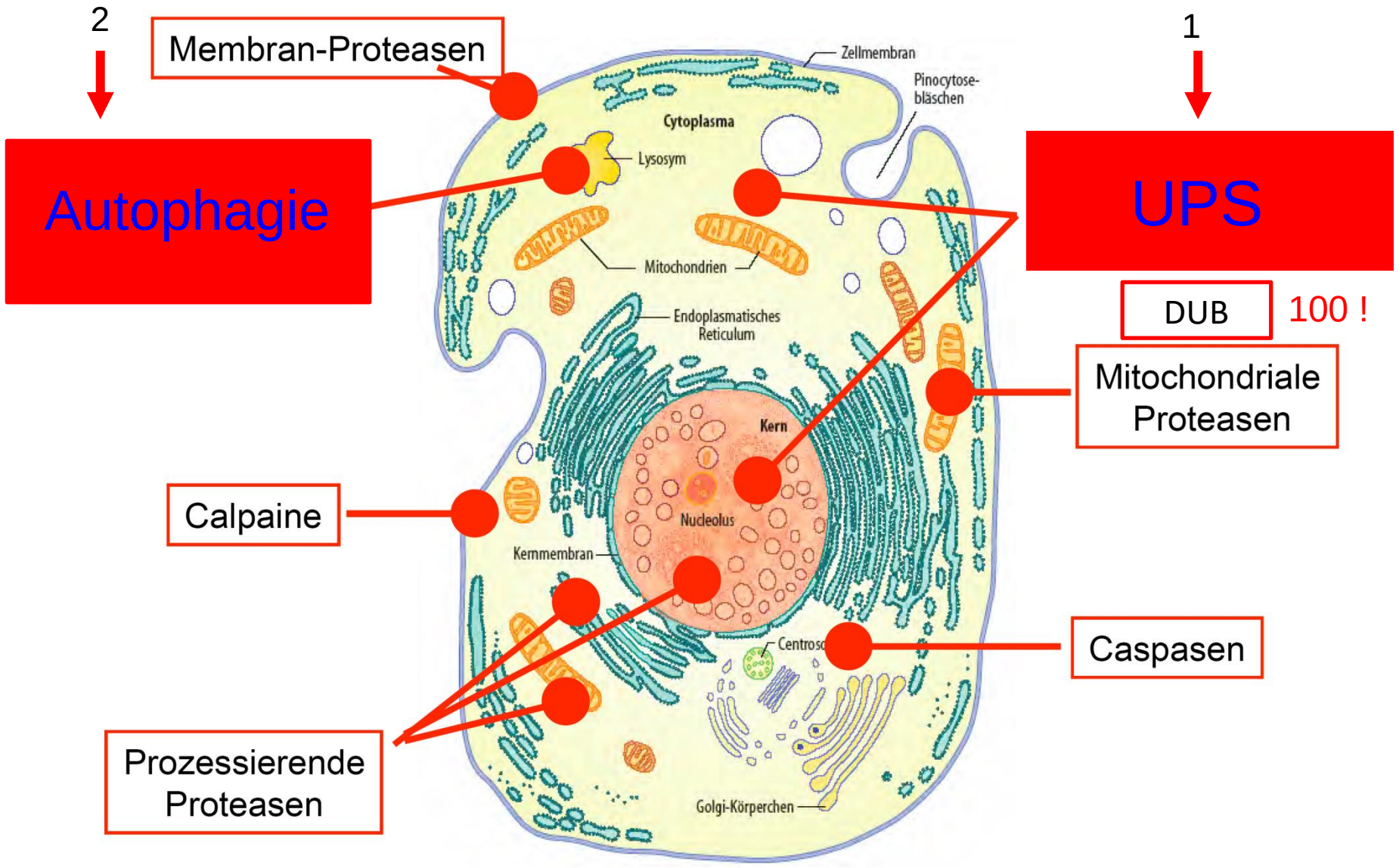
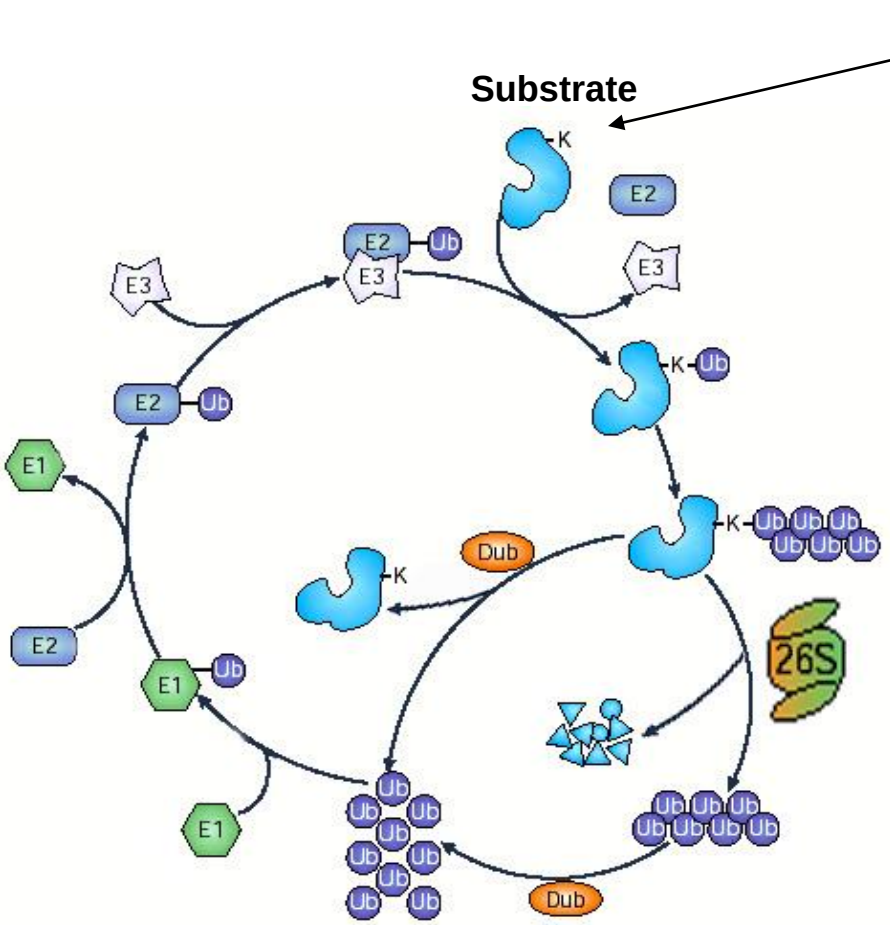
Localization of cellular proteases



The Ubiquitin (Ub) Proteasome System (UPS)

-Major proteolytic system in eukaryotic cells
-Ub is a signal for proteolysis

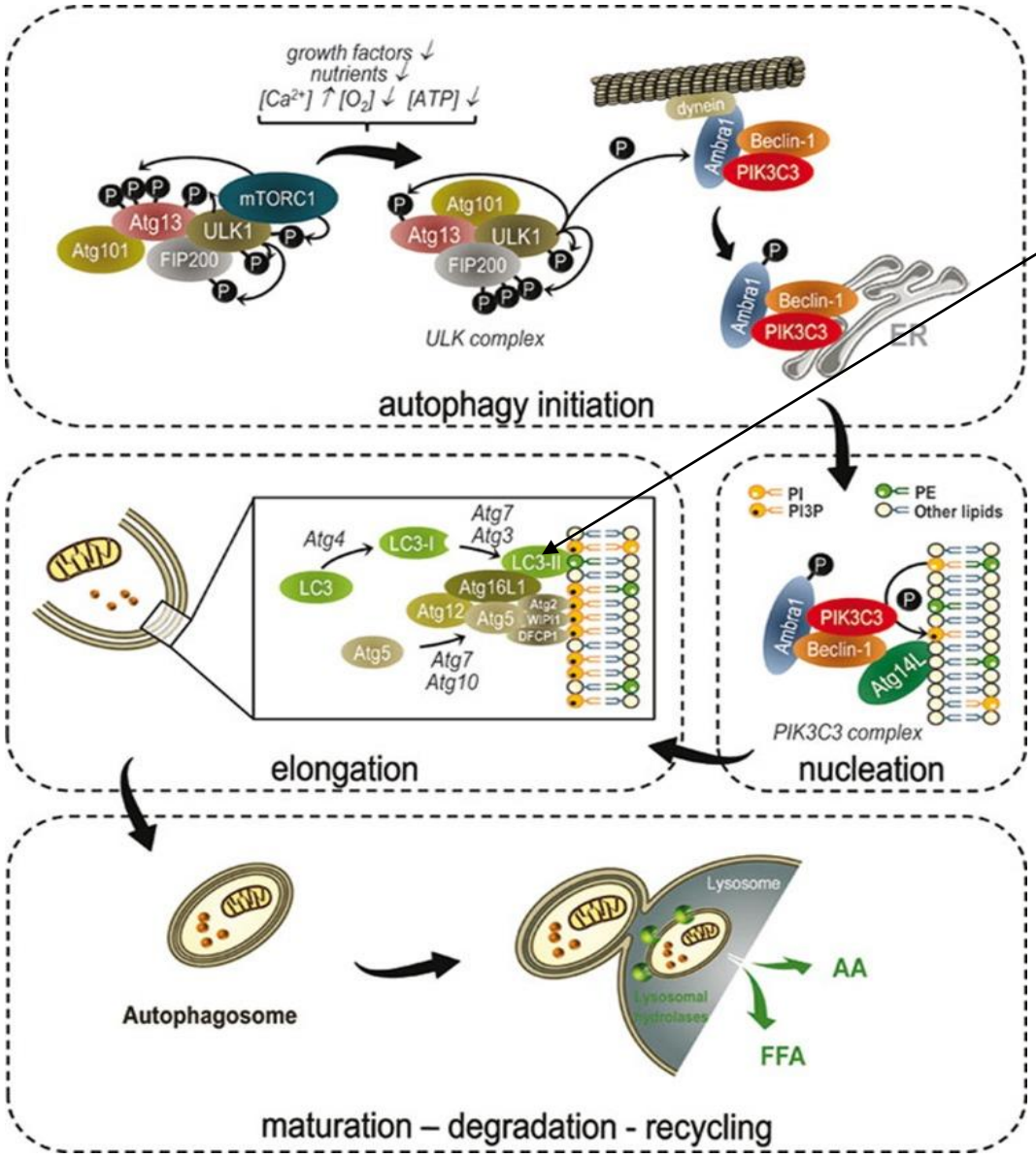


Components of the UPS

- E1 – Ub activating enzymes
- E2 – family of Ub conjugating enzymes
- E3 – families of Ub ligating enzymes
- The 26S proteasome
- Family of deubiquitinating enzymes (DUBs)

Modified from Sullivan et al., 2003

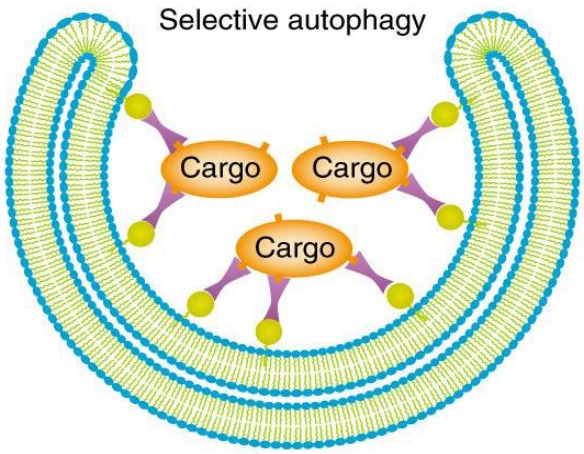
Macroautophagy steps



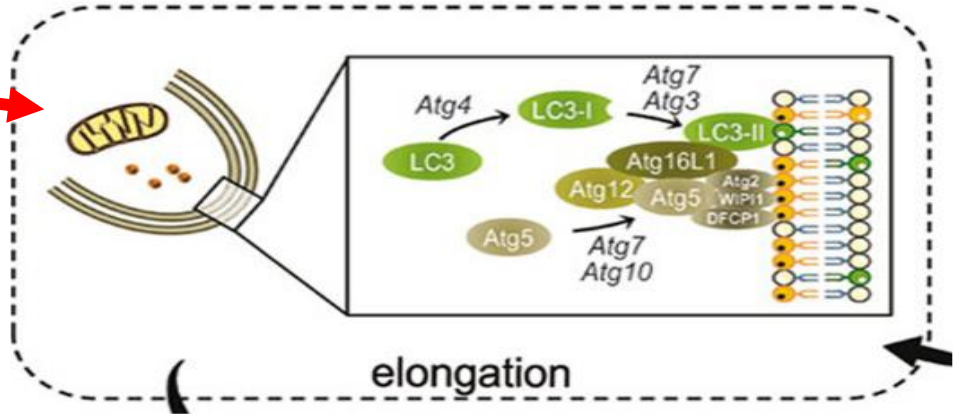
LC3=ATG8

Wirawan et al. *Cell Research* 2012

Selective Macroautophagy

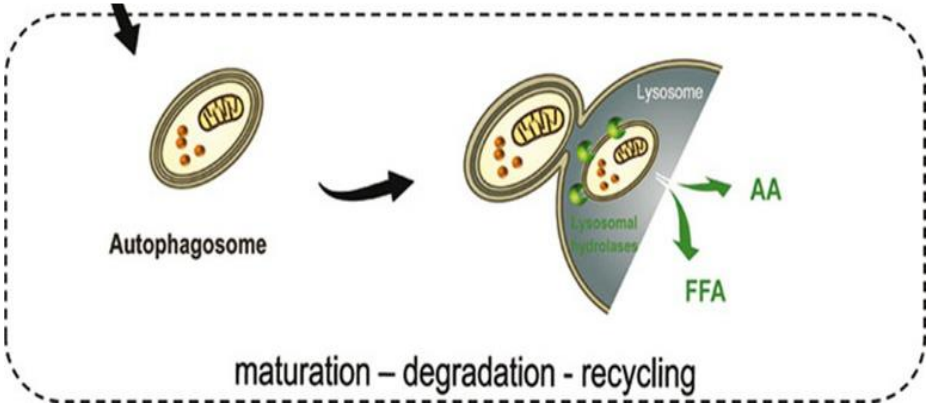


Formation of phagophore membranes from ER



1. Ubiquitination of substrates (cargos)
2. Transport of substrates into phagophore membrane

Formation of autophagosomes



Degradation of cargos in lysosome

Similarities and Differences of UPS and selective Macroautophagy

	UPS	Selective Macroautophagy
Abundance	All eucaryotic cells	All eucaryotic cells
Signal	Ubiquitin-Chain via Lys48 (min 4 Ub molecules)	Ubiquitin, ATG8
ATP consumption	Yes	Yes
Ub-like modifier conjugation system	Ubiquitination (Ub-conjugation, Ub.chain)	Ubiquitination, ATGylation: ATG8, ATG12-conjugation
Proteolytic machinery	Protein complex	Cell organelle: Lysosome
Enzymes	Protease: 26S proteasome	Proteases, Nucleases, Lipases
Selectivity	E3	Specific receptors, E3
Substrates	Proteins	Proteins, Protein complexes, Lipids, Nucleic acids, Cell organelles, Pathogens
Function	Proteolysis	Proteolysis, Lipolysis,

Antigenpresentation

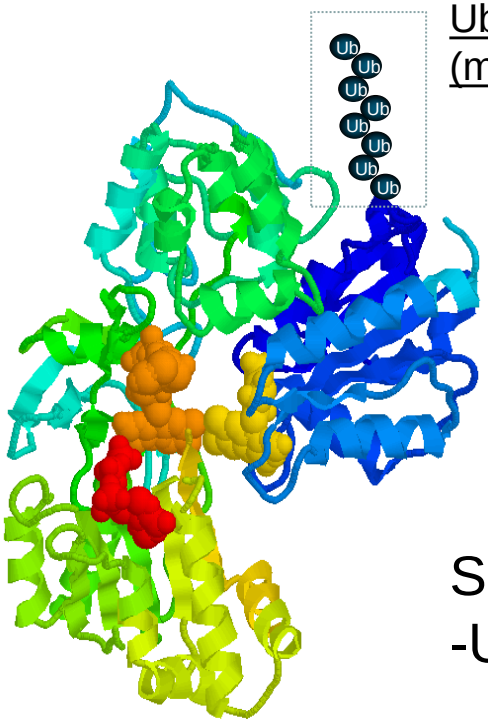
Antigenpresentation

Crosstalk between UPS and selective macroautophagy

- Ubiquitin-like proteins: Ubiquitin, ATG8, ATG12
- Ub-like protein conjugation system
- Ub or Ub-chains
- Ub E3 Ligases?

Signal of substrates:

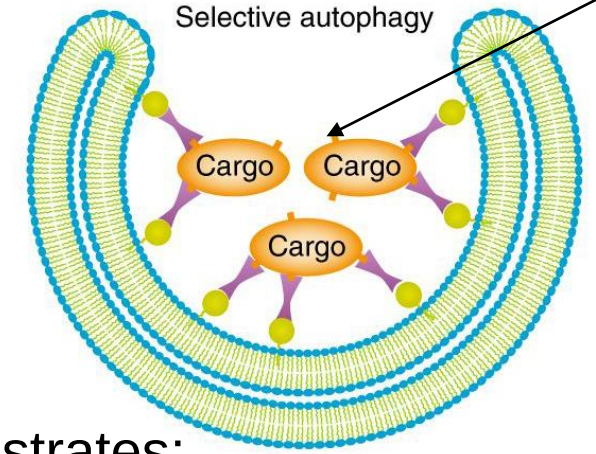
UPS



Ubiquitination
(min. 4 molecules)

(Lys 48 chain)

Selective macroautophagy



Ubiquitination
(1 or 2 molecules)

(Lys 63 chain)

Signal of substrates:
-Ubiquitination (Ub-chain or Mono-Ub, Di-Ub)

PE: Phosphatidylethanolamine

E3?

UPS

Selective macroautophagy

The Ub ligases (E3s) ligate specifically Ub to protein substrates. They determine the specificity of the UPS.

