

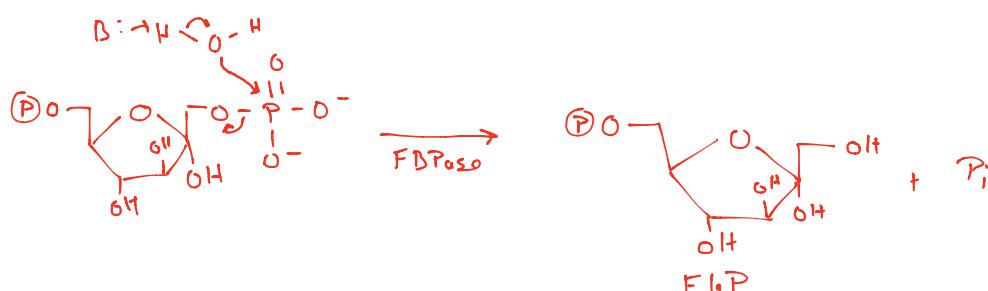
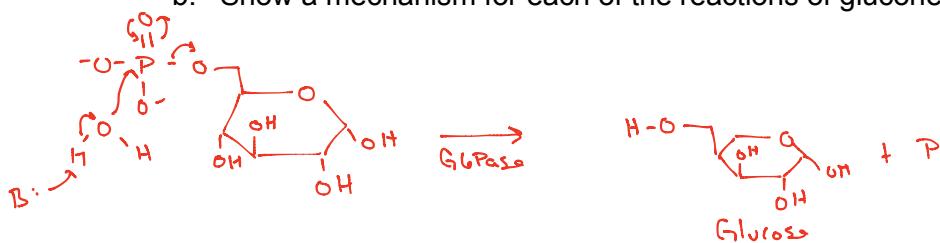
**Problem Set 6**

(Due March 4<sup>th</sup>)

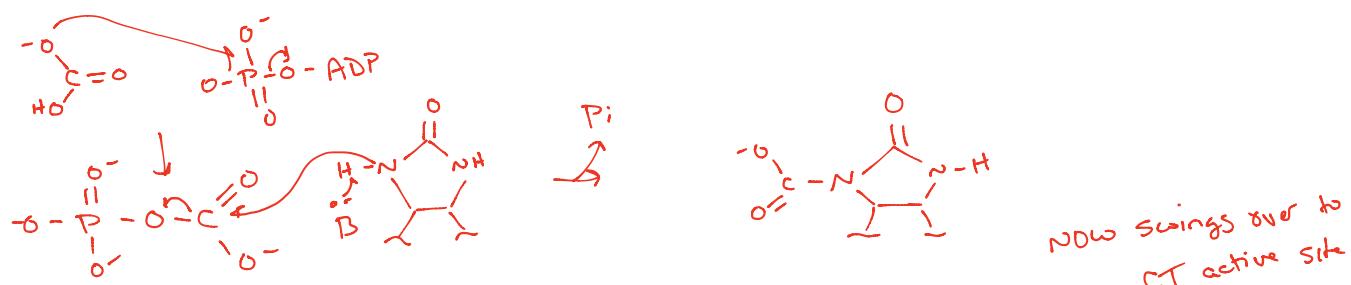
1. The synthesis of glucose from pyruvate only needs to bypass three reactions of glycolysis.

a. What are these steps and why only three? Hexokinase, phosphofructokinase, and pyruvate kinase - these are the 3 irreversible steps

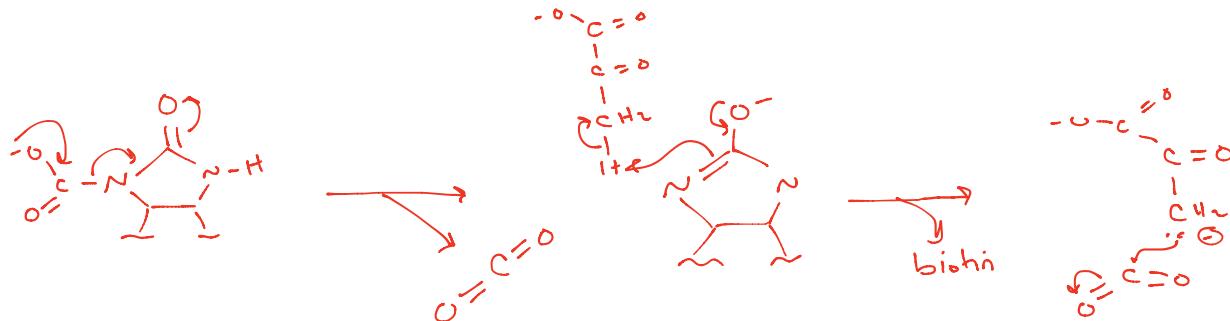
b. Show a mechanism for each of the reactions of gluconeogenesis that differ from glycolysis.



