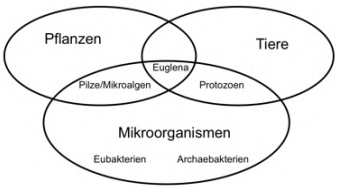
Cesare Primultini (pcesare@ethz.ch)

BIOENGINEERING_2020

CONTENTS

Cesare Primultini (pcesare@ethz.ch)

1. INTRODUCTION OF BIOLOGY



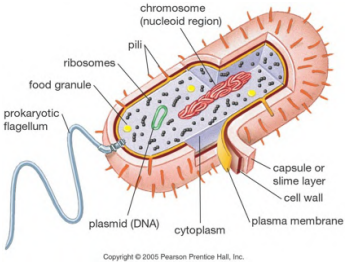
Three reigns of Biology: Plants, Animals, Microorganisms.

Further division of microorganisms:

- **Eukaryotes**: DNA in the nucleus of the cell organelles.
- **Prokaryotes**: DNA free in cell plasma, no nucleus and no organelles.

PROKARYOTES

- Small
- No internal structure
- No internal membranes (no nucleus)

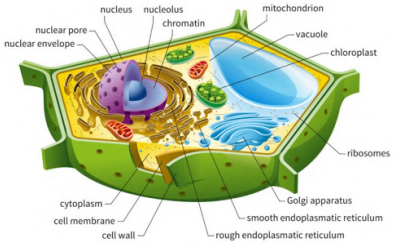


Structure:

- **Ribosomes** make protein synthesis.
- **Nucleolus** is the hotspot for transcribing DNA into mRNA.

EUKARYOTES

- Larger than prokaryotes
- Internal structures can be recognized
- Organelles and cell nucleus



Structures:

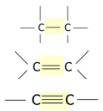
- **Ribosomes** make protein synthesis.
- **Nucleolus** is the hotspot for transcribing DNA into mRNA.
- **Nucleus** contains DNA.
- **Mitochondrion** is in charge of energy generation (ATP).
- **Cytoplasm** (similar to unboiled egg white) liquid in which cell chemistry happens.

2. CHEMICAL BASICS

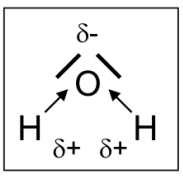
2.1. CHEMICAL BONDS

2.1.1. STRONG BONDS

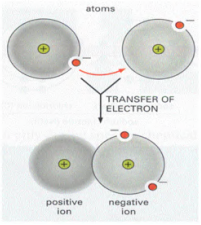
- **Covalent**
  - Pair of electrons (simple bond)
  - Double bond
  - Triple bond



Electrons are unevenly distributed in the atom, the atom with the strongest electronegativity attracts the electron more.
For example, in H2O, the O atom has a partial negative charge because is more electronegative. Hydrogen has a positive partial charge (less electrons). Consequence is that the molecule has a negative and a positive pole, the bond is called **polar**.
Between these kinds of molecules will form dipole forces.

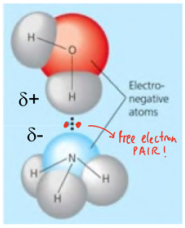


- **Ionic**
  - Cations (negatively charged): metallic ions (Na+, K+), hydrogen ion (H+), ammoniac (NH4+)
  - Anions (positively charged): nonmetals like phosphate (PO4^3-), chloride (Cl-)



In water: ions surrounded by a hydrate shell, separated from counterions.
In solid form, ion and counter ion are bound in high order ("crystal lattice") in a crystal.

2.1.2. WEAK INTERACTIONS



- **Hydrogen bonds**

Between dipole molecules there are also forces weaker than ionic ones. They are relatively strong if:

  - an H atom bonds with a very electronegative atom (F, O, N), so that becomes partially positive charged
  - in another molecule there's a free electron pair (usually N or O).

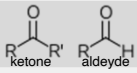
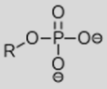
- **Van der Waals forces**

If two molecules get closer to a distance of 3-4 Å (1Å=10E-10m) interactions due to asymmetries in electron disposition happen. In every molecule there's always a time-changing dipole due to the nature of electron (free to move in the orbits, non-fixed -> can happen that there are more in one part of the molecule).

- **Hydrophobic interactions**

Water has a polar structure, if nonpolar molecules are put into water, they'll get together to minimize the interactions with it. These force that holds together the nonpolar molecules is more to optimize energy than an actual force.

2.2. FUNCTIONAL GROUPS

functional group name	structure	properties	common functions	commonly found in
Hydroxyl	R–OH	• polar • hydrophilic	tends to make things more soluble in water	• hugely abundant in sugars and alcohols
Carbonyl	 ketone aldeyde	• polar very reactive	the site of enzymatic c-c bond breaking/making	• every sugar has one
Carboxyl		• acidic • charged (-)	multifaceted, biological acid	• amino acids • fatty acids • acetic acids • other acids
Amino	$R–NH_2$	polar bond • basic • charged (+) free e- pair can bond with protons	biological base, maintains 3D structure of large molecules, defines base pairs in nucleic acids	• amino acids neurotransmitters • bases of nucleic acids
Thiol (Sulfhydryl)	R–SH	• polar	form di-sulfide bonds, enzymatic properties	• amino acid-cystine • acetyl-CoA
Phosphate		• acidic • charged (-) • hydrophilic	regulation, energy, structure	• phospholipids • DNA backbone • NTP • protein regulation

2.3. BUILDING-MATERIAL AND CONTENT OF CELLS

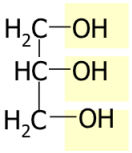
The first living beings where born in **water** and nowadays the most active cells are still in water. In cells, free hydrated ions of inorganic salts are always present (as in the ocean), as well as organic compounds. There are 3000-6000 different substances in cells.
Cells contain:

Water		77%
Hydrated ions		3%
Compounds of carbon	**Lipids**	2%
	Proteins	14%
	Carbohydrates	1%
	Nucleic acids	3%

2.3.1. TYPES OF LIPIDS

- Lipids from **alcohols**:

Molecules with 2 or 3 alcoholic OH-groups (bivalent, trivalent alcohols), for example Glycerin in the picture ->



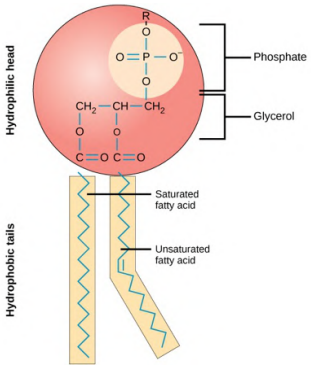


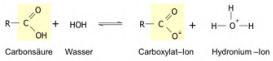
Image 1: common shape of a phospholipid.

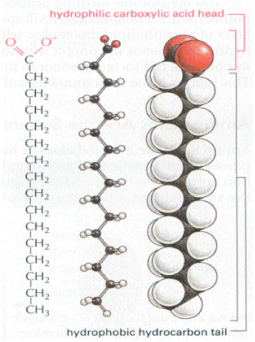
- Carbonic **acids**:

Formed by the carboxyl COOH-groups. For example:
- ethanoic acid (acidic acid), CH3-COOH.
- fat and acid fats (long C-chains, amphiphilic molecules, lipids)

2.3.2. CARBONIC ACIDS

Biggest part of carbonic acids are weak acids (they have only a slight tendency to split off the hydrogen of the carboxylic group as proton, H+, called protolysis reaction). PH is a measure of how concentrated the H3O+ ions are.



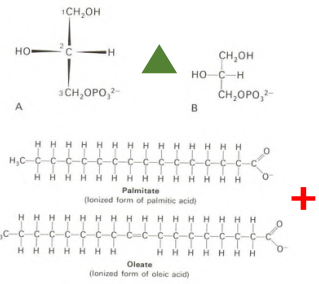


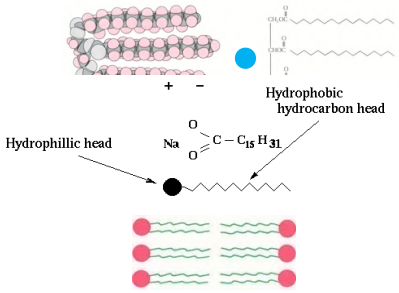
- **pH and pKa**: the pH is a measure of the concentration of hydrogen ions in an aqueous solution. pKa (acid dissociation constant) and pH are related, but pKa is more specific in that it helps you predict what a molecule will do at a specific pH. Moreover, pKa tells you what the pH needs to be in order for a chemical species to donate or accept a proton. *Essentially, pKa indicates whether an acid is a strong acid or a weak acid*. -> strong acids (Cl, pKa = -1), weak acid (acetic acid, pKa = 4.8).

2.3.3. LIPIDS (from alcohols)

They are often referred as the biologic lipids (**fats**) are **esters** (a chemical compound derived from an acid in which at least one –OH (hydroxyl) group is replaced by an –O–alkyl (alkoxy) group) of the trivalent alcohol glycerol with various fatty acids.

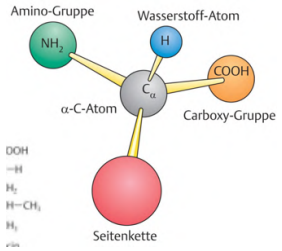
- Glycerophosphates ▲
- Fatty acids +
- Fats ●





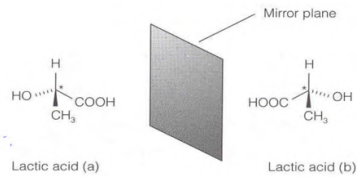
Lipids is a collective term for fat-soluble (**lipophilic**) compounds. These compounds are normally insoluble in water (**hydrophobic**).
Biologic lipids are often ***amphiphiles*** (**head** is hydrophilic, and **tail** is hydrophobic).
They are part of the **bacterial membrane**, which can selectively let things out and in.
Lipids are as well a **high-energy storage substance**.

2.3.4. PROTEINS

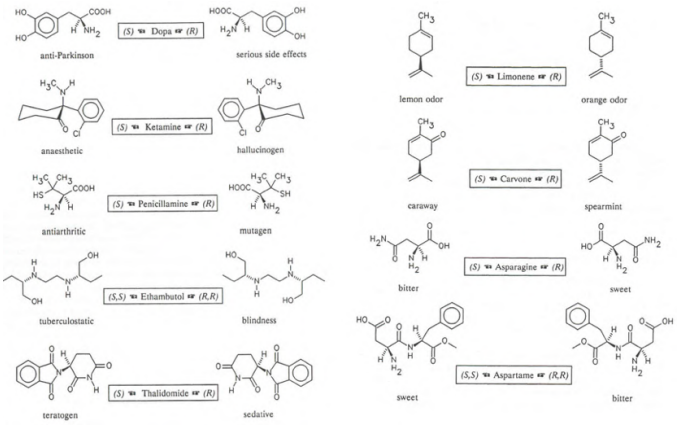


Proteins are formed from amino acids and all the amino acids have the same basic structure:

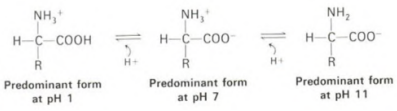
- Acidic group (e.g. carboxyl)
- Amino group
- H-atom
- Side chain R (whatever)



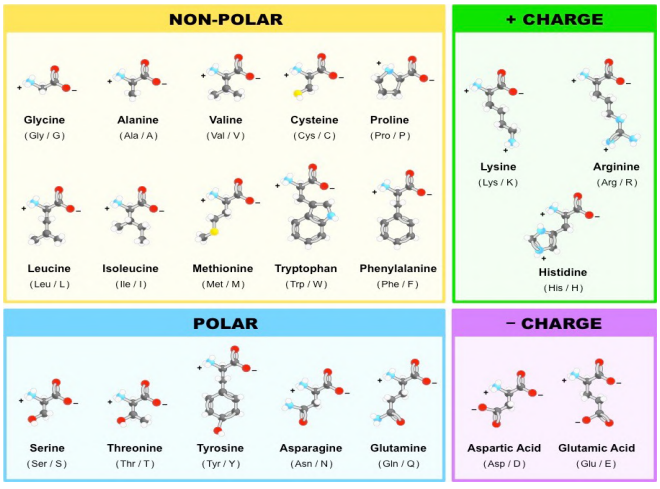
Amino acids are asymmetric. C-atom with 4 different boding partners is called **asymmetric**.
The mirrored molecule does not have the same properties of the original one (not congruent as well). For example, the molecule "**thialomide**" can be a sedative or a teratogen (causes malfunctions to the embryo). The scientific name has an R or an S in front of it depending if it's the mirrored or normal molecule.
Some examples:



The **physicochemical properties** of the amino acids depend on **pH** of the environment.



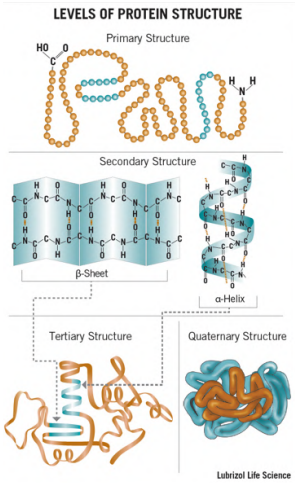
The amino acids *differ only from the atom chain R*, here beside some examples ->



"The cell works with 20 "proteinogenic" (protein-forming) amino acids, which differ chemically in their side chains R ("residues"). These 20 different side chains are the pool from which the amino acid polymers - the proteins - draw their (physicochemical) properties."

- **Formation of a protein**
  - **COOH-group** of an amino acid can bond with the **NH2-group** of another amino acid, with water condensation (H2O as secondary product of the reaction).
  - The formed molecule is called **dipeptide**, if another one attaches it becomes a tripeptide and so on…
  - They are called oligopeptides till 10 amino acids, and polypeptides till 50.
  - It can be called **PROTEIN** when the chain is > 50.
  - **Peptides** have a beginning (NH2-) and an end (COOH), which remains as amino acids attach.
  - After a certain length, weak bonds begin to form between amino acids of the chain, the chain starts folding up.
  - When folded correctly, we can speak of a **protein**.

- **Structure of a protein**
  - ***Primary structure*** is the sequence of amino acids (ala-val-gly-ala-etc…)
  - ***Secondary structure*** (repetitive, spatially structured peptide):
    - *Alpha helix*: formed due bonds between amino acids that are one over the other.
    - *Beta helix*: formed due to hydrogen bonds between amino acids close to each other.
  - ***Tertiary structure***: secondary structure one of top of the other (hydrogen bonds). Interactions of the polypeptide chains form a particular form of the protein. At this point the protein is functional and ready (monomer) but full capability can be reached if quarterly structure is reached.
  - ***Quarterly structure***: further interactions and bonds between monomers and polypeptide chains

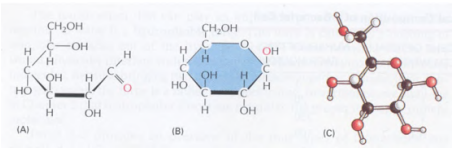


- **Cellular function of a protein:**
  - **Structure protein**: organize other macromolecules in specific forms (in ribosomes or cytoskeleton).
  - **Catalysators** (enzymes): direct chemical reactions.
  - **Pores**: in the membrane.
  - **Locomotion apparatus**.
  - **Antibodies**: they are formed by 4 protein chains connected by Sulphur bridges. For each "antigen" the body has the appropriate antibody. This specificity makes antibodies so valuable.

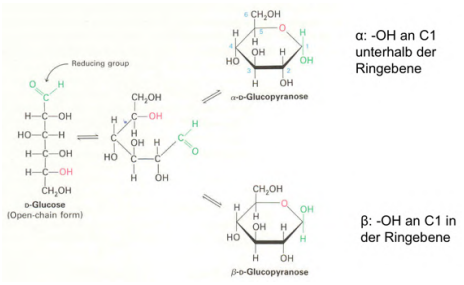
2.3.5. CARBOHYDRATES

General formula for carbohydrates is $(CH_2O)_x$. They can be very complex structures (even more that 100 **monosaccharides** bond together). Usually monosaccharides taste sweet, which is why they are often called simply **sugars**. Sugars can function as structural cells for the cell wall (support) or for the nucleic acids but as well as energy source (or transport).

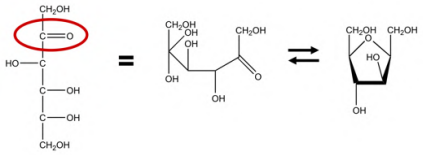
- **MONOSACCHARIDES IN GENERAL**
  - Monomers of polysaccarides.
  - Bond with C (from 3 to seven C-atoms), pentose (5C) and hexose (6C) are the most important.
  - They are polyalcohols (some hydroxyl groups present).
  - Good water-soluble molecules.
  - They can be found in ring or chain form.



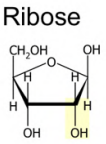
- **Monosaccharides I (hexose: GLUCOSE)**
  - Example given is glucose: $(CH_2O)_6$ which is a hexose.
  - It contains 5 hydroxyl groups and 1 aldehyde (**aldose**).
  - Can be both a ring of 5C, a chain of 4C or become a six-ring (**pyranose**).
  - The form of a ring is the form of the molecules when the sugar is crystalline. Helps to bond with other sugars as well.



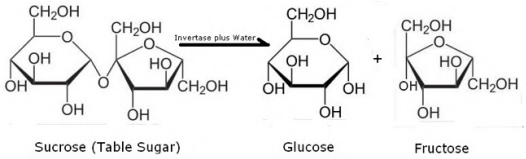
- **Monosaccharides II (hexose: FRUCTOSE)**
  - Hexose can be found in different forms; **fructose** is another one.
  - 5C ring: **furanose**.
  - The main difference is a carboxyl group in the 2C atom (**ketose**).



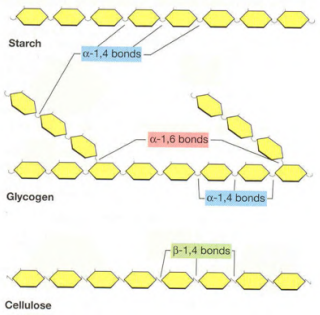
- **Monosaccharide III (pentose: RIBOSE)**
  - $(CH_2O)_5$ formula, called **ribose**.
  - It's a ketose as well (carbonyl in 2C).
  - 5C: **furanose**.



- **Disaccharides**
  - Aggregation of more monosaccharides by dehydration (one molecule of water in the products of the reaction).
  - Cane sugar has fructose and glucose as seen in the picture ->
  - 1,2-linkage, C position 1 with C in position 2 of the other molecule.



- **Polysaccarides**
  - Multiple molecules together (chains of monosaccharides)
  - Not soluble in cold water.
  - Difference between α *and* β molecules is small, but the effects are big:
    - If the hydroxyl group (-OH) in the first C atoms is in **position α**, the molecule will be spiral (**starch**). α molecules are more soluble but less stiff.
    - If it's in **position β**, then it's going to be a chain (**cellulose**). Stiffer but not much soluble.

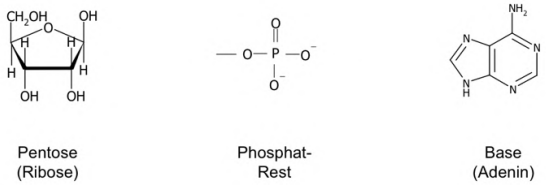


2.3.6. NUCLEIC ACIDS

Nucleic acids have different functions in the cell, but they're mainly **carriers for genetic information** (they play another important role in **protein synthesis** in ribosomes). Characteristics:

- Important in energy metabolism (ATP)
- Chain form
- Monomers are called **nucleotides**; therefore, nucleic acids are polynucleotides.
- They are often found in the cell nucleus.

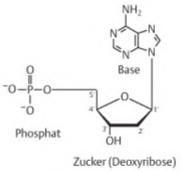
Nucleotides are formed by a ***pentose***, a ***phosphate-rest*** and a ***nitrogen-containing*** organic ring (called ***base***) compound as seen in the image ->



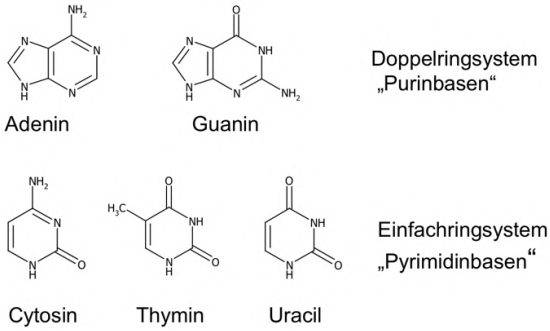
Cesare Primultini (pcesare@ethz.ch)

Structure is as follows:

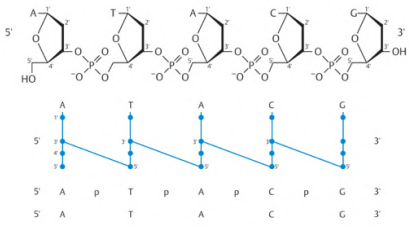
- The base is bonded to the **first** C-atom of the **pentose**.
- Phosphate-rest is bonded to the **fifth** C-atom.
- An example in the picture ->

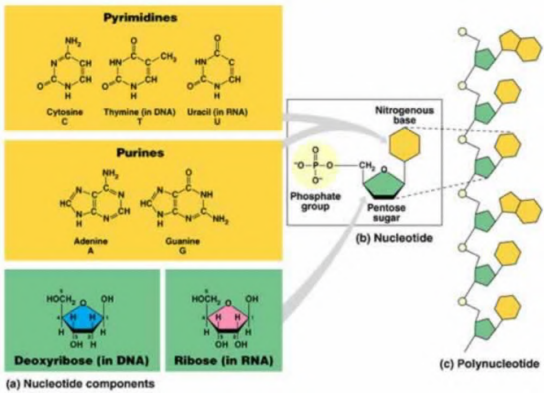


The BASIS are five:



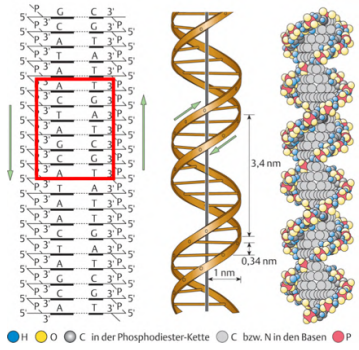
Concatenation of basis happens thanks to a **phosphate-bridge** bond between the **5th** atoms of a nucleotide and the **3rd** of another. The chain will always have a beginning and an end.





There are two main types of nucleic acids:

- **Ribonucleic acids** (**RNA**) containing ribose, has 4 bases: AGCU.
- **Deoxyribonucleic acid** (**DNA**) containing deoxyribose, has 4 bases: AGCT.



Two DNA chains can form the characteristic helix of the DNA. The two chains are attached following a systematic approach: the two nucleotide strings are complementary (same but mirrored, A attaches to T and C to G and opposite).
The roles of the nucleotides are the followings:

- Genetic material (**DNA**).
- Replicating material, mediator (**RNA**).
- As a **building block** for **protein synthesis** (ribosomes and RNA)
- As an **energy exchanger/carrier** and **chemical activator**.

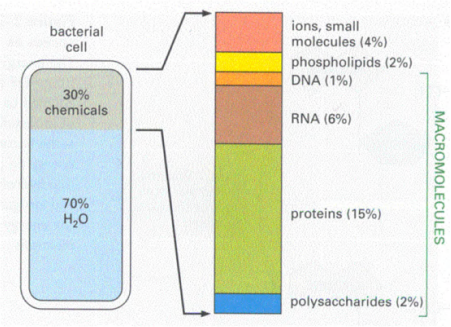
Summarising:

- Lipids are, among other things, components of the cell membrane.
- Proteins are a.o.t. catalysts of the cells.
- Carbohydrates are a.o.t. storage materials.
- Nucleic acids form RNA and DNA

Cesare Primultini (pcesare@ethz.ch)

3. CELL METABOLISM

3.1. CELL NUTRITION



Content of cells (see picture). Moreover, the cells must absorb a large quantity of elements such as C, O, H, N, S, Mg, Fe and many others.

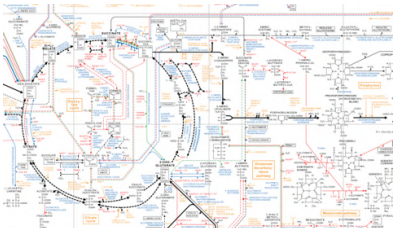
Where do these elements come from?

- Carbon (C):
  - From carbon dioxide (CO2), for plants and some bacteria.
  - From organic material (they always contain C).
- Oxygen (O):
  - From the air, water, carbon dioxide or sugars
- Sulfur (S):
  - From sulfates or sulfidic acids.
- Nitrogen (N):
  - From proteins and amino acids, air (N2) or ammonium.

How are these elements utilized?

- Synthesis of cell-building blocks (Proteins, DNA, …).
- Energy production: in order to make the synthesis cited above, to maintain the cell alive and to provide for movement.
- **Cell metabolism** is the set of life-sustaining chemical reactions in organisms. The three main purposes of metabolism are: the conversion of food to energy to run cellular processes; the conversion of food/fuel to building blocks for proteins, lipids, nucleic acids, and some carbohydrates; and the elimination of nitrogenous wastes.

3.1.1. OVERVIEW OF CELL METABOLISM



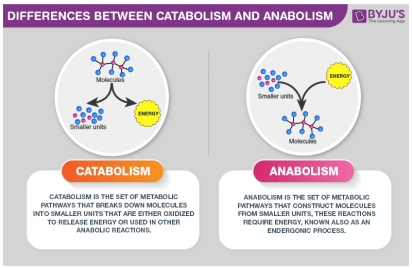
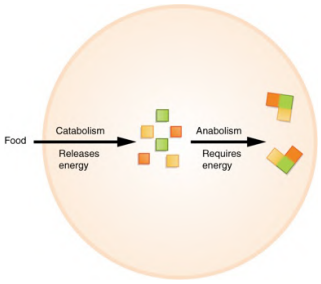


Figure 1: see also http://biochemical-pathways.com/#/map/1

- **Metabolism**: road map of thousands of chemical interactions (see figure ->). It's divided in two main processes:
  - **Catabolism**: degradation of energy-rich molecules to obtain energy and synthesis components (e.g. amino acids)
  - **Anabolism**: construction of cell components (e.g. proteins, nucleic acids) using energy



Important remarks:

- **metabolic pathways** gradually change a molecule.
- there is an energy transfer between anabolic and catabolic process.

3.2. PRINCIPLES OF CELL METABOLISM

3.2.1. THERMODYNAMIC BASICS

Remember that:

- Cell metabolism works for every cell (no matter if animal, plant or bacteria) with the same principles and mechanism.
- The differences can be found only on the spatial arrangements (e.g. bacteria don't have internal membrane systems).

Cesare Primultini (pcesare@ethz.ch)

- **First law of thermodynamics**:
  - Energy can be converted, but not created and destroyed
  - The energy of the universe is constant

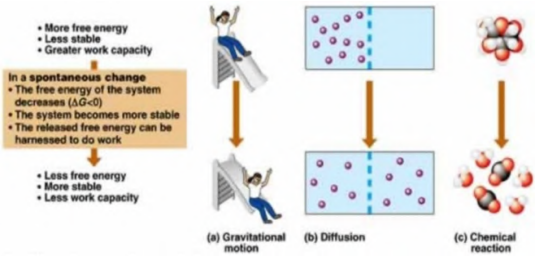
- **Second law of thermodynamics**:
  - Every voluntary (spontaneous) process makes the universe more messy
  - The disorder (entropy) of the universe is thus constantly increasing (disorder increases in the form of small molecules and heat).
  - Order can be created locally, but the universe as a whole becomes “untidier”.
  - Eventually everything becomes warm.
  - Heat can be defined as...
    - ..."messy" form of energy.
    - ... energy of the lowest quality (uncoordinated, random movement of molecules).

Therefore, the sum of the energy in the universe remain constant but the type of energy will not be constant. Thermodynamics is useful because cells exchange energy and heat with the environment, exactly like a thermodynamic machine.

Meaning of the first and second law for organisms: cells build **structured matter** (*reduce local entropy*), therefore they need energy to do so. This energy comes from **light** or **chemical components** (environment). Cells then release smaller and less structured molecules (H2O, CO2) at the cost of the environment which **increases** in **entropy**. Generally, **energy enters the system as light and leaves it as heat**.

Gibbs free energy: $\Delta G = \Delta H - T\Delta S$ (with T absolute temperature, H enthalpy and S entropy).

- Free energy (free enthalpy) is the amount of energy that the system can **transform into work** at a constant pressure and temperature (energy that is “free” to be used for work).
- Under **biological conditions** (T=const, P=const) free energy is a measure for the instability of a system.



- If $\Delta G < 0$
  - Free energy will be released from the reaction.
  - Reaction will run spontaneously (**exergonic**).
- If $\Delta G > 0$
  - Free energy will be used in the reaction.
  - Reaction will not be spontaneous (**endergonic**).
- If $\Delta G = 0$
  - Maximum stability has been reached (equilibrium).
  - No net reaction observable (e.g. products will not increase over educts).

Two reasons why a reaction can take place: **reduction** of the **bonding energy** in the molecules or **increase** in **disorder**.

- Reactions strive to reach equilibrium ($\Delta G = 0$)
- In reversible reactions, equilibrium is met when there’s no change in the concentrations of products and educts:

$$a\,A + b\,B \rightleftharpoons c\,C + d\,D$$

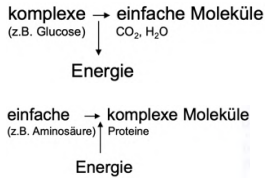
$$K = \frac{[C]^c \cdot [D]^d}{[A]^a \cdot [B]^b}$$

Summary of the Four Scenarios for Enthalpy and Entropy Changes

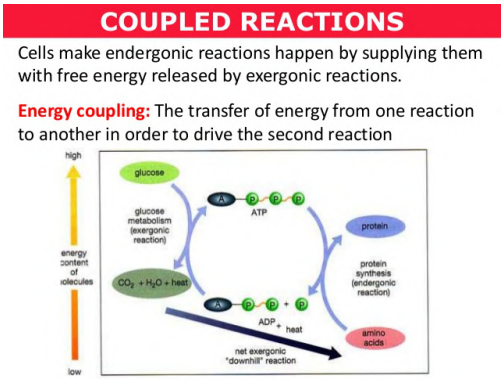
	ΔH > 0 (endothermic)	**ΔH < 0 (exothermic)**
ΔS > 0 (increase in entropy)	ΔG < 0 at high temperature ΔG > 0 at low temperature Process is spontaneous at high temperature	ΔG < 0 at any temperature Process is spontaneous at any temperature
ΔS < 0 (decrease in entropy)	ΔG > 0 at any temperature Process is nonspontaneous at any temperature	ΔG < 0 at low temperature ΔG > 0 at high temperature Process is spontaneous at low temperature

Cesare Primultini (pcesare@ethz.ch)

3.2.2. COUPLING OF REACTIONS IN CELL METABOLISM



- **Catabolism**: produces energy, it's exergonic (degrades high energy molecules as starch or amino acids). Catabolism in general is exergonic, but NOT EVERY catabolic reaction in itself is exergonic.
- **Anabolism**: needs energy, it's endergonic (e.g. to build proteins or nucleic acids).

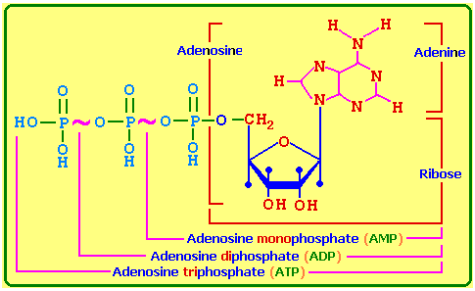


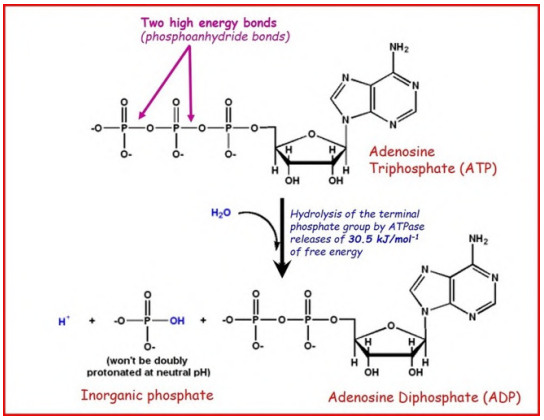
Energy transmission:

- **Energy** is not **produced** in a single reaction, but **gradually** (sugar is not transformed into CO2 and H2O in one big combustion, but in many steps - glycolysis and citric acid cycle).
- Part of the energy difference from step to step can be stored as chemical energy in the cell (ATP for example).
- Therefore: **exergonic** and an **endergonic** reaction are **coupled**!

ATP (energy storage)

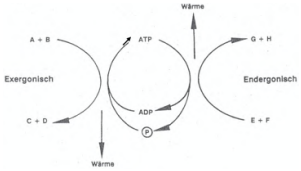
- In such couplings, the molecule that is used to **store energy** is called **adenosine triphosphate** (**ATP**) and it's a short-term energy storage for cells.
- In endergonic reactions, ATP is broken down in order to gain the energy needed for the reaction.
- Types of molecules: **ATP** (high energy), **ADP** (low energy) and **AMP** (no energy).
- Structure of ATP/ADP/AMP:
  - Organic base (**adenine**).
  - C-5-sugar (**ribose**).
  - **Phosphate-group**.





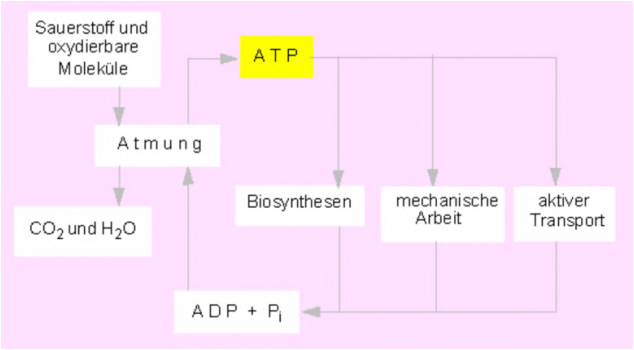
Operating principles of ATP and ADP:

- Hydrolysis of ATP (spin off of one phosphate group), releases 30.5 kJ/mol of energy ($\Delta G = -30.5\ kJ/mol$).
- This energy is then used for an endergonic reaction, some energy is lost as heat.



ATP provides:

- **Chemical work**: energy for endergonic reactions (e.g. synthesis of polymers from monomers).
- **Mechanical work**: muscle cells.
- **Transport work**: transport of substances across membranes against the concentration gradient.

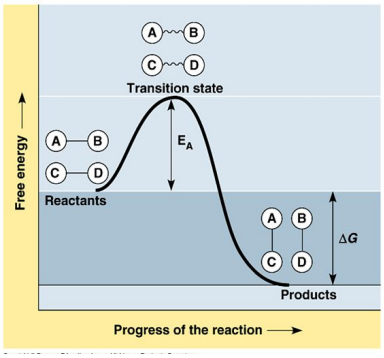


Cesare Primultini (pcesare@ethz.ch)

3.2.3. CATALYSIS OF REACTION IN CELLS

Thermodynamics tells us which reactions occur spontaneously and which do not. Although, it doesn't say anything about the **speed** of the reactions (kinetics).
The speed depends on **catalysts** that the cell produces itself: **enzymes** (consisting of **proteins** and sometimes other components).

- **Organic substances** (e.g. sugar) can be **converted into inorganic substances** (e.g. CO2, H2O) in the presence of **oxygen**.
- These reactions are always **exergonic** (the equilibrium is on the side of the low molecular weight compounds; *concentration of products is greater than the concentration of reactants*).
    - The speed at which the equilibrium is reached is extremely slow!
    - Only after supplying a certain amount of energy (**activation energy**), the organic substances react with oxygen!