HECT domain Ub ligases

1. E_2-S~Ub + Protein–NH_2 → E_3–SH + Protein–NH–CO–Ub

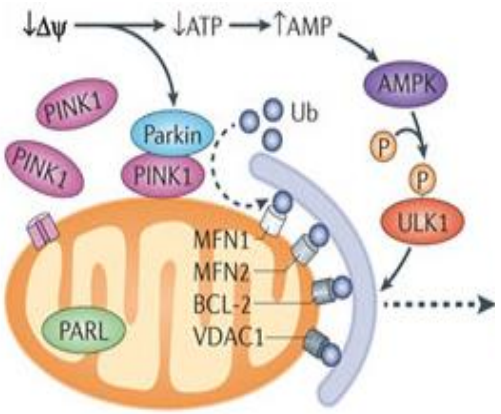
Isopeptide bond

RING domain Ub ligases

2. E_3•E_2–S–CO–Ub + Protein–NH_2 → E_3+E_2–SH + Protein–NH–CO–Ub

Hundreds E3s are known

Parkin: HECT-RING E3

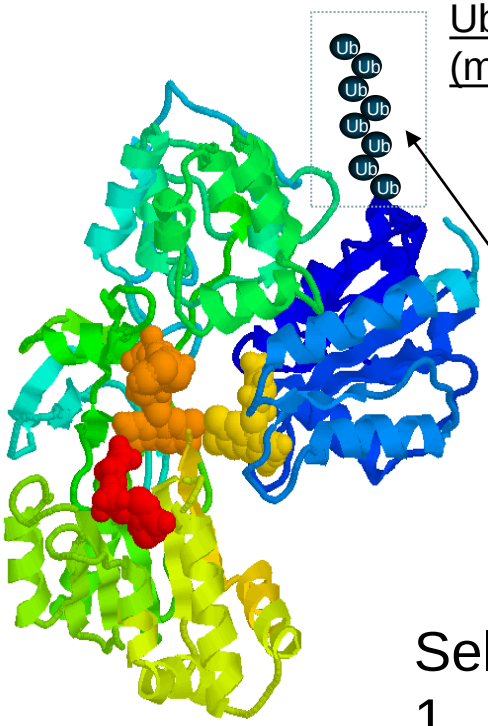


Very rare known about E3 ligases in auotphagy pathway

Selectivity of substrates:

Comparison between UPS and selective Macroautophagy

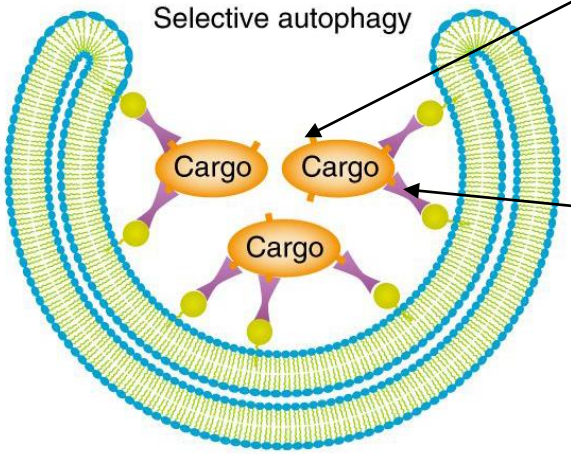
UPS



Ubiquitination
(min. 4 molecules)

Receptors
(Ub-chain and
Proteasome)

Selective macroautophagy



Ubiquitination
(1 or 2 molecules)

Receptors
(Ub and
ATG8)

Selectivity is determined by

1. Ub ligase E3s
2. Specific Receptors: binding to Ubl or Ub-chains

ATP consumption

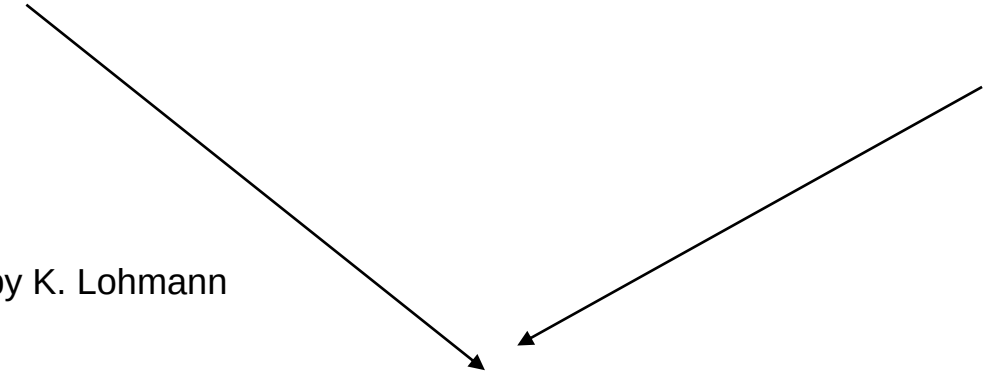
- UPS

Ubiquitination

- Selective macroautophagy

Ubiquitination

ATGylation



ATP consumption

ATP: is discovered by K. Lohmann

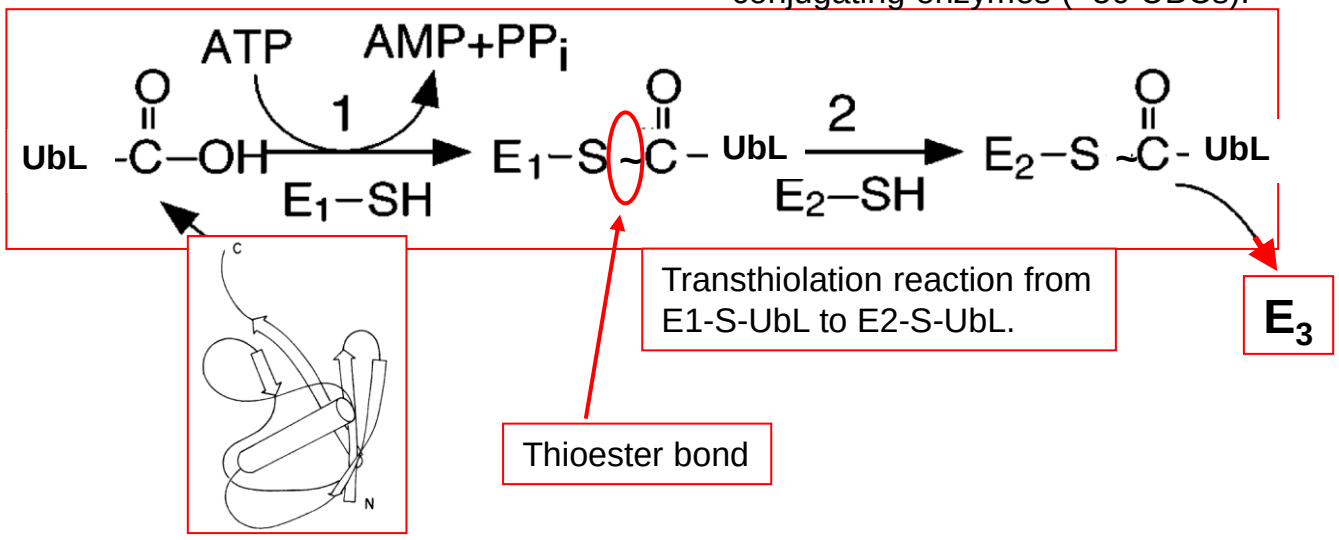
Activation and transfer of UbLs like Ub

E1 – UbL activating enzyme

There are few Ub activating enzymes in eukaryotic cells

E2s – UBCs – UbL conjugating enzymes

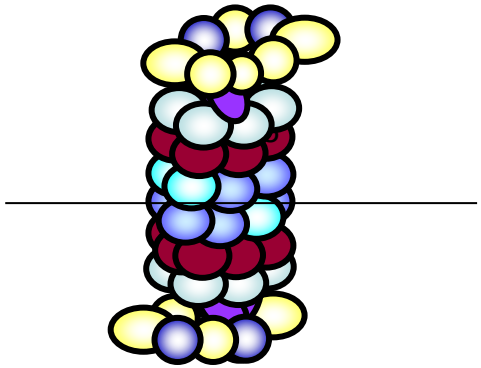
There is a large family of UbL conjugating enzymes (~50 UBCs).



proteolytic machineries

UPS

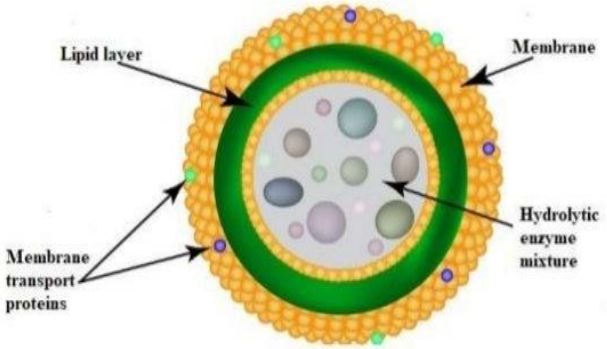
26S proteasome



Protein complex
Protease: 20S one molecule contains
6 active centres
(DUB activity)
Active sides occur at inner cavity
Why?
pH 7-7.5

Selective macroautophagy

Lysosomes



Organelle
Proteases, nucleases and lipases (60 enzymes)
enzymes are at inner of lysosomes, why?
pH 4.5-5

Substrates

Comparison between UPS and selective Macroautophagy

UPS

-Poly-Ub Proteins

Selective macroautophagy

-Organelles: Mitochondria

Perixosomes

Lipid Droplets

-Pathogens (Xenophagy): Bacteria (Bacteriophagy) Virus (Virophagy), Fungi (fungal autophagy)

-Misfolded proteins, protein aggregates, protein complexes e.g. 26S Proteasome

-Lipids

Stimulis and functions:

Comparison between UPS and selective Macroautophagy

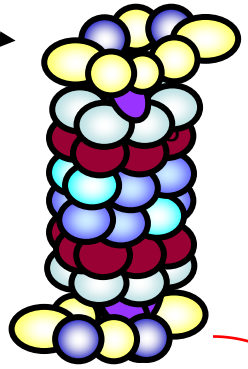
UPS

Hypoxia

ER stress

26S proteasome

Infection



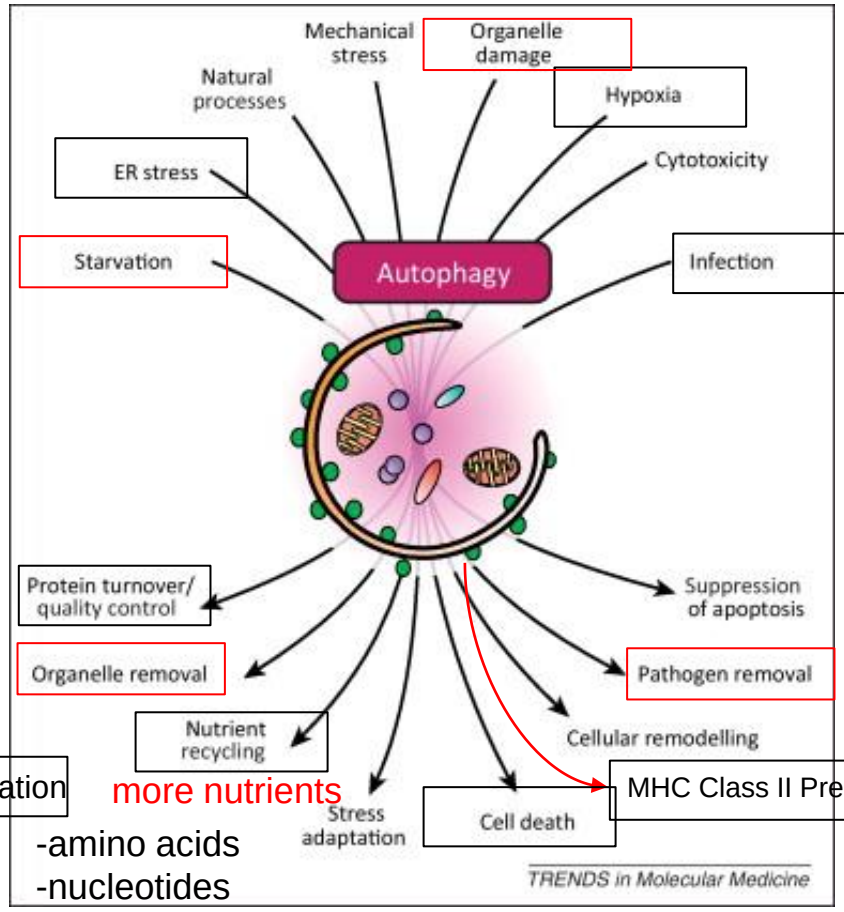
Protein turnover/ quality control

Nutrient recycling

Cell death

MHC Class I Presentation

Selective macroautophagy



more nutrients

-amino acids
-nucleotides
-carbon

MHC Class II Presentation

1. All nucleated cells, all somatic cells.

2. Endogenous antigenes
Viral proetins endogenous?

3. Peptide size: nonamer
Proteasome

4. apoptosis in infected or mutated cells

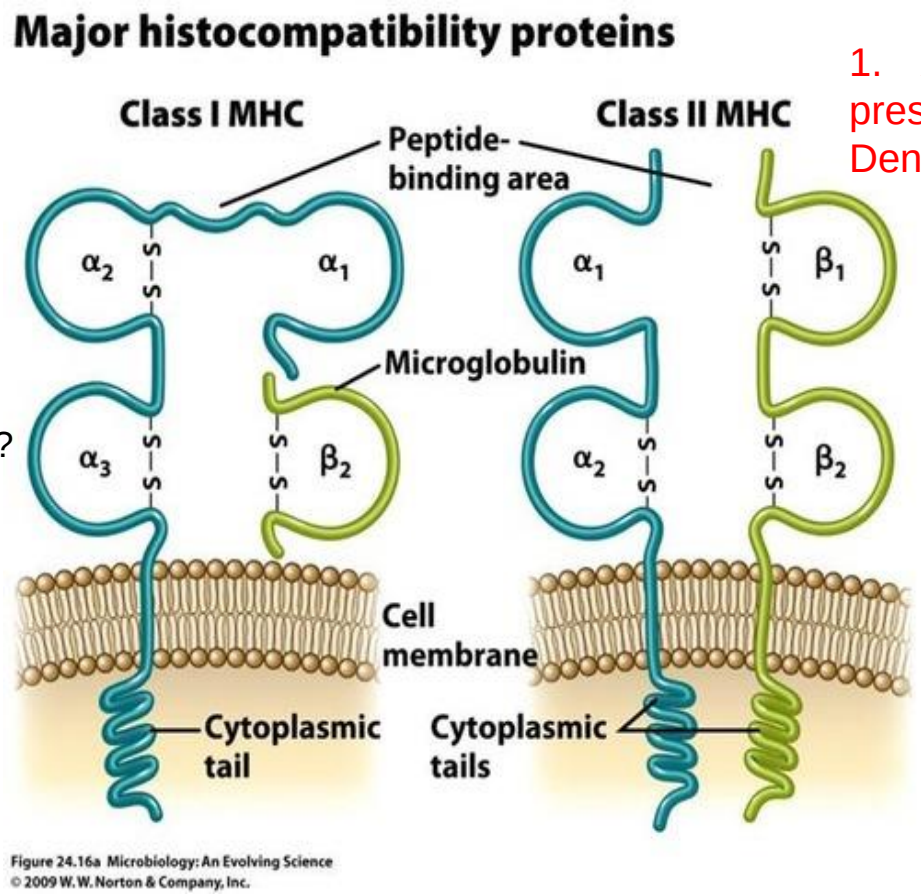


Figure 24.16a Microbiology: An Evolving Science
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1. All professional antigen presentig cells e.g. macrophages, Dentritic cells and B cells.

2.Exogenous antigenes

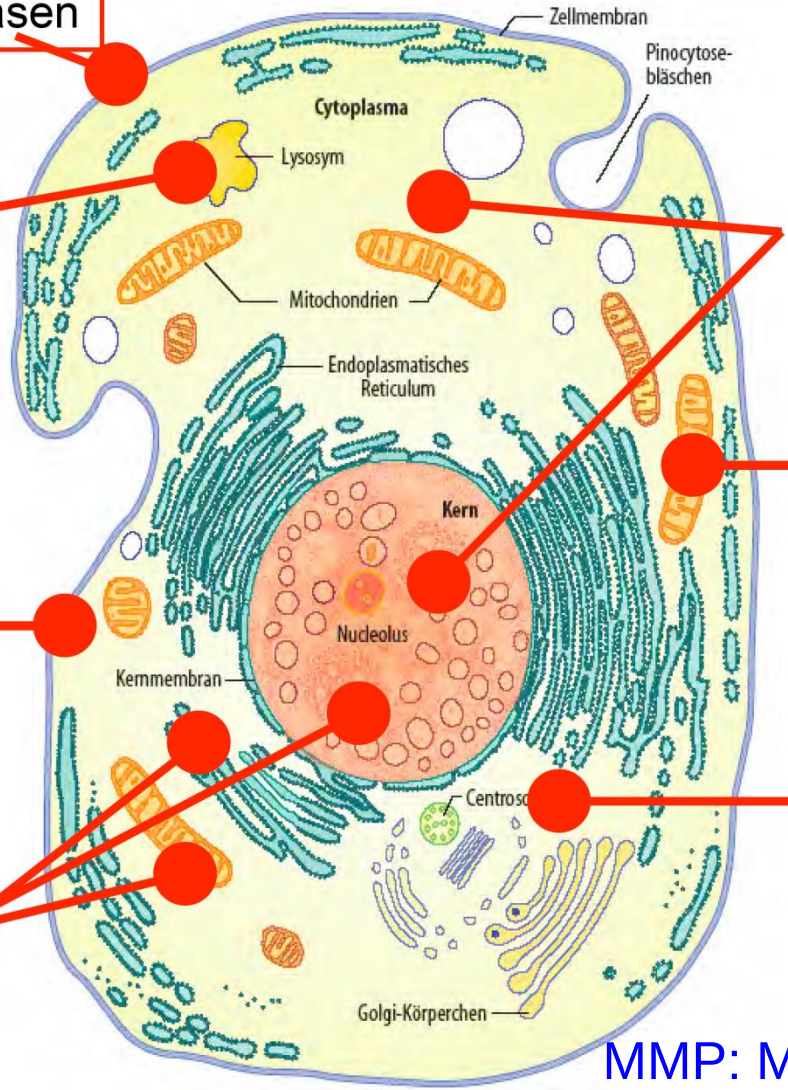
3. 18-20-mer
Lysosome

4. specific immune reaction: production of antibodies, formation of memory cells, apoptosis

Fragen:

- Autophagie-Typen
- Autophagie Signale: extra- und intrazellular
- Autophagie-Transkriptionsfaktoren
- Autophagie –Substrate
- Schritte von selektiver Makroautophagie
- Gemeinsamkeiten und Unterschiede zwischen UPS und selektiver Makroautophagie
- Autophagie-assoziierte Erkrankungen

Localization of cellular proteases



2

Membran-Proteasen

Autophagie

1

UPS

DUB

100 !