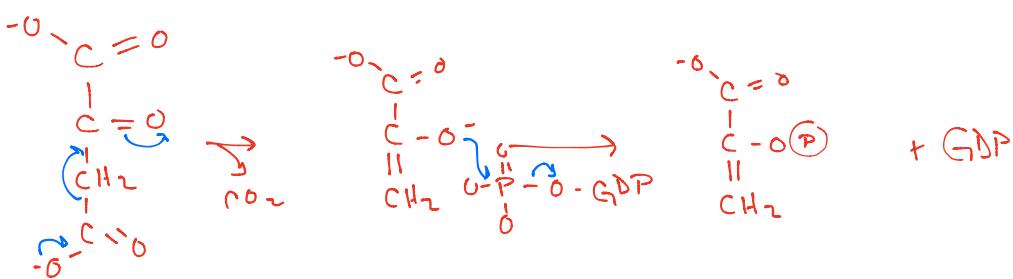
### Pyruvate Carboxylase - Phase I



### Phase II



### PEPCK



2. Discuss metabolic flux. Include as many key concepts as you can think of. Metabolic flux is the rate at which metabolites pass through a given pathway. Cells try to maintain a constant concentration of metabolites, but this does not mean that reaction are not occurring – similar to the idea of chemical equilibrium (an equilibrium is established with respect to reactant and product concentrations when the rate of the forward reaction equals the rate of the reverse reaction – both reactions are continuously happening, so the equilibrium is dynamic). When cellular conditions change, flux through a pathway changes to accommodate the change in the environment. The first irreversible step in a pathway is usually the rate limiting step and also the main regulatory step. Enzyme kinetics at this step can be controlled in a number of ways; the ones we talked about include allosteric activation or inhibition, covalent modification (phosphorylation, ubiquitination, etc), compartmentalization, and the presence of a regulatory protein (or coenzyme).
3. Adenylate Kinase is an important enzyme that serves to buffer the concentration of ATP in a cell. As we discussed in class,  $K = 0.44$  for the Adenylate Kinase reaction ( $2\text{ADP} \rightleftharpoons \text{ATP} + \text{AMP}$ ). Making the assumption that  $[\text{ATP}]$  is 10-fold higher than  $[\text{ADP}]$  and the total adenine nucleotide concentration in a cell is 5 mM and does not change, calculate the % change of  $[\text{AMP}]$  when  $[\text{ATP}]$  decreases by 10%. (If you do this properly, you should get ~450%).

$$A_T = [\text{ATP}] + [\text{ADP}] + [\text{AMP}] \quad \text{... as we discussed in class } [\text{AMP}] \approx \frac{[\text{ATP}]}{50}, \text{ so insignificant in +/- equation}$$

$$[\text{ADP}] = A_T - [\text{ATP}]$$

$$0.44 = \frac{[\text{ATP}][\text{AMP}]}{[\text{ADP}]^2}$$

$$0.44 (A_T - [\text{ATP}])^2 = [\text{ATP}][\text{AMP}]$$

$$\text{Condition 1: } 5\text{ mM} = [\text{ATP}] + [\text{ADP}] = [\text{ATP}] + \frac{[\text{ATP}]}{10} = \frac{11}{10} [\text{ATP}]$$

$$[\text{ATP}] = 4.545 \text{ mM}$$

$$[\text{AMP}] = \frac{(5 - 4.545)^2 (0.44)}{4.545} = 0.02 \text{ mM}$$

$$\text{Condition 2: } 10\% \text{ decrease in } [\text{ATP}] = 0.9 (4.545) = 4.091 \text{ mM}$$

