

MB&B 300a – Principles of Biochemistry – Fall 2015

Part 2: Metabolism – based on lectures by Prof. Michael Koelle
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Lecture 15: Principles of Metabolism

A. What is metabolism?

- a. Proteins operate together in series called pathways, and in macromolecular complexes
- b. The set of chemical reactions that (1) extract energy and (2) synthesize biomolecules
- c. Metabolism constitutes much of the minimal set of chemical reactions required for life:
In *Hemophilus influenzae*, 60% are known (minimal set). Half of those are metabolic enzymes.
- d. Longest studied and best understood aspect of biochemistry; linked to certain metabolic diseases.

B. Six principles of metabolism

- a. Metabolism is kinetically controlled by enzymes
- b. Metabolic reactions occur in “pathways” with many small steps
- c. A few important molecules carry “currencies” of metabolism
- d. Coupled reactions drive energy-requiring processes.
- e. Biosynthetic and degradative pathways are distinct
- f. Metabolic pathways are regulated and integrated

C. Principle 1: Metabolism is kinetically controlled by enzymes

- a. Comparison of organic chemistry and biology

Organic Chemistry	Biology
1 reaction per vessel	Hundreds of simultaneous reactions
Reactions go toward thermodynamic equilibrium	System maintained far from equilibrium
Reaction efficiency < 100%	Reactions highly efficient; No accumulation of intermediates or side products
Stereospecific reactions are rare	Stereospecific reactions are the rule
Harsh conditions	Constant mild conditions

- b. Most reactions are slow under biological conditions; over 500 different enzymes catalyze the needed reactions, controlling which reactions occur and how fast.

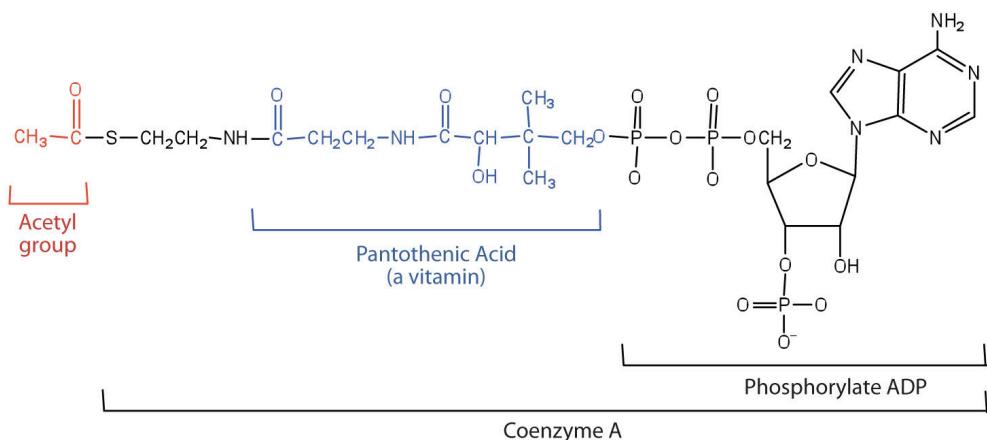
D. Principle 2: Metabolic reactions occur in “pathways” with many small steps

- a. Pathway: set of reactions coupled together for a common purpose
- b. Organic chemistry: direct combustion
 $C_6H_{12}O_6 + 6 O_2 \longrightarrow 6 CO_2 + 6 H_2O; \Delta G = -686 \text{ kcal/mol}$
- c. Biology: 24 serial steps divided into 3 pathways.
Glycolysis (10 steps), Citric acid cycle (9 steps), Oxidative phosphorylation (~5 steps)
- d. Why many steps?
 - i. Each enzyme performs a single chemical transformation (make/break a single bond)
 - ii. We can only efficiently input and capture energy in small amounts (via ATP)
 - iii. Allows different pathways to be integrated. Creates intermediates that function as convergence/divergence points- common products and building blocks.

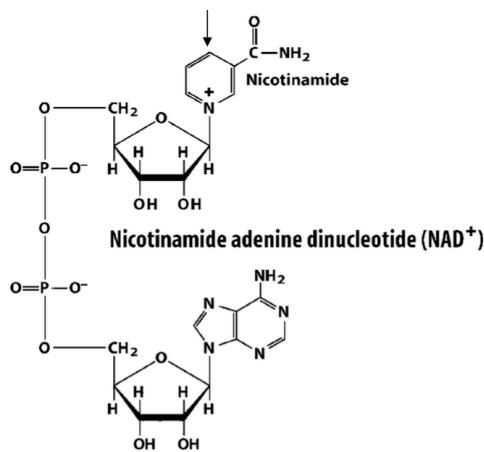
E. Principle 3: A few important molecules carry “currencies” of metabolism

- a. Common currencies allow for more efficient 1-size-fit-all-energy exchange (money > barter)
 - i. Small molecular components: Coenzyme A is the carrier for acetyl units
 - 1. Coenzyme: small molecules that help enzymes work
 - 2. Vitamins: cannot be synthesized; required in diet. Often coenzyme components.

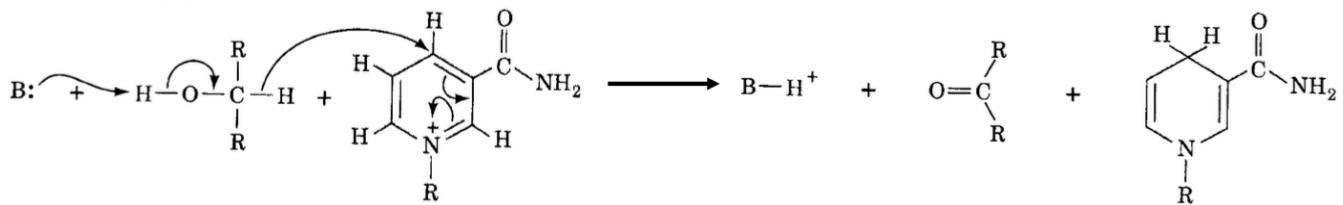
- ii. Reducing power packets (NAD^+) move electrons around
- iii. Energy packets (ATP) carry high-energy phosphates.
- b. Group transfer potential: energy released when a bond to a group is hydrolyzed.
Transfer from high potential to low potential is favorable, with $\Delta G < 0$.
 - i. Acetyl groups attached with a thioester bond.
 1. Coenzyme A: $\text{H}-\text{S}-\text{CoA}$
 2. Acetyl CoA: $\text{Ac}-\text{S}-\text{CoA}$
 - ii. The thioester linkage has a large energy of hydrolysis (high group transfer potential):
 $\text{Acetyl-CoA} + \text{H}_2\text{O} \rightleftharpoons \text{Acetate} + \text{CoA}; \Delta G = -7.5 \text{ kcal/mol}$
 - iii. The linkage is generally weaker in other molecules
 $\text{Acetyl-R} + \text{H}_2\text{O} \rightleftharpoons \text{Acetate} + \text{R}; \Delta G = -3 \text{ kcal/mol}$
 - iv. This means that transfer is generally favorable
 $\text{Acetyl-CoA} + \text{R} \rightleftharpoons \text{Acetyl-R} + \text{CoA}; \Delta G = -4.5 \text{ kcal/mol}$



- c. Reducing power packets: NAD^+ carries electrons
 - i. Redox reactions involve the transfer of electrons. NAD^+ oxidizes other molecules and carries the electrons released



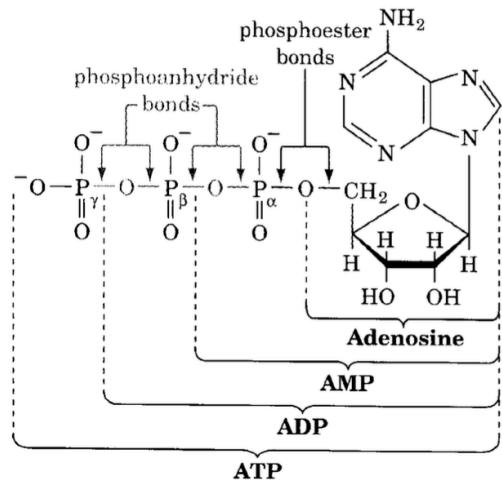
- ii. NAD^+ accepts a hydride ion (H^-)



- iii. NAD^+ can accept 2 electrons from compounds with higher electron transfer potentials.

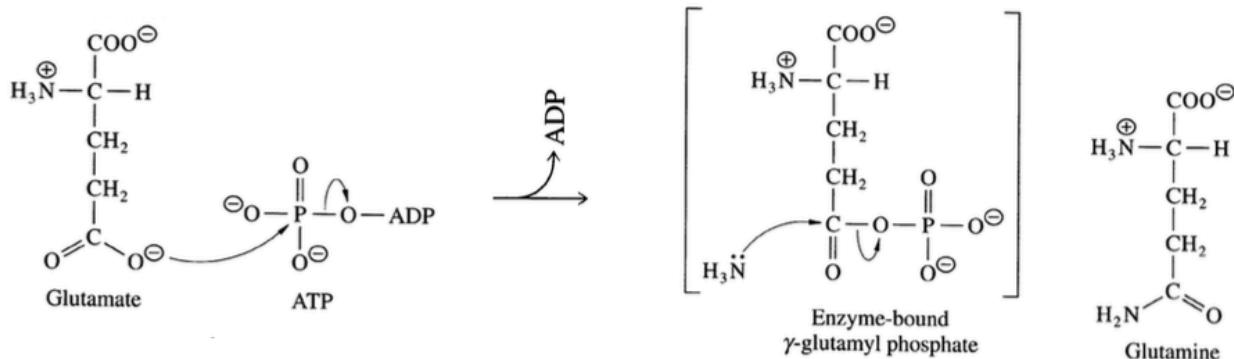
d. Energy packets: ATP carries P_i

- ATP is for immediate energy exchange
 - Typical half life < 1 minute
 - Long term energy storage: fats or carbohydrates
 - Money analogy: ATP = cash; glycogen/fat = gold bars
- ATP can be cleaved into ADP + P_i (-7.3 kcal/mol) or AMP + PP_i (-10.9 kcal/mol)
PP_i is inorganic pyrophosphate.
- Hydrolysis of phosphoanhydride bonds coupled to energy-requiring reactions.
- Creation of these bonds from energy-releasing reactions (e.g. oxidation of glucose yields ~30 ATP)



F. Principle 4: Coupled reactions drive energy-requiring processes

- a. Glutamate + ammonia + ATP \rightarrow glutamine + ADP + P_i



b. Thermodynamics of coupled reactions

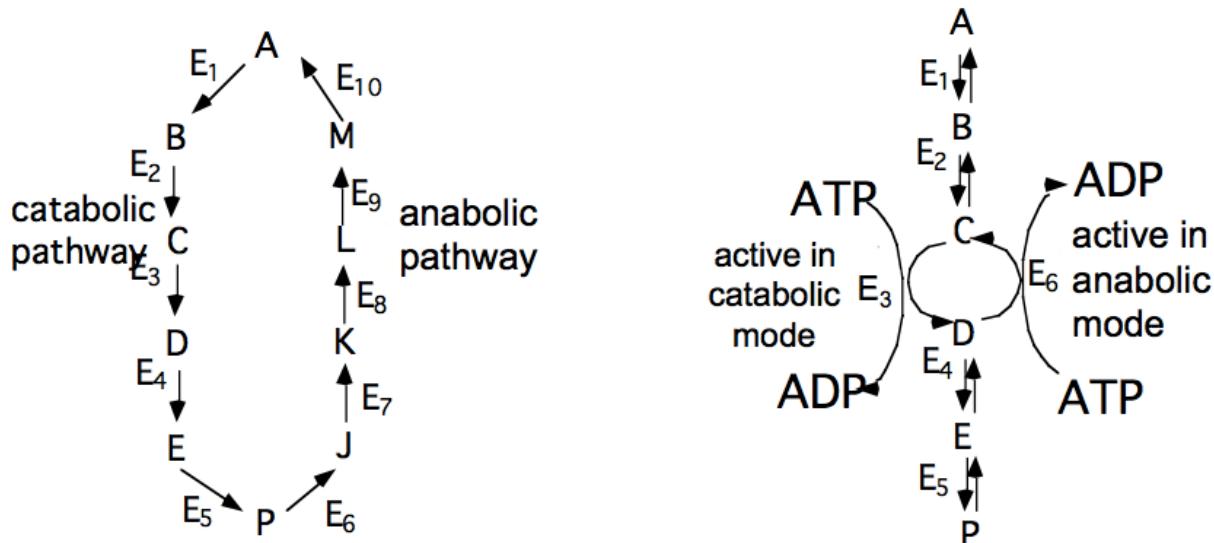
- If A \rightleftharpoons B; $\Delta G = 4$ kcal/mol; $K_{eq} = 1.15 \times 10^{-3}$
- A + ATP + H₂O \rightleftharpoons B + ADP + P_i + H⁺; $\Delta G = 4 - 7.3 = -3.3$ kcal/mol; $K_{eq} = 2.67 \times 10^2$
- Since [ATP]/[ADP][P_i] ≈ 500 in cells,
 $K_{eq} = 1/500 \times [B]/[A] = 267$; $[B]/[A] \approx 130,000$, 10⁸ shift.

- c. Metabolism often needs to drive $\Delta G > 7.3$ kcal/mol; ATP hydrolysis can still accomplish this in multiple small steps.

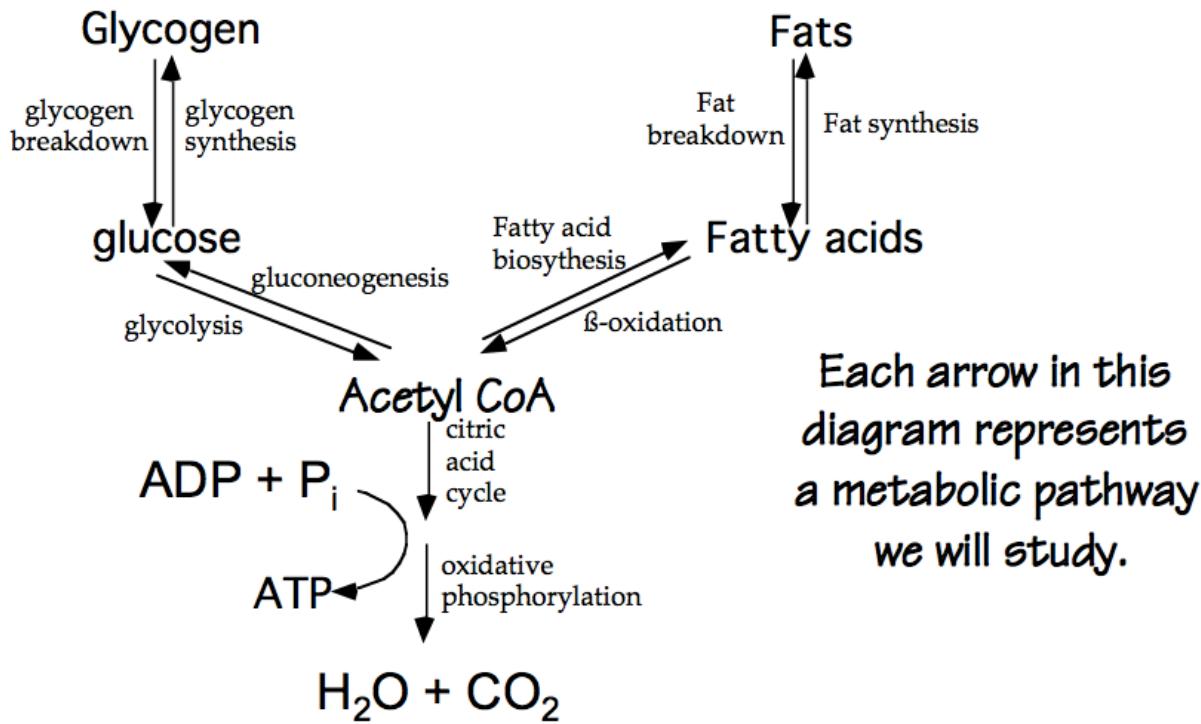
G. Principle 5: Biosynthetic and degradative pathways are distinct

- Certain pathways carry out opposite transformations:
 - Glycolysis v. gluconeogenesis
 - Beta-oxidation v. fatty acid biosynthesis
- Degradative pathways are catabolic: destructive, like mean cats.
- Biosynthetic pathways are anabolic: constructive, like a nice girl named Anna.
- Futile cycle: when opposite pathways operate simultaneously and achieve nothing.

This is avoided with distinct and separately controlled enzymes for catabolic/anabolic pathways.



H. Principle 6: Metabolic pathways are regulated and integrated



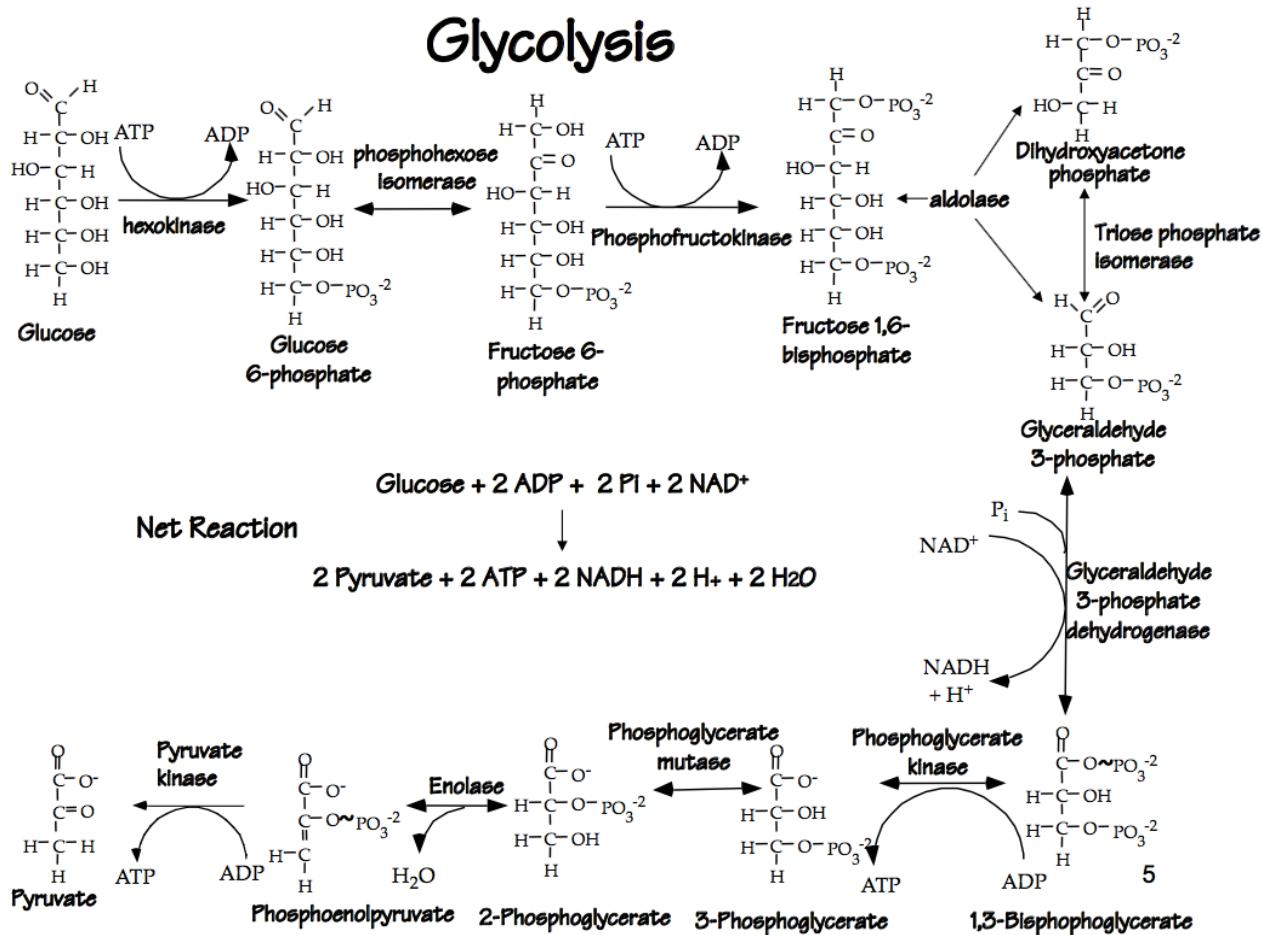
- Availability determines pathway activity
 - If ATP is low: glucose is oxidized.
 - If ATP is high: glycogen is synthesized
 - If lipids are low: glycolysis, then fatty acid biosynthesis
- Metabolic pathways are regulated (100x more glycolysis in working muscle v. resting muscle)
- Regulation is achieved by controlling:
 - Accessibility of substrates (e.g. insulin)
 - Amounts of enzymes
 - Catalytic efficiency of enzymes (phosphorylation; allosteric effectors)

Lecture 16: Glycolysis

A. Basics of Glycolysis

- Glycolysis: 10 step conversion of glucose to pyruvate
- Fermentation: anaerobic conversion of glucose to (1) ethanol + CO₂ or (2) lactate
- In aerobic conditions, glycolysis continues through acetyl CoA and the Krebs cycle, ending with oxidative phosphorylation. In anaerobic conditions, glycolysis ends one step short of acetyl CoA.

