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Molecular Cell Biology

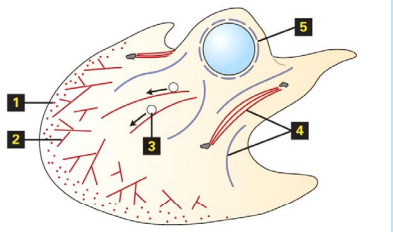
Fifth Edition

Chapter 19:
Cytoskeleton I: Microfilaments and Intermediate Filaments

Actin structure

- Forms very large structures- assembly or disassembly alters cell morphology or the morphology of specific compartments.
- Flexible cytoskeleton
- Monomers, filament, cross linked filaments, imperfect bundles and networks.
- An ancient highly conserved gene

Overview actin and intermediate filaments -functions



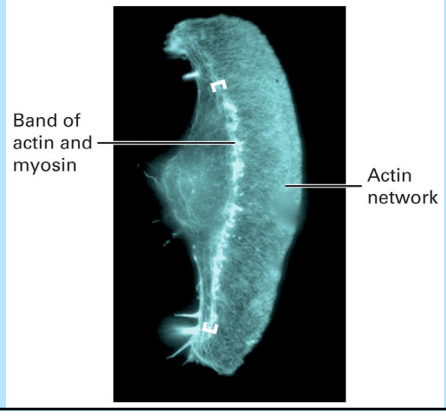
	CYTOSKELETAL COMPONENT	CELL FUNCTION
1	Actin dynamics	Membrane extension
2	Filament networks: bundles	Cell structure
3	Myosin motors	Contractility and vesicle transport
4	Actin bundles and intermediate filaments	Cell adhesion
5	Lamin network	Nuclear structure

- Actin is the most abundant intracellular protein
- The cytosolic concentration of non muscle actin is 0.1-0.5 mM.
- In microvilli 5mM
- Moderate size of MW 42.000.

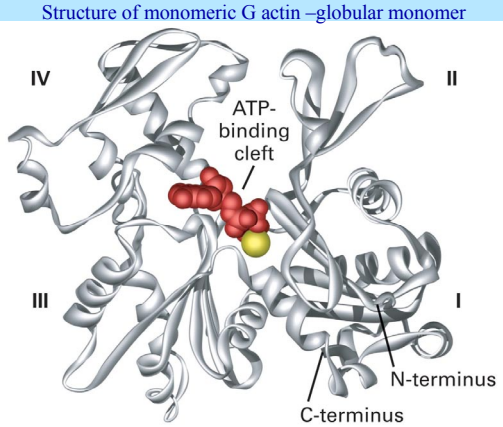
Actin a highly conserved gene

- In vertebrates four a-actin isoform in muscles .
- β and γ actin non muscle cells.
- β-actin in the leading edge of moving cells
- γ– actin form filaments
- the different isoforms differ in four of five positions

Two actin structure operate to generate the motion of keratinocytes. A network of actin pushes the membrane and myosin-actin generate the energy to pool the cell



Structure of monomeric G actin –globular monomer

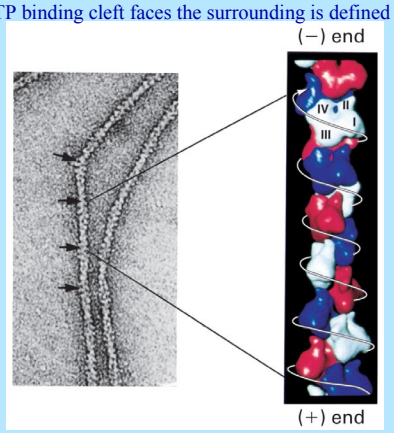


Addition of Mg, K or Na to G-actin solution induces polymerization of G-actin into F-actin

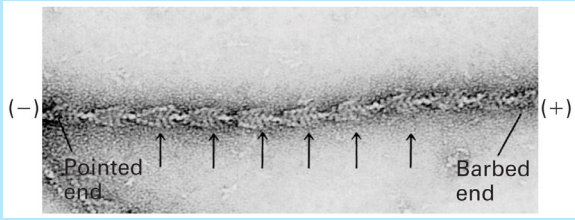
- The assembly of G-actin into F-actin is accompanied by the hydrolysis of ATP to ADP and Pi
- Hydrolysis effect the kinetics of polymerization but is not necessary.

- The reversible polymerization of G-actin into F-actin lies at the core of many cell movements

The ATP binding cleft faces the surrounding is defined as- end



F-actin has structural polarity



F-Actin forms networks with the help of actin cross linking proteins

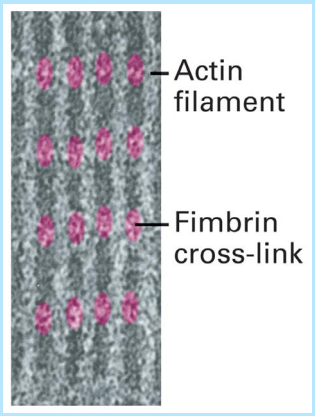
Actin cross linking proteins enable the formation of various forms of actin networks

- Monomeric-that contain two actin binding sites (finbrin fascin)
- Two polypeptide chains that each has a single actin binding site.
- The actin binding protein often bind membrane protein thus actin networks are generally found near the plasma membrane

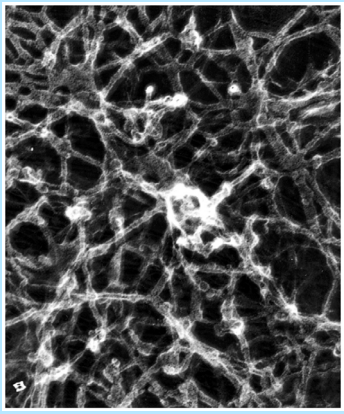
TABLE 19-1 Selected Actin-Binding Proteins

Protein	MW	Domain Organization*	Location
CH-DOMAIN SUPERFAMILY			
Fimbrin	68,000		Microvilli, stereocilia, adhesion plaques, yeast actin cables
α-Actinin	102,000		Filopodia, lamellipodia, stress fibers, adhesion plaques
Spectrin	α: 280,000 β: 246,000–275,000		Cortical networks
Dystrophin	427,000		Muscle cortical networks
Filamin	280,000		Filopodia, pseudopodia, stress fibers
OTHERS			
Fascin	55,000		Filopodia, lamellipodia, stress fibers, microvilli, acrosomal process
Villin	92,000		Microvilli in intestinal and kidney brush border

*Blue = actin-binding domains; red = calmodulin-like Ca^{2+}-binding domains; purple = α-helical repeats; green = β-sheet repeats; orange = other domains.

