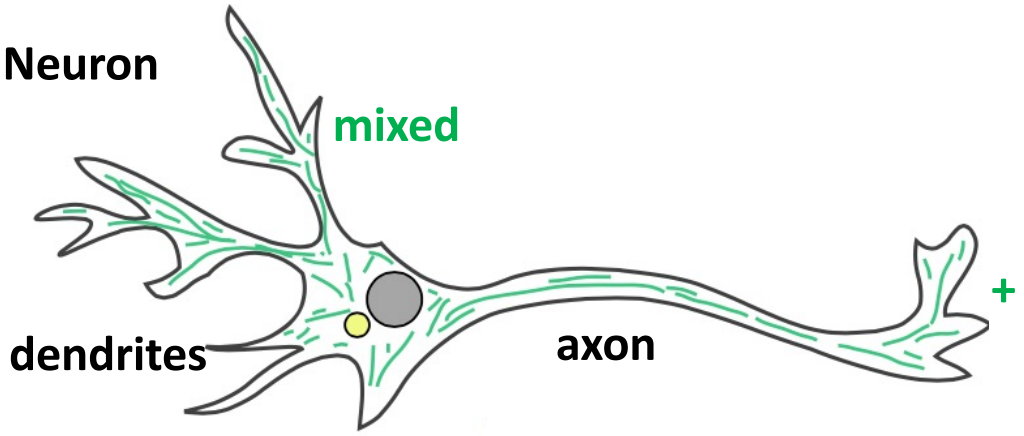






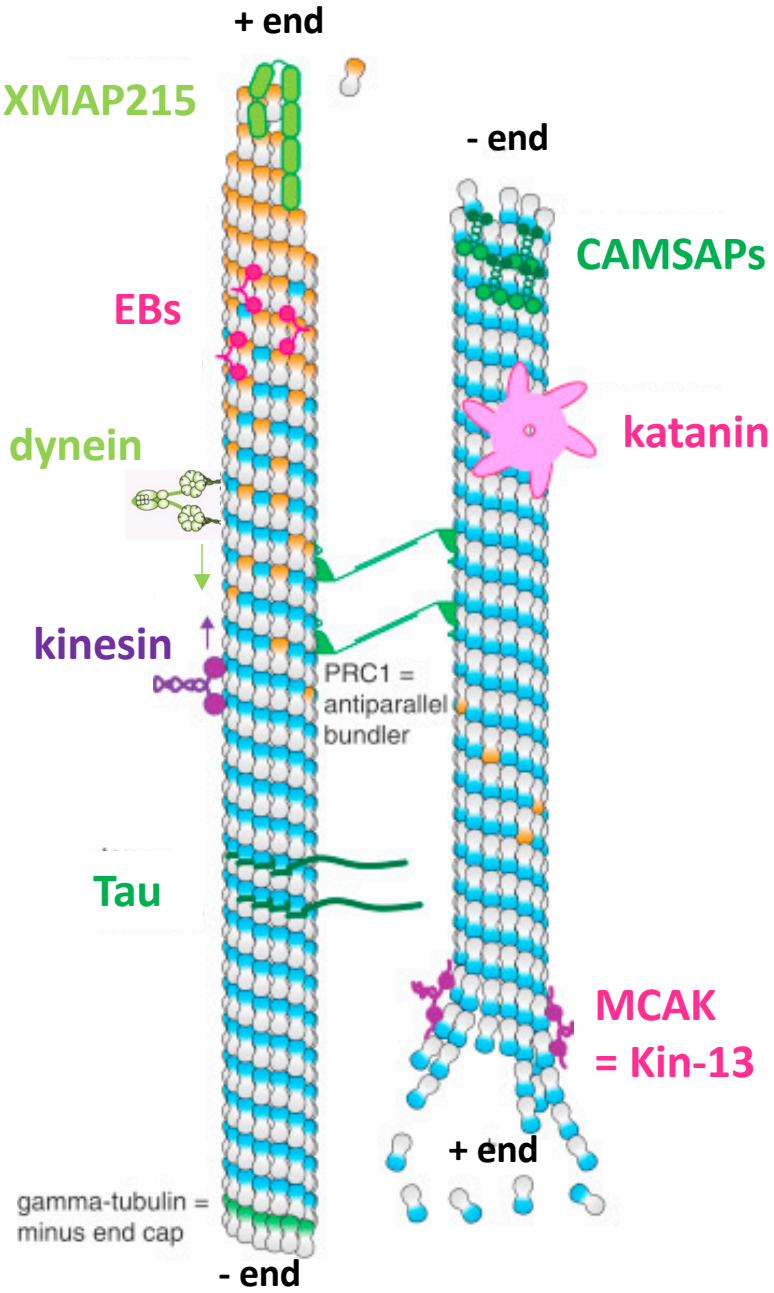






Adapted from Muroyama & Lechler, Dvot, 2017
https://en.wikipedia.org/wiki/File:Spindle_apparatus.svg
Figure 18-5 Molecular Biology of the Cell 6th

Microtubule-associated proteins (MAPs)



Structural MAPs
Tau, MAPs (bundling, crosslinking, stabilizing)

Severing MAPs
Katanin (cutting)

+TIPs
EBs, XMAP215 (assembly)
MCAK (disassembly), CLIP-170 (linker)

-TIPs
CAMSAPs

Molecular motors
Kinesin (+ end directed)
Cytoplasmic dynein (- end directed)

Tubulin dimer sequester
Stathmin

Straube, Encyclopedia Cell Biol., 2016

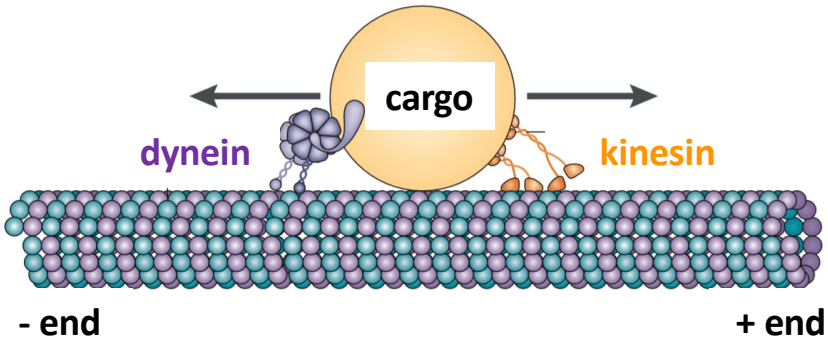
Microtubule functions

Interphase

Organelle positioning, membrane traffic, molecular transport : cell polarity, migration

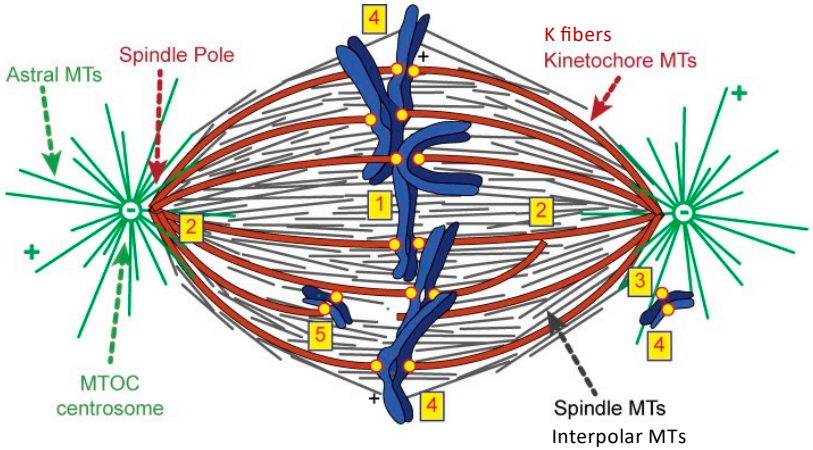
Ciliary / flagella motility : respiratory track / brain / sperm

