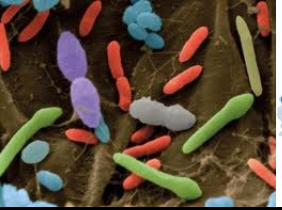
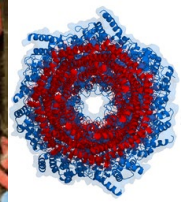
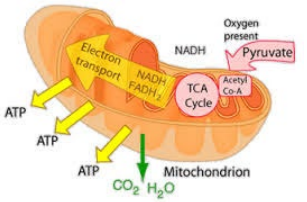
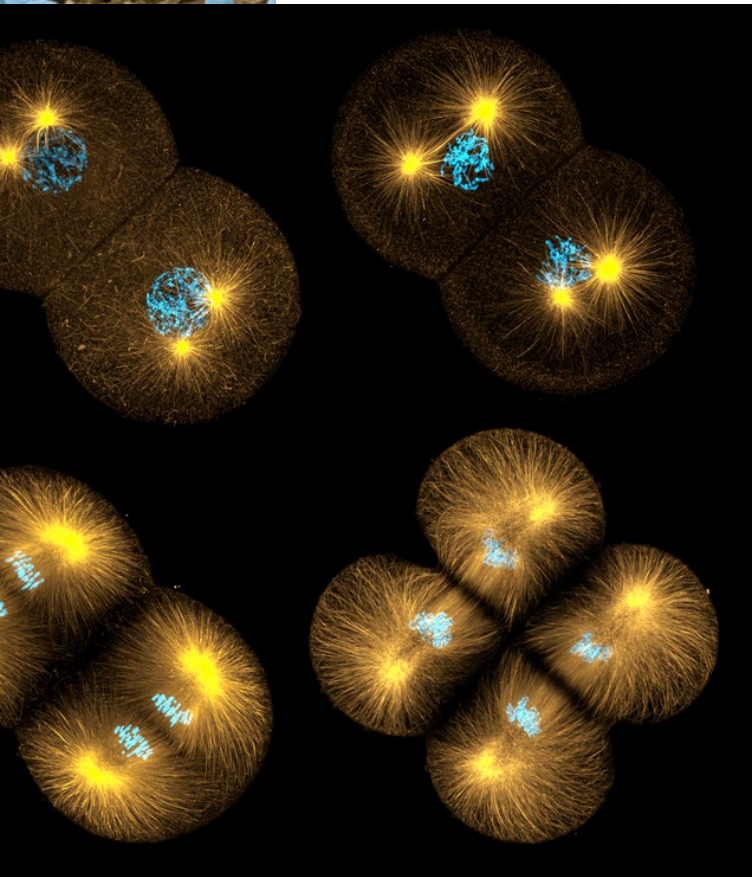








The Cell

Biochemical Engineering

ChE-311

Manfred Zinn

manfred.zinn@epfl.ch

Biochemical Engineering

ChE-311 / **3 credits**
Teacher(s): Crelier Simon, Zinn Manfred
Language: English

Summary

This course introduces the basic principles of bioprocess engineering and highlights the similarities and differences with chemical engineering. Without going into the fundamentals, it proposes an overview of the techniques for fermentation as well as product purification (DownStream Processing).

Exam

The exam will last 3 hours and be “open book”. Current discussion are ongoing about usage of Laptop.

Your lecturers

Manfred Zinn
manfred.zinn@epfl.ch
manfred.zinn@hevs.ch

Simon Crelier
simon.crelier@epfl.ch
simon.crelier@hevs.ch

Biochemical Engineering

- The cell as a biocatalyst, its needs and performance
- Bioreactor systems
- Bioprocess analytics and control
- Bioprocess design
- Batch, fed-batch, and continuous culture

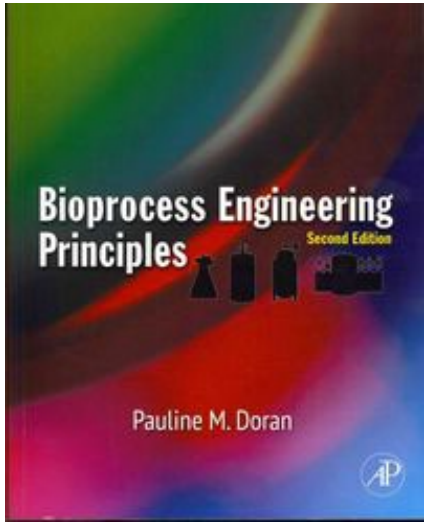
Downstream Processing (DSP)

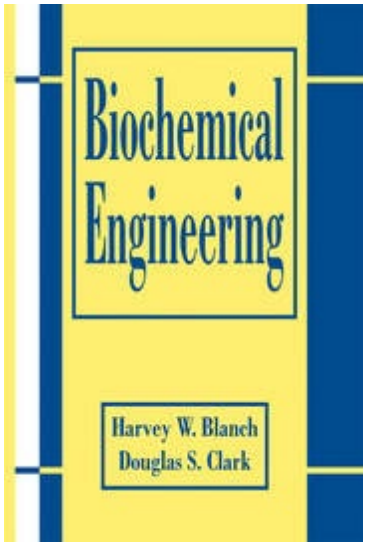
- Selection of a purification strategy
- Liquid/solid separations and cell lysis
- Liquid/liquid extraction and precipitation
- Adsorption and chromatography
- Membrane techniques
- Trends and trend-setters in DSP

Recommended literature

- "Biochemical Engineering", Harvey W. Blanch and Douglas S. Clark,2nd ed., Taylor & Francis, 1997

- "Bioprocess Engineering Principles", Pauline M. Doran, 2nd ed., Academic Press, 2013





By the end of the course, the student must be able to:

- ✓ Understand the cell physiology of microorganisms
- ✓ Distinguish the different types of bioreactors
- ✓ Dimension bioreactors and separation equipments
- ✓ Compare the various modes of fermentation
- ✓ Carry out calculations of yields in biomass or product
- ✓ Select appropriately a bioprocess configuration
- ✓ Interpret results based on taught concepts
- ✓ Propose adequate strategies for the development of bioprocesses or purification protocols
- ✓ Differentiate between chemical engineering and bioprocess engineering

Friday (after the lecture) is also Quiz Time!!!

Deadline Monday evening: 22:00h

Agenda

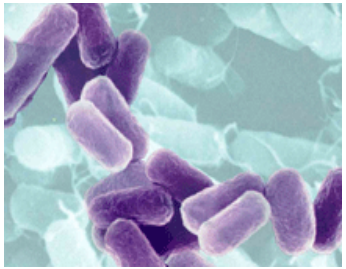
- Introduction to the course
- What is needed for successful bioprocesses?
- Selection and storage of cells
- Quick fresh-up in cell physiology
- Energy needs of a cell
- Biochemical pathways
- Basics in genetic engineering
- Exercises

Agenda for the course Biochemical Engineering ChE-311

Date	Topic and content (may be adapted during course)
21.2.	The Cell Bioprocesses, structure of the prokaryotic and eukaryotic cell, cell components, elemental cell composition, metabolic pathways (repetition), uptake systems, membrane potential
28.3.	Basic Properties of Bioreactors Basic function of a bioreactor, different types of bioreactors, materials used, agitation and energy input, scale-up aspects
7.3.	Advanced Bioreactors Control methods (off-line and online) for biomass and products, sterilization of bioreactors
14.3.	Medium Design Nutrient limitation, stoichiometry of reactions (e-balances), medium design, different C substrates, two-phase cultures
21.3.	Batch Culture Monod model, preparation of batch cultures
28.3.	Fed-Batch Cultivation Kinetics, feed strategy, product formation
4.4.	Continuous Culture Chemostat equations, X-D- diagram, maintenance energy, nutrient limitations

Questions concerning the exercises: Please contact the course assistant : eveline.mayner@epfl.ch

The basic tenet in industrial bioprocess engineering



- Genetic engineering
- Activity screening

- Bioprocessing
- Enzymatic catalysis

- Downstream processing

Chemical & Bioanalytics

Trends in biotechnology

White Biotechnology

- Improve chemical manufacturing efficiency
- Lower the cost & energy of cleaning clothes through temperature reductions
- Save money on manufacturing operation costs
- Minimize industry reliance on petrochemicals
- Reduce greenhouse gases
- Lower water usage and waste generation

Green Biotechnology

- Produce more crop yield with fewer resources
- Aid the environment by reducing the chemicals and runoff from crop production
- Create more resilient crops without the use of pesticides
- Alleviate vitamin and nutritional deficiencies to improve crops
- Alter the oil content in food to reduce heart health risks
- Create food without mycotoxins or allergens

Red Biotechnology

- Reduce infectious disease rates
- Change the probabilities of life-threatening conditions emerging for people around the world
- Create treatments specific to the individual to minimize health risk
- Help those with illnesses in the developing world

https://masschallenge.org/articles/trends-in-biotechnology/

Some cost/price indications in white biotechnology

- Citric acid 1,4 – 1,2 US$/kg
- Amylase 1 - 0,5 US$/kg liquid product
- Lysin 60 - 40 US$/kg
- Glutamate 1,7 – 1,1 US$/kg
- Vitamin B2 100 – 18 CHF/kg
- Alkaline Protease 22 – 50 US$/kg
- Lovastatin 300 – 70 US$/kg
- Cyclosporin 550 – 120 US$/kg
- Penicillin G 10 – 20 US$/kg
- Chymosin 100 - 600 US$/kg
- Vitamin B_{12} 7'200 – 500 US$/kg
- Small molecule pharma 50 – 5'000 CHF/kg
- Streptokinase 2'000'000 – 1'000'0000 .- /kg
- Insulin (bulk) 44'000 - CHF/kg
- Therapeutic proteins >100'000 CHF/kg

Expensive products can be produced at a smaller scale.

GRAS Certified

GENERALLY RECOGNIZED AS SAFE

New Culture Earns 'World's First' Self-Affirmed GRAS Status for Precision-Fermented Casein

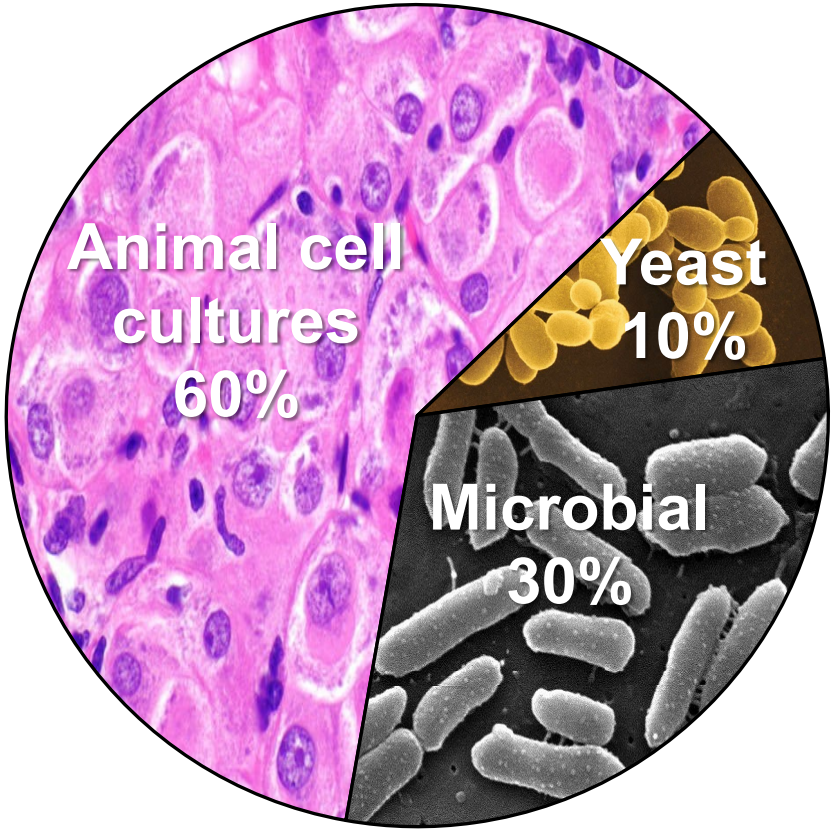
https://www.greenqueen.com.hk/new-culture-precision-fermentation-casein-self-affirmed-gras-fda/

Trends: fewer production hosts for rec. proteins

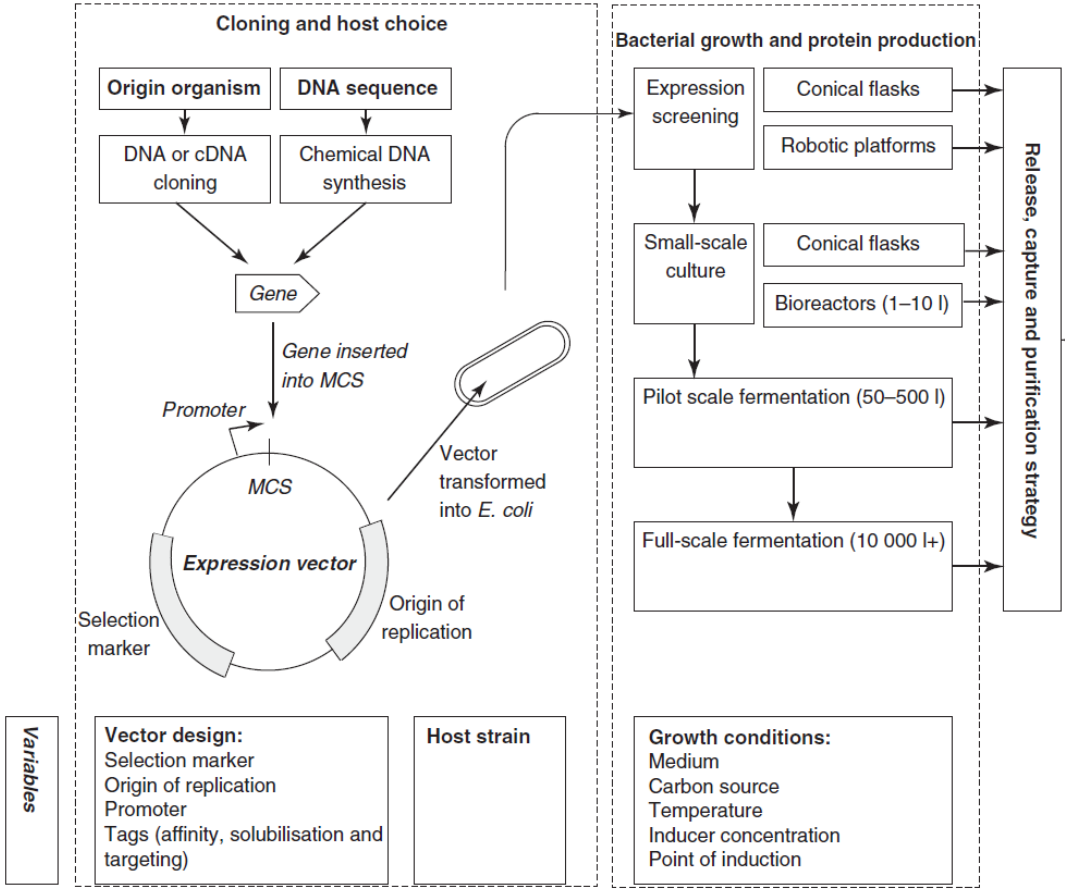
Host	*Advantage*	*Disadvantage*	*Product type*
E. coli	genetics, medium	secret. glycosyl.	small proteins
B. subtilis	GRAS, secretion	medium, protease	bulk proteins
Yeasts	genetics, GRAS	medium	food addit.
Asp. oryzae	secretion, GRAS	rheology	bulk proteins
Animal cells	postranslational modifications	expensive	large complex proteins
Plants	cheap	purification	bulk proteins
Mammals milk	cheap	etics? safety?	pharma prod.
Hen's egg	cheap, established		pharma. prod.

https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras

The majority of biotech products on the market are made in animal cells

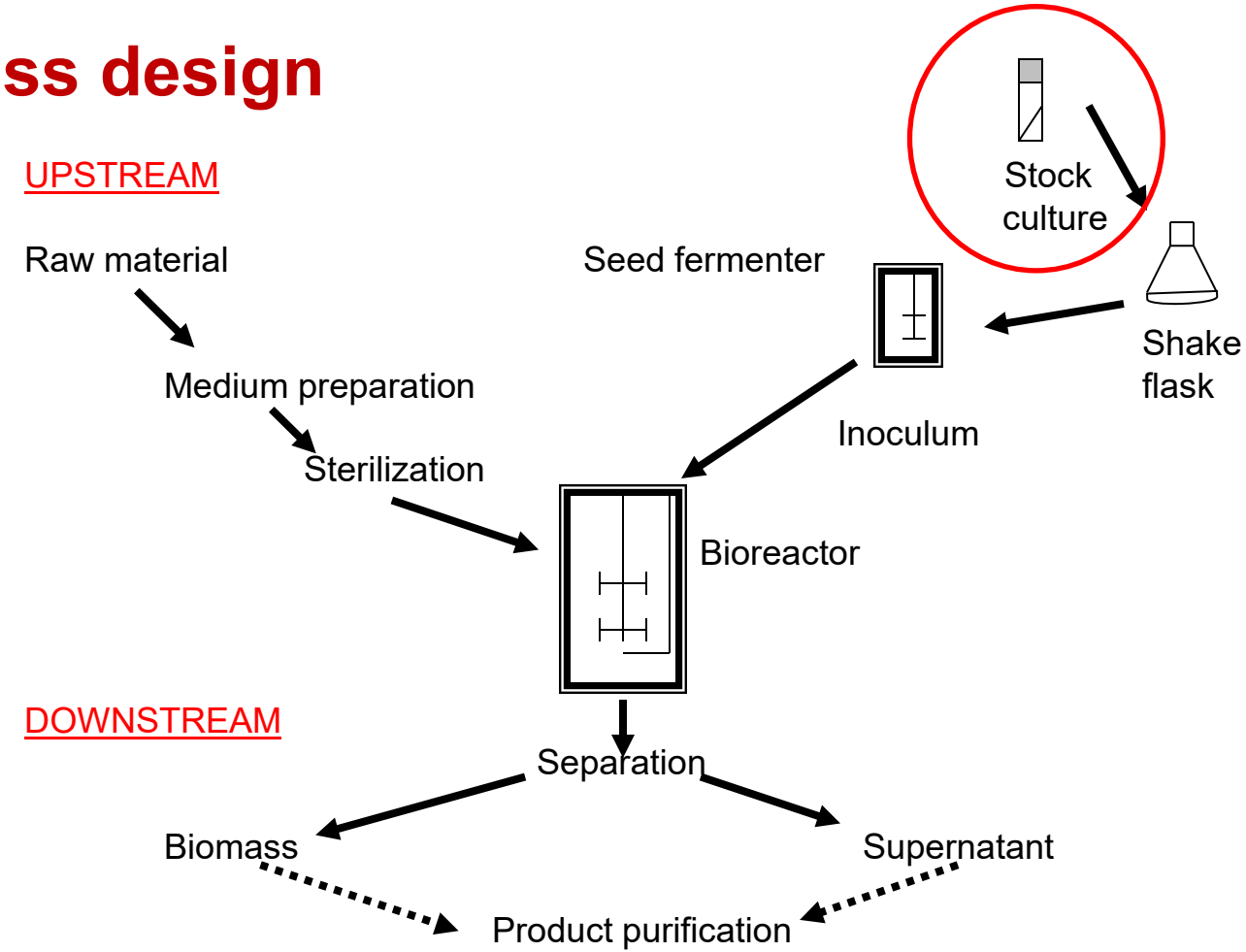


General approach to recombinant products



Overton TW. 2014. Recombinant protein production in bacterial hosts. Drug Discovery Today 19(5):590-601.

