Basic Seminar WS 2014

Cellular Signal Transduction

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Contents of the lecture can be found at:

http://www.meduniwien.ac.at/user/johannes.schmid/lectures.htm

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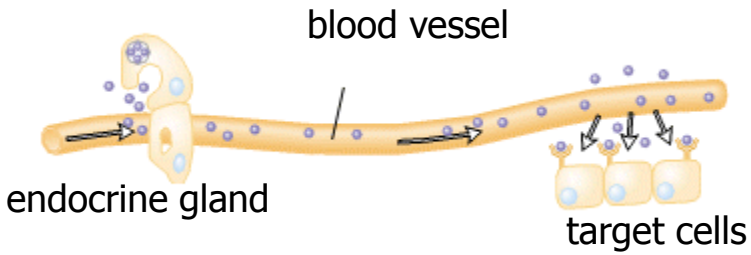
Signaltransduction Overview

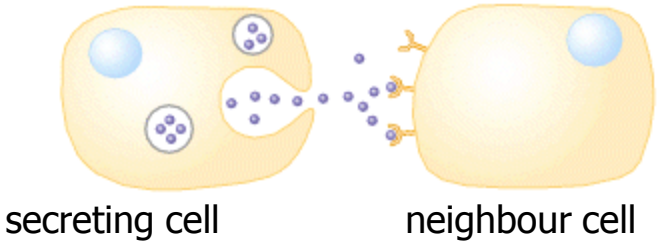
1. **synthesis of the mediator**: lipophilic: usually in the cytosol, peptide hormones or hydrophilic mediators usually in the ER
2. **release (secretion)**: lipophilic signaling molecules by diffusion through the membrane, hydrophilic by secretory granula
3. **transport to target cells**: a) via blood circulation
   b) by diffusion
4. **binding to specific receptors**: a) at the cell surface
   b) intracellularly (e.g. transcription factors such as Vitamin D3)
5. **activation of intracellular signal cascades**: in many cases via adaptor proteins, secundary mediators, signaling kinases…
6. **De-activation of the signaling** (and elimination of the mediator):
   a) Endocytosis and degradation of receptor and/or ligand
   b) Enzymatic inactivation of mediators and signaling molecules (e.g. dephosphorylation, hydrolysis of GTP...)

pathways of signaltransduction

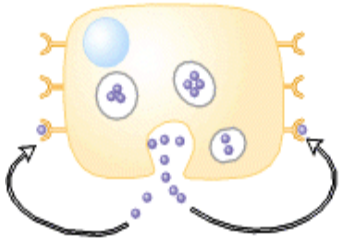
- endocrine:



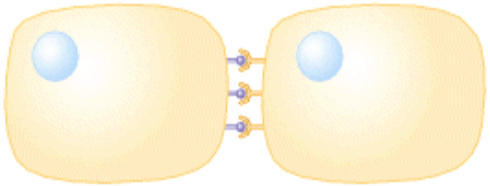
- paracrine:



- autocrine:



- direct cell contact:



Transmembrane Ser/Threonine Kinases: TGF-β signaling (transforming growth factor-beta)

- **Ligands**: TGF-β: large family of structurally related secreted dimers subgroups: TGF-β's themselves, activins, and bone morphogenetic proteins (BMPs). Ligands are usually secreted as pro-forms and have to be activated by cleavage.
- **Receptors**: Serine/Threonine kinases: types I and II (single-pass transmembrane proteins) Ligands bind to specific combinations of type I and II receptors – usually first on type II homodimer, which is then recruiting and phosphorylating a type I homodimer – which subsequently phosphorylates another signaling molecule of the Smad family (named after Sma in C. elegans and Mad in Drosophila). A phosphorylated R-Smad (receptor-Smad) dissociates from the receptor and binds to a co-Smad (e.g. Smad4)
- **R-Smad/Co-Smad complexes** then move into the nucleus, bind accessory proteins and transcription factors and activate TGF-β responsive genes

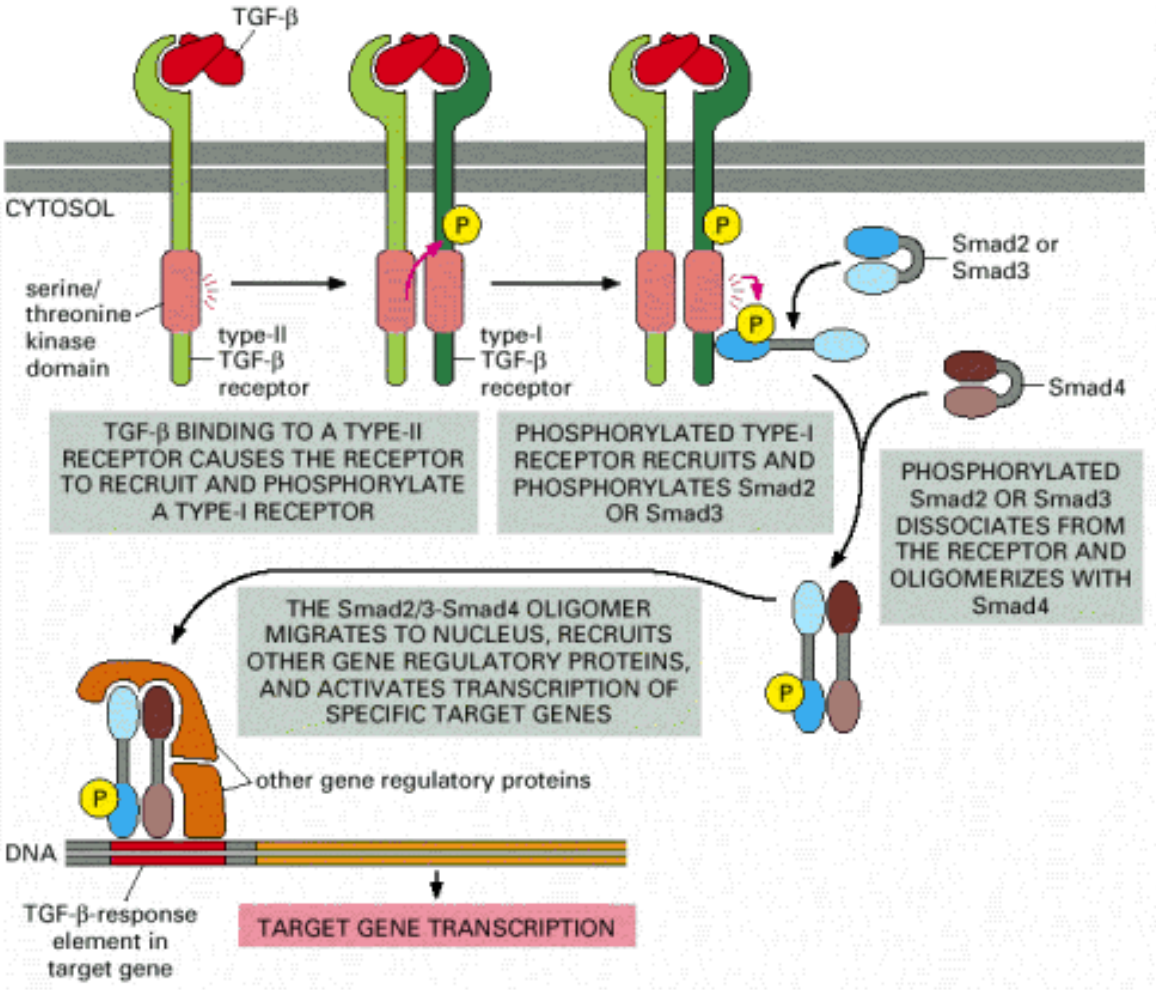
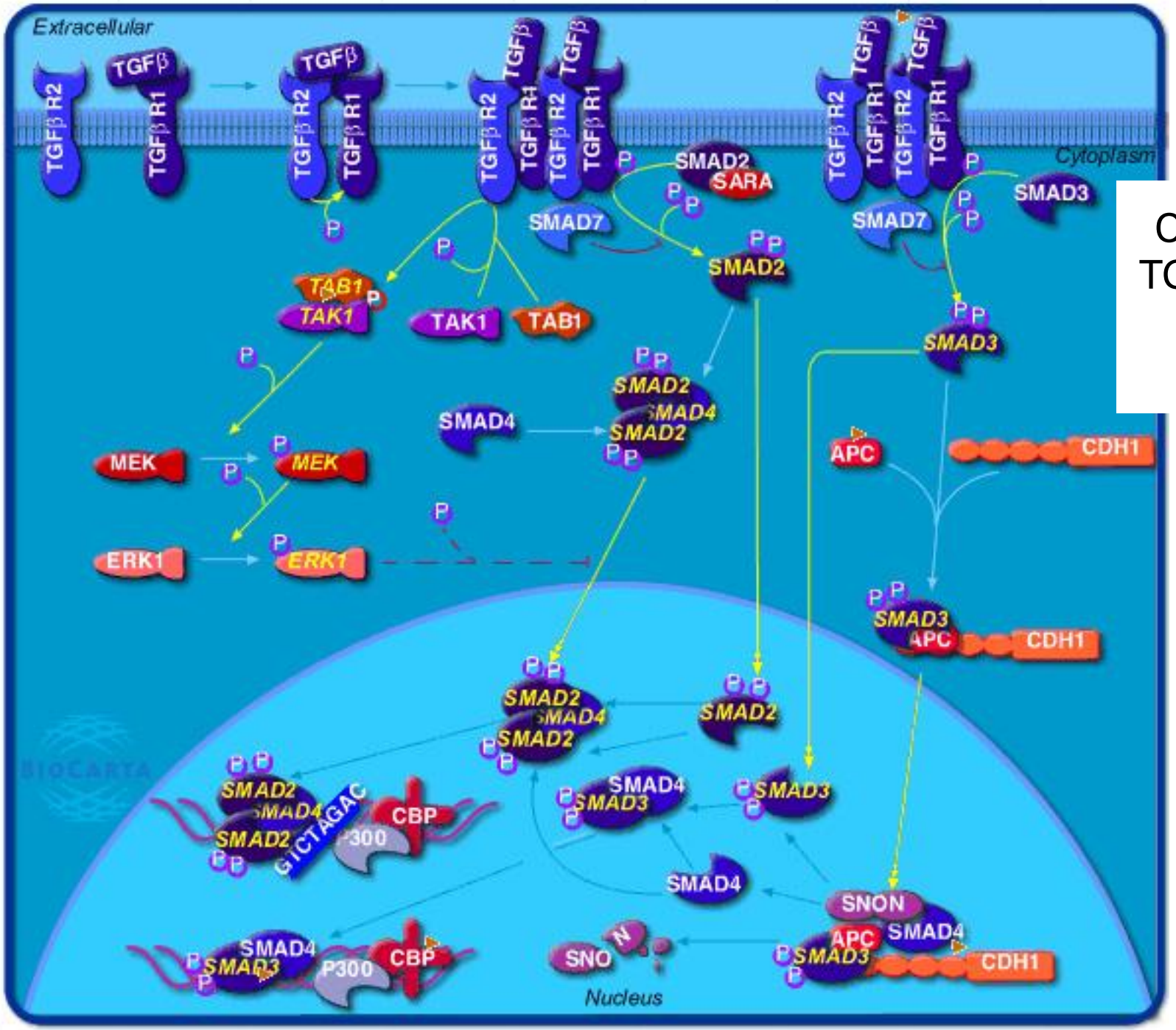
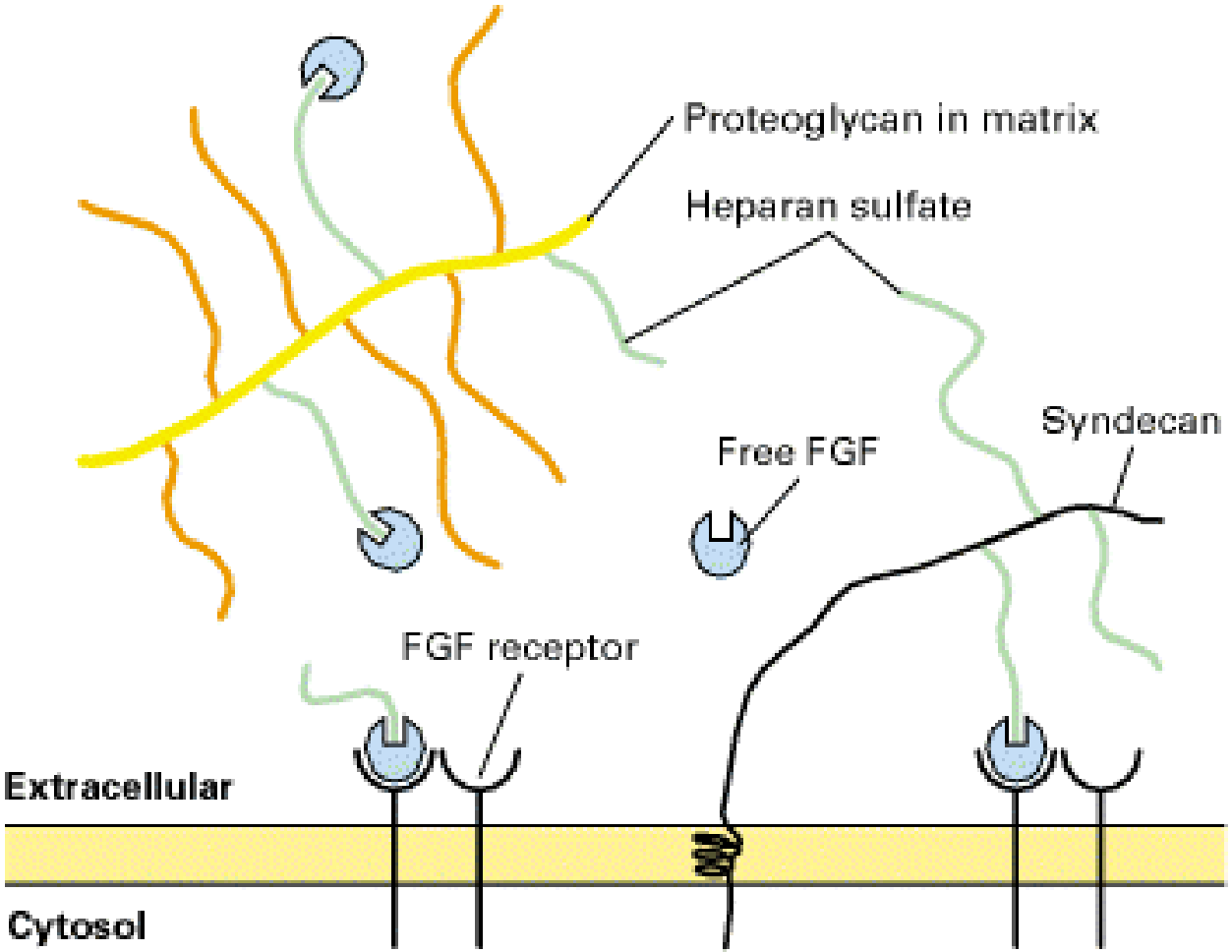


Figure 15-65. A model for the Smad-dependent signaling pathway activated by TGF-β. TGF-β is a dimer and Smads open up to expose a dimerization surface when they are phosphorylated. Several features of the pathway have been omitted for simplicity, including the following: (1) The type-I and type-II receptor proteins are both thought to be dimers. (2) The type-I receptors are normally associated with an inhibitory protein, which dissociates when the type-I receptor is phosphorylated by a type-II receptor. (3) The individual Smads are thought to be trimers. (4) An anchoring protein (called SARA, for *S*mad *a*nchor for *r*eceptor *a*ctivation) helps to recruit Smad2 or Smad3 to the activated type I receptor by binding to the receptor, to the Smad, and to inositol phospholipid molecules in the plasma membrane. (5) The function of certain Smads is regulated by enzymes that enhance their ubiquitylation and thereby their degradation.



Overview of TGFβ induced signaling pathways

Proteoglycanes in the extracellular matrix bind TGF-β and growth factors (like FGF)



presentation of ligands

storage of ligands

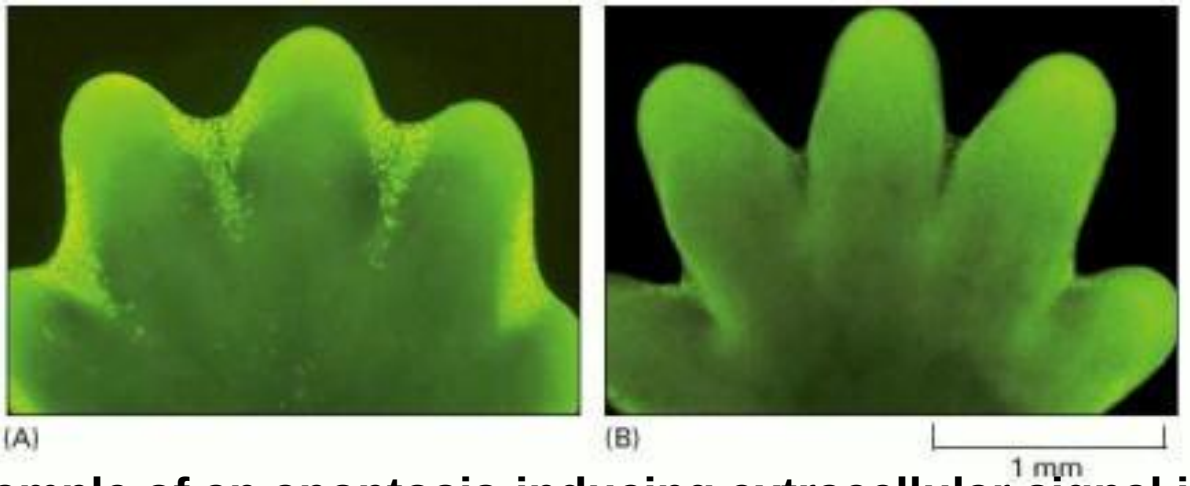
Targets and functions of TGF/Smad signaling

- Smads are transcriptional activators of Cdk inhibitors > inhibiting cell growth
- Smads can act as transcriptional repressors (e.g. of oncogenes such as Myc)
- Some TGF-β family members serve as graded morphogens during development, inducing different responses in a developing cell depending on their concentration

Feedback mechanisms

- Inhibitory Smads: Smad6 and Smad7: bind to activated type I receptors and prevent binding of other Smads
- The production of inhibitory Smads can be induced by other signaling pathways e.g. the interferon γ-pathway via activation of the Jak/STAT pathway and active STAT dimers
- extracellular inhibitors: bind to TGF-β and inactivate it (e.g. Noggin and chordin inhibit BMPs, and follistatin inhibits activins)

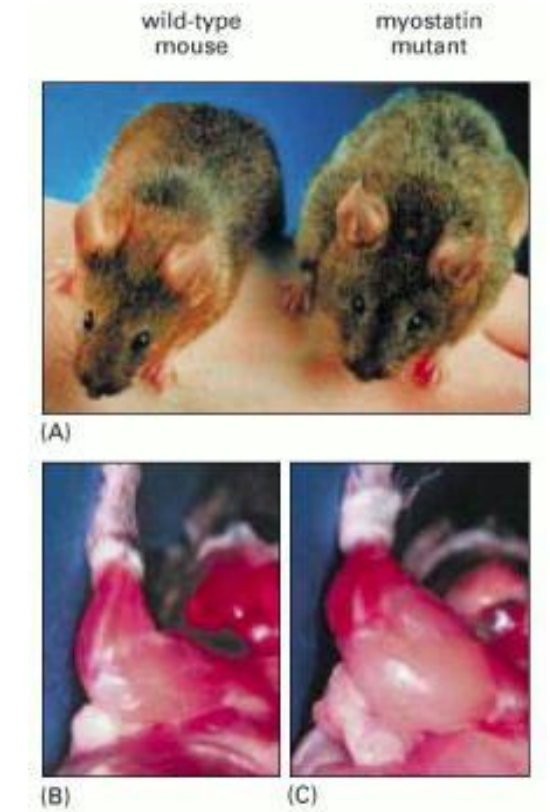
BMP's in the regulation of development through apoptosis



One example of an apoptosis-inducing extracellular signal is bone morphogenetic protein (BMP), a TGF-β family member. BMP helps trigger the apoptosis that removes the tissue between the developing digits in the mouse paw

Sculpting the digits in the developing mouse paw by apoptosis. (A) The paw in this mouse embryo has been stained with a dye that specifically labels cells that have undergone apoptosis. The apoptotic cells appear as bright green dots between the developing digits. (B) This interdigital cell death eliminates the tissue between the developing digits, as seen one day later, when few, if any, apoptotic cells can be seen. (From W. Wood et al., Development 127:52455252, 2000. © The Company of Biologists.)