Uphill - Reactants A & B must absorb enough energy from the surroundings to surmount the hill of activation energy and reach the unstable transition state.

Downhill – Bonds break, and new bonds form. Energy is released to surroundings during this process (EXERGONIC - ΔG negative) – products have less energy than reactants.

Without catalysts only high-energy molecules could react. There are only two ways to increase reaction speed:

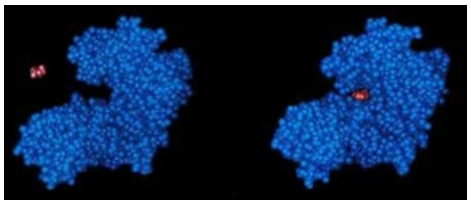
- Increase temperature (increase of kinetic energy inside the molecules).
- **Reduce the activation energy** thanks to **CATALYSTS**.

CATALYSTS reduce the activation energy of a reaction without altering the products and without being reduced by it.

Fact: a single enzyme molecule can convert up to 5,000,000 molecules of a substrate (educts) per minute.
Enzymes are **catalytically active proteins** (which are 3D-ordered chain of amino acids).

Types of ENZYMES:

- Enzymes made only by proteins.
- Enzymes made by a protein and a cofactor:
    - **Apoenzyme** (non-active proteins)
    - **Cofactor**: metal ions (Mg2+, Zn2+, etc.) or complex organic structures. If the cofactors are irreversibly bound to the apoenzyme, one speaks of a **prosthetic group**.

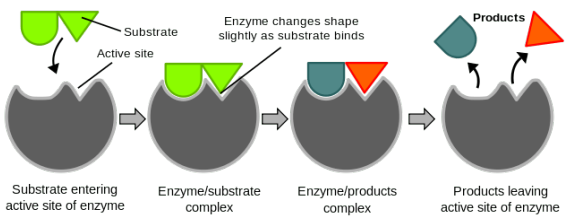


Mechanism of enzymatic conversion of substances:

- **Substrate binding** occurs at a **specific site** of the **enzyme** ("catalytic center" or "active center").
- Substrates diffuse to the enzyme and reach the **active center**. Only selected substrates can enter this center. There, is where the catalyzed reaction takes place. The **active center**:
    - can be on the surface, or deep inside the enzyme; usually a cavity or pocket in the protein.
    - is formed by interaction of amino acids that are not adjacent in the primary sequence.

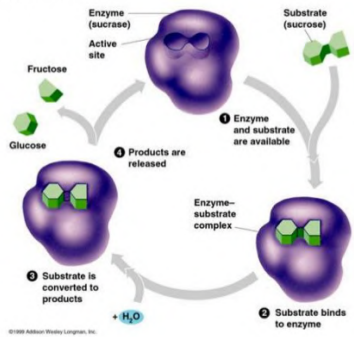
Bonding between substrate and enzyme can be described by two models:

- 1. **LOCK AND KEY MODEL**: "To use a picture, I want to say that enzyme and glucoside (substrate) must fit together like a lock and key to have a chemical effect on each other." (E. FISCHER).
- 2. **REALISTIC MODEL**: the catalytic center (and possibly the substrate) is noticeably altered by the binding of the substrate. Complementarity between the substrate and the active center is only established after the binding (this model comes much closer to reality).



Cesare Primultini (pcesare@ethz.ch)

Factors influencing the speed (kinetics) of an enzyme reaction:



- **<u>SUBSTRATE CONCENTRATION</u>** (educts):
  - Simplest enzyme-catalyzed reaction:

$$S \xrightarrow{E} P$$

S	Substrat (Edukt)
P	Produkt
E	Enzym

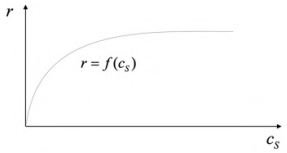
  - Reaction rate r (decrease in substrate concentration over time or increase in product concentration over time):

$$r = -\frac{dc_S}{dt} = \frac{dc_P}{dt}$$

c_S Substratkonzentration

c_P Produktkonzentration

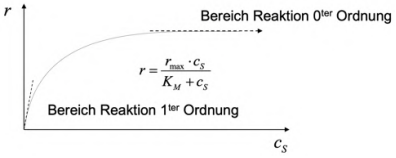
  - Relationship between reaction rate r and substrate concentration:
    - When c_s small: r increases linearly with the concentration.
    - When c_s big: r in doesn't depend on the concentration (saturated)



  - The **Michaelis–Menten** kinetics is one of the best-known models of enzyme kinetics. The model takes the form of an equation describing the rate of enzymatic reactions, by relating **reaction rate <u>v</u>** (rate of formation of product, **[P]**) to **[S]** (the **concentration** of a **substrate S**). The formula is the following:

$$\frac{d[P]}{dt} = \frac{k_2[E]_0[S]}{K_S + [S]} = \frac{r_{\max}[S]}{K_M + [S]}$$

With $\boldsymbol{r_{max}}$ the maximal reaction speed and $\boldsymbol{K_M}$ a measure for substrate affinity (the S concentration at which half-maximal reaction speed is reached).

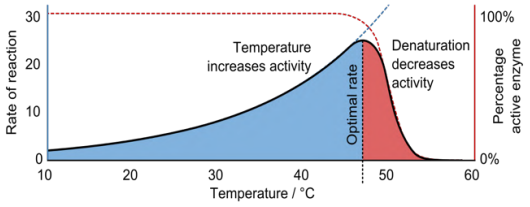


- **<u>TEMPERATURE</u>**:
  - Similar to chemical reactions, the higher the temperature the faster the enzyme reaction.
  - **<u>Arrhenius theorem</u>** to describe reaction rate and temperature:

$$r_{\max} = k_{+2}(T) \cdot c_{E0} = (k_{+2})_0 \cdot e^{-E_a/RT} \cdot c_{E0}$$

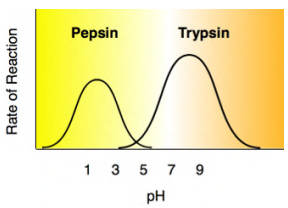
With E_a the activation energy, R the gas constant and T the absolute temperature.

  - An increase in temperature could deactivate the enzyme (**denaturation**).
    - Through denaturation processes a protein changes from the **soluble** form (native form) to the **insoluble form**. During this process, the ordered **secondary** and **tertiary structures** of the protein are **altered** or **destroyed**. As a result, among other things, the **<u>biological activity is lost</u>**.



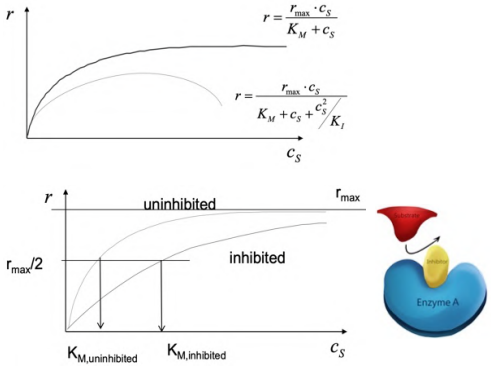
    - The optimal temperature for human enzymes is 37 °C, while for other organism can be very different (even 80 °C for heat resistant bacteria).

- **<u>PH-VALUE</u>**
  - $pH = -log(c_{H3O+})$
  - Reminder: an enzyme is a protein with a 3D ordered chain of amino acids.
  - Each enzyme has an optimal pH.
  - A change in pH can alter the **ionization** (gain or loss of electron, alters the electric charge) of the R groups of the amino acids. When the charges on the amino acids change,



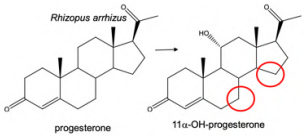
hydrogen bonding within the protein molecule (that make the 3D structure) change and the molecule changes shape. The new shape may not be effective.
  - The **substrate** can also change due to pH concentration. As a result, the **affinity** of the **enzyme** for a certain substrate can vary a lot (better or worse).
  - If the pH changes abruptly from its optimal value, the enzyme could **denature** (folding is irreparably damaged).

- INHIBITION
  - Enzymes can bind with different substances (despite their high specificity), either in the active center or elsewhere.
  - If the substrate and the inhibiting substances are present at the same time, the function of the enzyme could be blocked.
  - Inhibitors can attach to the active center, in the substrate or both locations of the enzyme.
  - Some examples of different types of inhibition:
    - **<u>Inhibition due to excess substrate</u>**: in addition to the already existing substrate (ES), another inactive substrate (ES2) can form. This second substrate blocks the active center of the enzyme.
    - **<u>Competitive inhibition</u>**: when a substrate and an inhibitor have a ***similar structure***, they can concur for the active center. The **maximum reaction speed** remains the **same**. In contrast, the **affinity** <u>between enzyme and substrate</u> is **reduced**, so that higher substrate concentrations are necessary to achieve the maximum speed. The Michaelis constant K_M increases.



Characteristics of ENZYMES:

- <u>Substrate specificity</u>: as a rule, each enzyme only acts on a **specific** substrate; however, the extent of substrate specificity is not the same for all enzymes.
- <u>Region-specificity</u>: property of an enzyme to act only on a specific group (of two similar ones) within a molecule. It is important here that the enzyme does NOT attack the red circled areas - it can therefore be chemically similar but positioned at different places in the molecule.
- <u>Reaction specificity</u>: of several possible reactions of the substrate, only one very specific reaction is catalyzed.
- <u>Enantiospecific and enantioselective</u>: property of an enzyme to convert only a specific optical form of a compound. Enantiomers are couples of molecules that are identic but mirrored.
  - Enantiospecific: from 2 enantiomers only one will be taken.
  - Enantioselective: from 2 enantiomers only one will be built.



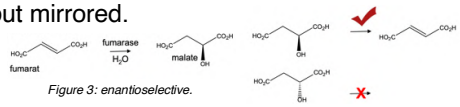


Figure 3: enantioselective.

Figure 2: enantiospecific.

Denomination of ENZYMES:

- Name of the substrate (a molecule with which the enzyme interacts) + "**-ase**" (sucrose is split by the enzyme "sucr**ase**").
- Enzymes with similar effects are grouped together. Group names are obtained by appending the **syllable "-ase**" to the name of the **reaction**:
  - *"Transferases*" transfer molecule groups.
  - *"Dehydrogenases"* have a dehydrating effect.

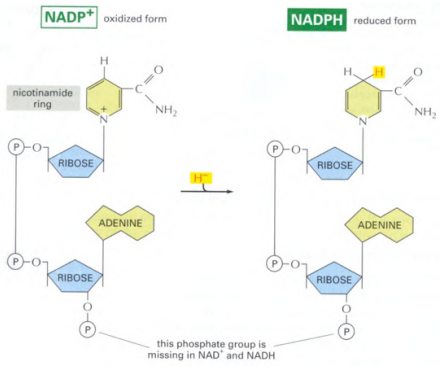
CLASS	DESIGNATION	FUNCTION
EC1	Oxidoreductases	catalyze oxidation/reduction reactions
EC2	Transferases	transfer a functional group (e.g. a methyl or phosphate group)
EC3	Hydrolases	catalyze the hydrolysis of various bonds
EC4	Lyases	cleave various bonds by means other than hydrolysis and oxidation
EC5	Isomerases	catalyze isomerization changes within a single molecule
EC6	Ligases	join two molecules covalent bonds.

Use of ENZYMES in industry:

- **Food industry** for the production of yoghurt, sourdough and for alcoholic fermentation.
- **Detergent industry** for the hydrolysis of proteins.
- **Pharmaceutical industry** for the production of enantiomerically pure active ingredients.
- Amylases are used in the **textile industry**.
- Lipases and proteases are used in the **leather industry** for cleaning leathers.
- Hydrolases and redox enzymes are used in **fine chemistry** for the **production** of **organic substances** (pharmaceutical intermediates) (enantioselective!).

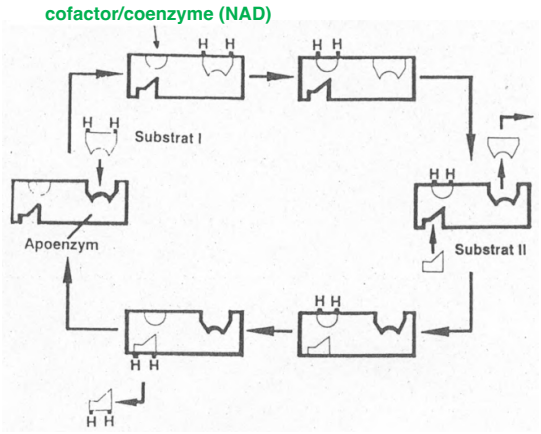
3.2.4. OXIDATION AND REDUCTION SYSTEMS

- The oxidation or reduction state (**redox** state) of an organic molecule can be read, e.g., from its hydrogen atom content. *The more H is present in a molecule per carbon, the more it is reduced.*
- Covalent bond electrons both migrate to the **more electronegative** bond partner, and the resulting "**charge state**" **is the redox state**.
  - CO2 is maximum oxidized carbon (oxidation state C: +4)
  - CH4 is maximum reduced carbon (oxidation state C: -4)
- The higher the **H content** of an organic molecule, the more energy can be gained from its oxidation.
- During the oxidation of **carbon compounds with oxygen**, the organic carbon is brought from a relatively high reduction level (carbohydrates: CH2O, C oxidation level: 0) to the maximum oxidized level of CO2 (+4). The hydrogen that is being released with the electrons is first **taken over by suitable *hydrogen-transferring* cofactors** (which play a central role in redox processes).
  - These can now transfer hydrogen (and electrons) back to other hydrogen-absorbing molecules, which are then reduced.
  - This is a process that happens parallel to ATP (which is a cofactor as well).



REMEMBER: in order to catalyze a reaction, there are two types of enzymes: **with or without cofactors**!!!
Such cofactors can be metal ions or complex organic molecules!!!!

Principle of the hydrogen-transferring process (NAD is part of the enzyme):



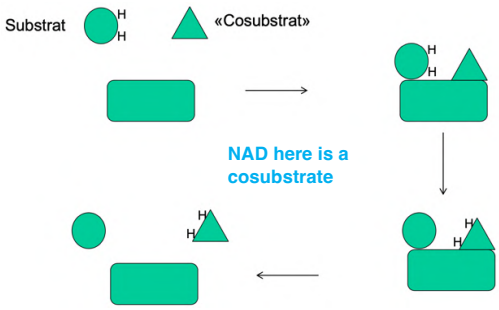
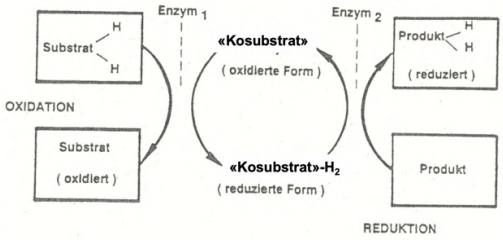


Figure 4: The "cofactor" here is independent from NAD, which acts as a second substrate (and is therefore not part of the protein)

Principle of delocalized hydrogen transfer through cosubstrates:

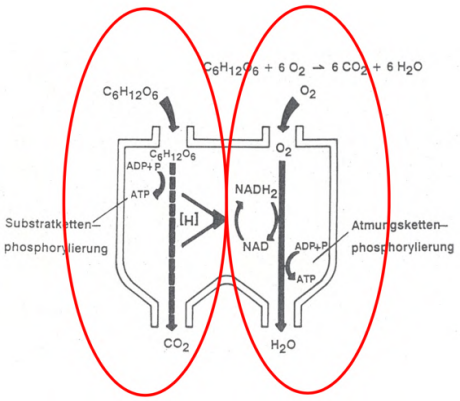
Hydrogen-transferring cofactors/co-substrates
are positively charged nicotinamide residues that can take up 2 electrons and 1 proton.



- There is a similarity with ATP/ADP system (which acts as an energy carrier). The fundamental difference is that:
  - NAD(P) transfers **H-molecules and electrons**
  - ATP transfers **phosphate groups**

Interaction of **energy storage** (ATP formation) and **hydrogen transfer**:

- Example of **glucose degradation** (redox) by aerobic microorganisms (whose basic principles are almost interchangeable with those of other cell types):
  - **Glucose is oxidized**: H is first bound to NAD+, CO2 is formed, a little amount of ATP is formed
  - **O2 is reduced**: H is transferred to O2, H2O is formed, a lot of ATP is formed



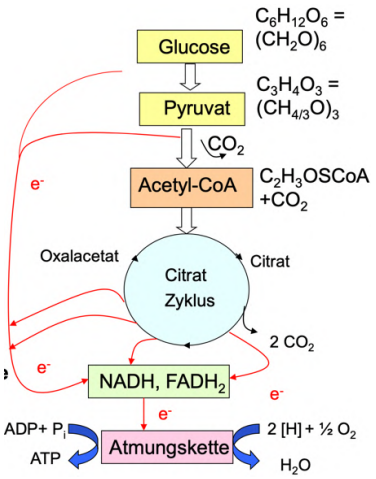
3.3. EXEMPLARY INSIGHT IN CELL METABOLISM

GLUCOSE BREAKDOWN AND CELLULAR RESPIRATION

3.3.1. OVERVIEW OF CELL RESPIRATION AND PRODUCTION OF BUILDING BLOCKS

- What happens to ingested carbohydrates:
  - The source of carbon (e.g., but not only: glucose) is first **broken down** into smaller molecules ("**building blocks**") and some is built up or converted into **new substances**. ("turning into the **anabolism**")
  - In the case of **aerobic degradation** (i.e. under oxygen consumption, oxidative), those **intermediate products** used for energy production are **degraded to the final products carbon dioxide** and **water**. ("passing through the catabolism")
- What is respiration?
  - In a very broad sense: when we breathe, **organic substrates** are **completely oxidized** to **CO2** and **water**, **generating energy**. Formula: C6H12O6 + 6O2 --------> 6CO2 + 6H2O
    - Oxygen as a "terminal" electron acceptor.
    - The energy released in stages is used for ATP formation.
  - To be more precise, the important part of respiration can be found the **sub-process of ATP production from reduced metabolic intermediates**. We will care only about respiration in the biochemical sense (no lungs).

- *Cell respiration and building block production are sub-processes in the complex network of cell metabolism*; **sub-processes**:
  - ***Glycolysis***: takes place in the cytoplasm.
  - ***Citrate cycle***: takes place in mitochondria (eukaryotes) or in the cytoplasm (prokaryotes).
  - ***Respiratory chain*** (respiration in the narrower sense): takes place in mitochondria (inner mitochondrial membrane, eukaryotes) or at the cell membrane (prokaryotes).



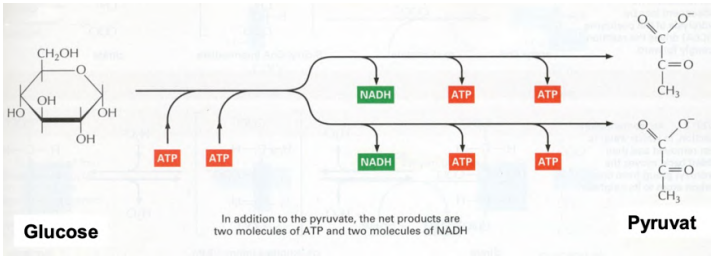
Generally, there are 3 PHASES:

- Phase 1: **Oxidation** of **glucose** to **pyruvate** under **NADH** and **ATP supply** (little) in **glycolysis**.
- Phase 2: Conversion of **pyruvate** into **acetyl-CoA**, introduction into the **citrate cycle**, there **oxidation** of **acetyl groups** and **reduction** of **NAD+** to **NADH** (FADH2) (a lot)
- Phase 3: In the **respiratory chain** the electrons of NADH and FADH2 are transferred to O2, with the **released energy ATP** is **produced.**

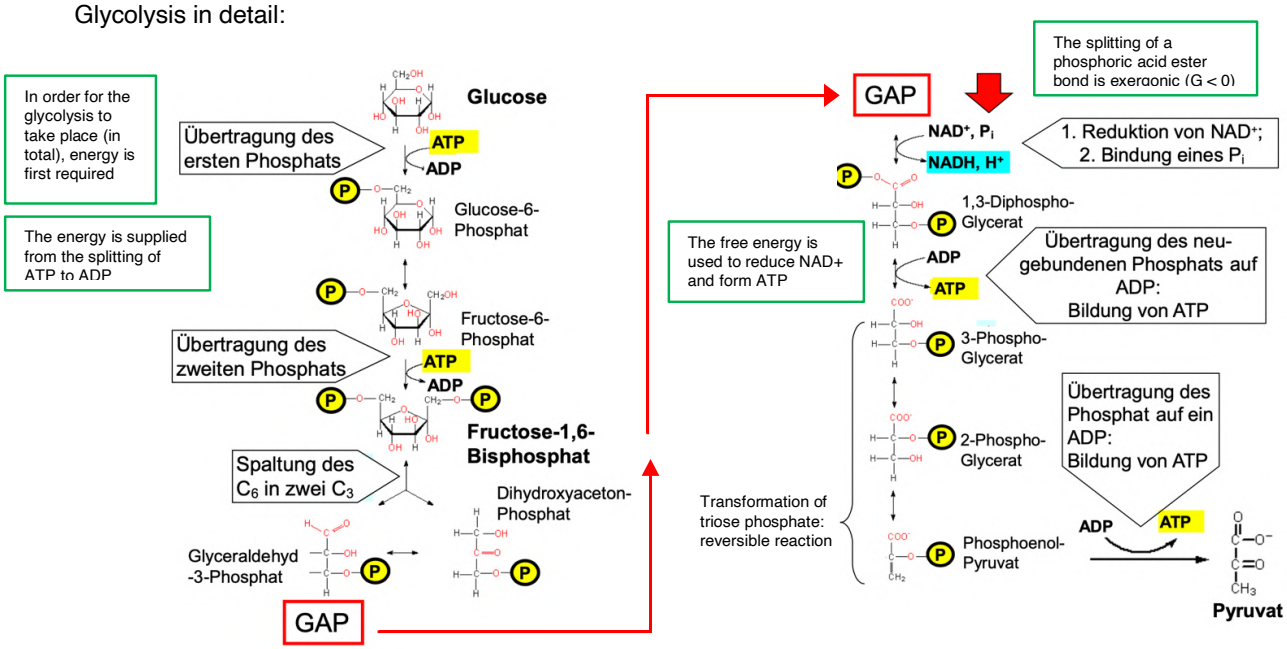
REMEMBER: intermediates from the glycolysis and the citrate cycle are used for the production of cell building blocks.

3.3.2. GLYCOLYSIS: DEGRADATION OF SUGAR

- One molecule of glucose is broken down into two molecules of pyruvate. Part of the released energy is stored in the form of ATP and NADH.
- For every molecule of glucose 2 ATP are produced and 2 NAD+ are reduced to 2 NADH.



Glycolysis in detail:



Seen as a formula, looks like this:

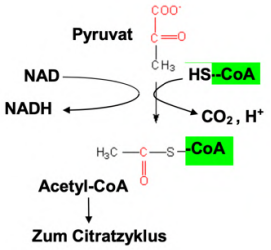
$$Glucose + 2NAD^+ + 2ADP + 2P_i \rightarrow 2\,Pyruvate + 2NADH + 2\boldsymbol{ATP} + 2H_2O$$

- The pyruvate will be used in the citrate cycle and the 2NADH in the respiratory chain.
- Here ATP is formed outside of the respiratory system. This is called substrate-level phosphorylation or substrate-chain phosphorylation (in contrast to respiratory chain phosphorylation, see later). Basically, a PO3-group (phosphonyl) is attached to ADP in order to create a higher-level molecule.

3.3.3. CITRATE/KREBS CYCLE: PRODUCTION OF NADH

The citrate cycle is a sequence of **9 reactions**. In the citrate cycle **all C-atoms** of the **original glucose** are **completely oxidized**. The **energy is stored** in the **form of NADH**, **FADH2**.
First of all:

- **Pyruvate** is **decarboxylated** (loses a CO2).
- This **exergonic reaction** (G < 0) is coupled with the **binding of CoA** (coenzyme A) **to pyruvate** and **NADH formation**.
- The resulting **C-S bond is very energy-rich**, the energy is further used in the citrate cycle



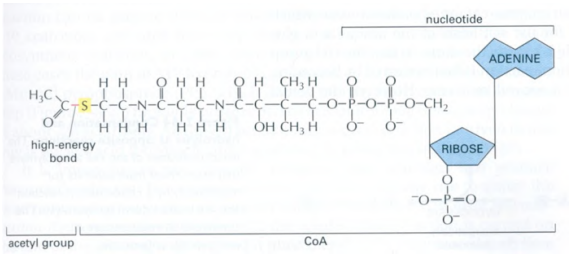
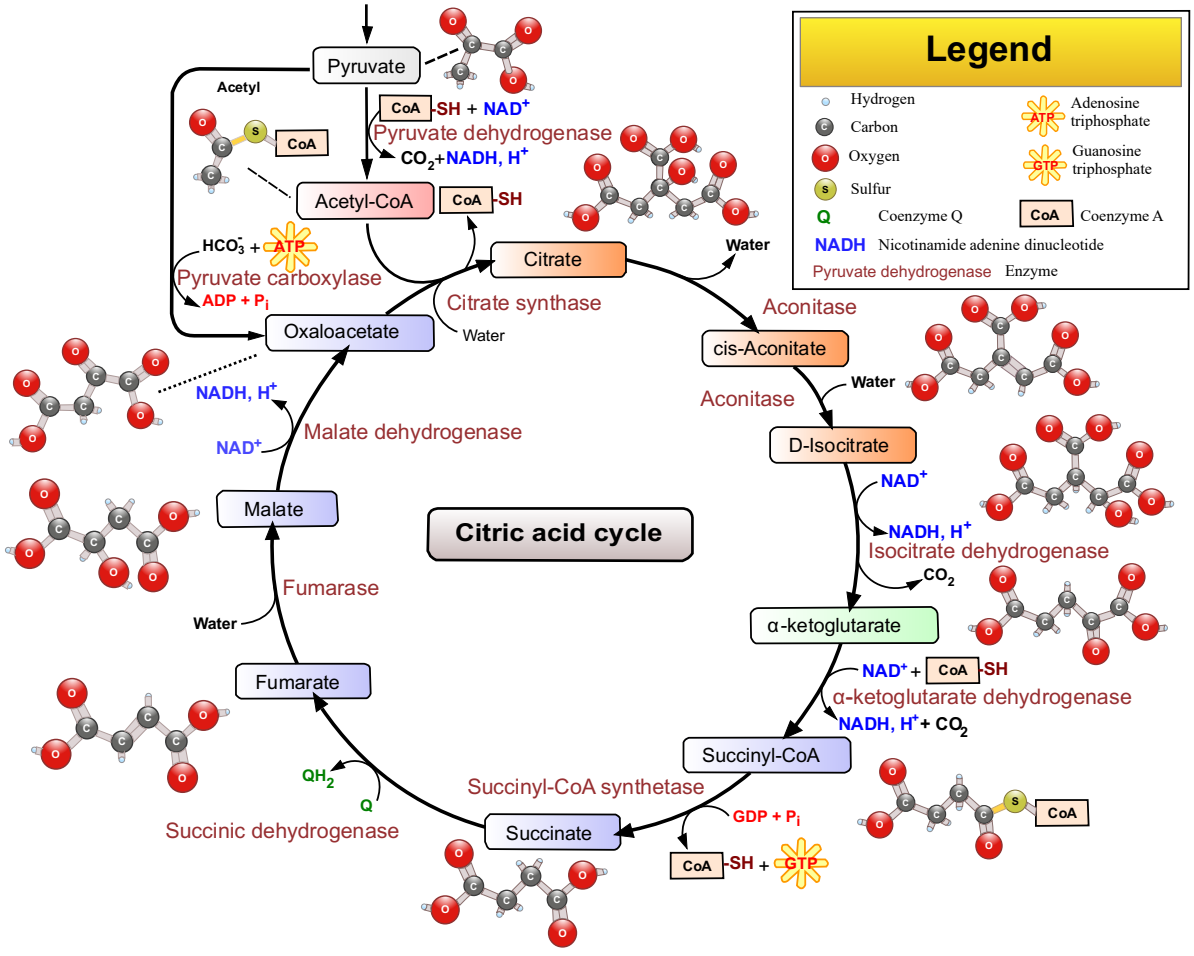


Figure 5: Acetyl-CoA.

- Before entering the actual cycle, pyruvate is converted to acetyl-CoA (1 CO2 is produced)
- **Each cycle produces one acetyl-CoA and 2 CO2**
- **Energy** is **conserved in hydrogen**, in the form of **FADH2** and **NADH**.
- At the end of the cycle the **initial product** of the cycle (**oxaloacetate**) is **regenerated** and **enters another cycle**.

The Krebs cycle in detail (here GTP = ATP, not in reality though):



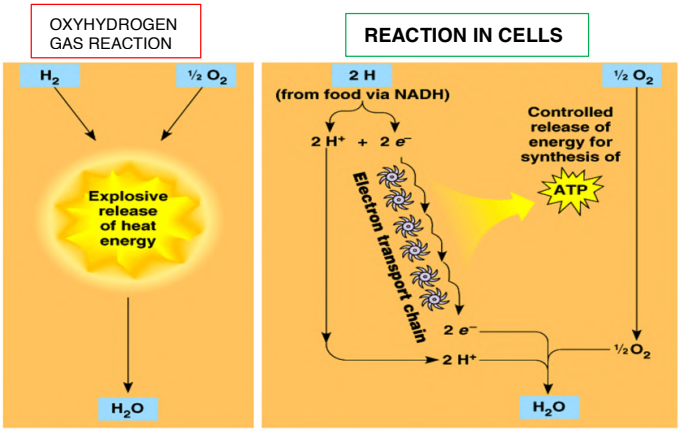
Details:

- Per cycle: **3 NADH** and **1 FADH2**.
- Per glucose molecule: **6 NADH** and **2 FADH2** are therefore formed.
- Almost **all the energy** from the **conversion of glucose** is thus **stored in the form of high-energy electrons**!
- The formula looks like this:

$$2\ Pyruvat + 8\ NAD^+ + 2\ FAD + 2\ ADP + 2\ P_i + 4H_2O$$
$$\rightarrow 6\ CO_2 + 8\ NADH_2 + 2\ FADH_2 + \mathbf{2\ ATP}$$

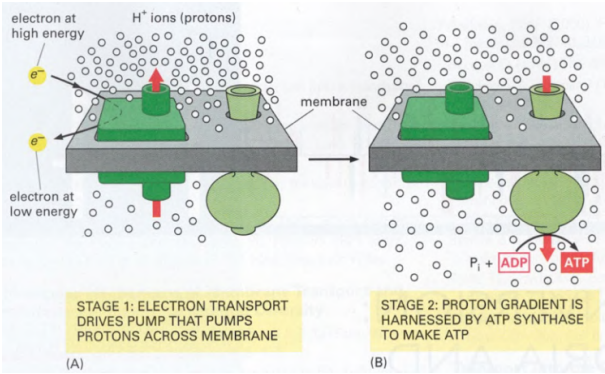
3.3.4. RESPIRATORY CHAIN: PRODUCTION OF ATP

- In the respiratory chain, stored **electrons** (hydrogen) are **combined with oxygen.** Therefore energy is obtained by the reduction of oxygen.
  - Compare:
    - **Oxyhydrogen gas reaction**: a lot of energy is released H2 +1/2O2 → H2O
    - **Reaction in the cell**: a lot of energy released NADH/H+ + 1/2 O2 → H2O + NAD+.
- The **reaction is so rich in energy** that **cells would explode**, so it is broken down into **several harmless partial steps** and the **energy** is "**dissipated**" in between.



Principle of "**CHEMIOSMOTIC COUPLING**" for stepwise energy generation:

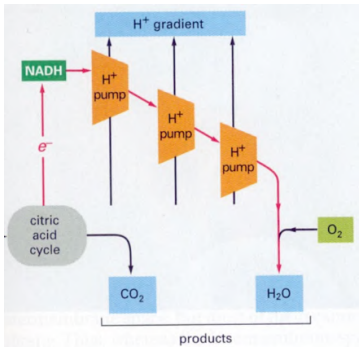
- A **proton gradient** is **produced** from the stored chemical energy (NADH electrons) via a membrane with a built-in **proton pump** (also a form of energy storage!)
- ATP synthase (**ATPase**, an enzyme) uses the **proton gradient** to **produce ATP**.

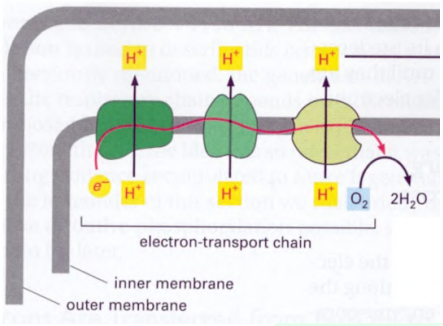


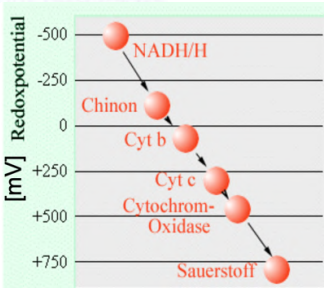
PROTON PUMP

- Electrons **gradually release energy** at **three proton pumps**, each of which is used to **bring protons** from one side of the membrane to the other.
- Afterwards the **electrons**, which are **no longer very energetic**, are **transferred** to the **oxygen**.

Cesare Primultini (pcesare@ethz.ch)



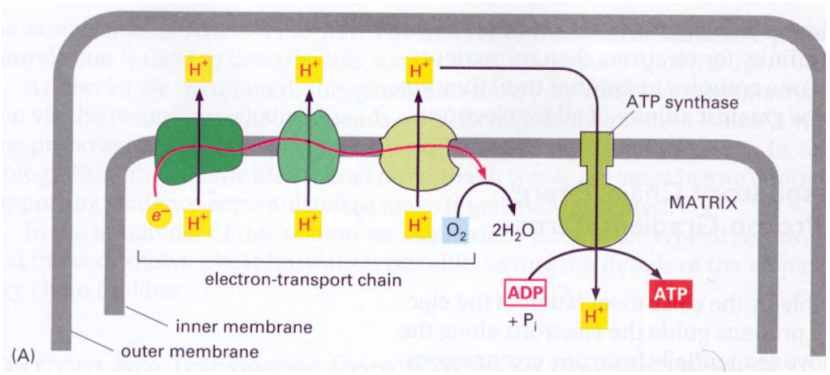


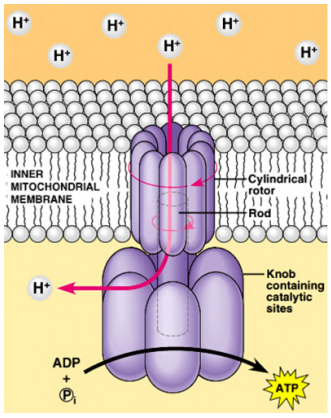


- **Between NADH/H+ and oxygen** there is a very big **difference** in the **redox potential** **(desire to release electrons)**: a lot of energy would be released directly.
- In contrast, the **enzyme complex quinone** has a **redox potential** which is only slightly lower than that of NADH/H+.
- Thus, during an **electron transport from NADH/H+ to quinone**, much **less energy** is **released** than during a (hypothetical) transfer to oxygen.

ATPase

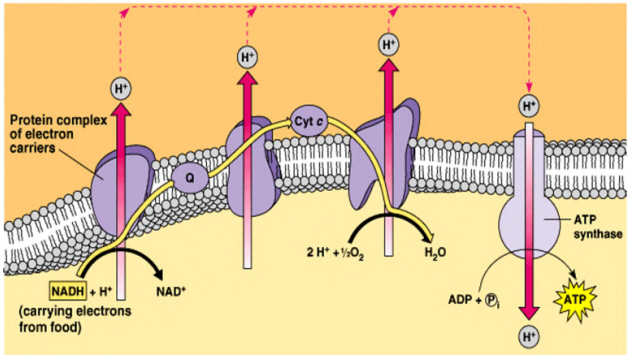
- **Protons diffuse inwards** (inside the cell) following their concentration gradient.
- They must **flow through the ATPase** and ***drive it like water drives a mill*** (quasi poetico)
- **Electrochemical gradient energy** drives **synthesis** of **ATP** from **ADP** and **Pi**.

