CHE 361
Bioprocess Engineering

Lecture 2: Gene Expression & Metabolic Regulation

* Some figures and schemes are adopted from *M.L. Shuler, F. Kargi, M. Delisa, Bioprocess Engineering. Basic Concepts, Prentice Hall, 2017*

Outline

- Biological Basics
  - ✓ Microbial Diversity
  - ✓ Cell Construction
  - ✓ Cell Nutrients
- Gene Expression
- Metabolic Regulation

Microbial Diversity

- Organisms that live in extreme environments (*extremophiles*) often provide us with important tools for processes to make useful chemicals and pharmaceutical products
- *Extremophiles* can exist and multiply in almost any environment on Earth, temperatures as low as -20 °C and as high as 120 °C, pH = 1 and pH > 9

Grand Prismatic Spring, Yellowstone Park

Microbial Diversity: Temperature and pH

*** With respect to temperature:**

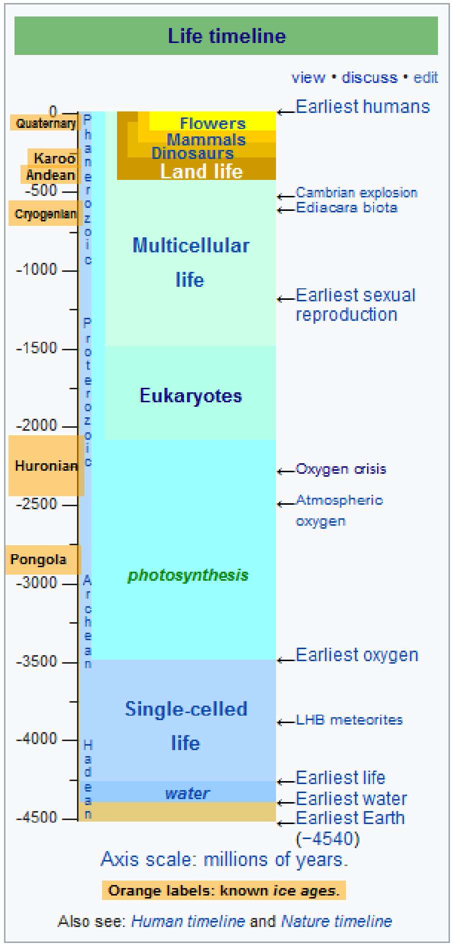
- ***Psychrophiles*** **(*cryophiles*)** are extremophilic organisms that are capable of growth and reproduction below 20 °C (down to -20 °C)
- ***Mesophiles*** have the optimum growth rate in the temperature range of 20-50 °C
- ***Thermophiles*** are extremophilic organisms that grow best at temperatures above 50 °C (up to 120 °C)

**** With respect to pH:**

- ***Acidophiles*** prefer pH values down to pH = 1-2
- ***Neutrophiles*** (most of organisms) grow best around neutral pH (pH = 6-8)
- ***Alkaliphiles*** grow best at pH > 9

Microbial Diversity (Cont'd)

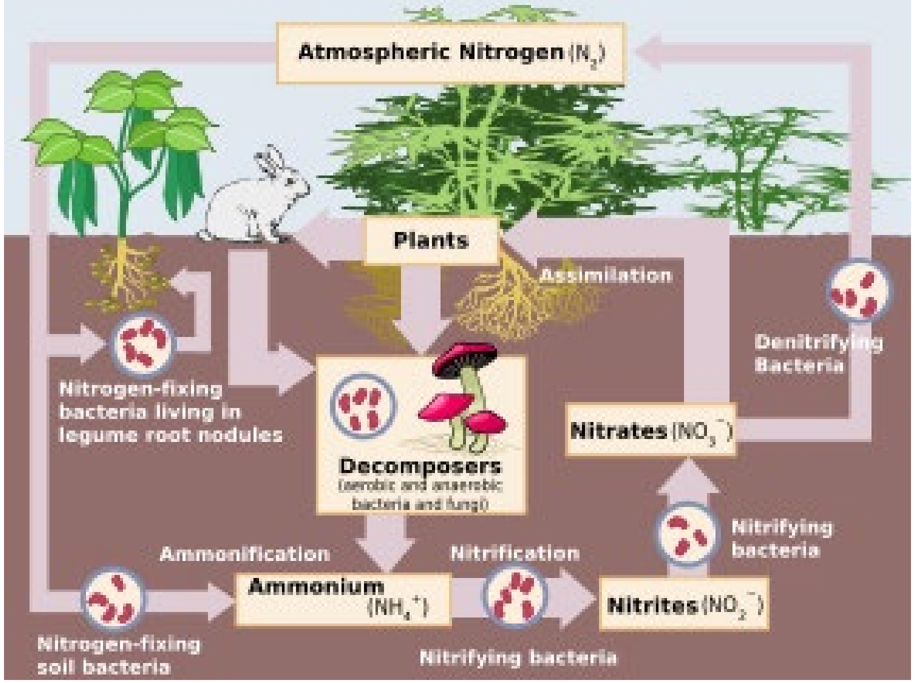
- ***Aerobic*** microorganisms require oxygen for growth and metabolism
- ***Anaerobic*** microorganisms does not require oxygen and their growth is inhibited in the presence of oxygen
- ***Halophiles*** grow in solutions with high salt concentration (at least 0.2M is required for growth) or on barely moist solid surfaces



https://en.wikipedia.org/wiki/Timeline_of_the_evolutionary_history_of_life

Microbial Diversity (Cont'd)

- ***Phototrophs*** convert CO_2 and H_2O into organic compounds using sunlight
- ***Diazotrophs*** convert N_2 into ammonia (NH_3) by an enzyme called a nitrogenase

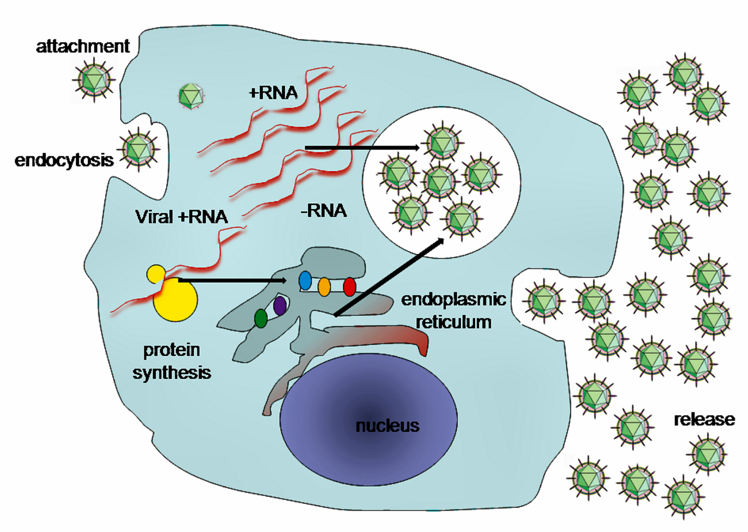


https://en.wikipedia.org/wiki/Nitrogen_fixation#Biological_nitrogen_fixation

Viruses

*** Viruses are small (typically 30-200 nm) infectious agents that can only replicate inside living cells. Viruses**

- ✓ cannot capture or store free energy
- ✓ are not functionally active outside host cells
- ✓ can infect all types of life forms ranging from microorganisms, including bacteria and archaea, to plants and animals



Life Properties Controversy:
Are viruses alive?

Viruses

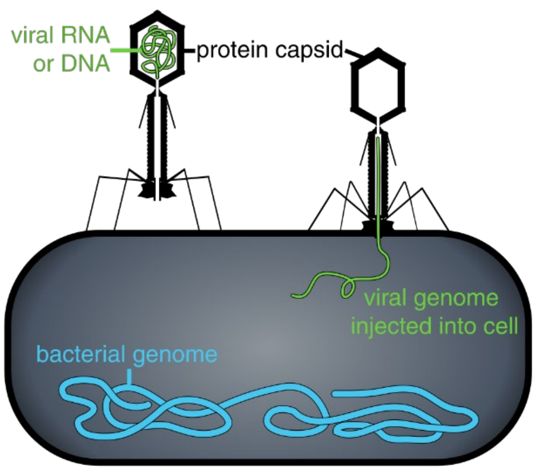
- ✓ have genes
- ✓ evolve by natural selection
- ✓ reproduce by self-replication (and self-assembly)

