

### 22.3.7. Animals Cannot Convert Fatty Acids into Glucose

It is important to note that *animals are unable to effect the net synthesis of glucose from fatty acids*. Specifically, acetyl

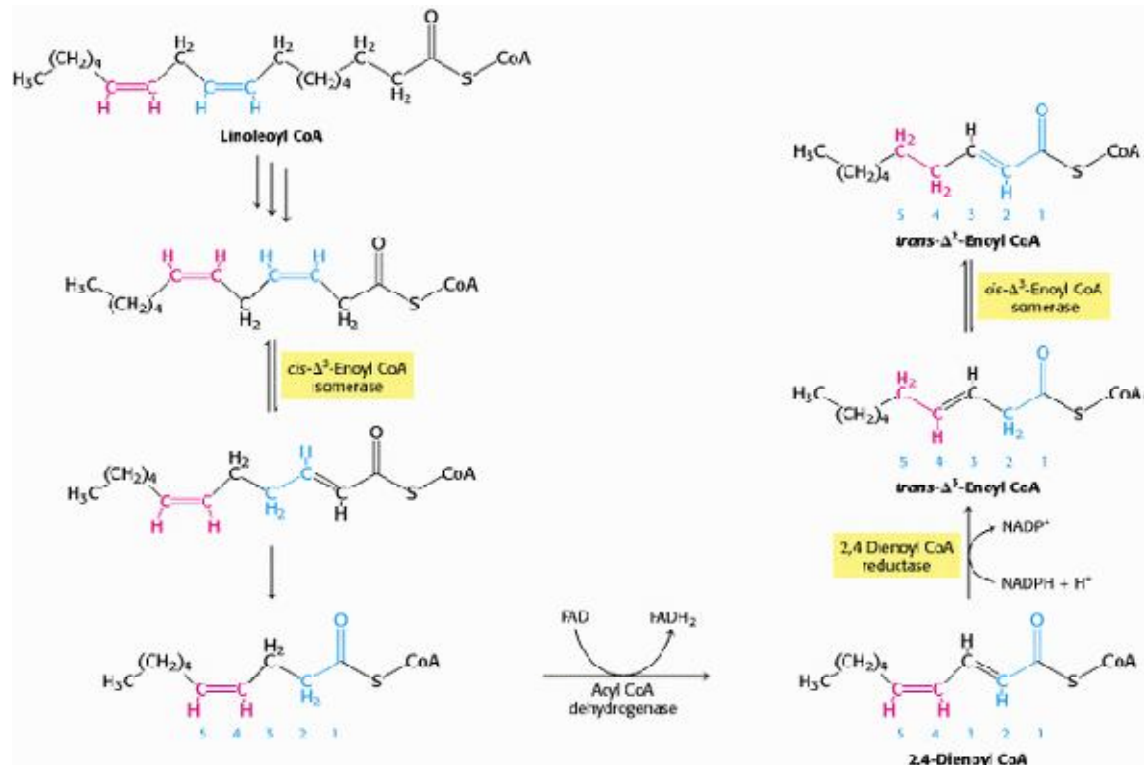
CoA cannot be converted into pyruvate or oxaloacetate in animals. The two carbon atoms of the acetyl group of acetyl

CoA enter the citric acid cycle, but two carbon atoms leave the cycle in the decarboxylations catalyzed by isocitrate

dehydrogenase and  $\alpha$ -ketoglutarate dehydrogenase. Consequently, oxaloacetate is regenerated, but it is not formed de

novo when the acetyl unit of acetyl CoA is oxidized by the citric acid cycle. In contrast, plants have two additional

enzymes enabling them to convert the carbon atoms of acetyl CoA into oxaloacetate (Section 17.4.).



**Oxidation of Linoleoyl CoA.** The complete oxidation of the diunsaturated fatty acid linoleate is facilitated by the activity of enoyl CoA isomerase and 2,4-dienoyl CoA reductase.

### Fatty Acids Are Synthesized and Degraded by Different Pathways

Fatty acid synthesis is not simply a reversal of the degradative pathway. Rather, it consists of a new set of reactions,

again exemplifying the principle that *synthetic and degradative pathways are almost always distinct*. Some important

differences between the pathways are:

1. Synthesis takes place in the *cytosol*, in contrast with degradation, which takes place primarily in the mitochondrial matrix.

2. Intermediates in fatty acid synthesis are covalently linked to the sulfhydryl groups of an *acyl carrier protein* (ACP),

whereas intermediates in fatty acid breakdown are covalently attached to the sulfhydryl group of coenzyme A.

3. The enzymes of fatty acid synthesis in higher organisms are joined in a *single polypeptide chain* called *fatty acid*

*synthase*. In contrast, the degradative enzymes do not seem to be associated.

4. The growing fatty acid chain is elongated by the *sequential addition of two-carbon units* derived from acetyl CoA.

The activated donor of twocarbon units in the elongation step is *malonyl ACP*. The elongation reaction is driven by the release of CO<sub>2</sub>.