$$[\text{AMP}] = \frac{(5 - 4.091)^2 (0.44)}{4.091} = 0.0899 \text{ mM}$$

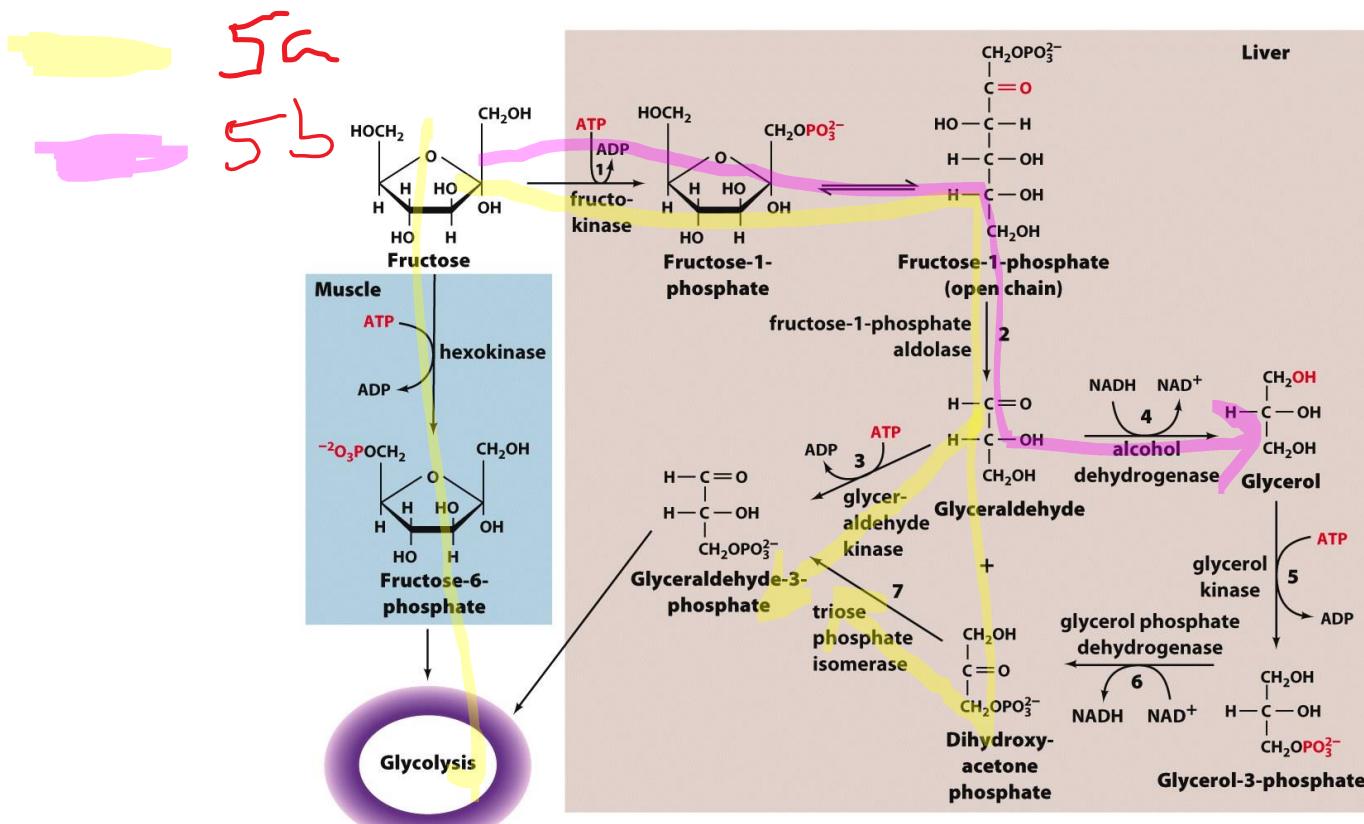
$$\frac{[\text{AMP}]_2}{[\text{AMP}]_1} = 4.44$$

444%

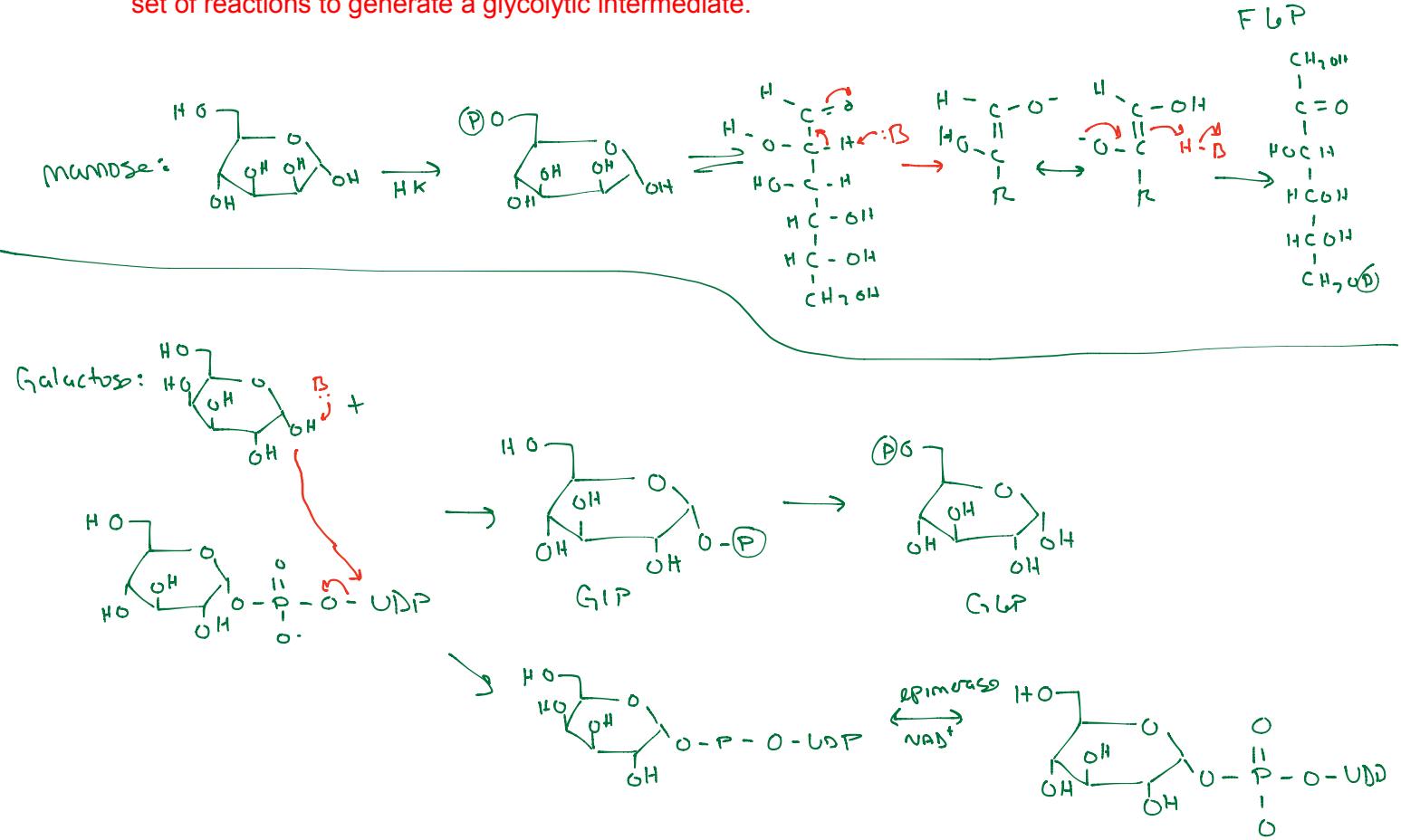
4. In glycolysis, two equivalents of ATP are consumed to make FBP, but gluconeogenesis fails to produce ATP when these steps are reversed. Why? Hydrolysis of the sugar-phosphate bond does not provide enough energy to drive the substrate level phosphorylation of ADP. Looking at the structures, the sugar phosphate ( $\text{R}-\text{O}-\text{PO}_3^{2-}$ ) does not have the electrostatic repulsion that the phosphodiester of ATP does. Additionally, the hydrolysis product of the sugar phosphate only gains one resonance structure (in the inorganic phosphate – no extra resonance in the newly formed  $-\text{OH}$ ) while the hydrolysis products of ATP gain two (one in the inorganic phosphate, one in ADP). Together, these strongly suggest that ATP hydrolysis is more exergonic than sugar phosphates)

