"Bioenergetics and Oxidative Phosphorylation"

I. Overview

- Bioenergetics
    - Describes the transfer and utilization of energy in biologic systems.
    - Concerns the initial and final energy states of the reaction components.
- Relation to Thermodynamics
    - Makes use of a few basic ideas from the field of thermodynamics.
    - Particularly involves the concept of free energy.
- Function of Free Energy
    - Changes in free energy provide a measure of the energetic feasibility of a chemical reaction.
    - Allows prediction of whether a reaction or process can take place.
- Important Distinction
    - Bioenergetics predicts if a process is possible.
    - Kinetics measures the reaction rate.

II. Free Energy

- Determinants of Reaction Direction and Extent
    - Determined by the degree to which two factors change during the reaction:
        - Enthalpy (ΔH): A measure of the change (Δ) in heat content of the reactants and products.
        - Entropy (ΔS): A measure of the change in randomness or disorder of the reactants and products.

- Limitations of Individual Quantities
    - Neither enthalpy (ΔH) nor entropy (ΔS) alone is sufficient to determine whether a chemical reaction will proceed spontaneously in the direction it is written.

- Definition of Free Energy (G)
    - When enthalpy and entropy are combined mathematically, they define a third quantity:
        - Free energy (G)
        - Predicts the direction in which a reaction will spontaneously proceed.

III. Free Energy Change

- Forms of Free Energy Change
    - Represented in two ways:
        - ΔG
        - ΔG^0 (with the superscript "0")
- ΔG (without superscript "0")
    - Represents the change in free energy.
    - Indicates the direction of a reaction at any specified concentration of products and reactants.
    - ΔG is a variable.
- ΔG^0 (standard free energy change)
    - The energy change when reactants and products are at 1 mol/l concentration.
    - Note: Proton concentration [H+][H+][H+] is assumed to be 10^{-7} mol/l → pH = 7.
        - This may be shown using a prime sign ['], e.g., $\Delta G^{0\prime}$.

- ΔG^0 is a constant and applies to nonphysiologic concentrations.
- Still useful for comparing energy changes of different reactions.
- ΔG^0 can be determined from measurement of the equilibrium constant.

A. ΔG and Reaction Direction

- ΔG sign indicates reaction direction at constant temperature and pressure.

- Example Reaction: $A \rightleftarrows B$

  - If ΔG is negative:
    - The reaction is exergonic.
    - There is a net loss of energy.
    - The reaction proceeds spontaneously as written, with A converted to B.

  - If ΔG is positive:
    - The reaction is endergonic.
    - There is a net gain of energy.
    - Energy must be added for the reaction from B to A to take place.

- If $\Delta G = 0$:
  - The reaction is in equilibrium.

- Spontaneous Reactions and Equilibrium

  - When ΔG is negative and the reaction proceeds spontaneously, it continues until:
    - ΔG reaches zero
    - Equilibrium is established

B. ΔG of the Forward and Reverse Reactions

- The free energy of the forward reaction ($A \rightarrow B$):

  - Is equal in magnitude but opposite in sign to that of the reverse reaction ($B \rightarrow A$).

- Example:

  - If ΔG (forward) = -5 kcal/mol, then:
    - ΔG (reverse) = +5 kcal/mol

- Units:
    - ΔG can be expressed in:
        - kcal/mol
        - kJ/mol
            - 1 kcal = 4.2 kJ

C. ΔG and Reactant and Product Concentrations

- ΔG of the reaction A → B depends on:
    - The concentrations of the reactant and
    - The concentration of the product.