Process design



Improvement of productivity

The optimization of a bioprocess can be improved at three levels:

- improvement of the **fermentation** (e.g. preculture, feeding strategy)
  - **medium** optimization
  - **strain** development

Engineering

This approach includes:

1. improvement of **functionality** of bioreactors for a maximal productivity. One has to assess the influence of pH, temperature, the transfer of biomass, the cell concentration, the morphology of the cells, the osmotic pressure, the rheology, etc.
2. the **design** of a bioreactor for a maximum productivity.
3. extraction and purification of a product (*downstream processing*)

Improvement of productivity

Composition of the growth medium

The medium composition plays a crucial role for an optimal production. The statistical approach (*design of experiments*) helps to determine the most significant parameters for cultivation in a most efficient way.

Improvement of cells

There is a possibility to increase the productivity by genetical engineering. The objectives are:
- Increase of the product yield
- The maintenance of the yield

Techniques:
- Selection
- Selection followed by a mutation: sequential mutagenesis
- Genetical engineering: directed evolution
- CRISPR/CAS19

Importance of maintaining and preservation of microorganisms

- There have been done significant efforts for isolating strains that are perfoming special tasks (*e.g.,* overexpression of enzymes) and have been adapted to particular growth conditions.

- An important goal is to preserve such strains for scientific but also for industrial applications. The strains have to be pure and should keep their properties (reduction of mutation).

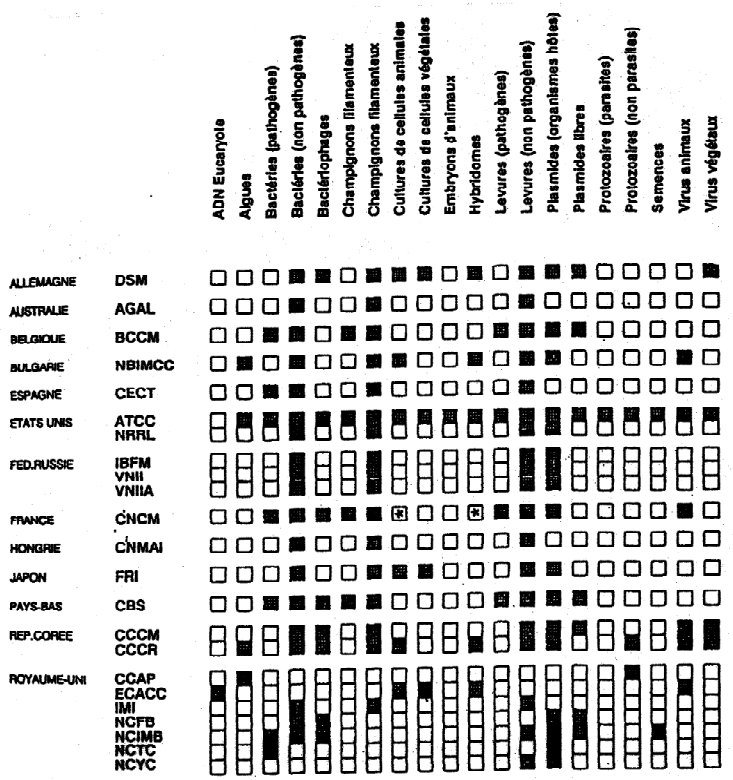
- Large strain collections have been established in industry and by academic and governmental organizations.

Public strain collections

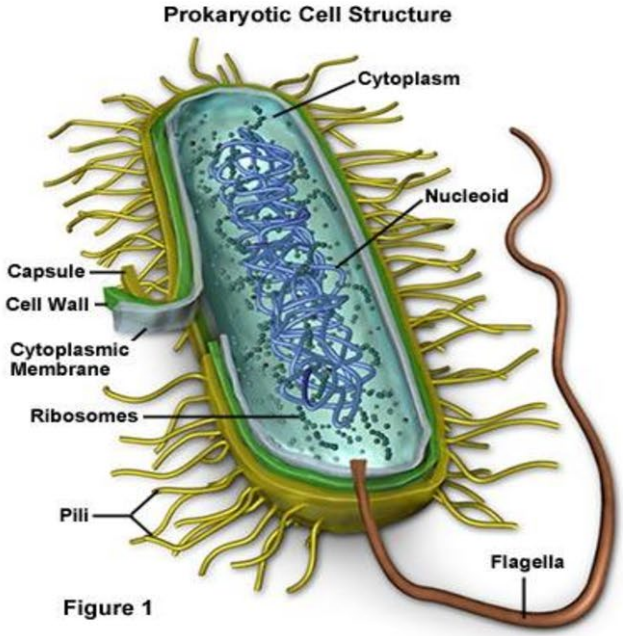
http://www.wfcc.info/ccinfo/home/

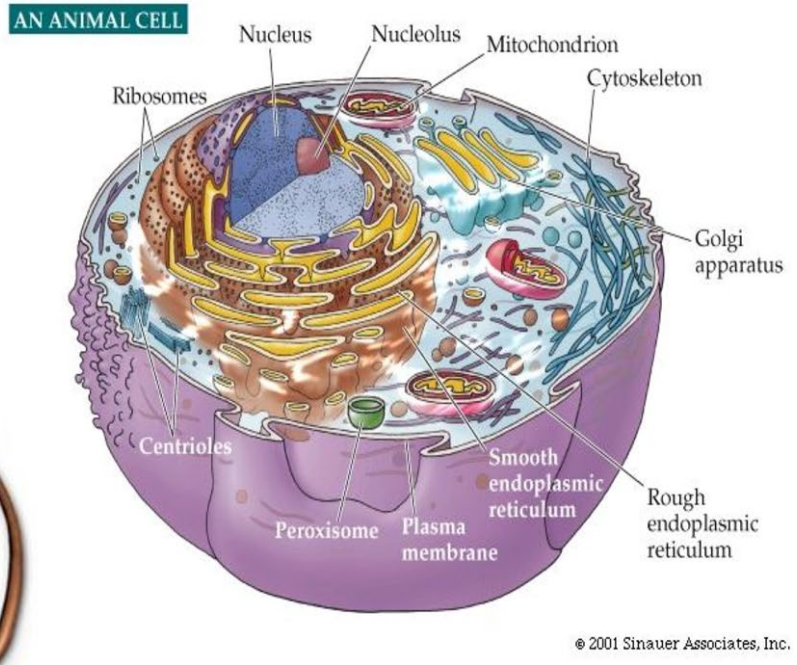
A large number of microorganisms will be conserved in public strain collections. The most important ones are:

- **ATCC** American Type Culture Collection (USA)
- **BCCM** Belgian Coordinated Collections of Microorganisms
- **CBS** Centralbureau voor Schimmelcultures (Niederland)
- **NRRL** Agriculturual Research Service Culture Collection (USA)
- **DSM** Deutsche Sammlung von Mikroorganismen und Zellkulturen (Deutschland)
- **CCoS** Culture Collection of Switzerland

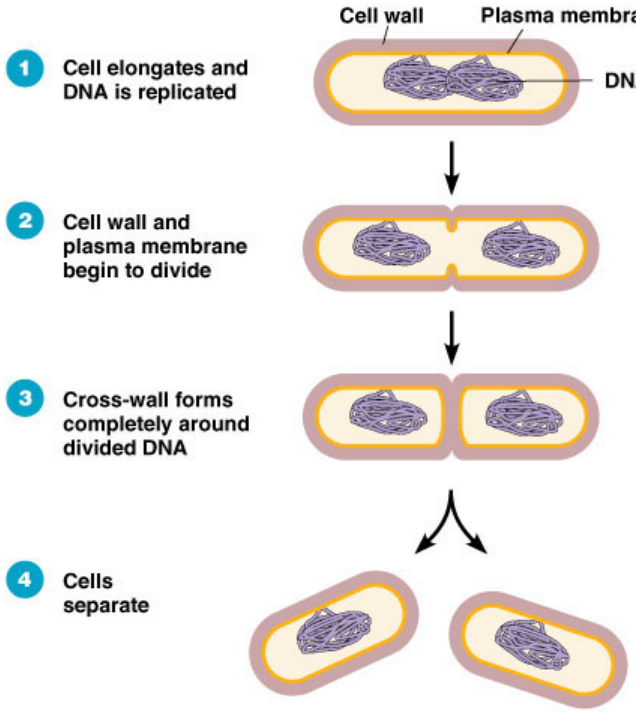


Prokaryotic vs Eukaryotic Cells

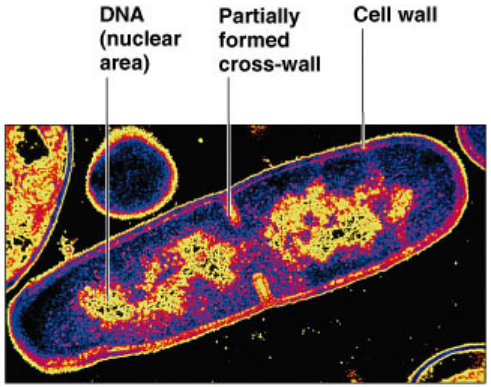




The prokaryotic cell cycle

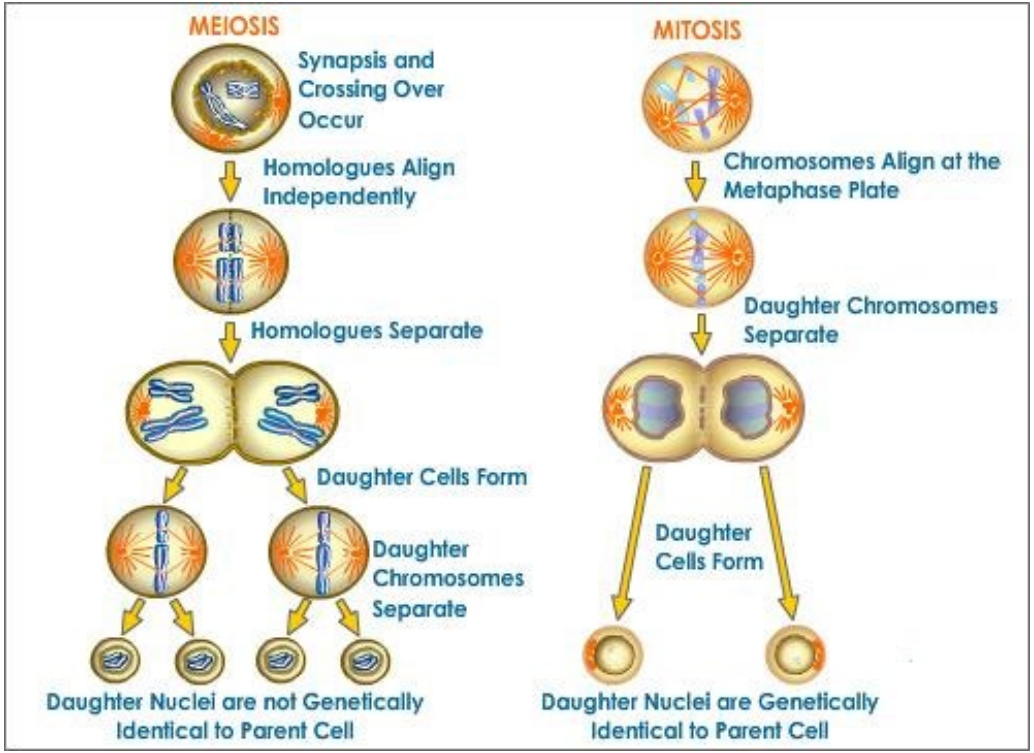


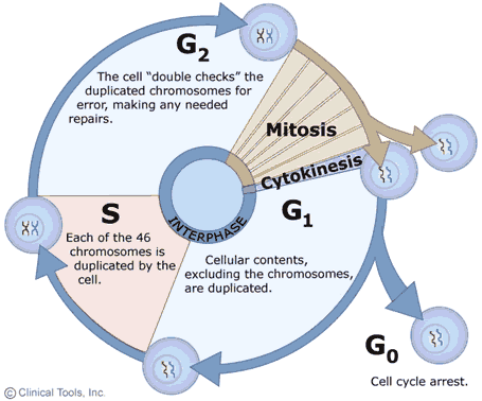
(a) A diagram of the sequence of cell division.



(b) A thin section of a cell of *Bacillus licheniformis* starting to divide.

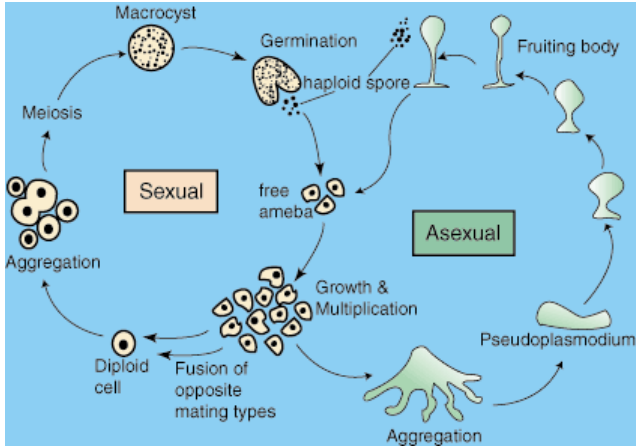
The cell cycle of eukaryotes

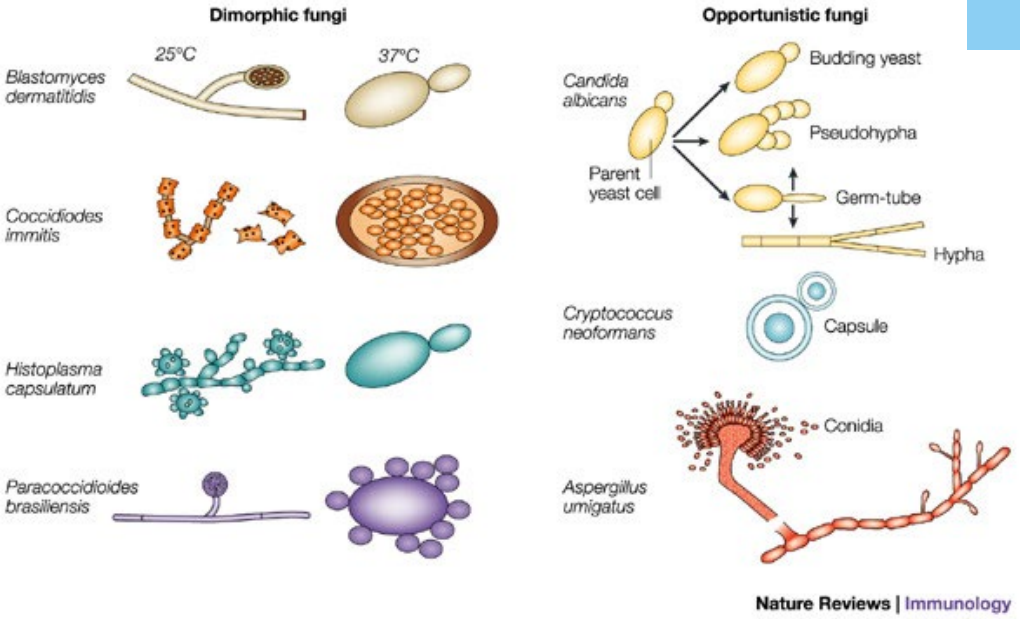


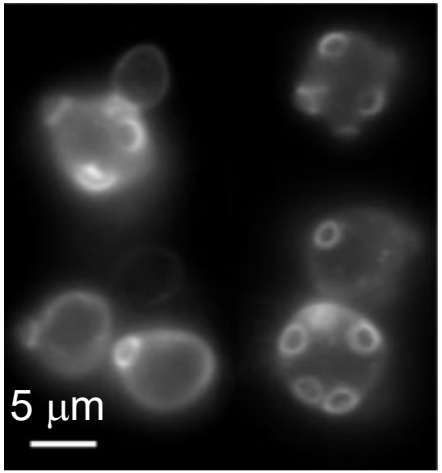


The complex world of fungi

The shape changes with the growth stage.







