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◉ Sheet ○ Slides

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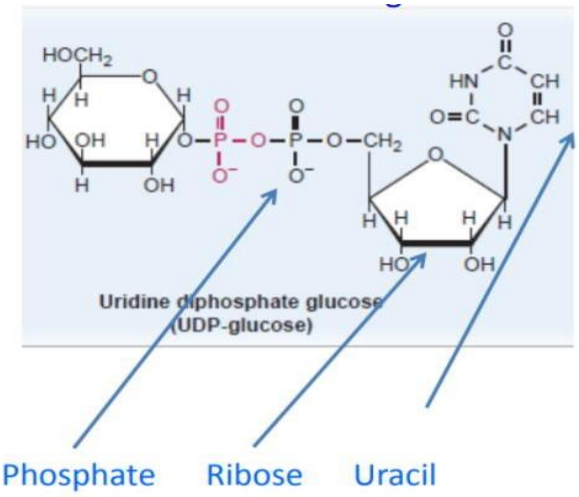
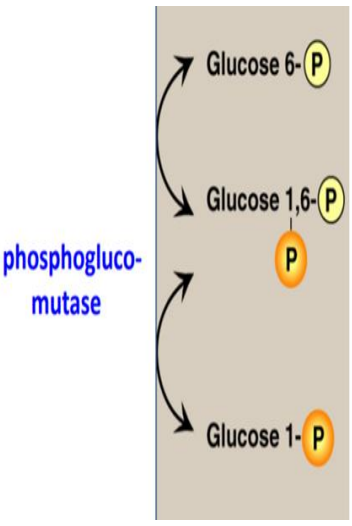
Formation of UDP-Glucose:

- Firstly, free glucose **can not** be added to glycogen directly because no enzyme is capable of adding glucose to glycogen.
- The glucose molecule must be added to a carrier (UDP), which assists in the addition of glucose to glycogen one by one (this is similar to coenzyme A which carries an acyl group to the Krebs cycle).
- UDP also adds sugar residues to proteins to form the glycoproteins.

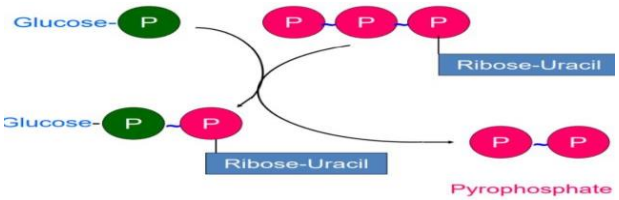
This figure shows the structure of UDP-Glucose



- Glucose is phosphorylated by **glucokinase** to form glucose-6-P, then glucose-6-P is converted to glucose-1-P by **phosphoglucomutase**. UTP can now react with glucose-1-P to form UDP-Glucose.
- Note that: phosphoglucomutase adds a phosphate group to glucose-6-P to form glucose-1,6-bisphosphate. The same enzyme then removes the phosphate group on carbon number 6 to produce glucose-1-P.
- Phosphoglucomutase is capable of catalyzing the reaction in both directions.



Mechanism of formation of UDP-Glucose:



From the figure on the right we notice that:

UDP-glucose pyrophosphorylase adds Glucose-1-P with its own phosphate to the first phosphate of UTP, the two terminal phosphates of the UTP molecules are subsequently released as pyrophosphate.

This reaction is reversible? why? →→→

when the high energy bonds between the first phosphate and the second phosphate break, and a high energy bond between the Phosphate group in glucose-1-Phosphate and the first phosphate in the UTP molecule simultaneously form, no net energy is released, making this reaction reversible (high energy bond break and high energy bond are formed so no net energy is released) . In this case, the reaction clearly has a low ΔG.

But this reaction occurs irreversibly inside the cell, why??

Because there is a rapid removal of pyrophosphate by enzyme pyrophosphatase, so the concentration of pyrophosphate decreases, and the equilibrium of this reaction shifts to the forward direction, forming more UDP-Glucose.