B. The 10 steps of glycolysis



C. Detailed mechanism

- Pre-reaction: glucose passively enters the cell by transmembrane glucose transporters.
- Reaction 1: hexokinase: phosphorylates the 6-carbon.
- Reaction 2: phosphohexose isomerase: moves the carbonyl to carbon-2.
- Reaction 3: phosphofructokinase: second phosphorylation at carbon-1.
- Reaction 4: aldolase: cleavage into two 3-carbon aldols (aldehyde + alcohol).
- Reaction 5: triose phosphate isomerase: converts DHAP side product into G3P.
- Reaction 6: G3P dehydrogenase: oxidizes carbonyl by addition of P_i, powers NAD⁺ → NADH.
- Reaction 7: phosphoglycerate kinase: harvests ATP via substrate-level phosphorylation.
- Reaction 8: phosphoglycerate mutase: rearranges phosphate down a carbon.
- Reaction 9: enolase: dehydrates to form an enol (phosphoenolpyruvate) and produce (~).
- Reaction 10: pyruvate kinase: Harvests ~P_i to make ATP. The high phosphoryl group transfer potential (-14.8 kcal/mol) comes from the unstable enol structure (contributes -10 kcal/mol)

D. The chemical logic of glycolysis

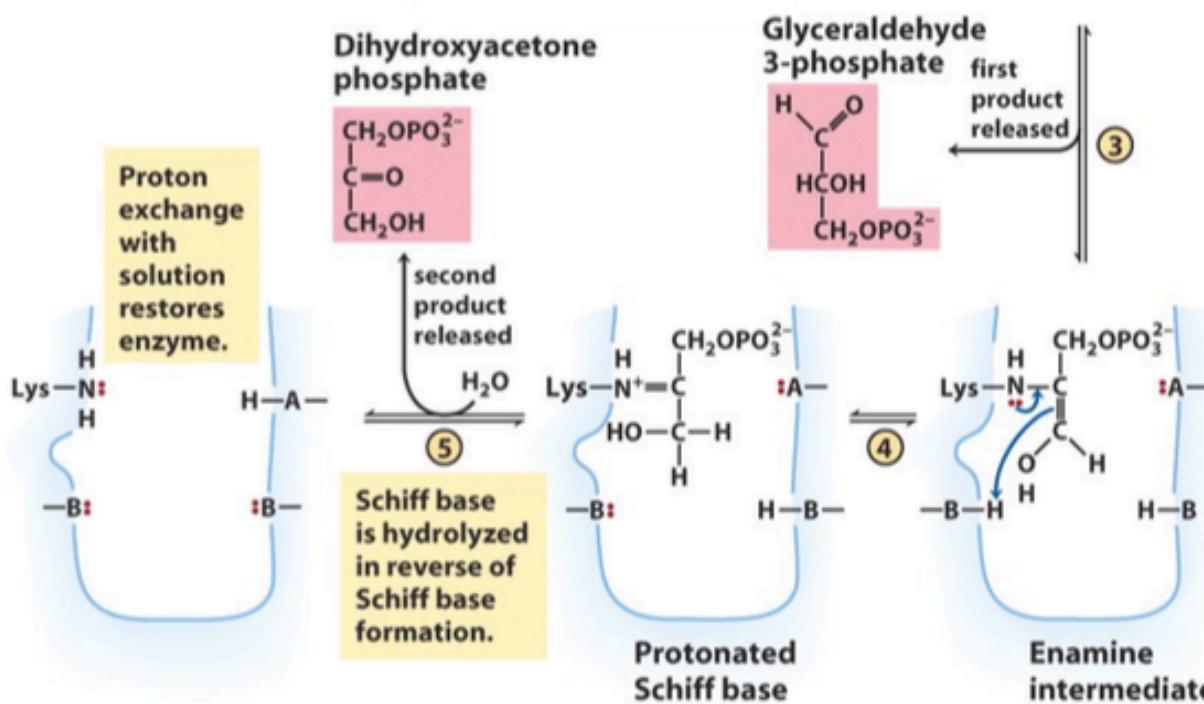
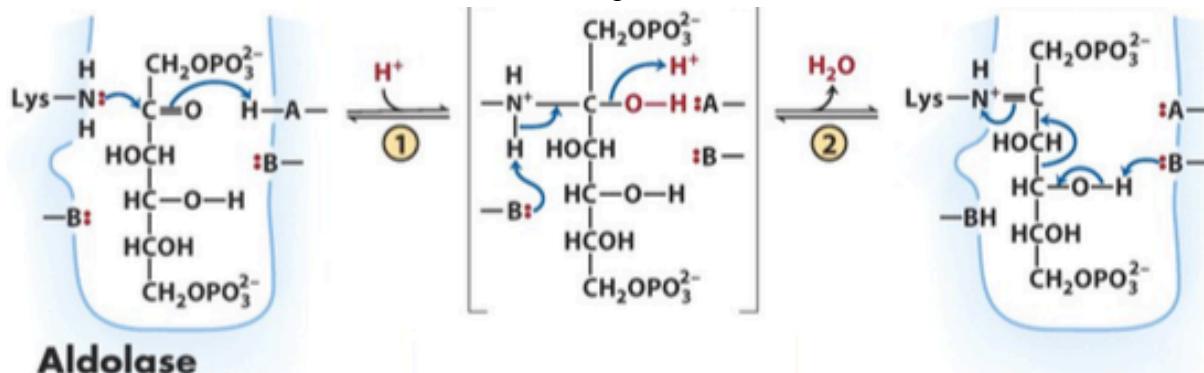
- Glycolysis splits a 6-carbon sugar into two 3-carbon sugars.
- 2 ATP invested, 4 ATP produced (2 net ATP).
- ATP production steps are fueled by oxidation (G3P dehydrogenase).

E. Minor principle of metabolism: a few chemical transformations are used over and over.

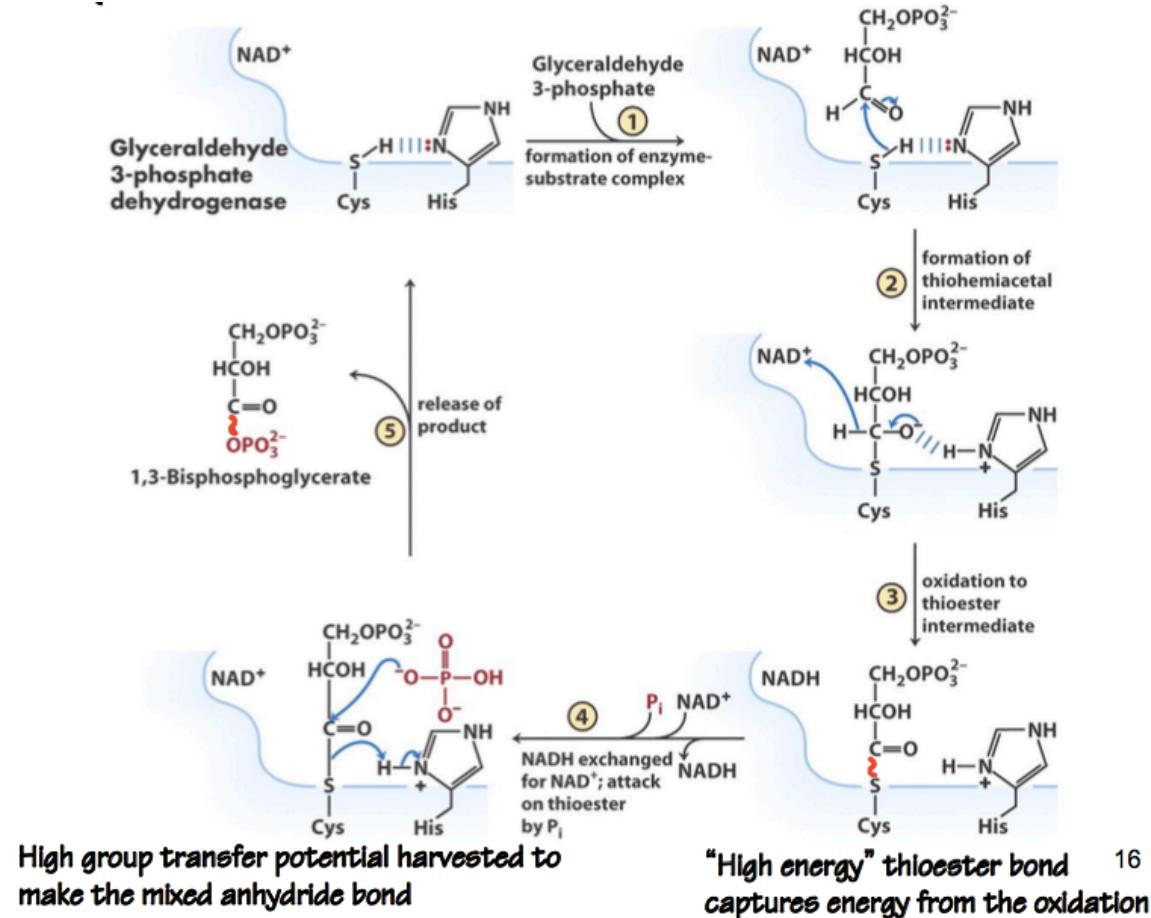
- Glycolysis has 2 isomerases, 4 kinases, and a dehydrogenase.
- Each class of enzyme uses similar mechanisms, and these reactions are in many other pathways.

F. Mechanisms of glycolytic enzymes

- Aldolase type I mechanism: aldol cleavage of C—C
 - A and B are general acidic and basic amino acids
 - The protonated Schiff base after (2) acts as an electron sink to cleave C—C.
 - G3P is released as the first product.
 - The protonated imine reforms and is hydrolyzed.
 - DHAP is released as the second product.



b. Energy coupling: G3P dehydrogenase



G. Regulating a metabolic pathway: theory

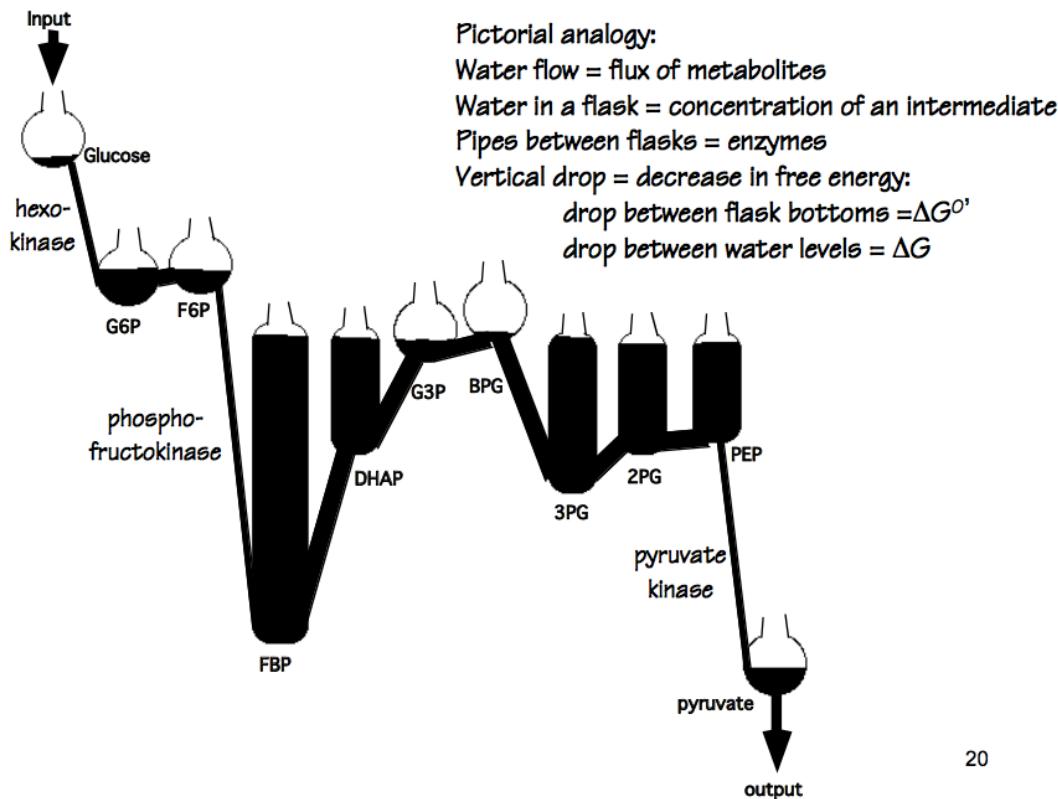
a. Control of flux

- i. Metabolic flux: number of metabolites going through a pathway per unit time.
Metabolites: intermediates/products of metabolic processes
- ii. Flux through glycolysis varies by >100-fold in muscle depending on need for ATP
- iii. Levels of glycolytic intermediates hardly change despite large changes in flux

b. Thermodynamics review:

- i. ΔG° is standard free energy change, ΔG is actual free energy change
- ii. For the reaction A \rightleftharpoons B, $\Delta G = \Delta G^{\circ} + RT \ln K$; $K = [B]/[A]$
- iii. Measuring steady-state levels of intermediates in glycolysis:

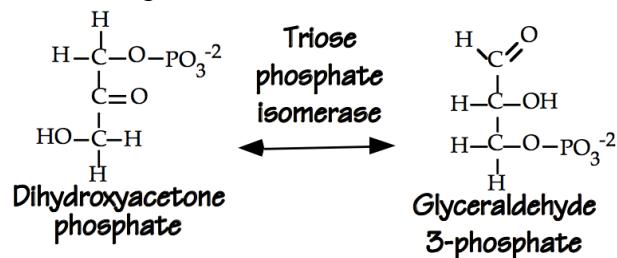
enzyme	ΔG° kcal/mol	ΔG kcal/mol
hexokinase	-4.0	-8.0
phosphohexose isomerase	+0.4	near equilibrium ¹
phosphofructokinase	-3.4	-5.3
aldolase	+5.7	near equilibrium
triose phosphate isomerase	+1.8	near equilibrium
glyceraldehyde 3-phosphate dehydrogenase	+1.5	near equilibrium
phosphoglycerate kinase	-4.5	near equilibrium
phosphoglycerate mutase	+1.1	near equilibrium
enolase	+0.4	near equilibrium
pyruvate kinase	-7.5	-4.0



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c. (Near) equilibrium reactions ($\Delta G \approx 0$)

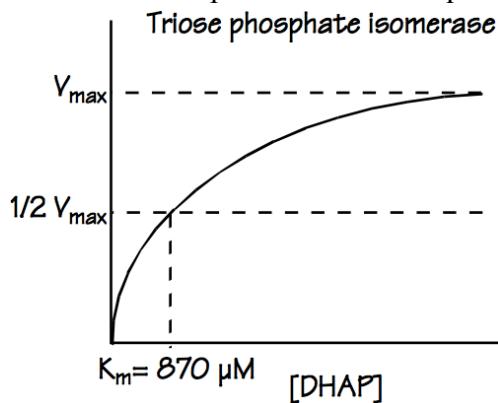
- Enzyme is present at high activity level: substrates and products stay at equilibrium despite changes in pathway flux. (The reaction “goes with the flow” no matter the flux)
- Equilibrium example:



1. $\Delta G^0 = +1.8 \text{ kcal/mol}$; $K_{eq} = 0.042$

2. But: cells have ~24-fold more DHAP than G3P, so $\Delta G \approx 0$

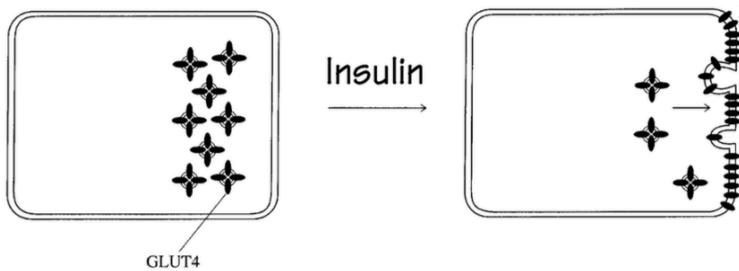
- In cells, $[DHAP] \approx 51 \mu\text{M}$. If flux increases and $[DHAP]$ increases, the catalytic rate increases to keep the reaction at equilibrium.



- $K_m > [S]$ so the initial slope is very steep; enzyme has large response to changes in $[S]$

H. Factors that limit the flux through glycolysis

- a. Supply of accessible glucose (low input)
- b. Transmembrane glucose passive transport proteins (GLUT4) amount is regulated by insulin.



- c. Capacity of glycolytic enzymes to process glucose (narrow pipes)
 - i. Three nonequilibrium reactions together can limit flux.
 - ii. Regulation by allosteric effectors (fast), modifications like phosphorylation (slower), and altering amount of enzyme (slow)

I. Maintaining homeostasis

- a. Narrowing one skinny pipe would back up some flasks, expanding one would drain others.
- b. Fluctuation results in osmotic problems and may accidentally interfere with connected pathways.
- c. Needs coordinated regulation of nonequilibrium reactions (narrow/expand all 3 pipes)
- d. Ex: In galactosemia, a missing enzyme causes accumulation of toxic substances.

J. Summary

- a. The 10 chemical steps of glycolysis
- b. The chemical logic of the 10 steps
- c. Mechanisms of some important glycolytic enzymes
- d. Regulating a metabolic pathway: theory

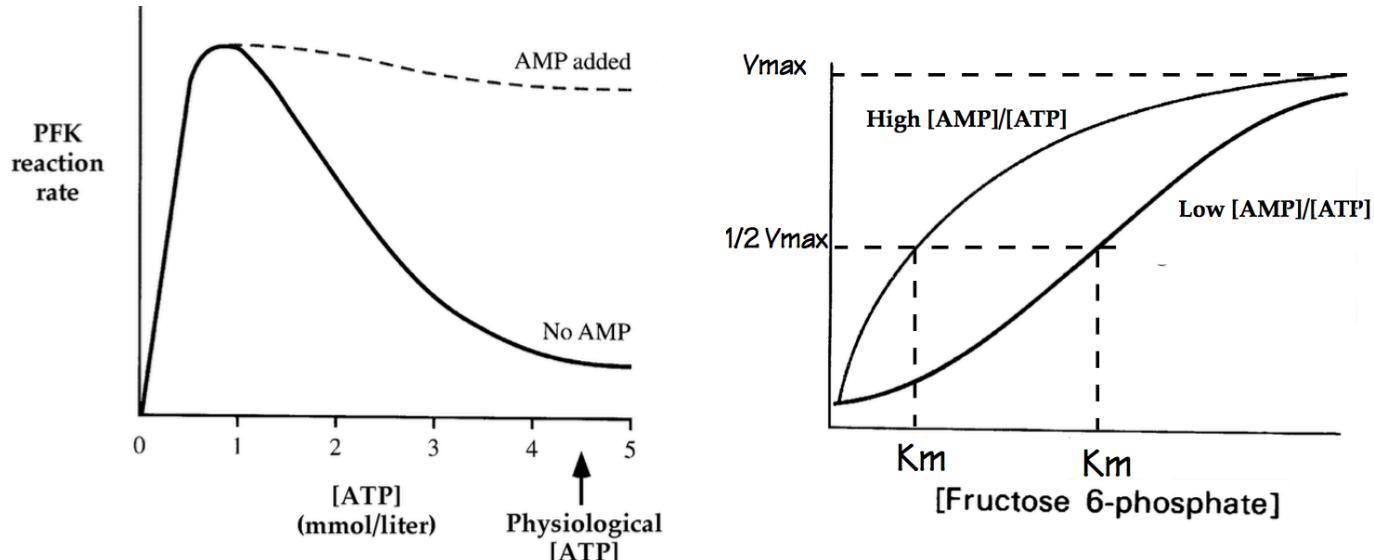
Lecture 17: Glycolysis II and Gluconeogenesis

A. Regulation by cellular energy charge

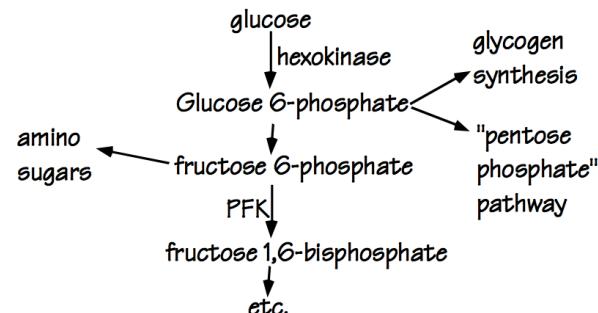
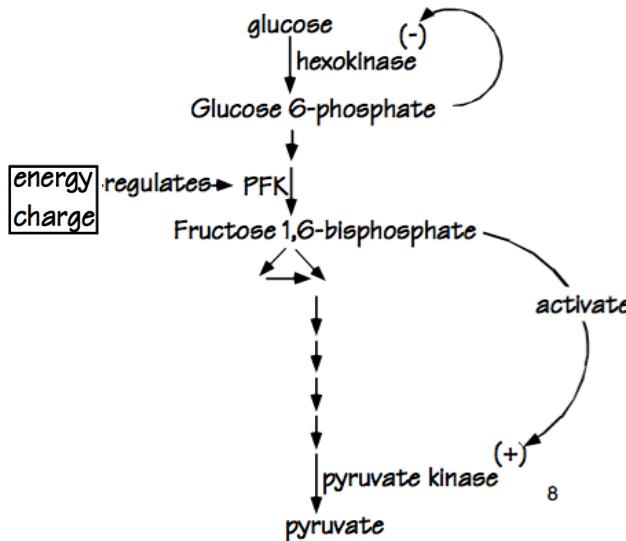
- a. Energy charge: $\frac{[ATP] + \frac{1}{2} [ADP]}{[ATP] + [ADP] + [AMP]}$; measure of energy status of biological cells
($\frac{1}{2}$ high-energy phosphate bonds per A?P)
- b. Adenylate kinase interconverts the A?Ps.
- c. Highly responsive: [ATP] varies by less than 10% despite large changes in utilization
- d. However, because [AMP], [ADP] \ll [ATP], large fluctuations in these indicate energy charge.

