Published in final edited form as:

Prog Mol Biol Transl Sci. 2014 ; 123: 103–125. doi:10.1016/B978-0-12-397897-4.00001-2.

Modeling Mitochondrial Function and Its Role in Disease

M. Saleet Jafri* and Rashmi Kumar†

*School of Systems Biology and Department of Molecular Neuroscience, George Mason University, Fairfax, VA, USA

†School of Systems Biology, George Mason University, Fairfax, VA, USA

Abstract

Many neurodegenerative diseases involve defects in mitochondrial function. These defects often arise from mutations carried in the genes that code mitochondrial proteins. Many of these defective proteins are involved in mitochondrial energy metabolism which is one of the primary roles of the mitochondria. However, others proteins have other roles that are related to signaling or mitochondrial structure. The interaction of the mitochondrial proteins is complex. Understanding these complex dynamics requires the use of computational models. Studies have started to exploit such models, but much further work is necessary to understand mitochondrial function and its role in neurodegenerative disease.

1. INTRODUCTION

Mitochondria are intracellular organelles that have multiple roles within cells. The mitochondria process metabolites to make ATP (adenosine triphosphate), the energy currency used by the cell, as is the case with cardiac muscle. In addition to this role, the mitochondria have a signaling role in cells. For example, they serve an essential role as a glucose sensor in pancreatic β -cells. Mitochondria also play a crucial role in apoptosis: programmed cell death. Finally, mitochondrial dysfunction has been implicated in a number of pathologies, including many neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. These diverse roles result from the complexity of the interaction of mitochondrial components which often cannot be directly measured by current experimental techniques. Hence, computational modeling of mitochondria has become an important tool to understand mitochondrial function. The complex physiology of the mitochondria and how modeling can be used to understand it will be discussed in this chapter.

Mitochondria are thought to have originated as a separate organism that was internalized early in evolution in a eukaryotic cell. Based on evolutionary and phylogenetic studies, many suggest that it was a protobacteria; however, there are some recent suggestions that it might have been a eukaryote.¹ As such, the mitochondria have an inner and outer membrane and mitochondrial DNA. The volume between the inner and outer membrane is the intermembrane space. The mitochondrial matrix is found within the inner membrane. Proteins in the mitochondrial matrix help to carry out mitochondrial function. The inner membrane forms tubular and lamellar structures called cristae. While the outer membrane is quite porous, the inner membrane is not. Instead, the large inner membrane surface area houses proteins that serve as transporters, exchangers, and ion channels that control the flow

of ions, metabolites, and signaling molecules across it. The cristae structures are also not static, changing under different physiological conditions and during disease (including neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease), suggesting that there are functional consequences of these structural changes.² In the following sections, some of the facets of mitochondrial function are described in more detail.

The approximately 1100 proteins that comprise the mitochondria arise from genes both in the nuclear and mitochondrial DNA. In most organisms, only a small number of mitochondrial proteins are encoded by the mitochondrial genome; whereas most are encoded by genes in the nucleus and transported to the mitochondria via the cytosol. The mitochondrial genome is small consisting of 37 genes. Of these, 13 code for proteins involved in the respiratory chain and oxidative phosphorylation and the remainder code for translation and transcription of the mitochondrial genome, RNA processing, protein import, and protein maturation.^{1,3} These genes would follow a maternal inheritance pattern as the mitochondria in the oocyte all come from the mother. The remaining polypeptides that comprise the mitochondria come from the nuclear genome. These would be contributed by both parents. Tables 5.1 shows the mitochondrial genes involved in neurodegenerative disease and the effect they have on mitochondrial function. Table 5.2 shows the nuclear genes involved in neurodegenerative diseases and the effect they have on mitochondrial function.

2. ENERGY METABOLISM

One of the primary functions of the mitochondria is catabolic energy metabolism; that is, substrates, such as carbohydrates, fatty acids, and proteins, are broken down to release energy that is stored in high-energy phosphate bonds in molecules such as ATP and CP (creatine phosphate). This occurs in multiple stages by multiple pathways. (1) The tricarboxylic acid (TCA) cycle breaks down small carbohydrates (acetyl-CoA and TCA cycle intermediates) to produce reducing equivalents, that store the released energy. (2) There are also pathways that bring energy substrates into the TCA cycle. (3) The reducing equivalents enter oxidative phosphorylation pathway to become ATP the energy substrate of the cell.

2.1. Tricarboxylic acid cycle

The TCA cycle is a set of eight catalyzed reactions and eight intermediates that break down hydrocarbon substrates into carbon dioxide (CO₂) and water (H₂O) using the energy released to protonate nicotinamide adenine dinucleotide converting from NAD⁺ to NADH or flavin adenine dinucleotide from FADH to FADH₂ (Fig. 5.1). Acetyl-CoA enters the TCA cycle to combine with oxaloacetate in a reaction catalyzed by citrate synthase to form citrate. Citrate is converted to isocitrate by aconitase liberating water. ISO dehydrogenase catalyzes the next reaction converting isocitrate to α-ketoglutarate while reducing NAD⁺ to NADH. This enzyme is activated by [Ca²⁺] and inhibited by [NADH] and succinyl-CoA (SCoA). The α-ketoglutarate dehydrogenase enzyme complex converts α-ketoglutarate to SCoA reducing NAD⁺ in the process. This enzyme is activated by mitochondrial [Ca²⁺] and [ADP] and inhibited by [NADH]. SCoA becomes succinate through a reaction catalyzed by succinate-CoA ligase while forming guanosine triphosphate or ATP. Succinate

dehydrogenase transforms succinate into fumarate and reduces FADH to FADH₂. This is followed by the conversion of fumarate to malate by fumarase. Completing the cycle, malate dehydrogenase creates oxaloacetate and NADH from malate and NAD⁺ in a reaction inhibited by oxaloacetate.

