Welcome to BCII lesson 6

Chimie Biologique II

Biological Chemistry II

BIO-213

Teachers

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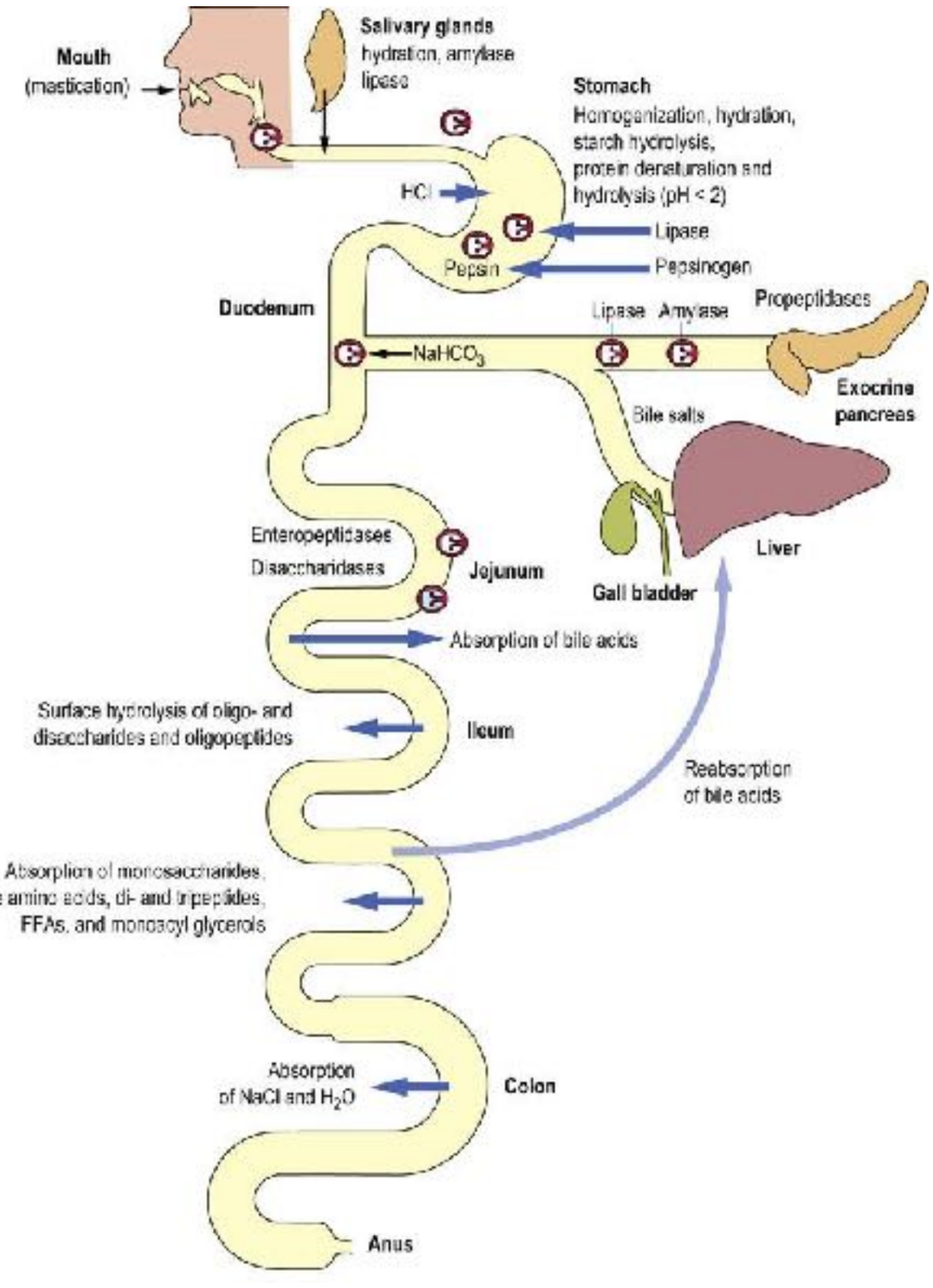
Lecture 6

The biosynthesis of carbohydrates

- gluconeogenesis
- glycogen metabolism

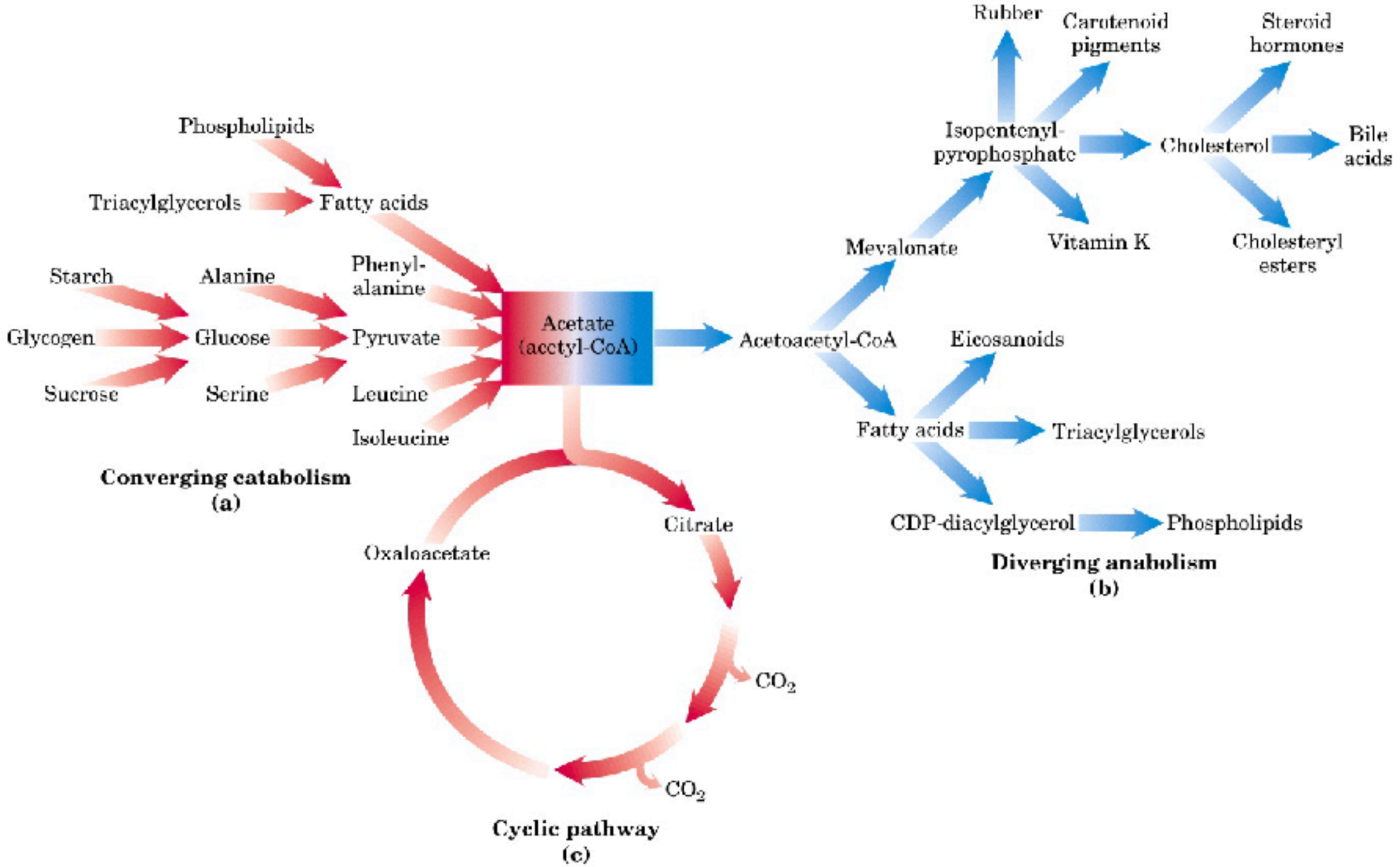
Recap Catabolism



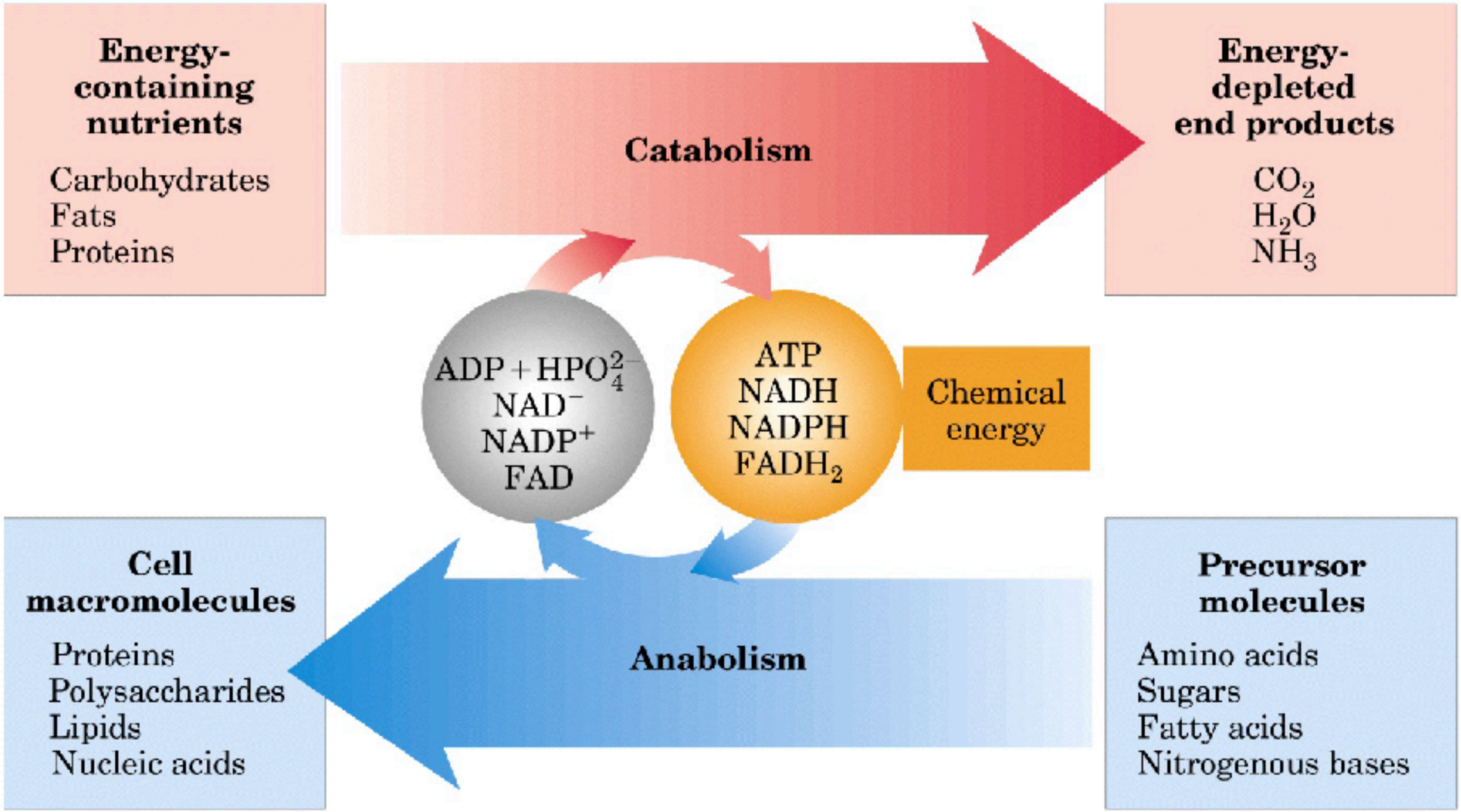
The food we ingest contains different nutrients including sugars, fats, and proteins. In our digestive tract, dedicated enzymes decompose these nutrients into simple constituents (complex sugars -> monosaccharides; fats -> fatty acids; proteins -> amino acids). These simple constituents are absorbed in our intestine and mobilised through our bloodstream. Once up-taken by cells monosaccharides can undergo glycolysis, fatty acids can undergo beta oxidation and amino acid can undergo aa oxidation. Following these catabolic steps, the products generated from these nutrients enter the TCA cycle for the production of NADH and FADH2. NADH and FADH2 enter the oxidative phosphorylation process to produce ATP (with consumption of O2 and release of CO2).

In all we use nutrients (food) and oxygen to produce energy (ATP) and CO2.

Recap Catabolism



Catabolism <=> Anabolism



Anabolic Pathways-General Concepts

- **Anabolic** pathways (as opposed to catabolic ones) use chemical energy from ATP, NADH, or NADPH to synthesise cellular components from simple precursor molecules.

- Anabolism and catabolism simultaneously operate in many cells in a **dynamic steady state** where the energy produced by catabolic reactions is used in biosynthetic processes.

- The biosynthetic and degradative pathways to/from a molecule differ **at least in one reaction** (*i.e.,* at least two of the enzymes in the pathway are specific for either the degradative of biosynthetic pathway).

- Corresponding catabolic and anabolic pathways are **controlled** through thee enzymes that are specific to **the one or the other pathway**.

- Biosynthetic processes require a consumption of chemical energy to be spontaneous that exceeds the energy produced in the corresponding catabolic processes.

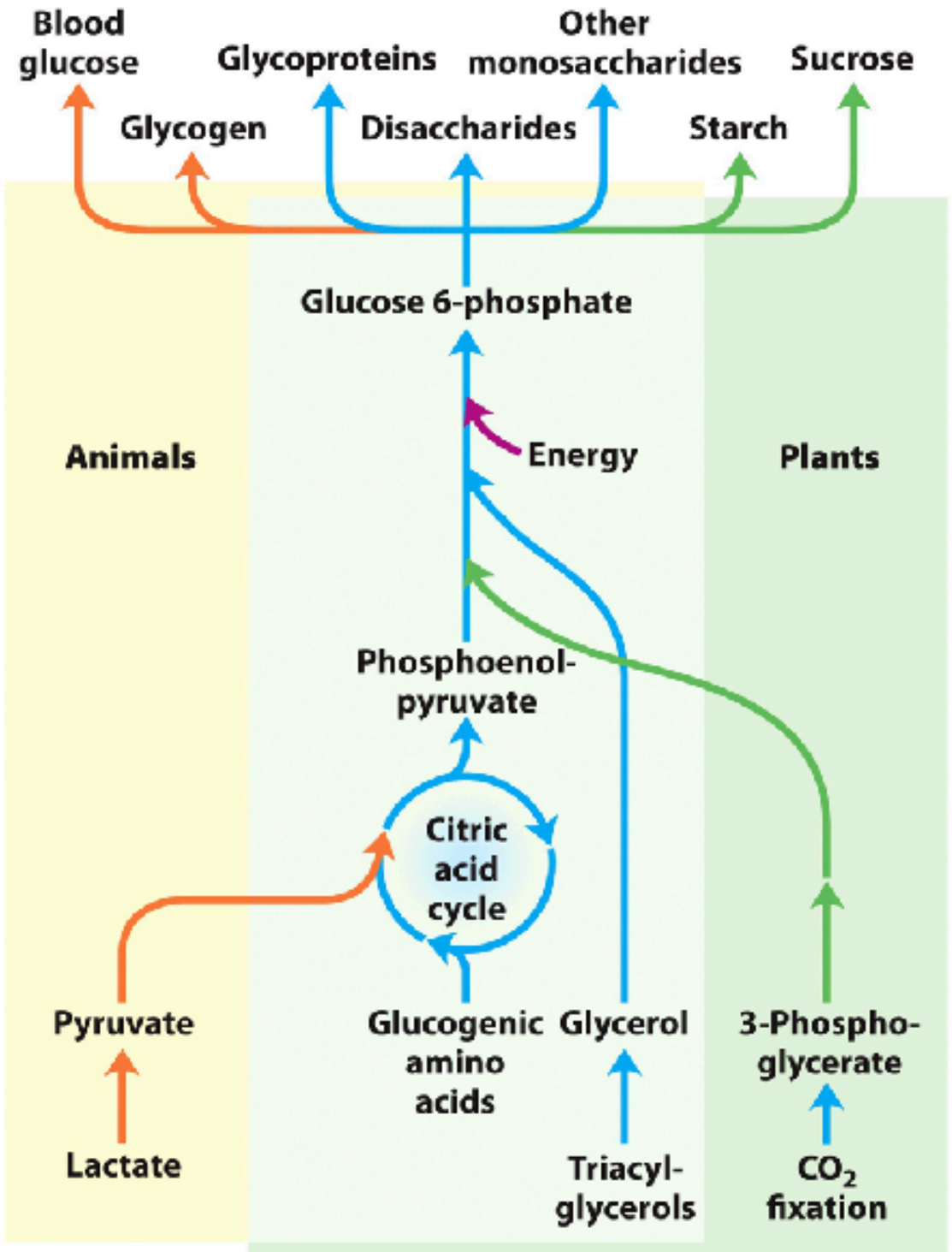
Gluconeogenesis

Gluconeogenesis (the make of new sugars) is the production of glucose from non-sugar precursors.

In its complete version it happens in the liver and kidney cortex.

Our body can store on average **210 g** of glucose (as a circulating molecule and as glycogen). The daily requirement for glucose is on average **160 g**. Some organs (CNS) use glucose as their energy source.
— in absence of gluconeogenesis our neural cells will stop functioning after 2 days in starving conditions.

The major non-sugar precursors used by mammals are **pyruvate**, **lactate**, **glycerol** and **amino acids**.

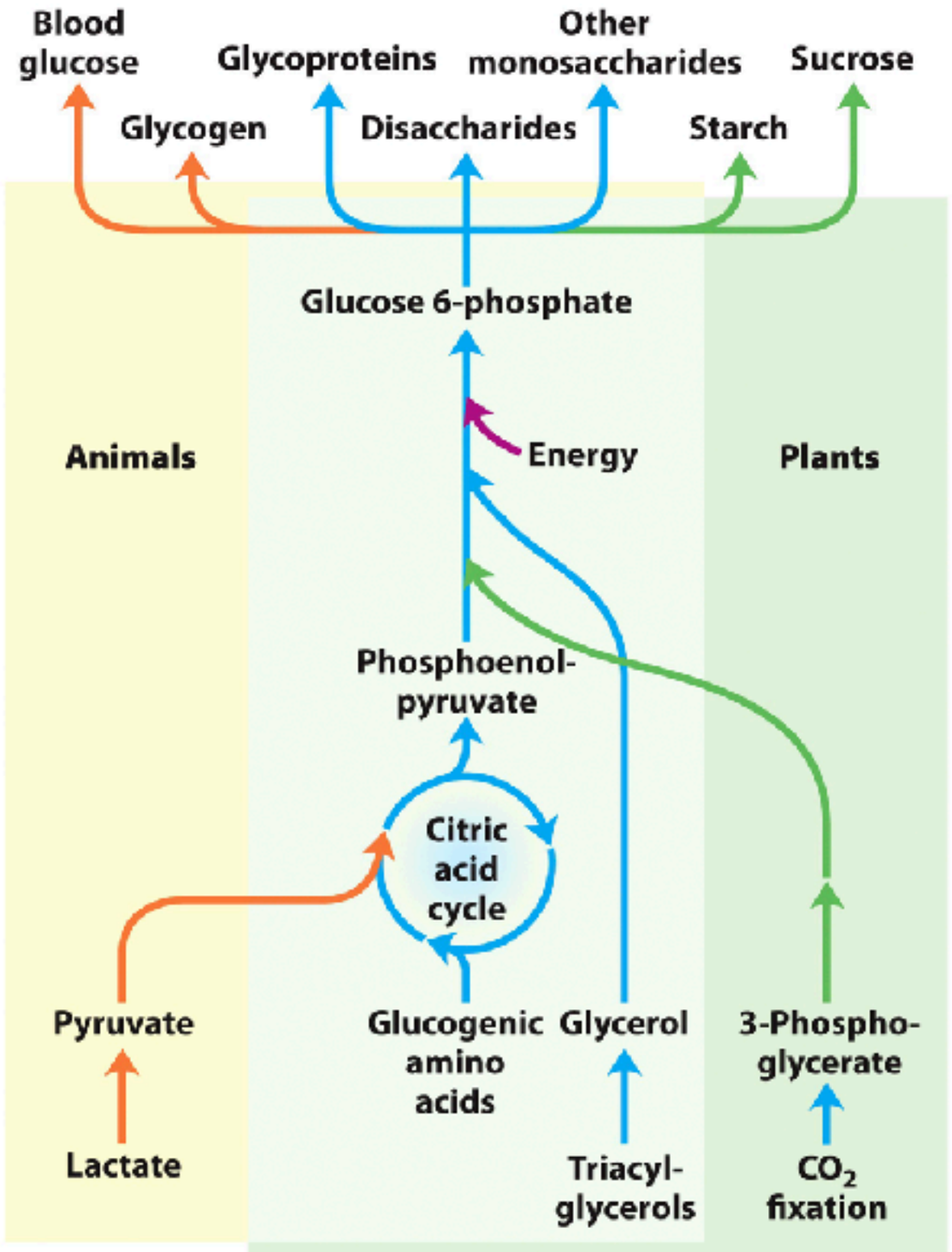


Gluconeogenesis

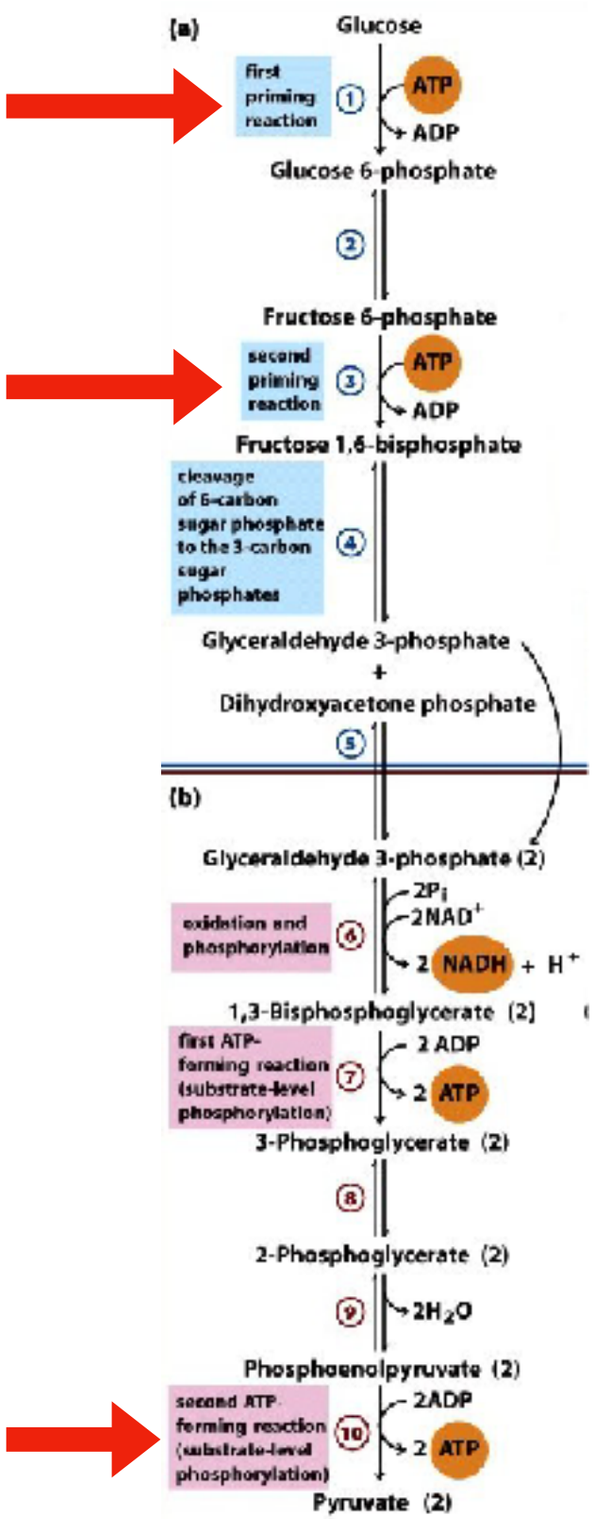
Lactate: produced by skeletal muscle cells under limiting oxygen conditions can be transformed into **pyruvate** by lactate dehydrogenase.

