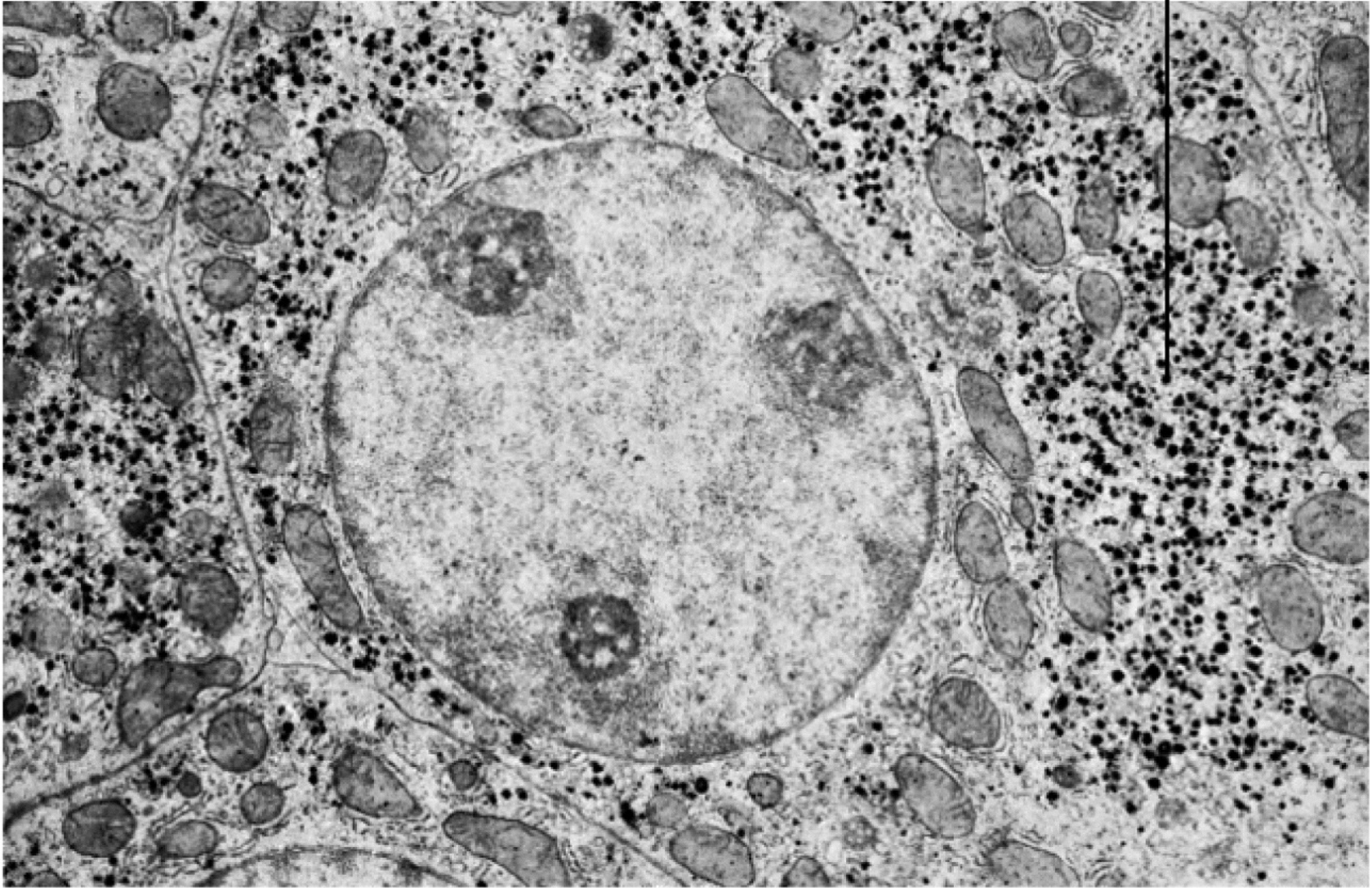


Biochemistry 2 Spr 2015 Lecture # Glycogen Metabolism



Glycogen granules



Glycogen Metabolism:

This is another reciprocal pathway for the sequestration or the rapid release of glucose. The presence of glycogen granules in the liver, about 21nm in size, form the beta particle. Each β particle has approx 55,000 glucose residues. Approx 20-40 β -particle cluster to form α -rosette particles. The amount of glucose bound in glycogen as free glucose would bring the glucose conc to 0.4M in the cytosol, while the bound macromolecules conc is less than 0.01 μ M.

The precise flux control of a pathway is possible when an enzyme functioning far from equilibrium is opposed by a separately controlled enzyme. Then, v_f & v_r vary independently & v_r can be larger or smaller than v_f , allowing control of both rate and direction. This is what occurs in glycogen metabolism due to there being two distinct enzymes, the phosphorylase & the synthase.

Why does the body preferentially utilize glycogen before fat since fat is more abundant in the body?

Muscle can not mobilize fat as efficiently as glycogen.

Fatty acid residues cannot be metabolize anaerobically

Animals can not convert fat to glucose

Utilization of Glucose;

Glucose is utilized in several pathways;

- glycogen synthesis ,
- pentose phosphate pathway
- Lipogenesis
- Amino acid synthesis
- Energy metabolism: both anaerobic & aerobic.

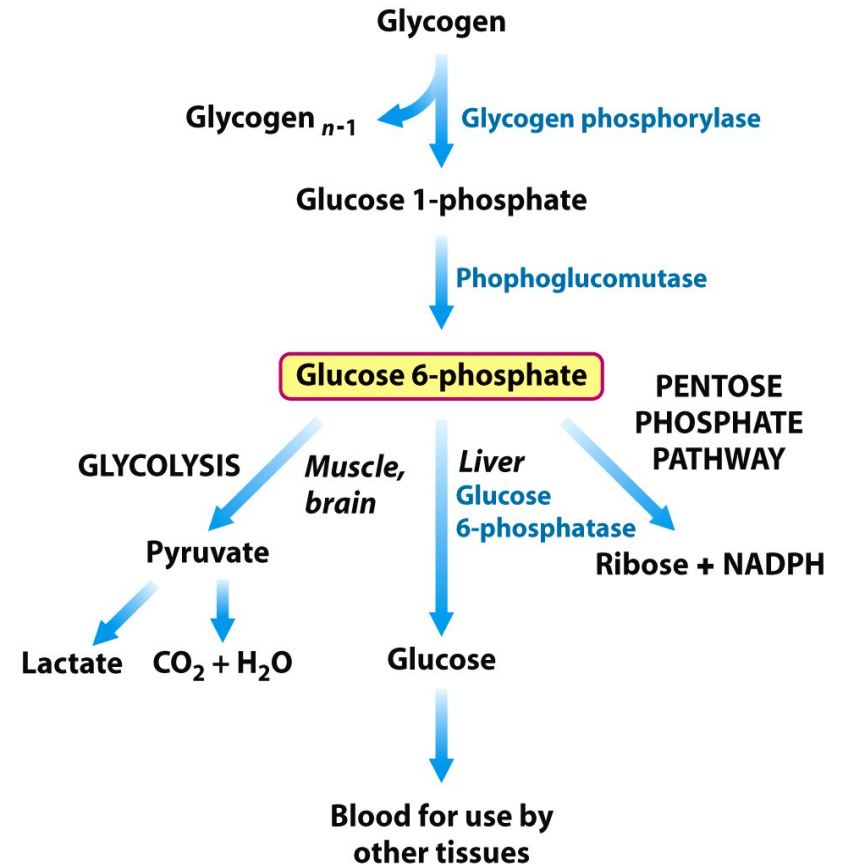
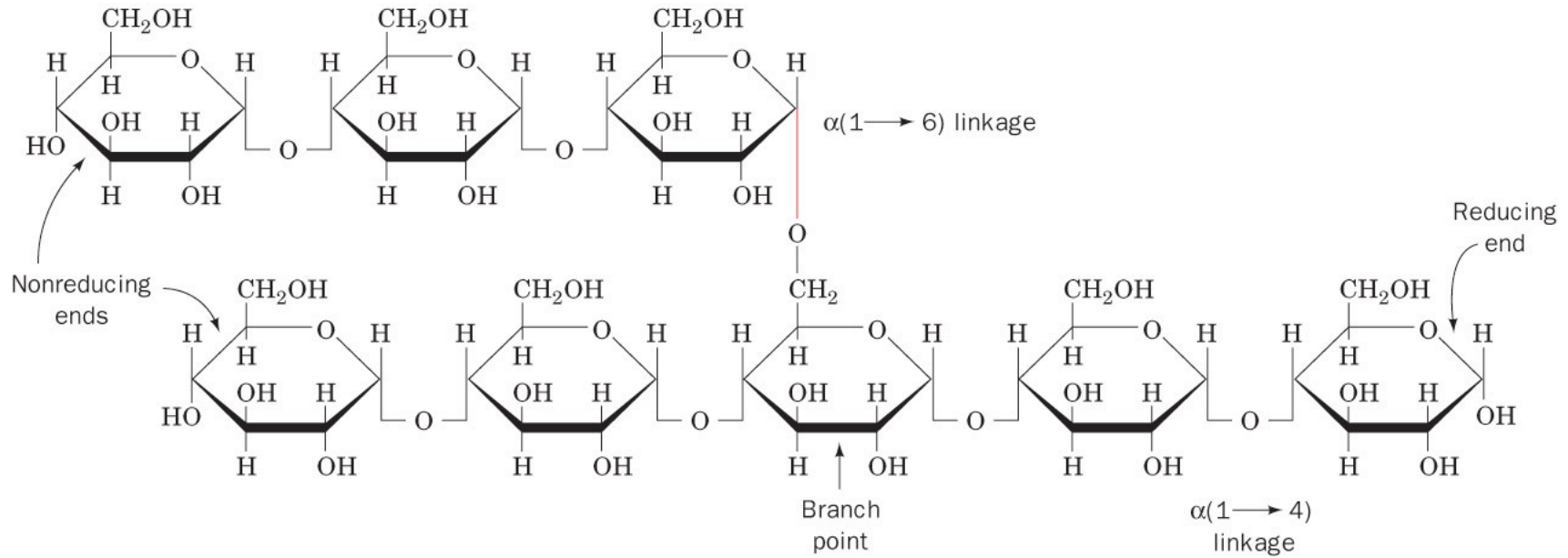
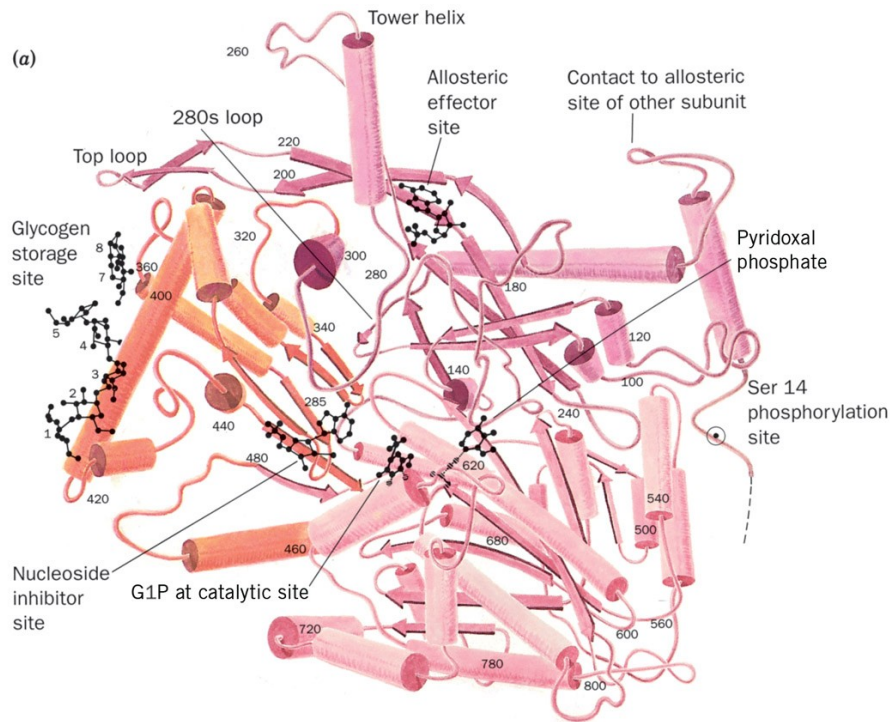


Figure 21-3
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Glycogen is a highly branched macromolecule that permits rapid hydrolysis (phosphorylatic cleavage) to rapidly release of glucose. Branch points are separated by 8 to 12 glucosyl residues. It can be hydrolysed from each branch of a glycogen particle simultaneously. In striated muscle glycogen is 1-2% of the cells dry weight while in liver its 10% dry weight, and in *Trichomonas vaginalis* or *Giardia intestinalis* it is 15%. Glycogen forms a left handed helix, 6.5 residues / turn. Glycogen breakdown depends on three enzymes; **glycogen phosphorylase**, **debranching enzyme** and **phosphoglucomutase**.



Structure of rabbit muscle glycogen phosphorylase, diagram of a phosphorylase b subunit.

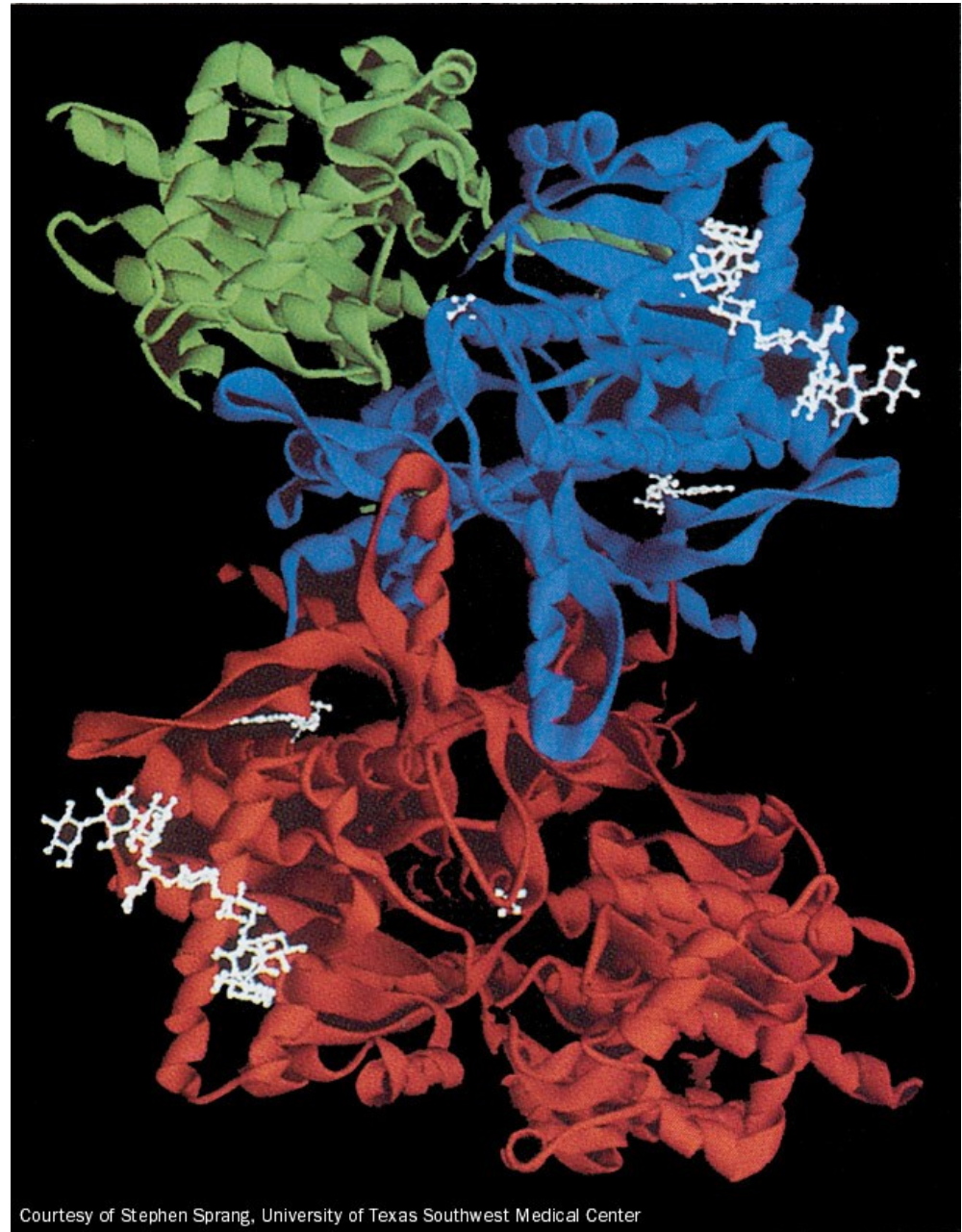
It is a homodimer of 97kD, 842 amino acid residues/ subunit.

