Theme 2

METABOLIC CONTROL

Included in this section:
Please use the study material provide as well as Matthews and van Holde – Biochemistry.
Page number may differ (3rd edition pp 463-467; pp 472-477; pp 504-506)

Gluconeogenesis and glycolysis both proceed largely in the cytosol. Because gluconeogenesis synthesizes glucose and glycolysis catabolizes glucose, it is evident that gluconeogenesis and glycolysis must be controlled in reciprocal fashion. If not for reciprocal control, glycolysis and gluconeogenesis would operate together as a giant futile cycle.

Conditions of low energy charge tend to activate the rate-controlling steps in glycolysis while inhibiting carbon flux through gluconeogenesis. Conversely, gluconeogenesis is stimulated at high energy charge, under conditions where catabolic flux rates are adequate to maintain sufficient ATP levels.

Regulation of the two pathways primarily is brought about by allosteric controls on the enzymes that differ between the two pathways. These enzymes are:
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<th>Glycolysis Enzyme</th>
<th>Gluconeogenesis Enzyme</th>
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<tr>
<td>Hexokinase</td>
<td>Glucose-6-phosphatase</td>
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<tr>
<td>Phosphofructokinase-1 (PFK-1)</td>
<td>Fructose 1,6 bisphosphatase</td>
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<td>Pyruvate kinase (note on isozymes)</td>
<td>1. Pyruvate Carboxylase</td>
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<td>2. Phosphoenolpyruvate carboxykinase (PEPCK)</td>
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1. **F2,6BP** and **AMP** activate **PFK-1** and inhibit **Fructose 1,6 bisphosphatase**

2. **G6P** substrate levels control **Hexokinase** and **Glucose-6-phosphatase**

3. **Acetyl-CoA** inhibits **Pyruvate kinase** and activates **Pyruvate Carboxylase**

Other control points on the two pathways are shown in **Figure 16.6**

The major allosteric regulatory factor of the two pathways is **Fructose 2,6 bisphosphate**. Note in **Figure 16.7** that **PFK-2** and **Fructose 2,6-bisphosphatase** are on the same peptide and are affected differently by phosphorylation (see below). Interconversion of **PFK-2** and **Fructose 2,6-bisphosphatase** depends on the level of **cAMP** (which is stimulated by **glucagon** and **epinephrine** and is inhibited by **insulin**). Increasing cAMP (glucagon/epinephrine) stimulates phosphorylation of **PFK-2** and **Fructose 2,6-bisphosphatase**, favoring the **Fructose 2,6-bisphosphatase**. Decreasing cAMP (insulin) stimulates dephosphorylation, favoring **PFK-2**.

Fructose-2,6-bisphosphatase is strongly inhibited by **fructose-6-phosphate**.

**Glucagon** represses transcription of **pyruvate kinase**.

**Glucagon** activates transcription of **phosphoenolpyruvate carboxykinase**

**Insulin** represses transcription of **phosphoenolpyruvate carboxykinase**.
Hormonal control of metabolism

- Glucagon, insulin and epinephrine (adrenalin) are particularly important in controlling fat and carbohydrate metabolism.
- Glucagon is produced in response to low blood glucose and insulin in response to high levels.
- Epinephrine is released from the adrenal gland and stimulates the release of food reserves, as does norepinephrine.

How do glucagon, epinephrine and insulin work?
- Only target cells respond to any given hormone. These hormones bind to extracellular receptors and produce their effect via an intracellular response.

What is a second messenger?
- This is a small intracellular regulatory molecule that changes in level when a hormone binds outside. It produces an intracellular response.

What is the second messenger for glucagon and epinephrine?
- This is cyclic AMP (cAMP), produced from ATP by the enzyme adenylate cyclase
- CAMP allosterically activates protein kinase A (PKA), which phosphorylates some enzymes at serine or threonine.
- CAMP binding to PKA causes the release of catalytically active C subunits.
- CAMP is hydrolysed to AMP by cAMP phosphodiesterase so that the effects of a hormone are transient unless it is continually present.