Glycerol: is produced by the breakdown of triglycerides in adipose tissue. It is converted into dihydroxyacetone phosphate (**DHAP**) (see Lecture 2 - slide 20).

Amino Acids: Obtained from the hydrolysis of proteins under starvation conditions. Some amino acids are converted to pyruvate others are converted to DHAP.



Gluconeogenesis



Gluconeogenesis is essentially the opposite of **glycolysis**. However it does not simply consist of of the reverse steps of glycolysis.

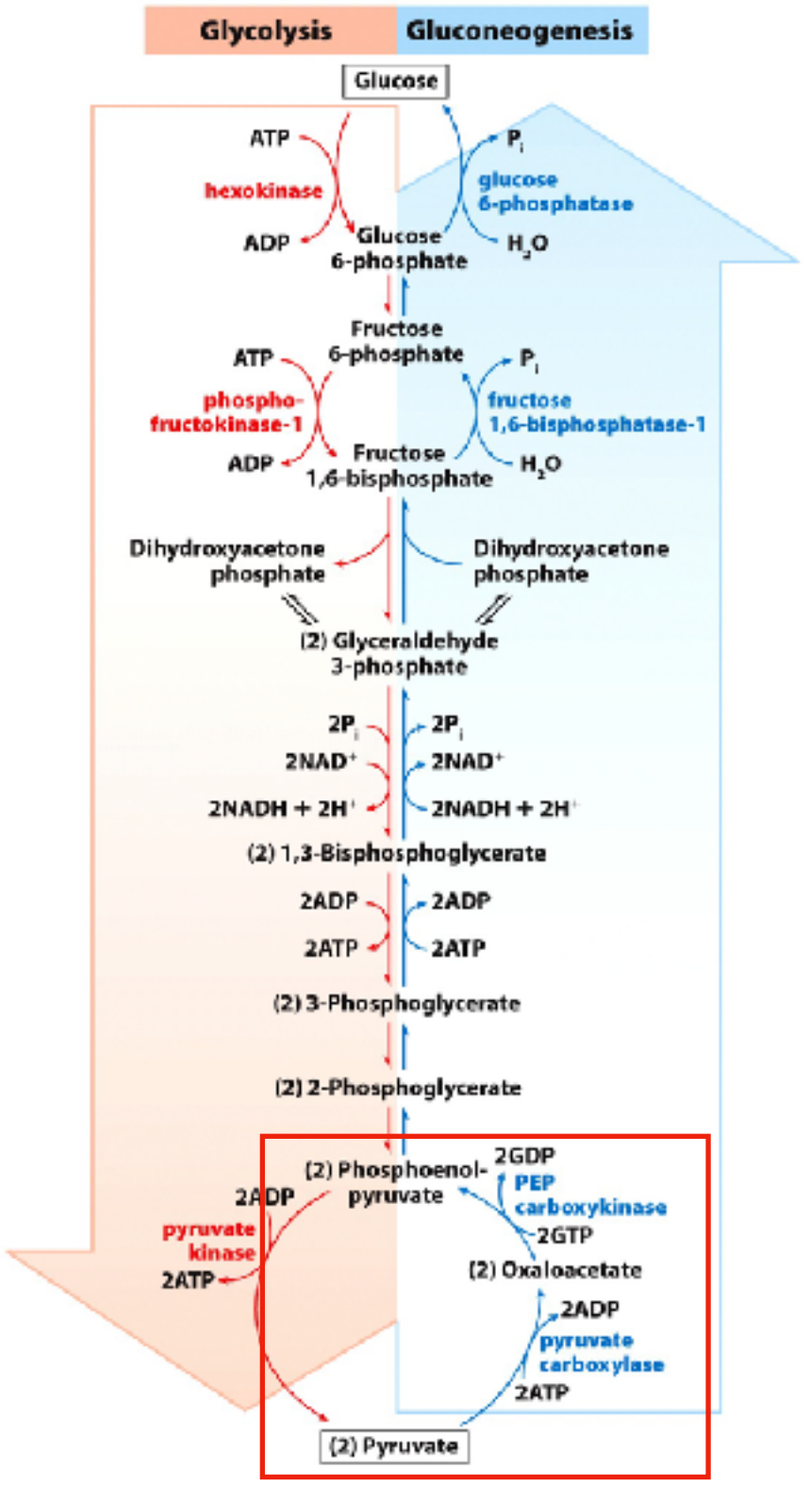
Three reactions in glycolysis are extremely exergonic and thus '**irreversible**'.

Gluconeogenesis circumvents these reactions by following different pathways

TABLE 14–2 Free-Energy Changes of Glycolytic Reactions in Erythrocytes

Glycolytic reaction step	$\Delta G'^{\circ}$ (kJ/mol)	ΔG (kJ/mol)
1 Glucose + ATP ⟶ glucose 6-phosphate + ADP	−16.7	−33.4
2 Glucose 6-phosphate ⇌ fructose 6-phosphate	1.7	0 to 25
3 Fructose 6-phosphate + ATP ⟶ fructose 1,6-bisphosphate + ADP	−14.2	−22.2
4 Fructose 1,6-bisphosphate ⇌ dihydroxyacetone phosphate + glyceraldehyde 3-phosphate	23.8	−6 to 0
5 Dihydroxyacetone phosphate ⇌ glyceraldehyde 3-phosphate	7.5	0 to 4
6 Glyceraldehyde 3-phosphate + P_i + NAD^+ ⇌ 1,3-bisphosphoglycerate + NADH + H^+	6.3	−2 to 2
7 1,3-Bisphosphoglycerate + ADP ⇌ 3-phosphoglycerate + ATP	−18.8	0 to 2
8 3-Phosphoglycerate ⇌ 2-phosphoglycerate	4.4	0 to 0.8
9 2-Phosphoglycerate ⇌ phosphoenolpyruvate + H_2O	7.5	0 to 3.3
10 Phosphoenolpyruvate + ADP ⟶ pyruvate + ATP	−31.4	−16.7

Gluconeogenesis



Gluconeogenesis bypasses

step 10 of glycolysis via a two step pathway that involves an oxaloacetate intermediate.

step 3 by the exergonic hydrolysis of Fructose 1,6-bisphosphate.

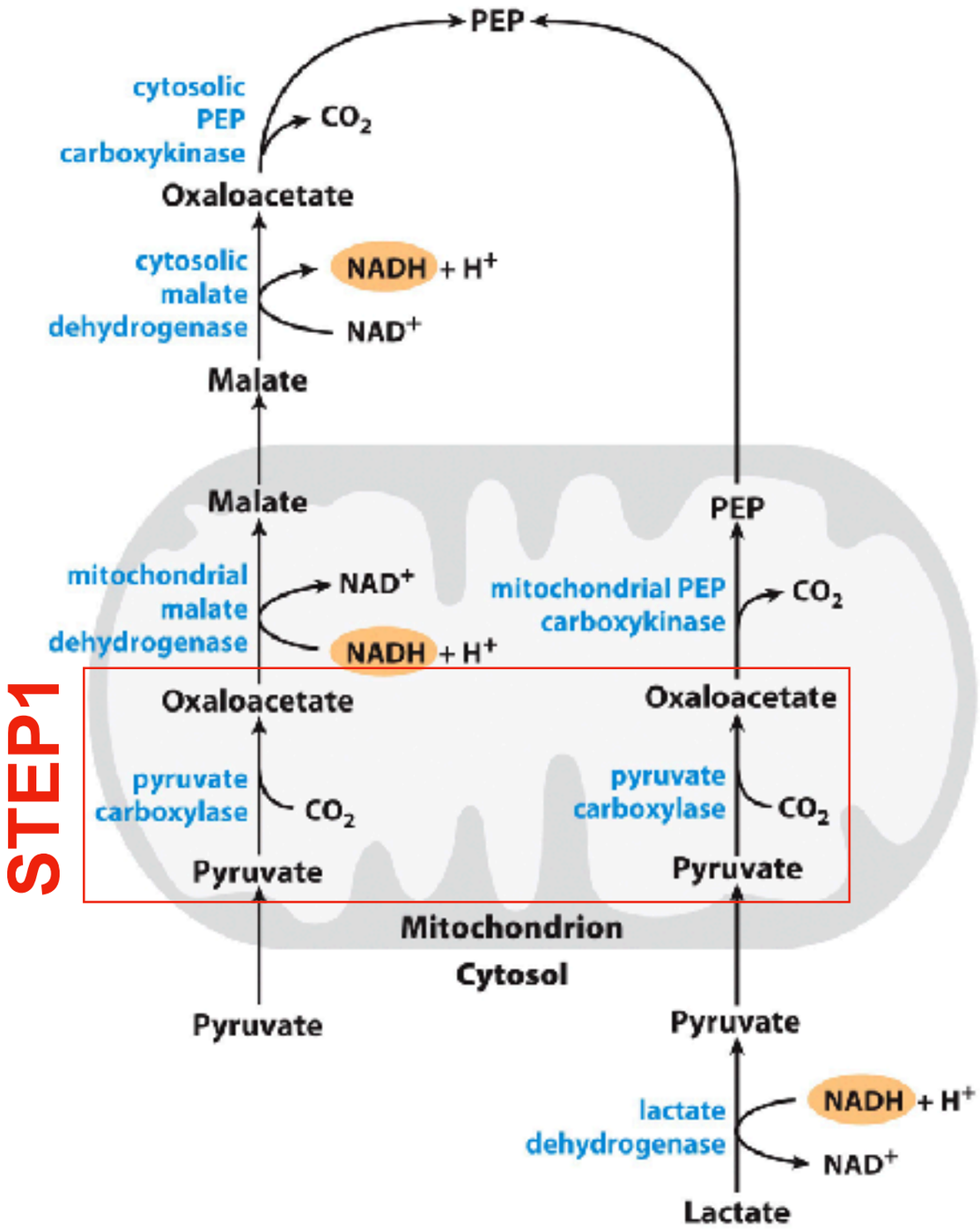
step 1 by the exergonic hydrolysis of Glucose 6-phosphate.

The enzymes that operate at these steps are different from the of glycolysis .

The catalysed reactions are ‘spontaneous’

The enzymes involved are subjected to regulation.

Gluconeogenesis - Step 1



1. **Pyruvate** makes it to the the mitochondrial matrix

2. In the mitochondria **Pyruvate** is carboxylated to **Oxaloacetate** by **pyruvate carboxylase**

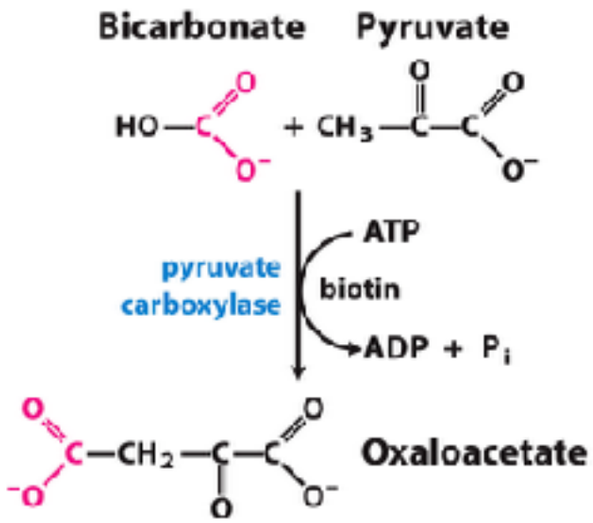
3. **Oxaloacetate** is either transformed to **PEP** by mitochondrial **PEP carboxylkinase** or converted to **malate** by **malate dehydrogenase**.

4. **Malate** and **PEP** are transported to the cytosol

5. **Malate** is reconverted into **Oxaloacetate** by cytosolic **malate dehydrogenase**

7. **Oxaloacetate** is transformed to **PEP** by cytosolic **PEP carboxylkinase**

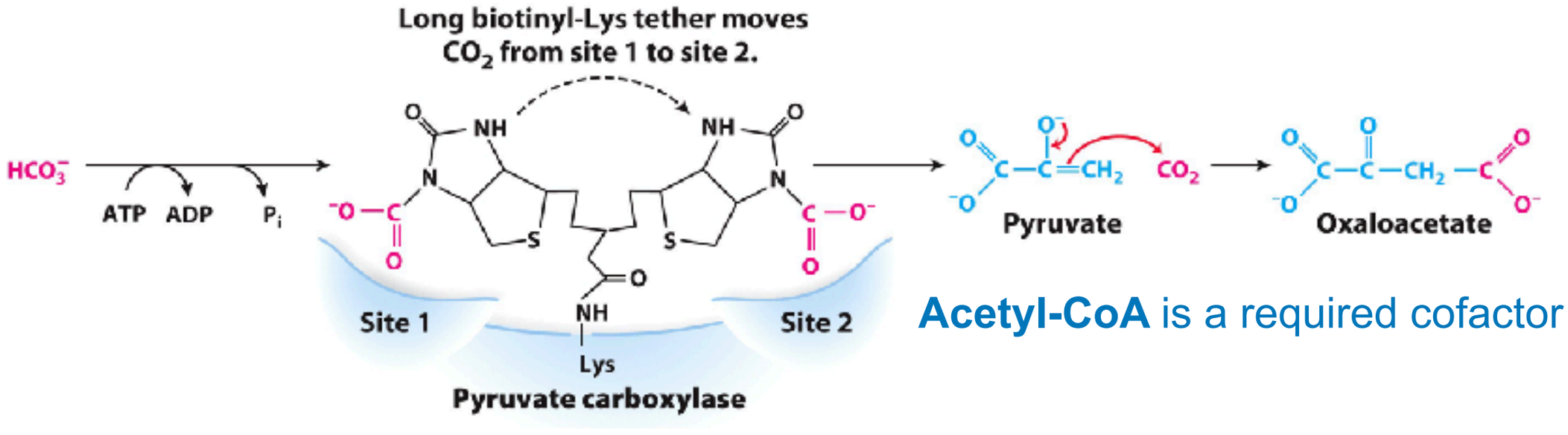
Gluconeogenesis - Step 1



Pyruvate carboxylase consists of 4 subunits that each have:

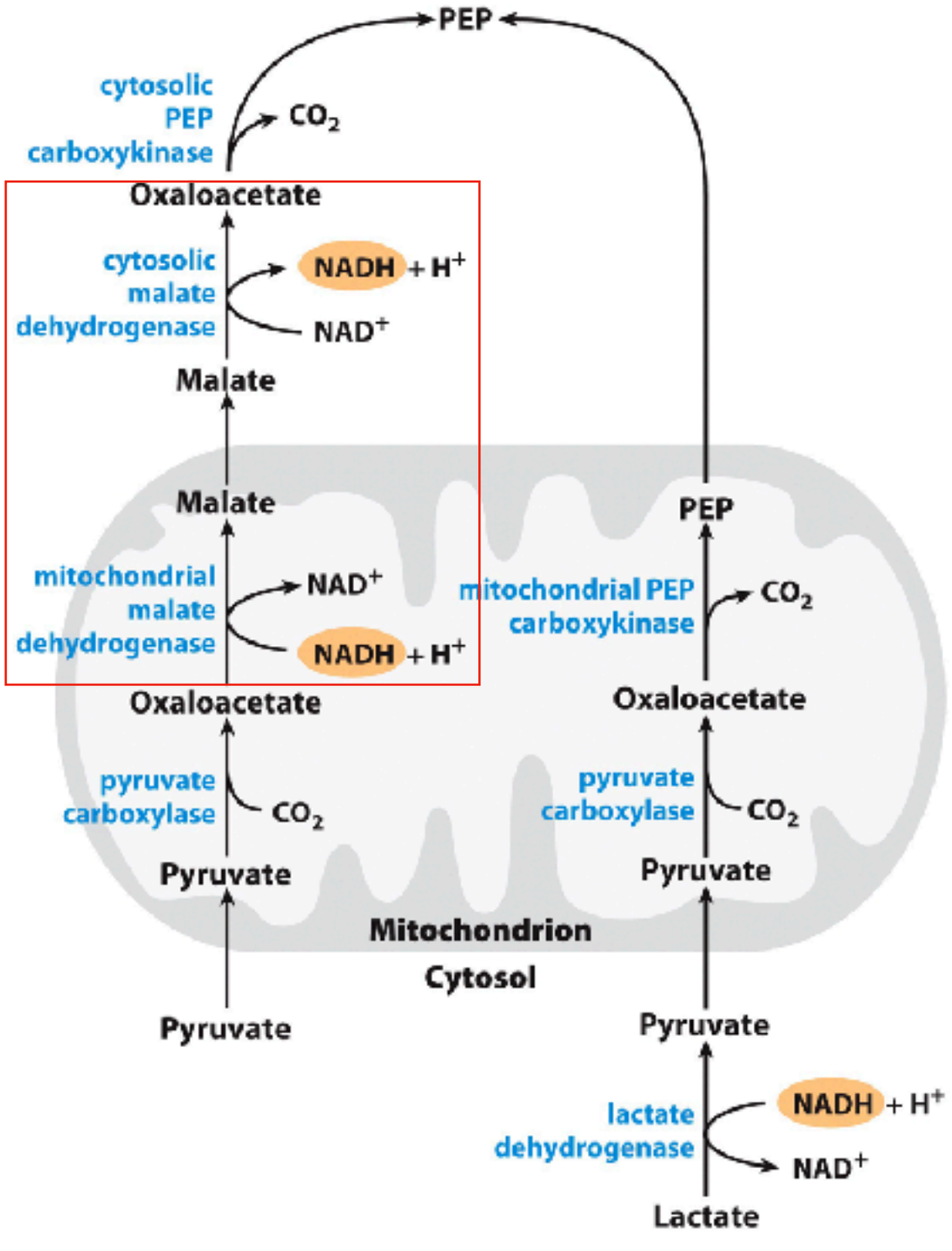
a) a biotin binding domain that complexes with the CO2 molecule and brings it into the active site
b) a domain that binds ATP that is needed to activate the CO2

Phase 1: ATP activates CO2 forming carboxylphosphate
Phase 2: Phosphorylated CO2 is attached to the biotin-enzyme with Pi release
Phase 3: The CO2 is transferred to pyruvate to to form oxaloacetate



