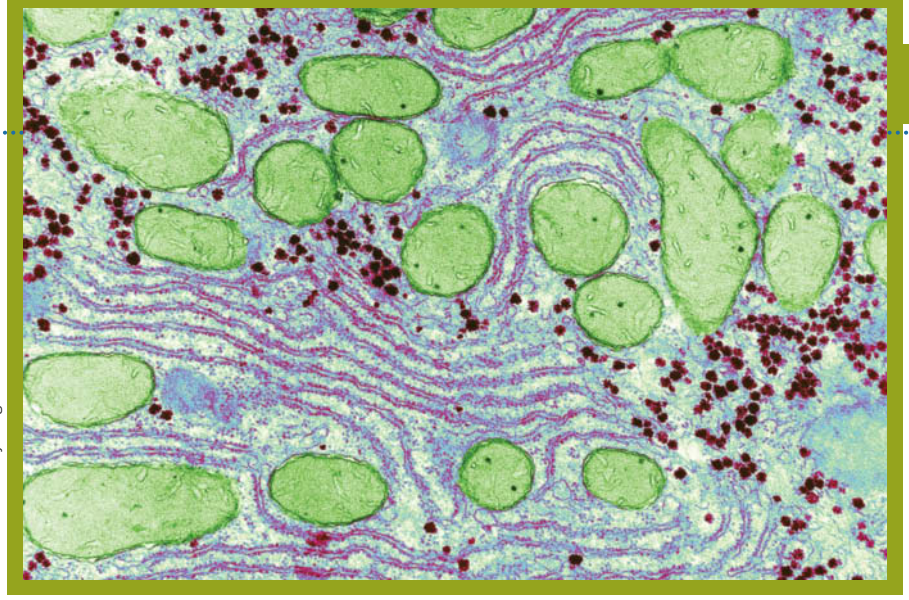
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Don W Fawcett / Getty Images

Glycogen particles, which consist of several enormous branched polymers of glucose residues, are stained green in this electron micrograph of salamander liver. In all animals, glycogen is a key form of stored metabolic energy that is built up and broken down according to the needs of the body.

CHAPTER 16

Glycogen Metabolism and Gluconeogenesis

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Glycogen (in animals, fungi, and bacteria) and starch (in plants) function to stockpile glucose for later metabolic use. In animals, a constant supply of glucose is essential for tissues such as the brain and red blood cells, which depend almost entirely on glucose as an energy source (other tissues can also oxidize fatty acids or amino acids for energy; Sections 20-2 and 21-4). The mobilization of glucose from glycogen stores, primarily in the liver, provides a constant supply of glucose (~5 mM in blood) to all tissues. When glucose is plentiful, such as immediately after a meal, glycogen synthesis accelerates. Yet the liver's capacity to store glycogen is sufficient to supply the brain with glucose for only about half a day. Under fasting conditions, most of the body's glucose needs are met by **gluconeogenesis** (literally, new glucose synthesis) from noncarbohydrate precursors such as amino acids. Not surprisingly, the regulation of glucose synthesis, storage, mobilization, and catabolism by glycolysis (Section 15-2) or the pentose phosphate pathway (Section 15-6) is elaborate and is sensitive to the immediate and long-term energy needs of the organism.

The importance of glycogen for glucose storage is plainly illustrated by the effects of deficiencies of the enzymes that release stored glucose. **McArdle's disease,** for example, is an inherited condition whose major symptom is painful muscle cramps on exertion. The muscles in afflicted individuals lack the enzyme required for glycogen breakdown to yield glucose. Although glycogen is synthesized normally, it cannot supply fuel for glycolysis to keep up with the demand for ATP.

Figure 16-1 summarizes the metabolic uses of glucose. Glucose-6-phosphate (G6P), a key branch point, is derived from free glucose through the action of hexokinase (Section 15-2A) or is the product of glycogen breakdown or gluconeogenesis. G6P has several possible fates: It can be used to synthesize glycogen; it can be catabolized via glycolysis to yield ATP and carbon atoms (as acetyl-CoA) that are further oxidized by the citric acid cycle; and it can be shunted through the pentose phosphate pathway to generate NADPH and/or ribose-5-phosphate. In the liver and kidney, G6P can be converted to glucose for export to other tissues via the bloodstream.

The opposing processes of glycogen synthesis and degradation, and of glycolysis and gluconeogenesis, are reciprocally regulated; that is, one is largely turned on while the other is largely turned off. In this chapter, we examine the enzymatic steps of glycogen metabolism and gluconeogenesis, paying particular attention to the regulatory mechanisms that ensure efficient operation of opposing metabolic pathways.

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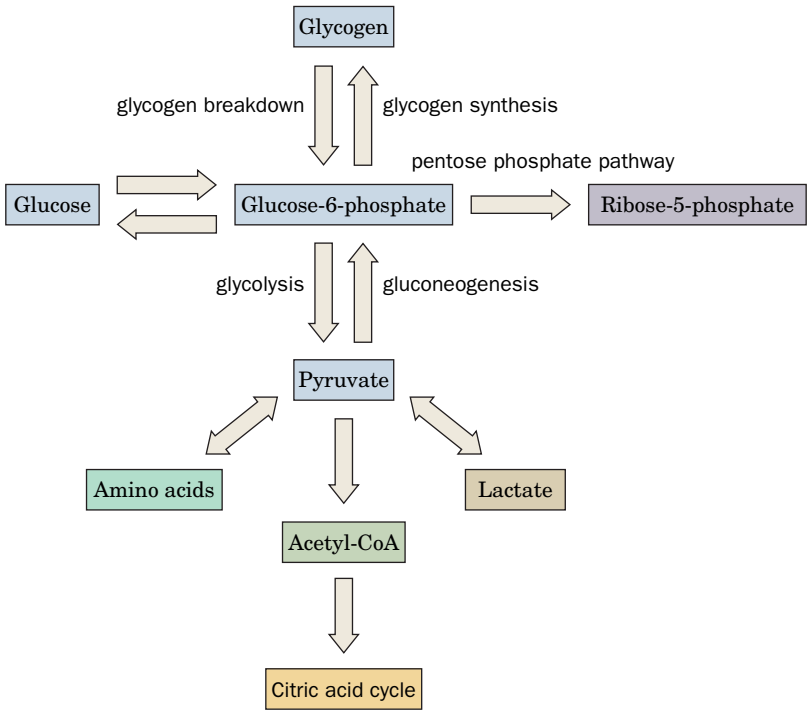


FIG. 16-1 Overview of glucose metabolism. Glucose-6-phosphate (G6P) is produced by the phosphorylation of free glucose, by glycogen degradation, and by gluconeogenesis. It is also a precursor for glycogen synthesis and the pentose phosphate pathway. The liver can hydrolyze G6P to glucose. Glucose is metabolized by glycolysis to pyruvate, which can be further broken down to acetyl-CoA for oxidation by the citric acid cycle. Lactate and amino acids, which are reversibly converted to pyruvate, are precursors for gluconeogenesis. **WPLS See the Animated Figures.**

? **Without looking at the figure, list the sources and fates of glucose-6-phosphate.**

1 Glycogen Breakdown

KEY CONCEPTS

- Glycogen, the storage form of glucose, is a branched polymer.
- Glucose mobilization in the liver involves a series of conversions from glycogen to glucose-1-phosphate to glucose-6-phosphate and finally to glucose.

Glycogen is a polymer of α(1→4)-linked D-glucose with α(1→6)-linked branches every 8–14 residues (Fig. 16-2*a*,*b* and Section 8-2C). Glycogen occurs as intracellular granules of 100- to 400-Å-diameter spheroidal molecules that each contain up to 120,000 glucose units (Fig. 16-2*c*). The granules are especially prominent in the cells that make the greatest use of glycogen: muscle (up to 1–2% glycogen by weight) and liver cells (up to 10% glycogen by weight; Fig. 8-11). Glycogen granules also contain the enzymes that catalyze glycogen synthesis and degradation as well as many of the proteins that regulate these processes.

Glucose units are mobilized by their sequential removal from the nonreducing ends of glycogen (the ends lacking a C1-OH group). Whereas glycogen has only one reducing end, there is a nonreducing end on every branch. *Glycogen's highly branched structure therefore permits rapid glucose mobilization through the simultaneous release of the glucose units at the end of every branch.*

Glycogen breakdown, or **glycogenolysis**, requires three enzymes:

1. **Glycogen phosphorylase** (or simply **phosphorylase**) catalyzes glycogen **phosphorolysis** (bond cleavage by the substitution of a phosphate group) to yield **glucose-1-phosphate (G1P):**

$$\underset{(n\ \text{residues})}{\text{Glycogen}} + P_i \rightleftharpoons \underset{(n-1\ \text{residues})}{\text{Glycogen}} + \text{G1P}$$

The enzyme releases a glucose unit only if it is at least five units away from a branch point.

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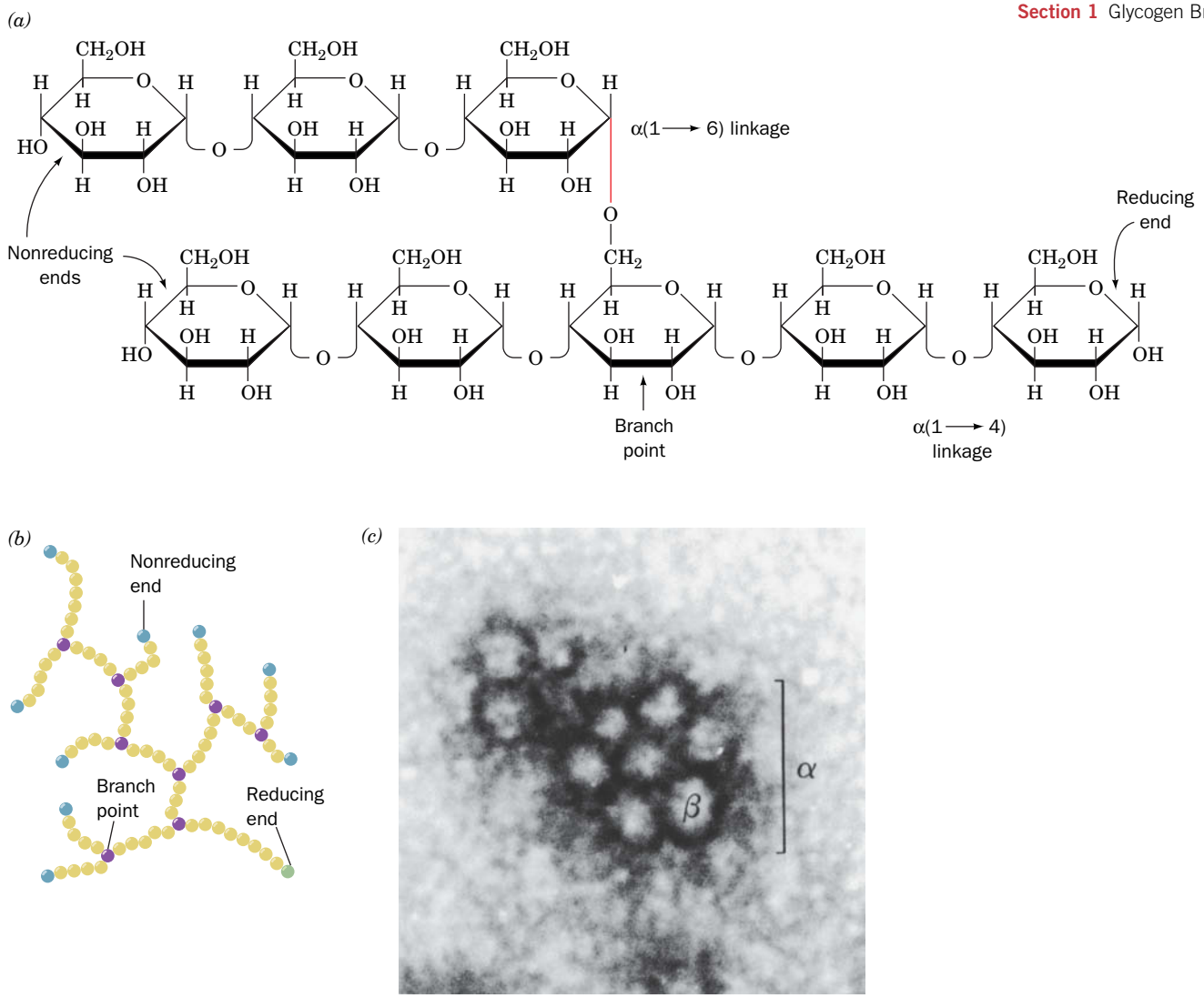


FIG. 16-2 **The structure of glycogen.** (*a*) Molecular formula. In the actual molecule, there are ~12 residues per chain. (*b*) Schematic diagram of glycogen's branched structure. Note that the molecule has many nonreducing ends but only one reducing end. *In vivo,* the reducing end is covalently linked to the protein glycogenin. (*c*) Electron micrograph of a glycogen granule from rat skeletal muscle. Each granule (labeled α) consists of several spherical glycogen molecules (β) and associated proteins. [From Calder, P.C., *Int. J. Biochem.* **23,** 1339 (1991). Copyright Elsevier Science. Used with permission.]

2. **Glycogen debranching enzyme** removes glycogen's branches, thereby making additional glucose residues accessible to glycogen phosphorylase.
3. **Phosphoglucomutase** converts G1P to G6P, which has several metabolic fates (Fig. 16-1).

Several key features of glycogen metabolism were discovered by the team of Carl and Gerty Cori (Box 16-1).

A Glycogen Phosphorylase Degrades Glycogen to Glucose-1-Phosphate

Glycogen phosphorylase is a dimer of identical 842-residue (97-kD) subunits that catalyzes the rate-controlling step in glycogen breakdown. It is regulated both by allosteric interactions and by covalent modification (phosphorylation

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Box 16-1 Pathways of Discovery Carl and Gerty Cori and Glucose Metabolism





Carl F. Cori (1896–1984)
Gerty T. Cori (1896–1957)

A lifelong collaboration commenced with the marriage of Carl and Gerty Cori in 1920. Although the Coris began their professional work in Austria, they fled the economic and social hardships of Europe in 1922 and moved to Buffalo, New York. They later made their way to Washington University School of Medicine in St. Louis, where Carl served as chair of the pharmacology department and, later, chair of the biochemistry department. Despite her role as an equal partner in their research work, Gerty officially remained a research associate.

The Coris' research focused primarily on the metabolism of glucose. One of their first discoveries was the connection between glucose metabolism in muscle and glycogen metabolism in the liver. The "Cori cycle" (Section 22-1F) describes how lactate produced by glycolysis in active muscle is transported to the liver, where it is used to synthesize glucose that is stored as glycogen until needed.

After describing the interorgan movement of glucose in intact animals, the Coris turned their attention to the metabolic fate of glucose, specifically, the intermediates and enzymes of glucose metabolism. In 1936, using a preparation of minced frog muscle, the Coris found glucose in the form of a phosphate ester (called the Cori ester, now known as glucose-1-phosphate). They traced the presence of the Cori ester to the activity of a phosphorylase (glycogen phosphorylase). This was a notable discovery, because the enzyme used phosphate, rather than water, to split glucose residues from the ends of glycogen chains. Even more remarkably, the enzyme could be made to work in reverse to elongate a glycogen polymer by adding glucose residues (from glucose-1-phosphate). For the first time, a large biological molecule could be synthesized *in vitro*.

During the 1940s, the Coris unraveled many of the secrets of glycogen phosphorylase. For example, they found that the enzyme exists in two forms, one that requires the activator AMP and one that is active in the absence of an allosteric activator. Although it was not immediately appreciated that the differences between the two forms resulted from the presence of covalently bound phosphate, this work laid the foundation for subsequent research on enzyme regulation through phosphorylation and dephosphorylation.

Carl and Gerty Cori also described phosphoglucomutase, the enzyme that converts glucose-1-phosphate to glucose-6-phosphate so that it can participate in other pathways of glucose metabolism. Over time, the Cori lab became a magnet for scientists interested in purifying and characterizing other enzymes of glucose metabolism.

Perhaps because of their experience with discrimination and — especially for Gerty — the lack of equal opportunity, the Cori lab welcomed a more diverse group of scientists than was typical of labs of that era. The Coris received the 1947 Nobel Prize in Physiology or Medicine. Several of their junior colleagues, Arthur Kornberg (see Box 25-1), Severo Ochoa, Luis Leloir, Earl Sutherland, Christian de Duve, and Edwin G. Krebs, later earned Nobel prizes of their own, quite possibly reflecting the work ethic, broad view of science and medicine, and meticulous work habits instilled by Carl and Gerty Cori.

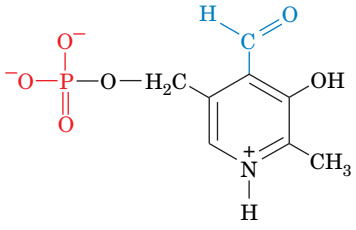
Cori, G.T., Colowick, S.P., and Cori, C.F., The activity of the phosphorylating enzyme in muscle extracts, *J. Biol. Chem.* **127,** 771–782 (1939).

Kornberg, A., Remembering our teachers, *J. Biol. Chem. Reflections,* www.jbc.org.

and dephosphorylation). Phosphorylase's allosteric inhibitors (ATP, G6P, and glucose) and its allosteric activator (AMP) interact differently with the phospho and dephosphoenzymes, resulting in an extremely sensitive regulation process. The structure of glycogen phosphorylase as well as its regulation by phosphorylation are discussed in Section 12-3B.

An ~30-Å-long crevice on the surface of the phosphorylase monomer connects the glycogen storage site to the active site (Fig. 12-15). *Since this crevice can accommodate four or five sugar residues in a chain but is too narrow to admit branched oligosaccharides, it provides a clear physical rationale for the inability of phosphorylase to cleave glycosyl residues closer than five units from a branch point.* Presumably, the glycogen storage site increases the catalytic efficiency of phosphorylase by permitting it to phosphorylyze many glucose residues on the same glycogen particle without having to dissociate and reassociate completely between catalytic cycles.

Phosphorylase binds the cofactor **pyridoxal-5′-phosphate** (**PLP;** *at left*), which it requires for activity. This prosthetic group, a **vitamin B_6** derivative, is covalently linked to the enzyme via a Schiff base (imine) formed between its aldehyde group and the ε-amino group of Lys 680. PLP also occurs in a variety of enzymes involved in amino acid metabolism, where PLP's conjugated ring system functions catalytically to delocalize electrons (Sections 21-2A and 21-4A). In phosphorylase, however, only the phosphate group participates in catalysis, where it acts as a general acid–base catalyst. Phosphorolysis of glycogen



Pyridoxal-5′-phosphate (PLP)

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proceeds by a Random mechanism (Section 12-1D) involving an enzyme · P_i · glycogen ternary complex. An oxonium ion intermediate forms during C1—O1 bond cleavage, similar to the transition state that forms in the reaction catalyzed by lysozyme (Section 11-4B). The phosphorylase reaction mechanism is diagrammed in **Fig. 16-3**, which shows how PLP's phosphate group functions as a general acid–base catalyst.

PROCESS DIAGRAM

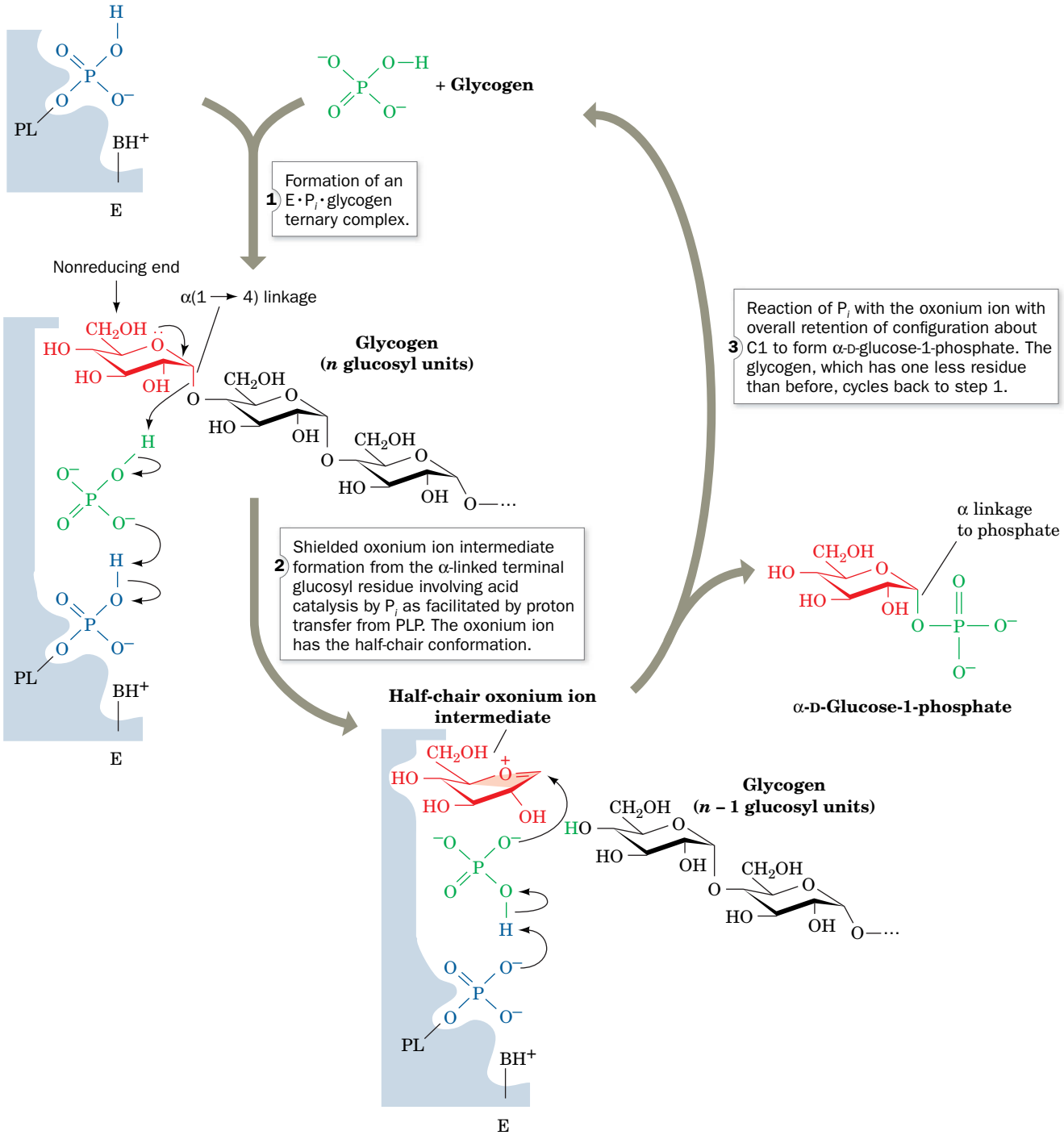


FIG. 16-3 The reaction mechanism of glycogen phosphorylase. PL is an enzyme-bound pyridoxal group; BH^+ is a positively charged amino acid side chain, probably that of Lys 568, necessary for maintaining PLP electrical neutrality. The reaction occurs via a shielded oxonium ion intermediate and involves acid catalysis by P_i as facilitated by proton transfer from PLP. **WPLS See the Animated Process Diagrams.**

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Glycogen Phosphorylase Undergoes Conformational Changes. The structural differences between the active (R) and inactive (T) conformations of phosphorylase (Fig. 12-16) are fairly well understood in terms of the symmetry model of allosterism (Section 7-1D). The T-state enzyme has a buried active site and hence a low affinity for its substrates, whereas the R-state enzyme has an accessible catalytic site and a high-affinity phosphate-binding site.

AMP promotes phosphorylase's T (*inactive*) → R (*active*) conformational shift by binding to the R state of the enzyme at its allosteric effector site. This conformational change results in increased access of the substrate to the active site by disordering a loop of residues (282–286) that otherwise block the active site. The conformational change also causes the Arg 569 side chain, which is located in the active site near the PLP and the P_i-binding site, to rotate in a way that increases the enzyme's binding affinity for its anionic P_i substrate (Fig. 12-16).

ATP also binds to the allosteric effector site, but in the T state, so that it inhibits rather than promotes the T → R conformational shift. This is because the β- and γ-phosphate groups of ATP prevent the alignment of its ribose and α-phosphate groups that are required for the conformational changes elicited by AMP.

Phosphorylation and dephosphorylation alter enzymatic activity in a manner reminiscent of allosteric regulation. The phosphate group has a double negative charge (a property not shared by naturally occurring amino acid residues) and its covalent attachment to Ser 14 causes dramatic tertiary and quaternary changes as the N-terminal segment moves to allow the phospho-Ser to ion pair with two cationic Arg residues. *The presence of the Ser 14–phosphoryl group causes conformational changes similar to those triggered by AMP binding, thereby shifting the enzyme's T ⇌ R equilibrium in favor of the R state.* This accounts for the observation that phosphorylase *b* (the unphosphorylated enzyme) requires AMP for activity and that the *a* form (the phosphorylated enzyme) is active without AMP. We return to the regulation of phosphorylase activity when we discuss the mechanisms that balance glycogen synthesis against glycogen degradation (Section 16-3).

B Glycogen Debranching Enzyme Acts as a Glucosyltransferase

Phosphorolysis proceeds along a glycogen branch until it approaches to within four or five residues of an α(1 → 6) branch point, leaving a "limit branch." Glycogen debranching enzyme acts as an **α(1 → 4) transglycosylase** (glycosyltransferase) by transferring an α(1 → 4)-linked trisaccharide unit from a limit branch of glycogen to the nonreducing end of another branch (**Fig. 16-4**). This reaction forms a new α(1 → 4) linkage with three more units available for phosphorylase-catalyzed phosphorolysis. The α(1 → 6) bond linking the remaining glycosyl residue in the branch to the main chain is hydrolyzed (not phosphorolyzed) by the same debranching enzyme to yield glucose and debranched glycogen. About 10% of the residues in glycogen (those at the branch points) are therefore converted to glucose rather than G1P. *Debranching enzyme has separate active sites for the transferase and the α(1 → 6)-glucosidase reactions.* The presence of two independent catalytic activities on the same enzyme no doubt improves the efficiency of the debranching process.

The maximal rate of the glycogen phosphorylase reaction is much greater than that of the glycogen debranching reaction. Consequently, the outermost branches of glycogen, which constitute nearly half of its residues, are degraded in muscle in a few seconds under conditions of high metabolic demand. Glycogen degradation beyond this point requires debranching and hence occurs more slowly. This, in part, accounts for the fact that a muscle can sustain its maximum exertion for only a few seconds.

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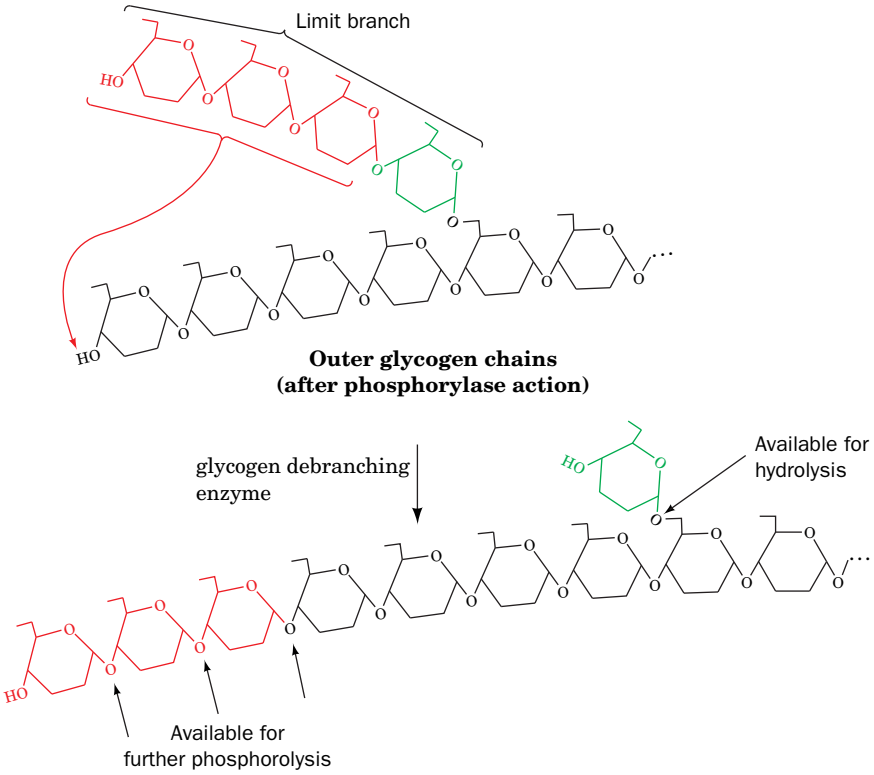


FIG. 16-4 The reactions catalyzed by debranching enzyme. The enzyme transfers the terminal three α(1 → 4)-linked glucose residues from a "limit branch" of glycogen to the nonreducing end of another branch. The α(1 → 6) bond of the residue remaining at the branch point is hydrolyzed by further action of debranching enzyme to yield free glucose. The newly elongated branch is subject to degradation by glycogen phosphorylase.

? **List the number and type of the monosaccharides produced by the complete degradation of the molecule shown here.**

C Phosphoglucomutase Interconverts Glucose-1-Phosphate and Glucose-6-Phosphate

Phosphorylase converts the glucosyl units of glycogen to G1P, which, in turn, is converted by phosphoglucomutase to G6P. The phosphoglucomutase reaction is similar to that catalyzed by phosphoglycerate mutase (Section 15-2H). A phosphoryl group is transferred from the active phosphoenzyme to G1P, forming **glucose-1,6-bisphosphate (G1,6P),** which then rephosphorylates the enzyme to yield G6P (Fig. 16-5; this near-equilibrium reaction also functions in reverse). An important difference between this enzyme and phosphoglycerate mutase is

PROCESS DIAGRAM

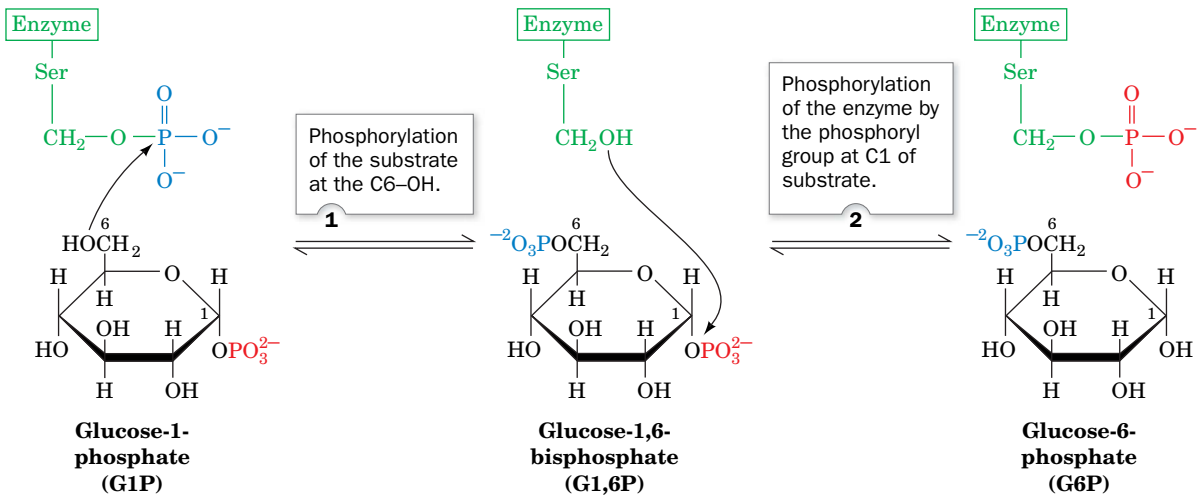


FIG. 16-5 The mechanism of phosphoglucomutase. WPLS See the Animated Process Diagrams.

? **Explain why this reaction is freely reversible.**

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Box 16-2 Biochemistry in Health and Disease Glycogen Storage Diseases

Glycogen storage diseases are inherited disorders that affect glycogen metabolism, producing glycogen that is abnormal in either quantity or quality. Studies of the genetic defects that underlie these diseases have helped elucidate the complexities of glycogen metabolism (e.g., McArdle's disease). Conversely, the biochemical characterization of the pathways affected by a genetic disease often leads to useful strategies for its treatment. The table on p. 531 lists the enzyme deficiencies associated with each type of glycogen storage disease.

Glycogen storage diseases that mainly affect the liver generally produce **hepatomegaly** (enlarged liver) and **hypoglycemia** (low blood sugar), whereas glycogen storage diseases that affect the muscles cause muscle cramps and weakness. Both types of disease may also cause cardiovascular and renal disturbances.

Type I: Glucose-6-Phosphatase Deficiency (von Gierke's Disease). Glucose-6-phosphatase catalyzes the final step leading to the release of glucose into the bloodstream by the liver. Deficiency of the enzyme results in an increase of intracellular [G6P], which leads to a large accumulation of glycogen in the liver and kidney (because G6P activates glycogen synthase) and to an inability to increase blood glucose concentration in response to the hormones glucagon or epinephrine. The symptoms of Type I glycogen storage disease include severe hepatomegaly and hypoglycemia and a general failure to thrive. Treatment of the disease has included drug-induced inhibition of glucose uptake by the liver (to increase blood [glucose]), continuous intragastric feeding overnight (again to increase blood [glucose]), surgical transposition of the portal vein, which ordinarily feeds the liver directly from the intestines (to allow this glucose-rich blood to reach peripheral tissues before it reaches the liver), and liver transplantation.

Type II: α-1,4-Glucosidase Deficiency (Pompe's Disease). α-1,4-Glucosidase deficiency is the most devastating of the glycogen storage diseases. It results in a large accumulation of glycogen of normal structure in the lysosomes of all cells and causes death by cardiorespiratory failure, usually before the age of 1 year. α-1,4-Glucosidase is not involved in the main pathways of glycogen metabolism. It occurs in lysosomes, where it hydrolyzes maltose (a glucose disaccharide) and other linear oligosaccharides, as well as the outer branches of glycogen, thereby yielding free glucose. Normally, this alternative pathway of glycogen metabolism is not quantitatively important, and its physiological significance is unknown.

Type III: Amylo-1,6-Glucosidase (Debranching Enzyme) Deficiency (Cori's Disease). In Cori's disease, glycogen of abnormal structure containing very short outer chains accumulates in both liver and muscle since, in the absence of debranching enzyme, the glycogen cannot be further degraded. The resulting hypoglycemia is not as severe as in von Gierke's disease (Type I) and can be treated with frequent feedings and a high-protein diet (to offset the loss of amino acids used for gluconeogenesis). For unknown reasons, the symptoms of Cori's disease often disappear at puberty.

Type IV: Amylo-(1,4 → 1,6)-Transglycosylase (Branching Enzyme) Deficiency (Andersen's Disease). Andersen's disease is one of the most severe glycogen storage diseases; victims rarely survive past the age of 4 years. Liver glycogen is present in normal concentrations, but it contains long unbranched chains that greatly reduce its solubility. The abnormal glycogen leads to tissue swelling and scarring (cirrhosis) and eventually liver failure.

Type V: Muscle Phosphorylase Deficiency (McArdle's Disease). The symptoms of McArdle's disease, painful muscle cramps on exertion, typically do not appear until early adulthood and can be prevented by avoiding strenuous exercise. This condition affects glycogen metabolism in muscle but not in liver, which contains normal amounts of a different phosphorylase isozyme.

Type VI: Liver Phosphorylase Deficiency (Hers' Disease). Patients with a deficiency of liver glycogen phosphorylase have symptoms similar to those with mild forms of Type I glycogen storage disease. The hypoglycemia in this case results from the inability of liver glycogen phosphorylase to respond to the need for circulating glucose.

Type VII: Muscle Phosphofructokinase Deficiency (Tarui's Disease). The result of a deficiency of the glycolytic enzyme PFK in muscle is an abnormal buildup of the glycolytic metabolites G6P and F6P. High concentrations of G6P increase the activities of glycogen synthase and UDP–glucose pyrophosphorylase (G6P is in equilibrium with G1P, which is a substrate for UDP–glucose pyrophosphorylase, an enzyme required for glycogen synthesis) so that glycogen accumulates in

that the phosphoryl group in phosphoglucomutase is covalently bound to a Ser hydroxyl group rather than to a His imidazole nitrogen.

Glucose-6-Phosphatase Generates Glucose in the Liver. The G6P produced by glycogen breakdown can continue along the glycolytic pathway or the pentose phosphate pathway (note that the glucose is already phosphorylated, so that the ATP-consuming hexokinase-catalyzed phosphorylation of glucose is bypassed). In the liver, G6P is also made available for use by other tissues. Because G6P cannot exit the cell, it is first hydrolyzed by **glucose-6-phosphatase (G6Pase):**

$$\text{G6P} + \text{H}_2\text{O} \rightarrow \text{glucose} + \text{P}_i$$

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muscle. Other symptoms are similar to those of muscle phosphorylase deficiency since PFK deficiency prevents glycolysis from keeping up with the ATP demand in contracting muscle.

Type VIII: X-Linked Phosphorylase Kinase Deficiency. Some individuals with symptoms of Type VI glycogen storage disease have normal phosphorylase enzymes but a defective phosphorylase kinase, which results in their inability to convert phosphorylase *b* to phosphorylase *a*. The α subunit of phosphorylase kinase is encoded by a gene on the X chromosome, so Type VIII disease is X-linked rather than autosomal recessive, as are the other glycogen storage diseases.

Type IX: Phosphorylase Kinase Deficiency. Phosphorylase kinase deficiency, an autosomal recessive disease, results from a mutation in one of the genes that encode the β, γ, and δ subunits of phosphorylase kinase. Because different tissues contain different phosphorylase kinase isozymes, the symptoms and severity of the disease vary according to the affected organs. Techniques for identifying genetic lesions are therefore more reliable than clinical symptoms for diagnosing a particular glycogen storage disease.

Type 0: Liver Glycogen Synthase Deficiency. Liver glycogen synthase deficiency is the only disease of glycogen metabolism in which there is a deficiency rather than an overabundance of glycogen. The activity of liver glycogen synthase is extremely low in individuals with Type 0 disease, who exhibit hyperglycemia after meals and hypoglycemia at other times. Some individuals, however, are asymptomatic, which suggests that there may be multiple forms of this autosomal recessive disorder.

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

Although G6P is produced in the cytosol, G6Pase resides in the endoplasmic reticulum (ER) membrane. Consequently G6P must be imported into the ER by a **G6P translocase** before it can be hydrolyzed. The resulting glucose and P_i are then returned to the cytosol via specific transport proteins. A defect in any of the components of this G6P hydrolysis system results in **type I glycogen storage disease** (Box 16-2). Glucose leaves the liver cell via a specific glucose transporter named **GLUT2** and is carried by the blood to other tissues. Muscle and other tissues lack G6Pase and therefore retain their G6P.

CHECKPOINT

- List the metabolic sources and fates of G6P.
- Describe the structure of a glycogen molecule. What is the advantage of its branched structure?
- List the three enzymes involved in glycogen degradation and describe the type of reactions they catalyze.
- List the activators and inhibitors of glycogen phosphorylase. How does phosphorylation affect its activity?

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2 Glycogen Synthesis

KEY CONCEPTS

- Liver glycogen synthesis involves a series of conversions from glucose to glucose-6-phosphate, to UDP–glucose, and finally to glycogen.
- UDP–glucose is an activated molecule.
- Glycogen is extended from a primer built on and by the protein glycogenin.

The $\Delta G^{\circ\prime}$ for the glycogen phosphorylase reaction is $+3.1$ kJ · mol^{-1}, but under physiological conditions, glycogen breakdown is exergonic ($\Delta G^{\circ\prime} = -5$ to -8 kJ · mol^{-1}). The synthesis of glycogen from G1P under physiological conditions is therefore thermodynamically unfavorable without free energy input. Consequently *glycogen synthesis and breakdown must occur by separate pathways.* This recurrent metabolic strategy—that biosynthetic and degradative pathways of metabolism are different—is particularly important when both pathways must operate under similar physiological conditions. This situation is thermodynamically impossible if one pathway is just the reverse of the other.

It was not thermodynamics, however, that led to recognition of the separation of synthetic and degradative pathways for glycogen, but McArdle's disease. Individuals with the disease lack muscle glycogen phosphorylase activity and therefore cannot break down glycogen. Yet their muscles contain moderately high quantities of normal glycogen. Clearly, glycogen synthesis does not require glycogen phosphorylase. In this section, we describe the three enzymes that participate in glycogen synthesis: **UDP–glucose pyrophosphorylase, glycogen synthase,** and **glycogen branching enzyme.** The opposing reactions of glycogen synthesis and degradation are diagrammed in Fig. 16-6.

A UDP–Glucose Pyrophosphorylase Activates Glucosyl Units

Since the direct conversion of G1P to glycogen and P_i is thermodynamically unfavorable (positive ΔG) under physiological conditions, glycogen biosynthesis requires an exergonic step. This is accomplished, as Luis Leloir discovered in 1957, by combining G1P with uridine triphosphate (UTP) in a reaction catalyzed by UDP–glucose pyrophosphorylase (Fig. 16-7). The product of this reaction, **uridine diphosphate glucose (UDP–glucose** or **UDPG),** is an "activated" compound that can donate a glucosyl unit to the growing glycogen chain. The formation of UDPG itself has $\Delta G^{\circ\prime} \approx 0$ (it is a phosphoanhydride exchange reaction),

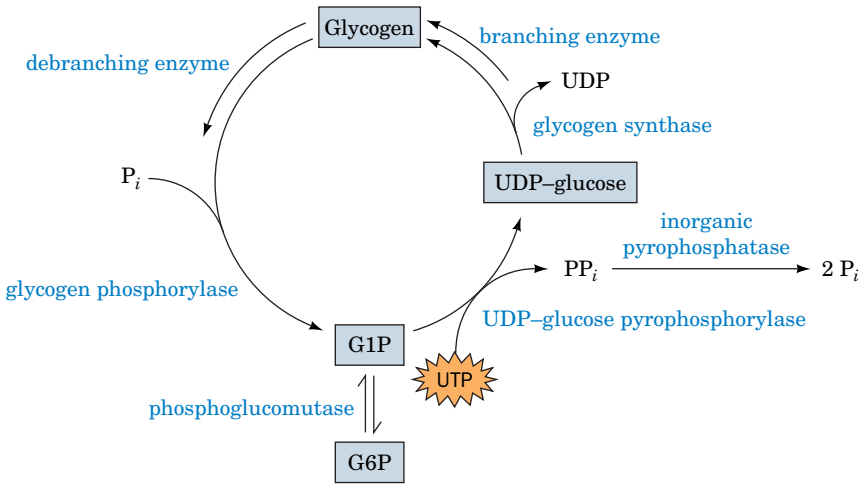


FIG. 16-6 Opposing pathways of glycogen synthesis and degradation. The exergonic process of glycogen breakdown is reversed by a process that uses UTP to generate a UDP–glucose intermediate.

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PROCESS DIAGRAM

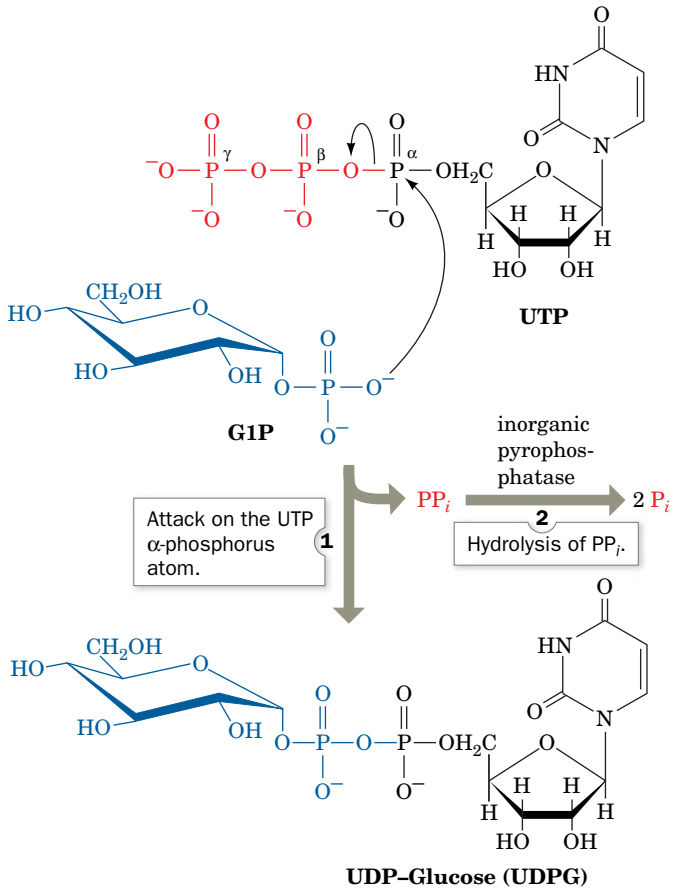


FIG. 16-7 The reaction catalyzed by UDP–glucose pyrophosphorylase. This enzyme, which is named for its reverse reaction, catalyzes a phosphoanhydride exchange reaction. The reaction is driven to completion by PP_i hydrolysis. **WPLS See the Animated Process Diagrams.**

? Identify the "high-energy" bonds that are broken and formed.

but the subsequent exergonic hydrolysis of PP_i by the omnipresent enzyme inorganic pyrophosphatase makes the overall reaction exergonic.

	$\Delta G^{\circ\prime}$ (kJ · mol^{-1})
G1P + UTP $\rightleftharpoons$ UDPG + PP_i	~0
H_2O + PP_i $\longrightarrow$ 2P_i	−19.2
Overall G1P + UTP $\longrightarrow$ UDPG + 2P_i	−19.2

This is an example of the common biosynthetic strategy of cleaving a nucleoside triphosphate to form PP_i. The free energy of PP_i hydrolysis can then be used to drive an otherwise unfavorable reaction to completion (Section 14-2B); the near total elimination of the PP_i by the highly exergonic (irreversible) pyrophosphatase reaction prevents the reverse of the PP_i-producing reaction from occurring.

B Glycogen Synthase Extends Glycogen Chains

In the next step of glycogen synthesis, the glycogen synthase reaction, the glucosyl unit of UDPG is transferred to the C4-OH group on one of glycogen's

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nonreducing ends to form an $\alpha(1 \rightarrow 4)$ glycosidic bond. The $\Delta G^{\circ\prime}$ for the glycogen synthase reaction

$$\underset{}{\text{UDPG}} + \underset{(n \text{ residues})}{\text{glycogen}} \rightarrow \underset{}{\text{UDP}} + \underset{(n+1 \text{ residues})}{\text{glycogen}}$$

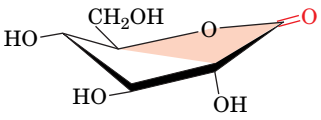
is $-13.4\ \text{kJ} \cdot \text{mol}^{-1}$, making the overall reaction spontaneous under the same conditions that glycogen breakdown by glycogen phosphorylase is also spontaneous. However, glycogen synthesis does have an energetic price. Combining the first two reactions of glycogen synthesis gives

$$\underset{(n \text{ residues})}{\text{Glycogen}} + \text{G1P} + \text{UTP} \rightarrow \underset{(n+1 \text{ residues})}{\text{glycogen}} + \text{UDP} + 2\text{P}_i$$

Thus, *one molecule of UTP is cleaved to UDP for each glucose residue incorporated into glycogen.* The UTP is replenished through a phosphoryl-transfer reaction mediated by nucleoside diphosphate kinase (Section 14-2C):

$$\text{UDP} + \text{ATP} \rightleftharpoons \text{UTP} + \text{ADP}$$

so that UTP consumption is energetically equivalent to ATP consumption.



1,5-Gluconolactone

The transfer of a glucosyl unit from UDPG to a growing glycogen chain involves the formation of a glycosyl oxonium ion by the elimination of UDP, a good leaving group (Fig. 16-8). The enzyme is inhibited by **1,5-gluconolactone** (*at left*), an analog that mimics the oxonium ion's half-chair geometry. The same analog inhibits both glycogen phosphorylase (Section 16-1A) and lysozyme (Section 11-4), which have similar mechanisms.

Human muscle glycogen synthase is a homotetramer of 737-residue subunits (the liver isozyme has 703-residue subunits). Like glycogen phosphorylase, it has two enzymatically interconvertible forms; in this case, however, the phosphorylated *b* form is less active, and the original (dephosphorylated) *a* form is more active. (Note: For enzymes subject to covalent modification, "*a*" refers to the more active form and "*b*" refers to the less active form.)

Glycogen synthase is under allosteric control; it is strongly inhibited by physiological concentrations of ATP, ADP, and P_i. In fact, the phosphorylated

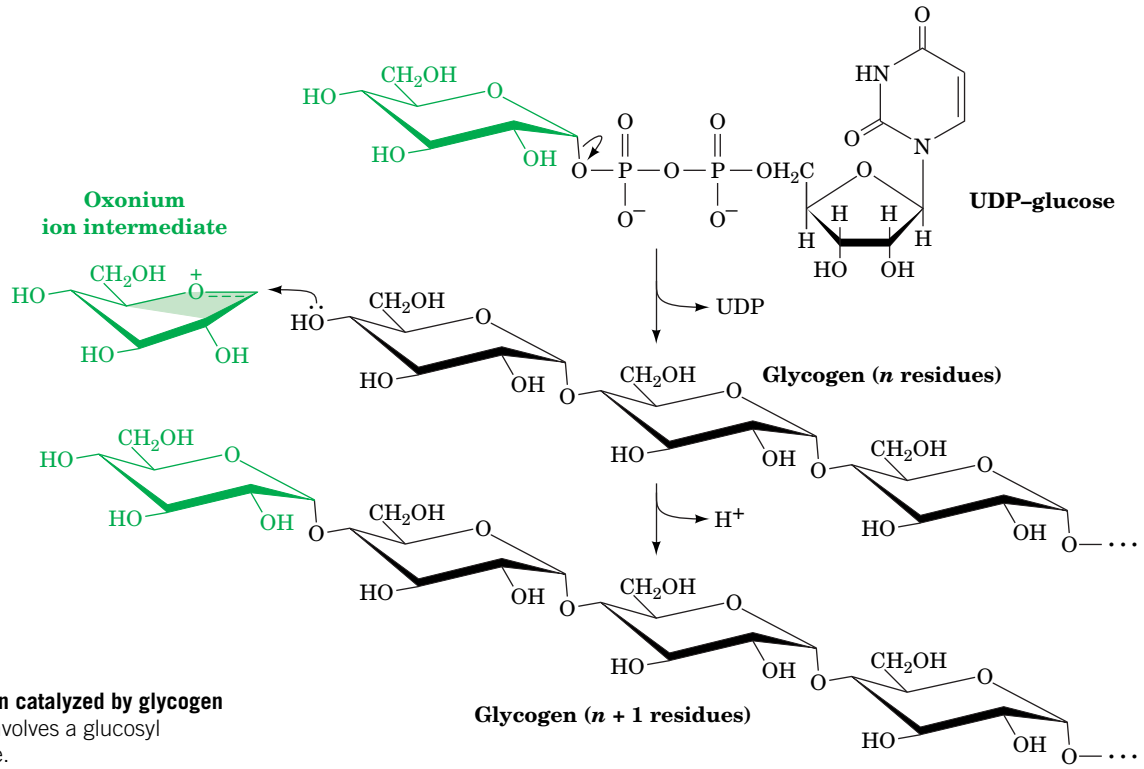


FIG. 16-8 The reaction catalyzed by glycogen synthase. This reaction involves a glucosyl oxonium ion intermediate.

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enzyme is almost totally inactive *in vivo*. The dephosphorylated enzyme, however, can be activated by G6P, so the cell's glycogen synthase activity varies with [G6P] and the fraction of the enzyme in its dephosphorylated form. The mechanistic details of the interconversion of phosphorylated and dephosphorylated forms of glycogen synthase are complex and are not as well understood as those of glycogen phosphorylase (for one thing, glycogen synthase has multiple phosphorylation sites). We discuss the regulation of glycogen synthase further in Section 16-3B.

Glycogenin Primes Glycogen Synthesis. Glycogen synthase cannot simply link together two glucose residues; it can only extend an already existing α(1 → 4)-linked glucan chain. How, then, is glycogen synthesis initiated? In the first step of this process, a 349-residue protein named **glycogenin,** acting as a glycosyltransferase, attaches a glucose residue donated by UDPG to the OH group of its Tyr 194. Glycogenin then extends the glucose chain by up to seven additional UDPG-donated glucose residues to form a glycogen "primer." Only at this point does glycogen synthase commence glycogen synthesis by extending the primer. Analysis of glycogen granules suggests that each glycogen molecule is associated with only one molecule each of glycogenin and glycogen synthase.

C Glycogen Branching Enzyme Transfers Seven-Residue Glycogen Segments

Glycogen synthase generates only α(1 → 4) linkages to yield α-amylose. Branching to form glycogen is accomplished by a separate enzyme, **amylo-(1,4 → 1,6)-transglycosylase (branching enzyme)**, which is distinct from glycogen debranching enzyme (Section 16-1B). A branch is created by transferring a 7-residue segment from the end of a chain to the C6-OH group of a glucose residue on the same or another glycogen chain (Fig. 16-9). Each transferred

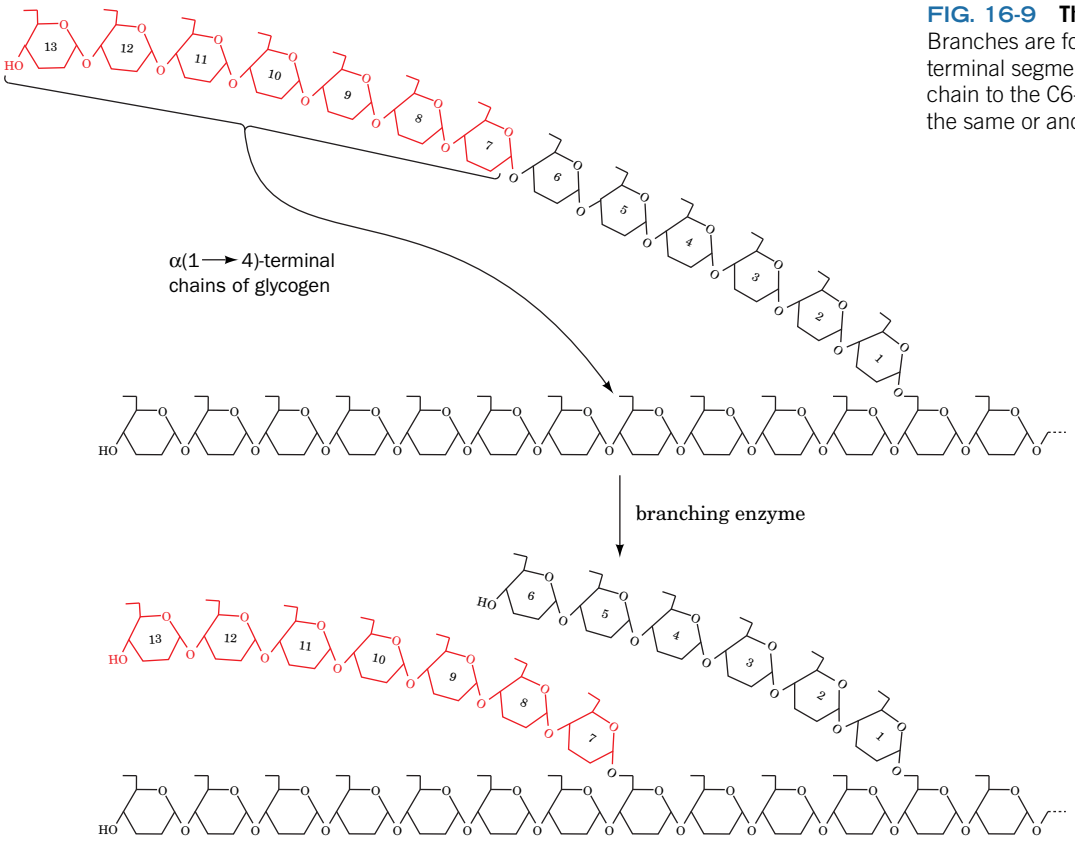


FIG. 16-9 The branching of glycogen. Branches are formed by transferring a 7-residue terminal segment from an α(1 → 4)-linked glucan chain to the C6-OH group of a glucose residue on the same or another chain.

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CHECKPOINT

- Why must opposing biosynthetic and degradative pathways differ in at least one enzyme?
- List the three enzymes involved in glycogen synthesis and describe the types of reactions they catalyze.
- What is the free energy source for glycogen synthesis?
- Describe the role of glycogenin.

segment must come from a chain of at least 11 residues, and the new branch point must be at least 4 residues away from other branch points. The branching pattern of glycogen has been optimized by evolution for the efficient storage and mobilization of glucose (Box 16-3).

3 Control of Glycogen Metabolism

KEY CONCEPTS

- The opposing processes of glycogen breakdown and synthesis are reciprocally regulated by allosteric interactions and the covalent modification of key enzymes.
- Glycogen metabolism is ultimately under the control of hormones such as insulin, glucagon, and epinephrine.

If glycogen synthesis and breakdown proceed simultaneously, all that is accomplished is the wasteful hydrolysis of UTP. Glycogen metabolism must therefore be controlled according to cellular needs. *The regulation of glycogen metabolism involves allosteric control as well as hormonal control by covalent modification of the pathway's regulatory enzymes.*

A Glycogen Phosphorylase and Glycogen Synthase Are under Allosteric Control

As we saw in Sections 14-1E and 15-4B, the net flux, J, of reactants through a step in a metabolic pathway is the difference between the forward and reverse reaction velocities, v_f and v_r. However, the flux varies dramatically with substrate concentration as the reaction approaches equilibrium ($v_f \approx v_r$). The flux through a near-equilibrium reaction is therefore all but uncontrollable. *Precise flux control of a pathway is possible when an enzyme functioning far from equilibrium is opposed by a separately controlled enzyme. Then, v_f and v_r vary independently and v_r can be larger or smaller than v_f, allowing control of both rate and direction.* Exactly this situation occurs in glycogen metabolism through the opposition of the glycogen phosphorylase and glycogen synthase reactions.

Both glycogen phosphorylase and glycogen synthase are under allosteric control by effectors that include ATP, G6P, and AMP. Muscle glycogen phosphorylase is activated by AMP and inhibited by ATP and G6P. Glycogen synthase, on the other hand, is activated by G6P. This suggests that when there is high demand for ATP (low [ATP], low [G6P], and high [AMP]), glycogen phosphorylase is stimulated and glycogen synthase is inhibited, which favors glycogen breakdown. Conversely, when [ATP] and [G6P] are high, glycogen synthesis is favored.

In vivo, this allosteric scheme is superimposed on an additional control system based on covalent modification. For example, phosphorylase *a* is active even without AMP stimulation (Section 16-1A), and glycogen synthase is essentially inactive (Section 16-2B) unless it is dephosphorylated and G6P is present. *Thus, covalent modification (phosphorylation and dephosphorylation) of glycogen phosphorylase and glycogen synthase provides a more sophisticated control system that modulates the responsiveness of the enzymes to their allosteric effectors.*

B Glycogen Phosphorylase and Glycogen Synthase Undergo Control by Covalent Modification

The interconversion of the *a* and *b* forms of glycogen synthase and glycogen phosphorylase is accomplished through enzyme-catalyzed phosphorylation and dephosphorylation (Section 12-3B), a process that is under hormonal control (Section 13-2). **Enzymatically interconvertible enzyme systems** can therefore respond to a greater number of effectors than simple allosteric systems.

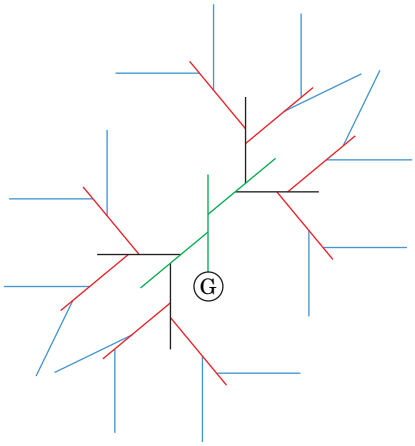
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Box 16-3 Perspectives in Biochemistry Optimizing Glycogen Structure

The function of glycogen in animal cells is to store the metabolic fuel glucose and to release it rapidly when needed. Glucose must be stored as a polymer, because glucose itself could not be stored without a drastic increase in intracellular osmotic pressure (Section 2-1D). It has been estimated that the total concentration of glucose residues stored as glycogen in a liver cell is ~0.4 M, whereas the concentration of glycogen is only ~10 nM. This huge difference mitigates osmotic stress.

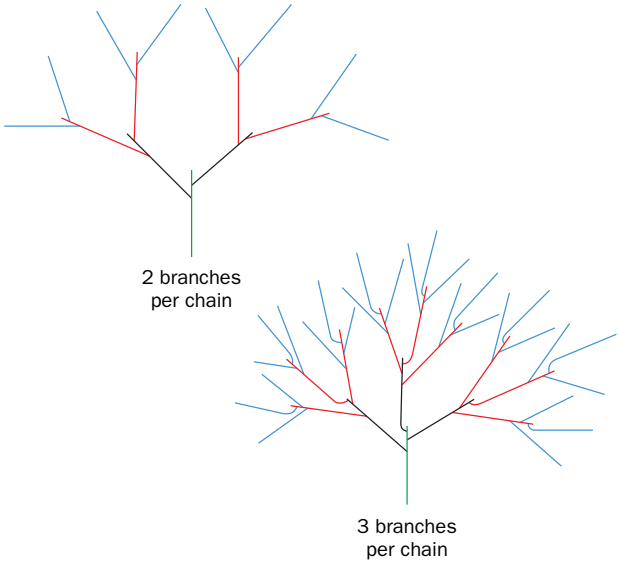
To fulfill its biological function, the glycogen polymer must store the largest amount of glucose in the smallest possible volume while maximizing both the amount of glucose available for release by glycogen phosphorylase and the number of nonreducing ends (to maximize the rate at which glucose residues can be mobilized). These criteria must be met by optimizing just two variables: the degree of branching and chain length.

In a glycogen molecule, shown schematically here,

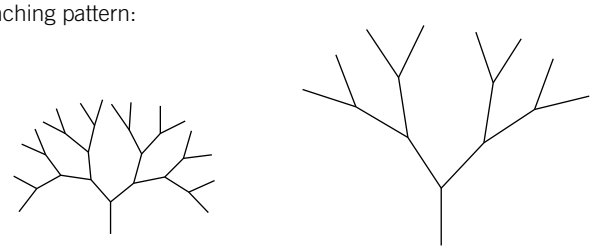


the glycogen chains, beginning with the innermost chain attached to glycogenin (G), each have two branches (the outermost chains are unbranched). The entire molecule is roughly spherical and is organized in tiers. There are an estimated 12 tiers in mature glycogen (only 4 are shown above).

With two branches per chain, the number of chains in a given tier is twice the number of the preceding tier, and the outermost tier contains about half of the total glucose residues (regardless of the number of tiers). When the degree of branching increases, for example, to three branches per chain, the proportion of residues in the outermost tier increases, but so does the density of glucose residues. This severely limits the maximum size of the glycogen particle and the number of glucose residues it can accommodate. Thus, glycogen has around two branches per chain.



Mathematical analysis of the other variable, chain length, yields an optimal value of 13, which is in good agreement with the actual length of glycogen chains in cells (8–14 residues). Consider the two simplified glycogen molecules shown below, which contain the same number of glucose residues (the same total length of line segments) and the same branching pattern:



The molecule with the shorter chains packs more glucose in a given volume and has more points for phosphorylase attack, but only about half the amount of glucose can be released before debranching must occur (debranching is much slower than phosphorolysis). In the less dense molecule, the longer chains increase the number of residues that can be continuously phosphorylyzed; however, there are fewer points of attack. Thirteen residues is apparently a compromise for mobilizing the largest amount of glucose in the shortest time.

Amylopectin (Section 8-2C), which is chemically similar to glycogen, is a much larger molecule and has longer chains. Amylose lacks branches altogether. Evidently, starch, unlike glycogen, is not designed for rapid mobilization of metabolic fuel.

[Figures adapted from Meléndez-Hevia, E., Waddell, T.G., and Shelton, E.D., *Biochem. J.* **295,** 477–483 (1993).]

Furthermore, a set of kinases and phosphatases linked in cascade fashion has enormous potential for signal amplification and flexibility in response to different metabolic signals. Note that the correlation between phosphorylation and enzyme activity varies with the enzyme. For example, glycogen phosphorylase is activated by phosphorylation ($b \rightarrow a$), whereas glycogen synthase is inactivated by phosphorylation ($a \rightarrow b$). Conversely, dephosphorylation inactivates glycogen phosphorylase and activates glycogen synthase.

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Glycogen Phosphorylase Is Activated by Phosphorylation. The cascade that governs the enzymatic interconversion of glycogen phosphorylase involves three enzymes (Fig. 16-10):

1. ***Phosphorylase kinase,*** which specifically phosphorylates Ser 14 of glycogen phosphorylase *b.*
2. ***Protein kinase A*** (***PKA;*** Section 13-3C), which phosphorylates and thereby activates phosphorylase kinase.
3. ***Phosphoprotein phosphatase-1*** (***PP1;*** Section 13-2D), which dephosphorylates and thereby deactivates both glycogen phosphorylase *a* and phosphorylase kinase.

Phosphorylase *b* is sensitive to allosteric effectors, but phosphorylase *a* is much less so, as discussed in Section 12-3B (Fig. 12-17). In the resting cell, the concentrations of ATP and G6P are high enough to inhibit phosphorylase *b.* The level of phosphorylase activity is therefore largely determined by the fraction of enzyme present as phosphorylase *a.* The steady state fraction of phosphorylated enzyme depends on the relative activities of phosphorylase kinase, PKA, and PP1. Recall that PKA is activated by **cAMP**, a second messenger that is made by **adenylate cyclase** on hormone-stimulated activation of a heterotrimeric **G protein** (Section 13-3C). Let us examine the factors that regulate the activities of phosphorylase kinase and PP1 before we return to the regulation of glycogen synthase activity.

Phosphorylase Kinase Is Activated by Phosphorylation and by Ca^{2+}. Phosphorylase kinase is a 1300-kD protein with four nonidentical subunits, known as α, β, γ, and δ. The γ subunit contains the catalytic site, and the other three subunits have regulatory functions. *Phosphorylase kinase is maximally activated by Ca^{2+} and by the phosphorylation of its α and β subunits by PKA.*

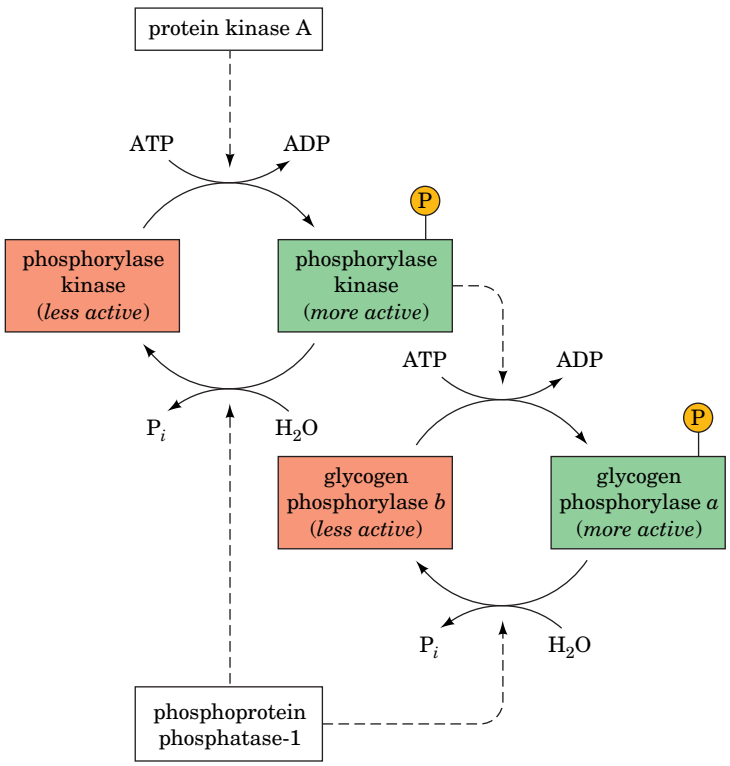


FIG. 16-10 The glycogen phosphorylase interconvertible enzyme system. Conversion of phosphorylase *b* (the less active form) to phosphorylase *a* (the more active form) is accomplished through phosphorylation catalyzed by phosphorylase kinase, which is itself subject to activation through phosphorylation by protein kinase A (PKA). Both glycogen phosphorylase *a* and phosphorylase kinase are dephosphorylated by phosphoprotein phosphatase-1.

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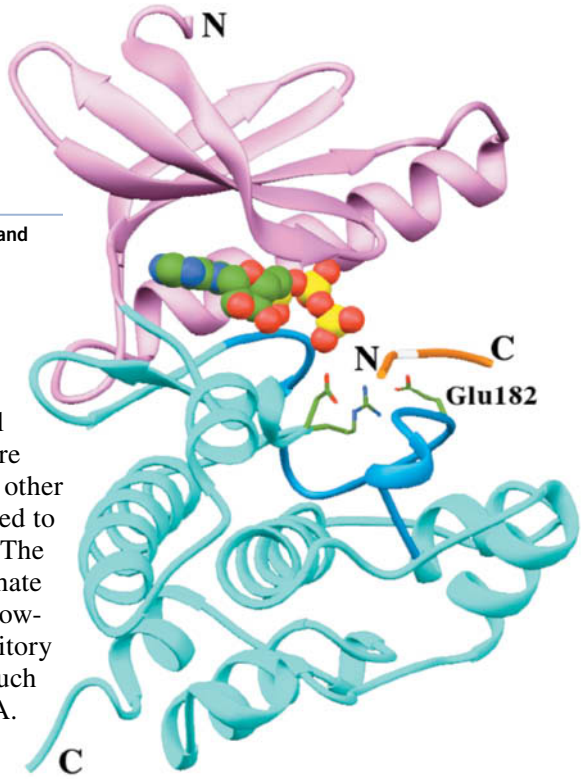


FIG. 16-11 X-Ray structure of the γ subunit of rabbit muscle phosphorylase kinase in complex with ATP and a substrate analog. The N-terminal domain is pink, the C-terminal domain is cyan, the activation loop is light blue, and the heptapeptide substrate analog is orange, with its residue to be phosphorylated (Ser) white. The ATP is shown in space-filling form and the side chains of the catalytically essential Arg 148, Asp 149, and Glu 182 are shown in stick form, all colored according to atom type (C green, N blue, O red, and P yellow). [After an X-ray structure by Louise Johnson, Oxford University, U.K. PDBid 2PHK.]

? Compare this structure to the structures of the C subunit of protein kinase A (Fig. 13-21) and the tyrosine kinase domain of the insulin receptor (Fig. 13-5).

The 386-residue γ subunit of phosphorylase kinase is 36% identical in sequence to the PKA C subunit (Fig. 13-21) and has a similar structure (**Fig. 16-11**). The γ subunit is not subject to phosphorylation, as are many other protein kinases, because the Ser, Thr, or Tyr residue that is phosphorylated to activate those other kinases is replaced by a Glu residue in the γ subunit. The negative charge of the Glu is thought to mimic the presence of a phosphate group and interact with a conserved Arg residue near the active site. However, full catalytic activity of the γ subunit is prevented by an autoinhibitory C-terminal segment, which binds to and blocks the kinase's active site, much like the R subunit blocks the activity of the C subunit of protein kinase A. An inhibitory segment in the β subunit may also block the activity of the γ subunit.

Autoinhibition of phosphorylase kinase is relieved by PKA-catalyzed phosphorylation of both the α and β subunits. This presumably causes the β inhibitor segment to move aside (the way in which phosphorylation of the α subunit modulates the enzyme's behavior is not understood). However, full activity of the γ subunit also requires Ca^{2+} binding to the δ subunit, which is **calmodulin** (**CaM**; Section 13-4B; CaM functions both as a free-floating protein and as a subunit of other proteins). Ca^{2+} concentrations as low as 10^{-7} M activate phosphorylase kinase by inducing a conformational change in CaM that causes it to bind to and extract the γ subunit's autoinhibitor segment from its catalytic site (Fig. 13-29).

The conversion of glycogen phosphorylase *b* to glycogen phosphorylase *a* through the action of phosphorylase kinase increases the rate of glycogen breakdown. The physiological significance of the Ca^{2+} trigger for this activation is that muscle contraction is also triggered by a transient increase in the level of cytosolic Ca^{2+} (Section 7-2B). The rate of glycogen breakdown in muscle is thereby linked to the rate of contraction. This is critical because glycogen breakdown provides fuel for glycolysis to generate the ATP required for muscle contraction. Since Ca^{2+} release occurs in response to nerve impulses, whereas the phosphorylation of phosphorylase kinase ultimately occurs in response to the presence of certain hormones, these two signals act synergistically in muscle cells to stimulate glycogenolysis.

Phosphoprotein Phosphatase-1 Is Inhibited by Phosphoprotein Inhibitor-1. A steady state for many phosphorylated enzymes is maintained by a balance between phosphorylation, as catalyzed by a corresponding kinase, and hydrolytic dephosphorylation, as catalyzed by a phosphatase. Phosphoprotein phosphatase-1 (PP1) removes the phosphoryl groups from glycogen phosphorylase *a* and the α and β subunits of phosphorylase kinase (Fig. 16-10), as well as those of other proteins involved in glycogen metabolism (see below).

PP1 is controlled differently in muscle and in liver. In muscle, the catalytic subunit of PP1 (called **PP1c**) is active only when it is bound to glycogen through its glycogen-binding **G_M subunit.** The activity of PP1c and its affinity for the G_M subunit are regulated by phosphorylation of the G_M subunit at two separate

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