Glycogen phosphorylase a has a PO_4 group esterified to ser 14 in each each subunit.

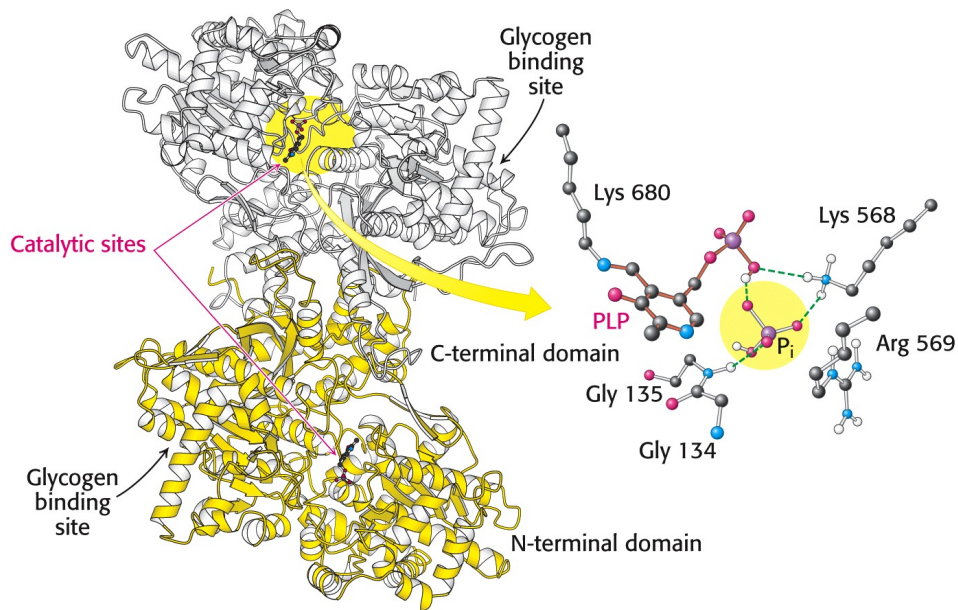
Phosphorylase is the rate limiting step in glycogen phosphoarlysis and is allosteric inhibitors ATP, G6P, glucose.
The major allosteric activator AMP.

Sensitivity of the phosphorylase to these modulators are dependent on whether the enzyme's subunits are phosphorylated.

X-Ray structure of rabbit muscle glycogen phosphorylase. A ribbon diagram of the glycogen phosphorylase a dimer. The dimer has a two fold axis of symmetry. In the top peptide, the N-terminal is in blue and the C-terminal in green. Glycogen is bound to the glycogen storage site, with a PO_4 in the catalytic site, and AMP in the allosteric site.

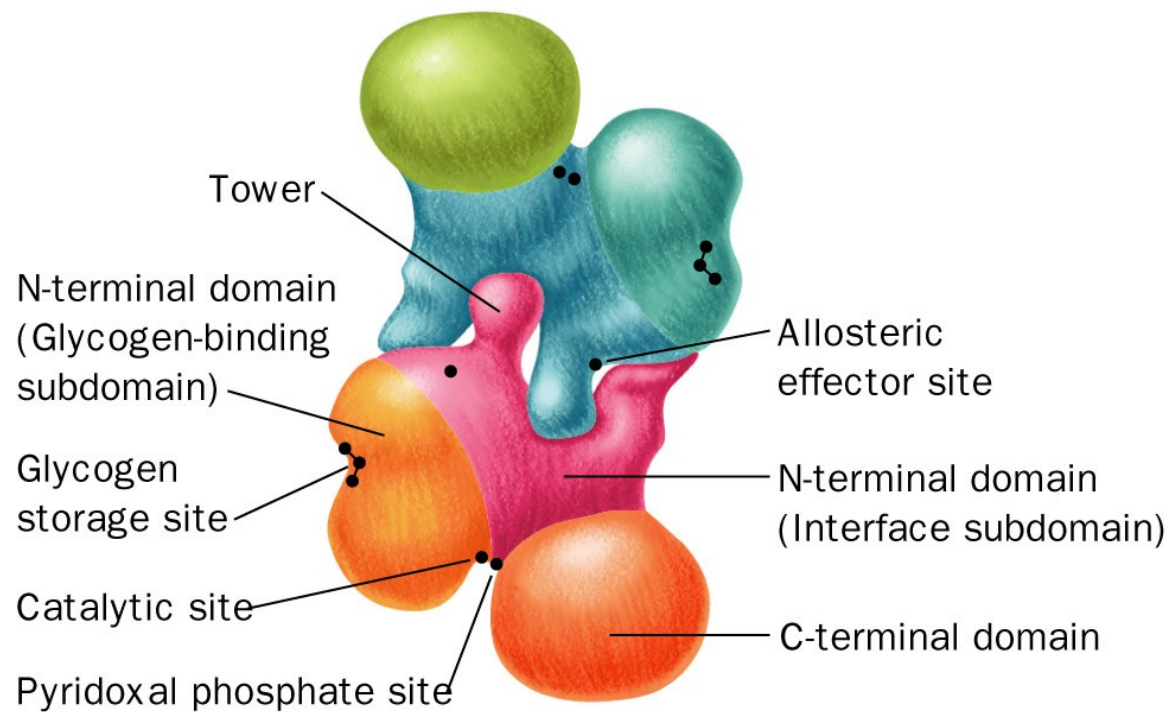


Courtesy of Stephen Sprang, University of Texas Southwest Medical Center

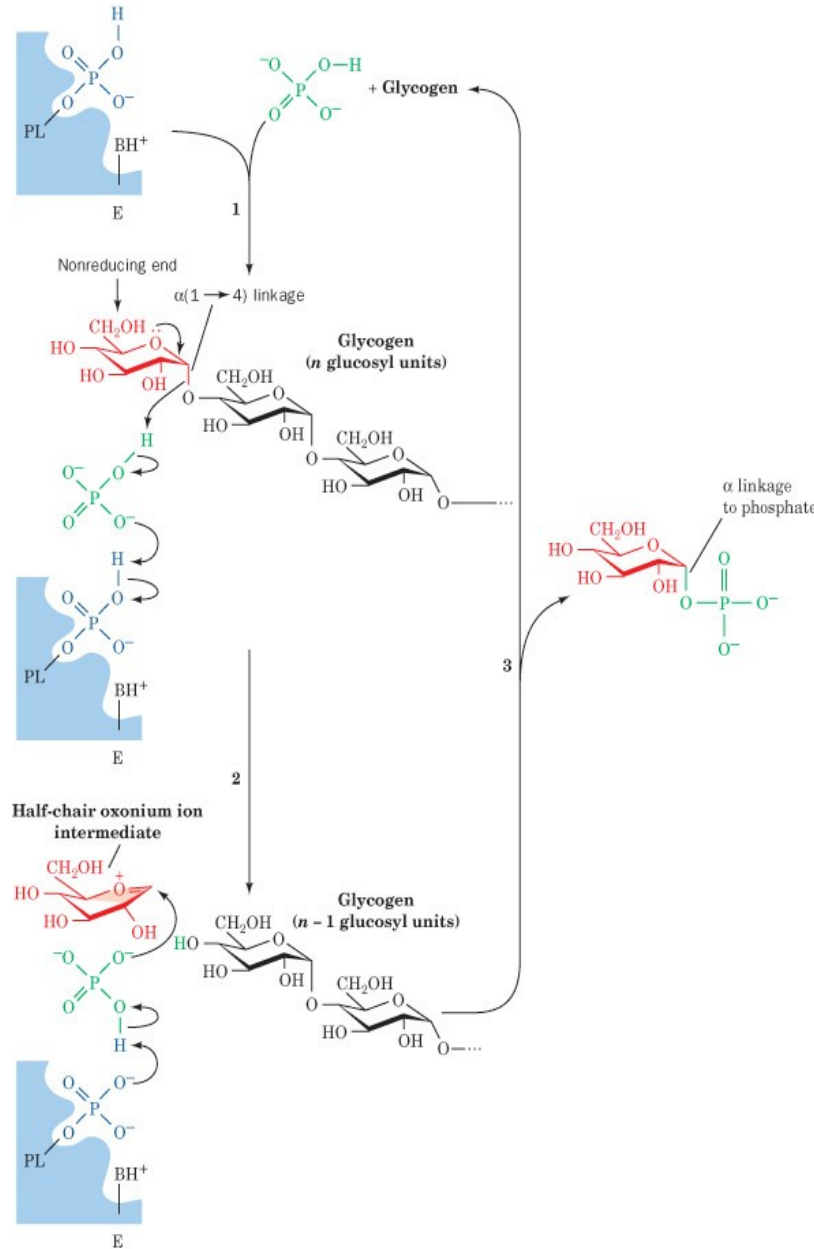


30Å crevice on the surface of each monomer has the same radius as the glycogen molecule. This crevice accommodates 4-5 glucose residues, connecting the binding site to the catalytic site. It is too narrow for branched oligosacch. ***What problem do you then see in the rapid hydrolysis of glycogen?***

PLP is covalently bound and functions in this reaction differently than in amino acid metabolism. The phosphoryl group will aid in the acid-base catalysis.



An interpretive “low-resolution” drawing showing the enzyme’s various ligand-binding sites. The glycogen storage site, which binds glycogen to the active site, increases the catalytic efficiency by of the phosphorylase by permitting it to phosphorylate many glucosyl residues on the same branch of a glycogen particle without having to dissociate and re-associate between catalytic cycles.



The reaction mechanism of glycogen phosphorylase.

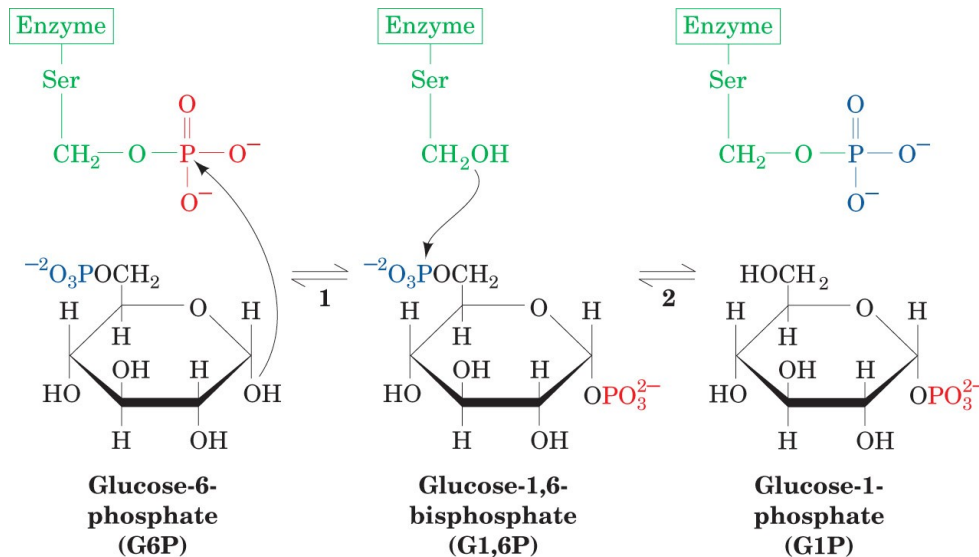
This reaction results in the cleavage of the C1-OH bond from the non-reducing terminal glucosyl residue to yield G1P.

The mechanism is very similar to that of **Lysozyme** and **MTA phosphorylase**.

1. Formation of a E-P_i-glycogen ternary complex.

2. Formation of an oxonium ion intermediate from the α -linked terminal glucosyl residue, acid catalysis by P_i facilitated by the proton transfer from PLP. Note half chair of oxonium ion intermediate.

3. Rxn with P_i forms G1P.
But how does a cell utilize G1P?



The mechanism of phosphoglucomutase. Phosphorylase converts glucosyl unit of glycogen to G1P. The rxn catalysed by phosphoglucomutase is similar to **phosphoglycerate mutase**, intermediate form is G1,6BP. The enzyme transfers a P_i to the 6 carbon and the E is re-PO₄ by the P_i on the C-1. G1,6BP dissociation leads to **phosphoglucomutase** inactivation. Small amounts of G1,6P are necessary to keep enzyme fully active. Phosphoglucokinase phosphorylates G-6-P in the 1 position to form G 1,6BP. Debranching enzyme acts as 1. α(1→4) transglycosylase, transferring oligosacch to non-reducing end. 2. α(1→6) glucosidase rxn, yeilding a glucose residue. Same enzyme has two different active sites.

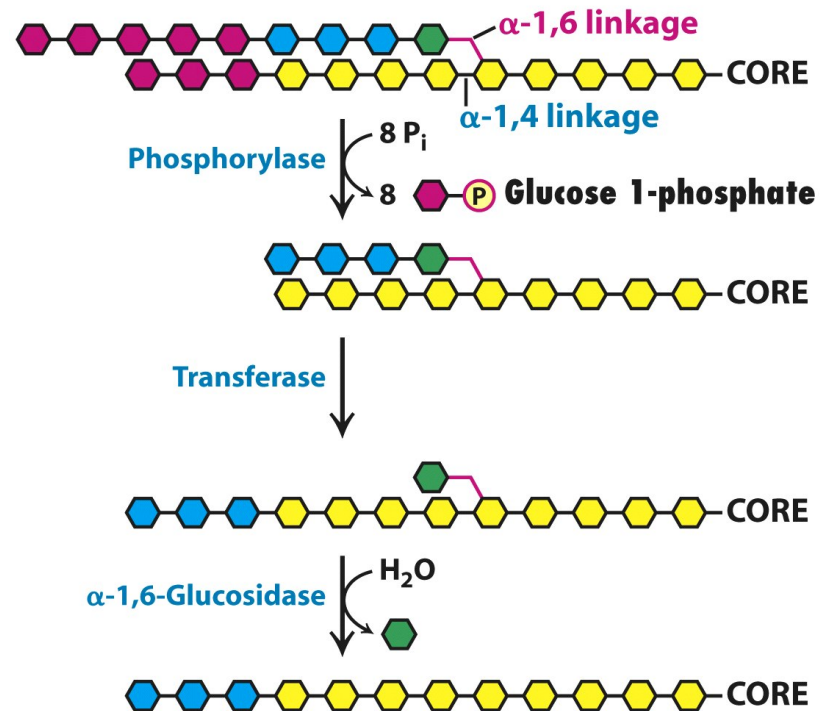
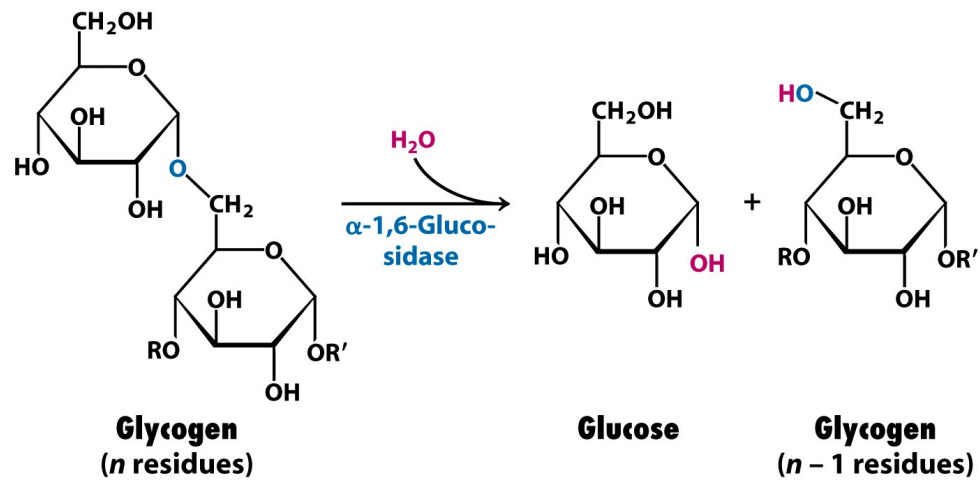


