ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Summary

Concept Course: Cell Biology

Dozenten:
Ulrich Suter, Ulrich Auf dem Keller, Werner Kovacs, Wilhelm Krek, Jaya Krishnan, Axel Niemann, Matthias Schäfer, Sabine Werner, Andrea Martina Kyburz Kooznetsoff

By Maik Müller
maimuell@student.ethz.ch

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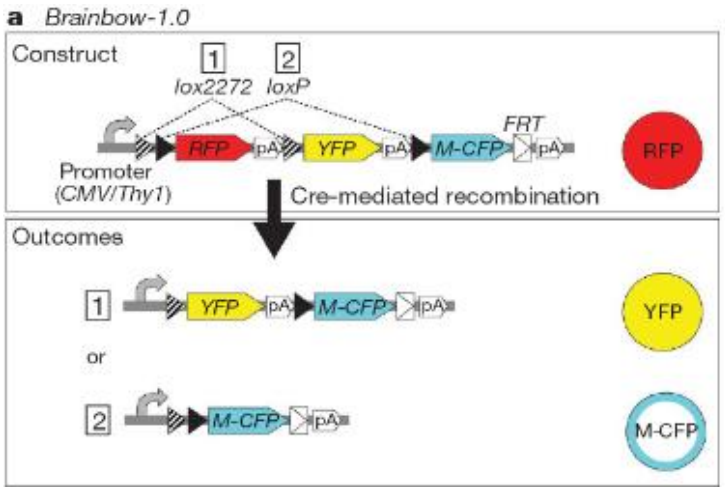
Multiple Sclerosis (US)

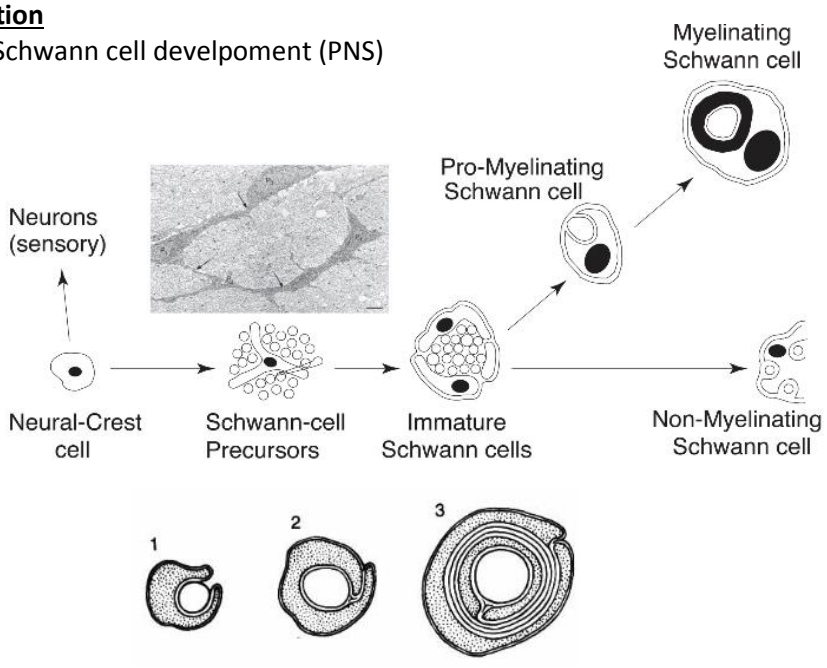
Introduction

- What organism to study myelination?
  Zebrafish: easy to keep, transparent during myelination development (one can follow same organism), BUT very diff immune system than human!
- Microglia in the nerve system:
  - features of macrophages → important in diseases
  - Functional in development and injury
  - Maintain a network of only active neurons! (others get destroyed)
- Brainbow Mice to follow single neurons in the brain of mice
  - Using diff. loxP-sites → no cross recombination possible
  - pA (poly-Adenylation site) after each XFP gene for the Pol II to stop transcription
  - In cortex high cell density → use only low expression of CRE recombinase
  - Need histology to look at cells (LMS) or if immunostaining fails use EMS
  - Can be used for 3D Imaging as well (looks amazing!)



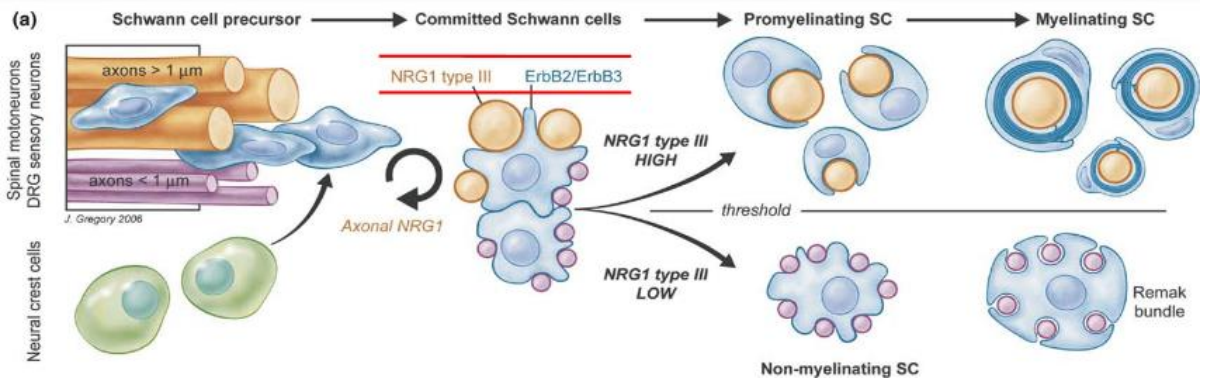
Myelination

- Schwann cell develpoment (PNS)



  - Myelinating and non-myelinating cells → How regulated?
  - Axon separation → How induced ?
  - Myelin thickness with respect to axon diameter →How regulated ?
  - Oligodendrocytes (CNS) myelinate multiple axons

- Myelination Signal
  - ErbB2/ErbB3 are Tyrosine-receptors on Schwann cells
  - NRG1 is ErbB3 Ligand and a survival factor for Schwann cells
  - Myelin thickness depends on NRG1 Type III expression (overexpression leads to degenerative disease ; cannot maintain such thick myelin sheets)
  - NRG1 binds ErbB3 → conf. Change → dimerization with ErbB2 → ErbB2 autophosphorylates itself and ErbB3 → Effector Proteins bind, signal transduction

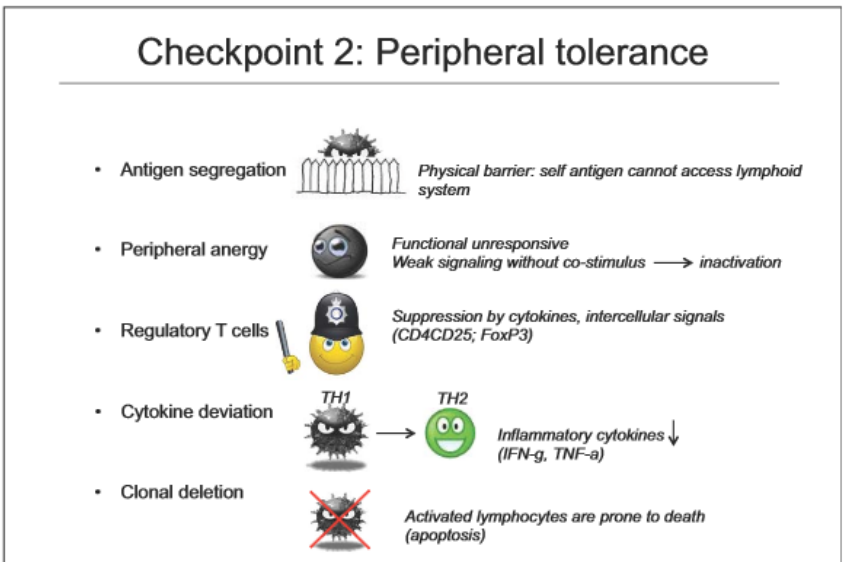


Multiple Sclerosis

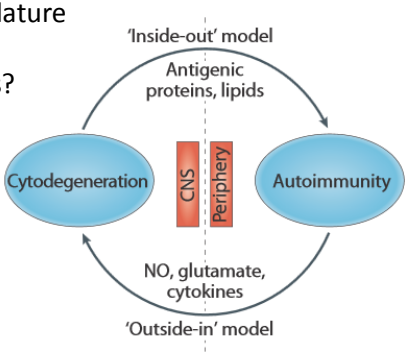
- Some Facts:
  - Most common neurodegenerative disorder among young people/adults
  - **Inflammatory** reaction in the CNS causing demyelination (myelin-auto reactive T cells)
  - **Loss of myelin** causes reduced or blocked nerve conduction resulting in attacks of numbness, loss of vision, weakness, bladder problems and ataxia (tremor)
  - Initially efficient functional recovery in 80% of patients (in 20%, illness is progressive from the onset, with or without preceding inflammatory phase)
  - **Gliosis** (Glia cells which form scars)
  - **Axonal damage/Loss**
  - Phenotype: Focal lesions in white matter (and grey matter), sclerotic plaques (dark points) in brain (can be seen by MRI with enhancing agents, since BBB is broken), PNS not affected
- Primary Cause /Trigger: Unknown
- Effectors of Damage: Inflammatory reaction in the CNS causing destruction of myelin and loss of oligodendrocytes ;
  T cells reactive to myelin protein epitopes, possibly as the result of molecular mimicry (autoimmune disorder ; pathogen mimicking body proteins)
- **Natalizumab (Tysabri)** inhibits trafficking and entering of Leukocytes through BBB:
  - Recombinant, humanized IgG4k monoclonal antibody
  - Binds α4-integrin subunit expressed on the surface of leukocytes, except neutrophils
  - Interaction disrupted with VCAM-1, MadCAM-1 receptors on activated endothelial cells
    ➔ No transmigration!
  - Problem with immunosurpressed patients: reactivation of human polyoma virus JC in CNS → progressive multifocal leukoencephalopathy (PML) → demyelination and oligodendrocyte damage → with Tysabri, no immune cells can invade, protect brain tissue!
  - Genome-wide association studies: variants of MHC gene associated with MS, and other 30-40 factors
- Effector-based MS Model in mice: Experimental Autoimmune Encephalomyelitis (EAE)
  - By active immunization (inject myelin-peptide e.g. Mog) or by adoptive Transfer (inject CD4-positive T-cells specific for myelin peptides)
  - Early break of the blood brain barrier, focal perivascular mononuclear cell infiltrates, gliosis and demyelination of CNS white matter.

- Mechanisms against CNS autoimmunity:

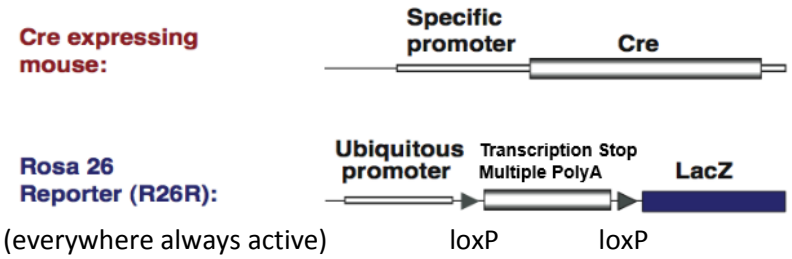
Checkpoint 1: T cell negative selection in thymus, elimination of self-reactive cell (T cell receptor / MHC)



- Pathological Heterogeneity in Multiple Sclerosis:
  - 1. T-cells, macrophages, myelin loss
    2. Antibodies and complement-mediated immune reactions against oligodendrocytes and Myelin (e.g. KIR4.1 (K^+ channel mainly on oligodendrocytes and astrocytes) antibodies in MS patients; do they play a pathogenic role?)
    3. Hypoxia-like damage (Hif1α), inflammation damage to vasculature
    4. Primary (?) Oligodendrocyte loss
- A Model for controlled cell death in adult myelinating oligodendrocytes?
  - Does primary ODC loss cause an immune response?
  - Are axons damaged if ODC die? Is the inflammation doing the damage to axons or the loss of support?
  - Production of transgenic mice:
    - Standard transgenic approach (DNA microinjection)
    - Gene-targeted transgenic approach (ES cells)
      → See "Concepts of Modern Genetics"



  - Conditional Gene Expression/Alteration:
    - Use prokaryotic CRE recombinase with tissue-specific Promoter
    - Also flox or frt Neo-Cassette (used for ES selection) by crossing with deleter-Cre mouse to get rid of possible interferences
    - Control by In-situ Hybridization (with use of negative control)
    - Fate Mapping using Cre/loxP system → if Cre expressed => blue



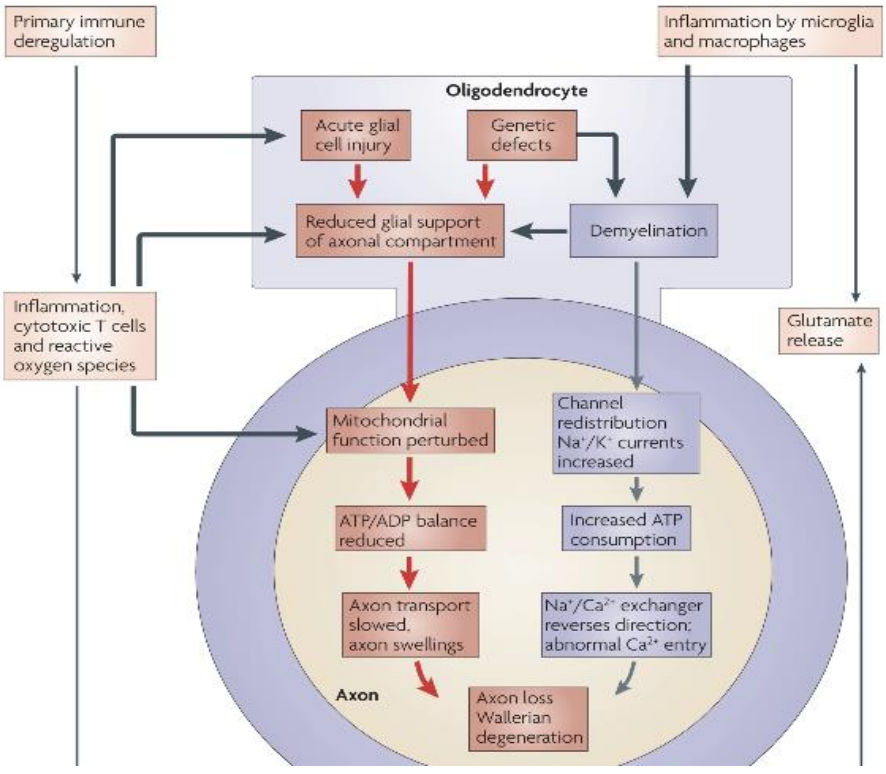
  - Protein Zero (P0) → Mutations cause **peripheral Neuropathies** (Charcot-Marie Tooth/CMT)
    → Most mutations are haplo-insufficient → Onion Bulbs
    → Homotetramer, major protein, which holds Myelin leaflets together
    → Similar to MS; Loss of Axons due to demyelination

- Beta-Catenin → Complete KO mouse: fail cell adhesion/differentiation, embryo dies
  → Regulates Sensory Neuron Differentiation of neural crest stem cells
  → Loss-of-function: No sensory neurons
  → Gain-of-function: Only sensory neurons (fate switch!), loss of other Derivatives of crest cells

- ODC cell death → Use mPLP promoter (ODC specific)
  → Use Cre fused with ERT2 (estrogen receptor); can be induced by Tamoxifen!
  → LacZ-STOP gene as marker for non-splicing
  → Expression of DT-A (Diphteria Toxin A-Domain) if spliced; cell death by blocking of protein synthesis (phosph. Of elongation factors)
  → Check symptomatic disease Development with RotaRod, etc. and MRI of brain and/or Histology
  → **Myelin disrupture , lesions, no immune cells enter brain (BBB intact)**
  --> <u>primary ODC loss does not induce anti-CNS immune reaction!</u>

<u>Multiple Sclerosis Progression</u>

- Current Hypothesis for Symptoms Progression in MS:
  - <u>First Phase</u>: dominating inflammation, variable in time (relapsing/remitting), Na^+ channel redistribution on axons, remyelination, axonal loss starts
  - <u>Second Phase (chronic)</u>: massive neurodegeneration and axonal loss without inflammation, same time rate in most patients, anti-inflammatory drugs have no effect
    → Neurodegeneration is the major cause of permanent neurological disability in MS
- Approaches/Experimental strategies:
  - Candidate Approach (gain information by studies and make educational guess)
  - Global Approaches (-omics with association studies)
    - Genomics, Transcriptomics, Proteomics (MS, 2D-SDS-GE), Lipidomics, Epigenomics,...
    - With Laser Capture Microdissection, FACS, Histology, etc.
- Brain Atrophy due to Demyelination and Axon Loss/Transection
  - Axons get damaged by loss of ODC (Mouse Model above) by neurofilament hypophosphorylation, Accumulation of versicles/organelles
  - Loss of Myelin → upregulation of Na^+ channel for compensation (temporarily) → Axon transection and formation of a blob at the cutted axon end
- Hypothetical Model of Axon degeneration:

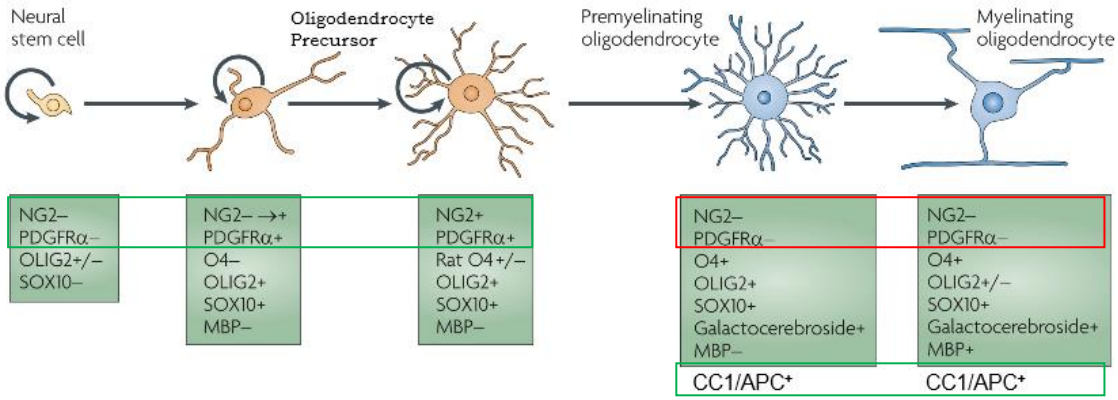


- Axonal Degeneration follows ODC defects with /without Myelin Loss
  - Red: Axonal Support System independent of Myelin, but involve metabolic coupling. Perturbation leads to reduced energy balance, slowed transport, axonal swelling and axonal degeneration
    (Lack of energy metabolites as glucose and lactate, provided my ODC, lead to axonal damage. Lactate is transported by MCT1 / SLC16A1, enriched in ODC, and their mutation cause axonal damage/loss)
  - Blue: Demyelination in the context of inflammation and Mt damage by ROS. Reorganization of ion channels, energy depletion, Ca^{2+}- mediated axonal decay follow up.

Remyelination in MS:

- Remyelination does not depend on age, gender or disease progression state
- Follows up induced adult ODC death
- Demyelinated axon either get remyelinated or declined
- Chronic Lesions in MS:
  - Loss of remyelination potential
  - Astrocytes hypertrophy, causing glial scar
  - Increased extracellular space due to axon loss
- Why does remyelination fails during disease Progression?
- How to enhance remyelination and inhibit concomitantly the destruction by immune system? (Pivot Point Model for CNS demyelinating Diseases)

- Where are the cells coming that remyelinate the axons?
  - ODC Lineage Markers:



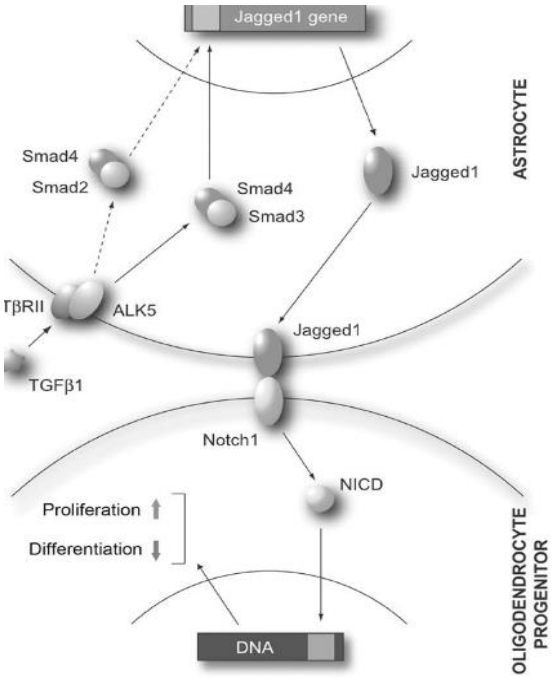
  - Study the problem with the Double Transgenic Mouse and Histology
    PDGFRalphaCreERT2 / ROSA26 lox STOP lox YFP
    → Adult ODC Precursors generate myelinating ODCs
    → Adult ODC Precursors generate remyelinating ODCs after demyelination (by the toxic compound lysolecithin; makes lysolecithin lesion)
    → Adult ODC Precursors generate remyelinating ODCs after demyelination (in EAE)
    → **BUT ODC Precursors are still present in chronic lesions but do not remyelinate! Why?**

- Differentiation of ODC Precursors to myelinating ODCs:
  - Notch Signaling Pathway responsible for neural development, especially the control of ODC Precursor differentiation:
    - Axons express Jagged 1 on their surface, which bind Notch 1 of ODC Precursors: Notch-ICD (NICD) gets cleaved off, gets imported into the nucleus by Importin-β, associate with CSL to express Hes, which inhibits in a complex with TLE the expression of Mash-1 and Math-1 (would activate ODC specific genes)
      → ODC Precursors kept in an immature state by Notch signalling!
    - If Jagged 1 is downregulated, inhibition gets releaved → mature myelinating ODCs

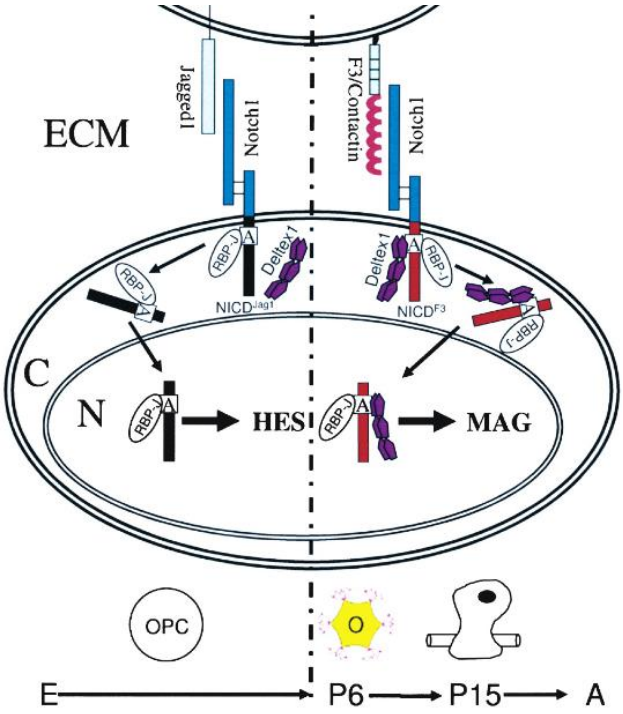
- Markers:
  - PLP (mRNA), MAG (Protein) for premyelinating ODCs
  - MBP (protein) for mature ODCs
  - TUNEL (labelling of fragmented DNA) for apoptotic cells

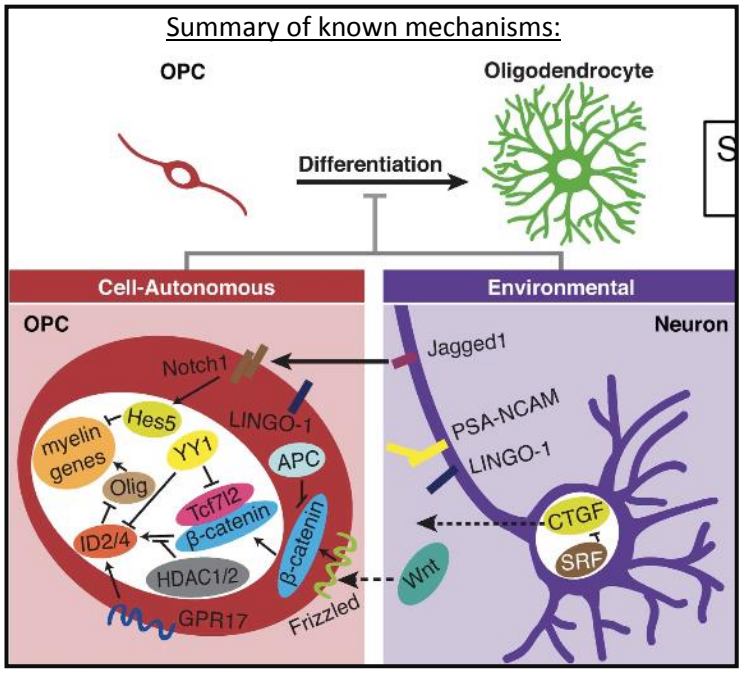
- Notch Signaling in MS: High Jagged 1 expression by hypertrophic Astrocytes in MS plaques lacking remyelination, Notch 1 and Hes5 localize to immature ODCs, and TGF-beta 1 was in the ECM of same area. Remyelinating areas show low jagged1 expression.
  - Proposed Signaling leading to Notch1 Activation by TGF-beta 1:

- ALK activation
- Smad2/3 phosphorylation
- Jagged 1 upregulation
- S3 Notch 1 cleavage
- Inhibits differentiation



- Antagonistic Effects of Notch 1 Signaling in ODC Precursor (OPC) differentiation:
  - Canonical Pathway: Jagged 1 as Ligand
    Maintenance of Precursor state and ODC Prec. Migration
  - Non-Canonical Pathway: F3/Contactin as Ligand
    Association with Deltex1
    Induce differentiation to mature myelinating ODCs
    Can be Inhibited by TIP30 (induced by stress?), which bind Importin-β associated with NICD → remyelination failure





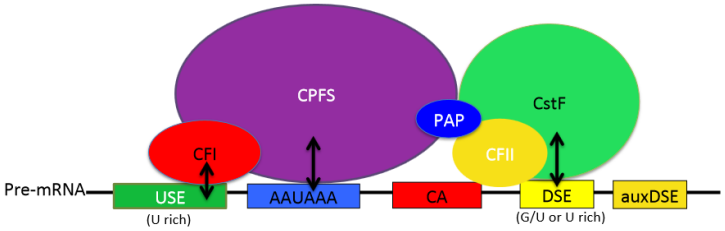
RNA Processing (AMK)

Introduction

- >28 different types of RNA:
  - mRNA: codes for proteins
  - tRNA: translation
  - rRNA : translation
  - snRNA : splicing
  - snoRNA : nucleotide modification of RNAs (mainly rRNAs)
  - RNaseP: tRNA maturation
  - Telomerase RNA: telomere synthesis
  - Long nc RNA: various
  - siRNA: gene Regulation
  - miRNA: gene regulation

3‘ End Processing

- Polyadenylation:
  - Poly(A) tails influence mRNA stability, Translation, mRNA transport, about 200 AA long; Polyadenylation is coupled to transcription /-termination, 5‘ capping and splicing
  - 3’end processing machinery and cis acting RNA signals are needed for cleavage and polyadenylation:

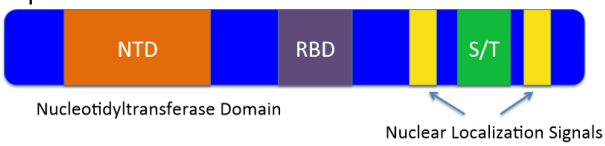


RNA secondary structures may also play a role; AAUAAA and DSE positioning can vary poly(A) site by several tens of nt!

  - Isolation of the complex with CPSF by
    - Mixing, Lysation, Centrifugation and Affinity chromatography or Gelfiltration
    - Using “hairpin” RNA and bacteriophage coat protein MS2, which bind them, fused to a Maltose-binding protein (MBP),fractionated by glycerol gradient sedimentation and affinity purification with Amylose beads
    - Purification by Flag tag fused to CPSF on a plasmid, which gets stably transfected into HEK293 cells with the use of markers, then affinity purification with anti-FLAG M2 monoclonal antibody fused to beads in a column

    (Activity assays and Western blots with radioactive mRNA help to determine conc.)

