from B. Alberts et al., Molecular Biology of the Cell
Garland, 1983. pp. 75-89

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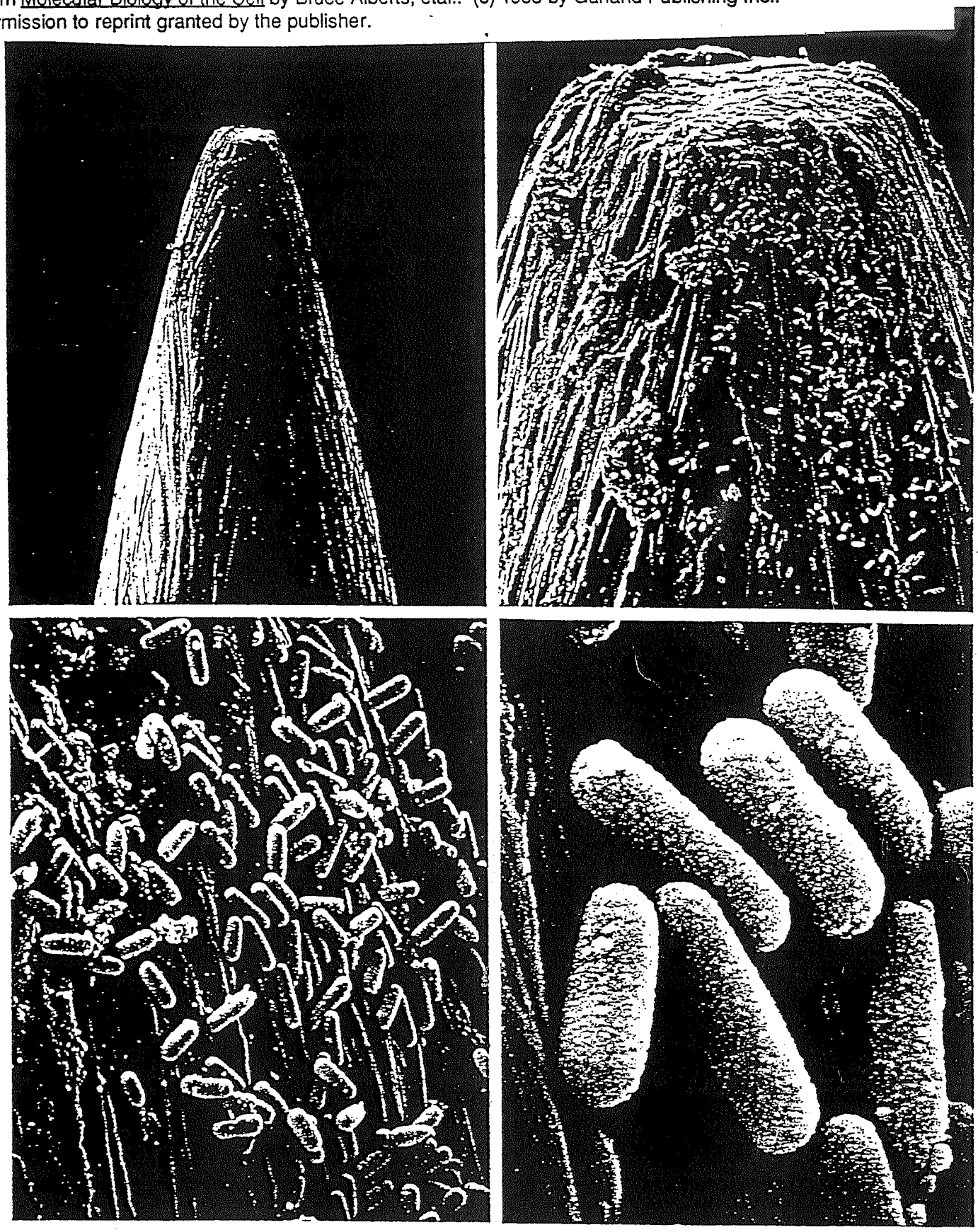

A sense of scale. These scanning electron micrographs, taken at progressively higher magnifications, show bacterial cells on the point of an ordinary domestic pin. (Courtesy of Tony Brain and the Science Photo Library.)

Summary

Animal cells can be considered to derive energy from food in three stages. In stage 1, proteins, polysaccharides, and fats are broken down by extracellular reactions to small molecules. In stage 2, these small molecules are degraded within cells to produce acetyl CoA and a limited amount of ATP and NADH. These are the only reactions that can yield energy in the absence of oxygen. In stage 3, the acetyl CoA molecules are degraded in mitochondria to give CO_2 and hydrogen atoms that are linked to carrier molecules such as NADH. Electrons from the hydrogen atoms are passed through a complex chain of carriers that leads eventually to the reduction of molecular oxygen to form water. Driven by the energy released in these electron-transfer steps, hydrogen ions (H^+) are transported out of the mitochondrion. The resulting electrochemical proton gradient across the inner mitochondrial membrane is harnessed to drive the synthesis of most of the cell's ATP.

Biosynthesis and the Creation of Order

Thousands of different chemical reactions are occurring in a cell at any instant of time. The reactions are linked together in chains in which the product of one reaction becomes the substrate of the next. It is possible in principle to go from any one compound to any other. However, like motor traffic along the routes of a major city, much of the metabolic traffic in a cell tends to be either "inward" or "outward." The inward traffic consists of catabolic reactions that convert food molecules into sugars and sugar phosphates, as described earlier. The outward traffic consists of biosynthetic reactions that begin with the intermediate products of glycolysis and the citric acid cycle (and their related compounds) and generate the larger and more complex molecules of the cell.

The Energy for Biosynthesis Comes from the Hydrolysis of ATP

Throughout the cell, reactions that would otherwise be unfavorable, including all biosynthetic reactions, are driven directly or indirectly by the hydrolysis of ATP molecules. The large amount of energy released in this reaction derives from several sources, including the high stability of phosphate (abbreviated as P_i or Ⓟ) in its free form and the release of unfavorable charge repulsion between two adjacent phosphates in an ATP molecule (Figure 2–27). An alternative pathway for ATP hydrolysis, in which two Ⓟ-Ⓟ bonds are hydrolyzed, releases about twice as much energy; in this case, ATP is hydrolyzed to AMP (*adenosine monophosphate*) and Ⓟ-Ⓟ (*pyrophosphate*), with subsequent hydrolysis of the released Ⓟ-Ⓟ to free phosphate (Figure 2–28).

We have thus far used the term "energy" quite loosely: what determines whether a reaction will occur is actually the change in **free energy.** As we have said earlier, the release of energy as heat, by increasing the violence of molecular motions and distorting molecules, creates disorder; and according to the Second Law of Thermodynamics, it is only those reactions that result in a net increase in disorder in the universe that can take place spontaneously. The change in free energy that occurs during a reaction, denoted ΔG, is defined in such a way that it provides a direct measure of the amount of disorder created in the universe when that reaction takes place. Reactions that release a large quantity of free energy are those that have a very large *negative* ΔG and create much disorder. Such reactions will have a strong tendency to

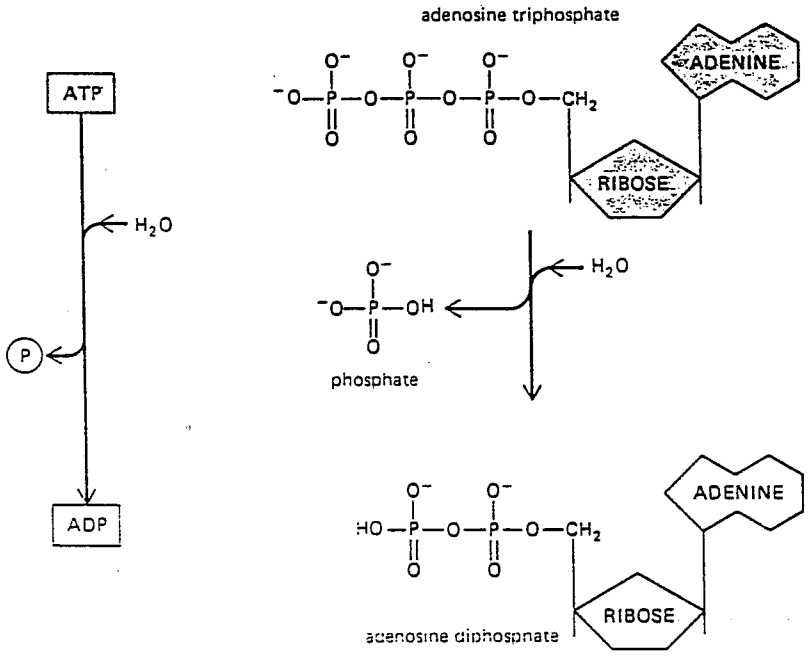

Figure 2–27 In the hydrolysis of ATP, the terminal phosphate can be cleaved to yield between 11 and 13 kilocalories per mole of usable energy, depending on intracellular conditions.

occur, although the rate at which they do so will depend on other factors, such as the availability of specific enzymes (see below). Conversely, reactions with a *positive* value of ΔG create net order in the universe and cannot occur spontaneously. Such energetically unfavorable reactions will happen only if they are coupled to a second reaction with a negative ΔG so large that the ΔG of the entire process is negative.

