CHAPTER 7

ENZYMES, METABOLISM, AND CELLULAR RESPIRATION

This file covers two lecture slots (about half for each)

Metabolism

- Sum total of all chemical reactions that occur within an organism
- Also refers to specific chemical reactions at the cellular level

Chemical reactions

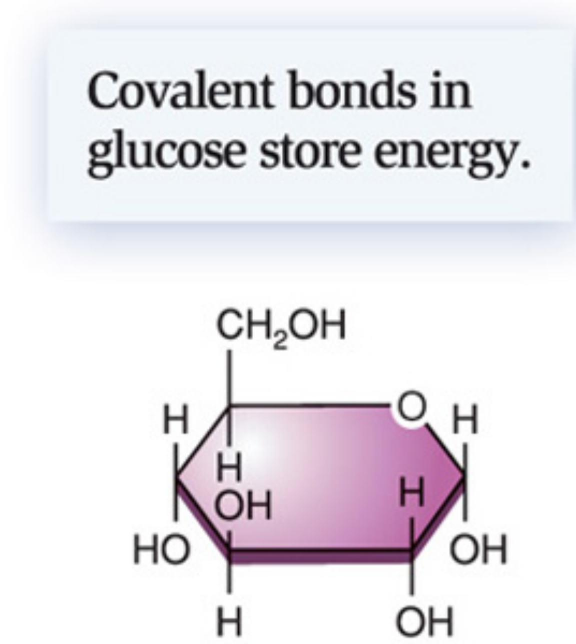
- 2 factors govern fate of a chemical rxn
  - Direction
    - Many cells use ATP to drive reactions in 1 direction
  - Rate
    - Catalysts called enzyme can speed the reaction rate

$$aA + bB \leftrightarrow cC + dD$$

Energy

-things tend towards disorder unless energy is put into system
-entropy: a measure of the disorder that cannot be harnessed to do work

(a) Kinetic energy



(b) Potential energy

- Ability to promote change
- 2 forms
  - Kinetic- associated with movement
  - Potential- due to structure or location
    - Chemical energy- energy in molecular bonds

Table 7.1

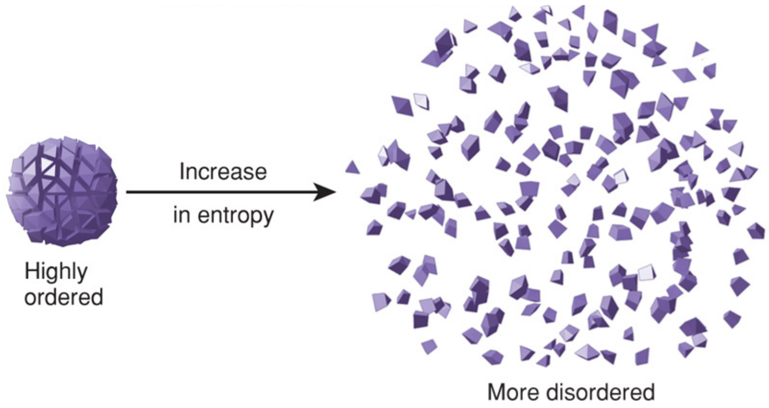
Table 7.1 Types of Energy That Are Important in Biology

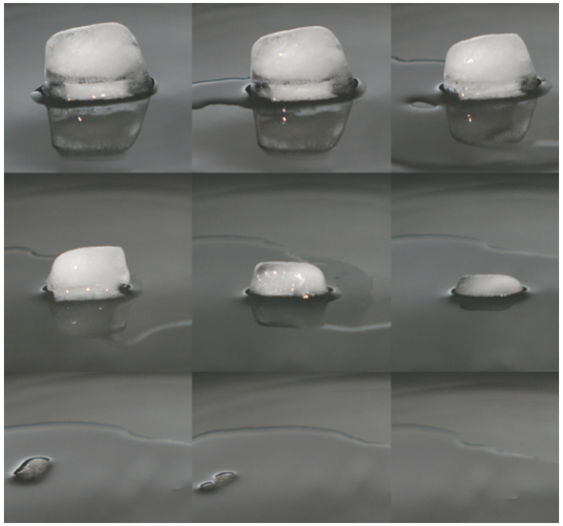
Energy type	Description	Biological example
Light	Light is a form of electromagnetic radiation that is visible to the eye. The energy of light is packaged in photons.	During photosynthesis, light energy is captured by pigments in chloroplasts (Chapter 8). Ultimately, this energy is used to reduce carbon, thus producing organic molecules.
Heat	Heat is the transfer of kinetic energy from one object to another or from an energy source to an object. In biology, heat is often viewed as energy that can be transferred because of a difference in temperature between two objects or locations.	Many organisms, such as humans, maintain their bodies at a constant temperature. This is achieved by chemical reactions that generate heat.
Mechanical	Mechanical energy is the energy that is possessed by an object because of its motion or its position relative to other objects.	In animals, mechanical energy is associated with movements caused by muscle contraction, such as walking.
Chemical	Chemical energy is energy stored in the chemical bonds of molecules. When the bonds are broken and rearranged, they can release large amounts of energy.	The covalent bonds in organic molecules, such as glucose and ATP, store large amounts of energy. When these bonds are broken, the chemical energy released can be used to drive cellular processes.
Electrical/ion gradient	The movement of a charge or the separation of a charge can provide energy. Also, a difference in ion concentration across a membrane constitutes an electrochemical gradient, which is a source of potential energy.	High-energy electrons can release energy (that is, drop down to lower energy levels). The energy that is released can be used to drive cellular processes, such as pumping H^+ across membranes (as discussed later in this chapter).

2 Laws of thermodynamics

1. First law
   - Law of conservation of energy
   - Energy cannot be created or destroyed
2. Second law
   - Transfer or transformation of energy from one form to another increases entropy or degree of disorder of a system

Change in free energy determines direction





Entropy - a measure of the disorder that cannot be harnessed to do work

$$H = G + TS$$

- H= enthalpy or total energy
- G= free energy or amount of energy for work
- S= entropy or unusable energy
- T= absolute temperature in Kelvin (K)

Spontaneous reactions?

- thermodynamically favoured
- Not necessarily fast
- Key factor is the free energy change

Biochemistry uses $\Delta G°'$: "standard" free energy change

$$\Delta G = \Delta H - T \Delta S$$

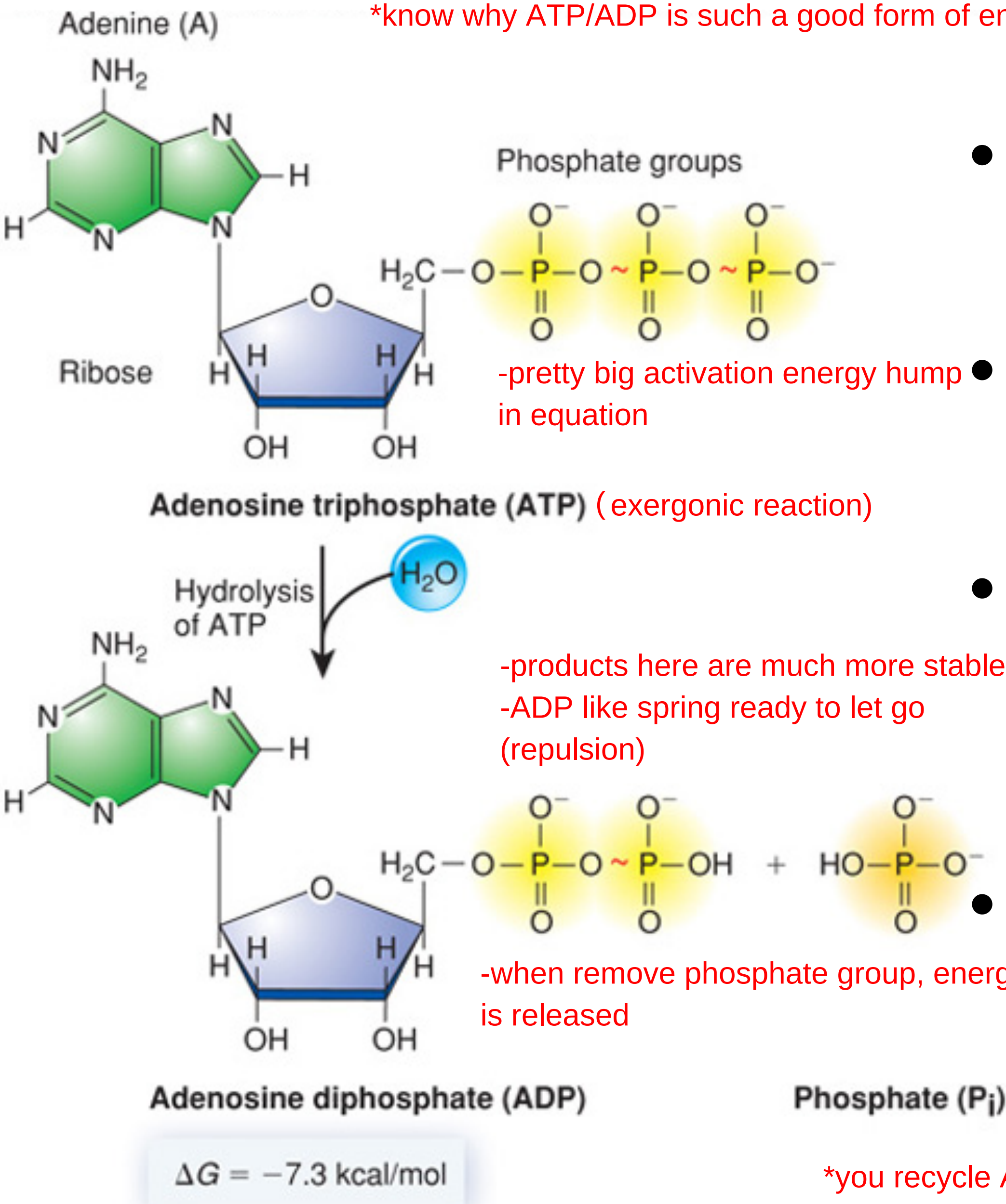
- specific conditions and concentrations