Adenine nucleotide	Concentration before ATP depletion (mM)		Concentration after ATP depletion (mM)		Relative change
ATP	5.0		4.5		10%
ADP	1.0		1.0		0
AMP	0.1		0.6		600%

- e. ATP and AMP allosterically regulate phosphofructokinase
- f. Physiological levels of [ATP] can completely block PFK, but this is relieved by AMP
Thus, this ratio [AMP]/[ATP] controls PFK activity by influencing K_m .



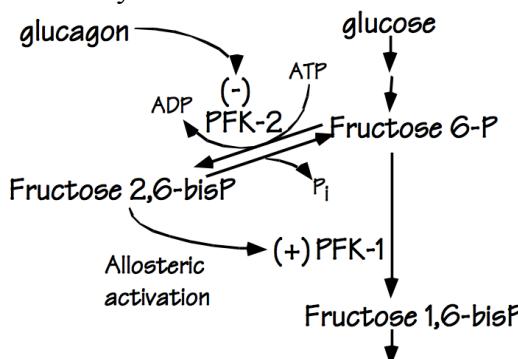
- i. PFK is an allosteric enzyme. Like hemoglobin, tetramer with cooperative binding.
- ii. Allosteric effectors bind remote from active site, shift equilibrium between high- and low- affinity conformations.
- iii. $[S] \approx K_m$ for allosteric enzymes. This is where the curves are separated the most and regulation is most effective.
- g. Hexokinase and pyruvate kinase are regulated to match PFK
 - i. Hexokinase is feedback inhibited by G6P (its own product)
 - ii. Pyruvate kinase is blocked by physiological levels of ATP, but this is relieved (feed-forward activated) by F1,6P (product of PFK)
 - iii. Together, these allow the three choke-points to match each other.



- h. PFK is the control point because it's the “first committed step” – first non-equilibrium enzyme NOT in other pathways. Also target of non-energy-charge regulation.

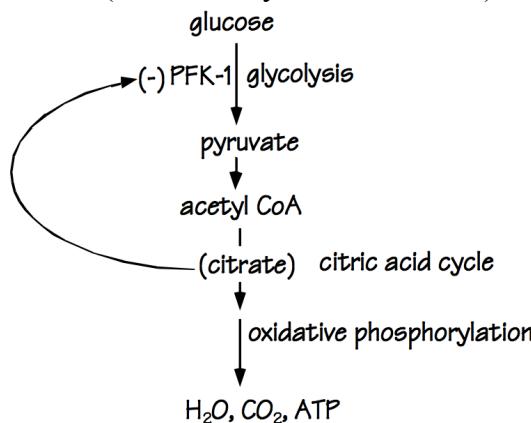
B. Regulation by glucagon hormone (liver only)

- Glucagon: hormone in mammals signaling low blood [glucose]
Inhibits glycolysis in the liver (which has the receptor), which provides glucose to other tissues.
- Glucagon mechanism: binds glucagon receptor; signal cascade activates a protein kinase.
This phosphorylates and inactivates PFK-2 → reduces synthesis of PFK-1 activator F2,6P.
- Bisphosphate is when the phosphate groups are attached to different carbons. Biphosphate is when they're on the same carbon.

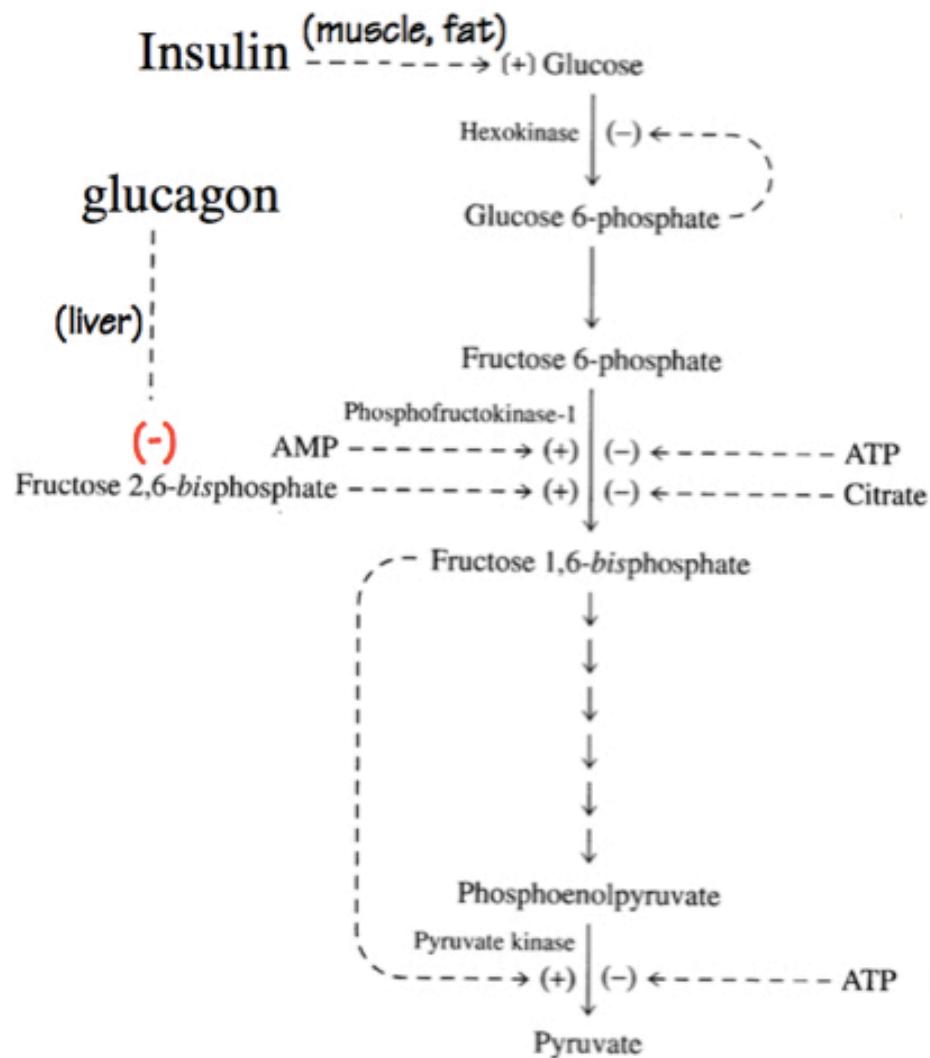


C. Regulation to coordinate glycolysis with other pathways

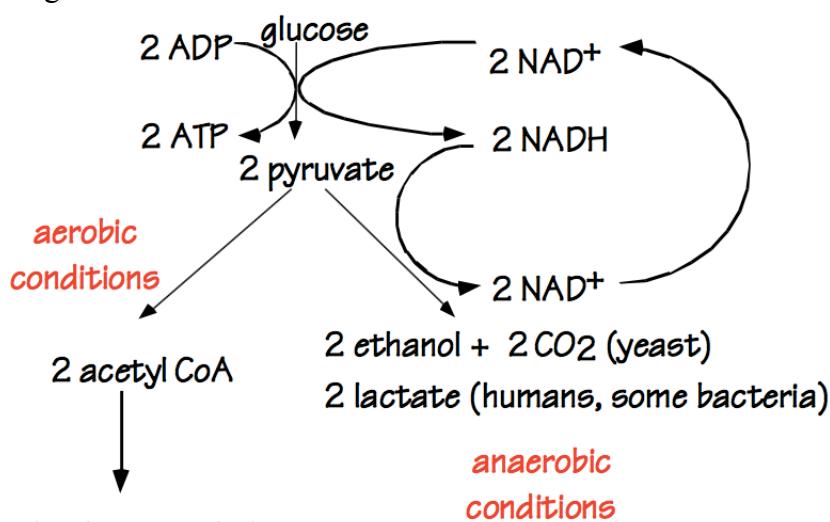
- Citrate (citric acid cycle intermediate) feeds back to allosterically inhibit PFK-1



b. Summary of glycolysis regulation



c. Regeneration of NAD^+

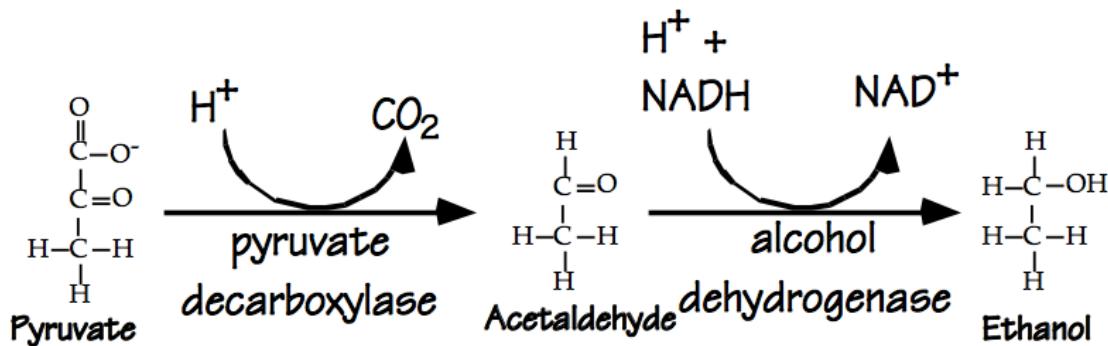


Further metabolism:

NADH reduces O_2

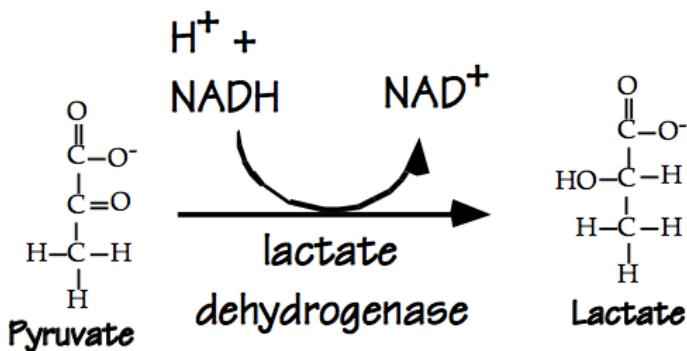
regenerating NAD^+

d. Alcoholic fermentation



- $\text{Glucose} + 2 P_i + 2 \text{ADP} + 2 H^+ \rightarrow 2 \text{EtOH} + 2 CO_2 + 2 \text{ATP} + 2 H_2O$
The point of fermentation is to recycle NAD^+ , so it is absent from the net equation
- Only 2 ATP per glucose, much lower than the ~30 from aerobic respiration.

e. Homolactic fermentation



- Net: $\text{Glucose} + 2 P_i + 2 \text{ADP} \rightarrow 2 \text{lactate} + 2 \text{ATP} + 2 H_2O$
 - Lactate: responsible for muscle ache; released to blood, processed by liver.
 - Microorganisms: released from cell; curdles milk, yogurt, and cheese.
- f. Aerobic metabolism: $\text{pyruvate} + \text{NAD}^+ + \text{CoA} \rightarrow \text{acetyl CoA} + \text{CO}_2 + \text{NADH}$
This uses NAD^+ (in oxidative phosphorylation) instead of recovering it.

Gluconeogenesis

A. Gluconeogenesis

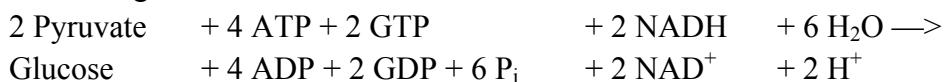
- Anabolic pathway; makes glucose from pyruvate; functional opposite of glycolysis
- Reversing glycolysis requires energy (ATP) to pump the non-equilibrium steps backwards.
- Note that the gluconeogenesis pathway is distinct from that of glycolysis.
 - Glycolytic steps that use ATP do not generate ATP in gluconeogenesis; it pops off P_i .
 - Similarly, ATP generation steps will consume ATP + GTP in gluconeogenesis.

B. Net reactions

- Glycolysis: $\Delta G^\circ = -20 \text{ kcal/mol}$



- Gluconeogenesis: $\Delta G^\circ = -9 \text{ kcal/mol}$

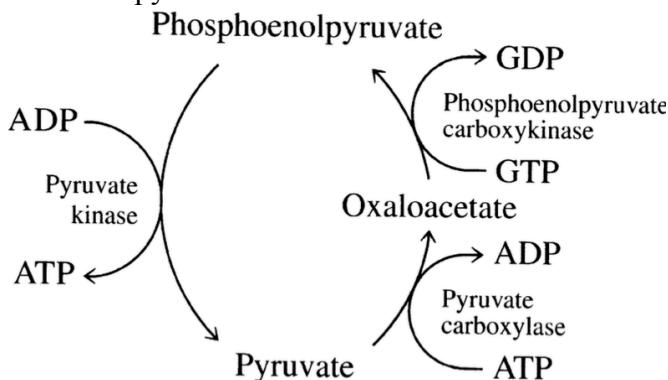


- 6 ATP/GTP equivalents drive gluconeogenesis, while glycolysis nets 2 ATP
The “cost” of gluconeogenesis is 4 ATP.

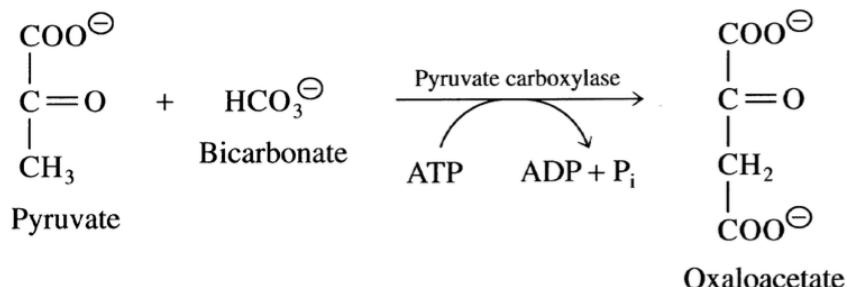
C. Enzymes

Glycolysis	Gluconeogenesis
Hexokinase	Glucose 6-phosphatase
Phosphofructokinase	Fructose 1,6-bisphosphatase
Pyruvate kinase	Pyruvate carboxylase Phosphoenolpyruvate carboxykinase

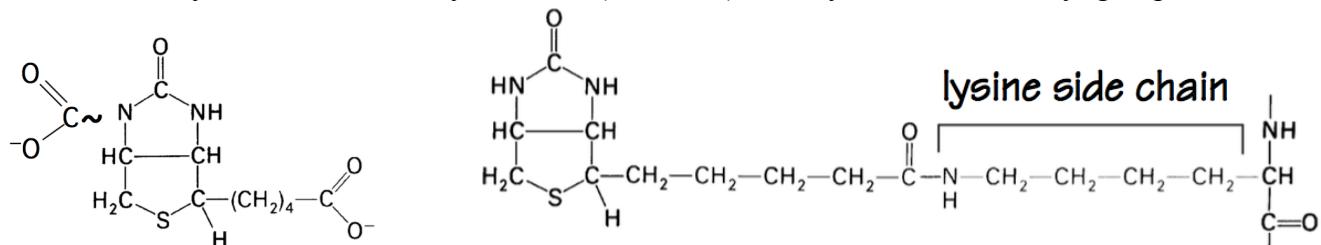
- Reversal of the pyruvate kinase reaction



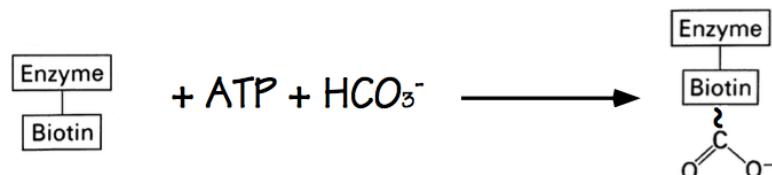
- Pyruvate enters the mitochondria
- Pyruvate carboxylase (only in mitochondria) catalyzes the following:



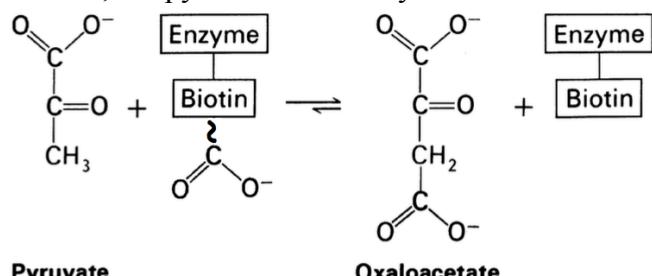
- b. Carboxylases use the coenzyme biotin (a vitamin) to carry activated carboxyl groups



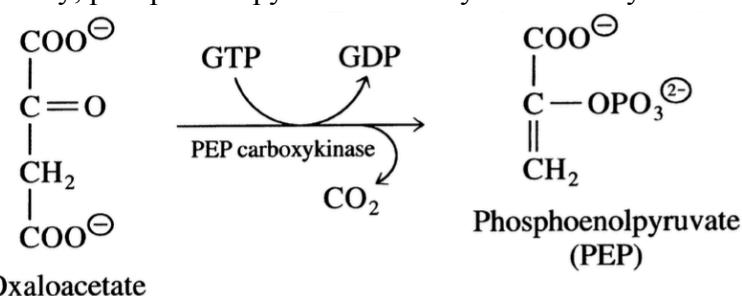
- i. Biotin is covalently attached to a Lys residue of pyruvate carboxylase (amide bond)
 - ii. Long arm allows biotin to swing between 2 active sites
 - iii. First, the ATP-bicarbonate site catalyzes the following:



- iv. Second, the pyruvate site catalyzes:



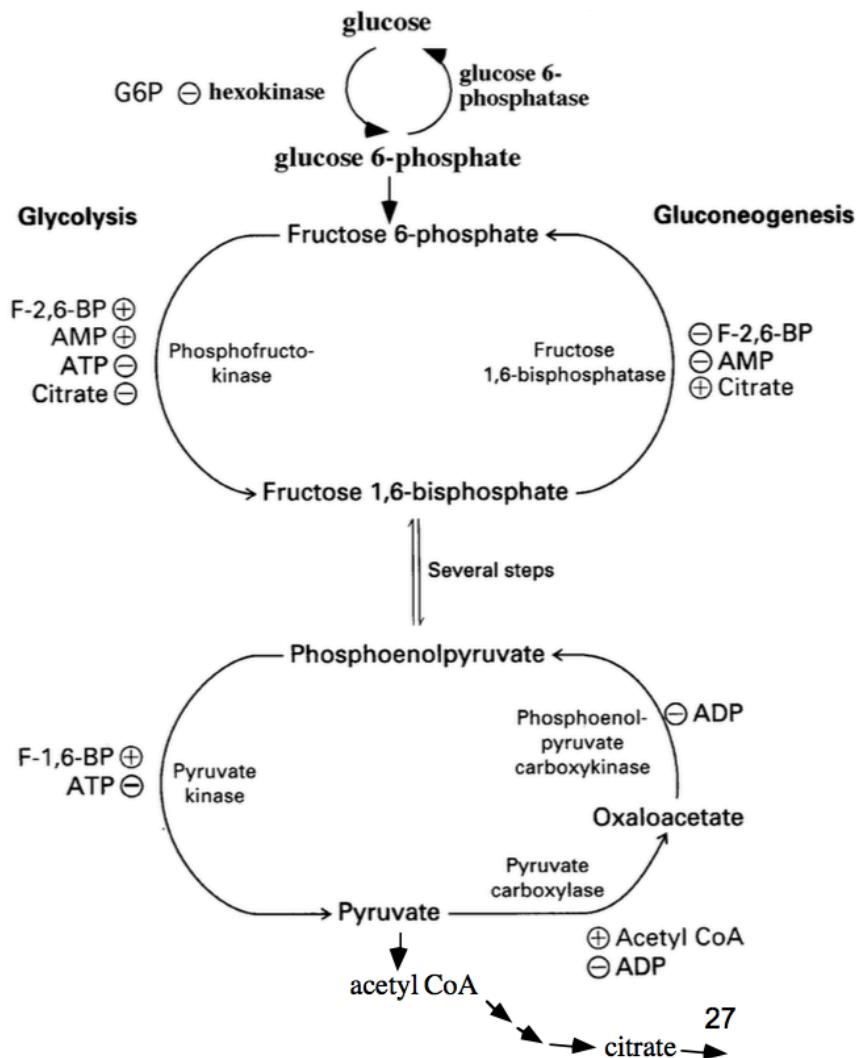
- c. The “oxaloacetate/malate shuttle” transports mitochondrial NADH to the cytosol for gluconeogenesis (see diagram on next page)
 - d. Oxaloacetate is formed in the mitochondria and shuttled back to the cytosol. Why?
 - i. Net effect: moves mitochondrial NADH (high) to the cytosol (low).
 - ii. Mitochondria sometimes need oxaloacetate for the citric acid cycle.
 - e. Finally, phosphoenolpyruvate carboxykinase catalyzes:



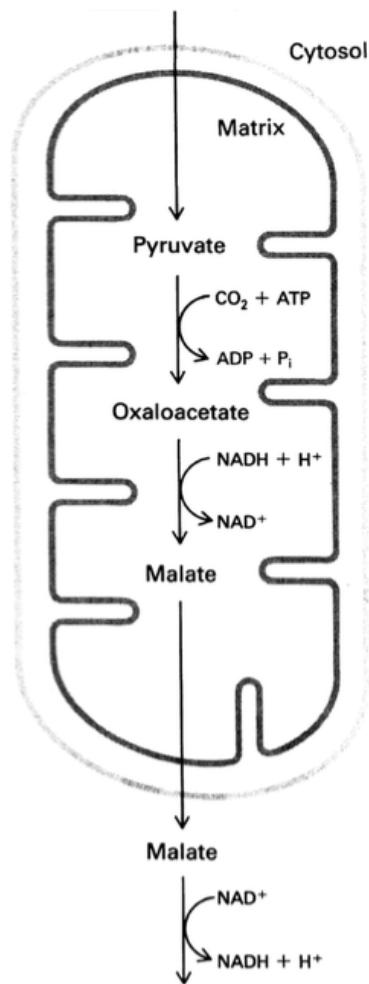
Oxaloacetate

D. Regulation

- a. Glycolysis and gluconeogenesis are reciprocally regulated
 - i. Low energy charge: -lysis up, -genesis down.
 - ii. Downstream metabolites: -lysis down, -genesis up
 - iii. Glucagon (liver, via F2,6bisP) -lysis down, -genesis up
 - iv. Insulin (muscle, fat): increases [glucose] by -lysis up
 - v. Homeostasis: feed-forward/back via F1,6P and G6P



Pyruvate

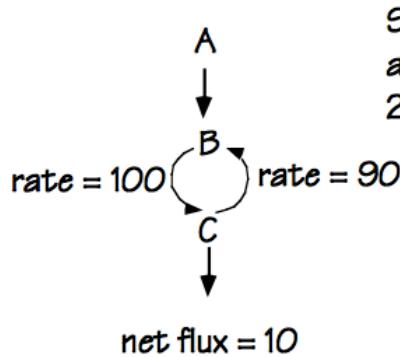


Oxaloacetate 25

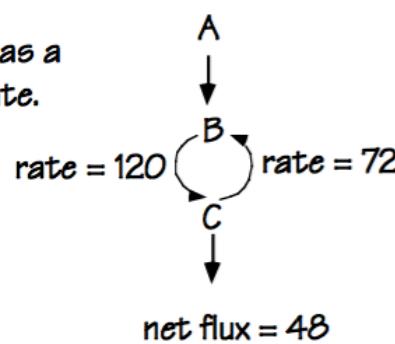
E. Substrate cycles amplify metabolic regulation

- Definition: interconversion of two metabolites by different enzymes (also: futile cycles)
- Glycolysis/gluconeogenesis have 3 substrate cycles
- Assume initial rates of 100 and 90, with net flux of 10.

If an allosteric effector increases the rate of B → C by 20% and decreases the rate of C → B by 20%, the overall flux goes to 48, which is 480% of the original value.

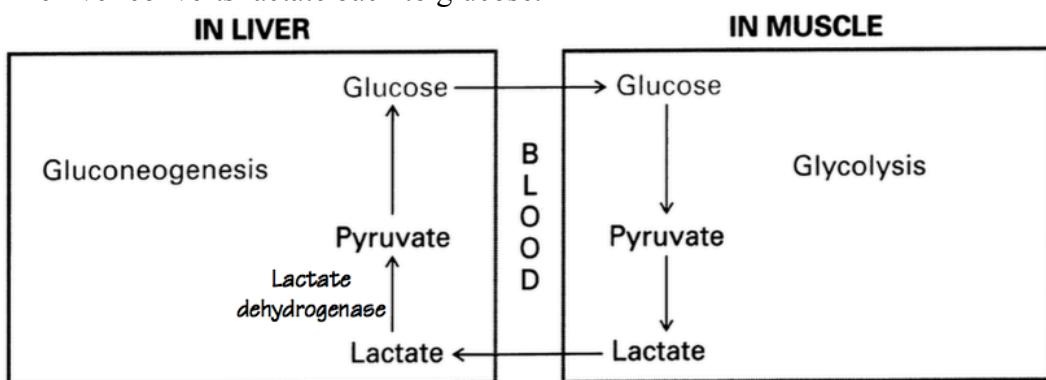


Suppose reciprocal allosteric regulation has a 20% effect on each rate.



F. The Cori cycle

- a. Homolactic fermentation builds up lactate in low-O₂ conditions.
- b. The liver converts lactate back to glucose.



G. Summary

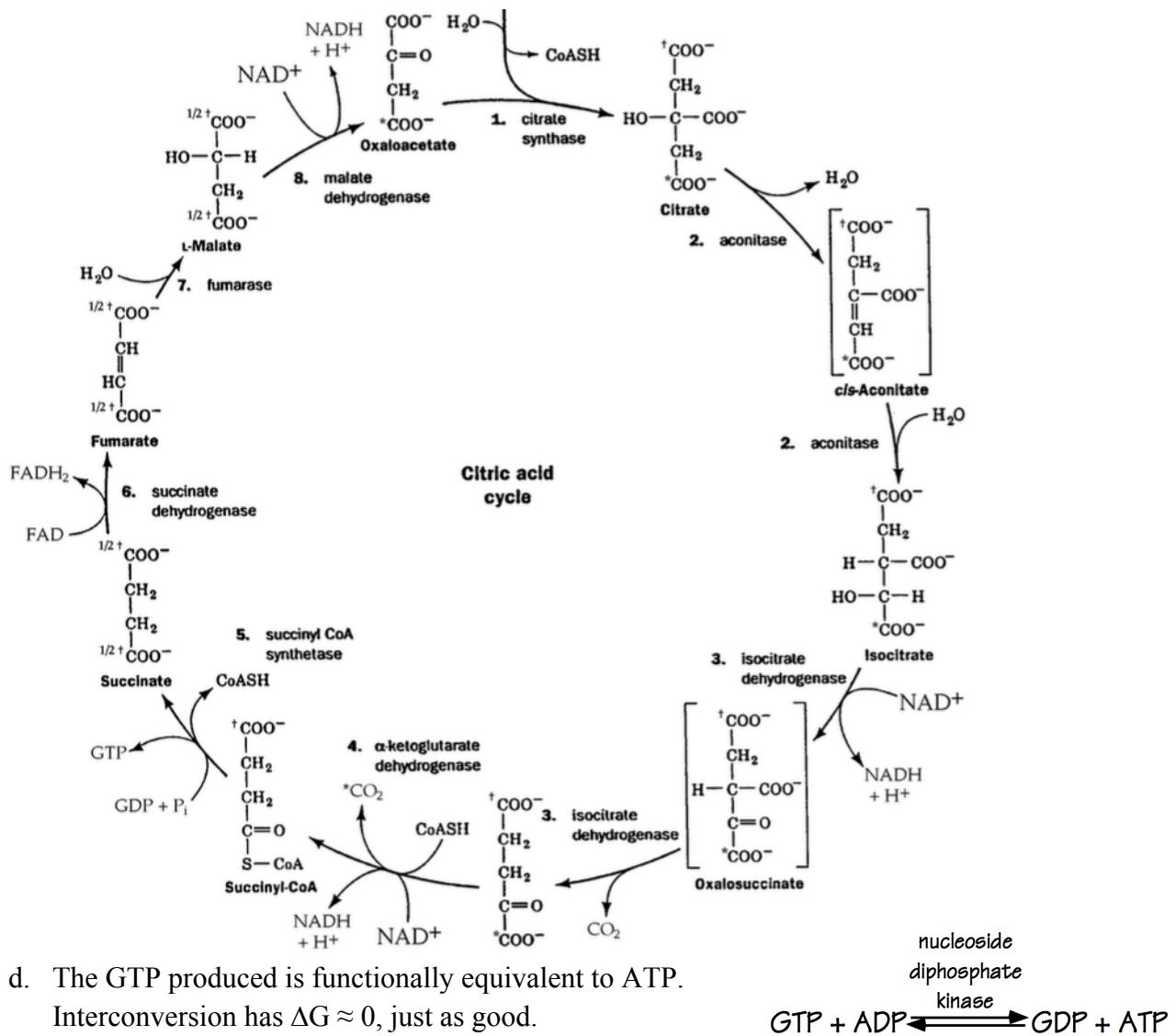
- a. Metabolic pathways have chemical logic
- b. Nonequilibrium reactions are regulated to control flux and homeostasis
- c. Reciprocal catabolic and anabolic pathways differ such that each is thermodynamically feasible, and they can be reciprocally regulated.

Lecture 18: Citric Acid Cycle

- A. Intro to the Citric Acid/Krebs/TCA (tricarboxylic acid) cycle
- Pathway that oxidizes acetyl CoA to 2 CO₂, driving ATP production
 - Breakdown products of glucose, fats, and amino acids are all oxidized via the cycle
 - The CAC is amphibolic: Intermediates are also source for many biosynthetic pathways

B. Overview of the Citric Acid Cycle

- For every turn of the cycle:
 - Joins an acetyl group to oxaloacetate
 - Oxidizes 2 carbons off as CO₂, leaving succinate
 - Converts succinate back to oxaloacetate
 - Produces 1 GTP and reduced cofactors (3 NADH and 1 FADH₂)
- Cycle intermediates act catalytically
(Adding a little oxaloacetate allows the cycle, but does not get consumed by it → ++CO₂)
- Net reaction: Acetyl CoA + 3 NAD⁺ + FAD + GDP + P_i + 2 H₂O → 2 CO₂ + CoA + 3 NADH + FADH₂ + GTP + 2 H⁺



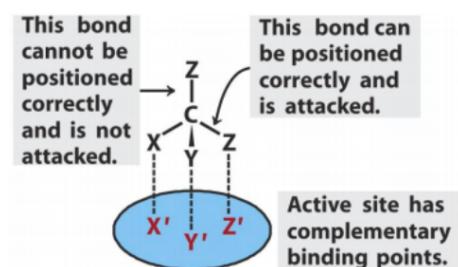
C. Isotopic labeling experiments

a. Steps

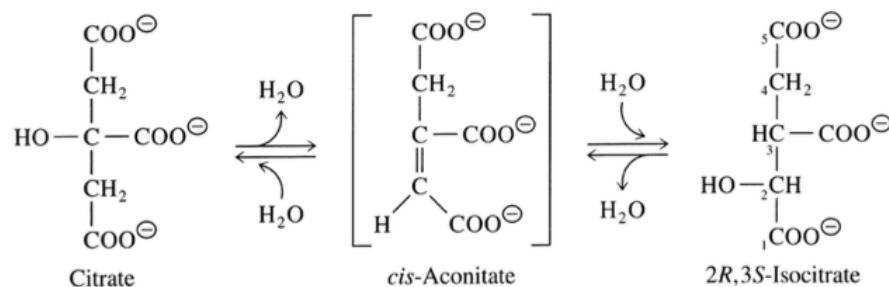
- Synthesize intermediates labeled with ^{14}C . Add to cell extract, isolate cyclic intermediates/products, and analyze for label
- First, ^{14}C -oxaloacetate was added with normal acetyl-CoA, so only 1 of the identical halves of citrate product was labeled. All of the ^{14}C came out in the first round.
- Second, ^{14}C -Acetyl-CoA was added with normal oxaloacetate, so only 1 of the identical halves of citrate product was labeled. Ordinarily, $\frac{1}{2} \text{CO}_2$ would have ^{14}C , because of the symmetry of the 2 acetyl groups. BUT, the signal was scrambled at succinate, and the signal was bled out over many cycles. None of the CO_2 released had ^{14}C in the first cycle.

b. Implication

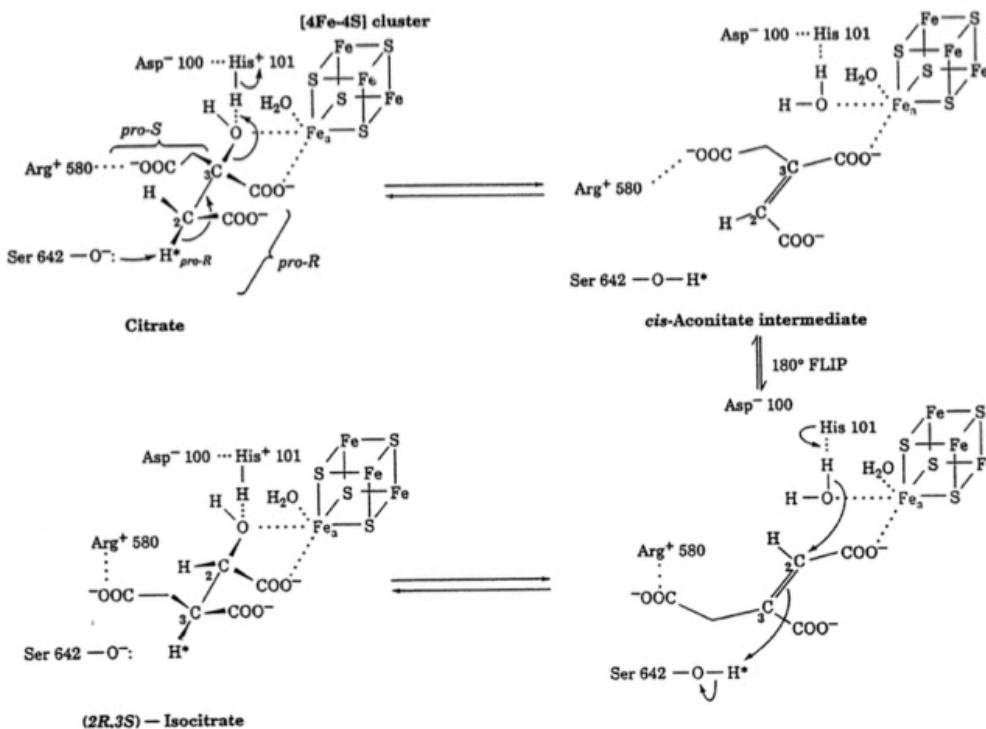
- Citrate has a prochiral carbon – one that can be converted from achiral to chiral in a single step.
- The trick is that the aconitase active site is asymmetric, and can act on citrate as though it were chiral. With at least 3 contact points, left and right acetyl groups can be distinguished.



D. Aconitase mechanism of action

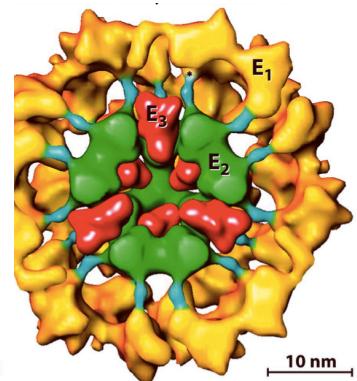
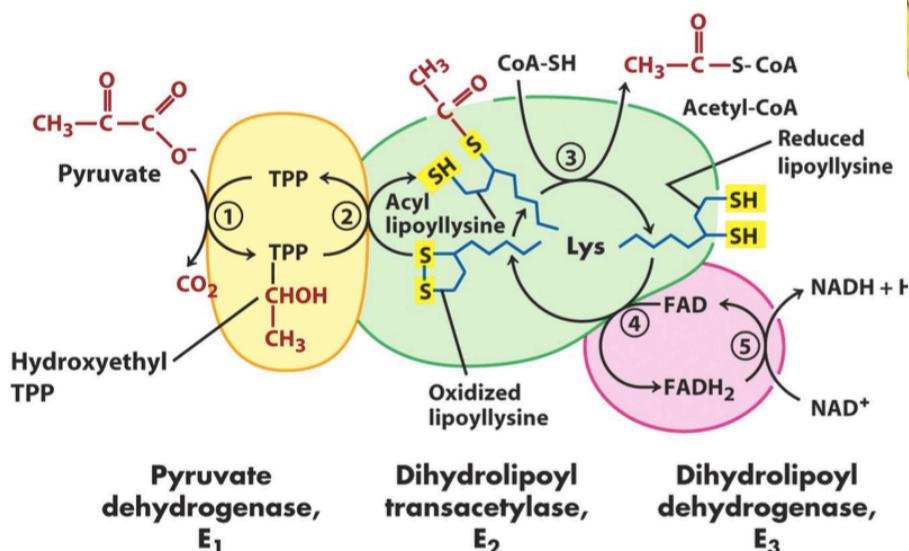


- There are ≥ 3 contacts with the enzyme.
- Mechanism: dehydrate, flip bound substrate 180°, stereospecifically add back H_2O



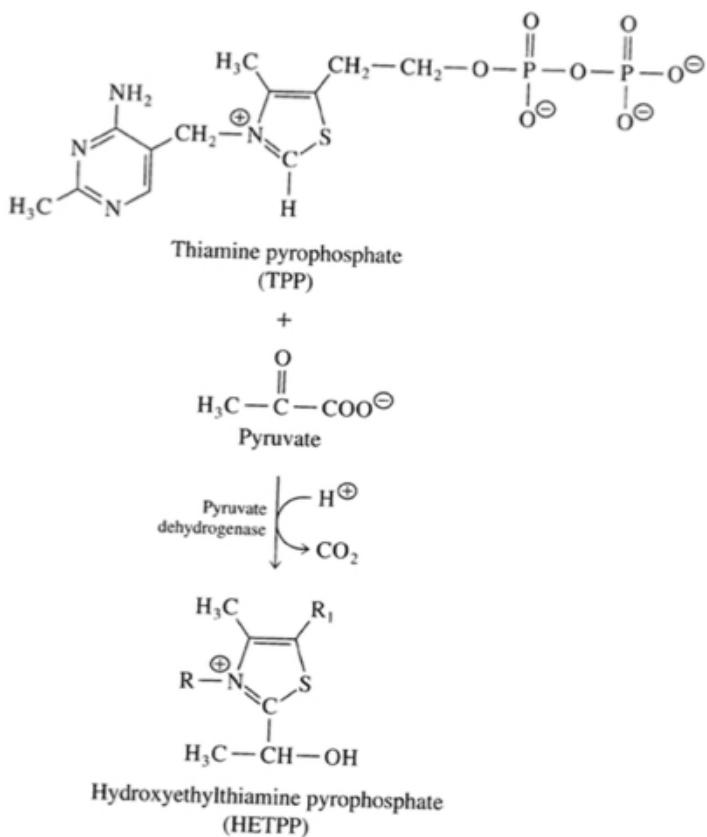
E. Pyruvate dehydrogenase (between glycolysis and CAC)

- Pyruvate + NAD⁺ + CoA → Acetyl CoA + CO₂ + NADH
- Purified enzyme is a huge protein complex ~500 Å across (100x bigger than a typical enzyme)
- In mammals: 3 polypeptides (E1, E2, E3)
 - Core of 60 E2 (20 trimers).
 - 12 E3, and 60 E1 are bound to the core.
 - E2 has a swinging arm that picks up substrate from E1 and moves it to E3.