As mentioned before, there are other biochemical pathways that allow the entry of substrate as intermediates of the TCA cycle. The cycle is split by a reaction catalyzed by aspartate amino transferase that converts oxaloacetate and glutamate to α -ketoglutarate and aspartate. In the brain under certain conditions, the top and bottom halves of the TCA cycle have actually been observed to occur at different rates.¹⁰

2.2. Substrate entry into the TCA cycle

There are several pathways by which different substrates enter the TCA cycle. For example, most carbohydrates are converted to glucose through digestion and postprocessing in the liver. The glucose absorbed by cells via the glucose transporter enters the glycolysis pathway in the cell cytoplasm to become pyruvate. Pyruvate enters the mitochondria via membrane transporters where it is metabolized by the pyruvate dehydrogenase complex to make acetyl-CoA. Pyruvate dehydrogenase complex is activated by mitochondrial calcium concentration ([Ca²⁺]) and inhibited when the [ATP]/[ADP] ratio, [NAD⁺]/[NADH] ratio, or [acetyl-CoA]/[CoA] ratio is increased. Acetyl-CoA enters the tricarboxylic acid cycle (also known as the TCA cycle, Krebs cycle, or the citric acid cycle).

Fatty acids in the cytoplasm are activated through a reaction catalyzed by fatty acid acyl-CoA synthetase and are then transported into the mitochondria across the inner membrane by a carnitine carrier system. Once in the mitochondrial matrix, β -oxidation of fatty acids catabolize the fatty acid into acetyl-CoA which then can enter the TCA cycle.

Proteins that are broken down into amino acids can enter the TCA cycle at different points depending upon the amino acid. Some glucogenic amino acids are deaminated and converted to glucose through gluconeogenic pathways. The glucose then enters glycolysis to form pyruvate and then acetyl-CoA. Other ketogenic amino acids are converted to acetyl-CoA or α -ketoglutarate which can enter the TCA cycle directly at different points of the cycle.

There are other anapleurotic pathways where substrates can enter the TCA cycle at additional loci. For example, pyruvate can be converted to oxaloacetate by pyruvate carboxylase found in mitochondria, aspartate can be converted to oxaloacetate through the transaminase reaction, glutamate can be converted to α -ketoglutarate by glutamate dehydrogenase, β -oxidation of fatty acids can result in SCoA, as well as others.

2.3. Oxidative phosphorylation

The reducing equivalents NADH and FADH₂ enter the electron transport chain to undergo a process called oxidative phosphorylation (Fig. 5.2). The steps of the electron transport chain are a series of reactions involving NADH-coenzyme Q oxidoreductase (Complex I), Succinate-Q oxidoreductase (Complex II), Electron transfer flavoprotein-Q oxidoreductase, Q-cytochrome c oxidoreductase (Complex III), Cytochrome c oxidase (Complex IV). This is

followed by a final step that makes ATP by the F_1F_0 -ATPase (Complex V). These protein complexes are found in the inner membrane distributed between the cristae membrane and the remaining inner membrane. In yeast, 60% of these proteins are in the cristae membrane while in cardiac mitochondria 94% are found in the cristae.² Note that the TCA cycle proteins are also clustered in a metabolon associated with the matrix side of the inner membrane so that isocitrate dehydrogenase can interact with Complex I and succinate dehydrogenase can interact with Complex II (Fig. 5.3).

Complex I is coupled to isocitrate dehydrogenase and is the point where NADH enters the electron transport chain. The electron released when NADH is oxidized is passed through the complex resulting in the translocation or pumping of four protons (H^+) across the inner membrane out of the mitochondrial matrix. Ubiquinone (coenzyme Q10 or Q) is also reduced to ubiquinol (QH_2) as it gains two protons.

Complex II is coupled to succinate dehydrogenase and is the entry point for $FADH_2$ into the electron transport chain. $FADH_2$ is oxidized and the two protons are used to reduce ubiquinone.

In Complex III, the ubiquinol is oxidized to reform ubiquinone. Four protons are extruded from the mitochondrial matrix across the inner membrane in this step. Energy stored as cytochrome C is reduced in this step.

In Complex IV, cytochrome C is oxidized to pump four protons across the inner membrane. Water is created in this step from protons and oxygen. This is the step that requires oxygen.

During electron transport, the respiration-driven proton pumps (the four steps) create a proton gradient across the mitochondrial inner membrane as well as a mitochondrial membrane potential (ψ_m). The membrane potential is extremely negative ranging from -160 to -180 mV. The proton gradient has been estimated to be between 0.1 and 0.5 pH units depending on the tissue type and conditions. This electrochemical gradient is then used by the F_1F_0 -ATPase (ATP synthase) to make ATP. As protons enter through a pore in the ATP synthase, ATP is created from ADP and free inorganic phosphate. The typical ratio is three protons for one ATP molecule created, although this might vary under different conditions. The ATP synthase is thought to be activated by mitochondrial matrix calcium.

2.4. Substrate transport

In order for energy metabolism to work effectively, substrates, ADP, and phosphate need to enter the mitochondria and ATP needs to exit. To this end, there exists a series of transport proteins in the form of carrier and shuttles. The ATP/ADP translocase, located in the inner membrane, transports ATP out of the matrix and ADP into the matrix. As ATP^{4-} and ADP^{3-} have different charges there is a net charge transfer during the translocation process. This net movement of charge generates an ionic current that dissipates some of the mitochondrial membrane potential. Phosphate is transported into the mitochondrial matrix by the phosphate carrier that exchanges phosphate and hydroxide (OH^-) so that there is no net charge carried (electroneutral). As OH^- exits the matrix when phosphate enters, this dissipates some of the proton gradient.