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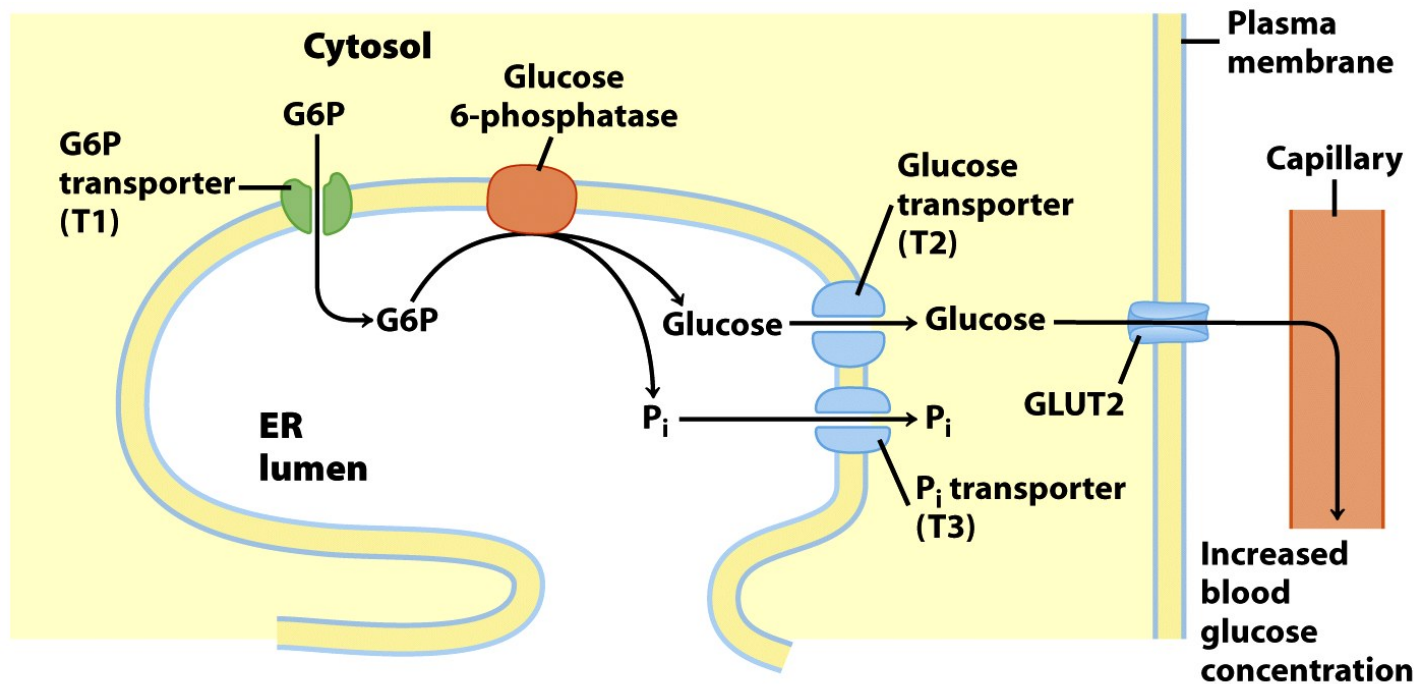
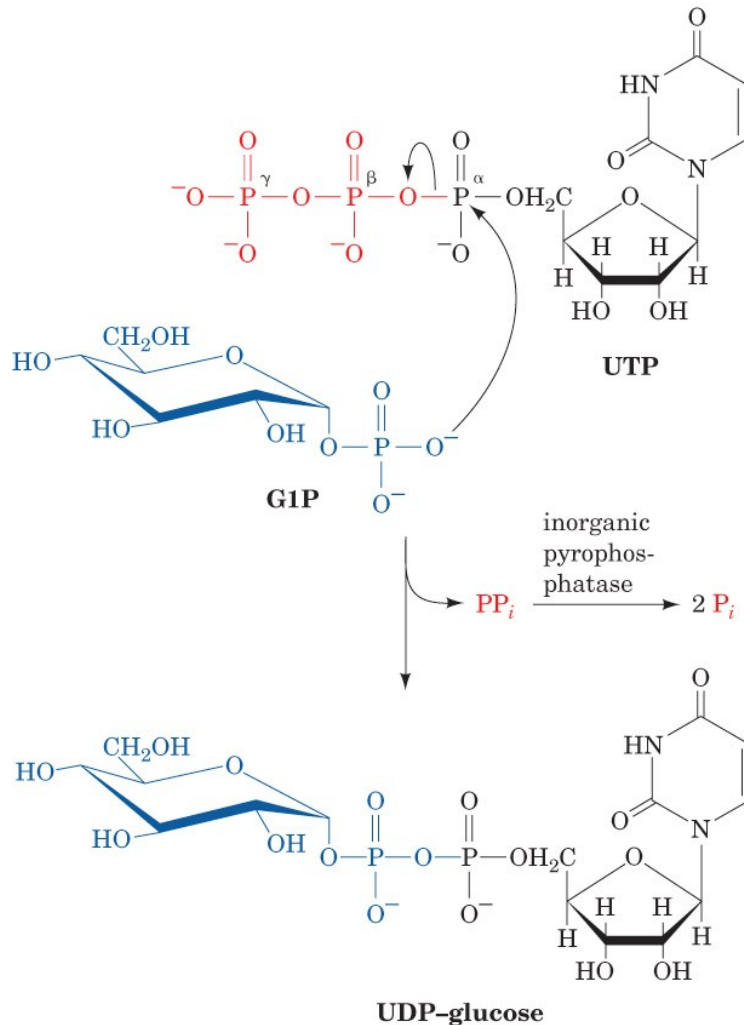


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Hydrolysis of glucose 6-phosphate by glucose 6-phosphatase of the ER. The catalytic site of glucose 6-phosphatase faces the lumen of the ER. A G6P transporter (T1) carries the substrate from the cytosol to the lumen, and the products glucose and P_i pass to the cytosol on specific transporters (T2 and T3). Glucose leaves the cell via the GLUT2 transporter in the plasma membrane.

Thermodynamics of glycogen metabolism

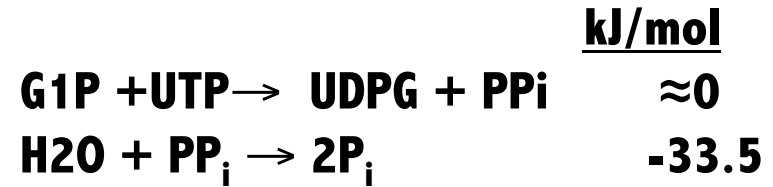
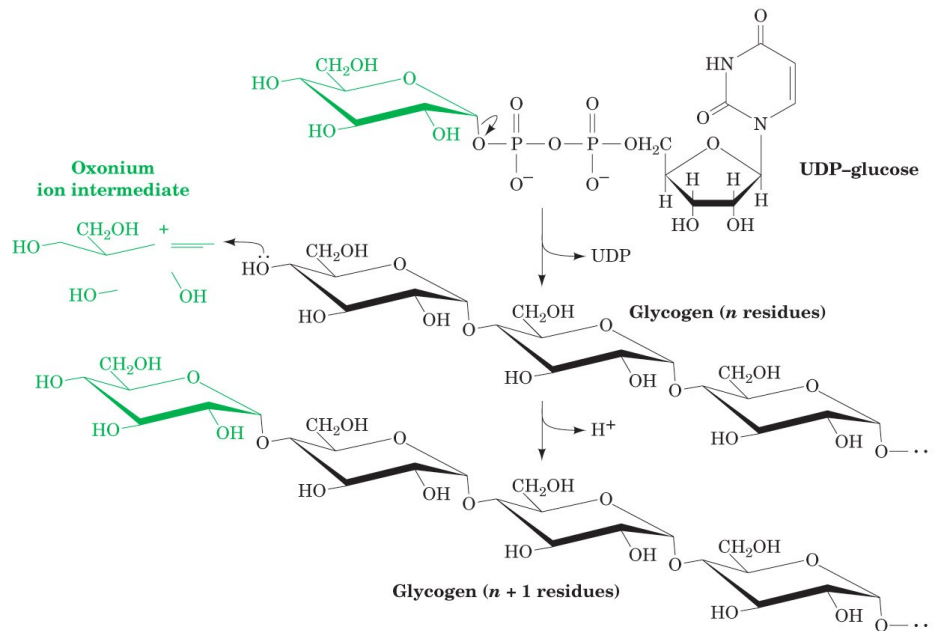
- Under physiological conditions phosphorylation of glycogen is exergonic, -5 to -8 KJ/mol
- The formation of G1P under physiological condition is unfavorable, requiring free energy input.
- Consequently breakdown and synthesis must be separate pathways.
- This allows reciprocal controls and independent regulation of each pathway.
- Since the synthesis of glycogen from G1P is thermodynamically unfavorable it requires a supply of energy.



Glycogen synthesis Reaction catalyzed by UDP–glucose pyrophosphorylase.

Glycogen synthesis requires an additional exergonic step, formation of UDP-glucose.

Three enzymes catalyse the formation of glycogen; ***UDP–glucose pyrophosphorylase, glycogen synthase & glycogen branching enzyme.***

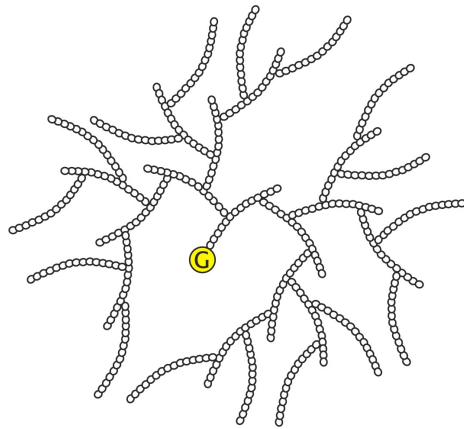


This rxn is catalysed by the enzyme UDP-glucose pyrophosphorylase.

This rxn is catalysed by pyrophosphate phospholytically cleaved from the UTP, this is energetically possible due to the hydrolysis of PP_i → 2P_i.

Reaction catalyzed by glycogen synthase. The UDPG is transferred to the C4-OH of the non-reducing end of glycogen forming an α(1→4) glycosidic bond. UDP is recycled to UTP by **nucleoside diphosphate kinase**.

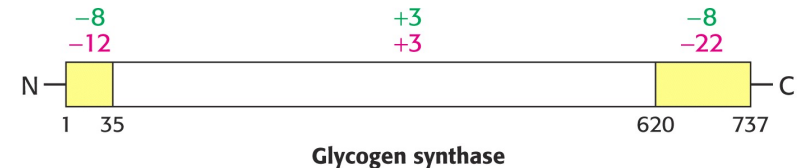
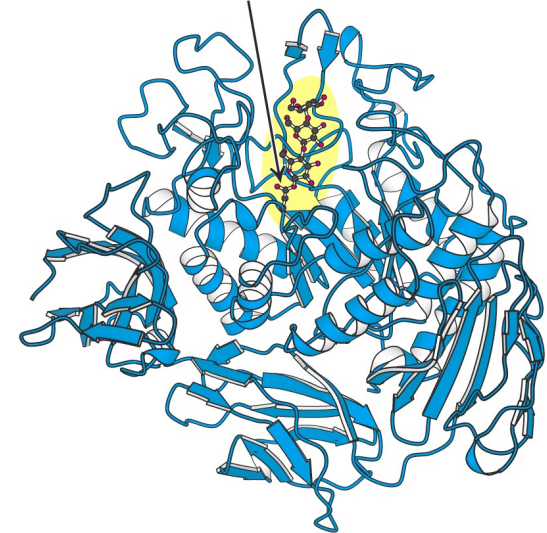
Glycogen synthase can only extend an existing glucan chain. What forms the primer? **Glycogenin**. It forms the heptamer needed as a primer to be extended by glycogen synthase.



Branching is accomplished by a separate enzyme, amylo(1,4→1,6) transglycosylase (branching enzyme). Breaking the $\alpha(1\rightarrow4)$ is **-15.5 kJ/ mol** & form the $\alpha(1\rightarrow6)$ is **-7kJ/mol**. Hydrolysis of the $\alpha(1\rightarrow4)$ drives the formation of $\alpha(1\rightarrow6)$ glycosidic bonds and branch transfer.

Branching causes increased solubility of glycogen & increases the rate of synthesis or hydrolysis. There is a conserved Asp residue found in the branching and debranching enzymes. The Asp residue may bind the oligosacch for transfer.

Carbohydrate linked to conserved Asp residue



Like the phosphorylase, the synthase is regulated by covalent modification. Phosphorylation has opposite effects on the glycogen phosphorylase & synthase. PO_4 converts active synthase a into inactive b form.

