

Bioengineering

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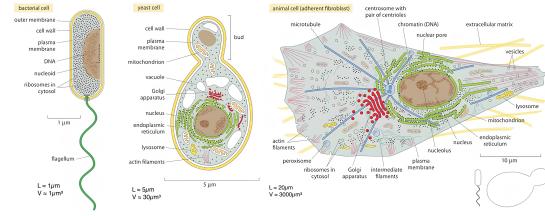
Tera	T	10^{12}	Kilo	k	10^3	Nano	n	10^{-9}
Giga	G	10^9	Milli	m	10^{-3}	Pico	p	10^{-12}
Mega	M	10^6	Mikro	μ	10^{-6}	Femto	f	10^{-15}

Orientation of the cell

Central Dogma of Molecular Biology



Cells:



nucleus: houses DNA for EK
nucleolus: produces ribosomes/rRNA
mitochondria: cellular respiration (prod. ATP)
ribosome: produces proteins from mRNA transcripts

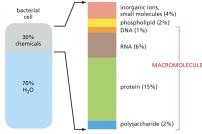
RER, SER and Golgi: involved in protein/lipid synthesis/processing

cytoskeleton: structure to cell, transport mol. in the cell or to enable the cell to move (cell migration)

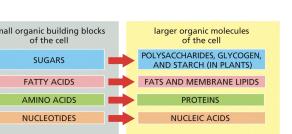
centrosome: organizes microtubules during cell division allows the mother cell to split into 2 cells

Building Blocks

Cell composition:



Main building blocks:



- Lipids (fatty acids):** long-term energy storage, cell membrane structure, signaling molecules.
- Proteins (amino acids):** perform most of the cell's functions, including catalyzing reactions, signaling, and structural support. amino group NH_2 , carboxyl group COOH
- Nucleic acids (nucleotides):** store and transmit genetic information (DNA, RNA), carry energy
- Carbohydrates (Sugars):** short-term energy storages and for structural support.

Bounds

$$\text{Covalent} \longleftrightarrow 100k_B T$$

$$\text{Ionic} \longleftrightarrow 1 - 10k_B T$$

$$\text{Hydrogen} \longleftrightarrow 1k_B T$$

$$\text{Van der Waals} \longleftrightarrow 0.1k_B T$$

$$\text{Electrostatic} \longleftrightarrow 0.1k_B T$$

K_D : Equilibrium const.; indicates the ratio of free & bound units

$$K_D = \frac{k_{off}}{k_{on}}$$

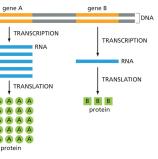
k_{off} : Dissociation rate constant; inverse of time protein dissociates from the ligand

k_{on} : Association rate constant, speed of the reaction

Enzymes (aka catalysts)

- Accelerate reaction by lowering the activation energy
- Are not consumed in the reaction
- Are specific to the reaction they catalyze
- Do not change the equilibrium point of the reaction.

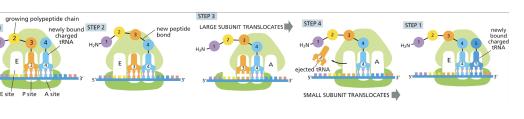
Biosynthesis



Genes are not always on and do not always produce the same number of transcripts or proteins.

Synthesis of proteins is a complicated and tightly regulated process.

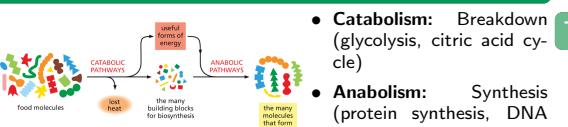
Translation by Ribosomes



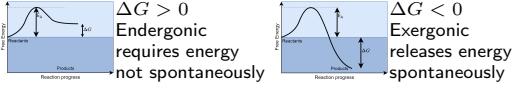
- On the mRNA, every three bases (= nucleotides) form one codon (needs ATP).
- A tRNA brings a matching amino acid. It has an anticodon that is complementary to the mRNA codon.
- The tRNA binds to the mRNA in the ribosome (at the A site).
- The amino acid is added to the growing polypeptide chain.
- The tRNA is ejected (from the E site), and the ribosome shifts forward by one codon.
- The process repeats until a stop codon is reached.

