

# "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 ( $\Delta H$ ): A measure of the change ( $\Delta$ ) in heat content of the reactants and products.
    - Entropy ( $\Delta S$ ): A measure of the change in randomness or disorder of the reactants and products.
- Limitations of Individual Quantities
  - Neither enthalpy ( $\Delta H$ ) nor entropy ( $\Delta S$ ) 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:
    - $\Delta G$
    - $\Delta G^0$  (with the superscript "0")
- $\Delta G$  (without superscript "0")
  - Represents the change in free energy.
  - Indicates the direction of a reaction at any specified concentration of products and reactants.
  - $\Delta G$  is a variable.
- $\Delta G^0$  (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  $\rightarrow$  pH = 7.
    - This may be shown using a prime sign [ $\prime$ ], e.g.,  $\Delta G^{0\prime}$ .



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

#### A. $\Delta G$ and Reaction Direction

- $\Delta G$  sign indicates reaction direction at constant temperature and pressure.
- Example Reaction:  $A \rightleftharpoons B$ 
  - If  $\Delta G$  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  $\Delta G$  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  $\Delta G$  is negative and the reaction proceeds spontaneously, it continues until:
    - $\Delta G$  reaches zero
    - Equilibrium is established

## B. $\Delta G$ 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  $\Delta G$  (forward) =  $-5 \text{ kcal/mol}$ , then:
    - $\Delta G$  (reverse) =  $+5 \text{ kcal/mol}$



- Units:
  - $\Delta G$  can be expressed in:
    - kcal/mol
    - kJ/mol
      - 1 kcal = 4.2 kJ

### C. $\Delta G$ and Reactant and Product Concentrations

- $\Delta G$  of the reaction  $A \rightarrow B$  depends on:
  - The concentrations of the reactant and
  - The concentration of the product.
- At constant temperature and pressure, the following relationship can be derived:
  - $\Delta G = \Delta G^0 + RT \ln([B]/[A])$ 
    - $\Delta G^0$ : 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  $\Delta G^0$  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  $\Delta G$  negative, allowing the reaction to proceed
- Example:
  - In the reaction: Glucose 6-phosphate  $\rightleftharpoons$  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  $RT \ln([fructose\ 6-phosphate]/[glucose\ 6-phosphate])$  becomes large and negative
    - This makes  $\Delta G$  negative even though  $\Delta G^0$  is positive
    - Thus, the reaction can still proceed in the forward direction



## D. Standard Free Energy Change

- Standard free energy change ( $\Delta G^0$ ) 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:
  - $\Delta G = \Delta G^0 + 0$

## 1. $\Delta G^0$ and Reaction Direction

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



- However,  $\Delta G^0$  cannot predict the reaction direction under physiologic conditions because:
  - It is composed solely of constants:
    - $R$  (gas constant)
    - $T$  (temperature)
    - $K_{eq}$  (equilibrium constant)
  - It is not altered by changes in product or substrate concentrations.

## 2. Relationship Between $\Delta G^0$ and $K_{eq}$

- In a reaction:  $A \rightleftharpoons B$ 
  - 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:

$$K_{eq} = [B]_{eq} / [A]_{eq}$$

- Where:
  - $K_{eq}$  = equilibrium constant
  - $[A]_{eq}$  and  $[B]_{eq}$  = concentrations of A and B at equilibrium



- If the reaction  $A \rightleftharpoons B$  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:

$$\Delta G^0 = -RT \ln K_{eq}$$

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

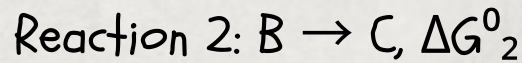
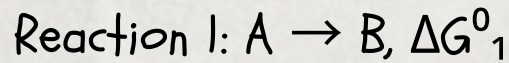
### 3. $\Delta G^0$ of Two Consecutive Reactions

- The  $\Delta G^0$  values are additive for any sequence of consecutive reactions, just like  $\Delta G$  values.

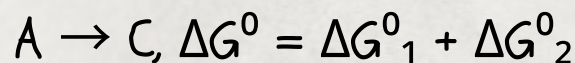


- For example:

- If:

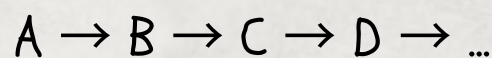


- Then total:



#### 4. $\Delta G$ of a Pathway

- The additive property of  $\Delta G$  is crucial in biochemical pathways such as:



- As long as the sum of the individual  $\Delta G$ s is negative, the overall pathway proceeds in that direction.
- Even if some steps have a positive  $\Delta G$ , the overall pathway can proceed if the total  $\Delta G$  is negative.



