Electron Transport & Oxidative Phosphorylation

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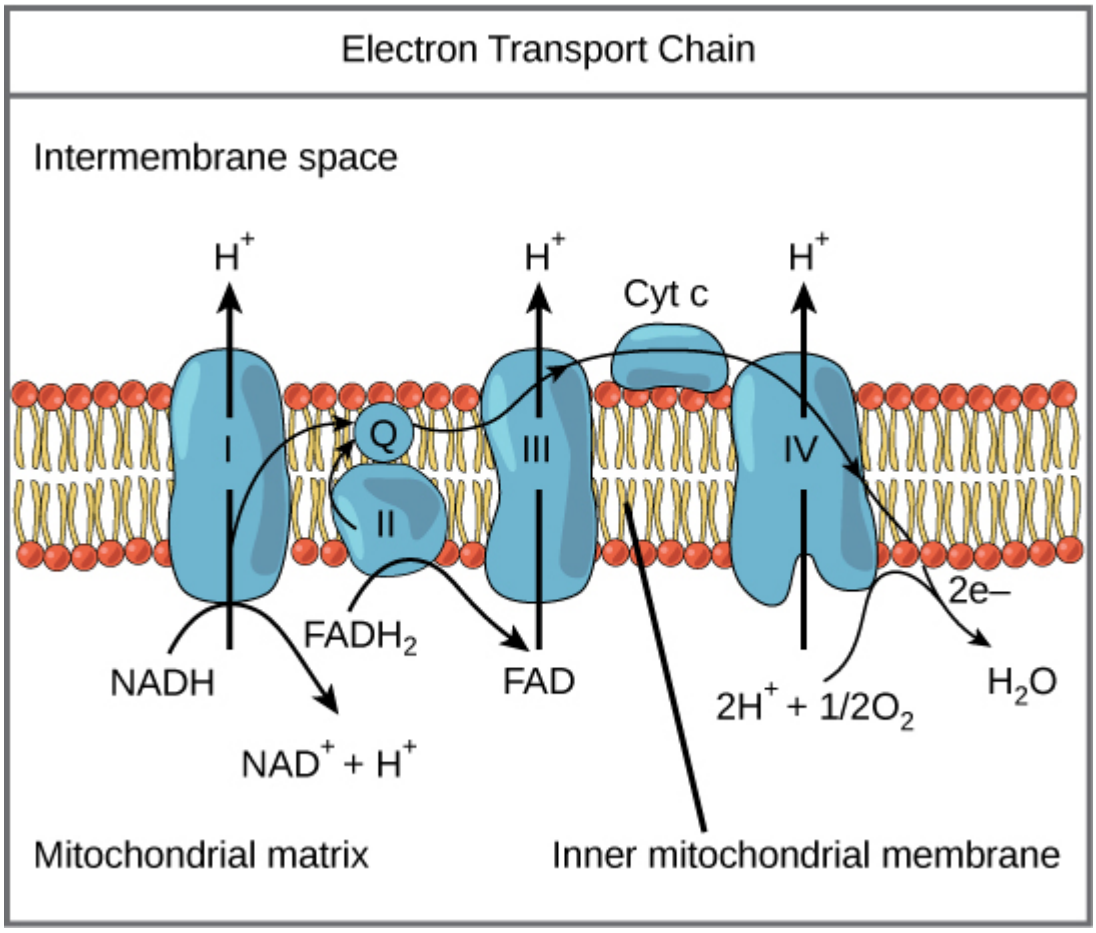
The complete aerobic catabolism of one molecule of glucose yields between 36 and 38 ATP; energy obtained mostly as the reduced coenzymes NADH and FADH2 are conveyed through the electron transport system. Three of the four respiratory complexes comprise the mitochondrial respiratory chain, as well as ATP synthase. The complexes are embedded in the inner mitochondrial membrane. Coenzyme Q and cytochrome c transfer electrons between complexes, which will ultimately meet oxygen, the terminal electron acceptor. Metabolic water is the product of oxygen reduction.



