16.3 Regulation of the Citric Acid Cycle

As we have seen in Chapter 15, the regulation of key enzymes in metabolic pathways, by allosteric effectors and by covalent modification, ensures the production of intermediates at the rates required to keep the cell in a stable steady state while avoiding wasteful overproduction. The flow of carbon atoms from pyruvate into and through the citric acid cycle is under tight regulation at two levels: the conversion of pyruvate to acetyl-CoA, the starting material for the cycle (the pyruvate dehydrogenase complex reaction), and the entry of acetyl-CoA into the cycle (the citrate synthase reaction). Acetyl-CoA is also produced by pathways other than the PDH complex reaction—most cells produce acetyl-CoA from the oxidation of fatty acids and certain amino acids—and the availability of intermediates from these other pathways is important in the regulation of pyruvate oxidation and of the citric acid cycle. The cycle is also regulated at the isocitrate dehydrogenase and α-ketoglutarate dehydrogenase reactions.

Production of Acetyl-CoA by the Pyruvate Dehydrogenase Complex Is Regulated by Allosteric and Covalent Mechanisms

The PDH complex of mammals is strongly inhibited by ATP and by acetyl-CoA and NADH, the products of the reaction catalyzed by the complex (Fig. 16–18). The allosteric inhibition of pyruvate oxidation is greatly enhanced when long-chain fatty acids are available. AMP, CoA, and NAD$^+$, all of which accumulate when too little acetate flows into the citric acid cycle, allosterically activate the PDH complex. Thus, this enzyme activity is turned off when ample fuel is available in the form of fatty acids and acetyl-CoA and when the cell's [ATP]/[ADP] and [NADH]/[NAD$^+$] ratios are high, and it is turned on again when energy demands are high and the cell requires greater flux of acetyl-CoA into the citric acid cycle.

In mammals, these allosteric regulatory mechanisms are complemented by a second level of regulation: covalent protein modification. The PDH complex is inhibited by reversible phosphorylation of a specific Ser residue on one of the two subunits of E$_1$. As noted earlier, in addition to the enzymes E$_1$, E$_2$, and E$_3$, the mammalian PDH complex contains two regulatory proteins whose sole purpose is to regulate the activity of the complex. A specific protein kinase phosphorylates and thereby inactivates E$_1$, and a specific phosphoprotein phosphatase removes the phosphoryl group by hydrolysis and thereby activates E$_1$. The kinase is allosterically activated by ATP: when [ATP] is high (reflecting a sufficient supply of energy), the PDH complex is inactivated by phosphorylation of E$_1$. When [ATP] declines, kinase activity decreases and phosphatase action removes the phosphoryl groups from E$_1$, activating the complex.

The PDH complex of plants, located in the mitochondrial matrix and in plastids, is inhibited by its products, NADH and acetyl-CoA. The plant mitochondrial

FIGURE 16–18 Regulation of metabolite flow from the PDH complex through the citric acid cycle. The PDH complex is allosterically inhibited when [ATP]/[ADP], [NADH]/[NAD$^+$], and [acetyl-CoA]/[CoA] ratios are high, indicating an energy-sufficient metabolic state. When these ratios decrease, allosteric activation of pyruvate oxidation results. The rate of flow through the citric acid cycle can be limited by the availability of the citrate synthase substrates, oxaloacetate and acetyl-CoA, or of NAD$^+$, which is depleted by its conversion to NADH, slowing the three NAD-dependent oxidation steps. Feedback inhibition by succinyl-CoA, citrate, and ATP also slows the cycle by inhibiting early steps. In muscle tissue, Ca^{2+} signals contraction and, as shown here, stimulates energy-yielding metabolism to replace the ATP consumed by contraction.
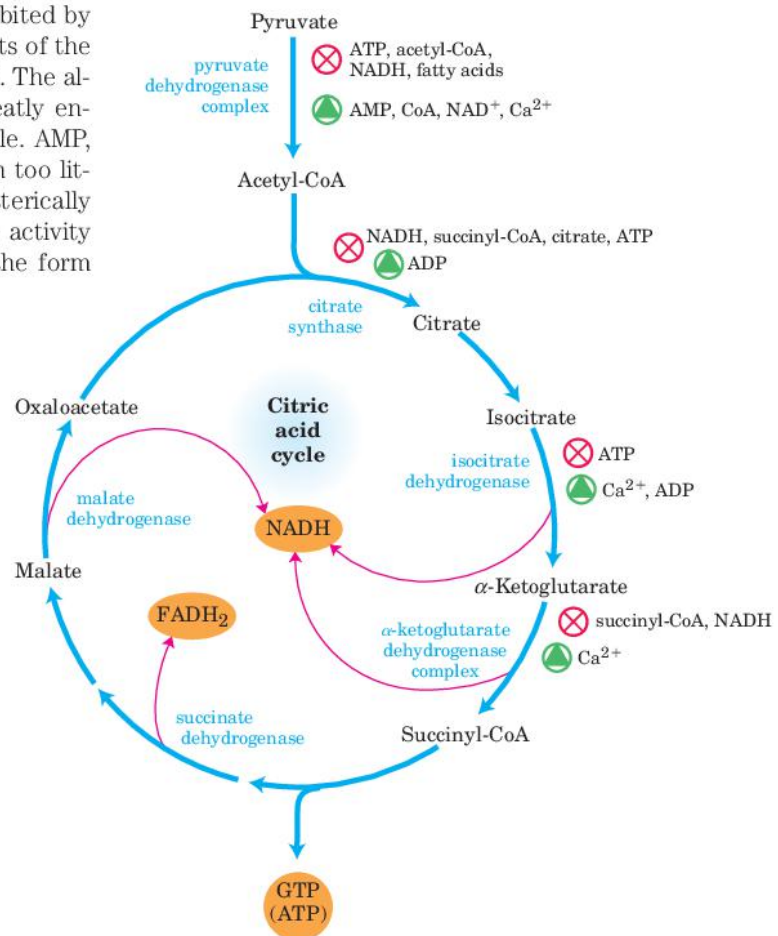
enzyme is also regulated by reversible phosphorylation; pyruvate inhibits the kinase, thus activating the PDH complex, and NH_4^+ stimulates the kinase, causing inactivation of the complex. The PDH complex of *E. coli* is under allosteric regulation similar to that of the mammalian enzyme, but it does not seem to be regulated by phosphorylation.