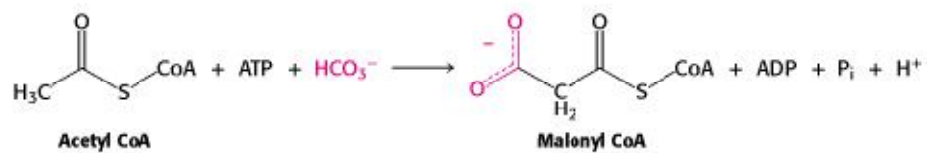
5. The reductant in fatty acid synthesis is *NADPH*, whereas the oxidants in fatty acid degradation are *NAD<sup>+</sup>* and *FAD*.

6. Elongation by the fatty acid synthase complex stops on formation of *palmitate* (C<sub>16</sub>). Further elongation and the insertion of double bonds are carried out by other enzyme systems.

## The Formation of Malonyl Coenzyme A Is the Committed Step in Fatty Acid Synthesis

### Synthesis

Fatty acid synthesis starts with the carboxylation of acetyl CoA to *malonyl CoA*. This irreversible reaction is the committed step in fatty acid synthesis.

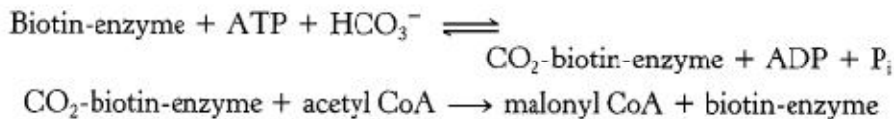


The synthesis of malonyl CoA is catalyzed by *acetyl CoA carboxylase*, which contains a biotin prosthetic group. The

carboxyl group of biotin is covalently attached to the  $\epsilon$  amino group of a lysine residue, as in pyruvate carboxylase

(Section 16.3.2) and propionyl CoA carboxylase. As with these other enzymes, a carboxybiotin intermediate is formed at the expense of the hydrolysis a molecule of ATP. The activated CO<sub>2</sub> group in this intermediate

is then transferred to acetyl CoA to form malonyl CoA.



This enzyme is also the essential regulatory enzyme for fatty acid metabolism (Section 22.5).

## Intermediates in Fatty Acid Synthesis Are Attached to an Acyl Carrier Protein

The intermediates in fatty acid synthesis are linked to an acyl carrier protein. Specifically, they are linked to the

sulfhydryl terminus of a phosphopantetheine group, which is, in turn, attached to a serine residue of the acyl carrier

protein (Figure 22.21). Recall that, in the degradation of fatty acids, a phosphopantetheine group is present as part of

CoA instead (Section 22.2.2). ACP, a single polypeptide chain of 77 residues, can be regarded as a giant prosthetic group, a "macro CoA."

## The Elongation Cycle in Fatty Acid Synthesis

The enzyme system that catalyzes the synthesis of saturated long-chain fatty acids from acetyl CoA, malonyl CoA, and NADPH is called the *fatty acid synthase*. The constituent enzymes of bacterial fatty acid synthases are dissociated when the cells are broken apart. The availability of these isolated enzymes has facilitated the elucidation of the steps in fatty acid synthesis (Table 22.2). In fact, the reactions leading to fatty acid synthesis in higher organisms are very much like those of bacteria.

The elongation phase of fatty acid synthesis starts with the formation of acetyl ACP and malonyl ACP. *Acetyl*

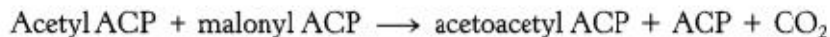
*transacylase* and *malonyl transacylase* catalyze these reactions.



Malonyl transacylase is highly specific, whereas acetyl transacylase can transfer acyl groups other than the acetyl unit, though at a much slower rate. Fatty acids with an odd number of carbon atoms are synthesized starting with propionyl ACP, which is formed from propionyl CoA by acetyl transacylase.

Acetyl ACP and malonyl ACP react to form acetoacetyl ACP (Figure 22.22). The *acyl-malonyl ACP condensing enzyme*

catalyzes this condensation reaction.