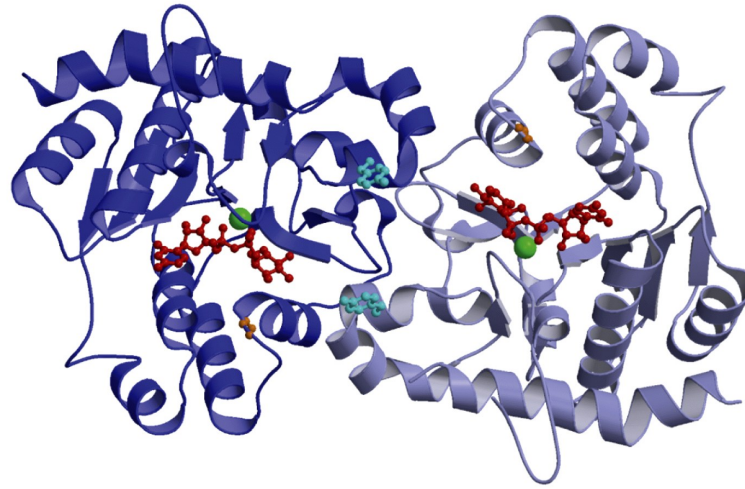
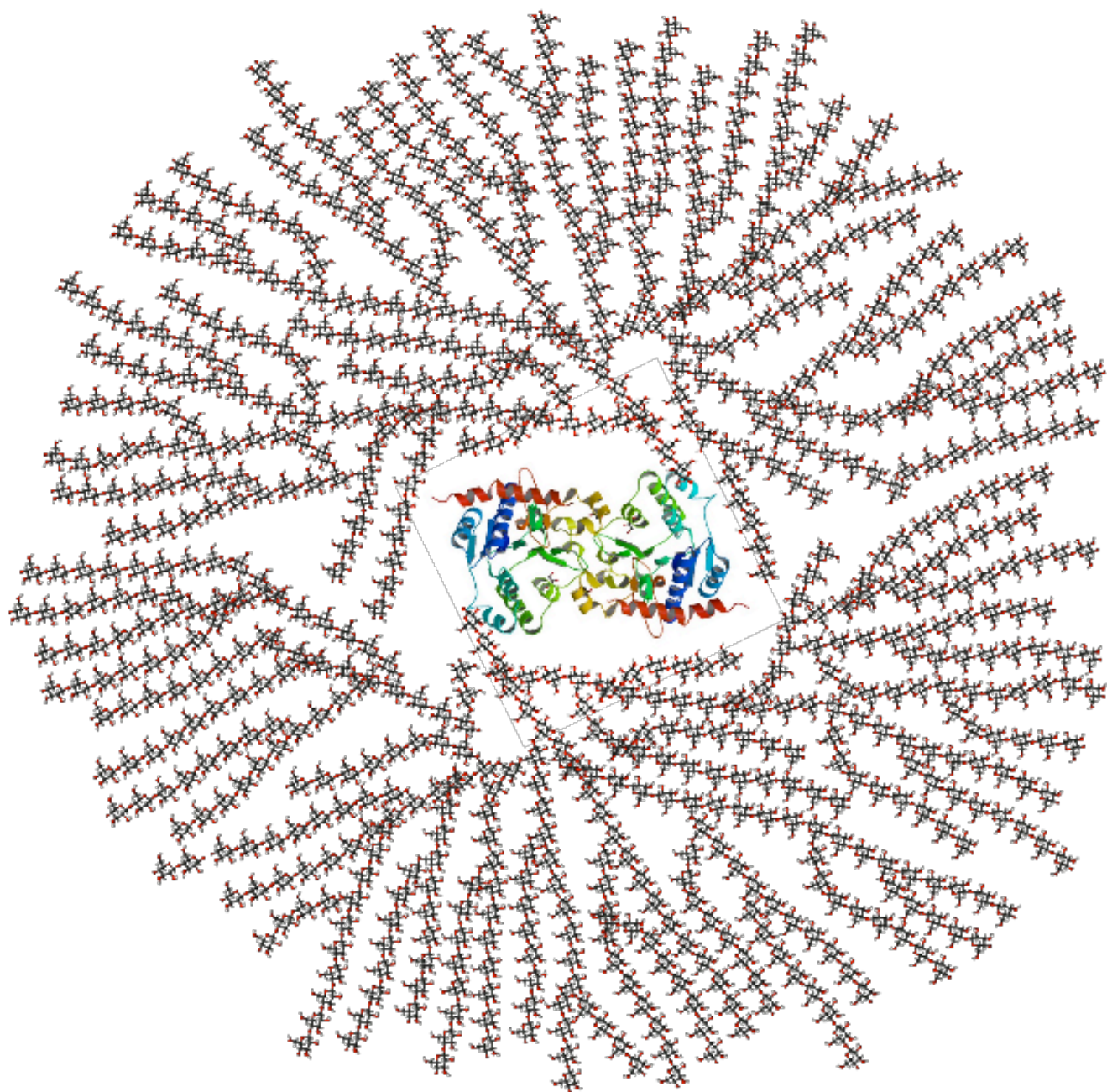
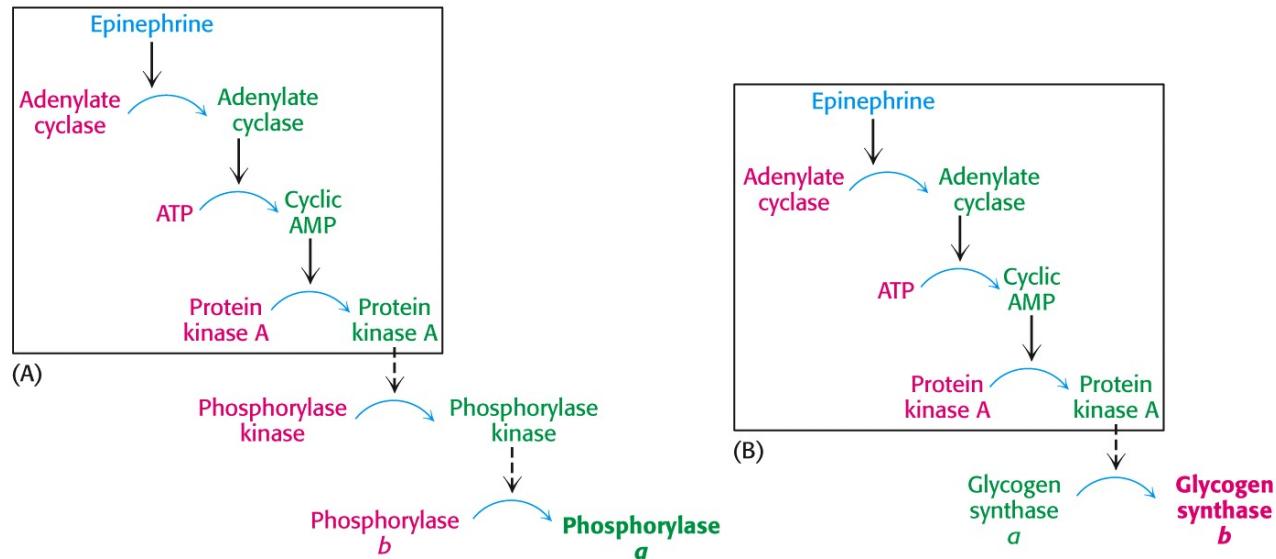


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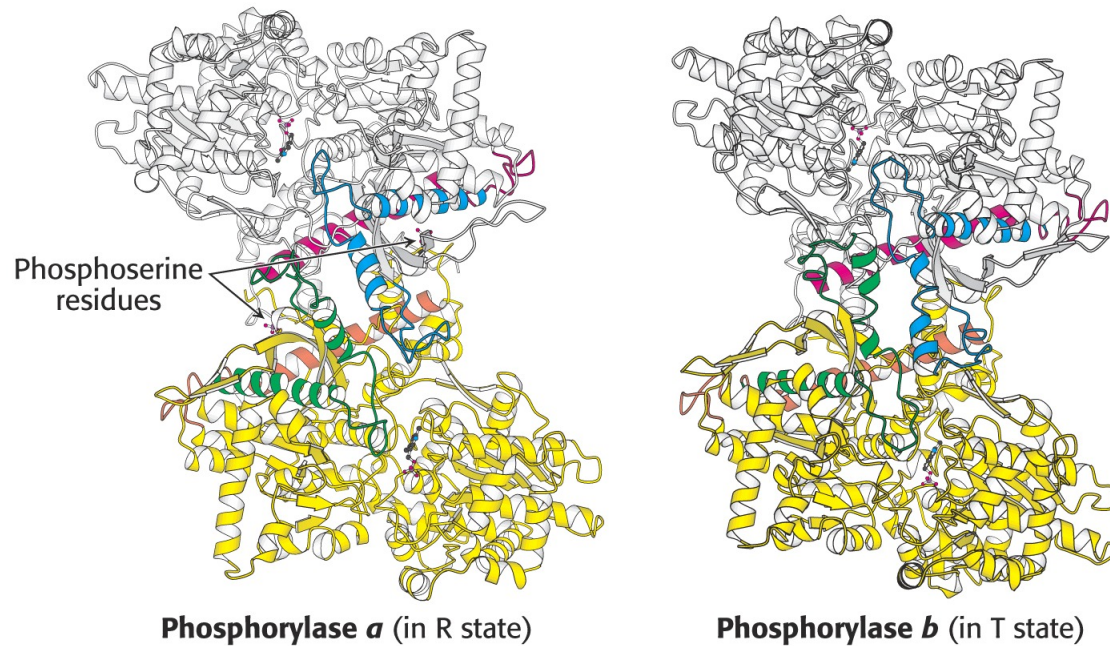
Muscle glycogenin (M_r 37,000) forms dimers in solution. Humans have a second isoform in liver, glycogenin-2. The substrate, UDP-glucose (shown as a red ball-and-stick structure), is bound to a Rossmann fold near the amino terminus and is some distance from the Tyr¹⁹⁴ residues (turquoise)—15 Å from the Tyr in the same monomer, 12 Å from the Tyr in the dimeric partner. Each UDP-glucose is bound through its phosphates to a **Mn²⁺ ion (green)** that is essential to catalysis. Mn²⁺ is believed to function as an electron-pair acceptor (Lewis acid) to stabilize the leaving group, UDP. The glycosidic bond in the product has the same configuration about the C-1 of glucose as the substrate UDP-glucose, suggesting that the transfer of glucose from UDP to Tyr¹⁹⁴ occurs in two steps. The first step is probably a nucleophilic attack by Asp¹⁶² (orange), forming a temporary intermediate with inverted configuration. A second nucleophilic attack by Tyr¹⁹⁴ then restores the starting configuration, this is accomplished by **tyrosine glucosyltransferase**. This forms a primer on glycogenin that can be extended by glycogen synthase.



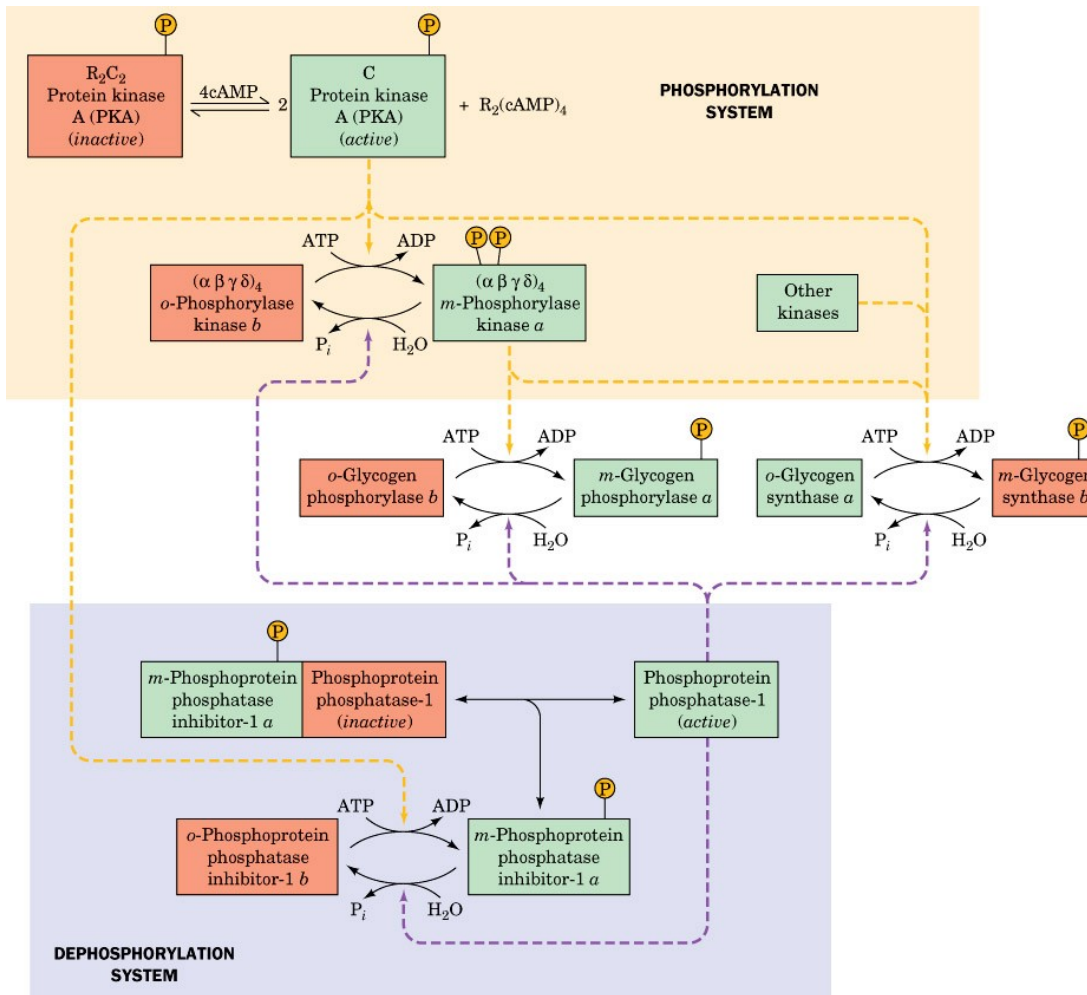
The control reciprocal pathways of glycogen synthesis and phosphoroylsis



As glycogen hydrolysis is activated, glycogen synthesis is being turned off, otherwise it would be a futile cycle and glycogen synthesis would be competing for glucose molecules that are for export to muscles and other tissues. These pathways are reciprocally regulated by hormone triggered **[cAMP]** cascades of PKA & IP₃ release of Ca⁺² with DAG triggered cAMP. The actions of the PKA are reversed by **protein phosphatase (PP1)**, which has a central role in the reciprocity of these pathways. PP1 inactivates phosphorylase kinase and **glycogen phosphorylase a** by dephosphorylating these enzymes. It removes PO₄ from inactive **glycogen synthase b** to convert it to the active **a** form. It accelerates glycogen synthesis.

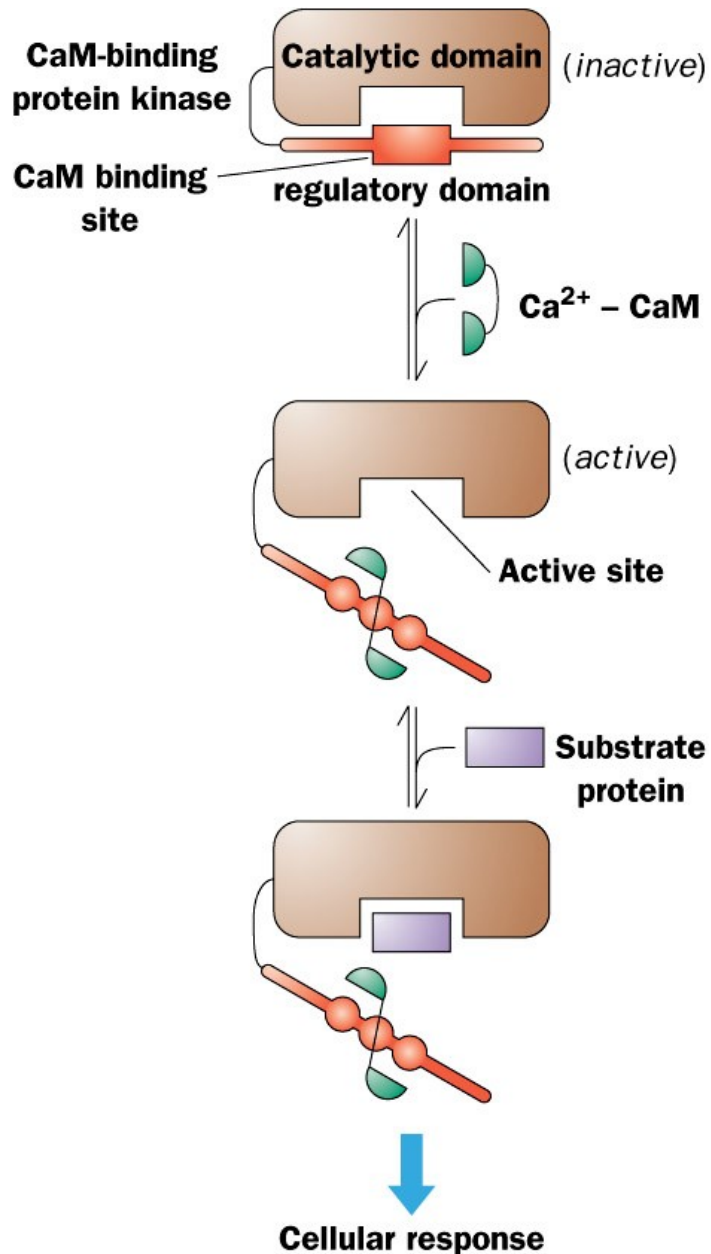


The structural difference between the R & T conformations are, in the T the state the enzyme active site is buried, hence the low affinity for the substrate, in the R state the enzyme has an accessible catalytic site and high affinity phosphate binding site. AMP promotes $T_{(inactive)} \rightarrow R_{(active)}$ conformational shift. ATP binds to the allosteric effector site in the T conformation and it inhibits the $T_{(inactive)} \rightarrow R_{(active)}$ shift.



Major enzymatic modification/demodification systems involved in the control of glycogen metabolism in muscle.

Phosphorylase kinase (PhK) is itself covalently modified. For it to be fully active Ca^{+2} must be present and it must be phosphorylated. cAMP activated **PKA**, that phosphorylates both **PhK** and **glycogen synthase**. The δ subunit of **PhK** is **calmodulin (CaM)**. Binding of Ca^{+2} to the **CaM** subunit caused conformation changes in **PhK** that leads to its activation which then phosphorylates glycogen phosphorylase increasing the breakdown of glycogen, increasing glycolysis activity and increasing ATP synthesis.



Schematic diagram of the Ca^{2+} -CaM-dependent activation of protein kinases. Auto-inhibited kinases have an N or C terminal pseudo-substrate sequence. It make the active site inaccessible to substrate. The **CaM** subunit binds near the auto-inhibitory sequence and the activation of **CaM** by binding Ca^{2+} , binds to the auto-inhibitory sequence and it opens up the access to the active site. It can now bind to proteins and phosphorylates them. The activity is dependent on the Ca^{2+} availability.