Control of carbohydrate metabolism

Control of glucose uptake into cells
- Glucose does not diffuse across membranes. It must be transported by proteins.
- This process is not insulin-responsive in brain or liver but is controlled by insulin in adipose cells and muscle.
- In these tissues insulin increases the number of transport proteins in the membrane.
- The increase occurs by the movement of transport proteins from within the cell to the membrane.
- Glucose transport occurs by facilitated diffusion.
Once glucose enters the cell it is rapidly phosphorylated by hexokinase (most tissues) or glucokinase (liver).

The lower affinity of the latter means that at times when blood glucose is low and the liver is releasing glucose it does not immediately take it up again.

Hexokinase is inhibited by glucose-6-phosphate at physiological concentrations but glucokinase is not. This allows the liver to take up glucose at high blood sugar levels and to synthesise glycogen-using glucokinase to phosphorylate glucose.

Not only does glucokinase only operate efficiently at high blood glucose but it is induced by insulin.

**Control of glycogen metabolism**

- Glycogen is made by glycogen synthase after feeding and broken down by glycogen phosphorylase during fasting.
- Muscle glycogen is used from glucose-6-phosphate, which in turn is used for ATP synthesis.
- AMP stimulates muscle phosphorylase and ATP inhibits it. AMP is formed from ADP by the enzyme adenylate kinase, which catalyses the formation of ATP and AMP from ADP.

**Activation of muscle phosphorylase by cAMP**

- In emergency situations adrenal release epinephrine and this causes the production of cAMP within muscle.
- Normally phosphorylase exists in the b form and is only active when it is allosterically activated by AMP.
- cAMP causes the conversion of phosphorylase b to the a form, through the action of phosphorylase kinase. The a form is active in the absence of AMP.
- The activation of phosphorylase kinase is via PKA. cAMP stimulates this enzyme and it phosphorylates phosphorylase kinase, thus activating it.
- This sequence of events amplifies the original signal of a relatively small number of epinephrine molecules binding to receptors and allows massive amounts of glucose to be mobilised.
- Muscle phosphorylase b kinase is also activated to some extent by calcium ions that are released during contraction. This does not involve phosphorylation of
phosphorylase kinase and is more rapidly reversed once calcium levels decrease. Calmodulin mediates this process.

Reversal of phosphorylase activation

- Phosphoprotein phosphatase 1 removes the phosphate group from phosphorylase a and converts it back to the b form.
- CAMP inhibits this process via PKA. PKA converts phosphatase inhibitor 1 from the inactive, dephosphorylated form to the active, phosphorylated form. Phosphorylated inhibitor 1 turns off phosphoprotein phosphatase 1.
- A summary of this control system is
  1. Phosphorylase b can be activated by AMP.
  2. Calcium ions partially activate phosphorylase b kinase, converting it to the a form.
  3. Epinephrine causes an increase in cAMP.
  4. cAMP activates PKA and this activates phosphorylates b kinase by phosphorylation. The result is the conversion of phosphorylase b to phosphorylase a.
  5. Phosphoprotein phosphatase 1 reverses this situation unless inhibitor 1 is present in the phosphorylated form.

Control of glycogen breakdown in the liver

- Liver releases glucose from glycogen breakdown into the blood. Glucagon stimulates this process and does so via cAMP as in the muscle (muscle does not have glucagon receptors and therefore does not respond in this way).
- Liver also releases glucose in response to epinephrine.
- Liver phosphorylase is inhibited by glucose.

Control of glycogen synthase

- Glycogen synthase is activated by insulin.
- It is inhibited by phosphorylation.
- Glycogen synthase has multiple phosphorylation sites, including a cluster of three sites towards the C-terminal end. These are dephosphorylated in response to insulin.
- Glycogen synthase kinase 3 (GSK 3) acts on these sites and is inactivated by insulin.
How does insulin inactivate GSK 3?
- Insulin binding to cell surface receptors activates protein kinase B (PKB).
- PKB inactivates GSK 3 by phosphorylation.
- Insulin also activates phosphoprotein phosphatase.
- Once insulin levels fall the process is reversed.