Glycogen synthesis:

- Firstly, you need to know that the enzyme, which is responsible for early synthesis of glycogen, is different than that used in adding glucose unit to glycogen.
- We now know that glycogen synthase makes an α(1→4) linkage in glycogen but this enzyme cannot initiate chain synthesis using free sugar molecule, it can only elongate already existing chain of glucose, therefore, it requires a primer (an already existing fragment of glycogen can serve as a primer).
- A protein called **Glycogenin** can serve as an acceptor of glucose residues from UDP-glucose. The side-chain hydroxyl group of a specific tyrosine in glycogenin serves as the site in which the initial glucosyl unit is attached. Because the reaction is catalyzed by glycogenin itself via autoglucosylation, **glycogenin is** an enzyme. Glycogenin then catalyzes the transfer of the next few molecules of glucose from UDP-glucose, producing a short, α (1→4)-linked glucosyl chain.
- This short chain serves as a primer that is able to be elongated by glycogen synthase.

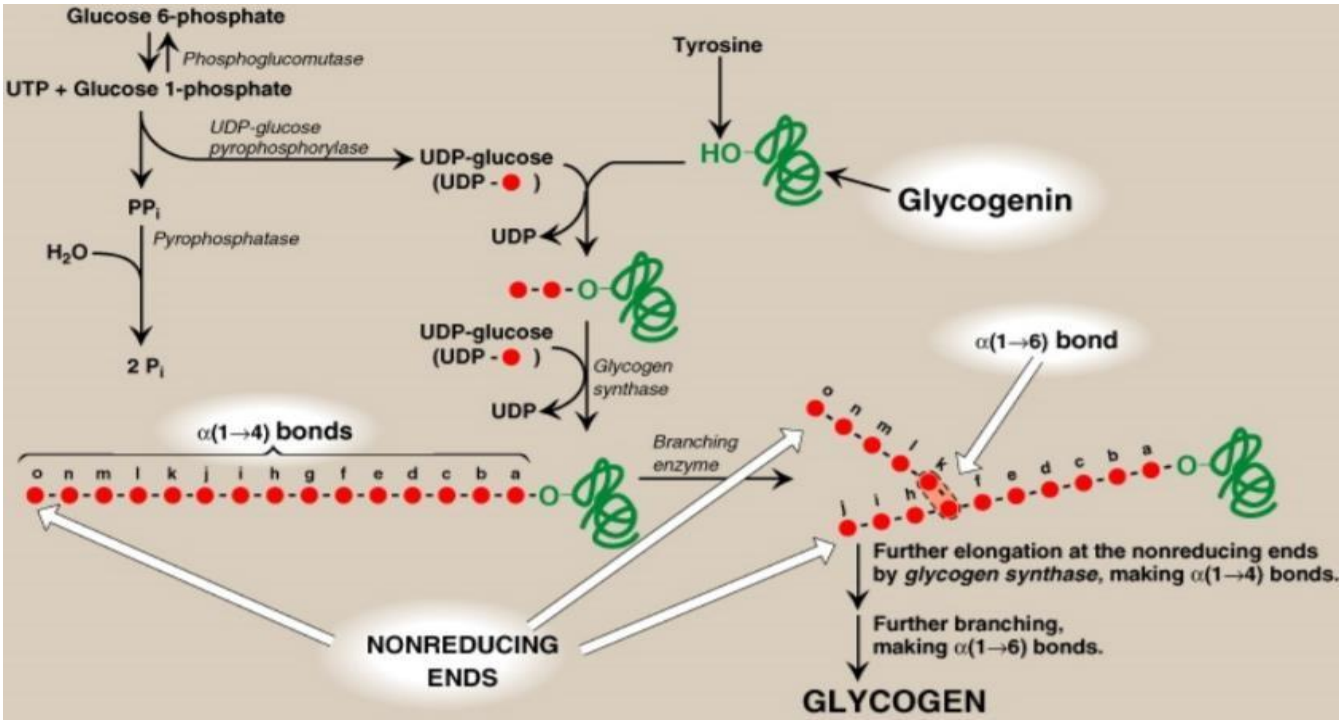
Glycogen elongation:

- Elongation of a glycogen chain involves the transfer of glucose from UDP-Glucose to the non-reducing end of the growing chain, forming a new glycosidic bond.
- The enzyme responsible for making the α(1→4) linkages in glycogen is known as **glycogen synthase.**

Synthesis of additional Branches:

- If no other enzymes acted on the chain, the resulting structure would be a linear (unbranched) chain of glucose residues attached by α (1→4) linkages, such as amylose, a starch found in plants. In contrast, glycogen is highly branched. Branching also increases the number of non-reducing ends to which new glucose residues can be added and more glucose residues can be removed from glycogen as well, so glycogen has high rate of synthesis and metabolism.
- After the elongation of linear chain, an enzyme called **branching enzyme** removes fragment from non-reducing end of glycogen chain (the terminal six to eight glucose residues) to make additional branches. This takes place by breaking an α(1→4) bond then attaching the fragment to a non-terminal glucose residue by an α(1→6) bond.
- Branching increases the number of non-reducing end in glycogen.
- Consecutive activity of both the glycogen synthase enzyme and the branching enzyme result in the elongation of the branches and the original glycogen chain (glycogen becomes larger and larger).