Acetyl-CoA is a required cofactor

Gluconeogenesis - Step 1



In the pathway involving cytosolic PEP carboxykinase

1. **Oxaloacetate** cannot be exported as such by mitochondria

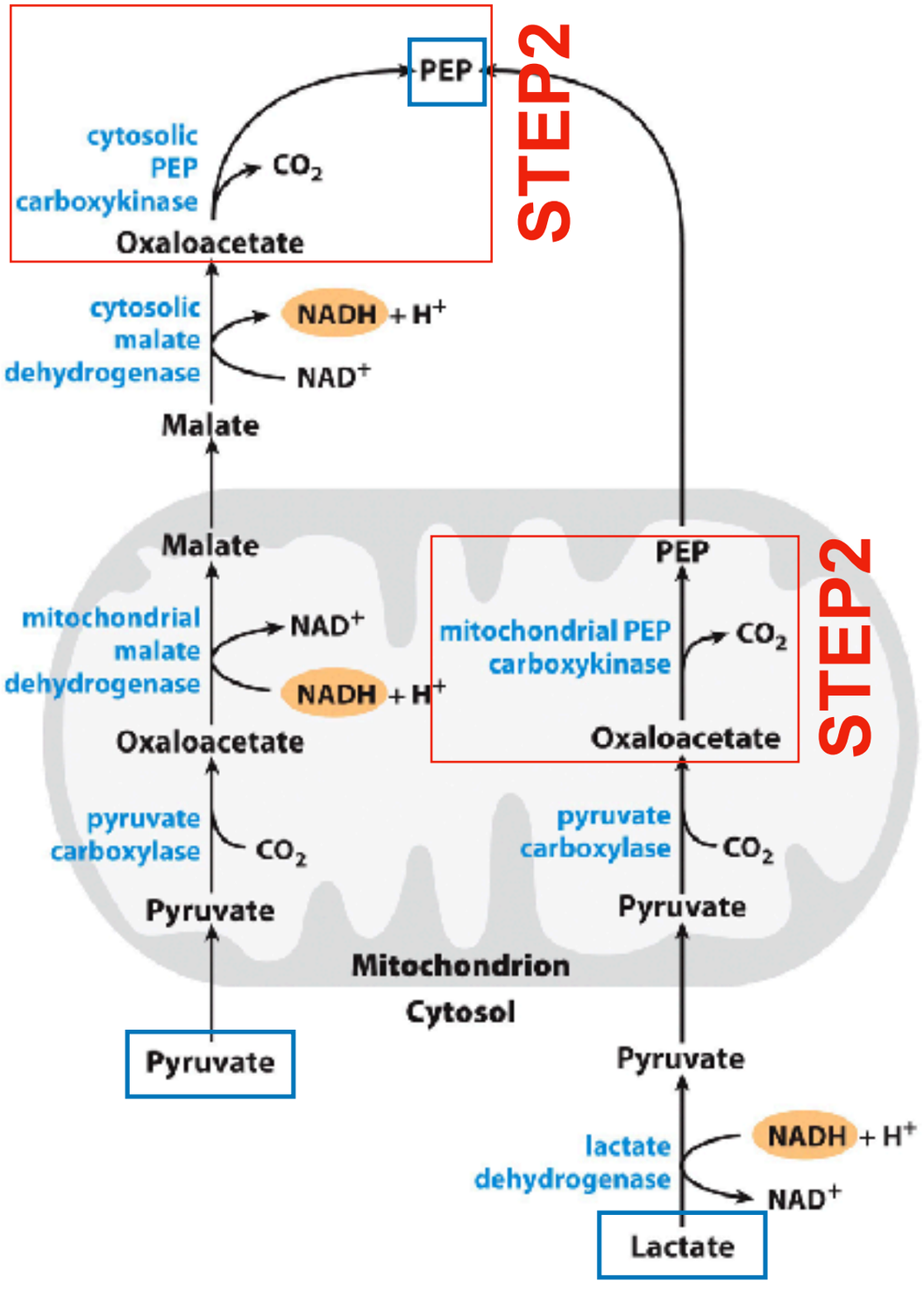
2. To be exported, **Oxaloacetate** is reduced to **Malate** with consumption of a NADH

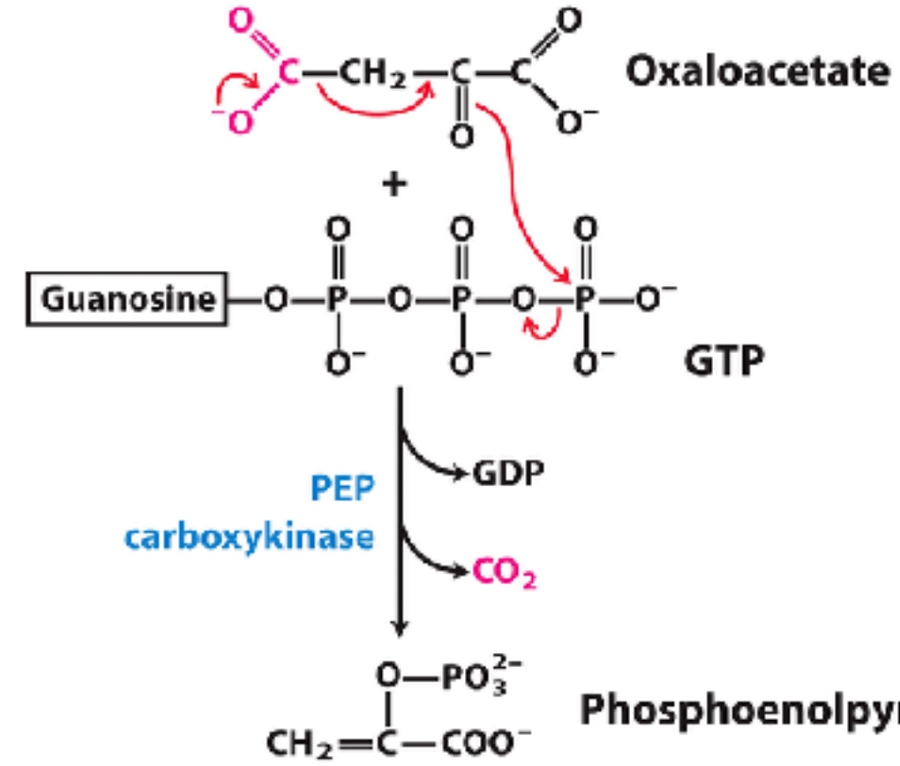
3. **Malate** is reconverted into **Oxaloacetate** with production of an NADH in the cytosol

4. this results in the net increase of NADH in the cytosol (reducing equivalents are required in later steps)

5. When **Lactate** is the source of **Pyruvate** NADH is formed in the cytosol by lactate dehydrogenase (no need to export oxaloacetate to increase cytosolic NADH)

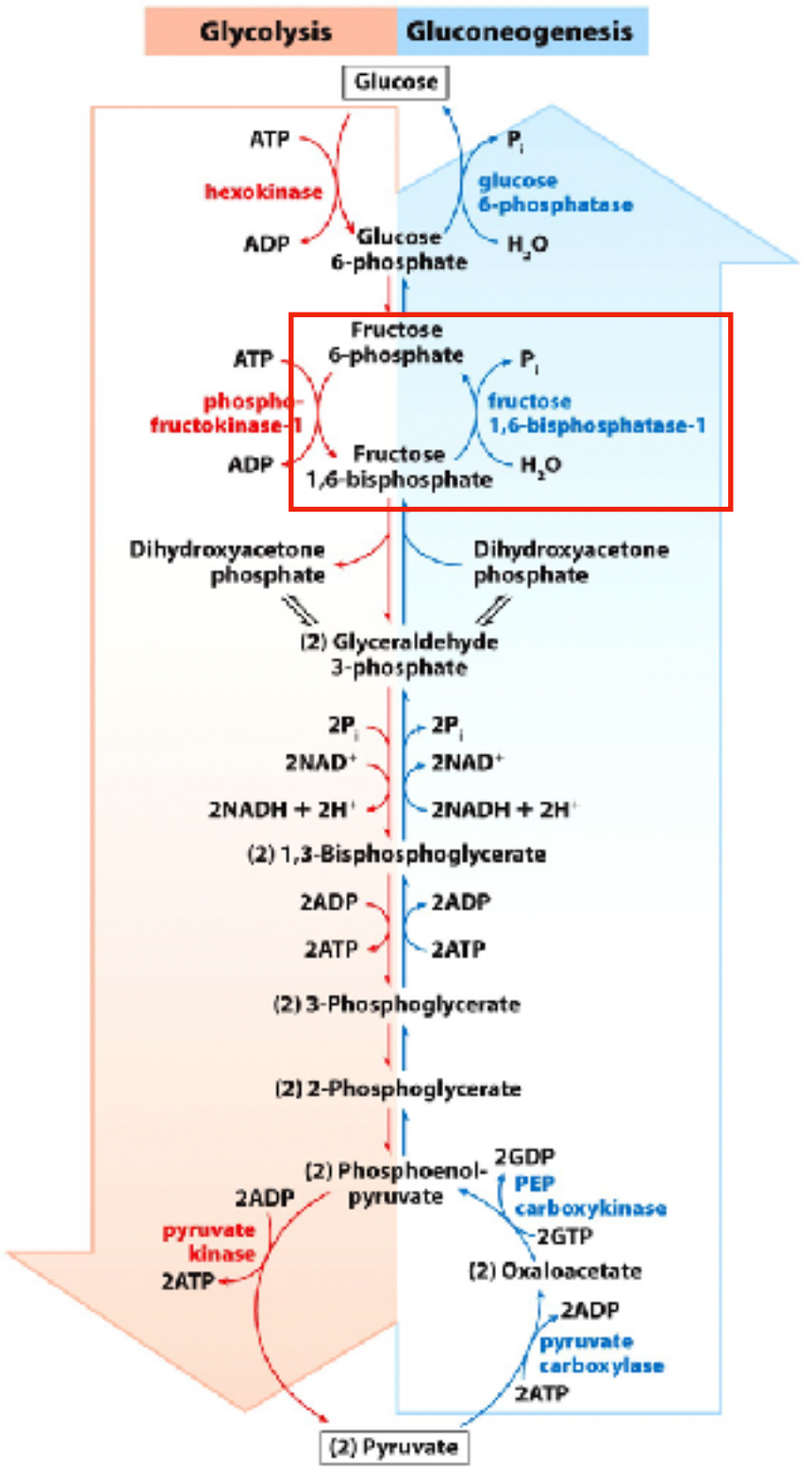
Gluconeogenesis - Step 2

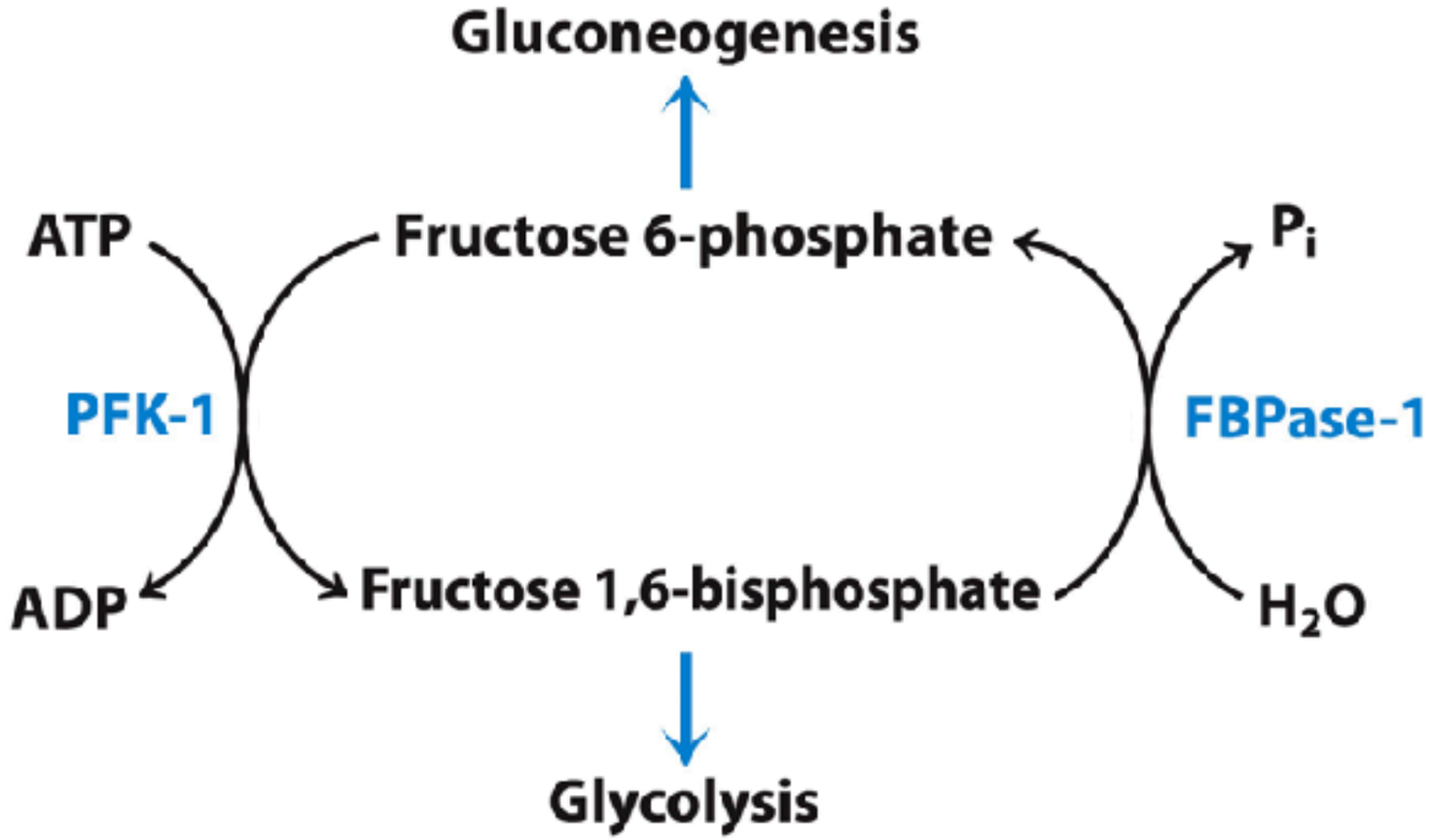




In the second step PEP carboxykinase coverts **oxaloacetate** into **PEP**. In this step the **highly endergonic** phosphorylation is coupled to the **exergonic** decarboxylation

Gluconeogenesis - Step8

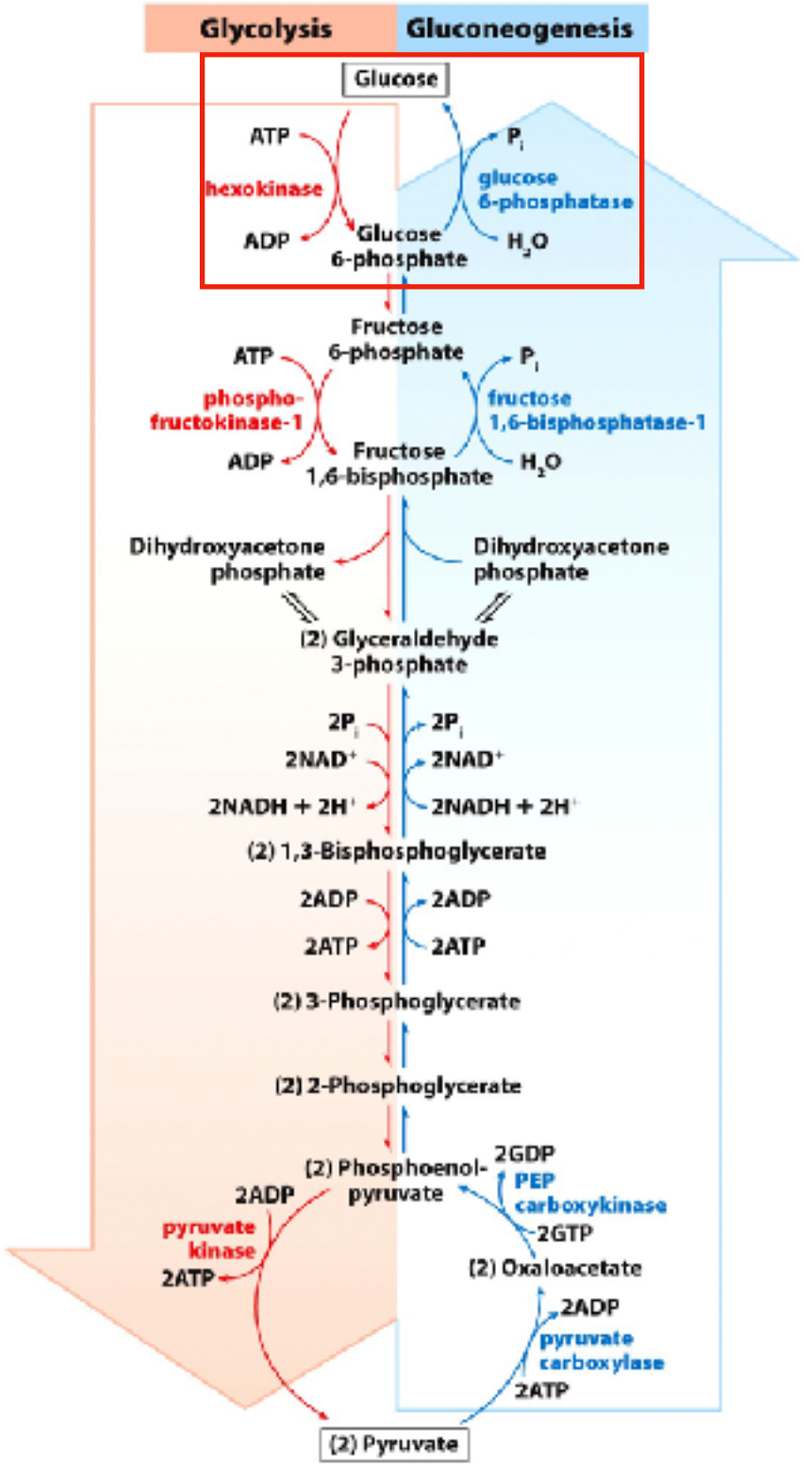




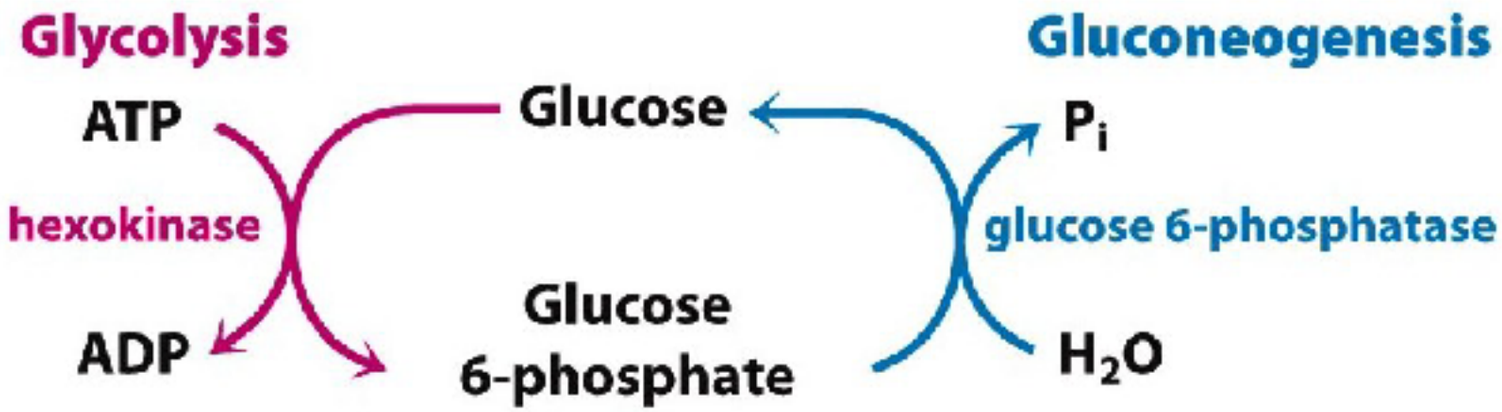
in the reaction catalysed by **Fructose 1,6-bisphosphatase-1**, Fru(1,6)P2 is dephosphorylated with the release of Pi. Note that this is different from the reverse reaction catalysed by PFK1 as this would imply the highly unfavourable transfer of Phosphate to ADP.