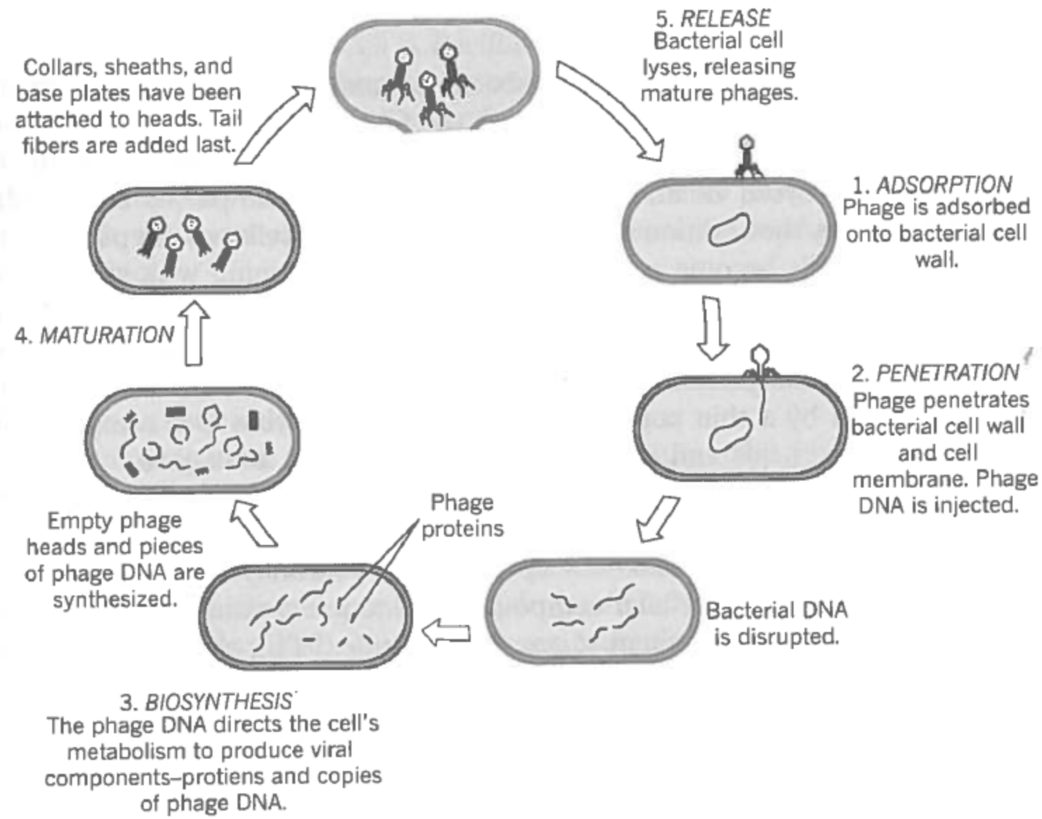
Viruses

- ➢ do not have a cellular structure
- ➢ do not have their own metabolism
- ➢ spontaneously assemble within cells (no cell division)

Bacteriophages

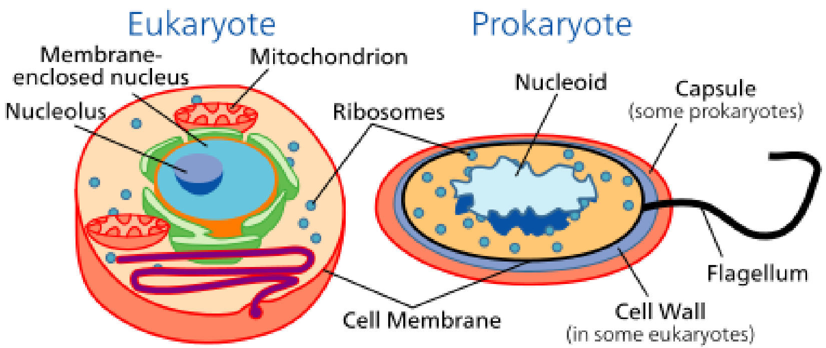
* ***Bacteriophages* can be used as agents to move desired genetic material into a bacterial cell**

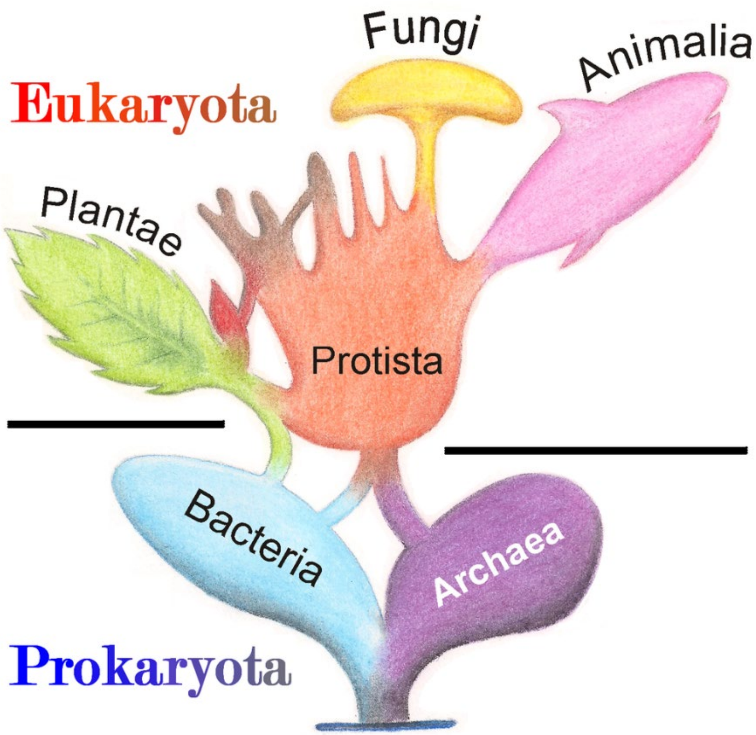




Prokaryotes

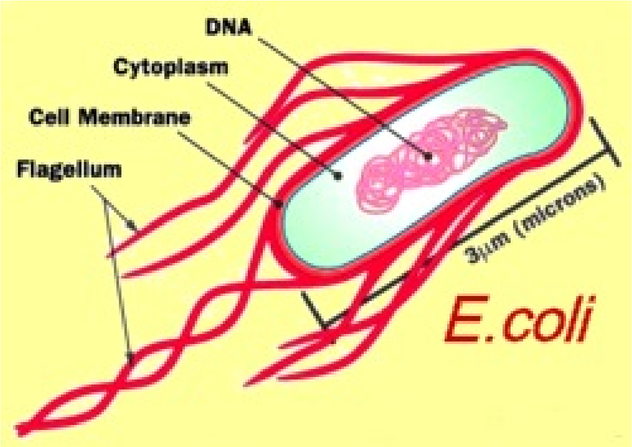
* ***Prokaryotes*** **are unicellular organisms (typically sized 0.5-3 μm) that lack a membrane-bound nucleus, mitochondria, or any other membrane-bound organelle**

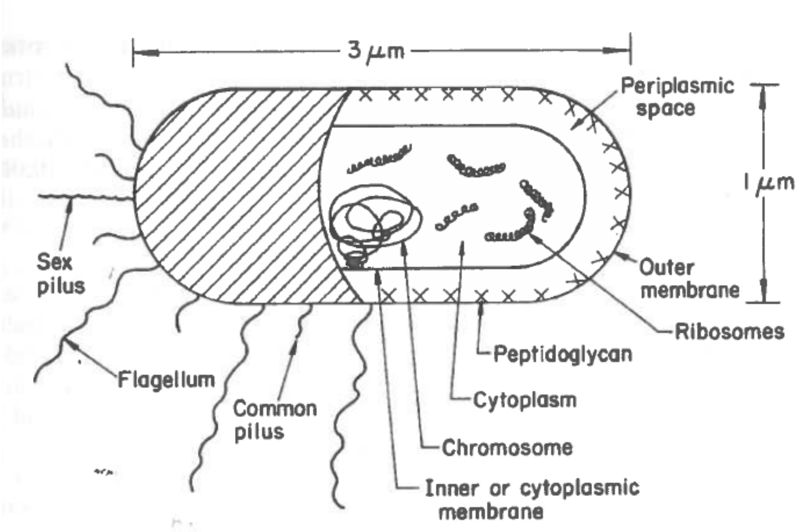




Prokaryotes (Cont'd)

- ✓ Prokaryotes can utilize a variety of nutrients as carbon source, including CO_2, hydrocarbons, carbohydrates, and proteins
- ✓ Prokaryotic cells grow rapidly, with typical doubling times of 30 minutes to several hours

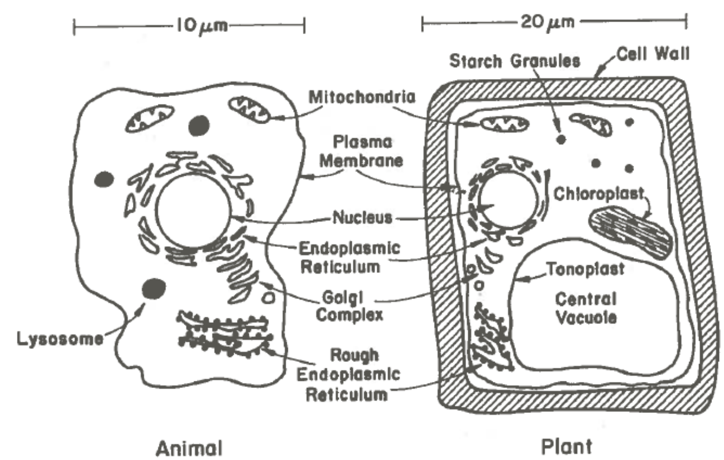




Eukaryotes

*** *Eukaryotes* are organism whose cells have a nucleus and other organelles enclosed within membranes**

- ✓ Eukaryotes have a true nucleus and a number of cellular organelles inside the cytoplasm
- ✓ Eukaryotic cells are five to ten times larger than prokaryotic cells, typically ranging from 5-20 μm in size



- ✓ Eukaryotic organisms include fungi (yeasts and molds), algae, protozoa, animals, and plants

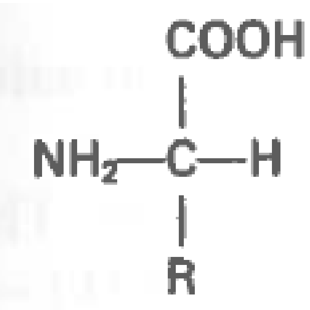
Cell Construction

- **Living cells are composed of high molecular weight polymeric compounds: nucleic acids, proteins, polysaccharides, lipids, and other storage materials (fats, glycogen, polyhydroxybutyrate)**
- **In addition to biopolymers, cells contain metabolites in the form of inorganic salts (e.g., NH_4^+, PO_4^{3-}, SO_4^{2-}, K^+, Ca^{2+}, Na^+), metabolic intermediates (e.g., pyruvate, acetate), and vitamins**
  - ✓ A typical bacterial cell is 50% carbon, 20% oxygen, 14% nitrogen, 8% hydrogen, 3% phosphorus, and 1% sulfur, with small amounts of K^+, Na^+, Ca^{2+}, Mg^{2+}, Cl^-, and vitamins