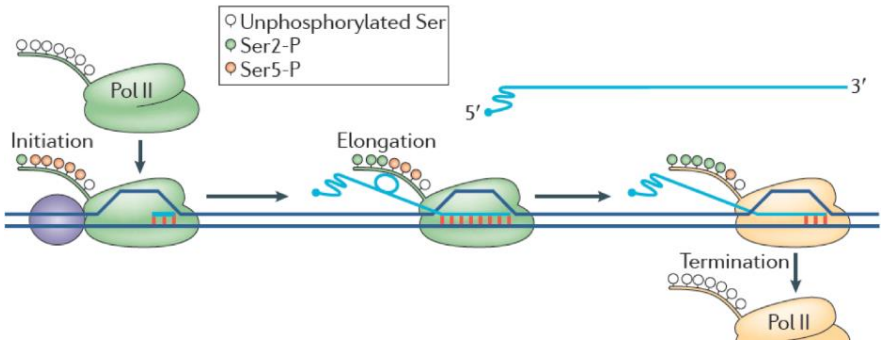
- Processing Factors:
  - Poly(A) Polymerase (PAP): RNA binding by RBD is not sequence specific, recruitment requires interactions with CPSF and CF1



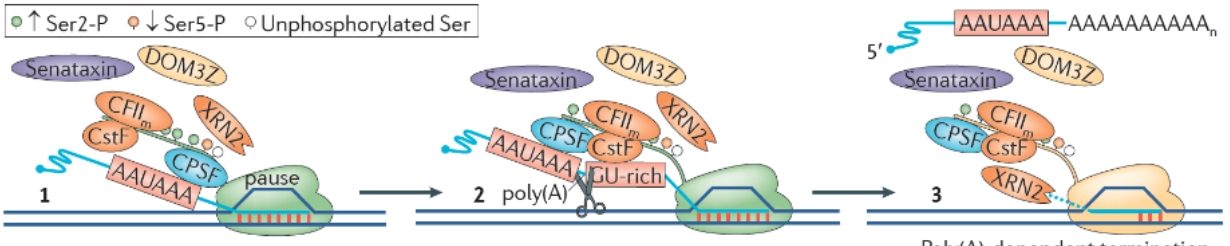
PAP CTD is hypophosphorylated during mitosis → PAP inhibition / protein synth. Inhibition
PAP CTD binds to 14-3-3ε, when phosphorylated → PAP inhibition, to cytoplasma
PAP CTD acetylation → inhibits association with CFI_m25, to nucleus
PAP CTD sumoylation → stabilizes PAP, to nucleus, inhibits enzymatic activity

  - Cleavage and polyadenylation specificity factor (CPSF): Required for cleavage and polyadenylation, recognizes AAUAAA, recruits other components, CPSF73 is endonuclease, binds CTD of RNAPII

- Cleavage stimulation factor (CstF): association to DSE needs cooperative binding of CPSF to AAUAAA, required for RNA cleavage but not polyadenylation, functions as a dimer, binds CTD of RNAPII
- Cleavage Factor I_m (CFI_m): binds UGUAN RNA motif (USE), required for RNA cleavage but not polyadenylation, functions as a dimer
- Cleavage Factor II_m ($CFII_m$): Yeast homolog of Pcf11 is involved in transcriptional termination, required for RNA cleavage but not polyadenylation, binds CTD of RNAPII
- Poly(A) binding Protein (PABPN1): Coats entire poly(A) tail, 5PABPs in humans (1 nuclear, 4 cytoplasmic), stimulates PAP activity, regulates tail length (approx. 250 nt, then stop), promotes mRNA export, cytoplasmic PABPs are involved in translation initiation
- RNA Polymerase II (RNAPII): Prior to initiation: CTD is not phosphorylated and interacts with a number of elongation factors; Transcription elongation: CTD 7 heptad repeats are phosphorylated at diff. serines, 3'end processing complex associates to CTD during elongation



- Transcripton termination:



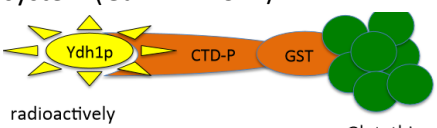
Pausing of Pol II is induced when CPSF recognizes the AAUAAA signal

Upon exposure of the GU rich binding site, CstF dislodges CPSF

Following cleavage at the poly(A) site, 5'-3' exoribonuclease (XRN2) degrades the downstream RNA product which may displace Pol II (Torpedo model).

Defective Termination → decrased splicing, increased degradation of mRNA, reduced initiation!

- Study function of processing factors:
  - Make cs or ts mutants of a gene (e.g. YDH1, part of CPSF) by error-prone PCR
  - Co-transform PCR product and linear vector with homolog. Sequences and LEU2 gene into haploid yeast strain (select for LEU2) → intacellular homol. recombination between them
  - Force loss of strain plasmid with URA3 and YDH1 gene (endog. YDH1 deleted!) by medium containing 5-FOA (5-Fluoro orotic acid)
  - Transfer on YPD plates, incubate at diff. Temperatures to see cs/ts mutants
  - Make Growth curves, cleavage assays, polyadenylation assays
  - Northern Blot with radioactive protein to have a look at stability of mRNAs (different times)
  - Test interactions (e.g. CTD RNAPII) by yeast two hybrid system (Gal4DBD-CTD/YDH1-Gal4AD →expression of HIS3) or make GST pull down:

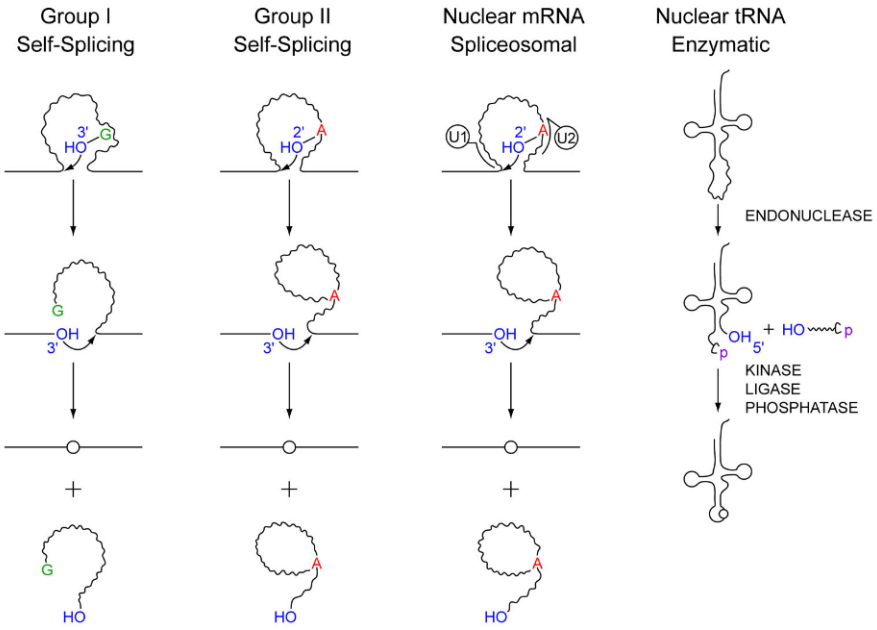


- Alternative Polyadenylation (APA):
  - Protein Regulators affecting APA:
    - Changes in expression/stoichiometry of the 3'end processing factors
    - Auxiliary factors, that bind pre-mRNA
    - Gene promoters recruiting factors that enhance PAS recognition (CPSF, ...)
    - Communication between Promoter and Terminator through gene loop formation
    - Specific pause sites within genes, which depend on cellular context
  - Pre-mRNA cis Sequences affecting APA:
    - RNA sequences direct binding of APA influencing factors
    - Affinity of CPSF and CstF complexes to RNA sequences modulated by PTM or other factors
    - Secondary structures (e.g. stem loops)
  - Chromatin and Epigenetics affecting APA:
    - Chromatin, Histone Modifications and nucleosome positioning may influence e.g. the rate of RNAPII elongation

  Switch from membrane-bound to secreted form of IgM Proteins in activated B-cells as an example of poly(A) site selection!

Splicing

- Overview:



Group I introns: Found in bacteria, lower eucaryotes and higher plants; requires exogenous G
Group II introns: Found in fungy, bacteria and plants. Spliceosomal way may have evolved from this

- Special SL trans-splicing in C.elegans:
  - SL upstream of ORF codes for a Leader RNA containing a mini-exon and a 3' intron
  - Intron can get picked off by a branch site A of endog. Intron
  - Mini-exon can now attack 3' Splice Acceptor Site (AU) of endog. Exon with its 5' Splice Donor Site (GU) leading to:
    - Capping of Pol I transcript
    - Resolving polycistronic pre-mRNA (replacing first upstream ORF)
    - Sanitizing 5'-UTR (replacing an early AUG upstream of ORF)
    - SL-enhanced translation (recruitment of ribosomal subunits upstream of ORF)

- Spliceosomal pre-mRNA Splicing:
  - Some Facts:
    - Human genome contains >200'000 different introns
    - Introns 30 to 800'000 nucleotides (average = 1800 nts)

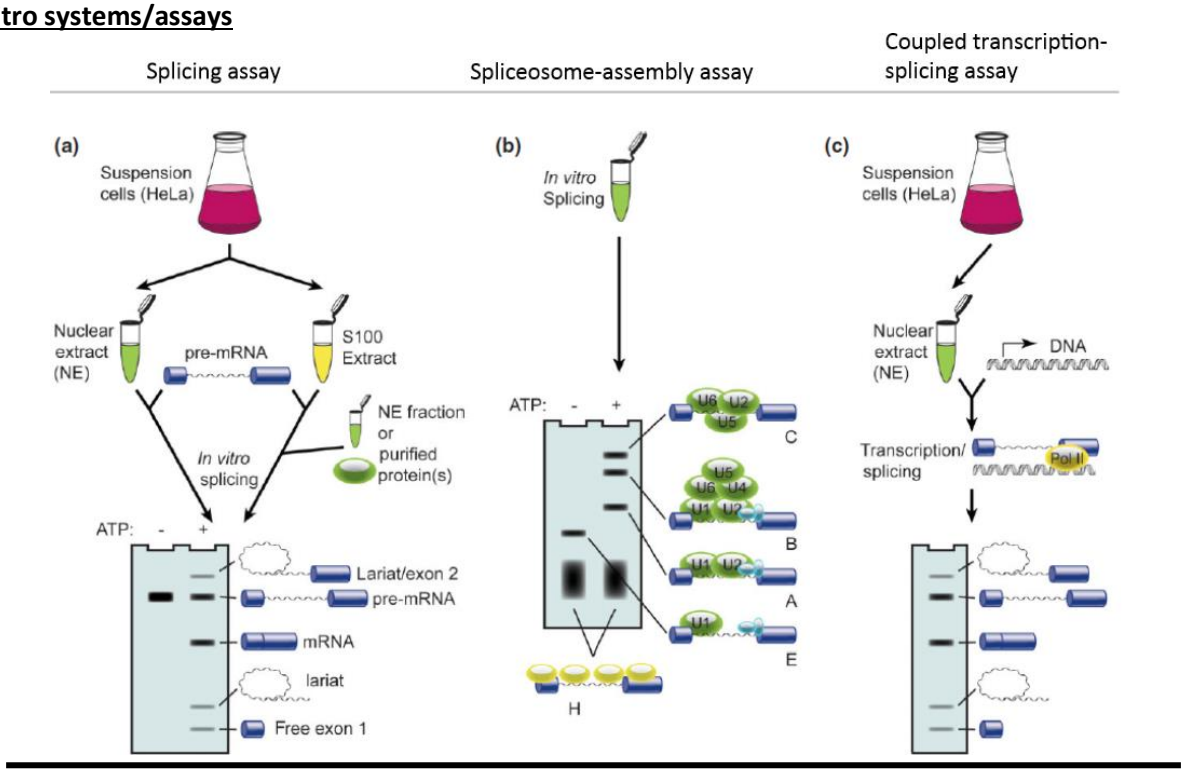
- Exons 6 – 400 nucleotides (average = 123 nts)
- Typically human genes contain seven to nine introns
- Average *in vivo* excision time for an intron is < 3min
- Splicing can occur co-transcriptionally!
- ATP is needed for conf. Changes of Spliceosome proteins and NOT splicing reaction

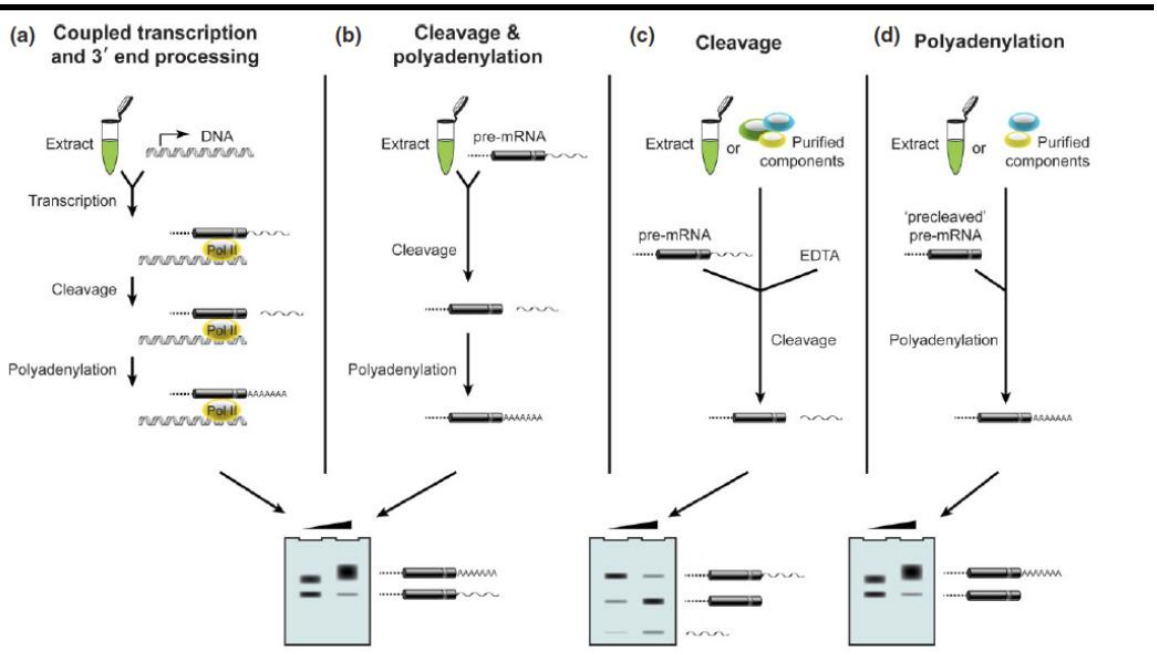
- Splicing regulatory factors:
  - SR and SR-related proteins: Typically activate splicing by recruiting components of the splicing machinery.
  - hnRNPs: Typically repress splicing by a variety of poorly understood mechanisms.
  - Other RNA-binding proteins: Activate or Repress splicing. U1 snRNP is essential for constitutive splicing but can also repress splicing
- Intron/Exon definition and The Splicing Machinery:
  - (SEE CORRESPONDING SECTION OF "CELLULAR BIOCHEMISTRY PART I" SUMMARY)
- The challenges faced by the Spliceosome:
  - exon length: 6 -400 nucleotides
  - intron length: in human up to 480,000 nucleotides (human neurexin pre-mRNA)
  - sequences that mark the splice sites are short and ill-defined
  - Positioning of the splice sites (which may lie tens of thousands nucleotides apart) within the atomic distance that allows the transesterification reactions to proceed.
- The Solutions:
  - → The reactive groups of the pre-mRNA are recognized multiple times by RNA or proteins to ensure the precision of the splicing reaction
  - → Important interactions in the spliceosome are weak, but are enhanced by multiple interactions. This also leads to higher flexibility
  - → RNA rearrangements (during assembly and catalytic activation) involve handing over of binding partners to new interaction partners
- Summary Pre-mRNA Splicing:
  - Involves **two coupled cleavage and ligation reactions**, which represent transesterification. This occurs in large complexes containing **five snRNAs** and more than **100 proteins**
  - The **snRNPs U1, U2, U4/U6 and U5** are essential. They associate with the pre-mRNA in a sequential fashion and recognize conserved sequences in the intron.
  - Splicing is thought to be **RNA catalyzed** (specially by U2 and U6)
  - Proteins have mainly **auxiliary functions** (Prp8 as cofactor and catalyst)
  - **RNA:RNA, RNA:protein, protein:protein interactions are dynamic**
- Alternative Splicing :
  - One of the main sources of protein diversity in multicellular eukaryotes
  - Strong Splicing silencers are only apparent when there is a competing splice site upstream of the affected site
  - Alternative Splicing can be influenced by:
    - Splicing factor concentrations (e.g. ASF/SF2)
    - The speed of the RNA Pol II (e.g. pause might allow alt. exon inclusion)
    - Transcriptional Activators and Promoter sequences
    - Histone modifications (H3 trimethylated → slow transcription, vice versa)
    - Extracellular Signals (e.g. depolarization siRNAs, ...)
    - Nucleosome remodelling factors
    - Cis acting elements (ESE, ESS, ISE, ISS) and RNA secondary structure
- Splicing mutations and disease :
  - At least 15% of human genetic diseases result from defects in splicing
    - Mutations in **cis** affect → constitutive splice sites (loss of function)
      → alternative splice sites (change isoform ratio, e.g. *Spinal muscular atrophy (SMA)*)
    - Mutation in **trans** affect → basic splicing machinery (*Retinitis pigmentosa*)
      → regulation of alt. Splicing

- Alternative splicing is an important mechanism **to increase the coding capacity** of the genome and **to regulate gene expression**, by generating multiple protein isoforms with different functions from a single gene.
- Alternative splice sites are usually selected at early stages of spliceosome assembly.
- **Regulatory sequences** can be found in exons or introns (splicing enhancers and silencers).
- The **relative concentrations of SR proteins** (= constitutive splicing factors) can regulate the use of duplicated splice sites by recruiting other splicing factors.
- Alternative splicing can also be regulated by proteins that are **specifically expressed in different tissues** or at **different developmental stages.**

- At least 15% of human genetic diseases result from defects in splicing, i.e. mutations of splice sites, branch site and splicing enhancers or silencers.
- Diseases can also be the result of mutations in splicing proteins.
- Approaches to correct miss-splicing by gene therapy are being developed.

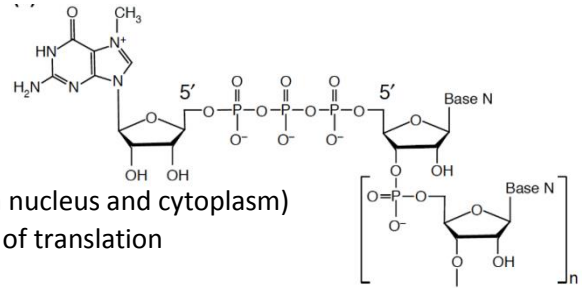
In vitro systems/assays





5' Capping

- Facts:
  - Capping occurs co-transcriptionally when pre-mRNA reaches length of 22-25 nts
  - Protects mRNA from degradation and promotes transcription, polyadenylation, splicing, nuclear export, translation



  - Nuclear cap binding complex (nCBC):
    - CPB20 and CPB80
    - Predominantly nuclear (shuttles between nucleus and cytoplasm)
    - Important for cytoplasmic pioneer round of translation
  - Cytoplasmic cap binding protein:
    - Eukaryotic translation initiation factor 4E (eIF4E)
    - Required for translation

The 3'UTR

- Length of the 3'UTR can effect:
  - Stability (in general: the longer the 3'UTR the less stable the mRNA)
  - Localization
  - Transport
  - Translational properties
- Poly(A) tail length and its role for mRNA recycling
  - Facts:
    - Increase in length generally correlates with translational activity
    - Cytoplasmic poly(A) tail length can be regulated by *cis* elements in the 3'UTR
    - The poly(A) tail appears to act trough PAPB

    (Regulation of translation via eukaryotic translation initiation factor complex eIF4E and the 43S pre-initiation complex containing 40S ribosomal subunit)
    - Increase in length generally correlates with translational activity
  - 3'UTR domains that affect poly(A) tail length:
  - (DA = Deadenylation, A = Adenylation)

• **ARE**:	a) TTP binds → CCR4-NOT complex → DA b) KRSP binds → PARN → DA c) HuR binds → Inhibit Exosome → stable mRNA
• **CPE**:	a)CREB1 binds → eIF4E-T → CCR4-NOT → DA b) CPEB1 binds → PARN → DA c) CPEB1 (phosph.) → CPSF → Gld2 → A (in cytoplasma)
• **Pumillo binding sites**:	a) PUF family proteins bind → CCR4-NOT / PARN → DA
• **EDEN:**	a) EDEN-BP bind → DA
• **miRNA sites**:	a) RISC binds → GW182 → CCR4-NOT → DA

  → TTP bound to ARE and miRNA mechanism can act together for deadenylation!
  - Deadenylated mRNAs are not necessarily degraded! They can be stable but translationally silent and can be reactivated by cytoplasmic polyadenylation!
    (α-subunit calcium/calmodulin dependent protein kinase II (αCaMKII) in neurons during learning and memory as an example)

RNA Editing

- Overview:

Type of Editing	Edited RNAs	Organisms	Genetic system
U insertion/ deletion	mRNA	protozoa	mitochondrial
N insertion (C,U, dinucleotides)	mRNA, rRNA, tRNA	Slime molds	mitochondrial
N replacement	tRNA	Amoebozoans, fungi, various animals	mitochondrial
C-to-U conversion	mRNA, tRNA	Various mammals, plants, protozoa	Nuclear, mitochondrial, chloroplast
U-to-C conversion	mRNA, rRNA	Mammals, plants	Mitochondrial, chloroplast
N substitution	mRNA , rRNA	dinoflagellates	Mitochondrial, chloroplast
A-to-I conversion	mRNA, miRNA, viral RNA	Metazoan animals	nuclear

- Why do RNA editing systems frequently emerge in mitochondria?
  → Mt encodes relatively few genes (<100), causing an advantageous less constrained gene expression out of which RNA editing could have evolutionary evolved
- Editing Effects:
  - On mRNA:
    - Creation/Removal of Start/Stop Codons by U insertion, C to U changes
    - Creation or completion of ORFs by nucleotide insertion
    - Changes in encoded AA or splice site choice by base conversion
  - On tRNA:
    - Correction of stem mismatches by base conversion
    - Creation of substrates for base modifications by base conversion
    - Changes in tRNA identity by base conversion in anticodon loop
    - Addition of conserved sequence elements by nucleotide insertion
  - On rRNA:
    - Potential Modulation of translational efficiency by base conversion
    - Creation of conserved structural elements by base conversion, nucleotide insertion
    - Changed translational fidelity and efficiency by nucleotide insertion
- C-to-U and A-to-I Editing are result of hydrolytic deamination (amino- to keto-group conversion):
  - **A-to-I RNA editing**: Main editing form in mammals, occurs in dsRNA, catalyzed by "adenosine deaminases that act on RNA" (ADARs), I behaves as G in translation, AMPA receptors as an example of critical A-to-I editing
  - **C-to-U RNA editing**: ApoB as an example of a crucial role for C-to-U editing

Mitochondria (AN)

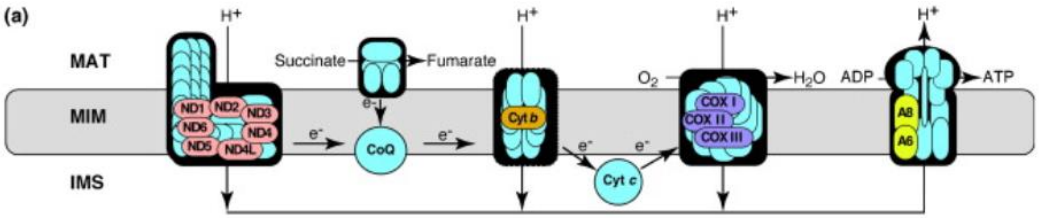
Introduction

- Mitochondria have their own genome, encode 13 proteins for Electron Transport Chain
- Most important function: Iron sulphur cluster biogenesis
- Are highly mobile although they produce very dangerous species (ROS)
- Curvature of IMM is important for formation of the respiratory chain complexes
- Mitochondria are not kidney shaped organelles!

Mitochondria and Diseases

- Mutations in the mtDNA are maternally inherited
- Same mutation can affect diff. regions in the CNS, PNS or muscle: Caused my **mitotic segregation**!
- Not all offspring/tissue develops a phenotype: Caused by **heteroplasmy and the threshold effect**!
- Tissues can show haplotype selectivity dependent on the energy demand and the dynamic range of mitochondria in the tissue

- Mitochondrial respiratory chain diseases:



Subunits	Complex I	Complex II	Complex III	Complex IV	Complex V
mtDNA-encoded:	7	0	1	3	2
nDNA-encoded:	~39	4	10	10	~14
"Assembly" factors:	~13	2	4	~20	2

Diseases	LHON MELAS LHON + dystonia Leigh syndrome			Encephalomyopathy	ALS–like syndrome Encephalomyopathy	NARP MILS FBSN
Mutated genes	ND1 – ND6			Cyt *b*	COX I – COX III	ATPase 6
Mutated genes	*NDUFA1* *NDUFS7* *NDUFS1* *NDUFS8* *NDUFS2* *NDUFV1* *NDUFS3* *NDUFV2* *NDUFS4* NDUFA12L *NDUFS6*	*SDHA* *SDHB* *SDHC* *SDHD*	*APTX* *COQ2* *PDSS1* *PDSS2*	*BCS1L* *UQCRB*	*COX10* *COX15* *LRPPRC* *SCO1* *SCO2* *SURF1*	*ATPAF2*
Diseases	Leigh syndrome Leukodystrophy	Leigh syndrome	Encephalo-myopathy Nephrosis	Leigh syndrome Encephalo-myopathy	Leigh syndrome Cardioencephalo-myopathy Leukodystrophy/ tubulopathy	Fatal infantile encephalomyopathy

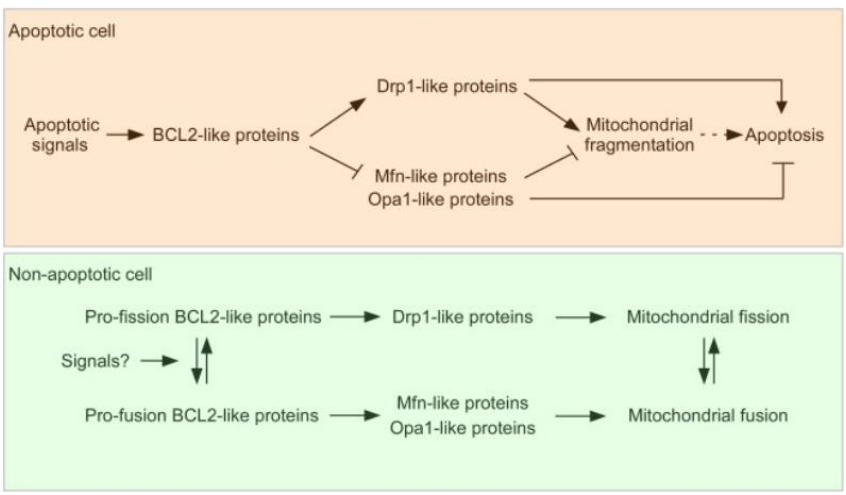
  - Less known mutations/diseases at the end of the RC, because alternative pathways (e.g. Krebs cycle, Beta-Oxidation, ...), which could compensate for the loss of complex efficiency are missing → lethal mutations.
  - There seems to be a correlation between Mt activity and morphology (Class I and II)
  - Mt morphology and localization is the sum of FUSION, FISSION, TRANSPORT and RESPIRATORY ACTIVITY interplay

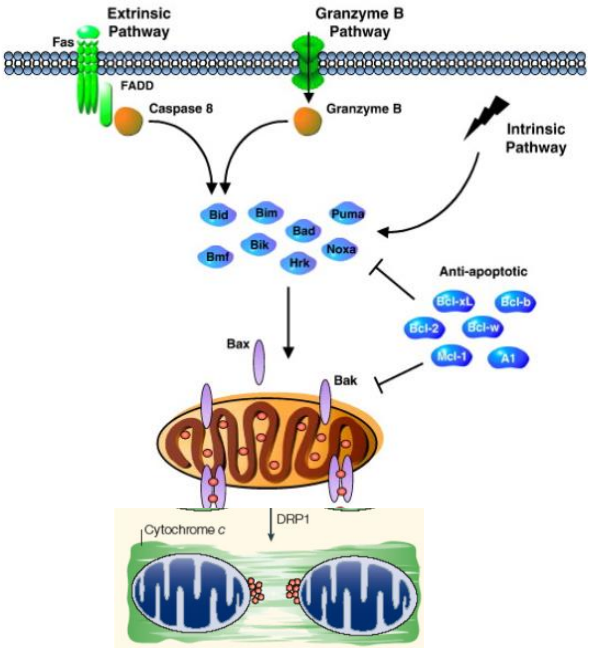
- Mitochondrial fusion and fission factors:

Protein	Localization	Function	Selected References
Mfn1	MOM	Fusion	Santel et al., J Cell Sci '03
Mfn2	MOM	Fusion	Chen et al., J Cell Biol '03
OPA1	MIM IMS	Fusion	Olichon et al., FEBS Lett '02
MICS1	MIM	Fusion	Oka et al., Mol Biol Cell '08
Bax	Cytosol/MOM	Fusion	Karbowski et al., Nature '06
Bak	MOM	Fusion	Karbowski et al., Nature '06
SLP-2	MIM	Fusion	Hajek et al., J Biol Chem '07
MICS1	IMM	Fusion	Oka et al., Mol Biol Cell '08
Drp1	Cytosol	Fission	Frank et al., Dev Cell '01
Fis1	MOM	Fission	James et al., J Biol Chem '03
GDAP1	MOM	Fission	Niemann et al., J Cell Biol '05
PINK1	MOM	Fission	Yang et al., PNAS '08
Parkin	Cytosol/MOM		Poole et al., PNAS '08
GDAP1L1	Cytosol/MOM	Fission	*unpublished*
MTP18	MIM	Fission	Tondera et al., J Cell Sci '05
Mff	MOM	Fission	Gandre-Babbe et al., MBC '08
MBP	Cytosol	Fission	Eura et al., J Cell Sci '06
Rab32	Cytosol	Fission	Alto et al., J Cell Biol '02
Endophilin B1	Cytosol	Fission	Karbowski et al., JCB '04
USP30	MOM	Fission	Nakamura et al., MBC '08
GGNBP1	IMS	Fission	Aihara et al., Biol Reprod '09
MARCH-5	MOM	Interaction with Drp1+Mfn2	Nakamura et al., EMBO R. '06
Or MITOL		Turnover of MOM proteins	Yonashiro et al., MBC '09
MIRO-1	MOM	Regulates Drp1	Saotome et al., PNAS'08
MIRO-2	MOM	Regulates transport	Fransson et al., BBRC '06
Bcl-X-l	MOM	Increases dynamics	Berman et al., J Cell Biol '09
SUMO1	Cytosol	Protects Drp1	Harder et al., Curr Biol '04
SENP5	Cytosol	Cleaves SUMO1	Zunino et al., J Cell Sci '07
DAP3	Matrix	Fission during apoptosis	Mukamel et al., JBC'04

  - Mfn1/Mfn2/Opa1 Fusion factors
    - All members of the dynamin super-family with a GTPase domain
    - Mfn1, Mfn2 are human homologs for the FZO (fuzzy-onion) in S.c., with TM domain
    - Mfn2 is important for the development of Purkinje cells in mice cerebellum (TUNEL and Anexin V mark apoptotic cells, Calbindin mark Purkinje cells)
    - Fusion deficient Mt often lack mtDNA
    - Mfn2 is mutated in peripheral neuropathies (CMT disease)
    - Mfn1 can bind to OPA1 and interact with other Mfn1 or Mfn2 by HR2 dimerization
  - Drp1/Fis1/Bcl-Xl Fission factors
    - Dynamin-related proteins with GTPase and GED domain
    - Initial constriction on Mt mediated by actin → Drp1 localization along constriction with the help of Mt surface protein Fis1 → Fission
    - Mt distribution reflects ability of neurons to react to external stimuli: Fission very important in neuronal cells!
    - Drp1 is important for mouse development (decreased body size, lack of TGC if KO)

- Mitochondrial Dynamics and Apoptosis:

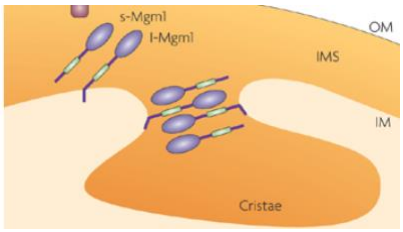




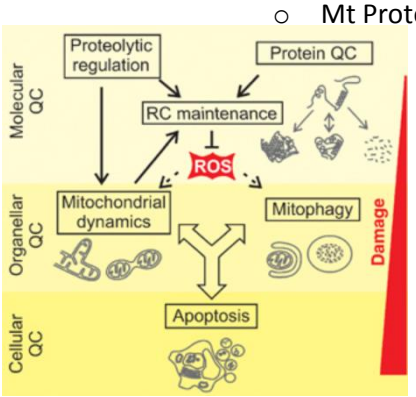
→ **The Induction of Apoptosis blocks fusion and increases fission!**

Protein	Mediated process (submitochondrial localization)	Protein characteristics
Bax/Bak	Proapoptotic (OMM)	Overexpression induces fragmentation of mitochondria; upon induction of apoptosis they coalesce into submitochondria punctate foci at the scission sites that colocalize with Drp1 and Mfn2
Bid	Proapoptotic (OMM)	Upon induction of apoptosis translocates to mitochondria and activates Bax and Bak; induces fusion of mitochondrial cristae; possesses lipid translocase activity
Bcl-2	Antiapoptotic (OMM)	Overexpression causes an increase in size and complexity of mitochondria
vMIA	Antiapoptotic (MITO)	Viral mitochondria-localized inhibitor of apoptosis, induces mitochondrial fragmentation
PB1-F2	Proapoptotic (MITO)	Viral product of influenza A PB1; induces mitochondrial fragmentation

- Stauder-Sporin, Etoposide (slow) and Actinomycin D as apoptosis inducing agents
- GDAP1 as an example for a Mt fission factor, which does not alter apoptotic response if KO
- OPA1 (Mgm1 in S.c.):
  - Dynamin related fusion factor as mentioned above
  - Mutated in dominantly inherited optical atrophy
    (Photodamage in retina with mutated OPA1 leads to rapid retina degeneration)
  - Overexpression protects against apoptosis by influencing cristae structure
    - Forms bottlenecks of cristae
    - If mutated: huge tubes
      → Easy release of cyt. C into cytoplasm!
    - (Also important for IM fusion)
    - Longer variants of OPA1 get processed by mitochondrial Proteases upon Stress



- Mitochondrial Proteases:
  - Form a **redundant system**:
    - m-AAA protease anchored in IMM pointing into Matrix (AFG3L2 (unique subunit), paraplegin (2 different subunits))
    - i-AAA protease anchored in IMM pointing into IMS
    - HTRA2/OMI pointing into IMS or diffuses free in IMS
    - PARL integrated into IMM
  - Prohibitins (PHB1, PHB2) form circular complexes and control/inhibit AAA-protease activity (both versions needed due to alternating binding in complex formation)
    → Prohibitins inhibit OPA1 processing
  - Mt Proteases act as **Quality Control (QC) complexes**:



<u>Functions:</u>

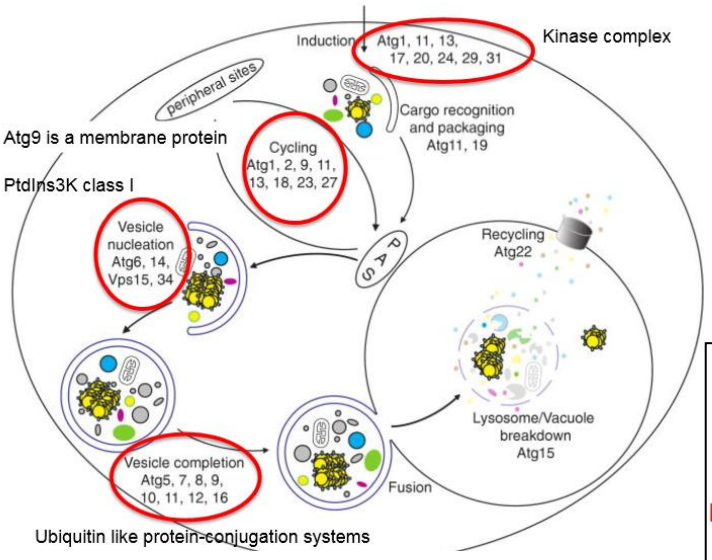
1. Process proteins for protein synthesis after imp. into Matrix (H^+ Gradient needed!) e.g. MRPL32 → subunit of Mt ribosome
2. Degrade missfolded proteins upon Stress
3. Influence Mt dynamics (e.g. OPA1 processing)
4. Ensure Mt transport
5. Responsible for assembly and stability of respiratory complexes I and III
6. Alter mtDNA amount upon m-AAA depletion or mutation
   → Paraplegin mutated in hereditary spastic paraplegia
   → AFG3L2 mutated in spinocebrellar ataxia

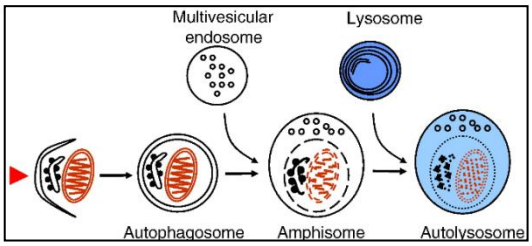
  - LON protease degrades Aconitase (FeS protein in Matrix), which is oxidatively modified and inactivated during aging (can lead to aggregation and Mt damage)
    - No ATP + many substrates → ATP/ADP-depleted form, which bind substrate, and mtDNA/RNA with ATP-binding site, to pause mitochondrial translation
    - ATP → sequestered DNA and RNA gets replaced and substrates are degraded upon ATP hydrolysis
  - MARCH V protease associate with ubiquitinated Mfn1, Mfn2, Fis1 and Drp1. Loss of MARCH V leads to branched Mitochondria. Specific function unknown.
- Mitochondrial Rescue:
  - Mt Fusion can rescue weak mitochondrias by providing ATP and functional proteins
  - Fusion does not work if membrane potential is lost! "Bad" cannot fuse back into the pool!

Autophagy/Mitophagy

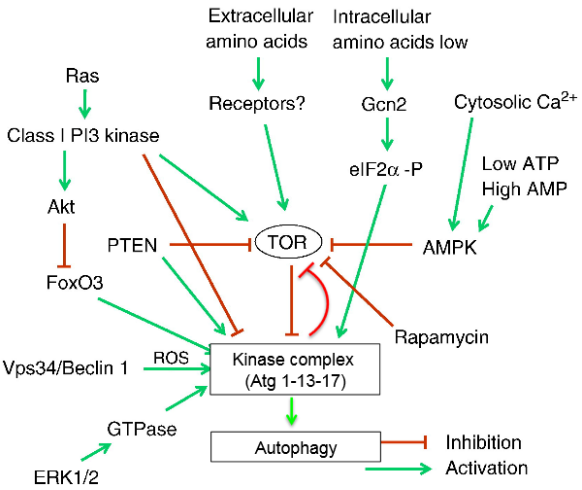
- Introduction:
  - Cellular Stress activates overlapping responses in a sequential way
    → Fusion/Fission → Mitophagy → Apoptosis → Necrosis
  - Autophagosomes form locally and mature to autolysosomes during transport to perinuclear region, where the new building blocks are needed for protein synthesis

- Identified autophagy relevant genes (ATGs) by yeast screens and their functional grouping:



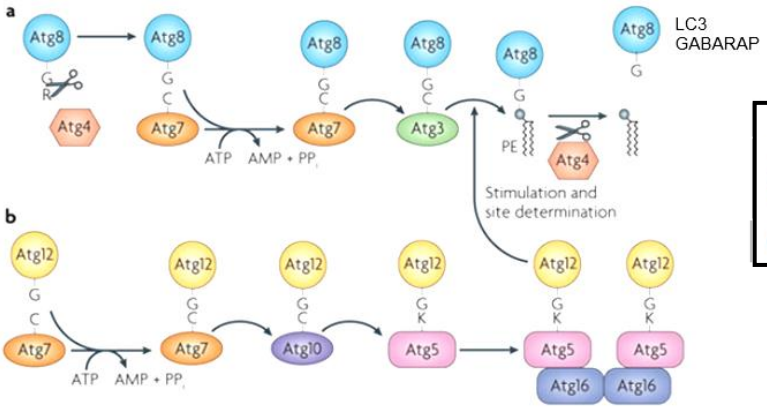


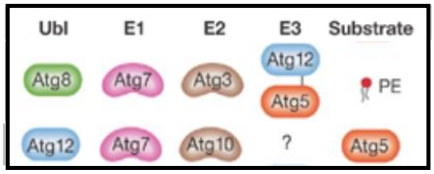
- Regulation of Autophagy:
  - Autophagy is a constant process at basal level, but can be induced for specific degradation
  - Autophagy can be induced by:



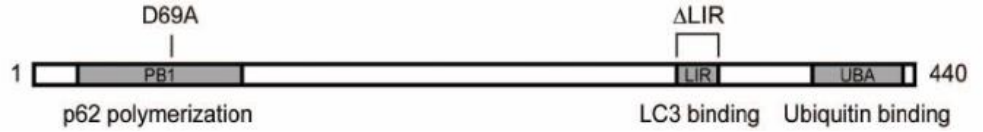
- Phospho-Inositol signalling
  (e.g. downregulation of IP_3)
- PI3K-mTOR Pathway
  (e.g. Inhibition of mTOR by rapamycin)
- Ca^{2+}-calpain-G_{SCL} pathway
  (e.g. calpain inhibition by calpastatin)
- cAMP-EPAC-PLC-IP_3 pathway
  (e.g. cAMP inhibition by clonidine)
- intracellular AA or ATP depletion

- Ubiquitin like conjugation system of Autophhagy:
  - Atg3,5,7,8,10,12 behave like an ubiquitin conjugation system (LC3, GABARAP are homologs for Atg8 in higher eukaryotes) and decorate PE of autophagosome membrane with Atg8!





- The procedure of Mitophagy:
  - RC Inhibition in mitochondria by MPP^+, Rotenone and Paraquat, DA metabolism and/or Mt depolarization by CCCP or Mt damage lead to oxidative stress
  - The proteins PINK1, Parkin, dLRRK, DJ-1 (discovered by genetic analyses of Parkinson patients) and antioxidants, SOD, GSTs counteract the formation of oxidative stress
    1. **PINK1** on the surface of Mt's (normally gets cleaved and degraded by UPS) is not cleaved anymore
    2. **Parkin** gets recruited by PINK1 to damaged Mt
    3. Parkin as a E3-Ligase ubiquitiates surface proteins (i.e. Mfn2)
    4. Proteasomal subunits are recruited to Mt and proteins degraded by **Rhomboid7**
    5. **p62** is recruited to Mt



       - UBA domain binds to ub. Surface proteins of damaged Mt
       - Polymerization of p62 and thereby aggregation of damaged Mt's
       - LIR domain binds LC3 (Atg8) and recruits autophagosomal membrane

       Deletion of p62 has no cellular effect → p62 and Atg7 are redundant!
       HDAC6 acts in same way for aggregated proteins as p62 for Mt's
    6. Mt aggregate around the nucleus (Aggresome)
       - **Miro** (OMM protein, 2 GTPase domains (Ras-family), binds Ca^{2+}) is bound to **milton**, which recruits **kinesin heavy chain** for + end transport of Mt's on microtubules
       - PINK1 phosphorylates Miro and Parkin ubiquitiates it for proteasomal degradation
       - Loss of interaction with milton and the kinesin heavy chain→ arrested Mt's
    7. Mt's get degraded

- The Role of Autophagy:
  - Plays a crucial role in development (many Atg KO lead to neonatal lethality)
    - Oocyte-to-embryo transition
    - Embryo-to-neonate transition
  - Important for cell differentiation (removal of organelles for blood cell formation)
  - Responsible for homeostasis and cell survival
    - Degradation of ubiquitinated proteins! (Lysosomal and proteasomal pathways are <u>not</u> separated)

- Induction of Autophagy may be a possible treatment for many neurological disease (Alzheimer, Parkinson, Huntington), which may come from protein aggregations

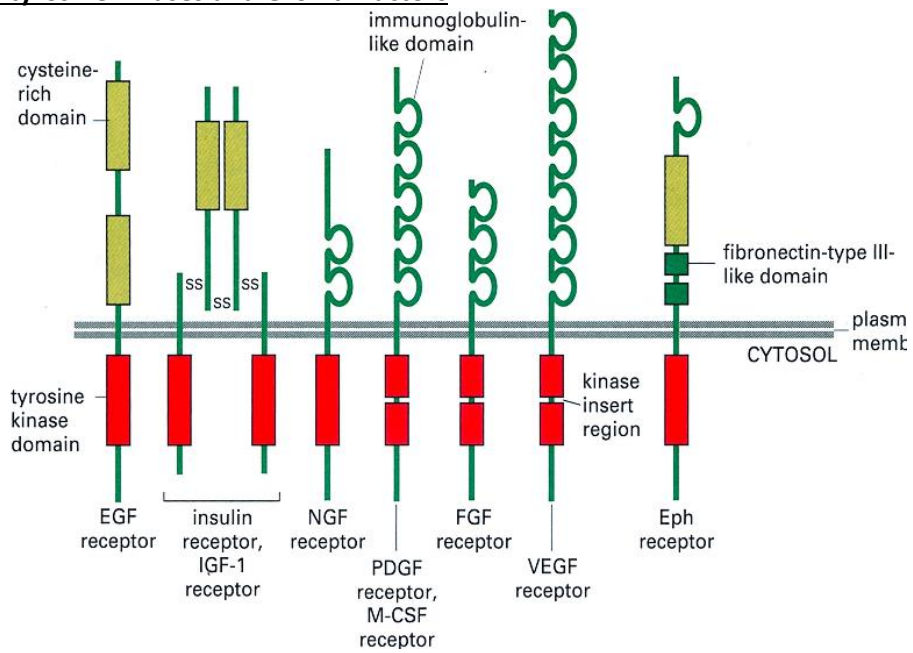
- Studies using Dox-induced Tet-On transcription system expressing Huntingtin fragments of different sizes revealed that aggregated fragments could be degraded by several Autophagy-inducing approaches

Growth factors and Cytokines (SW)

Definitions

- Cytokines: Molecules that regulate proliferation, differentiation, survival and other cellular functions at extremely low concentrations (includes "classical" growth factors)
- Growth factors: Usually small secreted peptides, produced by almost every cell, GF act on cells that have the appropriate receptor, can act in nanomolar to picomolar concentrations. Can act in a juxtacrine, paracrine, autocrine and endocrine mechanism. They regulate proliferation, differentiation, survival and migration. They are especially important during development, tissue repair and tumorigenesis. Cell. responses depend on receptors, cell-type and combination of ligand

Receptor tyrosine kinases and Growth factors



- Dimerization by ligand dimers (**PDGF**), proteoglycan associated ligands (bound via GAGs) (**FGF**), cell surface ligands (**ephrins**) OR activation of pre-formed dimer by conf. Change upon ligand binding (**EGF**)
- Dimerization leads to intrinsic (auto-)tyrosine phosphorylation and subsequent signalling
- Receptors can also form heterodimers, which bind a specific set of ligands (PDGF-, EGF, FGF-rec.)
- Mutation of one allele coding for a receptor has a dominant-negative effect (dimer formation!)

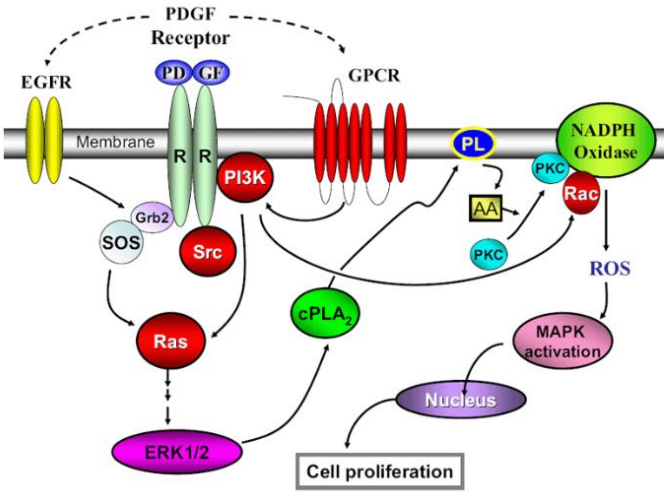
- Signal transduction:
  - Signalling molecules bind through SH2 or PTB domains; activation by
    - Tyrosine phosphorylation
    - Conf. Change upon binding
    - Recruitment to substrate location (membrane)
  - Phospholipase C-γ:
    - Activation by phosphorylation of SH2 domain
    - Cleaves PIP_2 to IP_3 and DAG
    - IP_3 releases Ca^{2+} from ER, which binds calmodulin → activates PKC (Ca^{2+} detection by cell permeable FURA-2 → red fluorescence upon binding)
  - Phosphatidylinositide-3-Kinase:
    - Binding of regulatory subunit → recruitment to substrate
    - Catalytic subunit phosphorylates PI's on 3. position (PTEN counteracts)
    - $PI(3,4,5)P_3$ binds PDK1, PKB (Akt)
    - Activation of Akt: Glycolysis, Glycogen production, prevents apoptosis

- Grb-2 and Ras-GEF:
  - Grb-2 adapter protein binds receptor via SH2 domain and recruits Ras-GEF
  - Production of Ras-GTP and activation MAP kinase signalling pathway after receptor endocytosis (via CME)
- C-Cbl:
  - E3 ubiquitin ligase, which catalyze monoub. of receptors
  - Monoub. receptor is targeted for lysosomal degradation

- Adaptation/Desensitation:
  - Delayed negative feedback due to
    - Receptor sequestration
    - Receptor downregulation
    - Receptor inactivation
    - Inactivation of signalling molecules
    - Production of inhibitory proteins

- Methods to analyze GF function:
  - Analysis of cell proliferation (incorporation of ^{3}H-thymidine, BrdU or cell counting)
  - Analysis of cell migration (scratch assay, Boyden Chamber / Transwell assay)
  - Analysis of cell survival (MTT reduction assay, TUNEL or cleaved caspase3 staining)

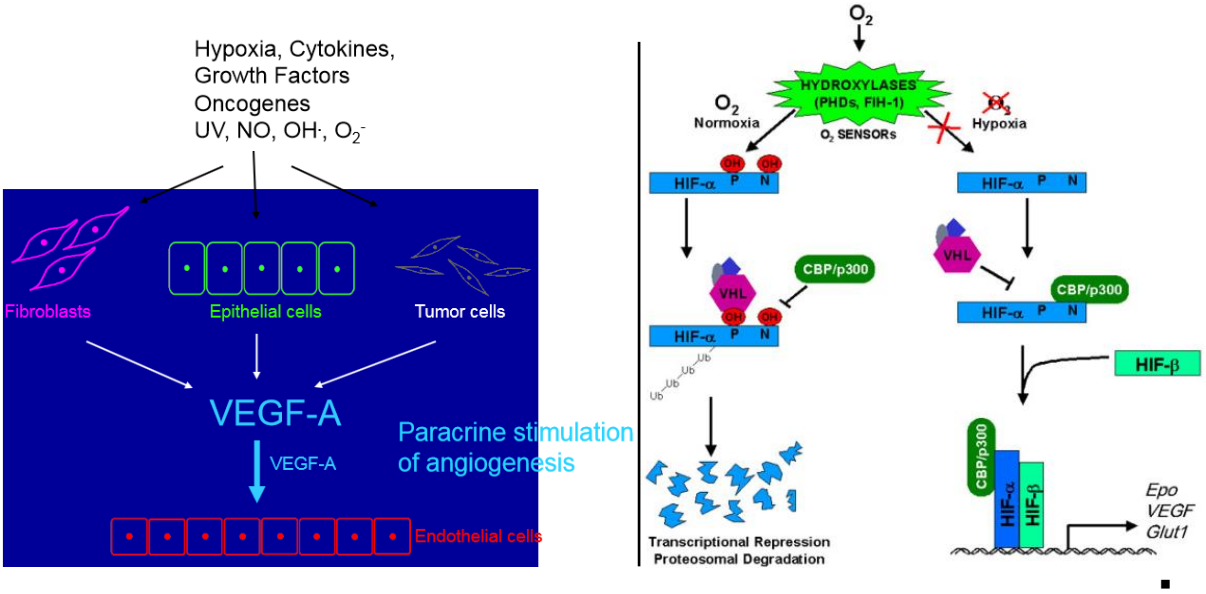
- **PDGF**s and their receptors:
  - Heterodimers of receptors and ligands possible
  - Functions of PDGF
    - Embryonic development (kidneys, blood vessels, lungs, CNS)
    - Stimulation of wound healing
    - Overexpressed in fibrotic diseases and many cancers
  - Signalling of PDGF
    - Multiple sites to activate MAP kinase pathway, Rho/Rac, Akt pathway
    - Special: Rac activates NADPH oxidase, which produces superoxide (O^{2-}). Superoxide gets then converted to hydrogen peroxide (H_2O_2), which acts as a signalling molecule



    - Intracellular ROS levels can be detected by DCF (gets fluorescent upon reaction) or HyPer (synth. Protein with an emission switch upon oxidation, specific for H_2O_2)
    - H_2O_2 secretion by wounded cells and tumor cells to attract immune cells!
    - ROS as signalling molecules, chemokines, damaging substances (against bacteria)
      → Cell need to balance concentration

- **EGF**s and their receptors:
  - Pre-formed Heterodimers of receptors possible, ligands are monomers
    - EGF receptor = HER1 = ErbB1
      - EGF, TGFα, Betacellulin, Heparin-binding EGF (HB-EGF), Amphiregulin, Epigen, Epiregulin
    - HER2 = ErbB2 (=NEU)
      - No ligands! Mainly for heterodimerization
    - HER3 = ErbB3
      - Heregulins 1 and 2
    - HER4 = ErbB4
      - Heregulins 1, 2, 3, Betacellulin, Epiregulin, HB-EGF
  - Ligands produced as membrane-anchored precursors, which can act in a juxtacrine manner or which are involved in cell-cell adhesion. Proteolytic cleavage leads to soluble factors, acting in a autocrine or paracrine manner
    (e.g. HB-EGF, which additionally can bind Diphteria Toxin with its EGF-domain)
  - EGF-Knock-out phenotypes (depend strongly on mouse strain!):
    - EGF receptor → survive until birth or later, defects in skin, intestine, pancreas, ...
    - HB-EGF → severe heart failure
    - EGF → no phenotype (BACK-UP system available)
    - TGFα → curly hair
    - Amphiregulin → underdeveloped mammary glands
    - EGF/TGFα/Amphiregulin → major mammary gland abnormalities
  - Pox viruses produce EGF-like growth factors:
    - Vaccinia Virus GF (VGF), Myxoma GF (MGF), Shope fibroma GF (SFGF)
    - Targets of various receptors with high affinity
    - Not required for viral replication, but stimulate cell proliferation at inf. Site
  - Signalling:
    - ErbB1, 3, 4 homodimers have weak or even no signalling (ErbB3 with dead kinase)
    - ErbB2 does not bind any Ligand but increases signalling by heterodimerization:
      - Stable complex → prolonged firing
      - Decreases Ligand dissociation rate
      - Decreases endocytosis rate
      - Relaxing ligand specificity
      - Target for fast recycling than degradation (as homodimers)
        (Heterodimers are unstable in endosomes, c-Cbl dissociates from complex)
  - EGFR signalling in Cancer:
    - HER2 overexpressed in 30% of all mammary carcinomas, this correlates with poor clinical prognosis (can be used as a marker), specially dangerous with pre-formed tumors during a hormone-therapy → faster cancer development.
      HER2_Overexpression also associated with increased malignancy and resistance to chemotherapy.
    - Therapy by antisense oligos, translation inhibitors (ribozymes), tyrosine kinase inhibitors or humanized monoclonal antibodies:
      - **HERCEPTIN** (Trastuzumab): Binds HER2 receptors, used for HER2-positive breast cancers; Flu-like symptoms and heart diseases as side-effects; early stage HER2-positive breast cancer treatment to avoid metastasis growth; paid by life insurance in switzerland
      - **Erbitux** (Cetuximab): Binds EGFR (overexpressed in colon cancer); often together with chemotherapy; Good success rate, well tolerated; skin rash and inflammation as side-effects (indicates that the patient responds)
      - **Iressa** (Gefitinib) and **Tarceva** (Erlotinib): Inhibitors of tyrosine kinase; used for locally advanced or metastatic lung cancer and for pantreatic cancer with EGFR mutations
    - Next generation drugs: Combination, which inhibit back-up pathways as well!

