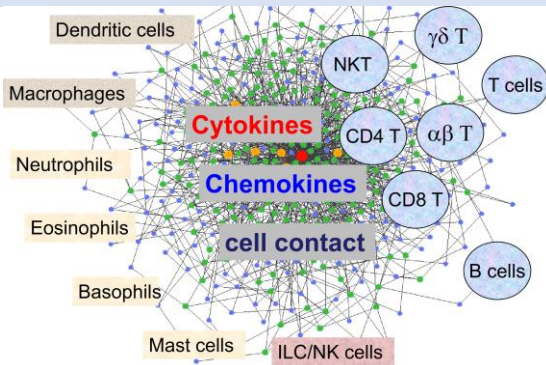# MOLECULAR DISEASE MECHANISMS

Summary 2019 - Carmen Joder

PART: KOPF
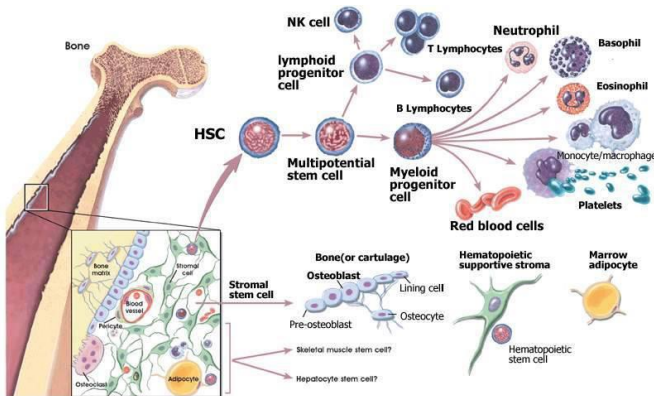
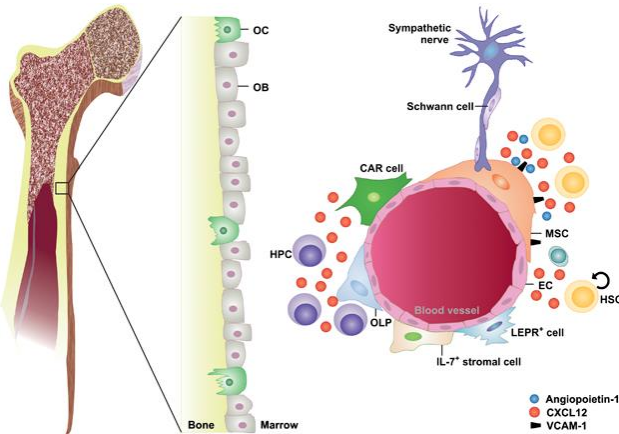## PART 1: IMMUNE SYSTEM AND ADAPTIVE IMMUNITY

IMMUNE CELLS

HEMATOPOIESIS

- Subdivisions into multipotential stem cells, myeloid and lymphoid progenitors
- Differentiation steps are regulated by transcription factors responding to signals from the environment

- Production of ≈ 10^{11} cells every day

- BM microenvironment: Various cell types including
  o HSCs
  o Osteoclasts (OCs)
  o Osteoblasts (OBs)
  o Osteolineage progenitor cells (OLPs)
  o Endothelial cells (ECs)
  o Mesenchymal stem/stromal cells (MSCs)
  o specialized CXCL12-abundant reticular (CAR) cells
  o Leptin receptor (LEPR)-positive cells
- HSC maintenance and differentiation are regulated through soluble factors such as CXCL12 and angiopoietin-1 or cell contact-dependent signals such vascular cell adhesion molecule-1 (VCAM1)

BLOOD CELL COUNTS

Cell type	cells/mm³ =µl	cells/µl	% leuko
Erythrocytes (M)	4.3 - 5.9 x10⁶		
(F)	3.5 - 5.0 x10⁶		
platelets	1.5 - 4 x10⁵		
leukocytes	5 - 7.5 x10³		
granulocytes		3200-6200	55-75%
lymphocytes		1500-3000	25-45%
monocytes		285- 500	3-7%
eosinophils		50-200	1-4%
basophils		15-50	< 1%

Erythrocytes: red blood cells (RBC)
Leucocytes: white blood cells (WBC)

- Eosinophil concentration increases during IR

TOLERANCE VS IMMUNITY

- Immune system needs to balance tolerance and immunity
  o Tolerant towards self
  o Immune towards tumors (the more mutations, the easier to recognize as foreign), pathogens, allergens (inappropriate), organ transplants

INNATE AND ADAPTIVE IMMUNITY

Non-specific Immunity = innate	Specific Immunity = adaptive
immediate response (minutes, hours)	lag time between exposure and maximal response (+/- 7 days).
Limited specificity (can distinguish between different types of pathogens)	highly antigen-specific (at the atomic level)
Exposure results in no immunologic memory (no difference betwee primary and secondary response)	Exposure results in immunologic memory (secondary response faster and stronger)

- Communication between innate and adaptive IS
- Innate cells are usually **very short-lived**, however may have some sort of **memory**
  o "Memory" turned out to be a product of epigenetic changes that last → changes take place in precursor cells, which can be passed on to daughter cells
- Long-lived T and B cells exist in the **periphery** → this is why vaccinations allow long term resistance

How long does it take until you can monitor the adaptive immune response?
After **7-8 days** one can begin to detect them (after expansion, they start dividing earlier)

ADAPTIVE IMMUNITY
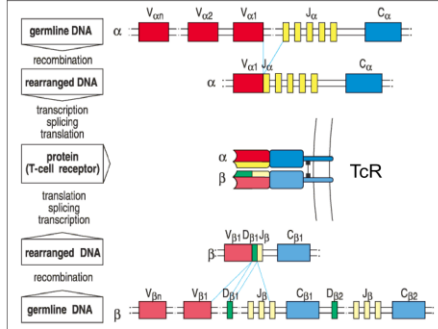
CARDINAL FEATURES OF ADAPTIVE IMMUNITY

- **Specificity**: Mediated by specific receptors expressed on B cells (BcR) and T cells (TcR)
- **Diversity**: B and T cell receptors have great variability due to DNA rearrangements → Every B and T cell have a unique antigen binding receptor
- **Clonal expansion**: B and T cells that recognize antigen become activated and proliferate
- **Self-limitation**: The response wanes elimination of antigen
- **Memory**: Second response is faster and stronger than the first
- **Self/non-self discrimination**

DIVERSITY
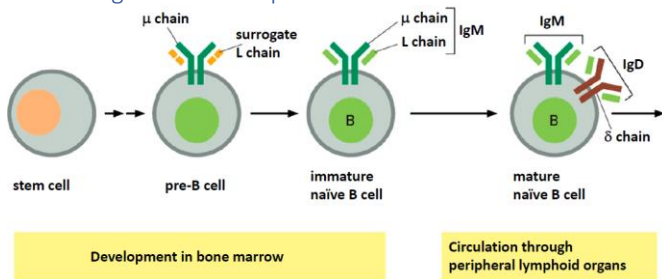
Diversity created by DNA rearrangement of BcR
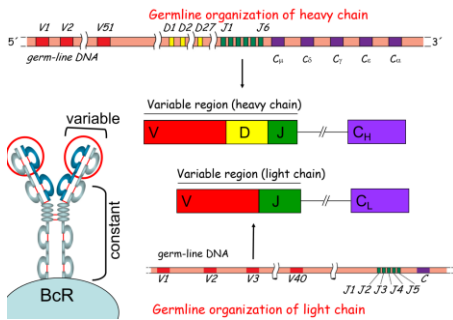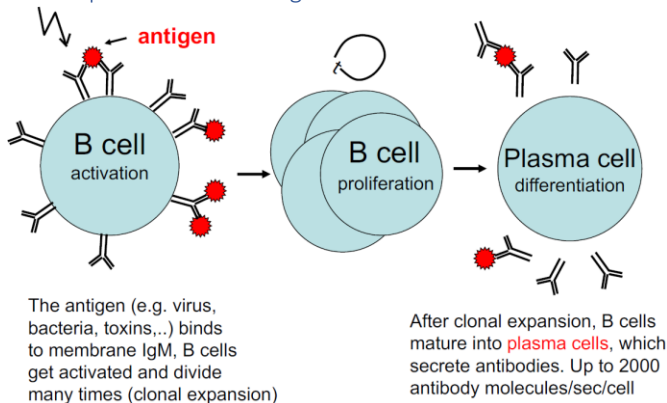

Diversity created by DNA rearrangement of TcR

B CELL DEVELOPMENT

Main Stages in Development

Development After Antigen Encounter

The antigen (e.g. virus, bacteria, toxins,..) binds to membrane IgM, B cells get activated and divide many times (clonal expansion)

After clonal expansion, B cells mature into plasma cells, which secrete antibodies. Up to 2000 antibody molecules/sec/cell
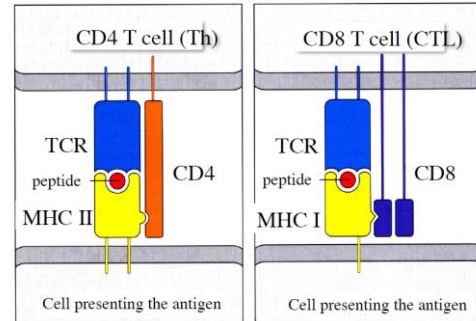
Antigen Recognition

- T and B cells recognize discrete sites on the antigen: **antigenic determinants** or **epitopes** (Epitopes are the immunologically active regions in an antigen)
  - o T cells recognize processed antigens (peptides of processed proteins) presented by self MHC molecules
  - o B cells recognize epitope of tertiary structure of a protein

CELLULAR IMMUNITY

- T-cells
- Antigen processing & presentation
- Major Histocompatibility Complex (MHC)

T CELLS

- TcR recognizes **small peptides** (8-20 amino acids) of proteins presented by **major histocompatibility complex (MHC) molecules** on **antigen presenting cells (APC):**


CD4⁺ T helper (Th) cells recognize peptide associated with MHC class II

CD8⁺ Cytotoxic T cells (CTL) recognize peptide associated with MHC class I
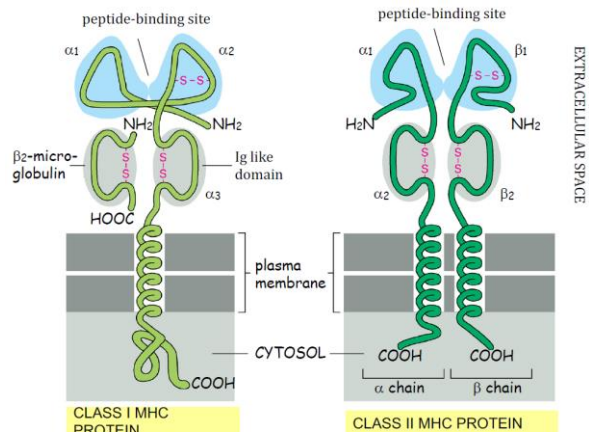
ANTIGEN PROCESSING & PRESENTATION

- **Fragmentation** of protein into peptides
- **Association** of peptide with an MHC molecule
- **Transport** to cell surface for expression
- **Different cellular pathways** for association of peptide with MHC class I and class II molecules
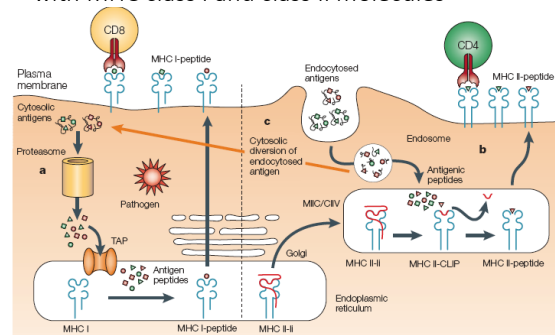
- There are **separate antigen-presenting pathways** for **endogenous** (are generated within a cell by normal metabolism or e.g. caused by a viral or intracellular bacterial infection) and **exogenous** (enter the body from the outside, are taken up by APCs) antigens:
  - o Intracellular proteins are processed in the ER and transported to the MHC I structures via the Golgi
  - o External proteins are cleaved in the lysosome and endosome, from where they are brought to the MHC II structure
- **Cross-presentation:** Ability of certain antigen-presenting cells to take up, process and present extracellular antigens with MHC class I molecules to CD8 T cells (cytotoxic T cells)

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

- <u>MHC I</u>: On **every cell** in the body with the exception of sperm and erythrocytes
- <u>MHC II</u>: Expressed only on **antigen presenting cells**

Diversity of MHCs

- Exists at the level of the **species** → not within an individual!
  - o The diversity is created in the population, we inherit the genes from our parents (while the T and B cell receptors diversity is generated in the individual)
  - o The chance that two people have the same combination of MHC genes is unlikely (5-10%).
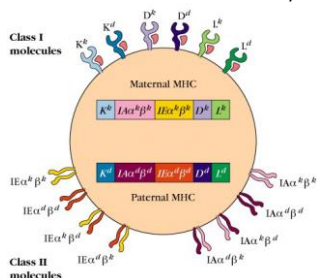- Arises from **polymorphism and polygeny**
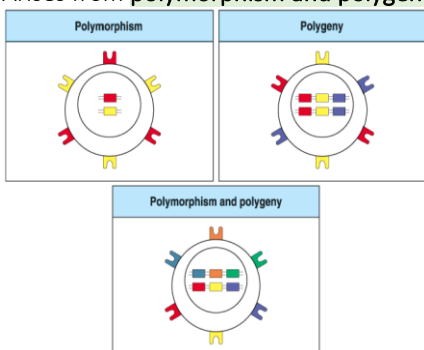
- MHC molecules are **highly polymorphic** and have **many variants**
  - o There are 3 genes for MHC I
  - o There are 3 genes for MHC II for alpha and beta chains each → total of 6
  - o There exist many variant alleles at class I and II loci
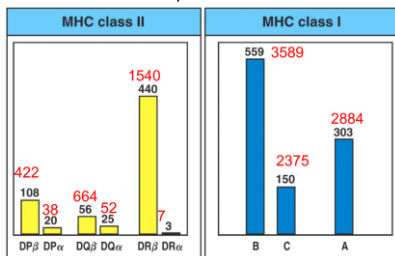

  - o Proteins are co-dominantly expressed

MHC molecules on a mouse antigen presenting cell

- Both the maternal and paternal molecules are expressed (6 different types)
- Class II MHCs are dimers, diffferent combinations are possible

<u>Advantages</u>: The more different the MHC genes of the parents, the better are the children protected from infectious disease → A broad spectrum of MHC genes provides greater chance to recognize diverse pathogen structures, which results in enhanced resistance

Self MHC Restriction

- T-cells recognize foreign antigen associated with <u>self</u> MHC → No value for an individual to have T cells that recognize foreign antigen associated with foreign MHC
- Self MHC restriction occurs in thymus (96% of cells die)
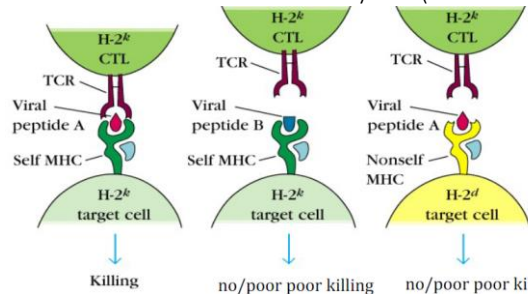
A particular TCR is specific for both, antigenic peptide and self MHC

- Foreign MHC alleles may enter the body via transplants → no response elicited

T CELL DEVELOPMENT IN THE THYMUS

- Immature T-cell **precursors** from bone marrow enter the thymus and leave it as **mature T-cells**
  - o Thymus is the main source of T-cells
- 96% of the cells die here because they either **do not recognize self MHC (first test)** or **recognize self-proteins** (second test, negative selection)
  - o T cells die by apoptosis without inducing inflammation → macrophages phagocytose apoptotic thymocytes
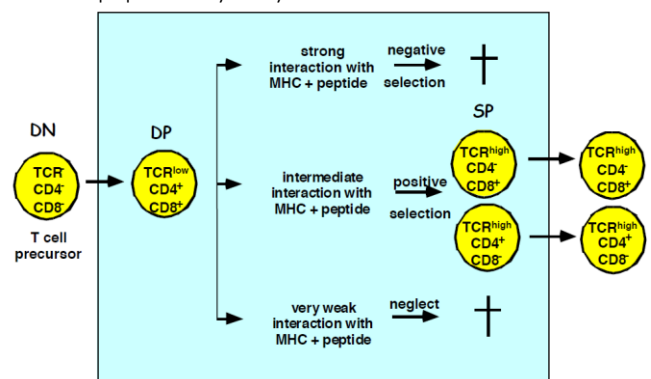
- T cell maturation and diversification
  - o TCR rearrangement
  - o Decision to become CD4 (T helper cell) or CD8 (Cytotoxic T cell) single positive
- Positive Selection:
  - o Eliminates T cells unable to recognize self-MHC
  - o Is the basis of MHC restriction
- Negative Selection:
  - o Eliminates thymocytes bearing high affinity TCR for MHC-self peptide complex produces self-tolerance

Central questions on central tolerance:

- *How can the thymus express all self-antigens – including self-antigens only made by specialized tissues?*
  → There exists a population of cells (**medullary epithelial cells, MEC**) in a specific lobe within the thymus that **expresses a plethora of tissue specific antigens represented in the body**

- *How do we become self-tolerant to these antigens?*
  → **AIRE** (autoimmune regulator) **Transcription Factor** is a "master" regulator of ectopic (out of place) expression of peripheral tissue-restricted antigens in stromal cells of the thymic medulla
  → **AIRE** enhances the expression of genes by **medullary epithelial cells (MECs)** that would normally only be expressed in specific tissues
  o Important gene for thymic selection
  o Mechanism prevents the autoimmune attack of peripheral organs
  o Is mutated in many autoimmune patients
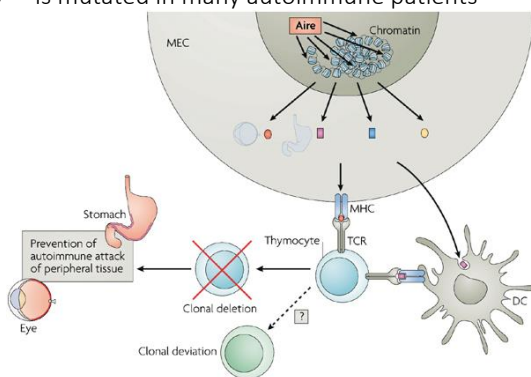
- Negative selection is central tolerance - not sufficient to prevent self-attack, peripheral tolerance also needed
-

T-CELL ACTIVATION AND PROLIFERATION

- CD4+ and CD8+ T cells leave thymus and enter circulation. They are in a resting (G$_0$ of cell cycle)
- Naive cells recirculate between blood and lymph system every 12-24h
- 90-95% of peripheral T cells express αβ-TCR (remaining are $\gamma\delta$ T cells)
- CD4:CD8 T cell ratio in lymphoid organs ≈ 2: 1
- Activation of naive T cell results in primary response: After 24 hours, initiation of repeated rounds of cell division and differentiation into:
  o Effector cells: cytokine producers (CD4, helper cells), cytotoxic killers (CD8, cytotoxic killers)
 o Memory cells – long lived, respond faster and with heightened activity in a secondary response
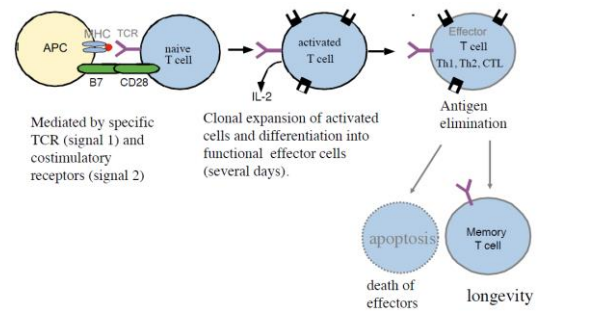
TWO SIGNAL MODEL FOR T CELL ACTIVATION

- **Two signals are required** for T-cell activation

- **Costimulatory molecules** are expressed by **most APC** including *dendritic cells, monocytes, macrophages, B cells* etc. but not by cells that have no immunoregulatory functions such as muscle, nerves, hepatocytes, epithelial cells etc.

The T-Cell Growth Factor IL-2

- Resting T-cells express receptor IL-2R β and γ chains but __no α chain or IL-2__
- Upon antigen encounter, the transcription factor NFAT binds to the promoter of IL-2Rα chain gene → α chain converts the low affinity IL-2R to a high affinity form
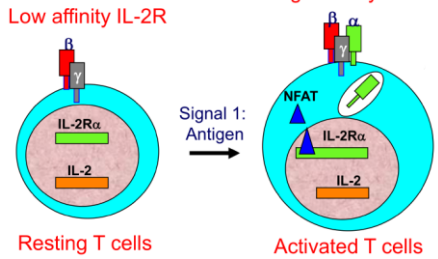

The CD28 Family

- CD28 is a family of **co-stimulatory molecules** for the interaction between T cells and APCs
  o CD28 and ICOS provide positive signals
  o CTLA-4 and PD-1 provide negative signals
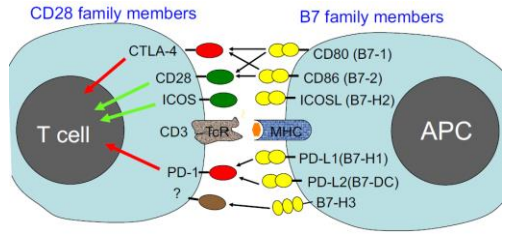
- The exchange of information between the APC and T-cell determines the state of the T cell → resting to active or off state
- This concept is important for immunotherapy

The TNF Receptor and Ligand Superfamily (CD40)

- Members of the **TNF receptor and ligand superfamily** are also essential for **crosstalk between APCs and T-cells**
- **CD40** is important in the **interaction of B-cells and T helper cells** → Th cells express CD40L after activation and co-stimulate B cells by triggering of CD40:
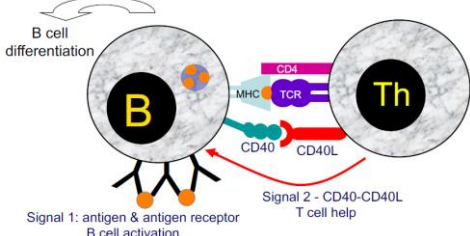
CD4+ TH-DEPENDENT B-CELL RESPONSES

1. B-cell activation by antigen mediated crosslinking of the B Cell Receptor → results in upregulation of MHC II and B7 molecules (CD80/CD86)
2. Antigen presentation to T-cell
3. T-cell activation, upregulation of CD40L, production of cytokines
4. CD40 and cytokine-mediated activation of the B cell
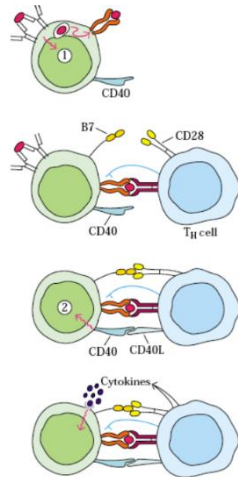
- Once the B-cell has been activated, there are **growth factors** which allow upregulation of certain receptors on the surface - sort of positive feedback loop
- These signals (e.g. T-cell cytokines) help the cells to decide on which constant region in the Igs (e.g. IgG, IgA, IgE) should be produced → **isotype switch** (involves **somatic hypermutation**)
- The type of Igs also determines the function / role of the B-cells
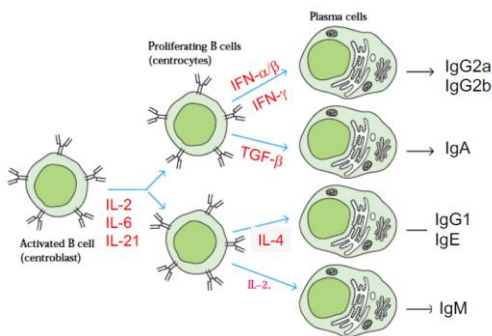
- Th cells promote survival of CD8 memory T cells

T HELPER CELLS

- CD4+ T cells play a central role in the control of **adaptive immune responses**
- CD4+ T cells can be activated to **secrete various cytokines** which in turn determines their function
- The correct activation of CD4+ T cells determines immunity or death following pathogen encounter
- CD4+ T cell differentiation is determined by the pathogen and the APC and the tissue environment
- Different subsets of Th cells exist, depending on which cytokines they secrete:
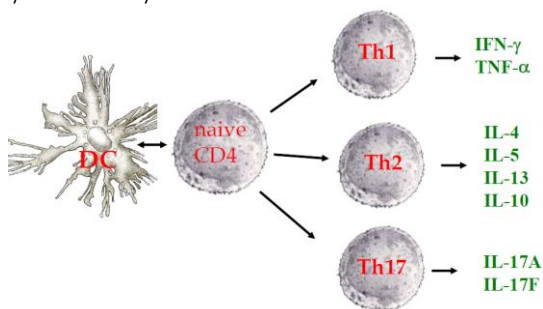

FACTORS AND CONDITIONS REGULATING T HELPER SUBSET DEVELOPMENT

- Cytokines (IL-4, IL-12, IFN-g, IL-6, TGF-ß)
- Strength of T cell stimulation (e.g. antigen dose, TCR affinity, costimulation)
- Type of antigen presenting cell
- Pathogen associated molecular patterns (PAMPs)
- Microenvironment (tissue)
- Epigenetics
- Diet (vitamins, lipids, oxidants)

- The **cytokine environment directs Th subset development:**
  o **IL-12** promotes Th1 and inhibits Th2 development
  o **IL-4** promotes Th2 development
  o **IL-6** and **TGF-β** promote Th17 development
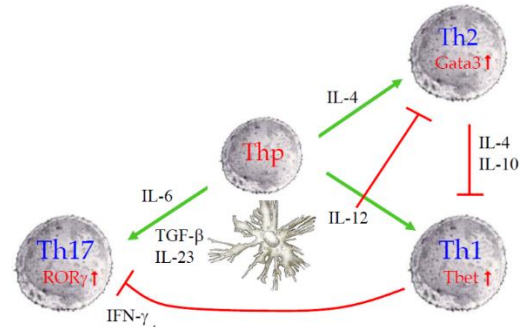

- Subset of **specific transcription factors** are the main regulators of Th subset development
  o Deletion or inhibition of the transcription factors **(Tbet, Gata3, RORγ)** abrogates development of the respective subsets

TH1 CELLS

- Associated with **intracellular infection/ microorganisms**
  o **protozoa**
  o **bacteria**
  o **viruses**
- Provide **protection from intracellular microorganisms** (e.g. *Mycobacterium tuberculosis* and other bacteria, viruses and protozoa e.g. *Leishmania*)
- **IL-12** is needed for the **activation** of Th1 cells
- **IFN-γ** is the **key cytokine** produced by Th1 cells, which is **absolutely essential for activation of macrophages**
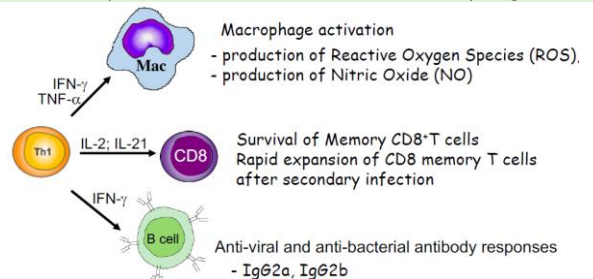
- Mice lacking **IL-12 or IFN-γ** (IFN-γ is more important than IL-12) are susceptible (anfällig) to infection with *Mycobacterium tuberculosis* and *Listeria infection*

$$\text{CD4+ T} \xrightarrow{IL-12} \text{TH1 cell} \rightarrow \text{INF-γ} \leftarrow \text{NK-cells}$$
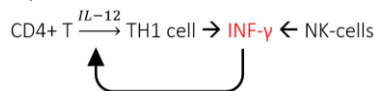
- *M. tuberculosis* infection in IFN-γ-deficient (IFN-γ -/-) mice leads to high bacterial load, increased lethality and impaired nitric oxide production
- IFN-γ defect children die from simple bacterial infection

LEISHMANIA SPP
- About 20 species and subspecies

Forms of Leishmaniasis:
- **Visceral**
  - o Parasite: *L. donovani, L. chagasi*
  - o Usually people become sick within several months
  - o Infects **retikuloendothelial system** (RES), **spleen**, **liver**, and **bone marrow**
  - o Prolonged **fever** and **weight loss**, **enlarged spleen and liver**, may cause **death** if not treated
- **Cutaneous**:
  - o Parasite: *L. major, L. mexicana, L. braziliensis*
  - o **Skin**, may spread to **nose**, **mouth** (mucocutaneous)
  - o Within a few weeks after bite, patients develop **single or multiple lesions**, which typically progress from **papules** to **nodules** to **non-ulcerated dry plaques** or **ulcers** → Will often heal on their own, but can take months to years and local trauma can activate latent infection

Transmittance:
- Bite of a **sandfly** (30 different, 1/3 of the size of a mosquito, usually are most active in twilight, evening and night-time hours)

Epidemiology:
- **90** countries worldwide affected
- 90% visceral Leishmaniasis mainly in India, Bangladesh, Nepal, Sudan, Brazil

- BALB/c mice are highly susceptible *(anfällig)* and mount **inappropriate IL-4 (Th2) response**
- :-) Knockout of **IL-4 or IL-4R** protects genetically susceptible BALB/c mice from disease
- Lack/neutralization of **IL-10** in susceptible mice (BALB/c) results in impaired parasite growth
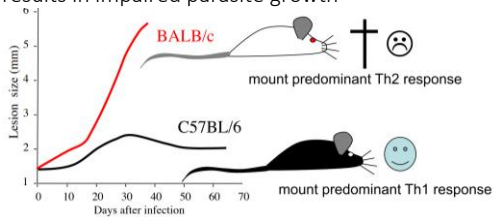
- C57BL/6 mice are protected from disease and mount predominant **IFN-γ (Th1) response**
- :-(Knockout of **IL-12 or IFN-γ** makes C57BL/6 mice **highly susceptible** to leishmaniasis

IFN-γ
- IFN-γ is essential for **activation of inflammatory macrophages** which are characterized by the production of **nitric oxide (NO)**
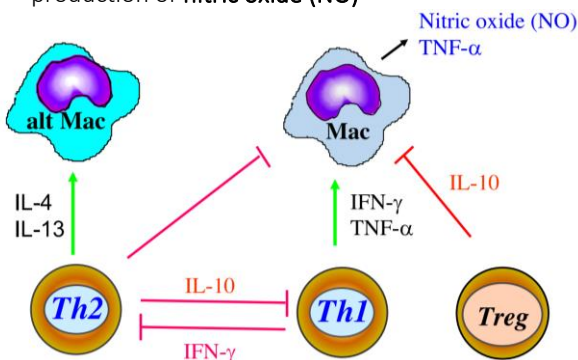

Activated Macrophage → NOS activation → NO
- **IFN-γ and TNF-α** produced by Th1 induce "classical" macrophages
- **IL-10** inhibits activation of "classical" macrophages (IL-10 is an **immunosuppressive** cytokine)
- **IL-4** and **IL-13** induce "alternative" macrophages

NO
- NO is **essential for defense** against *Leishmania* and other protozoa
- Enzyme: **Nitric oxid synthetase (NOS)** produces nitric oxide (NO) and citrulline by using **L-arginine** in the prescence of O_2 and **NADPH** with help of FAD and Tetrahydrobiopterin (BH4)
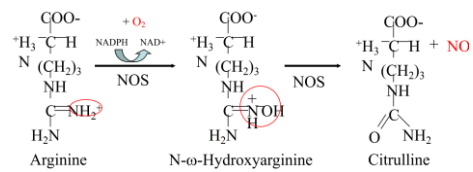
LEPROSY
- Causes **tissue and demyelinating lesions** in the peripheral nerves
- Infectious agent: *Mycobacterium leprae*, which infects **macrophages** as well as **dendritic** and **Schwann cells**
- Infection shows **two main clinical forms** associated with **Th1 and Th2 responses**:
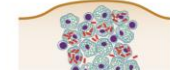
TH2 CELLS
- Associated with helminth infection (fight the worms) and allergies
- **IL-4** and **IL-13** mediated expulsion of nematodes
(i.e. Nippostrongylus, Trichuris, Trichinella, eligmosomoides)
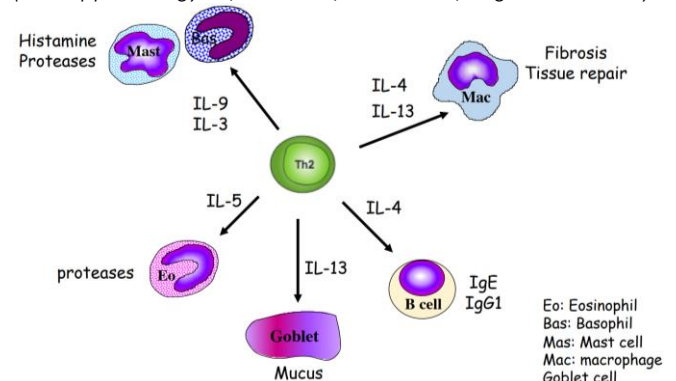
- Th2 response is responsible to take care of the tissue damage due to the nematodes → **Tissue repair by macrophages**
- **IL-13** is a very important one! → leads to **mucus production of goblet cells** and **peristaltic movement** → mucus protects the epithelia so that the worms cannot adhere to the epithelia → worms are cleared
- **IL-4 receptors:** There are two (3) types of receptors:
  o Type 1 IL-4R: binds only IL-4
  o Type 2 IL-4R: binds IL-4 and IL-13
  o IL-13 decoy receptor: binds IL-13 but doesn't trigger a reaction → competitive binding without answer
- IL-4-/- and IL-13-/- mice are susceptible to infection with *Trichuris muris*
  o Female IL-4-/- are resistant but if you block IL-13 the IL4-/- mice get susceptible again → Female can produce **IL-13 to compensate the IL-4**

→ IL-4 and IL-13 share activities → Redundancy
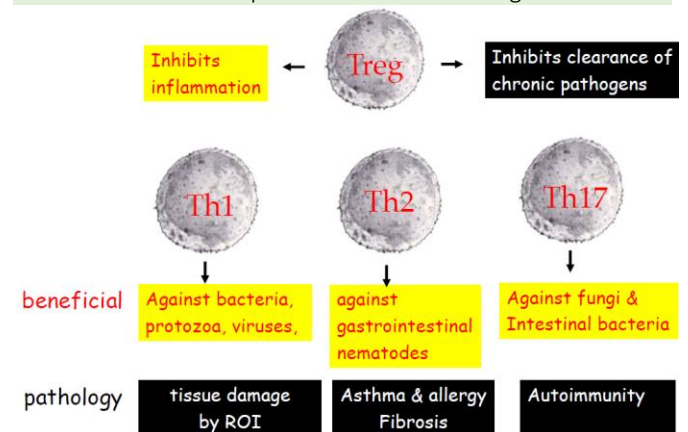
SCHISTOSOMA (ROUND WORM) INFECTION
- 200 million people are infected worldwide
- Disease: *Bilharziosis or Schistosomiasis*
- Adult schistosomes live in pairs in the portal system an in the mesenteric venules
- **Chronic disease** is the result of the ongoing host response to accumulation of tissue-trapped eggs in infection
- **Digestive** (damage in the intestine), **Lung, Liver** (many eggs are carried to the liver via blood flow → eggs die → granulomas resolve, leaving fibrotic plaques → liver becomes fibrotic), **increase of blood pressures**

- **IL-4/IL-4R** signaling in **macrophages** is essential to prevent mortality from *Schistosoma*
  o IL-4R signaling in macrophages prevents intestinal and liver damage
- IL-4/IL-13 inhibits chronic intestinal inflammatory responses
  o Lack IL-4: Mice dies after infection

IL-4 and IL-13 are cytokines secreted by Th2 cells and **induce alternatively activated macrophages** characterized by Arginase activity

- Macrophages get activated by IL-4 and IL-13 and have a repair function
  → Mice die due to the absence of the macrophages which leads to leakiness in the intestine → macrophages fail to heal the damage in the intestinal → bacterial shock

TH17 CELLS
- Promote clearance of <u>fungal and bacterial infection at barrier tissues</u> such as intestines, lung and skin
(*Klebsiella pneumonia, Bordetalla pertusis, Citrobacter rodentium*)

TH PATHOLOGY
Th cells also have the potential to harm the organism:
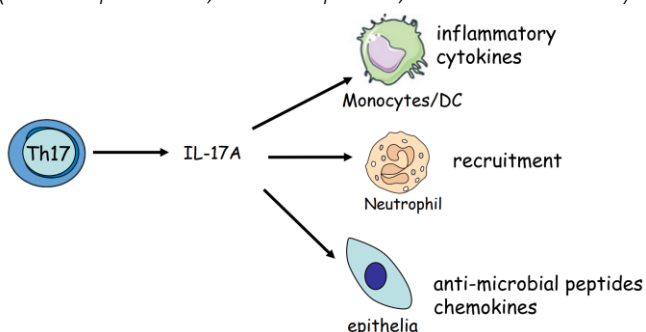
T CELL TOLERANCE AND AUTOIMMUNITY

CENTRAL TOLERANCE

Central tolerance: Process of **eliminating** developing T- or B-cells that are **reactive to self**

- This kind of tolerance is <u>not sufficient</u> because of the following reasons, **not all self-reactive T cells (and B cells) are deleted**:
  - o Peripheral tissue specific antigens not expressed in the thymus
  - o Expression of neo-antigens (tissue damage, puberty)
  - o Positive selection of specificities that exhibit weak self-reactivity but with the propensity for pathogenic autoreactivity (there is a threshold requirement for affinity to self-antigens before clonal deletion is triggered)
  - o Need for a peripheral repertoire that will protect from pathogens

CENTRAL AND PERIPHERAL TOLERANCE

<u>Central tolerance</u>:
Negative selection of autoreactive T cells in the thymus
→ is <u>not sufficient</u> → not all self-reactive T cells are deleted
→ Peripheral tolerance should compensate for this

<u>Peripheral tolerance:</u>
Unresponsiveness of peripheral T cells
- Suppression (or dominant regulation)
- Negative and anti-inflammatory signals

<u>Peripheral tolerance is mediated by:</u>
- Regulatory T cells (Treg)
- Anti-inflammatory cytokines
- Co-stimulatory & inhibitory surface receptors (signal 2)
- Dendritic cells (environmental cues)

ANTI-INFLAMMATORY AND IMMUNO-REGULATORY MICROENVIRONMENT

- **Anti-inflammatory factors**
  - o E.g. the cytokines Transforming Growth Factor-b (TGF-β) and Interleukin-10 (IL-10) can potently <u>inhibit</u> inflammation
- **Negative signals**
  - o E.g. the cell surface molecules CTLA4 and PD-1 expressed on T-cells <u>interfere</u> with **T-cell responses**
- **Expression of Death Receptors on T-cells**
  - o Cell surface or secreted molecules that bind to death receptors on T-cells <u>induce cell death</u> by apoptosis
  - o E.g. Fas-ligand binds to Fas-Receptor (CD95)
 - o Trail binds to death receptors DR4 and DR5

SUPPRESSION BY REGULATORY T-CELLS (TREGS)

- **Dominant regulation/ suppression:** Special T-cells (Regulatory T-cells/ Treg) prevent autoreactive T-cells in the host to react
- Regulatory T cells = **Treg**:
  - o Population of T-cells
  - o Express surface markers **CD4+** and **CD25+** and the transcription factor Foxp3
  - o Of thymic origin or generated in the periphery

FUNCTION OF TREGS

**Inhibit** proliferation & cytokine production of other T-cells
  - o Inhibitory signals mediated by **cytokine secretion (IL-10, TGF-b)** and/or **receptors (i.e. CTLA-4)** and many more
- Regulatory T-cells can **suppress** both effector **CD8+** and **CD4 T-cell subsets:**
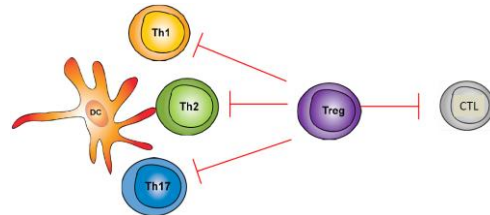
- The function of Tregs is to **suppress**:
  - o Autoimmunity
  - o Inflammatory bowel disease (colitis)
  - o Allergies
  - o Spontaneous abortion of the fetus
  - o Organ transplant rejection
  - o Responses to various pathogens (especially persistent)
  - o Recognition and rejection of tumor cells
- In vivo, the cells can **limit disease development** and **pathology**
- Function: A double edged sword:

HOW TREGS WORK
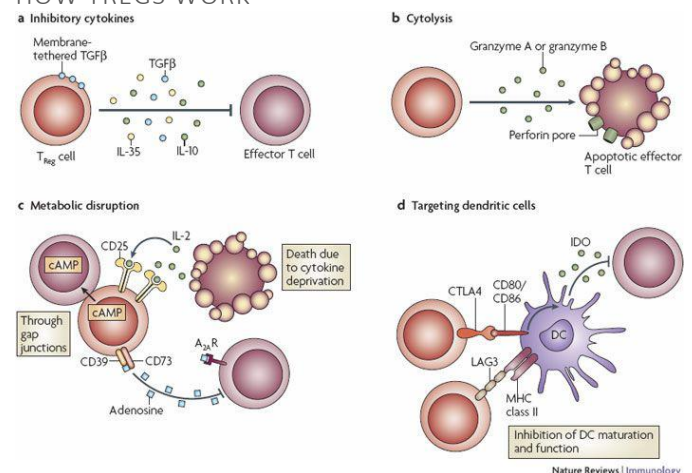
a) <u>Inhibitory cytokines:</u> Include interleukin-10 (IL- 10), IL-35 and transforming growth factor-β (TGFβ)
b) <u>Cytolysis:</u> Includes granzyme-A- and granzyme-B-dependent and perforin-dependent killing mechanisms
c) **Metabolic disruption**: Includes:
  - o **Apoptosis** → Caused by deprivation of High-affinity CD25 (also known as IL-2 receptor α)
  - o **Inhibition** → Mediated by Cyclic AMP (cAMP)
  - o **Immunosuppression** → CD39 converts ATP to AMP, CD73 AMP to adenosine → Adenosine binds to the receptor
d) **Targeting dendritic cells:** Includes mechanisms that modulate DC maturation and/or function
  - o Induction of IDO (=immunosuppressive molecule made by DCs)

IL-2
- IL-2 is essential for Treg-cell homeostasis/maintenance
- Mice lacking IL-2, IL-2Rα or IL2Rβ deelop fatal inflammatory disease

TRANSCRIPTION FACTOR FOXP3
- Foxp3 is essential for Treg cell function
- **Scurfy**: Spontaneous mutation in Foxp3 gene (introduces a premature stop codon) results in:
    o Presence of **activated lymphocytes infiltrating** multiple non-lymphoid organ systems such as liver and lung tissue
    o Severe gut-wasting syndrome
    o Production of autoantibodies
    o Lethality at 3-4 weeks of age
- Adoptive transfer of CD4$^+$ CD25$^+$ T$_R$-cells from WT-mice into neonatal scurfy mice protected them from disease

THE IPEX SYNDROME
- IPEX is caused by **mutations of Foxp3**
- Patients with **IPEX syndrome** (*Immune dysregulation, polyendocrinopathy, enteropathy, X-linked*) develop **disease similar to scurfy** mice:
    o Severe diarrhea, disorder of the intestines
    o Skin inflammation (dermatitis)
    o Multiple disorders of endocrine glands such as type-1 diabetes, thyroiditis
    o Autoimmune blood disorders including low levels of red blood cells (anemia), platelets (thrombocytopenia), white blood cells (neutropenia) because cells are attacked by the immune system
    o Early onset (age: 3-4 weeks) of autoimmune disease
    o Fatal in boys during early infancy or early childhood

AUTOIMMUNITY
= A **chronic inflammatory disease** that results from a **breakdown of tolerance**
- Increasing incidence in industrialized countries
- No cures! (Treatments now allow near normal life-span)
- Typical are **periods of flares and remissions**, which can last from days to months and occasionally years
- **Stress** and **infections** can cause flares in the disease
- Approximately **100 diseases**
- Top 10 autoimmune diseases include: *Rheumatoud artritis, Hashimoto's thyroiditis, Type 1 diabetes, Multiple sclerosis, Systemic lupus E*

RISK FACTOR GENS AND ENVIRONMENT:
- Autoimmunity is more common in females (x75% are women) (Is a major cause of death in women)
- There are also some **significant associations of MHC alleles** with increased risk for various autoimmune diseases
- Probability for **identical twins** to develop common autoimmune diseases is **"only" 20-40%**
    → **Environmental factors play an important role** in the development of autoimmune diseases
        o Pathogens
        o Nutrition/ Diet (life style)
        o Microbiota

- Autoimmunity occurs when the **different mechanisms of tolerance fail,** and the **immune system attacks self-tissue**
- The diseases are **chronic**, currently **no cures** are available, **women** are affected more than men
- The **release of sequestered antigens**, infections by **pathogens** and **genetic predisposition** play central roles in the induction of autoimmunity
- The diseases are typically mediated by **self-reactive T cells and autoantibodies**

INDUCTION OF AUTOIMMUNITY
- A **failure of any mechanism of tolerance**
- **Pathogens** may trigger autoimmunity = **molecular mimicry**
- **Modification of self-antigens**
- **Release** of antigen from **immunoprivileged** sites
→ Usually more than one factor will be involved in the initiation of the disease!!