Control of glycolysis and gluconeogenesis
Allosteric controls
- AMP activates phosphofructokinase (PFK) as well as glycogen phosphorylase. It also inhibits fructose-1:6-bisphosphatase.
- PFK is activates by fructose-6-phosphate from glycogen breakdown, thus increasing the level of fructose-1:6-bisphosphate and pyruvate kinase activity.
- ATP inhibits PFK.
- When ATP is high, citrate accumulates because of inhibition of the citric acid cycle and this also inhibits PFK.
- Pyruvate carboxylase is activated by acetyl-CoA since its accumulation is a signal that oxaloacetate is required.

Hormonal control of glycolysis and gluconeogenesis
- Glucagon causes liver to produce glucose both via gluconeogenesis and glycogenolysis.
- This is mediated via cAMP.
- cAMP does not stimulate glycolysis in liver.
- In muscle cAMP has the role of stimulating energy production, not glucose release.
- cAMP stimulates glycogenolysis and glycolysis.

How does cAMP control PFK?
- Glucagon, acting via cAMP, causes a decrease in another effector molecule, fructose-2:6-bisphosphatase.
- This effector is a powerful allosteric activator of liver PFK.
- Fructose-2:6-bisphosphate also inhibits fructose bisphosphatase, slowing gluconeogenesis.
• Fructose-2:6-bisphosphate is produced by a different type of phosphofructokinase, PFK₂.
• The 'normal' phosphofructokinase is labelled PFK₁ to signify that it was discovered first.
• cAMP inhibits PFK₂ by activating a kinase which phosphorylates it.
• This phosphorylation also has the effect of causing PFK₂ to destroy fructose-2:6-
bisphosphate by hydrolysis.
• Hormonal controls of this type do not occur for PFK in muscle.

Control of gluconeogenesis

REMEMBER HEXOKINASE VS. GLUCOSE-6-PHOSPHATASE

• Glucagon causes inhibition of PFK and activation of fructose-1:6-bisphosphatase.
• In liver cAMP causes phosphorylation of pyruvate kinase and inactivates it. This
decreases glucose utilisation and diverts PEP to glucose synthesis.

**Phosphofructokinase** (PFK) is a major control point for glycolysis. PFK is allosterically
inhibited by ATP and citrate, allosterically activated by AMP, ADP, and F2,6BP. Thus,
carbon movement through glycolysis is inhibited at PFK when the cell contains ample
stores of ATP and oxidizable substrates. Additionally, PFK is activated by AMP and
ADP because they indicate low levels of ATP in the cell. F2,6BP is the major
activator, though, because it reciprocally inhibits fructose 1,6 bisphosphatase,
which is the gluconeogenic enzyme that catalyzes the reversal of this step.

PFK-1 is a tetrameric enzyme that exist in two conformational states termed R and T
that are in equilibrium. ATP is both a substrate and an allosteric inhibitor of PFK-1.
Each subunit has two ATP binding sites, a substrate site and an inhibitor site. The
substrate site binds ATP equally well when the tetramer is in either conformation. The
inhibitor site binds ATP essentially only when the enzyme is in the T state. F6P is the
other substrate for PFK-1 and it also binds preferentially to the R state enzyme. At
high concentrations of ATP, the inhibitor site becomes occupied and shifting the
equilibrium of PFK-1 conformation to that of the T state decreasing PFK-1’s ability to
bind F6P. The inhibition of PFK-1 by ATP is overcome by AMP which binds to the R
state of the enzyme and, therefore, stabilizes the conformation of the enzyme capable of binding F6P. The most important allosteric regulator of both glycolysis and gluconeogenesis is fructose-2,6-bisphosphate, F2,6-BP, which is not an intermediate in glycolysis or in gluconeogenesis.