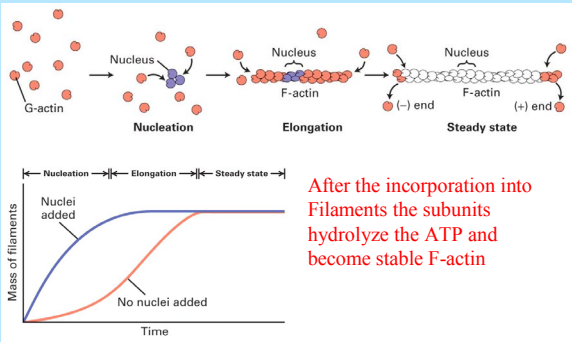


An actin network formed by flexible filamin cross linking

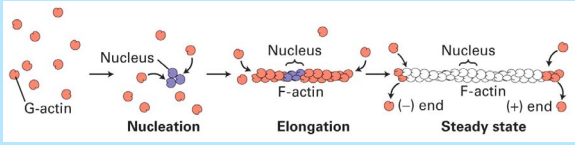


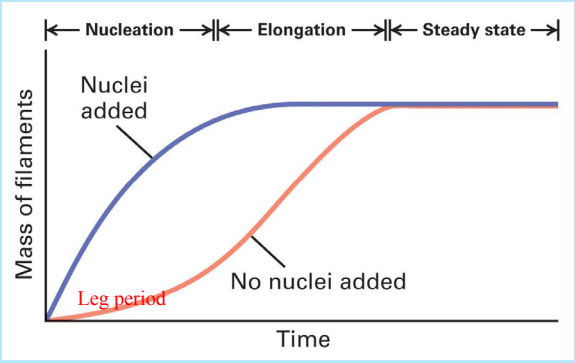
The dynamic nature of actin networks

Actin polymerization in vitro proceeds in 3 stages

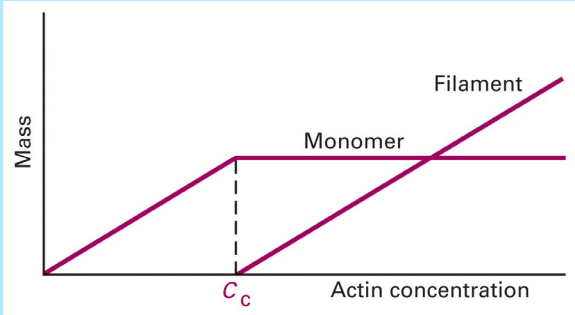


After the incorporation into Filaments the subunits hydrolyze the ATP and become stable F-actin

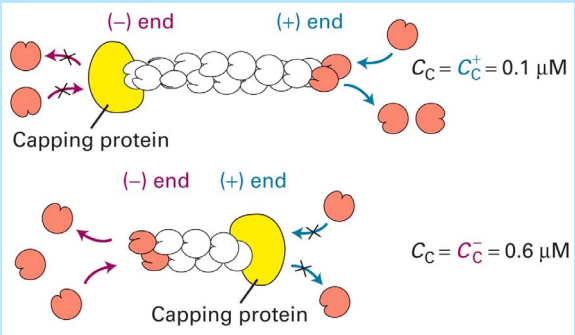




Concentration of G actin determines the filament formation

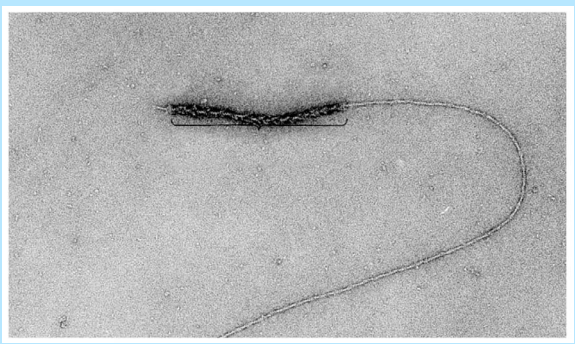


Actin filaments grow 5-10 times faster at the +end than the -end

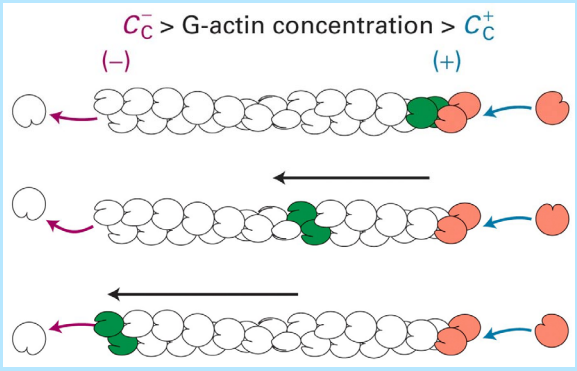


0.1μM G-actin=Cc

ATP hydrolysis is not necessary for polymerization G-actin containing ADP or nonhydrolyzable ATP analog polymerize actin



treadmilling



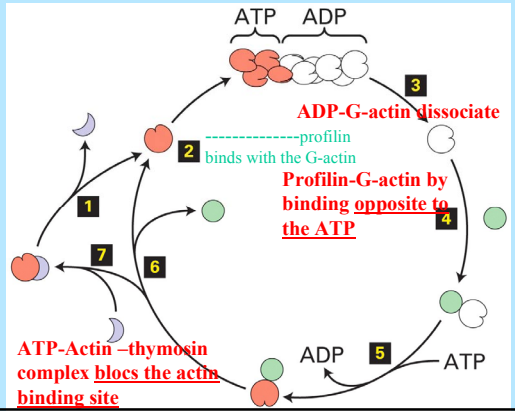
The design of actin!!!!!!!!

- A flexible cytoskeletal network
- Assembly
- Severing
- branching

Regulation of actin polymerization

- Actin binding proteins either promote or inhibit actin polymerization
- Calculations based on the actin Cc (0.1μM), intracellular concentration (0.5mM) and ionic strength- all G actin should exists as F-actin
- Nevertheless 40% of the actin is in the form of G-actin
- Actin sequestering proteins

Profilin and thymosin regulating polymerization



Severing proteins create new actin ends

Actin severing proteins gelsolin and cofilin

- After severing a fraction of a filament the protein remains attached at the +end (capping) and the minus end shortens rapidly

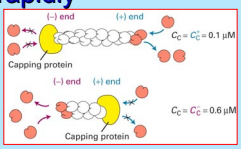


TABLE 19-2 Some Cytosolic Proteins That Control Actin Polymerization

Protein	MW	Activity
Cofilin	15,000	Dissociation from (−) end
Severin	40,000	Severing, capping [(+) end]
Gelsolin	87,000	Severing, capping [(+) end]
CapZ capping protein	36,000 (α) 32,000 (β)	Capping [(+) end]
Tropomodulin	40,000	Capping [(−) end]
Arp2/3 complex	200,000	Capping [(−) end], side binding and nucleation

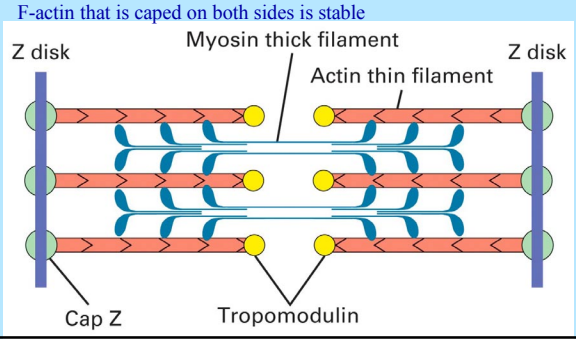
Signaling pathways that regulate actin polymerization

- Cofilin and Gelsolin bind PIP2 that inhibits their binding to F-actin. Hydrolysis of PIP2 by phospholipase C release the proteins and induce severing of F-actin.
- Phosphorylation of cofilin regulate its activity
- 1 μM Ca activate gelsolin.

