A Few Notes

Spinal Cord Regions & Myelination

Major Disease Caused by Faulty Myelination

Myelination Functions

2 *Antwort*

Spinal Cord Regions

- cervical - neck
- thoracic - upper back
- lumbar - lower back

Myelination

- increases speed and efficiency of neural impulses along myelinated fibers → improvement and regulation of information transfer
- **CNS**: Oligodendrocytes
- **PNS**: Schwann cells

1 *Antwort*

Hi there, fellow biologist!

I made these cards for the exam of the spring semester 2018 and thought I could improve my karma a bit by sharing them. They are probably not complete/won't entirely cover the next iteration of the course but they should be hella helpful. If you are wondering how i made these beauties, it's all made with LaTeX- which I would highly recommend you to take a look at. And if you want to expand or change them or just for telling me what a wonderful person I am for sharing my hard work, send me an email at reichlp@student.ethz.ch and I'll send you the original LaTeXfiles

Cheers and best of luck,

Pia

4 *Antwort*

- enhances nerve conduction velocity
- myelinating glia provide support to neurons/axons (trophic/metabolic)
- likely to restrict neural plasticity
- learning and training are accompanied by adaptive myelination
- regulation of neuronal network behavior through myelinating oligodendrocytes

3 *Antwort*

CNS:

- Multiple Sclerosis
- Leukodystrophies - genetic myelin deficiencies
- psychiatric disorders - schizophrenia
- memory decline in aging - correlation with reduced myelin
- Amyotrophic Lateral Sclerosis (ALS) - metabolic support of neurons/axons
- neonatal white matter disorders

PNS:

- peripheral neuropathies - affect motor and/or sensory systems, often progressing with age

Structure of Myelinating Cells

Main Effects of Myelination

Differentiation of Oligodendrocyte Progenitor Cells into Myelinating Oligodendrocytes

Model of how Oligodendrocytes/Myelin Wrap around Axons

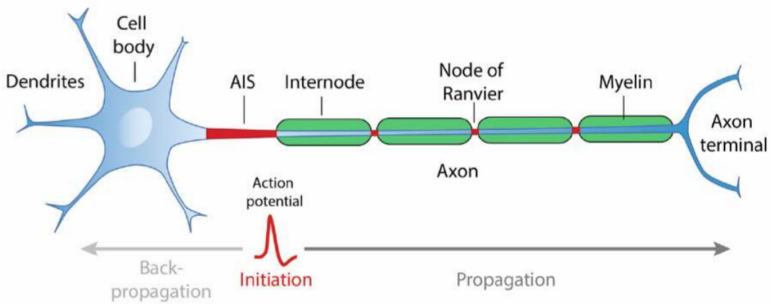
6 *Antwort*

→ **fast nerve conduction**

- in unmyelinated fibers: impulse conduction propagated propagated by local circuits
- in myelinated axons: excitable axonal membrane only exposed to extracellular space at nodes of Ranvier
- at nodes of Ranvier: Na^+-channels → restore action potential
- low capacity of myelin sheath → little energy required to depolarize membrane between nodes → increased speed of local circuit spreading → "saltatory conduction"

5 *Antwort*

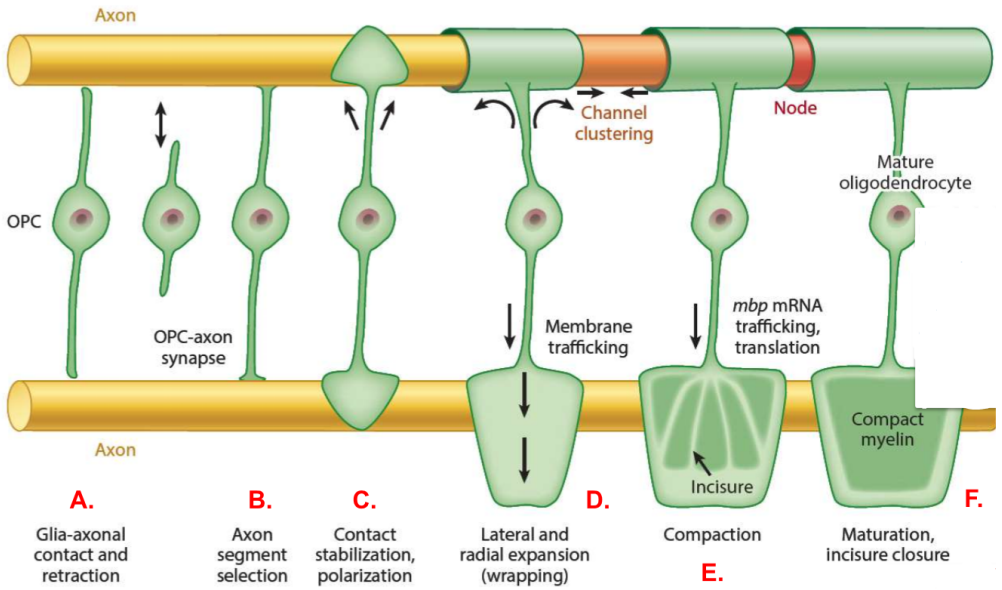
- alternating internodes and nodes of Ranvier
- Schwann cells form one internode
- Oligodendrocytes have 20-60 myelinating processes
- AIS: Axon initial segment
- red: unmyelinated axon areas enriched in voltage-gated sodium channels (Na_V)



8 *Antwort*

- myelin sheaths enormous compared to cells → metabolic challenge
- two distinct, but coordinated motions
  - wrapping of leading edges at inner tongue around axon **underneath** previously deposited membrane → build-up of more wraps
  - lateral extension of myelin membranbe layers toward nodal region → build-up of longer sheaths
- cytoplasmatic channels span through developing myelin → vesicles of membrane can be transported through to growing tip

7 *Antwort*



Steps Leading to CNS Myelination

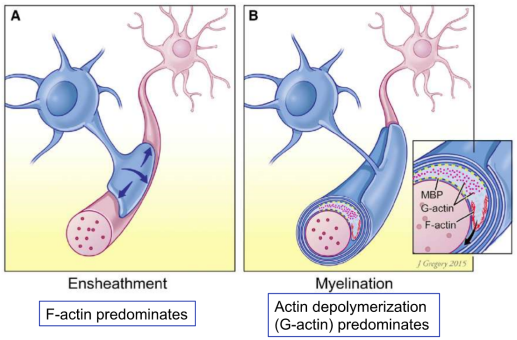
Actin Dynamics at Different Stages of CNS Myelination

Composition of PNS Myelin & Compaction

PNS Myelin Compaction

10 *Antwort*

- leading edge of inner turn expresses **F-actin**
- actin disassembly facilitates membrane spreading and supports iterative cycles of polymerization/depolymerization leads to protrusion
- ensheathment: F-actin predominates
- myelination: actin depolymerization, G-actin predominates

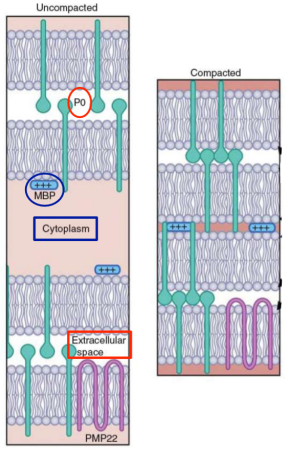


9 *Antwort*

1. proliferation and migration of oligodendrocyte precursor cells (OPCs) in white matter tracts
2. recognition of target axons and axon-glia signaling
3. differentiation of OPCs into myelinating oligodendrocytes
4. membrane outgrowth and axonal wrapping
5. trafficking of membrane components
6. myelin compaction → extrusion of cytoplasm
7. nodes of Ranvier formation

12 *Antwort*

- in cytoplasm: MBP (Myelin Basic Protein) - binds to opposing negatively charged cytoplasmic leaflets of myelin membrane
- in extracellular space: P0: Protein Zero (IgCAM) - homophilically interacts with other for compactions



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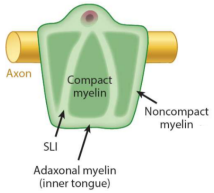


- IPL: intraperiod line
- MDL: major dense line - cytoplasm was originally here
- SLI: Schmidt-Lanterman Incisures - cytosolic channels of uncompacted myelin transversing compact myelin

CNS Myelin Compaction

Myelination - Composition and Effects

Functions and Interactions between Axons and Myelinating Cells at the Node of Ranvier

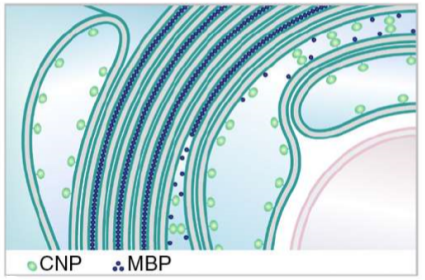
Node of Ranvier Pathologies in MS

14 *Antwort*

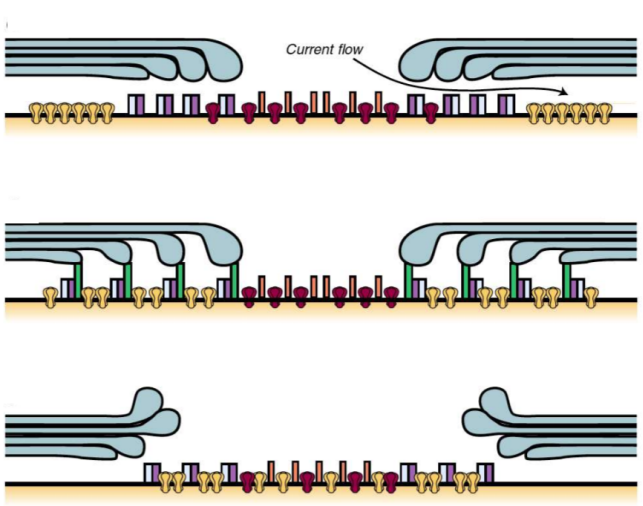
- membrane surface area has to expand several 1000x
- high lipid-to-protein ratio compared to other membranes
- $\approx 70 - 80\%$ lipids, 20-30% proteins by dry weight
- enriched in cholesterol and glycosphingolipids
- *de novo* in melinating glia synthesized cholesterol required for myelin growth and compaction → squalene synthase
- myelination leads to increased effective resistance, decreased effective capacitance → insulation of axon
- physical properties of myelinated axon fibers plus strict organization of nodal areas by myelinating glia-axon interactions

13 *Antwort*

- driven by increasing MBP concentration antagonizing actin-binding CNP (CNPase)
- compaction from outside to inside
- at increased concentrations MBP binds membranes together
- mechanism of extracellular membrane compaction remains unclear → **no P0 protein**



16 *Antwort*



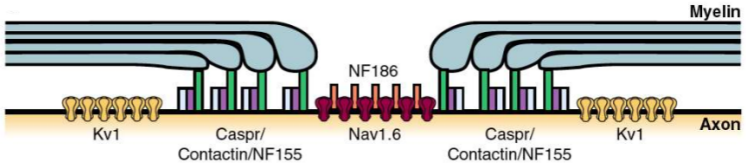
Multiple sclerosis: Node elongation with loss of NF155 may allow aberrant extracellular current flow to juxtaparanode (aberrant activation of Kv channels)

Multiple sclerosis: Node and paranode elongation with redistribution of Kv into paranode

Multiple sclerosis: Myelin retraction, paranodal loop eversion, node lengthening and mixing of Nav, Kv and Caspr

15 *Antwort*

- node is an area free of compact glial sheath
- voltage-gated Na^+ (Na_V) channels in axonal membrane at the node → demyelination causes re-destribution of Na^+ channels along axon and reduced nerve conduction
- axonal K_V channels on either side of nonde (critical)
- ends of myelin sheaths attached to axon on either side of node → current cannot easily pass under meylin (would negate membrane capacitance-reducing and resistance-increasing effects)
- paranodal junctions (with Caspr) segregate Na_V from K_V channels



Why and When Does Myelination Make Sense?

Charcot-Marie-Tooth Diseae
CMT

Molecular Control of PNS Myelination

Schwann Cell Lineage

18 *Antwort*

- reduced nerve conduction velocity
- autosomal dominant inheritance
- CMT Type 1A: duplication of 1.5 Mb on chromosome 17 → contains dosage-sensitive PMP22 gene → overexpression
- PMP22: abundant membrane component of compact PNS myelin, contributes to membrane organization
- mutant MPM22 is retained within cell → demyelination

17 *Antwort*

- increasing speed of nerve impulses without increasing axonal calibers tremendously
- evolutionary advantage: myelinated cells need a lesser fiber diameter for the same conduction velocity
- threshold of fiber calibers for usefulness of myelination: PNS: 1μm / CNS $0.4 - 0.8\mu$m
- myelin thickness roughly proportional to axon caliber
- adult neurons located in pyramidal neurons display different patterns of myelinated segments along axons → long unmeylinated segments
- axons in CNS seem to only be myelinated when it's beneficial → what drives the decision process?
- myelin sheath thickness in PNS fixed an highly optimized

20 *Antwort*

- neural crest → Schwann cell precursor → immature Schwann cell → promyelin Schwann cell → myelin Schwann cell
- Schwann cell precursor can also develop into melanocytes, endoneurial fibroblasts, parasympathetic neurons
- immature Schwann cells can also develop into Nonmyelin (Remak) Schwann cells that develop into Repair (Bungner) Schwann cells in response to injuries

19 *Antwort*

- receptor NRG1-III and NRG of axon activate PLCγ, MEK → Erk1/2, PI3K, SHP2 of Schwann cell →→ myelination
- axonal NRG1 Type III regulates successive steps in Schwann cell development
  - initiation of myelination: amount of NRG1 III present on accompanying axon → small caliber axons below NRG1 IIIU threshold don't need myelination
  - determination of myelin thickness: more NRG1 III (on larger caliber axons) → thicker myelin
- ErbB2/ErbB3: receptor tyrosine kinase pair, main glia receptors for NRG1
- Erb2 Null mutant (loss of function at birth) leads to neuronal cell death: nerves are hypomyelinated, lack Schwann precursors
- NRGI III acts juxtacrine: axon → Schwann cell
- NRG1 - likely via AKT/mTORC1 - controls both driving forces and brakes of myelination

NRG 1 Regulation

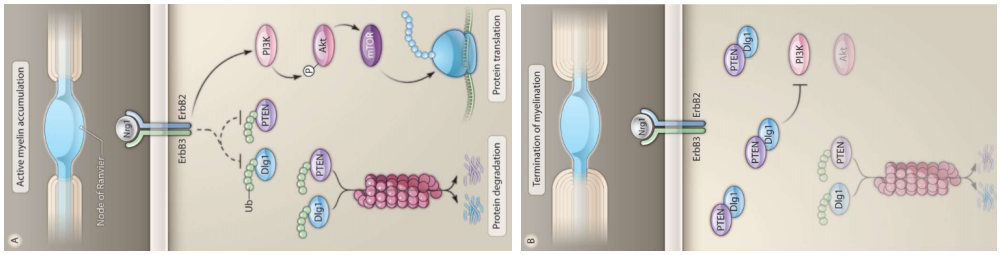
Regulation of Myelination Downstream of NRG1

Role of mTOR in Myelination

Niaspan

22 *Antwort*

- NRG1 III → ErbB2/3→PI3K
- PI3K phosphorylates PIP2 to PIP3 → PDK1 → PDK1 phosphorylates AKT to P-AKT → myelination ↑
- dephosphorylation of PIP3 to PIP2 → PTEN → Dlg interacts + stabilizes PTEN → myelination ↓
- in short: less AKT signaling → less myelin
- PTEN/Dlg1 regulates that myelin sheath is not growing too large → unstable



21 *Antwort*

- NRG1 = Neuregulin 1
- BACE1 cleavage generates a positive myelination signal (probably through processing of NRG1 III)
- BACE 1 KO mice display hypomyelination of peripheral nerves
- TACE cleavage generates negative myelination signal → TACE inhibits Schwan cell myelination
- TACE KO mice have hypermyelinated motor neurons

24 *Antwort*

- Niaspoan: TACE activator
- ameliorates hypermyelination in Pmp22+/- mouse nerves (→ HNPP model)
- HNPP: hereditary neuropathy with liability to pressure palsy - peripheral neuropathy, pressure on nerves can cause tingling, pain, muscle atrophy
- tomaculum = focal hypermyelination

23 *Antwort*

- mTOR = mechanisitic Target OF Rapamycin (serine/threonine kinase)
- mTORC1 inhibits lysosome biogenesis, autophagy
- mTORC1 activates glucose metabolism and mitochondrial metabolism
- mTORC2 activates cell survival/metabolism
- hypomyelination and shorter internodes in Raptor but not Rictor (subunit of mTORC2) mutants → PNS myelin growth dependent on mTORC
- SREBPs and targets (e.g. FASN - fatty acid synthase: synthesizes palmiate from Ac-CoA and malonyl-CoA) reduced in Raptor mutants → FASN only source of *de novo* fatty acids

Role of MEK/ERK1/2 in PNS Myelination

Summary of Myelination Control in PNS

Axon after PNS Injury - Regeneration

Axon after CNS Injury - No Regeneration

26 *Antwort*

- myelination initiation
- myelin growth
- restraining/contrl of myelin thickness
- remyelination after injury

are mainly controlled by **Neuregulin 1 (NRG1)**, which is mediated by

- PI3K / AKT / mTOR
- MEK-ERK (cell differentiation- and activation strength-dependent

25 *Antwort*

- ERK signaling required for PNS myelination in development
- moderate activation of MEK-ERK in developing Schwann cells caues hypermyelination
- strong activation of MEK-ERK in adult myelinating Schwann cells causes de-differentiation and demyelination

28 *Antwort*

1. myelin debris persists distal to site of injury
2. reactive astrocytes secrete CSPGs that wall of lesion site
3. oligodendrocytes undergo apoptosis or become quiescent
4. microglia regulate inflammatory milieu
5. overall anti-regenerative response of glia cells

27 *Antwort*

1. Schwann cell recruit macrophages into nerve
2. Schwann cells and macrophages clear myelin debris
3. Schwann cells secrete growth factors → autocrine survival, neuronal survival, axon outgrowth
4. Schwann cells increase secretion of pro-regenerative basal lamina
5. CSPGs (chondroitin sulfate proteoglycans → ECM molecules, highly inhibitory to axon outgrowth) upregulated, but are degraded by MMPs (matrix metallo-proteinases) and may be concealed by basal lamina

Multiple Sclerosis

Main Disease Developments in MS

Current Concepts of MS Initiation

Guarding Mechanisms Against CNS Autoimmunity (I/II)

30 *Antwort*

- **relapsing-remitting MS - RRMS**
  - most patients
  - often followed by secondary progressive form
  - during RRMS symptoms come and go reflecting partial remyelination
  - progression in severity and time is very variable
  - on average, a patient will have a relapse every two years
  - about 50-80% will develop a **secondary progressive phase - SPMS** -after - on average - 10 years
- **primary progressive course - PPMS**: without individual attacks but continuous progression of symptoms

29 *Antwort*

- most common neurodegenerative disorder in young people
- immune system attacks CNS myelin
- impaired sensory and motor nerve function and impaired cognitive function
- loss of myelin causes reduced or blocked nerve conduction → attacks of numbness, loss of vision, weakness, bladder problems, ataxia (tremors) and -quite often- visual problems
- focal lesions in myelin-rich white matter (but grey matter is also affected) due to myelin-autoreactive T-cells and microglia activation → inflammatory disease

32 *Antwort*

- Checkpoint 1: T cell negative selection in thymus, elimination of self-reactive cells
- some aggressive self-reactive T cells escape first checkpoint:
  - if antigen is separated from auto-reactive lymphocytes - e.g. blood-brain barrier - no attack
  - without danger signal-inducing activation (→ cytokines) → inactivation
  - regulatory T cells keep auto-reactive T cells in check
  - Th2 cells release cytokines to strengthen regulatory T cells → together they suppress auto-reactive T cells with cytokines
  - constantly present self-antigen causes death of activated cells

31 *Antwort*

1. a few (auto)antigen-specific T cells migrate through blood-brain barrier into perivascular space between capillary endothelium and glia limitans - the beginning of the CNS parenchyma (functional tissue)
2. T cells are confronted with dendritic cells that have been collecting (auto)antige and present them to T cells
3. auto-antigen-specific T cells start to proliferate and migrate to sites of primary inflammation
4. T cells mediate damage via direct cytotoxicity and secretion of cytokines
5. surrounding tissue cells such as neurotoxin-secreting (cytokines, ROS, glutamate, chemokines) microglial cells and astrocytes are activated, producing further damage

Guarding Mechanisms Against CNS Autoimmunity (II/II)

Today's Drugs Against MS

CNS Immune Privilege

Blood-Brain Barrier as a Drug Target

34 *Antwort*

- most act through immunomodulation or immunosuppression
- **IFN-β**: cytokine that increaes production of anti-inflammatory cytokines and reduces pro-inflammatory cytokines
- **Glatiramer acetate**: polymer of Ala, Lys, Glu, Tyr, competes with self-antigens for binding to MHC II, inhibits production of IFN-γ and increases production of anti-inflammatory cytokines
- **Fingolimod**: sphingosine-phosphate-1-receptor modulator - sphingosine-1-phosphate type 1 receptors (S1P1Rs) are expressed on lymphocytes, fingolimod binds receptors → internalized → prevents S1P1R-dependent release of lymphocytes from secondary lymphoid tissue
- problem: have benefical effects in relapsing-remitting stage, but fail in progressive stage → compartmentalization of inflammatory response within CNS behind closed/repaired blood-brain barrier with progressing axonal damage and loss
- problem: no drugs against PPMS
- 2017: first drug against PPMS → Ocrelizumab

33 *Antwort*

- Checkpoint 2: Peripheral Tolerance
  - antigen segregation: physical barrier, self antigen cannot access lymphoid system
  - peripheral anergy: functional unresponsive, weak signal without co-stimulus → inactivation
  - regulatory T cells: suppression by cytokines, intracellular signals
  - cytokine deviation: Th1 → Th2 → inflammatory cytokines ↓
  - clonal detection: activated lymphocytges are prone to apoptosis

36 *Antwort*

- how lymphocytes enter tissue in inflammation:
  - weak adhesion and rolling along endothelial cells: selectins on endothelial cells bind to oligosaccharides on white blood cells → selectin-dependent
  - strong adhesion and emigration: integrin activation (white blood cells) binding to Ig-CAMs on endothelial cells → integrin-dependent
- **Natalizumab/Tysabri** blocks CNS entry of lymphocytes by binding to α4-integrin → highly effective in reducing relapse rates, may effectively delay/stop disease progression
- rare side effect: **progressive multifocal leukoencephalopahty - PML**
  - JC polyomavirus (JVC) can be found in urinary tract in most adults → persistent but asymptomatic infection
  - in immuno-comprised individuals JCV can infect brain → PML: debilitating and frequently fatal disease → no treatments currently available for PML
  - antibodies against JCV can be used as biomarkers → if patient negative, drug risk-free

35 *Antwort*

- CNS is an immune privileged organ
- immune response within CNS restricted and graft rejections delayed
- blood-brain barrier prevents immune cells entry into parenchyma of healthy CNS
- disruption of barrier results in inflammation → often associated with neuronal cell death
- in MS: auto-immune inflammatory cells are thought to enter through barrier and play detrimental role
- **vascular blood-brain barrier**: double barrier → endothelium (→ tight junctions) + astrocytes (glia limitans)

Trigger-Based Model for MS

Effector-Based Model for MS

Hints for Specific Diseae Mechanisms: MS Pathologies

Inside-Out VS. Outside-In Model

38 *Antwort*

- inflammatory reaction in CNS causing demyelination via myelin-autoreactive T cells
- **EAE (Experimental Autoimmune Encephalomyelitis)** as MS Model
- EAE generally associated with early breach of blood-brain barrier, focal perivascular mononuclear cell infiltrates, gliosis, and demyelination of CNS white matter
- groundwork for Tysabri based on EAE
- infection of mice with MOG (myelin oligodendrcyte glycoprotein) peptide