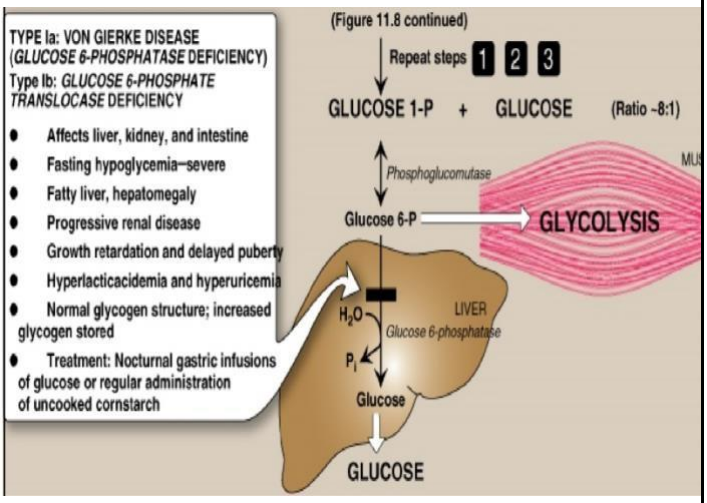
This figure recaps the glycogen synthesis. Take a look.



Glycogen storage diseases:

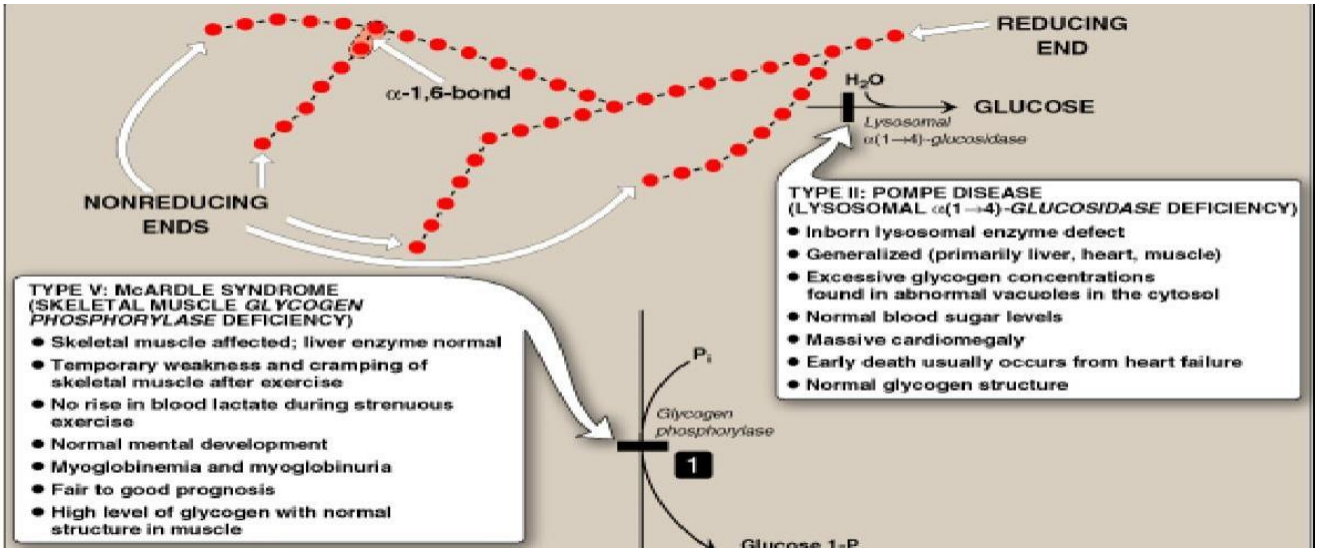
- Genetic diseases are usually caused by a mutation in a gene which results in the generation of inactive proteins. Some of these diseases are inherited.
- Defect in an enzyme required for synthesis or degradation.
- Accumulation of excessive amount of glycogen in one or more tissue.
- Severity: FATAL in Infancy……. Mild disorder (patient can cope with disease).

