Source – Lehninger (Principle of Biochemistry)

flux through one or more pathways. In contrast, the very rapid (milliseconds) allosteric changes in enzyme activity are generally triggered locally, by changes in the level of a metabolite within the cell. The allosteric effector may be a substrate of the affected pathway (glucose for glycolysis, for example), a product of a pathway (ATP from glycolysis), or a key metabolite or cofactor (such as NADH) that indicates the cell's metabolic state. A single enzyme is commonly regulated in several ways—for example, by modulation of its synthesis, by covalent alteration, and by allosteric effectors.

Yet another way to alter the effective activity of an enzyme is to change the accessibility of its substrate. The hexokinase of muscle 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 in the plasma membrane. Within cells, membrane-bounded compartments segregate certain enzymes and enzyme systems, and the transport of substrate into these compartments may be the limiting factor in enzyme action.

In the discussion that follows, 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.

SUMMARY 15.2 Regulation of Metabolic Pathways

- In a metabolically active cell in a steady state, intermediates are formed and consumed at equal rates. When a perturbation alters the rate of formation or consumption of a metabolite, compensating changes in enzyme activities return the system to the steady state.
- 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 storage of glycogen to the organism's changing needs.
- In multistep processes such as glycolysis, certain reactions are essentially at equilibrium in the steady state; the rates of these substrate-limited reactions rise and fall with substrate concentration. Other reactions are far from equilibrium; their rates are too slow to produce instant equilibration of substrate and product. These enzyme-limited reactions are often highly exergonic and therefore metabolically irreversible, and the enzymes that catalyze them are commonly the points at which flux through the pathway is regulated.
- The activity of an enzyme can be regulated by changing the rate of its synthesis or degradation, by allosteric or covalent alteration of existing enzyme molecules, or by separating the enzyme from its substrate in subcellular compartments.
- Fast metabolic adjustments (on the time scale of seconds or less) at the intracellular level are generally allosteric. The effects of hormones and growth factors are generally slower (seconds to hours) and are commonly achieved by covalent modification or changes in enzyme synthesis.

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. Gluconeogenesis employs most 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. 15–15). Three reactions of glycolysis are so exergonic as to be essentially irreversible: those catalyzed by hexokinase, PFK-1, and pyruvate kinase. Notice in Table 15–2 that all three reactions have a large, negative $\Delta G'$. 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; Fig. 15–15). Note that 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

