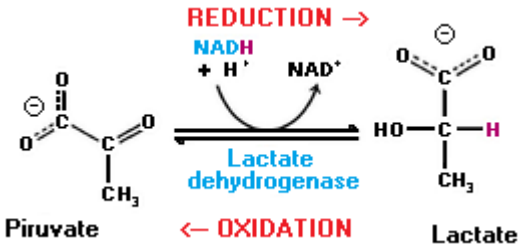


What is the difference between oxidation and reduction? What are they synonymous to in biological systems?

- Oxidation - describes the loss of an electron - either decreases the H content or increases the O content (methane can be oxidized)
- Reduction - describes the gain of an electron - either increases the H content or decreases the O content (CO_2 can be reduced).

In biological systems, oxidation is often synonymous with dehydrogenation and reduction with hydrogenation

Which ways are electrons transferred in biochemical reactions?

1) They are transferred as H atoms (1 proton (H+) + 1 electron)
2) They are transferred as a hydride ion :H- (transfer of a proton and 2 electrons).

How do NAD+/NADH, NADP/NADPH, FAD and FADH serve as electron carriers?

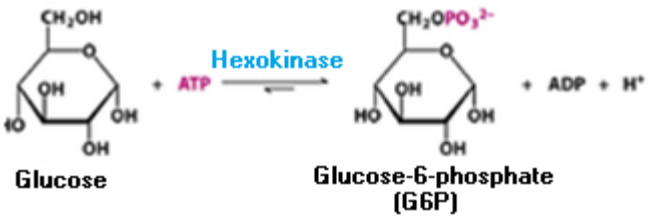
They are cofactors and they serve as electron carriers:

1) NAD+/NADH - electron transfers in catabolic reactions (fuel oxidation)
2) NADP/NADPH - electron transfers in anabolic reaction
3) FAD/FADH2 - electron transfers in catabolic reactions (fuel oxidation). Accepts 1 or 2 electrons in the form of 1 or 2 hydrogen atoms

What are the implications of free energy release upon oxidation (why are reduced carbons a form of stored energy?). Compare energy storage for fatty acids and glucose.

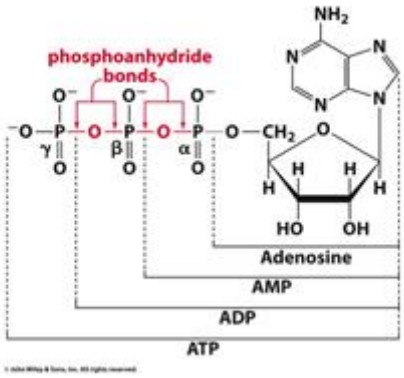
The reduction of CO_2 to more reduced forms requires energy input.
Therefore, reduced carbons represent a form of stored free energy. The more reduced a carbon is, the more energy from its oxidation.
Fatty acids are more reduced than glucose so fatty acids release more free energy.

- Most reduced state = most energy.
- Most oxidized state = least energy.



How does phosphorylation affect glucose in terms of cellular location? What is the role of hexokinase?

Hexokinase transfers a phosphoryl group to glucose.
This group traps glucose in the cell. The negative electric charges prevent G6P to cross the cell membrane



Why is ATP a high energy compound? Why are the products of ATP hydrolysis more stable than the reactants?

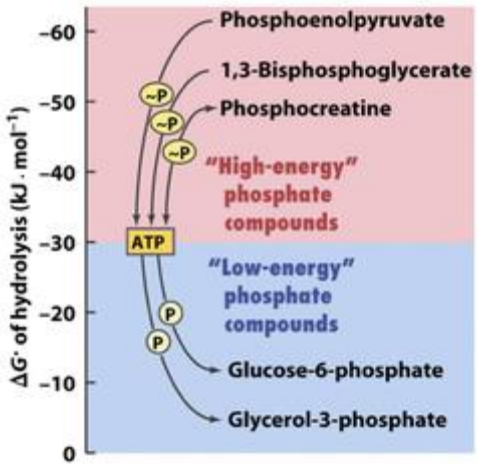
Phosphoryl group transfers from ATP are catalyzed by kinases.
A Phosphoryl group transfer involves the cleavage of the phospho-anhydride bond.
This cleavage results in a large release of free energy because the products are more stable than the reactants in many ways:

1) Phosphoryl groups of ATP have less resonance stabilization than those in the hydrolysis products.
2) ATP has four negative charges that repel each other. Removal of one phosphate group to produce ADP allows the charges to separate and relieve the repulsion
3) Products of hydrolysis (Pi and ADP) have greater solvation (hydration) relative to ATP – That is, they can interact more with water, making more bond and stabilizing themselves that way.
4) Products of hydrolysis have a higher entropic content than ATP. They have more degrees of freedom.

	$\Delta G'^{\circ}$
Glucose + Pi ⟶ Glucose 6- Phosphate + H_2O	+13.8 kJ/mole
ATP + H_2O ⟶ ADP + Pi	- 30.k kJ/mole
Glucose + ATP ⟶ Glucose 6- Phosphate + ADP	-16.7 kJ/mole

What does it mean when we say: "coupling to ATP hydrolysis can drive unfavourable reactions"?

It means that ΔG of ATP hydrolysis (negative) added to the unfavourable ΔG reaction (positive) gives a favourable total ΔG (negative).
I.e.: the summation of the phosphorylation of glucose and the hydrolysis of ATP results in an exergonic reaction. Small endergonic + high exergonic is exergonic.

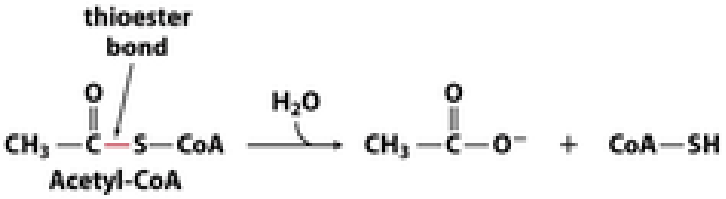


Why is ATP the universal energy currency?

ATP can receive energy from higher energy phosphate compounds (taking a Pi group from them) and carry it to lower energy phosphate compounds (giving the Pi group to them).

What is the difference between substrate level phosphorylation and oxidative phosphorylation.

- Substrate level phosphorylation is a direct transfer of P from a substrate to ATP. It is catalyzed by kinases.
- O.P is an indirect ATP generation using energy supplied by trans-membrane proton gradients in the electron transport chain. It is catalysed by ATP synthase.
-



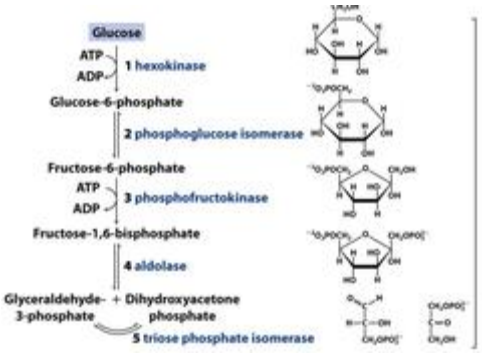
What are other important high energy group transfers?

The other important energy group transfer is the thioester bond of acyl-CoA (in Acetyl CoA and Succinyl CoA).
Coenzyme A is linked by a thioester bond to a carbonyl group with his sulfhydryl residue (SH).
Hydrolysis of the thioester bond is exergonic - products have more resonance stability than reactants.

What are the general energy characteristics of Stage 1 and Stage 2 of glycolysis? What is the energy yield of each stage?