Substrates enter the mitochondria through a number of pathways such as the malate–aspartate shuttle, the glutamine carrier, and the tricarboxylate carrier protein. The former exchanges malate for aspartate bringing malate into the matrix and extruding aspartate. The other two are unidirectional carriers of substrate.

2.5. Ionic and substrate homeostasis

As the mitochondria need to maintain homeostasis of its internal environment, the fluxes of ions and substrates across the inner membrane need to be balanced with the consumption or source fluxes. For example, the phosphate carrier flux must balance the consumption rate of phosphate by the F_1F_0 -ATPase or the rate of ADP consumed or ATP produced needs to match the flux transported by the ATP/ADP translocase.

This is also true of ions such as protons (H^+), calcium (Ca^{2+}), and sodium (Na^+). The number of protons extruded by the respiration-drive proton pumps in the electron transport chain need to be balance by the entry through the F_1F_0 -ATPase and the amount neutralizing the OH^- brought in by the phosphate carrier. This is complicated by proton buffering in the mitochondrial matrix.

This also extends to calcium which has thus far been presented as a regulator of energy metabolism. Calcium enters the mitochondrial matrix through the mitochondrial calcium uniporter (MCU). This ion channel open in response to extramitochondrial calcium (the outer membrane is leaky so the intermembrane space effectively has extramitochondrial calcium levels) and calcium enters driven by the electrochemical gradient created by the large mitochondrial membrane potential. The entry of positive charge generates a depolarizing current that partially dissipates the mitochondrial membrane potential. Calcium is buffered in the matrix by binding to proteins, ATP, and ADP. This calcium influx is balanced by the mitochondrial sodium–calcium (lithium) exchanger (NCLX) which extrudes one calcium ion for three sodium ions. This net charge difference creates a current that also helps to depolarize the mitochondrial membrane potential. The question of how much calcium is sequestered in the mitochondria and the resulting change in mitochondria is a topic of current debate. The interpretation of experiments from different groups suggest either little uptake of calcium by the mitochondria with a <100 nm change in mitochondrial $[Ca^{2+}]$ or a more significant contribution where mitochondrial $[Ca^{2+}]$ can change several micromolar.

3. MITOCHONDRIAL SIGNALING

The mitochondria also produce signaling molecules that have targets in the mitochondria as well as in the cell. One example of this is reactive oxygen species (ROS). ROS include superoxide (O_2^-), hydrogen peroxide (H_2O_2), and peroxynitrite ($ONOO^-$). Superoxide is created as byproduct of electron transport at Complex I and Complex III. Less than 1% of the electron transport flux results in the production of superoxide. It is highly reactive; hence, the mitochondria have a defense against it in the form of superoxide dismutase (SOD) which converts superoxide into hydrogen peroxide. In fact, the amount of mitochondrial superoxide dismutase is 4 times that found in the cytoplasm of heart. The hydrogen peroxide is converted to water and oxygen through the reaction with glutathions

(GSH) to form glutathione disulfide (GSSG). The glutathione disulfide is reduced to glutathione by glutathione reductase replenishing the antioxidant system.

ROS play a signaling role in the cell acting upon many targets. For example, ROS does oxidative damage to DNA which is considered to be one of the mechanisms of aging. It can also damage lipids and proteins. On the other hand, it can serve as a reversible signaling molecule. For example, ROS target the ryanodine receptor, a calcium release channel in the ER and increases its open probability which might have implications for calcium signaling.

The mitochondria are also a target of signaling molecules from the cell. In the discussion above, calcium signaling in the cell cytoplasm can result in a rise in mitochondrial calcium that can stimulate energy metabolism. Increases in cytosolic ADP levels can translate into increases of mitochondrial ADP and stimulate energy metabolism. Hence, if the cell is active (lots of calcium signaling) and consuming ATP (increase in ADP) the mitochondria can respond by increasing energy metabolism.

Another example of the mitochondria being a target of intracellular signaling is apoptosis. Apoptosis is a complex signaling set of pathways that can be triggered through a variety of cell stress stimuli. This results in the induction of apoptosis and cell death. One of these pathways targets mitochondrial function. Typically, apoptosis seems to be regulated by the balance between proapoptotic factors such as Bax, Bid, Bad, and Bak and anti-apoptotic pathways such as the Bcl-2 protein family in the mitochondrial outer membrane. However, there are different pathways that alter this balance. When the balance is shifted toward apoptosis there is an increase in mitochondrial permeability that allows efflux of small mitochondria-derived activators of caspases (SMAC) and cytochrome c. SMAC binds to and deactivates mitochondrial inhibitor proteins allowing apoptosis to proceed. Cytochrome c binds with another protein apoptotic protease activating factor (APAF) to form the apoptosome which then bind to caspase-9 which starts the cascade to apoptosis.

4. MITOCHONDRIA IN DISEASE

The two main types of neurological diseases that involve mitochondria are ischemia and neurodegenerative diseases.

4.1. Ischemic disease

Ischemia is the deprivation of tissue of oxygen and metabolic substrate. This is in contrast to hypoxia which is simply depriving the tissue of oxygen. In the case of oxygen deprivation Complex IV activity ceases. The result is that all the cytochrome c remains in the reduced state. This impairs Complex III and prevents the replenishment of ubiquinone from ubiquinol. Without ubiquinone, Complex I and II cannot function and NADH and FADH₂ accumulate. The accumulation of these reducing equivalents will inhibit enzymes in the TCA cycle and energy metabolism ceases. As a result, the cell must rely on anaerobic metabolism for minimal ATP production and cell acidification from the lactic acid produced. As ATP falls, cellular processes such as ionic transport ceases resulting in dysfunctional or no action potentials and impaired neurotransmitter release. With ischemia, substrate is not available and the cell has to use substrates already available in the cell which

quickly deplete. The result is that the crisis caused by low ATP occurs even sooner and is more severe. Prolonged ischemia/hypoxia can result in damage or death of neurons.