Multiple ribosomes can translate the same mRNA at the same time (making multiple proteins at the same time), forming a polyribosome (or polysome).

Energy and Metabolism

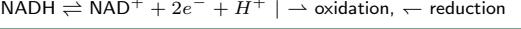


Free Energy

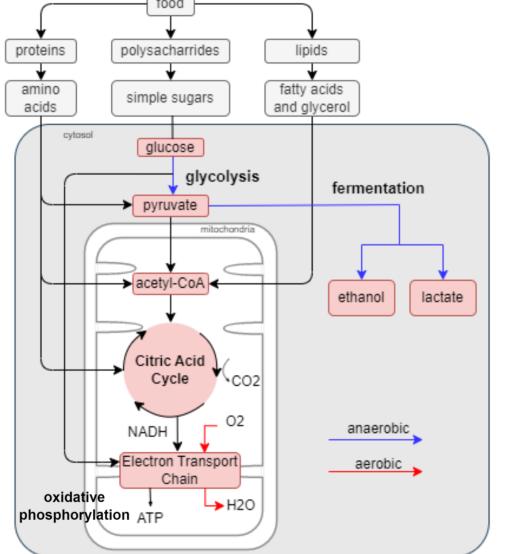


Redox

Oxidation loss of e^- ($H^+ + e^-$) | Oxidation of glucose
 Reduction gain of e^- ($H^+ + e^-$) | Reduction of pyruvate



Glucose Metabolism



Stage Molecules Glycolysis

Invest 2 ATP, 2 NAD^+ , 1 glucose
 Payoff 4 ATP (2 net), 2 NADH , 2 H^+ , 2 pyruvate
 Net gain 2 ATP, 2 NADH , 2 pyruvate

Stage Molecules Citric Acid Cycle

Invest 2 Acetyl-CoA, 6 NAD^+ , 2 FAD, 2 GDP (ADP)
 Payoff 6 NADH , 2 FADH_2 , 2 GTP (ATP), 4 CO_2
 Net gain 2 ATP (GTP), 6 NADH , 2 FADH_2 , 4 CO_2

Stage Molecules Oxidative Phosphorylation

Invest 10 NADH , 2 FADH_2 , 6 O_2
 Payoff 34 ATP, 6 H_2O
 Net gain 34 ATP, 6 H_2O

Transcription

- Small region of DNA opens and unwinds.
- RNA polymerase (catalytic enzyme) interacts with one strand in the open region of DNA and adds ribonucleotides to grow an RNA polymer.
- RNA polymerase further unwinds the DNA in the forward direction.

types of RNA

Type	Function
messenger RNA mRNA	code for proteins
ribosomal RNA rRNA	form core of ribosom structure and catalyze protein synthesis
micro RNA miRNA	regulate gene expression
transfer RNA tRNA	serve as adaptors between mRNA and amino acids during protein synthesis
other non-coding RNA	RNA splicing, gene regulation, telomere maintenance, etc.

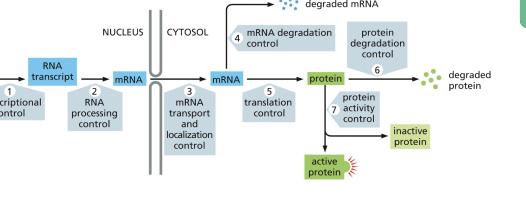
Eukaryotes vs Prokaryotes

- Multiple types of RNA polymerases transcribe different classes of RNA.
- Transcription initiation is more complex.
- mRNA molecules undergo splicing, where introns (non-coding regions) are removed and exons (coding regions) are joined together to form the final RNA.
- 1 type of RNA polymerase transcribes all types of RNA.
- Transcription initiation is a simpler process.
- mRNA molecules are translated immediately after transcription

Both use gene regulatory proteins that bind to specific sequences of DNA:

- Repressors: Bind to sequences to turn genes off. Make it more difficult for RNA polymerase to bind to DNA.
- Activators: Bind to sequences to turn genes on. Make it more favorable for RNA polymerase to bind to DNA.