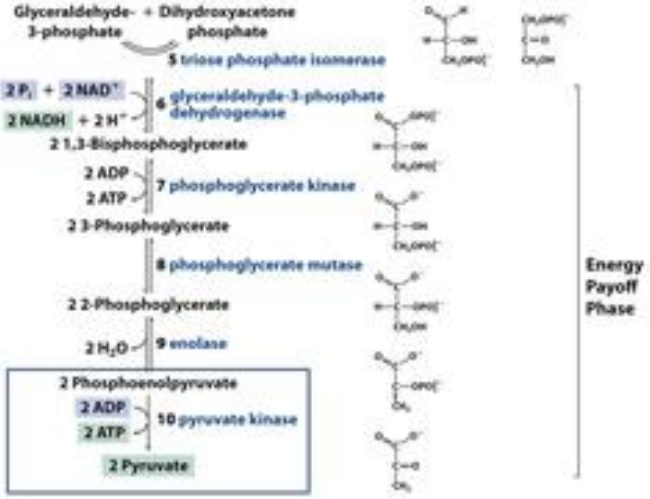
- Stage 1 - energy investment.
  Reactions 1-5. Glucose is phosphorylated and cleaved in half. 2 ATP are consumed.
- Stage 2 - energy payoff
  Reactions 6-10. Two pyruvate molecules are generated, 4 ATP are produced.

Net yield of glycolysis : 2 ATP.



What are the steps of glycolysis first stage?

1) ATP dependent phosphorylation of glucose - traps glucose - hexokinase. Glucose to glucose-6-phosphate.
2) Isomerization of glucose 6 phosphate to fructose 6 phosphate - phosphoglucose isomerase.
3) ATP dependent phosphorylation of F6-P to F1,6 Bisphosphate by phosphofructokinase.
4) Cleavage to yield 2 3-carbon molecules each with a phosphate - glyceraldehyde-3-phosphate + Dihydroxyacetone phosphate. Aldolase and Triose phosphate isomerase
5) Isomerization of DHAP and G3P via triose phosphate isomerase.

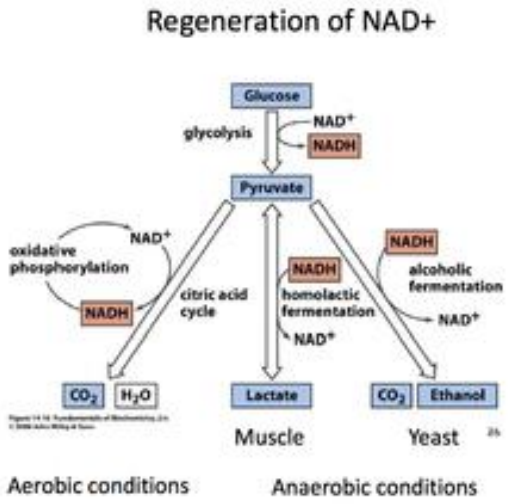


What happens during the 2nd stage of glycolysis?

Two molecules of glyceraldehyde-3-phosphate are converted to two molecules of pyruvate.
This means that for every molecule of glucose, glycolysis gives two pyruvates.

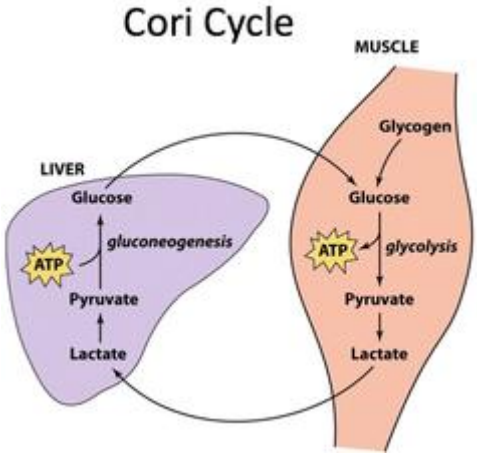
What are the products of glycolysis?

- ATP
  Investment of two ATP, generation of 4 ATP by substrate level phosphorylation, net of 2 ATP per molecule of glucose.
- NADH
  Glucose is oxidized reducing 2 NAD+ to 2 NADH. NADH must be oxidized to regenerate NAD+. Re-oxidation of NADH occurs through aerobic and anaerobic conditions.
- Two pyruvate molecules are produced per glucose molecule
  In aerobic condition: they are further oxidized to CO_2 and H_2O via citric acid cycle and produce more ATP.
  In anaerobic condition: they are metabolized to regenerate NAD+ in different ways (ex. Lactic fermentation, ethylic fermentation).



How is NAD+ regenerated in aerobic conditions? And in active muscle under anaerobic conditions?