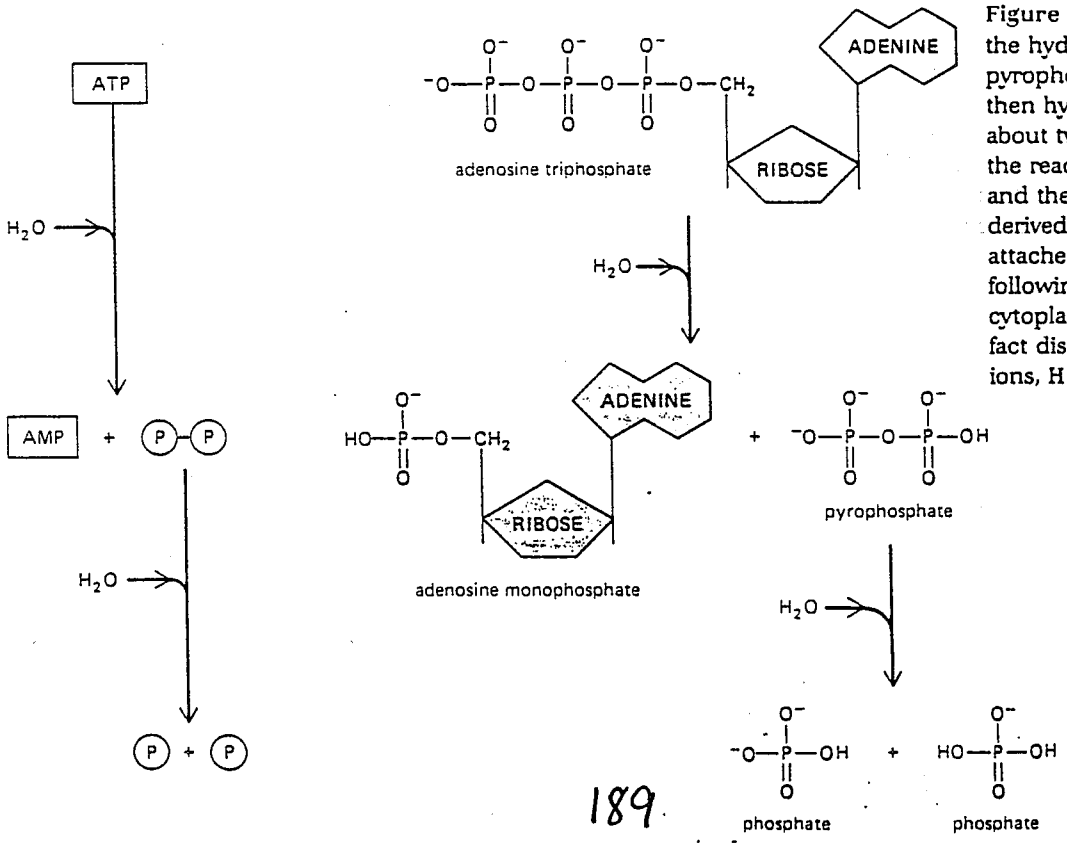

Figure 2–28 An alternative route for the hydrolysis of ATP, in which pyrophosphate is first formed and then hydrolyzed. This route yields about twice as much usable energy as the reaction in Figure 2–27. In this and the previous figure, the H atoms derived from water are shown attached to the phosphate groups following hydrolysis. At the pH of the cytoplasm, however, most of these in fact dissociate to form free hydrogen ions, H^+.

Biosynthetic Reactions Are Often Directly Coupled to ATP Hydrolysis

While enzymes speed up energetically favorable reactions, they cannot force energetically unfavorable reactions to occur. In terms of a water analogy, enzymes by themselves cannot make water run uphill. But in order to grow and divide, cells must do just that: they must build large and complex molecules from small and simple ones. We have seen that, in a general way, this is done through enzymes that couple the release of chemical energy (derived originally from the sun) to the completion of energetically unfavorable reactions. Let us examine in greater detail how such coupling is achieved.

Imagine a typical biosynthetic reaction in which two monomers, A and B, are to be joined in a *dehydration* (also called *condensation*) reaction, in which water is released:

$$\text{A-H} + \text{B-OH} \rightarrow \text{A-B} + \text{H}_2\text{O}$$

(For convenience, we shall refer to A-H and B-OH as Ⓐ and Ⓑ.) Almost invariably the reverse reaction (called *hydrolysis*), in which water breaks the covalently linked compound A-B, will be the energetically favorable one. This is the case, for example, in the hydrolysis of proteins, nucleic acids, and polysaccharides into their subunits.

The general strategy that allows the cell to make A-B from Ⓐ and Ⓑ is the same as that which allows it to make ATP from burning glucose: a multiple-step pathway couples the energetically unfavorable synthesis of the desired compound to an even more energetically favorable reaction (see Figure 2–17). In many cases the energetically favorable reaction exploited is the hydrolysis of an ATP molecule.

In the coupled pathway from Ⓐ and Ⓑ to A-B, energy from ATP hydrolysis first converts Ⓑ to a higher-energy intermediate compound, which then reacts directly with Ⓐ to give A-B. The simplest mechanism involves the transfer of a phosphate from ATP to Ⓑ to make Ⓑ-OPO_3^{2-} (or Ⓑ-Ⓟ), in which case the reaction pathway would contain only two steps:

1. Ⓑ + ATP → Ⓑ-Ⓟ + ADP
2. Ⓐ + Ⓑ-Ⓟ → A-B + Ⓟ

Since the intermediate Ⓑ-Ⓟ is formed and broken down again (possibly very quickly, while still bound to the surface of an enzyme molecule), the overall reactions that occur are

$$\text{Ⓐ} + \text{Ⓑ} \rightarrow \text{A-B} \qquad \text{and} \qquad \text{ATP} \rightarrow \text{ADP} + \text{Ⓟ}$$

Note that the first reaction has been forced to occur by being directly coupled to the second reaction.

The Outcome of Coupled Reactions Depends on the Total Change in Free Energy

The course of most reactions can be predicted quantitatively. A large body of thermodynamic data has been collected that makes it possible to calculate the change in free energy, ΔG, for most of the important metabolic reactions of the cell. The overall free-energy change for a pathway is then simply the sum of the energy changes in each of its component steps.

If we imagine two reactions

$$\text{A} \rightarrow \text{B} \quad \text{and} \quad \text{C} \rightarrow \text{D}$$

where the ΔG values are $+1$ and -9 kilocalories per mole, respectively, then if these two reactions can be coupled together, the ΔG for the coupled reaction

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will be −8 kilocalories per mole. (Recall that a mole is 6×10^{23} molecules of a substance.) This tells us that even a reaction with a positive ΔG, which will not occur spontaneously, can be driven by a second reaction. However, this requires that the latter reaction have a sufficiently large negative ΔG and that a mechanism exist by which the two reactions can be coupled together.

The ΔG for the hydrolysis of ATP to ADP and inorganic phosphate depends on the concentrations of all of the reactants (see p. 499), but under the usual conditions in a cell it is between −11 and −13 kilocalories per mole. In principle, this hydrolysis reaction can be used to drive an unfavorable reaction with a ΔG of, perhaps, +10 kilocalories per mole, provided that a suitable reaction path is available. For many biosynthetic reactions, however, even −13 kilocalories per mole is not enough, as in the synthesis of nucleic acids and in the activation of amino acids preparatory to protein synthesis. In these and other cases, the path of ATP hydrolysis is altered so that it initially produces AMP and Ⓟ-Ⓟ (pyrophosphate) (see Figure 2–28). Pyrophosphate is then itself hydrolyzed in a second step that makes an additional −13 kilocalories per mole available. Many biosynthetic pathways are effectively irreversible only because the pyrophosphate required for the reverse reaction is rapidly removed (Figure 2–29).