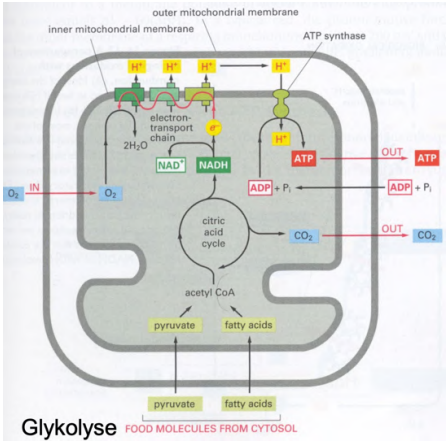




Multiple energy conversion in the ATPase:

- **electrochemical proton gradient** in...
  - **mechanical energy** (rotation of ATPase rotor) in ...
    - **chemical energy** (ATP)





Cesare Primultini (pcesare@ethz.ch)

Summary of the degradation of carbohydrates, seen in formulas:

- 1. Glycolyse (findet im Cytoplasma statt)
  - Glucose +2 NAD^+ + 2 ADP + 2P_i
    - → *2 Pyruvat* + *2 $NADH_2$* + ***2 ATP*** +2H_2O
- 2. Zitronensäurezyklus (findet in Mitochondrien statt)
  - *2 Pyruvat* + 8 NAD^+ + 2 FAD + 2 ADP + 2 P_i + 4H_2O
    - → *6 CO_2* + *8 $NADH_2$* + *2 $FADH_2$* + ***2 ATP***
- 3. Atmungskette (findet in Mitochondrien statt):
  - $6O_2$ +*10 $NADH_2$* + 2 $FADH_2$ + 34 ADP + 34 P_i
    - → 46 *H_2O* + *10 NAD^+* +*2 FAD* + ***34 ATP***

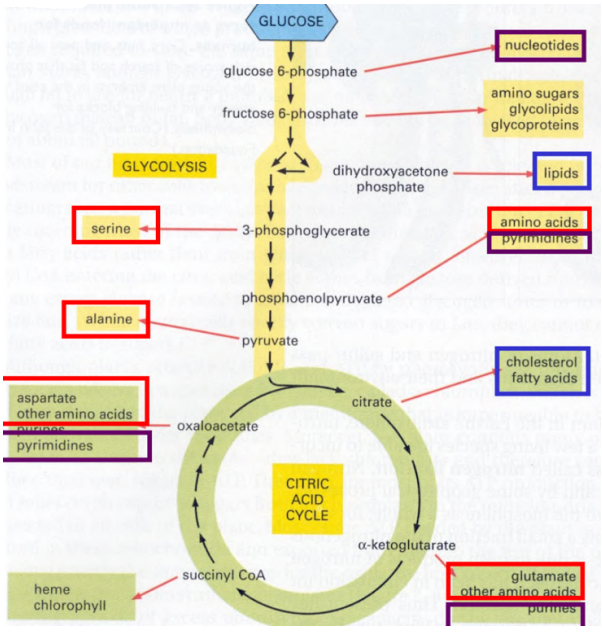
$$C_6H_{12}O_6 + 6O_2\,(+38ATP + 38P_i) \rightarrow 6CO_2 + 6H_2O\,(+38ATP + 38H_2O)$$

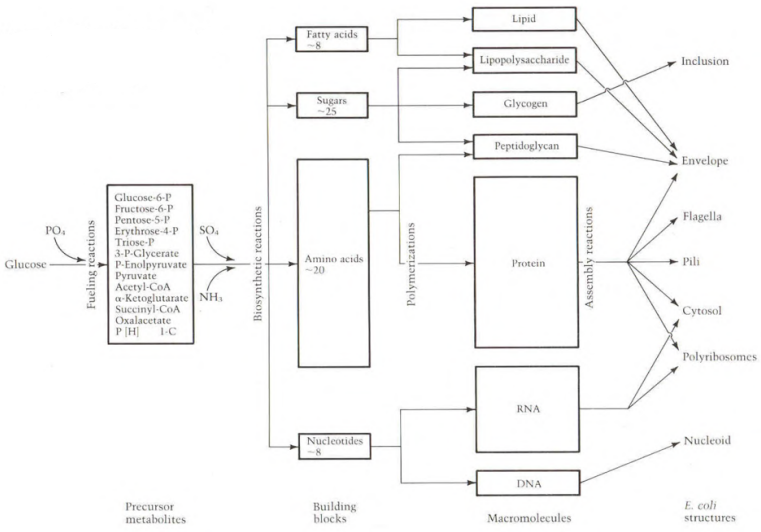
3.3.5. STRUCTURE OF CELL BUILDING BLOCKS

Most important building blocks of the cell:

- Amino acids → Proteins
- Nucleic acids → DNA
- Fatty acid → Membranes

How does the cell produce these building blocks?



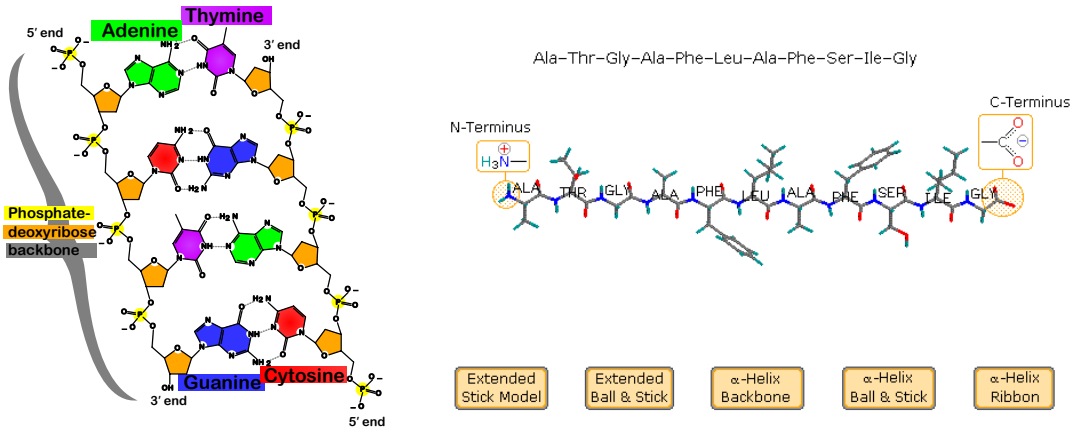


Cesare Primultini (pcesare@ethz.ch)

4. PROTEIN BIOSYNTHESIS

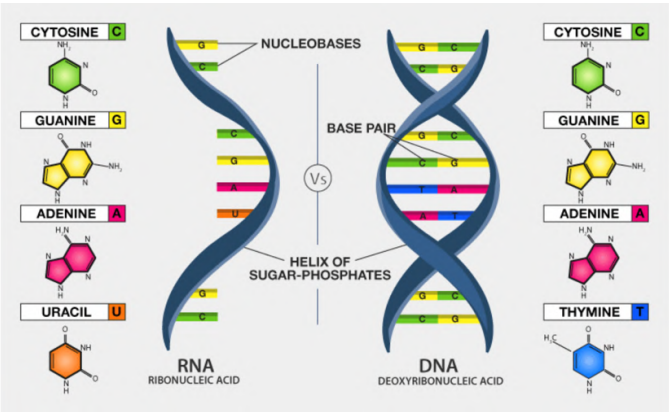
What are proteins?

- **Tools for the cell**: shaping, cell movement, metabolism, transport of metabolites, catalysts, ion pumps, signal transduction etc.
- Proteins consist of **chains of amino acids** put together. The information of this amino acid sequence is contained in the DNA via the base sequence of A, C, G and T (nucleobases).
    - In the picture DNA left and an example of a PROTEIN right:



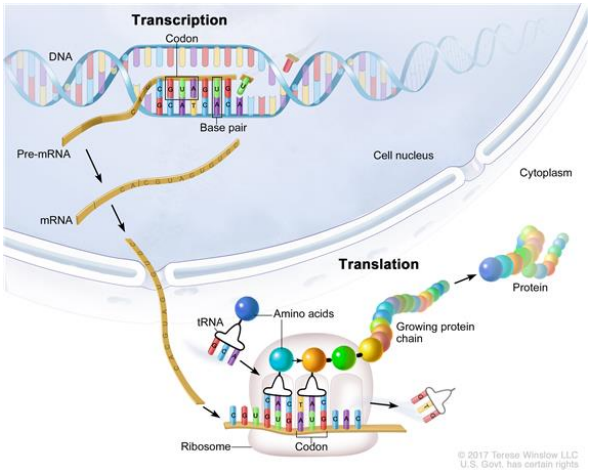
A gene is a DNA sequence that codes for a protein (contains all the DNA info to build a certain protein). DNA and RNA (another polymeric molecule essential for coding, decoding, regulating and express genes). Differences:

- DNA: Deoxyribonucleic acid with the sugar deoxyribose and the four bases, two helixes:
    - Adenine -> **Thymine**
    - Guanine -> Cytosine
- RNA: Ribonucleic acid with the sugar ribose and the four bases, one helix:
    - Adenine -> **Uracil**
    - Guanine -> Cytosine



Process to form a protein:

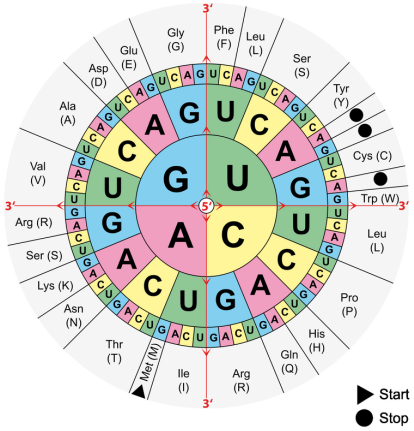
- **Transcription**: RNA-polymerase transfers the message of a gene (composed by DNA) into **mRNA** (a type of RNA that acts like a messenger).
- **Translation**: In the **ribosome** (part of the cell where proteins are built) the information of the mRNA is read. **Three mRNA bases** (***codons***) each encode the incorporation of a specific amino acid. Simply said, bases are seen as pairs of three (TTT, TCG, GTT, AAT etc.…), each pair encodes a specific type of amino acid (gly, cys, arg etc…. can be seen in a past chapter). The sequence of amino acids forms the protein.



The genetic code:

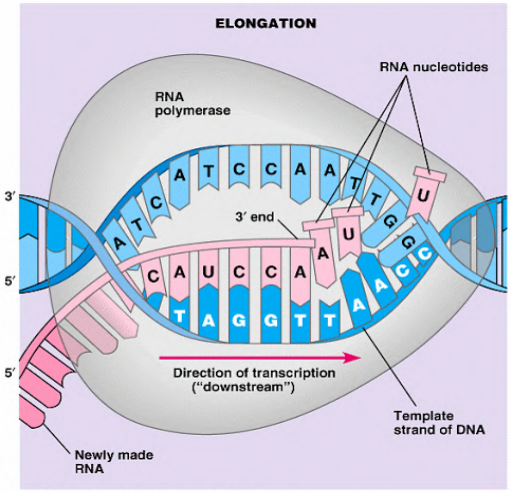
- Assigns the corresponding amino acid to each **triplet** (**codon**, **sequence** of **3 bases**) on the mRNA.
- There are **several codons for one amino acid**, but a **single sequence always codes for the same amino acid**!
- **Amino acids** are **supplied by *tRNAs*** and are strung together in the ribosome. The tRNAs contain the **anticodons** complementary to the mRNA codon (so that it can attach to form the protein).

This creates the **primary** structure (amino acid sequence) of the proteins. The *binding forces between the amino acids fold this amino acid chain into a three-dimensional body* (**secondary** structure, **tertiary** structure): a functional protein is completely assembled!



4.1. TRANSCRIPTION (DNA -> mRNA)

The nucleotide sequences in the genes of the DNA are transcribed into mRNA sequences by the enzyme **RNA polymerase**. The **RNA polymerase** copies the nucleotide sequence of one of the DNA strands according to the **rules of base pairing**. The DNA double strand is partially unwound, and ribonucleotides are attached to the end of the RNA chain.



RNA polymerase consists of 2 parts:

- **Core-enzyme** that *catalyzes* the mRNA-synthesis
- The **sigma factor** *detects* **promoters** (see below).

The RNA polymerase can synthesize mRNA only in 5' → 3' direction and therefore moves on the DNA strand that has direction 3' → 5' direction.

Promoters

- specific DNA sequences that are **located** at the **5' end in front of a gene** and signal the **starting point** of the transcription.
- These specific DNA sequences often have the **base sequence TATAAT**. The TATAAT sequence is **recognized by the sigma factor**.
- The RNA polymerase **starts transcription** as a **holoenzyme** (type of enzyme, all **subunits** are operating; subunits are parts of the RNA).
- After a certain transcribed distance, the **sigma factor decreases**, and the nuclear enzyme continues to transcribe alone (promoter defines the point from which mRNA is transcribed).

Terminators:

- Terminators are specific DNA sequences **located** at the **3' end** after a gene and are responsible for **stopping transcription**.
- They consist, for example, of **repeating DNA sequences**.
- After the RNA polymerase has passed the terminator sequence, **secondary structures** are formed **in the mRNA** produced, which **slow down** and **stop further transcription** by the RNA polymerase.
- The **nuclear enzyme falls off the DNA**.

4.2. TRANSLATION (mRNA -> PROTEIN)

The **mRNA sequences** are **translated** into **amino acid sequences** of proteins. The genetic code on the mRNA sequence determines the sequence of amino acids in the protein. **Ribosomes bring** the **mRNA together with the matching tRNAs**. Structure of the mRNA:

5' untranslated leader- AGGAG - (7-9 bp) - AUG - (AS-Codons für das Protein) - UAA / UAG / UGA - 3' untranslated leader

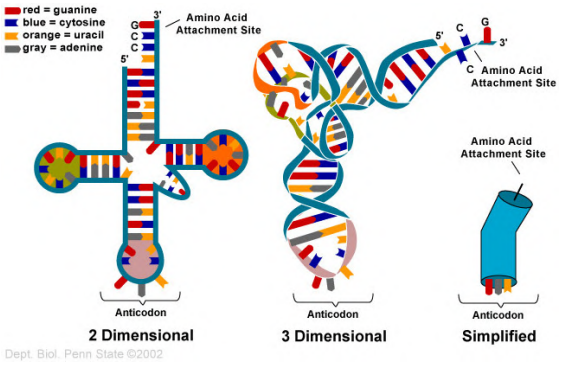
- The **ribosome binds to the base sequence AGGAG** (***Shine-Dalgarno*** sequence or *ribosome binding site*).
- The translation **always starts** with the **start codon AUG**, which codes for the amino acid ***methionine***.
- One amino acid after the other is then added to the amino acid methionine, matching the following codons of the mRNA. **UAA, UAG or UGA** are **stop codons**, which signal the *end of the translation*.

RIBOSOMES:

- Ribosomes consist of **two subunits**: 30S and 50S,
- The **complete and active ribosome** has a size of 70S (S: sedimentation coefficient).
- Each of these subunits consists of **ribosomal RNA** (**rRNA**) and **proteins**:
  - the **smaller** 30S subunit consists of 16S rRNA and 21 proteins
  - the **larger** 50S subunit consists of two different rRNAs (5S and 23S rRNA) and 34 proteins.

tRNA

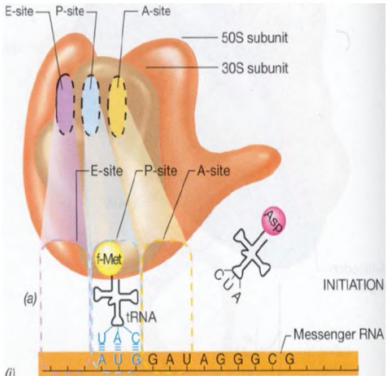
- tRNA is a **mediator between mRNA** and the **ribosome**. One part of the tRNA has a specific amino acid, in the other part, the anticodon (3 bases), which **perfectly matches** a **codon** on the **mRNA**.
- The tRNA is **single-stranded** and has a **characteristic secondary structure** (**cloverleaf**). The tRNA anticodon in the anticodon strain recognizes the codon on the mRNA. On the other side is the acceptor strain, which is loaded with the corresponding amino acid.



TRANSLATION is subdivided in 3 main parts:

- Initiation
- Elongation (polypeptide chain gets longer)
- Termination

4.2.1. INITIATION



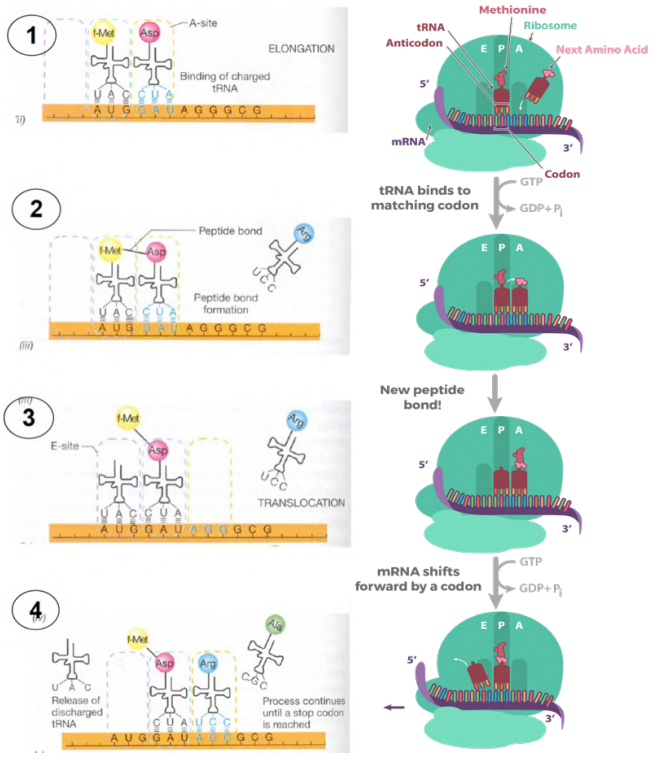
- Formation of the initiation complex, which consists of **30S subunit** (of the ribosome)**, mRNA and methionine tRNA**.
- The **Shine-Dalgarno** sequence **5'-AGGAG-3'** of the **mRNA** is **bound to the complementary** region (5'-CUCCU-3') **of the 30S subunit**.
- The **50S subunit** then **attaches** to the **initiation complex** and the **active 70S ribosome is formed**. With the *binding* of the *methionine tRNA* via the *anticodon (5'- CAU-3')* to the *start codon of the mRNA (5'- AUG-3')* in the **P-site** the initiation ends and goes into elongation.

4.2.2. ELONGATION

- **Three binding sites** for tRNAs are present on the 50S subunit:
  - A tRNA loaded with an amino acid is bound to the acceptor site (**A-site**).
  - At the peptide site (**P-site**) the growing peptide chain is bound to a tRNA.
  - At the exit site (**E-site**), the tRNAs that have already delivered their amino acid are released from the ribosome.

The elongation takes place in the following four steps:

- A **new tRNA loaded** with an **amino acid** (here asparagine) is **bound** at the **acceptor site**. The methionine tRNA is bound at the **peptide site** (where the proteins forms and gets longer).
- **Simultaneously** with the attachment of the **peptide bond**, the **ester bond** between **tRNA** and **amino acid** (**Met**) is **released**. The peptide now attaches to the tRNA, which is located at the acceptor site.
- The **loaded tRNA** (acceptor site) is shifted by 3 base pairs (1 triplet) to the peptide site (left) by shifting the ribosome along the mRNA. The **unloaded tRNA** migrates to the exit site and is **released**.
- The **acceptor site** is **now free again** for the binding of a **new loaded tRNA** (here alanine) and steps **1 to 3 are repeated until** there is a **stop codon** of the mRNA **in** the **acceptor site**.

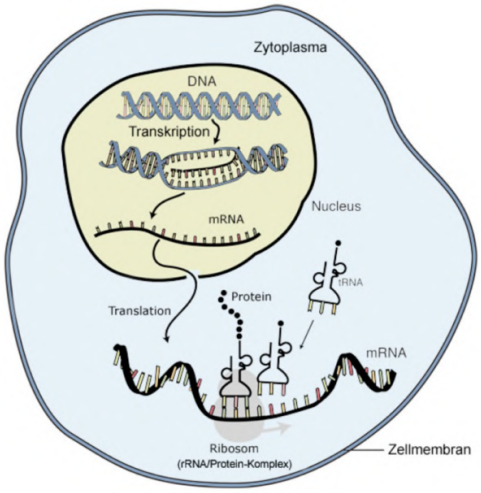


4.2.3. TERMINATION

- Termination occurs if a stop codon (UAA, UAG or UGA) of the mRNA is located at the acceptor site.
- For these **stop codons** there are **no tRNAs** and the **protein biosynthesis stops**.
- Certain proteins then cut the finished peptide from the last tRNA. Subsequently, the **active ribosome disintegrates** again **into its subunits**.

Table 14.2 Types of RNA

Type of RNA	Functions in	Function	Size
Messenger RNA (mRNA)	Nucleus, migrates to ribosomes in cytoplasm	Carries DNA sequence information to ribosomes	**very different, depends on the protein they are building (hundreds to thousands)**
Transfer RNA (tRNA)	Cytoplasm	Provides linkage between mRNA and amino acids; transfers amino acids to ribosomes	**80-95 nucleotides**
Ribosomal RNA (rRNA)	Cytoplasm	Structural component of ribosomes	**3 types: 120, 1540 or 2900 nucleotides**



Cesare Primultini (pcesare@ethz.ch)

5. REGULATION OF CELL METABOLISM

Regulation of cell metabolism is **essential** to **control** the many **chemical reactions** in a cell, to ensure **maximum use** of the **available resources** and to coordinate developmental processes. A **biological system** always tries to be reasonably **balanced**.

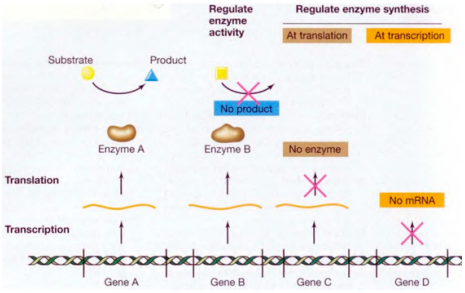
- **HOMEOSTASIS**: *self-regulation of a biological system in **dynamic equilibrium** to **protect** against **changing environmental** conditions.*

The **flow of substances** in the cell metabolic pathway is **mainly determined** by the **catalytic activities** of the **enzymes involved**. To **control the flow of substances**, it is sufficient to **change the catalytic activity** or ***concentration of the enzyme that catalyzes*** the decisive step in the reaction chain

the key enzyme = **regulatory enzyme**.

There are mainly two metabolic levels:

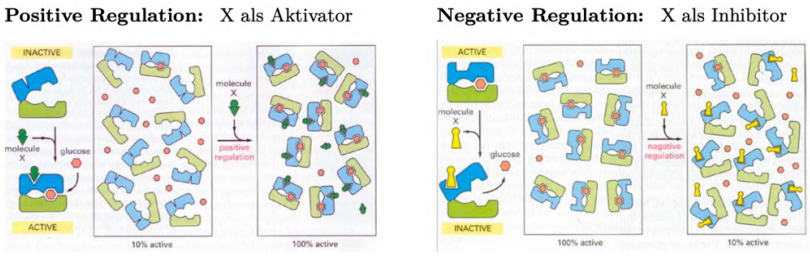
- 1st metabolic level: regulation of the **enzyme activity** of the already synthesized enzyme, serves to **fine-tune** the **metabolic processes** → ***fast!***
- 2nd genetic level: regulation of the **enzyme concentration** by **controlling** the **translation** and **transcription** of the **key enzymes** → ***slow!***



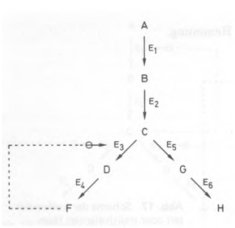
5.1. FIRST METABOLIC LEVEL (regulation of enzyme activity, fast)

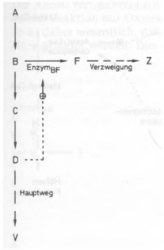
Allosteric enzymes:

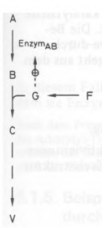
- Their **enzymatic activity** can be inhibited/activated by specific molecules (allosteric **inhibition** or **activation**).
- Inhibition or activation by **reversible attachment of effectors** (small molecules that selectively bind to a protein and regulate its biological activity) **to these enzymes** (inhibitors or activators).
  Allosteric enzymes have two specific binding sites:
  - 1. for **substrate** (*active center*)
  - 2. for **effector** (*regulatory center*)
- **Binding** of the **effector** (X in the drawing) leads to **positive** or **negative conformational changes** in the **protein**.



- ***Effectors** attach to a site in the enzyme and act as a button that **regulates enzyme activity**.*
- Allosteric enzymes have several **subunits** that provide mutual influence when binding with activator/inhibitor (cooperative allosteric change).







- **Left**: Redirection of the metabolic flow into a branching (pathway splits in 2 ways).
- **Middle**: Opening of a bifurcation in case of excess of the main pathway (too much V).
- **Right**: An allosteric activator (G) promotes the formation of a substance (B) which is required for its conversion.

Cesare Primultini (pcesare@ethz.ch)

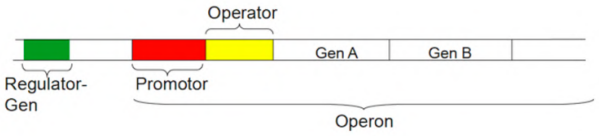
5.2. SECOND METABOLIC LEVEL (regulation of enzyme concentration, slow)

Only a fraction of all synthesizable proteins is needed by a given cell at any given time. Think about this: *DNA contains all the information to produce any type of molecule that the body needs; nevertheless, we don't want to produce hydrochloric acid in your eyes, that acid is useful only in the stomach. Therefore, we need to regulate the production of proteins by regulating the genes in the DNA.* **Here though, the regulation happens at enzymatic level, in order to speed up or slow down processes**.

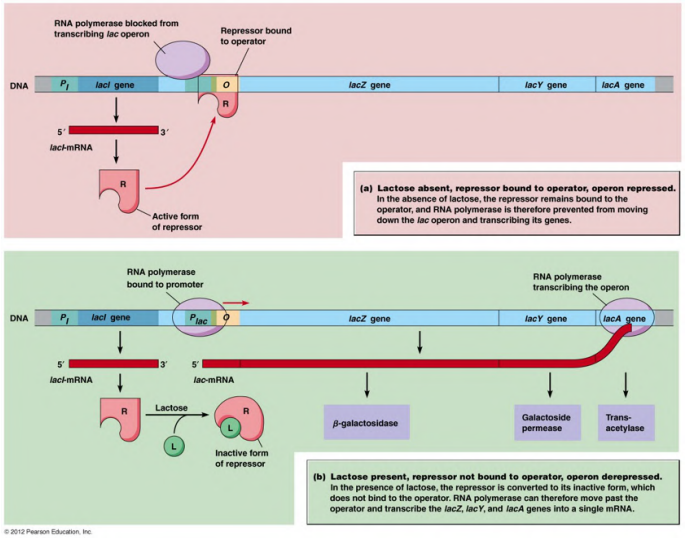
Enzymes can be seen as:

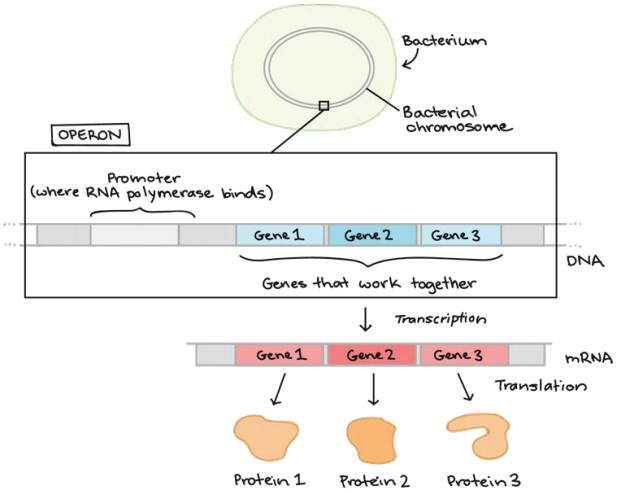
- **Constitutive enzymes,** that are always produced to the same extent regardless of external factors.
- **Adaptive enzymes**, which synthesis is initiated (induced) or interrupted (repressed) depending on external conditions.

An **operon** is a **complete unit of gene expression**, which contains the genes (gene A, gene B) coding for mRNA and thus for a specific enzyme, as well as the corresponding control elements (promoter, operator).



- **Promoters** are **regions of the operon** to which the **RNA polymerase can dock** and **start the synthesis** of **mRNA**. The synthesis of regulatory proteins is performed by the regulator gene.
- **Operators** (*control elements*) are **DNA sequences** (literally clusters of genes that produce certain proteins) to which **regulatory proteins** (repressors or activators) bind and thus **control the operon**. If an operator is bounded to a repressor, synthesis doesn't start and vice versa.



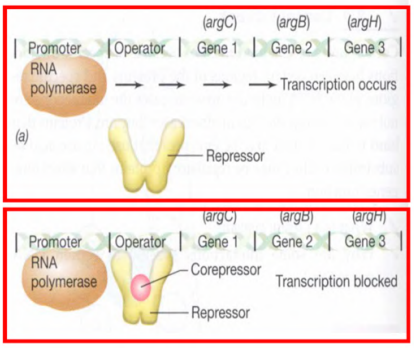


A distinction is made between 2 regulatory mechanisms: repression and induction:

5.2.1. REPRESSION

The **operon is transcribed** if the **repressor does not bind to the operator**. As soon as a **corepressor binds** to the **repressor**, the **repressor** can **bind** to the **operator** and suppress the transcription. The **mRNA** and the **corresponding protein cannot** be **synthesized**.

- **Corepressors** are usually **end products** of a biosynthesis (protein synthesis) or part of the nutrient supply.



Enzyme repression takes place **mainly in metabolic pathways** that **require many different enzymes** (e.g. *biosynthesis of amino acids*). The **repression is specific**, only this biosynthetic pathway is affected. Enzyme repression is very important because it ensures that no energy is wasted for the synthesis of unused enzymes.

Cesare Primultini (pcesare@ethz.ch)

5.2.2. INDUCTION

NEGATIVE:

An **inductor** can **bind to the repressor** and thus **cause its detachment from the operator**, so that the **RNA polymerase** is **no longer blocked** and the mRNA and the corresponding protein can be synthesized.

- Thus, an **induction of gene activity** leads to an **induction or increase** in the **formation** of an **enzyme** by an **inducer** (usually **in the nutrient supply**).

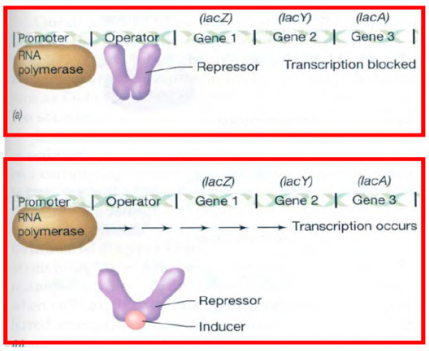
POSITIVE:

An **inductor** binds to **activator**, which can then **dock to the DNA**, which in turn **enables the RNA polymerase** to bind to the **promoter**, **transcription** happens.

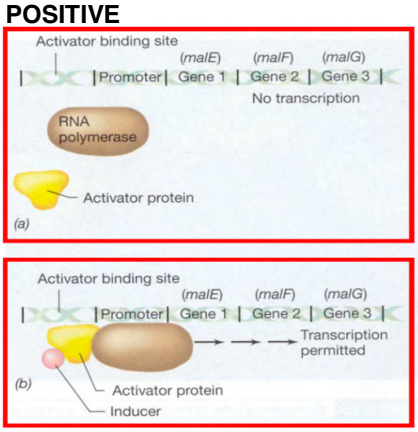
Example: regulation of maltose catabolism: Only when maltose is present and binds to activator can the activator bind to DNA and thus activate the synthesis of enzymes for the utilization of maltose

- Maltose acts as an inductor.
- Often happens that the **molecule,** that needs the enzymes produced by the operator in order to be broken down, **acts as the inductor** (to unblock or activate the enzyme production). So, it can activate only when is needed.

NEGATIVE



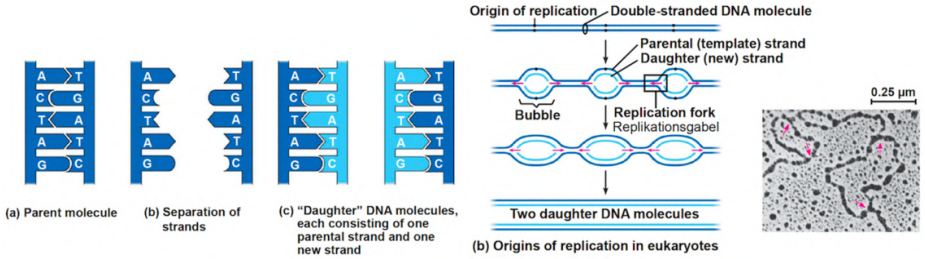
POSITIVE



Cesare Primultini (pcesare@ethz.ch)

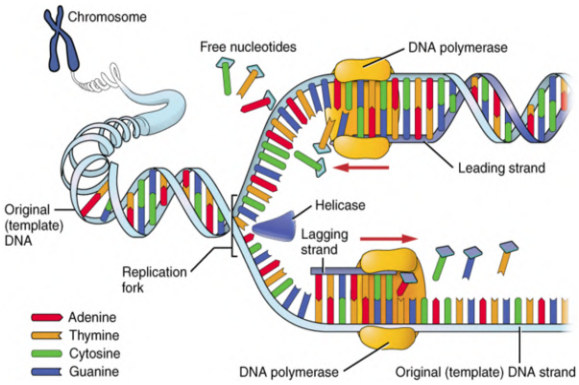
6. DNA REPLICATION

Semi-conservative model (Watson & Crick): DNA **double strand is separated** into single strands, on which new complementary strands are then formed.



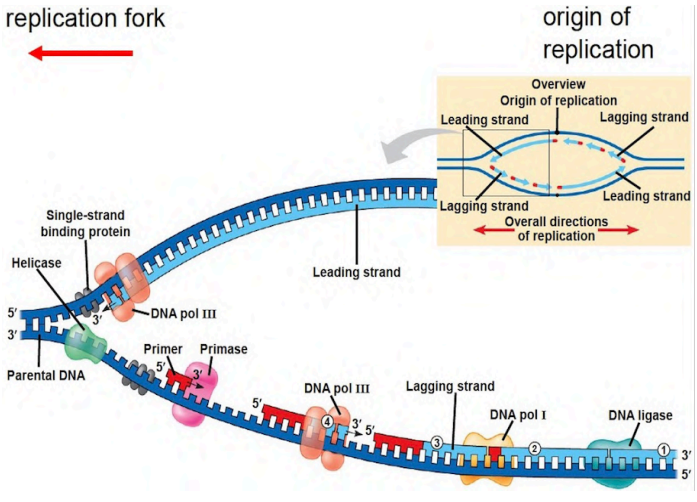
- The replication of DNA begins at the **replication origin** (eukaryotic chromosomes: hundreds of thousands of replication origins; prokaryotic chromosomes: usually only one).
- During the **separation of the DNA strands** a **replication bubble is formed**.
- Replication occurs in both directions in the bubble until the entire molecule is copied. The **Y-shaped area** where new DNA strands are formed is called the **replication fork.**

Antiparallel Elongation: strands of the double helix are oriented in opposite directions (5' to 3' and 3' to 5').