-change in free energy is greater in substrates than it is in products
-if not must figure out how to go "uphill"

$$A + B \rightarrow C + D$$

- Exergonic
  $\Delta G°'$ <0 or negative free energy change
  – Spontaneous (spontaneous does not mean it will happen quickly, it just means that products have more energy than substrates)
- Endergonic
  $\Delta G°'$ >0 or positive free energy change
  – Requires addition of free energy
  – Not spontaneous

*know why ATP/ADP is such a good form of energy



-pretty big activation energy hump in equation

(exergonic reaction)

-products here are much more stable
-ADP like spring ready to let go (repulsion)

-when remove phosphate group, energy is released

- Hydrolysis of ATP $\Delta G'^{o}$ = -7.3 kcal/mole
- favours formation of products
- Energy liberated can drive a variety of cellular processes
- ***Link*** exergonic rxn to drive endergonic

-forming of new bonds makes energy available, energy given off in new formation

*you recycle ADP (use, make, use, make...)
*keeps more ATP around than ADP (ATP is good fuel, keep more of it around than ADP

THIS REACTION IS DISPLACED FROM EQUILIBRIUM

Glucose + phosphate → glucose-phosphate + H_2O

$\Delta G'^{o}$ = +3.3 Kcal/mole

endergonic

-phosphoral transfer, by taking phosphate off ATP-temp./for a while and putting it on something else

-coupled means phosphoral transfer during ATP reaction

ATP + H_2O → ADP + P_i

$\Delta G'^{o}$ = -7.3 Kcal/mole

exergonic

Coupled reaction:

Glucose + ATP → glucose-phosphate + ADP

$\Delta G'^{o}$ = -4.0 Kcal/mole

Overall (sum) rxn is exergonic

*is there another way to drive an endergonic reaction? (yes!-see video on moodle)

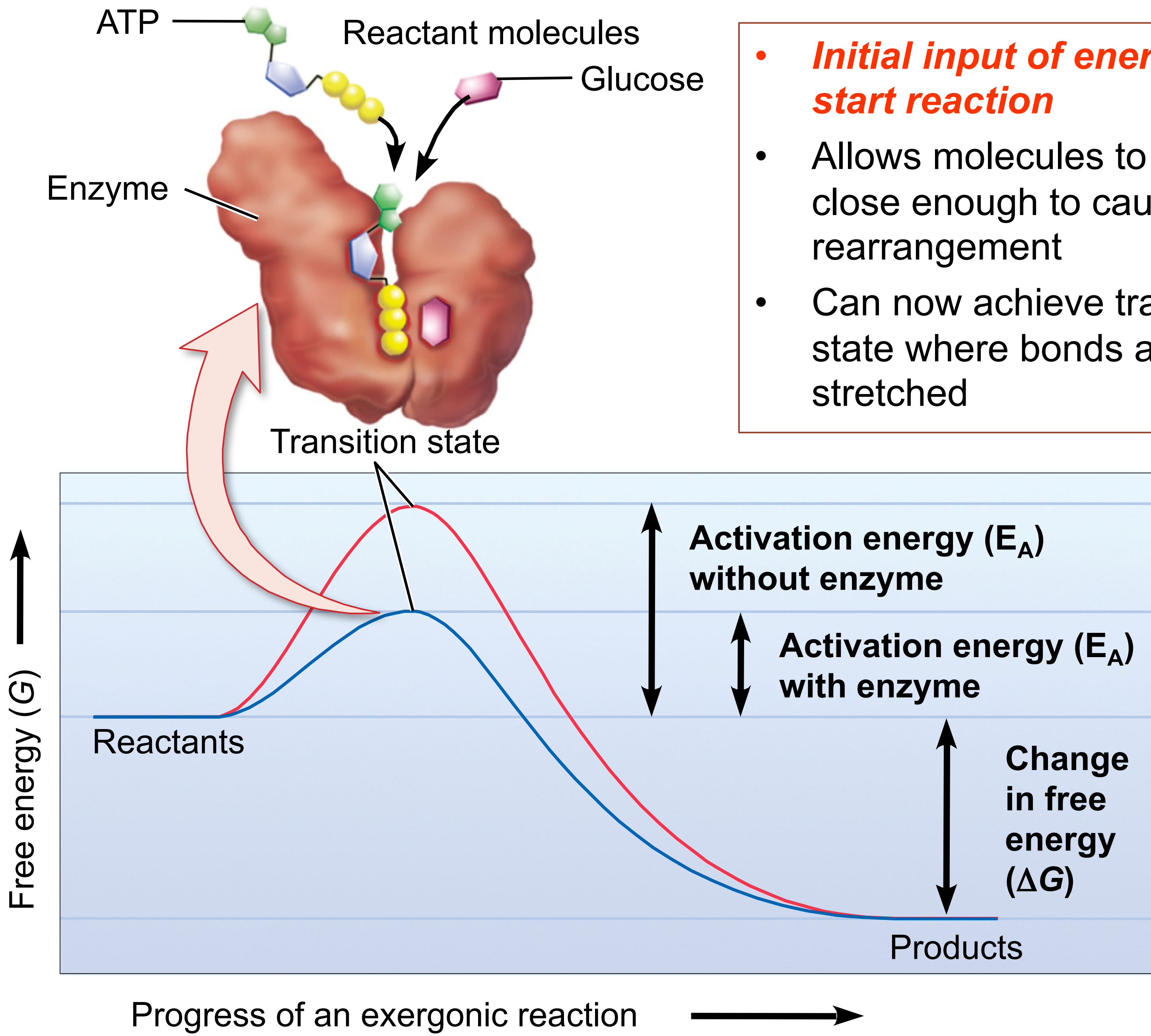
Enzymes

-most metabolic reaction in cell are catalysed (usually by enzyme)

- A spontaneous reaction is not necessarily a fast reaction
- Catalyst- speeds up the rate of reaction without being consumed
- Enzymes- protein catalysts in living cells
- May be multi-meric, complexes
- Often highly regulated (various levels)

Activation energy

- ***Initial input of energy to start reaction***
- Allows molecules to get close enough to cause bond rearrangement
- Can now achieve transition state where bonds are stretched



-difference between exothermic and exergonic?

Overcoming activation energy

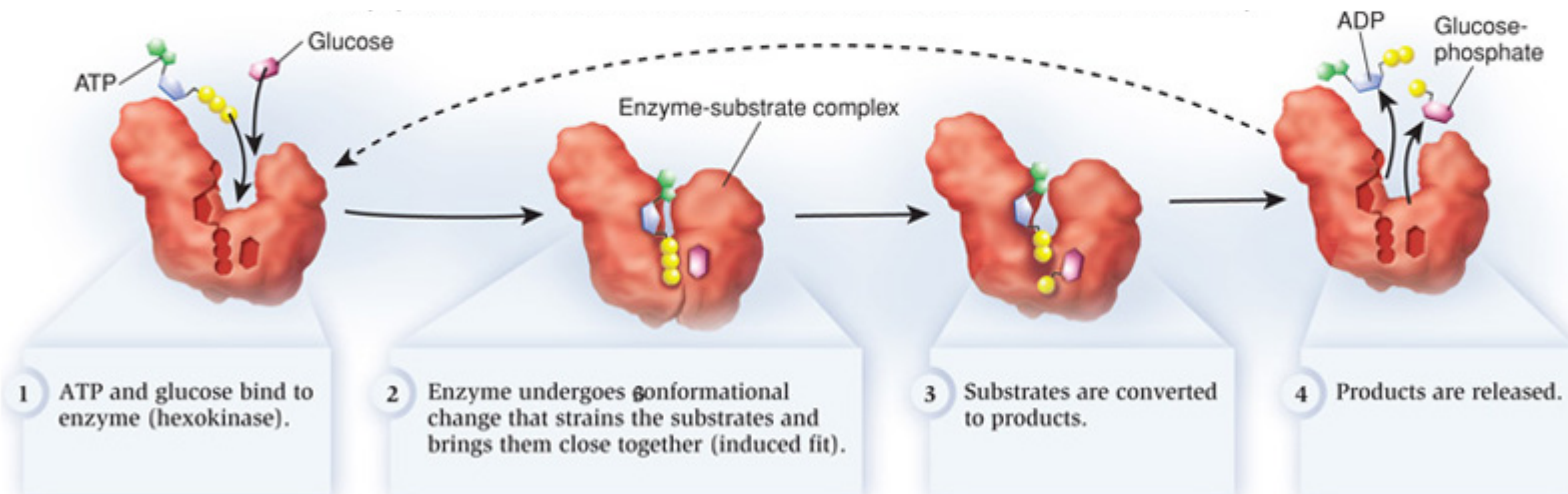
- 2 common ways
  - Large amounts of heat
  - Using ***enzymes*** to lower activation energy
    - Small amount of heat can now push reactants to transition state

Lowering activation energy

- Straining bonds in reactants to make it easier to achieve transition state
- Positioning reactants together to facilitate bonding
- Changing local environment
  - Direct participation through very temporary bonding

- ***<u>Active site</u>*** = where reaction takes place
- Substrate(s) = reactant(s) that bind to active site
- Enzyme-substrate complex formed when enzyme and substrate bind
- Optimal temp, pH, etc.