Regulation of glycolysis and gluconeogenesis by fructose-2,6-bisphosphate (F-2,6-BP). The major sites for regulation of glycolysis and gluconeogenesis are the phosphofructokinase-1 (PFK-1) and fructose-1,6-bisphosphatase (F-1,6-BPase) catalyzed reactions.

The synthesis of F-2,6-BP is catalyzed by the bifunctional enzyme PFK-2/F-2,6-BPase. In the nonphosphorylated form the enzyme is known as PFK-2 and serves to catalyze the synthesis of F-2,6-BP. The result is that the activity of PFK-1 is greatly stimulated and the activity of F-1,6-BPase is greatly inhibited.

Under conditions where PFK-2 is active, fructose flow through the PFK-1/F-1,6-BPase reactions takes place in the glycolytic direction, with a net production of F-1,6-BP.
When the bifunctional enzyme is phosphorylated it no longer exhibits kinase activity, but a new active site hydrolyzes F-2,6-BP to F6P and inorganic phosphate. The metabolic result of the phosphorylation of the bifunctional enzyme is that allosteric stimulation of PFK-1 ceases, allosteric inhibition of F-1,6-BPase is eliminated, and net flow of fructose through these two enzymes is gluconeogenic, producing F6P and eventually glucose.

The interconversion of the bifunctional enzyme is catalyzed by \textit{cAMP-dependent protein kinase (PKA)}, which in turn is regulated by circulating peptide hormones. When blood glucose levels drop, pancreatic insulin production falls, glucagon secretion is stimulated, and circulating glucagon is highly increased. Hormones such as glucagon bind to plasma membrane receptors on liver cells, activating membrane-localized \textit{adenylate cyclase} leading to an increase in the conversion of ATP to cAMP. cAMP binds to the regulatory subunits of PKA, leading to release and activation of the catalytic subunits. PKA phosphorylates numerous enzymes, including the bifunctional PFK-2/F-2,6-BPase. Under these conditions the liver stops consuming glucose and becomes metabolically gluconeogenic, producing glucose to reestablish normoglycemia.

\textbf{Control of pyruvate dehydrogenase, the citric acid cycle and oxidative phosphorylation}

- Pyruvate dehydrogenase is inhibited by acetyl-CoA and by NADH, both of which are products of the reaction. It is activated by CoA-SH and NAD$^+$.  
- High ATP:ADP levels activate a kinase that acts on pyruvate dehydrogenase to inactivate it by phosphorylation. The same kinase, which is part of the pyruvate dehydrogenase complex, is also activated by NADH and acetyl-CoA.  
- The key controls for the citric acid cycle and electron transport are substrate availability. If NADH exists primarily in the reduced form as shown, then the enzymes that require NAD$^+$ in the citric acid cycle cannot function.  
- Since electron transport and ATP synthesis are normally tightly coupled, lack of ADP availability slows electron transport and results in a build up of the proton gradient.  
- In addition, controls operate at the level of citrate synthase, \textit{\alpha}-ketoglutarate dehydrogenase and isocitrate dehydrogenase steps.
Regulation of the cellular ATP level by AMP-activated protein kinase

- AMP-activated protein kinase (AMPK) turns off anabolic reactions, thus preserving ATP when AMP levels are elevated.

A concluding note: metabolic control analysis

- The pathway of glycolysis from glucose-6-phosphate to lactate can be considered as an example of a pathway that can be analysed.
- Although the key control enzyme is phosphofructokinase, the rate of lactate production is not simply controlled by this enzyme because of the influence of all the other enzymes in the pathway. Feedback inhibition may operate and, in addition, metabolites may flow into other pathways.
- This means that a large number of parameters affect the rate of lactate production from glucose-6-phosphate.
- Metabolic control analysis uses the known parameters for all the enzymes in a pathway to predict the flow of material through that pathway. It also allows examination of the effect of changing a small number of parameters related to the pathway.
Regulatory mechanisms controlling glycolysis include allosteric and covalent modification mechanisms.