The TGFβ family member Myostatin in muscle growth control



The overall size of an organ may be limited in some cases by inhibitory signaling proteins. Myostatin, for example, is a TGF-β family member that normally inhibits the proliferation of myoblasts that fuse to form skeletal muscle cells. Its function, evidently, is to provide negative feedback to limit muscle growth When the gene that encodes myostatin is deleted in mice, muscles grow to be several times larger than normal. Both the number and the size of muscle cells increase.

Remarkably, two breeds of cattle that were bred for large muscles have both turned out to have mutations in the gene encoding myostatin

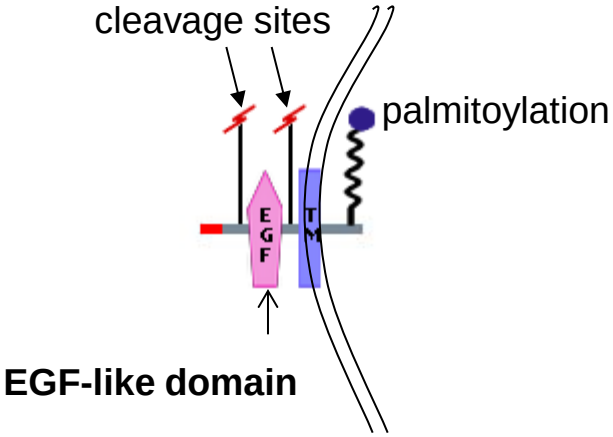
TGF-β in cancer

Loss of growth inhibition through TGFβ -mediated pathways contributes to the genesis of several types of human cancers. The receptor TGFβ-RII is found to be mutated in some cancers of the colon and Smad4, a key intracellular signal transducer in the pathway is inactivated in cancers of the pancreas and some other tissues.

Colorectal cancer GENE	CLASS	PATHWAY AFFECTED	TUMORS WITH MUTATIONS (%)
K-Ras	**oncogene**	**receptor tyrosine-kinase signaling**	**40**
β-catenin	**oncogene**	**Wnt signaling**	**5 - 10⁻**
p53	**tumor suppressor**	**stress/genetic-damage response**	**60**
APC	**tumor suppressor**	**Wnt signaling**	**> 60**
Smad4	**tumor suppressor**	**TGFβ signaling**	**30**
TGFβ receptor II	**tumor suppressor**	**TGFβ signaling**	**10**
***MLH1* and other DNA**	**tumor suppressor**	**DNA mismatch repair**	**15**

TGF-alpha in cancer

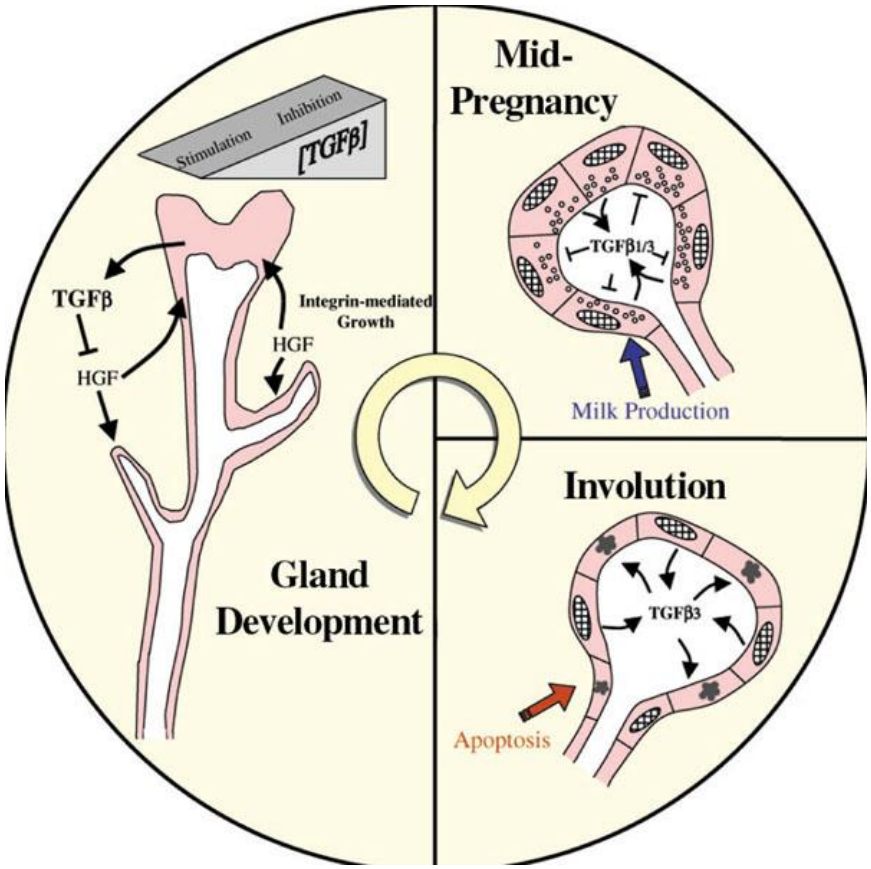
- In contrast to TGF-β, TGF-α (Transforming Growth Factor alpha) acts as an oncogene
- TGF-α is synthesized as a transmembrane protein, from which the extracellular domain can be cleaved – acting then as extracellular cytokine, similar to a growth factor
- TGF-α has a domain homologous to EGF and can bind to EGF-receptor and activate it > activation of cell proliferation



TGF-β in bone remodelling

- Through remodelling, bones are endowed with a remarkable ability to adjust their structure in response to long-term variations in the load imposed on them. This adaptive behavior implies that deposition and erosion of the matrix are somehow controlled by local mechanical stresses. The bone cells secrete signal proteins that become trapped in the matrix, which can then be released when the matrix is degraded or suitably stressed. The released proteins, especially members of the BMP subfamily of TGFβ proteins, apparently help to guide the remodelling process.

TGFβ in mammary gland control

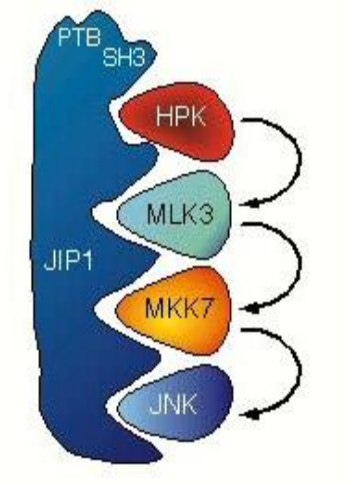


- When a baby is weaned and suckling stops, secretory cells of the mammary gland die by apoptosis, and most of the alveoli disappear. Macrophages rapidly clear away the dead cells, and the gland reverts to its resting state. This ending of lactation is abrupt and, unlike the events that lead up to it, seems to be induced by the accumulation of milk, rather than by a hormonal mechanism. If one subset of mammary ducts is obstructed so that no milk can be discharged, the secretory cells that supply it commit mass suicide by apoptosis, while other regions of the gland survive and continue to function. The apoptosis is triggered by a combination of factors including $TGF\beta_3$, which accumulates where milk secretion is blocked

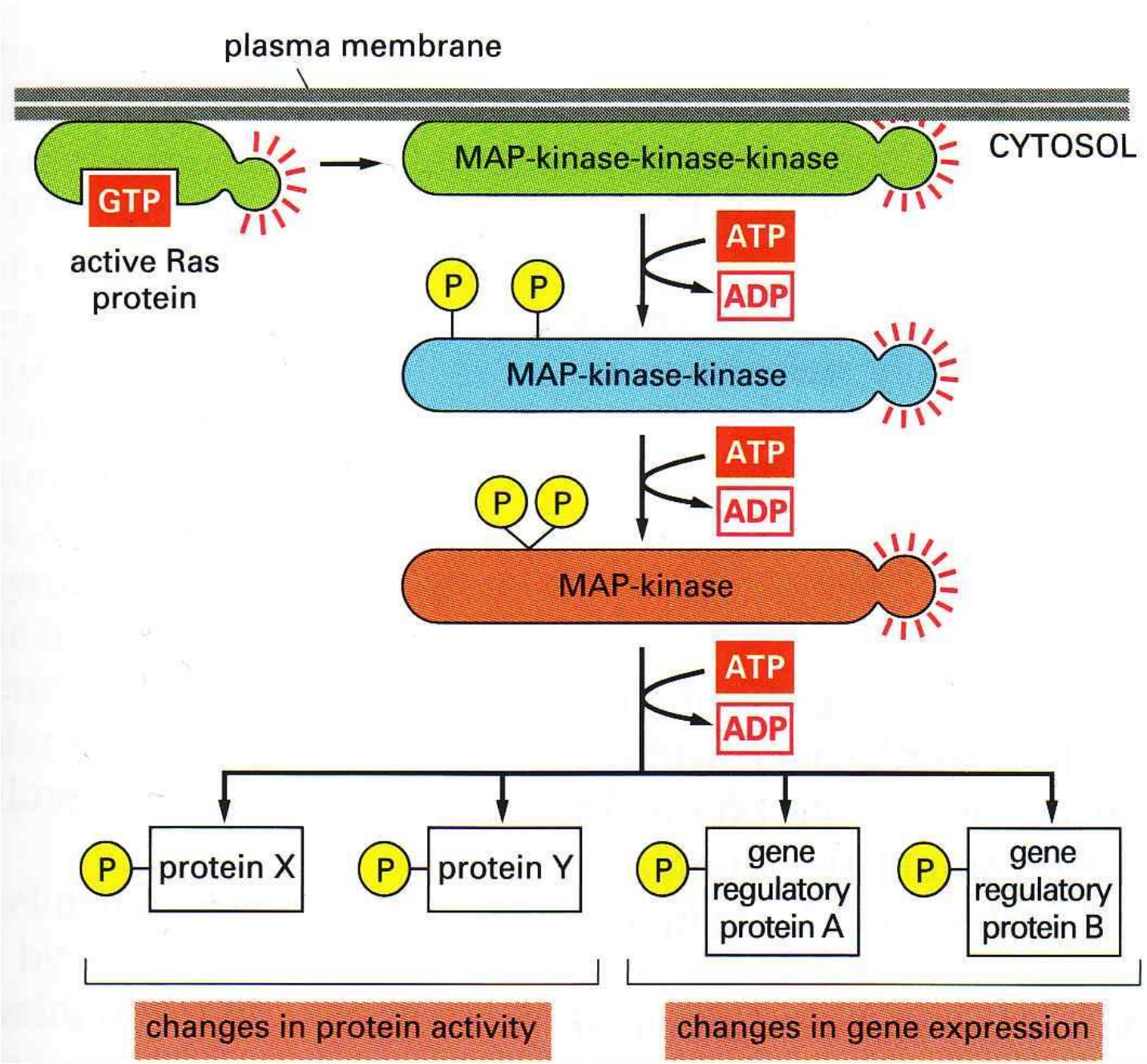
Paul Klee: Hauptwege und Nebenwege

Non-transmembrane serine/threonine-kinases

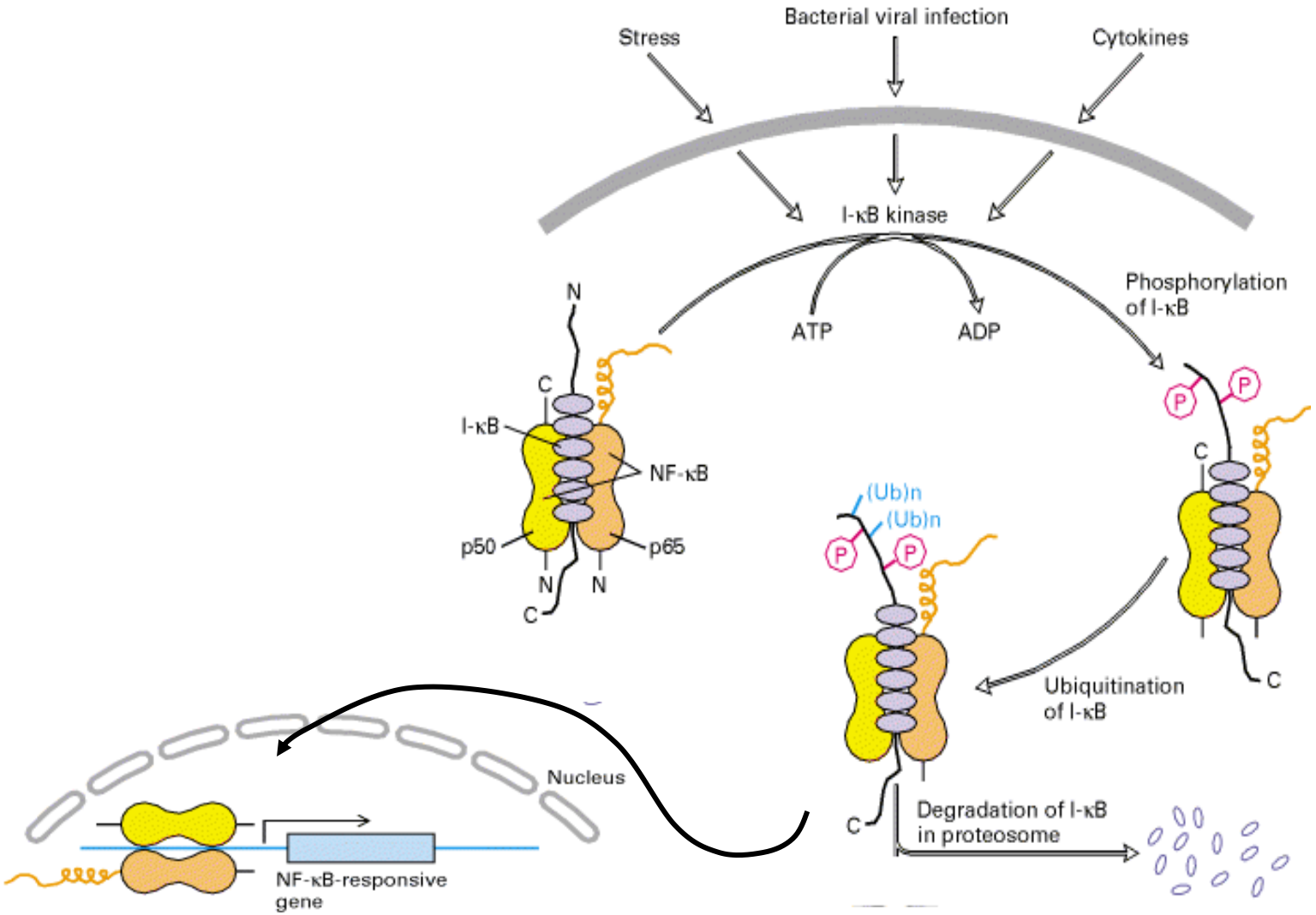
„Scaffold proteins“ bring various kinases in close proximity



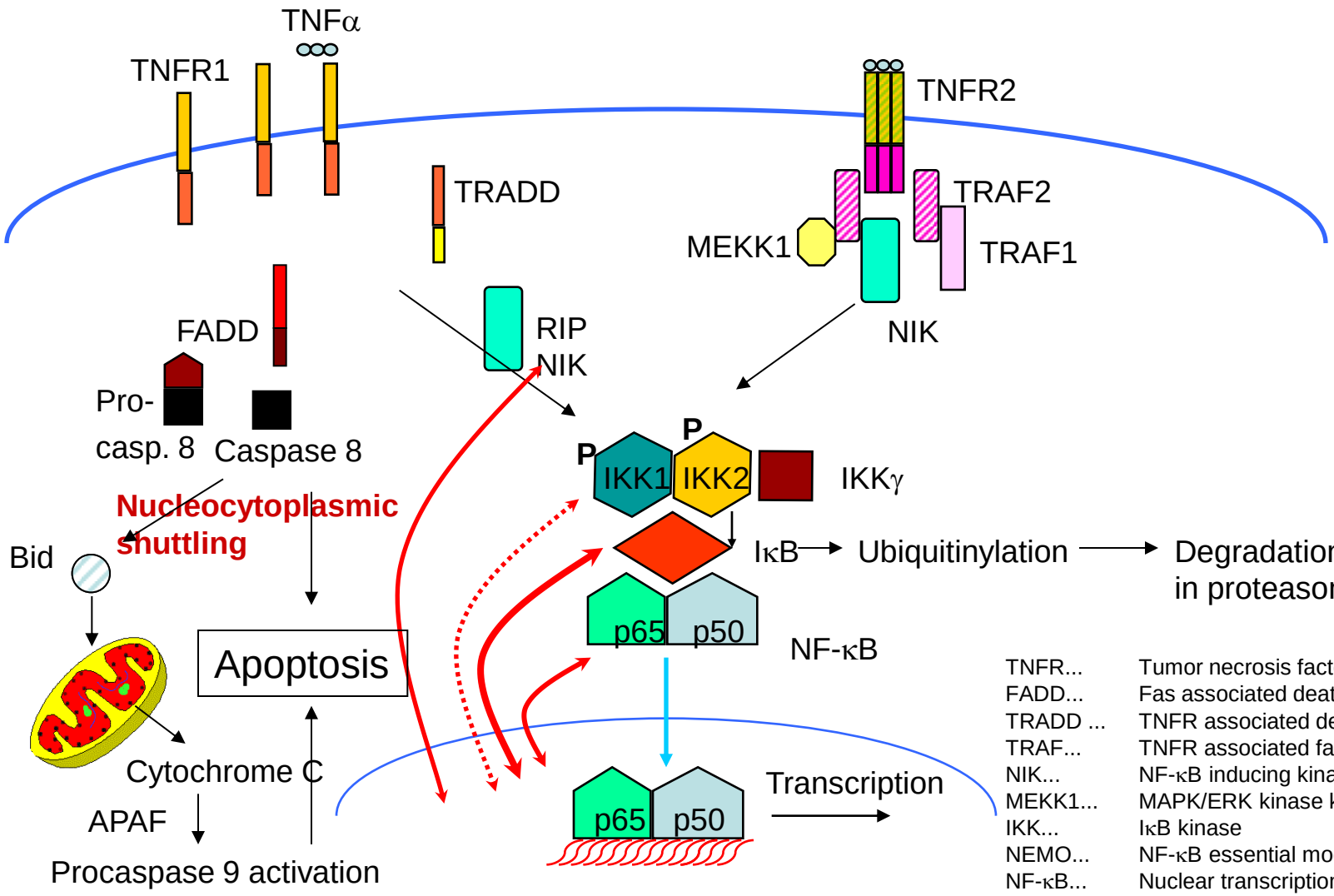
Activation of MAP Kinases



Serine/threonine kinases in other signaling pathways: The NF-κB signaling pathway

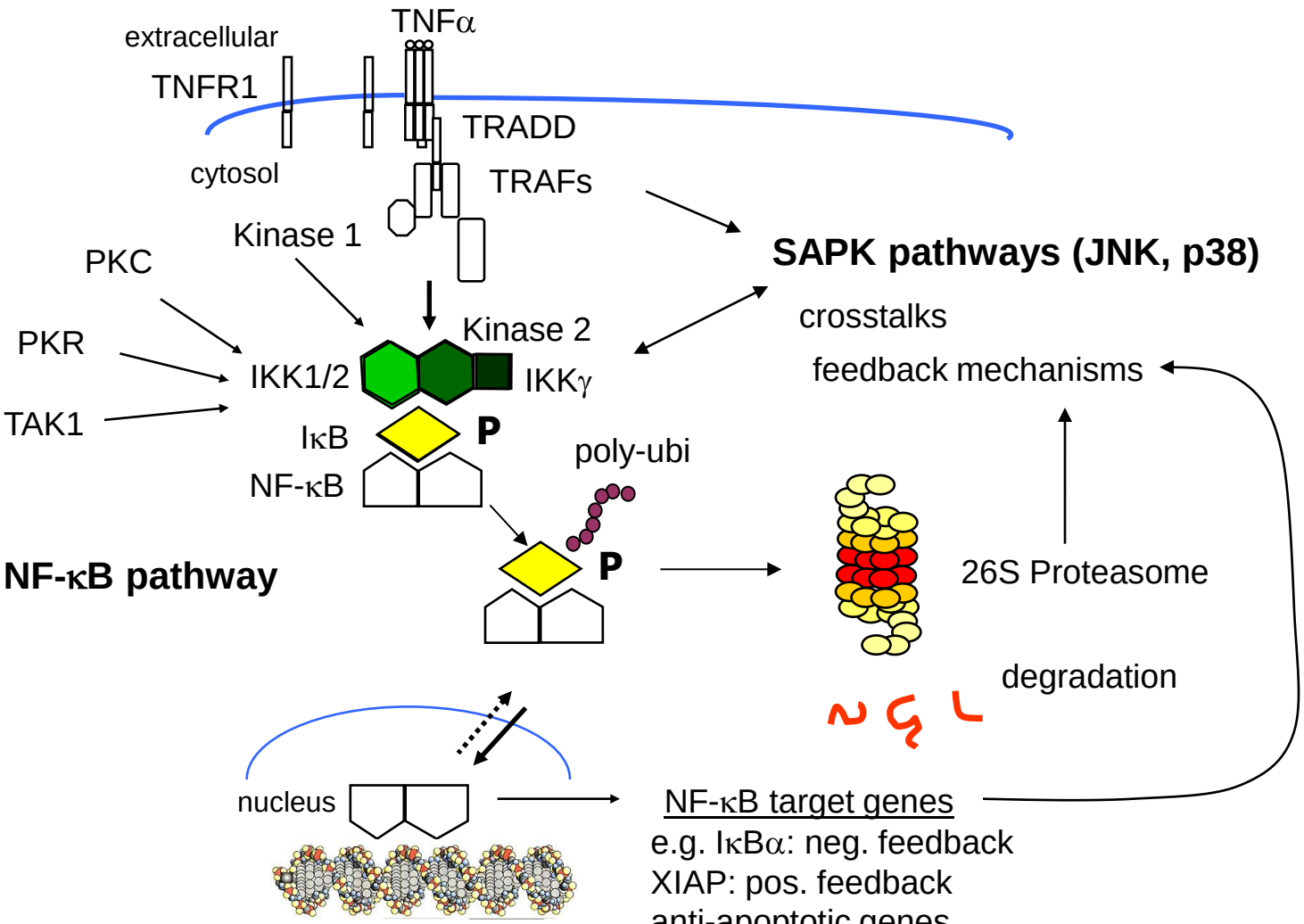


Dynamic view of the NF-κB signaling pathway and its connection to the apoptosis pathway