Leading strand: can be synthesized continuously because the **DNA polymerase works** in the **same direction as the replication fork opens**.
Lagging strand: has to be synthesized discontinuously because the **DNA polymerase** can **only work** in the **opposite direction to the opening of the replication fork**. This results in the formation of individual segments (**Okazaki fragments**), which are then **bound by the DNA ligase**.

Helicase: **unravels double helix**, separates double strand into single strands.
Topoisomerase: **prevents additional twisting** of the DNA.
Single strand binding proteins: bind and stabilize single strands.
Primase: **synthesizes RNA primers** at the **5'-end of the lead strand** and at the 5'-end of each Okazaki fragment of the subsequent strand (primase does not need a 3'-OH end as starting point).



DNA polymerase III: synthesizes complementary DNA strand to original strand, deoxyribonucleotides are placed at the 3' end. ***The DNA polymerase can only work in one direction (5' to 3')!***

DNA Polymerase I: **removes RNA primers** and **replaces them with DNA nucleotides**.

Cesare Primultini (pcesare@ethz.ch)

NORMAL CORRECTION AND REPAIR:

- **DNA polymerases check** and **correct** newly synthesized DNA, replacing wrong nucleotides. This correction is **usually error-free**, but if an error in base pairing should nevertheless occur (rarely), these are **corrected by mismatch repair enzymes**.

MISMATCH CORRECTION

- **DNA** can be **damaged by chemicals**, radioactive **emissions**, **X-rays**, **UV** light and certain molecules (e.g. in tobacco smoke). If the DNA is damaged in this way, during **nucleotide-excision-repair (NER)** the ***nuclease*** (enzyme capable of splitting bases of DNA) cuts out the damaged DNA strands.
- **DNA polymerase fills up missing nucleotides** and **DNA ligase connects** the **last newly inserted** nucleotide with the intact strand.

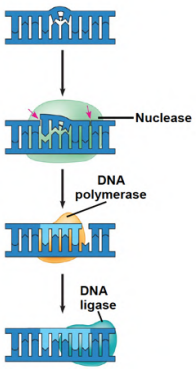
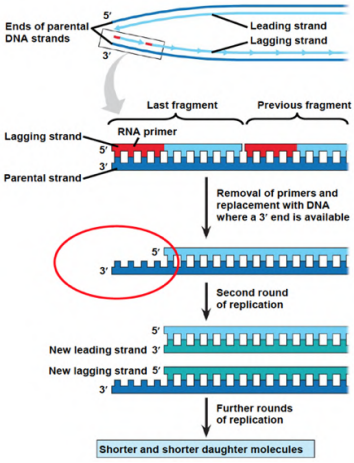


Figure 6: mismatch repair.

During **chromosome replication**, the DNA polymerase cannot continue their duplication of DNA all the way to the end of a chromosome (the 5' of DNA and 3' of the replica), so in **each duplication** the **end of the chromosome is shortened**.
This is because the **synthesis of Okazaki fragments** requires **RNA primers attaching ahead on the lagging strand**. To prevent gene and information loss telomeres come to help.



TELOMERES: ***non-coding, single-stranded ends of eukaryotic chromosomes***. With **each cell division**, the **telomeres shorten**, but this **delays the loss of genes near the ends of the chromosomes**.
Telomeres are associated with cell ageing and also with the development of cancer.

Telomerase:

- enzyme that **compensates for the shortening of telomeres**. However, it is **only active** in **germ cells**, **stem cells**, **cells of the immune system**, **cancer cells** and **unicellular organisms**. In germ cells (e.g. sperm cells),
- the **telomerase prevents** the **loss of essential genes** in the **gametes** (in bisexual organisms: egg cells and sperm cells).
- by **limiting** the **number of cell divisions**, the **shortening of the telomeres** could be a **protective mechanism against cancer growth**. In **cancer cells the telomerase is active** and thus **helps** the **cancer cell** to **divide infinitely often**.

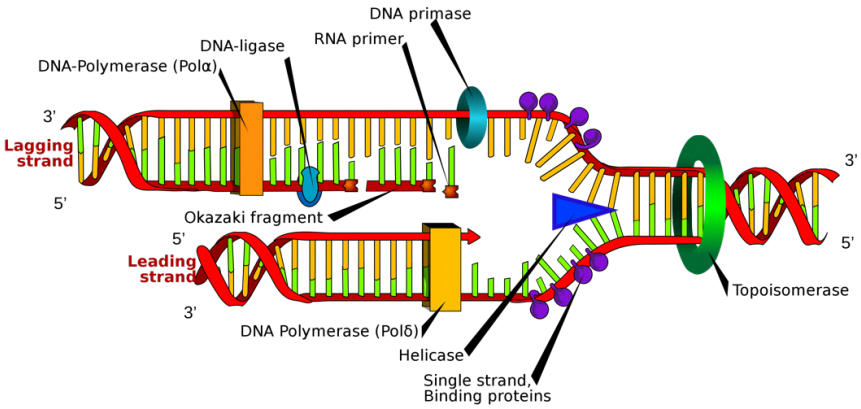


Table 16.1 Bacterial DNA Replication Proteins and Their Functions

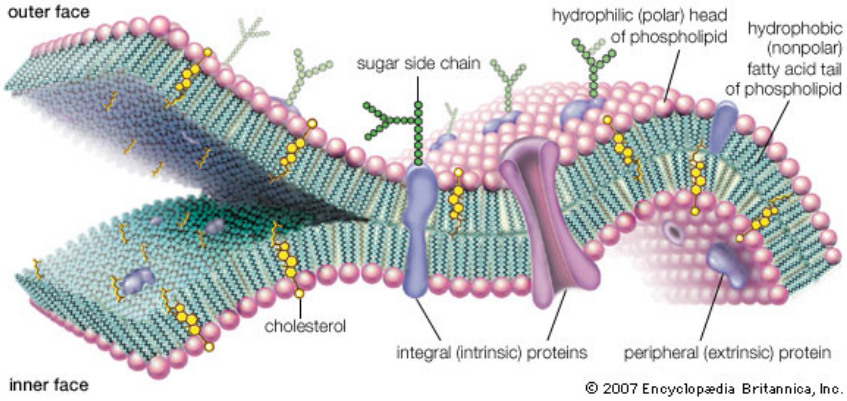
Protein	Function
Helicase	Unwinds parental double helix at replication forks
Single-strand binding protein	Binds to and stabilizes single-stranded DNA until it can be used as a template
Topoisomerase	Relieves "overwinding" strain ahead of replication forks by breaking, swiveling, and rejoining DNA strands
Primase	Synthesizes an RNA primer at 5′ end of leading strand and of each Okazaki fragment of lagging strand
DNA pol III	Using parental DNA as a template, synthesizes new DNA strand by covalently adding nucleotides to the 3′ end of a pre-existing DNA strand or RNA primer
DNA pol I	Removes RNA nucleotides of primer from 5′ end and replaces them with DNA nucleotides
DNA ligase	Joins 3′ end of DNA that replaces primer to rest of leading strand and joins Okazaki fragments of lagging strand

Cesare Primultini (pcesare@ethz.ch)

7. BIOLOGICAL MEMBRANES

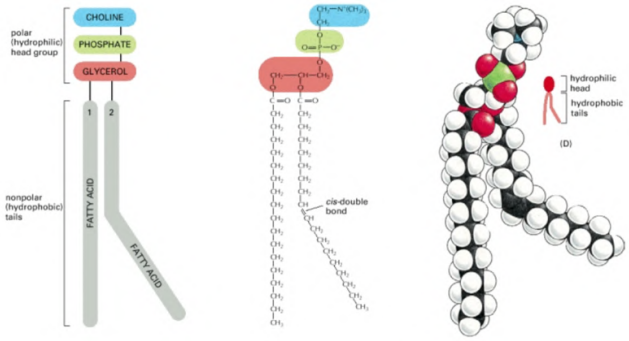
- **Uniform structure**:
  - They consist of a continuous (approx. 6 nm thick) **double layer** of **amphiphilic phospholipids** in which proteins are embedded.
  - Some membranes carry **carbohydrates bound to lipids** or **proteins** on the outside.
  - Membranes are **mobile (non-rigid and modifiable)**.

- **Liquid mosaic model** basically says that membranes are like a mosaic of different types of molecules (glycolipids, phospholipids, transmembrane proteins etc.…) that are not bound together in a rigid way but act more like an oily fluid (if a molecule pops out, the membrane will fill the gap).



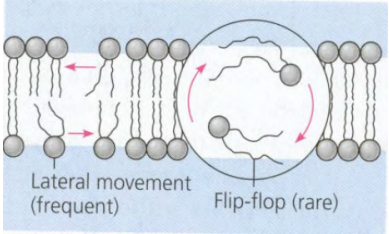
7.1. MEMBRANE LIPIDS (phospholipids, cholesterol and glycolipids)

7.1.1. PHOSPHOLIPIDS

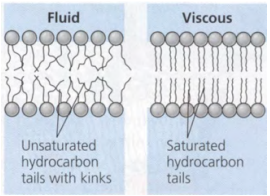


- Phospholipids are **complex esters** (derived from carboxylic acids -COOH with the H replaced by some kind of hydrocarbon group C_xH_y) and **highly amphiphilic** (strong hydrophilic and hydrophobic part) molecules:
  - **Polar hydrophilic head**: formed from glycerol, phosphate and an amino alcohol.
  - **Nonpolar, hydrophobic tail**: two fatty acids (saturated/unsaturated)
    - Saturated: double bond and bent.
    - Unsaturated: single bonds.

- They carry a phosphate group attached at the C3 atoms of glycerol (see picture).



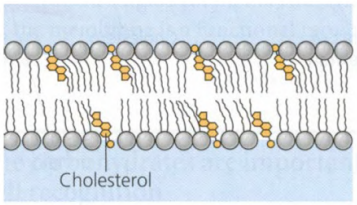
- Phospholipids form a **double layer** which is **hydrophobic on the inside**:
  - Membranes are formed by phospholipids which are **held together** by **hydrophobic and weak Van der Waals forces**.
  - Phospholipids are **not placed rigidly**, but they **can be moved** against each other.

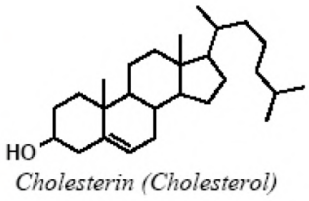


- **Membrane fluidity** is **regulated by phospholipids**:
  - Double bonds in the unsaturated fatty acids of the phospholipids make the membrane looser and thus more fluid. The bend makes the arrangement of phospholipids less dense.
  - Vice versa single bonds let a denser arrangement possible.

7.1.2. CHOLESTEROL

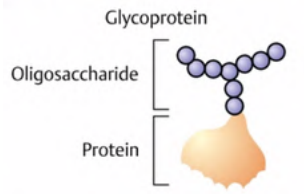
- In mammals plays the important role of **cell fluidity regulation** and **reduces the permeability** of the cell by packing phospholipids tighter.
- At **high temperatures**, **cholesterol reduces fluidity** (phospholipids become more rigid), whereas at **low temperatures**, cholesterol **prevents gel-like solidification** (due to disturbance of the regular phospholipids). Cholesterol thus **stabilizes membranes**.

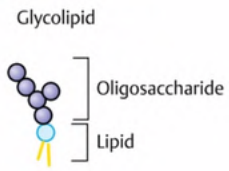


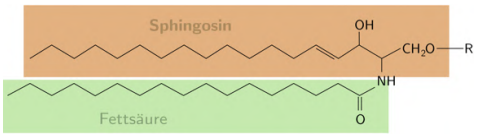


7.1.3. GLYCOLIPIDS

- They are present on the **outside of the membrane**, together **with the glycoproteins** form the **outer cell envelope**.
- Glycolipids consist of **sphingosine**, a **fatty acid** and a very large **oligosaccharide residue** (R) (→ green ball chains in the first chapter image).
- Chemical structure looks like this:







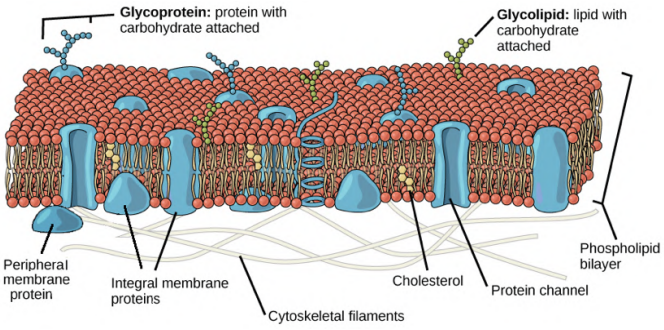
7.2. MEMBRANE PROTEINS

Integral proteins:

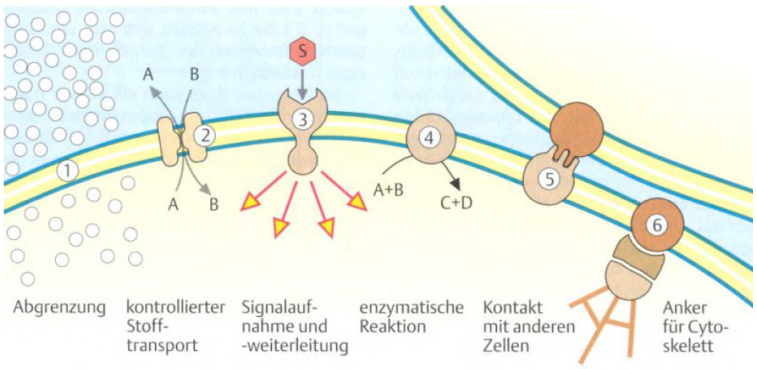
- They penetrate into the hydrophobic core of the phospholipid bilayer.
- They can be **integrated in one side** of the molecule or **span the membrane from in to out.**
- **Transmembrane proteins:**
  - They **span the double layer completely.**
  - Divided in two parts:
    - **hydrophobic, transmembrane α helices** in the **hydrophobic core** of the **membrane**
    - **non-helical, hydrophilic** segments of the protein in **contact with aqueous** phase on **both sides** of the **membrane**.

Peripheral proteins:

- They are associated with the **surface of the membrane** on the **intra- or extracellular side.**
- Binding can be achieved either by **interaction with integral membrane proteins** or with the **polar head groups** of the **membrane lipids**.

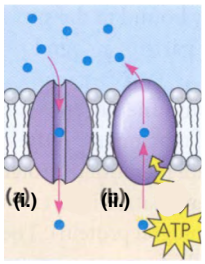


7.3. FUNCTIONS OF CELL MEMBRANES



a. **Boundary and insulation** provide mechanical and chemical protection against the cell environment. A must for **intra- and extracellular concentration differences**.

b. **Controlled mass transport through pores**, **channels** and **transporters** is responsible for the **composition** of the **internal environment**. This is a prerequisite for homeostasis (balance of substances within the body/cells). The **transport via membrane proteins** is further divided (next chapter):
   i. **Passive transport**: formation of a hydrophilic channel through the membrane.
   ii. **Active transport**: Hydrolysis of ATP for energy production to transport substances through the membrane.



c. **Recording of extracellular signals**: generally, **communication between cells/organelles**. ***Signal molecule*** binds to a ***specific binding site of a membrane protein***, which then changes its conformation and thereby ***triggers signal transmission*** into the cell interior.

d. **Enzymatic catalysis of reactions**: **localization of important enzymes on membranes** in the border area between lipid and water phase. The active center of the enzyme protrudes into adjacent aqueous solution. Those enzymes then control **reactions with non-polar substrates** (e.g. biosynthesis of lipids) and **reactions for energy conversion** (e.g. oxidative phosphorylation, photosynthesis).

e. **Interactions with other cells through intercellular compounds**. Membrane proteins can become **entangled** (formation of tissue) and **serve for cell-cell recognition**. ***Glycoproteins act as identification sites for other cells***.

f. **Anchoring of the cytoskeleton**: **microfilaments** or other elements of the **cytoskeleton** can **bind** to **membrane proteins** in order to **maintain cell shape** and **fix membrane proteins**. Coordination of extra- and intracellular changes (e.g. during cell division). Also, **interaction of the cell** with **extracellular matrix** (via membrane proteins).

7.4. TRANSPORT PROCESSES THROUGH THE MEMBRANE

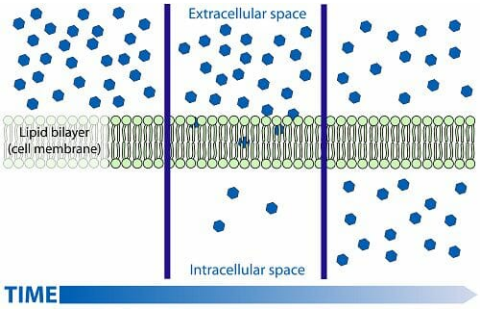
There is a **continuous transport** of **small molecules** and **ions across** the **plasma membrane** in **both directions**.

- Sugar, amino acids, O2 and other nutrients **enter the cell**.
- Excess metabolic products and CO2 **leave the cell**.

Membranes are **selectively permeable**, which means that **only certain molecules** can **pass the membrane**.

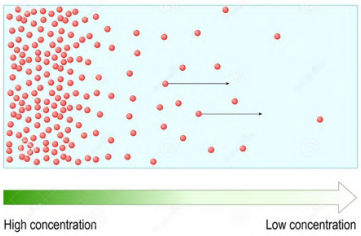
- **Hydrophobic molecules** such as hydrocarbons (C_xH_y), **CO2** and **O2** can **dissolve in the lipid bilayer** and **unrestrictedly diffuse through the membrane**.
- **H2O**, **glucose**, other **sugars** and **ions** with a **hydrate coat** (contains H and O) **cannot cross** the **hydrophobic part** of the membrane. They are therefore **transported through the membrane by transport proteins**.

7.4.1. PASSIVE TRANSPORT (along concentration gradient)

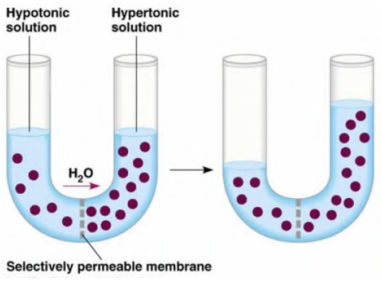


Molecules have **permanent kinetic energy**, which is **expressed in thermal movement**.
Brownian molecular movement describes the **tendency of molecules** to **distribute themselves** in **free space voluntarily** and **without the cell's energy input**.

- *Passive transport **describes the diffusion** of a **substance through** a **biological membrane along its concentration gradient without the cell having to expend energy for this**.*

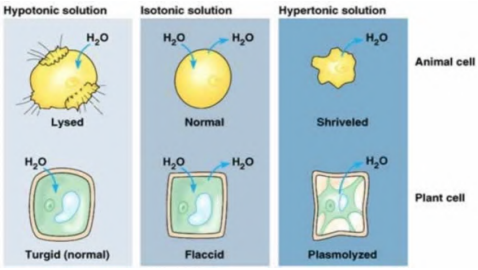


Law of DIFFUSION: *in the **absence of other forces, substances to which** the **membrane is permeable always diffuse from the higher to the lower concentration along their diffusion gradient**. This is a spontaneous process. The **diffusion** of substances **continues until a dynamic equilibrium** of substance concentrations is reached. Afterwards, the **number of diffusing molecules per second remains constant**. -> biology: OSMOSIS (diffusion of water)*



OSMOSIS: **diffusion of molecules** through a membrane along a concentration gradient. In biology, this term refers to the **diffusion of water molecules**.

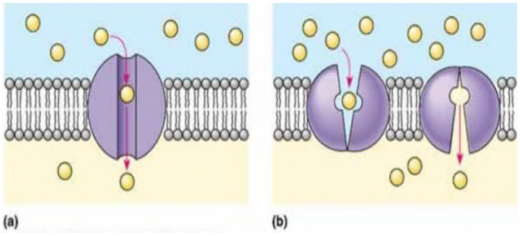
- **Two solutions** with **different concentrations** are **separated** by a **porous membrane** which is **only permeable for water**. Water diffuses from the less concentrated solution (**hypotonic**) into the more concentrated solution (**hypertonic**). Solutions with **equal concentrations are hot isotonic**.



The **ideal environment** for an **animal** (and human) cell is **isotonic**. For a **plant cell**, however, a **hypotonic environment is ideal** (pressure-stable cell wall). The **movement of water across** the **membrane** and the **balance between** the **cell** and its **environment** is **extremely important**.

FACILITATED DIFFUSION (another type o diffusion): **passive transport** of **polar molecules** and **ions** with the **help** of **transport proteins**. Similar to enzymes, they have a **specific binding site** for the substance and can be inhibited by similar substances. This therefore **corresponds** to a "**catalysis**" of physical processes (enzymes catalyze chemical processes). This can happen in two ways:

a. **Transport proteins** form a **channel** through which **H2O** or **specific solutes can pass** the membrane along its concentration gradient.
b. By **changing** the **conformation** of the **transport protein**, **solutes** are **transported** along their concentration gradient to the other side of the membrane

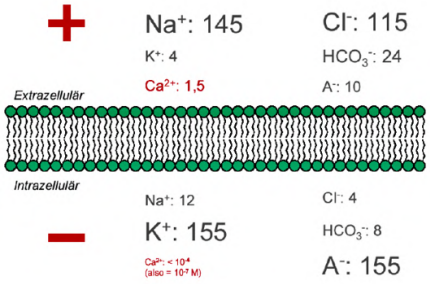


7.4.2. ACTIVE TRANSPORT (against concentration gradient)

Transport of a **molecule through the membrane against its concentration gradient** requires **metabolic energy** in the **form of ATP**.

- **Cations and anions** are **unequally distributed** on **both sides of the membrane** → **Concentration gradient**

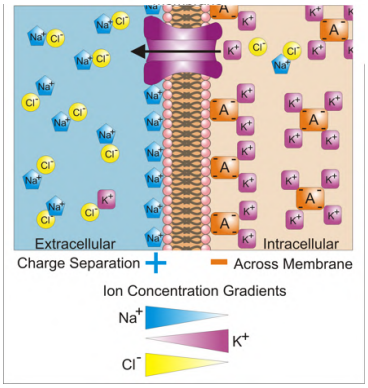
- This creates **negative charge** in the **cell interior** compared to the **positive charge** of the **cell environment**. This **charge difference** generates a **voltage across the membrane** → **Membrane potential**



The **membrane potential influences** the **transport of charged substances** across the membrane:

- The **electric force** drives the **passive transport** of **cations into the cell** and of **anions out** of the cell.
- In addition, a **chemical force** is built up by the **concentration gradient** of the **charged substances**, which **tries** to **balance** the **concentration** differences of the **ions**.

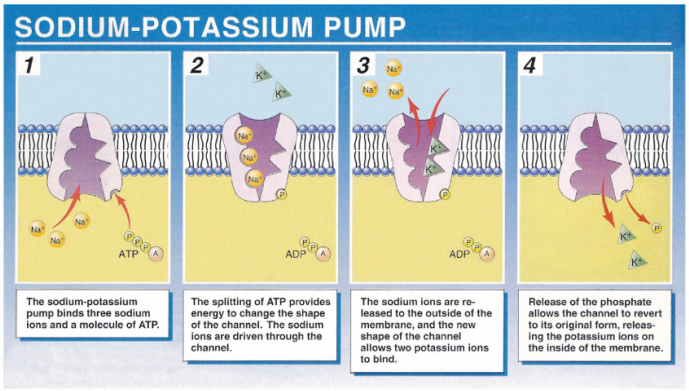
- These two forces acting on ions (electrical & chemical) are called **electrochemical gradients**.



The **cell must constantly maintain the electrochemical gradient** in order to **enable vital cell processes** (e.g. impulse conduction of **nerve cells** (Na+-K+ ion pump) or **cell respiration** (H+ ion pump)).
In order to achieve this, the cells have some membrane pumps that help ions transport and regulation:

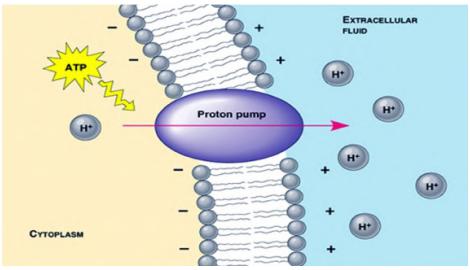
SODIUM-POTASSIUM PUMP:

- **K+ ions** are **constantly pumped in the interior** of animal/human cells **against their concentration gradient**.
- **Na+ ions** are **constantly pumped out** of animal cells **against their concentration gradient**.
- Energy is provided by the transfer of a terminal **phosphate group from** an **ATP to** the **transport protein**.



Cesare Primultini (pcesare@ethz.ch)

PROTON PUMP:



- Storage of energy achieved by generating a membrane voltage (a mini capacitor).
- To **transport positive charge** in the **form** of **H+ ions**, the **proton pump needs ATP** as an energy source.
- The **voltage** and the **H+ gradient** represent a **coupled energy source**, which is **used** for **other processes in the cell** (e.g. absorption of nutrients).

Cotransport: **coupling** of a **directed diffusion** of a **certain substance** (*along its concentration gradient*) and a **directed transport** of a **second substance** (*against its concentration gradient*):

- An **ATP-powered pump generates energy** by **accumulating H+** ions **on one side**.
- **H+** ions **diffuse back** through active transport and reenter the cell together with another molecule (usually more complex and bigger).

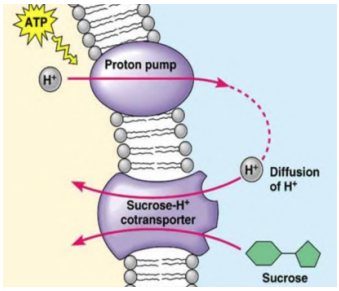
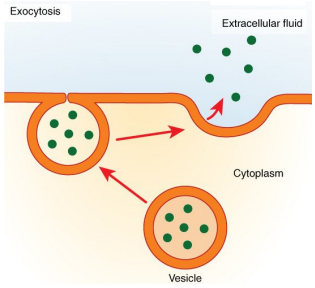


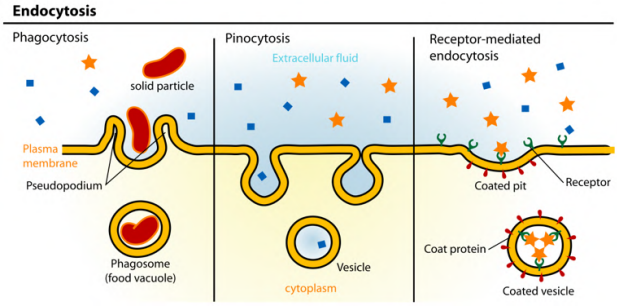
Figure 7: The transport of sucrose is coupled to the back diffusion of H+ ions.

VESICLE: a small structure within a cell, consisting of fluid enclosed by a lipid bilayer. Two processes happen thanks it:

- **EXOCYTOSIS**: **release** of **macromolecules from the cell** with the **help** of **vesicles**.



- **ENDOCYTOSIS**: **uptake** of **macromolecules into** the **cell** with the **help** of **vesicles**, two types:
  - Phagocytosis (large particles with the help of cell extensions in vesicles).
  - Pinocytosis (absorption of liquid or dissolved components in vesicles).



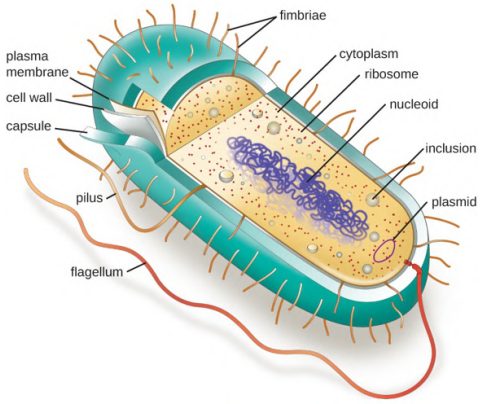
Fusion of vesicles with plasma membrane causes release/absorption of macromolecules.

Cesare Primultini (pcesare@ethz.ch)

8. STRUCTURE AND COMPOSITION OF CELLS

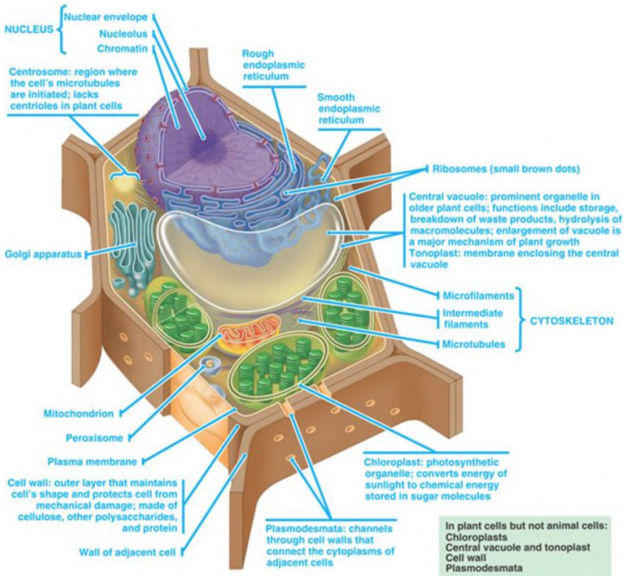
8.1. PROKARYOTIC CELLS

- **1-10** µm large
- Mostly **unicellular**
- Simple cell structure:
    - **Plasma membrane** (reinforcing cell wall and protective capsule)
    - Single organelles:
        - **ribosomes** (protein biosynthesis)
        - **pili** (attachment)
        - **flagella** for locomotion (flagella),
    - No membrane-enveloped cell nucleus
- **Nucleoid** (region in which DNA is concentrated), ring-shaped **plasmids** (extrachromosomal, additional DNA).

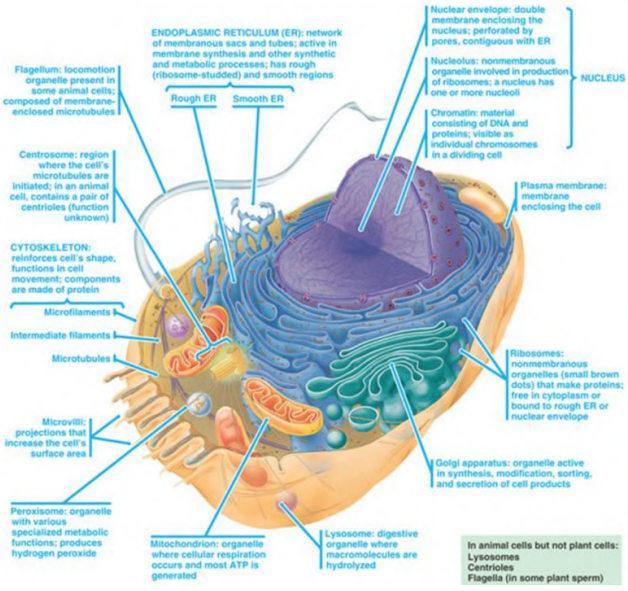


8.2. EUKARYOTIC CELLS

- 10 - 100 µm large
- Unicellular or multicellular
- Clearly **defined cell nucleus**
- Many cell organelles
- Differences between plant and animal cells:
    - Animal cell has different compartments, which are isolated from the rest of the cell by at least one selectively permeable membrane:
        - **Cytosol (part of cytoplasm)**: provides structure and it's a source of proteins, amino acids, mRNA, ribosomes, sugars, ions and more.
        - **ER** (endoplasmic reticulum): forms a series of flattened sacs within the cytoplasm of eukaryotic cells and serves multiple functions.
        - **Endosomes**: sorting and delivery of materials.
        - **Lysosomes**: remove waste.
        - **Peroxisomes**: break down fatty acids and transfer of hydrogen.

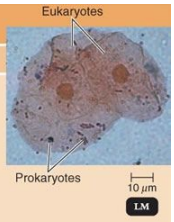


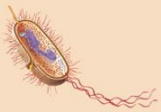
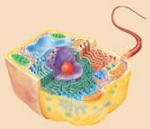
PLANT CELL



ANIMAL CELL

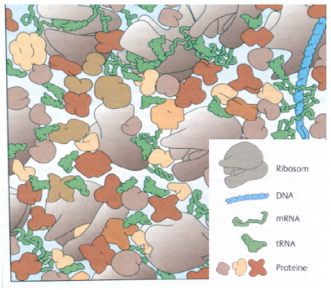
Table 4.2 Principal Differences between Prokaryotic and Eukaryotic Cells



Characteristic	Prokaryotic	Eukaryotic
		
Size of Cell	Typically 0.2–2.0 µm in diameter	Typically 10–100 µm in diameter
Nucleus	No nuclear membrane or nucleoli	True nucleus, consisting of nuclear membrane and nucleoli
Membrane-Enclosed Organelles	Absent	Present; examples include lysosomes, Golgi complex, endoplasmic reticulum, mitochondria, and chloroplasts
Flagella	Consist of two protein building blocks	Complex; consist of multiple microtubules
Glycocalyx	Present as a capsule or slime layer	Present in some cells that lack a cell wall
Cell Wall	Usually present; chemically complex (typical bacterial cell wall includes peptidoglycan)	When present, chemically simple (includes cellulose and chitin)
Plasma Membrane	No carbohydrates and generally lacks sterols	Sterols and carbohydrates that serve as receptors
Cytoplasm	No cytoskeleton or cytoplasmic streaming	Cytoskeleton; cytoplasmic streaming
Ribosomes	Smaller size (70S)	Larger size (80S); smaller size (70S) in organelles
Chromosome (DNA)	Usually single circular chromosome; typically lacks histones	Multiple linear chromosomes with histones
Cell Division	Binary fission	Involves mitosis
Sexual Recombination	None; transfer of DNA only	Involves meiosis

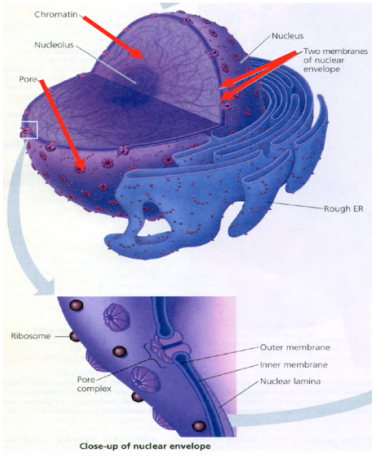
8.3. CELL ORGANELLES AND CELL STRUCTURE

8.3.1. CYTOPLASM



- It's the **most important component** in eukaryotes with more than 50% of the cell volume.
- It's also the **central reaction area of the cell** (e.g. glycolysis, gluconeogenesis, protein biosynthesis)
- Content: **macromolecules** for protein biosynthesis, **enzymes**, small **organic molecules**, **inorganic ions**, water.

8.3.2. CELL NUCLEUS



- **Largest cell** organelle in **eukaryotic** cells (5 µm diameter).
- Surrounded by **double membrane**, pores connect the inner and outer membrane and regulate the entry and exit of certain macromolecules,
- **Chromatin** = **DNA/protein complex** inside the cell nucleus, which **contracts** during **nuclear division** and thus forms the chromosomes.
- The **DNA** of the chromosomes contains the genetic information that is responsible for all processes of **storage, growth and development**.

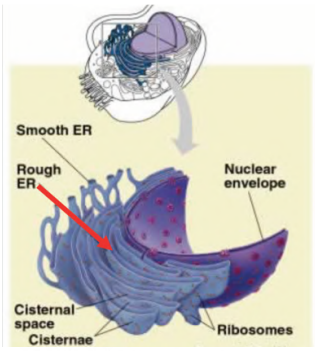
8.3.3. RIBOSOMES

- They **catalyze translation** in **protein biosynthesis**.
- They consist of **two differently sized subunits (small and large)** of **ribosomal RNA** (**rRNA**) and proteins. Only in the **presence of mRNA** do the **subunits come together** to form the **complete ribosome**.

