BIOC 2580 - Lecture notes All

Introduction to Biochemistry (University of Guelph)

BIOCHEMISTRY

W 17

Dawson, Wijekoon, Graether…

lipids • structurally diverse, defined by common chemical property: hydrophobicity
- not defined by chemical structure (like amnio acids)
- use organic solvents (non-polar) to dissolve lipids

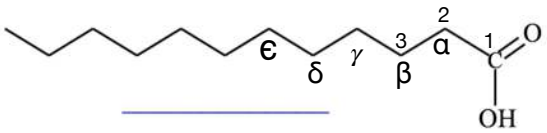
	Function	Example
Our focus	Energy storage	triacylglycerols - hydrophobic storage of energy good because elements do not dissolve in water, thereby do not increase osmotic pressure
	Structural elements of biological membranes	phospholipids and sterols
More 'active' functions	Signal transduction (cell-cell communication)	steroid hormones, prostoglandins
	Enzyme cofactors	Coenzyme Q: mitochondrial ETC
	Vitamins	A, D, E, K
	Light-absorbing pigments	carotene

Lipids can covalently bond to other classes of biomolecules:
glycolipids • carbohydrate + lipid, in a cell membrane, eg. blood antigens
lipoproteins • protein + lipid

Focus: **FATTY ACIDS, TRIACYLGLYCEROLS, PHOSPHOGLYCERIDES**

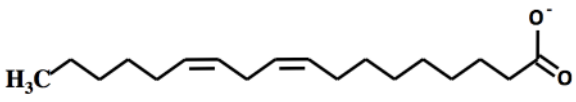
Fatty Acids • always found as part of a larger molecule, intermediate when energy storage is metabolized, free fatty acids present in *trace* quantities

- 4-36 C with carboxylic acid (the reactive functional group), even number of C
- saturated, monounsaturated, polyunsaturated fatty acids
- carboxyl C is always #1
- α C is always #2 (because it is attached to the carboxylic acid group like amino acids)



Naming:
delta (Δ) • number of carbon atoms in the chain : number of double bonds ($\Delta^{\text{position(s) of double bonds}}$)
- 18:2 ($\Delta^{9,12}$)

omega (ω) • ω position of the double bond relative to the methyl carbon • fatty acid
- ω6 fatty acid



- even number of C, unbranched, double bond in cis configuration
- double bonds in polyunsaturated fatty acids are **methyl bridged** (double-single-single-double), not **conjugated** (double-single-double-single) so you can determine the bond pattern from 1 double bond

Commonly occurring *saturated* fatty acids			
C	**common name**	**etymology**	determine the charge of the carboxylic acid group at different pH when pH = 7, $-COO^-$ and acidic: lauric acid, myristic acid…
12	laurate	bay, laurel	
14	myristate	myrtle, nutmeg	
16	palmitate	palm	
18	stearate	tallow	
20	arachidate	peanut	

Partial hydrogenation of unsaturated fatty acids generate 'trans fats' • the trans double bonds forms an extended conformation, oil to semi-solid, 'appears' saturated to the body

Melting point • increases as chain length increases
• increases with saturation
Solubility • decreases as chain length increases
• decreases with saturation
because chain length ∝ interactions between chains, saturation ∝ hydrocarbons - more non-polar
18:0 = 70°C 18:1 (cis) = 13.4°C 18:1 (trans) = 45°C trans acts similarly to saturated, but disorder from double bond

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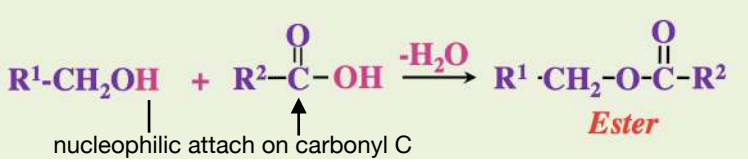
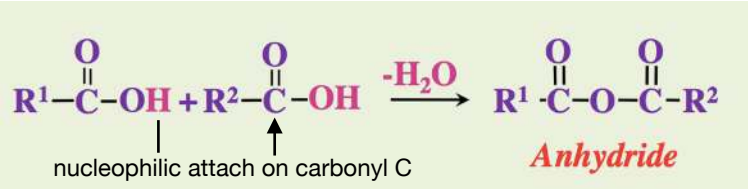
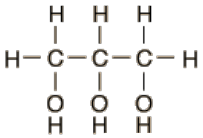
alcohol + carboxylic acid ⇀ ester + water

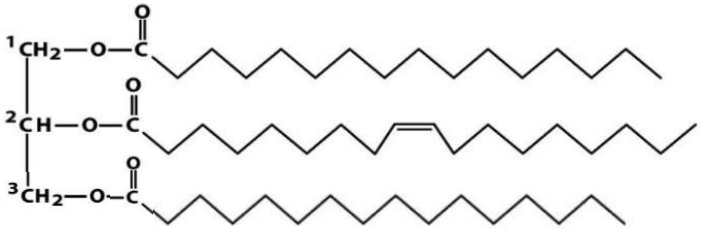


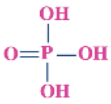
carboxylic acid + carboxylic acid ⇀ anhydride + water



Triacylglycerols • 'acyl' - acid derivative - TAGs; fats and oils, storage fat, how the majority of fatty acids are found in biological systems
• 3 fatty acids + glycerol ⇀ 3 ester linkages • highly hydrophobic because carboxylic acids are now (in less polar) esters, length of chain and degree of saturation of fatty acids involved determines melting point and solubility
• **simple** • same fatty acid in all 3 position
• **mixed** • 2 or 3 different fatty acids

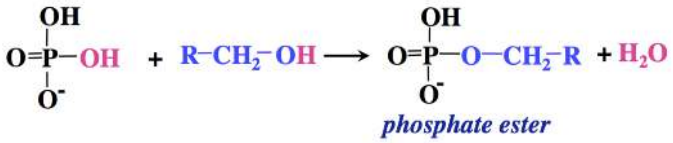




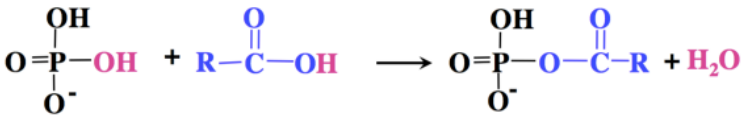
Phosphoglycerols • major lipid in membranes 

phosphoric acid (H_3PO_4) is a **triprotic acid** and exists in equilibrium with $H_2PO_4^-$ and HPO_4^{2-} at neutral pH
this **mixture** is represented by the notation ***P_i*** (inorganic phosphate)
• phosphorylation adds negative charge to molecules, leading to an increase in water solubility
• each -OH groups has a different pKa value

phosphoric acid + alcohol ⇀ phosphate ester + water



phosphoric acid + carboxylic acid ⇀ phosphoanhydride + water



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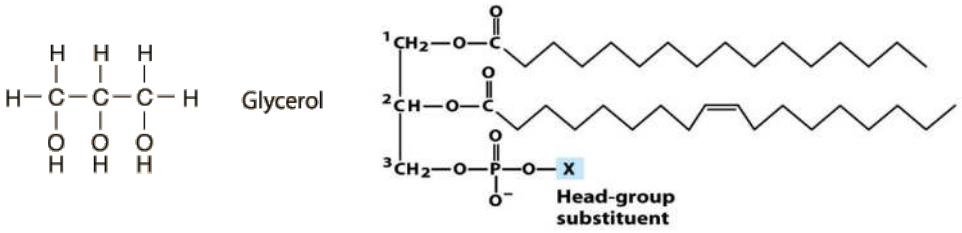
One kind of phospholipid is is phosphoglycerides • primary constituents of biological membranes because a highly polar or charged group (X) is attached through a phosphodiester linkage to the 3rd C atom - **amphipathic** • (unlike TAGs) so can form lipid bilayers



Classes of glycerophospholipids	Head group constituent	Formula of X	Net charge of molecule (including O^- from diester linkage)
Phosphatidylcholine (lecithin)	choline	$-CH_2-CH_2-N^+(CH_3)_3$	0
Phosphatidylethanolamine	ethanolamine	$-CH_2-CH_2-NH_3^+$	0
Phosphatidylserine	serine	$-CH_2-CH(NH_3^+)-COO^-$	-1
Phosphatidylglycerol	glycerol	$-CH_2-CH(OH)-CH_2-OH$	-1

*different combinations of fatty acids at R1 and R2 correspond to different molecule within each class

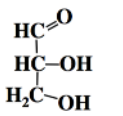
Lipids aggregate spontaneously to form complexes when dispersed in water:
Micelle • individual units, wedge-shaped, fatty acids
Bilayer • individual unit cylindrical, phospholipids (too bulky for micelles), which spontaneously form vesicles/liposomes (basics of cells) to protect hydrophobic interior

How to Analyze Lipids • lipids are very similar to each other (unlike proteins) so few characters used to distinguish between them
Two Phase Extraction • methanol (and water) - hydrophilic layer
• chloroform - hydrophobic layer
• progressively polar lipids elute from the column as solvents of increasing polarity are passed through it
• separate based on polarity on column of **silica gel** (elute neutral to charged) or **thin layer chromotography** (less polar lipids move farther than polar lipids - interact with silica)

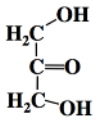
From either method, we know the lipids are in the form or triglycerides…
Identify fatty acids: break ester bonds to free fatty acids - **trans-esterification**, NaOH/methanol
fatty acids immediately react with methyl groups in solution so they are volatile (fatty acyl methyl esters) and volatile compounds escape from solution into gaseous phase
either: **gas-liquid chromotography** (mobile phase is gas) or
high performance liquid chromotography (chain length)
Separated fatty acids can be definitively identified through mass spectrometry.