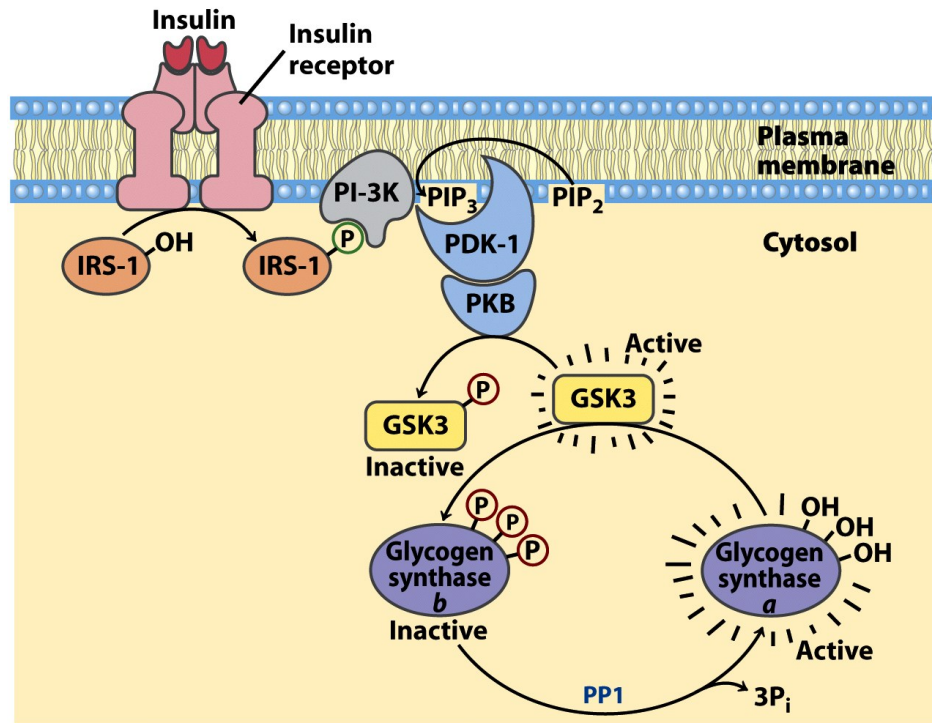


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The path from insulin to GSK3 and glycogen synthase. Insulin binding to its receptor activates a tyrosine protein kinase in the receptor, which phosphorylates insulin receptor substrate-1 (IRS-1). The phosphotyrosine in this protein is then bound by phosphatidylinositol 3-kinase (PI-3K), which converts phosphatidylinositol 4,5-bisphosphate (PIP₂) in the membrane to phosphatidylinositol 3,4,5-trisphosphate (PIP₃). A protein kinase (PDK-1) that is activated when bound to PIP₃ activates a second protein kinase (PKB), which phosphorylates glycogen synthase kinase 3 (GSK3) in its pseudosubstrate region. The inactivation of GSK3 allows phosphoprotein phosphatase 1 (PP1) to dephosphorylate and thus activate glycogen synthase. In this way, insulin stimulates glycogen synthesis.

Effects of GSK3 on glycogen synthase activity.

Glycogen synthase *a*, the active form, has three Ser residues near its carboxyl terminus, which are phosphorylated by glycogen synthase kinase 3 (GSK3). This converts glycogen synthase to the inactive (*b*) form. GSK3 action requires prior phosphorylation (priming) by casein kinase (CKII). Insulin triggers activation of glycogen synthase *b* by blocking the activity of GSK3 and activating a phosphoprotein phosphatase (PP1 in muscle, another phosphatase in liver). In muscle, epinephrine activates PKA, which phosphorylates the glycogen-targeting protein GM on a site that causes dissociation of PP1 from glycogen. Glucose 6-phosphate favors dephosphorylation of glycogen synthase by binding to it and promoting a conformation that is a good substrate for PP1. Glucose also promotes dephosphorylation; the binding of glucose to glycogen phosphorylase *a* forces a conformational change that favors dephosphorylation to glycogen phosphorylase *b*, thus relieving its inhibition of PP1.

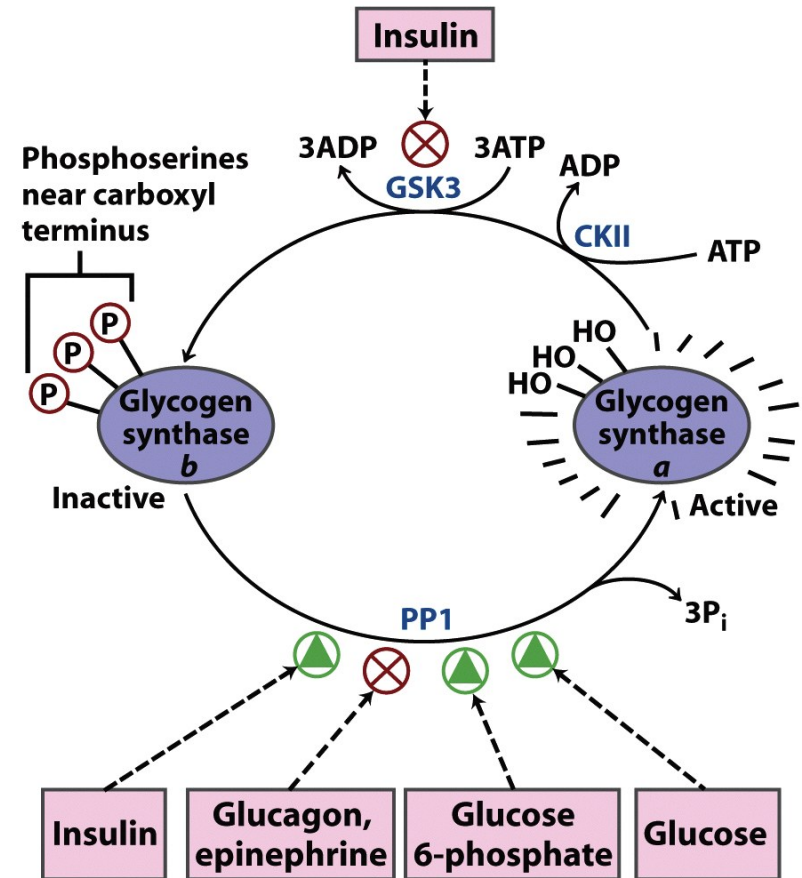
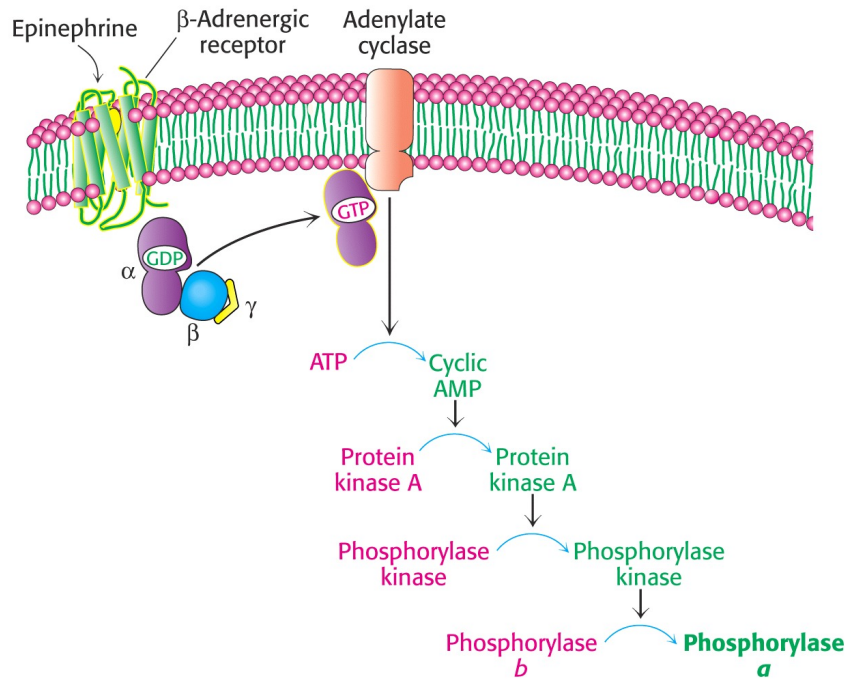


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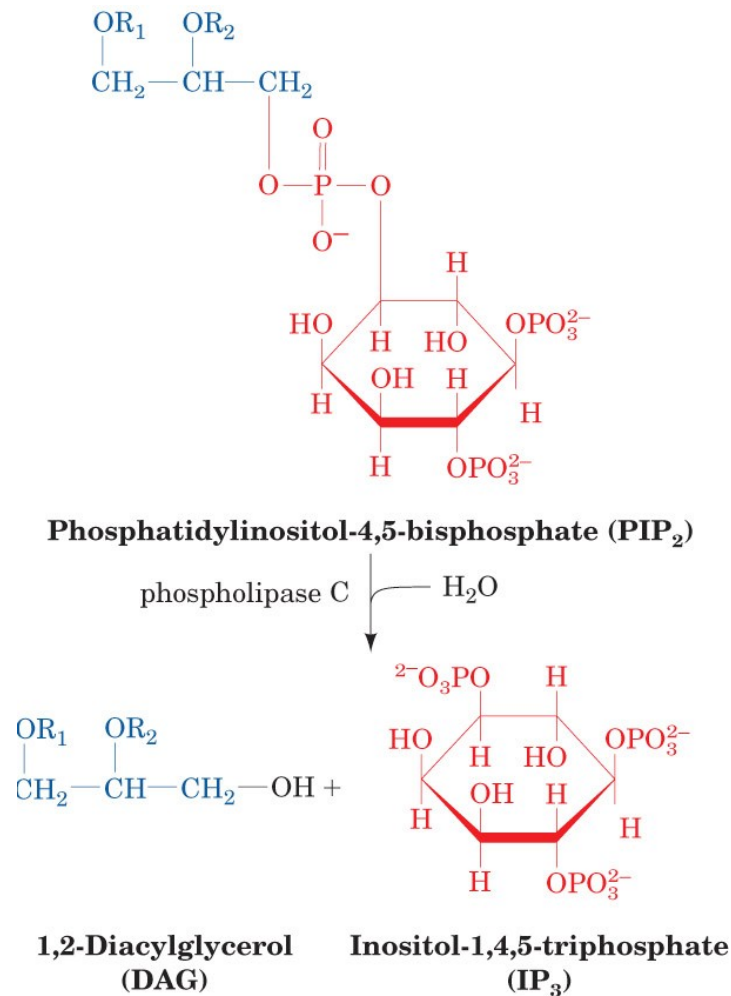


In muscle **Epi** & **Ins** have antagonistic effects on glycogen metabolism. **Epi** promotes glycogenolysis by activating cAMP dependent phosphorylation cascade, which stimulate glycogen hydrolysis & inhibits glycogen synthesis.

Ins activates **insulin-stimulated protein kinase** to PO_4 site1 on G_M subunit of **PP1**.

In liver, glucose & G6P inhibit the phosphorylase a by binding only to the active site of the enzymes inactive T state, the presence of glucose the shifts the $\text{T} \rightleftharpoons \text{R}$ equilibrium toward T, which causes ser 14 to be accessible to **PP1**. Release of **PP1** inhibition causes activation of **glycogen synthase**.

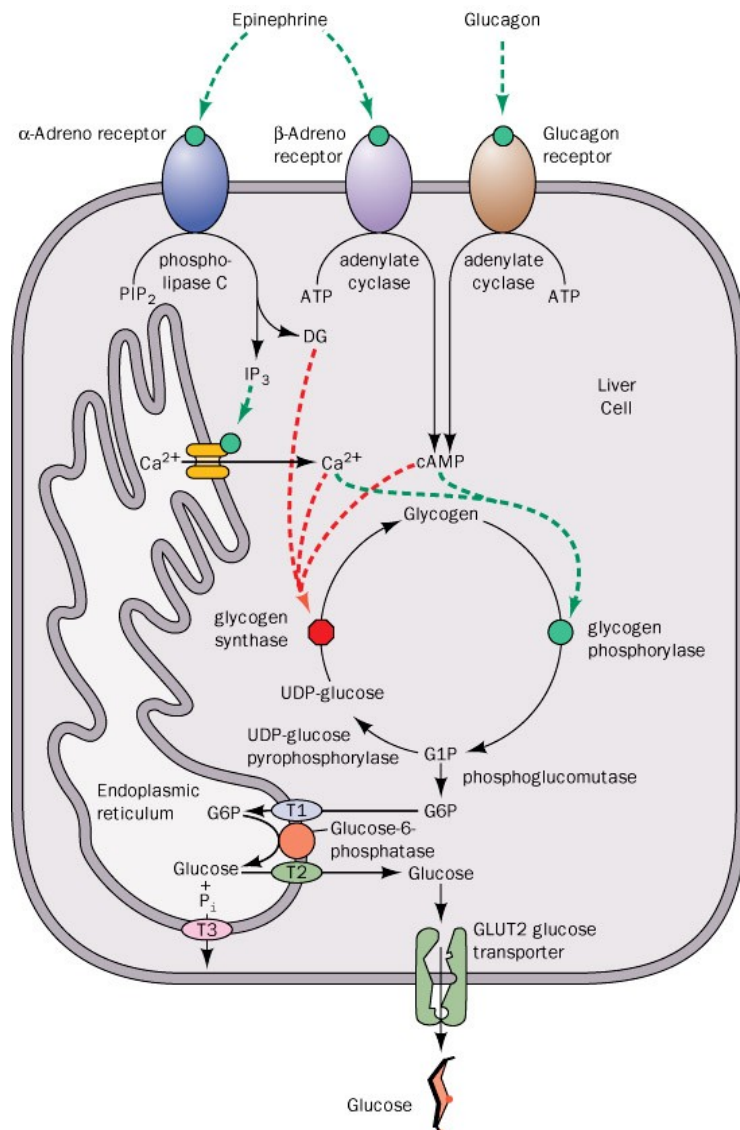
Glucokinase formation of G6P, causes further facilitation of conversion of glycogen synthase a to the active form.



Liver's response to stress. Stimulation of both the α & β -adrenoreceptors by epinephrine. **Epi** activates phospholipase C to hydrolyze PIP₂ to IP₃ and DAG.

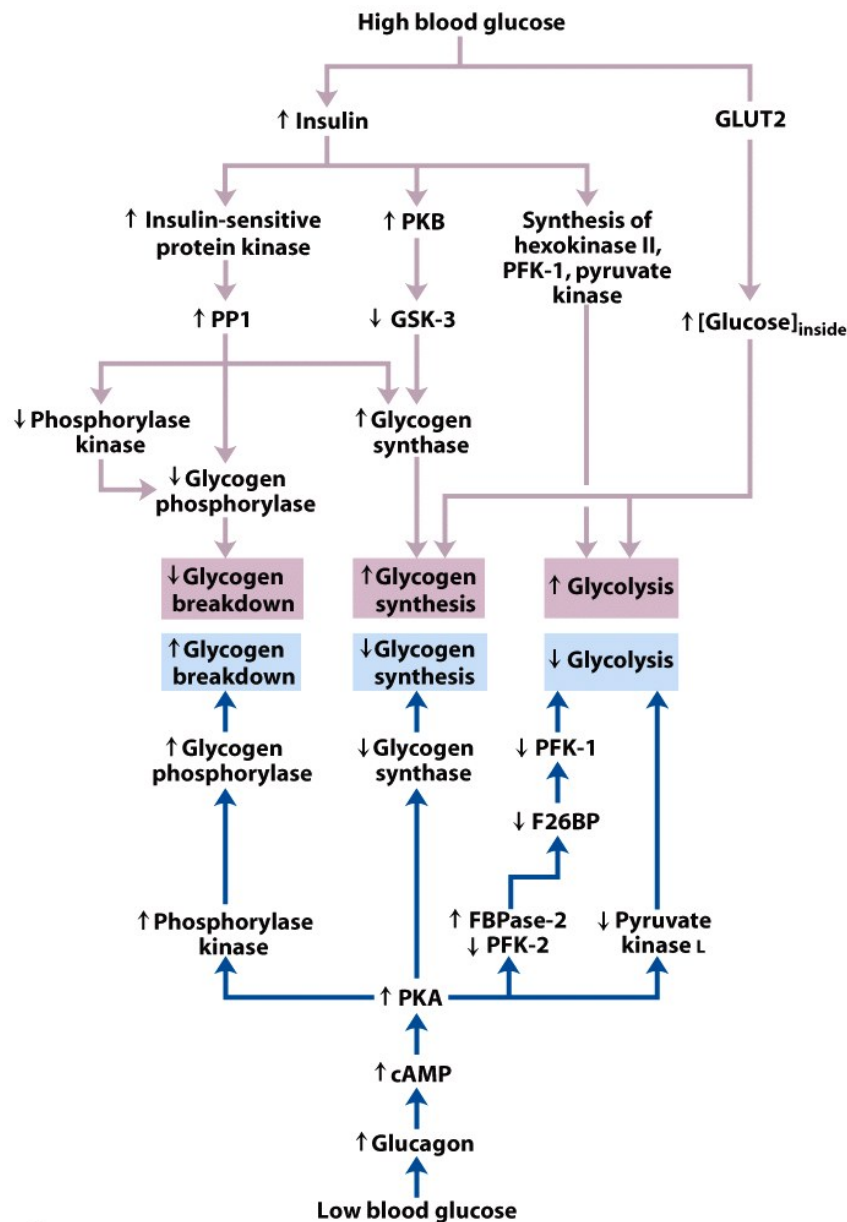
Both of these lead to rapid increases in [cAMP] & Ca⁺². The release of Ca reinforces the effects of cAMP. PhK which activates glycogen phosphorylase and inactivates glycogen synthase, is only fully active when it is phosphorylated and in the presence of Ca⁺².