S. cerevisiae stained with calcofluor white

Comparison of cell cycle times

Type of cell	G_1-Phase (pre-DNA Synthesis)	S-Phase (DNA Synthesis	G_2-Phase (Post-DNA synthesis)	M-Phase (Mitosis)	Total doubling time
Animal cell	8 – 10 h	8 – 10 h	4 h	1 h	ca. 24 h
Yeast	0.4 h	0.6 h	0.6 h	0.4 h	ca. 2 h
E. coli	24 min	36 min	0	0	ca. 1 h

Growth medium and physico-chemical growth conditions significantly influence the specific growth rate (see lecture on batch cultivation).

Cell composition of *Escherichia coli*

			C	H	N	O	P	S
Total [wt%]	Average	Range	47.82	6.38	14.36	24.49	2.74	0.75
Protein	55.0	15-75	61.17	61.55	67.93	46.35	0.00	100.00
RNA	20.5	5-30	15.17	10.38	24.29	28.62	71.25	0.00
DNA	3.1	1-5	2.45	1.70	3.66	3.92	11.31	0.00
Lipid	9.1	0-15	12.38	16.07	0.95	7.16	14.59	0.00
LPS	3.4	0-4	3.48	4.44	0.41	5.32	2.85	0.00
Peptidoglycan	2.5	0-20	2.57	2.57	1.88	3.61	0.00	0.00
Glycogen	2.5	0-50	2.32	2.43	0.00	5.03	0.00	0.00
Polyamines	0.4	0-10	0.47	0.86	0.87	0.00	0.00	0.00

(Composition of *E. coli* in exponential growth phase)

The total weight of an average cell is 9.5×10^{-13} g (containing 70% water). The cell dry weight of one *E. coli* cell is therefore 2.8×10^{-13} g.

Is the cell composition fixed?

During the cell cycle the composition of the cell changes but also during a batch cultivation!

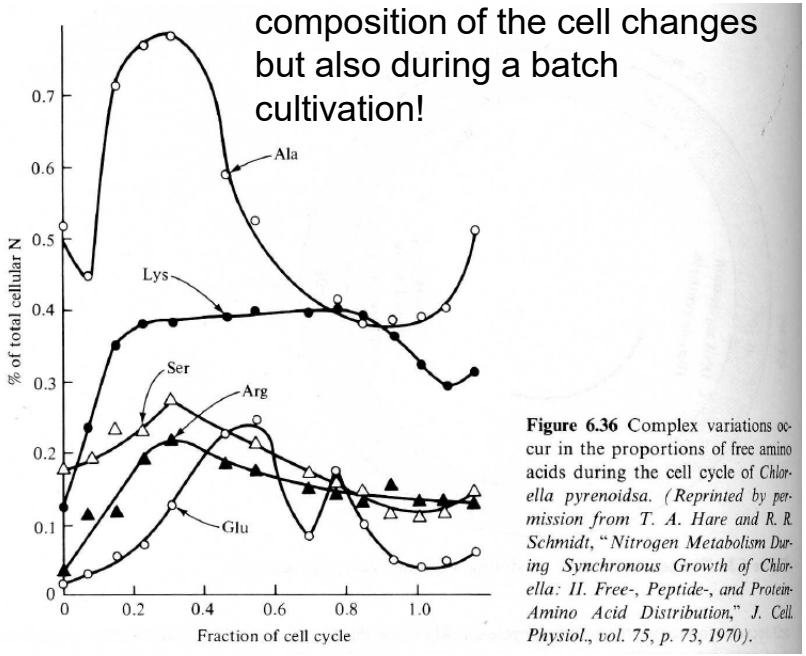


Figure 6.36 Complex variations occur in the proportions of free amino acids during the cell cycle of *Chlorella pyrenoidsa*. *(Reprinted by permission from T. A. Hare and R. R. Schmidt, "Nitrogen Metabolism During Synchronous Growth of Chlorella: II. Free-, Peptide-, and Protein-Amino Acid Distribution," J. Cell. Physiol., vol. 75, p. 73, 1970).*

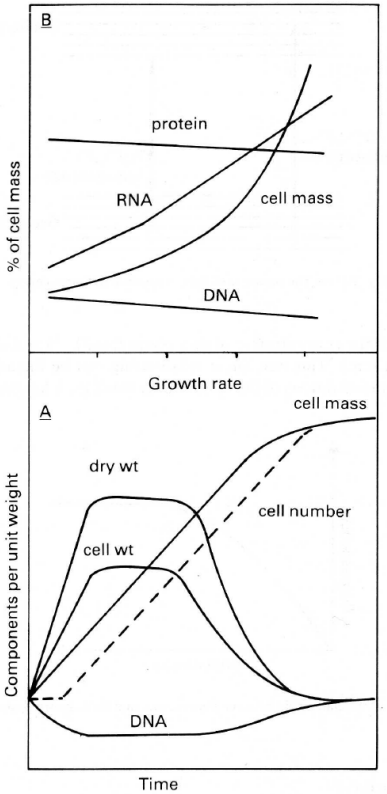


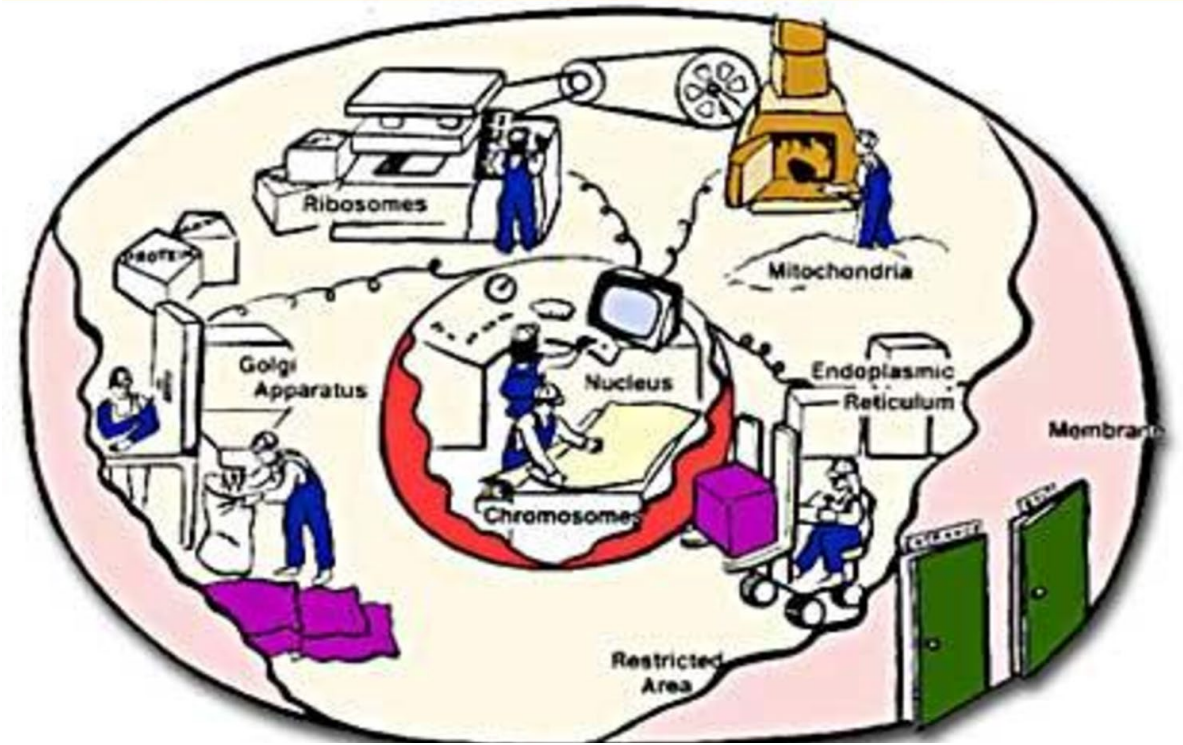
Fig. 2.10 — A; the changes in macromolecular composition of microbial cells during batch culture (redrawn from Herbert 1961). B; the variation in cellular composition of *Enterobacter aerogenes* grown in a chemostat at various growth rates (redrawn from Wang *et al.* 1979.

How surface area to volume ratio limits cell size

1. A cell is a **metabolic compartment** where a multitude of chemical reactions occur.

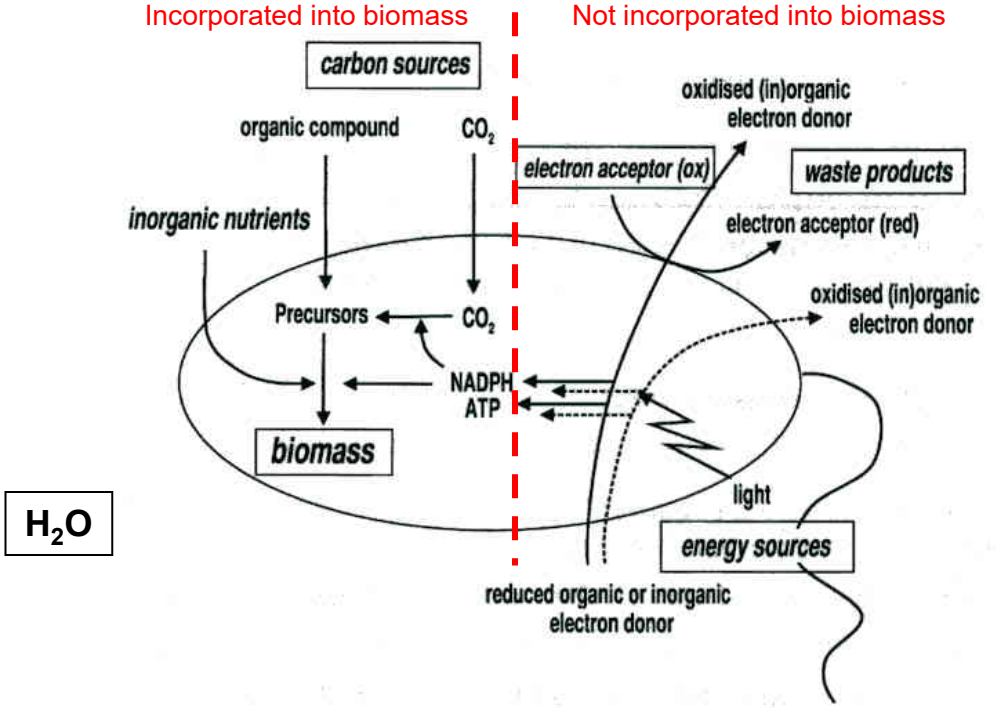
2. The **number of reactions increases** as the volume of metabolic volume within a cell increases (the larger the volume the larger the number of reactions).
3. All raw materials necessary for metabolism can **enter** the cell only through its **cell membrane**.
4. The greater the surface area the larger the amount of raw materials that can enter at only one time.
5. Each unit of volume requires a **specific amount of surface area** to supply its metabolism with raw materials. The amount of surface area available to each unit of volume varies with the size of a cell.
6. As a cell grows its **SA/V decreases**.
7. At some point in its growth its SA/V becomes so small that its surface area is too small to supply its raw materials to its volume. **At this point the cell cannot get larger.**

The Cell Factory



Energy is needed to keep the cell factory running!

Physiological function of nutrients

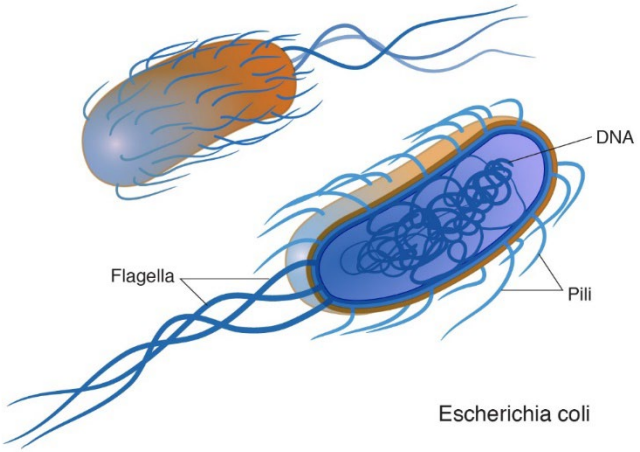


Simplified sketch of the physiological function of nutrients for the growth of microorganisms.

Energy (ATP) is required not only for anabolism but also for cell maintenance!

The cell needs maintenance energy for various activities:

- Transformation of cellular materials (recycling)
- Enzyme activation
- Maintenance of an electrochemical gradient between inside and outside of the cell
- Cell movement: World record owns *Vibrio cholerae:* 12 mm min^{-1} (ca. 50x body length/sec)



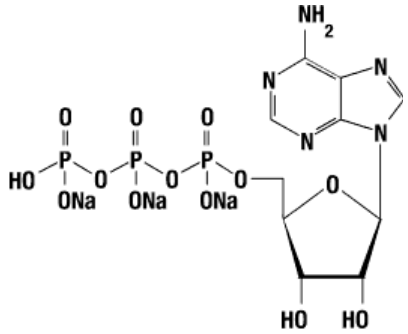
Escherichia coli

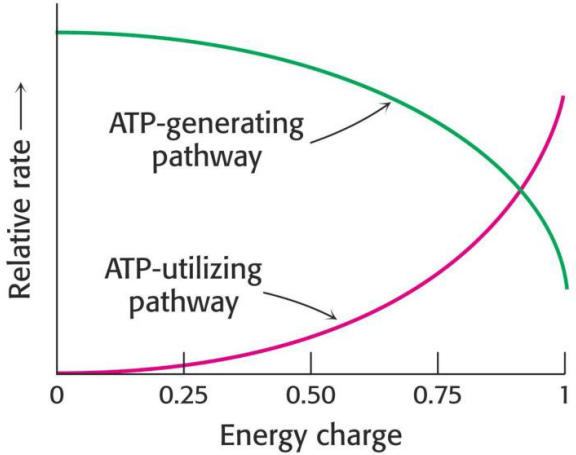
The energy charge

The energy unit in biochemistry: Adenosin triphosphate (ATP)

$$ATP \rightleftharpoons ADP + P_i$$

$$\Delta G = -30.6\ kJ$$





The energy charge:

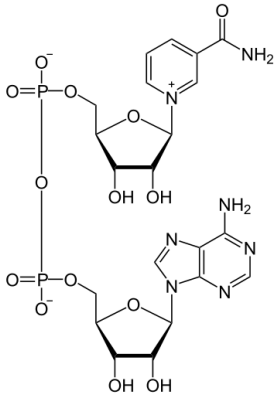
$$\frac{[ATP]+0.5[ADP]}{[ATP]+[ADP]+[AMP]}$$

High energy charge regulates metabolism.

High conc. of ATP inhibit the relative rates of a typical ATP-generating (catabolic) pathway and stimulate the typical ATP-utilizing (anabolic) pathway (The energy charge, like the pH of a cell, is 'buffered'!).

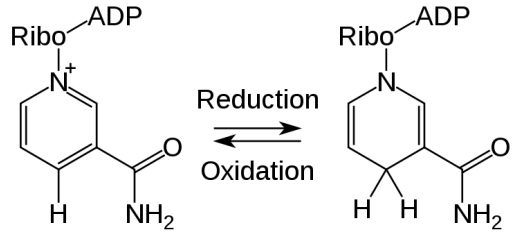
E. coli has an energy charge of 0.8 during exp. growth phase that slowly decreases to 0.5 during stationary growth phase. The cells die when the value is <0.5.

Nicotinamide adenine dinucleotide



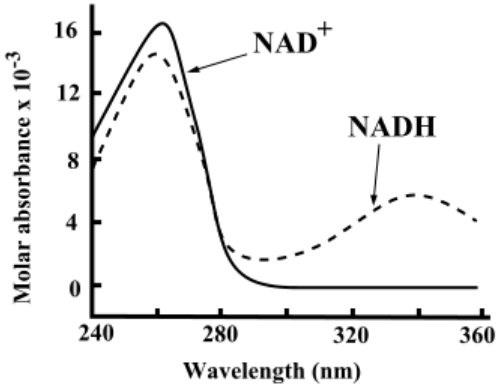
Nicotinamide adenine dinucleotide, abbreviated NAD^+, is a coenzyme found in all living cells. The compound is a dinucleotide, since it consists of two nucleotides joined through their phosphate groups. One nucleotide contains an adenine base and the other nicotinamide.

