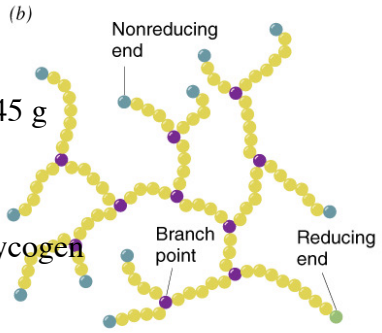
Glycogen metabolism

- Glycogen review - α 1,4 and 1,6 α -glycosidic links

~ every 10 sugars are branched - open helix with many non-reducing ends. Effective storage of glucose

- Glucose storage
  - Liver glycogen 4.0% 72 g
  - Muscle glycogen 0.7% 245 g
  - Blood Glucose 0.1% 10 g



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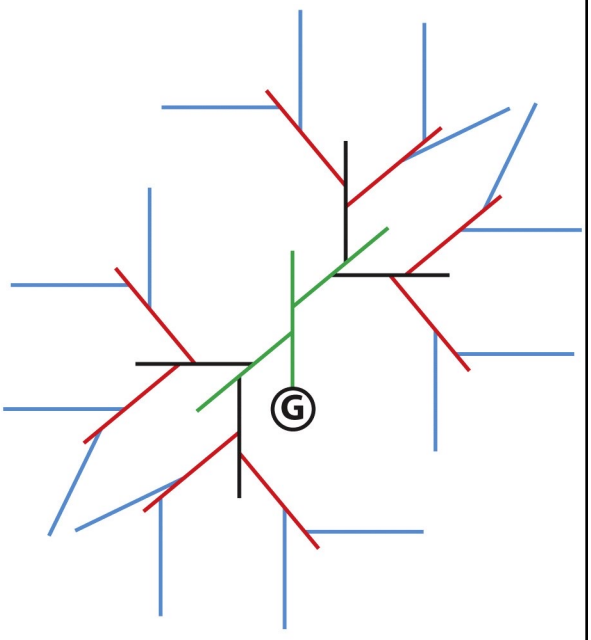
Large amount of water associated with glycogen - 0.5% of total weight

Glycogen stored in granules in cytosol w/proteins for synthesis, degradation and control

1

The function of glycogen is to store metabolic glucose
•Efficient release of glc from glycogen polymer requires many non-reducing ends for enzyme attack
•Must store largest amount of glycogen in smallest volume
•Chain length and branches support the "glucose bioavailability"
•G=glycogenin core with two branches, each continues to branch.
•Each layer (tier) of branches increase ends for enzymatic release of glucose



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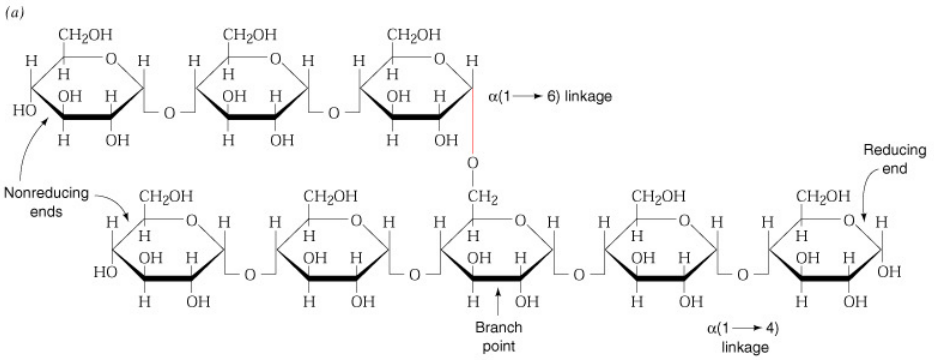

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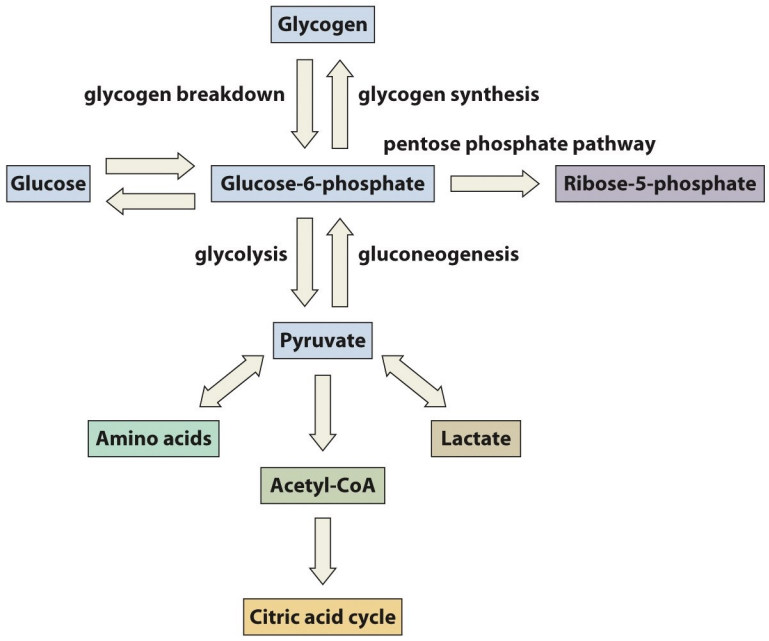

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4

Glycogen biosynthetic and degradative cycle

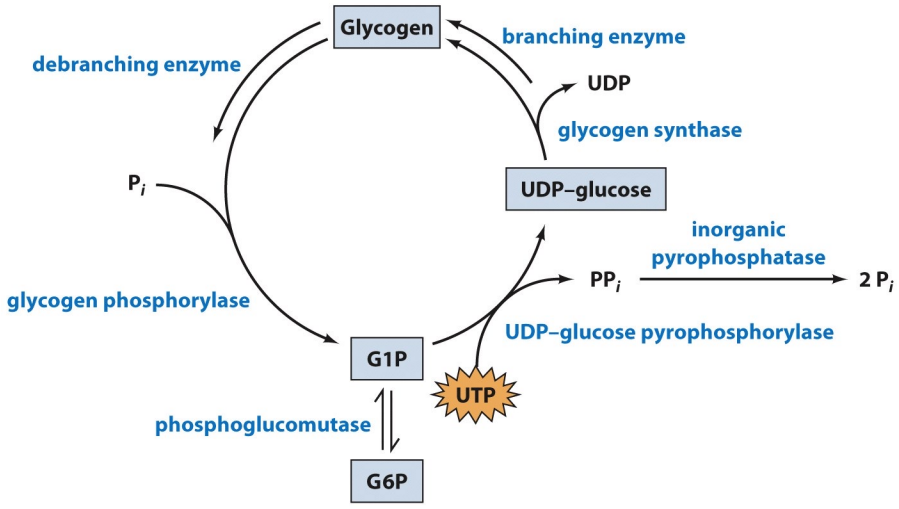
- There are very different means of control of glycogen metabolism between liver and muscle
- Two different pathways - do not share enzymes like glycolysis and gluconeogenesis

  glucose -> glycogen **glycogenesis - biosynthetic**

  glycogen -> glucose 1-P **glycogenolysis - breakdown**

- Evidence for two paths
  - Patients lacking phosphorylase can still synthesize glycogen
  - hormonal regulation of both directions
  - mass action

5



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6

Glycogenolysis (glycogen breakdown)-

Glycogen Phosphorylase

glycogen (n) + Pi -> glucose 1-p + glycogen (n-1)

- Enzyme binds and cleaves glycogen into monomers at the end of the polymer (reducing ends of glycogen)
- Dimmer interacting at the N-terminus.
- rate limiting - controlled step in glycogen breakdown
- glycogen phosphorylase - cleavage of 1,4 α glycosidic bond by Pi NOT H_2O
- Energy of phosphorolysis vs. hydrolysis
  - -low standard state free energy change -transfer potential
  - -driven by Pi concentration
  - -Hydrolysis would require additional step s/ cost of ATP
  - - Think of the difference between adding a phosphate group with hydrolysis

7

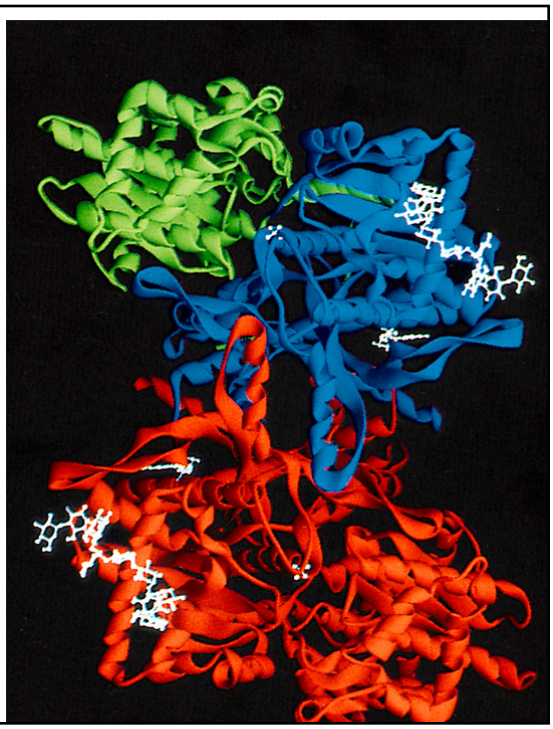
Glycogen Phosphorylase

- phosphorylation locks glucose in cell (imp. for muscle)
- Phosphorylase binds glycogen at storage site and the catalytic site is 4 to 5 glucose residues away from the catalytic site.
- Phosphorylase removes 1 residue at a time from glycogen until 4 glucose residues away on either side of 1,6 branch point – stericaly hindered by glycogen storage site
- Cleaves without releasing at storage site
- general acid/base catalysts

8

Glycogen Phosphorylase

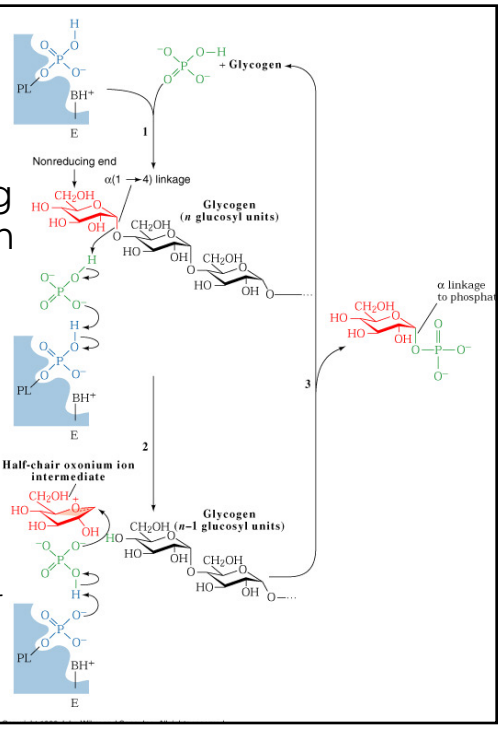
- Can only react until 4 or 5 glucoses away from branching enzyme.
- Glucose is held in place at a site away from catalytic site (more efficient than diffusion)
- Active site is a small crevice - too big for branched glycogen to fit - PLP is close in proximity. This results in keeping water out of the active site.



