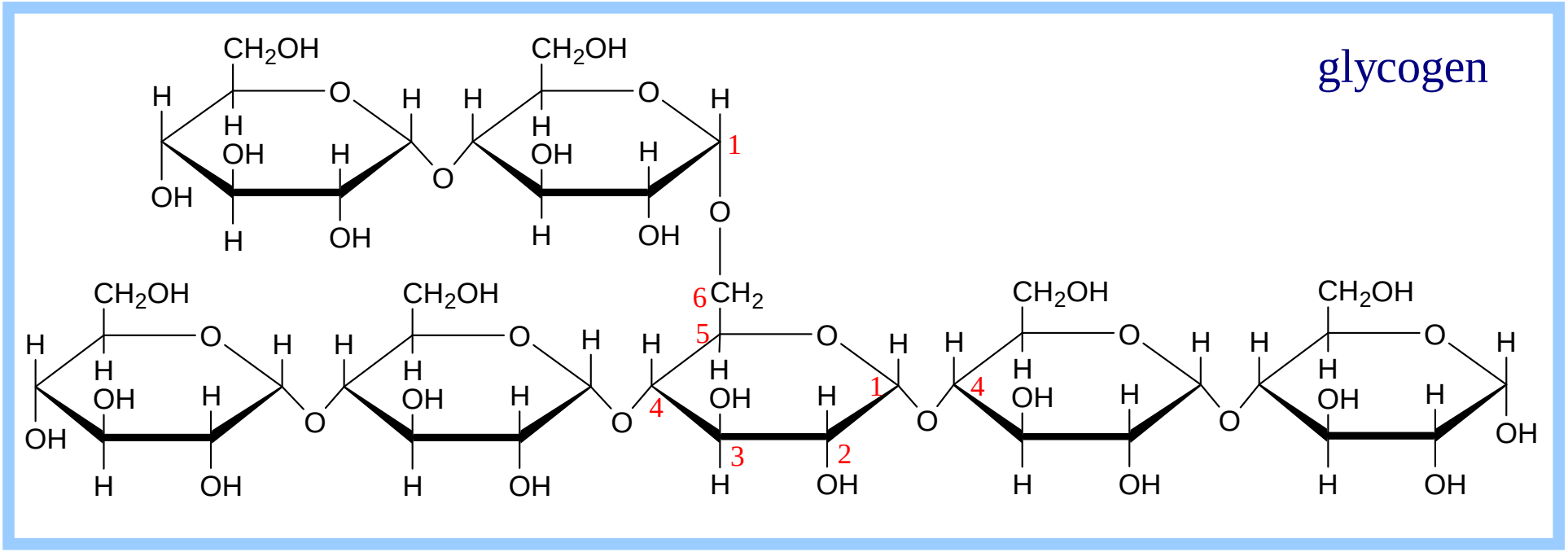
Glycogen Metabolism



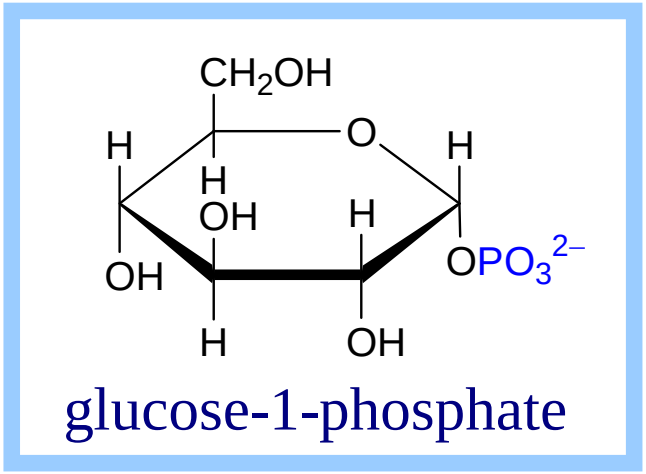
Glycogen is a polymer of **glucose** residues linked by

- α(1→4) glycosidic bonds, mainly
- α(1→6) glycosidic bonds, at branch points.

Glycogen chains & branches are longer than shown.

Glucose is stored as glycogen predominantly in **liver** and **muscle** cells.

Glycogen catabolism (breakdown):



glucose-1-phosphate

Glycogen Phosphorylase catalyzes phosphorolytic cleavage of the **α(1→4)** glycosidic linkages of glycogen, releasing glucose-1-phosphate as reaction product.

glycogen$_{(n\ residues)}$ + P_i →

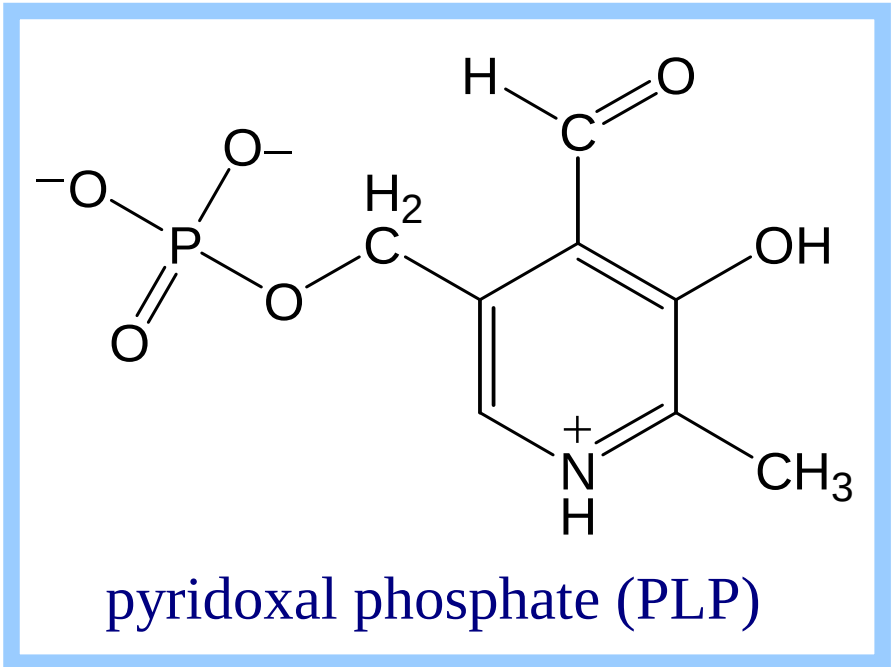
glycogen $_{(n–1\ residues)}$ + glucose-1-phosphate

This **phosphorolysis** may be compared to hydrolysis:

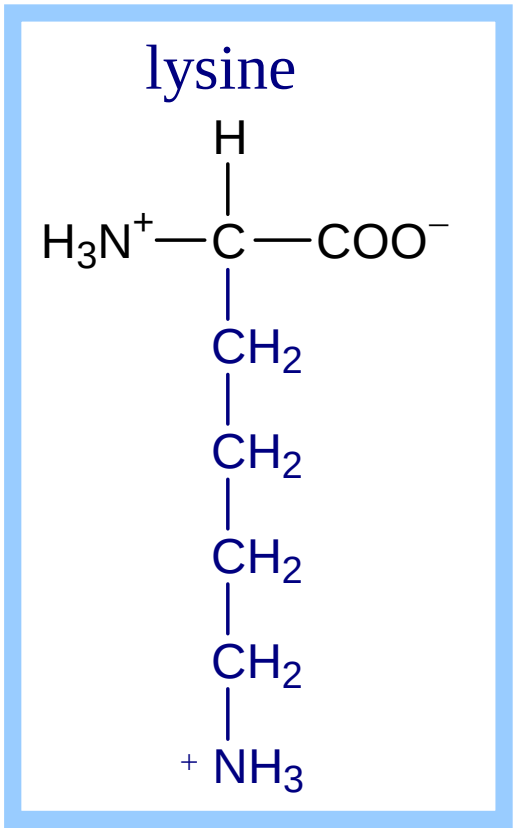
Hydrolysis: **R-O-R' + HOH → R-OH + R'-OH**

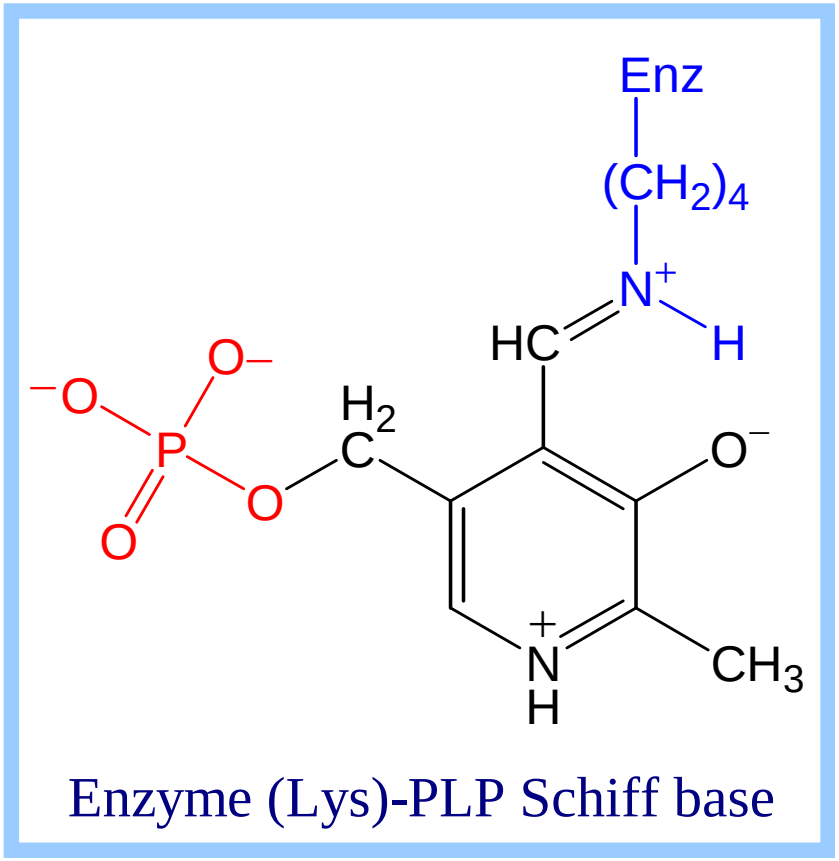
Phosphorolysis: **R-O-R' + HO-PO_3^{2-} → R-OH + R'-O-PO_3^{2-}**