37 *Antwort*

- initial trigger and risk factors for MS not yet found
- risk of MS correlated with residence in childhood (controversial) → high-risk to low-risk area migration in childhood reduces risk and vice versa
- living further from equator boosts risk, possibly because of reduced exposure to sunlight → Vit. D
- Epstein-Barr virus: few people that have been infected wit EPV develop MS, but nearly everyone with MS was been infected with the virus
- smoking: higher rates of MS in smokers than in non-smokers
- Female:Male / 2:1 affected in RRMS
- genders equally affected in PPMS
- genetic aspect: possibly an increase in susceptibility for the disease, but not necessarily causative

40 *Antwort*

Outside-In

- MS is an autoimmune disease
- auto-reactive T cells in periphery cross into CNS and - together with B cells and macrophages - proceed to destroy various CNS elememts → inflammatory reaction causes furhter demyelination and tissue injury

Inside-Out

- initial malfunction occurs in CNS, similarly to other neurodegenerative disorders like AD and Parkinson's
- primary cytodegeneration is initial event → be releasing highly antigenic constituents, secondarily promotes autoimmune and inflammatory response in predisposed host → possibly further driving degeneration

39 *Antwort*

- T cells, macrophages, myelin loss
- complement-mediated lysis of antibody-targeted myelin/oligodendrocytes
  - antibodies as a pathogenic player in MS highly disputed
- hypoxia-like damage of white matter: secondary to T cell-mediated inflammation damage to vasculature? → HIF1α is up
  - low oxygen in oligodendrocytes due to vascular damage → like angiogenesis
- primary oligodendrocyte loss (pyknotic nuclei) → does not trigger anti-CNS immunity alone or in conjunction with immue activation
- implications for animal models of MS: every model has its inherent limitations as long as we don't know the initial trigger → ideal model needs to be able to reproduce all different pathological MS-associated pattersn (might not even be possible) → select/generate appropriate models to address specific questions, e.g. EAE models for general neuroinflammation

Methods to Produce Transgenic Mice

Protein Zero (P0) Null Mice

Cre/LoxP System

Consequences of Genetically-Induced Adult Oligodendrocyte Cell Death

42 *Antwort*

- almost exclusively expressed in myelinating Schwann cells
- heterozygous P0 mutations can cause inherited demyelinating peripheral neuropathies (Charcot-Marie-Tooth Disease Type 1B, CMT1B) → haploinsufficiency: one copy not enough for normal phenotype

41 *Antwort*

- **standard technique**: transgene DNA is microinjected into male pronucleus of fertilized murine oocyte → injected oocytes are transferred to a 0.5-day pseudopregnant recipient mouse → offspring a screened for transgene by DNA analysis

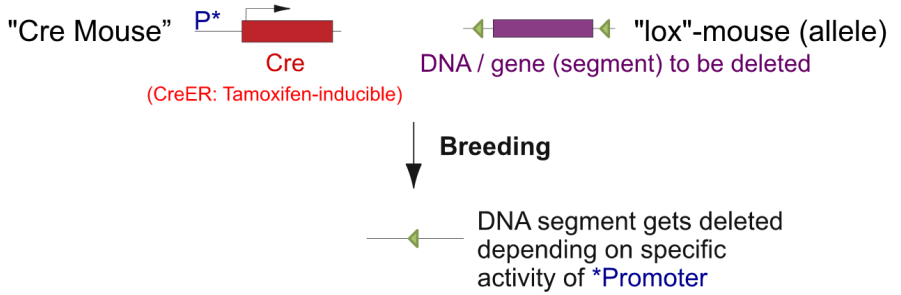
  BUT: random chromosomal integration, random transgene copy number
- **ES cell technology**: vector containing homologous pieces of DNA to target gene and desired DNA is introduced into ES cells (e.g. by electroporation) → positive-negative selection → proliferation of targeted ES cells → injection of ES cells into 3.5-day mouse blastocysts (mix and form mosaic of cells of inner cell mass) → blastocysts are transferred to a 2.5-day pseudopregnant recipient mouse → mosaic mice mate with normal mice to produce both gene targeted and normal offspring
- **CRISPR/Cas9**: injection of sgRNA into zygote → precise genome editing

44 *Antwort*

- massive myelin vacuolation and loss of myelin
- microglia activation in affected areas
- no significant influx of B and T cells
- pathology development independent of adaptive immune system
- no induction of anti-CNS immune response
- (cautious) conclusion: primary function of oligodendrocytes in supporting axonal integrity, very likely some protective role of living oligodendrocytes on axons
- in PLP Null Mice (PLP major protein component of compact CNS myelin) swelling and organelle accumulation in axons → axonal transport impeded → causes distal axonal degeneration

43 *Antwort*

- conditional DNA segment deletion
- breeding of Cre mouse (promotere with Cre = tamoxifen-inducible recombinase as target) and lox mouse (with DNA segement/ gene to be deleted between lox regions → serve as Cre target sequence)
- DNA segment gets deleted depending on specific activity of tissue-specific promoter



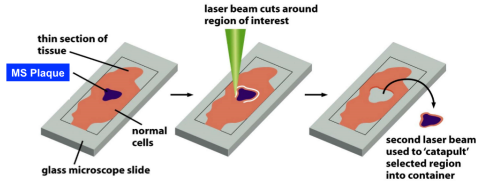
Symptoms Progression in RRMS

Laser Capture Microdissection & Krabbe's Disease

Role of Lactate Transporter in Axon Damage

Axonal Energy Management in MS

46 *Antwort*



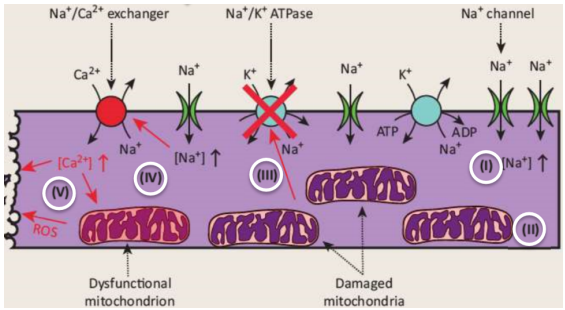
- autosomal-recessive leukodystrophy caused by loss-of-function of GALC (galactosylceramidase) - lysosomal enzyme → catabolizes *gala*-sphingolipids
- in Krabbe's disease, myelin defects lead to progressive and severe neurological deficiencies in PNS and CNS, symptoms include muscle rigidity and atrophy, ataxic movement, hearing and vision defects, rapid loss of cognitive and motor skills
- release of toxic lipid species from myelin such as psychosine spreading to axons where they cause damage

45 *Antwort*

- first phase dominated by inflammation, associated with damage to blood-CNS barrier → axonal damage starts
- second (chronic) phase dominated by neurodegeneration, blood-brain barrier repaired, some inflammation remains locally in parenchyma
- axonal degeneration continues in chronic MS lesions without major inflammation
- anti-inflammatory drugs: no effects in chronic progressive phase and primary progressive MS
- neurodegeneration is the major cause of permanent neurological disability in MS

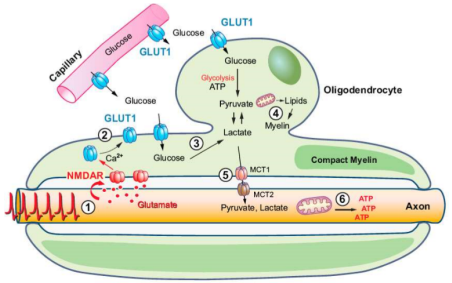
48 *Antwort*

- redistributed Na^+ channels, increase in intra-axonal Na^+ concentration. more ATP required to remove excess Na^+ by Na^+/K^+ ATPase
- mitochondria are damaged
- ATP production too low for Na^+/K^+ ATPase to remove excess intra-axonal Na^+
- rise of axonal Na^+, reversal of axolemmal Na^+/Ca^{2+} exchanger, transfer now mainly Na^+ out and Ca^{2+} into axon
- axonal Ca^{2+} triggers axonal degeneration



47 *Antwort*

- monocarboxylate transporter 1 (MCT1) highly enriched in oligodendroglia
- disruption of transporter from oligodendrcytes to axons → axon damage and neuron loss
- myelinating oligodendrcytes take up blood-derived glucose and deliver glycolysis products (lactate/pyruvate) via MCT1/2 to meylinated axons
- oligodendroglial NMDA receptor signaling senses axon energy: during myelination, respond to glutamate indiciating increased axonal electrical activity an energy needs → cause more glucose uptake via glucose transporters → glycolysis products are initally used for ATP and lipid synthesis → afterwards, myelinating oligodendrocytes release lactate (or pyruvate) to fuel axonal compartment for mitochondrial ATP produciton



Amyotrophic Lateral Sclerosis
ALS

Summary of Role of Myelin and Oligodendrocytes in MS

Treatment Strategies for MS

Oligodendrocyte Development

50 *Antwort*

- myelin and/or oligodendrocytes exert vital axon-protective and perhaps even neuro-protective functions that mediate long-term axonal integrity via glia-axon interactions maintaining axonal infrastructure and function (e.g. maintenance of axonal cytoskeleton, axonal transport, architecture of Nodes of Ranvier)
- primary demyelination in MS, possibly beggining with disruption to axo-glial apparatus and culminating in degeneration of myelin sheath is a major cause of degeneration of chronically demyelinated axons
- if intact oligodendrocytes are critical for axonal support → plausible that re-myelination in MS may be protective for axons and restore nerve conductivity (i.e. physical features of myelinated axons (partially) and integrity of Nodes of Ranvier, together with metabolic support of axons
- ergo: restoring myelin to demyelinated axons will reduce axonal loss and other deficits

49 *Antwort*

- fatal neurological disease
- clinic: progressive weakness
- pathology: cortical and spinal motoneuron degeneration
- pathogenesis of degeneration: only partially understood (specifics are unknown)
- late-onset (45-65 years)
- disease duration 1-5 years
- 90% sporadic, 10% familial
- 20% mutation in SOD1 (autophagosome), 40-50% mutation in C9ORF72 (RNA processing)

52 *Antwort*

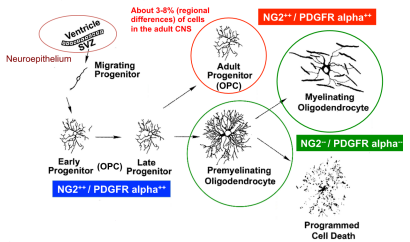
→ Neuroepithelium → migrating progenitor

→ early progenitor - NG2^{++}/PDGFR α^{++}

→ late progenitor - NG2^{++}/PDGFR α^{++}

→ adult progenitor (OPC) - NG2^{++}/PDGFR α^{++} → myelinating oligodendrocyte - NG2^{-}/PDGFR α^{--}

→ myelinating oligodendrocyte - NG2^{-}/PDGFR α^{--} OR programmed cell death



51 *Antwort*

- stop immune attack
- protect oligodendrocytes
- enhance remyelination → protection of axons

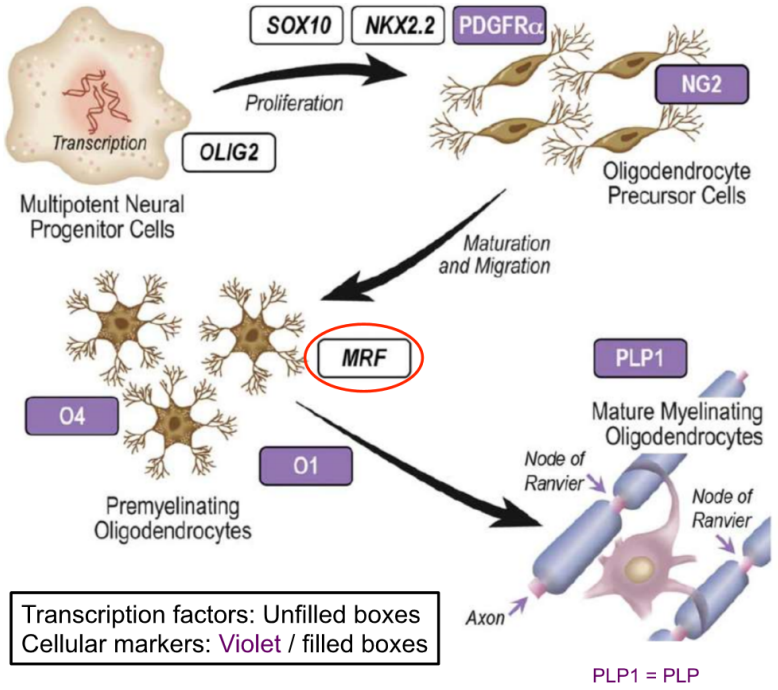
Oligodendrocyte Precursor Migration in Development

Molecular Regulators of Oligodendrocyte Lineage

Plastic Myelination

Role of Novel Activity in Stimulating OPC Differentiation

54 *Antwort*



53 *Antwort*

- OPCs migrate along brain microvessels to reach specific target areas
- migration directed by autocrine increased Wnt-induced expression of receptor Cxcr4 which binds to chemokine Sdf-1
- close interaction: cross talk between endothelial cells and OPCs may influence reciprocal function
- at arrival, OPCs detach from blood vessel and mature
- possible new concept: re-activated OPC migration plays a relevant role in repair in demyelinating disease in adults (like MS) → local alteration of vasculature may also alter capacity of repair

56 *Antwort*

- *Myrf* (=MRF) - transcription factor required for myelination
- MRF mutants have a detectable learning defect
- new neural connections are formed/existing connections strenghtened, in response to repetitive firing of neural circuits
- increased activity in circuits might simulate myelination of axons, myelin remodeling and making circuits more efficient
- neuronal activity-regulated oligodendrogenesis and myelination is associated with improved motor function

55 *Antwort*

- neuroimaging of white matter plasticity in mice and human suggest increase in myelination after skill learning
- different forms of adaptive myelination:
  - *de novo* myelination of unmyelinated axon segments
  - retraction or replacement of existing myelin segments
  - thickening/thinning and lengthening/shortening of existing myelin sheaths
  - adaptive changes at Node of Ranvier
- oligodendrocytes (and probably myelin) are generated throughout life in human CNS, but in low number after the age of 5
- turnover of myelinating oligodendrocytes quite low in adult mice, new oligodendrocytes seem to contribute to myelin adaption (not replacement of dying cells)
- development of new myelinating oligodend during adulthood required for motor learning

CNS Myelin Thickness Regulation on a Molecular Level

Effect of Neuronal Activity on Oligodendrocytes and Myelination

Summary of Role of Myelin in Learning

Modes of Myelination & Steps Required for Remyelination

58 *Antwort*

- neuronal activity promotes proliferation of neighbouring OPCs and thickening of old myelin sheats
- heterogeneity in electrical activity among subsets of axons biases axon selection for myelination to those that are more electrically active
- release of vesicles from active axons promote myelin sheaths from individual oligodendrocytes

57 *Antwort*

- NRG1 (Neuregulin-1) plays a more subtle role as in PNS (i.e. modulatory function in myelin formation, with other growth factors)
- MEK/ERK signaling and PI3K/AKT/mTOR signaling involved, acitvated by FGF-receptor 2, IGF-I-receptors and likely others
- increasing PIP3 levels can open channels through myelin, accompanied by increased myelination (in adults)
- mechanisms of interplay with electrical activity and relation to adaptive/plastic myelination unclear
- **similarities PNS/CNS**: PI3K/Akt/mTOR and MAPK crucial intracellular signaling paths, major upstream activators differ at least partially, pathways likely to regulate internodal length in coordinated way
- **difference PNS/CNS**: NRG1 chiefly regulating myelination initiation in PNS but not in CNS (CNS: axonal caliber sufficient)

60 *Antwort*

- **basal mode**: typical chronological and topographical sequence in mammal, process is devoted to establish basic homeostasis during development
- **targeted mode**: fine regulation of myelination according to needs of particular circuits and networks controlling more complex tasks later in life, potentially explaining part of (acquired) individual features → adaptation
- lesion → activation/recruitment of OPCs/oligodendrocytes → differentiation and remyelination
- promoting factors: semaphorin 3F, CXCL 1, 8, 10, PDGF
- inhibiting factor: semaphorin 3A
- in MS: oligodendrocyte progenitors remyelinate axons in early lesions
- in MS: OPCs are often still present, even in chronic lesions, but they do not remyelinate

59 *Antwort*

- synapses between neurons strenghten when circuits involving neurons are active → long-term potentiation → basis of learning
- additional refinement in active circuits learning needs active myelination, at least for some motor-related skills
- CNS myelination can be influenced by experience and this process is essential for behavioral changes and cognition
- cognition may depend as much on connectivity as on neuronal activity
- CNS myelination is adaptable and can change in response to experiences and electrical activity, modulating in turn the conductivity of neurons and neuronal network behavior

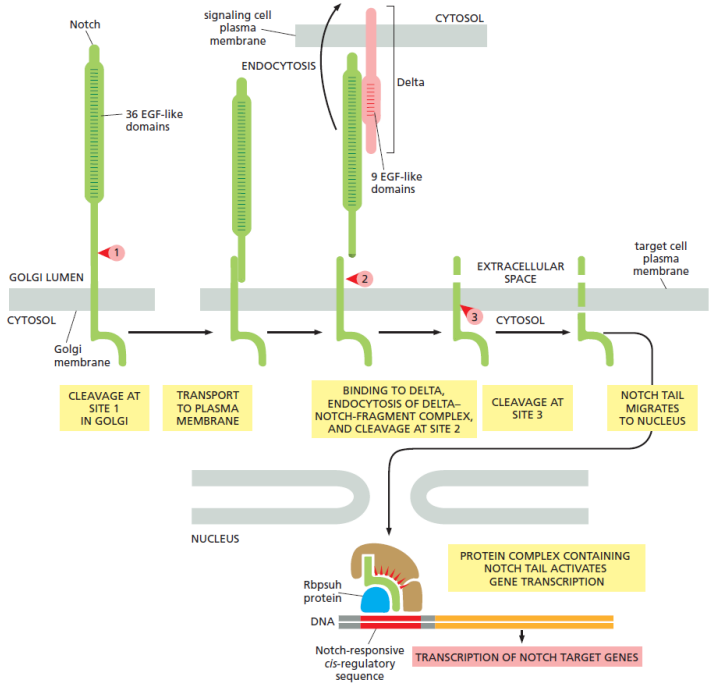
Developmental Myelin Formation VS Remyelination in Adults

Notch Signaling

Notch Signaling in Neural Development

Perinatal White Matter Injury
WMI

62 *Antwort*



61 *Antwort*

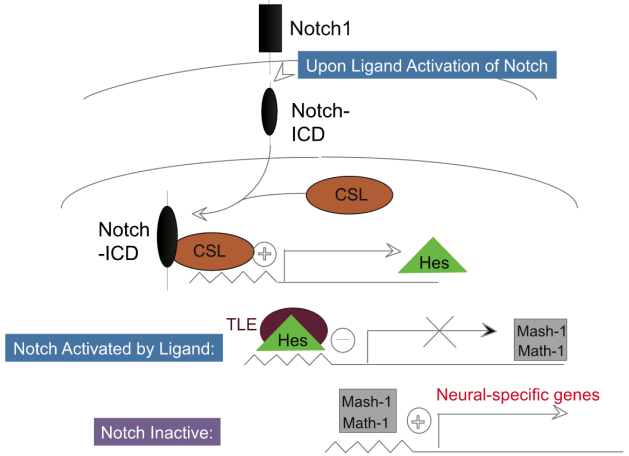
- stepwie development of oligodendrocytes
  - commitment of cells to oligodendrocyte lineage by production of OPC
  - differentiation into oligodendrocytes
  - productive oligodendrocyte-axon interaction
  - wrapping and compaction of oligodendroglial processes around axons to form myelin
- additional challenges in remyelination
  - axons may be injured or unhealthy + inflammation, which is detrimental, is present
  - growth factors may be expressed in inadequate amounts or at wrong time/place
  - recruitment and/or differentiation of OPCs may be impaired
  - accumulation of inhibitors of differentiation

64 *Antwort*

- cerebral palsy (CP) is a major course of chronic neurological morbidity and mortality in children
- white matter injury (WMI) most common finding in almost half of CP children
- periventricular leukomalacia (PVL) form of brain WMI, characterized by necrosis (more often coagulation) of white matter near lateral ventricles, can affect newborns and fetuses, premature infacts are at greatest risk of disorder
- affected individuals generally exhibit motor control problems or other developmental delays, often develop CP or epilepsy later in life
- initial cause is hypoxia or ischemia damaging developing oligodendrocytes

63 *Antwort*

- Notch activity commonly inhibitory for neural differentiation
- Notch1 controls oligodendrocyte differentiation *in vivo* and CNS remyelination after lysolecithin lesions



Role of Aging in Remyelination

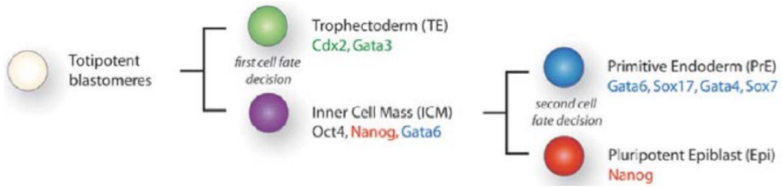
Preimplantation Development

Specification of Trophoblast and ICM

Cell Polarity in Preimplantation Embryos

66 *Antwort*

- zygote → cleavage stages - 2- and 4-cell embryo → morula → blastocyst
- differentiation potential of embryonic cells becomes restricted as development proceeds
- plulripotent potential is established in cells of epiblast
  - no guarantee for particular blastomere to become pluripotent
  - extraembryonic fate decisions are more likely at morula stage
  - hypoblast specification also prevents pluripotent cell fates
- after implantation pluripotential cells become quickly restricted
- Cdx2 is a marker gene for trophoectoderm



65 *Antwort*

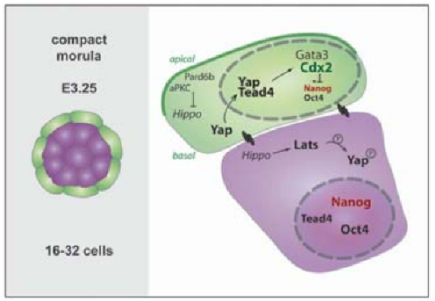
- regenerative processes decline with age → remyelination too
- mainly due to impaired differentiation of oligodendrocytes: regulated by intrinsic mechanisms (HDACs - histone deacetylases) and extrinsic mechanisms (macrophages, "circulatory factors")
- in older animals, recruitment of HDACs to promoters of inhibitory molecules is impaired → transcriptional enivironment skewed towards inhibition of myelin genes → impaired OPC differentiation and delayed remyelination
- resident old oligodendroglia retain remyelination potential with age, macrophage-mediated clearance of inhibitory myelin debris from lesion may become impaired
- M2 macrophages appear to not only be beneficial in removing myelin debris, but also produce remyelination factor: Activin A
- RXR agonist 9-cis-retinoic acid treatment enhances remyelination in aged adult rats
- Clemastine: antihistamine, induces oligodendrocyte differentiation and myelination, crosses blood-brain barrier

68 *Antwort*

- polar cells are identified by visualizing apical domains using ezrin/radixin/moesin (p-ERM) antibody staining
- pERM apically restricted
- other apical markers - e.g. PARD6B - weaker staining can be detected basolaterally
- ERM family proteins function as linkers between cell membrane proteins and F-actin cytoskeleton
- ezrin localizes to apical microvili in epithelia and polarized blastomeres in mous embryos
- cell adhesion not strictly required for cell polarity
  - Ca^{2+} required for adhesion of cells via adhesion molecules
  - E-cadherin (Cdh1) major cell adhesion molecule in earyl embryos → residual cell-cell contacts can be observed in Cdh1 -/- embryos

67 *Antwort*

- outer cells of morula assume polarity → inactivation of Hippo signaling pathway
- activator factor YAP enters nucleus and activates Tead4 bound genes: Gata3, Cdx2 specify TE fate
- inner cells: hippo is active leading to phosphorylation of YAP and preventing nuclear entry



Anaylsis of Singe Blastomeres from 8-Cell Embryo

Transcription Factors as Markers for Lineages & ES Stem Cell Cultivation

Caviation and Development of a Monolayer Epithelial Epiblast & Specification of Distal and Anterior Visceral Endoderm