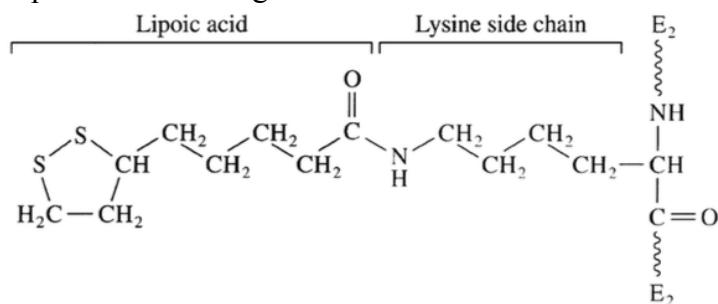
d. Reaction 1: E1-catalyzed

- Thiamine = vitamin B1; deficiency causes beriberi



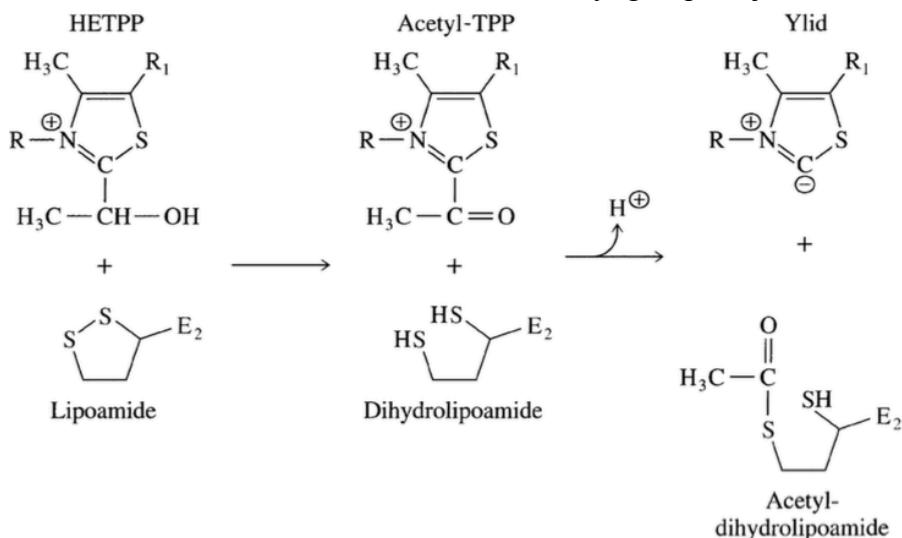
e. Reaction 2: E1-catalyzed

i. Lipoamide on a long flexible arm



ii. Lipoamides from different E2s can pass acetyl groups to each other

This allows all 60 E2 subunits to transfer acetyl groups to just 12 E3 units



iii. Oxidation to acetyl/Reduction to HS, then transfer of acetyl group.

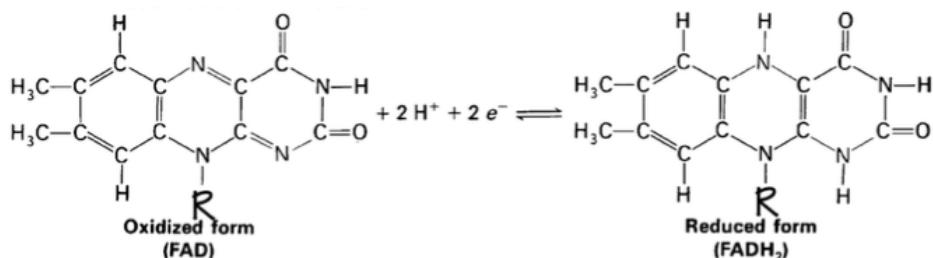
f. Reaction 3 ???

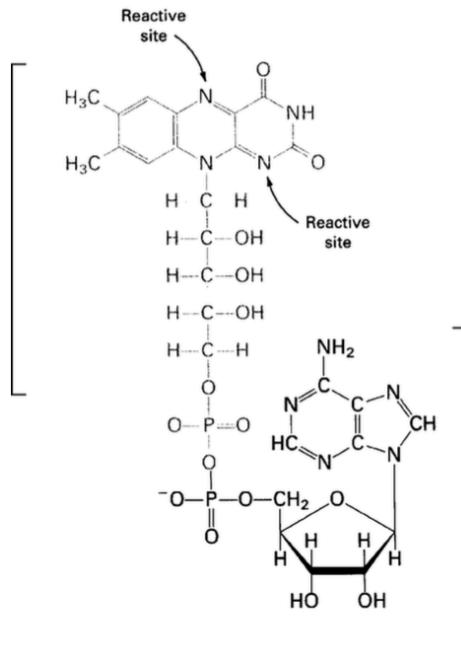
g. Reaction 4 and 5 use FAD

i. FAD: flavin adenine dinucleotide (electron carrier)

ii. Riboflavin (vitamin B2); integral part of FAD

iii. When bound to pyruvate dehydrogenase, FADH₂ has higher e- transfer potential than NADH. This justifies a separate electron carrier.





F. Multi-enzyme complexes can carry out a complex series of reactions:

- These channel intermediates from one protein to the next, increasing speed and efficiency while minimizing side reactions. Free pathways like glycolysis allow for side reactions.
- Ribosome: 55 polypeptides
- DNA replication complex: >20 polypeptides
- RNA transcriptional initiation complex
- Spliceosomes, Proteasomes

G. Alpha-ketoglutarate dehydrogenase reaction is analogous to pyruvate dehydrogenase reaction

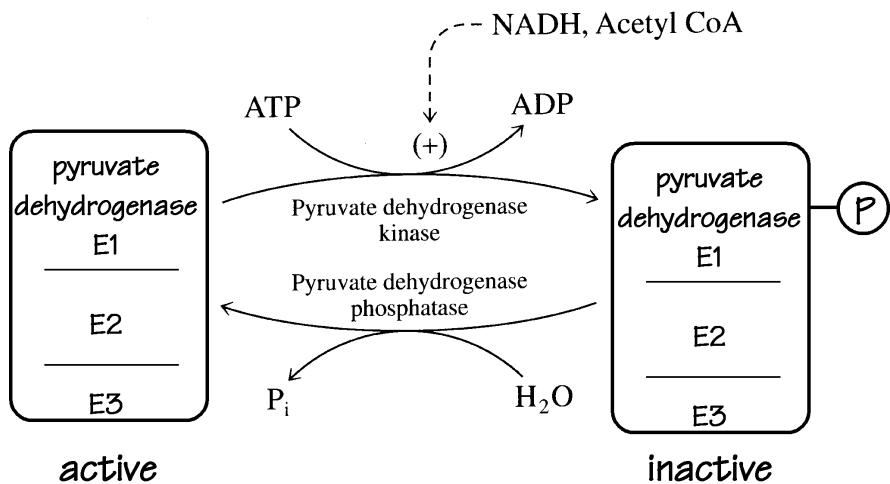
- Nearly identical multi-enzyme complex (same E3, different E1 and E2)

H. Regulation of the citric acid cycle

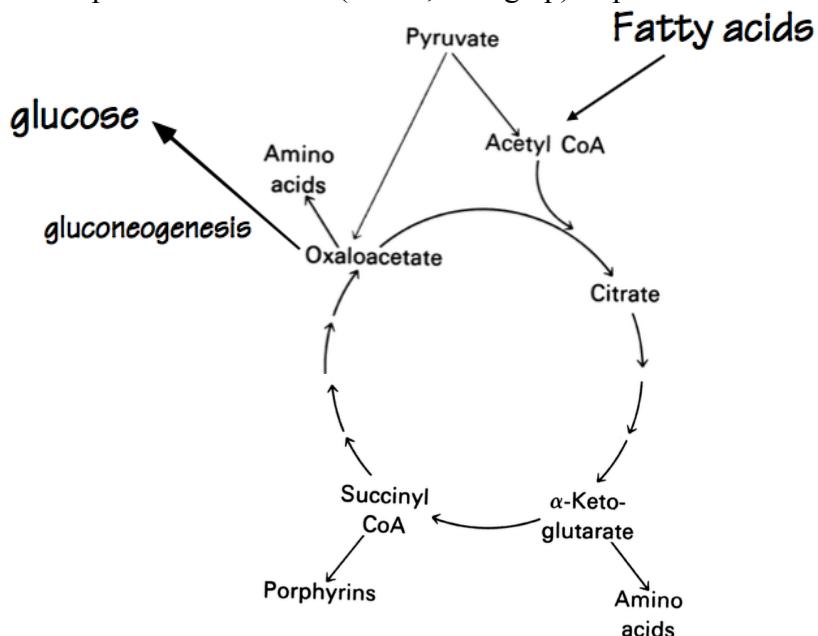
- Relatively simple: flux determined by availability of substrates (acetyl CoA, NAD⁺, FAD) and oxidants (NAD⁺ and FAD). The latter depends on regeneration by oxidative phosphorylation.
- Regulation may occur at the 3 non-equilibrium reactions:
Citrate synthase, isocitrate dehydrogenase, and alpha-ketoglutarate dehydrogenase.
- However, there's NO consensus on what the physiologically significant regulators are.

I. Regulation of pyruvate dehydrogenase

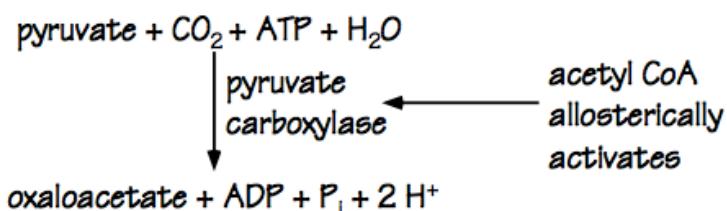
- End-product inhibition (not allosteric): high product affinity for active site, inhibiting turnover.
Also happens to hexokinase and G6P.
- Phosphorylation: activation of a kinase that inactivates the enzyme.
- Feedback inhibition by NADH and acetyl CoA:
Kinase and phosphatase are bound to E2 core, and these act on a specific Ser residue of E1



- J. The CAC is amphibolic: intermediates feed into biosynthetic pathways
- Anapleurotic reactions (Greek, filling up): replenish intermediates.

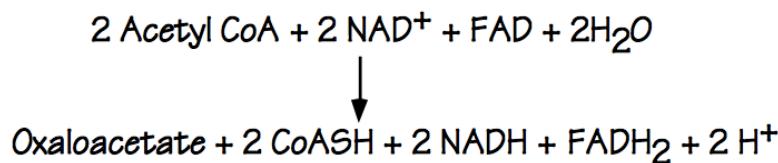


- If oxaloacetate runs out, the cycle stops, acetyl CoA builds up and activates pyruvate carboxylase to renew oxaloacetate from pyruvate.

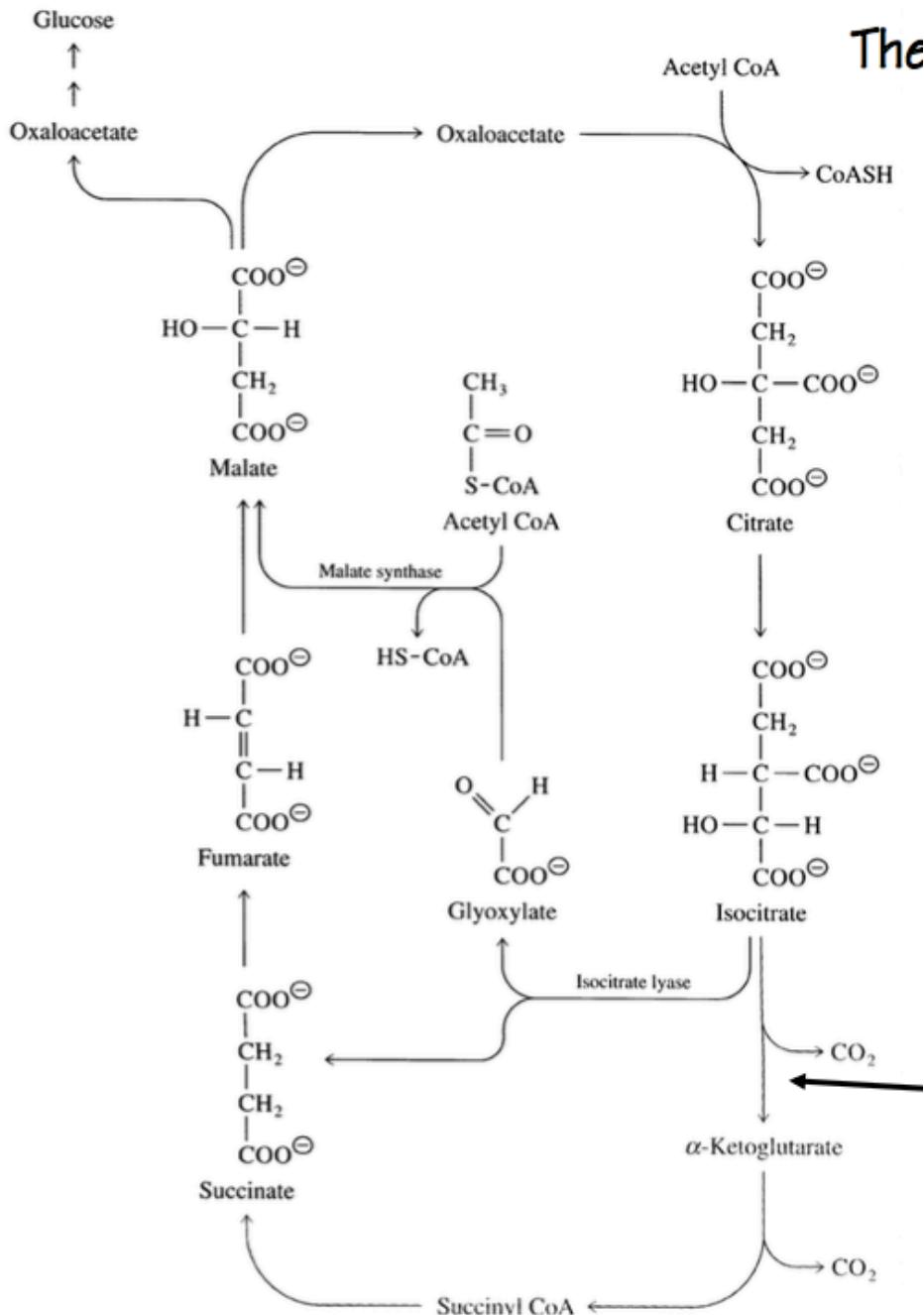


K. Fatty acids and the CAC

- Fatty acids can be broken into acetyl CoA and fed into the CAC. However, fatty acids cannot be used to synthesize carbohydrates because oxaloacetate is NOT produced (or consumed) with the addition of more reactant.
- Plants and bacteria use the glyoxylate cycle (modified CAC) to synthesize carbohydrates from acetyl CoA.



- c. Low [AMP] and high energy charge activates a kinase that inactivates isocitrate dehydrogenase, blocking CAC and starting the glyoxylate cycle. The arrow shows the regulation point.



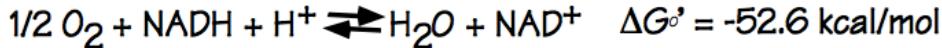
L. Main points

- CAC intermediates: needed in catalytic amounts, may be used up in biosynthetic reactions (and replaced by anapleurotic reactions).
- CAC regulated by availability of acetyl CoA and O_2 .
- Understand isotope labeling and prochirality.

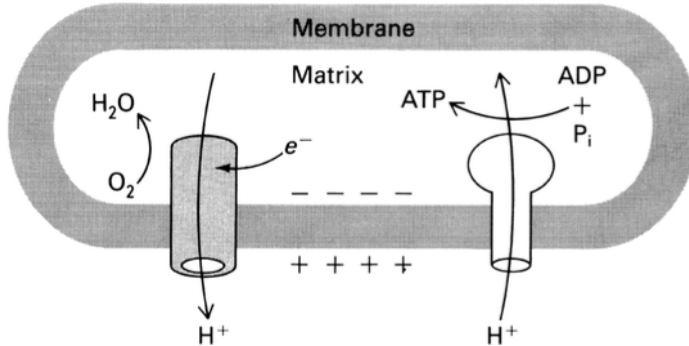
Lecture 19: Electron Transport & Oxidative Phosphorylation

Overview of the Electron Transport Chain (AKA Respiratory Chain)

- A. Summary of Energy Yield: 2 from glycolysis + 2 from CAC + (~)26 from ox-phos yields (~)30 total.
- B. Oxidative Phosphorylation: transfer of e- from NADH or FADH₂ to O₂ by electron carriers.



Stores this energy in an electrochemical proton gradient used to make ATP.

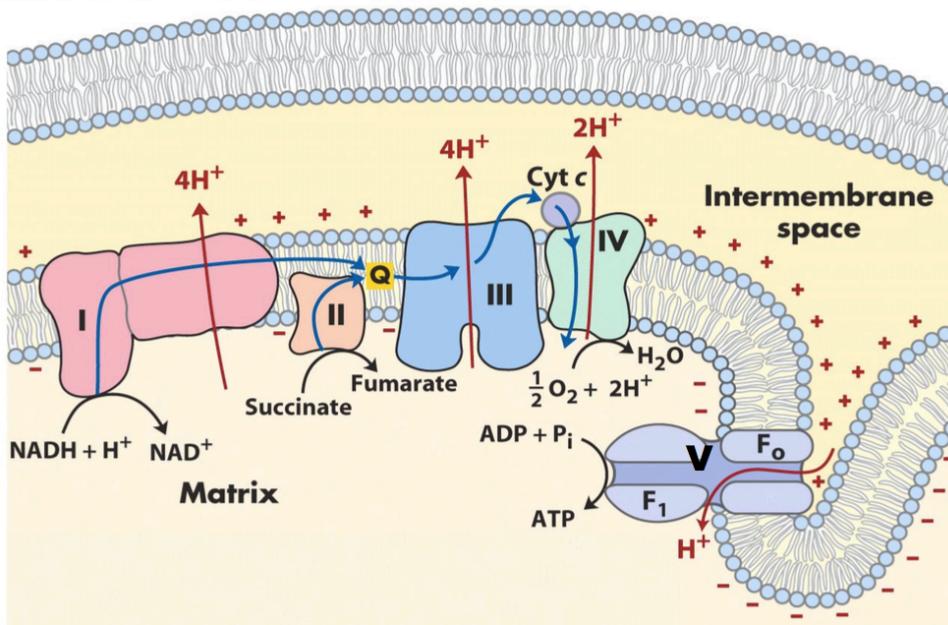


C. Mitochondrion

- a. Bacteria-sized, double-membrane organelle
- b. Outer membrane has porins (permeable to small metabolites like sugars, ions, and amino acids)
- c. Inner membrane is only permeable to very small molecules, e.g. O₂
 - i. Transporters required for ATP, P_i, pyruvate, etc.
 - ii. Higher protein/lipid than normal (80/20 > 50/50).
 - iii. Folding increases surface area for ox-phos machinery
- d. Matrix (innermost space) contains enzymes for CAC and fatty acid oxidation

D. Multiprotein complexes

5 Multiprotein complexes in the inner membrane



Succinate dehydrogenase is tied to complex II. It produces FADH₂, and the electrons are picked up by reducing coenzyme Q. This diffuses laterally and transfers them to complex III.