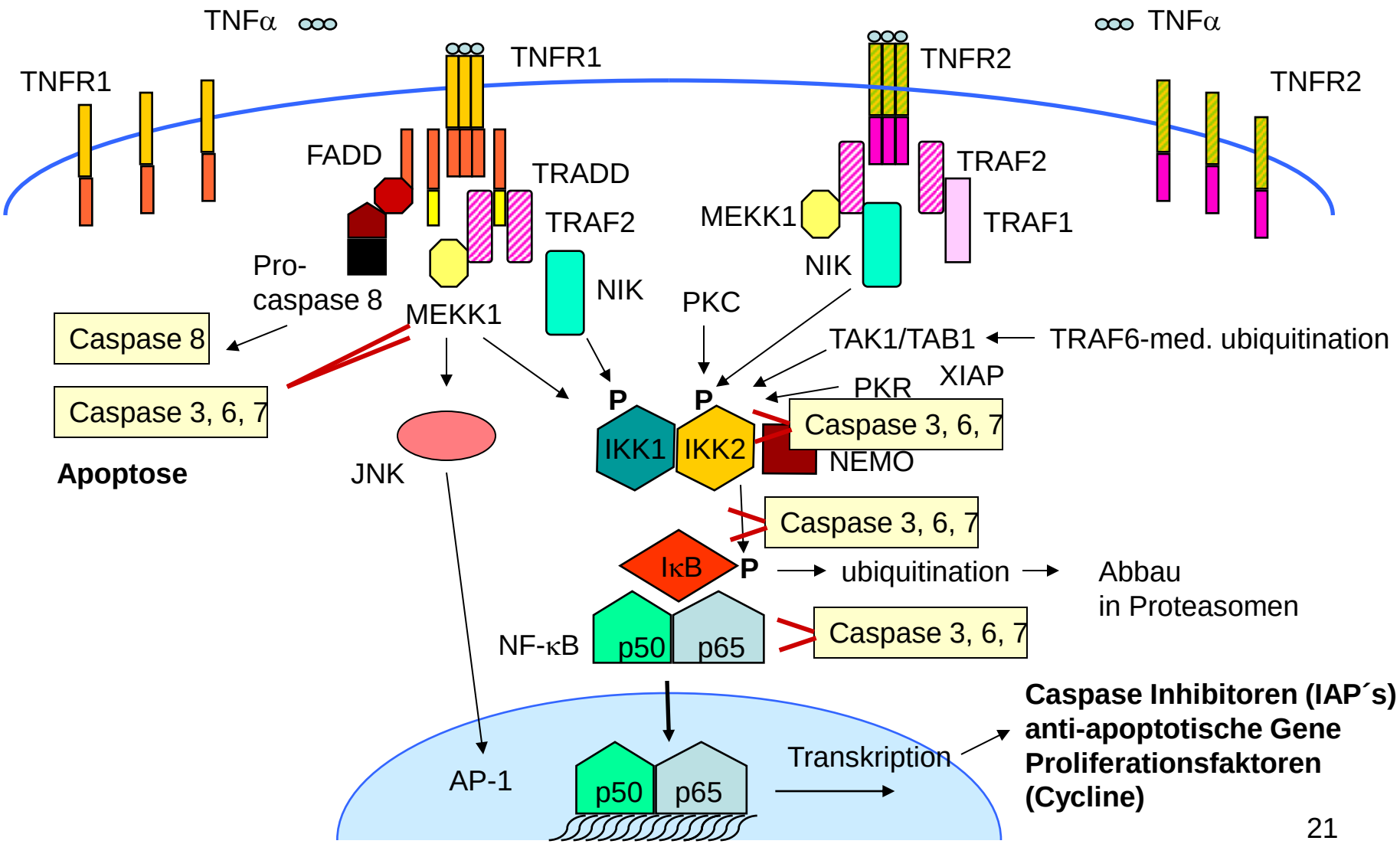


TNFR...	Tumor necrosis factor receptor
FADD...	Fas associated death domain protein
TRADD ...	TNFR associated death domain protein
TRAF...	TNFR associated factor
NIK...	NF-κB inducing kinase
MEKK1...	MAPK/ERK kinase kinase 1
IKK...	IκB kinase
NEMO...	NF-κB essential modulator
NF-κB...	Nuclear transcription factor κB
IκB...	Inhibitor of NF-κB

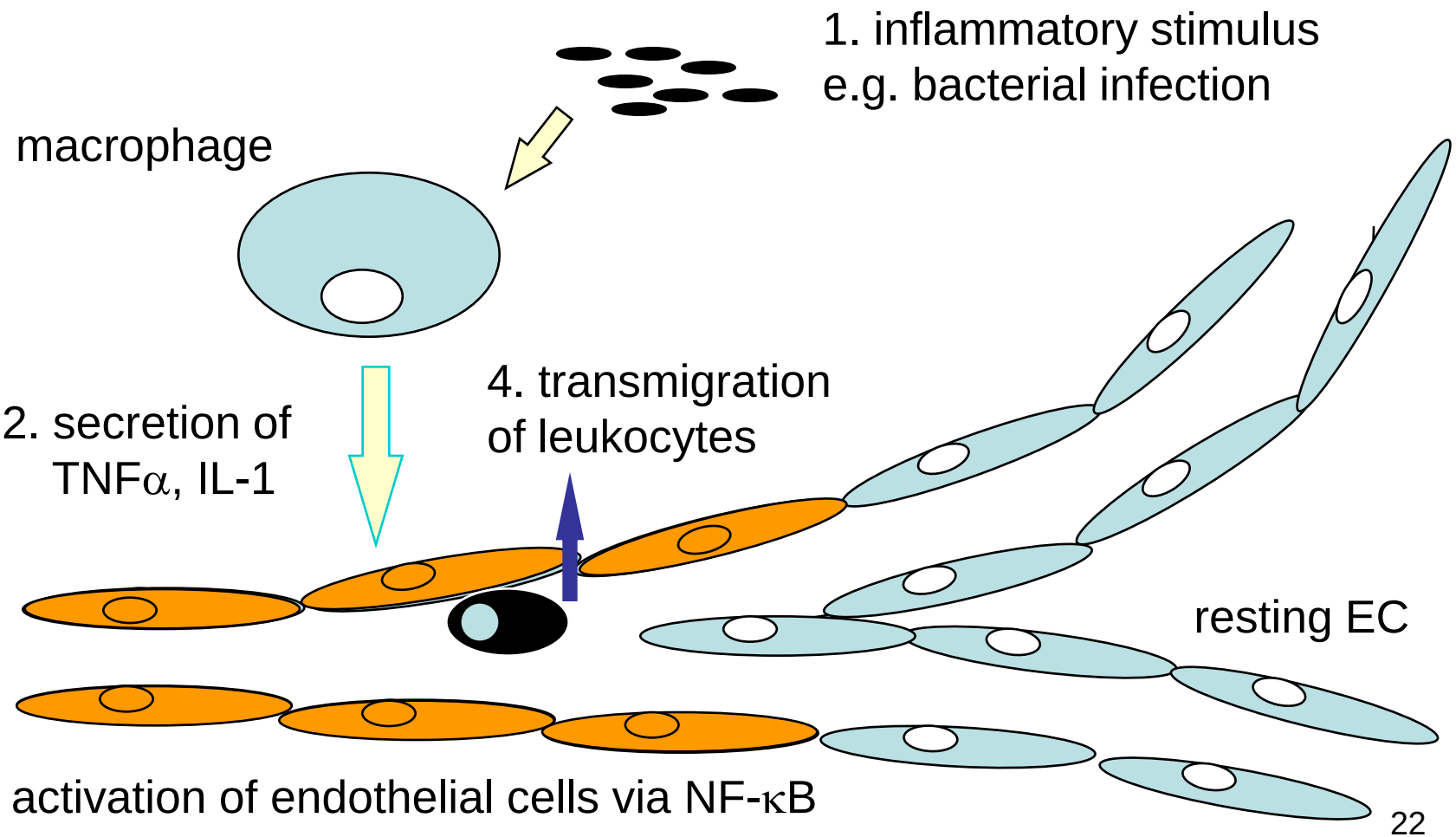
NF-κB signaling: crosstalks and feedbacks



Balance between apoptosis and survival signals



The role of NF-κB in inflammation



Cellular Signal Transduction WS 2014

Cytoskeleton and Cell Migration

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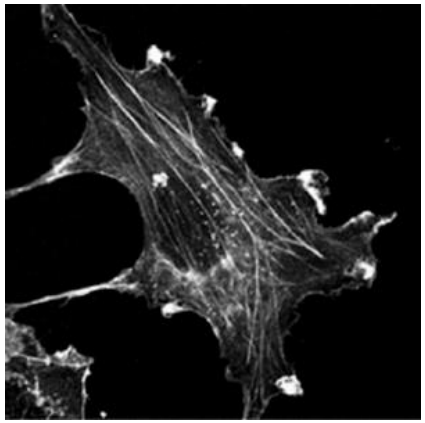
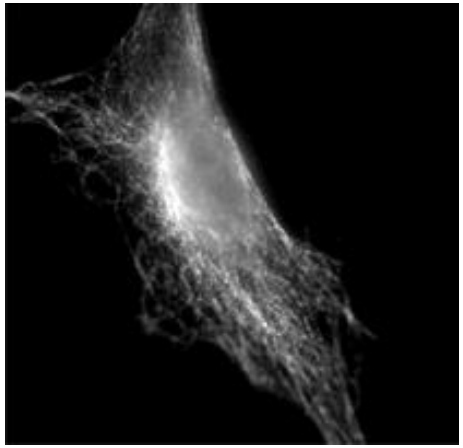
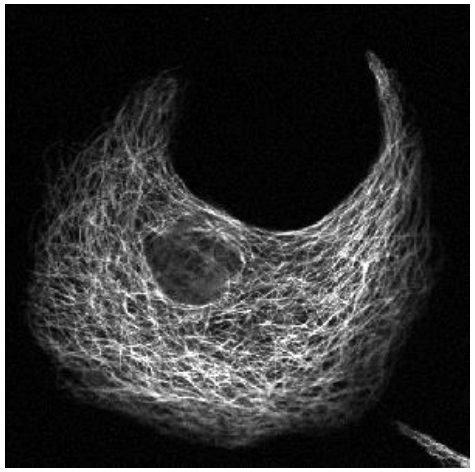
The Cytoskeleton

Microfilaments
(Actin filaments)



Microtubules





Intermediate Filaments

Actin Filaments

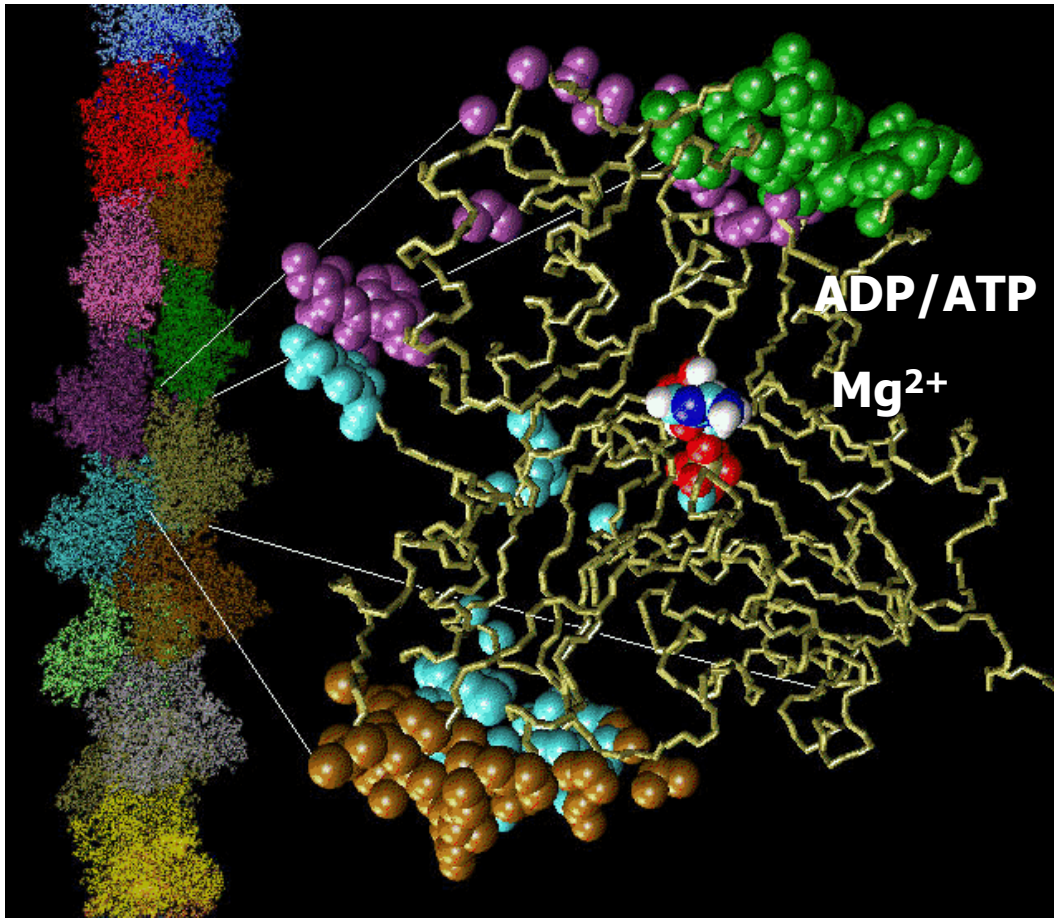
Composition and Structure:

Actin molecules (6 isoforms: α1-α4 in muscle, β- and γ-Actin in non-muscle cells)

G-Actin: globular, monomer (app. 40 kD)

Polymerisation to F-Actin (Ion-dependent): filamentous actin

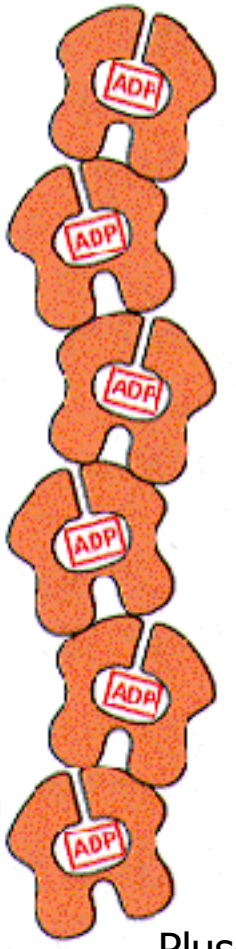
Actin content in non-muscle cells: 1 – 5 % (0.5 mM), in muscle: 10% of the proteins



7-9 nm

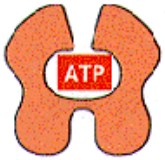
Polarity and Crosslinking of Actin Filaments

Minus-End



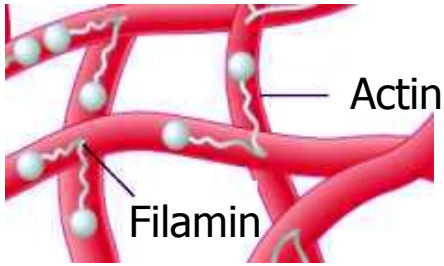
Plus-End

Polymer:
preferentially
in ADP-Form



Monomer:
preferentially in
ATP-Form

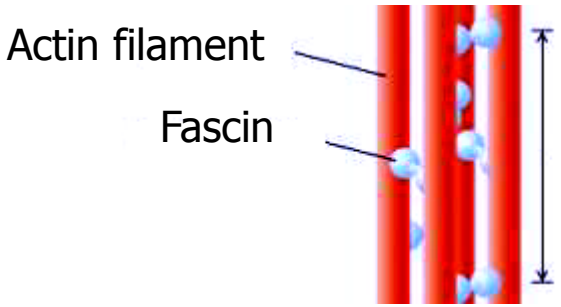
Network-like Crosslinking:



Crosslinking proteins:

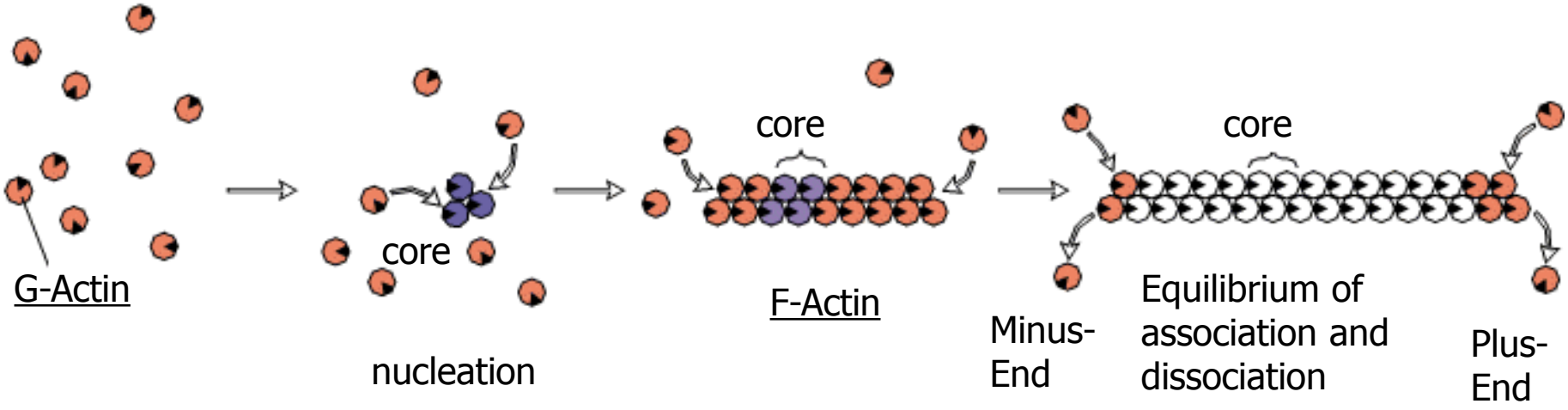
Filamin
Fascin
Villin
Spectrin
α-Actinin
Dystrophin