Capping proteins stabilize F-actin

CapZ binds to F-actin +end and stabilizes it

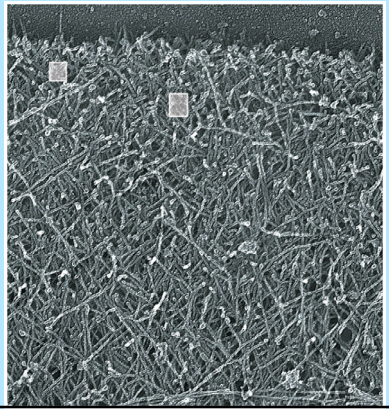
Tropomodulin caps the minus end of F-actin and stabilizes it
F-actin that is caped on both sides is stable

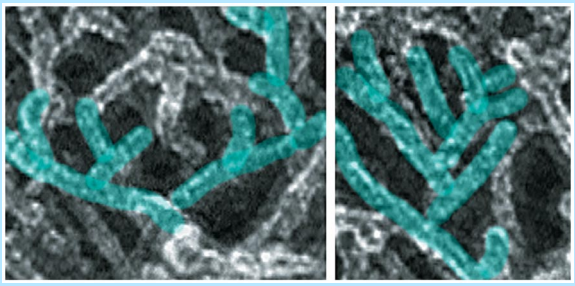


Actin-related –proteins Arp2/3

- The Arp2/3 complex binds at 70 ◊ to the side of an actin filament to nucleate a daughter filament.
- This creates a network in which the Arp2/3 complex is at the base of the branch
- This generate the force to push the membranes
- **Branching is stimulate by the Rho GTPase**

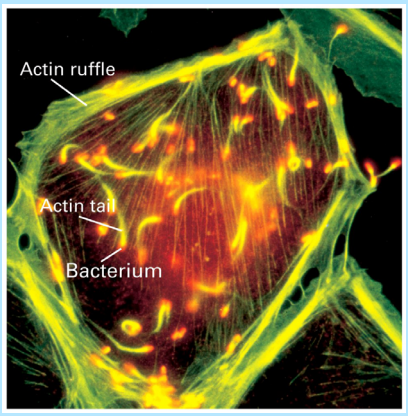
Arp2/3 Assembles Branched Filaments



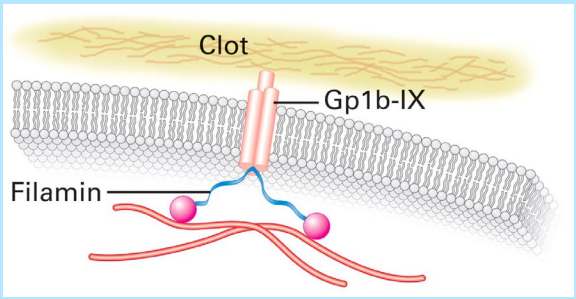


Intracellular movement and cell shape are driven by actin polymerization

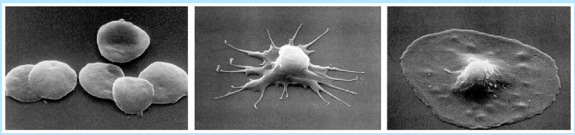
- Listeria –actin polymerize at the base of the bacterium and propel the bacterium through the cell and out of it.

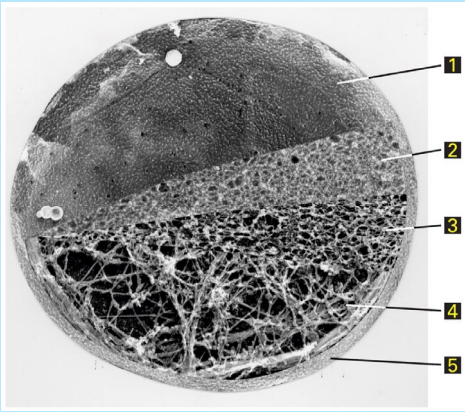


Cross linking of actin to the plasma membrane



Platelets change shape during blood clothing



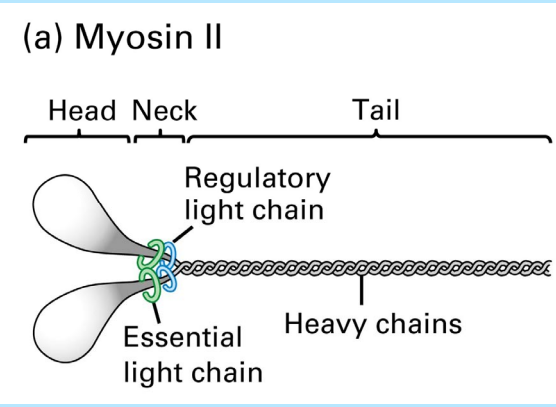


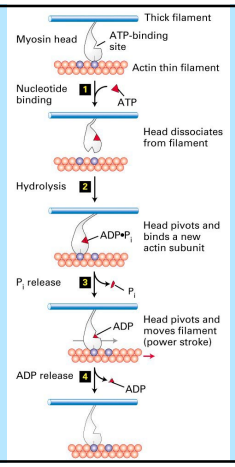
Myosin-Powered cell movements

- Myosins are mechanochemical motor proteins
- Myosin II powers muscle contraction
- Myosin I, and V powers cytoskeleton organelles interactions

Characteristic structure of myosin

- Head , neck and tail domains is found in all myosin heavy chains
- The head is an ATPase that couple hydrolysis with motion
- The activation of the ATPase is actin dependent (actin X5)
- The neck region is associated with the light chain. The light chain is necessary for the conversion of the small conformational changes to large steps.
- The tail domain contain the binding site of the particular myosin.





The role of myosin is related to the tail region. I, V binds to the plasma membrane

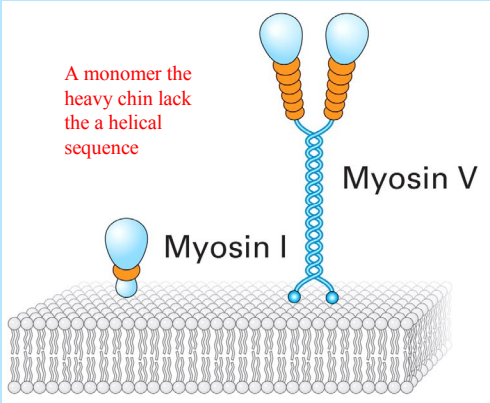
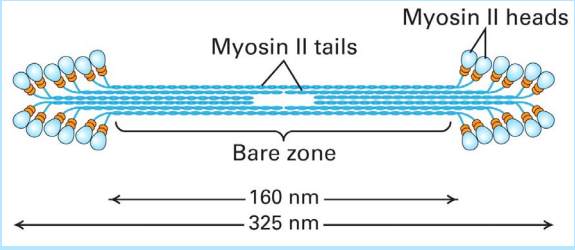
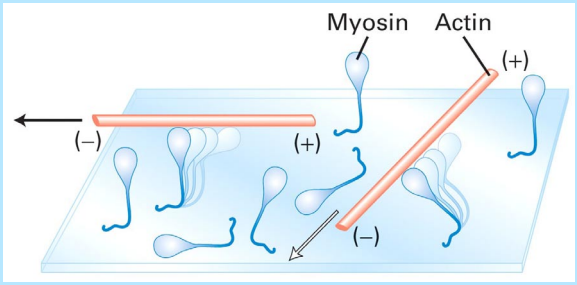


TABLE 19-3 Myosins

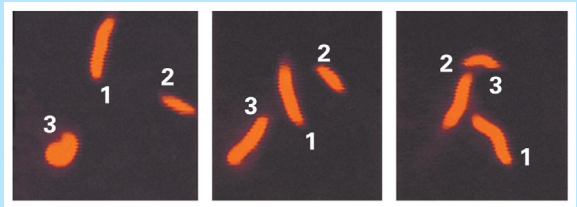
Type	Heavy Chain (MW)	Structure	Step Size (nm)	Activity
I	110,000–150,000		10–14	Membrane binding, endocytic vesicles
II	220,000		5–10	Filament sliding
V	170,000–220,000		36	Vesicle transport
VI	140,000		30	Endocytosis
XI	170,000–260,000		35	Cytoplasmic streaming