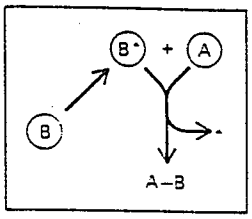
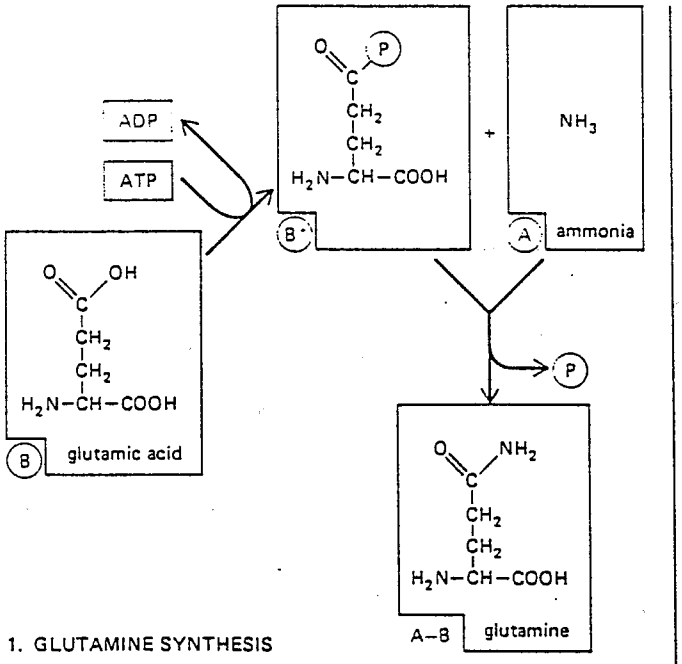
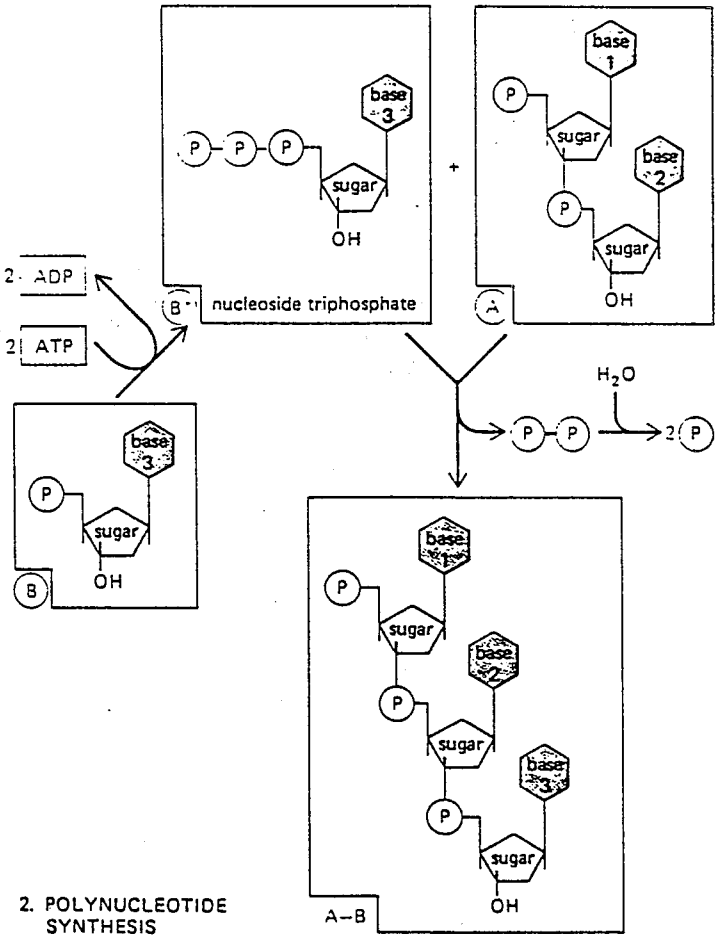


Figure 2–29 Examples of dehydration reactions of the type Ⓐ + Ⓑ → A–B. A schematic outline that applies in all cases is shown above; in general, nucleotide hydrolysis activates compound Ⓑ to Ⓑ* in order to drive an otherwise unfavorable reaction. In one of the two examples shown (below), the synthesis of the amino acid glutamine from glutamic acid and ammonia, a single phosphate bond is hydrolyzed. In the other example, two phosphate bonds are hydrolyzed in order to add each nucleotide to DNA or RNA (polynucleotide synthesis).



1. GLUTAMINE SYNTHESIS



2. POLYNUCLEOTIDE SYNTHESIS

Table 2–2 Some Coenzymes Involved in Group-Transfer Reactions

Coenzyme	Group Transferred
ATP	phosphate
NADH, NADPH	hydrogen & electron (hydride ion)
Coenzyme A	acetyl
Biotin	carboxyl
S-Adenosylmethionine	methyl
UDP-glucose	glucose

Coenzymes are small molecules that are associated with some enzymes and are essential for their activity. Each one listed is a carrier molecule for a small chemical group, and it participates in various reactions in which that group is transferred to another molecule. Some coenzymes are covalently linked to their enzyme; others are less tightly bound.

Coenzymes Are Involved in the Transfer of Specific Chemical Groups

ATP usually drives biosynthetic reactions by reacting with a second molecule to form a highly reactive phosphorylated intermediate, as we have just seen. Because the new phosphate linkage is easily cleaved with release of free energy, the second molecule can readily be joined to other molecules. This general principle is not confined to ATP-mediated reactions: a wide variety of other chemically labile linkages also work in this way. For example, specific carrier molecules are involved in the transfer of chemical groups such as acetyl groups or methyl groups (Table 2–2). The same carrier molecule will often participate in many different biosynthetic reactions in which its group is needed.

Acetyl coenzyme A (acetyl CoA), which is produced in the breakdown of glucose, is an example of such a carrier molecule. It carries an acetyl group

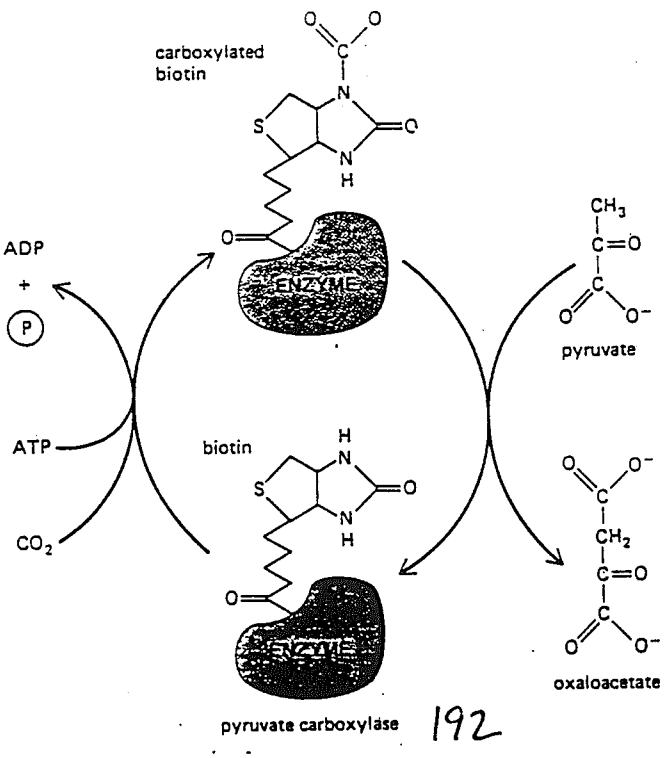


Figure 2–30 Transfer of a carboxyl group by the coenzyme biotin. Biotin acts as a carrier molecule for the carboxyl group (—COO^-). In the sequence of reactions shown, biotin is covalently bound to the enzyme pyruvate carboxylase. An activated carboxyl group derived from a bicarbonate ion (HCO_3^-) is coupled to biotin in a reaction that requires an input of energy from the hydrolysis of an ATP molecule. Subsequently, this carboxyl group is transferred to the methyl group of pyruvate to form oxaloacetate.

linked to CoA through a reactive thioester bond (see Figure 2–19). This acetyl group is readily transferred to another molecule, such as a growing fatty acid molecule. Another important example is biotin, which carries a carboxyl group in many biosynthetic reactions (Figure 2–30). Molecules such as acetyl CoA, biotin, and ATP are known as **coenzymes** because they are bound tightly to various enzyme surfaces and are essential for the activity of the enzyme. Many of the small molecules known as *vitamins*, which are required in trace amounts in the diet, are converted to coenzymes in the body.

