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Kirk's Amazing Exam 3 Review Notes

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Exam 3 Review

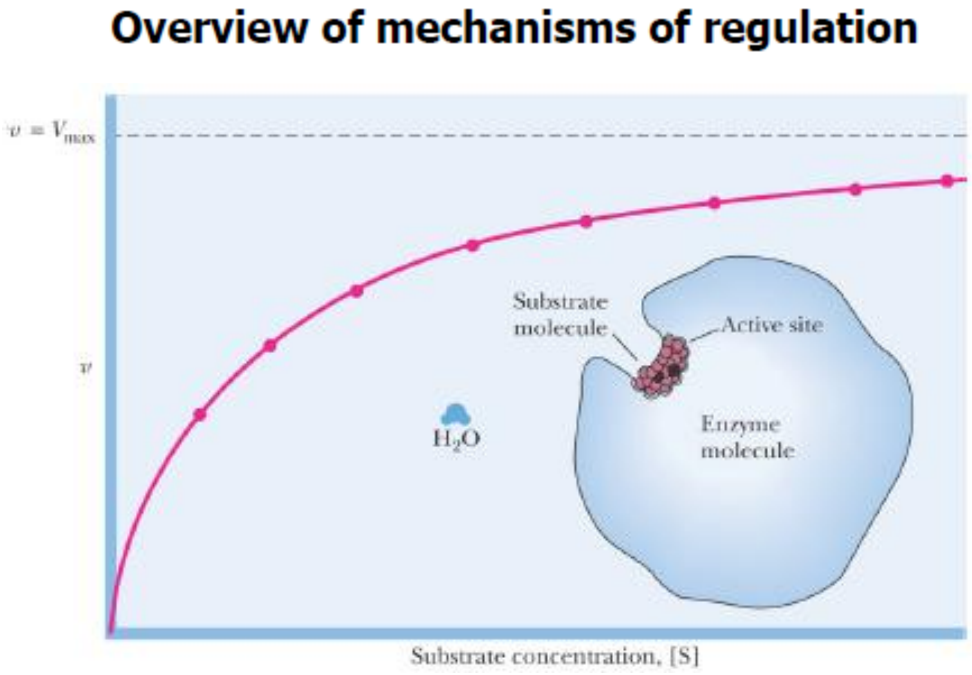
Enzyme Regulation

Chapter 15, pg. 452-462 and 467-76

Understand the different mechanisms by which enzyme activity can be regulated and know some examples of each

Regulation of activity by…

- Allosteric
- Covalent modification
- Zymogens
- Isozymes
- Modulator proteins



The velocity of a reaction is typically controlled by the concentrations of substrates and cofactors (cofactors: metal ions or organic coenzymes that participate in some enzyme reactions).

Understand the different mechanisms by which enzyme activity can be regulated and know some examples of each

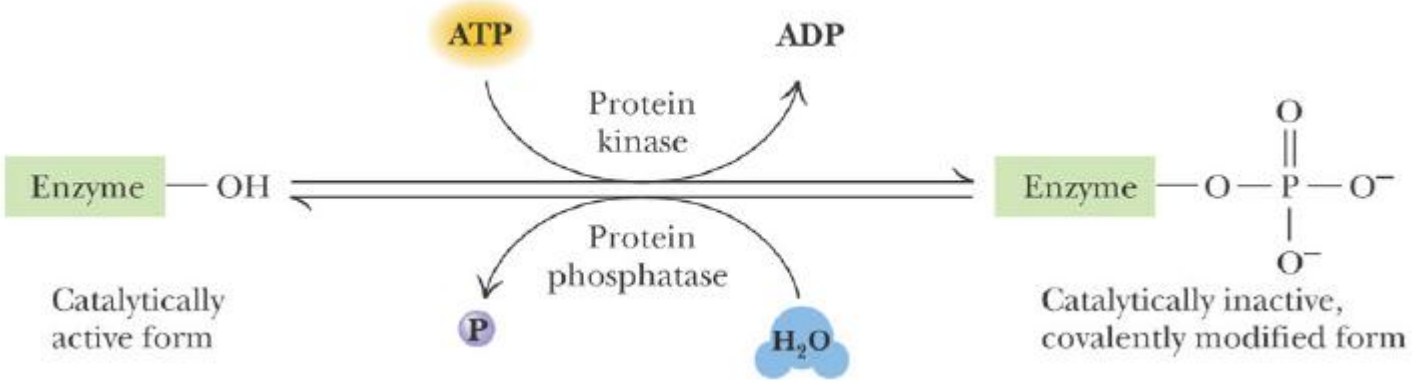
Allosteric regulation: activation or inhibition of enzyme activity via non-covalent interaction of the enzyme with small molecules other than the substrate

- Allosteric effector – are sterically different from the substrate, bind to a site other than the active site
- Positive heterotropic effector (allosteric activator) – an allosteric effector that activates the binding of substrate
- Negative heterotropic effector (allosteric inhibitor) – does the opposite
- Is a reversible process

Understand the different mechanisms by which enzyme activity can be regulated and know some examples of each

Regulation via covalent modification

- Reversible attachment of a chemical group
- Enzyme being modified is an interconvertible enzyme
- Catalyzed by converter enzymes
- An example is phosphorylation of an amino acid side chain that activates/inactivates the enzyme via protein kinases.
- Side chain targets for phosphorylation are serine, threonine, tyrosine, and aspartate.



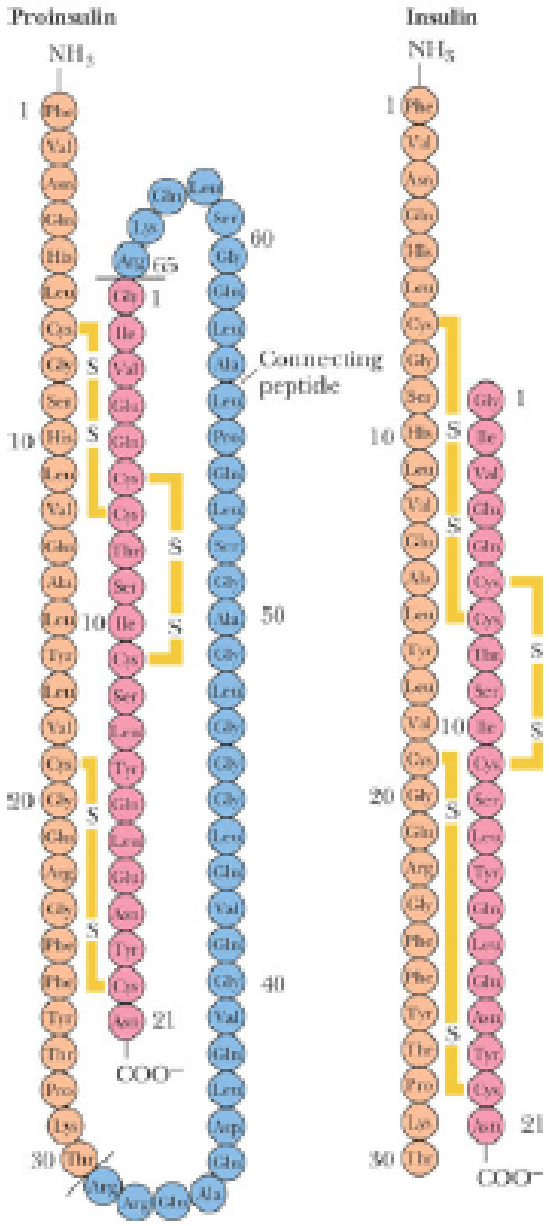
Understand the different mechanisms by which enzyme activity can be regulated and know some examples of each

Zymogens aka Proenzymes

- Inactive precursors that acquire full activity upon proteolytic cleavage of peptide bonds.
- It is an irreversible process.

Examples…

Insulin is made from the inactive precursor, proinsulin. Removing residues 31-65 makes insulin.



The hormone **insulin** is made as an inactive 86 amino acid precursor (proinsulin). Proteolytic removal of residues 31-65 generates the active form, which has two chains and three disulfide bonds

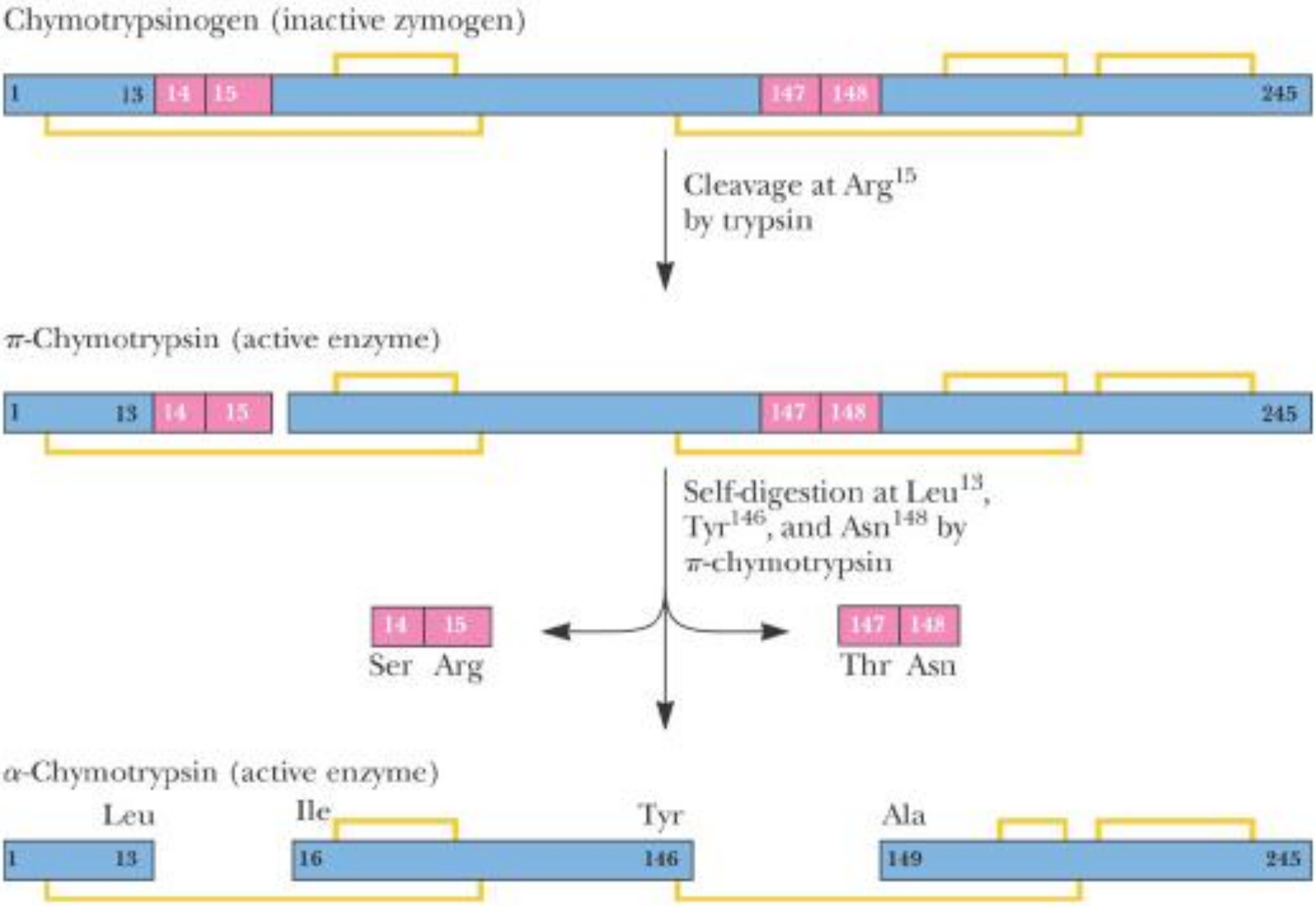
Understand the different mechanisms by which enzyme activity can be regulated and know some examples of each

Zymogen Examples Continued…

Proteolytic enzymes of the digestive tract: Chymotrypsinogen

Chymotrypsinogen is the zymogen of chymotrypsin

I. Trypsin cleaves chymotrypsinogen to make π-chymotrypsin
II. π-chymotrypsin cleaves other π-chymotrypsins
III. The mature α-chymotrypsin is formed


Chymotrypsinogen (inactive zymogen)
1
13
14
15
147
148
245
Cleavage at Arg15
by trypsin
π-Chymotrypsin (active enzyme)
1
13
14
15
147
148
245
Self-digestion at Leu13,
Tyr146, and Asn148 by
π-chymotrypsin
14
15
Ser Arg
147
148
Thr Asn
α-Chymotrypsin (active enzyme)
Leu
Ile
Tyr
Ala
1
13
16
146
149
245

Understand the different mechanisms by which enzyme activity can be regulated and know some examples of each

Zymogen Examples Continued…

Blood Clotting…

- Fibrinogen is a zymogen that is converted to fibrin by thrombin.
- Fibrin helps in blood clotting.

Understand the different mechanisms by which enzyme activity can be regulated and know some examples of each

Isozymes: Enzymes with different subunits…

- Differ in terms of relative affinities to substrates and sensitivity to inhibition by their product

Example… Lactate Dehydrogenase

- Has 5 different isozymes located in different areas in the body. Different tissues express different isozyme forms.

Another Example… Hexokinase IV ("Glucokinase") – Glycolysis Step 1

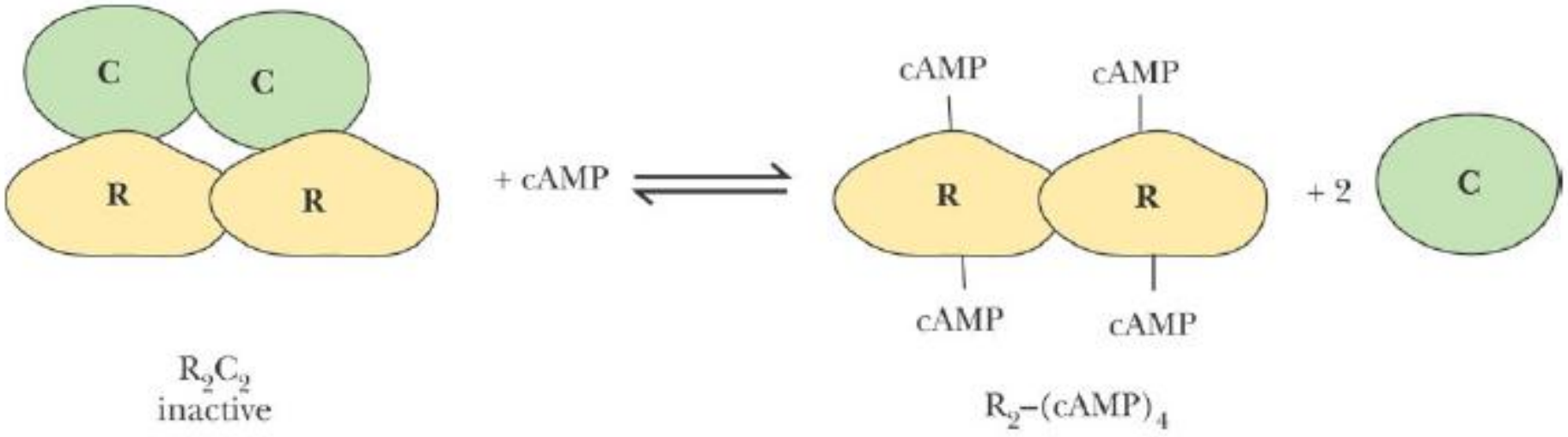
- Control of insulin release by β cells of pancreas
- Initiation of glycogen synthesis by liver cells

Understand the different mechanisms by which enzyme activity can be regulated and know some examples of each

Modulator Proteins…

Example: cAMP-dependent protein kinase (PKA)

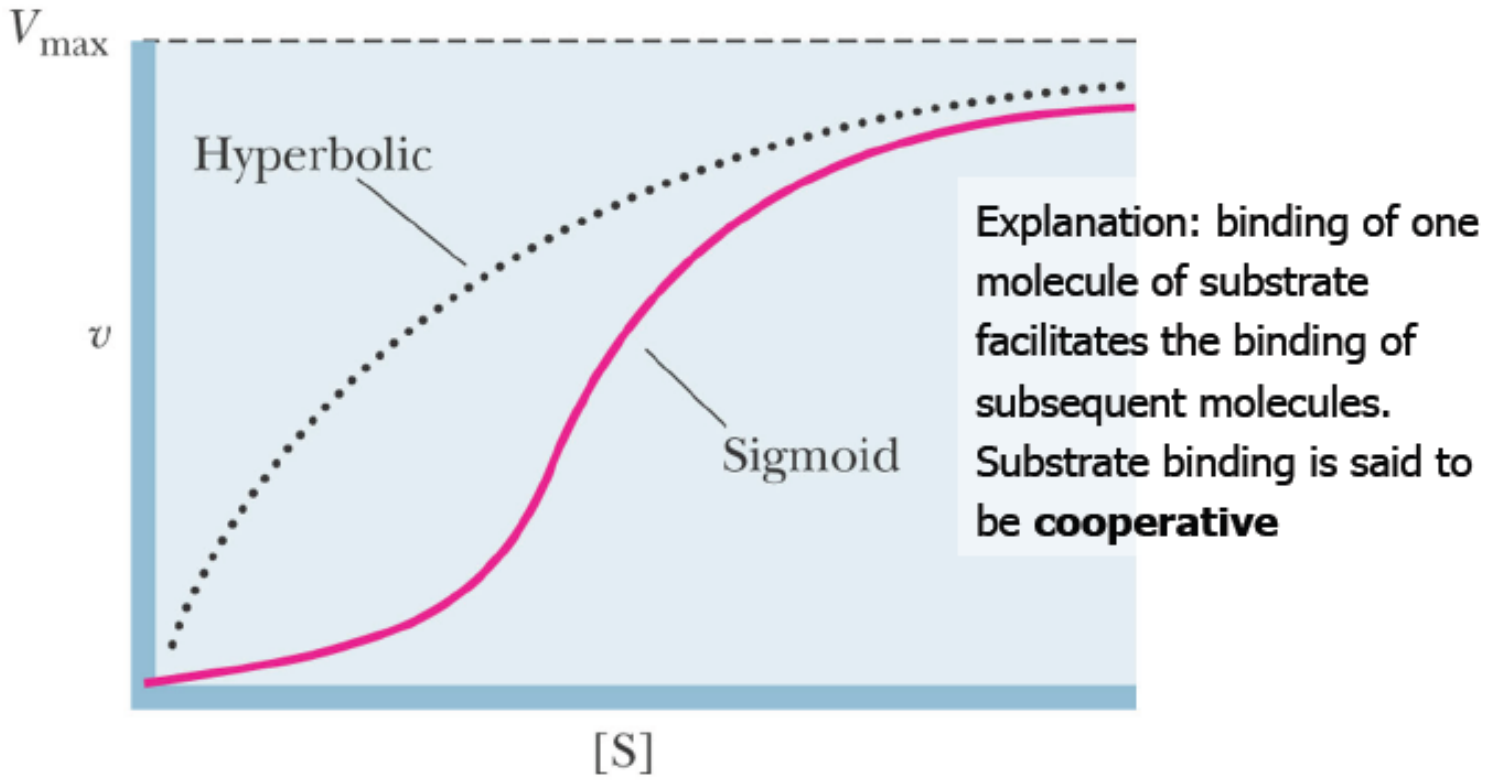
- Catalytic subunit, C, is kept inactive by binding of regulatory subunit, R. cAMP binding releases C, the active enzyme.



Regulatory enzymes typically show sigmoidal kinetics, indicative of cooperative substrate binding…

What the biochemistry does this mean?

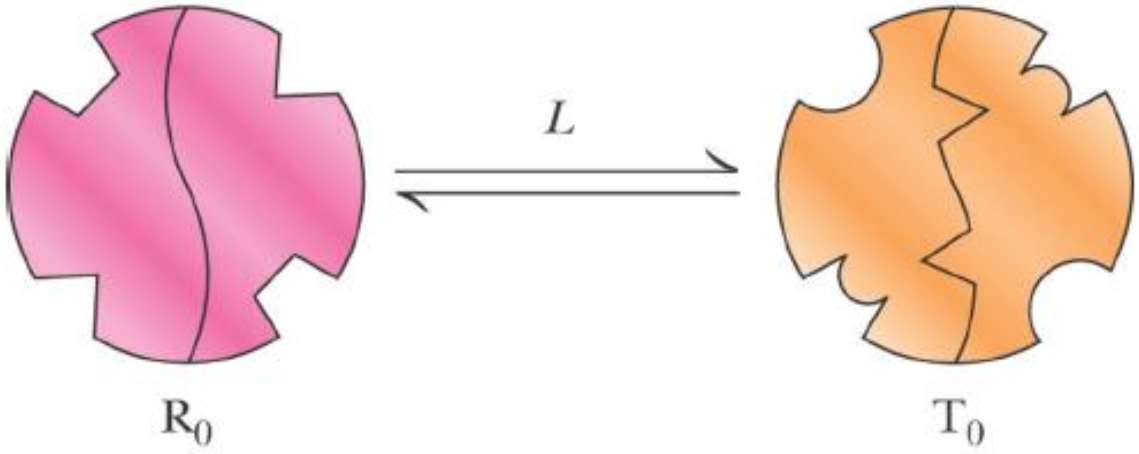
- Regulatory enzymes (allosteric enzymes) are oligomeric, meaning they have more than one subunit.
- Each subunit has a substrate binding site and a binding site for allosteric effectors.
- So first the velocity starts off slow, but binding of one substrate to the regulatory enzyme facilitates the binding of more molecules. This type of binding is cooperative.



- Allosteric effectors bind to sites distinct from the substrate binding sites.
- Allosteric enzymes are typically oligomers (>1 substrate binding site).
- Regulatory effects depend on **conformational changes** occurring in the enzyme as result of effector binding.

Understand how the MWC model explains cooperativity in substrate binding and allosteric regulation

The Monod-Wyman-Changeux (MWC) model to explain allosteric regulation



$$L = \frac{T_0}{R_0} \qquad L \text{ is large. } (T_0 >> R_0)$$

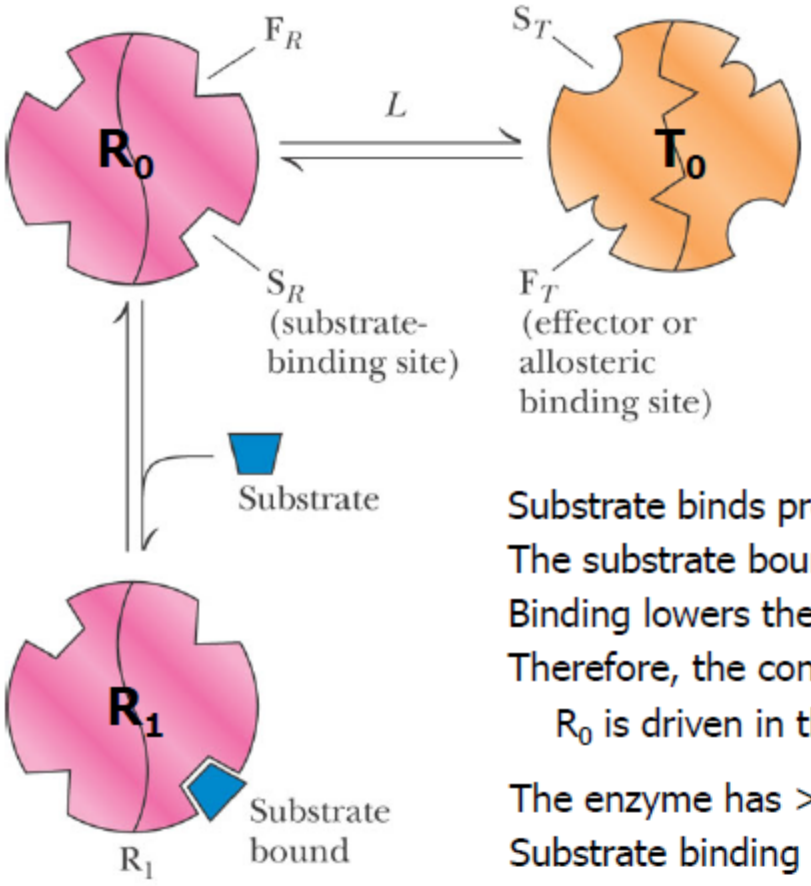
The affinity of each state of the enzyme for the substrate is described by a dissociation constant: K_R (for the relaxed form) and K_T (for the tense form)

The model supposes that K_T is much greater than K_R. That is, R_0 has a higher affinity for the substrate than does T_0

So, the substrate binds with higher affinity to the less abundant form of the enzyme

Understand how the MWC model explains cooperativity in substrate binding and allosteric regulation

The Monod-Wyman-Changeux (MWC) model explains cooperativity

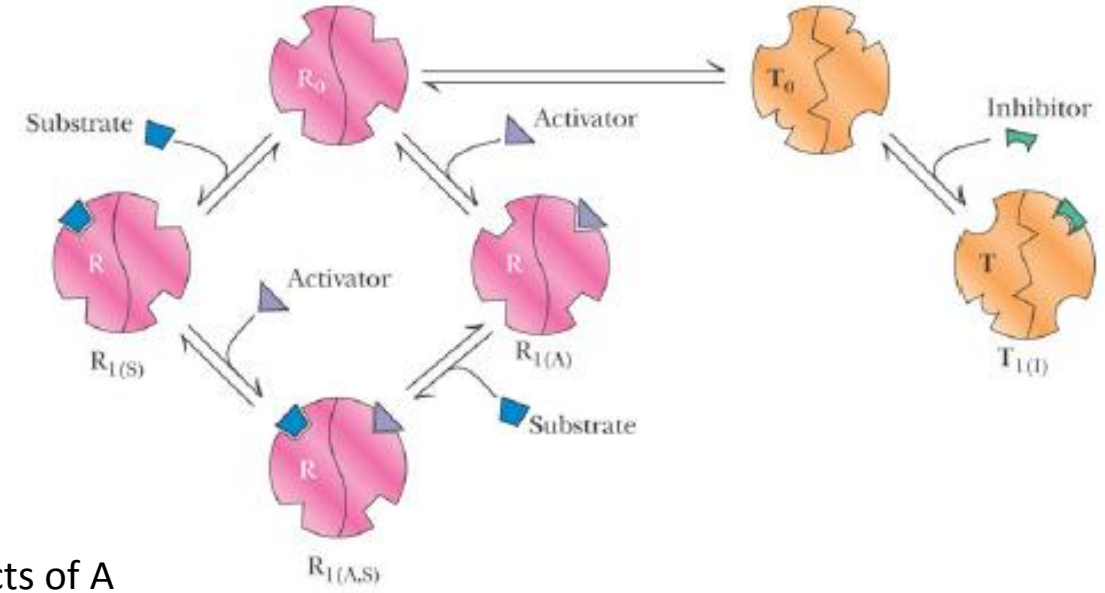


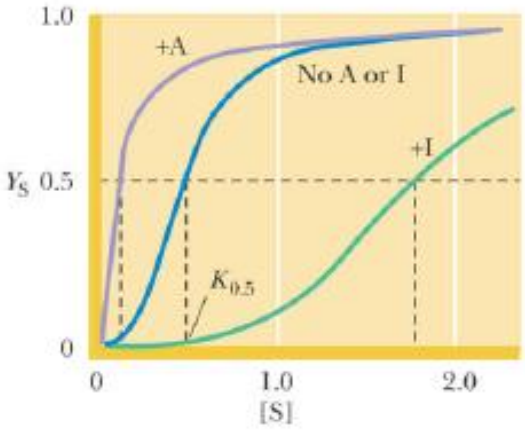
Substrate binds preferentially to R_0
The substrate bound form is called R_1
Binding lowers the abundance of R_0
Therefore, the conformational transition of T_0 to R_0 is driven in the direction of R_0

The enzyme has >1 substrate binding sites
Substrate binding increases the concentration of R_1 + R_0 (the total population of R confomers)
Therefore, the number of substrate binding sites increases
This explains positive cooperativity of substrate binding

Understand how the MWC model explains cooperativity in substrate binding and allosteric regulation

The Monod-Wyman-Changeux (MWC) model to explain allosteric activation





Effects of A

$A + R_0 \rightarrow R_{1(A)}$

Increase in number of R-conformers drives T_0 to R_0

1) More binding sites for S made available
2) Decrease in cooperativity in saturation curve

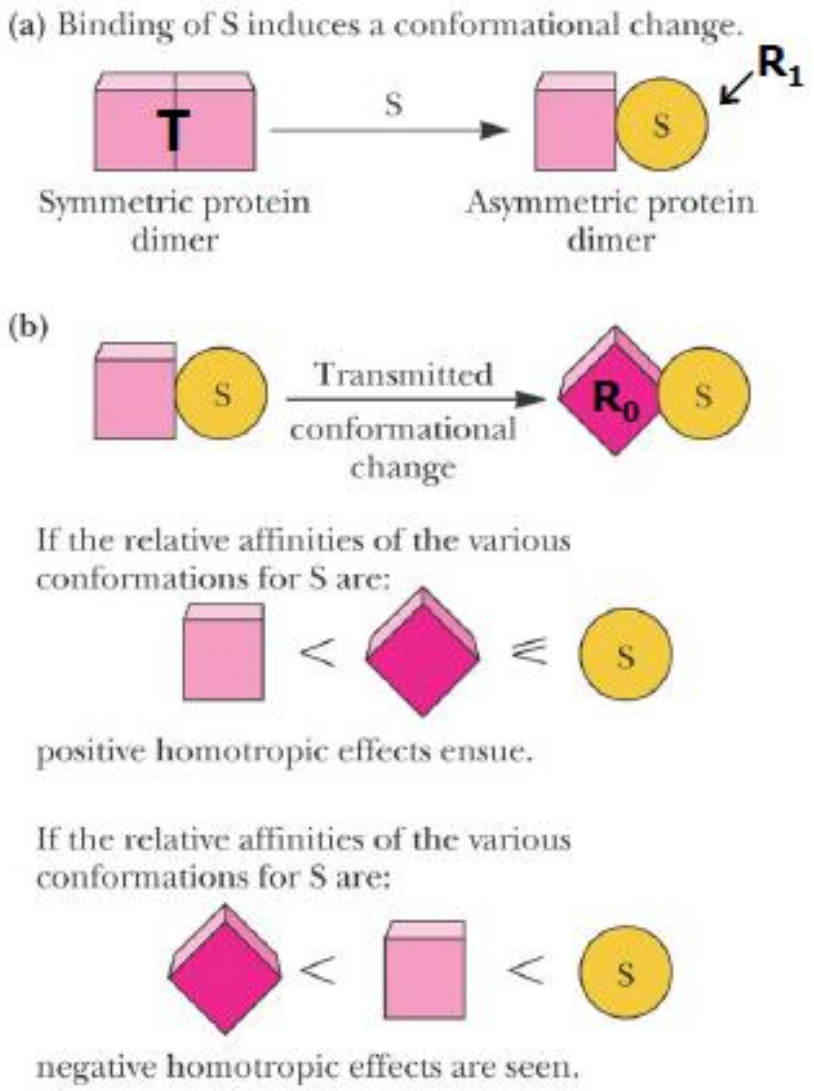
Effects of I

$I + T_0 \rightarrow T_{1(I)}$

1) Increase number of T-conformers (decrease in R_0 to restore equilibrium)
2) I inhibits association of S and A with R by lowering R_0
3) Thus, I increases cooperativity.

Understand how the KNF model explains cooperativity in substrate binding and allosteric regulation

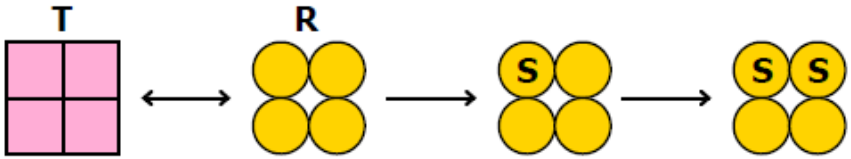
- In absence of S, there is no equilibrium between different conformers (R and T).
- S binding causes a conformational change, and the **subunits can adopt different conformations** (unlike MWC)
  - S binding causes the other subunit to undergo a conformation change to where it has a higher or lower affinity (thus it can explain negative cooperativity)
- Allosteric activator works like S, except it binds to a different site
- Inhibitor prevents transition from T to R that is induced by S binding



Know the key features that distinguish the two models…

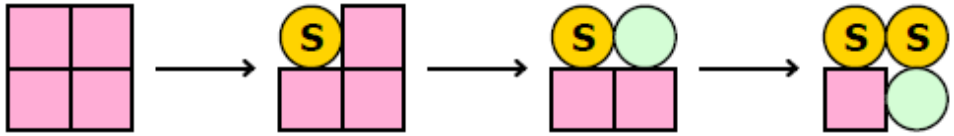
MWC Model

- There is a pre-existing equilibrium between R and T
- S or A binds preferentially to R form
- Only two possible conformations, and all subunits must be in the same conformation.
- Subunits change conformation together (**symmetry** model)



KNF Model

- Conformational change is induced by ligand binding
- Conformational change can be transmitted to a neighboring subunit
- Intermediate conformations are possible
- Subunits can be in different conformations and change sequentially (**sequential** model)



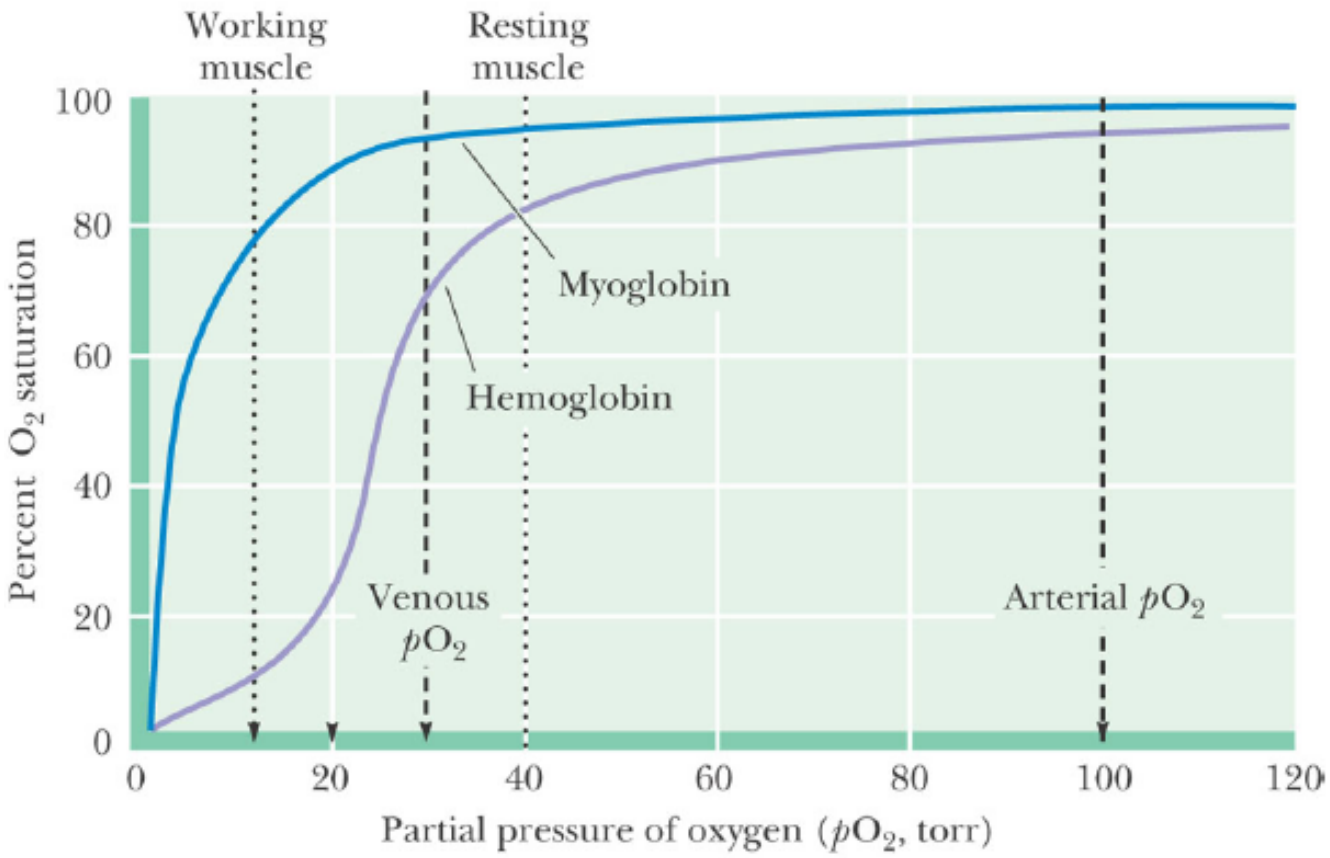
Understand that these models are not absolutes – different enzymes have features that are consistent (to a greater or lesser extent) with one or the other model, or with both.