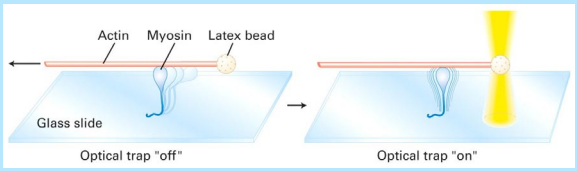


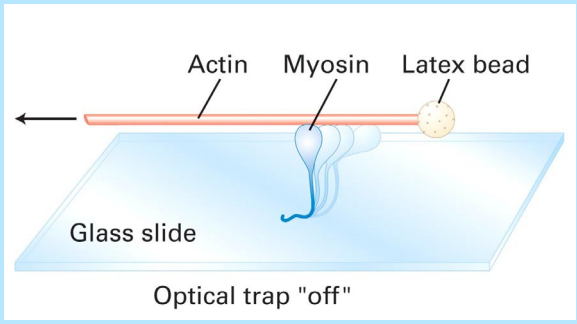
Myosin heads walk along actin filaments in the presence of ATP

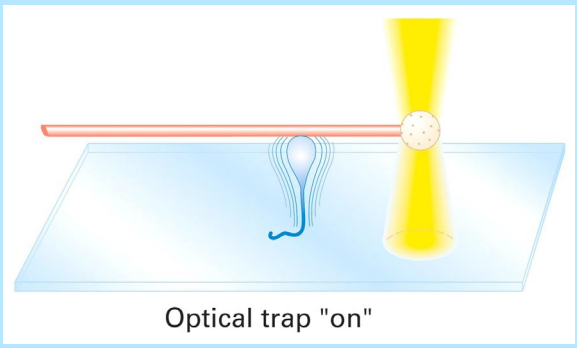


5-10 nm steps, the step size depends on the length of the neck 3-5 pN, one step /ATP

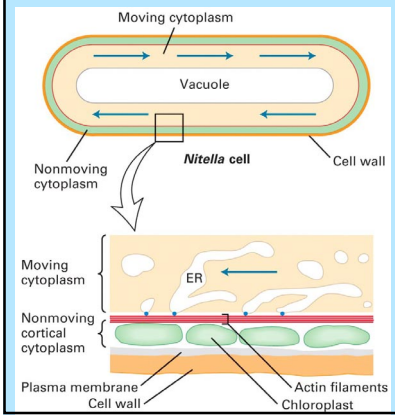




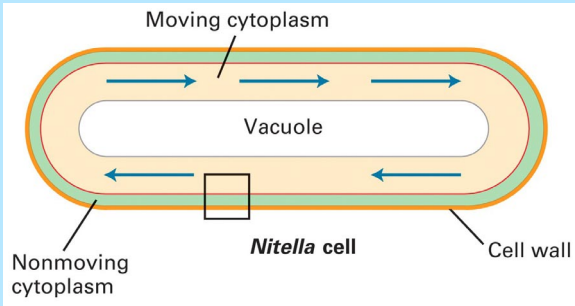


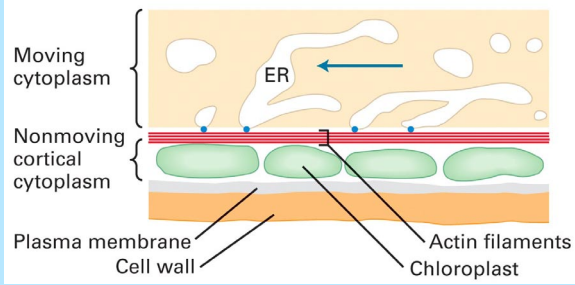


Nitella – a giant algae

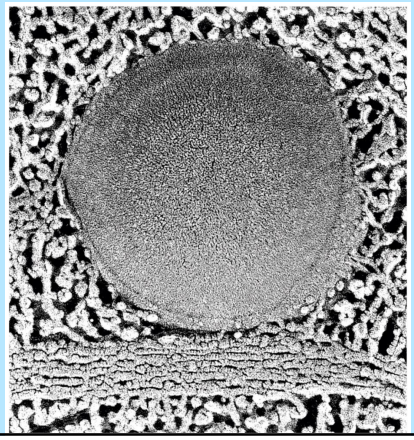


The cytosole flow at a rate of 4.5 mm/min.
The motors lie along the membranes
Large ER vesicles are propelled along the actin

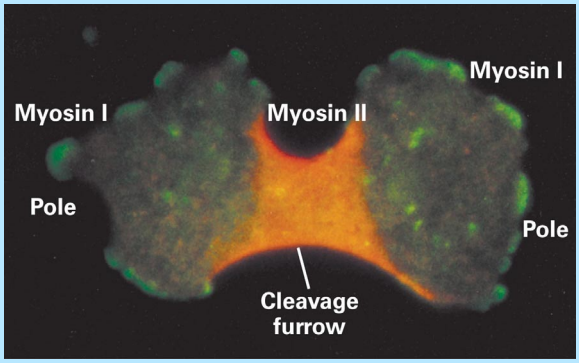


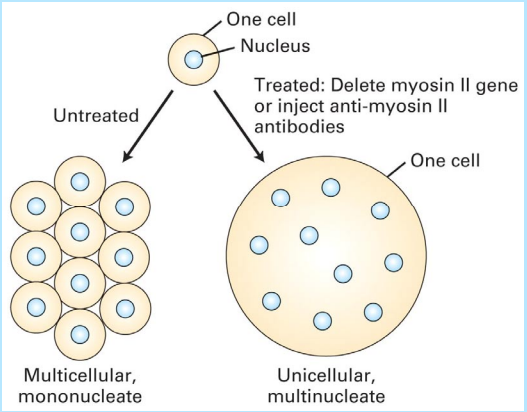


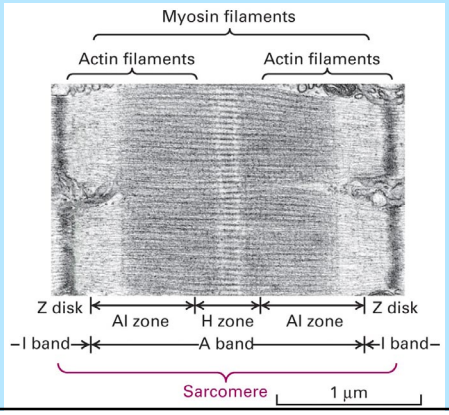
A large vesicle attached to actin filament

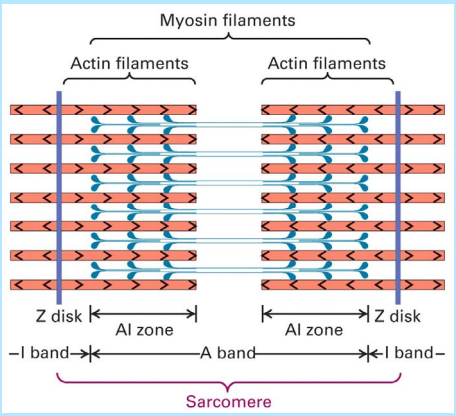


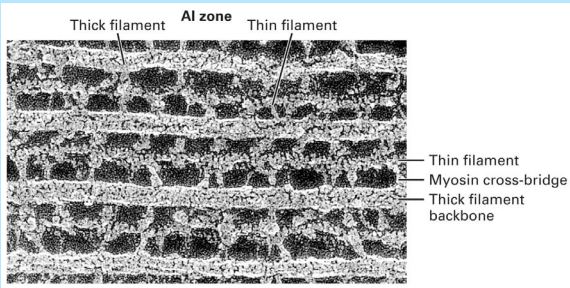
Contractile bundles in a non muscle cell-