In the condensation reaction, a four-carbon unit is formed from a two-carbon unit and a three-carbon unit, and CO<sub>2</sub> is released. Why is the four-carbon unit not formed from 2 two-carbon units? In other words, why are the reactants acetyl ACP and malonyl ACP rather than two molecules of acetyl ACP? The answer is that the equilibrium for the synthesis of acetoacetyl ACP from two molecules of acetyl ACP is highly unfavorable. In contrast, *the equilibrium is favorable if malonyl ACP is a reactant because its decarboxylation contributes a substantial decrease in free energy*. In effect, ATP drives the condensation reaction, though ATP does not directly participate in the condensation reaction. Rather, ATP is used to carboxylate acetyl CoA to malonyl CoA. The free energy thus stored in malonyl CoA is released in the decarboxylation accompanying the formation of acetoacetyl ACP. Although HCO<sub>3</sub><sup>-</sup> is required for fatty acid synthesis, its carbon atom does not appear in the product. Rather, *all the carbon atoms of fatty acids containing an even number of carbon atoms are derived from acetyl CoA*

The next three steps in fatty acid synthesis reduce the keto group at C-3 to a methylene group. First, acetoacetyl ACP is reduced to d-3-hydroxybutyryl ACP. This reaction differs from the corresponding one in fatty acid degradation in two respects:

- (1) the d rather than the l isomer is formed; and
- (2) NADPH is the reducing agent, whereas

NAD<sup>+</sup> is the oxidizing agent in  $\beta$  oxidation. This difference exemplifies the general principle that *NADPH is consumed in biosynthetic reactions, whereas NADH is generated in energy-yielding reactions*. Then d-3-hydroxybutyryl ACP is *dehydrated* to form crotonyl ACP, which is a *trans- $\Delta^2$* -enoyl ACP. The final step in the cycle *reduces* crotonyl ACP to butyryl ACP. NADPH is again the reductant, whereas FAD is the oxidant in the corresponding reaction in  $\beta$ -oxidation.

The enzyme that catalyzes this step, *enoyl ACP reductase*, is inhibited by triclosan, a broad-spectrum antibacterial agent. Triclosan is used in a variety of products such as toothpaste, soaps, and skin creams. These last three reactions a reduction, a dehydration, and a second reduction convert acetoacetyl ACP into butyryl ACP, which completes the first elongation cycle.

In the second round of fatty acid synthesis, butyryl ACP condenses with malonyl ACP to form a C6- $\beta$ -ketoacyl ACP.

This reaction is like the one in the first round, in which acetyl ACP condenses with malonyl ACP to form a C4- $\beta$ -ketoacyl ACP. Reduction, dehydration, and a second reduction convert the C6- $\beta$ -ketoacyl ACP into a C6-acyl ACP, which is ready for a third round of elongation. The elongation cycles continue until C16-acyl ACP is formed. This intermediate is a good substrate for a thioesterase that hydrolyzes C16-acyl ACP to yield palmitate and ACP. *The thioesterase acts as a ruler to determine fatty acid chain length.*

## Fatty Acids Are Synthesized by a Multifunctional Enzyme Complex in Eukaryotes

Although the basic biochemical reactions in fatty acid synthesis are very similar in *E. coli* and eukaryotes, the structure of the synthase varies considerably. The fatty acid synthases of eukaryotes, in contrast with those of *E. coli*, have the component enzymes linked in a large polypeptide chain.

Mammalian fatty acid synthase is a dimer of identical 260-kd subunits. Each chain is folded into three domains joined by flexible regions.

*Domain 1, the substrate entry and condensation unit*, contains acetyl transferase, malonyl transferase, and  $\beta$ -ketoacyl synthase (condensing enzyme).

*Domain 2, the reduction unit*, contains the acyl carrier protein,  $\beta$ -ketoacyl reductase, dehydratase, and enoyl reductase.

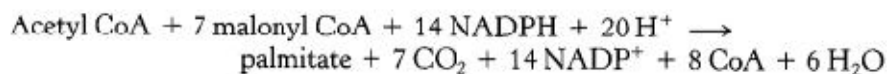
*Domain 3, the palmitate release unit*, contains the thioesterase.

Thus, *seven different catalytic sites are present on a single polypeptide chain*. It is noteworthy that many eukaryotic multienzyme complexes are multifunctional proteins in which different enzymes are linked covalently.

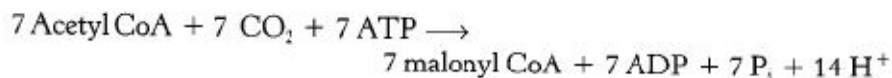
An advantage of this arrangement is that the synthetic activity of different enzymes is coordinated. In addition, a multienzyme complex consisting of covalently joined enzymes is more stable than one formed by noncovalent attractions. Furthermore, intermediates can be efficiently handed from one active site to another without leaving the assembly. It seems likely that multifunctional enzymes such as fatty acid synthase arose in eukaryotic evolution by exon shuffling (Section 5.6.2), because each of the component enzymes is recognizably homologous to its bacterial counterpart.