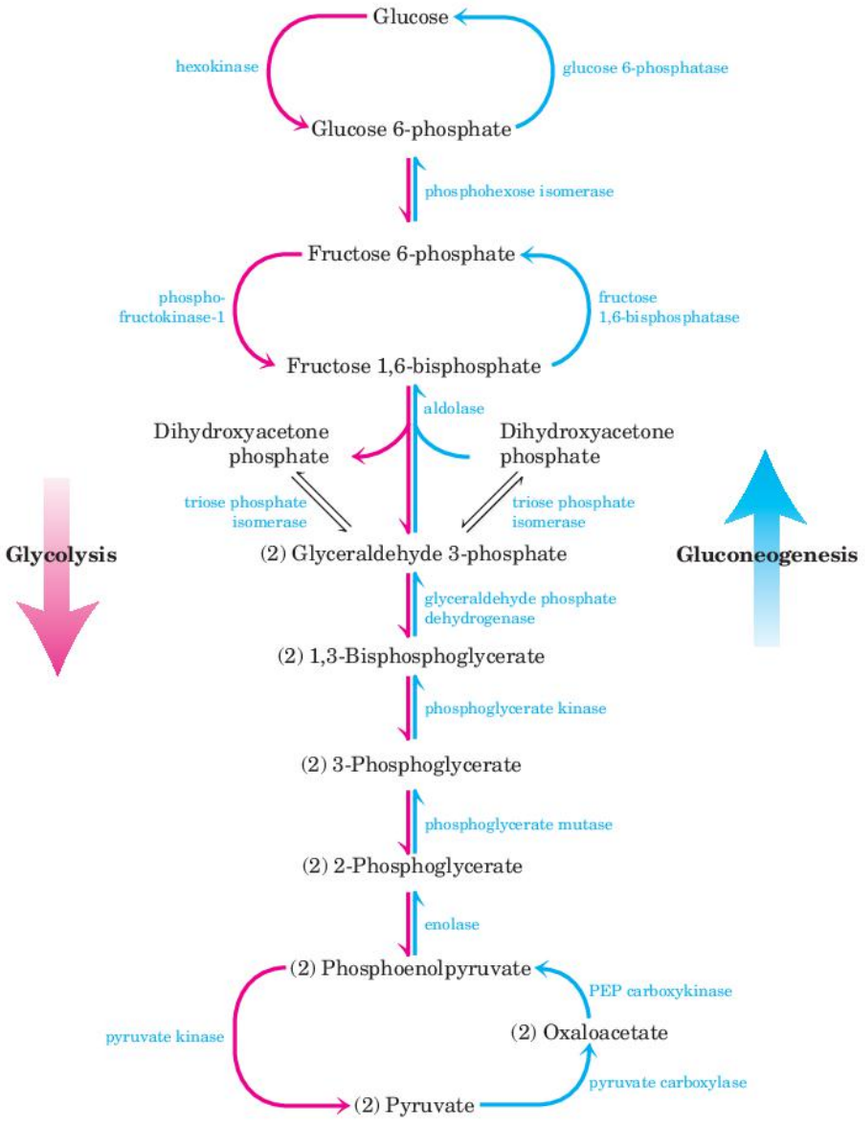

FIGURE 15–15 Glycolysis and gluconeogenesis. Opposing pathways of glycolysis (pink) and gluconeogenesis (blue) in rat liver. Three steps are catalyzed by different enzymes in gluconeogenesis (the "bypass reactions") and glycolysis; seven steps are catalyzed by the same enzymes in the two pathways. Cofactors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

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. 15–15).

We begin our examination of the coordinated regulation of glycolysis and gluconeogenesis by considering the regulatory patterns seen at the three main control points of glycolysis. We then look at the regulation of the enzymes of gluconeogenesis, leading to a consideration of how the regulation of both pathways is tightly, reciprocally coordinated.

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

Hexokinase, which catalyzes the entry of free glucose into the glycolytic pathway, is a regulatory enzyme. There are 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 hexokinases I and 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.

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 removing or producing glucose, depending on the prevailing glucose concentration. The

BOX 15–2 WORKING IN BIOCHEMISTRY

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, or even in the same cell. The different forms 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. 538), 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

These 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.

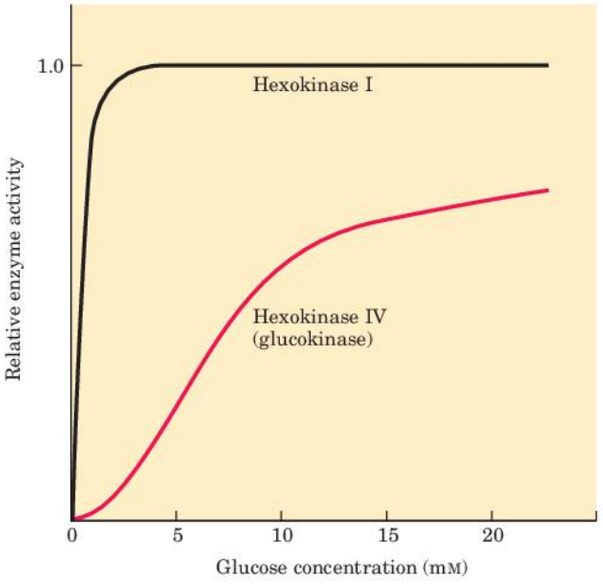

FIGURE 15–16 Comparison of the kinetic properties of hexokinase IV (glucokinase) and hexokinase I. Note the sigmoidicity for hexokinase IV and the much lower K_m for hexokinase I. When blood glucose rises above 5 mM, hexokinase IV activity increases, but hexokinase I is already operating near V_{max} at 5 mM glucose and cannot respond to an increase in glucose concentration. Hexokinase I, II, and III have similar kinetic properties.

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;** see Fig. 11–31) rapidly equilibrates the glucose concentrations in cytosol and blood, the high K_m of hexokinase IV allows its direct regulation by the level of blood glucose (Fig. 15–16). When the blood glucose concentration 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.

Second, hexokinase IV is subject to inhibition by the reversible binding of a regulatory protein specific to liver (Fig. 15–17). 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 hexokinase IV, 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 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. 15–17). When the glucose concentration in the cell 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.

Third, 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.

Phosphofructokinase-1 Is under Complex Allosteric Regulation

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

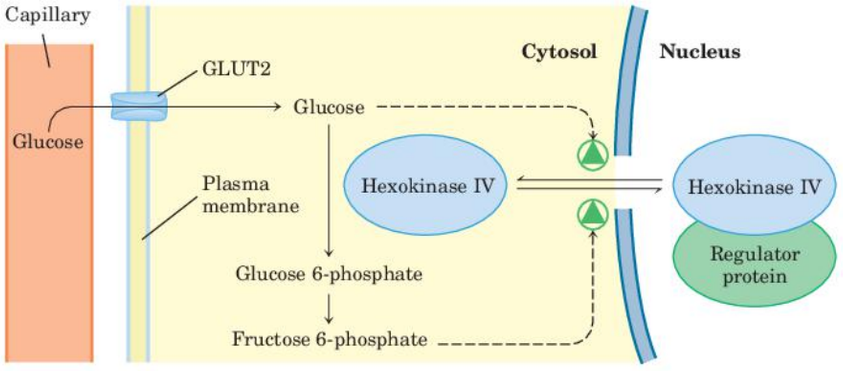

FIGURE 15–17 Regulation of hexokinase IV (glucokinase) by sequestration in the nucleus. The protein inhibitor of hexokinase IV is a nuclear binding protein that draws hexokinase IV into the nucleus when the fructose 6-0phosphate concentration in liver is high and releases it to the cytosol when the glucose concentration is high.