9

Glycogen Phosphorylase

- Inorganic phosphate attacks the terminal glucose residue passing through an oxonium ion intermediate.
- cofactor PLP pyridoxal 5' -phosphate
  - Covaliently bound by shiff base
  - Phosphate functional group of PLP acts a an acid/base catalyst
  - Allows the exclusion of water - replaced by Pi



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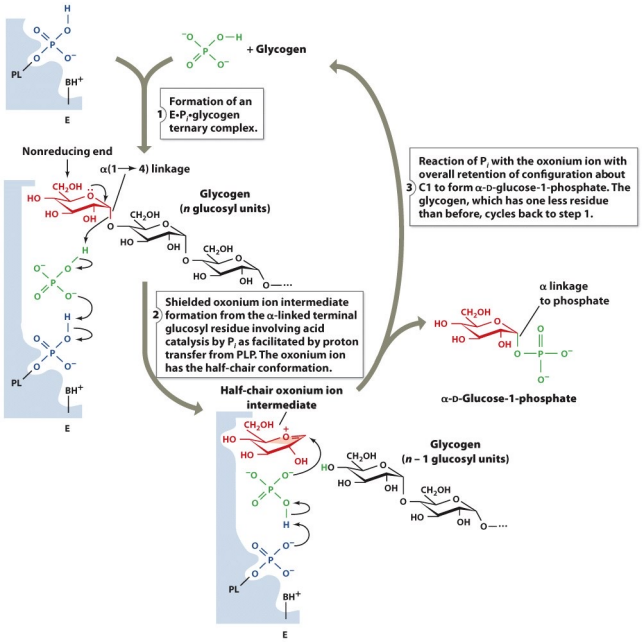

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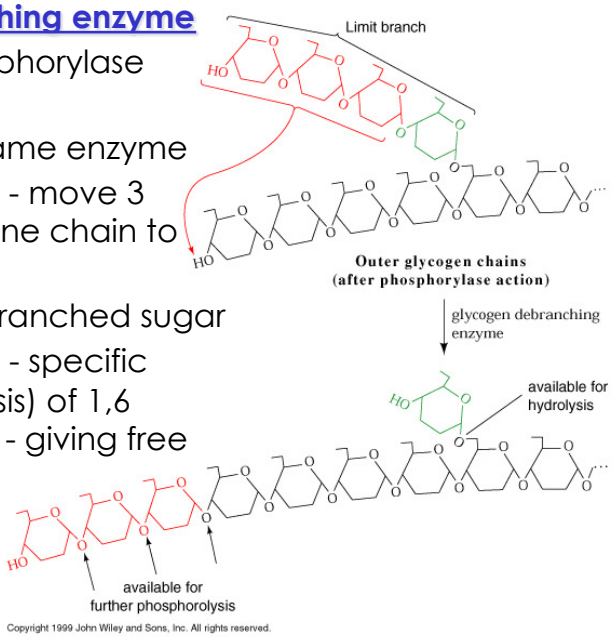
Transferase/debranching enzyme

- Finishes what phosphorylase cannot
- Two activities on same enzyme
  - 1st- transferase - move 3 sugars from one chain to another
  - exposes 1,6 branched sugar
- 2nd - debranching - specific cleavage (hydrolysis) of 1,6 branched glucose - giving free glucose



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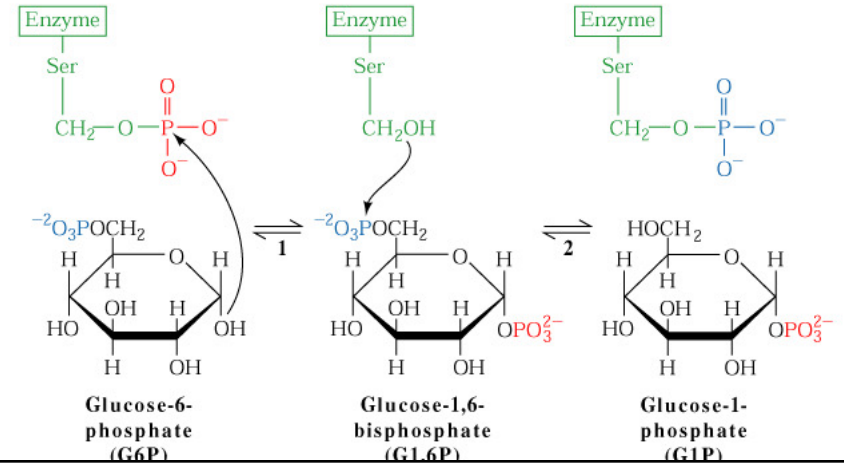
Phosphoglucomutase

glucose 1-P -> glucose 6-P

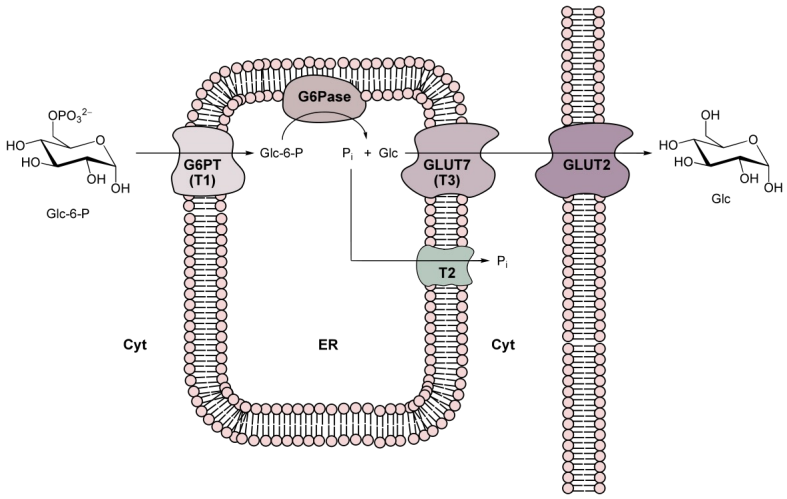
- mechanism involves phosphoryl shuttle from serine in active site to glucose hydroxyls
- no additional energy required - equilibrium far to the right



Glucose 6-phosphatase (G-6-Pase)

glucose 6P -> glucose + Pi

- Found mostly in Liver and Kidney NOT Muscle or Brain
- Allows gluconeogenic tissue to supply and glucose for body while muscle and brain keep it for glycolysis

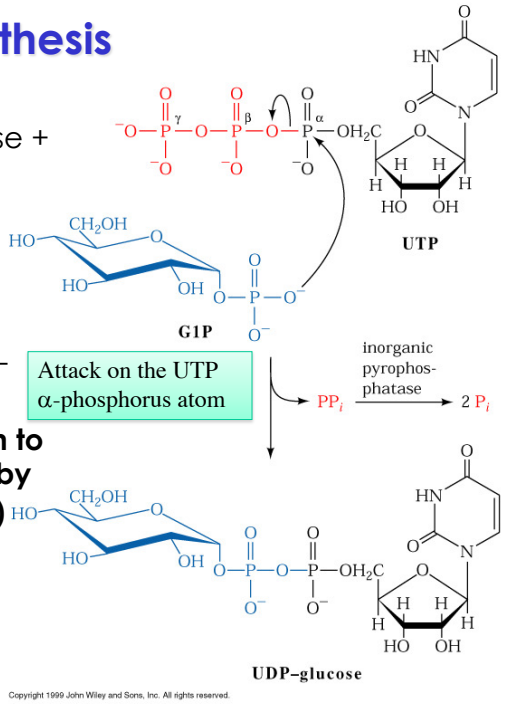


Glycogenesis - synthesis

UDP glucose transferase

Glucose 1 P + UTP ->UDPglucose + PPi

- UTP acts as a high energy handle for many sugar polymers
- This reaction is thermodynamically neutral – phosphoester transfer
- **PPi hydrolysis - pulls reaction to right of reaction (catalyzed by inorganic pyrophosphatase)**



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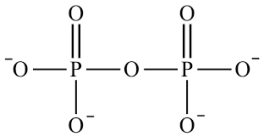
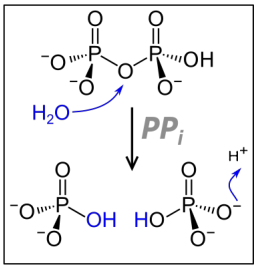
Pyrophosphate hydrolysis



Δg=-7 kcal/mol



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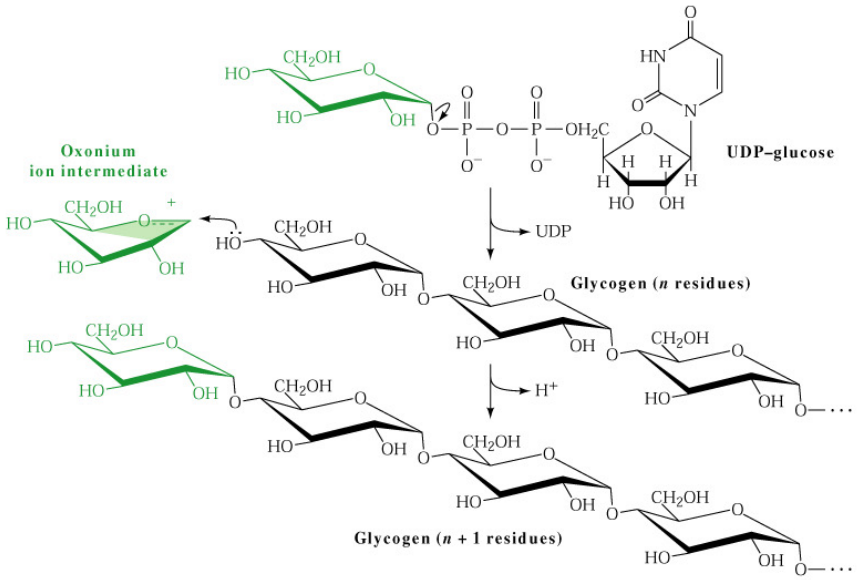
Glycogen synthase