MOLECULAR MIMICRY
Mimicry: Molecular structures of microorganisms have similarities to self-molecules → can lead to autoimmune disease → Immune system (cross-reactive T-cells or antibodies) mistakenly attacks self-molecules and cause autoimmune disease
EXAMPLES:
- Some antibodies raised against *Treponema pallidum* can cross-react with certain erythrocyte blood group antigens, leading to anemia
- Antigens common to **Trypanosoma cruzi** and some *Streptococcus A bacteria* cross react with human cardiac muscle
- Antigens from the bacteria *Borrelia burgdorferi* mimic a self- protein, which is expressed on the surface of most T cells, B cells, and APC
**Lyme disease**: chronic arthritis induced by infection with *Borrelia burgdorferi (*OspA is a molecule of Borrelia b. that mimics the self molecule LFA-1 (CD18) expressed by leucocytes). HLA-DR1 presents peptides of both OspA and also LFA-1. Individuals that encode HLA-DR1 molecule have been shown to develop arthritis

MULTIPLE SCLEROSIS (MS)

- Caused by an **immune response against myelin**
  o Myelin forms a sheath around certain neurons allowing fast conduction of nerve impulses
  o Demyelination slows nerve impulses
- The **HLA-DR2 haplotype** remains the strongest identified genetic risk factor for MS in Caucasians

GENERATION OF HUMANIZED MICE ENCODING A HUMAN SUSCEPTIBILITY MHC (DRB1*1501 = DR2B) AND T CELL RECEPTRO FROM A MS PATIENT:

HLA-DR2a molecule modifies multiple sclerosis-like disease mediated by the HLA-DR2b molecule by functional epistasis:
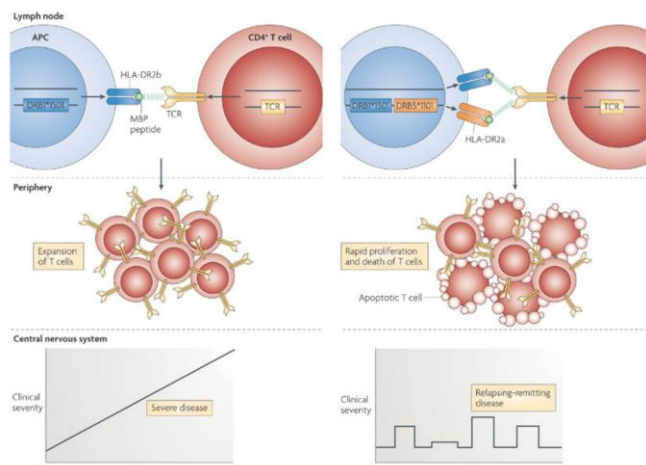

Left: MHC class II molecule HLA-DR2b was co-expressed with a CD4+ T-cell receptor (TCR) derived from a patient with multiple sclerosis → TCR recognizes an autoantigenic peptide from myelin basic protein (MBP) when presented by the HLA-DR2b molecule → Interaction leads to **severe, progressive multiple sclerosis**
Right: Additional expression of HLA-DR2a leads to more **rapid T-cell proliferation** and then deletion of T-cells in the periphery → Results in greatly moderated disease that resembles the most common form of multiple sclerosis, relapsing remitting disease

MODEL FOR MS

→ Experimental Autoimmune Encephalomyelitis (EAE)

- Mice were immunized with myelin basic protein (MBP, in other experiments MOG, another protein in the myelin sheath) and the adjuvant CFA
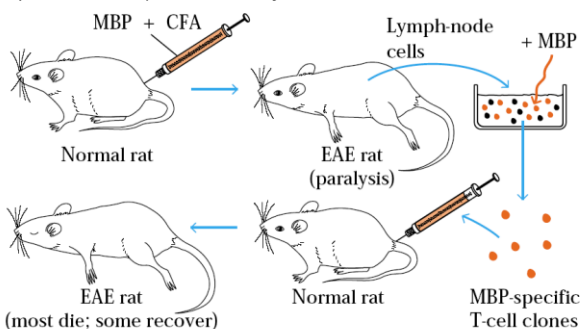
- **IL-6, IL-1** and **IL-23** are essential for development of MOG-induced EAE
- **RORγ knockout mice** are protected from EAE due to the absence of IL-17 producing Th17 cells → Th17 cells are essential for development of organ related autoimmune disease
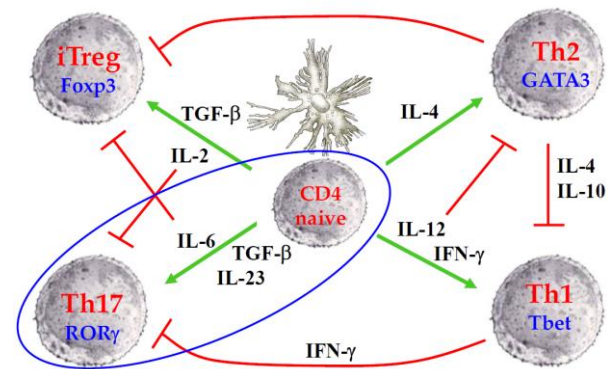
DIABETES MELLITUS

- Main symptom: **Hyperglycemia**
- The hormone **Insulin** plays a crucial metabolic role as a **mediator of glucose transport** across cell membranes and **inhibitor of gluconeogenesis**

Type 1 DM = insulin-dependent
- = "Juvenile diabetes"
- Lack of insulin due to destruction of pancreas islet cells.
- Mainly due to autoimmunity
  o Susceptibility to IDDM is polygenic

Type 2 DM = insulin-independent
- = "Maturity-onset diabetes"
- Insulin resistance (e.g. the number of free insulin receptors on a cell is reduced)

SPONTANEOUS AUTOIMMUNE DISEASE MODELS

- **Non-obese diabetic (NOD) mice:**
  o Develop **insulin-dependent** diabetes mellitus (IDDM)
  o NOD mice have a mutation in the CTLA-4 gene → isn't functioning properly → CTLA-4 T-cells attack insulin producing cells
  o Environment exerts a strong effect on gene penetrances → Housing conditions, health status, and diet all affect development of diabetes in the mice
  o Incidence is 60-80% in females and 20-30% in male
  o Incidence of disease is **linked to microbiome**
  o Susceptible to develop other autoimmune syndromes
  o Diabetes in these mice can be prevented by a single injection of mycobacterial adjuvants
- **New Zealand Black (MZB) mice:**
  o Display many autoimmune abnormalities
  o Are widely used as a model for **human systemic lupus erythematosus**

THERAPIES OF AUTOIMMUNE DISEASES

- **Conventional therapeutic approaches:**
  o Anti-inflammatory and immunosuppressive reagents (cortisone (naturally produced by adrenal gland) or methotrexate (antagonist of VitB9 = folic acid))
- **New therapies**:
  o Reagents blocking activity of TNF-α, IL-6, IL-1 with antibodies (anti-TNF- α, anti-IL-6R, anti-IL-1)
  o Depletion of B-cells with anti-CD20 (treatment of SLE)
- **Experimental/New approaches**
  o Antibodies against pro-inflammatory cytokines (IL-6R, IL-17)
  o Vaccination
  o Altered peptide ligands

ALLERGIES & ASTHMA

HYPERSENSITIVITY REACTIONS

Hypersensitivity refers to **undesirable** (discomfort producing and sometimes fatal) **reactions** produced by the **immune system**

- Hypersensitivity reactions **require a pre-sensitized (immune) state of the host**
- Hypersensitivity reactions can be divided into **four types,** based on the mechanisms involved and the time taken for the reaction:
  o <u>Immediate hypersensitivity reactions</u>: Types I, II, III
  o <u>Delayed hypersensitivity reaction</u>: Type IV

TYPE I HYPERSENSITIVITY

Also known as **immediate or anaphylactic hypersensitivity**

- Reaction may involve
  o skin (eczema)
  o eyes (conjunctivitis)
  o nasopharynx (rhinitis)
  o bronchopulmonary tissues (asthma)
  o gastrointestinal tract (gastroenteritis)
- Reaction may cause from minor inconvenience to death
- Reaction takes **15-30 minutes** from the time of antigen exposure (sometimes reaction have delayed onset, 10-12h)

Type I hypersensitivity is **IgE-mediated**

- Occurs in **three basic steps**:
1. Sensitization involves development of a **Th2- dependent IgE response** following encounter with an environmental antigen (allergen)
2. Subsequent exposure to the **same allergen** results in **cross-linking of specific IgE** present on the surface of mast cells and basophils → Cross-linking of the receptors induces signaling
3. Mast cells or basophils release preformed **pharmacologically active mediators**
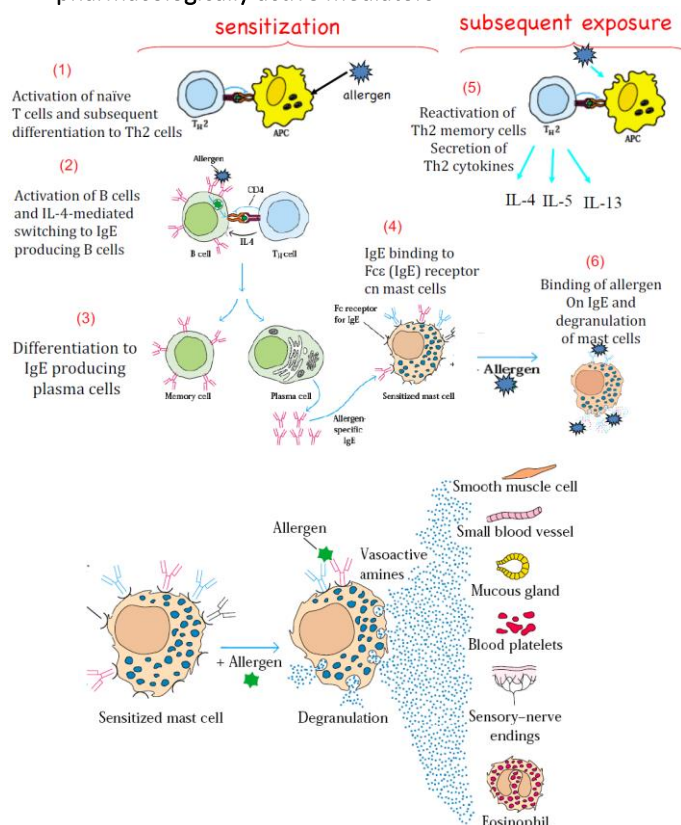
One of the released compounds is **histamine** which is responsible for:
- Dilated blood vessels and increased blood vessel permeability → Edema
- Activated endothelium → Cell influx
- Irritated nerve endings → Itching

CONSEQUENCES OF TYPE 1 HYPERSENSITIVITY REACTIONS:

<u>Systemic Anaphylaxis:</u>
- Shock like and often fatal systemic reaction that occurs within minutes induced by a wide range of antigens including
  o Venom from bee, wasp, or hornet
  o Drugs such as penicillin, insulin, and antitoxins
  o Seafood such as lobster
  o Nuts
- Mediated by endogenous substances mainly by **histamine and leukotrienes**, which **increase vascular permeability** and **induce smooth muscle contraction** → vascular collapse and cardiac failure

<u>Localized anaphylaxis (Atopy)</u>
- Restricted to a specific target tissue often involving surfaces at the site of allergen entry
- Atopy: A hereditary predisposition to mount localized anaphylactic reactions and an inappropriate high IgE response e.g. hay fever (rhinitis), asthma, atopic dermatitis (eczema), and food allergies

ALLERGIC REACTIONS

ACUTE PHASE ALLERGIC REACTIONS
- Occurs **within seconds to minutes** of **IgE receptor activation** (mast cell mediator release) and resolving within an hour
- Intense pruritus (itching), edema, erythema
- Almost all effects can be replicated with **histamine**

LATE PHASE ALLERGIC REACTIONS:
- A delayed inflammatory response (peaking at 4-8 hrs and persisting up to 24 hrs) following an intense acute phase reaction
  o Skin: erythema, induration, burning
  o Lungs: airway obstruction poorly responsive to bronchodilators
  o Nose/eyes: erythema, congestion, burning
- Histology: Mast cell degranulation followed by influx of first Neutrophils and eosinophils followed by mononuclear cells
- Major portion of effects replicated by TNF-α

ASTHMA

THE LUNG

- Lung surface area of a healthy human adult: **~90 m^2**, (Gut: ~10 m^2 ; Skin: ~2 m^2)
- Surface area of the pulmonary capillaries encompassing the alveoli: **~140m^2**
- Bronchioles & alveoli filter **~8500 liters of air** every day
- Continuous exposure to a variety of inhaled solid and liquid particles, allergens, and microbes


Bronchi, Bronchial Tree, and Lungs
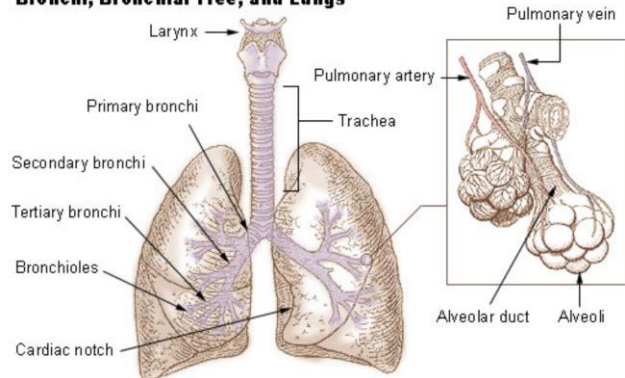
*Respiratory tract has a **tree-like organization** with a trachea connecting to branched airways that terminate in millions of highly vascularized & thin-walled alveoli, where gas exchange occurs*

THE MUCOCILIARY ESCALATOR

- Lower respiratory tract is lined with **tiny hair-like structures = Cilia**: Move in coordinated wave-like motion to help move debris upward & out of the lungs
- Once the debris reaches the larger bronchial tubes it can **stimulate the cough reflex**, which is designed to expel debris from the respiratory tract
- Filters particles between 2-10um
- Cilia are **killed by tobacco smoke** → smokers have few if any cilia remaining in their lungs
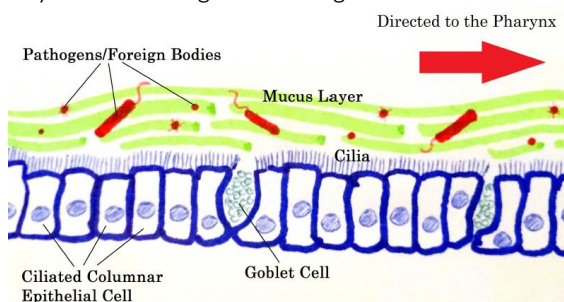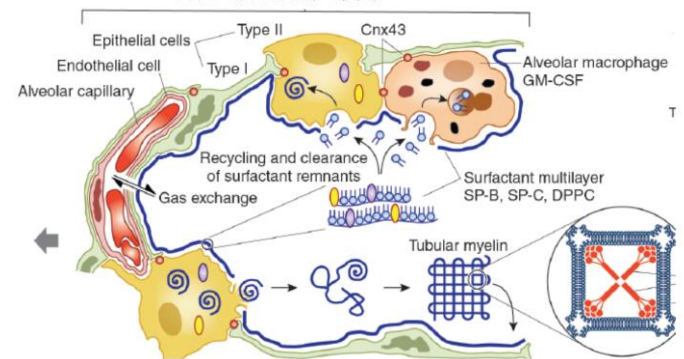

COMPONENTS OF LUNG IMMUNE SYSTEM
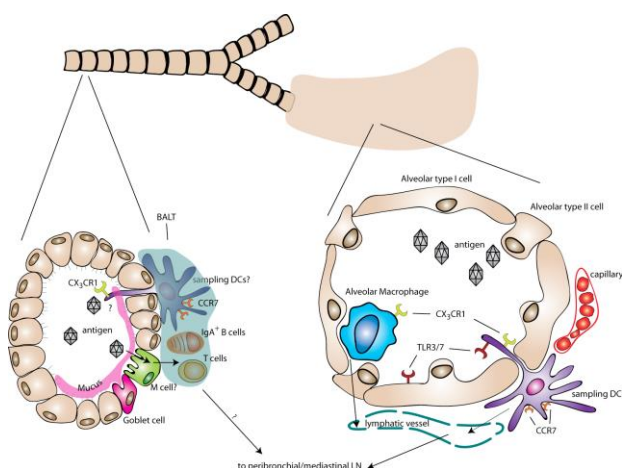
- DCs can reach antigens through the epithelial layer and bring them to the lymph nodes

- **Type II alveolar cells** produce **surfactant lipid** (phospholipid to create surface tension, prevents collapsing of alveoli)
- **Alveolar macrophages**:
  - First line of defense; uptake and catabolism of surfactant (is constantly renewed)
  - Are essential for **clearance of pulmonary surfactant** produced by type 2 alveolar epithelia
  - Make the decision whether inflammation should be induced or not

PULMONARY ALVEOLAR PROTEINOSIS (PAP):

- PAP is a rare human syndrome characterized by the **accumulation of surfactant material** within the alveoli
- PAP develops in mice and humans **with GM-CSF-deficiency** (or GM-CSF autoantibodies)
  - GM-CSF *(Granulocyte macrophage colony-stimulating factor)* induces differentiation to granulocytes and macrophages
 - GM-CSF is required for development of alveolar macrophages

ASTHMA

= An immunological airway disfunction
- Prevalence has risen to **true epidemic proportions**
  - Worldwide ~300 million affected people
  - In affluent societies up to 10 % of the population
- TRADITIONALLY: **Two forms** have been defined in the clinic: <u>allergic and non-allergic</u>
  - <u>Allergic</u> asthma coincides with **high levels of serum IgE** and/or **positive skin-prick test** to common allergens. Most children and about 50% of adults have allergic asthma
  - <u>Non-allergic</u> often develops later in life
- TODAY: different asthma phenotypes with distinct **pathophysiology** are defined as **asthma endotypes**
- Asthma comprises a range of **heterogeneous phenotypes** that differ in presentation, etiology and pathophysiology

TYPICAL SYMPTOMS:
  - Shortness of breath (Dyspnea)
  - Wheezing
  - Chest tightness
 - Persistent/recurrent cough (in the night and morning)

ASTHMA TRIGGERS
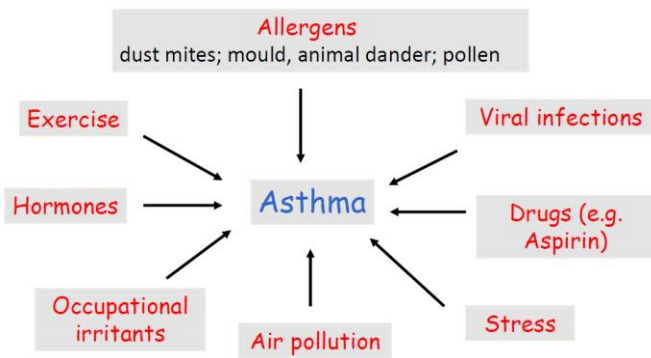

HALLMARKS OF ATOPIC ASTHMA

Atopy: Predisposition toward developing certain allergic hypersensitivity reactions

- **Chronic airway inflammation** (eosinophils, monocytes, Th2 cells)
- **Goblet cells hyperplasia** and **increased production of mucus**
- **Structural changes** (i.e. thickening of the airway smooth muscle, epithelial cell desquamation, disorganization of subbasement membrane)
- Unspecific **airway hyperresponsiveness** to irritants (i.e. tobacco, air pollution) and, in most cases, allergens, which cause acute airflow obstruction in the small airways (bronchospasms)
- Asthmatic lungs are densely populated with eosinophils
- Airway narrowing and goblet cell hyperplasia:
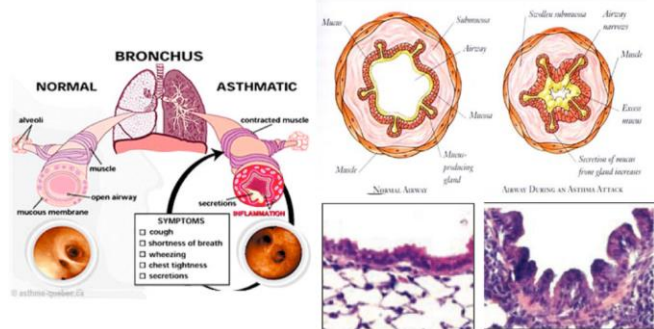
ASTHMA AND ALLERGY: ALLIANCE OF GENES AND ENVIRONMENT

- **Large regional differences** in the prevalence of asthma
  - Allergic disease is more common in **clean westernized environments**
- Asthma and atopy is **less common in:**
  - Younger siblings
  - Children attending day care < 1 year of life
  - Household with dogs and/or other pets
  - Children from farming households
  - Children exposed to bacterial endotoxin
  - Children with vaccinated against tuberculosis
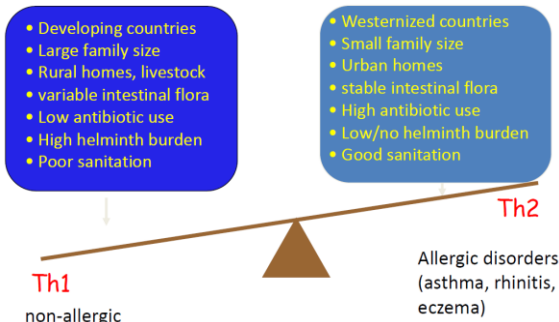
- Chronic LPS exposure protects mice from asthma

GENETIC BASIS

- Runs in families: Heritability as **high as 75%**
- Higher in monozygotic (75%) than in dizygotic (35%) twin
- Probably influenced by **few genes with a moderate effect** rather than many genes with a small effect or 1-2 genes with a dramatic effect

IDENTIFICATION OF ASTHMA-SUSCEPTIBILITY GENES

*Genetic association studies have identified and replicated susceptibility genes for asthma in **3 major types of studies**:*

1. Candidate gene studies
- Most frequently performed/ most common form to find asthma-susceptibility genes
- Based on known genes that could be related
- Are unlikely to find novel biology → limited to understanding molecular mechanisms related to known disease pathobiologic processes
- 53 genes have been identified: They are involved in a variety of biologic processes: **Th2 inflammation, T-reg cell function, the HLA locus/immunity, and IgE response of B cells**

2. Positional cloning using linkage studies
- Family-based method used to **map** a trait **to a genomic location**
- Useful for the identification of genes that are inherited in a Mendelian fashion
- Locations identified are more likely to contain a causal genetic variant
- Method limits investigation to specified chromosomal regions → is less likely to fully elucidate complex disorders such as asthma
- Suitable to find rare alleles with a high effect
- 6 novel genes were identified (ADAM33, VDR, DPP10, PHF11, HLA-G, GPR154)

3. Genome-wide association studies (GWAS)
  - Suitable to find **common genetic variations** associated with **complex disorders** such as asthma
  - Huge studies with >20'000 patients were performed (GABRIEL and EVE) and new genes could be identified (ORMDL3, IL1RL1,IL18, TSLP, IL33, SMAD3, HLA-DQ, ORMDL3, IL2RB, SLC22A5, IL13, and RORA). They are involved in disordered inflammatory / immunologic responses

ASTHMA MOUSE MODELS
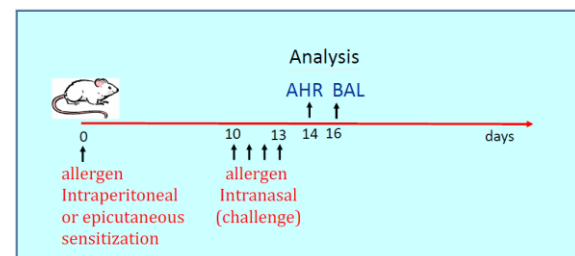
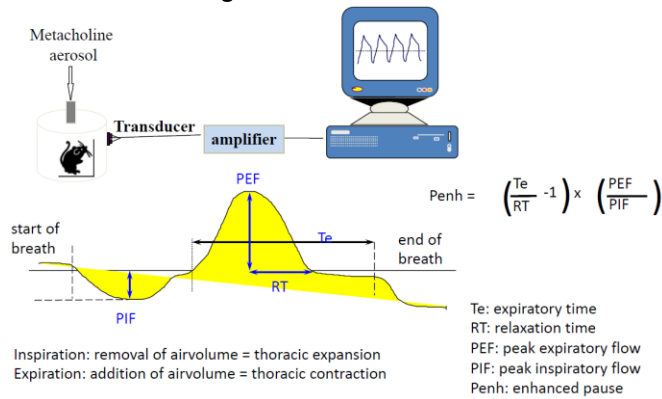
AHR: Airway hyperresponsiveness
BAL: Bronchoalveolar Lavage, medical procedure to collect fluids in lung
- BAL and the lung were analyzed by:
  - Flow cytometry for determination of cell populations
  - Cytokines / chemokines
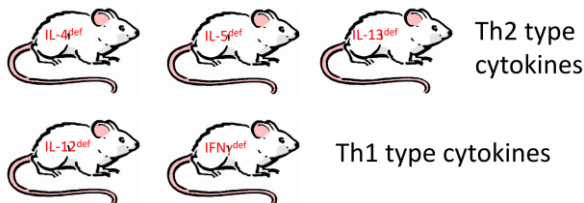 - Histology / inflammation
  - Antibody responses

Whole Body Plethysmography
- Measurement of airway obstruction in mice

A plethysmograph is an instrument for **measuring changes in volume within an organ** or whole bod.

$$Penh = \left(\frac{Te}{RT} - 1\right) \times \left(\frac{PEF}{PIF}\right)$$

Te: expiratory time
RT: relaxation time
PEF: peak expiratory flow
PIF: peak inspiratory flow
Penh: enhanced pause

Inspiration: removal of airvolume = thoracic expansion
Expiration: addition of airvolume = thoracic contraction

Some Findings
Key Th2 and Th1 type cytokines:

- **IL-4R-deficient** mice: Reduced airway hyperresponsiveness (AHR) and mucus production
- **Allergic IL-12 or IFN-α** deficient mice: Enhanced airway hyperresponsiveness
- Asthma features in mice **lacking Th2 cytokines:**

mice	eosinophils	mucus	AHR	Th2 cells
Wild-type	+++	+++	+++	+++
IL-5-/-	no	+++	+++	+++
IL-13-/-	+++	no	+	+++
IL-4-/-	+	+	+	+
IL-4 x IL-13 Double ko	no	no	no	no

- Mice **lacking IL-4 and IL-13** seem to do well
- There are two types of IL-4 receptor:
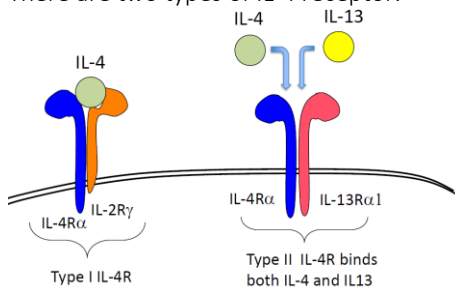

KEY PLAYERS IN DEVELOPMENT OF ASTHMA
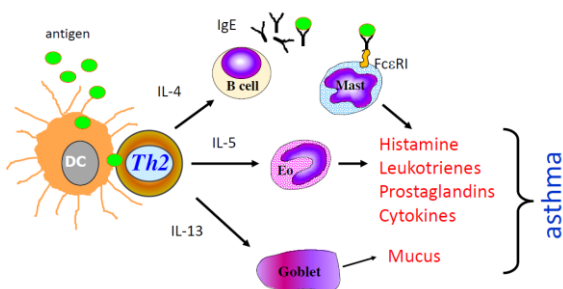
- **Th1 and Th2 balance** is central to the development of asthma
- The T$_R$S are necessary to keep the Th2 in check, without them there is a large increase in Th2
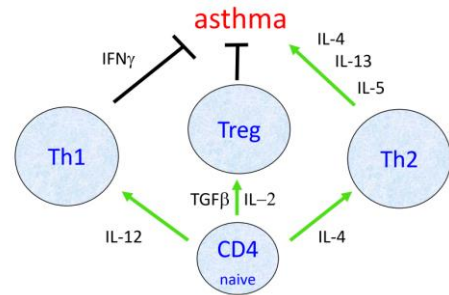

Type 2 Innate lymphocytes (ILC2) as instigators of asthma:
- ILC2 produce **IL-5, IL-9, and IL-13** in response to IL-**33 and TSLP** secreted by epithelial cells and can thereby **trigger key features of the asthma response**
- Specific T-cells for the antigens take a while to expand, whereas **ILCs respond immediately**
- By secretion of certain cytokines, the response to the subsets of ILCs is regulated
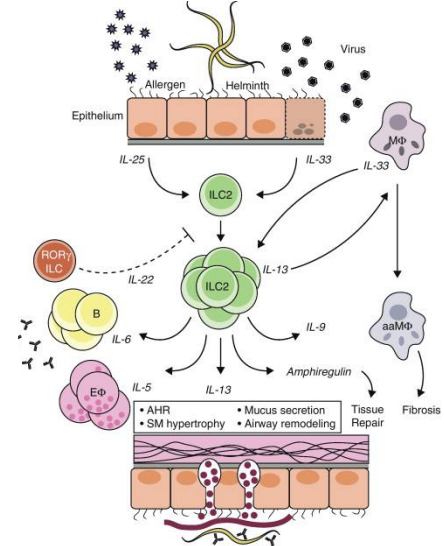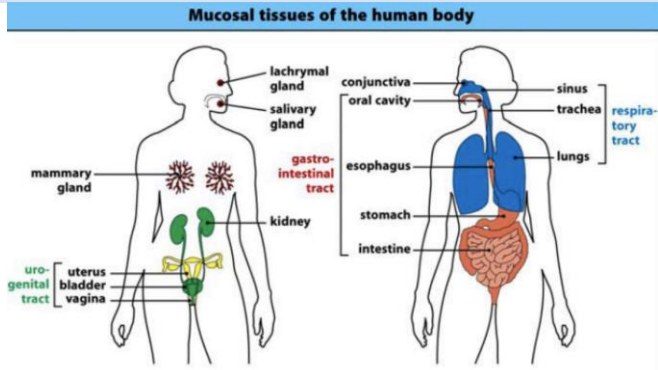

NOVEL ASTHMA THERAPIES
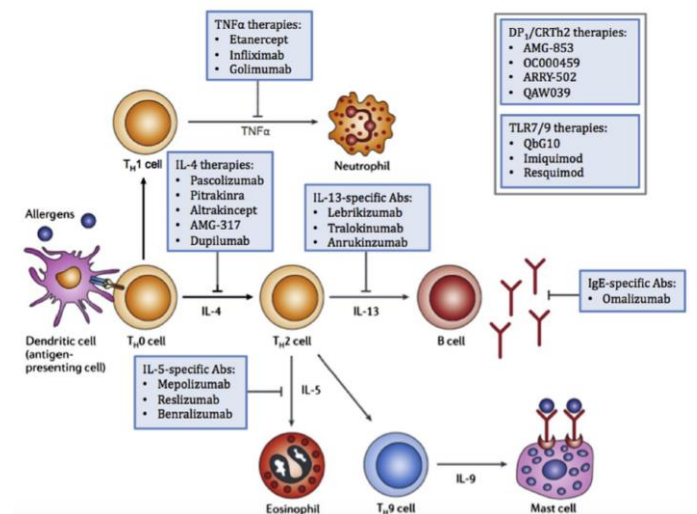
INTESTINAL IMMUNOLOGY AND INFLAMMATION

MUCOSAL SURFACES

Mucosal tissues of the human body

- **Mucosal surfaces** are particularly vulnerable and therefore **contain mucus-associated lymphoid tissue = MALT**
- Organized mucus-associated lymphoid tissue found in:
  - NALT - Nasal associated lymphoid tissue, i.e. tonsils, adenoid
  - BALT - Bronchial associated lymphoid tissue
  - GALT -Gut associated lymphoid tissue, e.g. intestines
- MALT may be present either as **single lymphoid follicles** embedded in the wall of the tissue or as **aggregated follicles** such as Peyer's patches in the intestines, a specialized epithelium

SMALL INTESTINES

Total surface area = 450 m²
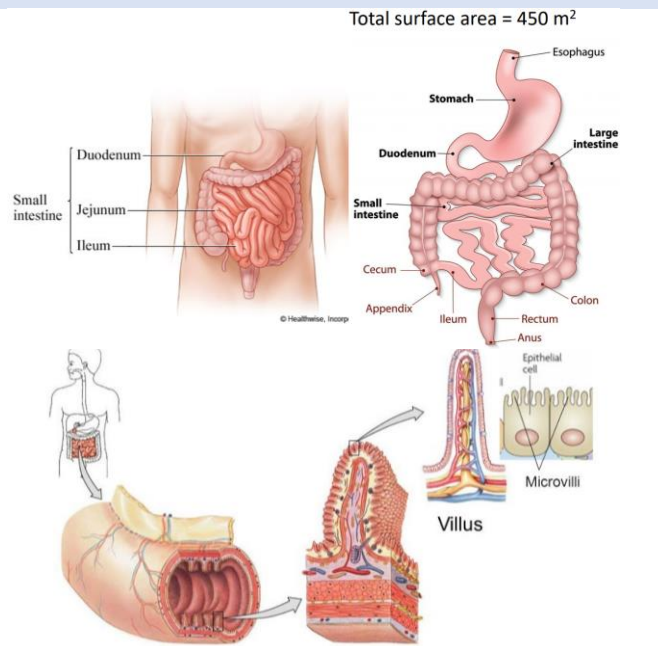
Villi and microvilli: increase surface area (450 m²) > maximize absorptio

INTESTINAL MICROFLORA

THE GUT ECOSYSTEM
- 100 Trillion (10^{14}) bacteria
- 500-1000 species
- Dominated by just 2 (out of > 30) phyla of bacteria (i.e *Bacteroides* and *Firmicutes*) and one member of **Archaea** (i.e. *Methanobrevibacter*)
- Most are uncultivable

- Characterized today by 16s RNA sequencing
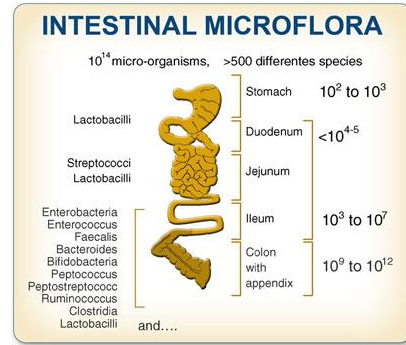
## GUT MICROBES INFLUENCE OBESITY
- Genetically obese mice (ob/ob): Obesity is associated with an **increase** in the relative abundance of the *Firmicutes* and a corresponding **decrease** in the relative abundance of the *Bacteroides*
- WT germ-free mice are fed a standard polysaccharide-rich chow diet with microbiota harvested from ob/ob mice → Resulted in **adiposity** → **Obese gut microbiota has an increased and transmissible capacity tom promote fat deposition**
- Gut microbiota was transplanted from normal mice into germ-free recipients → Increases their body fat without any increase in food consumption → **Composition of microbial community in the gut affects the amount of energy extracted from the diet**
- Experiment in humans: When changed to a fat- or carbohydrate-reduced diet, the relative abundances change → relative abundance of the Bacteroides progressively increased, proportional to the degree of weight loss

MAINTAINING UNRESPONSIVENESS TO INTESTINAL BACTERIA
- Oral gavage with *Enterobacteria cloacae* leads to the production of intestinal IgA only
- Intravenous injection with *E. cloacae* leads to the production of serum IgM and IgG
- The compartmentalization of the IS allows the ignorance of the systemic compartments - they do not come into contact with the bacteria in the gut most of the time
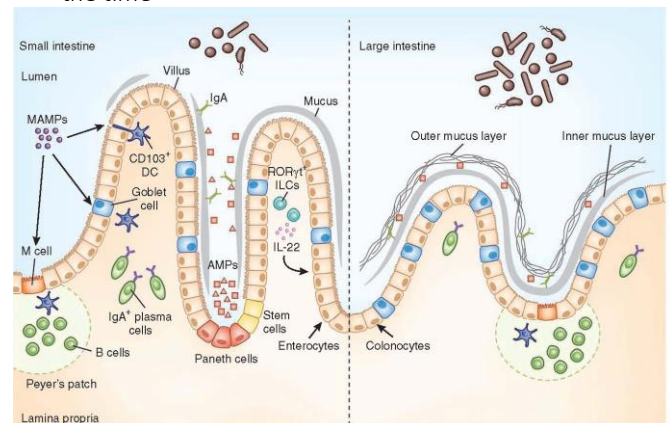
This is rather ignorance than tolerance

GALT

- Has the following challenges:
  o The mucosal membranes of the digestive tract must **allow absorption** of nutrients **without responding against them** and development of food allergies
  o Avoid responses against **commensal intestinal flora**
  o GALT has to mount an **effective response against a vast number of pathogens**, since mucosal surfaces are the most frequent entry of harmful microbes

### ORAL TOLERANCE

→ Refers to **unresponsiveness to food antigens**

- Systemic tolerance occurs after the oral administration of any soluble foreign protein given in the absence of adjuvant *(Hilfsmittel, Zusatz)*
- This likely occurs through the entry of such proteins directly into the bloodstream and their subsequent dispersal throughout lymphoid organs resulting in presentation by local tolergonic DC in the absence of pro-inflammatory signals
- Possible mechanisms of oral tolerance:
  o Deletion of specific T cells
  o Anergy of specific T cells
  o Induction of specific regulatory T cells
- Regulatory factors:
  o Antigen dose, nature of antigen, cytokine milieu, age

EXPERIMENT

Ovalbumin (OVA) is a commonly used antigen for studying antigen-specific responses in mice. Mice were fed with OVA to induce oral tolerance, control group was given PBA. Two weeks after feeding, OVA was injected together with CFA (adjuvant). The control group without induced oral tolerance showed stronger responses
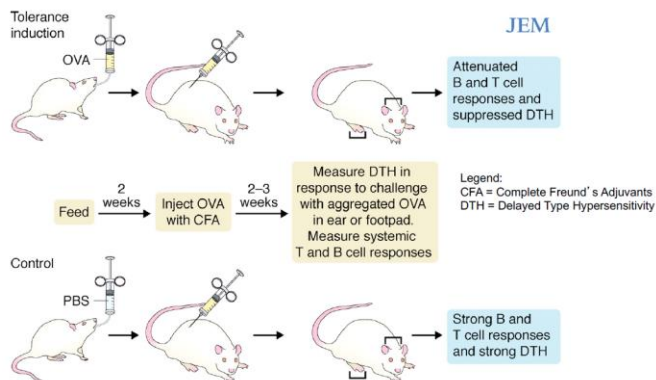

ANTIGEN ENTRY INTO GALT
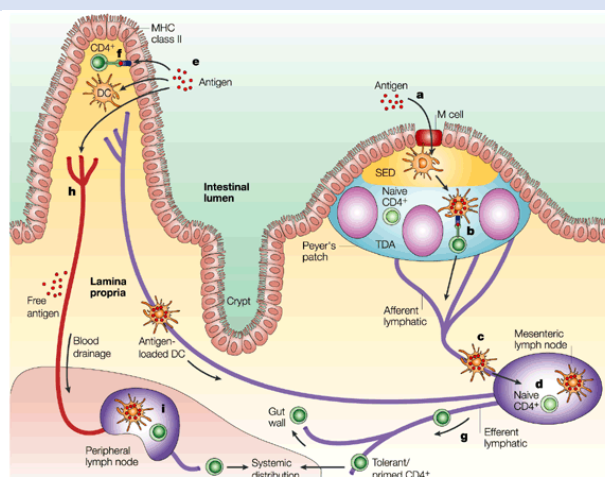
a) Antigen might enter through the M cells in the follicle-associated epithelium (FAE)
b) After transfer to local DCs, the antigen might then be presented directly to T cells in the peyer's patch
c) Alternatively, antigen or antigen-loaded DCs from the Peyer's patch might gain access to draining lymph
d) T-cell recognition in the mesenteric lymph nodes (MLN)
e) A similar process of antigen or antigen-presenting cell (APC) dissemination to MLNs might occur if antigen enters through the epithelium covering the villus lamina propria,
f) In this case, there is the further possibility that MHC class II positive enterocytes might act as local APCs. In all cases, the antigen-responsive CD4+ T cells acquire expression of the $\alpha 4 \beta 7$ integrin and the chemokine receptor CCR9, leave the MLN in the efferent lymph.
g) After entering the bloodstream through the thoracic duct, exit into the mucosa through vessels in the lamina propria. T cells which have recognized antigen first in the MLN might also disseminate from the bloodstream throughout the peripheral immune system.
h) Antigen might also gain direct access to the bloodstream from the gut
i) Interaction of the antigen with T cells in peripheral lymphoid tissues

COMPARTMENTALIZATION

- **Intestinal** and **systemic immune cells** are physically separated
- Commensal bacteria
  o Found in high densities within the lumen of the lower intestine (small and large intestine)
  o Are largely restricted from gaining access due to the **physical epithelial and mucus barriers**
- Small numbers of commensals are allowed to penetrate through the epithelial barrier into specialized inductive sites known as Peyer's patches or isolated lymphoid follicles → there they are picked up by DC or phagocytosed and destroyed by macrophages
- **DC presenting commensal bacterial antigens can traffic only as far as the mesenteric lymph nodes, which form the barrier between the mucosal and the systemic immune system**
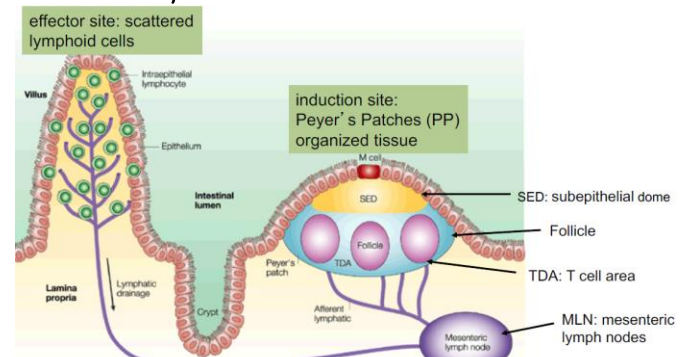
- The organized tissues of the PP and MLNs are involved in the induction of immunity and tolerance, whereas the effector sites are scattered throughout the lamina propria and epithelium of the mucosa
- Villus lamina propria and PP are drained by afferent lymphatics that go to the MLNs

INTESTINAL PATHOGENS

Bacteria
- Vibrio cholerae
- Enterotoxigenic Escherichia coli
- Campylobacter jejuni
- Yersinia enterocolitica
- Listeria monocytogenes
- Salmonella typhimurium
- Shigellen
- Helicobacter pylori
- Clostridium difficile

Viruses
- Rotavirus
- Poliovirus
- Hepatitis A virus
- HIV

Protozoa
- Entamoeba histolytica
- Giardia lamblia

Nematodes
- Trichinosis
- Trichuriasis
- Strongyloidiasis
- Ascariasis
- Hookworm
- Pinworm

Cestodes
- Tapeworm

Trematodes
- Schistosomiasis

PHYSICAL DEFENSE MECHANISMS

- Stomach acid
- Epithelial cell layer (enterocytes and tight junctions)
- Enzymes (e.g. lysozyme) & anti-microbial peptides (e.g. defensins produced by paneth cells)
- Mucus (produced by goblet cells)

DIFFERENT TYPES OF INTESTINAL EPITHELIAL CELLS

Cell Type		Function
Enterocytes	→	Absorption of nutrients
Goblet cells	→	Mucus production
Paneth cells	→	Production of anti-microbial compounds
Microfold (M) cells	→	Antigen uptake
Tuft cells	→	Pathogen sensing
Enteroendocrine cells	→	Hormone production

PEYER'S PATCHES

- PP are overlaid by a **specialized epithelium** called the follicle-associated epithelium (FAE)
- FAE is composed of **enterocytes** and **specialized M-cells**
- **Goblet cells** are rare within the FAE and hence the FAE is <u>not covered by a thick layer of mucus</u> and so **forms a direct contact** with the luminal contents

M-CELLS

- Peyer's patches contain **M-cells**:
  - o Are **specialized epithelial cells overlying lymphoid tissues**
  - o Are <u>lacking</u> glycocalyx or microvilli
  - o Take up luminal antigens by **endocytosis**
  - o Lymphocytes sit within 'pockets' at the base of M-cells
  - o M-cells release luminal antigens to underlying DC which go on to prime an immune response by activating naive T and B cells within the PP
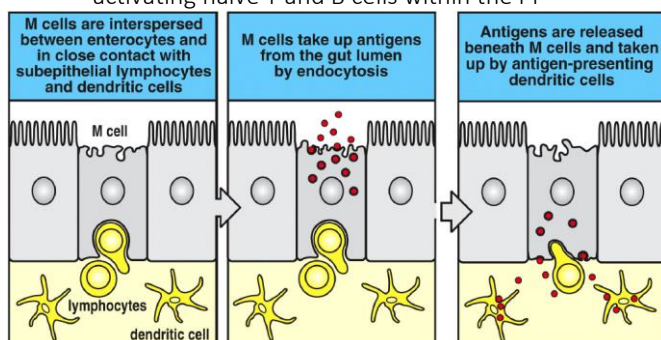

LYMPHOCYTE MIGRATION TO THE INTESTINES

Migration of lymphocytes to different tissues is regulated by different **adhesion molecules** and **chemokines**

- **T and B-cells** homing to intestines express $\alpha 4\beta 7$, which binds to MAdCAM on mucosa endothelial cells
  - o Otherwise activated T and B cells express $\alpha 4\beta 1$, which binds VCAM
- **Intestinal epithelial cells** produce chemokines CCL25 & CCL28 → recruit lymphocytes expressing CCR9 & CCR10
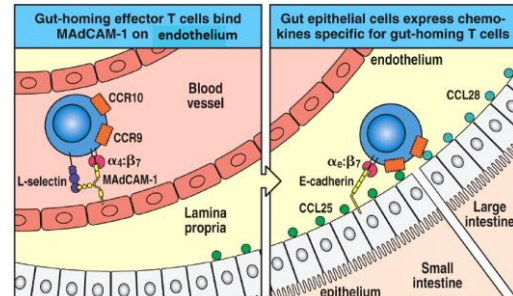
- Subset of **naive CD8+ T-cells** recognizing antigen in GALT but not peripheral LNs, selectively **retains** CCR9 **expression** and is targeted to the small intestinal epithelium (IE)
  - o Selective expression of adhesion molecules such as $\alpha 4\beta 7$ and $\alpha E\beta 7$ can contribute to this tropism
- Within the small intestine, intestinal epithelium (IE) produce CCL25 → mediates homing of effector CD8+ T-cells by promoting rapid adhesion, transmigration, and entry of cells into the IE
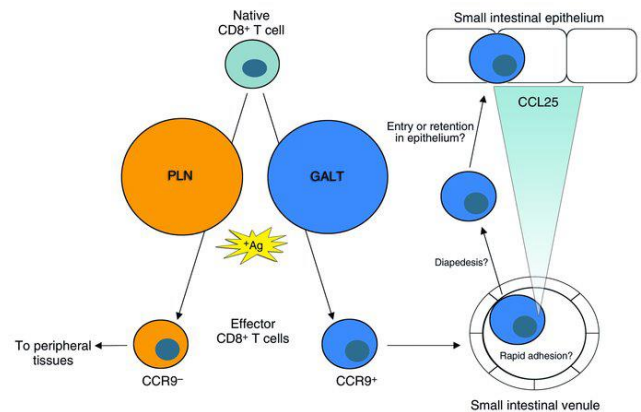
## ENVIRONMENTAL INFLUENCE

Vitamin A regulates intestinal T and B-cell responses

- Vitamin A reduces mortality from **persistent diarrhea** caused by infectious organisms
- Vitamin A deficient diet leads to:
  o Impaired migration of lymphocytes to intestinal mucosa
  o Decrease of IgA-secreting B-cells and CD4 T-cells in the ileum
- Responsible for these effects is the **vitamin A metabolite** Retinoic Acid (RA)
  o Enhances expression of gut-homing receptors ($\alpha4\beta7$ and CCR9) → imprints them with gut tropism
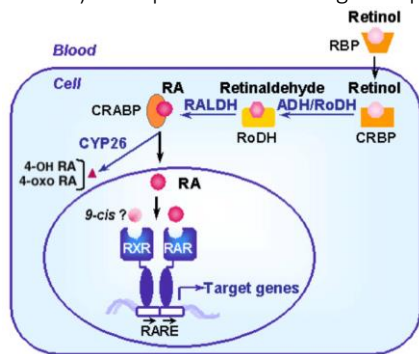
ADH: Alcohol dehydrogenase; RALDH: Retinal dehydrogenases

- Retinoic Acid Receptor (RAR) and Retinoic X Receptor (RXR) are ligand induced transcription factors that induce **Retinoic Acid Response Element (RARE)** which contains **genes for gut homing**
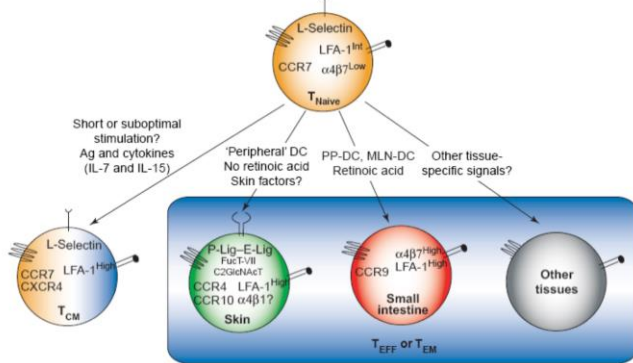
- RA also induces the **differentiation of FoxP3+ Treg cells** → prevents inflammation
- RA is secreted by a subset of DCs
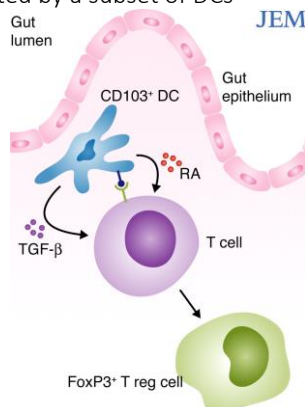
INFLAMMATORY BOWEL DISEASE

- IBD is a **chronic and relapsing inflammatory condition**
  o **Crohn's disease**: Distal small intestines and the colon are affected in a **transmural manner**
  o **Ulcerative colitis**: Only the colon is affected in a **superficial manner**
- Affects 1 in 500 people in westernized countries
- IBD results from **inappropriate inflammatory innate and adaptive immune responses** against intestinal commensal bacteria

EXPERIMENTAL MODELS OF IBD

- Conditional knockout mice <u>lacking</u> the transcription factor NF-kB selectively in intestinal epithelia develop spontaneous IBD due to **death of epithelial cells**
  o Treatment with **chemicals** (such as Dextran Sulfate Sodium, DSS) causing epithelial cell damage and innate inflammatory immune response
- **T-cell adoptive transfer** into lymphopenic mice
  o Mice lacking Treg cells also developed the disease:
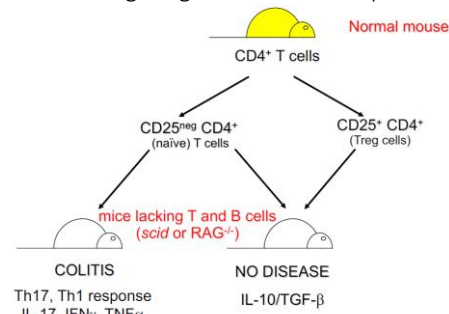
→ A disturbed balance of Th17 and Treg cells promotes IBD:
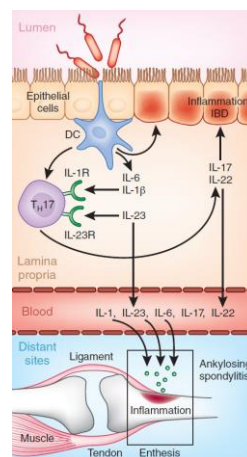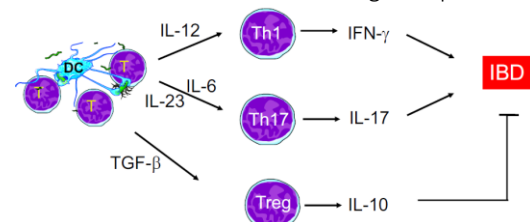
Dendritic cells and **macrophages** secrete IL-6 and IL-23 in response to commensal bacteria → These pro-inflammatory cytokines activate **Th17** cells (pathogenic IL-17 producing T-cells)→ induce chronic inflammation in the intestines (colitis)
When **inflammatory cytokines** and **Th17** cells enter the blood, they **may induce autoimmune responses** in other organs such as rheumatic diseases

CURRENT & FUTURE DIRECTIONS FOR TREATMENT

- Conventional treatments:
  o Broad spectrum immunosuppressive drugs
  o Antibiotics
  o Surgery
- New treatments:
  o Selective blockers of inflammation (antibodies against TNFα, IL-6, and IL-23
- Needed: Increased understanding of immune dysfunction leading to IBD

SUMMARY

- The intestine has developed **specialized and separate immune responses** due to the ubiquitous presence of intestinal bacteria
- We cope with food antigens by inducing **systemic tolerance**
- We cope with intestinal bacteria by **systemic ignorance** and the **induction of protective but local non-inflammatory responses**
- The gut creates a special microenvironment that 'imprints' T and B cells with a **non-inflammatory phenotype**
- Mounting an inappropriate immune response against intestinal bacteria leads to inflammatory bowel disease (IBD)

SEPSIS

- *Incidence: 500'000-750'000 Patients/Year*
- *Overall Mortality: 200'000-300'000 Patients/Year*

Most common cause of death in noncoronary intensive care unit

Bacterial products (toxins) mediate sepsis

- **Bacterial products interact** with host cells and serum proteins to initiate a series of reactions that ultimately may lead to cell injury and death
- Bacterial products themselves are **harmful**
- Widespread and **unregulated host response** to these substances results in amplification of chemical mediators that lead to further cell damage
- Circulatory insufficiency occurs

BACTERIAL TOXINS

Exotoxin (gram + or -)	Endotoxin (gram -)
secreted proteins	LPS in cell wall
often plasmid-encoded	chromosome-encoded
diverse modes of action	septic shock (conserved)
heat-sensitive	heat-stable
usually no fever	induce fever
neutralized effectively by Ab	weak immunogenicity

SENSITIVITY TO BACTERIAL LIPOPOLYSACCHARIDE

- 50 μg/kg are sufficient to kill a human

"When we sense LPS we are likely to turn on every defense at our disposal. We will bomb, defoliate, blockage, seal off and destroy all tissue in the area."