Gene Regulation

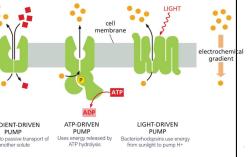


Facilitated Diffusion:

- selective to size and charge of solute
- bidirectional
- gated by external inputs
- Transporter good for large molecules

Active Transport

Pumps:



- Endocytosis: into cell
- Exocytosis: out of cell
- Pinocytosis: cell drinking
- Phagocytosis: cell eating
- Receptor-mediated endocytosis: specific uptake of molecules

Vesicular Transport

Intracellular Transport

- chemical energy (ATP) to mechanical work
- directed motion
- Kinesin: carry out microtubule-based retrograde transport (towards cell edge)
- Dynein: carry out retrograde transport (towards cell nucleus)

Growth and Differentiation

Cell Cycle

G1: Cell growth
S: DNA Synthesis
G2: Growth and preparation for Mitosis
M: Mitosis (cell division)

Quiescent cells: cells pause before replication. Reversible growth arrest (G0 phase)

Transfection

Insert DNA that codes for the wanted biomolecule into cell. Use:

- viruses
- electroporation
- carriers

store transfected cells in cryogenic conditions

Actin Filaments

- Provide support, change the cell shape (division) and drive movement
- Assemble from globular proteins ("G-Actin") like microtubules and form hierarchical structures by crosslinking
- Polar with no preferred direction
- Can form protrusions → exploring and sensing environment
- "Myosin motors" → participate in cargo transport and muscle contraction

Cell Sensing and Signaling

Cellular Communication

Long Range:
 Endocrine • into blood stream
 Neural • in neurons (electric)
 • affect whole organism

Short Range:
 Paracrine • affect local tissue
 Contact Dependent • direct binding

Signalizing

Cell Surface Receptors:

Extracellular signal molecule (hydrophilic or charged) causes receptor to release intracellular signal molecule.

Intracellular Receptors:

Signal molecule (hydrophobic) cross the membrane and act inside the cell.

Signal is:

- Amplified
- Distributed
- Integrated
- Modulated (feedback loop)

Receptors

Ion-channel-coupled

Enzyme-coupled

G-protein-coupled receptors

- Ligands (ex.: hormones, neurotransmitters) bind to GPCR (G-protein coupled receptor) which changes conformation
- Activated receptor causes G-protein to exchange its GDP for GTP gets activated
- G-protein modulates the activity of effector molecules generate intracellular second messenger

Stem Cells – Cell Differentiation

Differentiated cells in adult organisms contain all the genetic information to form a new organism. But, they express **only a fraction** of genes specific to their function.

Stem cells can differentiate to tissue-specific cell, but also renew themselves.

Pluripotent stem cells can give rise to all cell types.
Multipotent stem cells can give rise to a limited number of cell types tissue specific.

Bioprocesses

Bioprocesses rely on several key components, including biological components, such as the target molecule and the cells used to produce it; culture medium and one or more bioreactors; as well as a process.

Modern technology used:

- DNA sequencing and synthesis
- Precise gene editing
- Genetic circuit design

Cell source:

Mammalian cells:	E. coli:
- slow growth	+ fast
- complex growth media	+ simple growth media
+ proper folding	- refolding required
+ glycosylation	- no glycosylation

Culture medium

Source of nutrients to support the growth of cells.
 Composition:

- building blocks (sugars, aa)
- water
- salts/ions

Bioreactor

Carefully designed **culture medium** that provides **nutrients** (building blocks, water, ions, energy) and a **suitable environment** for cells to grow and generate biomolecules of interest.

Batch: Feed solution enters vessel containing cells. No addition.

Perfusion: Fresh medium enters vessel containing cells. Removal of product-rich culture broth.