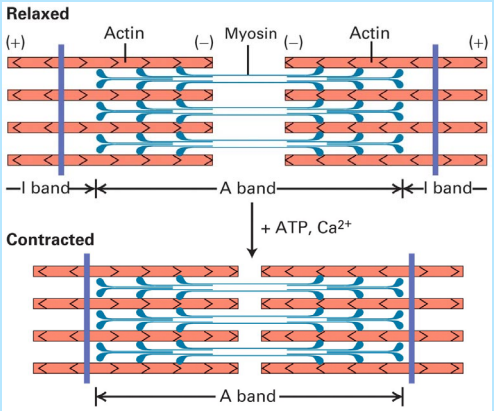


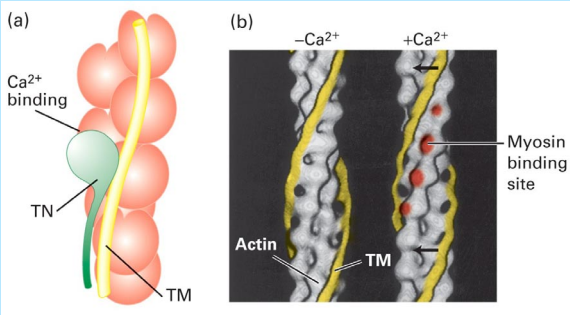


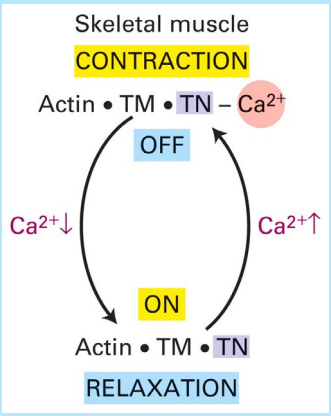


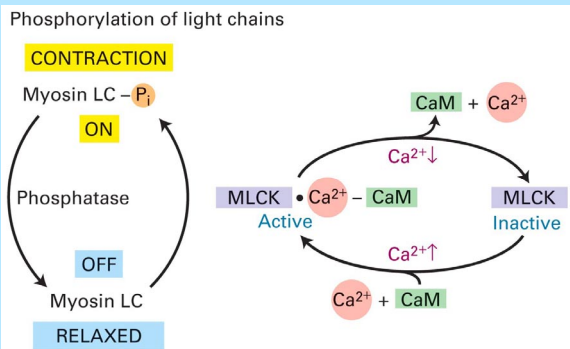

Myosin filaments
Actin filaments
Actin filaments
Z disk
AI zone
AI zone
Z disk
I band
A band
I band
Sarcomere

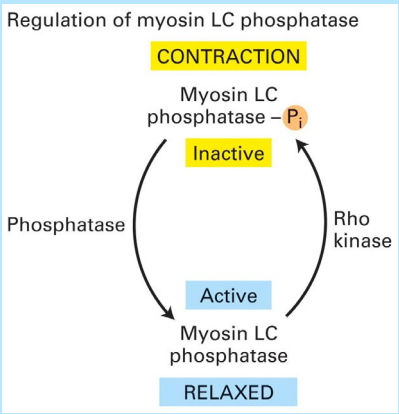

Thick filament
AI zone
Thin filament
Thin filament
Myosin cross-bridge
Thick filament backbone


Relaxed
(+)
Actin
(–)
Myosin
(–)
Actin
(+)
I band
A band
I band
+ ATP, Ca2+
Contracted
A band


(a)
Ca2+ binding
TN
TM
(b)
–Ca2+
+Ca2+
Myosin binding site
Actin
TM


Skeletal muscle
CONTRACTION
Actin • TM • TN – Ca2+
OFF
Ca2+↓
Ca2+↑
ON
Actin • TM • TN
RELAXATION

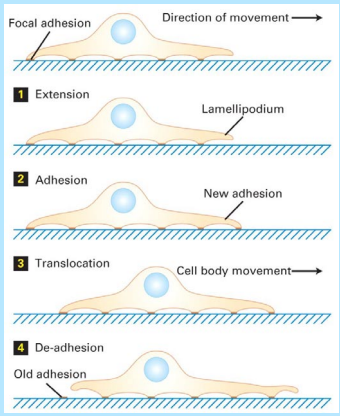

Phosphorylation of light chains
CONTRACTION
Myosin LC – Pi
ON
Phosphatase
OFF
Myosin LC
RELAXED
CaM + Ca2+
Ca2+↓
MLCK • Ca2+ – CaM
Active
MLCK
Inactive
Ca2+↑
Ca2+ + CaM



Cell locomotion

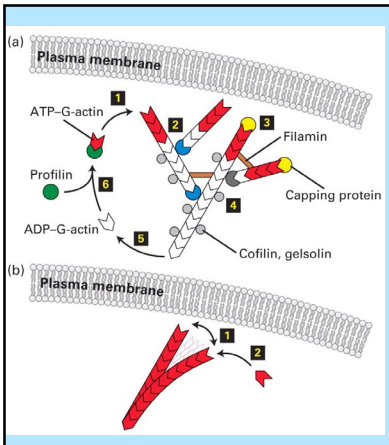
- Cells in motion are characterized by being polar.
- Cells extend a large membrane protrusion at the leading edge –the lamellipodium
- The lamellipodium the actin are crossed linked
- Finger like protrusions are formed the filopodia
- These structures form stable contact with the substrate to prevent the membrane from retracting

Force generation and cell adhesion

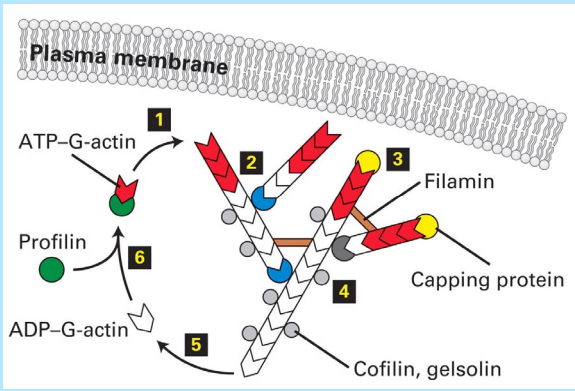


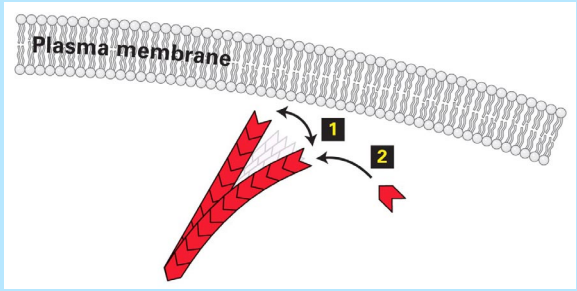
Membrane extension

- Actin polymerization pushes the membrane forward- addition of G-actin to F-actin
- The elastic Brownian ratchet model – explains the mechanism of G actin addition and force generation