Pyridoxal phosphate (**PLP**), a derivative of vitamin B_6, serves as prosthetic group for Glycogen Phosphorylase.



pyridoxal phosphate (PLP)

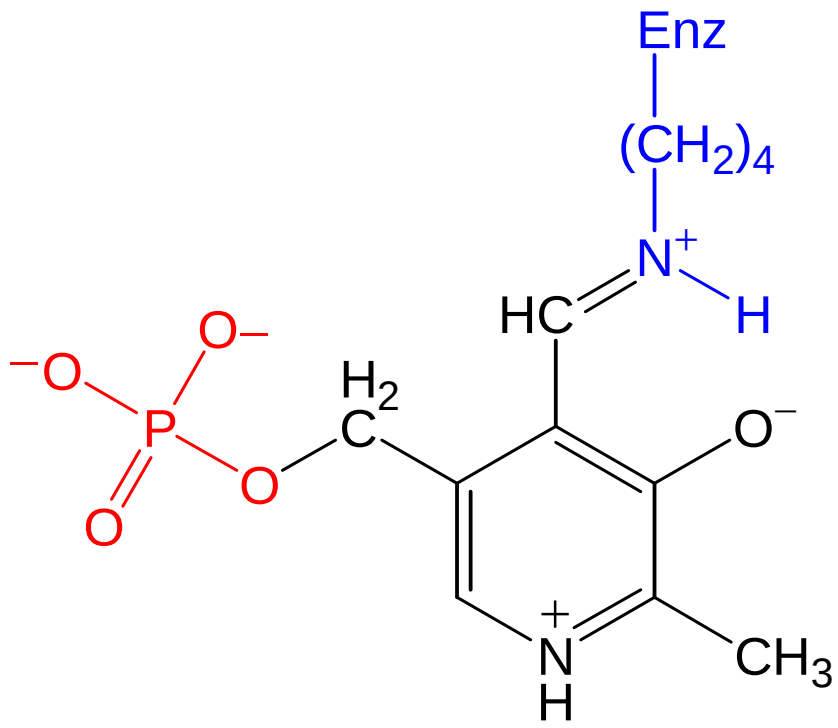




Pyridoxal phosphate (PLP) is held at the active site by a **Schiff base** linkage, formed by reaction of the aldehyde of PLP with the ε-amino group of a **lysine** residue.

In contrast to its role in other enzymes, the **phosphate** of PLP is involved in acid/base catalysis by Phosphorylase.

The **P_i substrate** binds between the phosphate of PLP and the glycosidic O linking the terminal glucose residue of the glycogen.



Enzyme (Lys)-PLP Schiff base

After the phosphate substrate donates **H^+** during cleavage of the glycosidic bond, it receives H^+ from the phosphate moiety of PLP.

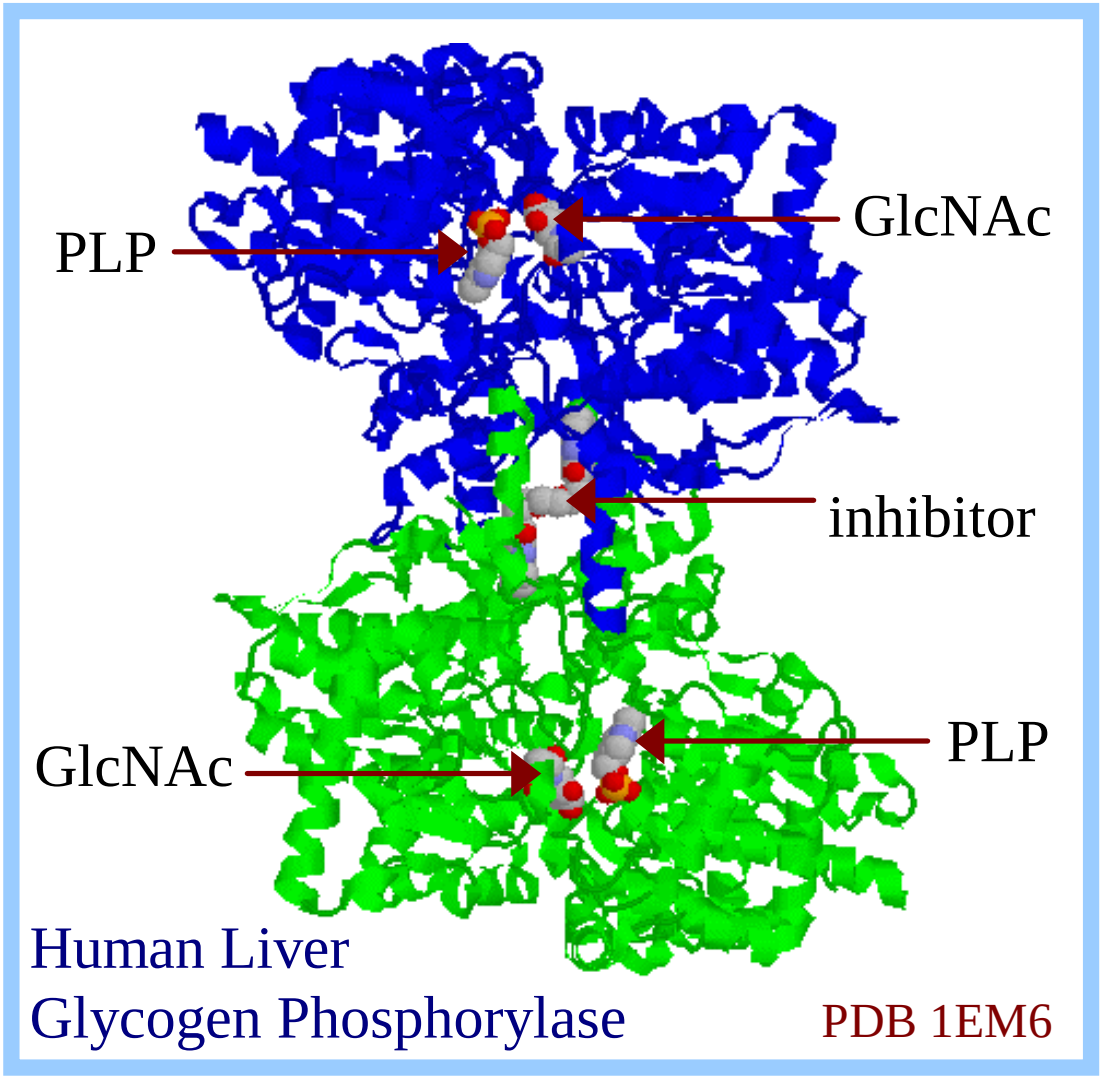
PLP then takes back the H^+ as the phosphate O attacks C1 of the cleaved glucose to yield glucose-1-phosphate.

Glycogen Phosphorylase:

a **homodimeric** enzyme, subject to allosteric control.

It transitions between "**relaxed**" (active) & "**tense**" (inhibited) conformations.

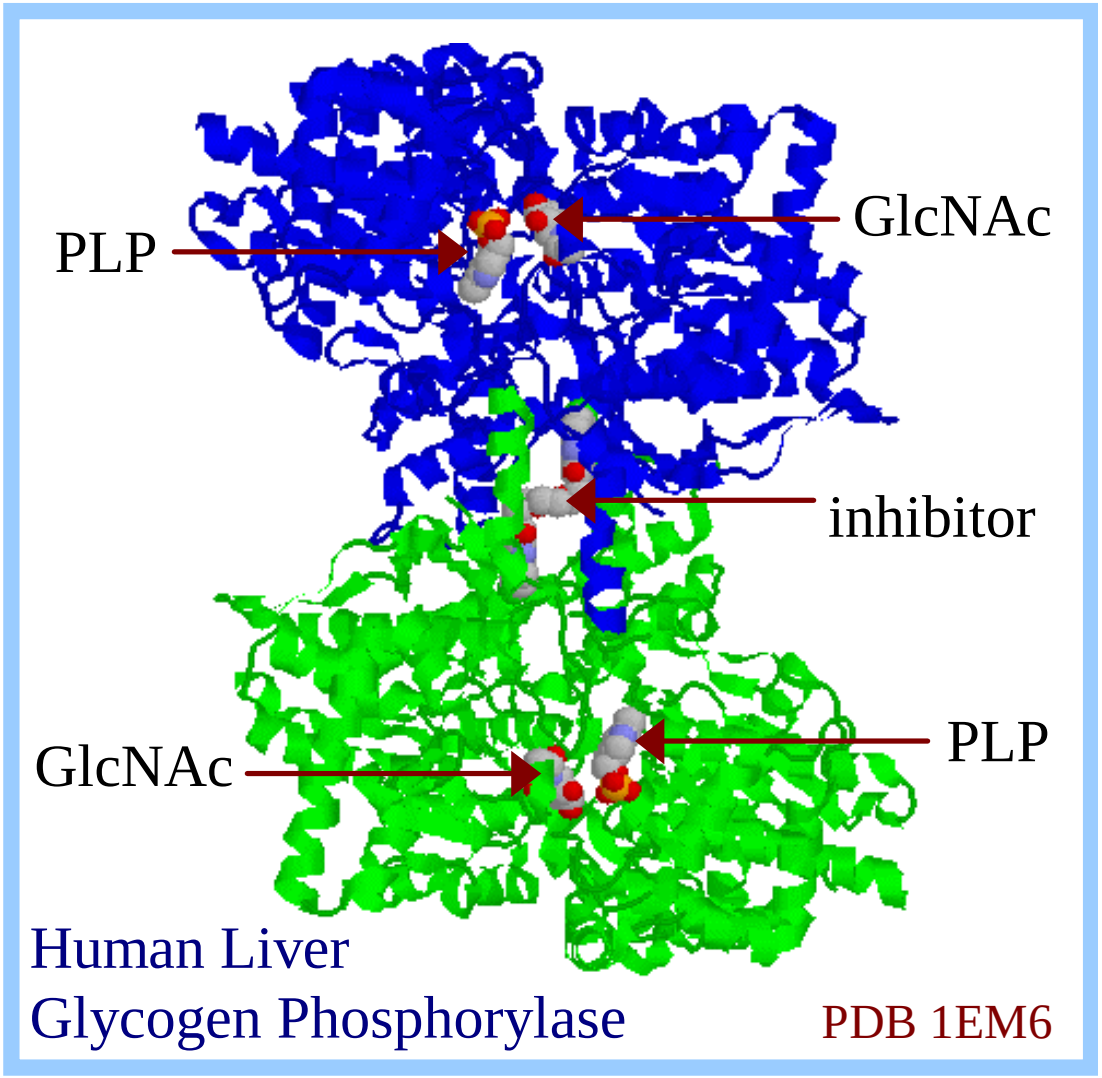
Diagram comparing relaxed and tense conformations.



