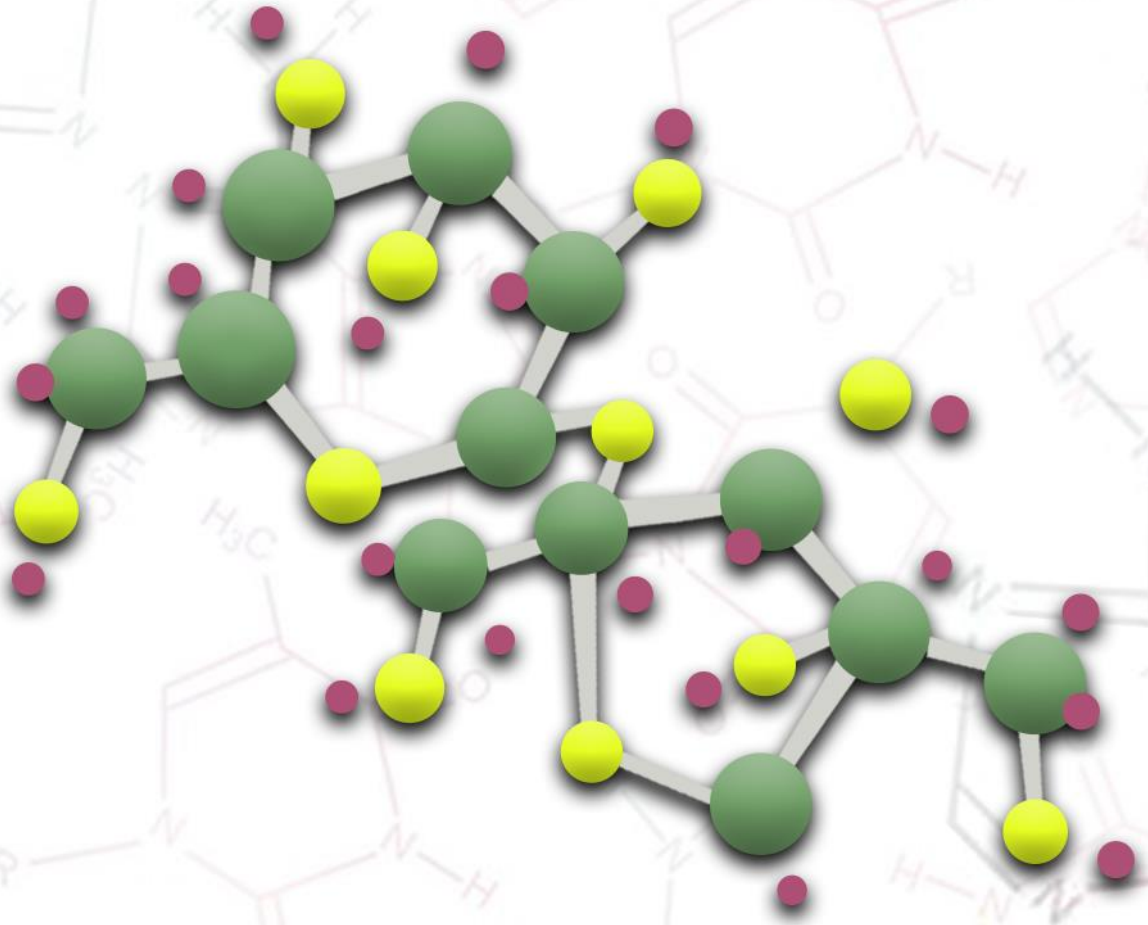
BIOCHEMISTRY

Second material



DONE BY :

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LECTURE :

Bioenergetics and ETC

02

Bioenergetics and Electron Transport Chain (ETC)

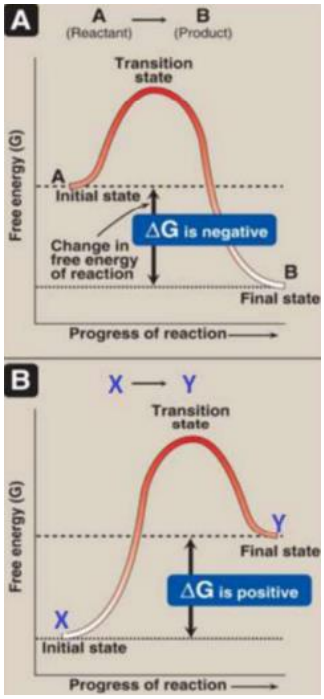
Bioenergetics overview

- Potential energy stored within the compound is called **Gibbs free energy (G)**
- As G increase → stability of the compound decrease
- **Bioenergetics**: is the study of potential energy changes (**ΔG**) in the chemical reaction between reactant (start point) and product (end point)