Know the quaternary structures of hemoglobin and myoglobin…

- Hemoglobin is an $\alpha_2\beta_2$ tetramer. Each of the alpha chains is in contact with both beta chains, but there are few alpha-alpha or beta-beta interactions.
- Myoglobin is monomeric.
- Both proteins are largely alpha-helical, subunits have similar tertiary structures. Each subunit contains a molecule of heme, a non-covalently bound prosthetic group that is the binding site for oxygen.
- Hb carries oxygen in the bloodstream.
- Mb stores oxygen in the muscle.

Understand the oxygen binding curves for Mb and Hb

Oxygen binding to Hb (but not Mb) shows sigmoidal kinetics



Explanation: O_2 binding to Hb (which has 4 binding sites) is positively cooperative

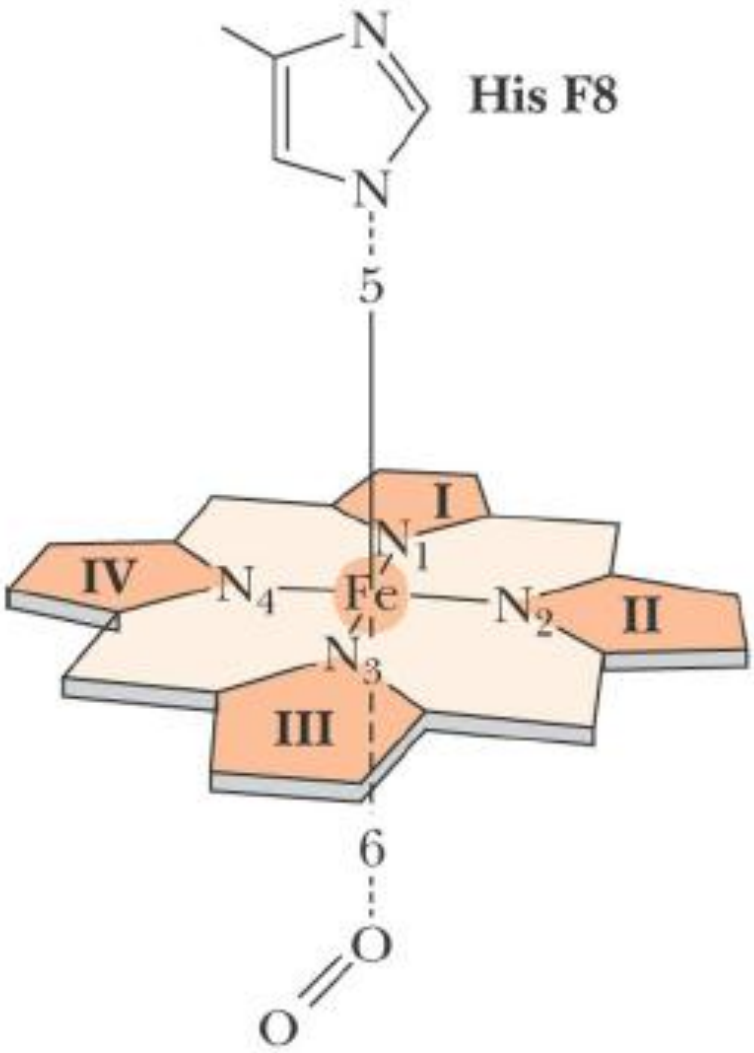
Understand how oxygen binds and how binding causes conformational changes…

- Heme is the oxygen carrier in Mb and Hb
- Heme contains an iron bound by four nitrogens of the porphyrin ring
- Fe exists in ferric (3+) state, and in ferrous (2+) oxidation states.
- Mb and Hb containing heme in the 2+ form are called deoxy-Mb and deoxy-Hb. Oxygen binds to this form to generate oxy-Mb and oxy-Hb
- Oxidation to the 3+ form generates met-Mb and met-Hb. Oxygen does not bind to this form.

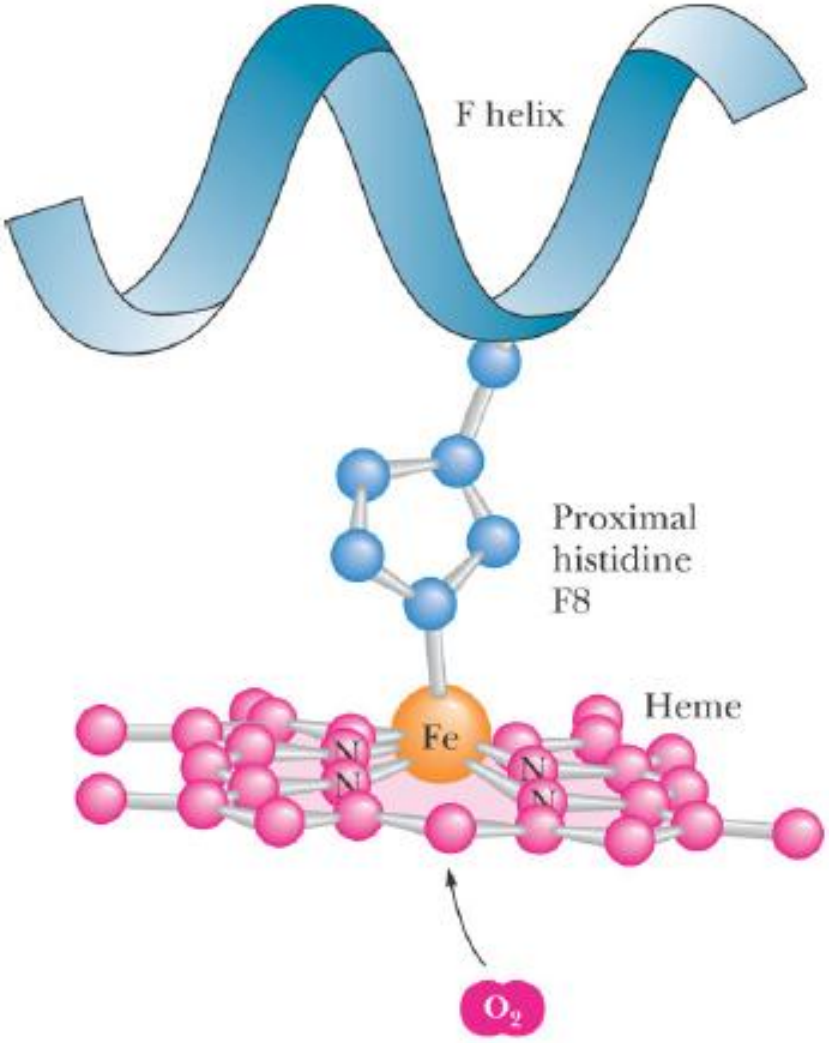
Understand how oxygen binds and how binding causes conformational changes…

Iron likes to have 5 or 6 ligands…

- Oxy form- 4 ligands from the porphyrin ring, 5th from histidine side chain, and 6th from O_2
- Deoxy form- (2+ oxidation) O_2 is not bound, hence, no sixth ligand
- Met form- (3+ oxidation) sixth ligand is water instead of O_2

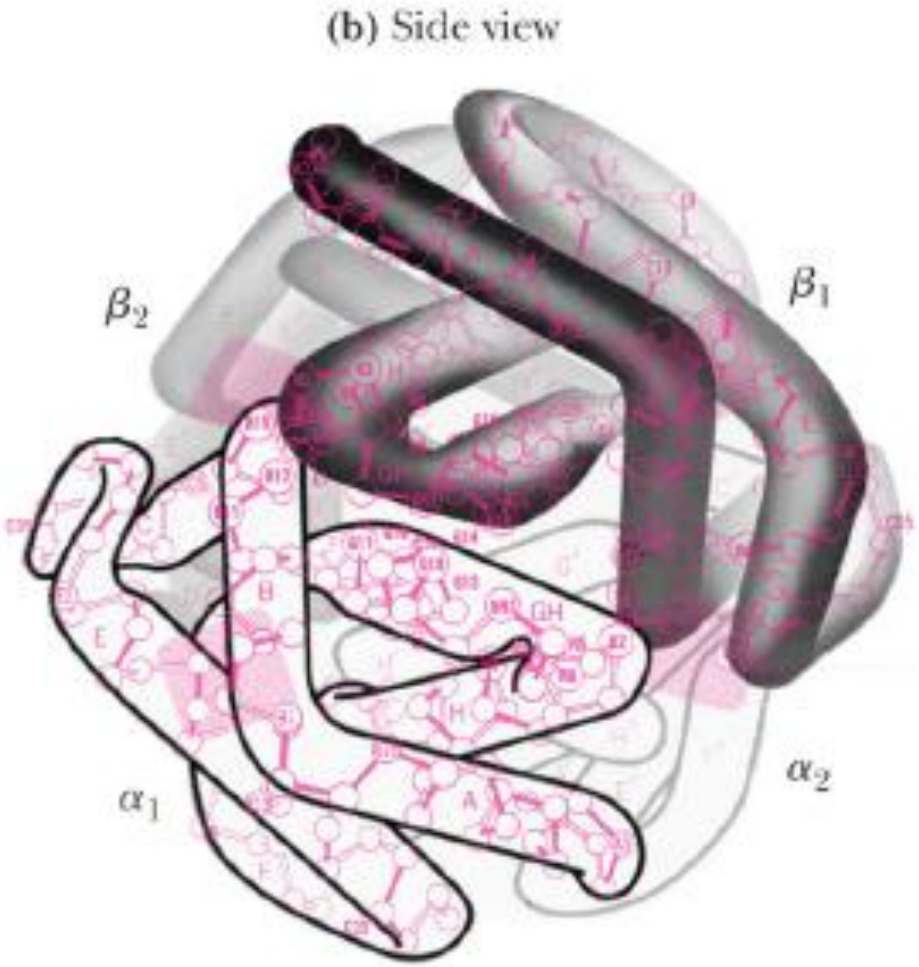


Understand how oxygen binds and how binding causes conformational changes…



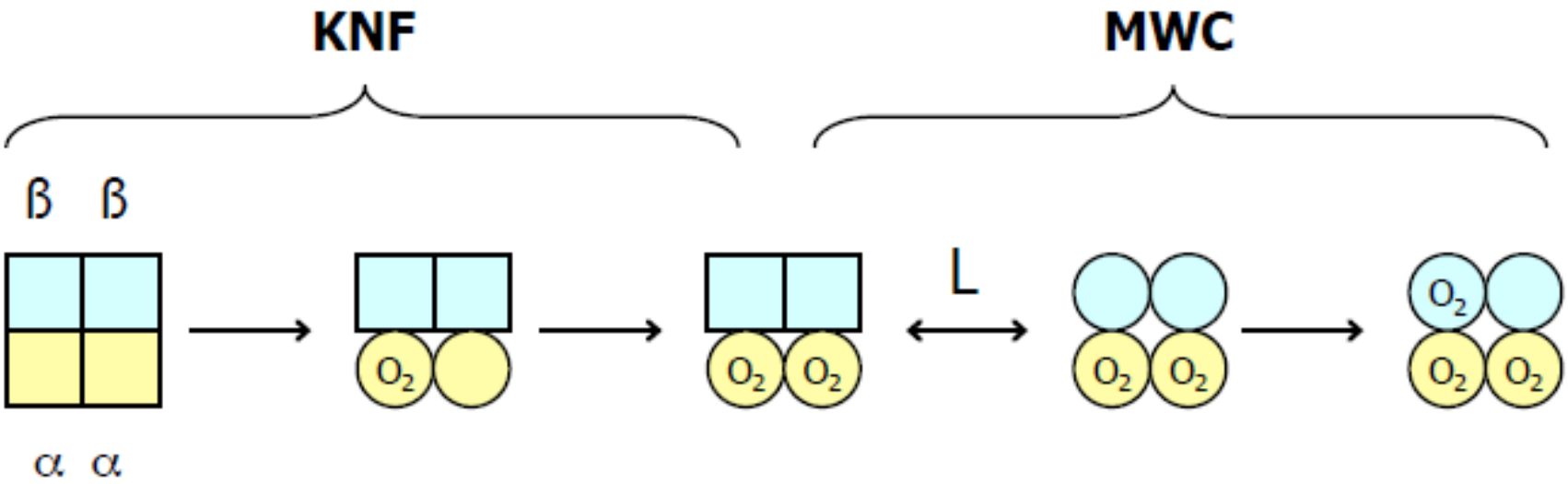
- Fe is pulled out of plane of porphyrin ring and towards histidine in deoxy-Mb form
- Binding of oxygen moves Fe back into plane and causes shift in position of F helix
- Movement of histidine F8 and F helix trigger series of movement that results in rupture of hydrogen bonds and inter-chain bridges

Understand how oxygen binds and how binding causes conformational changes…



- Hb is a symmetric $\alpha_2\beta_2$ tetramer
- Oxygen binding:
  - Cooperativity not explained by direct heme-heme interactions
  - Causes small changes in tertiary and large change in quaternary
  - Binds first to two α subunits, increases affinity of β subunits
- Each αβ moves as a rigid body, the two dimers rotate through 15° with respect to each other, comparing deoxy-Hb with oxy-Hb

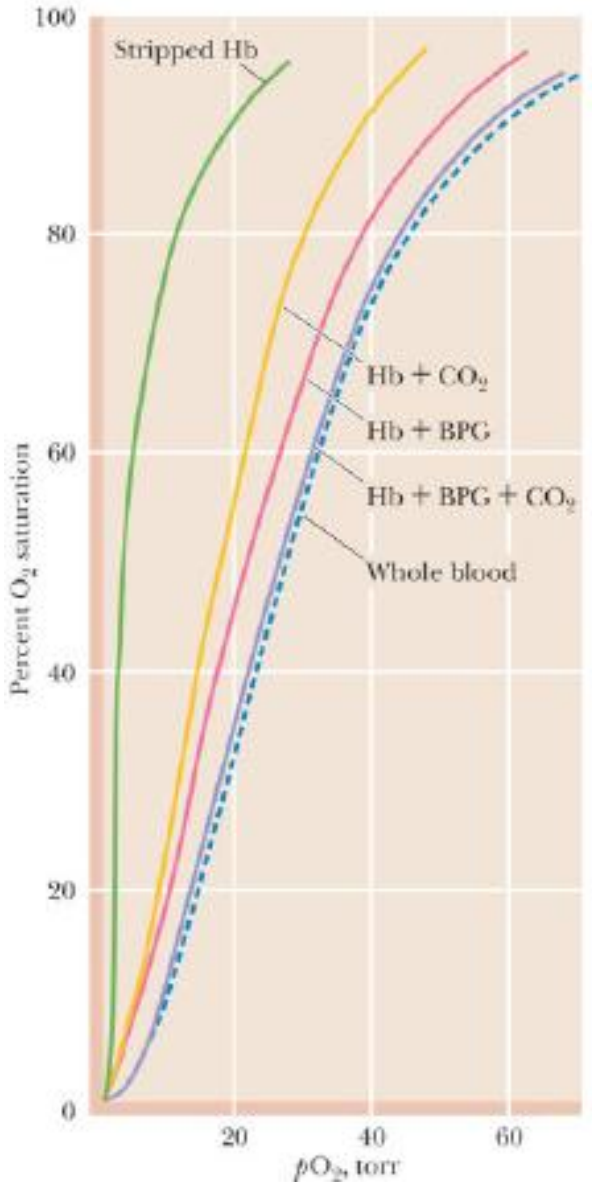
Binding causes small changes in tertiary structure and large changes in quaternary structure of Hb, cooperativity shows features of both models.

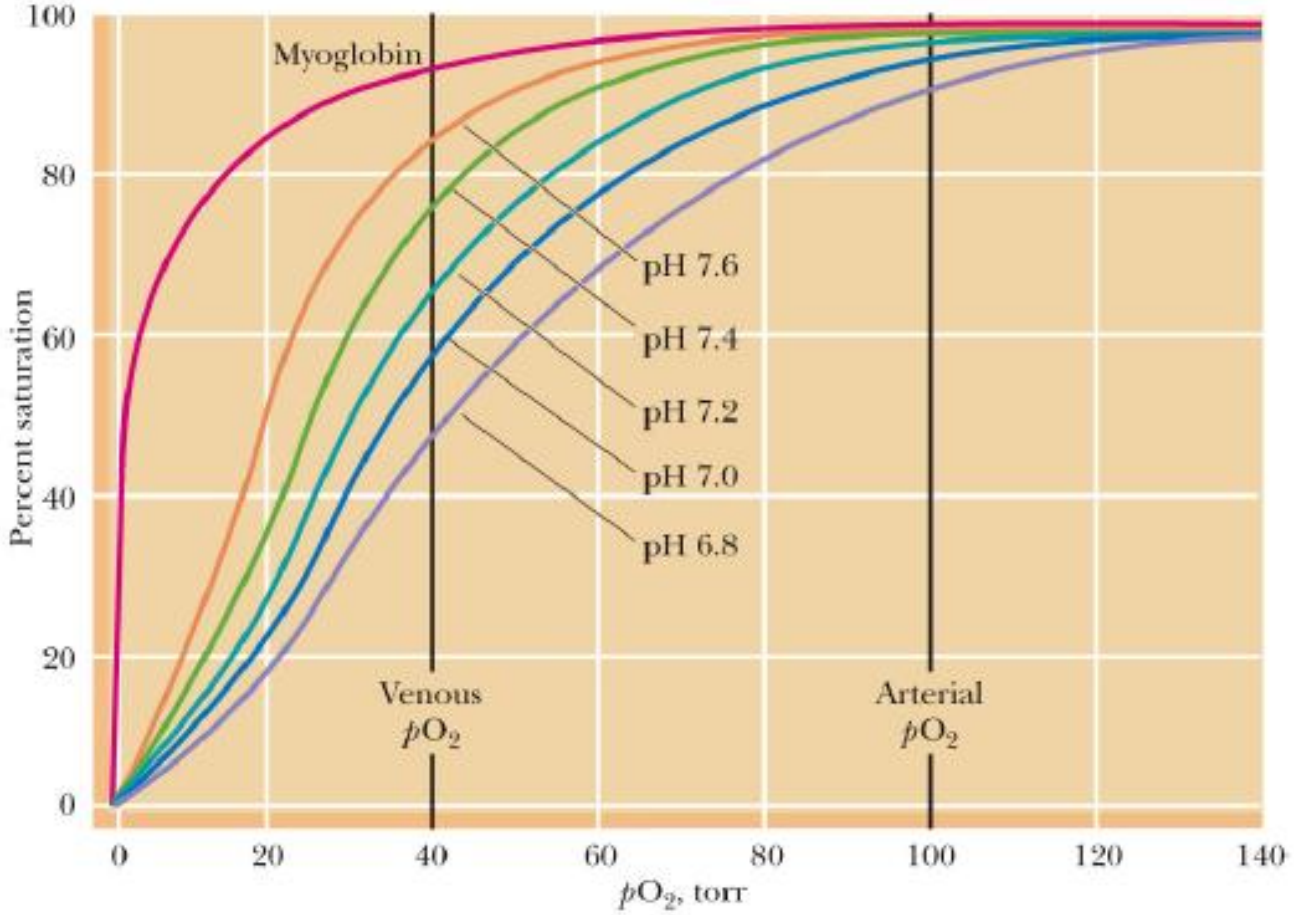


- At the beginning: deoxy-Hb has low affinity for oxygen
- β subunits are at first inaccessible to oxygen
- First oxygen binds to α subunit
- Small changes in tertiary structure of the other α subunit increases its affinity for oxygen 3-fold
- Between 3rd and 4th step, Hb with 2 oxygens is in equilibrium with a form of the protein in which all four subunits are in the R state
- Large change in quaternary structure occurs here at this step.

2,3-bisphosphoglycerate is an allosteric effector of Hb

- 2,3-BPG acts as a negative allosteric effector of Hb.
- The curve becomes more cooperative.
- 2,3-BPG stabilizes the deoxy form (T-state), reducing the affinity for oxygen.
- Fetal hemoglobin (α2γ2 structure) has a higher affinity for oxygen because it has a lower affinity for BPG. The curve should be less cooperative.





- Bohr effect: acidic solution promotes oxygen dissociation from Hb
- Acid produced when tissues metabolize → dissociation of O_2 at site of consumption

- $CO_2 + H_2O \xrightarrow{\text{carbonic anhydride}} HCO_3^- + H^+$
  - High CO_2 (in actively metabolizing tissues) also favor O_2 dissociation

Example Problem 1

The following are properties of allosteric enzymes EXCEPT:

A. They have multiple subunits.
B. The regulatory effect is by altering conformation and interaction with subunits.
C. Effectors may stimulate or inhibit activity.
D. They obey Michaelis-Menten kinetics.
E. Binding to one subunit affects binding of substrate to other subunits.

Example Problem 2

Proinsulin is converted into insulin by:

A. Allosteric binding of glucose
B. Phosphorylation to the active form
C. Proteolytic excision of a specific peptide
D. Removal of phosphate by converter enzymes
E. None of the above

Example Problem 3

Which of the following statements is correct regarding isozymes?

A. They catalyze the same reaction but have vastly different structures.
B. They often respond to different inhibitors and activators.
C. Their differences are based upon the type of tissue in which they are present.
D. They are always monomeric proteins.
E. B and C
F. A, B, and C

Example Problem 4

All are true about isozymes of lactate dehydrogenase (LDH) that are present in a number of different tissues EXCEPT:

A. They have different K_m values for pyruvate.
B. The amount of subunit phosphorylation differs.
C. The ratios of A and B subunits differ depending upon the tissue type.
D. They have different kinetic parameters.
E. They have different K_m values for lactate.

Example Problem 5

The presence of a negative allosteric effector on an allosteric protein would:

A. Cause a shift to the left in the sigmoidal curve
B. Increase the number of R conformations
C. Decrease the cooperativity of the substrate
D. Raise the apparent value of the equilibrium constant, L
E. Increase the likelihood of the binding of S

Example Problem 6

For an enzyme that shows negative cooperativity, the activity is _____ when the substrate is at concentrations _____ than the $K_{0.5}$ value as compared to enzymes that show no cooperativity.

A. Increased; greater
B. Increased; less
C. Decreased; greater
D. Decreased; less
E. Both B and C are correct.
F. Both A and D are correct.

Example Problem 7

When binding one equivalent of S to an allosteric protein enhances the binding of additional equivalents of S to the same protein molecule, it is termed a(n):

A. Positive homotropic effector
B. Positive heterotropic effector
C. Negative homotropic effector
D. Negative heterotropic effector
E. None of the above

Example Problem 8

Which statement below about contrasting Hb and Mb is FALSE?

A. Hb shows cooperativity, whereas Mb does not.
B. Hb binds O_2 more tightly than Mb.
C. Hb shows sigmoidal, whereas Mb shows hyperbolic oxygen saturation curves.
D. Oxygen binds to a ferrous ion in both proteins.
E. Hb-oxygen binding is dependent on physiological changes in pH, whereas Mb-oxygen binding is not.

Example Problem 9

When O_2 binds to _____ in Hb, the _____ ion is drawn into the plane of the _____ causing a conformational change that is transmitted to adjacent subunits enhancing the _____ for additional O_2 binding.

A. Porphyrin; Fe; heme; attraction
B. Porphyrin; Mg; heme; affinity
C. Heme; Fe; porphyrin; affinity
D. Heme; Mg; porphyrin; attraction
E. Fe; CO_2; porphyrin; affinity

Example Problem 10

BPG shifts the oxygen saturation curve of Hb to the _____ because BPG binds to _____ making Hb an O_2 delivery system eminently suited for _____.

A. Right; deoxyHb; humans and other primates
B. Right; deoxyHb; cattle, sheep and goats
C. Right; oxyHb; humans and other primates
D. Left; oxyHb; humans and other primates
E. Left; oxyHb; cattle, sheep and goats

Example Problem 11

All are true for the effect of protons on binding of O_2 by hemoglobin EXCEPT:

A. As the pH decreases, dissociation of O_2 from Hb is enhanced.
B. Actively metabolizing tissues produce acid which is an antagonist of oxygen binding by Hb.
C. The phenomenon is called the Bohr Effect.
D. The saturation curve of Hb for O_2 is displaced to the left (greater binding) as acidity increased.
E. All are true.

Questions?

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Problem Set #3: Due Friday 11/11 at 5:00PM in FO 3.602

Exam #3: Monday 11/14 at 10:00AM in normal classroom

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Exam 3 Review

Metabolism

Chapter 17

Know the classification of organisms by their energy source

Phototrophs: use light as energy source

Chemotrophs: use chemical compounds as energy source

- Chemoorganotrophs: use organic compounds
- Chemolithotrophs: use inorganic compounds

Know the classification of organisms by their carbon source

Autotrophs: use CO_2 as carbon source

Heterotrophs: use organic compounds as carbon source

Know the classification of organisms by their energy and carbon source

Classification	Energy Source	Carbon Source
Photoautotrophs – Green plants, algae, cyanobacteria, photosynthetic bacteria	Light	CO_2
Photoheterotrophs – Nonsulfur purple bacteria	Light	Organic Compounds
Chemoautotrophs – Nitrifying bacterial hydrogen, sulfur, and iron bacteria	Redox Chemical Compounds	CO_2
Chemoheterotrophs – All animals, most microorganisms, nonphotosynthetic plant tissue such as roots, photosynthetic cells in the dark	Redox Chemical Compounds	Organic Compounds

Understand the meanings of the words metabolism, catabolism, and anabolism…

- **Metabolism**: "the sum of chemical changes that convert nutrients … into energy and the chemically complex finished products of cells"
  - **Note**: nutrients are not converted into energy, rather metabolism allows the energy in nutrients to be captured in forms that can be used to do work in the cell
  - Serves two fundamentally different purposes:
    1. the generation of energy to drive vital functions
    2. the synthesis of biological molecules
  - Catabolism: energy-yielding
  - Anabolism: energy-requiring

Understand the meanings of the words metabolism, catabolism, and anabolism…

Catabolism: oxidative degradation of complex organic nutrients (polysaccharides, lipids, proteins, nucleic acids) to simpler oxidized energy-poor products (water, carbon dioxide, ammonia, lactate, ethanol)

- Oxidative, Exergonic
- Chemical energy released in form of ATP, NADH, NADPH, and proton motive force

Anabolism: a reductive synthetic process in which complex biomolecules (proteins, nucleic acids, polysaccharides, lipids) are synthesized from simple precursors

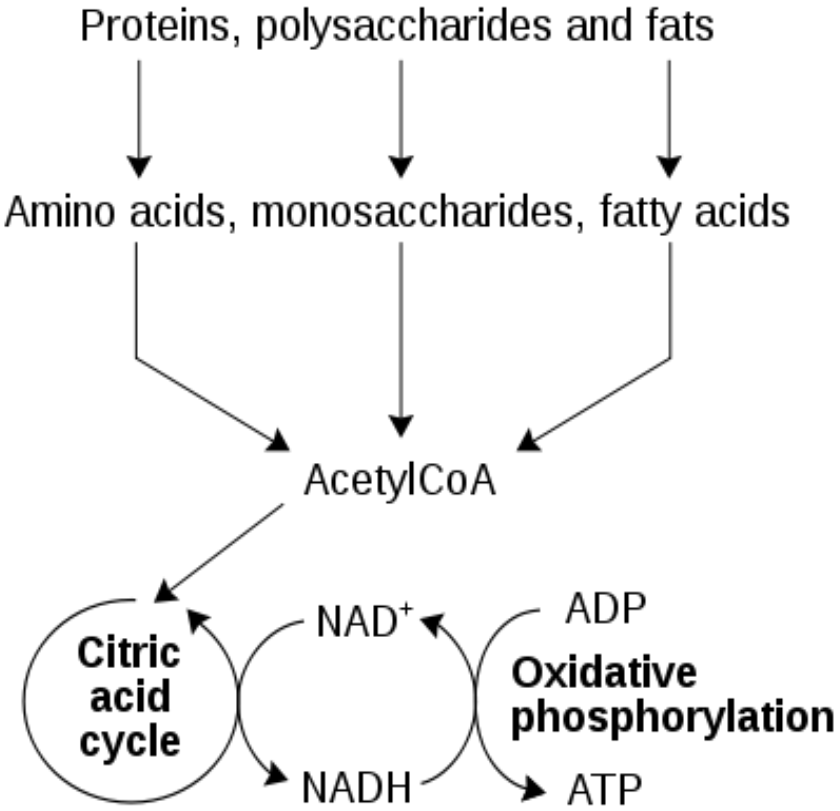
- Reductive, Endergonic
- ATP and NADPH provide the energy and reducing power

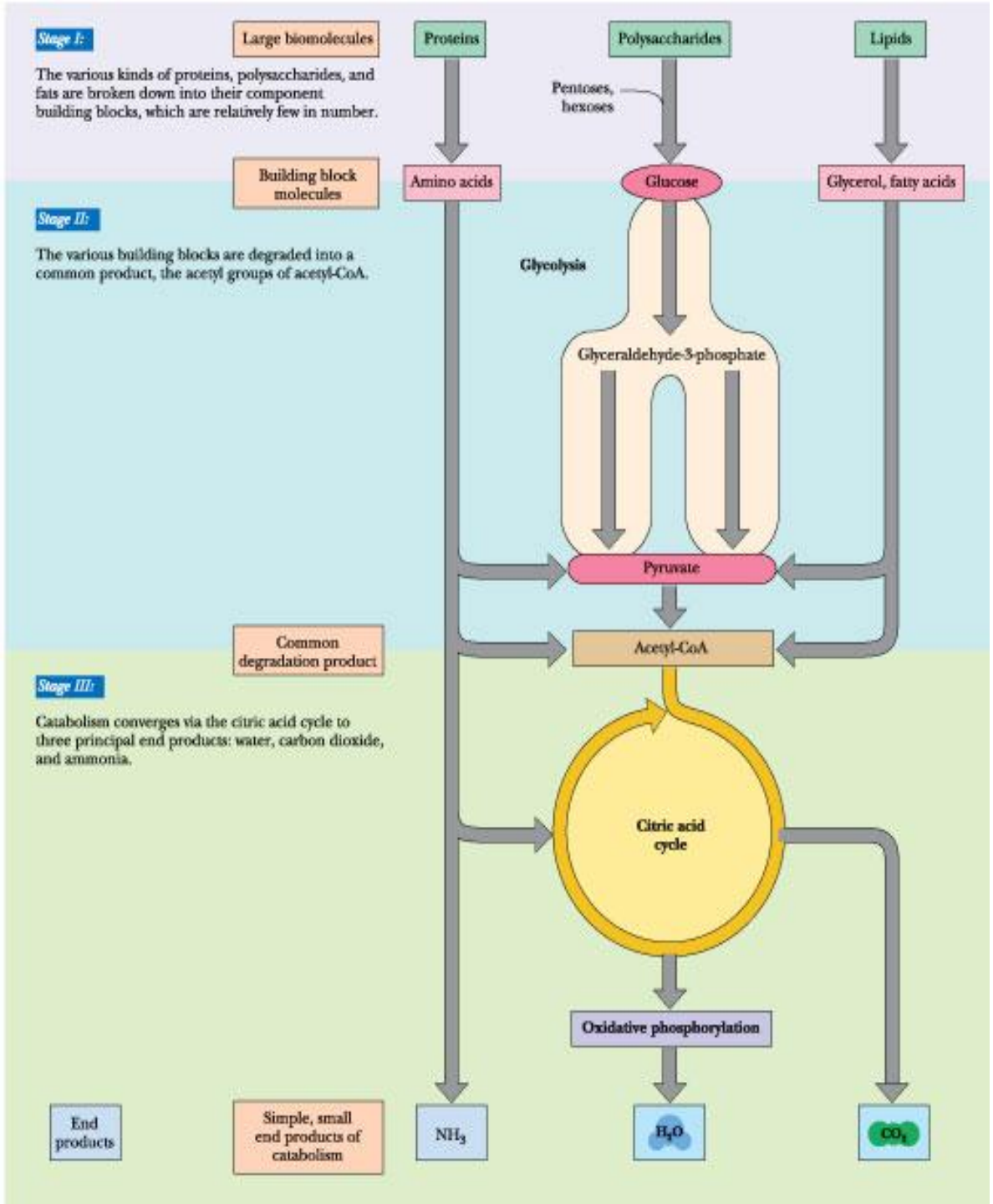
Catabolism allows the energy of nutrients to be captured in forms that can do work in the cell, principally ATP, NADH, NADPH and the proton motive force

Catabolic reactions are **exergonic** ($\Delta G < 0$), the chemical energy released is conserved in the form of…

- ATP
- NADH
- NADPH
- and the proton motive force

- Glycolysis, TCA cycle





The three stages of catabolism.

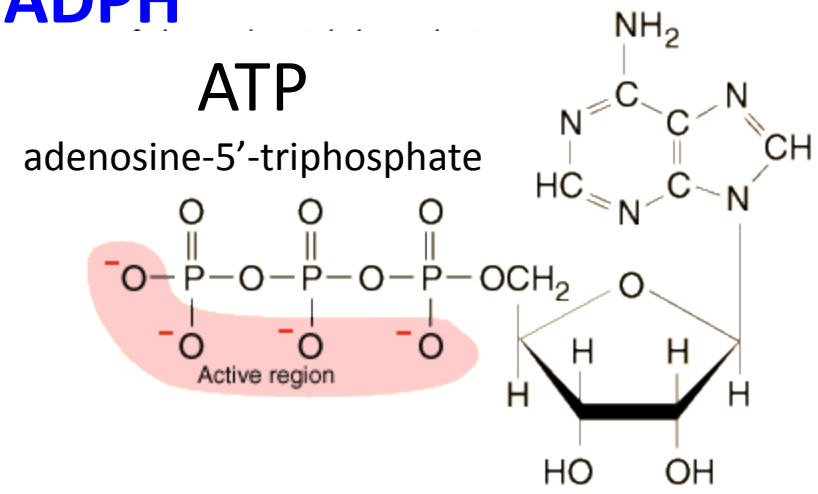
Stage I: Proteins, polysaccharides, and lipids are broken down into their component building blocks, which are relatively few in number.

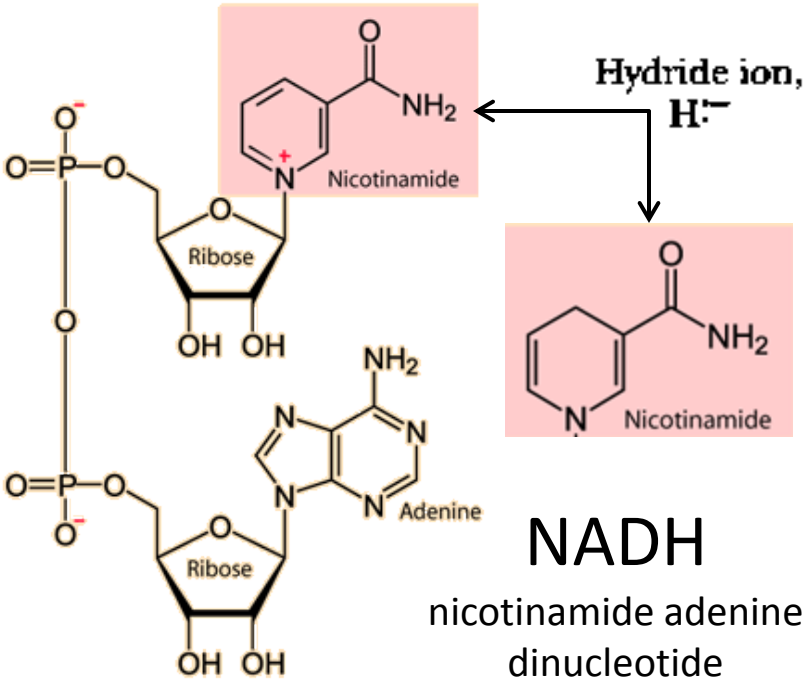
Stage II: The various building blocks are degraded into the common product, the acetyl groups of acetyl-CoA.