A **glucose analog**, *N*-acetylglucosamine (GlcNAc), is adjacent to pyridoxal phosphate at the **active site** in the crystal structure shown.

A class of drugs developed for treating the hyperglycemia of diabetes (chloroindole-carboxamides), inhibit liver Phosphorylase allosterically.

These **inhibitors** bind at the dimer interface, stabilizing the inactive (tense) conformation.



Question: Why would an inhibitor of Glycogen Phosphorylase be a suitable treatment for diabetes?

A **glycogen storage site** on the surface of the Phosphorylase enzyme binds the glycogen particle.

Given the distance between storage & active sites, Phosphorylase can cleave **α(1→4)** linkages only to within 4 residues of an **α(1→6)** branch point.

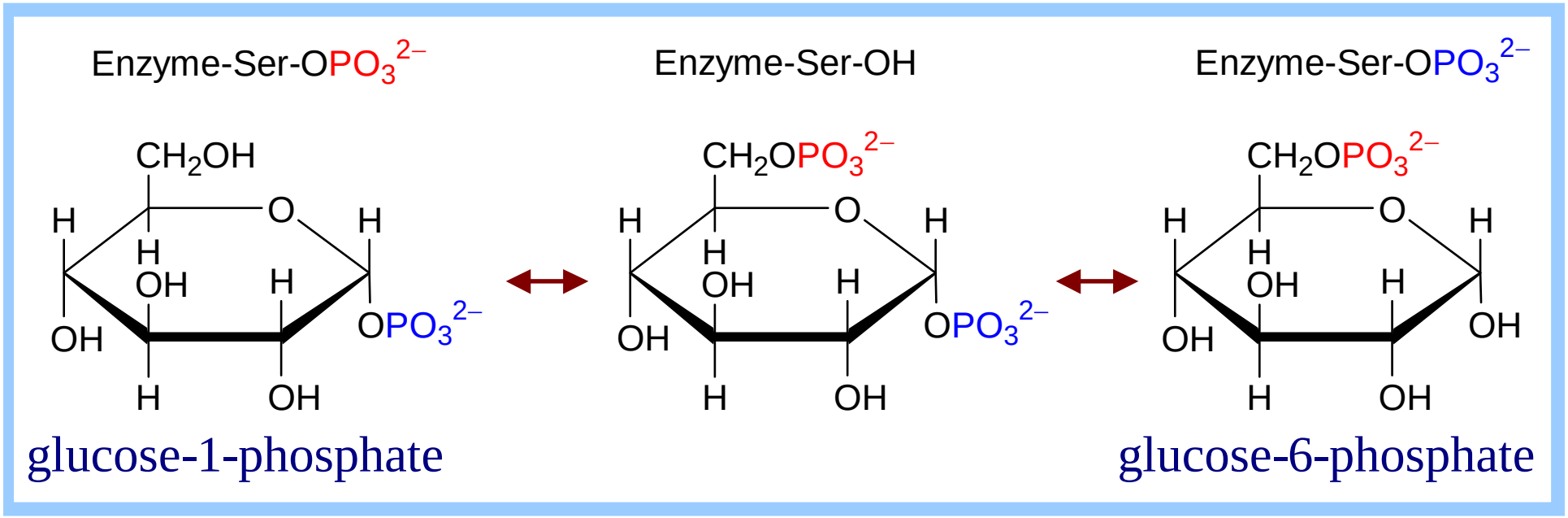
This is called a "**limit branch**".

Explore the structure of muscle Glycogen Phosphorylase with Chime.

Debranching enzyme has 2 independent active sites, consisting of residues in different segments of a single polypeptide chain:

- The **transferase** of the debranching enzyme transfers 3 glucose residues from a 4-residue limit branch to the end of another branch, diminishing the limit branch to a single glucose residue.
- The **α(1→6) glucosidase** moiety of the debranching enzyme then catalyzes hydrolysis of the **α(1→6)** linkage, yielding free **glucose**. This is a minor fraction of glucose released from glycogen.

The major product of glycogen breakdown is **glucose-1-phosphate**, from Phosphorylase activity.



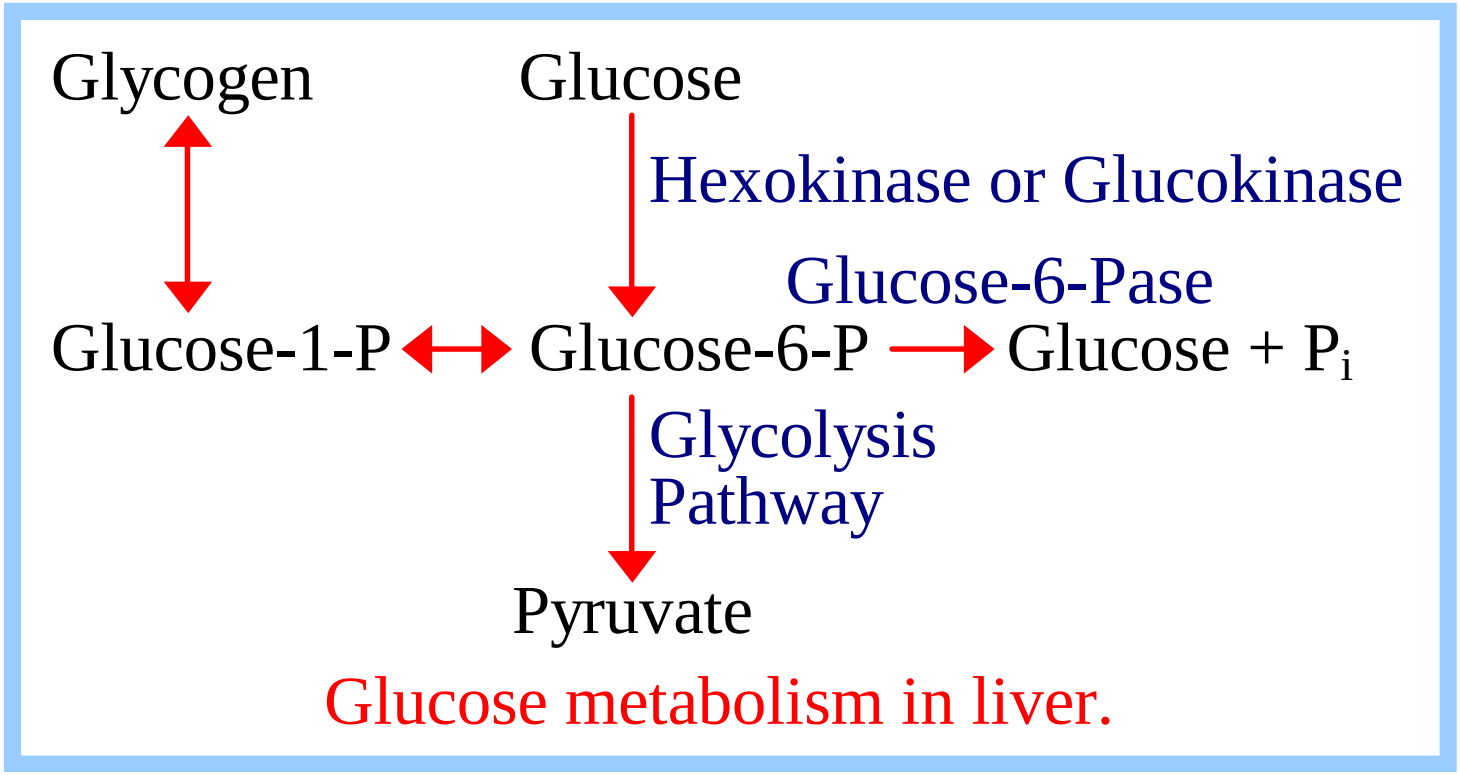
Phosphoglucomutase catalyzes the reversible reaction:

glucose-1-phosphate ←→ glucose-6-phosphate

A **serine OH** at the active site donates & accepts P_i.

The bisphosphate is not released.

Phosphoglycerate Mutase has a similar mechanism, but instead uses His for P_i transfer.



Glucose metabolism in liver.