UDPglucose -> 1,4 glycogen + UDP



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Glycogen synthase

- Highly regulated enzyme of this path
- requires a preexisting primer of glycogen
  - primer is glycogenin enzyme/protein
  - glycogenin glycosylates itself
  - glycogen "core"
- 1,4 glycogen addition until glycogen synthase looses contact with glycogenin core

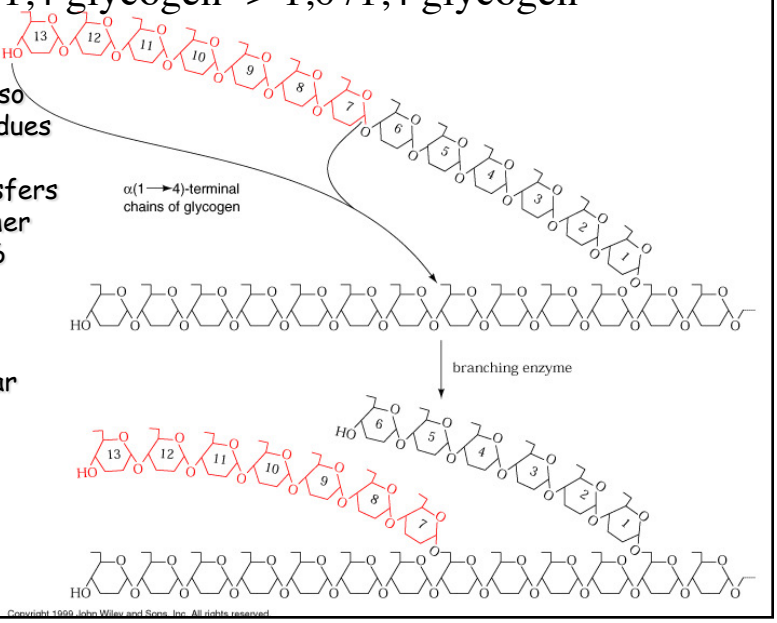
18

Branching enzyme

1,4 glycogen -> 1,6 /1,4 glycogen

- once 11 or so glucose residues have been added, transfers 1,4 to another chain in a 1,6 linkage

- transfers about 7 sugar residues



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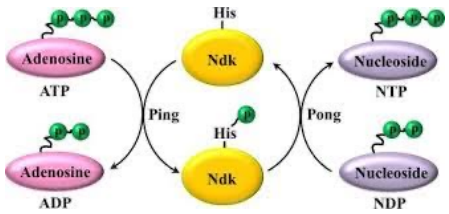
Nucleoside Diphosphate Kinase (NDPK)

- Responsible for the regeneration of UTP, but at the cost of an ATP
- This is the same enzyme discussed before, there are several forms of NDPK with varying substrate specificity



20

Energy cost of Glycogen synthesis

glucose 6-phosphate -> glucose 1 phosphate
glucose 1-phosphate + UTP -> UDP-Glucose + PPi
PPi + H20 -> 2 Pi
UDP-glucose + glycogen (n) -> glycogen (n+1) + UDP
ADP + ATP -> UTP + ADP
1 ATP cost / glycogen residue added

- The cost of storage of glucose in the form of glycogen is very small as compared to the production of ATP from glucose

Glycogen Metabolism Regulation Chpt 12 3B

Brain, liver muscle isoforms. – we will focus on muscle.

Three dimensional structure of glycogen phosphorylase

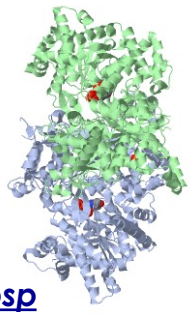
- 2 x 2 (heterotetramer)
- separate binding sites for each of the regulators, and glycogen particle site
- need to exclude water / active site (pyridoxal phosphate) interior of homoenzyme
- glycogen binding site distal (away) from the active site - permits several reactions before release of glycogen polymer
- concerted change to R active form through rotation of subunit and re-arraignment of active site

Phosphorylase regulation

- phosphorylase a – phosphorylated *at Ser **14 near N Terminus***
- phosphorylase b - is not –phosphorylated
- R form active / T form inactive
- Shifting between R and T forms alters activity.

T state malformed active site with surface loop blocking binding site access.

- N term has basic residues binding near active site acidic residues.



*R state has Arg to bind substrate phospho – ion position to bind (not in T state) and active site loop cover is moved away **when Ser 14 is phosp***

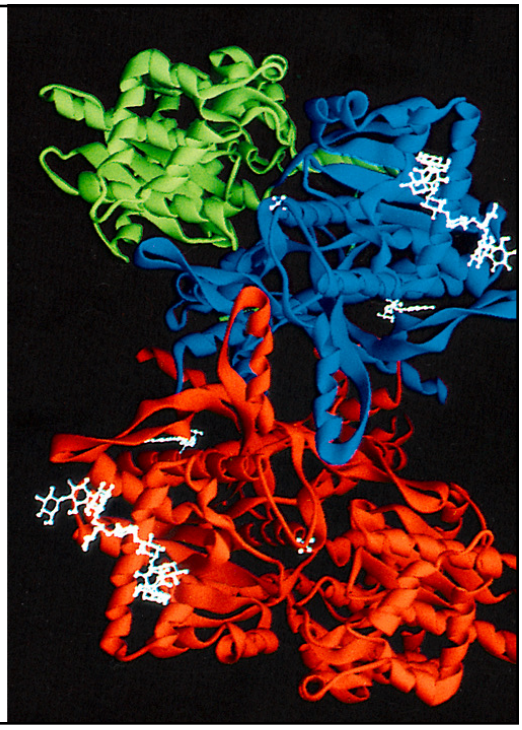
- phosphorylation state defines a or b but equilibrium between forms is also set by allosteric regulation

Five sites of regulation

1. Ser14 phosphate-recognition site
2. AMP activation / Glc-6-P inhibition site.
3. Catalytic site that binds glycogen, Glc-1-P
4. Inhibitor site, 12Å from catalytic site, binds caffeine and related compounds.
5. Glycogen storage site.

The active site in the T (b) form is hidden. AMP (NOT cAMP) binding moves Ser 14 similar to that seen when the Ser is phosphorylated.

AMP leads to the opening of the active site without the requisite phosphrylation, thus the conversion from b to a form of phosphorylase. (a and b mean active and less active, it does not discuss the phosphorylation state)



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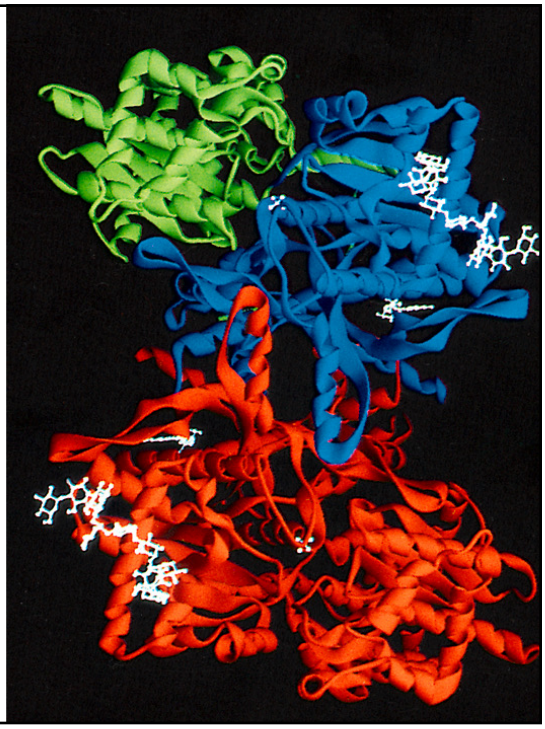
ATP binds to the same site but does NOT cause the same shifts, rather it tends to stabilize the T form, and is thus an inhibitor.

Thinks of the logic of the energy state and how AMP and ATP relate to the results of glycogenolysis



26

ATP, ADP & AMP

GLucose

↓

ATP -> **ADP**

At resting/ well fed state
- AMP is low conc

During exercise and starvation
- AMP increases as ADP->ATP

2ADP -> AMP + ATP

Adentylate kinase

ATP is ONE of the “high energy signals”
AMP is ONE of the ”low energy signals"

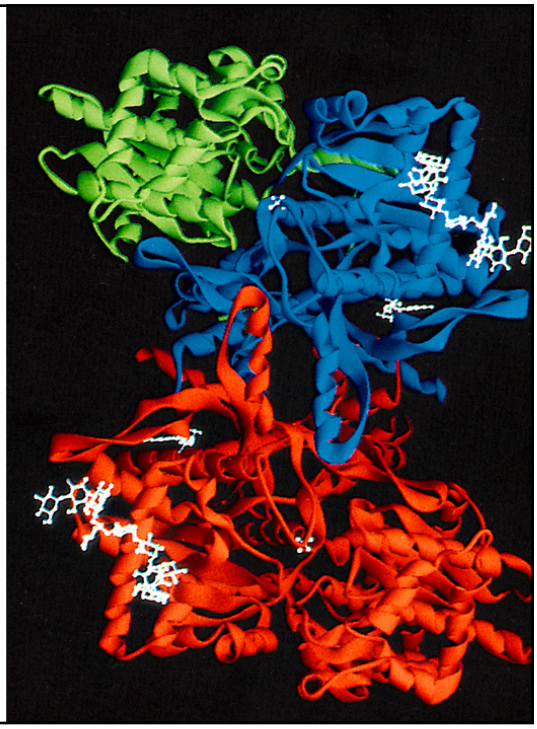
27

Phosphorylation of Ser 14 leads to a T to R conformation shift as the **negative charges of the phospho group interacts with positive charged Arg.**

This is similar to the changes in conformation found with AMP.