-if cell needs to slow down a reaction they can change it in some way in order for it to take longer to "fit into glove" (reduce affinity)



Substrate binding

- Enzymes have a high affinity and/or high degree of specificity for a substrate
- Induced fit - interaction also involves conformational changes
- Concepts exploited in pharmacology

HIGH AFFINITY = LOW *Km (Michaelis constant)*

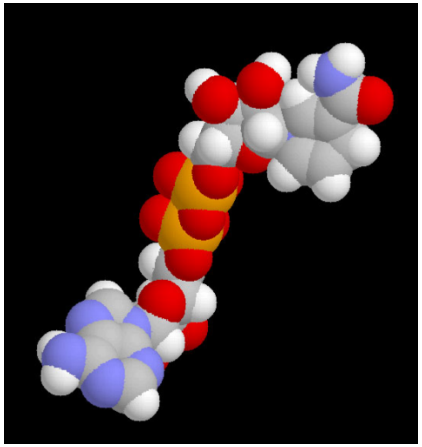
- ***expressed in mM, μM, nM…..***
- ***Km is substrate conc at half-max "speed"***

Other requirements for enzymes

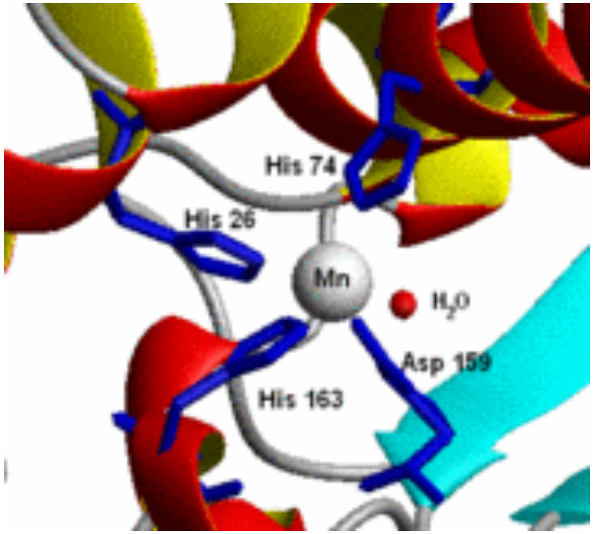
- **Prosthetic groups- small molecules (ion or organic) permanently attached to the enzyme**

- **Cofactor- usually inorganic ion that temporarily binds to enzyme (eg. Cu^{2+} in cytochrome oxidase, Zn^{2+} in ADH)**

- **Coenzyme- organic molecule that participates in reaction but left unchanged afterward (eg. NADH, vitamins)**



NADH

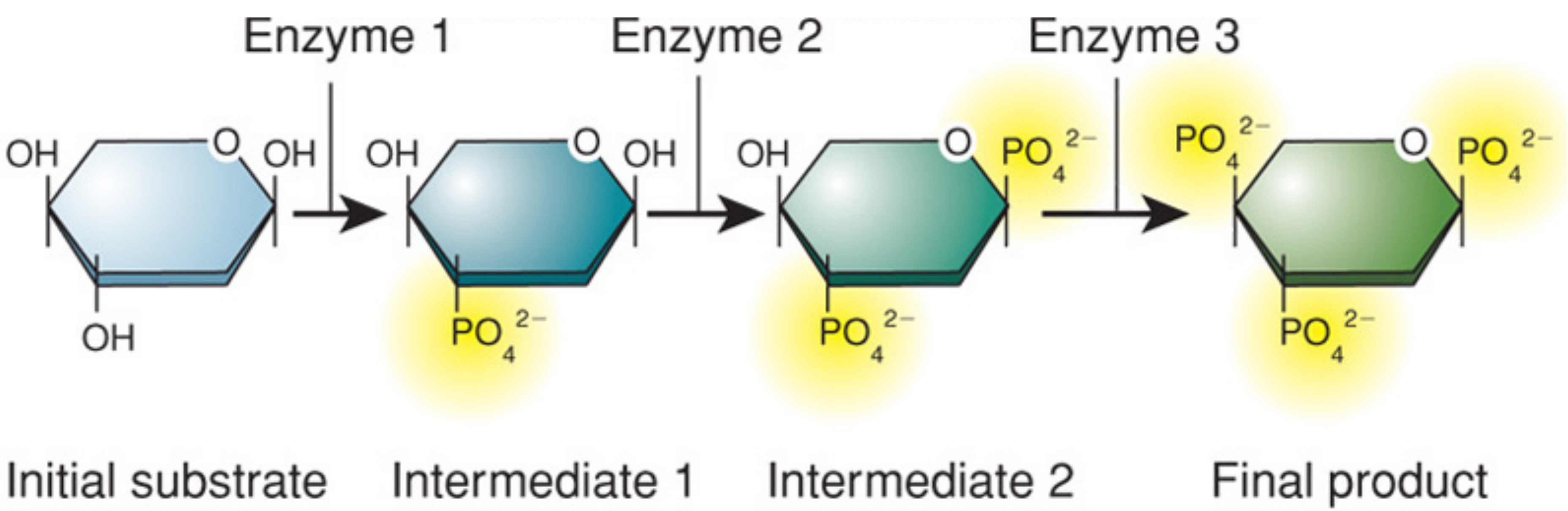


SOD2 with Mn cofactor

Overview of metabolism

-metabolism is series of controlled reactions (some you put energy in, some you get energy out)

- Chemical reactions occur in metabolic pathways
- Each step is coordinated by a specific enzyme
- Catabolic pathways: breakdown, exergonic
- Anabolic pathways: synthesis,endergonic
  - Must be coupled to exergonic reaction



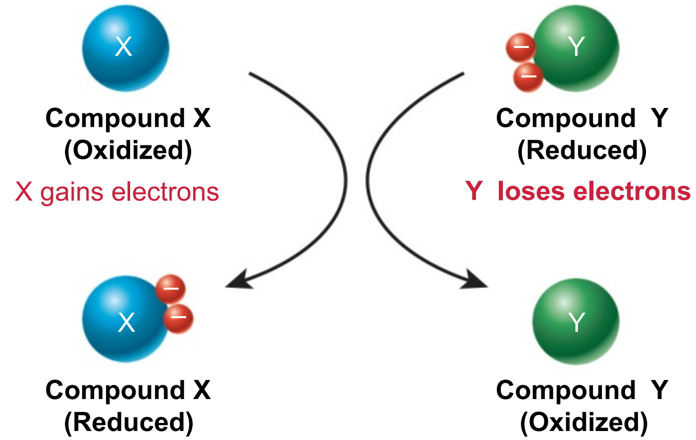
Catabolic reactions

- Breakdown of reactants (e.g. starch, glycogen)
- Used for recycling
- Used to obtain energy for endergonic reactions
  - Energy stored in energy intermediates
    - ATP, NADH
- 2 ways to make ATP
  - **Substrate-level phosphorylation**
  - **Chemiosmosis**

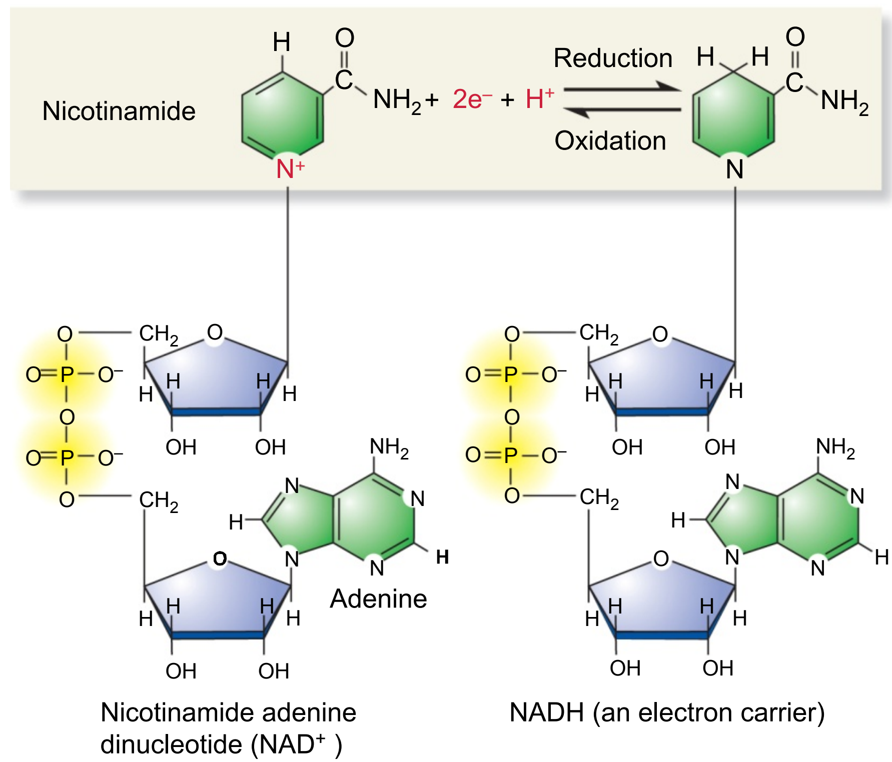
Energy intermediates

- Electrons removed by oxidation are used to create energy intermediates like NADH
- NAD^+ Nicotinamide adenine dinucleotide
- NADH, NADPH, $FADH_2$…
  - Oxidized to make ATP
  - Can donate electrons during synthesis reactions