PFK-1 and FBPase-1 are allosterically regulated enzymes

Gluconeogenesis - Step 10



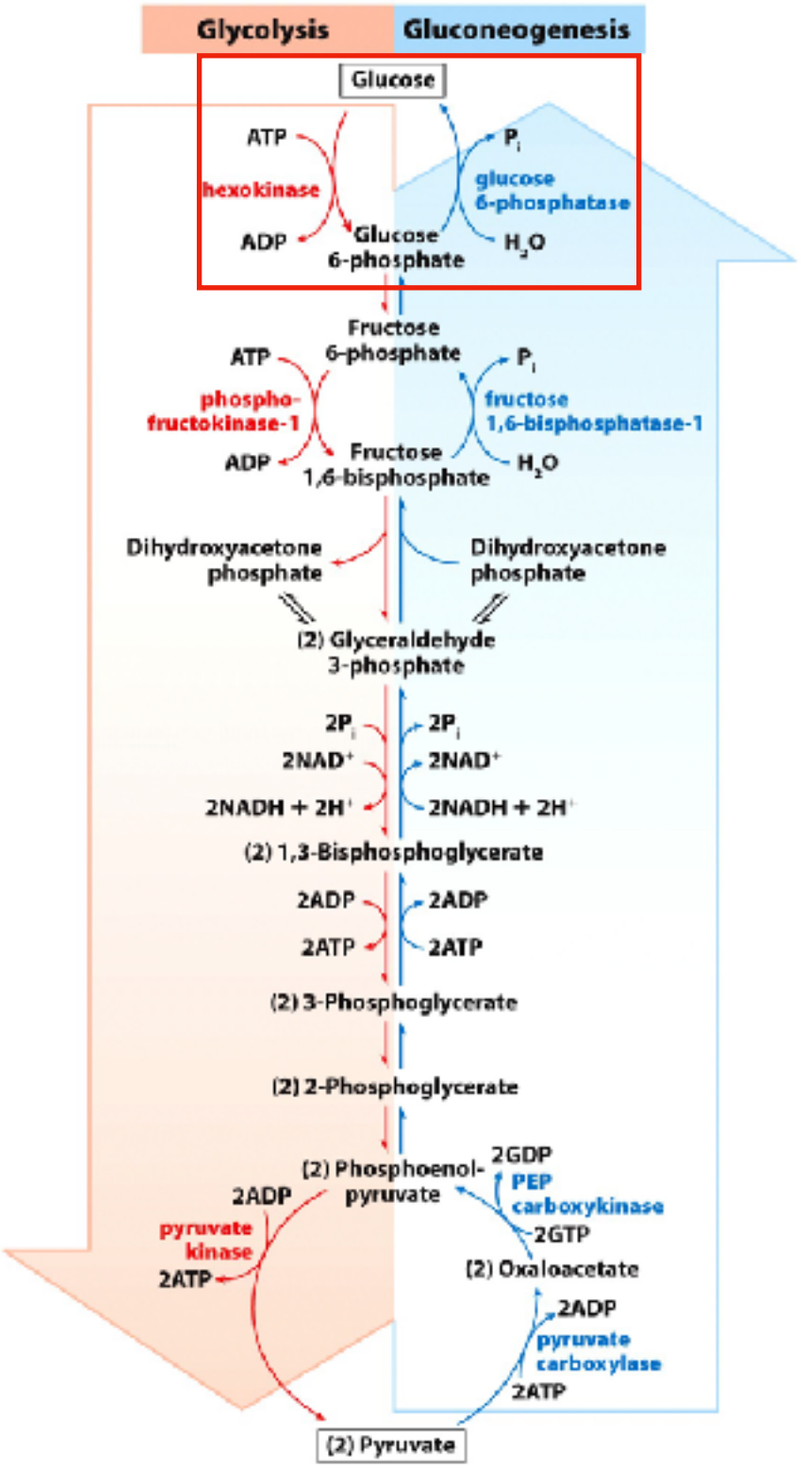
In most cells Gluconeogenesis stops at the Glucose-6-Phosphate level whereby this metabolite is used to initiate glycogen production. In liver and kidney cortex cells Glu(6)P is converted to Glucose that can leave the cells for the blood-stream.

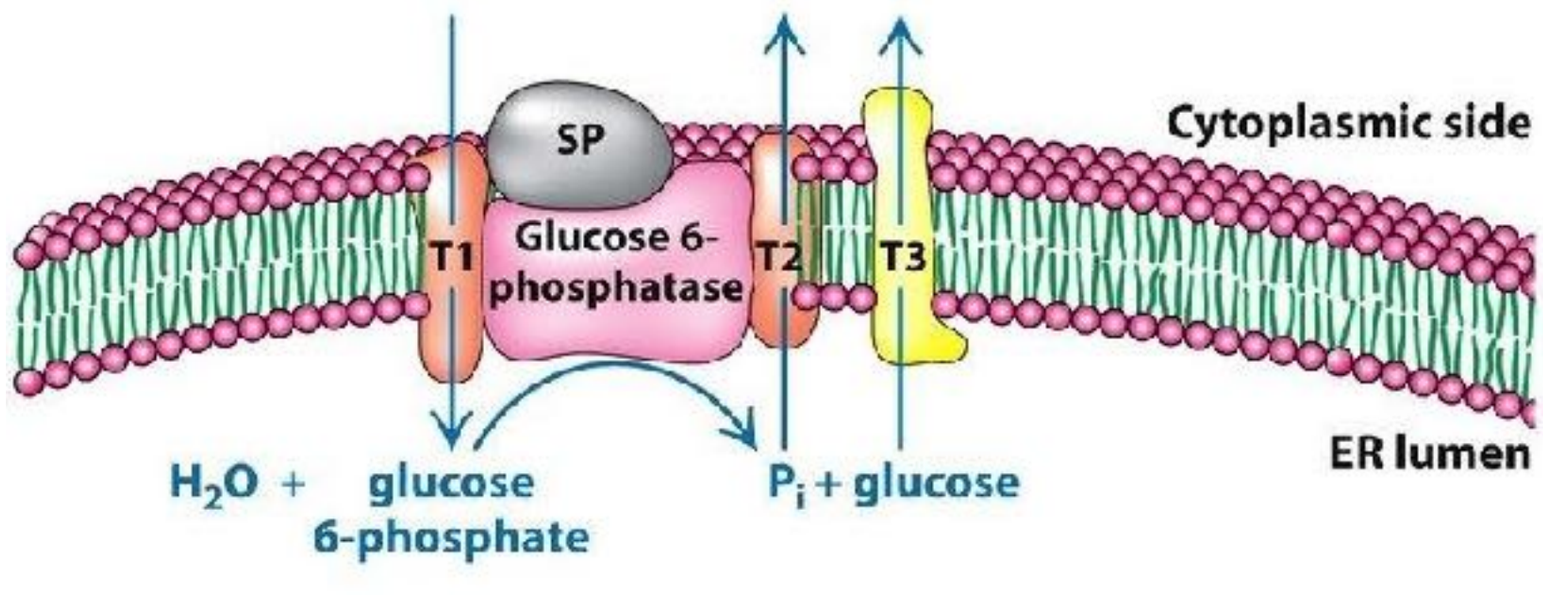


in the reaction catalysed by **Glucose 6-phosphatase**, Glu(6)Pis dephosphorylated with the release of Pi. Note that this is different from the reverse reaction catalysed by hexokinase as this would imply the highly unfavourable transfer of Phosphate to ADP.

This reaction happens with a peculiar topology

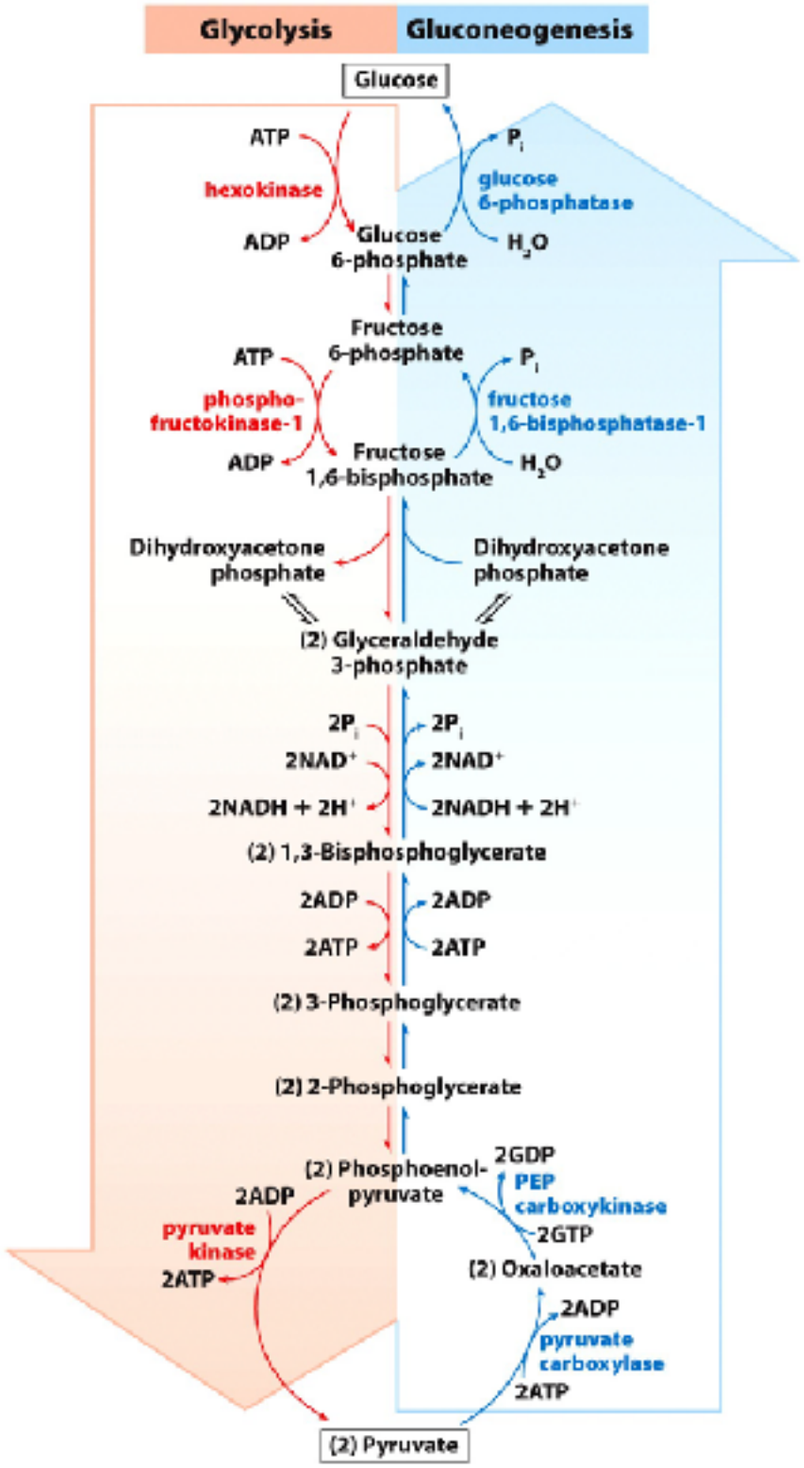
Gluconeogenesis - Step 10

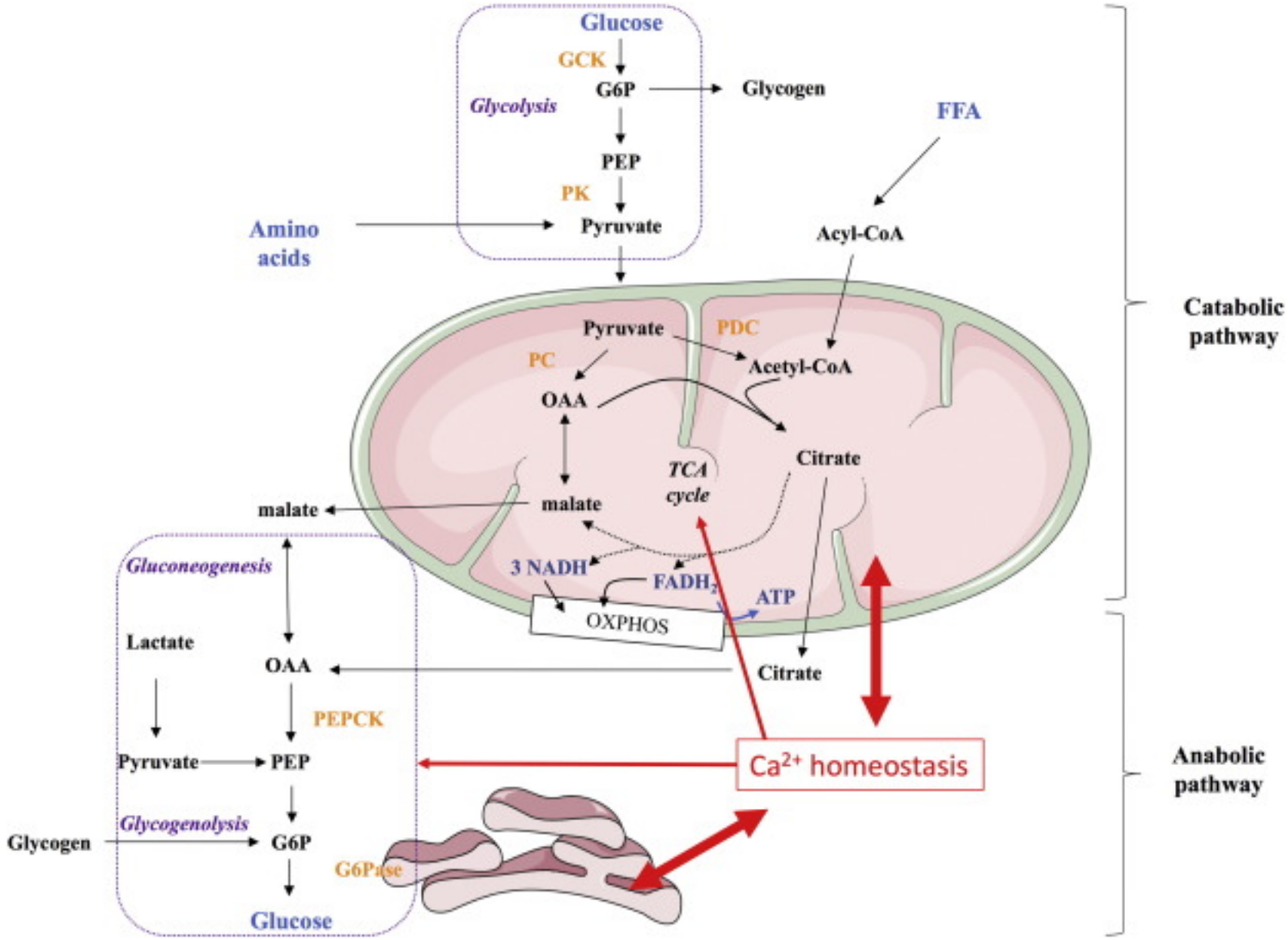




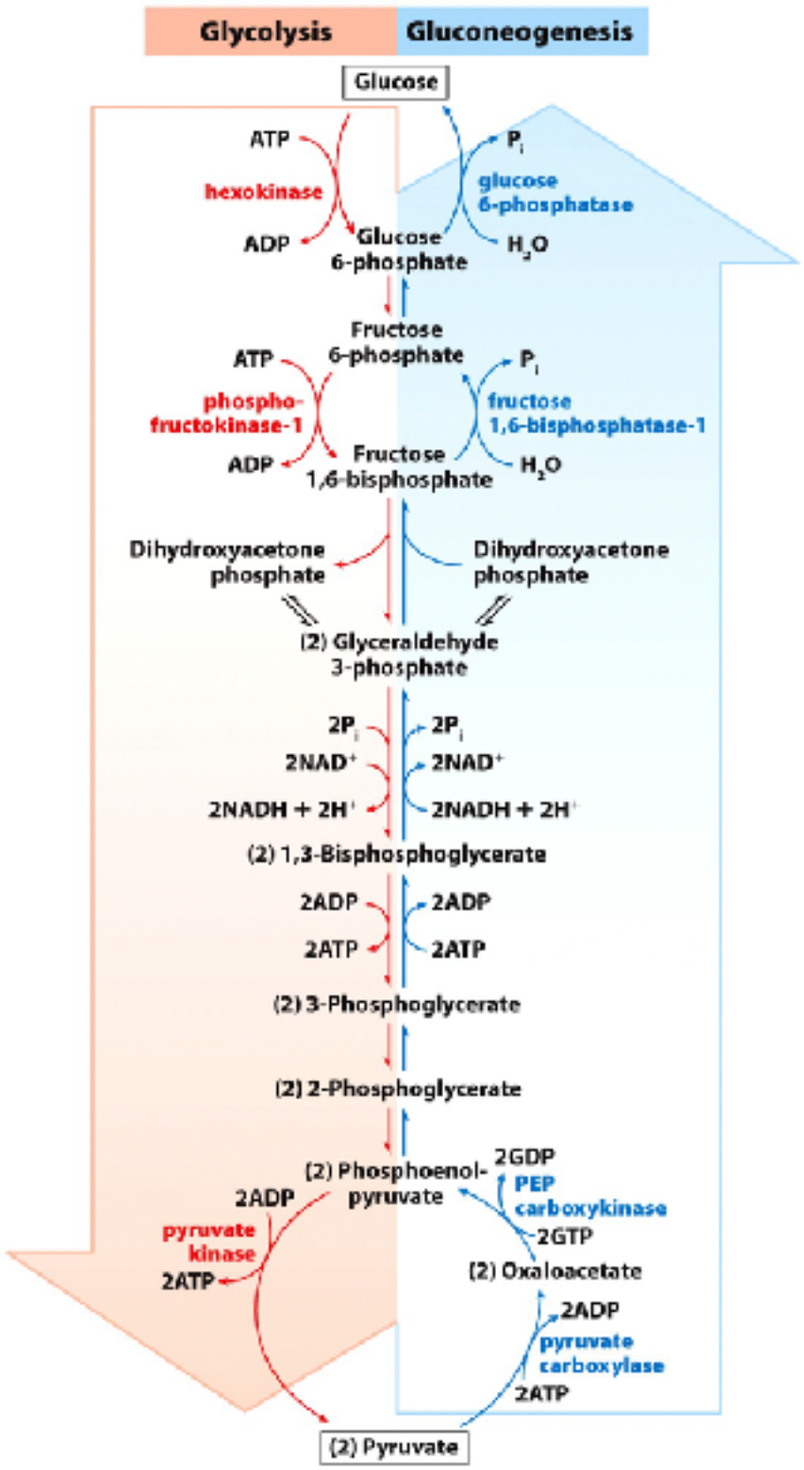
5 proteins are involved in Step 10: 1) **Glc(6)P transporter** (T1) imports Glc(6)P into the ER lumen; 2) **Glc(6)Ptase** converts Glc(6)P to glucose and Pi 3) with the help of the **Ca++ binding stabilising protein** (SP); 4) Pi is transported to the cytosol by the **Phosphate transporter** (T2); 5) Glucose is transported to the cytosol by the **Glucose transporter** (T3).

Gluconeogenesis





Gluconeogenesis



Sum of gluconeogenic reactions:

2 Pyruvate + 4 ATP + 2 GTP + 2 NADH + 2 H+ + 6 H2O → Glucose + 4 ADP + 2 GDP + 6 Pi + 2 NAD+

Clearly, this is not a simple reversal of glycolysis:

Glucose + 2 ADP + 2 Pi + 2 NAD+ → 2 Pyruvate + 2 ATP + 2 NADH + 2 H+ + 2 H2O

One gluconeogenic cycle costs more energy than is gained by a glycolytic cycle

Sum for one cycle of glycolysis and gluconeogenesis, per mol of glucose:

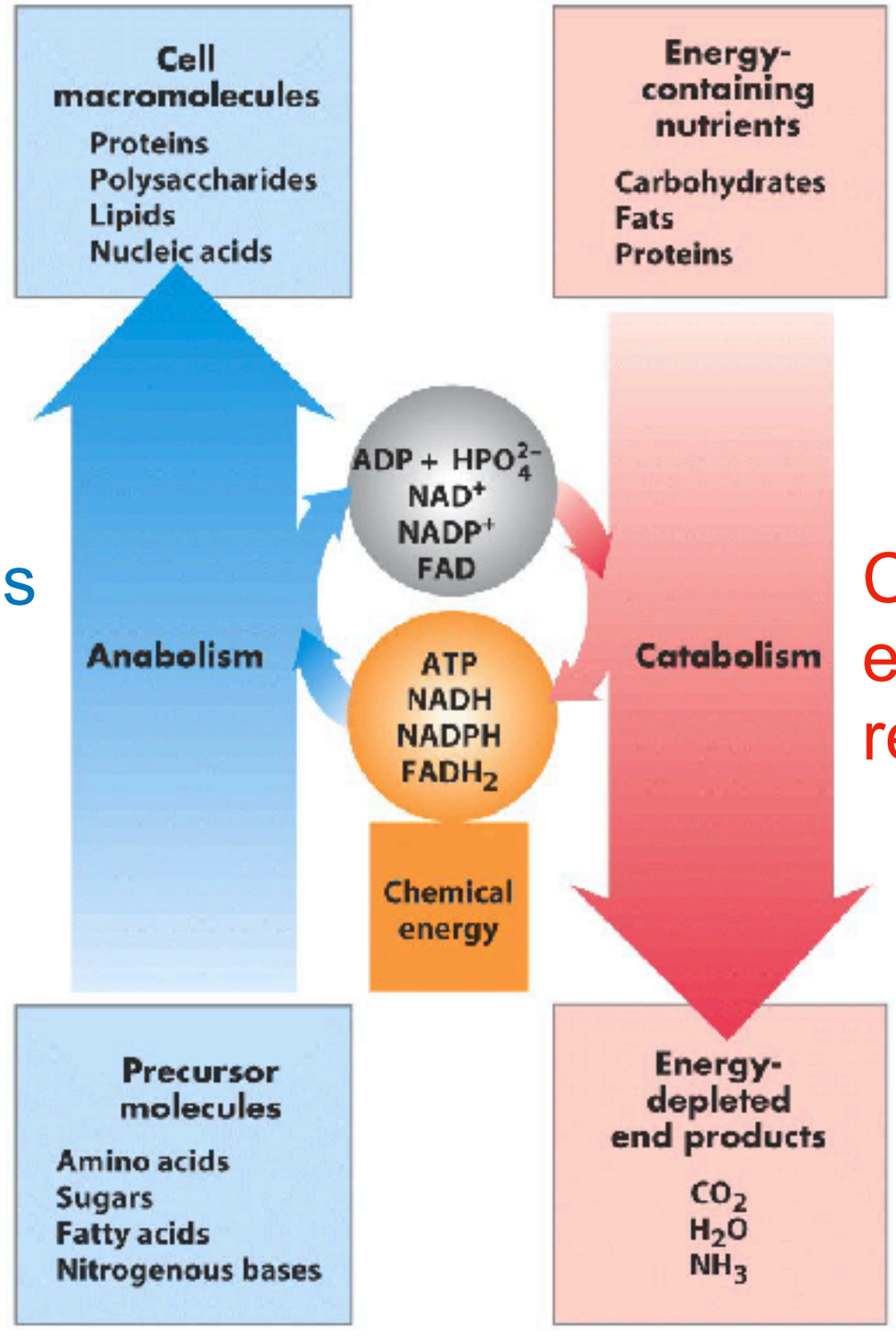
2 ATP + 2 GTP + 4 H2O → 2 ADP + 2 GDP + 4 Pi

Gluconeogenesis is expensive!