Thus the very low energy state of the cell can overcome covalent modification of the enzymes activity

KNOW the structural mechanics of this enzyme esp 12-3B!!!



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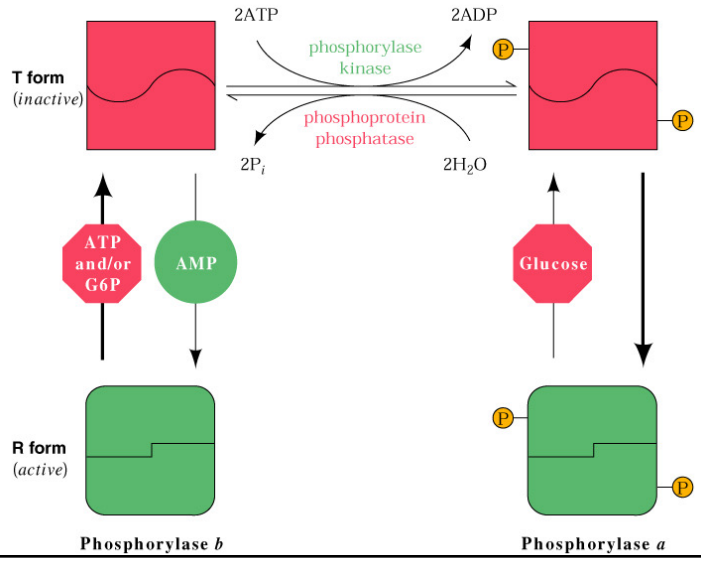
There are two levels of control of phosphorylase allosteric and covalent. Both are required for full activation

- **Covalent - Phosphorylation by PKA**
- **Allosteric -Phosphorylase activator - AMP**
- **Allosteric - Phosphorylase inhibitor - ATP, G6P and glucose**



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Two proteins modulate activity by covalent modification – phosphorylation

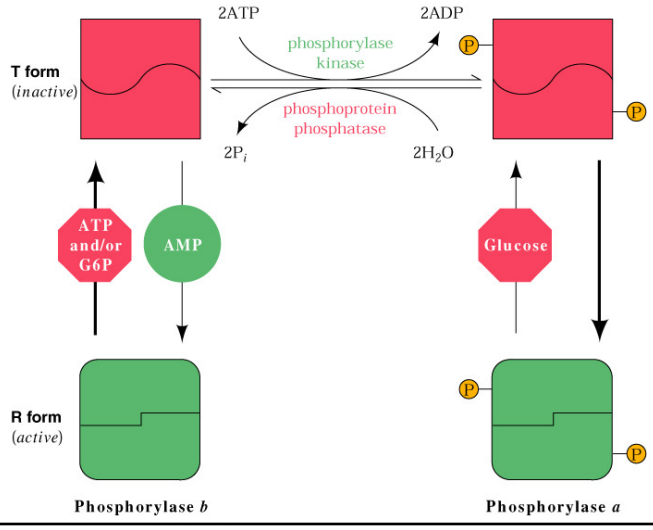
1 phosphorylase kinase

-phosphorylates 1 Ser / subunit

- activated by cAMP/PKA pathway (glucagon and epinephrine) and Calcium

2 protein phosphatase 1

- - general phosphatase under control of insulin



30

Differences between muscle and liver

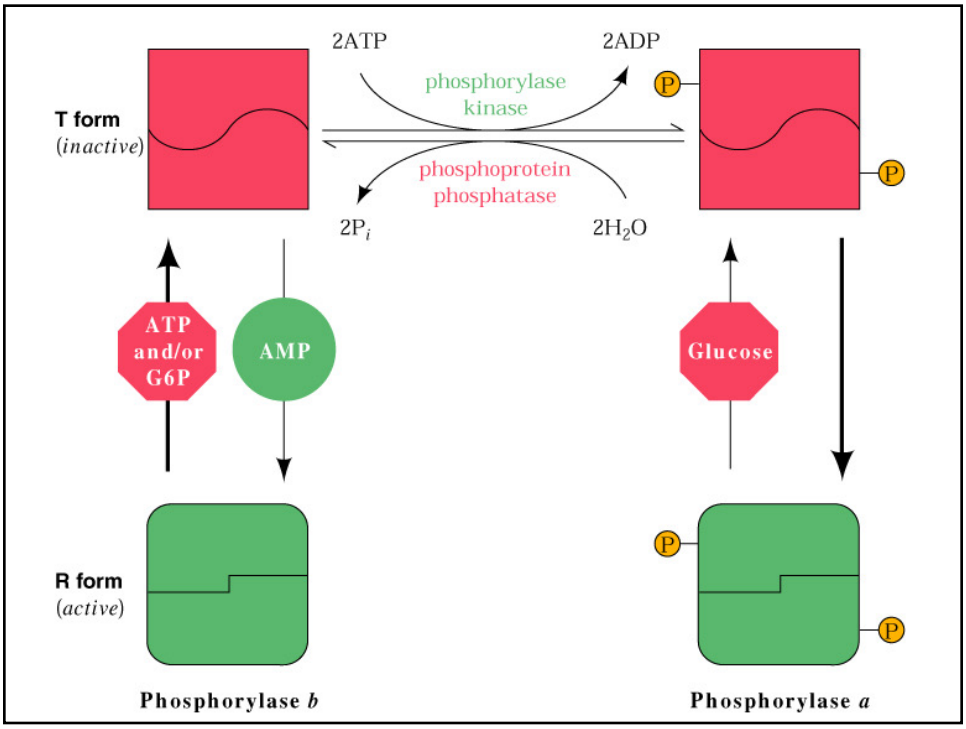
Muscle phosphorylase

- inactive b form activated by low energy signal AMP (leads to increased glucose for muscular activity)
- glucose 6-P and ATP (high energy signals) reverse b form activation

Liver phosphorylase

- active a form is converted to the b form by glucose (not muscle form)
- Therefore even with covalent modification when enough glucose is present in the liver cell, glycogenolysis stops, but not in the liver. You must remember that it is not easy to build up liver glucose levels due to G6Pase.

Allosteric and covalent modification regulation of both muscle and liver leads to use of glycogen glucose for muscle and liver glucose for export



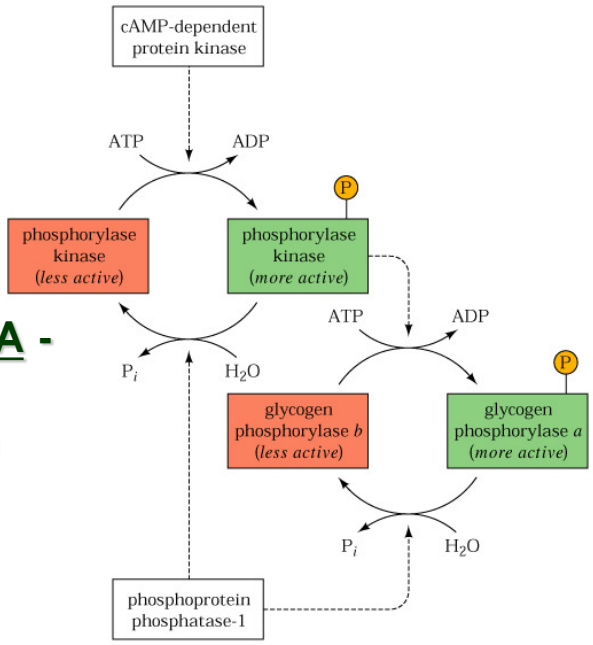
Glycogen Synthase Regulation

Glycogen synthase -

- phosphorylated at C and N terminals increases net charge from -13 to -31.
- active (a) form is dephosphorylated
- inactive (b) form is phosphorylated
- phosphorylation controlled via cAMP by PKA

Glycogen Synthase Regulatory proteins -

- **Protein Kinase A** - regulates the activity of both phosphorylase and synthase.



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Phosphorylase kinase -
phosphorylates phosphorylase

- Duel controlled enzyme
- Exact regulation is still not totally clear, but there are four different subunits some in different amounts. α, β, γ and δ.
- Both the α and the β subunits are phosphorylated by PKA - this leads to a highly active phosphorylase kinase when Ca^{+2} is also present.
- The gamma subunit is similar to a protein kinase and acts as a psuedosubstrate (kind of like the regulatory subunits of PKA) for phosphorylase kinase -key glutamate
- highly active form when phosphorylated by PKC

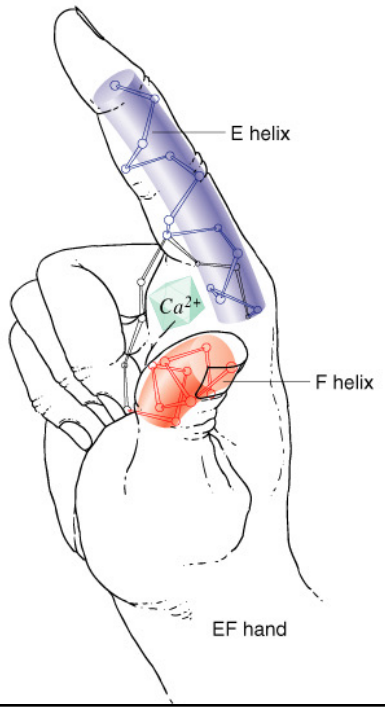
35

- Increased Ca^{+2} levels partially activate kinase (low active form) via nervous activity/muscle contraction/epinephrine

- Ca^{+2} activates by non-covalent interaction with a subunit of the phosphorylase kinase (calmodulin; CaM)
-CaM is a ubiquitous Ca^{+2} binding protein that interacts with many other proteins. Cytosolic Ca^{+2} levels are tightly controlled and only transiently increase.
-Most Ca^{+2} is stored in mitochondria and endoplasmic reticulum.
-Calmodulin is also a subunit of phosphorylase kinase (δ)

36

- Calmodulin binds Ca^{+2} in a central loop (EF hand) that causes the central helix to alter its conformation.