- At constant temperature and pressure, the following relationship can be derived:
    - $\Delta G = \Delta G^0 + RT \ln([B]/[A])$
        - ΔG^0: Standard free energy change (see section D)
        - R: Gas constant = 1.987 cal/mol·K
        - T: Absolute temperature (in Kelvin)
        - [A] and [B]: Actual concentrations of the reactant and product
        - ln: Natural logarithm

- A reaction with a positive ΔG^0 can still proceed in the forward direction if:
    - The ratio of products to reactants ([B]/[A]) is sufficiently small
    - That is, the ratio of reactants to products is large
    - This condition makes ΔG negative, allowing the reaction to proceed

- Example:
    - In the reaction: Glucose 6-phosphate $\rightleftarrows$ Fructose 6-phosphate
        - When the concentration of glucose 6-phosphate is high
        - And the concentration of fructose 6-phosphate is low
        - The ratio of product to reactant is small
        - As a result, the term $RT \ln([\text{fructose 6-phosphate}]/[\text{glucose 6-phosphate}])$ becomes large and negative
        - This makes ΔG negative even though ΔG^0 is positive
        - Thus, the reaction can still proceed in the forward direction

D. Standard Free Energy Change

- Standard free energy change (ΔG^0) is:
    - The free energy change under standard conditions
    - Standard conditions: Concentrations of reactants and products are 1 mol/l
- Under standard conditions:
    - The ratio [B]/[A] = 1
    - The natural logarithm of 1 = 0
    - ln(1) = 0
- Therefore, the equation becomes:
    - $\Delta G = \Delta G^0 + 0$

I. ΔG^0 and Reaction Direction

- Under standard conditions, ΔG^0 can be used to predict the direction a reaction proceeds.
    - This is because under standard conditions, ΔG^0 is equal to ΔG.

- However, ΔG^0 cannot predict the reaction direction under physiologic conditions because:
    - It is composed solely of constants:
        - R (gas constant)
        - T (temperature)
        - Keq (equilibrium constant)
    - It is not altered by changes in product or substrate concentrations.

2. Relationship Between ΔG^0 and Keq

- In a reaction: $A \rightleftarrows B$
    - A point of equilibrium is reached at which no further net chemical change takes place.
- At equilibrium, the ratio of [B] to [A] is constant, regardless of their actual concentrations:

$$Keq = [B]eq / [A]eq$$

    - Where:
        - Keq = equilibrium constant
        - [A]eq and [B]eq = concentrations of A and B at equilibrium

- If the reaction $A \rightleftarrows B$ is allowed to reach equilibrium at constant temperature and pressure, then:
    - At equilibrium, $\Delta G = 0$.
- Therefore, when [A] and [B] are at equilibrium concentrations:

$$\Delta G^0 = - RT \ln Keq$$

- This equation allows simple predictions:
    - If $Keq = 1$, then $\Delta G^0 = 0$
    - If $Keq > 1$, then $\Delta G^0 < 0$
    - If $Keq < 1$, then $\Delta G^0 > 0$

3. ΔG^0 of Two Consecutive Reactions

- The ΔG^0 values are additive for any sequence of consecutive reactions, just like ΔG values.

- For example:
    - If:

      Reaction 1: $A \rightarrow B$, ΔG^0_1

      Reaction 2: $B \rightarrow C$, ΔG^0_2

    - Then total:

$$A \rightarrow C, \Delta G^0 = \Delta G^0_1 + \Delta G^0_2$$

4. ΔG of a Pathway

- The additive property of ΔG is crucial in biochemical pathways such as:

$$A \rightarrow B \rightarrow C \rightarrow D \rightarrow \ldots$$

- As long as the sum of the individual ΔGs is negative, the overall pathway proceeds in that direction.

- Even if some steps have a positive ΔG, the overall pathway can proceed if the total ΔG is negative.

- However, the actual rate of each reaction depends on:
    - Lowering of activation energy (Ea)
    - This is achieved by enzymes that catalyze the reactions.

IV. ATP: An Energy Carrier

- Reactions with a large positive ΔG can occur if coupled with a spontaneous (exergonic) reaction having a large negative ΔG.
    - Example: Coupling with the hydrolysis of ATP.

A. Common Intermediates

- Two reactions share a common intermediate when:
    - The product of the first reaction becomes the substrate for the second.