Formation of Primitive Streak & Gastrulation

70 *Antwort*

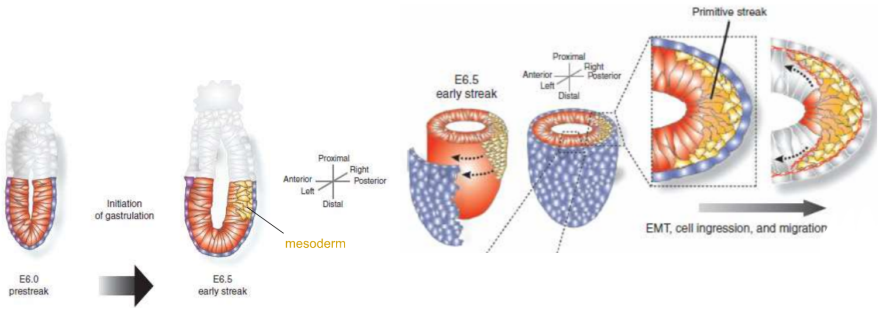
- blastocyst turns into trophoectoderm (TE) (*to* Cdx2 + Eomes) or inner cell mass (→ Oct4)
- ICM turns into hypoblast (→ Gata6) or epiblast (→ Oct4 + Nanog)
- pluripotent embryonic stem (ES) cells can be maintained in appropriate culture conditions
- mouse ES cells proliferate continuously and grow in colonies of tightly packed cells resembling ICM
- culture conditions often include a layer of embryonic fibroblasts as substrate and cytokine LIF - leukemia inhibitory factor, also called DIF = differntiation inhibitory factor
- LIF signaling leads to phosphorylation of STAT3 through activation of JAK kinases → activate self-renewal
- LIF also activates ERK-MAP kinase and PI3K pathways that appear to block self-renewal → blocking MAPK pathway useful for maintenance of ES cells
- inhibition of GSK3 helps ES cell culture maintenance by stabilizing β-catenin and inducing Wnt like signaling

69 *Antwort*

- transcripitonal coactivator YAP becomes phosphorylated and p-YAP localizes to cytoplasm in apolar cells
- single blastomeres divide and seem to attempt to reform basic structure of morula with polar outer cells and non-polar inner cells
- no differentiation → link between embryonic structure and cell differentiation
- remarkable plasticity: blastomeres from 4-cell stage can give rise to functional blastocysts
- elevated phosphorylation of myosin light chain II (p-MLCII) at cortex of apolar cells indicates enhanced cortical contractility of apolar cells

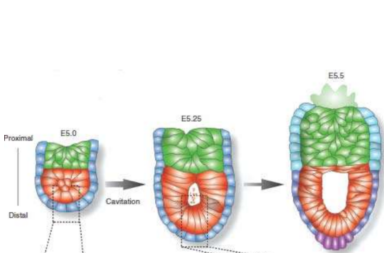
72 *Antwort*

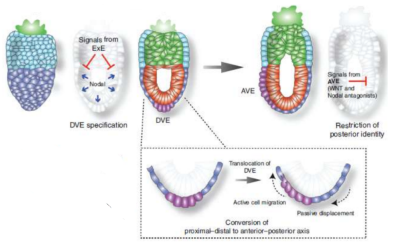
- Wnt and Nodal signaling required for streak formation → initiation of gastrulation
- Wnt3 expressed in posterior visceral endoderm and later in epiblast adjacent to extraembryonic ectoderm and opposite of anterior visceral endoderm
- mesoderm: at primitive streak, cells undergo epithelial to mesenchymal transition (EMT), ingress, migrate away
- endoderm: cells fated to form definitive endoderm undergo MET and subsequently egress into epithelium of embryonic visceral endoderm → changes its state by downregulation of visceral and upregulation of definitive endoderm markers

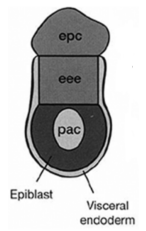


71 *Antwort*

- visceral endoderm sends cell death signal (BMP, TGF-β, whereas contact to the basement membrane confers cell survival of a single cell layer attached to the basement membrane
- anterior visceral endoderm: source of Cerberus (BMP and nodal inhibitor), Wnt inhibitors specify anterior part of axis allowing neuectoderm development







Gastrulation in Mouse Embryo

Cell Cultures from Early Embryo

Ectoderm Development and Repair Overview

Formation of Neural Tube

74 *Antwort*

- Embryonic Stem (ES) cells can differentiate into all cells of embryo including germline but not into extraembryonic trophectoderm
- Embryonic Germ (EG) cell lines from PGCs have similar characteristics dependent on Oct-4, STAT3, and LIF
- trophoblast stem (TS) cells : restricted to trophoblast differentiation FGF4 and heaprin, MEF cond. medium
- extra-embryonic endoderm (XEN) cells: restricted to extraembryonic endoderm differentiation, MEF cond. medium

73 *Antwort*

- 6 days after fertilization: mouse embryo consists of three layers
  - ICM cells in contact with blastocyst cavity differentiate into epithelial layer called extra-embryonic (primitive) endoderm
  - rest of ICM becomes epiblast (primitive ectoderm)
  - primordial germ cells (PGCs) arise from cell population in proximal epiblast adjacent to extra-embryonic ectoderm → they then pas through primitive streak and give rise to several extra-embryonic mesodermal lineages and to germ cells

76 *Antwort*

- elongation of neural plate → folding → elevation of neural crest → convergence of neural crests → closure of neural tube and formation of neural crest cells
- separation of neural tube from epidermal ectoderm involves expression of N-cadherin on neural ectoderm and E-cadherin on presumptive epidermis
- CNS development: patterning of neural ectoderm along anterior posterior axis causes developmeent of different brain regions and spinal cord
- Sonic hedgehog (SHH) from notochord - ventral to neural tube - induces Shh expression in floor plate - most ventral part of neural tube
- BMPs (bone morphogenic proteins) from epidermal ectoderm induce expression of TGF-β proteins in roof plate - most dorsal part
- opposed gradients of SHH (V) and BMP (D) induce different neural lineages in neural tube along dorso-ventral axis → very high Shh concentration in floor plate prohibits neural differentiation and maintenance of progenitor cell populations

75 *Antwort*

- early ectoderm development
  - specification of ectoderm
- neural tube - CNS: brain and spinal cord
  - neural tube and neural crest formation
  - specification of neural cell types
  - development of brain
- surface ectoderm development
  - epidermis - upper layer of skin and organs of skin
  - hair follicle: stem cell concept and hair regeneration

Formation of Neural Tube - Images

Development of Spinal Cord & Hair Follicle

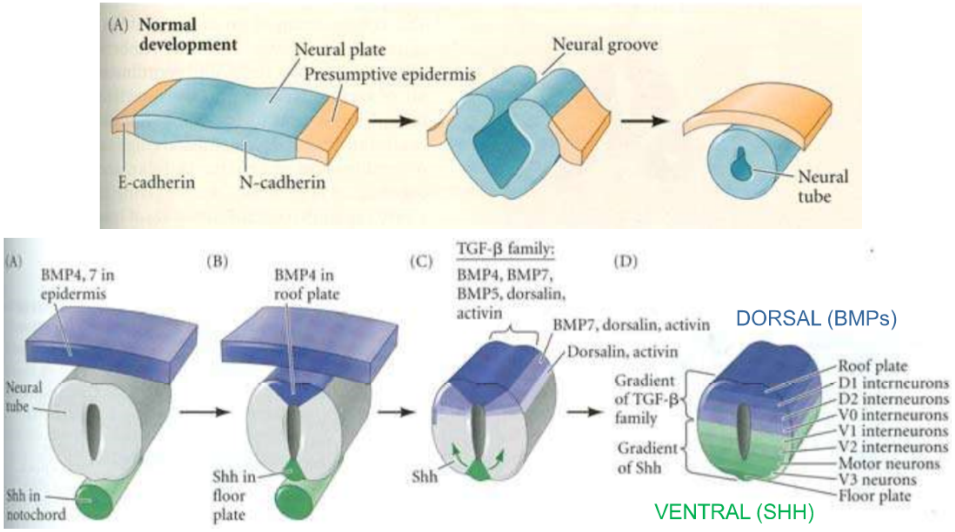
Development of Hair Follicle - Images

Development of Eye

78 *Antwort*

- neural tube forming spinal cord divided into ventral and dorsal part by sulcus limitans
- dorsal part of spinal cord receives information from ventral part which harbours cell bodies of motor neurons
- sensory sensory input into dorsal part of spinal cord
- longitudinally spinal cord is connected by tracks to brain stem
  - formation of hair follicle initiated by clustering of dermal fibroblasts - derived from dermis - to form dermal papilla
  - ectoderm above starts to involute and form hair placode
  - hair is derived from ectodermal stem cells residing in bulged region within lateral wall of hair follicle

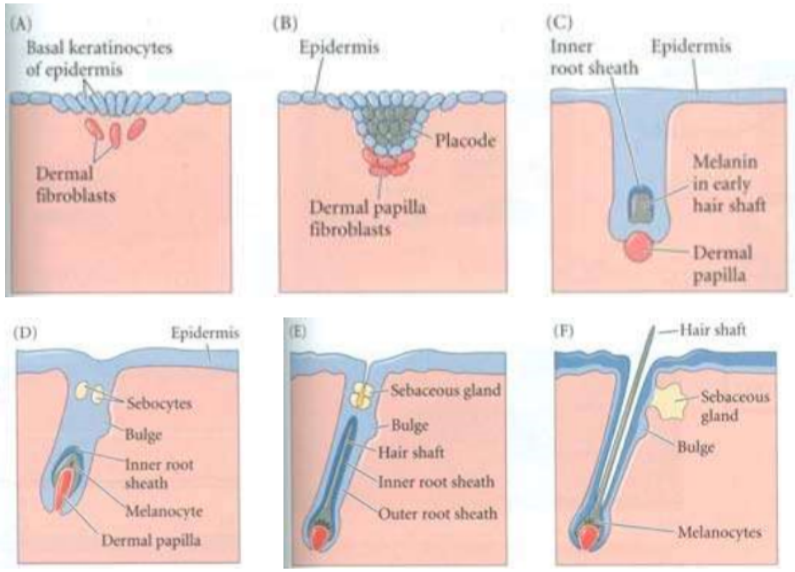
77 *Antwort*



80 *Antwort*

- develops primarily from ectoderm
- neural ectoderm in forebrain region begins to from eye field
- neural ectoderm then induces surface ectoderm to form lens placode
- neural ectoderm develops into retina including photoreceptor, neural cells, and optic nerve
  - inner layer of neural ectoderm forms neural retina → forming photoreceptors, glia cells, ganglion cells and interneurons
  - outer layer of neural ectoderm develops into retina pigment that produces melanin - light absorbing pigment
- Otx2 and Pax2 are transcription factors required for eye development and mark distinct stages of differentiation

79 *Antwort*



Age-Related Makular Degeneration
AMD

Differentiation of Human ES Cells into RPE Cells

Types of Pluripotent Cells

Gastrulation in Mouse Embryo

82 *Antwort*

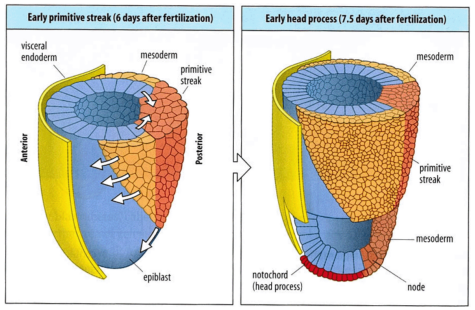
- clusters are formed from hESCs → embryoid bodies (EBs) in presence of nicotinamide (NIC) → NIC inhibits PARP/apoptosis required for mitochondrial ATP
- after 2 weeks Activin A is added → after another 2 weeks pigmented celsl are isolated
- marker proteins for RPE cultures: PAX6, OTX2...neuroectoderm, RPE65...RPE marker

81 *Antwort*

- most common cause of blindness and mild forms occur in nearly 30% of > 75 years old
- characterized by presence of cellular debris (drusen) in or under retinal pigment epithelium (RPE), irregularities in pigmentation or geographic atrophy
- once lost, RPE and retina cannot be restored
- risk factors: genetics, smoking, alcohol, diet, photo damage
- RPE cells proliferate + differentiate in development but not in adults
- RPE functions: provision of nutrients, growth factors, recycling of photopigments, phagocytosis, blood-retina barrier
- RCS (Royal Collge of Surgeosn) rat model has mutation in Mertk tyrosine kinase that impairs phagocytosis function in RPE cells → MERTK mutations in humans cause retinitis pigmentosa

84 *Antwort*

- mesoderm: at primitive streak, cells undergo EMT, incress, migrate away
- endoderm: cells fated to form derfinitive endoderm undergo MET and then egress into epithelium of embryonic visceral endoderm → changes its state by downregulation of visceral and upregulation of definite endoderm markers, possible that extraembryonically specified endoderm can contribute to endodermal tissue of embryo



83 *Antwort*

- naive ES
  - from pre-implantation blastocyste
  - LIF-responsive
- EpiSC
  - post-implantation embryo
  - Activin/FGF2-responsive
  - resembles human ESC more closely

Lateral Segregation of Mesoderm

Splanchnic Mesoderm Development

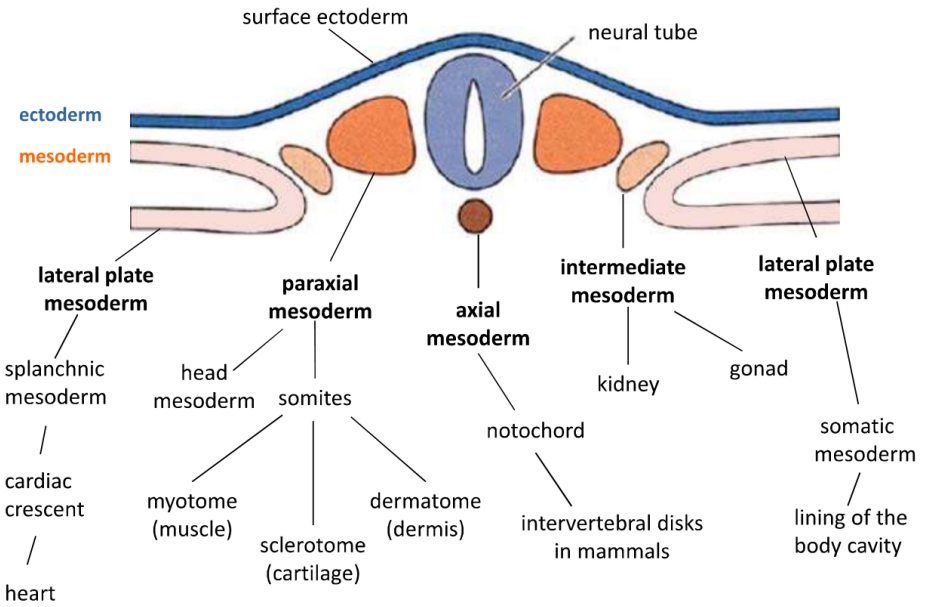
Somitogenesis

Summary of Mesoderm Development

86 *Antwort*

- splanchnic mesoderm develops into connective tissue and blood vessels of visceral organs + cartilage of lungs
- cardiac cresent also formed from anterior splanchnopleuric mesenchyme
- cardiogenic mesoderm develops into two heart tubes which fuse and fold to ultimately form embryonic heart
- cardiac muscle and endothelium are mesoderm derived, neural crest contributes to separation of heart chambers

85 *Antwort*



88 *Antwort*

- major subdivisions of mesoderm based on lateral position (from midline outwards)
- **axial mesoderm**: precordal plate (anterior) and notochord (corda dorsalis)
- **paraxial mesoderm**: presomitic plate mesoderm and somites
- **intermediate mesoderm**: gonads (mesonephros) and kidneys (metanephros)
- **lateral plate mesoderm**: initially continuous with extraembryonic mesoderm. embryonic part splits into splanchnic and somatic mesoderm (lines body cavity)

87 *Antwort*

- as AP-axis elongates and Hensen's node (point of germ layer formation) regresses towards posterior end, leaves behind presomitic mesoderm that consists of patterned areas → mature into somites (segmental plates)
- somites form from mesenchymal presomitic mesoderm cells by epithelialization of cells of segmental plate (=MET - mesenchymal-to-epithelial-transition)
- periodic waves of singlaing through posterior part of presomitic mesoderm correlate with formation of smites
- specification of somites is inhibited by FGF8 and Wnt signals at posterior end and induced by anterior retinoic acid signaling
- Hairy (Hes1 in mosue) is a transcription factor regulated Notch signaling (→ autoinhibition) → starts in posterior and progresses upward and narrows as it approaches anterior end
- cyclic expression patterns during somite formation → proposal of clock and wavefront models
- somites contribute to dermatome (forms dermis), myotome (forms skeletal muslce) and sclerotome (forms vertebrae, cartilage and bones)

Development of Circulatory System & Kidneys

Development of Endoderm
(I/II)

Development of Endoderm
(II/II)

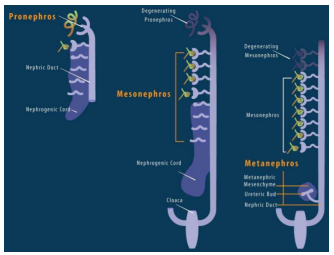
Development of Pharyngeal Pouches

90 *Antwort*

- mesoderm is open and continous with yolk sac
- primitive gut tube forms by lateral folding
- embryo resembles an assemblage of tubes that run longitudinal to anterior-posterior axis
- additional tubes are generated by folding up lateral parts of embryo
- transverese folding (also called flexion) generates right and left lateral folds
- lateral regions of embryonic disc roll ventrally rounding embryo towards midline → results in cylindrical shape of embryo where anterior-posterior axis is axis of cylinder
- yolk sac is constricted by involution of body wall, gut tube incorporates within embryo
- endoderm forms an epithelial lining of gut lumen surrounded by splanchnic mesoderm (→ froms connective tissue, muscle, blood vessels)
- endoderm tube divided into foregut, midgut, and hindgut → from specialized structures according to interactions with adjacent mesodermal structures

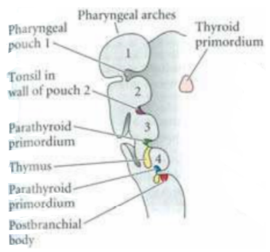
89 *Antwort*

- cardiac crescent → linear heart tube → looping → remodelling into heart
  - 3 stages - **Pronephros**: earliest nephric stage, completely disappears around 4. week, in most primitive vertebrates, pronephros constitutes kidney
  - **Mesonephros**: develops by formation of mesonephric tubules from intermediate mesoderm, principal excretory organ during early embryonic life (4-8 weeks), gradually degenerates, part of its duct system become associated with male reproductive organs
  - **Metanephros**: arises caudal to mesonephros at 5 weeks of development, permanent and functional kidney in higher vertebrates, derived from intermediate mesoderm, ureteric bud arises as a diverticulum from Wollfian duct close to entrance to cloace and grows towards and inside of metanephric mesenchyme



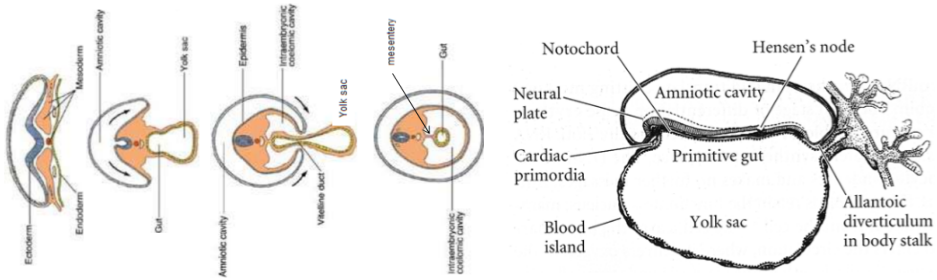
92 *Antwort*

- pouces give rise to several glands and the thymus
  - 1st pouch: auditory tube (inner ear)
  - 2nd pouch: tonsils
  - 3rd pouch: parathyroid gland (anterior), thymus (posterior)
  - 4th pouch: parathyroid gland, postbranhial body (part of thyroid gland)
  - thyroid gland develops from thyroid primordium in lateral wall



91 *Antwort*

- at level of midgut connection to yolk sac persists but becomes gradually more narrow → remaining connection to yolk sac becomes vitelline duct
- rapid extension of neural plate bends embryo inducing cranial and caudal folding
- further growth leads to narrowing of opening between gut and yolk sac, which becomes vitelline duct
- yolk sac is constricted by involution of body wall, gut tube becomes incorporated within embryo
- at level of midgut a connection to yolk sac persists but becomes gradually more narrow



Development of Respiratory Ducts and Lungs

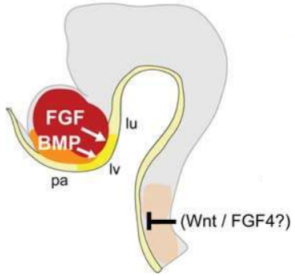
Development of Gut Endoderm

Summary of Endoderm Development

Extracellular Matrix
ECM

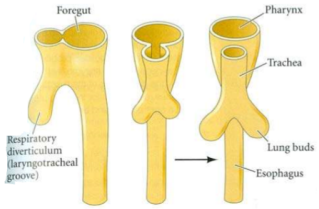
94 *Antwort*

- liver and pancreas develop as accessory organs from primitive gut tube
- hepatic region of gut endoderm - ventral foregut - expresses α-fetoprotein and albumin
- FGF signaling from cardiogenic mesoderm and BMPs from septum tranversum mesnchyme induces a portion of ventral foregut to differentiate into liver
- different doses of FGF appear to induce lung (lu) and ventral pancreas (pa)
- signals from axial mesoderm (candidates include FGF4 and Wnt ligands) repress hepatic fate in dorsal endoerm



93 *Antwort*

- respiratory diverticulum develops at end of pharynx on ventral side of foregut
- tracheal tube buds off from esophagus and elongates towards posterior
- repeated splitting of tracheal tube result in branched tubes of airway system
- tracheal bud develops into bronchi → branch further into bronchioli → alveoli form at ends of bronchioli
- epithelia derived from endoderm, mesenchyme from mesoderm -including blood vessels, connective tissue, cartilage of lungs and trachea



96 *Antwort*

- collection of extracell. secreted molecules providing structural + biochemical support
- signals coming from matrix influence all aspects of cell behaviour → survival, proliferation, migration, adhesion
- secretion of components via ER → Golgi → extracellular environment
- essential protein modification in secretory pathway:
  - disulfide bond formation in ER
    - intra- or inter-protein → stabilize tertiary or quaternary structure
  - protein glycosylation in ER and Golgi (N-GlcAc or O-GalNAc-linked)
    - supports proper protein folding and transport
    - limits protease accessibility
    - regulatory role in signaling

95 *Antwort*

- lateral folding of endoderm and attached splanchnic mesoderm produces an embryonic gut tube → subdived along AP-axis: pharynx, foregut, midgut and hindgut
- endoderm forms an epithelial lining and further receives inductive signals from interactions with mesodermal cells (or ectoderm → pharyngeal pouces) and posterior end
- splanchnic mesoderm contributes muscle, blood vessels, and connective tissue to gut
- dorsal mesentery connects digestive tract to body wall and blood circulation → connection essential for looping of intestine
- pharyngeal pouces give rise to inner parts of auditory ducts, parathyroid gland and thymus → thyroid gland develops from thyroid diverticulum and migrates posterior
- respiratory system and lungs form from respiratory diverticulum at position posterior of pharyngeal arches and ventral of esophagus → airways develop by repeated branching
- cardiogenic mesoderm induces liver formation from section of foregut, notochord blocks it
- pancreas develops from endoderm with same developmental competence

Function and Main Components of ECM

Proteoglycans

Collagen

Multiadhesive ECM Proteins

98 *Antwort*

- proteins covalently linked to glycosaminoglycans (GAGs) of variable length → up to 95% carbohydrates by weight
- long, unbranched glycosaminoglycan chains
- occupy space between cells and collagen
- form high viscosity environment (e.g. lubricating fluid in joints)
- formation of network by linkin collagen fibers
- sponge/shock absorber
- assist migration and adhesion by providing scaffold
- bind cytokines and chemokines to protect them from proteolysis
- form hydrated matrices

97 *Antwort*

- structural support
- biochemical support
- segregate tissues from each other
- regulate intercellular communication
- highly viscous proteoglycans (aggrecan, perlecan, syndecan, ...)
- insoluble collagen fibers
- multiadhesive ECM proteins (fibronectin, laminins, ...)