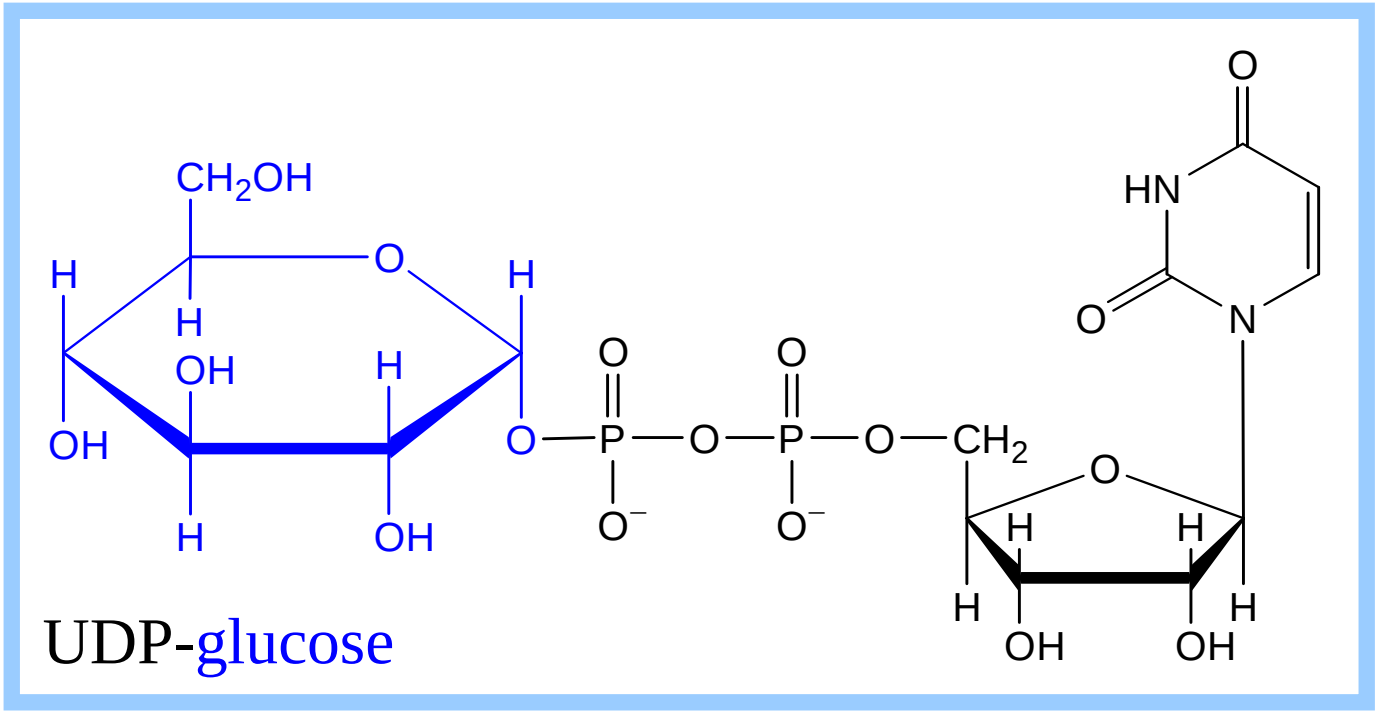
Glucose-6-phosphate may enter Glycolysis or (mainly in liver) be dephosphorylated for release to the blood.

Liver **Glucose-6-phosphatase** catalyzes the following, essential to the liver's role in maintaining blood glucose:

glucose-6-phosphate + H_2O → glucose + P_i

Most other tissues lack this enzyme.

Glycogen synthesis

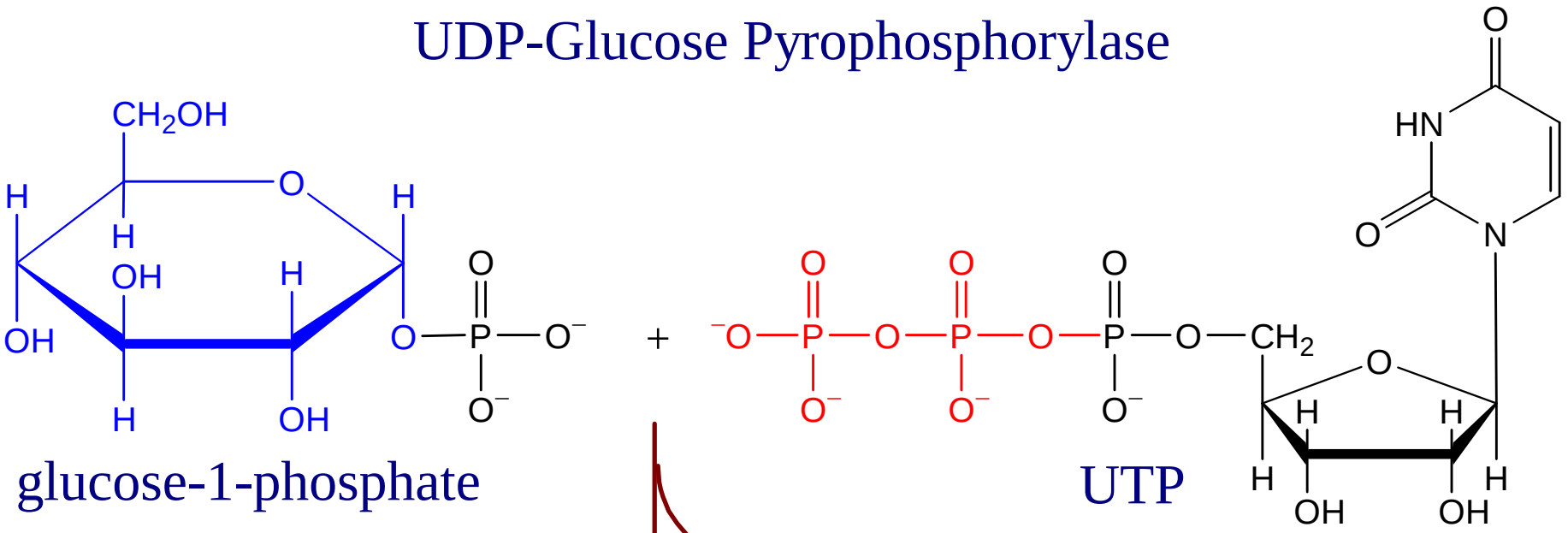


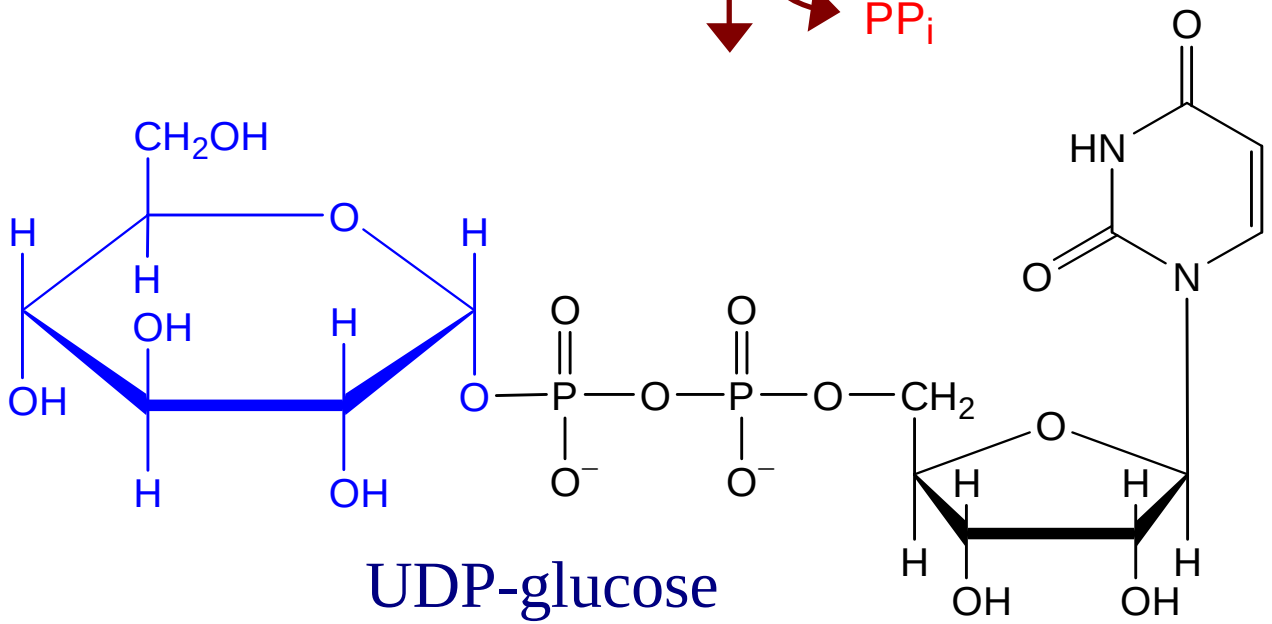
Uridine diphosphate glucose (UDP-glucose) is the immediate precursor for **glycogen synthesis**.

As glucose residues are added to glycogen, UDP-glucose is the substrate and UDP is released as a reaction product.

Nucleotide diphosphate sugars are precursors also for synthesis of other complex carbohydrates, including oligosaccharide chains of glycoproteins, etc.

UDP-Glucose Pyrophosphorylase





UDP-glucose is formed from glucose-1-phosphate:

- **glucose-1-phosphate + UTP → UDP-glucose + PP_i**
- **PP_i + H_2O → 2 P_i**

Overall:

- **glucose-1-phosphate + UTP → UDP-glucose + 2 P_i**

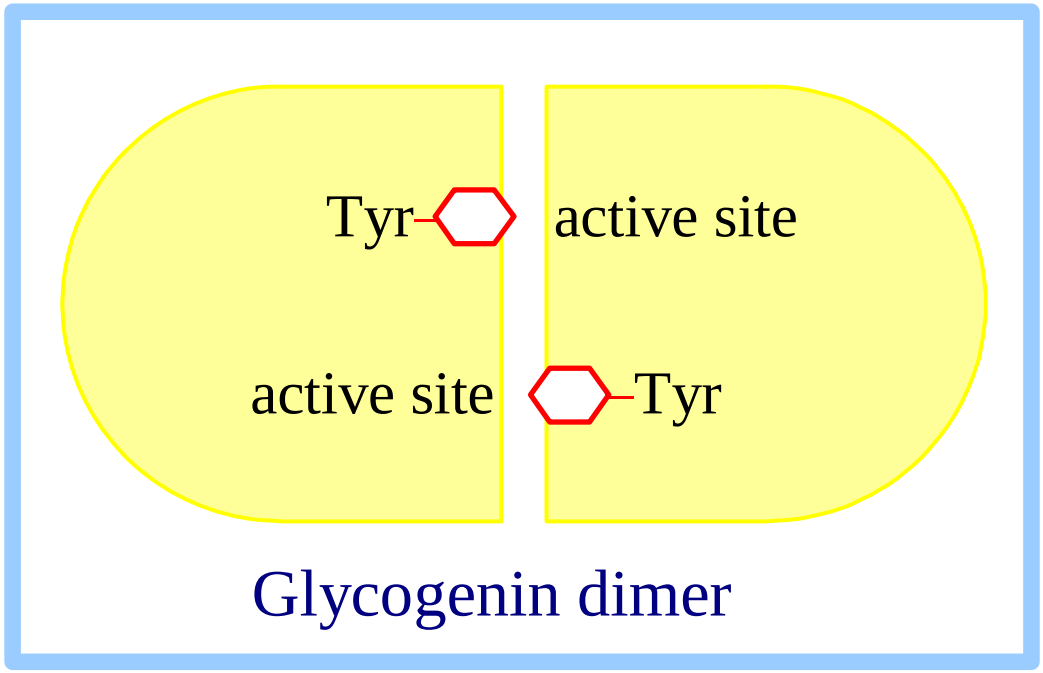
Spontaneous hydrolysis of the ~P bond in PP_i (P~P) drives the overall reaction.