5. Fructose metabolism is tissue specific.

- a. Discuss the two pathways for fructose metabolism that feed into glycolysis in the most direct way. In most tissues, fructose can be phosphorylated to F6P by hexokinase – this is due to the loose substrate specificity of this enzyme. In the liver, however, hexokinase is expressed in very low levels, so F6P is not readily produced in a one step process. Instead, fructose is modified at the C1 position to F1P by the action of Fructokinase. This, in turn, is converted to DHAP (glycolysis intermediate) and Glyceraldehyde through the action of a fructose-1-phosphate aldolase, a Type B Aldolase. The latter can be phosphorylated to G3P (glycolysis intermediate) by glyceraldehyde kinase. In the liver, this is the shortest pathway to fructose entering glycolysis.
- b. A rapid increase in [fructose] in the liver stimulates triacylglyceride biosynthesis. We will talk about this much more later in the term, but for now, think about why this may be. Note that F1P is often produced faster than the Type B Aldolase can cleave it, which leads to a drop in cellular ATP and Pi pools. The key to thinking about this problem is noting that ATP is not going to be very available, meaning that the activity of glyceraldehyde kinase will be dramatically decreased due to the lack of substrate availability. So, to maintain the homeostatic concentration of glyceraldehyde, flux through another pathway must increase. As we discussed in class, an alternate fate of glyceraldehyde is to be reduced to glycerol by alcohol dehydrogenase – this step does not require ATP, so it will be thermodynamically and kinetically favored (especially considering that flux through glycolysis is sure to be significantly upregulated due to the very low [ATP] and rapid regeneration of the NAD<sup>+</sup> pool is necessary to keep glycolysis moving efficiently). A sharp increase in glycerol ultimately signals hepatocytes to start making triacylglycerides – we'll cover this in a few weeks.



6. Comment on how hexokinase specificity plays a role in mannose, fructose, galactose, and glucose metabolism. For mannose and galactose, show how a glycolytic intermediate is made. Hexokinase has a loose specificity profile that allows for many glucose epimers and ketohexoses to be substrates. Of the sugars we discussed, mannose, fructose, and glucose can all be substrates; however galactose is not tolerated by the active site. This should give you some insight into the way that substrates interact with the active site. Since galactose cannot be directly phosphorylated, it undergoes a different set of reactions to generate a glycolytic intermediate.