- **VEGF, Angiopoietins, Ephrins** and Angiogenesis:
  - Vasculogenesis: de novo formation of vessels, wound healing and carcinogenesis
  - Angiogenesis: sprouting from pre-existing vessels, during late development, wounds, etc.
    - Pathological angiogenesis: wound healing, heart ischemia, diabetic retinopathy, rheumatoid arthritis, cancer
    - The different steps:
      1. Angiogenic signal: inflammation, hypoxia
      2. Degradation of ECM
      3. Migration and proliferation of endothelial cells (Tip)
      4. Contact to ECM
      5. Lumen formation (spontaneous)
      6. Stabilization by pericyte and smooth muscle cell association
  - <u>VEGF</u> and their receptors:
    - Heterodimerization of receptors and ligands possible
    - 7 Ligands: VEGF=VEGF-A, VEGF-B, VEGF-C, VEGF-D, PLGF, VEGF-E and VEGF-F
    - 3 Receptors: VEGFR1 (blood vessel endothelial cells), VEGFR2 (blood vessel and lympthatic endothelial cells), VEGFR-3 (lympthatic endothelial cells)
    - NRP-1,2 as co-/helper-receptors increase sensitivity
    - VEGF-A function and regulation:
      - Induces migration, proliferation of endoth. cells (act as survival factor), involved in vasculogenesis and angiogenesis, can also induce lymph-angiogenesis via VEGFR-2
      - Expression induced by hypoxia (via HIF), cytokines, GFs, ROS, UV, oncogene

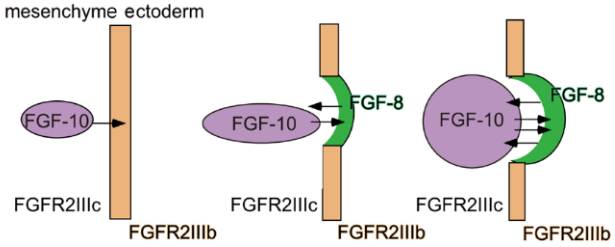


  - Lymphedema
    - Result from impaired lymphatic drainage (swollen extremities)
    - Congenital Lymphedema: VEGFR-3 mutation → reduced tyrosine kinase activity resulting in not enough lymph vessels
    - Acquired Lymphedema: Removal of lymph vessel without full recovery

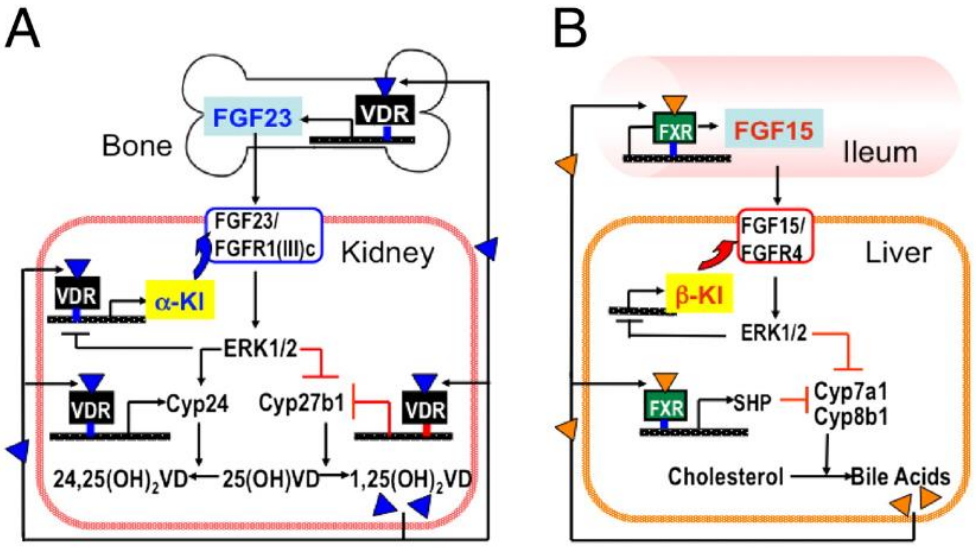
  - <u>Angiopoietins</u> and their receptors:
    - Bind to TIE-2 receptor; Agonists (Ang1,2,3,4) and Antagonists(Ang2,3) can bind to the same receptor; Act in concert with VEGF (e.g. stabilization of vessels); TIE-2 dimerises with TIE-1; important for vasculogenesis and angiogenesis
    - TIE-2/Ang1 → blood vessel stabilization by association of pericytes and sm cells (attraction by PDGF, TGF and secr. of Ang1 → double paracrine loop)
    - TIE-2/Ang2 → blood vessel regression and remodelling (in absence of VEGF)

- Ephrins and their receptors:
  - 14 Eph receptors and 8 ephrin in mammals
  - Eph receptors dimerize like other receptor tyrosine kinases
  - Ephrins are transmembrane proteins: juxtracrine mechanism, bidirectional signalling: EphrinA (GPI-anchor) and EphrinB (cytoplasmic tail) can signal upon receptor binding as well! Requires cell-cell contact.
  - Do not stimulate proliferation but regulate migration, cell-cell and cell-matrix cont.

- **FGF**s and their receptors:
  - Ligands: FGF1-23, mainly heparan sulfate binding proteins
    → **BUT: FGF 7, 19, 21, 23 act in a endocrine manner! (Need co-receptor Klotho)**
  - Receptors: FGFR1-4, but with different isoforms due to alternative splicing and polyadenylation, which have different ligand binding specificities
  - FGF5 is a negative regulator of hair growth
  - FGF10 responsible for limb development involving a double-paracrine loop:



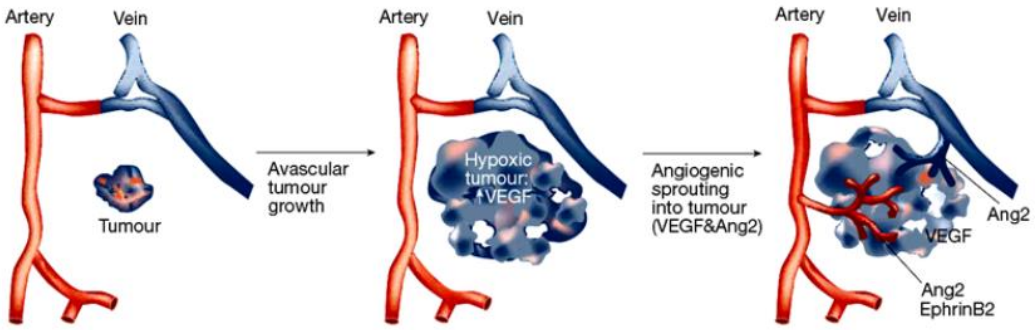
  - FGF7, FGF10, FGF22 stimulate keratinocyte proliferation and migration in wounds
  - FGFR1, FGFR2 are essential for hair regeneration and tight-junction formation in the epidermal barrier by expression of components (Claudin, Occludin, ...)
    - KO: impaired epidermal barrier function leads to skin dryness and inflammation, which causes a double paracrine loop! → Consequence: Inflammatory/hyperproliferative skin disease resembling Atopic Dermatitis
  - FGFR1, (FGFR2), FGFR3 affect skeletal development
    - Gain-of function mutations e.g. in FGFR3 lead to Achondroplasia (small bones)
  - **FGF23** regulates vitamin D metabolism and phosphate homeostasis in kidney **(A)**
    - Binding and rec. Activation needs co-receptor Klotho (excl. On kidney cells)
  - **FGF19** *(FGF15 in mice)* regulates bile acid synthesis and gall bladder filling **(B)**
  - **FGF21** regulates response to fasting by signalling to adipose tissue and brain

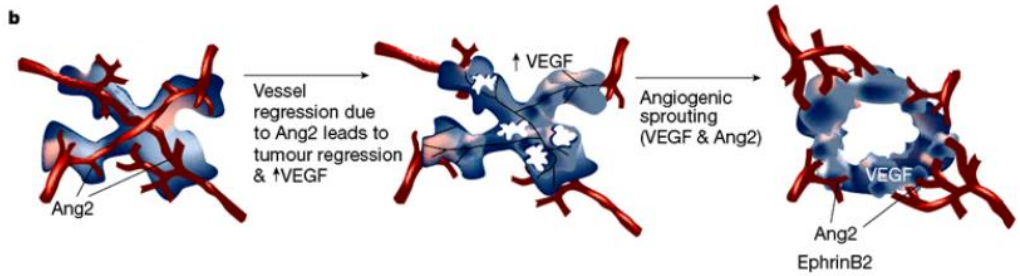


  - **FGF7 (= KGF)** acts as a cyto-protective factor
    - Good additional cancer-treatment to protect tissue from damages caused by chemotherapy and/or irradiation, ROS, etc. (test oxidative damage to proteins by OxyBlot assay)

Tumor Angiogenesis

- Tumors can only grow to a diameter of 0.2-0.4 mm → then angiogenic switch needed
- Tumor lives in a microenvironment with stromal cells (fibroblast, vessel cells, immune cells, etc.), which have been reprogrammed (epigenetically?) and support the tumor cells
- VEGF and Angiopoietin-2:
  - VEGF (see above) strongly upregulated; induces survival, migration and proliferation of endothelial and tumor cells, sprouting of new vessels; recruits endothelial progenitor cells from bone marrow (vasculogenesis); recruits inflammatory cells, stimul. tumor (autocrine)





- Anti-angiogenic tumor therapy:
  - Synthetic Inhibitors: Metalloprotease inhibitors, TNF (homologue of the antibiotic fumagillin), thalidomide
  - Endogenous inhibitors: Ang2, angiostatin, endostatin, IL-12, interferon-α
    - Endostatin: fragment of a collagen, inhibits angiogenesis and tumor growth, broad spectrum, very expensive
  - Biol. Antagonists: VEGF antibodies (Avastin), VEGF antisense RNA or ribozymes, VEGF receptor tyrosine kinase inhibitors
    - Avastin: hum. Monocl. Antibody against VEGF; for metastatic colon, rectal, lung and breast cancer; Bleeding, holes in colon, bad wound-healing as side-effects → VEGF inhibition alone may increase vessel maturation and tumor growth (by compensating mechanisms). Combination of VEGF and PDGF inhibition better!
    - SUTENT (Sunitinib): VEGFR1-3 and PDGF receptor tyrosine kinase Inhibitor; for breast cancer and neuroendrocrine tumors
    - Nexavar (Sorafenib): VEGFR2, PDGF receptor, Raf tyrosine kinase Inhibitor; for advanced kidney cancer
    - MACUGEN: anti-VEGF aptamer injected into eye against angiogenesis, macula deg.
    - RANIBIZUMAB: hum. Antibody fragment against VEGF; very expensive; macula deg.
  - Soluble VEGF receptors, soluble TIE-2 (as a ligand trap)

Tumor Lymphangiogenesis

- Tumors spread mainly via lymphatic vessels
- VEGF-C Overexpression → increased metastasis rate
- Inhibition of VEGF-C, VEGF-D or VEGFR3 suppress tumor and metastasis formation

Therapeutic Angiogenesis

- Use of GF or gene transfer encoding GF can treat coronary and peripheral vascular diseases
  - Virus mediated gene therapy
  - Direct application of GF (unstable, rapidly degraded)
  - Isolate cells of patient, Overexpression of GF and reinjection into affected tissue

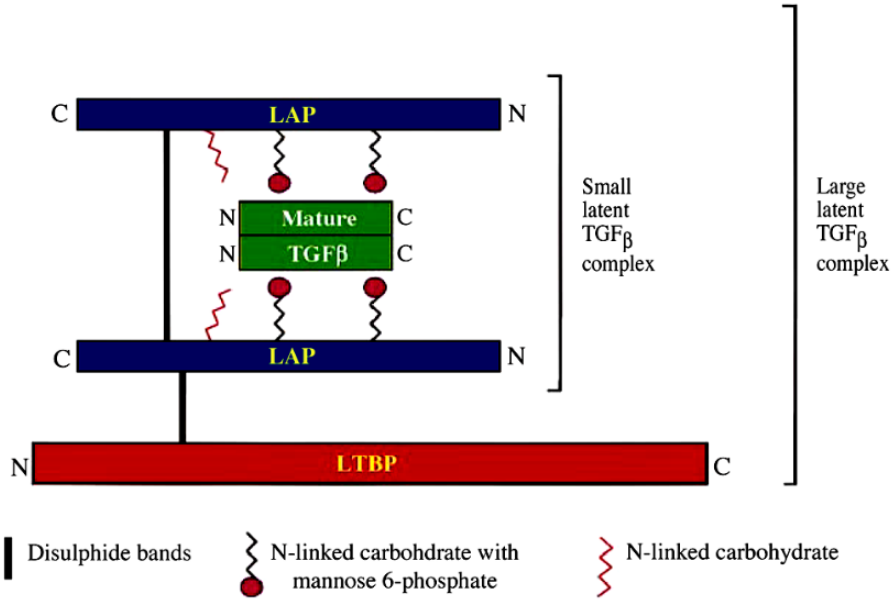
Angiogenesis Assays

- In vitro tube formation assay on Matrigel:
  - Coat wells with Matrigel (laminin, collagen IV, proteglycans, etc. → basement membrane), seed with endoth. cells, add factors, count number of formed capillary tubes
- Aortic ring assay:
  - Remove aorta of rats and cut into 1mm rings, embed rings in collagen polymerized agarose wells, add culture medium + factors, examine sprouting by phase contrast LM after 7-9 d → very reproductive, but one has to sacrifice a rat
- In vivo Matrigel plug assay:
  - Mix Matrigel and factors, inject cold liquid subcutaneously into mice → solidifies, count formed blood vessels in plug after several days. One can also add the factors later by insertion of a sponge into the plug, monitor new vessels by injection of FITC-dextran
- Chorioallantoic membrane (CAM) assay:
  - Incubate chicken egg, open window, place factors onto CAM, reseal and incubate, investigate vascularisation after some days
- Rabbit/Mouse Cornea assay:
  - Corne is avascular. Make a pocket into cornea, introduce factors by a sponge, monitor vascularisation with stereo-microscope or FITC-dextran

Other Receptors and Growth Factors

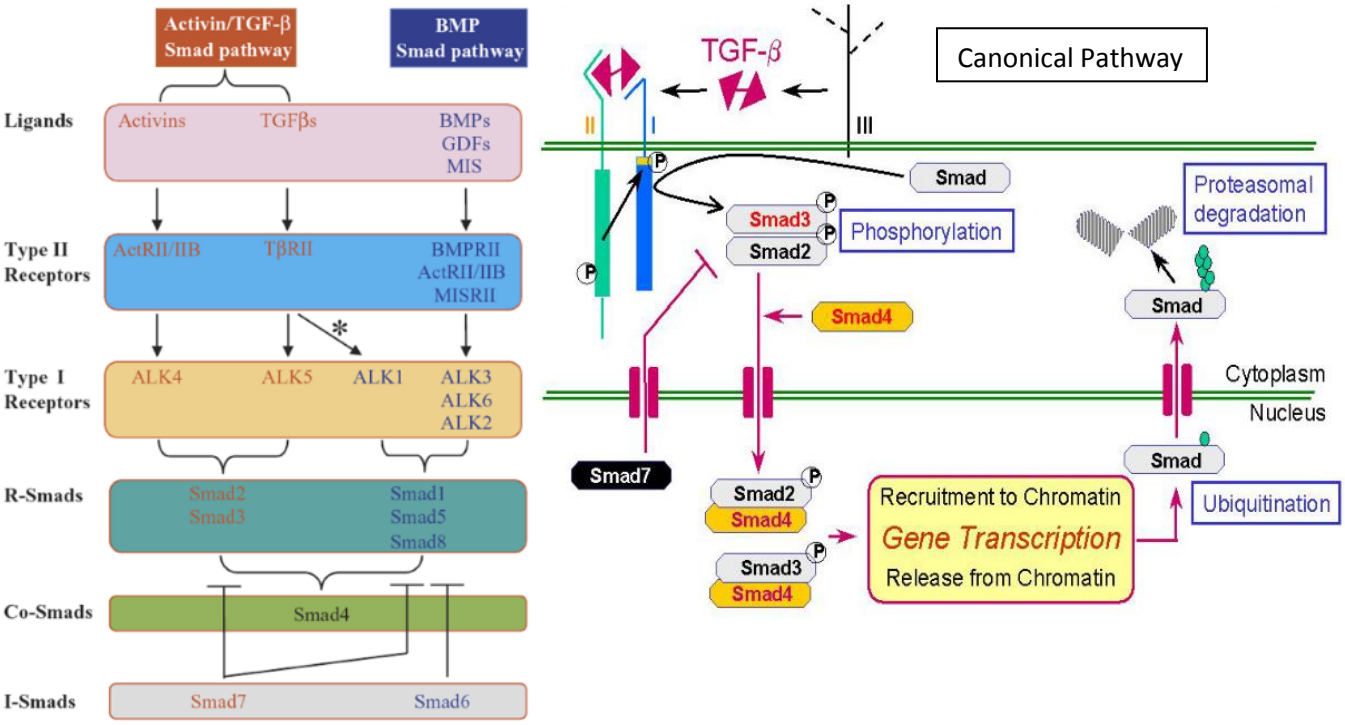
- **TGF-β Superfamily** and their receptors:
  - Include TGF-β 1-3, activins, inhibins, bone morphogenic proteins (BMPs), nodal, growth and differenciation factor (GDFs) and others

  - TGF-β 1-3
    - Produced as inactive precursor homodimer, associated with glycosylated LAP (latency associated peptide), or even additionally with LTBP (Latent TGF-β binding protein) for storage



    - Can be activated by:
      Proteases, low pH, ROS, binding of LAP to Mannose-6-phosphate receptors or integrin, mechanical tension

- TGF-β receptors:
  - Type III: TM protein with short cytoplasmic domain and no enzymatic activity, but large extracell. domain with many carbohydrate residues, promotes binding of the ligand to the signalling receptors
  - Type I and II: TM proteins with small extracellular domain, intracellular serine-threonine kinase domain, Dimerization of receptor dimers → tetramer;
    Type II receptor phosphorylates type I, which transduces signal
- Smad-Proteins:
  - Receptor Smads: Smad2 and Smad3 bind to TGF-β and activin receptors; Smad1, Smad5 and Smad8 bind to BMP receptors
  - Nuclear Smad: Smad4 bind receptor Smads
  - Smad6 and Smad7 are inhibitory Smads
- Signal Transduction:



  - Additional signalling pathways like Akt-pathway and MAP kinase pathway may play a role via proteins, which are recruited to the activated receptors (non-canonical)
- TGF-β family Inhibitors:
  - Decorin, biglycane: proteoglycans of the ECM that bind activated TGF-β
  - Noggin, chordin, Cerberus, DAN, gremlin: soluble proteins that bind BMPs
  - Follistatin, follistatin-like proteins: soluble glycoproteins that bin activin
  - Ski and Sno: Bind receptor Smads and Smad4 and block gene expression
- Biological functions of TGF-βs:
  - Inhibit proliferation of most cell types (mutations e.g. Smad4 deletion found in several epithelial cancers), exception: Proliferation in fibroblast by TGF-βs
  - Inhibition of autoimmune processes and inflammation (TGF-β1)
  - Stimulation of cell migration in palate development (TGF-β3)
  - Induction of migration and proliferation of fibroblast and ECM production
  - Promotes differentiation of fibroblasts into myofibroblasts (Overexpression leads to fibrotic diseases with hypertrophic scars and keloids, etc.)
- TGF-β in Cancers:
  - **Changes from tumor suppression to tumor promoting function** by induction of fibroblast proliferation, change of Macrophage polarization to degrade ECM for better proliferation and migration, inhibition of immune cells and induction of VEGF expression for angiogenesis!

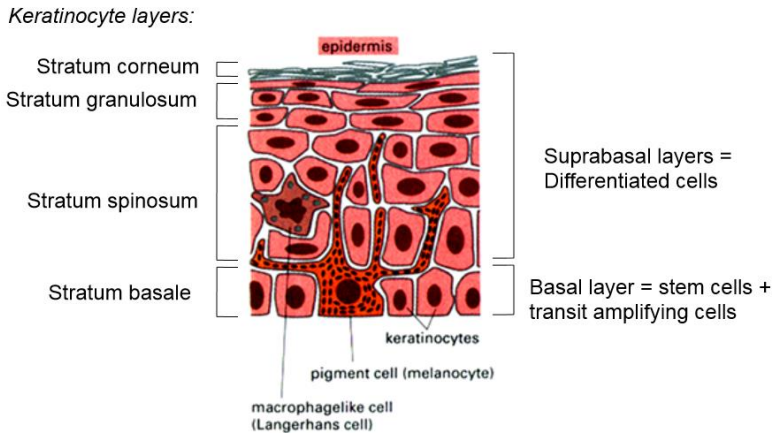
- Activins
  - Can form hetero- or homodimers of two β-chains (i.e. $\beta_A\beta_A$ = Activin A)
  - Acts via two different receptors (type I and II) with diff. splicing forms
  - Biologically active activin is secreted from keratinocytes during wound-healing
  - Activin is a novel tumor-promoting factor:
    - Inhibits proliferation of γδ T cells (suppress skin carcinogenesis)
    - Increases number of αβ T cells (promotes carcinogenesis) by chemokines
    - Activin Overexpression relevant for human squamous cell carcinoma (SCC)
  - ActRIIB or ActRIIA antagonists are in clinical trial for:
    - Myeloma-induced bone loss or bone loss in other cancers and osteoporosis
    - Chemotherapy-induced anemia
    - Cancer-induced muscle wasting and cachexia, muscular dystrophy

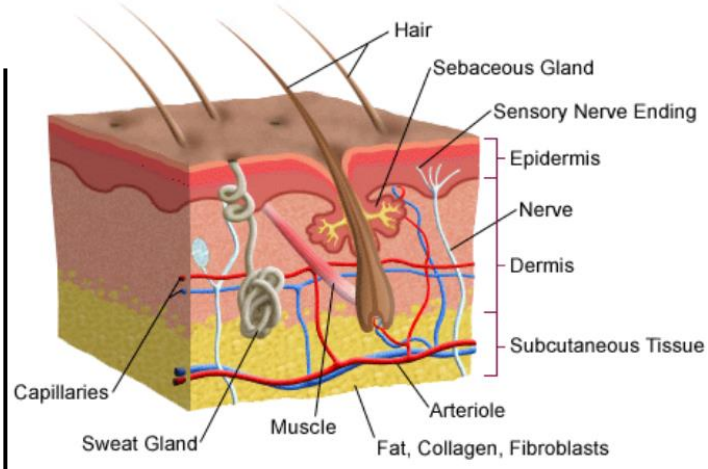
    => Also like TGF-β: Activin may also be a tumor suppressor for specific cell types! Careful application needed!

Cutaneous Wound Healing (MS)

Introduction

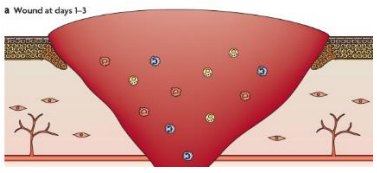
- Important **medical problem** (chronic wounds/ulcers, hypertrophic scars and keloids); Partial **recapitulation of developmental processes**; Important for **cancer research** ("Tumors are wounds that overheal")
- The mammalian skin and its functions:
  - Protection from UV, toxic, chemicals, pathogens
  - Regulation of temperature
  - Barrier against water loss
  - Sensory organ





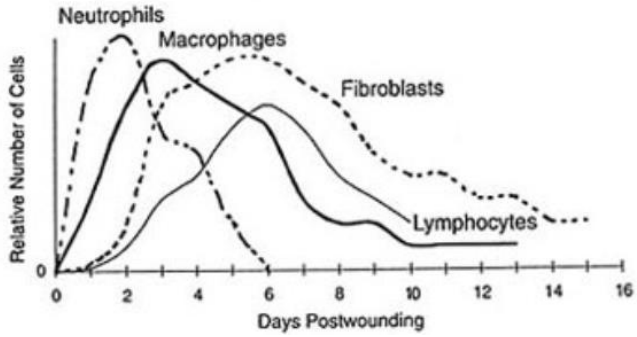
The 3 Phases of Wound Healing

- Inflammation Phase
  - 1. Blood clot formation:
    - Damage of blood vessels → platelets/thrombocytes bind to interstitial connective tissue
    - Aggregation of platelets → degranulation → release of GFs and chemotactic factors for neutrophils and macrophages as well as resident cells
    - Blood coagulation → formation of a fibrin clot → plugs the wound → serves as a provisional matrix



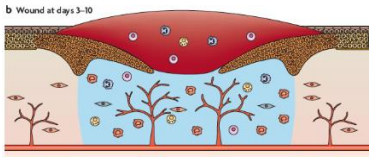
=> Protection of wound tissue (against invaders, water loss), Matrix for migrating cells, Reservoir of cytokines and GFs (recruit inflammatory cells,initiate reepithelialisation and formation of granulation tissue, connective tissue contraction).

- 2. Invasion of immune cells:



- Invasion facilitated by vasodilation (by mast cell histamines), vascular permeability
- Neutrophils and monocytes migrate concurrently into the wound
- Neutrophils arrive first due to their higher abundance in the circulation
- Neutrophils destroy bacteria (by enzymes, ROS); secrete pro-inflammatory cytokines (IL1α, IL1β, TNFα)→activation of macrophages, fibroblasts, keratinocytes; get entrapped in blood clot (neutrophils infiltration ceases within few days), become senescent, phagocytosed by macrophages
- Macrophages have antimicrobial functions (phagocytosis, NO, ROS); promote angiogenesis (by GFs bFGF, VEGF and cytokines TNFα); recruit and activate cells (GF TGFβ, PDGF, EGF, IGF and cytokines TNFα, IL1, IL6); Wound debridement (phagocytosis, enzymes collagenase, elastase); synthesise matrix (GF TGFβ, EGF, PDGF, cytokines TNFα, IL1, IFN-γ, enzymes collagenase, elastase)

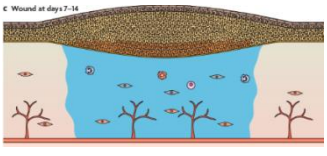
- Proliferation Phase
  - 1. Re-epithelialisation:
    - Keratinocyte (stem cells) migration and proliferation stimulated by
      - "Free edge" effect (missing neighbouring cells)
      - High concentration of EGF family members (EGF, HB-EGF, TGFα), HGF, KGF



    - Keratinocytes are connected to ECM laminin via receptors and interconnected via desmosomes → binding of ECM components fibronectin, tenascin, vitronectin, collagen I to the site of missing neighbour →desomosome degradation and actin reorganization → migration and secretion of gelatinaseA, collagenase1 and 3, stromelysin1, plasminogen activator
  - 2. Formation of Granulation tissue:
    - PDGF, TGFβ in blood clot → activation of fibroblasts → proliferation and migration → production of ECM (fibronectin, laminins, tenascin-C), collagen production → large collagen production → gradual replacement of provisional matrix by collagenous matrix → matrix remodelling begins
    - Wound contraction by formation of myofibroblasts by TGFβ and tension (can be stained by antibodies against α-smooth-muscle actin)
    - Neovascularization by bFGF, VEGF → proliferation and migration of endothelial cells (tube formation); secretion of plasminogen activator, MMP, procollagenase; deposition of own ECM, formation of new basement membrane around vessels

- Remodelling Phase

  Remodelling = transition from granulation tissue to mature scar



    - Apoptosis of fibroblasts/myofibroblasts
    - Regression of capillaries
    - Collagen remodelling (synthesis by fibroblasts; catabolism (hard regulated); MMPs and their inhibitors; formation of collagen bundles; alterations of intermolecular cross-links)
  - 1. Epithelium → Epidermis
  - 2. Granulation tissue → Dermis

- Outcome of the wound healing process:
  - Healed skin neither aesthetically nor functionally perfect
  - Loss of skin appendages (hair follicles, sweat glands, sebaceous glands →thermoregulation)
  - Reduced tensile strength
    - First 3 weeks: 20% of final tensile strength
    - Mature scar: 70% of tensile strength of uninjured skin

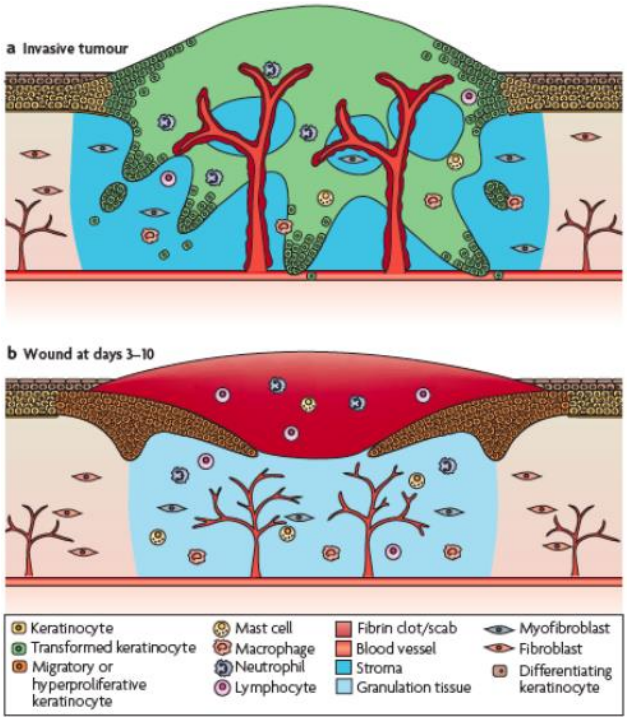
Fetal Wound Healing

- Fast reepithelialisation
- No myofibroblasts but actin cables
- Low inflammation
- No scarring (until third semester)
- Low levels of TGFβ, high levels of MMPs

Mice as a model organism for wound healing

- Incisional wounds and excisional wounds (e.g. full-thickness wounds)
- Require: Animal facilities, training, application and permission, regular reports
- Control mechanisms: written application, control of reports, unannounced lab controls
- Experiment: anaesthesia, including pain control, shaving, surgery, daily controls

Parallels between wound healing and cancer

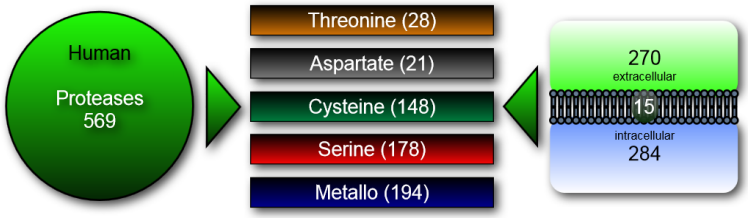


	Wound	Cancer
Fibrin matrix	**Blood clot formation** (damaged blood vessels)	**Chronic fibrin deposition** (hyperpermeability of vessels)
Inflammation	**transient**	**persistent** → protumorigenic → stimulates angiogenesis + ECM breakdown → enhances cancer cell motility + invasion → promotes malignancy (ROS, NOS)
Epithelial Proliferation + Migration	**transient**	**persistent**
Epithelial-mesenchymal transition (EMT)	**partial** → Remaining intercellular junctions + keratin expression	**complete** (metastasis) → Loss of cell-cell contacts → Fibroblast like morphology → Expression mesenchymal markers
	Stimulation: HGF, TGFβ, TNFα, MMPs, *only tumor:* Ras mutations	
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
	Stimulation: VEGFA, PLGF, FGF2 and others Inhibition: TSP1, IP10	

