## Glycogen

Earth organisms use three major forms of glucose polymers. **Cellulose** (illustrated below) is a β-1,4-glucose polymer used as a structural molecule in plants. Animals cannot cleave β-1,4-glucose bonds, and therefore cannot use cellulose as a source of energy (although microorganisms can, and ruminants can exist in a symbiotic relationship with cellulose-degrading microorganisms).
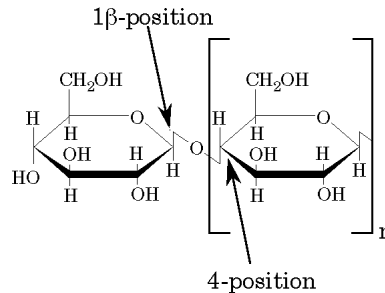


The other major glucose polymers are used as energy storage molecules. Plants produce **starch**; starch is a mixture combining a linear α-1,4-glucose polymer with an α-1,4-glucose polymer containing occasional (every 24-30 residues) α-1,6-branches. Animals and bacteria produce **glycogen**; glycogen is an α-1,4-glucose polymer with frequent (every ~10 residues) α-1,6-branches.



**Glycogen/Starch**
**(α-1,4-glucoside polymer, with α-1,6 branches)**
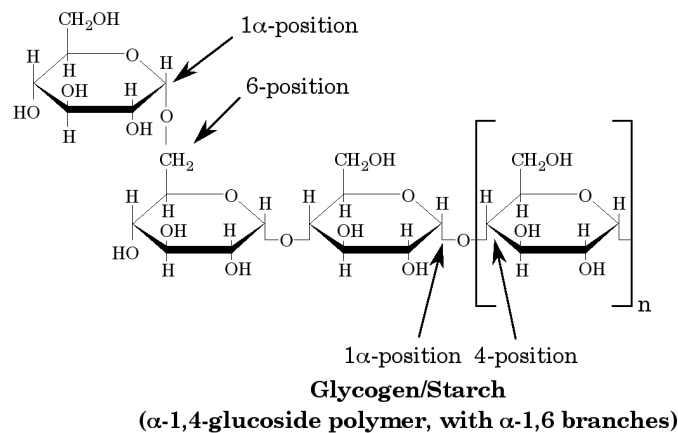
The branches in glycogen alter the structural characteristics of the molecule. The branches may also have a role in altering the rate of breakdown of the molecule; phosphorylase can only cleave to within about 5 residues of a branch-point, and therefore must coordinate with debranching enzyme to allow complete degradation of glycogen. (Note that roughly half of the glucose residues are found on the "edges" of the glycogen, and therefore phosphorylase can remove up to ~50% of the glucose before debranching enzyme action becomes necessary.)

Glycogen is found in a number of tissues, but is most important in muscle and liver. The muscle contains a maximum of ~2% glycogen by weight. The liver can contain up to 10% glycogen; this is enough to supply the body with glucose for at about 12 hours in the absence of food intake or gluconeogenesis. The muscle, being larger,

contains roughly three times more total glycogen than the liver, although each cell contains a smaller amount of the glucose polymer. Because glucose is removed from glycogen in phosphorylated form, the muscle (which lacks glucose-6-phosphatase) cannot release free glucose from glycogen into circulation.

The majority of glycogen metabolism involves addition or removal of glucose residues from preexisting glycogen molecules. Formation of new glycogen molecules requires a starting place. The starting place is provided by a protein called glycogenin discovered in the late 1980s by Bill Whelan. Glycogenin is glycosylated on the hydroxyl of a tyrosine residue; glycogenin then acts as an enzyme to add several glucose residues (using UDP-glucose as the substrate) to the chain until glycogen synthase takes over the process.

Glycogen molecules can be quite large, and frequently contain well over 100,000 glucose units. Because glucose can readily be both added and removed from glycogen, glycogen acts as a useful storage form for glucose. In principle, it would be possible to store free glucose; in practice storing glucose would result in severe complications due to the much higher osmotic pressure of vast numbers of small molecules compared to small numbers of large one, and to the fact that reducing sugars can covalently modify other molecules.