Biosynthesis Requires Reducing Power

We have seen that oxidation and reduction reactions occur continuously in cells. The chemical energy in food molecules is released by oxidative processes that are a form of combustion, while, in order to make biological molecules, the cell needs—among other things—to carry out a series of reduction reactions that require an input of chemical energy. By using the same principle of coupled reactions that operates in the synthesis of ATP, chemical energy is channeled into the synthesis of the high-energy bond between hydrogen and the nicotinamide ring in NADH. This high-energy bond then provides the energy for otherwise unfavorable enzyme reactions that transfer hydrogen (as a hydride ion) to another molecule. NADH, and the NADPH to which it can be readily converted, are therefore said to carry "reducing power."

To see how this works in practice, consider just one biosynthetic step: the last reaction in the synthesis of the lipid molecule *cholesterol*. In this reaction two hydrogen atoms are added to the polycyclic steroid ring in order to reduce a carbon-carbon double bond (Figure 2–31). As in most biosynthetic reactions, the constituents of the two hydrogen atoms required in this reaction are supplied as a hydride ion from NADPH and a proton (H^+) from the solution ($H:^- + H^+ = 2H\cdot$). As in NADH, the hydride ion to be transferred from NADPH is part of a nicotinamide ring and is easily lost because the ring can achieve a more stable aromatic state without it (see Figure 2–22). Therefore, NADH and NADPH both hold this hydride ion in a high-energy linkage from which it can be transferred to another molecule when a suitable enzyme is available to catalyze the transfer.

The difference between NADH and NADPH is trivial in chemical terms: NADPH has an extra phosphate group on a part of the molecule that is far from the active region (Figure 2–32). This phosphate group is of no importance to the reaction as such, but it serves as a handle for binding NADPH as a coenzyme to appropriate enzymes. As a general rule, NADH operates with

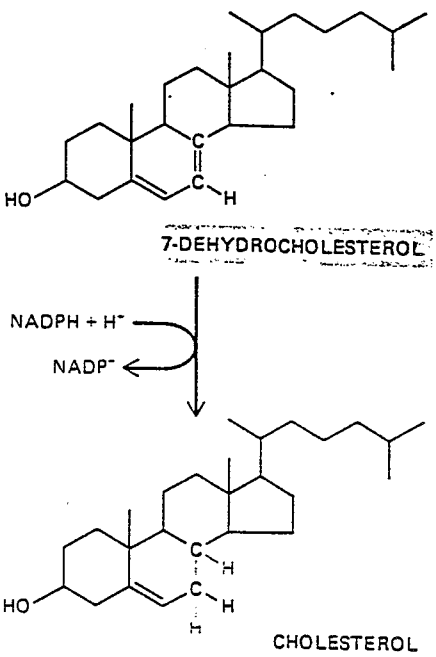


Figure 2-31 The final stage in one of the biosynthetic routes leading to cholesterol. The reduction of the C=C bond is achieved by the transfer of a hydride ion from the carrier molecule NADPH, plus a proton (H^+) from the solution.

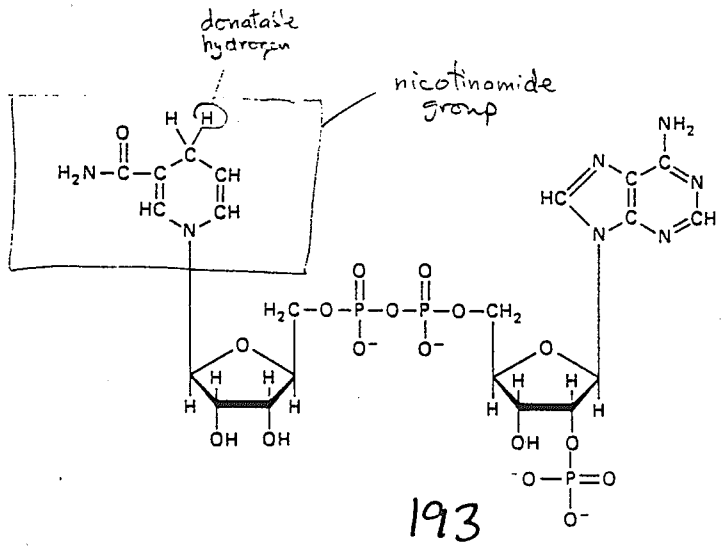


Figure 2–32 The structure of NADPH, which differs from NADH (Figure 2–22) only in the presence of an extra phosphate group that allows it to be selectively recognized by certain enzymes (usually those involved in biosynthesis).

enzymes catalyzing catabolic reactions, while NADPH operates with enzymes that catalyze biosynthetic reactions. This means that catabolic and biosynthetic pathways can be regulated separately by alterations in the levels of NADH and NADPH, respectively.

Biological Polymers Are Synthesized by Repetition of Elementary Dehydration Reactions

The principal macromolecules synthesized by cells are polynucleotides (DNA and RNA), polysaccharides, and proteins. They are enormously diverse in structure and include the most complex molecules known. Despite this, they are synthesized from a relatively small number of small molecules (referred to as either *monomers* or *subunits*) by a restricted repertoire of chemical reactions.

The addition of monomers to proteins, polynucleotides, and polysaccharides is shown in Figure 2–33. Although the synthetic reactions for each polymer involve a different kind of covalent bond and different enzymes and cofactors, there are strong underlying similarities. The addition of subunits in