Glycogen synthase is phosphorylated and inactivated by several other enzymes. In the presence of Ca⁺² & DAG PKC is activated and it will also phosphorylate the synthase.



The liver's response to stress. The participation of two second messenger systems. Stimulation of the α adrenoreceptor by epi activates **phospholipase C** to hydrolyse **PIP_2 to IP_3 & DAG**. The participation of two second message systems: the cAMP mediated glycogenolysis and inhibition of glycogen synthesis triggered by glucagon and the β adrenoreceptor activation; & **IP_3 , DAG and Ca^{2+}** mediated stimulation of glycogenolysis as well as inhibition of glycogen synthesis. DAG & Ca^{2+} activate PKC that PO_4 glycogen synthase causing inactivation.

G6Pase is an ER transmembr protein. **T1 G6P translocase(T1)** bring in the G6P, G6Pase metabolizes it to glucose + P_i and **T2 & T3** transport glucose & P_i repectively to cytosol. GLUT2 transports glucose into blood.



Regulation of carbohydrate metabolism in the liver. Arrows indicate causal relationships between the changes they connect. For example, an arrow from $\downarrow A$ to $\uparrow B$ means that a decrease in A causes an increase in B. Pink arrows connect events that result from high blood glucose; blue arrows connect events that result from low blood glucose.

Figure 15-41

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Lets take an overview, before
moving on to the TCA cycle

- The major carbohydrate pathways are the **Embden-Myerhoff-Pernas (glycolysis)**, **Pentose Phosphate** and **Entner Doudoroff** pathways.
- All 3 pathways convert glucose to **GAP**, although through different routes
- GAP and is oxidized to pyruvate via the same reactions.
- The importance of the **Pentose Phosphate** pathway is that it produces **NADPH and ribose 5 PO₄**.
- The PP pathway is the precursor of ribose 5 PO₄, precursor for nucleotide biosynthesis, histidine biosynthesis and several other pathways.
- Erythrose phosphate formed in the non-oxidative portion of the **PP** pathway, is the starting material for aromatic amino acids; phenylalanine, tyrosine and tryptophan.

Central Metabolic Pathways

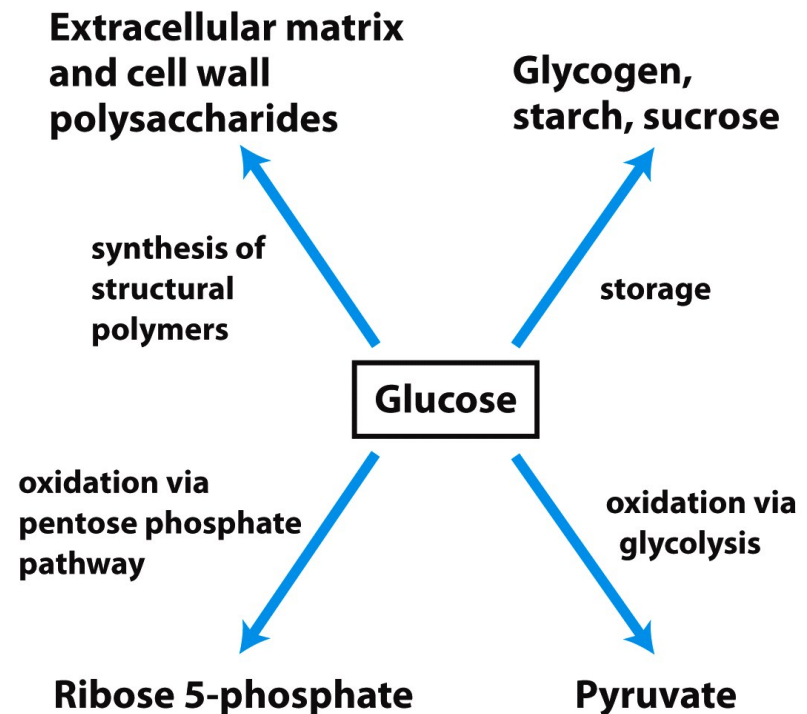


Figure 14-1
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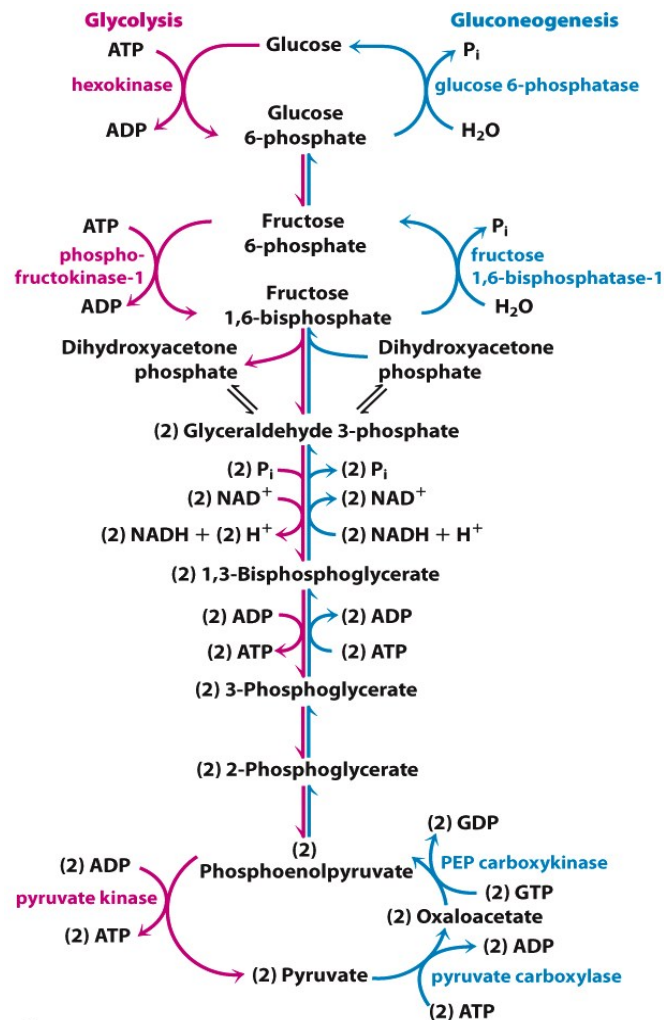


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Opposing pathways of glycolysis and gluconeogenesis in rat liver.

The reactions of glycolysis are on the left side, in red; the opposing pathway of gluconeogenesis is on the right, in blue. The major sites of regulation of gluconeogenesis are those glycolytic reactions that are thermodynamically irreversible. The $\Delta G^{\circ'}$ for these reactions combined = **- 22.6kJ/mole**.

The transcription of PEPCK is stimulated by glucagon and inhibited by insulin. PEPCK gene promoter contains a cAMP binding element (CRE) this is bound by a transcriptional factor called the CRE binding protein (CREB). The PEPCK promoter has other binding sites for other specific factors such as Thyroid Hormone Response Element. PEPCK transcription is repressed by protein factors phosphorylated by PI3K signal cascade initiated by the binding of insulin.

Alternative paths from pyruvate to phosphoenolpyruvate.

The relative importance of the two pathways depends on the availability of lactate or pyruvate (alanine deamination) and the cytosolic requirements for NADH for gluconeogenesis. The path on the right predominates when lactate is the precursor, because cytosolic NADH is generated in the lactate dehydrogenase reaction and does not have to be shuttled out of the mitochondrion.

Constitutive to several pathways is pyruvate carboxylase which produces OAA.