100 *Antwort*

- **laminin**
  - formed by three disulfid-bonded polypeptide chains
  - several specialized binding motifs for other proteins
  - main component of basal lamina
- **fibronection**
  - two polypeptide chains joined by disulfide bonds
  - each chain subdivided into domains
  - individual domains contain specialized modules

99 *Antwort*

- most abundant protein in body ($\approx 30\%$ of total protein mass)
- forms triple helices that self-assemble into fibrils
- main structural protein in connective tissue
- provides strength, support, flexibility and shape of tissue
- proper alignment of cells for proliferation and differentiation
- sequestering of soluble ECM cytokines
- activation of signaling cascades

Other ECM Components

Fibroblasts

ECM Remodeling During Fracture Repair & Enzymes for ECM Degradation

ECM Remodeling During Fracture Repair

102 *Antwort*

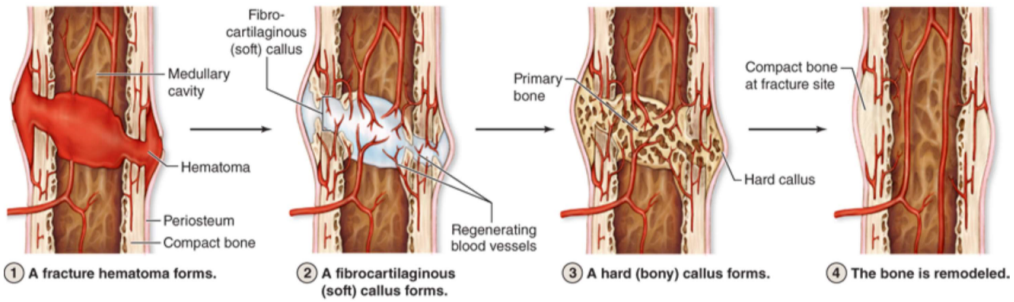
- major matrix producers
- highly heterogenous
- of different origin
- activation: fibroblast → myofibroblast
- bone marrow: fibrocyte
- vessel: pericyte/vascular smooth muscle cell

101 *Antwort*

- **matricellular proteins**
  - dynamically expressed, non-structural matrix proteins with regulatory roles
  - osteopontin, tenascin C, CCN, thromospondin
- **growth factors**
  - autocrine and paracrine signaling
  - elicit intracellular signaling cascades
  - FGFs, VEGFs, WNTs, BMPs
- **extracellular portion of transmembrane receptors**
  - involved in extra-/intracellular signaling and binding to extracellular components

104 *Antwort*

- composition of fracture matrix changes over time
  - loose fibrous matrix (collagen I and III, proteoglycans)
  - dense fibrocartilage
  - collagen II and cartialge specific protreoglycans



103 *Antwort*

- composition of fracture matrix changes over time
  - loose fibrous matrix (collagen I and III, proteoglycans)
  - dense fibrocartilage
  - collagen II and cartialge specific protreoglycans

◇ matrix metalloproteinases (MMPs)

◇ serine proteinases (e.g. plasmin)

◇ cysteine proteinases (e.g. cathepsin K)

◇ aspartic proteinases (e.g. pepsin)

Fibroproliferative Disease

Effects of Pro-Fibrogenetic TGF-β Signaling

Collagen Deposition in Fibrotic Tissue

Disease Associated with Liver Fibrosis

106 *Antwort*

- activated by angiotensin II, thrombospondin-1, platelets, macrophages, and mesenchymal cells
- inhibition of proteases
- inhibition of matrix degradation enzymes
- activation of matrix proteins
- differentiation of fibroblasts to myofibroblasts
- proliferation of fibroblasts
- epithelial-mesenchymal transition - epithelial cells lose cell polarity and adhesion and become mesenchymal stem cells

105 *Antwort*

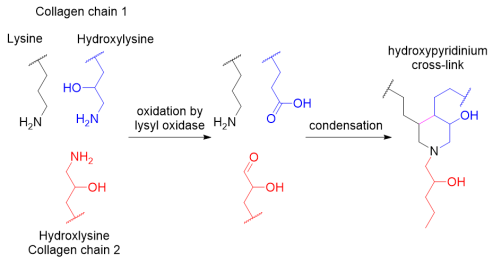
- characterized by abnormal matrix remodeling and deposition
- healty tissue:
  - slow metabolic turnover & limited proteolytic activity
  - quiescent fibroblasts
- fibrotic tissue:
  - immune cell infiltration
  - excessive deposition of collagens
  - increased collagen cross-linking
  - fibroblast activation by pro-fibrotic cytokines (myofibroblasts)
  - dysregulated MMP activity

108 *Antwort*

- NASH
- alcoholkic liver disease
- idiopathic portal hypertension
- congenital hepatic fibrosis
- HCV/HBV
- autoimmune hepatitis
- primary sclerosing cholangitis
- primary biliary cirrhosis

107 *Antwort*

- collagen triple helix formation
- secretion of pro-collagen
- N- and C-terminal cleavages
- organization in fibrils
- covalent cross-linking of fibirils
- increased collagen cross-linking in fiboritc tissue results in increased stiffness



Progession from Normal to Damaged Liver

Hepatic Stellate Cells
HSCs

Advantages of Animal Models

Disadvantages of Animal Models

110 *Antwort*

- also known as Ito cells
- key player in liver fibrosis
- in normal cell in quiescent stage
- activation when liver is damaged → promote ECM production, migration, proliferation, and contractility

109 *Antwort*

1. normal liver
2. steatosis: fatty liver - abnormal retention of lipids within cell
3. steatohepatitis: inflamed liver - inflammation and fat accumulation
4. fibrosis: ECM deposition (scar tissue accumulation)
5. cirrhosis: irreversibly advanced fibrosis
6. hepatocellular carcinoma

112 *Antwort*

- animals do not develop human diseae
- animals react diverently to various noxious agents → total aversion to alcohol in rodents
- some hepatic diseases do not exist in rodents → HCV only infects humans
- different timing in onset and progression of diseae → biliary cirrhosis develops in only a few weeks in rodents
- difficult/impossible to reproduce multifactor diseases

111 *Antwort*

Compared to clinical studies:

- collection of multiple samples at different time-points → possibility of sequential studies
- disease develops in relatively short time
- control and reduction of variables that cannot be closely followed in humans
- potential use of genetically modified animals

Compared to *in vitro*-studies:

- study of complete organ
- intact and dynamic cell-cell and cell-matrix interactions
- cross-talk with immune system/circulatory system

Mouse Models to Study Liver Fibrosis

Chimeric Liver Model

Treatment Approaches and Detection of Fibrosis

Growth Factors

114 *Antwort*

- HBV and HCV only infect human hepatocytes → no suitable mouse models
- solution: chimeric mouse with humanized liver → implantaton of human hepatocytes in mouse
- drawbacks:
  - no progression to fibrosis or HCC commonly seen in humans with HBV or HCV
  - cross-talk and interactions between human hepatocytes and resident murine non-parenchymal cells is suboptimal
  - missing immune system (ie. immunodeficient mice), which normally plays a fundamental role in progression of infection/disease → generation of double grafted mice: human immune system and human hepatocytes)

113 *Antwort*

- Hepatotoxin-induced liver fibrosis
  - models of post-necrotic fibrosis (killing of hepatocytes)
  - repeated injection of TAA (thioacetoamide), CCl_4, DMN, DEN, ... toxins
- biliary fibrosis
  - interruption of bile flow
  - common bile duct ligation
- autoimmune fibrosis
  - challenge lies in breaking immune tolerance and long-term maintenance of immune alterations OR use of genetic models
- NASH-associated fibrosis
  - dietary (hypercaloric), genetic (PTEN deletion in hepatocytes) or combined modekls

116 *Antwort*

- produced by almost every cell type
- usually small, secreted peptides
- every cell requires the presence of a certain set of growth factors
- act on cells that have appropriate receptors
- are active in nanomolar or picomolar concentrations
- regulate proliferation, differentiation, survival, migration
- are always required - in particular during development, tissue repair and tumorigenesis
- cellular responses depend on ligand, receptor, and cell type as well as on the combination of ligands

115 *Antwort*

- control or cure primary disease (e.g. Hepatitis B/C)
- block cellular differentiation (e.g. fibroblast activation)
- inhibit fibrogenesis
- promote resolution of fibrosis
  - Sirius Red staining: collagen staining, used for diagnosis of fibrosis
  - Masson's thrichrome staining: collagen green, ceratine red, cell nuclei brown, used for study of cardiac, muscular, renal and hepatic pathologies

Cytokines & Dimerization Patterns of Receptor Tyrosine Kinases by Ligands

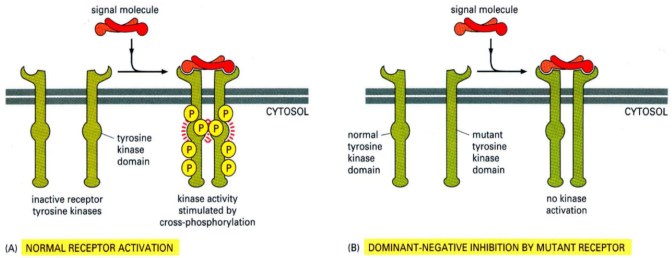
Signaling Between Ephrins and Eph Receptors, Heterodimers & Dominant-Negative Receptor Mutants

Signal Tranduction by receptor tyrosine kinases

Overview of Intra- and Extracellular Phosphorylation

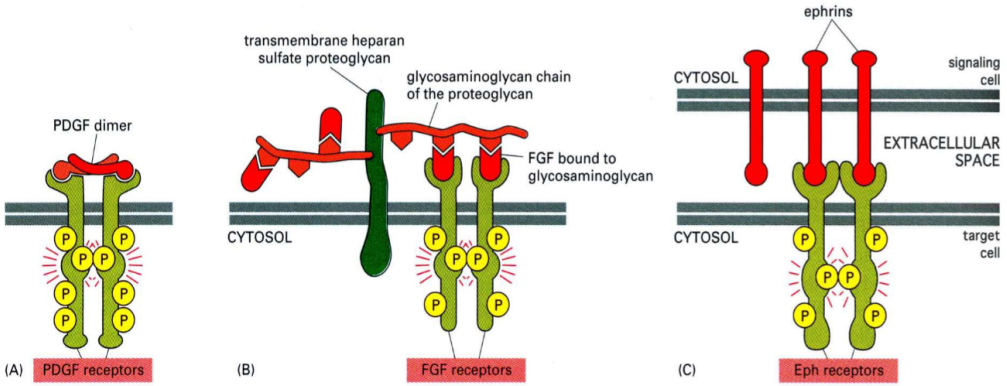
118 *Antwort*

- bidirectional: ephrins and Eph receptors can simultaneously act as ligands or receptors
- during development to prevent groups of cells to mix with each other
- heterodimerization is possible for growth factor receptors that belong to the same family
- each heterodimer binds a specific set of ligands
- e.g. PDGF receptor family, EGF receptor family, FGF receptor family



117 *Antwort*

Cytokines molecules that regulate proliferation, differentiation, survival and other cellular functions at extremely low concentrations



Ligand dimer

Ligands bound by proteoglycans

Cell-surface bound ligands

120 *Antwort*

Intracellular Phosphorylation	Extracellular Phosphorylation
well understood	mostly uncharacterized
many kinases reported	only two known kinases
fundamental biological process	of largely unknown significance
reversible	?
takes place in cytoplasma	?
known ATP otima	unknown ATP conc.
kinases as major drug targets	
well optimized experimental protocols	protocols still to be established

119 *Antwort*

- phosphorylation outside the kinase domain provide docking site for other proteins
- transautophosphorylation acts as swith to trigger assembly of transient intracellular signaling complex
- a variety of proteins bind phosphorylated tyrosines on RTKs
- common highly specific p-Tyr binding domains - e.g. SH2 or PTB

FAM20C & VLK

PDGF *in vivo* and in Disease

PDGF Antagonists

G-Protein-Coupled Receptors
GPCR

122 *Antwort*

- embryonic development
  - kidneys - mesangial cells, blood vessels - smooth muscle cells, pericytes, lungs - alveolar smooth muscle cells, CNS - oligodendrocytes
- stimulation of wound healing
- fibrotic conditions
  - lung fibrosis, liver cirrhosis, myelofibrosis, glomerulonephritis
- artherosclerosis
- cancer
  - autocrine stimulation - glioblastoma, sarcoma
  - paracrine stimulation - carcinomas

121 *Antwort*

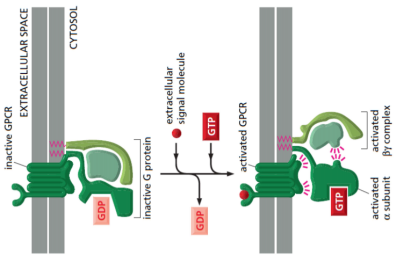
- localized to Golgi and then secreted outside of cell
- phosphorylizes casein and other extracellular proteins → 1/3 of all secreted proteins have bee shown to be tyrosine phosphorylated
- associated with Rain syndrome: rare autosomal recessive congenital (= congenital) disorder

VLK- vertebrate lonesome kinase:

- signal peptide required for secretion of VLK
- $VLK^{-/-}$ are smaller and die due to respiratory failure at birth
- platelets release VLK following degranulation
- VLK phosphorylates secreted proteins

124 *Antwort*

- binding of extracellular signal to GPCR changes conformation of receptor → alters conformation of G protein
- conformational change in α subunit allows for exchange of GDP to GTP → activation of α subunit and $\beta\gamma$ complex that regulate the activity of target proteins in the plasma membrane
- leads to increase in cAMP and activation of PKA
- leads to increase in cytosolic Ca^{2+} and activation of PKC



123 *Antwort*

- PDGF aptamer
  - DNA molecule that binds PDGF-B chain with high affinity
  - specific
  - expensive, cumbersome to administer
- STI571 (Gleevec)
  - small molecule inhibitor of PDGF receptors c-Kit, Bcr-Abl
  - not absolutely specific
  - inexpensive, easy to administer

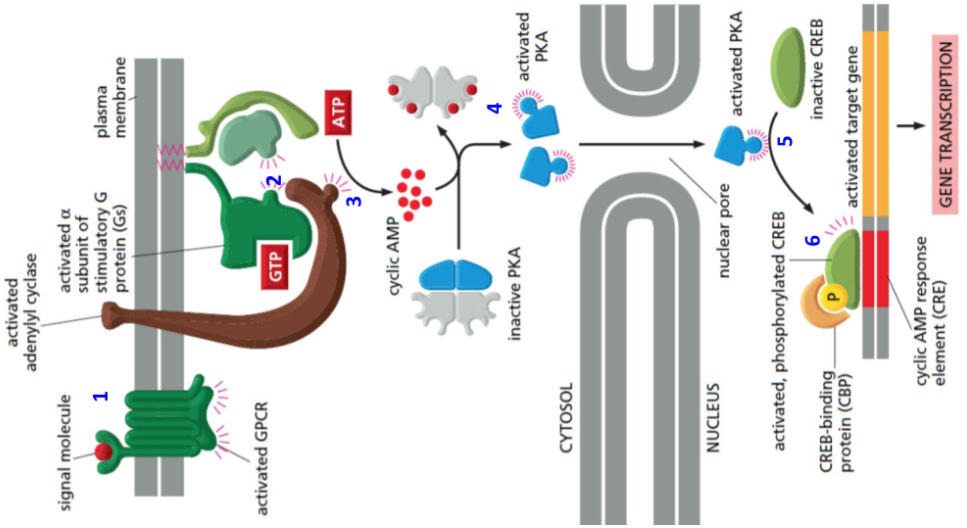
PKA Activation
(I/II)

PKA Activation
(II/II)

PKC Activation
(I/II)

PKC Activation
(II/II)

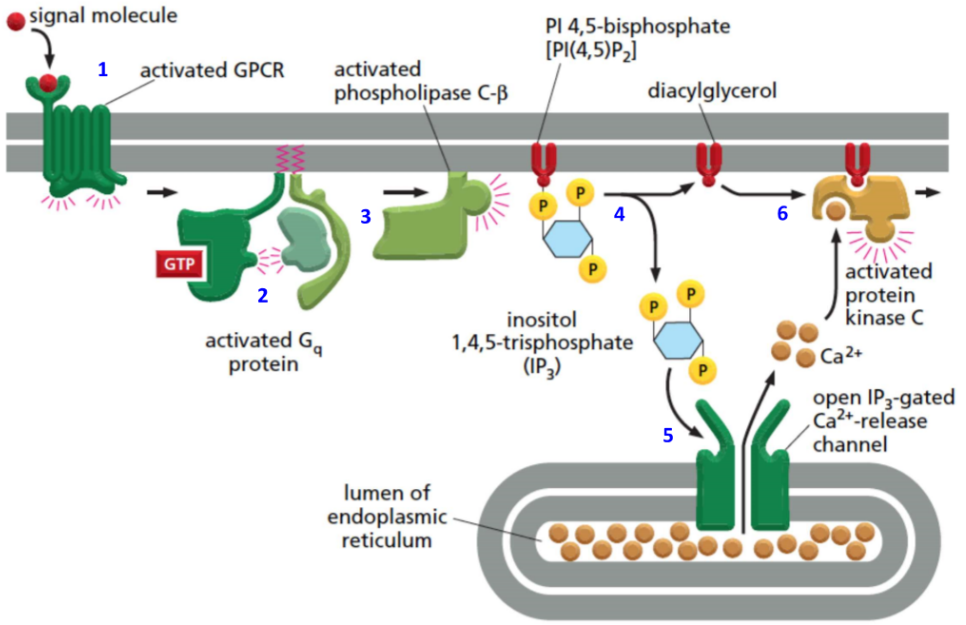
126 *Antwort*



125 *Antwort*

1. extracellular molecule binds GPCR
2. GDP → GTP → α subunit of G protein ("stimulatory G-protein") activates adenylyl cyclase
3. activated adenylyl cyclase increases levels of cAMP
4. binding of cAMP to regulatory subunits of PKA tetramer → dissociation of catalytic subunits
5. PKA catalytic subunits enter nucleus and phosphorylate CREB
6. phosphorylated CREB recruits co-activator CBP → activation of gene

128 *Antwort*



127 *Antwort*

1. extracellular molecule binds GPCR
2. GDP → GTP → G protein now active
3. activated G protein stimulates PLC-β
4. PLC-β hydrolyses $PI(4,5)P_2$ generating IP_3 and diacylglycerol (DAG)
5. IP_3 diffuses through cytosol and releases Ca^{2+} from ER by binding to IP_3 receptors in ER membrane
6. DAG - in plasma membrane - helps to activate protein kinase C - PKC

Receptor Tyrosine Kinases
RTKs

Phosphotyrosine-Binding Domains, Activation of Signaling Molecules by RTKs & Signaling Molecules

Phospholipase C-γ
PLC-γ

Ca^{2+} as a Second Messenger

130 *Antwort*

- signaling proteins bind to phosphorylated receptor via **SH2** or **PTB** domains
- **SH2**: Src homology domain 2: ≈ 100 aa that recognize phosphorylated tyrosines and their close vicinity $\rightarrow$ bind with high affinity (10^{-9}M)
- **PTB**: phosphotyrosine binding domain

- activation by tyrosine phosphorylation
- activation by conformational change without tyrosine phosphorylation
- activation through recruitment to membrane $\rightarrow$ close to substrates

- enzymes: PLC-γ, Src (cytoplasmic tyrosine kinase), c-cbl (Ubiquitin ligase), PI_3 - kinase
- structural proteins
- adaptor proteins (Grb-2, Shc, Eps15, Epsin)

129 *Antwort*

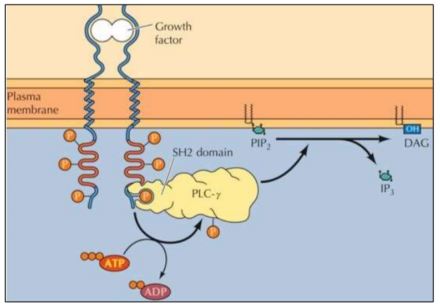
- extracellular ligand binding causes the receptor chains to dimerize $\rightarrow$ kinase domains of two receptor chains come together $\rightarrow$ both become active and cross-phosphorylate each other on multiple tyrosines - a process called **transphosphorylation**
- normal receptors: dimerize normally $\rightarrow$ cross-phosphorylation increases activity of kinase domains $\rightarrow$ can phosphorylate other sites on receptor
- mutant receptor with inactivated kinase: dimerize normally, but cannot cross-phosphorylate $\rightarrow$ if mutant receptors are present in excess, block signaling: **dominant-negative regulation**
- phosphorylation of tyrosines outside kinase domain creates high-affinity docking sites for intracellular signaling proteins $\rightarrow$ each binds to specific site on activated receptors as it contains a specific phosphotyrosine-binding domain
- RTKs: EGFm PDGF, FGF, VEGF, Eph, insulin, IGF-1 receptors

132 *Antwort*

- cytosolic Ca^{2+}: 10^{-7}M
- extracellular (and ER lumen) Ca^{2+}: 10^{-3}M
- opening Ca^{2+} channels after growth factor stimulation $\rightarrow$ 10-12 fold increase in cytosolic Ca^{2+}
- Ca^{2+} binds to different proteins (e.g calmodulin) that mediate Ca^{2+} signal
- determination of intracellular Ca^{2+} by FURA-2 - cell-permeable Ca^{2+} dye $\rightarrow$ calcium chelator that fluoresces red upon binding Ca^{2+}

131 *Antwort*

- increases cytosolic Ca^{2+} & activation of PKC
- PLC-γ binds receptor protein tyrosine kinases via SH2 domain $\rightarrow$ phosporylated (active)
- PLC-γ stimulates hydrolysis of PIP_2 to DAG and IP_3
- IP_3 regulates Ca^{2+}
- DAG activates PKC family



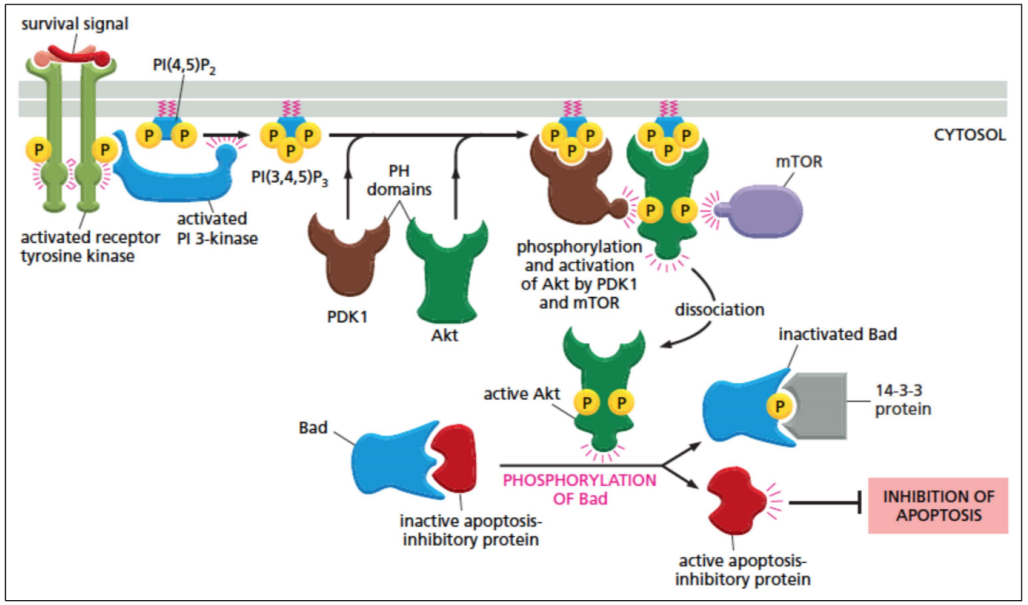
Phosphatidylinositide-3-Kinase
PI_3-Kinase

PI3K-AKT Signaling Pathway

Functions of AKT (PKB)

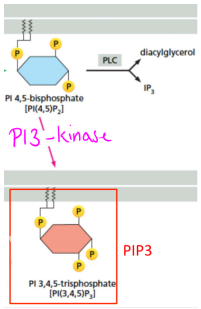
PTEN

134 *Antwort*



133 *Antwort*

- consists of regulatory and catalytic subunit
- regulatory subunit binds phosphorylated receptor tyrosine kinase → recruitment of enzyme to membrane where substrates are located
- catalytic subunit phosphorylates phosphoinositol-4,5-biphosphate at plasma membrane at positon 3