REDOX: LEO SAYS GER



(a) A coupled redox reaction

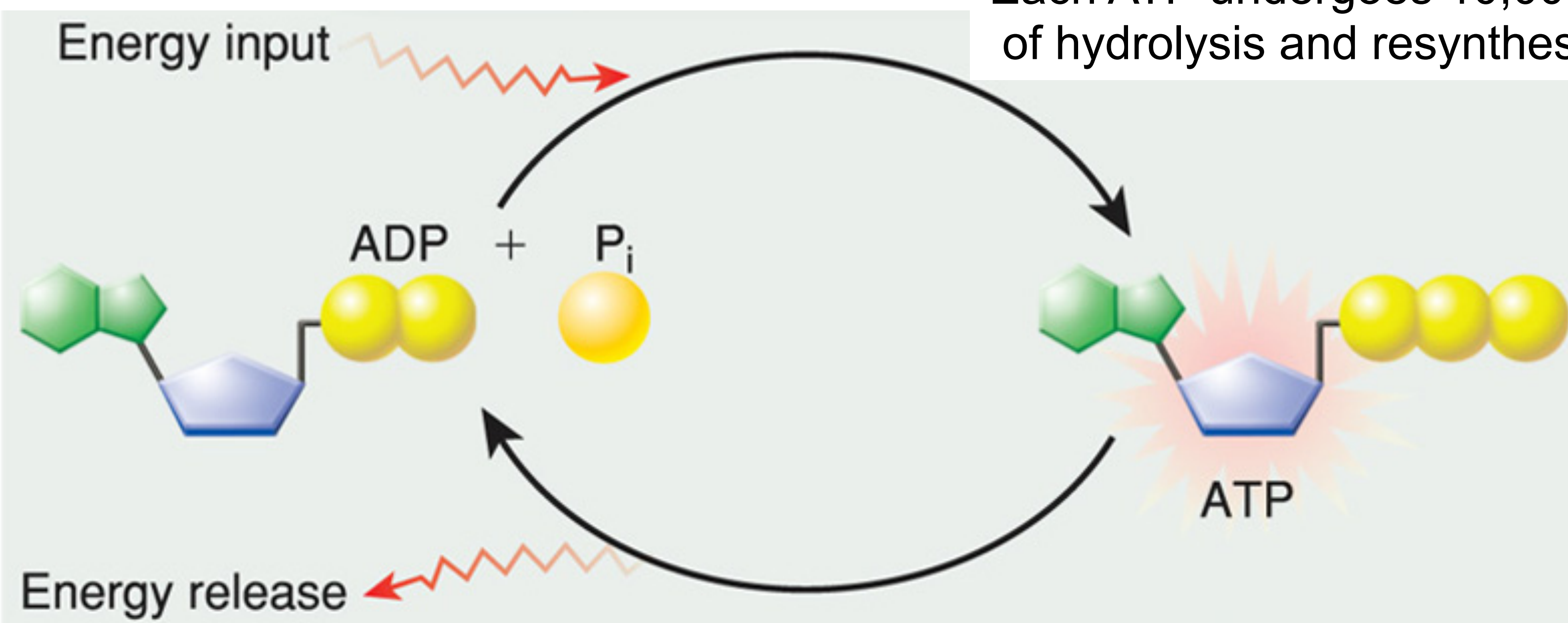


(b) The reduction of NAD^+ to create NADH

ANABOLIC REACTIONS

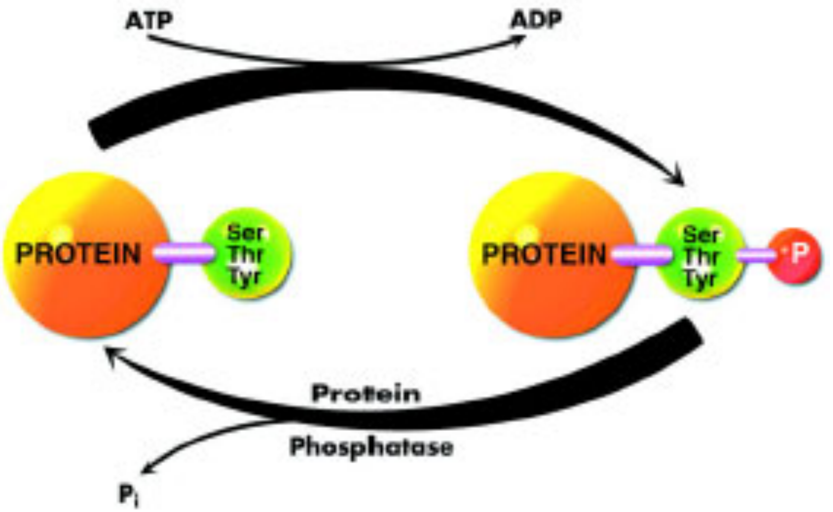
- Biosynthetic reactions
- Make large macromolecules, tissues, organs, etc or smaller molecules not available from food
- Powered by catabolism

Each ATP undergoes 10,000 cycles of hydrolysis and resynthesis every day



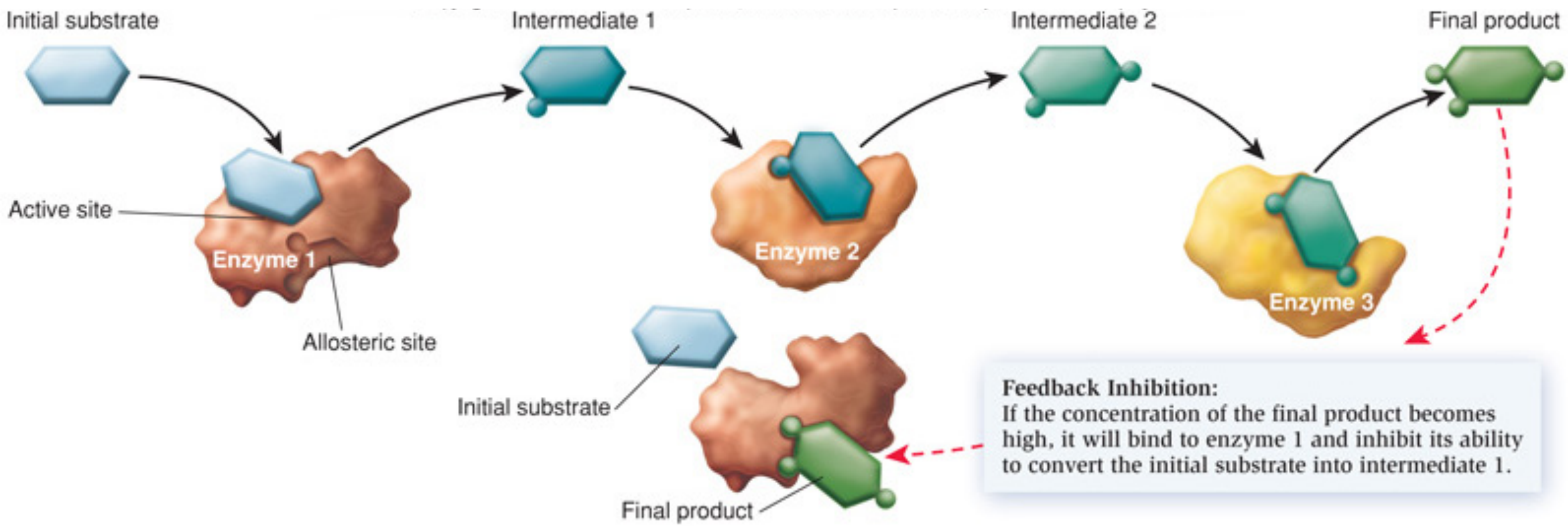
Regulation of metabolic pathways

1. Gene regulation (in Dr. Lefebvre's section)
2. Biochemical regulation



Biochemical regulation

- Competitive inhibitors-
- Uncompetitive inhibitors-
- Allosteric site- binding causes conformational change in enzyme active site inhibiting enzyme function
  - Feedback inhibition- product of pathway inhibits early steps to prevent overaccumulation of product
- Post-translational Modification
- Turnover (synthesis vs breakdown)
- Activators
- Substrate levels