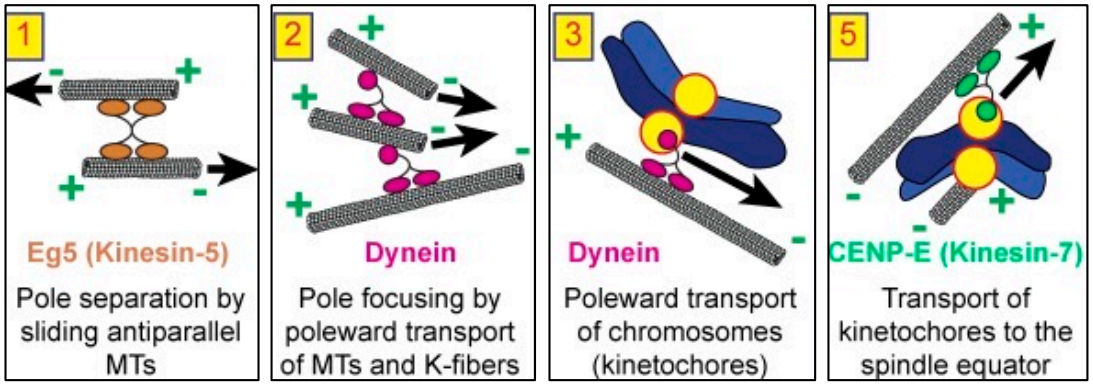
Primary cilia signaling



Mitosis

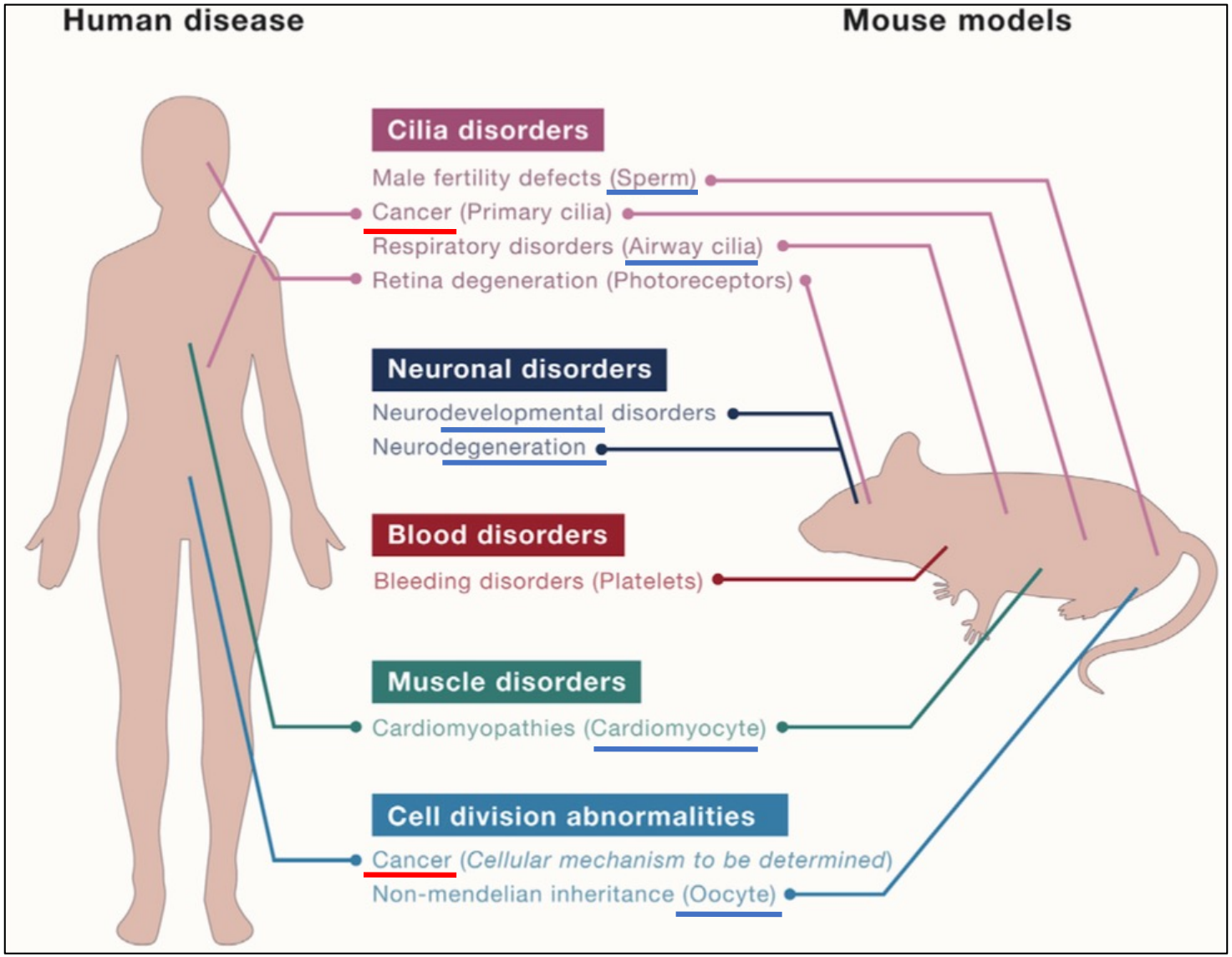
Chromosome alignment and separation





Heald & Khodjakov, J. Cell Biol. 2015
Hancock, Nat. Rev. Mol. Cell. Biol, 2014

Microtubules and diseases

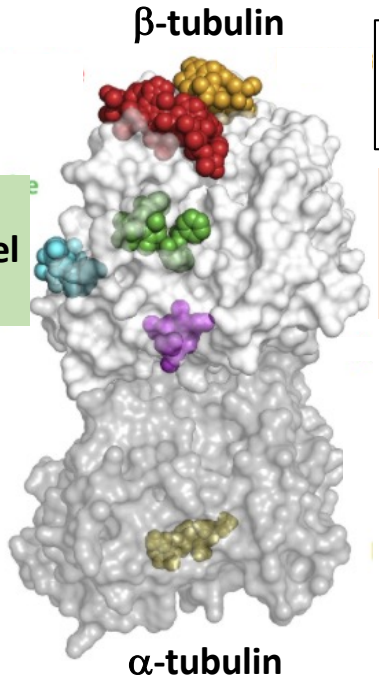


Magiera et al., Cell, 2018

Microtubules and cancer

MT-stabilizing agents (MSA)

Taxane site
paclitaxel, docetaxel
epothilone
Laulimalide/
Peloruside site



MT-destabilizing agents (MDA)

Vinca-alkaloid site
vinblastine, vincristine
eribulin
Maytansine site
Colchicine site
colchicine
nocodazole
Pironetin site

Tubulin / MAPs misregulation

- **Tubulin isoforms (β3 up)**
- **Tau, MAP2, MAP4 (up)**
- **Mitotic kinesins (up)**
  Eg5/kif11, CENP-E, KIF4A
- **Stathmin (up)**

2nd generation antimitotics

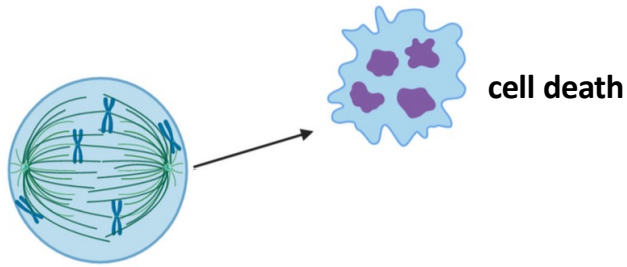
mitotic kinesins : Eg5/kif11, CENP-E
Limited efficacy in monotherapy

Taxanes "spindle poisons"

- **proliferation rate paradox** 1% of the cancer cells in mitosis
- **pro-apoptotic, anti-angiogenic, anticancer immunogenic ?**

Efficiently used in therapy, but :

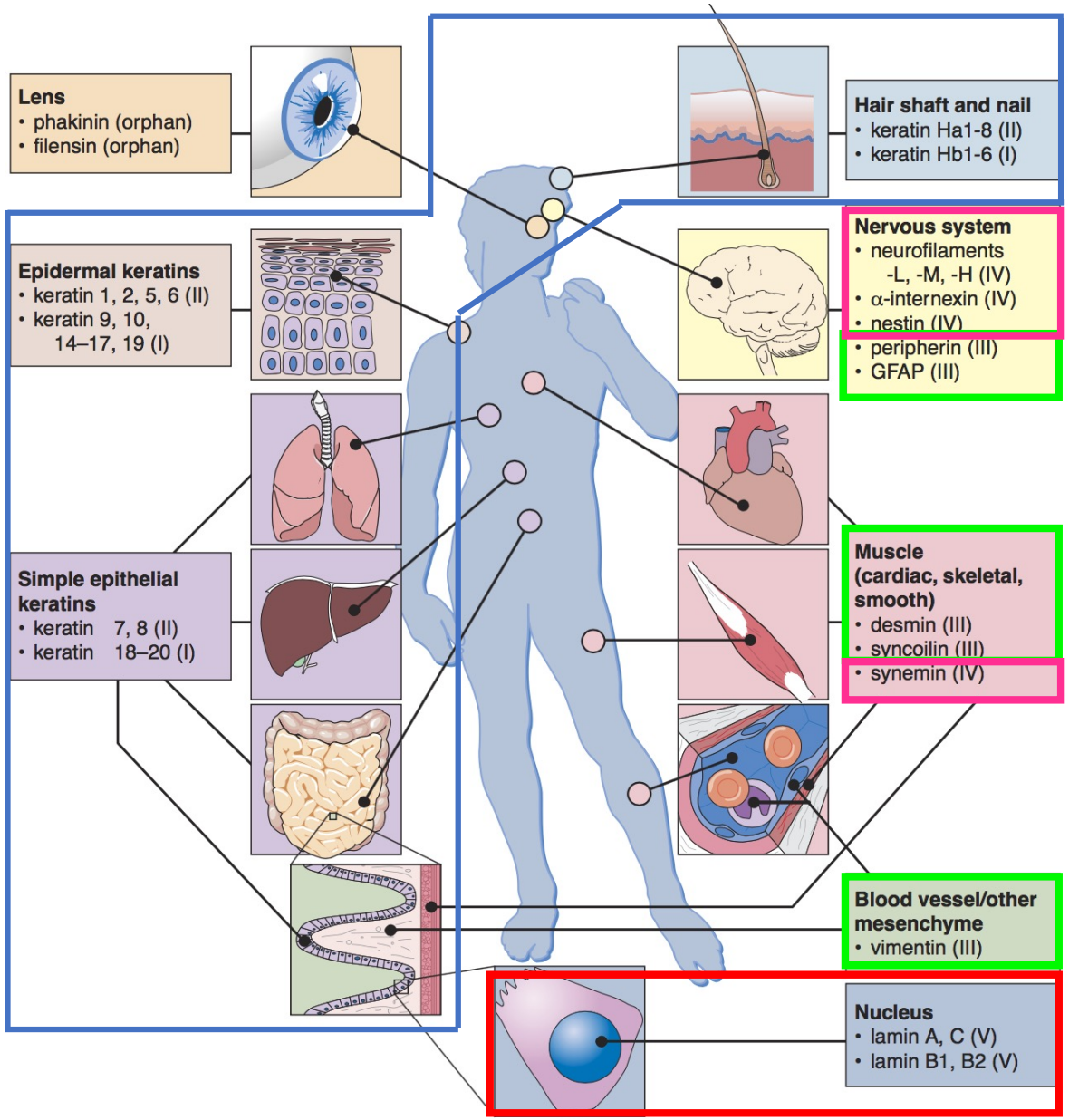
- **side effects (neuropathy, myelosuppression)**
- **resistance (intrinsic or acquired)**



Adapted from Steinmetz & Prota, Trends Cell Biol., 2018

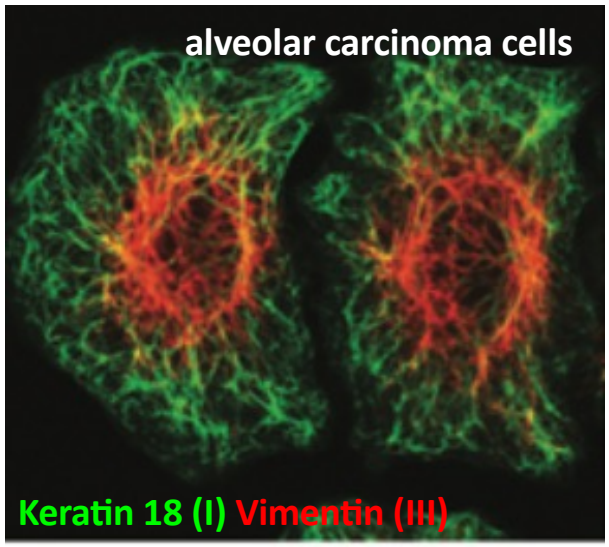
Intermediate filaments

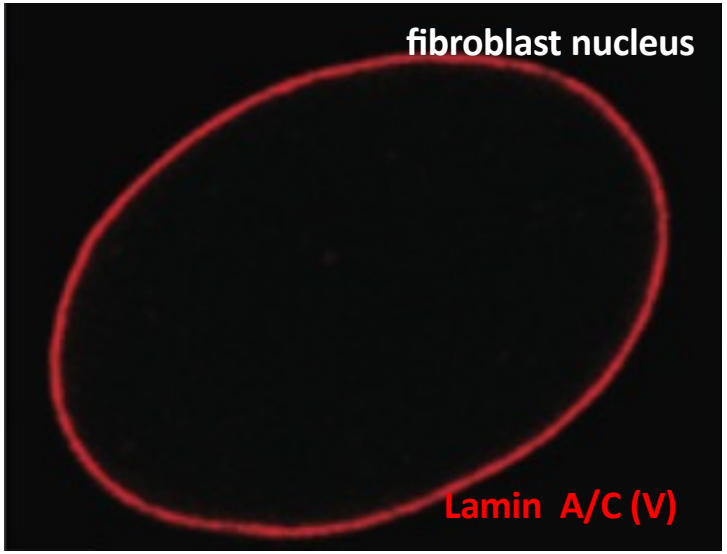
Diversity of IFs (70 genes) : cell specificity



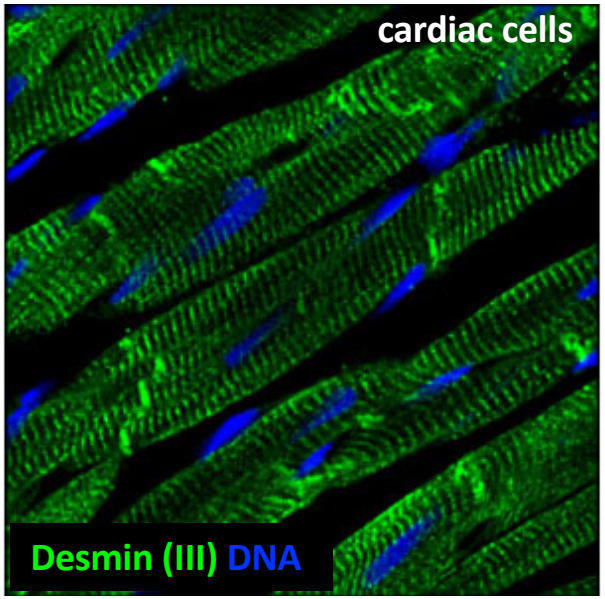
Toivoila et al., Trends Cell Biol, 2005

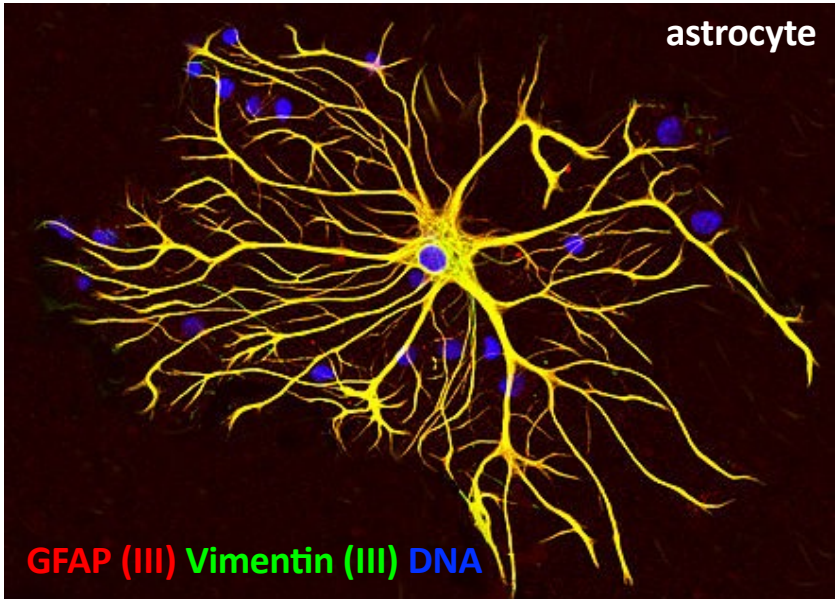
Intermediate filament networks





immunufluorescence





Cell Signaling Technology ; EnCor Biotec Inc
Eriksson et al., J. Clin. Inv., 2009; Wöll et al., Eur J Cell Biol, 2005