## The Stoichiometry of Fatty Acid Synthesis

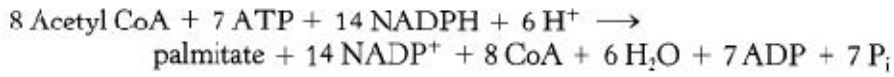
The stoichiometry of the synthesis of palmitate is



The equation for the synthesis of the malonyl CoA used in the preceding reaction is

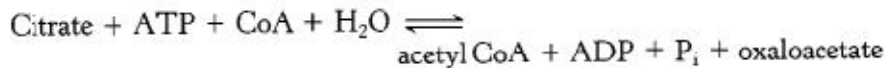


Hence, the overall stoichiometry for the synthesis of palmitate is



## Citrate Carries Acetyl Groups from Mitochondria to the Cytosol for Fatty Acid Synthesis

The synthesis of palmitate requires the input of 8 molecules of acetyl CoA, 14 molecules of NADPH, and 7 molecules of ATP. Fatty acids are synthesized in the cytosol, whereas acetyl CoA is formed from pyruvate in mitochondria. Hence, acetyl CoA must be transferred from mitochondria to the cytosol. Mitochondria, however, are not readily permeable to acetyl CoA. Recall that carnitine carries only long-chain fatty acids. *The barrier to acetyl CoA is bypassed by citrate, which carries acetyl groups across the inner mitochondrial membrane.* Citrate is formed in the mitochondrial matrix by the condensation of acetyl CoA with oxaloacetate (Figure 22.25). When present at high levels, citrate is transported to the cytosol, where it is cleaved by *ATP-citrate lyase*.



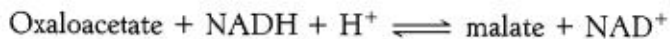
Thus, acetyl CoA and oxaloacetate are transferred from mitochondria to the cytosol at the expense of the hydrolysis of a molecule of ATP.

### Lyases-

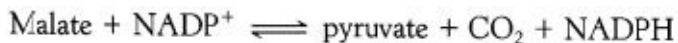
Enzymes catalyzing the cleavage of C-C, C-O, or C-N bonds by elimination. A double bond is formed in these reactions.

## Sources of NADPH for Fatty Acid Synthesis

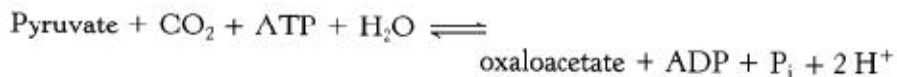
Oxaloacetate formed in the transfer of acetyl groups to the cytosol must now be returned to the mitochondria. The inner mitochondrial membrane is impermeable to oxaloacetate. Hence, a series of bypass reactions are needed. Most important, these reactions generate much of the NADPH needed for fatty acid synthesis. First, oxaloacetate is reduced to malate by NADH. This reaction is catalyzed by a *malate dehydrogenase* in the cytosol.



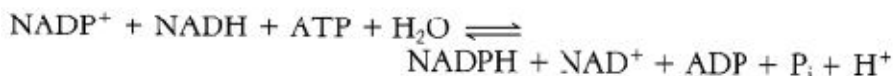
Second, malate is oxidatively decarboxylated by an *NADP* + -linked *malate enzyme* (also called *malic enzyme*).



The pyruvate formed in this reaction readily enters mitochondria, where it is carboxylated to oxaloacetate by pyruvate carboxylase.



The sum of these three reactions is

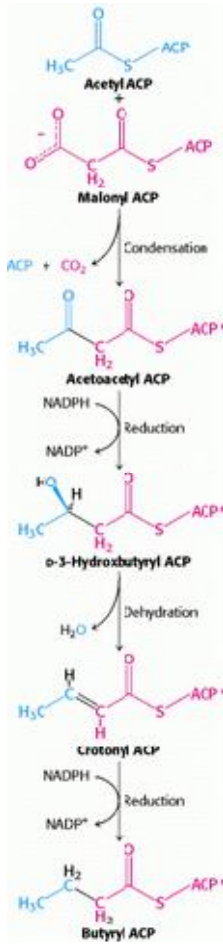


Thus, one molecule of NADPH is generated for each molecule of acetyl CoA that is transferred from mitochondria to the cytosol. Hence, eight molecules of NADPH are formed when eight molecules of acetyl CoA are transferred to the cytosol for the synthesis of palmitate. The additional six molecules of NADPH required for this process come from the pentose phosphate pathway.

