Oxidative Phosphorylation

Introduction:

Oxidative phosphorylation is the culmination of a series of energy transformations that are called *cellular respiration* or simply *respiration* in their entirety. Oxidative phosphorylation is a highly efficient method of producing large amounts of ATP, the basic unit of energy for metabolic processes. During this process electrons are exchanged between molecules, which creates a chemical gradient that allows for the production of ATP. The most vital part of this process is the electron transport chain, which produces more ATP than any other part of cellular respiration. In oxidative phosphorylation the oxidation of catabolic intermediates by molecular oxygen occurs via a highly ordered series of substances that act as hydrogen and electron carriers. They constitute the electron transfer system, or respiratory chain. In most animals, plants, and fungi, the electron transfer system is fixed in the membranes of mitochondria; in bacteria (which have no mitochondria) this system is incorporated into the plasma membrane. Sufficient free energy is released to allow the synthesis of ATP by a process described below. However, it is necessary to consider the nature of the respiratory chain. First, carbon fuels are oxidized in the citric acid cycle to yield electrons with high transfer potential. Then, this electron-motive force is converted into a proton-motive force and, finally, the proton-motive force is converted into phosphoryl transfer potential. The conversion of electron-motive force into proton-motive force is carried out by three electron-driven proton pumps—NADH-Q oxidoreductase, Q-cytochrome *c* oxidoreductase, and cytochrome *c* oxidase. These large transmembrane complexes contain multiple oxidation-reduction centers, including quinones, flavins, iron-sulfur clusters, hemes, and copper ions. The final phase of oxidative phosphorylation is carried out by *ATP synthase,* an ATP-synthesizing assembly that is driven by the

flow of protons back into the mitochondrial matrix. Components of this remarkable enzyme rotate as part of its catalytic mechanism. Oxidative phosphorylation vividly shows that *proton gradients are an interconvertible currency of free energy in biological systems.*

Oxidative phosphorylation is conceptually simple and mechanistically complex. Indeed, the unraveling of the mechanism of oxidative phosphorylation has been one of the most challenging problems of biochemistry. The flow of electrons from NADH or $FADH_2$ to O_2 through protein complexes located in the mitochondrial inner membrane leads to the pumping of protons out of the mitochondrial matrix. The resulting uneven distribution of protons generates a pH gradient and a transmembrane electrical potential that creates a *proton-motive force*. ATP is synthesized when protons flow back to the mitochondrial matrix through an enzyme complex. Thus, *the oxidation of fuels and the phosphorylation of ADP are coupled by a proton gradient across the inner mitochondrial membrane*.

Why do we need oxygen?

You, like many other organisms, need oxygen to live. As you know if you've ever tried to hold your breath for too long, lack of oxygen can make you feel dizzy or even black out, and prolonged lack of oxygen can even cause death. But have you ever wondered why that's the case, or what exactly your body does with all that oxygen?

As it turns out, the reason you need oxygen is so your cells can use this molecule during oxidative phosphorylation, the final stage of cellular respiration. Oxidative phosphorylation is made up of two closely connected components: the electron transport chain and chemiosmosis. In the electron transport chain, electrons are passed from one molecule to another, and energy released in these electron transfers is used to form

an electrochemical gradient. In chemiosmosis, the energy stored in the gradient is used to make ATP.

So, where does oxygen fit into this picture? Oxygen sits at the end of the electron transport chain, where it accepts electrons and picks up protons to form water. If oxygen isn't there to accept electrons (for instance, because a person is not breathing in enough oxygen), the electron transport chain will stop running, and ATP will no longer be produced by chemiosmosis. Without enough ATP, cells can't carry out the reactions they need to function, and, after a long enough period of time, may even die.

Cellular Redox Systems :

Reactive oxygen species (ROS) are products of normal metabolism and xenobiotic exposure, and depending on their concentration, ROS can be beneficial or harmful to cells and tissues. At physiological low levels, ROS function as "redox messengers" in intracellular signaling and regulation, whereas excess ROS induce oxidative modification of cellular macromolecules, inhibit protein function, and promote cell death. Additionally, various redox systems, such as the Glutathione, Thioredoxin, and Pyridine nucleotide redox couples, participate in cell signaling and modulation of cell function, including apoptotic cell death. Cell apoptosis is initiated by extracellular and intracellular signals via two main pathways, the death receptor- and the mitochondria-mediated pathways. Various pathologies can result from oxidative stress-induced apoptotic signaling that is consequent to ROS increases and/or antioxidant decreases, disruption of intracellular redox homeostasis, and irreversible oxidative modifications of lipid, protein, or DNA. In this review, we focus on several key aspects of ROS and redox mechanisms in apoptotic signaling and highlight the gaps in knowledge and potential

avenues for further investigation. A full understanding of the redox control of apoptotic initiation and execution could underpin the development of therapeutic interventions targeted at oxidative stress-associated disorders.

Oxidation-reduction status (redox) is an important regulator of various metabolic functions of the cell. Perturbations in the redox status of cells by external or internal stimuli elicit distinct responses, resulting in alteration of cell function. Glutathione and thioredoxin are two major reducing systems of the eukaryotic cell that maintain redox balance, as well as interact with various transducer and effector molecules to bring about specific responses. However, these two systems differ greatly in their functions and responses to various types of stress. Oxidative stress profoundly impacts them both by direct or indirect oxidation of sulfhydryl groups. Glutathione is a small tripeptide with a single cysteine residue that undergoes oxidation-reduction. Thioredoxin (Trx) is an approximately 12-kD protein that contains five cysteine residues (two catalytic and three structural). These cysteines undergo oxidation-reduction reactions in response to oxidants or reductants in the environment.

Mitochondria are the main intracellular location for fuel generation; however, they are not just power plants but involved in a range of other intracellular functions including regulation of redox homeostasis and cell fate. Dysfunction of mitochondria will result in oxidative stress which is one of the underlying causal factors for a variety of diseases including neurodegenerative diseases, diabetes, cardiovascular diseases, and cancer.

They are also involved in many other cellular functions including redox homeostasis maintenance. Mitochondria are the major sites for free radical species production, including both reactive oxygen species (ROS) and reactive nitrogen species (RNS). On one hand, free radical species are indispensable for proper cell signaling; on the other hand,

excessive generation of ROS results in cell/tissue injury and death. Since mitochondria are major sources for ROS production, it is not surprising that they are well equipped with antioxidant defenses, including a large pool of glutathione, glutathione peroxidase, glutathione reductase, MnSOD, catalase, and the thioredoxin system.