## Glycogen Synthesis
Glycogen is synthesized from glucose-6-phosphate. The process involves three reactions.
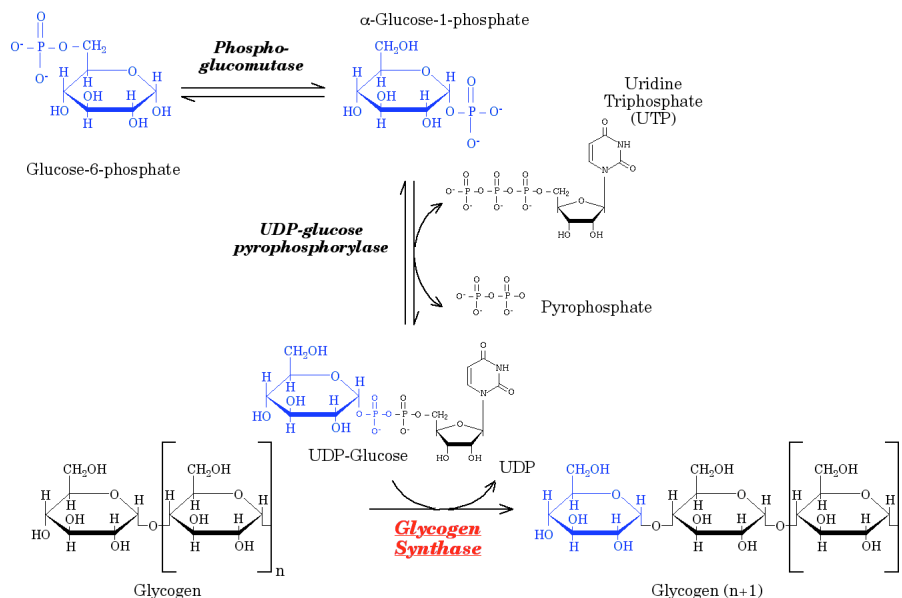


**Phosphoglucomutase** interconverts glucose-6-phosphate and glucose-1-phosphate. This process is completely reversible. When glycogen is broken down, phosphoglucomutase converts the glucose-1-phosphate released to glucose-6-phosphate, which can then be used for other processes. Phosphoglucomutase requires $\alpha$-glucose-6-phosphate as its substrate; this ensures that glycogen will be formed using $\alpha$-glycoside bonds.

**UDP-glucose pyrophosphorylase** also catalyzes a reversible reaction, the addition of UTP to the glucose-1-phosphate, with release of pyrophosphate. The reaction is driven physiologically by cleavage of pyrophosphate to inorganic phosphate by pyrophosphatase. The UDP-glucose pyrophosphorylase reaction acts as a priming step, and provides the energy required to form the glycoside bond in glycogen.

Note that UTP is thus acting as a metabolic energy-containing molecule in the same way that ATP does. Although ATP is most frequently used, all of the nucleotides normally present in RNA are also used for metabolic purposes. In contrast, deoxynucleotides are only used as building blocks of DNA.

The UDP glucose formed in the UDP-glucose pyrophosphorylase reaction is used for several different reactions (two of these, part of the galactose metabolic pathway, and glucuronic acid production, are discussed below). The majority of the UDP-glucose formed is used to synthesize glycogen, but because of the variety of roles for UDP-glucose, the synthesis of glycogen is not actually regulated until the next step.

**Glycogen synthase** uses UDP-glucose molecules as substrates for creating the α-1,4-glycoside bonds to the pre-existing glycogen molecule, releasing UDP and a slightly longer glycogen molecule. **Glycogen synthase is the major control point for glycogen synthesis in animals.** The regulation of glycogen synthase is discussed below in the section on regulation of glycogen metabolism.

A fourth enzyme, **branching enzyme**, catalyzes the transfer of short glucose polymers from α-1-4 glycoside to α-1-6 glycoside links. (Note: branching enzyme is important; the linear polymers of glucose due to lack of branching enzyme cause severe and usually fatal liver damage.)

## Glycogen and starch biosynthesis in bacteria and plants
Bacteria produce glycogen, and plants produce starch; both are branched polymers largely consisting of α-1,4-glycoside linkages. The difference is the degree of branching: starch contains fewer branch points than does glycogen. Starch is a mixture of amylose, a linear α-1,4-glucose polymer, and amylopectin, which contains α-1,6-bonds every 24-30 residues; in contrast, glycogen is branched every ~10 residues.