E. Electrochemical Gradient

- Electrical component: 0.14 V (outside is +)
- Chemical component: pH is 1.4 units < than inside (25x higher $[H^+]$)
- Combined proton-motive force: $0.224 \text{ V} = 5.2 \text{ kcal/mol H}^+ \text{ transported inside}$. Energy from 3 transported H^+ is more than enough to produce 1 ATP

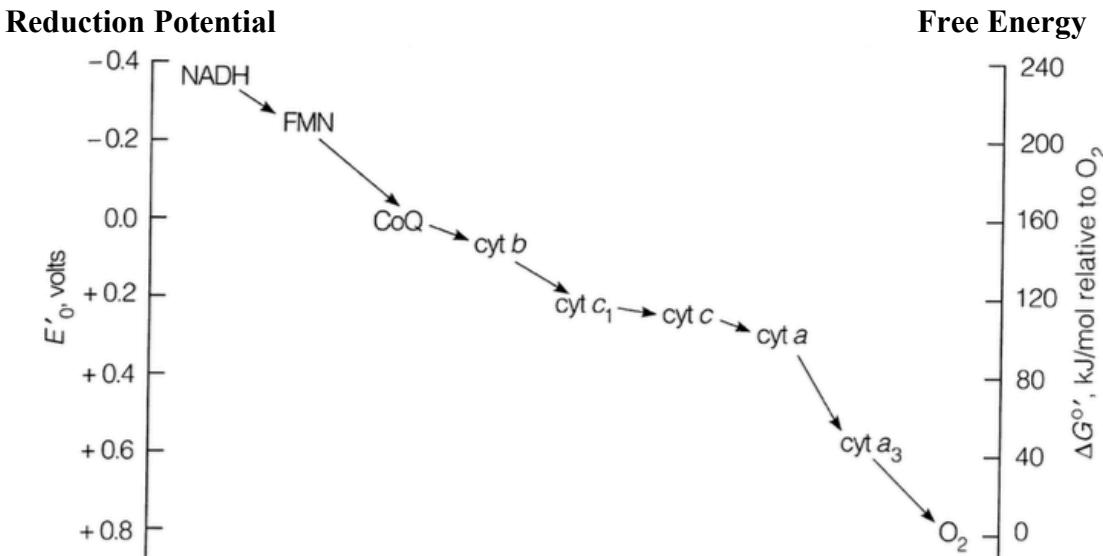
Electron Transport Chain Details

A. Redox components that carry electrons in a bucket brigade

	# polypeptide subunits	e^- carrying components
complex I (NADH-Q reductase)	42	FMN, 6-7 FeS
complex II (Succinate-Q reductase)	4	FAD, 3 FeS
coenzyme Q	0	itself
complex III (Cytochrome reductase)	11	Cyt b_H , Cyt b_L , FeS, Cyt $c1$
Cytochrome C	1	itself
complex IV (Cytochrome oxidase)	13	Cyt a , Cyt $a3$, Cu A , Cu B

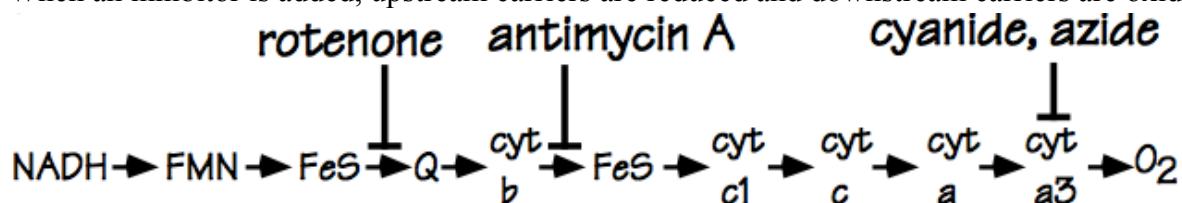
B. Reduction Potential and Direction of Flow

- Reduction potential (V) is directly related to affinity for electrons and free energy of transfer



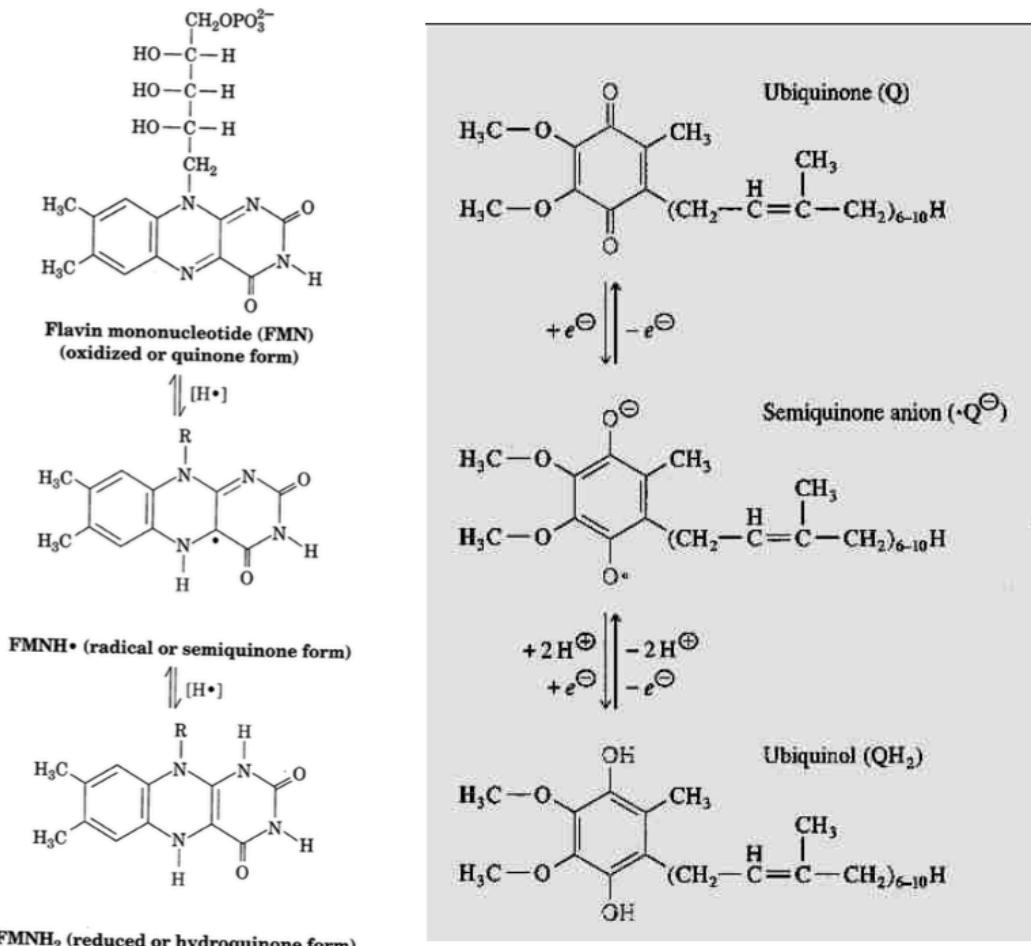
- b. Respiratory inhibitors confirm the order of carriers in the chain

When an inhibitor is added, upstream carriers are reduced and downstream carriers are oxidized.

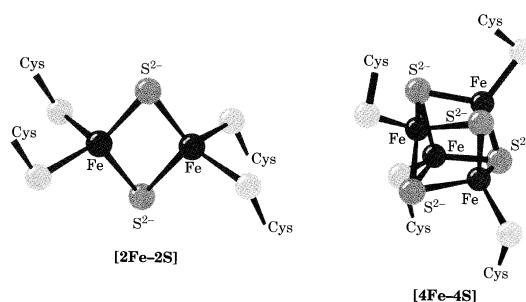


C. Electron Carriers

- FMN (complex 1) and coenzyme Q can each carry 1 or 2 electrons
- Q and QH₂ can freely move about the lipid bilayer to carry e⁻ from complexes I & II to III. The greasy alkene chain makes them hydrophobic.

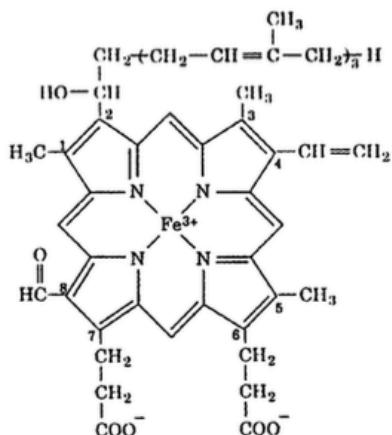


- Iron-sulfur clusters carry only 1 electron (in complexes I, II, and III)
 - Fe atoms are coordinated by S atoms (Cys side chains or free sulfide)

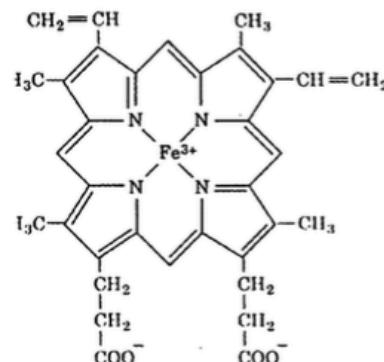


- Usually 2 Fe with 2 S, or 4 Fe with 4 S.
- Conjugated Fe are between the +2 and +3 oxidation states

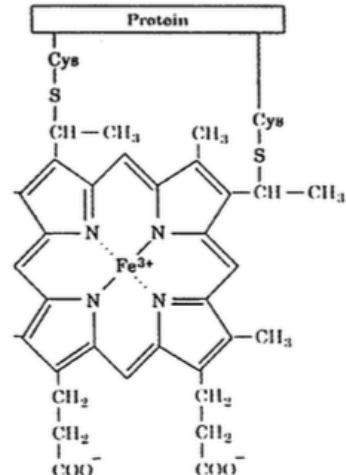
- d. Cu ions carry only 1 electron (in complex IV)
 - i. Alternate between +1 and +2 oxidation states
- e. Cytochromes carry 1 electron (complexes III, IV, and cytochrome C)
 - i. Small proteins with bound heme group. The heme iron alternates between +2 and +3.
 - ii. Each cytochrome has a different reduction potential, depending on heme conditions: Side chain structure, covalent bonds to protein, and protein environment.



Heme a



Heme b

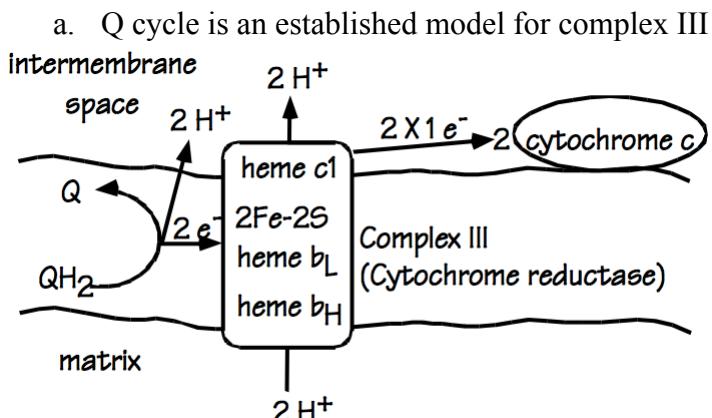


Heme c

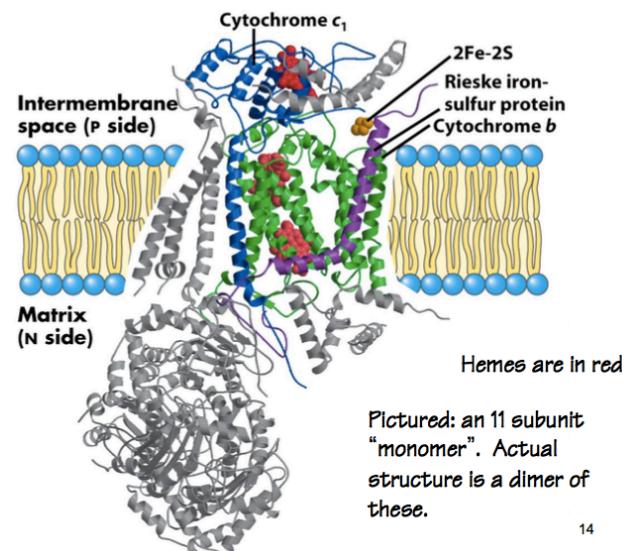
- D. Cytochrome C: peripheral membrane protein, shuttles e- between complexes III and IV.
Fe is liganded by His and Met side chains.

Note: e- carriers do NOT need to contact to transfer e- (???)

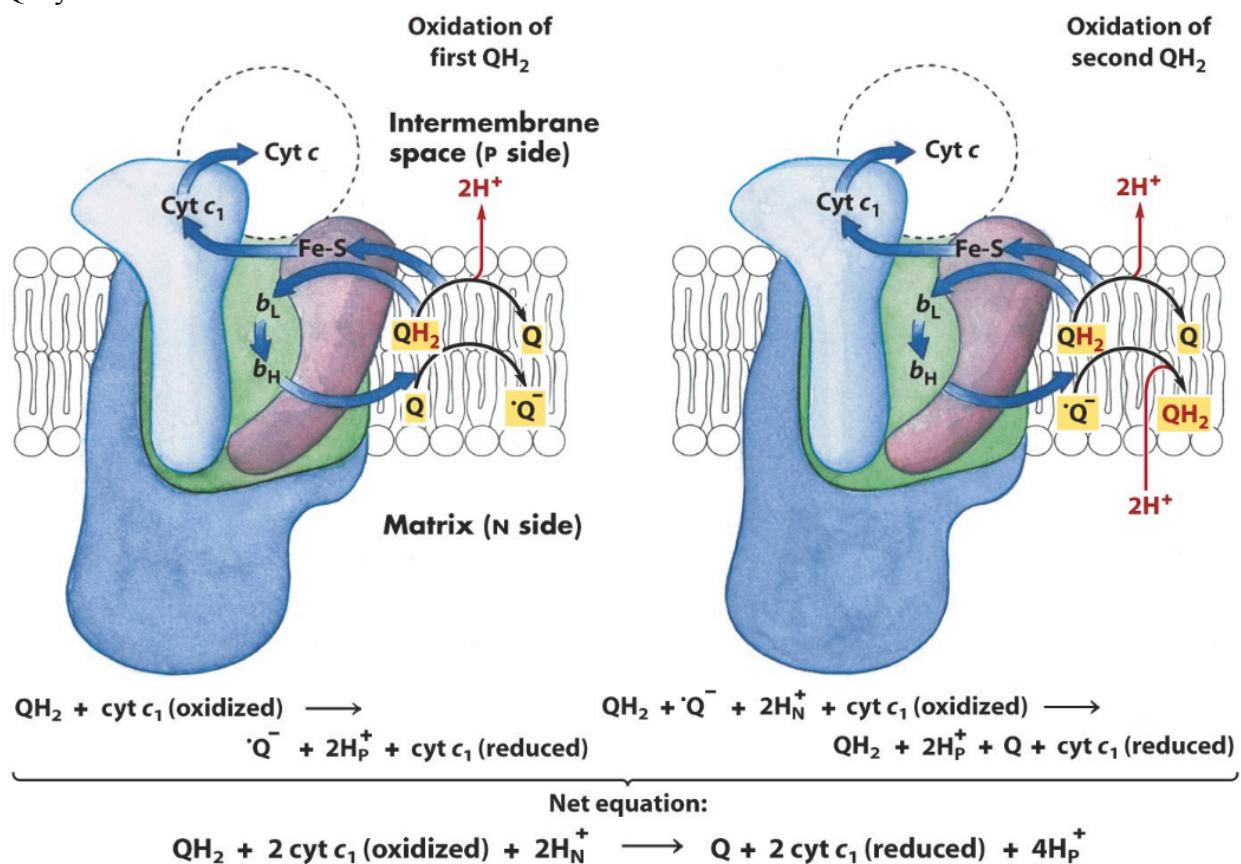
- E. Coupling e- transport to proton pumps



- b. Model must account for adapting 2 e- carrier (Q) to 1 e- carrier (Cyt C) when pumping H+.
- c. The large N-side blob is only present in mammalian cells, and is not necessary for function.

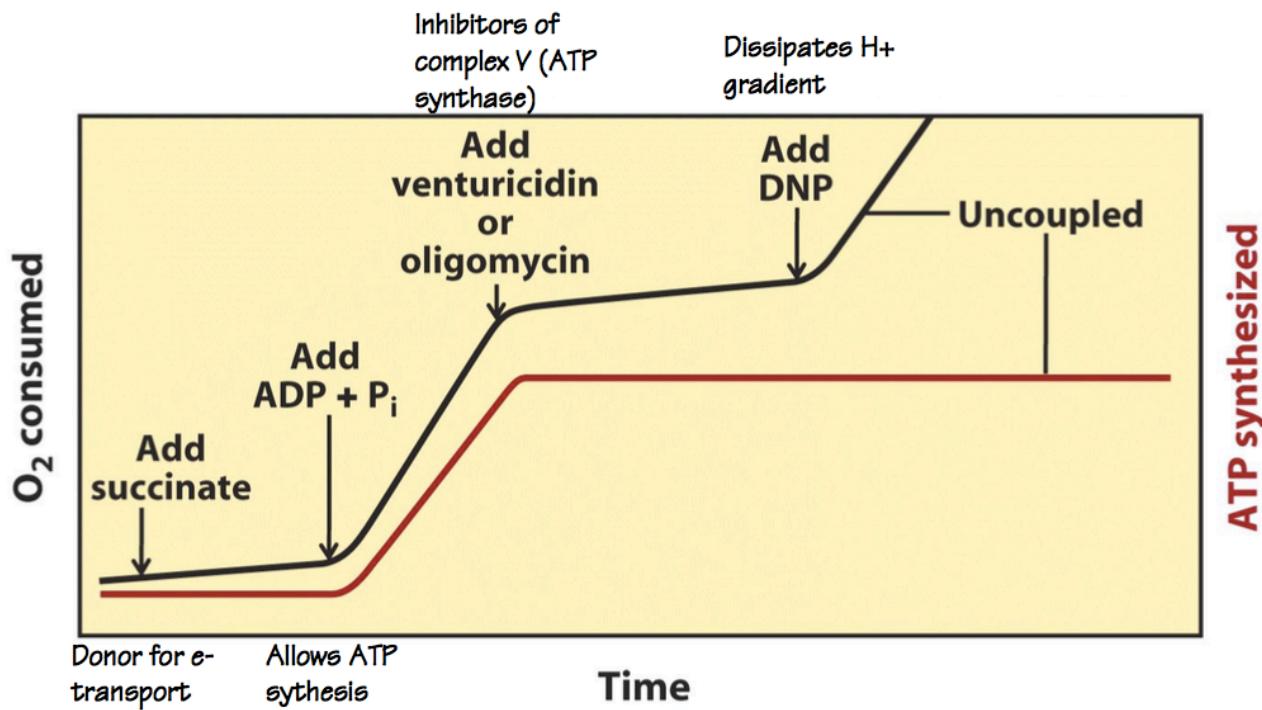


F. Q Cycle



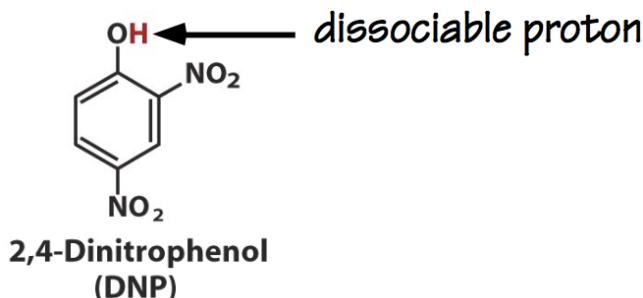
G. Dependence of e- transport ant ATP synthesis

Demonstrated by experiments with purified mitochondria in a test tube



H. Chemiosmotic hypothesis: energy from e- transport stored in a $[H^+]$ gradient is used to make ATP

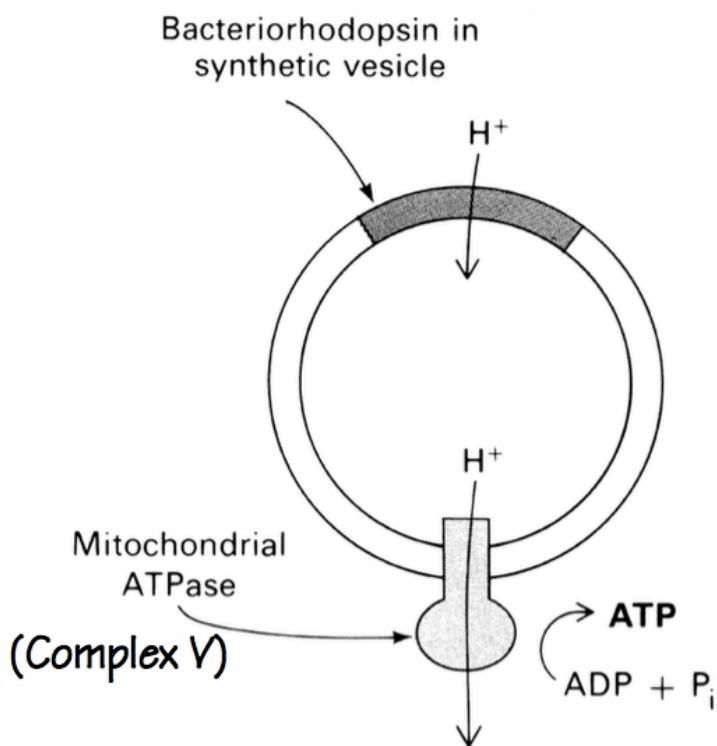
- a. DNP carries H^+ across the membrane, and thus dissipates the $[H^+]$ gradient.



- b. A $[H^+]$ gradient is sufficient to drive ATP synthesis:

Bacteriorhodopsin can replace the e- transport chain and drive ATP synthesis with light energy.

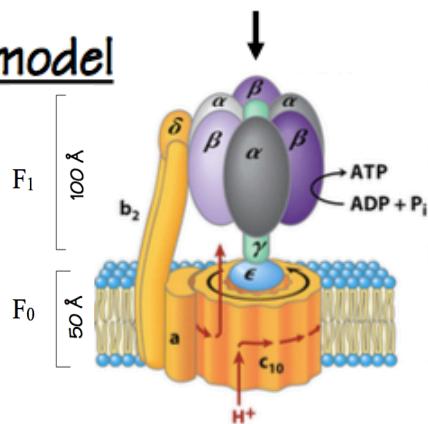
Difference: orientation of proton gradient is opposite of that in mitochondria.