Mitochondriale Proteasen

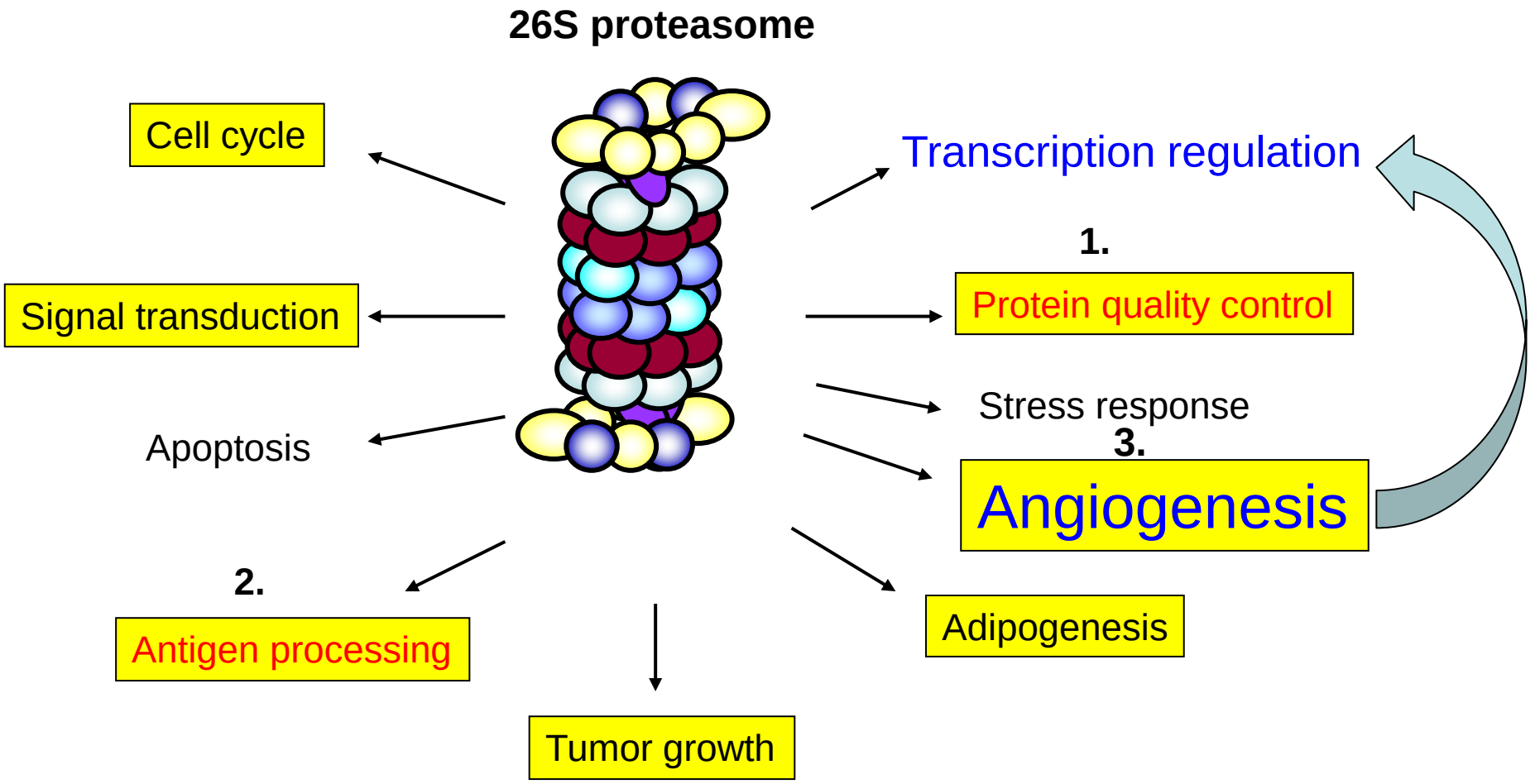
Calpaine

Caspasen

Prozessierende Proteasen

MMP: Matrix Metalloproteases
-extracellular
-collagens

Functions of the ubiquitin proteasome system (UPS) in cells



Institut für Experimentelle Innere Medizin
Medizinische Fakultät
Otto-von-Guericke-Universität Magdeburg

VL 3 (Dr. Dawadschargal Dubiel)

Angiogenesis

Outlines:

1. Definition
2. Angiogenesis types:
   - Normal angiogenesis
   - Tumor angiogenesis
3. Angiogenic factors:
   - Proangiogenic factors: e. g. VEGF
   - Transcription factors: e.g. HIF-1α, ß-catenin, c-Jun, NF-kB
4. Tumor angiogenesis phases:
   - Initiation
   - Proliferation invasion
   - Maturation
5. Desired and undesired angiogenesis
6. Treatment of cancer
7. Questions

What is angiogenesis?

Angio = tube, vessel (Gefäß)

Angiogenesis = blood vessel formation from preexisting tubes

distinct from angioneogenesis, tubulogenesis, vasculogenesis, formation of new blood vessels

Definition:

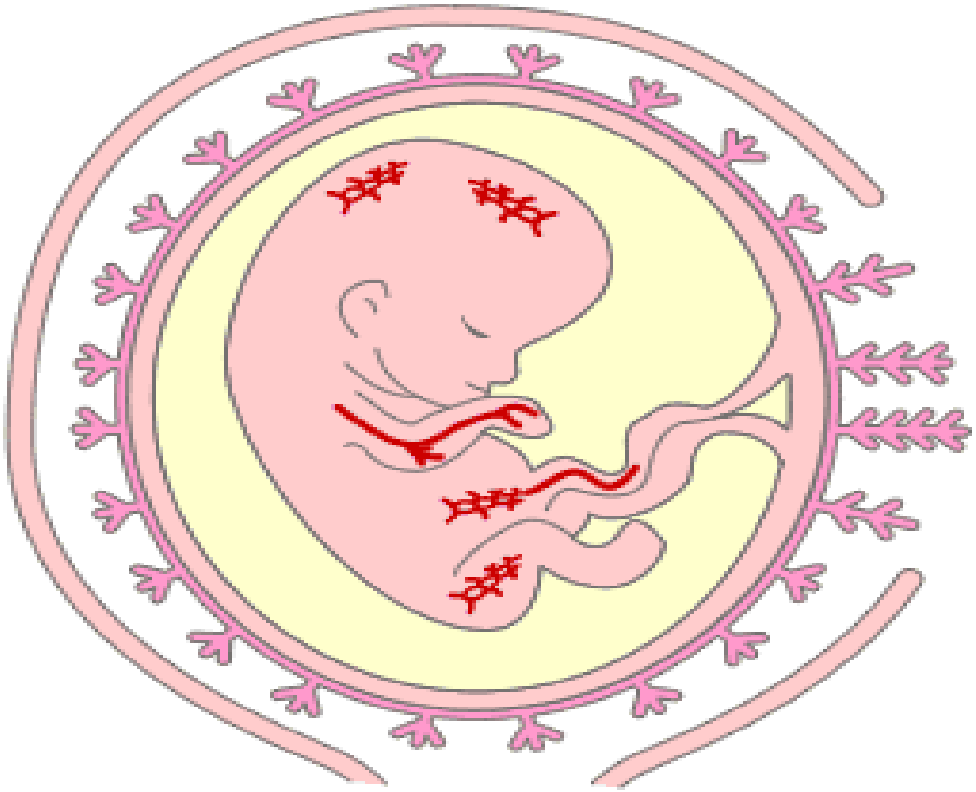
Angiogenesis describes a process of cell migration and cell differentiation that leads to the formation of blood vessels from preexisting tubes.

Under which conditions angiogenesis is switched on?

Normal angiogenesis:

1. **Embryogenesis**
2. **Menstruation**
3. **Wound healing**
4. **Obesity, adipose tissue expansion requires vascularization**

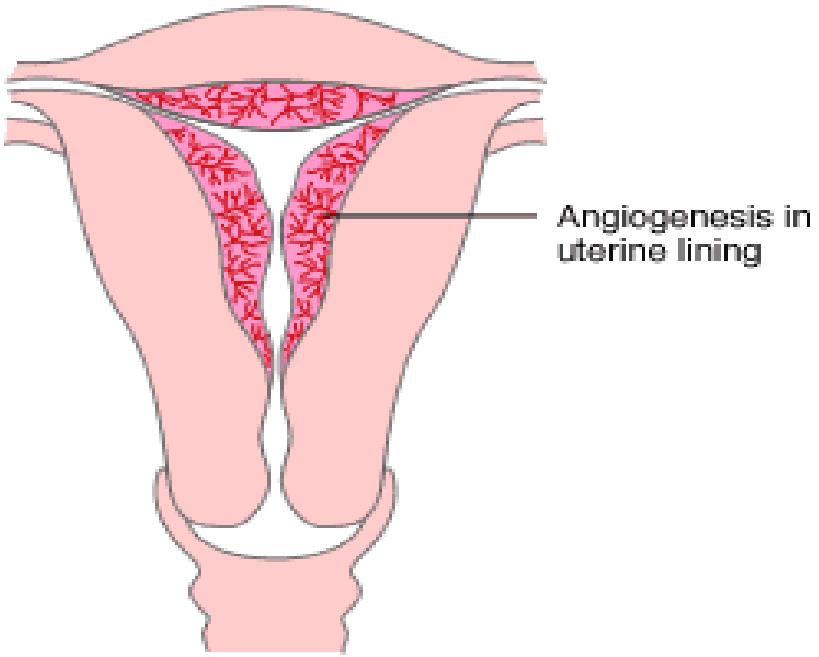
Embryogenesis

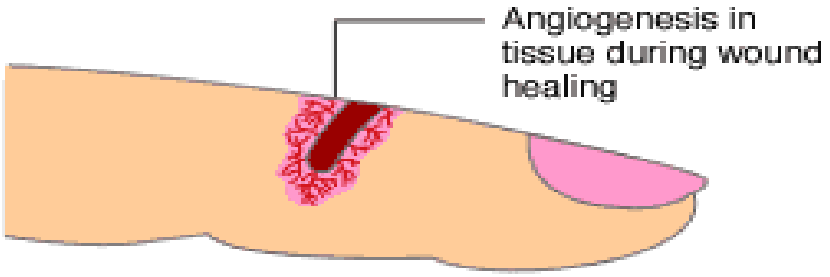


Normal Angiogenesis in Children - Angioneogenesis

In addition to its role in tumors, angiogenesis occurs normally in the human body at specific times in development and growth. For example, a developing child in a mother's womb must create the vast network of arteries, veins, and capillaries that are found in the human body. A process called ***vasculogenesis*** creates the primary network of vascular endothelial cells that will become major blood vessels. Later on, angiogenesis remodels this network into the small new blood vessels or capillaries that complete the child's circulatory system.

Menstruation and wound healing





Normal Angiogenesis in Adults

Proliferation of new blood vessels also takes place in adults, although it is a relatively infrequent event.

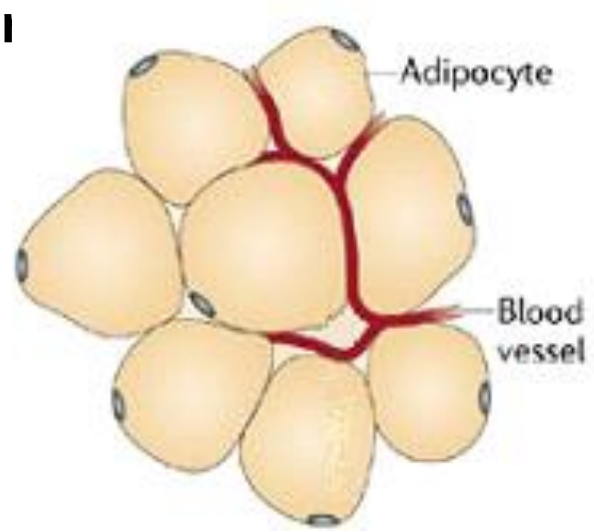
In women, angiogenesis is active a few days each month as new blood vessels form in the lining of the uterus during the menstrual cycle.

Also, angiogenesis is necessary for the repair or regeneration of tissue during wound healing.

Obesity needs angiogenesis

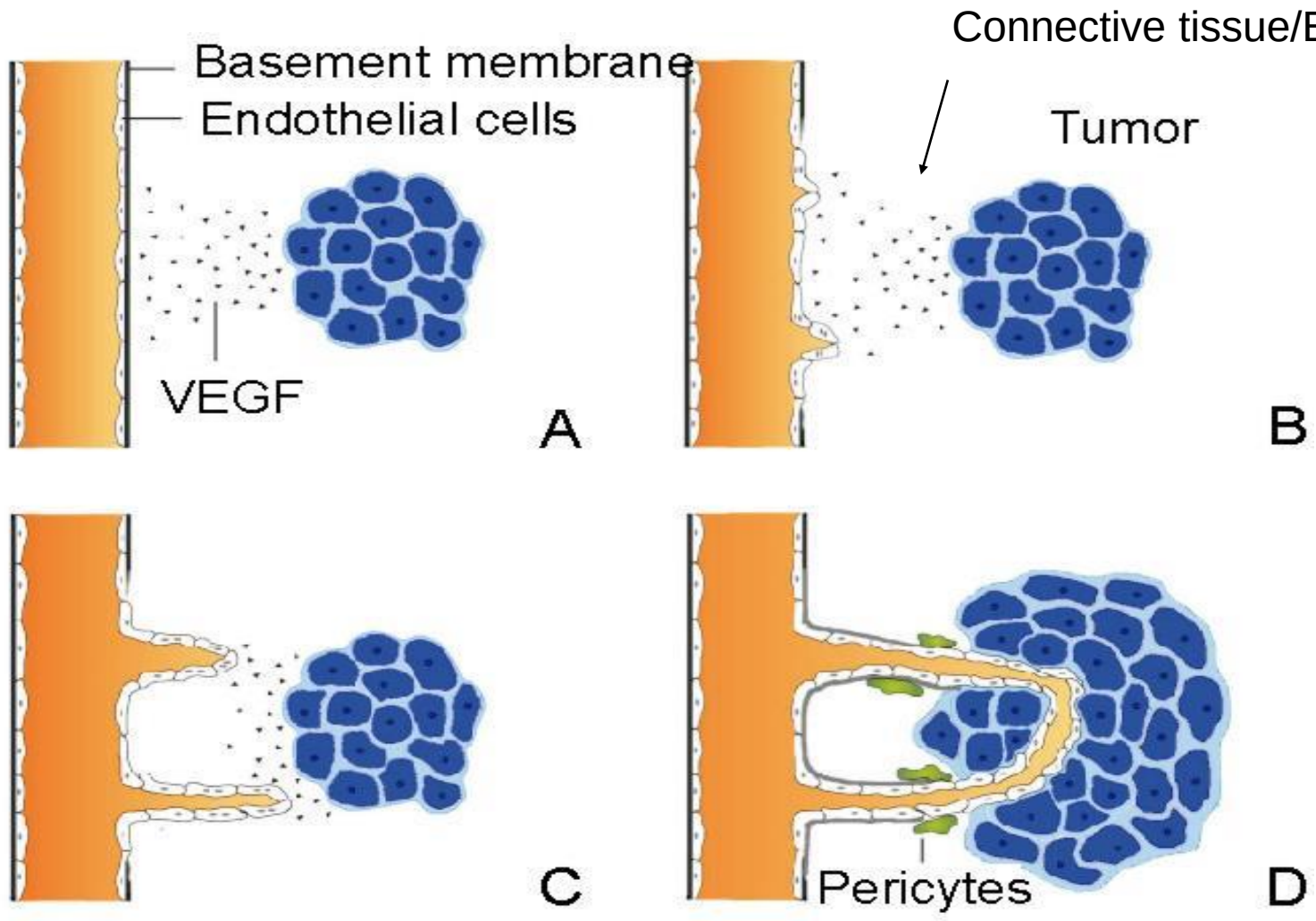
- **The adipose tissue growth by cell proliferatio differentiation of preadipocytes called adipogenesis**

- **Differentiating preadipocytes produce proangigenic factors such as VEGF**



❖ **Adipogenesis needs angiogenesis t**o supply growing adipose tissue with nutrients and oxygen, the vasculature responds by increasing the number and/or size of blood vessels.

Process of tumor angiogenesis, the formation of tubes from preexisting vessels



(Smooth muscle cells)

Factors necessary for angiogenesis

1. **Which factors are involved?**

Pro-angiogenic factors

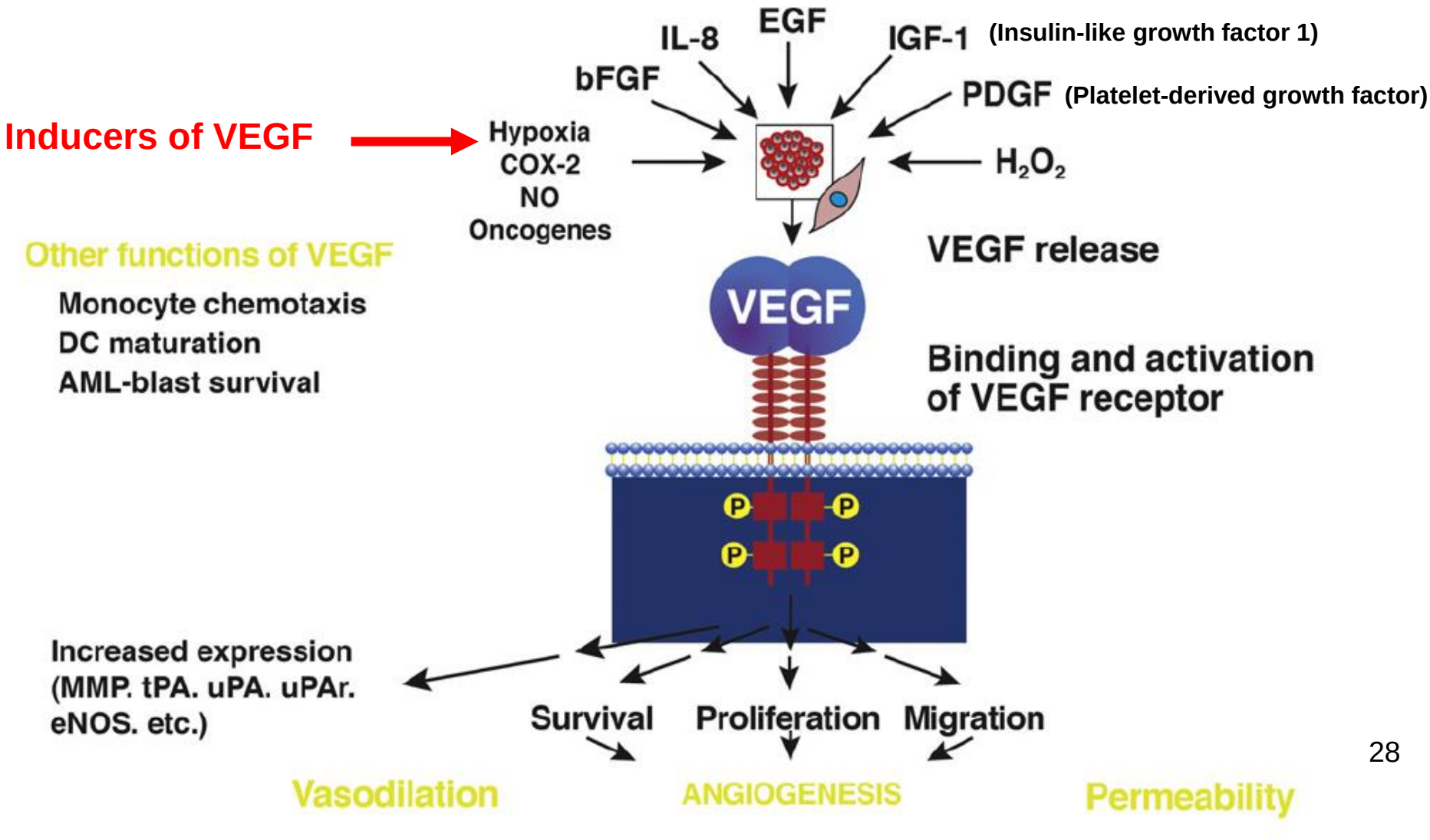
- FGF = fibroblast growth factor
- VEGF = vascular endothelial growth factor
- EGF = epithelial growth factor
- TNFα = tumor necrosis factor α
- Interleukins and prostaglandins
- HIF-1α
- β-catenin
- NF-κB
- c-Jun