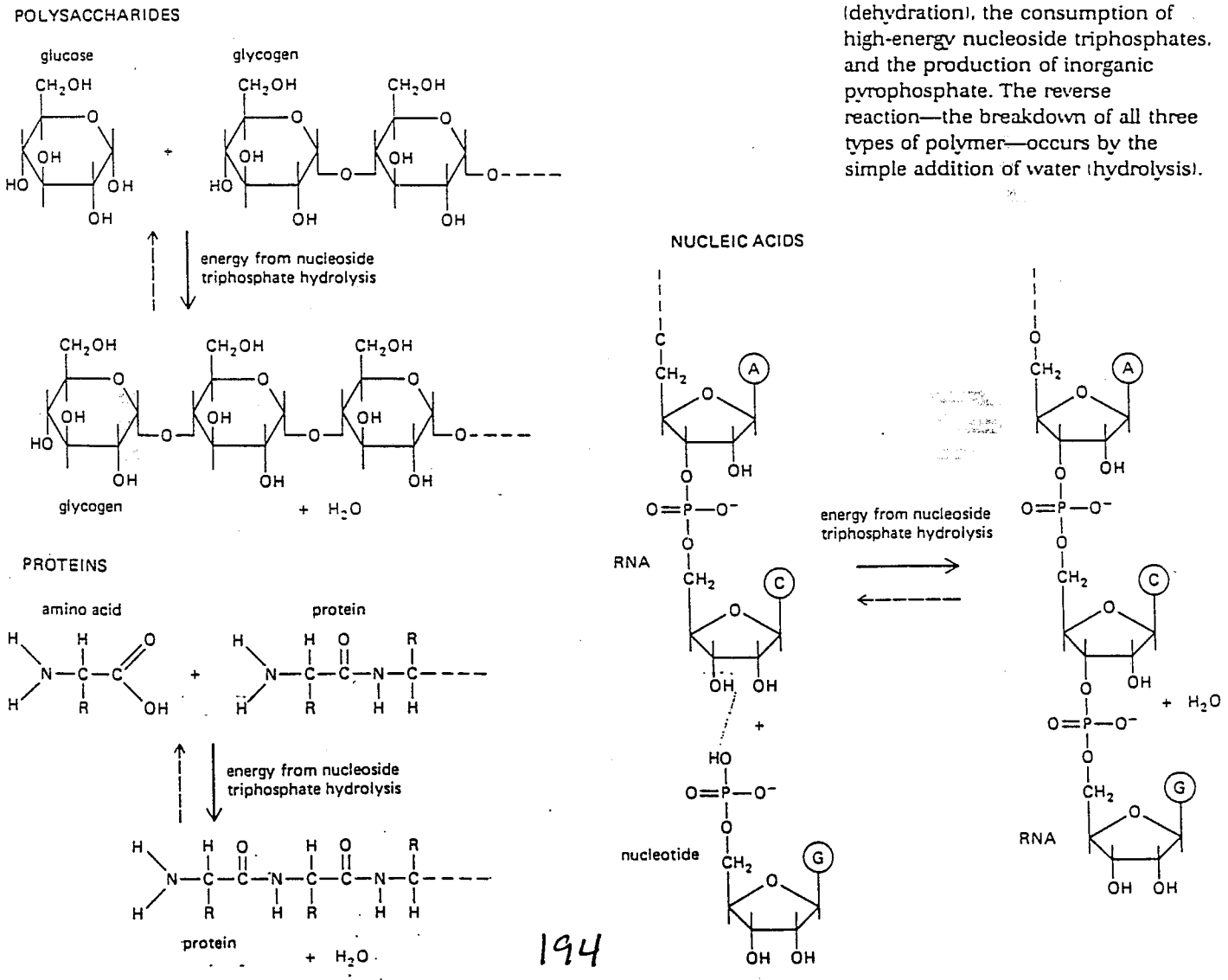


Figure 2–33 Schematic diagram of the polymerization reactions by which three kinds of biological polymer are synthesized. Although each reaction involves a number of different enzymes and other intermediates, there are underlying similarities. Synthesis in every case involves the loss of water (dehydration), the consumption of high-energy nucleoside triphosphates, and the production of inorganic pyrophosphate. The reverse reaction—the breakdown of all three types of polymer—occurs by the simple addition of water (hydrolysis).

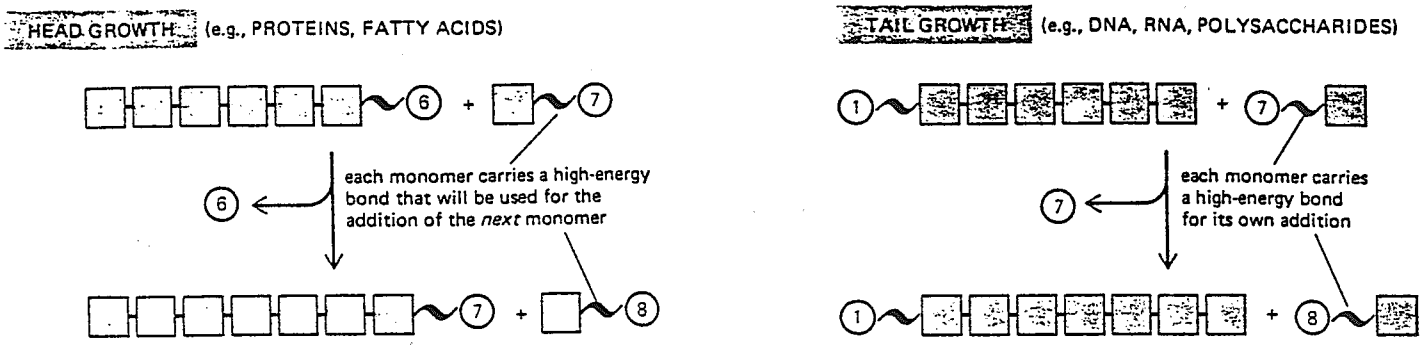


Figure 2–34 Head growth compared to the tail growth of polymers.

each case occurs by a dehydration reaction, involving the removal of a molecule of water from the reactants.

As in the more general case discussed previously (p. 77), the formation of these polymers requires the input of chemical energy, which is ultimately achieved by the standard strategy of coupling the biosynthetic reaction to the energetically favorable hydrolysis of a nucleoside triphosphate. In every case, at least one of the nucleoside triphosphates involved is cleaved to produce pyrophosphate, which is subsequently hydrolyzed to add extra driving force to the reaction (Figure 2–28).

The activated intermediates in the polymerization reactions can be oriented in one of two ways, giving rise to either head polymerization or tail polymerization. In *head polymerization*, the activated linkage is carried on the end of the growing polymer and must therefore be regenerated each time a monomer is added. In this case, each monomer brings with it the activated group that will be used to react with the next monomer in the series (Figure 2–34). In *tail polymerization*, the activated linkage carried by each monomer is used instead for its own addition. While the synthesis of polynucleotides and some simple polysaccharides occurs by tail polymerization, the synthesis of proteins occurs by head polymerization.

Summary

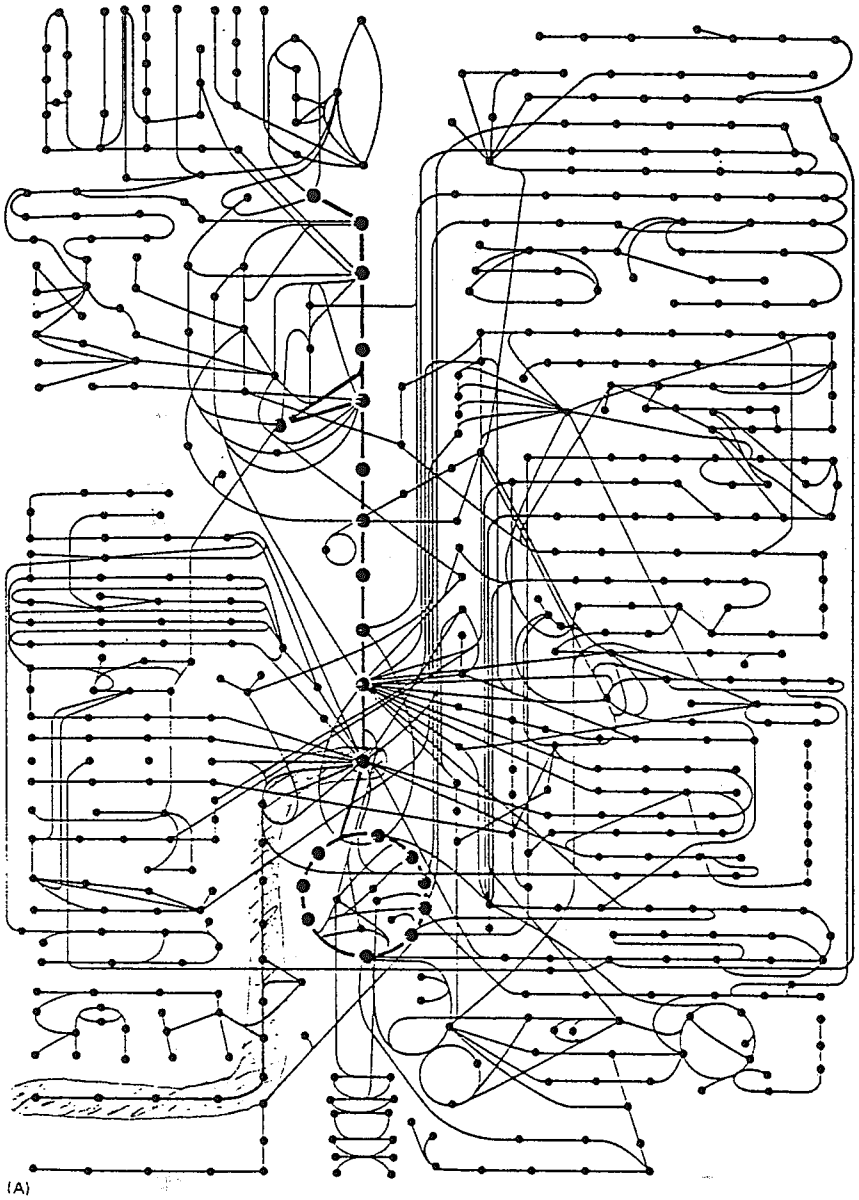
The hydrolysis of ATP is coupled to energetically unfavorable reactions, such as the biosynthesis of macromolecules, usually by the formation of reactive phosphorylated intermediates. Other reactive carrier molecules, called coenzymes, transfer other chemical groups in the course of biosynthesis: for example, NADPH transfers hydrogen as a proton plus two electrons (a hydride ion), while acetyl CoA transfers acetyl groups. Polymeric molecules such as proteins and nucleic acids are assembled from small activated precursor molecules by repetitive dehydration reactions.