- LPS binding protein (**LBP**) transfers **LPS to CD14** (which does not contain a signaling domain)
- **CD14** and **LPS** then complex with **MD2** and **TLR4** resulting in <u>TLR4 activation</u>:
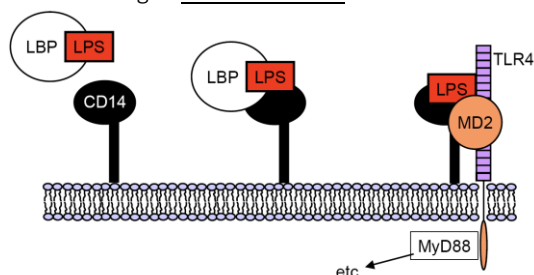
SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS)
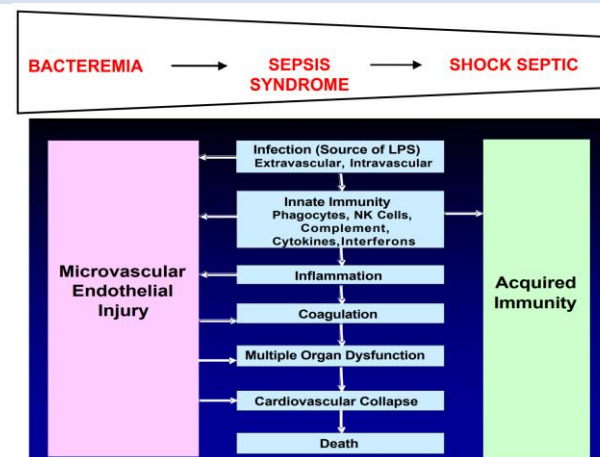

DEFINITIONS

- <u>SIRS = Systemic inflammatory response syndrome</u>
  Two or more changes in the following 4 factors:
  o Body temperature (>38; <36)
  o Heart rate (>90 beats/min)
  o Respiratory function (20 breath/min)
  o Peripheral leukocyte count (>12'000/mm^3, <4'000/mm^3)
- <u>Sepsis</u>: Systemic host response to infection with SIRS plus documented **infection**
- <u>Severe sepsis</u>: Sepsis plus **end-organ dysfunction or hypoperfusion**
- <u>Septic shock</u>: Sepsis with **hypotension** despite fluid resuscitation, evidence of **inadequate tissue perfusion**
  o Occurs in ~40% of patients with gram-negative bacteremia and in ~ 20% of patients with Staphylococcus aureus bacteremia

### LIPOPOLYSACCHARIDE-INDUCED MEDIATORS

- Pro-inflammatory cytokines
- Arachidonic acid metabolites (eg, leukotrienes, prostaglandins, thromboxanes)
- Complement system
- Coagulation cascade

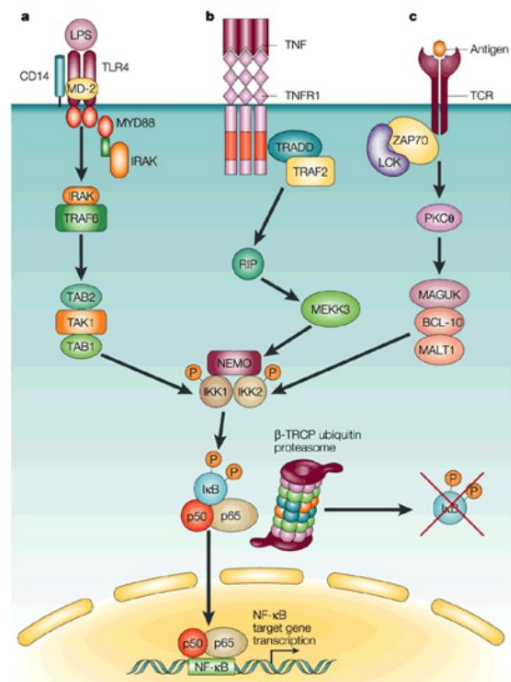*Constant and high levels of mediators lead to injurious effects:*
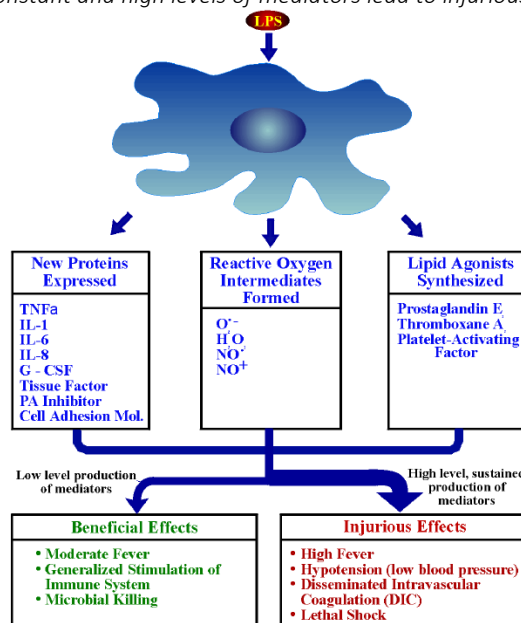
NF-κB PATHWAY

- NF-κB-binding activity in peripheral blood monocytes of septic patients is highly increased in non-survivors
- Two classes of NF-κB proteins exist:

Class	Protein	Aliases
I	NF-κB1	p105 → p50
	NF-κB2	p100 → p52
II	RelA	p65
	RelB	
	RelC	
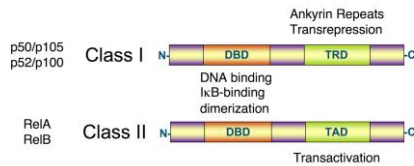
- Both classes: N-terminal DNA-binding domain (DBD)
  - o Dimerization interface to other NF-κB transcription factors
  - o Binds to inhibitory IκBα protein
- Different: C-terminus
  - o Class I protein: contains a number of ankyrin repeats and has transrepression activity
  - o Class II protein: Has a transactivation function

CANONICAL NF-κB PATHWAY

- IκB binds to the NF-κB proteins and inhibits them
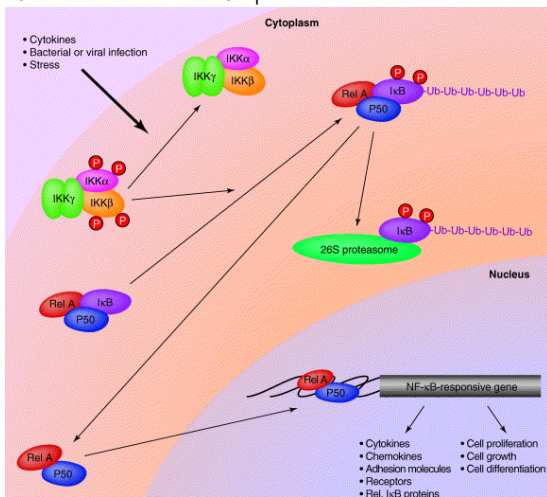
1. **IKK complex** gets activated by a variety of stimuli
   - o Cytokines: TNF-α, IL-1
   - o Lipopolysaccharids
   - o Antigen-binding on TCR (T-cell receptor (TCR) signalling)
   - o Bacterial or viral infection
   - o Stress
2. Activation of IKK complex leads to phosphorylation of **IκB proteins**
3. Phosphorylation leads to IkB polyubiquitination and its subsequent degradation by the 26S proteasome
4. **NF- kB proteins** (ReIA and P50) are now liberated from IkB and **translocate to the nucleus** where they bind to the promoter regions of NF- kB -responsive genes to result in increased gene expression
→ Stimuli leads to transcription of NF-κB responsive genes

a) LPS binds to Toll-like receptor 4 (TLR4)–CD14–MD-2 complex → activates intracellular signalling cascade that involves recruitment of MYD88 and IRAK → Activation of IRAK results in phosphorylation of TRAF6 → relay signals through TAK1–TAB1–TAB2 complex to IKK complexes → activates the NF-κB pathway
b) TNF receptor 1 (TNFR1) engagement by TNF results in **receptor trimerization** → recruitment of adaptor protein TRADD → interacts with TRAF2 → MAP/ERK kinase kinase 3 (MEKK3) and receptor-interacting serine/threonine kinase (RIP) probably link TNF signalling to IKK activation
c) T-cell stimulation: Antigen-presenting cells or anti-TCRCD3 antibodies → rapid translocation of protein kinase Cθ (PKCθ) to the plasma membrane

THE NON-CANONICAL NF-κB PATHWAY

- Is regulated by NF-κB-inducing kinase (NIK) and IKKa
- Is induced by ligands for receptors including the B-cell-activating factor receptor (BAFF-R), CD40 and the lymphotoxin-b receptor (LT-bR)
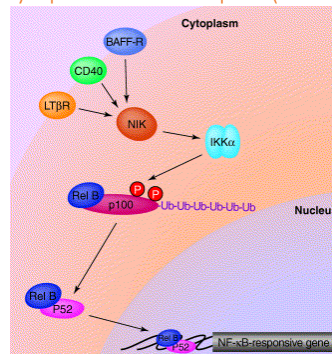
1. NF-κB inducing kinase (NIK) can be activated by various stimuli
2. NIK activates IKK-α
3. IKK-α phosphorylates p100 → induce processing to p52
4. Nuclear translocation of **RelB-p52 dimer** (are NF-κB proteins) which bind to the promoter regions of NF-kB-responsive genes → **increased gene expression**
→ Stimuli leads to transcription of NF-κB responsive genes.

DANGER AND INFLAMMATION

Pattern Recognition Receptors (PRRs) recognizing Pathogen Associated Molecuar Patterns (PAMP) involved in sensing danger to microbes and self

EXCOURSE: HISTORY AND BASICS

The Dirty Little Secret (Charley Janeway)

To induce an immune response, immunologists had to include crap along with non-self - the crappier the better, He proposed that that pathogen-associated molecular patterns are recognized by nonclonally distributed receptors found in the host

Danger Theory (Polly Matzinger)

This theory claims that immune responses are triggered by "danger signals," or "alarm signals," released by the body's own cells. According to the danger theory every immune response is not due to the presence of "nonself" (i.e., genetically foreign entities), but to the emission, within the organism, of "danger signals"

DROSOPHILA AND MAMMALS

DEFENSE IN DROSOPHILA

In Drosophila, the Toll and Imd pathways confer host defence against pathogens

- Toll pathway regulates production of antimicrobial peptides against fungi and Gram-positive bacteria
  - o Peptidoglycan Recognition Protein (PGRP)-SA is essential for activation of the Toll pathway in response to Gram-positive bacteria
  - o Persephone is involved in the activation of the Toll pathway in response to fungi
- PGRP-LC recognizes the invasion of Gram-negative bacteria and is required for activation of the Imd pathway, which is essential for responses against gram-negative bacteria
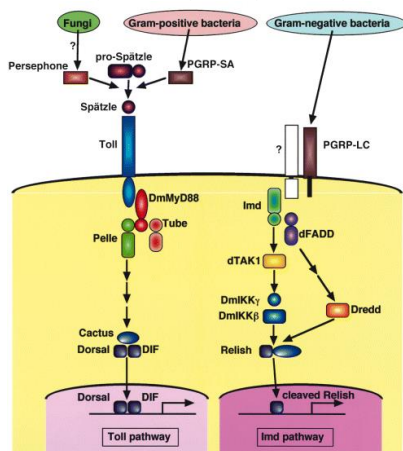
In Mice (Bruce Beutler)

- Mapped a candidate gene (=Toll-like receptor (TLR) 4) that renders particular strains of mice (i.e. C3H/HeJ or C57BL/10ScCr mice) highly resistant to bacterial Lipopolysaccharide (LPS)
  - o TLR4 mutant mice did not react to LPS
  - o TLR4 is a Lps gene product
  - o TLR4 directly interacts with bacterial LPS
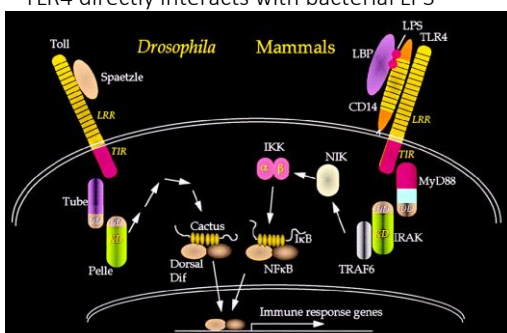
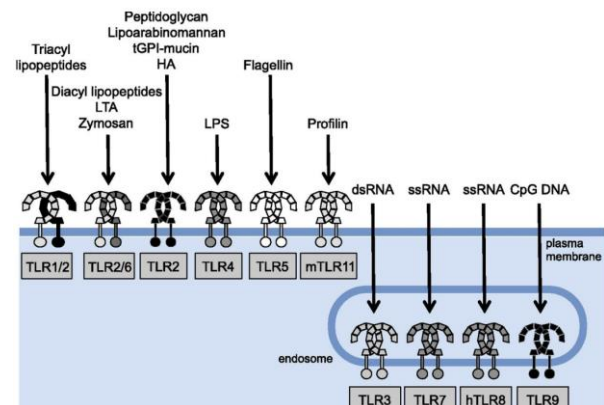## PATTERN RECOGNITION RECEPTORS

PRR are involved in sensing danger

- PAMP = Pathogen-associated molecular pattern
- PRR = Pattern Recognition Receptor
  - o Family of receptors that includes toll-like receptors
  - o Typically expressed mainly by innate immune cells
  - o Recognize PAMPs and certain endogenous stress molecules

PRRs can be grouped as:

- Membrane-bound PRRs, three subtypes:
  - o Toll-like receptors (TLRs)
  - o C-type lectin receptors (e.g. Dectin-1, DC-SIGN, Mannose receptor)
  - o Scavenger receptors
- Cytoplasmic PRRs:
  - o Nucleotide-binding Oligomerization Domain (NOD)-like receptors)
    - ▪ NLRPs (NOD subfamily, Leucine rich Repeat and Pyrin) also called NALP
  - o RNA Helicases including RIG-I and MDA5
- Secreted PRRs
  - o Collectins (e.g. mannan-binding lectin MBL)
  - o Pentraxins (e.g. SAP, CRP)

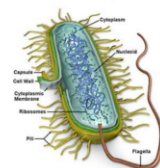## TOLL-LIKE RECEPTORS AND LIGANDS
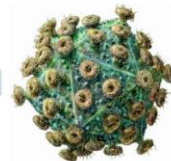
- Humans have 10 functional TLR genes (mice have 12):

TLR	Ligand
1+2	Lipopeptides
3	dsRNA
4	LPS
5	Flagellin
2+6	Lipopeptides
7	uncapped ssRNA
8	ssRNA
9	CpG DNA
10	unknown
11 (mouse only)	Flagellin, Profilin (mouse only)

TLR	Ligand
1+2	Lipopeptides
3	dsRNA
4	LPS
5	Flagellin
2+6	Lipopeptides
7	uncapped ssRNA
8	ssRNA
9	CpG DNA
10	unknown
11 (mouse only)	Flagellin, Profilin (mouse only)

bacteria

Virus

TLRs also bind endogenous danger molecules
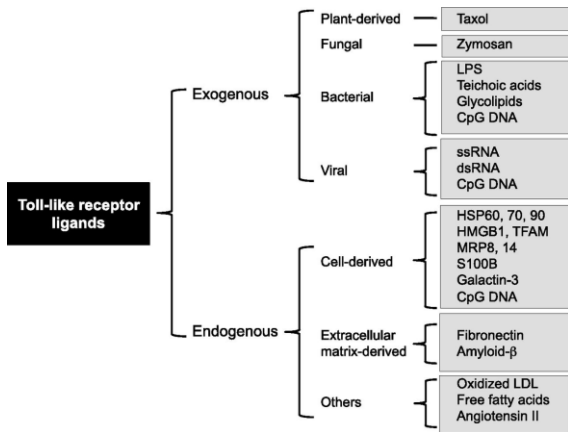
TLR SIGNALING PATHWAYS
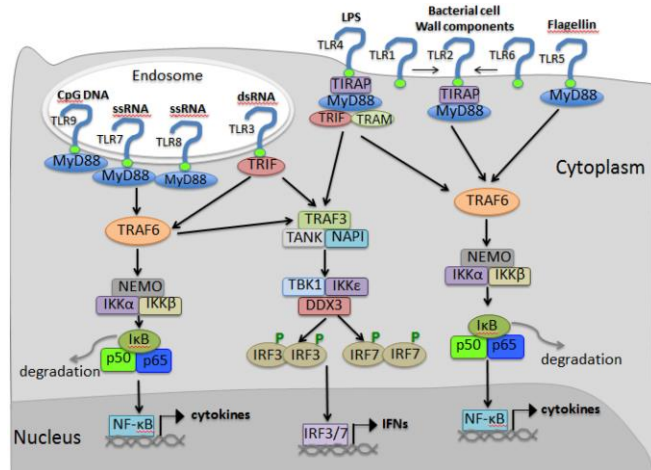
- Various **TIR domain-containing adaptors** such as MyD88, TIRAP, TRIF, TRAM become engaged or activated upon ligation of TLRs
  MyD88 is a **TLR signaling adaptor protein** → Interacts with **IRAK** family, leading to activation of **TRAF6** *(tumour-necrosis factor-receptor-associated factor 6)* → activation of **NF-kB** and **MAP kinases** and **JNK** → pathways lead to production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1
  (Corresponds to the canonical NF-κB pathway)
  o **Except for TLR3**, all TLR ligation events recruit the adaptor MyD88
- Binding of **TLR3** and **TLR4** ligands results in the recruitment of **TRIF**
  o TRIF adaptor allows activation of the IRF pathway
  o TLR3 signals through TRIF → interacts with kinase TBK1 → IRF3 activation → IFN-β production
  o Recruitment of TRIF by TLR4 needs **TRAM**

TOLL / IL-1 RECEPTOR SUPERFAMILY
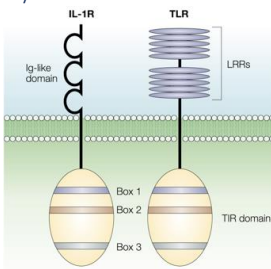
- Structure of the two receptors is practically identical → signaling is the same
- IL-1R also signals via MyD88
- Not only foreign PAMPs induce this pathway, cytokines indicating internal danger can have this effect as well
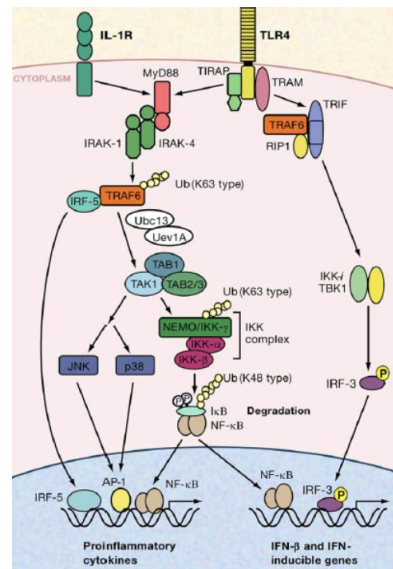
- Stimulation with their ligands recruits TIR-domain-containing adaptors including MyD88 and TIRAP
- Again, TLR4 can trigger the MyD88-independent, TRIF-dependent pathway via TRAM to induce type I IFNs

LOCALIZATION

TLRs are on the cell surface or in endosomes facing inwards (topologically outwards)
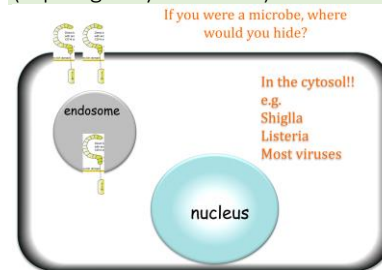
NOD-LIKE RECEPTORS (NLRS)

- **NLRs** = NOD-Like Receptors
  o **NOD** = Nucleotide Oligomerization Domain
  o Cytoplasmic proteins → are located in the cytosol!
  o Regulate inflammatory responses and cell death pathways
  o More than 20 of these proteins in the mammalian genome
  o Recognize microbial or endogenous danger molecules
  o Form oligomers that activate inflammatory caspases (e.g. caspase 1) causing cleavage and activation of important inflammatory cytokines such as IL-1β and IL-18, and activate the NF-κB signaling pathway

NLRs can be divided into **4 subfamilies** based on the type of **N-terminal domain:**
- **NLRA** (A for <u>acidic transactivating domain</u>): CIITA
- **NLRB** (B for <u>BIRs</u>): NAIP
- **NLRC** (C for <u>CARD</u>): NOD1, NOD2, NLRC3, NLRC4, NLRC5
- **NLRP** (P for <u>PYD</u>): NLRP1 to NLRP14

On the other hand, NLRs can be divided **into 3 subfamilies** with regard to their **phylogenetic relationships:**
- **NODs**: NOD1, NOD2, NOD3 (NLRC3), NOD4 (NLRC5), NOD5, (NLRX1), CIITA
- **NLRPs** (also called NALPs): NLRP1 to NLRP14
- **IPAF**: IPAF (NLRC4), NAIP

STRUCTURE OF NLRS

- In general, members of this family share **a tripartite domain structure** that consists of:
  - A **carboxy (C)-terminal Leucin-Rich-Repeat (LRR) domain** → Is involved in ligand recognition
  - A central **NACHT** (also known NOD) **domain** → for self-oligomerization and has ATPase activity
  - An **amino (N)-terminal domain** that is composed of protein-protein interaction cassettes, such as CARDs or pyrin domains
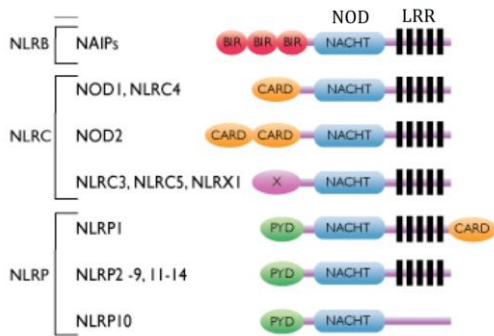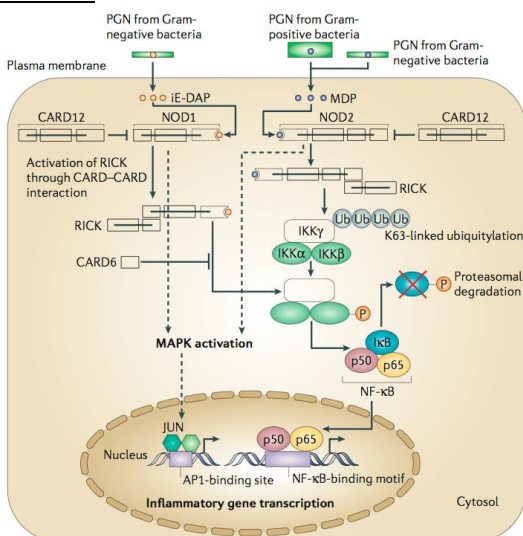

NOD1 and NOD2

NOD1 and NOD2 recognize **bacterial peptidoglycans in the cytosol**

- **NOD1** and **NOD2** are **caspase-independent NLRs**
  - NOD1: Binds DAP, which is found mainly in **gram-negative bacteria** and some gram positive such as *Listeria m.* and *bacillus spp.*
  - NOD2: Binds muramyldipeptide, which is the minimal motif found in **all gram-positive and gram-negative bacteria**
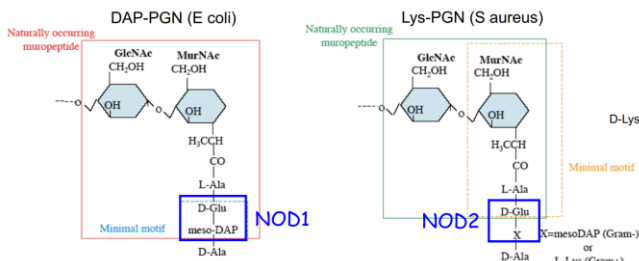
- NOD1 and NOD2 bind to RICK (RIP2) leading to **activation** of NF-κB:

Infectious and Autoimmune Diseases Associated with NOD1 and NOD2

Disease	Mutations*	Comments
NOD1		
Helicobacter pylori infection	No mutation	Delivery of PGN to epithelial cells through type IV secretion system
Chlamydophila pneumoniae infection	No mutation	Activation of NF-κB in endothelial cells
Inflammatory bowel disease	Deletion polymorphism in LRR domain	Risk factor for inflammatory bowel disease
Asthma and high IgE levels	Insertion polymorphism in LRR domain	Risk factor for asthma
NOD2		
Crohn's disease	Arg702Trp, Gly908Arg, Leu1007fsinsCys	Defective NF-κB activation in response to MDP
Blau syndrome	Arg334Trp, Arg334Gln, Leu469Phe	Constitutive NF-κB activation
Early-onset sarcoidosis	Arg334Trp, His496Leu, Thr605Pro	Constitutive NF-κB activation
Graft-versus-host disease	Arg702Trp, Gly908Arg, Leu1007fsinsCys	Risk factor for graft-versus-host disease

- Bacterial infections have been associated with the **increased risk of multiple sclerosis (MS) exacerbations**
- Peptidoglycan (PGN), a major bacterial cell wall component, has been detected in APCs located in the brains of **MS patients**
- Mice lacking NOD1-, NOD2-, RICK-, or TLR2-are resistant to EAE → Results indicate that both the NOD/RICK and TLR2 pathways are important for EAE pathogenesis

NOD2 Mutations and Crohn's Disease

- Crohn's disease is a consequence of a **disturbance in the normal immunological unresponsiveness to components of the intestinal microflora**
  - Hyperresponsiveness to these components gives rise to a Th17 and Th1 cell mediated inflammatory response that underlies all forms of disease
- There is a genetic linkage between NOD2 polymorphisms and Crohn's disease
- NOD2 is now known to be important for production of defensin's by Paneth Cells and macrophage activation

## CO-OPERATION OF NLR AND TLR PATHWAYS IN BACTERIAL DEFENSE
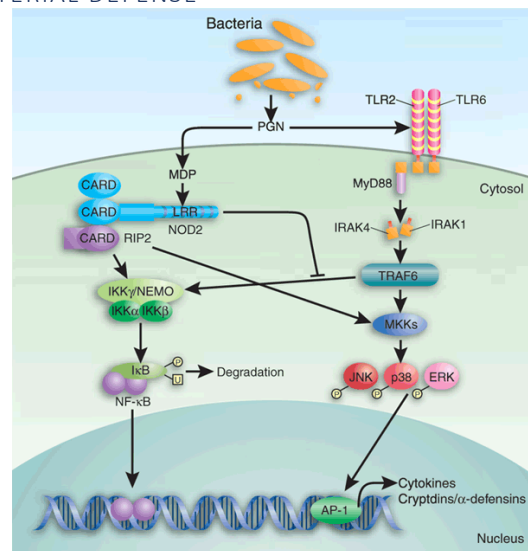
NLRP

- **NLRP** = NOD Leucine-Rich Repeat and Pyrin Domain Containing = **NALP**
- Type of **NOD like receptor (NLR)**
- Scaffolding proteins that are crucial for **aggregating other proteins** including inflammatory caspases → are together called **inflammasome**
  - Inflammasome activation is an innate **response to danger signals**
  - Inflammasomes are key for **activation of inflammatory** caspase-1 and caspase-11 (caspase-5 in mice) leading to **secretion of** IL-1β and IL-18 and eventually to **cell death** (pyroptosis)
- Unlike NOD1/NOD2, they do not (directly) induce a signaling pathway which alters gene expression
- Mainly active in **myeloid cells** (but not exclusively)

Composition of Inflammasomes

- **Canonical inflammasomes**: Convert procaspase-1 into the catalytically active enzyme **Caspase-1**
- **Noncanonical inflammasome**: Undefined, promotes activation of procaspase-11
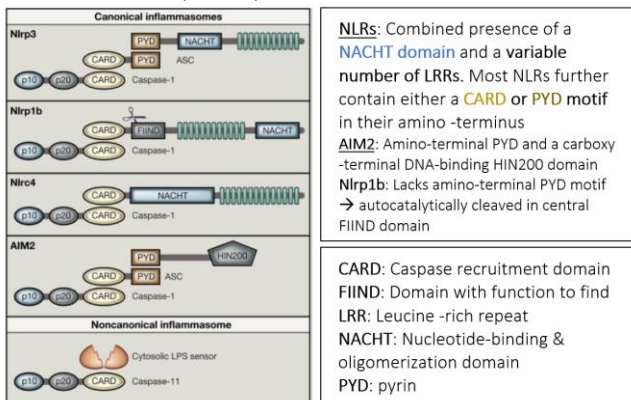
- **Nlrp3, Nlrp1b, Nlrc4 + AIM2** assemble canonical inflammasomes that **promote activation** of the **cysteine protease caspase -1**
  - Nlrp1b and Nlrc4 recruit caspase-1 via their CARD motifs
  - **Nlrp3** and **AIM2** require **ASC** (adaptor protein) for the assembly of AIM2-Nlrp3-inflammasomes and for the interaction with caspase-1→ ASC stabilizes interactions
- **NLRP3 activation** is critical for oligomerization, recruitment and activation of caspases-1 and 11, which is critical for processing and secretion of IL-1β and IL-18 prior to cell death by pyroptosis
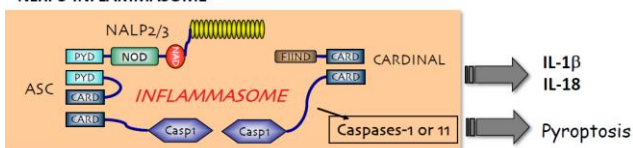
- **Pyrosis**: Highly inflammatory form of programmed cell death that occurs most frequently upon infection with intracellular pathogens and is likely to form part of the antimicrobial response. In this process, immune cells recognize foreign danger signals within themselves, release pro-inflammatory cytokines, swell, burst and die. The released cytokines attract other immune cells to fight the infection and contribute to the inflammation in the tissue

Activation of the NLRP3-Inflammasome

- Single NLRs are expressed in an **auto-repressed, inactive form** → NACHT-mediated oligomerization is blocked and thus inhibiting NLR auto-activation
- Binding of **ligands** → LRRs undergo conformational change that allows NACHT-mediated oligomerization and inflammasome assembly
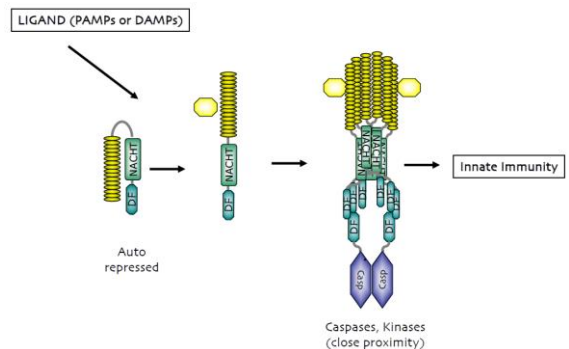
- Ligands can be of various origin:
  - **Direct activation**: Pannexin 1 pores allow entry of PAMPs/DAMPs that activate NLRP3 directly
  - **Lysosome rupture**: Crystalline structures are phagocytosed and lead to lysosome rupture → This causes the release of Cathepsin B that induces NLRP3 inflammasome
  - **Mitochondrial damage**: DNA released from a damaged mitochondrium induces NLRP3 inflammasome
  - **ROS**: Low intracellular K^+ levels trigger ROS generation that in turn lead to NLRP3 activation
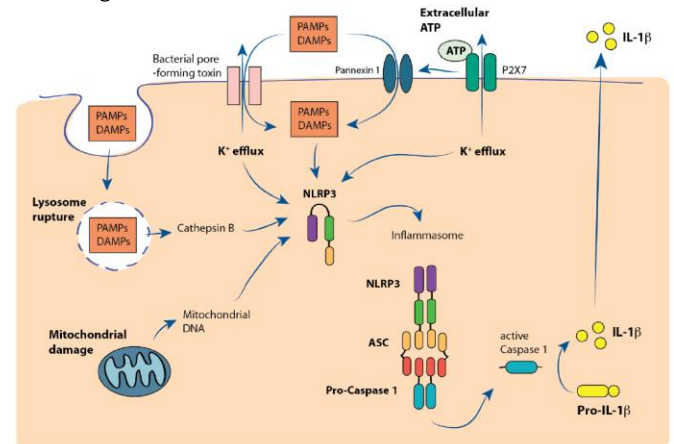
- **PAMPs** and **DAMPs** (Danger-associated molecular patterns) can lead to **ROS-production** and **NLRP3** activation:
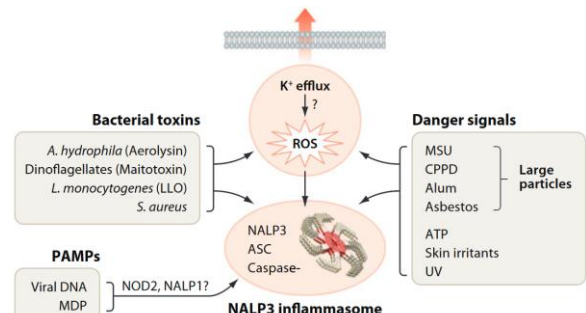

- **Uric acid** is considered to be an endogenous danger signal released from injured cells that can activate NLRP3. It also stimulates dendritic cell maturation and enhances $CD8^+$ T cell responses when co-injected with the antigen

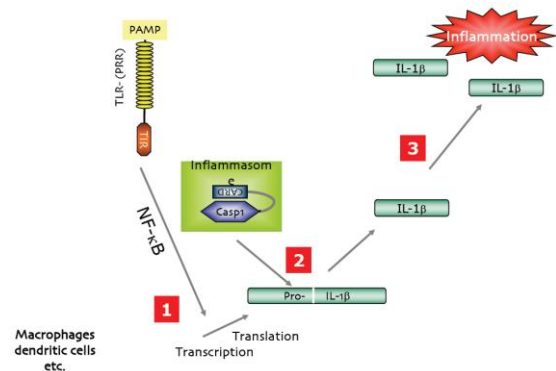Other NLRP3 inflammasome activators are listed in the following table:

Activator class	Activator	Disease associations
Whole pathogen	*Candida albicans*	Infection
	Saccharomyces cerevisiae[*]	Infection
	Staphylococcus aureus	Infection
	Listeria monocytogenes	Infection
	Influenza virus	Infection
	Sendai virus	Infection
	Adenovirus	Infection
Pathogen-associated molecules	Bacterial pore–forming toxins	Infection
	Hemozoin	Cerebral malaria
Environmental insults	Silica	Silicosis
	Asbestos	Asbestosis
	Skin irritants	Contact hypersensitivity reactions
	Ultraviolet light	Sunburn
Endogenous danger signals	ATP	Injury or necrotic cell dea
	Glucose	Metabolic syndrome
	MSU	Gout
	Calcium pyrophosphate dihydrate (CPPD)	Pseudogout
	Amyloid β	Alzheimer's disease
	Hyaluronan	Injury
Adjuvant	Alum	

[*]Viable (52) but not heat-killed (53) *S. cerevisiae* activates the NLRP3 inflammasome.
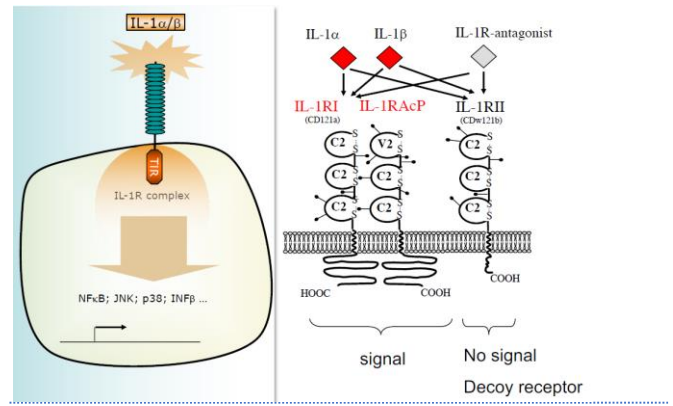
GOUT

- Is one of the most painful form of arthritis
- Occurs when uric acid builds up in the body (uric acid is generated in the breakdown of purines)
- Uric acid can accumulate in the body and form crystals which can lead to
  - Sharp uric acid crystal deposits in joints, often in the big toe
  - Deposits of uric acid (called tophi) that look like lumps under the skin
  - Kidney stones from uric acid crystals in the kidneys
- 1-3% of the population develop gout.

INTERLEUKIN 1

- General name for two distinct proteins **IL-1α and IL-1β**
- Both are **produced** during an **inflammatory response**
  - Mainly produced by **dendritic cells and macrophages**, but also epithelial cells, fibroblasts, keratinocytes, endothelial cells, hepatocytes, type II lung alveolar cells, osteoblasts, neutrophils, eosinophils, megakaryoctes, oligodendrocytes, neurons, Schwann cells
  - Are trimmed to active form by **caspase-1** (IL-1α has also a function in its uncleaved form)
- Main function of IL-1α/IL-1β:
  - **Immune system**: Defense against **bacterial infection**
  - **Cell migration**: Upregulate **adhesion molecules** on endothelial cells allowing transmigration of leucocytes (neutrophils, monocytes, T and B cells)
  - **Bone formation** and **remodelling**
  - **Fever induction** (endogenous pyrogen)
  - Appetite regulation
  - Insulin secretion

IL-1/IL-1 RECEPTOR SYSTEM

- IL-1α/IL-1β bind to the **receptor IL-1RI** and (together with RL-1RAcP) induces the already discussed signaling pathway that leads to the **expression of pro-inflammatory cytokines**
  - IL-1RAcP is required for signaling
  - IL-1R-antagonist (IL-1Ra) binds to IL-1RI and inhibits binding to IL-1RAcP, therefore inhibits signaling
  - IL-1RII is a decoy receptor, meaning it can bind the ligands but does not convey a signal

ACTIVATION OF IL-1

1. Binding of a PAMP to a TLR-receptor induces **NF-κB** signaling that leads to the expression of **Pro-IL-1β**
2. Inflammasome activated by a danger signal **activates caspase-1** → cleaves Pro-IL-1β to its active form
3. Active IL-1β promotes inflammation

- Uric acid (MSU) activates NLRP3 and therefore IL-1β
- Colchicine inhibits this process and is used to treat rheumatism, arthritis and gout

AUTOIMMUNE DISEASES RELATED TO NLRP3 AND IL-1B

- **Gain-of-function mutations** in the gene for NLRP3 are responsible for the following autosomal dominant diseases:
  o Muckle-wells syndrome (MWS)
  o Familial cold autoinflammatory syndrome (FCAS, FCU)
  o Chronic infantile neurological cutaneous and articular syndrome (CINCA, NOMID)

MUCKLE-WELLS SYNDROME (MWS)

- A missense mutation in NLRP3 leads to an **increased production of IL-1β** upon activation by e.g. peptidoglycan
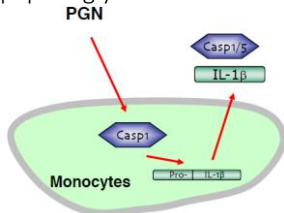
- It can be treated with IL-1Ra (Anakinra) (= competitive inhibitor of IL-1β)
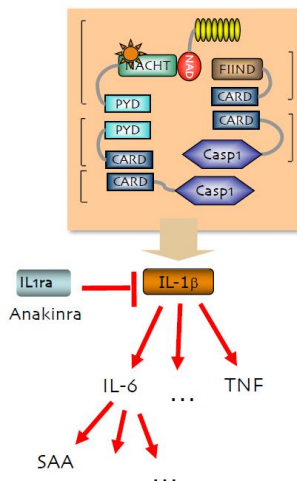
- Beside MWS, anakinra is also used to treat many other diseases with too active inflammasome

OBESITY-INDUCED INFLAMMATION AND INSULIN RESISTANCE

- The NLRP3 inflammasome initiates obesity-induced inflammation and insulin resistance
- Activation of the inflammasome is also responsible for obesity induced inflammation and insulin resistance
  o Treatment: restrict calories→ inhibition of IL-1β→ reduces inflammation
- The NLRP3 inflammasome induces insulin resistance and steatohepatitis in diet-induced obese mice
  o High fed diet → develop insulin resistance
  o NLRP3 deficient mice are resistant

ATHEROSCLEROSIS

- Chronic inflammatory disease affecting the **arterial vasculature** (myocardial infarction, stroke, cardiovascular disease)
- Main risc factors: hyperlipidemia, hypercholesterolemia, diabetes, smoking, hypertension, obesity
- Lipid deposition and oxidation in the artery wall
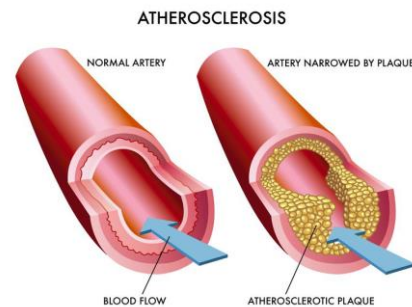
- Important contribution of inflammation and immune cells

PAPERS

NLRP3 IS ACTIVATED IN ALZHEIMER'S DISEASE AND CONTRIBUTES TO PATHOLOGY IN APP/PS1 MICE

ALZHEIMER'S DISEASE:
- Deposition of **amyloid-β peptide** drives cerebral **neuroinflammation** by activating microglia
- Active **caspase-1** expression is strongly enhanced in Alzheimer's disease → **inflammasome** plays a role in this neurodegenerative disease → NLRP3/caspase-1 axis plays an important role in the disease

NLRP3:
- NLRP3 inflammasome is implicated in several chronic inflammatory diseases
- **NLRP3** is <u>activated</u> in Alzheimer's disease
- Inflammasome activation might promote neuroinflammatory components in AD
- NLRP3/caspase-1 inflammasome activation seems to reduce amyloid-β phagocytosis by microglia
  - o NLRP3 activation negatively affects the microglial clearance function in Alzheimer's disease

MICROGLIA:
- Microglia clear pathological deposits of amyloid-β through **phagocytosis** and **degradation**
  - o Microglial phagocytosis is suppressed by proinflammatory cytokines
- In addition to phagocytosis, microglia also contribute to amyloid-β clearance through proteolytic enzymes, including insulin-degrading enzyme (IDE) and neprilysin (NEP)21
  - o Twofold increase of IDE expression is sufficient to reduce amyloid-β deposition strongly

MICE MODELS:
- **Nlrp3$^{-/-}$ or Casp1$^{-/-}$ mice:** (inflammasome deficient mice)
  - o largely protected from loss of spatial memory
  - o Reduced **caspase-1 and interleukin-1b** activation
  - o Decreased amyloid-β amounts and deposition and enhanced **amyloid-β clearance**
  - o Increased microglial amyloid-β phagocytosis
- **APP/PS1 mice**: Express 2 mutations associated with familial Alzheimer's disease, leading to the chronic deposition of amyloid-β, neuroinflammation and cognitive impairment
- **APP/PS1/ Nlrp3$^{-/-}$ mice:**
  - o Assess the contribution of the NLRP3 inflammasome to the pathogenesis of Alzheimer's disease (absent caspase-1 cleavage)
  - o Total volume of amyloid-b plaque was reduced → decreased amyloid-β deposition
  - o Microglial cells show increased amyloid-β phagocytosis
  - o Have increased IDE levels → might enhance the degradation of amyloid-β

THERAPEUTICS:
- NLRP3 inflammasome-inhibition represents a new therapeutic intervention for the disease
- Blocking the activity of NLRP3 inflammasome (or inflammasome derived cytokines) might effectively interfere with the progression of Alzheimer's disease

FAZIT:
- Amyloid-β induced activation of the NLRP3 inflammasome enhances Alzheimer's disease progression by mediating a harmful chronic inflammatory tissue response
- Amyloid-β activation of the NLRP3 inflammasome in microglia is fundamental for interleukin-1b maturation and subsequent inflammatory events

FATTY ACID–INDUCED MITOCHONDRIAL UNCOUPLING ELICITS INFLAMMASOME-INDEPENDENT IL-1A AND STERILE VASCULAR INFLAMMATION IN ATHEROSCLEROSIS

Hypothese: Atherogenesis is mediated by IL-1α
- **Chronic inflammation** is a fundamental aspect of metabolic disorders

Atherosclerosis
- Atherosclerosis is a chronic **inflammatory disease** that affects the **arterial vasculature**
- Development of atherosclerotic lesions is driven by the deposition and oxidation of cholesterol-rich lipoproteins in the subendothelial space
- Phagocytosis of oxidized lipoproteins limits the potential cytotoxic and proinflammatory properties of their lipid cargo but also transforms macrophages into cholesterol-loaded foam cells, which represent a hallmark of the atherosclerotic plaque
  - While macrophage foam cells initially contribute to removal of cholesterol from the plaque they also release proinflammatory cytokines, growth factors and matrix-modifying enzymes that sustain the local inflammatory milieu and promote disease progression

Interleukin 1
- Signaling through **receptor for interleukin 1 (IL-1R)** severely enhances vascular inflammation and atherogenesis
  - IL-1β has already been associated with various inflammatory conditions
  - IL-1α and IL-1β could initiate IL-1R-mediated vascular inflammation
  - **IL-1R-deficient mice** exhibit less atherosclerosis
  - **IL1Ra-deficient mice** display exacerbated atherosclerosis and fatal vascular inflammation

RESULTS:
- Macrophage-derived **IL-1α** but not IL-1β drives atherogenesis
  - Severity of atherosclerosis is significantly reduced in IL-1α-deficient mice
  - IL-1α, not IL-1β is the predominant inflammatory cytokine that exacerbated atherosclerosis via IL-1R signaling
- Oleic acid selectively elicits IL-1α secretion from macrophages
  - Fatty acids are potent inducers of IL-1-mediated vascular inflammation in atherogenesis
  - OA did not induce an IL-1β response
- IL-1α-inducing fatty acids are abundant in plaques
  - Fatty acid profiles changes with plaque progression → Over threefold greater abundance of OA and linoleic acid
  - Unsaturated fatty acids OA, linoleic acid and arachidonic acid selectively induce IL-1α production

  - Majority (74.5%) of fatty acids that accumulate in atherosclerotic lesions are selective inducers of IL-1α, but not of IL-1β
  - OA represent the most abundant and potent trigger of IL-1α
  - Fatty acids that accumulate in atherosclerotic plaques selectively stimulate macrophage foam cells to produce IL-1α
- Fatty acids elicit inflammasome-independent inflammation
  - OA-induced IL-1α is inflammasome independent
  - Injection of OA is characterized by massive infiltration of neutrophils and macrophages
  - IL-1α is the predominant cytokine of the IL-1 family that mediated the fatty acid–induced inflammation
- Dietary OA induces atherogenesis in vivo
  - Dietary OA induces foam-cell formation, vascular inflammation and atherogenesis in vivo
- OA-induced IL-1α secretion requires calcium signaling
  - Pro-IL-1α is processed into its secreted form by calcium-sensitive cysteine protease calpain → Activation of calpain is required for IL-1α secretion
  - Fatty acids act as **ionophores** able to dissipate the mitochondrial proton gradient, thereby uncoupling the respiratory chain from ATP synthesis → Mitochondrial uncoupler FCCP induces substantial calcium fluxes in primary macrophages consistent with a role for mitochondrial uncoupling in the elicitation of IL-1A by OA
  - Fatty acids induces the secretion of IL-1α via the release of intracellular calcium for subsequent calpain-mediated processing of pro-IL-1A → Fatty acid mediated mitochondrial uncoupling is required for OA-elicited production of IL-1α
  - Fatty acid–induced mitochondrial uncoupling is independent of PPAR and FFAR1 receptors for fatty acids
  - in dyslipidemic conditions, CC-exposed macrophages produced mainly IL-1α because of fatty acid–induced mitochondrial uncoupling
- Mitochondrial uncoupling controls IL-1 responses
  - Fatty acid–induced mitochondrial uncoupling and calcium-signaling deviates IL-1β responses toward IL-1α secretion
  - Exposure of macrophages to OA inhibited the ATP-induced IL-1β response
  - FCCP treatment alone elicited strong IL-1α production in primary macrophages. Notably, cotreatment of primary macrophages with FCCP suppressed the ATP-elicited IL-1β response
  - IL-1α -inducing fatty acids linoleic acid and arachidonic acid also inhibited the CCelicited IL-1β response, while palmitic acid showed no effect

- <u>Mitochondrial uncoupler UCP2 regulates OA-induced IL-1a</u>
  - o Fatty acid–mediated uncoupling has been proposed to involve UCPs
  - o UCP2 was the predominant uncoupling protein expressed by macrophage foam cells
  - o Mitochondrial uncoupling mediated the fatty acid–induced IL-1α response and suggest a role for UCP2 in this process
  - o OA-induced mitochondrial uncoupling, calcium fluxes and IL-1α production are regulated in part by mitochondrial UCP2

FAZIT:
- Fatty acid–induced mitochondrial uncoupling is required for the selective induction of inflammasome-independent, IL-1α-driven vascular inflammation in response to lipid overload
- Atherosclerosis in inflammasome-deficient mice does not necessarily link the IL-1β produced by foam cells to atherogenic vascular inflammation → impaired release of IL-1α in some Il1β$^{-/-}$ → results would suggest IL-1α and not IL-1β as the major IL-1 isoform involved in atherogenesis
- IL-1α drives vascular inflammation → predominant role of IL-1α in atherosclerosis
- Fatty acids are potent inducers of IL-1α driven vascular inflammation
  - o Fatty acids selectively stimulate the release of IL-1α but not of IL-1β by uncoupling mitochondrial respiration
  - o Fatty acid induced mitochondrial uncoupling abrogated IL-1β secretion, which deviated the cholesterol crystal–elicited response toward selective production of IL-1α
  - o IL-1α, not IL-1β, should be targeted in patients with cardiovascular disease

DR. HAILEY GAHLON

CANCER INCIDENCE & RISK FACTORS

- Cancer is defined as an **abnormal cell growth**

ORIGINE OF THE WORD *CANCER*

- ~ 400 BC the Greek physician Hippocrates: Named a mass of cancerous cells *karkinos*, which is the Greek word for **crab** (malignant tumor and swollen blood vessels around it reminded him of a crab dug in the sand with its legs spread in a circle)
- First recorded case of cancer was in **2500 BC** where an Egyptian physician Imhotep described a "bulging mass in the breast"

THE BEGINNING OF CANCER

- **Dysregulation**: Mutations occur which lead to uncontrolled **cell growth** (cells **divide uncontrollable**) → leads to a cell mass → leads to changes in environment (increased blood flow etc.) → invasive stage follows
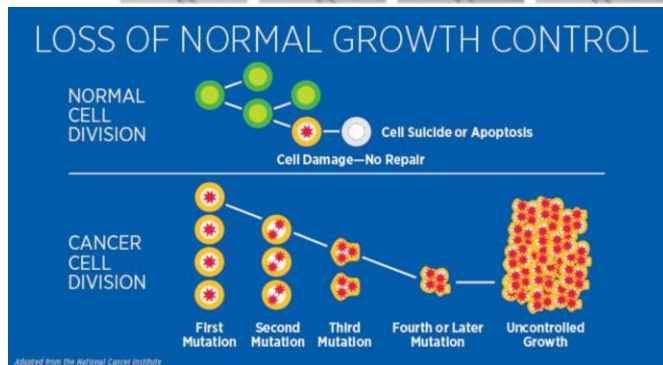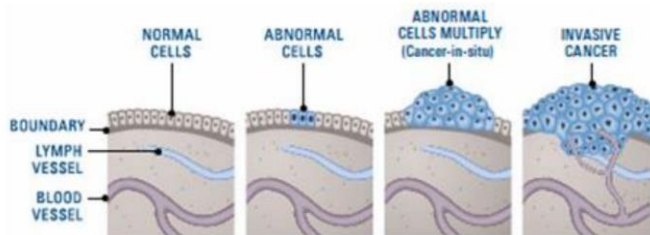
CANCER SPECIFICATION

- The six most common types of cancer: **Lung, Colon, Breast, Prostate, Stomach, Liver**
- Brain is not one of the 6 most common types of cancer

Four main types of cancers are classified in **tissue of origin**:
- **Carcinoma**: epithelial tissues found in the internal and external lining of the body
  - o Squamous cell carcinoma → develop in squamous epithelium of organs (skin, bladder, esophagus, lung)
  - o Adenocarcinoma develop in organ or gland (breast cancer)
- **Sarcoma**: Connective tissues from bone, tendons, cartilage, muscle and fat
- **Leukemia**: Blood cancers that originate in bone marrow
- **Lymphoma**: Lymphatic system cancers
-

TUMOR DEVELOPMENT

- Follows Darwinian evolution → Treelike accumulation of mutations and "survival of the fittest"
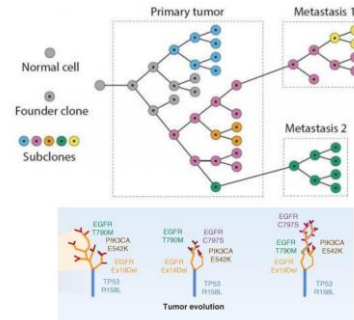
Challenge: **>1000 mutations/tumor** (not all mutations are cancer driver mutations)

STAGES OF CANCER

- There are **4 different stages** of cancer
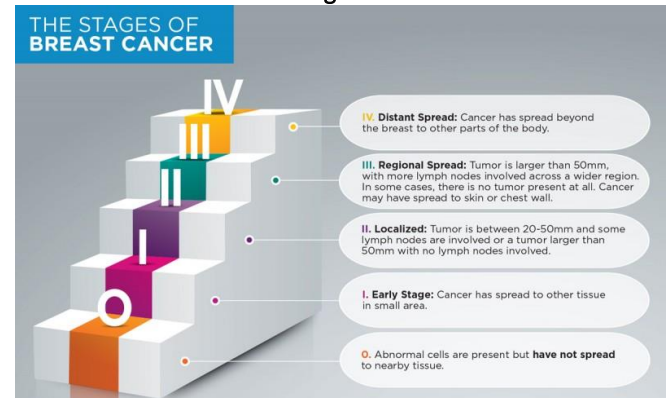
CANCER METASTASIS
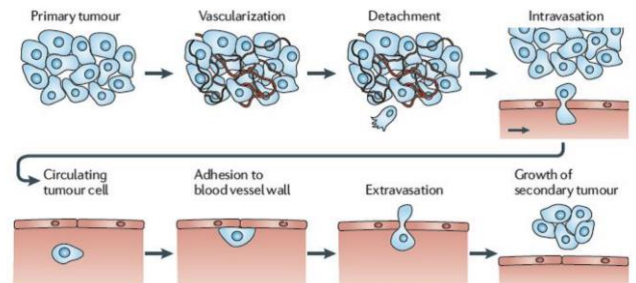
NUMBERS

Learn no exact numbers for the exam
- Cancer is one of the **leading causes of morbidity** and **mortality worldwide**
- Cancer is the **second leading cause** of death globally, and was responsible for **8.8 million deaths in 2015** (out of 54mio death per year)
- Nearly **1 in 6 deaths is due to cancer**
- Approximately 70% of deaths from cancer occur in **low- and middle-income countries**
- The **economic impact** of cancer is **significant** and is **increasing** → total annual economic cost of cancer in 2010 was estimated at approximately **US$ 1.16 trillion**
- CH: 35'000 cancer diagnoses/year, 16'000 cancer death/year (44 people each day)

GLOBAL TRENDS IN CANCER

GLOBAL TREND (COUNTRY, ETHNICITY)

- Cancer burden rises to 18.1 million new cases and **9.6 million cancer death** in 2018 (16%)
  - o Americans (21%), Europe (23.4%), Asia (48.4%), Oceania (1.4%) and Africa (5.8%)

Top cancer per country, estimated age-standardized incidence rates (World) in 2018, both sexes, all ages
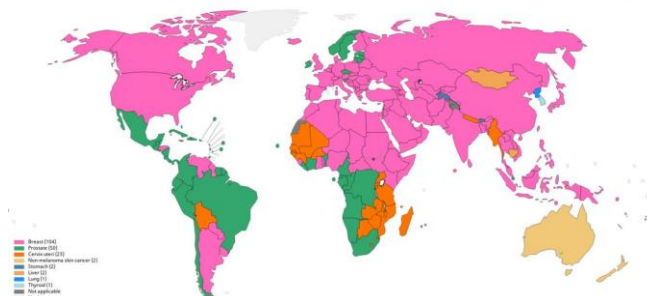
CH: low incidence of stomach cancer but high incidence of skin cancer (rank 4)

GENDER

- Cancer incidence based on gender
- In Switzerland:
- • Death frequency: men
  - – lung cancer 23%
  - – prostate cancer 15%
  - – colorectal cancer 10%
- • Death frequency: women
  - – breast cancer 13%
  - – lung cancer 13% (up 3%)
  - – colorectal cancer 11%

Men: All sites 854,790 (100%)
- Prostate (28%)
- Lung & bronchus (14%)
- Colon & rectum (9%)
- Urinary bladder (6%)
- Melanoma of the skin (5%)
- Kidney & renal pelvis (5%)
- Non-Hodgkin lymphoma (4%)
- Oral cavity & pharynx (3%)
- Leukemia (3%)
- Pancreas (3%)

Women: All sites 805,500 (100%)
- Female Breast (29%)
- Lung & bronchus (14%)
- Colon & rectum (9%)
- Uterine corpus (6%)
- Thyroid (6%)
- Non-Hodgkin lymphoma (4%)
- Melanoma of the skin (4%)
- Kidney & renal pelvis (3%)
- Pancreas (3%)
- Ovary (3%)

TIMELINE TREND

- 1975-2000: **cases doubled**
- 2020 another doubling predicted

- **Some major contributors**
  - o Adoption of **tobacco** use
  - o Increasingly high fat diets
  - o Demographic shift of population to less developed countries
  - o Unknown exposures
  - o Aging population

Cases — 14.1 million — New cases of worldwide cancer, 2012

Deaths — 8.2 million — Deaths from worldwide cancer, 2012

Smoking — Around 33% — Cancer cases linked to exposure to tobacco smoke, worldwide

Cases — 17 million — New cases of cancer worldwide, 2018

Deaths — 9.6 million — Deaths from cancer worldwide, 2018

Smoking — Around 33% — Cancer cases linked to exposure to tobacco smoke, worldwide

WHAT FACTORS CONTRIBUTE TO STOMACH CANCER MORTALITY DECLINE?