*** Living cell can be visualized as a very complex reactor in which more than 2000 interrelated and controlled reactions take place**

Amino Acids and Proteins

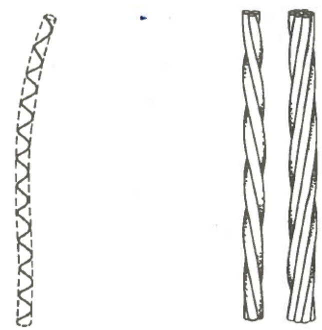
- **Proteins constitute 40-70% of a cell dry weight**
- **Proteins responsible for diverse biological functions:**
  - ✓ Structural (e.g., collagen, elastin, keratin)
  - ✓ **Catalytic** (e.g., amylase, catalase, lysozyme, pepsin)
  - ✓ Transport (e.g., hemoglobin, serum albumin, aquaporin)
  - ✓ Regulatory (e.g., transcription factors, growth hormones)
  - ✓ Protective (e.g., antibodies, thrombin)



- **While the *primary* protein's structure is determined by the sequence of amino acids, the *secondary* and *tertiary* structures are determined by weak interactions between the various side groups**

The 20 Encoded Amino Acids

* There are 22 amino acids that can be naturally incorporated into polypeptides: 20 encoded by the Universal Genetic Code; *selenocystein* and *pyrrolysine* are derivatives of *cysteine* and *lysine*



α-Helical coil

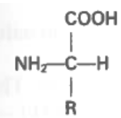
Supercoiling of α-helical coils to form ropes

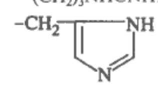
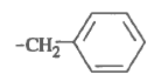
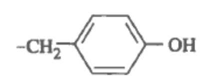
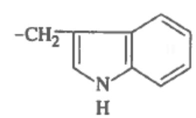
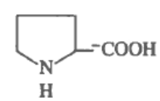
Fibrous Proteins

The tertiary structure of a single-chain globular protein

The quaternary structure of a multichain or oligomeric globular protein

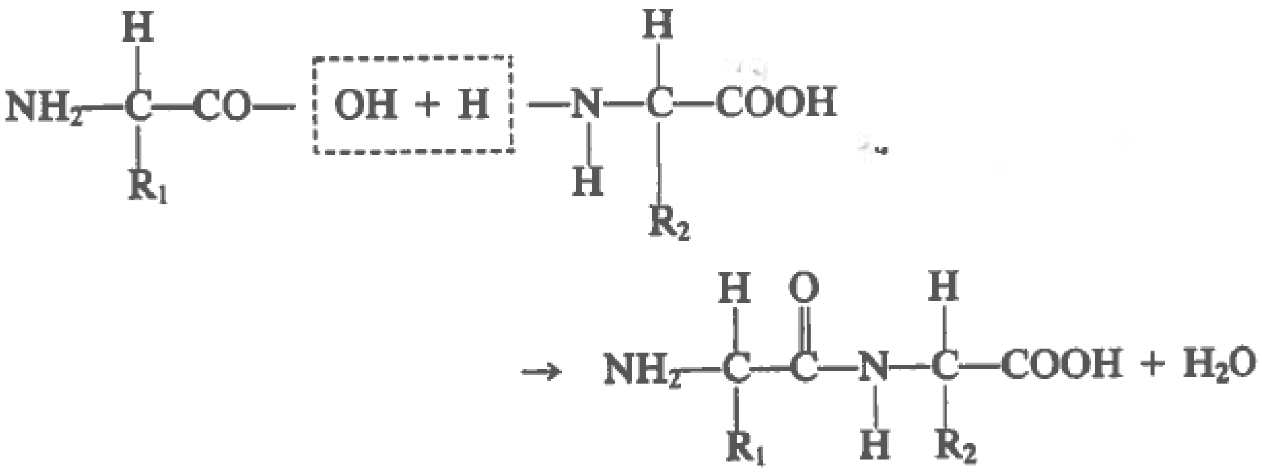
Globular Proteins



R Group	Name	Abbreviation	Symbol	Class
—H	Glycine	GLY	G	Aliphatic
$—CH_3$	Alanine	ALA	A	
$—CH(CH_3)_2$	Valine	VAL	V	
$—CH_2CH(CH_3)_2$	Leucine	LEU	L	
$—CHCH_3CH_2CH_3$	Isoleucine	ILU	I	
$—CH_2OH$	Serine	SER	S	Hydroxyl or sulfur containing
$—CHOHCH_3$	Threonine	THR	T	
$—CH_2SH$	Cysteine	CYS	C	
$—(CH_2)_2SCH_3$	Methionine	MET	M	
$—CH_2COOH$	Aspartic acid	ASP	D	Acids and corresponding amides
$—CH_2CONH_2$	Asparagine	ASN	N	
$—(CH_2)_2COOH$	Glutamic acid	GLU	E	
$—(CH_2)_2CONH_2$	Glutamine	GLN	Q	
$—(CH_2)_3CH_2NH_2$	Lysine	LYS	K	Basic
$—(CH_2)_3NHCNHNH_2$	Arginine	ARG	R	
	Histidine	HIS	H	
	Phenylalanine	PHE	F	Aromatic
	Tyrosine	TYR	Y	
	Tryptophan	TRP	W	
	Proline	PRO	P	Amino acid

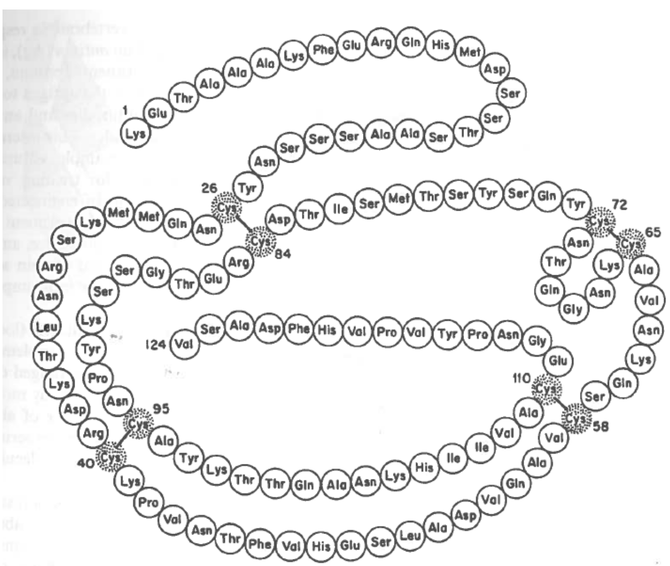
The Peptide Bond

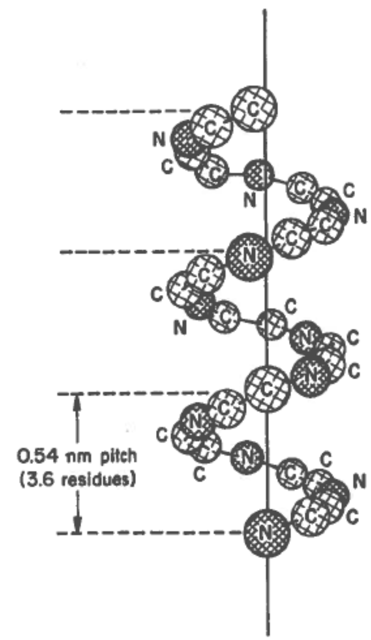
* **Proteins are linear chains of *covalently* linked amino acids. The condensation reaction between two amino acids results in the formation of a *peptide* bond**



The 3D Protein Structure

- The *Primary Structure* is a linear sequence of amino acids; each protein has a unique sequence that determine the 3D structure
- The *Secondary Structure* is a result of hydrogen bonding between adjacent residues: two major types are *helixes* and *sheets*



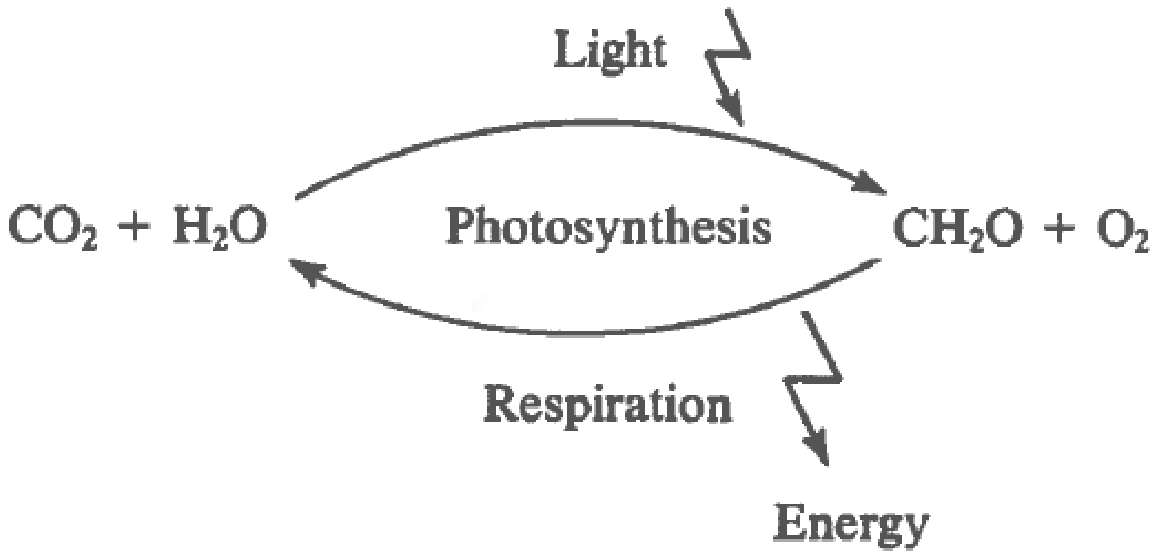


The 3D Protein Structure (Cont'd)