Glycolysis is regulated reciprocally from gluconeogenesis. Molecules, such as F2,6BP, that turn on glycolysis, turn off gluconeogenesis. Conversely, acetyl-CoA turns on gluconeogenesis, but turns off glycolysis.

The principle enzymes of glycolysis involved in regulation are hexokinase (reaction 1), phosphofructokinase (reaction 3), and pyruvate kinase (reaction 10):

1. **Hexokinase** is allosterically inhibited by glucose-6-phosphate (G6P). That is, the enzyme for the first reaction of glycolysis is inhibited by the product of the first reaction. As a result, glucose and ATP (in reactions 1 and 3) are not committed to glycolysis unless necessary.

- Passive Hexose Transporters
- Concentration Gradient
- GLUT – family coupled to insulin concentrations – why?

**REGULATION OF HEXOKINASES**

- HK I, II, III – allosterically inhibited by product
- GLUCOKINASE (HK IV) – LIVER!!
- Blood (Glc) → 5mM or ↑ 10mM after meals
HEXOKINASES \[ K_m > 0.1 \text{mM} \]

GLUCOKINASES \[ K_m = 2-5 \text{mM} \] and not inhibited

HOW IS THIS ACTION REGULATED?

Regulatory protein

Phosphofructokinase (PFK) is a major control point for glycolysis. PFK is allosterically inhibited by ATP and citrate, allosterically activated by AMP, ADP, and F2,6BP. Thus, carbon movement through glycolysis is inhibited at PFK when the cell contains ample stores of ATP and oxidizable substrates. Additionally, PFK is activated by AMP and ADP because they indicate low levels of ATP in the cell. F2,6BP is the major activator, though, because it reciprocally inhibits fructose 1,6 bisphosphatase, which is the gluconeogenic enzyme that catalyzes the reversal of this step.

Allosteric Regulation of Phosphofructokinase

PFK Activator = Fructose-2,6-bisphosphate (Figure 13.9)
Figure 13.9: Allosteric control of liver phosphofructokinase.
Phosphorylation/dephosphorylation

Other PFK Activators = \textbf{AMP, ADP}

PFK Inhibitors = \textbf{ATP} and Citrate
PFK is the enzyme through which adenylate energy charge is controlled

\textbf{More on the REGULATION OF PFK1:}

\begin{itemize}
  \item ALLOSTERIC REGULATION
  \item ATP $\uparrow K_m$ of PFK1 (allosteric inhibitor)
\end{itemize}
OR

- AMP = allosteric activator

- Citrate → feedback regulation

- Intracellular pH

- Fru–2,6–BP (Liver) activates PFK1
  - ↓→ Phosphorylation & Dephosphorylation

3. Pyruvate kinase is allosterically inhibited by acetyl-CoA, ATP, and Alanine; allosterically activated by F1,6BP, and inhibited by cAMP-dependent phosphorylation.

Note that several of the allosteric regulators are products of other metabolic pathways or are made in other metabolic pathways. These include acetyl-CoA, AMP, F2,6BP, and G1P, (readily converted into G6P). By having regulation dependent on other pathways, glycolysis is coordinately controlled with these pathways as well.
Catabolism of Polysaccharides

Hydrolytic and Phosphorolytic Cleavages (Figure 13.15)