Stage III: Catabolism converges to three principal end products: water, carbon dioxide, and ammonia.

Understand the different biological roles of ATP, NADH, and NADPH

- **ATP** is used directly for energy requiring processes. Provides the energy that drives the manifold activities of all living cells.
- **NADH** is used to drive further ATP synthesis in oxidative phosphorylation. NAD^+ collects electrons released in catabolism.
- **NADPH** is used as a source of reducing power for biosynthetic reactions (anabolism).





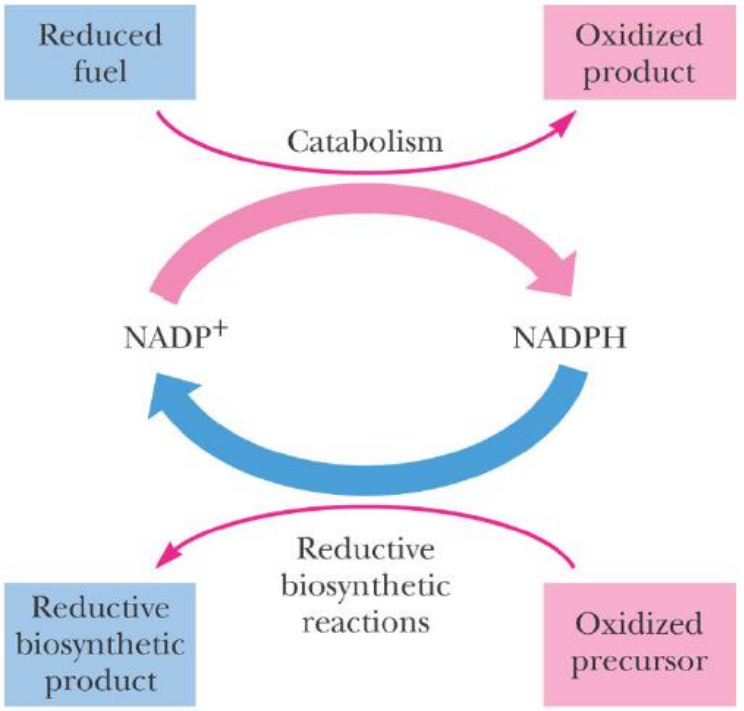
NADH

nicotinamide adenine dinucleotide

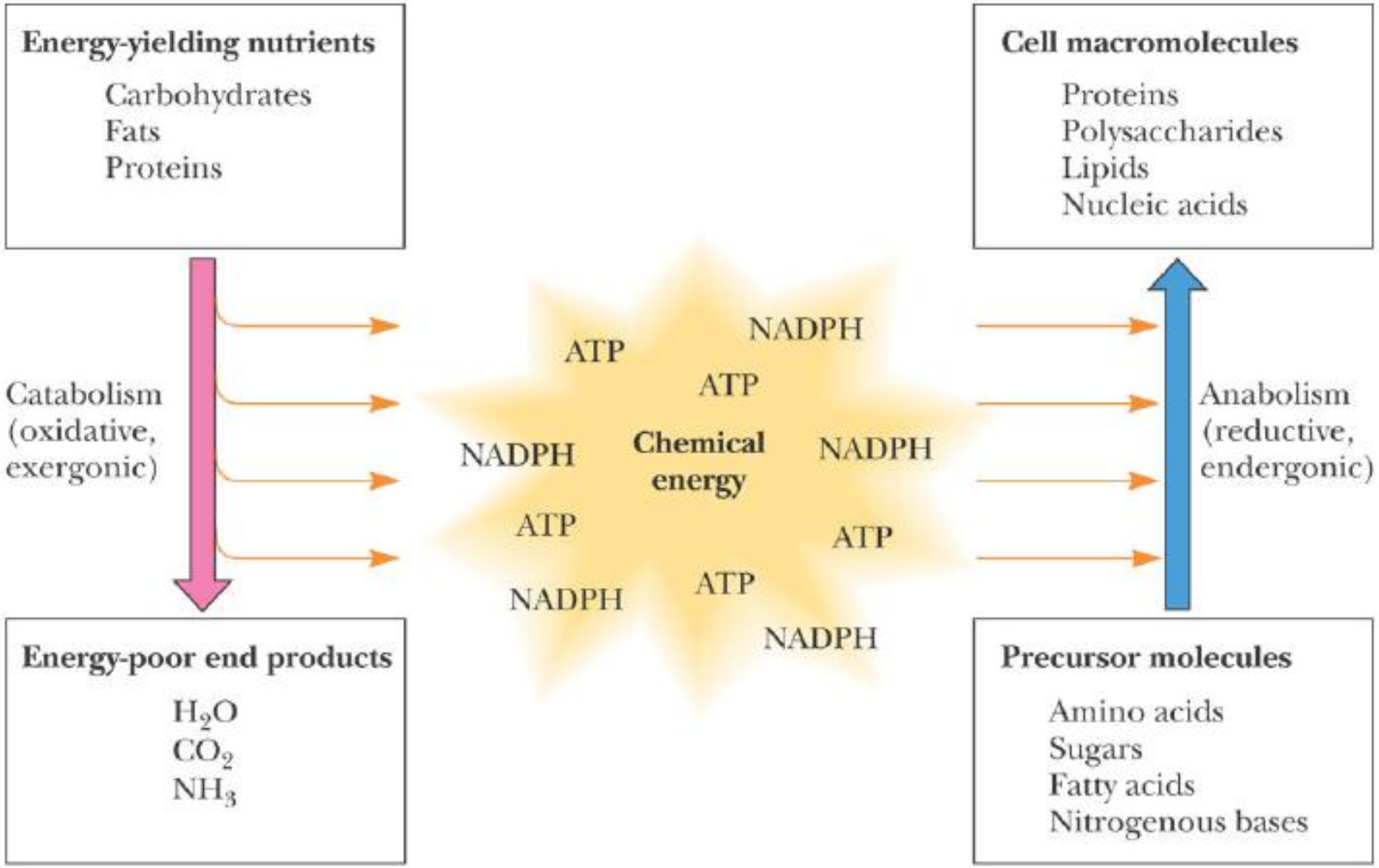
Anabolism is the synthesis of complex molecules, and requires energy and reducing power

- Anabolism is an **endergonic** process ($\Delta G > 0$)
  - The simple precursors used in anabolism to make complex molecules may be products of catabolism, acquired in the diet, or themselves synthesized by anabolic reactions from even simpler reactions…
- ATP and NADPH provide the energy and reducing power required for anabolism.

- Lipid synthesis
- Protein synthesis
- Glycogen synthesis
- DNA replication



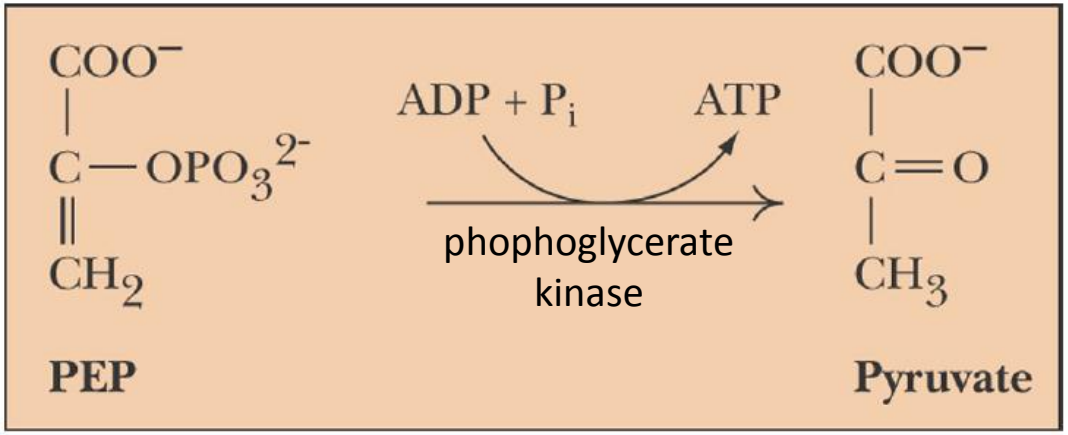
Catabolism and Anabolism

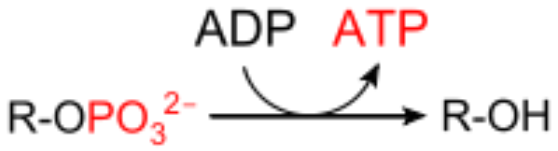


ATP can be synthesized by substrate level phosphorylation [and by oxidative phosphorylation]

Substrate level phosphorylation…

- Process in which a substrate, rather than an electron-transport chain or proton gradient, provides the energy for phosphorylation
- ADP/GDP can be converted to ATP/GTP when its formation is directly coupled to a reaction that has a phosphorylated reactive intermediate that transfers its phosphate group over to the ADP/GDP.
- **Example 1:** conversion of **1,3-BPG to 3-PG during glycolysis** (ATP)
- **Example 2**: conversion of **PEP to pyruvate during glycolysis** (ATP)
- **Example 3:** conversion of **succinyl-CoA to succinate during TCA cycle** (GTP)





ATP can be synthesized by substrate level phosphorylation [and by oxidative phosphorylation]

Oxidative Phosphorylation… (energy is captured in ATP)

- The exergonic oxidative reactions release energy that is coupled to the formation of ATP in a process called oxidative phosphorylation.
- The NAD^+ - NADH system acts as a shuttle that carries electrons released from catabolic substrate to the mitochondria, which are then transferred to O_2, the ultimate electron acceptor. The free energy released is captured in ATP.

- NAD^+ collects electrons → NADH is formed → O_2 accepts electrons from NADH → H_2O is formed → energy released makes ATP
- O_2 is the ultimate electron acceptor in catabolism (ultimate oxidizing agent).

Know, in general terms, at least one example of a substrate level phosphorylation

Glycolysis

- 1,3-BPG to 3-PG (ATP) – Phophoglycerate kinase
  - $\Delta G^{\circ\prime}$ = -18.9 kJ/mol
- PEP to pyruvate (ATP) – Pyruvate kinase
  - $\Delta G^{\circ\prime}$ = -31.7 kJ/mol

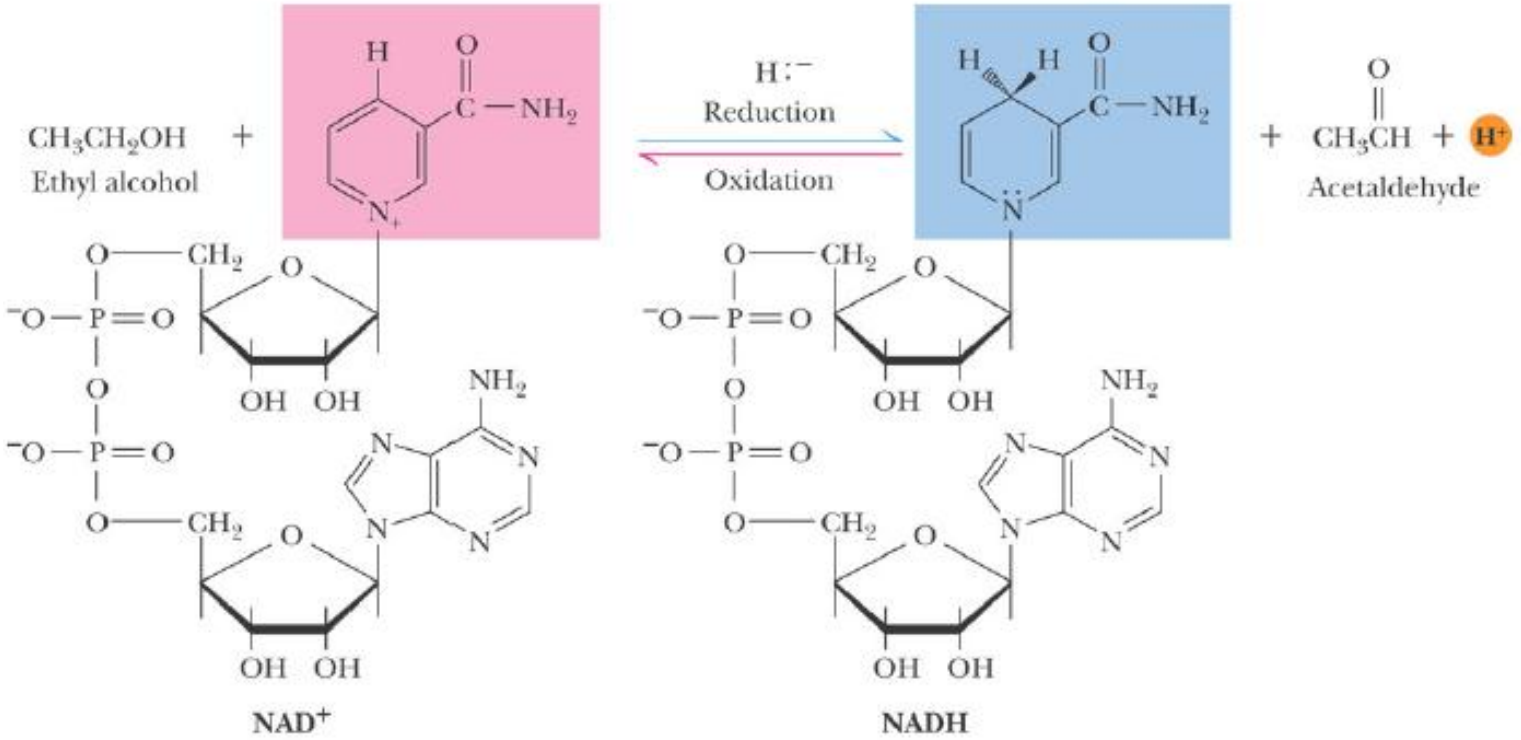
There are more examples you will learn about in the next unit.

TCA cycle

- Succinyl-CoA to Succinate (GTP) – Succinyl-CoA synthetase
  - $\Delta G^{\circ\prime}$ = -3.3 kJ/mol
  - GTP used to phosphorylate ADP by nucleoside diphosphate kinase

Understand how redox transformations of NADH/NAD$^+$ are coupled to the redox reactions of organic metabolites

Catabolic oxidation reaction coupled to the reduction of NAD$^+$ to NADH



$$CH_3CH_2OH \longrightarrow CH_3CHO + 2H^+ + 2e^-$$

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

$$CH_3CH_2OH + NAD^+ \longrightarrow CH_3CHO + NADH + H^+$$

Understand how catabolism and anabolism are linked

- They are linked by the fact that the chemical energy captured during catabolism can then be used in anabolism for biosynthesis.
- The energy is captured as ATP, NADH, and NADPH in catabolism.
- Anabolism will convert these back to ADP, NAD^+, and $NADP^+$.
- These will then be ready to use in capturing more energy during catabolism.

Catabolic and anabolic pathways that inter-convert the same metabolites cannot simply be the reverse of the other…

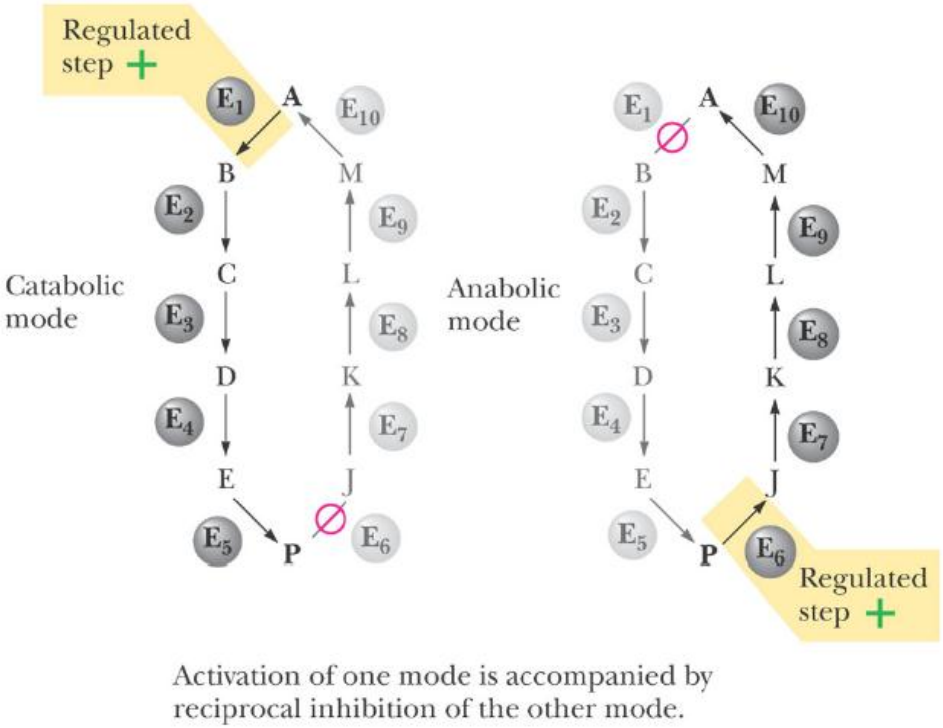
- The metabolites from catabolic and anabolic pathways may interconvert.
  - For example, gluconeogenesis synthesizes glucose and glycolysis breaks it down.
  - The two pathways cannot be the reverse, they require regulation.
  - If gluconeogenesis was simply the reverse, it would be strongly endergonic and would not proceed spontaneously.

- **Reciprocal regulation** between anabolic and catabolic pathways is done by using different enzymes or by compartmentalizing the pathways.

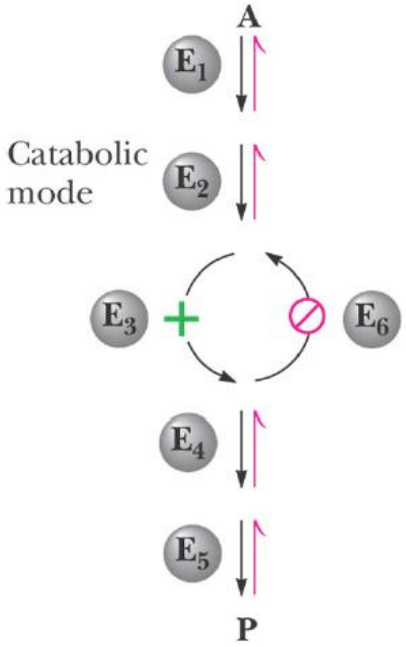
- Regulation must also be such that both pathways are not running at the same time. When glycolysis is occurring, gluconeogenesis is inhibited.

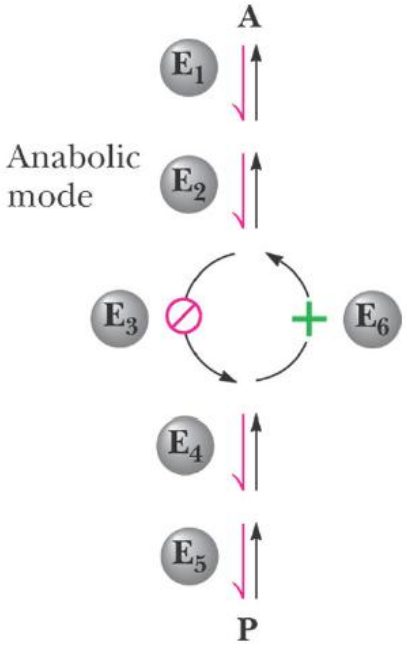
- These occur at regulated steps, where activation of one mode is accompanied by the reciprocal inhibition of the other mode.
  - Low energy state: glucose degraded to provide ATP (glycolysis)
  - High energy status: pyruvate synthesized to glucose and glycogen (gluconeogenesis)

Understand how such pathways can be reciprocally regulated

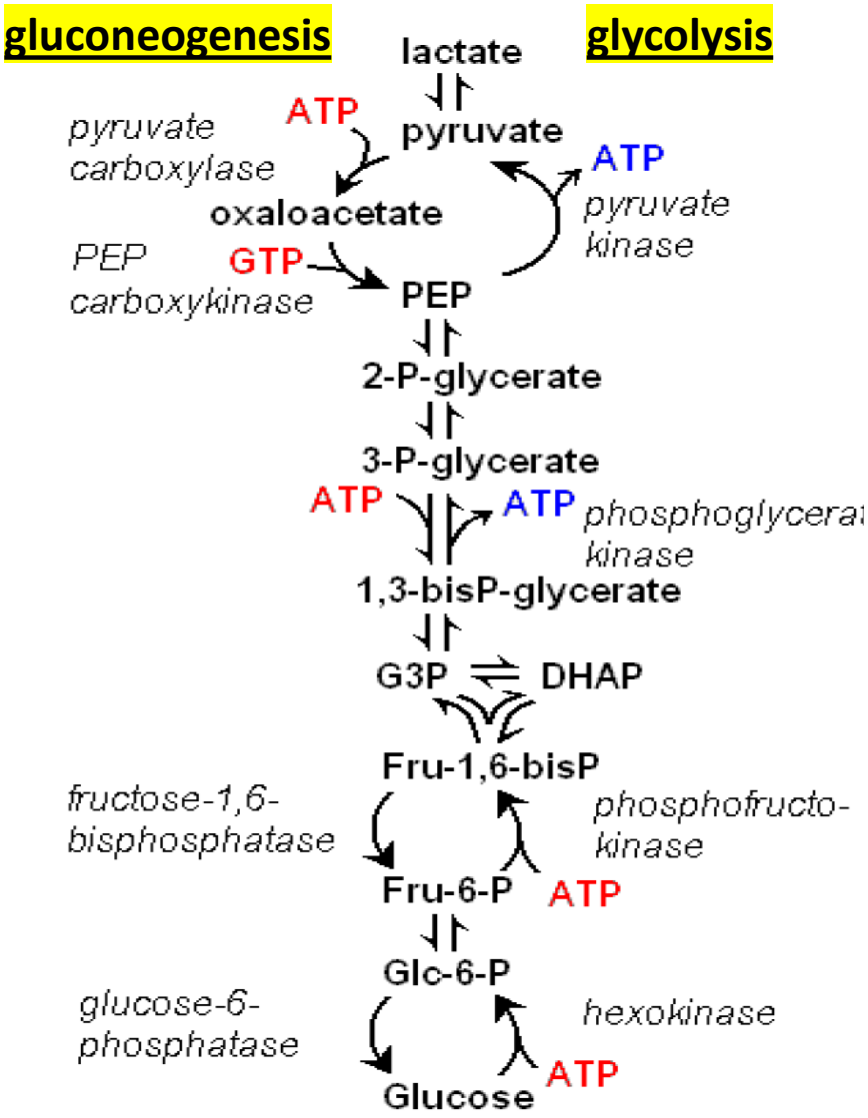


Activation of one mode is accompanied by reciprocal inhibition of the other mode.





Understand how such pathways can be reciprocally regulated



- Pathways like glycolysis and gluconeogenesis are reciprocally regulated by using different enzymes, so that they are not simply the reverse of each other.
- Therefore, when one of the pathways is taking place, the other is shut off.
- You can see that the enzyme used to break down PEP to pyruvate in glycolysis is different from the two enzymes used to convert pyruvate back to PEP in gluconeogenesis.

Understand that metabolism can be compartmentalized in eukaryotes

- From page 527 in the Grisham/Garrett text:

- Each compartment in eukaryotic cells is dedicated to specialized metabolic functions. The enzymes for these metabolic sequences occur together within the organelle membrane.

- Thus, the flow of metabolic intermediates in the cell is spatially as well as chemically segregated.

- Example: the 10 enzymes of glycolysis are found in the cytosol, but pyruvate, the product of glycolysis, is fed into the mitochondria. These organelles contain the citric acid cycle enzymes, which oxidize pyruvate to CO_2.

Know how glycolysis and the citric acid cycle are compartmentalized…

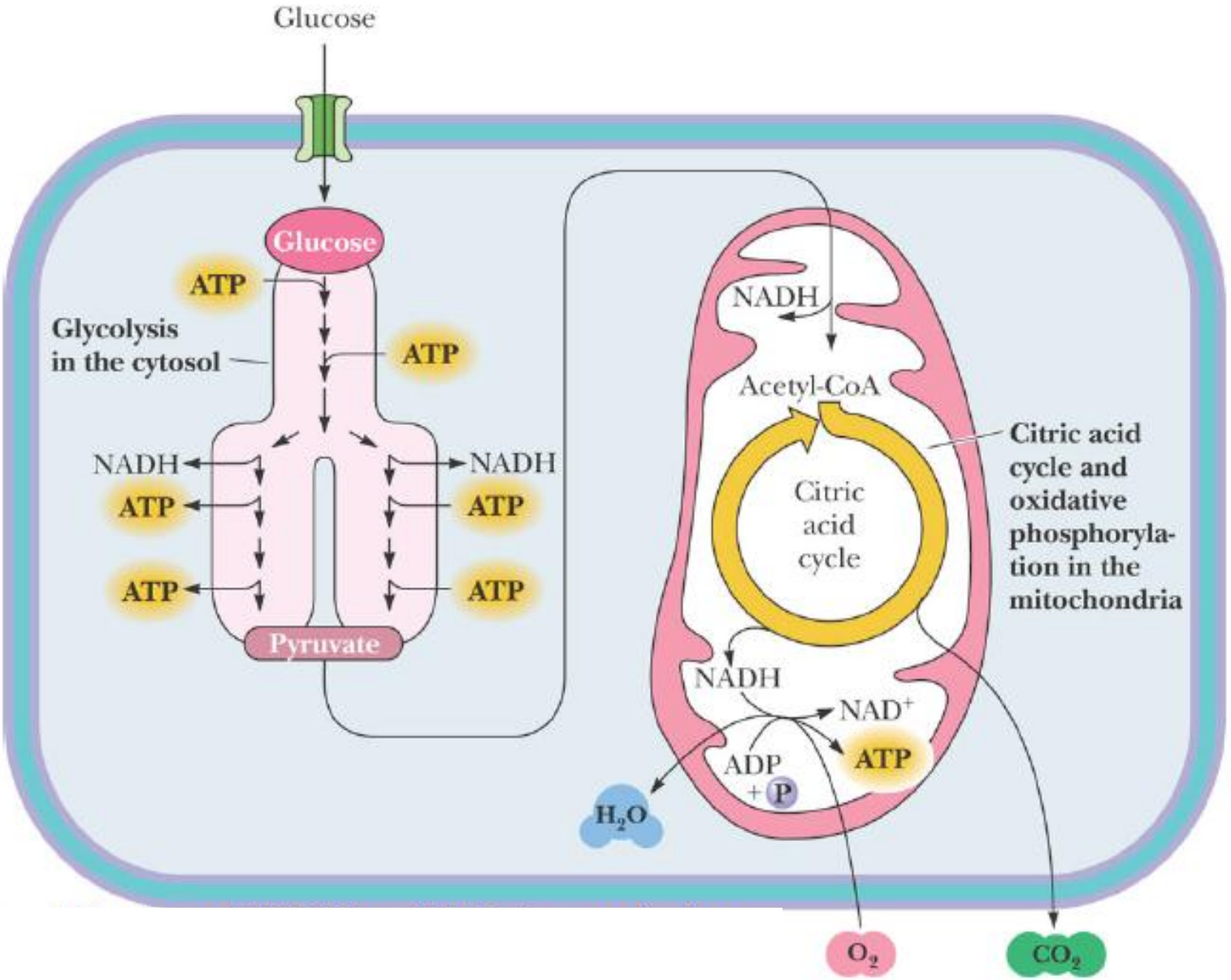
- From page 527 in the Grisham/Garrett text:

- The 10 enzymes of glycolysis are found in the cytosol, but pyruvate, the product of glycolysis, is fed into the mitochondria.

- These organelles contain the citric acid cycle enzymes, which oxidize pyruvate to CO_2.

- The great amount of energy released in the process is captured by the oxidative phosphorylation system of mitochondrial membranes and is used to drive the formation of ATP.

Know how glycolysis and the citric acid cycle are compartmentalized…



Example Problem 1

All are characteristics of metabolism EXCEPT:

A. A process responding to the momentary energy requirements of a cell
B. A process which synthesizes either energy or complex cellular substances
C. A process of intermediates
D. A free-flow unregulated process
E. The conversion of food energy into energy of motion

Example Problem 2

Chemoheterotrophs require:

A. Organic carbon sources and light
B. Organic carbon sources and oxidation-reduction reactions
C. CO_2 and light
D. CO_2 and oxidation-reduction reactions
E. None of the above

Example Problem 3

Anabolism is an _____ process, whereas catabolism is an _____ process. _____ and _____ provide the reducing power required for anabolism.

A. Exergonic; endergonic; ATP; NADPH
B. Exergonic; endergonic; NADH; ATP
C. Endergonic; exergonic; NADPH; GTP
D. Endergonic; exergonic; ATP; NADPH
E. Both A and D
F. None of the above

Example Problem 4

All of the following are substrate-level phosphorylation EXCEPT:

A. Succinyl-CoA to succinate in TCA cycle
B. 1,3-BPG to 3-PG in glycolysis
C. F-6-P to FBP in glycolysis
D. PEP to pyruvate in glycolysis
E. All are substrate-level phosphorylation..

Questions?

Kirk's contact email: knh093020@utdallas.edu

Problem Set #3: Due Friday 11/11 at 5:00PM in FO 3.602

Exam #3: Monday 11/14 at 10:00AM in normal classroom

Kirk Huynh

Contact email:
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Exam 3 Review

Carbohydrates

Chapter 7

Know the general features and roles of carbohydrate molecules

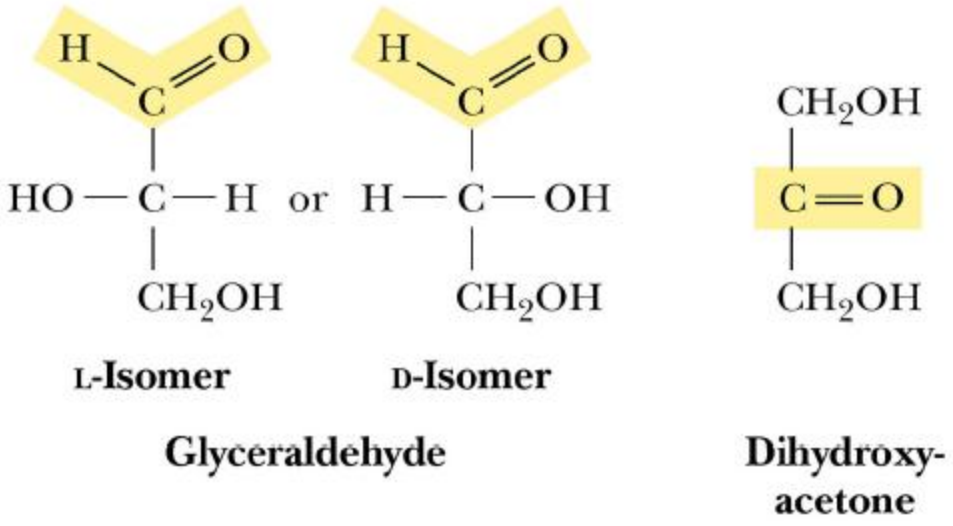
- Formula $(CH_20)_n$ n = 3 or more
- Most abundant organic molecule in nature
- Major form of stored energy – glycogen (animals) and starch (plants)
- Structural roles – cellulose (plants), chitin (insects), and peptidoglycan (bacteria)
- When linked to lipids – glycolipids
- When linked to proteins – glycoproteins
- Chemical features of carbohydrates
  - At least 1 asymmetic (chiral) center
  - Ability to exist in ring or linear structure
  - Can form polymers through **glycosidic** bonds
  - Can form multiple hydrogen bonds with water and other molecules

Know the general features and roles of carbohydrate molecules

- Four groups of carbohydrates:
  - Monosaccharaides: contain 3-7 carbons
    - Glucose
  - Disaccharides: contain two monosaccharaides
    - Sucrose
    - Lactose
    - Maltose and cellulobiose
  - Oligosaccharides: contain 2-10 monosaccharaides
  - Polysaccharides: contain many monosaccharaides (greater than 10)
    - Storage-related
      - Plants – Starch (amylose, amylopectin)
      - Animals – Glycogen
    - Structural-related
      - Plants – Cellulose
      - Insects – Chitin
      - Bacteria – Peptidoglycan

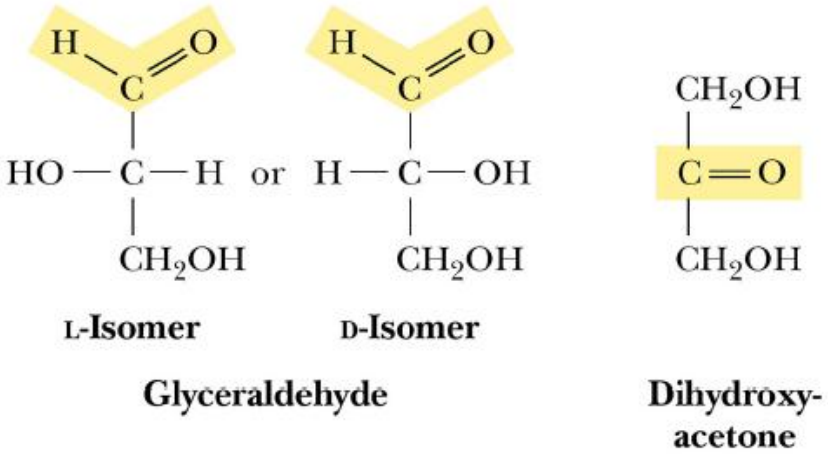
Know the difference between aldoses and ketoses

- Monosaccharides…
  - **Aldoses**: contain aldehyde function, simplest structure is glyceraldehyde
  - **Ketoses**: contain ketone function, simplest structure is dihydroxyacetone
    - These simple sugars are called trioses because they contain 3 carbons



Know the difference between aldoses and ketoses

- Examples…
  - Aldose:
    - Triose: glyceraldehyde
    - Tetrose: erythrose
    - Pentose: ribose
    - Hexose: glucose, mannose, galactose
  - Ketose:
    - Triose: dihydroxyacetone
    - Pentose: ribulose
    - Hexose: fructose



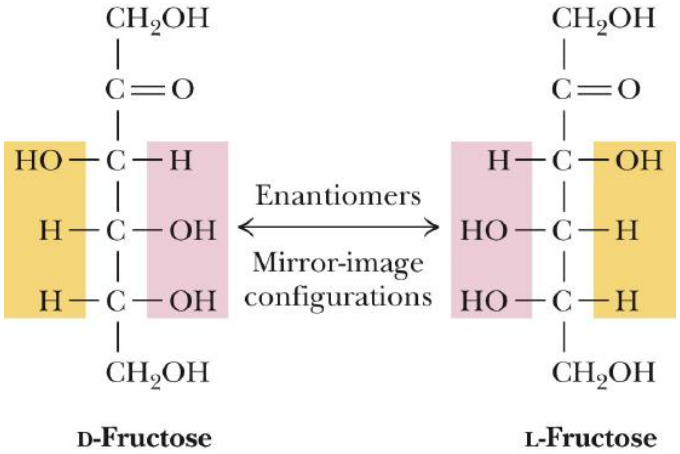
Understand how the D/L designation of sugars is derived

Stereochemistry

- Numbering for aldoses begin on the carbonyl carbon.
- Numbering for ketoses begin on the end closest to carbonyl carbon

- D/L refers to the configuration of the highest numbered asymmetric chiral carbon (usually hydroxyl); mirror images

- D conformation is when the –OH group on the highest numbered chiral carbon is drawn to the right on a flat Fischer projection.
- Most naturally occurring carbohydrates are D isomers.