4.2. Neurodegenerative disease

Dysfunction of the mitochondria has been implicated in many neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis (ALS). Accumulations of amyloid proteins have been found in the brain neurons of patients with Alzheimer's disease. However, symptoms are apparent before these plaques are observed. On the other hand, nonaggregated amyloid accumulation in mitochondria has been correlated with disease symptoms. Damage to the cell and mitochondria eventually lead to apoptosis.

In Parkinson's disease, Huntington's disease, and ALS excessive levels of ROS have been observed. In fact in some patients with these diseases, mutations in the ROS converting enzymes have been found. Excessive ROS levels can damage cell lipids, proteins, and DNA altering cell function and structure and eventually leading to cell death via apoptosis.

5. MODELS OF MITOCHONDRIAL ENERGY METABOLISM

Mitochondrial models have been an important tool in the understanding of mitochondrial function. The first computational model for mitochondrial energy metabolism was developed by Achs and Garfinkel^{11,12} to study ischemic metabolism. This model included glycolysis, the TCA cycle and associated pathways. It demonstrated the changes in metabolite concentrations and pH during ischemia in dog heart as well as suggested the mechanism of metabolic oscillations. Metabolic oscillations are oscillations in energy metabolism observed during ischemia. It should be noted that other oscillations of energy metabolism based upon enzyme dynamics, for example, phosphofructokinase in glycolysis, have been described.¹³ Metabolic oscillations caused by ischemia in heart and respiratory neurons do not rely on glycolytic oscillations. There have been other models based upon alternate mechanisms to explain this. These oscillations in heart have been shown to be blocked by antagonists of the mitochondrial inner membrane ion channel (IMAC). These two studies rely on two different mechanisms: The Jafri and Kotulska model relies on the regulation of IMAC by phosphate and magnesium while the model by Cortassa and coworkers predicts that redox sensitivity of the IMAC channel can allow for the oscillations.^{14,15} Note that both of these models include descriptions of oxidative phosphorylation.

The first model for oxidative phosphorylation was developed by Korzeniewski¹⁶ followed by improved versions. These models used empirical relations to describe the processes involved in oxidative phosphorylation and made predictions about the regulation of metabolism. One prediction was the idea of a “push-pull” mechanism in which energy metabolism was regulated both by the utilization of high-energy phosphates such as ATP and by factors that controlled the rate of energy production.

A model that tried to capture the mechanisms of energy metabolism and mitochondrial calcium dynamics in the pancreatic β -cell was developed by Magnus and Keizer.^{17,18} This

model predicted that calcium entry under certain conditions could be large enough to transiently depolarize the mitochondria and interfere with energy metabolism. A series of other models was developed based upon the Magnus–Keizer model. For example, Bertram¹⁹ created a simplified version of this model that was more amenable to mathematical analysis. The Bertram model was later used by Deikman and coworkers²⁰ in a model that integrates astrocyte membrane potential, ionic homeostasis, mitochondrial ATP production and calcium handling by mitochondria, and the endoplasmic reticulum. This model predicted how changes in IP₃-mediated calcium release affects mitochondrial energy production under different conditions and how certain signaling molecules (P2Y₁R) might protect astrocytes after ischemia.

Another series of models have integrated a model for the TCA cycle with the Magnus–Keizer model to describe energy metabolism in the cardiac myocyte.^{21–23} The models of Cortassa and coworkers have also integrated cardiac myocyte action potential and calcium dynamics with the mitochondrial model. The Nguyen–Dudycha–Jafri model focuses more on the regulation of mitochondrial energy metabolism and mitochondrial ionic homeostasis. In the sections that follow, a detailed description of the Nguyen–Dudycha–Jafri model is given as an example of how mitochondria in neurons might be modeled.

Computational modeling of mitochondrial function involves similar principles used for modeling excitable cell membranes, ionic homeostasis, and biochemical pathways. The inner membrane supports a membrane potential (ψ) determined by the ionic currents across it. These currents include the respiration driven proton pumps of the electron transport chain (J_{resp}), the F₁F₀-ATPase (J_{F1F0}), the mitochondrial calcium uniporter (J_{MCU}), the proton leak (J_{Hleak}), the mitochondrial sodium–calcium exchanger (J_{NCLX}), and the ATP/ADP antiporter (J_{ANT}). Hence the membrane potential is described by the ordinary differential equation:

$$-C_m \frac{d\psi}{dt} = J_{resp} + J_{F1F0} + J_{Hleak} + J_{MCU} + J_{NCLX} + J_{ANT}$$

where C_m is the mitochondrial membrane capacitance.

Each of these fluxes should have a representation based on experimental data. For example, the respiration driven proton pumps have been described in different ways in previous modeling efforts. In some studies, the whole electron transport chain is lumped into one flux.^{16,17} In the former, an empirical relation was derived for the flux through the electron transport chain. In the latter, a mechanistic state diagram of the electron transport chain is proposed and through mathematical analysis a flux equation is proposed. Others have developed descriptions of the individual complexes that form the electron transport chain.^{24–26} Korzeniewski uses empirical descriptions of each complex. On the other hand, Beard uses thermodynamics constraints to formulate descriptions of each complex based on first principles. Others have proposed detailed mechanistic models, such as Complex III.²⁷ In this model, the individual entities that comprise Complex III are represented with specific electron transfer between these entities described. All these approaches have their

applications depending upon the questions being asked. It is important that any of these approaches be constrained by available experimental data.