Carbohydrates (sugars) • most abundant biomolecule on Earth, important in energy metabolism
• essential compound of nucleic acids

Saccharides: store as polysaccharide so you do not alter osmotic pressure of cell - water soluble, colourless, sweet, generally $(CH_2O)_n$
monosaccharides • simple sugars, single sugar unit
oligosaccharides • short chains < 20 units, disaccharides • 2 units
polysaccharides • polymers > 20 units



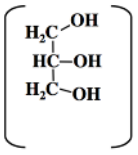
glyceraldehyde (aldose)



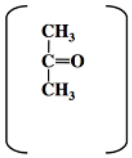
dihydroxyacetone (ketose)

Monosaccharide • 2 organic chemical functional groups:
1. carbonyl (C=O) group - aldehyde or ketone
2. at least 2 C with alcohol (OH) groups

Simplest monosaccharides are trioses, 3 C
triose, tetrose, pentose, hexose, heptose
Each C length has aldose ('aldotriose') and ketose ('ketotriose') forms



glycerol



acetone

Fisher Projection Formula • represent 3D structures - vertical bonds project behind the plane, horizontal bonds project out of the plane
Perspective Formula • represent 3D structures - solid wedge shapes bonds project out of the plane, dashed lines project behind the plane

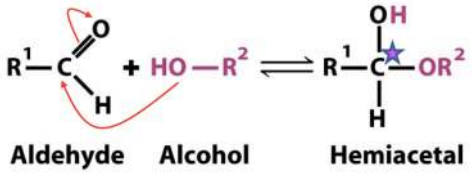
All monosaccharides (except dihydroxyacetone**)** contain one or more chiral C atoms

- produce optically active isometric forms, a type of stereoisomer: 2^n stereoisomers where n represents the number of chiral C atoms - half of the stereoisomers will be D sugars, half L sugars
- mirror images • enantiomers - differ in configuration at every chiral C atom
  left-handed and right-handed forms
  identical chemical properties but different optical activity - plane of polarization of light bent in opposite directions when passing through solutions of the 2 enantiomers
- diastereomers • differ in configuration at some chiral C atoms (1 or more)
  left-handed and right-handed forms
  do not have identical chemical properties, different spatial relationships between atoms
- epimers • differ in configuration at one chiral C atom (special diastereomer) - naming: C-3 epimers

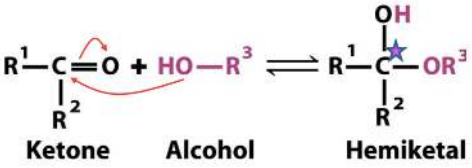
D and L designation • most naturally occurring sugars are D sugars, refers to chiral C atom furthest away from the carbonyl group: D • hydroxyl group on right
L • hydroxyl group on left

There are double the number of aldose stereoisomers than ketoses because in ketoses, 1 C atom is restricted due to the location of the carbonyl (C=O) group…
Consider a 4C aldose: 2 chiral C atoms - 2^2 = 4 stereoisomers
Consider a 4C ketose: 1 chiral C atom - 2^1 = 2 stereoisomers
recall that half will be D sugars, half will be L sugars

aldehyde + alcohol ⇌ hemiacetal



ketone + alcohol ⇌ hemiketal



The original carbonyl C becomes chiral upon formation of hemiacetals and hemiketals.

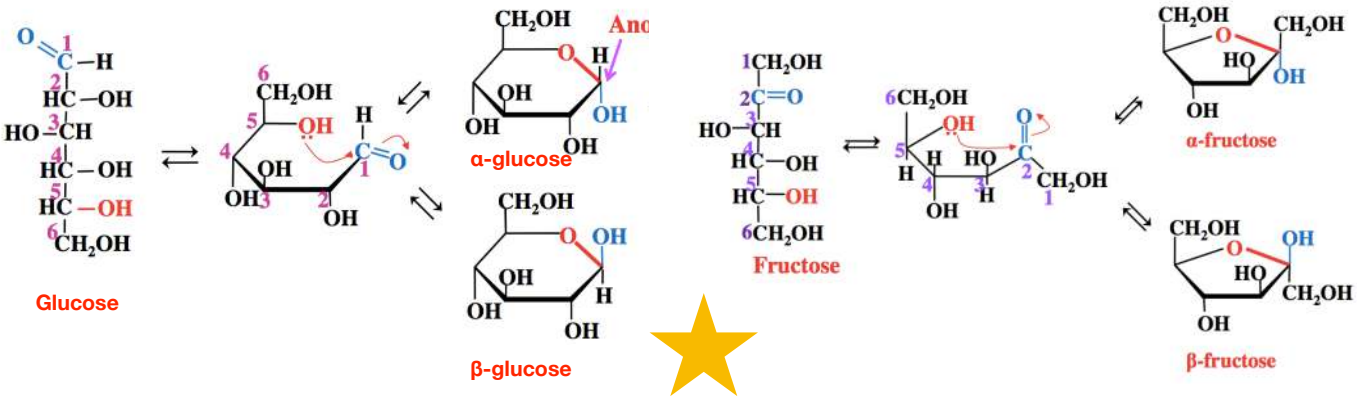
Cyclization of Sugars • sugars have both alcohol and aldehyde or ketone functional groups, so intramolecular formation of hemiacetals and hemiketals leads to the cyclization of sugars - most common way to find sugars

When sugars cyclize, they form one of 2 ring forms:
1. pyranose ring (6-membered) C1 becomes anomeric
2. furanose ring (5-membered) C2 becomes anomeric

The carbonyl C always becomes the anomeric C, and is the electrophile in reaction. Any OH group can act as a nucleophile.

Anomers • use a squiggly line to refer to anomers without specifying alpha or beta form
α • OH group below plane at anomeric C
β * OH group above plane at anomeric C

Mutarotation • of alpha and beta forms of hemiacetals and hemiketals when sugar is dissolved in water, converts to equilibrium - alpha, beta, and linear forms, the optical planes in solutions of alpha or beta forms of a sugar are identical



Haworth Projection • linear to ring structures (Fisher to Haworth)
OH groups LEFT ABOVE BETA
RIGHT BELOW ALPHA

Chemical property acts as a simple basis for detecting **presence** and **concentration** of sugars that are reducing:
Reducing sugars • **carbonyl carbon** (*only in linear* forms) of sugars can be oxidized to a carboxyl group by oxidizing agents like cupric ion (Cu2+) which is reduced to red precipitate Cu+
Non-reducing sugars • sugars that so not react with oxidants
Aldose or ketose group must be free to form reaction.

Glycosides: most important reactions of sugars…
condensation of anomeric C with nucleophilic OH group of alcohol or
nucleophilic NH group of amine
condensation reaction forms glycoside with glycosidic (C-O) or glycosilic (C-N) bond
the ring form can no longer open up to the linear form when the anomeric C is in a glycosidic or glycosilic bond so the sugar becomes a non-reducing sugar

Disaccharides: 2 monosaccharides form a glycosidic bond between anomeric C of one sugar (electrophilic C) and hydroxyl group (nucleophilic) of second sugar - many hydroxyl groups so many possibilities - glycosidic bond inhibits the mutarotation in the monomer but disaccharides can have a reducing end: when only 1 anomeric C is involved

Single-headed arrow from anomeric C to non-anomeric C

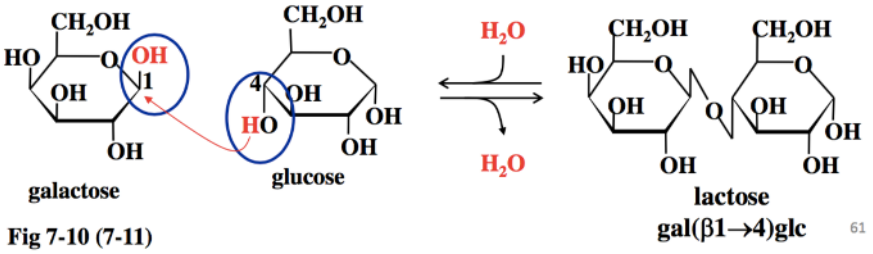
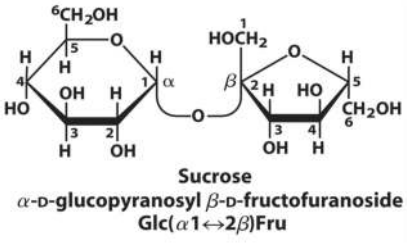


Fig 7-10 (7-11)

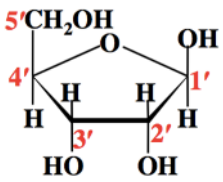
Double-headed arrow between anomeric Cs



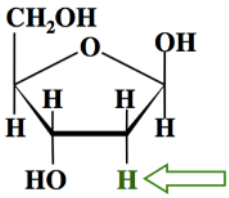
Polysaccharides • most natural carbohydrates, highly branches because many available OH groups per monomer to acts as nucleophile in glycosidic bond, differ in units/chain length/bond types/degree of branching
Homopolysaccharides • made from a single type of sugar monomer
glucans • glucose homopolymers • starch, cellulose, glycogen, chitin
mannans • mannose homopolymers
Heterosaccharides • made from 2 or more types of sugar monomers

Nucleic Acids • linear polymers of nucleotides connected via phosphodiester bonds • sugar phosphate backbones

Sugars • 2 kinds of pentose sugars - both in β furanose forms, C atoms numbered with **primes (‘)**