Glycolysis and gluconeogenesis are reciprocally regulated

Gluconeogenesis

Anabolism requires energy (ATP) and reducing power

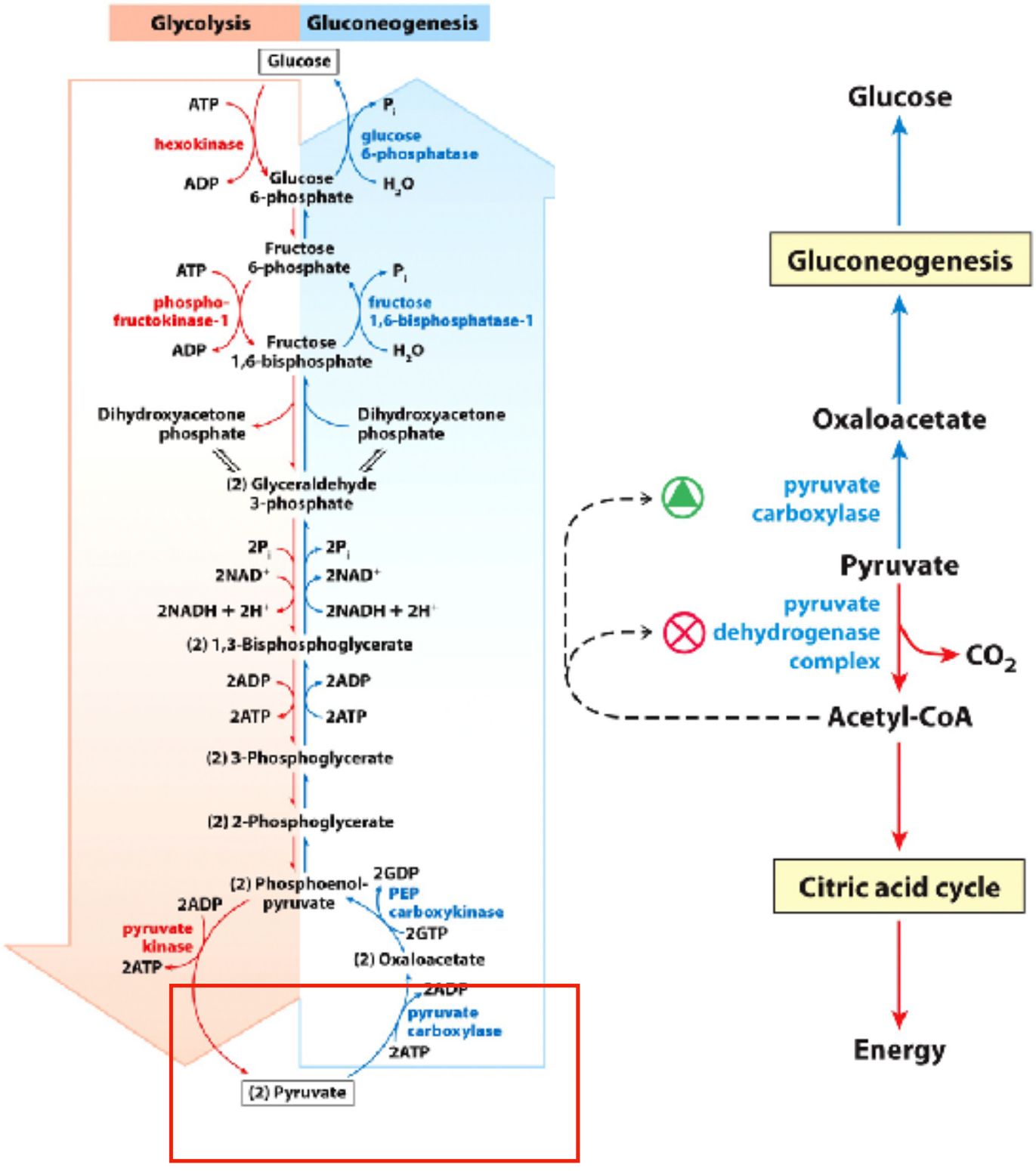


Catabolism generates energy (ATP) and reducing power

High energy charge: [ATP] >> [ADP] and [AMP] favors anabolic pathways such as gluconeogenesis

Low energy charge: [ATP] << [ADP] and [AMP] favors catabolic pathways such as glycolysis

Gluconeogenesis - regulation



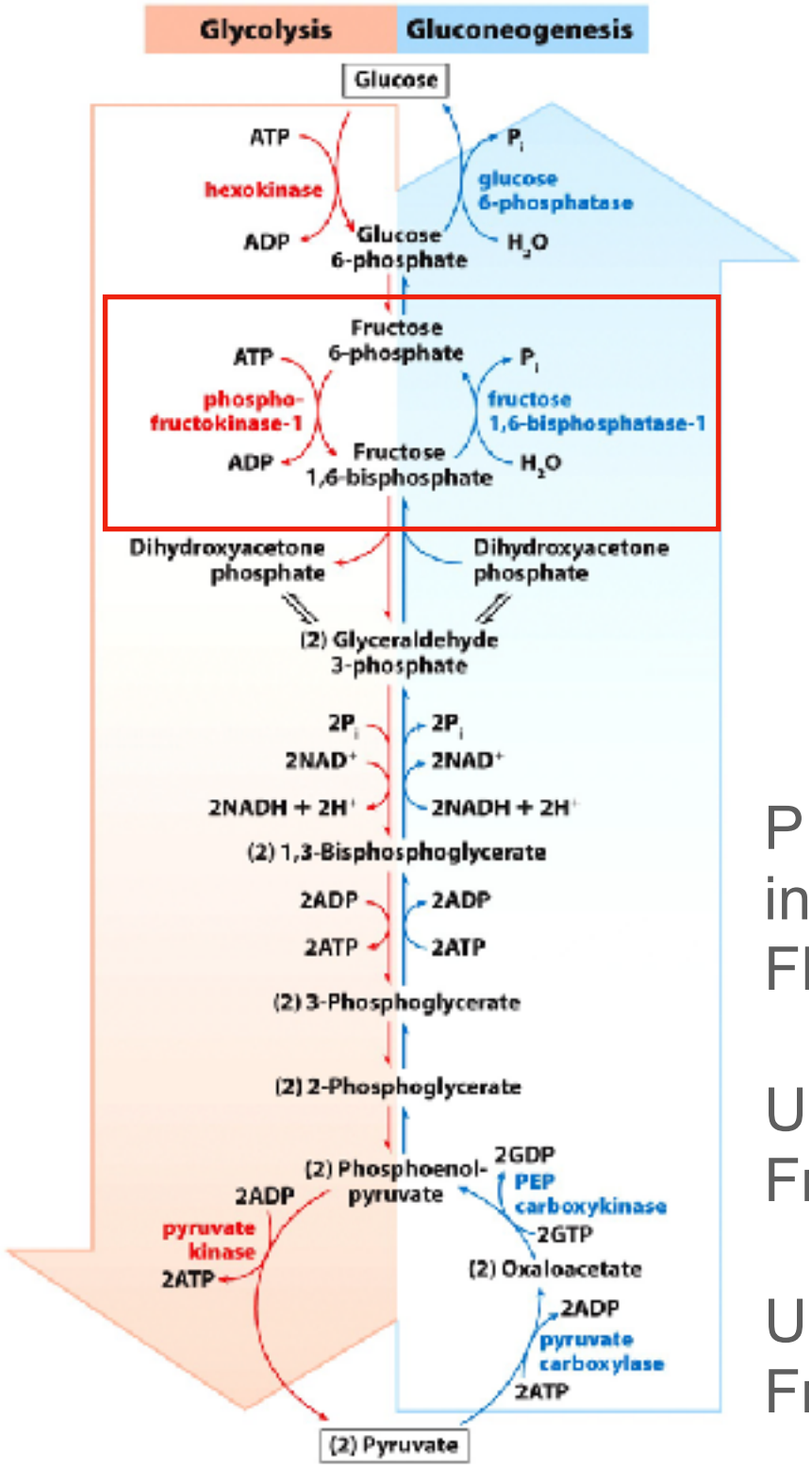
In the mitochondrial matrix Pyruvate is the common substrate of the pyruvate dehydrogenase complex (to form Acetyl-CoA) and of pyruvate carboxylase.

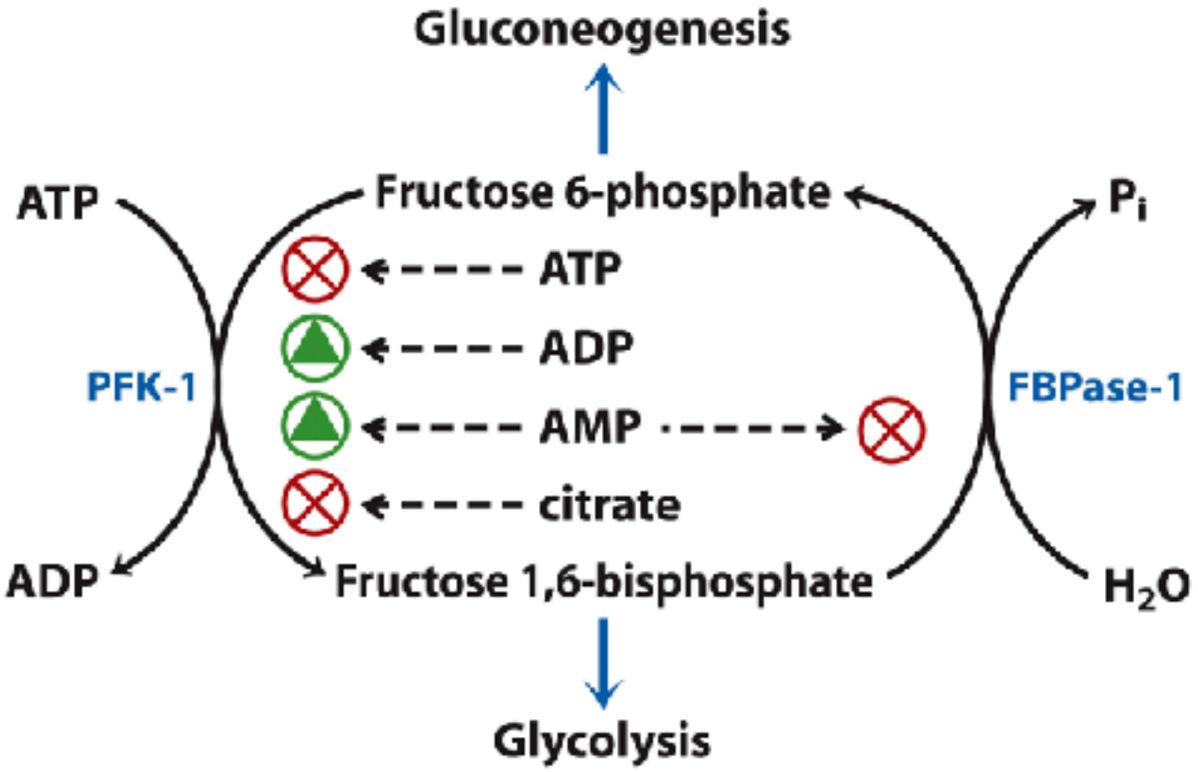
Acetyl-CoA is the entry point into the TCA cycle.

Acetyl-CoA is an allosteric inhibitor of pyruvate dehydrogenase complex, while it activates pyruvate carboxylase.

When the amount of Acetyl-CoA exceeds the TCA cycle capacity it fosters gluconeogenesis

Gluconeogenesis - regulation





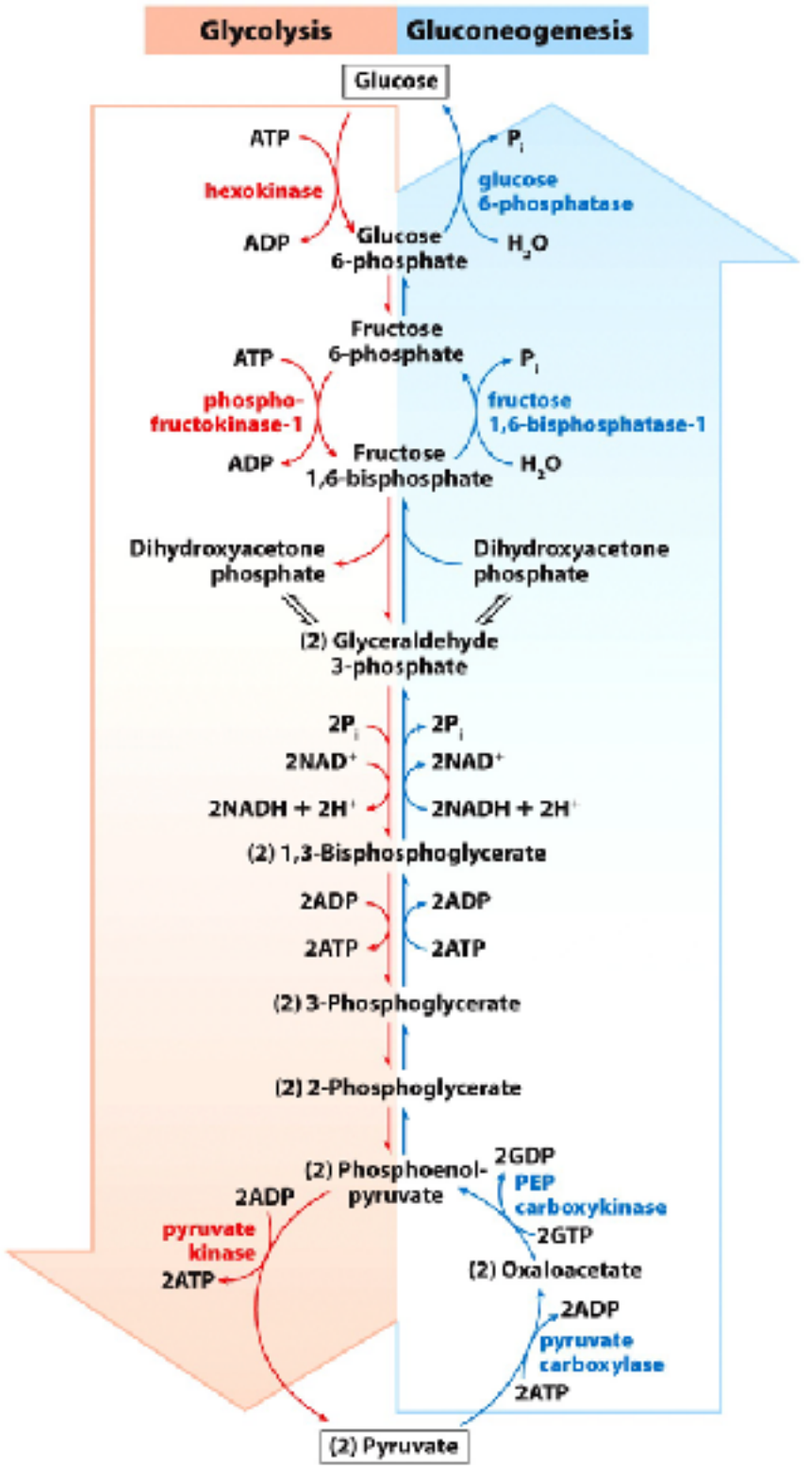
PFK-1 is inhibited by ATP and citrate (a TCA cycle intermediate) and stimulated by AMP and ADP, while FBPase-1 is inhibited by AMP.

Under **low energy charge** Fru(6)P is converted efficiently to Fru(1,6)P2.

Under **high energy charge** Fru(1,6)P2 is converted to Fru(6)P.

Break

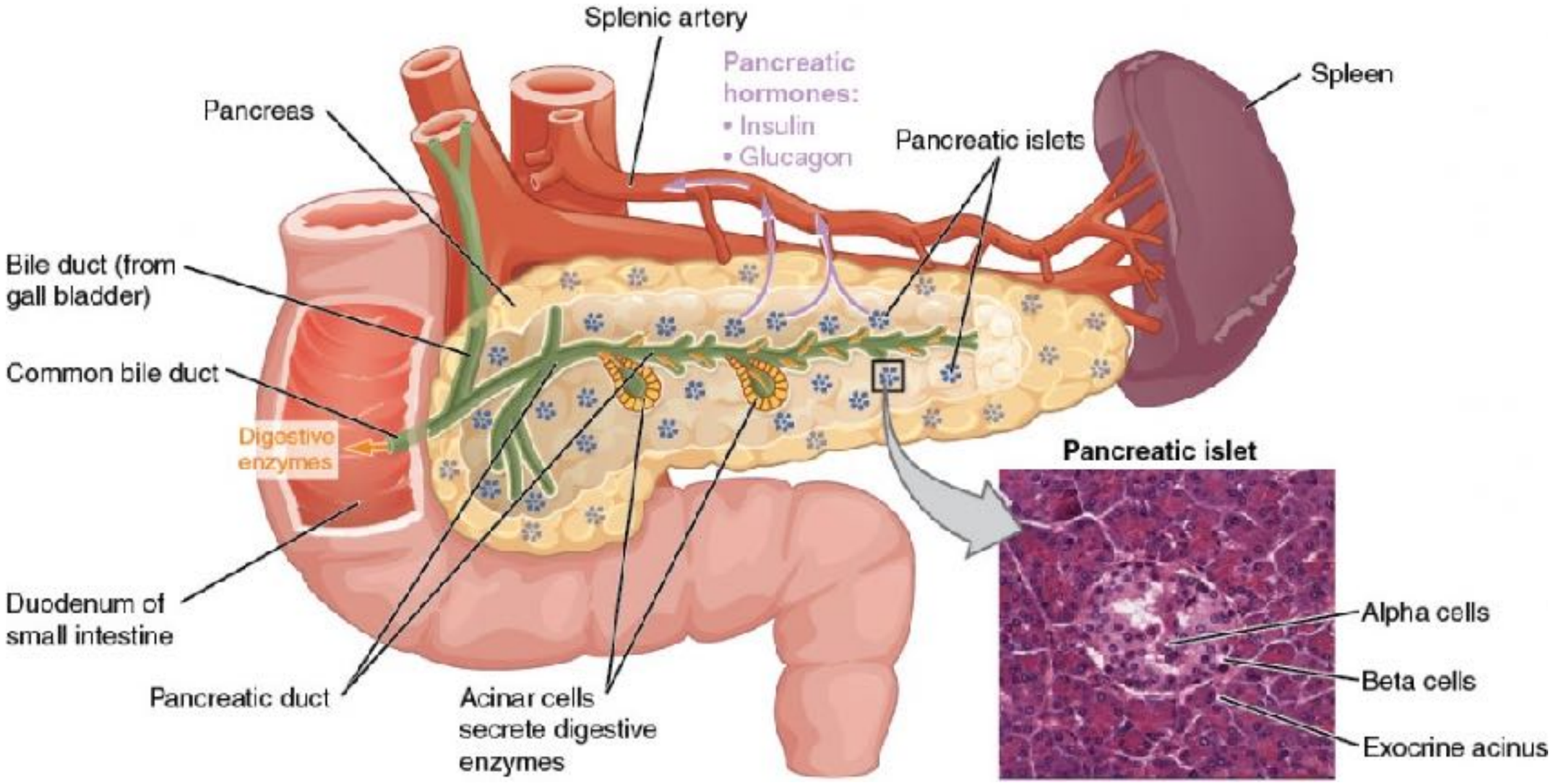
Gluconeogenesis - regulation



Glycolysis and **gluconeogenesis** are reciprocally regulated at the '*cell autonomous*' level (*i.e.*, individual cells are able to favour one of the two metabolic reactions depending on their energy charge status) .

Glycolysis and **gluconeogenesis** are reciprocally regulated also at the organism level whereby blood glucose is sensed and anabolism/ catabolism are fine tuned accordingly.