- Example:
    - $A + B \rightarrow C + D$
    - $D + X \rightarrow Y + Z$
    - Here, D is the common intermediate.
- D acts as a carrier of chemical energy between the two reactions.
    - (Note: The intermediate may be linked to an enzyme.)
- Many coupled reactions use ATP to create a common intermediate:
    - These may involve:
        - Transfer of phosphate from ATP to another molecule.
        - Or transfer of phosphate from an energy-rich intermediate to ADP, forming ATP.

B. Energy Carried by ATP

- ATP (Adenosine Triphosphate) consists of:
  - Adenosine (adenine + ribose sugar)
  - Three phosphate groups
- Phosphate removal:
  - Loss of one phosphate → ADP (Adenosine Diphosphate)
  - Loss of two phosphates → AMP (Adenosine Monophosphate)
- ΔG^0 of ATP hydrolysis:
  - $\approx$ -7.3 kcal/mol for each of the two terminal phosphate bonds
  - This large negative free energy makes ATP a high-energy phosphate compound
- Adenylate kinase interconverts adenine nucleotides:
  - $2\ ADP \rightleftharpoons ATP + AMP$

II. Free Energy

V. Electron Transport Chain (ETC)

- Energy-rich molecules like glucose are oxidized stepwise to yield:
    - $CO_2 + H_2O$
- Metabolic intermediates donate electrons to coenzymes:
    - $NAD^+ \rightarrow NADH$
    - $FAD \rightarrow FADH_2$
- These reduced coenzymes donate pairs of electrons to:
    - A specialized set of electron carriers called the Electron Transport Chain (ETC)

ETC Function and Energy Use

- As electrons pass through ETC, they:
    - Lose free energy gradually
    - This energy is used to pump H^+ across the inner mitochondrial membrane

- This creates a proton (H^+) gradient, which:
    - Drives ATP synthesis from ADP + Pi
    - This process is called oxidative phosphorylation (OXPHOS)
- OXPHOS occurs:
    - Continuously in all tissues that contain mitochondria
- Unused free energy is not wasted:
    - Drives other reactions like:
        - Calcium ion transport into mitochondria
        - Heat generation

A. Mitochondrial Electron Transport Chain (ETC)

- The ETC (except cytochrome c) is located in the inner mitochondrial membrane
- It is the final common pathway for electrons from various fuels of the body
- Electrons ultimately flow to oxygen (O_2), reducing it to H_2O

1. Mitochondrial Membranes

- Two membranes:
    - Outer membrane
        - Contains porin proteins → form specialized channels
        - Freely permeable to most ions and small molecules
    - Inner membrane
        - Impermeable to:
            - Most small ions (including H^+)
            - Small molecules (ATP, ADP, pyruvate, metabolites)
        - Transport proteins are required for movement across it
        - Protein-rich: Over half of the proteins are involved in oxidative phosphorylation
        - Contains cristae:
            - Folded structures that increase surface area

2. Mitochondrial Matrix

- The gel-like interior of mitochondria is called the matrix

- Rich in proteins and enzymes, including those for:
    - Pyruvate oxidation
    - Amino acid oxidation
    - Fatty acid β-oxidation
    - TCA (Krebs) cycle

- Partially occurs in the matrix:
    - Glucose synthesis
    - Urea synthesis
    - Heme synthesis

- Other matrix components:
    - NAD^+ and FAD → oxidized electron acceptors
    - ADP and Pi → required for ATP production
    - mtDNA, mtRNA, and ribosomes

B. Organization of the Electron Transport Chain (ETC)

- The inner mitochondrial membrane contains four protein complexes:
    - Complex I
    - Complex II
    - Complex III
    - Complex IV
- These complexes participate in the electron transport chain (ETC)
- Electrons are transferred between complexes by mobile electron carriers:
    - Coenzyme Q (CoQ) → lipid-soluble
    - Cytochrome c → protein-based
- Each carrier in the ETC:
    - Receives electrons from a donor
    - Donates electrons to the next acceptor
    - Final electron acceptor: O_2, which combines with H^+ to form H_2O