- All the reaction proceeds till they reach **equilibrium**:
  - Energy of reactants = energy of products (not necessarily concentrations)
  - Rate of forward reaction = rate of backward reaction (net reaction is zero)
- [products]/[reactants] **at equilibrium** is called **equilibrium constant Keq**
  - Each reaction has a specific Keq

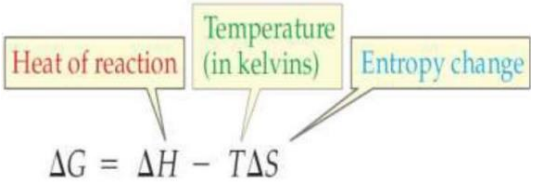
Reaction coordinate diagram (transition sate diagram)



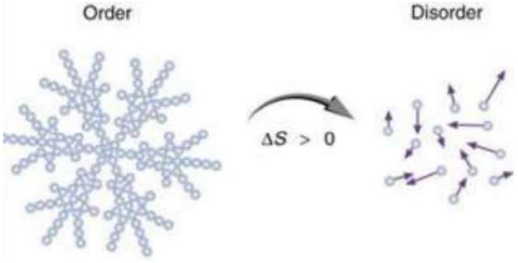
- It's a relationship between free energy (G) and reaction coordinate
- **ΔG = product energy – reactant energy**
- In the first reaction to the right, the reaction has a **negative ΔG** (product energy < reactant energy) → so this reaction is **favorable, spontaneous and exergonic** محرر للطاقة
  - This reaction will go **forward** to reach the equilibrium (ΔG = 0)
- In the second reaction to the right, the reaction has a **positive ΔG** (product energy > reactant energy) → so the reaction is **unfavorable, non-spontaneous and endergonic** مستهلك للطاقة
  - This reaction will go **backward** to reach the equilibrium (ΔG = 0)

❖ Notes:

- ✓ ΔG **tells nothing about the <u>rate of the reaction</u>**, it tells whether the reaction is spontaneous or not (the direction of the reaction), it predicts whether the reaction can take place or not
- ✓ **Spontaneous** means that the reaction will take place in the forward direction
- ✓ If ΔG = zero, then the reaction is at equilibrium (both direction at the same rate) → end of the reaction



Kelvin = Celsius + 273



- **H: Enthalpy** = Heat (total energy in the system)
  - If ΔH is –ve → Exothermic reaction محرر للحرارة
  - If ΔH is +ve → Endothermic reaction مستهلك للحرارة
- **S: Entropy** = freedom or randomness of the molecules
  - If ΔS is –ve → low entropy (freedom) → more order → less stable
  - If ΔS is +ve → high entropy (freedom) → less order → more stable

❖ Notes:

- ✓ Only ΔG determine whether the reaction is spontaneous or not
- ✓ ΔH or ΔS **alone cannot determine** the spontaneity of the reaction
- ✓ ΔG will surely be negative if ΔH is negative (exothermic) **And** ΔS is positive (high entropy)

- To compare reaction correctly, reactions must carried out at the same conditions called **standard conditions** where the **concentration of reactants and products equal = 1**
  - ΔG at standard conditions is **called ΔG°**
  - There is an equation for the relationship between ΔG and ΔG° for a reaction A→B below

$$\Delta G = \Delta G^0 + RT \ln \frac{[B]}{[A]}$$

ΔG: Gibb's free energy at any conditions
ΔG°: Gibb's free energy at standard conditions
R: Gas constant (1.987 cal/mol.K or 1.987 x 10^{-3} Kcal/mol.K)
T: absolute temperature in K

  - From the equation ΔG depends on **Temperature** and **Concentration of the reactants and products**

$$A + B \rightleftharpoons C + D$$

$$Keq = \frac{[Product]eq}{[Reactant]eq}$$

- **If Keq > 1 →** at equilibrium [Product] > [Reactant]
- **If Keq < 1 →** at equilibrium [Product] < [Reactant]
- **If Keq = 1 →** at equilibrium [Product] = [Reactant]

So if you know Keq for the reaction, you can determine which will be more when the reaction reach the equilibrium

At Equilibrium

- Rate of forward = Rate of Backward (net reaction is zero)
- Energy of Product = Energy of Reactant (ΔG = zero)
- $\frac{[Product]eq}{[Reactant]eq}$ is constant **Keq**