136 *Antwort*

- PIP_3 phosphatase
- dephosphorylates PIP_3 on position 3 to PIP_2
- classical tumor suppressor: somatic mutations in many human tumors (particularly prostate, thyroid, endometrium) causing increased AKT activity → mutated cells escape senescence
- KO mice die before birth, heterozygous mice have an increased tumor incidence
- Cowden diseae: genetic disease characterized by formation of multiple tumors → germ-line mutations in PTEN gene, patients have multiple skin lesions and develop polyps and adenomas in gut and thyroid, high breast cancer risk

135 *Antwort*

- a serine/threonine kinase
- phosphorylates transition initation factor → stimulation of translation
- phosphorylates phosphofructokinase-2 → increased production of frucotse-2,6-biphosphate → stimulation of glycolysis
- phosphorylates glycogen synthase kinase 3 (GSK3) and inactivates it → reduced phosphorylation of glycogen synthase (GS) → enhanced production of glycogen
- induces translocation of glucose transporters to plasma membrane
- prevents apoptosis via different mechanisms → increased cell survival under stress conditions
- involved in tumorigenesis → often overexpressed in malignant tumors

Adaptor Proteins

Ras

MAP Kinase Signaling Pathway
(I/II)

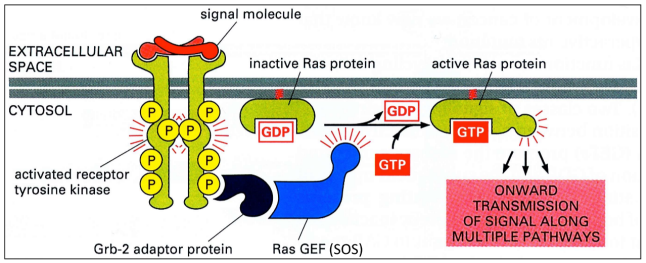
MAP Kinase Signaling Pathway
(II/II)

138 *Antwort*

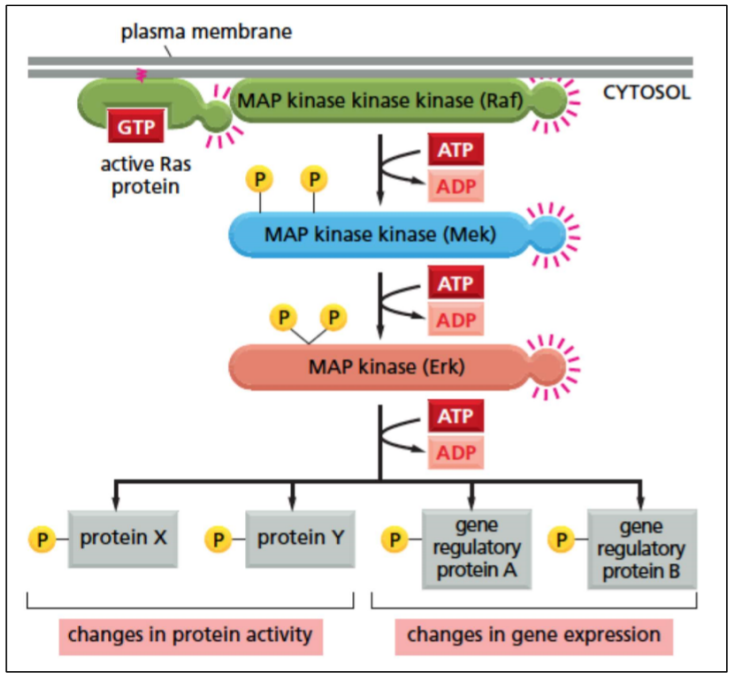
- monomeric GTPase
- belongs to large superfamily of monomeric GTPases, but only Ras and Rho relay signals from cell-surface receptors
- functions as a molecular switch, cycling between two conformational states: active when GTP is bound and inactive when GDP is bound
- Ras guanine nucleotide exchange factors (Ras-GEFs) stimulate the dissociation of GDP and subsequent uptake of GTP from cytosol → Ras activation
- Ras GTPase-activating proteins (Ras-GAPs) increase rate of hydrolysis of bound GTP by Ras → inactivating Ras
- hyperactive mutant forms of Ras are resistant to hydrolysis of GTP → locked permanently in GTP-bound active state → oncogenic!

137 *Antwort*

- lack catalytic domain
- have at least one SH2 domain or PTB domain + domains that interact with other proteins (e.g. SH3)
- e.g.: CRK, NCK, GRB-2, SHC
  - activation of Ras through RTKs and Grb2-SOS complex
  - SOS: Son of Sevenless, SOS1 and SOS2 are RasRas-GEFs that stimulate dissociation of GDP and then uptake of GTP from cytosol → Ras activation



140 *Antwort*



139 *Antwort*

- Raf (MAP3k) activation is initiated by Ras-GTP → conformational changes and recruitment to cell membrane promote RAF phosphorylation
- Raf then activates Mek (MAP2K) → activateds Erk (MAPK)
- Erk phosphorylates downstream proteins - including other protein kinases and gene regulatory proteins in nucleus
- resulting in changes in gene expression and protein activity
- 3 different MAPK cascades:
  - mitogenic factors activate growth, differentiation, development
  - stress, TNfα, IL-1 activate inflammation, apoptosis, growth, differentiation
  - UV, osmotic shock activate inflammation, apoptosis, growth, differentiation

Adaptation / Desensitation

c-CBL

Receptor Trafficking

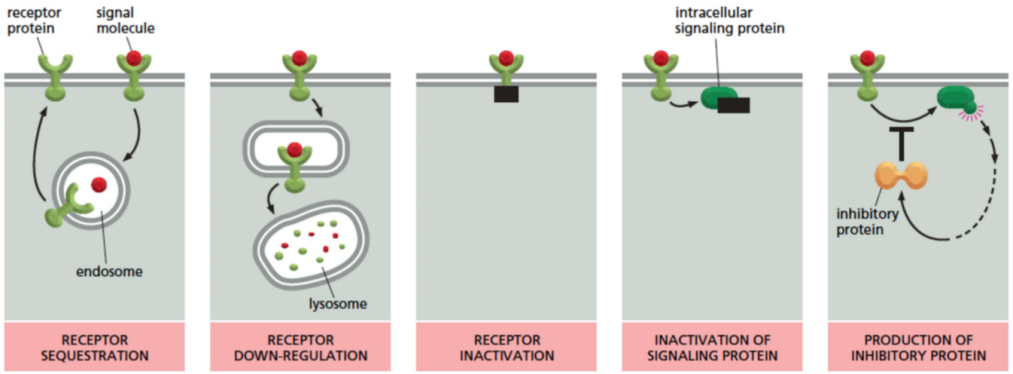
Methods to Analyze Growth Factor Function & MTT Assay

142 *Antwort*

- E3 ubiquitin-protein ligase
- c-Cbl docks on some activated receptors and catalyzes their ubiquitination by adding a single ubiquitin to one or more site on receptor → monoubiquitination
- monoubiquitination promotes endocytosis and degradation of receptors in lysosomes
- endcytoic proteins that contain ubiquitin-interaction motifs (UIMs) recognize monoubiquitylated RTKs and direct them into clathrin-coated vesicles and - ultimately - into lysosome
- mutations that inactivates c-Cbl dependent RTK down-regulation cause prolonged RTK signaling → promote development of cancer

141 *Antwort*

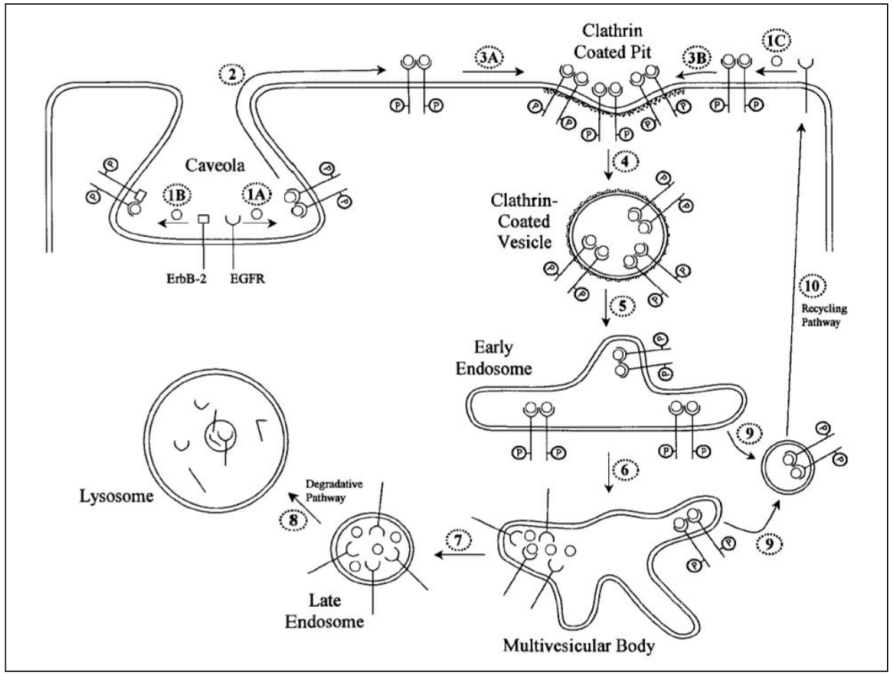
- prolonged exposure to a growth factor reduces the cellular response → reversible process
- advantage: cells can respond to changes in growth factor levels → delayed negative feedback



144 *Antwort*

- analysis of intracellular signalling cascades
- analysis of growth factor regulated gene expression
- analysis of cell proliferation
- analysis of cell migration
- analysis of cell survival
- generation of transgenic mice
- generation of KO mice
- MTT assay: quantitative assay for cell survival and proliferation - based on reduction of MTT (yellow) to formazan product (purple) in mitochondria by NAD(P)H-oxidoreductase → absorbance of purple solution can be quantified at 500-600 nm by spectrophotometer

143 *Antwort*



Methods to Determine Cell Proliferation

Dermatofibrosarcoma Protuberans
DFSP

ROS and their Detoxification

Role of Hydrogen Peroxide in PDGF Signaling

146 *Antwort*

- fibroblast-derived skin tumor of intermediate malignancy
- presently treated with surgery, but high recurrence rate
- characterized by collagen1A1/PDGF-B gene fusion
- fusion gene encodes precursor that is processed to PDGF-BB → high levels of this growth factor are produced in tumors
- Gleevec reduces growth of tumors and blocks PDGF receptor activation

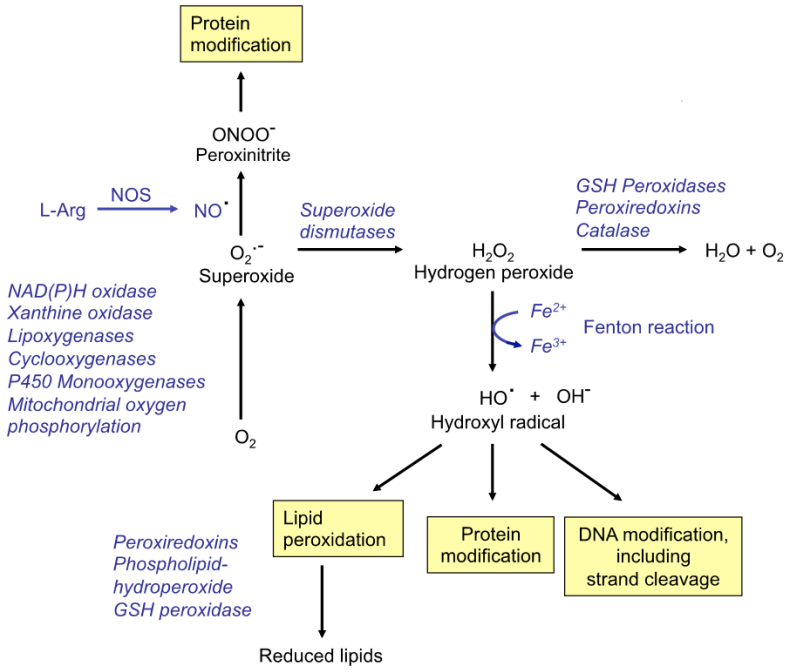
145 *Antwort*

- seed equal number of cells and count cells at different time points
- incorporation of ^{3}H-thymidine → measure incorporated radioactivity or identify labeled cells by autoradiography - almost not used anymore
- incorporation of 5-bromo-2'-deoxyuridine (BrdU, thymidine analogon) → identify labeled cells with antibody directed against BrdU
- BrdU commonly used to identify proliferating cells in living tissue
- antigen Ki-67 is a nuclear protein that is associated with and maybe necessary for cellular proliferation → marker for proliferation
- Ki-67 can be exclusively detected in nucleus during interphase and on surface of chromosomes during mitosis
- Ki-67 absent in resting (quiescent) cells (G_0) → fraction of Ki-67 positive tumor cells often correlates with clinical course of cancer

148 *Antwort*

- scavenging H_2O_2 by catalase inhibits PDGF receptor signaling - including MAPK activation, DNA synthesis, and cell migration
- H_2O_2 production is PI3K dependent → activation of Rac by PI3K
- activation of NADPH oxidase by Rac → production of O^{2-} → dismutation to hydrogen peroxide
- detection of hydrogen peroxide *in vivo* via HyPer (genetically encoded fluorescent sensor) or DCF (dichlorofluorescein)
- hydrogen peroxide produced at wound site attracts immune cells → chemokine

147 *Antwort*



Signaling by PDGF Receptors

Epidermal Growth Factor Receptor Family and Ligands

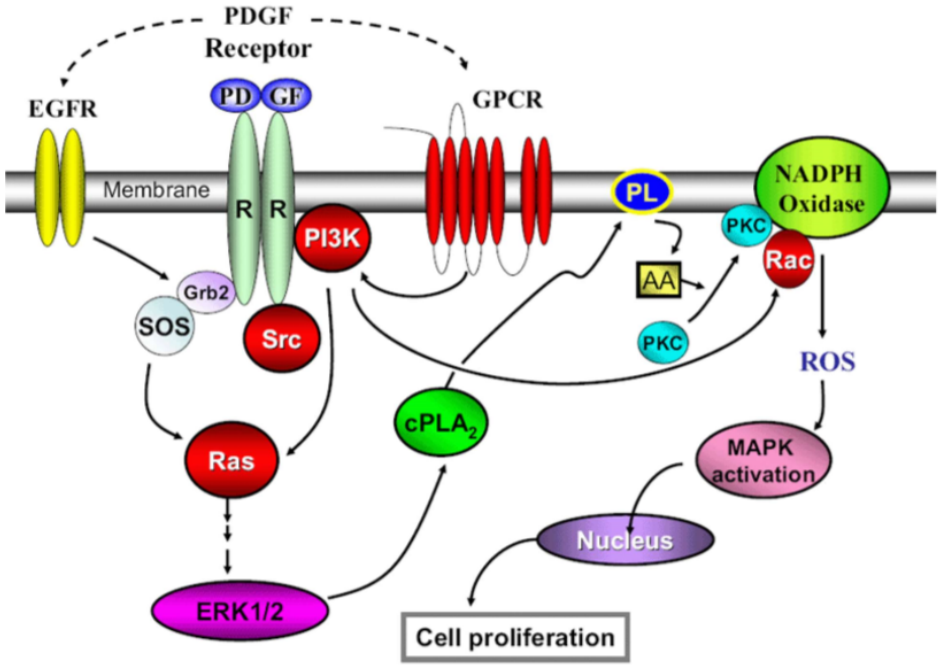
EGF Receptor Ligands & HB-EGF Functions

Phenotypes of EGF-KO Mice

150 *Antwort*

- EGF receptor = HER1 = ErbB1
  - EGF, TGFα - Transforming Growth Factor α, HB-EGF - Heparin-binding EGF, AR - Amphiregulin, Epigen, EPR - Epiregulin
- HER2 = ErbB2 = Neu
  - none identified yet
- HER3 = ErbB3
  - Heregulin 1 and 2 (Neu differentiation factor)
- HER4 = ErbB4
  - Heregulins 1, 2, 3, BTC - Betacellulin, EPR - Epiregulin,HB-EGF - Heparin-binding EGF

149 *Antwort*



152 *Antwort*

- **EGF receptor**: phenotype strain-dependent, only mice of certain genetic background survive until birth → defects in skin, intestine, pancreas, tooth, eyelid, brain
- **HB-EGF**: develop severe heart failure
- **EGF, Epigen**: no phenotype
- **TGF-α**: curly hair
- **AR**: underdeveloped mammary glands
- **EGF/TGF-α/AR**: survive to adulthood, but major mammary gland abnormalities

151 *Antwort*

- produced as membrane-anchored precursors that can act in a juxtacrine manner or that are involved in cell-cell adhesion
- soluble growth factor is produced by proteolytic cleavage → autocrine and paracrine mechanism of action
  - consisting of signaling sequence, heparin-binding domain and EGF domain on extracellular side
  - adhesion
  - proteolytic processing → paracrine growth factor stimulating cell migration and proliferation
  - binds diphteria toxin
  - juxtacrine growth factor

Virus-Encoded EGF-like Growth Factors

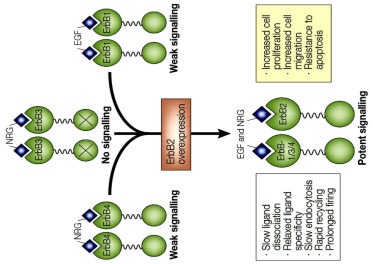
Signaling via ErbB Dimers

Role of c-Cbl in EGFR Degradation

Role of HER-2 in Cancer

154 *Antwort*

- ErbB2 (=HER2) forms heterodimers with ErbB1, ErbB3 and ErbB4
- potent signaling via ErbB2 heterodimers: stable signaling complexes with long half-life
- not a receptor for EGF, but can decreases rate of ligand dissoc. from EGFR
- undergo endocytosis at lower rate than EGFR homodimers
- HER2-EGFR heterodimers are targeted for recycling, while EGFR homodimers are destined for degradation



153 *Antwort*

- not required for viral replication, but enhance virulence
- Shope fibroma virus growth factor (SFGF)
- Myxoma virus growth factor (MGF)
- Vaccinia virus growth factor (VGF)
- produced by Pox virus

156 *Antwort*

- amplified and overexpressed in 30% of all mammary carcinomas
- overexpression correlates with poor clinical prognosis
- overexpression in transgenic mice: increased tumor malignancy, increased metastasis, increased resistance to chemotherapy, hormone-independent
- target for cancer therapy: inhibition by antisense strategy, ribozymes, tyrosine kinase inhibitors AND:
- recombinant, humanized monoclonal antibody (Herceptin): first approved recombinant protein for treatment of cancer, competitively binds HER2, so that EGF cannot bind

155 *Antwort*

- c-Cbl binds to phosphorylate Tyr1045
- ubiquitination of receptor targets for degradation in lysosomes
- in absence of c-Cbl or if c-Cbl binding site is mutated: receptor recycling, increased mitogenicity (substance that encourages mitosis)
- EGFR/Her2 heterodimers: unstable in endosome, causing c-Cbl to dissociate from receptor complex → recycling, no degradation

Use of Herceptin for Treatment of Breast Cancer

Erbitux & Iressa and Tarceva

Blood Vessel Formation

Steps of Angiogenesis &
VEGF Receptors and Ligands

158 *Antwort*

- monoclonal antibody against EGFR, also called Cetuximab
- EGFR often overexpressed in colon cancer
- approved for therapy of advanced colon and rectal carcinoma, often together with chemotherapy
- good success rate, relatively well tolerated
- side effects: skin rash and inflammation → indication that drug works
  - inhibitors of EGFR tyrosine kinase
  - used for treatment of locally advanced or metastatic lung cancer in patients that have received chemotherapy
  - clinical trails show Gefitinib (=Iressa) efficacy in esophageal cancer
  - Erlotinib (= Tarceva) also used for metastatic pancreatic cancer in combination with chemotherapy

157 *Antwort*

- used for treatment of advanced HER2-positive breast cancer
- unfortunately, rapid development of resistance
- side effects: flu-like symptoms, nausea, rash, low white or red blood cell count, in some patients: cardiotoxicity
- new stragtegy: treatment of patients with early stage HER2-positive breast cancer → 50% reduction in relapse
- current issue: worth the risk of developing heart disease? can treatment be shortened?

160 *Antwort*

- angiogenic signal: inflammation, hypoxia
- degradation of ECM
- migration of endothelial cells
- proliferation of endothelial cellks
- contact to ECM
- lumen formation
- stabilization by association with pericytes and smooth muscle cells
- 7 ligands: VEGF-A, -B, -C, -D, -E (produced by parapoxvirus Orf virus), -F (in snake venom), PLGF - placenta growth factor
- 3 receptors: VEGFR1 (blood vessel endothelial cells), VEGFR2 (lymphatic and blood vessel endothelial cells, stimulated by VEGF-A), VEGFR3 (lymphatic endothelial cells, stimulated by VEGF-C and -D

159 *Antwort*

Vasculogenesis

- during early development, de novo formation of vessels, can also occur in adult organism → wound healing and carcinogenesis

Angiogenesis

- late development and adulthood
- sprouting from preexisting vessels
- physiological: menstrual cycle, hair cycle, fat deposition, muscle growth
- pathological: wound healing, heart ischemia, psoriasis, diabetic retinopathy, rheumatoid arthritis, cancer
- growth factors involved: VEGF-A, Ang1, Ang2, Ephrin-B2

VEGF-A &
VEGF/VEGFR Mouse Mutants

Lymphatic Vessels

Angiopoietin

Ephrins and Eph Receptors

162 *Antwort*

- collect extravasated bloodless fluid from tissues and transfers it - as lymph - via collecting lymphatic vessels and thoracic duct back into venous circulation
- serve an immune function by transporting white blood cells and APCs to lymphoid organs
- lined by endothelium and surrounded by smooth muscle cells
- discontinuous or fenestrated basement membrane, lack of tight inter-endothelial junctions, permeable to interstitial fluid and cells
- low flow, low pressure
- less complex network than blood vessels with fewer sprouts
- lymphedema: result from impaired lymphatic drainage
- *congenital lymphedema*: mutations in VEGFR-3 → red. tyrosine kin activity

161 *Antwort*

- most important stimulus: hypoxia → activates HIF → activates VEGF expression
- other inducers; pro-inflammatory cytokines, growth factors, ROS, UV, oncogenes
- VEGFR2 KO: die *in utero*, no differentiation of endothelial cells, no blood vessel formation
- VEGFR1 KO: die between day 8 and 9 due to increase in number of endothelial progenitors → excessive proliferation of vasculature (negative regulator of VEGF-A?)
- VEGF-A heterozygous: die before birth from severe abnormalities in cardiovascular system
- VEGF-C KO: complete absence of lymphatic vessels
- overexpression of VEGF-C and -D in transgenic mice induces lymphangiogenesis

164 *Antwort*

- function: development of nervous system, vasculogenesis, angiogenesis, and others
- 14 Eph receptors, 9 ephrins in mammals
- remarkable: ephrins are transmembrane proteins → juxtacrine mechanism of action, bidirectional signaling, requires cell-cell contact
- EphR dimerize like other RTKs
- Type B ephrins: cytoplasmatic domain that becomes tyrosine phosphorylated upon receptor binding
- Type A ephrins: glycosyl-phosphatidyl inositol (GPI) anchor → recruit adaptor proteins and are able to signal
- ephrins regulate migration, cell-cell attachment, and cell-matrix contacts
- expressed in developing vasculature
- KO mice suggest role in vascular assembly and differentiation of perivascular cells

163 *Antwort*

- bind to TIE-2 receptors or TIE-1/TIE-2 heterodimers
- Angiopoietin-1 and -4 (human) are agonist for TIE-2
- Angiopoietin-2 and -3 (mouse) can be antagonists or agonists depending on cell type and growth conditions
- Ang-2 expressed at sites of vessel remodeling: co-expression with VEGF induces angiogenesis, in absence of VEGF blood vessel regression
- are not mitogenic for endothelial cells, but act in concert with VEGF
- TIE-2/Ang-1 KO: early stages of VEGF-dependent angiogenesis are normal, but vessels unstable → defect in association of pericytes and smooth muscle cells

Role of VEGF in Tumor Angiogenesis

Tumor-Microenvironment & Anti-Angiogenic Tumor Therapy

Inhibitors of Angigenesis & Endostatin

Avastin & SUTENT

166 *Antwort*

Tumor-Microenvironment tumor cells and and associated stromal cells → fibroblasts, vascular and lymphatix endothelial cells (and associated pericytes and smooth muscle cells), immune cells (macrophages, neutrophils, T-cells, B-cells, mast cells, ...)