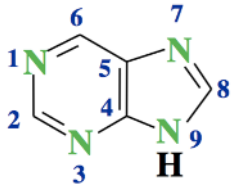
β-D-ribose (RNA)



β-D-2-deoxyribose (DNA)

Bases • 2 kinds of parent compounds - both heterocycles (contain non-C atoms) pyrimidines and purines (bicyclic) - note numbering convention

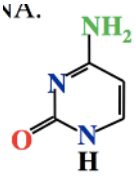
Pyrimidines



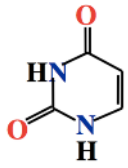
Purines

Pyrimidines

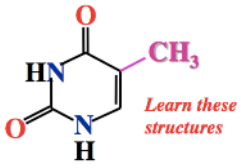
T has a higher fidelity, knows if it should be O or NH2, machinery to fix it is more efficient



cytosine



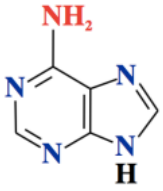
uracil (RNA)



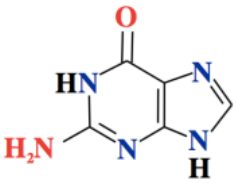
Learn these structures

thymine (DNA) *(5-methyluracil)*

Purines



adenine

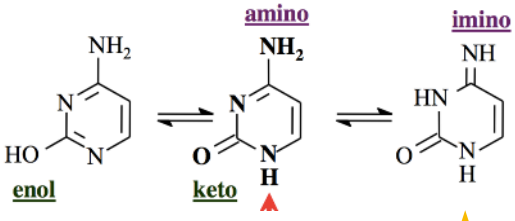


guanine

Tautomeric forms

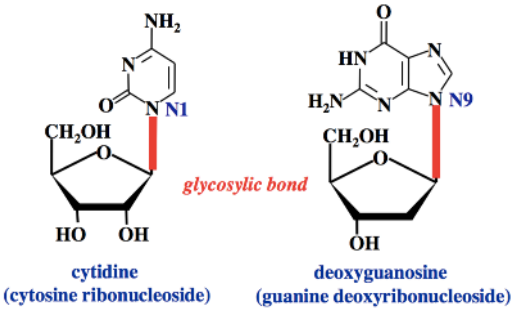
- isomers that differ by the shift of an H atom and a double bond
- OH group undergoes keto (=O) / enol (-OH) tautomerism
- NH2 group undergoes amino (-NH2) / imino (=NH) tautomerism

Predominant form is the keto-amino form



Nucleoside • base, and sugar (a special type of glycosidic bond - glycosilic because C-N linkage)

NH at position 1 of pyrimidines or
NH at position 9 of purines
and anomeric C of ribose or deoxyribose

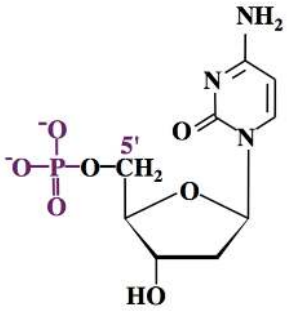


cytidine (cytosine ribonucleoside)

deoxyguanosine (guanine deoxyribonucleoside)

Base	Nucleoside (base + sugar)	Abbreviation (RNA/ DNA)
adenine	adenosine / deoxyadenosine	A / dA
guanine	guanosine / deoxyguanosine	G / dG
cytosine	cytodine / deoxycytodine	C / dC
thymine	thymidine	T (do not use ‘d’, assumed)
uracil	uridine	U

Nucleotide • base, sugar, phosphate group - phosphorylated nucleosides
- allow for phosphodiester linkages between 3'C and 5'C
  so 5' end and 3' end *which may or may not by phosphorylated*
- *phosphate groups are ionized and negatively charged at pH = 7*
- nucleotide sequence: 5'ATG3'



*** the 2' hydroxyl of RNA acts like a nucleophile and RNA will hydrolyze under alkaline conditions (pH > 7) so DNA is more stable!*

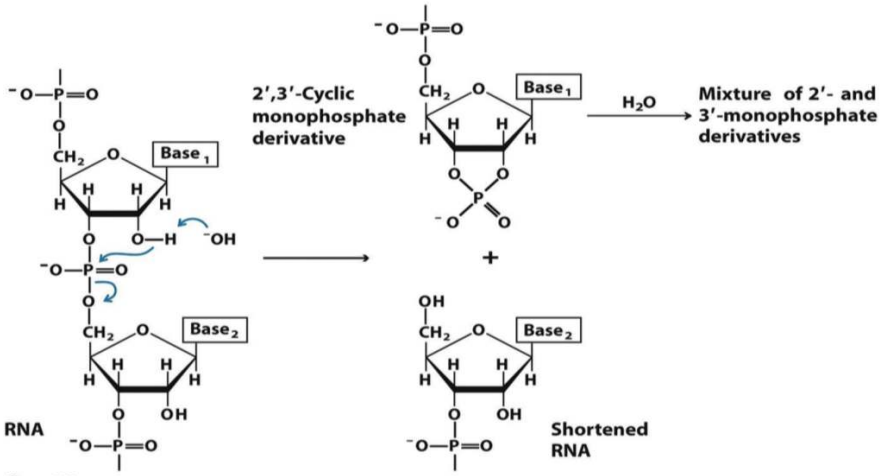
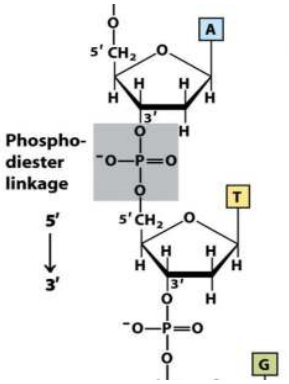


Figure 8-8



Chargaff's Rules: A% = T%, G% = C% but A+T ≠ G+C

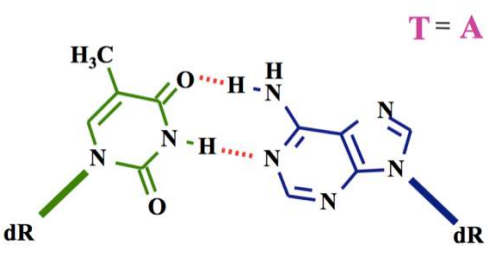
Franklin & Wilkins • process of purifying DNA causes sheer stress, fragments DNA
- but DNA can be drawn into long fibres - *not* crystalline (like protein) but can be diffracted
  - modern techniques produce small oligonucleotides which form well-ordered crystals, produce diffraction patterns like proteins
- Franklin - photo 51: diffraction image of DNA 'X'
  - helical, periodicities along long axis: primary 3.4 A°, secondary 34 A°

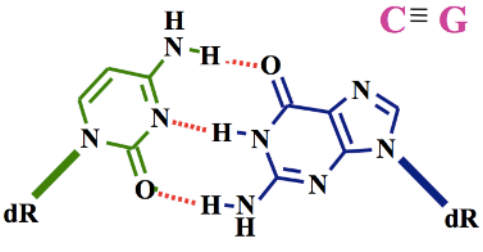
Watson & Crick • accumulated data on DNA structure, model for secondary structure: geometry
- right-handed double helix (right hand thumbs up), 2 strands antiparallel, self-complimentary (template)
- hydrophilic sugar phosphate backbone outside
- hydrophobic bases perpendicular to backbone inside
- vertically stacked base pairs **3.4 A°** apart, each turn of a helix **10 pairs or 34 A°**
- diameter **20 A°**

3 H bonds between G and C (if in higher ratio, harder to unwind strand)
2 H bonds between A and T (if in higher ratio, easier to unwind strand)

plectonemically coiled (duplex of strands twisted together)
'supercoiling', unwind strands from end

- Major Groove and Minor Groove • wider versus narrower gaps on side of helix appear to alternate consequence of glycosylic bond between base pairs, provide different degrees of accessibility for protein recognition

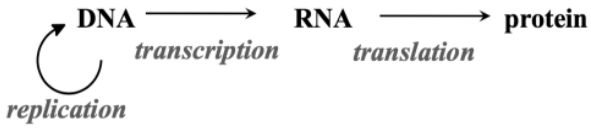




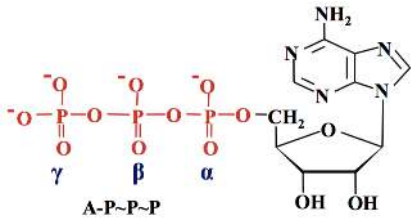
Secondary structure largely independent of sequence, 2 kinds of base pairs similar in shape and properties.