} **transcription factors**

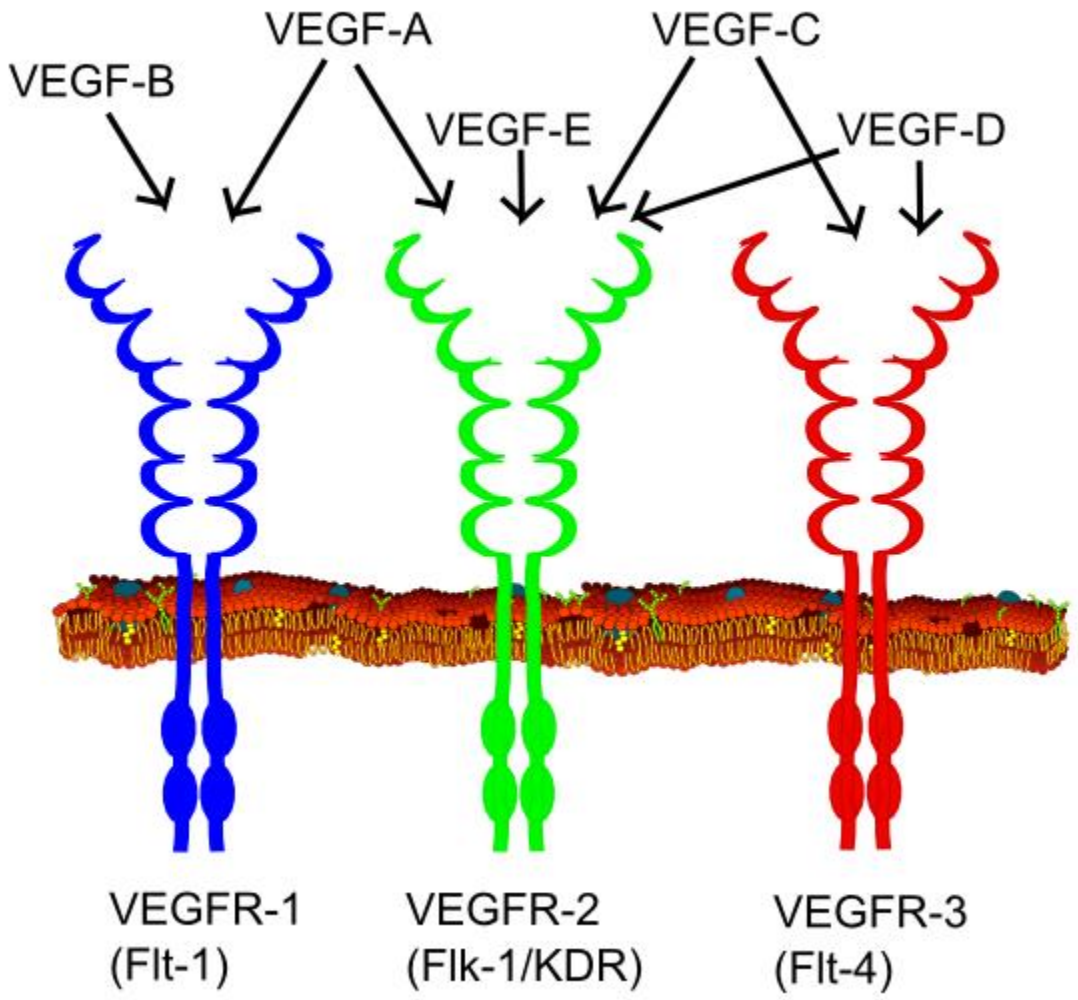
2. **Which cells are involved?**

- Endothelial cells
- Similar to smooth muscle cells (Pericytes)
- Fibroblasts (cells of the ECM)

The VEGF plays a central role in angiogenesis



The VEGF receptors



VEGF is induced by a number of transcription factors:

- HIF-1α (Hypoxia-inducible factor)

– β-catenin

- NF-κB

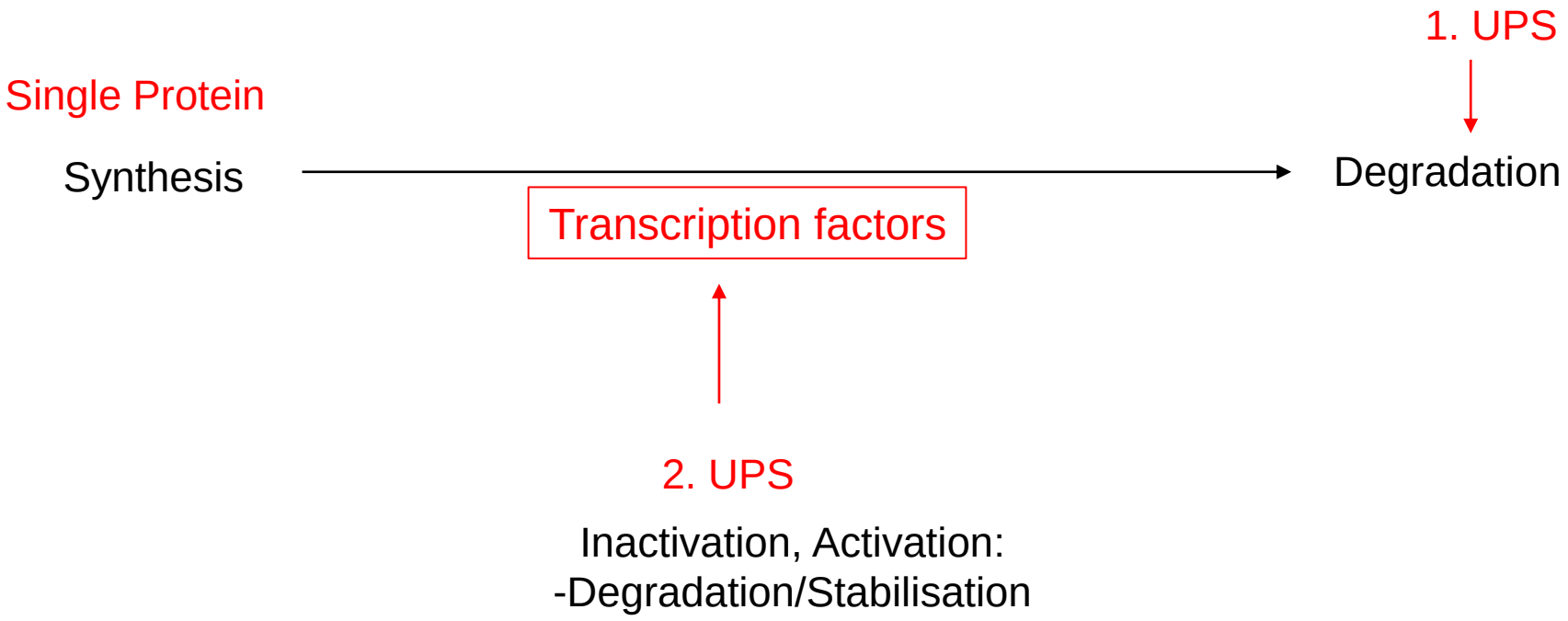
- c-Jun

Protein (Eiweß)_Turnover

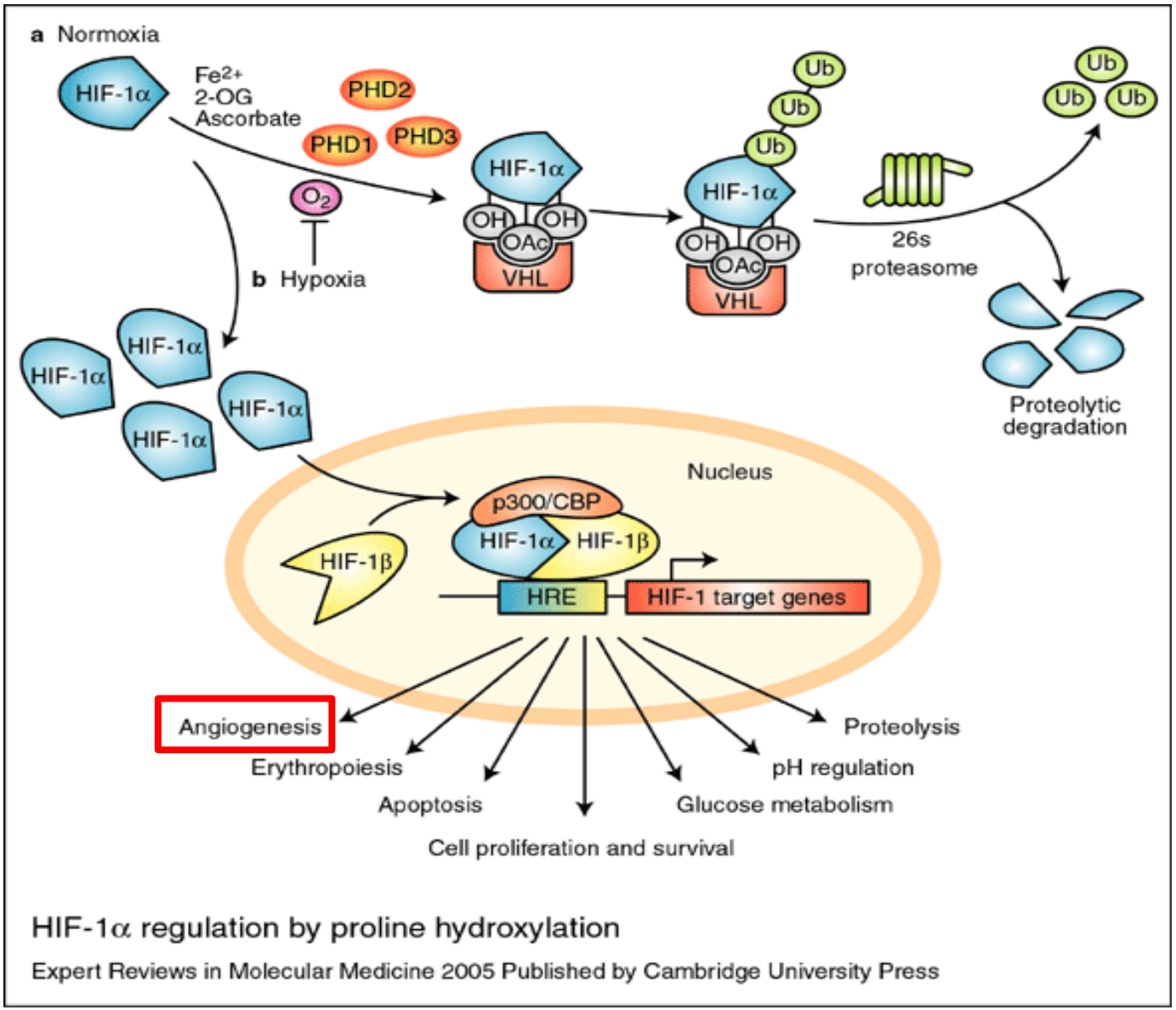
Definition: In cell biology, protein turnover refers to the **replacement of older proteins as they are broken down within the cell by new synthesized protein**.

Different types of proteins have very different turnover rates.

A rate of new synthesized protein is equal to rate of degrading protein: Steady state



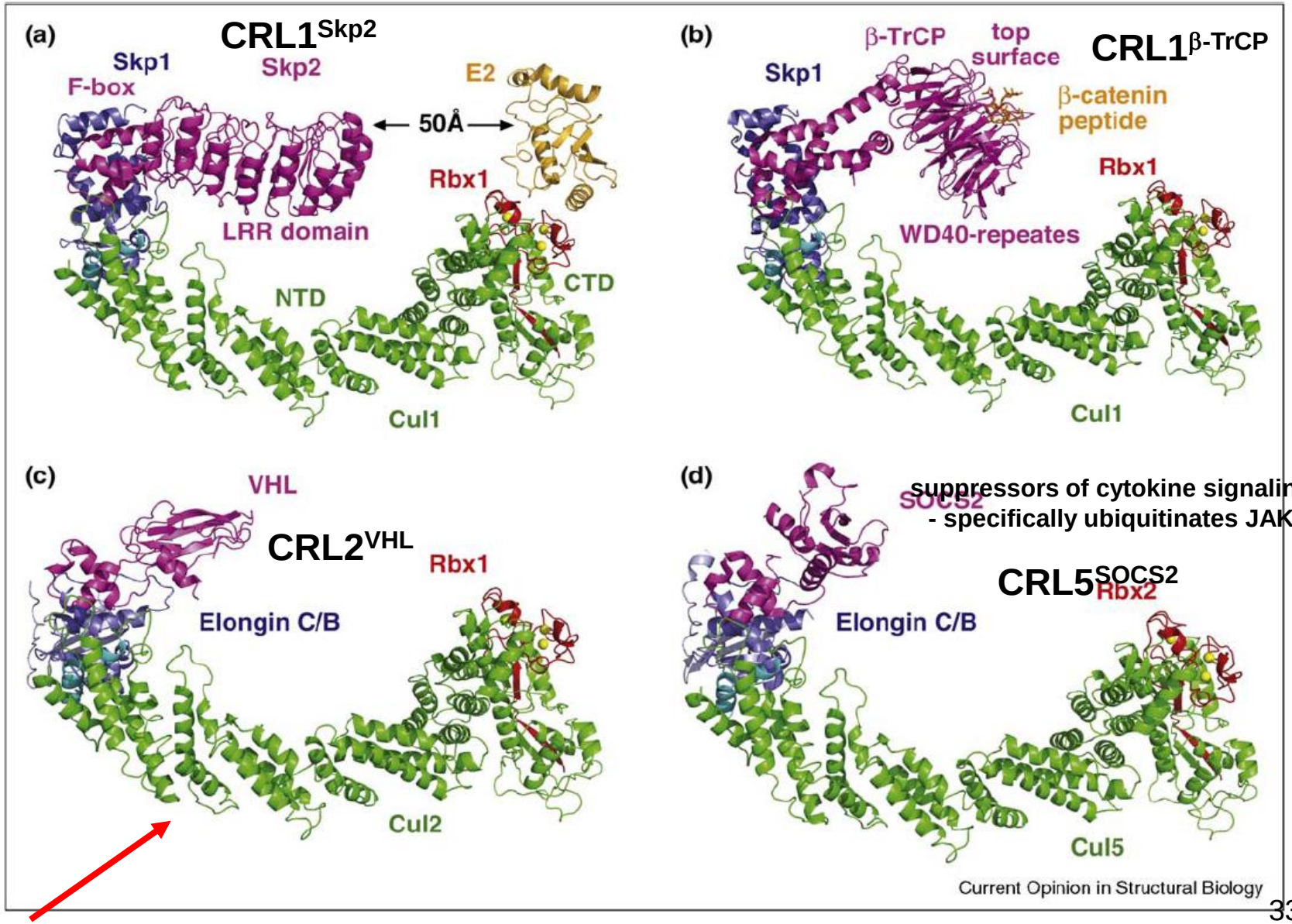
Regulation of HIF-1α by oxygen



HIF-1α regulation by proline hydroxylation

Expert Reviews in Molecular Medicine 2005 Published by Cambridge University Press

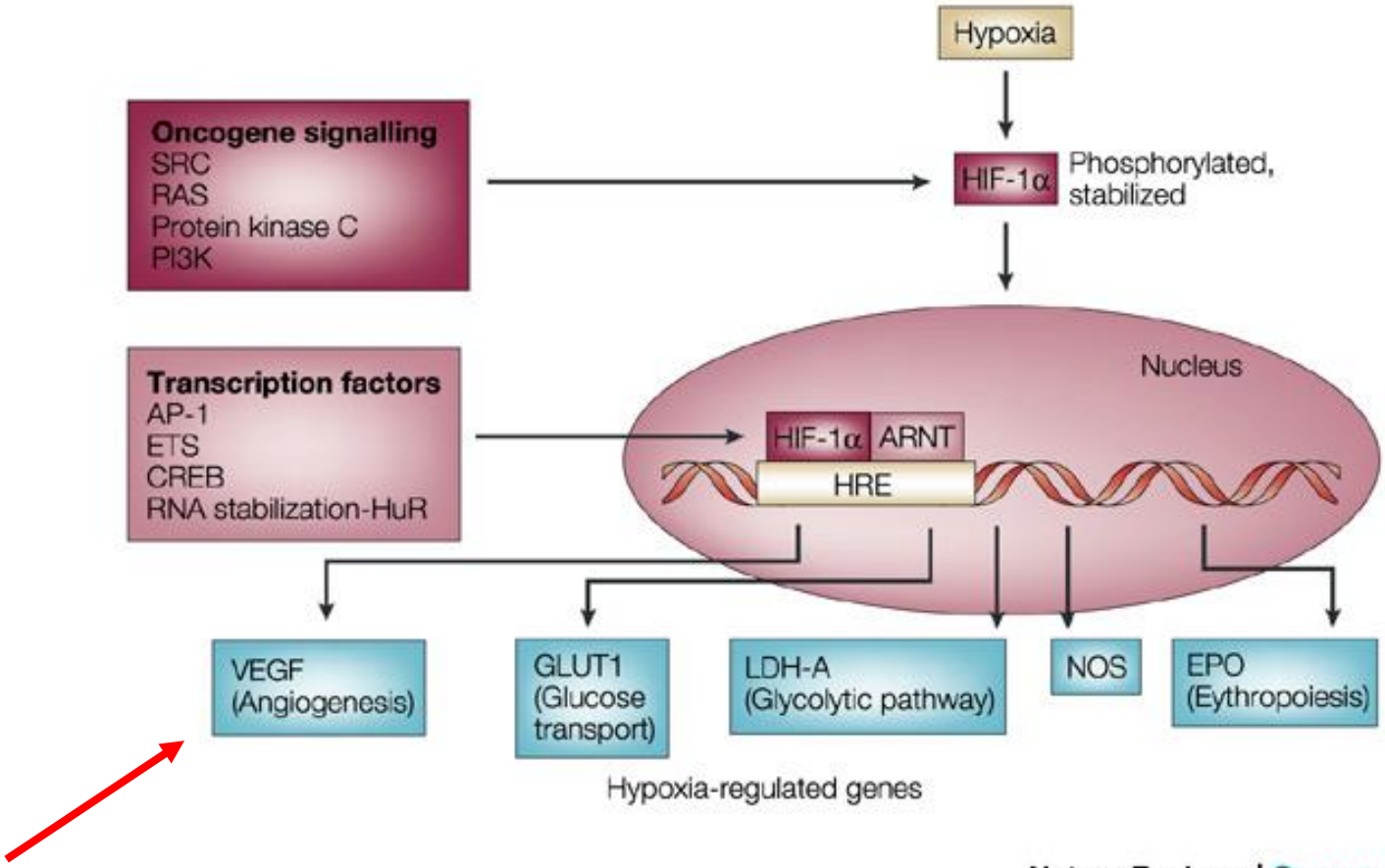
Structure of CRLs



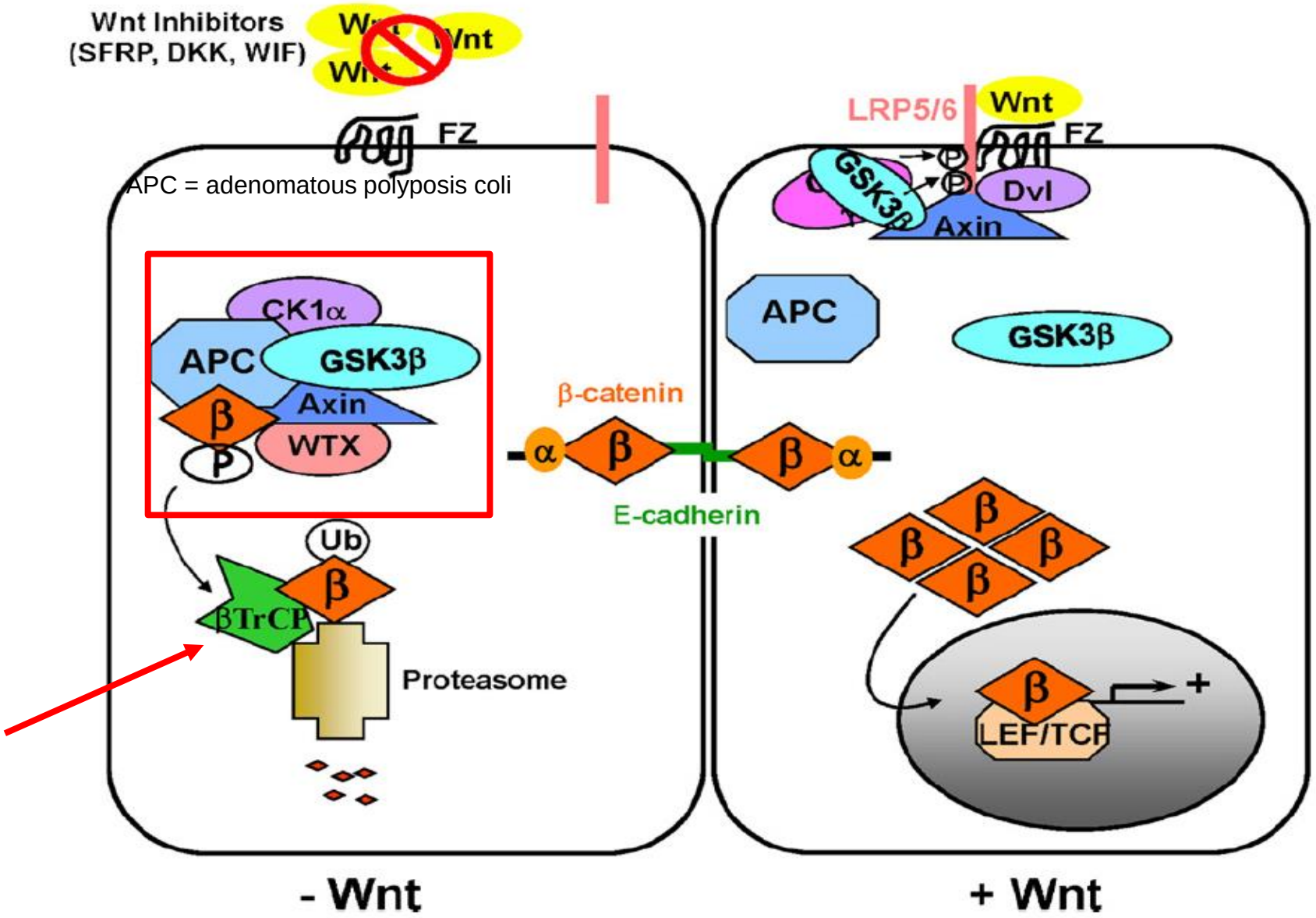
VHL (von Hippel Lindau protein) is the Ub E3 Ligase for HIF1 alpha