- observaton: frequent metastasis after removal of primary tumor
- hypothesis: primary tumor produces factors that inhibit growth of metastasis
- evidence: serum and urine of mice that suffer from lung cancer inhibit endothelial cell proliferation → purified: angiostatin - a proteolytic product of plasminogen

165 *Antwort*

- tumors can only grow to diameter of 0.2-0.4 mm when oxygen and nutrients are supplied by diffusion
- VEGF strongly upregulated in almost all tumors by hypoxia, inflammatory cytokines, oncogenes, ROS
- induces survival, migration, and proliferation of endothelial cells and sprouting of new vessels
- recruits endothelial progenitor cells from bone marrow that contribute to formation of new vessels
- recruits inflammatory cells and also stimulates some tumor cells directly
- VEGF antagonism may increase tumor growth: promotion of invasion and metastasis to provide access to normal tissue vasculature, upregulation of other angiogenic factors frequent → new strategy: combined blockage of VEGF and FGF

168 *Antwort*

- aka **Bevacizumab**, humanized monoclonal antibody against VEGF
- approved in combination with chemotherapy for treatment of metastatic colon and rectal cancer, non-small cell lung cancer and breast cancer
- side effects: bleeding, hypertension, holes in colon, impaired wound healing, kidney damage
- additional problem: endothelial cells become resistant → use other factors e.g. FGF2
- aka **Sunitinib**, tyrosine kinase inhibitor
- inhibits VEGFR1-3, PDGF receptors, KIT
- inhibits angiogenesis, reduces vessel stabil by pericytes + inhibits (some) tumors directly
- approved for treatment of advanced gastrointestinal stromal cancer, metastatic kidney cancer, pancreatic neuroendocrine cancer
- side effects: gastrointestinal problems, cardiac problems, anorexia, skin discoloration, mucositis, hypertension, fatigue, bleedings

167 *Antwort*

- synthetic inhibitors: metalloproteinase inhibitors, TNF-70, thalidomide
- endogenous inhibitors: Ang-2, angiostatin, endostatin, IL-12, interferon-α
- biological antagonists: VEGF antibodies (Avastin), VEGF antisense RNAs or ribozymes, VEGF RTK inhibitors
- soluble VEGF receptors, soluble TIE-2
  - naturally occurring C-terminal fragment from type XVIII collagen
  - inhibits angiogenesis and tumor growth in animal models
  - advantage: broad-spectrum inhibitor that blocks different angiogenic factors
  - disadvantage: expensive production, dosing is critical and difficult - low concentrations efficient, high not
  - initial clinical trails failed, recent trails in China more promising → approved in China for treatment of lung cancer in combination with chemotherapy

Nexavar, MACUGEN & RANIBIZUMAB

Lymphoangiogenesis in Cancer & Therapeutic Angiogenesis

Angiogenesis Assays

Fibroblast Growth Factors
FGFs

170 *Antwort*

- tumors spread via lymphatic vessels
- VEGF-C overexpression in mouse tumors increase rate of metastasis
- inhibiton of VEGF-C, -D or VEGFR3 can be used to suppress tumor formation and metastasis in experimental mouse models
  - use of growth factors or gene transfer of growth factors to promote development of collateral blood vessels → new approach for treatment of coronary artery and peripheral vascular disease
  - animal experiments and clinical trials with VEGF: arterial VEGF gene transfer, intramuscular gene therapy (limb muscle, myocardium)
  - healingof ischemic leg ulcers

169 *Antwort*

- aka **Sorafenib**, tyrosine kinse inhibitor
- inhibits VEGFR2, PDGFRβ and Raf kinase
- inhibits angiogenesis and some tumors directly
- approved for treatment of advanced kidney cancer, non-resectable hepatocellular carcinoma and metastatic thyroid cancer

- pegylated anti-VEGF aptamer
- direct injection into eye
- prevents excessive angiogenesis and vascular leakage from eye
- approved for treatment of wet form of macula degeneration
  - aka **LUCENTIS**, humanized antibody FAB fragment, affinity maturated (6 aa changed compared to WT protein), used for treatment of macular degeneration

172 *Antwort*

- identified as mitogens for fibroblasts
- most bind to heparin
- regulate proliferation, migration, differentiation and/or survival
- defined through:
  - sequence homology
  - conserved gene structure
  - binding to FGFR1-4 (except FGF11-14)
  - heparan sulfate proteogylcans (except FGF19,21,23 and FGF11-14
  - FGF-BP (FGF binding protein)
  - other transmembrane/extracellular proteins

171 *Antwort*

- ***in vitro* tube formatin assay on Matrigel**: coat wells with Matrigel (mixture of laminin, collagen IV, nidogen and proteogylcans → resembling basement membrane) → seed endothelial cells on surface → add angiogenesis factor/inhibitor → cells form capillary tubes within several hours → count tubes
- **aortic ring assay**: remove thoraci aorta of rats → cut into rings → embed rings in collagen → add culture medium plus test substance → examine by microscopy after 1 week
- ***in vivo* Matrigel plug assay**: mix Matrige and potential angiogenic factor/inhibitor → inject cold liquid mixture subcutaneously into mice → gel solidifies and permits penetration by host cells and formation of new blood vessels → count blood vessels after several days
- **chorioallantoic membrane (CAM) assay**: incubate chicken eggs in humidified chamber → at day 7-9: open window in egg shell *to* place tissue or organ graft or membrane with test substance directly onto CAM → seal window and re-incubate → recover grafts and score for vascularization
- **rabbit/mouse cornea assay**: cornea avascular → make pocket in cornea and introduce test substance in sponge or slow release material → monitor vascularization with microscope

FGFR

Atopic Dermatitis

Epidermal Barrier

Consequences of FGFR1/2 Loss in Keratinocytes

174 *Antwort*

- impaired barrier function
- severe skin dryness
- similar inflammatory infiltrate → very low number of neutrophils
- high serum IgG and IgE levels
- keratinocyte hyperproliferation
- epidermal thickening
- FGFR1/2 KO (in epidermis) mice show progressive hair loss and epidermal thickening → good animal model
- acceleration of symptoms by low humidity

173 *Antwort*

- different types of FGFR result from alternative splicing and polyadenylation
- differential splicing in extracellular domain of FGFR1 generates three receptor variants
- heparan sulfate proteoglycans regulate binding of FGFs to receptors

Knock-Out	Phenotype
FGF5	longer hair
FGF10	no limbs
FGFR1	die during gastrulation
FGFR2	die after implantation
FGFR3	bone deformation and extension of inner ear defect
FGFR4	liver phenotype

176 *Antwort*

- impaired epidermal barrier function through suppression of genes encoding tight junction components
- skin dryness and possibly exposure to foreign antigens cause progressive inflammation → initiation of double paracrine loop: inflammatory/hyerproliferative skin disease resembling Atopic Dermatitis
- high humidity rescues AD-like phenotype

175 *Antwort*

- protects from water loss, invasion of allergens, irritants, and bacteria
- defects in barrier cause inflammatory skin disease
- barrier mainly formed by cornified envelope → cross-linked proteins and lipids in outermost epidermal layer
- other component of barrier: tight junctions
- 20-30% of patients with Atopic Dermatitis have a mutation in a fiaggrin gene (component of cornified envelope) → not affected by loss of FGFR1/2

Chloride Channel Accessory 2
CLCA2

FGFR3 & FGF23

Endocrine FGFs

Role of FGFs in Liver Regeneration

178 *Antwort*

- FGFR 3 negative regulator of bone growth
- **Achondroplasia** = dwarfism, activating mutation in FGFR3 gene
- FGFs and BMPs have antagonistc effects on bone growth: FGF inhibits proliferation of chondrocytes and activates their differentiation, BMP inhibits differentiation, but activates proliferation
  - FGF23 present in circulation (→ endocrine acting growth factor)
  - produced in bone
  - regulates vitamin D metabolism and phosphate homeostasis in kidney
  - binding and receptor activation of FGF23 require transmembrane protein Klotho
  - KO of FGF23 or Klotho leads to premature aging

177 *Antwort*

- regulator of chloride channel activity
- involved in cell-cell adhesion, apoptosis, cell cycle control
- tumor suppressor function in breast cancer
- function in skin and keratinocytes is unknown
- upregulated at low humidity in organotypic cultures and in AD patients
- CLCA2 expression is upregulated by low humidity/hyperosmolarity via JNK /p38/ATF2 signaling pathway
- upregulation of CLCA2 promotes cell-cell adhesion, preserving epidermal integrity under dry/hyperosmotic conditions - like in AD patients

180 *Antwort*

- partial hepatectomy (PH) leads to complete regeneration of liver size
- FGFR4 KO in hepatocytes inhibits liver regeneration
- FGF15-FGFR4-STAT3-FoxM1 axis controls hepatocyte proliferation in injured liver

179 *Antwort*

- FGF19 (FGF15 in mice), FGF21 and FGF23 show reduced heparin binding → act as endocrine hormones
- receptor activation requires co-receptor protein Klotho or Klotho β
- FGF19
  - regulates bile acid synthesis and gall bladder filling
  - produced in intestine and stimulates hepatocytes in liver
  - involved in energy and lipid metabolism
- FGF21
  - produced in liver
  - regulates response to fasting by signaling to adipose tissue and brain
  - involved in energy, lipid and glucose homeostasis

KGF

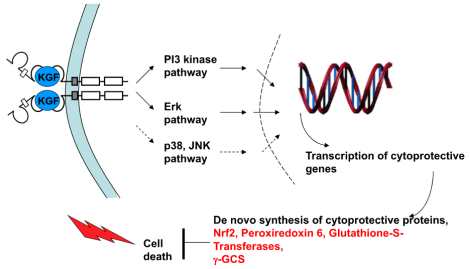
OxyBlot & FGF7 UV-Protection Mechanism

Transforming Growth Factor-β

TGF-β Receptors

182 *Antwort*

- investigation of protein oxidation through detection of carbonyl groups on proteins that result from several types of oxidative damage
- carbonyl groups are chemically converted to dinitrophenylhydrazone (DNP) derivatives
- protein samples are subjected to western blot analysis
  - synthesis of cytoprotective proteins: Nrf2, Peroxiredoxin 6, Glutathione-S-Transferase, γ-GCS



181 *Antwort*

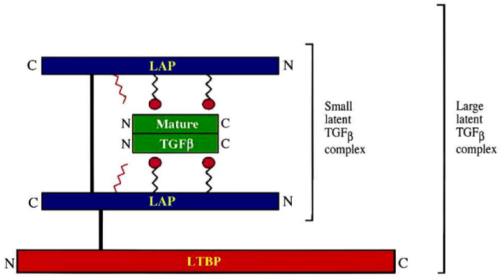
- = FGF7
- paracrine acting growth factor
- affects proliferation, differentiation, migration and has a protective effect:
  - cells of bladder: prevents ulcerative hemorrhagic cystitis after cyclophosphamide injection ($\rightarrow$ chemotherapy)
  - on alveolar cells; prevents lung injury in various model systems (e.g. hyperoxia, acid)
  - on cells of gastrointestinal tract: pretreatment of mice with recombinant FGF7 reduces injury induced by radiation and/or chemotherapy $\rightarrow$ increased mucosal thickness, increased crypt cell survival $\rightarrow$ used in patients for treatment of chemo and radiotherapy-induced mucositis
  - protects keratinocytes *in vitro* and skin *in vivo* from toxicity of UV radiation and treatment with ROS $\rightarrow$ reduced oxidative protein damage $\rightarrow$ reduced cell damage, reduced apoptosis

184 *Antwort*

- Type I and Type II receptors:
  - small, N-glycosylated extracellular domain
  - one transmembrane domain
  - intracellular serin-threonine kinase domain
  - dimeric ligands which combine type I with type II receptors $\rightarrow$ receptor dimerization
- Type III receptor (betaglycan)
  - transmembrane protein with short cytoplasmic part and large extracellular domain with carbohydrate residues
  - no enzymatic activity $\rightarrow$ does not transduce signal, but promotes binding of ligand to signaling receptors

183 *Antwort*

- 3 isoforms in mammals
- biologically active form: homodimer
- 1 cysteine for dimerization, 9 for intramolecular disulfid bridges
- produced as large inactive precursor $\rightarrow$ longer half-life compared to mature form
- LTBP - latent TGF-β binding protein, LAP - latency associated peptide



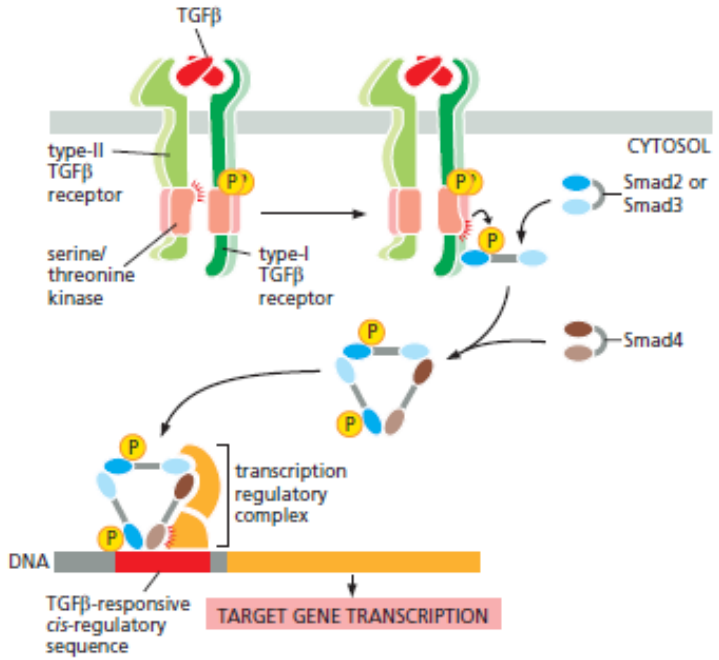
Activation of TGF-β

TGF-β Signaling Pathway

Smad Proteins & TGF-β1

Function of TGF-βs

186 *Antwort*



185 *Antwort*

***in vitro*:**

- low pH, proteinases, mechanical tension

***in vivo*:**

- proteinases
- thrombospondin (induces conformational change)
- locally low pH
- ROS
- binding of LAP to mannose-6-phosphate receptors
- integrin $\alpha v\beta 6$ mechanical tension (induces conformational change)

188 *Antwort*

- inhibit proliferation of most cell types, including epithelial cells
  - mutations in pathway frequently found in epithelial cancers
  - exception: proliferation of fibroblasts stimulated by TGF-β
- induce migration, proliferation of fibroblasts and matrix production by cells
- promote differentiation of fibroblasts into myofibroblasts - particularly in combination with mechanical tension $\rightarrow$ cell aquire contractile properties
- often overexpressed in fibrotic disease where functional epithelial tissue is replaced by non-functional connective tissue
  - e.g. lung fibrosis, liver cirrhosis, hypertrophic scars and keloids etc.
  - inhibition of TGF-β action for treatment of fibrotic disease $\rightarrow$ good results in animal models

187 *Antwort*

- receptor-Smads:
  - Smad2 and Smad3 bind to TGF-β and Activin receptors
  - Smad1, Smad5, and Smad8 bind BMP receptors
- Smad4 binds to receptor Smads
- Smad6 and Smad7 are inhibitory Smads

TGF-β1

- anti-inflammatory properites
- TGF-β1 KO mice suffer from severe inflammation
- possible use of TGF-β1 for treatment of MS via viral delivery of TGF-β producing T-cells

Skin Functions & Epidermis Composition

Cells of the Epidermis & Dermis

Phases of Inflammation (I/II)

Phases of Inflammation (II/II)

190 *Antwort*

- **keratinocytes**: formation of barrier against environmental damage
- **Merkel cells**: receptors connected to somatosensory nerve fibers, neuroendocrine function
- **immune cells**: e.g. Langerhans cells, T-cells
- **melanocytes**: produce melanin → protection from UV damage

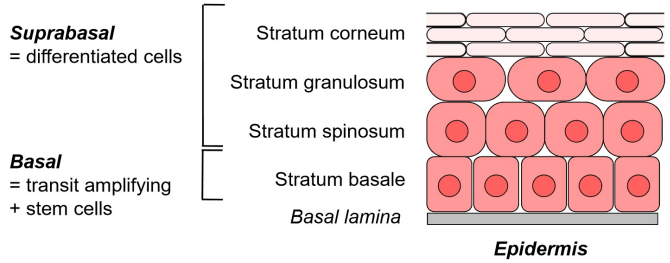
Dermis

- **fibroblasts**: synthesize ECM and collagen
- **immune cells**: e.g. T-cells, macrophages, neutrophils
- **endothelial cells**: form endothelium (interor surface of blood and lymphatic vessels
- **cutaneous nerves**: (also in epidermis) sensory innervation
- **smooth muscle cells**: form erector pili muscle and blood/lymphatic vessels

189 *Antwort*

Skin Functions

- protection form UV, toxins, chemicals, irradiation, pathogen, physical insults, etc.
- regulation of body temperature
- barrier against water loss
- sensory organ



192 *Antwort*

Invasion of Immune Cells

- neutrophils and monocytes migrate concurrently into wound
- neutrophils arrive first in larger number due to their higher abundance in circulation

191 *Antwort*

Blood Clot Formation

- damage of blood vessels
  - platelets bind to interstitial connective tissue
- aggregation of platelets
  - degranulation
  - release of growth factors and chemotactic factor for neutrophils and macrophages as well as for resident cells
- blood coagulation
  - formation of fibrin clot
  - plugs wound
  - serves as provisional matrix

Function of Blood Clot

Neutrophil Functions

Macrophage Functions

Stages of Proliferation
(I/III)

194 *Antwort*

- destroy bacteria
  - via phagocytosis
  - via enzymatic pathways and release of ROS
- secrete pro-inflammatory cytokines
  - IL1α, IL1β, TNFα
  - activation of macrophages, fibroblasts, keratinocytes
- neutrophil infiltration ceases within a few days
  - entrapped in blood clot, extruded with eschar (Schorf)
  - become senescent, phagocytosed by macrophages

193 *Antwort*

- protection of wound tissue → water loss, microorganisms
- matrix for migrating cells
- reservoir of cytokines and growth factors
  - recruit inflammatory cells
  - initiate reepithelialization and formation of granulation tissue
  - stimulate connective tissue contractions

196 *Antwort*

Re-Epithelialization

- keratinocyte migration and proliferationg
- stimulus: "free edge" effect → absence of neighbouring cells
- stimulus: high concentration of growth factors - EGF family, HGF, KGF
- fibronectin, tenascin, vitronectin & collagen 1 attach to keratinocyte → decompositon of desmosomes to neighbouring cells → accumulation of actin → expression and secretion of gelatinaseA, collagenase1 and 3, stromelysin1, plasminogen activator

195 *Antwort*

- antimicrobial function
  - phagocytosis, oxygen radicals, nitric oxide
- matrix synthesis
  - growth factors - TGFβ, EGF, PDGF - & cytokines - TNFα, IL1, IFN-γ
  - enzymes - collagenase, elastase
- wound debridement
- angiogenesis
  - growth factors - bFGF, VEGF - & cytokines - TNFα
- cell recruitment and activation
  - growth factors - PDGF, TGFβ. EGF, IGF - & cytokines - TNFα, IL1, IL6

Stages of Proliferation
(II/III)

Stages of Proliferation
(III/III)

Fetal Wound Healing

Diabetic Foot Ulcer - Disease Progression and
Histopathological Features

198 *Antwort*

Remodeling

- transition from granulation tissue to mature scar
- apoptosis of fibroblasts/myofibroblasts
- regression of capillaries
- collagen remodeling - formation of lager collagen bundles, alterations of intermolecular cross-links
- outcome:
  - healed skin neither aesthetically nor functionally perfect
  - loss of hair appendages (hair follicles, sweat and sebaceous glands
  - reduced tensile strength - mature scar: max. 70% of tensile strength of uninjured skin

197 *Antwort*

Granulation Tissue Formation

- fibroblast migration and proliferation:
  - PDGF, TGF-β $\rightarrow$ activation of fibroblasts $\rightarrow$ proliferation and migration $\rightarrow$ production of ECM (fibronectin, laminins, tenascin-C, onset of collagen production)
  - fibroblasts produce large amounts of collagen $\rightarrow$ gradual replacement of provisional matrix by collagenous matirx $\rightarrow$ matrix remodeling begins
- wound contraction
  - proto-myofibroblast $\rightarrow$ myofibroplast: synthesis of SM actin $\rightarrow$ increased contractile force
- neovascularization
  - bFGF, VEGF $\rightarrow$ proliferation and migration of endothelial cells (tube formation)
  - deposition of own ECM & formation of new basement membrane around vessels

200 *Antwort*

- trauma: peripheral neuropathy (nerve damage $\rightarrow$ reduced pain perception), ischemia (restricted blood supply $\rightarrow$ reduced nutrition and oxygenation), and impaired immune response $\rightarrow$ ulcer formation $\rightarrow$ infection & gangrene (=necrosis) $\rightarrow$ foot or leg amputation
- reduced migration and proliferation of keratinocytes and fibroblasts
- delay in mature granulation tissue formation
- reduced angiogenesis
- disturbed extracellular matrix formation and remodeling by MMPs
- reduced epidermal nerve formation

199 *Antwort*

- fast reepithelialization
- fetal fibroblasts
- no myofibroplasts but actin cable
- low inflammation
- nerve regeneration
- low levels of TGFβ1
- high levels of hyaluronic acid
- high levels of MMPs

$\rightarrow$ no scarring (until third trimester)

Diabetic Foot Ulcer - Molecular Changes

Diabetic Foot Ulcer - Standard Therapy & Clinical Trials

Parallels between Wound Healing and Cancer (I/II)

Parallels between Wound Healing and Cancer (II/II)

202 *Antwort*

1. removal of excessive cells and fluid: wound debridement (surgical removal of tissue), negative pressure
2. treatment of infection
3. correction of perfusion/oxygenation: hyperbaric oxygen therapy, negative pressure
4. enhancement of wound closure → wound dressing

- growth factors - PDGF-BB
- ECM proteins (collagen, hyaluronic acid)
- bioengineered skin
- cultured autologous bone-marrow cells

201 *Antwort*

- fibroblasts: senescent and reduced response to growth factors
- macrophages: reduced secretion of cytokines (IL-1β, VEGF)
- growth factors: trapped and reduced expression
- MMPs: excessive activation → impaired cell migration, degradation of ECM proteins and growth factors
- nitric oxidase: reduced levels → reduced fibroblast proliferation, collagen production and angiogenesis

204 *Antwort*

	Wound	**Cancer**
Epithelial-mesenchymal transition (EMT)	partial → remaining intercellular junctions + keratin expression	complete (metastasis) → loss of cell-cell contacts → fibroblast like morphology → expression of mesenchymal markers
	stimulation: HGF, TGFβ, TNFα, MMPs, only tumor: Ras	
Fibrous tissue	granulation tissue → fibrous tissue	persistent stroma formation microenvironment → tumor progression → cancer cell invasion
	fibroblast activation: PDGF, TGFβ and others	
Angiogenesis	transient	persistent + imperfect → essential for tumor growth
	stim:: VEGF-A, PLGF, FGF2 and others, inhib: TSP1, IP10	

203 *Antwort*

	Wound	**Cancer**
Fibrin matrix	blood clot formation (damaged blood vessels)	chronic fibrin deposition (hyperpermeability of vessels)
Inflammation	transient	persistent → protumerigenic → stimulates angiogenesis + ECM breakdown → enhances cancer cell motility + invasion → promotes malignancy (ROS, NOS)
Epithelial Proliferation + Migration	transient	persistent