- Stomach cancer was the leading cause of cancer deaths in the world until the 1980s when it was overtaken by lung cancer
- Incidence and mortality from stomach cancer have dropped dramatically over the last half century in most industrialized countries → Why?
  - o **decrease in the exposure** to Helicobacter pylori (H. pylori) infection
  - o **dietary** and other exogenous factors
  - o **better food preservation** and **refrigeration** (largely replacing salting, pickling, and smoking)
  - o Ready availability of **fresh foods and vegetables**
  - o **Improved detection** and **therapy** for stomach cancer and H. pylori infections

The scientist Barry Marshall proved that stomach ulcers are caused by bacteria by drinking a broth filled with bacteria. He got ulcers and then cured himself with antibiotics. He ended up winning a Nobel Price

RISKFACTORS

ENVIRONMENTAL FACTORS

- 90% of Cancers attributed to environmental factors
  → play a very high role as risk factors
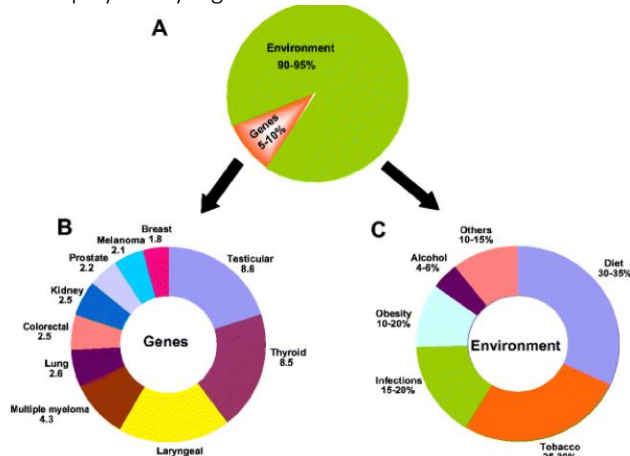
- Cancer causing infections, such as **hepatitis and human papilloma virus (HPV)**, are responsible for up to 25% of cancer cases in low- and middle-income countries
- Around 1/3 of deaths from cancer are due to the 5 leading **behavioral and dietary risks**: high body mass index, low fruit and vegetable intake, lack of physical activity, tobacco use and alcohol use
- **Tobacco** use is the most important risk factor for cancer and is responsible for approximately **20-30% of cancer deaths**

IARC CLASSIFICATION OF CARCINOGENS

IARC: International Agency research on cancer

- <u>Group 1</u>: The agent **is carcinogenic** to humans
- <u>Group 2A</u>: The agent is **probably** carcinogenic to humans
- <u>Group 2B</u>: The agent is **possibly** carcinogenic to humans
- <u>Group 3</u>: The agent **is not classifiable** as to its carcinogenicity to humans
- <u>Group 4:</u> The agent is **probably not carcinogenic** to humans

PANCREATIC CANCER RISK FACTORS

RED MEAT

Colorectal cancer risk and red meat:

- <u>Neu5GC</u> (sugar) is present in beef, pork, lamb but **humans can't synthesize it**
- Recent hypothesis: **N-glycolylneuraminic acid** (Neu5Gc - non human sugar in red meat) is incorporated in cell membrane, triggers an immune response that contribute to carcinogenesis (The chronic inflammation generates reactive oxygen species (ROS) that contributes to cancer and tumor progression)

ASBESTOS

- Group of oxidized silicate minerals that occurs naturally in the environment as bundles of fibers
- Classified as known human carcinogen
  o Cancer: lung and mesothelioma
- Basis of carcinogenesis
  o Fibre size and physical properties dictate degree of risk, Unique mode of physical interference with cell division process

OTHER CHEMICAL/ENVIRONMENTAL
- **Agriculture**
  - o Certain pesticides
  - o *Leukemia, lymphoma, multiple myeloma and soft tissue sarcoma, cancers of the skin, lip, stomach, brain and prostate*
- **Formaldehyde**
  - o Emitted from pressed wood building materials
  - o *Leukemia, particularly myeloid leukemia, possibly nasal cancers*
- **Chemicals used as dyes**, especially hair dyes before 1980
  - o *Leukemias and lymphomas*

ALCOHOL
- Classified as known **human carcinogen**
- Clear associations for several cancers
  - o *Head and neck, oesophageal, squamous cell carcinoma, liver cancer, breast cancer, colorectal cancer*
- How? Biotransformation of ethanol to acetaldehyde generation of reactive oxygen species that can damage DNA, proteins and lipids via oxidation, Inhibition of nutrient absorption, increase in blood oestrogen

HORMONES
- Pregnancy, Contraceptives and Menopausal hormone use
- Diethylstilboestrol (DES)
  - – Synthetic estrogen
  - – Prescribed to pregnant women in 40s-70s to prevent complications of pregnancy (until 1978 in europe)
  - – Prenatal exposure linked with clear cell adenocarcinoma: cervix and vagina
  - – DES is an endocrine-disrupting chemical
  - – Cancer-causing effects of endocrine disruption are most severe during fetal development
  - – So-called "DES daughters" have 40 times elevated risk, often developed very young
  - – After 40, about twice the risk of breast cancer

ADDITIONAL RISK FACTORS
- Infectious agents
  - o HIV infection
  - o HPV
  - o Helicobacter pylori
- Radiation
- Sunlight
- Tobacco

HAYFLICK LIMIT
The Hayflick limit or is the **number of times** a normal human cell population will **divide** before cell division stops
- Each time a cell undergoes mitosis, the telomeres on the ends of each chromosome shorten slightly. Cell division will cease once telomeres shorten to a critical length. Hayflick interpreted his discovery to be aging at the cellular level. The aging of cell populations appears to correlate with the overall physical aging of an organism

THE TUMOR MICROENVIRONMENT

Tumors increasingly being recognized as organs
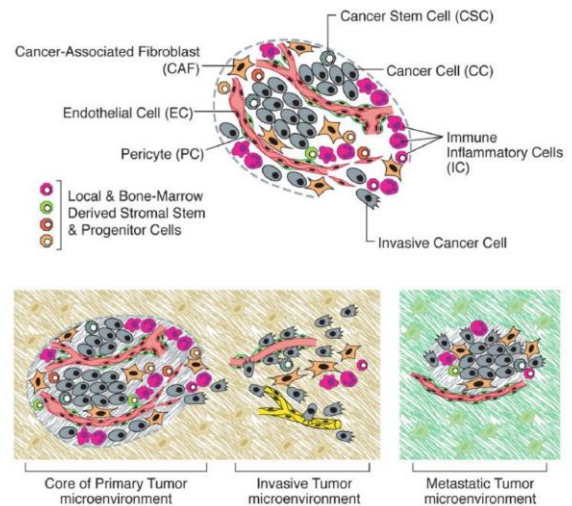
SIGNALING INTERACTIONS IN TUMOR MICROENVIRONMENT
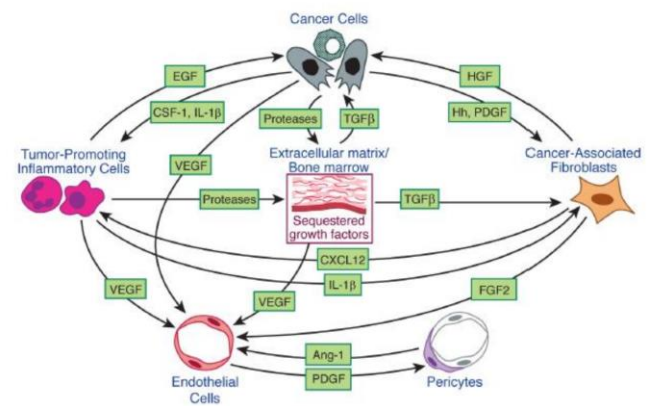
HALLMARK OF CANCERS
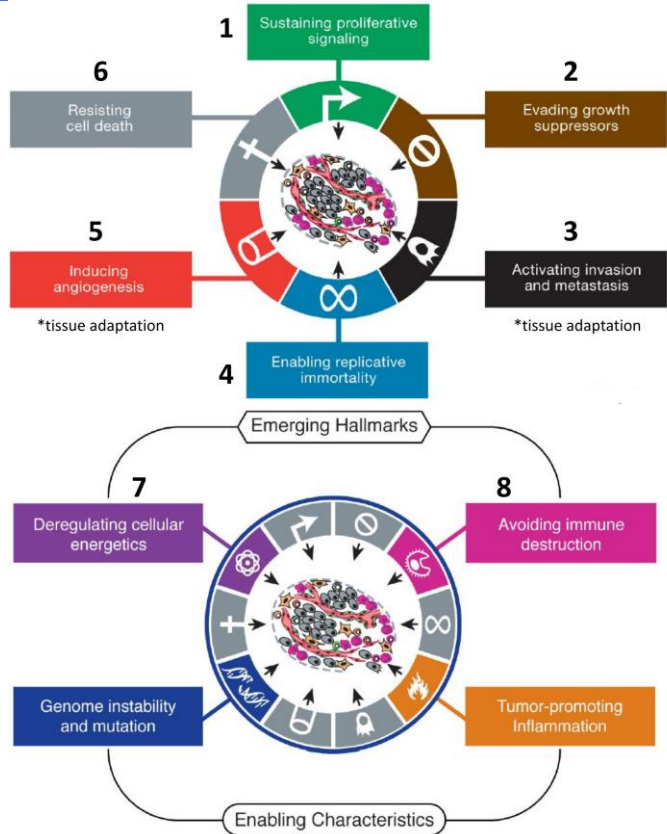
1: SUSTAINING PROLIFERATIVE SIGNALING

Supporting **chronic proliferation** (e.g. growth hormone release)

- o **Self-production** of **growth factors** (e.g. HGF)
- o **Self-production** of **cognate receptors** (e.g. Tyrosine kinases)
- o Somatic **mutations** that **activate signaling** circuits (e.g. PI3-kinase)
- o **Defects** in **negative-feedback loops** (e.g. **Ras**)

RAS SIGNALING PATHWAY

- Ras proteins are essential components of signaling networks controlling **cellular proliferation, differentiation, and survival**
- Oncogenic mutations of the H-ras, N-ras, or K-ras genes frequently found in human tumors (**~30%**)
- Oncogenic mutations are concentrated within 2 hotspots (around codons 12 and 61) of the primary nucleotide sequence of all Ras family members

Oncogenic mutations of Ras isoforms:

- o Mg^{2+}/nucleotide binding
- o Effector binding
- o Membranes binding/trafficking
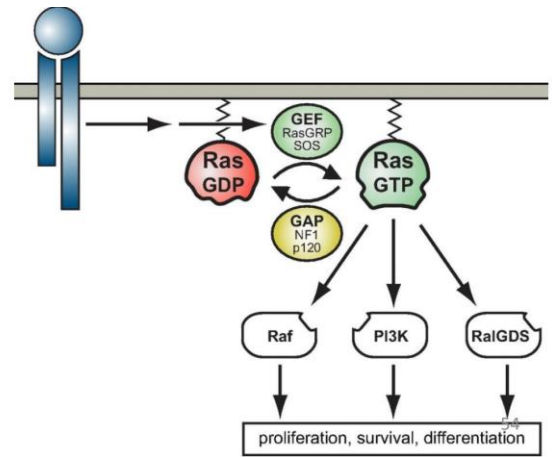
- Ras is a GTPase (Ras is bound to GTP if its turned on)
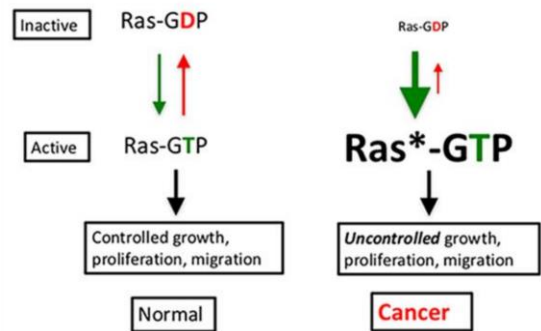
INHIBITION OF RAS
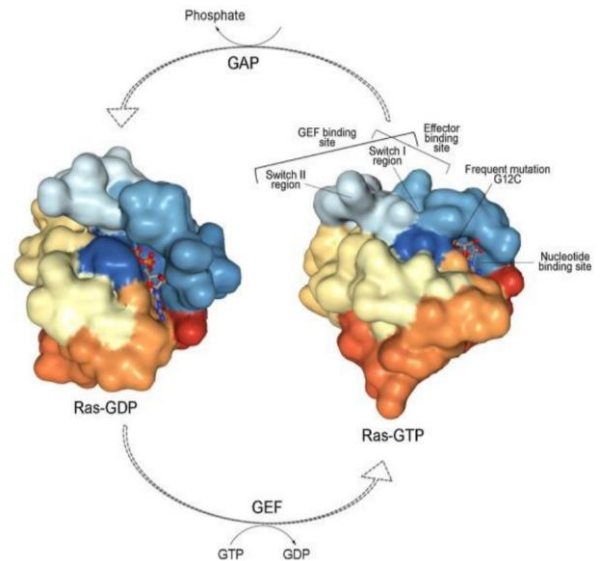
Examples of Ras inhibitors:

- **Nucleotide binding site inhibition**
  - o SML-8731, SML-10701, SML-11
  - o DABP-GTP
- **Post-translational modification inhibition**
  - o Spermatinamine
  - o S-farnesyl-thiopropionic acid

2: EVADING GROWTH SUPPRESSORS

Cancer cells **adapt ways** to **avoid signals that negatively regulate cell proliferation**
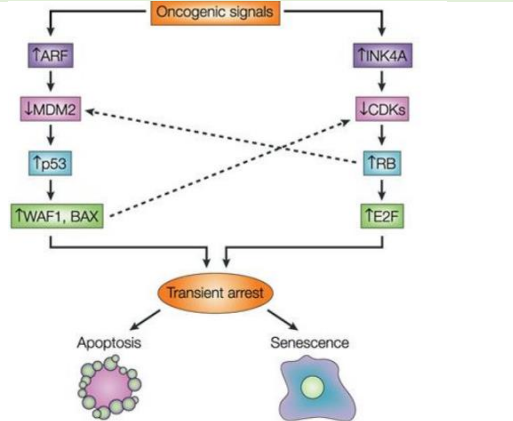
Some functional redundancy
RB null mice - free of proliferative abnormalities
P53 null mice - free of proliferative abnormalities

- This hallmark generally involves **tumor suppressor proteins** → modulate cell growth either through negative regulation of the cell cycle or by promoting apoptosis:
  o **RB** (Retinoblastoma) → inactivated by CDK phosphorylation
  o **TP53**

TUMOR SUPPRESSORS P53 AND RB
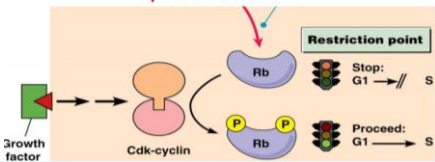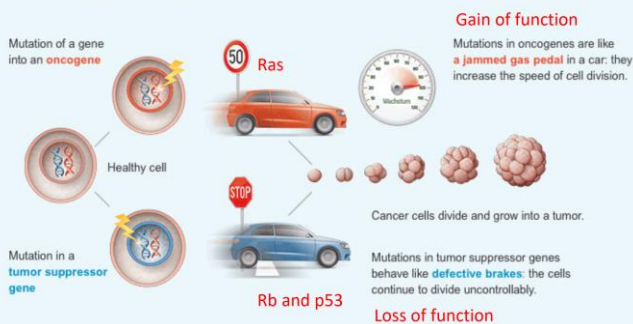
LOSS OF CONTACT INHIBITION IN CANCER
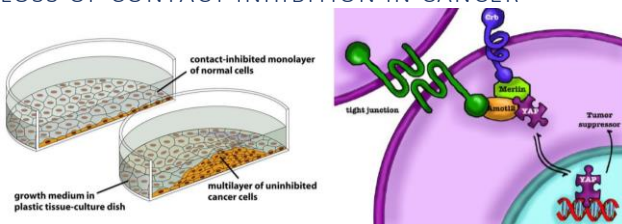
- **Merlin** is a **membrane scaffolding protein**
- Mediates **contact inhibition** by coupling cell surface adhesion molecules
- In various cancers there is a **loss of Merlin function**

3: ACTIVATING INVASION AND METASTASIS

Alterations in **cell-to-cell** or **cell-to-ECM adhesion molecules** (e.g. loss of E-cadherin)

- Traits of invasion and metastasis:
  o **Loss** of **adherent junctions**
  o Expression of **matrix degrading enzymes**
  o Increased **motility**
  o **Resistance to apoptosis**
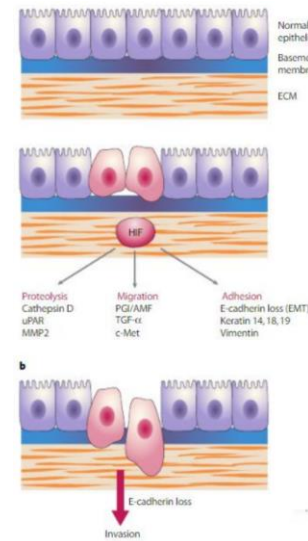
Hypoxia inducible factor (HIF) induces markers that activate proteolysis to stimulate migration

4: ENABLING REPLICATIVE IMMORTALITY

- Telomeres **protect the end of chromosomal DNA**
  o Composed of nucleotide repeats that shorten progressively after each round of DNA replication
- **Telomerase** is a DNA polymerase that **lengthens the ends of telomeres**
- Normal cells have **low levels of telomerase** and cancer cells have **significantly higher levels**
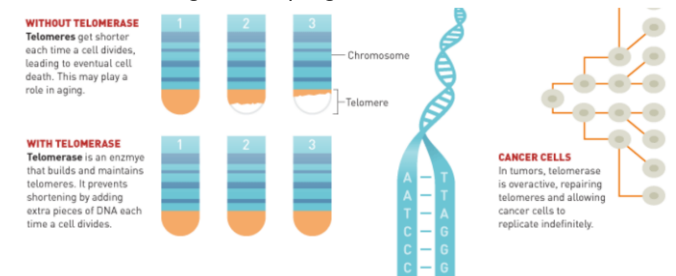
THE END REPLICATION PROBLEM
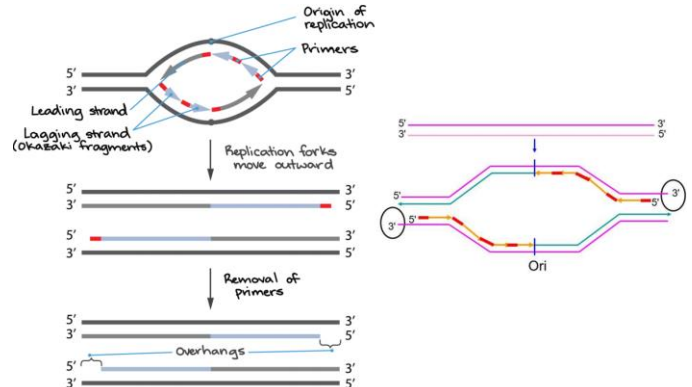
- Telomers shorten with each S phase
- DNA replication is bidirectional (leading and lagging)
- Polymerase moves 5' to 3'
- RNA primer used for initiation of synthesis
- Each round of DNA replication leaves 50-200 bp of DNA unreplicated at the 3' end

Humans could **circumvent the end replication problem with circular DNA**

- Why do we have linear chromosomes (advantage?)
- Hypothesis: small chance that all circular chromosomes are properly segregated during meiosis → telomere alignment
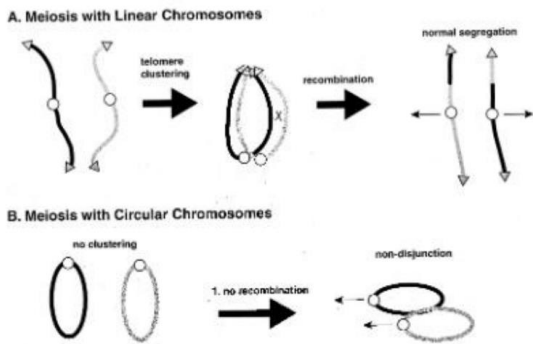
5: INDUCING ANGIOGENESIS

**Angiogenesis** is the **growth of blood vessels from pre-existing vasculature**

- Cancer cells need **high amounts of nutrients** and **oxygen** as well as the **ability to remove waste and CO_2**
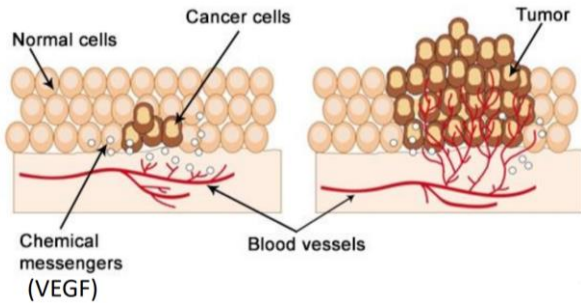

6: RESISTING CELL DEATH

Apoptosis = programmed cell death

- Cancer cells acquire mechanism that **evade apoptosis**
- <u>Bcl-2 family</u> of proteins are **inhibitors of apoptosis** (via binding to pro-apoptotic proteins like Bax and Bak) in the **outer mitochondrial membrane**
- <u>Bax and Bak</u> disrupt the mitochondrial membrane to release cytochrome c to activate proteases for apoptosis
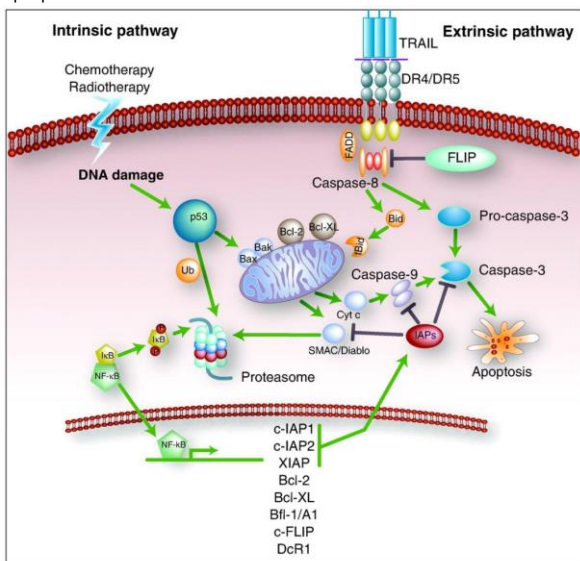
7: DEREGULATING CELLULAR ENERGETICS

- **Warburg effect:** Cancer cells **reprogram their energy production** by **limiting metabolism** largely to **glycolysis and lactic acid fermentation**
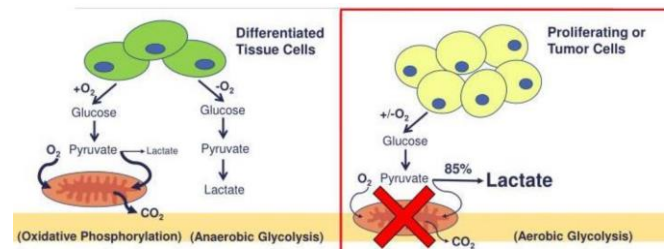
- Seems counterintuitive: **ATP production is ~18-fold less efficient** by glycolysis relative to oxidative phosphorylation → *Why?*
- Hypothesis: increased glycolysis allows for the molecular intermediates to be used in other biosynthetic pathways important for growth of new cells, e.g. generating nucleosides and amino acids

8: AVOIDING IMMUNE DESTRUCTION

- Immune system provides surveillance to **destroy abnormal/ cancer cells**
- Some cancer cells **avoid immune detection**, to evade eradication
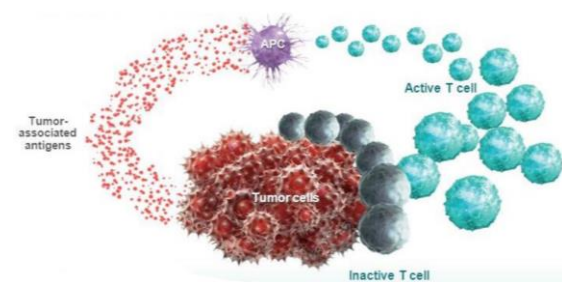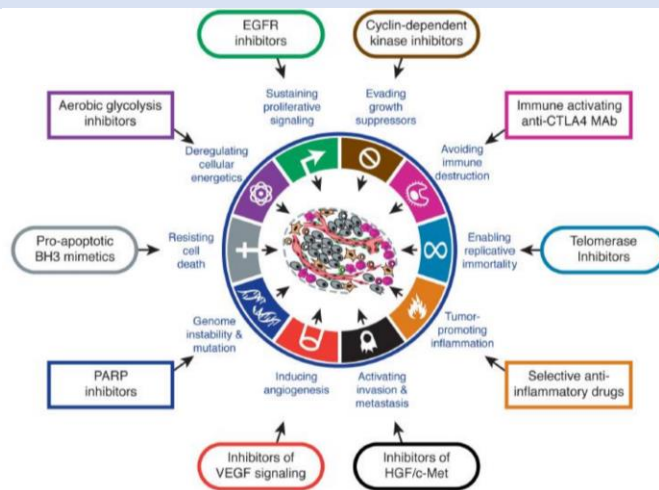

ENABLING CHARACTERISTICS
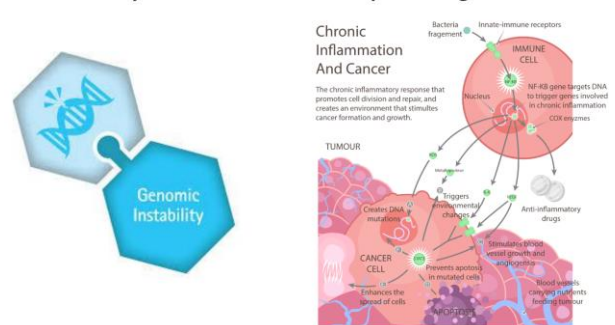
OVERVIEW
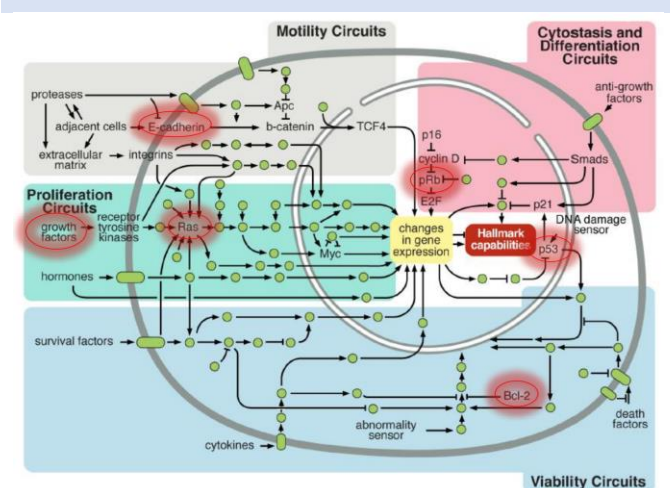
THERAPEUTIC TARGETS FOR CANCER

ONE EXAMPLE: TELOMERASE INHIBITION

Telomerase is a **ribonucleoprotein polymerase** that **maintains telomere** ends by addition of the telomere repeat TTAGGG (It binds to a ncRNA)

Clinical Trial GRN163L (Geron):
- One telomerase inhibitor on the market: **Imetelstat** (is used to treat hematologic myeloid malignancies)
- Imetelstat **binds** with **high affinity to the template region** of the RNA component of telomerase, resulting in direct, **competitive inhibition** of telomerase enzymatic activity

Imetelstat   Imetelstat Bound to Telomerase

Tips for cancer part of exam
- About half questions concern concepts from lecture and understanding in general context
- About half questions concern specific examples (i.e. carcinogenic factors, associated cancers, relevant mechanisms, important genes, key pathways)
- There will be no questions regarding global cancer incidence/statistics, often given for general interest and real world context of the molecular and mechanistic info

GENOTOXIC CARCINOGENESIS

Carcinogenesis is the **formation of a cancer**. It is a **multi-step process,** which is characterized by changes at cellular, genetic and epigenetic levels and abnormal cell division

CARCINOGENESIS

| INITIATION | PROMOTION | TRANSFORMATION | PROGRESSION |
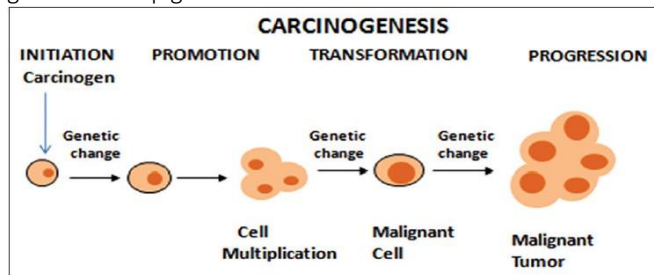
Initiation → Carcinogen (cancer-causing substances) induces a primary change in the cells

CARCINOGENESIS

• Genotoxic	• Non-Genotoxic
• Direct reaction with DNA to alter its structure	• Don't directly cause DNA mutation
• *Direct carcinogens:*	• Increased cell proliferation
• No metabolic activation (alkylating agents)	• Decreased apoptosis
• *Indirect carcinogens:*	• Induction of metabolic enzymes
• Require metabolic activation (aromatic amines, PAHs)	Example: endocrine disruptors

- Genotoxic carcinogens can be metabolized/ biotransformed to **proximal carcinogens**
- Proximal carcinogens are forms that **chemically react with DNA and damage it**
- This form of DNA damage is a **precursor to mutations**

SOME MAJOR EXAMPLES OF GENOTOXIC CARCINOGENS
- UV Radiation
- Reactive Oxygen Species
- Polycyclic Aromatic Hydrocarbons
- Mycotoxins/Aflatoxins
- Acrylamide, Alkenes and haloalkanes, N-Nitrosamines, Aromatic amines, Furans, Polycyclic Aromatic Amines

CONCEPTS IN XENOBIOTIC BIOTRANSFORMATION
Xenobiotic = chemical substance not naturally produced by an organism

(*Biotransformation* = *Metabolic conversion of endogenous and xenobiotic chemicals to more water-soluble compounds. Chemical modification of a xenobiotic by biotransformation may alter its biological effects*)
- Change in physical properties → Increase in **hydrophilicity**
- Metabolites as biologically reactive intermediates
- Enzyme levels depend on tissue type
- High polymorphism

MUTATION VS POLYMORPHISM

Both are **DNA sequence variations**
- A **mutation** is **any change in a DNA sequence** away from the normal, implying there is a normal allele prevalent in the population and that mutations are rare and are an abnormal variant
- A **polymorphism** is a DNA sequence variation **common in the population**
  - **No** single allele is regarded as **standard** sequence
  - **Many** are **neutral in effect**
  - Most polymorphic variants do not overtly cause a disease, impact characteristics like height, rather than disease
  - **Some contribute to disease susceptibility** and **influence drug responses** (i.e. high polymorphism in metabolic enzymes)

ROLE OF METABOLISM IN CARCINOGENESIS

METABOLIC ENZYME POLYMORPHISM IN CANCER SUSCEPTIBILITY (ANFÄLLIGKEIT)
- Considerable inter-individual genetic variability in metabolic pathways
- *Why is it important to understand?*
  - Identification of people at increased risk (association of genetically determined variants with risks of carcinogenesis)
  - **Personalized cancer prevention/therapy**
- Molecular genetic basis of differential enzyme activities involved in metabolism

**Example 1:** CYP2B6 has been identified in the **human brain**, the critical site in **CPS poisoning**
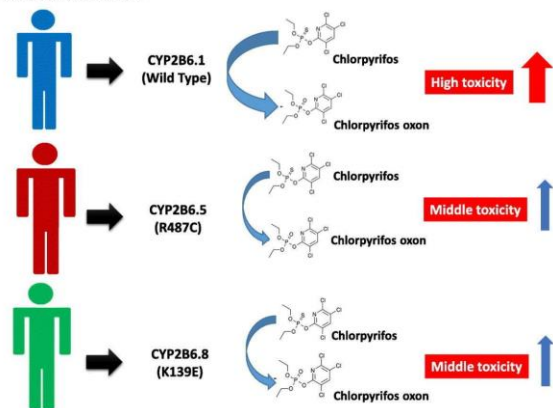
Allele CYP2B6.5 occurs more often in German people (10.9% of alleles) than in Japanese people (1.1% of alleles)

Example 2: Increased risk for **male bladder cancer** among a cohort of male and female hairdressers from geneva
- Significant increase in all cancers
- Proposed link to colouring agents in hair gels widely used in men's hairdressing in the period (2-Naphthylamine)
- **Polymorphisms** in NAT modulates **bladder cancer susceptibility** (evidence also in other cancers)
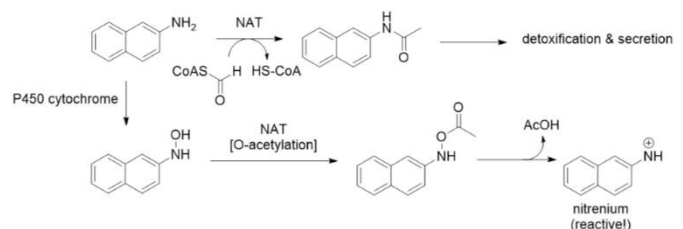
GENETIC VARIABILITY IN METABOLISM/ BIOTRANSFORMATION ENZYMES

- Genetic variability in **metabolism/ biotransformation enzymes** can influence susceptibility to the harmful effects of genotoxic carcinogens
- If the variant is associated with increased detoxification, the individual has a lower risk, and vice versa
- Two important polymorphic metabolism enzymes are **NAT and GST**
  - *What transformations do they catalyse?*
  - *Do the transformations involve activation or detoxification or both? If both, how is the balance shifted toward cancer?*
  - *What cancers are this relevant for? Thus, what cancers are those carcinogens associated with*

NAT POLIMORPHISM

Bladder cancer susceptibly is modulated by **N-Acetyl Transferase (NAT) polymorphism**

Associations between NAT **enzyme activities** and cancer risk:

- Evidence for **polymorphism** and myeloma, lung, bladder, lymphoma, liver, colorectal cancer
- NATs are involved in the **metabolic activation and detoxification of arylamines**
  - Located in the cytosol
  - Prevalent in liver in mammals
  - There are 2 well-characterized human variants
    → NAT1 & NAT2

ACTION OF NATS ON ARYLAMINES (AAS):

Catalyzes acetylation reaction

- Cofactor: acetyl-coenzyme A (Ac-CoA)
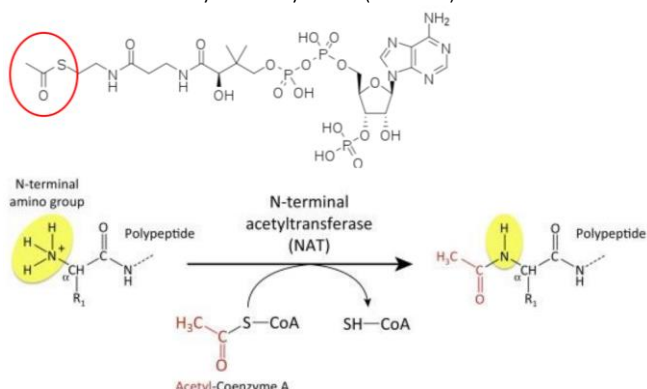
1. **Detoxification**
- N-acetylation
2. **Activation**
- N-hydroxy AAs by P450s
- NATs catalyze the formation of N-acetoxy esters of N-hydroxy AAs
- N-acetoxy esters cleave to yield reactive nitrenium
- Nitrenium is potent DNA alkylating agent
- ➔ NATs can both: **Activate** or **detoxify** xenobiotics

GENETIC POLYMORPHISM IN NATS:

- Extensive polymorphisms with **high frequency**
- Segregate human population into **rapid, intermediate, and slow acetylator phenotypes**
- **Slow acetylators** exhibit **increase in risk** of developing several cancers, including bladder cancer, as compared with rapid acetylators

GST POLYMORPHISMS

GST polymorphisms associated with **elevated cancer risk**

- GSTs are large multigene family of enzymes involved in **detoxification of potentially genotoxic chemicals**
  - High concentrations in liver, intestine, kidney
  - Account for ~10% of cellular proteins
  - GST Family: Mostly cytosolic (Kappa is mitochondrial)

GST-MEDIATED REACTION

- **Reduced form of glutathione (GSH)** conjugates to xenobiotic substrates for detoxification
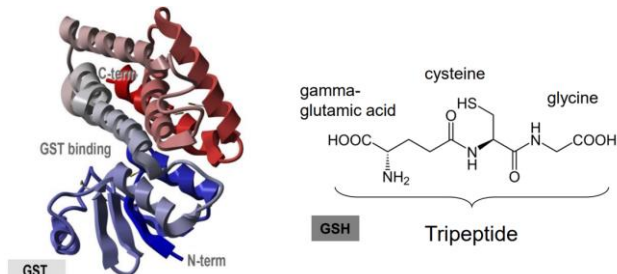
GSTs are involved in **detoxification of potentially genotoxic chemicals**
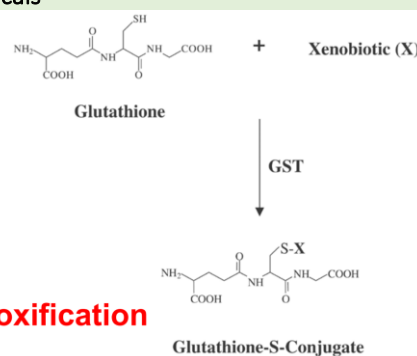
EXAMPLE OF ASSOCIATION STUDY

"Glutathione S-transferase M1, T1, P1 genotypes and risk for development of colorectal cancer"

- Tested relationship of GSTM1, GSTT1, and GSTP1 genes polymorphism, cigarette smoking and **colorectal cancer** incidence
- Results: (An OR greater than 1 indicates a **higher risk** of the disease in those exposed to the risk factor)
  - GSTM1 & GSTT1 Polymorphisms
    → OR for CRC: **1.62** & **1.64**
  - GSTT1 Polymophism
    → OR for CRC: **2.44** – in smokers

DNA DAMAGE (DNA ADDUCTS)

- A DNA adduct is not a mutation, it is **a precursor to a mutation** when it is a **substrate in DNA synthesis** and lead to cancer without proper DNA repair
- **Direct damage to DNA**, changes **chemical structure of DNA**
- Key basis of genotoxic mechanism of carcinogenesis
- Formation of adducts is marker for **exposure and risk**, therefore efforts to identify structures, properties and levels

HOW FREQUENT IS DNA DAMAGED?

Modification	Number*
8-Oxoguanine	~ 1000
Thymine glycol	~ 500
3-Methyladenine	~ 600
7-Methylguanine	~ 4000
O^6-Methylguanine	~ 200
Abasic site	~ 9000

Number of residues generated daily per human genome
- Adduct levels for many potent genotoxins are well below these numbers

COMMON REACTIONS YIELDING DNA ADDUCTS

- Oxidation
- Hydrolysis
- Photodimerization
- Alkylation
  o Methylation Adducts
  o Other Bulky Alkylation Products

OXIDATIVE DAMAGE TO THE DNA BASES

8-Oxogunanine (G) mutagenic (G->T)
FormAmidoPYrimidine (G) replication block
Thymidine glycol (T) replication block
5-Formyluracil (T) mutagenic (A->C,G)
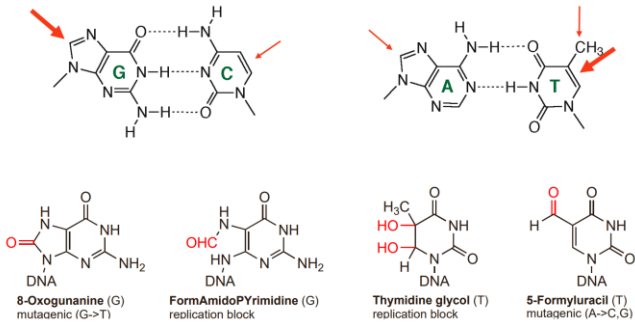
PHOTODIMERIZATION

DNA Photolesions induced by UV distort the DNA double helix → **Large distortion of the DNA backbone**
- UV-associated DNA damage structures:

Cyclobutane pyrimidine dimer (CPD)
CPD   (6-4) PP   (6-4) Dewar Dimer
adjacent thymine bases
Oxetane intermediate
(6-4)photoproduct (6-4PP)
Dewar photoproduct (DewarPP)
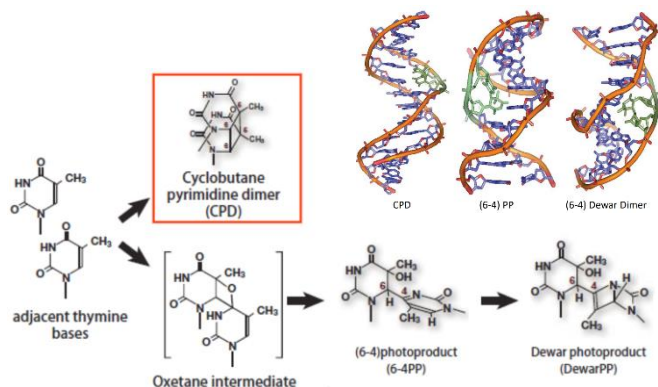
ALKYLATION

Frequent sites of alkylation in DNA:

7-Mehtylgunanine (G) relatively harmless
3-Mehtyladenine (A) replication block
O^6-Mehtylguanine (G) replication block mutagenic (G->A)
N^3-Mehtylcytosine (C) replication block
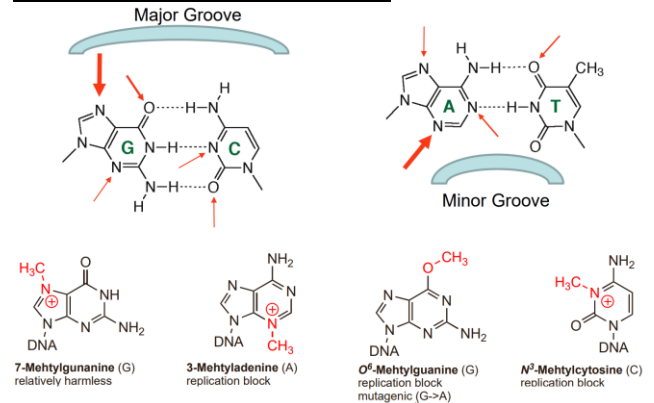
DNA ALKYLATION EXAMPLE 1: AFLATOXIN

- Produced by **fungi** (*Aspergillus sp.*) found in soil
- Dietary exposure: **Contamination of grains, seeds and nuts** in high temperature and **high humidity storage**
- **Liver carcinogen** → amongst most potent liver toxicants
  o Contributor to high incidences of liver cancer in china and west africa

N7-Aflatoxin Alkylation:

1. Metabolic activation
2. Alkylation of DNA
3. Two competing damage outcomes
   - Depurination (strongly promoted by alkylation)
   - Adduct Hydrolysis
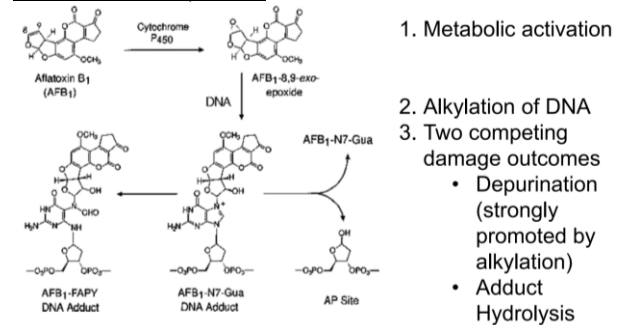
DNA ALKYLATION EXAMPLE 2: ACRYLAMIDE

- IARC Group 2A (Probable)
- Formation of acrylamide from asparagine and glucose increases by **increasing temperatures** (peak: 170°C) (Heated food can be dangerous)

Bioactivation and DNA Alkylation by Acrylamide:

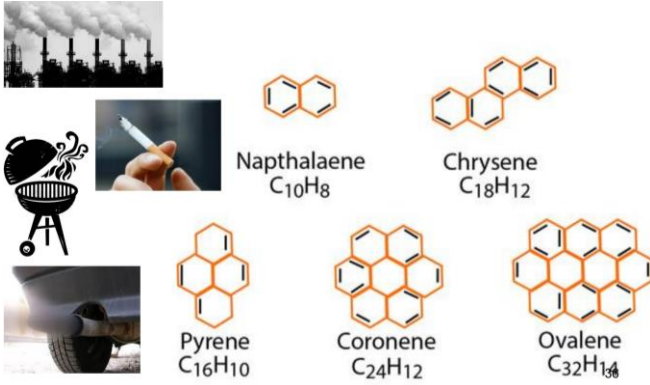o Acrylamide is metabolically activated by P450 to form **glycidamide** (DNA reactive epoxide)

acrylamide → CYP 2E1 → Glycidamide DNA Reactive Species
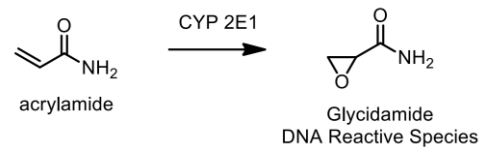
o Detoxification by GSH reaction
o DNA reacts at multiple positions and at both sides of the epoxide

Acrylamide Biomonitoring – Acrylamide Hb Adducts:

- Hemoglobin adducts (in blood) are markers of integrated acrylamide exposure over the preceding few months → levels increase with dietary intake
- Smokers have adduct levels **three to fourfold higher than non-smokers**; most non-smokers less than about 100 pmol/gram hemoglobin
- Levels are influenced by enzyme polymorphisms
- Younger children may have slightly higher levels possibly due to increased intake of acrylamide-containing foods relative to body size
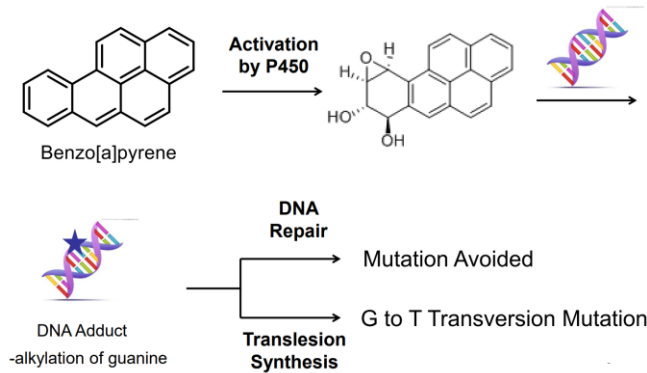
Example of a protein adduct
(Biomarker for exposure)
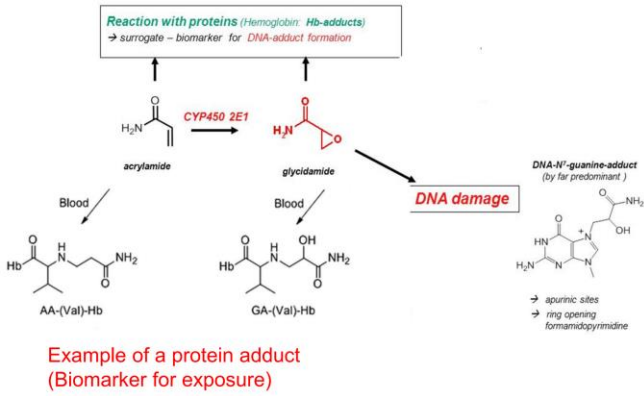
DNA ALKYLATION EXAMPLE 3: POLYCYCLIC AROMATIC HYDROCARBONS (PAH)

Napthalaene
$C_{10}H_8$

Chrysene
$C_{18}H_{12}$

Pyrene
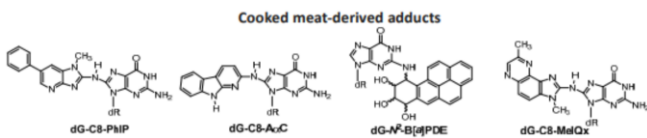$C_{16}H_{10}$

Coronene
$C_{24}H_{12}$

Ovalene
$C_{32}H_{14}$

- Can be found in cooked meat
- Lead to alkylation of guanine
  o Which leads to either DNA repair or translesion synthesis → transversion from G to T

How PAHs induce signature mutations:

Benzo[a]pyrene

DNA Repair → Mutation Avoided

DNA Adduct
-alkylation of guanine

Translesion Synthesis → G to T Transversion Mutation

DNA adducts from PAHs:

Cooked meat-derived adducts

dG-C8-PhIP   dG-C8-AαC   dG-N²-B[a]PDE   dG-C8-MeIQx

MUTAGENESIS (TRANSLESION DNA SYNTHESIS)

TYPES OF MUTATIONS INDUCED BY GENOTOXINS

- Point/Single Base Change
  - o Transversion
  - o Transition
- Frame shift-causing
  - o Deletion
  - o Insertion/Addition

Transition Purin
Transversion
Transversion
Transition Pyrimidin
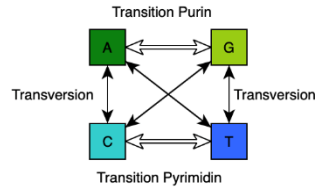
OUTCOMES OF MUTATIONS

- Lethality
- Translation into **mutant proteins**
- Mutant can be **recognized and repaired**
- **Cells** expressing mutant proteins can be **recognized and eliminated**
- Mutant proteins can **impart** *(vermitteln)* **cancer phenotype**

DNA POLYMERASES

- There are different family/kinds of polymerases
- In the clefts of Polymerases there are positively charged AA, to manage the negatively bound DNA
- **Replicative Polymerases** catalyze DNA replication
  - o They have the ability to **proofread** → mismatch of normal bases
- **Translesion DNA Sythesis (TLS) Polymerases** repair DNA damage of Bases that are <u>not normal</u>
- TLS Pols characteristics
  - o rates and fidelity
  - o active site properties
  - o proofreading
  - o processivity

Characteristic	B Family Polymerases (δ, ε)	Y Family Polymerases (η, ι, κ)
Error Rate	Low (< 10^{-10}/bp)	High (10^{-2} to 10^{-4})
Fidelity	High	Low
Proofreading	3' exonuclease	None
Processivity	High	Low
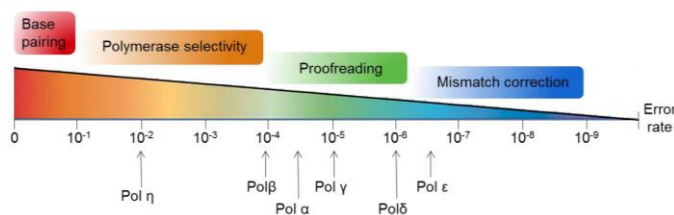
- *Processicity is the # of nucleotides that can be inserted in one binding event*
- Y-family polymerases catalyse **translesion DNA synthesis (TLS)** → **leads to mutations!**
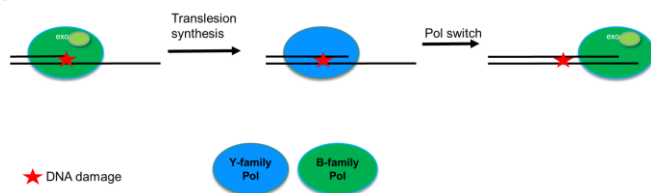
- In general Y-family Pol evolved to accommodate more distorted DNA

POLYMERASE SWITCHING MODELS

Cells contain several different DNA polymerases that transiently replace the replicative DNA polymerase

- Basic DNA Pol Switch Model → 2 relevant mechanisms for TLS, depending on the lesion structure and active enzymes

One TLS polymerase (red) responsible for insertion and extension.