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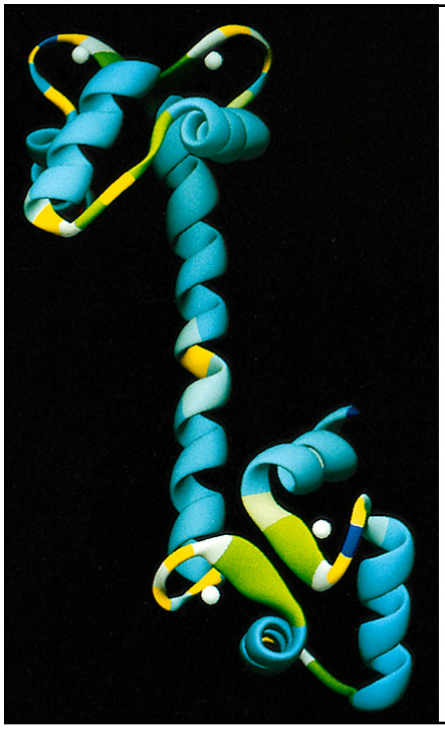

- When Ca^{+2} levels increase, calmodulin pulls the gamma subunit of phosphorylase away from the active site of phosphorylase kinase, allowing activation of the enzyme.

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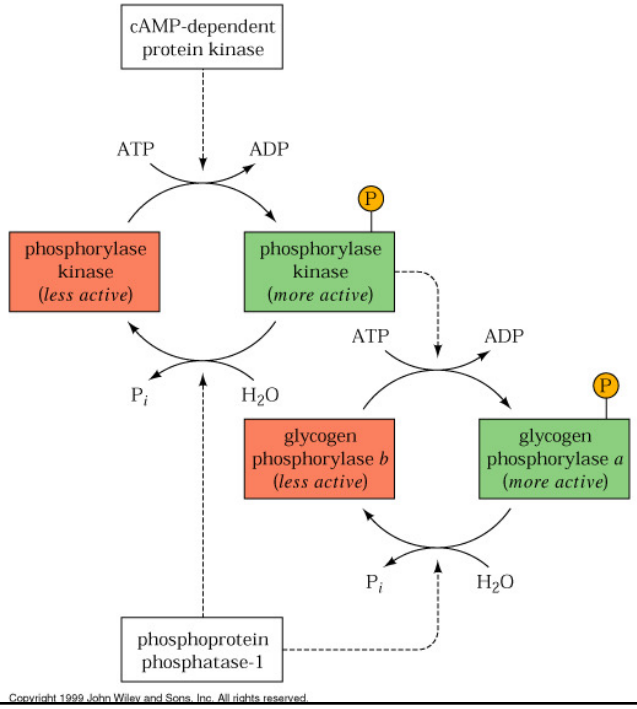

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Protein phosphatase 1 (PP1) *multiple phosphorylation sites*

- There are several protein phosphatases - most are many times more active (think about what that means) than the protein kinases. Generally these are not specific PPases and are not highly regulated. Except for…
- PP1 increases glycogen synthesis and inhibits glycogen phosphorylase
- PP1 removes the phosphoryl groups from phosphorylase kinase (α and β subunit)
- PP1 also removes the phosphoryl group from glycogen synthase (think of this consequence)

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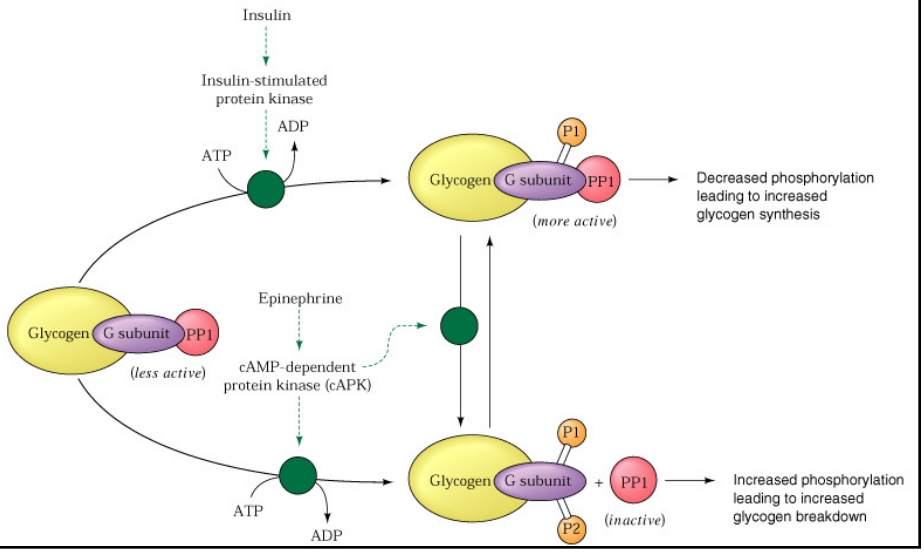
Regulation of PP1

- There are two subunits of PP1, the G protein (glycogen binding) and the catalytic domain.



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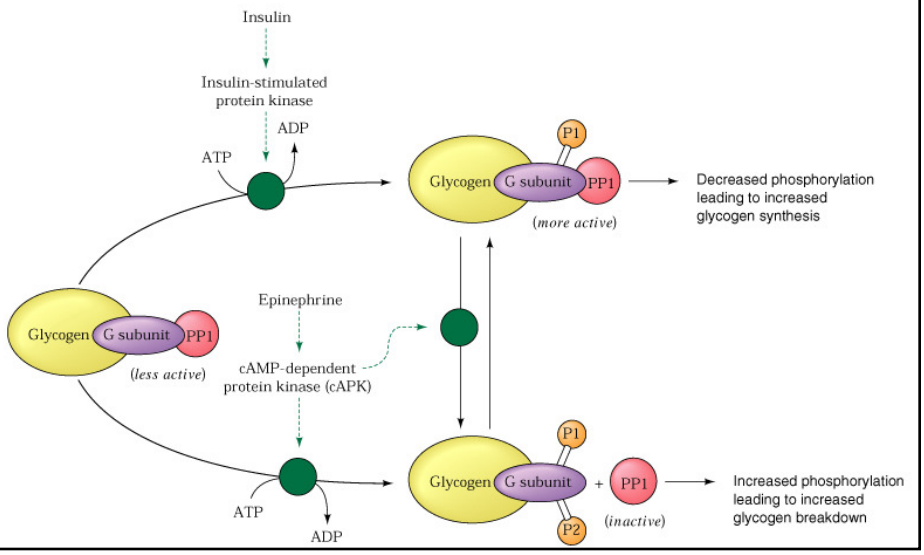
Regulation of PP1

- PP1, G protein and glycogen must be in a complex for phosphatase activity to occur.



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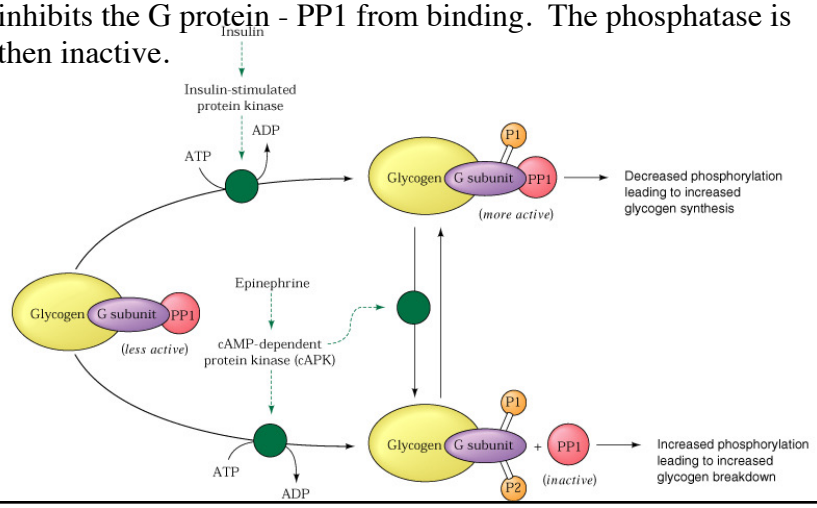
- G protein binds glycogen and acts to recruit PPI to glycogen complex
- Increases PPI/G protein interactions result when the G protein is phosphorylated at 1 residue (controlled by insulin)
- PKA also phosphorylates the G protein at a different site and inhibits the G protein - PP1 from binding. The phosphatase is then inactive.



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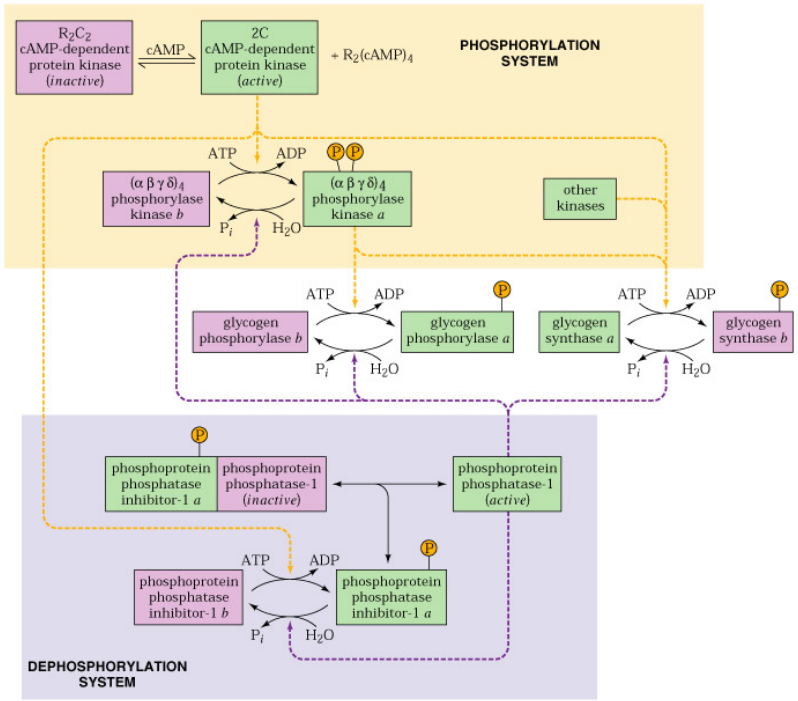