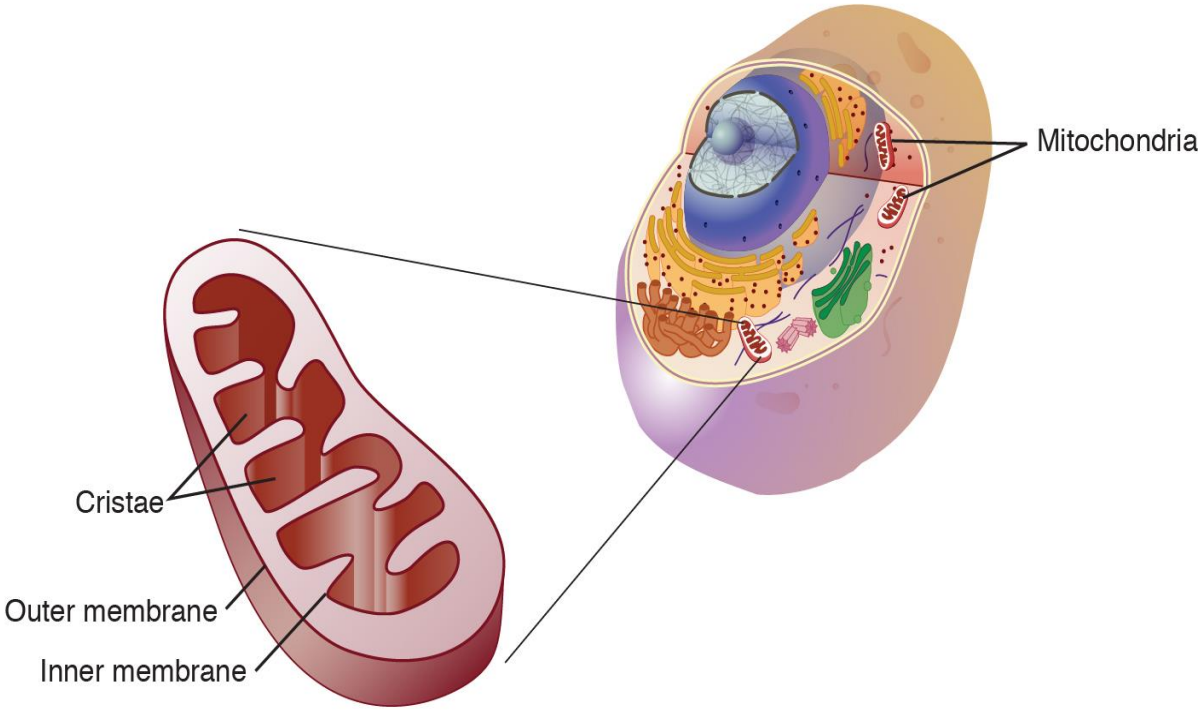
Although excessive levels of ROS will lead to protein oxidation and lipid peroxidation causing damage to mitochondrial membrane, proteins, and DNA, especially when the mitochondrial DNA is not protected with associated histones, lower levels of ROS have been demonstrated to be essential signaling molecules. A new concept is now emerging that mitochondrial ROS production is likely to be highly regulated as a part of physiological mitochondrial functions and the underlying molecular mechanisms are being gradually uncovered [7]. In this paper, a few mitochondrial proteins that act as redox regulators will be discussed as examples, including the antiapoptotic protein Bcl-2, cytochrome c oxidase (COX), and the small GTPase Rac1.

Mitochondria :

Mitochondria are membrane-bound cell organelles (mitochondrion, singular) that generate most of the chemical energy needed to power the cell's biochemical reactions. Chemical energy produced by the mitochondria is stored in a small molecule called adenosine triphosphate(ATP). Mitochondria contain their own small chromosomes. Generally, mitochondria, and therefore mitochondrial DNA, are inherited only from the mother.

Mitochondria are membrane-bound organelles, but they're membrane-bound with two different membranes. And that's quite unusual for an intercellular organelle. Those membranes function in the purpose of mitochondria, which is essentially to produce energy. That energy is produced by having chemicals within the cell go through pathways, in other words, be converted. And the process of that conversion produces energy in the form of ATP, because the phosphate is a high-energy bond

and provides energy for other reactions within the cell. So the mitochondria's purpose is to produce that energy. Some different cells have different amounts of mitochondria because they need more energy. So for example, the muscle has a lot of mitochondria, the liver does too, the kidney as well, and to a certain extent, the brain, which lives off of the energy those mitochondria produce. So if you have a defect in the pathways that the mitochondria usually functions with, you're going to have symptoms in the muscle, in the brain, sometimes in the kidneys as well; many different types of symptoms. And we probably don't know all of the different diseases that mitochondrial dysfunction causes.



Mitochondrial Respiratory Chain :

The **mitochondrial respiratory chain** (**electron transport chain)** is a series of proteins and organic molecules found in the inner membrane of the mitochondria. Electrons are passed from one member of the transport chain to another in a series of redox reactions. Energy released in these reactions is captured as a proton gradient, which is then used to make ATP in a process called **chemiosmosis**. Together, the electron transport chain and chemiosmosis make up **oxidative phosphorylation**. The key steps of this process, shown in simplified form in the diagram above, include:

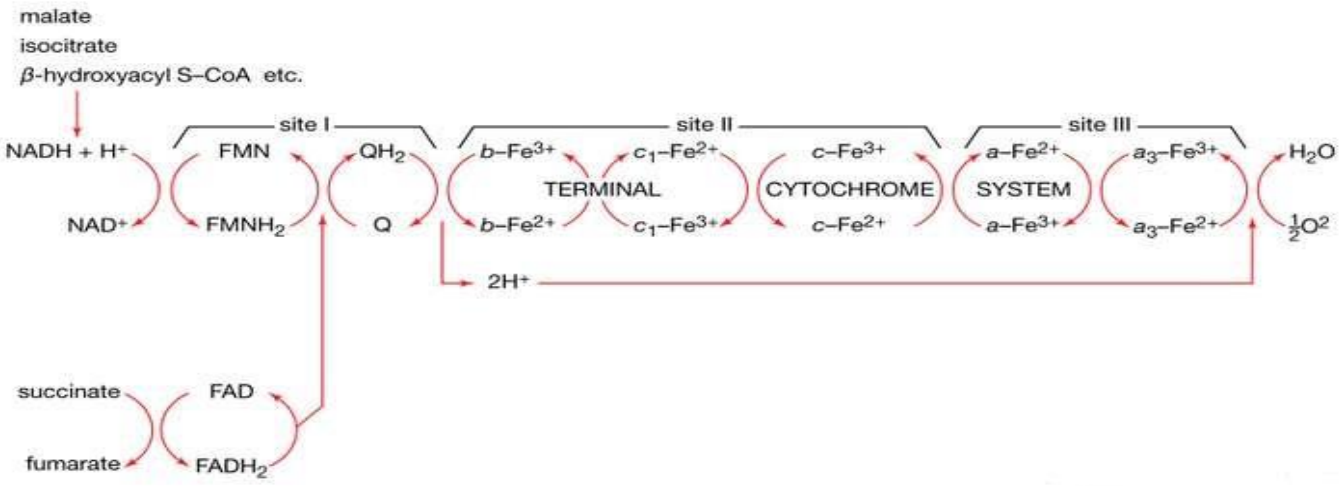
- **Delivery of electrons by NADH and $FADH_2$.** Reduced electron carriers (NADH and $FADH_2$) from other steps of cellular respiration transfer their electrons to molecules near the beginning of the transport chain. In the process, they turn back into NAD^+ and FAD, which can be reused in other steps of cellular respiration.
- **Electron transfer and proton pumping.** As electrons are passed down the chain, they move from a higher to a lower energy level, releasing energy. Some of the energy is used to pump H^+ ions, moving them out of the matrix and into the intermembrane space. This pumping establishes an electrochemical gradient.
- **Splitting of oxygen to form water.** At the end of the electron transport chain, electrons are transferred to molecular oxygen, which splits in half and takes up H^+ to form water.
- **Gradient-driven synthesis of ATP.** As H^+ ions flow down their gradient and back into the matrix, they pass through an enzyme called ATP synthase, which harnesses the flow of protons to synthesize ATP.

Four types of hydrogen or electron carriers are known to participate in the respiratory chain, in which they serve to transfer two reducing equivalents (2H) from reduced substrate (AH_2) to molecular oxygen; the products are the oxidized substrate (A) and water (H_2O).

The carriers are NAD^+ and the flavoproteins FAD and FMN (flavin mononucleotide); ubiquinone (or coenzyme Q); and several types of cytochromes. Each carrier has an oxidized and reduced form (e.g., FAD and $FADH_2$, respectively), the two forms constituting an oxidation-reduction, or redox, couple. Within the respiratory chain, each redox couple undergoes cyclic oxidation-reduction; i.e., the oxidized component of the couple accepts reducing equivalents from either a substrate or a reduced carrier preceding it in the series and in turn donates these reducing equivalents to the next oxidized carrier in the sequence. Reducing equivalents are thus transferred from substrates to molecular oxygen by a number of sequential redox reactions.

Most oxidizable catabolic intermediates initially undergo a dehydrogenation reaction, during which a dehydrogenase enzyme transfers the equivalent of a hydride ion ($H^+ + 2e^-$, with e^- representing an electron) to its coenzyme, NAD^+. The reduced NAD^+ thus produced (usually written as $NADH + H^+$ or $NADPH + H^+$) diffuses to the membrane-bound respiratory chain to be oxidized by an enzyme known as NADH dehydrogenase; the enzyme has as its coenzyme FMN. A few substrates (e.g., acyl coenzyme A and succinate) bypass this reaction and instead undergo immediate dehydrogenation by specific membrane-bound dehydrogenase enzymes. During the reaction, the coenzyme FAD accepts two hydrogen atoms and two electrons ($2H + 2e^-$). The reduced flavoproteins (i.e., $FMNH_2$ and $FADH_2$) donate their two hydrogen atoms to the lipid carrier ubiquinone, which is thus reduced.

The fourth type of carrier, the cytochromes, consists of hemoproteins—i.e., proteins with a nonprotein component, or prosthetic group, called heme (or a derivative of heme), which is an iron-containing pigment molecule. The iron atom in the prosthetic group is able to carry one electron and oscillates between the oxidized, or ferric (Fe^{3+}), and the reduced, or ferrous (Fe^{2+}), forms. The five cytochromes present in the mammalian respiratory chain, designated cytochromes *b*, c_1, *c*, *a*, and a_3, act in sequence between ubiquinone and molecular oxygen. The terminal cytochrome of this sequence (a_3, also known as cytochrome oxidase) is able to donate electrons to oxygen rather than to another electron carrier; a_3 is also the site of action of two substances that inhibit the respiratory chain, potassium cyanide and carbon monoxide. Special Fe-S complexes play a role in the activity of NADH dehydrogenase and succinate dehydrogenase. In each redox couple, the reduced form has a tendency to lose reducing equivalents (i.e., to act as an electron or hydrogen donor); similarly, the oxidized form has a tendency to gain reducing equivalents (i.e., to act as an electron or hydrogen acceptor). The oxidation-reduction characteristics of each couple can be determined experimentally under well-defined standard conditions.



The **electron transport chain** is the final component of aerobic respiration and is the only part of glucose metabolism that uses atmospheric oxygen. Electron transport is a series of redox reactions that resemble a relay race. Electrons are passed rapidly from one component to the next to the endpoint of the chain, where the electrons reduce molecular oxygen, producing water. The electron transport chain is a collection of membrane-embedded proteins and organic molecules, most of them organized into four large complexes labeled I to IV. In eukaryotes, many copies of these molecules are found in the inner mitochondrial membrane. In prokaryotes, the electron transport chain components are found in the plasma membrane.

As the electrons travel through the chain, they go from a higher to a lower energy level, moving from less electron-hungry to more electron-hungry molecules. Energy is released in these "downhill" electron transfers, and several of the protein complexes use the released energy to pump protons from the mitochondrial matrix to the intermembrane space, forming a proton gradient. A complex is a structure consisting of a central atom, molecule, or protein weakly connected to surrounding atoms, molecules, or proteins. The electron transport chain is an aggregation of four of these complexes (labeled I through IV), together with associated mobile electron carriers. The electron transport chain is present in multiple copies in the inner mitochondrial membrane of eukaryotes and the plasma membrane of prokaryotes.

All of the electrons that enter the transport chain come from NADH and $FADH_2$ molecules produced during earlier stages of cellular respiration: glycolysis, pyruvate oxidation, and the citric acid cycle.