1. **I (type 1) Glucose-6-phosphatase deficiency disease (Von Gierk's disease):**
   - Glucose-6-phosphatase is an enzyme required to produce glucose that can be exported outside the liver, and it is common in glycogen synthesis as well as glycogen degradation.
   - Affects the liver, kidney, and intestines.
   - Severe fasting hypoglycemia. (liver can't covert Glucose-6-phosphate to glucose to transport it to blood during fasting).
   - Hepatomegaly fatty liver. (fat synthesis in liver will be activated)
   - Normal glycogen structure when it is examined by microscope.
   - Progressive renal disease.
   - Growth retardation.
   - Muscles aren't affected by the disease because there is no Glucose-6-phosphatase in the muscles.



   - Treatment to avoid hypoglycemia: nocturnal gastric infusions of glucose or regular administration of uncooked cornstarch in order to provide glucose from the small intestines for longer time.

2. **V (type 5) muscle glycogen phosphorylase (McArdle syndrome):**
   - Only muscle is affected; normal in the liver.
   - Weakness and cramping of muscle after exercise.
   - no increase in [lactate] during exercise.
   - Normally, when we do exercises, lactic acid level in the blood will increase as a result of anaerobic glycolysis (there's no glycolysis because the muscle glycogen isn't degraded).



3. II (type 2) Lysosomes α (1→4) glucosidase (POMPE Disease):

- Lysosomes are organelles in the cell that degrade large molecules.
- Degradation of glycogen in the lysosomes.
- ≈ 3% of glycogen is degraded in the lysosomes.
- Affects liver, heart and muscle.
- Excessive glycogen in abnormal vacuoles in the lysosomes.
- Massive cardiomegaly (increased size of the cardiac muscle).
- Normal blood sugar, normal glycogen structure.
- Early death from heart failure.

- **Energy needed for glycogen synthesis:**

$$\text{Glucose} + \text{ATP} \longrightarrow \text{Glucose 6-phosphate} + \text{ADP}$$

$$\text{Glucose 6-phosphate} \rightleftharpoons \text{Glucose 1-phosphate}$$

$$\text{Glucose 1-phosphate} + \text{UTP} \rightleftharpoons \text{UDP-Glucose} + PP_i$$

$$PP_i + H_2O \longrightarrow 2P_i$$

$$\text{UDP-Glucose} + \text{Glycogen}_{(n)} \longrightarrow \text{UDP} + \text{Glycogen}_{(n+1)}$$

Any intermediate used by one step and produced by another step will be canceled out.

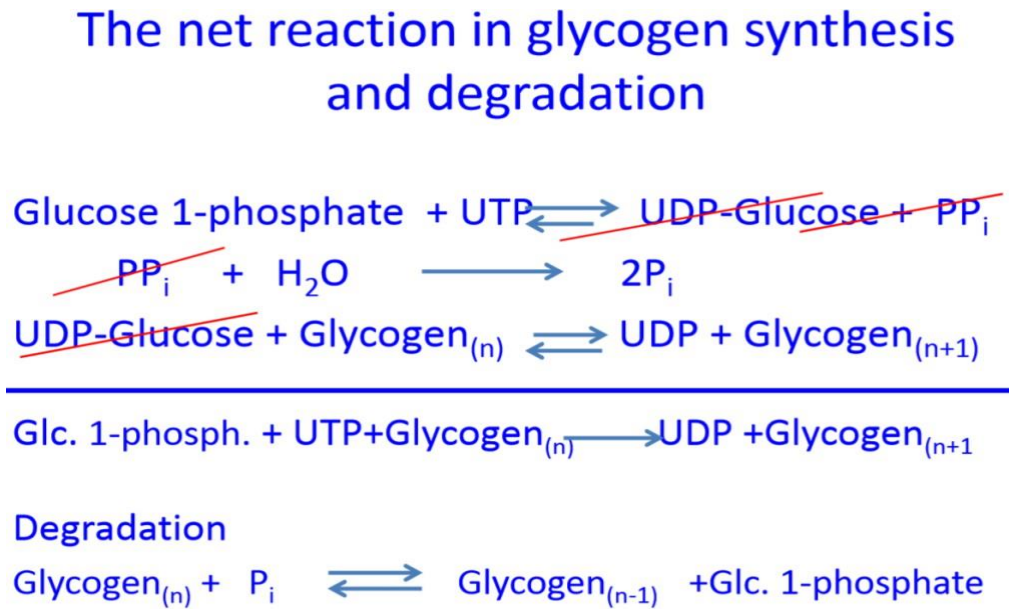
Rapidly hydrolyzed

$$\text{Glc.} + \text{ATP} + \text{UTP} + \text{Glycogen}_{(n)} \longrightarrow \text{ADP} + \text{UDP} + \text{Glycogen}_{(n+1)}$$

The net reaction.