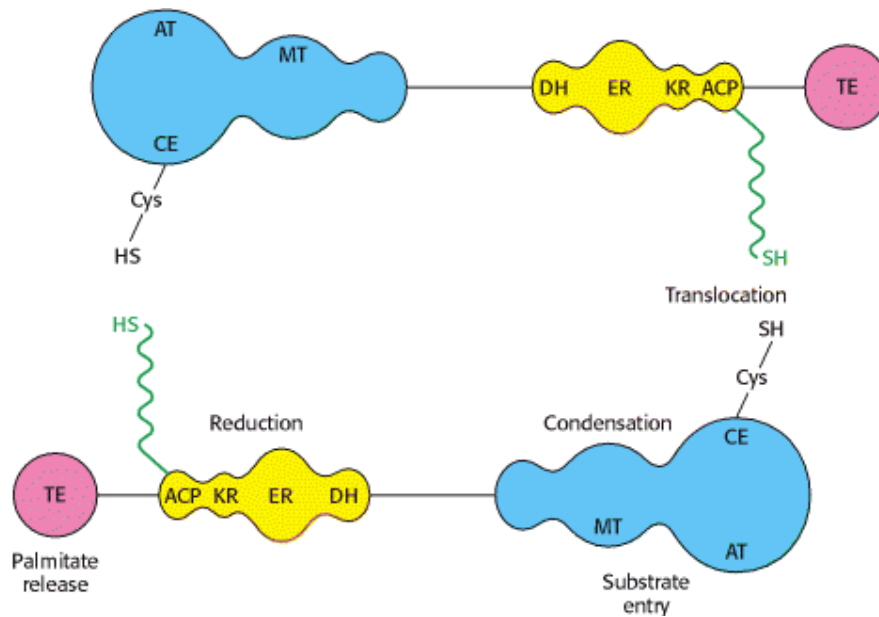
The accumulation of the precursors for fatty acid synthesis is a wonderful example of the coordinated use of multiple processes to fulfill a biochemical need. The citric acid cycle, subcellular compartmentalization, and the pentose phosphate pathway provide the carbon atoms and reducing power, whereas glycolysis and oxidative phosphorylation provide the ATP to meet the needs for fatty acid synthesis.

**Table. Principal reactions in fatty acid synthesis in bacteria**

Step	Reaction	Enzyme
1	Acetyl CoA + HCO <sub>3</sub> <sup>-</sup> + ATP----- malonyl CoA + ADP + Pi + H <sup>+</sup>	Acetyl CoA carboxylase
2	Acetyl CoA + ACP===== acetyl ACP + CoA	Acetyl transacylase
3	Malonyl CoA + ACP===== malonyl ACP + CoA	Malonyl transacylase
4	Acetyl ACP + malonyl ACP----- acetoacetyl ACP + ACP + CO <sub>2</sub>	Acyl-malonyl ACP condensing enzyme
5	Acetoacetyl ACP + NADPH + H <sup>+</sup> =====d-3-hydroxybutyryl ACP + NADP <sup>+</sup>	β-Ketoacyl ACP reductase
6	d-3-Hydroxybutyryl ACP===== crotonyl ACP + H <sub>2</sub> O	3-Hydroxyacyl ACP dehydratase
7	Crotonyl ACP + NADPH + H <sup>+</sup> ----- butyryl ACP + NADP <sup>+</sup>	Enoyl ACP reductase

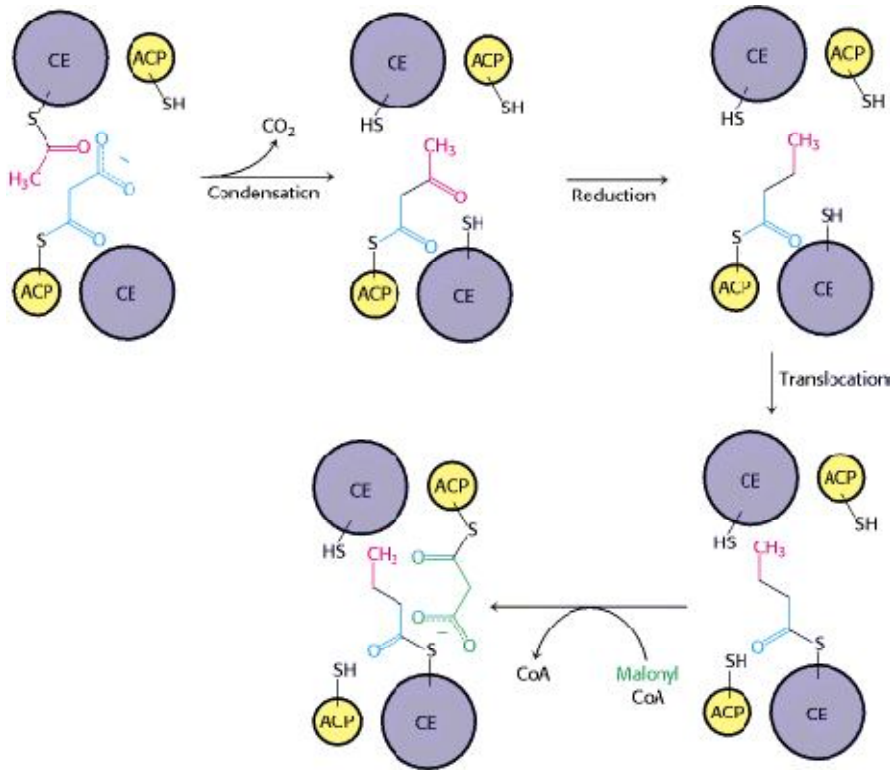


**Fatty Acid Synthesis.** Fatty acids are synthesized by the repetition of the following reaction sequence: condensation, reduction, dehydration, and reduction. The intermediates shown here are produced in the first round of synthesis.