The Coordination of Catabolism and Biosynthesis[8]

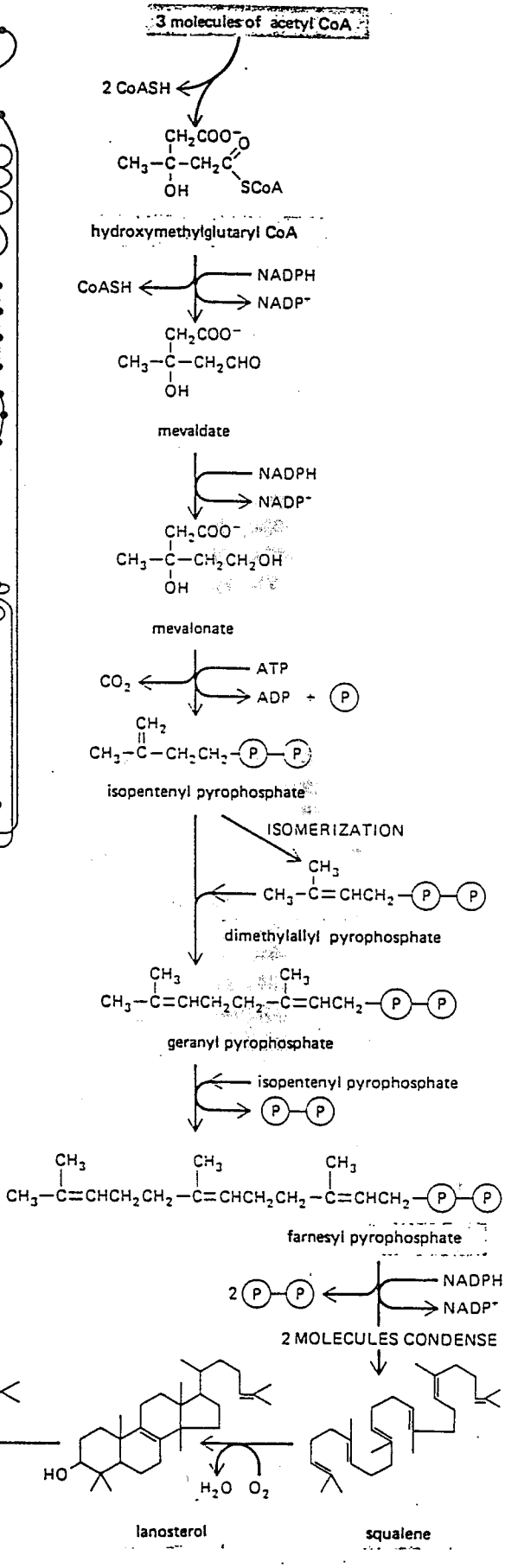
Metabolism Is Organized and Regulated

Some idea of how cleverly designed the cell is when viewed as a chemical machine can be obtained from Figure 2–35, which is a chart of a large number of the enzymatic pathways in a cell. All of these reactions occur in a cell that is less than 0.1 mm in diameter, and there are many enzymes that are not shown on this chart (especially those associated with the cytoskeleton and with cell membranes). Furthermore, each reaction requires a different enzyme

Figure 2–35 Some of the chemical reactions occurring in a cell. (A) Radiating from the glycolytic pathway and the citric acid cycle (shown in solid color) are about 500 common metabolic reactions. A typical mammalian cell synthesizes over 10,000 proteins, a major proportion of which are enzymes. In the arbitrarily selected segment of this metabolic maze that is color shaded, cholesterol is synthesized from acetyl CoA. To the right and below the maze, this segment is shown in detail in an enlargement (B). ▶



(A)


3 molecules of acetyl CoA
2 CoASH
hydroxymethylglutaryl CoA
CoASH
NADPH
NADP+
mevaldate
mevalonate
CO2
ATP
ADP + P
isopentenyl pyrophosphate
ISOMERIZATION
dimethylallyl pyrophosphate
geranyl pyrophosphate
isopentenyl pyrophosphate
farnesyl pyrophosphate
2 MOLECULES CONDENSE
HO
NADP+ NADPH
2CH3
CH3
H2O O2
cholesterol
7-dehydrocholesterol
lanosterol
squalene

196

(B)

that is itself the product of a whole series of information-transfer and protein-synthesis reactions.

The entire system is so complex that it seems like a metabolic jungle. Take any small molecule—the amino acid *serine*, for example—and there will be half a dozen or more enzymes that can modify it chemically in different ways: it can be linked to AMP (adenylated) in preparation for protein synthesis, or degraded to glycine, or converted to pyruvate in preparation for oxidation; it can be acetylated by acetyl CoA or transferred to a fatty acid to make phosphatidyl serine. All of these different pathways compete for the same serine molecule, and a similar struggle for thousands of other small molecules goes on at the same time. One might think that the whole system would need to be so finely balanced that any minor upset, such as a temporary change in dietary intake, would be disastrous.

In fact, the cell is amazingly stable. It can adapt and continue to function in a coherent way during starvation or disease. Mutations of many kinds can lead to the elimination of particular reaction pathways, and yet—provided that certain minimum requirements are met—the cell survives. It does so because an elaborate network of control mechanisms regulates the chemical reactions within cells. Some of the higher levels of control will be considered in later chapters. Here we are concerned only with the simplest mechanisms that regulate the flow of small molecules through the various metabolic pathways in a cell.

Metabolic Pathways Are Regulated by Changes in Enzyme Activity

The concentrations of the various small molecules in a cell are buffered against major changes by a process known as **feedback regulation.** This type of regulatory mechanism fine-tunes the flux of metabolites through a particular pathway by temporarily increasing or decreasing the activity of crucial enzymes. For example, the first enzyme of a series of reactions is usually inhibited by the final product of that pathway: thus, if large quantities of the final product accumulate, further entry of precursors into the reaction pathway is automatically inhibited (Figure 2–36). Where pathways branch or intersect, as they often do, there are usually multiple points of control by different final products. The complexity of such feedback control processes is illustrated in Figure 2–37, which shows the pattern of enzyme regulation observed in a set of related amino acid pathways.

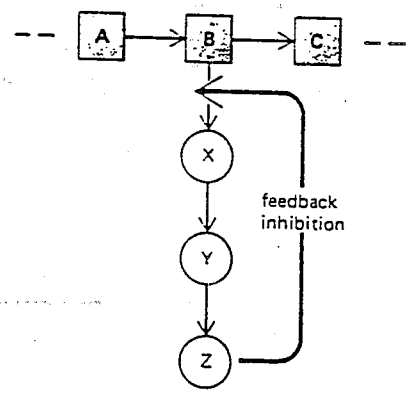


Figure 2–36 Feedback inhibition of a single biosynthetic pathway. The end product Z inhibits the first enzyme that is unique to its synthesis and thereby regulates its own level in the cell.

Feedback regulation can work almost instantaneously, and it may involve reversible enzyme activators as well as inhibitors. The molecular basis for this type of control in cells is well understood, but since an explanation requires some knowledge of protein structure, it will be deferred until Chapter 3.

Catabolic Reactions Can Be Reversed by an Input of Energy[9]

Large-scale changes that affect the metabolism of the entire cell can also be achieved by regulating a few enzymes. For example, a special pattern of feedback regulation enables a cell to switch from glucose degradation to glucose biosynthesis, or *gluconeogenesis*. The need for this reverse pathway is especially acute in periods of violent exercise, when the glucose needed for muscle contraction is generated by liver cells, and also in periods of starvation when glucose must be formed from fatty acids and amino acids for survival.

The normal breakdown of glucose to pyruvate during glycolysis is catalyzed by nine separate enzymes acting in series. The reactions catalyzed by most of these enzymes are readily reversible, but three reaction steps (numbers 1, 3, and 9 in the sequence of Figure 2–20) are effectively irreversible. In

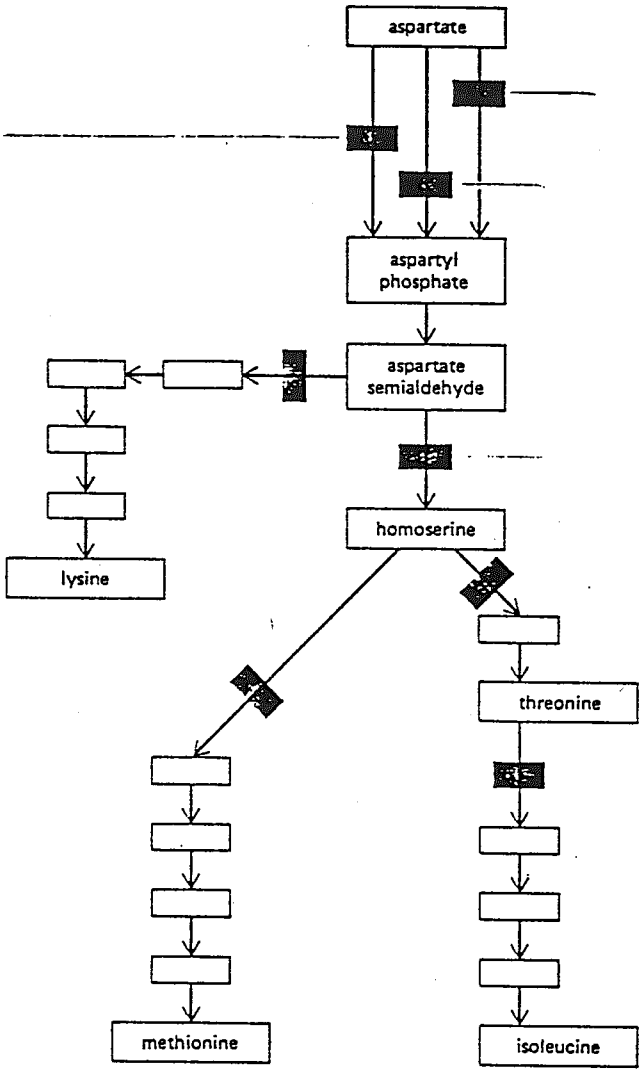


Figure 2–37 Feedback inhibition in the synthesis of the amino acids lysine, methionine, threonine, and isoleucine in bacteria. The colored arrows indicate positions at which products "feed back" to inhibit enzymes. Note that three different enzymes (called *isozymes*) catalyze the initial reaction, each inhibited by a different product.