So, how many ATP and UTP molecules are required for the addition of one glucose molecule to synthesize glycogen?

- 2; one from ATP, and the other from UTP (if we start with glucose).
- UDP can't be used, unless phosphate is transferred from ATP to UDP.



Here we start with glucose-1-phosphate, so we require only one high energy bond.

It is not logical to have synthesis and degradation occurring at the same time and in the same cell, because this achieves nothing, energy is wasted in the form of heat. So, these two processes should be regulated.

The process of Glycogen degradation:

- Glycogen degradation takes place when blood glucose levels are low, a condition known as hypoglycemia.
- When glucose levels are low, the level of **glucagon** in the blood increases.
- Glucagon will bind to its receptor on the surface of the liver cell. The glucagon receptor will activate the **G-protein** which is comprised of three subunits: Alpha, Beta, and Gamma. The alpha subunit will switch an already bound GDP molecule for a GTP molecule allowing for the dissociation of the alpha subunit from the rest of the protein.

- The **alpha subunit** will bind to a membrane enzyme known as **adenylate cyclase**, which as its name implies, allows for the cyclation of ATP to **cAMP** (3',5' cyclic AMP, two ester bonds are formed between the phosphate and the third and fifth carbon of ribose). Next, the cAMP will bind to a cytosolic enzyme, which is dependent on cAMP, called **Protein Kinase A** which is a tetrameric protein.

- Two of the four subunits are **regulatory subunits**, also known as R-subunits, which control the actions of the other two **catalytic subunits**, also known as C-subunits. Each R-subunit requires two cAMPs to be activated. Upon the binding of four cAMP to the two regulatory subunits, the dissociation takes place and the catalytic subunits are both free and activated. They remain activated until the cytosolic cAMP levels decrease. Once activated, the role of C-subunit is to phosphorylate and activate a multitude of enzymes. In the case of glycogen degradation, the enzyme which is phosphorylated is an enzyme known as **glycogen Phosphorylase Kinase B** (the enzyme's inactive form) which is converted to **glycogen Phosphorylase Kinase A** (the enzyme's active form).

- **Phosphorylase Kinase A** proceeds to activate its own substrate **Glycogen Phosphorylase B** (the enzyme's inactive form) which is converted to **Glycogen Phosphorylase A** (the enzyme's active form) which breaks down glycogen. The breakdown of glycogen can also take place during times of stress. In times of stress, we need a great quantity of energy. Epinephrine (which is a stress hormone) is released. This release of epinephrine can accelerate the degradation of glycogen by binding to surface receptors of the cells of the liver and the muscle, resulting in a cascade similar to the one above.

Enzymes of glycogen degradation:

The enzymes which participate in the breakdown of glycogen are catalytic in nature. This means that the enzyme will form many products from its own catalyzation reaction which will be utilized as substrates for each of the following reactions. For example, adenylate cyclase will form many cAMP molecules through its stimulation from the alpha subunit of the G-protein. Likewise, Protein Kinase A can phosphorylate a multitude of protein. The same idea is applied to phosphorylase Kinase. This implies that a small signal can result in dramatic results in relation to glycogen degradation which is known as signal amplification. Glycogen degradation continues until blood glucose levels are restored.

Removal of branches:

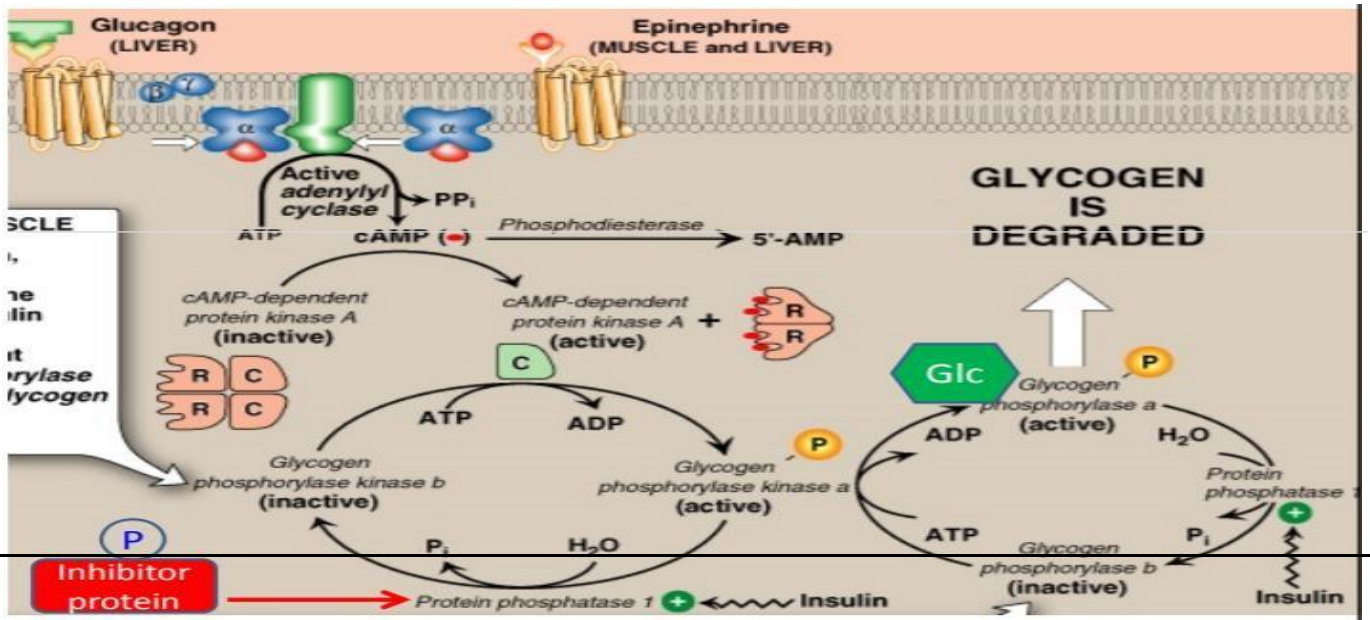
Note: Phosphorylase contains a molecule of covalently bound pyridoxal phosphate that is required as a coenzyme.] The resulting structure is called a limit dextrin, and phosphorylase cannot degrade it any further. Branches are removed by the two enzymic activities of a single bifunctional protein, the debranching enzyme. First, oligo-α(1→4)→α(1→4)- glucantransferase activity removes the outer three of the four glucosyl residues attached at a branch. It next transfers them to the nonreducing end of another chain, lengthening it accordingly. Thus, an α(1→4) bond is broken and an α(1→4) bond is made, and the enzyme functions as a 4:4 transferase. Next, the remaining glucose residue attached in an α(1→6) linkage is removed hydrolytically by amylo-α(1→6)- glucosidase activity, releasing free glucose. The glucosyl chain is now available again for degradation by glycogen phosphorylase until four glucosyl units in the next branch are reached.

Regulation of Glycogen Degradation Enzymes

Any regulatory function should easily be turned on and off. Adenylate cyclase, for example, is a major regulator of glycogen degradation because its sole purpose is the formation of cAMP for the activation of Protein Kinase A. We cannot use a molecule with a multitude of functions as a regulator because one cascade or cellular pathway may intersect with another. ADP is not a good regulator because it functions in a variety of pathways in the cell. Regulators should also be available and easily found in the cell. ATP is highly abundant in the cell meaning that cAMP can easily be made. Lastly, the regulators should be easily degraded. cAMP, for example, is easily degraded to 5'-AMP through the enzyme **Phosphodiesterase.**

Inhibition of Enzymes

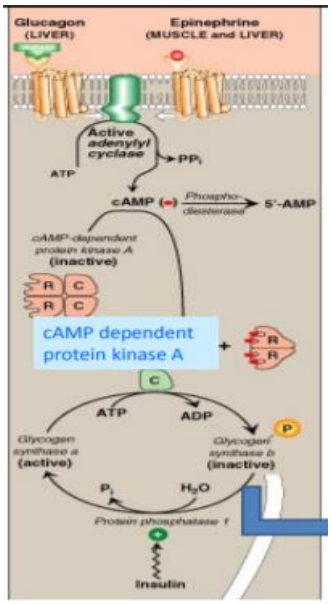
Enzymes involved in glycogen degradation are inhibited by many substances. For