- **NADH** is very good at donating electrons in redox reactions (that is, its electrons are at a high energy level), so it can transfer its electrons directly to complex I, turning back into NAD^++start superscript, plus, end superscript. As electrons move through complex I in a series of redox reactions, energy is released, and the complex uses this energy to pump protons from the matrix into the intermembrane space.
- **$FADH_2$** is not as good at donating electrons as NADH (that is, its electrons are at a lower energy level), so it cannot transfer its electrons to complex I. Instead, it feeds them into the transport chain through complex II, which does not pump protons across the membrane. Because of this "bypass," each $FADH_2$ molecule causes fewer protons to be pumped (and contributes less to the proton gradient) than an NADH.

Complex I

To start, two electrons are carried to the first complex aboard NADH. Complex I is composed of flavin mononucleotide (FMN) and an enzyme containing iron-sulfur (Fe-S). FMN, which is derived from vitamin B_2 (also called riboflavin), is one of several prosthetic groups or co-factors in the electron transport chain. A prosthetic group is a non-protein molecule required for the activity of a protein. Prosthetic groups can be organic or inorganic and are non-peptide molecules bound to a protein that facilitate its function.

Prosthetic groups include co-enzymes, which are the prosthetic groups of enzymes. The enzyme in complex I is NADH dehydrogenase, a very large protein containing 45 amino acid chains. Complex I can pump four hydrogen ions across the membrane from the matrix into the intermembrane space; it is in this way that the hydrogen ion gradient is established and maintained between the two compartments separated by the inner mitochondrial membrane.

Ubiquinone (Q) and Complex II

Complex II directly receives $FADH_2$, which does not pass through complex I. The compound connecting the first and second complexes to the third is ubiquinone (Q). The Q molecule is lipid soluble and freely moves through the hydrophobic core of the membrane. Once it is reduced to QH_2, ubiquinone delivers its electrons to the next complex in the electron transport chain. Q receives the electrons derived from NADH from complex I and the electrons derived from $FADH_2$ from complex II, including succinate dehydrogenase. This enzyme and $FADH_2$ form a small complex that delivers electrons directly to the electron transport chain, bypassing the first complex. Since these electrons bypass, and thus do not energize, the proton pump in the first complex, fewer ATP molecules are made from the $FADH_2$ electrons. The number of ATP molecules ultimately obtained is directly proportional to the number of protons pumped across the inner mitochondrial membrane.

Complex III

The third complex is composed of cytochrome b, another Fe-S protein, Rieske center (2Fe-2S center), and cytochrome c proteins; this complex is also called cytochrome oxidoreductase. Cytochrome proteins have a prosthetic heme group. The heme molecule is similar to the heme in hemoglobin, but it carries electrons, not oxygen. As a result, the iron ion at its core is reduced and oxidized as it passes the electrons, fluctuating between different oxidation states: Fe^{2+} (reduced) and Fe^{3+} (oxidized). The heme molecules in the cytochromes have slightly different characteristics due to the effects of the different proteins binding them, which makes each complex. Complex III pumps protons through the membrane and passes its electrons to cytochrome c for transport to the fourth complex of proteins and enzymes. Cytochrome c is the acceptor of electrons from Q; however, whereas Q carries pairs of electrons, cytochrome c can accept only one at a time.