- Aerobic: NADH is oxidized back to NAD+ in the Electron Transport Chain of Oxidative Phosphorylation.
  -Anaerobic: re-oxidation of NADH to NAD+ occurs in the muscle when pyruvate is converted to lactate.

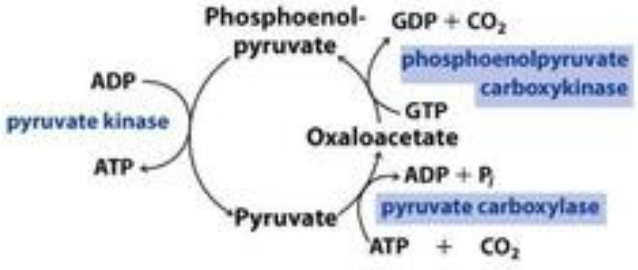


What is the Cori Cycle?

In the Cori cycle, lactate in the muscle is taken to the liver to be converted to glucose via gluconeogenesis, which requires ATP. The liver can then send the newly formed glucose back to the muscle to undergo glycolysis, which produces ATP.

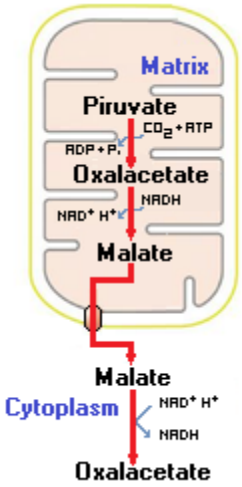
Where does gluconeogenesis occur? Which are the important enzymes involved in it? Which are the precursors of gluconeogenesis?

1 - In the liver and kidney
2 - Four unique enzymes are involved in gluconeogenesis. All the other enzymes are the same as in glycolytic steps.
Cytosolic enzymes are glucose-6-phosphatase and fructose-bisphosphatase.
Mitochondrial enzymes are phosphoenolpyruvate carboxykinase and pyruvate carboxylase
3 - Precursors for gluconeogenesis can come from amino acids, lactate, glycerol.



Describe the energy used for reactions that require PC (pyruvate carboxylase) and PEPCK (phosphoenolpyruvate carboxykinase). What is the function of the mitochondrial enzyme PC?

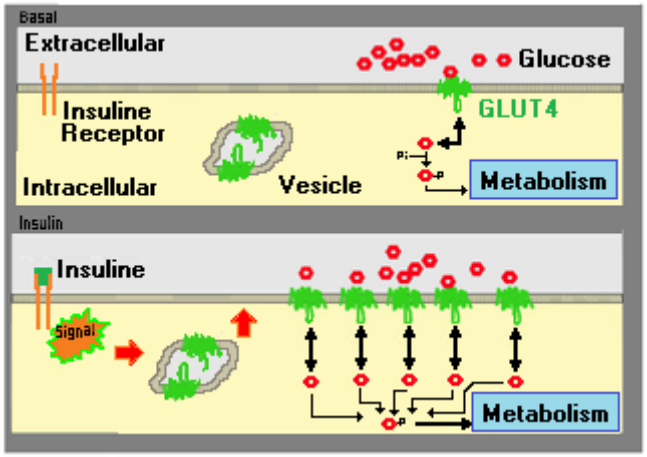
- Reactions of PC and PEPCK are energetically costly. Energy used is necessary to reverse the highly exergonic reaction catalysed by pyruvate kinase.
- Pyruvate carboxylase is a mitochondrial enzyme that takes part in gluconeogenesis by producing oxaloacetate. Since gluconeogenisis occurs in the cytosol, oxaloacetate must be transported from the mitrochondria to the cytosol.
- After PC produces OAA, OAA is then reduced to malate by mitochondrial malate dehydrogenase. Malate exits the mitrochondria through a transporter and is oxidized to OAA producing NADH.



Explain the reason for oxaloacetate is formed in the mitochondria but is then reformed in the cytosol. Which enzyme is responsible for forming oxaloacetate in the mitochondria?

The oxidation of malate to OAA in the cytosol generates NADH needed for gluconeogeneis. This process transfers reducing equivalents to the cytosol.