example, phosphodiesterase, breaks the bond between the third carbon in ribose and the phosphate, is inhibited by caffeine. Therefore, we feel alert when we drink caffeinated drinks like coffee, sodas and energy drinks. Insulin, which acts as a counterbalance for glucagon and exists only when blood glucose levels are high, inhibits a variety of phosphorylated enzymes by activating enzyme **phosphatases** which dephosphorylate phosphorylated enzymes. **Glycogen Phosphorylase Kinase a** is an example on an enzyme that is inhibited by insulin's activation of glycogen synthase. Phosphatase is inhibited by a **phosphorylated protein inhibitor** which is phosphorylated by the actions of the Protein Kinase A. Protein Kinase A phosphorylates the **inhibitor protein** so that the phosphatases can be inhibited just long enough so that blood glucose levels return to normal and insulin reactivates the glycogen synthase. Lastly, glycogen phosphorylase (liver) is inhibited by glucose-6-phosphate because its ultimate function is to break down glycogen into glucose. This is called feedback inhibition.

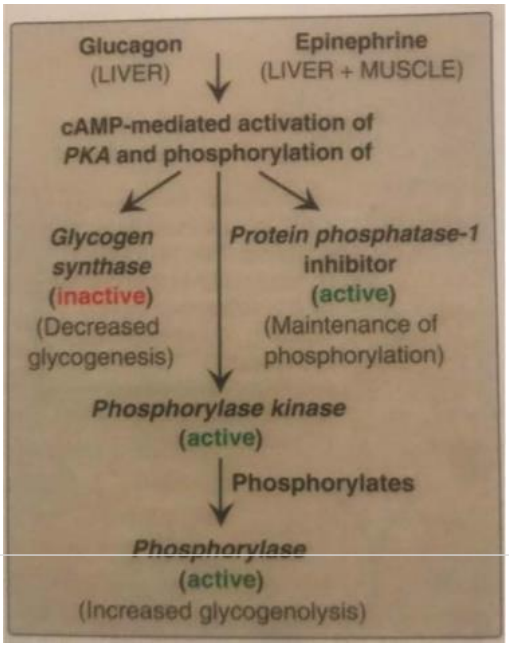
Glycogen Synthesis Inhibition

Glycogen synthesis could be inhibit in many cases. The first, and most obvious, glycogen synthesis is inhibited when degradation is active. The second method is the addition of a phosphate group to **Glycogen Synthase A** (active form) making it **Glycogen Synthase B** (inactive form). Phosphorylating many proteins not only activates degradation, but also activates inhibition. Many other methods could be found above under enzyme inhibition.



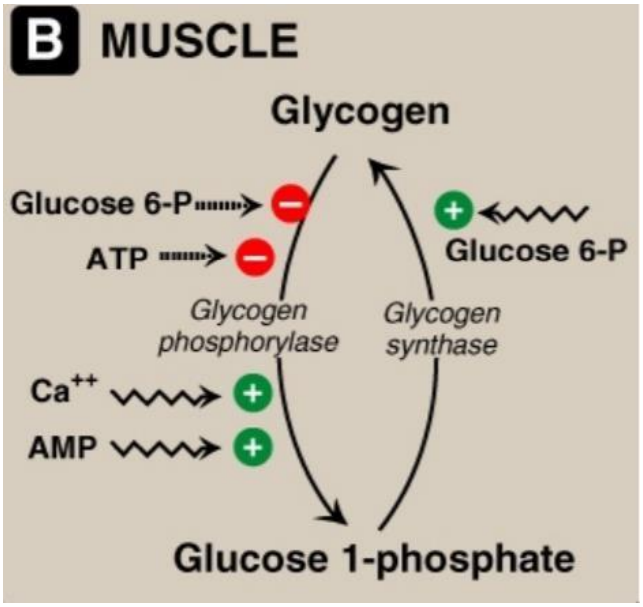
Note: The greater the phosphorylation is, the greater the inhibition.