8.3.4. ENDOMEMBRANE SYSTEM

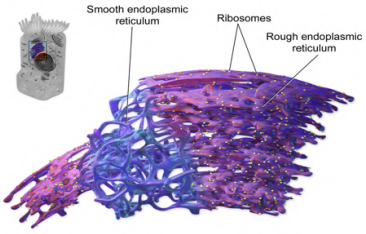
Closed membrane system consisting of tubular and sac-like structures, vesicles and lysosomes. Used for **intracellular transport** of **synthesized proteins**, **phospholipids** and **cholesterol**. Consists of:

- **Rough and smooth endoplasmic reticulum (ER)**
- The **Golgi** apparatus
- **Lysosomes**
- **Vesicles** and **vacuoles**



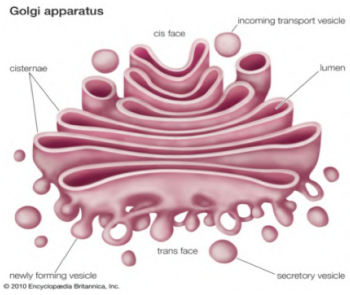
ROUGH ER (rER)

- ➔ **Membrane-bound ribosomes** on the **cytosolic surface**.
- ➔ Site of part of the **protein biosynthesis**:
  - The <u>proteins synthesized at the rER</u> are folded and <u>modified after translation</u> during the <u>maturation of the protein</u> and then <u>remain as membrane proteins in the rER</u> or reach the Golgi apparatus by means of transport vesicles (formed by constriction of membranes and disappear again by fusion with them).



SMOOTH ER (sER)

- ➔ Contains **no membrane-bound ribosomes**
- ➔ Made up of **branched**, self-contained **tubes**, containing membrane-bound enzymes that **catalyze** the **synthesis** of **lipids**: *<u>phospholipid synthesis, cholesterol synthesis</u>*.



GOLGI APPARATUS

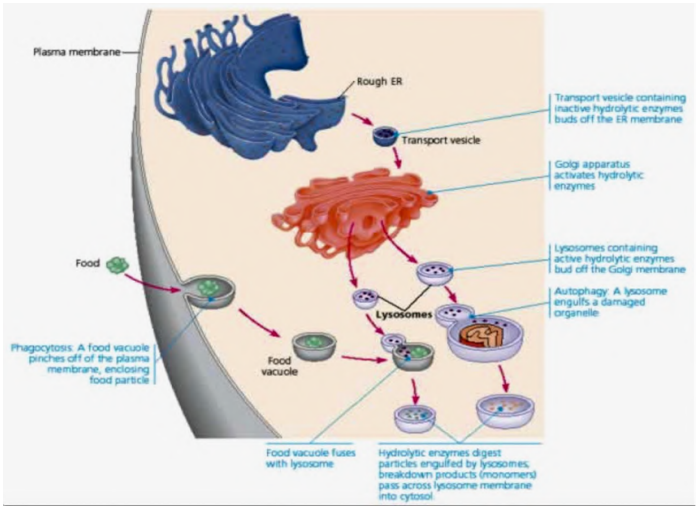
- ➔ Complex, closed system of flattened membrane bags, which are stacked on top of each other.
- ➔ Site of **final protein maturation**, **sorting**, and **packing** in vesicles and further transport in lysosomes or vesicles.

LYSOSOMES

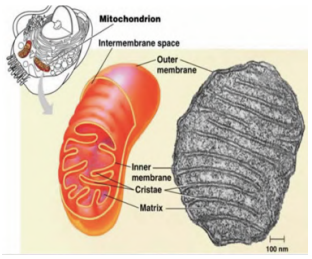
- ➔ They are used for the **enzymatic degradation** of **macromolecules** and **cell organelles**, which are delivered by different routes.

VACUOLES and VESCICLES

- ➔ Enveloped by a **membrane**, formed by **fusion** of **lysosomes**. Different types:
  - **<u>Food vacuoles</u>**: contain hydrolytic enzymes for digestion of food particles
  - **<u>Contractile vacuoles</u>**: pumps water out of the cell.
  - **<u>Central vacuole</u>**: makes up to 80% or more of the cell volume in plant cells
- ➔ **Storage** of various materials, **removal** of waste materials, **protection** and growth of the cell

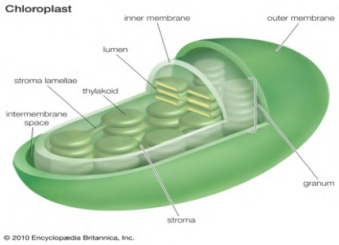


8.3.5. MITOCHONDRIA



- Cell **power plants,** they **produce** most of the **ATP** by oxidative phosphorylation.
- Two membranes: **outer smooth membrane** and **inner strongly folded membrane** (larger surface), contain their own DNA (mitochondrial DNA).

8.3.6. CHLOROPLASTS (ONLY PLANTS)



- The **site of photosynthesis in plant cells**, similar to mitochondria.
- **Two membranes** (inner and outer) **contain their own DNA**, the **interior** (**stroma**) contains the **thylakoids**, which are stacked into what it's called **granum**.

8.3.7. PEROXISOMES

- They are surrounded by a **single membrane**, **spherical organelles** with a **granular** or **crystalline core**.
- They contain **enzymes** that use **oxygen** to **produce peroxide** (**H2O2**) (strong oxidant), H2O2 serves to **break down fatty acids** so that they can be **transported** into the **mitochondria** and used for **respiration**.

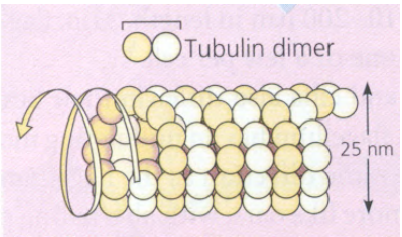
8.4. CYTOSKELETON

It has 3 important roles:

- **Mechanical framework** (typical shape, linking of organelles and membranes)
- **Motor for the movement of animal cells** (muscles, cell migration, plasma flow and cell division)
- **Rail for transport within the cell**: mechanical connection of the cell to its tissue ("mechanical signal transduction").

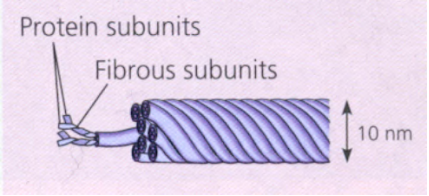
Some types of cytoskeleton:

MICROTUBULES (25 nm): point in all directions from the center near the **nucleus** (**centrosome**). Made of **hollow**, **tubular tubulin molecules**, stronger than actin (protein in muscles). Tasks:



- Maintenance of cell shape
- Cell motility (cilia and flagella)
- Chromosome movement during cell division
- Movement of organelles

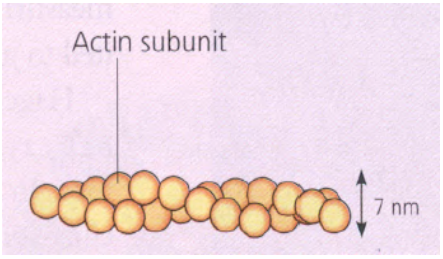
INTERMEDIATE FILAMENTS (8-12 nm): made of fibrillar **keratin proteins** (specific for certain cell types) wound into thicker **superhelices**. Tasks:

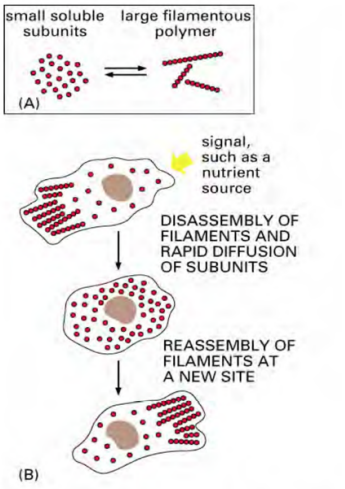


- Maintenance of cell shape
- Anchorage for the cell nucleus and other organelles
- Stabilization of the cell nucleus membrane

ACTIN FILAMENTS (5-9 nm): also called **microfilaments**, made of **two helically wound** actin **strands**, flexible. Structure: linear bundles, 2D networks or 3D gels. Distributed throughout the cell, densest in the **cortex** (**near plasma** membrane). Tasks:

- Maintenance of cell shape
- Changes in cell shape
- Muscle contraction
- Cytoplasmic flow
- Cell motility (pseudopodia)
- Cell division.





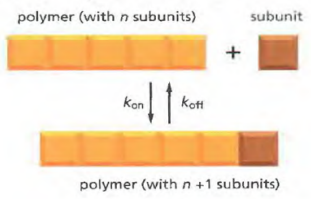
Each of these three families of cytoskeleton proteins has its **own specific functions**, **mechanical properties** and **different dynamic behavior**. They have some common features:

- Built from **chains** of **small subunits**
- **Fast assembly** and **disassembly** (**weak binding**)
- **Modulated by** accessory **proteins**

Cytoskeletal structures can extend throughout the cell (10-100 µm) but consist of individual proteins that are only about 10 nm long. These **subunits** can **disassemble**, **move** (thanks to diffusion) and **reassemble** at **desired locations**.

8.4.1. POLYMERIZATION

A **linear polymer** can **assemble** (polymerize) and **disassemble** (depolymerize) by **adding and removing subunits** (monomers) to one end of the polymer. The rate of addition is given by the **rate constant** k_{on}, the rate of removal by k_{off}.

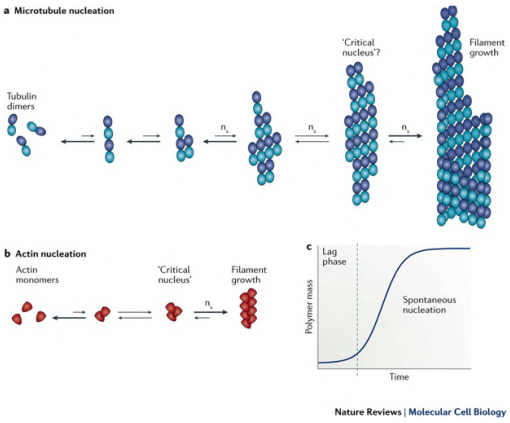


NUCLEATION

It's the **initial step** in polymerization. A **helical polymer** is stabilized through **many contacts** between **adjacent subunits**.

Example ACTIN: two actin molecules only bond relatively weakly, a third actin monomer creates a more stable trimer (nucleation nucleus) to which further monomers can attach themselves.

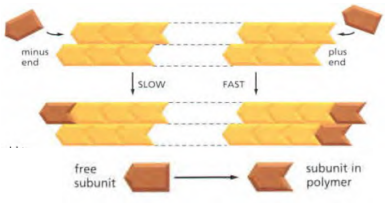
The **formation** of this **nucleation nucleus** is **relatively slow**, which explains the **delay phase** (lag phase) during polymerization. This delay can be reduced by adding more nuclei.



The **two ends** of the **actin filaments polymerize** at **different speeds**:

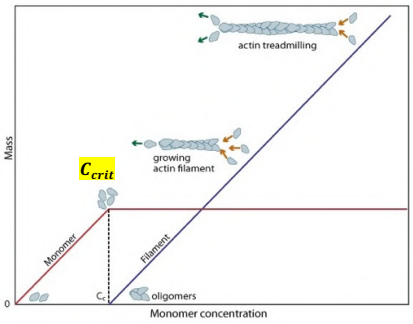
- The fast-growing end is called the **PLUS END**
- The slower growing end is called the **MINUS END**

These **speeds** are **determined** by the **conformational change** of the **subunits** when they bind to the polymer. At the **plus end** the k_{on} and the k_{off} rates are **higher than** the rates at the **minus end**. The **ratio** k_{off}/k_{on} is the **same at both ends** (the exact same bonds must be broken and the final state of the subunit after dissociation must be identical).



The number of **monomers** per second **binding** to the **polymer** is **proportional** to the **concentration** of free monomers ($k_{on} \cdot C$). However, the **number of monomers** that **dissolve from** the **polymer** is **independent** of the **monomer concentration** (C).

- As the **polymer grows**, more and more monomers are consumed
- The **monomer concentration decreases** to a certain **constant value**, which is the critical concentration C_{crit}. This is where the **rate of addition** and **removal** of monomers **balances out**. Then: $k_{on} \cdot C = k_{off}$
  - $C > C_{crit}$ both ends grow.
  - $C < C_{crit}$ both ends get shorter.



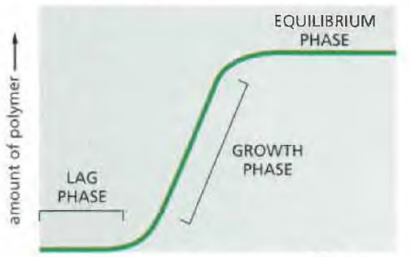
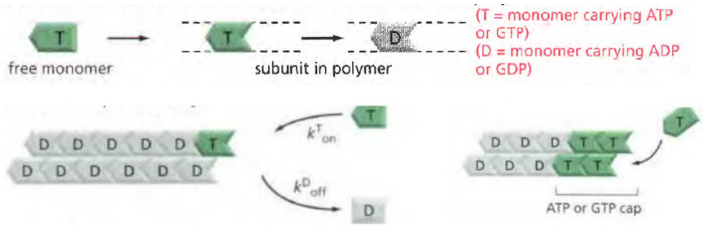
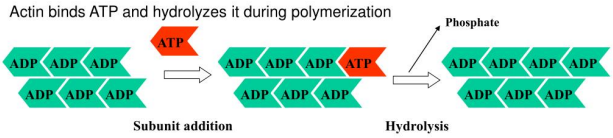


*Figure 8: **Lag** (time for nucleation), **Growth** (filament elongation), **Equilibrium** (constant building and breaking speed).*

NUCLEOTIDE HYDROLYSIS:

Each **actin molecule binds** an **ATP**, which is **hydrolyzed** to an **ADP** as soon as the actin binds to the polymer (**tubulin**: **same** but with **GTP**/**GDP**).



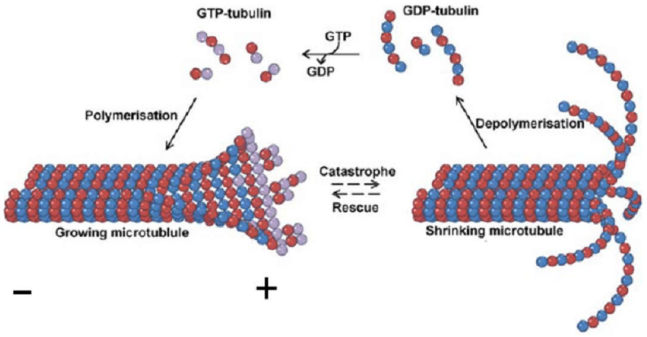


- If the **addition rate** of the monomers to the polymer is **faster than the hydrolysis** of the bound nucleotides, an **ATP cap** (or **GTP cap**) is **formed** → serves as a protection against degradation.

DYNAMIC INSTABILITY:

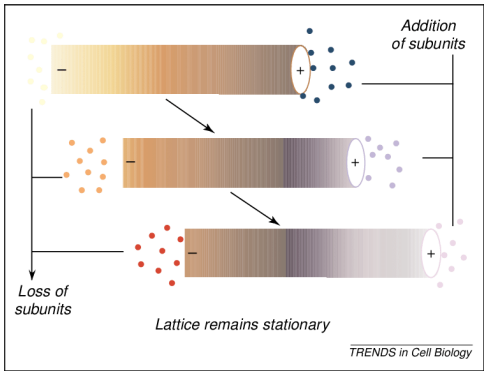
Microtubules depolymerize** about 100 times **faster** **from a tubulin end** bound with **GDP than bound** with **GTP: A **GTP cap promotes growth**, without a GTP cap depolymerization begins.

➔ A single **microtubule** can **alternate between slow growth** and **rapid degradation** (leads to instability).



Cesare Primultini (pcesare@ethz.ch)

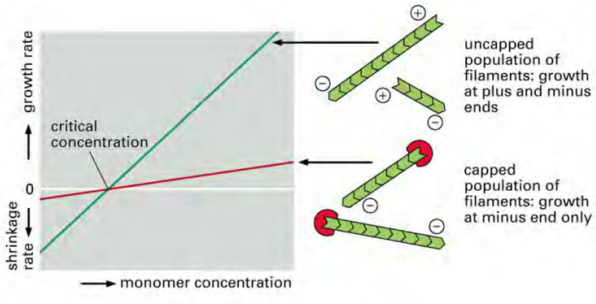
TREADMILLING:



Phenomenon **during** the **polymerization** of **actin filaments**. ***The critical concentration is changed by hydrolysis***.

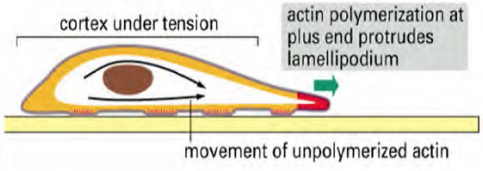
$$C_{crit}\ (minus\ end)\ >\ C_{crit}\ (plus\ end)$$

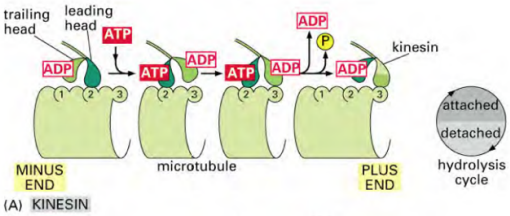
- ➔ **Concentration** of **free monomers higher than** C_{crit} for the **plus end** but **lower than** C_{crit} for the **minus end**.
- ➔ **Steady state** in which the speeds of **assembly at the plus end** and **disassembly** at the **minus end** are **equal**.
- ➔ The **length** of the **polymer remains constant**, although there is a **net flow** of **subunits through** the **polymer**.



Microtubule polymerization generates force, used for:

- **Separation of chromosomes** during cell division (in that case microtubules emerge from two opposite centrosomes)
- **Locomotion**: polymerization generates **protrusive force** (e.g. filopodial extension).





Cytoskeleton also provides a **framework** for **targeted active transport** by **molecular motors** that **transport** a **substance** (**cargo**).

- **Kinesin** (motor protein): transport towards the plus end of the microtubule
- **Dynein**: transport towards the minus end (coupled)

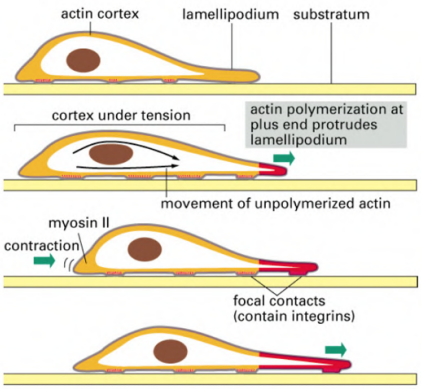
Finally:

- Cytoskeleton is **constantly remodeling** by **polymerization** of **protein monomers** which are assembled to form loadbearing and force-generating **filaments**.
- Cytoskeleton is crucial as a framework for **organelle transport** by **molecular motors** (**kinesin** to the outside, **dynein** to the nucleus).
- **Reactions** driven by ATP/ADP and GTP/GDP **shift** the **thermodynamic equilibrium** of **polymerization**, **storing elastic energy** in **protein form** (**change of conformation**), which drive many physical/mechanical processes.

Cesare Primultini (pcesare@ethz.ch)

9. FUNCTIONAL CELL BEHAVIORS

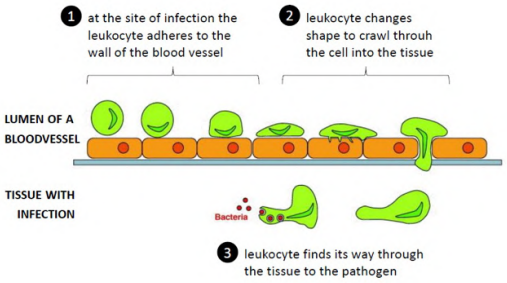
9.1. MIGRATION



Two key differences:

- ➔ **Cell migration**: is the active **relocation of cells**, **movement** of "**repair cells**" thro**ugh a blood vessel wall**, driven by **inflammation signals**.
- ➔ **Cell motility**: is the **mechanisms that enable cell migration**, it's the mechanical basis for cell spreading (spreading of the cell on a surface).

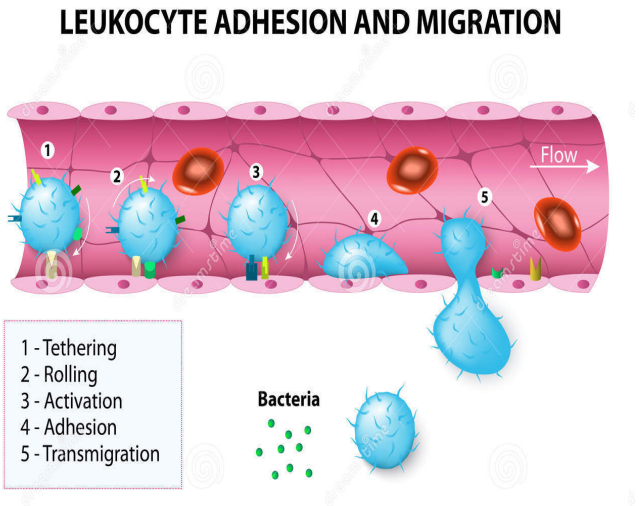
9.1.1. REPAIR CELL



Leukocyte migration (white blood cell): is the first thing and essential prerequisite of healing a wound. They migrate through activated blood vessel walls and anticipate the arrive of the repair tissue cells.
"**Polarized motility**" (directed movement) of leukocytes through vessel walls allows them to reach the outer part of the wound and start to combat bacteria. Stem cells for tissue repair also cross blood vessels in a similar manner.

This process works in different steps:

- **Chemoattraction/Tethering**: macrophages present at the site of the wound release **cytokines** (inflammatory signals) that enable cell adhesion in the endothelial cells of the blood vessels. These allow further attachment and transmembrane passthrough of leukocytes. Leukocyte are drawn toward the site of infection by the presence of chemokines.
- **Rolling adhesion**: leukocytes are attracted to the inner walls of the vessel by **carbohydrate ligands** that act like Velcro. Leukocytes slow down and start rolling along the surface.
- **Activation and adhesion**: leukocytes are bond strongly to the inner walls of the vessel by high-affinity molecules (**integrins**) that immobilize them to the surface.
- **Transmigration**: leukocytes change conformation, thanks to the cytoskeleton that reorganizes the shape of the cell, and pass through the endothelial walls of the blood vessel.



Once in the interstitial fluid, leukocytes **migrate along a chemotactic gradient** towards the site of injury or infection.

9.1.2. CELLULAR MOTILITY

Lamellipodia and **filopodia** are **plasma protuberances** (pseudopods) that are **important** for **cell migration**:

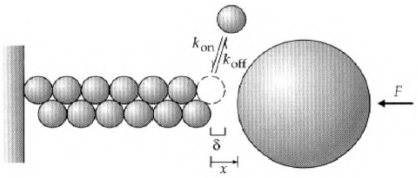
- **Rapid actin polymerization** takes place at the **anterior part of the cell**: dissolved actin monomers polymerize into filaments, which thus **push** the **leading cell front forward** (lamellipodial and filopodial extension).
- **The rear part** of the cell is **pulled forward** by **contractile elements of the cytoskeleton**. This is done by **actomyosin motors**, which are anchored to the attachment of the cell to the substrate.

*Growth or shortening of a polymer results from the **imbalance of addition and detachment** of **protein subunits** at the ends of the protofilaments.*

$$\frac{k_{\text{on}}}{k_{\text{off}}} = \exp(\Delta G / k_{\text{B}} T)$$

Ratio of addition rate k_{on} and replacement rate k_{off} depends on ΔG (Gibbs) which can be positive or negative. k_B: Boltzmann constant, T: absolute temp.

The following **maximum force** (**stall force**) can be generated by the assembly of a polymer consisting of **N-protofilaments** (δ: added polymer length). Which means: high monomer concentrations are related to more force:



$$F_{\text{stall}} = \frac{\Delta G}{\delta / N} = \frac{N k_{\text{B}} T}{\delta} \ln\left(\frac{k_{\text{on}}}{k_{\text{off}}}\right)$$

Polymerization is **favored towards the cell front** and disassembly occurs more frequently at the rear. Many important processes such as **tissue formation** during embryonic development, **wound healing**, **immune responses** etc. ***require finely tuned, coordinated movements*** of the cells.

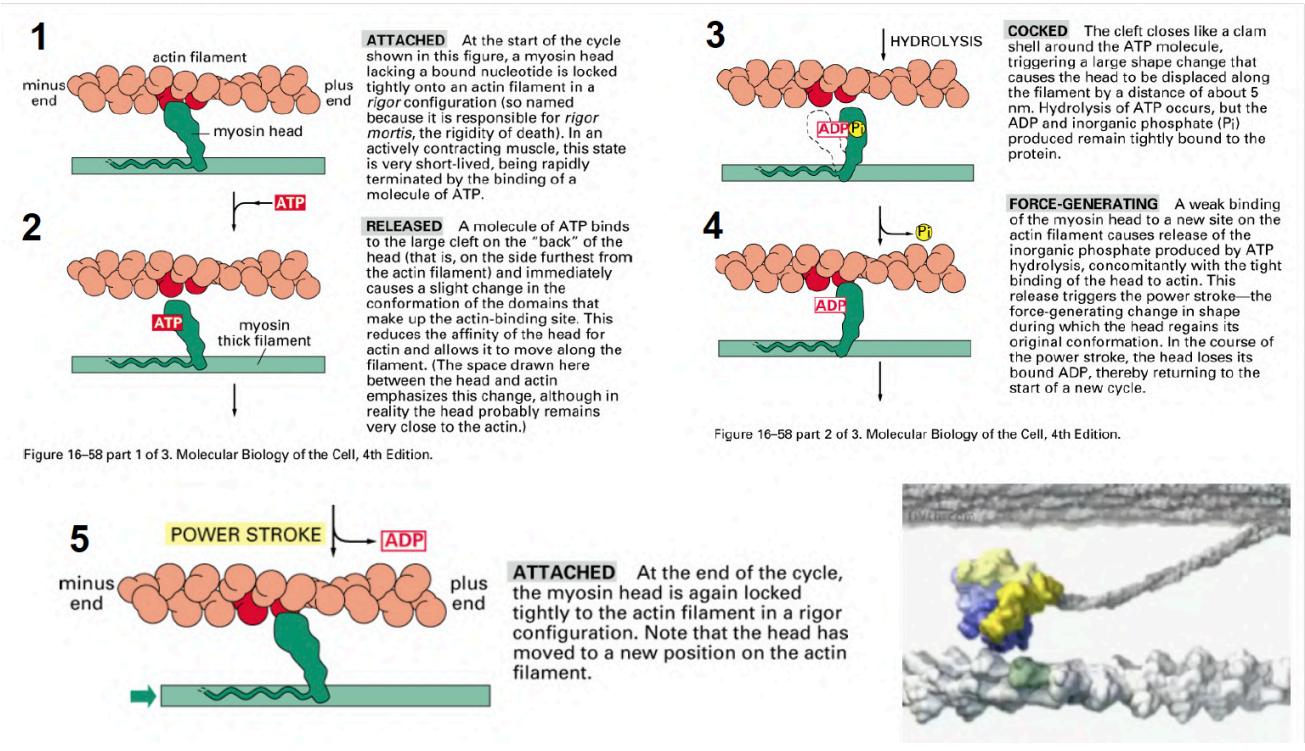
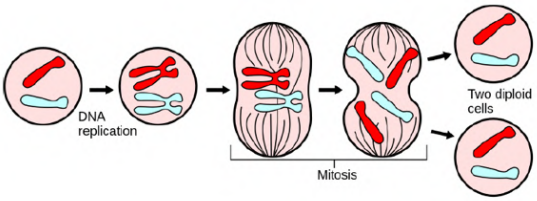


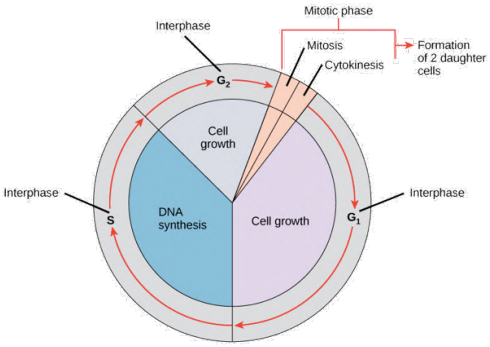
Figure 16–58 part 1 of 3. Molecular Biology of the Cell, 4th Edition.

Figure 16–58 part 2 of 3. Molecular Biology of the Cell, 4th Edition.

9.2. MITOSIS

It's a part of the process when a cell duplicates into two **genetically identical** daughter cells.





- **Sister chromatids** arise when a **single chromosome** is **replicated in two identical copies**.
- **Homologous chromosomes** are two **chromosomes with the same structure**, **one** comes **from** the **mother**, the **other from** the **father**.

The cell growth and division process have many phases, most of the time is spent on cell growth. The different phases are divided as follows:

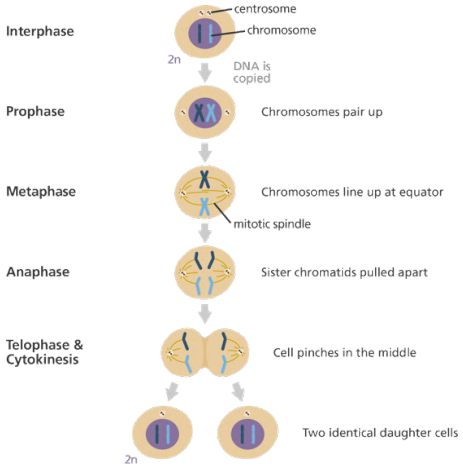
State			
quiescent/ senescent	Gap 0	G0	A resting phase where the cell has left the cycle and has stopped dividing.
Interphase	Gap 1	G1	Cells increase in size in Gap 1. The G1 checkpoint control mechanism ensures that everything is ready for DNA synthesis.
	Synthesis	S	DNA replication occurs during this phase.
	Gap 2	G2	During the gap between DNA synthesis and mitosis, the cell will continue to grow. The G2 checkpoint control mechanism ensures that everything is ready to enter the M (mitosis) phase and divide.
Cell Division	Mitosis	M	Cell growth stops at this stage and cellular energy is focused on the orderly division into two daughter cells. A checkpoint in the middle of mitosis (Metaphase Checkpoint) ensures that the cell is ready to complete cell division.

The processes of **nuclear division** (mitosis) and **cell division** (cytokinesis) are together called **M-phase** and are only a **small part of the cell cycle**.

The much longer part of the cell cycle is the **interphase**, which includes the **S-phase and the two G-phases**.

Mitosis is divided into **5 steps** (see picture). Important to know:

- During the **transition from metaphase to anaphase** an **abrupt biochemical change takes place**.
- A **cell can pause in the metaphase** before this transition point, but as soon as the cell has **passed this point**, the **mitosis comes** to an **end**.
- After that the **cytokinesis** leads to the **interphase**.



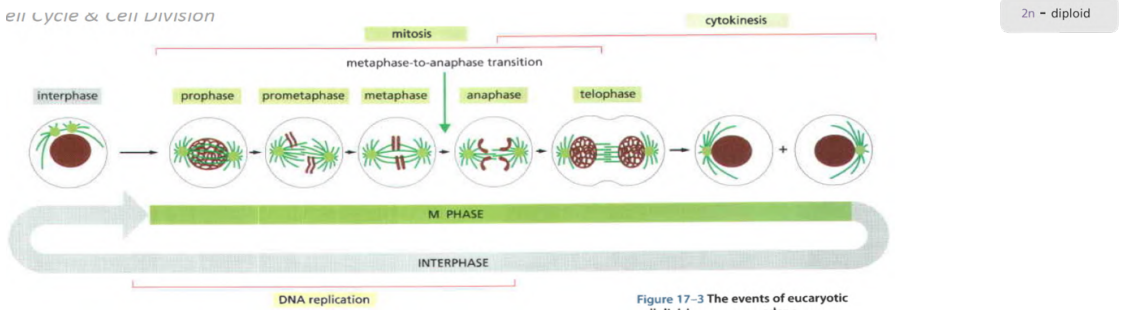
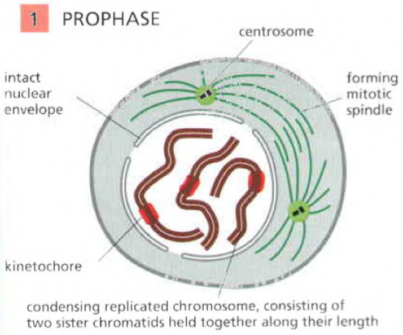


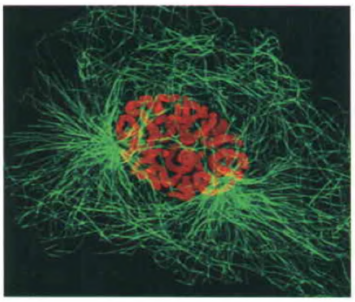
Figure 17–3 The events of eucaryotic

Cesare Primultini (pcesare@ethz.ch)

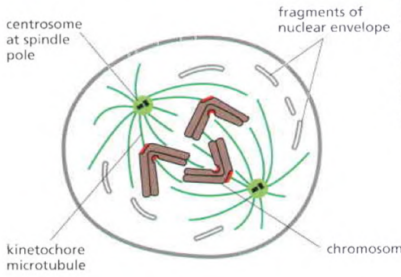
1 PROPHASE



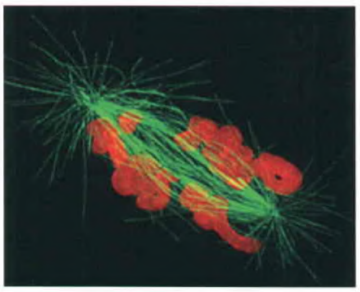
At prophase, the replicated chromosomes, each consisting of two closely associated sister chromatids, condense. Outside the nucleus, the mitotic spindle assembles between the two centrosomes, which have replicated and moved apart. For simplicity, only three chromosomes are shown. In diploid cells, there would be two copies of each chromosome present. In the photomicrograph, chromosomes are stained *orange* and microtubules are *green*.



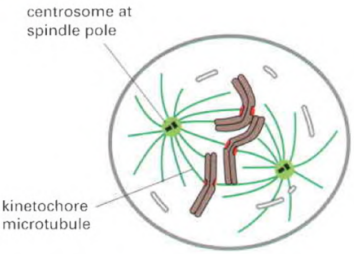
2 PROMETAPHASE



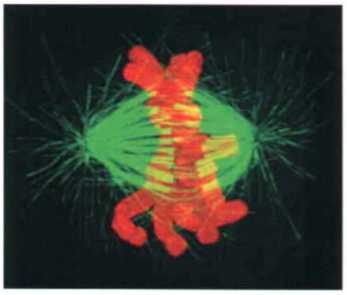
Prometaphase starts abruptly with the breakdown of the nuclear envelope. Chromosomes can now attach to spindle microtubules via their kinetochores and undergo active movement.



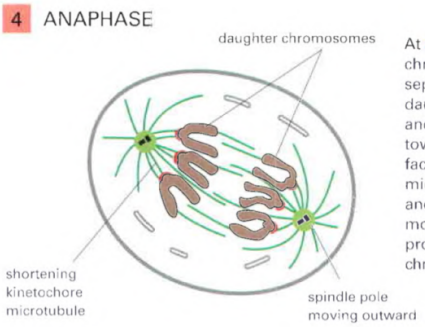
3 METAPHASE



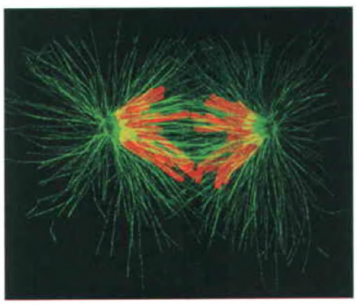
At metaphase, the chromosomes are aligned at the equator of the spindle, midway between the spindle poles. The kinetochore microtubules attach sister chromatids to opposite poles of the spindle.