Enzyme missing



How is glucose transport regulated into the cell, in muscle and in adipose tissue? How does this differ in the liver and the brain?

A passive glucose transporter (GLUT 4) imports glucose into the cells.
Elevated blood glucose and insulin levels lead to an increased number of GLUT4 transporters at the plasma membrane.
Absence of insulin - transporters are sequestered within the cell. Presence of insulin - GLUT4 moves to cell surface.
Liver and brain have insulin insensitive transporters

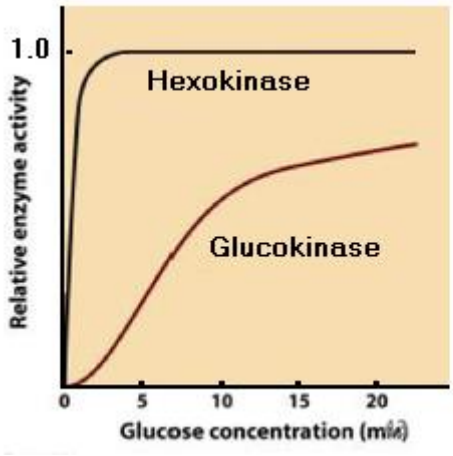
Which steps in glycolysis and gluconeogenesis are regulated?

Regulated steps are those whose reactions have largely negative ΔG.
Regulation also occurs where there is phosphorylation.
Regulated steps in glycolysis and gluconeogenesis are steps n° 1,3 and 10.

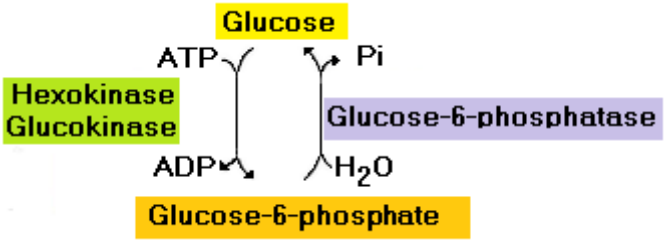
What is the first committed step in glycolysis in the liver? Why?

- Fructose 6 phosphate to fructose 1,6 bisphosphate is the first committed step because it is a far-equilibrium reaction, meaning it is largely irreversible as $\Delta G \ll 0$.
- Hexokinase converting glucose to glucose-6-phosphate is NOT the first committed step because G6P can follow different pathways.

Describe the differences in the kinetics between hexokinase and glucokinase.

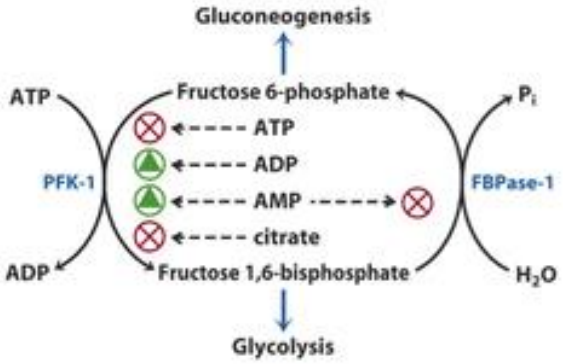


- Hexokinases are present in the muscle and have a very high affinity for glucose (Km=0.1 mM). This permits the initiation of glycolysis even when glucose is low. It is allosterically inhibited by its product, glucose 6 phosphate. This prevents the consumption of too much ATP when glucose concentrations are not limiting.
- Glucokinase is present in liver. It has a low affinity for glucose (Km of 10 mM). Its Km is higher than the concentration of blood glucose so it is rarely saturated. It is directly regulated by blood glucose. Glucokinase partakes in metabolism at high glucose levels but not necessarily at Low glucose levels. This allows glucose from gluconeogenesis to leave the liver before being phosphorylated. This process is further regulated by transcription of glucokinase.