Hypoxia

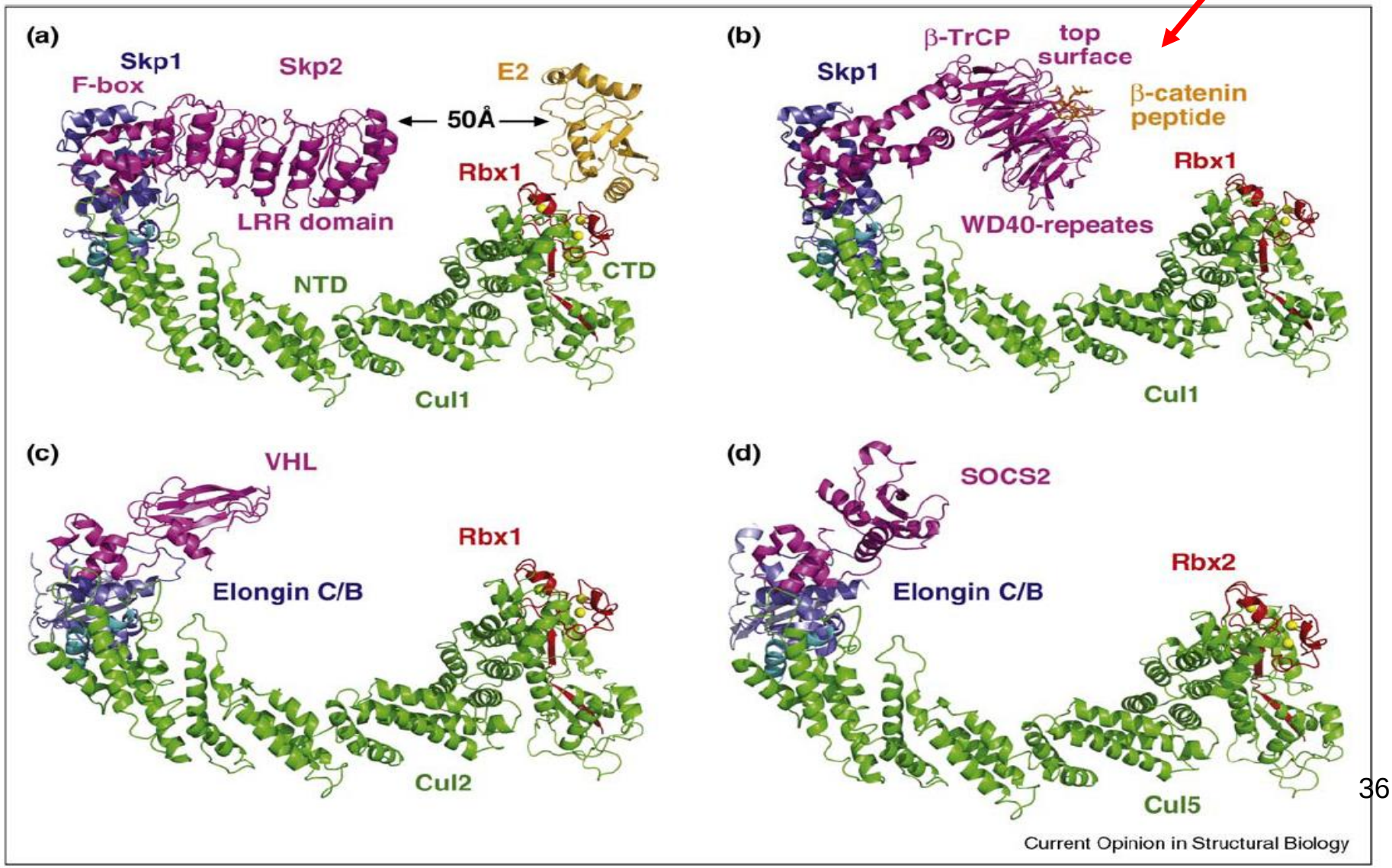
Target genes of HIF-1α



The Wnt/β-catenin signaling pathway

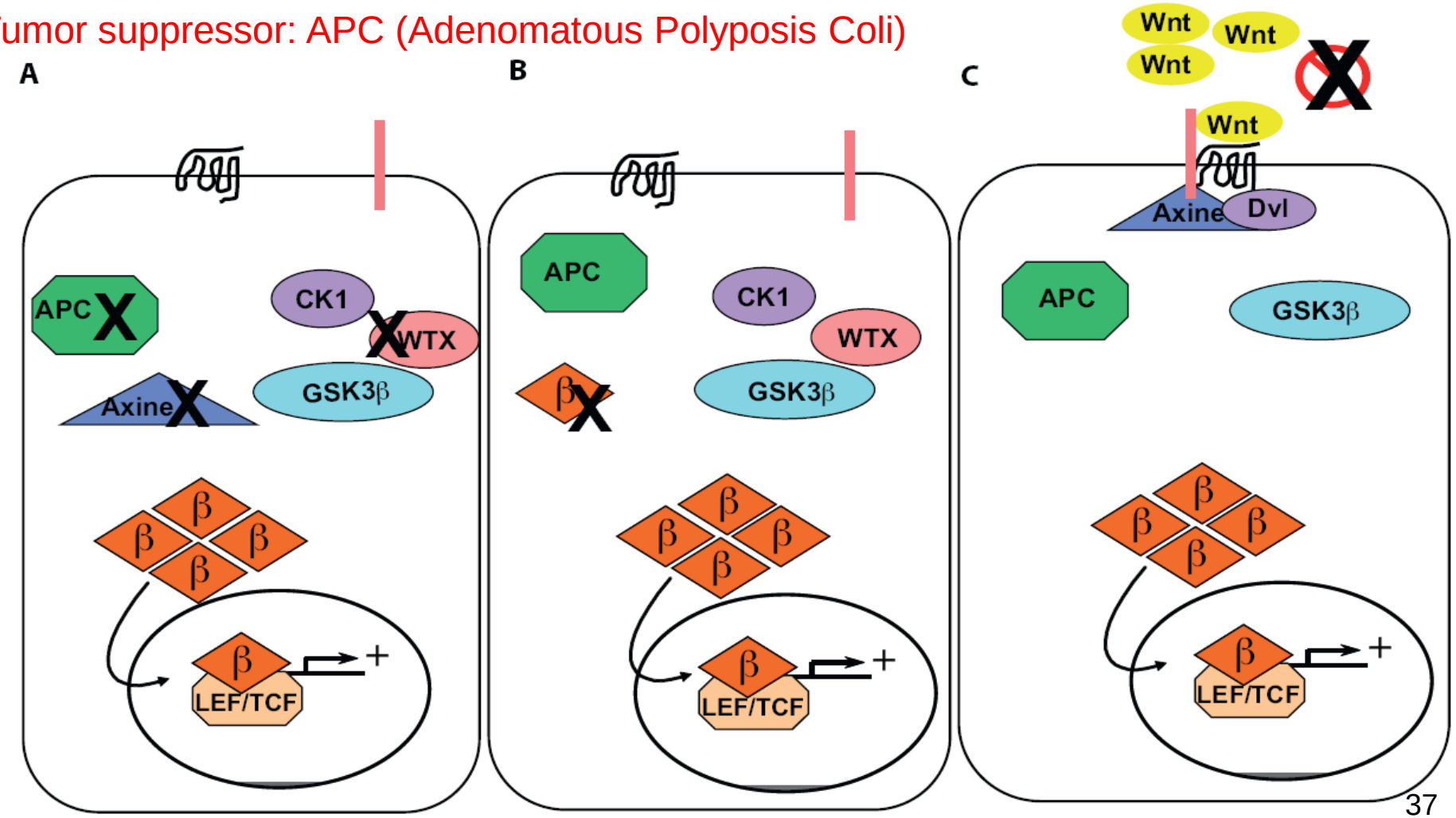


Beta-catenin is ubiquitinated by CRL1-β-TrCP



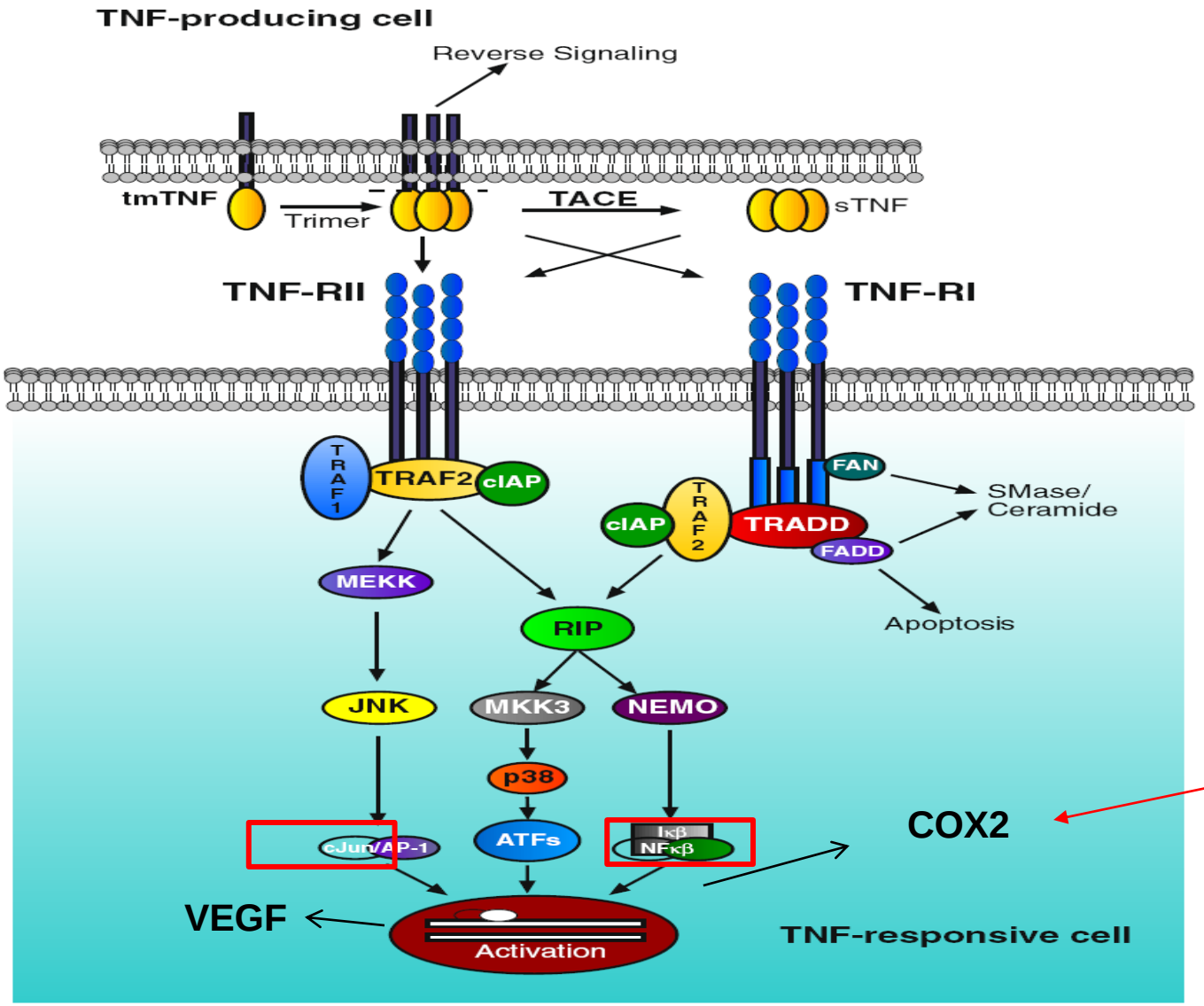
Mutations in components of the Wnt/β-catenin pathway result in cancer

Tumor suppressor: APC (Adenomatous Polyposis Coli)

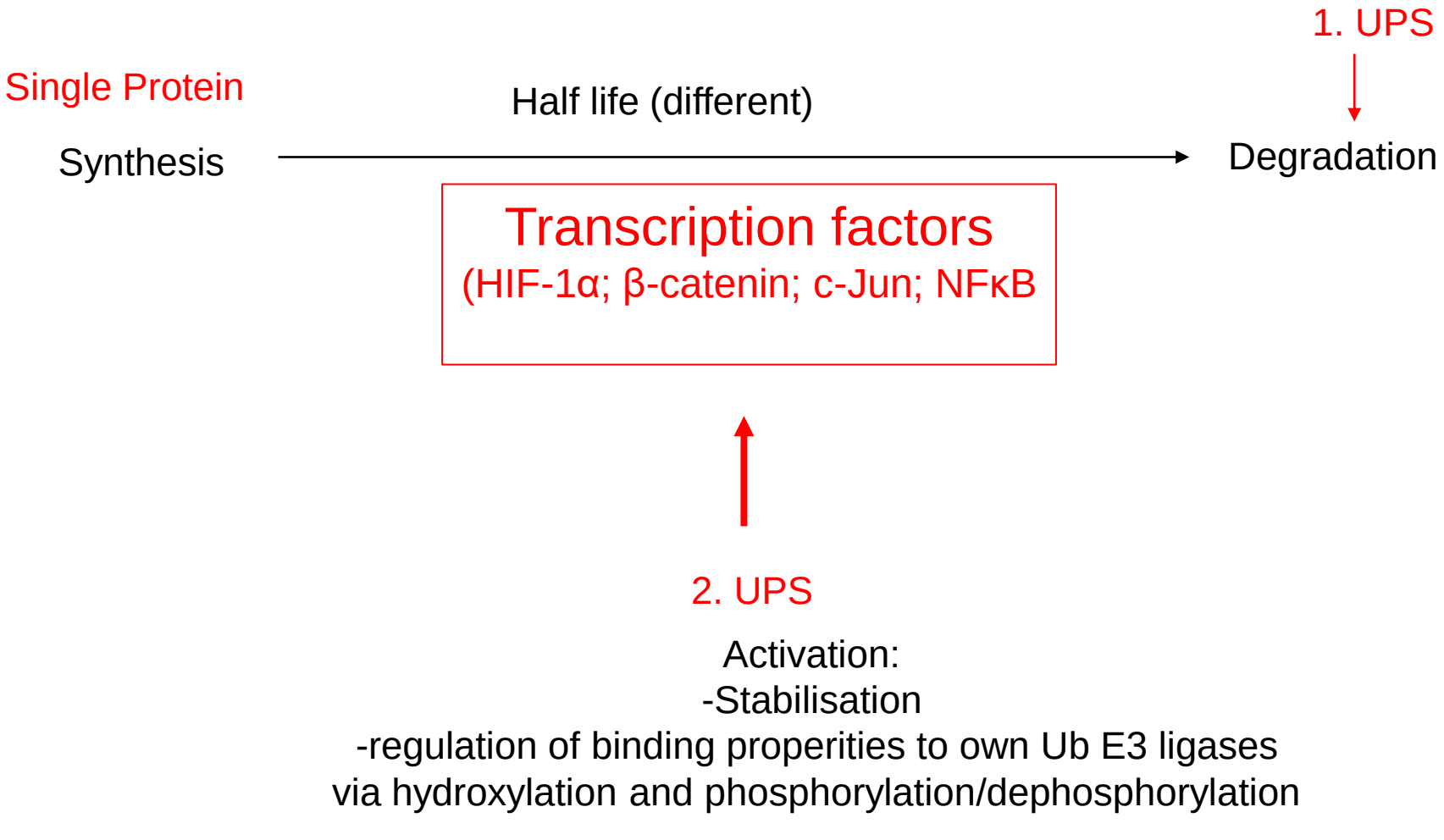


(WTX=Wilms tumor suppressor)

TNF increases the VEGF production via c-Jun and NF-κB

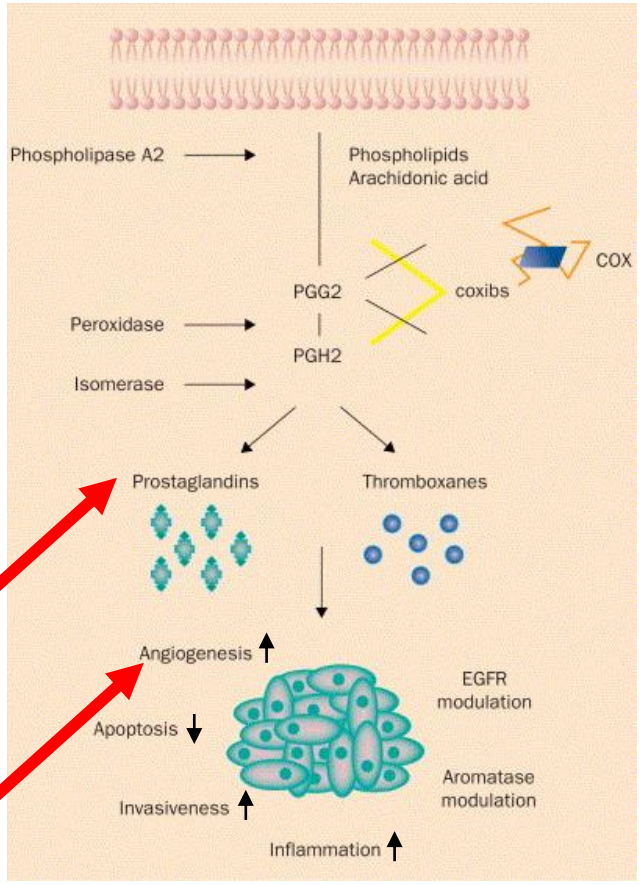


Transcription factors_Turnover



COX-2 is induced under conditions of inflamation and tumor growth

Gasparini et al. 2003 The Lancet Oncology 4, 605-615



Premalignant or malignant lesion	COX2 expression (%)
Colorectal	80–90
Gastric	80
Oesophageal	70
Hepatocellular (liver cirrhosis)	54 (81)
Pancreatic	67
Head and neck	80
Non-small-cell lung cancer	70
Breast (ductal carcinoma-in-situ)	40 (60)
Prostatic	83–93
Bladder	86
Cervix	43
Endometrial	37
Cutaneous basal cell	25
Cutaneous squamous cell	80
pPNET	100
Glioblastoma multiforme	71–74
Anaplastic astrocytoma (low grade)	44 (30)