The Citric Acid Cycle Is Regulated at Its Three Exergonic Steps

The flow of metabolites through the citric acid cycle is under stringent regulation. Three factors govern the rate of flux through the cycle: substrate availability, inhibition by accumulating products, and allosteric feedback inhibition of the enzymes that catalyze early steps in the cycle.

Each of the three strongly exergonic steps in the cycle—those catalyzed by citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase (Fig. 16–18)—can become the rate-limiting step under some circumstances. The availability of the substrates for citrate synthase (acetyl-CoA and oxaloacetate) varies with the metabolic state of the cell and sometimes limits the rate of citrate formation. NADH, a product of isocitrate and α-ketoglutarate oxidation, accumulates under some conditions, and at high [NADH]/[NAD$^+$] both dehydrogenase reactions are severely inhibited by mass action. Similarly, in the cell, the malate dehydrogenase reaction is essentially at equilibrium (that is, it is substrate-limited, and when [NADH]/[NAD$^+$] is high the concentration of oxaloacetate is low, slowing the first step in the cycle. Product accumulation inhibits all three limiting steps of the cycle: succinyl-CoA inhibits α-ketoglutarate dehydrogenase (and also citrate synthase); citrate blocks citrate synthase; and the end product, ATP, inhibits both citrate synthase and isocitrate dehydrogenase. The inhibition of citrate synthase by ATP is relieved by ADP, an allosteric activator of this enzyme. In vertebrate muscle, Ca^{2+}, the signal for contraction and for a concomitant increase in demand for ATP, activates both isocitrate dehydrogenase and α-ketoglutarate dehydrogenase, as well as the PDH complex. In short, the concentrations of substrates and intermediates in the citric acid cycle set the flux through this pathway at a rate that provides optimal concentrations of ATP and NADH.

Under normal conditions, the rates of glycolysis and of the citric acid cycle are integrated so that only as much glucose is metabolized to pyruvate as is needed to supply the citric acid cycle with its fuel, the acetyl groups of acetyl-CoA. Pyruvate, lactate, and acetyl-CoA are normally maintained at steady-state concentrations. The rate of glycolysis is matched to the rate of the citric acid cycle not only through its inhibition by high levels of ATP and NADH, which are common to both the glycolytic and respiratory stages of glucose oxidation, but also by the concentration of citrate. Citrate, the product of the first step of the citric acid cycle, is an important allosteric inhibitor of phosphofructokinase-1 in the glycolytic pathway (see Fig. 15–18).

Substrate Channeling through Multienzyme Complexes May Occur in the Citric Acid Cycle

Although the enzymes of the citric acid cycle are usually described as soluble components of the mitochondrial matrix (except for succinate dehydrogenase, which is membrane-bound), growing evidence suggests that within the mitochondrion these enzymes exist as multienzyme complexes. The classic approach of enzymology—purification of individual proteins from extracts of broken cells—was applied with great success to the citric acid cycle enzymes. However, the first casualty of cell breakage is higher-level organization within the cell—the noncovalent, reversible interaction of one protein with another, or of an enzyme with some structural component such as a membrane, microtubule, or microfilament. When cells are broken open, their contents, including enzymes, are diluted 100- or 1,000-fold (Fig. 16–19).

Several types of evidence suggest that, in cells, multienzyme complexes ensure efficient passage of the product of one enzyme reaction to the next enzyme in the pathway. Such complexes are called **metabolons.** Certain enzymes of the citric acid cycle have been isolated together as supramolecular aggregates, or have been found associated with the inner mitochondrial membrane, or have been shown to diffuse in the mitochondrial matrix more slowly than expected for the individual protein in solution. There is strong evidence for substrate channeling through multienzyme complexes in
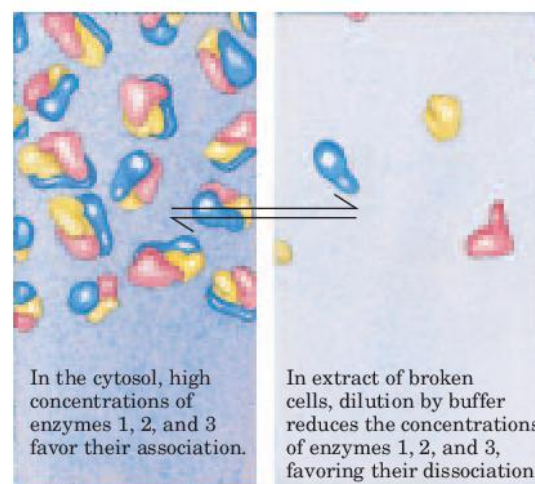

In the cytosol, high concentrations of enzymes 1, 2, and 3 favor their association.

In extract of broken cells, dilution by buffer reduces the concentrations of enzymes 1, 2, and 3, favoring their dissociation.

FIGURE 16–19 Dilution of a solution containing a noncovalent protein complex—such as one consisting of three enzymes—favors dissociation of the complex into its constituents.