Filamentous Crosslinking



network-like or filamentous crosslinking determine the mechanic properties

Dynamics of actin filaments

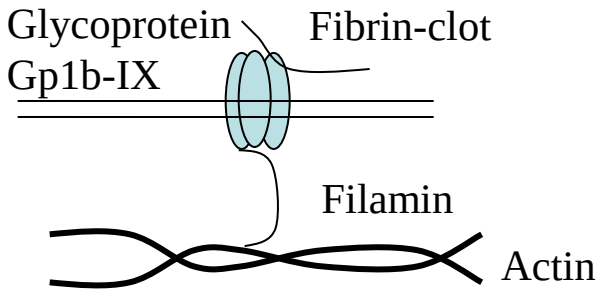


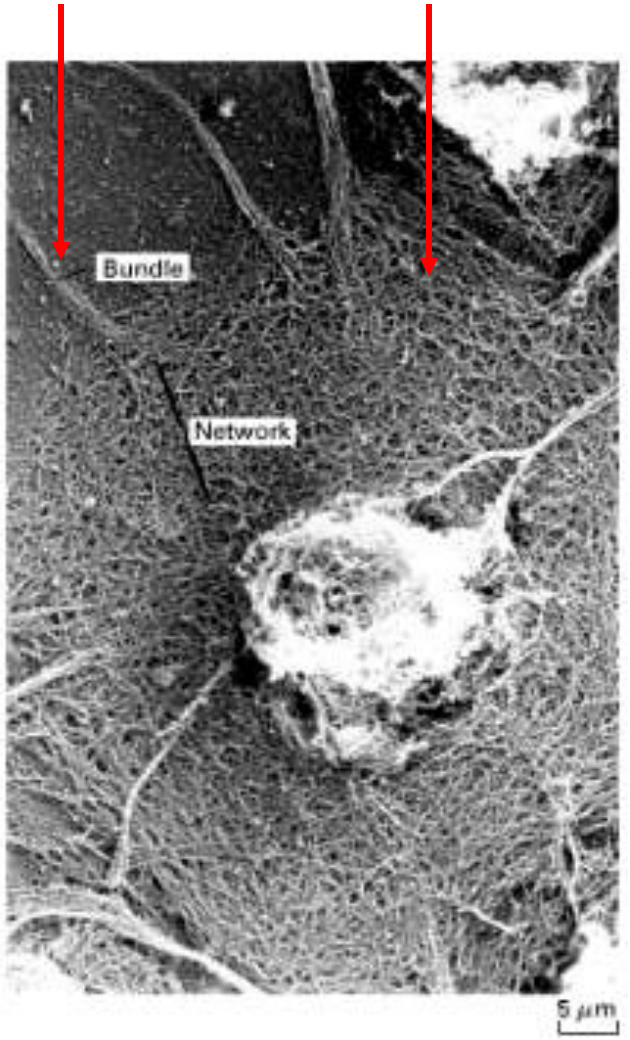
Growth (polymerisation) is 5 – 10x faster at the Plus-end

Capping proteins regulate association and dissociation of actin at the ends

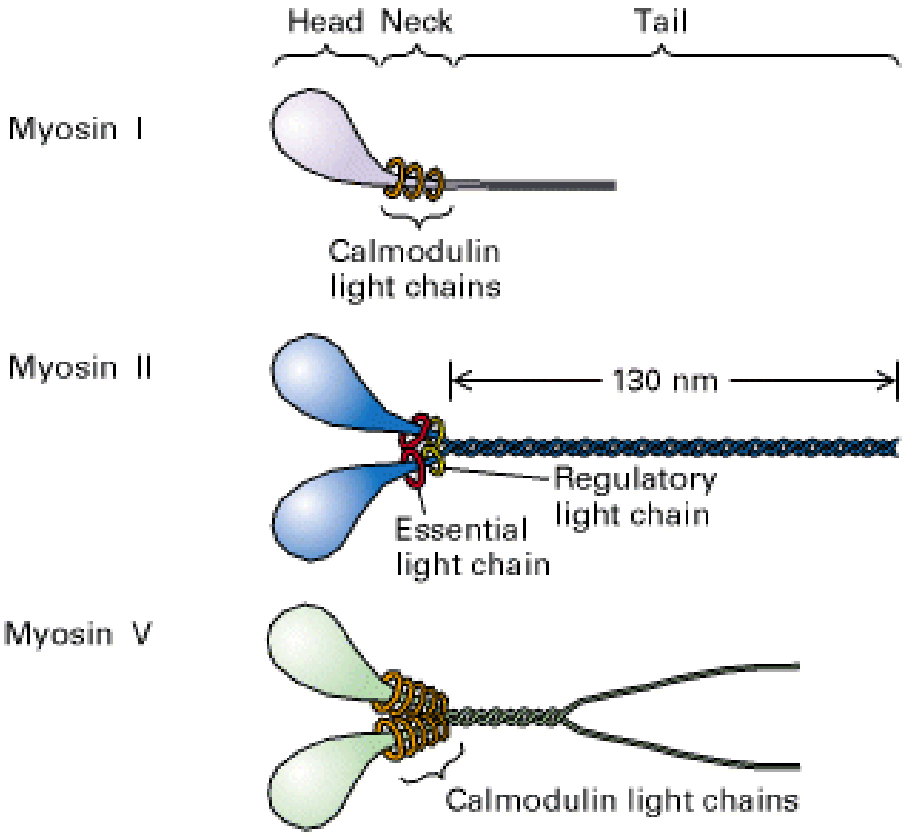
Filament Structures: Bundles and Networks

<u>Microfilaments of a platelet (thrombocyte)</u>
Role in blood coagulation: Stabilizing the thrombus via crosslinking of the intracellular cytoskeleton with the extracellular bood clot





Motor Proteins of Actin Filaments: Myosines

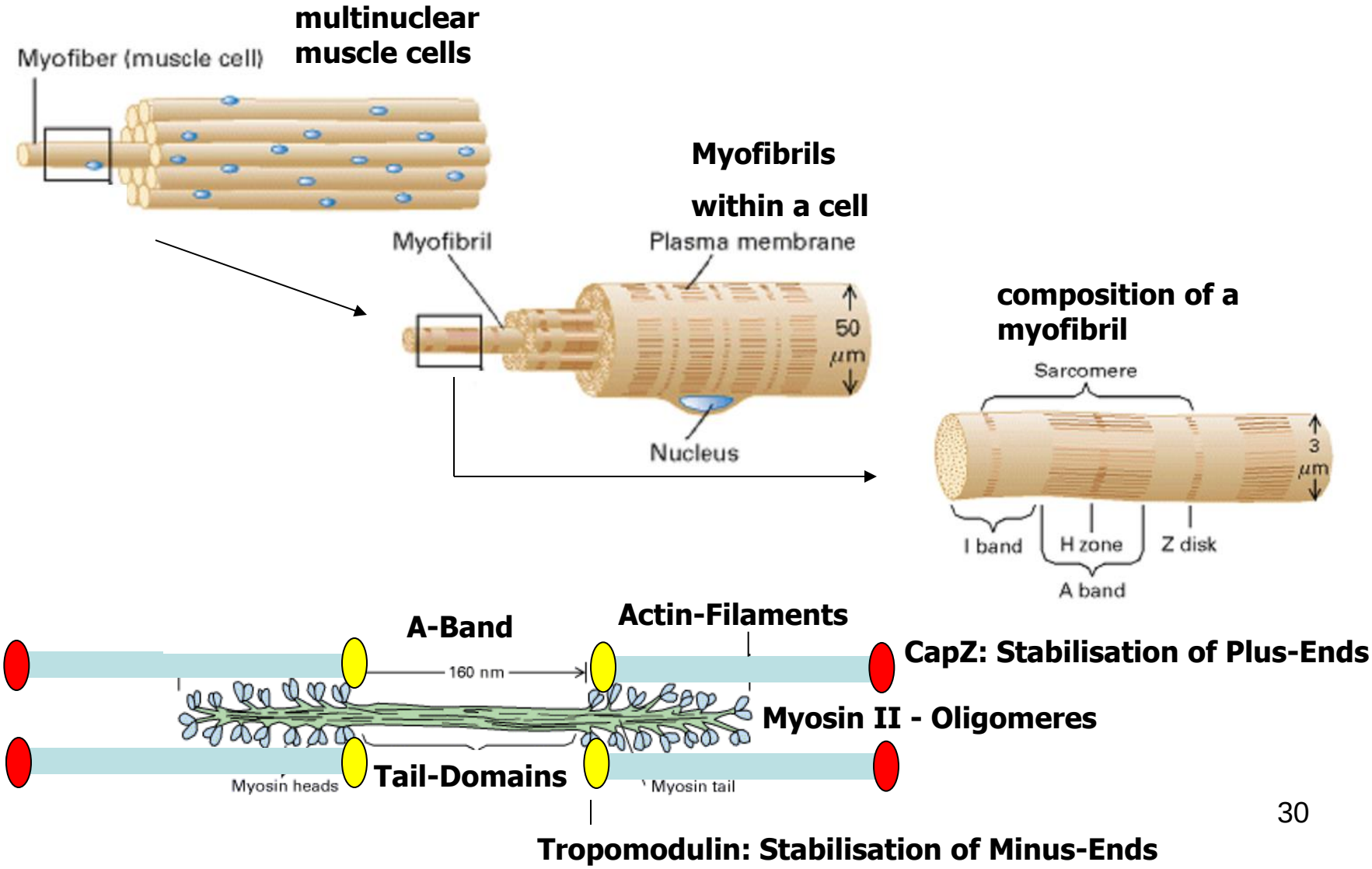


…mechano-chemical <u>ATPases</u>, converting the chemical energy of ATP into a conformational change (movement).

3 domains:
<u>Head</u>: Actin-binding, ATPase-Activity
<u>Neck</u>: Binding of regulatory light chains
<u>Tail</u>: specific binding sites dependent on function

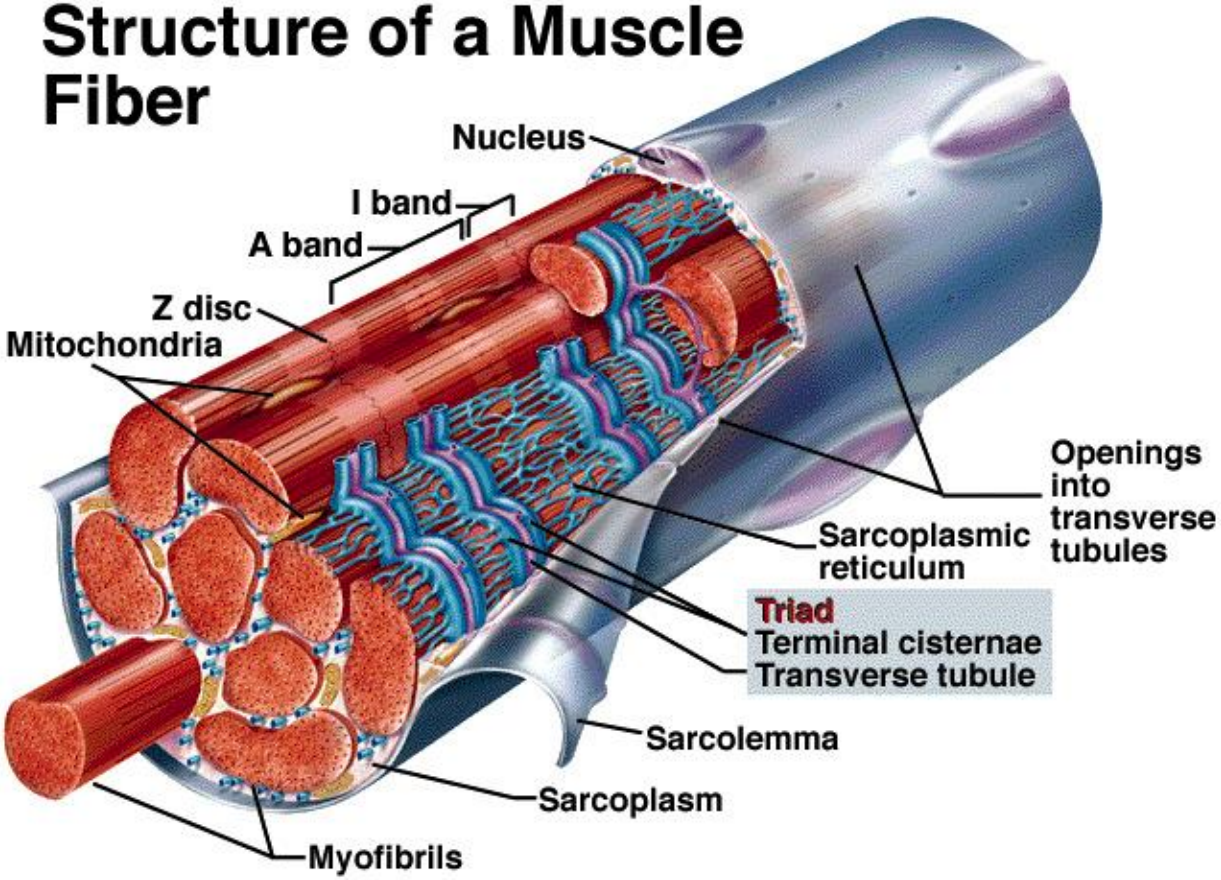
<u>Myosin I and Myosin V</u> are involved ininteractions between actin filaments and membranes (cytoplasmic membran or membrane vesicles) – and have functions in cell migration and in vesicular transport
<u>Myosin II</u> is important for muscle contraction and cell division (cytokinesis)

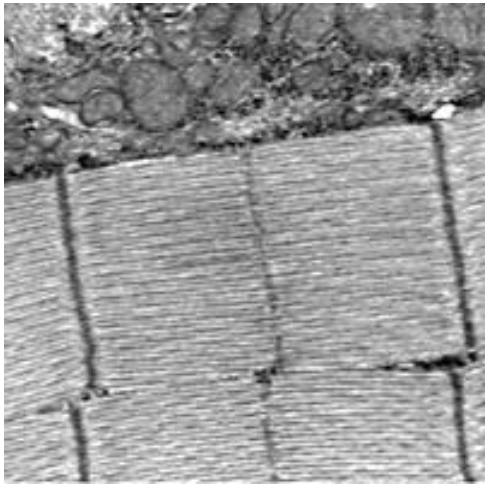
Actin Filaments in Striated Muscle



General Structure of Striated Muscle

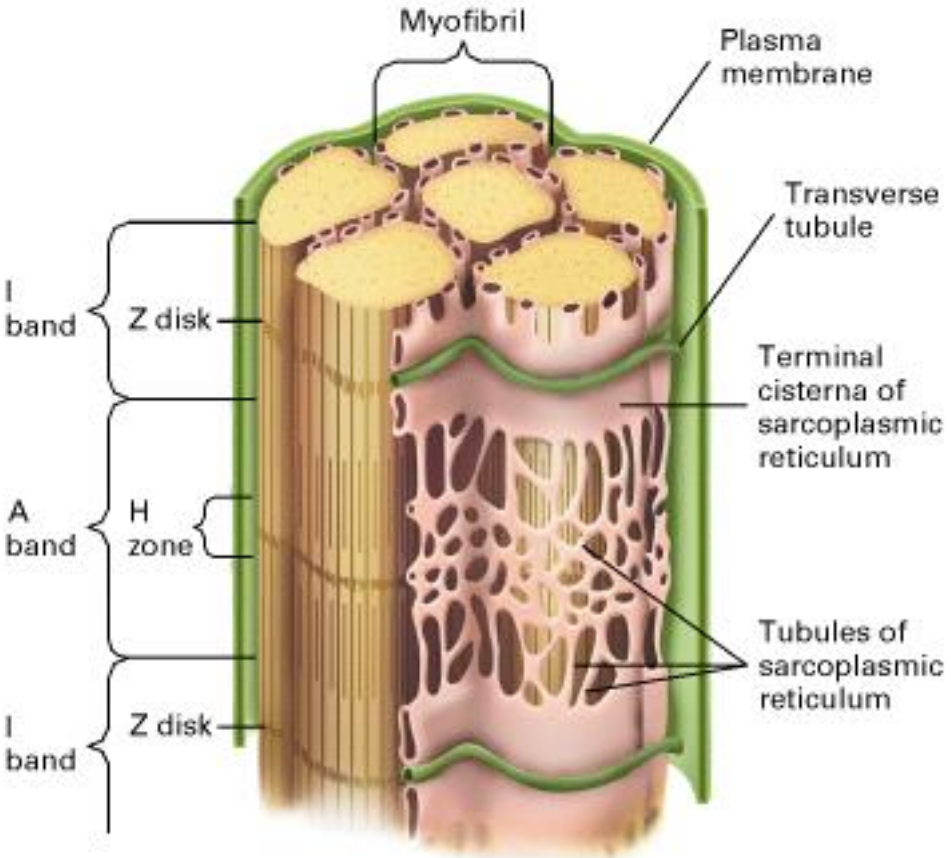
Muscle cells bild a syncytium (by fusion of several mononuclear cells)