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

#### IV. ATP: An Energy Carrier

- Reactions with a large positive  $\Delta G$  can occur if coupled with a spontaneous (exergonic) reaction having a large negative  $\Delta G$ .
  - 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  $\rightarrow$  ADP (Adenosine Diphosphate)
  - Loss of two phosphates  $\rightarrow$  AMP (Adenosine Monophosphate)
- $\Delta G^0$  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:
  - $2 \text{ ADP} \rightleftharpoons \text{ATP} + \text{AMP}$



## II. Free Energy

### V. Electron Transport Chain (ETC)

- Energy-rich molecules like glucose are oxidized stepwise to yield:
  - $\text{CO}_2 + \text{H}_2\text{O}$
- Metabolic intermediates donate electrons to coenzymes:
  - $\text{NAD}^+ \rightarrow \text{NADH}$
  - $\text{FAD} \rightarrow \text{FADH}_2$
- 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  $\text{H}^+$  across the inner mitochondrial membrane



- This creates a proton ( $H^+$ ) gradient, which:
  - Drives ATP synthesis from ADP +  $P_i$
  - 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  $\beta$ -oxidation
  - TCA (Krebs) cycle
- Partially occurs in the matrix:
  - Glucose synthesis
  - Urea synthesis
  - Heme synthesis
- Other matrix components:
  - $\text{NAD}^+$  and FAD  $\rightarrow$  oxidized electron acceptors
  - ADP and  $\text{P}_i$   $\rightarrow$  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)

### I. 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)

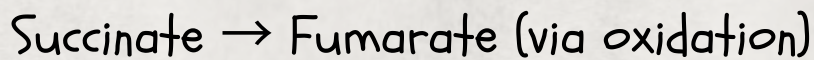
- NADH transfers its electrons to Complex I (NADH dehydrogenase)
- Complex I:
  - Embedded in the inner mitochondrial membrane
  - Contains:
    - Flavin mononucleotide (FMN)  $\rightarrow$  accepts 2 H atoms to form  $FMNH_2$
    - Fe-S peptide subunits
- Electron flow within Complex I:
  - $NADH \rightarrow FMN \rightarrow Fe-S \text{ centers} \rightarrow CoQ$
  - 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:



- Electron transfer path at Complex II:
  - $FADH_2 \rightarrow \text{Fe-S protein} \rightarrow \text{Coenzyme Q (CoQ)}$
- 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<sub>1</sub> 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<sup>3+</sup> (ferric) and Fe<sup>2+</sup> (ferrous) as it accepts and donates electrons



- Electron flow:

- Complex III (cytochrome  $bc_1$ )  $\rightarrow$  Cytochrome  $c$   $\rightarrow$  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  $O_2$

$\rightarrow$  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:
    - $CuA \rightarrow \text{cytochrome } a \rightarrow \text{cytochrome } a_3 \text{ (with } CuB) \rightarrow O_2$

## 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  $\text{NAD}^+$
  - FMN (prosthetic group) is reduced to  $\text{FMNH}_2$
- Each redox reaction can be written as:
  - Two half-reactions: One oxidation + One reduction
- Examples of redox pairs:
  - $\text{NAD}^+ / \text{NADH}$
  - $\text{FMN} / \text{FMNH}_2$
- 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 $\Delta G^\circ$ to $\Delta E^\circ$

- $\Delta G^\circ$  (standard free energy change) is directly related to the change in standard reduction potential ( $\Delta E^\circ$ ) by the equation:

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

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

### 4. $\Delta G^\circ$ of ATP Synthesis

- $\Delta G^\circ$  for phosphorylation of ADP + P<sub>i</sub> → ATP = +7.3 kcal/mol
- Energy released from the transfer of electrons from NADH to O<sub>2</sub> through the ETC = 52.6 kcal/mol



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

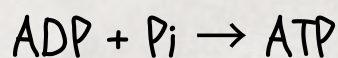
## VI. Phosphorylation of ADP to ATP

- Electron flow through ETC is energetically favorable:
  - Because NADH is a strong electron donor
  - And O<sub>2</sub> 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:



### 1. Proton Pump

- Electron transport is coupled to ADP phosphorylation via proton pumping across the inner mitochondrial membrane.
- $H^+$  is pumped from:
  - Mitochondrial matrix  $\rightarrow$  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  $\beta$  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  $\beta$  subunits of  $F_1$ , enabling:
    - i. Binding of ADP +  $P_i$
    - ii. Phosphorylation of ADP  $\rightarrow$  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  $\rightarrow$  ADP +  $P_i$
    - 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 +  $P_i$  in energy-requiring reactions:
    - More ADP and  $P_i$  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

### I. ATP and ADP Transport

- Adenine nucleotide antiporter:
  - Imports ADP into the mitochondrial matrix
  - Exports ATP into the cytosol (1:1 exchange)
- $\text{P}_i\text{-H}^+$  symporter:
  - Cotransports inorganic phosphate ( $\text{P}_i$ ) and  $\text{H}^+$  from cytosol into matrix
  - Supplies  $\text{P}_i$  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  $\text{FAD} \rightarrow \text{FADH}_2$
  - CoQ of ETC oxidizes  $\text{FADH}_2$
- 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 protein
  - 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:
  - ~40 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