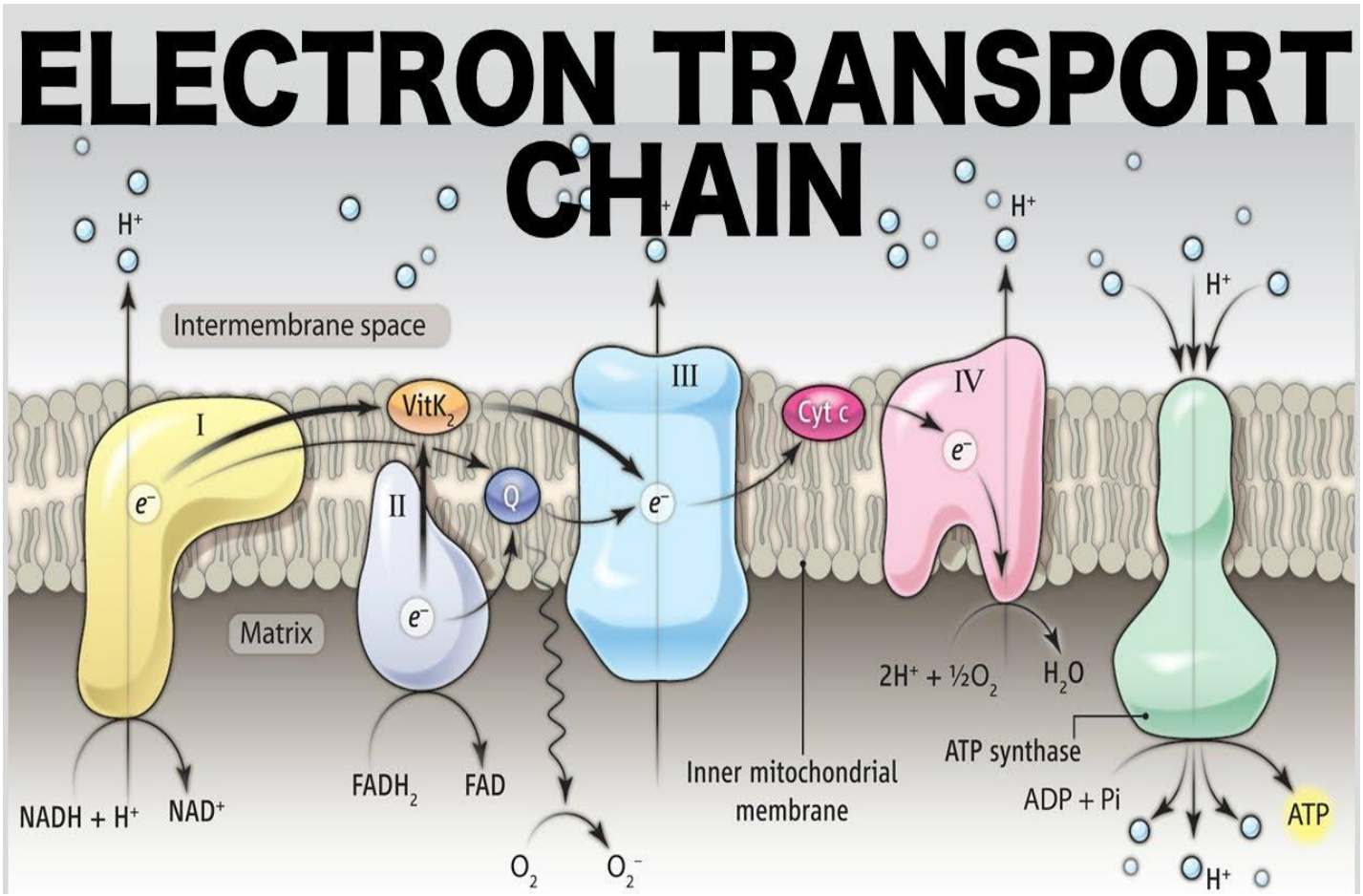
Complex IV

The fourth complex is composed of cytochrome proteins c, a, and a_3. This complex contains two heme groups (one in each of the cytochromes a and a_3) and three copper ions (a pair of Cu_A and one Cu_B in cytochrome a_3). The cytochromes hold an oxygen molecule very tightly between the iron and copper ions until the oxygen is completely reduced. The reduced oxygen then picks up two hydrogen ions from the surrounding medium to produce water (H_2O). The removal of the hydrogen ions from the system also contributes to the ion gradient used in the process of chemiosmosis.

Beyond the first two complexes, electrons from NADH and $FADH_2$ travel exactly the same route. Both complex I and complex II pass their electrons to a small, mobile electron carrier called **ubiquinone** (**Q**), which is reduced to form QH_2 and travels through the membrane, delivering the electrons to complex III. As electrons move through complex III, more H^+ ions are pumped across the membrane, and the electrons are ultimately delivered to another mobile carrier called **cytochrome C** (**cyt C**). Cyt C carries the electrons to complex IV, where a final batch of H^+ ions is pumped across the membrane. Complex IV passes the electrons to O_2, which splits into two oxygen atoms and accepts protons from the matrix to form water. Four electrons are required to reduce each molecule of O_2, and two water molecules are formed in the process.

Overall, what does the electron transport chain do for the cell? It has two important functions:

- **Regenerates electron carriers.** NADH and $FADH_2$ pass their electrons to the electron transport chain, turning back into NAD^+ and FAD. This is important because the oxidized forms of these electron carriers are used in glycolysis and the citric acid cycle and must be available to keep these processes running.
- **Makes a proton gradient.** The transport chain builds a proton gradient across the inner mitochondrial membrane, with a higher concentration of H^+ in the intermembrane space and a lower concentration in the matrix. This gradient represents a stored form of energy and it can be used to make ATP.



Chemiosmosis (Chemiosmotic Theory):

Chemiosmosis is the movement of ions across a selectively permeable membrane, down their electrochemical gradient. During chemiosmosis, electron carriers like NADH and FADH donate electrons to the electron transport chain. The electrons cause conformation changes in the shapes of the proteins to pump H+ across a selectively permeable cell membrane. The uneven distribution of H^+ ions across the membrane establishes both concentration and electrical gradients (thus, an electrochemical gradient) owing to the hydrogen ions' positive charge and their aggregation on one side of the membrane. If the membrane were open to diffusion by the hydrogen ions, the ions would tend to spontaneously diffuse back across into the matrix, driven by their electrochemical gradient. However, many ions cannot diffuse through the nonpolar regions of phospholipid membranes without the aid of ion channels. Similarly, hydrogen ions in the matrix space can only pass through the inner mitochondrial membrane through a membrane protein called ATP synthase. This protein acts as a tiny generator turned by the force of the hydrogen ions diffusing through it, down their electrochemical gradient. The turning of this molecular machine harnesses the potential energy stored in the hydrogen ion gradient to add a phosphate to ADP, forming ATP. Chemiosmosis is used to generate 90 percent of the ATP made during aerobic glucose catabolism. The production of ATP using the process of chemiosmosis in mitochondria is called oxidative phosphorylation. It is also the method used in the light reactions of photosynthesis to harness the energy of sunlight in the process of photophosphorylation. The overall result of these reactions is the production of ATP from the energy of the electrons removed from hydrogen atoms. These atoms were originally part of a glucose molecule. At the end of the pathway, the electrons are used to reduce an oxygen molecule to oxygen ions. The extra electrons on the oxygen attract hydrogen ions (protons) from the surrounding medium and water is formed.

Complexes I, III, and IV of the electron transport chain are proton pumps. As electrons move energetically downhill, the complexes capture the released energy and use it to pump H^+ ions from the matrix to the intermembrane space. This pumping forms an electrochemical gradient across the inner mitochondrial membrane. The gradient is sometimes called the **proton-motive force**, and you can think of it as a form of stored energy, kind of like a battery.