Aldoses

Carbons are numbered from the carbonyl carbon

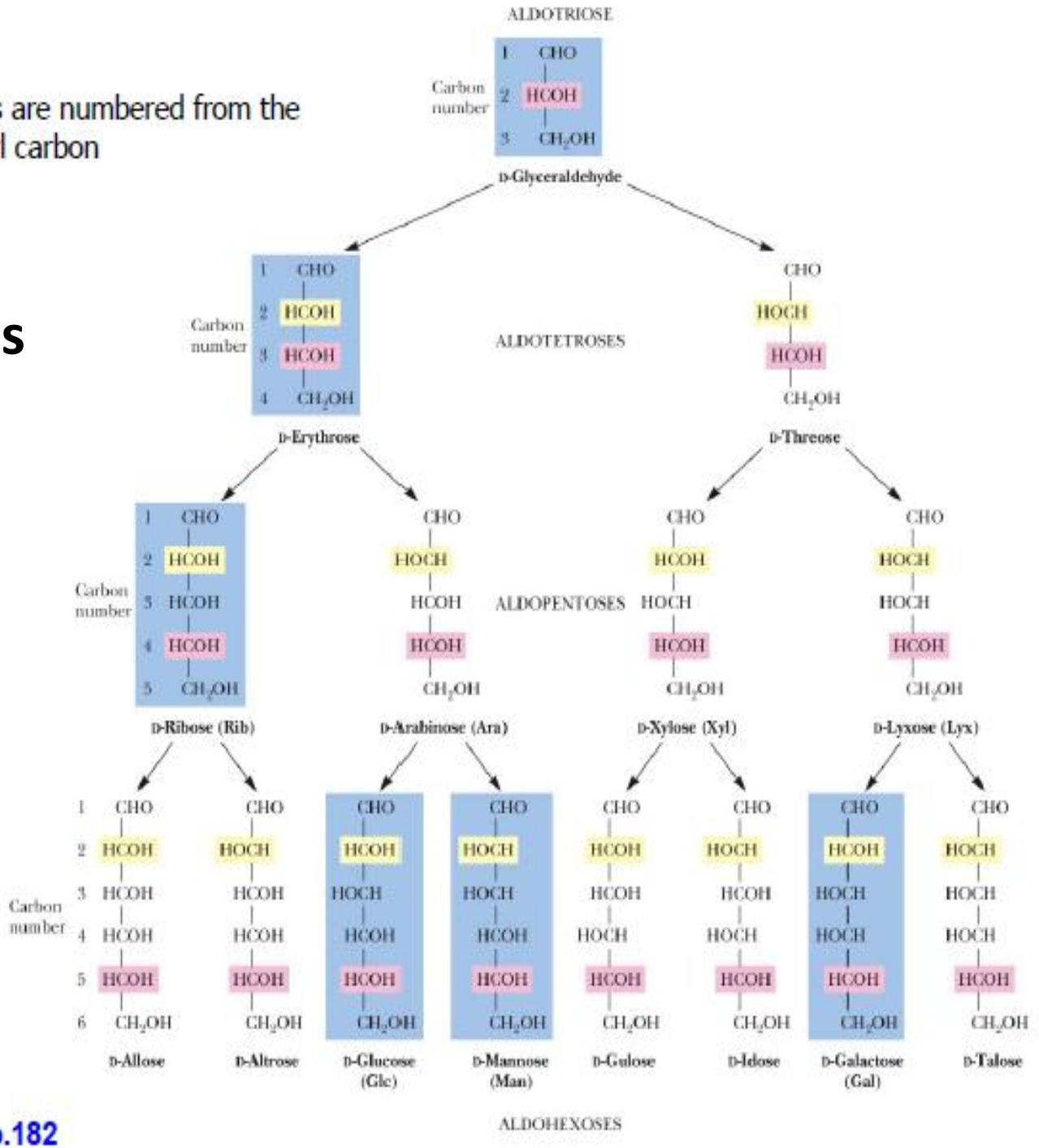


Fig. 7-2, p.182

Ketoses

Carbons are numbered from the end that is closer to the carbonyl carbon

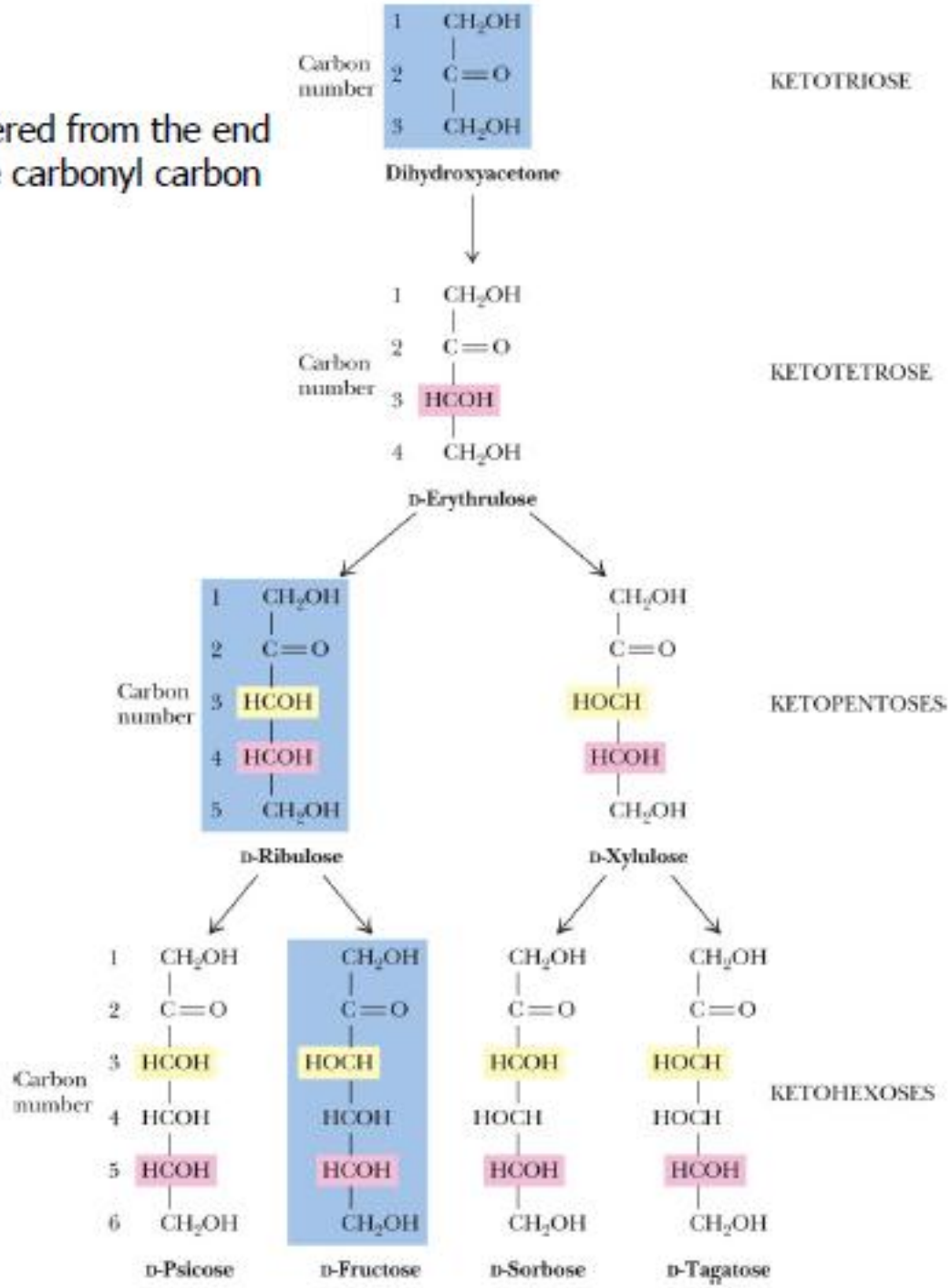
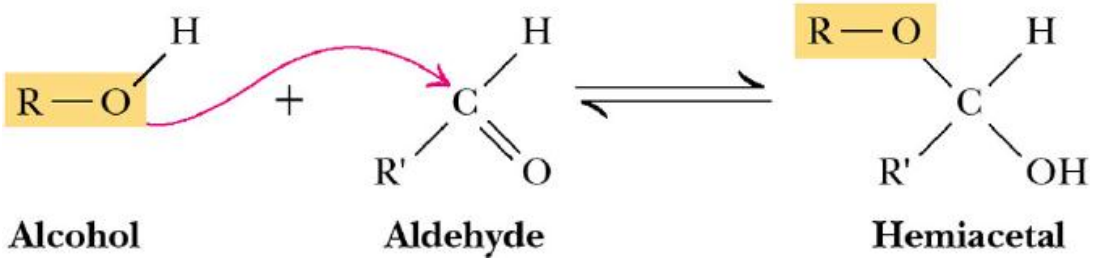


Fig. 7-2, p.182

Understand how linear forms can cyclize to form a pyranose or a furanose

- The alcohol group of a carbohydrate reacts with the aldehyde function to form a **hemiacetal**.



- **Hemiketal** is formed when alcohol group reacts with the ketone function.

- The hemiacetal/hemiketal cyclizes to generate an additional asymmetic center. The result forms either a pyranose or furanose.

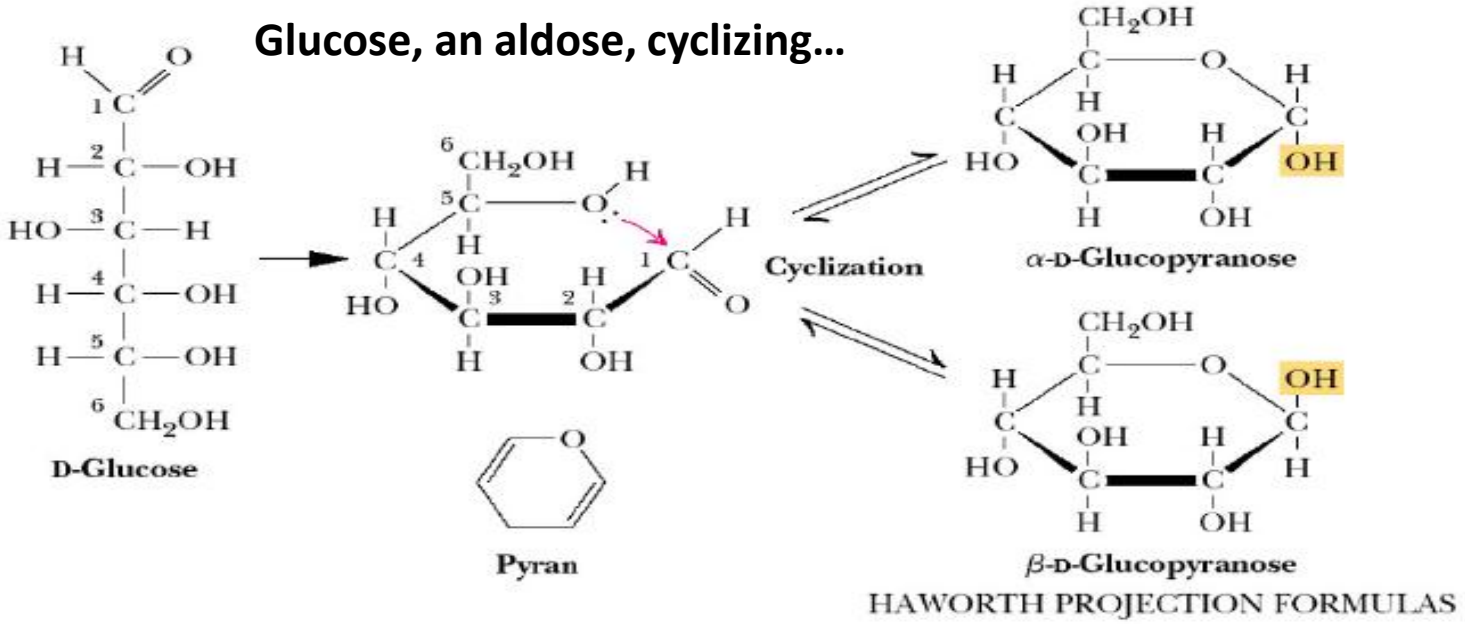
- **Pyranose**: 6-membered oxygen containing ring

- **Furanose**: 5-membered oxygen containing ring

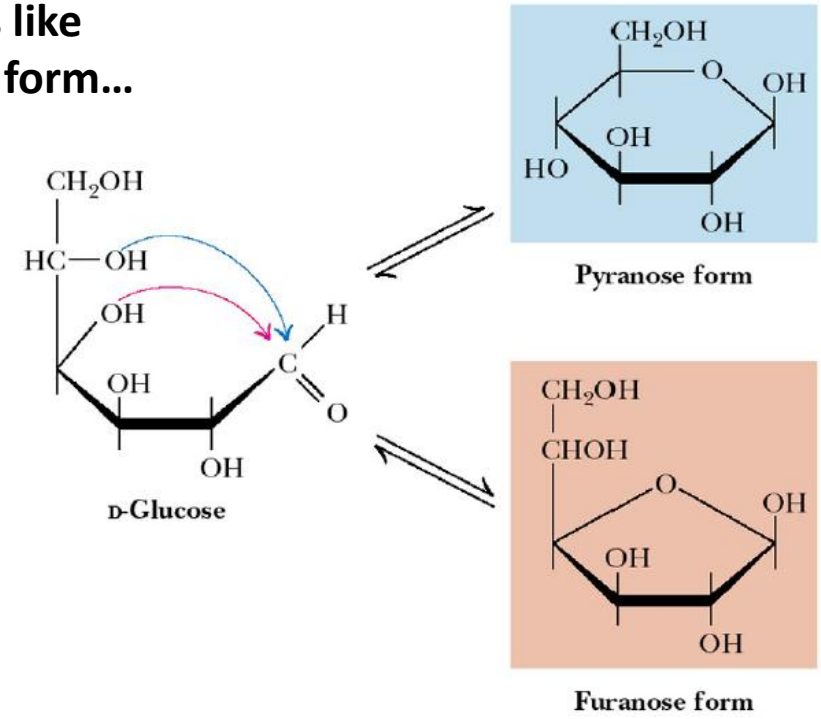
Understand how linear forms can cyclize to form a pyranose or a furanose

- Aldoses and ketoses can react with the –OH group to form pyranoses and furanoses.
- However, aldohexose sugars, such as D-glucose, prefer the pyranose form.
- Ketohexose sugars, such as D-fructose, prefer the furanose form.

Glucose, an aldose, cyclizing…

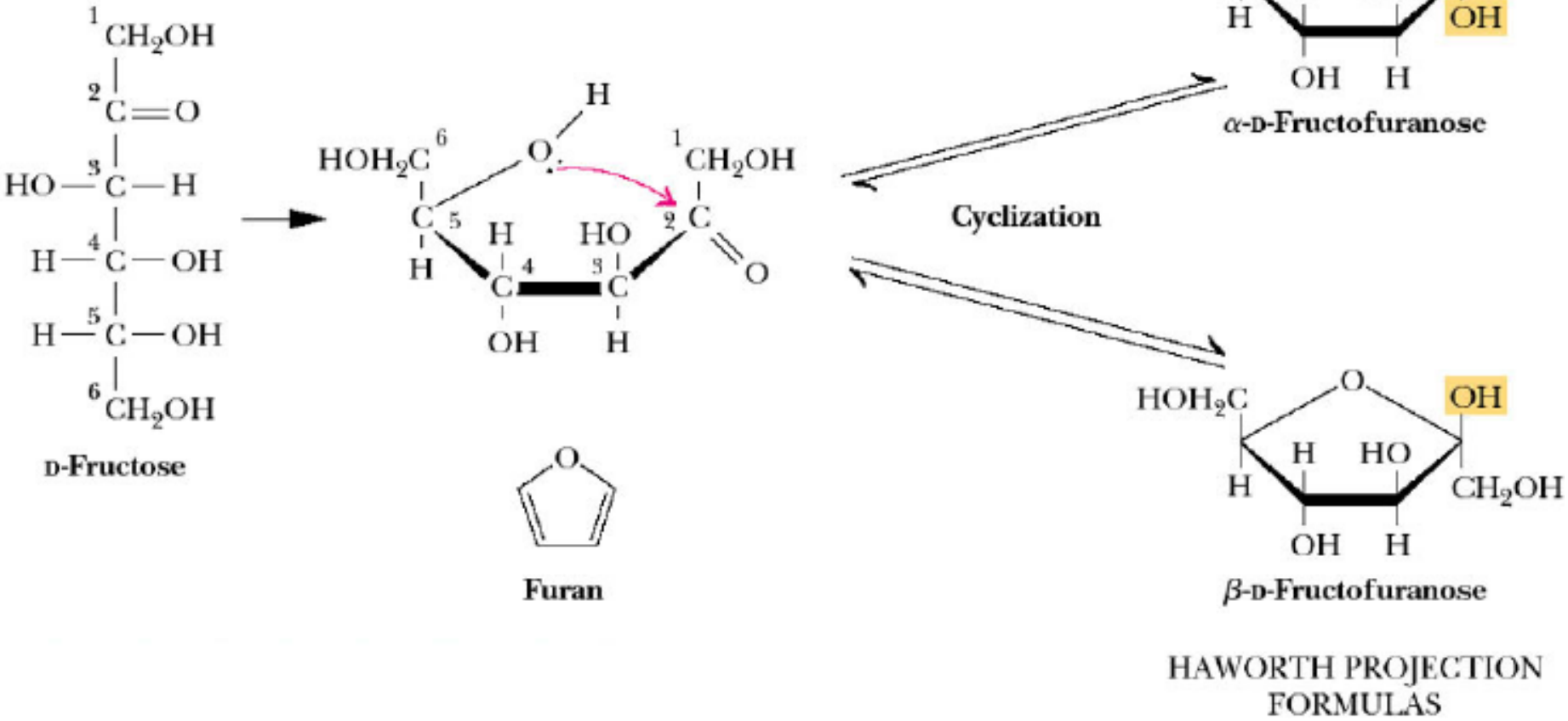


Note: aldohexose sugars like glucose prefer pyranose form…



Fructose, a ketose, cyclizing…

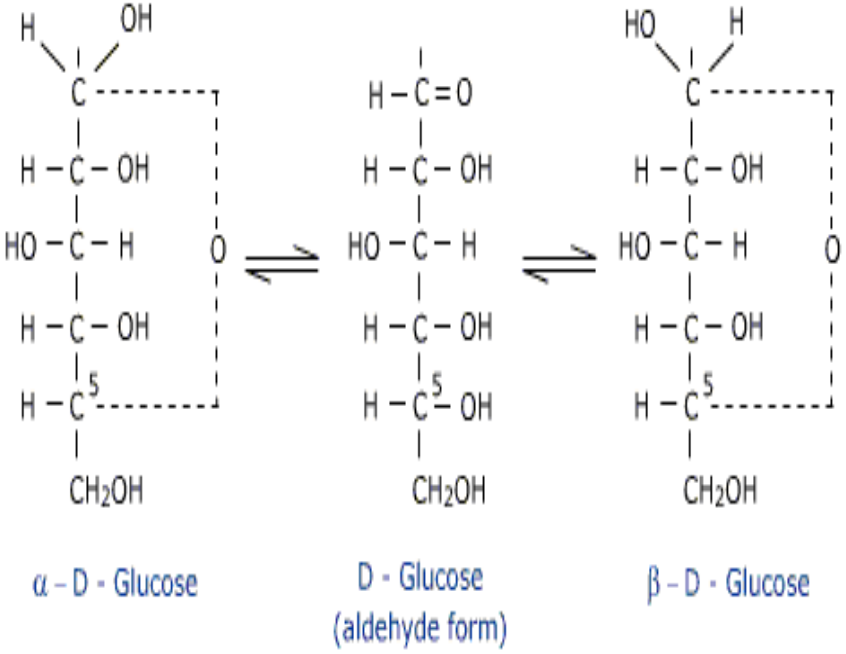
Note: ketohexose sugars like fructose prefer furanose form…



Cyclized forms may have two steroisomers called anomers

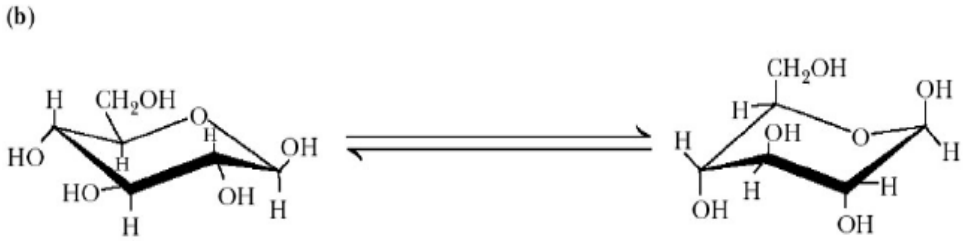
- **Anomer**: isoforms of monosaccharides that differ only in their configuration about the anomeric carbon
- The anomeric carbon is the carbonyl carbon (that was carbonyl before cyclization)
- α form occurs when the hydroxyl on the anomeric carbon is on the same side as of a Fischer projection as the oxygen atom at the highest numbered chiral carbon.
- β form occurs when the hydroxyl is on the opposite side.

α and β anomers of D-Glucose



Pyranoses typically adopt chair or boat configurations…

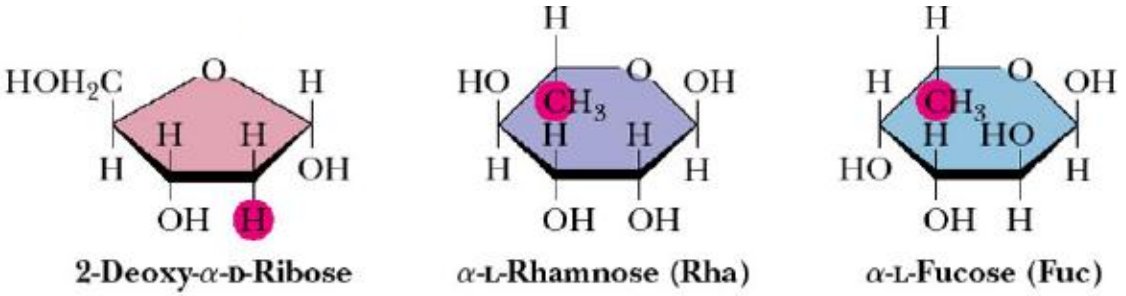
In three dimensions, monosaccharides are puckered rather than planar, and adopt either the **chair** or **boat** conformation (which is more stable depends upon the nature of the ring substituents)



Two possible chair configurations for ß-D-glucose

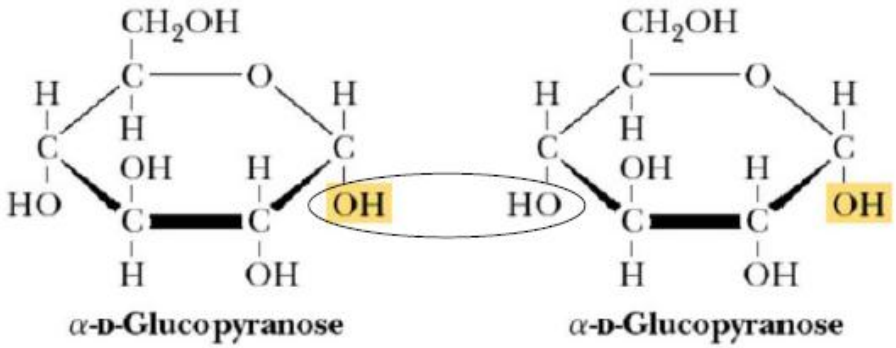
Sugars can be derivatized to form sugar alcohols, deoxy sugars, and so on…

- Conversion of monosaccharides into derivatives
  - Sugar acids: sugar with free anomeric carbon atoms can reduce H_20_2 and other metals
  - Sugar alcohol: prepared by addition of reducing agent $NaBH_4$
  - Deoxy sugars: 1 or more OH group is replaced with hydrogens (deoxyribose: DNA) the sugar part of ATP is furanose D-ribose
  - Sugar est



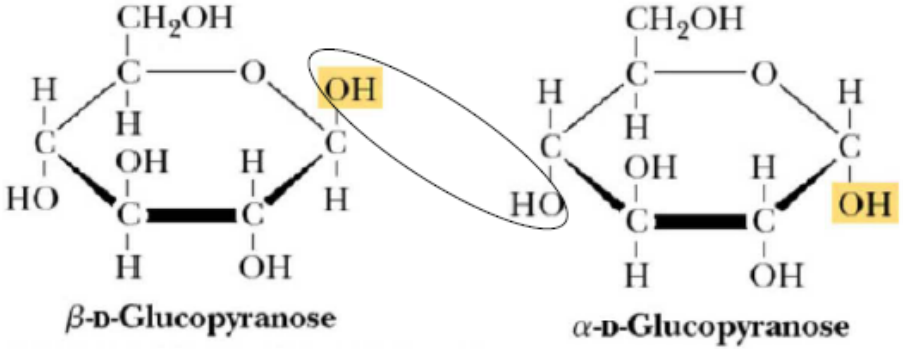
Elimination of water forms αor β glycosidic bond

Left sugar is α-anomer



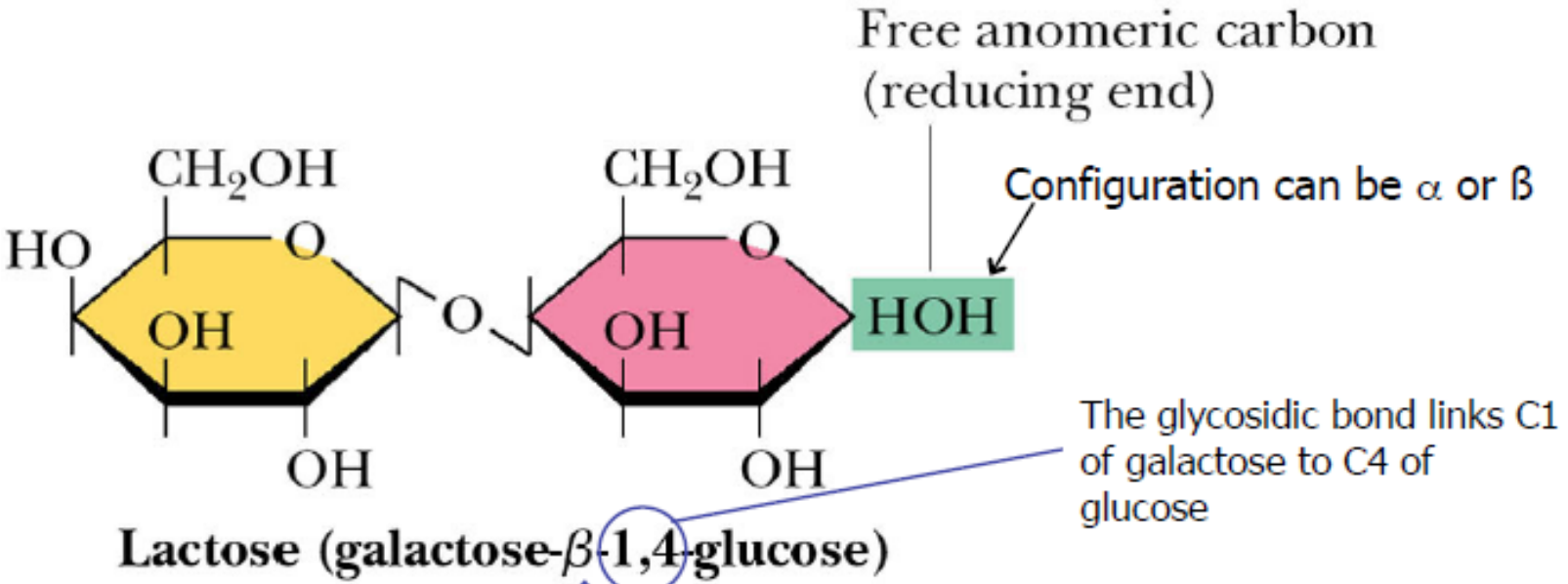
Product is an α glycosidic bond

Left sugar is β-anomer

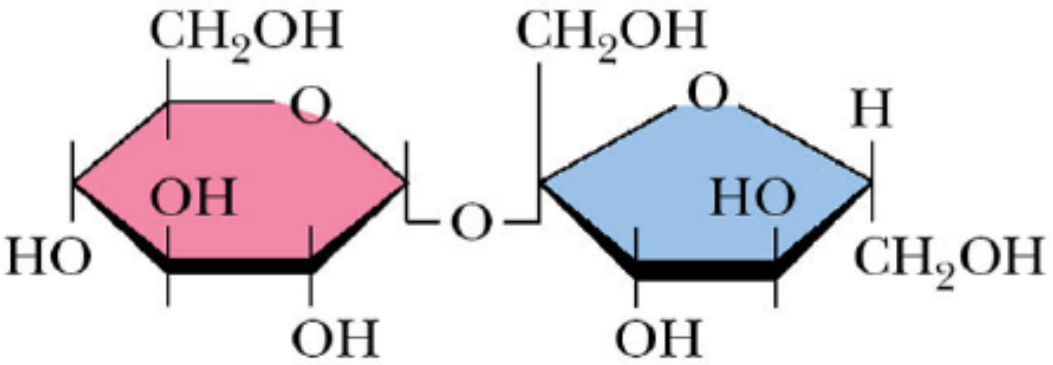


Product is a ß glycosidic bond

Know some examples of disaccharides…



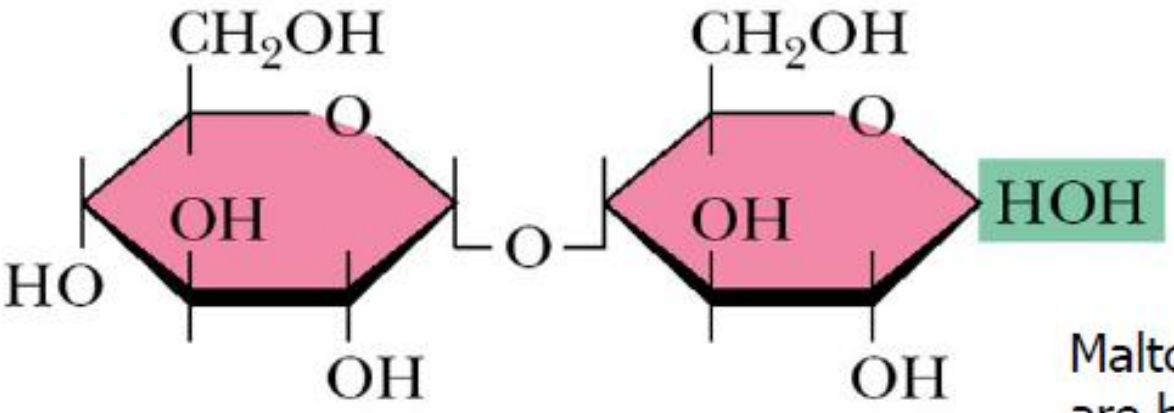
Lactose (galactose-β-1,4-glucose)



Sucrose (glucose-α-1,2-fructose)

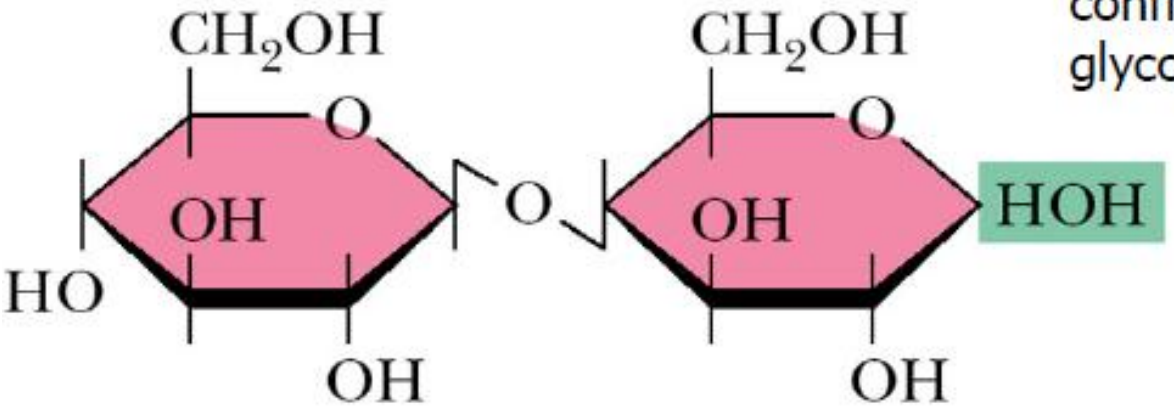
The glycosidic bond in lactose is hydrolyzed by **lactase**. **Lactose intolerance** is caused by lactase deficiency

Know some examples of disaccharides…



Maltose (glucose-α-1,4-glucose)

Maltose and cellobiose are both **homodisaccharides** of glucose. They are identical except for the configuration of the glycosidic bond

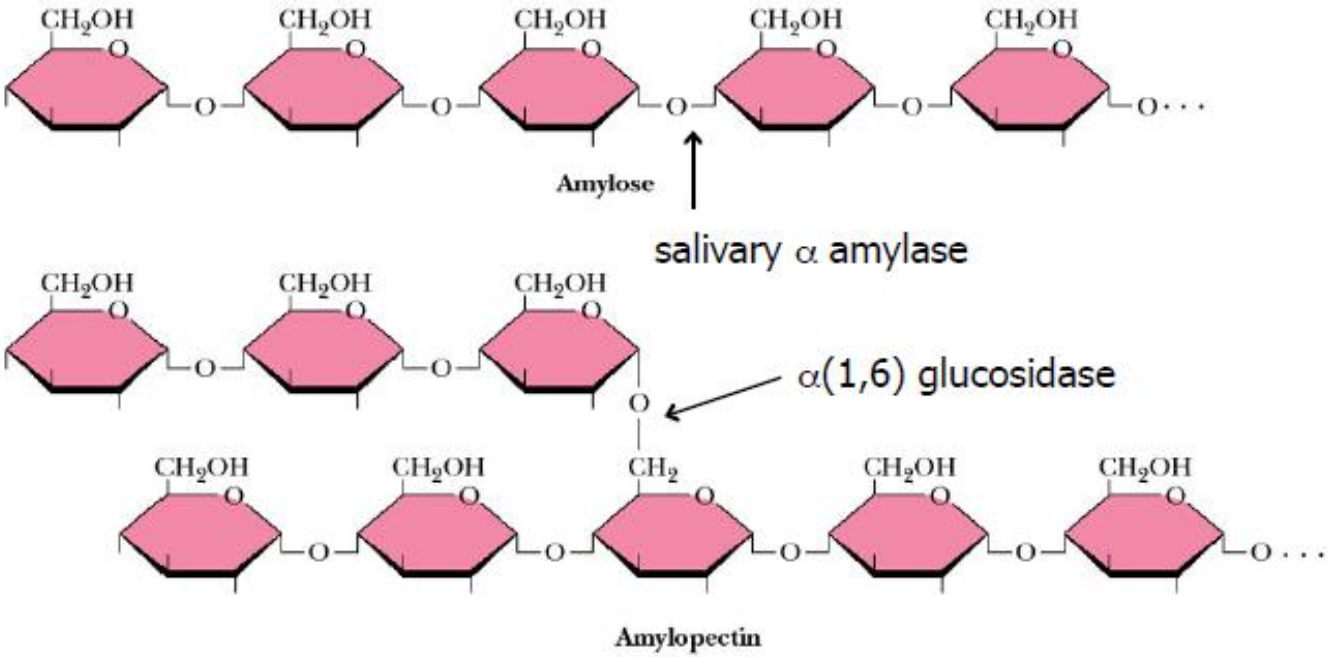


Cellobiose (glucose-β-1,4-glucose)

Starch is a polymer of amylose and amylopectin containing both 1,4 and 1,6 glycosidic bonds

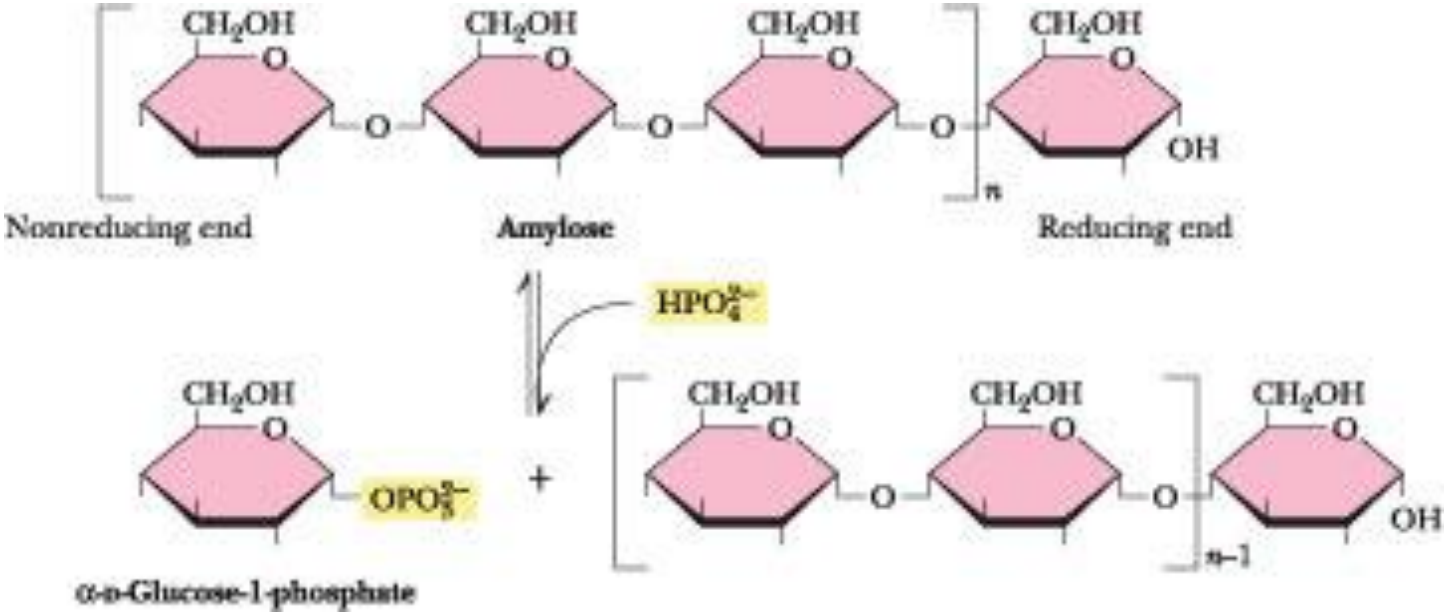
- Starch is a polysaccharide also called a **glycan**.
  - It is a **homopolysaccharid**e b/c it contains only one type of monosaccharide (glucose).
  - It is called a **glucan**, b/c the monosaccharide is glucose.
  - Starch is a mixture of two polysaccharides: amylose and amylopectin

- **Amylose**: linear chain of glucose units in α-1,4 linkages
- **Amylopectin**: a branched chain, in which branches are created by α-1,6 linkages. Branches occur every 12-30 residues. Average branch length is 24-30 residues.