In animals, glycogen synthase is the major regulated step for glycogen production, probably because its substrate, UDP-glucose, is also used for other purposes. In plants and bacteria, however, the primary regulated step is the activation of glucose-1-phosphate by a different enzyme: ADP-glucose pyrophosphorylase. This appears to be true because these organisms do not use the ADP-glucose for other purposes.

ADP-glucose pyrophosphorylase is primarily regulated by metabolic intermediates; the presence of these intermediates indicates that the cell has sufficient surplus energy available to allow energy storage for the future. The molecules used as regulators of ADP-glucose pyrophosphorylase depend on the glucose metabolic

pathways used by that species. Stimulators of ADP-glucose pyrophosphorylase include 3-phosphoglycerate, fructose-6-phosphate, fructose-1,6-bisphosphate, and pyruvate; inhibitors include AMP and phosphate.

### Side issue: galactose metabolism

Lactose is a major component of milk. Because lactose is a disaccharide comprised of galactose and glucose, animals need to be able to metabolize galactose.
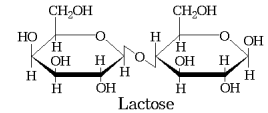
Lactose contains a β-1,4 galactoside bond. Humans produce an enzyme called lactase, which has β-galactosidase activity and is capable of cleaving β-<u>galacto</u>side bonds (note that many humans lose the ability to express lactase after the age of about five); in contrast, animals cannot cleave β-<u>gluco</u>side bonds. Complete lack of the ability to metabolize galactose has severely deleterious consequences, some of which can be alleviated by avoiding dietary galactose.

If galactose is being converted into glucose, the glucose leaves the pathway as glucose-1-phosphate, and therefore, the entry of galactose into the glycolytic pathway requires four enzymes (the three shown below and phosphoglucomutase).
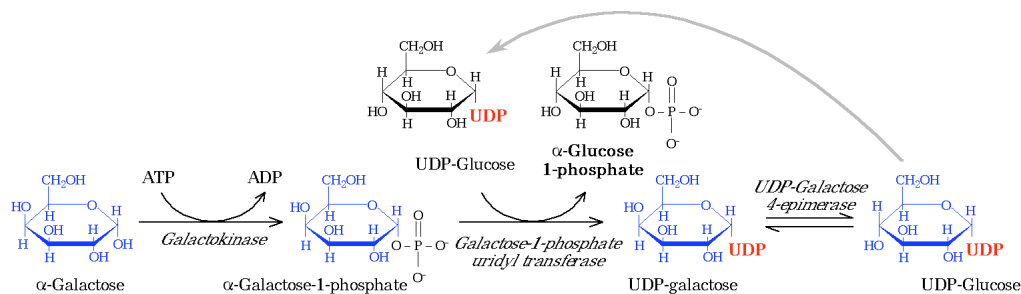


The first enzyme, **galactokinase**, phosphorylates galactose at the 1-position. As with most of the other enzymes, galactokinase is specific for the α-anomer of galactose.

**Galactose-1-phosphate uridyl transferase** uses UDP-glucose as a UMP donor. The result is the release of UDP-galactose and of glucose-1-phosphate (which can be converted to glucose-6-phosphate by phosphoglucomutase).

**UDP-galactose epimerase** alters the stereochemistry at the 4-position, converting galactose to its epimer, glucose. The reaction is reversible, and is used to synthesize galactose from glucose; galactose is a required component for some glycoproteins.

Note that UDP-glucose is used catalytically in the reaction scheme. The net reaction of the pathway is:

$$\text{galactose} + \text{ATP} \rightarrow \text{glucose-1-phosphate} + \text{ADP}$$
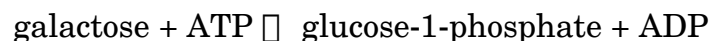
### Side issue: glucuronic acid

Glucuronic acid is produced from UDP-glucose by UDP-glucose dehydrogenase. The UDP-glucuronic acid is then used to produce glucuronic acid derivatives of other

compounds. Covalent modification of hydrophobic hormones and drugs with glucuronic acid makes these compounds more soluble in aqueous solution, and is critical for their excretion. Thus, while the amount of glucuronic acid produced is fairly small, the pathway is fairly important physiologically.
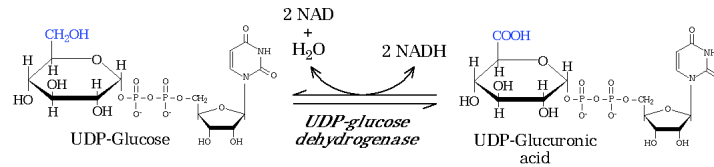