Intermediate filament protein subdomains

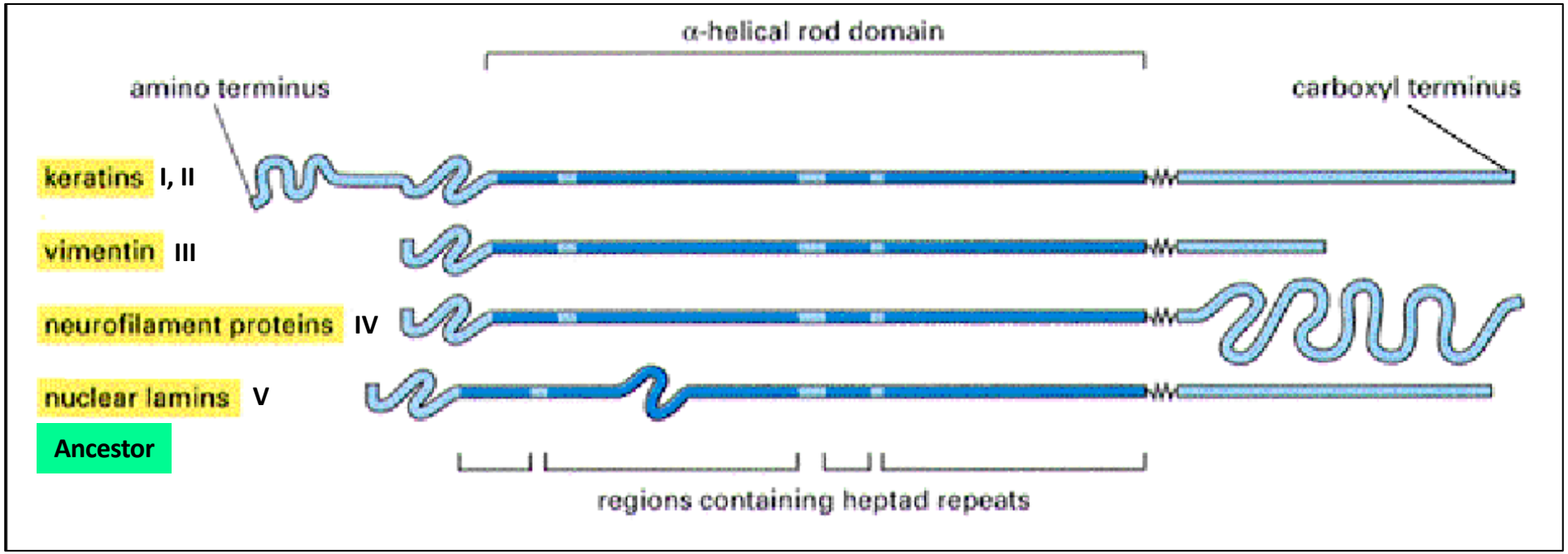
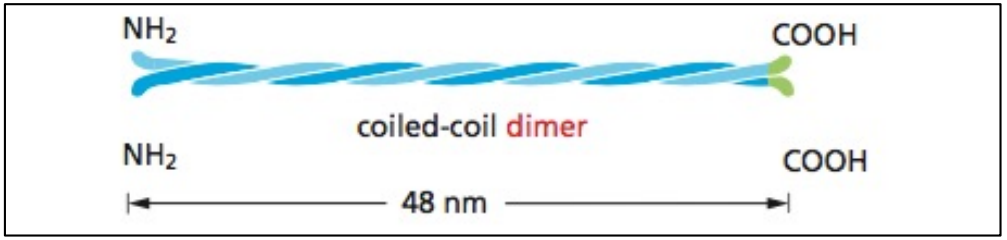
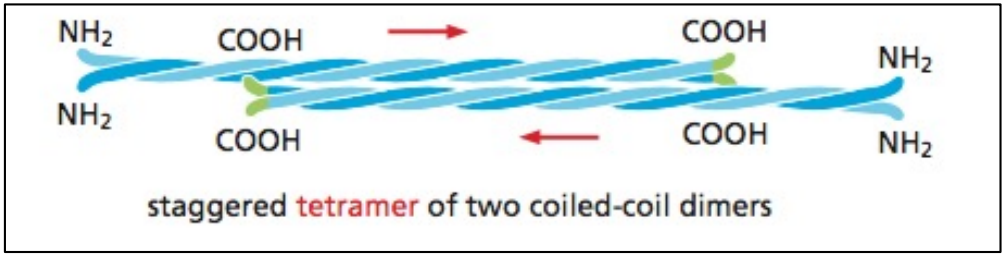
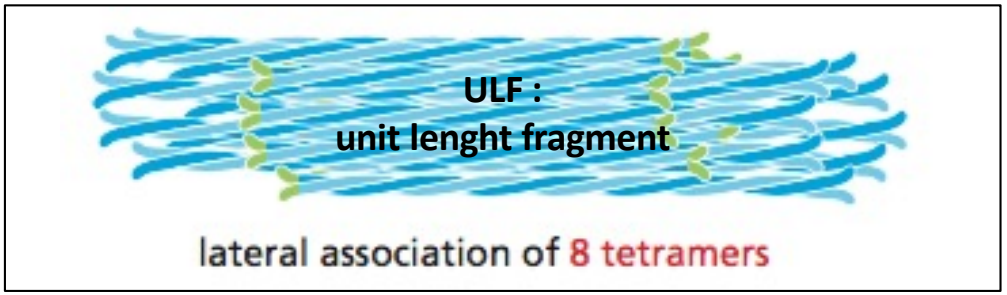


Figure 16-12 Biology of the Cell, 1996

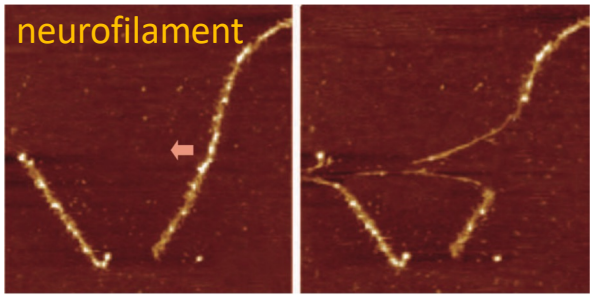
Intermediate filament structure



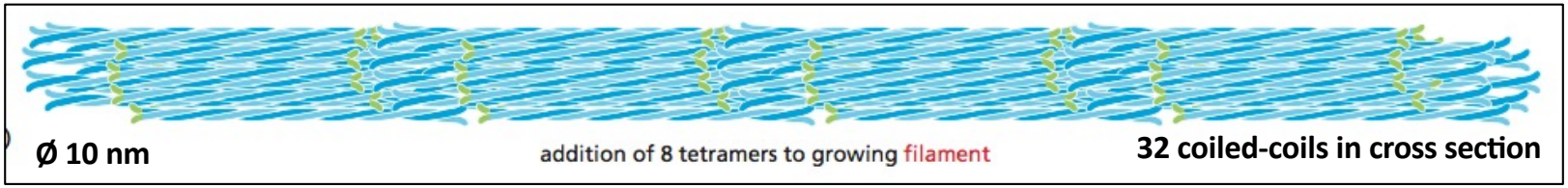




- **no polarity (no motor)**
- **no need of ATP, GTP**
- **low solubility (salt, detergent)**
- **elastic structure difficult to break**
- **binding partners not well known yet**