- The requirement of oxygen (O_2) makes this process the respiratory chain

- The ETC accounts for the majority of oxygen consumption in the body

C. Reactions in the ETC

- All ETC members (except CoQ) are proteins

- Components may include:

    - Enzymes (e.g., flavin-containing dehydrogenases)
    - Iron-sulfur (Fe-S) centers
    - Heme groups (in cytochromes with iron in a porphyrin ring)
    - Copper (Cu) (in cytochrome a + a_3 complex)

1. NADH Formation

- NAD^+ is reduced to NADH by dehydrogenases

- Dehydrogenases:
    - Remove 2 hydrogen atoms from a substrate
    - Transfer:
        - 2 electrons
        - Only 1 H^+ (as a hydride ion [:H^-]) to NAD^+
        - Result: NADH + free H^+

2. NADH Dehydrogenase (Complex I)

- NADH transfers its electrons to Complex I (NADH dehydrogenase)

- Complex I:
    - Embedded in the inner mitochondrial membrane
    - Contains:
        - Flavin mononucleotide (FMN) → accepts 2 H atoms to form $FMNH_2$
        - Fe-S peptide subunits

- Electron flow within Complex I:
    - NADH → FMN → Fe-S centers → CoQ
    - As electrons move, they lose energy

- This energy is used to pump 4 H^+ from the matrix to the intermembrane space

3. Succinate Dehydrogenase (Complex II)

- Catalyzes the reaction:

  Succinate → Fumarate (via oxidation)

- Electron transfer path at Complex II:
  - $FADH_2$ → Fe-S protein → Coenzyme Q (CoQ)
- No energy is lost in this process

→ No H^+ are pumped at Complex II

4. Coenzyme Q (CoQ / Ubiquinone)

- Structure:
  - Quinone derivative with a long hydrophobic isoprenoid tail
  - Synthesized from an intermediate of cholesterol synthesis

- Functions as a mobile electron carrier
- Accepts electrons from:
    - NADH dehydrogenase (Complex I)
    - Succinate dehydrogenase (Complex II)
    - Other mitochondrial dehydrogenases:
        - Glycerol 3-phosphate dehydrogenase
        - Acyl-CoA dehydrogenases
- Transfers electrons to:
    - Complex III (Cytochrome bc_1 complex)
- Function summary: Links flavoprotein dehydrogenases to cytochromes

5. Cytochromes

- Remaining ETC components are cytochrome proteins
- Each cytochrome contains a heme group:
    - Porphyrin ring + Iron (Fe)
    - Iron cycles between Fe^{3+} (ferric) and Fe^{2+} (ferrous) as it accepts and donates electrons

- Electron flow:
    - Complex III (cytochrome bc_1) → Cytochrome c → Complex IV (cytochromes a + a_3)
- H^+ pumping associated with cytochromes:
    - 4 H^+ pumped at Complex III
    - 2 H^+ pumped at Complex IV
- Cytochrome c:
    - Located in intermembrane space
    - Loosely associated with outer face of inner membrane
    - Functions as a mobile electron carrier (like CoQ)

6. Cytochrome a + a_3 (Complex IV / Cytochrome c Oxidase)

- Only electron carrier whose heme iron has a free coordination site for direct reaction with O_2

→ Hence called cytochrome c oxidase

- Final step in ETC:
    - Electrons + O_2 + free H^+ combine
    - O_2 is reduced to H_2O
- Reduction stoichiometry:
    - 4 electrons reduce 1 molecule of O_2 to 2 H_2O
- Structure and flow:
    - Contains copper (Cu) atoms essential for reaction
    - Electron transfer:
        - CuA → cytochrome a → cytochrome a_3 (with CuB) → O_2

7. Site-Specific Inhibitors of the Electron Transport Chain (ETC)

- Certain respiratory inhibitors bind to specific components of the ETC

→ Block electron flow by halting redox reactions

- Result of inhibition:
    - Electron carriers before the block → Fully reduced
    - Electron carriers after the block → Remain oxidized
- Inhibition of ETC also inhibits ATP synthesis