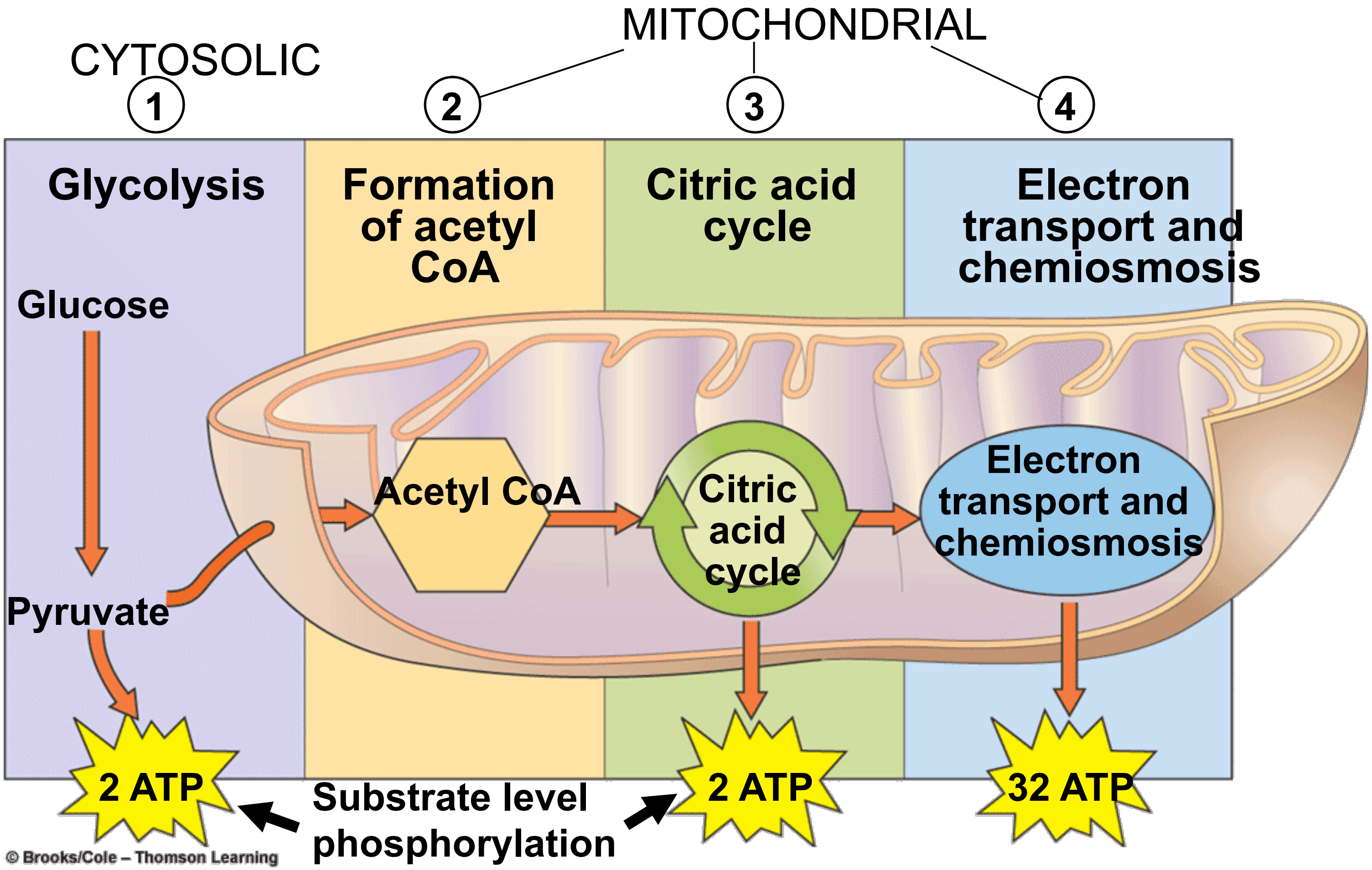

Initial substrate
Intermediate 1
Intermediate 2
Final product
Active site
Enzyme 1
Allosteric site
Enzyme 2
Enzyme 3
Initial substrate
Final product
Feedback Inhibition:
If the concentration of the final product becomes high, it will bind to enzyme 1 and inhibit its ability to convert the initial substrate into intermediate 1.

Cellular respiration

- Process by which living cells obtain energy from organic molecules
- Primary aim to make ATP and NADH
- Secondary role is anapleurotic
- Aerobic respiration uses oxygen
  - O_2 consumed and CO_2 released

Organic molecules + $O_2 \rightarrow CO_2 + H_2O$ + Energy

4 stages of aerobic respiration



FOR EACH PATHWAY/PROCESS:

WHERE? WHY? REGULATION? INPUT/OUTPUT?

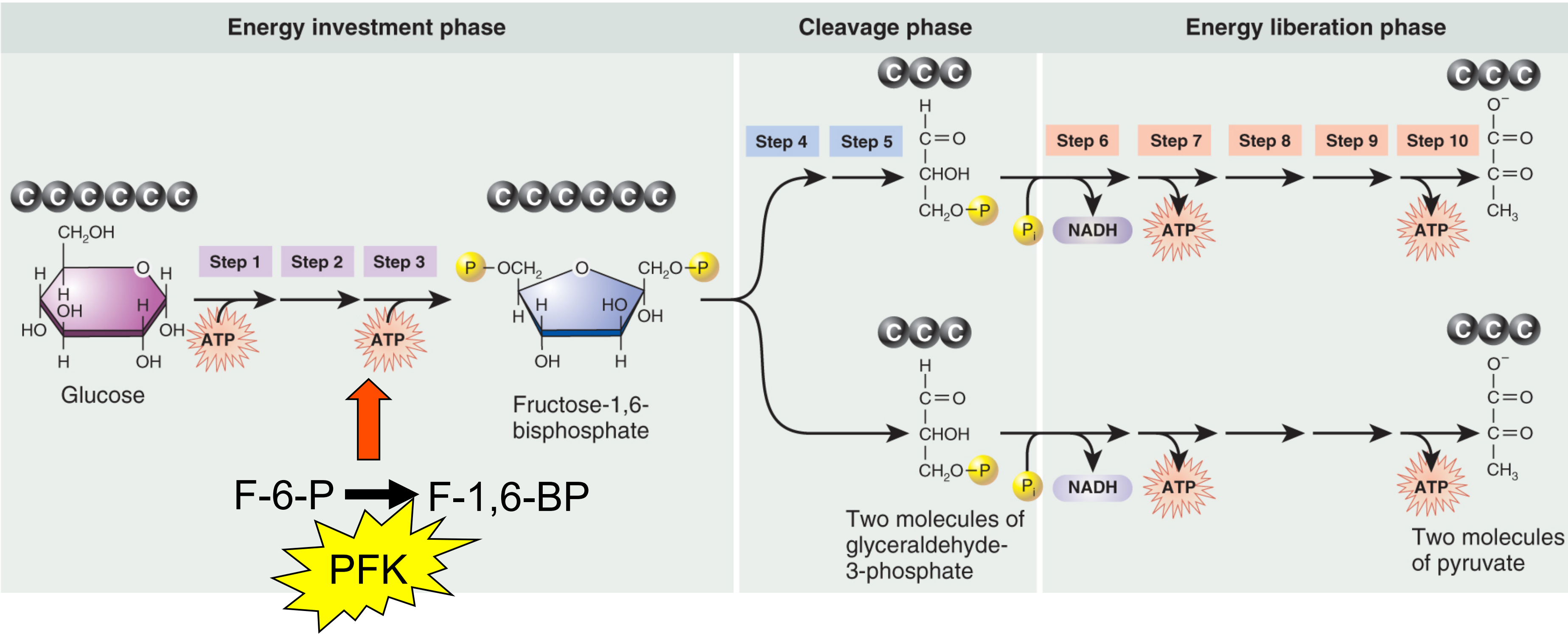
Stage 1: Glycolysis

- Glycolysis can occur with or without O_2
- Steps nearly identical across species
- 3 phases
  1. Energy investment
  2. Cleavage
  3. Energy liberation (substrate-level phosphorylation)

3 phases of glycolysis

1. Energy investment
   – Steps 1-3 (rearrangement and phosphorylation of sugar)
   – 2 ATP hydrolyzed to create fructose-1,6 bisphosphate
2. Cleavage
   – Steps 4-5
   – 6 carbon molecule broken into two 3 carbon molecules of glyceraldehyde-3-phosphate
3. Energy liberation
   – Steps 6-10
   – Two glyceraldehyde-3-phosphate molecules broken down into two pyruvate molecules producing 2 NADH and 4 ATP

- Net yield in ATP of 2



- Net yield of glycolysis: 2 ATP, 2 NADH, 2 pyruvate
- **Key regulation pt: feedback inhibition**