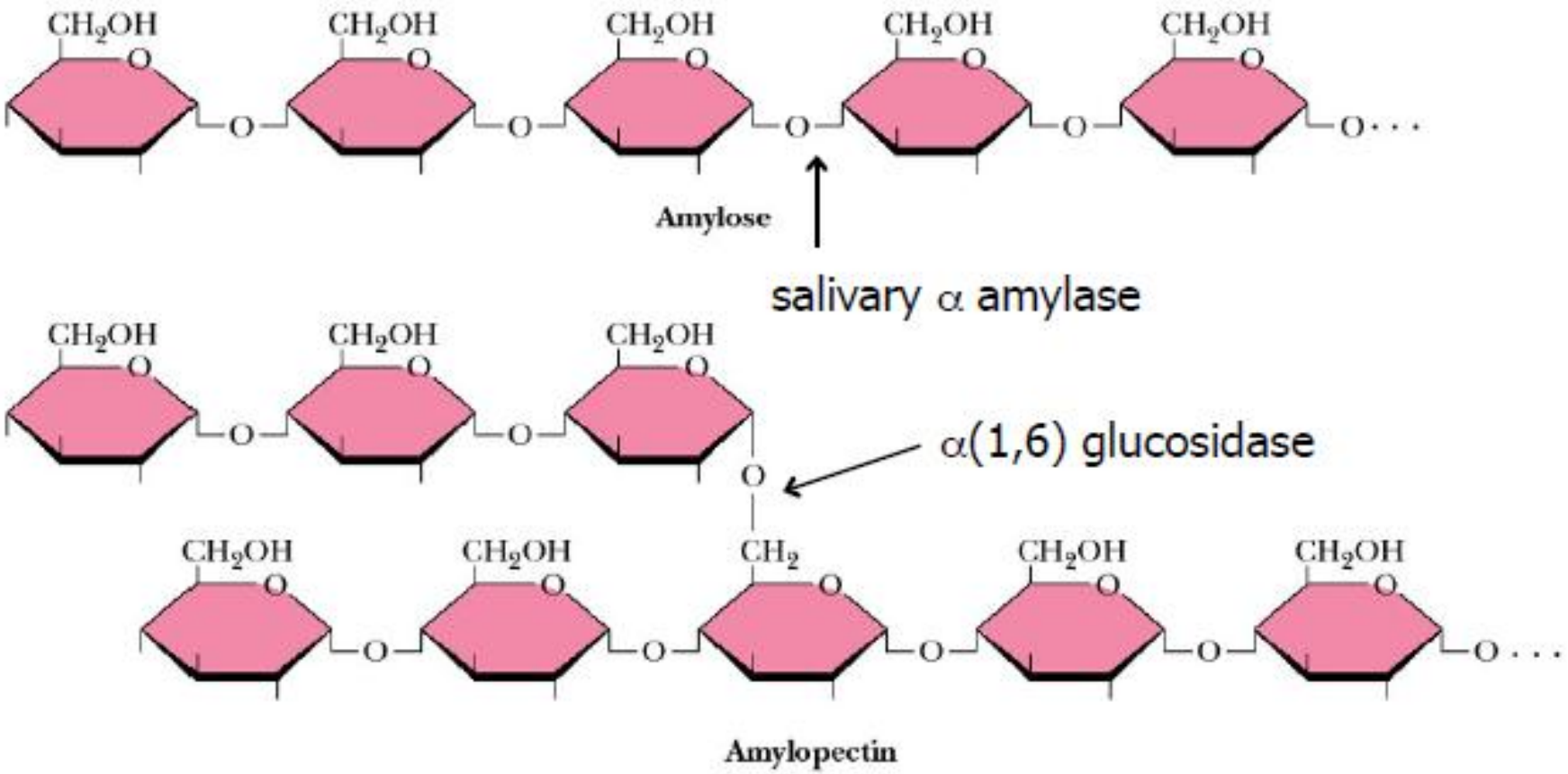


Plant starch phosphorylase releases glucose-1-phosphate from starch (following isomerization to glucose-6-phosphate, this can enter glycolysis)

- In plants, **starch phosphorylase** releases glucose phosphate, which can be catabolized by glycolysis (by isomerization to G6P).
- Cleavage at branch points requires **α(1,6) glucosidase**.
- One ATP is saved (compared to glycolysis) since glucose is already phosphorylated.

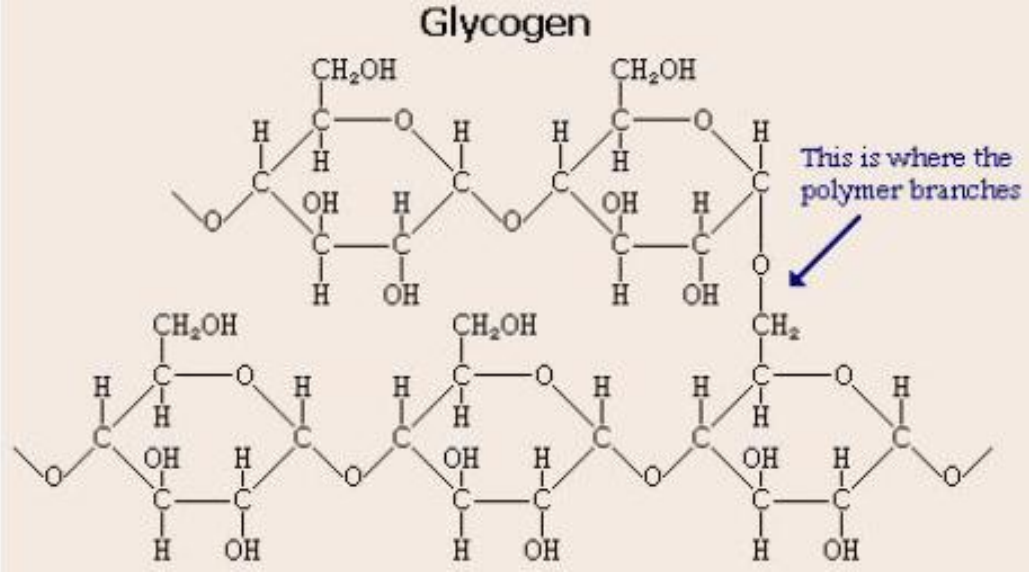


In animals, salivary amylase and α(1,6) glucosidase participate in starch catabolism...



Glycogen is identical to starch except for the degree of branching…

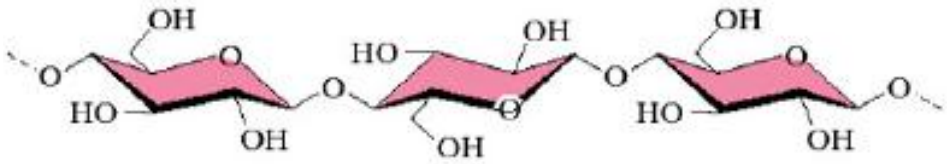
- Glycogen consists of glucose units joined by α1,4 linkages and α1,6 branches
- Starch and glycogen differ only in the degree of branching
- Branches in glycogen occur every 8-12 glucose units instead.



Cellulose is β(1,4) linked glucose units

- Cellulose differs from amylose only in the configuration of the glycosidic linkage, but has very different properties.
- Alternating 180^{o} flips in β(1,4) linkage creates an extended ribbon
- Cellulose is rigid and strong due to hydrogen bonding inherent in such extended structures

cellulose



β-1,4-Linked D-glucose units

(b)

The different nature of the glycosidic bond in amylose and cellulose has a profound effect on properties…

Cellulose has β(1,4) linked D-glucose units

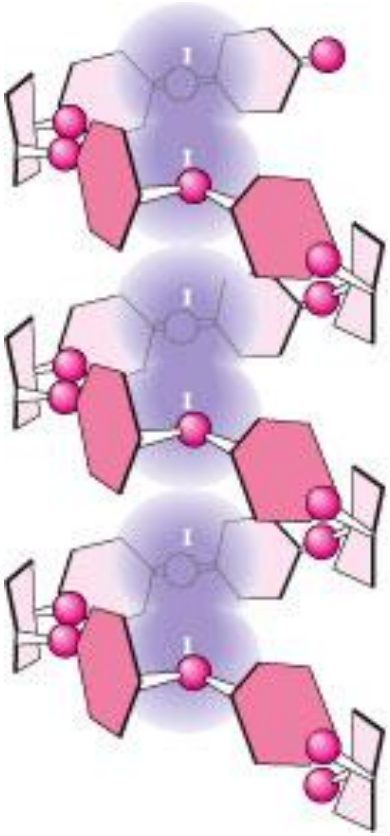
- Cellulose is a rigid structure, found in tree bark, and in the cell walls of nearly all plants.
- Resistant to hydrolysis by acid, and the amylases that degrade starch and glycogen
- In the rumen of ruminants, and the digestive tract of termites, bacteria expressing cellulase hydrolyze cellulose releasing glucose

Amylose has α(1,4) linked D-glucose units

- Found in starch, along with amylopectin.
- Forms micelles in water

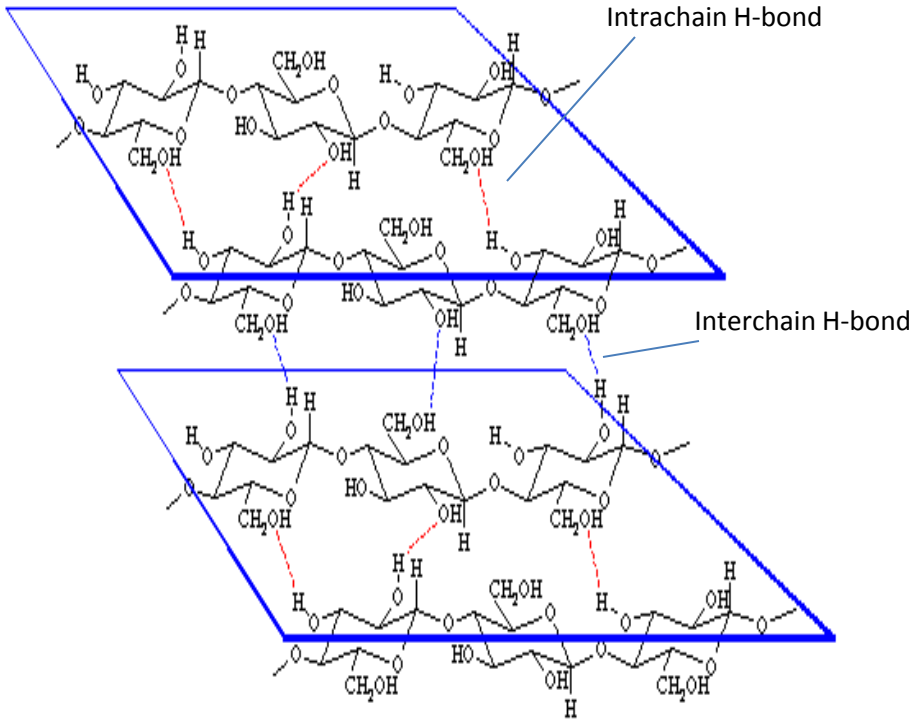
Understand the differences between amylose and cellulose in their 3D structures…

Amylose: adopts helical conformation

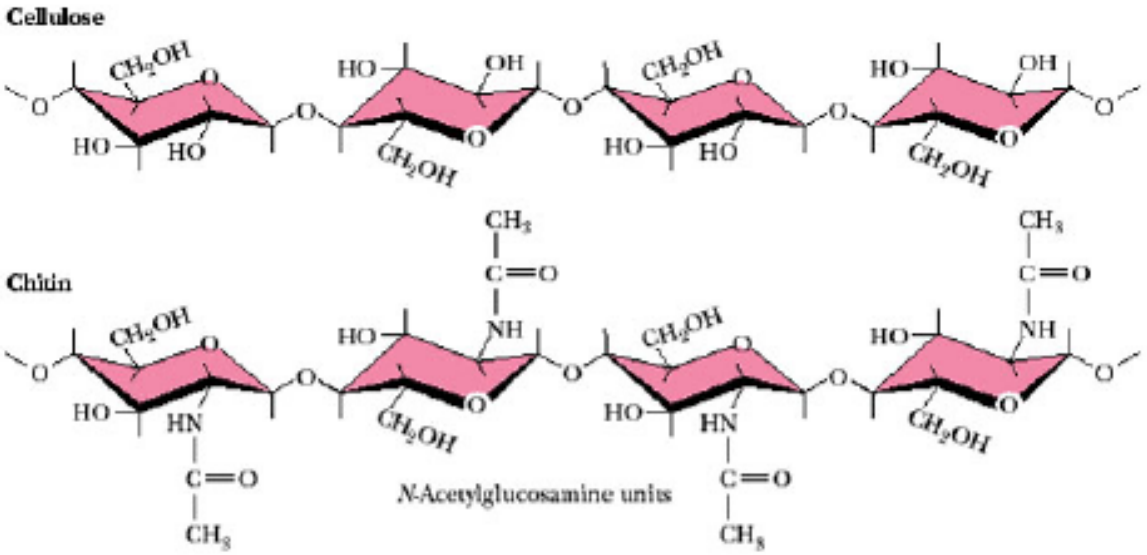


In suspension in water, amylose adopts a helical conformation

Cellulose: adopts ribbon structure. Intra and interchain H-bonding add to its rigidity.



Chitin (structural polysaccharide in insects) is similar to cellulose with the addition of N-acetyl units

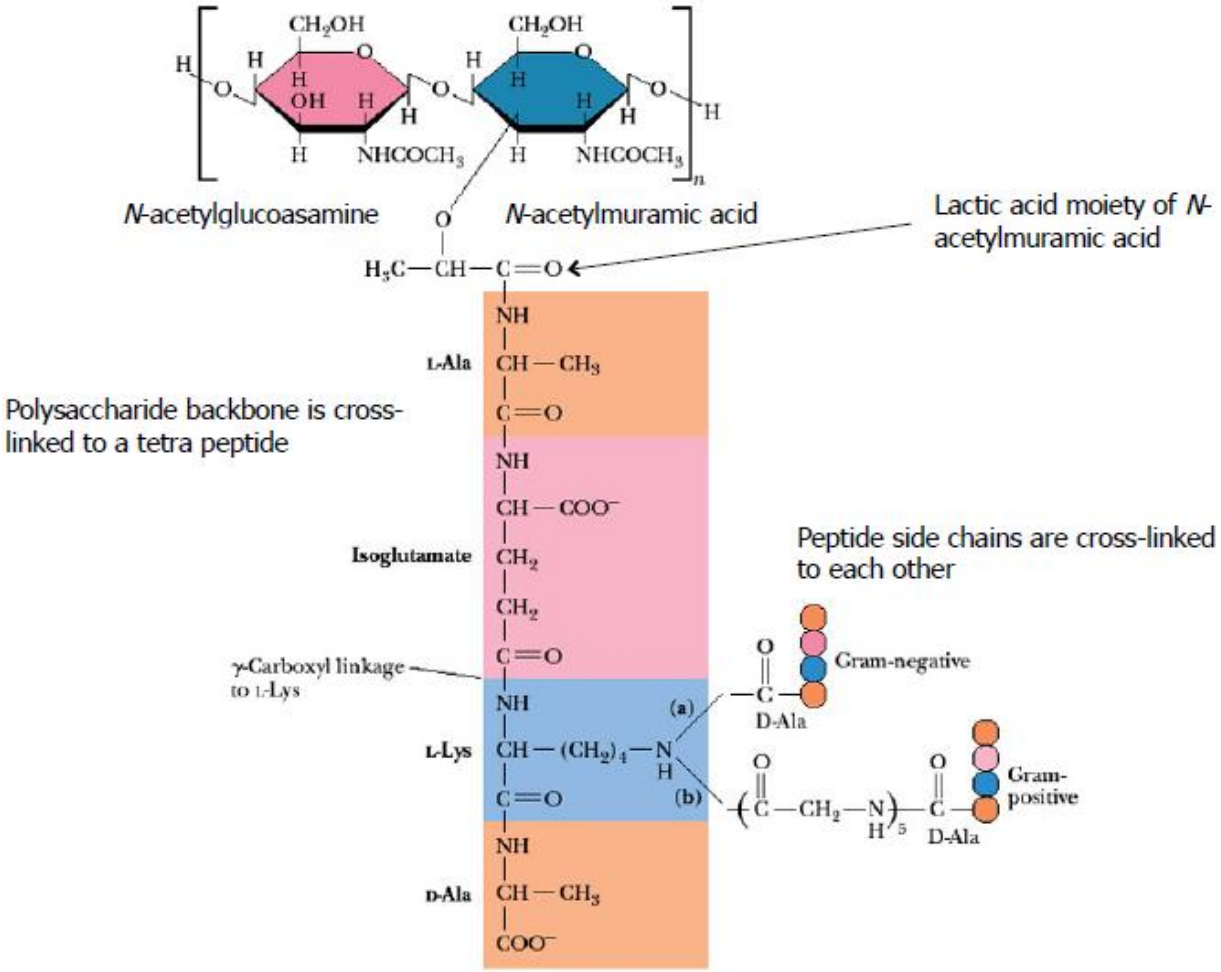


- **Chitin** is found in the cell walls of fungi, and the exoskeletons of crustacea and insects
- Chitin is identical to cellulose, except for the addition of $NHCOCH_3$ at the C2 position. The repeating unit is *N*-acetyl-D-glucosamine

Peptidoglycan (structural polysaccharide in bacteria) contains N-acetyl sugars with crosslinked peptide side chains.

Peptidoglycan

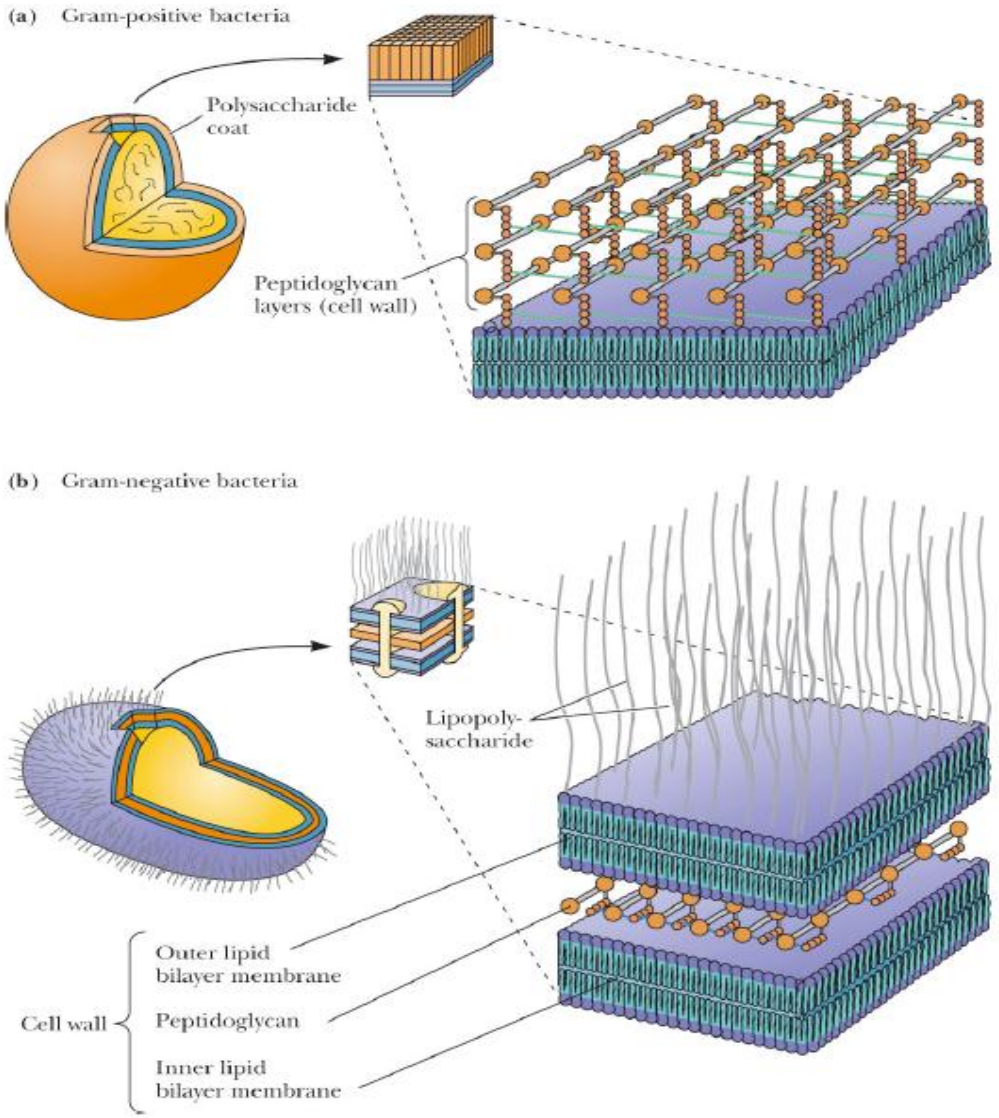
- A continuous cross-linked structure
- Backbone is made up of β(1,4) linked polymer of N-acetylglucosamine and N-acetylmuramic acid joined to tetrapeptides



Peptidoglycan in bacteria

Gram-positive bacteria: have a thick ~25nm cell wall with many layers of peptidoglycan

Gram-negative bacteria: have a thin ~2-3nm cell wall with one layer of peptidoglycan between lipid bilayer membranes



Questions?

Kirk's contact email: knh093020@utdallas.edu

Problem Set #3: Due Friday 11/11 at 5:00PM in FO 3.602

Exam #3: Monday 11/14 at 10:00AM in normal classroom

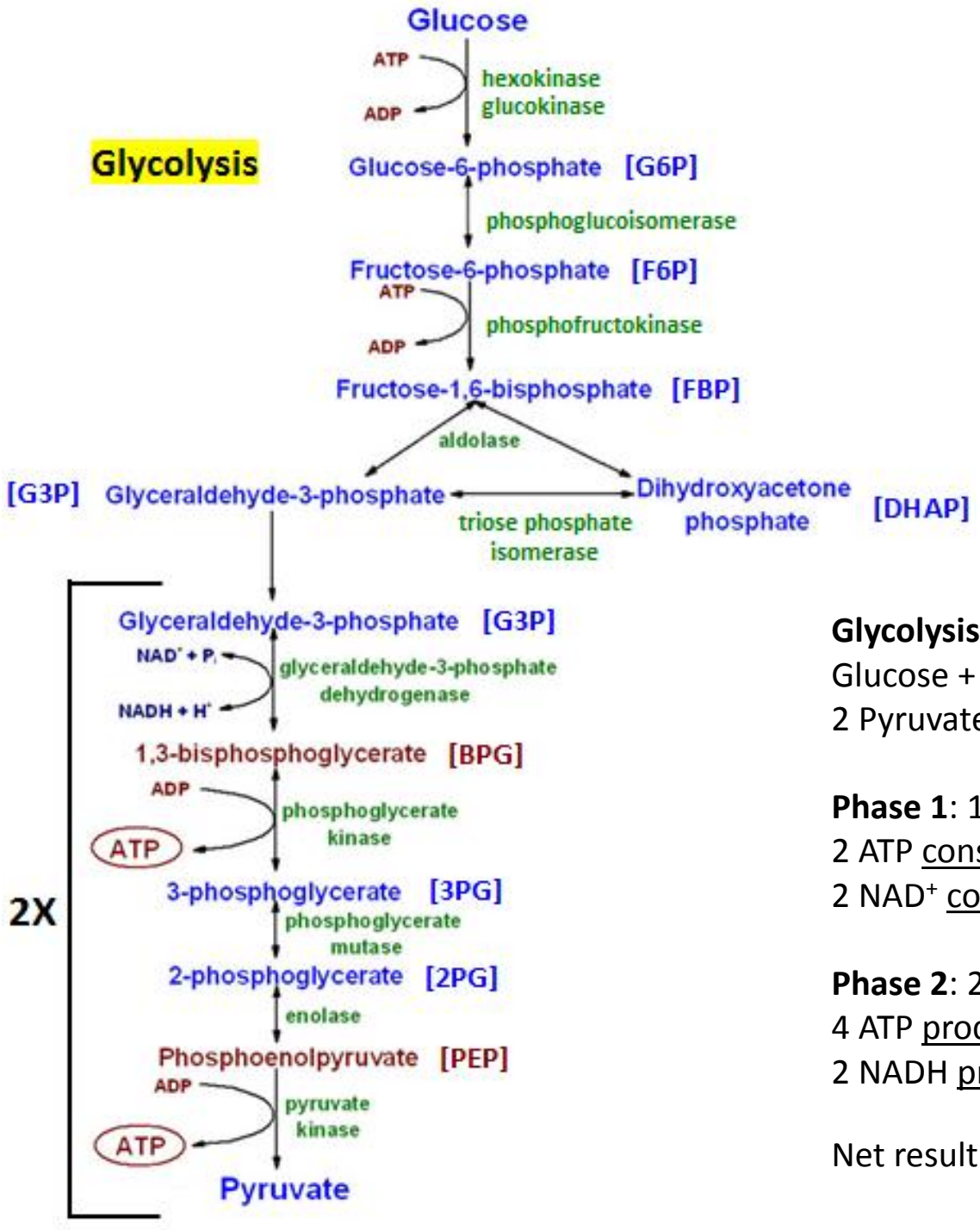
Kirk Huynh

Contact email:
knh093020@utdallas.edu

Exam 3 Review

Glycolysis Phase 1

Chapter 18



Glycolysis Pathway (cytoplasm)
Glucose + $2NAD^+$ + 2ADP + $2P_i$ →
2 Pyruvate + <u>2NADH</u> + <u>2ATP</u> + $2H^+$ + $2H_2O$

Phase 1: 1 Glucose → 2 G3P
2 ATP <u>consumed</u>
2 NAD^+ <u>consumed</u>

Phase 2: 2 G3P → 2 Pyruvate
4 ATP <u>produced</u>
2 NADH <u>produced</u>

Net result: 2 Pyruvate, 2 NADH, 2 ATP

Phosphorylation of glucose (by hexokinase or glucokinase) is a priming reaction required for the subsequent production of high energy phosphorylated intermediates

~ Reaction 1 ~

- Glucose is phosphorylated by **hexokinase** or **glucokinase** using ATP to create G-6-P (glucose-6-phosphate); priming the pump

- Glucokinase is in liver; K_m = 10 mM, acts on glucose only at high glucose concentrations (likes D-glucose). Controlled by insulin.

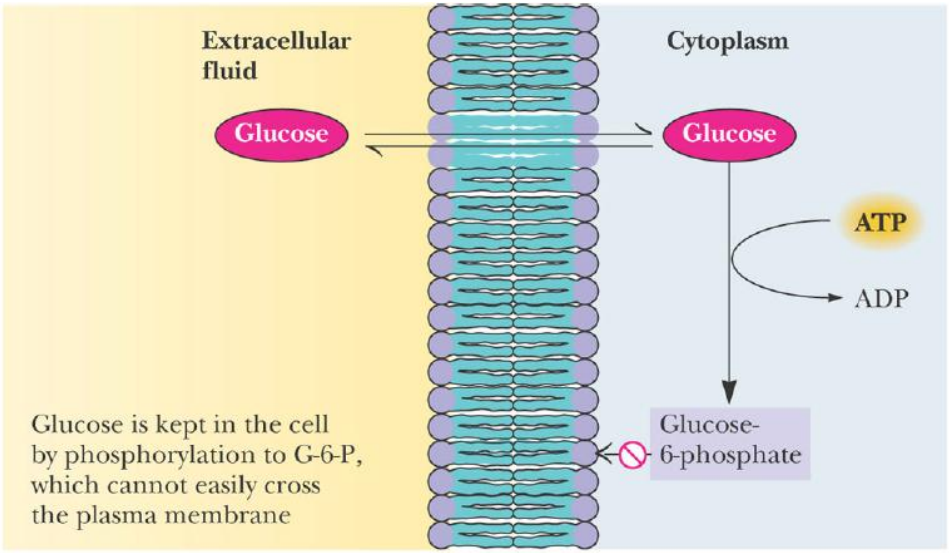
- Diabetes mellitus people do not make much insulin = low glucokinase = cannot tolerate high glucose diet.

- This makes G-6-P
  - Hydrolysis of ATP has a ΔG = -30.5 kJ/mol and making G-6-P uses +13.8 kJ so there is -16.7 kJ left over. In cell, ΔG = -33 kJ/mol (highly favorable).
  - Important step for regulation
  - Glucose is phosphorylated because the negative charge keeps it from crossing the cytoplasmic membrane, also phosphorylating it keeps intracellular concentration of glucose low, causing diffusion of glucose into the cell

Introduction of a charge helps to confine glucose-6-phosphate to the cytoplasm and helps to maintain a low concentration of glucose…

- In cells, the concentration of G-6-P is ~100 fold lower than the concentration of glucose.
- [ADP] is ~100 fold lower than [ATP]
- Phosphorylating glucose puts a -2 charge in glucose as G-6-P.
- When that happens, the glucose cannot get back out of the cytoplasm once it has entered into it.

$\Delta G^{\circ\prime}$ = -16.7 kJ/mol



Phosphoglucoisomerase catalyzes isomerization of glucose-6-phosphate (an aldose and a pyranose) to fructose-6-phosphate (a ketose and a furanose)

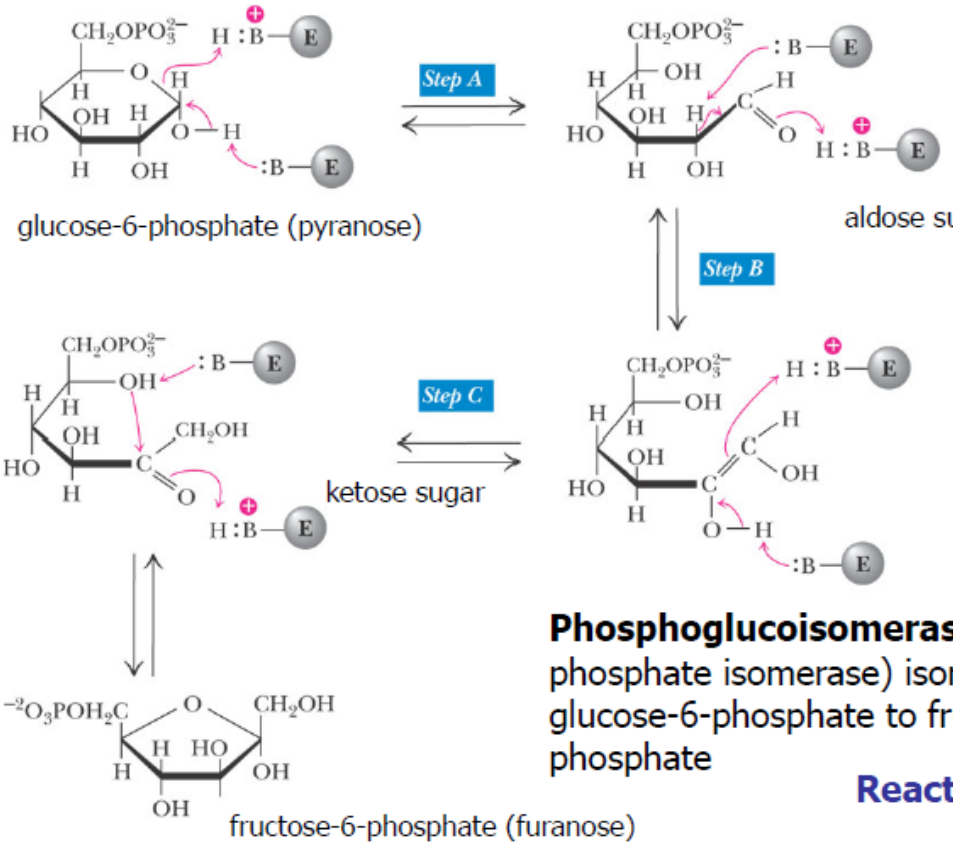
~ Reaction 2 ~

- **Phosphoglucoisomerase** catalyzes G-6-P to F-6-P (fructose-6-phosphate) and is necessary because…
- The next step is the phosphorylation at Carbon-1 position and F-6-P is easier to phosphorylate
- Isomerization activates Carbon-3 for cleavage in step 4
- Only a slightly negative $\Delta G^{\circ\prime}$ = -2.9 kJ/mol, so reaction is readily reversible.
- Sequence: Open pyranose ring (G6P) → Aldose forms, removal of C2 proton → Enediol is formed → Creates C2 carbonyl, ketose formed → Formation of furanose ring (F6P)

Phosphoglucoisomerase catalyzes isomerization of glucose-6-phosphate (an aldose and a pyranose) to fructose-6-phosphate (a ketose and a furanose)

Reaction Mechanism:

1. Open pyranose ring (G6P)
2. Aldose forms, remove C2 proton
3. Enediol forms
4. Creates C2 carbonyl, ketose forms
5. Formation of furanose ring (F6P)



Phosphoglucoisomerase (glucose phosphate isomerase) isomerizes glucose-6-phosphate to fructose-6-phosphate

Reaction 2

Isomerization is required for subsequent phosphorylation and ring cleavage reactions

- Isomerizing the phosphorylated glucose so that the C1 can stick out in the open upon conversion into fructose (a furanose) and this will make it easier to phosphorylate in the next step.