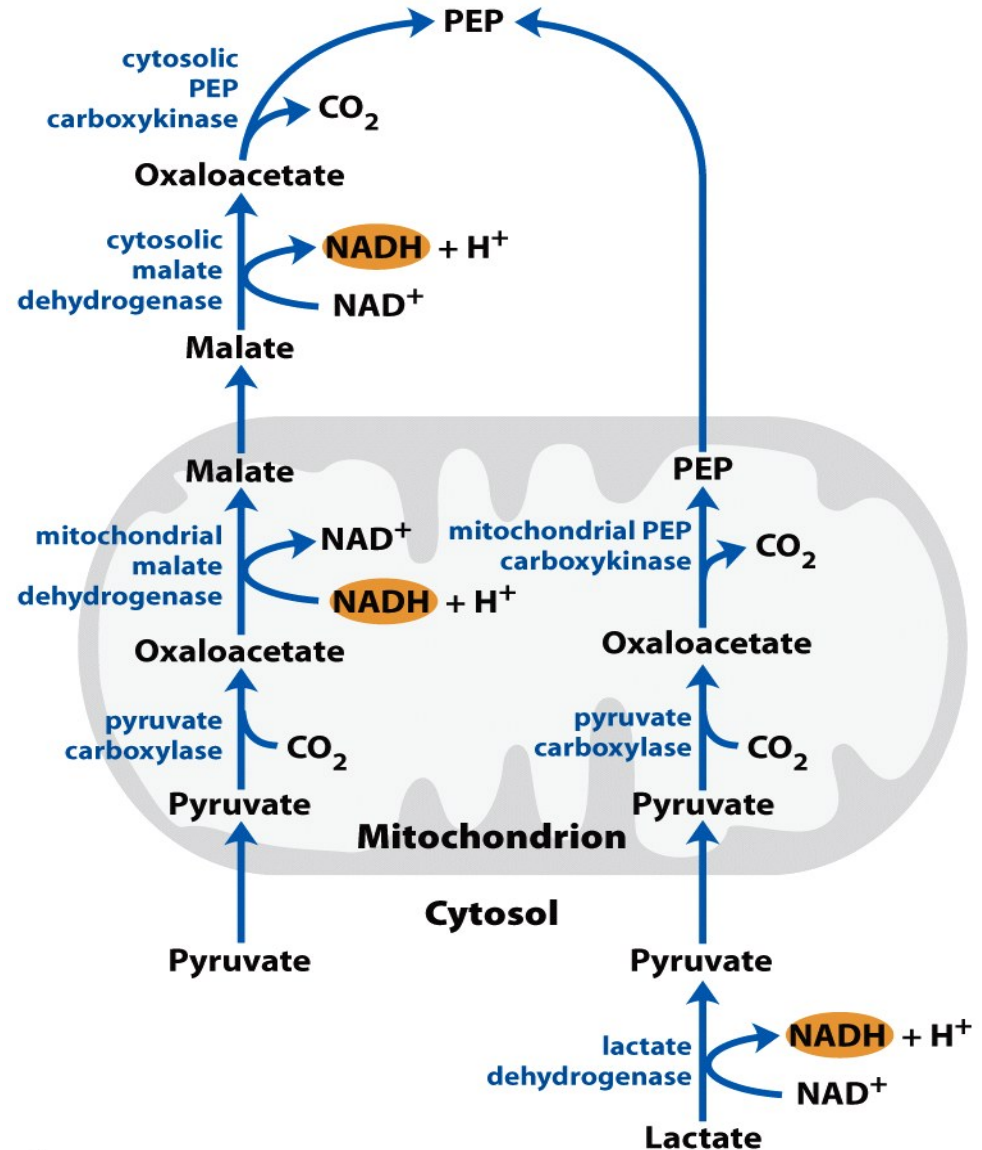


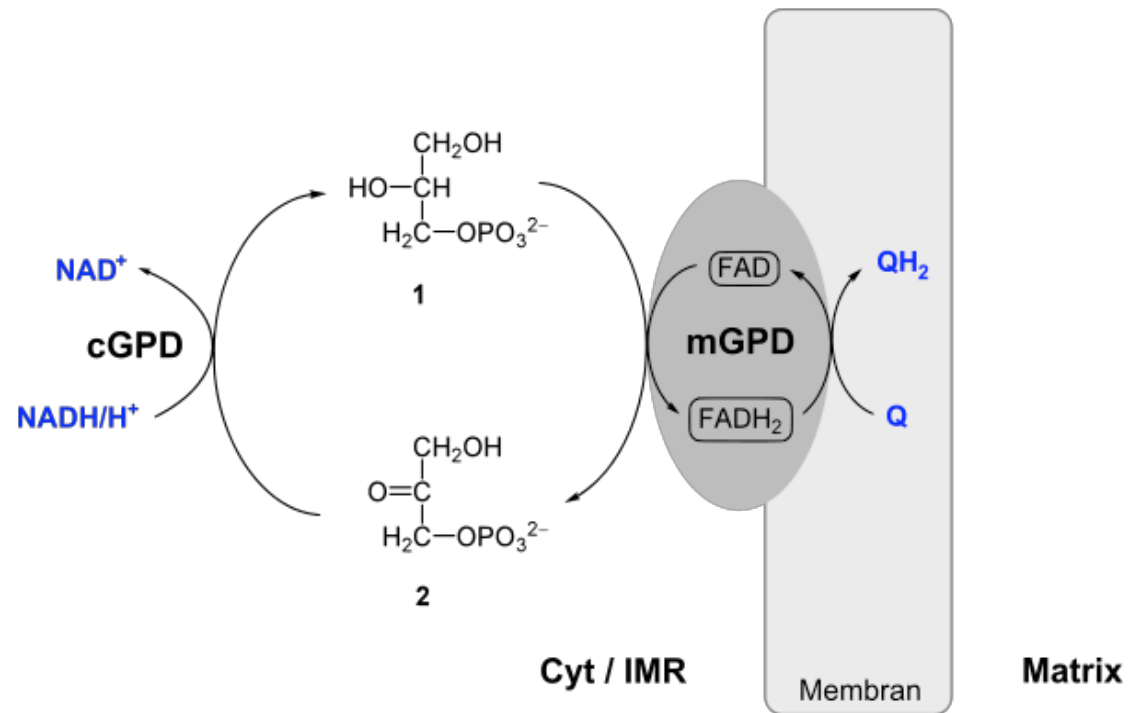
Figure 14-19

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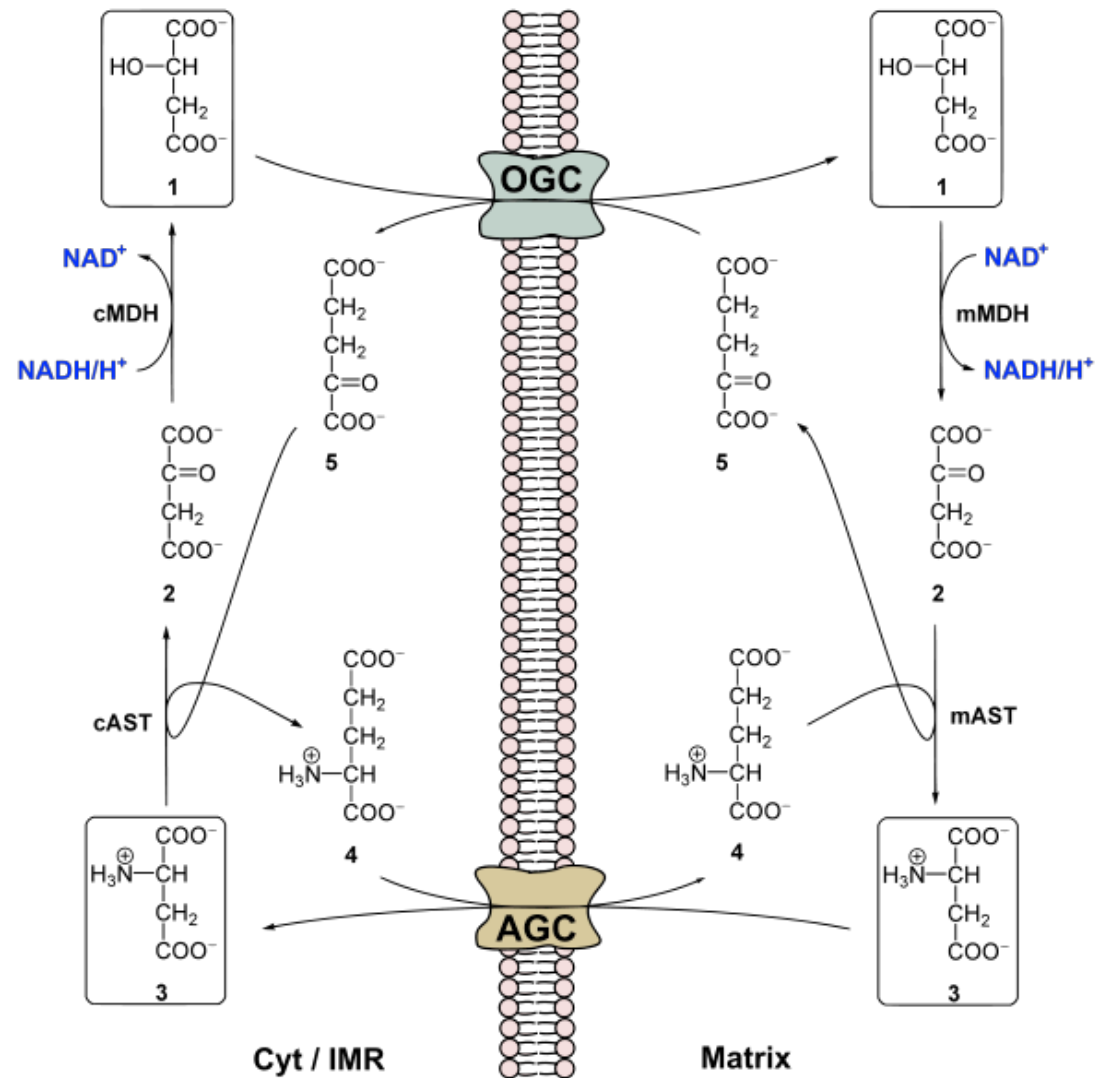
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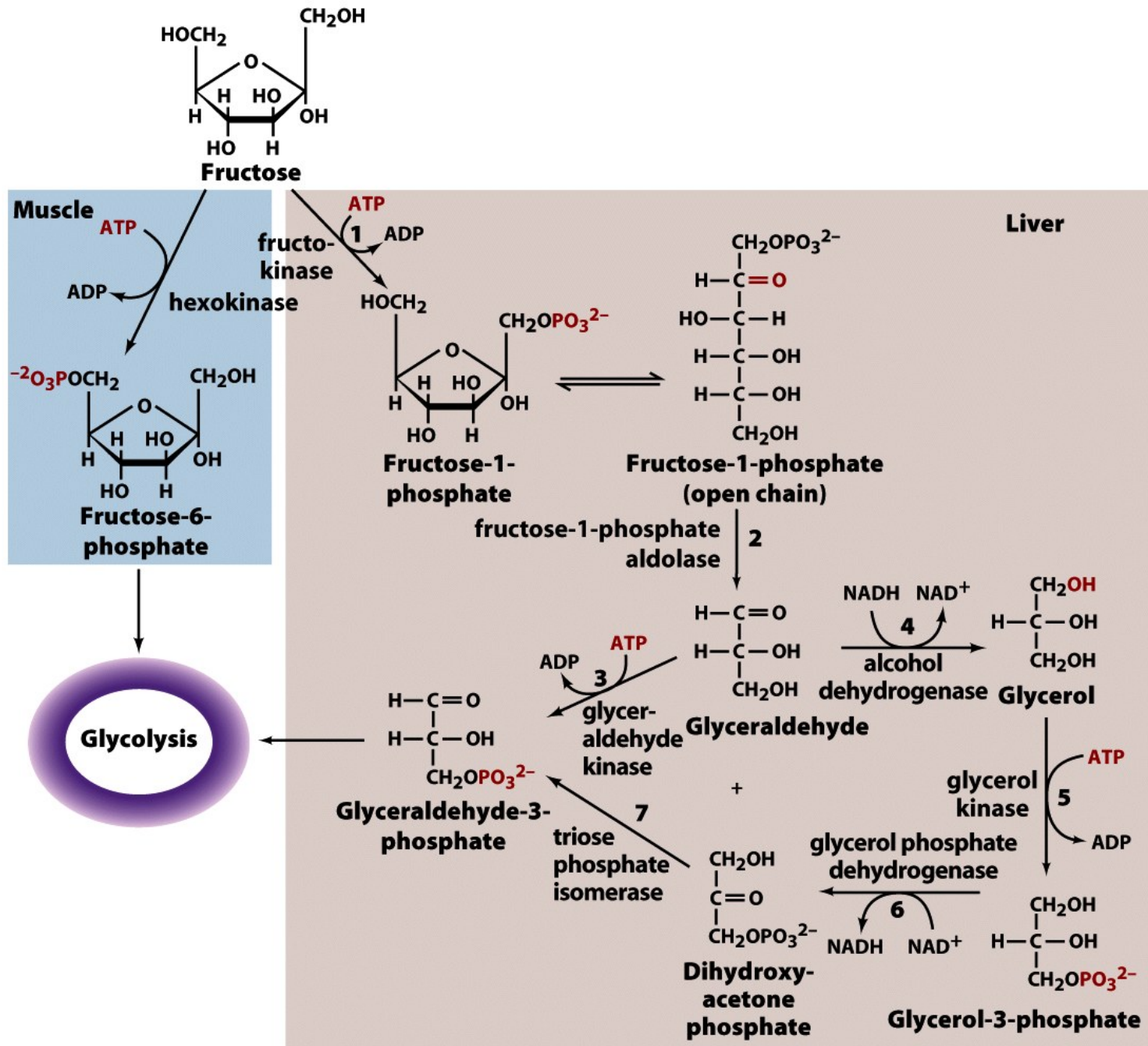
The glycerol-3-PO₄ Shuttle

- the enzyme called cytoplasmic glycerol-3-phosphate dehydrogenase (cGPD) converts DHAP to glycerol 3-phosphate by oxidizing one molecule of NADH to NAD⁺
- Glycerol-3-phosphate gets converted back to DHAP by a membrane-bound mGPD, this time reducing one molecule of enzyme-bound FAD to FADH₂. FADH₂ then reduces coenzyme Q (ubiquinone to ubiquinol) which enters into oxidative phosphorylation. This reaction is irreversible

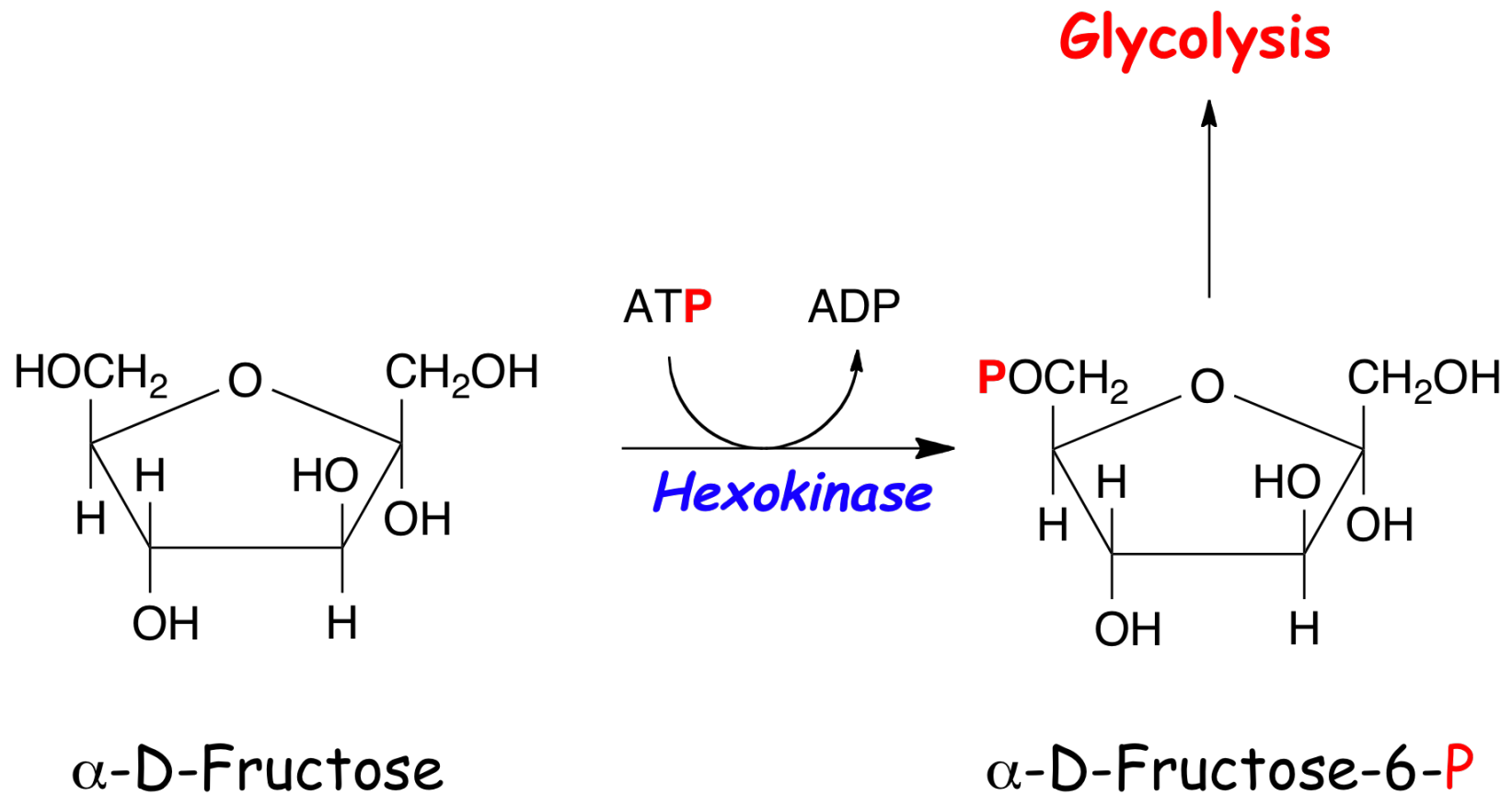


cMDH cyt malate
 dehydrogenase
 mMDH mitochondrial
 malate dehydrogenase
 cAST cyt aspartate
 transaminase
 mAST mitochondrial
 aspartate transaminase
 OGC malate/ α -
 ketoglutarate carrier
 AGC aspartate/glutamate
 carrier

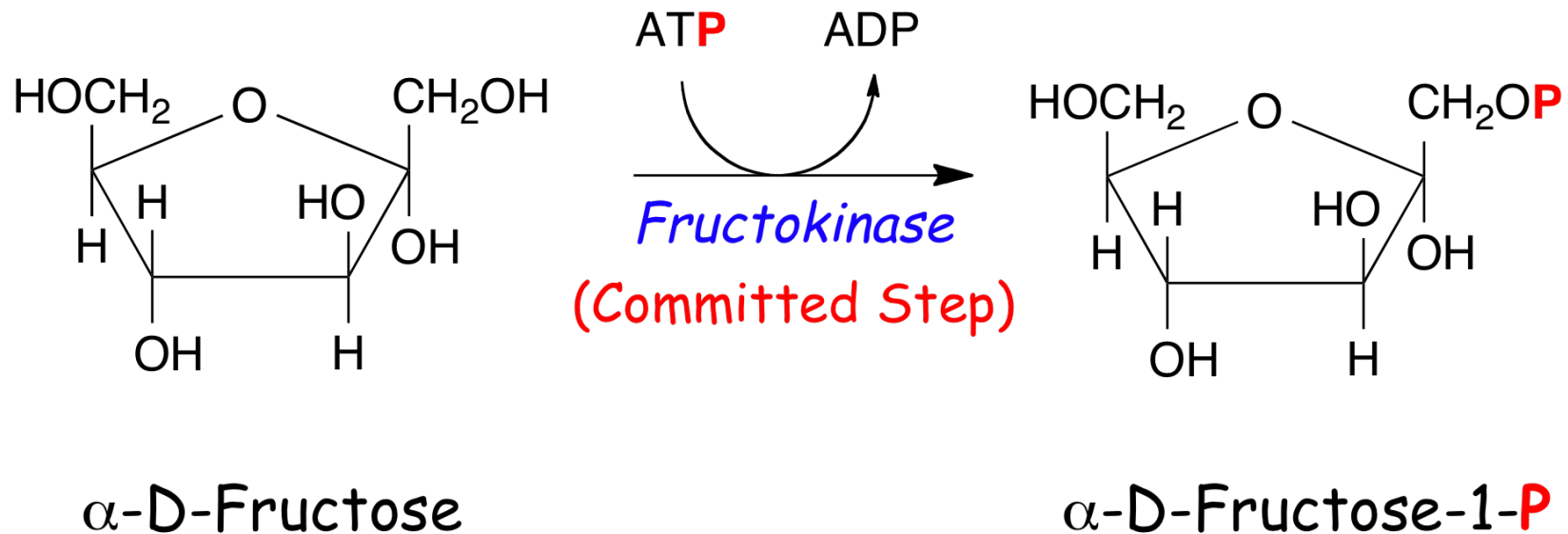




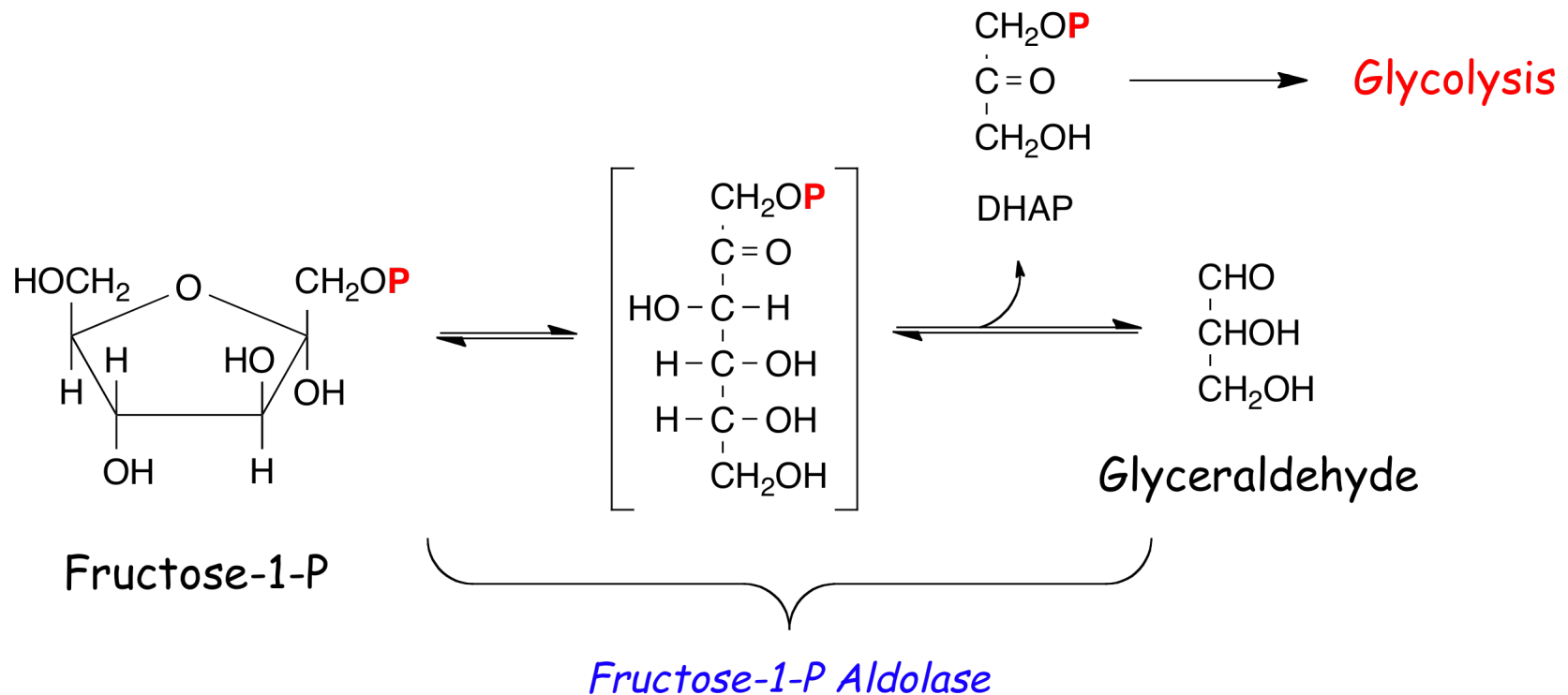
Muscle Metabolism of Fructose (Anaerobic Glycolysis) large amounts of hexokinase and do not contain glucokinase.