The chemical species must also be described by dynamic equations as well. For example, since Complex I is the point of entry for NADH into the respiratory chain, it must describe the consumption of NADH ($J_{\text{Complex I}}$) produced by the TCA cycle via isocitrate dehydrogenase, α -ketoglutarate dehydrogenase complex, and malate dehydrogenase (J_{IDH} , $J_{\alpha\text{KDHC}}$, and J_{MDH} , respectively).

$$\frac{d[NADH]}{dt} = J_{\text{IDH}} + J_{\alpha\text{KDHC}} + J_{\text{MDH}} - J_{\text{Complex I}}$$

The fluxes from the TCA cycle need to be described by principles from chemical kinetics as they are simply a set of catalyzed chemical reactions. There have been several efforts to model the cycle and its regulation by metabolites and ions such as calcium, protons, ADP, NADH, NAD^+ , and ATP. The first such work by Dudycha and Jafri²² included detailed descriptions of the regulation of citrate synthase, isocitrate dehydrogenase, α -ketoglutarate dehydrogenase complex, and malate dehydrogenase. These enzymes were based upon Michaelis–Menten kinetics which assumes that the reaction is at equilibrium. For example, the reaction velocity (v) of an enzyme (E) and substrate (S) reaction that irreversibly yields a product

can be described by $v = \frac{V_{\text{max}}[S]}{K_m + [S]}$, where $V_{\text{max}} = k_p [\text{E}]_{\text{total}}$ and $K_m = \frac{k_b + k_p}{k_f} [\text{E}]_{\text{total}}$ is the total concentration of enzyme. Michaelis–Menten formalism can also be used to derive expression for allosteric and competitive inhibitors and activators for the reactions. The bisubstrate reactions catalyzed by enzyme E with a random order of addition that can be used to describe the TCA cycle dehydrogenases is shown in Fig. 5.4.²⁸ This yields the following net reaction velocity equation

$$v = \frac{v_f \frac{[A]}{K_A} \frac{[B]}{K_B} - v_r \frac{[P]}{K_P} \frac{[Q]}{K_Q}}{1 + \frac{[A]}{K_A} + \frac{[B]}{K_B} + \frac{[A]}{K_A} \frac{[B]}{K_B} + \frac{[P]}{K_P} + \frac{[Q]}{K_Q} + \frac{[P]}{K_P} \frac{[Q]}{K_Q}}$$

where v_f and v_r are the maximal forward and reverse reaction rates, respectively, and the K_j where $j = A, B, P$, and Q are the Michaelis constants for the different reactants (A and B) and products P and Q.

The other reactions were described by the law of mass action which represents rate of change of the [A] (the concentration of A) governed by the chemical reaction equation

as

$$\frac{d[A]}{dt} = k_b [C] [D] - k_f [A] [B]$$

Similar equations can be derived for the concentration of the other chemical species involved.

This model set the stage for modeling by others who either adapted this model^{21,23} or made their own new implementations.²⁹ The common feature is that these models used Michaelis–Menten formalism for bisubstrate reactions to describe the regulation of the key regulator steps in the TCA cycle. The parameters for the binding constants for these reactions were fit to experimental data on initial reaction rates for these reactions as that is what is typically measured experimentally. The caveat with this is that the reverse reaction is not well constrained. Experimental measurement of reaction kinetics measures initial reaction rates, that is, reaction rates in the absence of product. For example, the work by Dudycha and Jafri constrained the model with this data and hence did not include reverse reactions. This however, under physiological conditions is not an issue as the change in free energy for these reactions made the reverse reaction flux negligible.

The model of mitochondrial energy metabolism developed by Kumar and Jafri²⁶ will be used here to give an example of how some of the genetic defects in energy metabolism can be modeled and what insights might be gained about how these mutations affect energy metabolism. The Kumar and Jafri model improves upon the Nguyen–Dudycha–Jafri model²³ which combines the Dudycha and Jafri²² model for the TCA cycle described above with the Magnus and Keizer model for mitochondrial energy metabolism and ionic homeostasis.¹⁷ The improvements in the most recent version of the model include improved calcium, sodium, potassium, and proton homeostasis as well as a mechanistic description of the complexes in the respiratory chain and the F₁F₀-ATPase.²⁶ This detailed description of the respiratory chain and oxidative phosphorylation includes the kinetic reactions for Complex I, II, and III and the F₁F₀-ATPase instead of steady-state solutions used earlier. It therefore allows accurate modeling of laboratory experimental manipulations that target these mechanisms and provides a means of validating the model.

Genetic mutations lead to changes in protein function. These can simply be a reduction in the proteins function, complete loss of the protein function, or a change in the protein characteristic, for example, the shifting of a dose–response curve. In the model, a mutant of a protein/protein complex can be developed to fit the data from the mutant gene if such data exists. Unfortunately, in many of the cases of neurodegenerative diseases, detailed characterization is not yet available. Table 5.3 shows examples demonstrating the effects of mutations that simply cause a reduction of function for three cases: (1) Complex I, (2)

Complex III, and (3) succinate dehydrogenase. In all these cases, NADH-linked substrates are used, that is, substrate enters the cycle as acetyl-CoA.

When Complex I is blocked, NADH cannot enter the respiratory chain. As a result, the NADH accumulates, inhibiting the TCA cycle. Without the entry of reduction equivalents, electron transport slows and mitochondrial membrane potential (ψ) drops. With entry of NADH and reduced membrane potential, ROS production also drops. The reduced membrane potential is also accompanied by a reduction in ATP production and a reduction in calcium uptake by the mitochondria.

The defect in Complex III is simulated by blocking the oxidation of ubiquinol to ubiquinone. The extent of disruption of the Q-cycle depends upon the exact part of the enzyme blocked. The result is accumulation of ubiquinol and depletion of ubiquinone. There is some decrease in mitochondrial membrane potential and this has decreases in calcium uptake. In spite of the decrease in membrane potential, ROS production increases as electrons in Complex I cannot be transferred to ubiquinone.