→ Because ETC and ATP synthesis are tightly coupled

- Electron leakage from ETC can generate Reactive Oxygen Species (ROS):
    - Examples of ROS:
        - Superoxide (O_2^-)
        - Hydrogen peroxide (H_2O_2)
        - Hydroxyl radical ($\cdot OH$)
- ROS effects:
    - Damages DNA, proteins, and lipids (lipid peroxidation)

- Cellular defense against ROS includes:
    - Superoxide dismutase (SOD)
    - Catalase
    - Glutathione peroxidase

D. Free Energy Release During Electron Transport

- As electrons move along the ETC from electron donor (reductant) to electron acceptor (oxidant):

→ Free energy is released

- This energy is used to pump H^+ across the inner mitochondrial membrane at:
    - Complex I
    - Complex III
    - Complex IV

- Electron donation types:
    - As hydride ions (H^-) to NAD^+
    - As hydrogen atoms ($H\cdot$) to FMN, CoQ, FAD
    - As electrons (e^-) to cytochromes

1. Redox Pairs

- Oxidation = Loss of electrons
- Reduction = Gain of electrons

→ Always occur together in redox reactions

- Example at Complex I:
  - NADH is oxidized to NAD^+
  - FMN (prosthetic group) is reduced to $FMNH_2$
- Each redox reaction can be written as:
  - Two half-reactions: One oxidation + One reduction
- Examples of redox pairs:
  - NAD^+ / NADH
  - FMN / $FMNH_2$
- Redox pairs differ in their tendency to lose or gain electrons

- This tendency is measured as:
    - E_0 (Standard Reduction Potential)
    - → Units: Volts

2. Standard Reduction Potential (E_0)

- Redox pairs can be ranked from most negative to most positive E_0
- Interpretation:
    - More negative E_0 → Greater tendency to lose electrons (strong reductant)
    - More positive E_0 → Greater tendency to accept electrons (strong oxidant)
- Electron flow direction:
    - From redox pairs with more negative E_0
    - → To redox pairs with more positive E_0
- ETC components are arranged in order of increasing E_0 values

→ Ensures unidirectional electron flow

3. Relationship of ΔG° to ΔE°

- ΔG° (standard free energy change) is directly related to the change in standard reduction potential (ΔE°) by the equation:

$$\Delta G^\circ = -nF\Delta E^\circ$$

- Where:
  - n = number of electrons transferred
    - 1 for cytochromes
    - 2 for NADH, $FADH_2$, and CoQ
  - F = Faraday constant = 23.1 kcal/volt·mol
  - $\Delta E^\circ = E^\circ(\text{acceptor}) - E^\circ(\text{donor})E^\circ\text{(acceptor)} - E^\circ\text{(donor)}E^\circ(\text{acceptor}) - E^\circ(\text{donor})$
  - ΔG° = Change in standard free energy (in kcal/mol)

4. ΔG° of ATP Synthesis

- ΔG° for phosphorylation of ADP + Pi → ATP = +7.3 kcal/mol

- Energy released from the transfer of electrons from NADH to O_2 through the ETC = 52.6 kcal/mol

- Therefore:
    - Energy available is more than enough to synthesize 3 ATP molecules
    - Energy required = 3 × 7.3 = 21.9 kcal/mol
- This is sometimes expressed as the P/O ratio:
    - NADH: P/O = 3:1
    - $FADH_2$: P/O = 2:1 (because Complex I is bypassed)
- Remaining energy is:
    - Used in ancillary reactions
    - Released as heat

VI. Phosphorylation of ADP to ATP

- Electron flow through ETC is energetically favorable:
    - Because NADH is a strong electron donor
    - And O_2 is a strong electron acceptor
- However, this electron flow does not directly produce ATP

A. Chemiosmotic Hypothesis (Mitchell Hypothesis)

- Explains how free energy from electron transport is used for ATP synthesis

- Mechanism:

    - Electrons moving through ETC release energy
    - This energy is used to pump H^+ ions across the inner mitochondrial membrane
    - This creates a proton gradient (electrochemical gradient)
    - ATP synthase uses the energy of H^+ flow back into the matrix (down its gradient) to convert:

$$ADP + Pi \rightarrow ATP$$

1. Proton Pump

- Electron transport is coupled to ADP phosphorylation via proton pumping across the inner mitochondrial membrane.