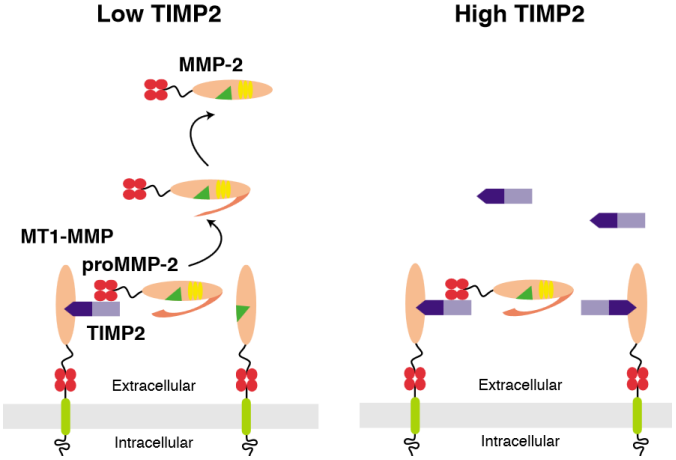
Proteases (UADK)

Introduction

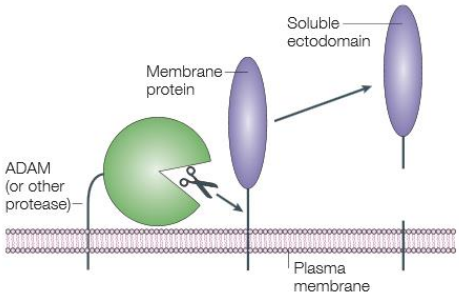
- Overview:

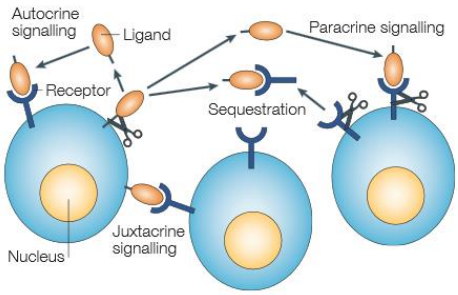


- Biological Consequences:
  - **Proteolytic Processing (precise)**
    - Removal initiator methionine, signal- and propeptides
      (protein maturation, stability, localization, zymogen activation by autoproteolysis or by other proteases)
      → Proteolytic systems form independent activation cascades
      → Zymogen activation tightly controlled
      Examples:
      - MMP-2 activation by MT1-MMP and its inhibitor TIMP2



    - Domain shedding
      (ligand release, receptor processing, trans-signalling)
      → Active GFs are shed from membrane bound precursors (change from juxtacrine to a paracrine, endocrine and/or autocrine mechanism)
      → Released ligands can be sequestered by shed receptors





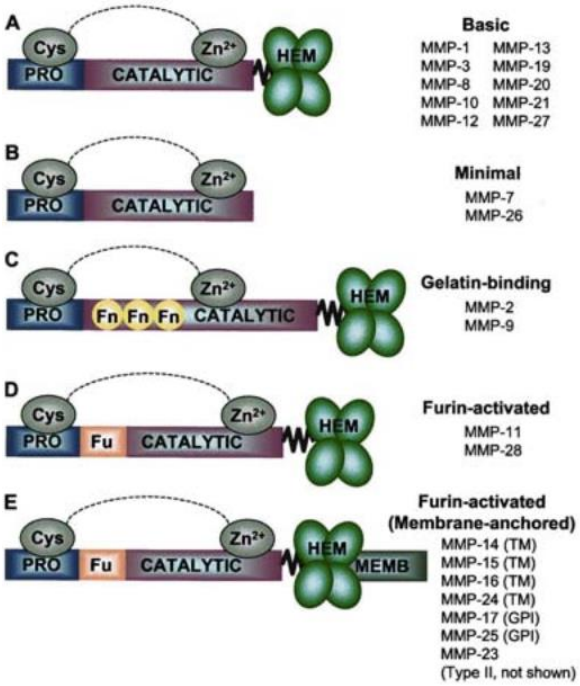
      Examples:
      - Shedding of TNF-α by TACE (ADAM17)
      - Shedding of EGF ligands and receptors by ADAMs upon activation by e.g. GPCRs. (cytoplasmic part of shed EGF ligand can enhance gene transcription and receptor shedding can lead to constitutive signalling!)
      - ADAMs release soluble IL-6 receptor fragment (sIL-6R), which can form a complex with IL-6 and can then bind to a receptor gp130

- Other precise cleavages
  (altered receptor binding upon ligand cleavage, control of growth factor and cytokine bioavailability, release from precursors)

- **Degradation**
  - Digestion of food (energy source)
  - Proteasome system (elimination of proteins, quality control)

Matrix Metalloproteinases (MMPs)

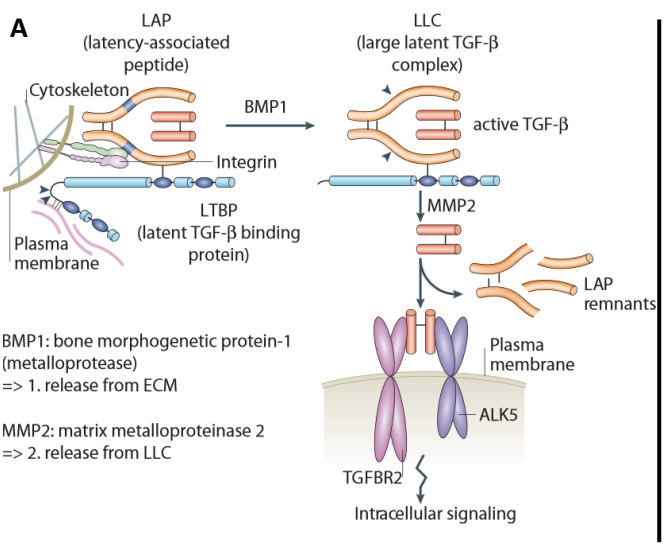
- Overview:

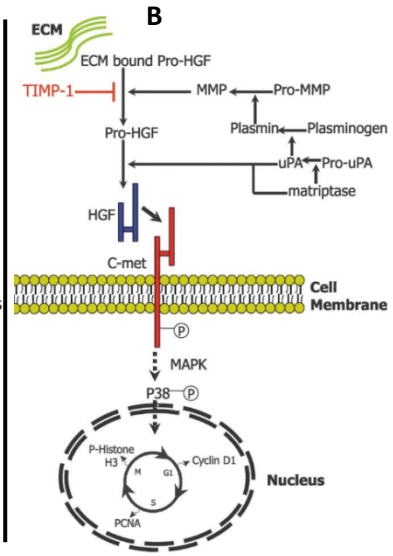


- Zn binding extracellular endopeptidases
- Highly expressed in wound healing and cancer
- Historically thought to predominantly degrade ECM

- HEM = Hemopexin domain important for specificity
- Fn = Fibronectin domains
- Fu = Furin activation domain

- Functions of MMPs in response to injury
  - MMPs important for metastasis and primary tumor growth in cancer
    → All trials to inhibit them failed, because of high inflammation rate!
  - Many different MMPs are expressed by all cell types in cancer and wounds (in cancer MMPs mainly by the stroma cells)
  - Modes of MMP action:
    - Degradation of ECM (in front of migrating cell)
    - Release of cryptic GFs from ECM molecules
    - Degradation of extracellular junctions (Desomosomes,...) and basement membrane
    - Activation of latent signals (shedding away of inhibitor domain)
    - Regulation of active signals (by degradation, ...)
  - Examples:
    - Release of angiogenesis inhibitors from collagens (endostatin, arrestin, etc.)
    - MMPs mediated basement membrane breakage during EMT upon soluble E-Cadherin (cleaved by activated cathepsin of a tumor cell)
    - Release of TGF-β by BMP1 and MMP2 (**A**)
    - Proteolytic cascade in liver regeneration with help of matriptases (Matrix-Spanning-Serine proteases) (**B**)
    - Release of active IGF from IGFBP to promote proliferation
    - Release of VEGF from HARP (heparin affin regulatory peptide) and CTGF (connective tissue growth factor) to promote angiogenesis
    - MMPs as modulators of chemokine signalling control influx and clearance of neutrophils and macrophages at sites of injury by chemokine processing (inhibition of MMPs → strong inflammation!)





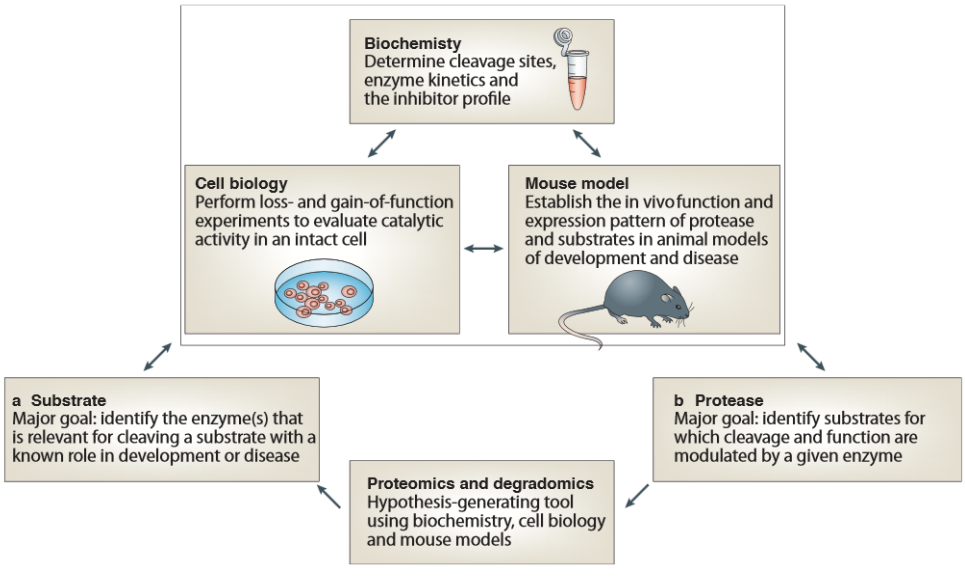
- MMPs release pro-HGF from the ECM
- urokinase plasminogen activator (uPA) or matriptase cleave and activate pro-HGF
- matriptase activates pro-uPA
- uPA activates plasminogen
- plasmin activates pro-MMPs

Mohammed et al

- MMP functions in wound healing:
  1. MMP7 sheds syndecan-1 (ED) → release of CXCL8 → immune cell recruitment
  2. MMP7 cleaves E-Cadherin (ED) → cell migration
  3. MMP7 sheds FASL (ED) → apoptosis → innate defense
  4. MMP9 cleaves inhibitor of neutrophils elastase (ED) → antimicrobial activity
  5. ADAM17, MMP7, MMP12 shed TNF (Dermis) → proinflammatory
  6. MMPs modulate chemokine activity (Dermis)
- MMP functions in cancer progression:
  - Tissue invasion and intravasation
  - Angiogenesis
  - Regulation of inflammation
  - Metastatic niche (form tumor-supporting stroma)

  → Many MMPs as targets and anti-targets in cancer therapy

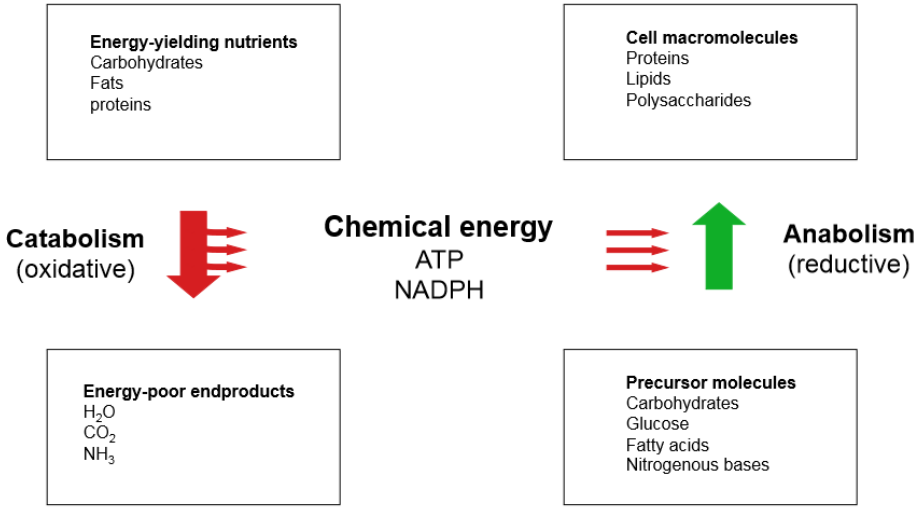
- MMP analyzing methods:
  - SDS-PAGE to see pro- and activated form (Zymogram)
  - Detection of proteolysis *in situ* with LM
  - Check protease substrates for cleavage by SDS-Page or Difference Gel Electrophoresis (DIGE):
    1. Two proteome samples one with and one without protease
    2. Samples labelled with fluorescent dyes (Cys3, Cys5)
    3. Pool samples and analyze by 2D-SDS-PAGE
    4. Unchanged proteins appear yellow and protease affected green or red
    5. Spots analyzed by MS/MS
  - Enrichment of protein N-termini (N-terminus and neo-N-terminus) and terminal amine isotopic labelling of substrates (TAILS) with iTRAQ labelling of NH_2 and MS/MS analysis



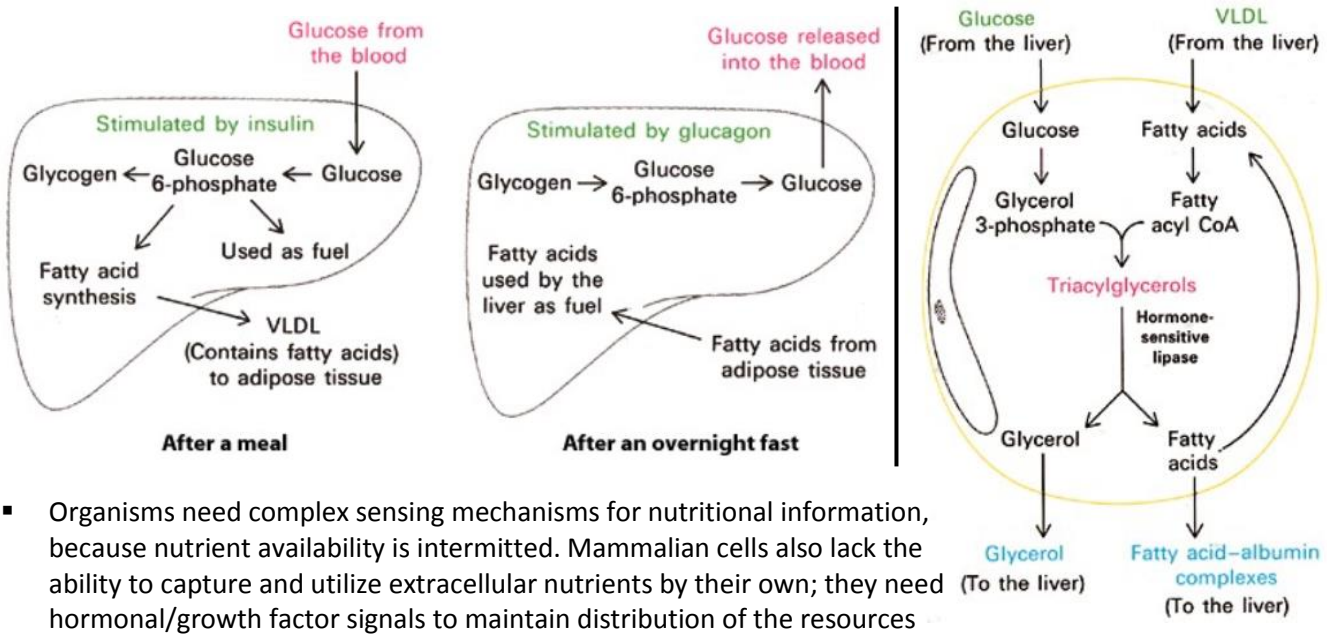
Metabolism: Signaling and sensing mechanisms (WKREK)

Introduction

- Metabolism is a broad term that includes all the chemical reactions that occur in the body
- Metabolism provides energy and basic components to support life-long homeostasis of cells and organsisms
- Metabolism consists of catabolism and anabolism
  - Catabolism is the degradation pathways to salvage components and energy from biomolecules such as proteins, lipids and polysacccharides. The processes generate energy.
  - Anabolism is the biosynthesis of biomolecules such as proteins, lipids and polysaccharides from simple precursor components. These processes require energy.



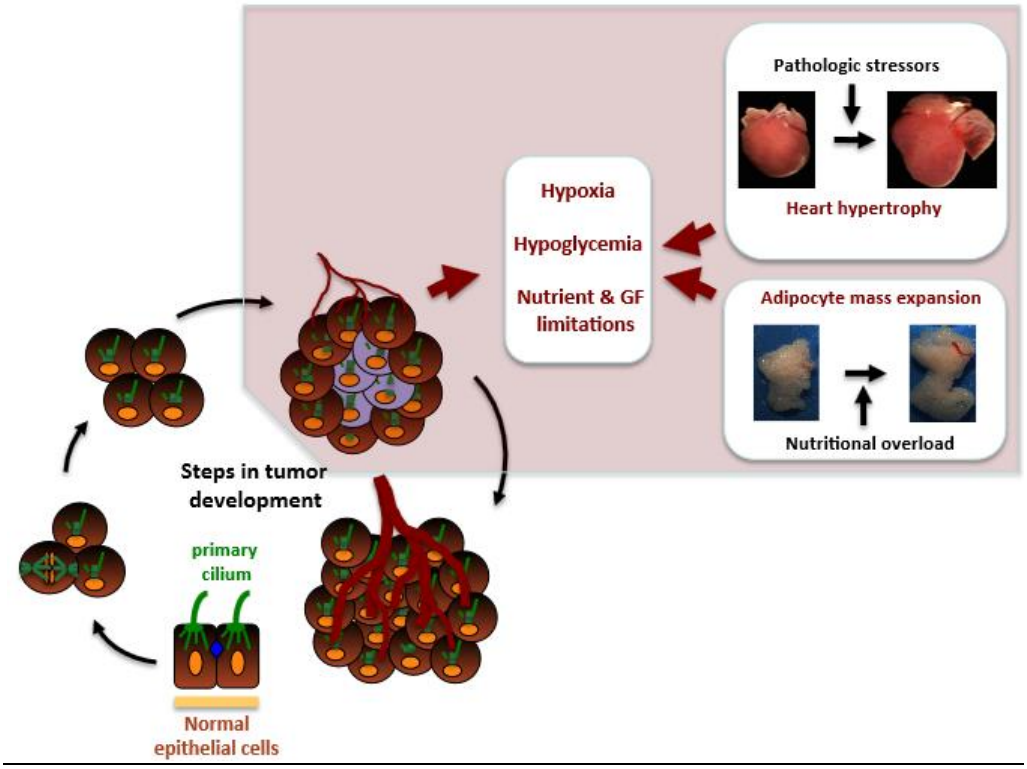
- Different organs or same organs at different time points have a different metabolism!
- Blood Glucose level control by liver and lipid storage in adipocytes:



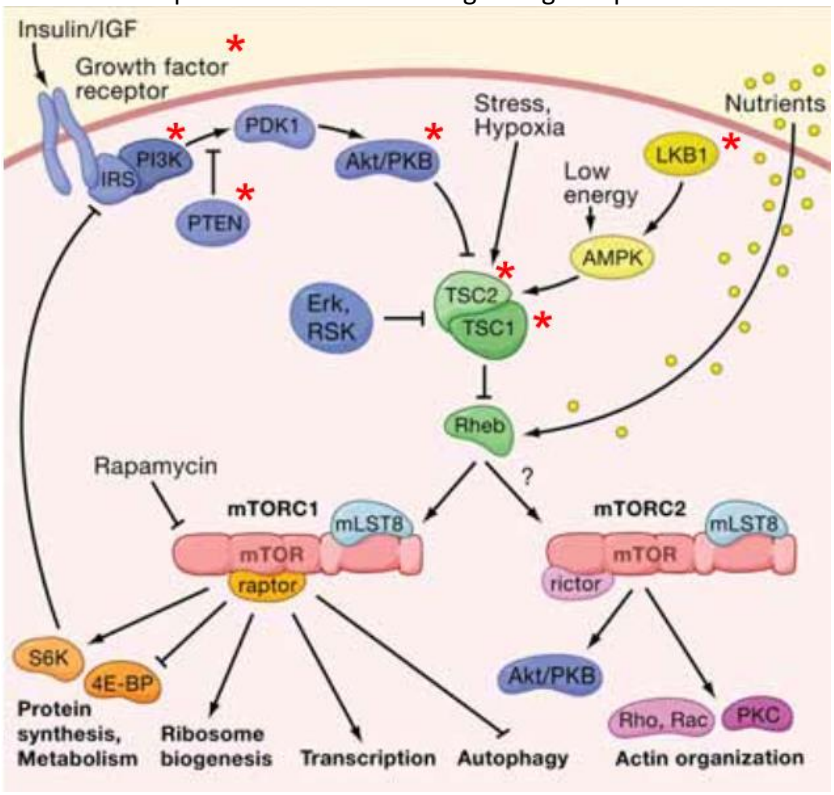
- Organisms need complex sensing mechanisms for nutritional information, because nutrient availability is intermitted. Mammalian cells also lack the ability to capture and utilize extracellular nutrients by their own; they need hormonal/growth factor signals to maintain distribution of the resources among the whole organism, cell growth, proliferation, survival and repair.

- Change from **traditional demand model** (GF→transcription→metabolism to cover energy demand) to a **supply-based model** (GF → metabolism→ transcription to adapt to new metabolism)

Metabolic reprogramming in physiology and pathophysiology



- Integration of nutrient- and hormonal/growth factor signalling: **The mTOR Pathway**
  - Rapamycin: Inhibitor of TOR/mTOR, anti-fungal macrolide antibiotic, Immunosuppressant, anti-cancer (derivatives in clinical trials)
  - TOR/mTOR is a member of the PI3K-related protein kinases
  - mTOR is a component of two distinct signalling complexes: mTORC1 and mTORC2:

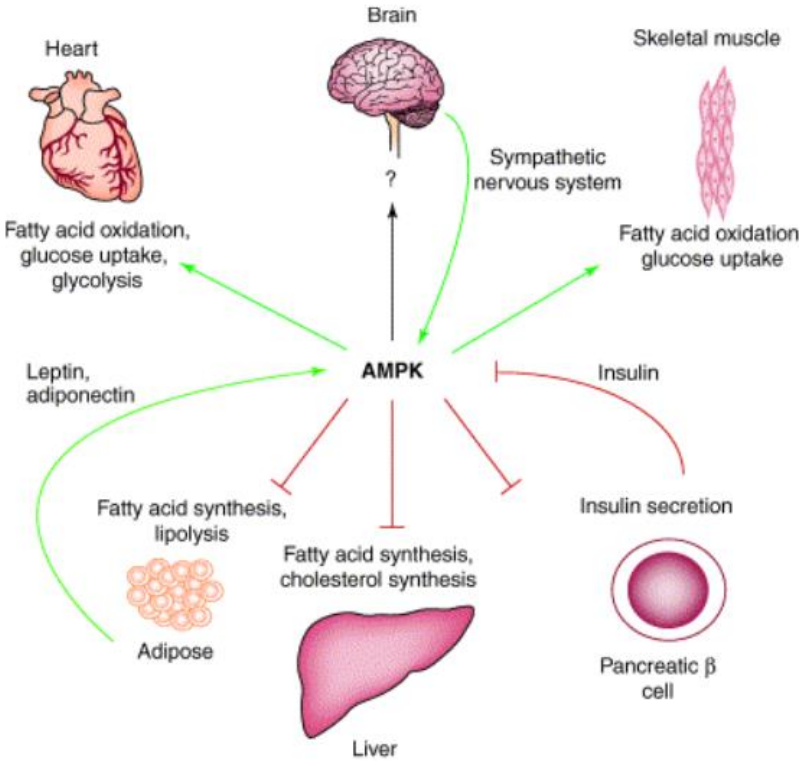


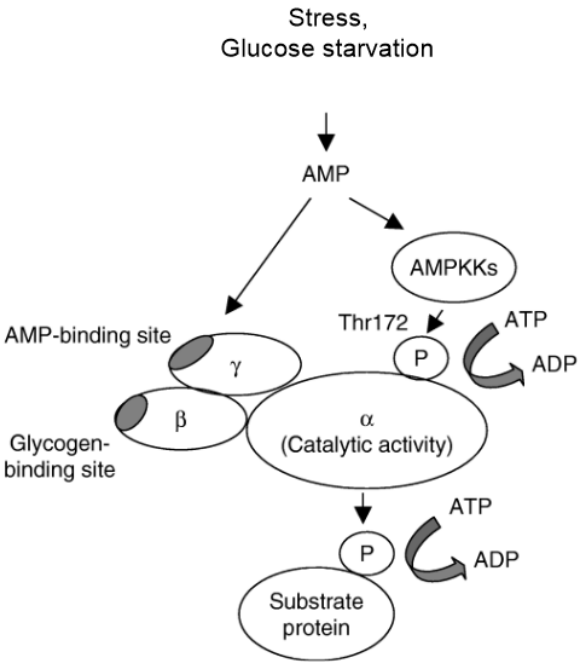
  - Rapamycin can inhibit mTORC1 by binding to the FRB domain of mTOR with FKBP12
  - Raptor and rictor both bind to HEAT repeats of mTOR

- Energy sensing mechanisms: **The AMPK Pathway**
  - Glucose metabolism and sensing mechanisms:
    - Functions of Glucose: Fuel for energy, substrate for biosynthesis, signalling molecule and regulator of transcription
    - Metabolic fate of glucose strongly depends on tissue and metabolic state (Glucose, Glycogen, Pyruvate, TCA-cycle)
    - Glucose sensing in pancreatic β-cells:
      - Glucokinase (GK) acts as a glucose sensor by direct proportionality between blood-glucose levels and formation of G-6-P
      - GLUT2 and GK are essential to sense changes in glucose metabolism
      - Insulin secretion AND production are coupled to glucose metabolism
      - Low glucose activates PDX1 transcription factor → Insulin expression
      - MODY2 (type of non-insulin-dependent diabetes):Mutation in 1 allele of GK
    - Hexokinases HK I and HK II couple intra- and extra-mitochondrial metabolism:
      - Associate with VDAC (ATP Transporter) on Mt and use ATP to convert glucose into G-6-P
      - G-6-P force HK I/II to dissociate from VDAC (negative feed-back)
      - GFs promote association via PKB for metabolism and cell survival (cancer!)
  - Intracellular energy sensing mechanisms:
    - AMP:ATP ratio is observed by AMPK (high → AMPK activation)
    - Interconverting reactions:

ATPases	ATP → ADP + Pi
Ligases	ATP → AMP + PPi
ATP synthases	ADP + Pi → ATP
Adenylate kinase	2ADP ↔ ATP + AMP

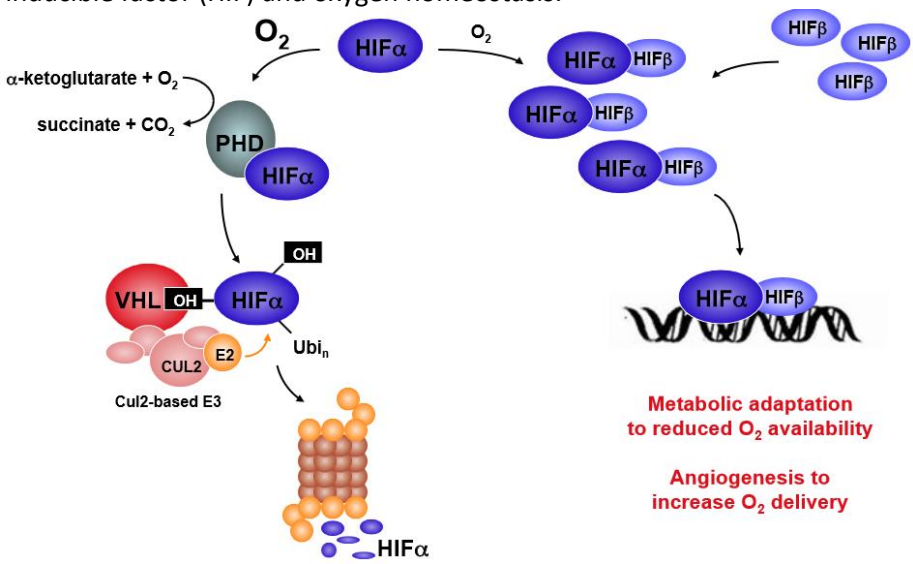
    - AMPK activation mechanism:





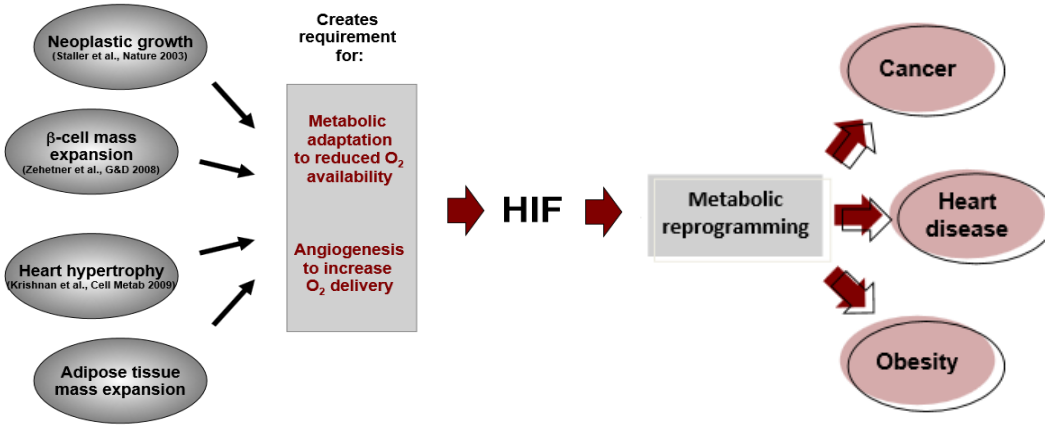
    - Effects of adipokines on AMPK strongly depends on the tissue:
      - Ghrelin in *Hypothalamus* activates AMPK → Food Intake
      - Leptin in *Hypothalamus* inhibits AMPK
      - Leptin/Adiponectin in *Skeletal Muscle* activates AMPK → FAO
      - Adiponectin in *Liver* activates AMPK → FAO, Gluconeogenesis
      - Resistin in *Liver* inhibits AMPK