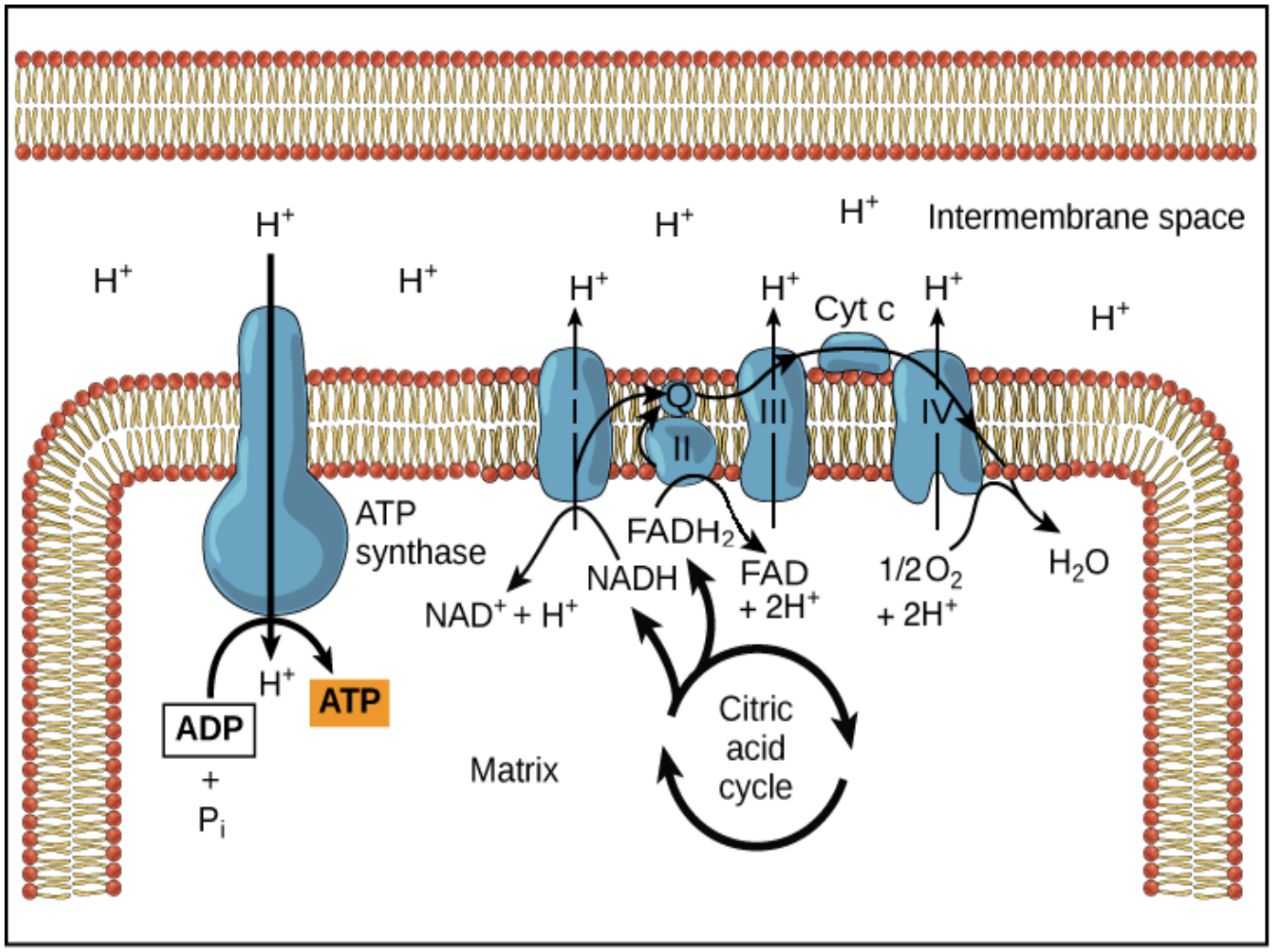
Like many other ions, protons can't pass directly through the phospholipid bilayer of the membrane because its core is too hydrophobic. Instead, H^+ ions can move down their concentration gradient only with the help of channel proteins that form hydrophilic tunnels across the membrane.

In the inner mitochondrial membrane, H^+ ions have just one channel available: a membrane-spanning protein known as **ATP synthase**. Conceptually, ATP synthase is a lot like a turbine in a hydroelectric power plant. Instead of being turned by water, it’s turned by the flow of H^+ ions moving down their electrochemical gradient. As ATP synthase turns, it catalyzes the addition of a phosphate to ADP, capturing energy from the proton gradient as ATP.

This process, in which energy from a proton gradient is used to make ATP, is called **chemiosmosis**. More broadly, chemiosmosis can refer to any process in which energy stored in a proton gradient is used to do work. Although chemiosmosis accounts for over 80% of ATP made during glucose breakdown in cellular respiration, it’s not unique to cellular respiration. For instance, chemiosmosis is also involved in the light reactions of photosynthesis.

What would happen to the energy stored in the proton gradient if it weren't used to synthesize ATP or do other cellular work? It would be

released as heat, and interestingly enough, some types of cells deliberately use the proton gradient for heat generation rather than ATP synthesis. This might seem wasteful, but it's an important strategy for animals that need to keep warm. For instance, hibernating mammals (such as bears) have specialized cells known as brown fat cells. In the brown fat cells, **uncoupling proteins** are produced and inserted into the inner mitochondrial membrane. These proteins are simply channels that allow protons to pass from the intermembrane space to the matrix without traveling through ATP synthase. By providing an alternate route for protons to flow back into the matrix, the uncoupling proteins allow the energy of the gradient to be dissipated as heat.



ATP synthesis in mitochondria :

In order to understand the mechanism by which the energy released during respiration is conserved as ATP, it is necessary to appreciate the structural features of mitochondria. These are organelles in animal and plant cells in which oxidative phosphorylation takes place. There are many mitochondria in animal tissues—for example, in heart and skeletal muscle, which require large amounts of energy for mechanical work, and in the pancreas, where there is biosynthesis, and in the kidney, where the process of excretion begins. Mitochondria have an outer membrane, which allows the passage of most small molecules and ions, and a highly folded inner membrane (crista), which does not even allow the passage of small ions and so maintains a closed space within the cell. The electron-transferring molecules of the respiratory chain and the enzymes responsible for ATP synthesis are located in and on this inner membrane, while the space inside (matrix) contains the enzymes of the TCA cycle. The enzyme systems primarily responsible for the release and subsequent oxidation of reducing equivalents are thus closely related, so that the reduced coenzymes formed during catabolism ($NADH + H^+$ and $FADH_2$) are available as substrates for respiration. The movement of most charged metabolites into the matrix space is mediated by special carrier proteins in the crista that catalyze exchange-diffusion (i.e., a one-for-one exchange). The oxidative phosphorylation systems of bacteria are similar in principle but show a greater diversity in the composition of their respiratory carriers.

The mechanism of ATP synthesis appears to be as follows. During the transfer of hydrogen atoms from $FMNH_2$ or $FADH_2$ to oxygen, protons (H^+ ions) are pumped across the crista from the inside of the mitochondrion to the outside. Thus, respiration generates an electrical potential (and in mitochondria a small pH gradient) across the membrane corresponding to 200 to 300 millivolts, and the chemical energy in the substrate is converted into

electrical energy. Attached to the crista is a complex enzyme (ATP synthetase) that binds ATP, ADP, and P_i. It has nine polypeptide chain subunits of five different kinds in a cluster and a unit of at least three more membrane proteins composing the attachment point of ADP and P_i. This complex forms a specific proton pore in the membrane. When ADP and P_i are bound to ATP synthetase, the excess of protons (H^+) that has formed outside of the mitochondria (an H^+ gradient) moves back into the mitochondrion through the enzyme complex. The energy released is used to convert ADP and P_i to ATP. In this process, electrical energy is converted to chemical energy, and it is the supply of ADP that limits the rate of this process. The precise mechanism by which the ATP synthetase complex converts the energy stored in the electrical H^+ gradient to the chemical bond energy in ATP is not well understood. The H^+ gradient may power other endergonic (energy-requiring) processes besides ATP synthesis, such as the movement of bacterial cells and the transport of carbon substrates or ions.