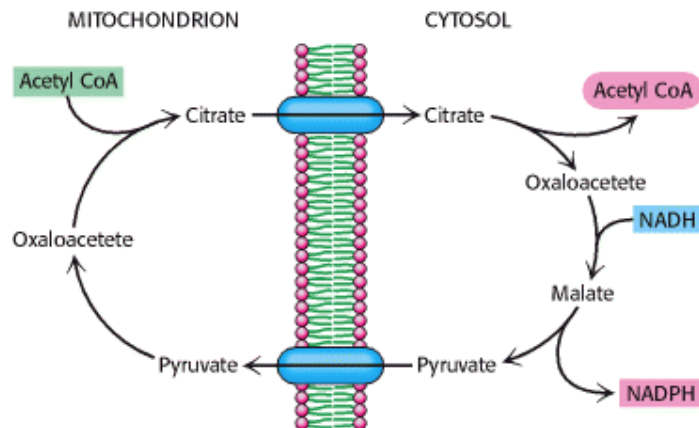




**Schematic Representation of Animal Fatty Acid Synthase.** Each of the identical chains in the dimer contains three domains. Domain 1 (blue) contains acetyl transferase (AT), malonyl transferase (MT), and condensing enzyme (CE). Domain 2 (yellow) contains acyl carrier protein (ACP),  $\beta$ -ketoacyl reductase (KR), dehydratase (DH), and enoyl reductase (ER). Domain 3 (red) contains thioesterase (TE). The flexible phosphopantetheinyl group (green) carries the fatty acyl chain from one catalytic site on a chain to another, as well as between chains in the dimer. [After Y. Tsukamoto, H. Wong, J. S. Mattick, and S. J. Wakil. *J. Biol. Chem.* 258(1983):15312.]



**Reactions of Fatty Acid Synthase.** Translocations of the elongating fatty acyl chain between the cysteine sulfhydryl group of the condensing enzyme (CE, blue) and the phosphopantetheine sulfhydryl group of the acyl carrier protein (ACP, yellow) lead to the growth of the fatty acid chain. The reactions are repeated until the palmitoyl product is synthesized.



**Transfer of Acetyl CoA to the Cytosol.** Acetyl CoA is transferred from mitochondria to the cytosol, and the reducing potential NADH is concomitantly converted into that of NADPH by this series of reactions.



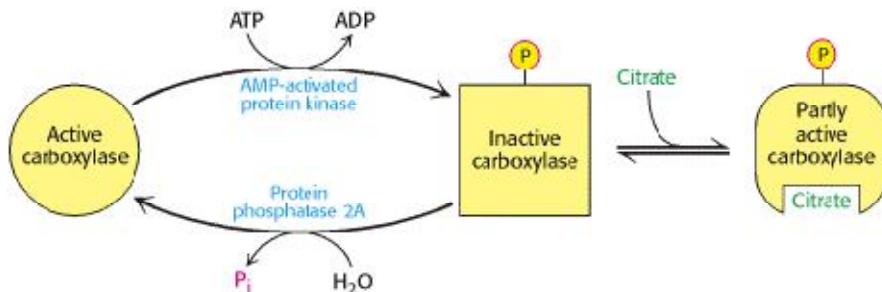
## Acetyl Coenzyme A Carboxylase Plays a Key Role in Controlling Fatty Acid Metabolism

Fatty acid metabolism is stringently controlled so that synthesis and degradation are highly responsive to physiological needs. Fatty acid synthesis is maximal when carbohydrate and energy are plentiful and when fatty acids are scarce.

*Acetyl CoA carboxylase plays an essential role in regulating fatty acid synthesis and degradation.* Recall that this enzyme catalyzes the committed step in fatty acid synthesis: the production of malonyl CoA (the activated two-carbon donor). The carboxylase is controlled by three global signals: glucagon, epinephrine, and insulin that correspond to the overall energy status of the organism. *Insulin stimulates fatty acid synthesis by activating the carboxylase, whereas glucagon and epinephrine have the reverse effect.* The levels of citrate, palmitoyl CoA, and AMP within a cell also exert control. *Citrate*, a signal that building blocks and energy are abundant, activates the carboxylase. Palmitoyl CoA and AMP, in contrast, lead to the inhibition of the carboxylase. Thus, this important enzyme is subject to both global and local regulation. We will examine each of these levels of regulation in turn.