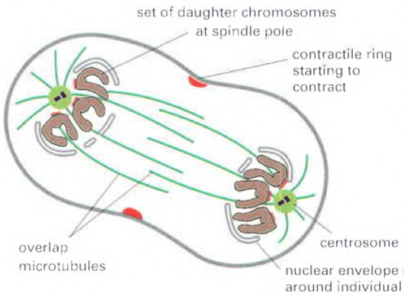
4 ANAPHASE



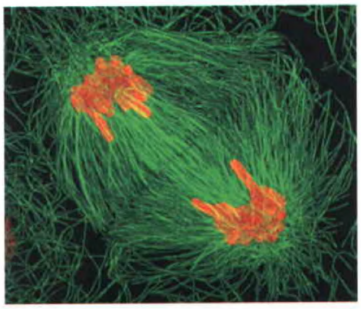
At anaphase, the sister chromatids synchronously separate to form two daughter chromosomes, and each is pulled slowly toward the spindle pole it faces. The kinetochore microtubules get shorter, and the spindle poles also move apart; both processes contribute to chromosome segregation.

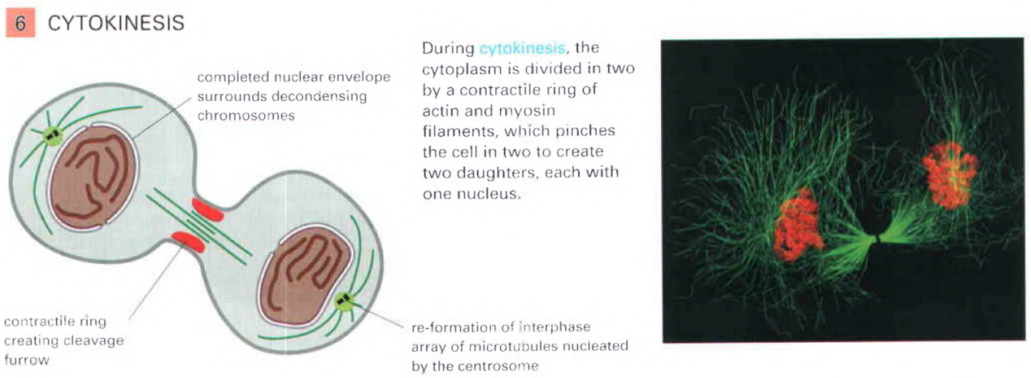


5 TELOPHASE



During telophase, the two sets of daughter chromosomes arrive at the poles of the spindle and decondense. A new nuclear envelope reassembles around each set, completing the formation of two nuclei and marking the end of mitosis. The division of the cytoplasm begins with contraction of the contractile ring.



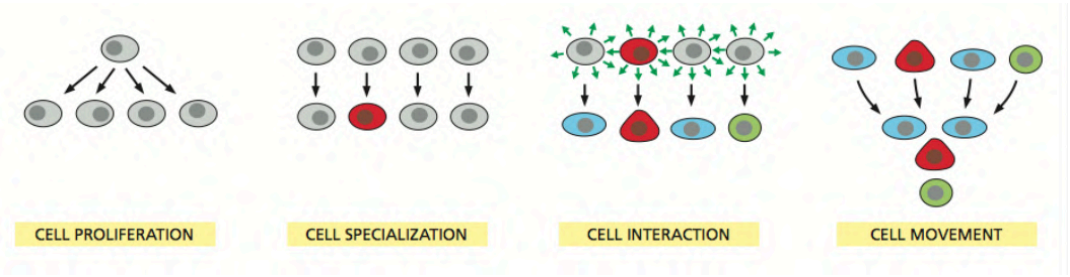


Further details:

- **Cell division** occurs as part of the **much larger cell cycle**, which consists **mainly of cell growth** and **DNA synthesis**.
- The **main goal** of cell division is to **maintain the original genome of the cell**. Before the cell can divide, the genetic information (stored in chromosomes) must be replicated and the duplicated genome must be shared between the cells.
- **Control points** in the cell cycle **regulate phase transitions** and **critical processes** (e.g. chromosome separation in metaphase). They serve to **protect the integrity of the genome** and prevent degeneration of the cell. *These processes are often disrupted in cancer*, leading to *uncontrolled, excessive cell division*.

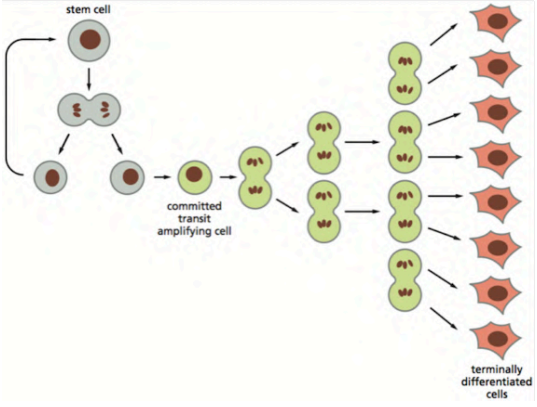
9.3. DIFFERENTIATION

The essential 4 processes by which a multicellular organism is created are:



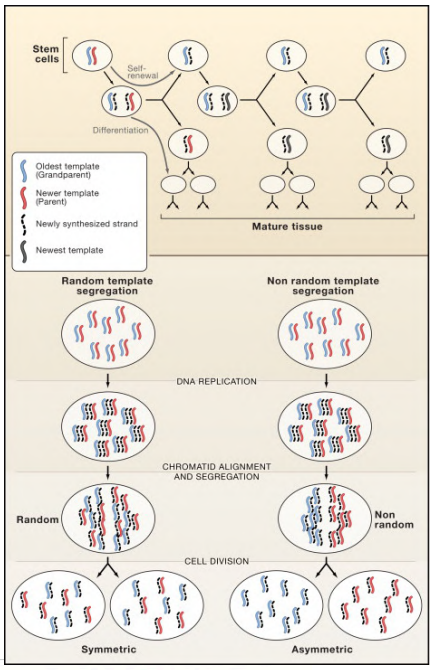
Proliferation: rapid growth/proliferation/proliferation of a tissue. These processes are also essential in wound healing but take a slightly different course (see next chapters).

Stem cells: every **daughter cell** of a **stem cell** can **either remain a stem cell** or **differentiate**. In many cases the daughter cell undergoes many additional cell divisions before terminal differentiation is complete.



- When **new cells are needed**, the **rate of stem cell division increases** dramatically.
- In many tissues, stem cells divide only rarely, but **produce transit-amplifying cells** (daughter cells, which still undergo a limited number of rapid cell divisions until they are fully differentiated).
- In this example, each stem cell division produces 8 fully differentiated offspring.

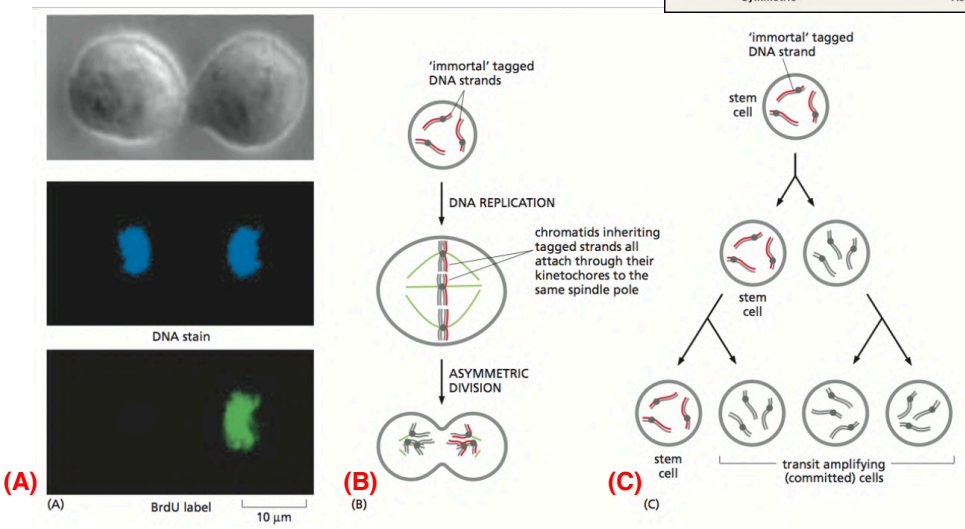
9.3.1. THE IMMORTAL STRAND HYPOTHESIS



(A) One of two sister cells has an **experimental marker**, the other does not. Thus, **daughter chromosomes** with DNA **strands synthesized** during cell division (in the **presence** of the **marker**) are all **inherited from one cell**. This phenomenon, in which **old and new DNA strands are distributed asymmetrically among the daughter cells**, is **only observed in cell populations with stem cells**.

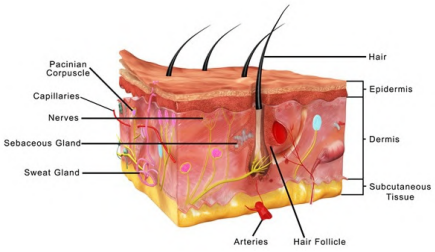
(B) This is the **pattern of DNA strand inheritance** in **stem cell**s according to the Immortal Strand Hypothesis. **One strand in each chromosome** of the **stem cell** is **marked** as a **human strand** and is **retained** by the **stem cell daughter** cell.

(C) This **original DNA strand** is **preserved through all subsequent stem cell generations** as a **template for the production of chromosomes from transit-amplifying cells** (see below for explanation).



This asymmetric division is intended to **prevent stem cell mutations**. Whether this mechanism actually occurs in stem cells in this way has **not yet been proven**.

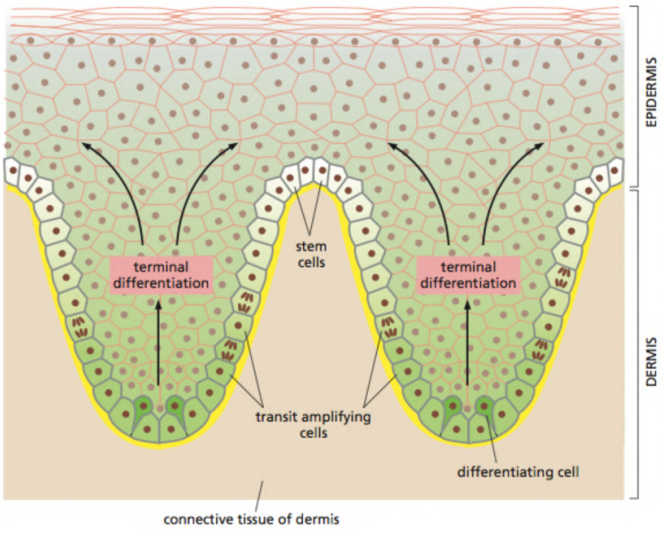
9.3.2. EPIDERMIS (**stem cell distribution** and **epidermal** cell **production**)

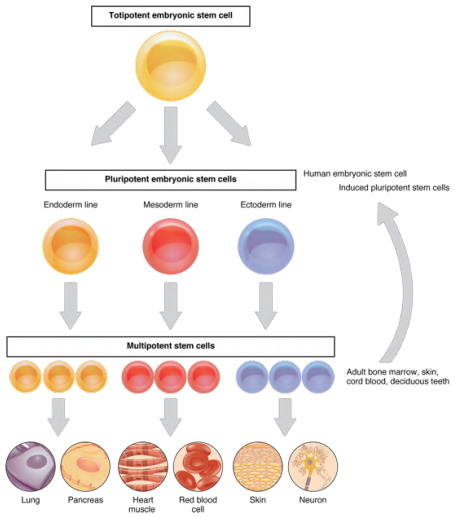


The **epidermis** is the **outermost of the three layers** that make up the **skin**, the inner layers being the dermis and hypodermis.

Epidermis has **many stem cells near the tips of the dermal papillae**. They **rarely divide but produce transit-amplifying cells**:

- ***Transit-amplifying cells possess the capacity to rapidly amplify the pool of differentiated cells produced at each stem cell division.***
- They **divide frequently**, but only **go through a limited number of cell cycles**. At the end of these cycles they **begin to differentiate** and **slip out of the basal cell layer**.





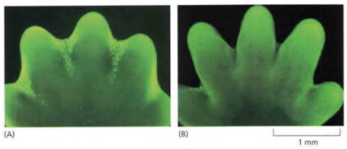
- **Differentiation** is a **change from one cell type to another**. This means a **mostly irreversible change** to a cell type with a specific function.
- **Stem cell proliferation (mitosis)** and **tissue-specific differentiation** play an **important role** in **embryonic development and pre-adult growth** of the organism.
- In **adult** organisms, **stem cells are important for tissue renewal and repair**.
- When a stem cell divides, it produces two daughter cells. **One daughter cell remains a stem cell**, while **the other takes the path of differentiation (asymmetrical division)**.
- In **many tissues stem cells rarely divide** but **produce transit-amplifying cells**.

9.4. APOPTOSIS (programmed death of a cell)

Apoptosis occurs in **multicellular organisms** and is **voluntarily used by the organism**.
In contrast, **necrosis** is a **traumatic cell death resulting from an acute cellular injury**.

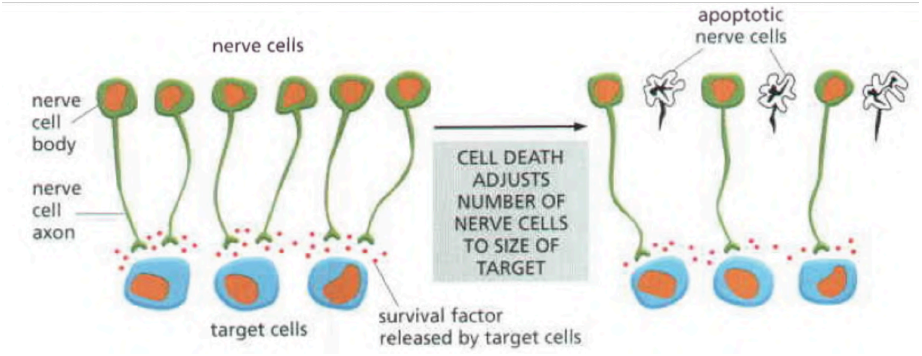
Example of apoptosis:

- the separation of fingers and toes during embryonic development is achieved by apoptosis of cells between the limbs.



A human adult consists of approximately **100 trillion** (10^{14}) **cells**. Of these, an **average of 50 million** cells **per second die by apoptosis** (50-70 billion cells per day).

- **Defects** in **apoptotic processes** play an **important role in many different diseases**:
  - **Excessive apoptosis** causes **atrophy** (**tissue loss**), **too little apoptosis** results in **uncontrolled** cell **proliferation** (e.g. **cancer**).



Cesare Primultini (pcesare@ethz.ch)

In nerve cells the **amount** of **survival factors** **released by the target cells** is **not sufficient** for all **nerve cells**, so that **some nerve cells** are **no longer able to prevent the process of apoptosis** (survival factors prevent apoptosis).

➔ This **strategy of overproduction** **followed by** **selection of nerve cells** ensures that **all target cells have contact with nerve cells** and that excess nerve cells are automatically eliminated.

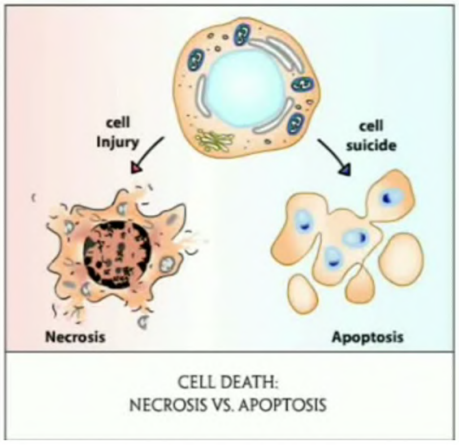
Many pathways and signals lead to apoptosis. When a cell receives an apoptosis stimulus, the cell organelles are degraded in a controlled manner by **proteolytic caspases** (enzyme with specific role of cell death).

During apoptosis a cell exhibits a characteristic morphology:

- The cell becomes **small and round** due to the **decay of the cytoskeleton**.
- The **cytoplasm** and **organelles** become more **densely packed**.
- The **chromatin condenses** to compact patches against the nuclear envelope, which becomes discontinuous.
- The **DNA** inside is **broken down into fragments**. The **nucleus breaks down** into a **few chromatin** bodies and **nucleic units** (DNA degradation).
- The cell **membrane** has **irregular** buds (membrane **vesicles**).
- Finally, the **decayed** cell is **eliminated** by **macrophages**.

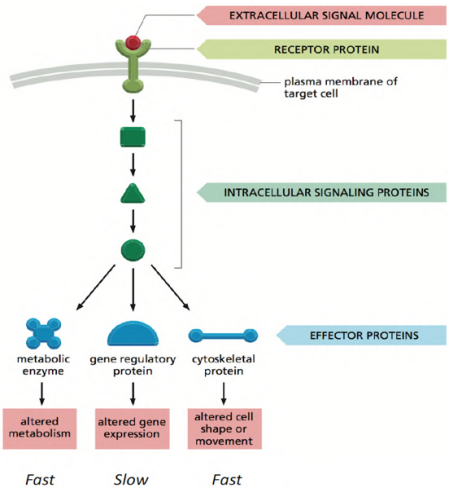
Necrosis and ***apoptosis*** are forms of **cell death**, but differ considerably:

- ***Necrosis*** occurs due to lack of nutrition or external trauma (bacterial infection, poisoning, injury). Necrosis is uncontrolled and induces an inflammatory response.
- ***Apoptosis*** is "**programmed**", is carried out carefully, **does not induce inflammation**.
    - ***Apoptosis limits/optimizes*** *the* ***number of cells in a tissue*** *and acts as a* ***quality control*** *in development and aging by* ***eliminating abnormal, (DNA-)damaged, misplaced or non-functional cells*** *that could pose a risk to the organism*.



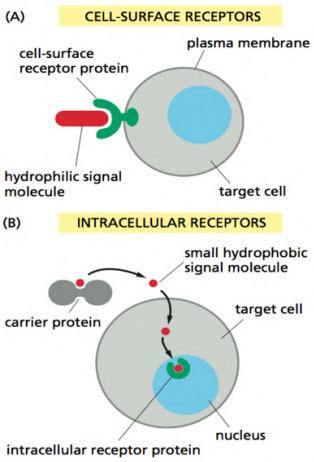
Cesare Primultini (pcesare@ethz.ch)

10. CELL-CELL INTERACTIONS (SIGNALING)



Extracellular signaling molecules can bind to a membrane protein of the **target cell** and thus **activate one or more signaling pathways**. The signaling proteins involved ultimately alter the activity of effector proteins (which regulate biological activity) and thus the **behavior of the cell**.

The **target cell** can receive the **signal molecules** in two ways: **via membrane-bound** or **intracellular receptors**, depending on whether the signal molecule is **hydrophilic** or **hydrophobic** in nature:

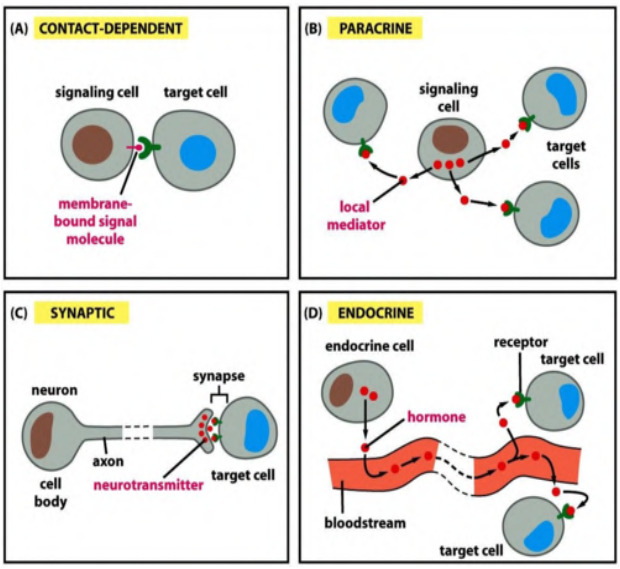


- **(A)** **Hydrophilic** signaling molecules ca**nnot pass the cell membrane directly**; they have to **bind to a membrane-bound receptor** on the cell surface, which then transmits signals into the cell.

- **(B)** Small, **hydrophobic** signal molecules, on the other hand, can **easily diffuse through the plasma membrane** and then bind to a receptor protein in the cytosol or in the cell nucleus. These water-insoluble molecules must be **transported** in **blood** and **other extracellular fluids** by **carrier proteins**.
  The signaling molecules then dissociate from these proteins before they can enter the target cell.

10.1. INTERCELLULAR SIGNALING

Can be split in 4 major types:



- **Contact-dependent signaling** requires direct membrane-membrane contact of the cells involved.

- **Paracrine signaling** is mediated by local signals (mediators) which are released into the extracellular space and act on cells in the environment.

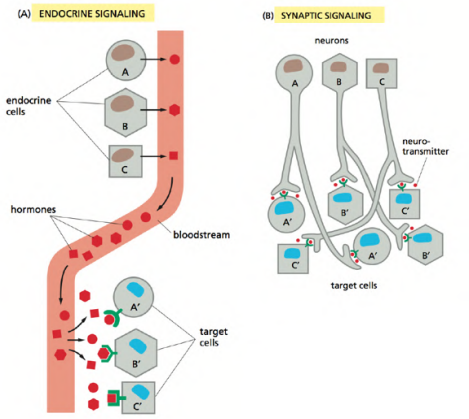
- **Synaptic signaling** is performed by neurons, which transmit signals electrically along their axons and then release neurotransmitters at the synapses (long axons (up to 1m) → long distance and fast signal conduction).

- **Endocrine signaling** requires endocrine cells that secrete hormones into the blood to distribute a signal throughout the body. Endocrine and neuronal cells work together to coordinate the activities of cells in widely separated parts of the body.

Cesare Primultini (pcesare@ethz.ch)

Many **signaling molecules in paracrine**, **synaptic** and **endocrine** signaling are **of the same type**, the important **differences being the speed** and **selectivity** with which the signals reach their targets.

Concentrating on the differences between synaptic and endocrine signaling:



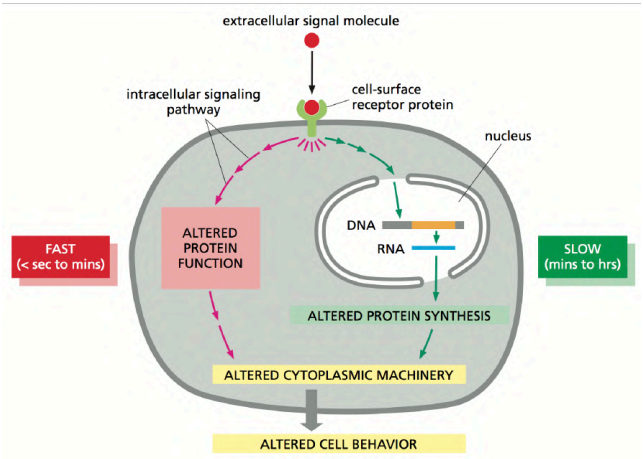
- **(A) Endocrine cells** secrete specific hormones into the blood, which only act on target cells with suitable receptors.
- **(B)** In **synaptic signaling**, on the other hand, **specificity** is **determined** by the **specific synaptic contacts** between a **nerve cell and the target cell**. Normally, the neurotransmitter only reaches the target cell which is in **synaptic contact with the neuron**. But there are also neurotransmitters which act as local mediators (**paracrine**) on several adjacent target cells.

10.1.1. FAST AND SLOW SIGNALS

- SLOW: Certain signal responses (e.g. **more cell growth and cell division**) require **changes** in **gene expression** and the **synthesis of new proteins** and are **therefore slow** (often after one or more hours).

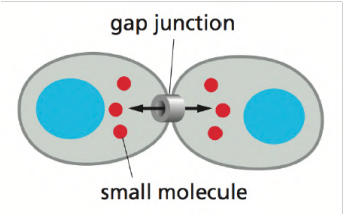
- FAST: Other signal responses (**changes in cell movement, secretion, metabolism**) **do not require changes in gene transcription** and are therefore **much faster** (sec/min). These occur for example through **rapid phosphorylation of effector** proteins in the cytoplasm.

- VERY FAST: **Synaptic responses** (by changing the membrane potential) **occur within milliseconds**.



10.1.2. GAP JUNCTIONS

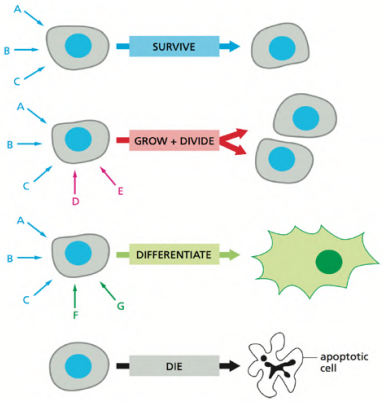
Gap junctions are **narrow**, **water-filled channels** that directly **connect the cytoplasm** of **neighboring cells** and **enable a flow of information**.



Only **small water-soluble molecules** can **move through gap junctions**. Macromolecules such as **proteins or nucleic acids are too large** for these channels and therefore **remain in their own cell**.

Gap junctions **enable a coordinated response** of **several cells** to **extracellular signals** (e.g. in epithelial cells) through these **cytoplasmic connections** (one signal to activate more cells).

10.1.3. RECEPTORS AND SIGNALS



Each cell type has a **set of receptors** that **enable** the cell to **respond** to a corresponding **set of signaling molecules**. These **signaling molecules** work in a **coordinated way** to regulate the **behavior of the cell**.

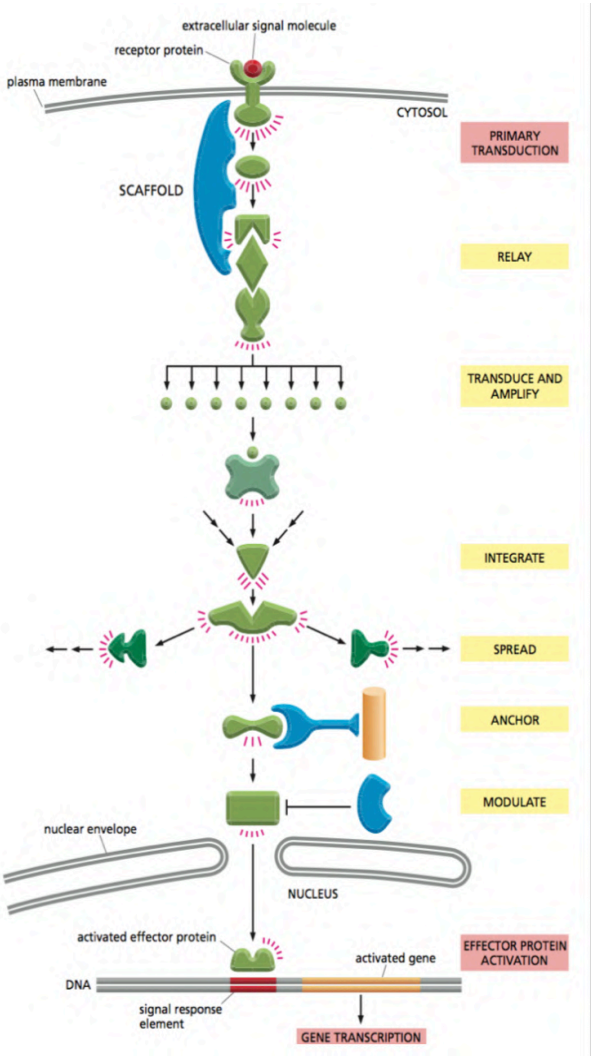
A cell often **receives many survival signals** and **additional signals for growth** and **division** or **differentiation**. If the **necessary survival signals are missing**, the **cell undergoes apoptosis**.

There are also **many inhibiting signaling molecules** that try to **avoid different cell behaviors** and also those that directly induce apoptosis.

10.2. TYPES OF INTRACELLULAR SIGNALING PROTEINS

Intracellular proteins can have the following functions:

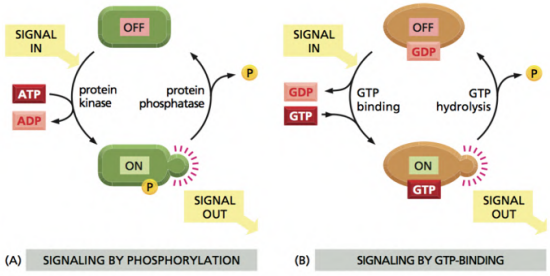
- **Relay protein**: simply pass the signal to the next component of the signaling pathway without any other involvement.
- **Scaffold Protein**: acts as a scaffold to bring two or more signaling proteins together so that they can interact quickly and efficiently. Specificity and signal complexity can be achieved this way, the scaffold hold together many types of signaling proteins in order to create a **signaling complex**. This way, undesired crosstalk (interaction) with other signaling pathways can be prevented (see figure next page@).
- **Transducer protein**: transforms the signal into another form, either suitable for transmission or to stimulate a cell response.
- **Amplifier protein**: amplifies the signal, either by producing a large number of small intracellular mediators or by activating many copies of a downstream signaling protein. By such amplification, a *few signaling molecules* can trigger a *large intracellular response*. A signaling pathway with many such amplification steps is also called a **signaling cascade**.
- **Integrator protein**: receives signals from two or more signaling pathways and integrates them before transmission. If such a protein needs two or more inputs to be activated, this is called a **coincidence detector** (simultaneity).
- **Spreading**: A protein can spread a signal from one signaling pathway to another. Such branching in the signal stream can increase the complexity of the signal response.
- **Anchoring protein**: attaches one or more signaling proteins to a desired structure of the cell.
- **Modulator protein**: modulates the activity of other signaling proteins and thereby regulates the strength of a signaling pathway.



Cesare Primultini (pcesare@ethz.ch)

These are two examples of **intracellular signaling proteins** that act as **molecular clocks**. In both, the addition of a phosphate group is crucial:

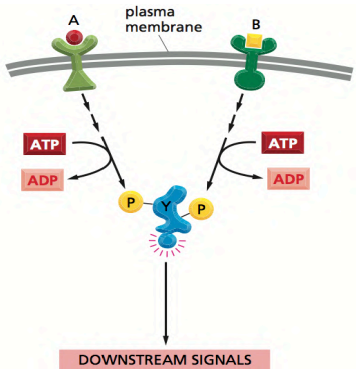
- **(A) Activation by direct phosphorylation** of the protein.
- **(B) Activation by replacing GDP with GTP** on the protein.



Many intracellular signaling proteins function as molecular clocks:

- When they receive a signal, they change from an **inactive** to an **active conformation**.
- They **remain active** until **another process inactivates them again**.
- **Inactivation** is as **important as activation**.
- Before a **pathway is ready to transmit a new signal**, **all activated molecules** of the pathway must return to their original, **inactive state**.

A cell must **integrate information from several signals** to produce an **appropriate response**. The **integration depends on intracellular coincidence detectors (integrator protein)**, which are only activated when they receive **two or more converging signals simultaneously**.



Many **cells need both soluble signals** (hydrophilic) and **signals from the extracellular matrix** (hydrophobic) to **grow** and **proliferate**.

A **single signal**, which only binds to one type of receptor, often **activates several parallel signaling pathways** so that completely different cell responses can be induced. **Signaling complexes** (formed by scaffolding proteins) prevent this as well as increasing the speed, efficiency and specificity of cell response:

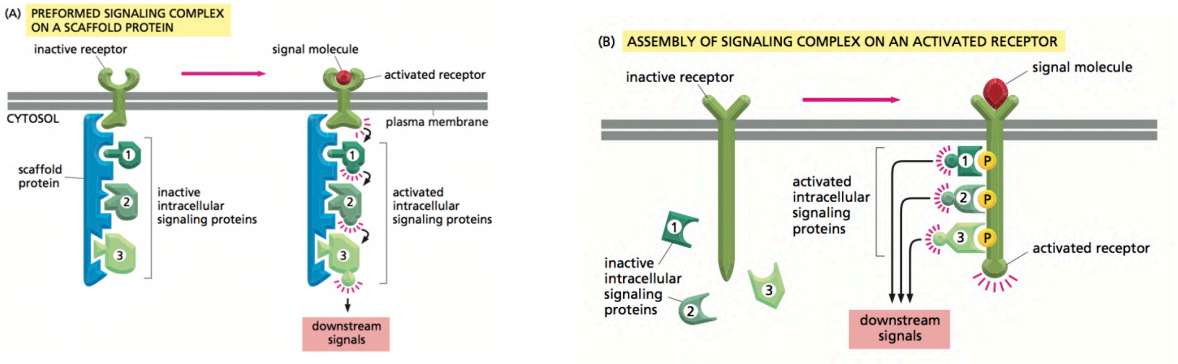
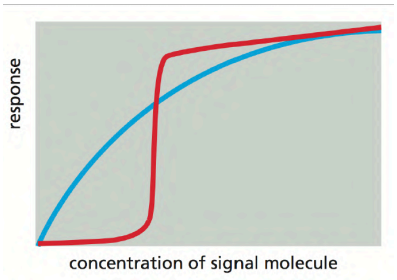


Figura 1: scaffolding proteins signaling complex.

10.2.1. SIGNAL RESPONSES

With **increasing concentration of the extracellular signal molecule**, **cell responses change in different ways**:

- Some cell responses **gradually increase** (blue).
- Others change **abruptly** (discontinuously) at a certain critical concentration of the signal molecule, according to the all-or-nothing principle (red).

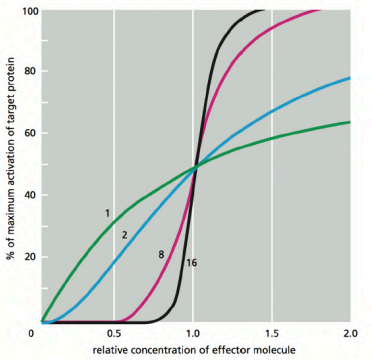


Three **possible molecular mechanisms** for **triggering a "sharpened" signal response**:

- ➔ The target protein needs more than one signal molecule to induce a response.
- ➔ The activation of an intracellular signaling protein requires phosphorylation at more than one site.
- ➔ An intracellular signaling molecule activates the enzyme that would favor the reaction needed, at the same time inhibits another enzyme that would catalyze the opposite reaction.

Activation curves for an **allosteric protein** as a **function** of the **concentration of the effector molecule**:

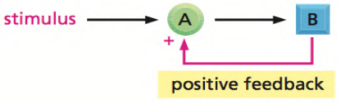
→ The **sharpness of the percentage activation** of the response **increases the more allosteric effector molecules** (here 1, 2, 8 or 16) **have to be bound simultaneously** to activate the target protein.

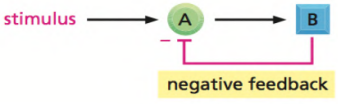


10.2.2. POSITIVE AND NEGATIVE FEEDBACK

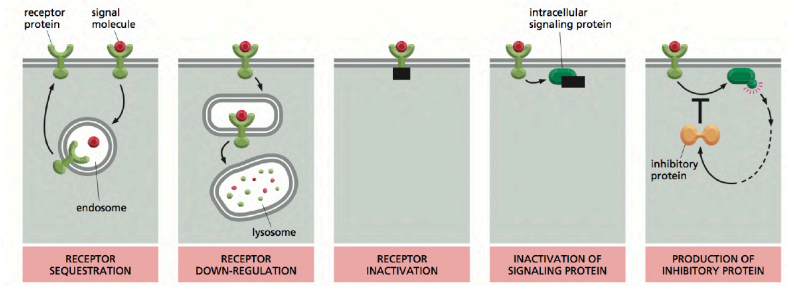
To get **correct all-or-nothing answers**, **positive feedback loops are needed**. Most intracellular signaling networks contain **feedback loops**.