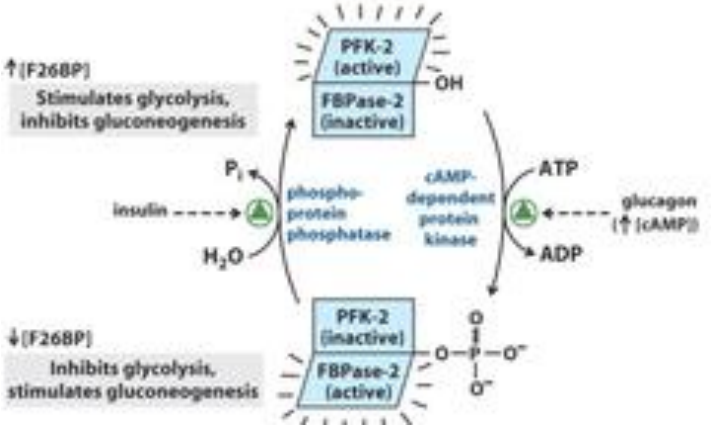
Describe the transcriptional regulation of glucokinase and glucose 6- phosphatase

When there is high blood glucose, there is increased transcription of glucokinase.
When there is low blood glucose, there is increase transcription of glucose-6-phosphatase in order to raise blood glucose levels.



Understand the various energy state signals (example, high ATP/ADP ratio) and how they affect overall metabolic pathways.

- High ATP concentration signals that ATP is being produced faster than it is being consumed. There is a decreased need for glycolysis and an increased need for glucose storage
- High citrate levels, an intermediate in TCA cycle, indicates that energy state is high and biosynthetic intermediates are abundant. Need for glycolysis decreases.
- Low ATP level (high AMP or ADP) signals that consumption of ATP outpaces production. There is an increased need for glycolysis, slowing of gluconeogenesis which requires ATP.



What is the role of fructose 2,6 bisphosphate in the allosteric and hormonal regulation of phosphofructokinase-1 and fructose bisphosphatase-1? What are its effects, where does it come from, and how are levels regulated?

- F-2,6-BP activates enzyme PFK1 (binds to allosteric site and increases its affinity for its substrate (Fructose-6-phosphate)
- F-2,6-BP inactivates Fructose-BisPhosphatase 1 and decreases its affinity for its substrate F-1,6-BP.

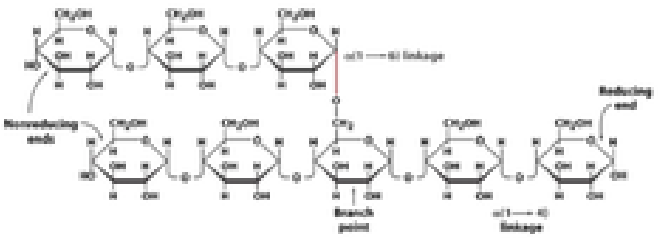
So an increased amount of F-2,6-BP stimulates glycolysis and inhibits gluconeogenesis.
Whereas its absence inhibits glycolysis and stimulates gluconeogeneis.

This is because PFK-2/FBPase 2 are one bifunctional enzyme with two separate activities.
Hormonal regulation of PFK-2/FBPase-2 is due to glucagon and insulin.
Glucagone → stimulates phosphorylation of PFK-2/FBPase-2 → Activation of FBPase-2 activity (phosphatase) → Reduction of F-2,6-BP → Decreases glycolysis and increased gluconeogeneis.
Insulin stimulates dephosphorylation of PFK-2/FBPase 2 → Activation of PFK2 activity (kinase) → increase of F-2,6-BP → Increased glycolysis and decreased gluconeogenesis.

What's the deal with high fructose corn syrup. Why is it so fattening?

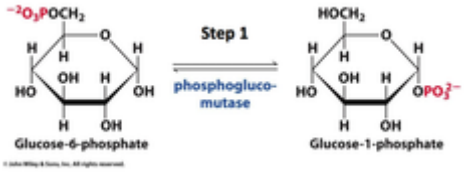
It bypasses a major regulatory step in glycolysis, entering at step 5. This disrupts fuel metabolism and increases productions of lipids since DAHP can be converted to glycerol 3 phosphate and then into triacylglycerols.