Two TLS polymerases (red and blue) responsible for insertion and extension
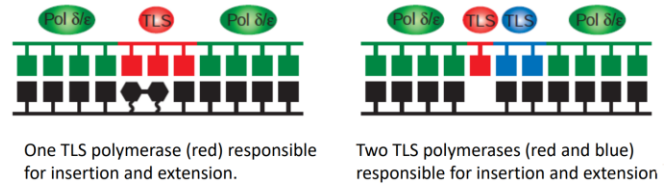
TLS is needed to prevent replication fork collapse. It overcomes the problem of needing to halt replication, since replicative polymerases do not accommodate damaged bases. Failure to restart after replication fork collapse could lead to double-strand breaks, chromosomal rearrangements, cell-cycle arrest and cell death. Thus, it is more beneficial to bypass replicative arrest

DETERMINANTS OF REPLICATION FIDELITY AND MUTATION RATES

- Biochemical factors related to the **polymerase enzyme**
  - o Expression levels
  - o Regulation
  - o Kinetic behavior/accuracy
- **Chemical Factors**
  - o Structure/size of the modification (this also dictates which polymerase is active)
  - o Watson-Crick H-bonding
  - o Base stacking interactions with neighboring bases as well as aromatic residues on the enzyme
  - o A-Rule (DNA polymerases insert Adenine opposite abasic sites)
- **Elimination of damage by Repair!**

MUTAGENESIS EXAMPLE

Oxidation-Induced Mutagenesis:

- Polymerase-mediated DNA replication of **8-oxoG** often **incorporates an A**
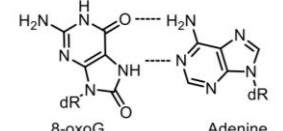

dR Guanine   dR 8-oxoG
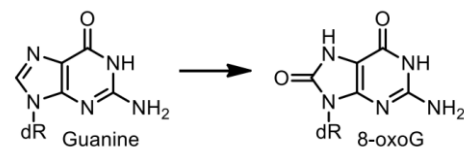
- This mismatch (8oxoG:A) is **not proofread efficiently**
- The poor proofreading can be attributed to the geometry of 8-oxo-G:A, which is similar to that of the correct base pair

Replication of the Lesion 8oxoG:
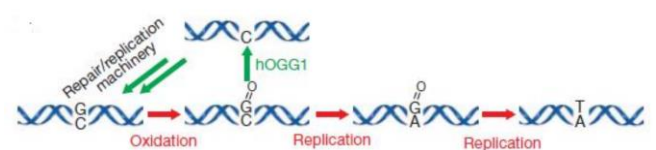

8oxoG causes G -> T transversions

8oxoG-A base pair is geometrically Similar to the natural G-C base pair

DNA REPAIR

Why is DNA repair important and why do we want to understand it?
- Mitigate (*mässigen*) damaging effects of endogenous DNA damage that is formed at high rates
  - **Oxidative lesions**
  - **Abasic sites**
- **Protect** against **carcinogenesis from xenobiotic agents**
- Is a significant source of **drug resistance in cancer chemotherapy**
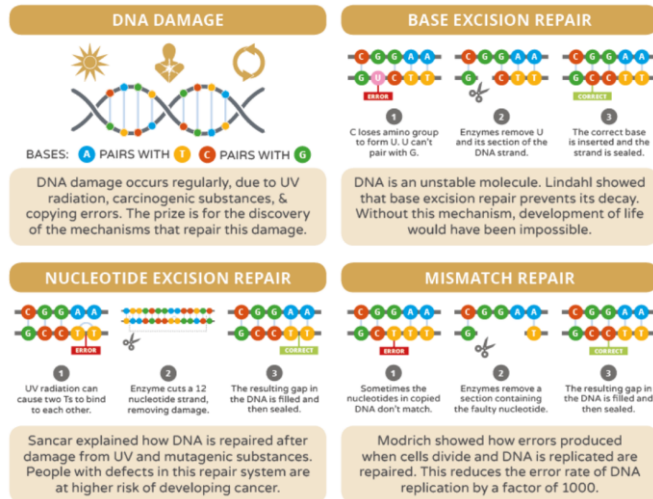
DNA REPAIR PATHWAYS

1. Nucleotide excision repair
2. Base excision repair
3. Mismatch repair
4. Homologous recombination
5. Non-homologous end-joining
6. Dealkylation (Direct reversion)

Study Tip: What to know for repair pathways:
- Similarities and differences in processes for recognition, removal, replacement synthesis, ligation
- Understand basic process and relevant enzyme types
- *Example:*
- → *In BER, small alkyl adduct is recognized and excised by a glycosylase*
- → *In BER, 3-methyl adenine is recognized and excised by alkyladenine DNA glycosylase (Aag)*
- Characteristics of damage – what pathway is relevant?

Important Proteins to remember:

NER: XP-proteins (XPC, XPF), TFIIH, Polymerase d/e/k

BER: First step needs glycosylate enzymes

MMR: Mut-proteins, RPA, EXO1

HR: No specific important proteins, but steps and enzymes that are important

NHEJ: Ku-proteins are important

DR: Important protein: MGMT (in humans!)

1. NUCLEOTIDE EXCISION REPAIR (NER)

NER substrates
- Bulky, helix-distorting DNA damage → big **distorted backbone**
- Thymine dimers & 6,4-photoproducts (UV Exposure)
- Chemotherapy-induced DNA crosslinks (Pt drugs as shown below-**Cisplatin**)
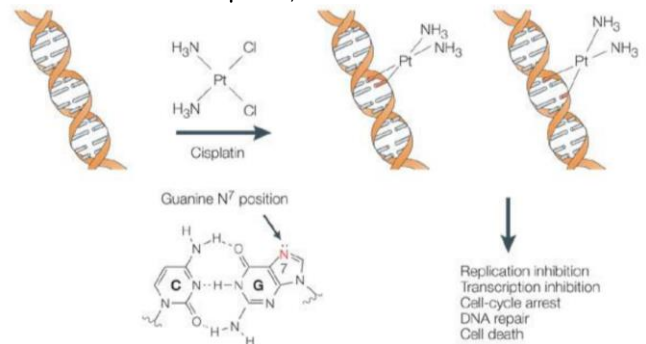
NER steps:

1. **Recognition** of damage
2. **Assembly** of multi-protein complex for remodelling and cutting
3. **Cut** into strand at both ends of the lesion
4. **Removal** of short single-stranded DNA segment containing the lesion
5. **DNA synthesis** (polymerase) to replace DNA
6. **Ligation** (ligase) of the remaining nick
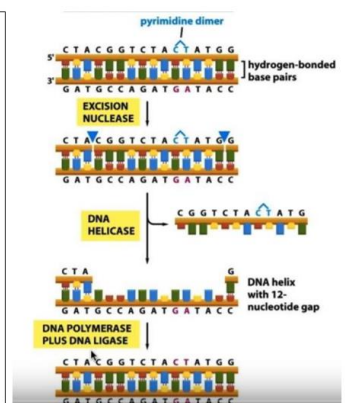
- NER occurs during **transcription coupled repair** (**TCR**) or **global genome repair** (**GGR**) → Damage recognition step is different in TCR (during transcription) and GGR (when proteins scan DNA). After the damage is recognized, the two mechanisms are very alike
- The steps of NER in Prokaryotes and Eukaryotes are more or less the same, only proteins are different
- **Prokaryotes: Uvr** (ultraviolet radiation) enzymes
  - In TCR, **Polymerase II** plays a role
  - In GGR, the **UvrA** and **UvrB** enzymes recognize the damage and flank it, allowing the formation of a repair bubble. **UvrC** is recruited and cuts flanking regions. **UvrD** & **Polymerase I** add missing nucleotides
- **Eukaryotes: XP Proteins**
  - **XPC** recognizes the damage, **TFIIH** (helicase= XPB&XPD) recognizes the site bound by XPC and forms the repair bubble. **XPF** (&XPG) cut the sequence so that the DNA **Polymerase d/e/k** can fill in the gap correctly
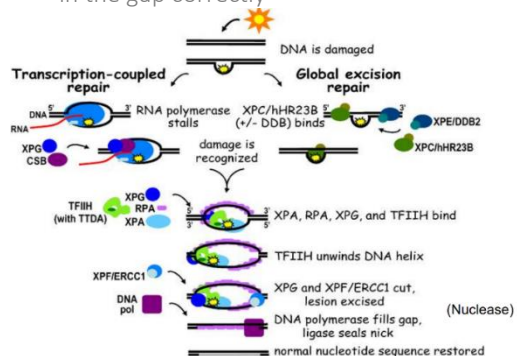
2. BASE EXCISION REPAIR (BER)
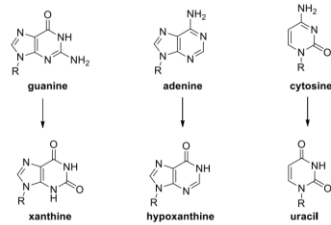
BER substrates:

Small, non-helix-distorting base adducts

- Alkylated bases:
  - 3-methyladenine
 - 7-methylguanine
- Oxidized bases:
  - 8-oxoguanine
- Deaminated bases:
  - xanthine
    - deamination of G
  - hypoxanthine
    - deamination of A
  - uracil
    - deamination of C
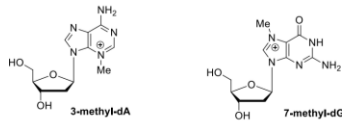
1. Initiation: DNA **glycosylases**
   (different glycosylases for different things)
   - o Recognize damage
   - o Remove altered bases
   - o Form AP sites
2. AP sites cleaved by **AP endonuclease**
3. New DNA synthesized by **polymerase**
   - o In short-patch single nucleotide is replaced
   - o In long-pach 2-10 nucleotides are synthesized and **flap endonuclease** trims displaced strand
4. Strand sealed by **ligase**
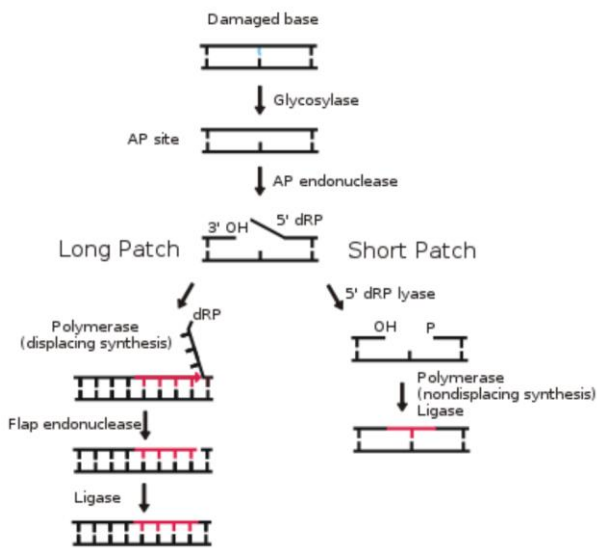
DNA DAMAGE INDUCED BY CHRONIC INFLAMMATION CONTRIBUTES TO COLON CARCINOGENESIS

- Chronic inflammation increases cancer risk
- ROS induce DNA damage recognized by alkyladenine DNA glycosylase (Aag)
- **Aag** recognition of adduct initiates base excision repair
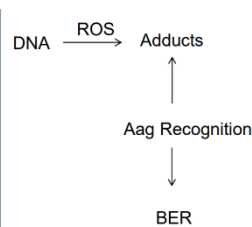
IMPORTANCE OF BER IN PROTECTION AGAINST COLON CARCINOGENESIS

- Impacts of Aag-mediated DNA repair (+Aag mice)
  - o Prevents colonic epithelial damage
  - o Reduces the severity of chemical–induced colon tumorigenesis
- Accumulation of DNA adducts in Aag-deficient mice following stimulation of colonic inflammation

3. MISMATCH REPAIR (MMR)

- DNA mismatch repair repairs **misincorporated bases** during DNA replication
1. **MutS** recognizes and binds mismatch
2. **MutL** makes incision, DNA contains nicks not yet sealed by DNA ligase
3. Patch is removed (**RPA**, replication protein A; **EXO1**, exonuclease 1) **RPA** stabilizes and protect single strand, **EXO1** removes the wrong nucleotides
4. DNA synthesis (polymerase δ) and ligation (ligase)
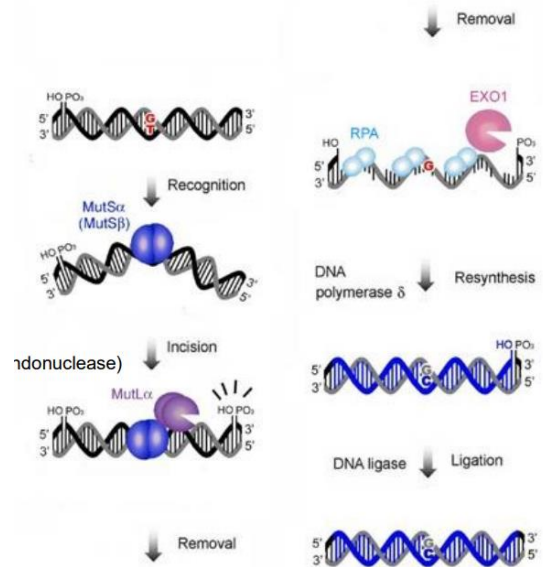
- Hereditary nonpolyposis colorectal cancers attributed to mutations in the genes encoding MSH2 and MLH1 Respectively
  - o MSH2 and MLH1 = tumour suppressor genes

STRATEGIES USED FOR DAMAGE RECOGNITION
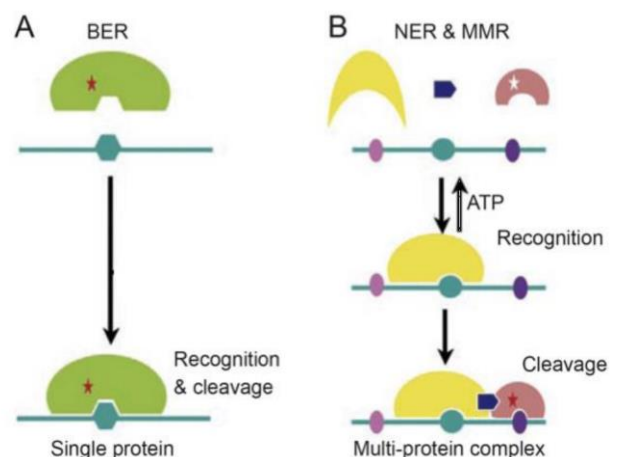
4. HOMOLOGOUS RECOMBINATION (HR)

- After **double-strand break**
1. Sections of DNA around the **5' ends** of the break are **cut away** in a process called resection → sticky 3'-ends
2. Overhanging 3' end of the broken DNA molecule "invades" a similar or identical DNA molecule that is not broken (sister chromatid in mitosis) → **Holiday junctions**
- After strand invasion:
  - ○ **SDSA** (synthesis-dependent strand annealing) or
  - ○ **DSBR** (double-strand break repair)
- **Resolvases**: Nucleases that resolve the holiday junctions, can cut either horizontally or vertically
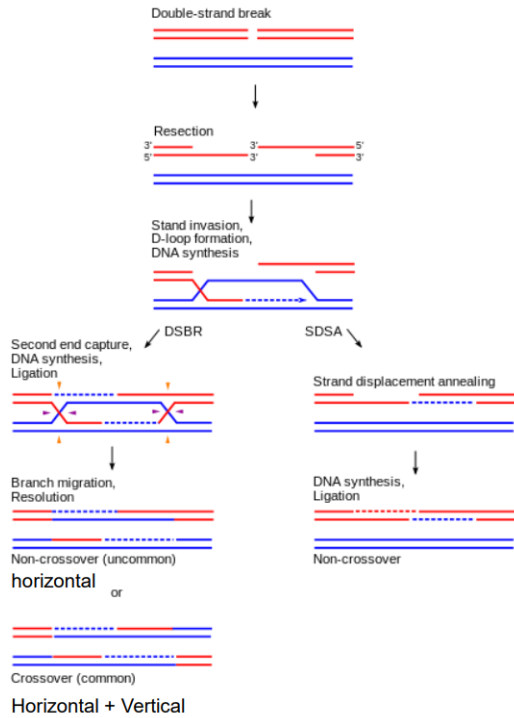
- Different cuts lead to different kind of products

5. NON-HOMOLOGOUS END-JOINING (NHEJ)

- NHEJ is more common than HR (NHEJ fast → 30 min, HR slow → 7 hrs), but NHEJ is more mutagenic
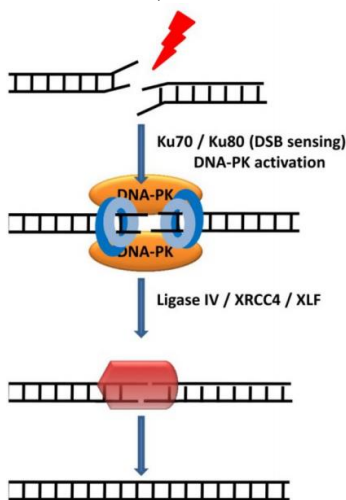
1. Recognition: **Ku70/Ku80** encircles duplex DNA at DSB
2. Ku70/Ku80 stabilizes/structurally aligns the two DNA ends and recruits **DNA-Protein Kinase (PK)**
3. DNA-PK phosphorylates and activates the **NHEJ effector complex** (**ligase IV**/XRC44/XLF) that ligates broken DNA

- Double strand break (DBS) can have different configurations → different NHEJ sub pathway dependent on the type of DBS
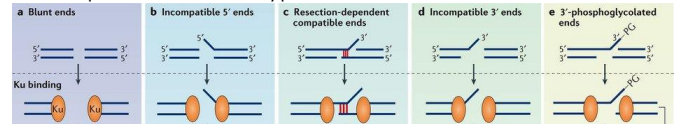
4. DIRECT REVERSION/DEALKYLATION REPAIR

- Has a **very narrow substrate selection**: O^6-methyl-dG
- Is a **low frequent** substrate but is **extremely mutagenic**
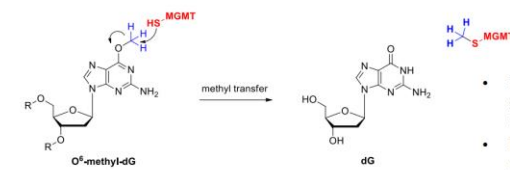
- **Repair protein: MGMT**
- O^6-methyl-dG is removed in a non-enzymatic reaction in which the **MGMT** protein is used up completely → "suicide" protein → can revert guanin back to its original structure

MGMT AND CANCER
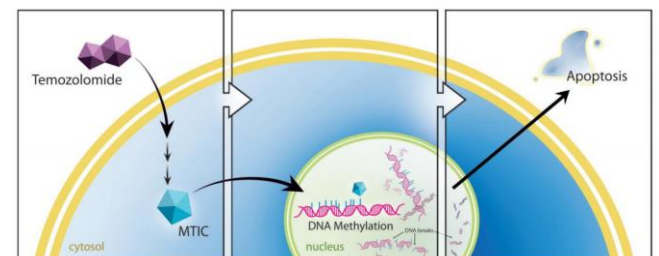
- **Temzolomide** is an **alkylating cancer drug**
- **MGMT inhibitors** sensitize cancer cells to temzolomide therapy (targeting DNA repair proteins in cancer therapy)→ Be a **Co-therapy** (Target a repair protein so that it cannot repair cancer cells)
- 438 known polymorphisms of MGMT:
  - ○ *Gly160Arg*: Here, Gly160 residue lies nearby the Cys145 active site (rare variant found in less than 1% of Caucasians, but it is expressed in approximately 15% of Japanese)
  - ○ This polymorphism has generated interest due to its **strong resistance to the MGMT inhibitor O^6-BG**
  - ○ Patients with this MGMT polymorphsims are not good candidates for O^6-BG therapy combined with alkylating agent treatment

OVERVIEW:

*Don't remember exact which polymerase but remember that they are important!*

DNA lesion	Single-strand break Single-base damage	Bulky lesions Crosslinks		O⁶MeG	Mismatch	Double-strand break	
Repair pathway	BER	NER		DR	MMR	HR	NHEJ
		GG-NER	TC-NER				
Proteins involved/biomarkers	OGG1 PARP1 PARP2	XPD, XPC DDB1/XPE	RNA pol β CSA, CSB	AGT	MSH2/MSH6 MLH1/PMS2	ATM MRN complex	KU70, KU80
	XRCC1	ERCC1/XPF			EXO1/PCNA/RCF	RPA BRCA2/FANCD RAD51, FANCF	DNA PKs Artemis XRCC4-XLF
	Pol β PCNA FEN 1	PCNA Pol δ Pol ε			Pol δ	Pol δ Pol ε	Pol μ
	Ligase III	Ligase I			Ligase I Ligase IV	Ligase I	Ligase IV

RECEPTOR-MEDIATED CARCINOGENESIS

ARYL HYDROCARBON RECEPTOR

- AHR protein is encoded by the AHR gene
- Is a **ligand-activated transcription factor**
- AHR is <u>inactive</u> in the **cytosol** → If a **ligand binds**, AHR **migrates** to the **nucleus** and gets <u>active</u>
- AHR activates the expression of multiple phase I and II **xenobiotic chemical metabolizing enzyme genes** (e.g. CYP1A1 gene)
- Ligands are generally **planar aromatic hydrocarbon** compounds
  - Plant flavonoids
  - Polyphenolic compounds
  - PAHs
  - Endogenous ligands of AhR include tryptophan-derived metabolites
  - **Dioxin**: Family of compounds with diverse chlorine substitutions at the benzene rings in PCDDs and PCDFs (210 possible types)
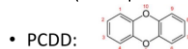
    • PCDD:
    polychlorinated dibenzo-p-dioxin (75)
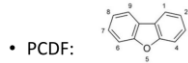
    • PCDF:
    polychlorinated dibenzofurans (135)

    TCDD – proto-typical example

    2,3,7,8 tetrachlorodibenzo-p-dioxine (TCDD)
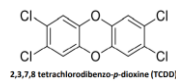
- **TCDD**: Industry byproduct, have been used as pesticides since before WWII
- Can be detected anywhere in the environment; most human exposure is from food (accumulate
- in fat due to their nonpolar/ high lipophilic structure, can stay there for a long time → high stability → half life TCDD ~ 7 years)
- Classified as known **human carcinogen**
- Classification based on mechanistic considerations focusing on the Ah receptor → receptor-mediated mechanism of carcinogenesis

EXCURSE: DIOXIN CONTAMINATION SCANDAL
Belgium 1999
- Farms in Belgium were ordered to destroy their livestock due to **feed contamination with dioxin**
- Contamination might come from tanks used to hold animal fats for producing animal feeds → Tanks previously used to hold **industrial oil containing dioxins** → Tanks not sufficiently cleaned so animal fats became **contaminated** with the dioxin-bearing oil residues

ARYL HYDROCARBON SIGNALING
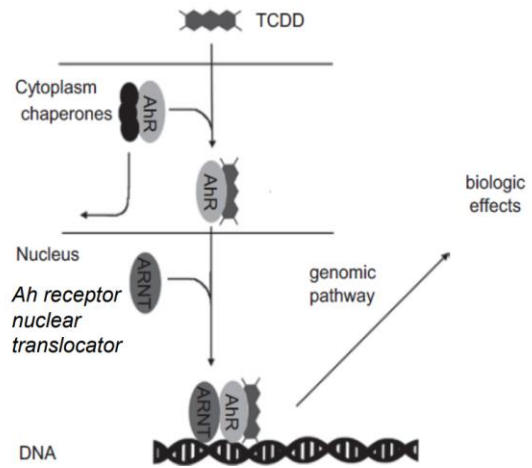
AHR ACTIVATION BY UV EXPOSURE
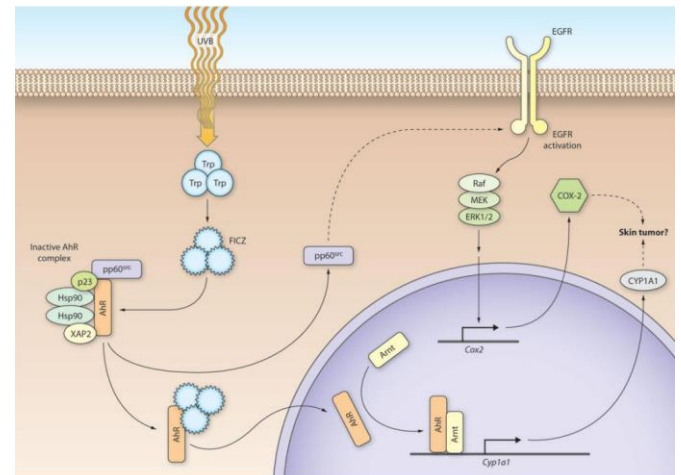
- AhR is normally **bound** to different proteins (heatshockproteins) → **inactivate**
- **UVB**-light enters the cell and can **react with Trp**
- **Tryptophan** is metabolized to **FICZ**
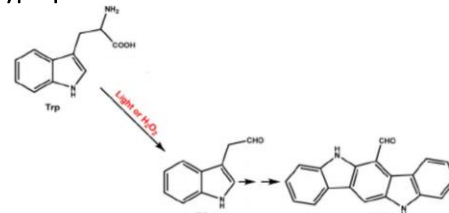
- **FICZ activates AhR** → gets transported into the **nucleus**
- In the nucleus, **AhR binds to ARNT** → leads to gene activation
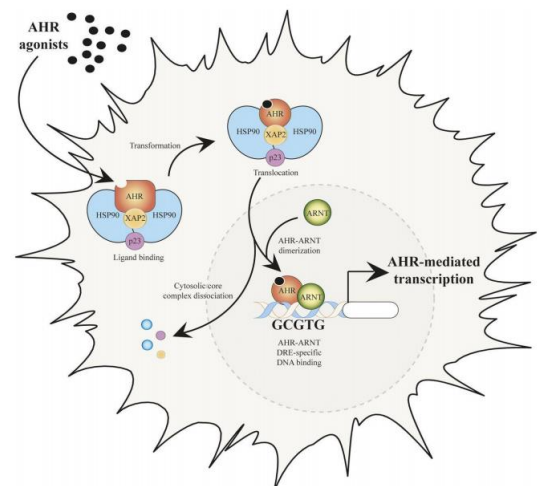
AhR's ROLE IN TUMORIGENESIS

- AhR **promotes tumorigenesis**
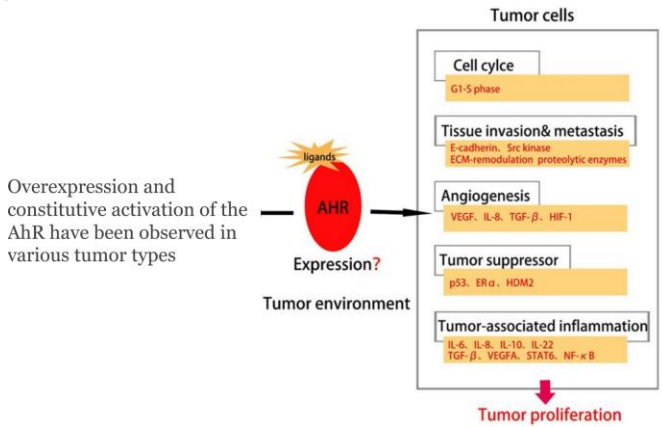
- AhR can activate a lot of hallmarks of tumor

Why can stimulating AhR be bad?
- Sustained **hyperactivation situation**:
  o Long human half-life (dioxin ~10 y)
  o High binding affinity
- Induces expression of enzymes that **bioactivate other carcinogens**
- Induces **growth factors**
- **Promotes inflammation signals**
- Overexpression of AhR and its target growth factors characterized in cancer cells
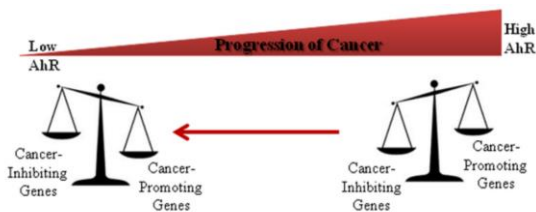
- Why does AhR-mediated expression of CYP enzymes increase BaP genotoxicity → **CYP metabolically activates BaP**
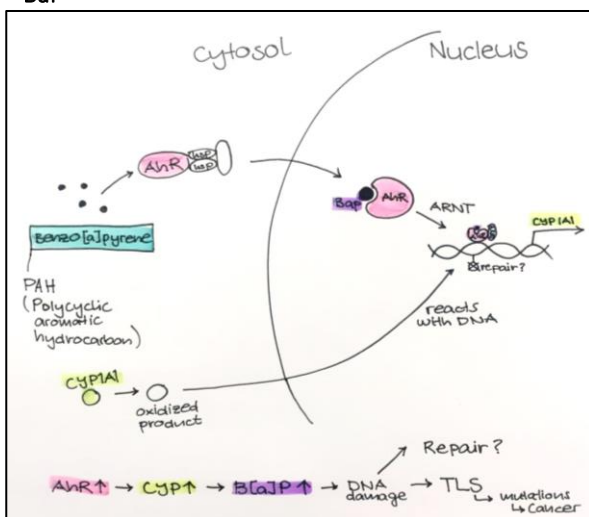
- Benzolpyremine activates receptor (AhR)
  → activates genexpression of CYP genes
  → CYP1A1 enzyme activates xenobiotics (BaP) that leads to DNA damage (can react with guanine)
  → TLS can lead to mutation that can drive cancer
  (Repair Pathways can remove DNA damage)
-

HORMONE-MEDIATED CARCINOGENESIS

Hormone dependent cancers: **Breast and Prostate**

SECRETION OF HORMONES

- Chemical messengers
- **Secreted into the blood** by specialized cells in **endocrine glands**
- Generally act on remote organ sites and **alter rates of processes** in target cells
- Act at very **low concentrations** (nano to picomolar)
- Control several processes
  o Growth
  o Development
  o Metabolism
  o Reproduction
  o Regulation of Homeostasis
- Hormones act by binding receptors on/in target cells:
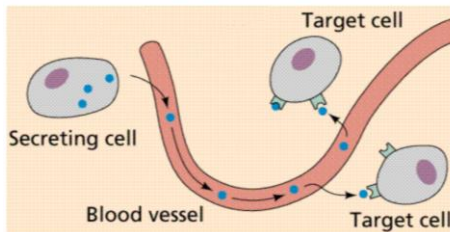
  o Controlling the **rates of enzymatic reactions**
  o Controlling the **movement** of ions or molecules **across membranes**
  o Controlling **gene expression** and **protein synthesis**

BREAST CANCER

RISK FACTORS
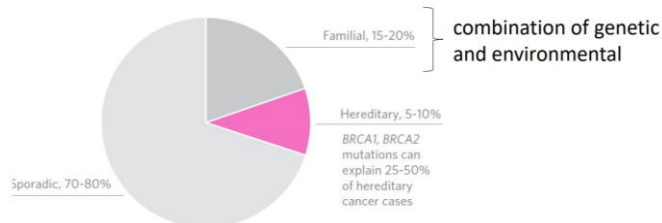
- Prolonged exposure to **high levels of estrogens**
- In **obese** women, adipose tissue is a major source of estrogens that increases the breast cancer risk (Also: Fat can accumulate bad substances)
- **Hormone replacement therapy** (HRT)
- Taking estrogens
- Higher levels of blood estrogen in post-menopausal

ESTROGEN AND ESTROGEN RECEPTOR (ER)

- Estrogen binds to the **ER Transcription Factor**
- There are two possible mechanisms:
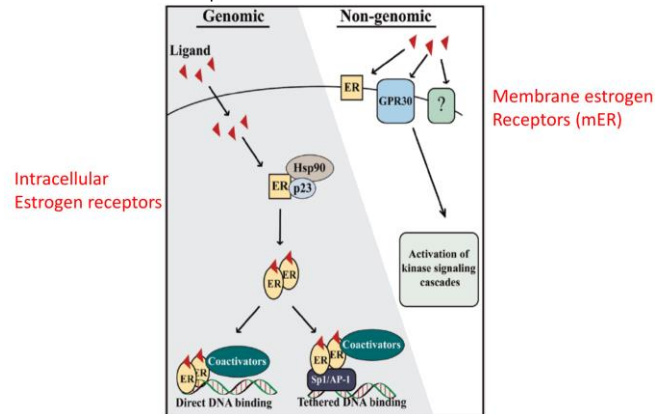
- **Estrogen receptor (ER)** is a **transcription factor** that regulates gene expression
- PR gene is a classic ER target → **Progesterone receptor (PR)** is used as biomarker for ER function
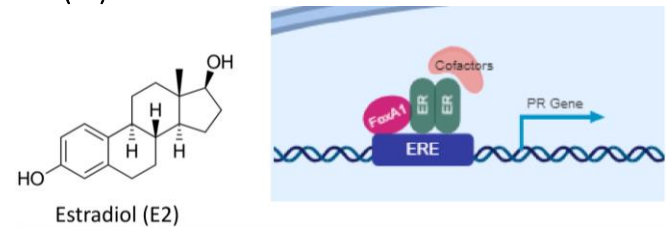
PIONEER FACTORS

Pioneer factors: FOXA1 and GATA3

FOXA1:
- FoxA1 leads oestrogen receptor to chromatinized DNA
- ER will not bind to chromatin (DNA+histones) without FoxA1 (ER will bind to DNA in a test tube)

GATA3:
- Helps in the recruitment of the estrogen receptor
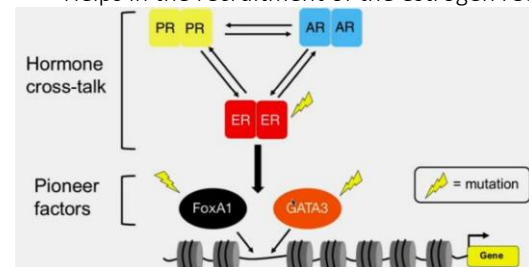
MUTATION IN GATA3:
- **C-terminal mutation: +1 insertion** causes a **frame shift** → changes stop codon → leads to an **enlarged protein** (adding 65aa to C-terminus)
- **Mutated Protein is stable**
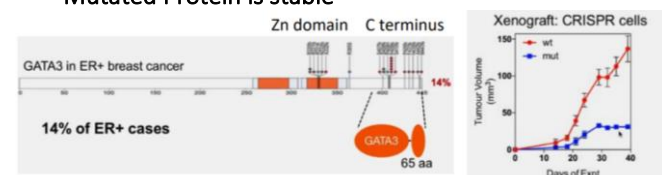
GATA3 mutant protein has a **protective function in cancer**!

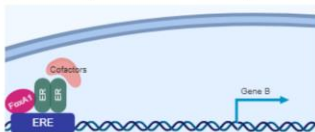## ER BINDING SITES: FORKHEAD MOTIFS
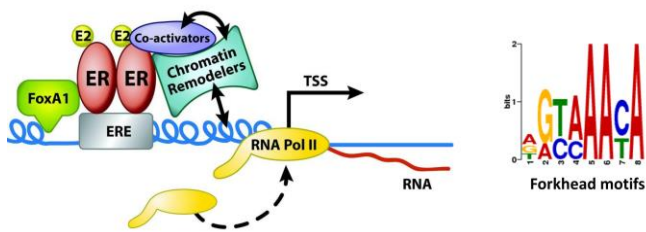
ER AND PROGESTERONE

- ER binds in different genomic regions depending on the cancer:

ER +, PR + (better outcomes)

The movement is not random: It moves from one Fox1 site to another

ER +, PR – (poor outcomes)

This was surprising as progesterone is usually linked to pro-proliferation

- Progesterone is generally (usually) pro-proliferative
- But in **cancer, progesterone** is often **antiproliferative!**
- Treating cancer cells with progesterone **changes where ER binds** → Leading to better clinical outcomes
- Progesterone brings **PR and ER together** for binding as TFs

TAMOXIFEN THERAPY

- Used to **prevent and treat breast cancer**
→ Tamoxifen blocks Estrogen Receptors
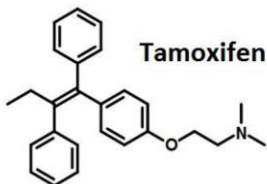
- **Competitive inhibitor of estrogen** → Blocks the action of estrogens in several tissues (most notably the breast)
- Therapeutic compound **acts as a ligand for estrogen receptors (ER)**, but has a clear different spectrum of activities from the natural hormone, **17-β-estradiol**
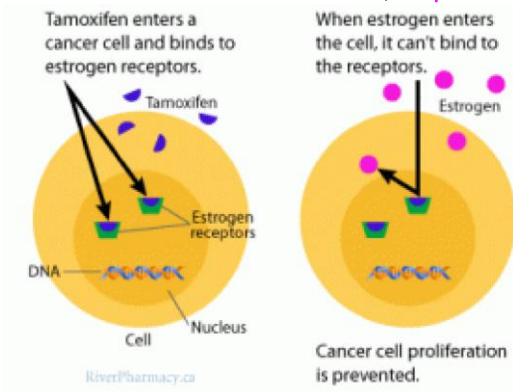
- Tamoxifen exhibits an **anti-estrogen activity** on mammary tissues in postmenopausal women

COMBINATION OF TAMOXIFEN AND PROGESTERONE
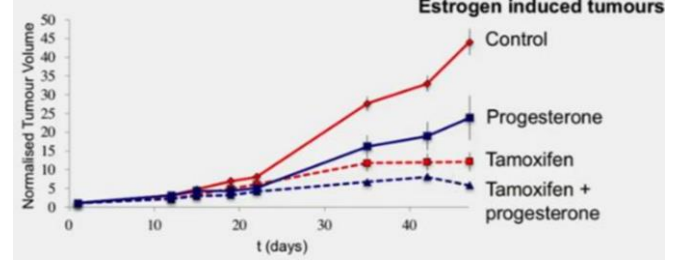
ER AND XENOBIOTICS (PCB)
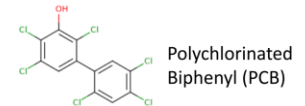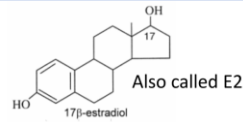
- PCB is a **synthetic hormone mimic**
  → is a similar binder like the natural ligand
- **PCBs** can bind to the **Estrogen Receptor**
- High fat solubility and stability can cause bioaccumulation in adipose tissue
  o **Very lipophillic and stable** (can accumulate in fat)

DIRECT INFLUENCE OF ENDOCRINE DISRUPTING COMPOUNDS AND ER SIGNALING

- Compete with endogenous estrogen for ER binding sites
- Shared E2 structural features determined by ER binding pocket
- Similar ligand binding pockets, but subtle differences in amino acids lining the pocket contribute to ligand selectivity

- Industrial phenolics
  o bisphenol A
  o Alkyl phenols
- Phytoestrogens (dietary estrogens)
  o Genistein
- Organochlorine pesticides
  o Methoxychlor
  o DDT
- Pharmaceutical agents
  o Tamoxifen
  o diethylstilbestrol (DES)

EPIGENETIC MECHANISMS OF CARCINOGENESIS

EPIGENETICS

Extra-layer of **control** for the gene expression
- Epigenetic changes **modify the activation of certain genes**
  o **Heritable** changes in gene expression
  o <u>No</u> changes in the genetic code/ **sequence of DNA**

EPIGENETIC INHERITANCE
- Epigenetic inheritance goes against the idea that inheritance happens only through transfer of the DNA code from parent to offspring
  o A parent's **epigenetic** tags can be passed down to future generations
  o Trans-generational epigenetic inheritance

THE CHANGING EPIGENOME
- <u>**Diet** and **environment**</u> are the biggest factors in epigenetic changes over lifespan

NUTRITION AND EPIGENETICS
What we can learn from bees:
- Queen bees and worker bees are **genetically similar**
- <u>Queen</u>
  o Fed **royal jelly** from larvae stage
  o Develops ovaries → are **fertile**
  o Large abdomen
- <u>Workers</u>
  o Fed nectar and pollen
  o Inactive ovaries, → Are **sterile**
  o Smaller in size

ENVIRONMENT AND EPIGENETICS
- Studies with identical twins
- The older the twins get, the more epigenetic tags are located differently → Epigenetic differences arise during the lifetime of monozygotic twins

EPIGENOME

Nucleosome
-147 bp DNA
-histones

Loosely packed
Active genes

Tightly packed
Inactive genes
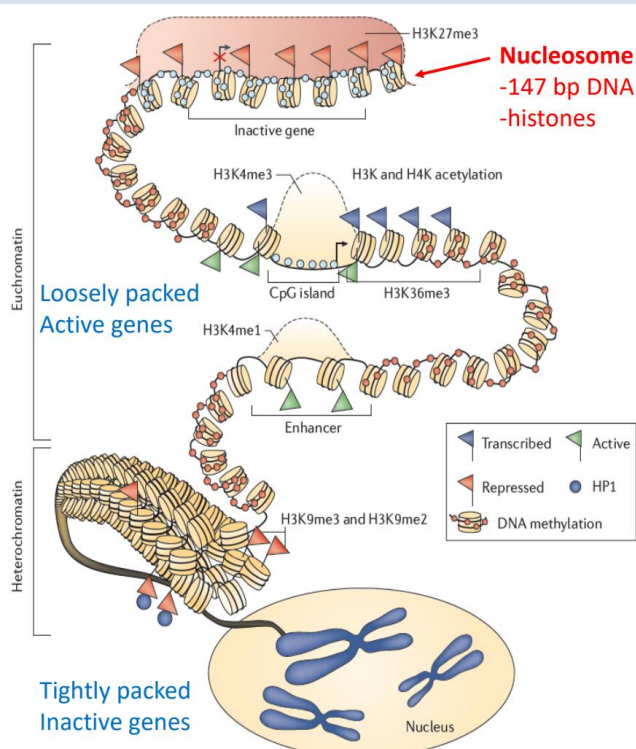
- Balanced state of chromatin, Nucleosome positioning and DNA methylation
  o **Repressive vs enhancing modifications** for histones and DNA → Methylation and acetylation of DNA and histones

MAIN EPIGENETIC PROCESSES

- DNA methylation
  - Genome wide hypomethylation → transcriptional **activation** (oncogenes)
  - Promoter-specific hypermethylation → transcriptional **silencing** (tumor suppressor genes)
- Chromatin modifications → Activation and Silencing
  - At histone lysine tails

Mediate reprogramming during development and maintenance of cell identity during the life of an organism

- *It's not black and white → Modification can be activating or deactivating depending on their region*

EPIGENETIC MODIFICATIONS
- DNA modification (4)
- Histone modification (16)
- ncRNA expression (functional RNA molecule, not translated to protein)

DNA MODIFICATIONS

CYTOSINE METHYLATION


Modifications are reversible
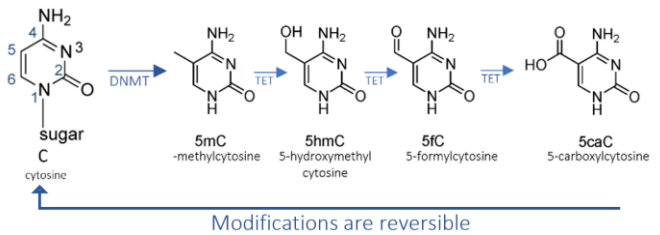
CPG ISLAND
- DNA region of >500 bp that has a **high CpG density**
- CpG islands are found upstream of many mammalian genes
- DNA methylation often <u>inhibits</u> the transcription of **genes**, usually around a promoter region (CpG islands occur near many gene promoters)
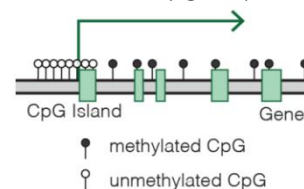

- There are about 29,000 regions in the human genome
- Potential biomarkers for cancer
- Decitabine is a hypomethylating agent for the treatment of acute myeloid leukemia (AML) (inhibits DNMT methylation)

DNA METHYLTRANSFERASES (DNMT)

- DNA methylation occurs in the **dinucleotide CpG**
- **Methyl groups added** to C-bases by the enzymes **DNMT3a** and **DNMT3b**
- When DNA is replicated, the methyl group on the template strand is recognized and a new one is introduced on the opposite (daughter) strand by the enzyme **DNMT1**
- DNMT1 associated with the replication machinery
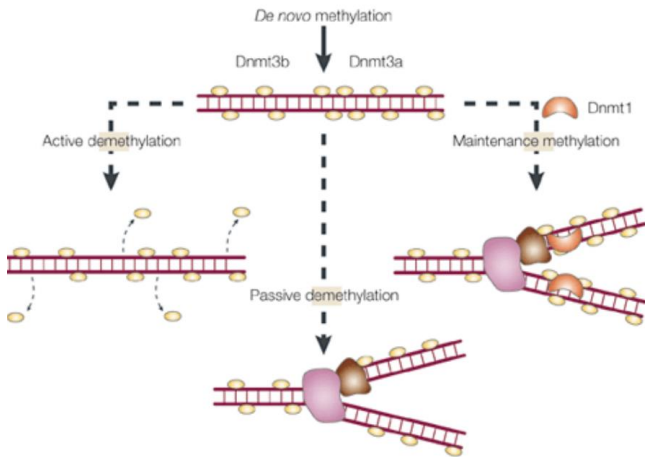
- DNA methylation patterns **tend to be maintained** (maintenance methylation)
- Demethylation can occur in absence of DNMT1 with:
  - Continued rounds of DNA replication (**passive demethylation**)
  - Without DNA replication (**active demethylation**) → TET enzymes
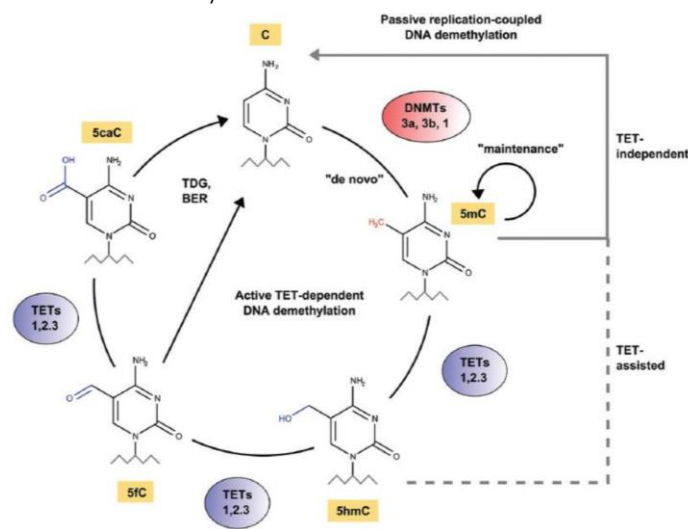
TET ENZYMES AND CANCER

- TET proteins **regulate gene transcription** by controlling 5-hmC, 5-formylC and 5-carboxyC levels
  - involved in modulating chromatin structure
- **TET2** is mutated in a **wide variety of cancers** → gives a non-functional enzyme which leads to methylation imbalance → hypermethylation at tumor suppressor genes
- TET1 and TET3 mutations are rare
- For drug development it is difficult to directly target TET for cancer treatment because they **are inactivated in cancer**

HISTON MODIFICATIONS:

NUCLEOSOME:
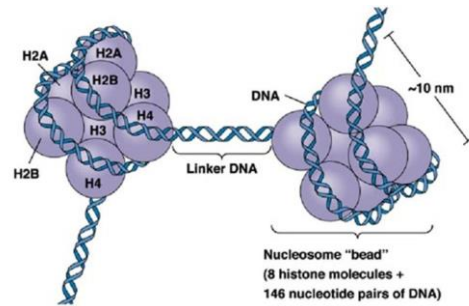
2x 4 different types of histons: H2a, H2b, H3, H4 (octamer)

ACETYLATION
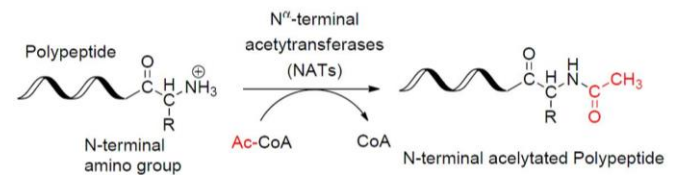
*What chemical interaction explains why histone acetylation opens chromatin?* → Electrostatics *(Changes in charge)*
- Primary amin is positively charged which interacts with the negative charged backbone of the DNA
- Acetylation changes the electrostatics (Group is no longer charged) → Backbone has not the same attraction anymore → DNA opens (Euchromatin)
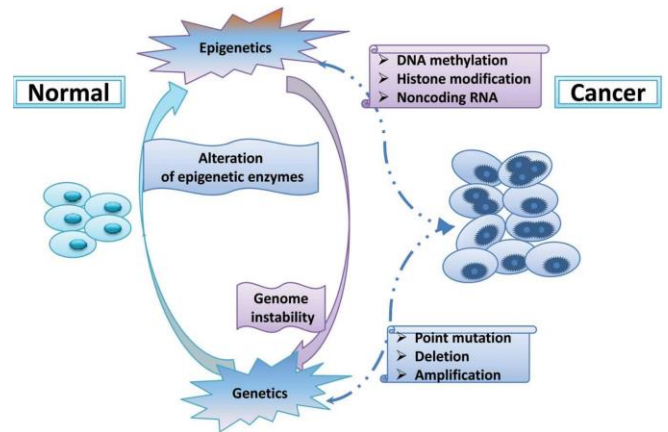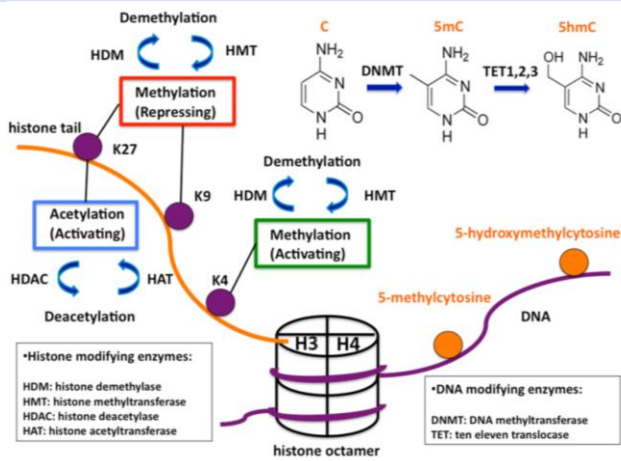

METHYLATION OF LYSIN RESIDUES
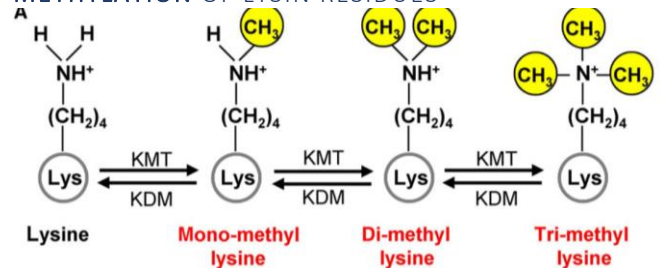
Can get methylated more than once!