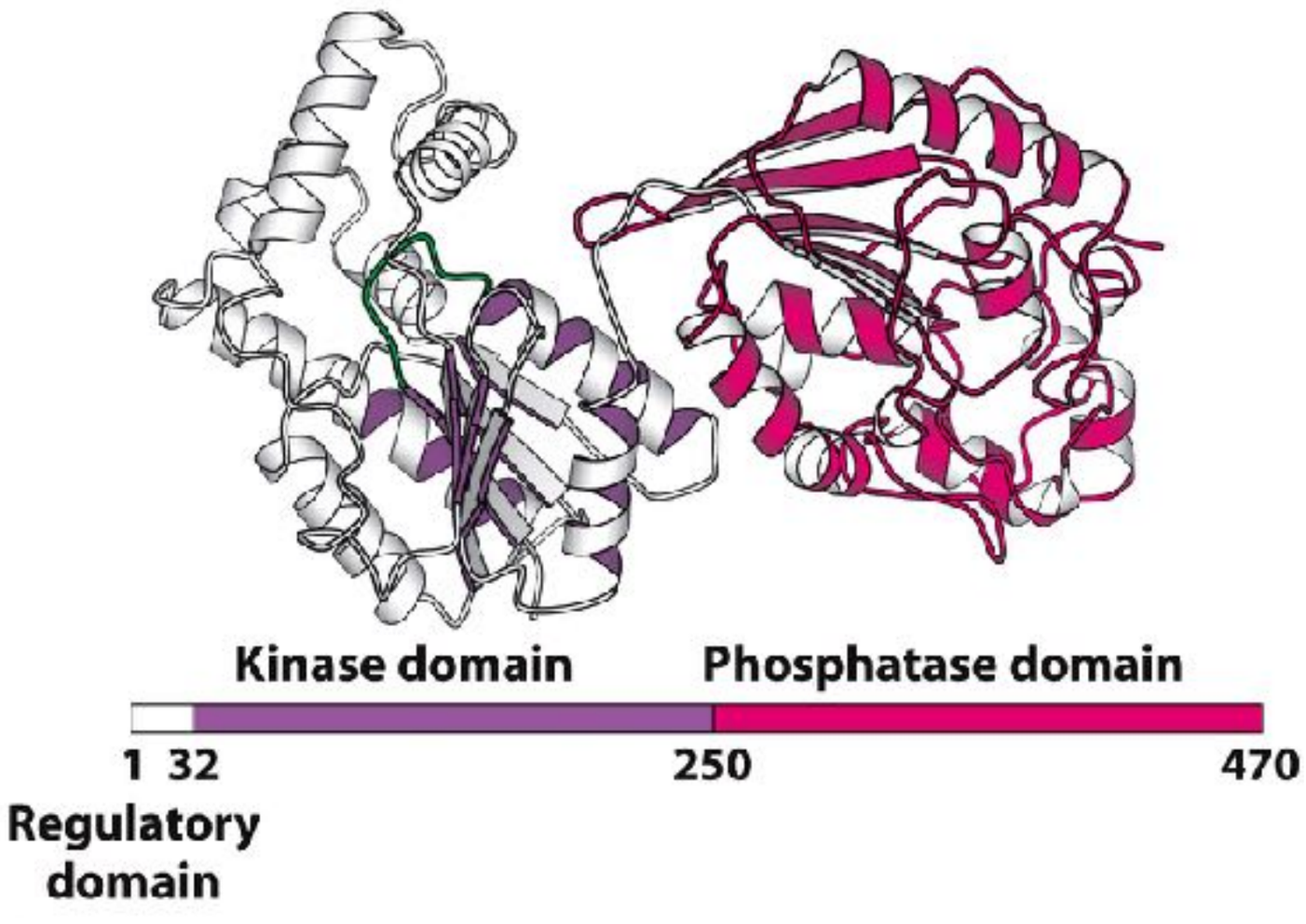
Gluconeogenesis - regulation



The pancreatic exocrine function involves the acinar cells secreting digestive enzymes that are transported into the small intestine by the pancreatic duct. Its endocrine function involves the secretion of **insulin** (produced by **beta cells** when blood glucose is high) and **glucagon** (produced by **alpha cells** when blood glucose is low) within the pancreatic islets. These two hormones regulate the rate of glucose metabolism in the body

Gluconeogenesis - regulation

PFK-2/ FBPase-2

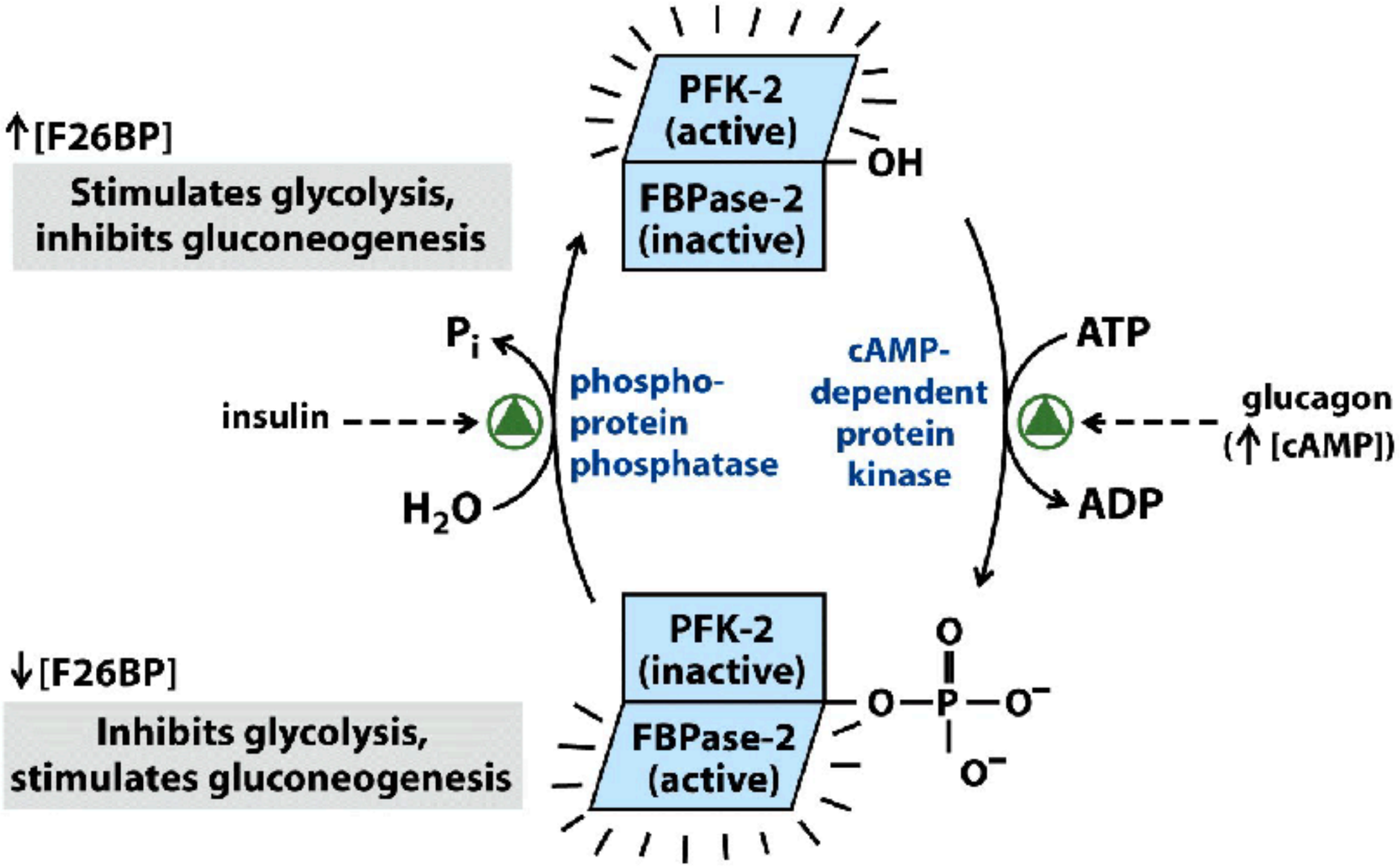


A key element in this regulatory pathway is a bifunctional allosteric enzyme that contains two different regulatory domains. One domain is **phosphofructokinase-2** (**PFK-2**) while the other domain is **fructose bisphosphatase-2** (**FBPase-2**). This bifunctional enzyme exists in two important states.

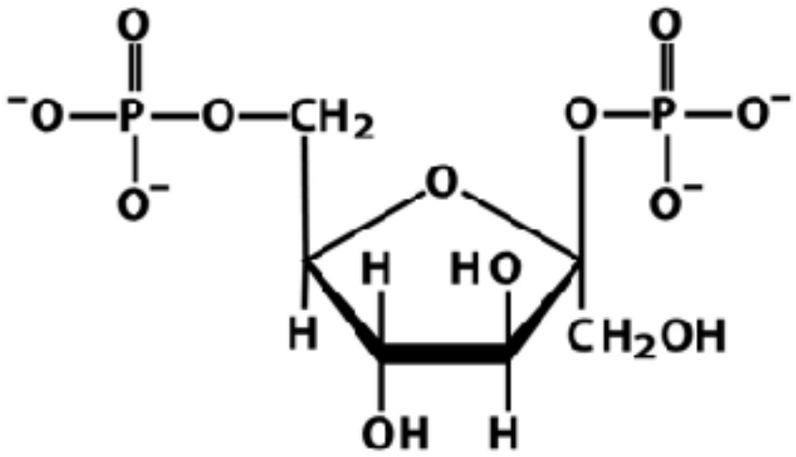
In one state, the **PFK-2** domain is unphosphorylated and active while the other domain is inactive. In this state, the PFK-2 domain will phosphorylate Fru(6)P to form Fru(2,6)P2.

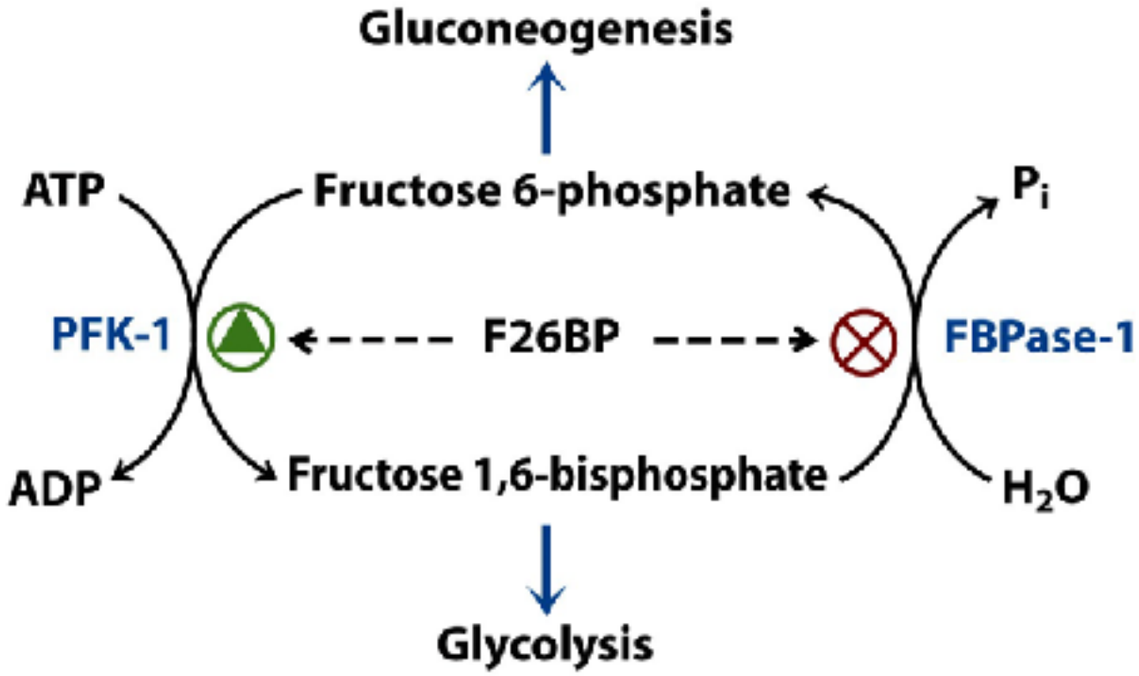
In the other state, a serine residue on the PFK-2 domain becomes phosphorylated and this inactivates it while activating the FBPase-2 domain.
FBPase-2 domain dephosphorylates the Fru(2,6)P2 to form Fru(6)P.

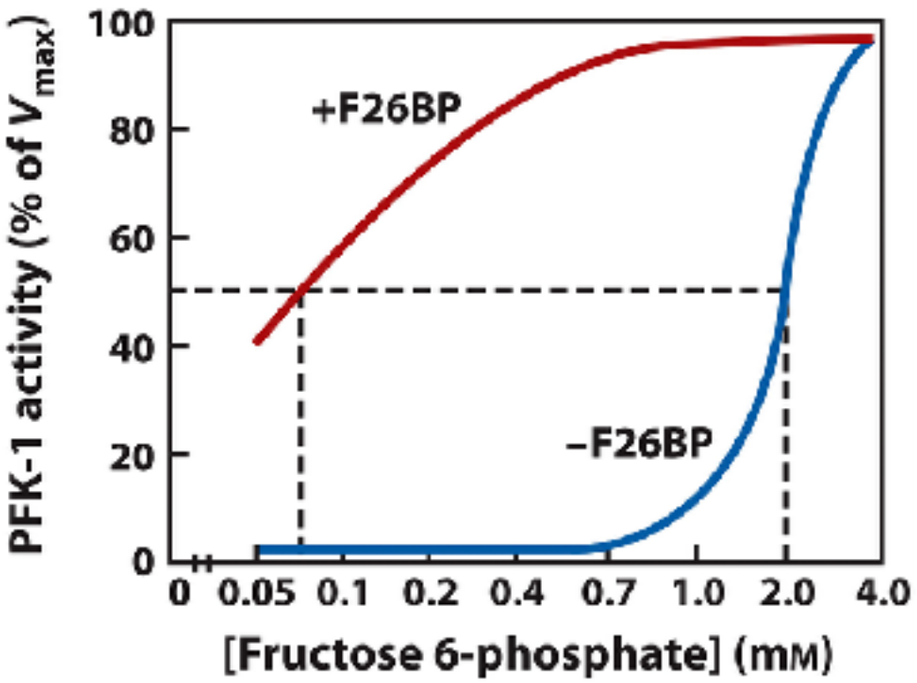
Gluconeogenesis - regulation

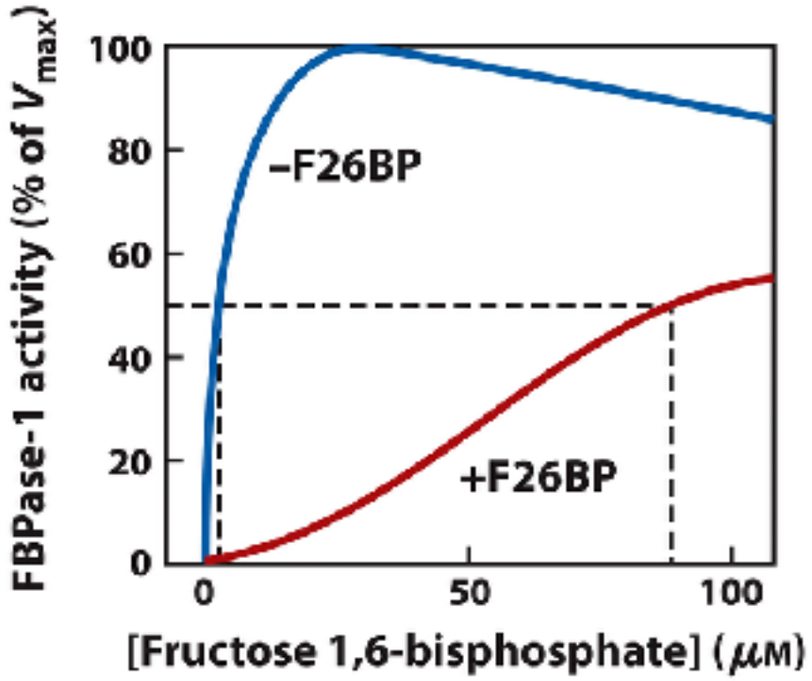


Gluconeogenesis - regulation

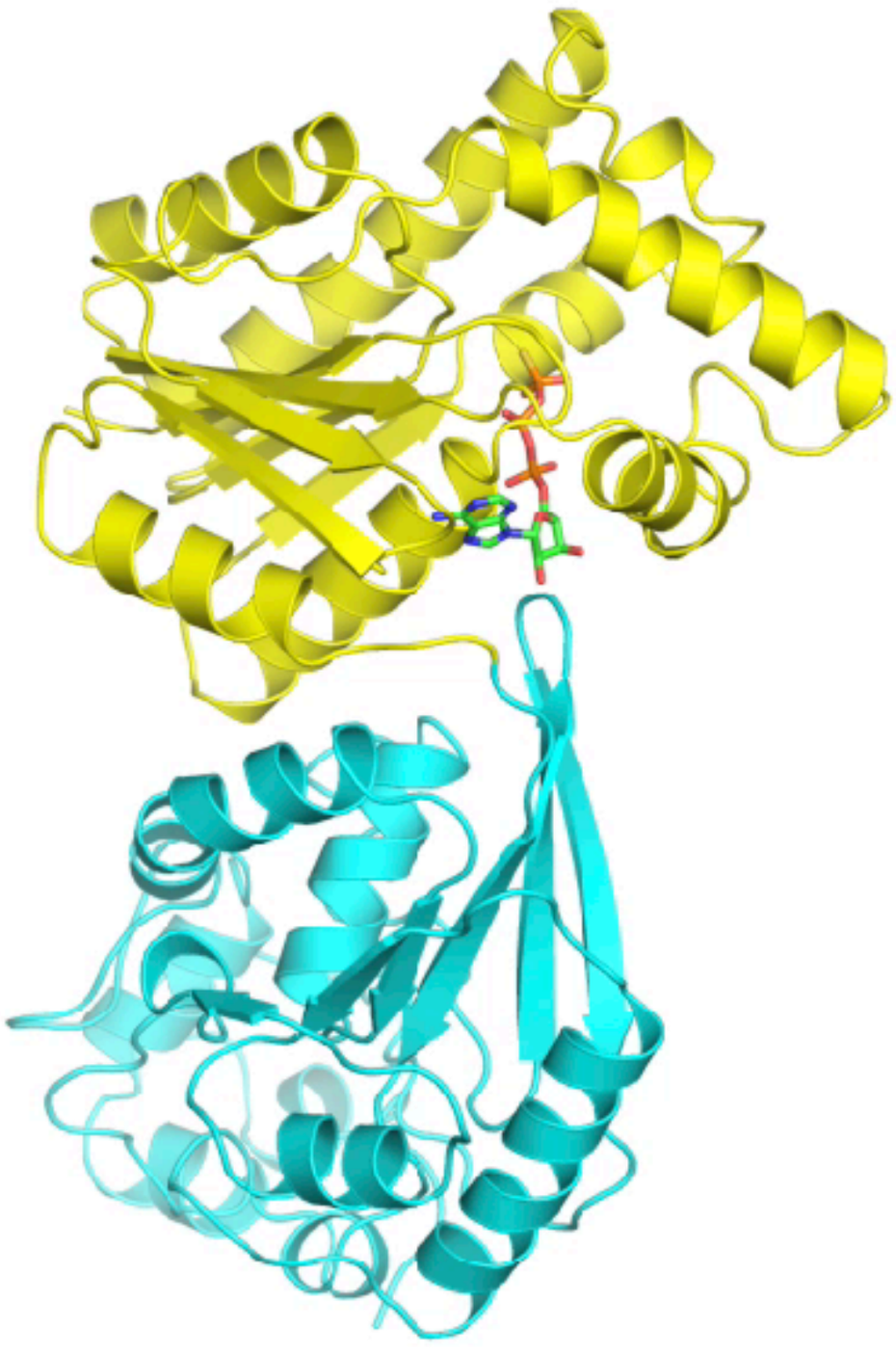






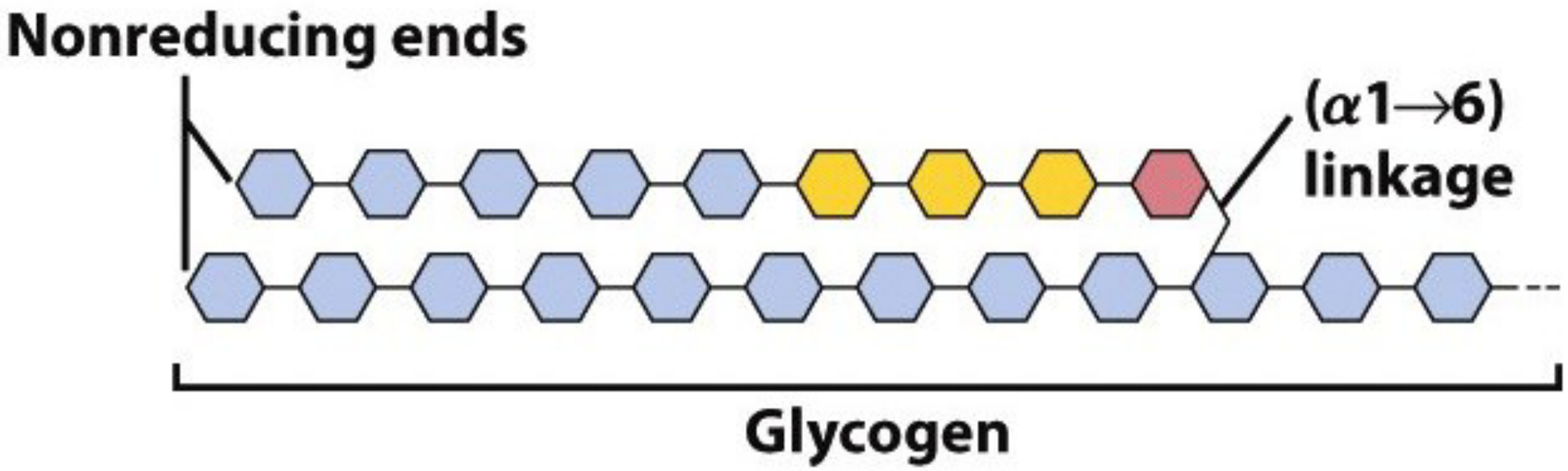


Gluconeogenesis - regulation



When blood glucose levels are high (after eating a meal), insulin will be released by the beta-cells of the pancreas, which will in turn stimulates glucose uptake into the cells. Insulin also helps activate the enzyme phosphoprotein phosphatase that is responsible for dephosphorylating the bifunctional enzyme. This actives the PFK-2 domain, which in turn stimulates the formation of fructose 2,6-bisphosphate, an allosteric activator of phosphofructokinase in glycolysis. This in turn stimulates the process of glycolysis and decreases the rate of gluconeogenesis. Under conditions of low blood glucose (during fasting periods), glucagon is activated, which in turn stimulates protein kinase A to turn on FBPase-2. This in turn transforms fructose 2,6-bisphosphate into its fructose 6-phosphate, which stimulates gluconeogenesis and decreases the rate of glycolysis.