- The *Tertiary Structure* is a result of interactions between R groups widely separated along the chain; R groups may interact by covalent, disulfide, or hydrogen bonds
- The *Quaternary Structure* can only be formed for proteins with more than one polypeptide chain; interactions among these chains results in a quaternary structure, e.g., hemoglobin has four subunits

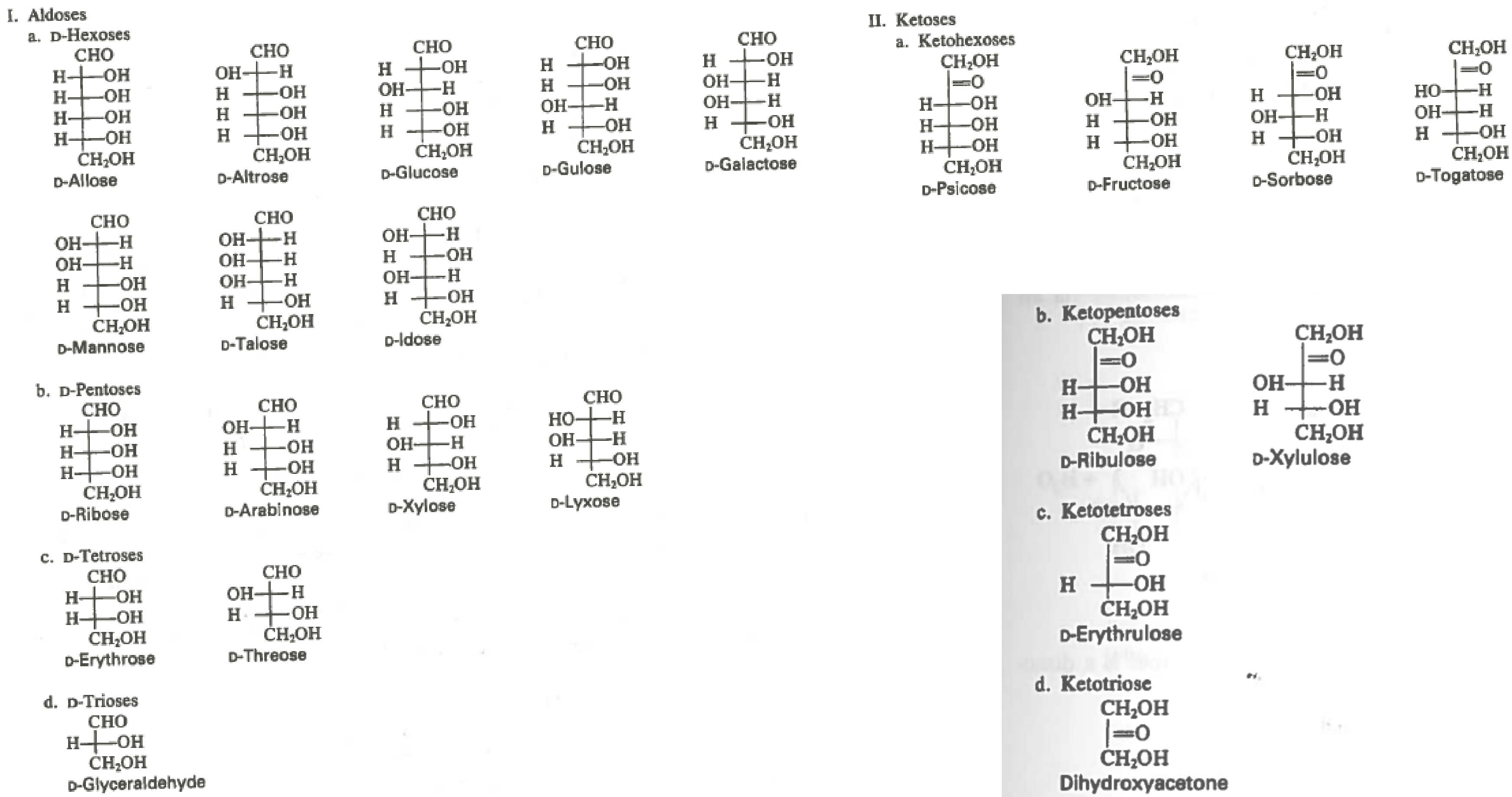
Carbohydrates

- ***Carbohydrates*** **(e.g., cellulose and starch) play key roles in *structural* and *storage* functions and also appear to play critical roles in modulating chemical signaling; the general formula is $(CH_2O)_n$ ($n \geq 3$)**



Mono-saccharides

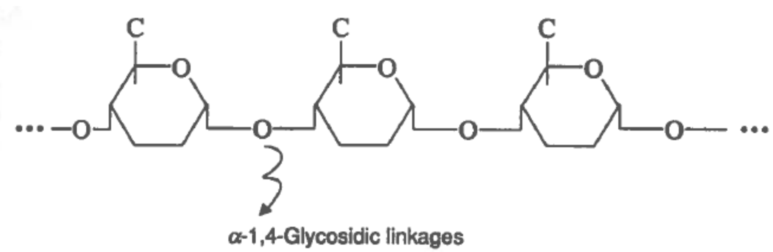
- *Mono-saccharides* are the smallest carbohydrates that contain three to nine carbon atoms

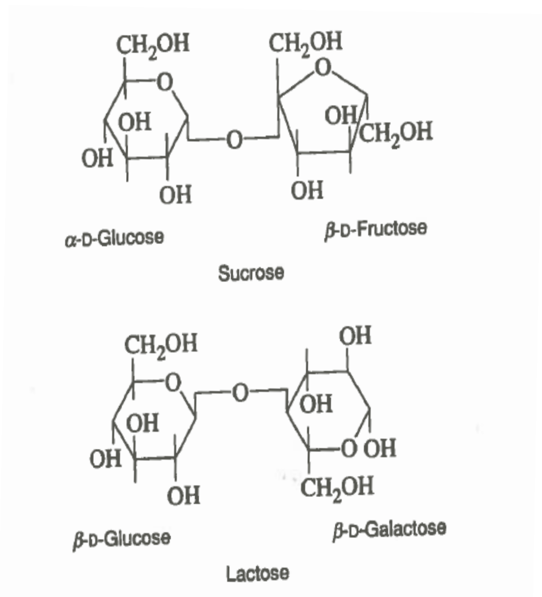


Poly-saccharides

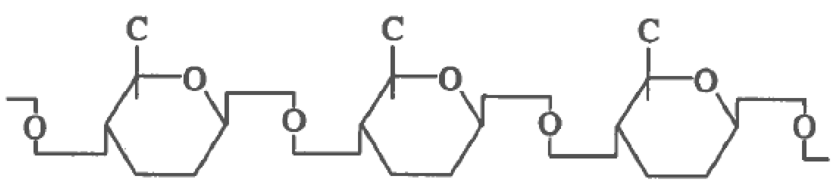
- ***Poly-saccharides*** **are formed by the condensation of more than two monosaccharides by glycosidic bonds in a linear or branched configuration**

Amylose is a straight chain of glucose molecules (20 %of starch):





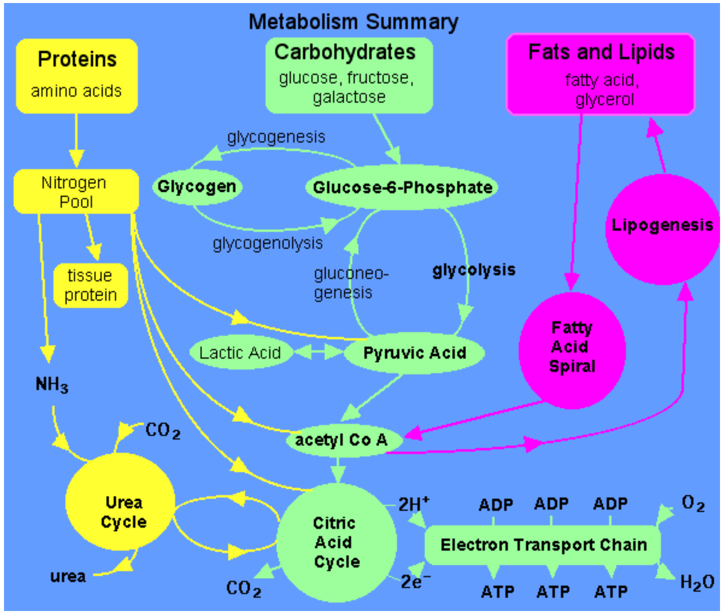
Cellulose is a long, unbranched chain of D-glucose with a MW ranging from 50000 – 1000000 Daltons:

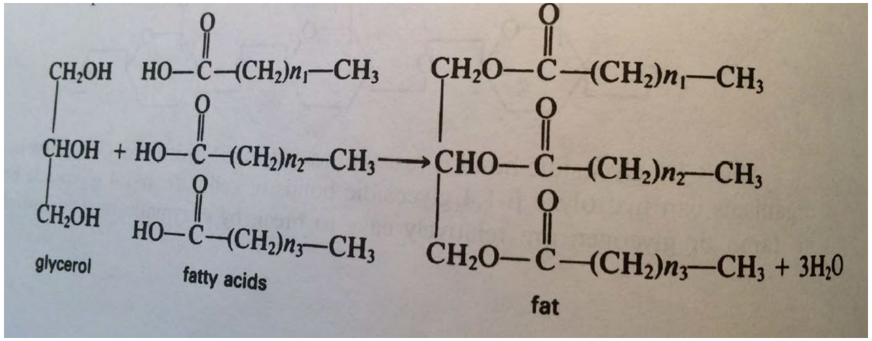


Lipids and Fats

- *Lipids* are water-insoluble biological compounds (plasma membranes and storage)
- *Lipoproteins* and *lipopolysaccharides* are types of lipids

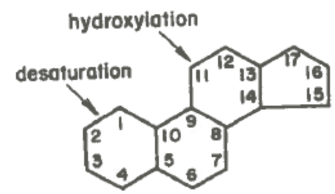
*** The formation of a fat molecule (lipogenesis):**

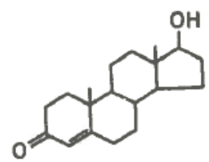




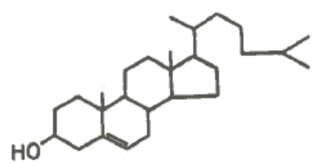
Steroids

- ***Steroids*** **(can be classified as lipids) are important regulators (hormones) of animal development and metabolism**

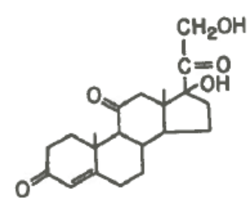




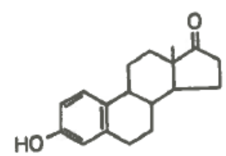
testosterone (a male sex hormone)



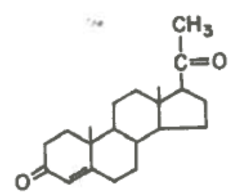
cholesterol (precursor for many steroids including testosterone and cortisone)



cortisone (an adrenocortical hormone)



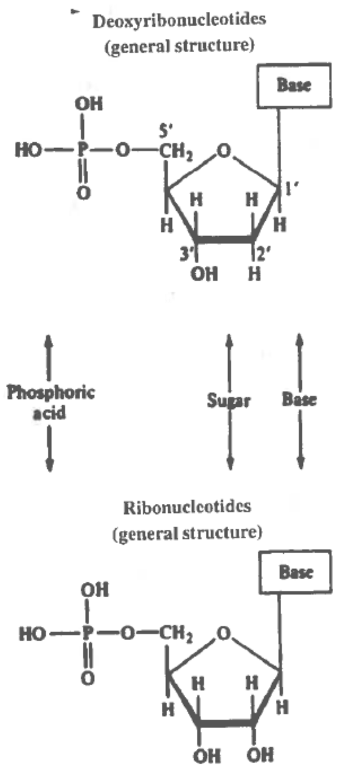
estrone (a female sex hormone)

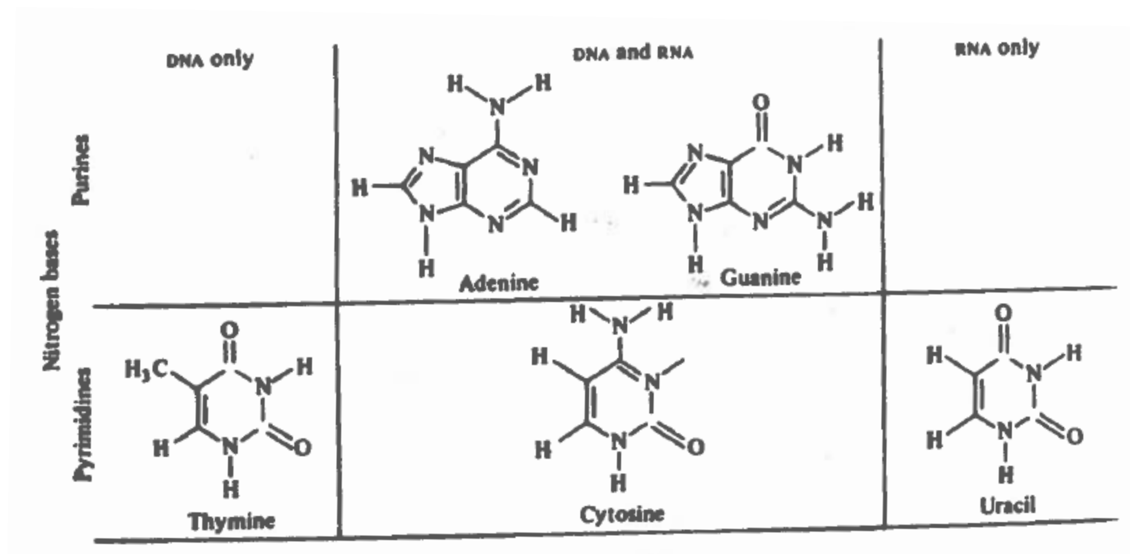


progesterone

RNA and DNA

- ***Ribonucleic Acid*** **(RNA) and** ***Deoxyribonucleic Acid*** **(DNA) are large polymers made of nucleotides; DNA codes genetic information, RNA plays a central role in protein synthesis**

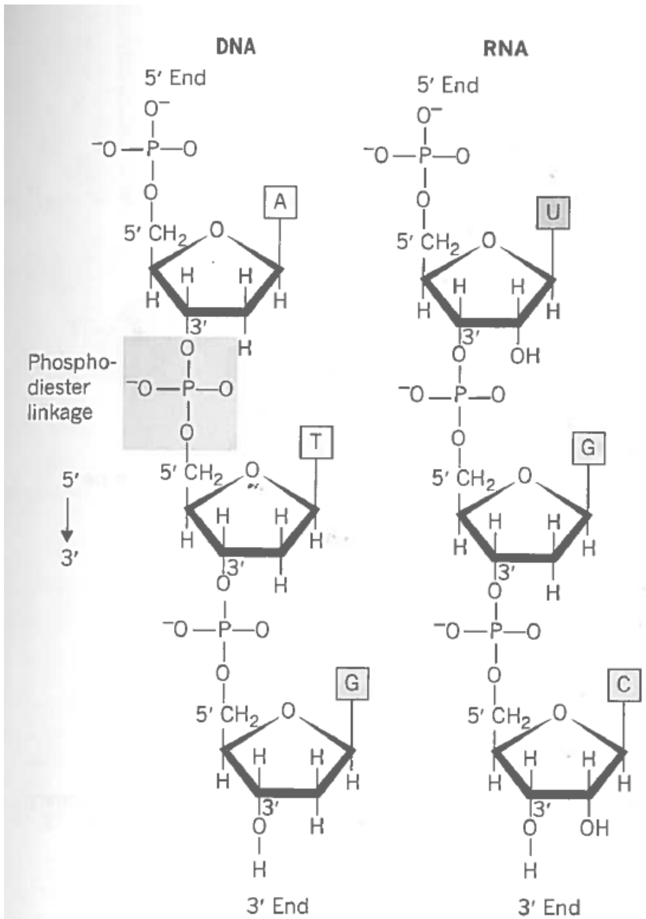


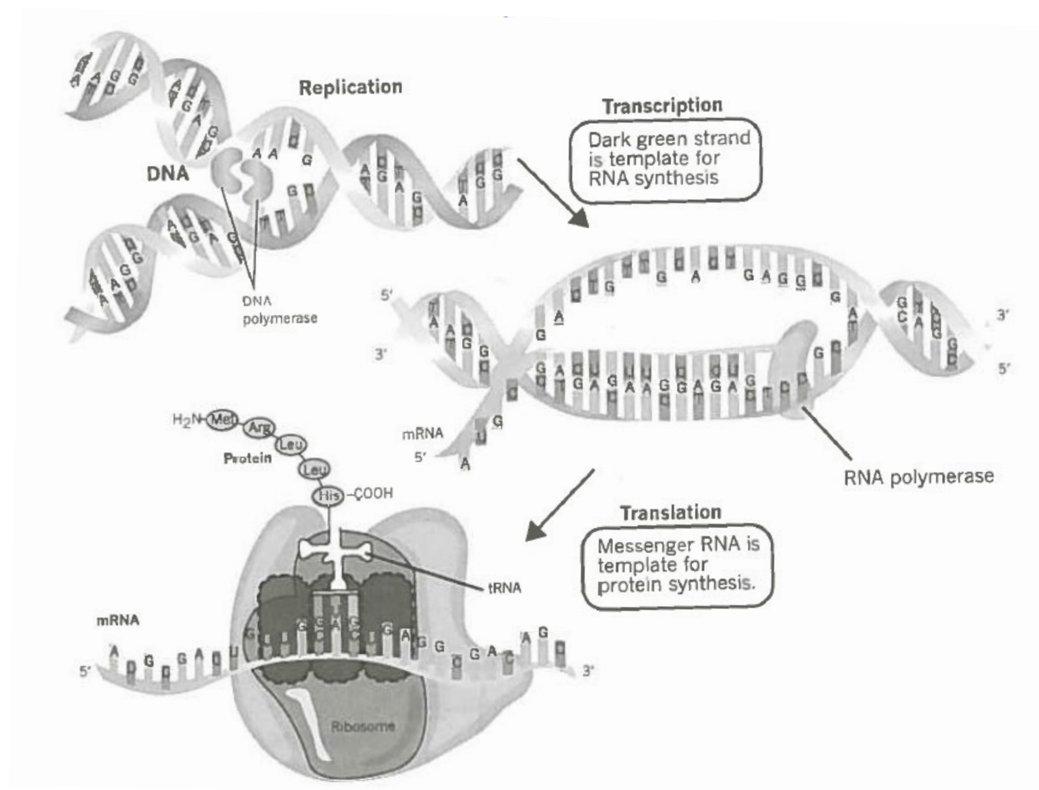


(b)