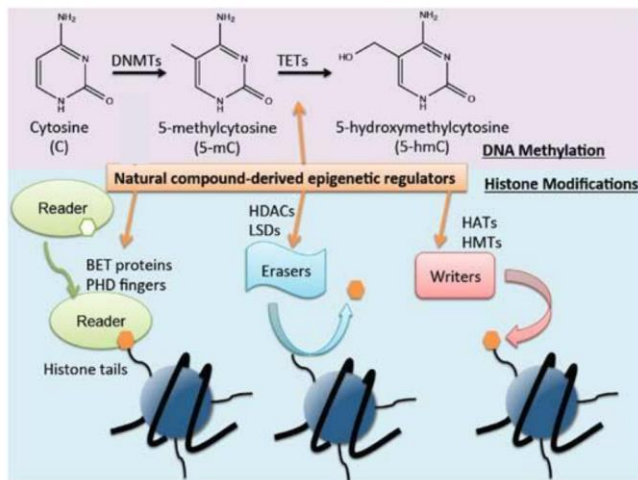### 2 METHYLATIONS WITH VARYING BIOLOGICAL EFFECTS
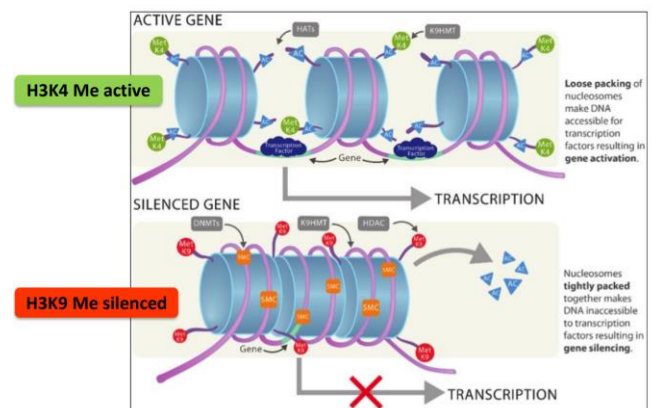
- Histone methylation can be associated with either transcriptional repression or activation

EPIGENETIC MODIFICATIONS ARE DYNAMIC

WRITERS, READERS AND ERASERS

Readers: Read what modifications are → to have an effect
Erasers: Take modifications away
Writer: Add modifications (e.g. add methyl group)

CANCER EPIGENOME (REPROGRAMMING)

- Bottom line for cancer: Altered DNA and histone modification status **influence transcriptional regulation** of **oncogene and tumor suppressor genes**
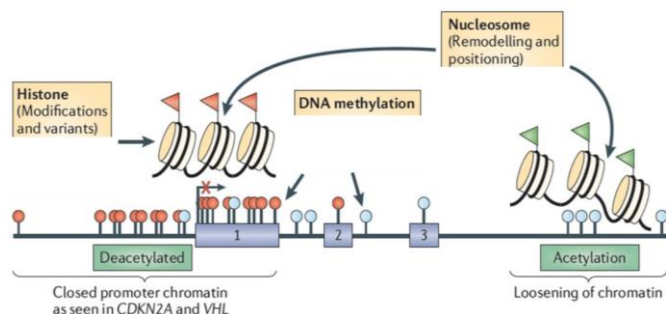

- Cancer epigenome is characterized by **global losses in DNA methylation** (pale blue circles)
- Certain genes have abnormal gains of DNA methylation (red circles) and repressive histone modifications (red flags) in promoter region CpG islands (→ tumor suppressor regions)

VIRAL CARCINOGENESIS

CANCER CAUSED BY INFECTIOUS AGENTS

- 14 million new cancer cases in 2012 → **2.2 million** were attributable to **carcinogenic infections (15.4%)**
- The most important infectious agents worldwide are
  - *Helicobacter pylori (770 000 cases)*
  - *Human papillomavirus (640 000)*
  - *Hepatitis B virus (420 000)*
  - *Hepatitis C virus (170 000)*
  - *Epstein-Barr virus (120 000)*

Attributable fraction of cancer related to infection, 2012
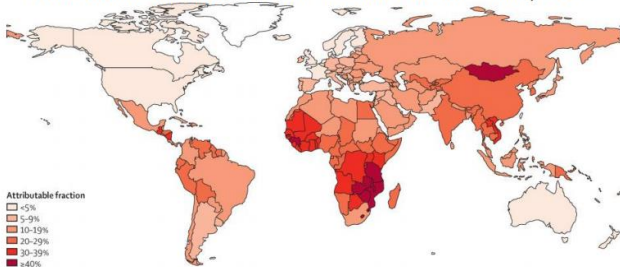
VIRUS BASICS

- Virus: **Infectious particles** consisting of **RNA or DNA** molecules packaged in a **protein capsid**
- Can only multiply inside a host cell
- Outcome of viral infection:
  - **Lysis** of **infected cells** with release of viral particles
 - **Integration** of **nucleic acid** sequence into host chromosome

EXCURSE: HOW DID WE DISCOVER THE LINK BETWEEN VIRUSES AND CANCER?

Rous Peyton (American virologist):
- Studies in chickens → Showed the role of a virus in cancer transmission *(Übertragung)*
- **Sarcoma** in chickens was **transmissible** to other chickens

The Rous Sarcoma Virus (RSV)

**A virus can transform a normal cell into a tumor**
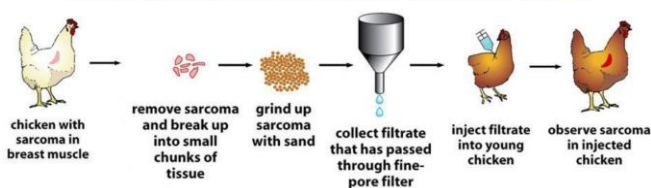
He took a tumor extract that was passed through filter (too fine to contain chicken cells or bacteria) and it caused cancer in a healthy chicken → tumorogenic agent was a **virus**, later known as **RSV**

RSV - ROUS SARCOMA VIRUS

- RSV is a virus with a **RNA genome**
- RSV has **four genes**:
  - **gag** – encodes the capsid proteins
  - **pol** – encodes for reverse transcriptase
  - **env** – encodes for the envelope gene
  - **src** – encodes a tyrosine receptor kinase that attaches phosphate groups to the amino acid tyrosine in the host cells proteins

EXCURSE: RSV HOST INFECTION

But how does this lead to cancer?
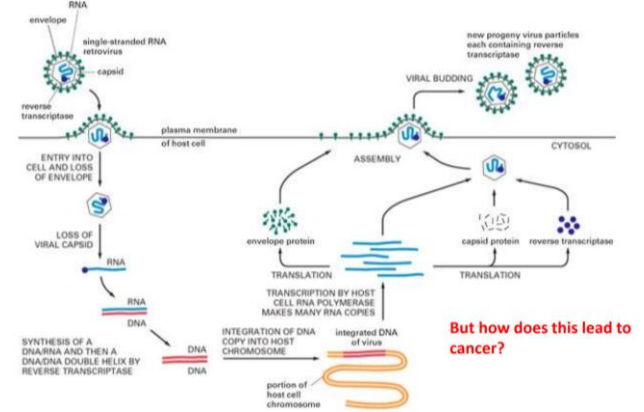
VIRAL ONCOGENES

- Viral oncogenes are 80-99% **homologous** to **cellular proto-oncogenes**
- Viral oncogenes in general are **copies of cellular mRNA** and **lack introns**

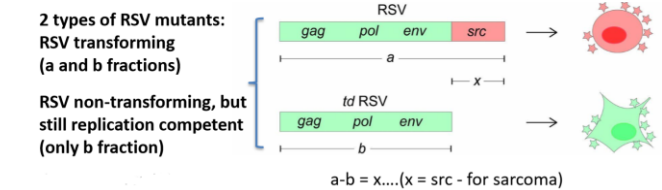Retrovirus oncogenes derived from normal cellular genes:

Retrovirus	Viral oncogene	Cellular proto-oncogene
Rous sarcoma virus	v-src	c-src (src)
Simian sarcoma	v-sis	c-sis (sis)
Harvey murine sarcoma	v-H-ras	c-H-ras (H-ras)
Kirsten murine sarcoma	v-K-ras	c-K-ras (K-ras)
FBJ murine osteosarcoma	v-fos	c-fos (fos)
Avian myelocytomatosis	v-myc	c-myc (myc)
Abelson leukemia virus	v-abl	c-abl (abl)
Avian erythroblastosis	v-erbB	c-erbB (erbB)

First oncogene identified → Rous sarcoma virus (v-src)

- 1964: First oncogenic human virus was observed → **Epstein Barr virus**

WHAT IS THE CAUSE OF THE TRANSFORMING ABILITY IN THE RSV VIRUS?

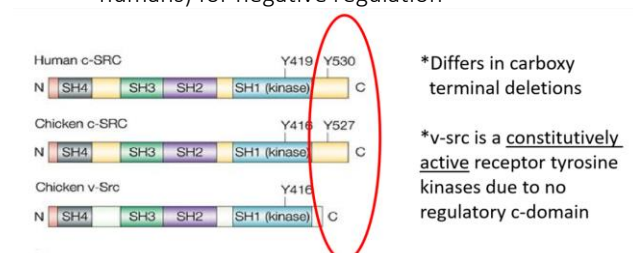→ **v-src** is involved in the **transformation to cancer**
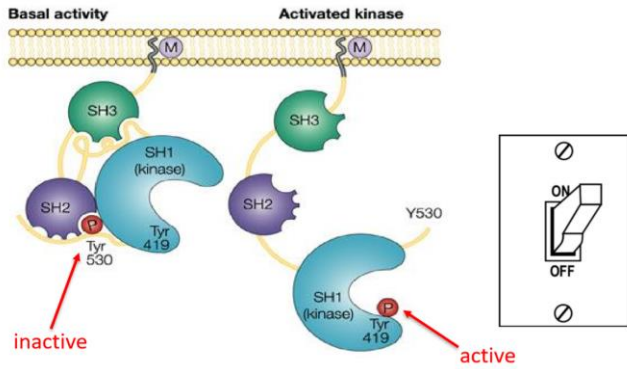- It is the first discovered oncogene

2 types of RSV mutants:
RSV transforming (a and b fractions)

RSV non-transforming, but still replication competent (only b fraction)

a-b = x....(x = src - for sarcoma)

v-src/c-src relationship: src gene of **RSV (v-src)** is a transduced allele of a **cellular gene (c-src)** that the virus picked up by recombination during the retroviral life cycle

C-SRC

- **Non-receptor tyrosine kinase overexpressed in many cancers**
- Most tyrosine kinases phosphorylate serine and threonine, Src **phosphorylates tyrosine residues**
- SRC structure
  - 4 Src homology domains (SH)
    - SH1: autophosphorylation site
    - SH2: interacts SH1 (negative regulator)
    - SH3: interacts SH1 (kinase domain)
    - SH4: lipid motif for membrane localization
  - C terminus: site of phosphorylation (Tyr530 in humans) for negative regulation

*Differs in carboxy terminal deletions

*v-src is a **constitutively active** receptor tyrosine kinases due to no regulatory c-domain

- Effect of src on tumor behaviour:
  - **ECM degradation** ($\uparrow$ invasiveness)
  - **Adheren junction degradation** (E-Cadherin endocytosis) → $\uparrow$ motility and invasiveness
  - Stimulated **angiongenesis** (VEGF)
  - Inhibition of Integrin function & changes in actin cytoskeleton → Focal-adhesion disruption → $\uparrow$ motility and invasiveness
- → Increased cell motility and invasiveness

VIRUSES CARCINOGENIC TO HUMAN

- *Epstein-Barr virus*
- *Hepatitis B virus*
- *Hepatitis C virus*
- *Kaposi's sarcoma herpes virus*
- *Human immunodeficiency virus type 1 (HIV-1)*
- *Human T cell lymphotropic virus type 1 (HTLV-1)*
- *Human papilloma virus*

CAUSAL RELATIONSHIP BETWEEN PUTATIVE CANCER-CAUSING VIRUS AND HUMAN CANCER:

1. Epidemiological evidence
2. Serological evidence (presence of certain antibodies)
3. Insertion of viral genome into host genome
4. Consistent chromosomal translocation
5. Experimental evidence of viral-induced transformation

EPSTEIN BARR VIRUS (EBV)

- Was first discovered in cultured tumor cells derived from African **Burkitt's lymphoma** tissue (Endemic in equatorial Africa) → First human tumor shown to be associated with a virus
- Possible prevention by vaccine
- EBV leads to transformation of B-cells (Cancer that start in immune B-cells recognized as fastest growing human tumor)
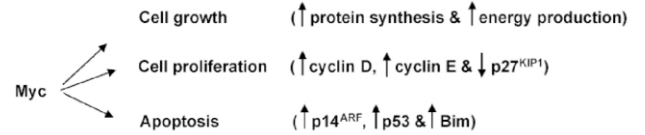
MYC TRANSLOCATION IN THE PATHOGENESIS OF BURKITT'S LYMPHOMA

Chromosomal translocation identified; shown to juxtapose Ig sequences to *myc*

-translocation of myc puts it under the <u>control</u> of IgH (highly active) promoter
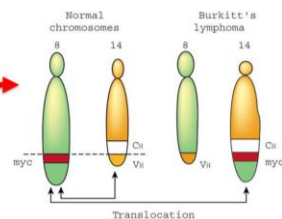
- Reciprocal **chromosomal translocation** <u>activates</u> the **Myc oncogene** by juxtaposing it to an immunoglobulin gene loci → **Juxtaposing** brings the proto-oncogene under the control of a transcriptionally **active Ig locus**

→ leads to **deregulated constitutive expression of the translocated Myc gene** → **Myc protein accumulates** to higher levels than in normal B cells

Myc → Cell growth ($\uparrow$ protein synthesis & $\uparrow$ energy production)
Myc → Cell proliferation ($\uparrow$ cyclin D, $\uparrow$ cyclin E & $\downarrow$ p27^{KIP1})
Myc → Apoptosis ($\uparrow$ p14^{ARF}, $\uparrow$ p53 & $\uparrow$ Bim)

HUMAN PAPILLOMAVIRUS (HPV)

- **DNA virus** from the papillomavirus family (Group of more than 200 related viruses, ~40 spread sexually)
- **High-risk HPVs** cause **several types of cancer** → ~5% of worldwide cancers
  - **Cervical cancer**: All cases are caused by HPV, 2 HPV types (16 & 18) are responsible for ~70% of all cases (cervix cancer is the 4. cancer mortality cause in women, but new cases are decreasing)
  - **Anal cancer**: ~95% of anal cancers are caused by HPV, most of these are caused by HPV type 16
  - **Oropharyngeal cancers**: ~70% are caused by HPV
  - **Rarer cancers**: HPV causes about **65% of vaginal cancers**, **50% of vulvar cancers**, and **35% of penile cancers** (Most of these are caused by HPV type 16)

FROM INFECTION TO MALIGNANT TUMOUR
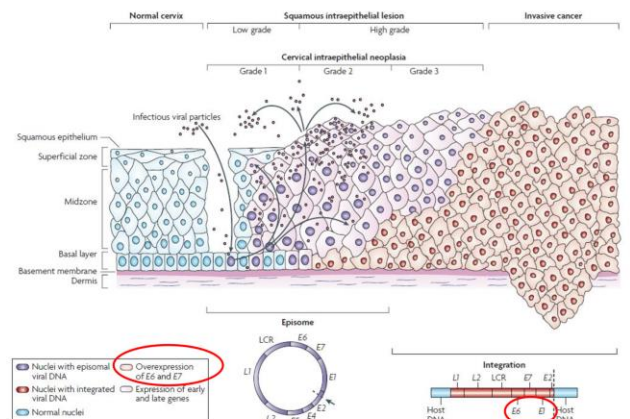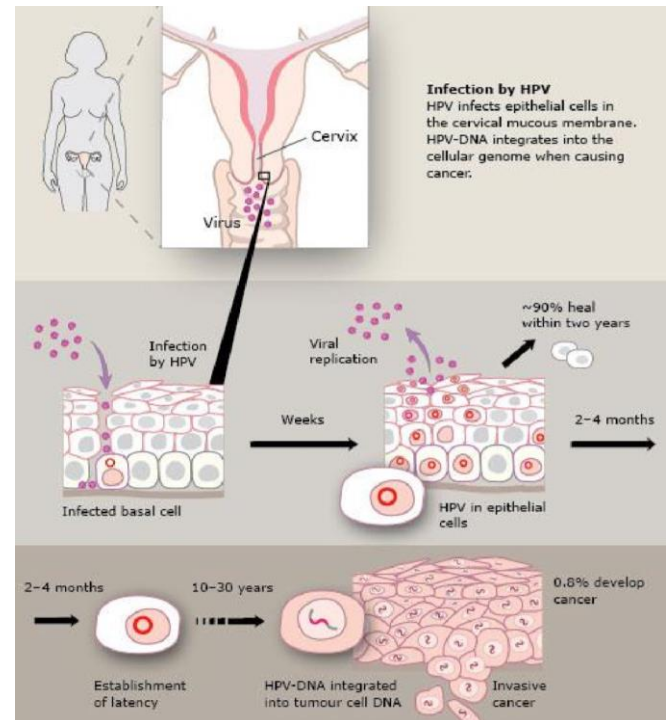
VIRAL ONCOGENES E6 AND E7

- These oncogenes **inhibit Rb and p53 tumor suppressors**
  - o **E6 decreases apoptosis**: E6 is expressed in the host and interacts with p53 leading to it's degradation (ubiquitination)
  - o **E7 increases proliferation**: Rb binds E2F keeping it from going into the nucleus. E7 binds Rb, releasing the TF E2F and allowing it to enter the nucleus
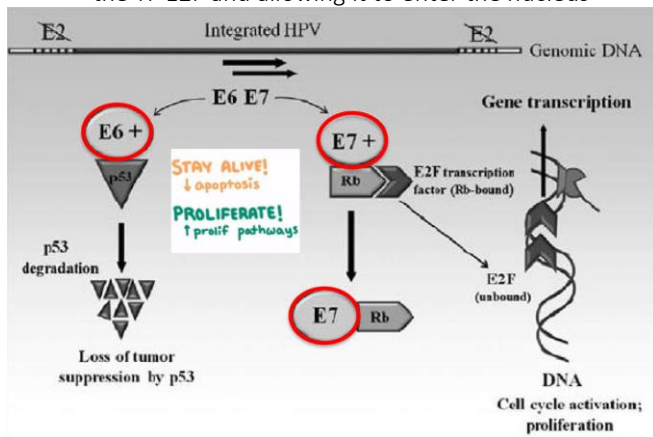
Oncogenes E6 and E7 important in HPV, they drive tumorigenesis once inserted

PREVENTING HPV CANCERS WITH VACCINATION

- 2006 first vaccination for HPV available to the public
- Hela cells are HPV18 positive

VIRAL TRANSFORMING GENES

- Two general strategies:
  - o Permanent activation of cellular signal transduction cascades
  - o Disruption of cell cycle regulation

IMPORTANT DISCOVERIES & EVENTS IN TUMOR VIROLOGY

RADIATION-INDUCED CANCER

- **Nuclear fallout** → residual radioactive material in the upper atmosphere after a nuclear blast
- **UV**

IONIZING AND NON-IONIZING RADIATION

- **'Non-ionizing Radiation'**: Radiation that does not have enough energy to break chemical bonds but can **vibrate atom** (e.g. radiowaves, microwaves, infrared, visible light etc.)
- **'Ionizing Radiation'**: Radiation that **has enough energy to break chemical bonds** (e.g. alpha particles, beta particles, gamma rays etc.)
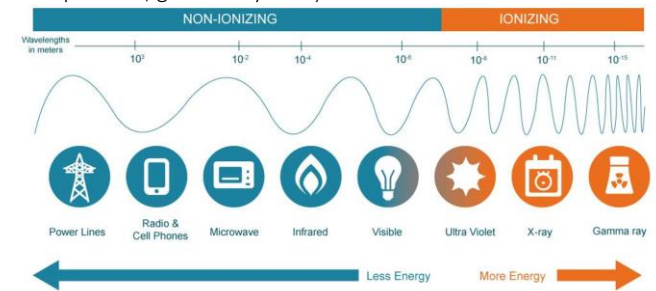
UV - ULTRAVIOLET RADIATION

- **Sun exposure** is linked to **skin cancer**
- UV of solar spectrum is divided into 3 wavelengths:
  - o **UVA** (320 to 480 nm), (95% of sun) → production of reactive oxygen species
  - o **UVB** (280 to 320 nm) → most correlated with cutaneous cancer
 - o **UVC** (200 to 280 nm) → most dangerous
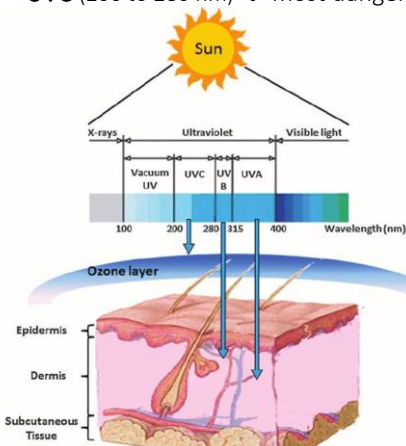
  ⇒ UVA has more depth penetration than UVB
- **Ozone layer** shields from much effects of UV radiation
- European with fair skin have higher incidence of skin cancer
- Persons of darker skin are **protected by the pigment melanin** (derived from tyrosine) that **absorbs ultraviolet radiation**

CANCER SITES AND TISSUE RADIOSENSITIVITY

Proliferation
High

- LYMPHATIC TISSUE, ESPECIALLY LYMPHOCYTES
- WHITE BLOOD CELLS AND IMMATURE RED CELLS OF MARROW
- CELLS LINING GASTRO-INTESTINAL TRACT
- GONADIC CELLS
- SKIN, ESPECIALLY THE PROLIFERATING LAYER
- BLOOD VESSALS AND BODY CAVITY LININGS
- TISSUES OF GLANDSA ND LIVER
- CONNECTIVE TISSUE
- MUSCLE
- NERVES

Low

DIRECT AND INDIRECT DNA DAMAGE

- Radiation interacting with DNA (direct and/or indirect)

Indirect Route: Radiation →water →free radical →DAMAGE
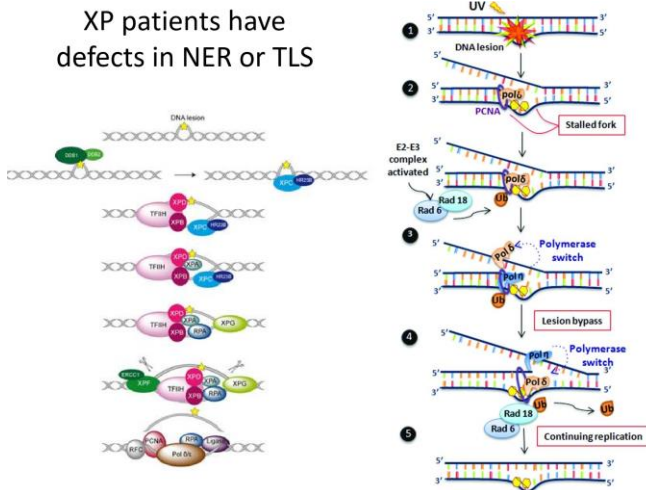
Direct Route: Radiation → DAMAGE
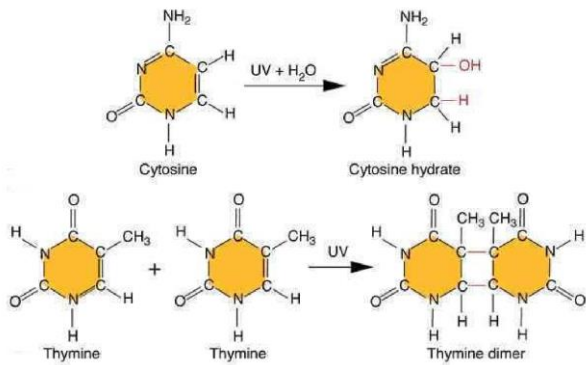
Remember which repair pathways are connected with the damage

DNA REPAIR DEFECTS AND UV-INDUCED CANCER

- **Xeroderma Pigmentosum (XP)**: Rare genetic disorder
  - o Increase in skin cancer risk 1000-2000 fold
  - o Defects in DNA repair (TT dimers)
  - o Defective XP proteins in NER

XP patients have defects in NER or TLS

NUCLEAR FALLOUT RADIATION

- Exposure to ionizing radiation causes **damage to living cells**, especially to **DNA in the cell nucleus**
- Radiation-induced **chromosomal aberration** after atomic bomb

AGENTS THAT CAN CAUSE CANCER

chemicals   infectious agents   radiation

CANCER AND INFLAMMATION

IMMUNE SYSTEM

- 2 types of defense systems
  - o **Innate**: Response <u>after</u> an infection, no prior exposure, does not require the presentation of an antigen, does not lead to immunological memory
  - o **Adaptive**: Immune defense <u>later</u> in infection that is <u>highly specific</u> to pathogen (immunological <u>memory</u>)

### IMMUNITY AND CANCER: A DOUBLE EDGED SWORD

- Cancer can be promoted and/or exacerbated by inflammation and infection
- Tumor cells **produce various cytokines** & **chemokines** that **attract leukocytes** (→ growth advantage) as well as to prevent immune cell detection (→ surveillance)
- Problem: **Chronic** inflammation (vs acute)
- Inflammation has a **vital and complex role** in driving tumorigenesis
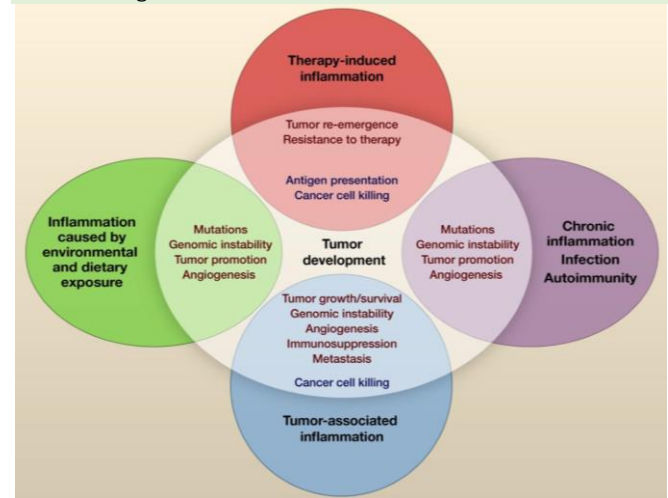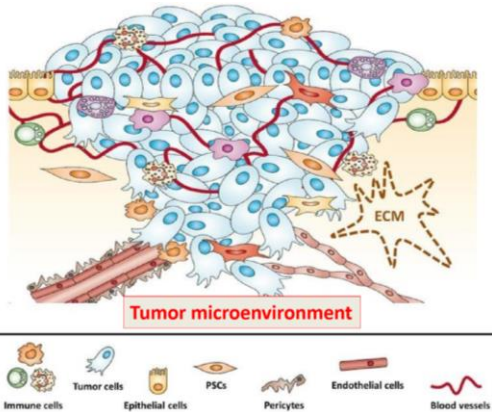

THE INFLAMMATORY RESPONSE
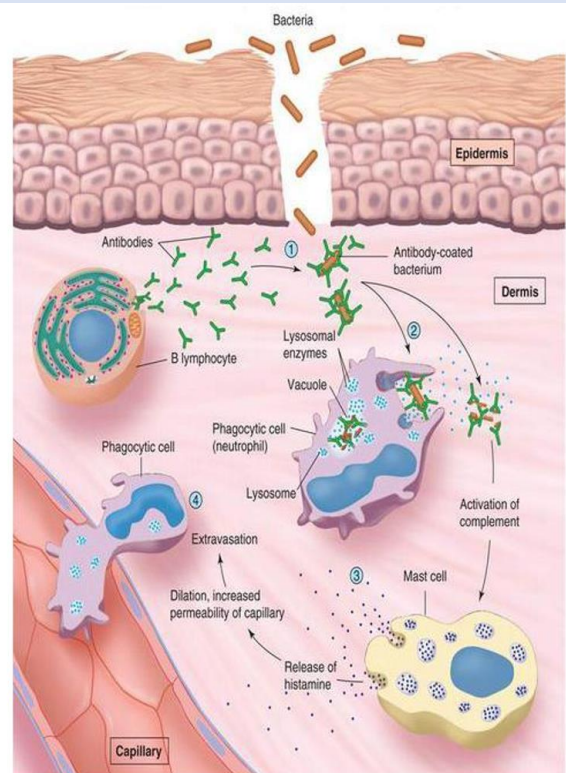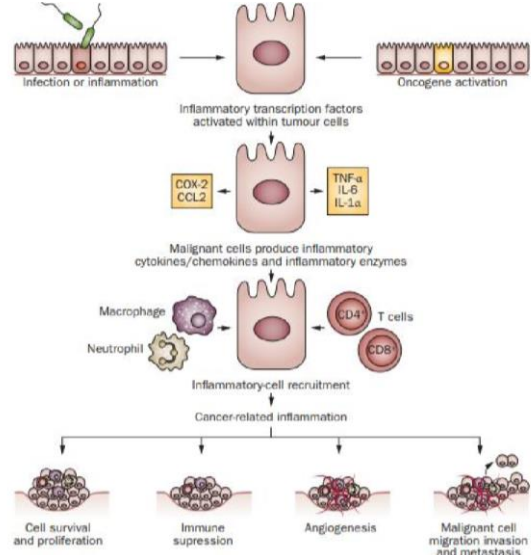
WOUND HEALING VS TUMOR GROWTH
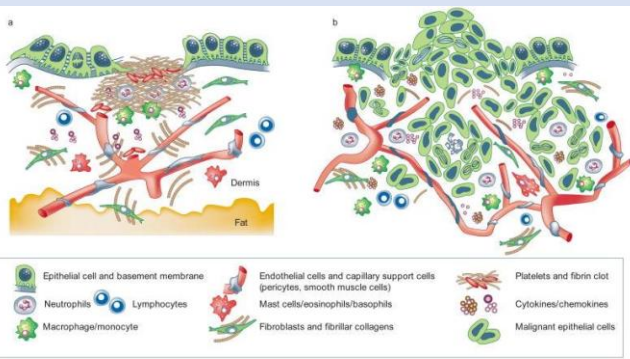
Tumors act as wounds that fail to heal

- Tumor environment similar to a wound that won't heal/chronic inflammation
- A lot of immune cells can exacerbate the problem

TUMOR MICROENVIRONMENT


MOLECULAR MECHANISM OF INFLAMMATION-INDUCED CANCER

1. **Inflammation** or **oncogene activation** results in the expression of vital **proinflammatory transcription factors** within tumour cells (e.g. NF-κB, STAT3, HIF1α)
2. Activated transcription factors mediate the expression of **key cytokines and chemokines** (including TNFα &IL-6) as well as inflammatory enzymes (such as COX-2), forming a rich and complex network of inflammatory responses within the tumour microenvironment
3. Host **leukocytes** (macrophages, dendritic cells, mast cells and T cells) are **recruited by chemokines**, and function within the tumour stroma to **mediate the immune response**
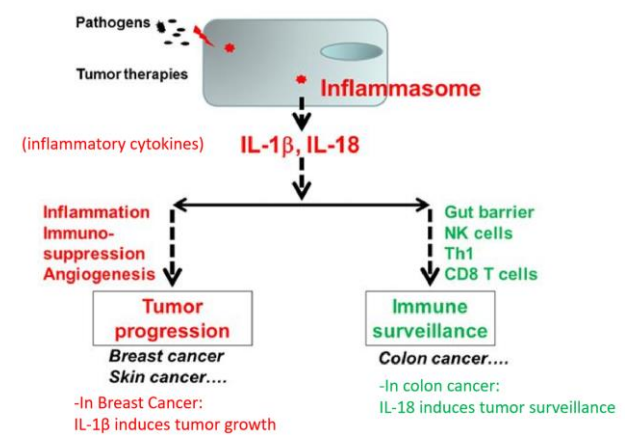
CYTOKINES

- Broad category of **small proteins** (~5–20 kDa)
- Important in **cell signaling**
- Cytokines include chemokines, interferons (IF), interleukins (IL), lymphokines, and tumor necrosis factors (TNF)

CYTOKINE PLAYERS IN CANCER

- There are cytokine players in cancer
  → Supressors and promotors
  o **IL-12** often associated with suppression of tumours
  o **TNF-α** and **TGF-β** often pop up in cancer
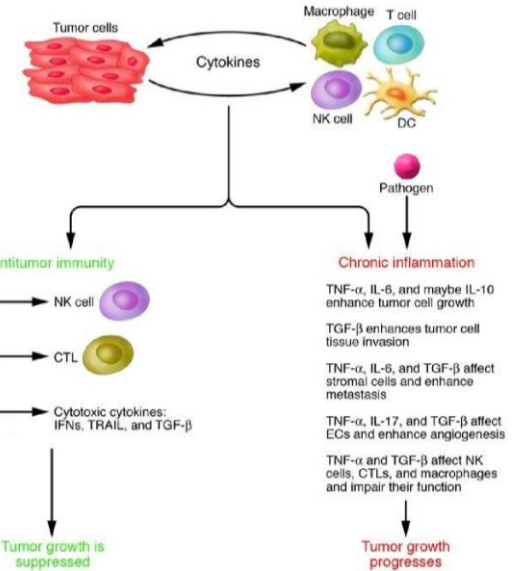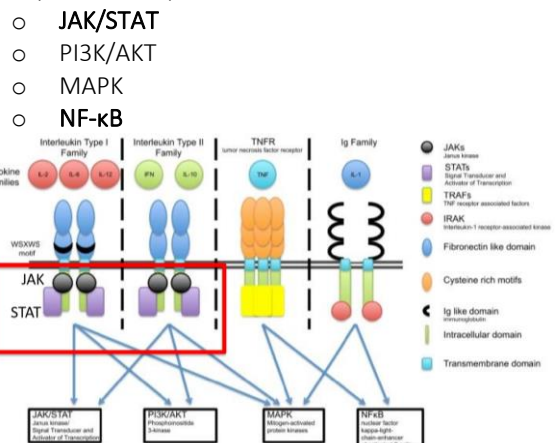

INFLAMMASOME AND CANCER

- The inflammasomes can **promote or inhibit** tumor progression depending on context

CYTOKINE RECEPTORS

- Cytokine receptor families:
  o **JAK/STAT**
  o PI3K/AKT
  o MAPK
 o **NF-κB**

ACTIVATION OF **JAK-STAT3** IN CANCER

1. Cytokines bind to receptor and activates it
2. Recruitment and activation of JAKs by phosphorylation → docking-site for STAT
3. Phosphorylation of STATs by JAKs
4. Dissociation and dimerization of STATs → Translocation into nucleus → activation of gene transcription
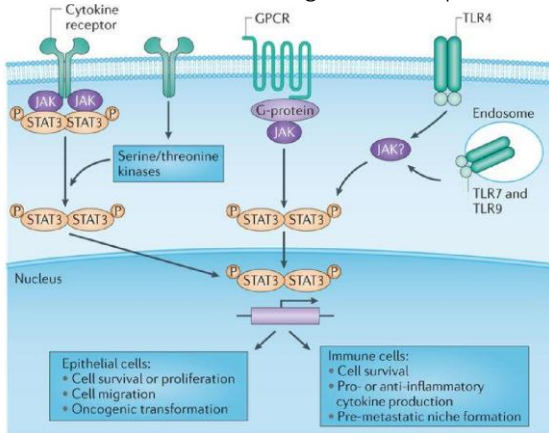
- Pancreatic Cancer Therapeutics: **STAT3 inhibition**
  o Small Molecule that activates Transcription-3 (STAT3) Protein Inhibitors → binds to the SH2 domain of STAT
- Orally administered small molecule **JAK inhibitors**:
  o Bind to kinase domain of JAK and prevents activation
 o Reduction of pro-inflammatory cytokines, e.g. IL-17

NF-KB PATHWAY

- Inflammation triggers the **NF-kB pathway**
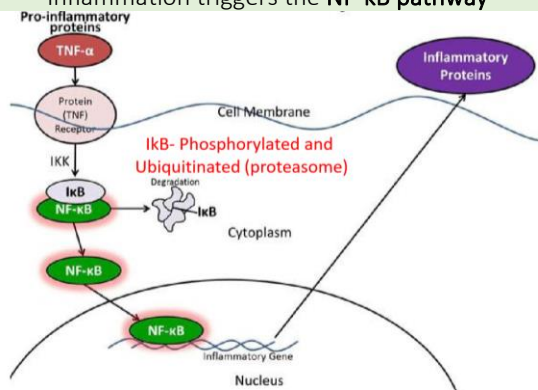
BORTEZOMIB

- First **proteasome inhibitor anticancer drug**
  o Treatment of relapsed multiple myeloma
- **Suppression of the NF-κB signaling pathway** resulting in the **down-regulation of its anti-apoptotic target genes**
  o Is a **reversible inhibitor** of the **proteasome**
  o **Boronic acid group** can bind and complex to the active site of threonine hydroxyl group (β5subunit) and block the chymotrypsin-like activity of the proteasome
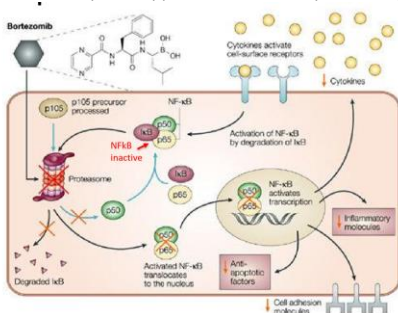

NF-kB involved in B cell maturation - Bortezomib treats B cell malignancies

CANCER IMMUNOTHERAPY

- Immunotherapy treatment uses a **person's immune system to fight cancer**
  o **Stimulating** the **own immune system** to attack cancer cells
  o **Administering** immune system components (such as manmade immune system proteins)

CAR-T

- CAR-T: **Chimeric antigen receptor T-cell therapy**
- **Engineering patients' immune cells** to treat their cancer
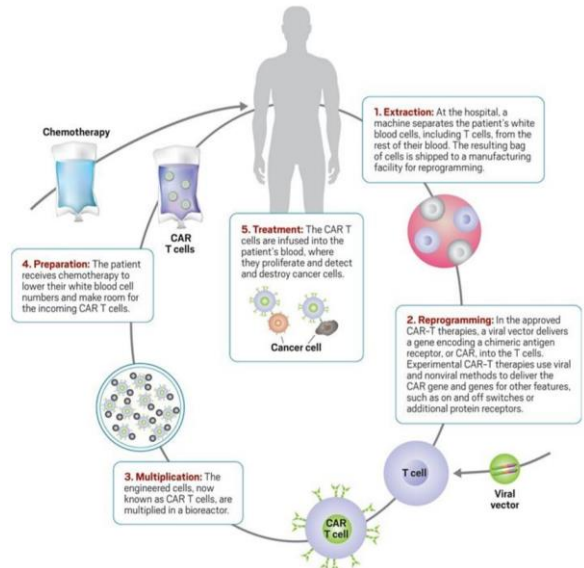
CHALLENGES WITH CAR T-CELL THERAPY

- **Dosing**: No uniform consensus on the dose
  o Small dose may not obtain ideal curative effect
  o Large dose can increase cytokine release syndrome and tumor-lysis syndrome
  o Traditional drugs are ephemeral → begin to break down as soon as they enter the bloodstream
    BUT: CAR-T is a living therapy → **multiplies exponentially** once the cells spot their cancer target in the blood
- **Toxicity**: Both, *Kymriah* and *Yescarta*, come with **warnings for cytokine release syndrome** → severe, body-wide immune reaction after injection of the drug
  o *"It's not an exaggeration to say that it almost kills you before it helps you"*
- **Treatment**: Approved for **only a small subset of cancers**
  o *Kymriah*: Treats people **up to 25 years** old with **acute lymphoblastic leukemia of B-cell origin** who are resistant to treatment or have relapsed twice
  o *Yescarta*: Treats **large B-cell lymphoma in adults** after **two other treatments have failed**
- **Price**:
  o Kymriah's (Novartis) one-time cost of $475,000
  o Yescarta's (Kite Pharma) costs $373,000

CAR-T 2.0

- **Brain swelling** commonly occurs with CAR-T cell therapy
- Making a kill switch:
  o Two engineered proteins located inside the CAR-T cell that **dimerize** when exposed to a small-molecule drug called **rimiducid**
  o Rimiducid activates a protein called caspase-9, which kick-starts the process of **CAR T-cell suicide**
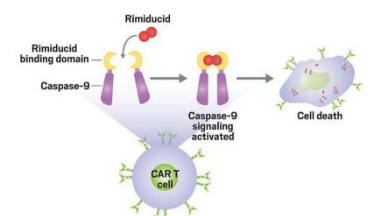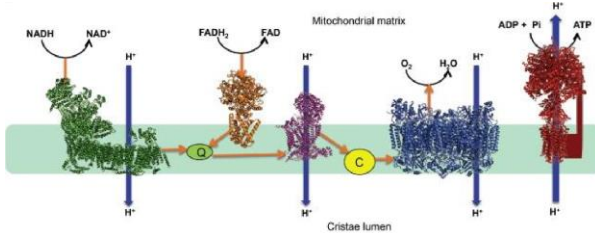
CANCER AND MITOCHONDRIA

MITOCHONDRIA

ANATOMY:
- o Size of bacteria (0.5 μm diameter, 1.0 μm length)
- o Smooth outer membrane, **folded inner membrane** (invaginations = cristae)
- o Proteins for:
  - **OXPHOS**: Bound to inner membrane
 - **TCA**: Within inner membrane space

ENERGY METABOLISM
- *"The Power House"*
- Glycolysis: Cytosol, Pyruvate is produced
- TCA cycle: matrix of mitochondria
  - o Inner membrane space
  - o <50% water with high [protein] for TCA cycle
  - o Electron transfer sites forming NADH and $FADH_2$
- Oxidative phosphorylation (OXP)
  - o Metabolic pathway: **Enzymes oxidize nutrients to release energy** needed for **ATP production**
  - o Takes place in the **inner mitochondrial matrix**
  - o Electron transport chain: Free energy of **electron transfer from NADH and $FADH_2$ to O_2** through protein bound redox centers coupled to **ATP synthesis**
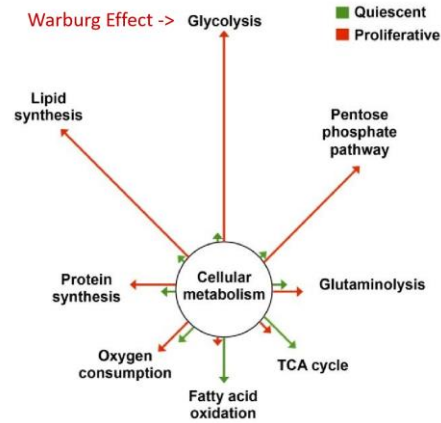 - o 4 enzyme complexes and ATP synthase

MITOCHONDRIA AND CANCER
- Nuclear-Mitochondria Crosstalk (PolG mutations)
- Organelle dynamics
- Apoptosis
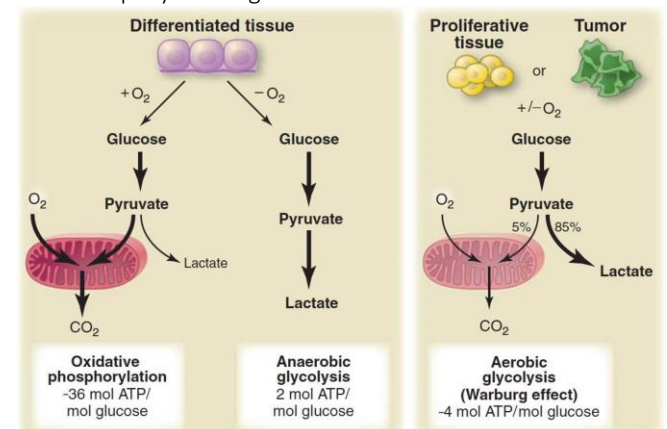- Metabolic reprogramming
- mtDNA genome
- Reactive oxygen species
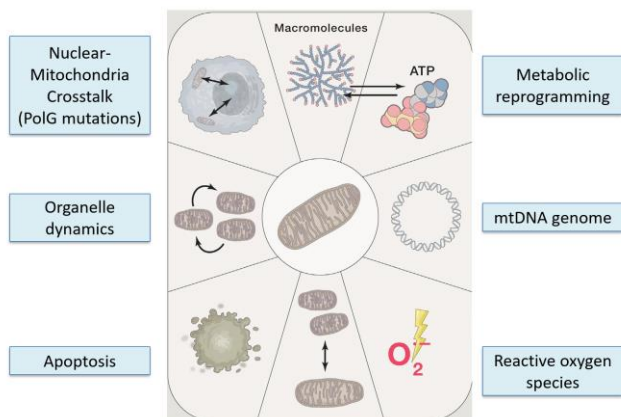

METABOLIC REPROGRAMMING

WARBURG EFFECT
- Shift to aerobic glycolytic pathways in cancer
  - o Hypothesis for shift: Generation of more molecular intermediates for protein synthesis, since cells are rapidly dividing

→ Alterations in glucose utilization

ATP SYNTHASE AND CANCER
- ATP synthase is **down regulated in cancer** via the **upregulation of IF1 (Inhibitor Factor 1)**
- IF1 is the physiological inhibitor of ATP synthase
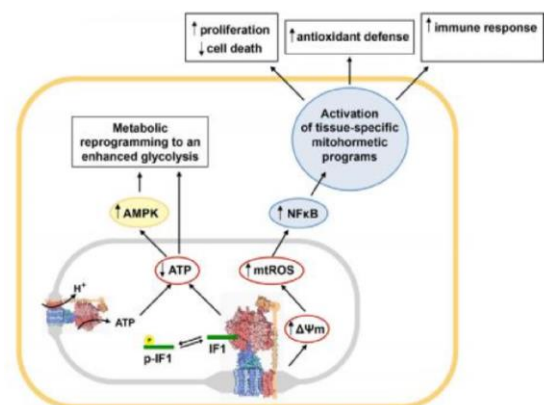
MITOCHONDRIA GENOME

- Mitochondria have their own DNA (multiple copies of circular genome)

| mtDNA	nDNA
ds circular	ds linear
Single chromosome	Several chromosomes
16,569 bp -37 proteins	3.3 billion bp -30,000 proteins
No histones	Histone packing
No introns (non coding)	Many introns
Cell cycle independent replication	S-phase replication
Maternal inheritance	Maternal and Paternal Inheritance (Recombination events)

mtDNA MUTATIONS AND CANCER

- Several **mtDNA mutations** have been identified in various types of human cancer
- Mutations have been found to be present in both the **non-coding region** and **coding regions** of the mtDNA
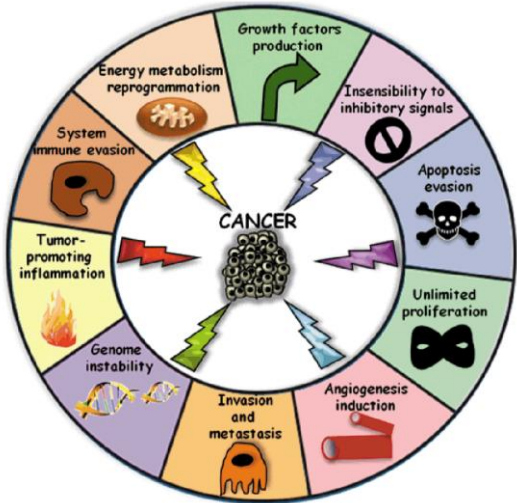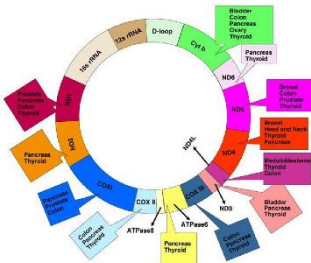
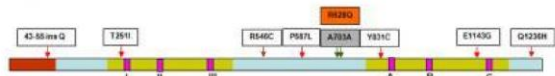### mtDNA REPLICATION: DNA POL GAMMA

- DNA Pol Gamma is involved in mtDNA replication
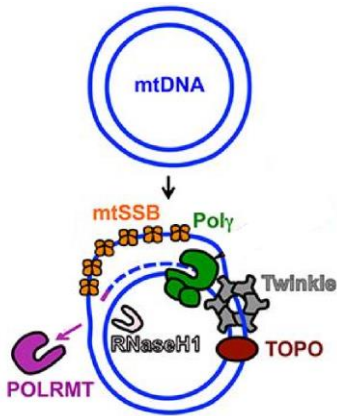
PolG expressed in nucleus and transported to mitochondria for the replication of its genome

- Mutations in mitochondrial DNA polymerase y promotes breast tumorigenesis

Arrows indicate PolG disease associated mutations

- Breast tumors contained mutations in mtDNA
- Mutations in POLG can cause mutations in mtDNA
- mtDNA mutator mice that harbor the mutation in the exonuclease domain (that abolishes the POLG proof reading activity) show a marked **reduction in lifespan** due to the **increased rate of mtDNA mutation**

- It is possible that mtDNA mutations do not initiate tumorigenesis, i.e., transform normal cells, but rather are **involved in the promoting tumorigenesis**
- POLG gene mutations in human cancer suggest a role for POLG in human tumorigenesis
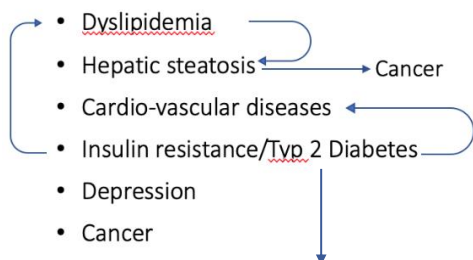
CONCLUDING REMARKS ON CANCER

PART WOLFRUM

OBESITY

- 50% of USA people have a BMI higher than BMI > 30
- Extremely large increase in obesity over last 20 years
- Today more people die from obesity than from malnutrition
- If you understand changes over the years you can infer causality → Change is probably **not genetic** → need to understand molecular drivers!

CONSEQUENCE OF OBESITY: CO-MOBIDITIES

Obesity itself is not the problem, it is not the disease but it can lead to many of them:

- Dyslipidemia
- Hepatic steatosis → Cancer
- Cardio-vascular diseases
- Insulin resistance/Typ 2 Diabetes
- Depression
- Cancer

Diabetes and Adipositas: For genetic/epigenetic reasons, diabetes rates are higher in India vs. the US, although though they have the same prevalence of obesity

Lipodystrophy: Is the inability to produce and maintain healthy fat tissues. This disease has the same co-morbidites as obesity→ co-morbidities are dependent on adipose tissue!

CAUSE OF OBESITY
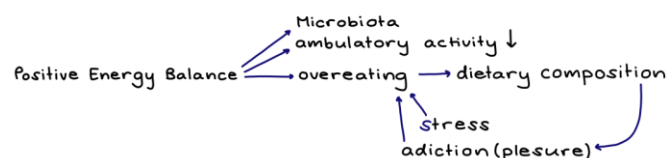
- Environment → Effects ampulatory activity, dietary composition and stress
- Genetics → Basal metabolic rate (BMR)
  - o Heritability of obesity is between 60-80%
- Epigenetics → Effects obesity on a shorter time-scale

ENERGY METABOLISM

ENERGY EXPENDITURE
- **Voluntary energy expenditure (activity)**
  - o Exercise
  - o Movement
  - o Brain activity
- **Involuntary Energy expenditure**
  - o Twitching
} (15-20%)
- **BMR (60%)**
  - o Temperature
  - o Muscle mass
  - o Hormones
  - o **Gender**
  - o **Age**
  - o (Effected by environment)
- **Thermal effects of food** (diet induced thermogenese)
- ➔ 8kg weight gain per year with ΔE of 200 kcal/day

ENERGY INTAKE
- **Hunger:** Sensation associated with the drive to eat
- **Appetite:** Psychological desire to eat
- **Satiation:** Termination of eating after hunger has been satisfied

Feeding cycle:
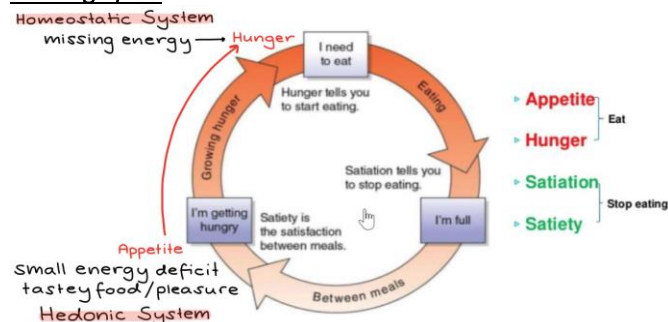
- **Homeostatic system:** short term survival (missing energy → hunger), energy sensors in body (see later)
- **Hedonic system:** Reward based regulation (appetite, pleasure), makes sure the body builds Energy reservoirs for homeostatic system when needed (energy dense food) → long-term survival

HOMEOSTATIC SYSTEM – ENERGY HOMEOSTASIS

SET POINT THEORY
Suggests that the body has a weight it tries to keep in a homeostatic manner
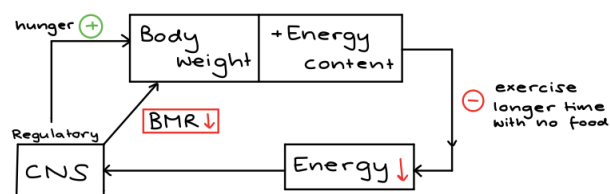
ENERGY HOMEOSTASIS IN THE HYPOTHALAMUS
The brain **(hypothalamus)** is a key player in the control of energy homeostasis:
- **Arcuate nucleus (ARC):** Receptors for hormones and neuropeptides that regulate feeding
- **Paraventricular nucleus (PVN):** Integrates signals from ARC, the **MC4 Receptor** is located here.
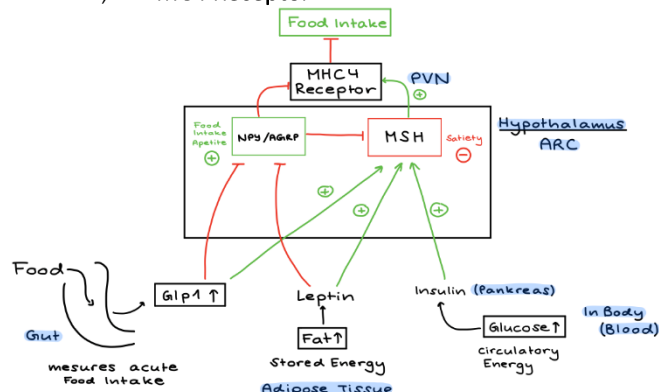
- The brain integrates incoming information in the form of hormonal and neural signals with data on energetic needs or anticipated needs.
- The physiological regulation of food intake is a complex homeostatic process that is regulated by many endocrine and metabolic factors in a combination with visual, olfactory, taste sensation, emotions, memory and the life conditions.