- C3 will also be activated for cleavage in step 4

- $\Delta G^{\circ\prime} = -2.9$ kJ/mol
  - reaction operates close to equilibrium and is readily reversible

Phosphofructokinase phosphorylates fructose-6-phosphate to fructose-1,6-bisphosphate, the second priming reaction

~ Reaction 3 ~

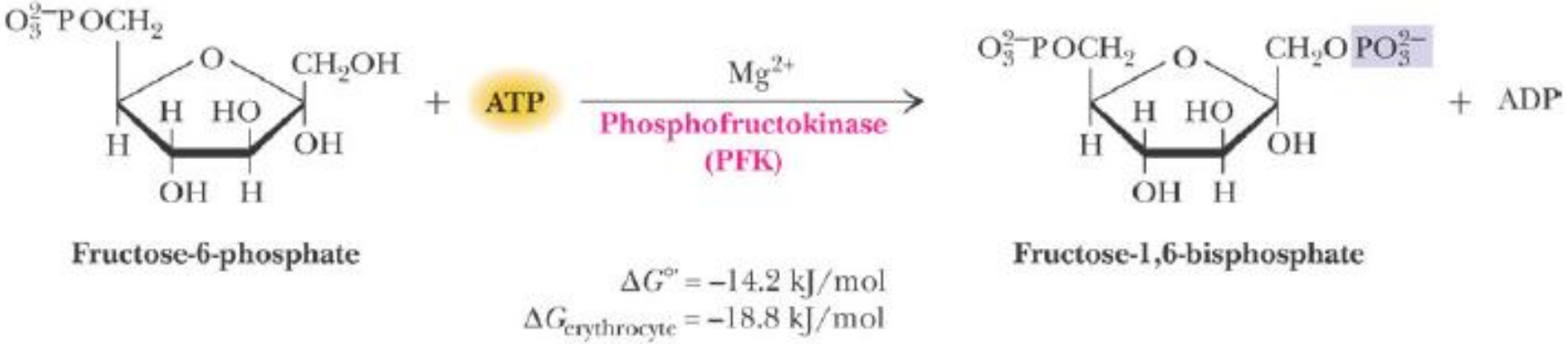
- **Phosphofructokinase** phosphorylates F-6-P at C1 position, the second priming step, which makes FBP (fructose-1,6-bisphosphate).
- This reaction commits the cell to metabolizing glucose rather than storing it or converting to another sugar.
- It is exergonic due to coupling with ATP hydrolysis; therefore, it's an important step for regulation.
- Has 2 binding sites for ATP: a high-affinity substrate site, and another low-affinity regulatory site, because ATP is also an allosteric inhibitor
  - High [ATP]: K_m of PFK is increased and reaction rate decreases; AMP reverses ATP inhibition, low [ATP] increases glycolysis
  - In aerobic conditions, pyruvate is sent to the citric acid cycle, when TCA cycle is saturated, glycolysis is slowed because citrate is also an inhibitor of PFK.
  - F-2,6-biphosphate is an activator: PFK is activated when F-6-P and glucose are high, feed forward.
    - » Also decreases inhibitory effects of ATP
    - » AMP also reverses inhibition by ATP

$\Delta G^{\circ\prime}$ = -14.2 kJ/mol

Phosphofructokinase phosphorylates fructose-6-phosphate to fructose-1,6-bisphosphate, the second priming reaction

Reaction 3

Irreversible



PFK commits glucose to glycolysis and is a key point for regulation – know what regulates PFK and how…

- PFK has two binding sites for ATP: a high-affinity substrate site and a low affinity regulatory site

- High ATP concentration will allow ATP to bind to the regulatory site; therefore, PFK will behave more cooperatively. Its K_m for F-6-P will be increased, the curve will be sigmoidal.

- AMP reverses ATP inhibition… High AMP levels indicate low ATP levels due to action by the enzyme, **ADENYLATE KINASE**, which catalyzes the reaction below…

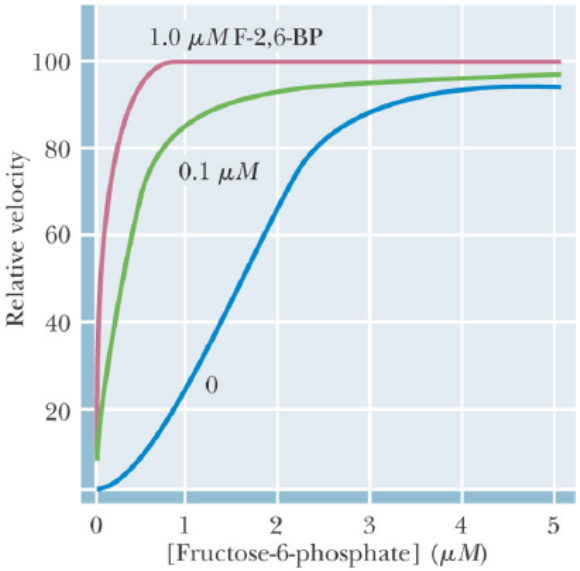
(KNOW THIS)

- PFK couples glycolysis and the citric acid cycle… Thus, citrate, an intermediate of the citric acid cycle, is an allosteric inhibitor of PFK.

- Fructose-2,6-bisphosphate acts as an allosteric activator by increasing the affinity of PFK for fructose-6-phosphate (**feed-forward** regulation, and restores hyperbolic kinetics).

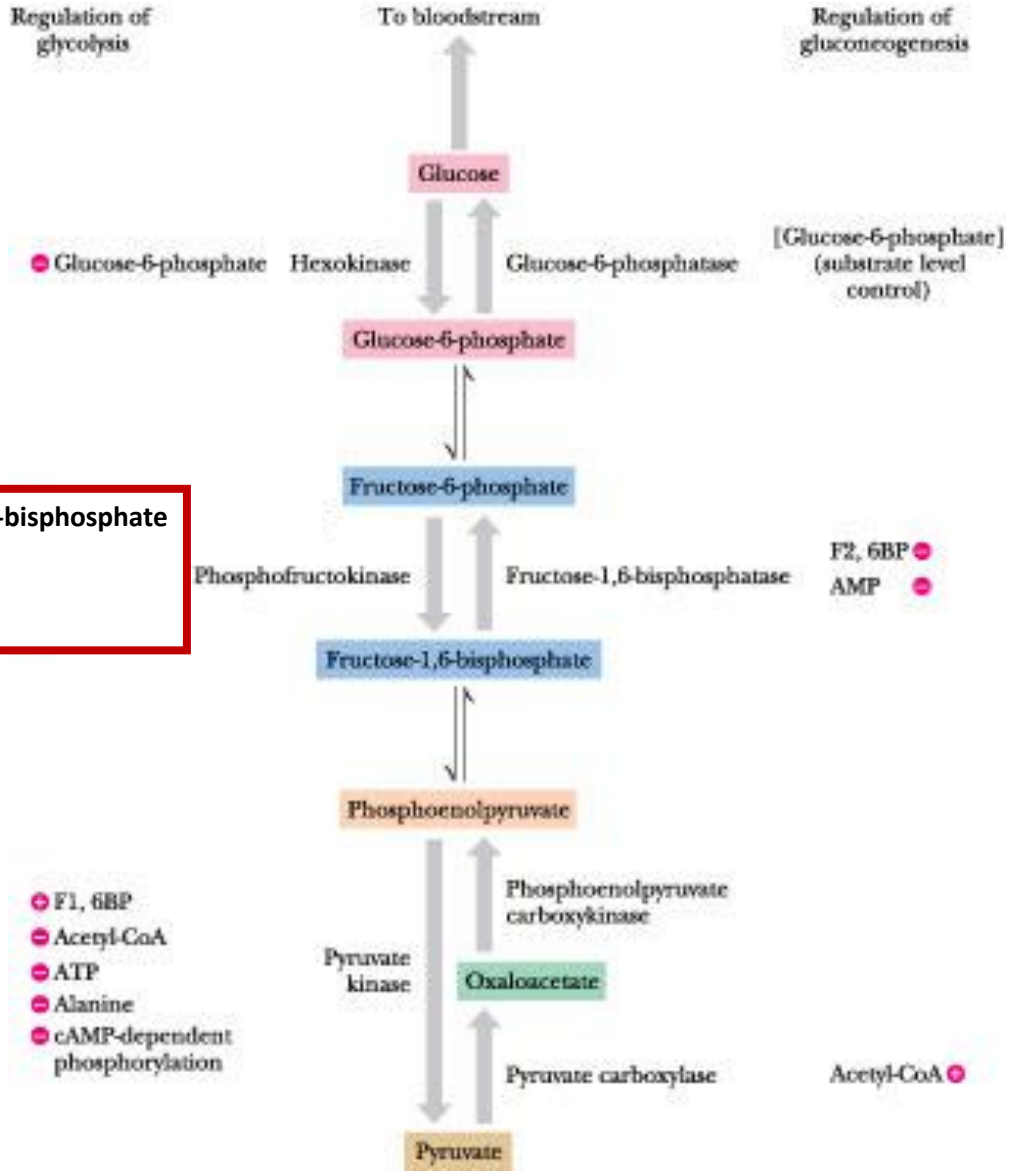
PFK commits glucose to glycolysis and is a key point for regulation – know what regulates PFK and how…

- Allosteric Activators
  - Fructose-2,6-bisphosphate
  - AMP
- Allosteric Inhibitors
  - ATP
  - Citrate



Fructose-2,6-bisphosphate reverses the inhibition by ATP and restores hyperbolic kinetics

+ Fructose-2,6-bisphosphate
+ AMP
- ATP
- Citrate



Fructose bisphosphate aldolase catalyzes a ring cleavage reaction, products are dihydroxyacetone phosphate and glyceraldehyde-3-phosphate

~ Reaction 4 ~

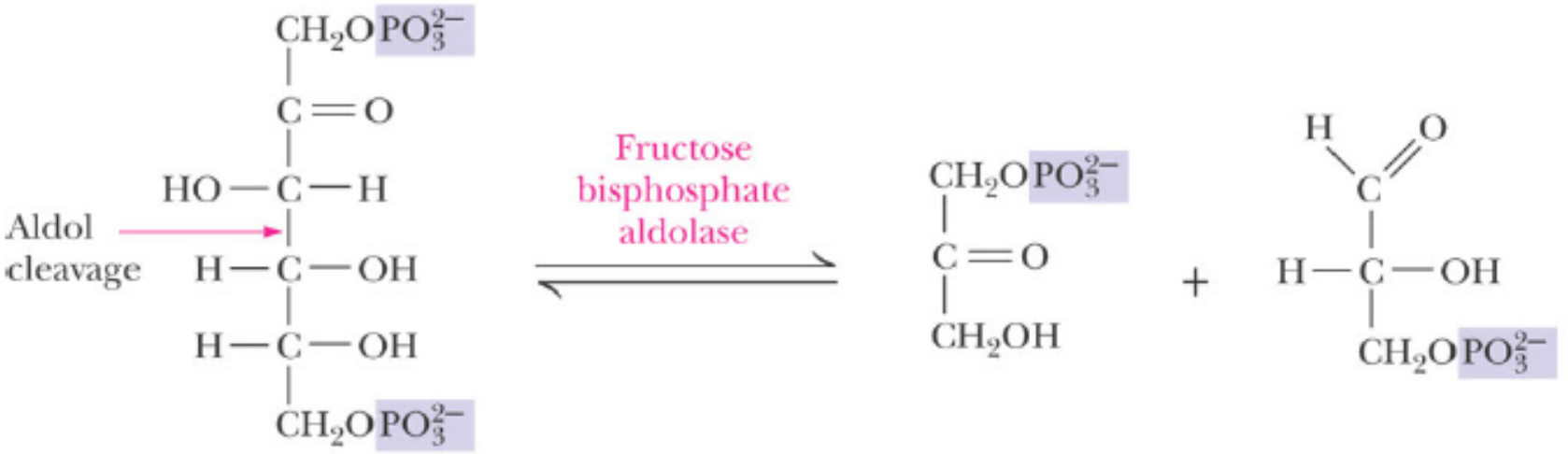
- **Fructose bisphosphate aldolase** cleaves FBP between C3 and C4 to yield two triose phosphates: Dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G-3-P)
  - cleaves the ring

- In animals, the reaction proceeds through formation of a Schiff base between the carbonyl group and active site lysine.

- Reaction is endergonic, but close to equilibrium at physiological concentrations of reactants; therefore, reversible.

Fructose bisphosphate aldolase catalyzes a ring cleavage reaction, products are dihydroxyacetone phosphate and glyceraldehyde-3-phosphate

Reaction 4



D-Fructose-1,6-bisphosphate (FBP)

Dihydroxyacetone phosphate (DHAP)

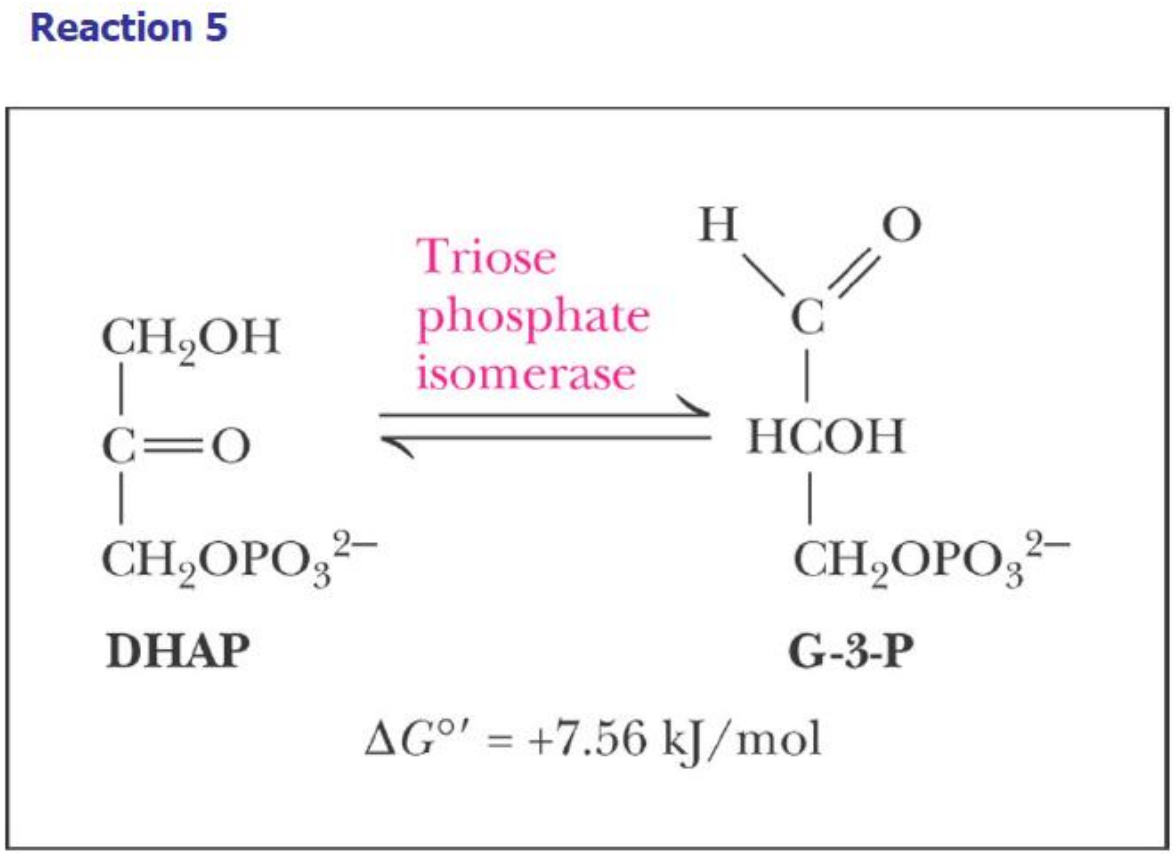
D-Glyceraldehyde 3-phosphate (G-3-P)

$\Delta G^{\circ\prime} = 23.9$ kJ/mol

Triose phosphate isomerase interconverts DHAP to G-3-P

~ Reaction 5 ~

- **Triose Phosphate Isomerase** converts DHAP into G-3-P to complete Phase 1 of glycolysis.



Phase 1 has a negative ΔG under physiological cellular conditions, so it proceeds in the forward direction.

Glycolysis Pathway (cytoplasm)

Glucose + $2NAD^+$ + 2ADP + $2P_i$ → 2 Pyruvate + 2NADH + 2ATP + $2H^+$ + $2H_2O$

Phase 1: 1 Glucose → 2 G3P

2 ATP consumed

2 NAD^+ consumed

Phase 2: 2 G3P → 2 Pyruvate

4 ATP produced

2 NADH produced

Net result: 2 Pyruvate, 2 NADH, 2 ATP

Know the sequence of reactions, the enzymes that catalyze them, and the substrates/products (no structures necessary)

Kirk Huynh

Contact email:
knh093020@utdallas.edu

Exam 3 Review

Glycolysis Phase 2

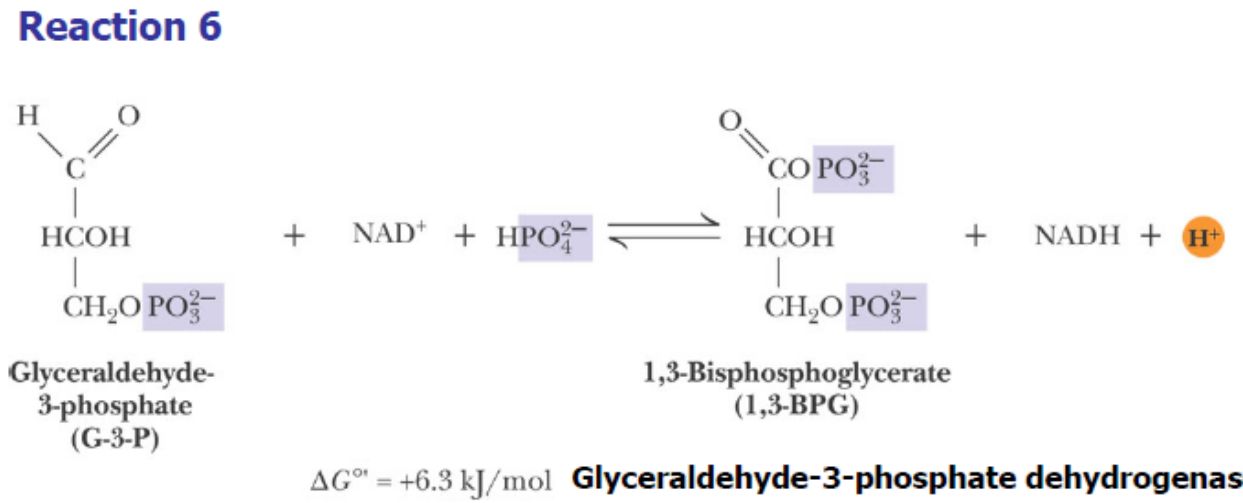
Chapter 18

The reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase is unique to glycolysis, because (a) it is an oxidation, and (b) it used inorganic phosphate to phosphorylate substrate

~ Reaction 6 ~

- **G-3-P dehydrogenase** oxidizes G-3-P to 1,3-BPG (1,3-bisphosphoglycerate)
  - The aldehyde group gets **oxidized** to carboxylic acid
  - Uses **inorganic** phosphate to phosphorylate the substrate
  - NAD^+ is oxidizing agent and gets reduced to NADH

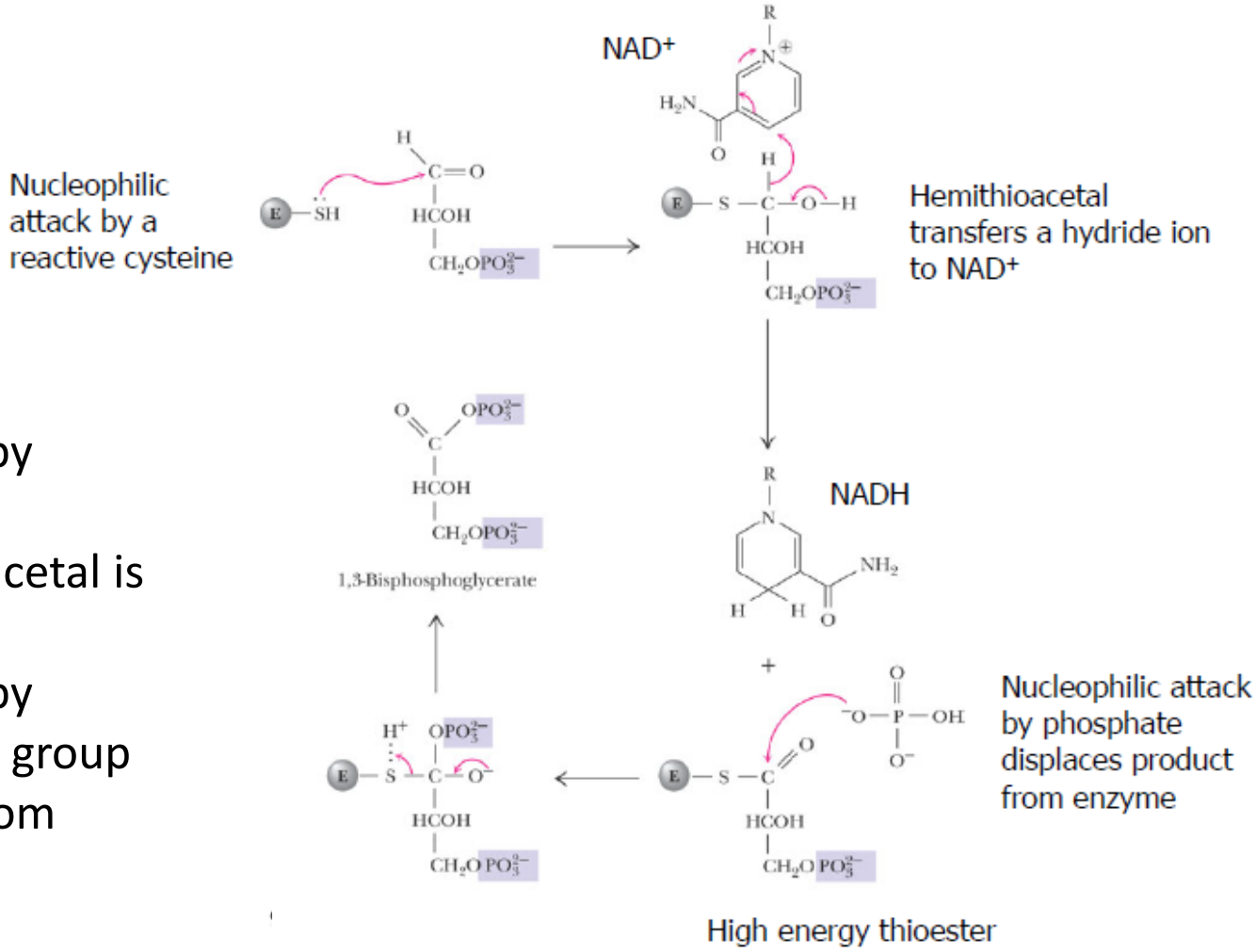
- Sequence: Nucleophilic attack by cysteine –SH group → a H^+ from hemithioacetal is transferred to NAD^+ → Nucleophilic attack by inorganic phosphate group displaces 1,3-BPG from enzyme



The reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase is unique to glycolysis, because (a) it is an oxidation, and (b) it used inorganic phosphate to phosphorylate substrate

Reaction mechanism:

1. Nucleophilic attack by cysteine –SH group
2. A H^+ from hemithioacetal is transferred to NAD^+
3. Nucleophilic attack by inorganic phosphate group displaces 1,3-BPG from enzyme



Understand how glycolysis yields ATP despite using two ATP in phase 1…

- Two G-3-P's enter phase 2 from phase 1.
- There are two substrate level phosphorylation reactions in phase 2 that yield a total of 4 ATP.
- Hence, the net yield of glycolysis after phase 2 is 2 ATP.

Phase 1: 1 Glucose → 2 G3P

2 ATP consumed

2 NAD^+ consumed

Phase 2: 2 G3P → 2 Pyruvate

4 ATP produced

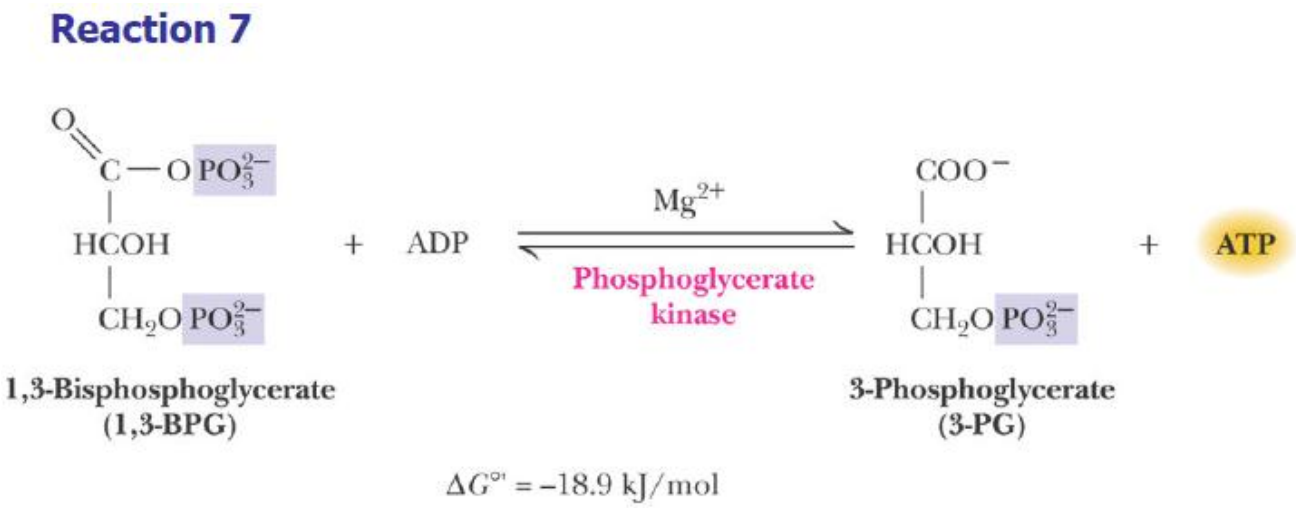
2 NADH produced

Net result: 2 Pyruvate, 2 NADH, 2 ATP

Phosphoglycerate kinase catalyzes a substrate level phosphorylation to yield ATP

~ Reaction 7 ~

- **Phosphoglycerate kinase** transfers 1 phosphoryl group from 1,3-BPG to ADP and makes 2 ATP and 3-PG (3-phosphoglycerate).
  - This is the break even reaction.
- This is a TRANSFERASE ENZYME!
- ATP is synthesized at expense of substrate, i.e. substrate level phosphorylation
- ***1,3-BPG* is the first intermediate in glycolysis with a free energy of hydrolysis that is more negative than ATP!**

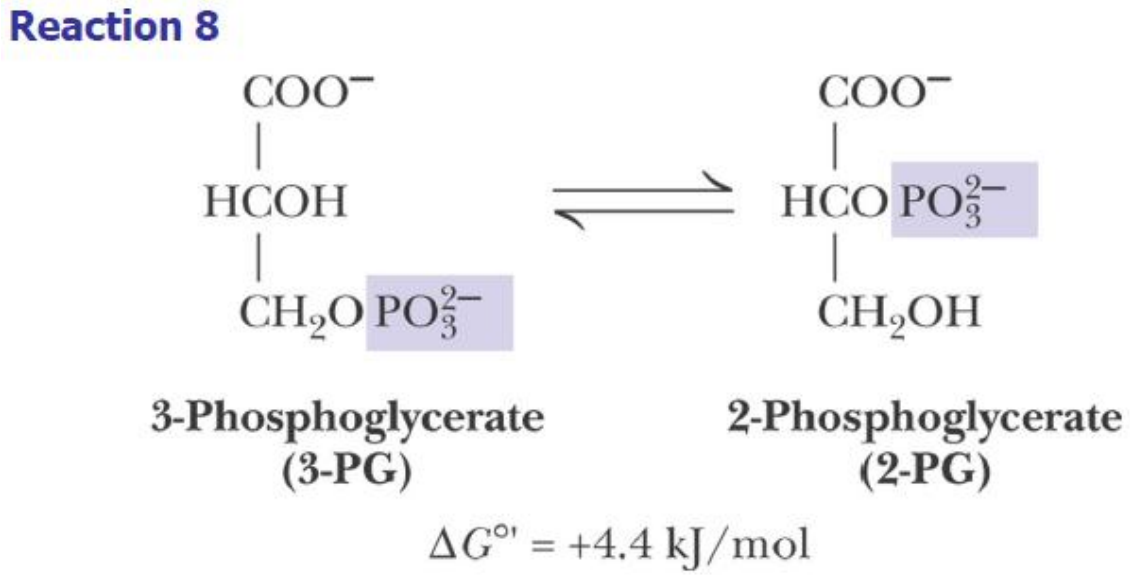


Phosphoglycerate mutase isomerizes 3-PG to 2-PG, to prepare for the next steps…

~ Reaction 8 ~

- **Phosphoglycerate mutase** moves the phosphoryl group from 3 to 2 position (3-PG → 2-PG).

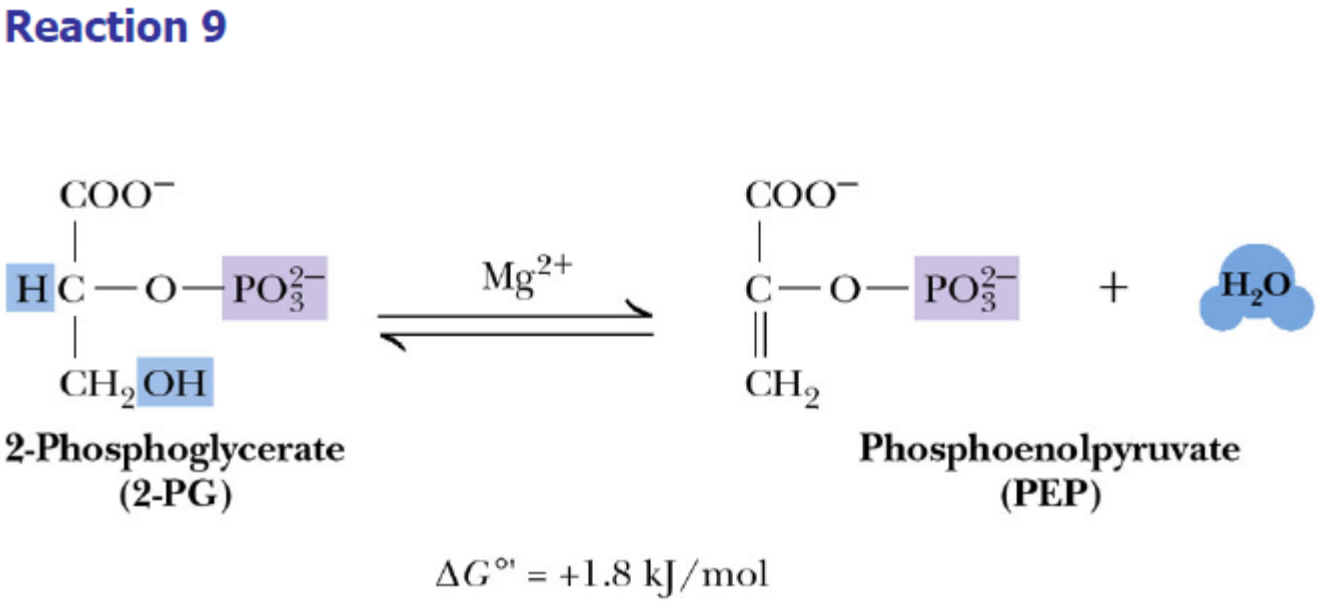
- Mutases are a type of isomerase…the functional group is moved within the substrate.



Enolase removes water from 2-PG to form phosphoenol pyruvate. This rearrangement makes more energy available when PEP is hydrolyzed…

~ Reaction 9 ~

- **Enolase** dehydrates 2-PG to form PEP (phosphoenolpyruvate)
- Generates a high-energy phospho compound



Pyruvate kinase catalyzes a substrate level phosphorylation

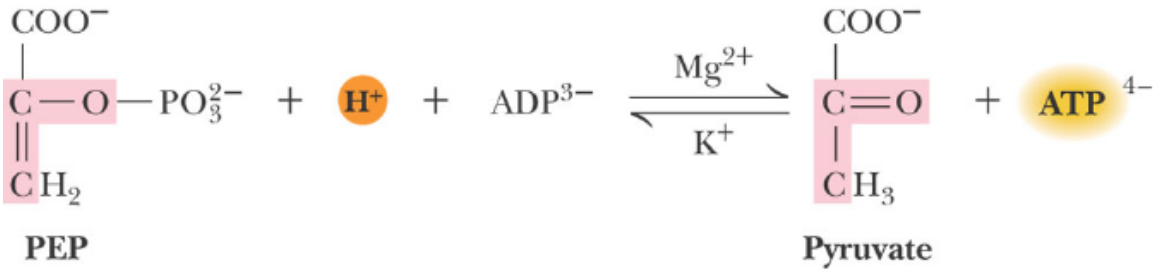
~ Reaction 10 ~

- Second substrate level phosphorylation: **pyruvate kinase** transfers phosphoryl group from PEP to ADP and makes Pyruvate
- Spontaneous conversion from enol to keto tautomer of pyruvate is very favorable and drives reaction forward;
- Pyruvate kinase is a point where glycolysis is regulated
  - PK is activated by AMP and F 1,6 BP
  - Inhibited by ATP and alanine, alanine made in 1 step from pyruvate
  - Liver PK is activated by glucagon

ΔG°' = -31.7 kJ/mol

Reaction 10

Irreversible

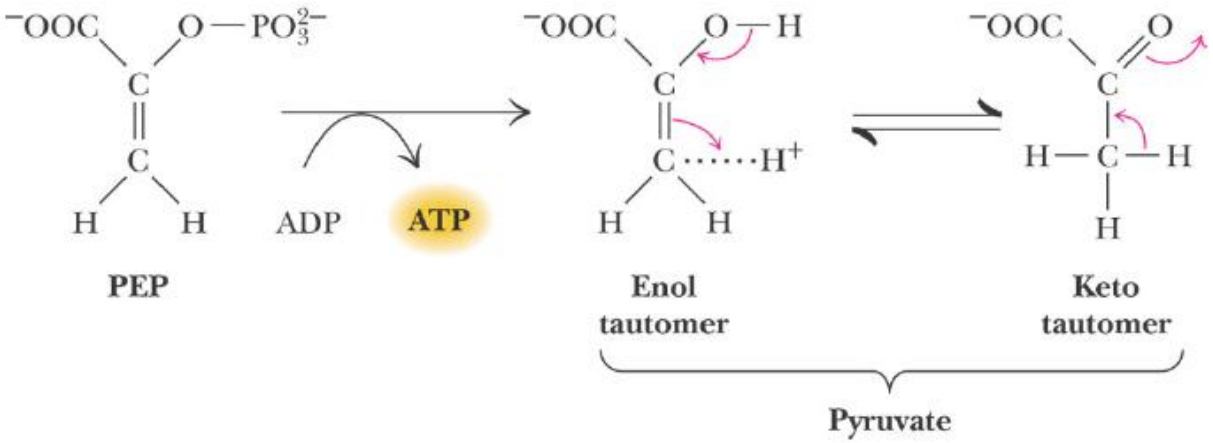


$\Delta G^{\circ\prime} = -31.7$ kJ/mol

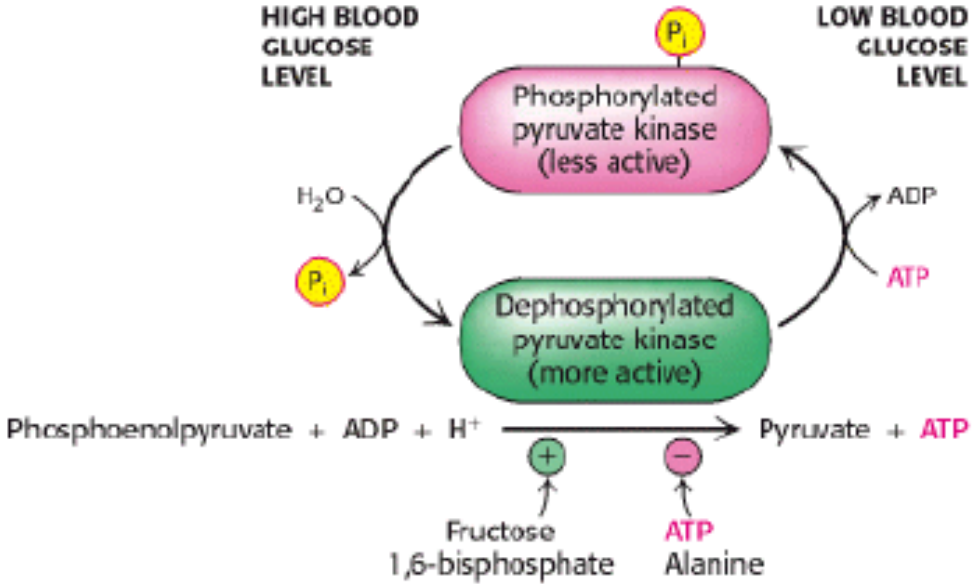
Understand, in general terms, how enol-keto tautomerism drives the PK reaction forwards

The conversion of PEP to pyruvate has a high free energy change ($\Delta G^{\circ\prime}$ = -31.7 kJ/mol), this is because….