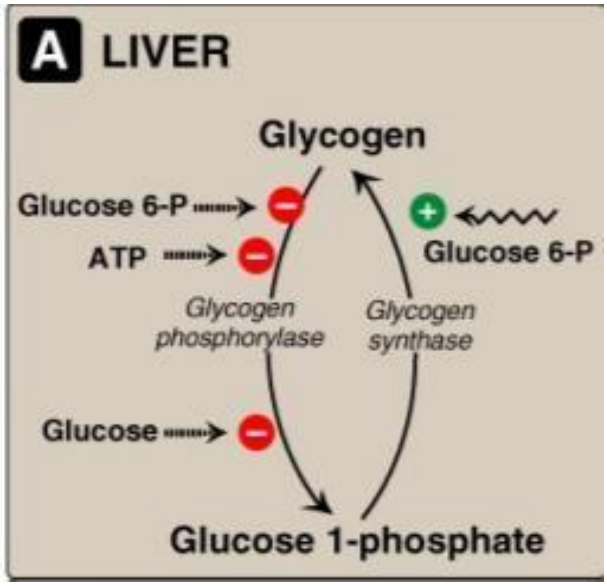
The following image summarizes everything that was previously discussed.



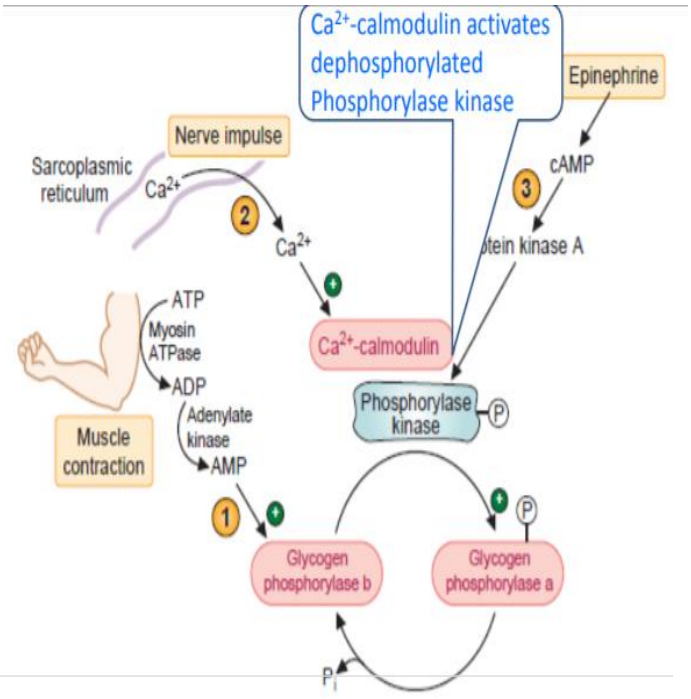
Allosteric Regulation

Glycogen degradation is a biological process that take place in order to benefit the organism as a whole not just a single cell for instance. Glycogen degradation may take place for example, if there is a depletion of glucose in the brain or the muscles. But what about cellular needs? Within the cell itself there are allosteric regulations that take place in which certain molecules bind to the enzymes and the enzymes give a rapid response that is extremely transient and depends on the concentration of glucose.

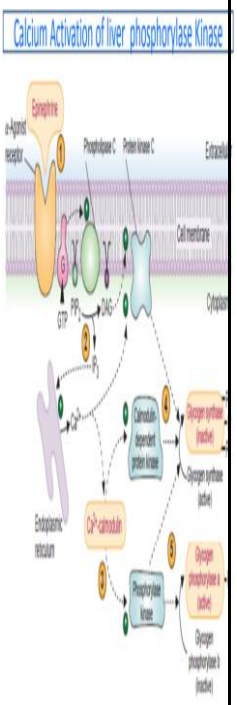




Note: glycogen degradation and synthesis are similar in the muscle & liver. However, high levels of AMP activate glycogen degradation, because high levels of AMP indicate that energy reserves are depleted, and that glycogen degradation should start before hormones reach the cell. On the other hand, high levels of calcium could activate glycogen degradation for two reasons. The first is that calcium could bind to calmodulin and activate Phosphorylase Kinase. The second is that high levels of calcium as we all know, indicate powerful contractions. So, energy must be made to accommodate these powerful contractions.



Note: the accumulation of phosphorylated sugar is harmful because it inhibits ATP production. This is why the phosphorylated sugar inhibits glycogen degradation and activates glycogen production.



Note: The release of epinephrine can accelerate the degradation of glycogen by binding to surface receptors of the cells of the liver and the muscle, resulting in a cascade similar to the one above. In muscles, the binding of epinephrine results in the release of Calcium ions, which bind to calmodulin and activate phosphorylase kinase. cAMP binds to glycogen phosphorylase allowing glycogen degradation.

Good luck!