I. Complex V (ATP synthase, F_0F_1 -ATPase)

- a. Can also catalyze reverse reaction! $3H^+$ per ATP.
- b. Crystal structure: three $\alpha\beta$ pairs with ADP, ATP, empty.

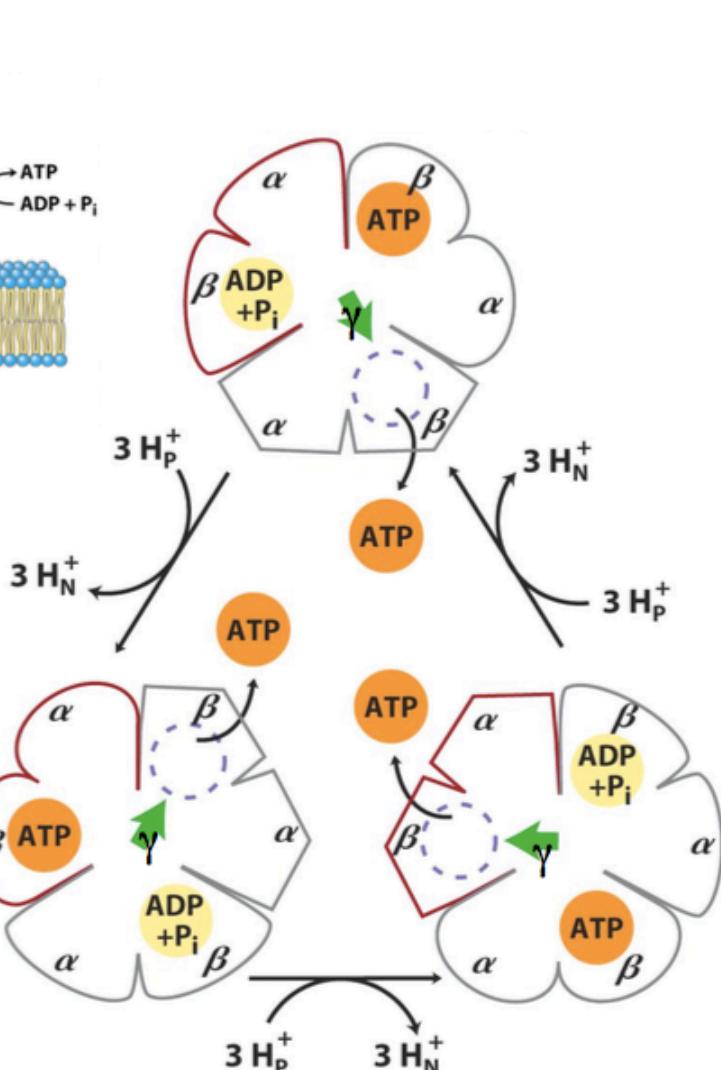
J. Binding change model

Binding change model

H⁺ passage makes γ spin within αβ "head"

Shifting contacts w/ γ make αβ pairs shift through 3 conformations that:

1. Bind ADP + Pi
2. Stabilize transition state for phosphoanhydride bond formation
3. Release ATP

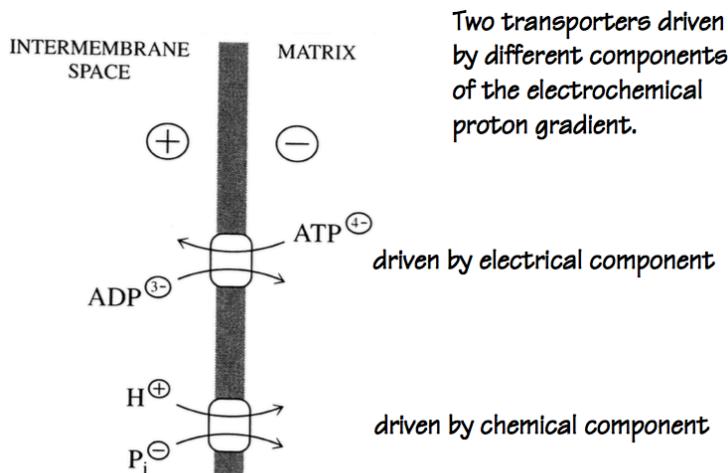


K. Control of Oxidative Phosphorylation (Limiting flux)

- a. Source of e- (NADH, FADH₂)
- b. Sink for e- (O₂)
- c. Substrates for ATP synthesis (ADP, P_i)

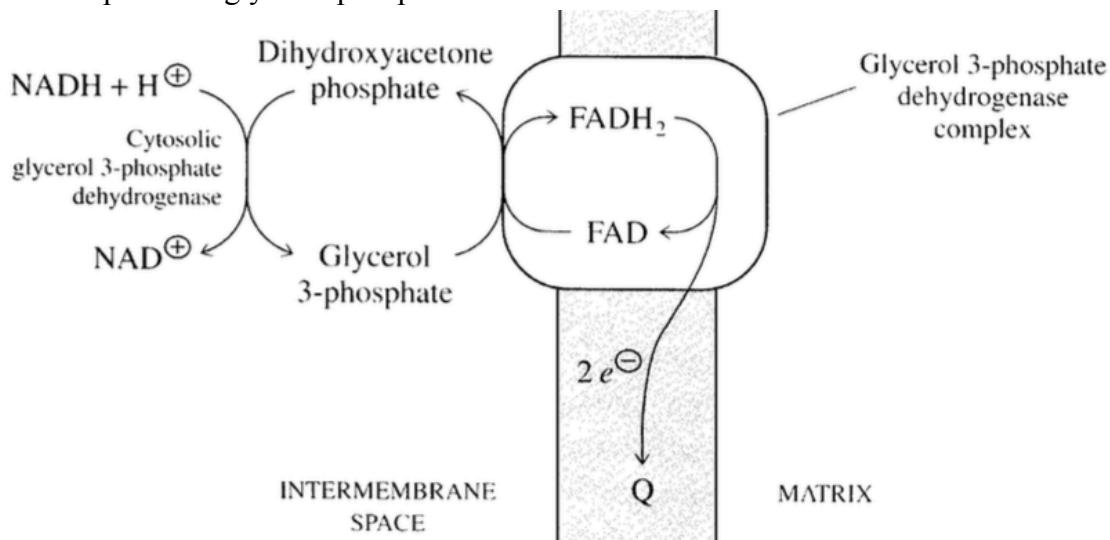
This is typically limiting. Energy use → higher [ADP] → increased ox-phos flux

L. Transport of ATP out of the mitochondria



M. Transport of Cytosolic NADH to the Mitochondrial e- Transport Chain

- a. 2 shuttles carry the e- into the mitochondria.
- b. Simpler one: glycerol phosphate shuttle



N. Yield of ATP from Oxidation of Glucose

substrate level phosphorylation

Glycolysis - 2 ATP: Citric acid cycle - 2 GTP (=2ATP): Subtotal = 4 ATP
oxidative phosphorylation

Glycolysis: 2 cytosolic NADH, shuttled to mito. as 2 QH₂
 12 H⁺ pumped out in e- transport.

Pyruvate dehydrogenase: 2 mitochondrial NADH - 20 H⁺ pumped.

Citric acid cycle

6 mitochondrial NADH - 60 H⁺ pumped.

2 mitochondrial FADH₂ - 12 H⁺ pumped.

Subtotal = 104 H⁺ pumped out of mitochondria

ATP synthase

lets 3 H⁺ back in to mito. to make 1 ATP

P_i transporter - also requires 1 H⁺ back in / ATP made

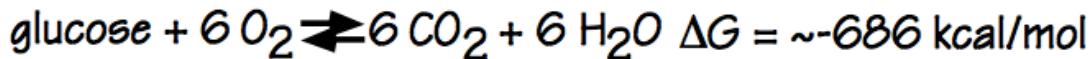
Subtotal = 4 H⁺ into mitochondrion / ATP made

Total for ox. phos. = 104/4 = 26 ATP

Grand total = 30 ATP

27

O. Efficiency of Glucose Oxidation



Efficiency is 359/686 = 52%. This is much better than that of an automobile engine (<30%)

P. ATP Production by Photosynthesis is Analogous to Oxidative Phosphorylation

- a. Similarities: e- transport through complexes to pump protons and power ATP synthase.
- b. Difference: ox-phos gets high-energy e- from NADH, and photosynthesis gets high energy e- from heme-like chlorophyll when excited by light.

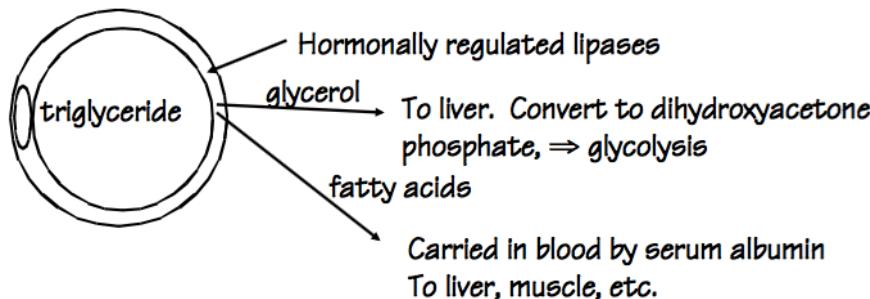
Q. Summary

- a. Oxidative phosphorylation makes most of the ATP (at least when O₂ is present)
- b. Multiprotein complexes in the inner membrane carry out electron transport. This is coupled to ATP synthesis by an electrochemical proton gradient across the membrane.
- c. The Q cycle explains the proton pumping mechanism of complex III.
- d. The binding change model explains the mechanism of ATP synthesis.

Lecture 20: Lipid Metabolism

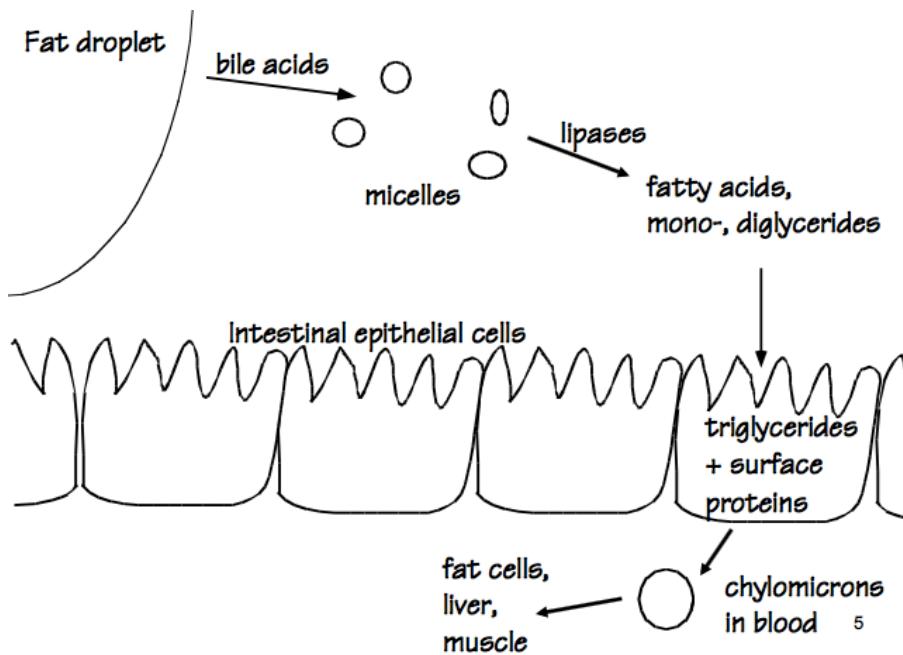
A. Overview of Triacylglycerols/Triglycerides/Fats

- a. Description
 - i. Major form of energy storage in animals
 - ii. Three fatty acids ester linked to glycerol.
 - iii. Palmitate (16-C saturated) is most common
- b. Energy Reserves:
 - i. 0.5% carbohydrates (glycogen and glucose)
 - ii. 15% protein (muscle, last resort!)
 - iii. 85% fat (40% of calories in Western diet)
- c. Advantages
 - i. 6-times more energy dense by mass.
 - ii. Separates from aqueous phase (insoluble)
- d. Localization:
 - i. Fat cells surround a fat droplet with a thin rim of cytoplasm and nucleus.

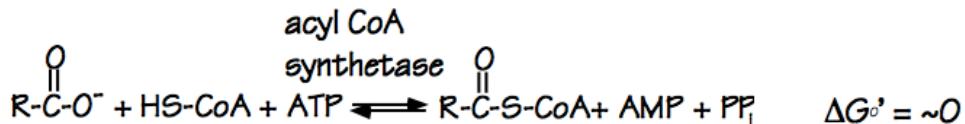


B. Metabolism of Fats

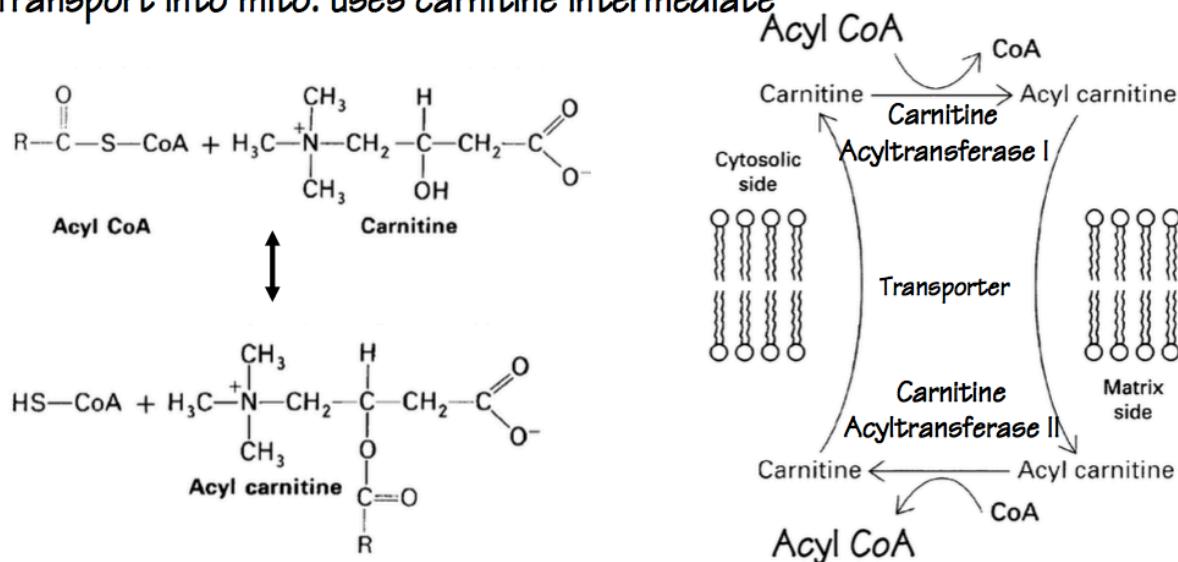
- a. Natural detergents (e.g. bile acids from cholesterol) help metabolize fats by increasing surface/volume ratio.



- b. Once in the cell, it is transported to mitochondria,



- Transport into mito. uses carnitine intermediate

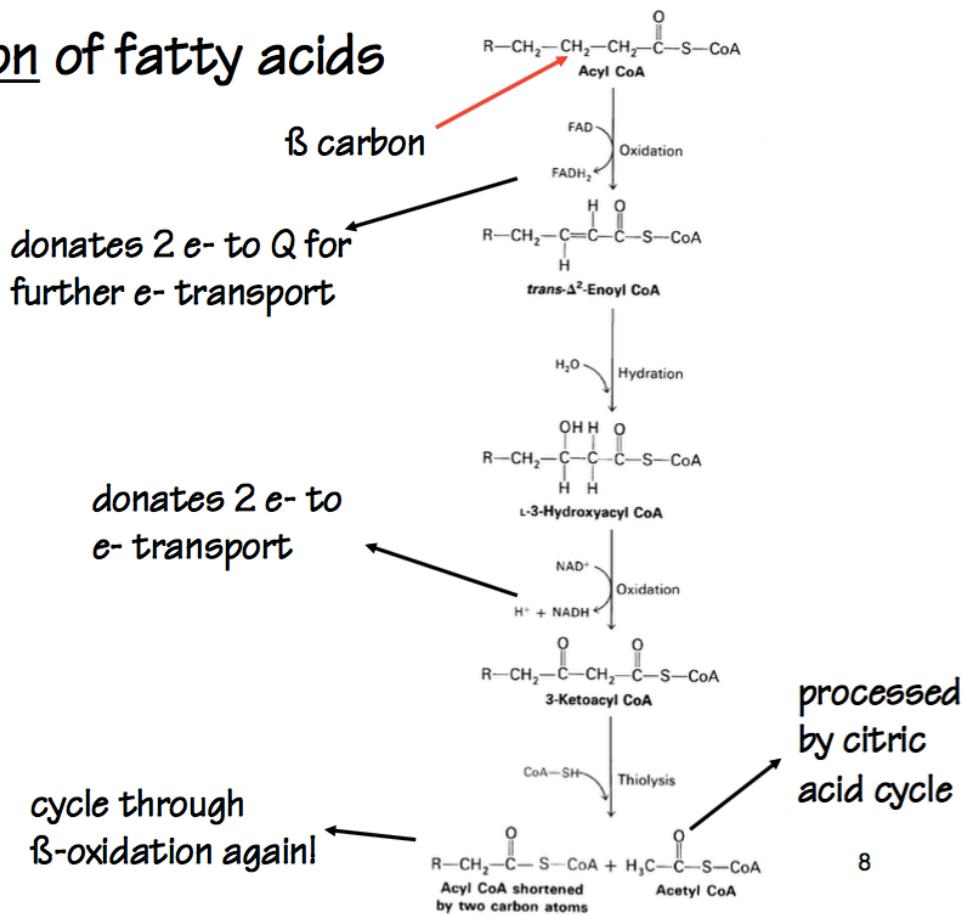


- c. Then, it is beta-oxidized to acetyl CoA (while producing NADH/FADH₂), and fed into the citric acid cycle and electron transport chain.

- d. High [malonyl CoA] inhibits carnitine acyltransferase I.

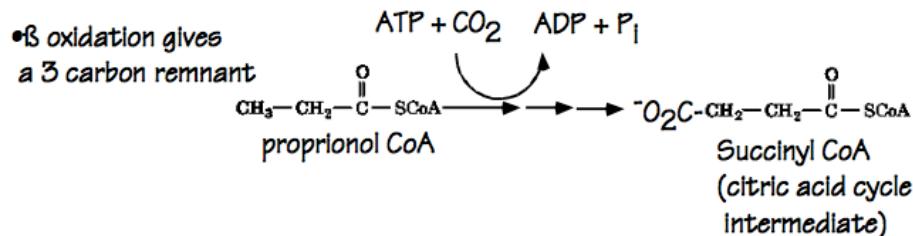
This keeps fatty acids out of the mitochondria, and thus stops beta oxidation.

B-oxidation of fatty acids



- e. Odd chain and unsaturated fatty acids are oxidized differently:

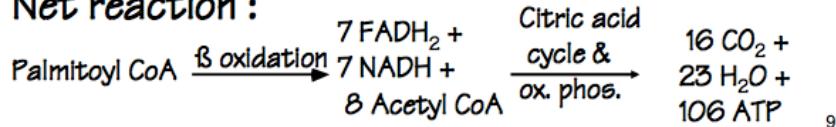
What about odd chain fatty acids?



What about unsaturated fatty acids?

Double bonds dealt with by additional enzymes

Net reaction :



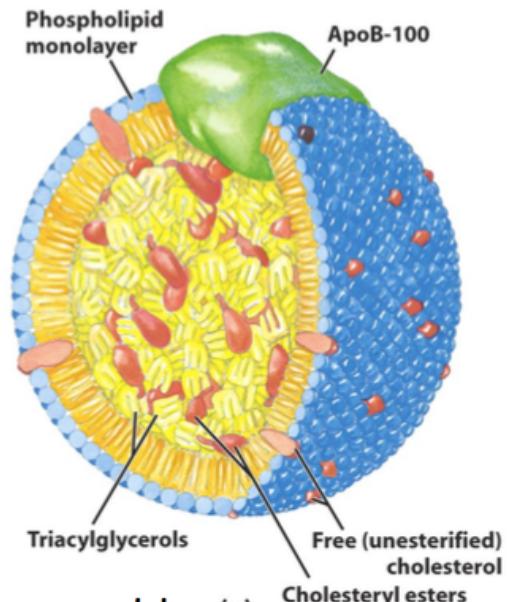
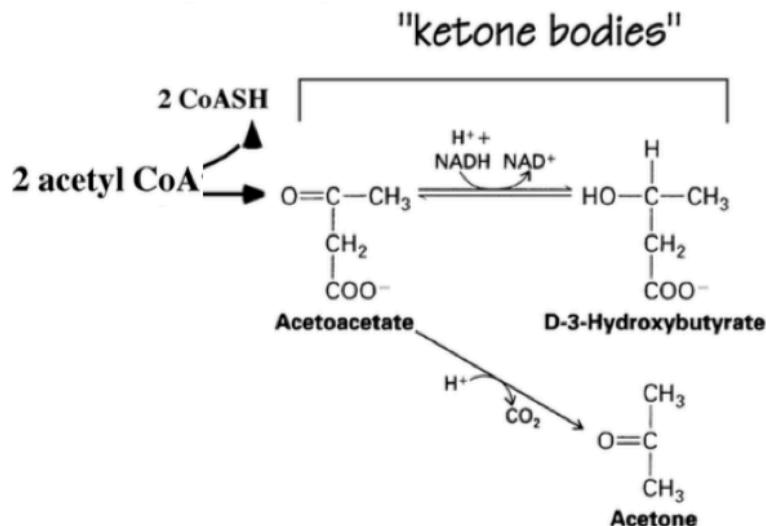
C. Lipid Transport

- a. Ingestion, synthesis, storage, and utilization occur in different sites; this necessitates transport.
 - b. Lipoproteins: spherical particles with a hydrophobic lipid core surrounded by polar lipids and specialized proteins.