## Glycogen breakdown

Glycogen breakdown (glycogenolysis) is the removal of glucose monomers (in the form of glucose-1-phosphate) from glycogen by *glycogen phosphorylase*. Phosphorylase, like glycogen synthase, is a heavily regulated enzyme.
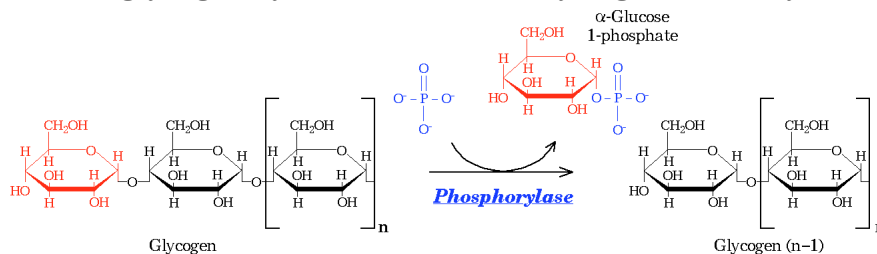


Phosphorylase is a pyridoxal phosphate-dependent enzyme; the pyridoxal phosphate present in phosphorylase accounts for ~75% of the vitamin in the body. The pyridoxal phosphate forms a Schiff-base with the enzyme; the phosphate portion of the cofactor is thought to have a role in the catalytic process.

Phosphorylase uses inorganic phosphate as one substrate. Because the concentration of inorganic phosphate in the cell tends to be fairly high, the phosphorylase reaction is irreversible under physiological conditions.

### Debranching enzyme

Debranching enzyme cleaves 1-6 links (the protein also has a glucose polymer transferase activity that moves 1-4 linked oligosaccharides chains from one chain to another). Because phosphorylase cannot remove glucose units closer than about 5 residues from a branch point, debranching enzyme is necessary for removal of glucose residues near these branch points.

Both branching enzyme and debranching enzyme appear to be unregulated enzymes.

## Regulation of glycogen metabolism

### Glycogen synthase

Glycogen synthase is **inactivated by phosphorylation** by a number of protein kinases including $Ca^{2+}$/calmodulin-dependent protein kinase and cAMP-dependent protein kinase. High levels of glycogen also inhibit glycogen synthase. Glycogen synthase is activated by glucose-6-phosphate even when phosphorylated, and

therefore will synthesize glycogen if glucose-6-phosphate is not being used for other purposes.

### Phosphorylase isozymes

**Liver phosphorylase** is activated by phosphorylation by phosphorylase kinase; phosphorylase kinase is activated by phosphorylation by cAMP-dependent protein kinase, or by $Ca^{2+}$/calmodulin-dependent protein kinase. Dephosphorylated liver phosphorylase is inactive; a specific phosphatase (activated by insulin) inactivates phosphorylase. (A cAMP-mediated process inhibits the phosphatase; cAMP therefore both stimulates phosphorylase phosphorylation and inhibits the phosphate removal).

**Muscle phosphorylase** is activated either by phosphorylation or by AMP (a signal that ATP levels have decreased; in other words, exercise has a direct effect on muscle glycogen breakdown.) Muscle phosphorylase kinase is activated by calcium (a direct result of muscle contraction) and by cAMP-dependent protein kinase. Muscle phosphorylase is inhibited by ATP and by glucose-6-phosphate (both are signals that the cell has sufficient energy).

### Glycogen storage diseases

Lack of glucose-6-phosphatase, debranching enzyme, phosphorylase, and phosphorylase kinase all result in the accumulation of glycogen in the liver and in some other tissues. These enzyme deficiencies usually resulting in increased risk for hypoglycemia; depending on the isozyme deficiency, they may also result in exercise intolerance.

Lack of branching enzyme is fatal early in life due to the accumulation of straight-chain glucose polymers, especially in the heart and liver.