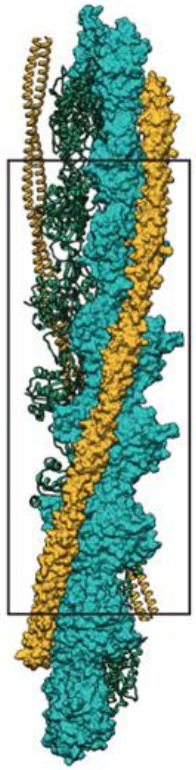


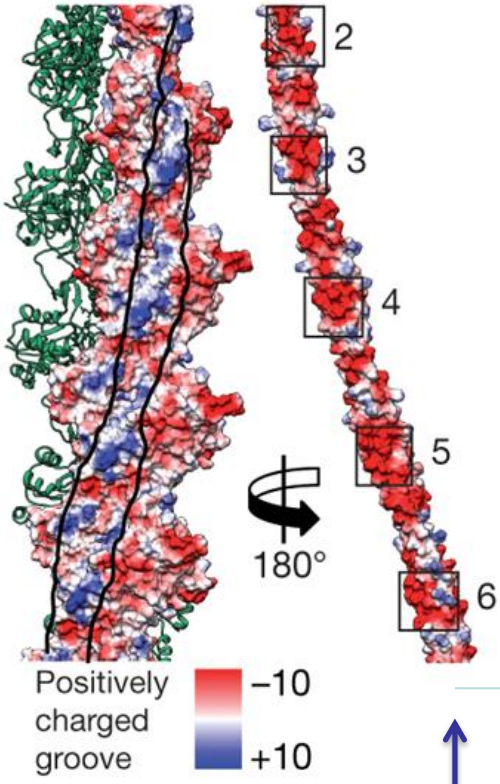


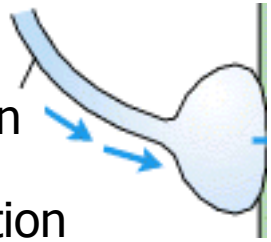
Regulation of the Muscle Contraction

J von der Ecken *et al. Nature* (2014)
doi:10.1038/nature14033







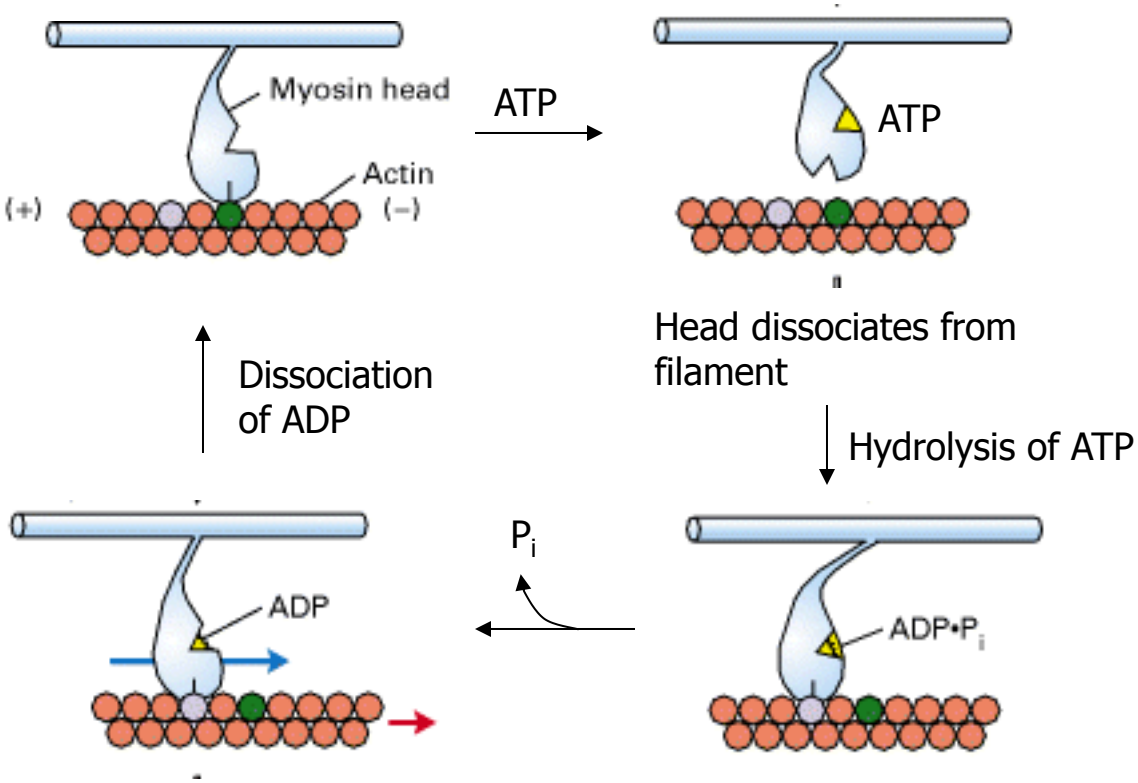


Depolarisation

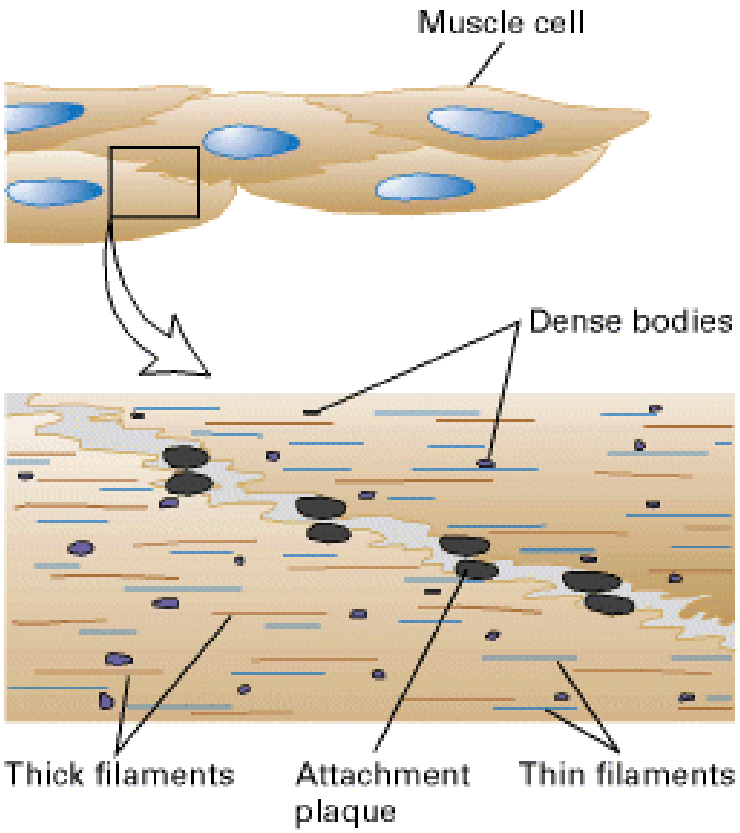
Ca^{2+}-release from Reticulum

Dislocation of Tropomyosin and unmasking of Myosin binding sites

Molecular Mechanism of the Muscle Contraction



General Structure of Smooth Muscles

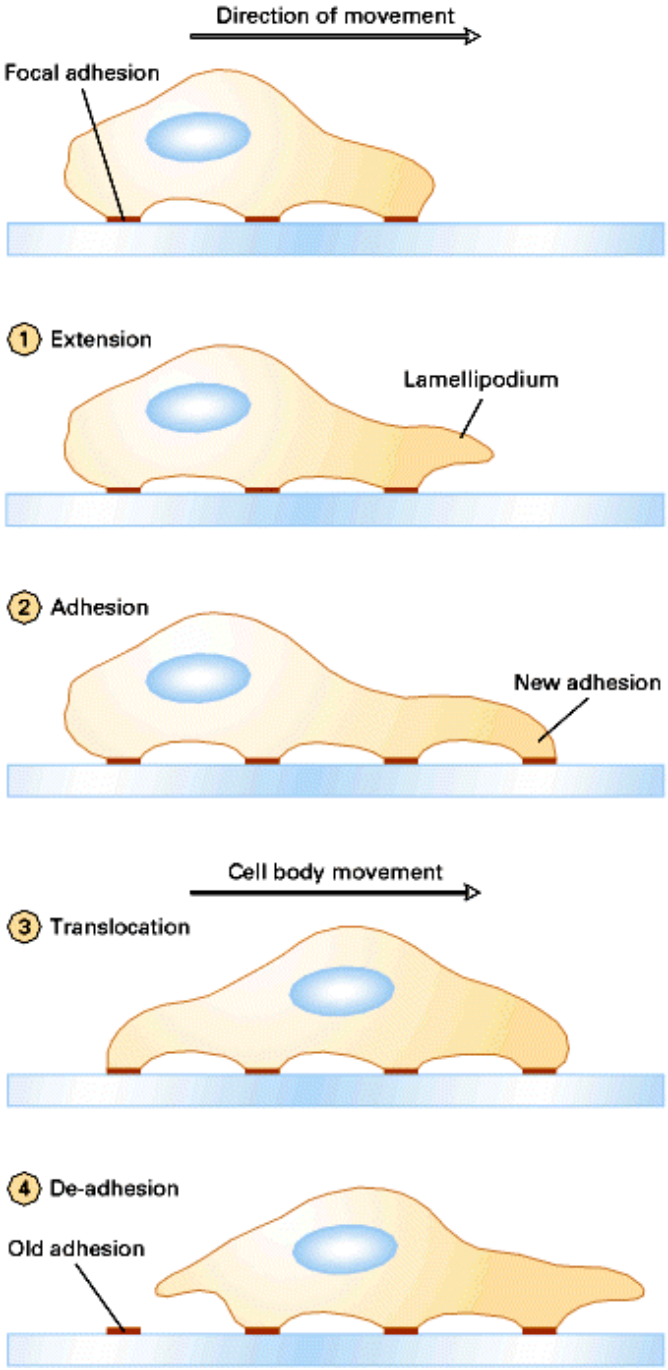


Mononuclear cells contain actin-myosin filaments, which are anchored to protein complexes (dense bodies) within the cell and also to adhesion sites of the cell membrane (adhesion plaques).

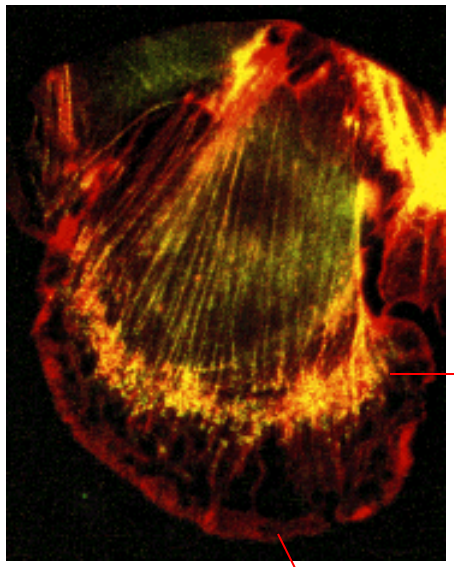
Myosin-Actin-mediated contraction leads to transmission of mechanical force via these anchoring points. Smooth muscles can transmit less maximum force but are more persistent than striated muscles, because of better energy supply (more mitochondria per volume unit)

Functions of Actin in Non-Muscle Cells

- Cellular Movements, cell migration
- Cell adhesion: Actin and Myosin II (e.g. adhesion belts in epithelial cells)
  Stress fibers are filaments, which are linked with the cell surface at adhesion sites (adhesion plaques)
- Actin and Myosin II are essential for the physical separation of daughter cells after cell division (Cytokinesis)
- Transport of vesicles along actin filaments (Myosin I und V)



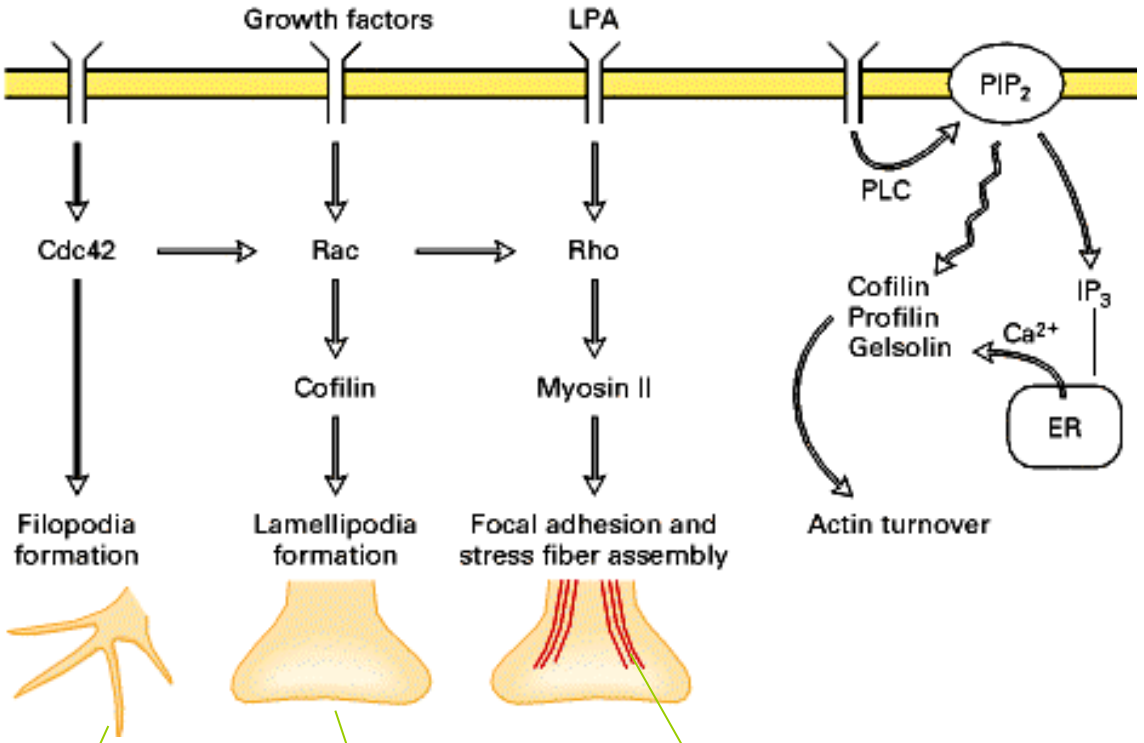
Cell Migration

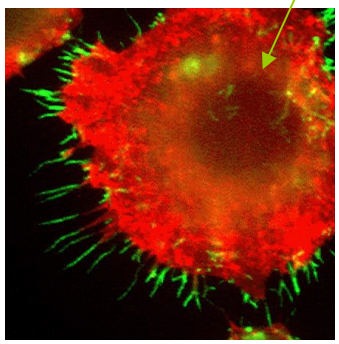


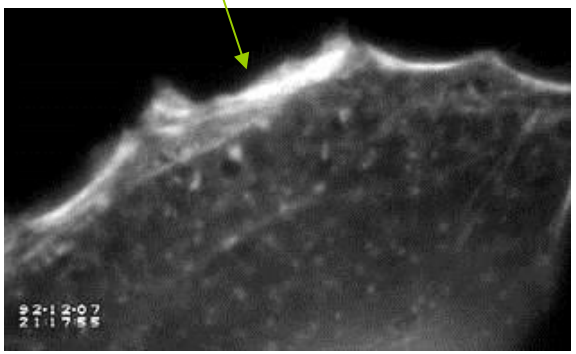
Myosin II-Band

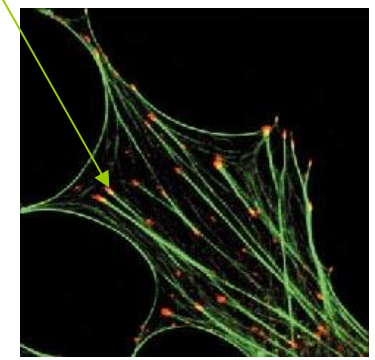
Actin-Front (also contains Myosin I)

Siganling pathways influence actin filaments



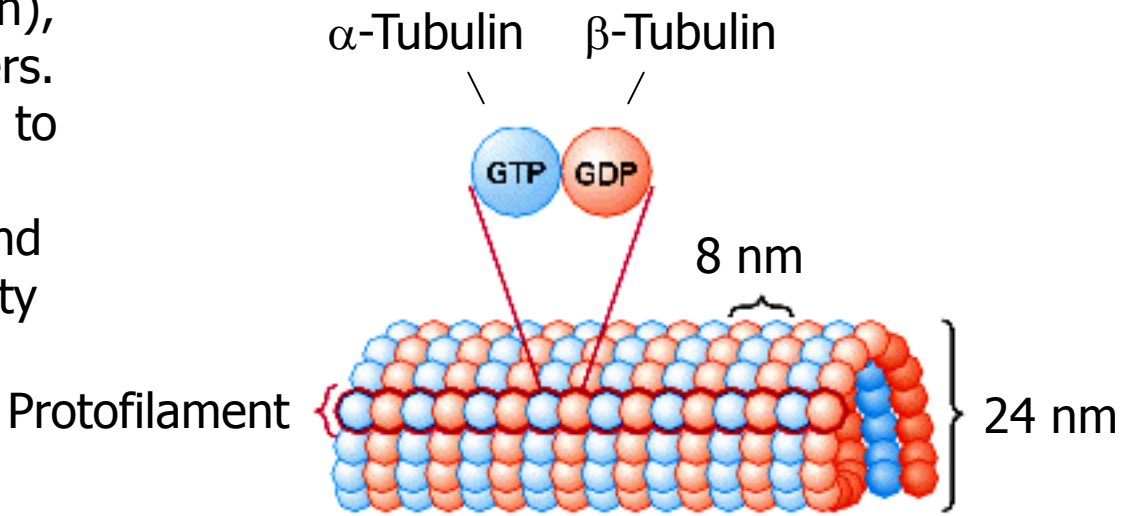




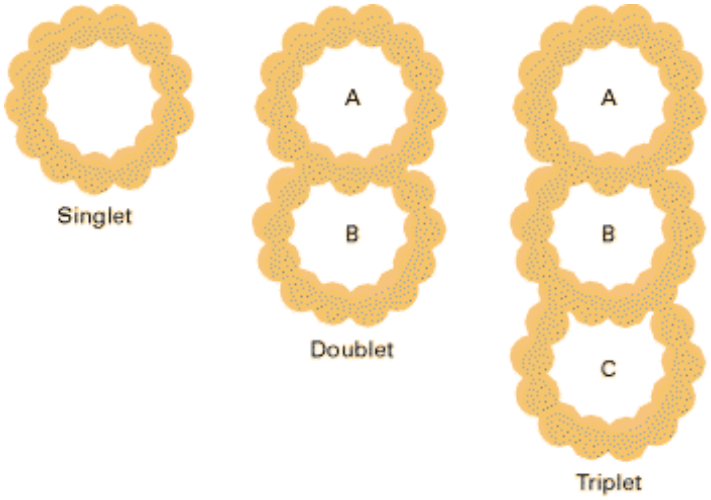


Microtubules

... consist of α-Tubulin and β-Tubulin-units (app. 55 kD each), which form stable heterodimers. α-Tubulin is linked irreversibly to GTP, β-Tubulin is coupled reversibly to GDP oder GTP and has an inherent GTPase activity

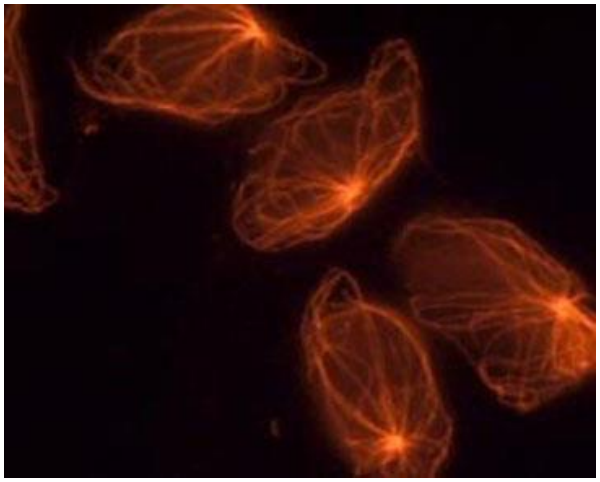


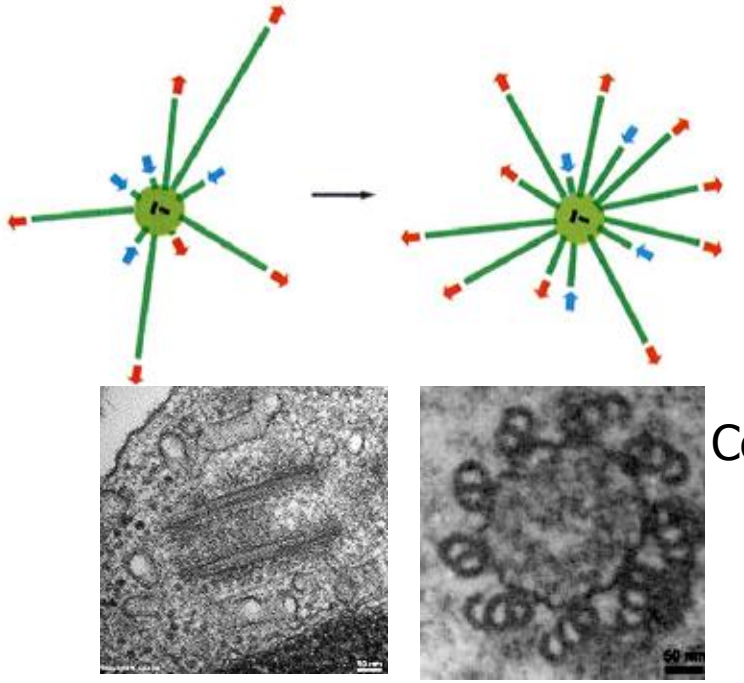
Linear assembly of the dimers leads to the formation of a protofilament, which then assemble to singulet-, Doublet- or Triplet-microtubules



The microtubuli-organizing center (MTOC)

Growth of microtubules starts at MTOC's (Centrosomes), a structure in the center of the cell close to the nucleus, in which γ-Tubulin is concentrated. In human cells it contains a pair of *Centrioles* (two rings out of triplett-microtubules). Ring complexes of γ-Tubulin act as core for the nucleation and polymerization of microtubules (with the minus end at the centrosome), from which the grow towards the periphery (the Plus-end of microtubules).

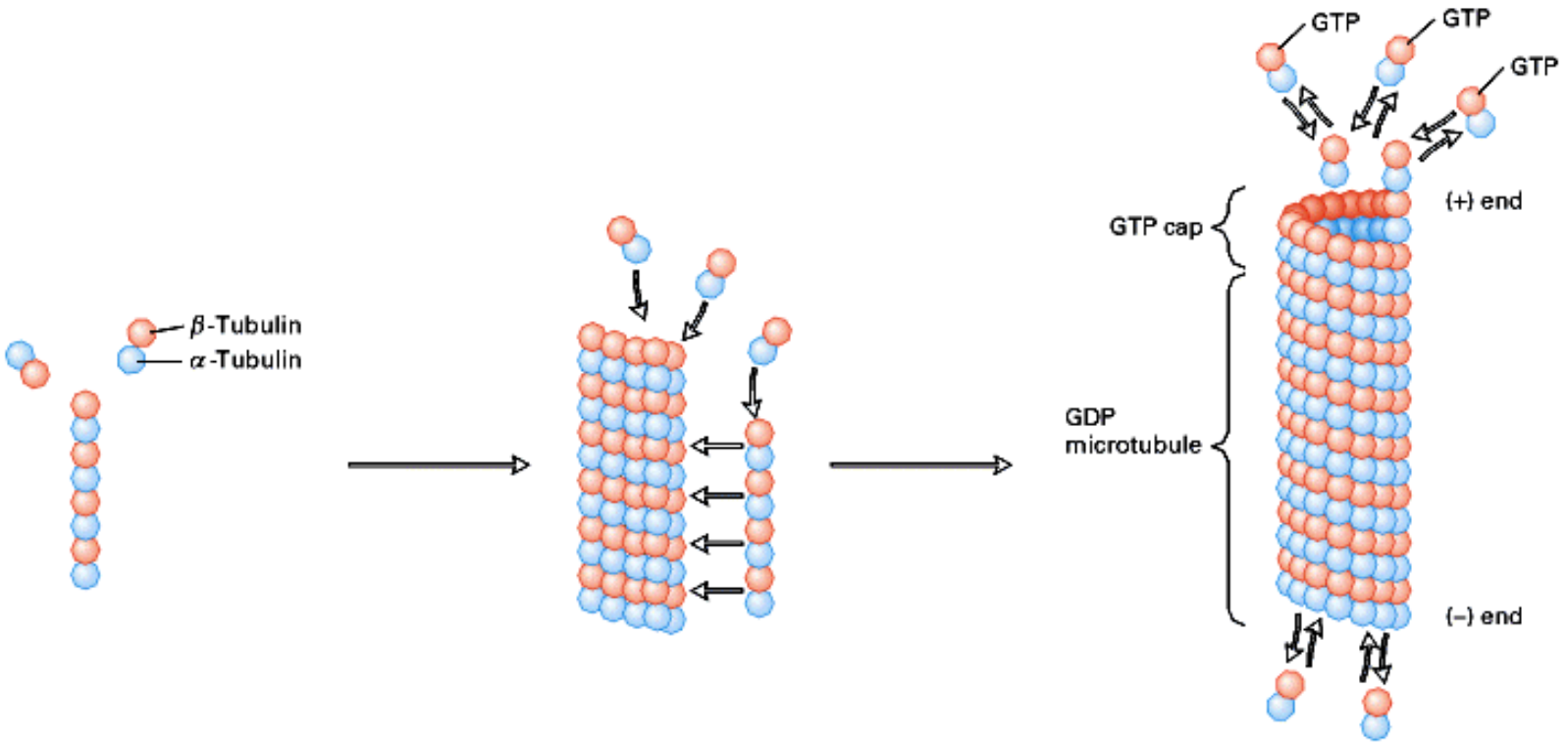




Centrioles

Dynamics of microtubules

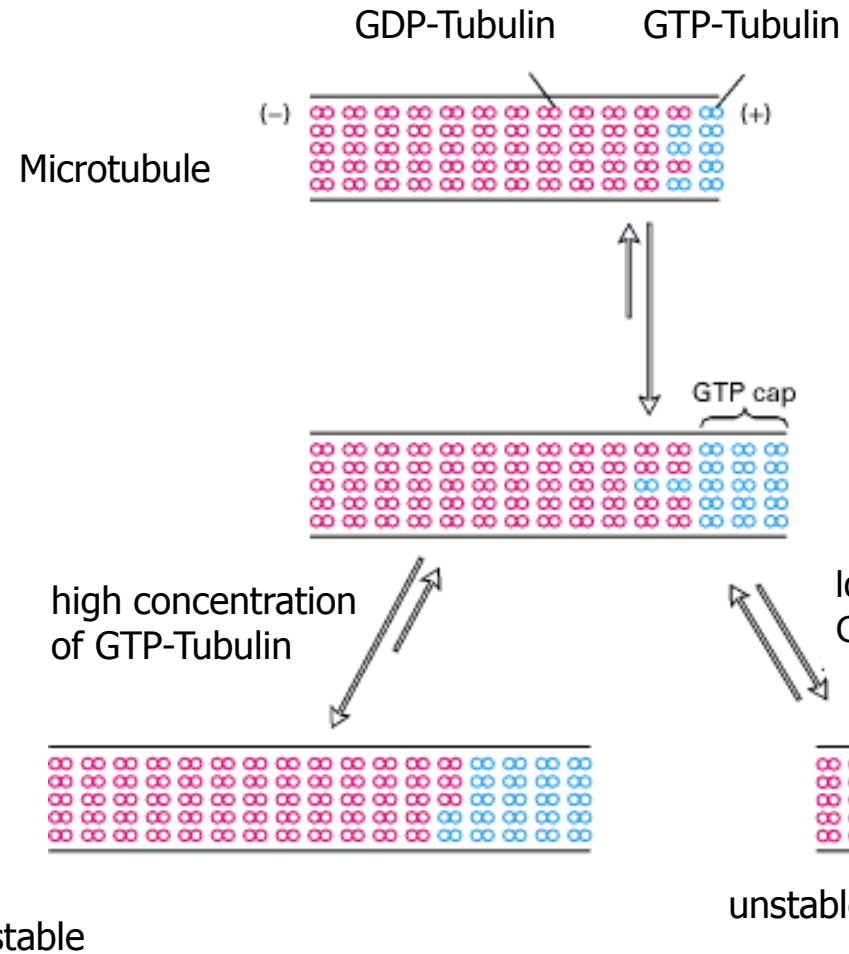
Association and dissociation of tubulin dimers happens mainly at the plus-ends.