7. PFK is similar to hemoglobin in many ways.

- Discuss the similarities and why this is important. PFK is a tetrameric protein that is subject to allosteric regulation. Both proteins have two common structural forms, the R and T states, which promote or prevent O<sub>2</sub> binding or substrate turnover.
- List all allosteric regulators and comment on their role – does it make sense that it is an activator or inhibitor? Inhibitors: ATP (signals sufficient energy levels), citrate (signals abundance of TCA intermediates), PEP (signals elevated glycolytic intermediate levels). All of these are products of glycolysis or subsequent pathways and all suggest that glycolysis should slow down. Activators: ADP, AMP → signal low cellular energy levels. cAMP and F2,6P → key regulatory molecules that are products of extracellular signals. F6P → buildup of substrate.
- How does ATP inhibit PFK? ATP binding in the allosteric site on an adjacent protomer results in the flipping of an arginine residue out of the active site. This Arg is important in substrate binding because it forms a very stable salt bridge with the phosphate on C6 of F6P. With the rotated

8. How are flux through glycolysis and gluconeogenesis controlled in a coordinated manner? This occurs through a key regulatory molecule (F26P) and protein (PFK-2/FBPase2). F2,6P is the product of an isoform of PFK (called PFK-2) that generates a structural isomer of FBP. F26P is an allosteric activator of PFK – when it's around, flux through glycolysis is increased. F26P is a potent inhibitor of FBPase, so its presence prevents flux through glycolysis. Activation of PKA through a signal transduction pathway, in response to low blood glucose, results in phosphorylation of the PFK2/FBPase2 protein. This inhibits the PFK2 activity and activates the FBPase2 domain. F26P is hydrolyzed - PFK is no longer activated and FBPase is no longer inhibited.

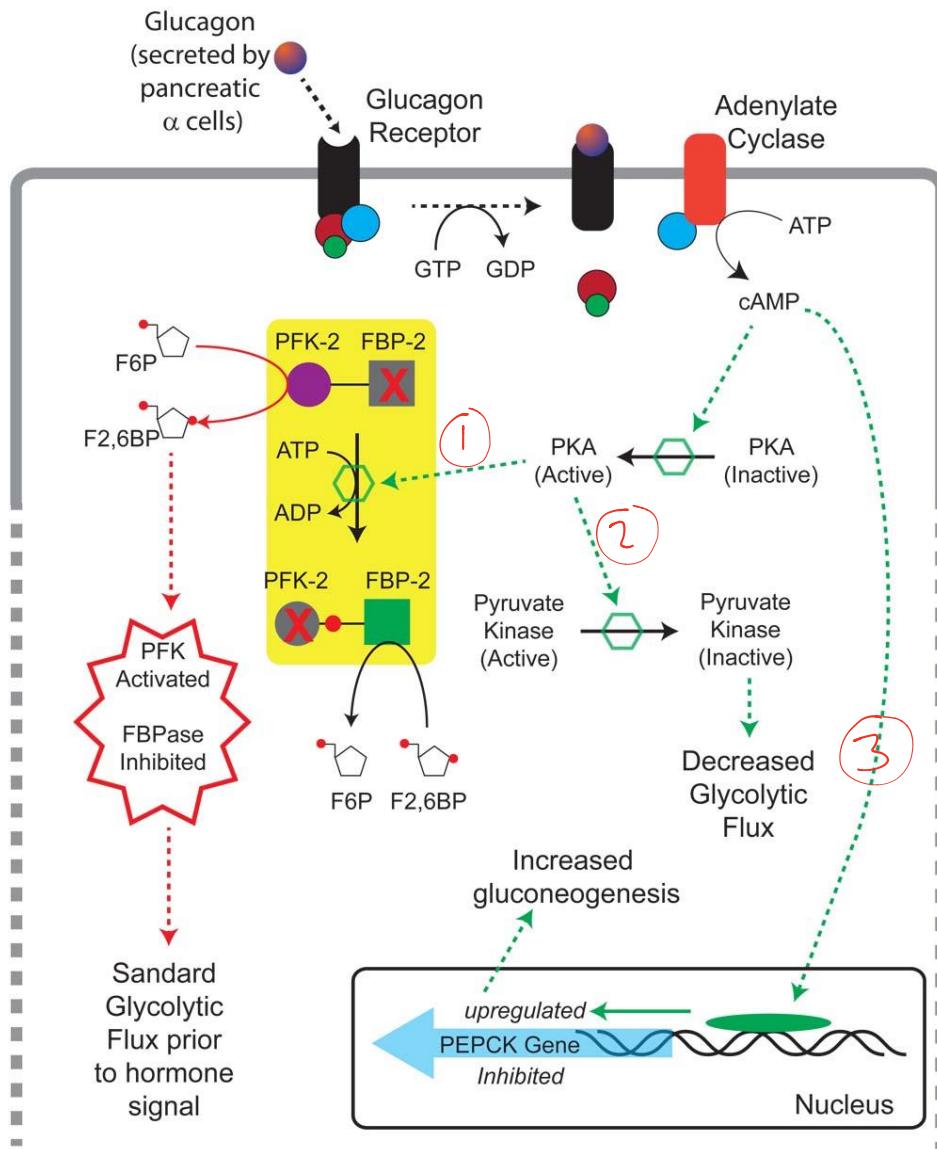
Additionally, activation of PKA results in inactivation of Pyruvate kinase (decreased flux through glycolysis) due to phosphorylation. Increased cAMP levels lead to transcriptional activation of PEPCK (increased flux through gluconeogenesis)