Phosphorylase vs. phosphatase

Energy considerations

Digestion:
- **α-Amylase** cleaves internal α(1 → 4) linkages of starch and glycogen. In the intestine, digestion continues, aided by **α-amylase** secreted by the pancreas. α-Amylase degrades amylose to **maltose** and a little **glucose**. However, it only partially degrades **amylopectin** and **glycogen**, as shown in Figure 13.16, because it cannot cleave the α(1 → 6) linkages found at branch points. The product of exhaustive digestion of amylopectin or glycogen by α-amylase is called a limit dextrin; its continued degradation requires the action of a "debranching enzyme," α(1 → 6)-glucosidase (also called isomaltase). This action exposes a new group of α(1 → 4)-linked branches, which can be attacked by α-amylase until a new set of α(1 → 6)-linked branches is reached. The end result of the sequential action of these two enzymes is the complete breakdown of starch or glycogen to maltose and some glucose. Maltose is cleaved hydrolytically by maltase, yielding 2 moles of glucose, which is then absorbed into the bloodstream and transported to various tissues for utilization.

**Glycogen**

Glycogen is a branched polymer of **glucose**, consisting of main branches of
glucose units joined in α(1->4) linkages. Every 7-20 residues, α(1->6) branches of glucose units are also present. Glycogen is a primary energy storage material in muscle. Individual glucose units are cleaved from glycogen in a phosphorolytic mechanism catalyzed by glycogen phosphorylase.

The storage polysaccharides, such as glycogen, are admirably designed to serve their function. Glucose and even maltose are small, rapidly diffusing molecules, which are difficult to store. Were such small molecules present in large quantities in a cell, they would give rise to a very large cell osmotic pressure, which would be deleterious in most cases. Therefore, most cells build the glucose into long polymers, so that large quantities can be stored in a semi-insoluble state. Whenever glucose is needed, it can be obtained by selective degradation of the polymers by specific enzymes.

Figure 13.17: The debranching process in glycogen catabolism.
**Phosphoglucomutase**

(Also called glucose phosphomutase) Phosphoglucomutase catalyzes the interconversion of glucose-1-phosphate (G1P) and glucose-6-phosphate (G6P).

\[ \text{G1P} \leftrightarrow \text{G6P} \]

This reaction is important in glycogen biosynthesis and galactose metabolism.

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**Glycogen Breakdown Regulation**

Glycogen breakdown (and synthesis) is regulated by hormones.

**Epinephrine** (also called adrenalin) and **glucagon** stimulate breakdown. Insulin stimulates synthesis.

Breakdown occurs as a result of a **kinase cascade** that arises from binding of the appropriate hormone to the appropriate cell surface receptor. Recall that a regulatory cascade is a process in which the intensity of an initial regulatory signal is amplified manyfold through a series of enzyme activations.

The cascade depicted in **Figure 13.18** provides a way for cells to rapidly turn on glycogen breakdown and release of glucose. This is useful in emergency situations (e.g., the need to catch prey or the need to avoid being caught).
Figure 13.18: The regulatory cascade controlling glycogen breakdown
Figure 13.7
Activation of glycogen phosphorylase and inactivation of glycogen synthase.
Phosphorylation reactions catalyzed by protein kinase A increase glycogen degradation.
A green background indicates activated enzymes; a red background, inactivated enzymes. Green arrows signify activation; red arrows, inactivation.

Figure 13.8

**Pyruvate Oxidation**

One source of acetyl-CoA molecules for the citric acid cycle is via oxidation of pyruvate in a reaction catalyzed by the pyruvate dehydrogenase complex. The process of converting pyruvate to acetyl-CoA is an oxidative decarboxylation. In the overall reaction (below), the carboxyl group of pyruvate is lost as CO2, while the remaining two carbons form the acetyl moiety of acetyl-CoA.

\[
\text{Pyruvate + NAD}^+ + \text{CoASH} \leftrightarrow \text{Acetyl-CoA + NADH} + \text{CO}_2 \quad (\Delta G^{\circ} = -33.5 \text{ kJ/mol})
\]

Enzymatic activities contained in the pyruvate dehydrogenase complex include:

- **Pyruvate decarboxylase (E1)**
- **Dihydrolipoamide transacetylase (E2)**
- **Dihydrolipoamide dehydrogenase (E3)**

and five coenzymes

- **Thiamine Pyrophosphate (TPP)**
- **Lipoic Acid-Lipoamide**
- **FAD**
- **NAD^+**
- **CoASH**