When succinate dehydrogenase is blocked there are two results. The first is disruption of the bottom half of the TCA cycle. This results in reduced NADH and FADH₂ production. It also prevents entry of FADH₂ into the respiratory chain. The membrane potential drops resulting in a decrease in ROS production and calcium uptake.

The simulations above suggest how mitochondrial concentrations and fluxes change. These changes are likely to have impact on neuronal properties as well. For example, reduction in ATP production can affect ionic homeostasis and neurotransmitter release/reuptake. Further exploration of the impact of these mutations is possible when the mitochondrial model is coupled to a neuronal model. For example, if the aforementioned changes are coupled with a model for neuronal excitability that includes the ATP usage by pumps and other processes, the effect on neural excitability can be assessed. Coupling this to a model of apoptosis can show how this might make the neuron susceptible to programmed cell death.

6. MODELS OF MITOCHONDRIAL SIGNALING

Mitochondria are both the target of cellular signaling pathways, as in the case of apoptosis, and are the source of signaling molecules such as ROS. One computational study that explores the bistability caused by the interplay of Bax and Bcl-2, by Bagci and coworkers³⁰ suggested that the cooperative formation of the apoptosome is the key mechanism for achieving this bistability. The combination of this type of mechanistic model with other pathways will be an essential step for understanding apoptosis. One recent effort to do so by Hong and colleagues³¹ developed a large-scale literature based model to describe the signaling pathways involved in cisplatin-induced apoptosis. The model suggests that the pathways involve activation of the ER-stress pathway, increased cytosolic calcium and activation of the TNF (tumor necrosis factor) pathway that reduces expression of Bcl-2. This tips the balance that allows Bax activation of the apoptosis. However, this result is controversial, because in experimental studies TNF increases Bcl-2 and Bax expression. This demonstrates the difficult and complexity of understanding the system and emphasizes the need for more computational modeling.

ROS are produced by the mitochondria as a byproduct of electron transport. ROS have many cellular targets such as the ryanodine receptor, DNA, RNA replication, and lipids. ROS also triggers apoptosis. One possible pathway is the increase in TNF expression resulting from high levels of ROS. TNF is known to be involved in the induction of apoptosis. ROS might also act directly on mitochondria to induce cytochrome c release. This area is mostly unexplored by computational modeling studies and presents an open area for such modeling work.

There are also other possible mechanisms for apoptosis. While calcium is an essential signaling molecule, prolonged levels of high calcium is toxic to the cell. Not only does calcium overload activate calcium dependent processes, it results in increased mitochondrial calcium. With mitochondrial calcium overload, permeability transition occurs resulting in membrane depolarization and release of cytochrome c into the cytosol. Cytochrome c release then leads to activation of apoptosis.

Computational modeling can help understand calcium overload and how it impacts mitochondrial calcium levels. Under normal conditions calcium transients of approximately 1.0 μM in amplitude occur. Modeling constrained by experiments indicates that the resulting mitochondrial calcium changes are relatively small.²³ However, for mitochondria near release sites that experience higher local calcium levels (~20 μM in amplitude) uptake can be much higher. These responses are transient, making them insufficient to cause mitochondrial dysfunction. Alzheimer's disease has been characterized by neurons with higher than normal calcium levels, presumable through calcium entry from the extracellular space. However, the mode of calcium entry is still an open question. Modeling can be used to explore the mode of calcium entry and how it must be localized with respect to the mitochondria to induce mitochondrial calcium overload.

These examples emphasize that mitochondria play an essential role in the induction of apoptosis. The resulting death of neurons, via apoptosis, results in permanent impaired function of neural networks, such as those involved in motor control, memory, and cognitive function. However, even prior to apoptosis, the changes to neural function by activation of signaling pathways that affect mitochondria impair energy metabolism resulting in neural dysfunction. It stresses the importance of developing an understanding of the pathways regulating and leading up to apoptosis which will require more advances in computational modeling in this area.

7. CONCLUDING REMARKS

Mitochondria play a critical role in the development and progression of neurodegenerative diseases through complex mechanisms. Computational modeling of mitochondria energy metabolism and signaling can be a powerful tool to understand these complex systems. Efforts have started to apply these models to neuroscience. However, there are many opportunities for the development and application of such models and their integration with experimental work. For example, connection of mitochondrial metabolism to signaling pathways will be an essential function of modeling. Modeling will also be crucial to explore

functional implications of the high degree of spatial compartmentalization caused by complex cristae structure and their role in neurodegenerative diseases.

Acknowledgments

We would like to thank Aman Ullah and Hoang-Trong Minh Tuan for their helpful comments. This work was supported by the National Institutes of Health 5R01AR057348 and 5R01HL105239 and the National Science Foundation (DMS 0443843).