Electron carrier

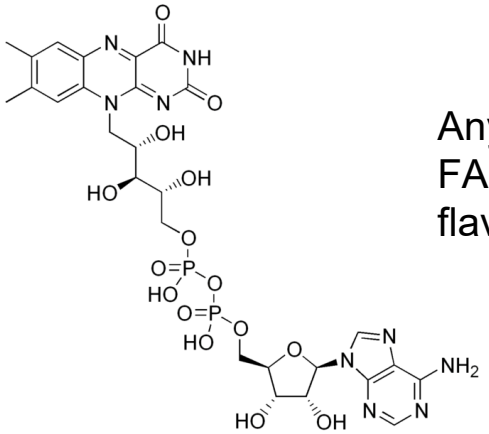


$NAD^+ + H^+ + 2e^- \longrightarrow NADH$

259 nm 339 nm

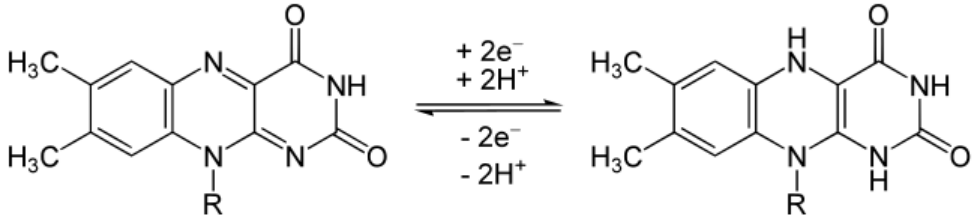


Flavin adenine dinucleotide (FAD)

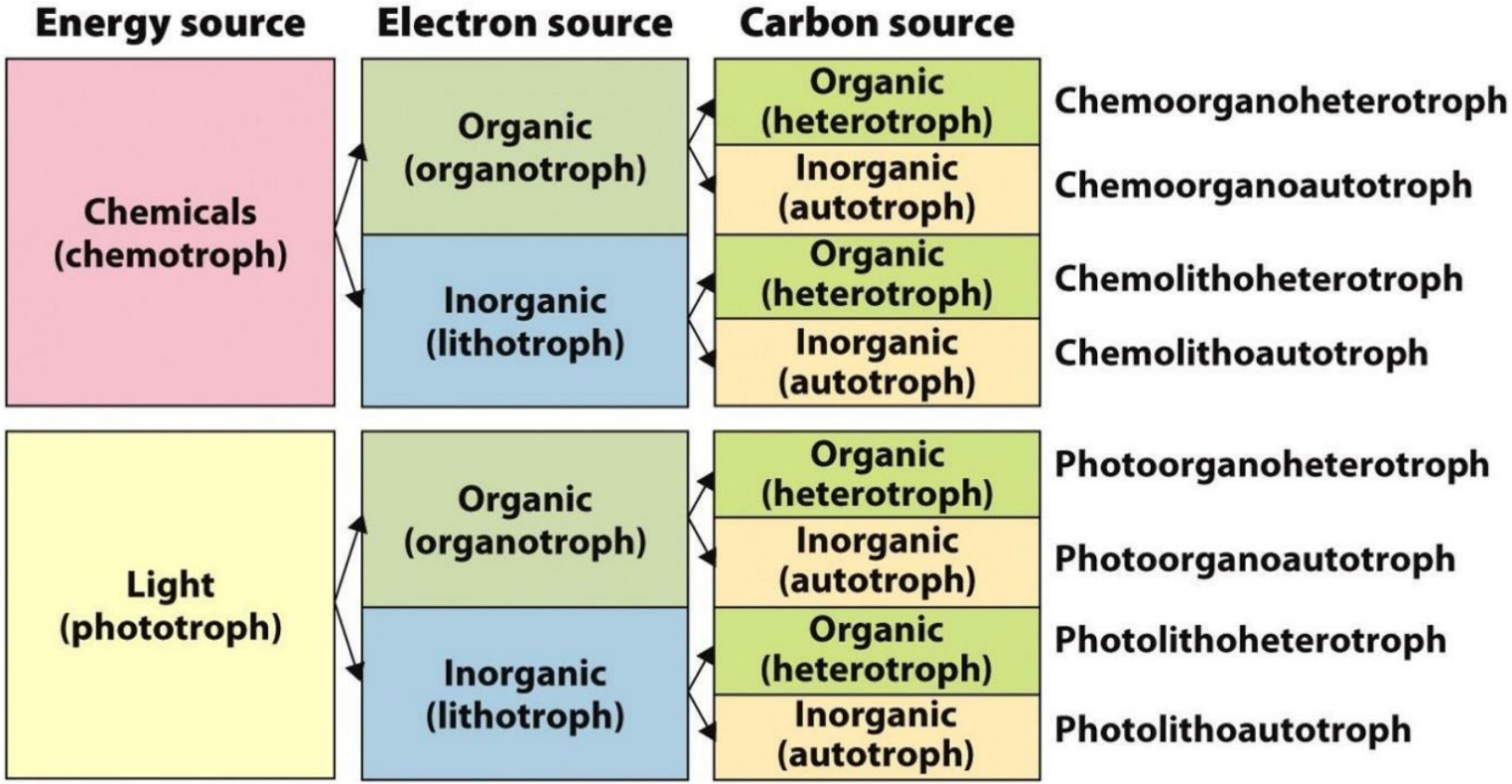


Any oxidoreductase enzyme that uses FAD as an electron carrier is called a flavoprotein.

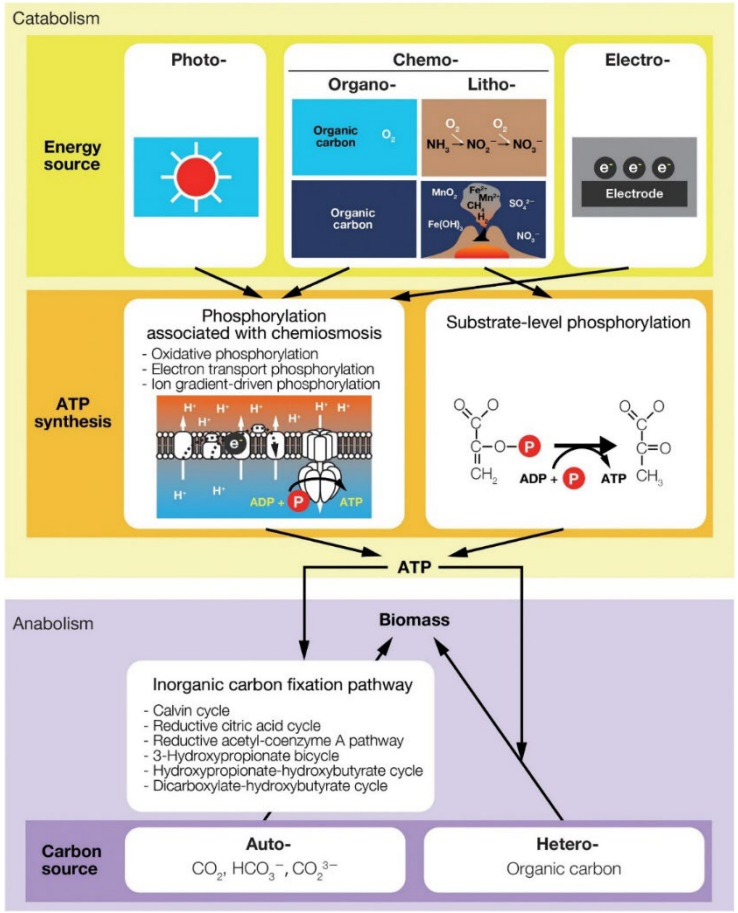
Electron carrier

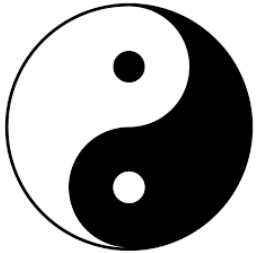


Energy sources for microorganisms



The Yin and Yang of metabolism





Dissimilation: The oxidation of a reduced (in)organic compound to provide energy for biosynthesis and cell maintenance.

Assimilation: The incorporation of a compound into biomass.

Seto M, Iwasa Y. How Thermodynamics Illuminates Population Interactions in Microbial Communities. Frontiers in Ecology and Evolution. 2020;8.

A few definitions

Metabolism:	Transformation of substances in the cell to gain precursors for cell components and energy
Catabolism:	Breakdown of nutrients into smaller fragments (ev. energy gain)
Amphibolism:	Intermediary metabolism (synthesis of organic acids and phosphate esters = building blocks); *anaplerotic sequences* when no transfer from catab. to anab. is possible
Anabolism:	Synthetic metabolism (synthesis of polymers from building blocks)

Metabolism = (Catabolism + Amphibolism) + Anabolism

Amphibolism has 13 key compounds

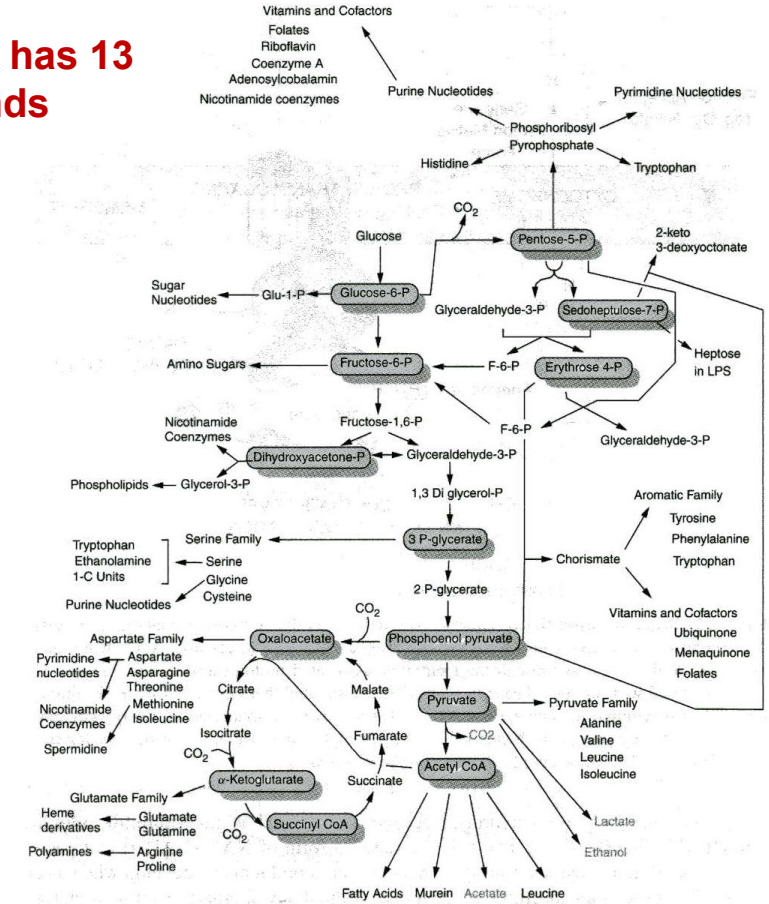
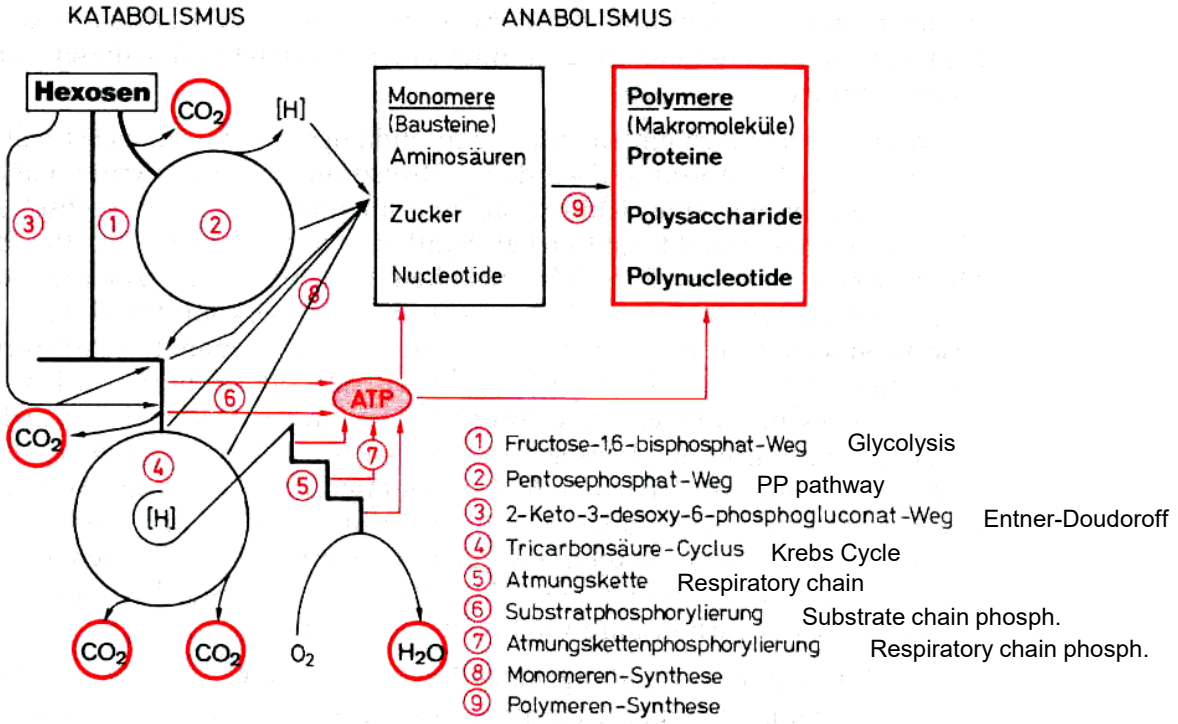


Fig. 1-11. Biosynthetic pathways leading to the amino acids and related compounds. The oblong-circled intermediates are the 13 key compounds that serve as biosynthetic precursors for a variety of essential end products.

A. G. Moat, J.W. Foster, M.P. Spector, 2002. Microbial Physiology ISBN 0-471-39483-1

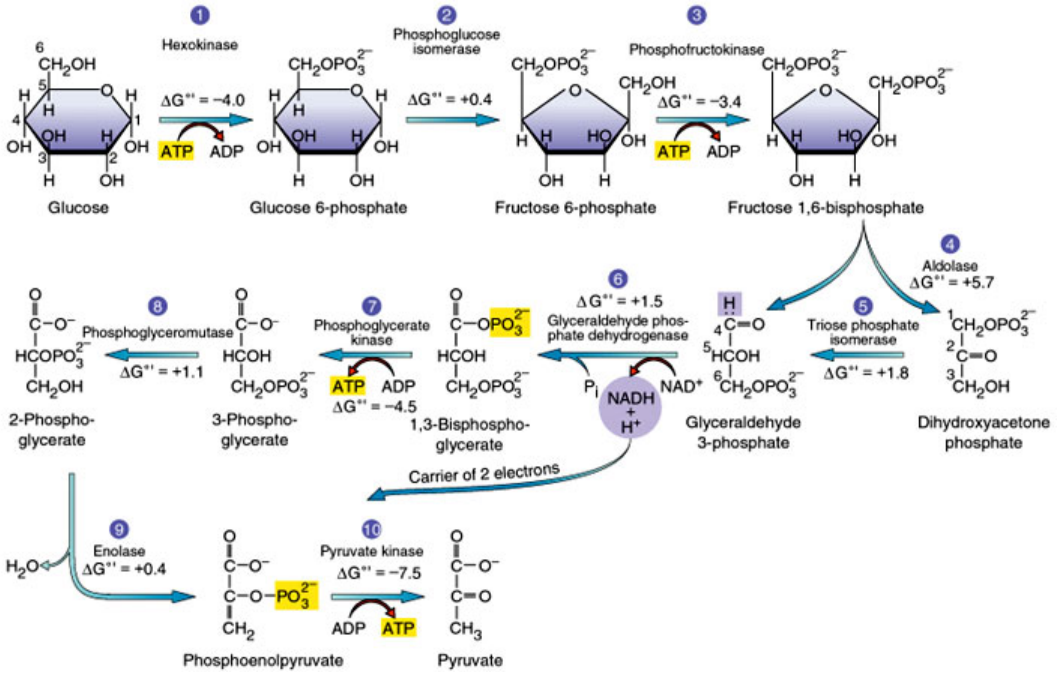
Metabolism in aerobic cells



Schlegel, 7. überarbeitete Auflage

Fructose-1,6-bisphosphate pathway

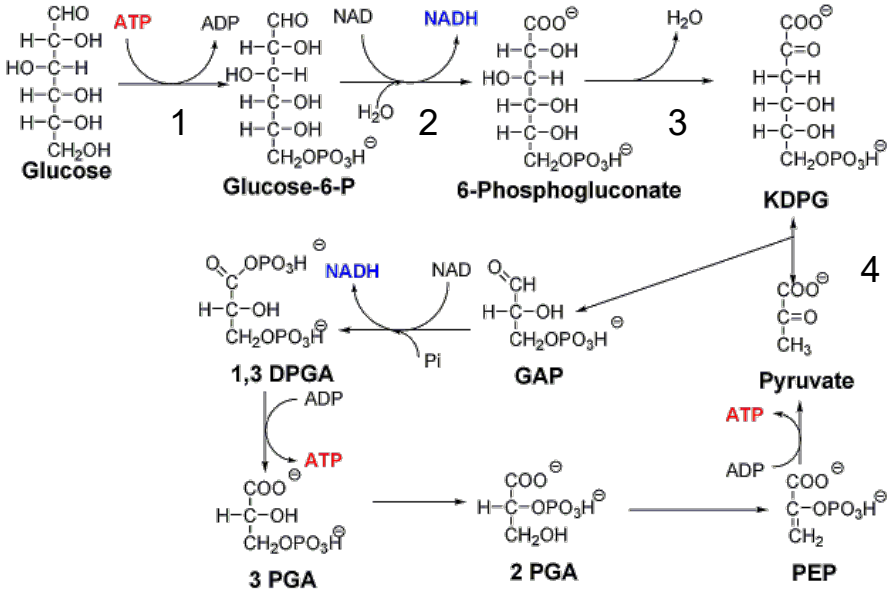
(Embden-Meyerhof-Parnas pathway, Glycolysis)



$$\text{Glucose} + 2NAD^+ + 2ADP + 2P_i \rightarrow 2\text{Pyruvate} + 2NADH + 2ATP$$

http://4.bp.blogspot.com/_t38RPovEIS8/TRYWdZrSt7I/AAAAAAAAADA/O_h0g2Fve9Q/s1600/glycolysis_pathway.jpg