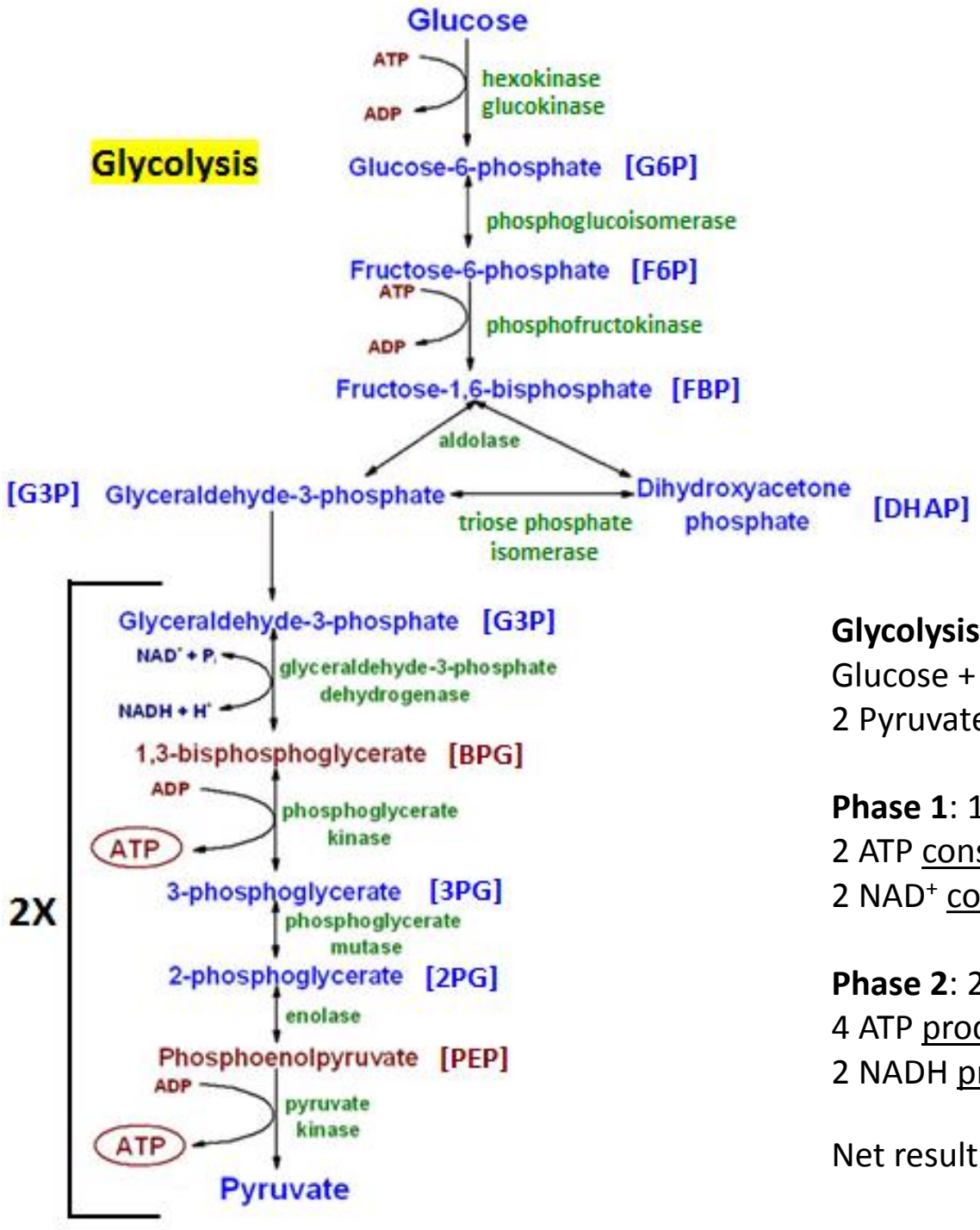
- Pyruvate has tautomer forms: enol and keto.
- The conversion from the enol form to the keto form is HIGHLY FAVORABLE.
- This is because the KETO FORM IS MORE STABLE.
- The phosphoryl transfer step follows.



Understand, in general terms, how enol-keto tautomerism drives the PK reaction forwards



Liver PK is phosphorylated by a **protein kinase** which is activated by **glucagon**, a hormone that signals glucose status.
Phosphorylated enzyme has a higher K_m for PEP and is more strongly inhibited by ATP and alanine



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Phase 1: 1 Glucose → 2 G3P
2 ATP <u>consumed</u>
2 NAD^+ <u>consumed</u>

Phase 2: 2 G3P → 2 Pyruvate
4 ATP <u>produced</u>
2 NADH <u>produced</u>

Net result: 2 Pyruvate, 2 NADH, 2 ATP

Understand the possible fates of pyruvate, and why there is a need to reoxidize NADH…

NADH must be reoxidized to NAD^+ (redox balancing)

Aerobic conditions (presence of oxygen):

- TCA cycle: pyruvate reoxidized to CO_2, produces additional NADH and $FADH_2$
- Electron-transport chain: NADH reoxidized to NAD^+, drives ATP synthesis

Pyruvate dehydrogenase reaction (mitochondrial matrix):

2 Pyruvate + $2NAD^+$ + CoASH → 2 Acetyl-CoA + <u>2NADH</u> + $2CO_2$

TCA cycle/Krebs cycle (mitochondrial matrix):

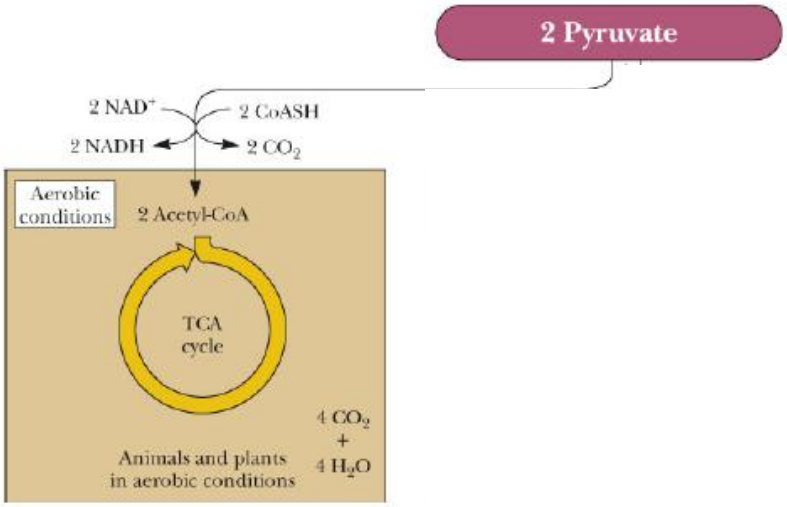
2 Acetyl-CoA + $6NAD^+$ + 2FAD + 2ADP + $2P_i$ + $4H_2O$ → <u>6NADH</u> + <u>$2FADH_2$</u> + <u>2ATP</u> + $4CO_2$ + $6H^+$ + CoASH

Electron Transport Chain/Oxidative Phosphorylation (mitochondrial inner membrane):

$10NADH + 2FADH_2$ → <u>32 ATP</u>

$2NADH + 2H^+ + O_2 \rightarrow 2NAD^+ + 2H_2O$

Net ATP production: 36 ATP!



Understand the possible fates of pyruvate, and why there is a need to reoxidize NADH…

NADH must be reoxidized to NAD^+ (redox balancing)

Anaerobic conditions (absence of oxygen):

- **Fermentation** (coupled to reduction of catabolic product)
  - Yeast: pyruvate reduced to ethanol, produces CO_2
  - Other microorganisms and animals: pyruvate reduced to lactate

Alcoholic Fermentation:

Glucose + 2ADP + $2P_i$ → 2 Ethanol + <u>2ATP</u> + $2CO_2$ + $2H_2O$

or

2 Pyruvate + 2NADH → 2 Ethanol + $2NAD^+$ + $2CO_2$

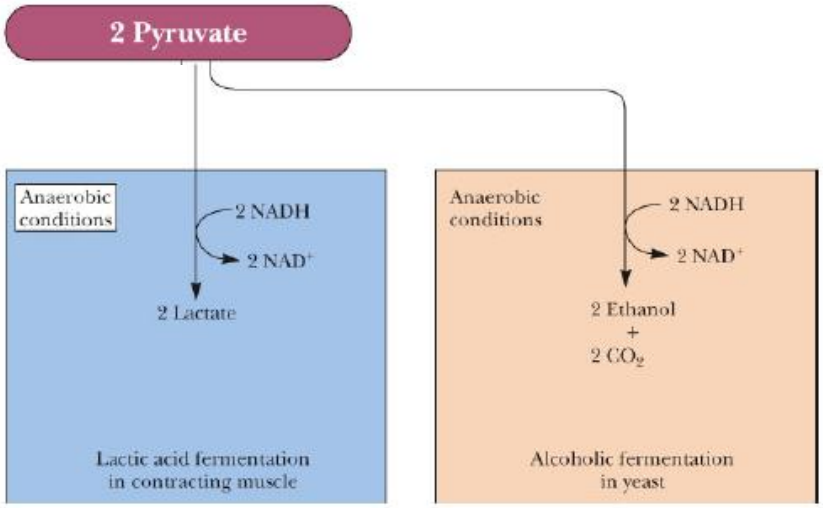
Lactate Fermentation (homolactic):

Glucose + 2ADP + $2P_i$ → 2 Lactate + <u>2ATP</u> + $2H^+$ + $2H_2O$

or

2 Pyruvate + 2NADH → 2 Lactate + $2NAD^+$

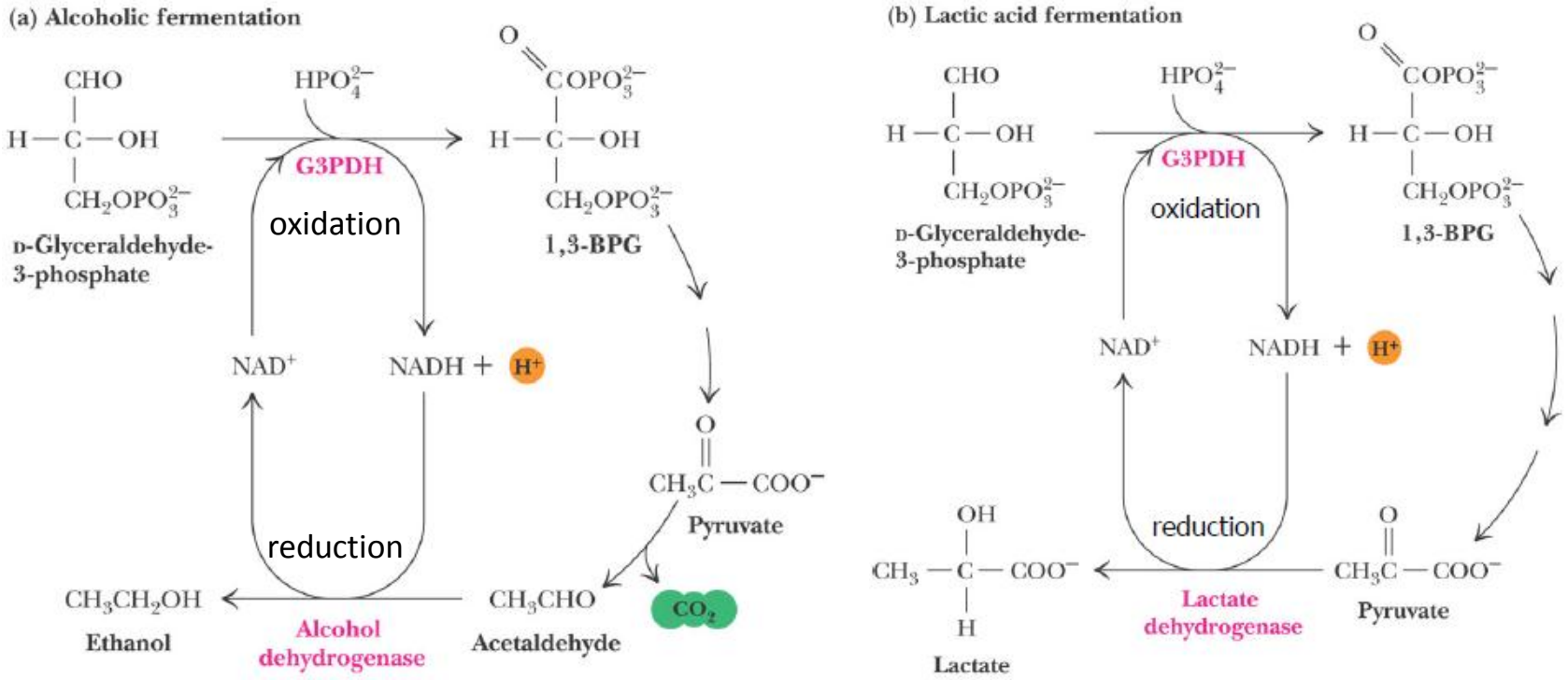
Net ATP production: 2 ATP!



Understand the nature of fermentation

Fermentation: the production of ATP energy by reaction pathways in which organic molecules function as donors and acceptors of electrons

- In either aerobic or anaerobic conditions, pyruvate is reduced to reoxidize the NADH produced in step 6 (G3P → 1,3-BPG) of glycolysis.
- Step 1: Pyruvate is decarboxylated to acetaldehyde by **pyruvate decarboxylase** in an irreversible reaction
- Step 2: **Alcohol dehydrogenase** (ADH) or **lactate dehydrogenase** (LDH) catalyzes the reduction of acetaldehyde to ethanol by NADH.



Understand, in general terms how fermentation (eg lactate and ethanol fermentations) reactions allow redox balancing

NADH must be reoxidized to NAD^+ (redox balancing)

- In either aerobic or anaerobic conditions, pyruvate is reduced to reoxidize the NADH produced in step 6 (G3P → 1,3-BPG) of glycolysis.
- More specifically, fermentation reoxidizes the two NADH produced in glycolysis by coupling it with a reduction process of reducing pyruvate to ethanol/lactate.

Understand that fermentation of glucose to lactate or ethanol is redox neutral

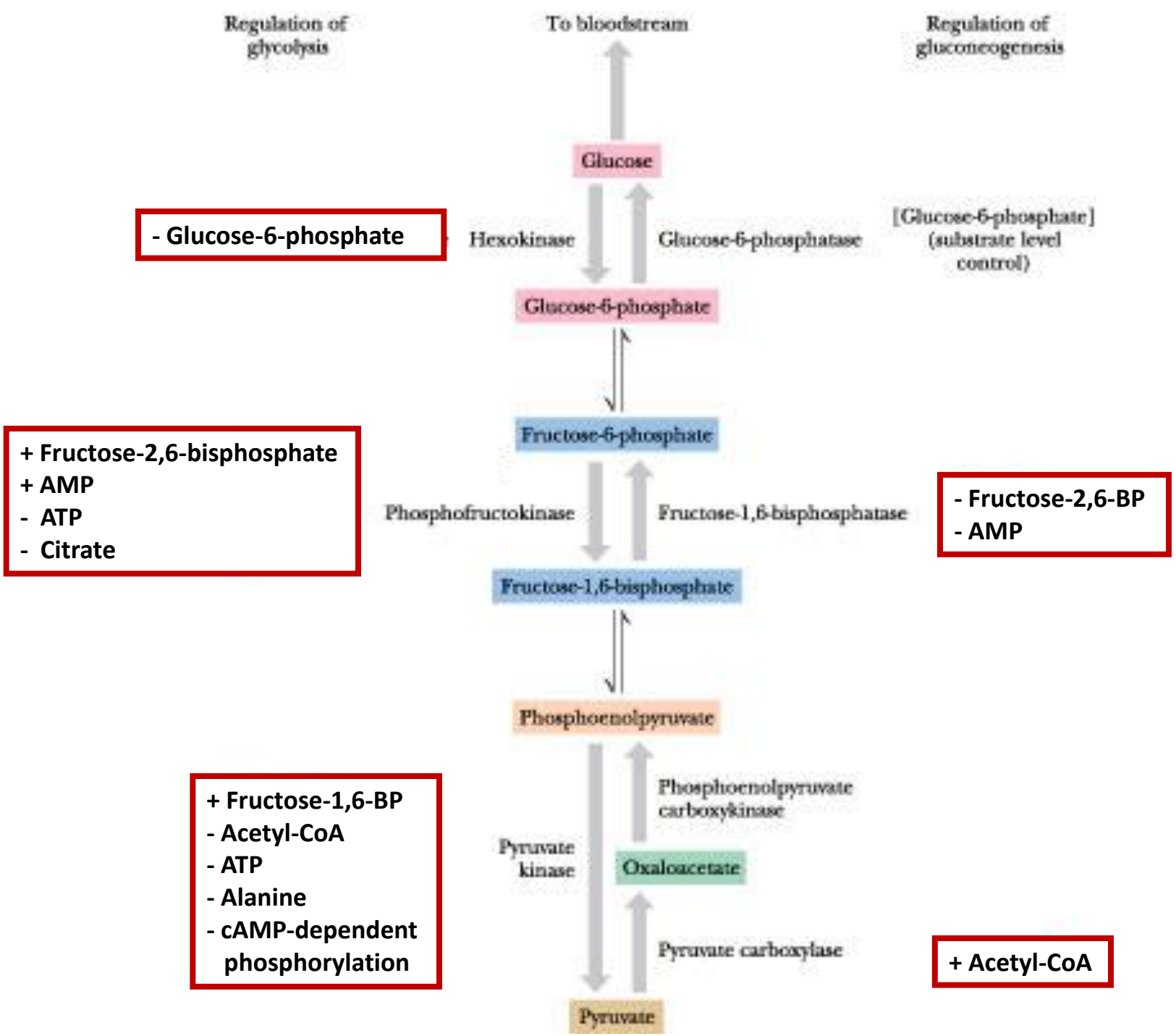
- Net yield of 0 NADH in glycolysis/fermentation
  - 2 NADH produced in glycolysis
  - 2 NADH reoxidized to NAD^+ in fermentation
- Alcoholic fermentation: 2 Pyruvate + 2NADH → 2 Ethanol + $2NAD^+$ + $2CO_2$
- Lactate fermentation: 2 Pyruvate + 2NADH → 2 Lactate + $2NAD^+$

Understand that regulation of glycolysis is exerted at the exergonic steps

The three exergonic steps of glycolysis are:

- Reaction 1: **Hexokinase**; ΔG = -16.7 kJ/mol
  - Inhibitory: G-6-P
- Reaction 3: **Phosphofructokinase** (PFK); ΔG = -14.2 kJ/mol
  - Activator: Fructose-2,6-bisphosphate, AMP
  - Inhibitory: ATP, Citrate
- Reaction 10: **Pyruvate kinase** (PK); ΔG = -31.7 kJ/mol
  - Activator: Fructose-1, 6-BP
  - Inhibitory: Acetyl-CoA, ATP, Alanine, cAMP-dependent phosphorylation

- Reaction 11: **Lactate dehydrogenase** (LDH); ΔG = -25.2 kJ/mol


Regulation of glycolysis
To bloodstream
Regulation of gluconeogenesis
Glucose
- Glucose-6-phosphate
Hexokinase
Glucose-6-phosphatase
[Glucose-6-phosphate] (substrate level control)
Glucose-6-phosphate
Fructose-6-phosphate
+ Fructose-2,6-bisphosphate
+ AMP
- ATP
- Citrate
Phosphofructokinase
Fructose-1,6-bisphosphatase
- Fructose-2,6-BP
- AMP
Fructose-1,6-bisphosphate
Phosphoenolpyruvate
Phosphoenolpyruvate carboxykinase
+ Fructose-1,6-BP
- Acetyl-CoA
- ATP
- Alanine
- cAMP-dependent phosphorylation
Pyruvate kinase
Oxaloacetate
Pyruvate carboxylase
+ Acetyl-CoA
Pyruvate

Understand that other sugars can enter glycolysis (at different points) with galactose as an example…

Leloir Pathway:

Galactose + ATP → Galactose-1-phosphate + ADP + H^+

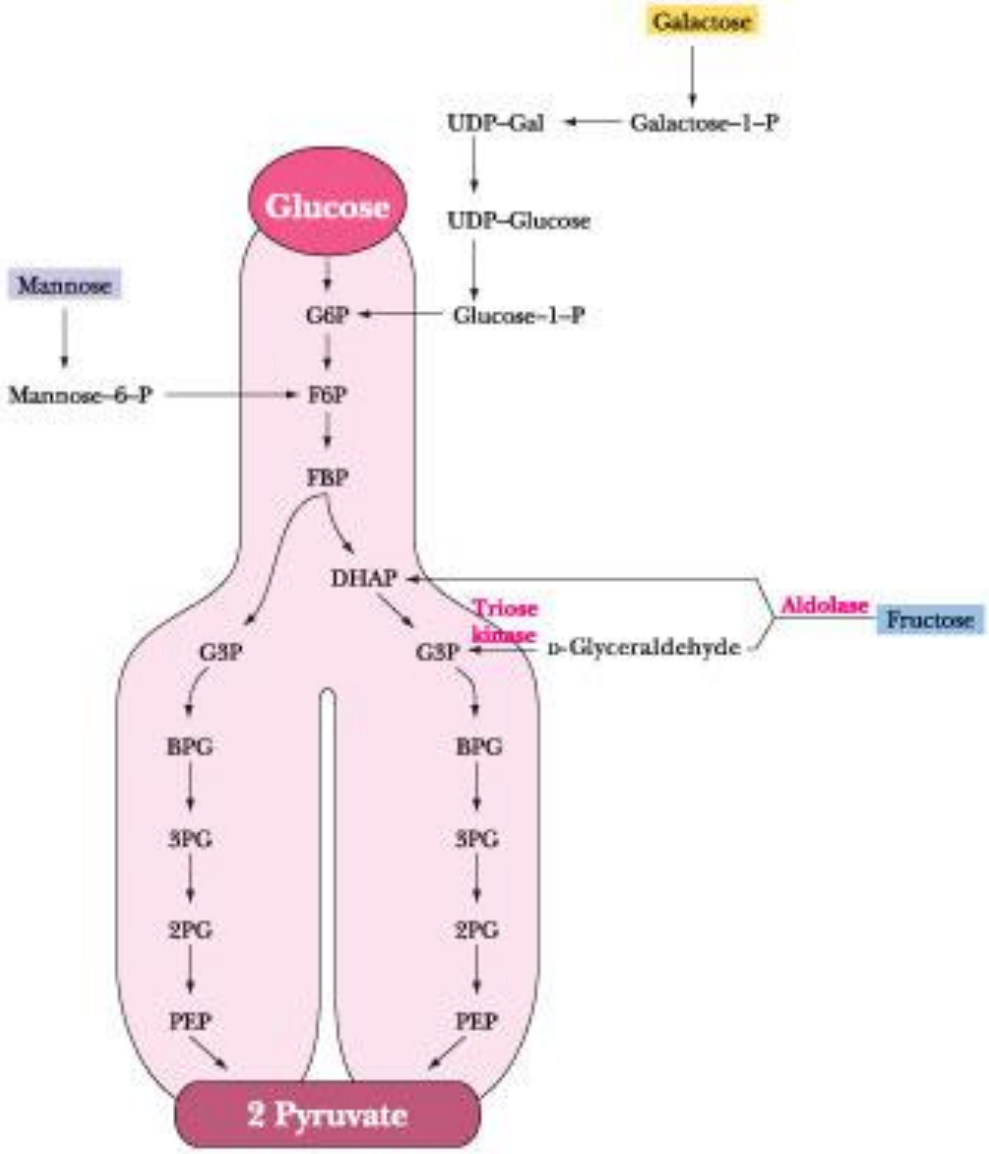
Mannose:

1. Mannose + ATP → Mannose-6-phosphate + ADP + H^+
2. Mannose-6-phosphate → F-6-P

Fructose:

Fructose + ATP → F-1-P → Glyceraldehyde + DHAP

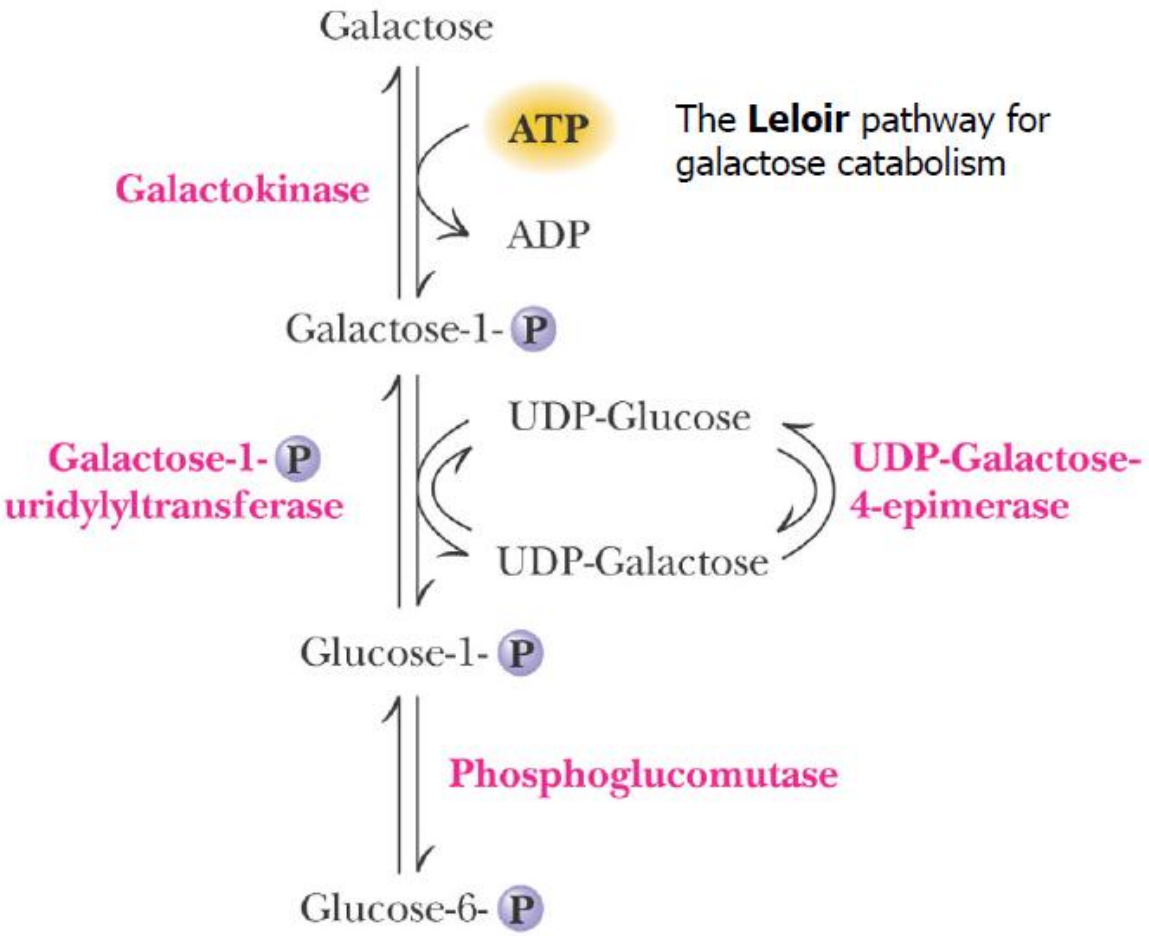
Fructose + ATP → F-6-P + ADP + H^+



Understand that other sugars can enter glycolysis (at different points) with galactose as an example…

Leloir Pathway:

Galactose + ATP → Galactose-1-phosphate + ADP + H^+



Know the sequence of reactions, the enzymes that catalyze them, and the substrates/products

(no structures necessary)

Example Problem 1

All are characteristics of the conversion of glucose to lactate EXCEPT:

A. Anaerobic pathway with no net oxidation
B. “primed” by ATP phosphorylation
C. Located in the cytosol
D. Approximately 50% efficient in erythrocytes
E. Net production of four ATP per glucose

Example Problem 2

For the first five steps of glycolysis, the appropriate sequence of enzymes is:

A. Phosphofructokinase (PFK)
B. Hexokinase/glucokinase
C. Fructose biphosphate aldolase
D. Phosphoglucoisomerase
E. Triose phosphate isomerase

Example Problem 3

All are important reasons to phosphorylate glucose in the first step of glycolysis EXCEPT:

A. The large positive energy is important in getting the pathway started.
B. Regulatory control can be imposed only at a reaction not at equilibrium.
C. Fructose phosphorylation keeps the glucose in the cell
D. The concentration of free glucose in the cell is lowered favoring influx of glucose.
E. Glucose-6-phosphate (G-6-P) has a negative charge preventing transport out of the cell.

Example Problem 4

Glucokinase has a K_m value of 10.0 mM, whereas hexokinase has a K_m value of 0.1 mM. This is consistent with which of the following?

A. Glucokinase acts on glucose only at high glucose concentrations.
B. Glucokinase acts on glucose only at low glucose concentrations.
C. Glucokinase phosphorylation of most of the glucose at low glucose levels.
D. Hexokinase acting on glucose at high levels of glucose.
E. Hexokinase acting at about half-maximal velocity at glucose concentrations of 4-5 mM.

Example Problem 5

All are true for the isomerase reaction of glucose-6-phosphate to fructose-6-phosphate EXCEPT:

A. The reaction is reversible with a slightly negative delta G.
B. It is an aldose to ketose isomerization.
C. “Moving” the carbonyl from C-1 to C-2 creates a new primary alcohol group at C-1.
D. The reaction is irreversible with a large negative delta G.
E. The enzyme belongs to the isomerase class of enzymes.

Example Problem 6

The step that commits glucose to glycolysis is catalyzed by:

A. Hexokinase/Glucokinase
B. Phosphoglucoisomerase
C. Phosphofructokinase (PFK)
D. Fructose bisphosphate adolase
E. None of the above

Example Problem 7

All are characteristics of the phosphofructokinase (PFK) catalyzed reaction EXCEPT:

A. Commits the cell to metabolize glucose
B. “Valve” controlling the rate of glycolysis
C. “Priming reaction”
D. Exergonic
E. All are true.

Example Problem 8

All are allosteric regulators of phosphofructokinase (PFK) EXCEPT:

A. AMP by activation
B. ATP by inhibition
C. Glucose-6-phosphate (G-6-P) by inhibition
D. Fructose-2,6-bisphosphate by activation
E. Citrate by inhibition
F. All are true.

Example Problem 9

Adenylate kinase catalyzes the reaction:

ADP + _____ → _____ + AMP

A. ATP; ADP
B. ADP; ATP
C. AMP; ATP
D. ATP; AMP
E. None of the above.

Example Problem 10

In the second half of the glycolytic pathway, _____ ATP molecules are produced and with the offset of _____ ATPs consumed in the first half, the net yield is _____ ATPs per molecule of glucose.

A. Two; zero; two
B. Two; one; one
C. Four; two; two
D. Four; zero; two
E. None of the above.

Example Problem 11

All of the following enzymes of glycolysis are allosterically regulated EXCEPT:

A. Hexokinase
B. Phosphofructokinase (PFK)
C. Fructose-1,6-bisphosphotase
D. Pyruvate carboxylase
E. Pyruvate kinase (PK)
F. Both C and D.
G. All of the above.

Questions?

Kirk's contact email: knh093020@utdallas.edu

Problem Set #3: Due Friday 11/11 at 5:00PM in FO 3.602

Exam #3: Monday 11/14 at 10:00AM in normal classroom

Kirk Huynh

Contact email:
knh093020@utdallas.edu

Exam 3 Review

Gluconeogenesis

Chapter 22.1-22.2

Understand why a pathway for glucose synthesis is required

- Pyruvate, lactate, most amino acids, glycerol and citric acid cycle intermediates can all be converted to glucose.
  - During exercise : pyruvate → lactate
  - Gluconeogenesis salvages the pyruvate and lactate and reconverts it back into glucose.

- If glucose is not obtained in diet, body must produce new glucose from non-carbohydrate precursors.

- Majors sites are in the liver (90%) and kidney (10%).

Know some of the important substrates for gluconeogenesis

- The substrates for gluconeogenesis include pyruvate, lactate, and amino acids.