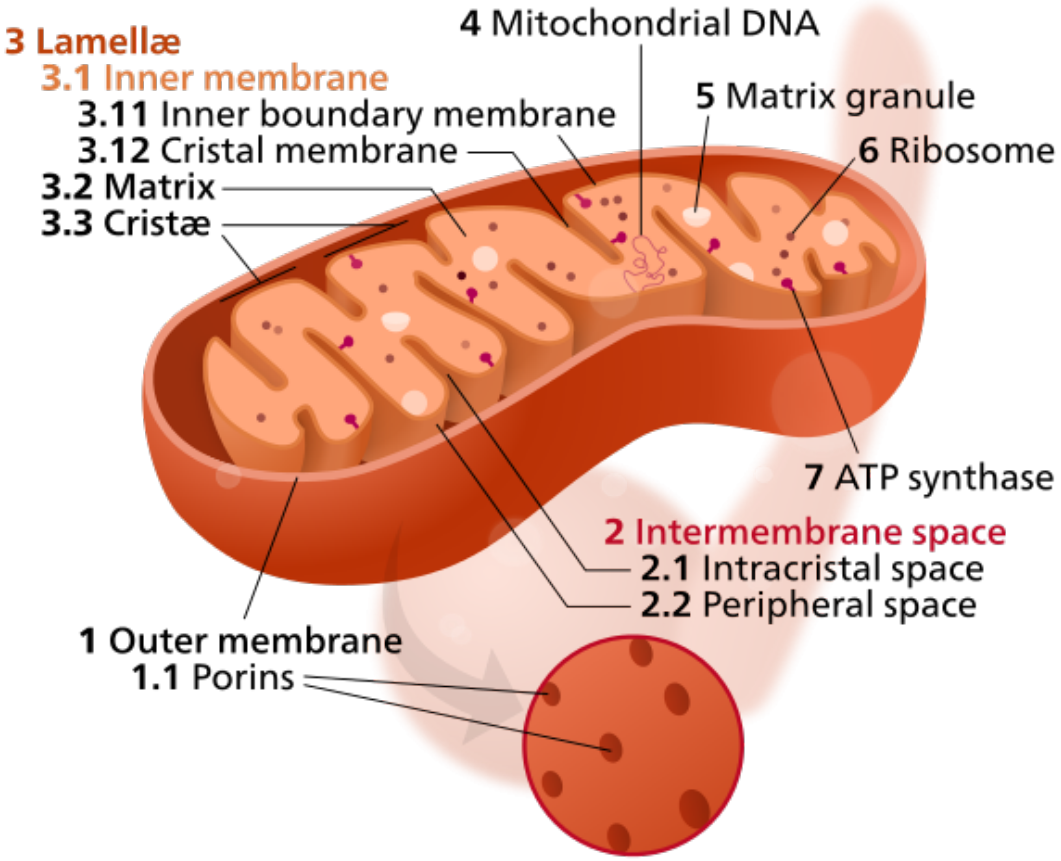
The Structure of Mitochondria

The inner and outer membranes surround the mitochondria. The inner membrane has invaginations, called cristae, which house respiratory complexes. These invaginations increase the inner membrane's surface area and effectively increase the mitochondria's respiratory capability. Many of the enzymes and intermediates of the tricarboxylic acid

(TCA) cycle and β-oxidation, which occur in the mitochondria matrix, are localized nearby. Other pathways, such as glycolysis, occur in the cytosol, and the nicotinamide adenine dinucleotide (NAD) + hydrogen (H) (NADH) produced there must be transported to the matrix in the form of reducing equivalents to participate in electron transport and adenosine triphosphate (ATP) synthesis via oxidative phosphorylation.

The mitochondrial outer membrane contains porins that permit diffusion of ions and metabolites, but the inner membrane is only freely impermeable to O2, CO2, and H2O. The passage of metabolites, such as ATP, adenosine diphosphate (ADP), Ca2+, and phosphate, is a process mediated by transport proteins, which permit the generation of ion gradients. 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 transported explicitly into the matrix as the newly synthesized ATP is transported out.