Entner-Doudoroff pathway in many Pseudomonads



(1) Hexokinase; (2) glucose-6-phosphate dehydrogenase; (3) phosphogluconate dehydrase; (4) 2-keto-3-deoxy-6-phosphogluconate aldolase.

$$\text{Glucose} + 2NAD^{+} + 1ADP + 1P_i \rightarrow 2\text{Pyruvate} + 2NADH + 1ATP$$

Pentose-phosphate pathway

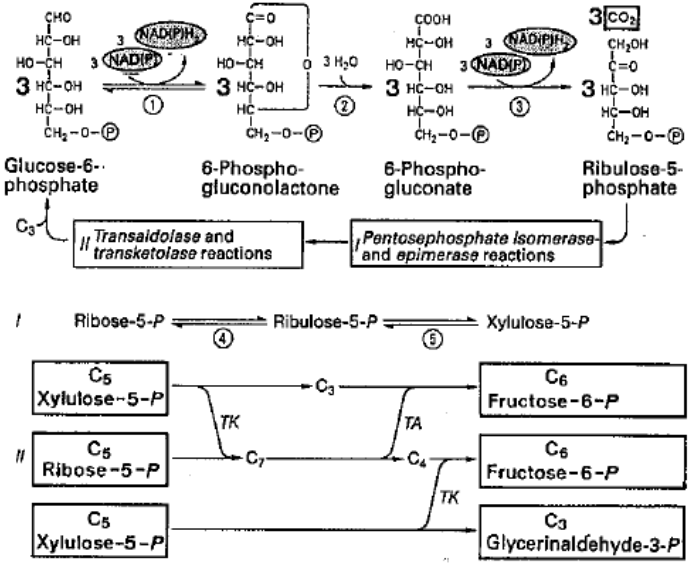


Fig. 7.4. *The pentose-phosphate pathway for the oxidative catabolism of glucose-6-phosphate.*

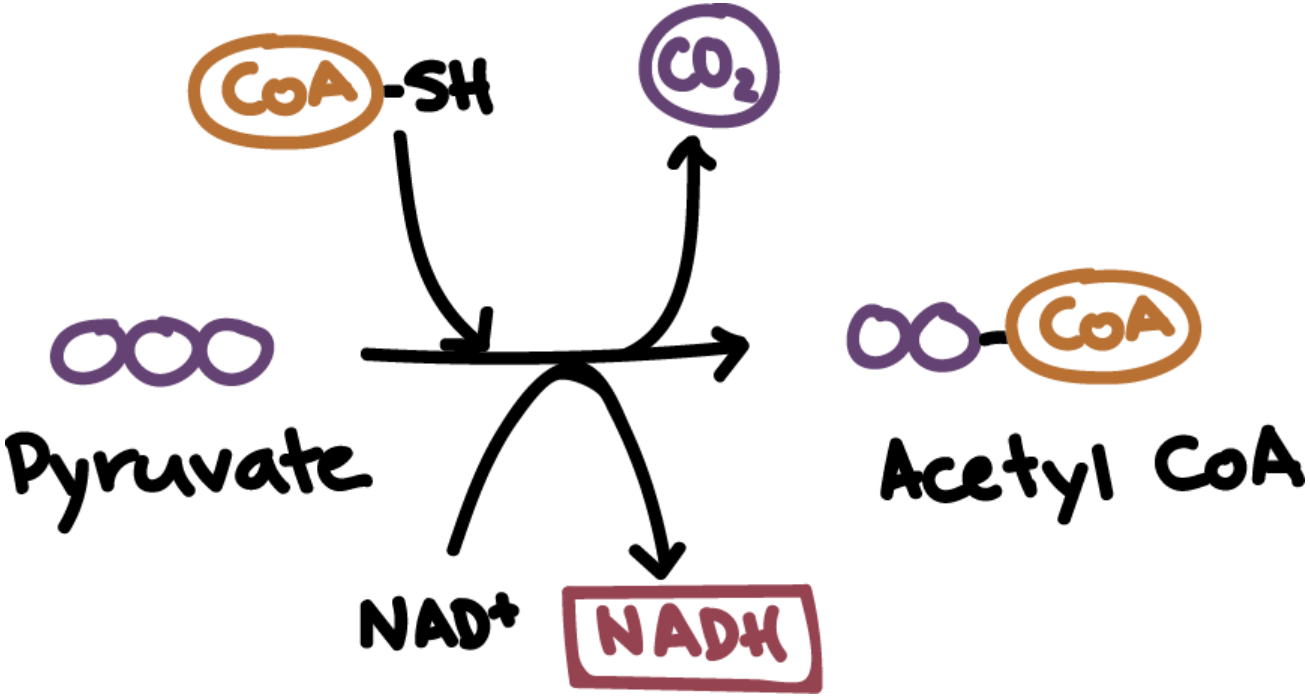
The oxidative steps culminate in the formation of ribulose-5-phosphate. The ribulose-5-phosphate exists in an enzyme-catalysed equilibrium with ribose-5-phosphate and xylulose-5-phosphate. The pentose-phosphates are converted to two fructose-phosphates and one glyceraldehyde-phosphate by the actions of transketolase and transaldolase. These reactions are completely reversible; in the reverse direction, they participate in the ribulose-monophosphate cycle of formaldehyde fixation and in the ribulose-bisphosphate cycle of carbon dioxide fixation, as well as in other cyclical processes. The enzymes involved are: (1) glucose-6-phosphate dehydrogenase; (2) lactonase; (3) 6-phosphogluconate dehydrogenase; (4) phosphoribose isomerase; (5) ribulose-5-phosphate-3-epimerase; TK, transketolase; TA, transaldolase.

Glucose + 1ATP + 6NADP$^+$ → Glyceraldehyde 3-P + ADP + 6NADPH + 3CO_2

Summary: PPP vs. Glycolysis & ED Pathway

Feature	Pentose Phosphate Pathway (PPP)	Glycolysis (EMP)	Entner-Doudoroff (ED)
Main Purpose	NADPH & biosynthesis	ATP & pyruvate	ATP & pyruvate
ATP Yield	None directly	2 ATP per glucose	1 ATP per glucose
NADPH Production	High	None	Some (but mostly NADH)
Key Metabolites	Ribose-5-phosphate, NADPH	Pyruvate, ATP	Pyruvate, ATP
Flexibility	Highly adaptable	Less flexible	Less flexible
Best for...	Biosynthesis, nucleotide production, oxidative stress response	Energy generation, fermentation	Sugar acid metabolism, aerobic bacteria

Pyruvate oxidation



https://www.khanacademy.org/science/biology/cellular-respiration-and-fermentation/pyruvate-oxidation-and-the-citric-acid-cycle/a/pyruvate-oxidation

Krebs cycle

(Citric acid cycle)

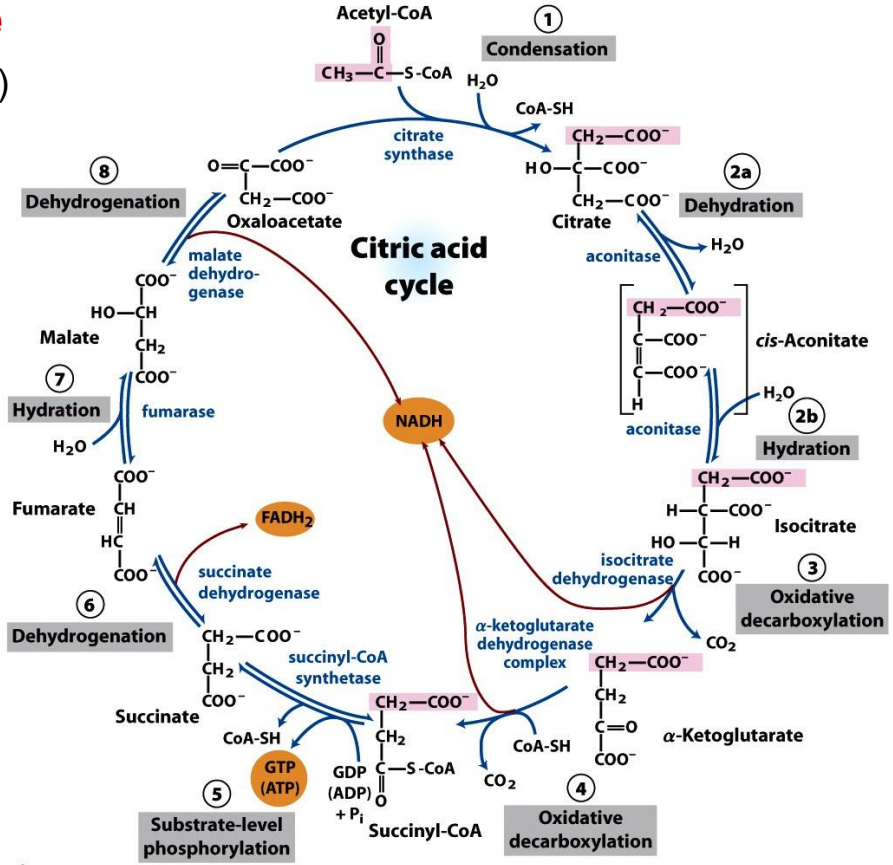
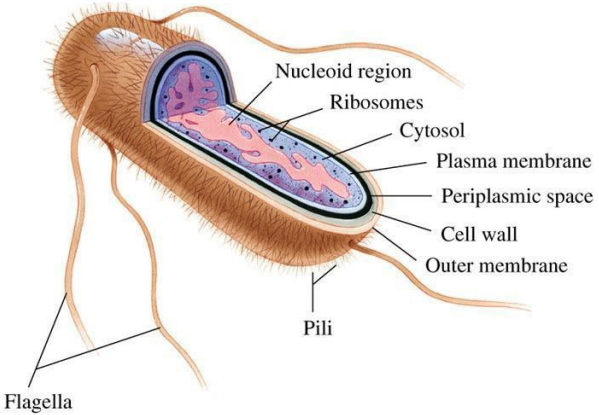


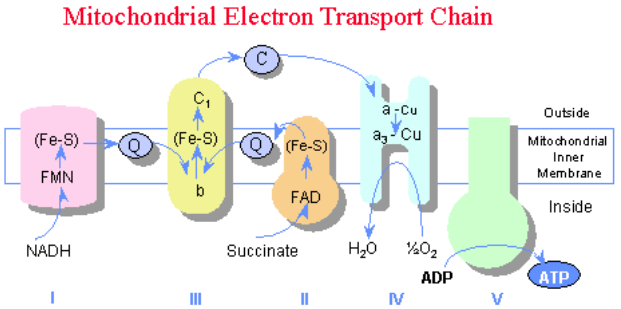
Figure 16-7

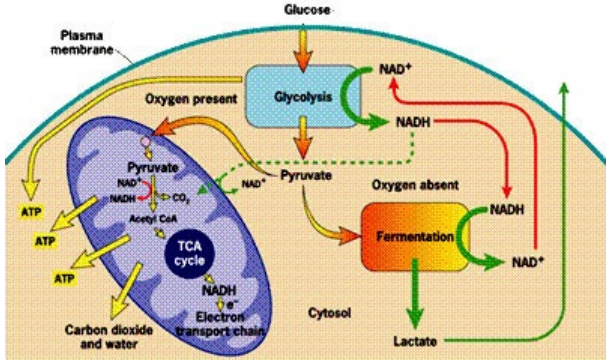
Lehninger Principles of Biochemistry, Fifth Edition

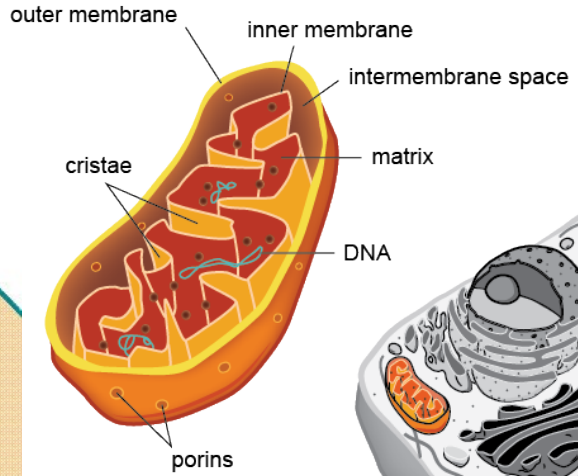
$$\text{Acetyl-CoA} + 3NAD^+ + FAD + GDP(ADP) \rightarrow 2CO_2 + \text{coenzyme A} + 3NADH + FADH^+ + GTP(ATP)$$

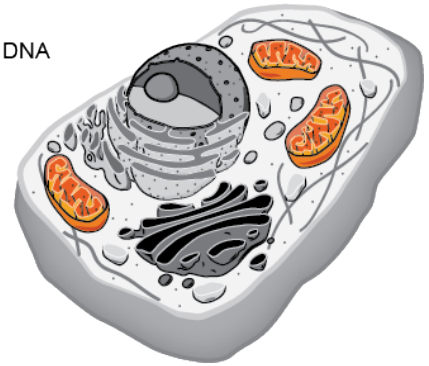
Two very close relatives: The prokaryote and the mitochondria





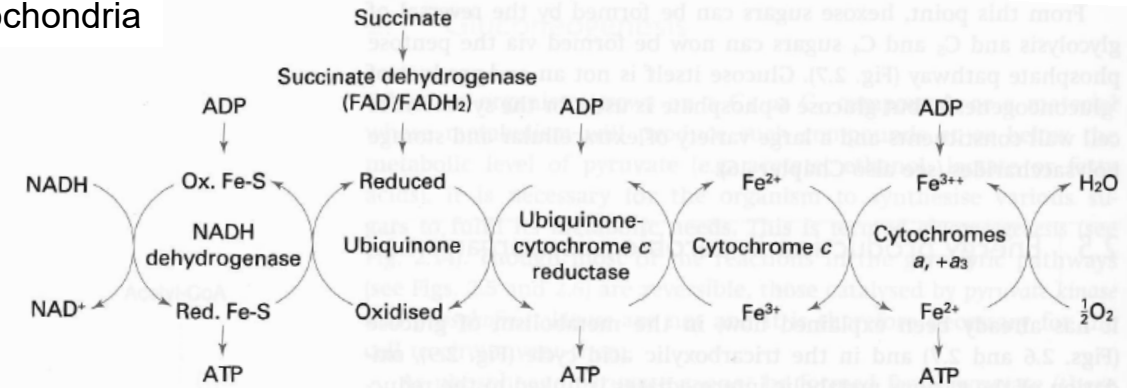




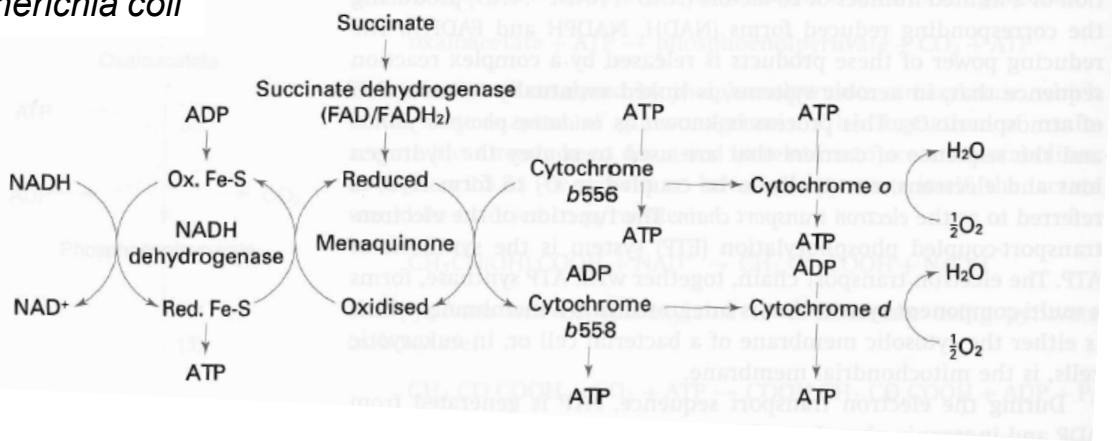


Electron-transport-coupled phosphorylation (ETP) System

Mitochondria



Escherichia coli



Oxidative and substrate-level phosphorylation

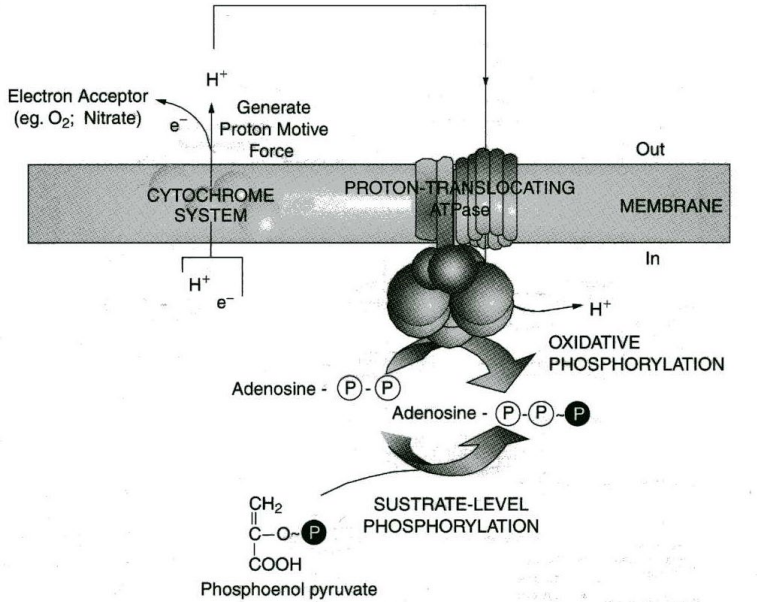


Fig. 1-12. Reactions essential to energy production. Oxidative phosphorylation. The energy that comprises the proton motive force can be harnessed and used to generate ATP when protons from outside the cell pass through the membrane-associated proton-translocating ATPase. The energy released will run the ATPase in reverse. It is estimated that passage of three H^+ through the ATPase is required to generate one ATP. Substrate-level phosphorylation. Energy contained within high-energy phosphate bonds of certain glycolytic intermediates can be transferred to ADP, forming ATP. The example shows phosphoenolpyruvate.