Glycogen metabolism

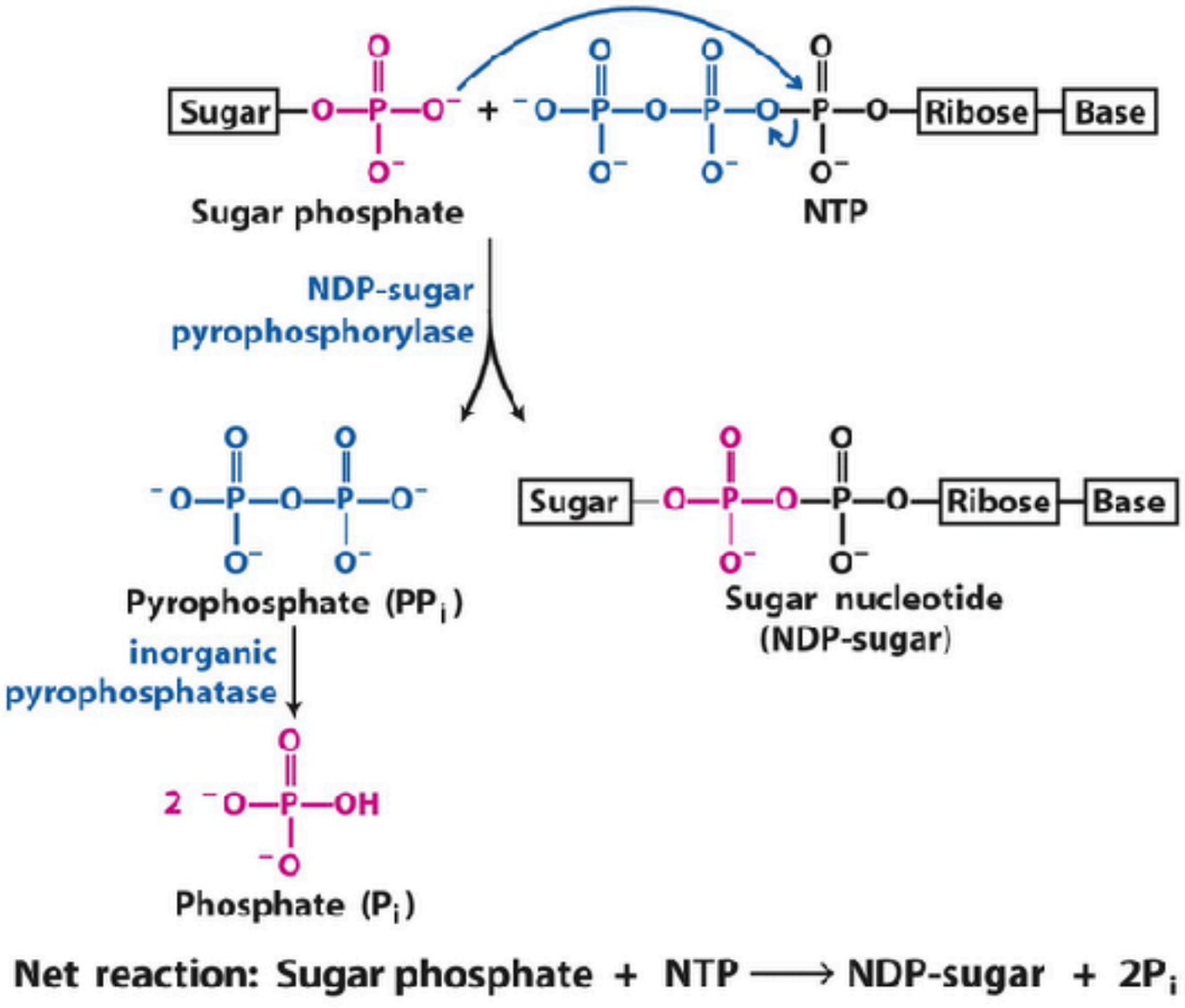


Glycogen is the storage form of glucose that is stored within tiny cytoplasmic granules found predominantly in liver and skeletal muscle cells.

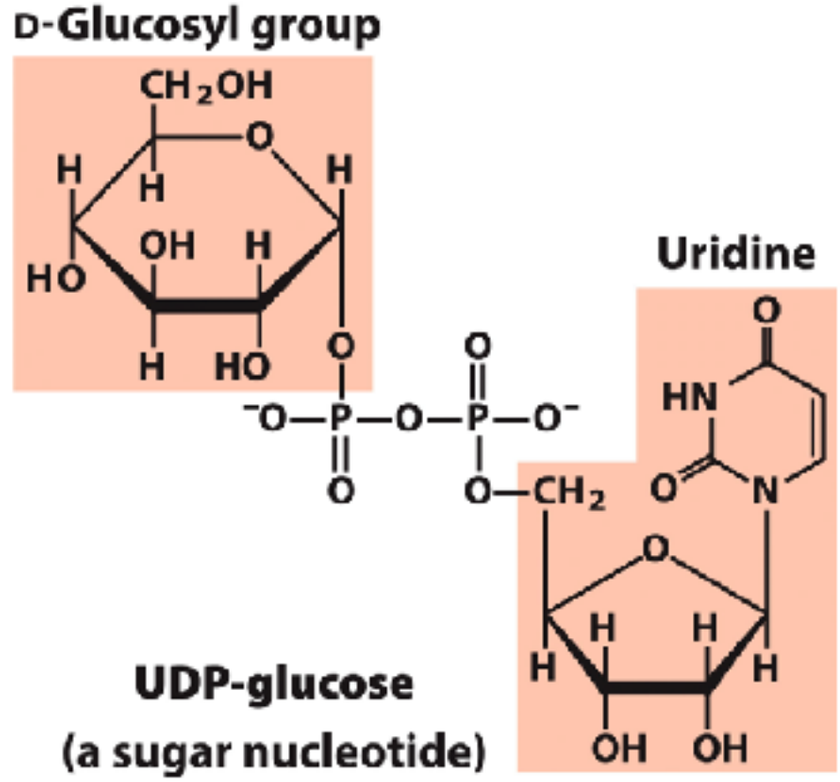
Glycogen is a branched polymer of glucose

The liver uses glycogen to **regulate blood glucose** levels while our skeletal muscle cells use glycogen for **energy**

Glycogenesis

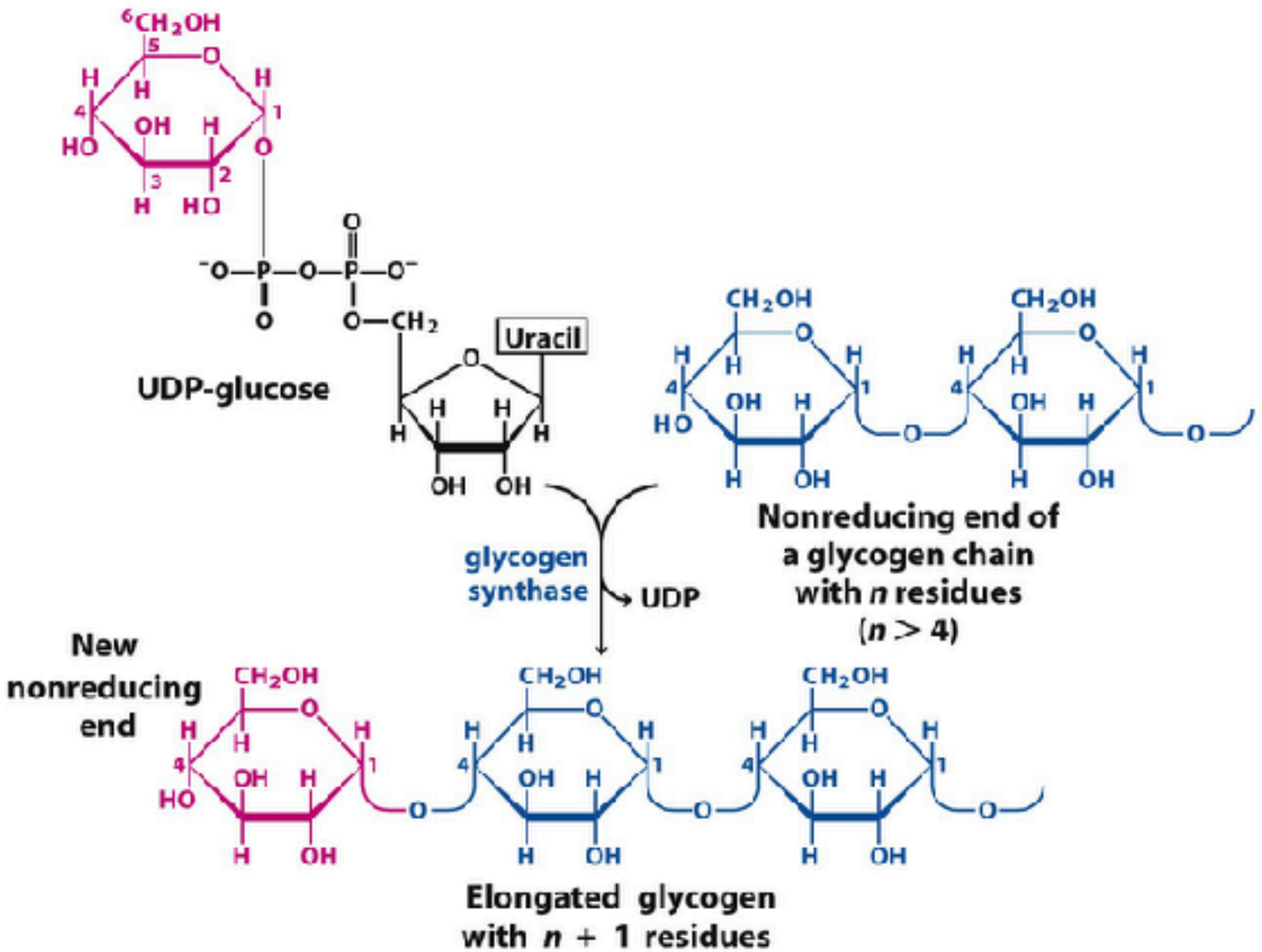


In order to attach a glucose molecule onto a growing polysaccharide chain, that glucose molecule must be activated



Our cells use an enzyme called **UDP-glucose pyrophosphorylase** to transform a glucose 1-phosphate into a uridine diphosphate glucose. This reaction produces a pyrophosphate, which then is hydrolyzed by water to form two orthophosphate molecules. This second step drives the reaction forward.

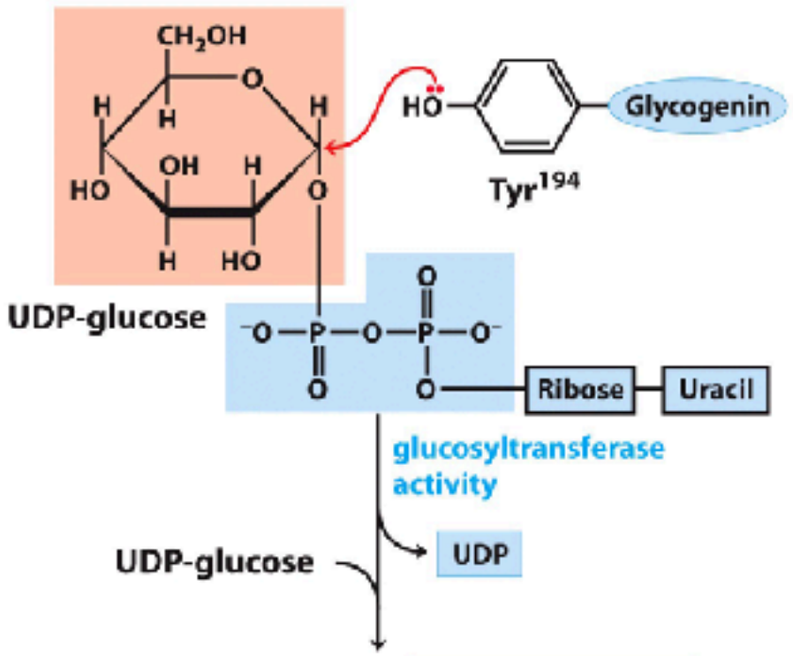
Glycogenesis

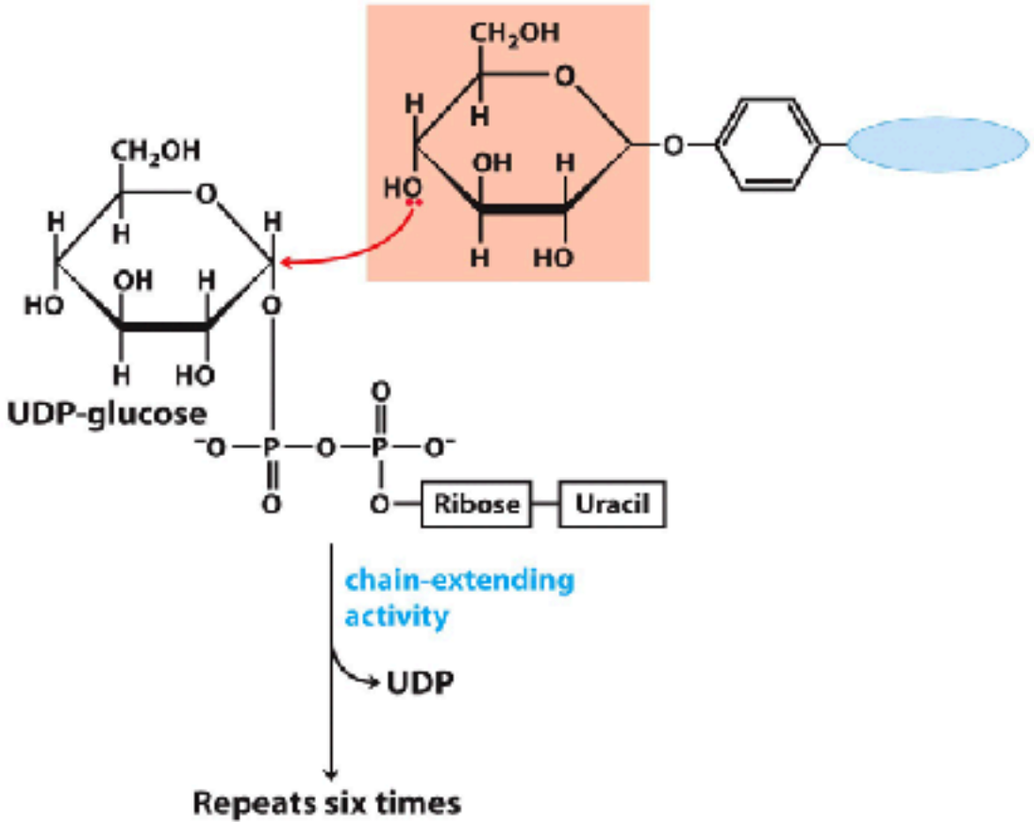


Once the activated UDP-glucose is formed, an enzyme called **glycogen synthase** catalyzes its attachment onto the growing glycogen chain. Glycogen synthase catalyzes the formation of **alpha-1,4-glycosidic bonds** and requires a **primer** to actually begin.

How is the glycogen primer synthesised?

Glycogen-Priming





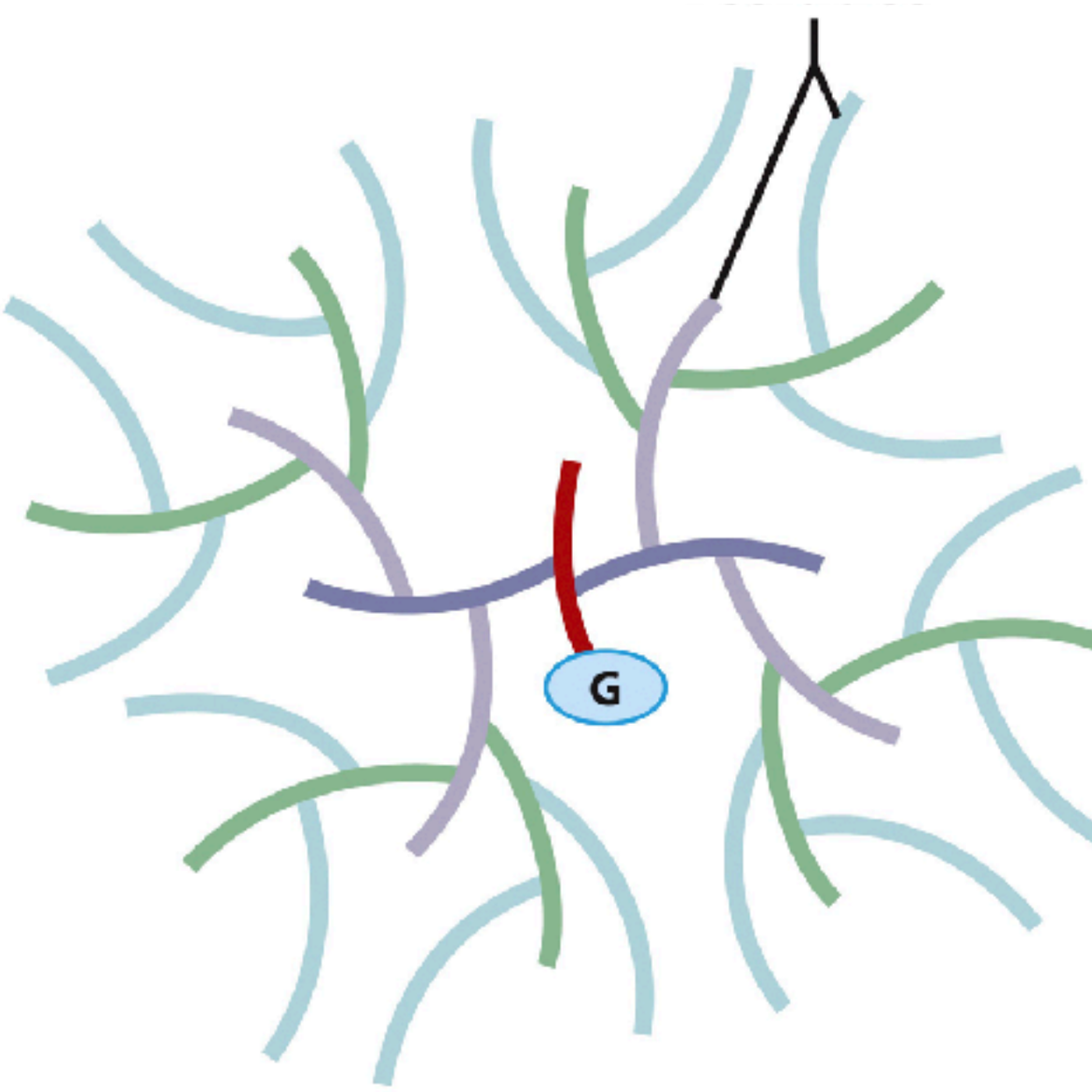
Glycogen synthase can only add the activated glucose molecules to a pre-existing primer.
This primer is created by an enzyme called **glycogenin** in a 3 Steps prrocess.

Step 1:
Transfer of a glucose residue from UDP-glucose to a tyrosine residue of glycogenin, catalyzed by glycogenin itself.

Step 2:
Sequential addition of seven more glucose units, catalyzed by glycogenin

Step 3:
Complex formation with glycogen synthase, glycogen synthase further extends the glycogen molecule!

Glycogen-Branching



Once the primer is formed, the glycogen synthase begins the elongation process. However, glycogen synthase can only create alpha-1,4-glycosidic bonds. Another enzyme called the **glycogen branching enzyme** is responsible for catalyzing the formation of alpa 1,6-glycosidic bonds.

But what is branching for?

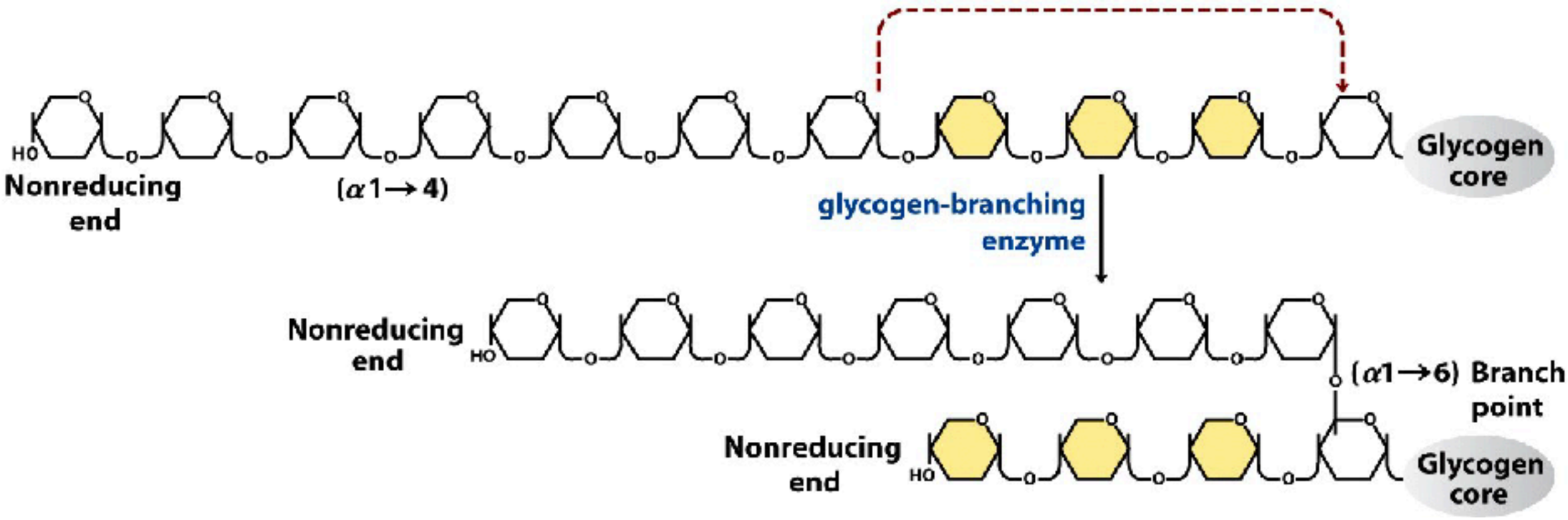
1. increases the **solubility** in water

2. increases the **rates** at which glycogen synthesis and breakdown can take place (because we have more terminal non reducing ends.