- H^+ is pumped from:

    - Mitochondrial matrix → Intermembrane space
    - At Complexes I, III, and IV

- For every 2 electrons transferred from NADH to O_2:
    - 10 H^+ ions are pumped
- This generates two gradients:
    - Electrical gradient:
        - More positive charges on cytosolic (intermembrane space) side
        - Matrix side becomes more negative
    - pH (chemical) gradient:
        - Cytosolic side becomes more acidic (lower pH)
        - Matrix remains alkaline (higher pH)
- Combined gradient = Proton-motive force
    - Drives ATP synthesis
    - H^+ gradient acts as the common intermediate coupling oxidation to phosphorylation

2. ATP Synthase (Complex V)

- ATP synthase = Multisubunit enzyme responsible for synthesizing ATP
    - Also called F_1F_0-ATPase

- Structure:
    - F_0 domain:
        - Embedded in inner mitochondrial membrane
        - Contains H^+ channel and c ring
    - F_1 domain:
        - Projects into the mitochondrial matrix
        - Contains three β subunits involved in ATP synthesis

- Mechanism (Chemiosmotic Hypothesis):
    - H^+ ions reenter the matrix through the H^+ channel in F_0
    - This drives rotation of the c ring in F_0
    - Rotation causes conformational changes in the three β subunits of F_1, enabling:
        i. Binding of ADP + Pi
        ii. Phosphorylation of ADP → ATP
        iii. Release of ATP

- Yield:
    - One complete rotation of the c ring = Three ATP molecules synthesized
- Additional Note:
    - ATP synthase can also catalyze the reverse reaction:
        - ATP hydrolysis → ADP + Pi
        - Hence called ATPase

A. Coupling in Oxidative Phosphorylation

- In normal mitochondria, there is tight coupling between:
    - Electron transport (ETC)
    - ATP synthesis via ATP synthase
- This coupling occurs through the H^+ gradient:
    - Any increase or decrease in one process affects the other equally.

- Example of coupling:
    - When ATP is hydrolyzed to ADP + Pi in energy-requiring reactions:
        - More ADP and Pi become available for ATP synthase
        - This increases H^+ flow through ATP synthase
        - As a result, ETC activity increases to pump more H^+ and maintain the proton gradient

B. Oligomycin (Inhibitor of ATP Synthase)

- Oligomycin:
    - Binds to the F_0 domain (membrane portion) of ATP synthase
    - Closes the H^+ channel in F_0
- Effect of binding:
    - Prevents reentry of H^+ into the mitochondrial matrix
    - Inhibits phosphorylation of ADP to ATP

- Consequences:
    - H^+ gradient builds up (cannot be dissipated)
    - Electron transport halts:
        - Because pumping more H^+ becomes energetically unfavorable
        - Due to the steep gradient already present

- Key concept: Respiratory Control:
    - The dependency of cellular respiration on the ability to phosphorylate ADP to ATP is known as respiratory control
    - Reflects the tight coupling between oxidation and phosphorylation

C. Uncoupling Proteins (UCPs)

- Location: Inner mitochondrial membrane of mammals (including humans)

- Function:
    - Form H^+ channels allowing protons to reenter the matrix
    - Bypass ATP synthase, so no ATP is produced

- Energy from H^+ gradient is instead released as heat

- Process name:
  - Nonshivering thermogenesis

- Key protein:
  - UCP1 (thermogenin):
    - Present in brown adipose tissue
    - Responsible for heat production
    - Activated by cold exposure via catecholamine signaling

- Brown vs White Fat:
  - Brown fat:
    - Rich in mitochondria
    - ~90% of respiratory energy used for heat (thermogenesis)
    - Especially important in infants
  - White fat:
    - Specialized for energy storage
  - (Note: Brown fat depots also exist in adults, though less abundant)