Environmental pH is important

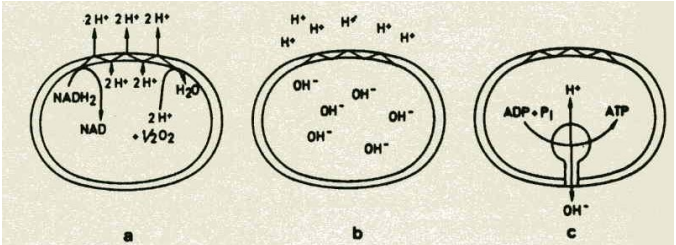


Fig. 7.8. *The respiratory chain and electron-transport phosphorylation in and at the cytoplasmic membrane of protocytes and the inner membrane of mitochondria.*

(a) $NADH_2$ oxidation and proton extrusion; (b) electrochemical gradient between inner and outer surfaces; (c) regeneration of ATP as a consequence of the backflow of protons.

$$\Delta p = \frac{\Delta \mu_{H^+}}{F} = \Delta\Psi - Z * \Delta pH$$

Both the pH gradient and the electrical membrane potential gradient exert a pull in the direction of the cell interior on the extruded protons = proton motive force Δp.

$\Delta\Psi$: Electrical membrane potential

Z=2.3 RT/F (59mV at 25°C)

The proton potential can be based entirely on the H difference or on the membrane potential or on both.

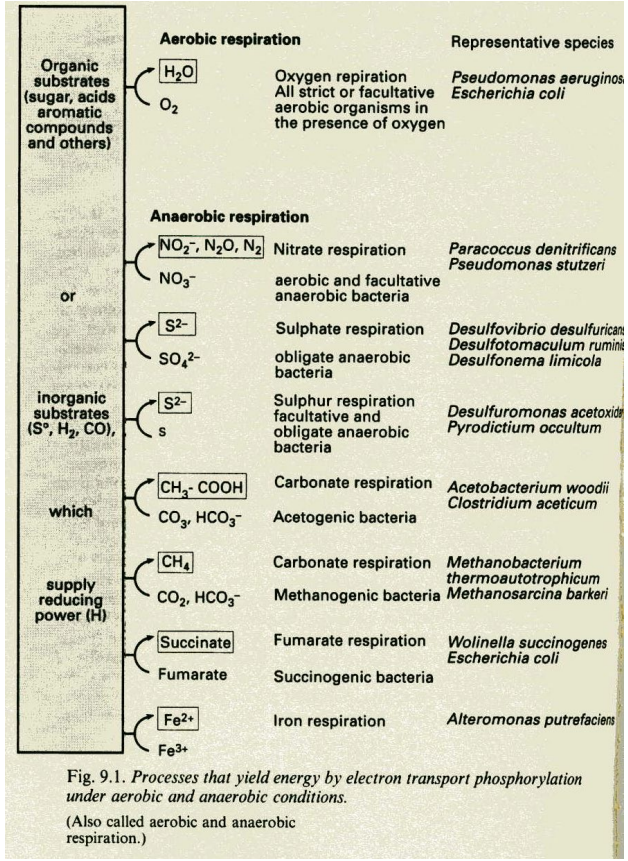
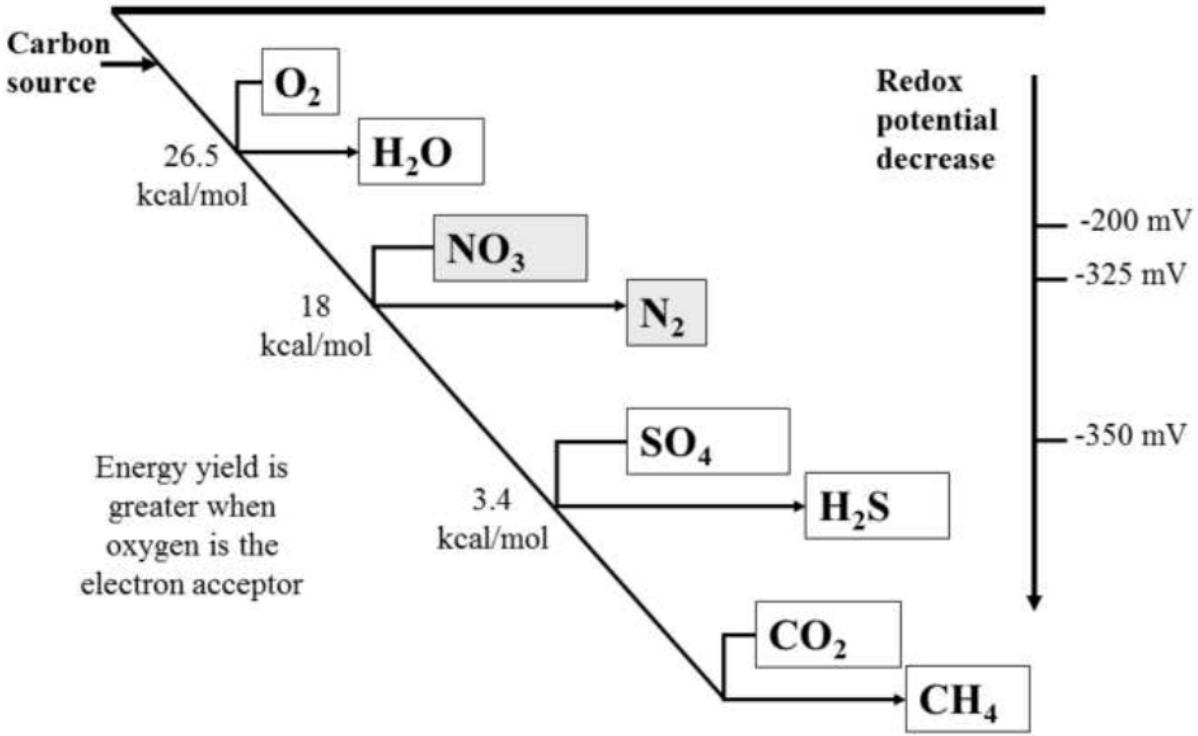


Fig. 9.1. *Processes that yield energy by electron transport phosphorylation under aerobic and anaerobic conditions.*

(Also called aerobic and anaerobic respiration.)

Respiratory processes



Combined energy gain

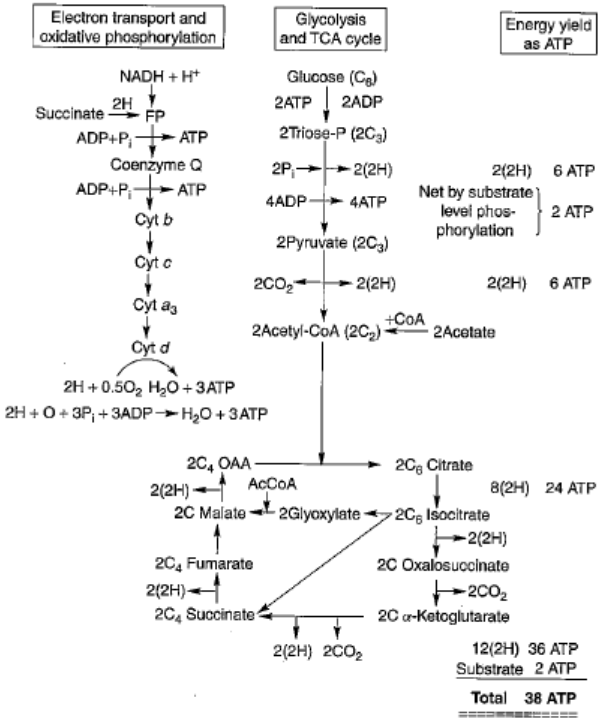
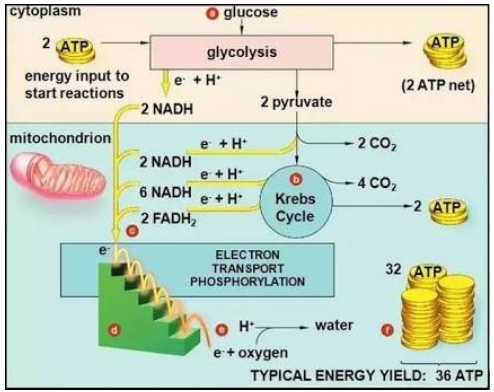


Fig. 9-9. Theoretical energy yield as ATP from glycolysis and the TCA cycle. The calculations shown here assume that each pair of hydrogen atoms (2H) released from the substrate yields 3 ATP. The reaction shown as $ADP + P_i \rightarrow ATP$ represents the action of ATP synthase. Two turns of the TCA cycle are required to completely oxidize the 2 acetyl-CoA derived from glucose. Each 2H generated by the system yields 1 molecule of water ($2H + 0.5O_2 \rightarrow H_2O$). Overall reaction: $C_6H_{12}O_6 + 38P_i + 38ADP + 6O_2 \rightarrow 6CO_2 + 6H_2O + 38ATP$. Total ATP from TCA cycle: $12(2H) + 6O_2 + 36P_i \rightarrow 6H_2O + 36ATP$.



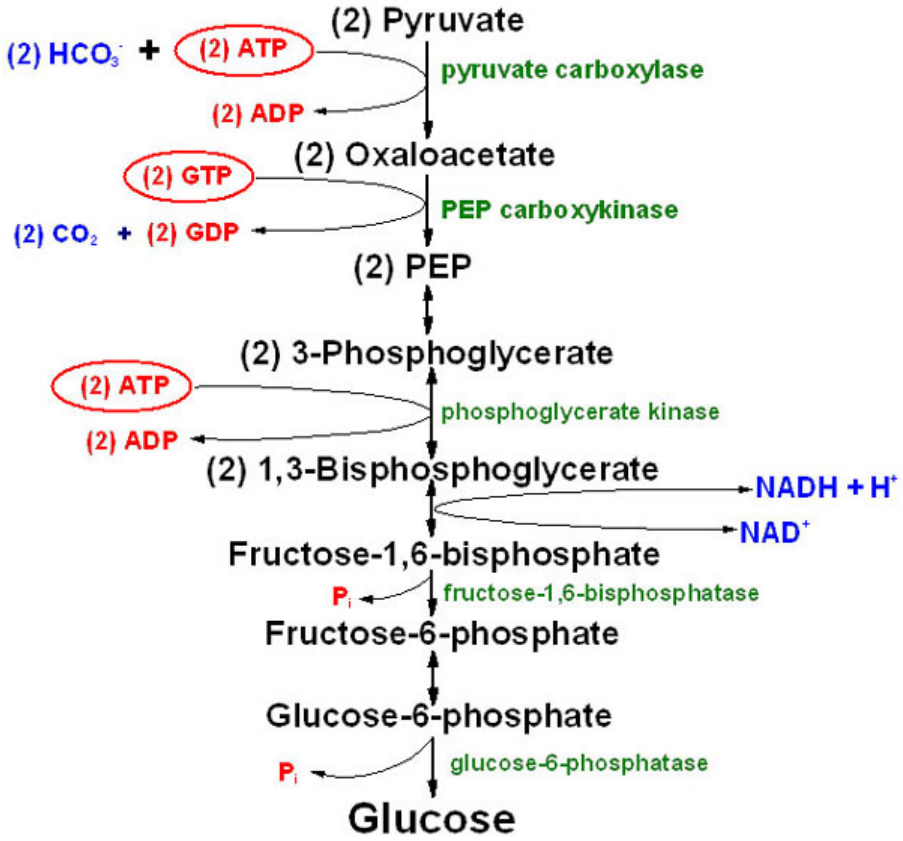
P/O ratios:

$NADPH + 3ADP + 3P_i + 0.5O_2 \rightarrow NADP^+ + 3ATP + H_2O$

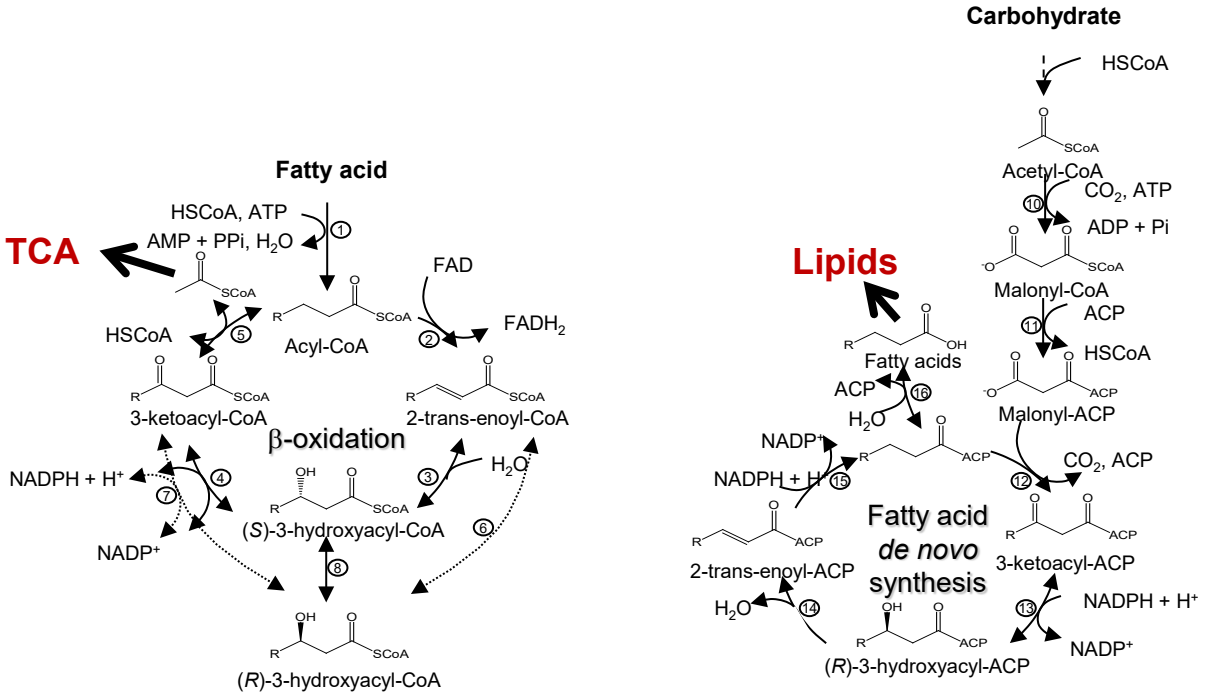
$NADH + 3ADP + 3P_i + 0.5O_2 \rightarrow NAD^+ + 3ATP + H_2O$

$FADH + 2ADP + 2P_i + 0.5O_2 \rightarrow FAD^+ + 2ATP + H_2O$

Gluconeogenesis



Fatty acid degradation and fatty acid *de novo* synthesis



The capacity of bacteria for biosynthesis

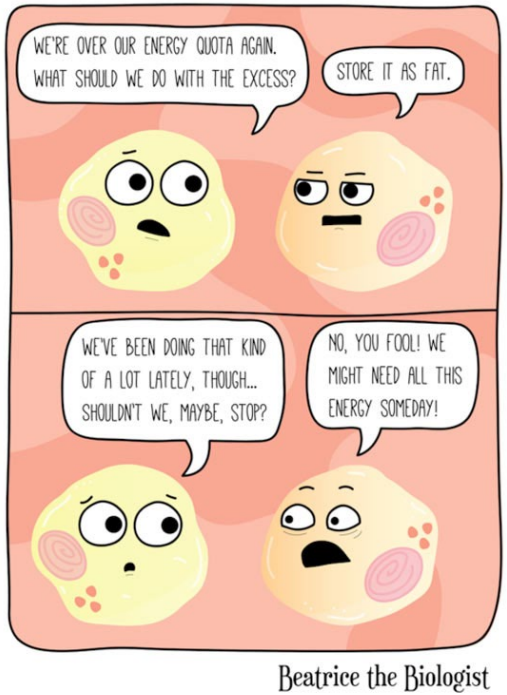
TABLE 5.2 The Biosynthetic Capabilities of a Bacterial Cell[a]

Chemical component	Percent of dry weight	Approximate molecular weight	Number of molecules per cell	Number of molecules synthesized per second	Number of molecules of ATP required to synthesize per second	Percent of total synthetic energy required
DNA	5	2,000,000,000	1	0.00083	60,000	2.5
RNA	10	1,000,000	15,000	12.5	75,000	3.1
Protein	70	60,000	1,700,000	1,400	2,120,000	88.0
Lipids	10	1,000	15,000,000	12,500	87,500	3.7
Polysaccharides	5	200,000	39,000	32.5	65,000	2.7

[a] Reprinted with permission from Lehninger (1971). *Escherichia coli* is about $1 \times 1 \times 3\ \mu m$ in size; it has a volume of $2.25\ \mu m^3$, a total weight of 10×10^{13} g, and a dry weight of 2.5×10^{-13} g. The rates of biosynthesis were averaged over a 20 minute cell division cycle.