Liver Metabolism of Fructose, has very little Hexokinase (1,2 & 3) and the specificity of Glucokinase of the major liver enzyme for glucose metabolism, that will not phosphorylate fructose.

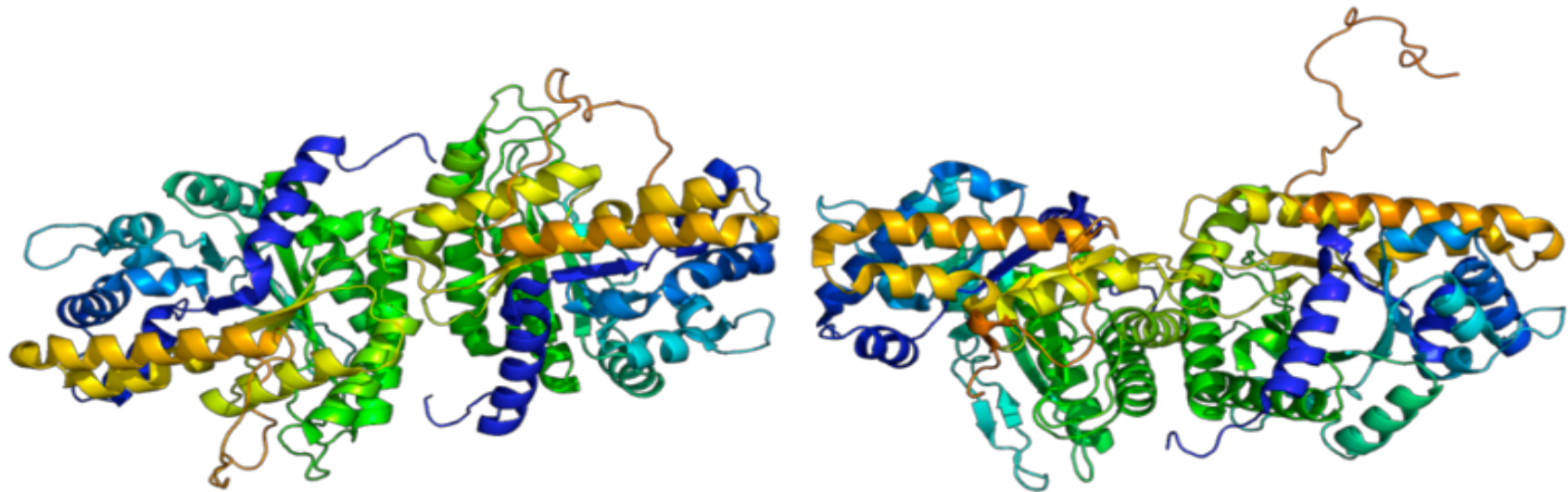


Liver Metabolism of Fructose-1-P

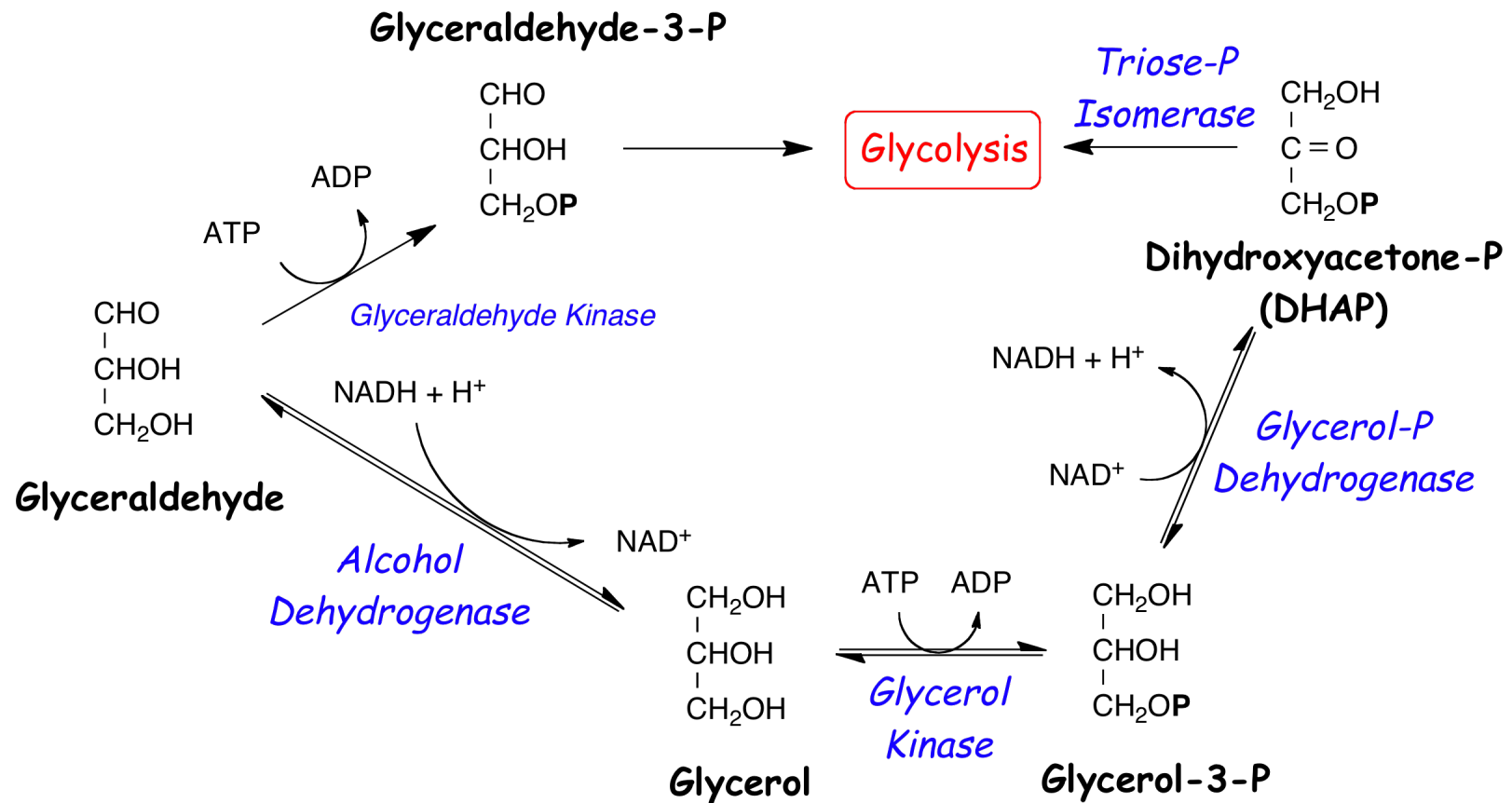


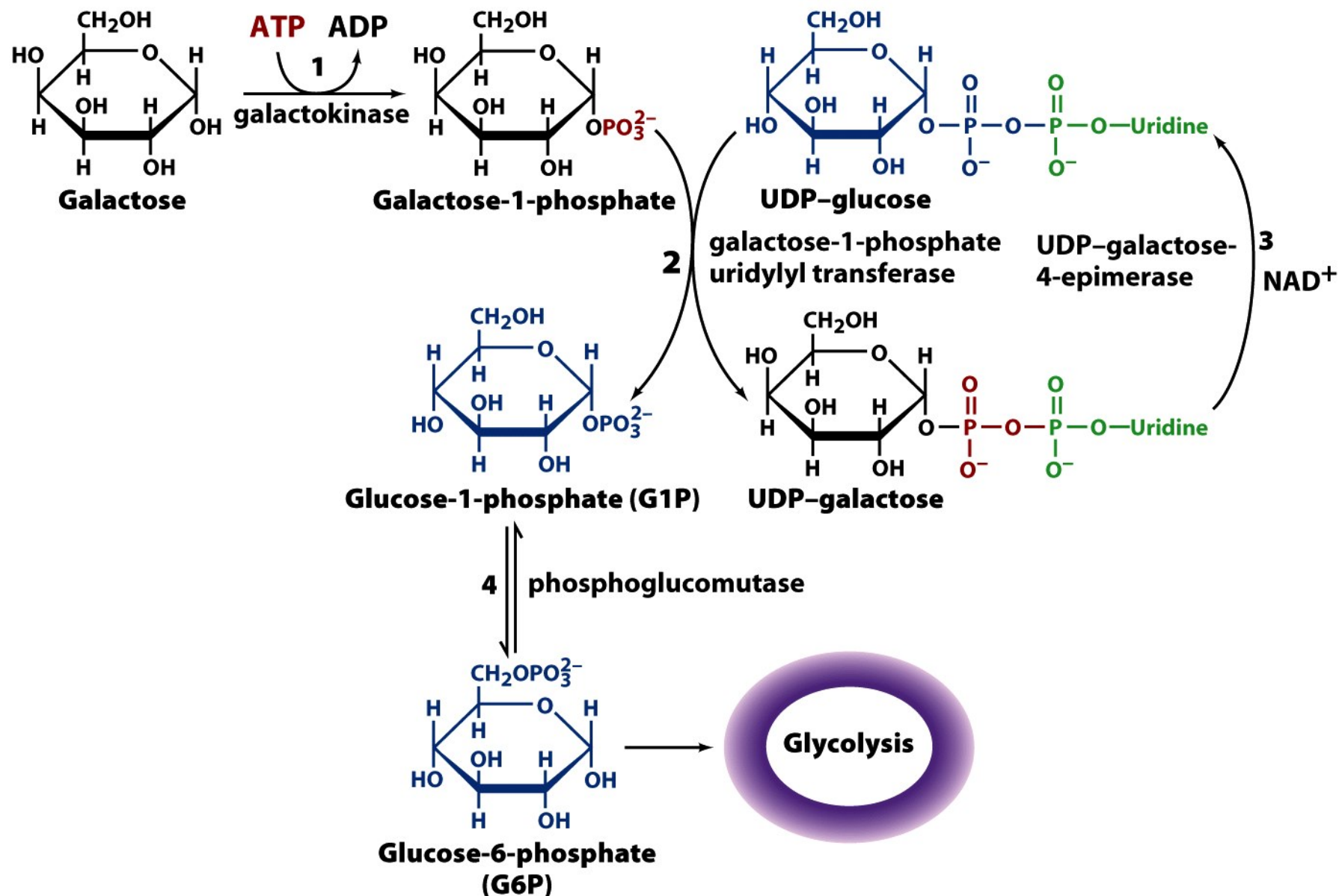
Rate-limiting Step!

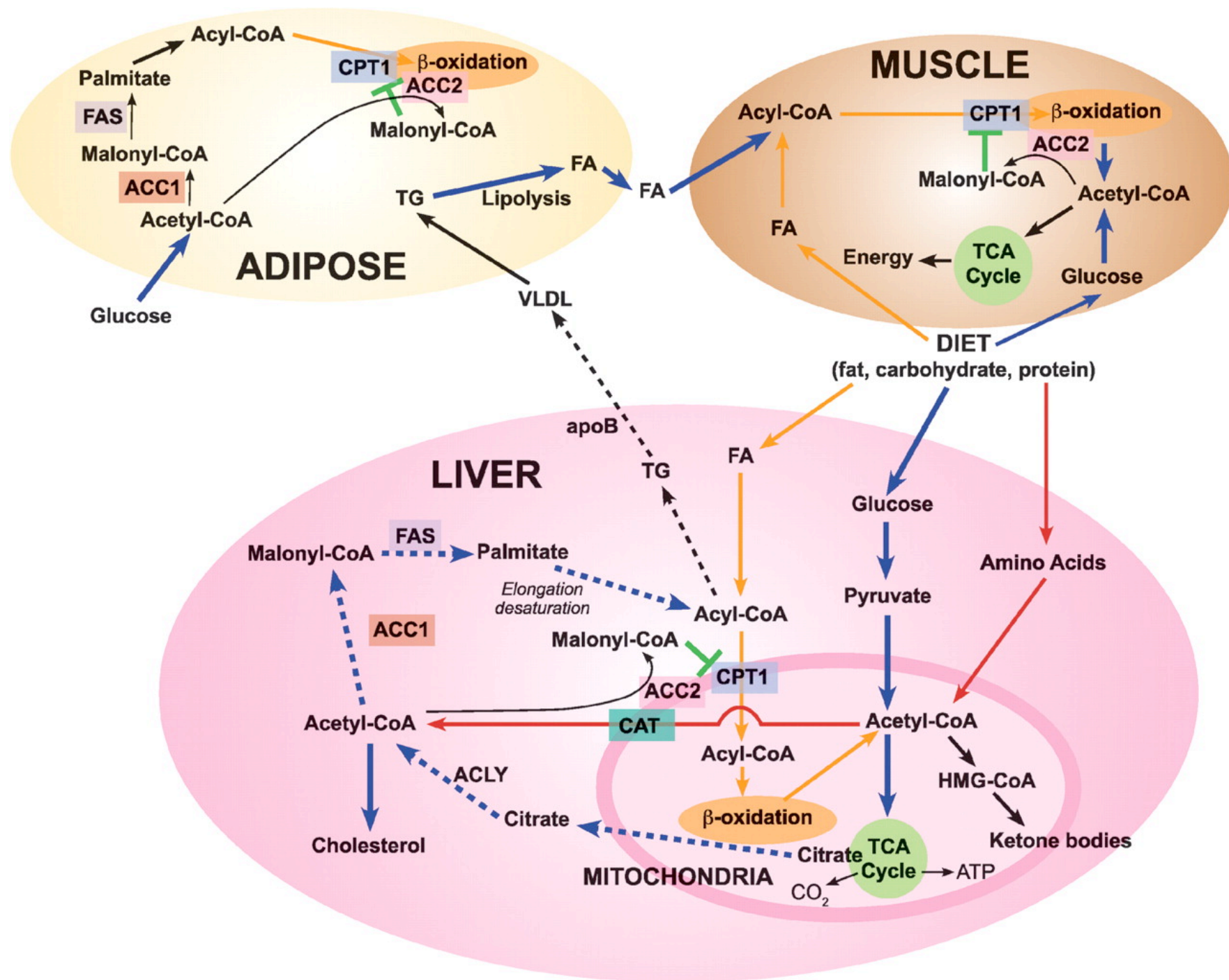
Aldolase B also known as **fructose-bisphosphate aldolase B** or **liver-type aldolase** is one of three isoenzymes (A, B, and C) of the class I fructose 1,6-BP aldolase enzyme, and plays a key role in both glycolysis and gluconeogenesis. The generic fructose 1,6-BP aldolase enzyme catalyzes the reversible cleavage of fructose 1,6-bp into GAP & DHAP as well as the reversible cleavage of fructose 1-p into glyceraldehyde and DHAP. In mammals, aldolase B is preferentially expressed in the liver, while aldolase A is expressed in muscle and erythrocytes and aldolase C is expressed in the brain. Slight differences in isozyme structure result in different activities for the two substrate molecules: FBP and fructose 1-p. Aldolase B exhibits no preference and thus catalyzes both reactions, while aldolases A and C prefer F 1,6BP.



Liver Metabolism of Glyceraldehyde







Schematic representation of the pancreatic β -cell metabolic stimulus–secretion showing the involvement of glucose, alanine and glutamine in insulin secretion, together with the involvement of the malate–aspartate