Cell and tissue architecture

Cytoskeleton – "Bones and muscles of the cell"

- Resistance to deformation
- Drives movement
- Organizes the cell interior (shape and cargo)
- Physical interactions with the environment
- Present in all eukaryotic cells

Intermediate Filaments

- Toughest and most durable filaments
- Primary function: **Withstanding mechanical stress**
- Assembled from α -helical proteins → Rope-like structures
- Without preferred direction (Diameter 10nm)
- Major types: Keratins, Vimentins, Neurofilaments, Lamins

Microtubules

- Essential for spatial organization
- Polar, have a distinct orientation, centrosome → cell membrane
- Are assembled from globular proteins: α - and β -tubulins that assemble in tubulin dimers (25nm diameter)
- Dynamic → constantly grow or shrink
- Can form Cilia, help in cell division or transport

Muscle contraction:

In a **sarcomere**, myosin II binds to actin and proceeds towards the **plus** side. This sliding of myosin on actin filaments **shortens** the strands and leads to muscle contraction

Extracellular Matrix (ECM)

Fibrous elements outside the cell that hold cells and tissues together

Functions:

- Structural support and mechanical scaffold
- Resistance to stretch and compression
- Boundary between tissues
- Water retention
- Reservoir for signaling molecules

Plants have cell walls (cellulose and pectin) instead of ECM.

Composition of ECM

Protein fibres:

Collagen and **Elastin** provide strength and elasticity

Glycoproteins: Fibronectin and Laminin provide adhesion and signaling

Glycosaminoglycans (GAGs) and Proteoglycans:

- Linear, rigid polysaccharide chains → form large volumes of porous gels
- They carry **negative charges** → **retention of water**
- Often covalently linked to protein cores called **proteoglycans** that also provides lubrication
- Resistance to compression**

All components are **produced** and **matured** in the cells then **secreted** in extracellular environment
 → Cells engineer their local extracellular matrix

Physical Cell-Cell and Cell-ECM Interactions

Mechanical, electrical, metabolic coupling at cell-cell junctions (desmosome). Physical cell-cell and cell-ECM communication (e.g. via integrins)

Woundhealing and Tissue Engineering

Circulatory system

The circulatory system consists of cardiovascular system and the lymphatic system.

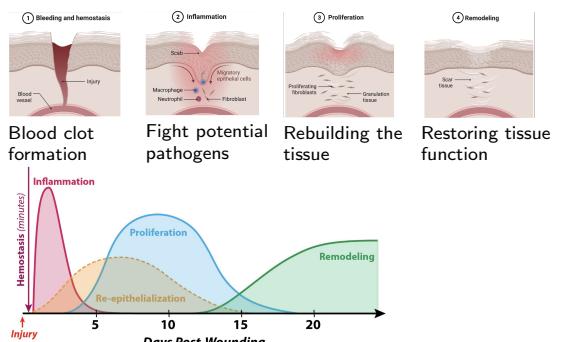
Blood cell types:

- Enucleated: **Erythrocytes** (red blood cells) and platelets
- Nucleated: **Leukocytes** (white blood cells) and other immune cells

Vascular Structure:

Artery, Arteriole, Capillary bed, Venule, Vein

Woundhealing



Tissue Engineering

Mimic in vivo conditions so that cells can grow.

- scaffolds
- genetic tools
- biomaterials
- bioreactors

Hydrogel as ECM mimics:

- Hydrophilic networks with tunable mechanical, biochemical and physiochemical properties.
- Matrices can be natural, engineered or hybrid.
- Body tissues have different matrix properties.

Microphysiological Systems and Immune Engineering

Organoids

Organoids are "mini organs" built in the lab from stem cells. Organoids **mimic geometric features** and **cell organization** of the original organ (tissue replica). The surrounding **ECM composition** is **custom** designed.

Matrigel: matrix scaffold for organoids, provides **mechanical support** and **adhesion sites** for cells.

Cell source: induced pluripotent SC (iPSC) terminally differentiated cells reprogrammed back to pluripotency.

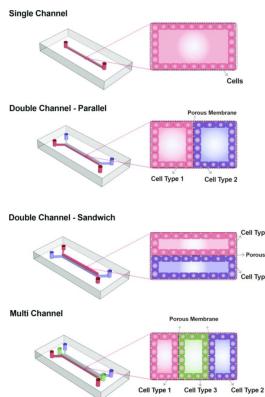


Possibility to model genetic diseases such as Parkinson in cerebral organoids.

Limited to small size, nutrient and oxygen supply is limited by diffusion.

Organs on a Chip

Micro-tissues grown in a controlled microfluidic device where **physical and mechanical stimulation** can be applied. OoC are patient-specific (gender, age, history of disease, etc).