How can the cell control its cell physiology?

- The cell needs to react quickly to changes of the chemical environment. During evolution many different methods and techniques have been established.
- Prime goal for all activities is to optimize the energy gain but also to provide always enough building blocks (anabolism).



Compartimentalisation

- The separation of chemical reactions by organelles enables the increase of the chance for reaction because the concentration of the reactants are elevated (e.g. TCA in mitochondria).
- Another example for compartimentalisation is the phosphorylation of substrates: These compounds cannot diffuse through cell membranes and therefore remain inside the cell. One of the typical examples is the phosphorylation of glucose.
- Storage compounds are stored in granules. Typical examples are polyphosphates, glycogen, lipids and polyhydroxyalkanoates.

Nutrient uptake

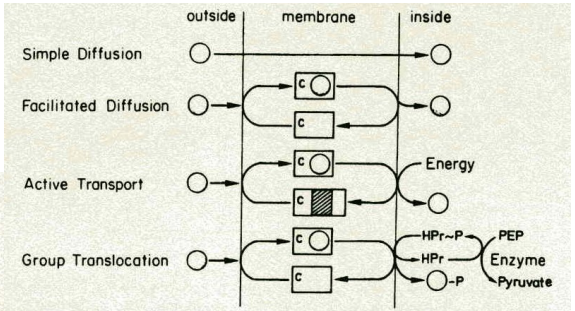


Fig. 7.19. *Schematic representation of the four mechanisms for transport of materials into the cell.*

Circle, substrate to be transported; C, permease(carrier)-protein; C with grey hatching, energised carrier; PEP, phosphoenolpyruvate; HPr, heat stable protein. See text for details.

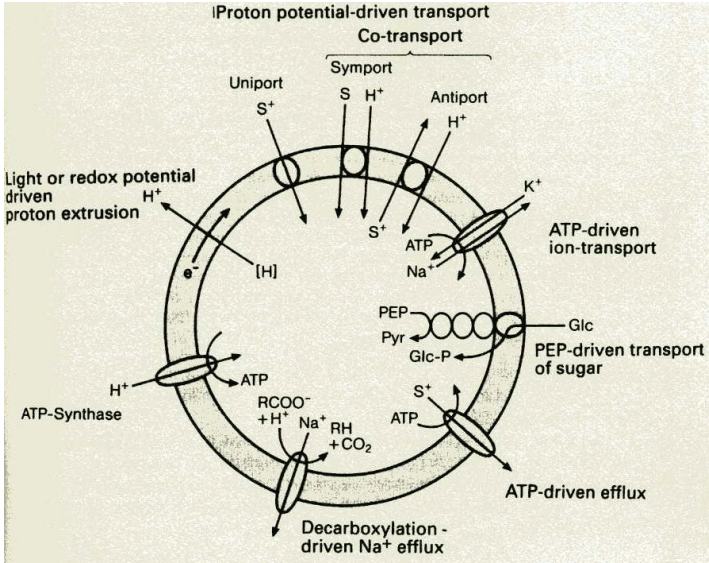


Fig. 7.21. *Summary of systems that transport ions and other metabolites through the cytoplasmic membrane.*

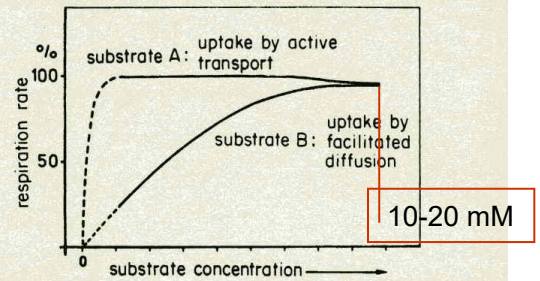
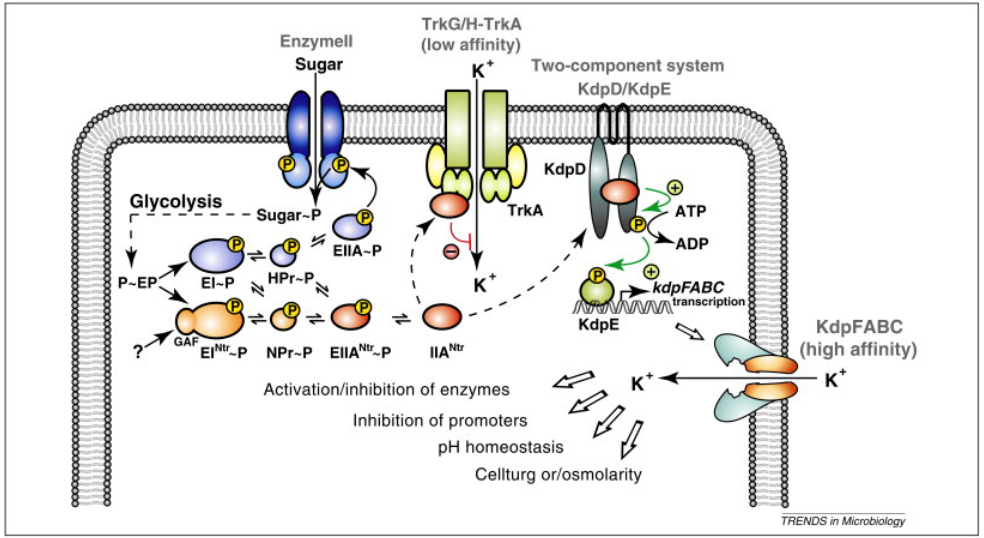


Fig. 7.20. *Substrate saturation curves for the uptake of two substrates by intact bacterial cells measured by oxygen consumption (respiration rate).*

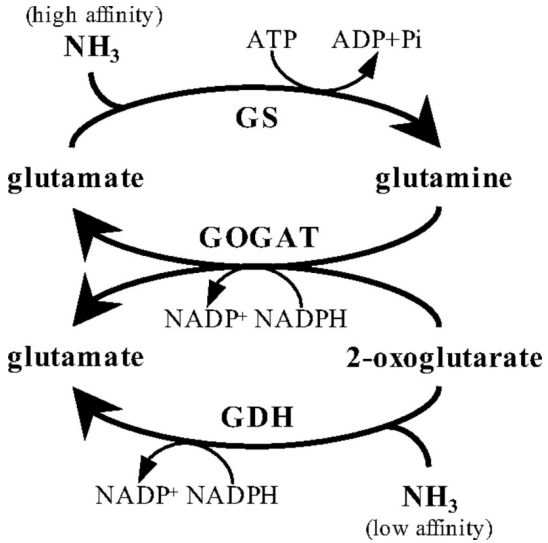
High and low affinity potassium transporter in *E. coli*



Regulation of potassium transport by PTS^{Ntr} in *E. coli*. In *E. coli* and many other bacteria, K^+ uptake is basically achieved via the Trk and the Kdp systems. **Trk is a low-affinity transporter** and sufficient as long as environmental K^+ concentrations are high. At low concentrations, K^+ uptake is achieved by the **high-affinity K^+-transporter KdpFABC**. Although the *trk* genes are constitutively expressed, transcription of the *kdpFABC* operon is positively controlled by the two-component system KdpD–KdpE in response to low K^+ concentrations. Both transporters are regulated by $EIIA^{Ntr}$ through protein–protein interactions. $EIIA^{Ntr}$ directly binds subunit TrkA of the Trk system and thereby inhibits K^+ uptake. Moreover, $EIIA^{Ntr}$ stimulates autophosphorylation activity of sensor kinase KdpD by direct interaction, which in turn enhances KdpFABC synthesis through increased *kdp* promoter activity. Therefore, $EIIA^{Ntr}$ limits uptake of K^+ at high concentrations and stimulates uptake when K^+ concentrations are low. $EIIA^{Ntr}$ is a member of PTS^{Ntr} (orange–red), which works in parallel to the PTS catalyzing sugar uptake (blue). In both systems, EI- and HPr-like proteins transfer phosphoryl groups from PEP to EIIA-like domains. Although in the canonical PTS these phosphoryl groups are transferred to the sugars via EII activities, they are utilized in PTS^{Ntr} to control activity of $EIIA^{Ntr}$. Non-phosphorylated $EIIA^{Ntr}$ (but not its phosphorylated form) binds TrkA and KdpD. As cross-phosphorylation occurs between both PTSs, the phosphorylation state of $EIIA^{Ntr}$ and thereby K^+ transport activity is, at least in part, also determined by activity of the sugar PTS (i.e. by carbon source availability). However, a hypothetical, unidentified signal might enter this regulatory circuit through the modulation of EI^{Ntr} activity by binding to its GAF domain.

Trends in Microbiology; Vol. 18, Issue 5, May 2010, Pages 205–214

The central nitrogen metabolic circuit in enteric bacteria

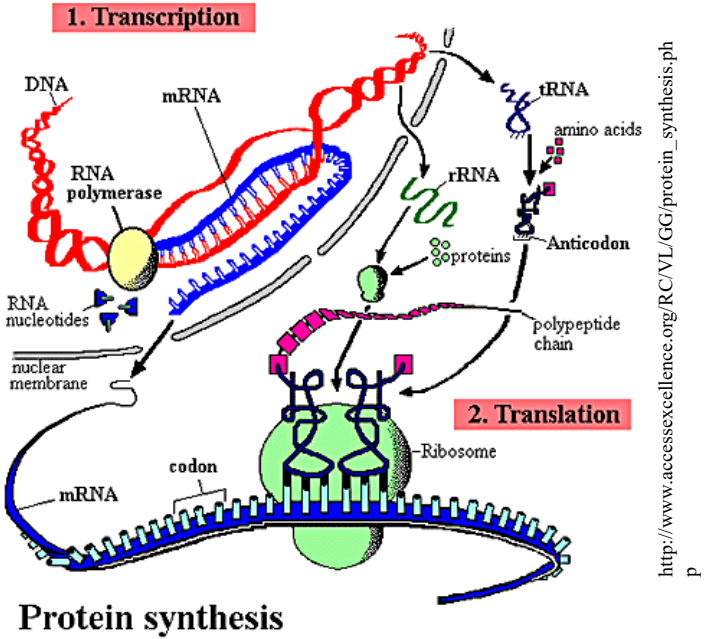


GS: Glutamine synthetase
GOGAT: Glutamine:2-oxoglutarate aminotransferase
GDH: Glutamate dehydrogenase

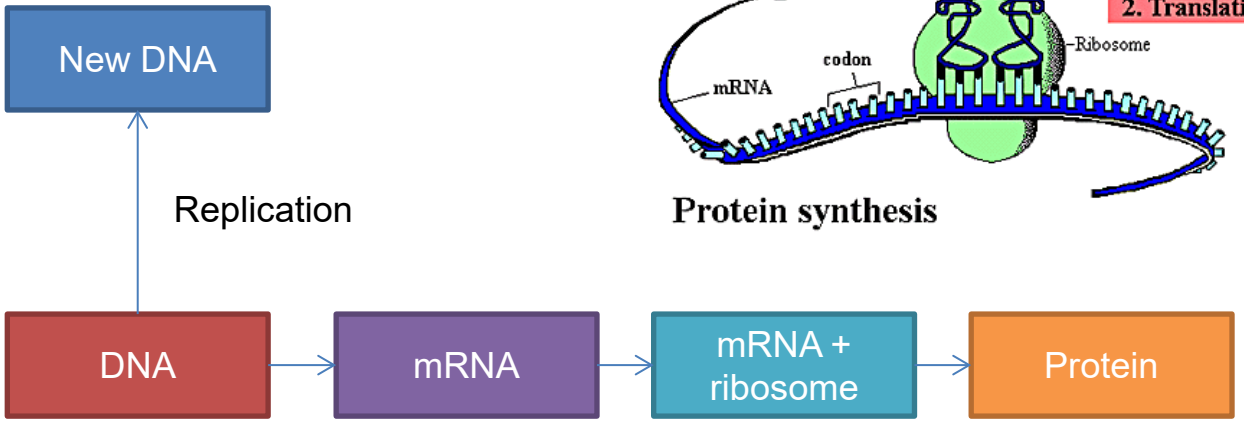
The three-enzyme circuit assimilates NH_4^+ and produces two central intermediates, **glutamine** and glutamate. GS catalyzes glutamine synthesis. Glutamate can be synthesized by the action of either GS/GOGAT or GDH, respectively, with high or low affinity for NH_4^+. The two glutamate molecules shown have no known functional difference.

Control of metabolic processes

- Need for control of the new protein level



http://www.accessexcellence.org/RC/VL/GG/protein_synthesis.php



Control of enzyme activity

- Regulation of DNA expression (induction, repression)
- Posttranscriptional control
- Modification of enzyme activity
- Degradation of enzymes

Catabolite repression

Diauxic growth of *E. coli*

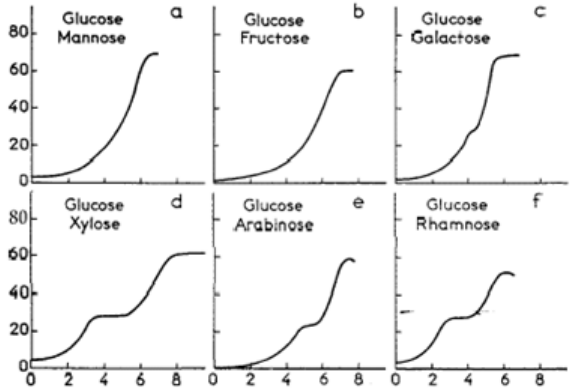
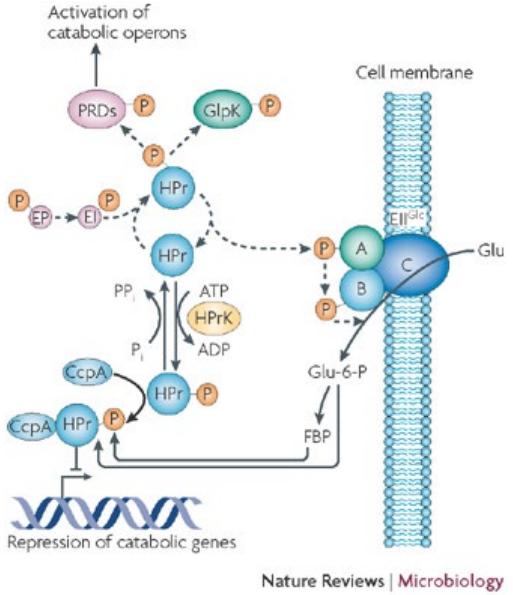
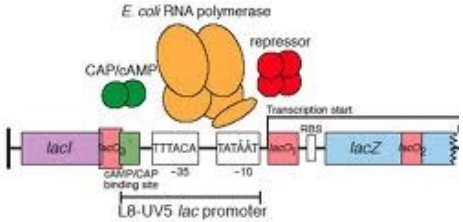


Fig.1. Growth of *Escherichia coli* in the presence of different carbohydrate pairs serving as the only source of carbon in a synthetic medium[30].



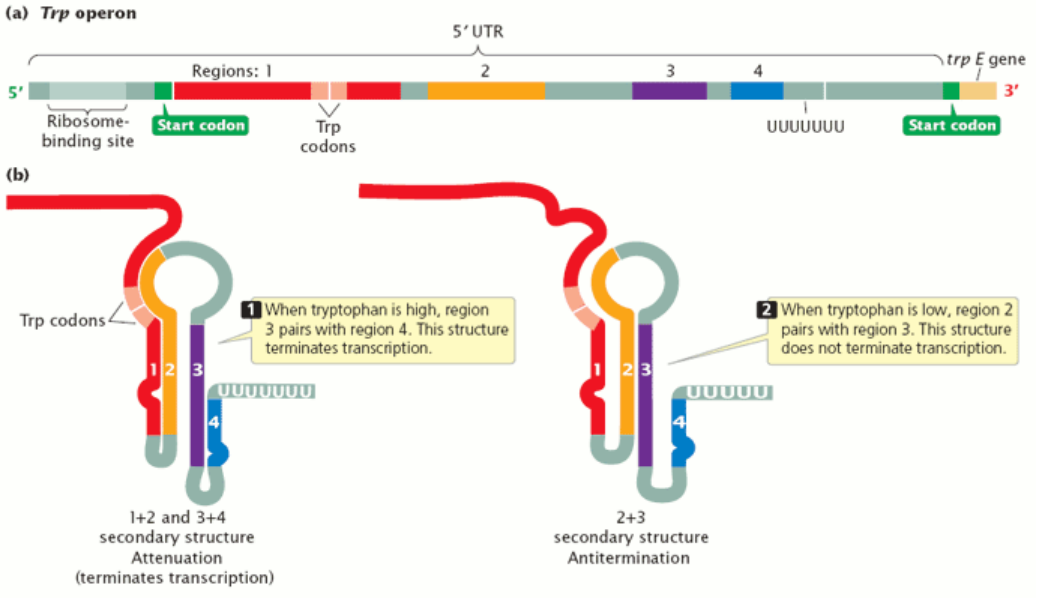
Typical example is the lac operon:



See also exercises!