How many ATP do we get per glucose in cellular respiration? The most current sources estimate that the maximum ATP yield for a molecule of glucose is around 30-32 ATP. This range is lower than previous estimates because it accounts for the necessary transport of ADP into, and ATP out of, the mitochondrion.

Where does the figure of 30-32 ATP come from? Two net ATP are made in glycolysis, and another two ATP (or energetically equivalent GTP) are made in the citric acid cycle. Beyond those four, the remaining ATP all come from oxidative phosphorylation. Based on a lot of experimental work, it appears that four H^+ ions must flow back into the matrix through ATP synthase to power the synthesis of one ATP molecule. When electrons from NADH move through the transport chain, about 10 H^+ ions are pumped from the matrix to the intermembrane space, so each NADH yields about 2.5 ATP. Electrons

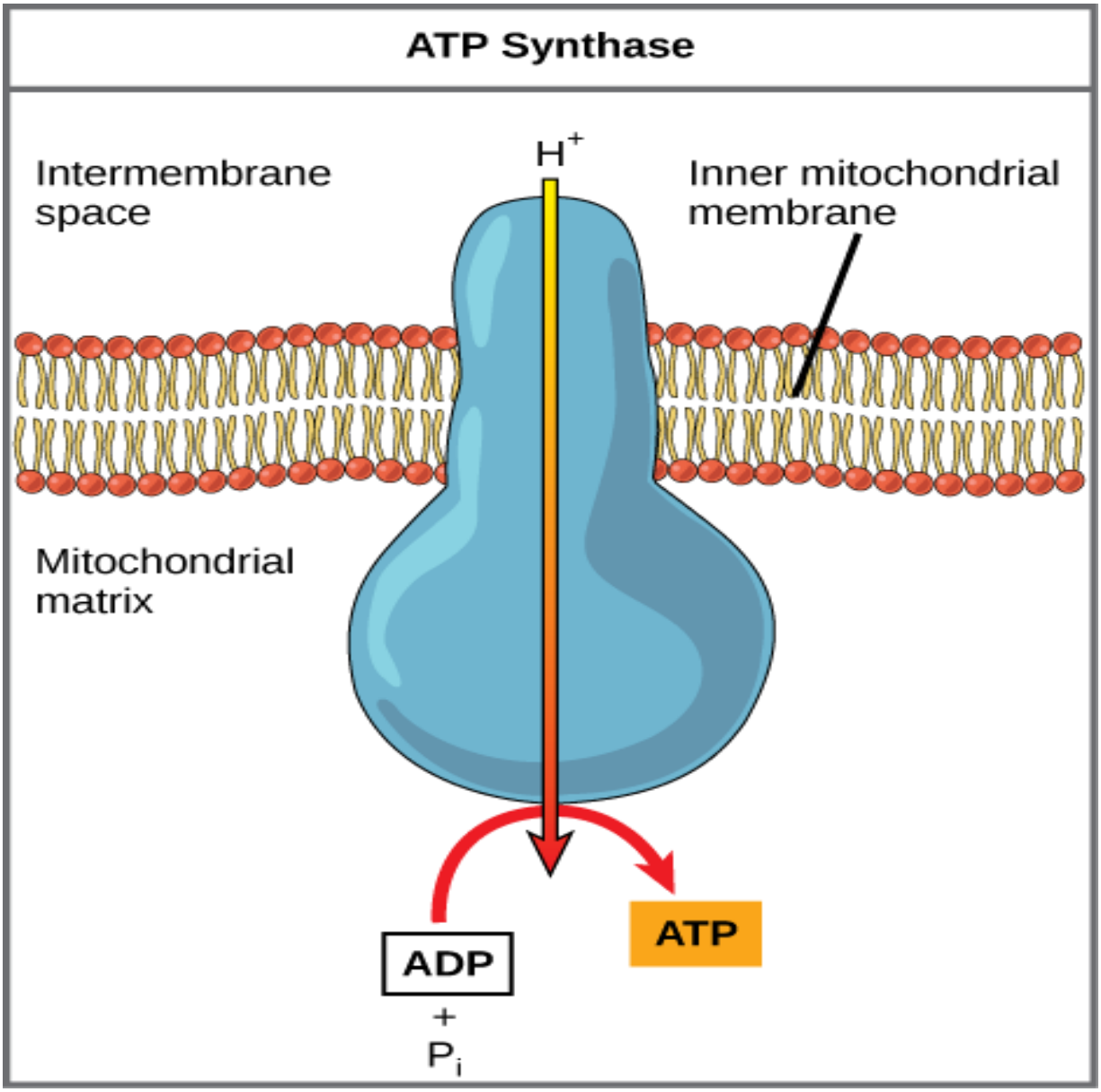
from $FADH_2$, which enter the chain at a later stage, drive pumping of only 6 H^+, leading to production of about 1.5 ATP.

ATP Synthase:

ATP is synthesized from its precursor, ADP, by ATP synthases. These enzymes are found in the cristae and the inner membrane of mitochondria. ATP synthesis is the most widespread chemical reaction inside the biological world. ATP synthase is the very last enzyme in oxidative phosphorylation pathway that makes use of electrochemical energy to power ATP synthesis. ATP synthase is one of the most ubiquitous and plentiful protein on the earth, accountable for the reversible catalysis of ATP to ADP and Pi. The mitochondrial ATP synthase is a multi-subunit protein complex having an approximate molecular weight of 550 kDa. The human mitochondrial ATP synthase or F1/ F0 ATPase or complex V is the fifth component of oxidative phosphorylation chain. This enzyme is the smallest known biological nanomotor and plays a crucial role in ATP generation.