- Mutations in MC4R (e.g. lower activity) can be advantageous from an evolutionary point of view → eating more is less likely to be problematic than eating too little

LEPTIN
- Adipocyte derived hormone
- Increases metabolic rate/energy expenditure
- Decreases food intake

Historical Parabiosis Experiment (Jeffrey Friedmann):
- Observation: Interbreeding of heterozygous mice → ¼ obese → hyperphagia (over eating)
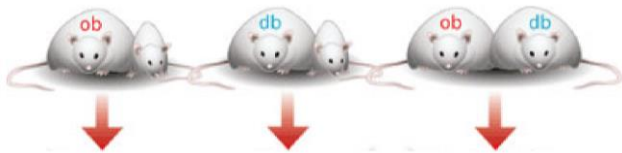

Obese ob/ob mouse loses weight (eats less). Normal mouse stays the same.	Nothing happens with obese db/db mouse. Normal mouse stops eating and dies.	Nothing happens with obese db/db mouse. Obese ob/ob mouse stops eating and loses weight.

- Ob-gene encodes for a circulating factor and binds to a receptor encoded by the db-gene. This receptor signals to stop eating.
    o Ob-gene → Leptin
    o Db-gene → Leptin receptor
➔ First Experiment: The circulating factor, leptin was missing
➔ Second Experiment: Db-mouse induces ob-gene to activate pathway → compensation → resistance. The normal mouse reacts to the leptin and is never hungry.

Leptin Signaling pathway:
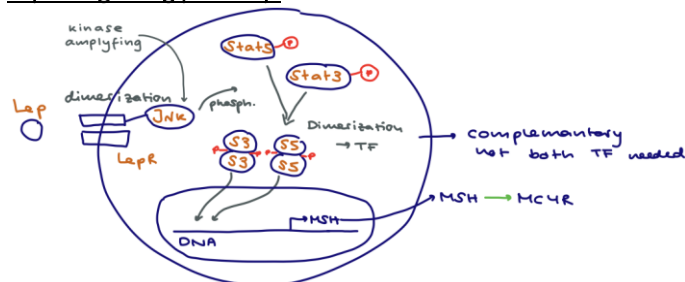
Leptin inhibits NPY/AGRP neurons that increase NPY and results in inhibition of food intake.
Resistance:
BUT: leptin treatment does not work for obesity because of **resistance.** Resistance means that there is a lot of leptin but no signaling, why?[1]
- No LepR dimerization
    o Inhibited by interacting protein
    o Inhibited by post-translation modification, e.g. phosphorylation
    o LepR expression reduced

[1] Resistence is not yet completely understood.

- Reward and craving depends on satiety state → Food deprivation strongly augments the reward value
- Different reward from different foods → shift towards highly palatable, calorie-dense foods
    o With salad satiety sets in quicker but reward takes longer

## HEDONIC PROCESSING IS AN INTEGRAL PART OF THE HOMEOSTATIC REGULATORY SYSTEM
Hedonic and homeostatic neural circuitries are not separate entities but are part of the same regulatory system. They are connected via neurons and affect each other but sensors also directly affect the reward system. Neural circuitries for internal nutrient sensing and hedonic processing act in concert to control eating and body weight and the hedonic system becomes part of the homeostatic system.

Integrative Model:
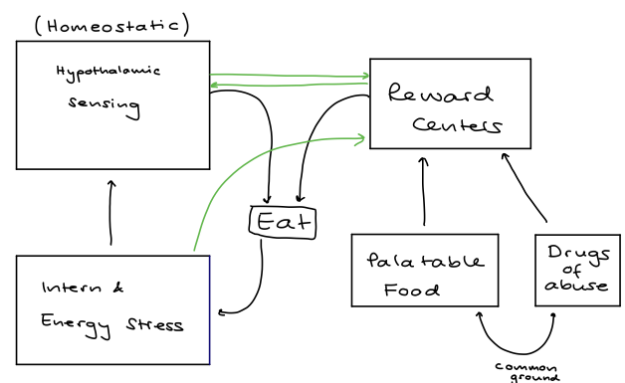
CONNECTION OF BOTH SYSTEMS – MECHANISMS
- **VTA (ventral tegmental area):** Part of midbrain
- **LH or LHA (Lateral hypothalamus):** Feeding centre
- **NAc (Nucleus Accumbens):** Region in basal forebrain
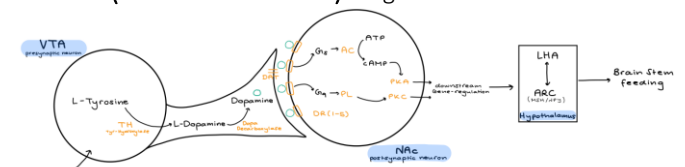
- **DAT:** Dopamine transporter
- **DR (1-%):** Dopamine receptors
    o G-Protein coupled receptors → two different signaling pathways → G_s and G_q
    o Have different affinity → slow and quick rxn, compartmentalization
- LH integrates reward-related input from NAc with information related to energy homeostasis from ARC neurons
- VTA → NAc pathway may promote consumption of palatable food involves projections to the LH
- The LH contains neurons that potently stimulate food intake and is supplied by fibres not only from striatum and orbitofrontal cortex, but also from the ARC
- LH area neurons supplying the NTS may, in addition, attenuate the response to satiety signals, increasing the amount of food consumed during a meal

INSULIN RESISTANCE AND TYPE 2 DIABETES

GLUCOSE METABOLISM

Why is it important to regulate blood glucose levels?
- Number 1 energy source of the Brain → needs constant blood glucose levels
- High glucose levels can be **toxic**
- → Blood glucose levels need to be tightly regulated

How is the blood glucose level regulated?
- By the hormones **glucagon and insulin**: Beta cells in the Pancreas secrete insulin in response to increased glucose levels and alpha cells secrete glucagon in response to a low blood glucose level
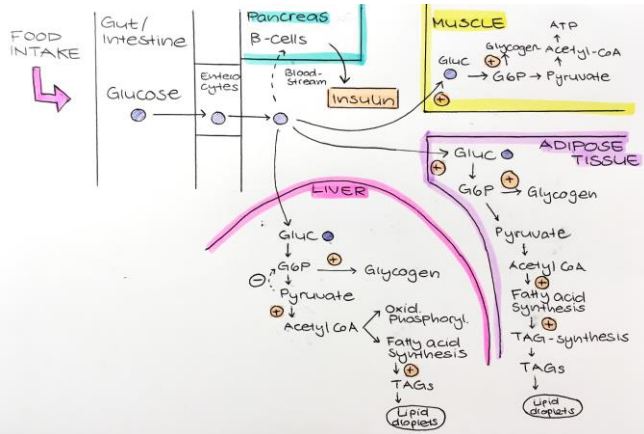
INSULIN
- Is an **anabolic hormone** (promotes the synthesis of carbohydrates, proteins, lipids and nucleic acids)
- Most important target organs for insulin action are:
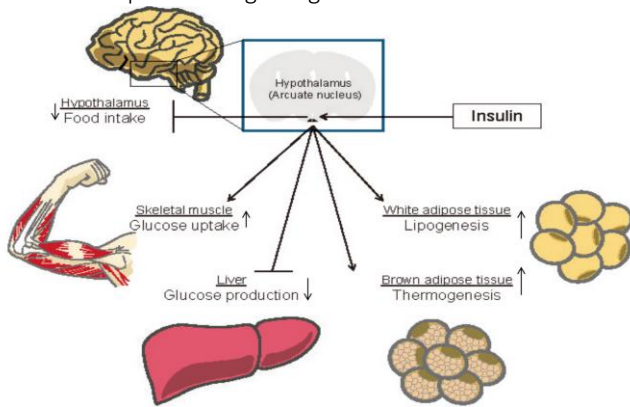
FOOD INTAKE/ FED
- Increased levels of insulin

LIVER

Is the glucose transport into the liver insulin dependent?
- Transport is only **indirect insulin dependent** → insulin shifts the metabolisms to glycogen synthesis → glucose gets metabolized and used up → glucose import is dependent on its concentration gradient and gets therefore transported into the liver

What happens with the glucose in the liver?
- Glycogen synthesis
- Metabolized zo pyruvate
  - Oxidative phosphorylation
  - Lipid synthesis

What happens with the pyruvate in the liver?
- Pyruvate gets metabolized to Acetyl CoA
  - Acetyl CoA can enter the TCA cycle → Oxidative phosphorylation → ATP production

  - Acety CoA can be used for fatty acid synthesis (positively regulated through Insulin)
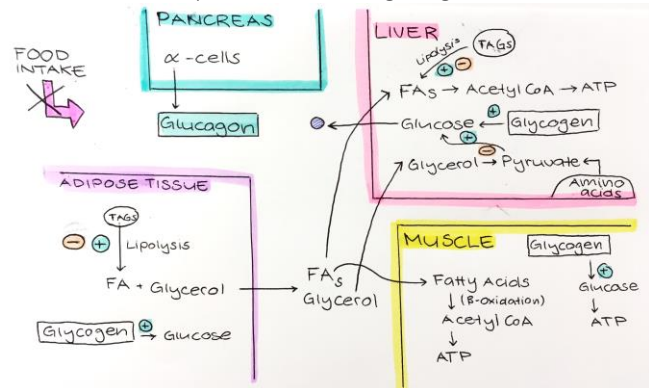
MUSCLE
- Glucose import/uptake is **completely dependent on insulin** (the liver is the only indirect dependent tissue)

ADIPOSE TISSUE
- Glucose import/uptake is **completely dependent on insulin**
- Has no mitochondria! → no oxidative phosphorylation!

FASTED
- Little or no insulin but **high levels of glucagon**
- α-cells of the pancreas secrete glucagon

ADIPOSE TISSUE
- **Lipolysis**: Triacylglycerol's are broken down in **fatty acids** and **glycerol** (both exported)
  - Lipolysis is **dependent on glucagon** and **inhibited by insulin**

MUSCLE:
- **β-oxidation**: Fatty Acids → Acetyl CoA → further processed to gain ATP
- **Glycogenolysis**: Glycogen → Glucose → ATP
  - Is **dependent on glucagon**
  - Muscle uses glucose to function (will not secrete it into the blood stream!)

LIVER
- Can take up **FAs** and **glycerol** from the adipose tissue
- TAGs from the own store: Lipolysis → FAs
- **β-oxidation**: FAs → Acetyl CoA → ATP
- **Glycogenolysis**: Glycogen → Glucose → released in the blood stream
- **Gluconeogenesis**: (Aminoacids →) Pyruvate → Glucose
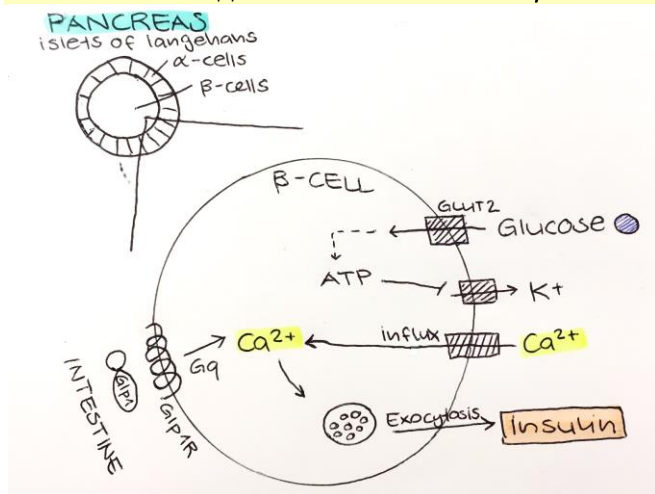 - Insulin massively supresses Gluconeogenesis

Remember: You cannot easily make Pyruvate out of Acetyl-CoA!

MOLECULAR PHYSIOLOGY OF GLUCOSE METABOLISM

B-CELLS

How is insulin secreted?

Insulin secretion happens via Ca²⁺ mediated Exocytosis

1. Glucose enters β-cells via **Glut2** transporter
2. Glucose is metabolized to ATP → [ATP]↑
3. ATP inhibits opening of potassium channels (K⁺)
4. Depolarization → Opening of voltage-gated calcium channels → [Ca²⁺]↑
5. Ca²⁺ leads to vesicular secretion of insulin (Exocytosis)

Further: Intestine secretes Glp1 → Glp1R → Gq → [Ca²⁺]↑

DRUG TARGETS FOR TYPE 2 DIABETES:

1. Glp1 → supply with another peptide with a longer half live than Glp1 → activate signalling → increase insulin secretion
2. K⁺-blockers: Induces Ca²⁺ influx → increases insulin secretion

DIABETES MELLITUS (DM)

Increase of blood glucose levels

Is an endocrine disease, which develops due to *absolute* (type 1) or *relative* (type 2) insulin insufficiency

- Global prevalence: **8.3%** (366 million, with increasing tendency!)
- Term DM refers to **excretion of large quantities of sweet urine** → Greek: *diabaino = siphon* and *mellitus = honey,"sweet" taste*

PLASMA INSULIN RESPONSES TO ORAL AND INTRAVENOUS GLUCOSE
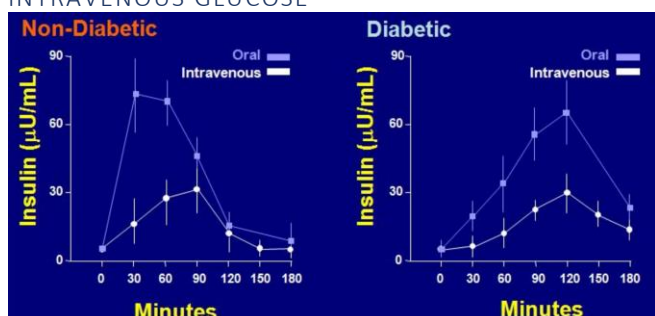

STAGES OF DM DEVELOPMENT

1. Prediabetes (risk factors or predispose factors)
2. Impaired glucose tolerance (latent DM)
3. Clinical manifestation of DM

PREDIABETES - RISK FACTORS/PREDISPOSE FACTORS

- Obesity
- Positive family history of DM
- Persons which were born with weight more than 4,0 kg
- Women who had children with weight more than 4,0 kg
- Endocrine disorders

Relationship between BMI and Risk of Type 2 DM:
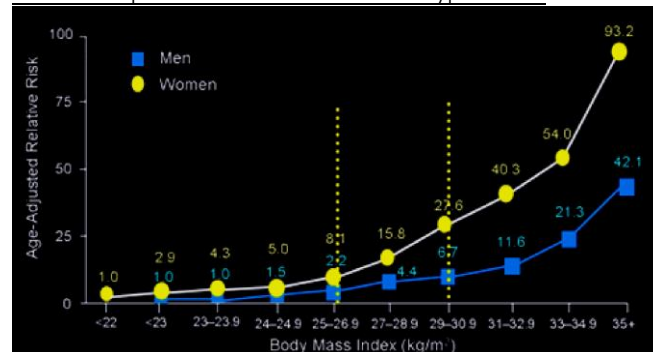
Sugar: For OGTT → 75 g for adults → ~10-11 teaspoons

WHEN SHOULD WE SCREEN FOR DIABETES:

- All patients ≥ 45 years of age.
- All patients with BMI ≥ 25 and any of the following:
  - Hypertension of ≥140/90 mmHg
  - HDL < 35 mg/dL, or Triglycerides > 250 mg/dL
  - Clinical insulin resistance (severe visceral obesity, acanthosisnigricans)
  - History of cardiovascular disease.
  - Gestational DM, or delivered a baby > 9 lbs
  - African/Lationo/Native/Asian American or Pacific islander
  - First degree relative with diabetes
  - Physically inactive
- If testing is normal, then repeat screening in 3 years

DIAGNOSTIC CRITERIA FOR DM

- **A1c ≥ 6.5% (new)**
  - or
- **FPG ≥ 126 mg/dL** – fasting for ≥ 8 hours
  - or
- **75 gr, 2hr OGTT ≥ 200 mg/dL**
  - or
- **Random Glucose ≥ 200 mg/dL + symptoms of hyperglycemia**

  - **Repeat testing if uncertain.**

TYPES

### ETIOLOGIC CLASSIFICATION OF DM (1999)

- **Type 1 DM** → **Destruction** of β-cells which mostly leads to **absolute insulin insufficiency**
  - o Autoimmune
  - o Idiopathic
- **Type 2 DM** → **Resistance** to insulin and **relative insulin insufficiency** or defect of insulin secretion with or without **resistance to insulin**
- **Other specific types**
  - o Genetic defects of β-cells function
  - o Genetic defects of insulin action
  - o Pancreatic diseases (chronic pancreatitis; trauma, pancreatectomy; tumor of pancreatic gland; fibrocalculosis; hemochromatosis)
  - o Endocrine disease (acromegaly, thyrotoxicosis, Cushing's syndrome)
  - o Drug exposures
  - o Infections and others
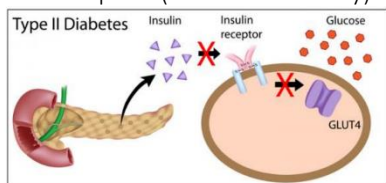- **Gestation diabetes** (*Schwangerschaftsdiabetes*)

INSULIN INSUFFICIENCY
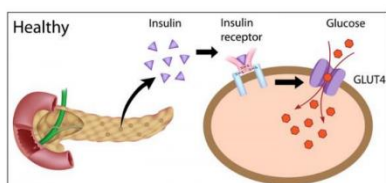
Absolute Insulin insufficiency:
1. Genetic disorders
2. Autoimmune damaging of β-cells
3. Viral damage
4. Toxic influence on β-cells
5. Diseases of pancreatic gland
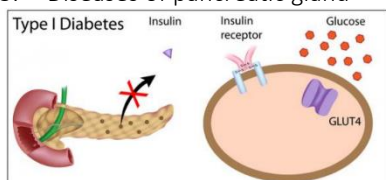
Relative Insulin insufficiency:
1. β-cells
2. Insulin transport
3. Receptors (tissue insensitivity)

PATHOGENETIC AND CLINICAL DIFFERENCES

| N	Signs	Type 1	Type 2
1	Age	Young (under 35)	Old, middle
2	Beginning of disease	Acute	Gradual
3	Duration	Labile	Stable
4	Ketosis, ketoacidosis	Often develops	Rarely develops
5	Body weight	Decreased or normal	Obesity in 80-90 % of patients

EPIDEMIOLOGY

- **Type 1**: Complications from end stage **renal disease** are major cause of death
- **Type 2:** More likely to have **macrovascular diseases** leading to **myocardial infarction** and **stroke** as main causes of the death

TYPE 1 DM

Type 1 DM = **Insulin-dependent diabetes mellitus (IDDM)**
- Characterized by pancreatic islet **beta-cell destruction** and **absolute deficiency**
- Onset of the disease is **generally in youth**, but it can occur at any age
- Patients have dependence on daily insulin administration for survival
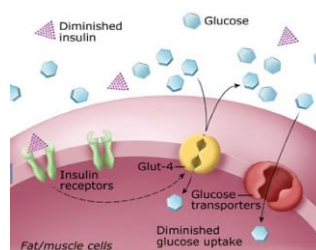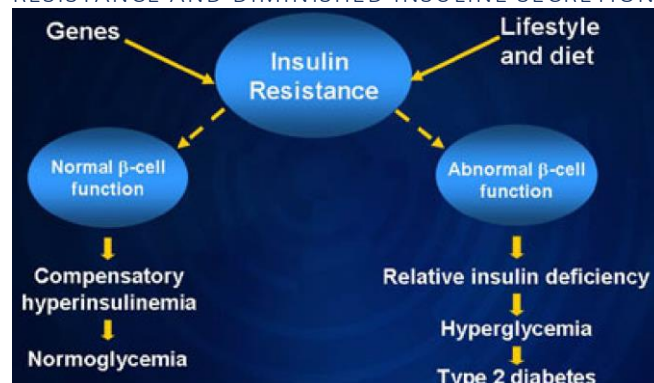

TYPE 2 DM

Type 2 = **Noninsulin-dependent diabetes mellitus (NIDDM)**
- **Most common form of diabetes** → 90-95% of the diabetic population
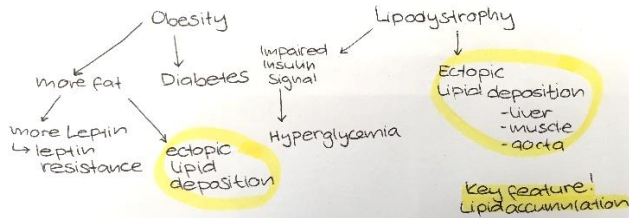- Most investigators agree that **genetic factors underlie Type 2 DM**

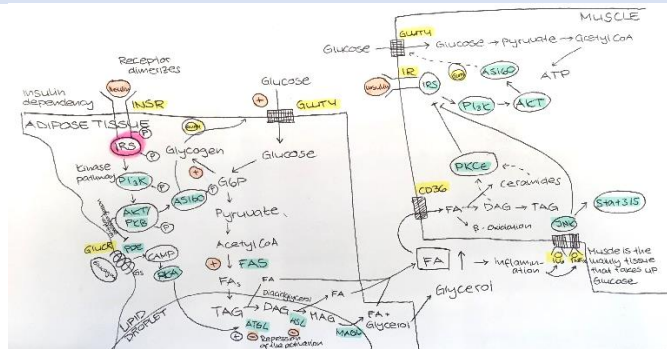### ETIOLOGY OF TYPE 2 DIABETES: INSULIN RESISTANCE AND DIMINISHED INSULINE SECRETION

1. Insulin Resistance (not yet diabetes = high blood sugar levels)
- Pancreas produces more insulin
- High insulin, normal blood sugar levels
- Normal β-cell function
2. β-cells get exhausted
- dysfunction → relative insulin deficiency → hyperglycemia → type 2 diabetes

CONNECTION BETWEEN OBESITY AND DIABETES

ADIPOSE TISSUE AND MUSCLE

ADIPOSE TISSUE:

Insulin: Is an endocrine hormone → Has a receptor signalling pathway followed by a kinase pathway

What happens with glucose in the adipose tissue?
- Glucose gets metabolized to Triacylglycerols and stored in lipid droplets

How is the glucose uptake regulated?
→ via Insulin:
1. **Insulin** binds to Adipocyte Insulin Receptor
2. **IRS** gets mobilized and phosphorylated through dimerization of the INS-R
3. **IRS** → PI3K → AKT (=PKB) →AS160
4. AS160 activates GLUT4 transport to the membrane → Enhances glucose uptake

How is the glucose metabolism regulated by insulin?
- Insulin activates glucose metabolism via
  o Glycogen synthesis: G6P → Glycogen
  o Lipid synthesis: Acetyl CoA → Fatty acids via the **FAS enzyme** → Triacylglycerides in stored lipid droplets

What happens with the TAG in the lipid droplets?
- Insulin inhibits ATGL and HSL over inhibition *(activates?)* of PDE (AKT phosphorylates & inactivates *(activates?)* PDE → no cAMP → inactive PKA → no ATGL activation) and therefore inhibits the TAG breakdown to FA and glycerol
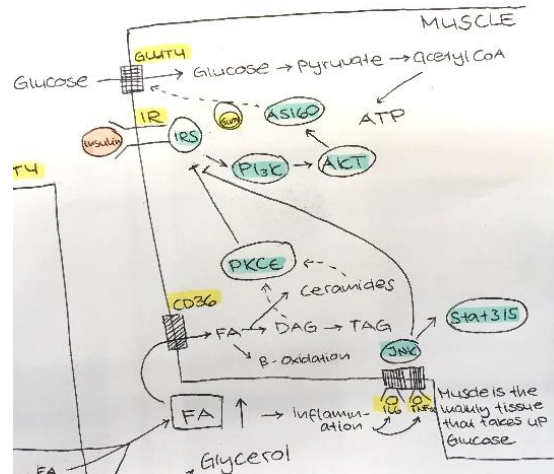
- Glucagon activates *(inhibits?)* PDE → cAMP → PKA → activates ATGL → leads to TAG breakdown to FA and glycerol which both get exported
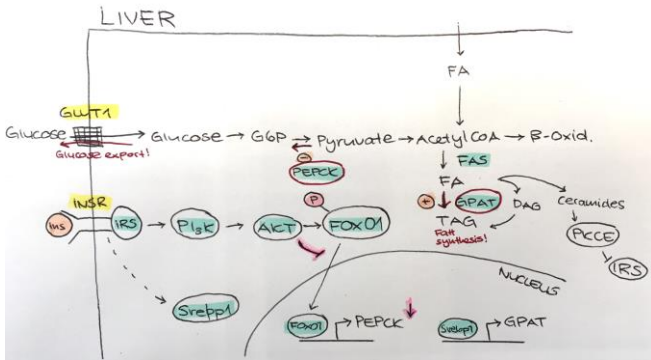*#This makes no sense; PDE should lower cAMP levels!!*

MUSCLE:

How is the glucose uptake regulated?
- Same as in the adipose tissue (via IRS and GLUT4)
What happens with glucose in the muscle?
- Glucose is mainly used for **ATP production**
What happens with FA in the muscle?
- FA are taken up from the system (mainly from adipose tissue) through the CD36 transporter and further processed to Triacylglycerides or used via β-Oxidation
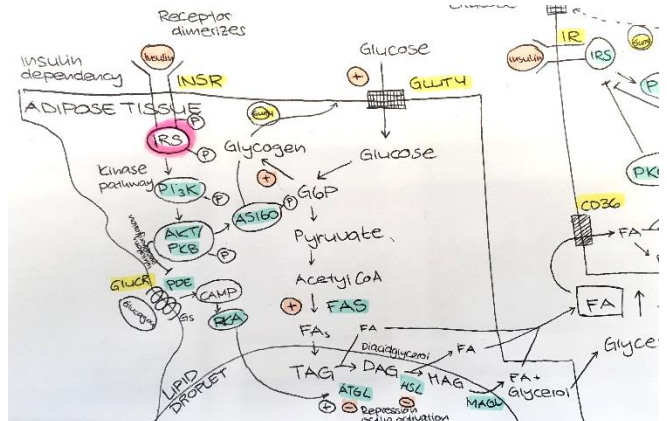
REGULATION MECHANISMS:
There exist two feedback regulation mechanisms in the muscle for glucose uptake

1. Lipids vs glucose pathway:
   → Feedback mechanism via PKCɛ
   → Prohibiting uptake of too much nutrients
Muscle doesn't want to take up glucose if to many FA/TAG are stored → If there are a lot of FA/TAG, the muscle wants first to use up the lipids before taking up more glucose
- Excess of FA/TAG in the muscle → Activation of PKCɛ → Inhibition of IRS = Block insulin signalling → Insulin pathway doesn't work properly anymore (no GLUT4) → Impaired glucose uptake → Hyperglycaemia

What happens if you have a long time lipid overload
FA/Lipids can lead to **subchronic inflammation**
→ Cell reacts to an overload of lipids by inflammation

2. Inflammatory pathway:
   → Longterm pathway
If you have **more FA** → You have **more inflammation**
→ **TNF-α and IL6** are very important **inflammatory cytokines**
- Inflammatory cytokines lead to JNK activation → JNK inhibits IRS and therefore **inhibits glucose uptake** in the muscle
Why does the muscle reacts to inflammation by decreased glucose uptake?
- Immune cells need ATP as energy source and thus are dependent on glucose → system inhibits glucose uptake in the muscle, so that the immune-cells have enough glucose and therefore energy to work properly

LIVER:

- **Akt** phosphorylates & inactivates **FOX01** to prevent it from translocating into the nucleus and activating **PEPCK genes expression**
  Akt → inactive FOX01 → PEPCK ↓

- Under normal condition, glucose is taken up through **GLUT1** (insulin independent) and gets further metabolized

What happens, if there is too much glucose e.g. to much FA/TAG in the cell?
Too much glucose/TAG in the cell → Overflow of substrates (Ceramides, DAG) → activation of **PKCε** → inhibition of **IRS** = inhibition of Insulin signalling → no Akt → Fox01 is active, translocates into the nucleus and activates expression of PEPCK → **PEPCK** ↑→ Gluconeogenesis/Production of glucose → release of glucose into the system
OUTCOME: Cell get rid of energy in the cells by producing and pushing out glucose
➔ LIVER gets insulin resistant and starts to produce and export glucose

FAZIT: All cells try to safe themselves from high toxic glucose concentration → Muscle doesn't takes up glucose anymore, liver produces glucose and secretes it

What is the connection with Srebp1?
- Lipid angle is **not** insulin resistant (→ selective hepatic insulin resistance = lipid synthesis is not uncoupled)
  → Srebp1 is still working → liver will not only **produce glucose** but also **produce lipids** → Fatty liver

ADIPOSE TISSUE INSULINE RESISTANCE
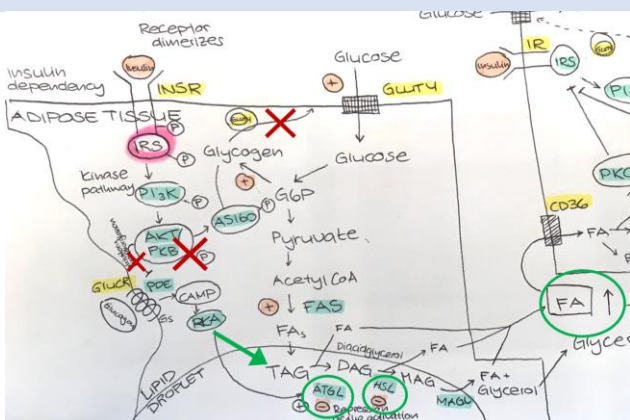
What happens to glucose in the adipose tissue if it becomes resistant?
- Glucose isn't taken up anymore
What will happen to the lipids by an insulin resistance?
- Lipolysis will be upregulated → more fatty acids in the serum → cause insulin resistance to the other organs

- Adipose tissue is actually the **first tissue/organ to become insulin resistance** → spillover of FA → muscle and liver develops an insulin resistance → impaired glucose uptake and further production/secretion of glucose in the liver leads finally to **Hyperglycemia** → Diabetes is formed by cascade → progressing
- Furthermore, adipocytes themselves can produce inflammatory cytokines → protection from excess lipids → protects itself from getting insulin resistance

DRUGS TARGETS

For diabetes: You want to decrease glucose levels
Concept: You want to **increase insulin sensitivity**
- **Increasing insulin secretion** or **give extra insulin**
- **Increasing insulin sensitivity** in the liver and muscle
- **Inhibition of FA secretion** by adipose tissue
  You don't want lipolysis to occur!
Metformin: Multi drug target → inhibits PKCε
- Increases insulin sensitivity in the liver which leads to reduced glucose output
- Increased glucose uptake in the muscle
Glitazones:
- Activates **PPARγ**
  o PPAR8 is one of the strongest regulator of lipolysis
- Reduces FA spill-over
- **Side-effects**
  o Overweight → increase in fat mass → Push all the fat into the adipose tissue
  o Heart droplets and heart failure: PPAR8 also exists in the heart → fat accumulation in the heart
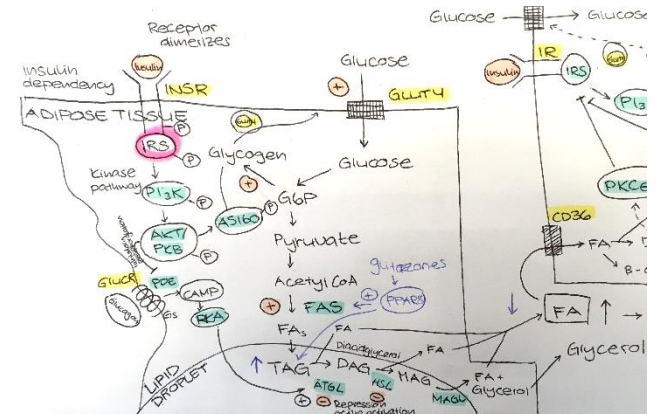
QUIZ:

|	Richtig	Falsch	
Insulin inhibits glucose output from liver by inhibiting gluconeogenesis.	☑	☐	✓
Insulin induces glucose uptake in muscle.	☑	☐	✓
Insulin induces fatty acid release from adipose tissue by activating lipolysis.	☐	☑	✓
Insulin inhibits glucose uptake into adipose tissue.	☐	☑	✓
Insulin inhibits hepatic gluconeogenesis by reducing PEPCK expression.	☑	☐	✓
Insulin stimulates muscle glucose uptake through Glut4.	☑	☐	✓
Liver is stimulated to take up glucose through induction of Glut1	☐	☑	✓
Insulin inhibits adipose tissue lipolysis by blocking HSL function.	☑	☐	✓
Insulin resistance can be induced by inhibitory phsophorylation of Irs proteins.	☑	☐	✓
Insulin resistance can be induced by inflammatory signals.	☑	☐	✓
Ceramides and DAGs can induce insulin resistance by activating PKC isoforms.	☑	☐	✓
TNFa activates IRS activity.	☐	☑	✓

Explain in a few sentences the physiological changes in glucose levels in a patient with an inhibitory mutation of the PEPCK gene in the fasted and the fed state.

Fed state: GLucose -> insulin is up -> insulin inhibits PEPCK and therefore gluconeogenesis
PEPCK is inactive anyway and therefore the mutation does not have any impact -> normal increase in glucoselevels

Fasted state: Glucose goes down -> insulin goes down -> Gluconeogenesis should appear but cannot function properly -> blood sugar levels stay low -> hypoglycemia

HYPERTENSION

= High blood pressure
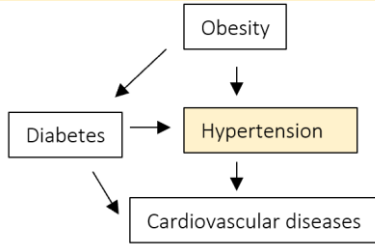
Hypertension is one of the main drivers of CVD → the higher your blood pressure, the higher is the risk of CVD
- It's not even clear whether obesity drives hypertension

FACTORS INFLUENCING THE BLOOD PRESSURE:

3 main driving forces:
- Vascular Resistance (diameter changes)
- Blood volume
- Cardiac output

DEFINITION OF BLOOD PRESSURE

- **Systolic blood pressure =** Contraction of the heart (Out of the heart)
- **Diastolic blood pressure** = Pressure into the heart

| Systolic blood pressure		Diastolic blood pressure
< 120	Normal	< 80
120-140	Pre-hypertension	< 90
140-160	Hypertension stage I	< 100
> 160	Hypertension stage II	> 100

PRIMARY AND SECONDARY HYPERTENSION

- **Primary Hypertension**: Without other disease
  - o Nearly 90%
  - o If you have obesity, it's primary! (obesity is not a disease) → Multifactorial diseases: We are not 100% sure why hypertension develops
- **Secondary Hypertension**: Consequence of another disease
  - o 10%

Why is it important to distinguish between this two?
In primary hypertension we don't really fully understand what drives the hypertension → Otherwise we know exactly what the cause is and how to treat it

FACTORS DRIVING HYPERTENSION

- Environment
- Genetics (high degree of heritability)
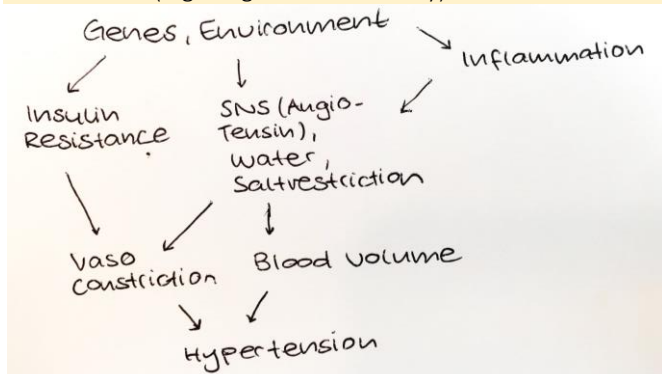

WHY DO WE NEED HIGH PRESSURE?

Evolutionary: If a lot of energy is needed

ASSOCIATED RISKS OF HYPERTENSION

- Hypertension causes **endothelial stress** due to too high blood pressure
  - o Can lead to stroke (blood vessels break)
- Promotes **plaque deposition** (beacause the damage allows infiltration of specific cells which leads to deposistion)
  - o Can lead to **cardiac infarctions**
  - o **Enhances pressure** further→ again leads to endothelial stress (cycle!)
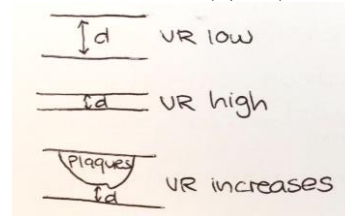
- Blood pressure drives **cardiovascular diseases**

### MECHANISM/REGULATION BEHIND HYPERTENSION
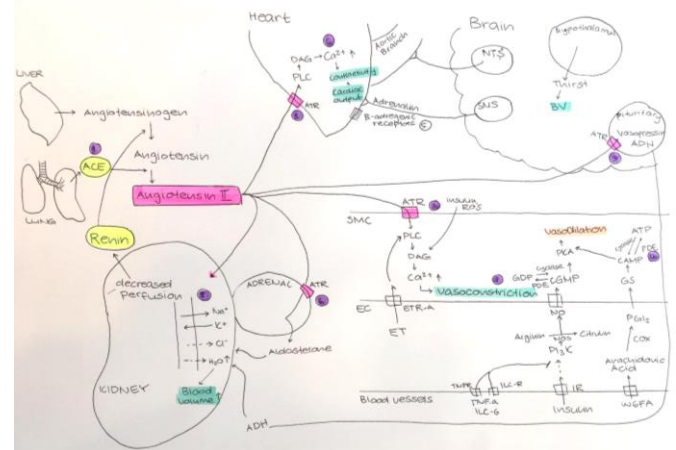
- **Blood volume** (Increased water re-absorption in the kidney leads to increase of circulating blood and thirst from hypothalamus)
- **Vasoconstriction** (smooth muscle cells)
- **Contracting & Cardiac output** (heart)

How to sense the blood pressure?
- Pressure system sits in aortic branch
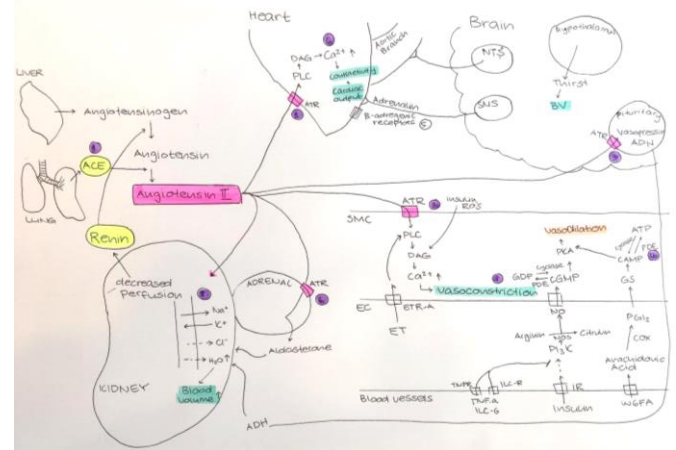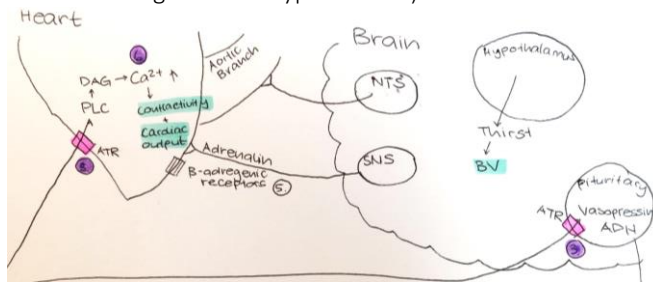- Decreased perfusion in the kidney

ANGIOTENSIN SYSTEM:

- Requires two proteases: ACE (lung) and Renin (kidney)
- **Angiotensin II** regulates a lot of proceses in different organs
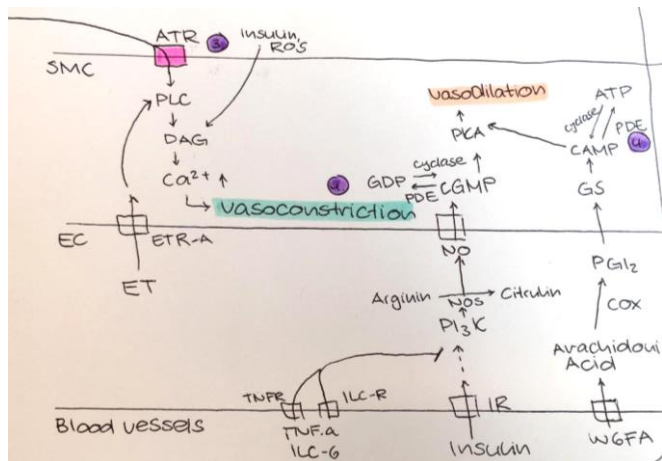 - o Direct **binding interaction with ATR** (receptor)
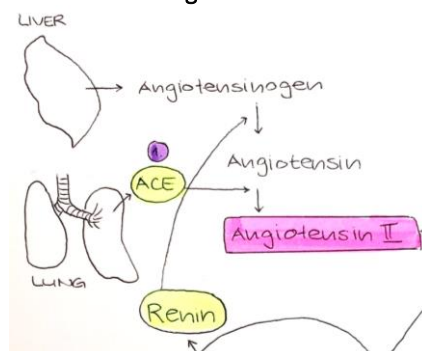
HEART AND BRAIN

- SNS = sympatric nerve system
  - o Can get activated upon stress (partial link to a driving factor of hypertension)
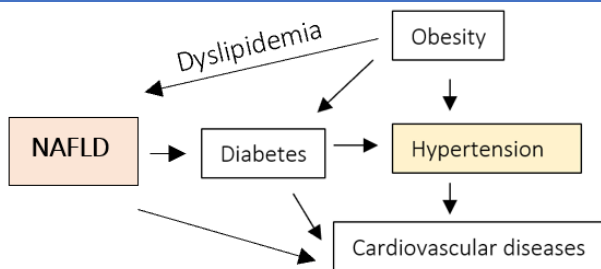

BLOOD VESSELS

- TNF-α and IL-6 → Inflammation cytokines: Inhibit insulin signaling (insulin resistance) → cannot induce vasodilation anymore
- Most of the pathology comes from the endothelial cells (EC) and smooth muscle cells (SMC)
- Insulin resistance: Main drivers for insulin resistance are lipid accumulation and inflammation

DRUGS TO PREVENT HYPERTENSION

- Different treatments act on different organs
1. **Reduce amount of Angiotensin II** → inhibit Renin & ACE
   - o Blockage of proteases → can turn off the signal very fast since Angiotensin II has a really short half-life
   - o ACE inhibitors → would lead to reduced formation of Angiotensin II which means less water retention, less cardiac output, less vasoconstriction and indirectly blood volume control
2. **Diuretics**
   - o Water will not be reabsorbed → decrease blood volume (block Na^+ and thus water retention)
3. **Block Angiotensin II-ATR interaction**
   - o Direct binding (Angiotensin II to ATR)
4. **Inhibit PDE** → longer half-life of cGMP → leads to vasodilation
5. Anti-stress response → **Block all the SNS-output** (Beta-blockers)
6. **Calcium channel blockers**
   - o Act directly on the heart

*ET= Endothelin, *W6FA= omega-6 FA, *PGL₂= Prostaglandine
(Angiotensin II promotes Na⁺ retention, Aldosterone Cl⁻ retention and ADH water retention and thirst by hypothalamus → all leading to blood volume increase)

NON ALCOHOLIC FATTY LIVER DISEASE

- Don't' mix it with the alcohol liver disease! Alcohol will lead to a fatty liver for sure

TASKS OF THE LIVER IN THE BODY

- Detoxification
- Glucose homeostasis
- Hämoglobin homeostasis
- Cholesterol/sterol metabolism
- Portal and enterohepatic circulation

PORTAL CIRCULATION

- Hepatic portal system which directs **nutrient rich blood from the intestines to the liver**
- Liver also drains venous blood from pancreas
- The liver has highest insulin levels of all organ systems

ENTEROHEPATIC CIRCULATION
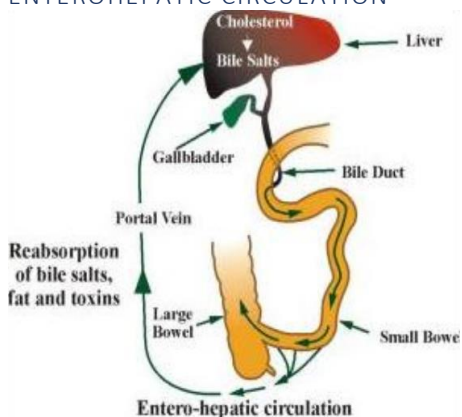
- Hepatocytes metabolize **cholesterol** to **cholic acid and chenodeoxycholic acid**
- These lipid-soluble bile acids are conjugated mainly to glycine of taurine to form water soluble primary conjugated bile acids
- 95% of the bile acids which are delivered to the duodenum will **reabsorbed in the ileum** and recycled by the enterohepatic circulation
- 5% are lost in the feces
- Bile acids form micelles with ingested lipids and are important for lipid absorption
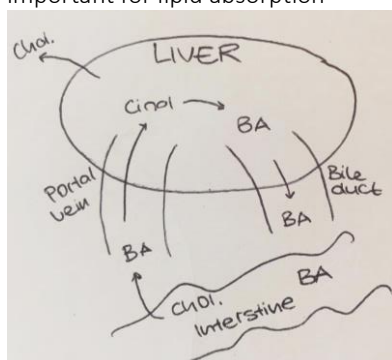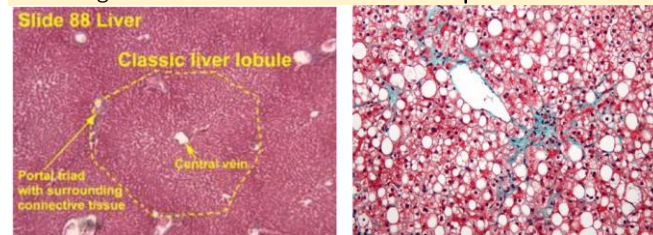

NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

Describes a spectrum of **steatotic liver disease** generally associated with **obesity** and **metabolic syndrome**
HTN, dyslipidemia, increased waist circumference, insulin resistance

➔ Progression of **liver diease** based on **lipid accumulation**

Healthy liver left, NAFLD liver right

- Fat deposition (steatosis) in the liver (Lipid accumulation!)
  - o Not due to excessive alcohol use
- Related to **insulin resistance** and the **metabolic syndrome** ➔ There is probably already a insulin resistance but it can also be a driver of diabetes
- Can be obesity related but doesn't have to be!
  ➔ Is a disease by itself!
- May respond to treatments for **T2D, weight loss, metformin and thiazolidinediones** (βγ-activator ➔ pushes lipids in adipose tissue)
- Genetic forms exist

SIGNS AND SYMPTOMS

- Most patients with NAFLD have **few or no symptoms**
- Patients may complain of **fatigue**, can exhibit dull right-upper-quadrant abdominal discomfort
- Mild **jaundice** *(Gelbsucht)* may be noticed (although rare)
- More commonly NAFLD is diagnosed following **abnormal liver function tests during routine blood tests**
  - o By definition, alcohol consumption of over 20 g/day (about 25 ml/day of net ethanol) **excludes the condition**

NAFLD is associated with **insulin resistance** and **metabolic syndrome** (obesity, combined hyperlipidemia, diabetes mellitus (type II) and high blood pressure)

PREVALENCE OF NAFLD

- Most common pediatric chronic liver disease in US and globally (2.6% - 9.6%)
- The prevalence of non-alcoholic fatty liver disease ranges from **9 to 36.9% of the population** in different parts of the world
- Approximately **20%** of the United States population suffers from non-alcoholic fatty liver, and the prevalence of this condition is increasing
- More common in boys vs. girls

RISK FACTORS ASSOCIATED WITH NAFLD

Conditions with established association	Conditions with emerging association
Obesity	Polycystic ovary syndrome
Type 2 diabetes mellitus	Hypothyroidism
Dyslipidemia	Obstructive sleep apnea
Metabolic syndrome	Hypopituitarism
	Hypogonadism
	Pancreato-duodenal resection

PATHOPHYSIOLOGY OF HEPATIC STEATOSIS
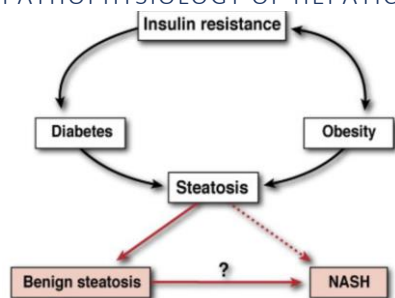
PROGRESSION OF FATTY LIVER DISEASE

- Is a complex disease
- Consider it as a progression:

1. **Nonalcoholic fatty liver (NAFL):** Progression of hepatic steatosis (lipids in the liver) with no evidence of hepatocellular injury
2. **Nonalcoholic steatohepatitis (NASH):** Advanced form; Presence of hepatic steatosis with hepatocellular injury (liver damage) (ballooning) with or without **fibrosis**
3. **Cirrhosis**: Extreme fibrosis, scarred liver, non-functional: Hepatocellular Carcinoma
- Once the liver has reached the stage of cirrhosis, the only chance left is a liver plantation (non-reversible)
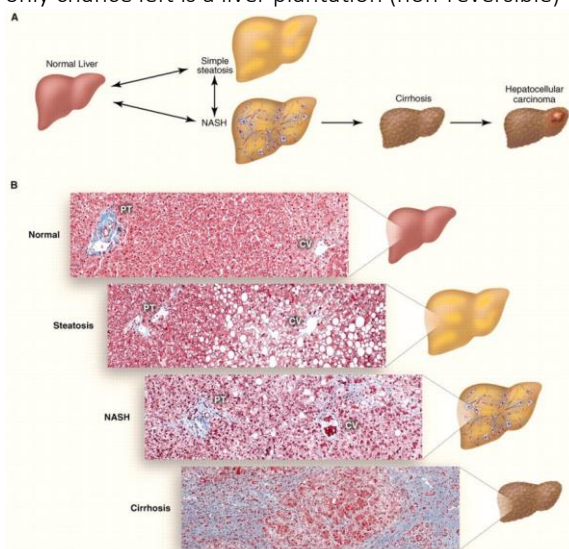
How do we progress to NASH?
➔ **"Two Hit" Theory**
- The first "hit" involves the **accumulation of fat** in the hepatocytes (lipid partitioning)
- Subsequent "hits" involve:
  o Chronic oxidative stress with the production of Reactive oxygen species (ROS)
  o Secretion of pro-inflammatory cytokines
  o Mitochondrial dysfunction
  o Liver injury, hepatic apoptosis (liver cell death) and fibrosis

INFLAMMATION AND FIBROSIS

- **Pro-inflammatory cytokines (TNF-α)** are produced directly by hepatocytes in response to an **increased supply of FFA** and/or by adipose tissue **macrophages** that increase during obesity
- Fibrosis is thought to arise as part of the normal healing response to **inflammation and injury**

CYTOKINES AND OXIDATIVE STRESS

- **Adiponectin**: Inversely associated with obesity, BMI, metabolic syndrome, visceral adiposity, NAFLD
- **IL-6:** Implicated in insulin resistance, NASH
- **TNF-α:** Elevated levels with insulin resistance, metabolic syndrome
- **CRP**: May be a marker for hepatic steatosis --- but not of severity of NAFLD

GENETIC DETERMINANTS OF HEPATIC STEATOSIS

Genetics may play an **important role in etiology of NAFLD**, particularly at early onset disease
- Hepatic lipid export (MTTP, ApoB)
- Hepatic lipid uptake (APOCIII)
- Hepatic lipid synthesis (DGAT2, SLC25A13)
- Insulin resistance (AKT2, ADIPOQ, IRS1, PPARs)
- Hepatic TAG hydrolysis (ATGL, LIPA)
- FA oxidation disorders (MCAD, LCAD, VLCAD)
- Lipodystrophies (LMNA, PPARg)

GENE POLYMORPHISMS ASSOCIATED WITH NAFLD

- Several gene polymorphisms are associated with NAFLD & genes that influence:
  o Insulin signaling and regulation of fat metabolism
  o Oxidative stress
  o Responses to endotoxins
  o Release of cytokines
  o Severity of fibrosis
- Genetic factors may also **predispose** certain individuals **to environmental influences** that promote the development of NAFLD
- Use of genetic analysis and genotyping has the potential to become an important noninvasive tool for the screening and diagnosis of NAFLD

DIETARY CHARACTERISTICS

- Over-consumption of **fructose & soft drinks**
- Lower consumption of **fiber**
- Over-consumption of **meat/saturated fat/cholesterol**
- Lower consumption of **fish**, **omega-3 fatty acids**, and **some vitamins (vitamin E)**

ROLE OF CHO:

- **Sucrose**: Increases hepatic TG synthesis
- **Fructose**: Increases de novo lipogenesis (DNL) & insulin resistance in animal models

Fructose overfeeding **increases fasting and postprandial plasma TG** ➔ hepatic DNL, VLDL-TG secretion & decreased VLDL-TG clearance

A possible explanation: Insulin resistance and hyperglycemia develops primarily in presence of sustained fructose exposures associated with changes in body composition
- **Sugar sweetened beverages:** Increased soda consumption in US children and adults
  o ~175 calories/day
- **Caramel colouring**: Contains advanced glycation end products which can increase insulin resistance and inflammation
- **Polyunsaturated n-3 and n-6 fatty acids:**
  o Animal models: Reduction of steatosis
  o Studies in adults with NAFLD: Improved lipid profiles, reduced inflammation
  o 2 gm fish oil (6 mos, n=40 adults) reduced serum TG, liver enzymes, and TNF-α; regression of steatosis

CLASSIFICATION

Features

Histological	Anthropometric Findings
Classification of steatosis	Overweight, obesity, abdominal obesity
Biochemical Insulin resistance, Hypertriglyceridemia, elevated ALT	**Clinical Features** Acanthosis nigricans
	Diagnostic Imaging MRI, CT, ultrasound

ANTHROPOMETRIC FEATURES

Overweight
BMI >95th percentile

Abdominal obesity
WC > 90th percentile

Obesity
BMI >95th percentile

CLINICAL FEATURES

Acanthosis nigricans
Hepatomegaly on palpation

HISTOLOGICAL

- **NAFLD**: Presence of hepatic steatosis with no evidence of hepatocellular injury in the form of ballooning of the Hepatocytes
- **NASH**: Hepatic steatosis and inflammation with hepatocyte injury (ballooning) with or without fibrosis
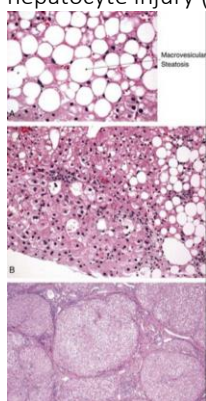
Spectrum of disease in NAFLD

A: Nonalcoholic fatty liver (NAFL)

B: Nonalcoholic steatohepatitis (NASH)

C: Cirrhosis

- Histological is the <u>only way</u> **to grade:**

Steatosis Grades

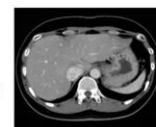| Grade 0 (normal)	up to 5% of hepatocytes affected
Grade 1 (mild)	5 - 33% of hepatocytes affected
Grade 2 (moderate)	34 - 66% of hepatocytes affected
Grade 3 (severe)	≥ 67% of hepatocytes affected

DIAGNOSIS

- **Liver biopsy**: Invasive, risks, expensive
- **Diagnostic Imaging:**
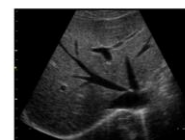
Computerized Tomography (CT)
- exposure to ionizing radiation
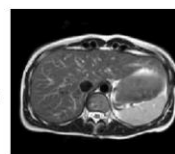

CT image of the Liver

Ultrasound
- accessible, no ionizing radiation exposure
- low sensitivity: mild-moderate steatosis
- limited beam penetration in obese individuals
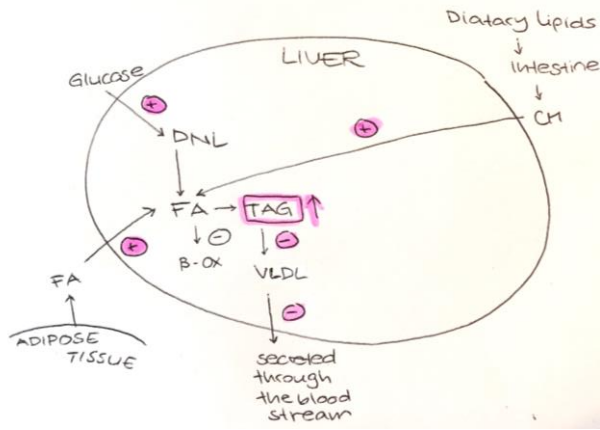

Ultrasound image of the Liver

Magnetic Resonance Imaging (MRI)
- no exposure to ionizing radiation expensive!