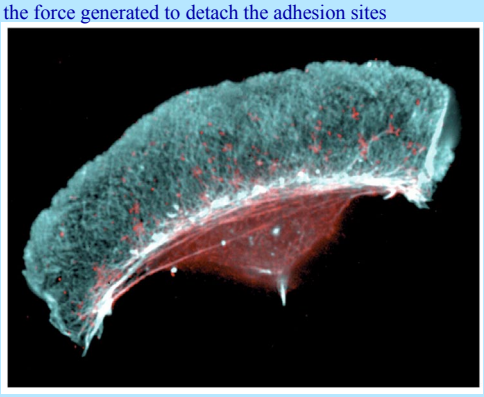


1-ATP- G actin pushes the membrane
2-Arp2/3 complex bines and form a branch
3- capping proteins are added
4-ATP actin convert to ADP actin and dissociate through the action of gelsolin cofilin
5- the released ADP-actin regenerate new ATP-actin profilin complex

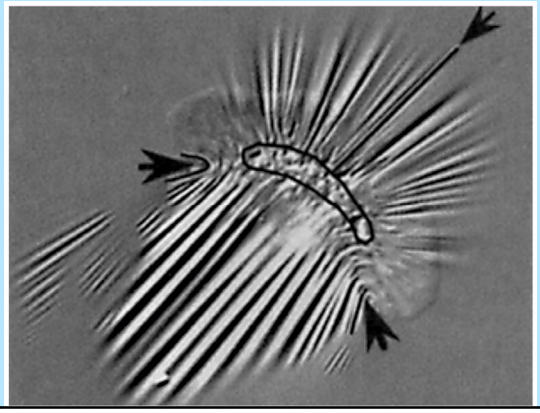




Detachment of the ell body by actin myosin contraction
Cell motility is controlled by a delicate balance between adhesion and the force generated to detach the adhesion sites



A keratinocyte generate force on a thin silicon membrane



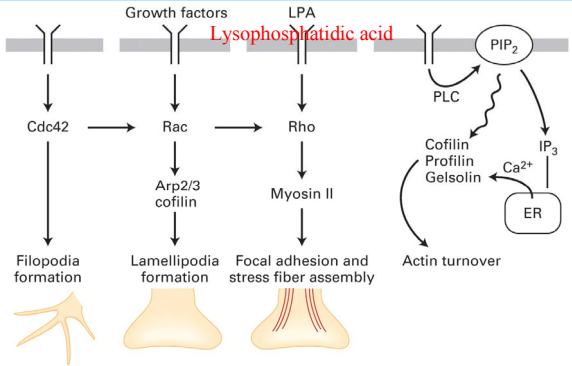
Reversible Gel – sol transition in amoeboid movement

- Ectoplasm-gel- viscous
- Endoplasm- fluid
- Profilin at the front of the cell promotes actin polymerization. Filamin form gel like actin network in the more viscous ectoplasm
- Cofilin sever actin filaments to form the more fluid endoplasm

Growth factors control cell motility

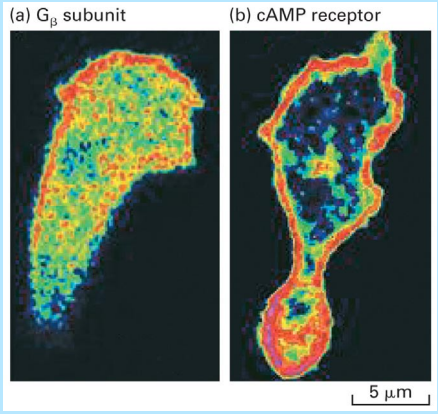
- Wound healing (fibroblasts) embryonic development and metastasis of cancer cells
- The signaling are initiated by binding of growth factors to tyrosine kinases receptor molecules

GTPase superfamily of switch proteins

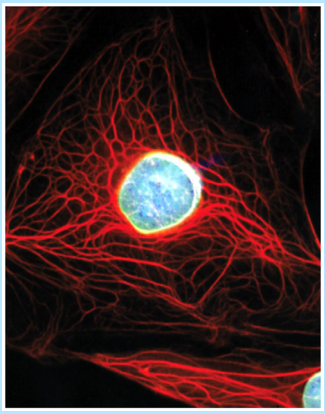


Directional motility -Navigation

- Redistribution of receptors
- Calcium concentration gradients- first increase than a gradient low in the leading edge and high on the other side. Theis probably regulate the sol-gel gradients in the cell



Intermediate filaments- The principal function is structural

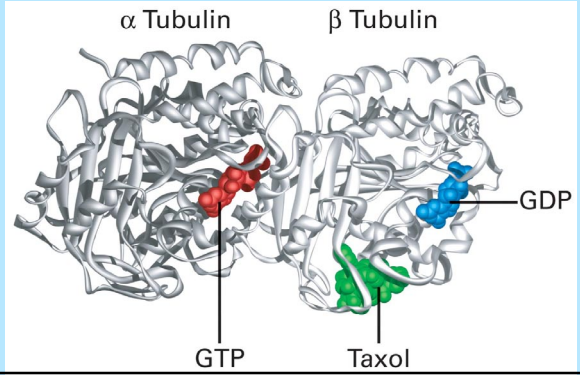


Unlike F-actin (microfilaments) and MTs, IF are not associated with motility

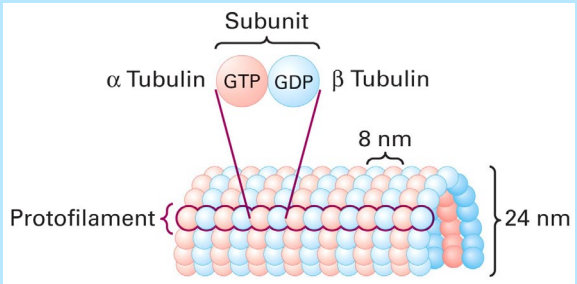
IFs

- In contrast to Actin-F and MTs Intermediate filaments are stable even after extraction
-
- MTs 24nm, IF 10nm, microfilaments = actin 7nm
- Unlike MTs and AF, IF are helical rods
- Assembly does not involve ATP or GTP hydrolysis
- The assembly mechanisms of IF are not understood

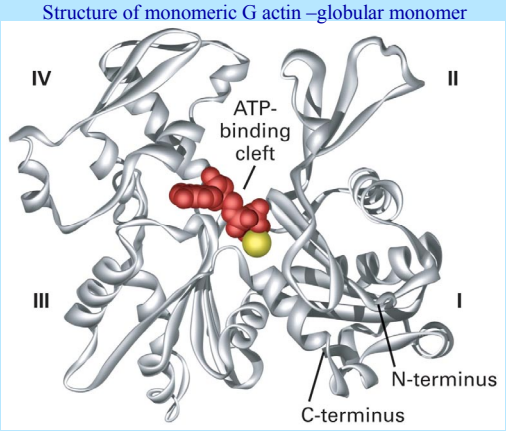
Building blocks are α and β-tubulin bound together by noncovalent bonds 55,000 MW. GTP-binding sites: α tubulin irreversibly, β tubulin binds GTP reversibly and serves as a GTPase. The GDP modulate the addition of tubulin subunits.