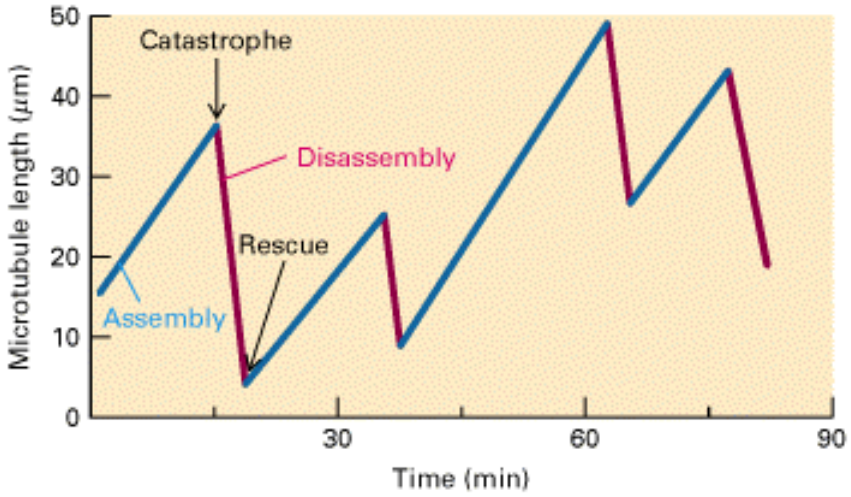


Formation of Protofilaments **assembly to tubules** **growth of tubules**

The dynamic instability of microtubules

The stability depends on local concentrations of GTP-Tubulin. There is often an alternating polymerization / depolymerization process

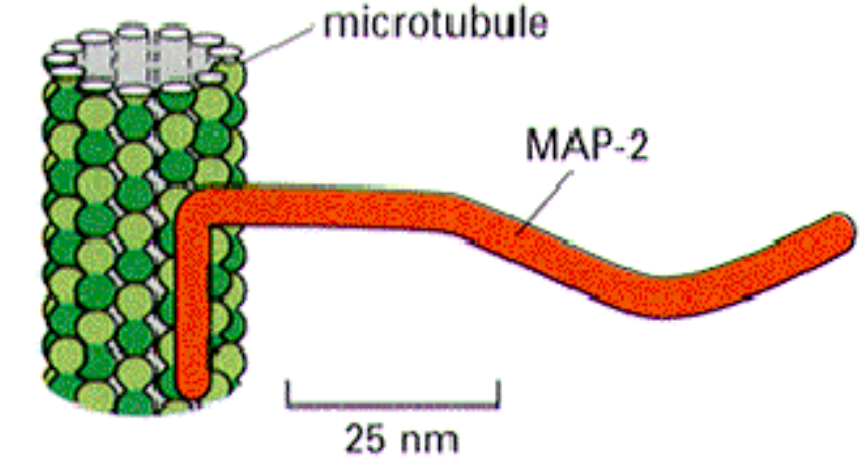




Mikrotubuli-associated Proteins (MAP´s)

…crosslink microtubules with each other and with other cellular structures. They have a micrutubuli-binding domain and a 2nd domain binding either intermediate filaments, cell membranes or other microtubules.

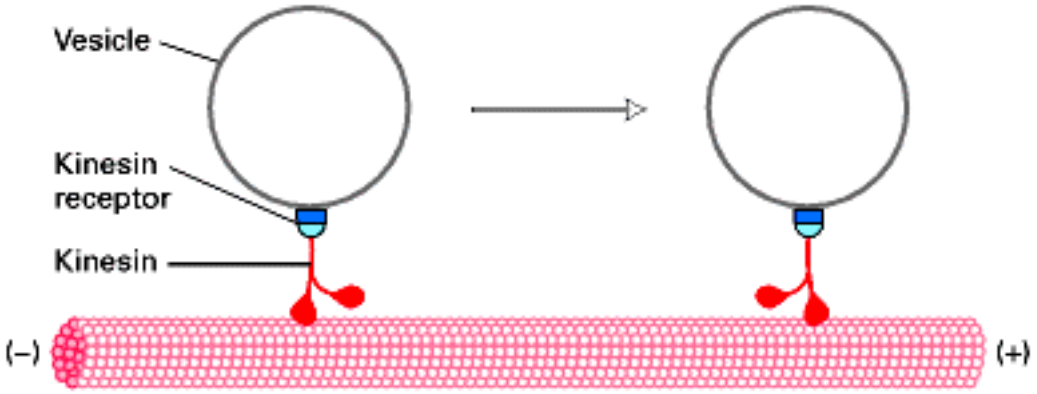
Binding of MAPs usually stabilizes microtubules (inhibits tubulin dissociation)

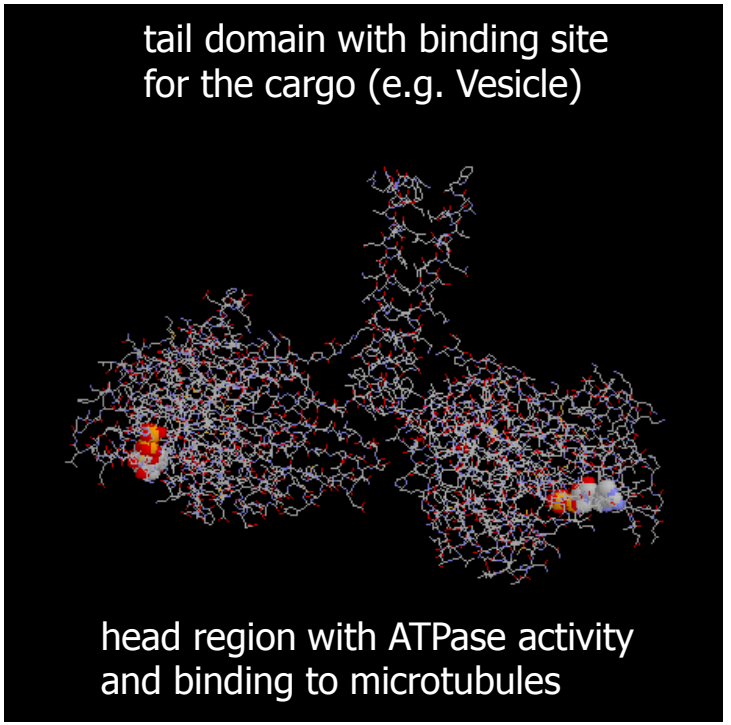


(B)

Microtubular motor proteins: Kinesines and Dyneins

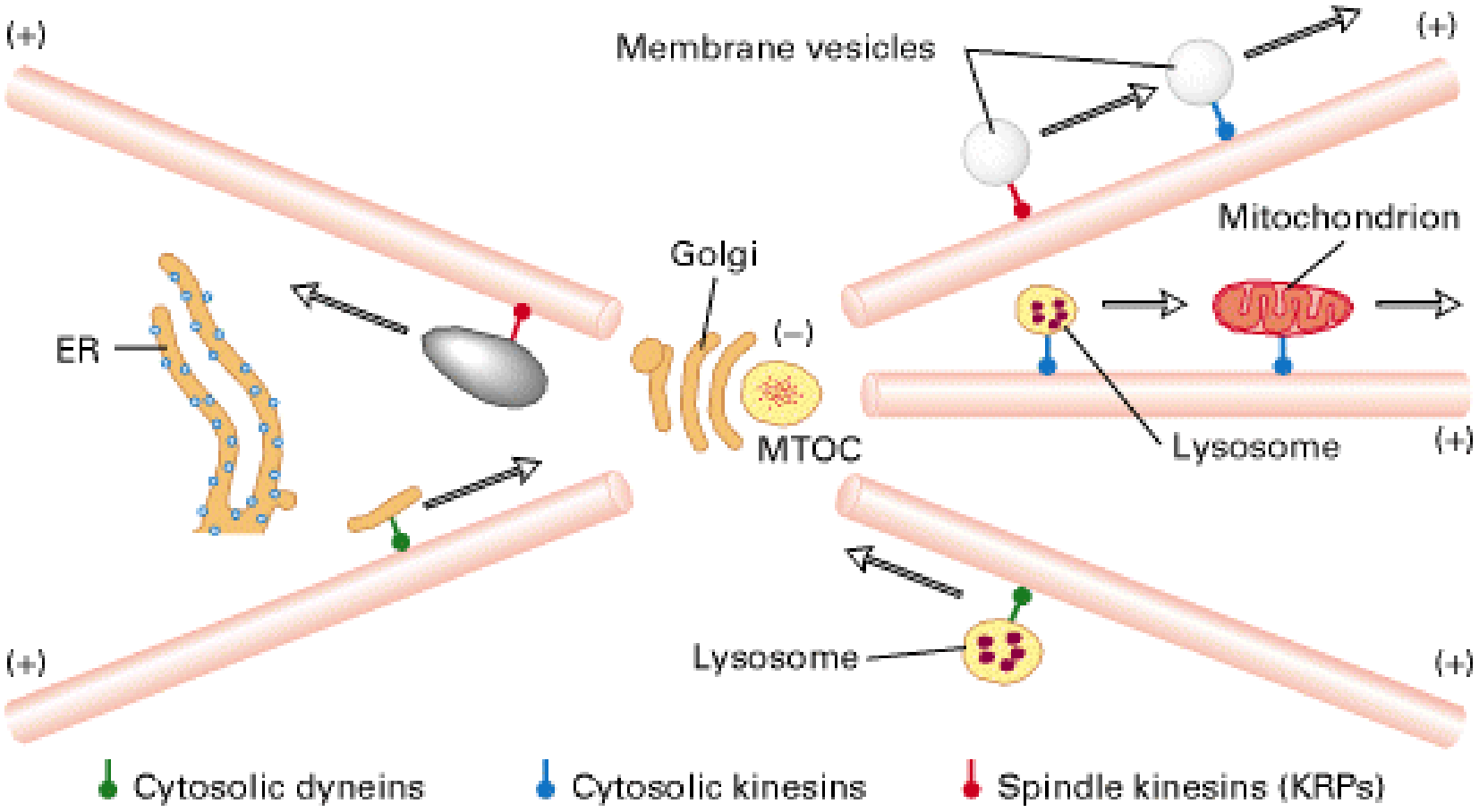
Kinesins are motor proteins, which drive movement towards the Plus end by hydrolysis of ATP.





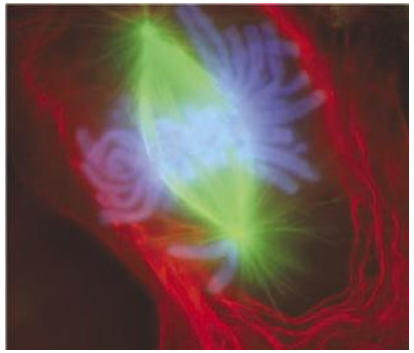
Dyneins are motor proteins migrating towards the Minus end. They have to form complexes with other microtubuli binding proteins for their activity.

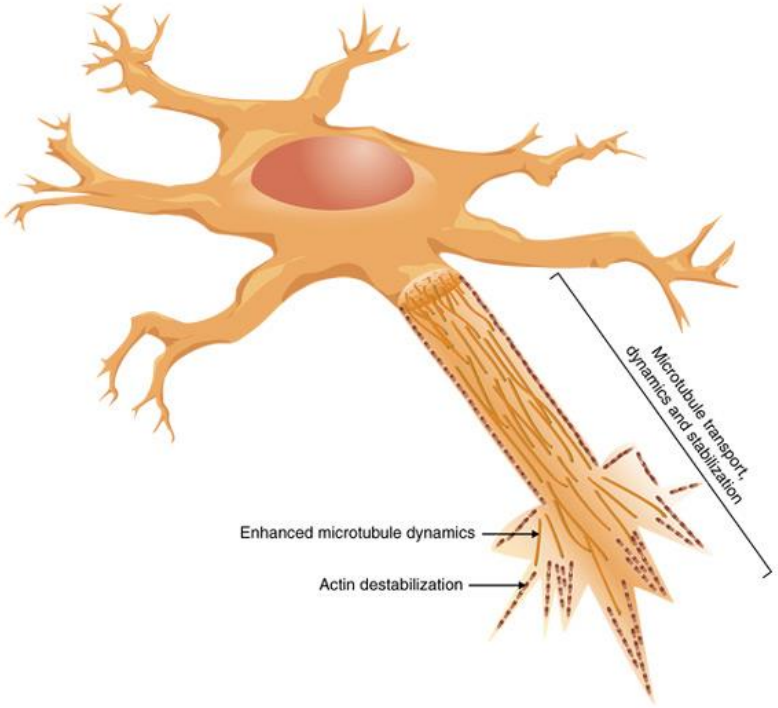
Roles of microtubules for intracellular transport



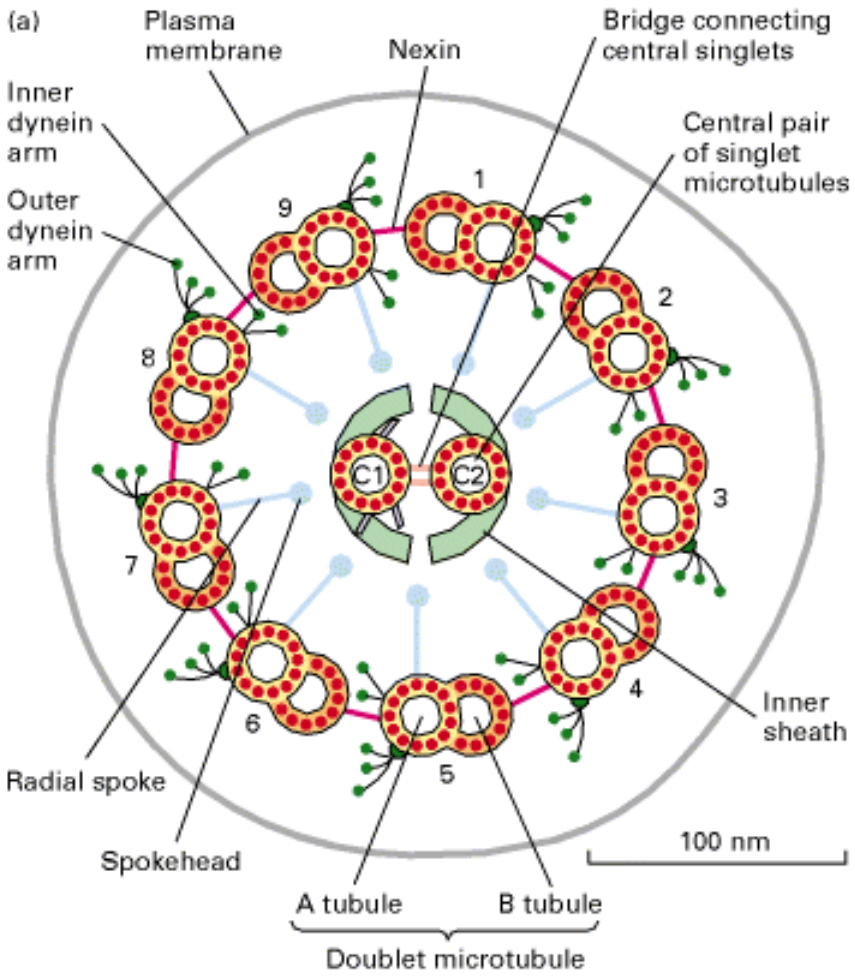
Some Functions of Microtubules in Multicellular Organisms

- Seggregation of chromosomes in M-phase of cell division (mitotic spindle)
- Transport of organelles: e.g. of vesicles along axons in neurons (to transport secretory vesicles containing neurotransmitters)
- Movement of cilia (e.g. to keep the bronchial system clean)

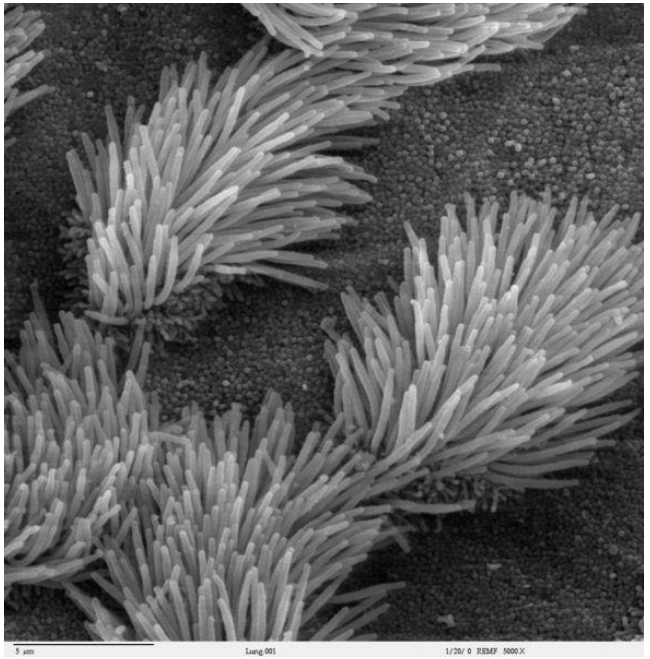




Special Microtubular Structures: Cilia of bronchiolar epithelium



The movement of cilia occurs by forces between the outer doublet microtubuli initiated by dynein motor proteins



Intermediate Filaments

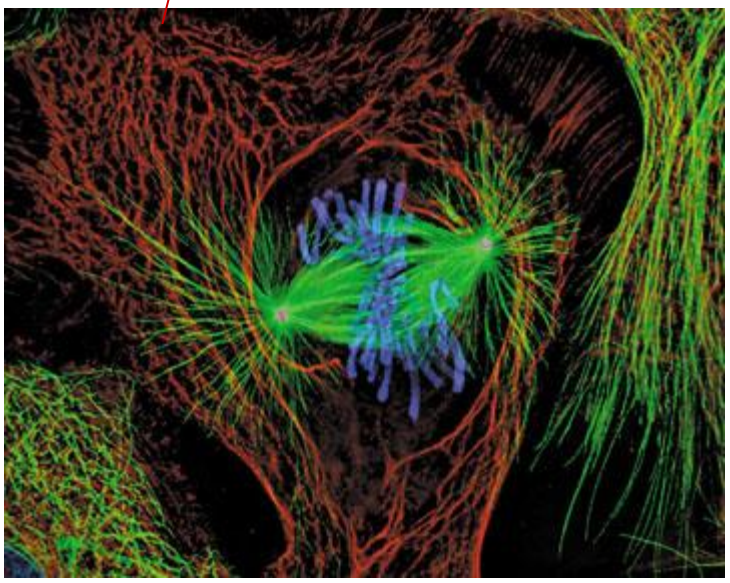
...are very stable filaments (diameter in between microtubules and microfilaments: d = 10 nm) composed of helical subunits, which build filaments. They do not bind ATP- or GTP-Nucleotides and there are no motor proteins known for them. Their primary task is apparently the maintenance of mechanical stability.