fact, it is the large negative free-energy change that occurs in these reactions that normally drives the breakdown of glucose. For the reactions to proceed in the opposite direction and make glucose from pyruvate, each of these three reactions must be bypassed. This is achieved by substituting three alternate enzyme-catalyzed bypass reactions that are driven in the uphill direction by an input of chemical energy (Figure 2–38). Thus, while two ATP molecules are generated as each molecule of glucose is degraded to two molecules of pyruvate, the reverse reaction during gluconeogenesis requires the hydrolysis of four ATP and two GTP molecules. This is equivalent, in total, to the hydrolysis of six molecules of ATP for every molecule of glucose synthesized.

The bypass reactions in Figure 2–38 must be closely controlled so that glucose is broken down only when energy is needed and is synthesized only when the cell is nutritionally replete. If both forward and reverse reactions were allowed to proceed without restraint, they would shuttle metabolites backward and forward in futile cycles that consumed large amounts of ATP to no purpose.

The elegance of these control mechanisms can be illustrated by a single example. Step 3 of glycolysis is one of the reactions that must be bypassed during glucose formation. Normally the step involves the addition of a phosphate group to fructose 6-phosphate from ATP and is catalyzed by the enzyme *phosphofructokinase*. This particular enzyme is activated by AMP and ADP

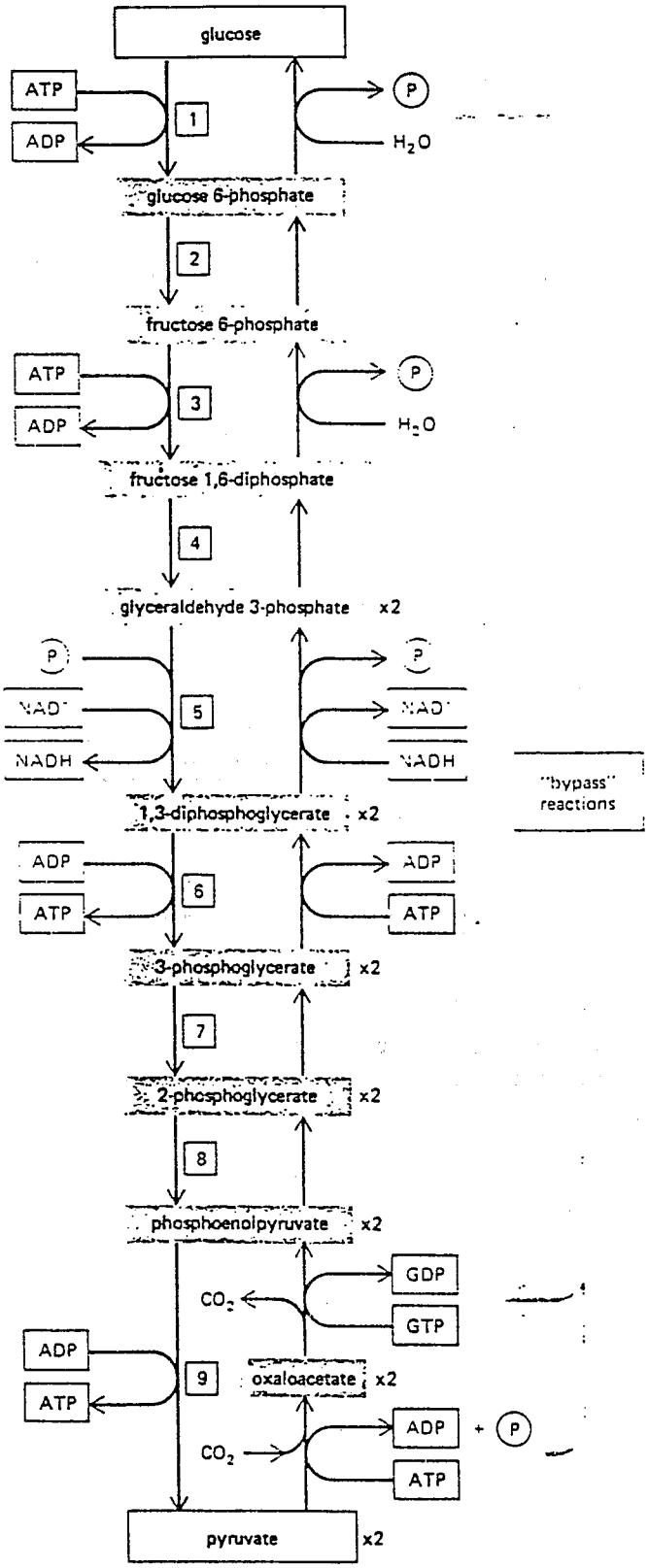


Figure 2–38 Comparison of the reactions that produce glucose during gluconeogenesis with those that degrade glucose. The degradative (glycolytic) reactions are energetically favorable (the free-energy change is less than zero), while the synthetic reactions require an input of energy. To synthesize glucose, different "bypass enzymes" are needed that bypass reactions 1, 3, and 9 of glycolysis. The overall flux of reactants is determined by feedback control mechanisms that operate at these crucial steps.

and inhibited by ATP, citrate, and fatty acids. In other words, the enzyme is activated when energy supplies are low and AMP and ADP accumulate, and it is inactivated when energy (in the form of ATP) or food supplies such as fatty acids or citrate (derived from amino acids) are abundant. The enzyme that catalyzes the reverse (bypass) reaction that leads to the formation of glucose is *fructose diphosphatase.* This enzyme is regulated in the opposite way by the same feedback control molecules, so that it works when the phosphofructokinase does not.

Note that phosphofructokinase is activated by ADP, which is a product of the reaction it catalyzes (ATP + fructose 6-P → ADP + fructose 1,6-diphosphate), and is inhibited by ATP, which is one of its substrates. As a result, this enzyme is subject to a complex form of positive feedback control. Under certain circumstances such feedback control gives rise to striking oscillations in the activity of the enzyme, causing corresponding oscillations in the concentrations of various glycolytic intermediates (Figure 2–39). While the physiological significance of these particular oscillations is not known, they illustrate how a biological oscillator can be produced by a few enzymes. In principle, such oscillations could provide an internal clock, enabling a cell to "measure time" and, for example, to perform certain functions at fixed intervals.

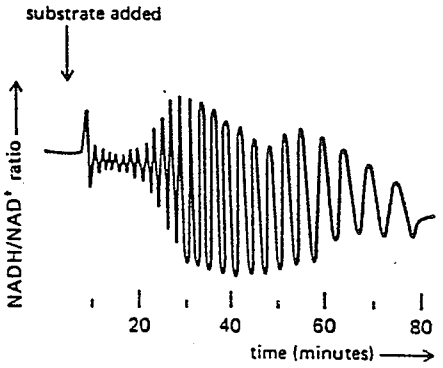


Figure 2–39 The abrupt addition of glucose to an extract containing the enzymes and cofactors required for glycolysis can produce large cyclic fluctuations in the levels of intermediates such as NADH. These metabolic oscillations arise, in part, from the positive feedback control of the glycolytic enzyme phosphofructokinase.