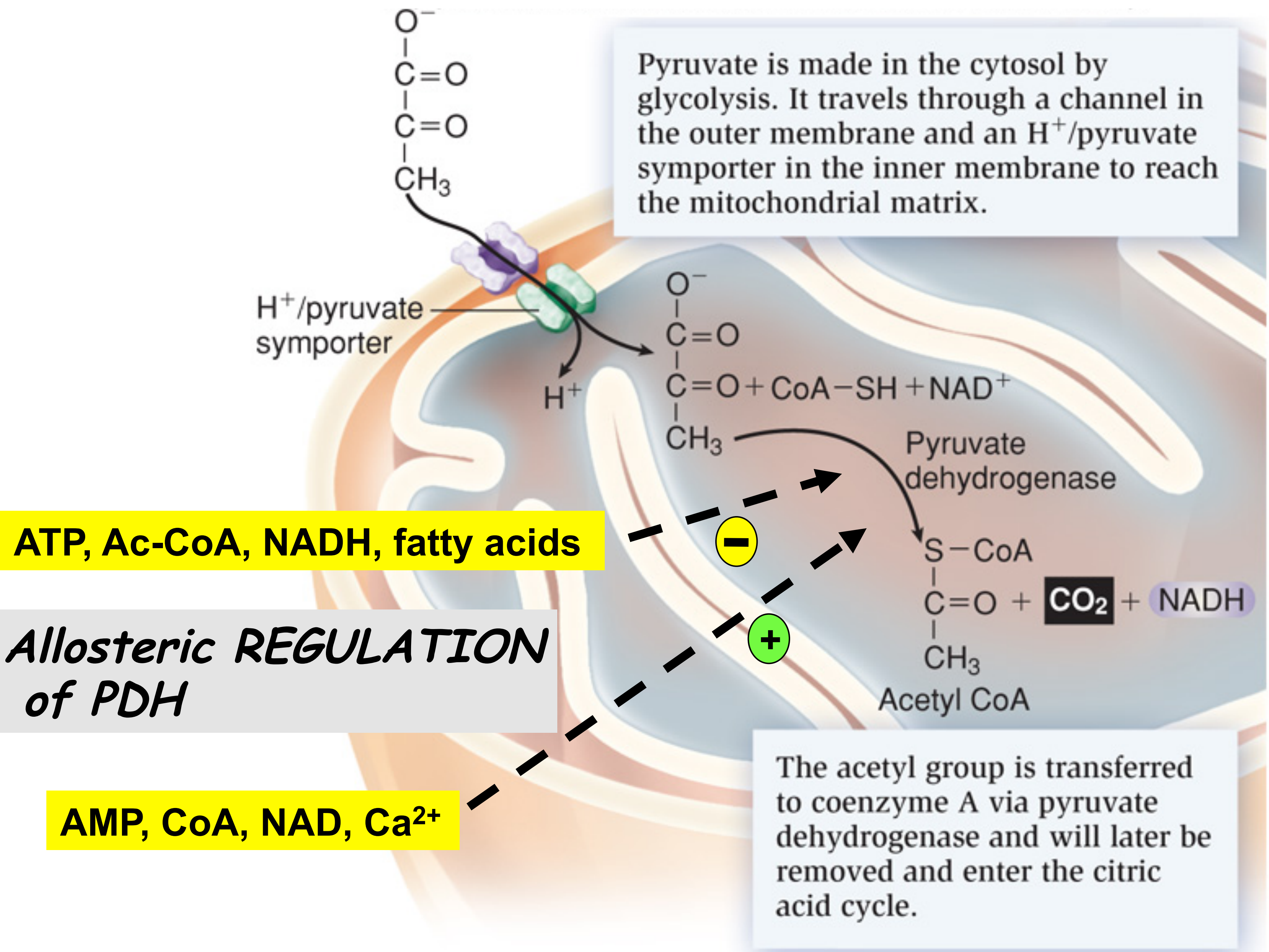
- PFK (phosphofrucktokinase) has allosteric site for inhibition by ATP, citrate, activation by **F2,6BP**

    - PFK considered rate-limiting step (in animals)
    - PFK rxn is irreversible

Stage 2: Breakdown of pyruvate to an acetyl group

- In eukaryotes, pyruvate transported to the mitochondrial matrix
- Broken down by pyruvate dehydrogenase (PDH)
- Molecule of CO_2 removed from each pyruvate
- Remaining acetyl group attached to CoA
- 1 NADH is made for each pyruvate
- Regulation: PDH allosteric inhib/activation

Allosteric REGULATION of PDH



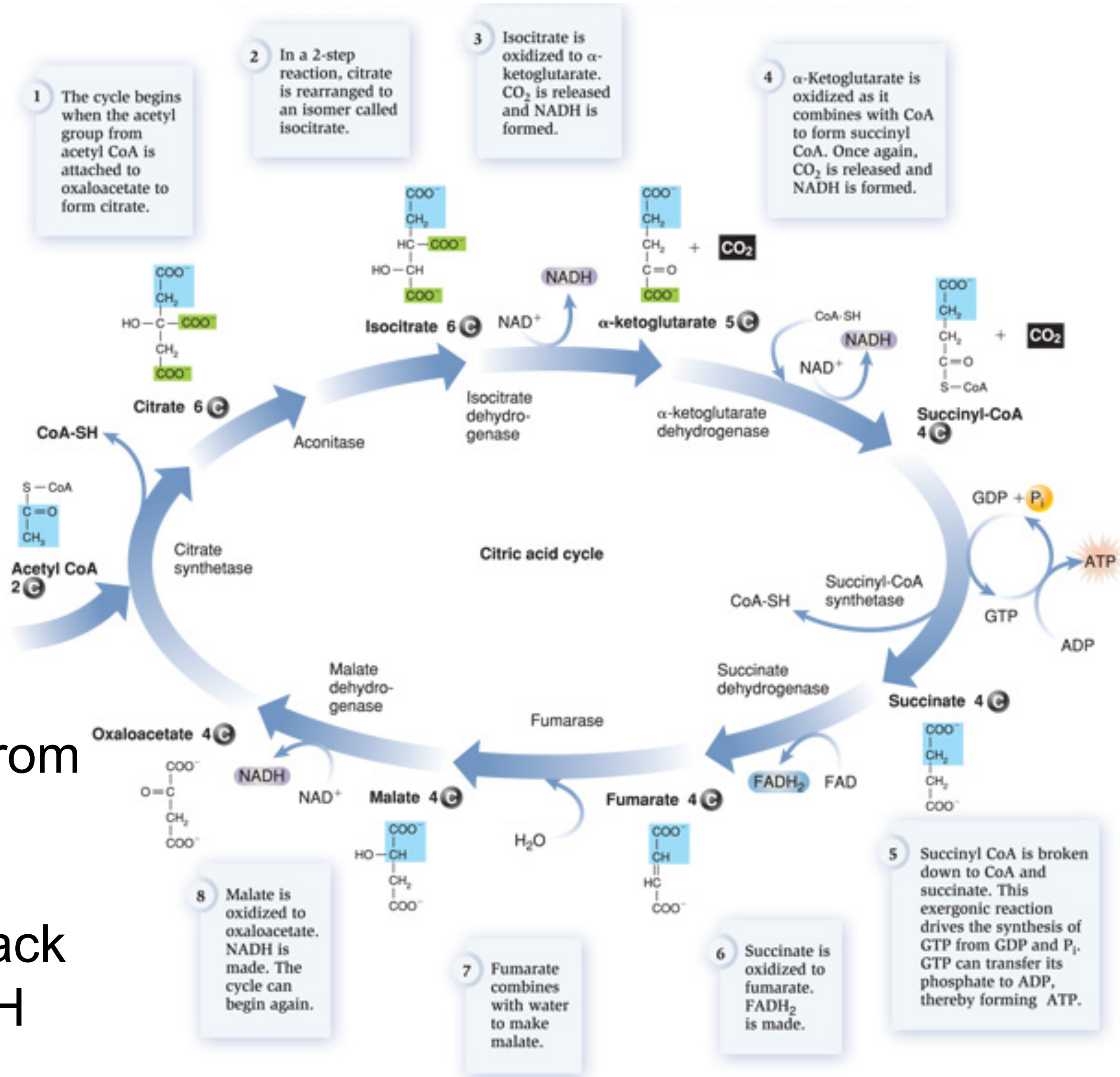
Stage 3: Citric acid cycle

- Metabolic ***cycle*** *(regenerative)*

- **Acetyl** is removed from Acetyl-CoA and attached to oxaloacetate to form citrate
- Series of steps releases 2CO_2, 1NTP, 3NADH, and 1 $FADH_2$
- Oxaloacetate is regenerated to start the cycle again

Regulation:

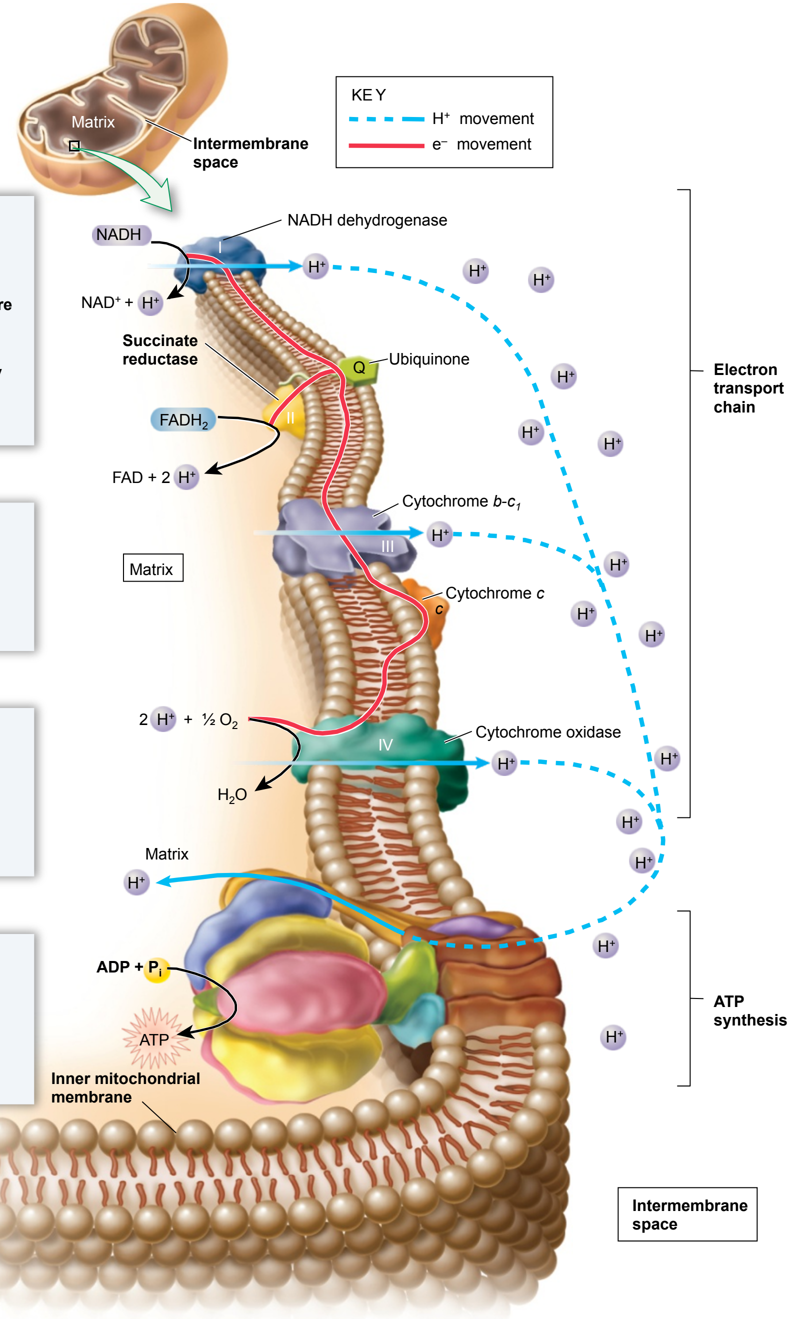
- at PDH
  - substrate availability from PDH
- various steps: negative feedback from ATP, NADH