Examples for intermediate filament proteins: sind z.B. die

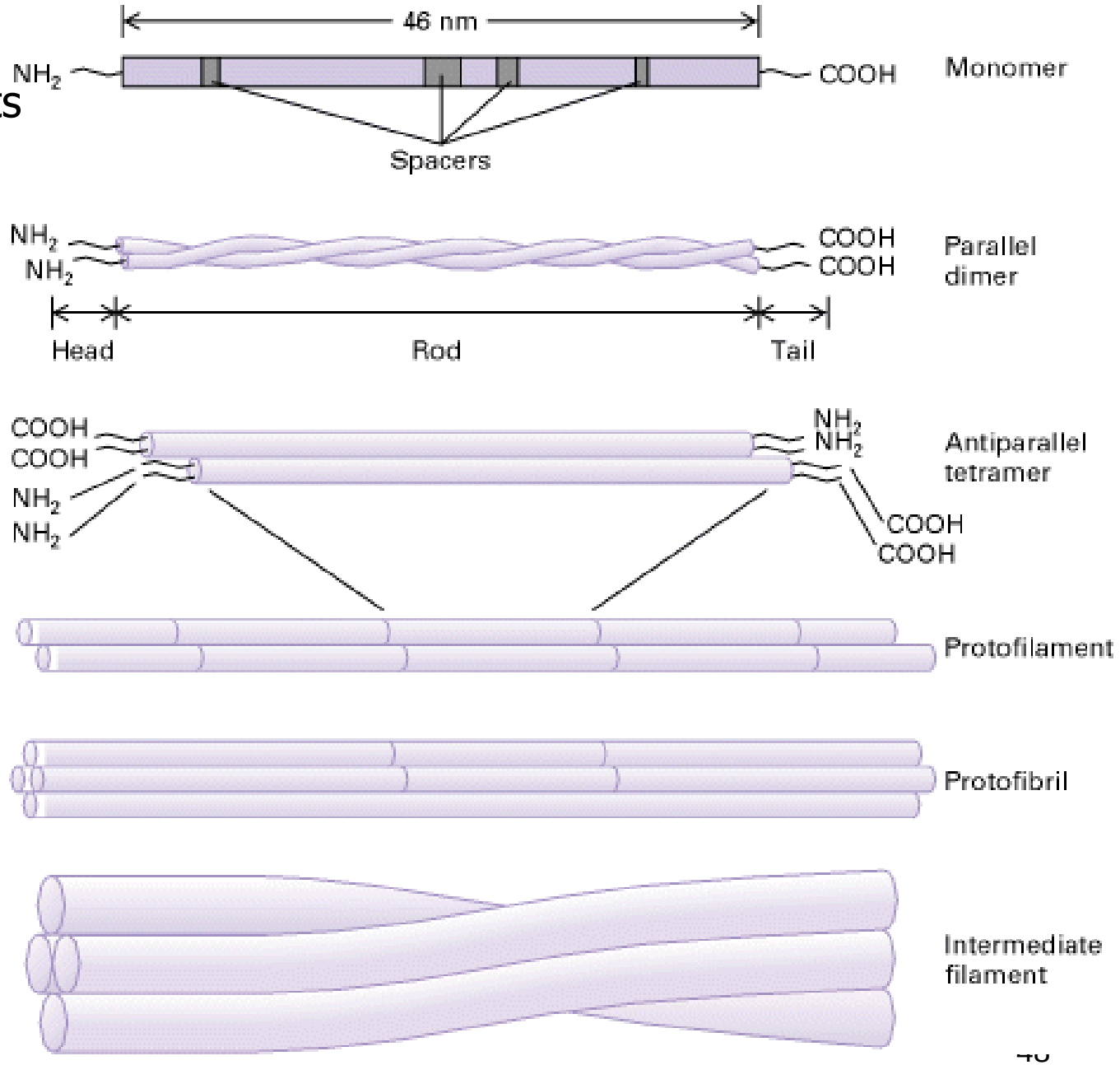
Cytokeratins (e.g. in keratinocytes of the skin) and in various epithelia),

Lamins (A, B and C), build the filaments of the inner side of the nuclear membrane

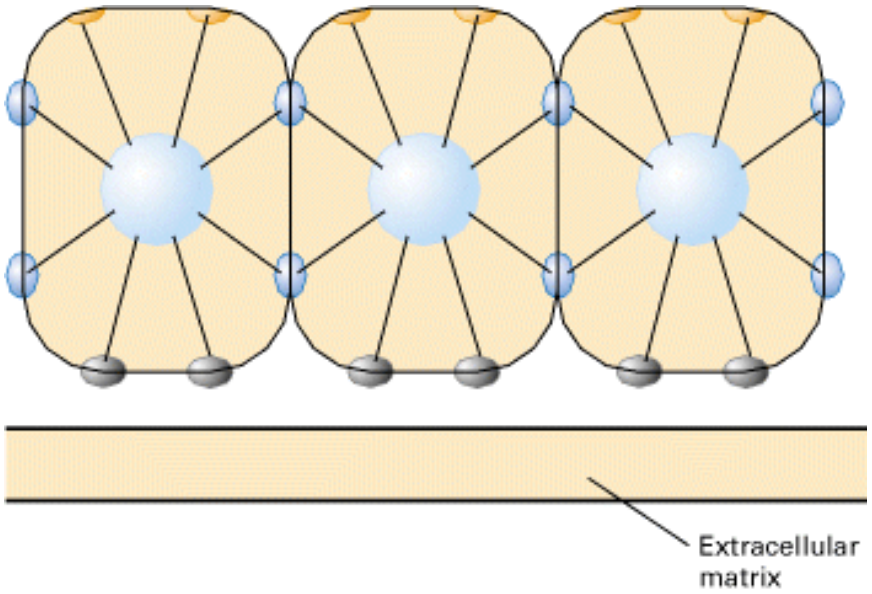
Vimentins: cytoskeleton elements, Neurofilaments, stabilize the long axons of neurons.



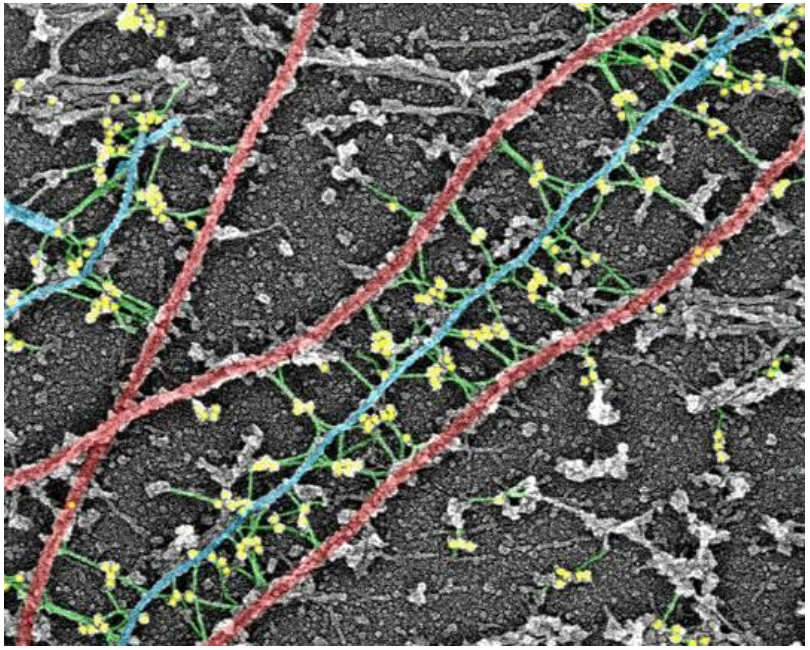
General structure of intermediate filaments



Intermediate Filaments link the cytoskeleton of neighbouring cells (via desmosomes) or provide a link to the basal membrane (in hemidesmosomes)



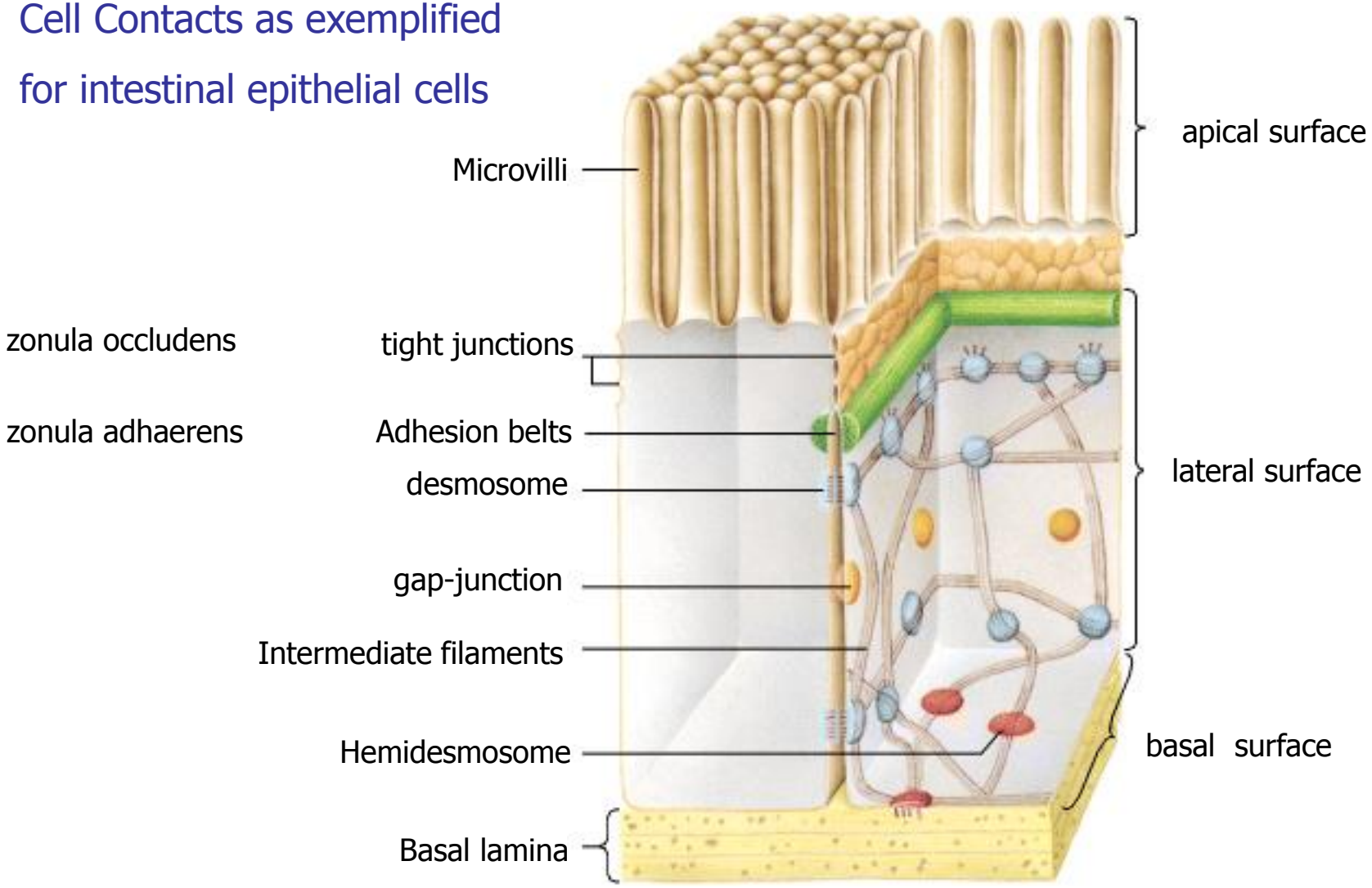
Plectin (yellow) crosslinks intermediate filaments (blue) with microtubules (red)



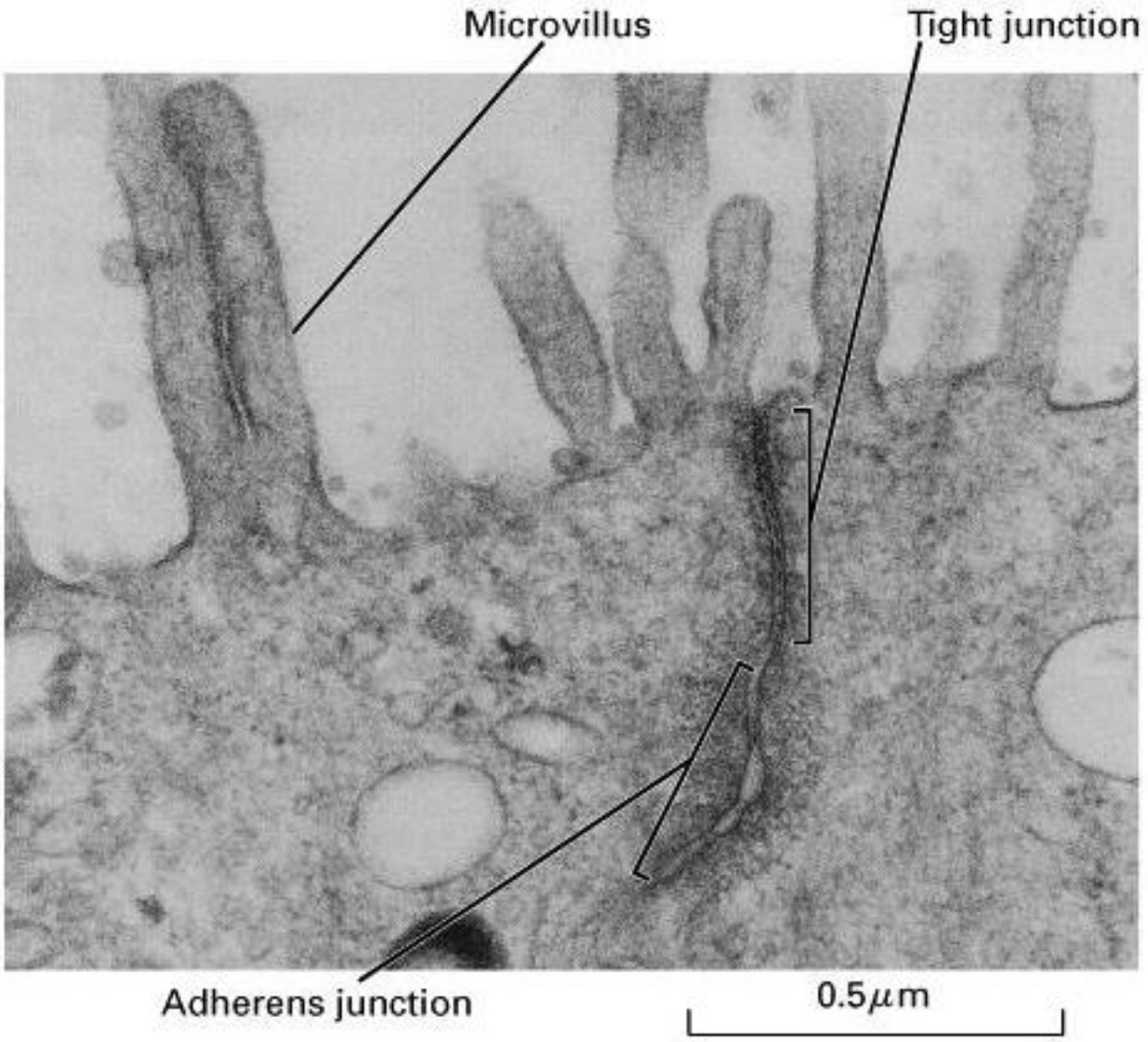
Polarized Cells Build Supra-Cellular Structures such as Epithelia

Cell Contacts as exemplified

for intestinal epithelial cells



Structure of Tight Junctions I

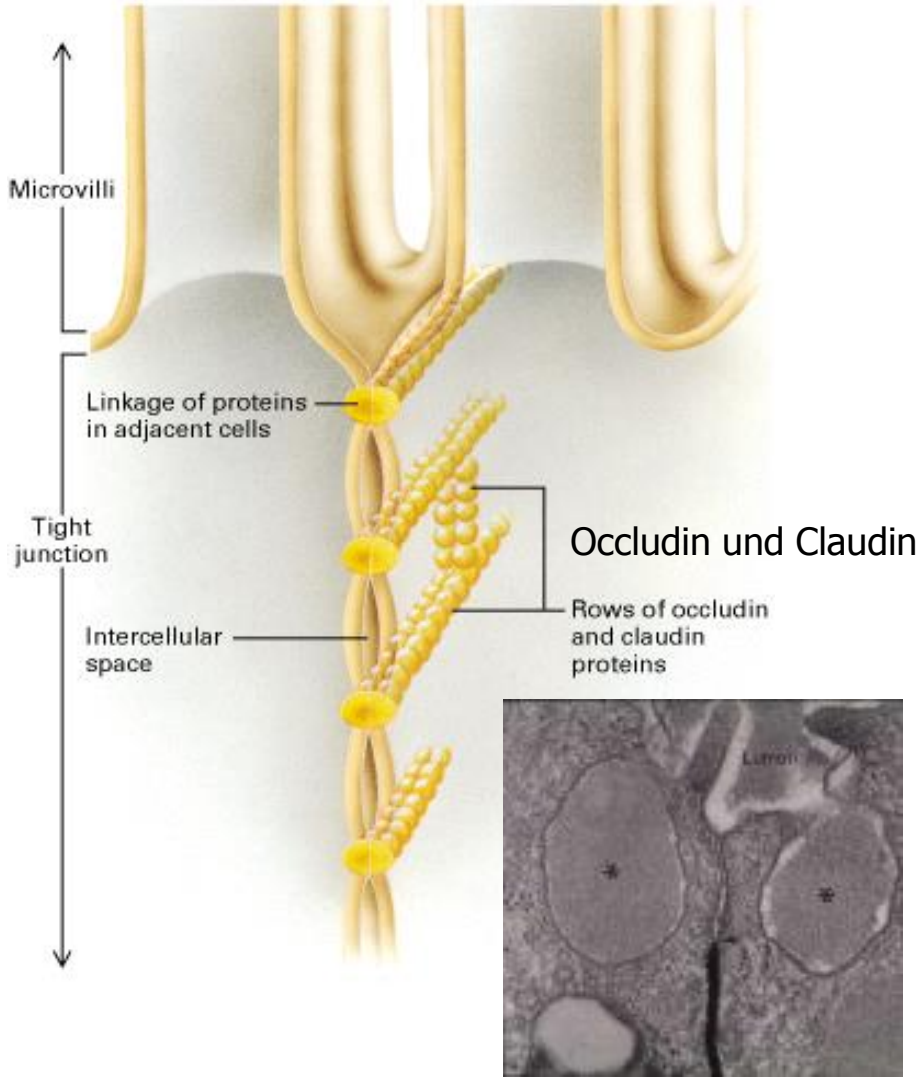


web.mit.edu (Lectures)