References available at http://image.thelancet.com/extras/03oncl205webfr.pdf

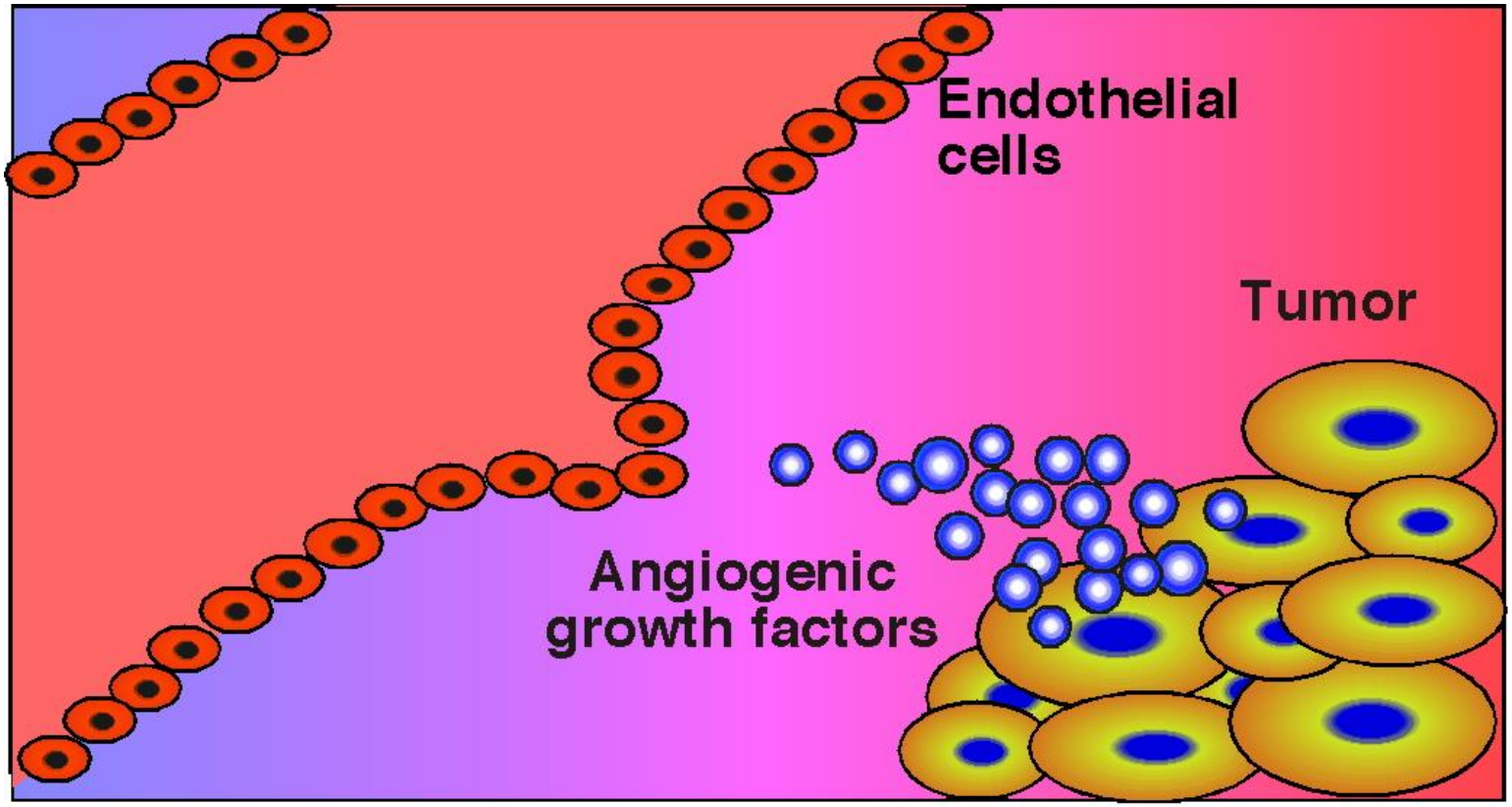
Specific inhibition or degradation of COX-2 has high anti-tumor therapeutic significance

Phases of Tumor Angiogenesis

1. Initiation
2. Proliferation and invasion
3. Maturation

1. Initiation of tumor angiogenesis

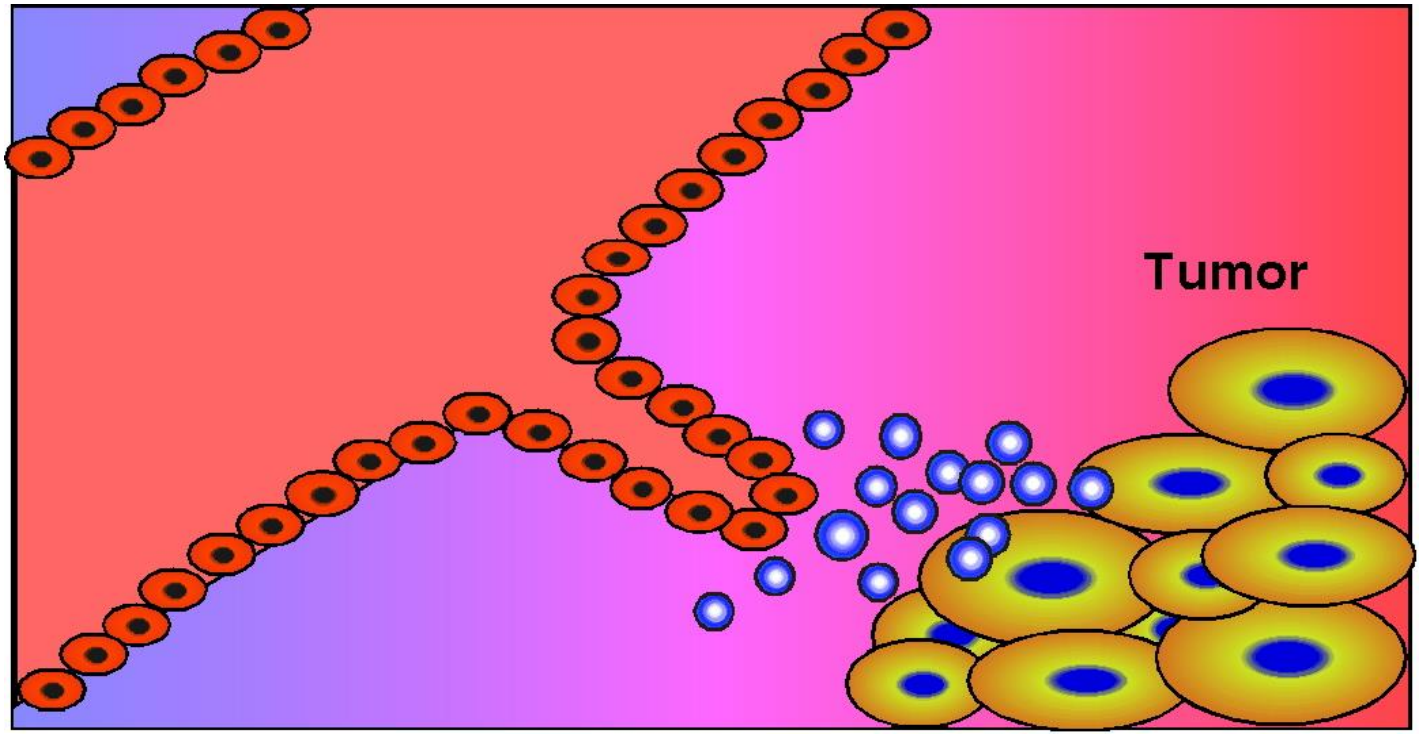
The angiogenic switch



Characteristics of human dormant tumors

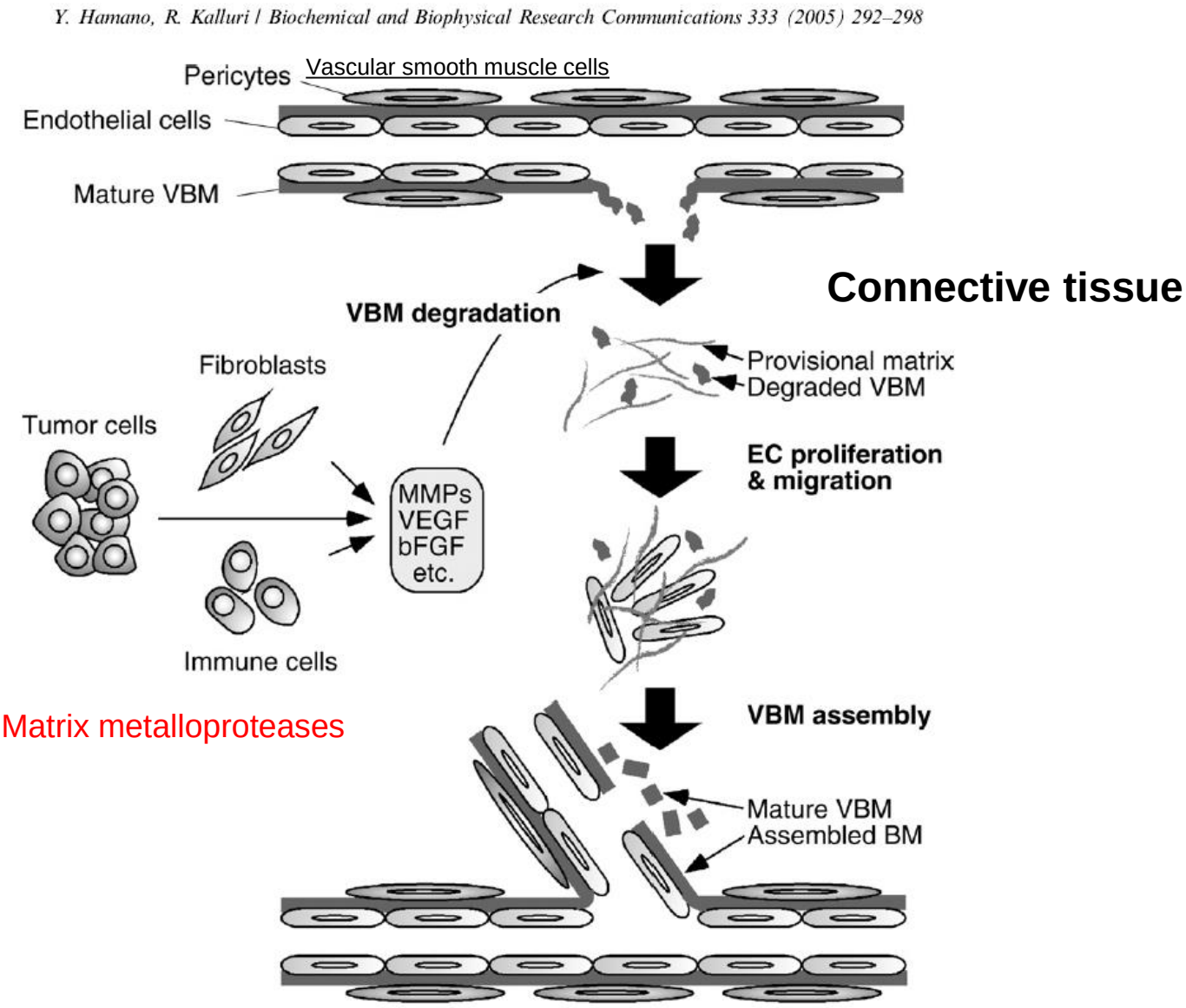
- Possess no angiogenic activity
- Tumors remain limited in size (Ø = 1 mm), because of restricted supply of oxygen and nutrients
- Tumor cell proliferation index can be as high as that of large vascularized tumors
- can persist for long periods of time as microscopic lesions and remain harmless to the host
- The angiogenic switch

2. Proliferation and invasion



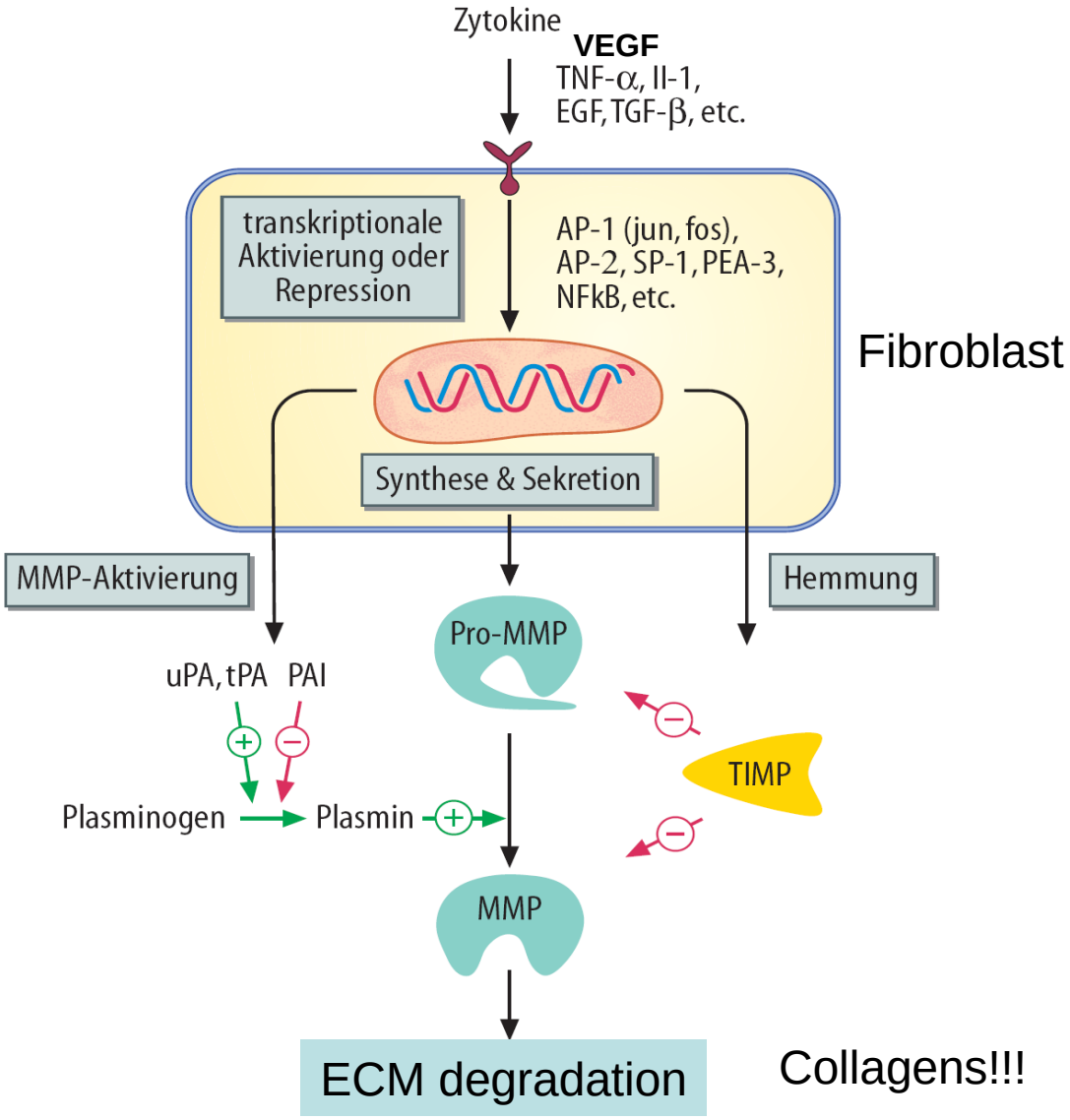
- Endothelial cells: VBM degradation, proliferation and migration of endothel cells, ECM degradation

Role of the Extra Cellular Matrix (ECM)