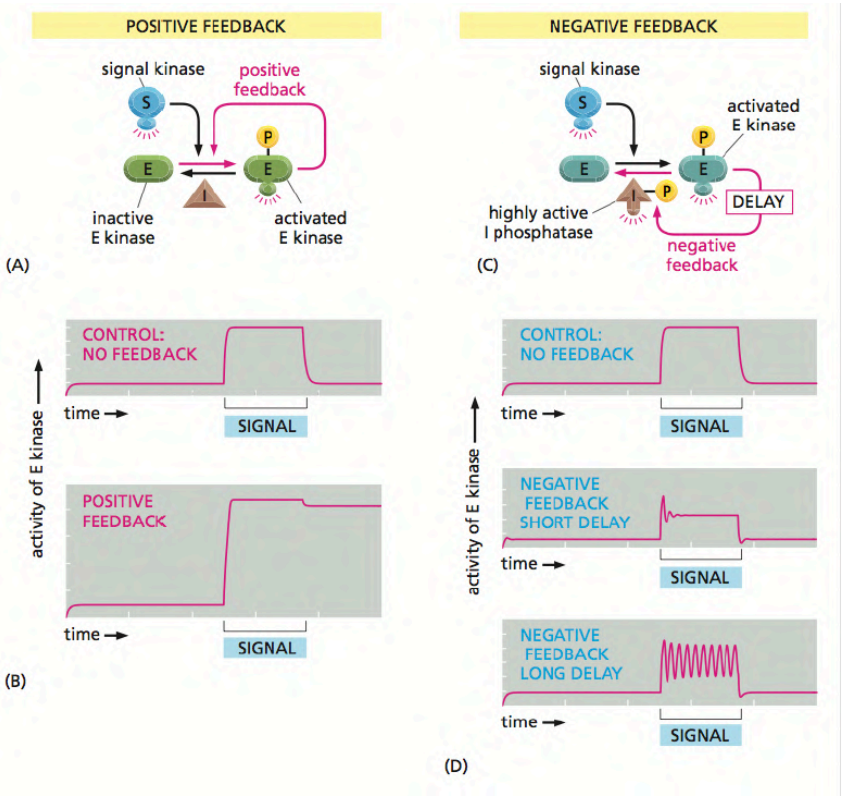
- Positive feedback allows cells to **maintain the response to a signal**.
- Negative feedback allows cells to **reduce their sensitivity to a signal** (desensitization).





Cells are able to **detect the same percentage change** in a signal over a very **wide range of stimulus strength**. This is made possible by a **reversible adaptation process** (**desensitization**), where a **sustained stimulus reduces the associated cell response**. This is a **negative feedback mechanism** that sets in **with a short delay**. The signaling pathway is modified so that the cell reacts less responsively to the same signal.



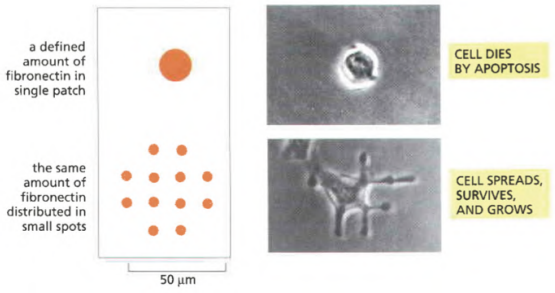


11. CELL-MATRIX SIGNALING

11.1. INTRODUCTION

Mastering the **interaction between cells and extracellular environment** is a fundamental prerequisite in order to engineer **functional biomaterial interfaces** able to instruct cells with specific commands. Such advanced biomaterials might find relevant application in **prosthesis design**, **tissue engineering**, **diagnostics** and **stem cell biology**.

The physical contact between a cell and its local environment strongly influences the behavior of the cell. Cell spreading on a substrate is essential for the survival of a cell.



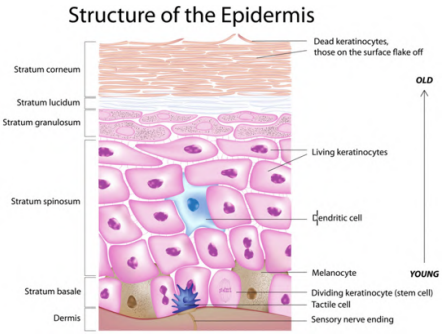
Signals displayed by the ECM (extracellular matrix) can be in the form of:

- **biochemical signals** (fixed proteins or diffusible factors).
- **mechanical stimuli** (hard/elastic, soft/compliant or gel-like tissues).
- **topographic signals** (fibrils, fibers, pores, meshes, protrusions).

Cesare Primultini (pcesare@ethz.ch)

Four **basic animal tissues**:

- ➔ **Epithelial tissue** (cell-cell): found in the outer surfaces of organs and blood vessels as well as on inner organ cavities.
- ➔ **Connective tissue** (cell-matrix): bone and cartilage tissue, fatty tissue and connective tissue in the narrow sense
- ➔ Muscle tissue.
- ➔ Nerve tissue.

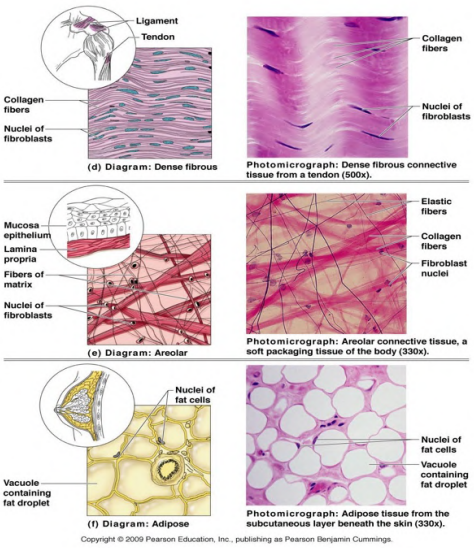


The two **main classes of physical contact** are:

- **(A) Cell-cell contact** (e.g. epithelial cells)
- **(B) Cell-matrix contact** (e.g. mesenchymal cells like fibroblasts)

Cells	Location	Function
Simple squamous epithelium	Air sacs of lungs and the lining of the heart, blood vessels, and lymphatic vessels	Allows materials to pass through by diffusion and filtration, and secretes lubricating substance
Simple cuboidal epithelium	In ducts and secretory portions of small glands and in kidney tubules	Secretes and absorbs
Simple columnar epithelium	Ciliated tissues are in bronchi, uterine tubes, and uterus; smooth (nonciliated tissues) are in the digestive tract, bladder	Absorbs; it also secretes mucous and enzymes
Pseudostratified columnar epithelium	Ciliated tissue lines the trachea and much of the upper respiratory tract	Secretes mucus; ciliated tissue moves mucus
Stratified squamous epithelium	Lines the esophagus, mouth, and vagina	Protects against abrasion

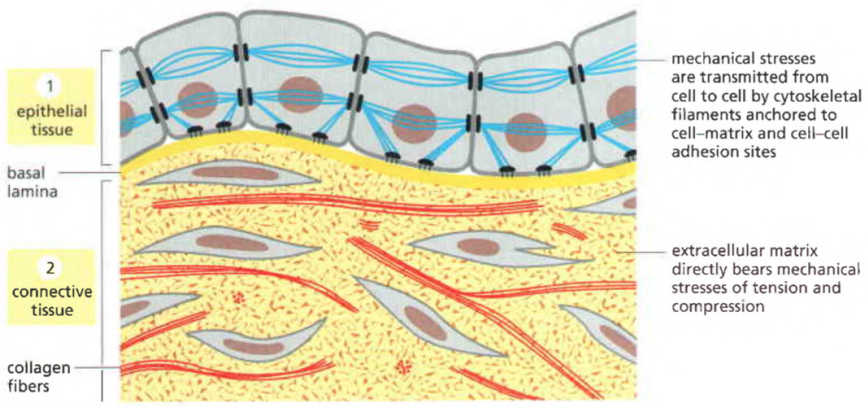
***(A)** different types of epithelium.*



***(B)** different types of connective tissue.*

In **connective tissue**, the **main component** that **carries the mechanical load** (stress-bearing) is the **extracellular matrix**. In **epithelial tissue**, however, this is the **cytoskeleton of the epithelial cells**, which is **connected from cell to cell by anchoring junctions** (black line pairs).

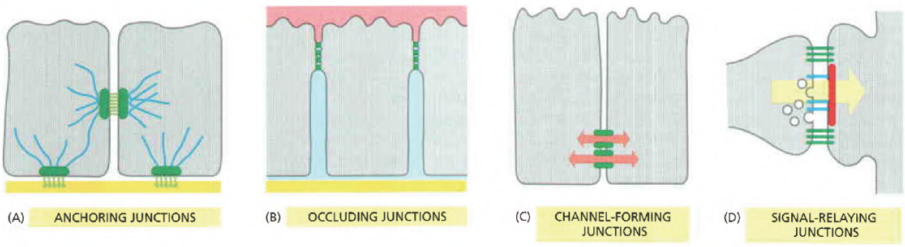
- ➔ *Cell-matrix junctions connect the epithelial tissue with the underlying connective tissue.*



11.2. FOUR DIFFERENT CONNECTIONS

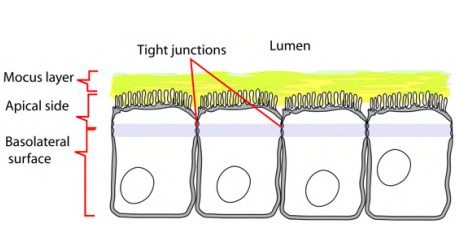
Summarizing:

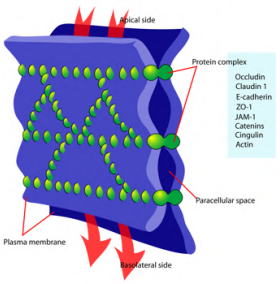
- **Anchoring Junctions**: mechanical stability, sensing physical forces.
- **Occluding Junctions** / Tight Junctions: physical sealant.
- **Channel forming connection**: Fast intracellular transport (also Ca2+). Chapter 10.
- **Signal transmission**: fast intracellular signaling (also chapter 10).

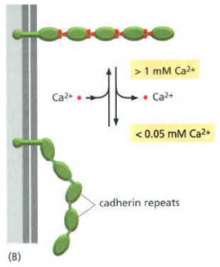


11.2.1. OCCLUDING JUNCTIONS

Physical sealant that allows an **epithelium to act as a barrier** against dissolved substances.



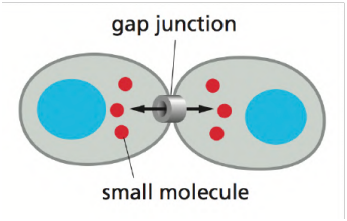




11.2.2. CHANNEL-FORMING JUNCTIONS

Mainly focuses on transport and signaling. **Gap junctions** are **specialized intracellular channels** between many animal cell-types. Direct **cytoplasmic connection** of adjacent cells allows **free movement of small molecules** and ions.

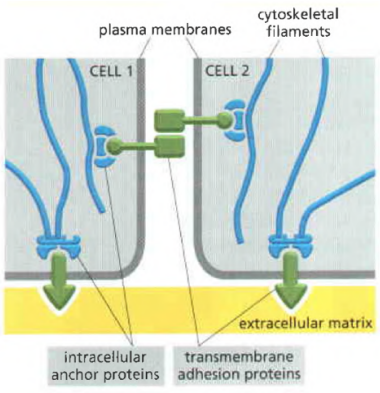
➔ Important function in **electrical and metabolic signaling**, functional grouping into cellular networks (cardiac depolarization wave, apoptosis).



11.2.3. ANCHORING JUNCTIONS

They give **mechanical strength and integrity**. Formed by **transmembrane adhesion proteins**: **cadherins** (cell-cell) and **integrins** (cell-matrix), which **connect the cytoskeleton with extracellular** structures.

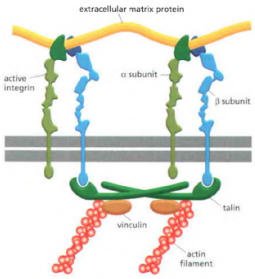
The extracellular part of the cadherin consists of **cadherin-repeats**, which are connected by **flexible joint regions** *(which wouldn't be enough to hold the cells together).* **Ca2+ binds to each joint** so that **it cannot bend**. Without Ca2+ the molecule has no more tension and adhesion can no longer take place.



In general, **specific combinations of extracellular signals are necessary** (instead of just one signal alone) to stimulate **complex cell behavior**.

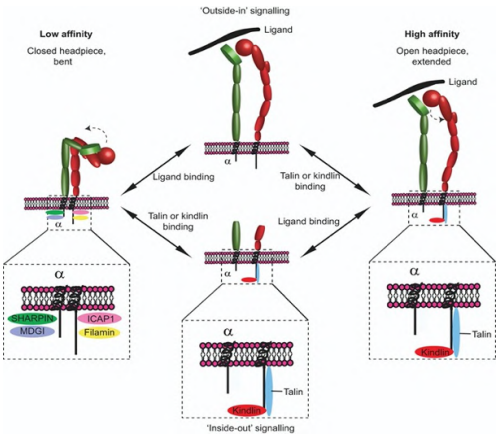
11.3. FUNCTIONAL PARTS OF JUNCTIONS

11.3.1. INTEGRINE



Integrins are **transmembrane proteins** that **connect the cell and the matrix**. Understanding their function is important for the design of biomaterials.

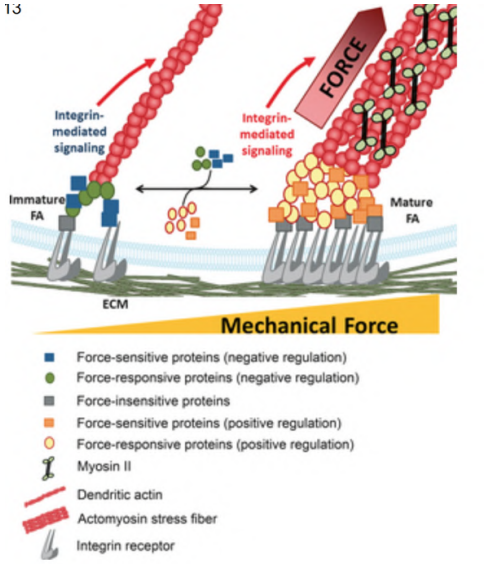
- The **head of the integrin** molecule attaches directly to a protein of the extracellular matrix, such as fibronectin.
- The **intracellular part** of the integrin binds to **talin**, which in turn binds to **actin** (cytoskeleton).
- Other intracellular anchor proteins such as **α-actinin**, **filamin** and **vinculin** help to **strengthen the binding.**



Mechanical principle of integrin: conformational changes at opposite ends of the molecule are **coupled together** and tensile forces tighten the bond:

- In the **absence of an extracellular ligand**, the integrin molecule appears small and **tightly folded**.
- When an **interaction with an RGD peptide** can take place, the **integrin unfolds** into an elongated structure with two units. **RGD** refers to an **amino acid sequence** (R: arginine, G: glycine and D: aspartic acid) which is found on certain ECM proteins such as **fibronectin**.
- Cells thus bind via integrins to the RGD sequence of ECM proteins.

11.3.2. FOCAL ADHESIONS



Focal adhesions are **anchoring cell connections** that **mechanically couple the actin cytoskeleton** of a cell to the **substrate (ECM).** They transfer **matrix forces to the cytoskeleton** (important in cell-biomaterial interactions).

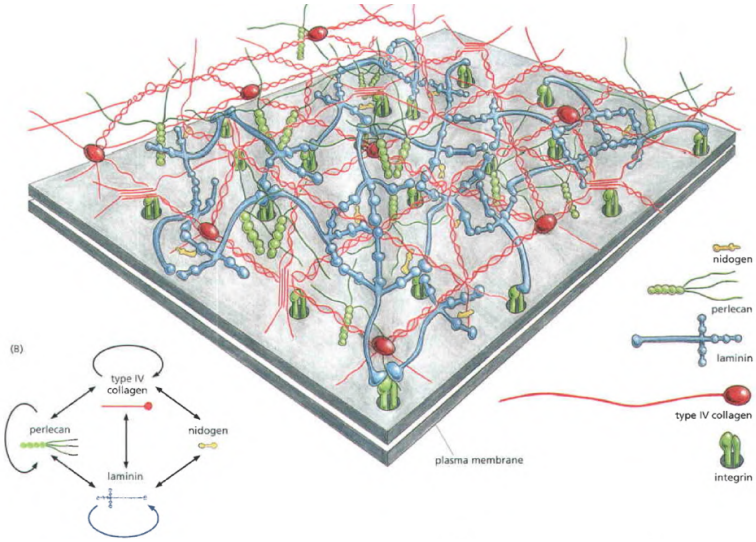
➔ *Mechanotransduction (converts mechanical stimulus into electrochemical activity) through focal adhesion: focal adhesions not only **serve to anchor the cell**, but **also act as signal transmitters** that **inform the cell about the state of the ECM** and thus influence its behavior.*

11.4. EXTRACELLULAR MATRIX (ECM)

ECM **consists of all macromolecules in the intercellular space**, it is a **dynamic structure** with important **chemical** and **mechanical properties**.

Many **cellular processes** involve **interactions of cells with the ECM** (e.g. cell cycle, migration, differentiation, apoptosis). The extracellular matrix not only **connects cells with each other** in a tissue, it also **guides processes in wound healing** and **embryonic development**.

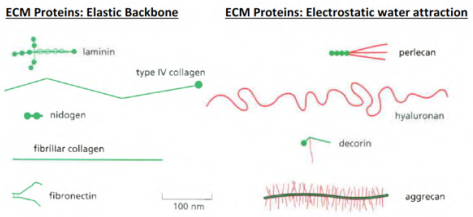
The **basement membrane** is a **thin layer between epithelium and adjacent tissue** and consists of the **linkages** of the **protein** laminin, collagen, nidogen and the proteoglycan perlecan (PLC).



The **most important ECM proteins**:

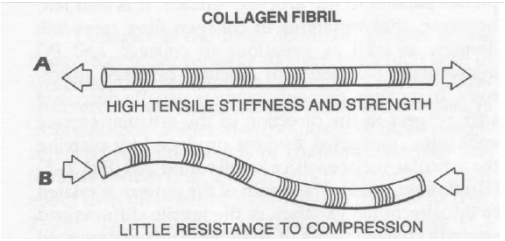
- **Proteoglycans** (heparan sulfate, chondroitin sulfate, keratan sulfate).
- **Non-proteoglycan** polysaccharides (hyaluronic acid).
- **Fibronectin** (fibrous).
- **Collagen** (fibrous, large family).
- **Laminin.**

The **combination** of **collagen and proteoglycan** results in a **stretchable and pressure-resistant ECM.**



The **viscoelasticity of tissue** depends on:

- **fibrillar structures** (elastic)
- **proteoglycan portion** (viscous)



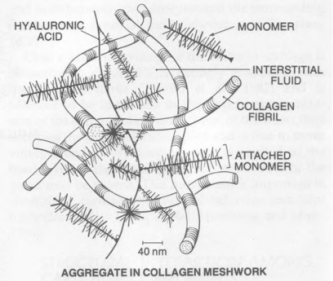
Cell-matrix interactions are important for cell behavior:

- **biomechanically** (especially collagen and proteoglycans).
- **biochemically** (all ECM ligands).

→ **ECM macromolecules** have **very different shapes** and **sizes** (protein in green, glycosaminoglycan in red).

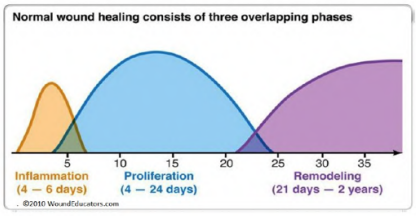
Cesare Primultini (pcesare@ethz.ch)

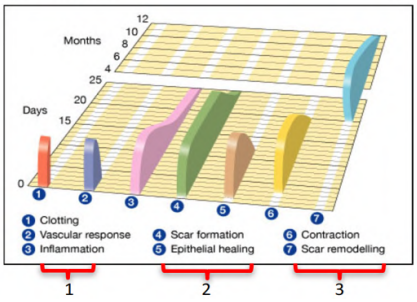
11.4.1. CARTILAGE: MOLECULAR ORGANIZATION



Structural components of cartilage are **collagen and proteoglycan**. These **interact with each other** and form a **solid**, **porous**, organic matrix with a **high content of water** (~70%).

The **composition of the ECM** reflects the status of wound healing:

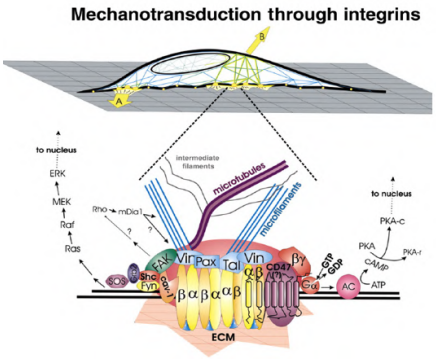


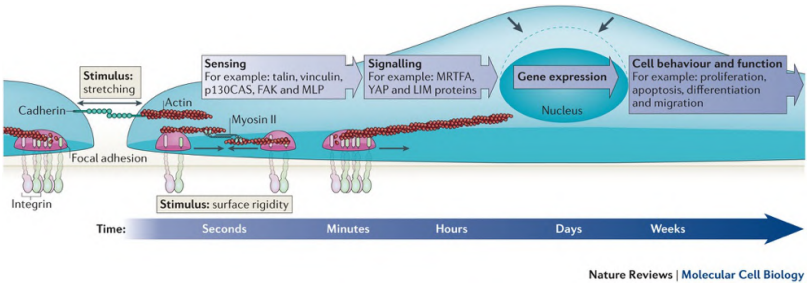


- **Phase 1**: blood cell products (platelets), stimulating proteins.
- **Phase 1-2**: granulation tissue (fibronectin, small collagen & proteoglycans).
- **Phase 2-3**: revascularization and vascular remodeling (collagen, laminin)
- **Phase 3**: scar tissue remodeling (small amounts of fibronectin and small collagen, proteoglycan, with time also larger collagens and elastin)

Cell-Matrix signaling (Junctions, Integrin and Focal Adhesions): **ECM binding and/or mechanical forces activate enzymes** (protein kinases or phosphatases) which **phosphorylate or dephosphorylate signaling proteins**, thus activating or deactivating it, depending on the signaling pathway.

Cell-matrix activity can also mediate signaling via **GTP-binding** (serve as activation signals, if a GTP protein is bound to a pathway protein that the signal is “ON”).



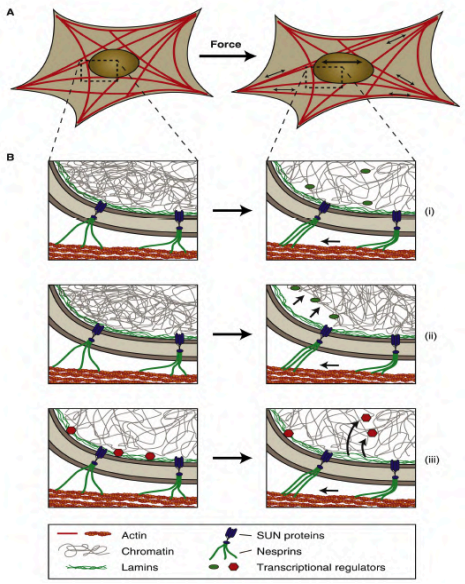


Nature Reviews | Molecular Cell Biology

Cesare Primultini (pcesare@ethz.ch)

Nuclear Deformation: **force-induced nuclear deformations** can **modulate the gene expression** of a **cell**.

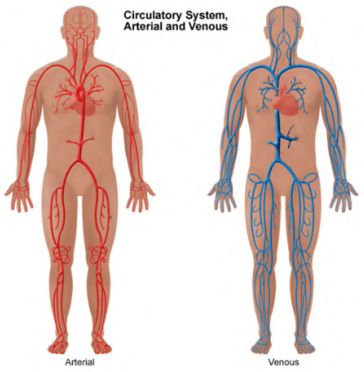
- **(A) Tensile forces** can cause **nuclear deformation** by being **transmitted from focal adhesions** via the **(actin) cytoskeleton** to the nucleus.
- **(B)** Potential **molecular mechanisms** for **<u>nuclear mechanosensing</u>** *(ability of a cell to sense mechanical cues of its microenvironment):*
    - **(i)** Opening of chromatin structures under force allows access of **transcriptional regulators**.
    - **(ii) Chromatin detaches** from the **lamina**, **exposing genes** of the nuclear periphery (these are otherwise often transcriptionally inhibited).
    - **(iii) Conformational changes** or partial unfolding of the nuclear envelope alter the interaction with transcriptional regulators.



➔ **Mechanical and biochemical** (ligand/receptor) **contacts between** a **cell and its environment** (cell-cell and cell-ECM) are **<u>crucial for cell behavior</u>**.

➔ **Activation and occupation** of cytoskeleton/Focal Adhesion-associated signal receptors. Many of these are **sensitive to traction** (force-regulated protein conformation). **Transfer of cell forces** to the **nuclear membrane** (and chromatin) and **regulation of gene transcription**.

12. BLOOD

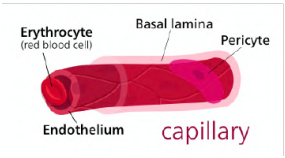


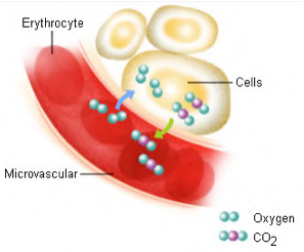
The **circulatory system distributes nutrients** (e.g. glucose, O2) **and hormones** throughout the body, **removes waste products** from the tissues (e.g. CO2) and is **able to regulate body temperature** and remove heat generated by metabolic activities of the internal organs.

Every living cell in the body is **no more than 10-100µm away from a capillary** (small blood vessel with **walls 8µm in diameter**, about the **size of a red blood cell**). This short distance allows a **diffusion** of O2, CO2 and many other small dissolved substances into the capillaries and then into the cells.

➔ The **direction of the diffusion** is **determined by concentration** and **partial pressure gradients**.

On average a person has **5.2 L of blood**, which corresponds to **8 percent (+/- 1%) of the total body weight**. The blood is a **complex heterogeneous suspension** of **solid elements** (blood cells or hematocytes) **in a light liquid** (plasma). The plasma has a **mass density of 1.057 g/cm3**, so basically like water, but is **6 times more viscous**.



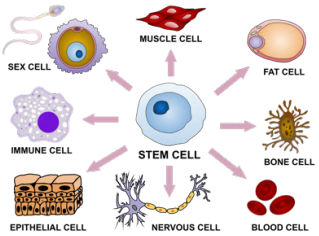


Blood cells (hematocytes) include **3 basic cell types**:

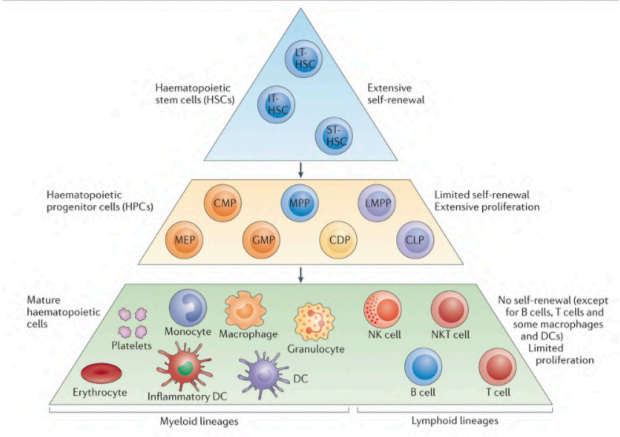
- **red blood cells** (erythrocytes, almost **95%** of the solid components)
- **white blood cells** (leukocytes, less than **0.15%** of the blood cells)
- **platelets** (thrombocytes, about **5%** of the blood cells).

12.1.1. HEMATOPOIESIS

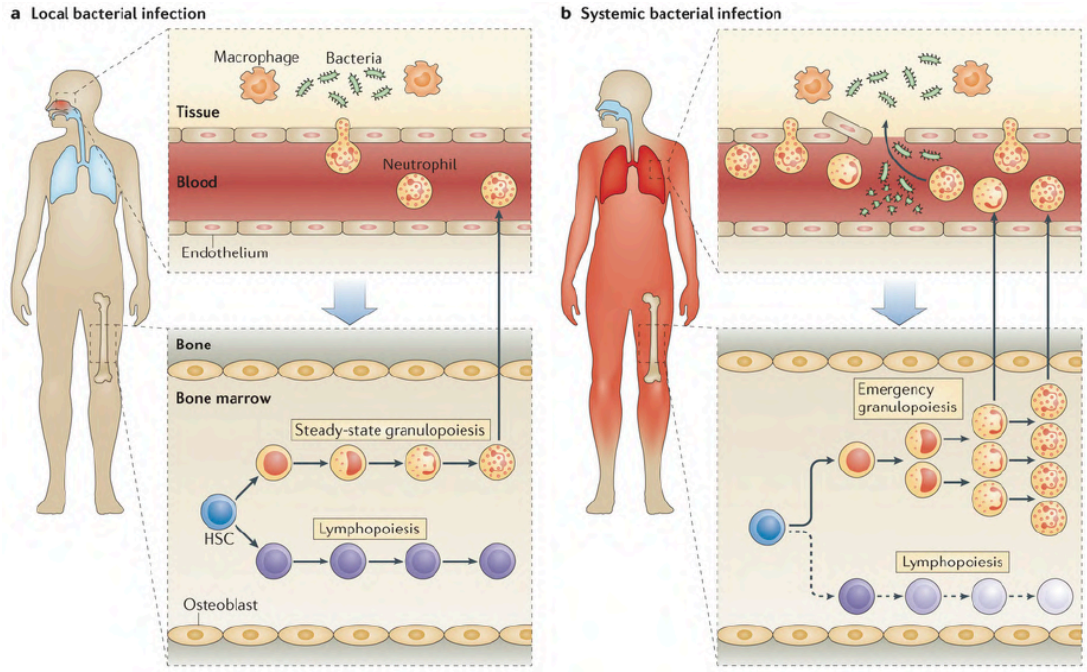
Hematopoiesis is a **complex biological process** that **physiologically takes place mainly in the bone core** (marrow) and ensures the **continuous supply of blood cells**.



In hematopoiesis, **cell divisions** and **increasing differentiation** turn **multipotent hematopoietic stem cells** into **mature blood cells** (hematocytes).



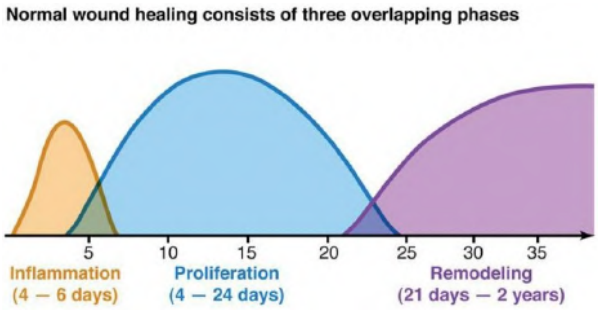
- The main function of **erythrocytes** (red blood cells) is to **transport blood gases**.
- The main function of **leukocytes** (white blood cells) is the **recognition and elimination of foreign substances** (such as infectious agents). These are divided into two parts as follows:
    - **Agranulocytes** (lymphocytes and monocytes) are responsible for **recognition**.
    - **Granulocytes** (neutrophils, basophils and eosinophils) for **elimination**.
- The main function of **platelets** is their contribution in the **process of blood coagulation**.



13. WOUND HEALING

13.1. PHASES OF WOULD HEALING

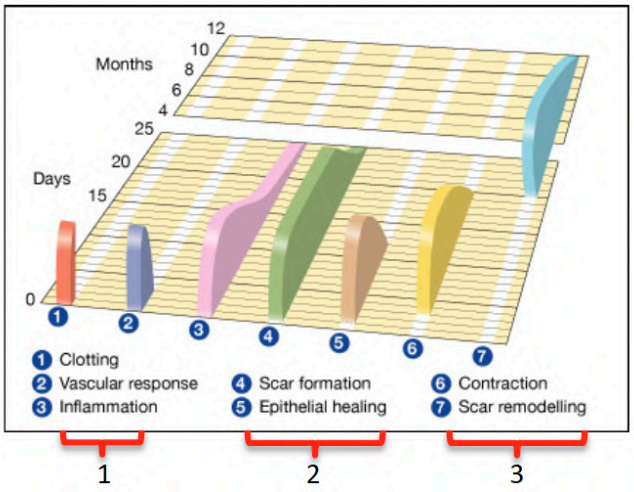
Hemostasis & Inflammation → Tissue Modeling → Tissue Remodeling



- **PHASE I**: **Hemostasis and inflammation**: hemostasis, lay the **groundwork for repair**.
- **PHASE II**: **Cell proliferation / tissue modeling**: **recruiting necessary cell populations**, creating a template for tissue architecture, **restoring tissue function** as quickly as possible.
- **PHASE III**: **Tissue Remodeling**: **Optimization of the tissue** (if possible, as before injury).

The following cellular processes take place in the three phases of wound healing:

- IN PHASE I:
  - **blood cell products** (platelets),
  - **temporary matrix scaffold** (fibrin),
  - **stimulating proteins** (recruit vascular and other tissue-related stem cells, macrophages and immune cells, fibroblasts).
- IN PHASES I-II:
  - **granulation tissue is formed** (mixture of all necessary players: stem cells, immune cells, other cells, fibronectin, smaller collagens and proteoglycans)
- IN PHASES I-III:
  - **revascularization** (vascular modeling) and **vascular remodeling** (larger collagens, lamin).
- IN PHASE III:
  - **scar tissue remodeling** towards normal tissue:
    - **optimal cell types**,
    - **optimal matrix** (usually *little or no fibronectin, few small collagens and proteoglycans, more larger collagens and elastin).*



13.1.1. TISSUE MODELING

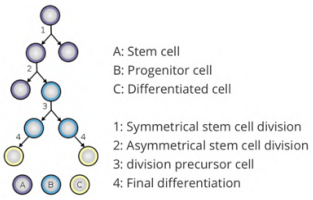
Gaps in tissue are **filled with cells**, **matrix** and **cytokines** for tissue generation. This **temporary tissue** (cells and matrix) is called **granulation tissue**. The function of the **emergency tissue** is essentially **sealing** and **connecting** the **loose ends**. Important steps are:

- **ANGIOGENESIS**: **formation** of **new vessels** from **pre-existing blood vessels**. Endothelial cells (lining blood vessels) migrate, proliferate and form new blood vessels.
  - **New blood vessels** are **needed** to **bring oxygen**, **nutrients**, **cells** and important **growth factors to the site of injury**.
  - **Insufficient angiogenesis** (growth of new blood vessels) is a **characteristic of chronic wounds**.
  - The following factors often **restrict angiogenesis**: **old age**, **diabetes**, **high cholesterol** level, high **alcohol** consumption, **tobacco** consumption.

- **MATRIX DEPOSITION**: **fibroblasts** (form matrix fibers) **migrate within the wound tissue** where they deposit proliferated and extracellular matrix.

13.1.2. STEM CELLS AND PROGENITOR CELLS

After the **formation of the blood clot** and the **infiltration of immune cells**, **stem cells are recruited** to the **site of injury** (via blood circulation or from surrounding tissue). **Stem** cells **multiply** and **differentiate into required cell types** (vascular and other tissue-specific cells).