REFERENCES

1. Gray MW. Mitochondrial evolution. *Cold Spring Harb Perspect Biol.* 2012; 4(9):a011403. [PubMed: 22952398]
2. Mannella CA, Lederer WJ, Jafri MS. The connection between inner membrane topology and mitochondrial function. *J Mol Cell Cardiol.* 2013; 62:51–57. [PubMed: 23672826]
3. Schon EA, Manfredi G. Neuronal degeneration and mitochondrial dysfunction. *J Clin Invest.* 2003; 111(3):303–312. [PubMed: 12569152]
4. Tanji K, Vu TH, Schon EA, DiMauro S, Bonilla E. Kearns-Sayre syndrome: unusual pattern of expression of subunits of the respiratory chain in the cerebellar system. *Ann Neurol.* 1999; 45(3): 377–383. [PubMed: 10072053]
5. Gonzalez-Cabo P, Palau F. Mitochondrial pathophysiology in Friedreich's ataxia. *J Neurochem.* 2013; 126(Suppl. 1):53–64. [PubMed: 23859341]
6. Goizet C, Depienne C, Benard G, et al. REEP1 mutations in SPG31: frequency, mutational spectrum, and potential association with mitochondrial morpho-functional dysfunction. *Hum Mutat.* 2011; 32(10):1118–1127. [PubMed: 21618648]
7. Fink JK. Hereditary spastic paraplegia: clinico-pathologic features and emerging molecular mechanisms. *Acta Neuropathol.* 2013; 126:307–328. [PubMed: 23897027]
8. Magnoni R, Palmfeldt J, Christensen JH, et al. Late onset motoneuron disorder caused by mitochondrial Hsp60 chaperone deficiency in mice. *Neurobiol Dis.* 2013; 54:12–23. [PubMed: 23466696]
9. OMIM. Online Mendelian Inheritance in Man. The Johns Hopkins University; Baltimore, MD: 2013.
10. Yudkoff M, Nelson D, Daikhin Y, Erecinska M. Tricarboxylic acid cycle in rat brain synaptosomes. Fluxes and interactions with aspartate aminotransferase and malate/aspartate shuttle. *J Biol Chem.* 1994; 269(44):27414–27420. [PubMed: 7961653]
11. Achs MJ, Garfinkel D. Metabolism of totally ischemic excised dog heart. I. Construction of a computer model. *Am J Physiol.* 1979; 237(5):318–326. [PubMed: 40439]
12. Garfinkel D, Achs MJ. Metabolism of the acutely ischemic dog heart. II. Interpretation of a model. *Am J Physiol.* 1979; 236(1):R31–R39. [PubMed: 434185]
13. Goldbeter A, Caplan SR. Oscillatory enzymes. *Annu Rev Biophys Bioeng.* 1976; 5:449–476. [PubMed: 182066]
14. Jafri MS, Kotulska M. Modeling the mechanism of metabolic oscillations in ischemic cardiac myocytes. *J Theor Biol.* 2006; 242(4):801–817. [PubMed: 16814324]
15. Zhou L, Cortassa S, Wei AC, Aon MA, Winslow RL, O'Rourke B. Modeling cardiac action potential shortening driven by oxidative stress-induced mitochondrial oscillations in guinea pig cardiomyocytes. *Biophys J.* 2009; 97(7):1843–1852. [PubMed: 19804714]
16. Korzeniewski B, Froncisz W. An extended dynamic model of oxidative phosphorylation. *Biochim Biophys Acta.* 1991; 1060(2):210–223. [PubMed: 1657162]
17. Magnus G, Keizer J. Model of beta-cell mitochondrial calcium handling and electrical activity. I. Cytoplasmic variables. *Am J Physiol.* 1998; 274(4 Pt 1):C1158–C1173. [PubMed: 9575813]
18. Magnus G, Keizer J. Model of beta-cell mitochondrial calcium handling and electrical activity. II. Mitochondrial variables. *Am J Physiol.* 1998; 274(4 Pt 1):C1174–C1184. [PubMed: 9575814]
19. Bertram R, Gram Pedersen M, Luciani DS, Sherman A. A simplified model for mitochondrial ATP production. *J Theor Biol.* 2006; 243(4):575–586. [PubMed: 16945388]

20. Diekman CO, Fall CP, Lechleiter JD, Terman D. Modeling the neuroprotective role of enhanced astrocyte mitochondrial metabolism during stroke. *Biophys J.* 2013; 104(8):1752–1763. [PubMed: 23601322]
21. Cortassa S, Aon MA, O'Rourke B, et al. A computational model integrating electro-physiology, contraction, and mitochondrial bioenergetics in the ventricular myocyte. *Biophys J.* 2006; 91(4): 1564–1589. [PubMed: 16679365]
22. Dudycha, SJ. A Detailed Model of the Tricarboxylic Acid Cycle in Heart Cells. Biomedical Engineering, The Johns Hopkins University; Baltimore, MD: 2000.
23. Nguyen MH, Dudycha SJ, Jafri MS. Effect of Ca^{2+} on cardiac mitochondrial energy production is modulated by Na^{+} and H^{+} dynamics. *Am J Physiol Cell Physiol.* 2007; 292(6):C2004–C2020. [PubMed: 17344315]
24. Beard DA. A biophysical model of the mitochondrial respiratory system and oxidative phosphorylation. *PLoS Comput Biol.* 2005; 1(4):e36. [PubMed: 16163394]
25. Korzeniewski B. Regulation of oxidative phosphorylation through parallel activation. *Biophys Chem.* 2007; 129(2–3):93–110. [PubMed: 17566629]
26. Kumar R, Jafri MS. Mechanism of reactive oxygen species generation in cardiac mitochondria a computational approach. *Biophys J.* 2011; 100(3 Suppl. 1):460.
27. Demin OV, Kholodenko BN, Skulachev VP. A model of O_2 -generation in the complex III of the electron transport chain. *Mol Cell Biochem.* 1998; 184(1–2):21–33. [PubMed: 9746310]
28. Alberty RA. Enzyme kinetics. *Adv Enzymol Relat Areas Mol Biol.* 1956; 17:1–64.
29. Wu F, Yang F, Vinnakota KC, Beard DA. Computer modeling of mitochondrial tricarboxylic acid cycle, oxidative phosphorylation, metabolite transport, and electrophysiology. *J Biol Chem.* 2007; 282(34):24525–24537. [PubMed: 17591785]
30. Bagci EZ, Vodovotz Y, Billiar TR, Ermentrout GB, Bahar I. Bistability in apoptosis: roles of bax, bcl-2, and mitochondrial permeability transition pores. *Biophys J.* 2006; 90(5):1546–1559. [PubMed: 16339882]
31. Hong JY, Kim GH, Kim JW, et al. Computational modeling of apoptotic signaling pathways induced by cisplatin. *BMC Syst Biol.* 2012; 6:122. [PubMed: 22967854]

Figure 5.1. Tricarboxylic acid cycle schematic

The enzymes shown in bold text have been previously modeled in detail (see Dudycha and Jafri, 2001). The influx of acetyl-CoA (ACoA) to the system reflects the breakdown of substrate from the catabolism of glucose, fatty acids, and proteins.