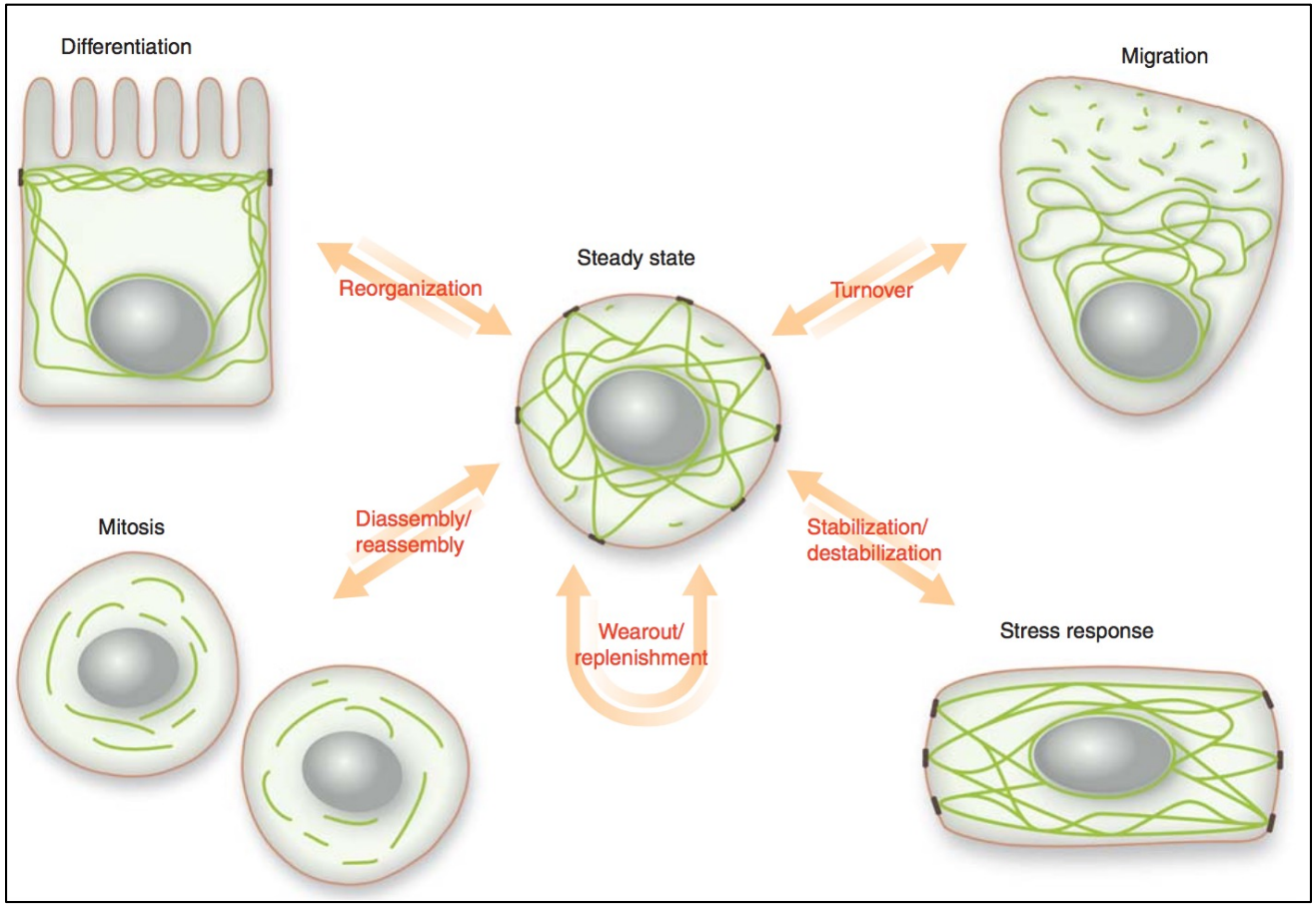


atomic force microscope



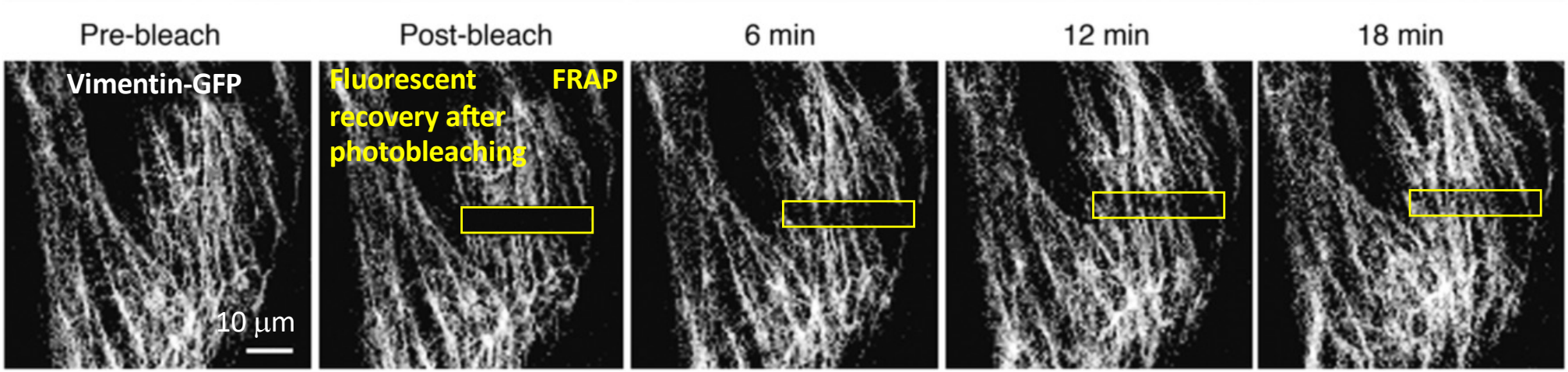
Figures 16-6 & 67 , Molecular Biology of the Cell 6th

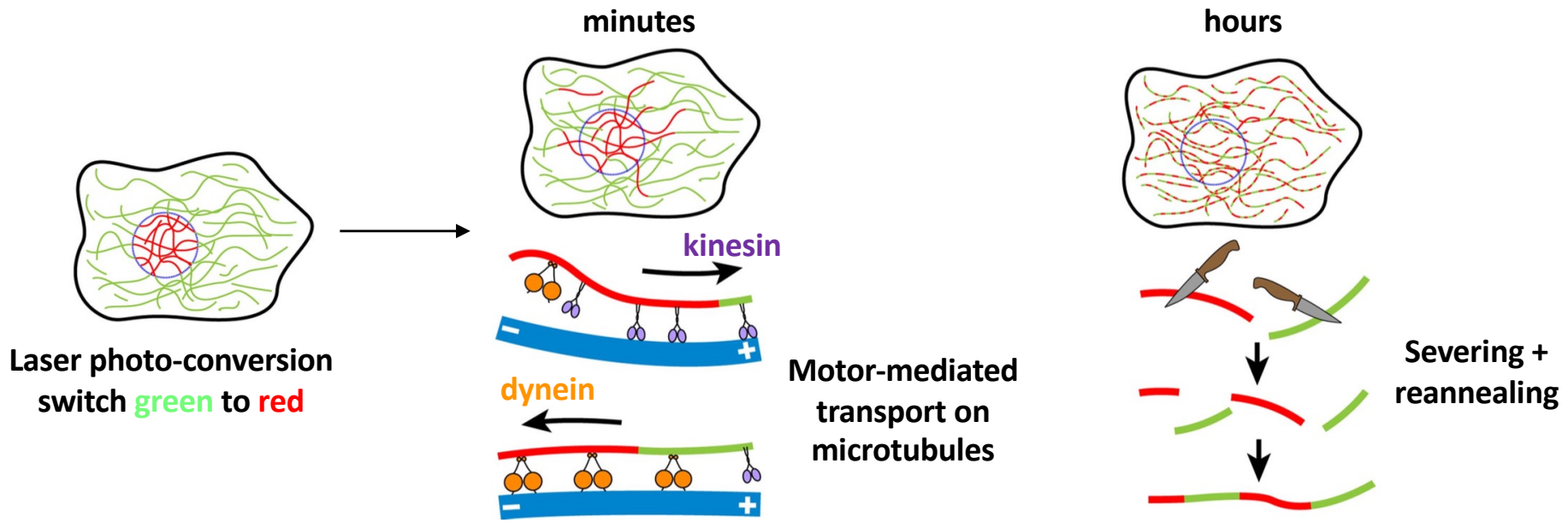
Intermediate filament dynamic restructuration



Leube et al., CSH perspectives Biol, 2020

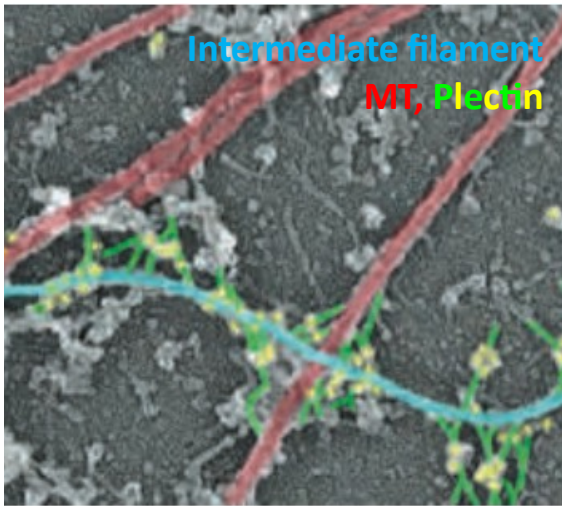
Intermediate filament dynamics



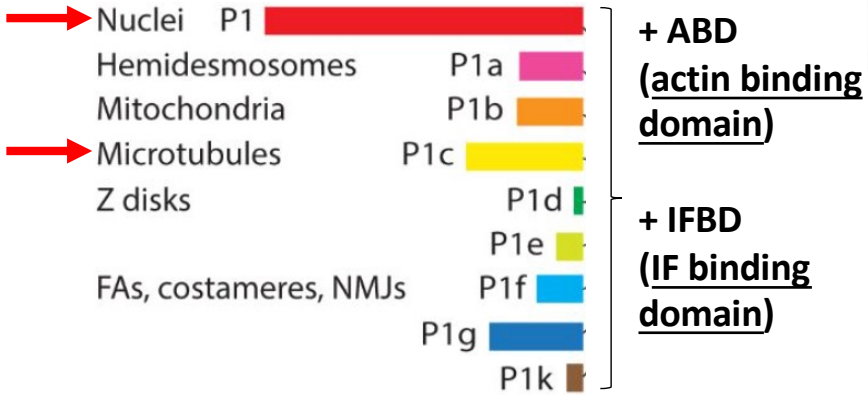


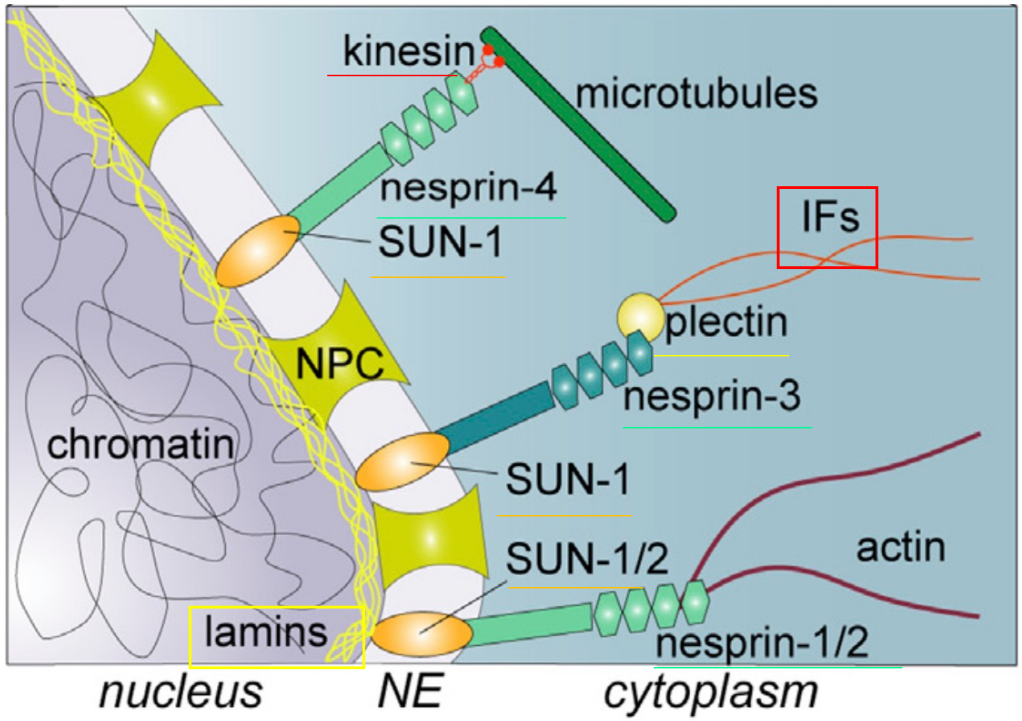
Eriksson et al., J. Clin. Inv., 2009; Hookway et al. MBoC, 2015

Intermediate filament-associated proteins (IFAPs) linkers : plectin, SUNs, nesprin