Immune Engineering

Consists of using engineering tools and principles to investigate and modulate the **immune system**.

Applications:

- Evade or delay immune response
- Shut off auto-immunity in auto-immune diseases
- Stimulate immune response (e.g. vaccines)
- Multiply native immune response (T cell activation)

Immune Response

Self: (part of organism)

- Own organs, cells and proteins
- Commensal bacteria

Non-self (not part of organism):

- Non-harmful particles of food or pollen
- Pathogens (bacteria, viruses...)

Innate: Unspecific and immediate (hours)

Adaptive: Specific and slow (days) Lymphocytes (B and T cells)

Evading (block reaction):

- Avoid cell attachments or phagocytosis (for example through "self"-markers) – physical
- Avoid protein adsorption with hydrophilic, non-fouling coatings – biochemical

Activate:

- Delivery of **cytokines** → stimulate immune cell proliferation and recruitment
- **Vaccines** that expose the immune system to specific antigens
- **Hydrogels** loaded with immune-stimulatory substances

Immunotherapies

- use engineered **antibodies** to boost the immune response
- antibodies can be **modified** and mass-produced to trigger an amplified and **targeted therapeutic response**
- CAR-T cells: New form of cancer therapy where T cells from patient's blood are **genetically modified** to attack specific proteins on **cancer cells**

Foodprocessing



Meat alternatives

Plant based meat alternatives, from **pea protein**.

Lab grown meat alternatives, cell cultures grow into synthetic muscle tissue.

Microbiome

All the bacteria and microbes within the GI tract.

Functions:

- Barrier integrity
- Mucus production
- Food metabolism
- Transform food products into chemicals that act as **signaling molecules**.

Dysregulation:

- Leads to diseases (ex: anxiety, depression, insulin resistance, etc)

Solutions:

- **Probiotics**: bacteria that metabolize food sources into signals that regulate **homeostasis** in body.
- Design **microphysiological models** (eg. OoC) that capture the interactions between the gut microbiome intestinal cells.

Drug Delivery

Diagnostics

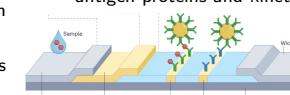
RT-PCR tests: real time polymerase chain reaction

- Amplification of nucleic acids is done through cycles of **DNA elongation** using **DNA polymerase** (temperature controlled)
- Steps: DNA denaturation, primer annealing, elongation of new DNA strand
- At each cycle, the number of DNA fragments doubles (measured optically with **fluorescent dye**)
- Higher **sensitivity** than lateral flow tests

Lateral flow tests:

rapid antigen tests

- **Detection of proteins** (antigens) through immobilization of a receptor-nanoparticle complex on a substrate.
- **Antibodies bind to antigens** in the sample and **antigens simultaneously bind to capture antibodies** immobilized on test line.
- The **antigen** is sandwiched between the **capture antibody** and the **detection complex**.
- Signal depends on: the amount of virus, flow, diffusion of antigen proteins and kinetics of reception-antigen binding.



Prophylactics

Vaccine breakthrough (COV 19):

Requirements:

mRNA sequences:

- increased stability
- longer half-life
- higher translation efficiency

mRNA sequence carrier:

- Protect cargo
- Carry it across the tissue barrier
- Target specific cells
- Allow the mRNA to escape the endosome

Which **barriers** need to be crossed?

- Extracellular barriers (blood vessels)
- Intracellular barriers (LNPs endocytosis)
- Endosome and LNP degradation
- mRNA free in cytoplasm

Solution → LNPs

Lipid Nanoparticles (LNPs)

Needed to **avoid digestion of mRNA** by nucleases (foreign body response) and to facilitate endocytosis.

LNP include 4 **main components**:

- **phospholipids**: stabilize shape
- **ionizable lipids**: help RNA to escape endosome
- **cholesterol**: reduce permeability
- **PEGylated lipids**: help avoid immune response

How are **LNPs assembled**?

- **Lipids and cholesterol** are dissolved in an **organic phase** (e.g. ethanol)
- **mRNA** is dissolved in an **aqueous buffer** (low pH)
- **Rapid mixing** of both phases leads to LNPs assembly