The inner membrane space (IM space) separates the inner membrane from the matrix and has a high concentration of protons. The passage of electrons through the chain provides the energy required to translocate protons against their concentration gradient.



Electron Transport

The inner mitochondrial membrane is mostly impermeable to molecules and ions, such as (H^+), except for rare species that pass this membrane using specialized carriers.

The mitochondrial matrix is made up of the pyruvate dehydrogenase complex and the citric acid cycle enzymes, the fatty acid *β*-oxidation pathway, and other pathways involved in amino acid oxidation.

The semipermeable inner mitochondrial membrane separates the intermediates and enzymes of the metabolic pathways of those in the cytosol from those occurring in the mitochondrial matrix. The inner mitochondrial membrane hosts cofactors that were reduced throughout catabolic pathways occurring in different cellular compartments. The inner membrane bears the compartment of the respiratory chain and the ATP synthase. Here, the conveying of electrons through three respiratory complexes is coupled to the outward pumping of protons into the intermembrane space.

Oxidative Phosphorylation- Two Thought Questions

[1] How many ATP are produced from the complete oxidation of 1mol of glucose to CO2 and H2O?

A net of two ATP is produced during glycolysis, where a six-carbon glucose molecule is anaerobically catabolized into two three-carbon pyruvate molecules. Two NAD^+ are reduced to NADH as well during this process.

These two pyruvate molecules are then used to produce two Acetyl-CoA molecules by pyruvate dehydrogenase, which produces one NADH each.

The two Acetyl-CoA molecules enter the TCA Cycle, where they condense with oxaloacetate and generate two GTP (which are converted to two ATP), six NADH, and two flavin adenine dinucleotide hydrogen, or $FADH_2$.

Each NADH enters the electron transport chain at complex I, where it is re-oxidized and passes its electrons to CoQ. Electrons flow from CoQ to complex III, which relays them through cytochrome c complex IV. Here, they are accepted by O_2. Both Complex I and Complex IV convey four protons each into the IM space, whereas complex III pumps two.

The six NADH produced during the TCA Cycle yield 60 protons in the IM space.

During glycolysis, two NADH are reduced, and two more generate during the pyruvate dehydrogenase reaction. When added to the 60 protons produced during TCA, there are 100 protons in the IM space.

Two $FADH_2$ molecules enter ETS through complex II, which does not pump protons into the IM space. Therefore, the two $FADH_2$, produced in the TCA cycle, account for 12 protons; accordingly, there are 112 protons in the IM space.

ATP synthase produces one ATP per three protons pumped from the IM space into the matrix, so the protons pumped during electron transport will generate 37 ATP. There were two ATP generated during glycolysis, and two produced in TCA (as GTP). Thus, glucose oxidation produces 41.3 ATP rather than 32.

Pi must be transported into the matrix, which requires a proton to accomplish. Also, two other contributing factors are that protons can exit through the ATPase, and some leak out of the F0 subunit.

It takes four protons to synthesize one ATP molecule. Dividing the total number of protons from NADH (100) and FADH2 (12) (=112) yields 28 ATP. Adding the four ATP produced as a result of glycolysis and TCA yields 32 ATP produced per molecule of glucose.

[2] Compare this to the amount produced during the complete oxidation of a C16 fatty acid, such as palmitic acid, or an odd-numbered fatty acid such as C17 margaric acid.

The C16 fatty acid is broken down through β-oxidation to eight acetyl-CoA molecules, which condense with oxaloacetate to form citrate. β-oxidation also produces 7 NADH and 7 $FADH_2$ molecules. As explained in the above example, one turn of the cycle produces 3 NADH, 1 $FADH_2$, and 1 GTP. The activation of palmitate to palmitoyl-CoA consumes two ATP equivalents as well. In all, C16 palmitoyl-CoA produces 31 NADH, 15 $FADH_2$, and 8 GTP. The subsequent electron transport of the coenzymes yields a total of 400 protons in the matrix (10 for each NADH, and 6 for each $FADH_2$. 400 total protons), yielding 100 ATP. Adding the 8 GTP that is produced and subtracting the two ATP for the activation of palmitate to palmitoyl-CoA yields 106 ATP produced for the complete oxidation of C16 palmitate.