Plectin variable N-term domain



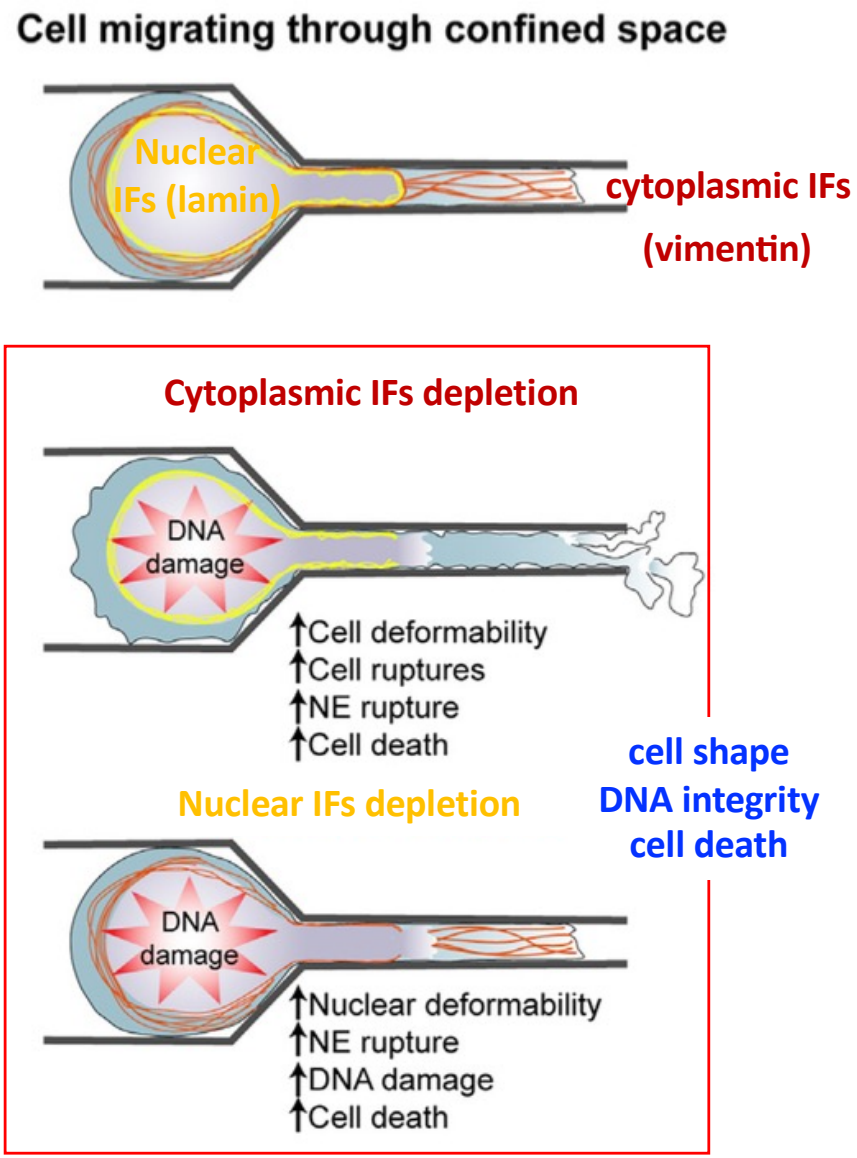


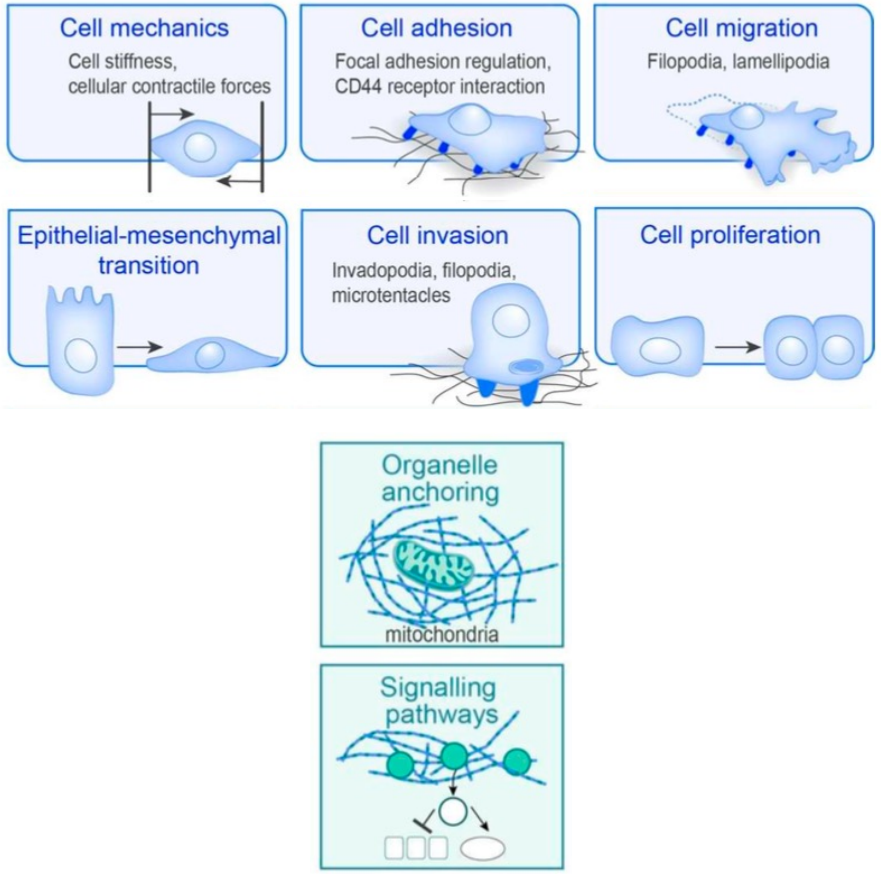
Nuclear envelope

Roles in cell adhesion, migration, division mechanotransduction

Svitkina et al., JCB, 1996, Wiche, Cells, 2021
Piccus & Brayson, Biol. Letters, 2020

Intermediate filament cellular functions

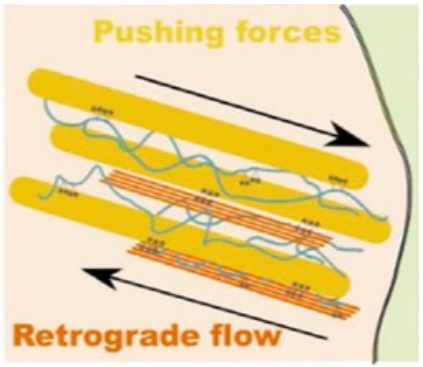


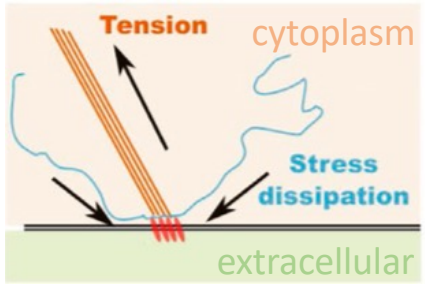


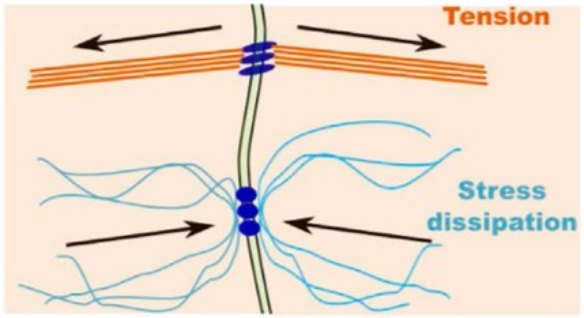
Infante & Etienne-Manneville, Front. Cell Dev. Biol., 2022
Danielsson et al. Cells, 2018

Intermediate filaments in mechanic-stress response

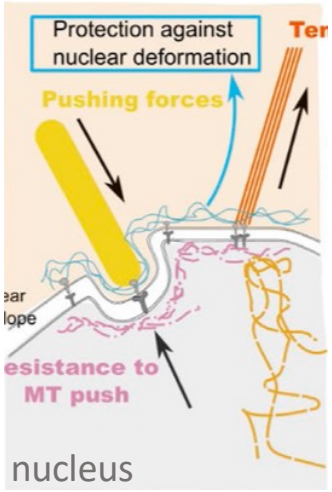
Plasma membrane

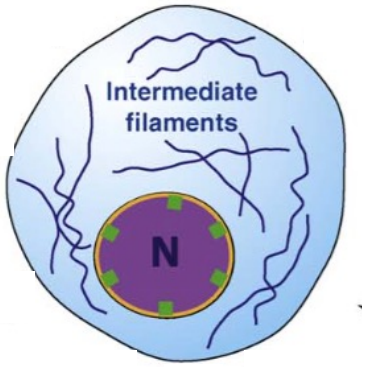






Nuclear membrane



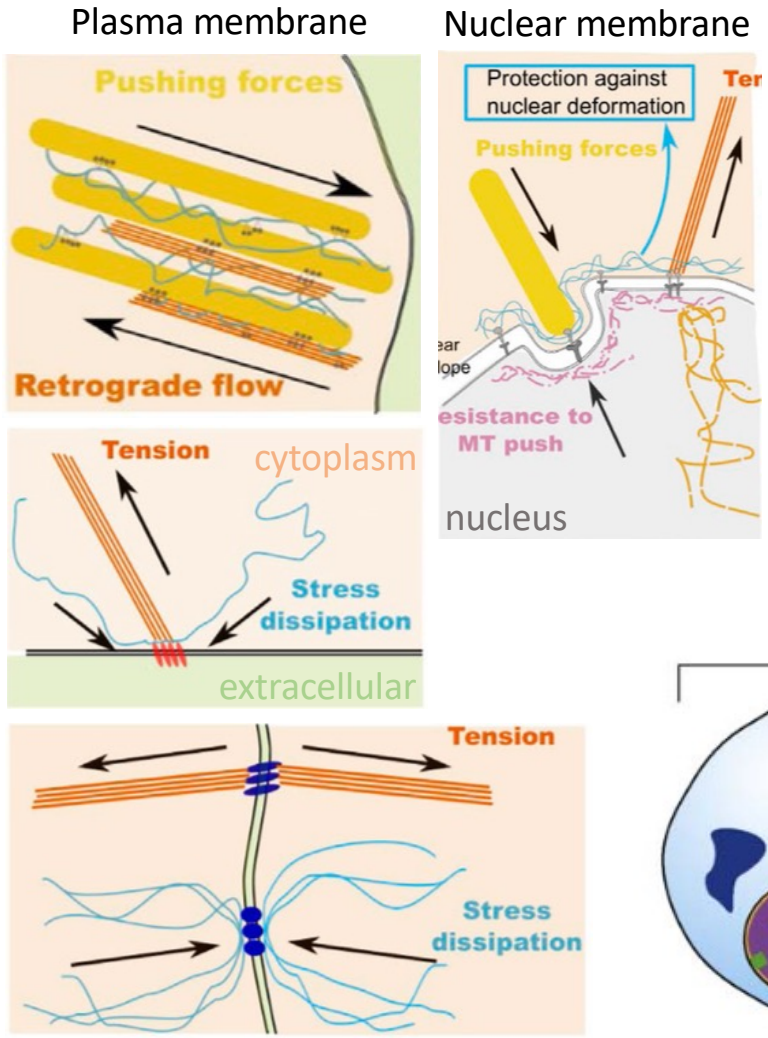


Stress
Mechanic (tension, shear, compression)

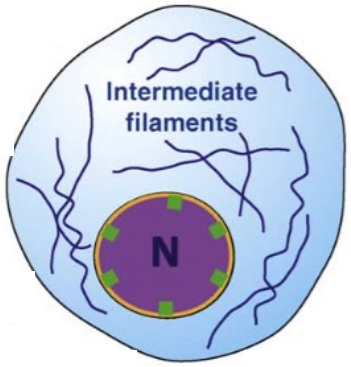
Intermediate filament (cytoplasm)
Intermediate filament (nucleus)
Actin
Microtubule

Ndiaye et al., Front. Cell Dev. Biol., 2022
Adapted from Toivoila et al., Trends Cell Biol, 2010

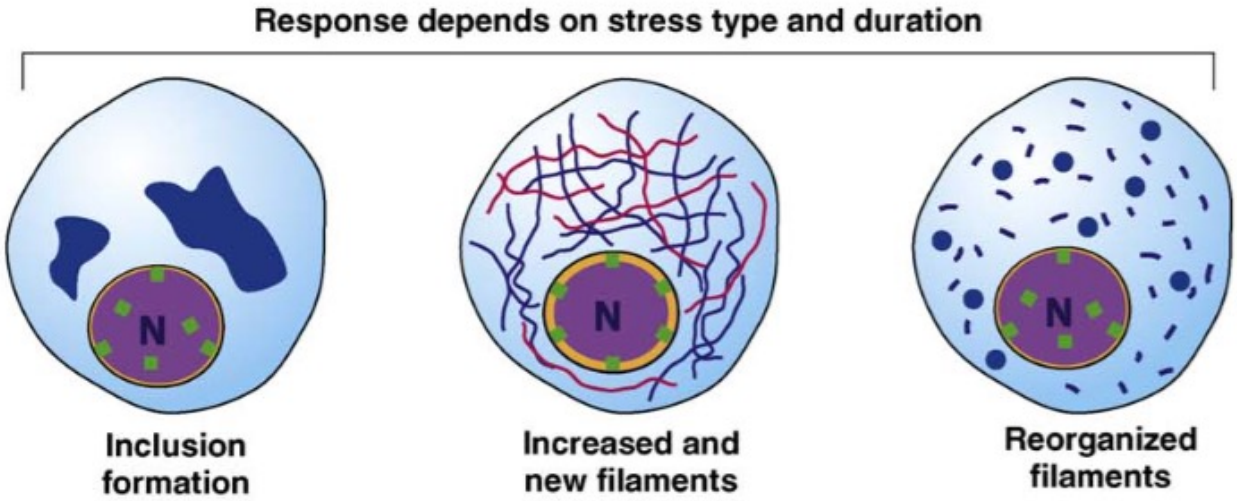
Intermediate filaments in cell-stress response