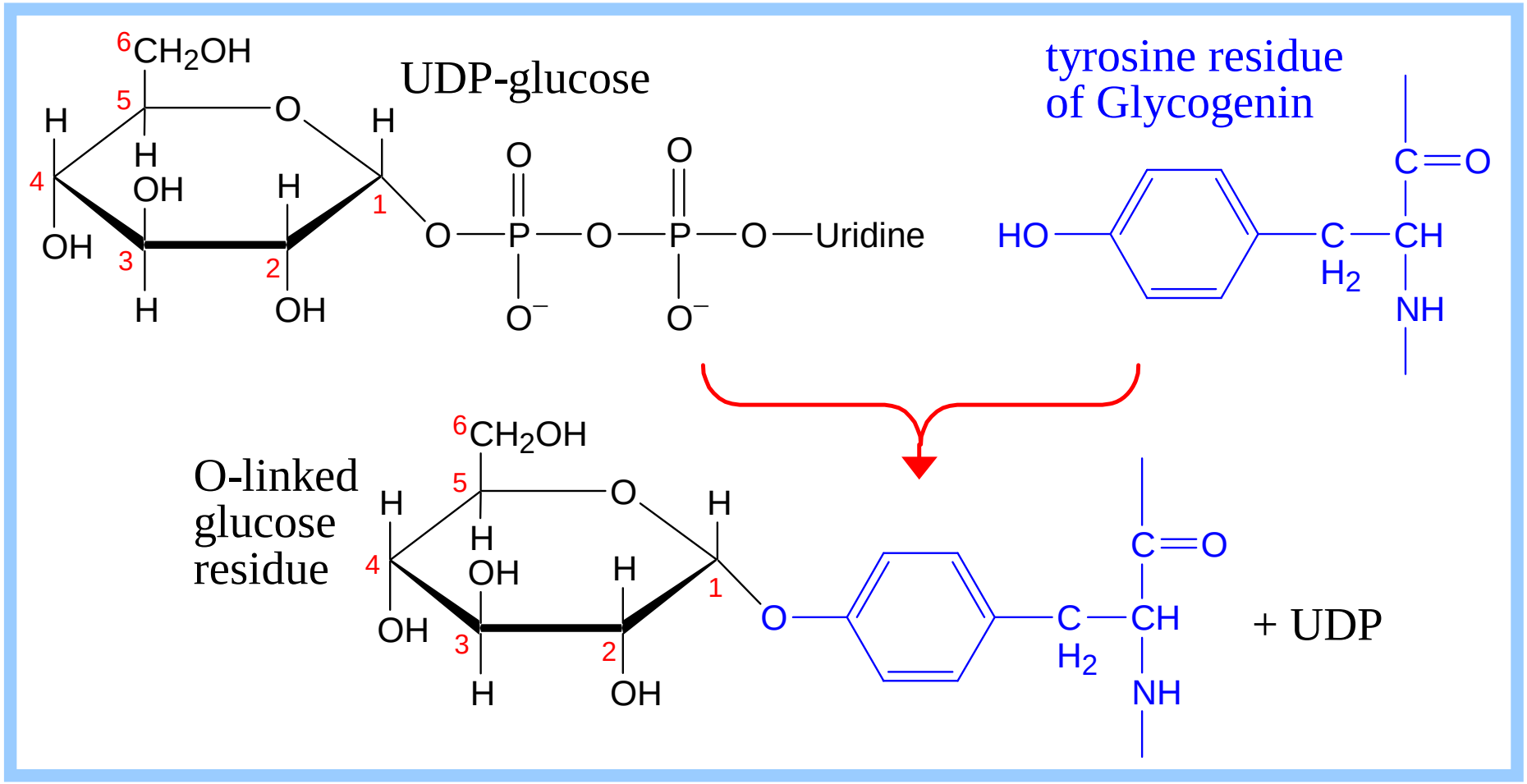
Cleavage of PP_i is the only energy cost for glycogen synthesis (<u>one</u> ~P bond per glucose residue).

Glycogenin initiates glycogen synthesis.

Glycogenin is an enzyme that catalyzes attachment of a **glucose** molecule to one of its own **tyrosine** residues.

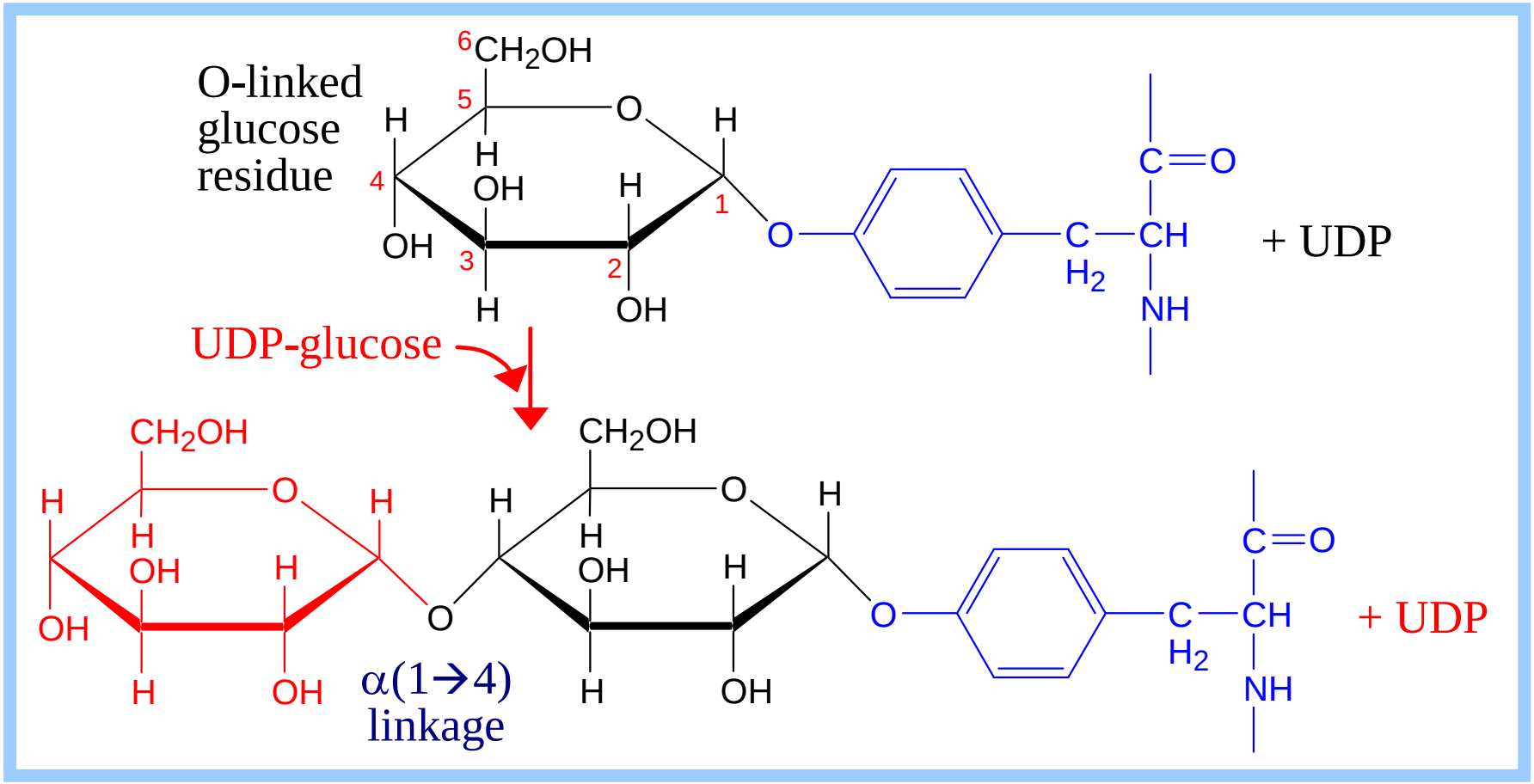
Glycogenin is a **dimer**, and evidence indicates that the 2 copies of the enzyme glucosylate one another.





A **glycosidic bond** is formed between the anomeric C1 of the glucose moiety derived from UDP-glucose and the hydroxyl oxygen of a **tyrosine** side-chain of **Glycogenin**.

UDP is released as a product.



Glycogenin then catalyzes glucosylation at C4 of the attached glucose (UDP-glucose again the donor), to yield an O-linked disaccharide with α(1→4) glycosidic linkage.

This is repeated until a **short linear glucose polymer** with α(1→4) glycosidic linkages is built up on Glycogenin.

Glycogen Synthase then catalyzes **elongation** of glycogen chains initiated by Glycogenin.

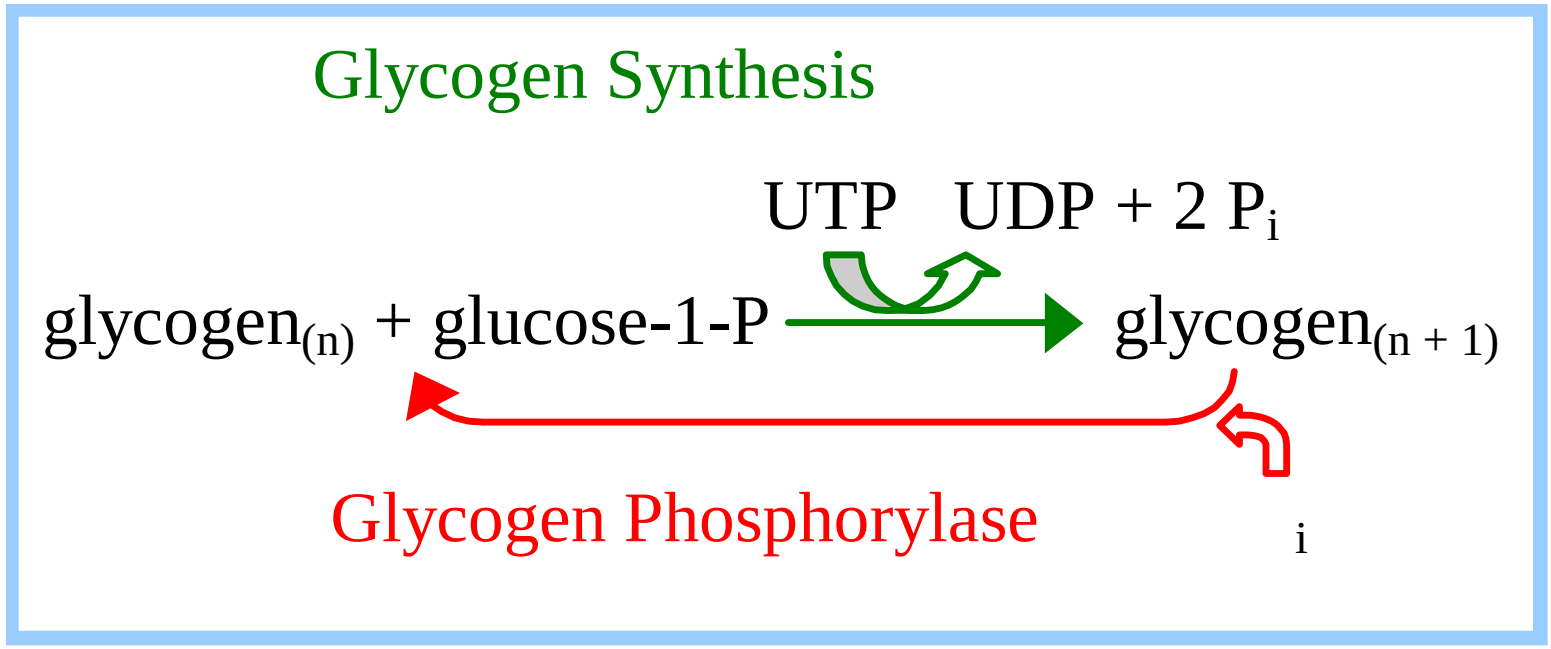
Question: Where would you expect to find Glycogenin within a cell?

Answer: Most of the Glycogenin is found associated with **glycogen particles** (branched glycogen chains) in the cytoplasm.

Glycogen Synthase catalyzes transfer of the glucose moiety of UDP-glucose to the hydroxyl at C4 of the terminal residue of a glycogen chain to form an **$\alpha(1\rightarrow 4)$** glycosidic linkage:

$$\textbf{glycogen}_{\textbf{(n residues)}} + \textbf{UDP-glucose} \rightarrow \textbf{glycogen}_{\textbf{(n +1 residues)}} + \textbf{UDP}$$

A **branching enzyme** transfers a segment from the end of a glycogen chain to the C6 hydroxyl of a glucose residue of glycogen to yield a branch with an **$\alpha(1\rightarrow 6)$** linkage.



Both synthesis & breakdown of glycogen are spontaneous.

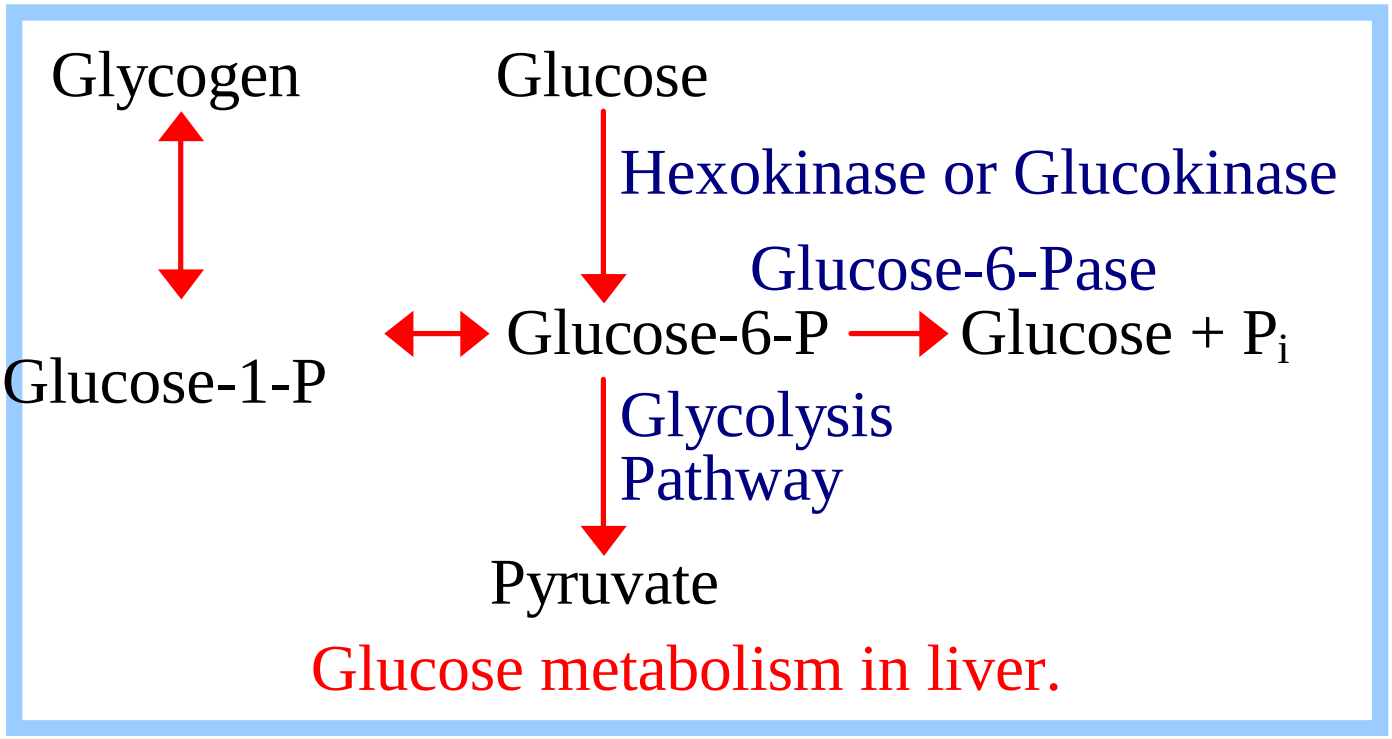
If both pathways were active simultaneously in a cell, there would be a "**futile cycle**" with cleavage of **one ~P bond per cycle** (in forming UDP-glucose).

To prevent such a futile cycle, Glycogen Synthase and Glycogen Phosphorylase are **reciprocally regulated**, by allosteric effectors and by phosphorylation.

Glycogen Phosphorylase in **muscle** is subject to allosteric regulation by AMP, ATP, and glucose-6-phosphate.

A separate isozyme of Phosphorylase expressed in liver is less sensitive to these allosteric controls.

- **AMP** (present significantly when ATP is depleted) **activates** Phosphorylase, promoting the relaxed conformation.
- **ATP** & **glucose-6-phosphate**, which both have binding sites that overlap that of AMP, **inhibit** Phosphorylase, promoting the tense conformation.
- Thus glycogen breakdown is inhibited when ATP and glucose-6-phosphate are plentiful.



Glucose metabolism in liver.

Glycogen Synthase is allosterically **activated** by **glucose-6-P** (opposite of effect on Phosphorylase).

Thus Glycogen Synthase is active when high blood glucose leads to elevated intracellular glucose-6-P.

It is useful to a cell to store glucose as glycogen when the input to Glycolysis (glucose-6-P), and the main product of Glycolysis (ATP), are adequate.

Regulation by covalent modification (phosphorylation):

The hormones **glucagon** and **epinephrine** activate G-protein coupled receptors to trigger **cAMP cascades**.

- Both hormones are produced in response to **low blood sugar**.
- Glucagon, which is synthesized by α-cells of the pancreas, activates cAMP formation in liver.
- Epinephrine activates cAMP formation in muscle.

The cAMP cascade results in **phosphorylation** of a serine hydroxyl of Glycogen Phosphorylase, which promotes transition to the **active** (relaxed) state.

The phosphorylated enzyme is **less sensitive to allosteric inhibitors**.

Thus, even if cellular ATP & glucose-6-phosphate are high, Phosphorylase will be active.

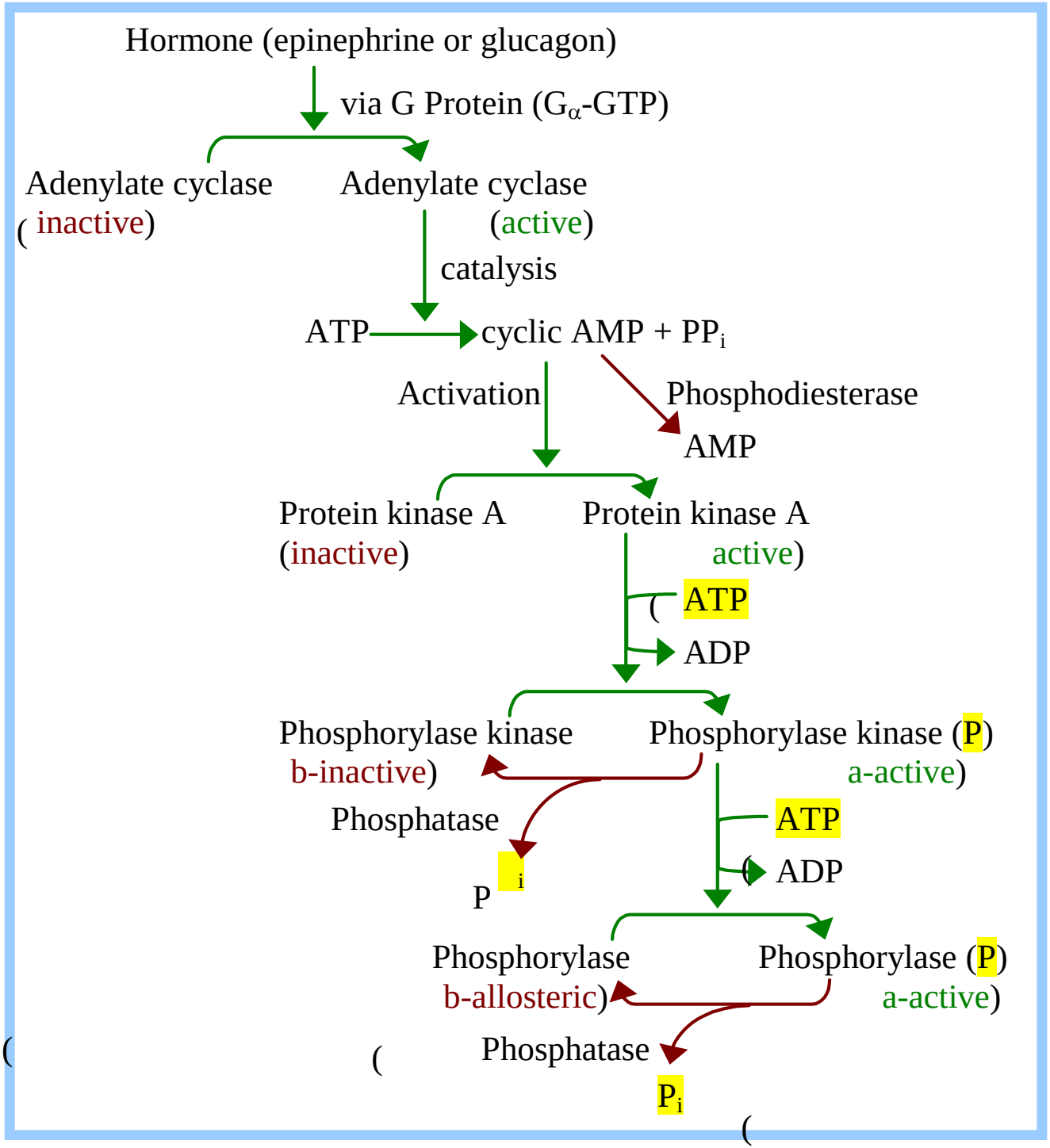
The glucose-1-phosphate produced from glycogen in liver may be converted to free **glucose** for release to the blood.

With this hormone-activated regulation, the needs of the organism take precedence over needs of the cell.

Commonly used terminology:

- "**a**" is the form of the enzyme that tends to be **active**, and **independent** of allosteric regulators (in the case of Glycogen Phosphorylase, when phosphorylated).
- "**b**" is the form of the enzyme that is **dependent** on local allosteric controls (in the case of Glycogen Phosphorylase when dephosphorylated).

Signal cascade by which Glycogen Phosphorylase is activated.



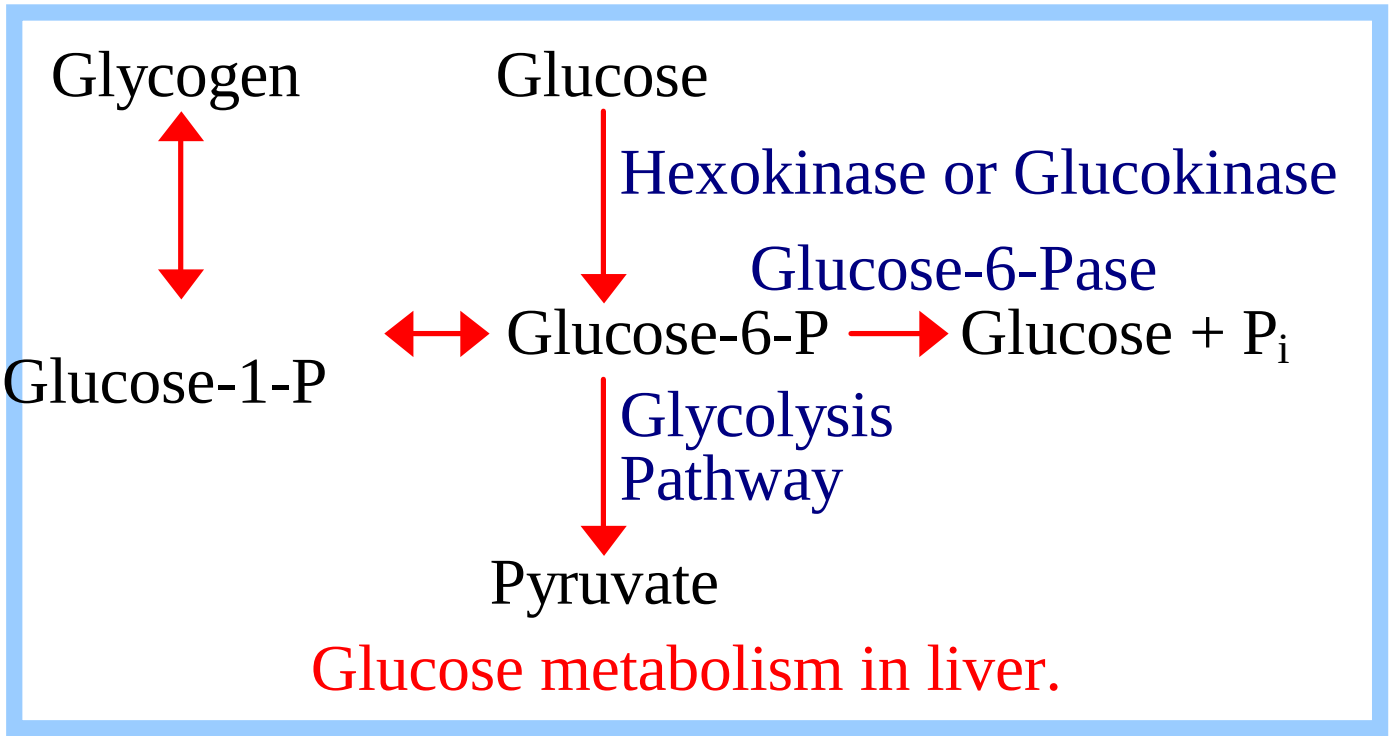
The **cAMP cascade** induced in liver by glucagon or epinephrine has the **opposite effect on glycogen synthesis**.

Glycogen Synthase is phosphorylated by Protein Kinase A as well as by Phosphorylase Kinase.

Phosphorylation of Glycogen Synthase promotes the "**b**" (less active) conformation.

The cAMP cascade thus **inhibits glycogen synthesis**.

Instead of being converted to glycogen, glucose-1-P in liver may be converted to glucose-6-P, and dephosphorylated for release to the blood.



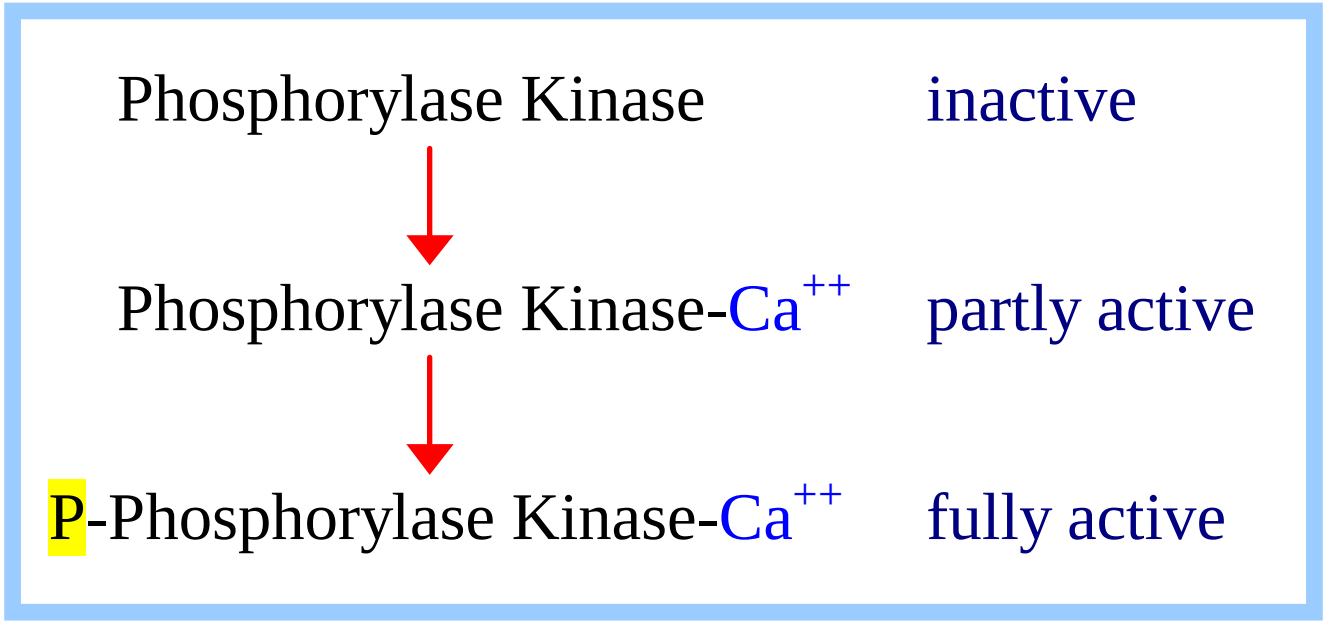
High cytosolic glucose-6-phosphate, which would result when blood glucose is high, turns off the signal with regard to glycogen synthesis.

The conformation of Glycogen Synthase induced by the allosteric activator glucose-6-phosphate is susceptible to dephosphorylation by Protein Phosphatase.

Insulin, produced in response to **high blood glucose**, triggers a separate signal cascade that leads to **activation of Phosphoprotein Phosphatase**.

This phosphatase catalyzes removal of regulatory phosphate residues from Phosphorylase, Phosphorylase Kinase, & Glycogen Synthase enzymes.

Thus **insulin antagonizes** effects of the cAMP cascade induced by **glucagon** & **epinephrine**.

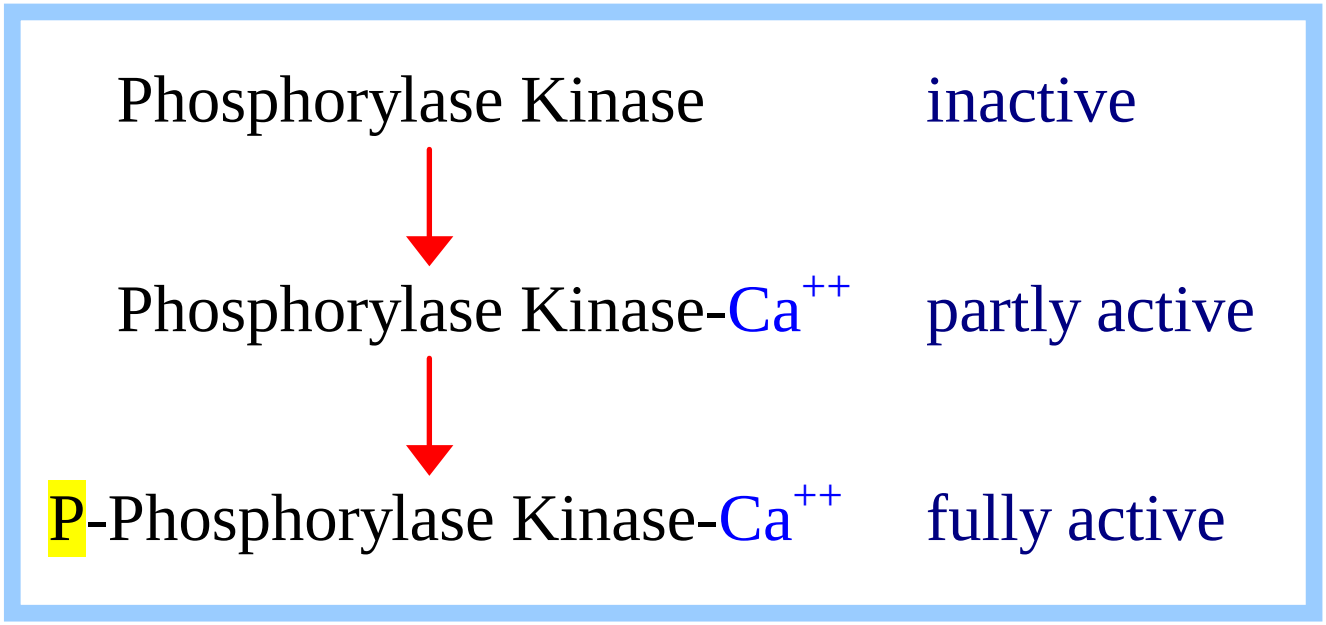


Ca^{++} also regulates glycogen breakdown in **muscle**.

During activation of contraction in skeletal muscle, **Ca^{++}** is released from the sarcoplasmic reticulum to promote actin/myosin interactions.

The released **Ca^{++}** also **activates Phosphorylase Kinase**, which in muscle includes **calmodulin** as its **δ subunit**.

Phosphorylase Kinase is partly activated by binding of **Ca^{++}** to this subunit.



Phosphorylation of the enzyme, via a cAMP cascade induced by epinephrine, results in further activation.

These regulatory processes ensure release of phosphorylated glucose from glycogen, for entry into **Glycolysis** to provide **ATP** needed for muscle contraction.

During **extended exercise**, as glycogen stores become depleted, muscle cells rely more on glucose uptake from the blood, and on fatty acid catabolism as a source of ATP.

A **genetic defect** in the isoform of an enzyme expressed in **liver** causes the following **symptoms**:

- **After eating** a **CHO** meal, **elevated** blood levels of **glucose**, **lactate**, & lipids.
- **During fasting**, **low blood glucose** & high ketone bodies.

Which liver enzyme is defective? **Glycogen Synthase**

Explain Symptoms:

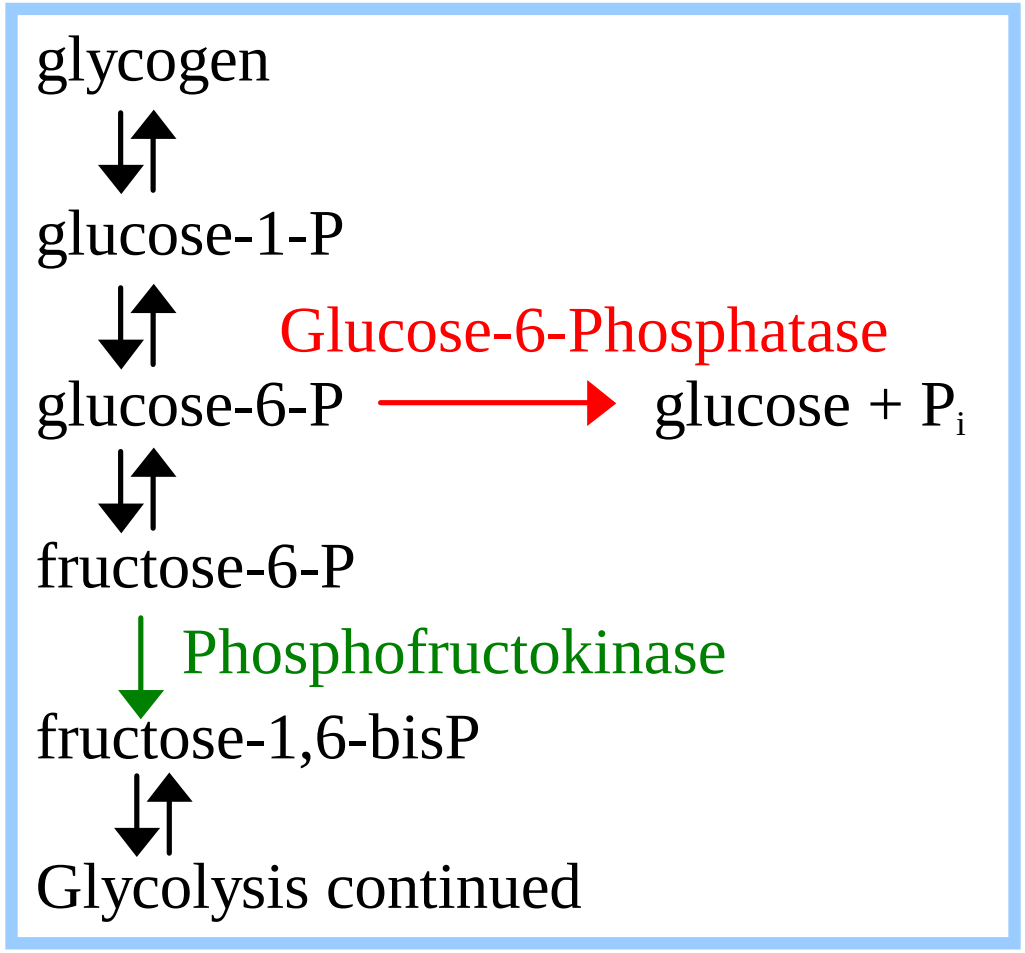
- **After eating**, blood **glucose** is high because liver cannot store it as glycogen. Some excess glucose is processed via **Glycolysis** to produce **lactate** & fatty acid precursors.
- **During fasting**, **glucose** is low because the liver lacks glycogen stores for generation of glucose.
  Ketone bodies are produced as an **alternative fuel**.

Question: How would you nutritionally treat deficiency of liver Glycogen Synthase?

- Frequent meals of **complex carbohydrates** (avoiding simple sugars that would lead to a rapid rise in blood glucose)
- Meals high in **protein** to provide substrates for **gluconeogenesis**.

Glycogen Storage Diseases are genetic enzyme deficiencies associated with **excessive glycogen accumulation** within cells.

Some enzymes whose deficiency leads to glycogen accumulation are part of the inter-connected pathways shown here.



Symptoms in addition to excess glycogen storage:

- When a genetic defect affects mainly an isoform of an enzyme expressed in **liver**, a common symptom is **hypoglycemia**, relating to impaired mobilization of glucose for release to the blood during fasting.
- When the defect is in **muscle** tissue, **weakness** & **difficulty with exercise** result from inability to increase glucose entry into Glycolysis during exercise.
- Additional symptoms depend on the particular enzyme that is deficient.

Glycogen Storage Disease	**Symptoms**, in addition to glycogen accumulation
Type I, liver deficiency of **Glucose-6-phosphatase** (von Gierke's disease)	**hypoglycemia** (low blood glucose) when fasting, liver enlargement.
Type IV, deficiency of **branching enzyme** in various organs, including liver (Andersen's disease)	**liver dysfunction** and early death.
Type V, muscle deficiency of **Glycogen Phosphorylase** (McArdle's disease)	**muscle cramps** with exercise.
Type VII, muscle deficiency of **Phosphofructokinase**.	**inability to exercise**.