processes involve the flow of electrons through a chain of membrane-bound carriers. (2) The free energy made available by this "downhill" (exergonic) electron flow is coupled to the "uphill" transport of protons across a proton-impermeable membrane, conserving the free energy of fuel oxidation as a transmembrane electrochemical potential (p. 391). (3) The transmembrane flow of protons down their concentration gradient through specific protein channels provides the free energy for synthesis of ATP, catalyzed by a membrane protein complex (ATP synthase) that couples proton flow to phosphorylation of ADP.

We begin this chapter with oxidative phosphorylation. We first describe the components of the electron-transfer chain, their organization into large functional complexes in the inner mitochondrial membrane, the path of electron flow through them, and the proton movements that accompany this flow. We then consider the remarkable enzyme complex that, by "rotational catalysis," captures the energy of proton flow in ATP, and the regulatory mechanisms that coordinate oxidative phosphorylation with the many catabolic pathways by which fuels are oxidized. With this understanding of mitochondrial oxidative phosphorylation, we turn to photophosphorylation, looking first at the absorption of light by photosynthetic pigments, then at the light-driven flow of electrons from H_2O to $NADP^+$ and the molecular basis for coupling electron and proton flow. We also consider the similarities of structure and mechanism between the ATP synthases of chloroplasts and mitochondria, and the evolutionary basis for this conservation of mechanism.

OXIDATIVE PHOSPHORYLATION

19.1 Electron-Transfer Reactions in Mitochondria

The discovery in 1948 by Eugene Kennedy and Albert Lehninger that mitochondria are the site of oxidative phosphorylation in eukaryotes marked the beginning of the modern phase of studies in biological energy transductions. Mitochondria, like gram-negative bacteria, have two membranes (Fig. 19–1). The outer mitochondrial membrane is readily permeable to small molecules (M_r <5,000) and ions, which move freely through transmembrane channels formed by a family of integral membrane proteins called porins. The inner membrane is impermeable to most small molecules and ions, including protons (H^+); the only species that cross this membrane do so through specific transporters. The inner membrane bears the components of the respiratory chain and the ATP synthase.

Albert L. Lehninger, 1917–1986

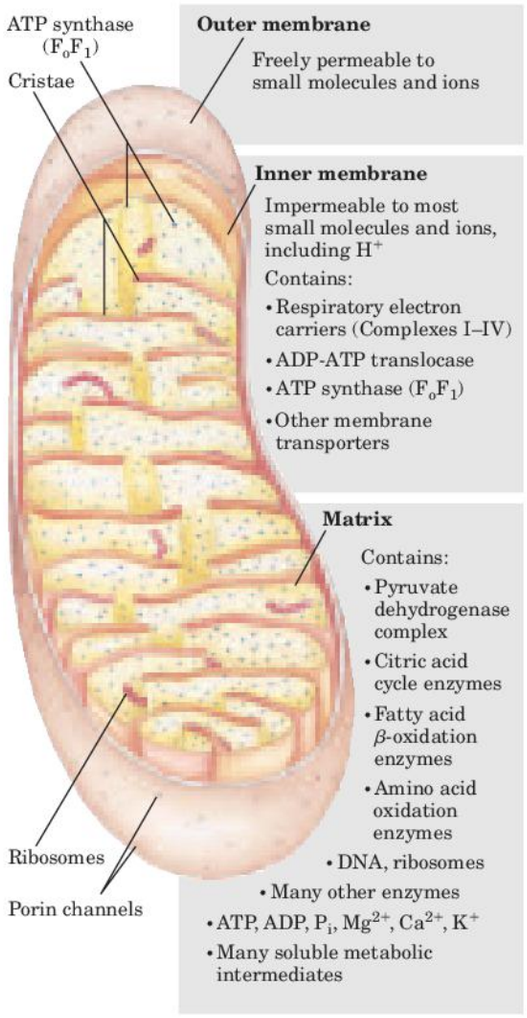

FIGURE 19–1 Biochemical anatomy of a mitochondrion. The convolutions (cristae) of the inner membrane provide a very large surface area. The inner membrane of a single liver mitochondrion may have more than 10,000 sets of electron-transfer systems (respiratory chains) and ATP synthase molecules, distributed over the membrane surface. Heart mitochondria, which have more profuse cristae and thus a much larger area of inner membrane, contain more than three times as many sets of electron-transfer systems as liver mitochondria. The mitochondrial pool of coenzymes and intermediates is functionally separate from the cytosolic pool. The mitochondria of invertebrates, plants, and microbial eukaryotes are similar to those shown here, but with much variation in size, shape, and degree of convolution of the inner membrane.