Intermediate filament (cytoplasm)
Intermediate filament (nucleus)
Actin
Microtubule



Stress
Mechanic (tension, shear, compression)
Wound closure
Hypoxia
Osmotic, oxydative
Protein misfolding
Toxin, radiation
DNA damage
Pathogens (virus, bacteria)
Heat



Ndiaye et al., Front. Cell Dev. Biol., 2022
Adapted from Toivoila et al., Trends Cell Biol, 2010

Intermediate filaments and diseases

Keratins I, II : skin diseases (epidermolysis bullosa simplex EBS), predisposition for liver diseases (steatosis), cancer markers

Vimentin-like III : myopathies, Alexander disease, cataract, metastasis

Neurofilaments IV : neuropsychiatric diseases (Charcot-Marie-Tooth, Alzheimer, amyotrophic lateral sclerosis, Parkinson)

Lamins V : laminopathies (progeria / precocious aging)

Orphan VI : cataract

Ashwagandha *Withania somnifera*

wikipedia

withaferin A :
Disrupts Vimentin, Keratin, NF networks
Grin et al., PLOS ONE, 2012
Phase I trial osteosarcoma
Pires et al., J. A. Int. Med, 2020

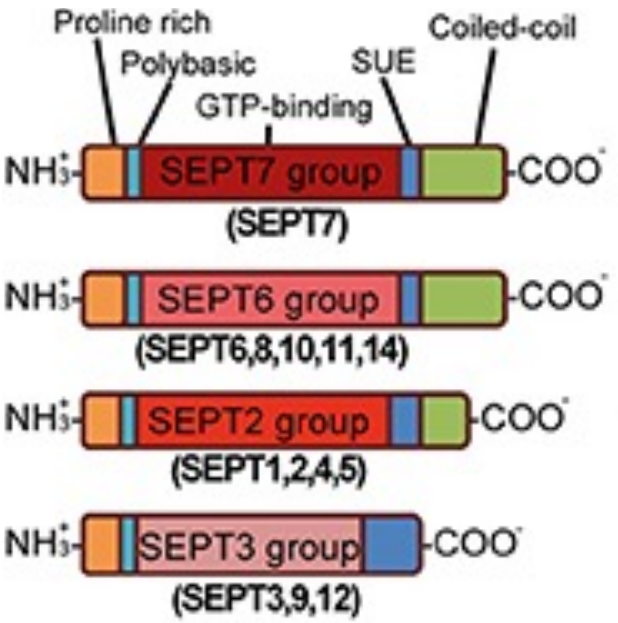
statin (simvastatin) :
anti-cholesterol
Disrupts Vimentin network
Trogden et al., Faseb J, 2018
Phase II trial (combination therapy in cancer) in progress

Adapted from Eriksson et al., J. Clin. Inv., 2009

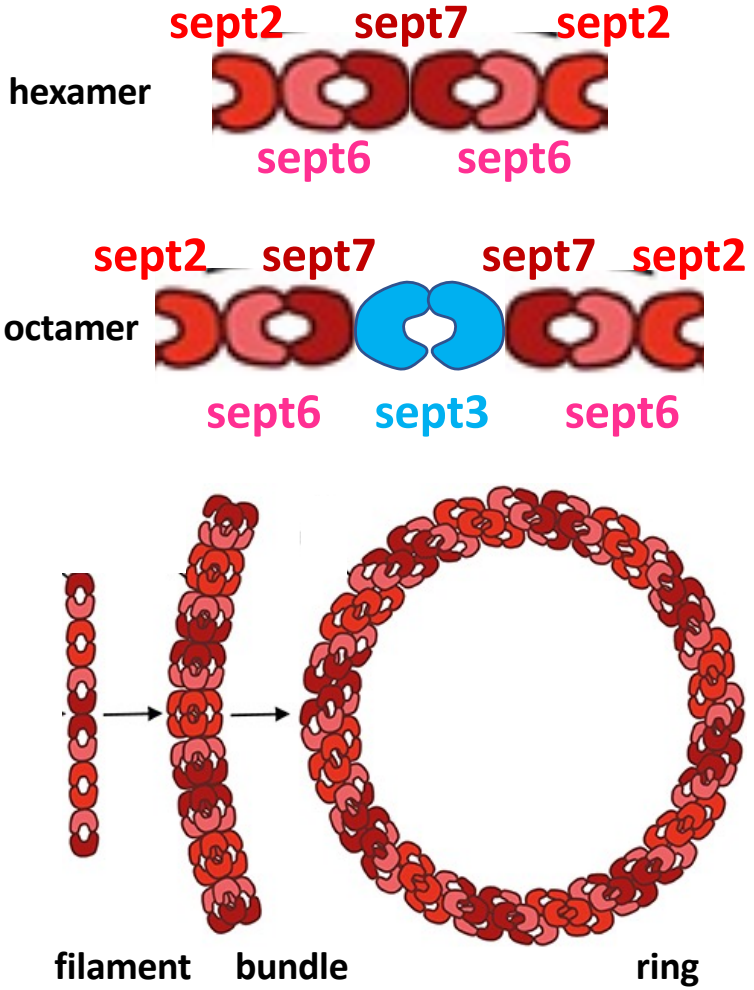
Septin filaments

Septin filament structure

GTPase proteins

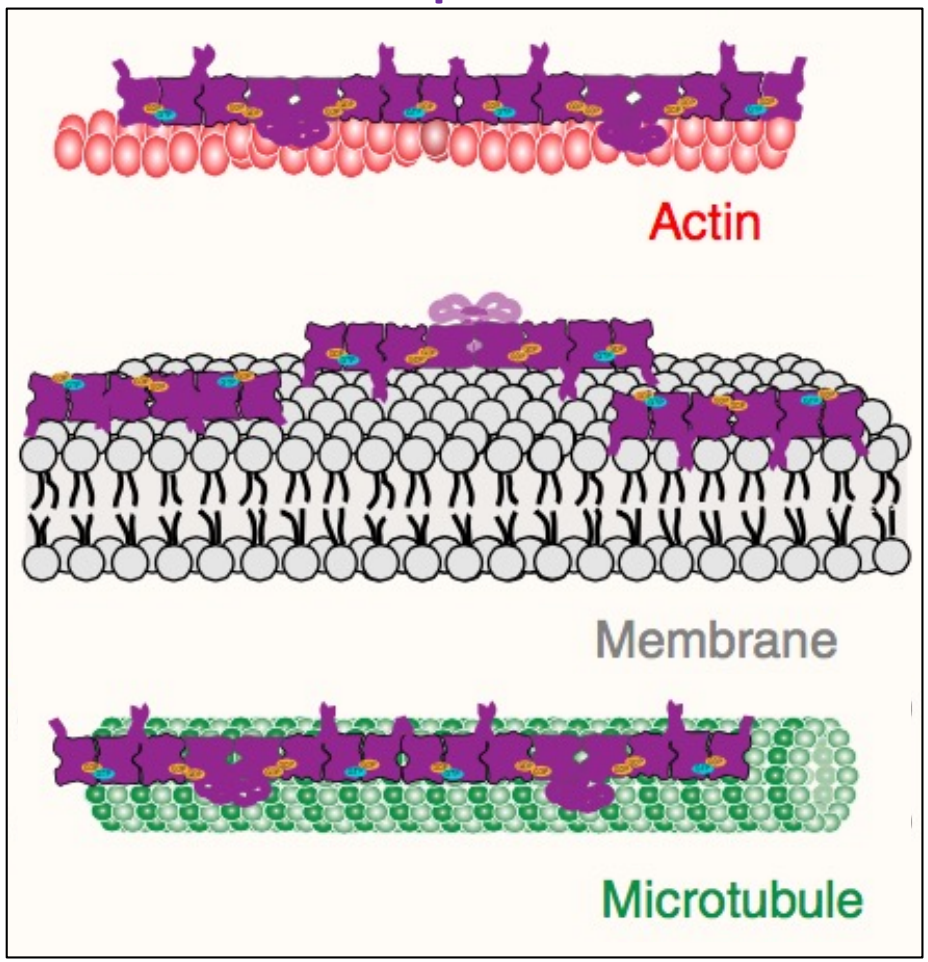


Palindromic hetero-oligomers of ~5 nm diameter



Adapted from Torraca & Mostowy, Front. Cell Dev. Biol., 2016
& Woods and Gladfelter, Curr. Opi. Cell Biol., 2021

Septin filament association



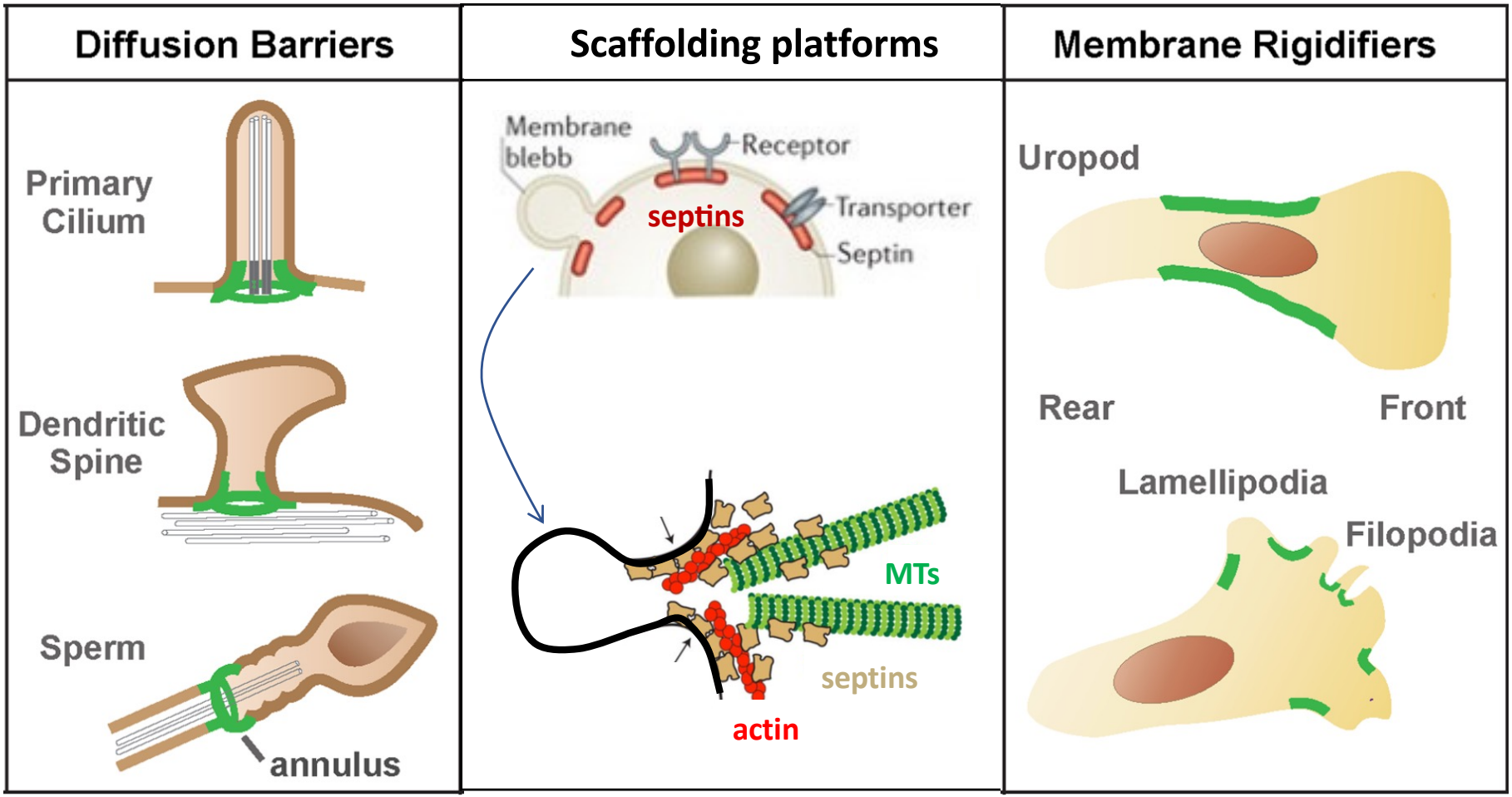
Linker proteins
(Arp2/3, myosin II, anillin, BORG)