- Oxygen sensing and metabolism: **The HIF Pathway**
  - Differentiated cells make oxidative phosphorylation (38 ATP) or anaerobic glycolysis (2 ATP)
  - Proliferating cells (cancer cells) do aerobic glycolysis (4 ATP) although there is oxygen! (**Warburg-Effect**)
  - Oxygenated atmosphere allowed much more complex reactions to take place in organisms
  - Hypoxia-inducible factor (HIF) and oxygen homeostasis:



    - Hydroxylases (2-oxogluterate (= α-ketoglutarate) oxygenases)
      - Require 2-oxogluterate as substrate (from TCA)→ succinate as side-product
      - Ascorbate as cofactor (can be limiting in some cells)
      - Catalytic site contains Fe(II), can be chelated or substituted by Co(II) → ($CoCl_2$ hypoxia mimic)
      - Relative high Km for O_2
      - Hydroxylation of Pro-402 and Pro-564 on Hif → association with VHL (and hydroxylation of Asn-803 by asparagines-hydroxylase → no p300 binding)
    - HIF induced expression of Glycolytic genes →Warburg-Effect
    - VHL-HIF regulates glucose metabolism and insulin secretion in pancreatic beta cells

- Metabolism-related disorders: Cancer and Heart Disease
  - VHL-defective mutations in cancer display constitutive activation of HIF → Adaption of metabolism, proliferation, differentiation, survival, apoptosis, etc.
  - Cardiac Hypertrophy: A compensatory response to stress to maintain heart function
    - Physiological hypertrophy: long-term exercise
      - Improvement of cardiac performance
    - Pathological hypertrophy: ischemia, pressure overload, hypertension
      - Cardiac dysfunction and failure
      - Metabolic reprogramming (shift from fatty acids to glucose and lipid acc.)
      - Activation of embryonic gene expression program
    - HIF1α activation is restricted to the diseased heart in humans and mice
  - HIF1α and PPARγ activation result in glucose-to-lipid conversion as well → lipid accumulation (→ Myocardial steatosis in heart)

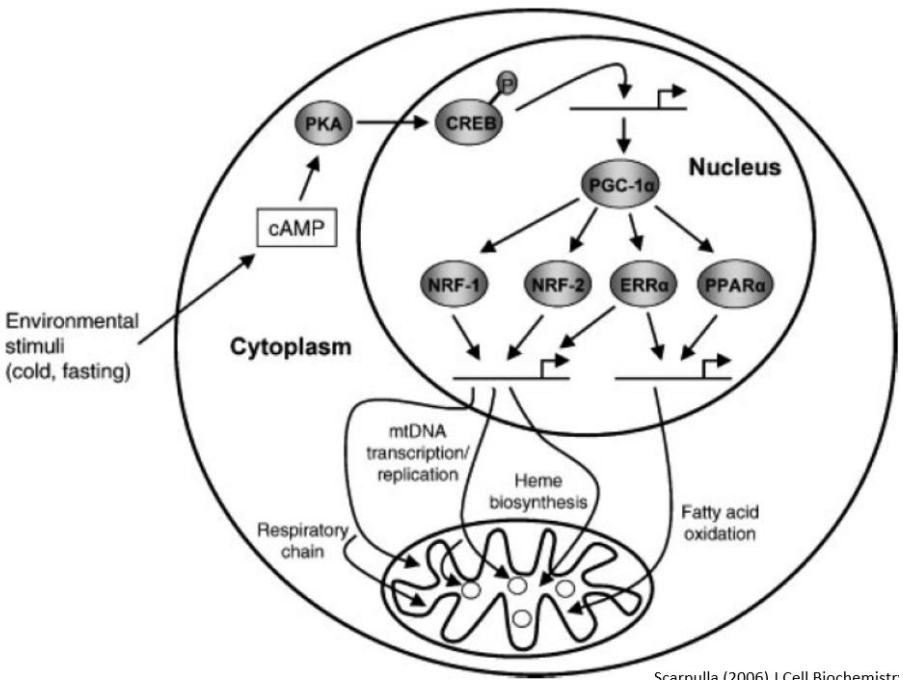


Energy Homeostasis and Oxygen signalling (JK)

Oxygen signalling in physiology and organ development

- ATP-producing pathways:
  - Glucose → Glycolysis, TCA Cycle, ETC → ATP
  - Fats → β-Oxidation, TCA Cycle, ETC → ATP
  - Proteins → Deamination, TCA Cycle, ETC → ATP
- ATP is used for Mechanical work (muscle contraction), chemical work (Na/K pump) and synthetic work (production of macromolecules like nucleic acids, proteins, lipids and complex carbohydrates)

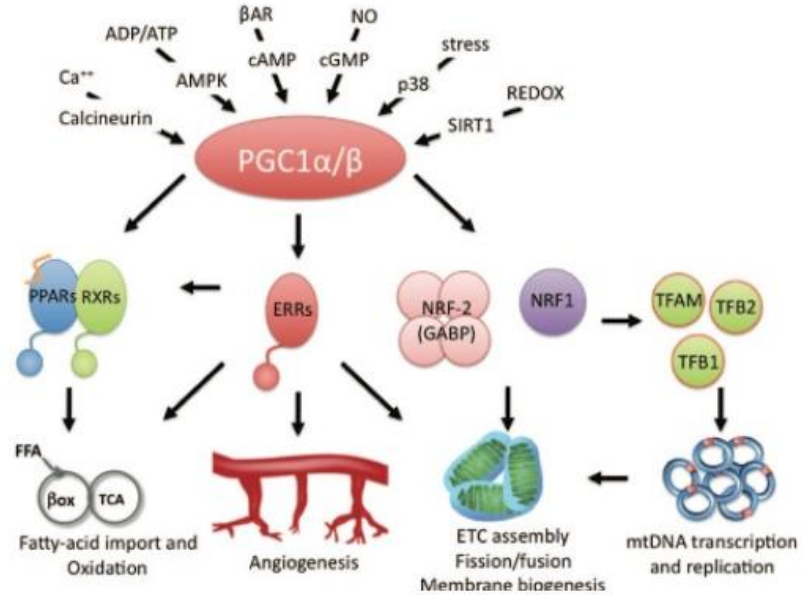
- Mitochondria and Oxidative Phosphorylation:
  - Symbiotic relationship with nuclear DNA products
    - Replication of mtDNA is controlled by nuclear DNA products
    - Transcription of mRNA is polycistronic and RNA endonucleases come from nDNA
    - DNA repair mechanism are nuclear DNA products
  - Mt Replication is not strictly coupled to S phase
  - Mutation Index is likely higher for Mt DNA due to
    - Oxidative processes, which generate free radicals that can damage DNA
    - Few introns, therefore mutational damage to exons are greater
    - Fewer DNA repair mechanisms
  - Multiple copies of mtDNA genome per mitochondrion (2-10 copies)
  - Threshold (energy minimum) at which a cell or organ or organism can function
    - Changes during development
    - Changes during stress or illness
    - Varies between organs (e.g. brain activity vs. mature epidermal cell)
  - Stochastic Redistribution
    - Random at cell division
    - Changes the degree of heteroplasmy from cell generation to cell generation
    - Non-dividing cells (brain, muscle) do not change their heteroplasmy and collect abnormal mitochondria
  - Organ Susceptibility
    - Dividing cell select against abnormal Mts
    - Non-dividing cells accumulate abnormal Mts
    - High energy demanding cells/organs are more sensitive to heteroplasmy
    - Growth, stress and illness lower threshold for bioenergetic diseases
  - Mitochondrial Biogenesis



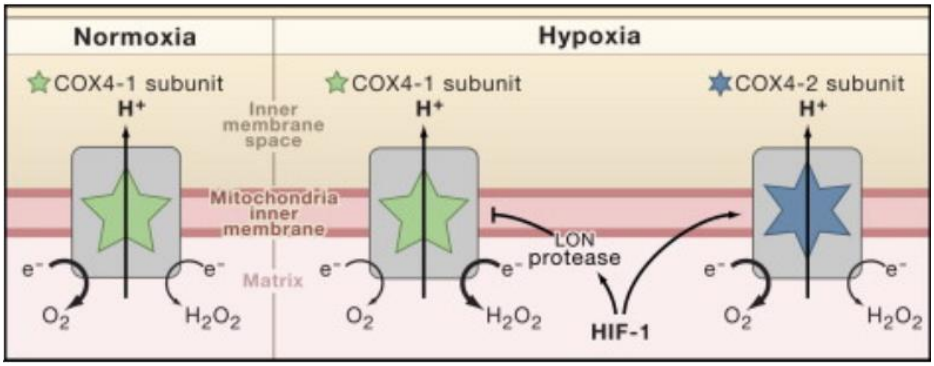
Scarpulla (2006) J Cell Biochemistr

- What happens in ATP or energy insufficiency:
  - Brain dysfunction (seizure, mental retardation, cognitive and psych. dysfunction)
  - Cardiac dysfunction (cardiac hypertrophy, heart failure, death)
- In Adult cells: Mainly fatty acid oxidation (however, need glycolysis to proliferate)

- Cardiac development and regulation:
  - Heart in terms of cancer: Undergo "cell division" only without cytokinesis cause of sarcomers! Can act like cancer tissue under stress conditions
  - Pathological stress →hypertrophy→cells die or get replaced by fibroblasts (cardiac fibrosis) (Also here: VHL → HIF → Growth/Hypertrophy)
  - Estrogen-related-receptor-γ (ERRγ)
    - Transcription factor, which binds to ERRE element on DNA
    - Directs and maintains transition to oxidative metabolism in postnatal heart
    - KO → inhibited Mt biogenesis → multiplying Mt DNA instead
    - KO lead to decreased pyruvate consumption by TCA Cycle but rather conversion to lactate
  - PPAR-γ-related-coactivator-1α (PGC-1α)
    - Drives the formation of dark red muscle tissue (= ox. phosph. fibers)
      → Change of metabolism changes nature of cells!
    - PGC-1α normally activated by exercise/motor nerve activity and induces expression of type I myofibres, Mt biogenesis and genes for oxidative metabolism:



  - HIF-1
    - Regulates cytochrome oxidase subunits to optimice efficancy of respiration in hypoxic cells:

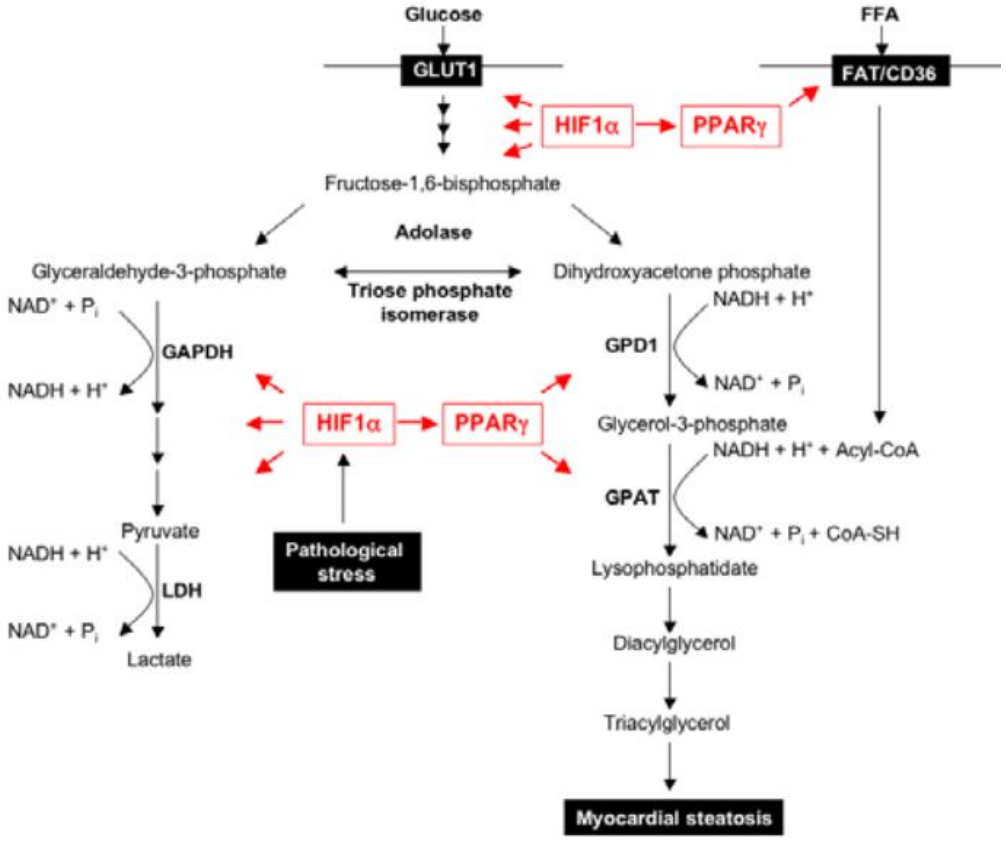


    - COX4-2 has an increased sensitivity for O_2 (more efficient in anoxia)
    - Even in anoxia cells need little O_2 to create needed metabolites → COX4-1/2 switch (=> Mt's also needed for glycolysis!)

- Cardiac development
  - Cardiogenesis occurs in hypoxia and leads to HIF1α accumulation
  - Hypoxia/ HIF1α is needed for cardiac development (Differentiation, Hypertrophy and Myofibrillar gene expression)!
  - KO → peristaltic motion (not fully differentiated heart cells)
  - HIF1α is necessary for cardiogenic transcription factor expression, which share often promoter sequence ACGTG

Oxygen signalling in non-neoplastic tissue pathology

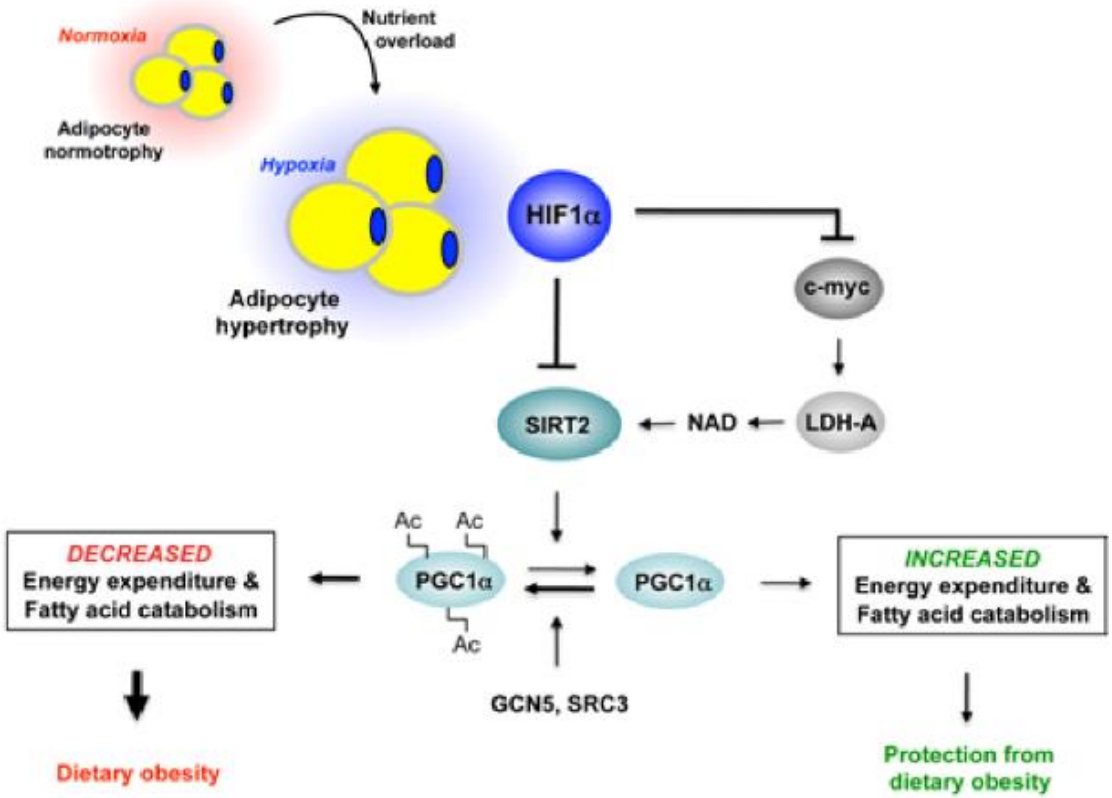
- Pathological cardiac Hypertrophy:
  - PGC-1α
    - Controls the energy state and contractile function of cardiac muscle
    - TAC (Trans-Aorta-Constriction: compress aorta→more work needed→hypoxia) in PGC-1α$^{-/-}$ (KO) mice:
      - Cardiac dysfunction and clinical heart failure
      - Develop dilated cardiomyopathy (enlarged, weak, hypertrophic heart)
      - Downregulation of PGC-1α genes and ETC
      - PE inhibits PGC-1α and its target genes in cardiomyocites
        → ectopic PGC-1α expression rescues phenotype
  - HIF1α
    - Pressure Overload/Hypertension → HIF1α → TAG synthesis, glycolytic genes, embryonic expression, inhibition of Mt biogenesis/activity and oxidative catabol.
    - VHL inactivation is sufficient to induce cardiac hypertrophy
    - HIF1α regulates PPARγ transcription and both induce metabolic switch:



- Nutritional overload and obesity:
  - Adipocytes undergo hypertrophy by nutritional overload, which lead to pathologies:
    - Glucose intolerance
    - Insulin resistance
    - Cardiovascular diseases
    - Cancer

- Adipose tissue expansion is characterised by hypoxia and HIF1α accumulation
- Tissue-specific inactivation of HIF1α gives protection against dietary overload and improves glucose homeostasis
- Tamoxifen-induced HIF1α inactivation:
  - Accelerates weight loss
  - Decelerate weight gain
  - Improves glucose homeostasis
  - Attenuates progression to diabetic cardiomyopathy and heart failure
  - Inhibits lipid accumulation and adipocytes growth
  - Promotes FAO (specially in white adipocytes) and energy expenditure
  - Promote Mt biogenesis in white adipocytes
  - Augments PCG1α deacetylation by SIRT2 (NAD^+ dependent deacetylase)
    - Oxidative phosphorylation
    - Uncoupling
    - Mt biogenesis

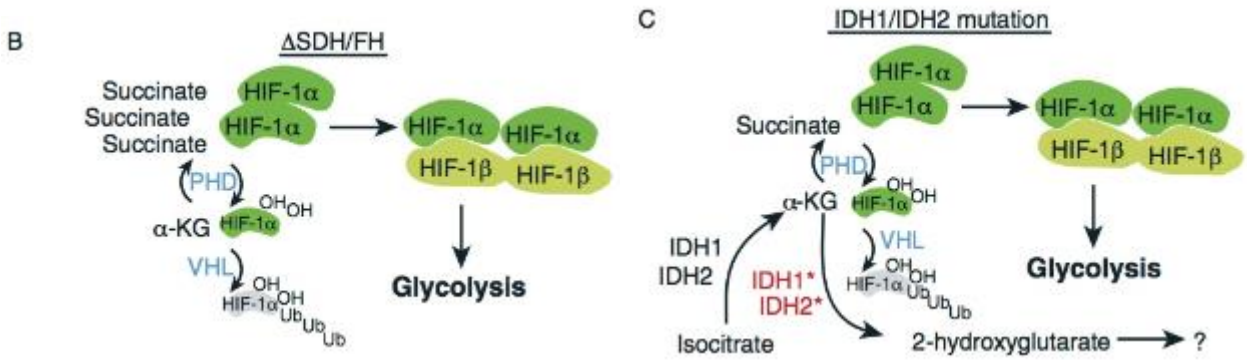
    (HIF1α represses SIRT2 transcription)
  - Promotes NAD^+ regeneration
  - Promotes c-myc and LDH expression
- Human obesity correlates with adipose HIF1α accumulation, and inversely correlates with SIRT2 and CPT1 expression
- Mode of HIF1α-mediated metabolic reprogramming in adipocytes growth:



Oxygen signalling in non-neoplastic and neoplastic tissue pathology

- Regulation of HIF1α in response to mutations in metabolic enzymes:
  - Inhibition of SDH (TCA Cycle enzyme in Mt) increases HIF1α levels and activity
  - HIF proteins are overexpressed in FH renal tumors
  - FH inhibition coupled with fumarate addition upregulates and stabilizes HIF protein

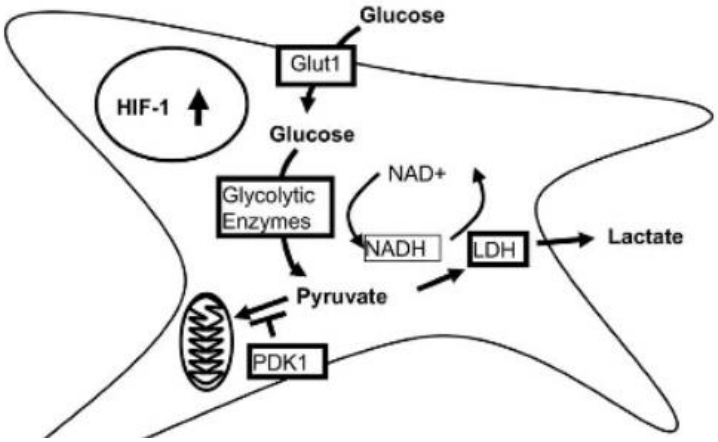
- Explanation:



- **B)** Mutations in SDH (succinate → fumarate) or FH (fumarate → malate) enzymes result in accumulation of succinate impairing HIF hydroxylation and glycolysis activation
- **C)** Mutations of IDH1 or IDH2 may reduce availability of α-ketoglutarate, restricting HIF hydroxylation → glycolysis activation. Alternative Model: Mutated IDH1 or IDH2 gain new function in which they produce 2-hydroxyglutarate

- Tumorigenic cells have increased glucose-dependency and LDH-A activity compared to immortalized non-transformed cells
- LDH-A knockdown decreases tumor growth and enhance respiration rate

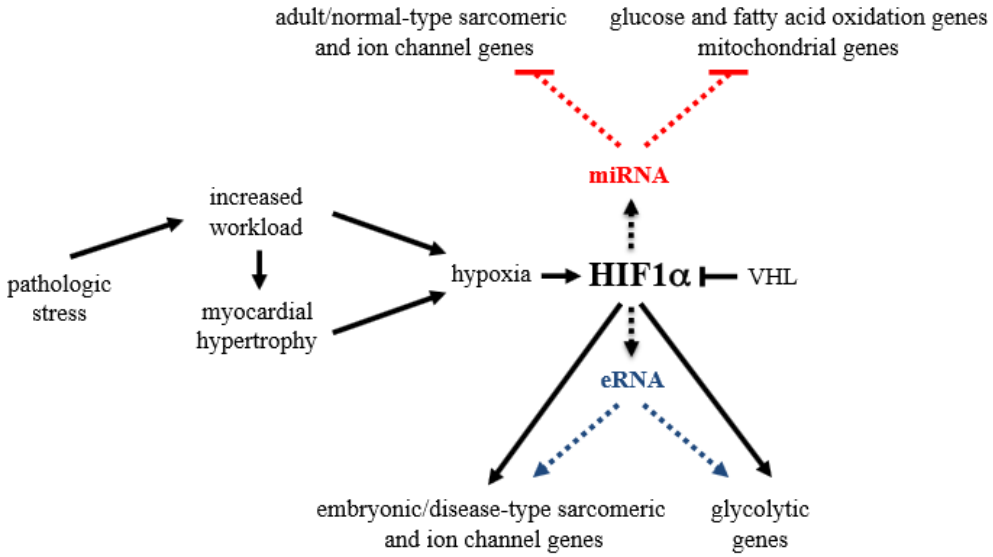
- Downreglation of mitochondrial oxygen consumption by HIF1α:
  - PDK1 (PDH-Kinase1) is upregulated in hypoxia in a HIF1α dependent manner
  - PDK1 expression directly regulates cellular oxygen consumption rate
  - Explanation:



- The Effect of Dichloracetate (DCA) on cancer cells:
  - DCA reverses the glycolytic phenotype and depolarizes Mts (but not in healthy cells!)
  - DCA induces apoptosis and reduces proliferation in cancer cells → inhibits tumor growth
  - DCA-mediated pyruvate dehydrogenase kinase (PDK) inhibition suppresses tumor growth
  - DCA-mediated pyruvate dehydrogenase kinase (PDK) inhibition augments cardiac function

- HIF1α-dependent miRNA and enhancer RNA (eRNA) in cardiac metabolic remodelling:
  - miRNAs are sufficient and necessary for pathological cardiac hypertrophic development
  - many possible HIF1α- and PPARγ-direct target miRNAs identified
  - A subset of VHL-dependent miRNAs contain cross-species conserved HREs in their promoters (e.g. mir-27b, which control many mitochondrial genes)

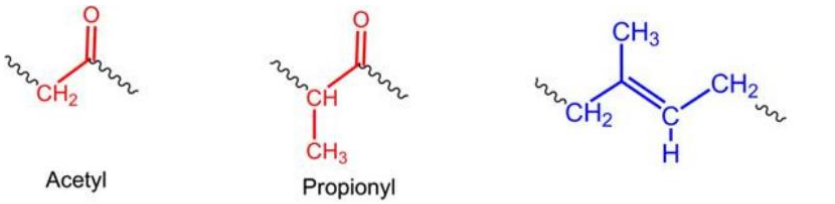
- Possible alternative mechanism of HIF1α-mediated gene induction:



Lipid Metabolism (WEKO)

Basics of lipid metabolism

- Defitintion of a lipid:
  Lipids may be broadly defines as hydrophobic or amphipathic small molecules that originate entirely or in part from two distinct biochemical subunits or "building blocks": **ketoacyl** and **isoprene** groups. Using this approach, lipids may be divided into eight categories:
  - Fatty acyls (FA), glycerolipids (GL), glycerophospholipids (GP), sphingolipids (SP), saccharolipids (SL) and polyketides (PK)
  - Sterol lipids (ST) and prenol lipids (PR)



Ketoacyl "building blocks" **Isoprene "building block"**

- How many lipids are there?