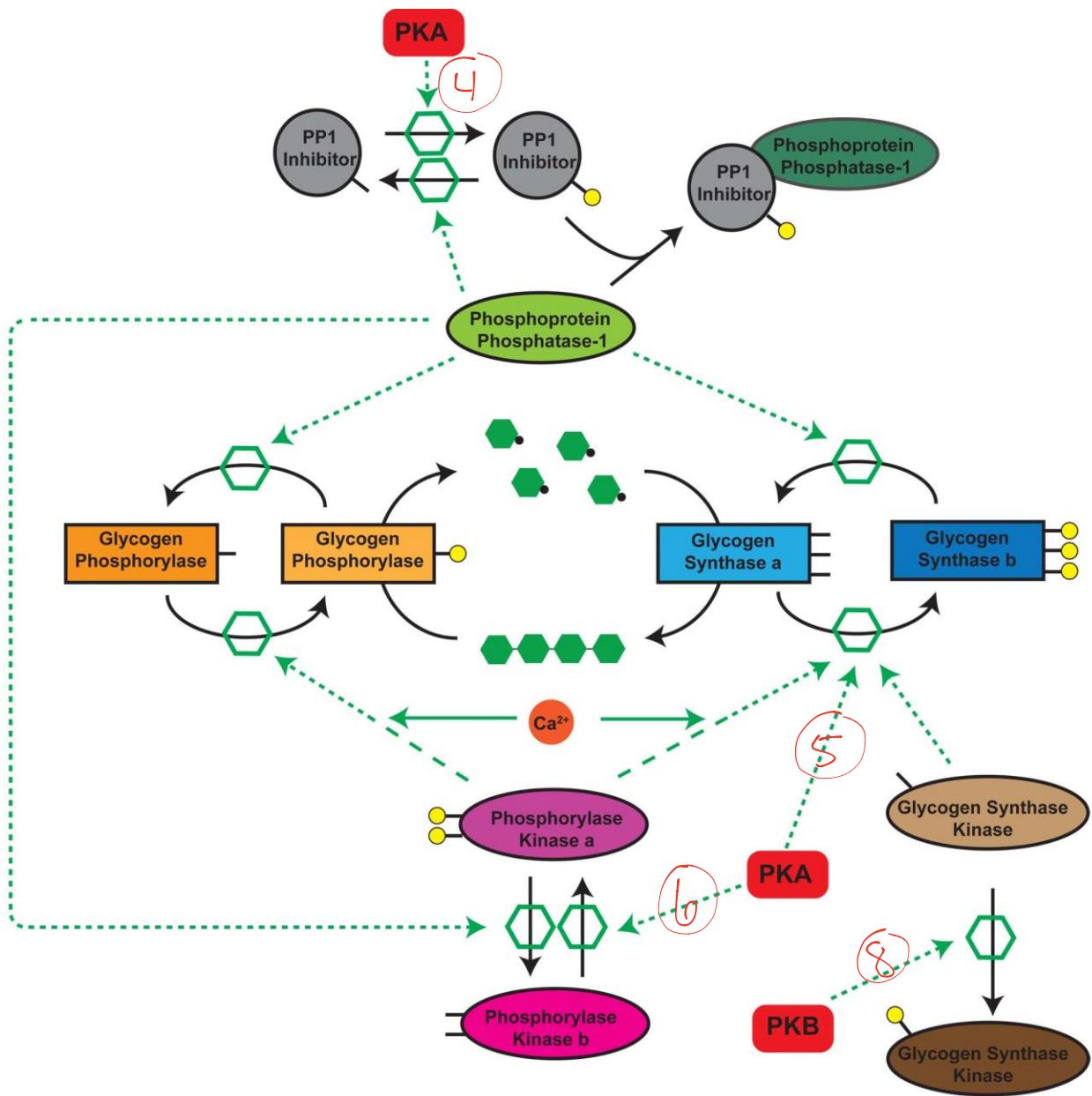
9. High blood glycerol levels (a result of fat breakdown...and other things) have been shown to promote glucagon release from pancreatic  $\alpha$  cells. What result does this have on glucose metabolism in hepatocytes (liver cells)? Glucagon stimulates PKA through a GPCR-Adenylate cyclase signal transduction pathway. PKA activation aggressively influences the activity of many enzymes in the sugar metabolism pathways. Each is highlighted below (Numbers on the images) with a brief explanation. All of these lead to production of glucose so that it can be transported into the blood.

