Electron Transport & Oxidative Phosphorylation

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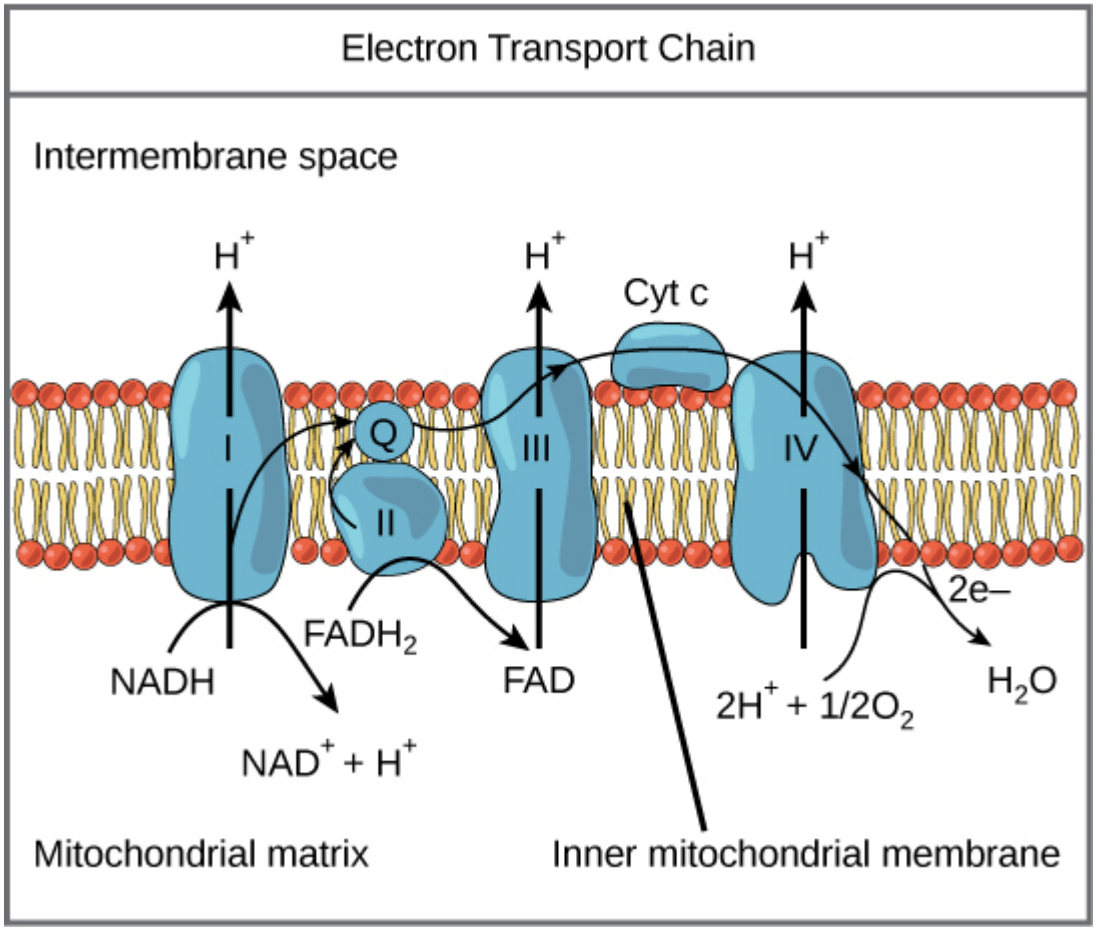
The complete aerobic catabolism of one molecule of glucose yields between 36 and 38 ATP; energy obtained mostly as the reduced coenzymes NADH and FADH2 are conveyed through the electron transport system. Three of the four respiratory complexes comprising the mitochondrial respiratory chain, as well as ATP synthase, 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.