Stabilize double helix: H bonding - between bases
Van der Waals - stacking of bases
Hydrophobic effect

Central Dogma: predominantly unidirectional flow



Adenosine Triphosphate ATP • nucleotide: base (adenosine), sugar (ribose), 3 phosphate groups link between catabolism and anabolism (not energy storage): high turnover
free energy from catabolic / exergonic processes synthesizes ATP from ADP
free energy used for anabolic / endergonic processes breaks down ATP to ADP

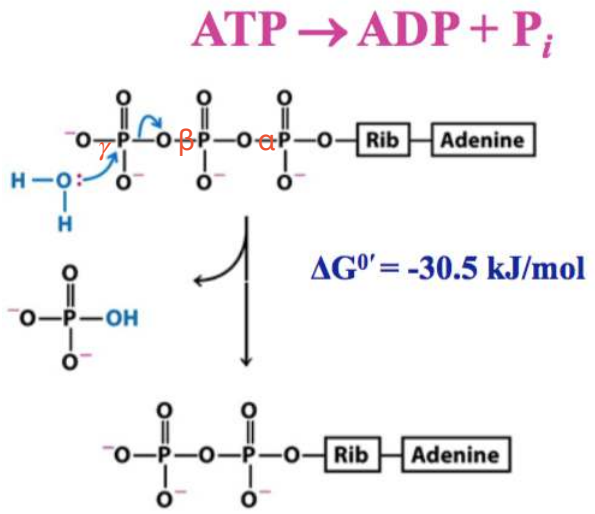


$\Delta G_{hydrolysis}$ for ATP = -50 kJ/mol - phosphate bonds hydrolyzed

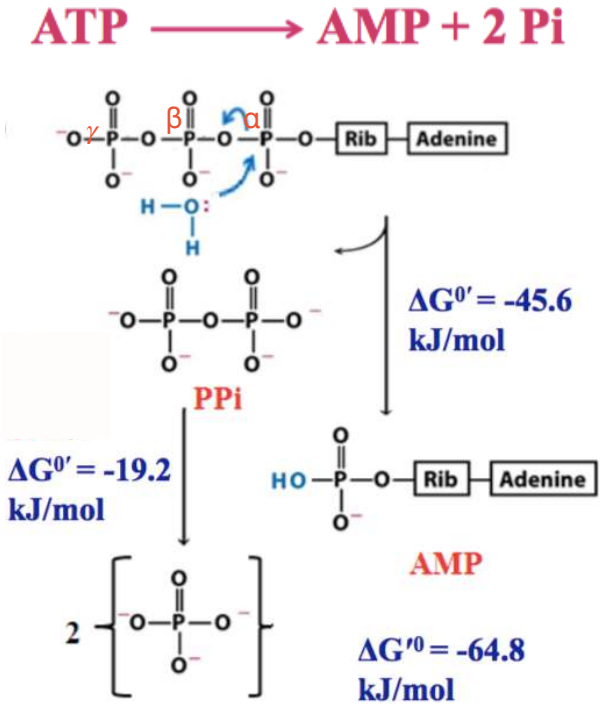
Chemical Basis:
1. hydrolysis releases electrostatic repulsion among negative charges
2. product inorganic phosphate has greater resonance stabilization than ATP
3. ADP^{2-} product ionizes, releases proton into low [H+] environment

2 high energy bonds in ATP: phosphoanhydride linages…

hydrolysis of link between γ and β phosphate, nucleophilic attack of γ phosphate

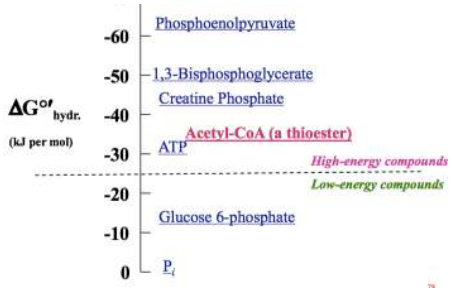


hydrolysis of link between α and β phosphate, nucleophilic attach of α phosphate



How does the hydrolysis of ATP drive unfavourable reactions?

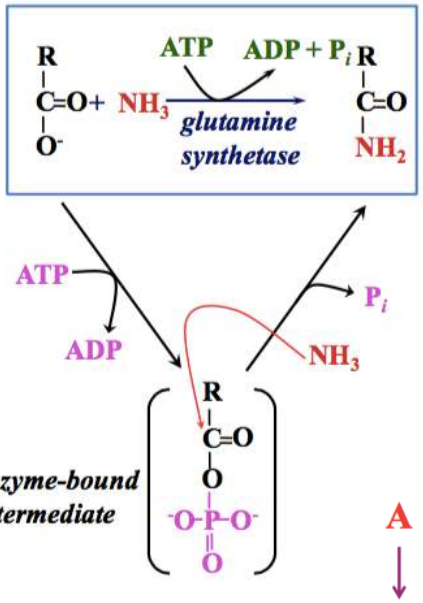
- couple enzyme with ATP hydrolysis to drive reaction forward
- ATP provided energy *not* by simple hydrolysis but by **group transfer**
- chemically versatile: transfer pyrophosphate (PPi), adenylate (AMP) moiety, or phosphoryl group (Pi)



ATP can be formed from AMP 'using' compounds of greater free energy (above) ATP (more negative)

Example of how ATP drives unfavourable reactions using 2 steps:

1. ATP reacts with glutamate to produce a covalent intermediate, a mixed anhydride, of phosphate and glutamate - high energy intermediate
2. NH3, nucleophile, reacts with carbonyl C, electrophile, and Pi, leaving group, is displaced



Metabolism • anabolism and catabolism

Anabolic pathways diverge • Acetyl CoA can be used to build everything

Catabolic pathways converge • carbon skeletons converted into Acetyl CoA

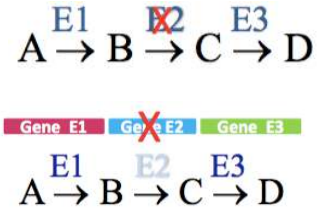
Acetyl CoA - acetyl coenzyme A - **carrier of 2 C units (acetate)**

metabolite • series of intermediates in a metabolic pathways, Acetyl CoA

Metabolic pathways usually contain as least one thermodynamically favourable reaction (irreversible) and are regulated - transcriptional control of enzyme level, or inhibition / activation of enzyme activity eg. feedback inhibition

How metabolic Pathways are understood:

1. Use metabolic inhibitors • chemical approach • if E2 inhibited, B accumulates and can be isolated and identified
2. Biochemical genetics • biochemical approach
   a) genetic diseases • where enzyme not created, eg. black pee
   b) auxotrophic mutants • expose e.coli or yeast to a mutagen to inactivate gene encoding for a specific enzyme - phototroph to auxotroph, identified by end product of affected pathway
3. Radioactive-labelled substrates • 14C radioactive, traceable (as opposed to 12C) and any products detected



Oxidation-Reduction Reactions: where equilibrium lies indicates which species has greater tendency to accept available electrons, use Standard Reduction Potential (at pH 7 E°') of 2 half reactions
if sum is positive, spontaneous reaction
if sum is negative, non-spontaneous reaction

$\Delta G° = -nF\Delta E°'$ F • Faraday's constant • charge on 1 mole of electrons, 96.5 x 10^3 Coul/mol

$\Delta G° \propto \Delta E°'$ n • number of electrons transferred

Electrons flow from redox pair with lower E°' to higher E°'

FADH2 and NAD(P)H like to reduce things!

	Half-reaction	E'°(V)
Strong oxidants	$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.816
	$Fe^{3+} + e^- \longrightarrow Fe^{2+}$	0.771
	$NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O$	0.421
	Cytochrome f (Fe^{3+}) + $e^- \longrightarrow$ cytochrome f (Fe^{2+})	0.365
	$Fe(CN)_6^{3-}$ (ferricyanide) + $e^- \longrightarrow Fe(CN)_6^{4-}$	0.36
	Cytochrome a_3 (Fe^{3+}) + $e^- \longrightarrow$ cytochrome a_3 (Fe^{2+})	0.35
	$O_2 + 2H^+ + 2e^- \longrightarrow H_2O_2$	0.295
	$FAD + 2H^+ + 2e^- \longrightarrow FADH_2$	−0.219
	Glutathione + $2H^+ + 2e^- \longrightarrow$ 2 reduced glutathione	−0.23
	$S + 2H^+ + 2e^- \longrightarrow H_2S$	−0.243
	Lipoic acid + $2H^+ + 2e^- \longrightarrow$ dihydrolipoic acid	−0.29
Strong reductants	$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
	$NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	−0.324

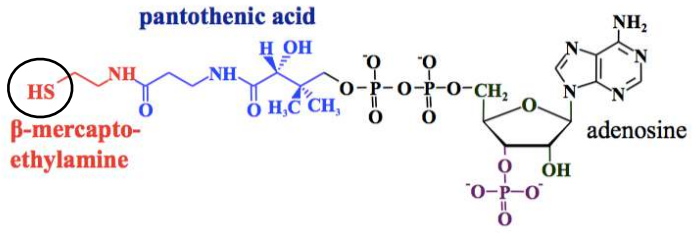
Enzyme CoFactors • compounds that help enzymes carry out their functions

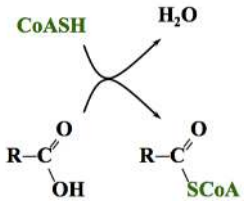
i. inorganic ions • Fe2+, Mg2+, Mn2+, Zn2+, Cu2+

ii. Coenzymes • complex organic or metalloorganic compounds that acts as **carriers of functional groups**, *many are derivatives of adenosine:*
CoA, ATP, pyridine nucleotides, flavin nucleotides

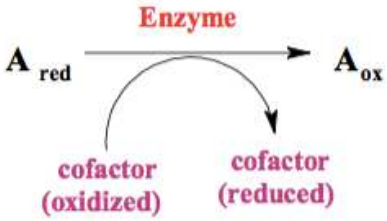
CoA (CoASH, Coenzyme A) • carrier of acyl (acid) groups • pantothenic acid (B5) vitamin
forms **thioester** (sulphur analogue of ester) derivatives with organic acids, "acyl CoA", special case: derivative with acetic acid, "acetyl CoA"

chemically reactive end





ATP • carrier of phosphate groups • adenosine derivative



Vast majority of coenzymes use 4 Cofactors • universal electron carriers, electron acceptors to be later reduced to conserve energy of oxidation