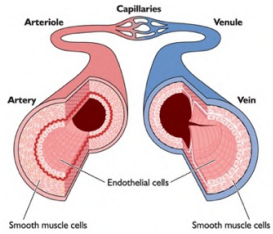


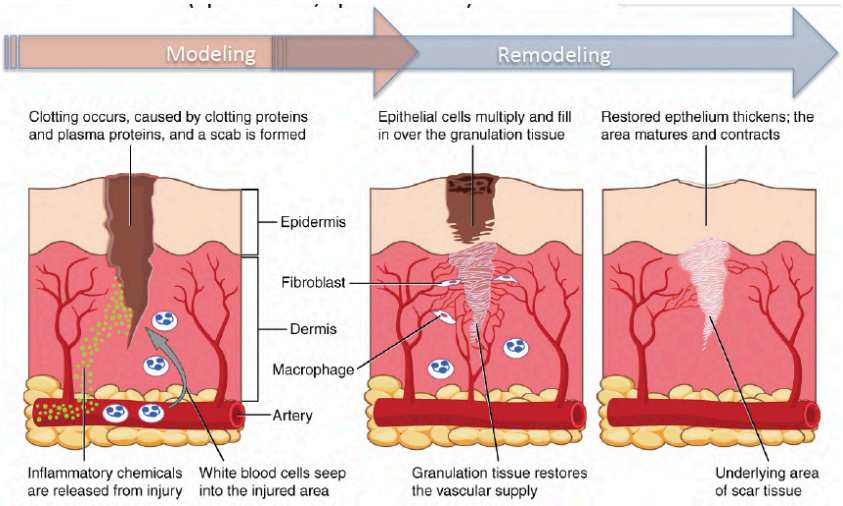
13.1.3. MACROPHAGES

Macrophages (Greek for "big eaters"), belong to the **white blood cells** that **devour and digest cellular residues**, **foreign substances** and **microbes** (phagocytosis). Macrophages mediate many cellular processes through **<u>paracrine signaling</u>** (local signals released into extracellular space).

13.1.4. ENDOTHELIAL CELLS

They **cover blood vessels** and thus form the **endothelium**. The **<u>formation of new capillaries</u>** is one of their main tasks.

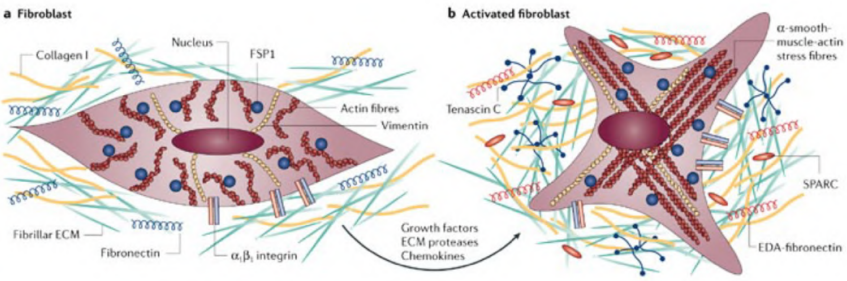




13.1.5. FIBROBLASTS

Those are **cells that form the extracellular matrix** (produce **fibronectin** and **collagen**) and thus give a tissue **hold and structure**.

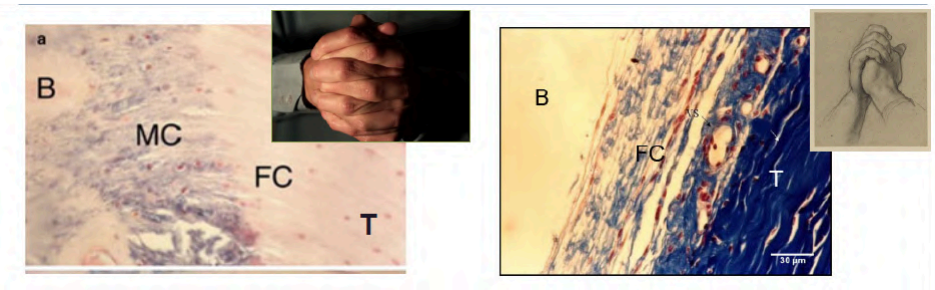
- ➔ **Activated fibroblasts or myofibroblasts generate** strong **contractile forces** to **arrange and rearrange the matrix**.
- ➔ Fibroblasts can **migrate as mature cells** from **surrounding tissue** into the **endothelium** or **differentiate** from a precursor cell (stem cell).



Cesare Primultini (pcesare@ethz.ch)

13.1.6. WOUND HEALING EXAMPLES

Tissue often heals incorrectly and therefore **often cannot completely restore** the **original mechanical strength**. Typical example of a tendon growing back to the bone after a tear (B: Bone, MC: Mineralized Fibrocartilage, FC: Fibrocartilage, T: Tendon):



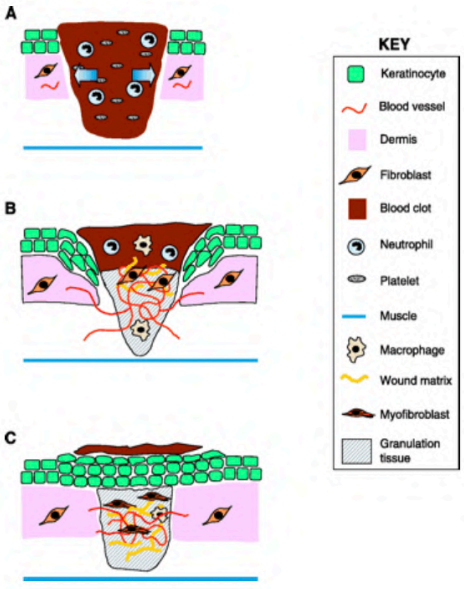
Schematic representation of the different stages of wound healing (skin):

(A) **<u>12 - 24 hours</u>** after the injury a blood clot has formed. **Neutrophils** are the **first immune cells** to **enter** the **blood clot**.

(B) **<u>3 - 7 days</u>** after injury most **neutrophils have undergone apoptosis**. But now many **macrophages are present**. Endothelial precursor cells migrate into the blood clot, proliferate and **form new blood vessels**.

- **FIBROBLASTS** or their precursors **migrate** into the **sore tissue** where they **proliferate**, differentiate and **deposit extracellular matrix**. **<u>Granulation tissue</u>** is generated.
- **KERATINOCYTES** (**skin cells**) **proliferate** and migrate along the injured dermis and over this provisional dressing of cells and matrix.

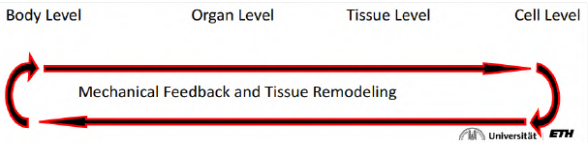
(C) **<u>1 - 2 weeks</u>** after the injury, the **wound is completely filled** with **granulation tissue**. **Fibroblasts** have transformed into **myofibroblasts** (highly contractile cells), resulting in **wound contraction** and deposition of collagen. The **wound** is now **completely covered with a neo-epidermis** (new skin surface).



14. MECHANOBIOLOGY

Mechanobiology is an emerging field that integrates biology and engineering. It involves the way in which physical forces and changes in cell- or tissue-mechanics contribute to development, physiology and disease. Improvements in mechanobiology suggest that **changes** in **cell mechanics**, **ECM structure** or **mechanotransduction** have an **impact** on the **development** of **many diseases** (atherosclerosis, asthma, osteoporosis, heart failure, cancer).

Cells **optimize tissues according to the load acting** at each level:



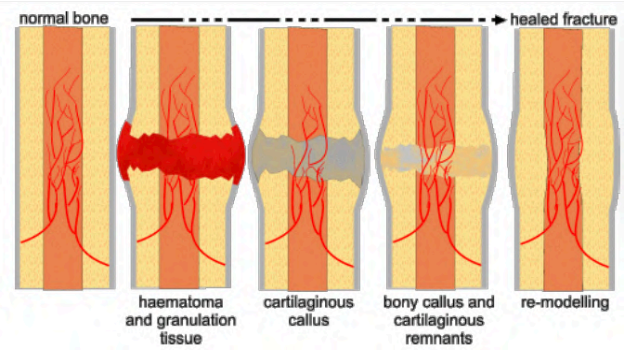
14.1. TISSUE HEALING (E.G: BONE FRACTURE)

Within a few hours after a bone fracture:

- The extravascular blood cells form a **blood clot** called a **hematoma**.
- All cells in the blood clot degenerate and die over time.
- **Fibroblasts** and **stem cells**, on the other hand, **can survive, divide and spread** (proliferate). They **form** a loose cell aggregate with small blood vessels called **granulation tissue**.
- **Tissue healing** is a **mechanically driven process**, from initial scar formation to long-term remodeling.

A few days after the fracture:

- Many of the **skeletal progenitor cells** in the wound and the tissue around the fracture site develop into **osteoblasts**, which form a **very unstructured bone**.
- **Fibroblasts** in granular tissue **develop** into **chondroblast-like cells** that **can form hyaline cartilage**.
- These **two new tissues** grow until they **merge with their counterparts from other parts** of the fracture, which is called **fracture callus**.
- Finally, the **fracture gap is bridged** with **hyaline cartilage** and **braided bone** (gives some strength).
- Then the hyaline cartilage and braided bone are replaced by better organized bone and a supporting vascular system. The strength of the bone is now almost restored.



14.1.1. PSEUDOARTHROSIS

It **occurs** when, **6 months** after a fracture, the **segments have not fully hardened**. There is a **pathological mobile connection** between two or more bone fragments.

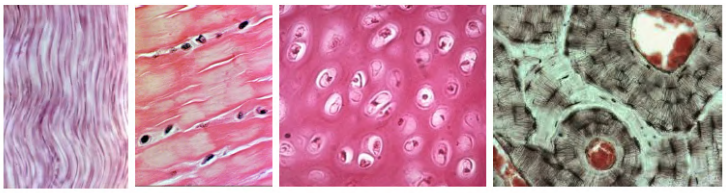
➔ ***Bone healing is strongly influenced by mechanical tension in the tissue***, which **drives cell differentiation**, **proliferation** and **matrix formation**.

14.1.2. TYPES OF TISSUES IN BONE HEALING

- **Fibrous connective tissue**:
  - cells: fibroblasts.
  - ECM: mainly type I collagen with fibers arranged in the direction of stress.
- **Cartilage tissue**:
  - (Cartilage) **cells**: chondroblasts and chondrocytes.
  - **ECM**: especially type II collagen in a highly hydrogenated matrix (rich in proteoglycan, which gives the viscosity of the matrix).

- **Fibrocartilage**: mix of the **two upper tissues**, occurs in **poorly healed skeletal tissue** and in tissues with **very high mechanical demands** (temporomandibular joint).

- **Bone tissue**: osteoblasts and osteocytes, mineralized type I collagen matrix.



Fibrous → Fibrocartilgenous → Cartilagenous → Ossified

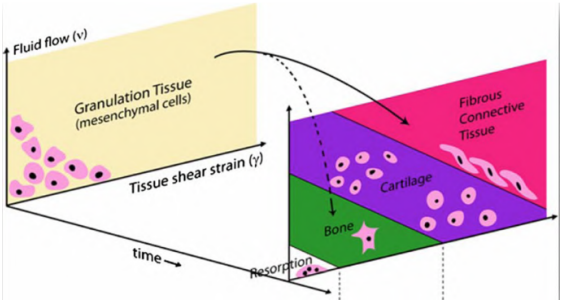
Key points:

- ➔ **Cell behavior**, especially in the **musculoskeletal system** (musculoskeletal tissue), is **strongly regulated by mechanical forces**. Important role in **tissue healing** and **tissue homeostasis** (cell differentiation, cell matrix production).
- ➔ After a bone fracture, the **wound is first filled with soft tissue**, which **becomes increasingly mineralized**. Strong **control through mechanical feedback**.
- ➔ **Maintenance of bone** is also constantly **regulated by mechanical feedback**.

14.1.3. MECHANOTRANSDUCTION

An important **challenge in the field of mechanobiology** is the **understanding of mechanotransduction**, the **molecular mechanisms** by which **cells can perceive and respond to mechanical signals**.

In addition to **many molecular structures** and **signaling molecules**, **cell-generated tensile forces** also **contribute to cellular mechanotransduction by modulating the tension in cells**, **tissues** and **organs**.



This **tension regulates the mechanical stability** of the **cell** as well as the **mechanical signal transduction** from the **macro to the nanoscale**.

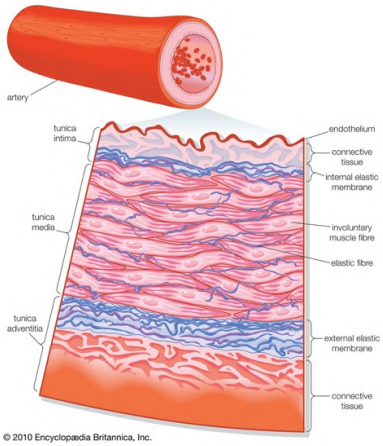
14.2. MECHANOBIOLOGY IN ARTHEROSCLEROSYS

Healthy **arteries are flexible** and **elastic**, but over time the **artery walls can harden**, arterial hardening is called **arteriosclerosis**.

Atherosclerosis is a form of arteriosclerosis and **refers to the abnormal accumulation** of **cholesterol ester** (a dietary lipid) and **other fats** in the **inner wall layer** of arterial blood vessels.

- The deposition of such plaques can **restrict the blood flow**.
- When these **plaques rupture**, a **blood clot forms**, further restricting blood flow.
- Although atherosclerosis is often thought to be a **heart problem** (coronary artery), it **can occur** in arteries **anywhere in the body**.

Micrograph of an artery that supplies the heart showing significant atherosclerosis and marked luminal narrowing.

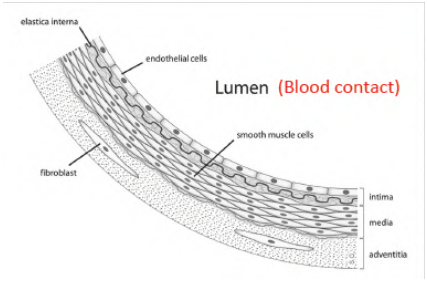


The arteries have a few layers, starting from the outside:

- **Tunica adventitia**: is the **outermost layer of a blood vessel**. Consists of **connective tissue** containing collagen.
- **Tunica media**: consists of **smooth muscle cells** and **elastic tissue** (actual connective tissue).
- **Tunica intima**: is the **innermost layer**, in direct contact with the blood flow. Consists of **endothelial cells**.
- **Lumen**: the cavity in which the blood flows.

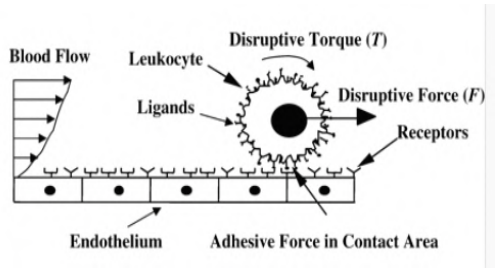
The **endothelium** is the **innermost thin layer of cells** that shapes the inner surface of blood vessels and lymphatic vessels. It forms an **interface between the circulating blood** (or lymphatic fluid) in the lumen and **the rest of the vessel wall**.

➔ *Many biological responses of these endothelial cells are highly dependent on fluid shear stress*.



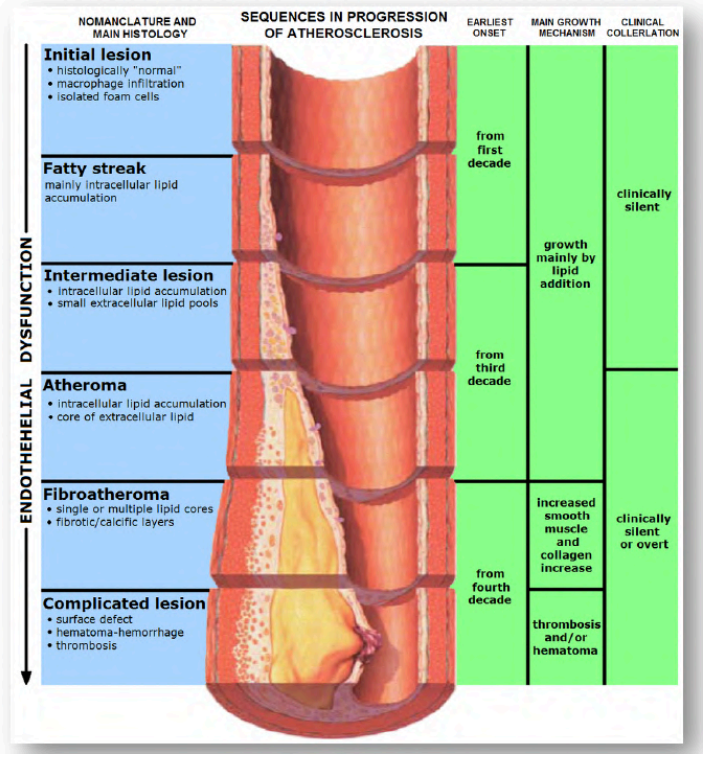
Leukocyte rolling and cell-cell contact (signaling):

A fine balance between **hydrodynamic forces** and **protein adhesion** forces



Atherosclerosis is a **slow progressive disease** that can begin in childhood. It can **begin with an injury to the inner layer of an artery**. Such injury can be **caused by the following factors**:

- **Mechanical factors**: **high blood pressure.**
- **Biochemical factors**: too much **cholesterol** (used to build cells and certain hormones), **triglycerides** (unused calories that provide the body with energy) in the blood, **diabetes**.
- **Biological factors**: inflammation (arthritis, skin tuberculosis, infections).



Faulty cell-matrix remodeling in atherosclerosis.

Circulating cells: white blood cells, monocytes (develop into macrophages in tissue).

Resident tissue cells (in intima): endothelial cells, smooth muscle cells (media), fibroblasts (externals)

Processes in atherosclerosis:

- Lipid **deposition**,
- **Oxidation** and **inflammation**,
- **Activation** of **macrophages**: foam cells,
- **Plaques** initiation: foam cell, lipid core, fibrous cap (collagen & elastin, calcium),
- Arterial **remodeling**,
- **Rupture** of the plaque,
- **Contact** between lipid core and blood,
- **Formation** of blood clots,
- **Wound healing processes**, alternating repair and rupture.

Inflammation in atherosclerosis: **immune cells** are **recruited by cytokines** and **surface adhesins** through a **highly regulated multistage process**.

14.2.1. ARTERIAL STENT (current standard treatment)

A **stent** is a **mesh tube** used to **treat narrowed or weakened arteries**. **Percutaneous coronary intervention** (PCI) is the procedure of **placing a stent in an artery**, sometimes called **coronary angioplasty**.

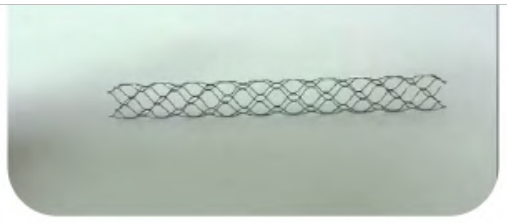
PCI restores blood flow through a narrowed or blocked artery by **supporting the inner wall** of the artery months to years after PCI. Stents can also be inserted into weak arteries to improve blood flow and prevent the formation of an aneurysm (arterial sacculation).

- Stents are usually made of **metal mesh**, sometimes of **softer materials**.
- Stent grafts are **coated stents** and are **used in larger arteries**.
- Some stents are **coated with a drug** that is released slowly and continuously into the artery (drug-eluting stents).
  - ***The drug prevents the artery from re-occluding (prevents restenosis, frequent complications).***

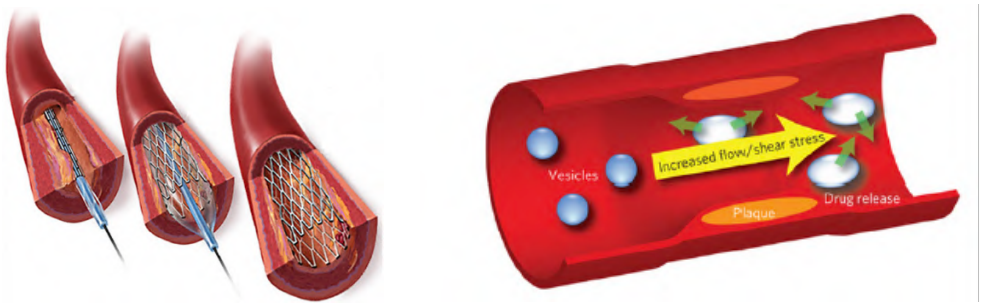
In angioplasty, a **small balloon** is **inserted into the artery** and **inflated so that the artery is stretched**. The **reticular stent is also inflated** and then ensures that the artery remains open.

A shape-memory alloy ("smart metal") stent:

Such materials can "remember" a pre-programmed shape, for example when brought to body temperature (37°C).



Through **highly targeted drug delivery**, side effects can be reduced, and the drug can be used more efficiently. **Shear stress** in **constricted areas** of the blood vessel wall become **great** and the blood flow can even become **turbulent**. This can be used to **trigger mechanically sensitive drug delivery** at these points.



14.3. PATHOLOGICAL MECHANOBIOLOGY IN CANCER

14.3.1. CANCER GROWTH AND DEVELOPMENT

Cancer **can start** in (**almost**) **all cells of the body**. Malignant transformations (acquisition of cancerous characteristics) occur due to **one or many gene mutations**. Cancer cells acquire the following characteristics:

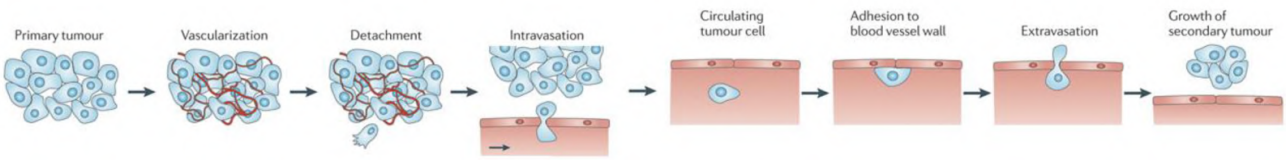
- **Insensitivity** to **growth inhibitory** signals (e.g. cell contact inhibition),
- **Apoptosis resistance**,
- Unlimited replication potential,
- **Invasion**,
- Ability to **bypass the immune system**,
- Activation of **vascularization mechanisms**.

The process of **tumorigenesis** has many **similarities with defective healing processes** in connective tissue:

- excessive matrix formation,
- misdirected tissue remodeling,
- chronic inflammation,
- uncontrolled and/or inappropriate angiogenesis (blood vessel formation and remodeling).

These **similarities have led to the following question**:

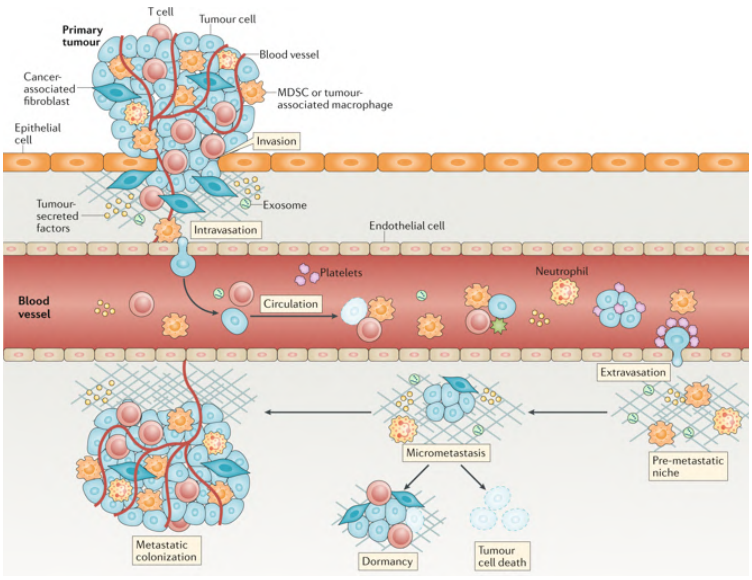
➔ Are **epithelial cells** (as previously suspected) or **stroma-supporting cells** (**fibroblasts**) that **initiate** and **maintain disease evolution** (neoplasia = new tissue formation)?



Cesare Primultini (pcesare@ethz.ch)

During **development and metastatic spread**, **cancer cells go through** the following **steps**:

- Growth and vascularization of the primary tumor
- Invasion into adjacent tissue
- Intravasation into the vascular or lymphatic system
- Survival in circulating phase
- Adhesion to endothelium and extravasation into the target organ
- Growth of secondary malignancy

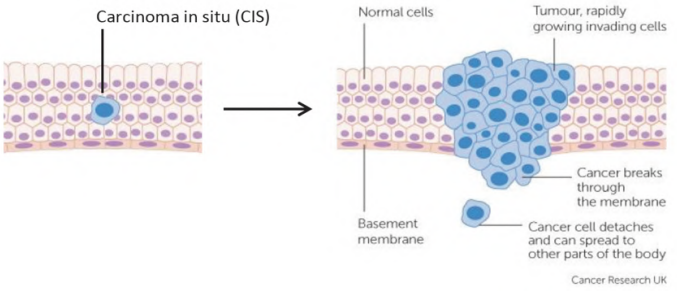


Tumors of average size **consist of several billion cells** and can release up to **about one million cells per day into circulation**. Only those cells that have already adapted their metabolism and physical properties can survive in the blood.

➔ **Metastasis**, i.e. the **formation** of **tumors** at **distant sites** in the body, is the **deadliest phase** in cancer development and is **responsible for almost 90%** of all **cancer-related deaths**.

The **main causes of cancer** are:

- **Genetic changes**: either **inherited** or **spontaneously** occurring after birth.
- **Chemicals**: in environment, mutagenic (**tobacco**) and non-mutagenic (**alcohol**) chemicals.
- **Nutrition and lifestyle**: **low vegetable** intake, highly processed and **salty diets** are associated with an increased risk of cancer.
- **Infections**: Oncoviruses cause tumorigenesis of the infected cell (e.g. papillomavirus)
- **Radiation**: causes mutations that can cause cancer



A **solid tumor** is **limited in phases 0 and 1** and **begins to invade** neighboring tissue in **phase 2**. After **extensive invasion**, in **phase 3**, tumor cells detach and enter the lymphatic or blood system. In **phase 4** secondary tumors (**metastases**) **have formed**.

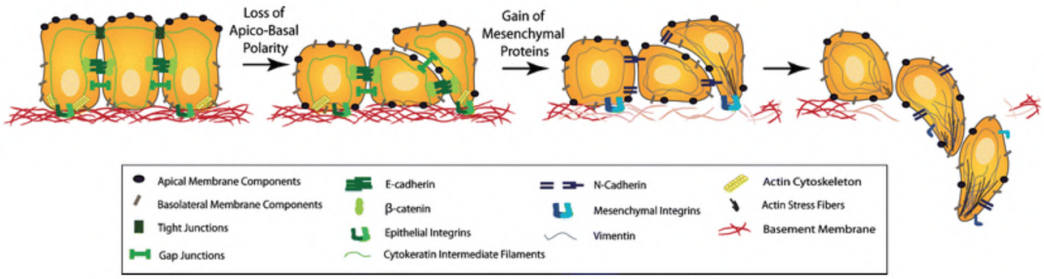
Tumors exhibit increased stiffness compared to the **surrounding tissue**. This *property is used in the detection of cancer* (manual palpitation, sonoelastography). However, **malignant tumor cells** are **softer** than their healthy counterparts.

Epithelial mesenchymal transition (EMT) is a process in which **epithelial cells acquire mesenchymal properties (become stem cells → differentiate into a variety of cell types)**:

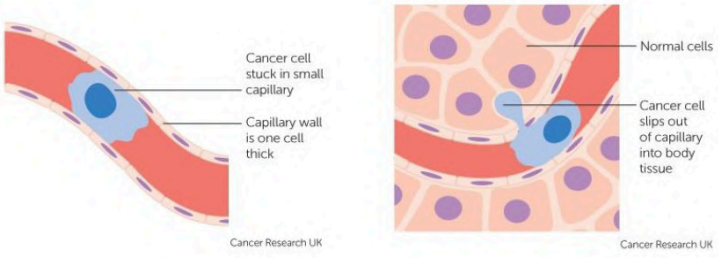
- **loss** of **cell-cell connections** and apical-basal polarities,
- **reorganization** of the **cytoskeleton**,
- **adaptation** of their **signaling** and **shift** in **gene expression**,
- **increased mobility** of individual cells and development of an invasive phenotype.

EMT occurs in 3 different situations: **embryonic development**, **wound healing/remodeling** and **cancer**.

The **structure of healthy epithelium** is **maintained by cell-cell** and **cell-matrix interactions**. In contrast, **cells of mesenchymal origin (stem cells) do not form fixed cell-cell contacts** and are **able to move in three-dimensional matrices**.



The **SEED-AND-SOIL THEORY** states that **circulating tumor cells** ("seed") **form metastases** at these sites, where **particularly favorable conditions** ("soil") **prevail**. In addition, anatomical differences in the vasculature of the different organs and the resulting different blood flow play an important mechanical role in the selection of the metastasis site.



CELL MECHANICS plays an **important role** in many aspects of **tumor development**. Well described are the changes in cell phenotype associated with **epithelial-mesenchymal transition**.

➔ *Originally **dormant, inactive epithelial cells** adopt a **migrating, invasive phenotype** with increased nuclear deformability, **allowing the cells to pass through narrow spaces** in the **ECM** and thus **enter the bloodstream** and **re-enter the tissue** at **other sites** (metastasis).*

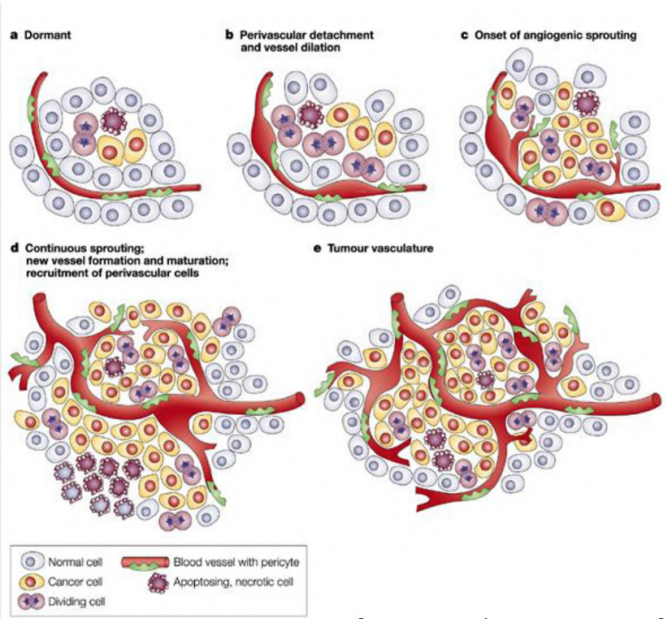
The **VASCULARIZATION** of a tumor is characterized by the formation of fragile, leaking and disorganized blood vessels. Solid tumors can be neo-vascularized by (at least) 4 different mechanisms:

Host-derived:

- **Angiogenesis**: **dilation** of the **existing vessel**.
- **Vasculogenesis**: new **formation** of **blood vessels** from circulating endothelial progenitor cells.

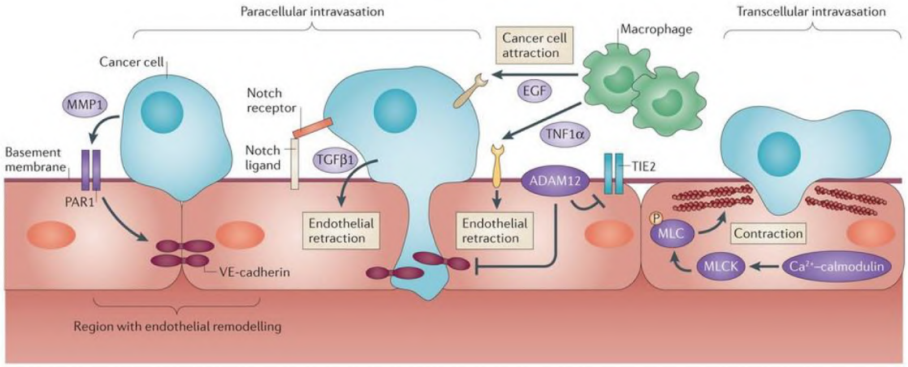
Tumor-derived:

- **Transdifferentiation**: functionally **defective endothelial cells develop** from cancer cells.
- **Vascular imitation**: cancer cells form **capillary-like structures** without endothelial cells.



INTRAVASATION: in order for **cancer cells** to **enter the blood**, they have to **move to blood vessels** in or near the tumor. **Invasion** through the stroma to the blood vessel is **promoted by tumor-associated macrophages (TAMs)** and **cancer-associated fibroblasts (CAFs)**.

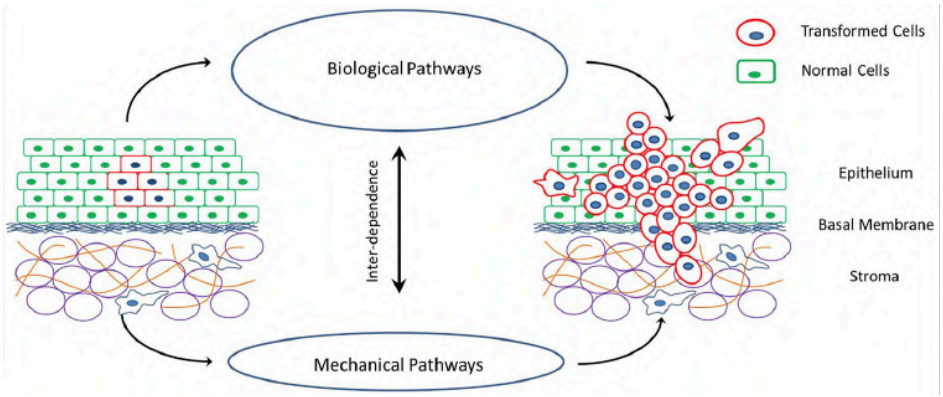
➔ Cancer cells can then **enter the circulation** either **paracellularly** (between endothelial cells) or **transcellularly** (through endothelial cells).



CIRCULATING TUMOR CELLS (CTCs) are cells that **detach from a primary tumor** either as single cells or as hetero-cellular clusters and **enter the bloodstream**.

- The more CTCs are present, the higher the expression of the **adhesion protein plakoglobin**.
- The more **plakoglobin** is present, the **worse the prognosis for the patient**.

CTCs must be **able to survive without contact** to other cells or tissues (triggers apoptosis in healthy epithelial cells). The **majority of CTCs** are **eliminated by immune cells** (NK/T cells) or **cannot withstand shear stress**. CTCs **adapt** their **morphology** and **mechanical properties** accordingly: **smaller size**, **larger nucleus-cytoplasmic ratio** and **softer actomyosin (muscle protein) cortex**.



14.3.2. CANCER TREATEMENTS

OPERATION: partial or complete removal of the solid malignoma. **Very efficient**, especially when combined with chemotherapy (before, after or both).

RADIATION: can be applied **externally** or **internally**.

CHEMOTHERAPY: **different compounds**, **selective activity** against cancer cells.

IMMUNOTHERAPHY: cancer treatment using **antibodies**, **cytokines**, **vaccines**.

Cesare Primultini (pcesare@ethz.ch)

The treatment of cancer is often **much more efficient at the beginning of the disease**. Therefore, early detection is crucial. **Liquid biopsy** is a **blood analysis** in which an **attempt is made to detect proteins**, **DNA** or **cells** released by the **primary tumor** as early as possible.

- **Inadequate** or **pathological cell-matrix interactions play** an important role in many diseases such as **atherosclerosis** and **cancer**.
- **Changes in cell mechanics**, **composition** and **structure** of the extracellular matrix and **mechanotransduction characterize these diseases**.
- The **vicious circle** of **pathological tissue remodeling** is an important research topic and of **crucial importance** in **biomedical development**.