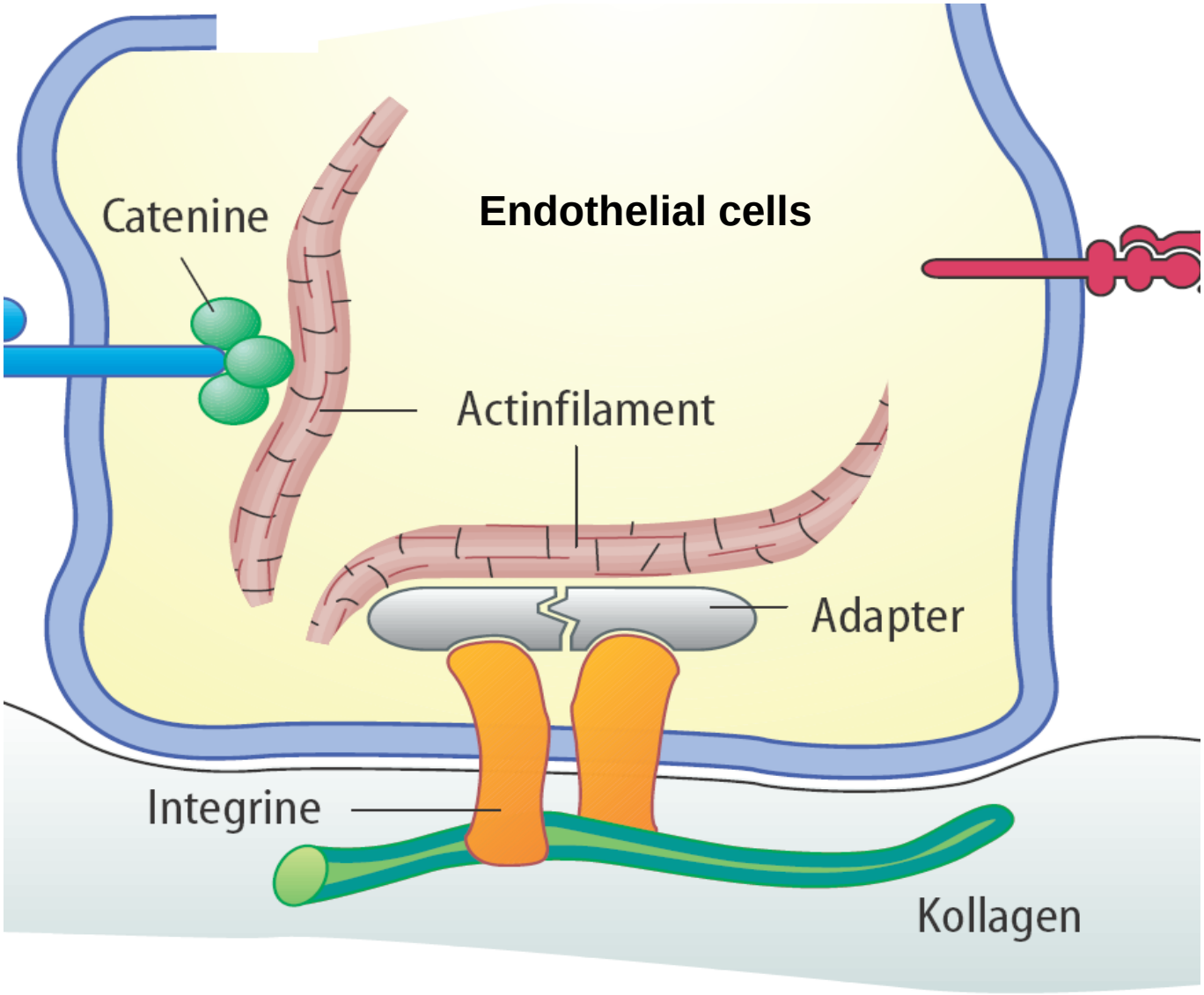


MMPs - Matrix metalloproteases

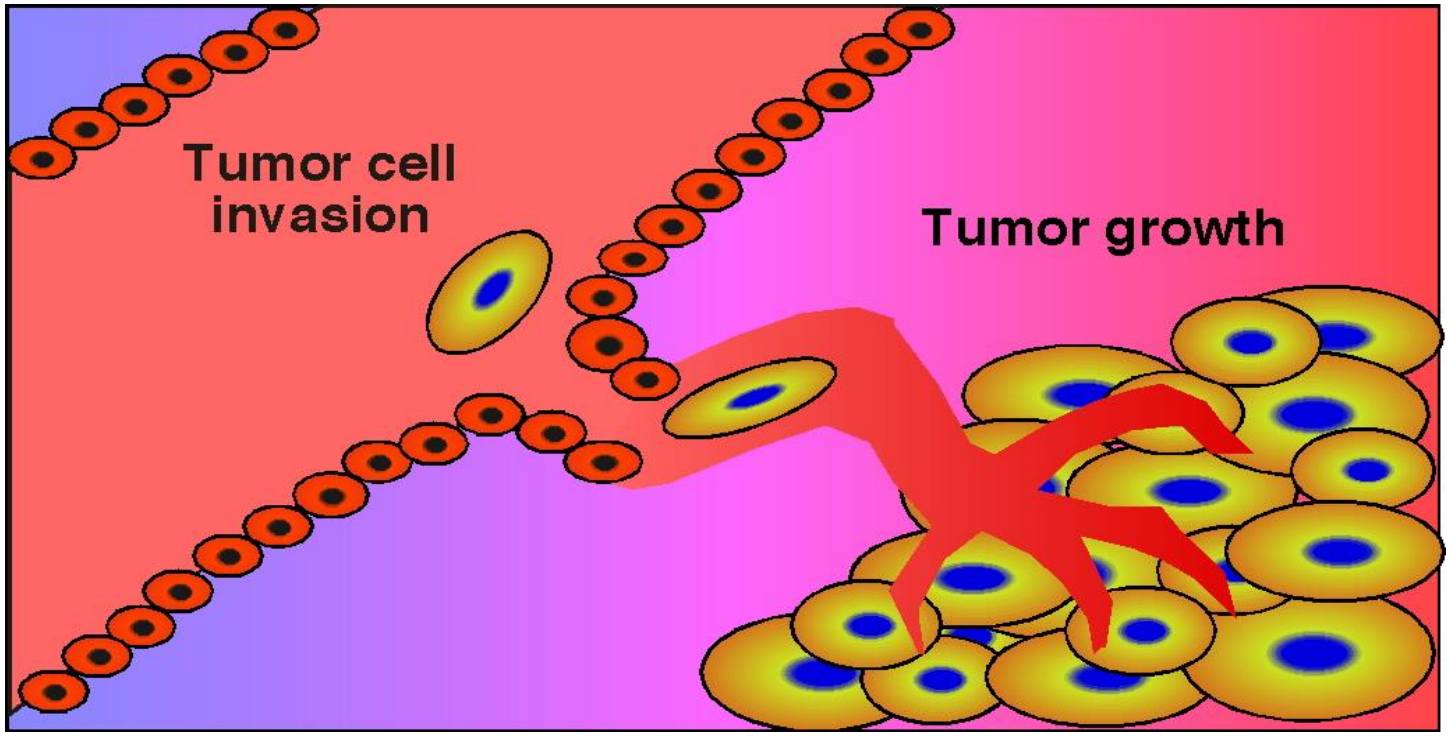
Degradation/Reassembly of the ECM



Mobility of endothelial cells



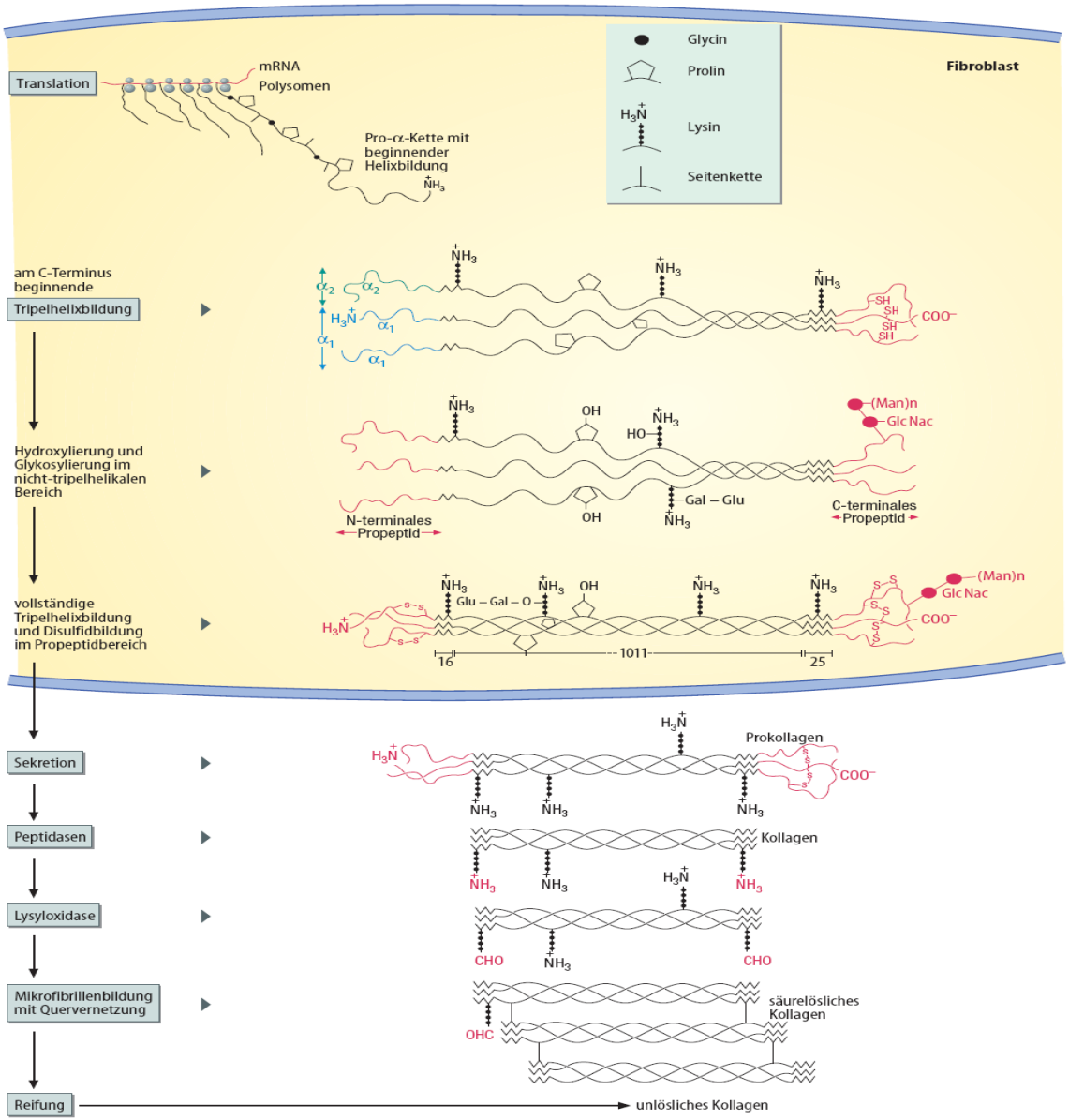
3. Maturation



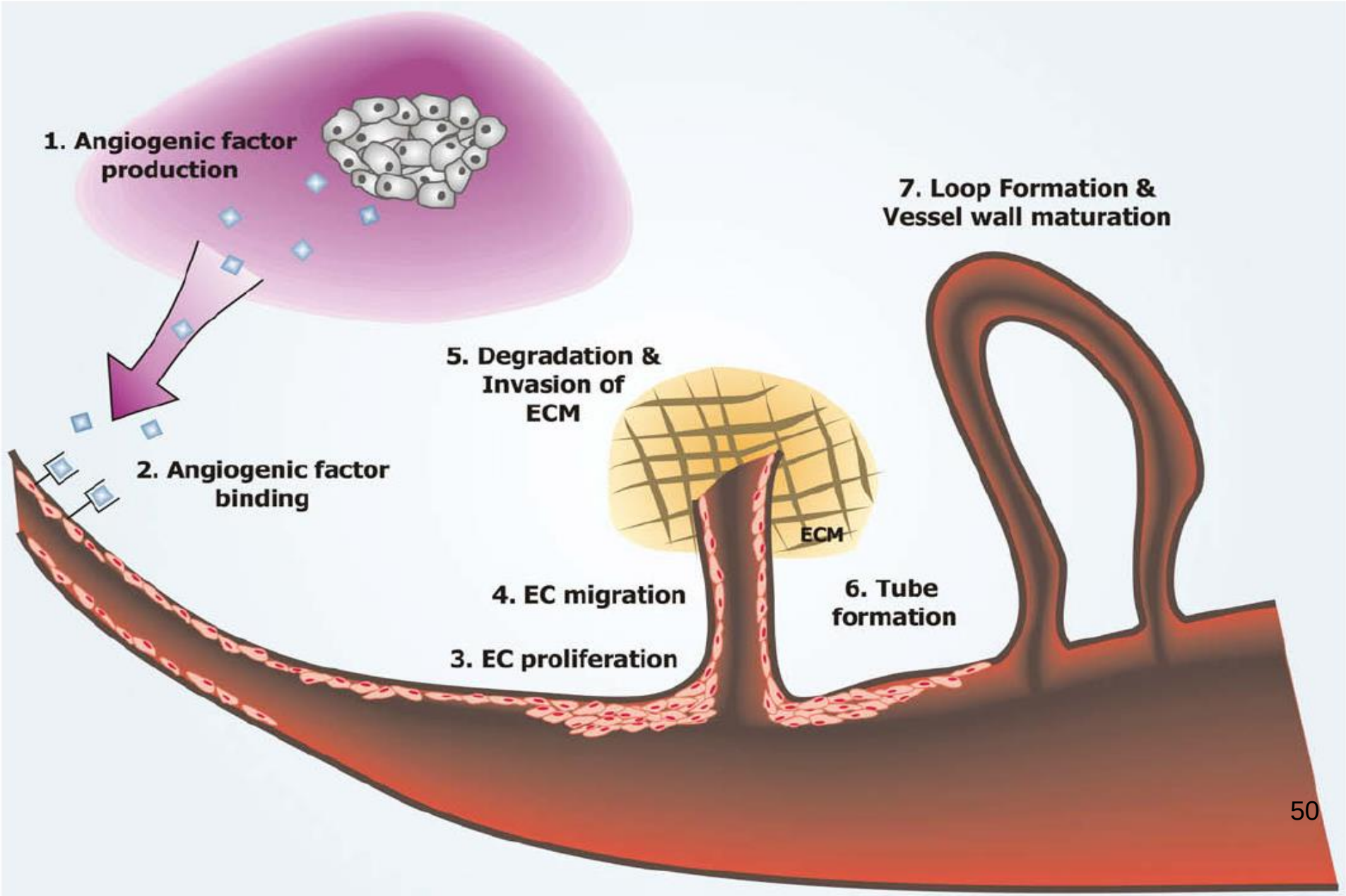
- ECM remodeling (new synthesis),

new synthesis of VBM, pericytes prolifreation, tumor cells migration (metastasis!)

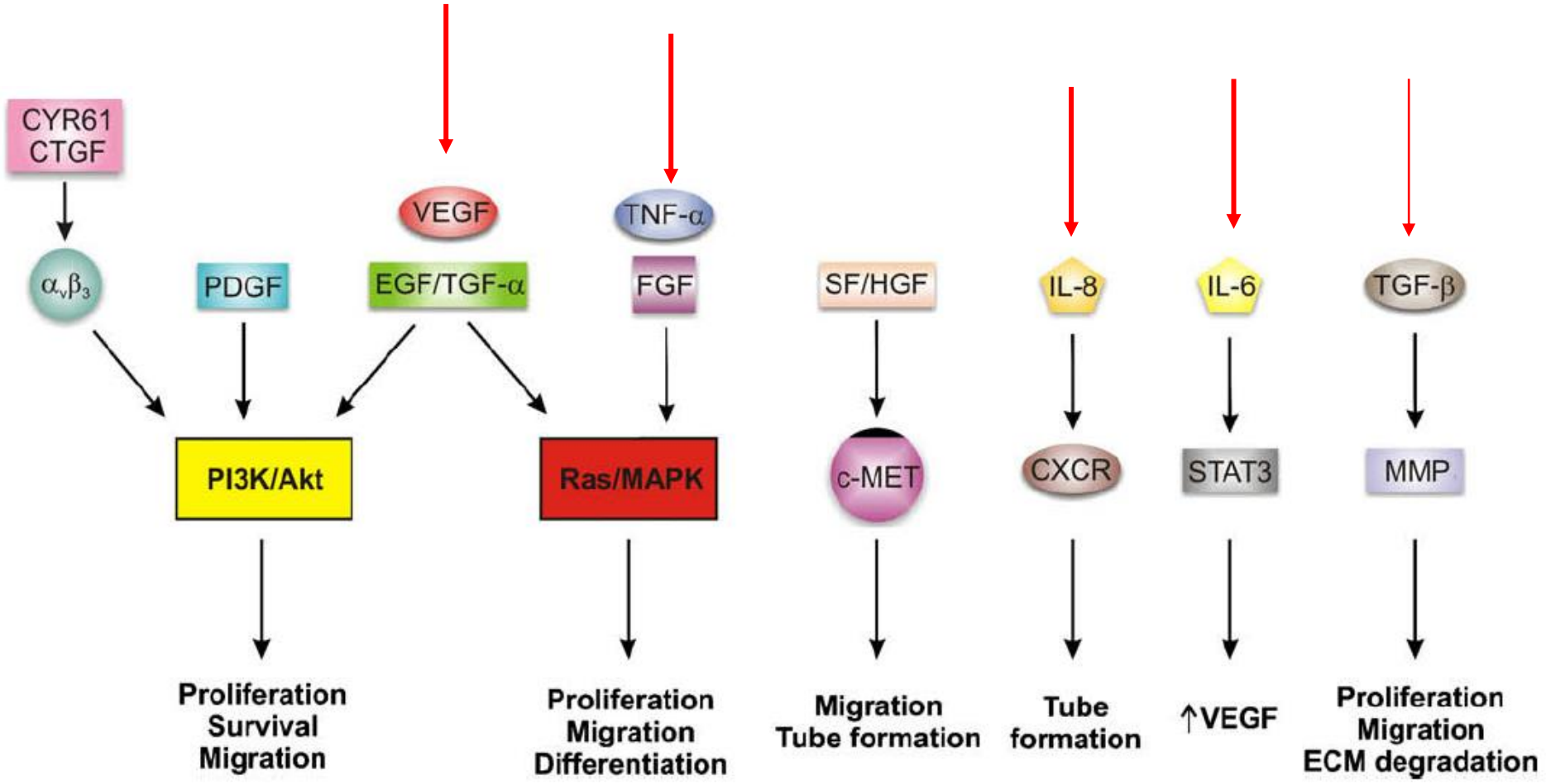
Synthesis of collagen



Tumor Angiogenesis



Angiogenic mediators



HGF=hepatocyte growth factor

Which cells are involved?

- Endothelial cells (Proliferation, migration)
- Visceral smooth muscle cells (Pericytes: vitality of tubes)
- Fibroblasts (ECM remodeling, angiogenesis stimulation)
- By inflammation: immune cells (ECM remodeling, angiogenesis stimulation)
- By tumor angiogenesis: tumor cells (angiogenesis stimulation)
- By obesity: adipocytes, immune cells (angiogenesis stimulation)

Undesired angiogenesis during pathological processes

Undesired: 1. Angiogenesis during inflammation in the muscular-skeletal-system

During degenerative and inflammatory diseases of the muscular-skeletal-system (Arthrose, rheumatoid Arthritis) pro-angiogenic factors (cytokines, prostaglandins) are produced, which induce migration of cells and formation of vessels that support inflammatory processes and degradation of the tissue.

2. Angiogenesis in solid tumours

Desired angiogenesis during pathological processes

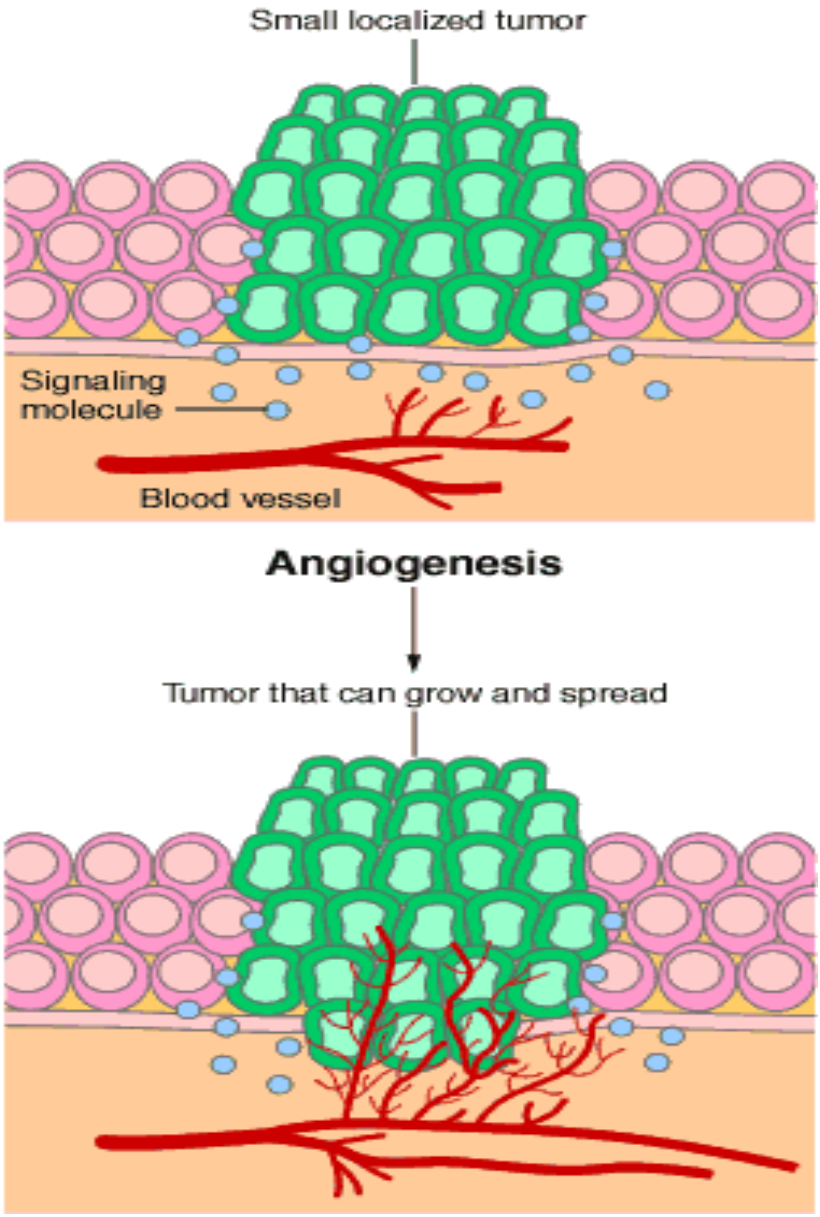
Desired:

1. Angiogenesis after heart attack
   bypass the plug

2. Chronic peripheral arterial occlusive disease (PAOD), leads to amputation of lower extremity

periphere arterielle Verschlusskrankheit

Undesired angiogenesis in solid tumors



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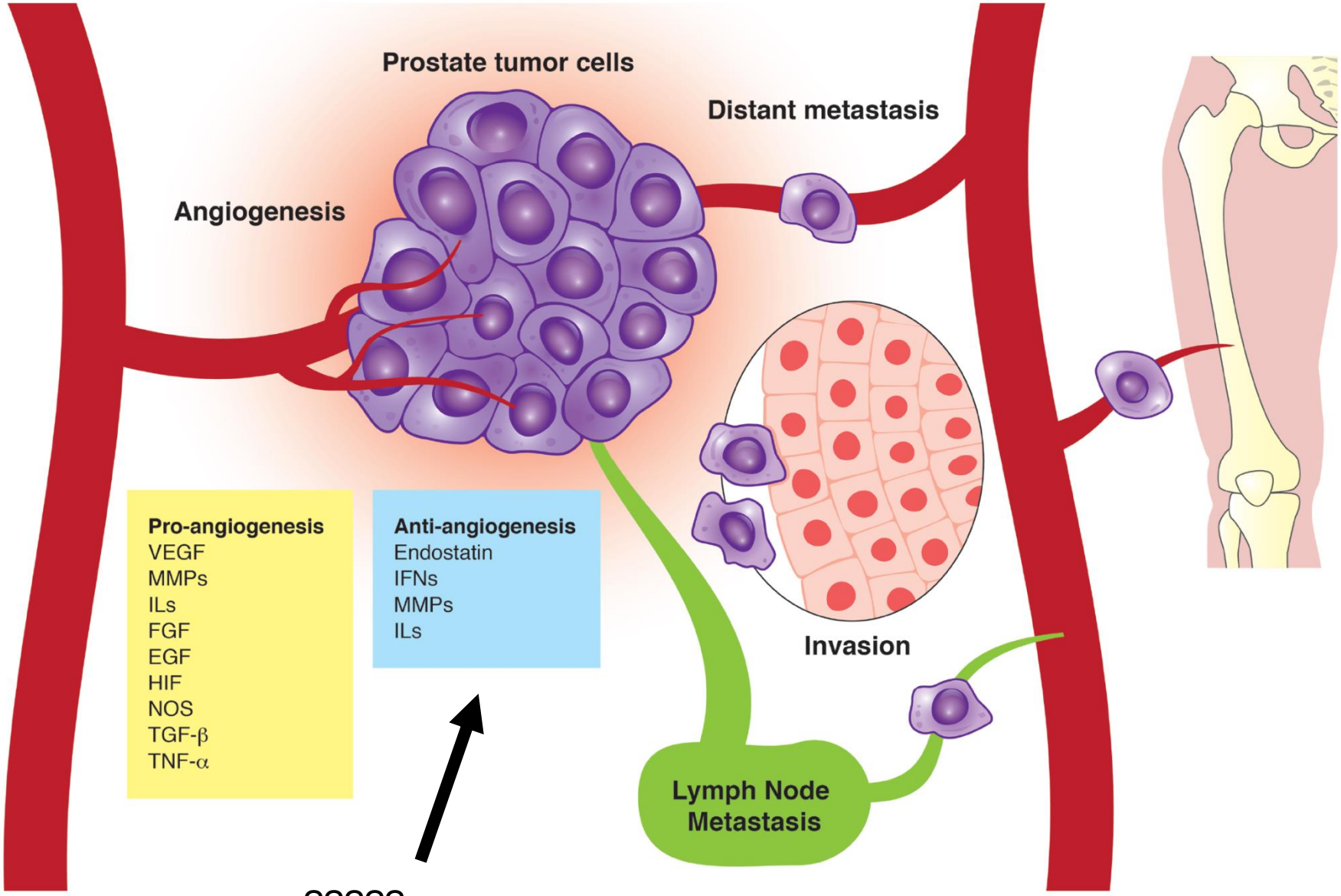
What Is Tumor Angiogenesis?

Tumor angiogenesis is the proliferation of a network of blood vessels that penetrates into cancerous growths, supplying nutrients and oxygen and removing waste products. Tumor angiogenesis actually starts with cancerous tumor cells releasing molecules that send signals to surrounding normal host tissue. This signaling activates certain genes in the host tissue that, in turn, make proteins to encourage growth of new blood vessels.

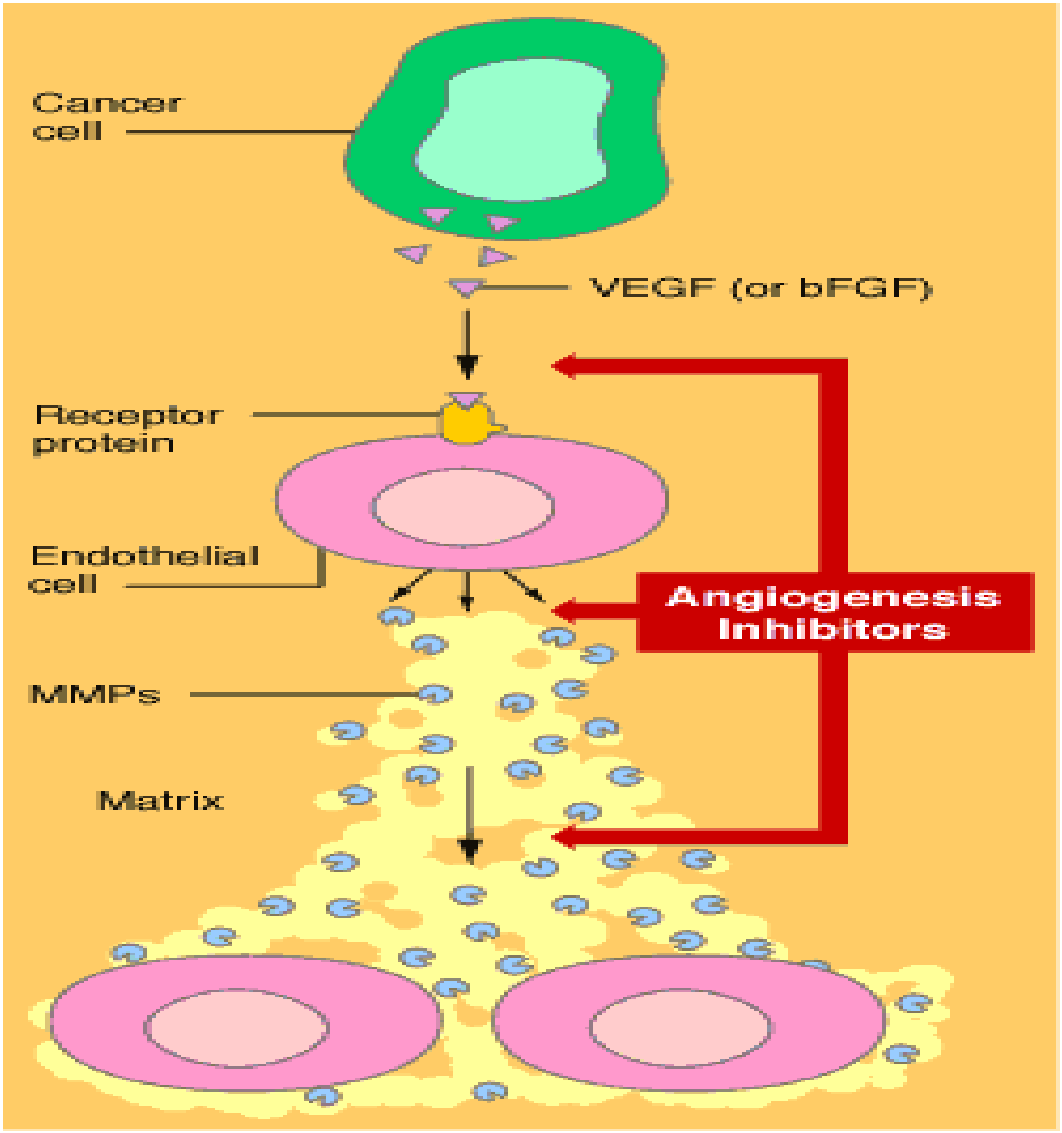
The role of angiogenesis in tumor progression



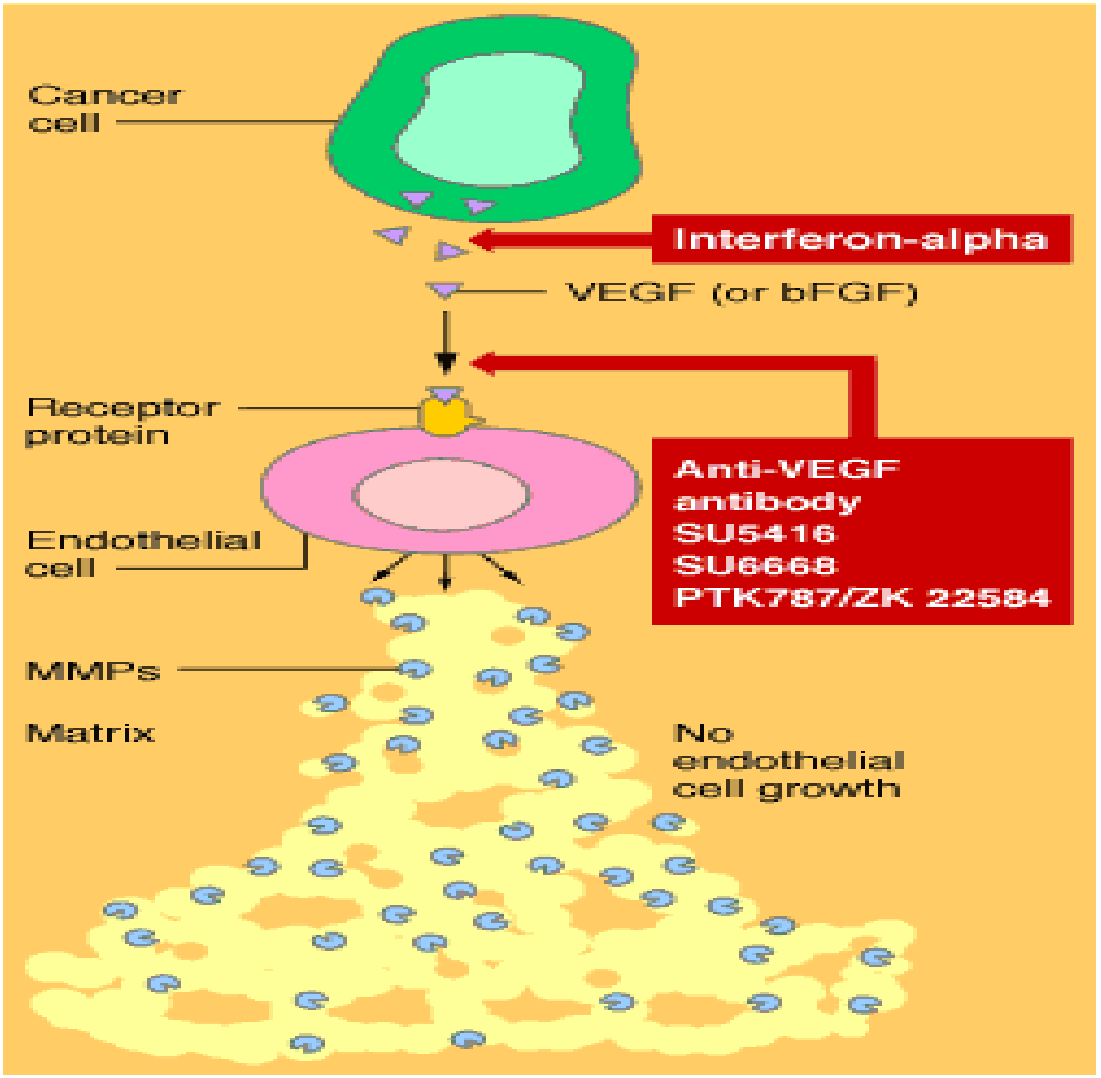
Novel strategy in tumor therapy: by using inhibitors of tumor angiogenesis the solid tumor is dried out (1970).

Judah Folkman

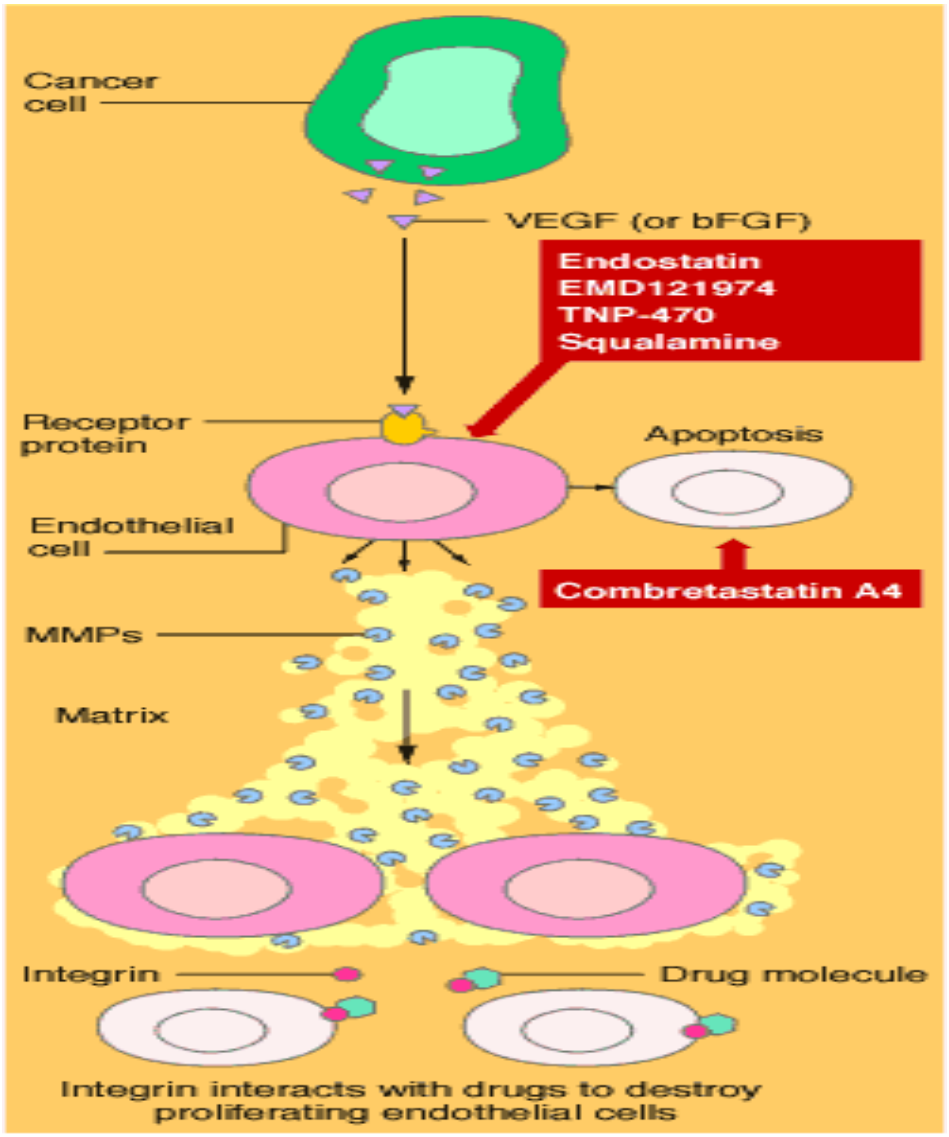
At which points tumor angiogenesis can be blocked?



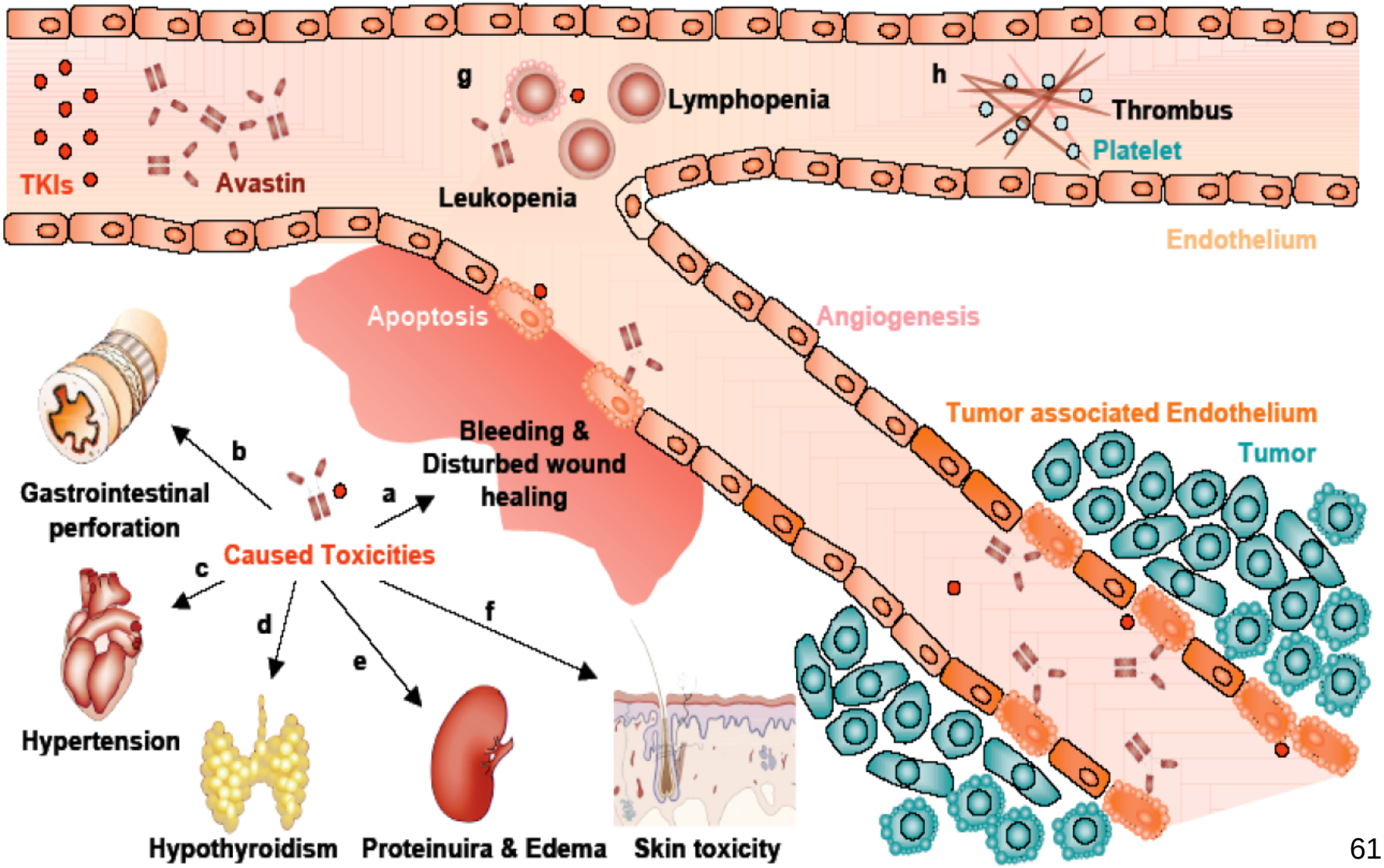
Substances that block the production or the action of VEGF



Inhibition of endothelial cells



Risks of antiangiogenic therapy



Heart attack

Contergan: Schlafmittel, Beruhigungsmedikament, Morgenübelkeit

Fragen zur Angiogenese