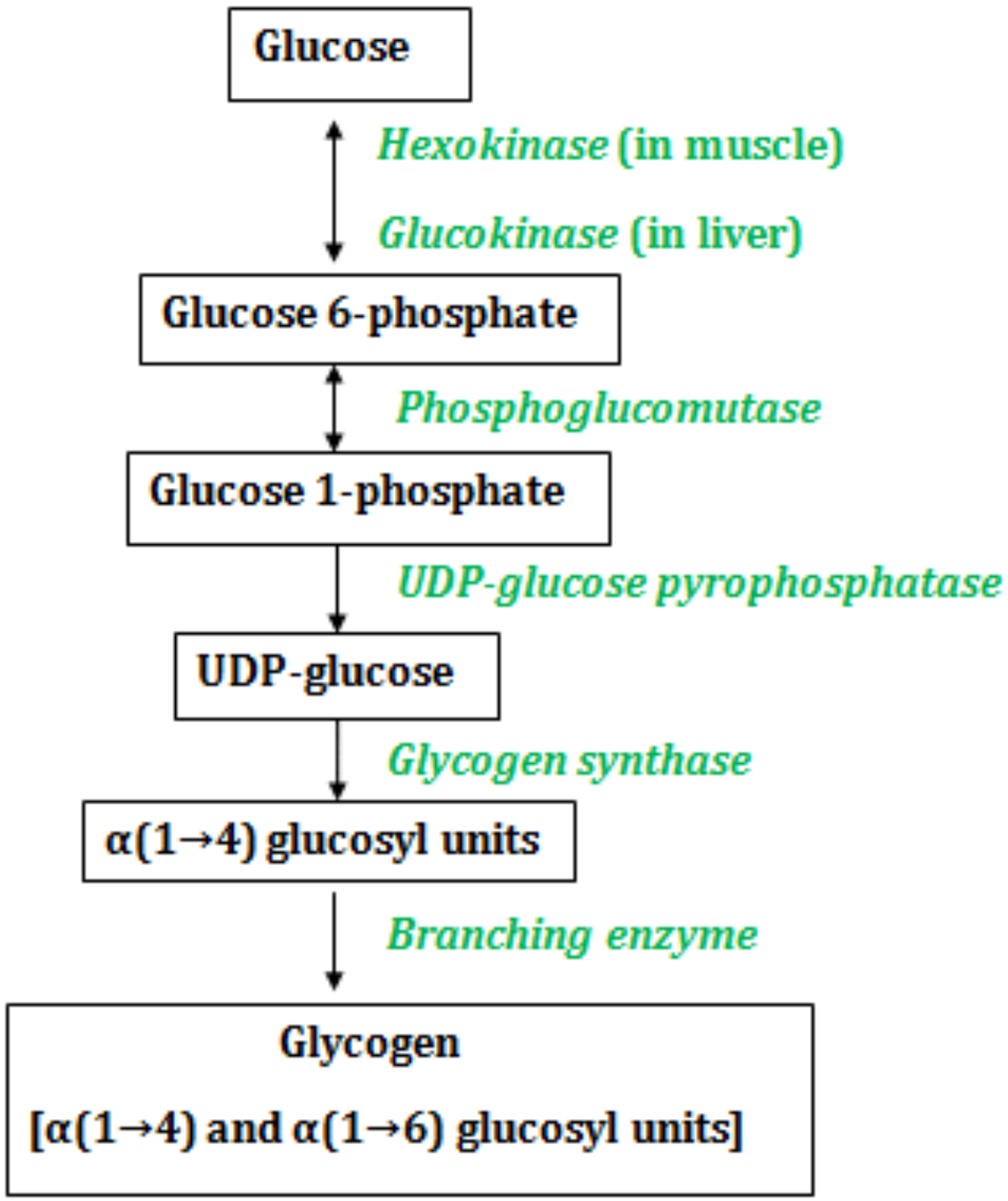
Glycogen-Branching

Glycogen-branching enzyme catalyzes:

The transfer of a terminal fragment (6 or 7 residues long) from the nonreducing end of a branch (at least 11 residues long) to the C-6 hydroxyl group of a glucose residue on the same chain or another chain creating a branch with an (α1→6) linkage



Glycogenenesis



After eating a meal, our blood glucose level rise. To maintain a proper glucose level, our liver cells uptake glucose.

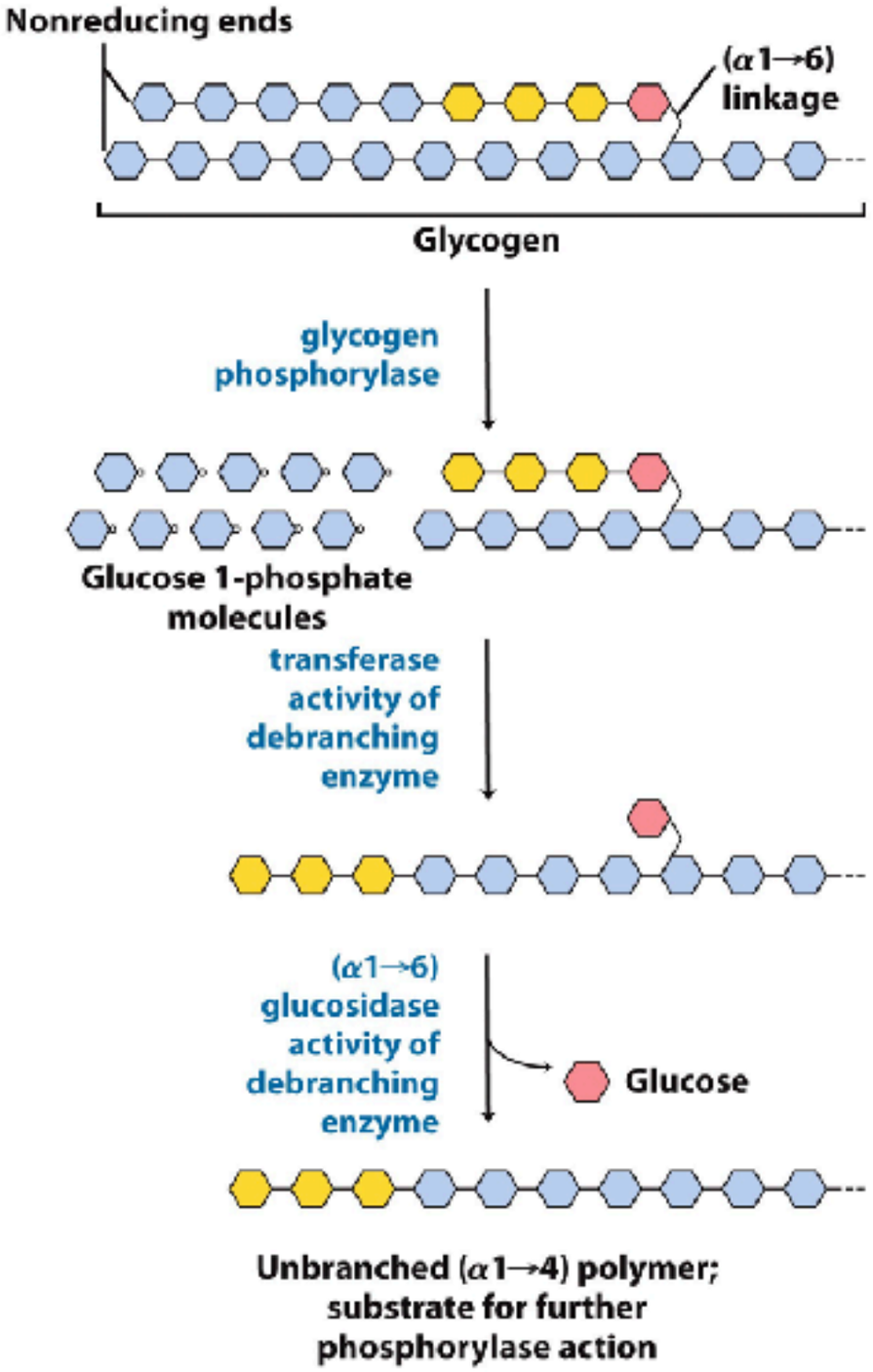
Cells trap the glucose by transforming it into glucose 6-phosphate by **hexokinase**.

The glucose 6-phosphate is then transformed into glucose 1-phosphate by **phosphoglucomutase**. Glucose 1-phosphate is activated by **UDP-glucose pyrophosphorylase**.

Glycogenin then uses these activated glucose molecules to build a primer that is used by **glycogen synthase** to begin extending and elongating the glycogen chain.

The branching points are created by **glycogen branching enzyme**. This enzyme cleaves alpha-1,4-glycosidic bonds and forms alpha-1,6-glycosidic bonds.

Glycogen-Breakdown



Glycogen breakdown consists of three steps

The **first** step is **phosphorolysis**, which is catalyzed by **glycogen phosphorylase**. This enzyme uses an orthophosphate to cleave a glycosidic bond between a terminal glucose with a free hydroxyl group . The products of this reaction are a glucose 1-phosphate and a glycogen that contains one less glucose.

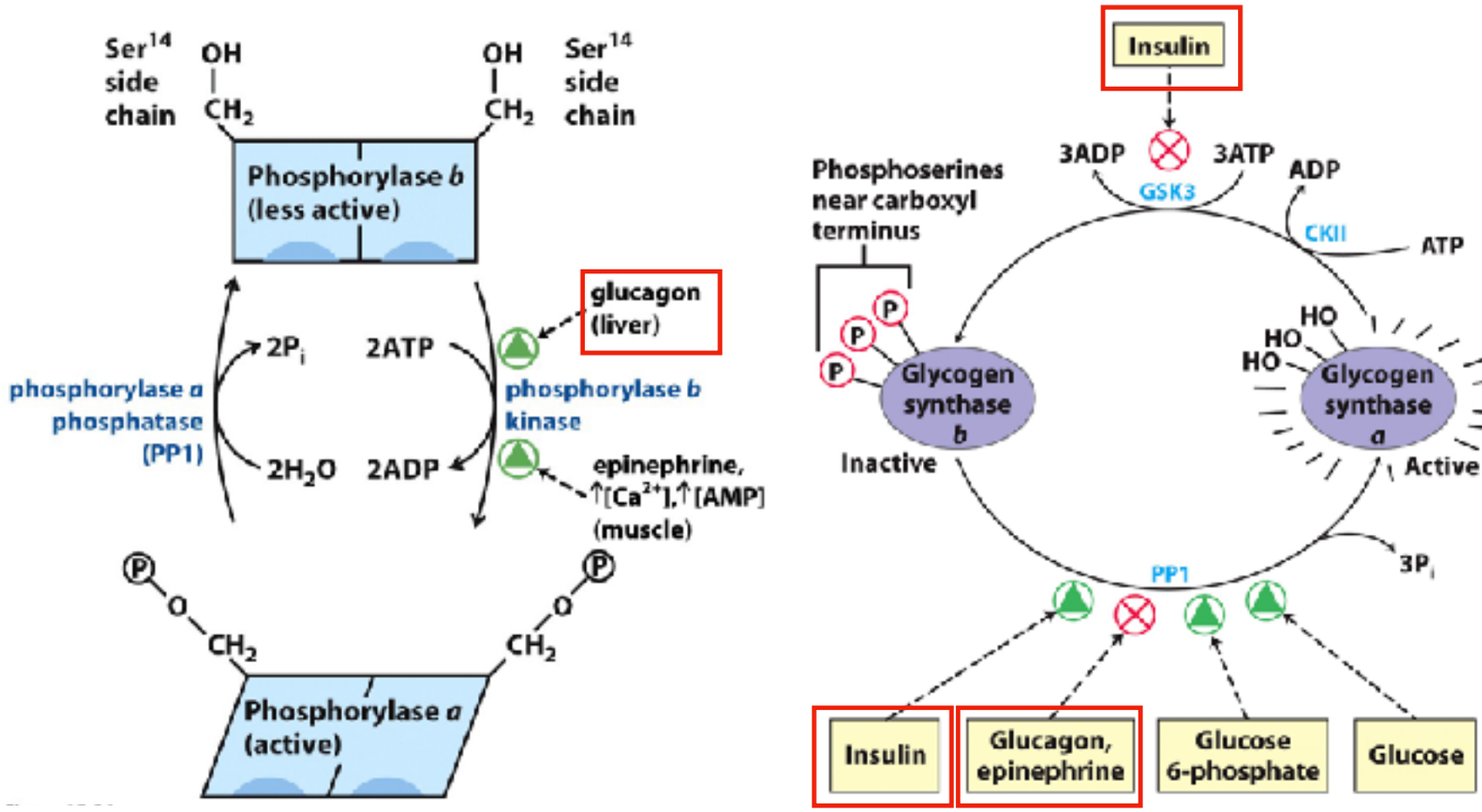
Glycogen phosphorylase cannot cleave the alpha 1,6 glycosidic bonds that make up the branching points. In fact, glycogen phosphorylase stops cleaving the glycogen four glucose residues away from a branching point.

In the **second** step **transferase** removes a group of three glucose molecules and transfers it onto the other branch of glycogen. **Alpha 1,6 glucosidase** removes the remaining glucose molecule by cleaving the alpa 1,6 glycosidic bond.

In the final step, an enzyme called **phosphoglucomutase** converts the glucose 1-phosphate into glucose 6-phosphate.

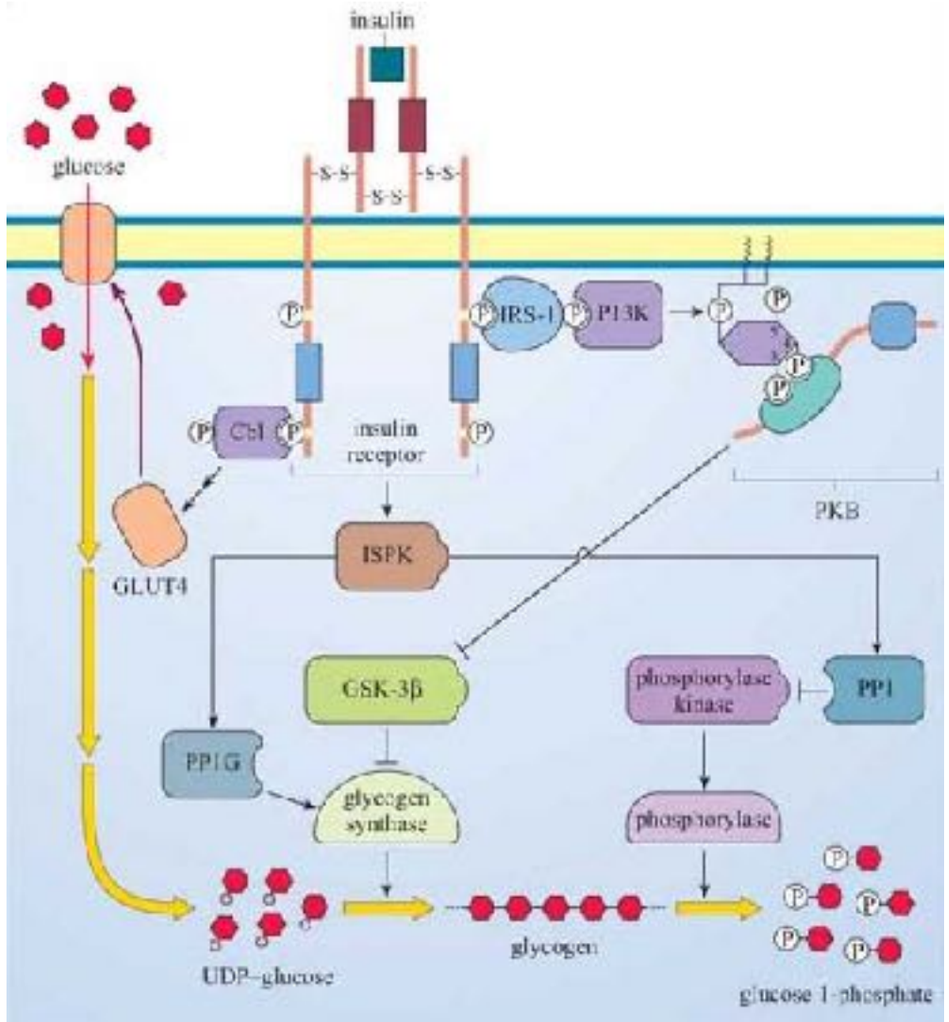
Regulation of Glycogen Metabolism

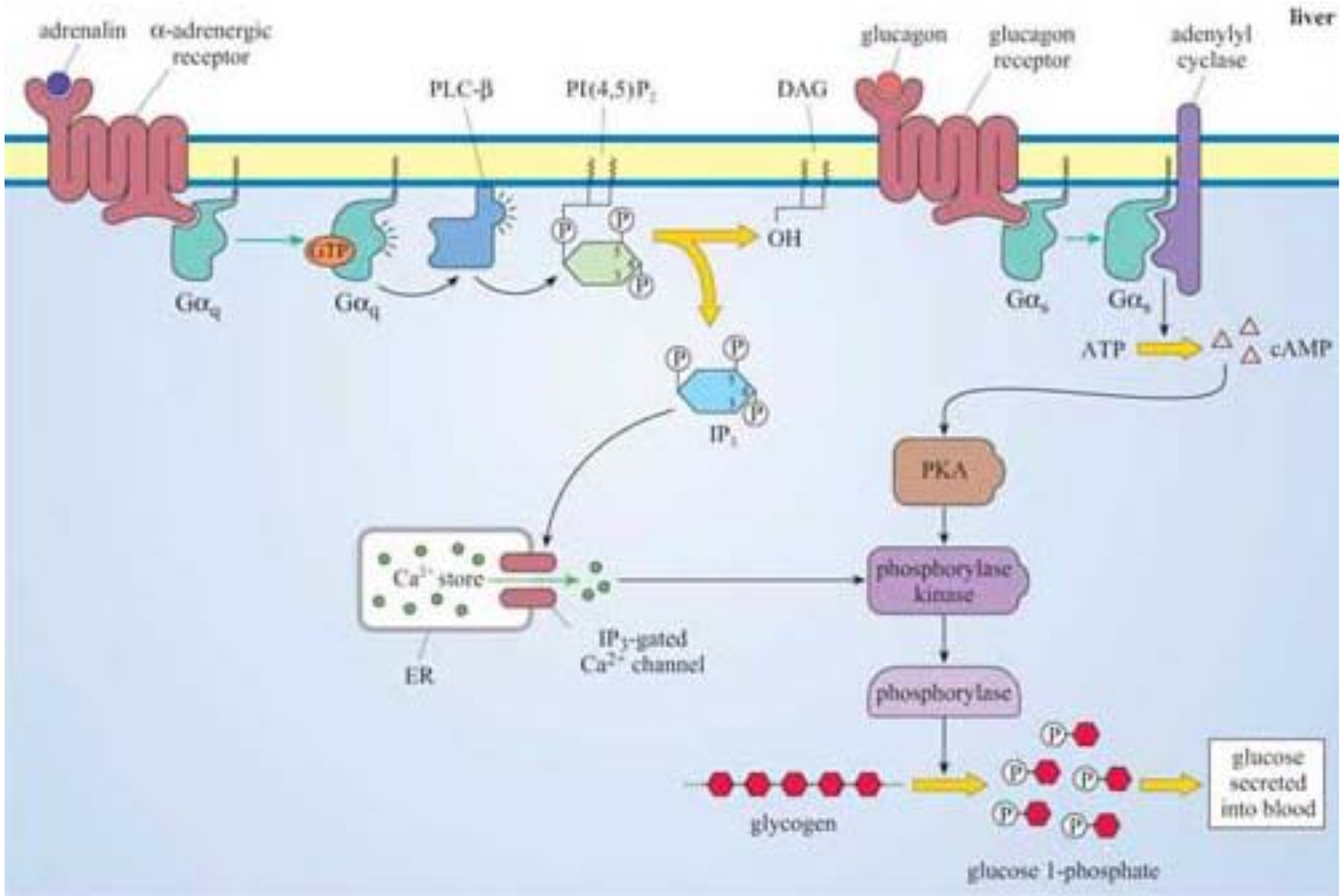
Glycogen metabolism consists of two processes - glycogen synthesis and glycogen degradation. These two processes however do not take place the same moment in time. In fact, our body has a mechanism in place that regulates them in a reciprocal fashion - when one process is on, the other process is off.



Glycogen phosphorylase and glycogen synthase are reciprocally regulated

Regulation of Glycogen Metabolism





Take home messages

Gluconeogenesis is the production of glucose from non-sugar precursors.

It is essentially the opposite of **glycolysis** but it does not simply consist of of the reverse steps of glycolysis.

3 reactions in **glycolysis** are '**irreversible**'. **Gluconeogenesis** circumvents these reactions by following different pathways

Gluconeogenesis and **glycolysis** are reciprocally regulated both at the cell and organism levels

Glycogen is the storage form of glucose and it is branched polymer of glucose

Glycogen **biosynthesis** and **breakdown** are reciprocally regulated and coordinated with **glycolysis** and **gluconeogenesis**