All of these entities together make up the pyruvate dehydrogenase complex. An overview of the process is shown in Figure 14.4
Figure 14.4: Overview of the reactions of the pyruvate dehydrogenase complex.
**Allosteric Regulation**
- Pyruvate dehydrogenase is a major regulatory point for entry of materials into the citric acid cycle. The enzyme is regulated allosterically and by covalent modification.
- E2 - inhibited by acetyl-CoA, activated by CoA-SH
- E3 - inhibited by NADH, activated by NAD^+.
- ATP is an allosteric inhibitor of the complex, and AMP is an activator. The activity of this key reaction is coordinated with the energy charge, the [NAD^+]/[NADH] ratio, and the ratio of acetylated to free coenzyme A.

**Covalent Regulation**
- Part of the **pyruvate dehydrogenase complex**, pyruvate dehydrogenase kinase, phosphorylates three specific E1 serine residues, resulting in loss of activity of pyruvate dehydrogenase. NADH and acetyl-CoA both activate the kinase. The serines are dephosphorylated by a specific enzyme called pyruvate dehydrogenase phosphatase that hydrolyzes the phosphates from the E1 subunit of the **pyruvate dehydrogenase complex**. This has the effect of activating the complex. The phosphatase is activated by Ca^{2+} and Mg^{2+}. Because ATP and ADP differ in their affinities for Mg^{2+}, the concentration of free Mg^{2+} reflects the ATP/ADP ratio within the mitochondrion. Thus, pyruvate dehydrogenase responds to ATP levels by being turned off when ATP is abundant and further energy production is unneeded.
- In mammalian tissues at rest, much less than half of the total pyruvate dehydrogenase is in the active, nonphosphorylated form. The complex can be turned on when low ATP levels signal a need to generate more ATP. The kinase protein is an integral part of the pyruvate dehydrogenase complex, whereas the phosphatase is but loosely bound.
Regulation of Citric Acid Cycle

Because the citric acid cycle is a source of biosynthetic intermediates, as well as a route for generating metabolic energy, regulation of the cycle is somewhat more complex than if it were solely an energy-generating pathway. The citric acid cycle is regulated in two primary ways, by controlling the entry of fuel into the cycle and by controlling key reactions within the cycle (Figure 14.16).

1. The entry of fuel into the cycle: Substrate level regulation occurs by limiting amounts of acetyl-CoA (see here for regulation of pyruvate oxidation), oxaloacetate, citrate, and NAD+/NADH. The latter is the most important and common substrate-level regulation. NAD+ is an essential substrate for three enzymes in the cycle as well as the pyruvate dehydrogenase complex.

Conditions which reduce NAD+ concentration include lack of oxygen, because the electron transport system, which converts NADH back to NAD+ usually depends upon oxygen. Oxygen shortage may occur during heavy exercise when the blood is unable to deliver oxygen as fast as it is needed in muscle cells.

2. Key reactions within the cycle: Movement of materials through the citric acid cycle is also regulated allosterically. Enzymes susceptible to allosteric regulation include isocitrate dehydrogenase and α-ketoglutarate dehydrogenase:

Isocitrate dehydrogenase: Activated by ADP, inhibited by NADH (apart from substrate level regulation of NAD+). Phosphorylation of one serine residue in the enzyme prevents binding of isocitrate.

α-Ketoglutarate dehydrogenase: Inhibited by succinyl-CoA and NADH.

To summarize, the citric acid cycle is responsive to the energy state of the cell, through allosteric activation of isocitrate dehydrogenase by ADP; to the redox state of the cell, through flux rate limitation caused when intramitochondrial
[NAD$^+$] decreases; and to the availability of energy-rich compounds, through inhibition of relevant enzymes by acetyl-CoA or succinyl-CoA.