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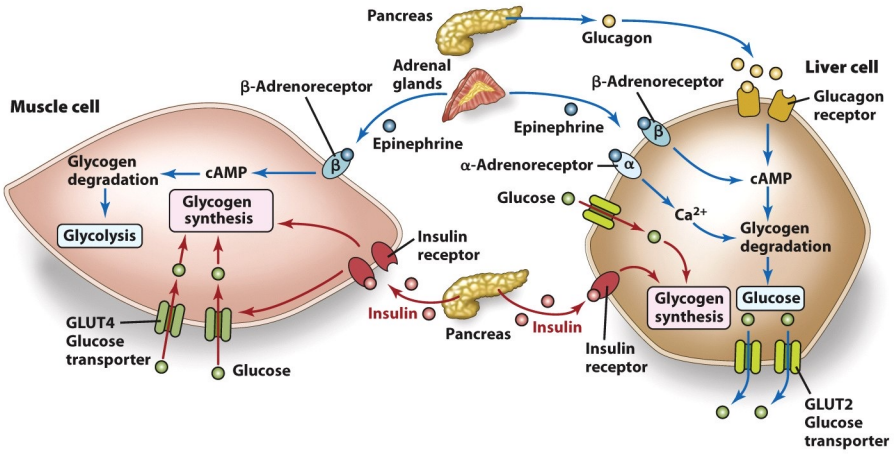

Figure 16-14
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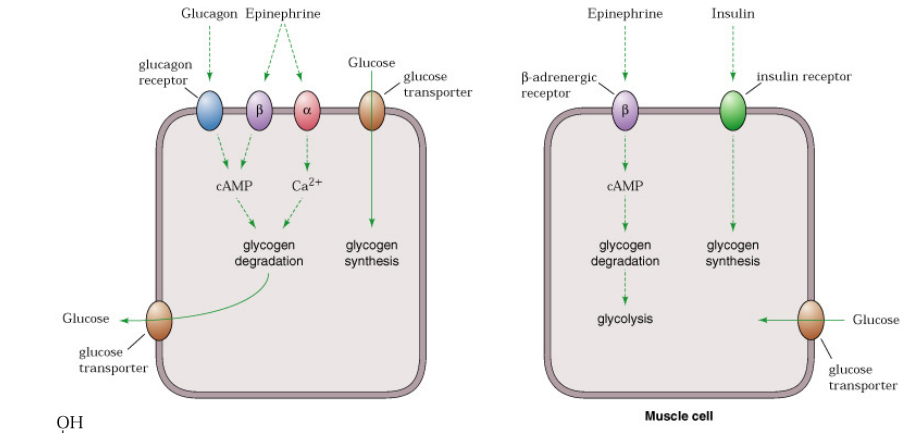
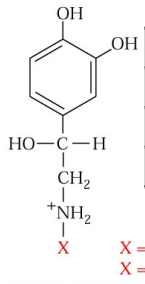


	Released by	Affects	Results on Blood Glucose Levels
Insulin -			
Glucagon -			
Epinephrine -			



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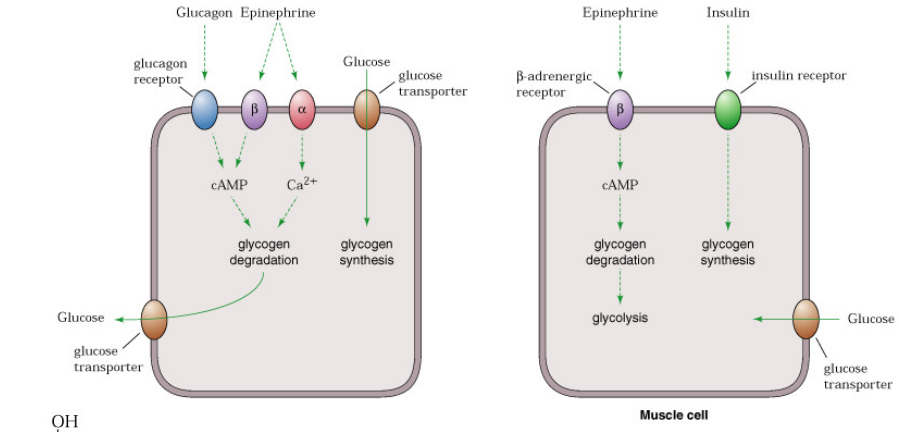

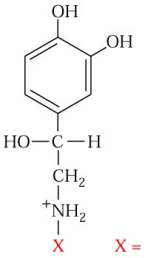

X = CH_3 **Epinephrine**
X = H **Norepinephrine**

	Released by	Affects	Results on Blood Glucose Levels
Insulin -	ß pancreatic cells		
Glucagon -	α pancreatic cells		
Epinephrine -	Adrenal gland		

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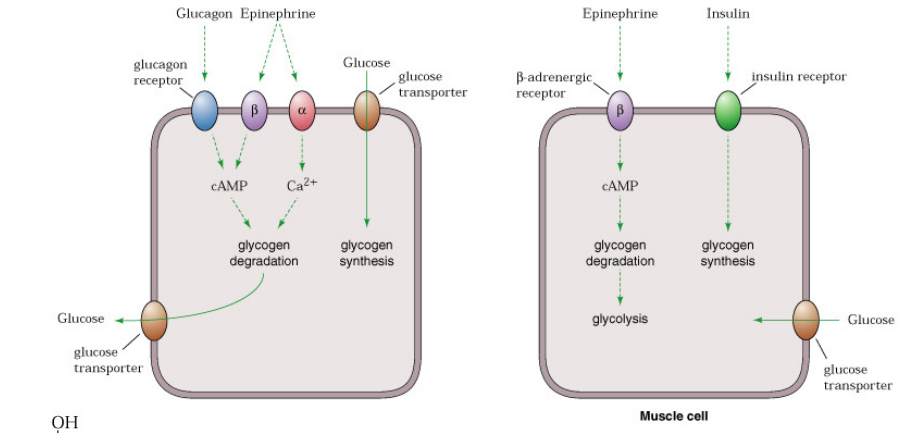

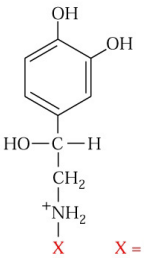

X = CH_3 **Epinephrine**
X = H **Norepinephrine**

	Released by	Affects	Results on Blood Glucose Levels
Insulin -	ß pancreatic cells	Decrease cAMP Increase Protein Pase2	
Glucagon -	α pancreatic cells	Increase cAMP	
Epinephrine -	Adrenal gland	Increase cAMP Increase Ca^{+2}	

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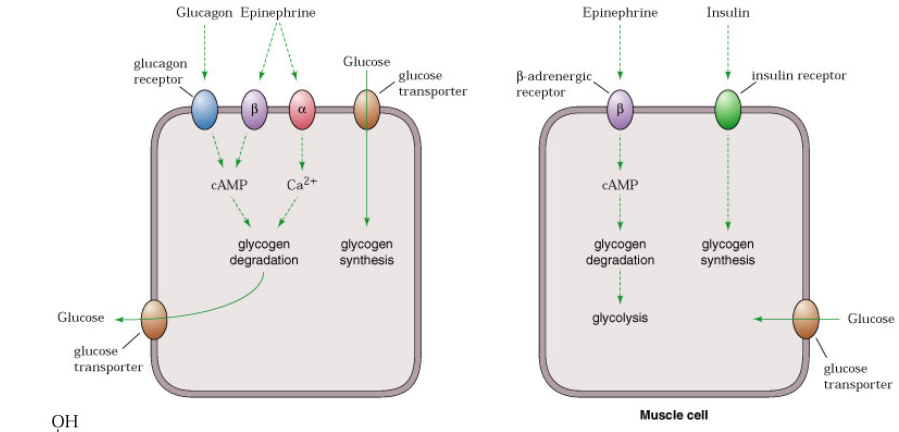

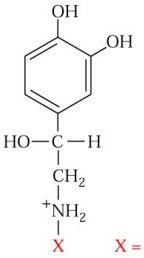

X = CH_3 **Epinephrine**
X = H **Norepinephrine**

	Released by	Affects	Results on Blood Glucose Levels
Insulin -	β pancreatic cells	Insulin receptor	
Glucagon -	α pancreatic cells	Glucagon receptor	
Epinephrine -	Adrenal gland	α, β adrenergic receptor	

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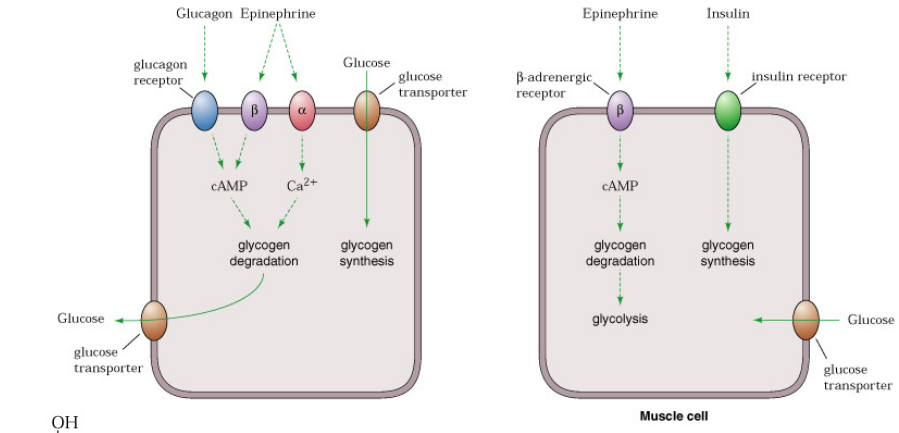

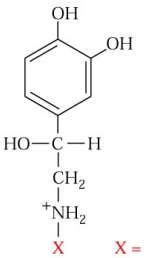

X = CH_3 **Epinephrine**
X = H **Norepinephrine**

	Released by	Affects	Results on Blood Glucose Levels
Insulin -	β pancreatic cells	Insulin receptor	Reduce
Glucagon -	α pancreatic cells	Glucagon receptor	Increase
Epinephrine -	Adrenal gland	α, β adrenergic receptor	Increase