Structure of Tight Junctions II

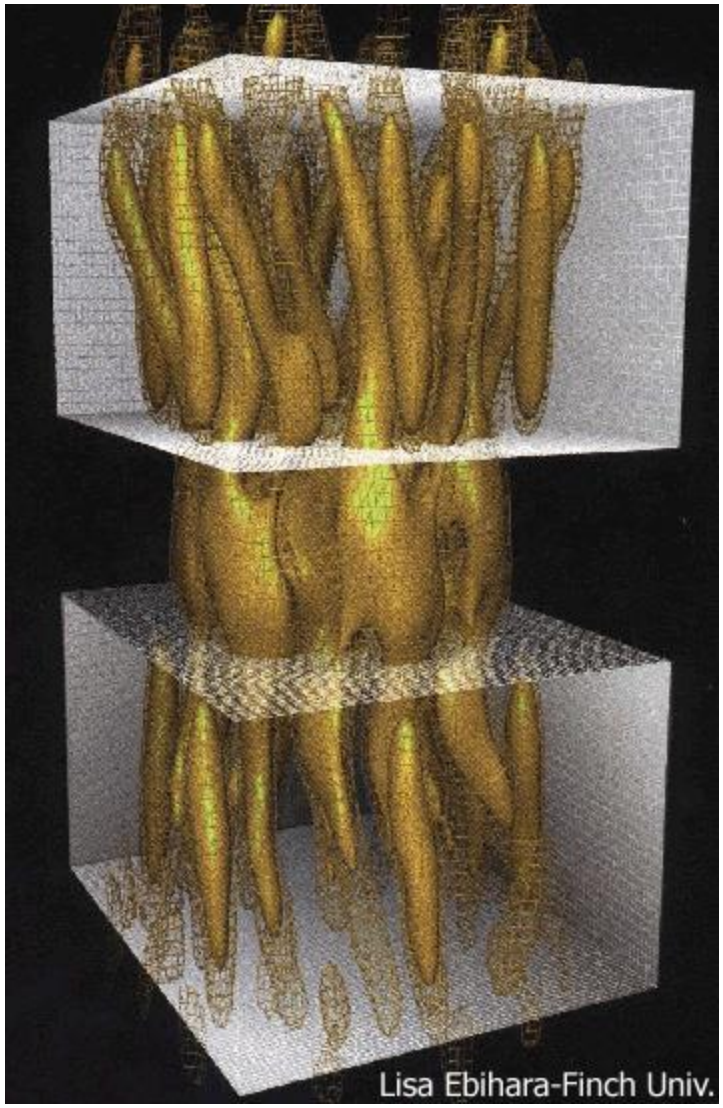
web.mit.edu (Lectures)

Molecular Structure and Functions of „Tight Junctions“

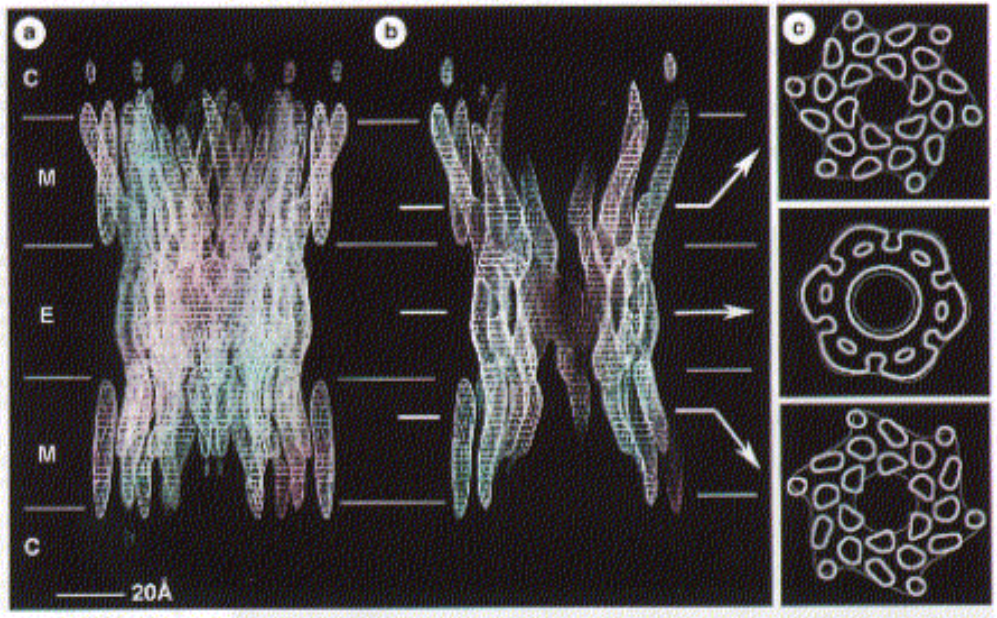


- Tight Junctions are built by membrane protein complexes (consisting of Occludin and Claudin), which link neighbouring cells
- this results in an impermeable junction, which prevents transport processes in between the cells (paracellular transport)
- the junction acts as a diffusion barrier for membrane proteins and lipids preventing diffusion from the apical to the basolateral side and vice versa
- this is essential for maintaining the cellular polarity

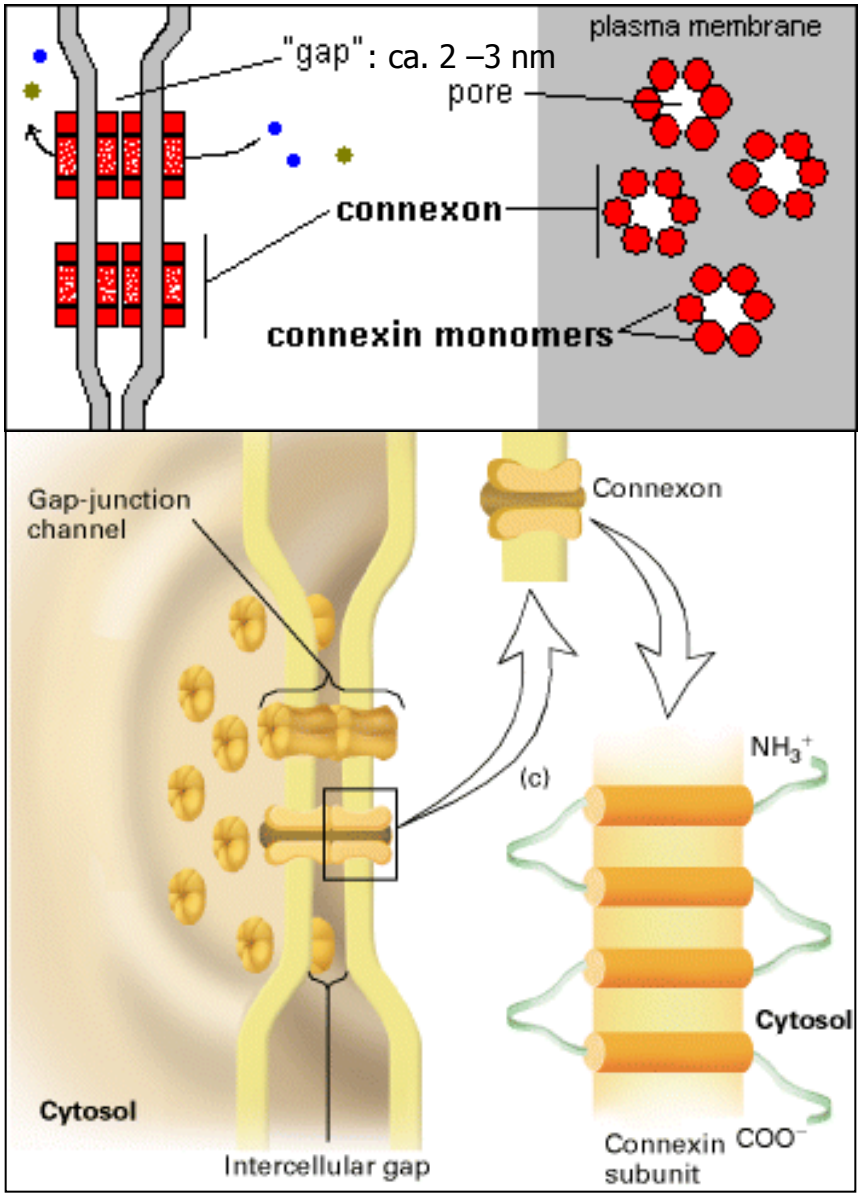
Gap Junctions (open junctions)



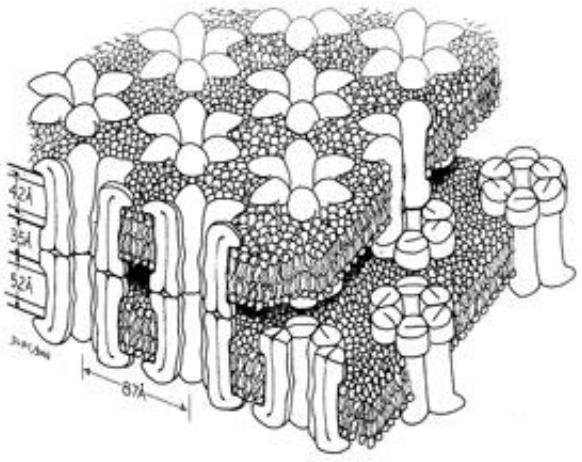
... are pores between neighbouring cells, composed of protein complexes that form a channel. Molecules up to 1.2 m diameter (app. 2 kd) can diffuse through these pores. This is important for the diffusion if ions, metabolites (e.g. glucose) and second messengers (e.g. calcium, cAMP...)



Structure of Gap Junctions

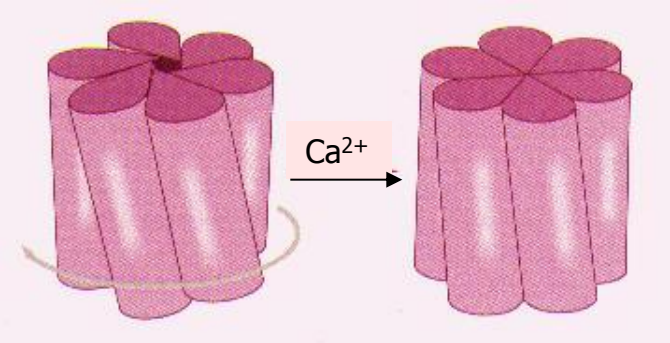


Gap Junctions are formed by proteins of the Connexin-family. These build a hexameric ring with a central channel. The hexamer of one cell (called Connexon) interacts with the hexamer of a connected cell building a functional channel between the cells (consisting of 12 connexins in total). 12 Genes of the Connexin family have been identified so far. Hetero-oligomers of these can have quite different transport characteristics.



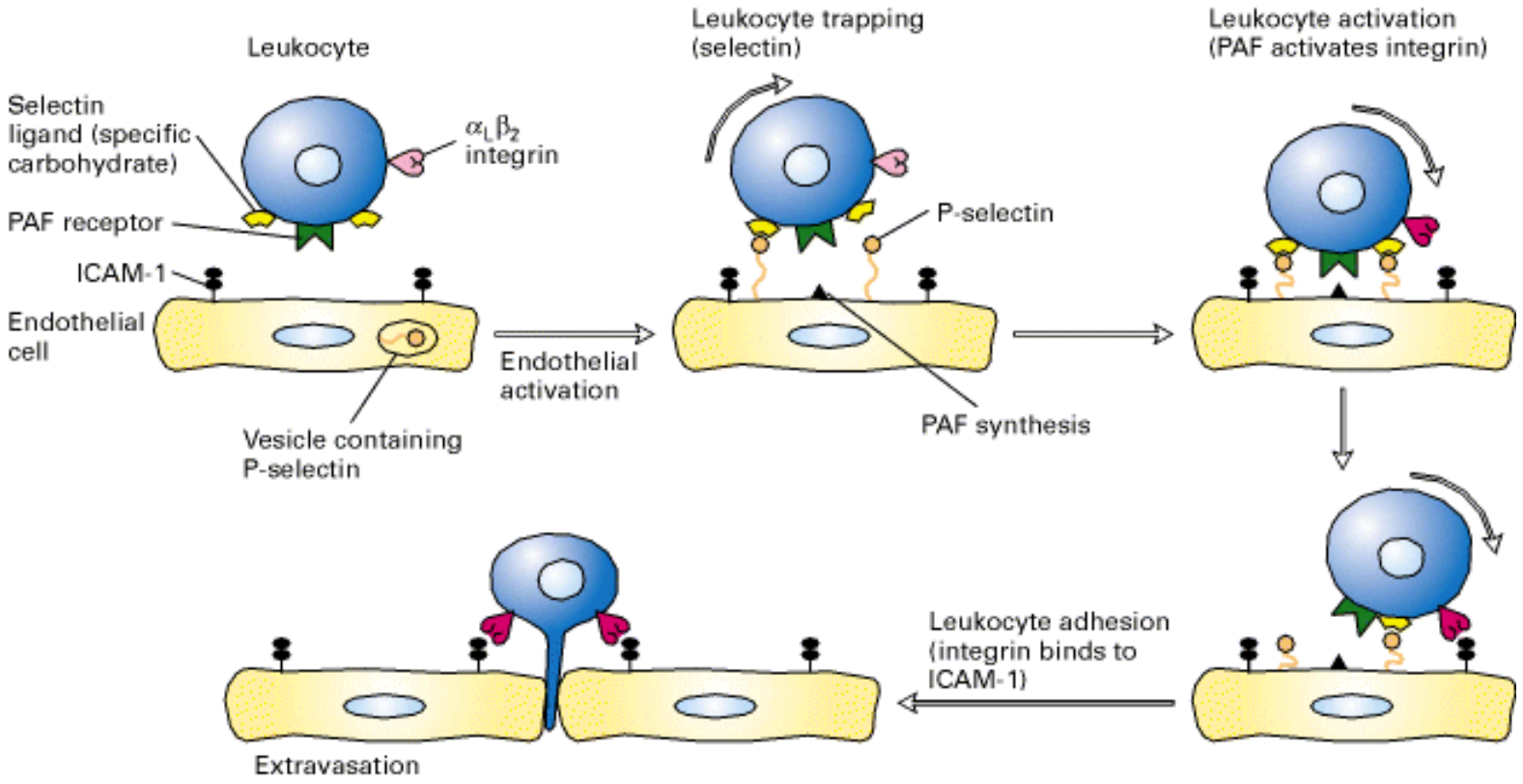
Functions of Gap Junctions

- Metabolic coupling (Metabolite Transfer): e.g. Nucleotides
- Intercellular Coomunication via second messengers: cAMP, Ca^{2+} etc: e.g. increase of the Ca^{2+}-concentration in muscle cells leads to stimulation of neighbouring cells and to synchronisation of the contraction.
- electrical coupling of neurons (electrical synapses: fast signal transmission within several µsec; in chemical synapses with transmission via neurotransmitters: approx. 0.5 msec)
- The pore function is variable and can be modulated: e.g.: permeability can be regulated by the calcium concentration

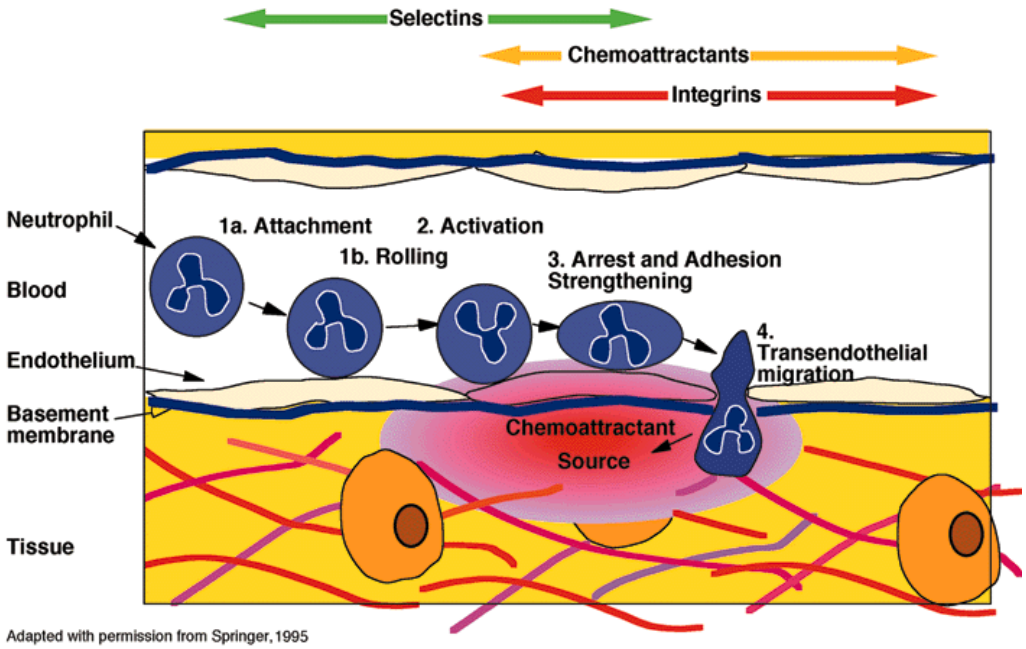


Scheme of the calcium dependent conformational change of gap junctions

Specific transient Cell-Cell Contacts: Binding of leukocytes to endothelial cells before transmigration from the blood circulation to the tissue



Leukocyte binding to blood vessels and transmigration

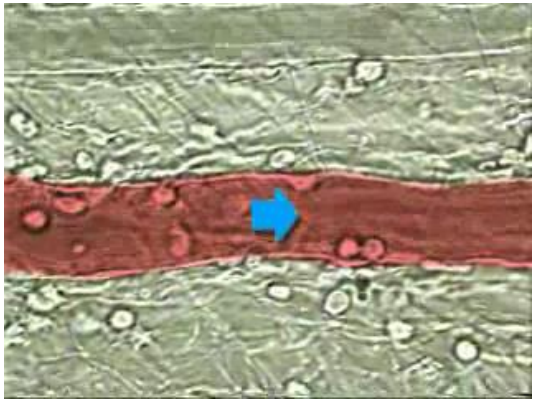


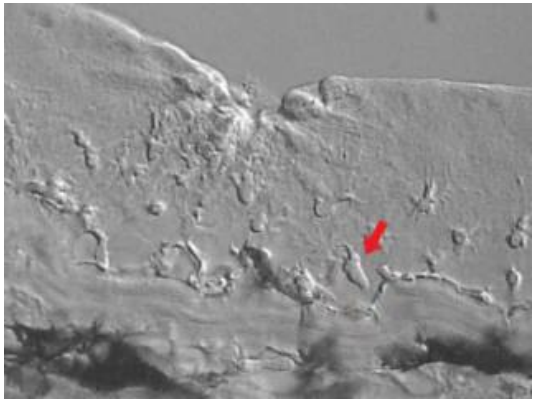
Adapted with permission from Springer, 1995

Activated endothelial cells (e.g. by IL-1, TNF) express P-Selectin on the surface, which binds glycans on the surface of leukocytes. This leads to loose adherence „leukocyte rolling". The adhesion is strengthened by binding of PAF (platelet activating factor – on EC) and PAF-receptors (on leukocytes). Interaction between integrins of leukocytes and ICAM-1 of EC leads to firm adhesion (arrest) and transmigration

Video: leukocyte rolling

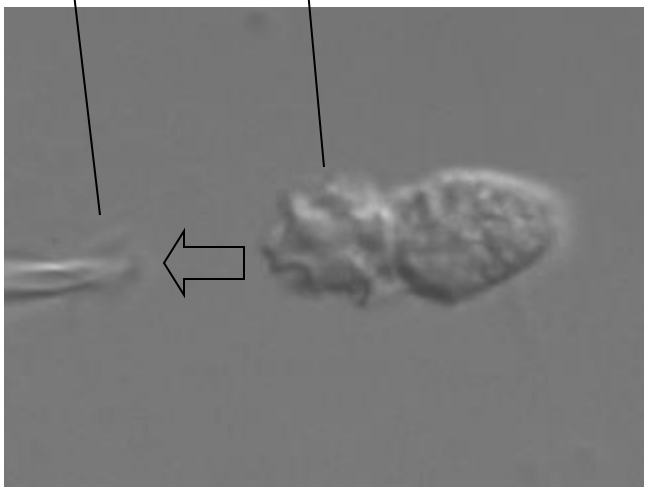
Video: lymphocyte homing

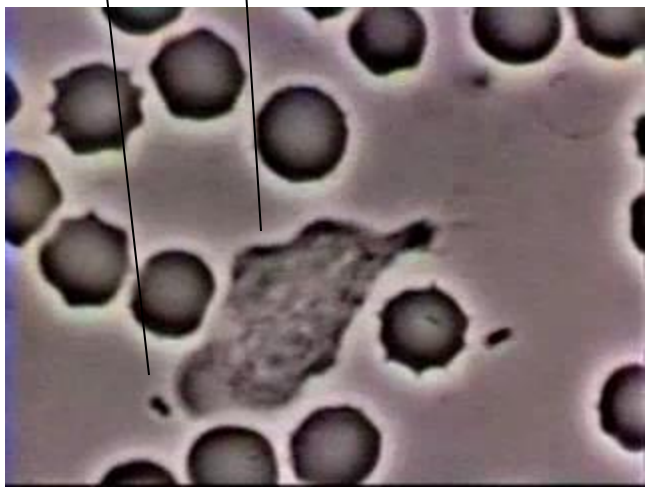




Control of cell migration by chemotaxis

Certain bacterial molecules (e.g. peptides like formyl-Met-Leu-Phe) act as chemoattractants for leukocytes (binding to specific receptors). Leukocytes can sense a gradient of these molecules and migrate along the gradient towards the source of the chemoattractants, the bacteria (using lamellipodia and actin filaments).





Videos: neutrophil chemotaxis 1 and 2