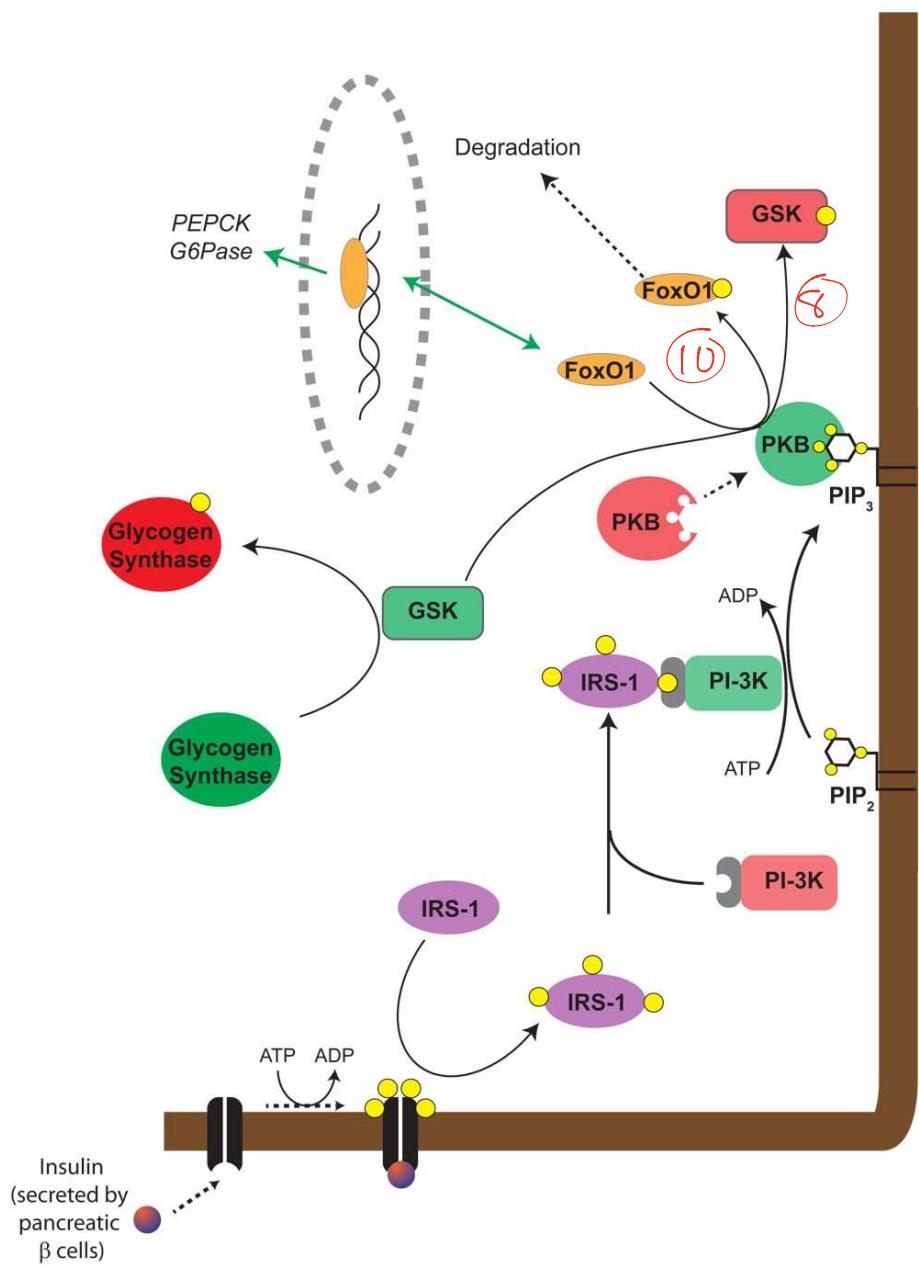
1. PFK2 inhibited, FBP2 activated. Flux through glycolysis decreased, gluconeogenesis increases
2. Pyruvate Kinase inactivated – Glycolysis decreased
3. cAMP triggered upregulation of PEPCK – gluconeogenesis increased
4. PP1 inhibitor phosphorylated – PP1 inhibited. Glycogen breakdown increased.
5. Glycogen Synthase phosphorylated – Glycogen synthesis decreased
6. Phosphorylase Kinase activated – Glycogen Phosphorylase activated – Glycogen breakdown increased
7.  $G_M$  phosphorylated @ 2<sup>nd</sup> site – PP1 inactivated – Glycogen breakdown increased