D. Synthetic Uncouplers

- Mechanism:
    - Disrupt the H^+ gradient by shuttling protons across the inner mitochondrial membrane
    - Allow electron transport to continue, but no ATP is made
    - Energy is released as heat
- Classic example:
    - 2,4-Dinitrophenol (DNP):
        - Lipophilic H^+ carrier (ionophore)
        - Easily diffuses through the mitochondrial membrane
        - Uncouples ETC from phosphorylation, just like UCPs
- Clinical note:
    - High doses of aspirin and other salicylates:
        - Act as uncouplers
        - Cause fever due to heat release instead of ATP synthesis

B. Membrane Transport Systems

- Barrier:
    - Inner mitochondrial membrane is impermeable to most charged/hydrophilic molecules
- Solution:
    - Specialized transport proteins facilitate selective transport of essential molecules

1. ATP and ADP Transport

- Adenine nucleotide antiporter:
    - Imports ADP into the mitochondrial matrix
    - Exports ATP into the cytosol (1:1 exchange)
- P_i–H^+ symporter:
    - Cotransports inorganic phosphate (P_i) and H^+ from cytosol into matrix
    - Supplies P_i for ATP synthesis

2. Reducing Equivalent Transport

- Problem:
  - Cytosolic NADH (e.g., from glycolysis) cannot cross the inner mitochondrial membrane directly
- Solution:
  - Use substrate shuttles to transfer reducing equivalents indirectly

A. Glycerol 3-Phosphate Shuttle

- Steps:
  - NADH reduces dihydroxyacetone phosphate (DHAP) to glycerol 3-phosphate (cytosolic enzyme)
  - Glycerol 3-phosphate is oxidized by mitochondrial glycerol 3-phosphate dehydrogenase
  - This reduces FAD → $FADH_2$
  - CoQ of ETC oxidizes $FADH_2$
- ATP yield:
  - 2 ATP per cytosolic NADH

B. Malate-Aspartate Shuttle

- Steps:
    - NADH reduces oxaloacetate → malate (in cytosol)
    - Malate is transported into matrix by a transport portein
    - In matrix, malate is oxidized back to oxaloacetate, regenerating NADH
- ATP yield:
    - 3 ATP per cytosolic NADH

C. Inherited Defects in Oxidative Phosphorylation

- Protein origin:
    - ~90 proteins required for oxidative phosphorylation
    - 13 polypeptides encoded by mtDNA, synthesized within mitochondria
    - Remaining proteins encoded by nuclear DNA, synthesized in cytosol, then imported into mitochondria

- Mutation risk:
    - mtDNA has 10× higher mutation rate than nuclear DNA → more likely cause of defects
- Affected tissues:
    - Tissues with high ATP demand are most vulnerable:
        - Brain, nerves, retina, skeletal muscle, heart, liver
- Clinical features:
    - Lactic acidosis, especially in muscles, CNS, and retina
    - Symptoms may include:
        - Seizures
        - Ophthalmoplegia
        - Muscle weakness
        - Cardiomyopathy

- Medication caution:
    - Some drugs impair mitochondrial function and should be avoided in mitochondrial disorders
- Inheritance:
    - mtDNA is maternally inherited
        - Sperm mitochondria do not survive fertilization
        - Only oocyte mitochondria persist in embryo and adult

D. Mitochondria and Apoptosis

- Trigger:
    - Apoptosis initiated via intrinsic (mitochondrial) pathway
    - In response to irreparable cell damage
- Key steps:
    - Bax or Bak proteins inserted into outer mitochondrial membrane
    - Cytochrome c released from intermembrane space into cytosol

Apoptosome formation:

- Cytochrome c + proapoptotic factors → apoptosome

- Caspase activation:
  - Apoptosome activates caspases (proteolytic enzymes)
  - Caspases cleave key cellular proteins → leads to morphologic & biochemical signs of apoptosis