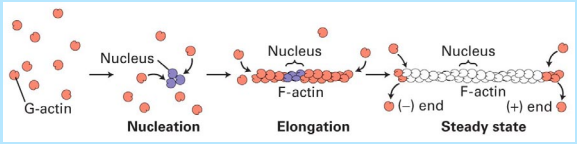
Protofilaments longitudinal interactions α- α, β- β
Cylindrical –tube, lateral interactions of 13 protofilaments
Because of the structure addition of new dimers takes place only at the + or – ends



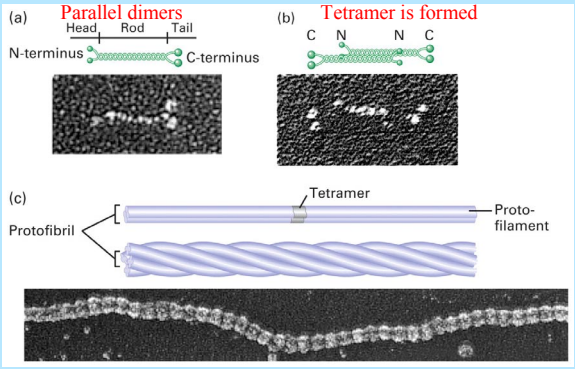
Structure of monomeric G actin –globular monomer

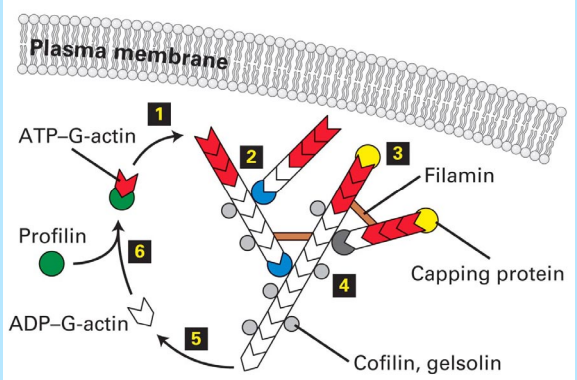


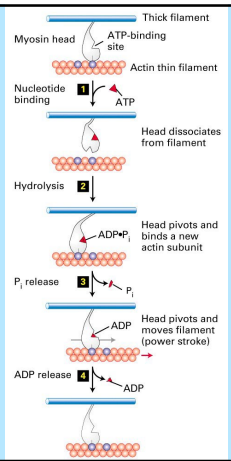
Addition of Mg, K or Na to G-actin solution induces polymerization of G-actin into F-actin



Basic structure – No polarity







Classification of IF
Unlike actin and tubulin isoforms the IF proteins are widely divergent

- Epithele cells express acidic and basic keratins which form heterodimeres
- the keratins is the most diverse IF
- 10 keratins are specific for hard tissues- nails hair
- Lamins- exclusively in the nucleus
- Other epithelial cells

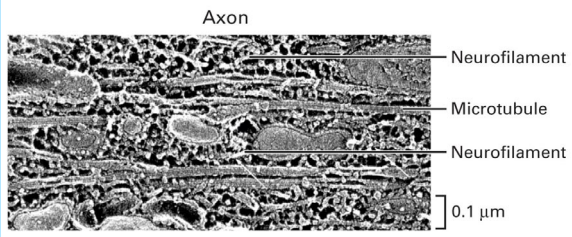


TABLE 19-4 Primary Intermediate Filaments in Mammals

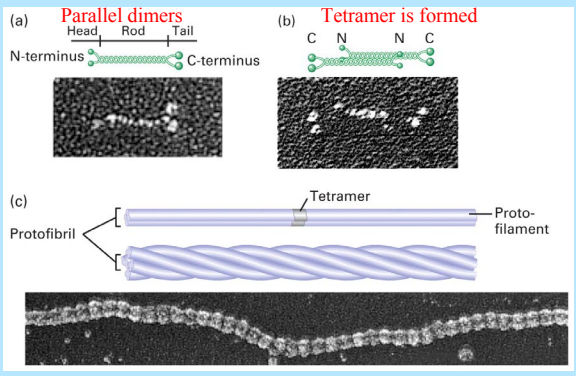
IF Protein	MW (10^{-3})*	Filament Form	Tissue Distribution
NUCLEAR LAMINS			
Lamin A	70	Homopolymer	Nucleus
Lamin B	67	Homopolymer	Nucleus
Lamin C	67	Homopolymer	Nucleus
KERATINS†			
Acidic keratins	40–57	Heteropolymers	Epithelia
Basic keratins	53–67	Heteropolymers	Epithelia
TYPE III INTERMEDIATE FILAMENTS			
Vimentin	57	Homo- and heteropolymers	Mesenchyme (fibroblasts)
Desmin	53	Homo- and heteropolymers	Muscle
Glial fibrillary acidic protein	50	Homo- and heteropolymers	Glial cells, astrocytes
Peripherin	57	Homo- and heteropolymers	Peripheral and central neurons
NEUROFILAMENTS			
NF-L	62	Homopolymers	Mature neurons
NF-M	102	Heteropolymers	Mature neurons
NF-H	110	Heteropolymers	Mature neurons
Internexin	66	—	Developing CNS

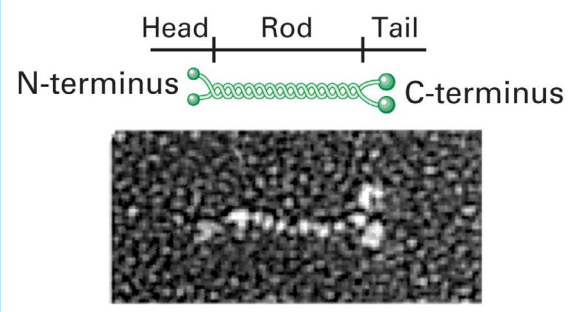
*Intermediate filaments show species-dependent variations in molecular weight (MW).
†More than 15 isoforms of both acidic and basic keratins are known.

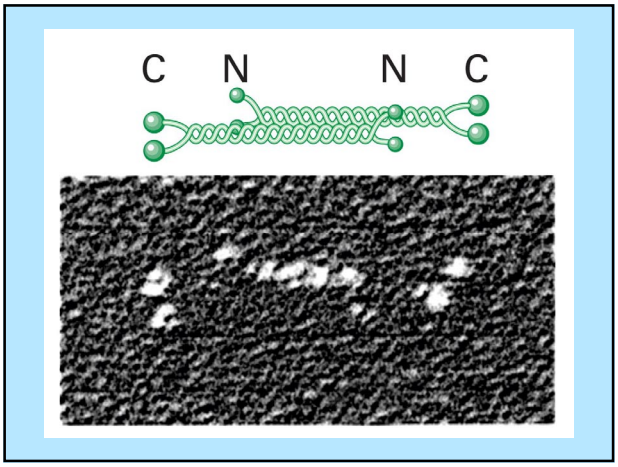
Conserved core domain of IF

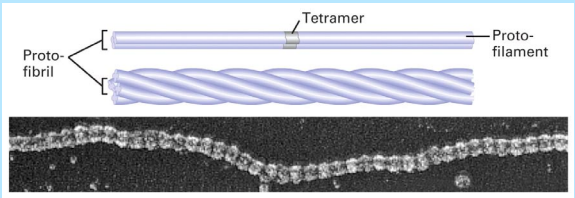
- All Ifs have a central α helical core flanked by globular N- and C- terminal domain
- Formation of dimers
- Formation of symmetric tetramers- No polarity
- Tetrameres bind end to end to form a profilament 2-3 nm thick
- Four protofibriles form a 10nm intermediate filament