The ATP synthase, also called Complex V, has two major subunits designated F0 and F1. The F0 part, bound to inner mitochondrial membrane is involved in proton translocation, whereas the F1 part found in the mitochondrial matrix is the water soluble catalytic domain. F1 is the first factor recognized and isolated from bovine heart mitochondria and is involved in oxidative phosphorylation. It was named so from the term ‘Fraction 1’. F0 was named so as it is a factor that conferred oligomycin sensitivity to soluble F1. The structure of enzyme ATP synthase mimics an assembly of two motors with a shared common rotor shaft and stabilized by a peripheral stator stalk. The F1 part of ATP synthase is made up of 8 subunits, 3α, 3β, γ, δ and ε, where the γ, δ and ε subunits add up to the central stalk (or the rotor shaft) and an alternate arrangement of 3α and 3β form a hexameric ring with a central cavity. The γ subunit inserted in the central cavity protrudes out to meet ε which binds on its side and together they bind the F0. Eukaryotic F0 has

several subunits including d, F6 and the oligomycin sensitivity-conferring protein (OSCP). Subunits b, d, F6 and OSCP form the peripheral stalk, which connect both F1 and F0 and keep the stators (F1-αβ3 and F0a) from spinning along with the rotor (γδε and F0c). Other additional subunits such as subunit e, f, g, and A6L extending over the membrane cohort with F0



Inhibitors of Electron Transport Chain:

- Inhibitors are the inhibiting agents. They bind with the specific electron carriers.
- The inhibitors bind to different components of the electron transport chain and block the carrier. After binding with the carrier it does not allow to change in a reversible form from an oxidized state to a reduced state. It leads to and this results in the accumulation of reduced forms prior to the inhibitor point, and oxidized forms of the components of the ETC down the line of inhibition point.
- The synthesis of ATP stops due to cease of energy release. The most important known inhibitors of the ETC are Amytal, Rotenone, Antimycin A, CO, Sodium Azide, and Cyanides.
- Site specific inhibitors of ETC have been identified. These compounds prevent the transfer of electrons by binding to a various complex of the chain which blocks the redox reactions.
- ROTENONE: Rotenone is a plant product. Rotenone is extracted from roots of plant Derris elliptica. It is an insecticide. It is a strong inhibitor of complex I of the electron transport chain. It inhibits the transport of electron through the NADH-CoQ reductase complex. Certain tribes use it as a fish poison which paralyse the fish.
- Amobarbital (Amytal) : Amytal is a barbiturate. It inhibits the transport of electron through the NADH-CoQ reductase complex.
- Demerol: Demerol also inhibits complex I.
- Piericidin A: It is an antibiotic of microbial origin. Its mode of action is similar to Rotenone.

- Antimycins: It is an antibiotic from Streptomyces species. It blocks the electron flow at complex III of electron transport chain. It inhibits the flow of electrons from cytochrome b to cytochrome c1.
- Cyanide: It is a respiratory inhibitor which blocks the complex IV of the electron transport chain. It blocks at cytochrome oxidase which prevents both coupled and uncoupled respiration. Cyanide binds with iron within this protein complex and prevent the regular activity of the complex system. It blocks the transport of electron to oxygen which stops the further passage of electron through the electron transport chain. As a result, the person is deprived of energy to carry out the many numerous processes that sustain life and the person dies.
- Carbon monoxide: It is a respiratory inhibitor which blocks the complex IV of the electron transport chain. It binds with the cytochrome oxidase (Complex IV) which blocks the transfer of electrons to oxygen. Carbon monoxide binds with the reduced form of iron in the hem groups (Fe++) in Cytochrome Oxidase (Complex IV).
- Azide: It is a respiratory inhibitor which blocks the complex IV of the electron transport chain. It blocks the electron flow between the cytochrome oxidase complex and oxygen. It reacts with the ferric form (Fe3+) of the complex IV of electron transport chain.
- Hydrogen Sulphide: It is a respiratory inhibitor which blocks the complex IV of the electron transport chain. It is toxic. Inhibitors of ATP synthase complex (Phosphorylation inhibitor)
- These inhibitors prevent the synthesis of ATP by binding to the ATP synthase complex. It prevents the inflow of protons. Example: Oligomycin and dicyclo hexyl carbo diimide (DCCD). o Oligomycin is polypeptide in nature act as an antibiotic which is obtained from the Streptomyces species. It stops the transfer of

high energy phosphate group to adenine diphosphate (ADP). Oligomycin binds to a 23kd polypeptide in the F0 base plate and blocks ATP synthesis by the F0 /F1 ATPase. It prevents respiration in mitochondria and all ATP-driven functions in sub-mitochondrial particle. Diclyclohexyl carbo diimide (DCCD): It makes covalent bonds with amino acid glutamate of the c subunit of F0. When it binds covalently, it stops the proton channel, which prevents the formation ATP synthesis.

Uncouplers of Electron Transport Chain:

- In intact mitochondria and in special preparations of sub mitochondrial particles, the transport of electrons and the phosphorylation of ADP are tightly coupled reactions.
- In damaged mitochondria, respiration (i.e., electron transport) may occur unaccompanied by oxidative phosphorylation. When this happens the mitochondria are said to be uncoupled.
- Chemical agents which uncouple the electron transport with ATP synthesis is known as uncouplers of electron transport chain. Such agents will stop the synthesis of ATP but transport of electron will continues. Example: Dintrophenol
- These uncoupling agents are lipid-soluble. These agents bind with the hydrogen ions and transport them across the membranes. During electron transport hydrogen ions are pumped across the mitochondrial membrane but these uncoupling agents carries it back to the mitochondria. It will stop the generation of proton gradient. It will stop the synthesis of ATP by oxidative phosphorylation. Rather the energy derived from electron transport is released as heat.

- Energy derived from electron transport is released as heat during uncoupling. Such kind of heat generation is known as nonshivering thermogenesis. It is vital in many biological situations. For example, uncoupling occurs naturally in brown adipose tissue. The inner mitochondrial membranes of such tissue contain a protein called thermogenin (uncoupling protein). Thermogenin is an endogenous protein found in the brown adipose tissue. Thermogenin act as an uncoupler which uncouples the ATP synthesis from ETC by creating a passive proton pump (UCP-1) with in the inner mitochondrial membrane. E.g. Such tissues found in new born animals and hibernating animals. These uncouplers have hydrophobic nature which makes them soluble in the lipid bilayer of membrane. These uncouplers have dissociable protons which allow them to bring protons from the inter membrane space to the matrix. It will disintegrate pH gradient. This energy lost as heat.

- Examples of uncouplers:

  Chemical Uncouplers - 2,4-Dinitrophenol, dicumarol , CCCP (carbonyl cyanide m-chloro phenyl hydrazone) and FCCP (p-trifluoromethoxy carbonyl cyanide phenyl hydrazone). CCCP is a lipid-soluble weak acid. CCCP is a very potent mitochondrial uncoupling agent.

  Physiological uncouplers - It includes long chain fatty acids, Thyroxin, Thermogenin (protein), Calcium ions.