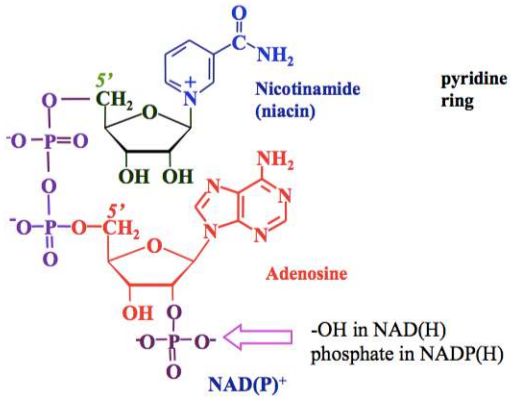
Pyridine Nucleotides • carrier of1 electron • niacin (B3) vitamin

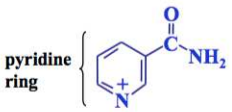
redox reactions occur at the nicotinamide ring, 2 H atoms are removed from the substrate, pyridine accepts **hydride ion** (**:H-** the equivalent of 1 proton and 2 electrons) and

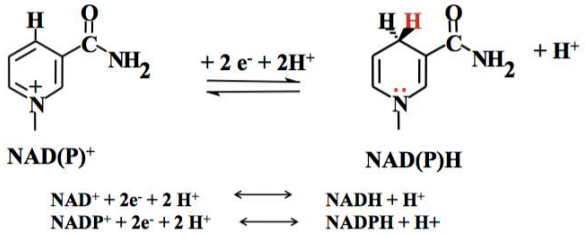
is reduced to NADH or NADPH - the other proton is released into the environment

nicotinamide adenine dinucleotide (NAD+) - oxidizing agent

nicotinamide adenine dinucleotide phosphate (NADP+)







Flavin Nucleotides • carrier of electron (1 or 2) • riboflavin (B2) vitamin

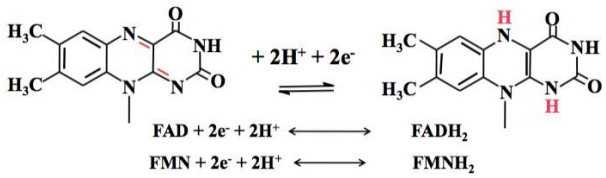
usually act as prosthetic groups: found tightly bound to enzyme (not covalently)

flavin adenine dinucleotide (FAD)

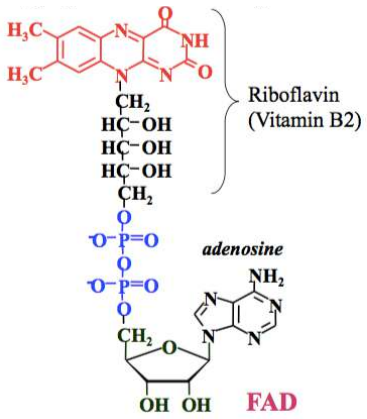
flavin mononucleotide (FMN)

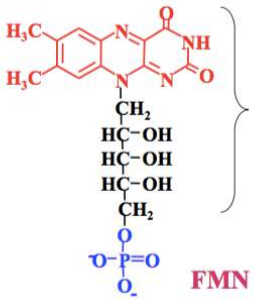
can accept either one 1 or 2 electrons in the form of 1 or 2 H atoms from substrates being oxidized

when only 1 electron is accepted, formation of **semiquinone radicals** - FADH• and FMN•



greater diversity of reaction than pyridines because flavins can participate in either 1 or 2 electrons transfers





(recognize, but do not need to reproduce these structures)

Catabolism of Fats

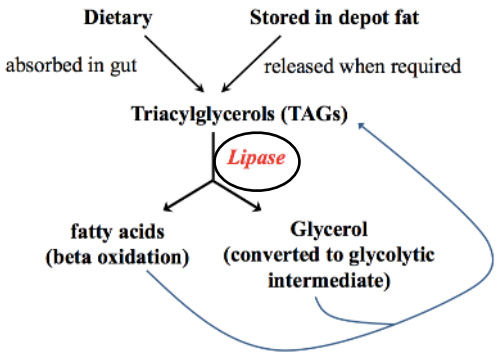
Fat is the most concentrated store of metabolic energy (per gram).

1. Chemically very reduced, most C are CH2, so releases maximum amount of free energy when oxidized to CO2.
2. Hydrophobic: can be stored nearly water-free, does not impact osmotic pressure of cell.

Consituent	Energy (kJ per g dry weight)	Human energy reserves (kJ)
protein	17	100,000
carbohydrate	16	glucose: 150 glycogen: 2,500
fat	37	500,000

C-C bonds reduced, C-O bonds oxidized more oxidation step = more energy released

Metabolize readily available to reservoirs of energy.



Stage 0: Activation of Fatty Acids to Fatty Acyl CoA
Stage 1: Beta Oxidation
Stage 2: Citric Acid Cycle
Stage 3: Electron Transport Chain

synthetase • enzyme that combines 2 small molecules to form a larger molecule ***using ATP***

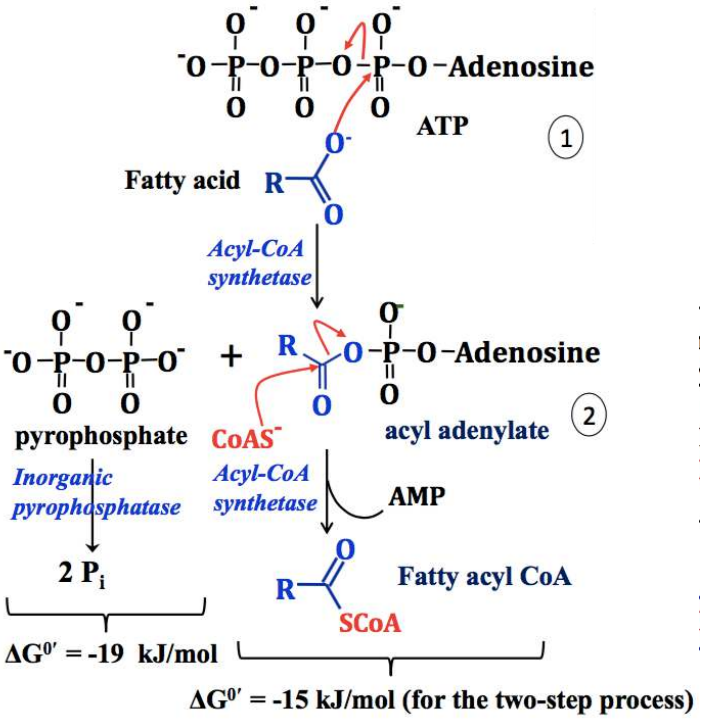
sythase • enzyme that combines 2 small molecules to form a larger molecule ***without using ATP***

Stage 0: Activation of Fatty Acids to Fatty Acyl CoA • outer mitochondrial membrane

Fatty Acid + CoA + ATP ⇀ Fatty Acyl-CoA + AMP +2Pi (equivalent to the hydrolysis of 2 ATP to ADP + Pi)

Step 1: nucleophilic oxygen atom of fatty acid attacks the electrophilic α phosphate of ATP. This forms PPi (inorganic pyrophosphatase) and an acyl adenylate. PPi is immediately hydrolyzed to 2Pi (inorganic phosphate).

Step 2: nucleophilic thiolate anion form of CoA reacts with acyl adenylate. This forms fatty acyl CoA (thioester) and AMP is the leaving group.



Overall $\Delta G^{0'} = -34$ kJ/mol

Stage 1: Beta Oxidation • **mitochondrial matrix**

Fatty acids catabolized 2 C atoms at a time (an acetyl moiety in the form of acetyl CoA); no mechanism to to remove *only* 1 C (Knoop tagged the ω (terminal C) end of even and odd-chained fatty acids with phenyl to dogs, collected aromatic products in urine).

OMM highly permeable to small molecules and ions but IMM highly impermeable to most solutes. This creates an environmental gradient between the cytosol and mitochondrial matrix.

Fatty acids >12 C are transported into the mitochondrial matrix via acyl-carnitine/carnitine transporter in the form of fatty acyl-carnitine esters.

Once in the mitochondrial matrix, fatty acyl CoA molecules are committed to the beta oxidation pathway.

Step 1: oxidation • acyl-CoA dehydrogenase • FAD cofactor reduced to FADH2 • formation of double bond between α and β C of fatty acyl CoA (alkane to alkene)

Step 2: hydration • enoyl-CoA hydratase • H2O added across the double bond, add H to α and alcohol OH to β (alkene to alcohol)

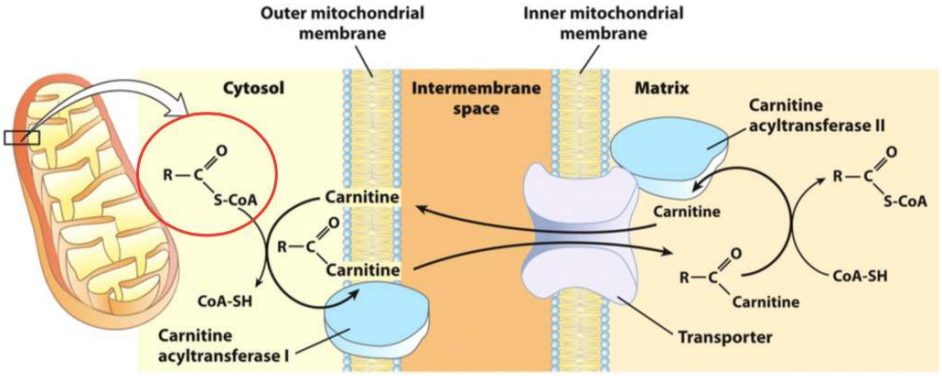
Step 3: oxidation • β-hydroxyacyl-CoA dehydrogenase • NAD+ cofactor reduced to NADH + H+ (alcohol to ketone)