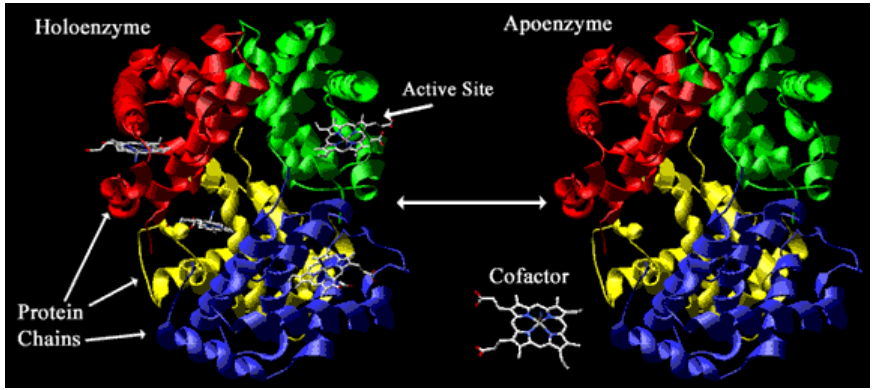
Posttranscriptional control

The tryptophane operon



http://www.nature.com/scitable/topicpage/simultaneous-gene-transcription-and-translation-in-bacteria-1025

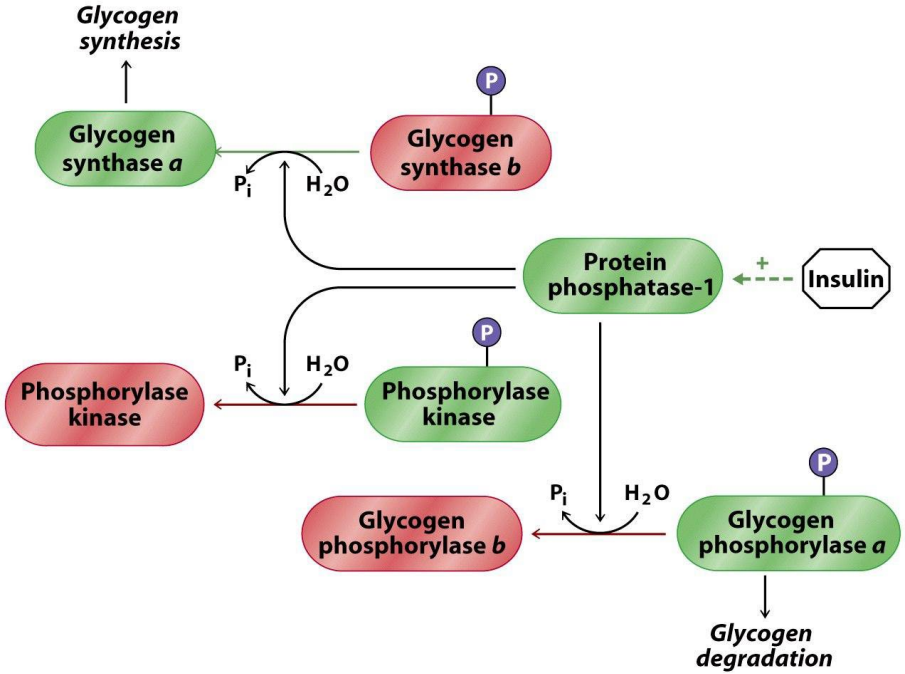
Modification of enzyme activity



Binding of the substrate to the enzyme changes the enzyme's shape, a phenomenon called **induced fit**. The active site's embrace of the substrate brings chemical groups of the active site into positions that enhance their ability to work on the substrate and to catalyze the chemical reaction. When the reaction is complete, the product of catalyzed reaction is released and the enzyme resumes its previous shape and is ready to catalyze another chemical reaction. For convenience the active enzyme is termed as a **holoenzyme**. It may in turn comprise of major protein part of enzyme the apoenzyme. A number of other chemical substances help the enzyme to be functional. These chemicals are termed as prosthetic group and may be coenzyme, cofactors.

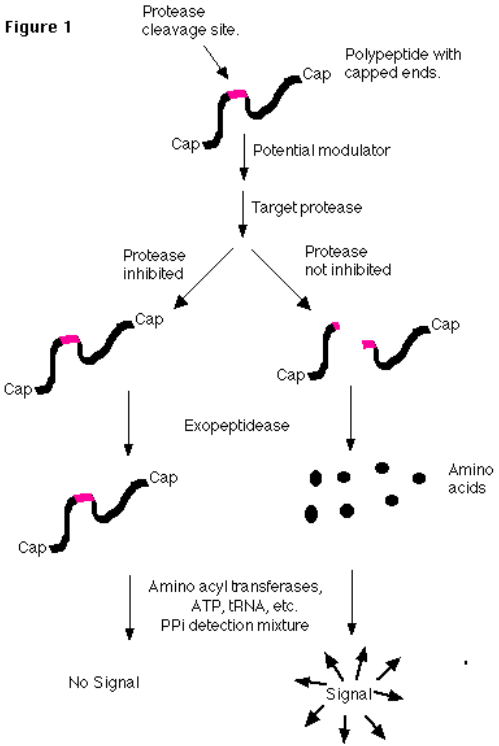
http://notesforpakistan.blogspot.ch/2010/12/enzymes-and-factors-affecting-enzyme.html

Modification of enzyme activity



Principles of Biochemistry, 4/e

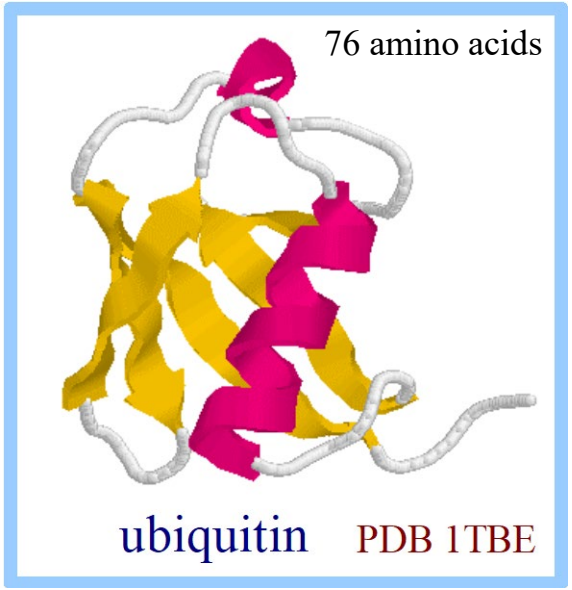
Degradation of enzymes



Intracellular proteases are especially expressed under nitrogen starvation. Proteolytic degradation is a fast reaction to the cellular needs. Important peptides can be protected from exopeptidases by a cap.

N-end rule: On average, a protein's **half-life** correlates with its **N-terminal residue**:

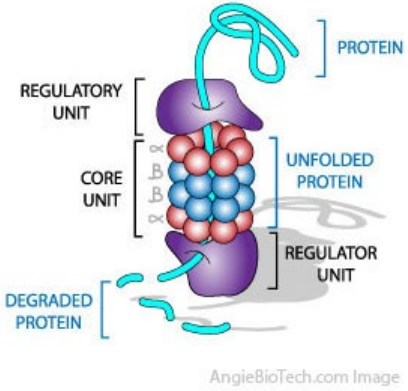
- Proteins with N-terminal Met, Ser, Ala, Thr, Val, or Gly have half lives greater than 20 hours.
- Proteins with N-terminal Phe, Leu, Asp, Lys, or Arg have half lives of 3 min or less.
- **PEST** proteins, rich in Pro (P), Glu (E), Ser (S) and Thr (T), are more rapidly degraded than other proteins.

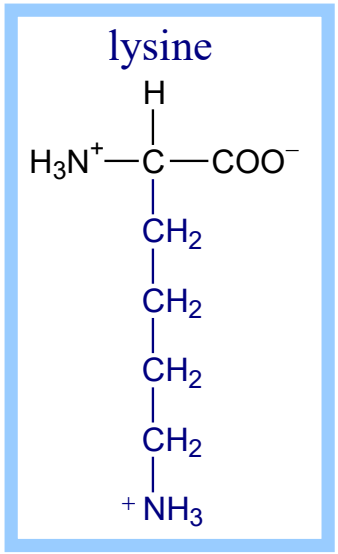


Ubiquitin determines the fate of proteins

Proteins are usually **tagged** for selective destruction in proteolytic complexes called **proteasomes** by covalent attachment of **ubiquitin**, a small, compact, highly conserved protein.

However, some proteins may be degraded by proteasomes (right) without ubiquitination.





An isopeptide bond links the **terminal carboxyl** of **ubiquitin** to the **ε-amino** group of a **lysine** residue of a "condemned" protein.

Summary

- The production strain is the most valuable part in biotechnology industry. A thorough understanding of the strain is essential for designing optimal bioprocesses.

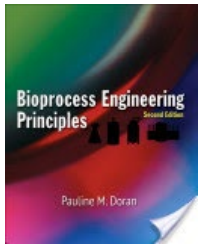
- Although eukaryotes and prokaryotes have similar ways of energy gain, they differ fundamentally in mode of reproduction, genetical organization and compartimentalization.

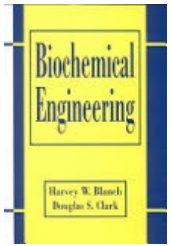
- The cell has to control the metabolic activity by adjusting the flux of metabolites, the synthesis and activation of enzymes as well as their degradation.

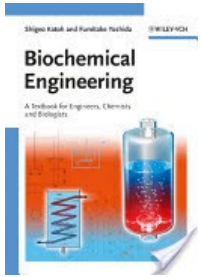
Further reading (M. Zinn)

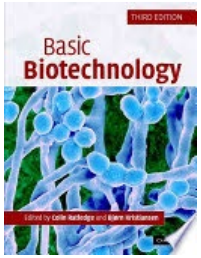
On the web:
Classification of enzymes:
https://www.ebi.ac.uk/intenz/advice.jsp#:~:text=The%20enzyme%20classification%20system,-Each%20enzyme%20is&text=Each%20enzyme%20is%20allocated%20a,uniquely%20defines%20the%20reaction%20catalysed.
Enzyme data base: http://www.brenda-enzymes.org/index.php
Bioinformatics resource portal: http://www.expasy.org/
Cell metabolism: http://www.genome.jp/kegg/kegg2.html
Genomic pathways: http://biocyc.org/
Energy cycle: http://hyperphysics.phy-astr.gsu.edu/hbase/biology/etrans.html

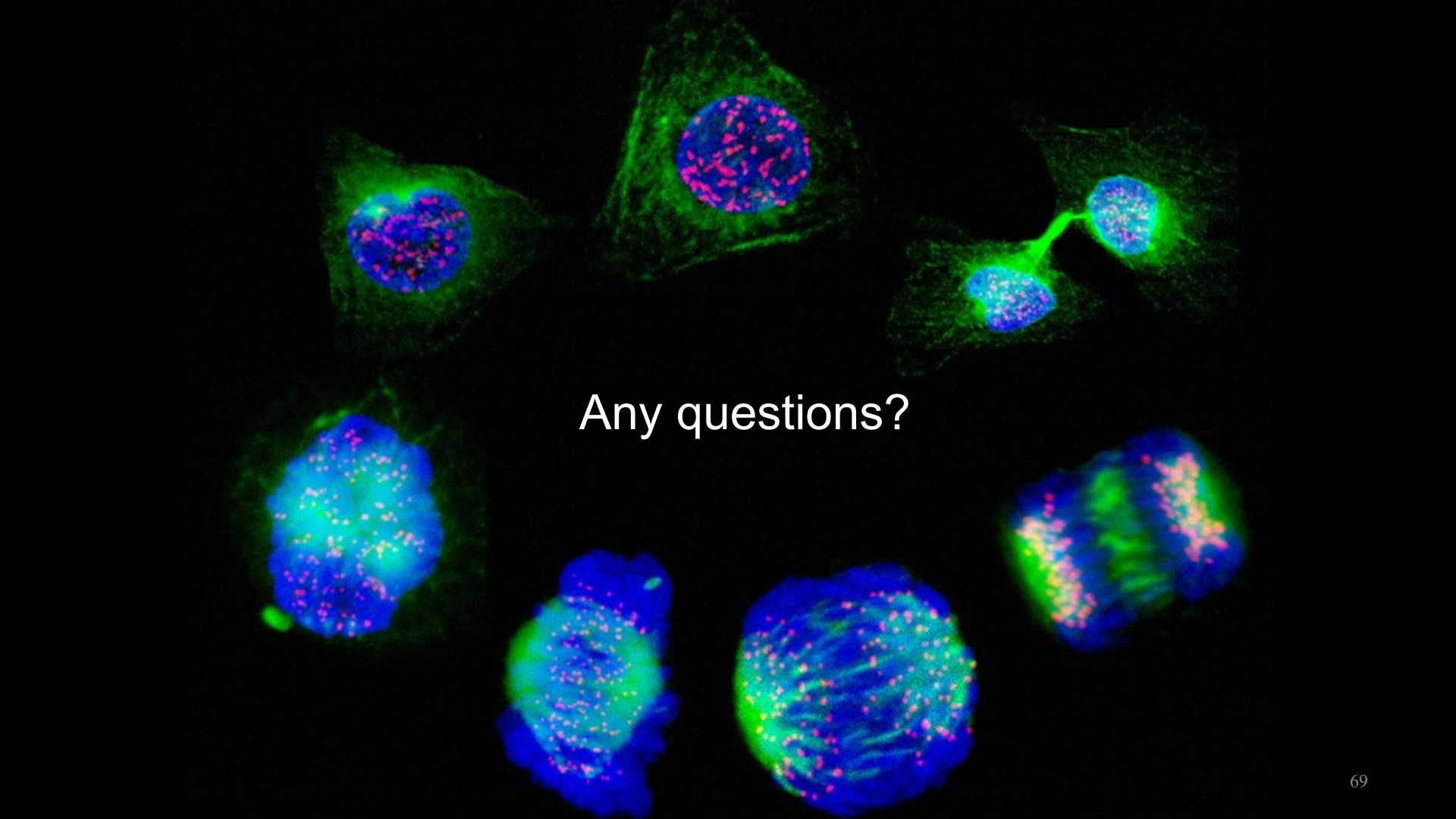
Books:
Online: http://www.freebookcentre.net/Biology/BioTechnology-Books.html

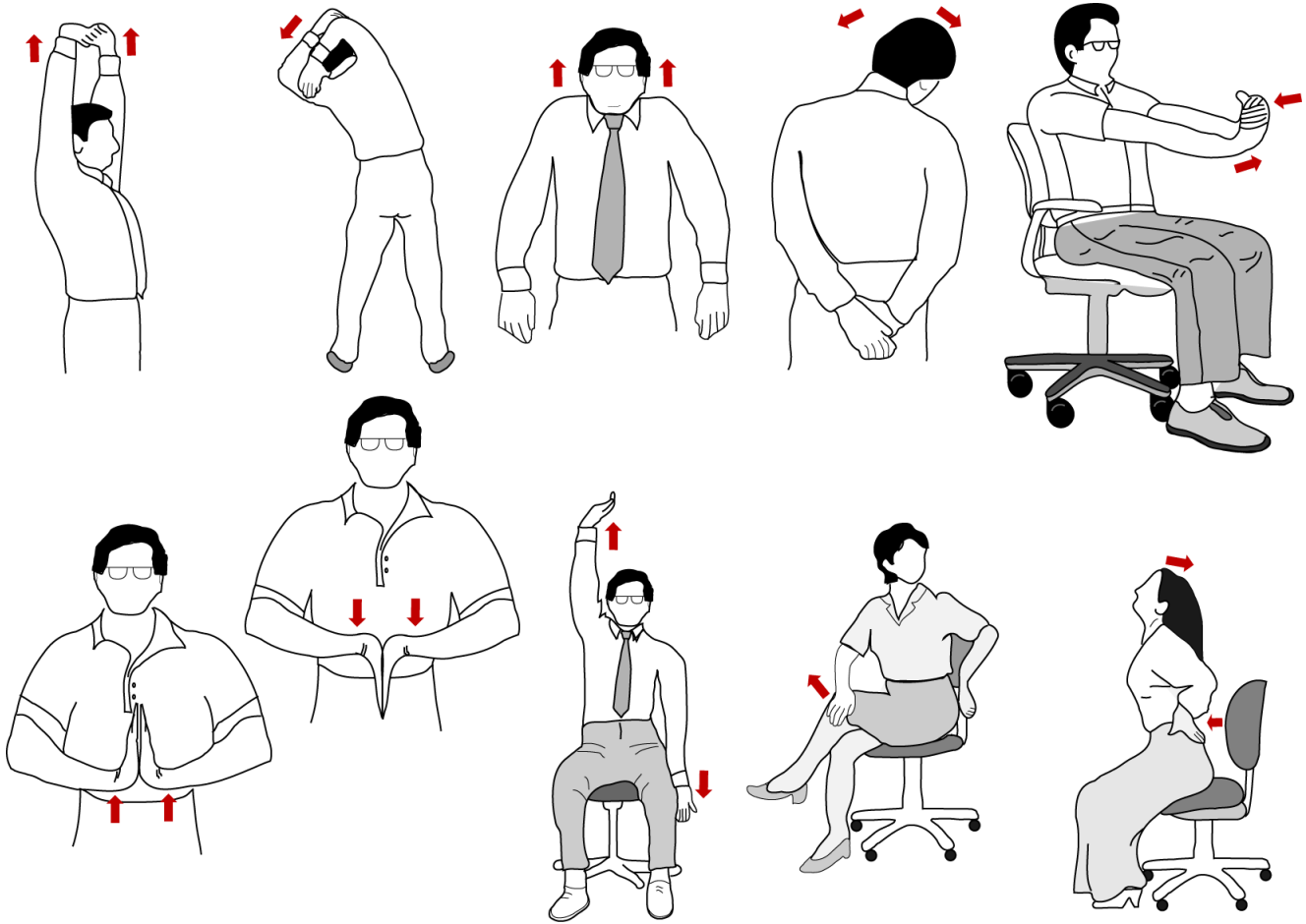









Any questions?



Exercises with Evie!