The mitochondrial matrix, enclosed by the inner membrane, contains the pyruvate dehydrogenase complex and the enzymes of the citric acid cycle, the fatty

acid β-oxidation pathway, and the pathways of amino acid oxidation—all the pathways of fuel oxidation except glycolysis, which takes place in the cytosol. The selectively permeable inner membrane segregates the intermediates and enzymes of cytosolic metabolic pathways from those of metabolic processes occurring in the matrix. However, specific transporters carry pyruvate, fatty acids, and amino acids or their α-keto derivatives into the matrix for access to the machinery of the citric acid cycle. ADP and P_i are specifically transported into the matrix as newly synthesized ATP is transported out.

Electrons Are Funneled to Universal Electron Acceptors

Oxidative phosphorylation begins with the entry of electrons into the respiratory chain. Most of these electrons arise from the action of dehydrogenases that collect electrons from catabolic pathways and funnel them into universal electron acceptors—nicotinamide nucleotides (NAD^+ or $NADP^+$) or flavin nucleotides (FMN or FAD).

Nicotinamide nucleotide–linked dehydrogenases catalyze reversible reactions of the following general types:

$$\text{Reduced substrate} + NAD^+ \rightleftharpoons \text{oxidized substrate} + NADH + H^+$$

$$\text{Reduced substrate} + NADP^+ \rightleftharpoons \text{oxidized substrate} + NADPH + H^+$$

Most dehydrogenases that act in catabolism are specific for NAD^+ as electron acceptor (Table 19–1). Some are in the cytosol, others are in mitochondria, and still others have mitochondrial and cytosolic isozymes.

NAD-linked dehydrogenases remove two hydrogen atoms from their substrates. One of these is transferred as a hydride ion ($:H^-$) to NAD^+; the other is released as H^+ in the medium (see Fig. 13–15). NADH and NADPH are water-soluble electron carriers that associate *reversibly* with dehydrogenases. NADH carries electrons from catabolic reactions to their point of entry into the respiratory chain, the NADH dehydrogenase complex described below. NADPH generally supplies electrons to anabolic reactions. Cells maintain separate pools of NADPH and NADH, with different redox potentials. This is accomplished by holding the ratios of [reduced form]/[oxidized form] relatively high for NADPH and relatively low for NADH. Neither NADH nor NADPH can cross the inner mitochondrial membrane, but the electrons they carry can be shuttled across indirectly, as we shall see.

Flavoproteins contain a very tightly, sometimes covalently, bound flavin nucleotide, either FMN or FAD (see Fig. 13–18). The oxidized flavin nucleotide can accept either one electron (yielding the semiquinone form) or two (yielding $FADH_2$ or $FMNH_2$). Electron transfer occurs because the flavoprotein has a higher reduction potential than the compound oxidized. The standard reduction potential of a flavin nucleotide, unlike that of NAD or NADP, depends on the protein with which it is associated. Local interactions with functional groups in the protein distort the electron orbitals in the flavin ring, changing the relative stabilities of oxidized and reduced forms. The relevant standard reduction

TABLE 19–1 Some Important Reactions Catalyzed by NAD(P)H-Linked Dehydrogenases

Reaction[*]	*Location*[†]
NAD-linked	
α-Ketoglutarate + CoA + $NAD^+ \rightleftharpoons$ succinyl-CoA + CO_2 + NADH + H^+	M
L-Malate + $NAD^+ \rightleftharpoons$ oxaloacetate + NADH + H^+	M and C
Pyruvate + CoA + $NAD^+ \rightleftharpoons$ acetyl-CoA + CO_2 + NADH + H^+	M
Glyceraldehyde 3-phosphate + P_i + $NAD^+ \rightleftharpoons$ 1,3-bisphosphoglycerate + NADH + H^+	C
Lactate + $NAD^+ \rightleftharpoons$ pyruvate + NADH + H^+	C
β-Hydroxyacyl-CoA + $NAD^+ \rightleftharpoons$ β-ketoacyl-CoA + NADH + H^+	M
NADP-linked	
Glucose 6-phosphate + $NADP^+ \rightleftharpoons$ 6-phosphogluconate + NADPH + H^+	C
NAD- or NADP-linked	
L-Glutamate + H_2O + $NAD(P)^+ \rightleftharpoons$ α-ketoglutarate + NH_4^+ + NAD(P)H	M
Isocitrate + $NAD(P)^+ \rightleftharpoons$ α-ketoglutarate + CO_2 + NAD(P)H + H^+	M and C

[*] These reactions and their enzymes are discussed in Chapters 14 through 18.
[†] M designates mitochondria; C, cytosol.