MRI of the Liver

BIOCHEMICAL FEATURES

Elevated serum TG
> 150 mg/dL

Elevated ALT
- **No universal standards**
- 30-45 U/L most commonly used cutoff for abnormal ALT
- ALT may be **normal**
- Current standards controversial—range may be too high

- ALT is a marker for liver damage
- Not a clear biochemical definition
- Insulin resistance:
  - Homeostatic Model Assessment Insulin Resistance (HOMA- IR):

$$\frac{\text{fasting glucose (mg/dl)} \times \text{fasting insulin (µU/ml)}}{405} \quad \mathbf{>3}$$

TAG IN THE LIVER

Where can the liver get lipids from (uptake/production)?
- Liver takes up **fatty acids** that are secreted from the adipose tissue
- Glucose uptake followed by **de novo lipogenesis**
- **Diatarry lipids** → Interstine → Chylomicrons → fueling the liver lipid cycle

How does the liver get rid of lipids (secretion/consuption)?
- FA → TAG → **VLDL secretion** in the blood stream
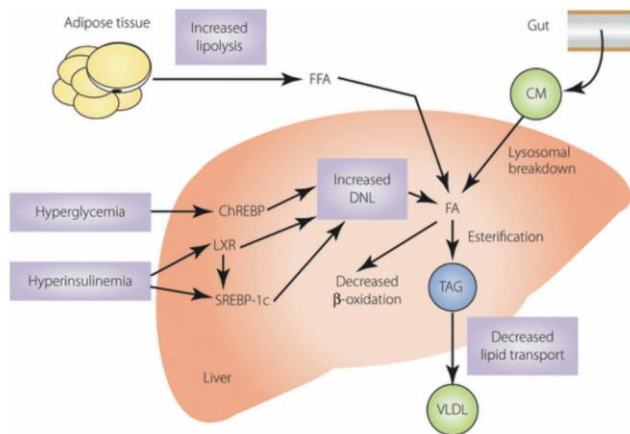- FA → **β-oxidation**

How can lipids accumulate (TAG↑)?

If lipid uptake/production is enhanced (+) or liver secretion/consumption is inhibited (-)
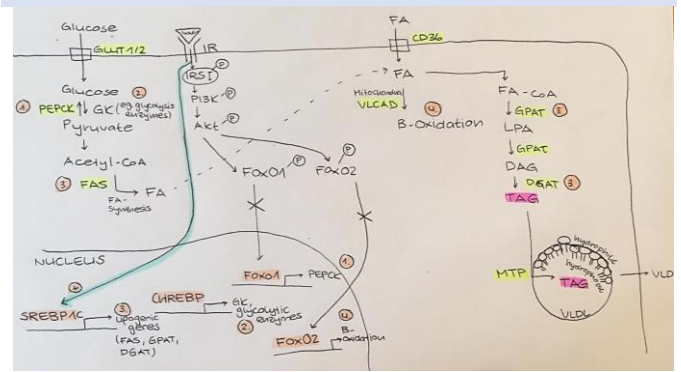- ➔ There are 3 major sources for the increased TG deposition in the liver:
  - o **Rate of FFA uptake and synthesis** (Need for FFA for essential functions)
  - o **Impaired VLDL export**
  - o **Increased de novo lipogenesis (DNL) synthesis of FA from CHO in the liver**

LIPID HOMEOSTASIS IN THE LIVER

LIPID METABOLISM IN THE LIVER


Lipid/TAG production:
- Glucose uptake through GLUT1/2 → Pyruvate → Acetyl-CoA → FA
- FA acid uptake mainly from adipose tissue → FA-CoA → LPA → DAG → TAG

LIPID/TAG consumption:
- FA → **β-oxidation**
- TAG export → Packed into VLDL
  - o Lipids are rarely free in the blood → are very hydrophobic → are transported in lipoproteins

TRANSCRIPTOR FACTORS
- 4 trancriptor factor that regulate this system
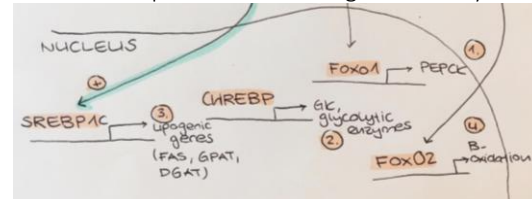
- **Fox01** → Transcription factor for PEPCK (gluconeogenesis enzyme)
- **Fox02** → Transcriptionfactor for β-oxidation
- **CHREBP**: Carbohydrate responsive element-binding protein → Transcription factor that responses to glucose → GK and other glycolytic enzymes
- **SREBP1c**: Sterol regulatory element-binding protein 1c → Transcriptionfactor for genes required for de novo lipogenisis
  - o SREBP is a key regulator in hepatic fat metabolism

INSULIN SIGNALLING:
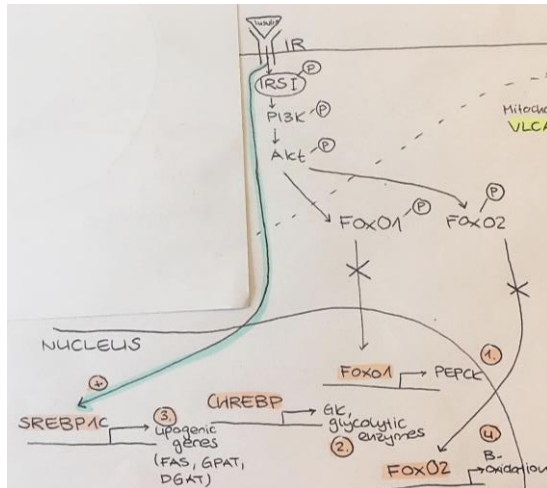
- Meal with glucose → insulin signaling
- Insulin binds to IR → IRS1 gets phosphorylated → PI3K gets phosphorylated → Akt gets phosphorylated → phosphorylates Fox01/2 thus inhibiting them from entering the nucleus (inhibition of gluconeogenesis and β-oxidation)
- Insulin positively stimulates SREBP1c → pushes lipgenesis

Insulin signalling inhibits gluconeogenesis (phosphorylates FoxO1 → no PEPCK transcribition) and shifts the glucose away (lipogenesis) via SREBP1 stimulation
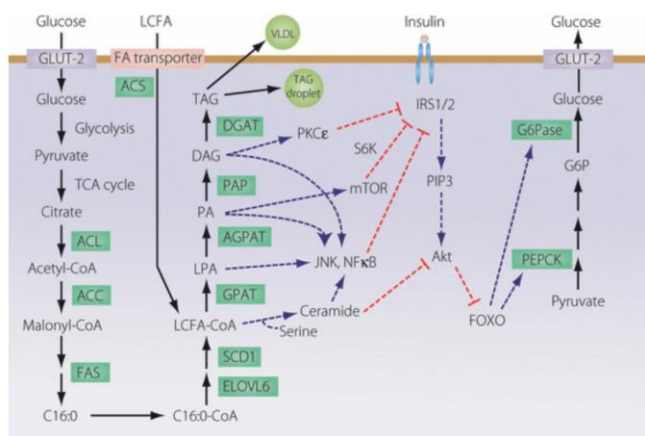
INSULIN RESISTANCE:

1. High levels of glucose
2. Insulin signaling doesn't work anymore
3. Foxo1/2 gets active → increased β-oxidation
- **Hypothetical**: Less SREBP1c → Less lipogenesis → Thus we would not expect any lipid accumulation in the liver!

How does lipid accumulation occur in the liver in NAFLD?
- FA are coming from the adipose tissue → Huge amount/ influx → sufficient even if the pathways are downregulated
- Insulin resistance doesn't happen to all pathways
- → Selectiv hepatic insulin resistance

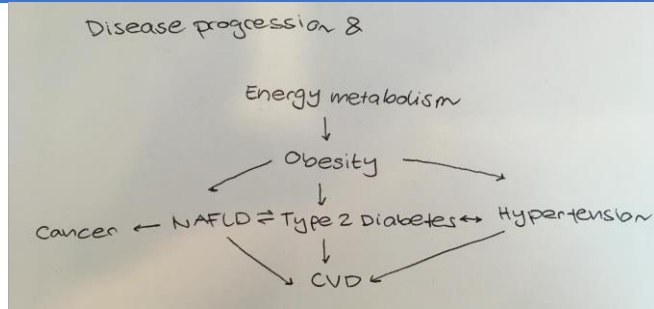## SELECTIV HEPATIC INSULIN RESISTANCE

- **Selectiv hepatic insulin resistance:** SREBP1c pathway remains active while the glycolysis pathway (Fox01/2) is turned off → The liver pushes everything that is left and that is entering into lipogenesis

| Normal liver Metabolism	Type 2 Diabetes - selective insulin resistance
1. Dietary glucose 2. Insulin production: Phosphorylation of Fox01 → decrease of gluconeogenesis → decrease in [glucose] - Activation of Srebp1c → Induce TG → secretion in blood → fat and muscle uptake - Insulin induces glucose uptake in the muscle and fat	- Fox01 is NOT phosphorylated → gluconeogenesis → glucose gets produced and secreted - Insulin still pushes the SREBP-1c pathway → TG are formed and get enriched in the blood - Glucose is not sufficiently taken up from muscles and fat

QUIZ:

LIPID METABOLISM AND CARDIOVASCULAR DISEASE

IMPORTANCE:

Age standardized death rate:
- Cancer had its peak at the end of the 1990 → is now declining
- Cardiovascular disease has increased!
  - o Declined a bit on the end of 1990 (medication)
  - o than increasing
 - o Now again decreasing (medication, treatments)
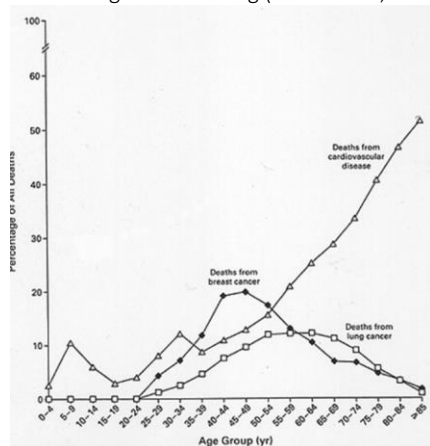
CARDIOVASCULAR DISEASE OVERVIEW

- Refers to diseases of the **heart** ("*cardio*") and **blood vessels** ("*vascular*")

Typically affects (one or both):
- The ability of the **heart to pump**
- The ability of the **blood vessels to deliver blood**
  - o Arteries bring oxygenated/nutrient rich blood to where it is required
  - o Coronary arteries provide the heart with blood
  - o If this is not working → **Necrosis**

CARDIOVASCULAR SYSTEM
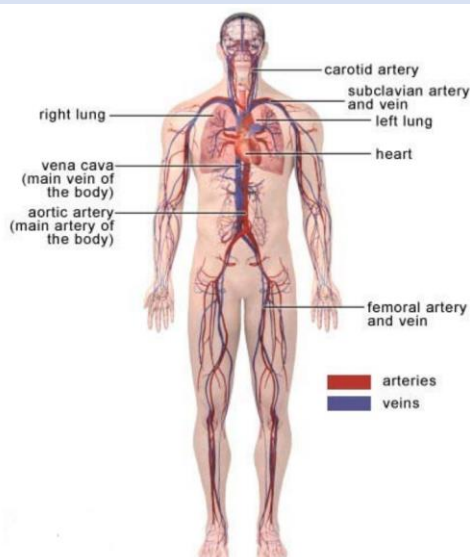
CV: we can have a problem everywhere in this system!

MAJOR FORMS OF CARDIOVASCULAR DISEASE (CVD)

- **Atherosclerosis**: Progressive **narrowing of the arteries** typically caused by fatty deposits (ongoing chronic form)
- **Coronary Artery Disease (CAD)/ coronary heart disease (CHD)**: Atherosclerosis of the coronary artery → effecting especially the heart → disfunction of the heart → can lead to heart failure
- **Heart Failure**
- **Hypertension** (high blood pressure)
- **Cerebrovascular disease** (Stroke)

ATHEROSCLEROSIS

HISTORY

- **1883** (*Lobstein JF*) Arteriosklerose: Alterung und hämodynamisch bedingte Veränderungen der Arterienwand, an erster Stelle die **Kalzifizierung** (Heute: Nicht Kalziumablagerung!)
- **1904** (*Marchand F.*) Atherosklerose: Bezeichnet die Arteriosklerose begleitende **Intimaverfettung** der Arterienwand (von Verkalkung zu Verfettung √)

PROGRESSION

Healthy artery

Lesion

Smooth muscle cells

Plaque filled with ox-LDL, debris, calcium and platelets
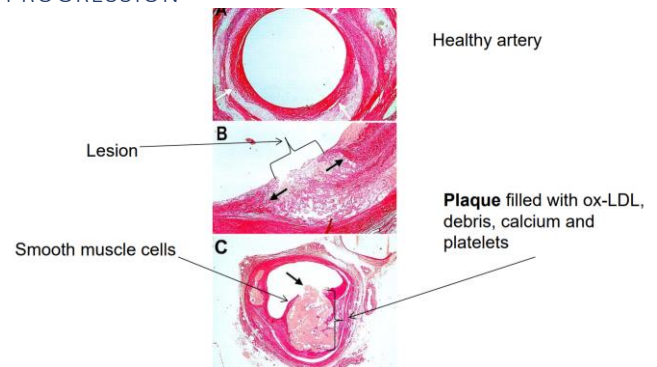
Hypertension or something else leads to rupture of endothelial layer → **Infiltration of lipids and inflammatory cells** which in long term lead to plaque formation
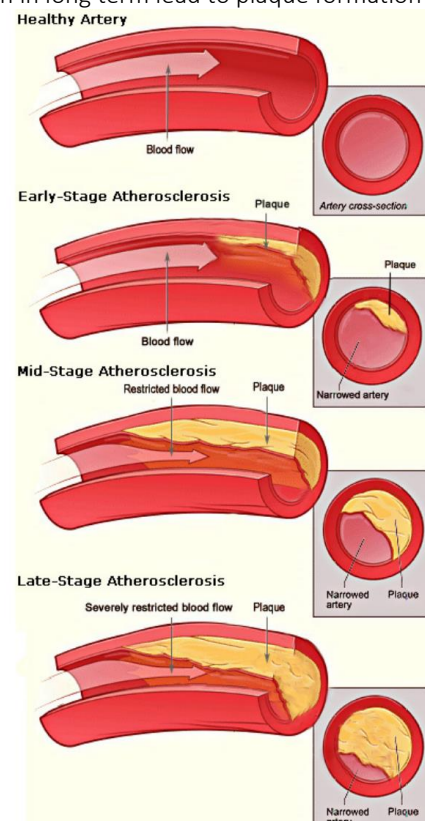
- Artery progression is asymptomatic
  o Only complete blocking will lead to symptoms
- Unstable plaque → plaque-rupture → gets stuck in the already narrow artery → acutely blocks it completely
  o Can also be a blood clot or sth. else that gets stuck

CHOLESTERIN DEPOSITION IN VESSEL

- Plaques are lipid depositions!
  o Contain on the long term cholesterol crystals
- One driving force are the **foam cells** = Macrophages
  o don't fulfil their normal role as protector of pathogens, but now protect the body from the lipids → Respond that is physiological becomes pathophysiological over time

Atherosclerosis   Cholesterin Crystals   Foam Cells

MAJOR CONSEQUENCES OF ATHEROSCLEROSIS

1. Atherosclerosis can **reduce the elasticity** of the arteries, making them less able to respond to demand and putting more strain on the heart
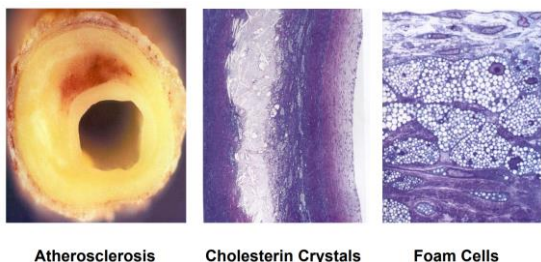2. Atherosclerosis can cause an **aneurism**, a dilation of the artery, which can eventually rupture, leaking blood into the surroundings (**haemorrhage**)
   o Artery cannot flex anymore and instead breaks
3. Atherosclerosis **reduces blood flow** and can completely **block blood flow** if a **thrombus** (blood clot) gets lodged there

TWO MAIN DISEASES

MYOCARDIAL INFARCTION (MI)

- **Myocardial Infarction (heart attack)** can occur when there is an absence of blood flow to the heart
- This is most often caused by **coronary artery disease** (atherosclerosis of an artery in the heart)
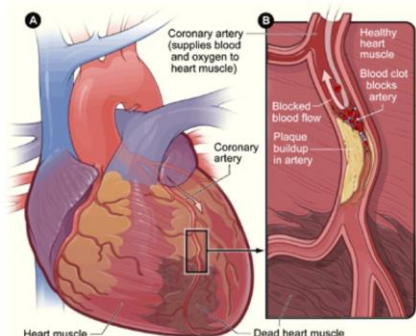
STROKE

Ischemic strokes:
- (**80%**) Caused by lack of blood flow to the *brain*
- Typically due to **thrombus + atherosclerosis**
1. **Thrombotic stroke:** Thrombus starts in artery near brain
2. **Embolic stroke:** Thrombus develops somewhere else in body and travels to the brain

Transient ischemic attack (TIA):
- Is caused by a ***temporary* disruption of blood flow** to brain; 'mini-stroke'; warning sign
  o Happens if there is a small clot → short time blocking or only partial blocking
    → higher pressure will flush thrombus away
  o Aspirin inhibits blood clotting

Hemorrhagic stroke:
- (**20%**) Caused by **uncontrolled bleeding in the brain**
  o Disrupts normal blood flow, kills brain cells
  o Can be caused by weakness of the artery wall
- **Aneurysm:** Weakened vessel full of blood
- (**Arteriovenous malformation (AVM):** Malformed blood vessels in brain that make the artery weak; typically present at birth)

LIPIDS AND THEIR TRANSPORT

- **Lipid accumulation** as primary factor
  o Lipid deposition happens thereby outside the cell

How do lipids circulate in the body?
- **Lipoprotein:** Structure with hydrophilic outer layer (phospholipid) and hydrophobic core
- Core is transporting:
  o **Cholesterol/ Cholesterol esters**
  o **TAG**
- (Fatty acids are the only lipids that can be excreted in the blood without being transported in a lipoprotein!)

How would the body know what exactly is transported?
There are a lot of proteins attached to lipoproteins
- **Apolipoproteins** (*"no lipids", are proteins*)
  o Main function: Signalling → Tells the rest of the body what is carried inside
  o **Lipoprotein receptors** then recognize it
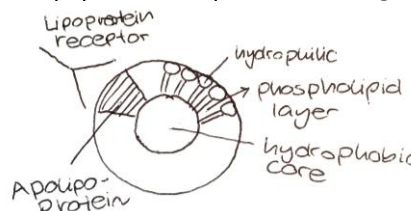
TYPES OF LIPOPROTEINS

Lipoproteins	Lipids	Origin	Apoliprotein
Chylo-microns	TAG, cholesterol (only a bit)	Intestine	ApoB48
VLDL	TAG (mostly), cholesterol (only a bit)	Produced by the Liver *Provides the rest of the body with lipids*	ApoB100
LDL	Cholesterol (mostly), TAG (only a bit)	Produced from VLDL	ApoB100
HDL	Cholesterol (mainly)	Liver, Kidney	ApoA1

RISK FACTORS

FRAMINGHAM STUDY:

- Goal: Identifying the risk factors for CVD
- Begun in 1948 in Framingham, Mass
- 5000+ volunteers
- On 3rd generation now!
- Monitored at 2-year intervals
- Made associations between CVD death and lifestyle behaviours/ non-changeable (genetics) factors
- Measurements
  - o Standard risk factors since inception of study, except HDL-C began around 1970
  - o Serial ECGs (first to document high rate of unrecognized MIs)
  - o M-mode echocardiograms in 1980's, first large study to show prognostic importance of increased LV mass
  - o Newer measures done in subsets include: Carotid ultrasound, bone denistometry, coronary artery calcium, and other novel risk factors and biomarkers (e.g. natriuretic peptides)
- Milestones:
  - • **1960** Cigarette smoking found to increase the risk of heart disease
  - • **1961** Cholesterol level, blood pressure, and electrocardiogram abnormalities found to increase the risk of heart disease
  - • **1967** Physical activity found to reduce the risk of heart disease and obesity to increase the risk of heart disease
  - • **1970** High blood pressure found to increase the risk of stroke
  - • **1976** Menopause found to increase the risk of heart disease
  - • **1978** Psychosocial factors found to affect heart disease
  - • **1988** High levels of HDL cholesterol found to reduce risk of death
  - • **1994** Enlarged left ventricle (one of two lower chambers of the heart) shown to increase the risk of stroke
  - • **1996** Progression from hypertension to heart failure described
  - o Age-adjusted analyses showed risk of reinfarction to be **positively associated** with **blood pressure** and **serum cholesterol**
  - o Risk of coronary death was strongly associated with **blood sugar level, systolic blood pressure, serum cholesterol, heart rate, diabetes, and interim infarction**
  - o In multivariable analyses, systolic pressure, serum cholesterol, and diabetes were predictive of reinfarction
  - o Relative weight was inversely associated with reinfarction
  - o Systolic pressure, serum cholesterol, and the prevalence of diabetes persisted as independent predictors of coronary death
  - o Women were at only half the risk of coronary death compared with men

STATISTICS

- Risk is twice as high if you are smoking

- The higher the cholesterol the higher heart attack risk
- The higher the HDL, the lower the heart attack risk
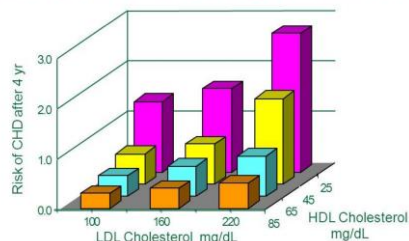
- LDL seems to be a predictor!
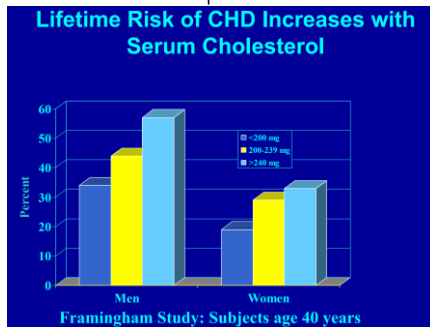
Cholesterol seems to be an important driving force!
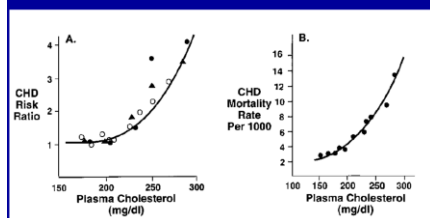
- From 200 to 300 → you quadruple the risk!

IMPORTANT to understand:
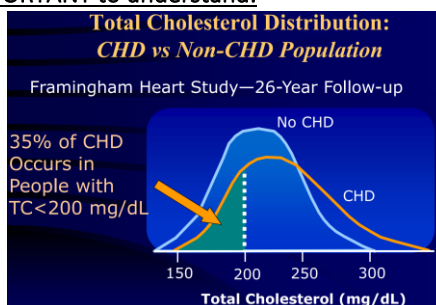
- We have a distribution which is shifted to the right (to higher cholesterol) → That does NOT mean that everyone with high cholesterol will develop a heart disease and not everybody below 200 is safe from a heart disease!
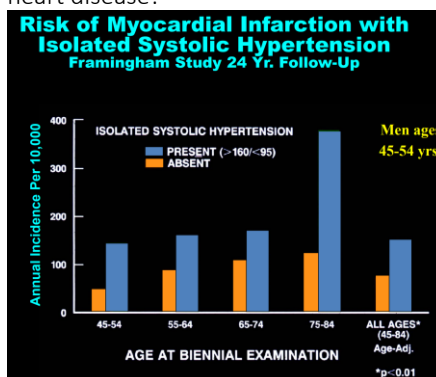
- Age is also a risk factor
- Systolic blood pressure seems to drive cardiac diseases
  - o High pulse correlates with high blood pressure

Joint Influences of SBP and Pulse Pressure on CHD Risk

RISK FACTORS:

	Major	Minor
Changeable	- Smoking - Diabetes - Hypertension - Blood cholesterol levels - Physical inactivity - Obesity	- Stress - Low Omega 3 fatty acids - High alcohol intake
Fixes	- Sex (male, menopause) - Age - Heredity - Ethnicity	

- Physical activity and high levels of HDL cholesterol reduce risk of death
- Lipids and **cholesterol** as one of the main drivers of CVD

HYPERTENSION
= High arterial blood pressure
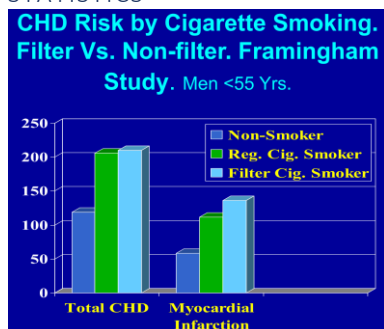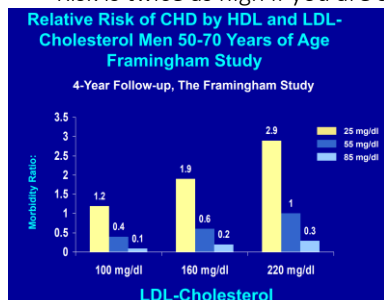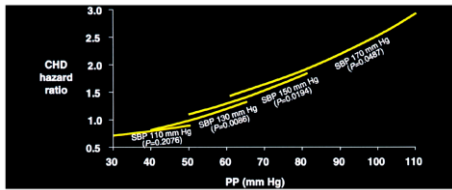- Can cause damage to blood vessels → puts extra strain on the heart
- Cause of hypertension can be unknown. However, high body fat, high salt intake, lack of exercise are known risk factors

HIGH SERUM (BLOOD) CHOLESTEROL
- Typically caused by eating too much **saturated fat**
- Can deposit in artery walls
  - LDL/VLDL = "bad" cholesterol
    - Recall: ox-LDL deposits in artery walls, forms plaque
  - HDL = "good" cholesterol
    - Lowers ox-LDL deposition in artery walls!

TOBACCO SMOKE
- Nicotine can cause **lesions in the artery wall**
- Carbon monoxide in cigarette smoke is doubly damaging
  - Causes lesions in the artery wall
  - Decreases ability of the blood to transport oxygen
- Smoking inhibits NO release

OTHER RISK FACTORS:
- **Exercise**: Can lower blood pressure, increase HDL and lower LDL and VLDL, reduce stress, maintain body weight and control type II Diabetes.
- **Obesity/overweight:** Especially abdominal obesity
  - Can lead to hypertension, low HDL, type II diabetes
- **Diabetes Mellitus:** Impaired ability of the blood to store glucose (sugar)

- **Stress**: Increases blood pressure, increases blood clotting, can increase cholesterol levels
- **Low Omega-3 fatty acid intake:** Found in cold water fish fat, inverse correlation with CVD
- **Alcohol**: Low daily intake (1-2 glasses per day) of
- alcohol has been associated with lower risk of CVD! However, high intake can damage the heart muscle and increase CVD risk
- **Age**: The older you are, the higher the risk
- **Gender**: Males are at higher risk than females
  - Biological difference or cultural difference?
- **Ethnicity**: Higher risk in African Canadians, Latinos, Aboriginals and South Asians

POSSIBLE STRATEGIES TO TREAT
Prevention of CVD absolutely unrelevant for the exam!

CVD PATHOPHYSIOLOGY

OVERVIEW
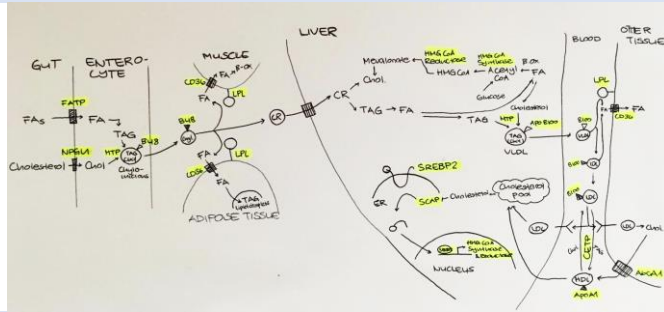
CHYLOMICRONS

- From the Gut, FAs and cholesterol are transported into enterocytes, where they are further packed (via MTP) into **chylomicrons** (B48)
- Chylomicrons transport TAG (FA) to mucsle and adipose tissue
  - o LPL (Lipoprotein Lipase): Converts TAG in FA
  - o Uptake of FA via Transporter: CD36
  - o Chylomicrons → CR (have higher cholesterol levels since TAGs are broken down and taken up)

CHOLESTEROL SYNTHESIS & DISTRUBUTION

- CR are transported into the liver → Cholesterol and TAG
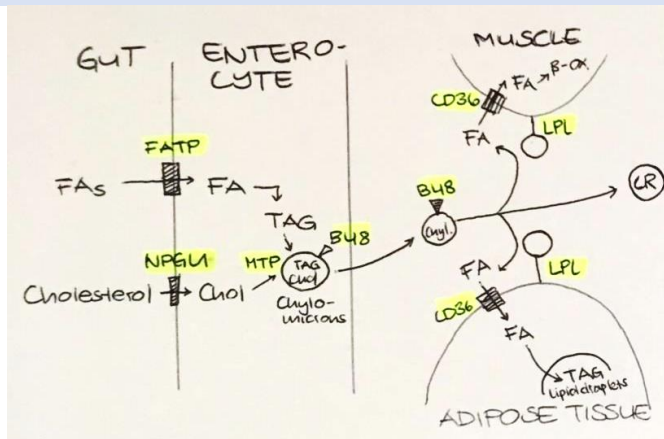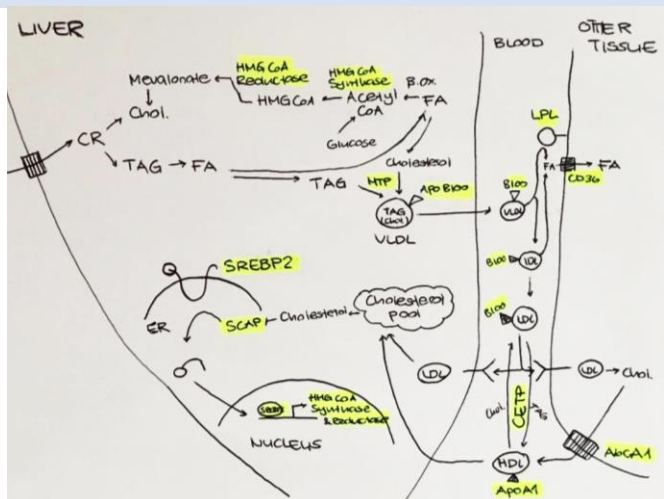- Liver is the main organ that controls cholesterol levels! → Responsible for cholesterol **production** (only liver!) and **distribution**
1. Liver: Cholesterol uptake and cholesterol synthesis
2. Liver packs cholesterol into VLDL and secretes them
3. VLDL releases TAG (→IDL) → TAGs get converted to FA (by LPL) and taken up by other tissues
4. IDL again release TAGs and become LDLs

5. LDLS are taken up vie LDLR and thus import/release cholesterol into other tissues
6. Cholesterol can be exported again (if needed) via AbcA1, packed into HDL and transported back into the liver (cholesterol pool)
- LDL = Pool of circulating cholesterol → important since you need to distribute cholesterol to the other tissues than the liver

CHOLESTEROL SYNTHESIS
- Only in the liver!
- FA → Acetyl CoA → HMG CoA → Mevalonate → Cholesterol
- Two rate limiting enzymes: HGM CoA Synthase and HGM CoA reductase
- Main source of cholesterol → NOT the from the diet up taken lipids!

CETP
- Re-equalization
  - o Transfers TAGs from LDL to HDL and cholesterol from HDL to LDL
- Treatment: You want to inhibit CETP → cholesterol will stay in HDL and LDL will not additionally receive cholesterol → shift the balance
  - o Drug failed → we might miss something...

REGULATION
How does the body know when to produce cholesterol?
- Regulatory system is based on TF → SREBP2
  - o Low cholesterol → SCAP (protease) active → cuts SREBP2 → translocases into the nucleus → activates transcription of HMG CoA Synthase & Reductase → cholesterol production → [Cholesterol]↑
Further regulatory system:
- SREBP2 positively induces PCSK9 translation → If cholesterol level in the blood Is low, you want more PCSK9! → less recycling of LDLR → higher LDL-levels in the blood
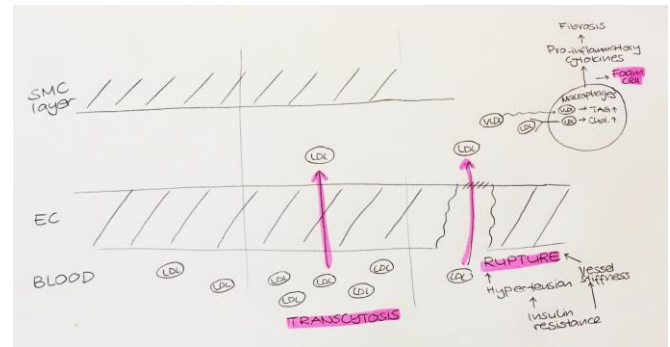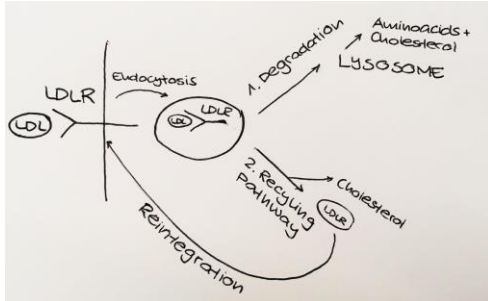
MUTATIONS:
LDLR-mutation
- LDL is constantly produced but not cleared → no feedback to liver and no LDL uptake into other tissues → High LDL level in the blood (increased formation and reduced uptake of LDL)

ABCA1 mutation
- People have very low HDL levels → massive coronary hear disease risk!

LDL UPTAKE

- LDL should get into the cell: LDL binds to LDLR and gets internalized → **Receptor mediated endocytosis**
- There are two possible ways:
  o Degradation of LDLR
  o Recycling of LDLR (reintegration into the membrane) → Recycling leads to higher number of LDLR in the membrane and thus higher LDL uptake into the cell → lowers blood LDL levels

PCSK9

- PCSK9 will lead to **degradation** of LDLR
  o Is co-internalized into the endosome
  o Leads to lower number of LDLR and thus less LDL uptake → higher blood LDL level
  o Without PCSK, the LDLR gets recycled

What could you do if you want to lower blood LDL-levels?
Block PCSK9 (PCSK9-AB) → More efficient clearance of LDL

AORTIC SYSTEM AND PLAQUE FORMATION

Plaque formation:
- More LDL in the blood → more **transcytosis**
- **Disruption** of endothelial cell layer
1. **Transcytosis**:
   o Concentration dependent
   o If LDL levels in the blood are high, more LDL will pass the endothelial barrier layer
   o Without disruption! → the layer is completely intact
2. **Rupture/ Break of endothelial barrier layer**
   → Allows LDL to enter
   o Hypertension can lead to a rupture
   o Insulin resistance makes vessels stiff → Rupture
→ Insulin resistance can cause a rupture directly (vessel stiffness) or as driving factor for hypertension which in turn can lead to a rupture
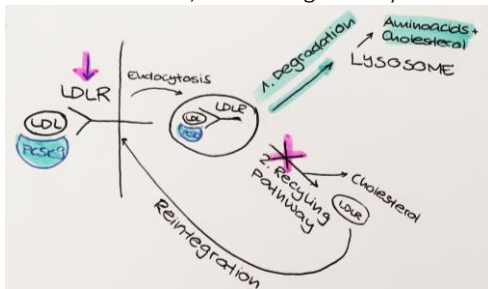

1. LDL enter the extracellular space
2. Enhanced LDL levels lead to **migration of macrophages** → Sense high levels of lipids and tries to clear them
3. Macrophages take up LDL and VLDL via receptors → Leads to higher levels of cholesterol and TAG inside the macrophages
4. Macrophages differentiates into **foam cells** (Foam cell = Macrophages + Lipid → bigger in size)
5. Macrophages will release **pro-inflammatory cytokines** which in turn recruits more macrophages and in the long-term leads to a wound healing event ending in **fibrosis**

PROBLEM: Chronic stimulus when macrophages are constantly overloaded with lipids → Inflammation → Fibrosis
Role of **LDL, Hypertension** and **Insulin resistance** in this disease!

RISKFACTORS

- **Hypertension** → Endothelial damage → LDL accumulation → Macrophage invasion
- **Insulin resistance**
  o Linked to hypertension
  o Stiffness of vessels → rupture/ endothelial damage
  o Adipose tissue secretes more FA → Excess of FA → enhanced FA uptake in the liver → Excess of substrate for cholesterol synthesis (system cannot control synthesis efficiently anymore) → increased cholesterol levels (LDL) in the blood
- **Type II diabetes** → Pushes CVD
- **NAFLD**: Liver constantly produces TAG (due to FA↑)

TREATMENTS:

- **Reduce hypertension**
- **Treat insulin resistance**
- If the patient has no hypertension or insulin resistance:
  o **Statins** → Reduce cholesterol synthesis in the liver
  o **Ezetimibe**
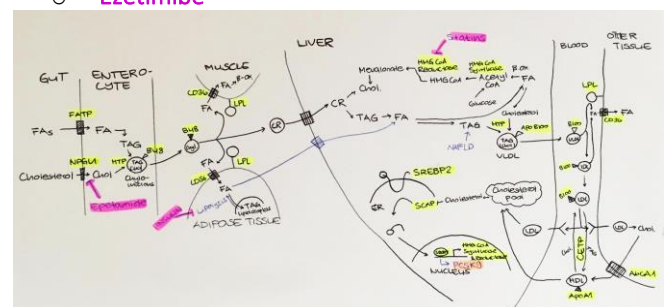
QUIZ:

	Richtig	Falsch
Lipids are removed from lipoproteins through lipases.	☑	✗
LDLR levels in the cell membrane is dependent on receptor endocytosis, degradation and recycling.	☑	✗
LDL is formed by degradation of lipids from HDL.	✗	☑
PCSK9 inibits LDLR recycling.	☑	✗

3. Is not degradation → HDL lipids are clearly not degraded to directly form LDL!!

	Richtig	Falsch
Foam cell formation is due to uncontrolled de novo lipogenesis.	✗	☑
HDL is responsible for the reverse cholesterol transport.	☑	✗
Lipid transfer between HDL and LDL is regulated by CETP.	☑	✗
Most cholesterol in the body is taken up directly from the diet.	✗	☑

1. Foam cell formation is due to uncontrolled de novo lipogenesis: Is only in part true (one possibility but not only)

A patient has a heterozygous missense mutation in the LDLR gene.
1. Please explain what you expect to see in LDL, HDL and VLDL levels.
2. What are the long-term consequences of such a mutation.
3. What treatment suggestion would you suggest and why.

1. Less LDLR (is not produced) -> no LDL uptake -> higher LDL levels in the blood, VHDL levels might go up a little bit, HDL levels might lower a bit or stay unchanged

2. Plaque formation (artherosclerosis) --> Cardio vasculaire disease

3. inhibit cholesterol synthesis in the liver (Statins), PCSK9 inhibitors (only heterozygous mutation!)

PAPER

*Mechanism by which **Fatty Acids Inhibit Insulin Activation** of Insulin Receptor Substrate-1 (IRS-1)-associated Phosphatidylinositol 3-Kinase Activity in Muscle*

Hypothesis: An increase in plasma fatty acid concentration results in an increase in intracellular **fatty acyl-CoA and DAG concentrations**, which results in **activation of PKC-ϴ** leading to increased **IRS-1 Ser307 phosphorylation**. This in turn leads to **decreased IRS-1 tyrosine phosphorylation** and **decreased activation of IRS-1-associated PI3-kinase activity** resulting in **decreased insulin-stimulated glucose transport activity**

Insulin resistance
- Insulin resistance in skeletal muscle is a major factor in the pathogenesis of type 2 diabetes
- Infusions of lipid emulsions with herparin (acutely raise plasma [fatty acid]) cause profound insulin resistance in rat and human skeletal muscle within 4–6 h
    o Fatty acids induce insulin resistance in skeletal muscle
- Increase in plasma fatty acid concentration results in lower intramyocellullar glucose 6-phosphate and glucose concentrations → fatty acids inhibit insulin-stimulated glucose transport activity
    o Fatty acids cause insulin resistance through inhibition of insulin signaling

METHODS/MODELS*:*
- Rats were infused with a **lipid emulsion** (mostly 18:2 fatty acids) or **glycerol (→** Lipid infusion)
    o Fatty acid metabolites at different time intervals were measured in relation to insulin stimulation: (i) insulin receptor tyrosine phosphorylation, (ii) IRS-1 tyrosine phosphorylation, (iii) IRS-1-associated PI3-kinase activity as well as PKC-ϴ translocation

RESULTS:

Lipid/heparin infusion → Basal plasma fatty concentration increases rapidly
    o **Increase** in both intramuscular **LCACoAs (C18:2 CoA)** and **DAG concentration**
    o No change in intramyocellullar ceramide or muscle triglyceride → metabolites do not play a major role in mediating fatty acid-induced insulin resistance in skeletal muscle
- ↑ intracellular [LCACoA] & [DAG] → **PKC-ϴ activation**
    o Less PKC-ϴ in the cytosol & more membrane-associated PKC-ϴ
    o Reduction of total PKC-ϴ content
    o Higher PKC-ϴ distribution
- ↑ intracellular fatty acyl-CoA & PKC-ϴ activation → impairment in **insulin-stimulated IRS-1 tyrosine phosphorylation** and **IRS-1- associated PI3-kinase activity**
    o 30% reduction in insulin activation of IRS-1 tyrosine phosphorylation
    o 50% reduction in IRS-1- associated PI3-kinase activity

    o 1.6-fold increase in **IRS-1 Ser307** phosphorylation
    o no effect on insulin receptor tyrosine phosphorylation
- Lipid infusion induced a profound **defect in insulin-stimulated glucose uptake**
    o Reductions in insulin-stimulated IRS-1 tyrosine phosphorylation and IRS-1-associated PI3-kinase activity across all insulin concentrations
    o no change in insulin-stimulated IR tyrosine phosphorylation

IRS-1 Ser307
- IRS-1 Ser307 phosphorylation = critical site in mediating TNF-induced insulin resistance
    o IRS-1 Ser307 mutation to IRS-1 Ala307 → protection from TNF-induced insulin resistance
- 1.6-fold increase in IRS-1 Ser307 phosphorylation → fatty acids may mediate insulin resistance through the same common final pathway as TNF

SUMMARY/CONCLUSION:
- **Fatty acids induce insulin resistance** in skeletal muscle by **blocking insulin activation** of insulin receptor substrate-1 (IRS1)-associated phosphatidylinositol 3-kinase (**PI3-kinase**)
- Fatty acid-induced inhibition of insulin-stimulated glucose transport activity in muscle can be explained for the most part by **decreased activation of PI3-kinase at the level of IRS-1 tyrosine phosphorylation,** which cannot be overcome with supraphysiologic concentrations of insulin
- Plasma [fatty acid] ↑ → intracellular [fatty acyl-CoA] & [DAG] ↑ → active PKC-ϴ ↑→ IRS-1 Ser307 phosphorylation ↑ → IRS-1 tyrosine phosphorylation & IRS-1-associated PI3-kinase activity ↓ → insulin-stimulated glucose transport ↓

*1. Why is the study being conducted (Rationale)?*
2. What is the Aim of the published study?
To find the link between increased circulating lipids and increased insulin resistance (mostly in the muscle).
The aim of the study is to examine the mechanism by which fatty acids induce insulin resistance in skeletal muscle by blocking insulin activation of insulin receptor substrate-1 (IRS1)-associated phosphatidylinositol 3-kinase (PI3-kinase)

3. Which model system does the study employ and does it fit with the research question?
Rats were infused with a **lipid emulsion** (mostly 18:2 fatty acids) or **glycerol** (→ Lipid infusion)

4.   *What are the main results of Fig.1?*
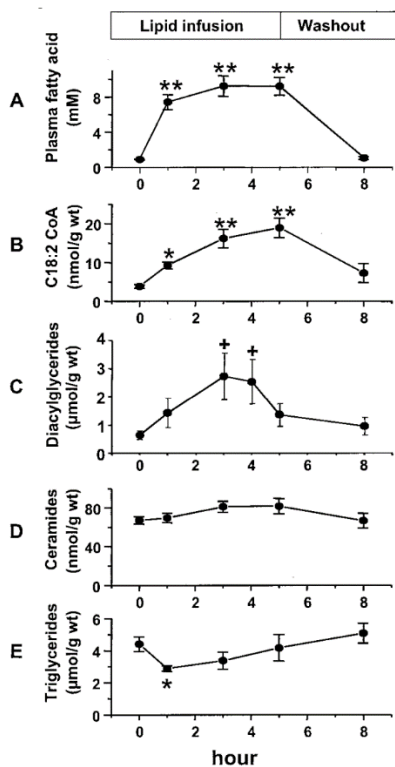
Different fatty acid metabolites were measured at different time intervals after rats have been infused with a lipid emulsion. It can be seen, that **basal plasma fatty** (A) concentration increases rapidly. Further, there is an increase in both intramuscular LCACoAs (C18:2 CoA, B) and DAG (C) concentration but no changes in intramyocellular ceramide (D) or muscle triglyceride (E) are visible (→ Those two metabolites seems not to play a major role in mediating fatty acid-induced insulin resistance in skeletal muscle)

- Plasma peaks first (A), the intracellular levels peak later (B) (make sense)
- Interesting: Diacylglycerides peak earlier, whilst C18:2 peak later
- TAG take longer to be formed → lipids must get stuck somewhere before…

5. Are the conclusions, which are made for Fig.1, warranted?