Stage 4: Oxidative phosphorylation

- High energy electrons removed from NADH and $FADH_2$ to make ATP
- Typically requires oxygen
- Oxidative process involves electron transport chain
- Phosphorylation occurs by ATP synthase

Fig. 7.16



1a **NADH is oxidized to NAD⁺. High-energy electrons are transferred to NADH dehydrogenase. Some of the energy is harnessed to pump H⁺ into the intermembrane space. Electrons are then transferred to ubiquinone.**

1b **$FADH_2$ is oxidized to FAD. High-energy electrons are transferred to succinate reductase and then to ubiquinone.**

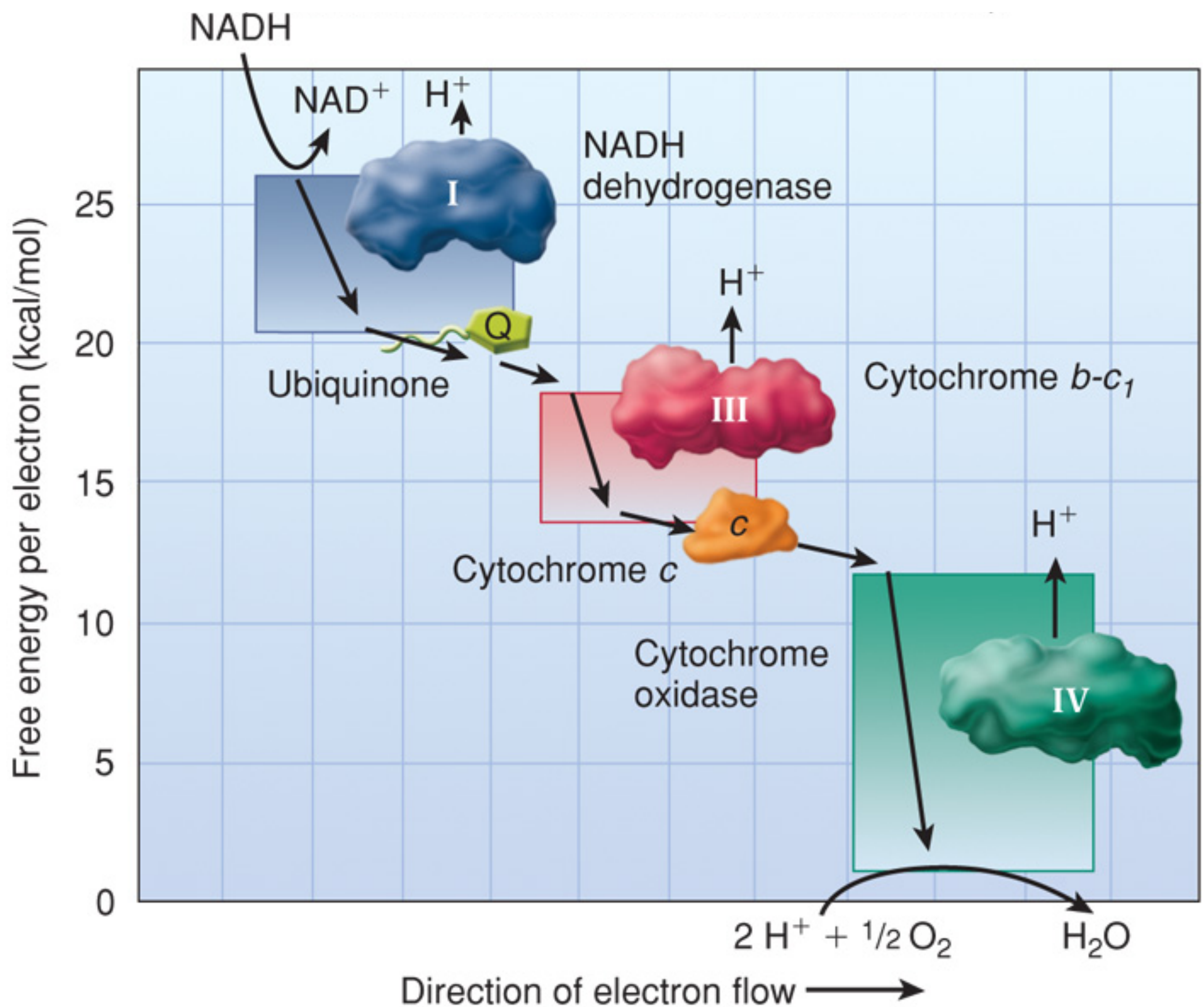
2 From ubiquinone, electrons travel to cytochrome *b*-c_1. Some of the energy is harnessed to pump H⁺ into the intermembrane space. Electrons are transferred to cytochrome *c*.

3 From cytochrome c, electrons are transferred to cytochrome oxidase. Some of the energy is harnessed to pump H⁺ into the intermembrane space. Electrons are transferred to oxygen, and water is produced.

4 Steps 1–3 produce a H⁺ electrochemical gradient. As H⁺ flow down their electrochemical gradient into the matrix through ATP synthase, the energy within this gradient causes the synthesis of ATP from ADP and P_i.

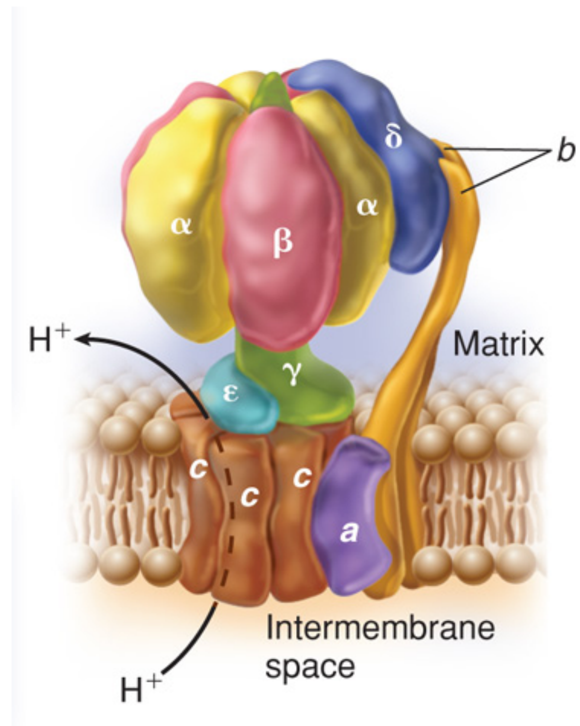
Free energy change

- Movement from NADH to O_2 is a very negative free energy change
  - Spontaneous in forward direction
- Highly exergonic
- Some energy used to pump H^+ across inner mitochondrial membrane and create H^+ electrochemical gradient