### *Response to Diet.*

Fatty acid synthesis and degradation are reciprocally regulated so that both are not simultaneously active. *In starvation, the level of free fatty acids rises because hormones such as epinephrine and glucagon stimulate adipose-cell lipase. Insulin, in contrast, inhibits lipolysis.* Acetyl CoA carboxylase also plays a role in the regulation of fatty acid degradation. Malonyl CoA, the product of the carboxylase reaction, is present at a high level when fuel molecules are abundant. *Malonyl CoA inhibits carnitine acyltransferase I, preventing access of fatty acyl CoAs to the mitochondrial matrix in times of plenty.* Malonyl CoA is an especially effective inhibitor of carnitine acyltransferase I in heart and muscle, tissues that have little fatty acid synthesis capacity of their own. In these tissues, acetyl CoA carboxylase may be a purely regulatory enzyme. Finally, two enzymes in the  $\beta$ -oxidation pathway are markedly inhibited when the energy charge is high. NADH inhibits 3-hydroxyacyl CoA dehydrogenase, and acetyl CoA inhibits thiolase. *Long-term control is mediated by changes in the rates of synthesis and degradation of the enzymes participating in fatty acid synthesis.* Animals that have fasted and are then fed high-carbohydrate, low-fat diets show marked increases in their amounts of acetyl CoA carboxylase and fatty acid synthase within a few days. This type of regulation is known as *adaptive control*.



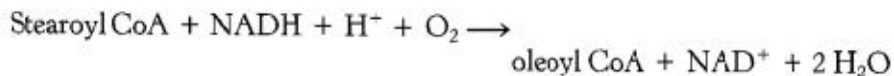
**Control of Acetyl CoA Carboxylase.** Acetyl CoA carboxylase is inhibited by phosphorylation and activated by the binding of citrate.

## Elongation and Unsaturation of Fatty Acids Are Accomplished by Accessory Enzyme Systems

The major product of the fatty acid synthase is palmitate. In eukaryotes, longer fatty acids are formed by elongation reactions catalyzed by enzymes on the cytosolic face of the *endoplasmic reticulum membrane*. These reactions add twocarbon units sequentially to the carboxyl ends of both saturated and unsaturated fatty acyl CoA substrates. Malonyl CoA is the two-carbon donor in the elongation of fatty acyl CoAs. Again, condensation is driven by the decarboxylation of malonyl CoA.

## Membrane-Bound Enzymes Generate Unsaturated Fatty Acids

Endoplasmic reticulum systems also introduce double bonds into long-chain acyl CoAs. For example, in the conversion of stearoyl CoA into oleoyl CoA, a *cis*- $\Delta$  9 double bond is inserted by an oxidase that employs *molecular oxygen* and *NADH* (or *NADPH*).



This reaction is catalyzed by a complex of three membrane-bound enzymes: *NADH-cytochrome b5 reductase*, *cytochrome b5*, and a *desaturase*. First, electrons are transferred from NADH to the FAD moiety of *NADH-cytochrome b5 reductase*.

The heme iron atom of *cytochrome b5* is then reduced to the Fe<sup>2+</sup> state. The nonheme iron atom of the *desaturase* is subsequently converted into the Fe<sup>2+</sup> state, which enables it to interact with O<sub>2</sub> and the saturated fatty acyl CoA substrate. A double bond is formed and two molecules of H<sub>2</sub>O are released. Two electrons come from NADH and two from the single bond of the fatty acyl substrate.

A variety of unsaturated fatty acids can be formed from oleate by a combination of elongation and desaturation reactions.

For example, oleate can be elongated to a 20:1 *cis*- $\Delta$  11 fatty acid. Alternatively, a second double bond can be inserted to yield an 18:2 *cis*- $\Delta$  6, $\Delta$  9 fatty acid. Similarly, palmitate (16:0) can be oxidized to palmitoleate (16:1 *cis*- $\Delta$  9), which can then be elongated to *cis*-vaccenate (18:1 *cis*- $\Delta$  11).

Unsaturated fatty acids in mammals are derived from either palmitoleate (16:1), oleate (18:1), linoleate (18:2), or linolenate (18:3). The number of carbon atoms from the  $\omega$  end of a derived unsaturated fatty acid to the nearest double bond identifies its precursor.

*Mammals lack the enzymes to introduce double bonds at carbon atoms beyond C-9 in the fatty acid chain.* Hence, mammals cannot synthesize linoleate (18:2 *cis*- $\Delta$  9,  $\Delta$  12) and linolenate (18:3 *cis*- $\Delta$  9,  $\Delta$  12,  $\Delta$  15). *Linoleate and linolenate are the two essential fatty acids.* The term *essential* means that they must be supplied in the diet because they are required by an organism and cannot be endogenously synthesized. Linoleate and linolenate furnished by the diet are the starting points for the synthesis of a variety of other unsaturated fatty acids.