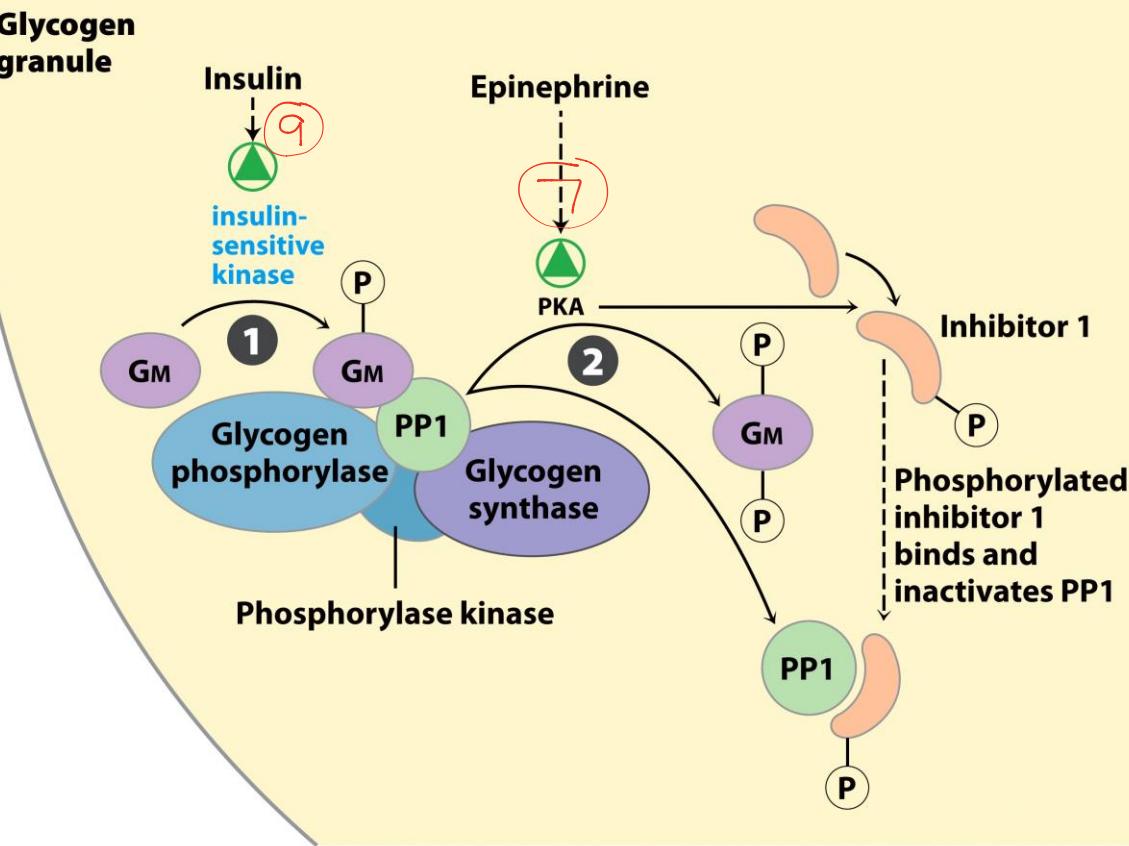
10. Insulin plays a major role in sugar metabolism in hepatocytes. Based on our discussions in class, describe all the ways it can influence flux through these pathways. Insulin leads to activation of PKB through the phosphoinositide pathway. Like PKA, PKB activity has a significant impact on sugar metabolism. All of these lead to storage of glucose. Intracellular energy demand would have a significant impact on flux through glycolysis and gluconeogenesis.

8. Glycogen Synthase Kinase inactivated – Glycogen synthesis activated.
9.  $G_M$  phosphorylated @ 1<sup>st</sup> site – PP1 activated, glycogen Phosphorylase inactivated, Glycogen Synthase activated. Glycogen synthesis increased, glycogen breakdown decreased.
10. FoxO1 inactivated. Decreased transcription of PEPCK and G6Pase – both are gluconeogenesis enzymes, so flux through gluconeogenesis is decreased









11. G<sub>M</sub> is an important adaptor protein that can exist in two different phosphorylation states (as well as unmodified). Describe the metabolic result of each phosphorylation event. In addition, comment on the extracellular conditions that would trigger each phosphorylation event.

- The first phosphorylation of G<sub>M</sub> leads to localization of PP1 to a glycogen granule. PP1 phosphorylates glycogen synthase, which is an activating modification, and glycogen phosphorylase, an inhibitory modification. So glycogen synthesis is stimulated and glycogen degradation is prevented. This suggests that conditions are sufficient for packing glucose into a storage form, so this must represent a situation where the blood-glucose levels are too high. Indeed, the first phosphorylation event is catalyzed by PKB, which is activated in response to insulin.
- The 2<sup>nd</sup> phosphorylation event causes PP1 to dissociate from the glycogen granule. It is no longer able to modify glycogen phosphorylase or synthase, the extracellular conditions must be representative of a glucose-depleted blood. This modification is catalyzed by PKA, which is activated by glucagon, so the result matches the demand.

12. We did not talk about the role AMPK has on global regulation of metabolism throughout all tissues. Please read the attached review and summarize the role this protein plays. There are a large number of topics you could have selected to focus on here – but the most important point to pick out is that AMP Kinase is activated by AMP, so as the [AMP] increases, the activity of AMK increases. This increase in [AMP] can be triggered by depletion of the nutrient supply or by vigorous muscle activity. When activated, AMPK suppresses energy requiring processes and activates energy producing pathways (e.g. glycogen breakdown and glycolysis).