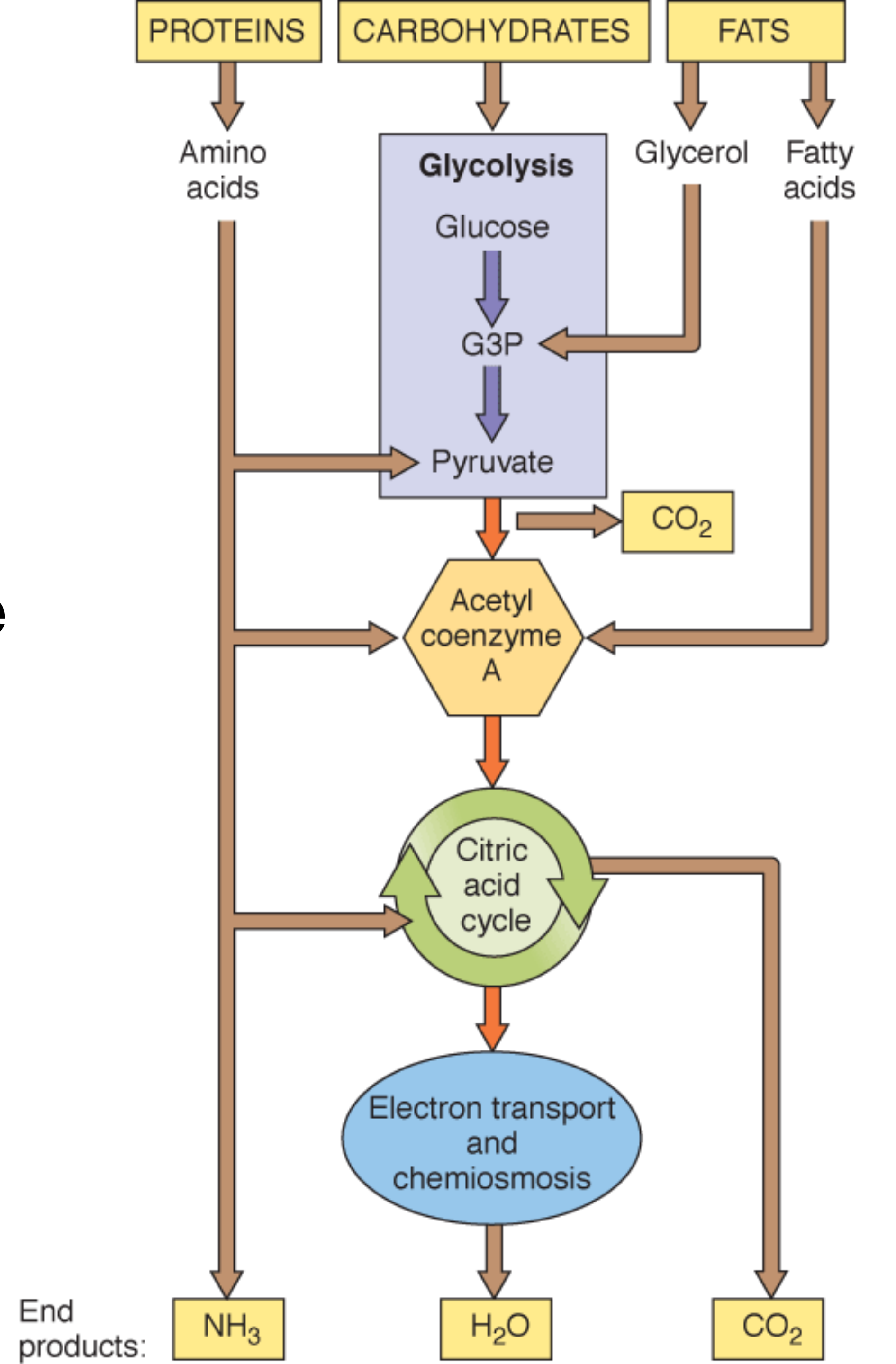

NADH
NAD+
H+
NADH dehydrogenase
I
25
20
15
10
5
0
Free energy per electron (kcal/mol)
Q
Ubiquinone
H+
III
Cytochrome b-c1
c
Cytochrome c
H+
Cytochrome oxidase
IV
2 H+ + 1/2 O2
H2O
Direction of electron flow

ATP synthase

- Enzyme harnesses free energy as H^+ flow through membrane embedded region
- Energy conversion- H^+ electrochemical gradient or proton motive force converted to chemical bond energy in ATP
- Racker and Stoeckenius confirmed used of an H^+ electrochemical gradient
- Rotary machine that makes ATP as it spins



- Many organisms depend on nutrients other than glucose
- Products of protein and lipid catabolism enter same metabolic pathways as glucose (efficient)
- Amino acids are deaminated

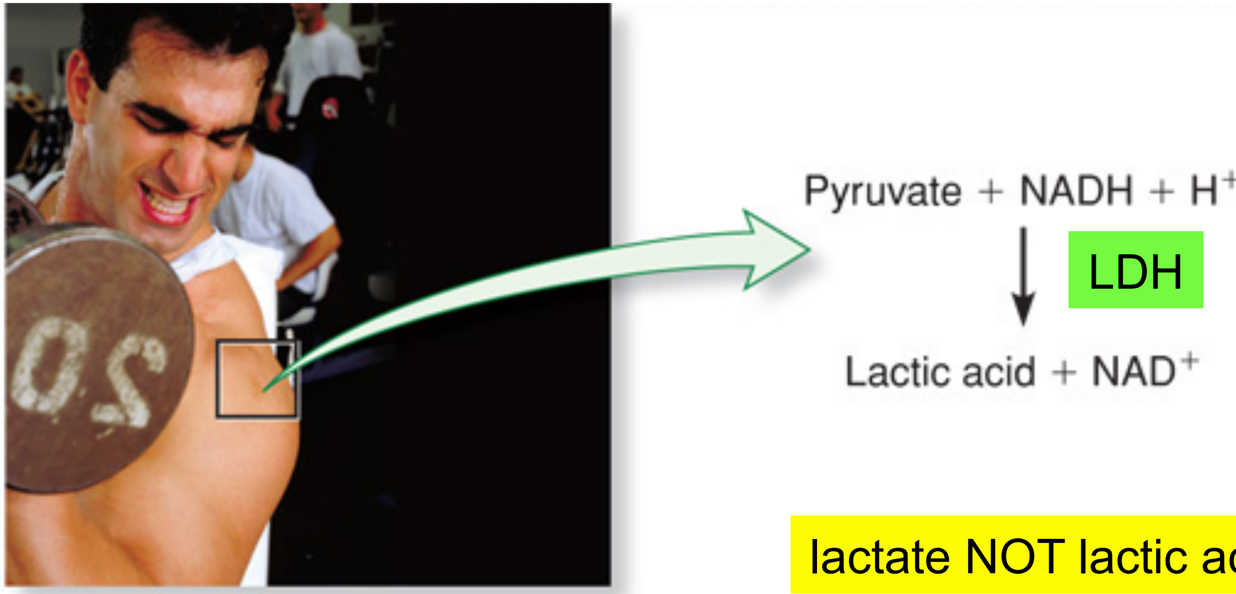


Anaerobic metabolism

- 2 strategies
  - Use substance other than O_2 as final electron acceptor in electron transport chain
  - If confined to using O_2, carry out glycolysis only
    - Pyruvate converted to lactate (muscles) or ethanol (yeast, plants)
    - Fermentation – produces far less ATP

AEROBIC → **~ 35 ATP via glycolysis + ETC**
~50% of total energy recovered

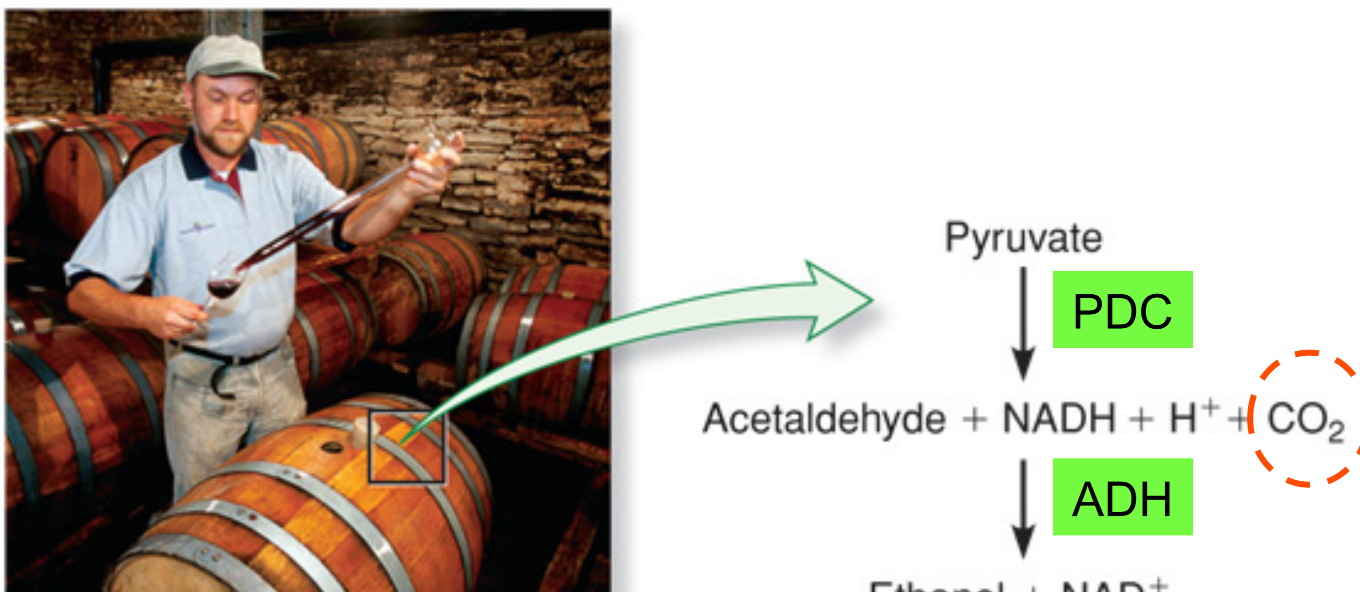
ANAEROBIC → **~ 2 ATP via glycolysis only**
~4% of total energy recovered



lactate NOT lactic acid!

(a) Production of lactic acid

WHY?



(b) Production of ethanol

Secondary Metabolism

- Primary - essential for cell structure and function
- Secondary - synthesis of metabolites that are not necessary for cell structure and growth
- 2º metabolites unique to a species or group
- Roles in defense, attraction, protection, competition

4 categories

- Phenolics
  - Antioxidants with intense flavors and smells
- Alkaloids
  - Bitter-tasting molecules for defense
- Terpenoids
  - Intense smells and colors
- Polyketides
  - Chemical weapons

There are thousands (!!) of 2º metabolites…..