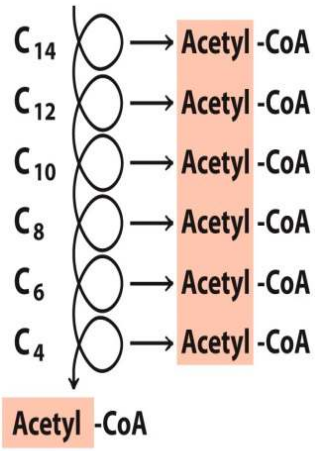
Step 4: thiolysis (thiol analogue of hydrolysis) • thiolase • lysis of α and β C bond • formation of fatty acyl-CoA thioester and acetyl CoA (ketone to acetyl CoA)

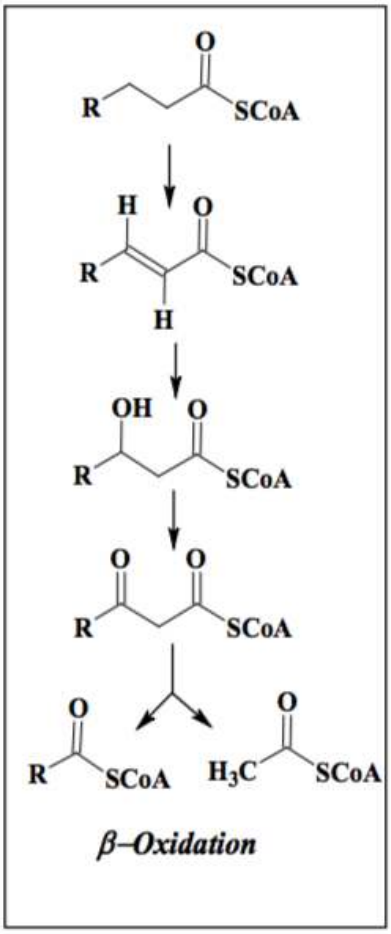
Fatty acyl CoA returns to start of beta oxidation sequence.

Overall stoichiometry: complete oxidation of 16 C fatty acid into 8 molecules of acetyl CoA requires 7 passes through beta oxidation sequence. 1FADH2 and 1NADH (and H+) formed per sequence.

palmitoyl CoA (C16:0) + 7 CoASH + 7FAD + 7NAD+ + 7H2O → 8 acetyl CoA + 7NADH2 + 7(NADH + H+)





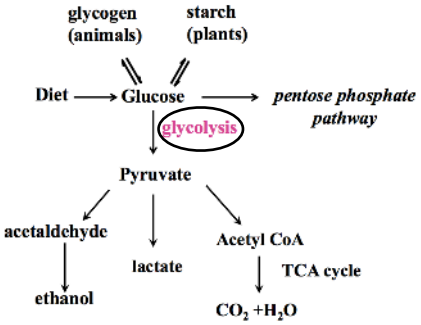


Catabolism of Carbohydrates

Glucose is highly polar and cannot enter cells by passive diffusion across the membrane. Glucose Transporter proteins (GLUTs) import glucose. Insulin hormone stimulates GLUT glucose uptake into skeletal muscle and adipose tissue. In diabetes, blood glucose is not taken up into cells adequately.

Fasting glucose [blood glucose] = 5 mM. Some tissues solely dependent on glycolysis for energy (not fat) - red blood cells, renal medulla, brain, sperm.

Only pathway that can provide energy under **anaerobic conditions**.



Stage 1: Glycolysis + Pyruvate Dehydrogenase
Stage 2: Citric Acid Cycle
Stage 3: Electron Transport Chain

synthetase • enzyme that combines 2 small molecules to form a larger molecule ***using ATP***

sythase • enzyme that combines 2 small molecules to form a larger molecule ***without using ATP***

Stage 1: Glycolysis + Pyruvate Dehydrogenase • cytosol

Preparatory Phase (Steps 1 through 5) • 2 ATP used to phosphorylate and activate glucose to 2 triose molecules

Step 1: phosphorylation • hexokinase (**four** different **isozymes** - two or more enzymes that catalyze the same reaction but are encoded by different genes) • ATP reduced to ADP (phosphorylation of glucose at position 6)

Step 2: isomerization, reorganization • phosphohexose isomerase • (aldose to ketose, glucose to fructose)

Step 3: phosphorlyation • phosphofructokinase-1 • ATP reduced to ADP (phosphorylation of fructose at position 1)

Step 4: cleavage of C-C bond • aldolase • (6 C fructose is split into two 3 C units (DHAP and G3P) that are each phosphorylated)

Step 5: isomerization, reorganization • triose phosphate isomerase (same mechanism as phosphohexose isomerase) • DHAP isomerized to G3P

Pay Off Phase (Steps 6 through 10) • 2 ATP and 2 NADH for each triose molecule

Step 6: oxidation and phosphorylation • glyceraldehyde 3-phosphate dehydrogenase • NAD+ cofactor reduced to NADH + H+ (forms a mixed anhydride)

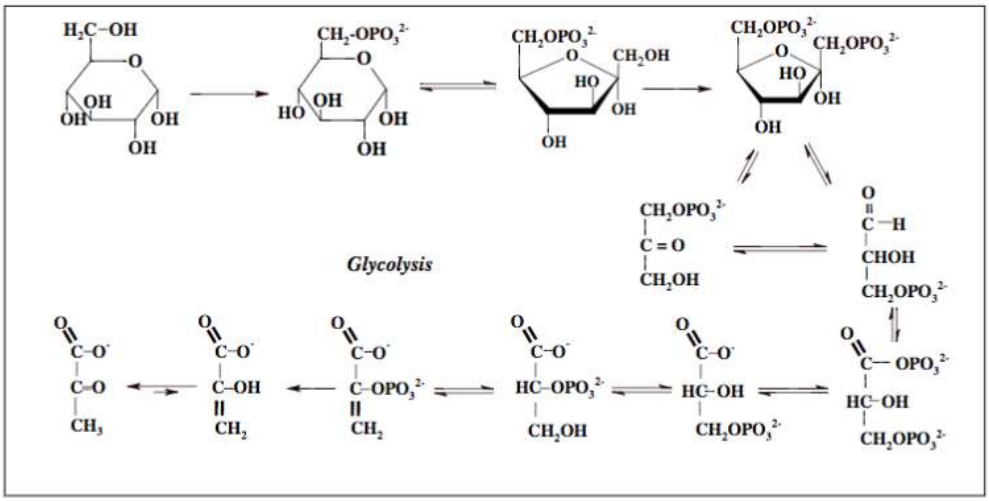
Step 7: hydrolysis • phosphoglycerate kinase • ADP phosphorylated to ATP • free energy from hydrolysis of anhydride bond recovered as ATP

Step 8: isomerization, reorganization • phosphoglycerate mutase (**mutase** - catalyze reaction in which a functional group is shunted between different position in a molecule) • conversion of 3-phosphoglycerate to 2-phosphoglycerate

Step 9: dehydration • enolase • H2O released, dehydration to form double bond (forms phosphoenolpyruvate)

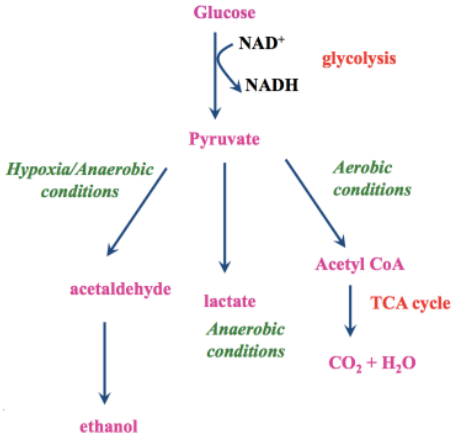
Step 10: hydrolysis • pyruvate kinase • ADP phosphorylated to ATP • free eneergy from hydrolysis of phosphoenolpyruvate recovered as ATP (forms enolpyruvate - non-enzymatic tautomerization between enol and keto (tautomer) forms driving reaction forward)

For each molecule of glucose, 1 Preparatory Phase and 2 Pay Off Phases (with 2 instances of substrate-level phosphorylation each) - net product: 2 ATP + 2 NADH

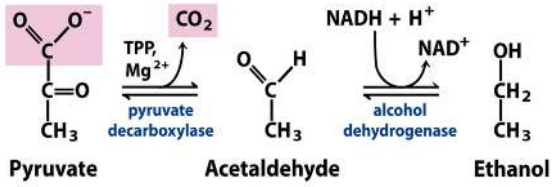


Continuation of Stage 1: Glycolysis + Pyruvate Dehydrogenase • cytosol

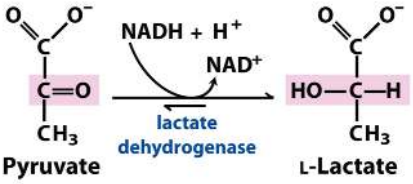
Pyruvate is further metabolized via 1 of 3 different routes depending on physiological conditions in order to oxidize the NADH formed in glycolysis. This ensures glycolysis can function under aerobic or anaerobic conditions (when it is not possible to oxidize NADH through the ETC).

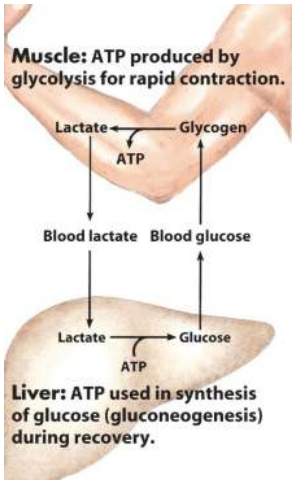


HYPOXIA/ANAEROBIC CONDITIONS: yeast and other microorganisms convert pyruvate to ethanol and CO_2 in a process called alcohol fermentation. Animals do not have pyruvate decarboxylase and while animals possess alcohol dehydrogenase, the reaction proceeds in the opposite direction (and is NAD+ dependent).