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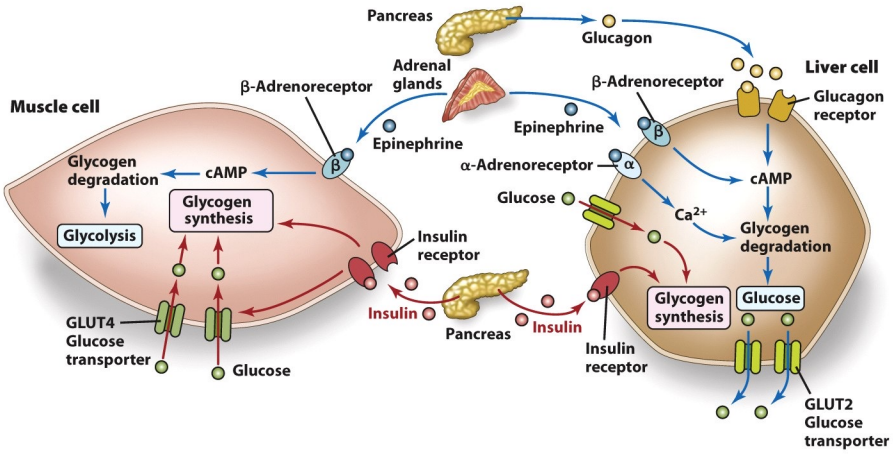

Figure 16-14
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Glycogen storage disorders

Hereditary Glycogen Storage Diseases

Type	Enzyme Deficiency	Tissue	Common Name	Glycogen Structure
I	Glucose-6-phosphatase	Liver	von Gierke's disease	Normal
II	α-1,4-Glucosidase	All lysosomes	Pompe's disease	Normal
III	Amylo-1,6-glucosidase (debranching enzyme)	All organs	Cori's disease	Outer chains missing or very short
IV	Amylo-(1,4→1,6)-transglycosylase (branching enzyme)	Liver, probably all organs	Andersen's disease	Very long unbranched chains
V	Glycogen phosphorylase	Muscle	McArdle's disease	Normal
VI	Glycogen phosphorylase	Liver	Hers' disease	Normal
VII	Phosphofructokinase	Muscle	Tarui's disease	Normal
VIII	Phosphorylase kinase	Liver	X-Linked phosphorylase kinase deficiency	Normal
IX	Phosphorylase kinase	All organs		Normal
0	Glycogen synthase	Liver		Normal, deficient in quantity

Box 16-2 table
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Glycogen storage disorders

- clinical manifestations is fatty liver -> distended abdomen
- many different kinds depending on mutated enzyme
- Von Gierke's Disease - G 6-Pase or transporters missing
- normal glycogen but high levels of trapped phospho-sugars
- surgery to liver and controlled feedings treat this disease. One of the patients was discovered in Fargo. Dr. Nordlie at UNDSM is the leader in the study of this disease.

McArdle's disease - Found after cramps at onset of exercise

- ADP concentrations increase initially and decrease w/ more exercise
- phosphorylase kinase missing in muscle but liver present (isozymes) - what is happening? Muscle glycogen is NOT available. The muscles are probably damaged due to lack of ATP. With lower levels of activity, glucose (exported from liver) can enter and take the place of glycogenesis.

Pompe's Disease - Missing glucosidase activity, usually found in lysosomes.

Leads to large increases in glycogen found in lysosomes in nearly every tissue in the body. Once the glycogen particles are in the lysosome it can no longer function normally, although extralysosomal glycogene acts as normal. The reason for this is not known, but results in cardiomegaly and death occurs at an early age from heart failure.

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Gluconeogenesis - Generation of glucose from pyruvate

- **The principle noncarbohydrate precursors of glucose are lactate, pyruvate, and amino acids. Lactate and amino acids can be converted to glucose by this pathway (glucogenic) - NOT Fatty acids**
- **Most amino acids (except leucine and lysine) are converted into oxalacetate and then metabolized to glucose.**
- **This is only in part a reversal of glycolysis. Remember the irreversible reactions of glycolysis.**
  - **Liver and kidney are only real gluconeogenic tissues**
    - **This is how liver can provide most of glucose during fasting state - i.e. after the glycogen is gone.**

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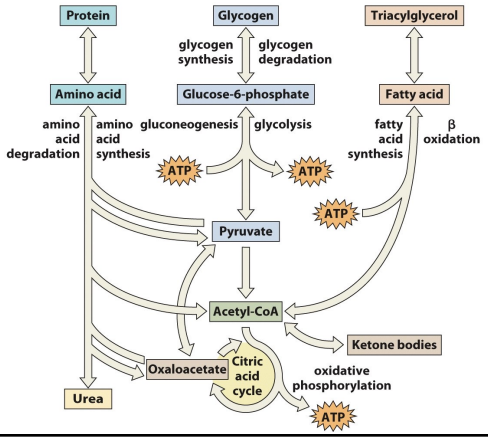
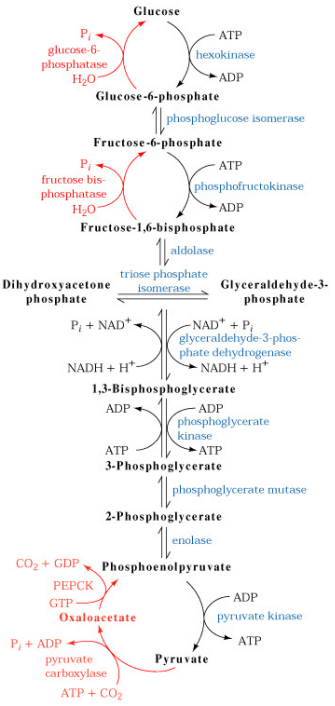
Pathway and Tissues of gluconeogenesis

ONLY Liver and Kidney have all gluconeogenic enzymes expressed!





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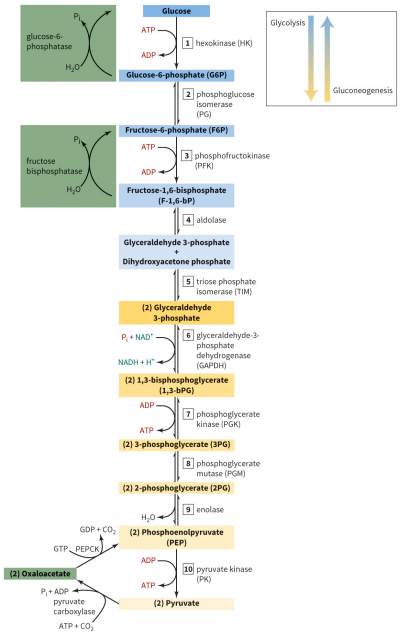
Gluconeogenesis

Reactions of gluconeogenesis.

- Gluconeogenesis differs from glycolysis by four different reactions (three steps).
- Pyruvate is converted to oxaloacetate by pyruvate carboxylase.
- Phosphoenolpyruvate is formed from oxaloacetate by phosphoenolpyruvate carboxykinase (PEPCK).
- The steps of glycolysis now proceed in the reverse direction until fructose-1,6-bisphosphate is synthesized.
- Removal of the last two phosphates to yield glucose is accomplished by fructose bisphosphatase and glucose-6-phosphatase.



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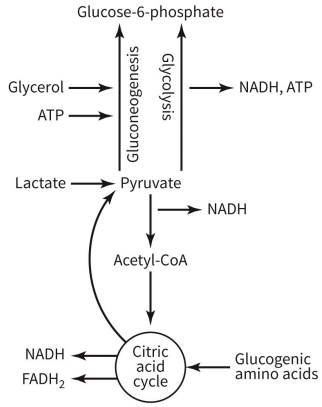
Gluconeogenesis



Pathways that flow through gluconeogenesis. Glucogenic precursors include lactate, pyruvate, glycerol, and glucogenic amino acids.

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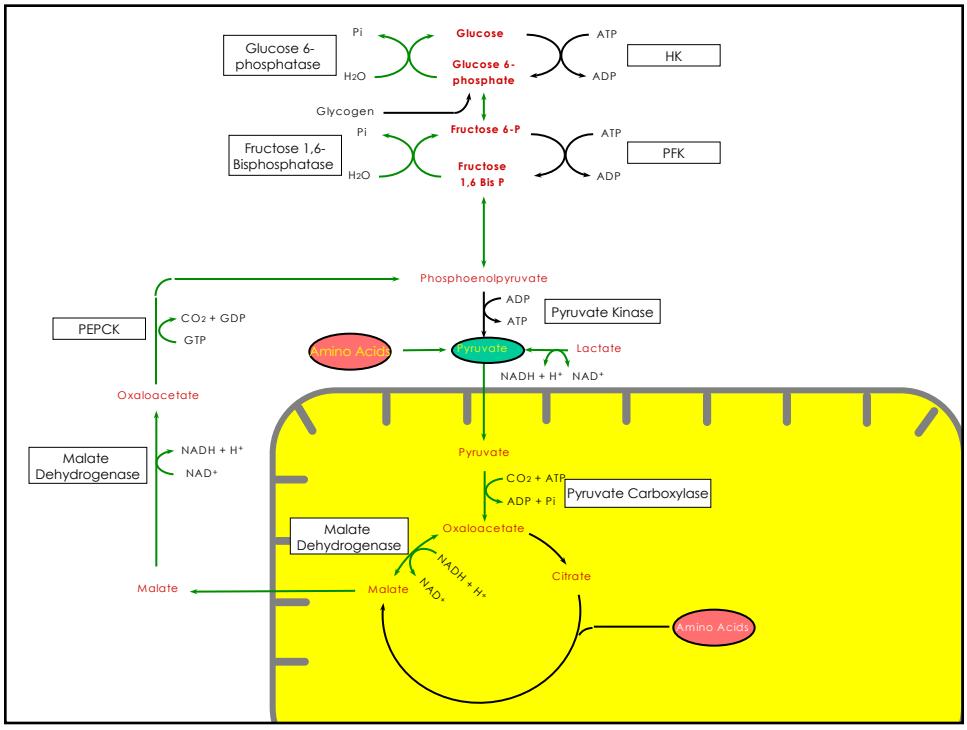

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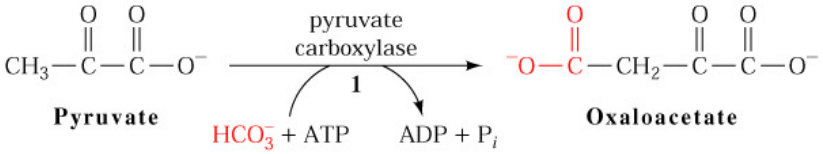
There are three steps and four enzymes involved.