Describe the general structure of glycogen and why branches are important



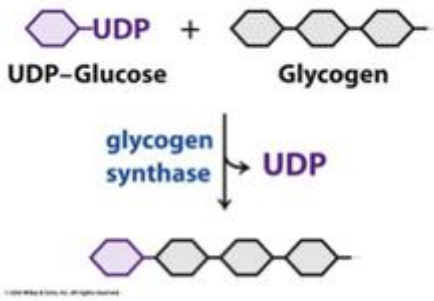
- Found, primarily in liver and muscle, some in kidney. Not in brain.
- Polymer of alpha (1-4) linked D glucose with alpha (1-6) linked branches every 8-14 residues.
- Only 1 reducing end but many non-reducing ends - 1 on every branch.
- Glucose is removed from non-reducing ends – Branching allows simultaneous release of glucose.

What is the precursor for glycogen synthesis?
How is the precusor activated to partake in gluconeogenesis?



The precursor to glycogen is glucose-6-phosphate, which is a key metabolite with several different fates in cell. In the glicogenesis pathway, it is activated with UTP giving UDP-glucose.
The released PPi is then hydrolyzed to 2 Pi. This is highly exergonic, which drives the reaction forward.

What is the role of glycogen synthase in the synthesis of Glycogen?



It adds UDP-glucose to glycogen chain.
Then a separate branching enzyme cleaves off a segment and reattaches it to a glucose C6-OH group to generate an alpha (1-6) branch point.

What is the role of glycogen phosphorylase in the breakdown of glycogen? How do you break it down?

It adds a phosphate group to glycogen to make it glucose 1 phosphate thus committing it to become glucose.

Understand the organ-specific expression of glucose-6-phosphatase in glycogen breakdown

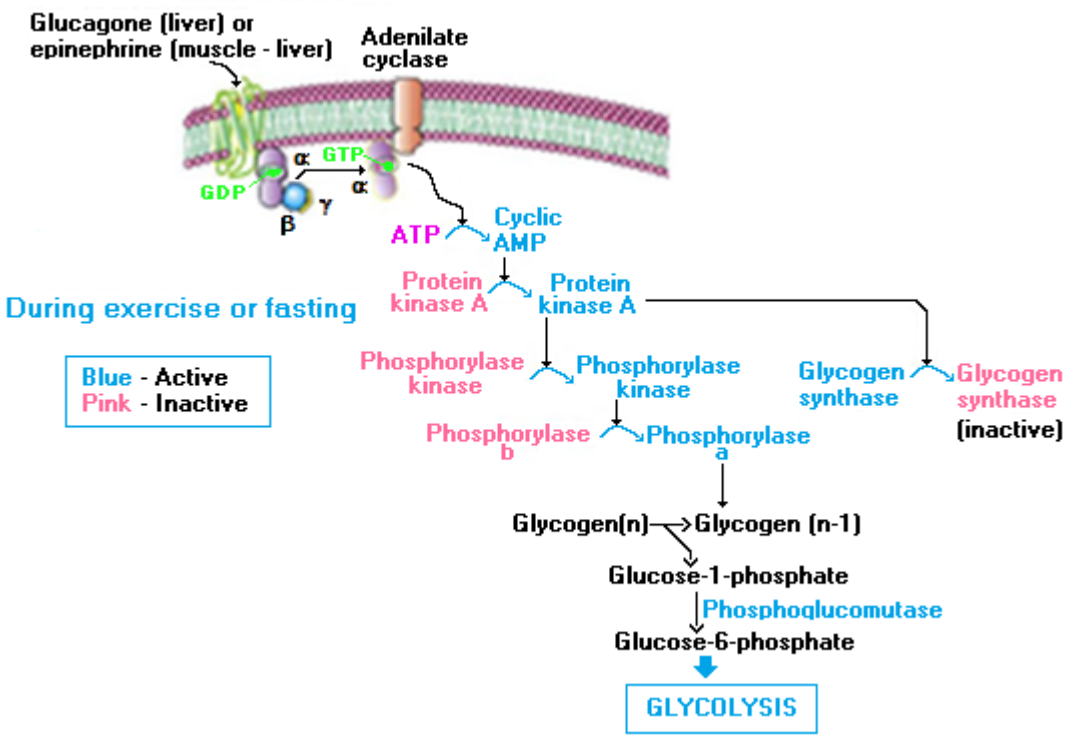
That enzyme removes the phosphate group in the liver to allow it to leave the liver to the rest of the body.