## Eicosanoid Hormones Are Derived from Polyunsaturated Fatty Acids

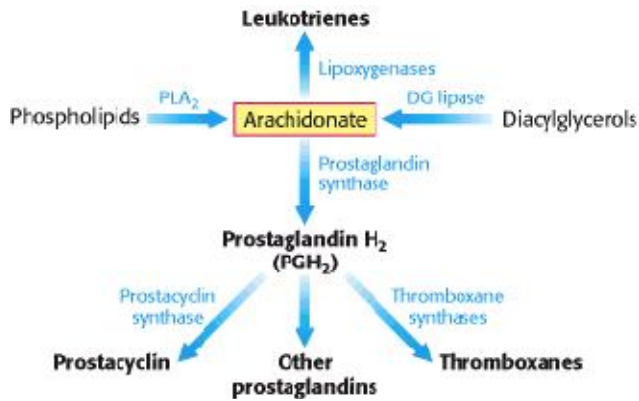
*Arachidonate*, a 20:4 fatty acid derived from linoleate, is the major precursor of several classes of signal molecules: prostaglandins, prostacyclins, thromboxanes, and leukotrienes.

*A prostaglandin is a 20-carbon fatty acid containing a 5-carbon ring.* A series of prostaglandins is fashioned by reductases and isomerases. The major classes are designated PGA through PGI; a subscript denotes the number of carbon-carbon double bonds outside the ring. Prostaglandins with two double bonds, such as PGE<sub>2</sub>, are derived from arachidonate; the other two double bonds of this precursor are lost in forming a five-membered ring.

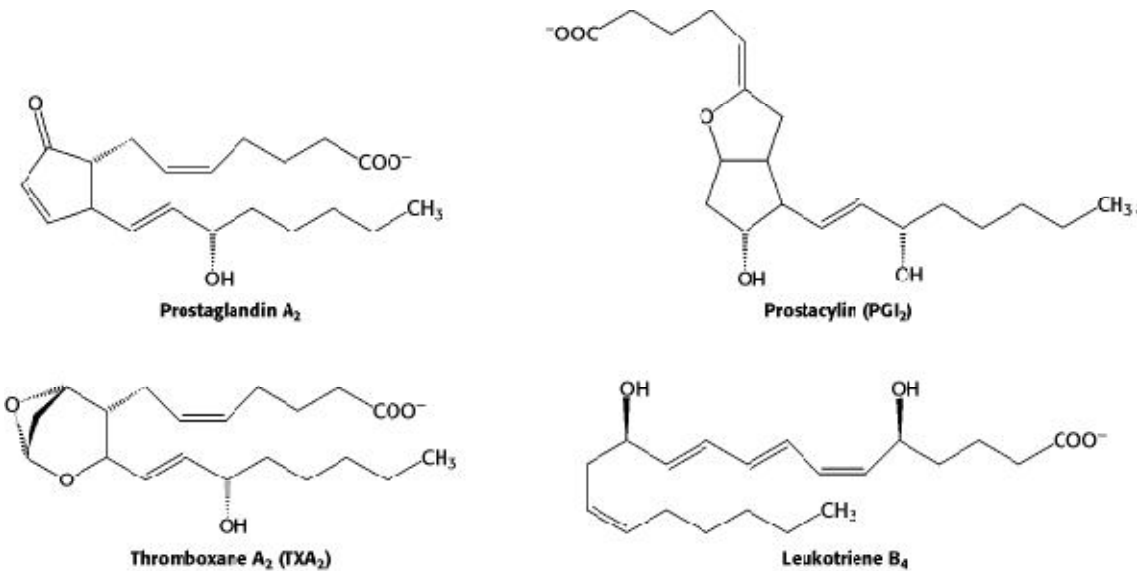
*Prostacyclin* and *thromboxanes* are related compounds that arise from a nascent prostaglandin. They are generated by *prostacyclin synthase* and *thromboxane synthase* respectively. Alternatively, arachidonate can be converted into *leukotrienes* by the action of *lipooxygenase*. These compounds, first found in leukocytes, contain three conjugated double bonds hence, the name. Prostaglandins, prostacyclin, thromboxanes, and leukotrienes are called *eicosanoids* because they contain 20 carbon atoms.

Prostaglandins and other eicosanoids are *local hormones* because they are short-lived. They alter the activities both of the cells in which they are synthesized and of adjoining cells by binding to 7TM receptors. The nature of these effects may vary from one type of cell to another, in contrast with the more uniform actions of global hormones such as insulin and glucagon. Prostaglandins stimulate inflammation, regulate blood flow to particular organs, control ion transport across membranes, modulate synaptic transmission, and induce sleep.

Recall that aspirin blocks access to the active site of the enzyme that converts arachidonate into prostaglandin H<sub>2</sub>. Because arachidonate is the precursor of other prostaglandins, prostacyclin, and thromboxanes, blocking this step affects many signaling pathways. It accounts for the wide-ranging effects that aspirin and related compounds have on inflammation, fever, pain, and blood clotting.



**Arachidonate Is the Major Precursor of Eicosanoid Hormones.** Prostaglandin synthase catalyzes the first step in a pathway leading to prostaglandins, prostacyclins, and thromboxanes. Lipoxygenase catalyzes the initial step in a pathway leading to leukotrienes.



**Structures of Several Eicosanoids.**