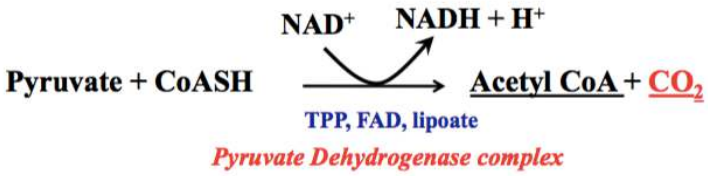
ANAEROBIC CONDITIONS: animals reduce pyruvate to lactate using the Cori Cycle: exchange of metabolites between organs. Tissues switch to using pyruvate itself to re-oxidize NADH. Pyruvate is reduced to lactate (lactic acid) by lactate dehydrogenase. After strenuous exercise, lactate is exported from muscle tissue to blood to liver. The liver recycles lactate by converting it back to glucose via gluconeogenesis (requires ATP). Glucose is exported back to muscles.





AEROBIC CONDITIONS: all organisms, pyruvate is transported into the mitochondria through a transporter. **Pyruvate dehydrogenase (PDH)** occurs in the mitochondrial matrix and catalyzes an irreversible oxidate decarboxylation. PDH is the link between glycolysis and the citric acid cycle! Catalyzes an analgous reaction to alpha-ketoglutarate dehydrogenase in TCA.

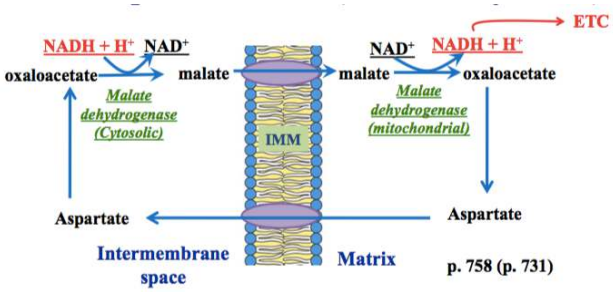
Pyruvate dehydrogenase complex requires 5 coenzymes: NAD+, FAD, CoA (B vitamin derivatives), TPP (Thiamine pyrophosphate (B1 vitamin; thiamine)) and lipoate (lipotic acid).



Under aerobic conditions, NADH is oxidized by the Electron Transport Chain. Glycolysis occurs in the cytosol but ETC is in the matrix. Shuttle system carries ***reducing equivalents (electrons in the form of: e-, H+ + e-, or H-)*** from the mitochondrial NADH to the mitochondria because the IMM is impermeable to NADH.

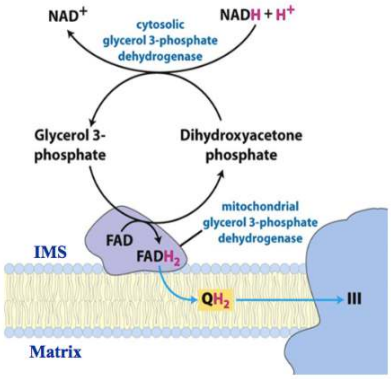
Malate-aspartate shuttle (liver, kidney, and heart)

oxaloacetate - malate - oxaloacetate
aspartate aspartame



Glycerol 3-phosphate shuttle (skeletal muscle and brain)

glycerol 3-phosphate - dihydroxyacetone
reducing equivalents from cystolic NADH are passed onto FADH2 and are directly delivered to coenzyme Q of the ETC (bypassing complex I)



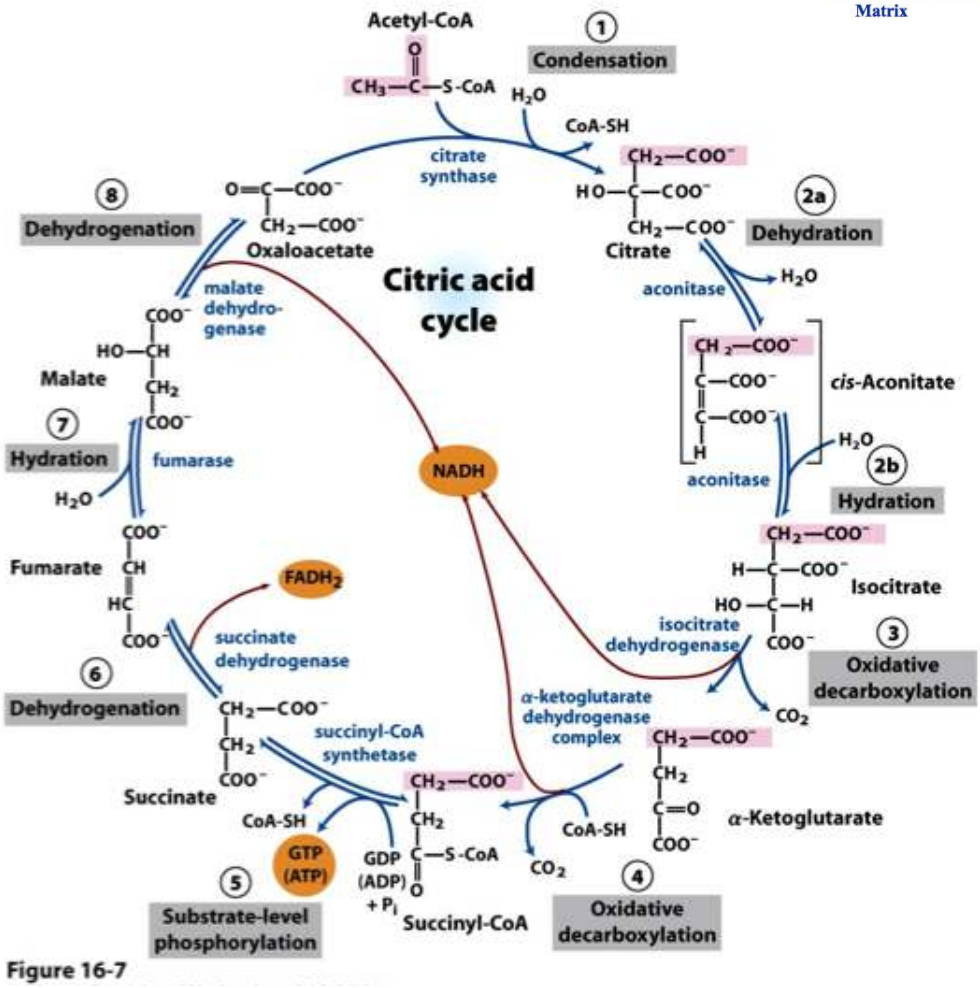


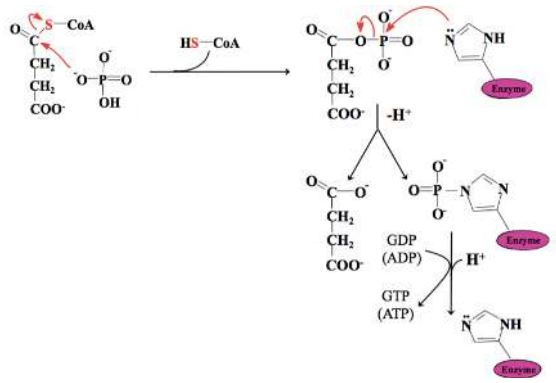
Figure 16-7

When Acetyl CoA is formed, it will enter into the Citric Acid Cycle (CAC, Tricarboxylic Acid Cycle, TCA, Krebs Cycle)
The TCA is indirectly dependent on O_2. because PDH Complex function under aerobic conditions.

Stage 2: Citric Acid Cycle • mitochondrial matrix, O2 dependent - reoxidizing cofactors in ETC
Takes acetate moieties derived (from catabolism of fats, carbohydrates, and certain amino acids) in the form of Acetyl CoA and oxidizes them completely to carbon dioxide.
Step 1: condensation • citrate synthetase • acetyl CoA and oxaloacetate condensed to citrate, ONLY TCA CYCLE STEP THAT FORMS C-C BOND, methyl of acetyl CoA acts as the nucleophile when it is deprotonated by the enzyme.
Step 2: isomerization • aconitase
- 2a: dehydration • citrate is a tertiary alcohol - poor substrate for oxidation, remove H2O for C=C bond to cis-aconitate
- 2b: hydration • hydration of cis-aconitate to a secondary alcohol - good substrate for oxidation, add H2O for isocitrate

Step 3: oxidative decarboxylation • isocitrate dehydrogenase • NAD+ cofactor reduced to NADH • oxidation of alcohol to ketone through transfer of a hydride from C-H bond to NAD+ followed by the loss of -COO group as CO2 (oxidative decarboxylation)
Step 4: oxidative carboxylation • alpha ketoglutarate dehydrogenase complex - NAD+ cofactor reduced to NADH • reaction mechanism identical to PDH • alpha ketoglutarate converted to high energy thioester succinyl CoA
Step 5: substrate level phosphorylation • succinyl CoA synthetase • GTP (ATP) cofactor phosphorylated to GTP (ATP) • high energy thioester bond of succinyl CoA hydrolyzed and CoASH released • involves a phosphoenzyme intermediate

Reaction mechanism of Succinyl CoA synthetase: creating a phosphoenzyme intermediate
1. inorganic phosphate displace CoA from succinyl CoA and forms high energy acyl-phosphate (succinyl phosphate)
2. a reactive histidine residue at the active site of the enzyme accepts the phosphate, releasing succinate
3. the phosphate is transferred from phosphoenzyme to ADP (or GDP), releasing the free enzyme