Enzymes Can Be Switched On and Off by Covalent Modification[10]

The types of feedback control just described permit the rates of reaction sequences to be continuously and automatically regulated in response to second-by-second fluctuations in metabolism. Cells have different devices for regulating enzymes when longer lasting changes in activity, occurring over minutes or hours, are required. These involve reversible covalent modification of enzymes, which is often, but not always, accomplished by the addition of a phosphate group to a specific serine, threonine, or tyrosine residue in the enzyme. The phosphate comes from ATP and its transfer is catalyzed by enzymes known as *protein kinases.*

We shall describe in the following chapter how phosphorylation alters the shape of an enzyme in such a way as to increase or inhibit its activity. The subsequent removal of the phosphate group, which reverses the effect of the phosphorylation, is achieved by a second enzyme, called a *phosphoprotein phosphatase.* Covalent modification of enzymes adds another dimension to metabolic control, because it allows specific reaction pathways to be regulated by signals (such as hormones) that are unrelated to the metabolic intermediates themselves.

Reactions Are Compartmentalized Both Within Cells and Within Organisms[11]

Not all of a cell's metabolic reactions occur within the same subcellular compartment. Because different enzymes are found in different parts of the cell, the flow of chemical components is physically as well as chemically channeled.

The simplest form of such spatial segregation occurs when two enzymes that catalyze sequential reactions form an enzyme complex, and the product of the first enzyme does not have to diffuse through the cytoplasm to encounter the second enzyme. As soon as the first reaction is over, the second begins. Some large enzyme aggregates carry out whole series of reactions without losing contact with the substrate. For example, the conversion of pyruvate to acetyl CoA proceeds in three chemical steps, all of which take place on the same large enzyme complex (Figure 2–40), and in fatty acid

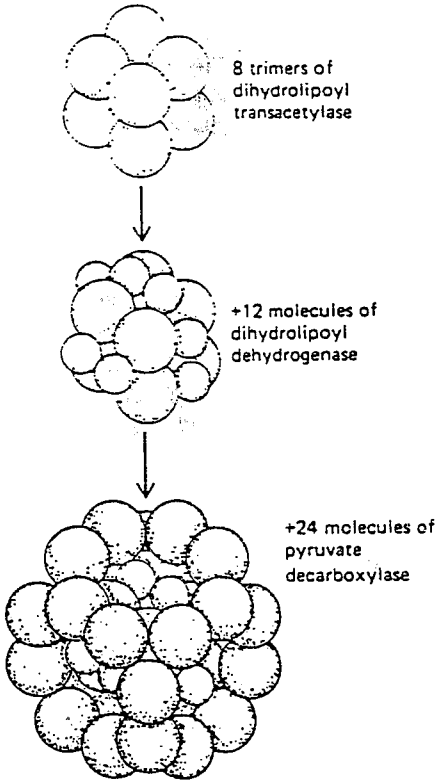


Figure 2–40 The structure of pyruvate dehydrogenase—an example of a large multienzyme complex in which reaction intermediates are passed directly from one enzyme to another. This enzyme complex catalyzes the conversion of pyruvate to acetyl CoA.

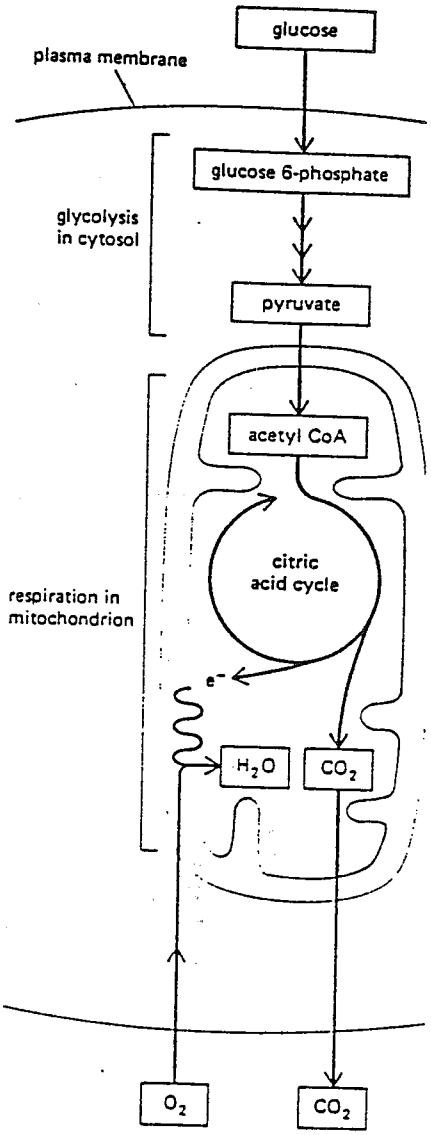


Figure 2–41 Segregation of the various steps in the breakdown of glucose in the eucaryotic cell. Glycolysis occurs in the cytosol, whereas the reactions of the citric acid cycle and oxidative phosphorylation take place only in mitochondria.

synthesis an even longer sequence of reactions is catalyzed by a single enzyme assembly. Not surprisingly, some of the largest enzyme complexes are concerned with the synthesis of macromolecules such as proteins and DNA.

The next level of spatial segregation in cells involves the confinement of functionally related enzymes within the same membrane or within the aqueous compartments of organelles that are bounded by membranes. The oxidative metabolism of glucose is a good example (Figure 2–41). After glycolysis, pyruvate is actively taken up from the cytosol into the inner compartment of the mitochondrion, which contains all of the enzymes and metabolites involved in the citric acid cycle. Moreover, the inner mitochondrial membrane itself contains all of the enzymes that catalyze the subsequent reactions of oxidative phosphorylation, including those involved in the transfer of electrons from NADH to O_2 and in the synthesis of ATP. The entire mitochondrion can therefore be regarded as a small ATP-producing factory. In the same way, other cellular organelles, such as the nucleus, the Golgi apparatus, and the lysosomes, can be viewed as specialized compartments where functionally related enzymes are confined to perform a specific task. In a sense, the living cell is like a modern city, with many specialized services concentrated in different areas that are extensively interconnected by various paths of communication.

Spatial organization in multicellular organisms extends beyond the individual cell. The different tissues of the body have different sets of enzymes and contribute in distinct ways to the survival of the organism as a whole. In addition to differences in specialized products such as hormones or antibodies, there are significant differences in the "common" metabolic pathways between various types of cells in the same organism. Although virtually all cells contain the enzymes of glycolysis, the citric acid cycle, lipid synthesis and breakdown, and amino acid metabolism, the levels of these processes in different tissues are subject to fine-tuning in response to the needs of the organism. Nerve cells, which are probably the most fastidious cells in the body, maintain almost no reserves of glycogen or fatty acids and rely almost entirely on a supply of glucose from the bloodstream. Liver cells supply glucose to actively contracting muscle cells and recycle the lactic acid produced by muscle cells back into glucose (Figure 2–42). All types of cells have their distinctive metabolic traits and cooperate extensively in the normal state as well as in response to exercise, stress, and starvation.

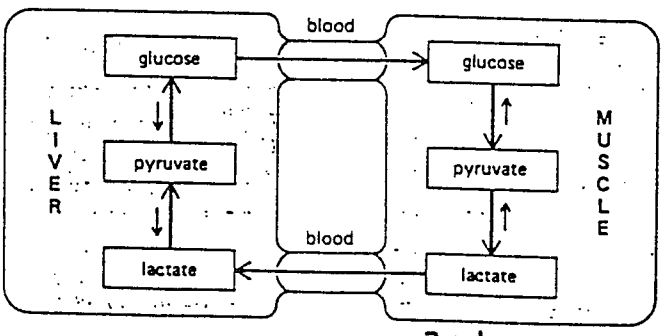


Figure 2–42 Schematic view of the metabolic cooperation between liver and muscle cells. The principal fuel of actively contracting muscle cells is glucose, much of which is supplied by liver cells. Lactic acid, the end product of anaerobic glucose breakdown in muscle, is converted back to glucose in the liver.

Summary

The many thousands of distinct chemical reactions carried out simultaneously by a cell are closely coordinated. A variety of control mechanisms regulate the activities of key enzymes in response to the changing conditions in the cell. One very common form of regulation is a rapidly reversible feedback inhibition exerted on the first enzyme of a pathway by the final product of that pathway. A longer-lasting form of regulation involves the chemical modification of one enzyme by another, often by phosphorylation. Combinations of regulatory mechanisms can produce major and long-lasting changes in the metabolism of the cell. Not all cellular reactions occur within the same intracellular compartment, and spatial segregation by internal membranes permits organelles to specialize in their biochemical tasks.

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