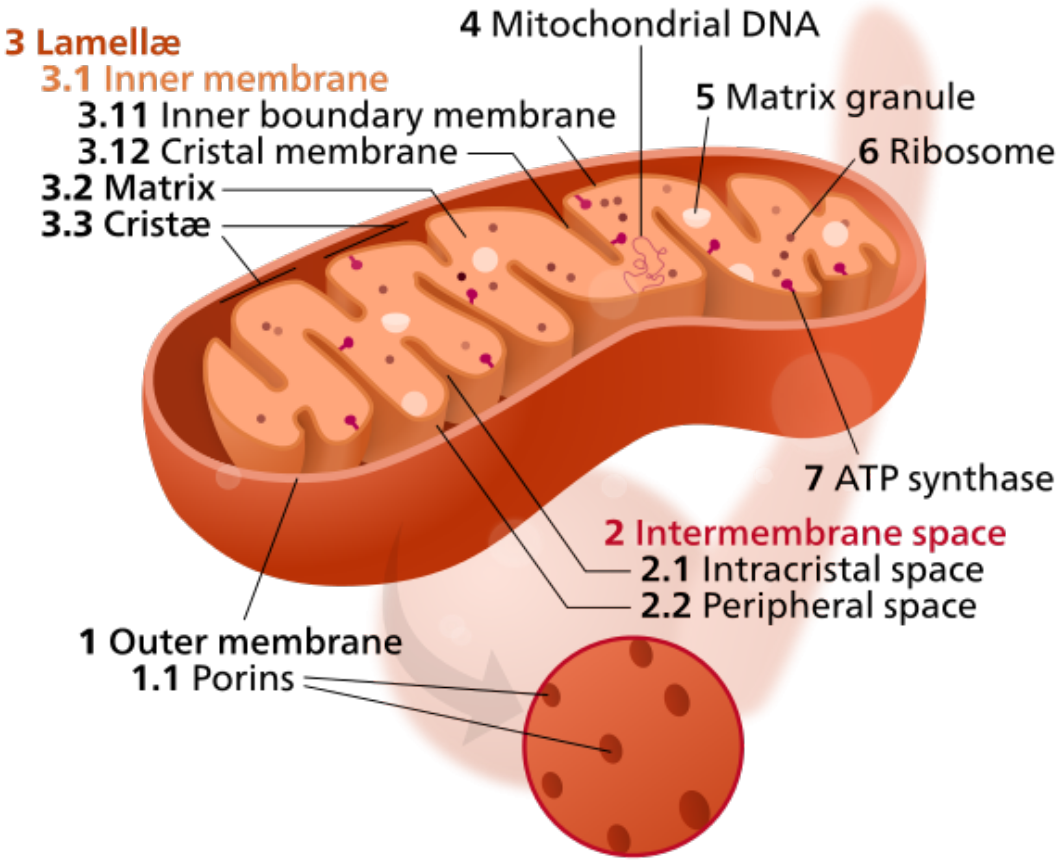
The Structure of Mitochondrion

The inner and outer membranes surround mitochondria, and the former possesses invaginations, called cristae, which house respiratory complexes. These infoldings increase the surface area of the inner membrane and effectively increase the respiratory

capability of mitochondria. Localized nearby are many of the enzymes and intermediates of the TCA Cycle and β-oxidation, which occur in the matrix of the mitochondria. Other pathways, for example, glycolysis, occur in the cytosol, and NADH produced there must be transported to the matrix in the form of reducing equivalents to participate in electron transport and 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. Passage of metabolites, such as ATP, 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 specifically transported into the matrix as newly synthesized ATP is transported out.

The inner membrane space (IM space) separates the inner membrane from the matrix and possesses a high concentration of protons. The passage of electrons through the chain provides the energy required to translocate protons against their concentration gradient; later as we discuss oxidative phosphorylation, the logic of this will become clear.



Electron Transport

The inner mitochondrial membrane is mostly impermeable to molecules and ions such as (H^+), with an exception of rare species that pass this membrane by means of specialized carriers.

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 metabolic pathways of those in the cytosol from those occurring in the mitochondrial matrix. The inner mitochondrial membrane plays host to 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

Earlier I discussed differential ATP production on electrons originating from NADH and $FADH_2$; which now presents us with an excellent opportunity to tie together several key concepts by exercising our knowledge of metabolism.

[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 1 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 $FADH_2$.

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 sixty protons in the IM space.

During glycolysis two NADH were reduced, and there were two more generated during the pyruvate dehydrogenase reaction; four total. When added to the sixty protons produced during TCA, we now have a total of one hundred 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 twelve protons. So far we have one hundred and twelve 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 thirty-seven ATP. There were two ATP generated during glycolysis, and two produced in TCA (as GTP). Now we can say that Glucose oxidation produces 41.3 ATP.

So, why do textbooks say that there are 32 produced and not 41.3?

Pi needs to be transported into the matrix, which requires a proton to accomplish. Also,

two other contributing factors are that the only point that protons can exit is the ATPase (some miss the bus because they are hanging out elsewhere), and some leak out of the F0 subunit.

If we now take the transport of Pi into consideration, it takes four protons to synthesize one ATP molecule. We divide the total number of protons from NADH (100) and FADH2 (12) (=112) to get 28 ATP produced. When we add the four ATP produced as a result of glycolysis and TCA, we get 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 a 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. Also produced during β-oxidation are 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 will produce 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. If you add in the 8 GTP that is produced and subtract 2 ATP for the activation of Palmitate to palmitoyl-CoA, you will get 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; which enters the TCA cycle as succinyl-CoA. This means that it will 'miss out' on the isocitrate dehydrogenase and α-ketoglutarate dehydrogenase steps, and convey twenty fewer protons into the IM space as a result.