Step 6: dehydrogenation • succinate dehydrogenase • FAD cofactor reduced to FADH2 • reaction analogous to acyl-CoA dehydrogenase reaction in beta oxidation - oxidize succinate to fumarate
Step 7: hydration • fumarase • fumarate hydrated to alcohol, malate • reaction analogous to enoyl-CoA hydratase reaction in beta oxidation
Step 8: dehydrogenation • malate dehydrogenase • NAD+ cofactor reduced to NADH • reaction analogous to hydroxyacyl-CoA dehydrogenase reaction of beta oxidation - dehydrogenate malate to oxaloacetate

Net Effect of CAC:
- 2 carbons of acetyl group of acetyl-CoA oxidized to CO2
- electrons from oxidation reduce 3 NAD+ and 1 FAD (indirect energy)
- one GTP (or ATP) is formed per cycle (direct energy)
- intermediates in the cycle are not depleted

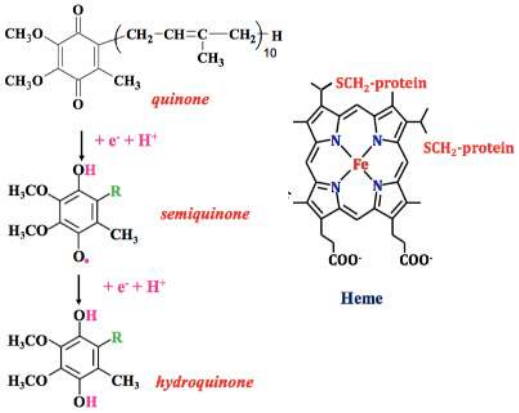
Stage 3: Electron Transport Chain • **inner mitochondrial matrix, IMM, and mitochondrial matrix**

Oxidative phosphorylation oxidizes NADH and FADH2 which is used to synthesize ATP.
(the direct oxidation of NADH ($\Delta G^{\circ\prime}$ = -220 kJ/mol) and FADH2 ($\Delta G^{\circ\prime}$ = -200 kJ/mol) by O2 energetically wasteful because no covalent bond can contain more than a fraction of energy released. Enough energy for ~10 covalent bonds could be made to synthesize ATP, so release energy in several small steps.

Reducing equivalents from reduced cofactors are passed to O2 indirectly along the ETC - several distinct processes with smaller free energy changes re-oxidize NADH and FADH2.

Set of electron carriers of increasing reduction potential: reducing equivalents passed down to O2, the terminal electron acceptor. Four enzymes (electron carrier complexes) catalyze the transfer of electrons.

Complex I (NADH dehydrogenase)
Complex II (succinate dehydrogenase)
Coenzyme Q (ubiquinone, CoQ) • can function at junctions between 1-electron and 2-electron donors, freely moves in the membrane
lipid-soluble isoprenoid tail (R)
accepts one electron (and proton) to form a semiquinone radical (•QH)
or two electrons (and protons) to form an alcohol, ubiquinol (QH2)
Complex III (cytochrome C reductase)
Cytochrome C • can function only with 1 electron at a time, freely moves in the membrane - soluble protein in the mitochondrial intermembrane space
iron-containing heme
iron atom of heme acts as redox active component
Complex IV (cytochrome oxidase)



Proton Motive Force: harnesses the stored energy, proton pump

1. Chemical potential energy • due to difference in concentration of H+
2. Electrical potential energy • due to the separation of charges (voltage)

The free energy change fro the transfer of 1 mole of H+ across the inner mitochondrial membrane 20 kJ/mol • therefore ~200 kJ of available energy from NADH oxidation is conserved in the proton gradient

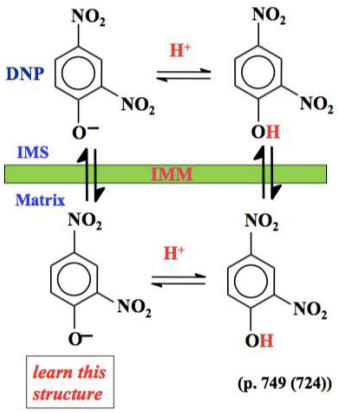
Chemiosmotic Theory • free energy liberated by the redox reactions i used by the ETC to pump protons from the matrix to the inter-membrane space, energy stored as electrochemical gradient (specifically, proton motive force), as protons flow down the gradient into the mitochondrial matrix, energy of the electrochemical gradient is released and used for the generation of ATP by ATP synthase

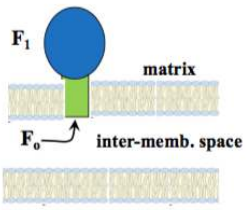
Electron transfer and ATP synthesis are coupled pathways; neither reaction occurs without the other.

1. An inhibitor of electron transfer will inhibit both the oxygen consumption and the ATP synthesis - oxidation process is the source of energy for ATP sythesis.
2. Inhibition of ATP synthase also blocks the electron transport chain - energy to pump protons against gradient will exceed energy available from NADH oxidation. Blocked by venturicidin or oligomycin.

Uncoupling oxidative phosphorylation:
if the integrity of the inner mitochondrial membrane is disrupted, proton gradient eliminated, ETC continues and ATP synthesis stops.

Produce similar effect (of disrupting the gradient) with chemicals:
2,4-DNP collects proton to form DNPH and transports it across the IMM to the matrix. DNP- will cross the intermembrance space because the negative charge is delocalized given the aromatic ring so it is still hydrophobic in character. Proton gradient collapses.

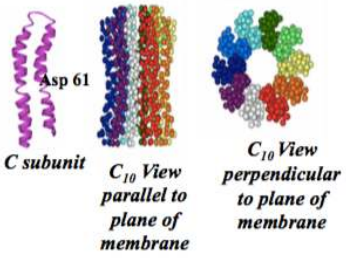


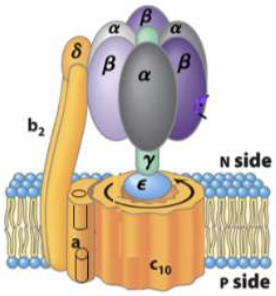


Mitochondrial ATP synthase/ATPase has 2 functional domains:

1. F1 (peripheral membrane protein) GREEK
   9 subunits $\alpha_3\beta_3\gamma\delta\varepsilon$:

   3 α subunits are identical, 3 β subunits are identical
   each β subunit contains a catalytic site for ATP synthesis
   γ subunit forms the stalk in the centre of the $\alpha_3\beta_3$ complex and acting through ε, attaches F1 to the membrane embedded 'C' ring of Fo
2. Fo (integral membrane protein - o: oligomycin-binding) LATIN
   $ab_2C_{10\text{-}12}$:
   multiple C subunits, each a hairpin of 2 hydrophobic alpha helices, assemble the transmembrane C10 ring where the number of C units varies among different kingdoms
   each C subunit contains a conserved aspartic acid residue (Asp 61 -) in the middle of one of its helices
   2 b subunits act through δ to associate firmly with $\alpha_3\beta_3$
   a subunit resides in the membrane and wraps partially around C10 ring - contains 2 half-channels for the movement of protons: one half-channel from inter-membrane space into a subunit, one half-channel from a subunit to matrix - not a direct route. Protons 'jump from a to C to a.





Equilibrium constant to synthesize ATP is close to 1 when the reaction occurs in the active site of ATP synthase (free energy change for the reaction is about 0). But ATP production would require energy? Yes, but formed ATP remains tightly bound to the active site. The energy required for the release of the formed ATP is provided by the proton gradient (energy barrier is *not* reaching transition state but releasing formed ATP from the enzyme).

ATP synthase overcomes large energy barrier to release ATP: Rotational Catalysis
ATP synthase active site cycles between conformation states: tight binding of ATP, release of ATP

Each β subunit of F1 assumes 3 conformations because the γ subunit interacts asymmetrically with the $\alpha_3\beta_3$ complex which causes the β subunits to have conformation that each associate with differences in ADP/ATP binding site: Loose (ADP + Pi bound), Tight (ATP formed), Open (ATP released).

γ subunit rotates in the centre of the $\alpha_3\beta_3$ complex, each 120° turn of the γ subunit cycles between interactions with the β subunits.

a, b and δ subunits for the stator arm, remaining fixed with respect to the membrane and holds the $\alpha_3\beta_3$ complex in place.

The ring of C subunits rotates with respect to the stator arm, powered by the proton gradient,
γ and ε rotate with the C subunits.
C10 ring held in place by ionic interaction between Asp61 (-) of C and 2 conserved Arg (+) residues of a.
When a proton 'jumps' form a to C and protonates Asp 61, ionic bond is broken, C10 ring rotates so that the protonated c moves away from a and into the hydrophobic milieu of the membrane. Simultaneously, another c subunit (AspH) is forced into contact with the second half-channel. The proton carried by that Asp is released into the matrix.
10 protonation and deprotonation events completes one revolution of C10 ring, each β subunit cycles through all 3 conformations and the release of 3 ATPs.

Overall efficiency of oxidative phosphorylation:
x(ADP + Pi) + NADH + H+ + 1/2O2 > NAD+ + H2O + xATP
x = P/O ratio - the number of moles of ATP synthesized per mole of O reduced to water (2 e- passed along ETC, per mole of NADH/FADH2 oxidized)
P/O NADH = 2.5 (not the simple 3!)
P/O FADH2 = 1.5
Chemiosmotic coupling allows for non-integral values as the ratio depends on the detailed mechanism by which the proton gradient energy is used to power ATP synthesis.

Knowing P/O ratios, we can calcualte total energy yield from ctabolism of nutrient molecules.