Understand allosteric control of the phosphorylase and synthase

Phosphorylase: breaks down - activated by AMP, inhibited by ATP, G6P
Synthase - synthesis - activated by G6P.

Regulation by allosteric and hormonal control (covalent modification).

-When there is increased demand for ATP, phosphorylase is active and synthase is inactive.
When there is plenty of G6P and ATP, phosphorylase is inactive and synthase is active.



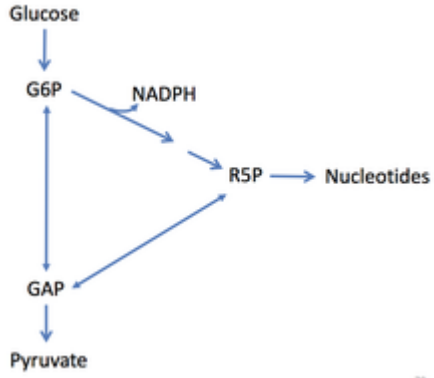
Understand the hormonal regulation of glycogen breakdown and synthesis. What is the role of cAMP? What is the role of phosphorylation /dephosphorylation?

1) Glucagon or epinephrine binds to a receptor which activates GDP to become GTP.
2) GTP Activates adenylate cyclase which activates cyclic AMP
3) Cyclic AMP activates protein kinase A
4) PKA phosphorylates the enzyme and increases phosphorylase activity to break down glycogen.

-Phosphorylation inactivates synthase and activates phosphorylase to break down glycogen
-Dephosphorylation activates synthase and inactivates phosphorylase to generate glycogen.

Where does the pentose phosphate pathway occur? What are the products of the pentose phosphate pathway? Under what conditions would you find high levels of PPP?

- Active in tissue heavily involved in lipid biosynthesis (ex. lipid and adipose tissue.) active in tissue with rapidly dividing cells which need high quantities of RNA and DNA (bone marrow, skin).
- Two major products of PPP are NADPH and Ribose 5 phosphate.
- NADPH is necessary for reductive biosynthesis of fatty acids and cholesterol. Provides reducing power and Energy currency. This is not interchangeable with NADH.
- Ribose 5 phosphate is a precursor for ribose unit of nucleotide biosynthesis.



How is the PPP related to glycolysis and how is this relationship beneficial?

Ribose 6 phosphate and intermediates of glycolysis can be funneled from one pathway to another to make pyruvates or nucleotides or glucose.

List the steps of glycogen synthesis

1) Glucose 6 phosphate is converted to glucose 1 phosphate by phosphoglucomutase
2) Glucose 1 phosphate is activated with UTP (highly exergonic driving reaction forward)
3) UDP glucose is added to the glycogen chain
4) A separate branching enzyme cleaves off a segment and reattaches it to glucose C6-OH group to create an alpha (1-6) branch point

List the steps of glycogen breakdown

1) Glucose in alpha1>4 linkage at the nonreducing end is phosphorylized to yield glucose 1 phosphate
2) G1P is converted to G6P, which can enter glycolysis skipping energy dependent hexokinase step.
3) In the liver, phosphate is removed from G6P to form glucose to leave cell for work

What is the role of PP1 (protein phosphatase-1)? What activates/regulates it?

1) PP1 dephosphorylates and inactivates glycogen phosphorylase. It is regulated by insulin. glucagon activates it.

2) Insulin activates PP1 which Increases PP1 activity and decreases phosphorylated glycogen phosphorylase. This means there is decreased glycogen phosphoylase activity and decreased breakdown of glycogen.

How is glycogen synthase regulated?

- When glycogen synthase is phosphorylated by PKA and phosphorylase kinase, it is inactivated. Meaning glucose concentration will increase.
- When it is dephosphorylated by PP1, it is activated