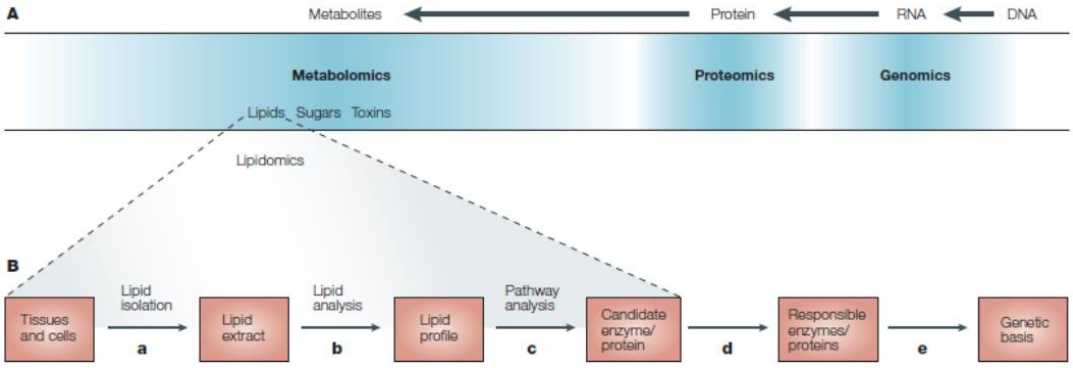
Types of lipid	Number of lipids in class
40 common FA	40
1,2,3 TAG	40*40*40 = 64000
1-, 2-, and 3-MAG	40+40+40 = 120
40 FA acyl CoA	40
1,2 + 2,3 + 1,3 DAG	(40*40)*3 = 4800
SUM	69000

FA, fatty acid; TAG, triacylglycerol; MAG, monoacylglycerol; DAG, diacylglycerol

Glycerophospholipids:
1. Position: saturated FA, 2. Position: unsaturated FA, 3. Position: P-group

- Principle roles of lipids:
  - Whole body energy homeostasis (i.e. storage)
  - Transcription and signal transduction (i.e. ligands of nuclear receptors, PI3 kinase pathway)
  - Environment sensing
  - Protein modification
  - Generation of permeability barriers
  - Protection against reactive metabolites
  - Structural components of membranes

- Experimental approaches:

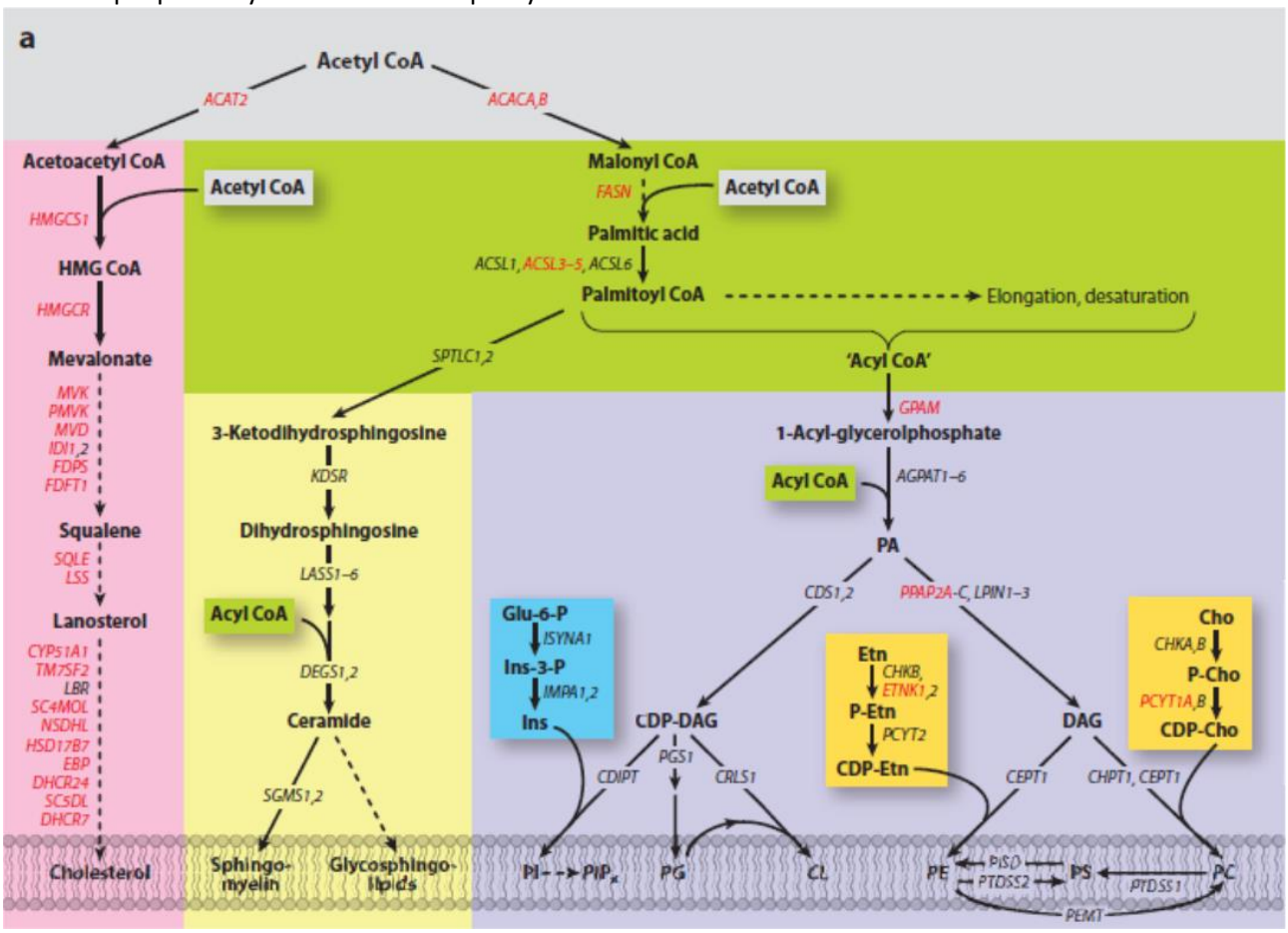


Experimental approach	Lipid classes covered	Advantages	Disadvantages
Chromatography			
Thin-layer chromatography (TLC)	Solvent systems established for most lipid classes	Very established technique; technically relatively easy; does not require sophisticated instrumentation; spot chromatograms allow for rapid screening of mutant extract libraries.	Low resolution and sensitivity limits many lipidomic applications; detection of lipid by iodine vapour and (class-specific) dyes and radioactivity.
High-performance liquid chromatography (HPLC)	Many lipids, including sterols, GP, TG, DG, FA and lipid headgroup derivatives.	Well established with worked out reverse- and normal-phase conditions available; ease of automation; very quantitative.	Detection by refractive index or mass detector (lipids, as defined here, in general do not absorb visible and UV light effectively); medium sensitivities in general.
Gas chromatography (GC)	Non-polar compounds such as TG; derivatized FA and sterols.	Very widely used for determination of fatty-acid composition, detection generally by MS.	Requires volatile compounds or derivatization of polar lipids.
Mass spectrometry			
ESI	Polar compounds such as GP (more apolar compounds can be analysed by APCI)	Direct detection by *m/z*; high sensitivity and resolution; direct profiling of complex lipid mixtures; ease of automation; compatible with upfront LC separation.	Supression of ionization, in particular in the case of crude extracts and when low-abundance species are to analysed; absolute quantification requires considerable efforts (for example, class and mass dependent internal standards).
MALDI	Many lipids including complex glycolipids.	Direct detection by *m/z*; buffer and salt contaminants generally well tolerated; can be combined with prior TLC separation.	Supression of ionization, in particular in the case of crude extracts and when low-abundance species are to analysed; matrix backgrounds.
NMR			
^{31}P	Phospholipids.	Direct measurement; non-destructive; quantitative.	Line broadening of lipids in aqueous solutions; low sensitivity.
^{1}H	All lipids.	Direct measurement; non-destructive; powerful technique for structural analysis of purified compounds.	low sensitivity, spectra dominated by very abundant lipids (cholesterol, PC).
Biochemistry			
Assays using immobilized lipids (for example, monolayer adsorption, langmiur blodget films, immobilized lipid biosensors, lipid blots/beads)	Many lipids both in pure form or mixtures.	Sensitive approaches which allow determination of interaction of ligands with lipids.	Functional immobilization of lipids difficult; technically tedious; automation and throughput generally limited.
Assays using lipid in solution/suspension (for example, optical, calorimetric, radiometric approaches using liposomes and micelles)	Many lipids both in pure form or mixtures.	Solution conditions; binding studies; often quantitative; enzyme assays, some which of high throughput.	Often experimentally challenging; optimization of conditions can require significant effort.
Lipid antibodies	Very few.	Cell biological studies (for example, subcellular localization) possible.	Specificity of antibodies.
Reactive lipids (for example, photoactivatable)	Few.	Identification of lipid-binding proteins.	Specificity; probes limited.

DG, diacylglycerol; ESI, electrospray ionization; FA, fatty acid; GP, glycerophospholipids; LC, liquid chromatography; MALDI, matrix-assisted laser desorption/ionization; MS, mass spectrometry; TG, triacylglycerol.

- Lipid composition of membranes:
  (SEE CORRESPONDING SECTION OF "CELLULAR BIOCHEMISTRY PART I" SUMMARY)

- Principle pathways for membrane lipid synthesis:



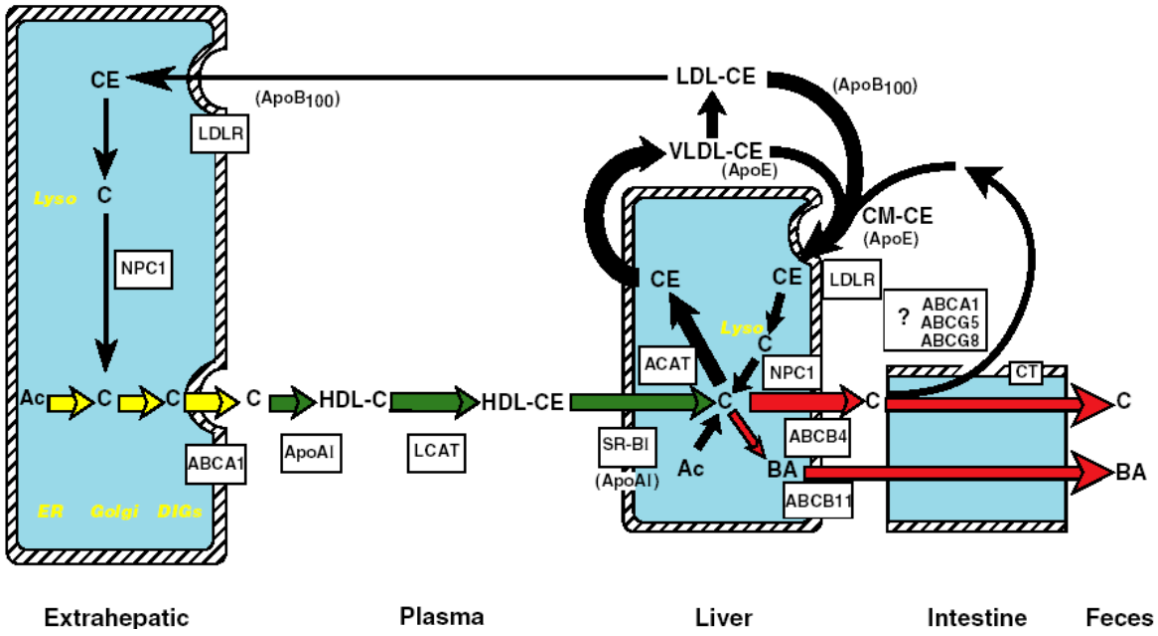
  - Membrane biogenesis is elemental to cell growth, proliferation and differentiation:
    - Outgrowth of neurites, formation of myelin sheaths, biogenesis of lung surfactant, expansion of the endomembrane system by professional secretory cells
    - Membrane growth in response to certain forms of stress (enlargement of ER in response to excess of unfolded proteins)
    - Generation of bile, the epidermal permeability barrier, and the formation of neutral lipid assemblies found in lipoproteins, milk fat globules and cellular lipid droplets

  - Key to adjusting the concentrations of membrane components:
    - 1) Multiple feedback loops, wherein individual lipids throttle supply through inhibition of synthesis and uptake or by promoting their own storage, export, metabolism
    - 2) Cross-regulation, whereby one class of lipids affects the synthesis or disposition of lipids in a different metabolic branch (e.g. cholesterol and phospholipids, cholesterol and sphingolipids)

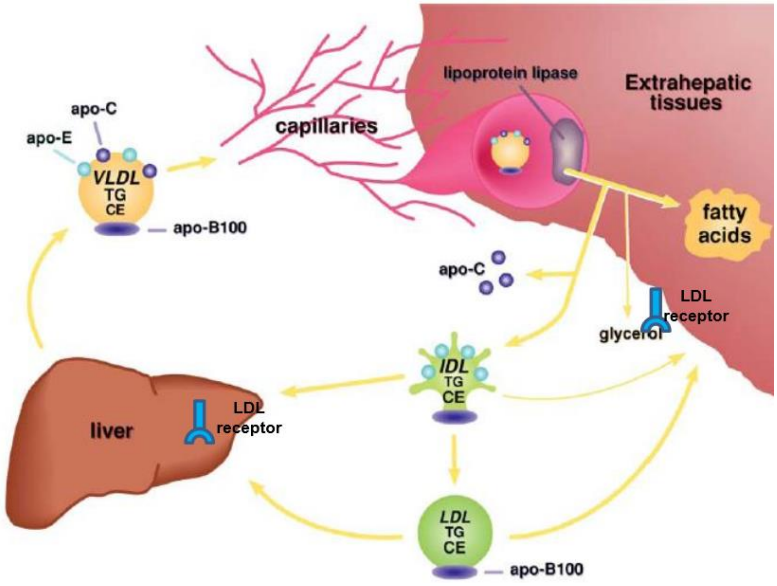
  - Principal means for global regulation:
    - Centrally acting transcription factors
    - Uptake of membrane precursors through endocytosis of lipoproteins
    - Inactivation of certain phospholipases
    - Regulation of kinases

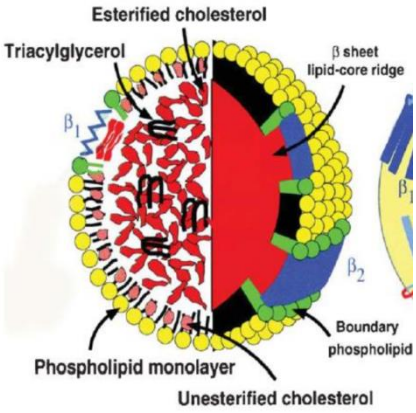
Cholesterol Homeostasis

- Introduction:
  - Cholesterol (C) biosynthesis is among the most intensely regulated processes in biology
  - Various pathways for uptake of C from LDL and export to HDL
  - Intracellular transport: vesicular (or membrane) and non-vesicular mechanisms
  - C provides membranes with special physical properties
  - Metabolites of C (steroids, oxysterols and bile acids) have important biological functions (signal transducer, solubilizer of other lipids, ...)
  - C is critical for embryonic development
  - Alterations in C homeostasis involved in pathogenesis of brain and cardiac vascular diseases, dementias, diabetes and cancer
  - Diseases caused by a defect in C trafficking (e.g. Niemann-Pick Type C, Tangier disease)
- Cholesterol flow through major tissue compartments:



- Intercellular transport of "free" cholesterol, cholesteryl esters, and other lipids by lipoproteins
- 4 major classes of lipoproteins defined by density state:
  - **LDL**: Low-density lipoprotein (BAD: dyslipidemia → risk for atherosclerosis)
  - **HDL**: High-density lipoprotein (GOOD)
  - **VDL**: Very-low-density lipoprotein (triglyceride carrier from liver)
  - **CM**: Chylomicrons (dietary lipid carrier synthesised in intestine)





- LDL receptor:
  - Binding, internalization, disassembly (, recycling)
  - Regulated by cellular cholesterol
  - Determines the LDL level in blood
  - Induced by "Statin" drugs

- Monogenic disorders causing hypercholesterolemia:
  - familial hypercholesterolemia (FH) *(defective LDL-receptor)*
  - familial ligand-defective apoB-100 (FDB) *(apoB100 defective → weaker LDLR binding)*
  - Sitosterolemia *(defective ABCG5/8 → lower LDLR expression)*
  - Autosomal recessive hypercholesterolemia (ARH) (*weaker LDLR internalization)*
  - Tangier disease *(Loss of functional ABCA1)*

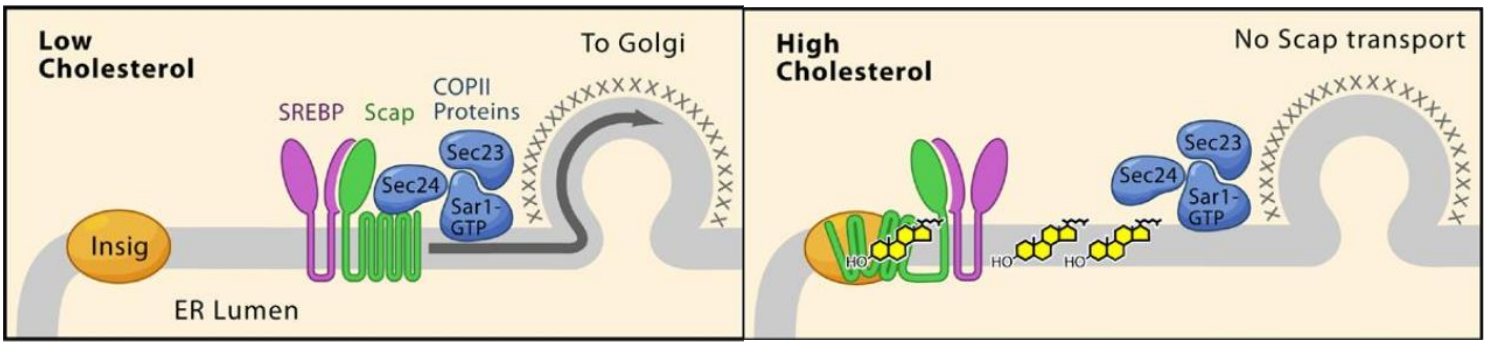
- **Lipoprotein-Cell exchange of Cholesterol**
  - Via LDL (liver → peripheral tissue):
    - Receptor-mediated endocytosis and receptor recycling
  - Via HDL (peripheral tissue → liver or steroidogenic tissues):
    - SR-BI-mediated selective uptake (CE) and efflux/loading of HDL (free C); Cholesterol-loading of HDL by LCAT; HDL can transfer CE to other lipoproteins by CETP (cholesteryl ester transfer protein)
      Secretion of apoA-1 via ABCA1 transporter into interstitial fluid → nascent HDL → Loading cholesterol, secreted by transporter ABCG1, into HDL by LCAT

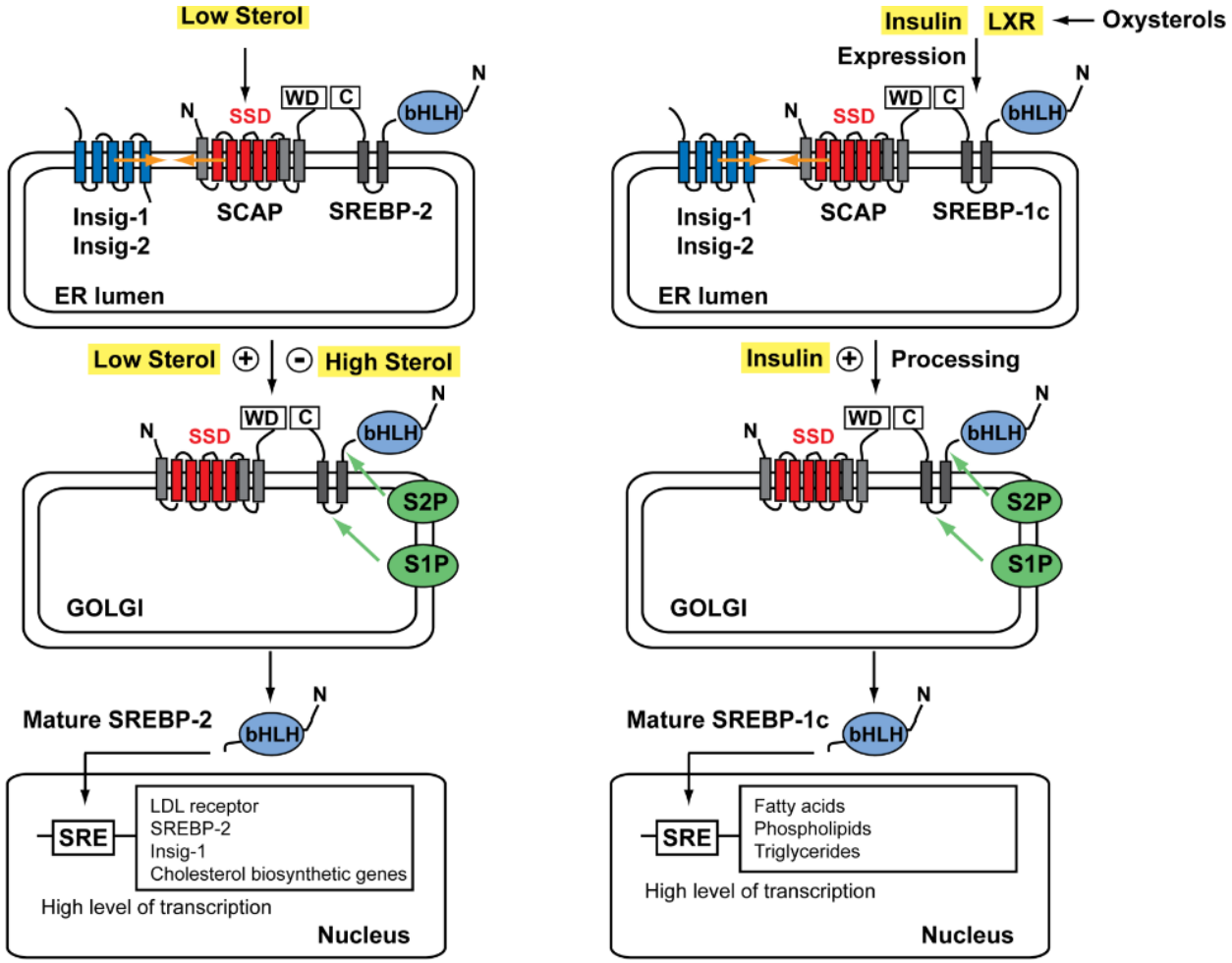
- The PCSK9 decade
  - Activation by self-cleavage of the Pro-domain in the ER (keeps associated)
  - Gets secreted and binds LDLR in complex with LDL (or already binds in Golgi)
  - Internalization with LDLR/LDL via CME and degradation in Lysosome
    → PCSK9 inhibits recycling of LDL receptors → higher LDL level in blood! (BAD)
- Therapeutic approaches to inhibit PCSK9:
  - Gene silencing (anti-sense oligonucleotides (ASO), locked nucleic acids (LNA), siRNA)
  - Antibodies (anti-PCSK9 monoclonal Ab (Amgen, Pfizer-Rinat) with high dose of statin)

- Statins block HMG-CoA reductase (TM-enzyme of C synthesis in peroxisomes and ER) and induce LDL receptor expression
  (Myopathy as a known but rare side-effect)

SREBPs: Activators of the complete program of cholesterol and fatty acid synthesis (LEARN!)

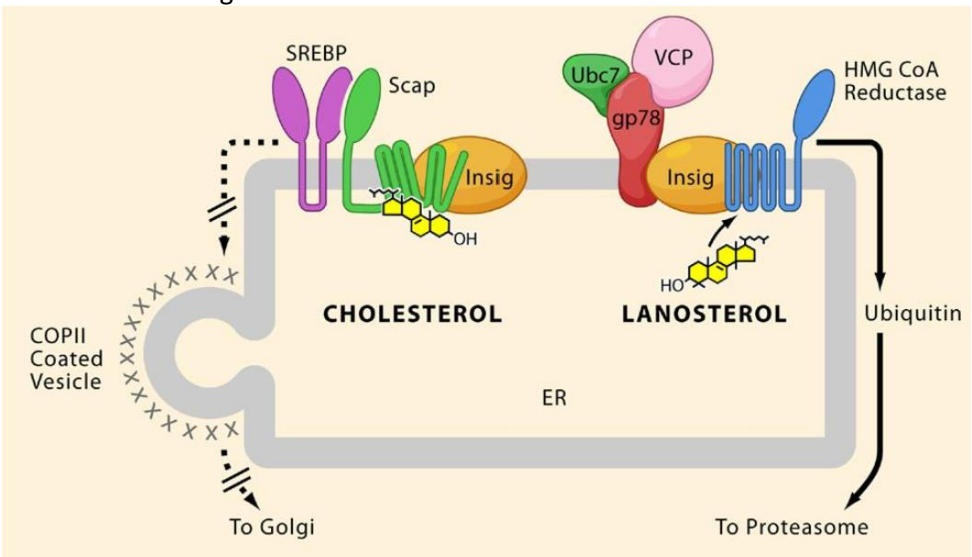
- Lipid homeostasis in vertebrate cells is regulated by a family of membrane-bound transcription factors designated sterol regulatory element-binding proteins (SREBPs)
- The mammalian genome encodes three SREBP isoforms, designated SREBP-1a, SREBP-1c, and SREBP2
- SREBP-1a and -1c are derived from a single gene through the use of alternative transcription start sites that produce alternate forms of exon 1, designated 1a and 1c
- At normal levels of expression, SREBP-1c favors the fatty acid biosynthetic pathway and SREBP-2 favors cholesterologenesis
- SREBPs comprise a subfamily of bHLH leucine zipper (bHLH-LZ) proteins. SREBPs bind both the canonical inverted-repeat E-box site, characteristic for most bHLH proteins, and the SREBP-specific direct-repeat-binding element (SRE)
- ER-cholesterol homeostasis at 5 mole%





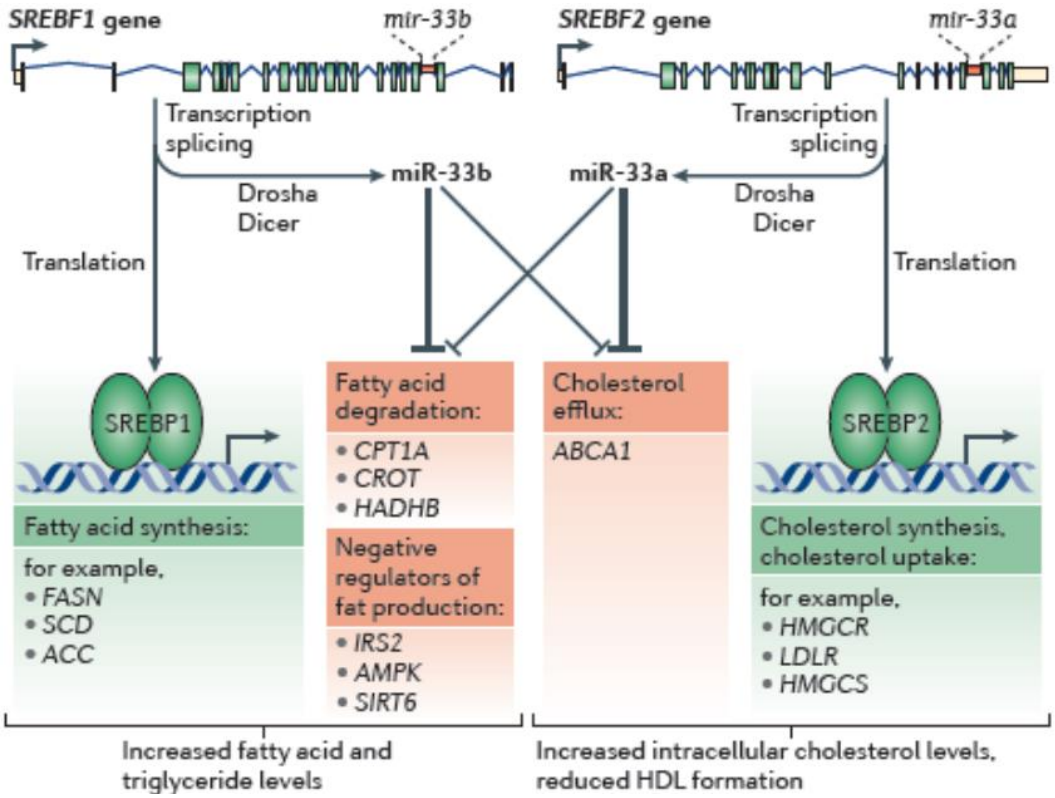
- Model for convergent feedback inhibition of cholesterol synthesis and uptake:
  → SREBP-2 produced Insig-1 and Cholesterol inhibit further SREBP-2 activation

- Two actions of Insigs in cholesterol homeostasis:



- Cholesterol and lipid regulation by miRNA:
  - miR-122
    - primarily expressed in the liver
    - Silencing of miR-122 itself led to decreased cholesterol and FA biosynthesis and an increase in FA β-oxidation with a reduction of cholesterol and triglyceride in blood
    - Targeted genes do not seem to be direct targets of miR-122

- miR-33 a/b
  - coexpressed with SREBPs; located in introns
  - downregulation of cholesterol transporter ABCA1 and therefore HDL

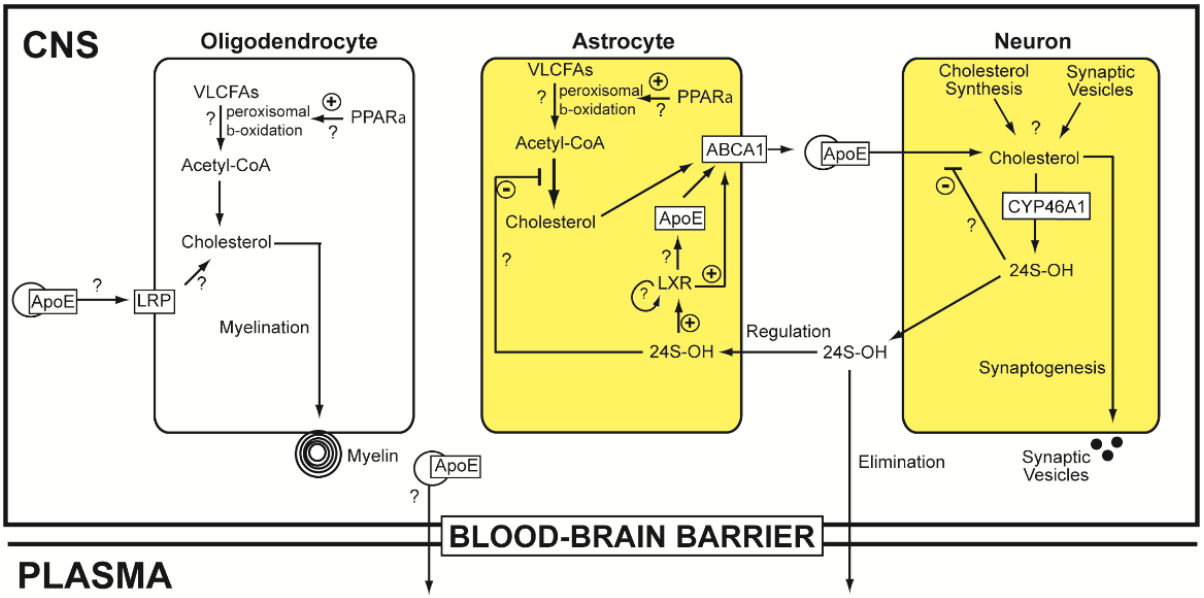


- miRNAs have recently be seen to be transported by HDL as well! (communication with liver!)