Protons conveyed into the IM space as a result of oxidation of one molecule of palmitoyl-CoA to CO_2 and H_2O		
Enzyme catalyzing oxidation step	Number of NADH or $FADH_2$ formed	Number of protons ultimately translocated into IM Space
Acyl-CoA dehydrogenase	7 $FADH_2$	42
β-Hydroxyacyl-CoA dehydrogenase	7 NADH	70
Isocitrate dehydrogenase	8 NADH	80
α-Ketoglutarate dehydrogenase	8 NADH	80
Succinate dehydrogenase	8 $FADH_2$	48
Malate dehydrogenase	8 NADH	80
Total		400

A C17 fatty acid, such as margaric acid, will produce five fewer ATP because the product of its β-oxidation's last round is a C3 unit of propionyl-CoA. This unit enters the TCA cycle as succinyl-CoA. This means that it will not be involved in the isocitrate dehydrogenase and α-ketoglutarate dehydrogenase steps. As a result, it will convey 20 fewer protons into the IM space as a result.

Control of Oxidative Phosphorylation

An interlocking regulation mechanism controls the rate of glycolysis, the citric acid cycle, pyruvate oxidation, and oxidative phosphorylation by the relative concentrations of ATP, ADP, AMP, and NADH. Glycolysis, fatty acid degradation, and the TCA Cycle provide the primary sources of electrons that enter the mitochondrial electron-transport chain. Not surprisingly, control of glycolysis and the citric acid cycle is coordinated with the demand for oxidative phosphorylation. Oxidative phosphorylation is maintained by cellular energy requirements. The intracellular [ADP]and the [ATP] are measures of a cell's energy status. An adequate supply of electrons, which feeds the electron transport chain, is provided by regulation of the control points of glycolysis and the citric acid cycle (phosphofructokinase, pyruvate dehydrogenase, citrate synthase, isocitrate dehydrogenase, and α -ketoglutarate dehydrogenase) by NADH and/or certain metabolites.

The interlocking of the citric acid cycle and glycolysis by citrate, which hinders glycolysis, facilitates the mode of action of the adenine nucleotide system. Additionally, increased levels of NADH and acetyl-CoA hinders the oxidation of pyruvate to acetyl-CoA, and a relatively high [NADH]/ [NAD^+] ratio hinders the citric acid cycle's dehydrogenase reactions.

Interestingly, another important regulatory effect is citrate's inhibition of phosphofructokinase. ATP and ADP concentrations determine the electron transport rate through the respiratory series of metabolic reactions via a system of interlocking controls on glycolysis and the citric acid cycle. When the demand for ATP decreases, [ATP] increases, and [ADP] decreases. ADP activates isocitrate dehydrogenase, and ATP inhibits α -ketoglutarate dehydrogenase, so the citric acid cycle slows down. This causes the citrate concentration to build up. Citrate leaves the mitochondrion via a specific transport system and, once in the cytosol, further restrains the carbohydrate breakdown by inhibiting PFK.

The citrate concentration also builds up when the acetyl-CoA concentration increases, which occurs during fatty acid oxidation. The inhibition of glycolysis by fatty acid oxidation is called the **glucose–fatty-acid cycle.** The pathway allows fatty acids to be used as the primary fuel for oxidative metabolism in heart muscle while conserving glucose for organs that require it, such as the brain.

Several chemical compounds inhibit the electron transport chain at different points. Rotenone and Amytal inhibit complex I, Antimycin A at complex III, and CN^- blocks complex IV. 2,3-Dinitrophenol doesn't directly inhibit the chain but dissipates the proton gradient.

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Correct answers: 1B; 2A; 3B

Notes