. Curvature (radius 0,5-1,5 µm positive curvature)
. lipids (PIPs = phosphatidyl inositol phosphate, cardiolipin)

. Sept9 (Nterm)
. Tubulin isotype (TUBB2)
. Tubulin PTM (polyGlu, Tyr)
. Tubulin GTP

Adapted from Spiliotis & Nakos, Curr. Biol. 2021

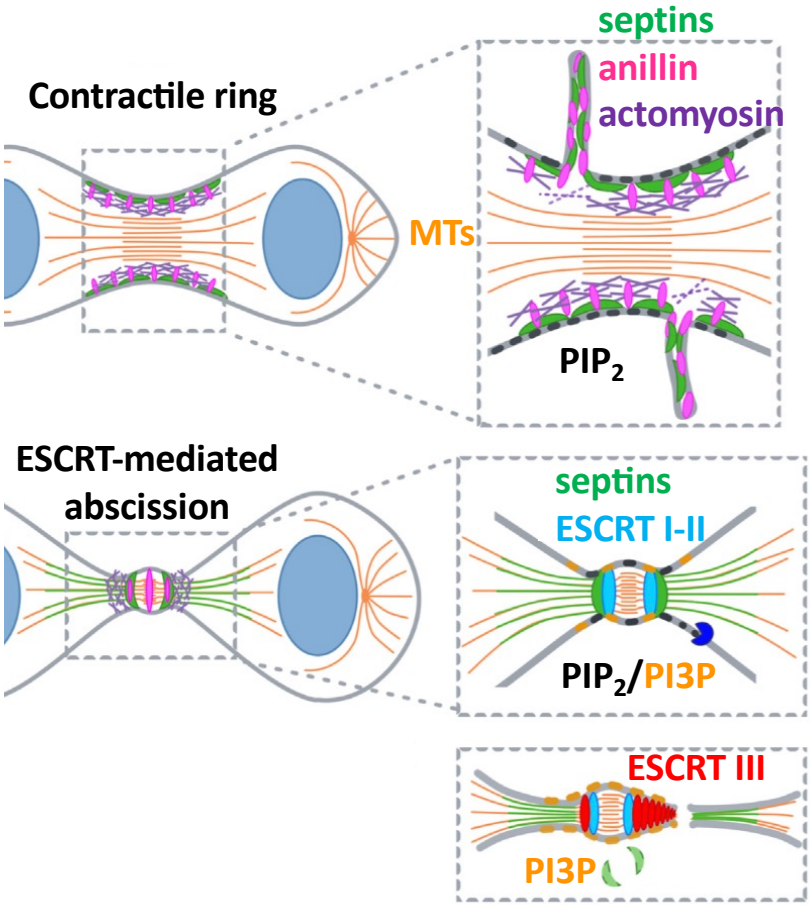
Septin filament functions



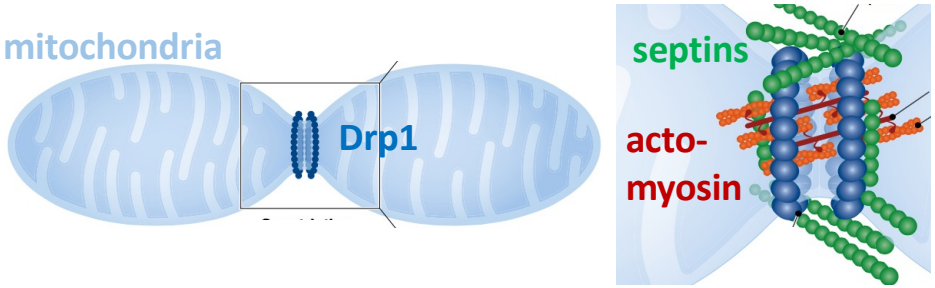
Spiliotis & McMurray, MBOC, 2020
Mostowi & Cossart, Nat. Rev. Mol. Cell Biol., 2012
Spiliotis J. Cell sci., 2018

Septin filament functions

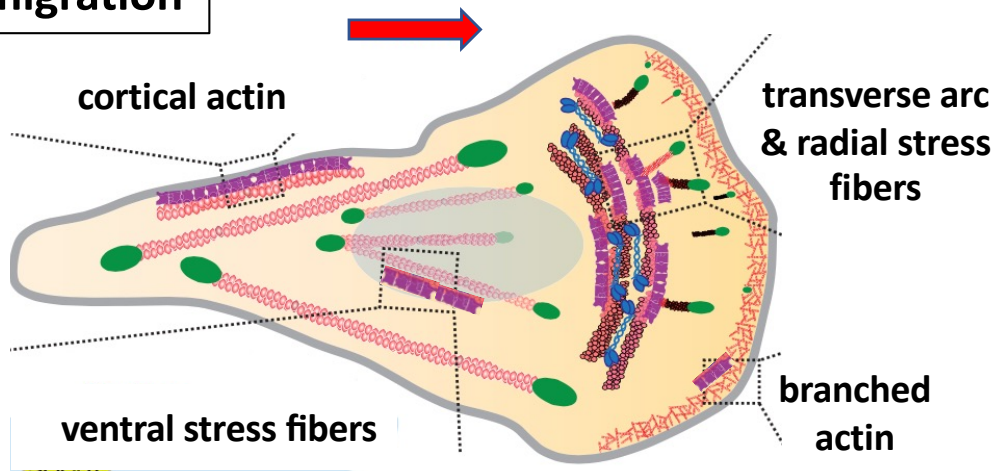
cytokinesis



mitochondrial fission



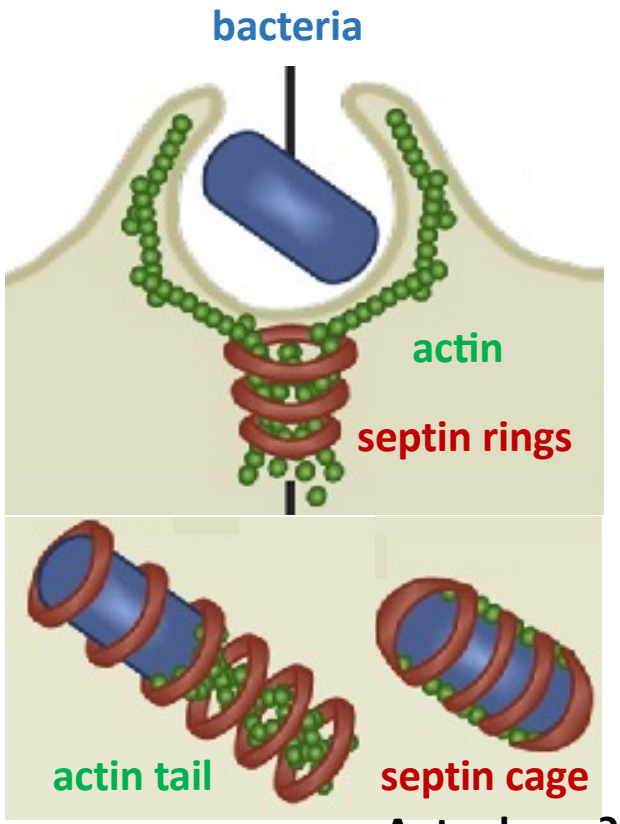
migration



Russo & Krauss, Front. Cell Dev. Biol.., 2021
Spiliotis & Dolat, Embo Rep., 2016
Adapted from Spiliotis & Nacos Curr. Biol., 2021

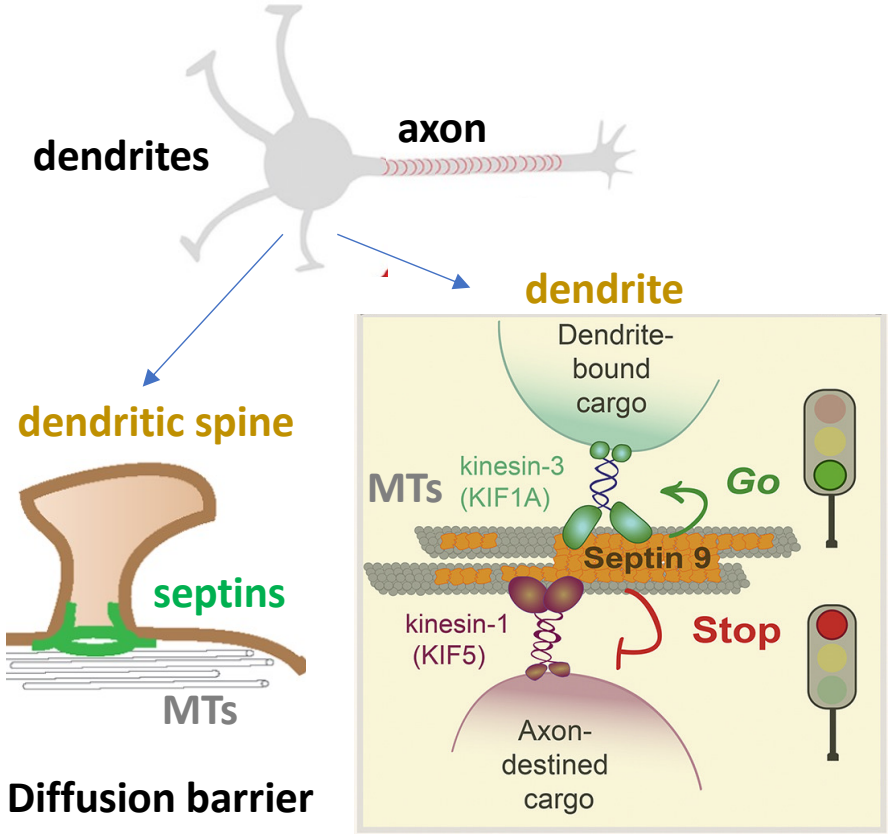
Septin filament functions

infection



Autophagy ?