Regulation of Hypoxia-Inducible Factor-α Subunits

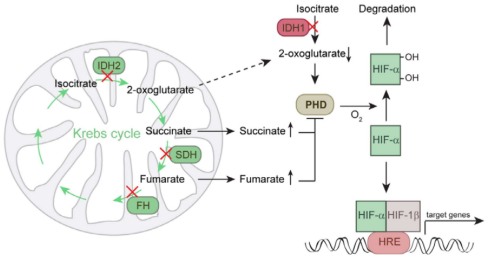
Pseudohypoxia

Role of Pyruvate Kinase M in Metabolism & Glutaminolysis

Cholesterol

206 *Antwort*

- stabilization of HIF-α subunits under non-hypoxic conditions
- e.g. mutations in IDH1/2 lead to depeltion of 2-oxoglutarate and instead accumulation of 2-hydroxyglutarate → oncogenic metabolite in leukemia and brain cancer that dysregulates epigenetics and cell differentiation
- 2-hydroxyglutarate inhibits JHDM (demethylates histones) and TET1/2 (oxidates 5-mehtylcytosine (5-mC) to hydroxymethylcytosine (5-hmC)
- inhibitors specific to mutated forms of IDH1/2 are under clinical investigation



205 *Antwort*

- under normoxic condtions: HIF-α gets hydroxylated on proline by PHD - prolyl-hydroxylase - using O_2 and 2-oxogluterate
- leads to recogniton by VHL (von Hippel-Lindau) tumor suppressor protein, which is part of a E3 ubiquitin ligase complex
- under hypoxic conditions: HIF-α forms a heterodimer with HIF-β that acts as a promoter
- activated genes include:
  - EPO → erythropoiesis
  - VEGF → angiogenesis
  - glycolytic enzymes → metabolism, energy
  - BNIP3/BNIP3é → cell death, mitophagy
- regulates mitochondrial metabolism, biogenesis, and selective autophagy (mitophagy

208 *Antwort*

- isolated from gallstones in 1789
- its biosynthesis amongst most intensely regulated processes in biology
- various pathways for uptakle of cholesterol form low-density lipoproteins (LDL) and export to high-density lipoproteins (HDL)
- intracellular cholesterol transport: vesicular and non-vesicular mechanisms
- provides membranes with special physical properties
- metabolites of Chol- steroids, oxysterols and bile acids - have significant roles as signal transducers and solubilizers of other lipids
- critical for embryonic development
- aberrant cholesterol homeostasis involved in pathogenesis of cardiac and brain vascular diseases, dementias, diabetes and cancer
- disease caused by defect in cholesterol trafficking (e.g. Niemann-Pick type C, Tangier)

207 *Antwort*

- PKM2 tetramers: PEP → pyruvate → respiratory chain
- PKM2 dimers: PEP → pyruvate → lactate (via LDH)
- independent from oxygen supply → allows survival under hypoxic conditions
- facilitates macromolecular synthesis - nucleotides and amino acids

1. leads to generation of NADPH
2. deamidation of glutamine to glutamate
3. transamidation of glutamine to glutamate through enzyme of nucleotide biosynthesis
4. transamination of glutamate to α-ketoglutarate via transaminases (alanine aminotransferase)
5. mitochondrial metabolism of α-ketoglutarate to malate and oxidation of malate to pyruvate via malic enzyme (ME)

Chloesterol - Structure

Dyslipidemia

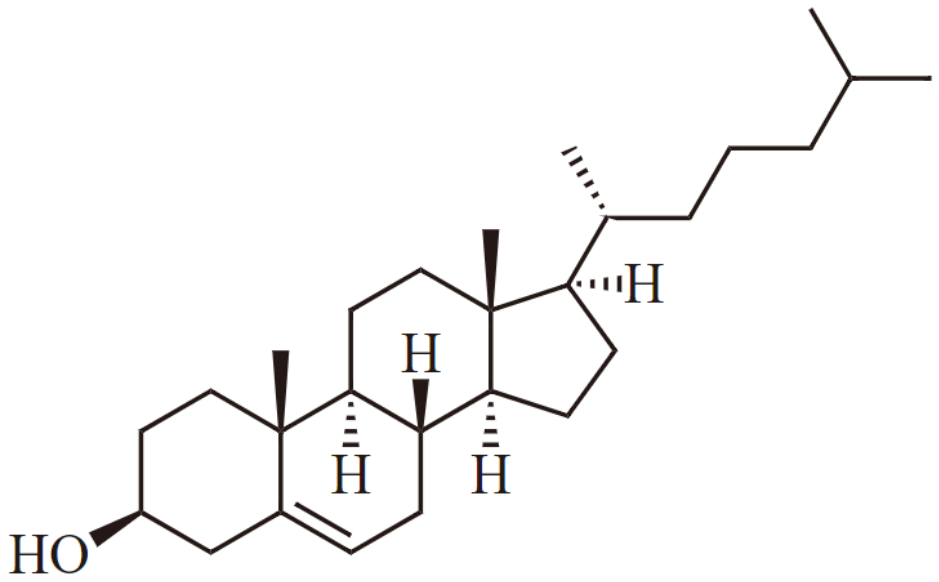
Transport of Lipoproteins into Cell

Reverse Cholesterol Transport (RCT)

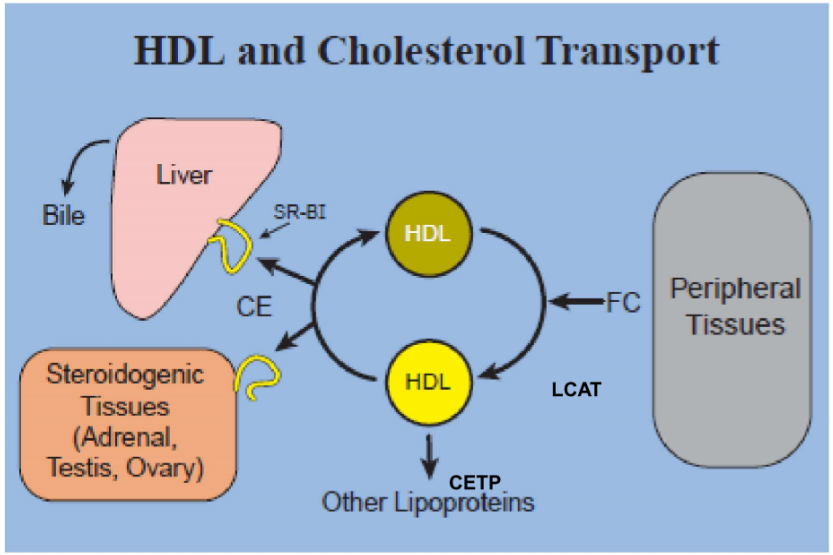
210 *Antwort*

- abnormal amounts of lipids in blood
- major risk factor for atherosclerosis
- packing of cholestrol, cholesteryl esters, and other lipids into lipoproteins for transport in blood
- types of lipoproteins:
  - LDL: low-density lipoprotein
  - HDL: high-density lipoprotein
  - VLDL: very low-density lipoprotein → triglyceride carrier from liver
  - chylomicrons: dietary lipid carrier synthesized in intestines

209 *Antwort*



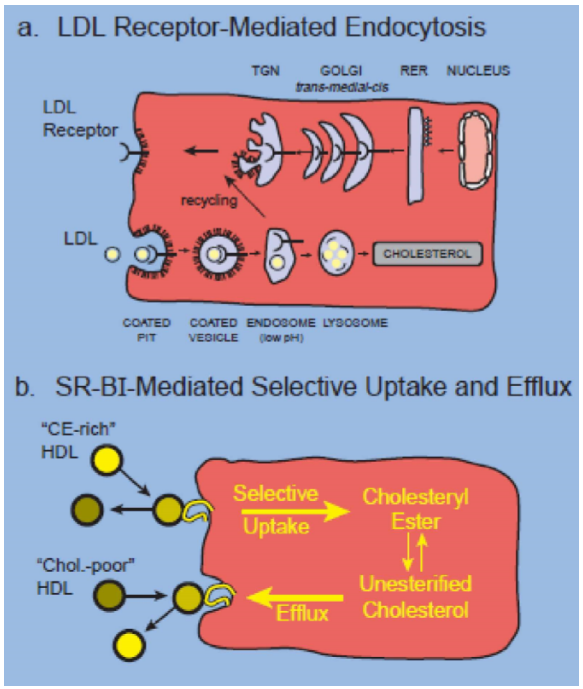
212 *Antwort*



SR-BI: scavenger receptor class B type I
LCAT: lecithin:cholesterol acyltransferase
CETP: cholesteryl ester transfer protein
CE: cholesteryl ester

Krieger M, Annu. Rev. Biochem. (1999)

211 *Antwort*



PCSK9

SREBPs

SREBP Activation through Insig

Regulated Degradation of HMG-CoA Reductase

214 *Antwort*

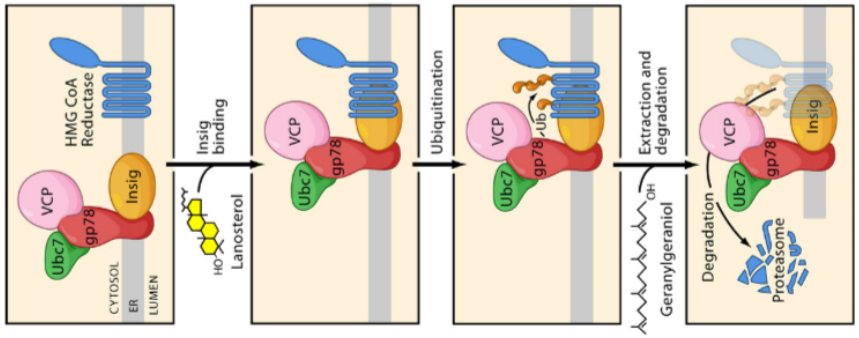
- lipid homeostasis in vertebrate cells is regulated by family of membrane-bound transcription factors designated sterol regulatory element-binding proteins (SREBPs)
- directly activate expression of genes dedicated to synthesis and uptake of cholesterol, fatty acids, TAGs, phospholipids, and NADPH cofactor required for synthesize of former molecules
- mammalian genome encodes three SREBP isoforms → SREBP-1a, SREBP-1c, SREBP-2
- SREBP-1a und -1c derived from same gene through use of alternative transcription site
- at normal levels, SREBP-1c favors FA biosynthetic pathways and SREBP-2 favors cholesterologenesis item SREBP-2 activated by low sterol → activates LDL receptor, Insig-1, cholesterol biosynthetic genes transcription
- SREBP-1c activated by insulin and oxysterols → activates genes for fatty acid, phospholipid and TAG synthesis
- **statins** inhibit of HMG-CoA reductase and induce expression of LDL receptors → increase in LDL uptake

213 *Antwort*

- destroyer of LDL receptors
- gain-of-function mutations lead to hypercholesterolemia
- loss-of-function: no apparent health problems, very low plasma levels of LDL-C
- clinical trails for antibodies against PCSK9, gene silencing, or inhibiton of autocatalytic site

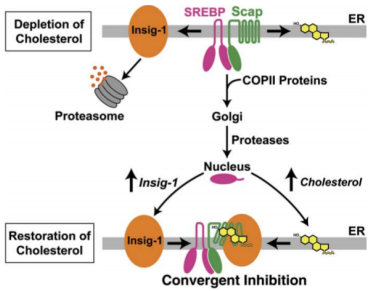
216 *Antwort*

- lanosterol activates Insig binding to HMG-CoA reductase
- HMG-CoA gets ubiquitinated
- geranylgeraniol leads to extraction and degradation of HMG-CoA reductase via proteasome



215 *Antwort*

- SREBP is synthesized as ER membrane protein → remains anchored by interaction with SCAP (SREBP cleavage activation protein) if there is sufficient cholesterol and Insig-1 in membrane → SCRAP binds cholesterol and Insig-1
- low cholesterol: cholesterol binding site in SCAP is empty and Insig-1 gets degraded by proteasome → SCAP changes conformation and is packaged together with SREBP into transport vesicles to Golgi
- Golgi proteases cleave SREBP → release of cytosolic domain → moves to nucleus and binds promoters of genes involved in cholesterol biosynthesis
- tipping scale at 5% cholesterol in ER membrane



Role of miRNAs in Control of Cholesterol Metabolism & Niemann-Pick Disease Type C

CNS Disorders - The Cholesterol Connection

Cholesterol in CNS

Role of (Maternal) Cholesterol in Embryogenesis

218 *Antwort*

- increased cholesterol turnover in neurogenerative disorders e.g. AD and NPC
- patients with elevated plasma cholesterol levels have increased susceptibility to AD
- hypercholesterolemia is associated with increased brain amyloid β (Aβ) immunoreactivity
- Apolipoprotein E (ApoE) is the major cholesterol transporter in brain → increased expression of ApoE4 allele is associated with increased risk of AD development at a younger age
- determinants of cardiovascular health - especially midlife dyslipidemia - are associated with an increased risk of dementia
- statins might have beneficial effects in several neurological disease (e.g. MS, AD, ischemic stroke)

217 *Antwort*

- miRNAs are regulators of mRNA stability and translation; reduce translation and/or lead to degradation by binding to partially complementary sites in 3' untranslated region (3'UTR) of mRNA transcript
- intronic miRNA typically coordinately expressed and processed with precursor mRNA
- SREBP intronic miRNAs: 33a/b → coexpressed with SREBP
- inhibition of miR-33a leads to elevated HDL levels
  - neurovisceral disorder → lysosomal storage disease
  - children with NPC show delays in normal developmental milestones before developing cognitive decline → decline, ataxia (unsteady walking), epilepsy
  - cholesterol sequestration/accumulation in late endosome and lysosome of NPC1-deficient cells

220 *Antwort*

- membrane formation and maintenance of integrity → structure and function of membrane-bound proteins
- part of lipid rafts and caveolae → critical for directing location and activity of proteins in lipid-rich or -poor membrane microdomains → many signaling pathways start in lipid microdomains (i.e. insulin signaling)
- Hedgehog processing
- precursor for bile acid, steroid hormones and oxysterols
- maternal transfer of cholesterol does occur but significance still in debate
- early development depends on maternal cholesterol, later on endogenous cholesterol synthesis
- women with lower plasma cholesterol concentration have newborns with lower birth weights, correlation of low plasma cholesterol and microcephaly

219 *Antwort*

- plasma lipoproteins cannot cross blood-brain barrier → brain depends on intracerebral de novo synthesis of cholesterol
- brain most cholesterol-rich organ in body (25% of unesterified cholesterol in whole body) → cholesterol concentration in brain is 15-20mg/g tissue (compared to 2mg/g in average tissue)
- 2 major cholesterol pools in CNS: myelin sheaths (70% lipids and 30% proteins) and plasma membranes of astrocytes and neurons
- cholesterol predominantly found in white matter
- cholesterol is an essential signal for synaptogenesis and formation, function and stability of synapses are sensitive to disturbances in cholesterol metabolism
- half-life of 4-6 months in rat brain

Smith-Lemli-Opitz Syndrome
SLOS

Perturbation of Hedgehog Signaling

Hedgehog Signaling

Sterol Intermediates - Toxicity or Gain of Function?

222 *Antwort*

- Hedgehog family is comprised of three different proteins; Sonic Hedgehog (SHH), Indian Hedgehog (IHH) and Desert Hedgehog (DHH)
- cholesterol is a covalent ligand of hedgehog family of developmental patterning proteins → essential for proper maturation of hedgehog proteins
- Shh-deficient mice: cyclopic (one eye), defects in ventral neural tube, somite, and foregut patterning, later defects: distal limb malformation, absence of vertebrae and ribs, failure of lung branching → all consequences of dysfunction in patterning during early embryogenesis
- hypothesis that cholesterol biosynthesis phenotypes are phenocopies of hedgehog defects

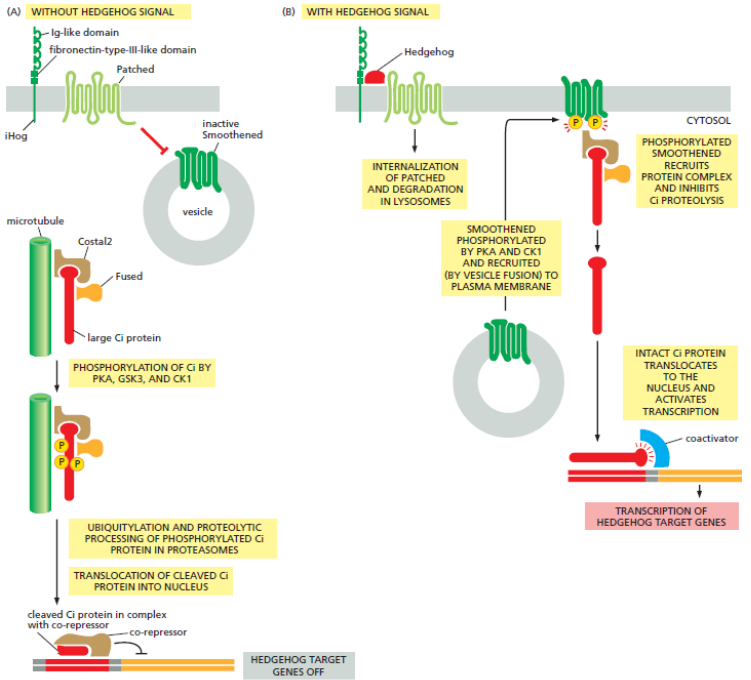
221 *Antwort*

- mutation in Δ^{7-} reductase: reduces 7-dehydrocholesterol to cholesterol
- microcephaly
- ptosis (drooping or falling of upper or lower eyelid)
- small upturned nose
- micrognathia (undersized jaw)
- hand malformations (polydactyly, short thumb)
- retinal degeneration

224 *Antwort*

- inhibition of enzymes early in sterol biosynthetic pathway leads to early embryonic lethality
- most fetuses with defects in sterol biosynthesis are viable until late in gestation or until birth
- early inhibition of cholesterol synthesis pathways results in additional lack of isoprenoids
- late inhibition leads to buildup of intermediates
- lack of isoprenoids occurs in reactions prior to farnesol synthesis → isoprenoids - including geranylgeraniol and farnesol - essential for basic cellular processes (e.g. cell proliferation) → modified proteins include ras, rab, rho families, GTP-binding proteins, G proteins

223 *Antwort*



Functions of Bile Acids

Fat Distrution & Lipid and Glucose Homeostasis during Satieity

Lipid and Glucose Homeostasis during Starvation

Selective Insulin Resistance

226 *Antwort*

- fat distribution influences risks associated with obesity
  - pear shaped obesity (increased subcutaneous fat): low risk of DM and metabolic syndrome
  - apple-shaped obesity (increased visceral fat): high risk of DM and metabolic syndrome

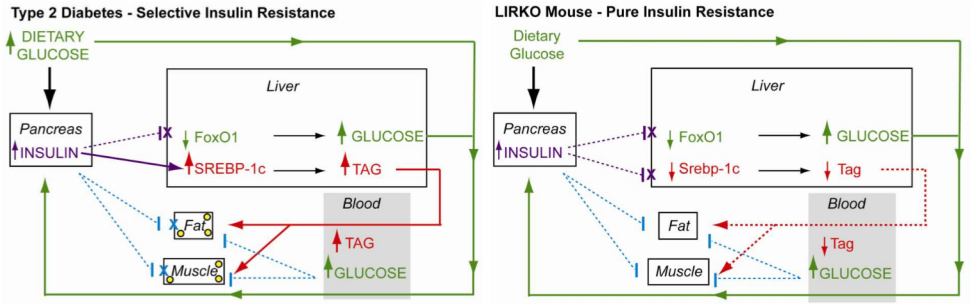
Satiety (→ Insulin)	**heart**	**skeletal muscle**	**adipose tissue**	**liver**
Glycolysis	low	high	high	high
Gluconeogenesis	-	-	-	low
Glycogen Synthesis	-	high	-	high
Glycogenolysis	-	low	-	low
Lipolysis	-	-	low	-
TAG Synthesis	-	-	high	high
FFA Synthesis	high	high	high	high
FFA β-oxidation	high	high	-	low
Keton Body Formation	-	-	-	low

225 *Antwort*

- dietary lipid absorption
- cholesterol homeostasis
- inherited mutations that impair bile acid synthesis cause spectrum of human disease - from liver failure in early childhood to progressive neuropathy in adults
- role as signaling molecules → activate MAPK pathways, ligands for GPCR TGR5, activate nuclear hormone receptors such as FXR

228 *Antwort*

- selective insulin resistance more severe metabolic defect than total resistance
- SREBP-1c still active → synthesis of TAGs in liver → accumulation of lipid droplets in muscle and fat



227 *Antwort*

- starvation/exercise/ fight or flight
- glucagon and/or adrenaline

Starvation (Glucagon/Adrenaline)	**heart**	**skeletal muscle**	**adipose tissue**	**liver**
Glycolysis	low	high	low	low
Gluconeogenesis	-	-	-	high
Glycogen Synthesis	-	low	-	low
Glycogenolysis	-	high	-	high
Lipolysis	-	-	high	-
TAG Synthesis	-	-	low	low
FFA Synthesis	low	low	low	low
FFA β-oxidation	high	high	-	high
Keton Body Formation	-	-	-	high

Model of SREBP and miR-33 Circuit

Nutrient/Horomone-Mediated Changes in Protein Phosphorylation Determine ChREBP Activity

Metabolism in Cancer

Role of Adipocytes in Pancreatic Cancer

230 *Antwort*

fating/starvation

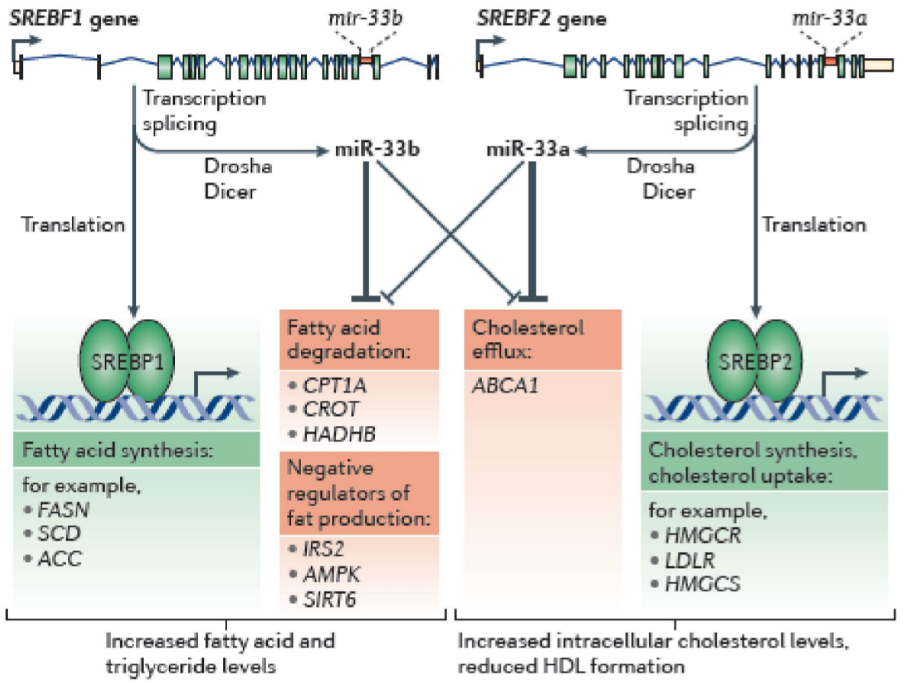
- low glucose
- glucagon, epinephrine → PKA
- ATP deletion, FFA oxidation → AMPL
- lead to phosphorylation → ChREBP inactivation

fed

- glucose
- glucose → HMP shunt (= pentose phosphate pathway) → xylulose-5-phosphate → PP2Aδ
- leads to dephosphorylation → ChREBP activation

ChREBP KO in liver leads to hyperlipidemia and hyperglycemia

229 *Antwort*



232 *Antwort*

- adipocytes remodel microenviroment around pancreatic tumoers
- adipocytes, immune cells and pancreatic stellate cells signal through IL-β and AT1 angiotensin receptor to drive migration of neutrophils to tumor microenvironment
- increases inflammatory and fibrotic response in tumor microenviroment
- denser cellular microenvironment puts extra mechanical tension on tissue and may restrict bloodvessel perfusion
- associated with poor response to chemotherapy and poor prognosis
- depletion of neutrophils or blocking activity of IL-β reduces cancer progression