Structure of DNA and RNA

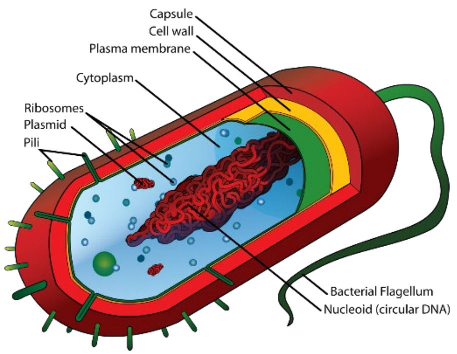
- **Nucleotides are linked together by phosphodiester bonds**

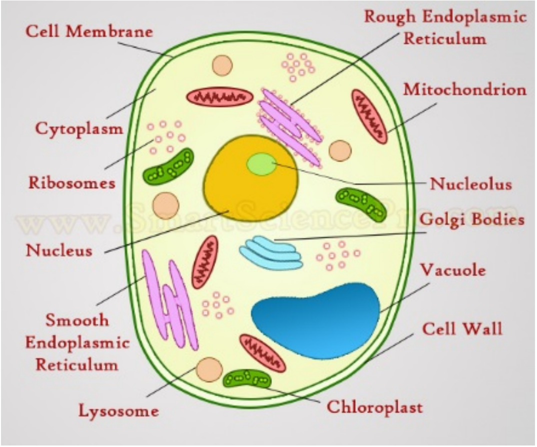


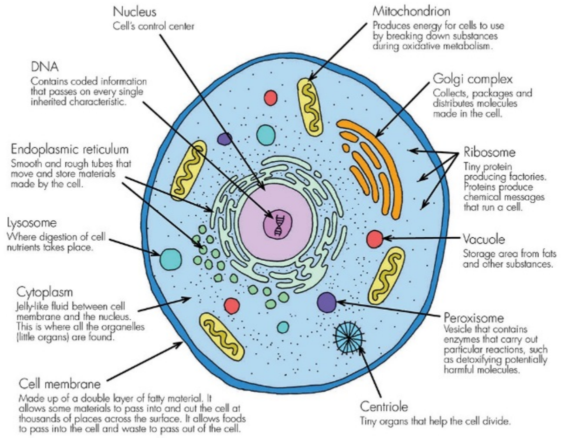


Cell Nutrients

- A living cell exists far from the thermodynamic equilibrium
- Thermodynamic equilibrium is equivalent to death for a cell
- A cell must selectively remove desirable compounds from the extracellular environment and retain other compounds intracellularly
- The selectivity is achieved by a semipermeable membrane







Cell Nutrients (Cont'd)

*** Cell composition and typical culture population**

TABLE 2.7. Chemical Analyses, Dry Weights, and the Populations of Different Microorganisms Obtained in Culture

	Composition (% dry weight)			Typical Population in Culture (numbers/ml)	Typical Dry Weight of This Culture (g/100 ml)
Organism	Protein	Nucleic Acid	Lipid		
Viruses	50–90	5–50	<1	10^8–10^9	0.0005[a]
Bacteria	40–70	13–34	10–15	2×10^8–2×10^{11}	0.02–2.9
Filamentous fungi	10–25	1–3	2–7		3–5
Yeast	40–50	4–10	1–6	1–4×10^8	1–5
Small unicellular algae	10–60(50)	1–5(3)	4–80(10)	4–8×10^7	0.4–0.9

- ***Macronutrients*** **(carbon, nitrogen, oxygen, hydrogen, sulfur, phosphorus, magnesium, and potassium) are needed in concentrations larger than 10^{-4} M**
- ***Micronutrients*** **(Mo^{2+}, Zn^{2+}, Cu^{2+}, Mn^{2+}, Na^{+}, vitamins, hormones, and metabolic precursors) are needed in concentrations less than 10^{-4} M**

Macronutrients

- ***Carbon*** **compounds are major sources of cellular carbon and energy**
  - ✓ ***Autotrophs*** use CO_2 as a carbon source
    - ➢ ***Chemoautotrophs*** obtain energy from the oxidation of inorganic compounds
    - ➢ ***Photoautotrophs*** utilize light as an energy source
  - ✓ ***Heterotrophs*** use organic compounds such as carbohydrates, lipids, and hydrocarbons as a source of carbon
- ***Nitrogen*** **constitutes 10-14% of cell dry weight in the from of proteins and nucleic acids; nitrogen sources include ammonia, ammonium salts, proteins, peptides, and amino acids**

Macronutrients in Fermentation Industry

TABLE 2.8. Some Carbon and Nitrogen Sources Used by the Fermentation Industry

Carbon Sources	Nitrogen Sources
Starch waste (maize and potato)	Soya meal
Molasses (cane and beet)	Yeast extract
Whey	Distillers solubles
n-Alkanes	Cottonseed extract
Gas oil	Dried blood
Sulfite waste liquor	Corn steep liquor
Domestic sewage	Fish solubles and meal
Cellulose waste	Groundnut meal
Carbon bean	

Macronutrients

- ***Oxygen*** is present in all cellular components and cellular water constituting 15-20% of cell dry weight; molecular oxygen is required as electron acceptor in the aerobic metabolism
- ***Hydrogen*** constitutes about 8% of cell dry weight, primarily derived from carbon-based compounds (e.g., carbohydrates); some bacteria (e.g., methanogens) can utilize hydrogen as an energy source
- ***Phosphorus*** constitutes 2-3% of cell dry weight, mainly as nucleic acids, and in the cell walls of some bacteria
- ***Sulfur*** constitutes 0.5-1% of cell dry weight, mainly in proteins and some coenzymes
- ***Magnesium*** (usually supplied as $MgSO_4$ and $MgCl_2$) is a cofactor for some enzymes and is present in cell walls and membranes
- ***Potassium*** (supplied as K_2HPO_4, KH_2PO_4, and K_3PO_4) is a cofactor for certain enzymes and is required for carbohydrates metabolism

Macronutrients Summary

Element	Physiological Function	Required Concentration (mol/l)
Carbon	Constituent of organic cellular material. Often the energy source	$>10^{-2}$
Nitrogen	Constituent of proteins, nucleic acids, and coenzymes	10^{-3}
Hydrogen	Organic cellular material and water	—
Oxygen	Organic cellular material and water. Required for aerobic respiration	—
Magnesium	Cofactor for many enzymes and chlorophylls (photosynthetic microbes) and present in cell walls and membranes	10^{-4} to 10^{-3}
Phosphorus	Constituent of nucleic acids, phospholipids, nucleotides, and certain coenzymes	10^{-4} to 10^{-3}
Potassium	Principal inorganic cation in the cell and cofactor for some enzymes	10^{-4} to 10^{-3}
Sulfur	Constituent of proteins and certain coenzymes	10^{-4}

Micronutrients

- **Micronutrients are essential to microbial nutrition**
- **Lack of micronutrients increases the lag phase**

- ***Commonly* added:** Fe, Zn, and Mn are important enzymatic cofactors
- ***Sometimes* added:** Cu, Co, Mo, Ca, Na, Cl, Ni, and Se are required as cofactors for certain enzymes
- ***Rarely* added:** B, Al, Si, Cr, V, Sn, Be, F, Ti, Ga, Ge, Br, Zr, W, Li, and I are required in concentrations less than 10^{-6} M (toxic otherwise)

- ***Growth factors*** (vitamins, hormones, and amino acids) stimulate the growth and synthesis of some metabolites

