Glycogen Metabolism
Glycogen is a polymer of glucose residues linked by
- $\alpha(1\rightarrow4)$ glycosidic bonds, mainly
- $\alpha(1\rightarrow6)$ 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):

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

$$\text{glycogen}_{(n \text{ residues})} + P_i \rightarrow \text{glycogen}_{(n-1 \text{ residues})} + \text{glucose-1-phosphate}$$

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

**Hydrolysis:** $R-O-R' + HOH \rightarrow R-OH + R'-OH$

**Phosphorolysis:** $R-O-R' + HO-PO_3^{2-} \rightarrow R-OH + R'-O-PO_3^{2-}$
**Pyridoxal phosphate (PLP)**, a derivative of vitamin B\textsubscript{6}, serves as prosthetic group for Glycogen Phosphorylase.
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.

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 $\alpha(1\rightarrow4)$ linkages only to within 4 residues of an $\alpha(1\rightarrow6)$ 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:

\[
\text{glucose-1-phosphate} \rightleftharpoons \text{glucose-6-phosphate}
\]

A *serine* OH at the active site donates & accepts P\textsubscript{i}. The bisphosphate is not released.

Phosphoglycerate Mutase has a similar mechanism, but instead uses His for P\textsubscript{i} transfer.
**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:

\[ \text{glucose-6-phosphate} + \text{H}_2\text{O} \rightarrow \text{glucose} + \text{P}_i \]

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

glucose-1-phosphate + UTP → UDP-glucose + PPi
UDP-glucose is formed from glucose-1-phosphate:
- glucose-1-phosphate + UTP $\rightarrow$ UDP-glucose + PP$_i$
- PP$_i$ + H$_2$O $\rightarrow$ 2 P$_i$

Overall:
- glucose-1-phosphate + UTP $\rightarrow$ UDP-glucose + 2 P$_i$

Spontaneous hydrolysis of the $\sim$P bond in PP$_i$ (P$\sim$P) drives the overall reaction.

Cleavage of PP$_i$ is the only energy cost for glycogen synthesis (one $\sim$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 $\alpha(1\rightarrow4)$ glycosidic linkage.

This is repeated until a **short linear glucose polymer** with $\alpha(1\rightarrow4)$ 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:

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

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 $\alpha(1\rightarrow6)$ linkage.
Glycogen Synthesis

$$\text{UTP} \quad \text{UDP} + 2 \text{P}_i \quad \text{glycogen}_n + \text{glucose-1-P} \quad \text{glycogen}_{n+1}\quad \text{Glycogen Phosphorylase}$$

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 \sim 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.
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 $\alpha$-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).
Hormone (epinephrine or glucagon) via G Protein (G_α-GTP)

Adenylate cyclase (inactive) → Adenylate cyclase (active) → catalysis → ATP → cyclic AMP + PP_ı

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 interconnected pathways shown here.

Glycogen
↓↑
glucose-1-P
↓↑
Glucose-6-Phosphatase
glucose-6-P → glucose + P_i
↓↑
fructose-6-P
↓↑
Phosphofructokinase
fructose-1,6-bisP
↓↑
Glycolysis continued
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

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
<th>Symptoms</th>
</tr>
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<tbody>
<tr>
<td>Type I</td>
<td>Liver deficiency of Glucose-6-phosphatase (von Gierke's disease)</td>
<td>Hypoglycemia (low blood glucose) when fasting, liver enlargement.</td>
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<tr>
<td>Type IV</td>
<td>Deficiency of branching enzyme in various organs, including liver (Andersen's disease)</td>
<td>Liver dysfunction and early death.</td>
</tr>
<tr>
<td>Type V</td>
<td>Muscle deficiency of Glycogen Phosphorylase (McArdle's disease)</td>
<td>Muscle cramps with exercise.</td>
</tr>
<tr>
<td>Type VII</td>
<td>Muscle deficiency of Phosphofructokinase</td>
<td>Inability to exercise.</td>
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