Figure 5.2. Mitochondrial energy metabolism

Energy substrates enter in the form of glucose or fatty acids that are converted to acetyl-CoA. The acetyl-CoA enters the tricarboxylic acid (TCA) or Krebs cycle to form NADH and FADH₂ which enter the electron transport chain. The electron transport chain generates a membrane potential and proton gradient across the mitochondrial inner membrane. These gradients are then used to make ATP.

Figure 5.3. Mitochondrial schematic

The mitochondrion has an inner membrane with a large surface area that define the cristae (in oval) and mitochondrial matrix (top). The circled region (top) is enlarged in the bottom panel. The large surface area serves as the site for energy metabolism with the proteins for the respiratory chain and oxidative phosphorylation located in the membrane and the tricarboxylic acid (TCA) cycle proteins clustering on the matrix side of the membrane. There are also the proteins involved in transport of substances in and out of the mitochondria. (MCU, mitochondrial calcium uniporter; NCLX, mitochondrial sodium–calcium–lithium exchanger; PIC, phosphate carrier; NHE, sodium hydrogen exchanger; KHE, potassium hydrogen exchanger.)

Figure 5.4. Bisubstrate reaction schemes

The top shows the binding of enzyme (E) to substrates A and B with random order of addition to form products P and Q. The bottom shows a scheme where A binds the enzyme first.

Table 5.1

Summary of mitochondrial genes, their function, and their involvement in disease

Disease	Mitochondrial gene	Defect
Kearns–Sayre syndrome	Subunits of Complexes I, II, and IV	Respiratory chain dysfunction ⁴
Leber hereditary optic atrophy	Subunits of Complexes I, II, III, and IV	Impaired oxidative phosphorylation/electron transport chain
	MT-ATP6—ATP synthase subunit 6	Decreased ATP production
Leigh's syndrome	Subunits of Complex I	Decreased Complex I activity
Maternally inherited Leigh's syndrome (MILS)	MT-ATP6—ATP synthase subunit 6	Decreased ATP production
Mitochondrial Complex I deficiency	Many subunit of Complex I	Impaired Complex I function
Neuropathy, ataxia, and retinosa pigmentosa (NARP)	MT-ATP6—ATP synthase subunit 6	Decreased ATP production
Spinocerebellar ataxia	ATXN7—ataxin 7	Abnormally large mitochondria with irregular cristae

Table 5.2

Summary of nuclear genes, their function, and their involvement in disease

Disease	Gene	Defect
Alzheimer's disease	APP—amyloid β precursor protein	Impaired succinate dehydrogenase
Adrenoleukodystrophy	ABCD1—ATPase binding cassette I	Defect in β -oxidation of fatty acids
Amyotrophic lateral sclerosis	SOD1—Cu, Zn superoxide dismutase I	Increased cellular ROS
Encephalopathy (lethal)	DNM1L—dynamin 1 like	Mitochondrial and peroxisome fusion
Friedreich ataxia	FXN—frataxin	Increased ROS production through interaction at Complex II ⁵
Hereditary spastic paraplegia	REEP1—receptor expression-enhancing protein-1	Abnormal mitochondrial network organization and impaired mitochondrial energy production ⁶
	SPG7—paraplegin-7 and other SPG proteins	Abnormal mitochondria and dysfunction ⁷
	HSPD1—heat-shock 60 KD protein-1 and other HSP proteins	Morphological changes in mitochondria, deficient ATP synthesis, defect in assembly of Complex III ^{7,8}
Huntington's disease	HTT—huntingtin	Mitochondria have lower membrane potential, have more ROS production, and are more sensitive to depolarization due to high-cellular calcium
Leigh's syndrome	Complex I subunits from nuclear DNA	Decreased oxygen consumption, decreased ATP production, and increased ROS and lactic acid production
	SDH1—succinate dehydrogenase complex subunit A in Complex II	Decreased succinate dehydrogenase and Complex II activity
	SURF1 SCO2 in Complex IV	Decreased cytochrome C oxidase activity
	Pyruvate dehydrogenase E1- α subunit	Decreased pyruvate dehydrogenase activity
Mohr-Tranebjaerg syndrome	TIMM8A—translocase of the inner mitochondrial membrane	Decreased NADH levels by calcium binding aspartate-glutamate shuttles
Parkinson's disease	PARK2—parkin	Damaged mitochondria not degraded
	SNCA— α -synuclein	Mitochondrial fragmentation
	LRRK2—leucine-rich repeat kinase 2	Increases peroxiredoxin (PRDX3) phosphorylation which reduces its peroxidase activity in mitochondria
Spongiform encephalitis	PRNP—prion protein	Fewer mitochondria, with abnormal morphology, and increased ROS production
Supranuclear palsy	MAPT—microtubular associated protein Tau	Mitochondria do not enter neurites
Spinocerebellar ataxia	ATXN7—ataxin 7	Large mitochondria with no cristae

Data gathered from Refs. 3,9.

Table 5.3

Changes in mitochondrial species due to mutation

Mutation	ψ	ROS production	[NADH]	[Ca ²⁺]	ATP production
Complex I	–	–	+	–	–
Complex III	–	+	0	–	0
Succinate dehydrogenase	–	–	0	+	0

Key: +, indicates increase; –, indicates decrease; 0, indicates little change.