Control of Oxidative Phosphorylation

Interlocking regulation mechanism exists to control the rate of glycolysis, the citric acid cycle, pyruvate oxidation and oxidative phosphorylation by the relative concentrations of ATP, ADP, and AMP, and by 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 requirement. The intracellular [ADP]and the [ATP] are measures of a cell's energy status. An adequate supply of electrons to feed 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.

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 dehydrogenase reactions of the citric acid cycle.

Interestingly, another important regulatory effect is the inhibition of phosphofructokinase by citrate. ATP and ADP concentrations determine the rate of electron transport 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. Because ADP activates isocitrate dehydrogenase and ATP inhibits α -ketoglutarate dehydrogenase, 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, acts to restrain further carbohydrate breakdown by inhibiting PFK.

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

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.

Review Questions

1. Which of the following would not increase as a result of a substantial increase in work performed by the cell?

A. The utilization of oxygen
B. The ratio of ATP to ADP in the cell
C. The rate of utilization of ADP by ATP synthase
D. The entrance of H^+into the mitochondrial matrix through ATP synthase
E. The pumping of H^+into the IM space of the mitochondria by the electron transport chain

2. Which of the following would not increase in rate as a consequence of subjecting the cell to Aspirin, T4 or some other chemical uncoupler of oxidative phosphorylation?

A. ATP synthesis
B. Proton pumping by the electron transport chain

C. Heat generation by the mitochondria
D. Oxygen utilization by the cell
E. NADH oxidation by NADH dehydrogenase

3. All of the following statements regarding electron transport in mitochondria are correct, except for:

A. It is located in the mitochondrion inner membrane.
B. Cytochrome c accepts electrons from complex II.
C. Cytochrome oxidase (complex IV) accepts electrons from Cytochrome c.
D. Complex I is called NADH dehydrogenase.
E. Coenzyme Q accepts electrons from complex I and complex II.

References

Boyer, P.D., Catalytic site forms and controls in ATP synthase catalysis, Biophys. Acta 1458, 252–262 (2000). [A description of the steps of ATP synthesis and hydrolysis, along with experimental evidence and alternative explanations, by the author of the binding change mechanism.]

Brzezinski, P. and Johansson, A.-L., Variable proton-pumping stoichiometry in structural variants of cytochrome c oxidase, Biophys. Acta 1797, 710–723 (2010). Crofts, A.R., The cytochrome bc1 complex: Function in the context of structure, Annu. Rev. Physiol. 66, 689–733 (2004).

Efremov, R.G., Baradaran, R., and Sazanov, L.A., The architecture of respiratory complex I, Nature 465, 441–445 (2010).

Frey, T.G. and Mannella, C.A., The internal structure of mitochondria, Trends Biochem. Sci. 23, 319–324 (2000).

Goodsell, D.S., Mitochondrion, Mol. Biol. Educ. 38, 134–140 (2010). [An illustrated guide to the mitochondrion.]

Hinkle, P.C., P/O ratios of mitochondrial oxidative phosphorylation, Biophys. Acta 1706, 1–11 (2005).

Hosler, J.P., Ferguson-Miller, S., and Mills, D.A., Energy transduction: Proton transfer through the respiratory complexes, Rev. Biochem. 75, 165–187 (2006). [A review that focuses on cytochrome c oxidase.]

Johnson, D.C., Dean, D.R., Smith, A.D., and Johnson, M.K., Structure, function, and formation of biological iron–sulfur clusters, Rev. Biochem. 74, 247–281 (2005) Kühlbrandt, W., Bacteriorhodopsin—the movie, Nature 406, 569–570 (2000). Lanyi, J.K., Bacteriorhodopsin, Annu. Rev. Physiol. 66, 665–688 (2004).

Nicholls, D.G. and Ferguson, S.J., Bioenergetics 3, Academic Press (2002). [An authoritative monograph devoted almost entirely to the mechanism of oxidative phosphorylation and the techniques used to elucidate it.]

Noji, H. and Yoshida, M., The rotary machine in the cell, ATP synthase, Biol. Chem. 276, 1665–1668 (2001).

Pebay-Peyroula, E., Dahout-Gonzalez, C., Kahn, R., Trézéguet, V., Lauquin, G.J.-M., and Brandolin, G., Structure of mitochondrial ADP/ATP carrier in complex with carboxyatractyloside, Nature 426, 39–44 (2003).

Schultz, B.E. and Chan, S.I., Structures and proton-pumping strategies of mitochondrial respiratory enzymes, Rev. Biophys. Biomol. Struct. 30, 23–65 (2001).

Correct answers: 1B; 2A; 3B

Notes