Growth Media

TABLE 2.10. Compositions of Typical Defined and Complex Media

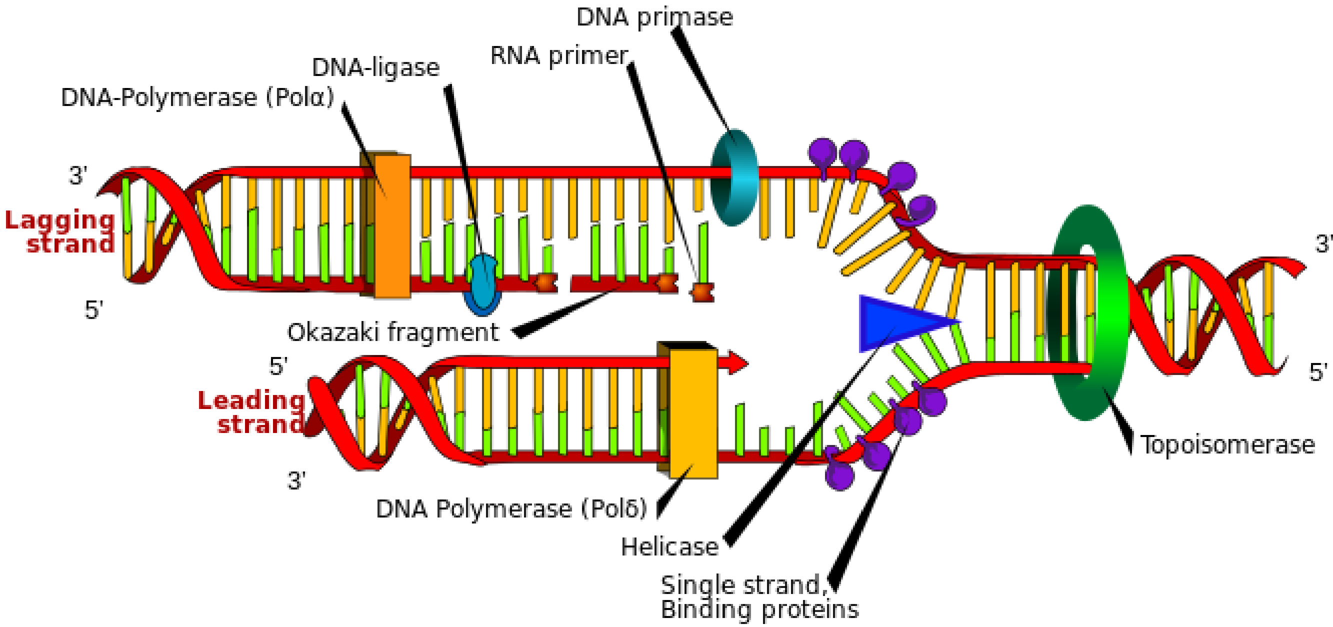
Defined Medium		
Constituent	Purpose	Concn (g/l)
Group A		
Glucose	C, energy	30
KH_2PO_4	K, P	1.5
$MgSO_4 \cdot 7H_2O$	Mg, S	0.6
$CaCl_2$	Ca	0.05
$Fe_2(SO_4)_3$	Fe	15×10^{-4}
$ZnSO_4 \cdot 7H_2O$	Zn	6×10^{-4}
$CuSO_4 \cdot 5H_2O$	Cu	6×10^{-4}
$MnSO_4 \cdot H_2O$	Mn	6×10^{-4}
Group B		
$(NH_4)_2HPO_4$	N	6
$(NH_4)H_2PO_4$	N	5
Group C		
$C_6H_5Na_3O_7 \cdot 2H_2O$	Chelator	4
Group D		
Na_2HPO_4	Buffer	20
KH_2PO_4	Buffer	10

Complex Medium Used in a Penicillin Fermentation	
Glucose or molasses (by continuous feed)	10% of total
Corn steep liquor	1–5% of total
Phenylacetic acid (by continuous feed)	0.5–0.8% of total
Lard oil (or vegetable oil) antifoam by continuous addition	0.5% of total
pH to 6.5 to 7.5 by acid or alkali addition	

Gene Expression: DNA Replication

- **Preserving and Propagating the Message**

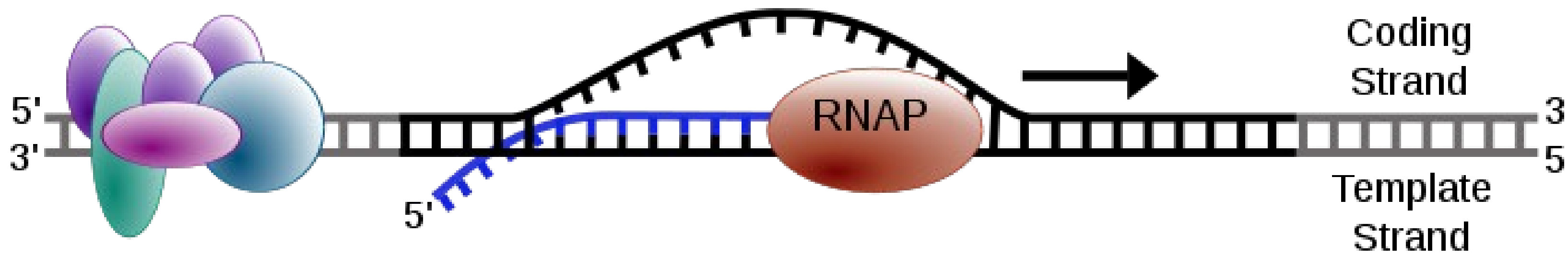
DNA replication is the biological process of producing one identical replicas of DNA from one original DNA molecule. This process is the basis for *biological inheritance*.



Gene Expression: Transcription

- **Sending the Message**

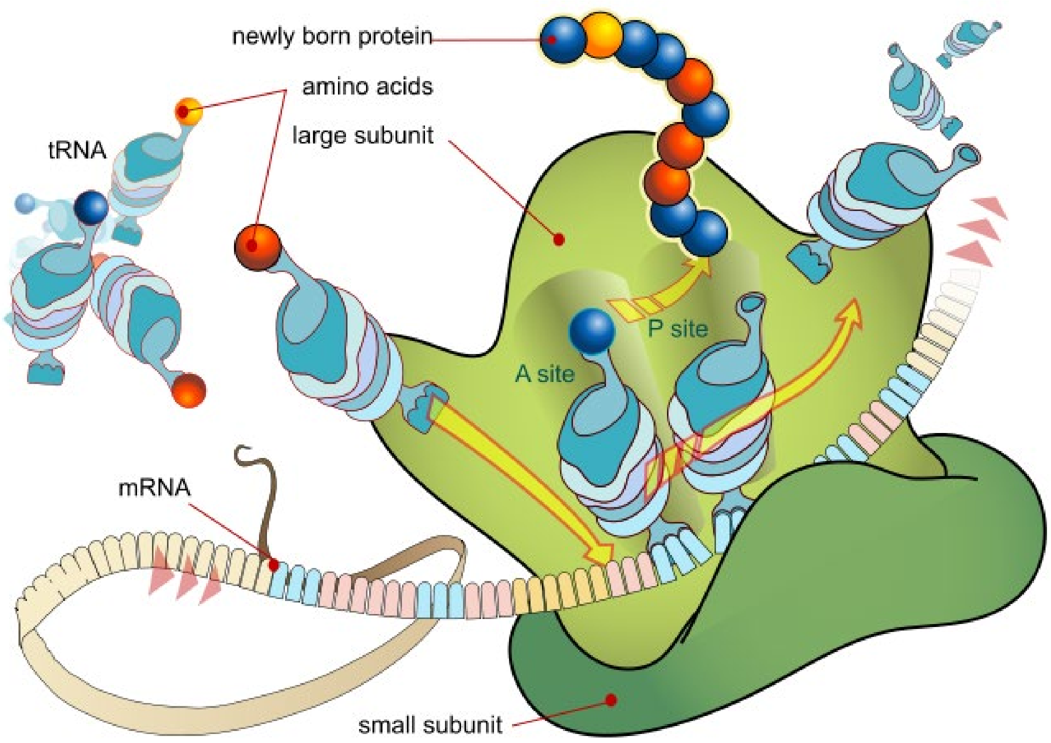
Transcription is the first step of gene expression, in which a particular segment of DNA is copied into RNA (especially mRNA) by the enzyme ***RNA polymerase***. During transcription, a DNA sequence is read by an RNA polymerase, which produces a complementary, antiparallel RNA strand called a primary transcript.



Gene Expression: Translation

- **Going from Message to Product**

Translation is the process in which *ribosomes* in a cell's cytoplasm create proteins, following transcription of DNA to RNA in the cell's nucleus



Gene Expression: The Genetic Code

- The genetic code is a *blueprint* of any living cell; more than one codon can specify a particular amino acid

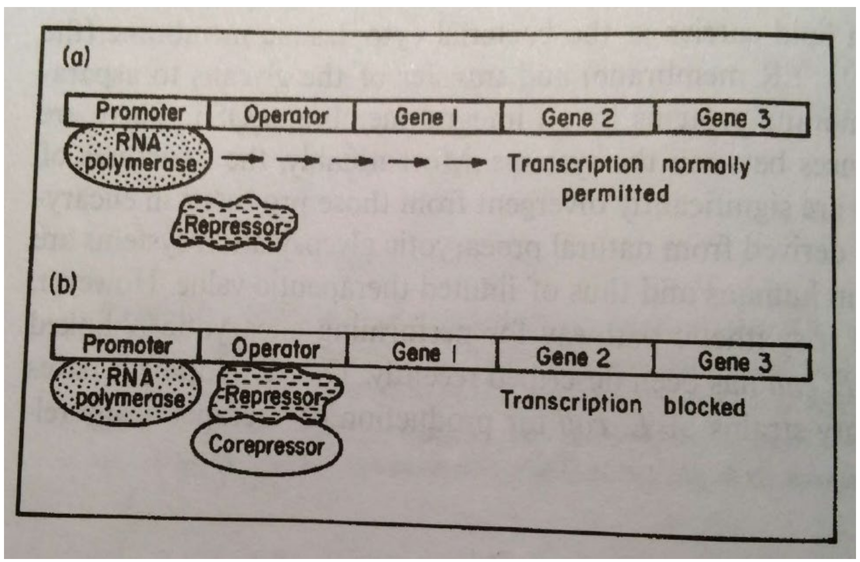
	Second Bases							
First Base	U		C		A		G	
U	UUU	phe[a]	UCU	ser	UAU	tyr	UGU	cys
	UUC	phe	UCC	ser	UAC	tyr	UGC	cys
	UUA	leu	UCA	ser	UAA	(none)[b]	UGA	(none)[b]
	UUG	leu	UCG	ser	UAG	(none)[b]	UGG	try
C	CUU	leu	CCU	pro	CAU	his	CGU	arg
	CUC	leu	CCC	pro	CAC	his	CGC	arg
	CUA	leu	CCA	pro	CAA	glu-N	CGA	arg
	CUG	leu	CCG	pro	CAG	glu-N	CGG	arg
A	AUU	ileu	ACU	thr	AAU	asp-N	AGU	ser
	AUC	ileu	ACC	thr	AAC	asp-N	AGC	ser
	AUA	ileu	ACA	thr	AAA	lys	AGA	arg
	AUG	met	ACG	thr	AAG	lys	AGG	arg
G	GUU	val	GCU	ala	GAU	asp	GGU	gly
	GUC	val	GCC	ala	GAC	asp	GGC	gly
	GUA	val	GCA	ala	GAA	glu	GGA	gly
	GUG	val	GCG	ala	GAG	glu	GGG	gly