Step 1 Pyruvate to Phosphoenolpyruvate (2 enzymes)

***1. Pyruvate carboxylase* (PC)**

- **pyruvate + CO_2 + ATP-> oxaloacetate + ADP +Pi**
- **Pyruvate is carboxylated in the mitochondria and the reaction is driven by ATP hydrolysis**



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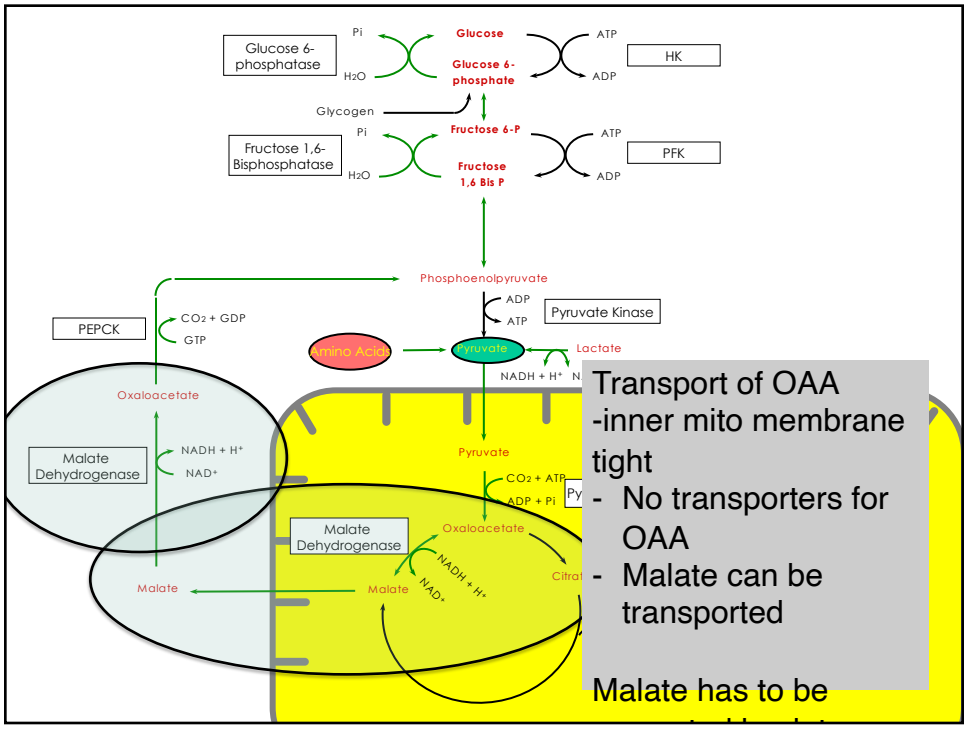

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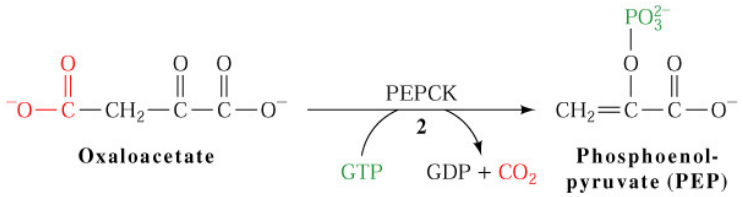
2. *Phosphoenolpyruvate kinase* (PEPCK)

- 2 isozymes cytosolic/mitochondrial
- Found in liver, kidney and adipose
- OAA + GTP -> PEP + GDP + Pi
- Potential involvement in SIDS
- In adipose (fat cells) glycerol is produce rather than glucose (via - glycerol 3-phosphate). Used for glycerol backbone of triacylglycerol
- In kidney PEPCK is responsible for decrease ammonia produced via the krebs cycle. Ultimately responsible for acid base regulation,)



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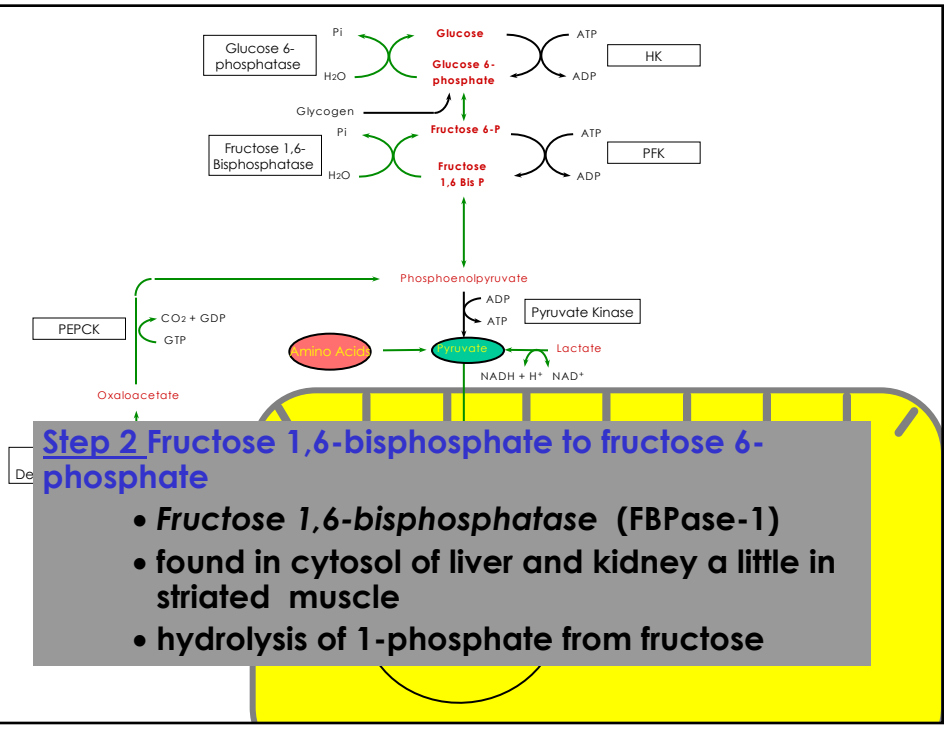

Step 2 Fructose 1,6-bisphosphate to fructose 6-phosphate

- *Fructose 1,6-bisphosphatase* (FBPase-1)
- found in cytosol of liver and kidney a little in striated muscle
- hydrolysis of 1-phosphate from fructose

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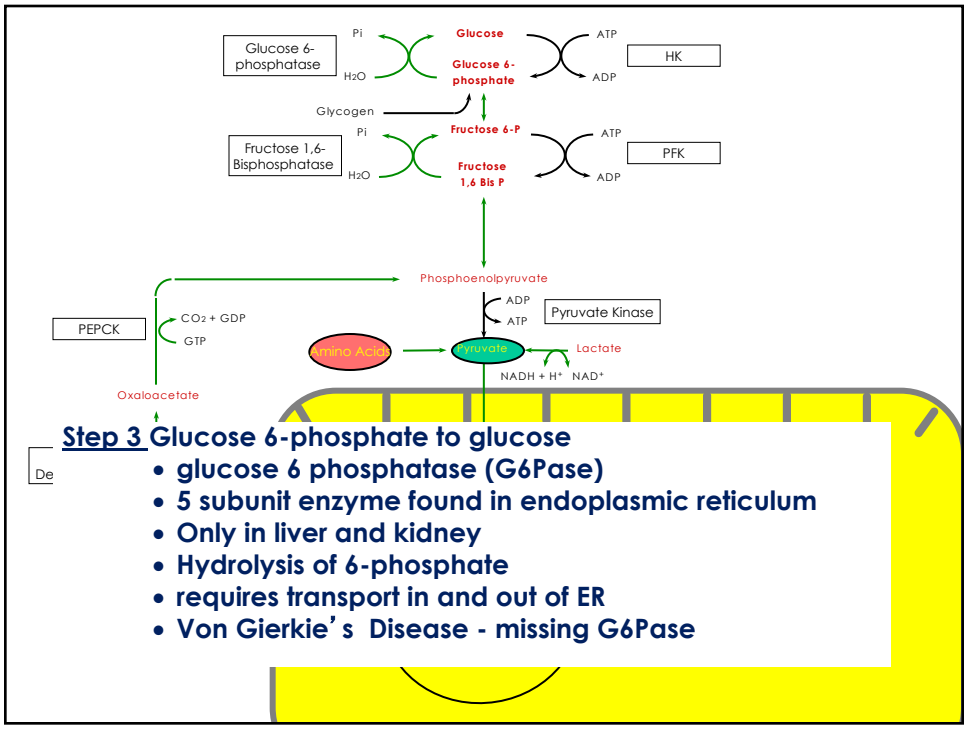

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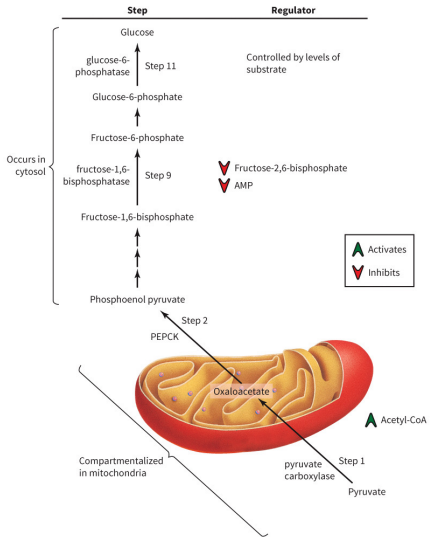
Regulation of Gluconeogenesis

The regulation of gluconeogenesis is in many ways the reciprocal of glycolysis. In addition, the first two reactions of gluconeogenesis are compartmentalized to the mitochondria.

- *Occurs through compartmentalization*
- *Allosteric regulation*
- *Phosphorylation*
- *Changes in gene expression*



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Control of Gluconeogenesis

- **Several common factors that increase one pathway will shut off the other.**
  - **High energy state -> ATP, citrate**
  - **Low energy state -> ADP, AMP**
  - **Fructose 2,6 bisphosphate -> increase blood [glucose]**
  - **Starvation increases gluconeogenesis**
  - **High carbo reduces gluconeogenesis while low carbo diet increases.**
- **In general, the well feed state decreases gluconeogenesis and increases glycolysis**