neuron

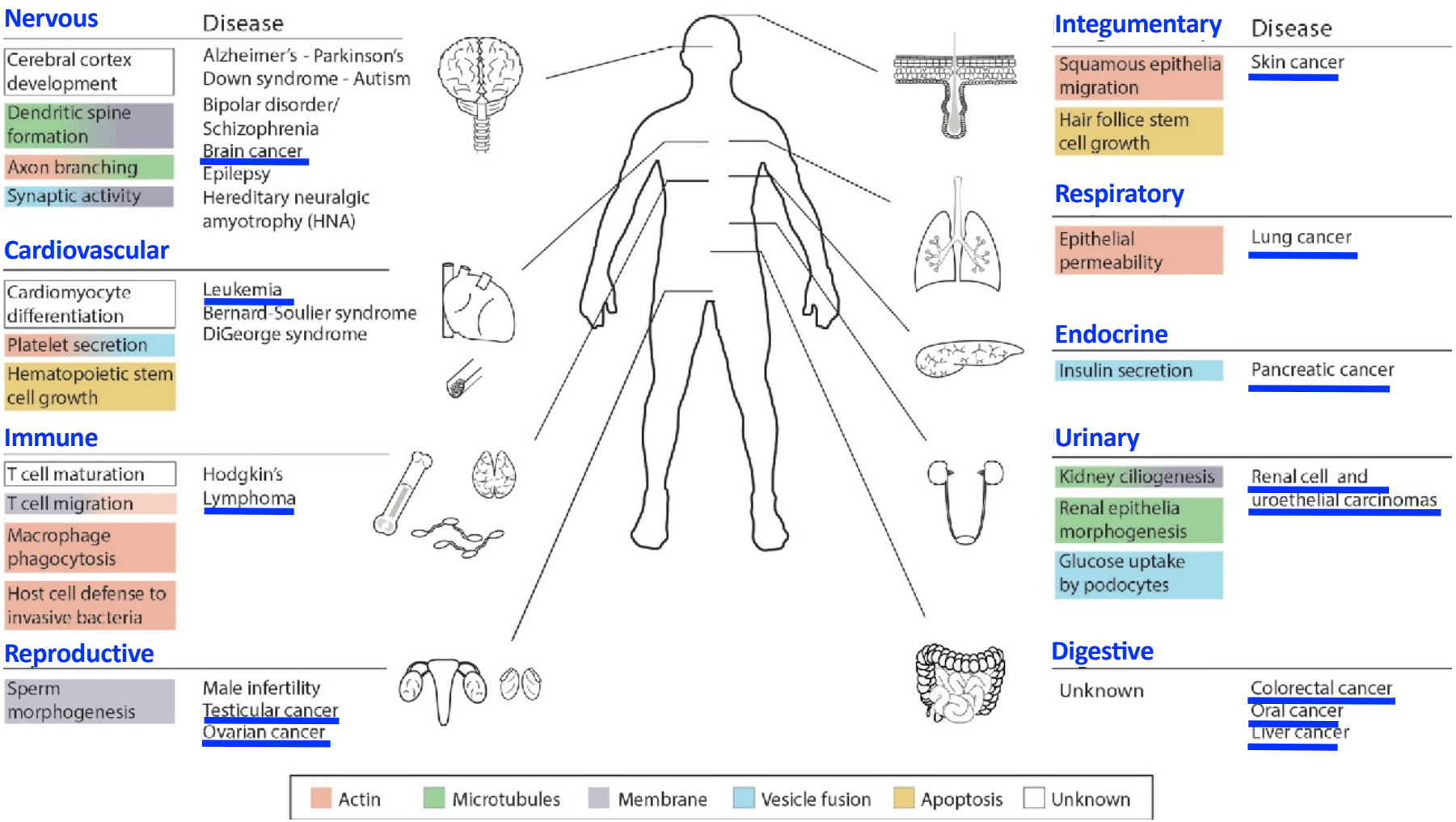


Diffusion barrier

Diffusion barrier on MTs

Adapted from Krokowski & Mostowi, Meth. Cell Biol., 2016
Karasmanis et al., Dvpt Cell, 2018

Septin filaments : diseases / cancer

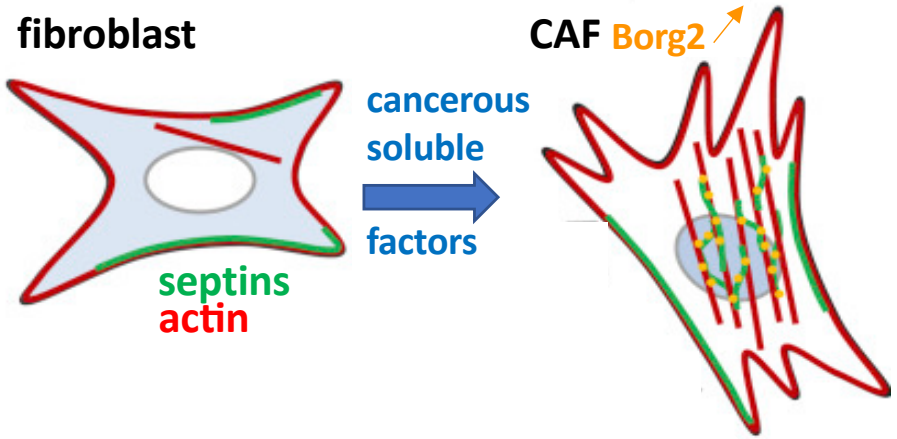


Septin 9 methylated DNA in a blood test for colorectal cancer
(Septin 4 isoform in a urinary test for urothelial cancer)

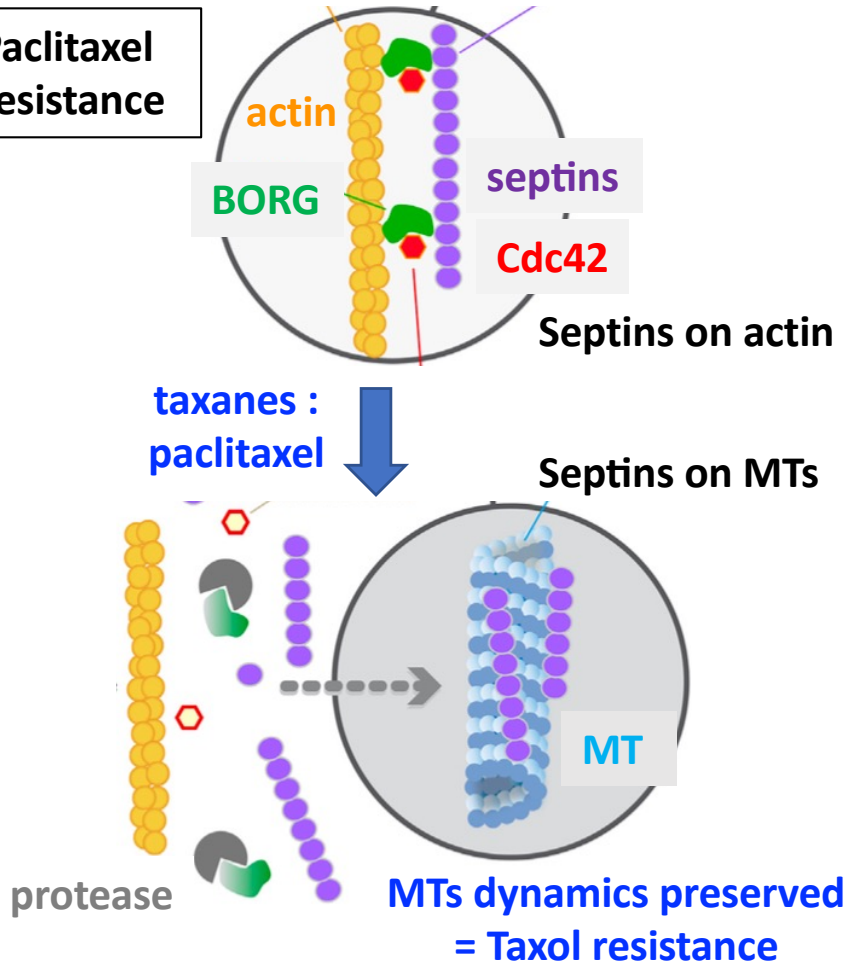
Dolat et al., Biol. Chem., 2014

Septin filaments / BORG and cancer

Cancer-associated fibroblast (CAF)
= favor migration of cancer cells
(tumor microenvironment = TME)



Paclitaxel resistance

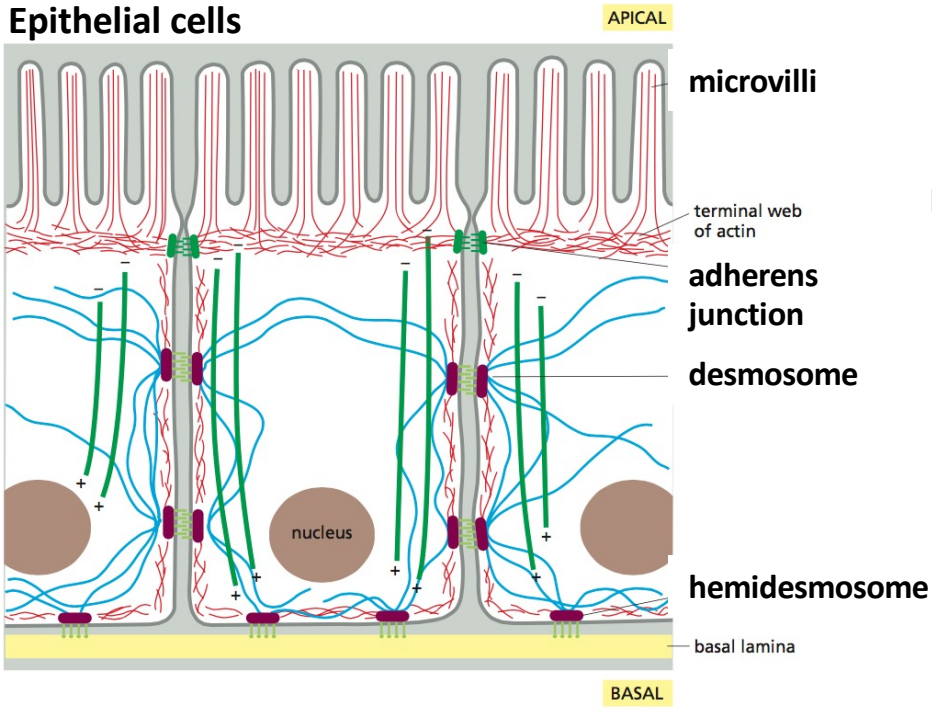


BORG overexpression is pro-metastatic / active BORG on actin is required for taxanes efficiency

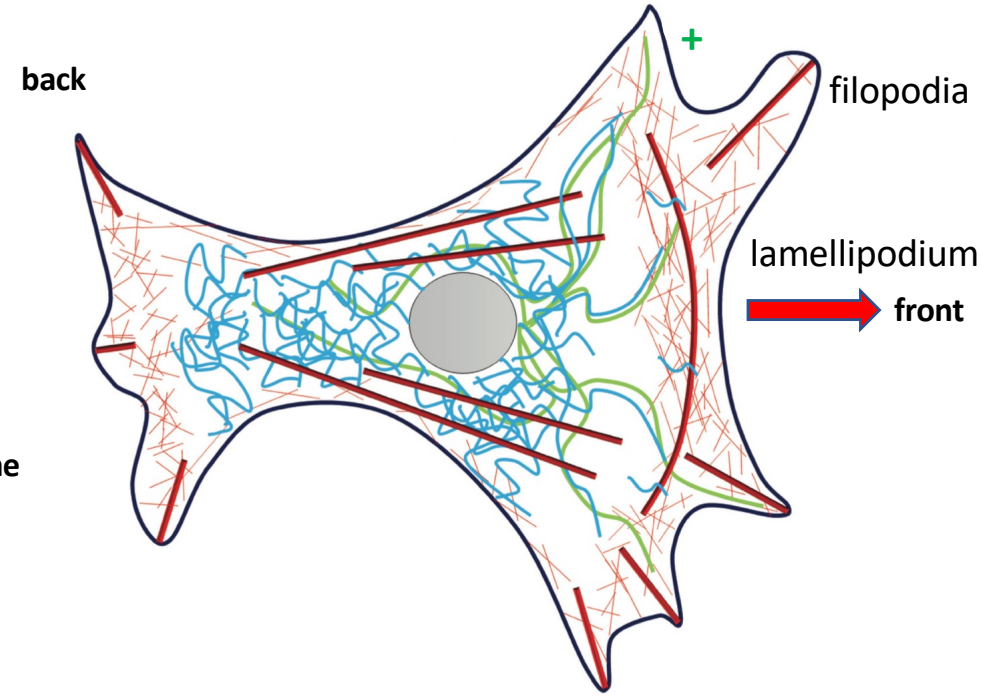
Adapted from Calvo et al., Cell Reports., 2015
Adapted from Salameh et al., Curr. Biol., 2021

Cytoskeleton dynamic crosstalks …

Epithelial cells



Mesenchymal migration



Actin microfilaments / (septins)
Microtubules Intermediate filaments

Adapted from figure 16-4, Molecular Biology of the Cell 6th
Battaglia et al., F1000 Res., 2018