Class	major lipid carried	origin
chylomicrons	dietary triacylglycerols	Intestine
VLDL	endogenous triacylglycerols	liver
LDL	endogenous cholesterol	liver
serum albumin	fatty acids	fat cells

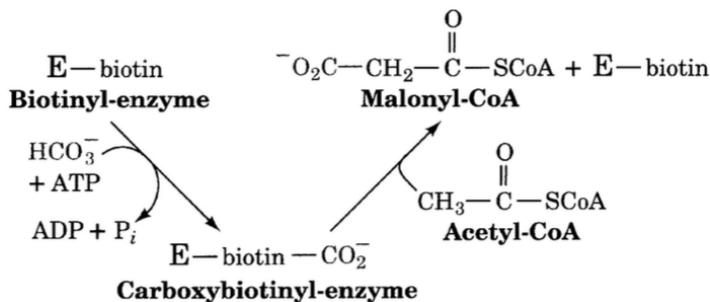
D. Ketogenesis

- a. The liver can convert fatty acids → acetyl CoA → soluble “ketone bodies”
 - b. These are exported to the blood and converted back to acetyl CoA in destination tissues
 - c. Major fuel for brain and heart during fasting/starvation (when glucose is unavailable).



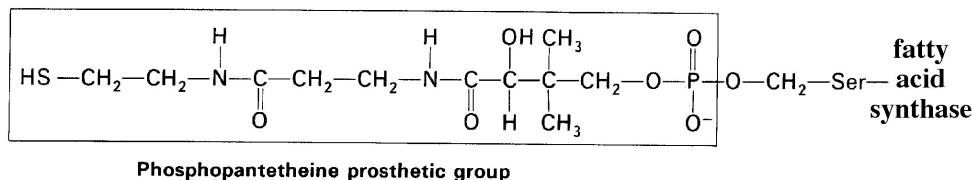
E. Fatty Acid Biosynthesis

- Occurs in the cytosol of liver cells
- Different enzymes/control from beta oxidation
- Acetyl CoA carboxylase converts acetyl CoA → malonyl CoA



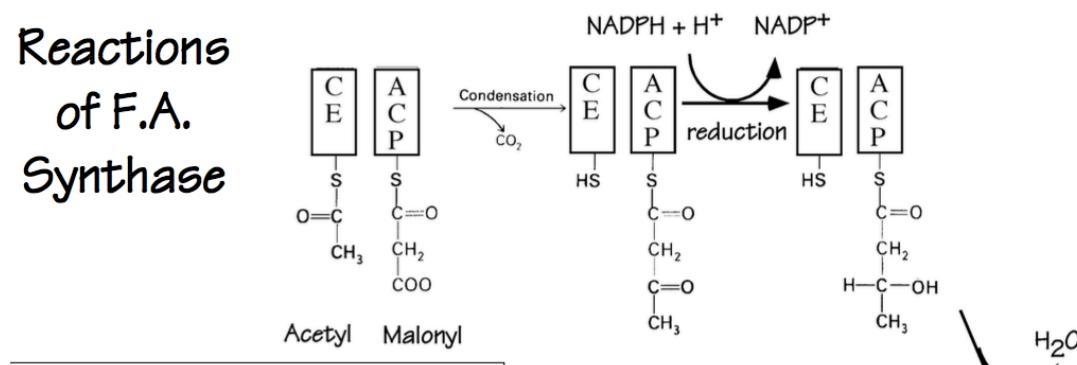
- Fatty acid synthase: polymerizes activated malonyl CoA → fatty acids
 - In mammals: single dimer with 7 enzymatic activities
In prokaryotes: 7 separate peptides
 - The growing fatty acid chain is attached to synthase by thioester linkages via Cys or a phosphopantetheine prosthetic group (the same as in CoA)

fatty
HS - Cys - acid
synthase

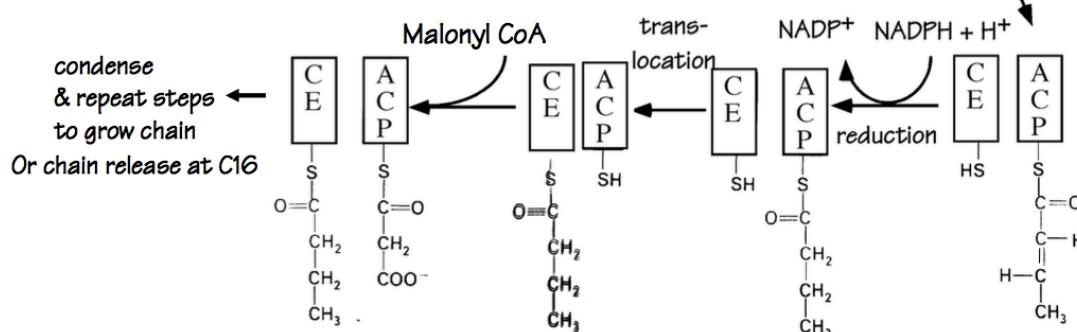


iii. Mechanism

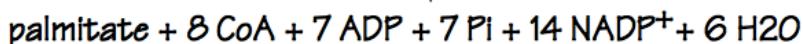
Reactions of F.A. Synthase



CE = condensing enzyme domain
 ACP = acyl carrier protein domain



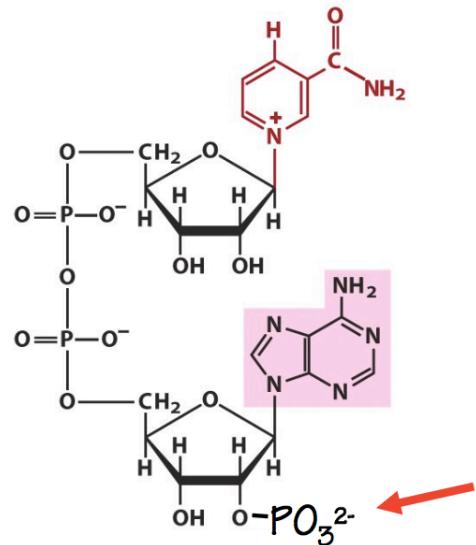
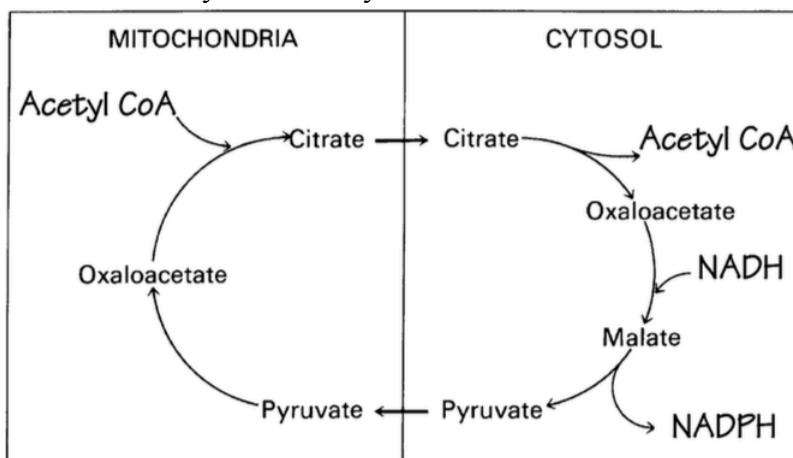
e. Net reaction:



F. NADP⁺ as an Energy Carrier

- a. NAD⁺ with a P_i tag, which does not affect the business end and is recognizable to enzymes.
- b. This separates pools of reducing power (NADH for catalysis, NADPH for biosynthesis).

G. Production of cytosolic acetyl CoA and NADPH

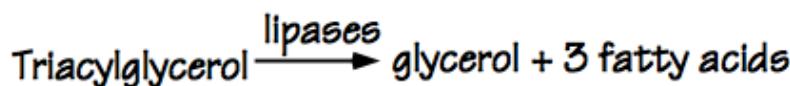


*NADPH is also created by the pentose phosphate shunt pathway.

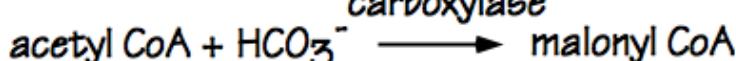
H. Regulation of fatty acid metabolism

Committed steps:

β oxidation

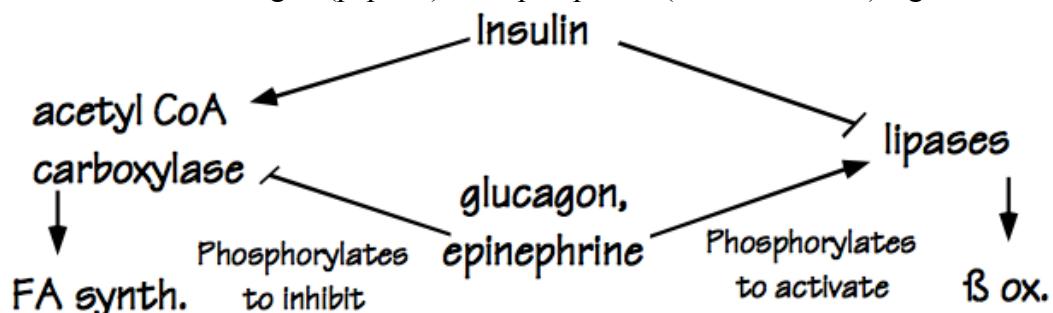


FA biosynthesis



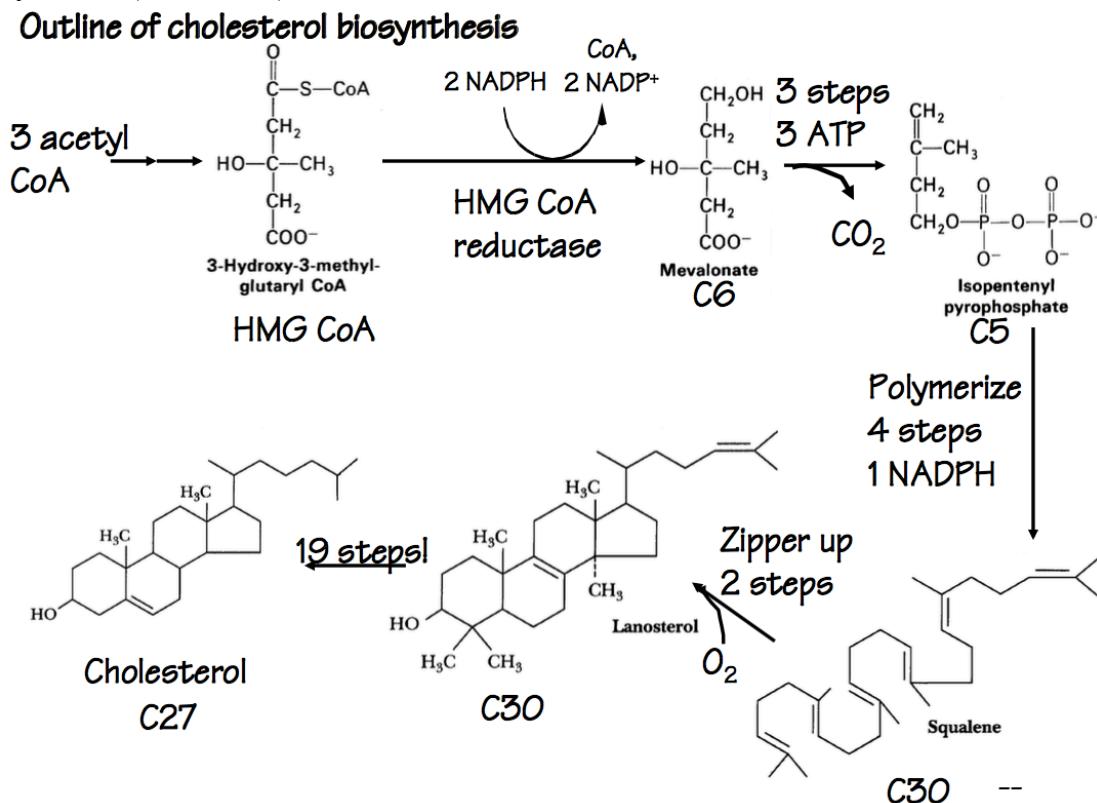
- a. Hormone control:

- i. Insulin (peptide) signals fed state
- ii. Glucagon (peptide) and epinephrine (small molecule) signal fasted (hungry) state.



I. Cholesterol

- a. Function: component of plasma membrane, precursor for steroid hormones and bile salts
- b. Synthesis (in the liver):



J. Regulation of cholesterol biosynthesis

- a. HMG CoA reductase catalyzes a non-equilibrium committed step.
- b. Feedback inhibition: cholesterol itself reduces HMG CoA reductase in the cell (>200-fold).
- c. Mechanism:
 - i. Cholesterol inhibits enzyme gene expression
 - ii. Pathway metabolites inhibit translation of enzyme mRNA
 - iii. Cholesterol activates a protease targeting the enzyme.

K. Cholesterol Transport

- a. LDL particles deliver liver-synthesized cholesterol throughout the body.
- b. LDL receptor on cell surfaces bind a protein component of LDL to internalize it.
- c. Familial hypercholesterolemia: missing LDL receptors. Intracellular cholesterol levels are low, so HMG CoA reductase is constitutively active.
- d. [Cholesterol] in mg/100mL = Healthy: 175, Homozygotes: 600, Heterozygotes: 300
- e. Rational medicine: understanding biology at the biochemical level can help deduce primary causes of disease and link to symptoms, allowing rational treatments.
 - i. Ex: Lovastatin is a competitive inhibitor of HMG CoA reductase (mimics substrate)
 - ii. This blocks cholesterol biosynthesis and lowers serum cholesterol.

L. Summary

- a. Lipids are solubilized for transport between intestines, liver, fat cells, and tissues via lipoprotein particles, by binding to albumin, or being converted to ketone bodies.
- b. Beta oxidation burns fatty acids to make ATP. Fatty acid biosynthesis generates fats.
- c. Cholesterol biosynthesis is regulated at HMG CoA reductase.

Lecture 21: Glycogen Metabolism

A. Review of glycogen:

- a. Polymer of glucose: α -1,4 linkages with α -1,6 branching.
- b. 0.5% of energy reserves; found in muscles and liver.
- c. ~55,000 glucose units, ~2,000 nonreducing ends, only 1 reducing end.

B. Advantages over fats (RAM v. hard drive)

- a. Metabolized faster and more efficiently
- b. Can be metabolized anaerobically
- c. Can provide glucose for the brain.

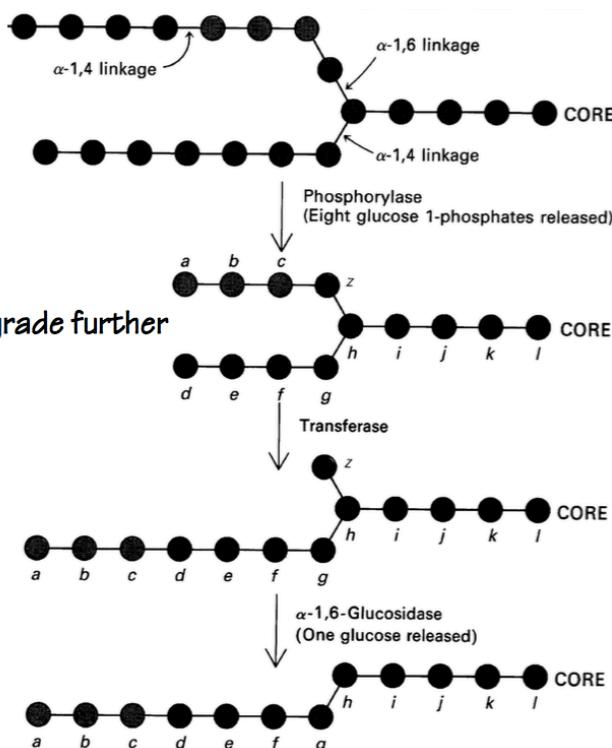
C. Glycogen Breakdown

a. Extraction by Phosphorolysis

- i. Glucose units removed one at a time from nonreducing ends ($\Delta G^\circ = \sim 0$)
- ii. Reaction driven forward by removal of G1P.

b. Debranching enzyme

Removing α -1,6 branches requires an additional enzyme



"Debranching enzyme" - one polypeptide with both transferase and α -1,6-glucosidase activities

- c. In muscles: phosphoglucomutase converts G1P \rightarrow G6P for glycolysis
- d. In liver: glucose 6-phosphatase (last enzyme in gluconeogenesis, not in muscle, brain, etc) dephosphorylates G6P + H₂O \rightarrow Glucose + P_i, allowing circulatory export.

D. Glycogen synthesis

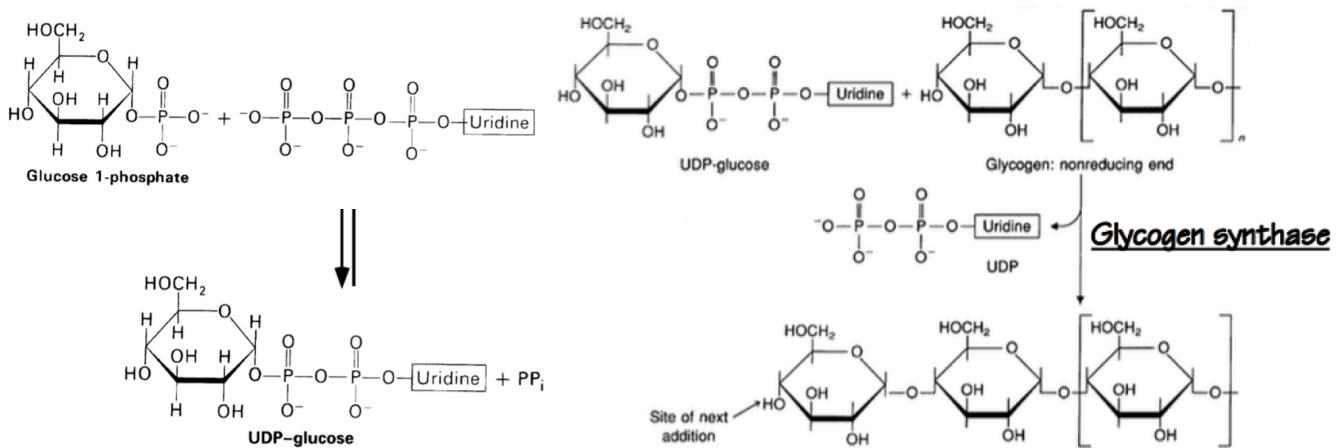
- a. Glucose is activated as UDP-glucose before polymerization to glycogen.

Hexokinase

Phosphoglucomutase

- b. Glucose $\xrightarrow{\text{-----}}$ G6P $\xrightarrow{\text{-----}}$ G1P + UTP $\xrightarrow{\text{-----}}$ UDP-glucose + PP_i

- i. Liver hexokinase has a higher K_m (needs more glucose to work properly).
- ii. Reaction driven by low [PP_i] maintained by pyrophosphatase: PP_i + H₂O \rightarrow 2P_i



- c. Glycogenin uses UDP-glucose to O-glycosylate its own Tyr residue, then adds 7 more α-1,4 glucoses. The result – glycogenin-[glucose]₈ – is the primer for glycogen synthase.
 - d. Branching enzyme removes 6-7 residues from chains of over 11 glucoses.
- These are added back as branches least 3 (average 10) residues later.

E. Control of glycogen metabolism

- a. Hormones regulate synthesis/degradation by controlling (de)phosphorylation of enzymes.
- b. Glucagon and epinephrine: phosphorylase++, synthase--
- c. Insulin: synthase++, phosphorylase--
- d. Diseases from faulty control: enlarged liver, liver failure, hypoglycemia, muscle cramps.

F. Summary

**The pathways
we've covered**