<u>Cholesterol in the central nervous system</u>

- Steady-state cholesterol pools:
  - <u>Whole mouse: 2100 mg/kg</u>
  - Intestine: 130 mg/kg
  - Liver 132 mg/kg
  - **CNS** **314 mg/kg** (produced mainly by glia cells)
  - Skeletal 1524 mg/kg
- CNS disorders due to cholesterol:
  - Increased cholesterol turnover in neurodegenerative disorders such as Alzheimer‘s disease and Niemann-Pick type C disease
  - Patients with elevated cholesterol levels have increased susceptibility to Alzheimers disease
  - Hypercholesterolemia is associated with increased brain immunoreactivity
  - Statins have beneficial effects in several neurologic diseases (e.g. multiple sclerosis, AD, ischaemic stroke)
- Cholesterol in the CNS:
  - Plasma lipoproteins **do not** cross the blood-brain barrier
  - Brain depends on intracerebral de novo synthesis of cholesterol by astrocytes
  - Mature neurons shut down C synthesis to save energy (get C from astrocytes)
  - Brain is the most cholesterol-rich organ in the body (25% of free cholesterol in the body)
  - 2 major cholesterol pools in the CNS:
    - Myelin sheaths (i.e. oligodendroglia)
    - Plasma membranes of astrocytes and neurons
  - Cholesterol is found predominantly in white matter
  - Up to 70% of the brain cholesterol is associated with myelin

- Model of Cholesterol Homeostasis in Brain:



- Role of cholesterol in the embryo and fetus:
  - Maintains membrane integrity and consequently the structure and function of membrane-bound proteins
  - Cholesterol is part of lipid rafts and caveolae, which are critical for directing the location and thereby activity of proteins into lipid-rich or -poor membrane microdomains. Numerous signaling processes originate in lipid microdomains, including those related to growth (i.e., insulin signaling).
  - Hedgehog processing
  - Precursor for bile acids, steroid hormones, and oxysterols. Bile acids are key integrators of metabolism in addition to being involved in lipid absorption. Some steroid hormones are essential for normal development of the fetus (i.e. lack of estrogen affects morphology of the gonads)

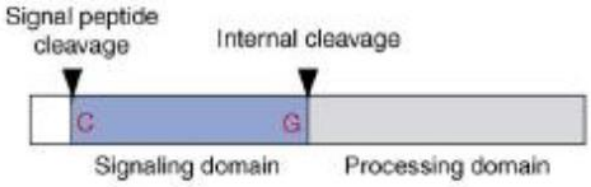
Inborn errors of cholesterol synthesis

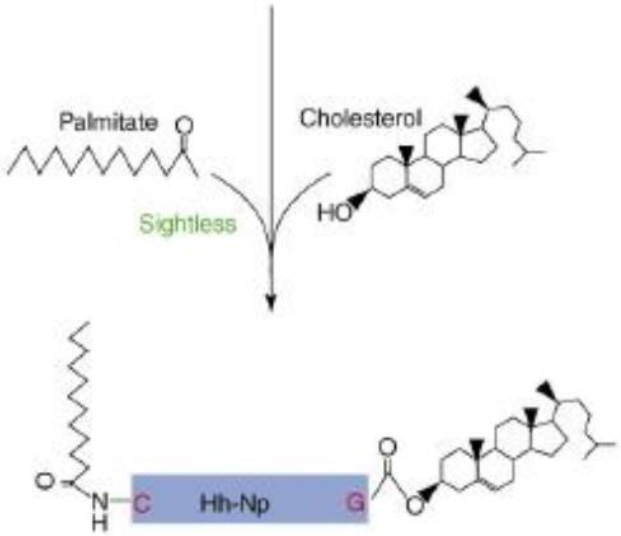
- Eight distinct inherited disorders:
  - Inborn error of presqualene cholesterol synthesis (i.e., mevalonate kinase deficiency)
  - Inborn error of postqualene cholesterol synthesis
- The sooner a gene acts in cholesterol synthesis, the earlier the phenotype occurs.
- Dependence of fetal development on endogenous cholesterol synthesis
- Inhibitors of cholesterol biosynthetic enzymes are potentially teratogenic.
- Potential consequence of accumulation of bioactive or toxic precursor sterols
- Multiple morphogenic and congenital anomalies (internal organ, skeletal and/or skin abnormalities)
- Defects in cholesterol synthesis are generally lethal in mice, while humans with impaired later steps of the pathway can survive with severe malformations.

- Biological basis of cholesterol phenotypes:
  1. Deficiency of the final product cholesterol
  2. Excess or deficiency of sterol intermediates, which are precursors in cholesterol synthesis
  3. Modification of hedgehog signalling
- Perturbation of Hedgehog signalling:
  - Cholesterol is a covalent ligand of the hedgehog family of developmental patterning proteins (sonic hedgehog, desert hedgehog, indian hedgehog)
  - Shh-deficient mice: cyclopic, defects in the ventral neural tube, somite, and foregut patterning. Later defects include distal limb malformation, absence of vertebrae and ribs, and failure of lung branching.

- These phenotypes are consequences of dysfunction in patterning during early embryogenesis.
- Possibility that congenital abnormalities associated with defects in cholesterol synthesis arise because the autocatalytic processing of hedgehog proteins is disrupted.
- Hypothesis that cholesterol biosynthesis phenotypes are phenocopies of hedgehog defects, in which the properties of hedgehog are altered when processed with sterols other than cholesterol

  → **Cholesterol is essential for the proper maturation of hedgehog proteins!**



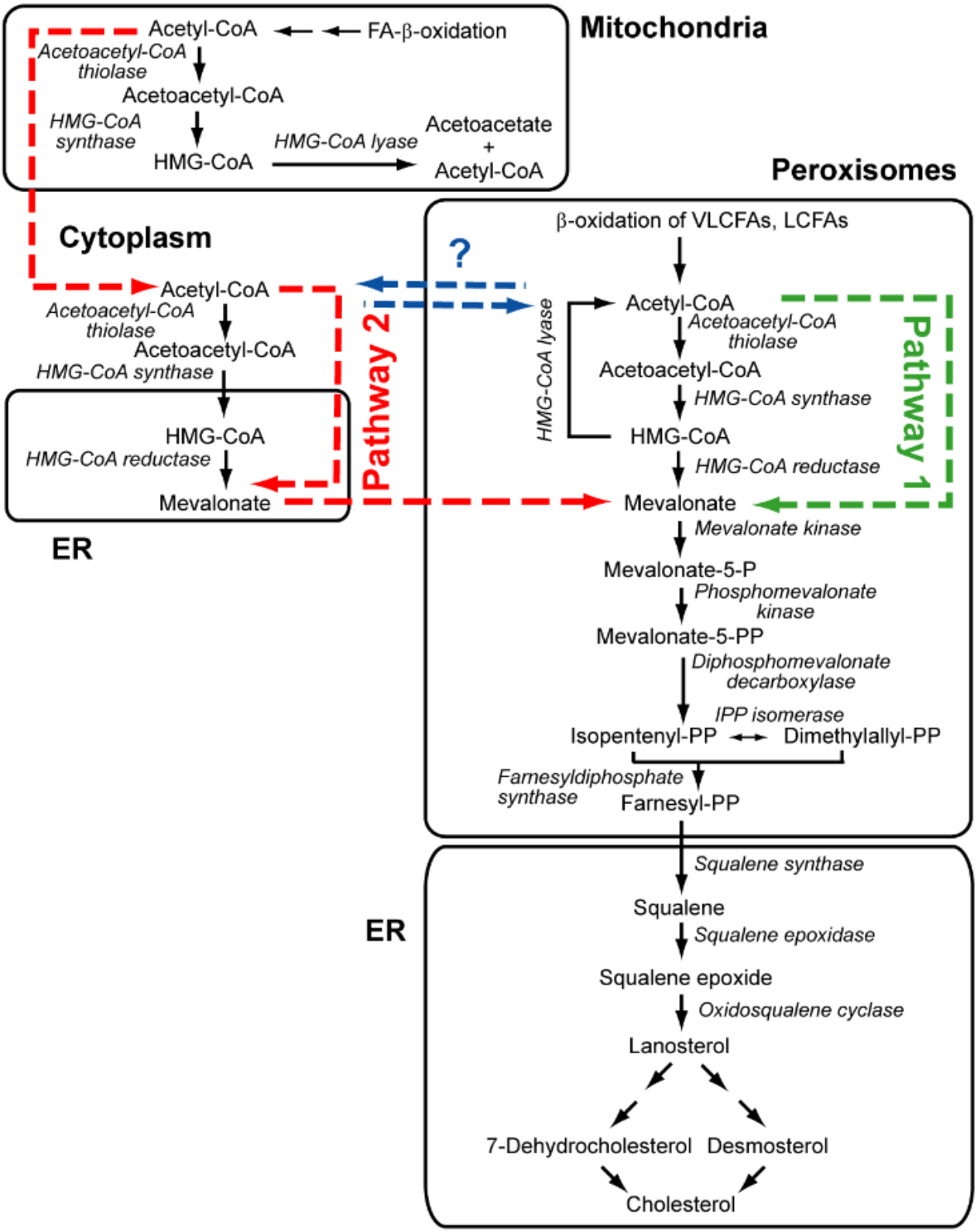


- Sterol-sensing domain (SSD)-containing proteins:
  - HMG-CoA Reductase
  - Patched (Hedgehog protein)
  - SCAP

- The role of maternal cholesterol:
  - Maternal transfer of cholesterol to the fetus does occur, but the significance of the maternal contribution of cholesterol and other sterols is still in debate
  - Early development depends on maternal cholesterol, while later on, endogenous cholesterol synthesis capacity is indispensable
  - Women with lower plasma cholesterol concentrations had smaller newborns

- Sterol intermediates: toxicity or gain of function:
  - Inhibition of enzymes early in the sterol biosynthetic pathway leads to early embryonic lethality (e.g., HMGCR, MVK, SREBP-2, squalene synthase).
  - Most fetuses with defects in sterol biosynthesis late in the pathway are viable until late in gestation or until just after birth.
  - Even though the endpoint of all reactions is cholesterol, early inhibition of the process results in the lack of isoprenoids as well as a lack of cholesterol.
  - Isoprenoids, including geranylgeraniol and farnesol, are essential for basic cellular processes (e.g., cell proliferation). The proteins modified by isoprenoids include proteins of the the ras, rab and rho families; GTP-binding proteins; and G proteins. Farnesyl pyrophosphate is a precursor for dolichol, which is essential for survival of blastocysts past implantation

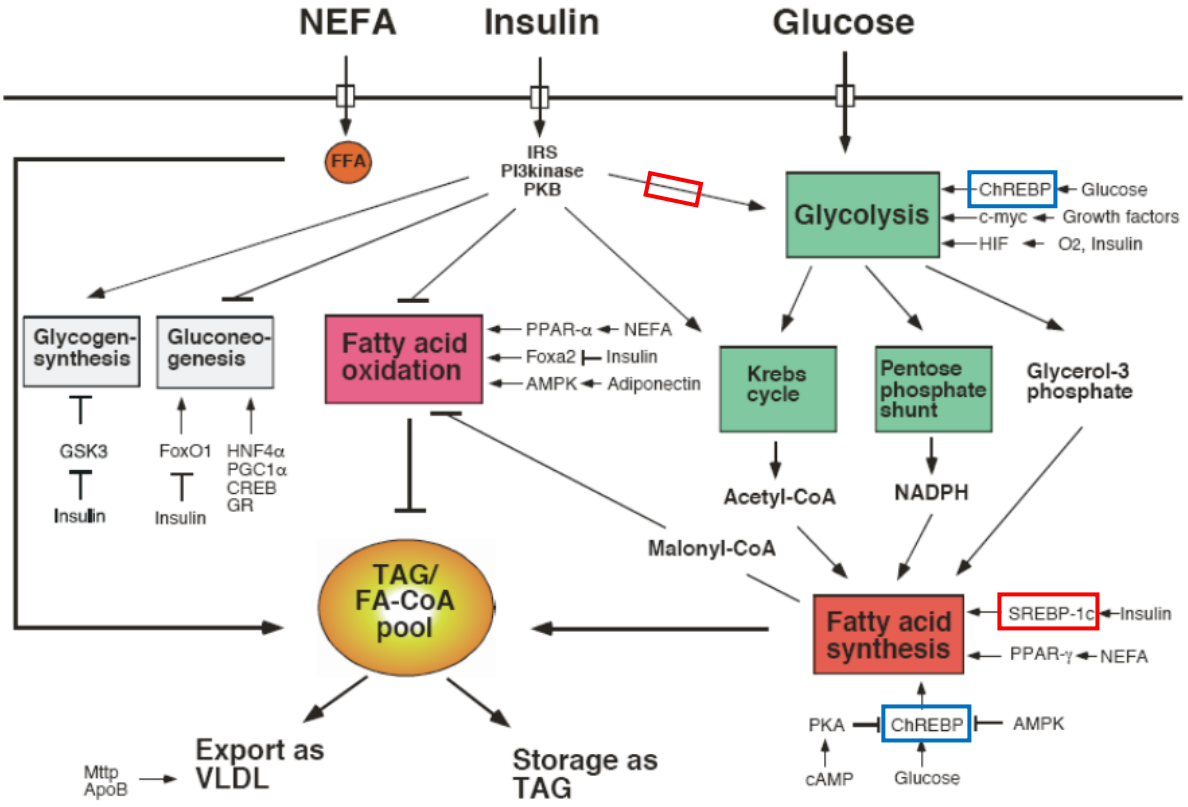
Subcellular localization of cholesterol synthesis in mammalian cells



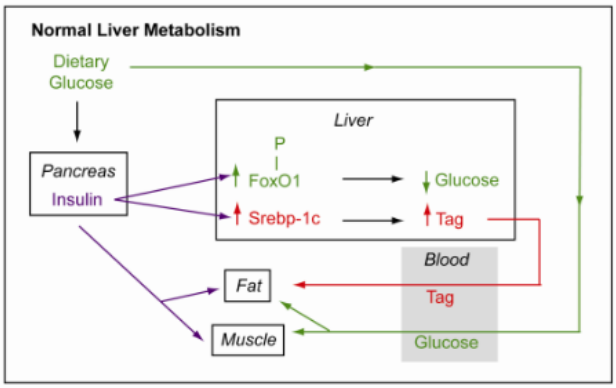
Metabolic dysfunction and hepatic (liver) steatosis (lipid accumulation)

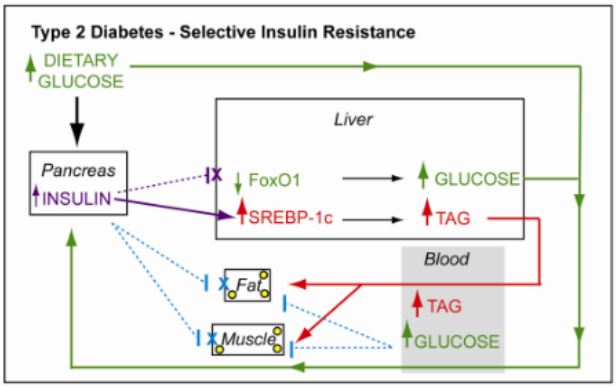
- Obesity leads to simple liver steatosis → NASH → liver cirrhosis → hepatocellular carcinoma
- Lipids stored in adipocytes in the form of TAG (triacylglycerides) and are released in form of free FA to get oxidized in liver and muscle or stored as lipid droplets in liver if excess

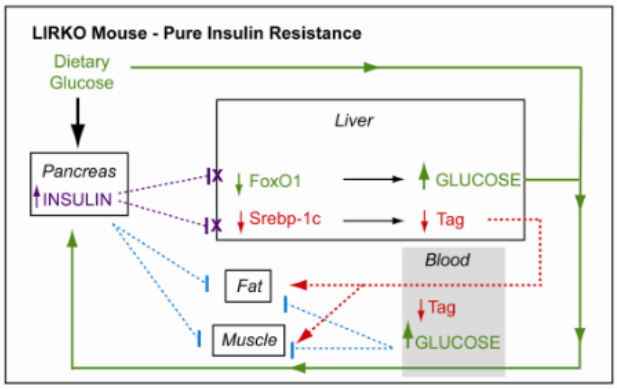
- Effects of Insulin and Glucose on hepatocytes:
  - Insulin→inhibits FAO and gluconeogenesis, promotes Glycolysis, Glycogen and FA synthesis
  - Glucose → promotes Glycolysis and FA synthesis



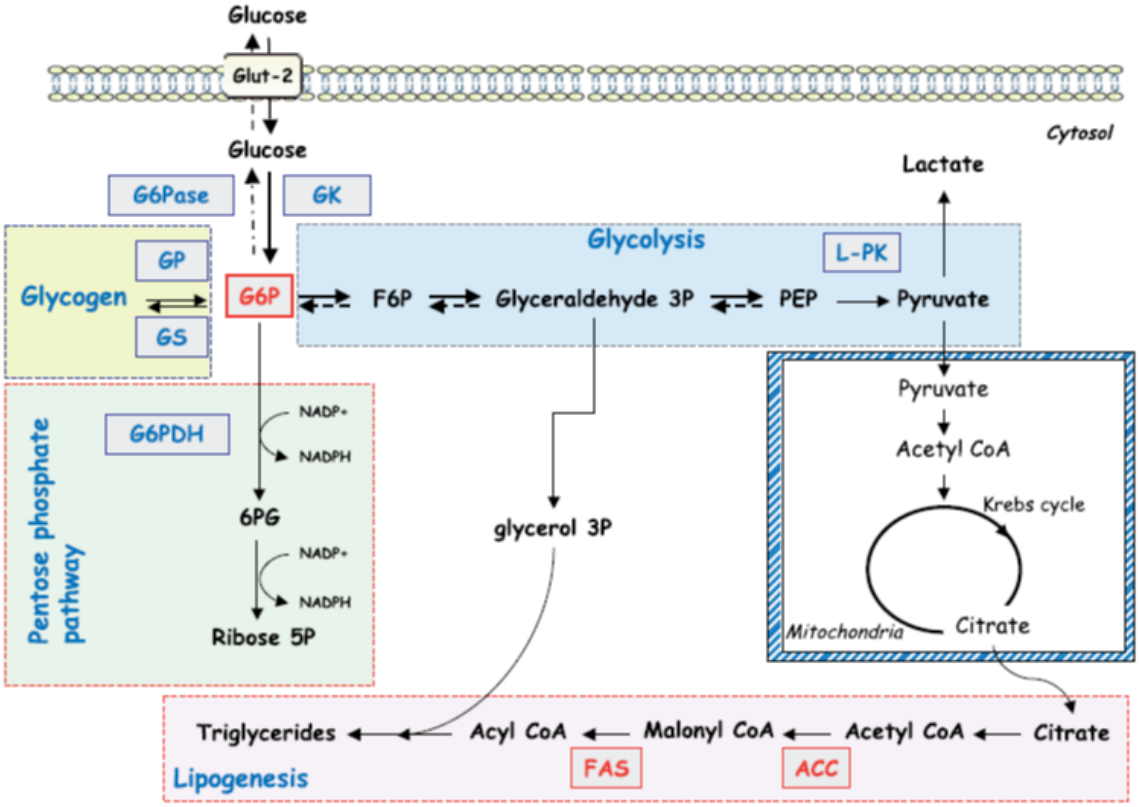
  - Selective Insulin resistance is much worse than total insulin resistance:
    → Insulin still promotes TAG synthesis, what leads to massive lipid accumulation/steatosis!
    (Double-Function of Insulin: mTORC1 only needed for stimulation of lipogenesis)





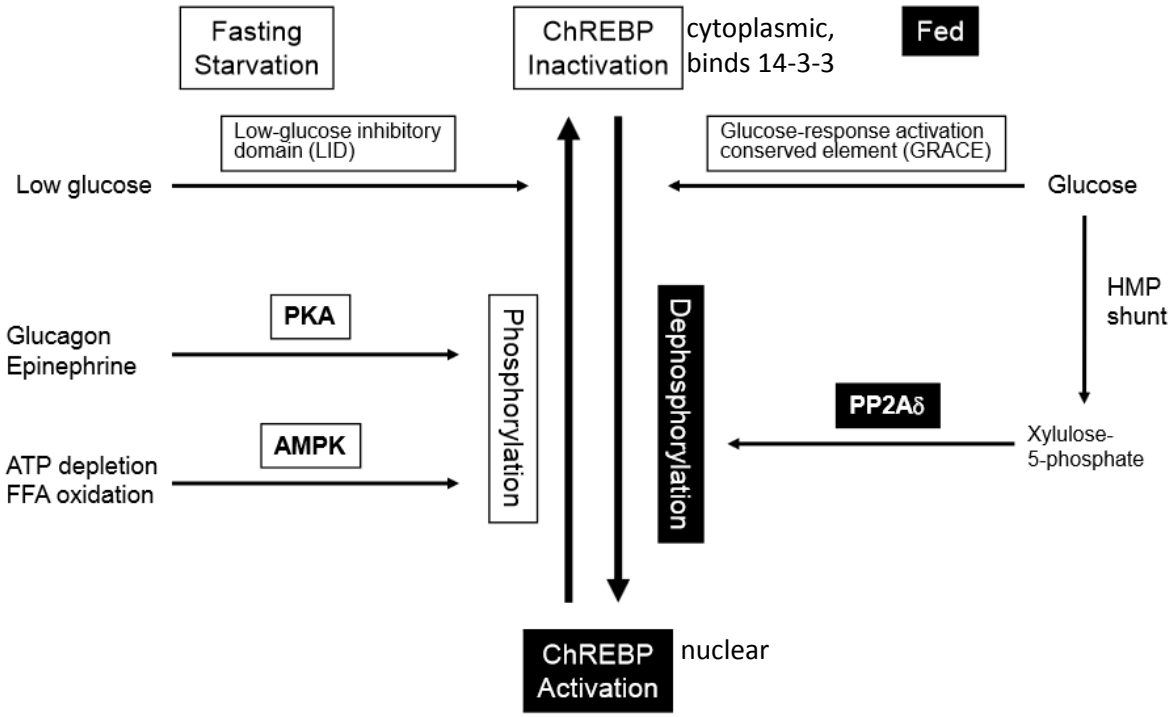


- Metabolic pathways leading to the synthesis of TAG in the liver:



  - Multiple isoforms catalyze each step of the biosynthesis
  - Transcriptional regulation by:
    - Insulin: Activates SREBP-1c→ expression of TAG synthesis enzymes
    - LXR: Activates SREBP-1c, ChREBP→ expression of TAG synth. enzymes
    - Glucose: Activates ChREBP→ expression of TAG synthesis enzymes

- ChREBP forms heterodimers with Mlx (unknown function)
- Nutrient/hormone-mediated changes in protein phosphorylation alter ChREBP activity:

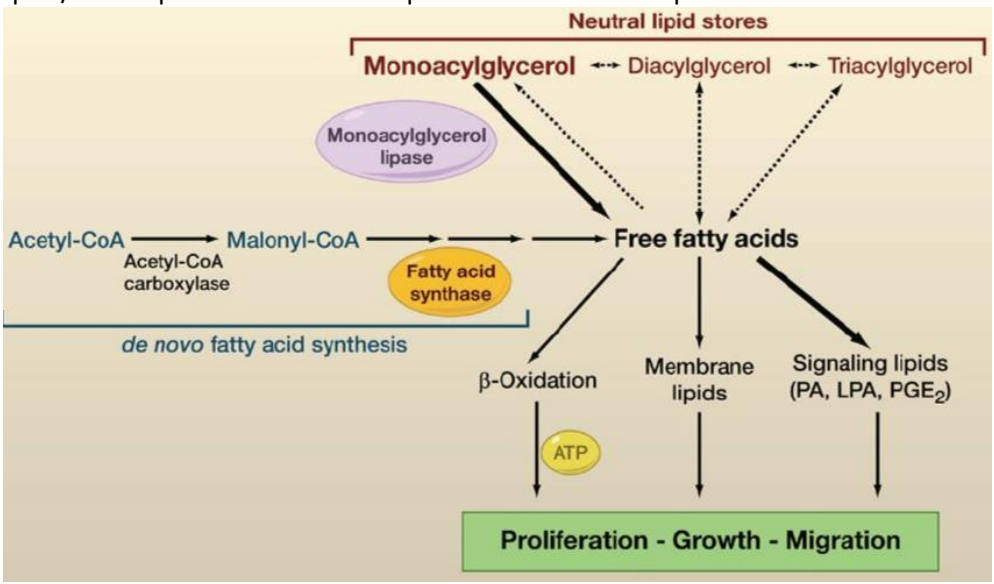


- ChREBP links glycolysis and lipogenesis in hepatocytes, by expression of Glycolysis enzymes!

- KO of SREBP-1a (Splicing-isoform of SREBP-1c) leads to massive liver with a lot of stored lipids (very light)
- Poly-unsaturated FA (PUFA) inhibit ChREBP and SREBP-1c expression and activation

Lipid Metabolism in Cancer

- Lipids/FA can promote different aspects of cancer development:



→ Adipose Lipases contribute to the formation of cancer!
→ Cancer profiteers of increased insulin level in blood (due to obesity or diabetes-2) by proliferation signalling and inflammation conditions

- FAs in physiology:
  - Two sources:
    - exogenously-derived (dietary) FAs
    - endogenously-synthesized FAs
  - Biosynthesis by the multifunctional, homodimeric fatty acid synthase (FAS or FASN)
  - Predominant product of FASN is palmitate (C16:0)
  - FASN gets ubiquitinated by USP2a and degraded by proteasome
  - 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 the synthesis of new structural lipids.
  - In normal conditions FASN converts excess carbohydrate into FAs that are then esterified to storage TAGs.
  - De novo FA synthesis is very active during embryogenesis and in fetal lungs (production of lung surfactant)
- FAs in cancer:
  - Tumors undergo de novo biogenesis of FAs irrespective of the levels of circulating lipids.
  - Neoplastic lipogenesis by significantly increased activity and expression of several lipogenic enzymes [e.g., FASN, ATP citrate lyase (ACLY), acetyl-CoA carboxylase (ACACA)].
  - Upregulation of FASN represents a nearly-universal phenotype in cancer cells
  - FAs synthesized in cancer cells are esterified predominantly to phospholipids and incorporated into membrane lipids
  - Increased FA synthesis in tumor cells seems to involve the modulation of multiple lipogenic enzymes at various levels (e.g., increased transcription, enhanced protein stabilization).
- Expression of FAS and thereby FA synthesis is promoted by SREBP-1c, which gets expressed upon Insulin induced PI3K/Akt and MAPK pathway.
- In tumor cells these pathways are also activated by GFs like oestrogen, androgen, progestin
  → Increased SREBP-1c expression and FA synthesis!

- Inhibition of FASN in cancer cell:
  - Disturbance of membrane function (GF signalling, etc)
  - Inhibition of DNA replication
  - p53: senses lipogenic stress → growth arrest
  - mutated p53: doesn't sense lipogenic stress →apoptosis
  - Inhibition of anti-apoptotic proteins (like Akt)
  - End-product starvation (phospholipids)
  - Toxic accumulation of malonyl-CoA → apoptosis

- Common gene network link cancer with lipid metabolism:
  - Many drugs used for treatment of diabetes and cardiovascular diseases inhibit transformation and tumor growth.
  - Lipid metabolism genes are important for transformation and are upregulated in cancer
  - As in atherosclerosis, oxidized LDL and its receptor OLR1 activate the inflammatory pathway through NF-κB, leading to transformation.
  - OLR1 is important for maintaining the transformed state in diverse cancer cell lines and for tumor growth, suggesting a molecular connection between cancer and atherosclerosis.

  → Drugs designed for treatment of metabolic diseases inhibit transformation and tumor growth!
  → OLR1 is important for tumor growth and overexpressed in late breast and prostate cancer!

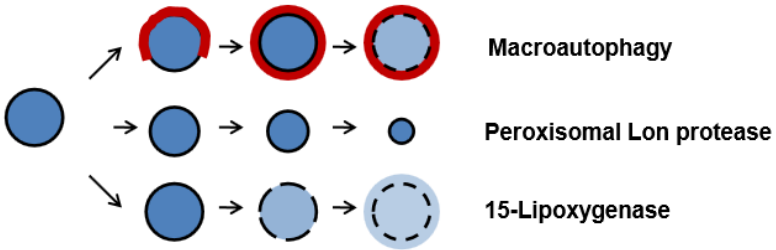
Peroxisomes

- Peroxisomal metabolism:
  - Enzymes involved in lipid and ROS metabolism depend on O_2!
  - Major metabolic pathways:
    - β-Oxidation (similar to mitochondrial β-Oxidation, but not complete)
    - Important steps of cholesterol synthesis
    - Bile acids conversion
    - H_2O_2 degradation
    - Ether lipids synthesis
    - PUFA synthesis and degradation
  - Peroxisomal deficiency would lead to
    - FA alterations
    - Oxidative stress
    - Missing Cholesterol precursors (in young)
    - Less steroid hormones, protein modifications, lipoproteins, ...
    - Dysregulated BA synthesis

    → ER stress/ISR → SREBP-2 activation → expression of C biosynthesis enzymes!

- Peroxisomal biogenesis:
  (SEE CORRESPONDING SECTION OF "CELLULAR BIOCHEMISTRY PART I" SUMMARY)
  - Peroxisomes and Mitochondria are divided by similar machineries
  - Defect of peroxisomal and mitochondrial fission is lethal

- Peroxisomal turnover:



  - Autophagy:
    (SEE CORRESPONDING SECTION OF "CELLULAR BIOCHEMISTRY PART I" SUMMARY)
  - Selective Peroxisomal Autophagy: Marco-/Micropexophagy

- Peroxisomal diseases: