15

Principles of Metabolic Regulation

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Metabolic regulation, a central theme in biochemistry, is one of the most remarkable features of living organisms. Of the thousands of enzyme-catalyzed reactions that can take place in a cell, there is probably not one that escapes some form of regulation. This need to regulate every aspect of cellular metabolism becomes clear as one examines the complexity of metabolic reaction sequences. Although it is convenient for the student of biochemistry to divide metabolic processes into "pathways" that play discrete roles in the cell's economy, no such separation exists in the living cell. Rather, every pathway we discuss in this book is inextricably intertwined with all the other cellular pathways in a multidimensional network of reactions **(Fig. 15–1)**. For example, in Chapter 14 we discussed four possible fates for **glucose 6-phosphate** in a hepatocyte: breakdown by glycolysis for the production of ATP, breakdown in the pentose phosphate pathway for the production of NADPH and pentose phosphates, use in the synthesis of complex polysaccharides of the extracellular matrix, or hydrolysis to glucose and phosphate to replenish blood glucose. In fact, glucose 6-phosphate has other possible fates in hepatocytes, too; it may, for example, be used to synthesize other sugars, such as glucosamine, galactose, galactosamine, fucose, and neuraminic acid, for use in protein glycosylation, or it may be partially degraded to provide acetyl-CoA for fatty acid and sterol synthesis. And the bacterium *Escherichia coli* can use glucose to produce the carbon skeleton of *every one* of its several thousand types of molecules. When any cell uses glucose 6-phosphate for one purpose, that "decision" affects all the other pathways for which glucose 6-phosphate is a precursor or intermediate: any change in the allocation of glucose 6-phosphate to one pathway affects, directly or indirectly, the flow of metabolites through all the others.

Such changes in allocation are common in the life of a cell. Louis Pasteur was the first to describe the more than 10-fold increase in glucose consumption by a yeast culture when it was shifted from aerobic to anaerobic conditions. This "Pasteur effect" occurs without a significant change in the concentrations of ATP or most of the hundreds of metabolic intermediates and products derived from glucose. A similar effect occurs in the cells of skeletal muscle when a sprinter leaves the starting blocks. The ability of a cell to carry out all these interlocking metabolic processes simultaneously—obtaining every product in the amount needed and at the right time, in the face of major perturbations from outside, and without generating leftovers—is an *astounding* accomplishment.

In this chapter we use the metabolism of glucose to illustrate some general principles of metabolic regulation. First we look at the general roles of regulation in achieving metabolic homeostasis and introduce metabolic control analysis, a system for analyzing complex metabolic interactions quantitatively. We then describe the specific regulatory properties of the individual enzymes of glucose metabolism; for glycolysis and gluconeogenesis, we described the catalytic activities of the enzymes in Chapter 14. Here we also discuss both the catalytic and regulatory properties of the enzymes of glycogen synthesis and breakdown, one of the best-studied cases of metabolic regulation. Note that in selecting carbohydrate metabolism to illustrate the principles of metabolic regulation, we have artificially separated the metabolism of fats and carbohydrates. In fact, these two activities are very tightly integrated, as we shall see in Chapter 23.

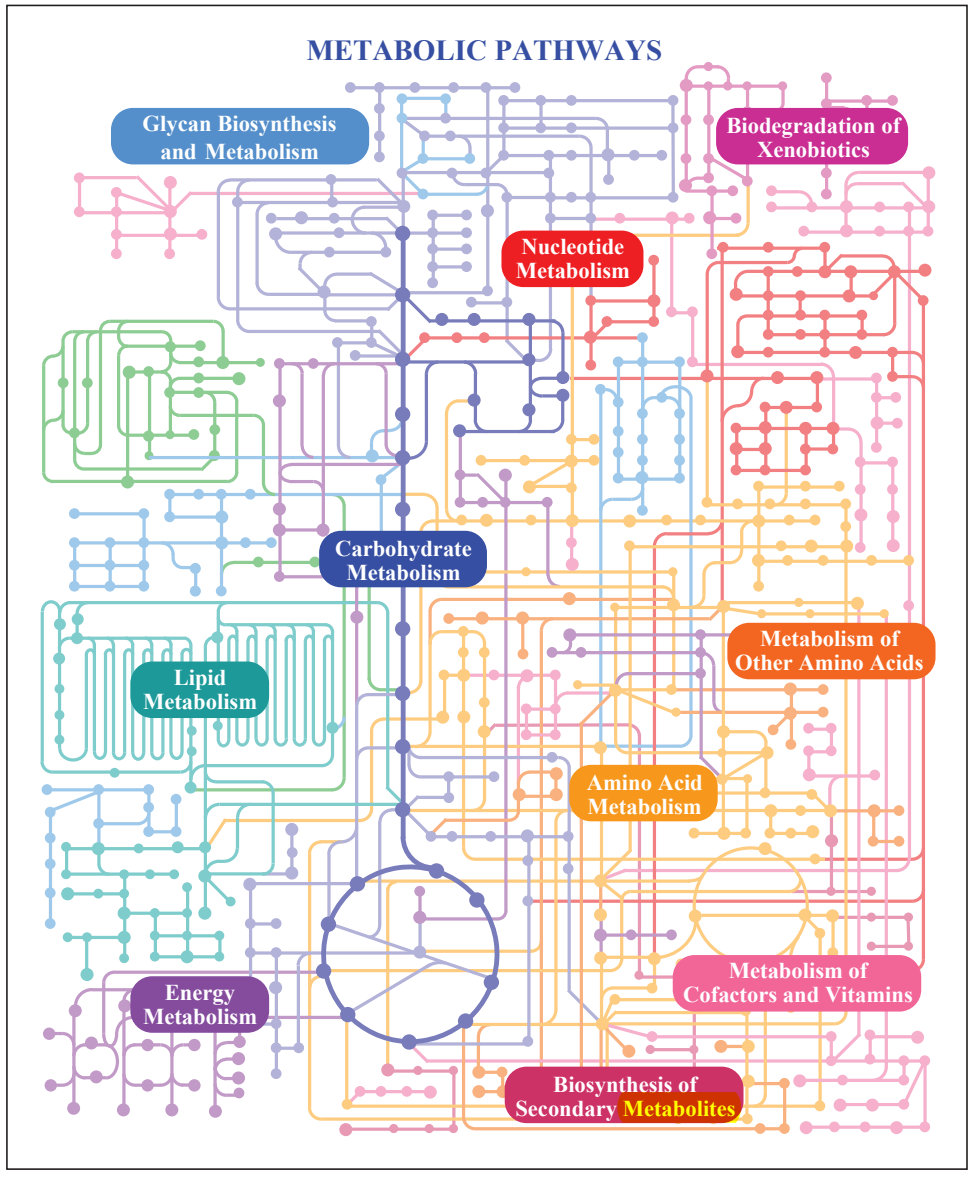


FIGURE 15–1 Metabolism as a three-dimensional meshwork. A typical eukaryotic cell has the capacity to make about 30,000 different proteins, which catalyze thousands of different reactions involving many hundreds of metabolites, most shared by more than one "pathway." In this much-simplified overview of metabolic pathways, each dot represents an intermediate compound and each connecting line represents an enzymatic reaction. For a more realistic and far more complex diagram of metabolism, see the online KEGG PATHWAY database (www.genome.ad.jp/kegg/pathway/map/map01100.html); in this interactive map, each dot can be clicked to obtain extensive data about the compound and the enzymes for which it is a substrate. The cover of this book shows the interlocking reactions that occur in the mitochondrion.

15.1 Regulation of Metabolic Pathways

The pathways of glucose metabolism provide, in the catabolic direction, the energy essential to oppose the forces of entropy and, in the anabolic direction, biosynthetic precursors and a storage form of metabolic energy. These reactions are so important to survival that very complex regulatory mechanisms have evolved to ensure that metabolites move through each pathway in the correct direction and at the correct rate to match exactly the cell's or the organism's changing circumstances. By a variety of mechanisms operating on different time scales, adjustments are made in the rate of metabolite flow through an entire pathway when external circumstances change.

Circumstances do change, sometimes dramatically. For example, the demand for ATP in insect flight muscle increases 100-fold in a few seconds when the insect takes flight. In humans, the availability of oxygen may decrease due to hypoxia (diminished delivery of oxygen to tissues) or ischemia (diminished flow of blood to tissues). The relative proportions of carbohydrate, fat, and protein in the diet vary from meal to meal, and the supply of fuels obtained in the diet is intermittent, requiring metabolic adjustments between meals and during periods of starvation. Wound healing

requires huge amounts of energy and biosynthetic precursors.

Cells and Organisms Maintain a Dynamic Steady State

Fuels such as glucose enter a cell, and waste products such as CO_2 leave, but the mass and the gross composition of a typical cell, organ, or adult animal do not change appreciably over time; cells and organisms exist in a dynamic steady state. For each metabolic reaction in a pathway, the substrate is provided by the preceding reaction at the same rate at which it is converted to product. Thus, although the rate (v) of metabolite flow, or **flux**, through this step of the pathway may be high and variable, the concentration of substrate, S, remains constant. So, for the two-step reaction

$$A \xrightarrow{v_1} S \xrightarrow{v_2} P$$

when $v_1 = v_2$, [S] is constant. For example, changes in v_1 for the entry of glucose from various sources into the blood are balanced by changes in v_2 for the uptake of glucose from the blood into various tissues, so the concentration of glucose in the blood ([S]) is held nearly constant at 5 mM. This is **homeostasis** at the molecular level. The failure of homeostatic mechanisms is often at the root of human disease. In diabetes mellitus, for example, the regulation of blood glucose concentration is defective as a result of the lack of or insensitivity to insulin, with profound medical consequences.

When the external perturbation is not merely transient, or when one kind of cell develops into another, the adjustments in cell composition and metabolism can be more dramatic and may require significant and lasting changes in the allocation of energy and synthetic precursors to bring about a new dynamic steady state. Consider, for example, the differentiation of stem cells in the bone marrow into erythrocytes. The precursor cell contains a nucleus, mitochondria, and little or no hemoglobin, whereas the fully differentiated erythrocyte contains prodigious amounts of hemoglobin but has neither nucleus nor mitochondria; the cell's composition has permanently changed in response to external developmental signals, with accompanying changes in metabolism. This **cellular differentiation** requires precise regulation of the levels of cellular proteins.

In the course of evolution, organisms have acquired a remarkable collection of regulatory mechanisms for maintaining homeostasis at the molecular, cellular, and organismal levels, as reflected in the proportion of genes that encode regulatory machinery. In humans, about 4,000 genes (~12% of all genes) encode regulatory proteins, including a variety of receptors, regulators of gene expression, and more than 500 different protein kinases! In many cases, the regulatory mechanisms overlap: one enzyme is subject to regulation by several different mechanisms.

Both the Amount and the Catalytic Activity of an Enzyme Can Be Regulated

The flux through an enzyme-catalyzed reaction can be modulated by changes in the *number* of enzyme molecules or by changes in the *catalytic activity* of each enzyme molecule already present. Such changes occur on time scales from milliseconds to many hours, in response to signals from within or outside the cell. Very rapid allosteric changes in enzyme activity are generally triggered locally, by changes in the local concentration of a small molecule—a substrate of the pathway in which that reaction is a step (say, glucose for glycolysis), a product of the pathway (ATP from glycolysis), or a key metabolite or cofactor (such as NADH) that indicates the cell's metabolic state. Second messengers (such as cyclic AMP and Ca^{2+}) generated intracellularly in response to extracellular signals (hormones, cytokines, and so forth) also mediate allosteric regulation, on a slightly slower time scale set by the rate of the signal-transduction mechanism (see Chapter 12).

Extracellular signals (**Fig. 15–2**, ❶) may be hormonal (insulin or epinephrine, for example) or neuronal (acetylcholine), or may be growth factors or cytokines. The number of molecules of a given enzyme in a cell is a function of the relative rates of synthesis and degradation of that enzyme. The rate of synthesis can be adjusted by the activation (in response to some outside signal) of a transcription factor (Fig. 15–2, ❷; described in more detail in Chapter 28). **Transcription factors** are nuclear proteins that, when activated, bind specific DNA regions (**response elements**) near a gene's promoter (its transcriptional starting point) and activate or repress the transcription of that gene, leading to increased or decreased synthesis of the encoded protein. Activation of a transcription factor is sometimes the result of its binding of a specific ligand and sometimes the result of its phosphorylation or dephosphorylation. Each gene is controlled by one or more response elements that are recognized by specific transcription factors. Genes that have several response elements are therefore controlled by several different transcription factors responding to several different signals. Groups of genes encoding proteins that act together, such as the enzymes of glycolysis or gluconeogenesis, often share common response element sequences, so that a single signal, acting through a particular transcription factor, turns all of these genes on and off together. The regulation of carbohydrate metabolism by specific transcription factors is described in Section 15.3.

The stability of messenger RNAs—their resistance to degradation by cellular ribonucleases (Fig. 15–2, ❸)—varies, and the amount of a given mRNA in the cell is a function of its rates of synthesis and degradation (Chapter 26). The rate at which an mRNA is translated into a protein by ribosomes (Fig. 15–2, ❹) is also regulated, and depends on several factors described in detail in Chapter 27. Note that an n-fold increase in an mRNA

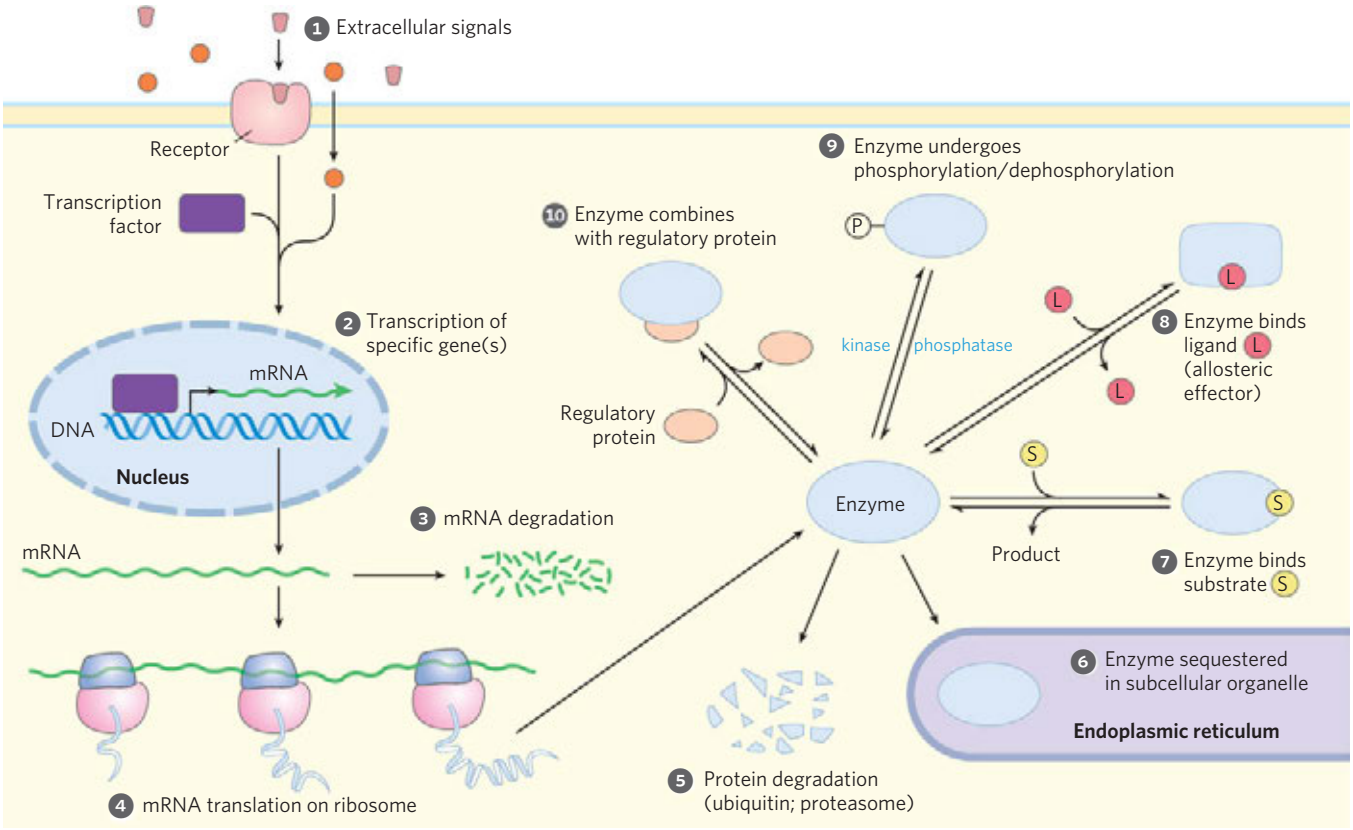


FIGURE 15–2 Factors affecting the activity of enzymes. The total activity of an enzyme can be changed by altering the *number* of its molecules in the cell, or its *effective* activity in a subcellular compartment (❶ through ❻), or by modulating the *activity* of existing molecules (❼ through ❿), as detailed in the text. An enzyme may be influenced by a combination of such factors.

does not always mean an n-fold increase in its protein product.

Once synthesized, protein molecules have a finite lifetime, which may range from minutes to many days (Table 15–1). The rate of protein degradation (Fig. 15–2, ❺) differs from one enzyme to another and depends on the conditions in the cell. Some proteins are tagged by the covalent attachment of ubiquitin for degradation in proteasomes, as discussed in Chapter 27 (see, for example, the case of cyclin, in Fig. 12–47). Rapid **turnover** (synthesis followed by degradation) is energetically expensive, but proteins with a short half-life can reach new steady state levels much faster than those with a long half-life, and the benefit of this quick responsiveness must balance or outweigh the cost to the cell.

TABLE 15–1 Average Half-Life of Proteins in Mammalian Tissues

Tissue	Average half-life (days)
Liver	0.9
Kidney	1.7
Heart	4.1
Brain	4.6
Muscle	10.7

Yet another way to alter the *effective* activity of an enzyme is to sequester the enzyme and its substrate in different compartments (Fig. 15–2, ❻). In muscle, for example, hexokinase cannot act on glucose until the sugar enters the myocyte from the blood, and the rate at which it enters depends on the activity of glucose transporters (see Table 11–3) in the plasma membrane. Within cells, membrane-bounded compartments segregate certain enzymes and enzyme systems, and the transport of substrate across these intracellular membranes may be the limiting factor in enzyme action.

By these several mechanisms for regulating enzyme level, cells can dramatically change their complement of enzymes in response to changes in metabolic circumstances. In vertebrates, liver is the most adaptable tissue; a change from a high-carbohydrate to a high-lipid diet, for example, affects the transcription of hundreds of genes and thus the levels of hundreds of proteins. These global changes in gene expression can be quantified by the use of DNA microarrays (see Fig. 9–23) that display the entire complement of mRNAs present in a given cell type or organ (the **transcriptome**) or by two-dimensional gel electrophoresis (see Fig. 3–21) that displays the protein complement of a cell type or organ (its **proteome**). Both techniques offer great insights into metabolic regulation. The effect of changes in the proteome is often a change in the total ensemble

of low molecular weight metabolites, the **metabolome (Fig. 15–3)**. The metabolome of *E. coli* growing on glucose is dominated by a few classes of metabolites: glutamate (49%); nucleotides (mainly ribonucleoside triphosphates) (15%); intermediates of glycolysis, the citric acid cycle, and the pentose phosphate pathway (central pathways of carbon metabolism) (15%); and redox cofactors and glutathiones (9%).

Once the regulatory mechanisms that involve protein synthesis and degradation have produced a certain number of molecules of each enzyme in a cell, the activity of those enzymes can be further regulated in several other ways: by the concentration of substrate, the presence of allosteric effectors, covalent modifications, or binding of regulatory proteins—all of which can change the activity of an individual enzyme molecule (Fig. 15–2, ❼ to ❿).

All enzymes are sensitive to the concentration of their substrate(s) (Fig. 15–2, ❼). Recall that in the simplest case (an enzyme that follows Michaelis-Menten kinetics), the initial rate of the reaction is half-maximal when the substrate is present at a concentration equal to K_m (that is, when the enzyme is half-saturated with substrate). Activity drops off at lower [S], and when $[S] << K_m$, the reaction rate is linearly dependent on [S].

The relationship between [S] and K_m is important because intracellular concentrations of substrate are often in the same range as, or lower than, K_m. The activity of hexokinase, for example, changes with [glucose], and intracellular [glucose] varies with the concentration of glucose in the blood. As we will see, the different forms (isozymes) of hexokinase have different K_m values and are therefore affected differently by changes in intracellular [glucose], in ways that make sense physiologically. For a number of phosphoryl transfers from ATP, and for redox reactions using NADPH or NAD^+, the metabolite concentration is well above the K_m **(Fig. 15–4)**; these cofactors are not likely to be the limiting factors in such reactions.

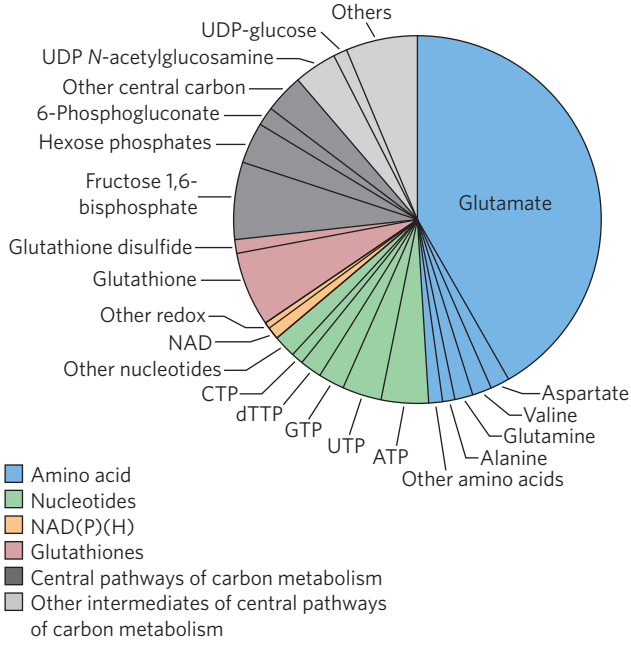


FIGURE 15–3 The metabolome of *E. coli* growing on glucose. Summary of the amounts of 103 metabolites measured by a combination of liquid chromatography and tandem mass spectrometry (LC-MS/MS).

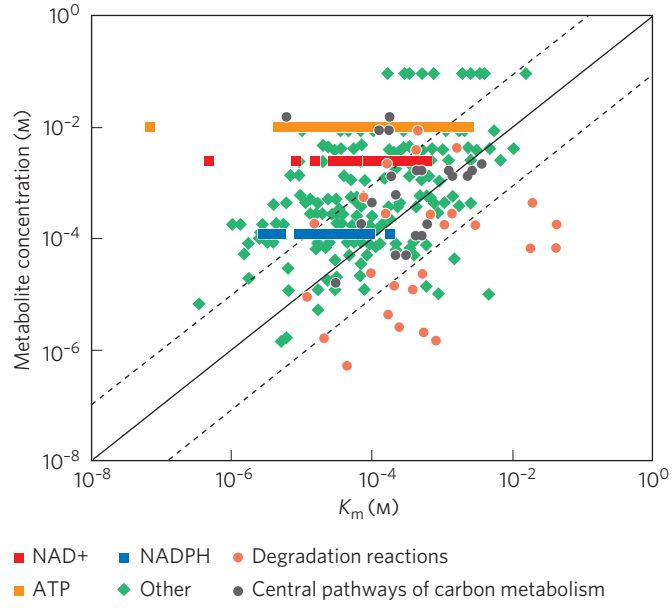


FIGURE 15–4 Comparison of K_m and substrate concentration for some metabolic enzymes. Measured metabolite concentrations for *E. coli* growing on glucose are plotted against the known K_m for enzymes that consume that metabolite. The solid line is the line of unity (where metabolite concentration = K_m), and the dashed lines each denote a tenfold deviation from the line of unity.

WORKED EXAMPLE 15–1 Activity of a Glucose Transporter

If K_t (the equivalent of K_m) for the glucose transporter in liver (GLUT2) is 40 mM, calculate the effect on the rate of glucose flux into a hepatocyte of increasing the blood glucose concentration from 3 mM to 10 mM.

Solution: We use Equation 11–1 (p. 406) to find the initial velocity (flux) of glucose uptake.

$$V_0 = \frac{V_{max}[S]_{out}}{K_t + [S]_{out}}$$

At 3 mM glucose

$$V_0 = V_{max}\ (3\ mM)/(40\ mM + 3\ mM)$$
$$= V_{max}\ (3\ mM/43\ mM) = 0.07\ V_{max}$$

At 10 mM glucose

$$V_0 = V_{max}\ (10\ mM)/(40\ mM + 10\ mM)$$
$$= V_{max}\ (10\ mM/50\ mM) = 0.20\ V_{max}$$

So a rise in blood glucose from 3 mM to 10 mM increases the rate of glucose influx into a hepatocyte by a factor of $0.20/0.07 \approx 3$.

Enzyme activity can be either increased or decreased by an allosteric effector (Fig. 15–2, ❽; see Fig. 6–34). Allosteric effectors typically convert hyperbolic kinetics to sigmoid kinetics, or vice versa (see Fig. 15–16b, for example). In the steepest part of the sigmoid curve, a small change in the concentration of substrate, or of allosteric effector, can have a large impact on reaction rate. Recall from Chapter 5 (p. 167) that the cooperativity of an allosteric enzyme can be expressed as a Hill coefficient, with higher coefficients meaning greater cooperativity. For an allosteric enzyme with a Hill coefficient of 4, activity increases from 10% V_{max} to 90% V_{max} with only a 3-fold increase in [S], compared with the 81-fold rise in [S] needed by an enzyme with no cooperative effects (Hill coefficient of 1; Table 15–2).

Covalent modifications of enzymes or other proteins (Fig. 15–2, ❾) occur within seconds or minutes of a regulatory signal, typically an extracellular signal. By far the most common modifications are phosphorylation and dephosphorylation **(Fig. 15–5)**; up to half the proteins in a eukaryotic cell are phosphorylated under some circumstances. Phosphorylation by a specific protein kinase may alter the electrostatic features of an enzyme's active site, cause movement of an inhibitory region of the enzyme out of the active site, alter the enzyme's interaction with other proteins, or force conformational changes that translate into changes in V_{max} or K_m. For covalent modification to be useful in regulation, the cell must be able to restore the altered enzyme to its original activity state. A family of phosphoprotein phosphatases, at least some of which are themselves under regulation, catalyzes the dephosphorylation of proteins.

Finally, many enzymes are regulated by association with and dissociation from another, regulatory protein (Fig. 15–2, ❿). For example, the cyclic AMP–dependent protein kinase (PKA; see Fig. 12–6) is inactive until cAMP binding separates catalytic from regulatory (inhibitory) subunits of the enzyme.

These several mechanisms for altering the flux through a step in a metabolic pathway are not mutually exclusive. It is very common for a single enzyme to be regulated at the level of transcription and by both allosteric and covalent mechanisms. The combination provides fast, smooth, effective regulation in response to a very wide array of perturbations and signals.

TABLE 15–2 Relationship between Hill Coefficient and the Effect of Substrate Concentration on Reaction Rate for Allosteric Enzymes

Hill coefficient (n_H)	Required change in [S] to increase V_0 from 10% to 90% V_{max}
0.5	×6,600
1.0	×81
2.0	×9
3.0	×4.3
4.0	×3

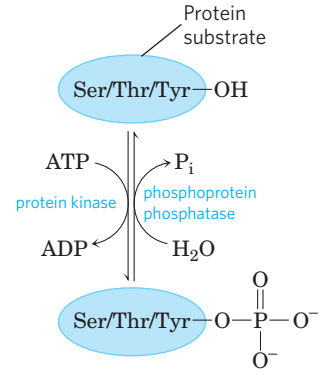


FIGURE 15–5 Protein phosphorylation and dephosphorylation. Protein kinases transfer a phosphoryl group from ATP to a Ser, Thr, or Tyr residue in an enzyme or other protein substrate. Protein phosphatases remove the phosphoryl group as P_i.

In the discussions that follow, it is useful to think of changes in enzymatic activity as serving two distinct though complementary roles. We use the term **metabolic regulation** to refer to processes that serve to maintain homeostasis at the molecular level—to hold some cellular parameter (concentration of a metabolite, for example) at a steady level over time, even as the flow of metabolites through the pathway changes. The term **metabolic control** refers to a process that leads to a change in the output of a metabolic pathway over time, in response to some outside signal or change in circumstances. The distinction, although useful, is not always easy to make.

Reactions Far from Equilibrium in Cells Are Common Points of Regulation

For some steps in a metabolic pathway the reaction is close to equilibrium, with the cell in its dynamic steady state **(Fig. 15–6)**. The net flow of metabolites through these steps is the small difference between the rates of the forward and reverse reactions, rates that are very similar when a reaction is near equilibrium. Small changes in

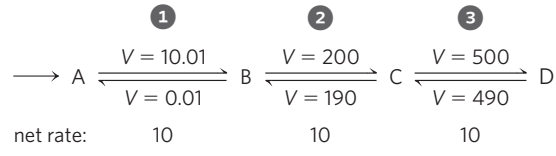


FIGURE 15–6 Near-equilibrium and nonequilibrium steps in a metabolic pathway. Steps ❷ and ❸ of this pathway are near equilibrium in the cell; for each step, the rate (V) of the forward reaction is only slightly greater than the reverse rate, so the net forward rate (10) is relatively low and the free-energy change, ΔG, is close to zero. An increase in [C] or [D] can reverse the direction of these steps. Step ❶ is maintained in the cell far from equilibrium; its forward rate greatly exceeds its reverse rate. The net rate of step ❶ (10) is much larger than the reverse rate (0.01) and is identical to the net rates of steps ❷ and ❸ when the pathway is operating in the steady state. Step ❶ has a large, negative ΔG.

TABLE 15–3 Equilibrium Constants, Mass-Action Coefficients, and Free-Energy Changes for Enzymes of Carbohydrate Metabolism

Enzyme	K'_{eq}	Mass-action ratio, Q		Reaction near equilibrium in vivo?*	$\Delta G'^{\circ}$ (kJ/mol)	ΔG (kJ/mol) in heart
		Liver	Heart			
Hexokinase	1×10^{3}	2×10^{-2}	8×10^{-2}	No	−17	−27
PFK-1	1.0×10^{3}	9×10^{-2}	3×10^{-2}	No	−14	−23
Aldolase	1.0×10^{-4}	1.2×10^{-6}	9×10^{-6}	Yes	+24	−6.0
Triose phosphate isomerase	4×10^{-2}	—†	2.4×10^{-1}	Yes	+7.5	+3.8
Glyceraldehyde 3-phosphate dehydrogenase + phosphoglycerate kinase	2×10^{3}	6×10^{2}	9.0	Yes	−13	+3.5
Phosphoglycerate mutase	1×10^{-1}	1×10^{-1}	1.2×10^{-1}	Yes	+4.4	+0.6
Enolase	3	2.9	1.4	Yes	−3.2	−0.5
Pyruvate kinase	2×10^{4}	7×10^{-1}	40	No	−31	−17
Phosphoglucose isomerase	4×10^{-1}	3.1×10^{-1}	2.4×10^{-1}	Yes	+2.2	−1.4
Pyruvate carboxylase + PEP carboxykinase	7	1×10^{-3}	—†	No	−5.0	−23
Glucose 6-phosphatase	8.5×10^{2}	1.2×10^{2}	—†	Yes	−17	−5.0

Source: K'_{eq} and Q from Newsholme, E.A. & Start, C. (1973) *Regulation in Metabolism*, Wiley Press, New York, pp. 97, 263. ΔG and $\Delta G'^{\circ}$ were calculated from these data.

*For simplicity, any reaction for which the absolute value of the calculated ΔG is less than 6 is considered near equilibrium.

†Data not available.

substrate or product concentration can produce large changes in the net rate, and can even change the direction of the net flow. We can identify these near-equilibrium reactions in a cell by comparing the **mass-action ratio**, Q, with the equilibrium constant for the reaction, K'_{eq}. Recall that for the reaction A + B → C + D, Q = [C][D]/[A][B]. When Q and K'_{eq} are within 1 to 2 orders of magnitude of each other, the reaction is near equilibrium. This is the case for 6 of the 10 steps in the glycolytic pathway (Table 15–3).

Other reactions are far from equilibrium in the cell. For example, K'_{eq} for the phosphofructokinase-1 (PFK-1) reaction is about 1,000, but Q ([fructose 1,6-bisphosphate][ADP]/[fructose 6-phosphate][ATP]) in a hepatocyte in the steady state is about 0.1 (Table 15–3). It is *because* the reaction is so far from equilibrium that the process is exergonic under cellular conditions and tends to go in the forward direction. The reaction is held far from equilibrium because, under prevailing cellular conditions of substrate, product, and effector concentrations, the rate of conversion of fructose 6-phosphate to fructose 1,6-bisphosphate is limited by the activity of PFK-1, which is itself limited by the number of PFK-1 molecules present and by the actions of allosteric effectors. Thus the net forward rate of the enzyme-catalyzed reaction is equal to the net flow of glycolytic intermediates through other steps in the pathway, and the reverse flow through PFK-1 remains near zero.

The cell *cannot* allow reactions with large equilibrium constants to reach equilibrium. If [fructose 6-phosphate], [ATP], and [ADP] in the cell were held at typical levels (low millimolar concentrations) and the PFK-1 reaction were allowed to reach equilibrium by an increase in [fructose 1,6-bisphosphate], the concentration of fructose 1,6-bisphosphate would rise into the molar range, wreaking osmotic havoc on the cell. Consider another case: if the reaction ATP → ADP + P_i were allowed to approach equilibrium in the cell, the actual free-energy change ($\Delta G'$) for that reaction (ΔG_p; see Worked Example 13–2, p. 519) would approach zero, and ATP would lose the high phosphoryl group transfer potential that makes it valuable to the cell. It is therefore essential that enzymes catalyzing ATP breakdown and other highly exergonic reactions in a cell be sensitive to regulation, so that when metabolic changes are forced by external circumstances, the flow through these enzymes will be adjusted to ensure that [ATP] remains far above its equilibrium level. When such metabolic changes occur, the activities of enzymes in all interconnected pathways adjust to keep these critical steps away from equilibrium. Thus, not surprisingly, many enzymes (such as PFK-1) that catalyze highly exergonic reactions are subject to a variety of subtle regulatory mechanisms. The multiplicity of these adjustments is so great that we cannot predict by examining the properties of any one enzyme in a pathway whether that enzyme has a strong influence on net flow through the entire pathway. This complex problem can be approached by metabolic control analysis, as described in Section 15.2.

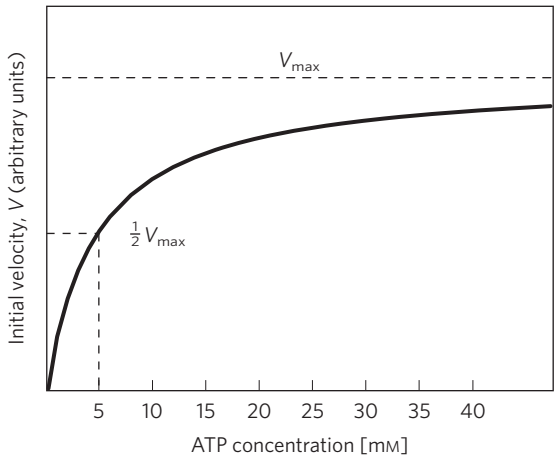


FIGURE 15–7 Effect of ATP concentration on the initial reaction velocity of a typical ATP-dependent enzyme. These experimental data yield a K_m for ATP of 5 mM. The concentration of ATP in animal tissues is ~5 mM.

Adenine Nucleotides Play Special Roles in Metabolic Regulation

After the protection of its DNA from damage, perhaps nothing is more important to a cell than maintaining a constant supply and concentration of ATP. Many ATP-using enzymes have K_m values between 0.1 and 1 mM, and the ATP concentration in a typical cell is about 5 to 10 mM (Fig. 15–4). If [ATP] were to drop significantly, these enzymes would be less than fully saturated by their substrate (ATP), and the rates of hundreds of reactions that involve ATP would decrease **(Fig. 15–7)**; the cell would probably not survive this *kinetic* effect on so many reactions.

There is also an important *thermodynamic* effect of lowered [ATP]. Because ATP is converted to ADP or AMP when "spent" to accomplish cellular work, the [ATP]/[ADP] ratio profoundly affects all reactions that employ these cofactors. (The same is true for other important cofactors, such as NADH/NAD^+ and NADPH/$NADP^+$.) For example, consider the reaction catalyzed by hexokinase:

$$\text{ATP} + \text{glucose} \longrightarrow \text{ADP} + \text{glucose 6-phosphate}$$

$$K'_{eq} = \frac{[\text{ADP}]_{eq}[\text{glucose 6-phosphate}]_{eq}}{[\text{ATP}]_{eq}[\text{glucose}]_{eq}} = 2 \times 10^3$$

Note that this expression holds true *only* when reactants and products are at their *equilibrium* concentrations, where $\Delta G' = 0$. At any other set of concentrations, $\Delta G'$ is not zero. Recall (from Chapter 13) that the ratio of products to substrates (the mass action ratio, Q) determines the magnitude and sign of $\Delta G'$ and therefore the driving force, $\Delta G'$, of the reaction:

$$\Delta G' = \Delta G'^\circ + RT \ln \frac{[\text{ADP}][\text{glucose 6-phosphate}]}{[\text{ATP}][\text{glucose}]}$$

Because an alteration of this driving force profoundly influences every reaction that involves ATP, organisms have evolved under strong pressure to develop regulatory mechanisms responsive to the [ATP]/[ADP] ratio.

AMP concentration is an even more sensitive indicator of a cell's energetic state than is [ATP]. Normally cells have a far higher concentration of ATP (5 to 10 mM) than of AMP (<0.1 mM). When some process (say, muscle contraction) consumes ATP, AMP is produced in two steps. First, hydrolysis of ATP produces ADP, then the reaction catalyzed by **adenylate kinase** produces AMP:

$$2\text{ADP} \longrightarrow \text{AMP} + \text{ATP}$$

If ATP is consumed such that its concentration drops 10%, the *relative* increase in [AMP] is much greater than that of [ADP] (Table 15–4). It is not surprising, therefore, that many regulatory processes are keyed to changes in [AMP]. Probably the most important mediator of regulation by AMP is **AMP-activated protein kinase (AMPK)**, which responds to an increase in [AMP] by phosphorylating key proteins and thus regulating their activities. The rise in [AMP] may be caused by a reduced nutrient supply or by increased exercise. The action of AMPK (not to be confused with the *cyclic* AMP–dependent protein kinase; see Section 15.5) increases glucose transport and activates glycolysis and fatty acid oxidation, while suppressing energy-requiring processes such as the synthesis of fatty acids, cholesterol, and protein **(Fig. 15–8)**. We discuss AMPK further, and the detailed mechanisms by which it effects these changes, in Chapter 23.

In addition to ATP, hundreds of metabolic intermediates also must be present at appropriate concentrations in the cell. To take just one example: the glycolytic intermediates dihydroxyacetone phosphate and 3-phosphoglycerate are precursors of triacylglycerols and serine, respectively. When these products are needed, the rate of glycolysis must be adjusted to provide them without reducing the glycolytic production of ATP. The same

TABLE 15–4 Relative Changes in [ATP] and [AMP] When ATP Is Consumed

Adenine nucleotide	Concentration before ATP depletion (mM)	Concentration after ATP depletion (mM)	Relative change
ATP	5.0	4.5	10%
ADP	1.0	1.0	0
AMP	0.1	0.6	600%

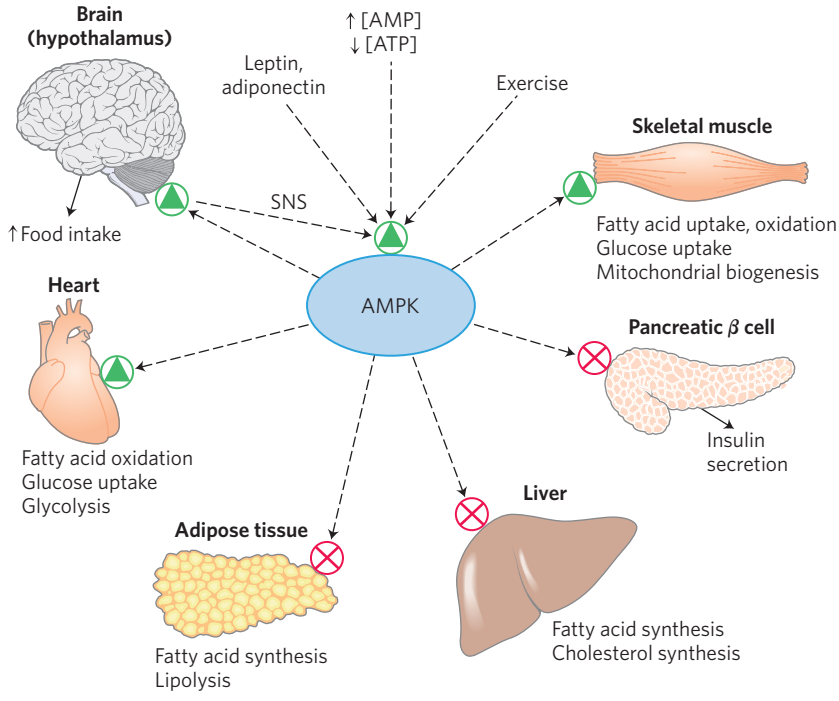


FIGURE 15–8 Role of AMP-activated protein kinase (AMPK) in carbohydrate and fat metabolism. AMPK is activated by elevated [AMP] or decreased [ATP], by exercise, by the sympathetic nervous system (SNS), or by peptide hormones produced in adipose tissue (leptin and adiponectin, described in more detail in Chapter 23). When activated, AMPK phosphorylates target proteins and shifts metabolism in a variety of tissues away from energy-consuming processes such as the synthesis of glycogen, fatty acids, and cholesterol; shifts metabolism in extrahepatic tissues to the use of fatty acids as a fuel; and triggers gluconeogenesis in the liver to provide glucose for the brain. In the hypothalamus, AMPK stimulates feeding behavior to provide more dietary fuel.

is true for maintaining the levels of other important cofactors, such as NADH and NADPH: changes in their mass action ratios (that is, in the ratio of reduced to oxidized cofactor) have global effects on metabolism.

Of course, priorities at the *organismal* level have also driven the evolution of regulatory mechanisms. In mammals, the brain has virtually no stored source of energy, depending instead on a constant supply of glucose from the blood. If blood glucose drops from its normal concentration of 4 to 5 mM to half that level, mental confusion results, and a fivefold reduction in blood glucose can lead to coma and death. To buffer against changes in blood glucose concentration, release of the hormones insulin and glucagon, elicited by high or low blood glucose, respectively, triggers metabolic changes that tend to return the blood glucose concentration to normal.

Other selective pressures must also have operated throughout evolution, selecting for regulatory mechanisms that accomplish the following:

1. Maximize the efficiency of fuel utilization by preventing the simultaneous operation of pathways in opposite directions (such as glycolysis and gluconeogenesis).
2. Partition metabolites appropriately between alternative pathways (such as glycolysis and the pentose phosphate pathway).
3. Draw on the fuel best suited for the immediate needs of the organism (glucose, fatty acids, glycogen, or amino acids).
4. Slow down biosynthetic pathways when their products accumulate.

The remaining chapters of this book present many examples of each kind of regulatory mechanism.

SUMMARY 15.1 Regulation of Metabolic Pathways

- In a metabolically active cell in a steady state, intermediates are formed and consumed at equal rates. When a transient perturbation alters the rate of formation or consumption of a metabolite, compensating changes in enzyme activities return the system to the steady state.
- Cells regulate their metabolism by a variety of mechanisms over a time scale ranging from less than a millisecond to days, either by changing the activity of existing enzyme molecules or by changing the number of molecules of a specific enzyme.
- Various signals activate or inactivate transcription factors, which act in the nucleus to regulate gene expression. Changes in the transcriptome lead to changes in the proteome, and ultimately in the metabolome of a cell or tissue.

- In multistep processes such as glycolysis, certain reactions are essentially at equilibrium in the steady state; the rates of these reactions rise and fall with substrate concentration. Other reactions are far from equilibrium; these steps are typically the points of regulation of the overall pathway.
- Regulatory mechanisms maintain nearly constant levels of key metabolites such as ATP and NADH in cells and glucose in the blood, while matching the use or production of glucose to the organism's changing needs.
- The levels of ATP and AMP are a sensitive reflection of a cell's energy status, and when the [ATP]/[AMP] ratio decreases, the AMP-activated protein kinase (AMPK) triggers a variety of cellular responses to raise [ATP] and lower [AMP].

15.2 Analysis of Metabolic Control



Eduard Buchner, 1860–1917

Detailed studies of metabolic regulation were not feasible until the basic chemical steps in a pathway had been clarified and the responsible enzymes characterized. Beginning with Eduard Buchner's discovery (c. 1900) that an extract of broken yeast cells could convert glucose to ethanol and CO_2, a major thrust of biochemical research was to deduce the steps by which this transformation occurred and to purify and characterize the enzymes that catalyzed each step. By the middle of the twentieth century, all 10 enzymes of the glycolytic pathway had been purified and characterized. In the next 50 years much was learned about the regulation of these enzymes by intracellular and extracellular signals, through the kinds of allosteric and covalent mechanisms described in this chapter. The conventional wisdom was that in a linear pathway such as glycolysis, catalysis by one enzyme must be the slowest and must therefore determine the rate of metabolite flow, or flux, through the whole pathway. For glycolysis, PFK-1 was considered the rate-limiting enzyme, because it was known to be closely regulated by fructose 2,6-bisphosphate and other allosteric effectors.

With the advent of genetic engineering technology, it became possible to test this "single rate-determining step" hypothesis by increasing the concentration of the enzyme that catalyzes the "rate-limiting step" in a pathway and determining whether flux through the pathway increases proportionally. Most often it does not; the simple solution (a single rate-determining step) is wrong. It has now become clear that in most pathways the control of flux is distributed among several enzymes, and the extent to which each contributes to the control varies with metabolic circumstances—the supply of the starting material (say, glucose), the supply of oxygen, the need for other products derived from intermediates of the pathway (say, glucose 6-phosphate for the pentose phosphate pathway in cells synthesizing large amounts of nucleotides), the effects of metabolites with regulatory roles, and the hormonal status of the organism (such as the levels of insulin and glucagon), among other factors.

Why are we interested in what limits the flux through a pathway? To understand the action of hormones or drugs, or the pathology that results from a failure of metabolic regulation, we must know where control is exercised. If researchers wish to develop a drug that stimulates or inhibits a pathway, the logical target is the enzyme that has the greatest impact on the flux through that pathway. And the bioengineering of a microorganism to overproduce a product of commercial value (p. 321) requires a knowledge of what limits the flux of metabolites toward that product.

The Contribution of Each Enzyme to Flux through a Pathway Is Experimentally Measurable

There are several ways to determine experimentally how a change in the activity of one enzyme in a pathway affects metabolite flux through that pathway. Consider the experimental results shown in **Figure 15–9**. When a sample of rat liver was homogenized to release all soluble enzymes, the extract carried out the glycolytic conversion of glucose to fructose 1,6-bisphosphate at a measurable rate. (This experiment, for simplicity, focused on just the first part of the glycolytic pathway.) When increasing amounts of purified hexokinase IV

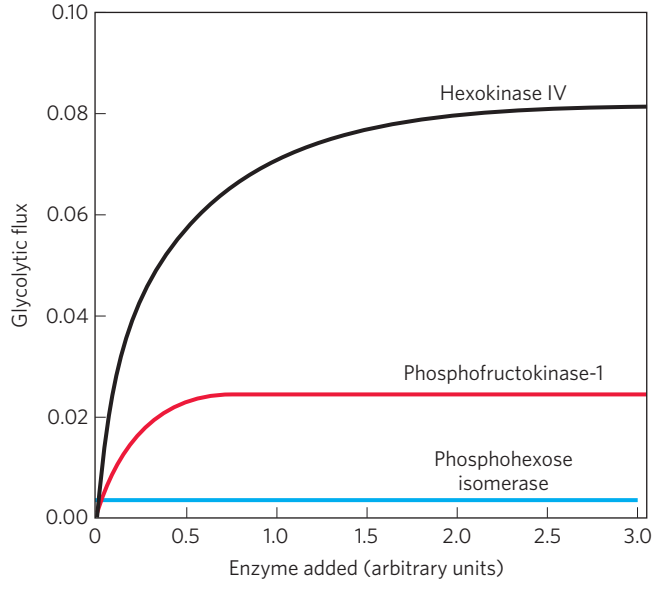


FIGURE 15–9 Dependence of glycolytic flux in a rat liver homogenate on added enzymes. Purified enzymes in the amounts shown on the *x* axis were added to an extract of liver carrying out glycolysis in vitro. The flux through the pathway is shown on the *y* axis.

(glucokinase) were added to the extract, the rate of glycolysis progressively increased. The addition of purified PFK-1 to the extract also increased the rate of glycolysis, but not as dramatically as did hexokinase. Addition of purified phosphohexose isomerase was without effect. These results suggest that hexokinase and PFK-1 both contribute to setting the flux through the pathway (hexokinase more than PFK-1), and that phosphohexose isomerase does not.

Similar experiments can be done on intact cells or organisms, using specific inhibitors or activators to change the activity of one enzyme while observing the effect on flux through the pathway. The amount of an enzyme can also be altered genetically; bioengineering can produce a cell that makes extra copies of the enzyme under investigation or has a version of the enzyme that is less active than the normal enzyme. Increasing the concentration of an enzyme genetically sometimes has significant effects on flux; sometimes it has no effect.

Three critical parameters, which together describe the responsiveness of a pathway to changes in metabolic circumstances, lie at the center of **metabolic control analysis**. We turn now to a qualitative description of these parameters and their meaning in the context of a living cell. Box 15–1 provides a more rigorous quantitative discussion.

The Flux Control Coefficient Quantifies the Effect of a Change in Enzyme Activity on Metabolite Flux through a Pathway

Quantitative data on metabolic flux, obtained as described in Figure 15–9, can be used to calculate a **flux control coefficient**, ***C***, for each enzyme in a pathway. This coefficient expresses the relative contribution of each enzyme to setting the rate at which metabolites flow through the pathway—that is, the **flux**, ***J***. C can have any value from 0.0 (for an enzyme with no impact on the flux) to 1.0 (for an enzyme that wholly determines the flux). An enzyme can also have a *negative* flux control coefficient. In a branched pathway, an enzyme in one branch, by drawing intermediates away from the other branch, can have a negative impact on the flux through that other branch **(Fig. 15–10)**. C is not a constant, and it is not intrinsic to a single enzyme; it is a function of the whole system of enzymes, and its value depends on the concentrations of substrates and effectors.

When real data from the experiment on glycolysis in a rat liver extract (Fig. 15–9) were subjected to this kind of analysis, investigators found flux control coefficients (for enzymes at the concentrations found in the extract) of 0.79 for hexokinase, 0.21 for PFK-1, and 0.0 for phosphohexose isomerase. It is not just fortuitous that these values add up to 1.0; we can show that for any complete pathway, the sum of the flux control coefficients must equal unity.

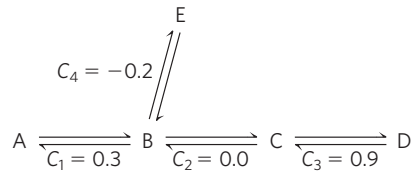


FIGURE 15–10 Flux control coefficient, *C*, in a branched metabolic pathway. In this simple pathway, the intermediate B has two alternative fates. To the extent that reaction B → E draws B away from the pathway A → D, it controls that pathway, which will result in a *negative* flux control coefficient for the enzyme that catalyzes step B → E. Note that the sum of all four coefficients equals 1.0, as it must for any defined system of enzymes.

The Elasticity Coefficient Is Related to an Enzyme's Responsiveness to Changes in Metabolite or Regulator Concentrations

A second parameter, the **elasticity coefficient**, ε, expresses quantitatively the responsiveness of a single enzyme to changes in the concentration of a metabolite or regulator; it is a function of the enzyme's intrinsic kinetic properties. For example, an enzyme with typical Michaelis-Menten kinetics shows a hyperbolic response to increasing substrate concentration **(Fig. 15–11)**. At low concentrations of substrate (say, 0.1 K_m), each increment in substrate concentration results in a comparable increase in enzymatic activity, yielding an ε near 1.0. At relatively high substrate concentrations (say, 10 K_m), increasing the substrate concentration has little effect on the reaction rate, because the enzyme is already saturated with substrate. The elasticity in this case approaches zero. For allosteric enzymes that show positive cooperativity, ε may exceed 1.0, but it cannot exceed the Hill coefficient, which is typically between 1.0 and 4.0.

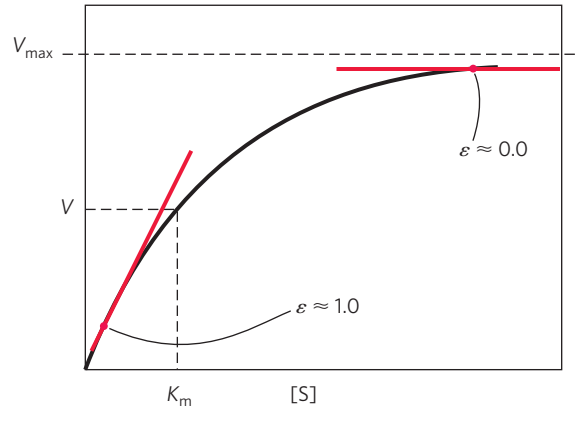


FIGURE 15–11 Elasticity coefficient, ε, of an enzyme with typical Michaelis-Menten kinetics. At substrate concentrations far below the K_m, each increase in [S] produces a correspondingly large increase in the reaction velocity, V. For this region of the curve, the enzyme has an ε of about 1.0. At [S] >> K_m, increasing [S] has little effect on V; ε here is close to 0.0.

BOX 15–1 METHODS Metabolic Control Analysis: Quantitative Aspects

The factors that influence the flow of intermediates (flux) through a pathway may be determined quantitatively by experiment and expressed in terms useful for predicting the change in flux when some factor involved in the pathway changes. Consider the simple reaction sequence in Figure 1, in which a substrate X (say, glucose) is converted in several steps to a product Z (perhaps pyruvate, formed glycolytically). An enzyme late in the pathway is a dehydrogenase (ydh) that acts on substrate Y. Because the action of a dehydrogenase is easily measured (see Fig. 13–24), we can use the flux (J) through this step (J_{ydh}) to measure the flux through the whole path. We manipulate experimentally the level of an early enzyme in the pathway (xase, which acts on the substrate X) and measure the flux through the path (J_{ydh}) for several levels of the enzyme xase.

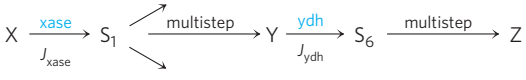


FIGURE 1 Flux through a hypothetical multienzyme pathway.

The relationship between the flux through the pathway from X to Z in the intact cell and the concentration of each enzyme in the path should be hyperbolic, with virtually no flux at infinitely low enzyme activity and near-maximum flux at very high enzyme activity. In a plot of J_{ydh} against the concentration of xase, E_{xase}, the change in flux with a small change in enzyme level is $\partial J_{ydh}/\partial E_{xase}$, which is simply the slope of the tangent to the curve at any concentration of enzyme, E_{xase}, and which tends toward zero at saturating E_{xase}. At low E_{xase}, the slope is steep; the flux increases with each incremental increase in enzyme activity. At very high E_{xase}, the slope is much smaller; the system is less responsive to added xase because it is already present in excess over the other enzymes in the pathway.

To show quantitatively the dependence of flux through the pathway, ∂J_{ydh}, on ∂E_{xase}, we could use the ratio $\partial J_{ydh}/\partial E_{xase}$. However, its usefulness is limited because its value depends on the units used to express flux and enzyme activity. By expressing the *fractional* changes in flux and enzyme activity, $\partial J_{ydh}/J_{ydh}$ and $\partial E_{xase}/E_{xase}$, we obtain a unitless expression for the **flux control coefficient**, $\boldsymbol{C}$, in this case $C^{J_{ydh}}_{xase}$:

$$C^{J_{ydh}}_{xase} \approx \frac{\partial J_{ydh}}{J_{ydh}} \Big/ \frac{\partial E_{xase}}{E_{xase}} \qquad (1)$$

This can be rearranged to

$$C^{J_{ydh}}_{xase} \approx \frac{\partial J_{ydh}}{\partial E_{xase}} \cdot \frac{E_{xase}}{J_{ydh}}$$

which is mathematically identical to

$$C^{J_{ydh}}_{xase} \approx \frac{\partial \ln J_{ydh}}{\partial \ln E_{xase}}$$

This equation suggests a simple graphical means for determining the flux control coefficient: $C^{J_{ydh}}_{xase}$ is the slope of the tangent to the plot of ln J_{ydh} versus ln E_{xase}, which can be obtained by replotting the experimental data in Figure 2a to obtain Figure 2b. Notice that $C^{J_{ydh}}_{xase}$ is not a constant; it depends on the starting E_{xase} from which the change in enzyme level takes place. For the cases shown in Figure 2, $C^{J_{ydh}}_{xase}$ is about 1.0 at the lowest E_{xase}, but only about 0.2 at high E_{xase}. A value near 1.0 for $C^{J_{ydh}}_{xase}$ means that the enzyme's concentration wholly determines the flux through the pathway; a value near 0.0 means that the enzyme's concentration does not limit the flux through the path. Unless the flux control coefficient is greater than about 0.5, changes in the activity of the enzyme will not have a strong effect on the flux.

The **elasticity**, $\boldsymbol{\varepsilon}$, of an enzyme is a measure of how that enzyme's catalytic activity changes when the concentration of a metabolite—substrate, product, or effector—changes. It is obtained from an experimental plot of the rate of the reaction catalyzed by the enzyme versus the concentration of the metabolite, at metabolite concentrations that prevail in the cell. By arguments analogous to those used to

The Response Coefficient Expresses the Effect of an Outside Controller on Flux through a Pathway

We can also derive a quantitative expression for the relative impact of an outside factor (such as a hormone or growth factor), which is neither a metabolite nor an enzyme in the pathway, on the flux through the pathway. The experiment would measure the flux through the pathway (glycolysis, in this case) at various levels of the parameter P (the insulin concentration, for example) to obtain the **response coefficient**, $\boldsymbol{R}$, which expresses the change in pathway flux when P ([insulin]) changes.

The three coefficients C, ε, and R are related in a simple way: the responsiveness (R) of a pathway to an outside factor that affects a certain enzyme is a function of (1) how sensitive the pathway is to changes in the activity of that enzyme (the flux control coefficient, C) and (2) how sensitive that specific enzyme is to changes in the outside controlling factor (the elasticity, ε):

$$R = C \cdot \varepsilon$$

Each enzyme in the pathway can be examined in this way, and the effects of any of several outside factors on flux through the pathway can be separately determined.

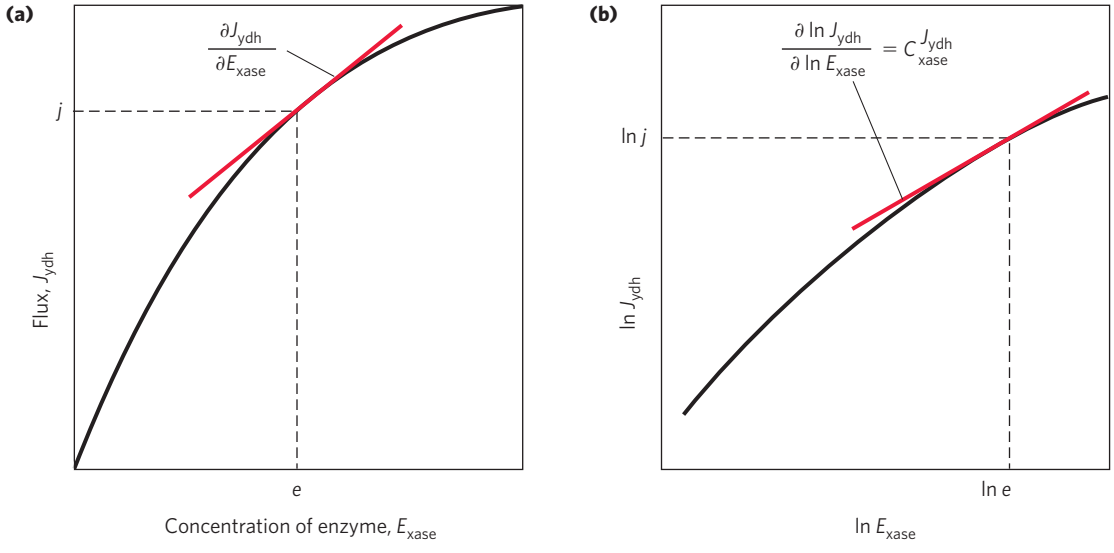


FIGURE 2 The flux control coefficient. **(a)** Typical variation of the pathway flux, J_{ydh}, measured at the step catalyzed by the enzyme ydh, as a function of the amount of the enzyme xase, E_{xase}, which catalyzes an earlier step in the pathway. The flux control coefficient at (*e*,*j*) is the product of the slope of the tangent to the curve, $\partial J_{ydh}/\partial E_{xase}$, and the ratio (scaling factor) *e*/*j*. **(b)** On a double-logarithmic plot of the same curve, the flux control coefficient is the slope of the tangent to the curve.

derive C, we can show ε to be the slope of the tangent to a plot of ln V versus ln [substrate, or product, or effector]:

$$\varepsilon_S^{xase} = \frac{\partial V_{xase}}{\partial S} \cdot \frac{S}{V_{xase}} = \frac{\partial \ln |V_{xase}|}{\partial \ln S}$$

For an enzyme with typical Michaelis-Menten kinetics, the value of ε ranges from about 1 at substrate concentrations far below K_m to near 0 as V_{max} is approached. Allosteric enzymes can have elasticities greater than 1.0, but not larger than their Hill coefficient (p. 167).

Finally, the effect of controllers outside the pathway itself (that is, not metabolites) can be measured and expressed as the **response coefficient**, ***R***. The change in flux through the pathway is measured for changes in the concentration of the controlling parameter P, and R is defined in a form analogous to that of Equation 1, yielding the expression

$$R_P^{J_{ydh}} = \frac{\partial J_{ydh}}{\partial P} \cdot \frac{P}{J_{ydh}}$$

Using the same logic and graphical methods as described above for determining C, we can obtain R as the slope of the tangent to the plot of ln J versus ln P.

The three coefficients we have described are related in this simple way:

$$R_P^{J_{ydh}} = C_{xase}^{J_{ydh}} \cdot \varepsilon_P^{xase}$$

Thus the responsiveness of each enzyme in a pathway to a change in an outside controlling factor is a simple function of two things: the control coefficient, a variable that expresses the extent to which that enzyme influences the flux under a given set of conditions, and the elasticity, an intrinsic property of the enzyme that reflects its sensitivity to substrate and effector concentrations.

Thus, in principle, we can predict how the flux of substrate through a series of enzymatic steps will change when there is a change in one or more controlling factors external to the pathway. Box 15–1 shows how these qualitative concepts are treated quantitatively.

Metabolic Control Analysis Has Been Applied to Carbohydrate Metabolism, with Surprising Results

Metabolic control analysis provides a framework within which we can think quantitatively about regulation, interpret the significance of the regulatory properties of each enzyme in a pathway, identify the steps that most affect the flux through the pathway, and distinguish between *regulatory* mechanisms that act to maintain metabolite concentrations and *control* mechanisms that actually alter the flux through the pathway. Analysis of the glycolytic pathway in yeast, for example, has revealed an unexpectedly low flux control coefficient for PFK-1, which, as we have noted, has been viewed as the main point of flux control—the "rate-determining step"—in glycolysis. Experimentally raising the level of PFK-1 fivefold led to a change in flux through glycolysis of less than 10%, suggesting that the real role of PFK-1

regulation is not to control flux through glycolysis but to mediate metabolite homeostasis—to prevent large changes in metabolite concentrations when the flux through glycolysis increases in response to elevated blood glucose or insulin. Recall that the study of glycolysis in a liver extract (Fig. 15–9) also yielded a flux control coefficient that contradicted the conventional wisdom; it showed that hexokinase, not PFK-1, is most influential in setting the flux through glycolysis. We must note here that a liver extract is far from equivalent to a hepatocyte; the ideal way to study flux control is by manipulating one enzyme at a time in the living cell. This is already feasible in many cases.

Investigators have used nuclear magnetic resonance (NMR) as a noninvasive means to determine the concentrations of glycogen and metabolites in the five-step pathway from glucose in the blood to glycogen in myocytes **(Fig. 15–12)** in rat and human muscle. They found that the flux control coefficient for glycogen synthase was smaller than that for either the glucose transporter (GLUT4) or hexokinase. (We discuss glycogen synthase and other enzymes of glycogen metabolism in Sections 15.4 and 15.5.) This finding contradicts the conventional wisdom that glycogen synthase is the locus of flux control and suggests that the importance of the phosphorylation/dephosphorylation of glycogen synthase is related instead to the maintenance of metabolite homeostasis—that is, *regulation*, not *control*. Two metabolites in this pathway, glucose and glucose 6-phosphate, are key intermediates in other pathways, including glycolysis, the pentose phosphate pathway, and the synthesis of glucosamine. Metabolic control analysis suggests that when the blood glucose level rises, insulin acts in muscle to (1) increase glucose transport into cells by conveying GLUT4 to the plasma membrane, (2) induce the synthesis of hexokinase, and (3) activate glycogen synthase by covalent alteration (see Fig. 15–41). The first two effects of insulin increase glucose flux through the pathway (control), and the third serves to adapt the activity of glycogen synthase so that metabolite levels (glucose 6-phosphate, for example) will not change dramatically with the increased flux (regulation).

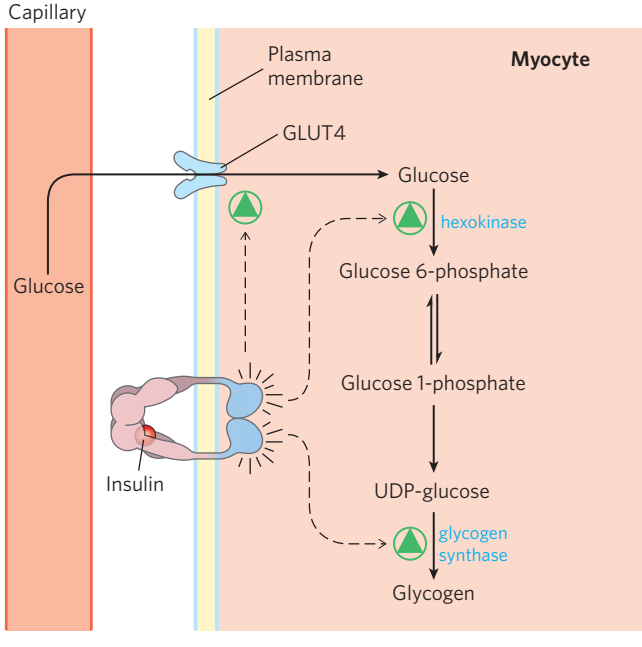


FIGURE 15–12 Control of glycogen synthesis from blood glucose in muscle. Insulin affects three of the five steps in this pathway, but it is the effects on transport and hexokinase activity, not the change in glycogen synthase activity, that increase the flux toward glycogen.

Metabolic Control Analysis Suggests a General Method for Increasing Flux through a Pathway

How could an investigator engineer a cell to increase the flux through one pathway without altering the concentrations of other metabolites or the fluxes through other pathways? More than three decades ago Henrik Kacser predicted, on the basis of metabolic control analysis, that this could be accomplished by increasing the concentrations of every enzyme in a pathway. The prediction has been confirmed in several experimental tests, and it also fits with the way cells normally control fluxes through a pathway. For example, rats fed a high-protein diet dispose of excess amino groups by converting them to urea in the urea cycle (Chapter 18). After such a dietary shift, the urea output increases fourfold, and the amount of all eight enzymes in the urea cycle increases two- to threefold. Similarly, when increased fatty acid oxidation is triggered by activation of peroxisome proliferator-activated receptor γ (PPARγ, a ligand-activated transcription factor; see Fig. 21–22), synthesis of the *whole set* of fatty acid oxidative enzymes is increased. With the growing use of DNA microarrays to study the expression of whole sets of genes in response to various perturbations, we should soon learn whether this is the general mechanism by which cells make long-term adjustments in flux through specific pathways.

SUMMARY 15.2 Analysis of Metabolic Control

- Metabolic control analysis shows that control of the rate of metabolite flux through a pathway is distributed among several of the enzymes in that path.
- The flux control coefficient, C, is an experimentally determined measure of the effect of an enzyme's concentration on flux through a multienzyme pathway. It is characteristic of the whole system, not intrinsic to the enzyme.
- The elasticity coefficient, ε, of an enzyme is an experimentally determined measure of its responsiveness to changes in the concentration of a metabolite or regulator molecule.
- The response coefficient, R, is a measure of the experimentally determined change in flux through

a pathway in response to a regulatory hormone or second messenger. It is a function of C and ε: $R = C \cdot \varepsilon$.

- Some regulated enzymes control the flux through a pathway, while others rebalance the level of metabolites in response to the change in flux. The first activity is *control*; the second, rebalancing activity is *regulation*.
- Metabolic control analysis predicts, and experiments have confirmed, that flux toward a specific product is most effectively increased by raising the concentration of all enzymes in the pathway.

15.3 Coordinated Regulation of Glycolysis and Gluconeogenesis

In mammals, **gluconeogenesis** occurs primarily in the liver, where its role is to provide glucose for export to other tissues when glycogen stores are exhausted and when no dietary glucose is available. As we discussed in Chapter 14, gluconeogenesis employs several of the enzymes that act in glycolysis, but it is not simply the reversal of glycolysis. Seven of the glycolytic reactions are freely reversible, and the enzymes that catalyze these reactions also function in gluconeogenesis **(Fig. 15–13)**. Three reactions of glycolysis are so exergonic as to be essentially irreversible: those catalyzed by hexokinase, PFK-1, and pyruvate kinase. All three reactions have a large, negative $\Delta G'$ (Table 15–3 shows the values in heart muscle). Gluconeogenesis uses detours around each of these irreversible steps; for example, the conversion of fructose 1,6-bisphosphate to fructose 6-phosphate is catalyzed by fructose 1,6-bisphosphatase (FBPase-1). Each of these bypass reactions also has a large, negative $\Delta G'$.

At each of the three points where glycolytic reactions are bypassed by alternative, gluconeogenic reactions, simultaneous operation of both pathways would consume ATP without accomplishing any chemical or biological work. For example, PFK-1 and FBPase-1 catalyze opposing reactions:

$$\text{ATP} + \text{fructose 6-phosphate} \xrightarrow{\text{PFK-1}} \text{ADP} + \text{fructose 1,6-bisphosphate}$$

$$\text{Fructose 1,6-bisphosphate} + \text{H}_2\text{O} \xrightarrow{\text{FBPase-1}} \text{fructose 6-phosphate} + \text{P}_\text{i}$$

The sum of these two reactions is:

$$\text{ATP} + \text{H}_2\text{O} \longrightarrow \text{ADP} + \text{P}_\text{i} + \text{heat}$$

that is, hydrolysis of ATP without any useful metabolic work being done. Clearly, if these two reactions were allowed to proceed simultaneously at a high rate in the same cell, a large amount of chemical energy would be dissipated as heat. This uneconomical process has been called a **futile cycle**. However, as we shall see later, such cycles may provide advantages for controlling pathways, and the term **substrate cycle** is a better description. Similar substrate cycles also occur with the other two sets of bypass reactions of gluconeogenesis (Fig. 15–13).

We look now in some detail at the mechanisms that regulate glycolysis and gluconeogenesis at the three points where these pathways diverge.

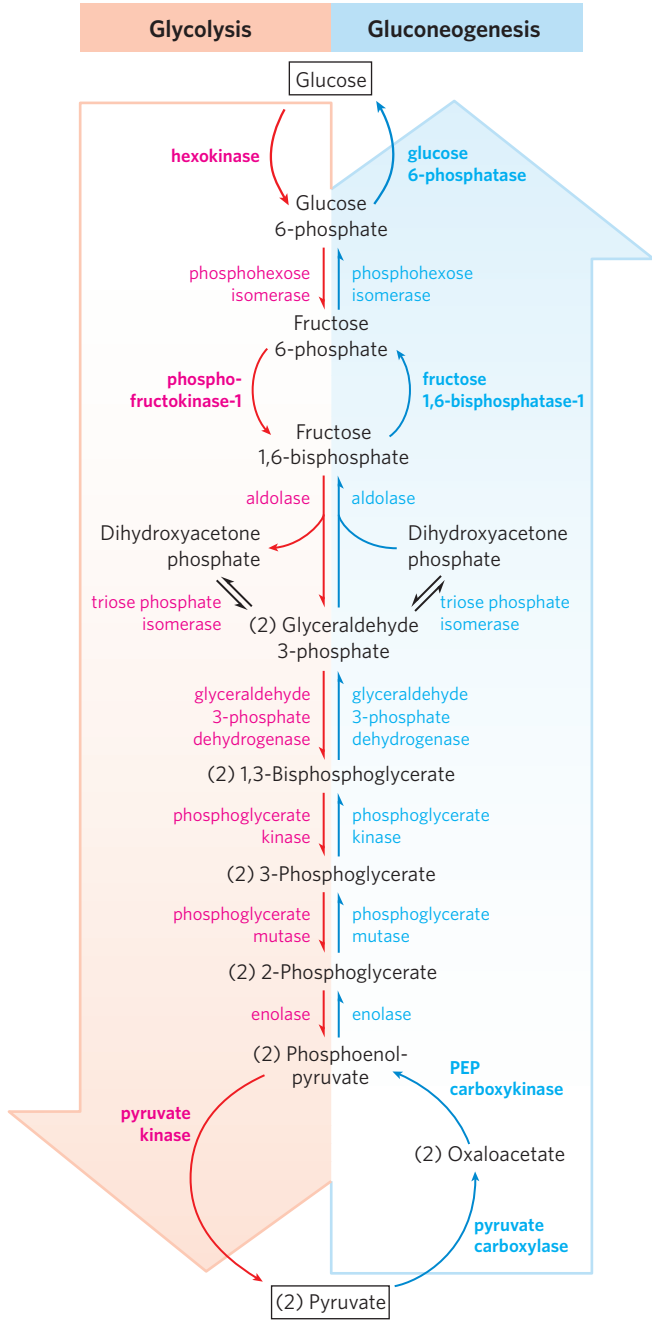


FIGURE 15–13 Glycolysis and gluconeogenesis. Opposing pathways of glycolysis (pink) and gluconeogenesis (blue) in rat liver. Three steps are catalyzed by different enzymes in gluconeogenesis (the "bypass reactions") and glycolysis; seven steps are catalyzed by the same enzymes in the two pathways. Cofactors have been omitted for simplicity.

Hexokinase Isozymes of Muscle and Liver Are Affected Differently by Their Product, Glucose 6-Phosphate

Hexokinase, which catalyzes the entry of glucose into the glycolytic pathway, is a regulatory enzyme. Humans have four isozymes (designated I to IV), encoded by four different genes. **Isozymes** are different proteins that catalyze the same reaction (Box 15–2). The predominant hexokinase isozyme of myocytes (**hexokinase II**) has a high affinity for glucose—it is half-saturated at about 0.1 mM. Because glucose entering myocytes from the blood (where the glucose concentration is 4 to 5 mM) produces an intracellular glucose concentration high enough to saturate hexokinase II, the enzyme normally acts at or near its maximal rate. Muscle **hexokinase I** and hexokinase II are allosterically inhibited by their product, glucose 6-phosphate, so whenever the cellular concentration of glucose 6-phosphate rises above its normal level, these isozymes are temporarily and reversibly inhibited, bringing the rate of glucose 6-phosphate formation into balance with the rate of its utilization and reestablishing the steady state.

BOX 15–2 Isozymes: Different Proteins That Catalyze the Same Reaction

The four forms of hexokinase found in mammalian tissues are but one example of a common biological situation: the same reaction catalyzed by two or more different molecular forms of an enzyme. These multiple forms, called isozymes or isoenzymes, may occur in the same species, in the same tissue, even in the same cell. The different forms (isoforms) of the enzyme generally differ in kinetic or regulatory properties, in the cofactor they use (NADH or NADPH for dehydrogenase isozymes, for example), or in their subcellular distribution (soluble or membrane-bound). Isozymes may have similar, but not identical, amino acid sequences, and in many cases they clearly share a common evolutionary origin.

One of the first enzymes found to have isozymes was lactate dehydrogenase (LDH; p. 563), which in vertebrate tissues exists as at least five different isozymes separable by electrophoresis. All LDH isozymes contain four polypeptide chains (each of M_r 33,500), each type containing a different ratio of two kinds of polypeptides. The M (for muscle) chain and the H (for heart) chain are encoded by two different genes.

In skeletal muscle the predominant isozyme contains four M chains, and in heart the predominant isozyme contains four H chains. Other tissues have some combination of the five possible types of LDH isozymes:

Type	Composition	Location
LDH_1	HHHH	Heart and erythrocyte
LDH_2	HHHM	Heart and erythrocyte
LDH_3	HHMM	Brain and kidney
LDH_4	HMMM	Skeletal muscle and liver
LDH_5	MMMM	Skeletal muscle and liver



The differences in the isozyme content of tissues can be used to assess the timing and extent of heart damage due to myocardial infarction (heart attack). Damage to heart tissue results in the release of heart LDH into the blood. Shortly after a heart attack, the blood level of total LDH increases, and there is more LDH_2 than LDH_1. After 12 hours the amounts of LDH_1 and LDH_2 are very similar, and after 24 hours there is more LDH_1 than LDH_2. This switch in the $[LDH_1]/[LDH_2]$ ratio, combined with increased concentrations in the blood of another heart enzyme, creatine kinase, is very strong evidence of a recent myocardial infarction. ■

The different LDH isozymes have significantly different values of V_{max} and K_m, particularly for pyruvate. The properties of LDH_4 favor rapid reduction of very low concentrations of pyruvate to lactate in skeletal muscle, whereas those of isozyme LDH_1 favor rapid oxidation of lactate to pyruvate in the heart.

In general, the distribution of different isozymes of a given enzyme reflects at least four factors:

1. *Different metabolic patterns in different organs.* For glycogen phosphorylase, the isozymes in skeletal muscle and liver have different regulatory properties, reflecting the different roles of glycogen breakdown in these two tissues.
2. *Different locations and metabolic roles for isozymes in the same cell.* The isocitrate dehydrogenase isozymes of the cytosol and the mitochondrion are an example (Chapter 16).
3. *Different stages of development in embryonic or fetal tissues and in adult tissues.* For example, the fetal liver has a characteristic isozyme distribution of LDH, which changes as the organ develops into its adult form. Some enzymes of glucose catabolism in malignant (cancer) cells occur as their fetal, not adult, isozymes.
4. *Different responses of isozymes to allosteric modulators.* This difference is useful in fine-tuning metabolic rates. Hexokinase IV (glucokinase) of liver and the hexokinase isozymes of other tissues differ in their sensitivity to inhibition by glucose 6-phosphate.

The different hexokinase isozymes of liver and muscle reflect the different roles of these organs in carbohydrate metabolism: muscle consumes glucose, using it for energy production, whereas liver maintains blood glucose homeostasis by consuming or producing glucose, depending on the prevailing blood glucose concentration. The predominant hexokinase isozyme of liver is **hexokinase IV** (glucokinase), which differs in three important respects from hexokinases I–III of muscle. First, the glucose concentration at which hexokinase IV is half-saturated (about 10 mM) is higher than the usual concentration of glucose in the blood. Because an efficient glucose transporter in hepatocytes (**GLUT2**) rapidly equilibrates the glucose concentrations in cytosol and blood (see Fig. 11–31 for the kinetics of the same transporter, GLUT1, in erythrocytes), the high K_m of hexokinase IV allows its direct regulation by the level of blood glucose **(Fig. 15–14)**. When blood glucose is high, as it is after a meal rich in carbohydrates, excess glucose is transported into hepatocytes, where hexokinase IV converts it to glucose 6-phosphate. Because hexokinase IV is not saturated at 10 mM glucose, its activity continues to increase as the glucose concentration rises to 10 mM or more. Under conditions of low blood glucose, the glucose concentration in a hepatocyte is low relative to the K_m of hexokinase IV, and the glucose generated by gluconeogenesis leaves the cell before being trapped by phosphorylation.

Second, hexokinase IV is not inhibited by glucose 6-phosphate, and it can therefore continue to operate when the accumulation of glucose 6-phosphate completely inhibits hexokinases I–III. Finally, hexokinase IV is subject to inhibition by the reversible binding of a regulatory protein specific to liver **(Fig. 15–15)**. The binding is much tighter in the presence of the allosteric effector fructose 6-phosphate. Glucose competes with fructose 6-phosphate for binding and causes dissociation of the regulatory protein from the hexokinase, relieving the inhibition. Immediately after a carbohydrate-rich meal, when blood glucose is high, glucose enters the hepatocyte via GLUT2 and activates hexokinase IV by this mechanism. During a fast, when blood glucose drops

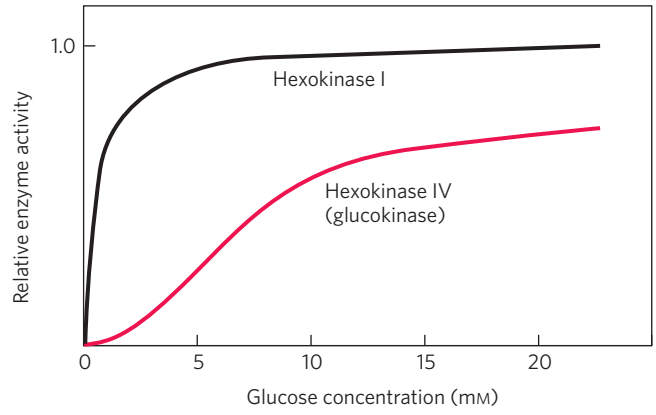


FIGURE 15–14 Comparison of the kinetic properties of hexokinase IV (glucokinase) and hexokinase I. Note the sigmoidicity for hexokinase IV and the much lower K_m for hexokinase I. When blood glucose rises above 5 mM, hexokinase IV activity increases, but hexokinase I is already operating near V_{max} and cannot respond to an increase in glucose concentration. Hexokinases I, II, and III have similar kinetic properties.

below 5 mM, fructose 6-phosphate triggers the inhibition of hexokinase IV by the regulatory protein, so the liver does not compete with other organs for the scarce glucose. The mechanism of inhibition by the regulatory protein is interesting: the protein anchors hexokinase IV inside the nucleus, where it is segregated from the other enzymes of glycolysis in the cytosol (Fig. 15–15). When the glucose concentration in the cytosol rises, it equilibrates with glucose in the nucleus by transport through the nuclear pores. Glucose causes dissociation of the regulatory protein, and hexokinase IV enters the cytosol and begins to phosphorylate glucose.

Hexokinase IV (Glucokinase) and Glucose 6-Phosphatase Are Transcriptionally Regulated

Hexokinase IV is also regulated at the level of protein synthesis. Circumstances that call for greater energy production (low [ATP], high [AMP], vigorous muscle contraction) or for greater glucose consumption (high blood glucose, for example) cause increased transcription of the hexokinase IV gene. Glucose 6-phosphatase, the

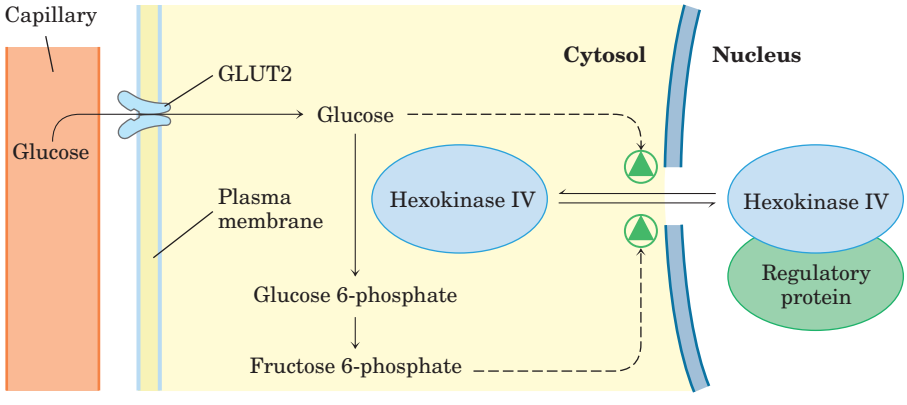


FIGURE 15–15 Regulation of hexokinase IV (glucokinase) by sequestration in the nucleus. The protein inhibitor of hexokinase IV is a nuclear binding protein that draws hexokinase IV into the nucleus when the fructose 6-phosphate concentration in liver is high and releases it to the cytosol when the glucose concentration is high.

gluconeogenic enzyme that bypasses the hexokinase step of glycolysis, is transcriptionally regulated by factors that call for increased production of glucose (low blood glucose, glucagon signaling). The transcriptional regulation of these two enzymes (along with other enzymes of glycolysis and gluconeogenesis) is described below.

Phosphofructokinase-1 and Fructose 1,6-Bisphosphatase Are Reciprocally Regulated

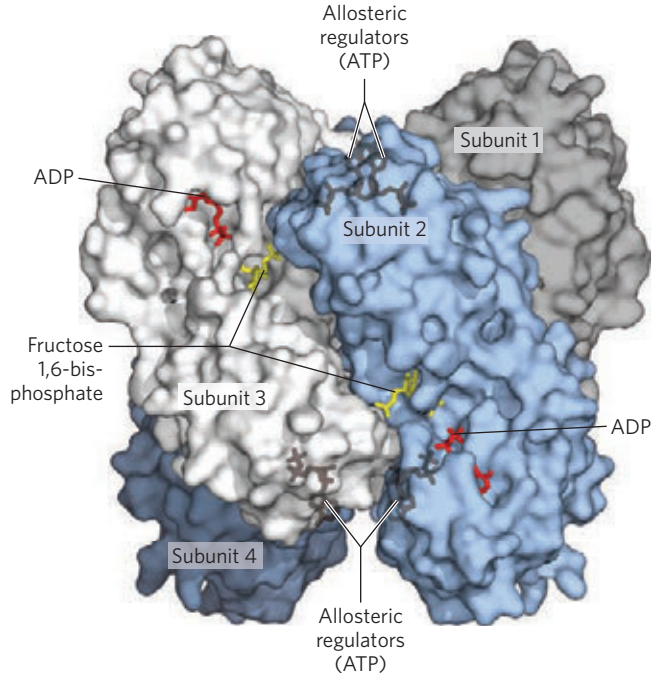
As we have noted, glucose 6-phosphate can flow either into glycolysis or through any of several other pathways, including glycogen synthesis and the pentose phosphate pathway. The metabolically irreversible reaction catalyzed by PFK-1 is the step that commits glucose to glycolysis. In addition to its substrate-binding sites, this complex enzyme has several regulatory sites at which allosteric activators or inhibitors bind.

ATP is not only a substrate for PFK-1 but also an end product of the glycolytic pathway. When high cellular [ATP] signals that ATP is being produced faster than it is being consumed, ATP inhibits PFK-1 by binding to an allosteric site and lowering the affinity of the enzyme for its substrate fructose 6-phosphate **(Fig. 15–16)**. ADP and AMP, which increase in concentration as consumption of ATP outpaces production, act allosterically to relieve this inhibition by ATP. These effects combine to produce higher enzyme activity when ADP or AMP accumulates and lower activity when ATP accumulates.

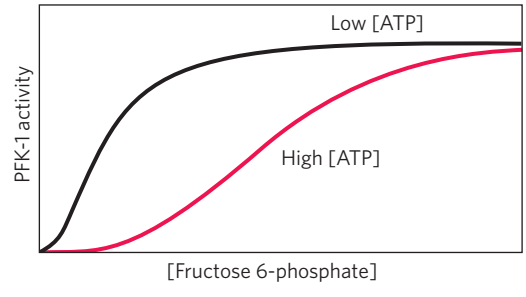
Citrate (the ionized form of citric acid), a key intermediate in the aerobic oxidation of pyruvate, fatty acids, and amino acids, is also an allosteric regulator of PFK-1; high citrate concentration increases the inhibitory effect of ATP, further reducing the flow of glucose through glycolysis. In this case, as in several others encountered later, citrate serves as an intracellular signal that the cell is meeting its current needs for energy-yielding metabolism by the oxidation of fats and proteins.

The corresponding step in gluconeogenesis is the conversion of fructose 1,6-bisphosphate to fructose 6-phosphate **(Fig. 15–17)**. The enzyme that catalyzes this reaction, FBPase-1, is strongly inhibited (allosterically) by AMP; when the cell's supply of ATP is low (corresponding to high [AMP]), the ATP-requiring synthesis of glucose slows.

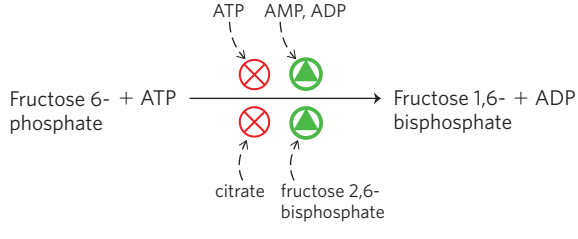
Thus these opposing steps in the glycolytic and gluconeogenic pathways—those catalyzed by PFK-1 and FBPase-1—are regulated in a coordinated and reciprocal manner. In general, when sufficient concentrations of acetyl-CoA or citrate (the product of acetyl-CoA condensation with oxaloacetate) are present, or when a high proportion of the cell's adenylate is in the form of ATP, gluconeogenesis is favored. When the level of AMP increases, it promotes glycolysis by stimulating PFK-1 (and, as we shall see in Section 15.5, promotes glycogen degradation by activating glycogen phosphorylase).



(a)



(b)



(c)

FIGURE 15–16 Phosphofructokinase-1 (PFK-1) and its regulation. **(a)** Surface contour image of *E. coli* PFK-1, showing portions of its four identical subunits (PDB ID 1PFK). Each subunit has its own catalytic site, where the products ADP and fructose 1,6-bisphosphate (red and yellow stick structures, respectively) are almost in contact, and its own binding sites for the allosteric regulator ATP, buried in the protein in the positions indicated. **(b)** Allosteric regulation of muscle PFK-1 by ATP, shown by a substrate-activity curve. At low [ATP], the $K_{0.5}$ for fructose 6-phosphate is relatively low, enabling the enzyme to function at a high rate at relatively low [fructose 6-phosphate]. (Recall from Chapter 6 that $K_{0.5}$ is the K_m term for regulatory enzymes.) When [ATP] is high, $K_{0.5}$ for fructose 6-phosphate is greatly increased, as indicated by the sigmoid relationship between substrate concentration and enzyme activity. **(c)** Summary of the regulators affecting PFK-1 activity.

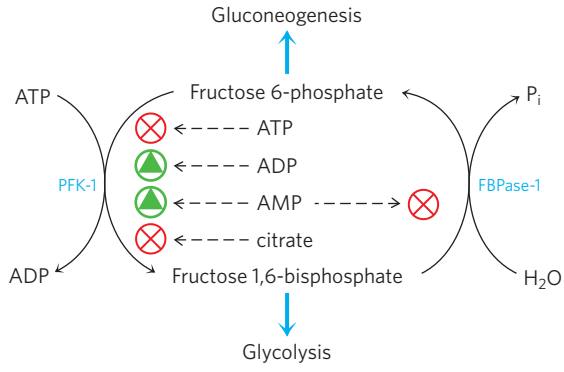
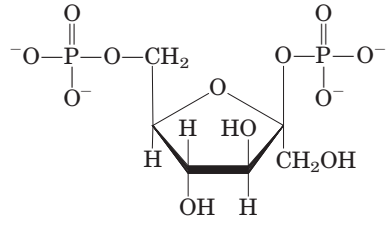


FIGURE 15–17 Regulation of fructose 1,6-bisphosphatase (FBPase-1) and phosphofructokinase-1 (PFK-1). The important role of fructose 2,6-bisphosphate in the regulation of this substrate cycle is detailed in subsequent figures.

Fructose 2,6-Bisphosphate Is a Potent Allosteric Regulator of PFK-1 and FBPase-1

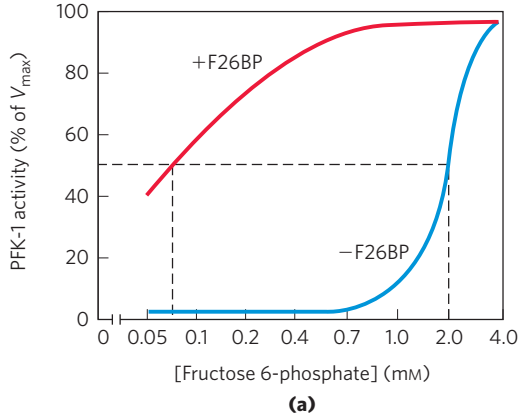
The special role of the liver in maintaining a constant blood glucose level requires additional regulatory mechanisms to coordinate glucose production and consumption. When the blood glucose level decreases, the hormone **glucagon** signals the liver to produce and release more glucose and to stop consuming it for its own needs. One source of glucose is glycogen stored in the liver; another source is gluconeogenesis, using pyruvate, lactate, glycerol, or certain amino acids as starting material. When blood glucose is high, insulin signals the liver to use glucose as a fuel and as a precursor for the synthesis and storage of glycogen and triacylglycerol.

The rapid hormonal regulation of glycolysis and gluconeogenesis is mediated by **fructose 2,6-bisphosphate**, an allosteric effector for the enzymes PFK-1 and FBPase-1:

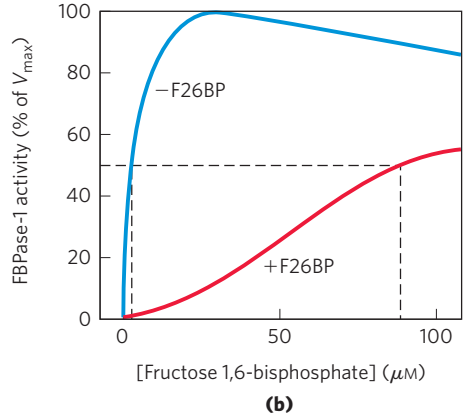


Fructose 2,6-bisphosphate

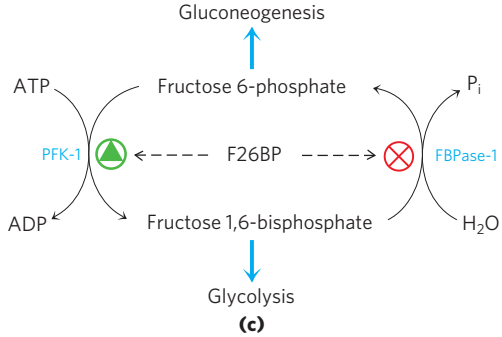
When fructose 2,6-bisphosphate binds to its allosteric site on PFK-1, it increases the enzyme's affinity for its substrate fructose 6-phosphate and reduces its affinity for the allosteric inhibitors ATP and citrate **(Fig. 15–18)**. At the physiological concentrations of its substrates, ATP and fructose 6-phosphate, and of its other positive and



(a)



(b)



(c)

FIGURE 15–18 Role of fructose 2,6-bisphosphate in regulation of glycolysis and gluconeogenesis. Fructose 2,6-bisphosphate (F26BP) has opposite effects on the enzymatic activities of phosphofructokinase-1 (PFK-1, a glycolytic enzyme) and fructose 1,6-bisphosphatase (FBPase-1, a gluconeogenic enzyme). **(a)** PFK-1 activity in the absence of F26BP (blue curve) is half-maximal when the concentration of fructose 6-phosphate is 2 mM (that is, $K_{0.5}$ = 2 mM). When 0.13 μM F26BP is present (red curve), the $K_{0.5}$ for fructose 6-phosphate is only 0.08 mM. Thus F26BP activates PFK-1 by increasing its apparent affinity for fructose 6-phosphate (see Fig. 15-16b). **(b)** FBPase-1 activity is inhibited by as little as 1 μM F26BP and is strongly inhibited by 25 μM. In the absence of this inhibitor (blue curve) the $K_{0.5}$ for fructose 1,6-bisphosphate is 5 μM, but in the presence of 25 μM F26BP (red curve) the $K_{0.5}$ is >70 μM. Fructose 2,6-bisphosphate also makes FBPase-1 more sensitive to inhibition by another allosteric regulator, AMP. **(c)** Summary of regulation by F26BP.

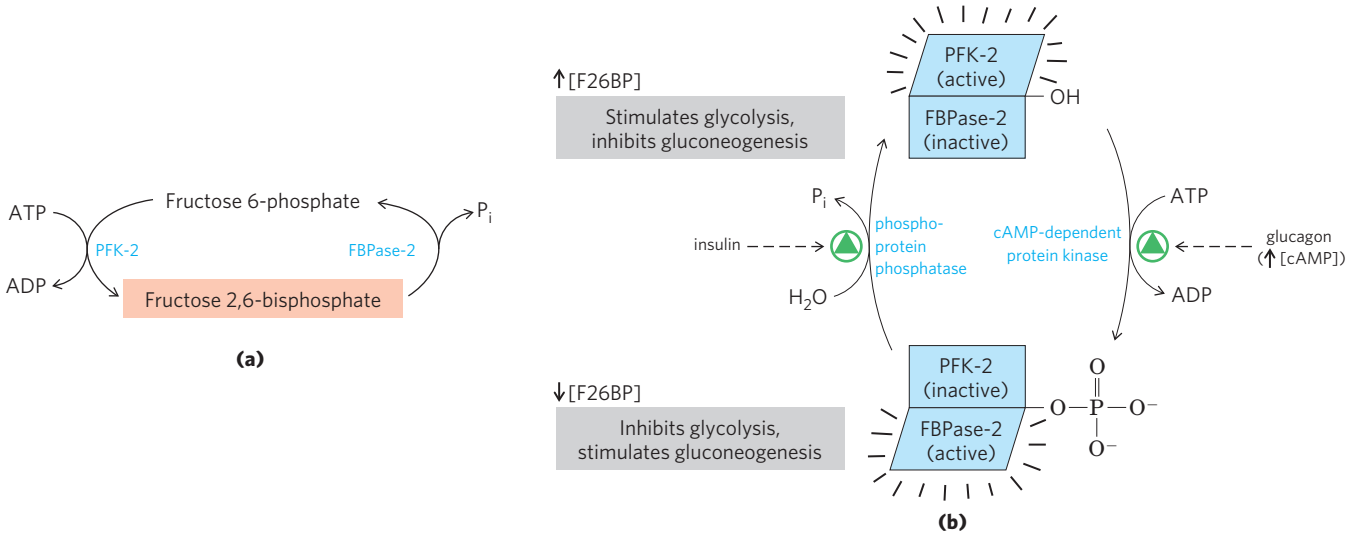


FIGURE 15–19 Regulation of fructose 2,6-bisphosphate level. (a) The cellular concentration of the regulator fructose 2,6-bisphosphate (F26BP) is determined by the rates of its synthesis by phosphofructokinase-2 (PFK-2) and its breakdown by fructose 2,6-bisphosphatase (FBPase-2). **(b)** Both enzyme activities are part of the same polypeptide chain, and they are reciprocally regulated by insulin and glucagon.

negative effectors (ATP, AMP, citrate), PFK-1 is virtually inactive in the absence of fructose 2,6-bisphosphate. Fructose 2,6-bisphosphate has the opposite effect on FBPase-1: it reduces its affinity for its substrate (Fig. 15–18b), thereby slowing gluconeogenesis.

The cellular concentration of the allosteric regulator fructose 2,6-bisphosphate is set by the relative rates of its formation and breakdown **(Fig. 15–19a)**. It is formed by phosphorylation of fructose 6-phosphate, catalyzed by **phosphofructokinase-2 (PFK-2)**, and is broken down by **fructose 2,6-bisphosphatase (FBPase-2)**. (Note that these enzymes are distinct from PFK-1 and FBPase-1, which catalyze the formation and breakdown, respectively, of fructose 1,6-bisphosphate.) PFK-2 and FBPase-2 are two separate enzymatic activities of a single, bifunctional protein. The balance of these two activities in the liver, which determines the cellular level of fructose 2,6-bisphosphate, is regulated by glucagon and insulin (Fig. 15–19b).

As we saw in Chapter 12 (p. 446), glucagon stimulates the adenylyl cyclase of liver to synthesize 3′,5′-cyclic AMP (cAMP) from ATP. Cyclic AMP then activates cAMP-dependent protein kinase, which transfers a phosphoryl group from ATP to the bifunctional protein PFK-2/FBPase-2. Phosphorylation of this protein enhances its FBPase-2 activity and inhibits its PFK-2 activity. Glucagon thereby lowers the cellular level of fructose 2,6-bisphosphate, inhibiting glycolysis and stimulating gluconeogenesis. The resulting production of more glucose enables the liver to replenish blood glucose in response to glucagon. Insulin has the opposite effect, stimulating the activity of a phosphoprotein phosphatase that catalyzes removal of the phosphoryl group from the bifunctional protein PFK-2/FBPase-2, activating its PFK-2 activity, increasing the level of fructose 2,6-bisphosphate, stimulating glycolysis, and inhibiting gluconeogenesis.

Xylulose 5-Phosphate Is a Key Regulator of Carbohydrate and Fat Metabolism

Another regulatory mechanism also acts by controlling the level of fructose 2,6-bisphosphate. In the mammalian liver, xylulose 5-phosphate (p. 577), a product of the pentose phosphate pathway (hexose monophosphate pathway), mediates the increase in glycolysis that follows ingestion of a high-carbohydrate meal. The xylulose 5-phosphate concentration rises as glucose entering the liver is converted to glucose 6-phosphate and enters both the glycolytic and pentose phosphate pathways. Xylulose 5-phosphate activates phosphoprotein phosphatase 2A (PP2A; **Fig. 15–20**), which dephosphorylates the bifunctional PFK-2/FBPase-2 enzyme (Fig. 15–19). Dephosphorylation activates PFK-2 and inhibits FBPase-2, and the resulting rise in fructose 2,6-bisphosphate concentration stimulates glycolysis and inhibits gluconeogenesis. The increased glycolysis boosts the production of acetyl-CoA, while the increased flow of hexose through the pentose phosphate pathway generates NADPH. Acetyl-CoA and NADPH are the starting materials for fatty acid synthesis, which has long been known to increase dramatically in response to intake of a high-carbohydrate meal. Xylulose 5-phosphate also increases the synthesis of *all* the enzymes required for fatty acid synthesis, meeting the prediction from metabolic control analysis. We return to this effect in our discussion of the integration of carbohydrate and lipid metabolism in Chapter 23.

The Glycolytic Enzyme Pyruvate Kinase Is Allosterically Inhibited by ATP

At least three isozymes of pyruvate kinase are found in vertebrates, differing in their tissue distribution and their response to modulators. High concentrations of

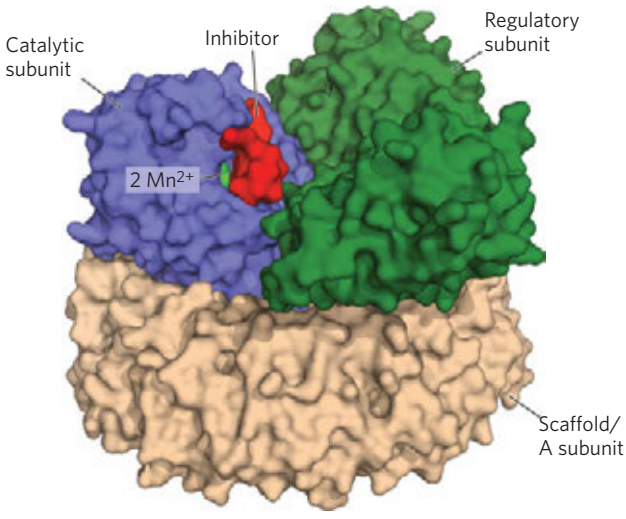


FIGURE 15–20 Structure and action of phosphoprotein phosphatase 2A (PP2A). (a) The catalytic subunit has two Mn^{2+} ions in its active site, positioned close to the substrate-recognition surface formed by the interface between the catalytic subunit and the regulatory subunit (PDB ID 2NPP). Microcystin-LR, shown here in red, is a specific inhibitor of PP2A. The catalytic and regulatory subunits rest in a scaffold (the A subunit) that positions them relative to each other and shapes the substrate-recognition site. **(b)** PP2A recognizes several target proteins, its specificity provided by the regulatory subunit. Each of several regulatory subunits fits the scaffold containing the catalytic subunit, and each regulatory subunit creates its unique substrate-binding site.

ATP, acetyl-CoA, and long-chain fatty acids (signs of abundant energy supply) allosterically inhibit all isozymes of pyruvate kinase **(Fig. 15–21)**. The liver isozyme (L form), but not the muscle isozyme (M form), is subject to further regulation by phosphorylation. When low blood glucose causes glucagon release, cAMP-dependent protein kinase phosphorylates the L isozyme of pyruvate kinase, inactivating it. This slows the use of

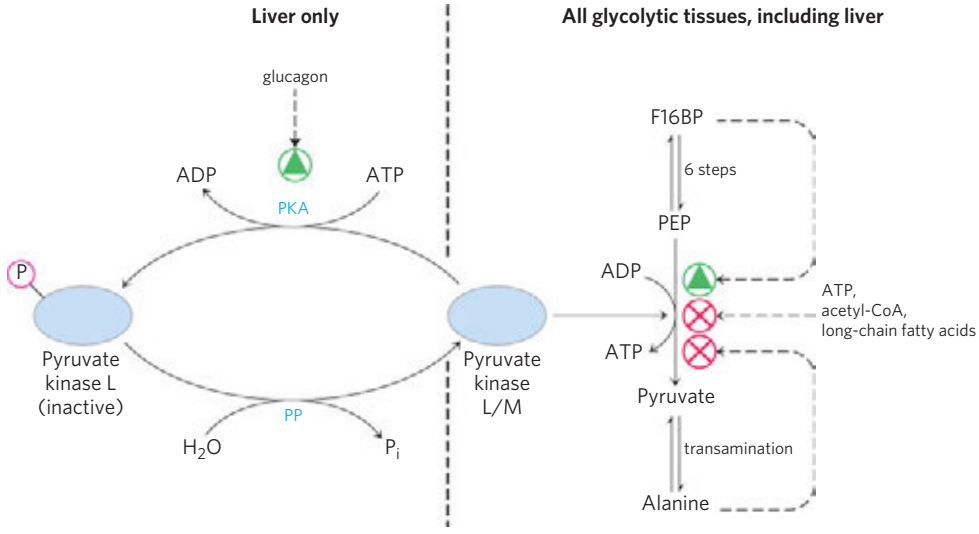


FIGURE 15–21 Regulation of pyruvate kinase. The enzyme is allosterically inhibited by ATP, acetyl-CoA, and long-chain fatty acids (all signs of an abundant energy supply), and the accumulation of fructose 1,6-bisphosphate triggers its activation. Accumulation of alanine, which can be synthesized from pyruvate in one step, allosterically inhibits pyruvate kinase, slowing the production of pyruvate by glycolysis. The liver isozyme (L form) is also regulated hormonally. Glucagon activates cAMP-dependent protein kinase (PKA; see Fig. 15–37), which phosphorylates the pyruvate kinase L isozyme, inactivating it. When the glucagon level drops, a protein phosphatase (PP) dephosphorylates pyruvate kinase, activating it. This mechanism prevents the liver from consuming glucose by glycolysis when blood glucose is low; instead, the liver exports glucose. The muscle isozyme (M form) is not affected by this phosphorylation mechanism.

glucose as a fuel in liver, sparing it for export to the brain and other organs. In muscle, the effect of increased [cAMP] is quite different. In response to epinephrine, cAMP activates glycogen breakdown and glycolysis and provides the fuel needed for the fight-or-flight response.

The Gluconeogenic Conversion of Pyruvate to Phosphoenolpyruvate Is under Multiple Types of Regulation

In the pathway leading from pyruvate to glucose, the first control point determines the fate of pyruvate in the mitochondrion: its conversion either to acetyl-CoA (by the pyruvate dehydrogenase complex) to fuel the citric acid cycle (Chapter 16) or to oxaloacetate (by pyruvate carboxylase) to start the process of gluconeogenesis **(Fig. 15–22)**. When fatty acids are readily available as fuels, their breakdown in liver mitochondria yields acetyl-CoA, a signal that further oxidation of glucose for fuel is not necessary. Acetyl-CoA is a positive allosteric modulator of pyruvate carboxylase and a negative modulator of pyruvate dehydrogenase, through stimulation of a protein kinase that inactivates the dehydrogenase. When the cell's energy needs are being met, oxidative phosphorylation slows, [NADH] rises relative to [NAD^+] and inhibits the citric acid cycle, and acetyl-CoA accumulates. The increased concentration of acetyl-CoA inhibits the pyruvate dehydrogenase complex, slowing the formation of acetyl-CoA from pyruvate, and stimulates gluconeogenesis by activating pyruvate carboxylase, allowing conversion of excess pyruvate to oxaloacetate (and, eventually, glucose).

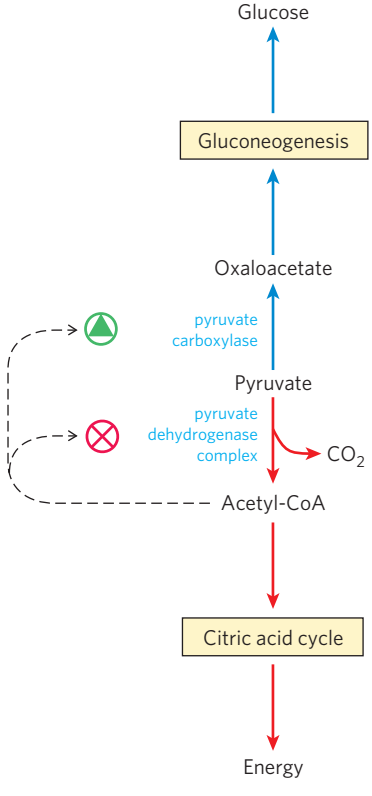


FIGURE 15–22 Two alternative fates for pyruvate. Pyruvate can be converted to glucose and glycogen via gluconeogenesis or oxidized to acetyl-CoA for energy production. The first enzyme in each path is regulated allosterically; acetyl-CoA, produced either by fatty acid oxidation or by the pyruvate dehydrogenase complex, stimulates pyruvate carboxylase and inhibits pyruvate dehydrogenase.

Oxaloacetate formed in this way is converted to phosphoenolpyruvate (PEP) in the reaction catalyzed by PEP carboxykinase (Fig. 15–13). In mammals, the regulation of this key enzyme occurs primarily at the level of its synthesis and breakdown, in response to dietary and hormonal signals. Fasting or high glucagon levels act through cAMP to increase the rate of transcription and to stabilize the mRNA. Insulin, or high blood glucose, has the opposite effects. We discuss this transcriptional regulation in more detail below. Generally triggered by a signal from outside the cell (diet, hormones), these changes take place on a time scale of minutes to hours.

Transcriptional Regulation of Glycolysis and Gluconeogenesis Changes the Number of Enzyme Molecules

Most of the regulatory actions discussed thus far are mediated by fast, quickly reversible mechanisms: allosteric effects, covalent alteration (phosphorylation) of the enzyme, or binding of a regulatory protein. Another set of regulatory processes involves changes in the number of molecules of an enzyme in the cell, through changes in the balance of enzyme synthesis and breakdown, and our discussion now turns to regulation of transcription through signal-activated transcription factors.

In Chapter 12 we encountered nuclear receptors and transcription factors in the context of insulin signaling. Insulin acts through its receptor in the plasma membrane to turn on at least two distinct signaling pathways, each involving activation of a protein kinase. The MAP kinase ERK, for example, phosphorylates the transcription factors SRF and Elk1 (see Fig. 12–15), which then stimulate the synthesis of enzymes needed for cell growth and division. Protein kinase B (PKB; also called Akt) phosphorylates another set of transcription factors (PDX1, for example), and these stimulate the synthesis of enzymes that metabolize carbohydrates and the fats formed and stored following excess carbohydrate intake in the diet. In pancreatic β cells, PDX1 also stimulates the synthesis of insulin itself.

More than 150 genes are transcriptionally regulated by insulin; humans have at least seven general types of insulin response elements, each recognized by a subset of transcription factors activated by insulin under various conditions. Insulin stimulates the transcription of the genes that encode hexokinases II and IV, PFK-1, pyruvate kinase, and PFK-2/FBPase-2 (all involved in glycolysis and its regulation); several enzymes of fatty acid synthesis; and glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, enzymes of the pentose phosphate pathway that generate the NADPH

TABLE 15–5 Some of the Genes Regulated by Insulin

Change in gene expression	Pathway
Increased expression	
Hexokinase II	Glycolysis
Hexokinase IV	Glycolysis
Phosphofructokinase-1 (PFK-1)	Glycolysis
Pyruvate kinase	Glycolysis
PFK-2/FBPase-2	Regulation of glycolysis/gluconeogenesis
Glucose 6-phosphate dehydrogenase	Pentose phosphate pathway (NADPH)
6-Phosphogluconate dehydrogenase	Pentose phosphate pathway (NADPH)
Pyruvate dehydrogenase	Fatty acid synthesis
Acetyl-CoA carboxylase	Fatty acid synthesis
Malic enzyme	Fatty acid synthesis (NADPH)
ATP-citrate lyase	Fatty acid synthesis (provides acetyl-CoA)
Fatty acid synthase complex	Fatty acid synthesis
Stearoyl-CoA dehydrogenase	Fatty acid desaturation
Acyl-CoA–glycerol transferases	Triacylglycerol synthesis
Decreased expression	
PEP carboxykinase	Gluconeogenesis
Glucose 6-phosphatase (catalytic subunit)	Glucose release to blood

required for fatty acid synthesis. Insulin also slows the expression of the genes for two enzymes of gluconeogenesis: PEP carboxykinase and glucose 6-phosphatase (Table 15–5).

One transcription factor important to carbohydrate metabolism is **ChREBP** (**carbohydrate response element binding protein**; **Fig. 15–23**), which is expressed primarily in liver, adipose tissue, and kidney. It serves to coordinate the synthesis of enzymes needed for carbohydrate and fat synthesis. ChREBP in its inactive state is phosphorylated, and is located in the cytosol. When the phosphoprotein phosphatase PP2A (Fig. 15–20) removes a phosphoryl group from ChREBP, the transcription factor can enter the nucleus. Here, nuclear PP2A removes another phosphoryl group, and ChREBP now joins with a partner protein, Mlx, and turns on the synthesis of several enzymes: pyruvate kinase, fatty acid synthase, and acetyl-CoA carboxylase, the first enzyme in the path to fatty acid synthesis.

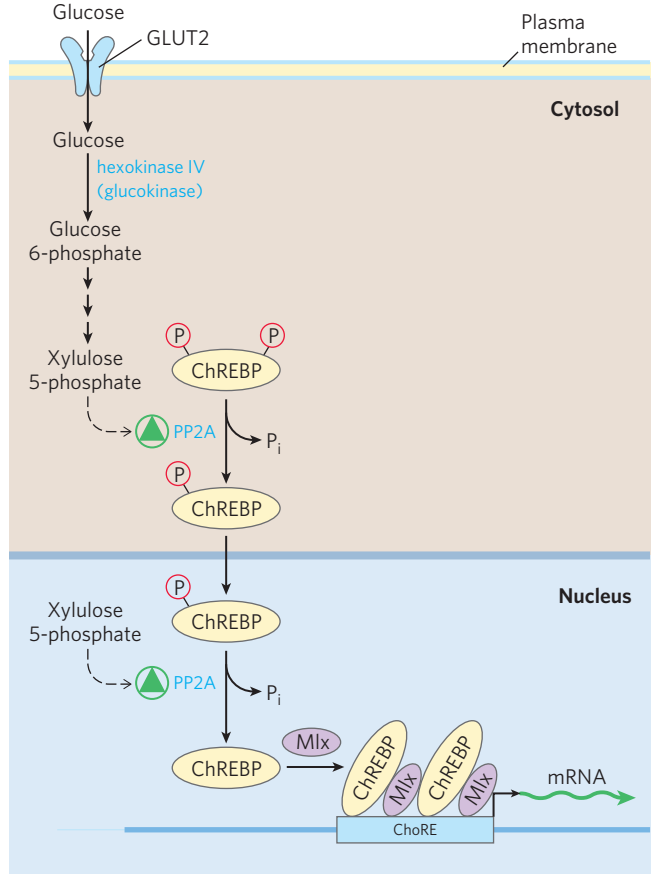


FIGURE 15–23 Mechanism of gene regulation by the transcription factor ChREBP. When ChREBP in the cytosol of a hepatocyte is phosphorylated on a Ser and a Thr residue, it cannot enter the nucleus. Dephosphorylation of Ⓟ-Ser by protein phosphatase PP2A allows ChREBP to enter the nucleus, where a second dephosphorylation, of Ⓟ-Thr, activates ChREBP so that it can associate with its partner protein, Mlx. ChREBP-Mlx now binds to the carbohydrate response element (ChoRE) in the promoter and stimulates transcription. PP2A is allosterically activated by xylulose 5-phosphate, an intermediate in the pentose phosphate pathway.

Controlling the activity of PP2A—and thus, ultimately, the synthesis of this group of metabolic enzymes—is xylulose 5-phosphate, an intermediate of the pentose phosphate pathway (Fig. 14–23). When blood glucose concentration is high, glucose enters the liver and is phosphorylated by hexokinase IV. The glucose 6-phosphate thus formed can enter either the glycolytic pathway or the pentose phosphate pathway. If the latter, two initial oxidations produce xylulose 5-phosphate, which serves as a signal that the glucose-utilizing pathways are well-supplied with substrate. It accomplishes this by allosterically activating PP2A, which then dephosphorylates ChREBP, allowing the transcription factor to turn on the expression of genes for enzymes of glycolysis and fat synthesis (Fig. 15–23). Glycolysis yields pyruvate, and conversion of pyruvate to acetyl-CoA provides the starting material for fatty acid synthesis: acetyl-CoA carboxylase converts acetyl-CoA to malonyl-CoA, the first committed intermediate in the path to fatty acids. The fatty acid synthase complex produces fatty acids for export to adipose tissue and storage as triacylglycerols (Chapter 21). In this way, excess dietary carbohydrate is stored as fat.

Another transcription factor in the liver, **SREBP-1c**, a member of the family of **sterol regulatory element binding proteins** (see Fig. 21–44), turns on the synthesis of pyruvate kinase, hexokinase IV, lipoprotein lipase, acetyl-CoA carboxylase, and the fatty acid synthase complex that will convert acetyl-CoA (produced from pyruvate) into fatty acids for storage in adipocytes. The synthesis of SREBP-1c is stimulated by insulin and depressed by glucagon. SREBP-1c also suppresses the expression of several gluconeogenic enzymes: glucose 6-phosphatase, PEP carboxykinase, and FBPase-1.

The transcription factor **CREB** (**cyclic AMP response element binding protein**) turns on the synthesis of glucose 6-phosphatase and PEP carboxykinase in response to the increase in [cAMP] triggered by glucagon. In contrast, insulin-stimulated *inactivation* of other transcription factors turns off several gluconeogenic enzymes in the liver: PEP carboxykinase, fructose 1,6-bisphosphatase, the glucose 6-phosphate transporter of the endoplasmic reticulum, and glucose 6-phosphatase. For example, **FOXO1 (forkhead box other)** stimulates the synthesis of gluconeogenic enzymes and suppresses the synthesis of the enzymes of glycolysis, the pentose phosphate pathway, and triacylglycerol synthesis **(Fig. 15–24)**. In its unphosphorylated form, FOXO1 acts as a nuclear transcription factor. In response to insulin, FOXO1 leaves the nucleus and in the cytosol is phosphorylated by PKB, then tagged with ubiquitin and degraded by the proteasome. Glucagon prevents this phosphorylation by PKB, and FOXO1 remains active in the nucleus.

Complicated though the processes outlined above may seem, regulation of the genes encoding enzymes of carbohydrate and fat metabolism is proving far more complex and more subtle than we have shown here. Multiple transcription factors can act on the same gene promoter; multiple protein kinases and phosphatases can activate or inactivate these transcription factors; and a variety of protein accessory factors modulate the action of the transcription factors. This complexity is apparent, for example, in the gene encoding PEP carboxykinase, a very well-studied case of transcriptional control. Its promoter region **(Fig. 15–25)** has 15 or more response elements that are recognized by at least a dozen known transcription factors, with more likely to be discovered. The transcription factors act in combination on this promoter region, and on hundreds of other gene promoters, to fine-tune the levels of hundreds of metabolic enzymes, coordinating their activity in the metabolism of carbohydrates and fats. The critical importance of transcription factors in metabolic regulation is made clear by observing the effects of mutations in their genes. For example, at least five different types of maturity-onset diabetes of the young (MODY) are associated with mutations in specific transcription factors (Box 15–3).

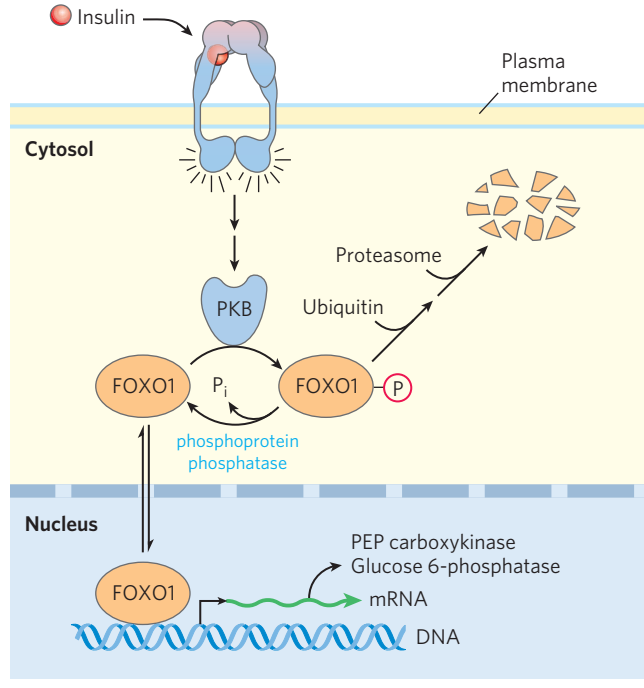
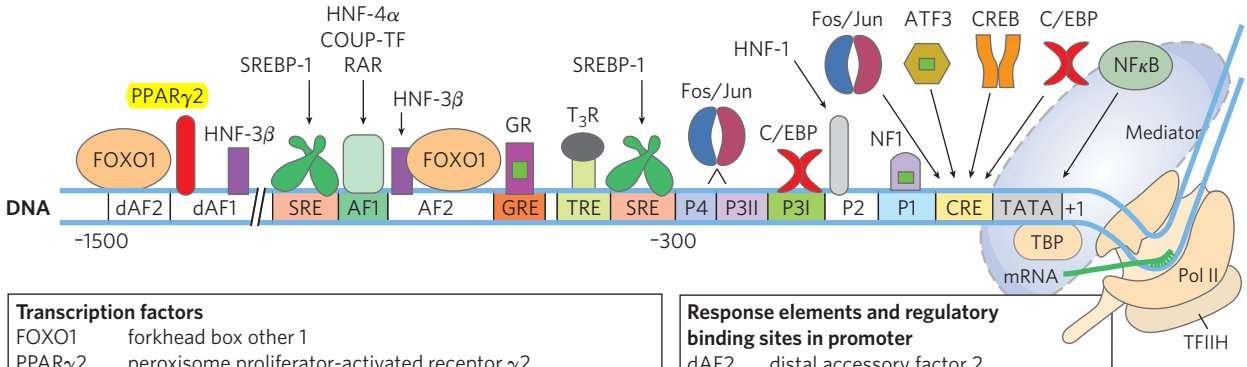


FIGURE 15–24 Mechanism of gene regulation by the transcription factor FOXO1. Insulin activates the signaling cascade shown in Figure 12–16, leading to activation of protein kinase B (PKB). FOXO1 in the cytosol is phosphorylated by PKB, and the phosphorylated transcription factor is tagged by the attachment of ubiquitin for degradation by proteasomes. FOXO1 that remains unphosphorylated or is dephosphorylated can enter the nucleus, bind to a response element, and trigger transcription of the associated genes. Insulin therefore has the effect of turning *off* the expression of these genes, which include PEP carboxykinase and glucose 6-phosphatase.



Transcription factors	
FOXO1	forkhead box other 1
PPARγ2	peroxisome proliferator-activated receptor γ2
HNF-3β	hepatic nuclear factor-3β
SREBP-1	sterol regulatory element binding protein-1
HNF-4α	hepatic nuclear factor-4α
COUP-TF	chicken ovalbumin upstream promoter–transcription factor
RAR	retinoic acid receptor
GR	glucocorticoid receptor
T_3R	thyroid hormone receptor
C/EBP	CAAT/enhance binding protein
HNF-1	hepatic nuclear factor-1
NF1	nuclear factor 1
ATF3	activating transcription factor 3
CREB	cAMP response element binding protein
NFκB	nuclear factor κB
TBP	TATA-box binding protein
TFIIH	transcription factor IIH

Response elements and regulatory binding sites in promoter	
dAF2	distal accessory factor 2
dAF1	distal accessory factor 1
SRE	sterol regulatory element
AF1	accessory factor 1
AF2	accessory factor 2
GRE	glucocorticoid regulatory element
TRE	thyroid hormone regulatory element
CRE	cAMP regulatory element

FIGURE 15–25 The PEP carboxykinase promoter region, showing the complexity of regulatory input to this gene. This diagram shows the transcription factors (smaller icons, bound to the DNA) known to regulate the transcription of the PEP carboxykinase gene. The extent to which this gene is expressed depends on the combined input affecting all of these factors, which can reflect the availability of nutrients, blood glucose level, and other circumstances that affect the cell's need for this enzyme at any particular time. P1, P2, P3I, P3II, and P4 are protein-binding sites identified by DNase I footprinting (see Box 26–1). The TATA box is the assembly point for the RNA polymerase II (Pol II) transcription complex.

BOX 15–3 MEDICINE Genetic Mutations That Lead to Rare Forms of Diabetes

The term "diabetes" describes a variety of medical conditions that have in common an excessive production of urine. In Box 11–1 we described diabetes insipidus, in which defective water reabsorption in the kidney results from a mutation in the gene for aquaporin. "Diabetes mellitus" refers specifically to disease in which the ability to metabolize glucose is defective, due either to the failure of the pancreas to produce insulin or to tissue resistance to the actions of insulin.

There are two common types of diabetes mellitus. Type 1, also called insulin-dependent diabetes mellitus (IDDM), is caused by autoimmune attack on the insulin-producing β cells of the pancreas. Individuals with IDDM must take insulin by injection or inhalation to compensate for their missing β cells. IDDM develops in childhood or in the teen years; an older name for the disease is juvenile diabetes. Type 2, also called non–insulin-dependent diabetes mellitus (NIDDM), typically develops in adults over 40 years old. It is far more common than IDDM, and its occurrence in the population is strongly correlated with obesity. The current epidemic of obesity in the more developed countries brings with it the promise of an epidemic of NIDDM, providing a strong incentive to understand the relationship between obesity and the onset of NIDDM at the genetic and biochemical levels. After completing our look at the metabolism of fats and proteins in later chapters, we will return (in Chapter 23) to the discussion of diabetes, which has a broad effect on metabolism: of carbohydrates, fats, and proteins.

Here we consider another type of diabetes in which carbohydrate and fat metabolism is deranged: mature onset diabetes of the young (MODY), in which genetic mutation affects a transcription factor important in carrying the insulin signal into the nucleus, or affects an enzyme that responds to insulin. In MODY2, a mutation in the hexokinase IV (glucokinase) gene affects the liver and pancreas, tissues in which this is the main isoform of hexokinase. The glucokinase of pancreatic β cells functions as a glucose sensor. Normally, when blood glucose

(*Continued on the next page*)

BOX 15–3 MEDICINE Genetic Mutations That Lead to Rare Forms of Diabetes (*Continued*)

rises, so does the glucose level in β cells, and because glucokinase has a relatively high K_m for glucose, its activity increases with rising blood glucose levels. Metabolism of the glucose 6-phosphate formed in this reaction raises the ATP level in β cells, and this triggers insulin release by the mechanism shown in Fig. 23–27. In healthy individuals, blood glucose concentrations of ~5 mM trigger this insulin release. But individuals with inactivating mutations in both copies of the glucokinase gene have very high thresholds for insulin release, and consequently, from birth, they have severe hyperglycemia—permanent neonatal diabetes. In individuals with one mutated and one normal copy of the glucokinase gene, the glucose threshold for insulin release rises to about 7 mM. As a result these individuals have blood glucose levels only slightly above normal: they generally have only mild hyperglycemia and no symptoms. This condition (MODY2) is generally discovered by accident during routine blood glucose analysis.

There are at least five other types of MODY, each the result of an inactivating mutation in one or another of the transcription factors essential to the normal development and function of pancreatic β cells. Individuals with these mutations have varying degrees of reduced insulin production and the associated defects in blood glucose homeostasis. In MODY1 and MODY3, the defects are severe enough to produce the long-term complications associated with IDDM and NIDDM—cardiovascular problems, kidney failure, and blindness. MODY4, 5, and 6 are less severe forms of the disease. Altogether, MODY disorders represent a small percentage of NIDDM cases. Also very rare are individuals with mutations in the insulin gene itself; they have defects in insulin signaling of varying severity.

SUMMARY 15.3 Coordinated Regulation of Glycolysis and Gluconeogenesis

- Gluconeogenesis and glycolysis share seven enzymes, catalyzing the freely reversible reactions of the pathways. For the other three steps, the forward and reverse reactions are catalyzed by different enzymes, and these are the points of regulation of the two pathways.
- Hexokinase IV (glucokinase) has kinetic properties related to its special role in the liver: releasing glucose to the blood when blood glucose is low, and taking up and metabolizing glucose when blood glucose is high.
- PFK-1 is allosterically inhibited by ATP and citrate. In most mammalian tissues, including liver, fructose 2,6-bisphosphate is an allosteric activator of this enzyme.
- Pyruvate kinase is allosterically inhibited by ATP, and the liver isozyme also is inhibited by cAMP-dependent phosphorylation.
- Gluconeogenesis is regulated at the level of pyruvate carboxylase (which is activated by acetyl-CoA) and FBPase-1 (which is inhibited by fructose 2,6-bisphosphate and AMP).
- To limit substrate cycling between glycolysis and gluconeogenesis, the two pathways are under reciprocal allosteric control, mainly achieved by the opposing effects of fructose 2,6-bisphosphate on PFK-1 and FBPase-1.
- Glucagon or epinephrine decreases [fructose 2,6-bisphosphate], by raising [cAMP] and bringing about phosphorylation of the bifunctional enzyme PFK-2/FBPase-2. Insulin increases [fructose 2,6-bisphosphate] by activating a phosphoprotein phosphatase that dephosphorylates and thus activates PFK-2.
- Xylulose 5-phosphate, an intermediate of the pentose phosphate pathway, activates phosphoprotein phosphatase PP2A, which dephosphorylates several target proteins, including PFK-2/FBPase-2, tilting the balance toward glucose uptake, glycogen synthesis, and lipid synthesis in the liver.
- Transcription factors including ChREBP, CREB, SREBP, and FOXO1 act in the nucleus to regulate the expression of specific genes coding for enzymes of the glycolytic and gluconeogenic pathways. Insulin and glucagon act antagonistically in activating these transcription factors, thus turning on and off large numbers of genes.

15.4 The Metabolism of Glycogen in Animals

Our discussion of metabolic regulation, using carbohydrate metabolism as the primary example, now turns to the synthesis and breakdown of glycogen. In this section we focus on the metabolic pathways; in Section 15.5 we turn to the regulatory mechanisms.

In organisms from bacteria to plants to vertebrates, excess glucose is converted to polymeric forms for storage—glycogen in vertebrates and many microorganisms, starch in plants. In vertebrates, glycogen is found primarily in the liver and skeletal muscle; it may repre-

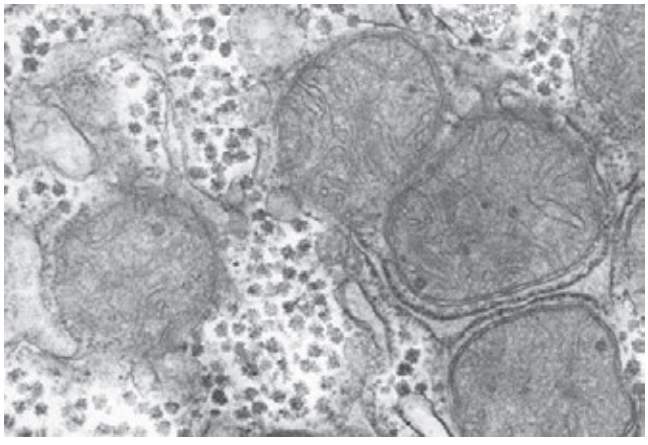


FIGURE 15–26 Glycogen granules in a hepatocyte. Glycogen, a storage form of carbohydrate, appears as electron-dense particles, often in aggregates or rosettes. In hepatocytes glycogen is closely associated with tubules of the smooth endoplasmic reticulum. Many mitochondria are also evident in this micrograph.

sent up to 10% of the weight of liver and 1% to 2% of the weight of muscle. If this much glucose were dissolved in the cytosol of a hepatocyte, its concentration would be about 0.4 M, enough to dominate the osmotic properties of the cell. When stored as a large polymer (glycogen), however, the same mass of glucose has a concentration of only 0.01 μM. Glycogen is stored in large cytosolic granules. The elementary particle of glycogen, the β-particle, is about 21 nm in diameter and consists of up to 55,000 glucose residues with about 2,000 nonreducing ends. Twenty to 40 of these particles cluster together to form α-rosettes, easily seen with the microscope in tissue samples from well-fed animals **(Fig. 15–26)** but essentially absent after a 24-hour fast.

The glycogen in muscle is there to provide a quick source of energy for either aerobic or anaerobic metabolism. Muscle glycogen can be exhausted in less than an hour during vigorous activity. Liver glycogen serves as a reservoir of glucose for other tissues when dietary glucose is not available (between meals or during a fast); this is especially important for the neurons of the brain, which cannot use fatty acids as fuel. Liver glycogen can be depleted in 12 to 24 hours. In humans, the total amount of energy stored as glycogen is far less than the amount stored as fat (triacylglycerol) (see Table 23–5), but fats cannot be converted to glucose in mammals and cannot be catabolized anaerobically.

Glycogen granules are complex aggregates of glycogen and the enzymes that synthesize it and degrade it, as well as the machinery for regulating these enzymes. The general mechanisms for storing and mobilizing glycogen are the same in muscle and liver, but the enzymes differ in subtle yet important ways that reflect the different roles of glycogen in the two tissues. Glycogen is also obtained in the diet and broken down in the gut, and this involves a separate set of hydrolytic enzymes that convert glycogen to free glucose. (Dietary starch is hydrolyzed in a similar way.) We begin our discussion with the breakdown of glycogen to glucose 1-phosphate (**glycogenolysis**), then turn to synthesis of glycogen (**glycogenesis**).

Glycogen Breakdown Is Catalyzed by Glycogen Phosphorylase

In skeletal muscle and liver, the glucose units of the outer branches of glycogen enter the glycolytic pathway through the action of three enzymes: glycogen phosphorylase, glycogen debranching enzyme, and phosphoglucomutase. Glycogen phosphorylase catalyzes the reaction in which an (α1→4) glycosidic linkage between two glucose residues at a nonreducing end of glycogen undergoes attack by inorganic phosphate (P_i), removing the terminal glucose residue as **α-D-glucose 1-phosphate (Fig. 15–27)**. This *phosphorolysis* reaction is

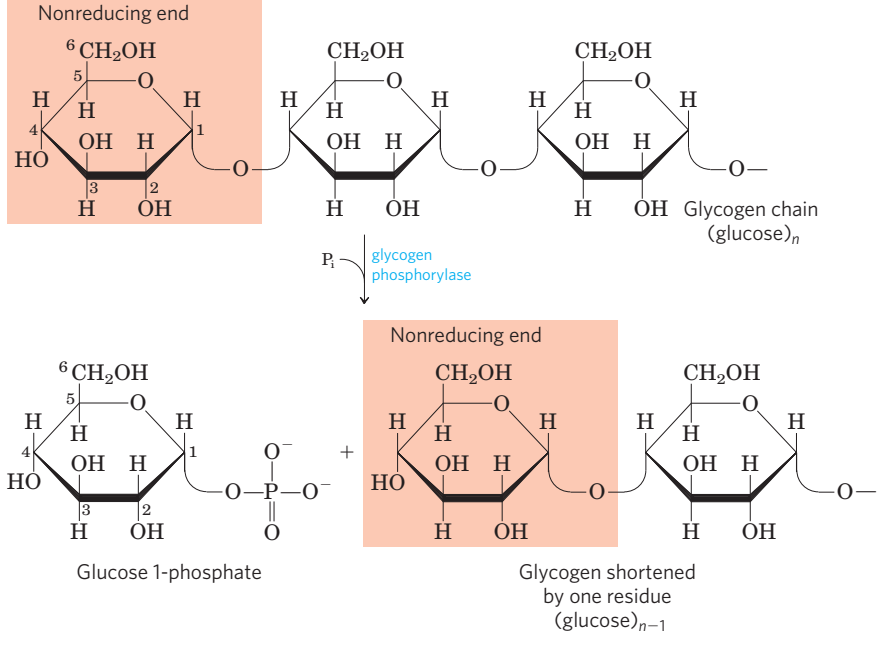


FIGURE 15–27 Removal of a glucose residue from the nonreducing end of a glycogen chain by glycogen phosphorylase. This process is repetitive; the enzyme removes successive glucose residues until it reaches the fourth glucose unit from a branch point (see Fig. 15–28).

different from the *hydrolysis* of glycosidic bonds by amylase during intestinal degradation of dietary glycogen and starch. In phosphorolysis, some of the energy of the glycosidic bond is preserved in the formation of the phosphate ester, glucose 1-phosphate (see Section 14.2).

Pyridoxal phosphate is an essential cofactor in the glycogen phosphorylase reaction; its phosphate group acts as a general acid catalyst, promoting attack by P_i on the glycosidic bond. (This is an unusual role for pyridoxal phosphate; its more typical role is as a cofactor in amino acid metabolism; see Fig. 18–6.)

Glycogen phosphorylase acts repetitively on the nonreducing ends of glycogen branches until it reaches a point four glucose residues away from an (α1→6) branch point (see Fig. 7–13), where its action stops. Further degradation by glycogen phosphorylase can occur only after the **debranching enzyme**, formally known as **oligo (α1→6) to (α1→4) glucantransferase**, catalyzes two successive reactions that transfer branches **(Fig. 15–28)**. Once these branches are transferred and the glucosyl residue at C-6 is hydrolyzed, glycogen phosphorylase activity can continue.

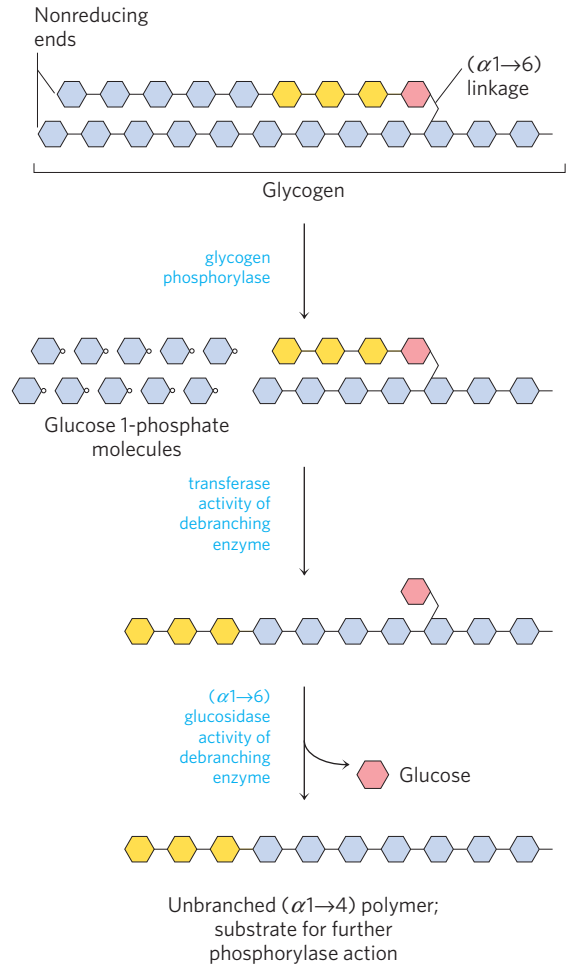


FIGURE 15–28 Glycogen breakdown near an (α1→6) branch point. Following sequential removal of terminal glucose residues by glycogen phosphorylase (see Fig. 15–27), glucose residues near a branch are removed in a two-step process that requires a bifunctional debranching enzyme. First, the transferase activity of the enzyme shifts a block of three glucose residues from the branch to a nearby nonreducing end, to which they are reattached in (α1→4) linkage. The single glucose residue remaining at the branch point, in (α1→6) linkage, is then released as free glucose by the debranching enzyme's (α1→6) glucosidase activity. The glucose residues are shown in shorthand form, which omits the —H, —OH, and —CH_2OH groups from the pyranose rings.

Glucose 1-Phosphate Can Enter Glycolysis or, in Liver, Replenish Blood Glucose

Glucose 1-phosphate, the end product of the glycogen phosphorylase reaction, is converted to glucose 6-phosphate by **phosphoglucomutase**, which catalyzes the reversible reaction

$$\text{Glucose 1-phosphate} \rightleftharpoons \text{glucose 6-phosphate}$$

Initially phosphorylated at a Ser residue, the enzyme donates a phosphoryl group to C-6 of the substrate, then accepts a phosphoryl group from C-1 **(Fig. 15–29)**.

The glucose 6-phosphate formed from glycogen in skeletal muscle can enter glycolysis and serve as an energy source to support muscle contraction. In liver, glycogen breakdown serves a different purpose: to release glucose into the blood when the blood glucose

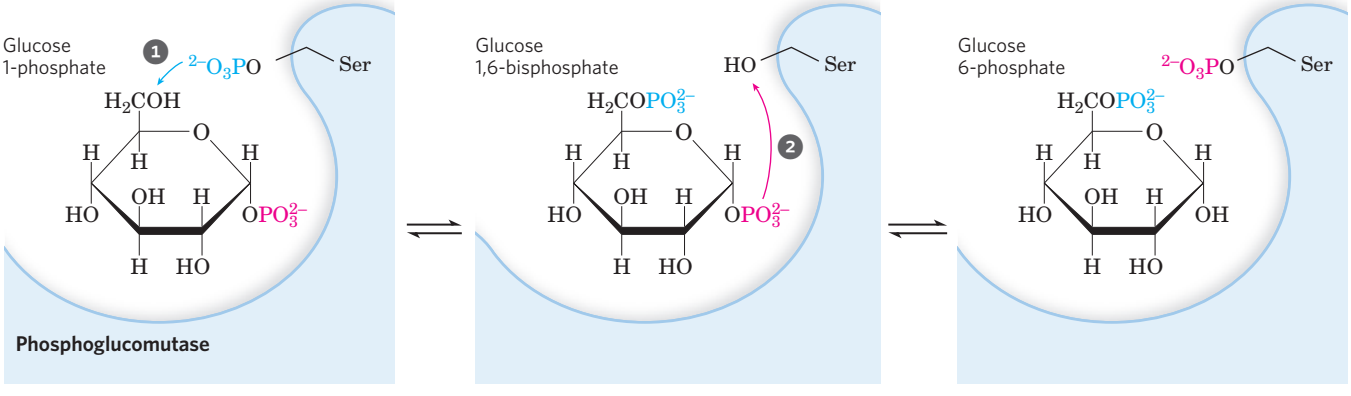


FIGURE 15–29 Reaction catalyzed by phosphoglucomutase. The reaction begins with the enzyme phosphorylated on a Ser residue. In step ❶, the enzyme donates its phosphoryl group (blue) to glucose 1-phosphate, producing glucose 1,6-bisphosphate. In step ❷, the phosphoryl group at C-1 of glucose 1,6-bisphosphate (red) is transferred back to the enzyme, reforming the phosphoenzyme and producing glucose 6-phosphate.

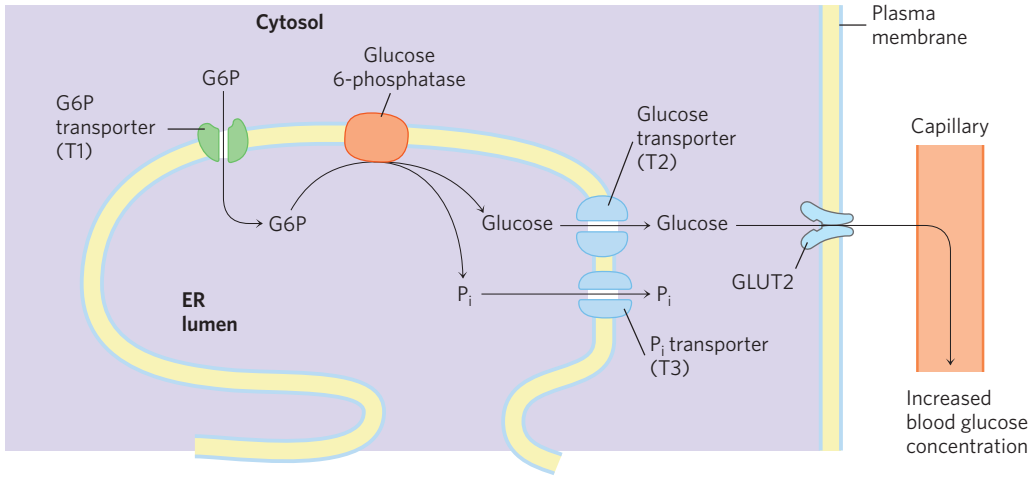


FIGURE 15–30 Hydrolysis of glucose 6-phosphate by glucose 6-phosphatase of the ER. The catalytic site of glucose 6-phosphatase faces the lumen of the ER. A glucose 6-phosphate (G6P) transporter (T1) carries the substrate from the cytosol to the lumen, and the products glucose and P_i pass to the cytosol on specific transporters (T2 and T3). Glucose leaves the cell via the GLUT2 transporter in the plasma membrane.

level drops, as it does between meals. This requires the enzyme glucose 6-phosphatase, present in liver and kidney but not in other tissues. The enzyme is an integral membrane protein of the endoplasmic reticulum, predicted to contain nine transmembrane helices, with its active site on the lumenal side of the ER. Glucose 6-phosphate formed in the cytosol is transported into the ER lumen by a specific transporter (T1) **(Fig. 15–30)** and hydrolyzed at the lumenal surface by the glucose 6-phosphatase. The resulting P_i and glucose are thought to be carried back into the cytosol by two different transporters (T2 and T3), and the glucose leaves the hepatocyte via the plasma membrane transporter, GLUT2. Notice that by having the active site of glucose 6-phosphatase inside the ER lumen, the cell separates this reaction from the process of glycolysis, which takes place in the cytosol and would be aborted by the action of glucose 6-phosphatase. Genetic defects in either glucose 6-phosphatase or T1 lead to serious derangement of glycogen metabolism, resulting in type Ia glycogen storage disease (Box 15–4).

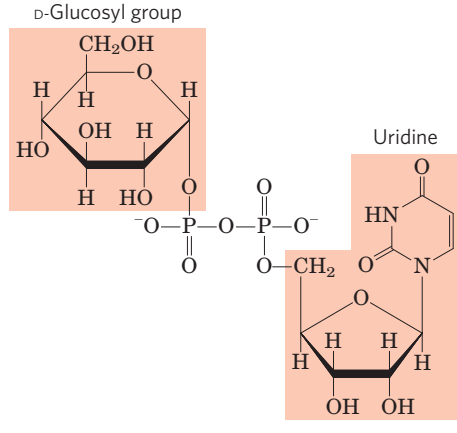
Because muscle and adipose tissue lack glucose 6-phosphatase, they cannot convert the glucose 6-phosphate formed by glycogen breakdown to glucose, and these tissues therefore do not contribute glucose to the blood.

The Sugar Nucleotide UDP-Glucose Donates Glucose for Glycogen Synthesis



Luis Leloir, 1906–1987

Many of the reactions in which hexoses are transformed or polymerized involve **sugar nucleotides**, compounds in which the anomeric carbon of a sugar is activated by attachment to a nucleotide through a phosphate ester linkage. Sugar nucleotides are the substrates for polymerization of monosaccharides into disaccharides, glycogen, starch, cellulose, and more complex extracellular polysaccharides. They are also key intermediates in the production of the aminohexoses and deoxyhexoses found in some of these polysaccharides, and in the synthesis of vitamin C (l-ascorbic acid). The role of sugar nucleotides in the biosynthesis of glycogen and many other carbohydrate derivatives was discovered in 1953 by the Argentine biochemist Luis Leloir.



UDP-glucose
(a sugar nucleotide)

The suitability of sugar nucleotides for biosynthetic reactions stems from several properties:

1. Their formation is metabolically irreversible, contributing to the irreversibility of the synthetic pathways in which they are intermediates. The condensation of a nucleoside triphosphate with a hexose 1-phosphate to form a sugar nucleotide has a small positive free-energy change, but the reaction releases PP_i, which is rapidly hydrolyzed by inorganic pyrophosphatase **(Fig. 15–31)**, in a reaction that is strongly exergonic ($\Delta G'^\circ = -19.2$ kJ/mol). This keeps the cellular concentration of PP_i low, ensuring that the actual free-energy change in the cell is favorable. In effect, rapid removal of the product, driven by the large, negative free-energy change of PP_i

BOX 15–4 Carl and Gerty Cori: Pioneers in Glycogen Metabolism and Disease

Much of what is written in present-day biochemistry textbooks about the metabolism of glycogen was discovered between about 1925 and 1950 by the remarkable husband and wife team of Carl F. Cori and Gerty T. Cori. Both trained in medicine in Europe at the end of World War I (she completed premedical studies and medical school in one year!). They left Europe together in 1922 to establish research laboratories in the United States, first for nine years in Buffalo, New York, at what is now the Roswell Park Memorial Institute, then from 1931 until the end of their lives at Washington University in St. Louis.

In their early physiological studies of the origin and fate of glycogen in animal muscle, the Coris demonstrated the conversion of glycogen to lactate in tissues, movement of lactate in the blood to the liver, and, in the liver, reconversion of lactate to glycogen—a pathway that came to be known as the Cori cycle (see Fig. 23–19). Pursuing these observations at the biochemical level, they showed that glycogen was mobilized in a phosphorolysis reaction catalyzed by the enzyme they discovered, glycogen phosphorylase. They identified the product of this reaction (the "Cori ester") as glucose 1-phosphate and showed that it could be reincorporated into glycogen in the reverse reaction. Although this did not prove to be the reaction by which glycogen is synthesized in cells, it was the first in vitro demonstration of the synthesis of a macromolecule from simple monomeric subunits, and it inspired others to search for polymerizing enzymes. Arthur Kornberg, discoverer of the first DNA polymerase, said of his experience in the Coris' lab, "Glycogen phosphorylase, not base pairing, was what led me to DNA polymerase."



The Coris in Gerty Cori's laboratory, around 1947.

Gerty Cori became interested in human genetic diseases in which too much glycogen is stored in the liver. She was able to identify the biochemical defect in several of these diseases and to show that the diseases could be diagnosed by assays of the enzymes of glycogen metabolism in small samples of tissue obtained by biopsy. Table 1 summarizes what we now know about 13 genetic diseases of this sort. ■

Carl and Gerty Cori shared the Nobel Prize in Physiology or Medicine in 1947 with Bernardo Houssay of Argentina, who was cited for his studies of hormonal regulation of carbohydrate metabolism. The Cori laboratories in St. Louis became an international center of biochemical research in the 1940s and 1950s, and at least six scientists who trained with the Coris became Nobel laureates: Arthur Kornberg (for DNA synthesis, 1959), Severo Ochoa (for RNA synthesis, 1959), Luis Leloir (for the role of sugar nucleotides in polysaccharide synthesis, 1970), Earl Sutherland (for the discovery of cAMP in the regulation of carbohydrate metabolism, 1971), Christian de Duve (for subcellular fractionation, 1974), and Edwin Krebs (for the discovery of phosphorylase kinase, 1991).

hydrolysis, pulls the synthetic reaction forward, a common strategy in biological polymerization reactions.

2. Although the chemical transformations of sugar nucleotides do not involve the atoms of the nucleotide itself, the nucleotide moiety has many groups that can undergo noncovalent interactions with enzymes; the additional free energy of binding can contribute significantly to catalytic activity (Chapter 6; see also pp. 306–307).
3. Like phosphate, the nucleotidyl group (UMP or AMP, for example) is an excellent leaving group, facilitating nucleophilic attack by activating the sugar carbon to which it is attached.
4. By "tagging" some hexoses with nucleotidyl groups, cells can set them aside in a pool for one purpose (glycogen synthesis, for example), separate from hexose phosphates destined for another purpose (such as glycolysis).

TABLE 1 Glycogen Storage Diseases of Humans

Type (name)	Enzyme affected	Primary organ affected	Symptoms
Type 0	Glycogen synthase	Liver	Low blood glucose, high ketone bodies, early death
Type Ia (von Gierke)	Glucose 6-phosphatase	Liver	Enlarged liver, kidney failure
Type Ib	Microsomal glucose 6-phosphate translocase	Liver	As in type Ia; also high susceptibility to bacterial infections
Type Ic	Microsomal P_i transporter	Liver	As in type Ia
Type II (Pompe)	Lysosomal glucosidase	Skeletal and cardiac muscle	Infantile form: death by age 2; juvenile form: muscle defects (myopathy); adult form: as in muscular dystrophy
Type IIIa (Cori or Forbes)	Debranching enzyme	Liver, skeletal and cardiac muscle	Enlarged liver in infants; myopathy
Type IIIb	Liver debranching enzyme (muscle enzyme normal)	Liver	Enlarged liver in infants
Type IV (Andersen)	Branching enzyme	Liver, skeletal muscle	Enlarged liver and spleen, myoglobin in urine
Type V (McArdle)	Muscle phosphorylase	Skeletal muscle	Exercise-induced cramps and pain; myoglobin in urine
Type VI (Hers)	Liver phosphorylase	Liver	Enlarged liver
Type VII (Tarui)	Muscle PFK-1	Muscle, erythrocytes	As in type V; also hemolytic anemia
Type VIb, VIII, or IX	Phosphorylase kinase	Liver, leukocytes, muscle	Enlarged liver
Type XI (Fanconi-Bickel)	Glucose transporter (GLUT2)	Liver	Failure to thrive, enlarged liver, rickets, kidney dysfunction

Glycogen synthesis takes place in virtually all animal tissues but is especially prominent in the liver and skeletal muscles. The starting point for synthesis of glycogen is glucose 6-phosphate. As we have seen, this can be derived from free glucose in a reaction catalyzed by the isozymes hexokinase I and hexokinase II in muscle and hexokinase IV (glucokinase) in liver:

$$\text{D-Glucose} + \text{ATP} \longrightarrow \text{D-glucose 6-phosphate} + \text{ADP}$$

However, some ingested glucose takes a more roundabout path to glycogen. It is first taken up by erythrocytes and converted to lactate glycolytically; the lactate is then taken up by the liver and converted to glucose 6-phosphate by gluconeogenesis.

To initiate glycogen synthesis, the glucose 6-phosphate is converted to glucose 1-phosphate in the phosphoglucomutase reaction:

$$\text{Glucose 6-phosphate} \rightleftharpoons \text{glucose 1-phosphate}$$

The product of this reaction is converted to UDP-glucose by the action of **UDP-glucose pyrophosphorylase**, in a key step of glycogen biosynthesis:

$$\text{Glucose 1-phosphate} + \text{UTP} \longrightarrow \text{UDP-glucose} + PP_i$$

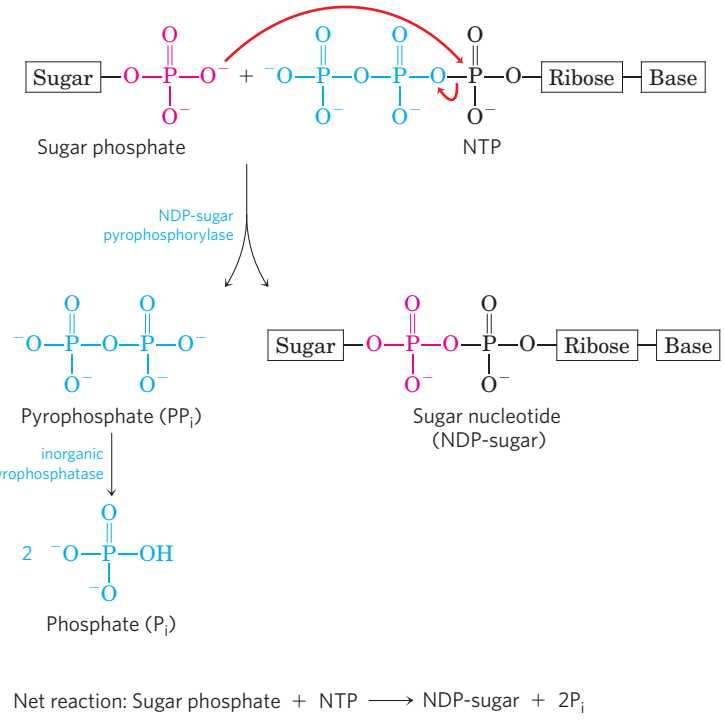


FIGURE 15–31 Formation of a sugar nucleotide. A condensation reaction occurs between a nucleoside triphosphate (NTP) and a sugar phosphate. The negatively charged oxygen on the sugar phosphate serves as a nucleophile, attacking the α phosphate of the nucleoside triphosphate and displacing pyrophosphate. The reaction is pulled in the forward direction by the hydrolysis of PP_i by inorganic pyrophosphatase.

Notice that this enzyme is named for the reverse reaction; in the cell, the reaction proceeds in the direction of UDP-glucose formation, because pyrophosphate is rapidly hydrolyzed by inorganic pyrophosphatase (Fig. 15–31).

UDP-glucose is the immediate donor of glucose residues in the reaction catalyzed by **glycogen synthase**, which promotes the transfer of the glucose residue from UDP-glucose to a nonreducing end of a branched glycogen molecule **(Fig. 15–32)**. The overall equilibrium of the path from glucose 6-phosphate to glycogen lengthened by one glucose unit greatly favors synthesis of glycogen.

Glycogen synthase cannot make the (α1⟶6) bonds found at the branch points of glycogen; these are

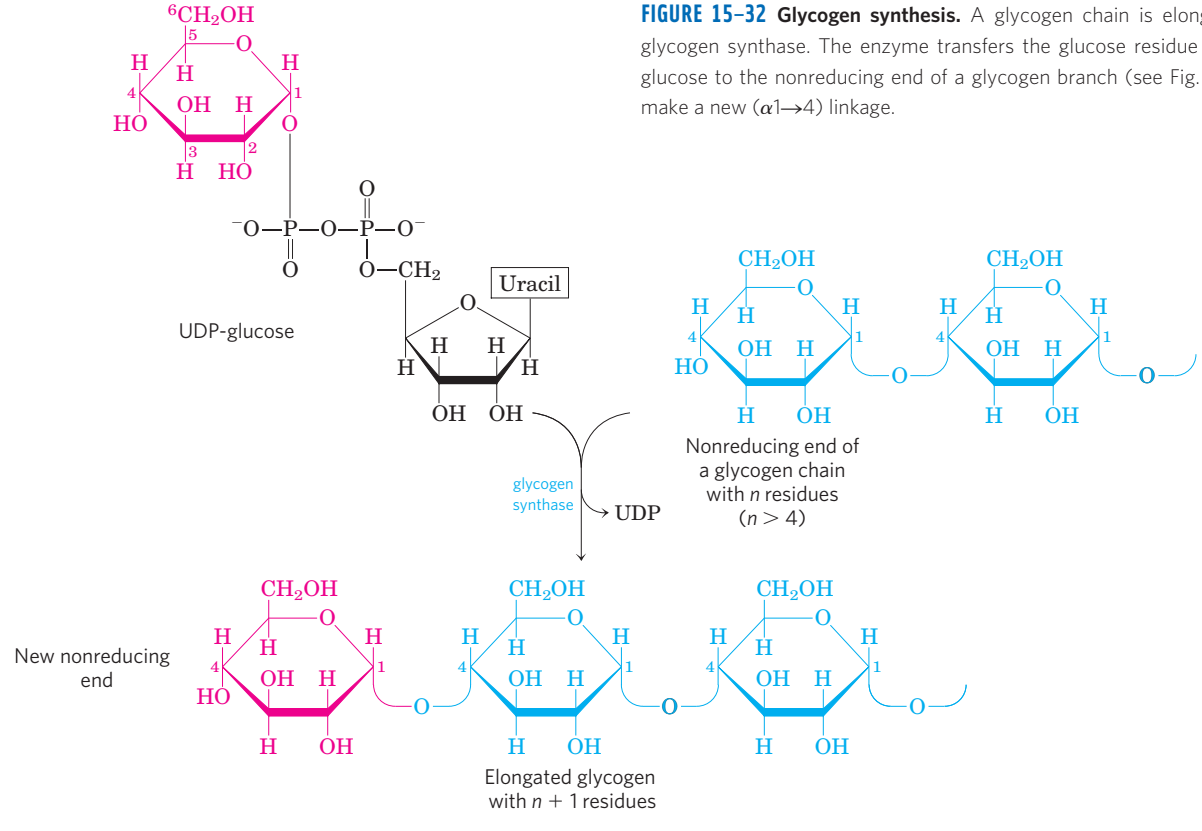


FIGURE 15–32 Glycogen synthesis. A glycogen chain is elongated by glycogen synthase. The enzyme transfers the glucose residue of UDP-glucose to the nonreducing end of a glycogen branch (see Fig. 7–13) to make a new (α1⟶4) linkage.

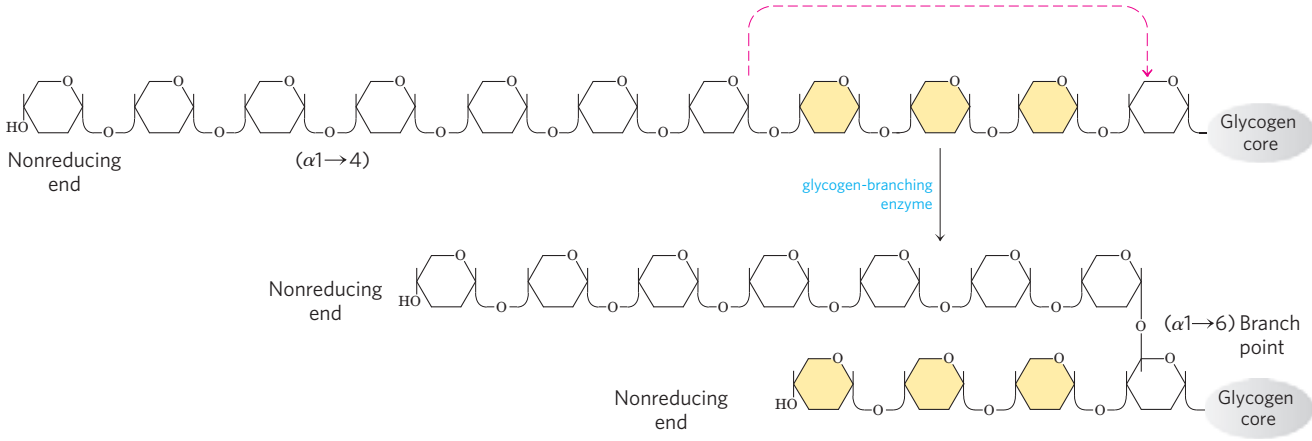


FIGURE 15–33 Branch synthesis in glycogen. The glycogen-branching enzyme (also called amylo (1→4) to (1→6) transglycosylase, or glycosyl-(4→6) transferase) forms a new branch point during glycogen synthesis.

formed by the glycogen-branching enzyme, also called **amylo (1→4) to (1→6) transglycosylase**, or glycosyl-(4→6) transferase. The glycogen-branching enzyme catalyzes transfer of a terminal fragment of 6 or 7 glucose residues from the nonreducing end of a glycogen branch having at least 11 residues to the C-6 hydroxyl group of a glucose residue at a more interior position of the same or another glycogen chain, thus creating a new branch **(Fig. 15–33)**. Further glucose residues may be added to the new branch by glycogen synthase. The biological effect of branching is to make the glycogen molecule more soluble and to increase the number of nonreducing ends. This increases the number of sites accessible to glycogen phosphorylase and glycogen synthase, both of which act only at nonreducing ends.

Glycogenin Primes the Initial Sugar Residues in Glycogen

Glycogen synthase cannot initiate a new glycogen chain de novo. It requires a primer, usually a preformed (α1→4) polyglucose chain or branch having at least eight glucose residues. So, how is a *new* glycogen molecule initiated? The intriguing protein **glycogenin** **(Fig. 15–34)** is both the primer on which new chains are assembled and the enzyme that catalyzes their assembly. The first step in the synthesis of a new glycogen molecule is the transfer of a glucose residue from UDP-glucose to the hydroxyl group of Tyr^{194} of glycogenin, catalyzed by the protein's intrinsic glucosyltransferase activity **(Fig. 15–35)**. The nascent chain is extended by the sequential addition of seven more glucose residues, each derived from UDP-glucose; the reactions are catalyzed by the chain-extending activity of glycogenin. At this point, glycogen synthase takes over, further extending the glycogen chain. Glycogenin remains buried within the β-particle, covalently attached to the single reducing end of the glycogen molecule (Fig. 15–35b). Medical consequences of a mutation in the gene for glycogenin that knocks out that protein's polymerizing activity include muscle weakness and fatigue, depleted glycogen in the liver, and an irregular heartbeat (cardiac arrhythmia).

SUMMARY 15.4 The Metabolism of Glycogen in Animals

- Glycogen is stored in muscle and liver as large particles. Contained within the particles are the

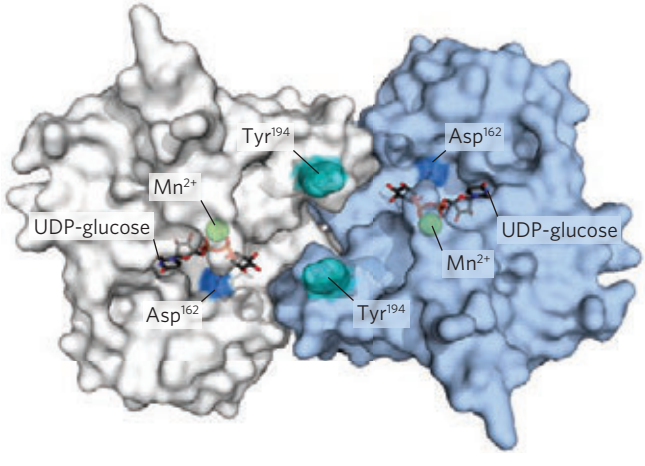


FIGURE 15–34 Glycogenin structure. (PDB 1D 1LL2) Muscle glycogenin (M_r 37,000) forms dimers in solution. Humans have a second isoform in liver, glycogenin-2. The substrate, UDP-glucose, is bound to a Rossmann fold near the amino terminus and is some distance from the Tyr^{194} residues—15 Å from the Tyr in the same monomer, 12 Å from the Tyr in the dimeric partner. Each UDP-glucose is bound through its phosphates to a Mn^{2+} ion, which is essential to catalysis. Mn^{2+} is believed to function as an electron-pair acceptor (Lewis acid) to stabilize the leaving group, UDP. The glycosidic bond in the product has the same configuration about the C-1 of glucose as the substrate UDP-glucose, suggesting that the transfer of glucose from UDP to Tyr^{194} occurs in two steps. The first step is probably a nucleophilic attack by Asp^{162}, forming a temporary intermediate with inverted configuration. A second nucleophilic attack by Tyr^{194} then restores the starting configuration.

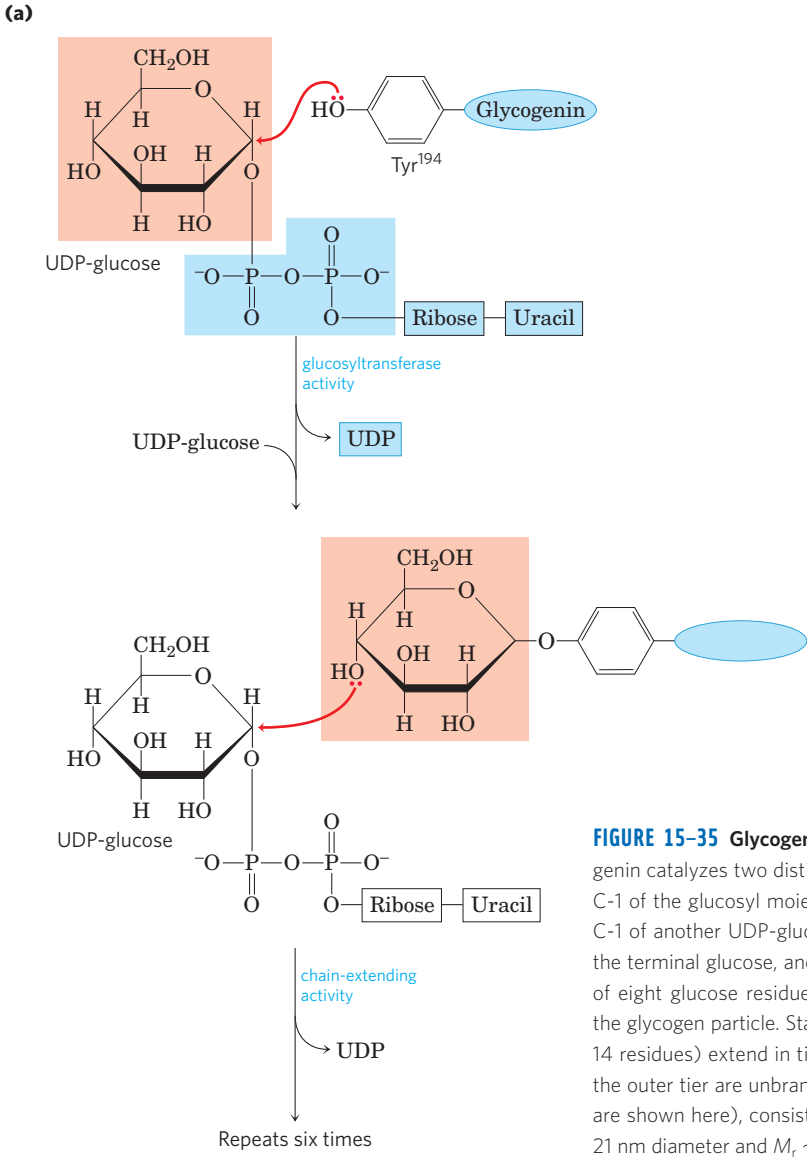


FIGURE 15–35 Glycogenin and the structure of the glycogen particle. (a) Glycogenin catalyzes two distinct reactions. Initial attack by the hydroxyl group of Tyr^{194} on C-1 of the glucosyl moiety of UDP-glucose results in a glucosylated Tyr residue. The C-1 of another UDP-glucose molecule is now attacked by the C-4 hydroxyl group of the terminal glucose, and this sequence repeats to form a nascent glycogen molecule of eight glucose residues attached by (α1→4) glycosidic linkages. **(b)** Structure of the glycogen particle. Starting at a central glycogenin molecule, glycogen chains (12 to 14 residues) extend in tiers. Inner chains have two (α1→6) branches each. Chains in the outer tier are unbranched. There are 12 tiers in a mature glycogen particle (only 5 are shown here), consisting of about 55,000 glucose residues in a molecule of about 21 nm diameter and M_r ~1 × 10^7.

enzymes that metabolize glycogen, as well as regulatory enzymes.

- Glycogen phosphorylase catalyzes phosphorolytic cleavage at the nonreducing ends of glycogen chains, producing glucose 1-phosphate. The debranching enzyme transfers branches onto main chains and releases the residue at the (α1→6) branch as free glucose.
- Phosphoglucomutase interconverts glucose 1-phosphate and glucose 6-phosphate. Glucose 6-phosphate can enter glycolysis or, in liver, can be converted to free glucose by glucose 6-phosphatase in the endoplasmic reticulum, then released to replenish blood glucose.
- The sugar nucleotide UDP-glucose donates glucose residues to the nonreducing end of glycogen in the reaction catalyzed by glycogen synthase. A separate branching enzyme produces the (α1→6) linkages at branch points.
- New glycogen particles begin with the autocatalytic formation of a glycosidic bond between the glucose of UDP-glucose and a Tyr residue in the protein glycogenin, followed by addition of several glucose residues to form a primer that can be acted on by glycogen synthase.

15.5 Coordinated Regulation of Glycogen Synthesis and Breakdown

As we have seen, the mobilization of stored glycogen is brought about by glycogen phosphorylase, which degrades glycogen to glucose 1-phosphate (Fig. 15–27). Glycogen phosphorylase provides an especially instructive case of enzyme regulation. It was one of the first known examples of an allosterically regulated enzyme and the first enzyme shown to be controlled by reversible phosphorylation. It was also one of the first allosteric

enzymes for which the detailed three-dimensional structures of the active and inactive forms were revealed by x-ray crystallographic studies. Glycogen phosphorylase is also another illustration of how isozymes play their tissue-specific roles.

Glycogen Phosphorylase Is Regulated Allosterically and Hormonally



Earl W. Sutherland, Jr., 1915–1974

In the late 1930s, Carl and Gerty Cori (Box 15–4) discovered that the glycogen phosphorylase of skeletal muscle exists in two interconvertible forms: **glycogen phosphorylase *a***, which is catalytically active, and **glycogen phosphorylase *b***, which is less active **(Fig. 15–36)**. Subsequent studies by Earl Sutherland showed that phosphorylase *b* predominates in resting muscle, but during vigorous muscular activity epinephrine triggers phosphorylation of a specific Ser residue in phosphorylase *b*, converting it to its more active form, phosphorylase *a*. (Note that glycogen phosphorylase is often referred to simply as phosphorylase—so honored because it was the first phosphorylase to be discovered; the shortened name has persisted in common usage and in the literature.)

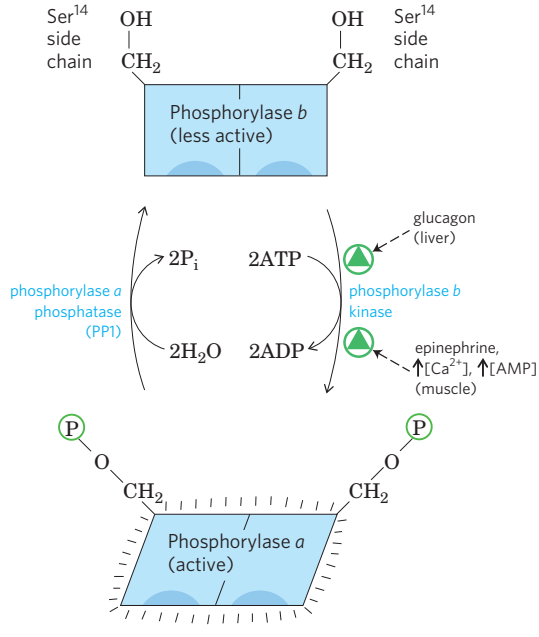


FIGURE 15–36 Regulation of muscle glycogen phosphorylase by covalent modification. In the more active form of the enzyme, phosphorylase *a*, Ser^{14} residues, one on each subunit, are phosphorylated. Phosphorylase *a* is converted to the less active form, phosphorylase *b*, by enzymatic loss of these phosphoryl groups, catalyzed by phosphorylase *a* phosphatase (also known as phosphoprotein phosphatase 1, PP1). Phosphorylase *b* can be reconverted (reactivated) to phosphorylase *a* by the action of phosphorylase *b* kinase. (See also Fig. 6–42 on glycogen phosphorylase regulation.)

The enzyme (phosphorylase *b* kinase) responsible for activating phosphorylase by transferring a phosphoryl group to its Ser residue is itself activated by epinephrine or glucagon through a series of steps shown in **Figure 15–37**. Sutherland discovered the second messenger cAMP, which increases in concentration in response to stimulation by epinephrine (in muscle) or glucagon (in liver). Elevated [cAMP] initiates an **enzyme cascade**, in which a catalyst activates a catalyst, which activates a catalyst (see Section 12.1). Such cascades allow for large amplification of the initial signal (see pink boxes in Fig. 15–37). The rise in [cAMP] activates cAMP-dependent protein kinase, also called protein kinase A (PKA). PKA then phosphorylates and activates **phosphorylase *b* kinase**, which catalyzes the phosphorylation of Ser residues in each of the two identical subunits of glycogen phosphorylase, activating it and thus stimulating glycogen breakdown. In muscle, this provides fuel for glycolysis to sustain muscle contraction for the fight-or-flight response signaled by epinephrine. In liver, glycogen breakdown counters the low blood glucose signaled by glucagon, releasing glucose. These different roles are reflected in subtle differences in the regulatory mechanisms in muscle and liver. The glycogen phosphorylases of liver and muscle are isozymes, encoded by different genes and differing in their regulatory properties.

In muscle, superimposed on the regulation of phosphorylase by covalent modification are two allosteric control mechanisms (Fig. 15–37). Ca^{2+}, the signal for muscle contraction, binds to and activates phosphorylase *b* kinase, promoting conversion of phosphorylase *b* to the active *a* form. Ca^{2+} binds to phosphorylase *b* kinase through its δ subunit, which is calmodulin (see Fig. 12–11). AMP, which accumulates in vigorously contracting muscle as a result of ATP breakdown, binds to and activates phosphorylase, speeding the release of glucose 1-phosphate from glycogen. When ATP levels are adequate, ATP blocks the allosteric site to which AMP binds, inactivating phosphorylase.

When the muscle returns to rest, a second enzyme, **phosphorylase *a* phosphatase**, also called **phosphoprotein phosphatase 1 (PP1)**, removes the phosphoryl groups from phosphorylase *a*, converting it to the less active form, phosphorylase *b*.

Like the enzyme of muscle, the glycogen phosphorylase of liver is regulated hormonally (by phosphorylation/dephosphorylation) and allosterically. The dephosphorylated form is essentially inactive. When the blood glucose level is too low, glucagon (acting through the cascade mechanism shown in Fig. 15–37) activates phosphorylase *b* kinase, which in turn converts phosphorylase *b* to its active *a* form, initiating the release of

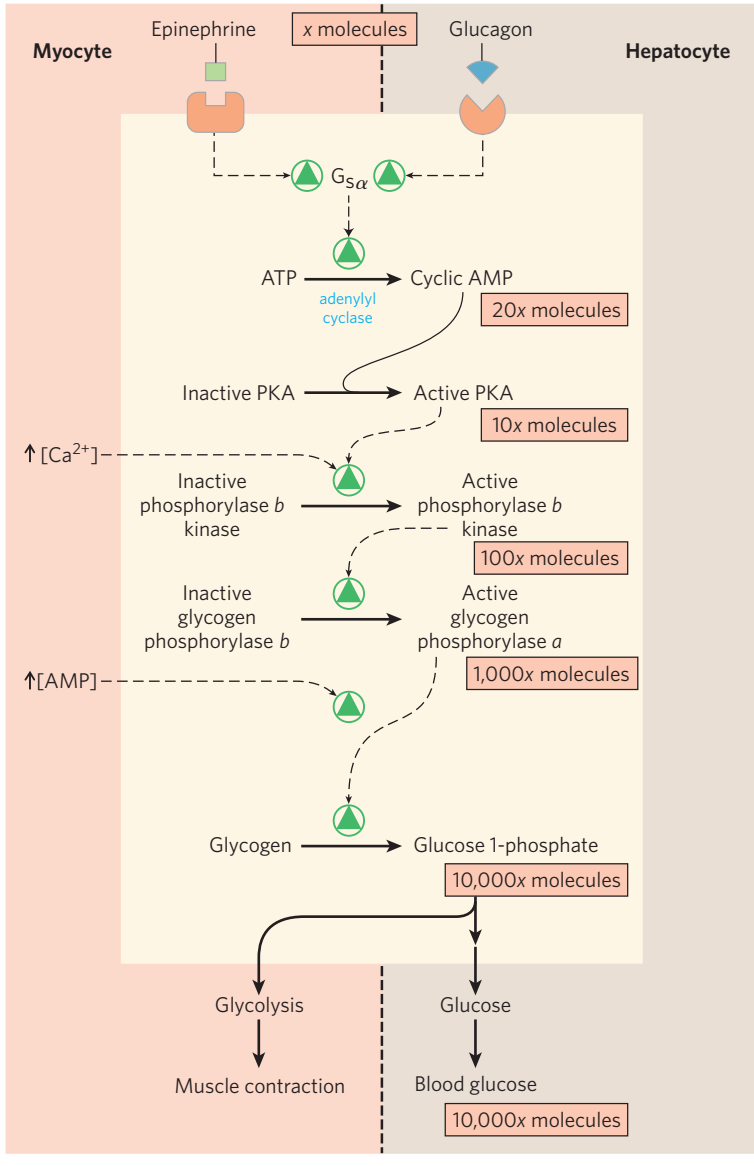


FIGURE 15–37 Cascade mechanism of epinephrine and glucagon action. By binding to specific surface receptors, either epinephrine acting on a myocyte (left) or glucagon acting on a hepatocyte (right) activates a GTP-binding protein, $G_{s\alpha}$ (see Fig. 12–4). Active $G_{s\alpha}$ triggers a rise in [cAMP], activating PKA. This sets off a cascade of phosphorylations; PKA activates phosphorylase *b* kinase, which then activates glycogen phosphorylase. Such cascades effect a large amplification of the initial signal; the figures in pink boxes are probably low estimates of the actual increase in number of molecules at each stage of the cascade. The resulting breakdown of glycogen provides glucose, which in the myocyte can supply ATP (via glycolysis) for muscle contraction and in the hepatocyte is released into the blood to counter the low blood glucose.

glucose into the blood. When blood glucose levels return to normal, glucose enters hepatocytes and binds to an inhibitory allosteric site on phosphorylase *a*. This binding also produces a conformational change that exposes the phosphorylated Ser residues to PP1, which catalyzes their dephosphorylation and inactivates the phosphorylase **(Fig. 15–38)**. The allosteric site for glucose allows liver glycogen phosphorylase to act as its own glucose sensor and to respond appropriately to changes in blood glucose.

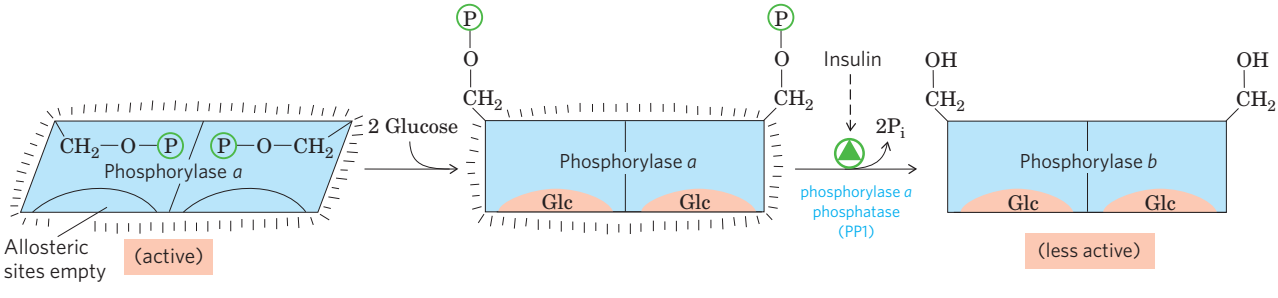


FIGURE 15–38 Glycogen phosphorylase of liver as a glucose sensor. Glucose binding to an allosteric site of the phosphorylase *a* isozyme of liver induces a conformational change that exposes its phosphorylated Ser residues to the action of phosphorylase *a* phosphatase (PP1). This phosphatase converts phosphorylase *a* to phosphorylase *b*, sharply reducing the activity of phosphorylase and slowing glycogen breakdown in response to high blood glucose. Insulin also acts indirectly to stimulate PP1 and slow glycogen breakdown.

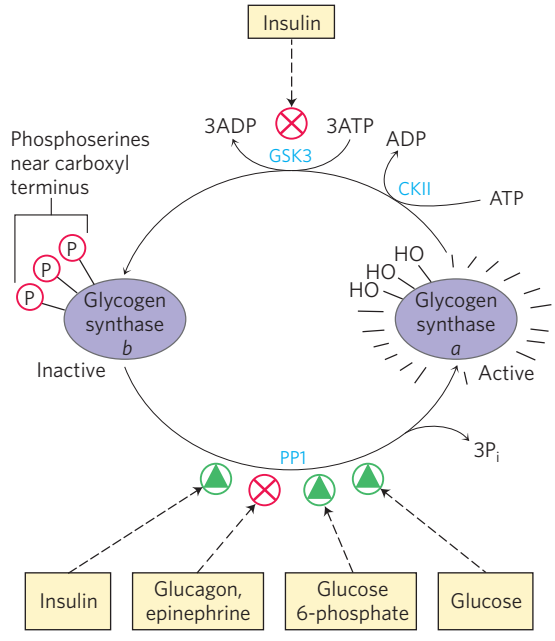


FIGURE 15–39 Effects of GSK3 on glycogen synthase activity. Glycogen synthase *a*, the active form, has three Ser residues near its carboxyl terminus, which are phosphorylated by glycogen synthase kinase 3 (GSK3). This converts glycogen synthase to the inactive (*b*) form. GSK3 action requires prior phosphorylation (priming) by casein kinase (CKII). Insulin triggers activation of glycogen synthase *b* by blocking the activity of GSK3 (see the pathway for this action in Fig. 12–16) and activating a phosphoprotein phosphatase (PP1 in muscle, another phosphatase in liver). In muscle, epinephrine activates PKA, which phosphorylates the glycogen-targeting protein GM (see Fig. 15–42) on a site that causes dissociation of PP1 from glycogen. Glucose 6-phosphate favors dephosphorylation of glycogen synthase by binding to it and promoting a conformation that is a good substrate for PP1. Glucose also promotes dephosphorylation; the binding of glucose to glycogen phosphorylase *a* forces a conformational change that favors dephosphorylation to glycogen phosphorylase *b*, thus allowing the action of PP1 (see Fig. 15–41).

Glycogen Synthase Is Also Regulated by Phosphorylation and Dephosphorylation

Like glycogen phosphorylase, glycogen synthase can exist in phosphorylated and dephosphorylated forms **(Fig. 15–39)**. Its active form, **glycogen synthase *a***, is unphosphorylated. Phosphorylation of the hydroxyl side chains of several Ser residues of both subunits converts glycogen synthase *a* to **glycogen synthase *b***, which is inactive unless its allosteric activator, glucose 6-phosphate, is present. Glycogen synthase is remarkable for its ability to be phosphorylated on various residues by at least 11 different protein kinases. The most important regulatory kinase is **glycogen synthase kinase 3 (GSK3)**, which adds phosphoryl groups to three Ser residues near the carboxyl terminus of glycogen synthase, strongly inactivating it. The action of GSK3 is hierarchical; it cannot phosphorylate glycogen synthase until another protein kinase, **casein kinase II (CKII)**, has first phosphorylated the glycogen synthase on a nearby residue, an event called **priming (Fig. 15–40a)**.

In liver, conversion of glycogen synthase *b* to the active form is promoted by PP1, which is bound to the glycogen particle. PP1 removes the phosphoryl groups from the three Ser residues phosphorylated by GSK3. Glucose 6-phosphate binds to an allosteric site on

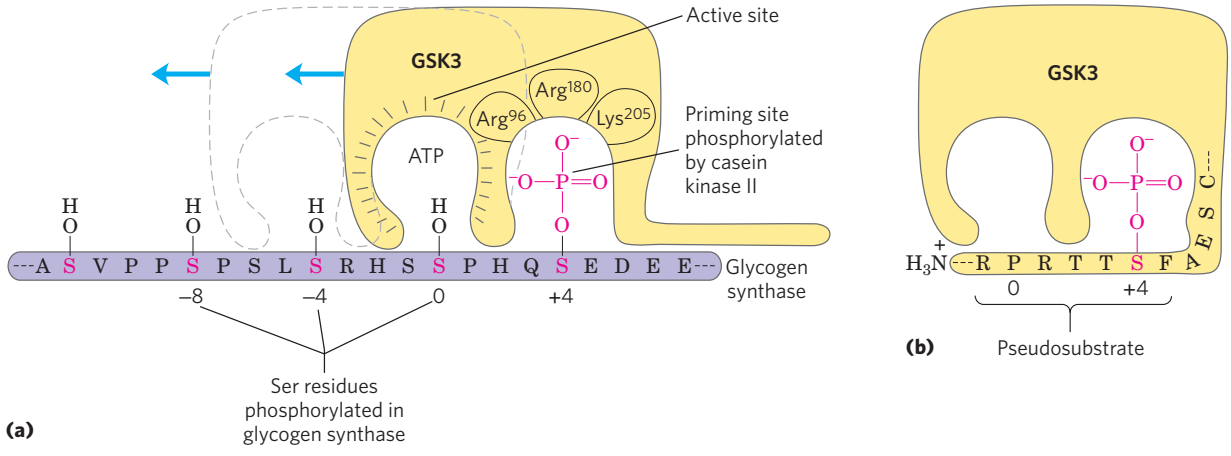


FIGURE 15–40 Priming of GSK3 phosphorylation of glycogen synthase. **(a)** Glycogen synthase kinase 3 first associates with its substrate (glycogen synthase) by interaction between three positively charged residues (Arg^{96}, Arg^{180}, Lys^{205}) and a phosphoserine residue at position +4 in the substrate. (For orientation, the Ser or Thr residue to be phosphorylated in the substrate is assigned the index 0. Residues on the amino-terminal side of this residue are numbered −1, −2, and so forth; residues on the carboxyl-terminal side are numbered +1, +2, and so forth.) This association aligns the active site of the enzyme with a Ser residue at position 0, which it phosphorylates. This creates a new priming site, and the enzyme moves down the protein to phosphorylate the Ser residue at position −4, and then the Ser at −8. **(b)** GSK3 has a Ser residue near its amino terminus that can be phosphorylated by PKA or PKB (see Fig. 15–41). This produces a "pseudosubstrate" region in GSK3 that folds into the priming site and makes the active site inaccessible to another protein substrate, inhibiting GSK3 until the priming phosphoryl group of its pseudosubstrate region is removed by PP1. Other proteins that are substrates for GSK3 also have a priming site at position +4, which must be phosphorylated by another protein kinase before GSK3 can act on them. (See also Figs 6–37 and 12–22b on glycogen synthase regulation.)

glycogen synthase *b*, making the enzyme a better substrate for dephosphorylation by PP1 and causing its activation. By analogy with glycogen phosphorylase, which acts as a glucose sensor, glycogen synthase can be regarded as a glucose 6-phosphate sensor. In muscle, a different phosphatase may have the role played by PP1 in liver, activating glycogen synthase by dephosphorylating it.

Glycogen Synthase Kinase 3 Mediates Some of the Actions of Insulin

As we saw in Chapter 12, one way in which insulin triggers intracellular changes is by activating a protein kinase (PKB) that in turn phosphorylates and inactivates GSK3 (**Fig. 15–41**; see also Fig. 12–16). Phosphorylation of a Ser residue near the amino terminus of GSK3 converts that region of the protein to a pseudosubstrate, which folds into the site at which the priming phosphorylated Ser residue normally binds (Fig. 15–40b). This prevents GSK3 from binding the priming site of a real substrate, thereby inactivating the enzyme and tipping the balance in favor of dephosphorylation of glycogen synthase by PP1. Glycogen phosphorylase can also affect the phosphorylation of glycogen synthase: active glycogen phosphorylase directly inhibits PP1, preventing it from activating glycogen synthase (Fig. 15–39).

Although first discovered in its role in glycogen metabolism (hence the name glycogen synthase kinase), GSK3 clearly has a much broader role than the regulation of glycogen synthase. It mediates signaling by insulin and other growth factors and nutrients, and it acts in the specification of cell fates during embryonic development. Among its targets are cytoskeletal proteins and proteins essential for mRNA and protein synthesis. These targets, like glycogen synthase, must first undergo a priming phosphorylation by another protein kinase before they can be phosphorylated by GSK3.

Phosphoprotein Phosphatase 1 Is Central to Glycogen Metabolism

A single enzyme, PP1, can remove phosphoryl groups from all three of the enzymes phosphorylated in response to glucagon (liver) and epinephrine (liver and muscle): phosphorylase kinase, glycogen phosphorylase, and glycogen synthase. Insulin stimulates glycogen synthesis by activating PP1 and by inactivating GSK3.

Phosphoprotein phosphatase 1 does not exist free in the cytosol, but is tightly bound to its target proteins by one of a family of **glycogen-targeting proteins** that bind glycogen and each of the three enzymes, glycogen phosphorylase, phosphorylase kinase, and glycogen synthase **(Fig. 15–42)**. PP1 is itself subject to covalent and allosteric regulation: it is inactivated when phosphorylated by PKA and is allosterically activated by glucose 6-phosphate.

Allosteric and Hormonal Signals Coordinate Carbohydrate Metabolism Globally

Having looked at the mechanisms that regulate individual enzymes, we can now consider the overall shifts in carbohydrate metabolism that occur in the well-fed state, during fasting, and in the fight-or-flight response—signaled by insulin, glucagon, and epinephrine, respectively. We need to contrast two cases in which regulation serves different ends: (1) the role of hepatocytes in supplying glucose to the blood, and (2) the selfish use of carbohydrate fuels by nonhepatic

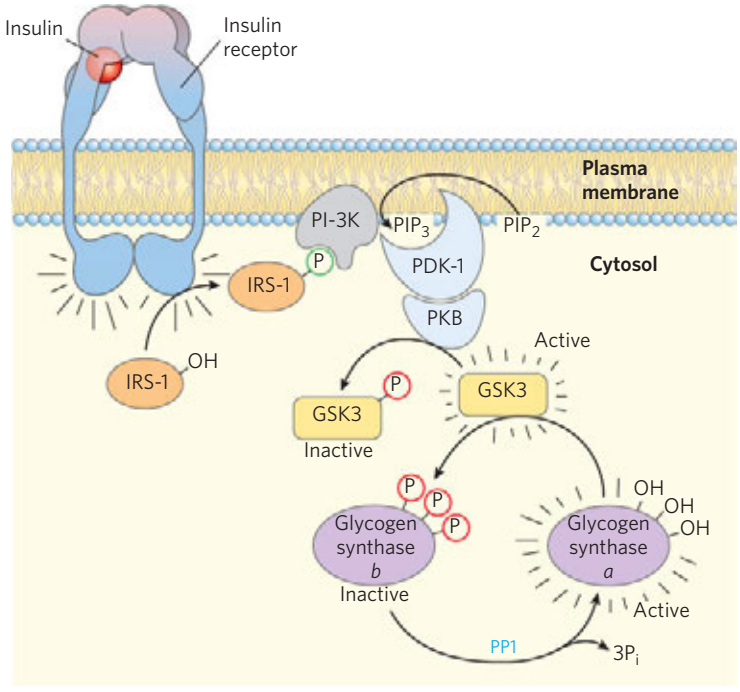


FIGURE 15–41 The path from insulin to GSK3 and glycogen synthase. Insulin binding to its receptor activates a tyrosine protein kinase in the receptor, which phosphorylates insulin receptor substrate-1 (IRS-1). The phosphotyrosine in this protein is then bound by phosphatidylinositol 3-kinase (PI-3K), which converts phosphatidylinositol 4,5-bisphosphate (PIP_2) in the membrane to phosphatidylinositol 3,4,5-trisphosphate (PIP_3). A protein kinase (PDK-1) that is activated when bound to PIP_3 activates a second protein kinase (PKB), which phosphorylates glycogen synthase kinase 3 (GSK3) in its pseudosubstrate region, inactivating it by the mechanism shown in Figure 15–40b. The inactivation of GSK3 allows phosphoprotein phosphatase 1 (PP1) to dephosphorylate and thus activate glycogen synthase. In this way, insulin stimulates glycogen synthesis. (See Fig. 12–16 for more details on insulin action.)

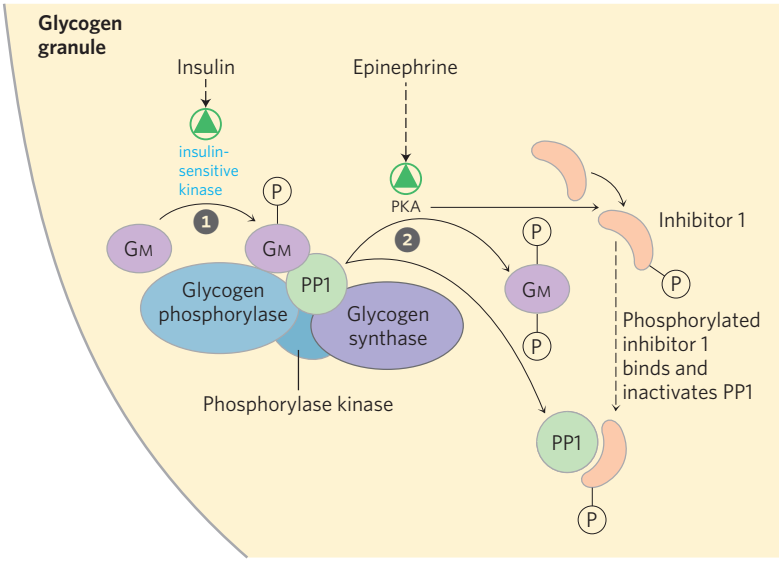


FIGURE 15–42 Glycogen-targeting protein GM. The glycogen-targeting protein GM is one of a family of proteins that bind other proteins (including PP1) to glycogen particles. GM can be phosphorylated at two different sites in response to insulin or epinephrine. ❶ Insulin-stimulated phosphorylation of GM site 1 activates PP1, which dephosphorylates phosphorylase kinase, glycogen phosphorylase, and glycogen synthase. ❷ Epinephrine-stimulated phosphorylation of GM site 2 causes dissociation of PP1 from the glycogen particle, preventing its access to glycogen phosphorylase and glycogen synthase. PKA also phosphorylates a protein (inhibitor 1) that, when phosphorylated, inhibits PP1. By these means, insulin inhibits glycogen breakdown and stimulates glycogen synthesis, and epinephrine (or glucagon in the liver) has the opposite effects.

tissues, typified by skeletal muscle (myocytes), to support their own activities.

After ingestion of a carbohydrate-rich meal, the elevation of blood glucose triggers insulin release (**Fig. 15–43**, top). In a hepatocyte, insulin has two immediate effects: it inactivates GSK3, acting through the cascade shown in Figure 15–41, and activates a protein phosphatase, perhaps PP1. These two actions fully activate glycogen synthase. PP1 also inactivates glycogen phosphorylase *a* and phosphorylase kinase by dephosphorylating both, effectively stopping glycogen breakdown. Glucose enters the hepatocyte through the high-capacity transporter GLUT2, always present in the plasma membrane, and the elevated intracellular glucose leads to dissociation of hexokinase IV (glucokinase) from its nuclear regulatory protein (Fig. 15–15). Hexokinase IV enters the cytosol and phosphorylates glucose, stimulating glycolysis and supplying the precursor for glycogen synthesis. Under these conditions, hepatocytes use the excess glucose in the blood to synthesize glycogen, up to the limit of about 10% of the total weight of the liver.

Between meals, or during an extended fast, the drop in blood glucose triggers the release of glucagon, which, acting through the cascade shown in Figure 15–37, activates PKA. PKA mediates all the effects of glucagon (Fig. 15–43, bottom). It phosphorylates phosphorylase kinase, activating it and leading to the activation of glycogen phosphorylase. It phosphorylates glycogen synthase, inactivating it and blocking glycogen synthesis. It phosphorylates PFK-2/FBPase-2, leading to a

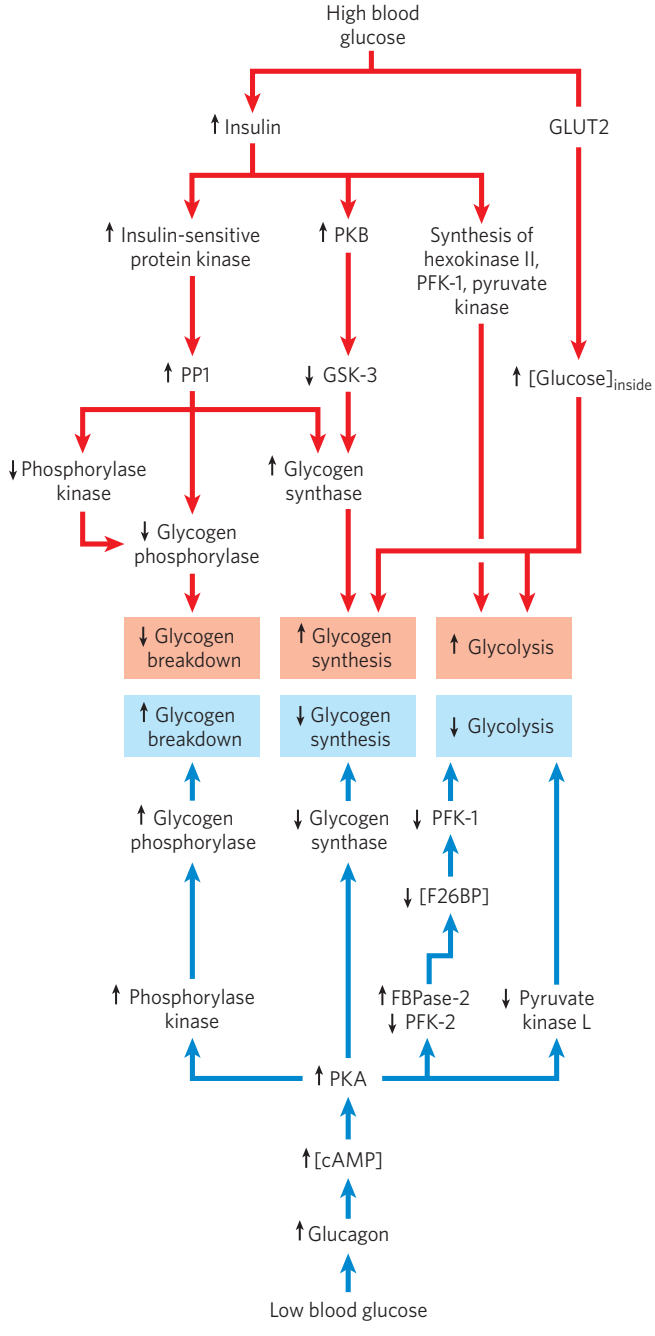


FIGURE 15–43 Regulation of carbohydrate metabolism in the liver. Arrows indicate causal relationships between the changes they connect. For example, an arrow from ↓A to ↑B means that a decrease in A causes an increase in B. Red arrows connect events that result from high blood glucose; blue arrows connect events that result from low blood glucose.

drop in the concentration of the regulator fructose 2,6-bisphosphate, which has the effect of inactivating the glycolytic enzyme PFK-1 and activating the gluconeogenic enzyme FBPase-1. And it phosphorylates and inactivates the glycolytic enzyme pyruvate kinase. Under these conditions, the liver produces glucose 6-phosphate by glycogen breakdown and by gluconeogenesis, and it stops using glucose to fuel glycolysis or make glycogen, maximizing the amount of glucose it can release to the blood. This release of glucose is possible only in liver and kidney, because other tissues lack glucose 6-phosphatase (Fig. 15–30).

The physiology of skeletal muscle differs from that of liver in three ways important to our discussion of metabolic regulation **(Fig. 15–44)**: (1) muscle uses its stored glycogen only for its own needs; (2) as it goes from rest to vigorous contraction, muscle undergoes very large changes in its demand for ATP, which is supported by glycolysis; (3) muscle lacks the enzymatic machinery for gluconeogenesis. The regulation of carbohydrate metabolism in muscle reflects these differences from liver. First, myocytes lack receptors for glucagon. Second, the muscle isozyme of pyruvate kinase is not phosphorylated by PKA, so glycolysis is not turned off when [cAMP] is high. In fact, cAMP *increases* the rate of glycolysis in muscle, probably by activating glycogen phosphorylase. When epinephrine is released into the blood in a fight-or-flight situation, PKA is activated by the rise in [cAMP] and phosphorylates and activates glycogen phosphorylase kinase. The resulting phosphorylation and activation of glycogen phosphorylase results in faster glycogen breakdown. Epinephrine is not released under low-stress conditions, but with each neuronal stimulation of muscle contraction, cytosolic [Ca^{2+}] rises briefly and activates phosphorylase kinase through its calmodulin subunit.

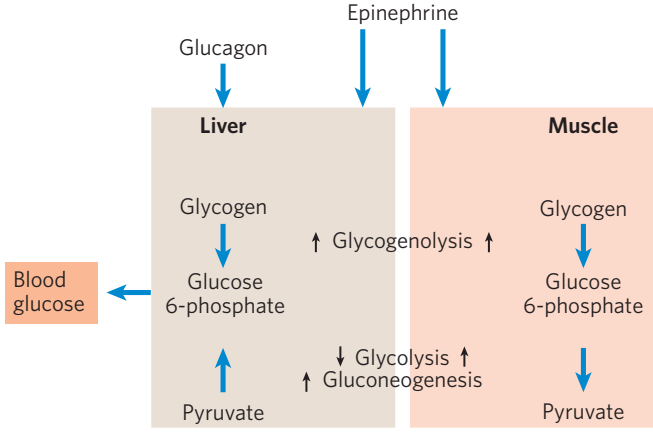


FIGURE 15–44 Difference in the regulation of carbohydrate metabolism in liver and muscle. In liver, either glucagon (indicating low blood glucose) or epinephrine (signaling the need to fight or flee) has the effect of maximizing the output of glucose into the bloodstream. In muscle, epinephrine increases glycogen breakdown and glycolysis, which together provide fuel to produce the ATP needed for muscle contraction.

Elevated insulin triggers increased glycogen synthesis in myocytes by activating PP1 and inactivating GSK3. Unlike hepatocytes, myocytes have a reserve of GLUT4 sequestered in intracellular vesicles. Insulin triggers their movement to the plasma membrane (see Fig. 12–16), where they allow increased glucose uptake. In response to insulin, therefore, myocytes help to lower blood glucose by increasing their rates of glucose uptake, glycogen synthesis, and glycolysis.

Carbohydrate and Lipid Metabolism Are Integrated by Hormonal and Allosteric Mechanisms

As complex as the regulation of carbohydrate metabolism is, it is far from the whole story of fuel metabolism. The metabolism of fats and fatty acids is very closely tied to that of carbohydrates. Hormonal signals such as insulin and changes in diet or exercise are equally important in regulating fat metabolism and integrating it with that of carbohydrates. We return to this overall metabolic integration in mammals in Chapter 23, after first considering the metabolic pathways for fats and amino acids (Chapters 17 and 18). The message we wish to convey here is that metabolic pathways are overlaid with complex regulatory controls that are exquisitely sensitive to changes in metabolic circumstances. These mechanisms act to adjust the flow of metabolites through various metabolic pathways, as needed by the cell and organism, and to do so without causing major changes in the concentrations of intermediates shared with other pathways.

SUMMARY 15.5 Coordinated Regulation of Glycogen Synthesis and Breakdown

- Glycogen phosphorylase is activated in response to glucagon or epinephrine, which raise [cAMP] and activate PKA. PKA phosphorylates and activates phosphorylase kinase, which converts glycogen phosphorylase *b* to its active *a* form. Phosphoprotein phosphatase 1 (PP1) reverses the phosphorylation of glycogen phosphorylase *a*, inactivating it. Glucose binds to the liver isozyme of glycogen phosphorylase *a*, favoring its dephosphorylation and inactivation.
- Glycogen synthase *a* is inactivated by phosphorylation catalyzed by GSK3. Insulin blocks GSK3. PP1, which is activated by insulin, reverses the inhibition by dephosphorylating glycogen synthase *b*.
- Insulin increases glucose uptake into myocytes and adipocytes by triggering movement of the glucose transporter GLUT4 to the plasma membrane.
- Insulin stimulates the synthesis of hexokinases II and IV, PFK-1, pyruvate kinase, and several

enzymes involved in lipid synthesis. Insulin stimulates glycogen synthesis in muscle and liver.

- In liver, glucagon stimulates glycogen breakdown and gluconeogenesis while blocking glycolysis, thereby sparing glucose for export to the brain and other tissues.
- In muscle, epinephrine stimulates glycogen breakdown and glycolysis, providing ATP to support contraction.

Key Terms

Terms in bold are defined in the glossary.

glucose 6-phosphate 587
flux, J 589
homeostasis 589
cellular differentiation 589
transcription factor 589
response element 589
turnover 590
transcriptome 590
proteome 590
metabolome 591
metabolic regulation 592
metabolic control 592
mass-action ratio, *Q* 593
adenylate kinase 594
AMP-activated protein kinase (AMPK) 594
flux control coefficient, C 597, 598
elasticity coefficient, ε 597, 598
response coefficient, R 598
gluconeogenesis 601
futile cycle 601
substrate cycle 601
hexokinase II 602
hexokinase I 602
hexokinase IV 603
GLUT2 603
glucagon 605
fructose 2,6-bisphosphate 605
phosphofructokinase-2 (PFK-2) 606
fructose 2,6-bisphosphatase (FBPase-2) 606
carbohydrate response element binding protein (ChREBP) 609
sterol regulatory element binding protein (SREBP) 610
cyclic AMP response element binding protein (CREB) 610
forkhead box other (FOXO1) 610
glycogenolysis 613
glycogenesis 613
glucose 1-phosphate 613
debranching enzyme 614
oligo (α1→6) to (α1→4) glucantransferase 614
phosphoglucomutase 614
sugar nucleotides 615
UDP-glucose pyrophosphorylase 617
amylo (1→4) to (1→6) transglycosylase 619
glycogenin 619
glycogen phosphorylase a 621
glycogen phosphorylase b 621
enzyme cascade 621
phosphorylase b kinase 621
phosphoprotein phosphatase 1 (PP1) 621
glycogen synthase a 623
glycogen synthase b 623
glycogen synthase kinase 3 (GSK3) 623
casein kinase II (CKII) 623
priming 623
glycogen-targeting proteins 624

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Problems

1. Measurement of Intracellular Metabolite Concentrations Measuring the concentrations of metabolic intermediates in a living cell presents great experimental difficulties—usually a cell must be destroyed before metabolite concentrations can be measured. Yet enzymes catalyze metabolic interconversions very rapidly, so a common problem associated with these types of measurements is that the findings reflect not the physiological concentrations of metabolites but the equilibrium concentrations. A reliable experimental technique requires all enzyme-catalyzed reactions to be instantaneously stopped in the intact tissue so that the metabolic intermediates do not undergo change. This objective is accomplished by rapidly compressing the tissue between large aluminum plates cooled with liquid nitrogen (−190 °C), a process called **freeze-clamping**. After freezing, which stops enzyme action instantly, the tissue is powdered and the enzymes are inactivated by precipitation with perchloric acid. The precipitate is removed by centrifugation, and the clear supernatant extract is analyzed for metabolites. To calculate intracellular concentrations, the intracellular volume is determined from the total water content of the tissue and a measurement of the extracellular volume.

The intracellular concentrations of the substrates and products of the phosphofructokinase-1 reaction in isolated rat heart tissue are given in the table below.

Metabolite	Concentration (μM)*
Fructose 6-phosphate	87.0
Fructose 1,6-bisphosphate	22.0
ATP	11,400
ADP	1,320

Source: From Williamson, J.R. (1965) Glycolytic control mechanisms I: inhibition of glycolysis by acetate and pyruvate in the isolated, perfused rat heart. *J. Biol. Chem.* **240**, 2308–2321.

*Calculated as μmol/mL of intracellular water.

(a) Calculate Q, [fructose 1,6-bisphosphate][ADP]/[fructose 6-phosphate][ATP], for the PFK-1 reaction under physiological conditions.

(b) Given a $\Delta G'^{\circ}$ for the PFK-1 reaction of −14.2 kJ/mol, calculate the equilibrium constant for this reaction.

(c) Compare the values of Q and K'_{eq}. Is the physiological reaction near or far from equilibrium? Explain. What does this experiment suggest about the role of PFK-1 as a regulatory enzyme?

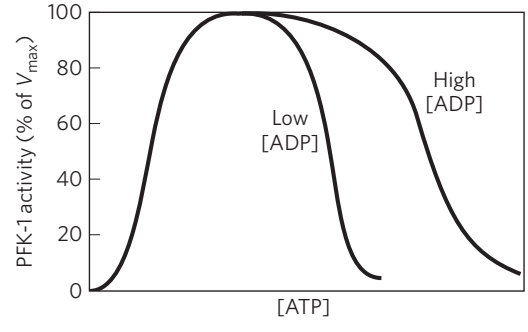
2. Are All Metabolic Reactions at Equilibrium?

(a) Phosphoenolpyruvate (PEP) is one of the two phosphoryl group donors in the synthesis of ATP during glycolysis. In human erythrocytes, the steady-state concentration of ATP is 2.24 mM, that of ADP is 0.25 mM, and that of pyruvate is 0.051 mM. Calculate the concentration of PEP at 25 °C, assuming that the pyruvate kinase reaction (see Fig. 13–13) is at equilibrium in the cell.

(b) The physiological concentration of PEP in human erythrocytes is 0.023 mM. Compare this with the value obtained in (a). Explain the significance of this difference.

3. Effect of O_2 Supply on Glycolytic Rates The regulated steps of glycolysis in intact cells can be identified by studying the catabolism of glucose in whole tissues or organs. For example, the glucose consumption by heart muscle can be measured by artificially circulating blood through an isolated intact heart and measuring the concentration of glucose before and after the blood passes through the heart. If the circulating blood is deoxygenated, heart muscle consumes glucose at a steady rate. When oxygen is added to the blood, the rate of glucose consumption drops dramatically, then is maintained at the new, lower rate. Explain.

4. Regulation of PFK-1 The effect of ATP on the allosteric enzyme PFK-1 is shown below. For a given concentration of fructose 6-phosphate, the PFK-1 activity increases with increasing concentrations of ATP, but a point is reached beyond which increasing the concentration of ATP inhibits the enzyme.



(a) Explain how ATP can be both a substrate and an inhibitor of PFK-1. How is the enzyme regulated by ATP?

(b) In what ways is glycolysis regulated by ATP levels?

(c) The inhibition of PFK-1 by ATP is diminished when the ADP concentration is high, as shown in the illustration. How can this observation be explained?

5. Cellular Glucose Concentration The concentration of glucose in human blood plasma is maintained at about 5 mM. The concentration of free glucose inside a myocyte is much lower. Why is the concentration so low in the cell? What happens to glucose after entry into the cell? Glucose is administered intravenously as a food source in certain clinical situations. Given that the transformation of glucose to glucose 6-phosphate consumes ATP, why not administer intravenous glucose 6-phosphate instead?

6. Enzyme Activity and Physiological Function The V_{max} of the glycogen phosphorylase from skeletal muscle is much greater than the V_{max} of the same enzyme from liver tissue.

(a) What is the physiological function of glycogen phosphorylase in skeletal muscle? In liver tissue?

(b) Why does the V_{max} of the muscle enzyme need to be greater than that of the liver enzyme?

7. Glycogen Phosphorylase Equilibrium Glycogen phosphorylase catalyzes the removal of glucose from glycogen. The $\Delta G'^{\circ}$ for this reaction is 3.1 kJ/mol.

(a) Calculate the ratio of [P_i] to [glucose 1-phosphate] when the reaction is at equilibrium. (Hint: The removal of glucose units from glycogen does not change the glycogen concentration.)

(b) The measured ratio [P_i]/[glucose 1-phosphate] in myocytes under physiological conditions is more than 100:1. What does this indicate about the direction of metabolite flow through the glycogen phosphorylase reaction in muscle?

(c) Why are the equilibrium and physiological ratios different? What is the possible significance of this difference?

8. Regulation of Glycogen Phosphorylase In muscle tissue, the rate of conversion of glycogen to glucose 6-phosphate is determined by the ratio of phosphorylase *a* (active) to phosphorylase *b* (less active). Determine what happens to the rate of glycogen breakdown if a muscle preparation containing glycogen phosphorylase is treated with (a) phosphorylase kinase and ATP; (b) PP1; (c) epinephrine.

9. Glycogen Breakdown in Rabbit Muscle The intracellular use of glucose and glycogen is tightly regulated at four points. To compare the regulation of glycolysis when oxygen is plentiful and when it is depleted, consider the utilization of glucose and glycogen by rabbit leg muscle in two physiological settings: a resting rabbit, with low ATP demands, and a rabbit that sights its mortal enemy, the coyote, and dashes into its burrow. For each setting, determine the relative levels (high, intermediate, or low) of AMP, ATP, citrate, and acetyl-CoA and describe how these levels affect the flow of metabolites through glycolysis by regulating specific enzymes. In periods of stress, rabbit leg muscle produces much of its ATP by anaerobic glycolysis (lactate fermentation) and very little by oxidation of acetyl-CoA derived from fat breakdown.

10. Glycogen Breakdown in Migrating Birds Unlike the rabbit with its short dash, migratory birds require energy for extended periods of time. For example, ducks generally fly several thousand miles during their annual migration. The flight muscles of migratory birds have a high oxidative capacity and obtain the necessary ATP through the oxidation of acetyl-CoA (obtained from fats) via the citric acid cycle. Compare the regulation of muscle glycolysis during short-term intense activity, as in the fleeing rabbit, and during extended activity, as in the migrating duck. Why must the regulation in these two settings be different?



11. Enzyme Defects in Carbohydrate Metabolism Summaries of four clinical case studies follow. For each case determine which enzyme is defective and designate the appropriate treatment, from the lists provided at the end of the problem. Justify your choices. Answer the questions contained in each case study. (You may need to refer to information in Chapter 14.)

Case A The patient develops vomiting and diarrhea shortly after milk ingestion. A lactose tolerance test is administered. (The patient ingests a standard amount of lactose, and the glucose and galactose concentrations of blood plasma are measured at intervals. In individuals with normal carbohydrate metabolism, the levels increase to a maximum in about 1 hour, then decline.) The patient's blood glucose and galactose concentrations do not increase during the test. Why do blood glucose and galactose increase and then decrease during the test in healthy individuals? Why do they fail to rise in the patient?

Case B The patient develops vomiting and diarrhea after ingestion of milk. His blood is found to have a low concentration of glucose but a much higher than normal concentration of reducing sugars. The urine tests positive for galactose. Why is the concentration of reducing sugar in the blood high? Why does galactose appear in the urine?

Case C The patient complains of painful muscle cramps when performing strenuous physical exercise but has no other symptoms. A muscle biopsy indicates a muscle glycogen concentration much higher than normal. Why does glycogen accumulate?

Case D The patient is lethargic, her liver is enlarged, and a biopsy of the liver shows large amounts of excess glycogen. She also has a lower than normal blood glucose level. What is the reason for the low blood glucose in this patient?

Defective Enzyme

(a) Muscle PFK-1
(b) Phosphomannose isomerase
(c) Galactose 1-phosphate uridylyltransferase
(d) Liver glycogen phosphorylase
(e) Triose kinase
(f) Lactase in intestinal mucosa
(g) Maltase in intestinal mucosa
(h) Muscle debranching enzyme

Treatment

1. Jogging 5 km each day
2. Fat-free diet
3. Low-lactose diet
4. Avoiding strenuous exercise
5. Large doses of niacin (the precursor of NAD^+)
6. Frequent feedings (smaller portions) of a normal diet



12. Effects of Insufficient Insulin in a Person with Diabetes A man with insulin-dependent diabetes is brought to the emergency room in a near-comatose state. While vacationing in an isolated place, he lost his insulin medication and has not taken any insulin for two days.

(a) For each tissue listed below, is each pathway faster, slower, or unchanged in this patient, compared with the normal level when he is getting appropriate amounts of insulin?

(b) For each pathway, describe at least one control mechanism responsible for the change you predict.

Tissue and Pathways

1. Adipose: fatty acid synthesis
2. Muscle: glycolysis; fatty acid synthesis; glycogen synthesis
3. Liver: glycolysis; gluconeogenesis; glycogen synthesis; fatty acid synthesis; pentose phosphate pathway

13. Blood Metabolites in Insulin Insufficiency For the patient described in Problem 12, predict the levels of the following metabolites in his blood *before* treatment in the emergency room, relative to levels maintained during adequate insulin treatment: (a) glucose; (b) ketone bodies; (c) free fatty acids.

14. Metabolic Effects of Mutant Enzymes Predict and explain the effect on glycogen metabolism of each of the following defects caused by mutation: (a) loss of the cAMP-binding site on the regulatory subunit of protein kinase A (PKA); (b) loss of the protein phosphatase inhibitor (inhibitor 1 in Fig. 15–42); (c) overexpression of phosphorylase *b* kinase in liver; (d) defective glucagon receptors in liver.

15. Hormonal Control of Metabolic Fuel Between your evening meal and breakfast, your blood glucose drops and your liver becomes a net producer rather than consumer of glucose. Describe the hormonal basis for this switch, and explain how the hormonal change triggers glucose production by the liver.

16. Altered Metabolism in Genetically Manipulated Mice Researchers can manipulate the genes of a mouse so that a single gene in a single tissue either produces an inactive protein (a "knockout" mouse) or produces a protein that is always (constitutively) active. What effects on metabolism would you predict for mice with the following genetic changes: (a) knockout of glycogen debranching enzyme in the liver; (b) knockout of hexokinase IV in liver; (c) knockout of FBPase-2 in liver; (d) constitutively active FBPase-2 in liver; (e) constitutively active AMPK in muscle; (f) constitutively active ChREBP in liver?

Data Analysis Problem

17. Optimal Glycogen Structure Muscle cells need rapid access to large amounts of glucose during heavy exercise. This glucose is stored in liver and skeletal muscle in polymeric form as particles of glycogen. The typical glycogen particle contains about 55,000 glucose residues (see Fig. 15–35b). Meléndez-Hevia, Waddell, and Shelton (1993) explored some theoretical aspects of the structure of glycogen, as described in this problem.

(a) The cellular concentration of glycogen in liver is about 0.01 μM. What cellular concentration of free glucose would be required to store an equivalent amount of glucose? Why would this concentration of free glucose present a problem for the cell?

Glucose is released from glycogen by glycogen phosphorylase, an enzyme that can remove glucose molecules, one at a time, from one end of a glycogen chain. Glycogen chains are branched (see Figs 15–28 and 15–35b), and the degree of branching—the number of branches per chain—has a powerful influence on the rate at which glycogen phosphorylase can release glucose.

(b) Why would a degree of branching that was too low (i.e., below an optimum level) reduce the rate of glucose release? (Hint: Consider the extreme case of no branches in a chain of 55,000 glucose residues.)

(c) Why would a degree of branching that was too high also reduce the rate of glucose release? (Hint: Think of the physical constraints.)

Meléndez-Hevia and colleagues did a series of calculations and found that two branches per chain (see Fig. 15–35b) was optimal for the constraints described above. This is what is found in glycogen stored in muscle and liver.

To determine the optimum number of glucose residues per chain, Meléndez-Hevia and coauthors considered two key parameters that define the structure of a glycogen particle: t = the number of tiers of glucose chains in a particle (the molecule in Fig. 15–35b has five tiers); g_c = the number of glucose residues in each chain. They set out to find the values of t and g_c that would maximize three quantities: (1) the amount of glucose stored in the particle (G_T) per unit volume; (2) the number of unbranched glucose chains (C_A) per unit volume (i.e., number of chains in the outermost tier, readily accessible to glycogen phosphorylase); and (3) the amount of glucose available to phosphorylase in these unbranched chains (G_{PT}).

(d) Show that $C_A = 2^{t-1}$. This is the number of chains available to glycogen phosphorylase before the action of the debranching enzyme.

(e) Show that C_T, the total number of chains in the particle, is given by $C_T = 2^t - 1$. Thus $G_T = g_c(C_T) = g_c(2^t - 1)$, the total number of glucose residues in the particle.

(f) Glycogen phosphorylase cannot remove glucose from glycogen chains that are shorter than five glucose residues. Show that $G_{PT} = (g_c - 4)(2^{t-1})$. This is the amount of glucose readily available to glycogen phosphorylase.

(g) Based on the size of a glucose residue and the location of branches, the thickness of one tier of glycogen is $0.12\ g_c$ nm + 0.35 nm. Show that the volume of a particle, V_s, is given by the equation $V_s = \frac{4}{3}\pi t^3(0.12g_c + 0.35)^3\ \text{nm}^3$.

Meléndez-Hevia and coauthors then determined the optimum values of t and g_c—those that gave the maximum value of a quality function, f, that maximizes G_T, C_A, and G_{PT}, while minimizing V_s: $f = \frac{G_T C_A G_{PT}}{V_S}$. They found that the optimum value of g_c is independent of t.

(h) Choose a value of t between 5 and 15 and find the optimum value of g_c. How does this compare with the g_c found in liver glycogen (see Fig. 15–35b)? (Hint: You may find it useful to use a spreadsheet program.)

Reference

Meléndez-Hevia, E., Waddell, T.G., & Shelton, E.D. (1993) Optimization of molecular design in the evolution of metabolism: the glycogen molecule. *Biochem. J.* **295**, 477–483.

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