231 *Antwort*

- Warburg effect: aerobic glycolysis - cancer cells consume high amounts of glucose and produce lactic acid, provides cancer cells growth advantage in tumor microenvironment
- increased glutamine metabolism, glutamine-derived α-ketoglutarat contibutes to production of citrate
- high rate of energy-consuming processes driving increased protein synthesis (e.g. mTOR) and more active DNA synthesis
- increased *de novo* fatty acid synthesis, which is functionally related to glycolytic pathway (glycolysis provides energy and precursors for FA synthesis)

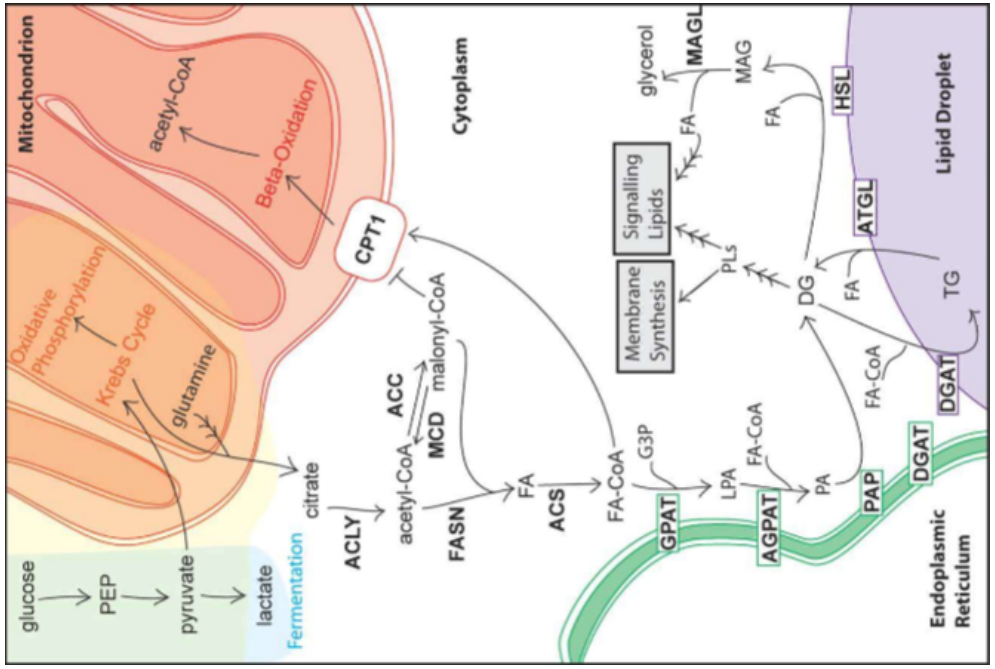
Role of Fatty Acids in Cancer Development

Cellular Fatty Acid Metabolism
(I/II)

Cellular Fatty Acid Metabolism
(II/II)

de novo Fatty Acid Synthesis in Health

234 *Antwort*



233 *Antwort*

- membrane synthesis → cell growth and proliferation
- membrane saturation → oxidative stress resistance
- lipid droplet formation → survival under energy stress
- NADPH oxidation → redox balance
- cholesterol lipid hormones → proliferation and invasion

236 *Antwort*

- two sources: exogenously-derived (dietary) and endogenously-synthesized FAs
- biosynthesis catalyzed by homodimeric fatty acid synthase (FASN)
- FASN activated by PI3K and MEK/ERK signaling
- perdominant product of FASN is palmitate (C16:0)
- in well-nourished individuals the role of FASN is of minor importance owing to sufficient levels of dietary fat
- most normal cells and tissues, even those with high cellular turnover, seem to preferentially use circulating lipids for synthesis of new structural lipids
- normally: FASN: excess carbohydrates into FAs → esterified to storage TAGs
- *de novo* FA synthesis is very active during embryogenesis and in fetal lungs (production of lung surfactant)

235 *Antwort*

FA, fatty acid
LPA, lysophosphatidic acid
PA, phosphatidic acid
MAG, monoacylglycerol
DG, diacylglycerol
TG, triacylglycerol

ACLY: ATP citrate lyase
ACC: acetyl-CoA carboxylase
FASN: fatty acid synthase
ACS: fatty acid-CoA ligase
MCD: malonyl-CoA decarboxylase
CIC, citrate carrier

CPT1: carnitine palmitoyl transferase

GPAT: glycerol-3-phosphate acyltransferase
AGPAT: acylglycerolphosphate acyltransferase
PAP: phosphatidic acid phosphohydrolase
DGAT: diacylglycerol acyltransferase

ATGL: adipose triglyceride lipase
HSL: hormone sensitive lipase
MAGL: monoacylglycerol lipase

de novo Fatty Acid Synthesis in Cancer

Link of Cancer and Lipid Metabolism

Link of Cholesterol/Isoprenoid Biosynthesis and Cancer

Role of p53 in Cancer

238 *Antwort*

- transcriptional profiling of two isogenic models of transformation identified a gene signature linking cancer with inflammatory and metabolic disease
- many drugs used for treatment of diabetes and CVD inhibit transformation and tumor growth
- lipid metabolsim genes are important for transformation and are upregulated in cancer tissue
- as in atherosclerosis, oxidized LDL and its receptor OLR1 activate inflammatory pathway through NF-κB, leading to transformation
- OLR1 is important for maintaining transformed state in diverse cancer cell lines and for tumor growth, suggesting a molecular connection between cancer and atherosclerosis

237 *Antwort*

- increased *de novo* biosynthesis of FAs in a wide variety of tumors and their precursor lesions irrespective of levels of circulating lipids
- neoplastic lipogenesis is reflected by significantly increased activity and coordinate expression of several lipogenic enzymes in tumor cells (e.g. FASN, ATP citrate lyase (ACLY), acetyl-CoA carboxylase (ACACA))
- upregulation of FASN represents a nearly-universal phenotypic alteration in most human malignancies
- FAs synthesized in cancer cells are esterified predominantly to phospholipids and incorporated into membrane lipids by proliferating cells
- many of the genes that encode enzymes of FA biosynthetic pathway, including ACYL, ACACA, FASN, reside on human chormosome 17q $\rightarrow$ common site for gene rearrangement and location of many oncogene amplifications
- increased FA synthesis in tumor cells seems to involve modulation of multiple lipogenic enzymes at various levels (e.g. increased transcription, enhanced protein stabilization)

240 *Antwort*

- p53 can be activated by a number of cellular stressors, including DNA damage, radiation, chemical agents, hypoxia, and oncogene deregulation
- in stress response, p53 functions as transcription factor to suppress cell cycle progression, promote senescence, or to induce apoptosis
- majority of cancer-associated mutations in *TP53* are missense mutations that result in translation of different full-length protein
- mutant p53 can override protective effect of remaining wild-type allele
- mutations of *TP53* not equivalent to simply losing wild-type p53 function, which normally prevents tumorigenesis, instead certain p53 mutants may acquire a tumor-promoting function
- mutant p53 can transcriptionally enhance expression of many mevalonate pathway genes

239 *Antwort*

- cancer cells have a deficient feedback control of HMGCR or increased HMGC expression $\rightarrow$ dysregulation of mevalonate pathway might drive malignant transformation
- statins might exert anticarcinogenic activity, but mechanisms do not necessarily involve cholesterol lowering
- mevalonate is a precursor of several products regulating cell cycle, including dolichol, geranylgeranyldiphosphate (GGPP) and farnesyldiphosphate (FFP)
- dolichol has a stimulatory effect on DNA synthesis
- GGPP and FPP cause isoprenylation of intracellular G proteins Ras and Rho $\rightarrow$ regulate signaling transduction of several membrane receptors crucial for transcription of genes involved in cell proliferation, differentiation, and apoptosis

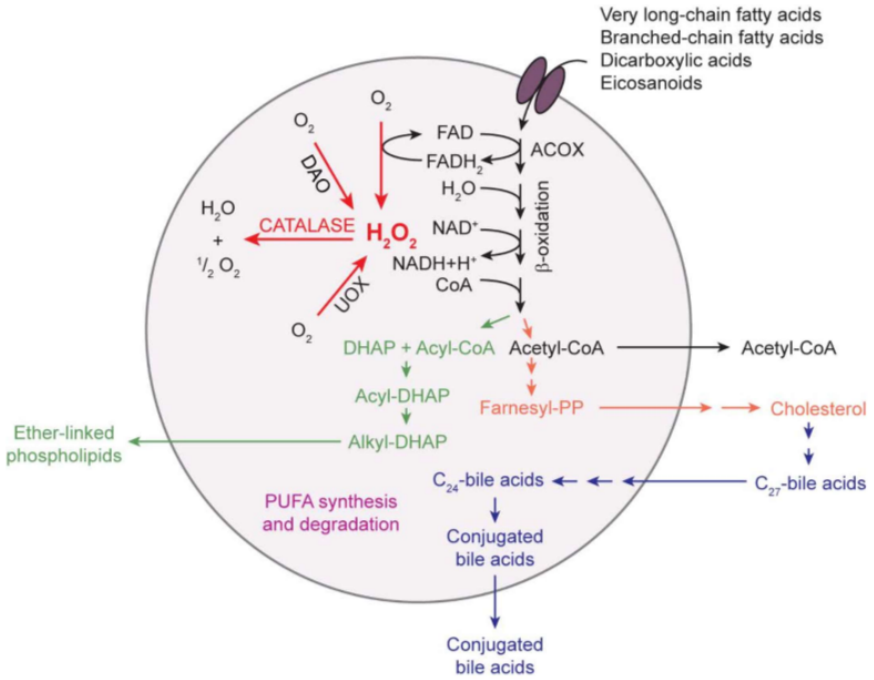
Cholesterol and Cancer

Metabolic Pathways in Mammalian Peroxisomes

Metabolic Functions of Peroxisomes in Mammals

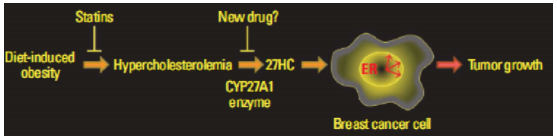
Novel Peroxisomal Functions

242 *Antwort*



241 *Antwort*

- environmental factors play a large role in prostate cancer (PC) risk (e.g. Western diet)
- epidemiological studies suggest that men with hypercholesterolemia are at increased risk for prostate cancer or late stage, aggressive disease
- cholesterol-sensitive mechanisms in PC: cell proliferation, inflammation, steroidogenesis
- hypercholesterolemia and metabolic syndrome are risk factors for breast cancer
- cholesterol metabolite 27-hydroxycholesterol (27-HC) mimics estrogen in certain tissues, estrogen-driven breast cancer may rely on 27-HC to grow wwhen estrogen isn't available
- aggressive breast tumors have higher levels of CYP27A1, which converts cholesterol into 27-HC, breast cancer patients with low tumor levels of CYP7B1 - enzyme that breaks down 27-HC - don't live as long as women with highest levels
- 27-HC may play role in other hormone-driven cancers (e.g. endometrial cancer)



244 *Antwort*

- viral innate immune defense
- GPI-anchor biosynthesis
- H_2O_2 signaling in hypthalamic neurons
- platform for cytomegalovirus evasion from cellular immune response
- peroxisome inheritance coupled with mitosis to balance growth + different.
- peroxisomal ether lipid synthesis regulates inflammation by sustained neutrophil membrane composition and viability
- peroxisome proliferation contributes to physiological response to sound exposure, impaired in patients with pejvakin mutations (lead to deafness)
- PEX13 required for selective autophagy of Sindbis virus (virophagy) and damaged mitochondria (mitophagy)

243 *Antwort*

- β-oxidation of FAs (e.g. very long-chain, branched-chain & dicarboxylic FAs)
- α-oxidation of FAs (e.g. phytanic acid, 2-hydroxylated FAs)
- ether phospholipid (plasmalogen) syntehsis
- cholesterol and isoprenoid synthesis
- bile acid synthesis (i.e. β-oxidation of cholesterol side chain
- synthesis (e.g. docosahexaenoic acid (DHA)) and degradation of polyunsaturated FAs
- H_2O_2 degradation by catalase
- degradation of eicosanoids, amino acids, polyamine, and purine
- metabolism of ROS
- synthesis of pyrimidines and purines

Difference Mitochondrial/Peroxisomal β-Oxidation & Peroxisomal Steps in Bile Acid Synthesis

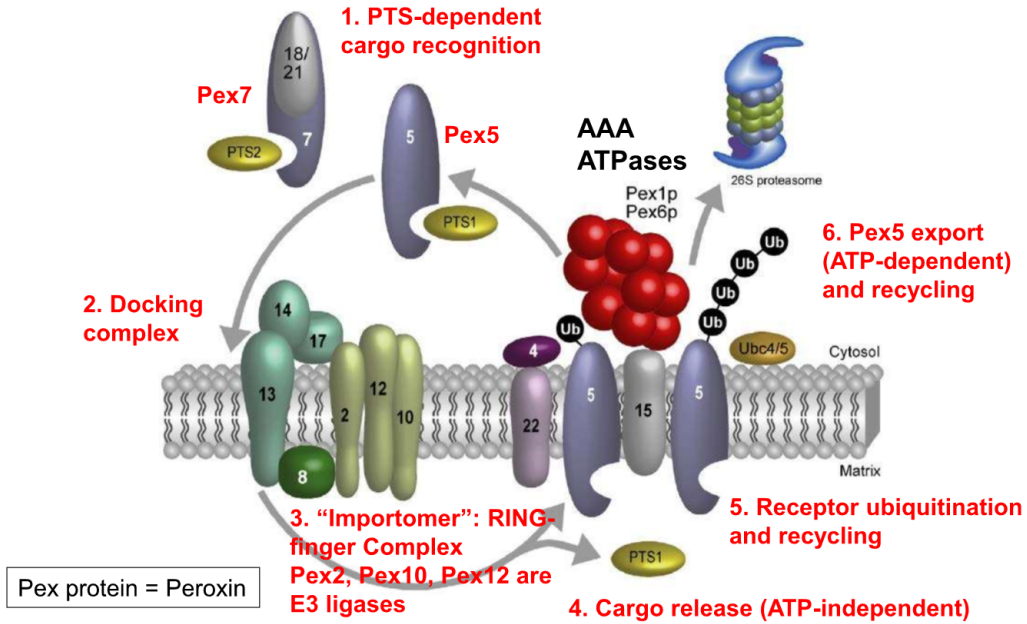
Peroxisomal ROS Homeostasis

Peroxisomal Matrix Protein Import Cascade (I/II)

Peroxisomal Matrix Protein Import Cascade (II/II)

Type of ROS/RNS produced	Generating reaction	Produced in PO by	Scavenged in PO by
Hydrogen peroxide (H_2O_2)	$O_2^{\bullet-} + H^+ \rightarrow HO_2^{\bullet}$, $2HO_2^{\bullet-} \rightarrow H_2O_2 + O_2$	Acyl-CoA oxidase (several types) Urate oxidase Xanthine oxidase D-amino acid oxidase D-aspartate oxidase Pipecolic acid oxidase Sarcosine oxidase L-alpha-hydroxy acid oxidase Polyamine oxidase	Catalase Glutathione Peroxidase Peroxiredoxin I PMP20
Superoxide anion ($O_2^{\bullet-}$)	$O_2 + e^- \rightarrow O_2^{\bullet-}$	Xanthine oxidase	MnSOD CuZnSOD
Nitric oxide ($^{\bullet}NO$)	L-Arg + NADPH + H^+ + O_2 → NOHLA + $NADP^+$ + H_2O, NOHLA + ½ NADPH + ½ H^+ + O_2 → L-citrulline + ½ $NADP^+$ + $^{\bullet}NO$ + H_2O	Nitric oxide synthase	

- difference between mitochondrial and peroxisomal β-oxidation: Acetyl-CoA $\rightarrow$ Enoyl-CoA
- mitochondrial: Acyl-CoA dehydrogenase with reduction of FAD to $FADH_2$
- peroxisomal: Catalase and ACOX 1, 2 & 3
- malfunction of peroxisome leads to decreased levels of mature C_{24}-bile acid and increased levels of C_{27}-bile acid intermediates in blood, urine, and tissue



1. proteins harboring a peroxisomal targeting sequence are recognized and bound by import factros Pex5 and Pex7 in cytosol
2. cargo-loaded receptor is directed to peroxisomal membrane and binds to docking complex (Pex13/Pex14/Pex17)
3. import receptr assembles with Pex14 to form a transient pore and cargo proteins are transported into peroxisomal matrix in an unknown manner, cargo release might involve function of Pex8 or Pex14
4. import receptor is monoubiquitinated at a conserved cysteine by E2-enzyme complex Pex4/Pex22 in tandem with E3-ligases of RING-complex (Pex2/Pex10/Pex12)
5. ubiquitinated receptor is released from peroxisomal membrane in an ATP-dependent manner by AAA-peroxins Pex1 and Pex6, which are anchored to peroxisomal membrane via Pex15, as the last step of the cycle, ubiquitin moiety is removed and receptor enters new round of import

Models for Peroxisome Multiplication & Peroxisomal Disorders

Principles of Selective Autophagy (I/II)

Principles of Selective Autophagy (II/II)

Types of Selective Autophagy in Mammals

250 *Antwort*

- 4 key steps: induction → cargo tagging → sequestration → degradation of cargo and receptor
- central to selectivity is LC3-interacting region (LIR) motif, which ensures targeting of autophagy receptors to LC3 (or other ATG8 family proteins) anchored in phagophore membrane
- every selective autophagy pathway requires a specific cargo receptor
- cargo receptors usually have a tripartite role in cargo binding, interaction with Atg11, and interaction with Atg8 (LC3 in mammals) via an Atg8-interaction motif (LIR motif)

249 *Antwort*

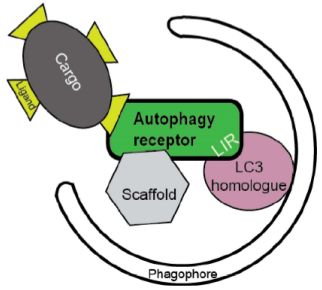
- vesicle fusion model
- growth and division model
- reintroduction of peroxisome - import of matrix proteins and membrane proteins into one vesicle
- shared components of peroxisomal and mitochondrial division machinery in mammals
  - peroxisome biogenesis disorders: Zellweger spectrum disorders → nervous system affected
  - single peroxisomal enzyme deficiencies

252 *Antwort*

- lipophagy → lipid droplets & glycophagy → protein glycosides
- aggrephagy → protein aggregates
- ribophagy → ribosomes
- reticulophagy → parts of ER membranes
- granulophagy → stress granules
- pexophagy → peroxisomes
- zymophagy → zymogen particles
- mitophagy → mitochondria
- midbody degradation → cellular midbody
- xenophagy → microbes and viruses

251 *Antwort*

- autophagy receptors are often synthesized under contitions where cargoes are not degraded → receptor activation relies on protein modifications - e.g. phosphorylation or ubiquitination
- specialized membrane structures - e.g. MIPA (micropexophagy-specific membrane apparatus) - needed for micropexophagy, but not macropexophagy
- receptors are generally degraded in vacuole (lysosome) along with cargo



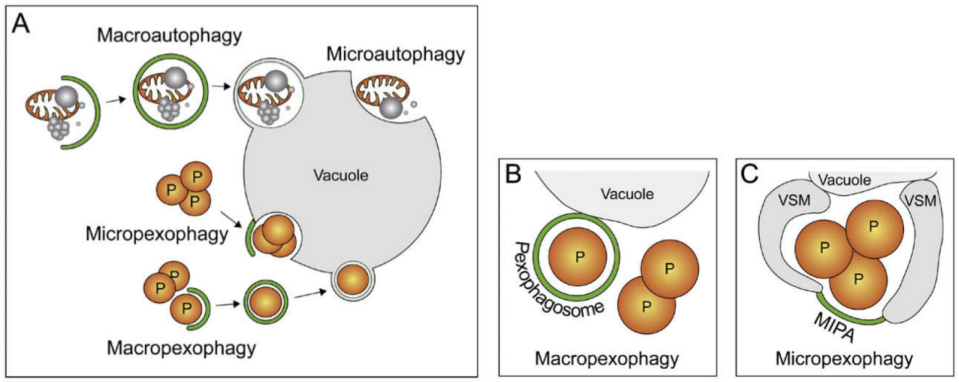
Mechanisms of Organellophagy

Modes of General Autophagy and Pexophagy

Peroxisomes in $Vhl^{-/-}$ Mice

Influence of Hif-2α on Peroxisomes

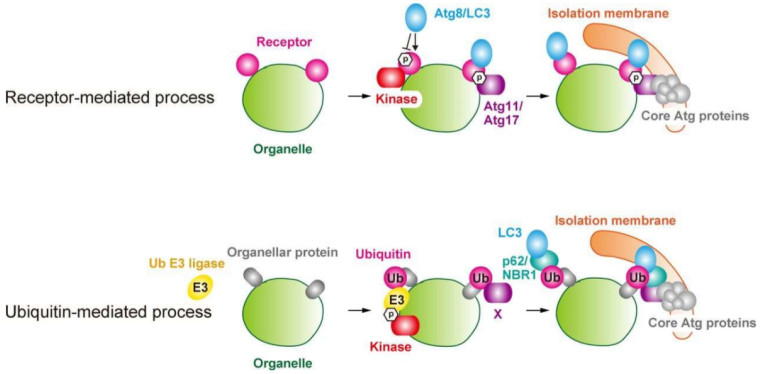
254 *Antwort*



- macro → with own vacuole
- micro → only associated to a membrane and fuse with lysosome

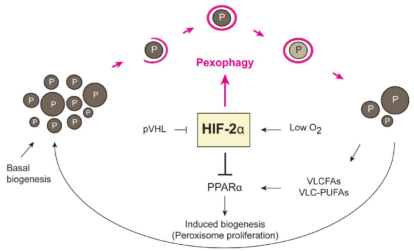
253 *Antwort*

- **receptor-mediated**: Atg8/LC3 inhibits receptor and a kinase phosphorylates it → Atg8/LC3 and Atg11/17 bind phosphorylated receptor → core Atg proteins bind Atg11/17 bound to isolation membrane
- **ubiquitin-mediated**: ubiquitin binds to an organellar protein and recruits Ub E3 ligase which is phosphorylated by a kinase → ligase releases the complex and ubiquitin recruits p62/NBR1 and LC3 to itself → this complex binds to core Atg proteins bound to isolation membrane



256 *Antwort*

- Hif-α is a negative regulator of peroxisome abundance and metabolism
- loss of tumor suppressor *Vhl* in hepatocytes decreases peroxisome number
- Hif-2α promotes mammalian pexophagy
- Hif-2α mediates changes in lipid composition reminiscent of peroxisomal disorders
- human ccRCCs (clear cell renal cell carcinoma) with high Hif-2α levels have decreased peroxisome abundance
- Hif-2α might trigger pexophagy by activating the E3-ligase OR by activating the kinase phosphorylating NBR1/inhibiting phosphatase OR both



255 *Antwort*

- hypothesis: under hypoxic conditions, O_2-dependent peroxisomal metabolic pathways must be inhibited or peroxisome abundance must be decreased by Hif-α signaling to minimize O_2 consumption
- $Vhl^{-/-}$ causes hepatic steatosis
- peroxisomes are reduced in liver, but intact peroxisomes exist
- Hif-2α, not Hif-1α mediates decrease of peroxisome abundance in Vhl KO livers
- C_{27}-bile acid intermediates are increased in plasma of $Vhl^{-/-}$ and $Vhl^{-/-}$/Hif-$1\alpha^{-/-}$ mice
- peroxisome biogenesis machinery functional
- peroxisomes are sequestered in autophagosomes in $Vhl^{-/-}$ livers
- inhibition of autophagy increases peroxisome abundance in $Vhl^{-/-}$ livers
- protein levels of Nbr1 and p62 are decreased in $Vhl^{-/-}$ and $Vhl^{-/-}$/Hif-$1\alpha^{-/-}$ → part of pexophagy pathway

Peroxisome Abundance in Tumors

- Hif-2α stabilization is observed in vast majority of solid tumors and might lead to reduced peroxisome abundance in other cancers
- excessive peroxisome proliferation leads to hepatocellular carcinomas in rodents
- peroxisomal branched-chain fatty acid β-oxidation enzymes are induced in prostate cancers
- peroxisomes are essential for ether lipid synthesis - aggressive cancers have high levels of ether lipids and inhibition of ether lipid synthesis reduces tumor growth
- decrease in peroxisome abundance has been observed in other tumors such as hepatocellular carcinoma, colon carcinoma, breast cancer