- Other non-carbohydrate precursors:
  - Amino acids, glycerol, and TCA cycle intermediates
  - Animals: cannot convert fatty acids to glucose
    - Acetyl-CoA cannot by synthesized to sugars

Understand the reasons why gluconeogenesis cannot be the reverse of glycolysis

- Glycolysis is exergonic, so the reverse pathway cannot happen. The reverse of glycolysis would consume only 2 ATP and would be endergonic ($\Delta G^\circ = +74$ kJ/mol).
- Six nucleoside triphophates (4 ATP + 2 GTP) are hydrolyzed as 2 pyruvate are converted to glucose
  - exergonic, $\Delta G^\circ = -37.7$ kJ/mol
- Different enzymes are required to allow separate regulation of the two pathways (reciprocal regulation, compartmentalized).
- ATP consumed, NADH reoxidized to NAD^+

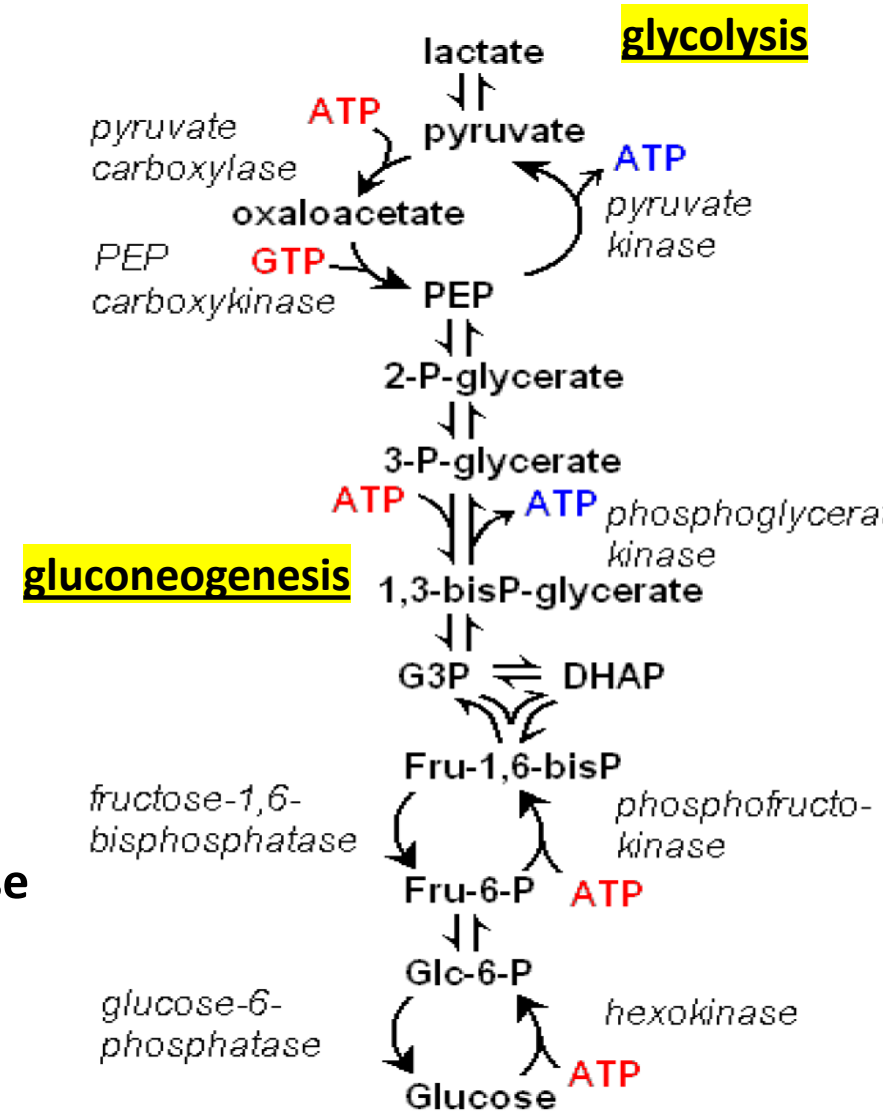
Understand the reasons why gluconeogenesis cannot be the reverse of glycolysis

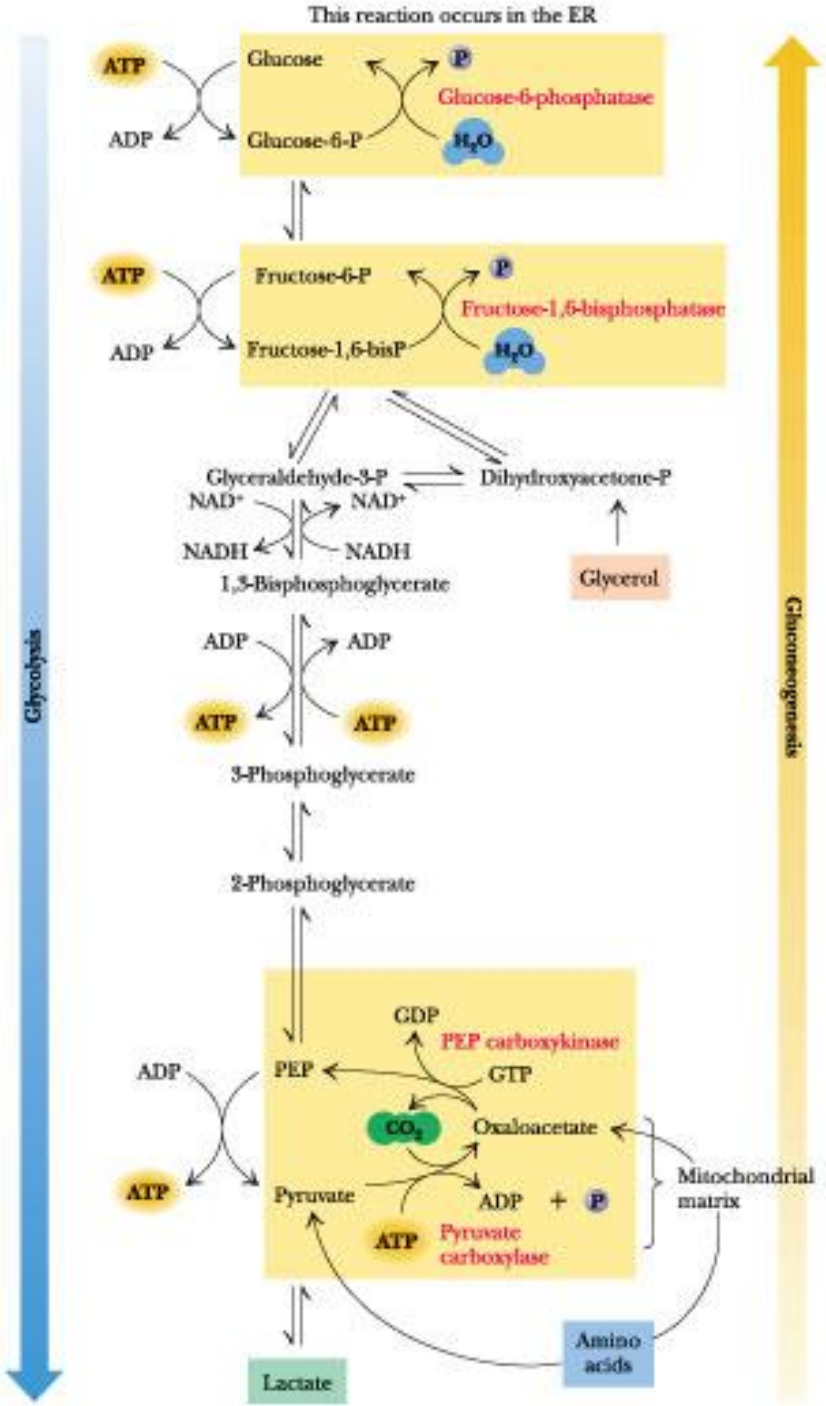
The three exergonic steps of glycolysis are:

- Reaction 1: **Hexokinase**
  - Phosphorylates glucose to G-6-P
- Reaction 3: **Phosphofructokinase** (PFK)
  - Phosphorylates F-6-P to FBP
- Reaction 10: **Pyruvate kinase** (PK)
  - Dehydrates 2-PG to PEP

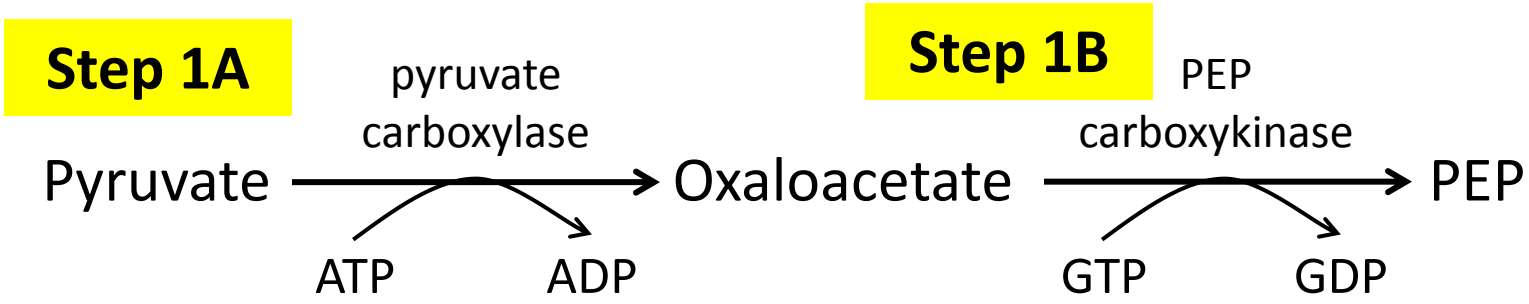
Replaced in gluconeogenesis…

- Reaction 1A: **Pyruvate carboxylase**
  - Converts pyruvate to oxaloacetate
- Reaction 1B: **PEP carboxykinase**
  - Catalyzes oxaloacetate to PEP
- Reaction 8: **Fructose-1,6-bisphosphatase**
  - Converts FBP to F-6-P
- Reaction 10: **Glucose-6-phosphatase**
  - Hydrolysis of G-6-P to glucose



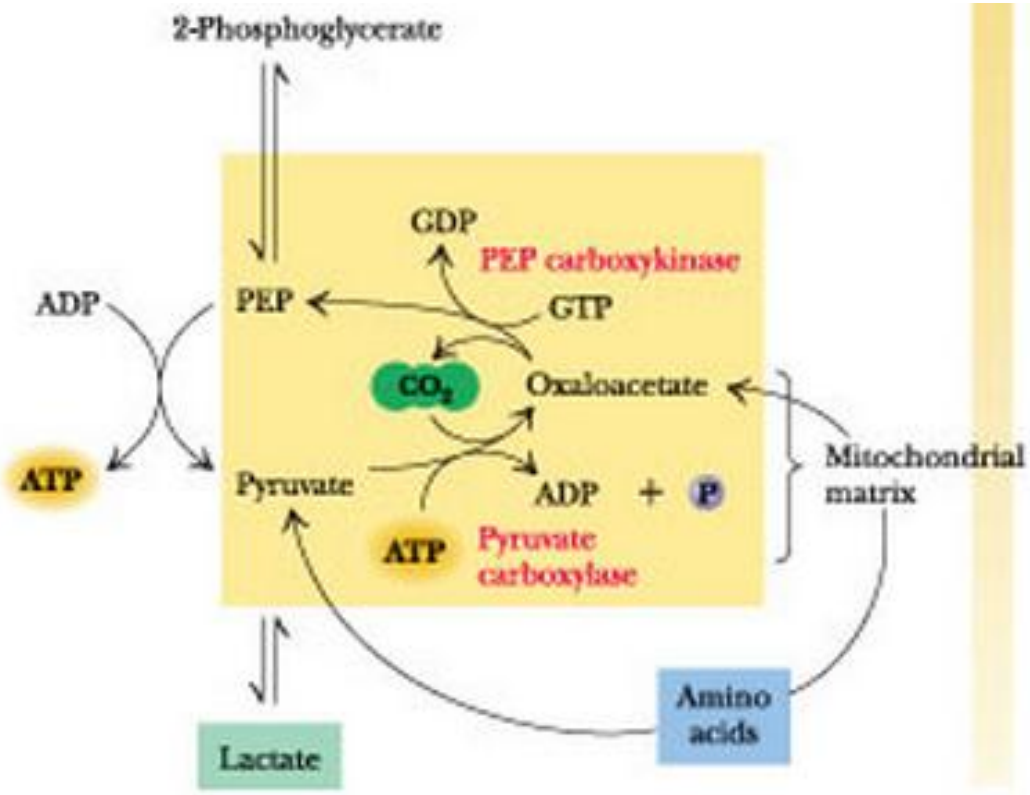

This reaction occurs in the ER
ATP
ADP
Glucose
Glucose-6-P
P
Glucose-6-phosphatase
H_2O
ATP
ADP
Fructose-6-P
Fructose-1,6-bisP
P
Fructose-1,6-bisphosphatase
H_2O
Glyceraldehyde-3-P
Dihydroxyacetone-P
NAD^+
NADH
NAD^+
NADH
1,3-Bisphosphoglycerate
Glycerol
ADP
ATP
ADP
ATP
3-Phosphoglycerate
2-Phosphoglycerate
GDP
PEP carboxykinase
ADP
PEP
GTP
CO_2
Oxaloacetate
ATP
Pyruvate
ADP + P
ATP
Pyruvate carboxylase
Mitochondrial matrix
Amino acids
Lactate
Glycolysis
Gluconeogenesis

Pyruvate carboxylase and PEP caryboxykinase convert pyruvate to PEP, replacing the pyruvate kinase reaction of glycolysis

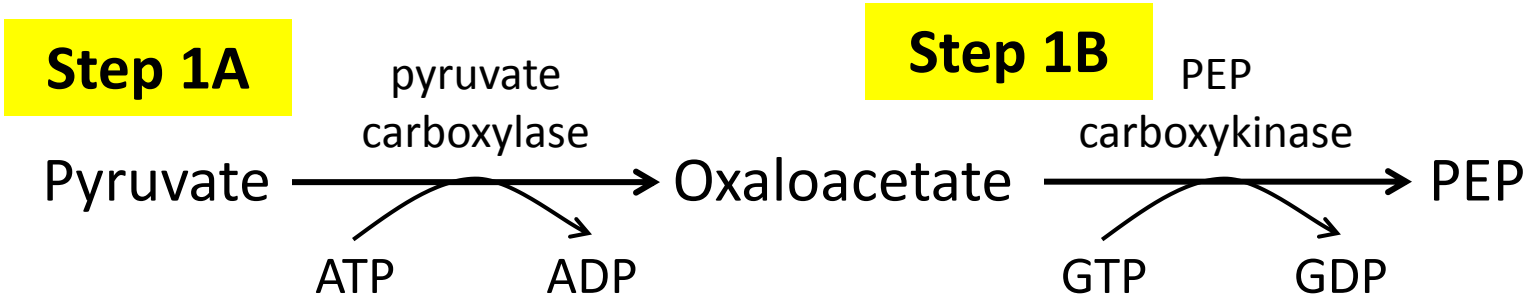


Pyruvate carboxylase converts pyruvate to oxaloacetate.

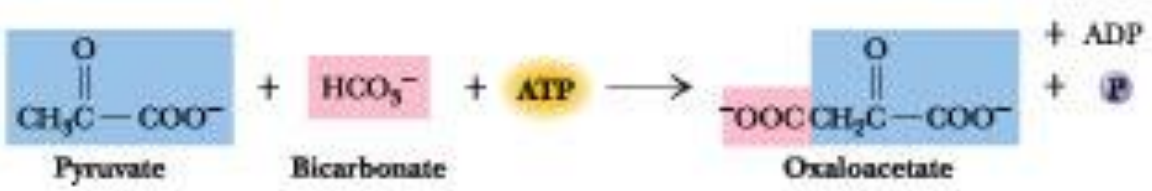
PEP carboxykinase converts oxaloacetate to PEP.



Pyruvate carboxylase and PEP caryboxykinase convert pyruvate to PEP, replacing the pyruvate kinase reaction of glycolysis



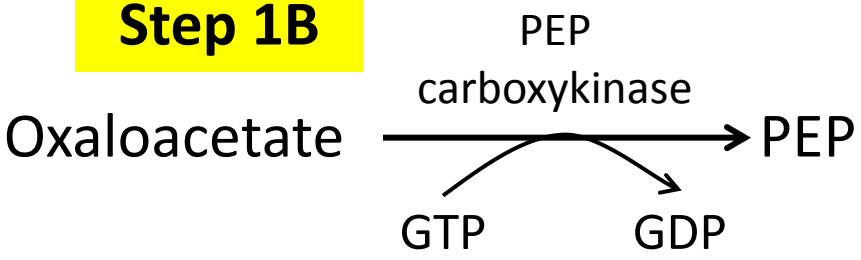
Pyruvate carboxylase converts pyruvate to oxaloacetate. ATP activates bicarbonate group and transfers to biotin.



PEP carboxykinase converts oxaloacetate to PEP.

Decarboxylation of oxaloacetate provides the driving force, two high energy NTPs are consumed

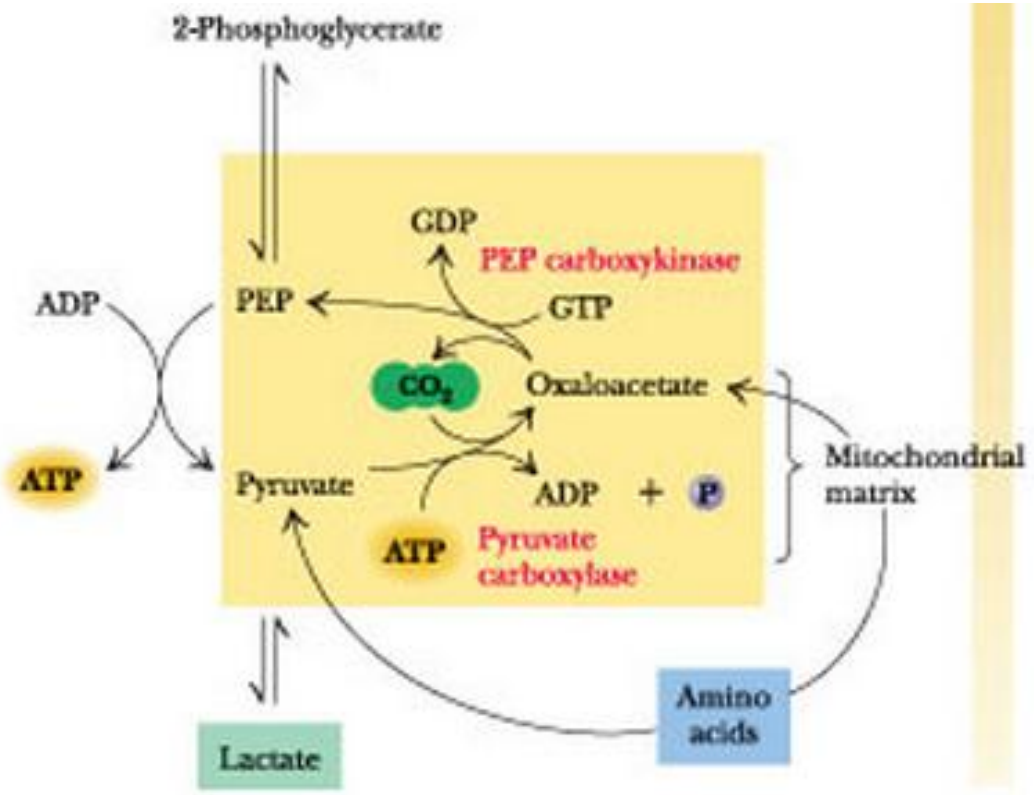
Step 1B



Energetic requirements (2 steps):

1. The CO_2 added to pyruvate in step 1A is removed in step 1B – called a decarboxylation
2. The decarboxylation drives formation of high-energy PEP

ΔG°' = -22.6 kJ/mol

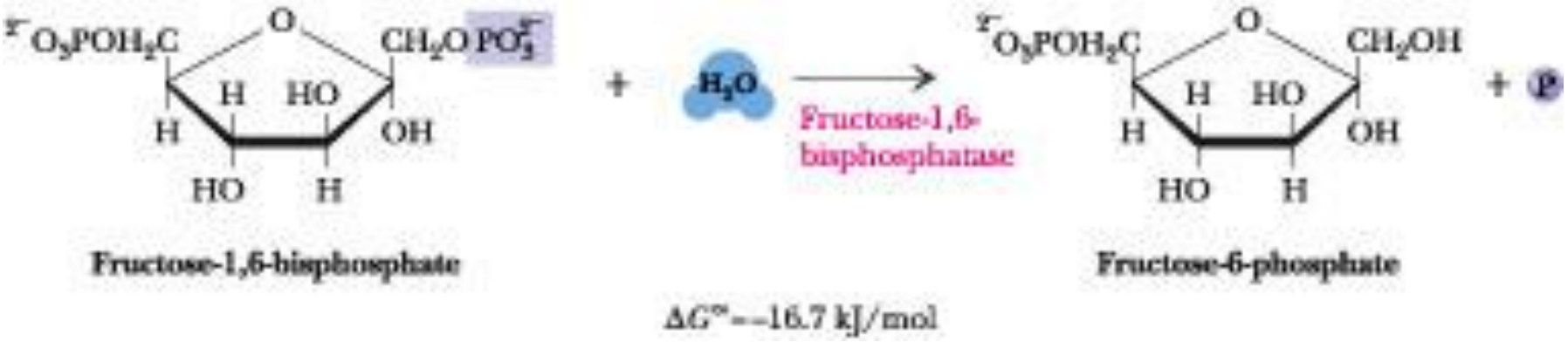


Fructose-1,6-bisphosphatase converts fructose-1,6-bisphosphate to fructorse-6-phosphate

~ Reaction 8 ~

- **Fructose-1,6-bisphosphatase** hydrolyzes FBP (fructose-1,6-bisphosphate) to F-6-P (fructose-6-phosphate).
- Thermodynamically favorable (exergonic, **ΔG°' = -16.7 kJ/mol**)

- Allosteric enzyme regulation:
  - Activator: citrate
  - Inhibitor: fructose-2,6-bisphospate

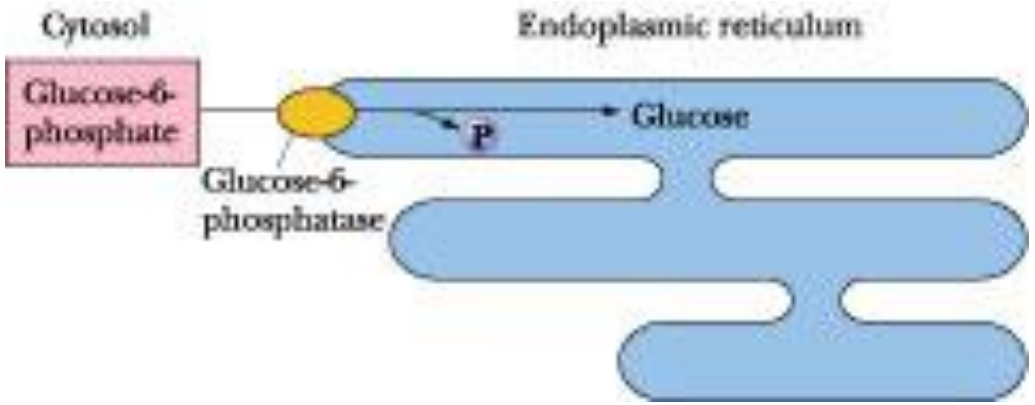


Glucose-6-phosphatase converts glucose-6-phosphate to glucose; enzyme is associated with the endoplasmic reticulum (why?)

~ Reaction 10 ~

- **Glucose-6-phosphatase** converts G-6-P (glucose-6-phosphate) to glucose
- Present in the endoplasmic reticulum (ER) of liver and kidney cells but absent in muscle and brain
- Involves G6Pase enzyme itself and three transport proteins (T1, T2, T3)
  - T1: hydrolyzes G-6-P to glucose and P_i
  - T2/T3: export to cytosol
  - Glucose then exported to circulation

ΔG°' = -5.1 kJ/mol



Gluconeogenesis consumes 4 ATP and 2 GTP and 2 NADH for each glucose molecule synthesized

Coupling with Hydrolysis of ATP and GTP Drives Gluconeogenesis

- Gluconeogenesis
  - 2 Pyruvate + 4 ATP + 2 GTP + 2 NADH + 2 H^+ + 6 H_2O → glucose + 4 ADP + 2 GDP + 6 P_i + 2 NAD^+
- Net free energy change: $\Delta G^{\circ\prime}$ = -37.7 kJ/mol
- Net result of gluconeogenesis:
  - 4 ATP consumed
  - 2 GTP consumed
  - 2 NADH consumed

Understand the Cori cycle for recycling lactate in liver…

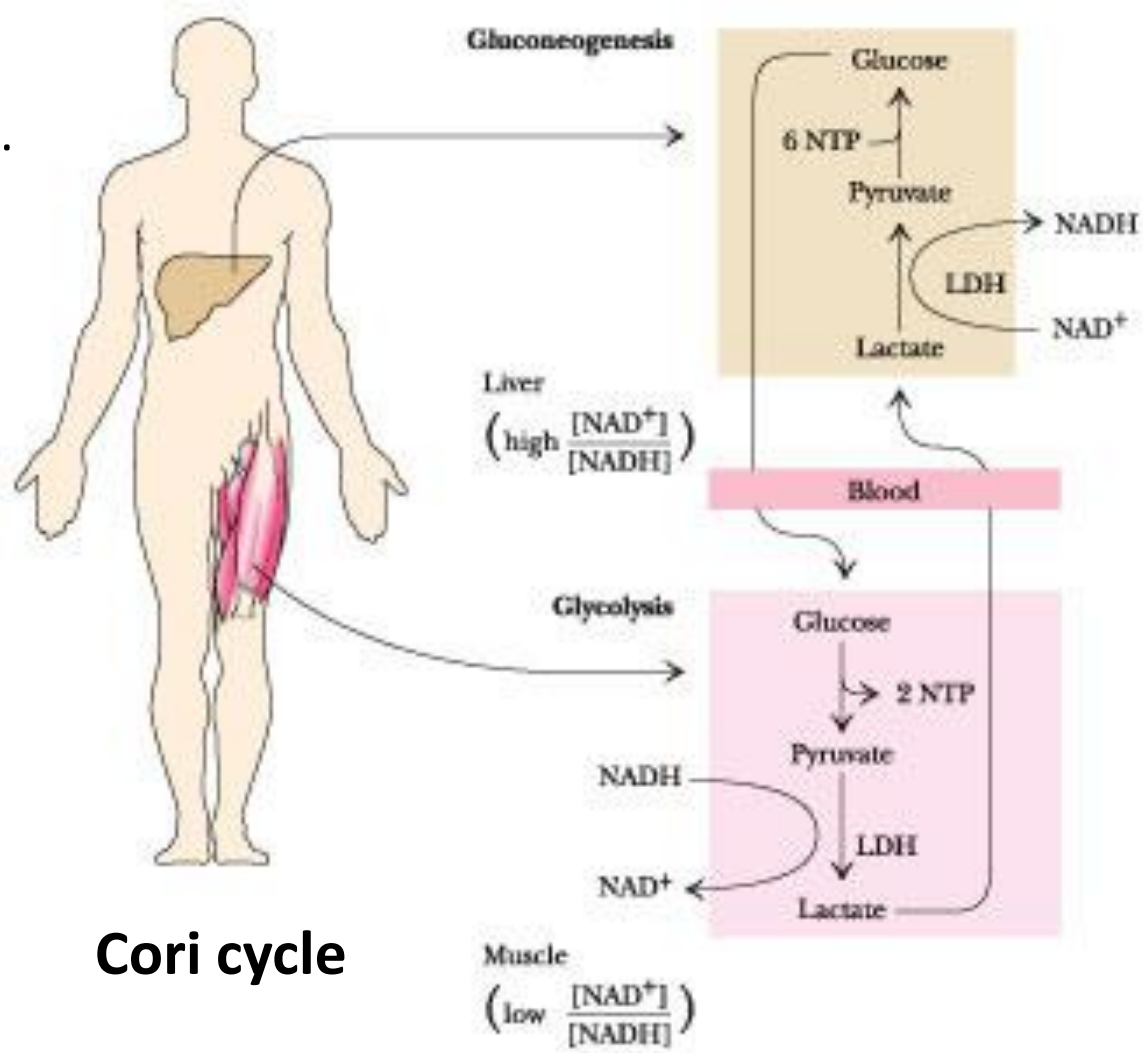
<u>Lactate Formed in Muscles Is Recycled to Glucose in the Liver</u>

Glycolysis converts NAD^+ to NADH.

Oxygen deficiency during exercise causes formation of lactate coupled with large reoxidation of NADH (no O_2 available for cellular respiration).

Lactate is transported to liver where it is reoxidized to yield pyruvate via LDH.

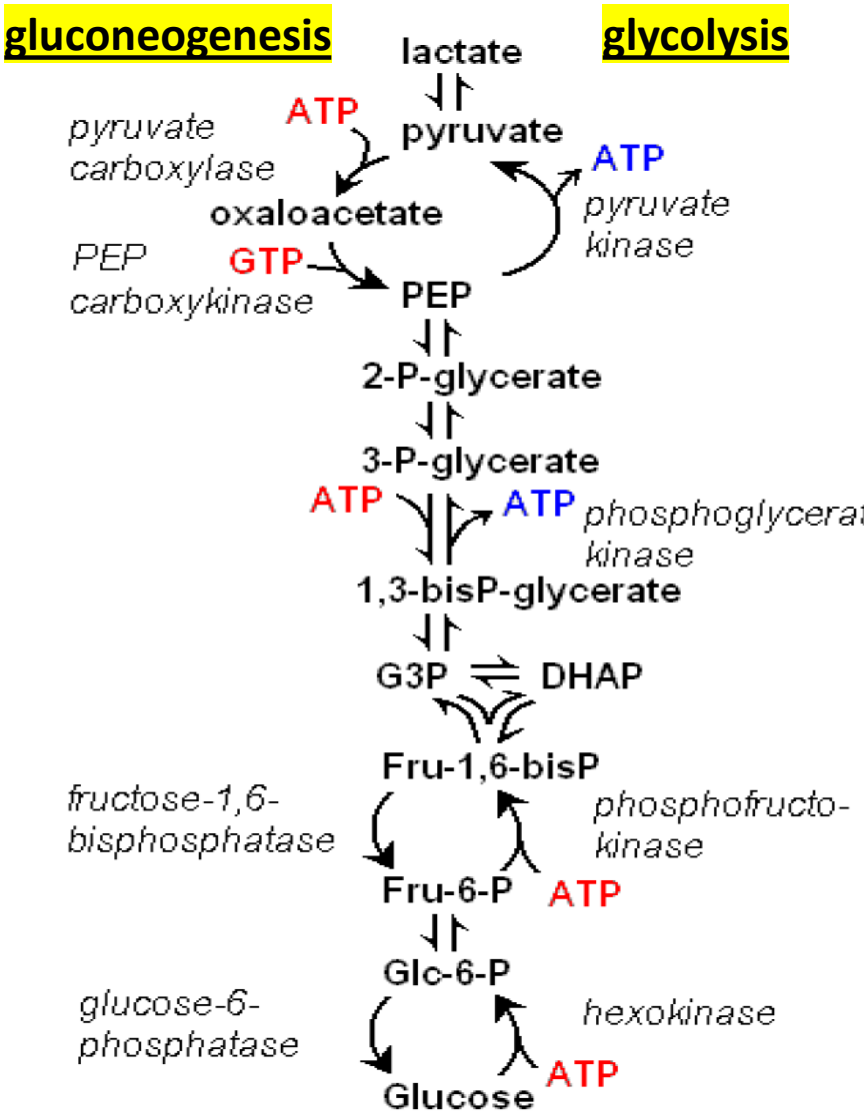
Metabolic burden of exercise on the muscles and liver.

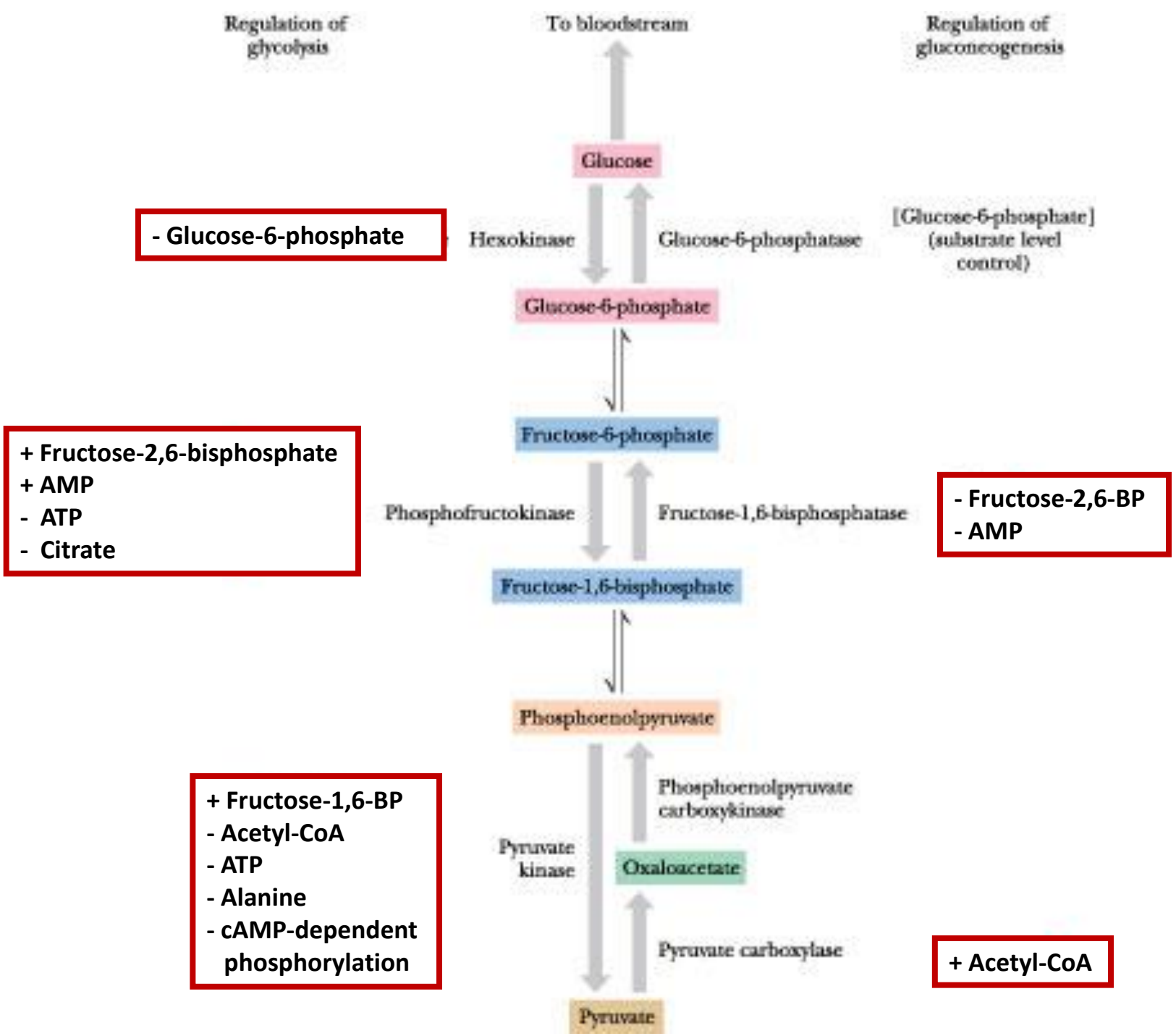


Cori cycle

Understand that glycolysis and gluconeogenesis are subject to reciprocal regulation

- Glycolysis and gluconeogenesis are subject to reciprocal control, such that one is inhibited when the other is active.
  - Gluconeogenesis synthesizes glucose and glycolysis breaks it down.
- Without this control, there would be a futile cycle and massive ATP consumption.
- **Low energy state**: glucose degraded to provide ATP (glycolysis)
- **High energy status**: pyruvate synthesized to glucose and glycogen (gluconeogenesis)




Regulation of glycolysis
To bloodstream
Regulation of gluconeogenesis
Glucose
- Glucose-6-phosphate
Hexokinase
Glucose-6-phosphatase
[Glucose-6-phosphate] (substrate level control)
Glucose-6-phosphate
Fructose-6-phosphate
+ Fructose-2,6-bisphosphate
+ AMP
- ATP
- Citrate
Phosphofructokinase
Fructose-1,6-bisphosphatase
- Fructose-2,6-BP
- AMP
Fructose-1,6-bisphosphate
Phosphoenolpyruvate
Phosphoenolpyruvate carboxykinase
+ Fructose-1,6-BP
- Acetyl-CoA
- ATP
- Alanine
- cAMP-dependent phosphorylation
Pyruvate kinase
Oxaloacetate
Pyruvate carboxylase
+ Acetyl-CoA
Pyruvate

Example Problem 1

Gluconeogenesis is the synthesis of:

A. Glucose from fatty acids
B. Fatty acids from glucose
C. Glycogen from glucose
D. Glucose from non-carbohydrate precursors
E. Pyruvate from glucose

Example Problem 2

All are substrates for gluconeogenesis EXCEPT:

A. Glycerol
B. Acetate
C. Pyruvate
D. Lactate
E. Most amino acids

Example Problem 3

The major tissues carrying out gluconeogenesis are the _____ and _____.

A. Brain; muscles
B. Muscles; kidneys
C. Liver; kidneys
D. Liver; red blood cells
E. Red blood cells; brain

Example Problem 4

Seven of the ten reactions of glycolysis are reversible (Delta G near zero) and can be used in reverse of glycolysis for gluconeogenesis. The 3 irreversible reactions are catalyzed by:

A. Hexokinase/Glucokinase
B. Phosphofructokinase (PFK)
C. Phosphoglycerate kinase
D. Fructose-1,6-bisphosphatase
E. Pyruvate kinase (PK)
F. A, B, and E
G. A, C, and D
H. All of the above

Example Problem 5

Which of the following reactions represents the gluconeogenic reversal of PFK in glycolysis?

A. Fructose-6-P + ADP → fructose + ATP
B. Fructose-6-P + ATP → fructose-1,6-bisphosphate + ADP
C. Fructose-6-P + H_20 → fructose + P_i
D. Fructose-1,6-bisphosphate + ADP → fructose-6-P + ATP
E. Fructose-1,6-bisphosphate + ATP → fructose-6-P + ADP
F. Fructose-1,6-bisphosphate + H_20 → fructose-6-P + P_i
G. None of the above

Example Problem 6

The endoplasmic reticulum (ER) bound enzyme that hydrolyzes glucose-6-phosphate to glucose in liver is:

A. Glucokinase
B. Glucose oxidase
C. Fructose-1,6-bisphosphotase
D. Phosphoglucomutase
E. Glucose-6-phosphatase
F. Phosphoglycerate kinase
G. None of the above

Example Problem 7

All of the following enzymes are unique to gluconeogenesis EXCEPT:

A. Fructose-1,6-bisphosphatase
B. PEP carboxykinase
C. Phosphoglycerate kinase
D. Pyruvate carboxylase
E. Phosphoglucoisomerase
F. Glucose-6-phosphatase
G. Both C and E
H. Both E and F
I. None of the above

Example Problem 8

All of the enzymes of gluconeogenesis may be found in the cytosol/ER EXCEPT _____ which is only found in the mitochondria.

A. PEP carboxykinase
B. Pyruvate carboxylase
C. Phosphoglycerate kinase
D. Glucose-6-phosphatase
E. Fructose-1,6-bisphophatase
F. All are found in the cytosol.

Example Problem 9

In the Cori cycle, the liver _____ because it has typically high _____.

A. Shares the load of exercising muscle; ADP/ATP ratio
B. Converts lactate to pyruvate; $NADH/NAD^+$ ratio
C. Shares the load of exercising muscle; $NAD^+/NADH$ ratio
D. Burns fat; ATP
E. Converts glucose to lactate; $NAD^+/NADH$ ratio
F. None of the above

Questions?

Kirk's contact email: knh093020@utdallas.edu

Problem Set #3: Due Friday 11/11 at 5:00PM in FO 3.602

Exam #3: Monday 11/14 at 10:00AM in normal classroom