Metabolic Regulation

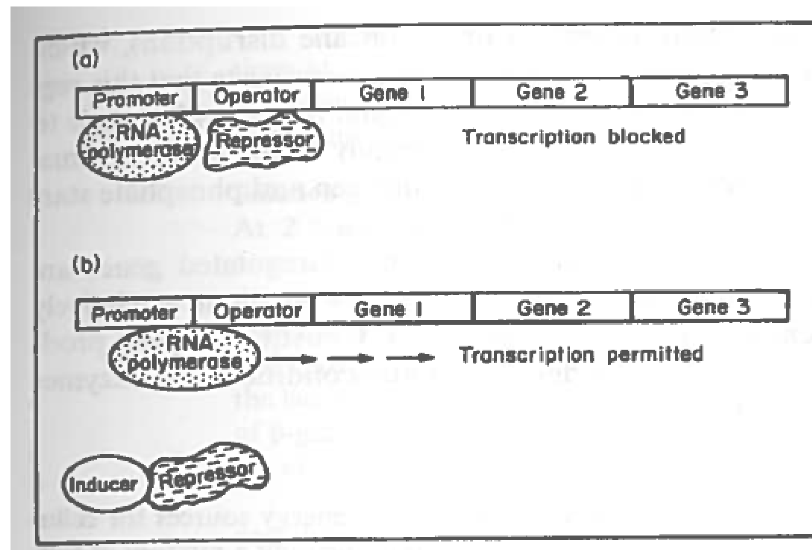
* Metabolic regulation takes place at the genetic level (via gene expression control) and at the cellular level (control of enzyme activity through cell surface receptors)

* Genetic-Level Control

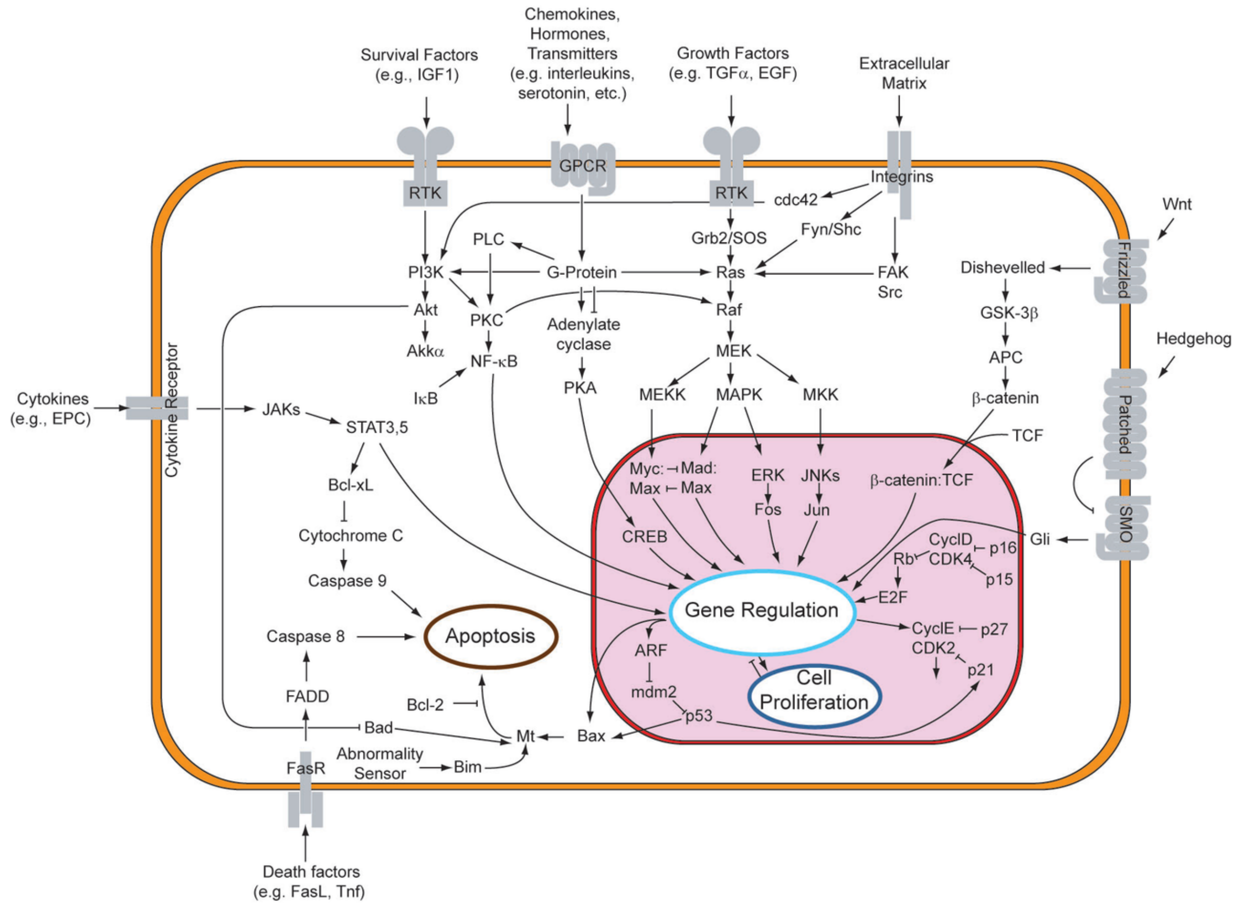
Enzyme Repression



Enzyme Induction

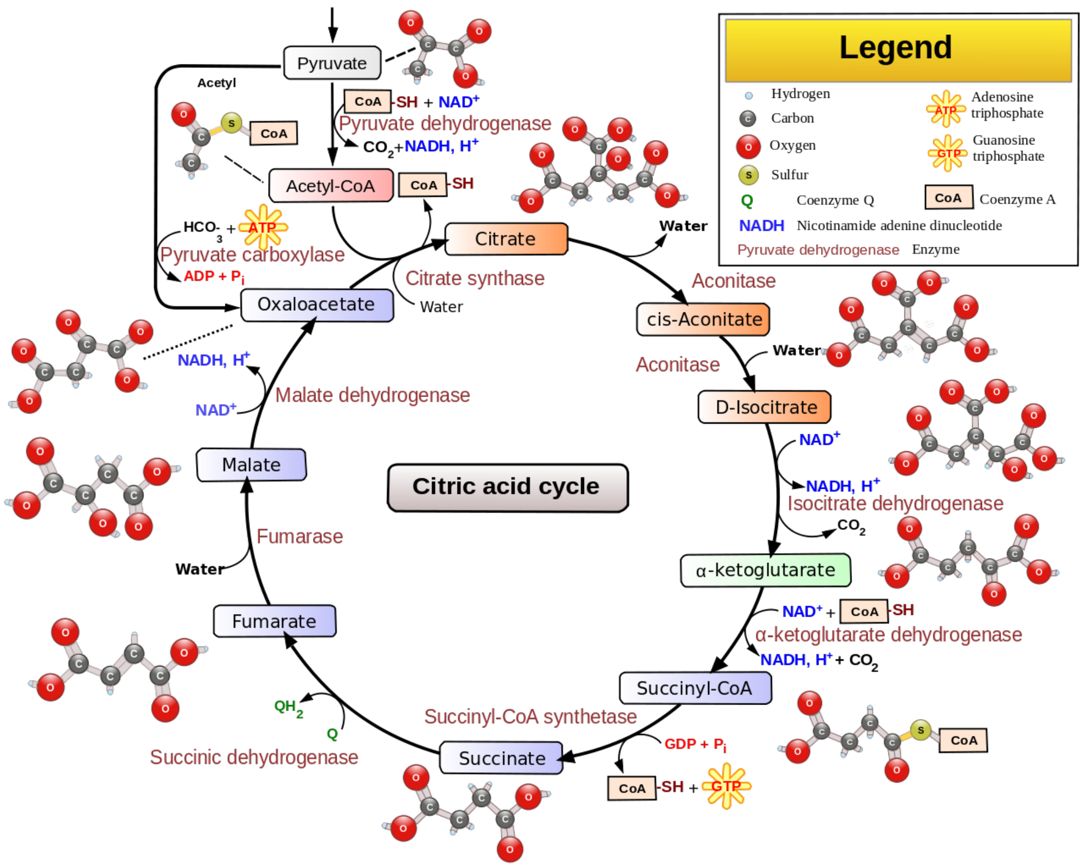


Metabolic Regulation: Cell Signaling



Example of Metabolic Pathway

Krebs Cycle (conversion of pyruvate to CO_2 and NADH)



Summary

- Microbes can grow over a wide range of environmental conditions
- All cells contain proteins, RNA, DNA, carbohydrates, and lipids
- Proper nutrients are essential for microbial culture growth
- Metabolism can be regulated by gene expression and intercellular signaling