Basic structure – No polarity

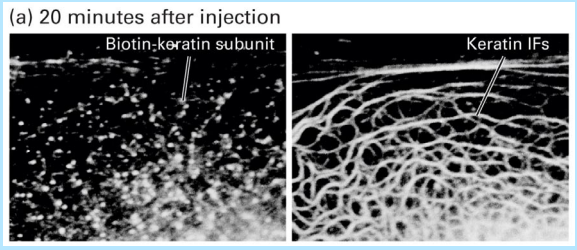


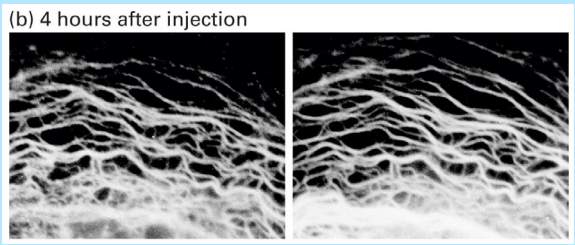






IF are dynamic





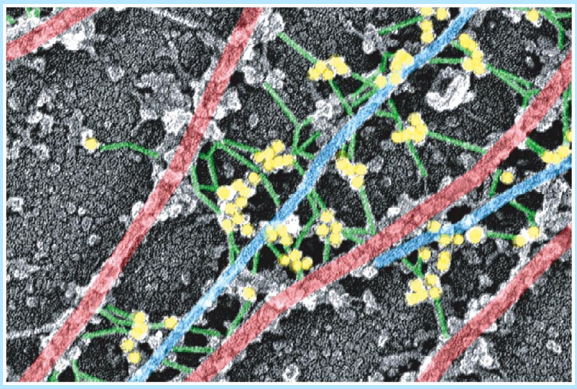
The IFs are stable

- Nevertheless, in mitotic cells-
- Break down of the nuclear envelop early in mitosis
- lamin filaments forms a meshwork supporting the nuclear membrane
- cyclin dependent kinase Cdc2 underlie the dissociation of the lamin network. A phospahatase reform the network

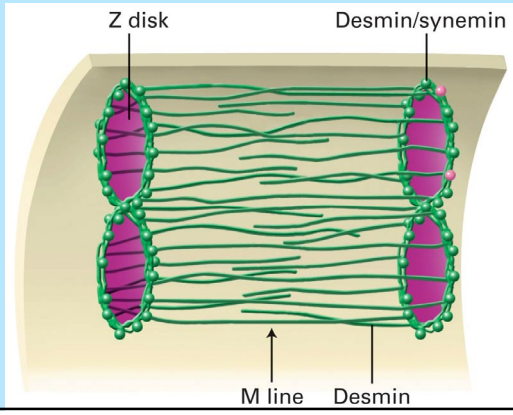
Intermediate Filaments Associated Proteins

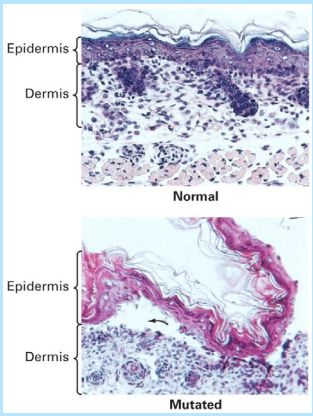
- Unlike in MTs and actin filaments the IFAP do not serve as cap, sequestering proteins, or act as motor proteins
- Rather they link IFs to IFs, to MTs, actin filaments and membranes

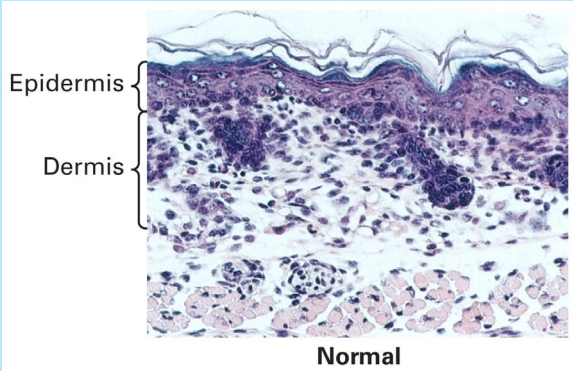
Gold label of plectin cross linking between MTs and IFs

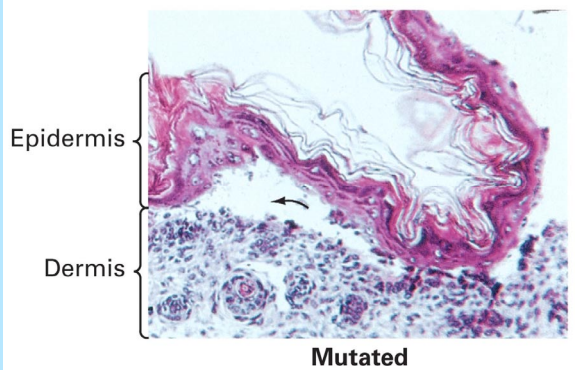


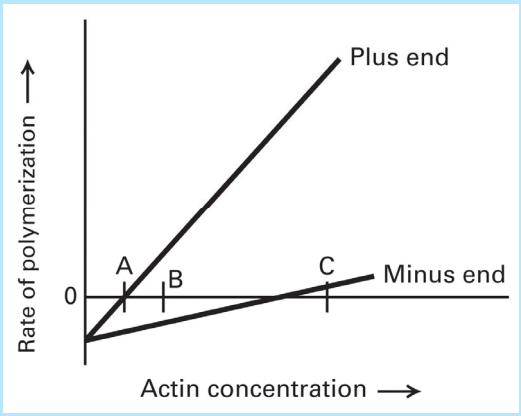
Desmin filaments in muscle cell

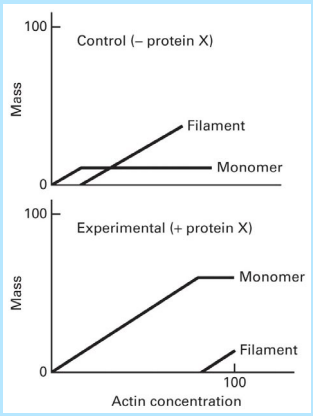


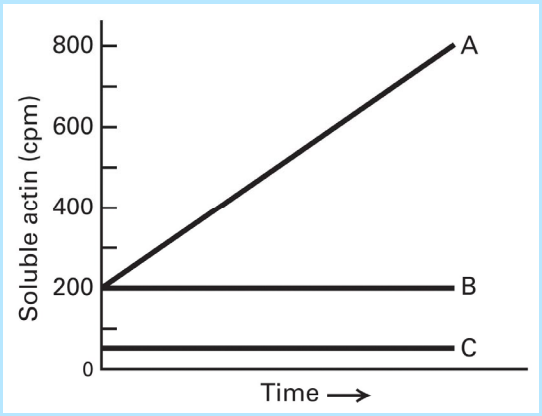


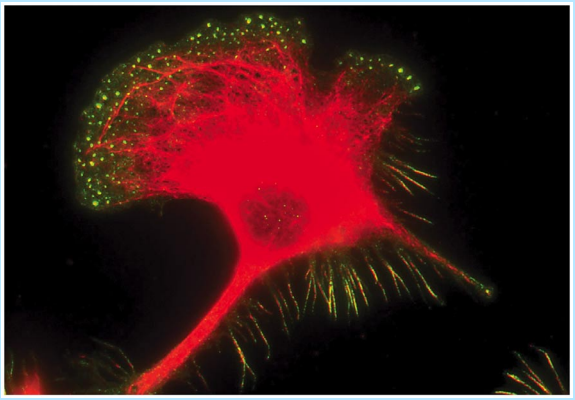












Myosin heads walk along actin filaments in the presence of ATP