So, $\Delta G = \Delta G° + R\,T \ln \frac{[Product]}{[Reactant]}$

Zero= ΔG° + R T ln Keq

ΔG° = - R T ln Keq

At Equilibrium

- If [Product] > [Reactant] → Keq > 1

Ln Keq → positive , then ΔG° will be negative

A ⇌ B

- If [Product] < [Reactant] → Keq < 1

Ln Keq → negative , then ΔG° will be Positive

A ⇌ B

- If [Product] = [Reactant] → Keq = 1

Ln 1 → zero , then ΔG° will be zero

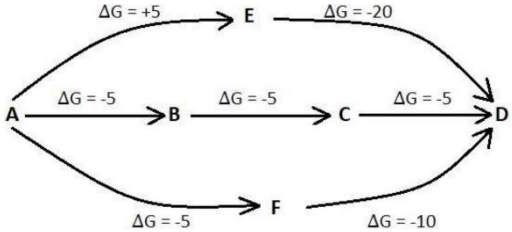
A ⇌ B

Energy coupling

- If you have a reaction with positive ΔG (endergonic) and you want the reaction to proceed forward, **we couple this reaction with another that has negative ΔG (exergonic)**
- The energy released from the second reaction is paid for the first reaction
- The energy released from the exergonic reaction **must be enough** for the endergonic reaction (more or at least equal) and **this is what happens in the metabolic pathways**

$$T \xrightarrow{\Delta G1} A \xrightarrow{\Delta G2} R \xrightarrow{\Delta G3} E \xrightarrow{\Delta G4} Q$$

- ΔG is additive → overall ΔG of the pathway equals the sum of ΔG of all individual steps of that pathway (overall ΔG T→Q = ΔG1 + ΔG2 + ΔG3 + ΔG4)
- We care about the overall ΔG of the pathway to determine whether its spontaneous or not
- ΔG depends only on the **initial and final state**, **independent on the path taken of the reaction**
- If A→B ΔG = -X then B→A ΔG = +X

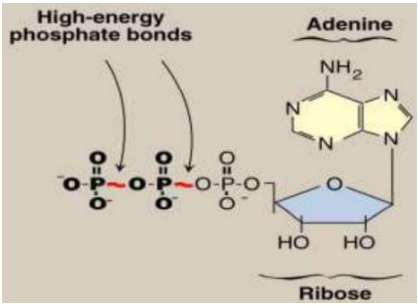


Q: if A→B ΔG = -5 and C→B ΔG = -3 calculate ΔG for A→C

Ans: we need to know the ΔG of A→B→C pathway and we have A→B ΔG but not B→C ΔG. although we have C→B ΔG = -3 so B→C ΔG = +3. Now overall ΔG = -5+3 = -2

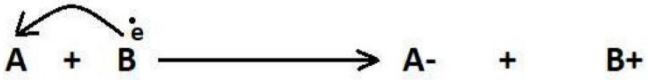
ATP

- Adenosine Tri Phosphate (ATP) is a high energy compound
- Hydrolysis of ATP provides energy for most processes that need energy in the body
- ATP → ADP + Pi ΔG = -7.3
- ADP → AMP + Pi ΔG = -7.3
- ATP → AMP +2Pi ΔG = -14.6 (which equals losing 2 ATP molecules to ADP)
- To regenerate ATP (ADP + Pi →ATP ΔG = +7.3)
  - So phosphorylation of ADP to ATP requires energy that is **provided by catabolism of organic fuel (carbohydrate, lipids and proteins)**

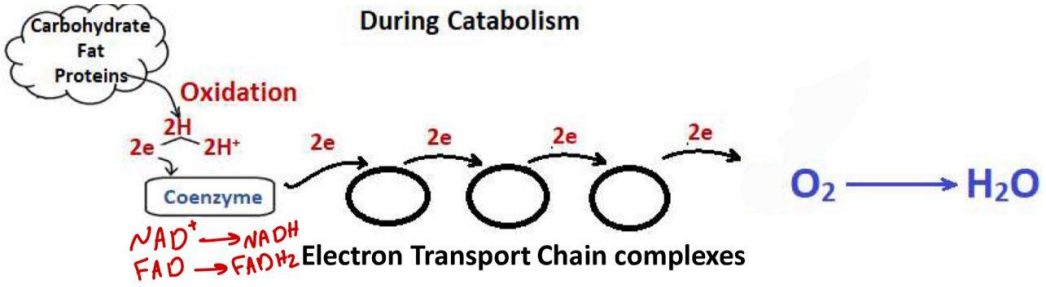


Redox reaction

- **Oxidation**: loss of electrons/ Hydrogen or gain of O2
- **Reduction**: gain of electrons/ Hydrogen or loss of O2
- Oxidation and reduction **must occur together**; one substance lose electrons (oxidation) while other substance accept electrons (reduction)
- **Oxidizing agent (oxidant)**: Reduction مادة يحصل لها
- **Reducing agent (reductant)**: Oxidation مادة يحصل لها
- **Reducing potential (E°)**: the tendency of a compound to accept electrons (to be reduced)



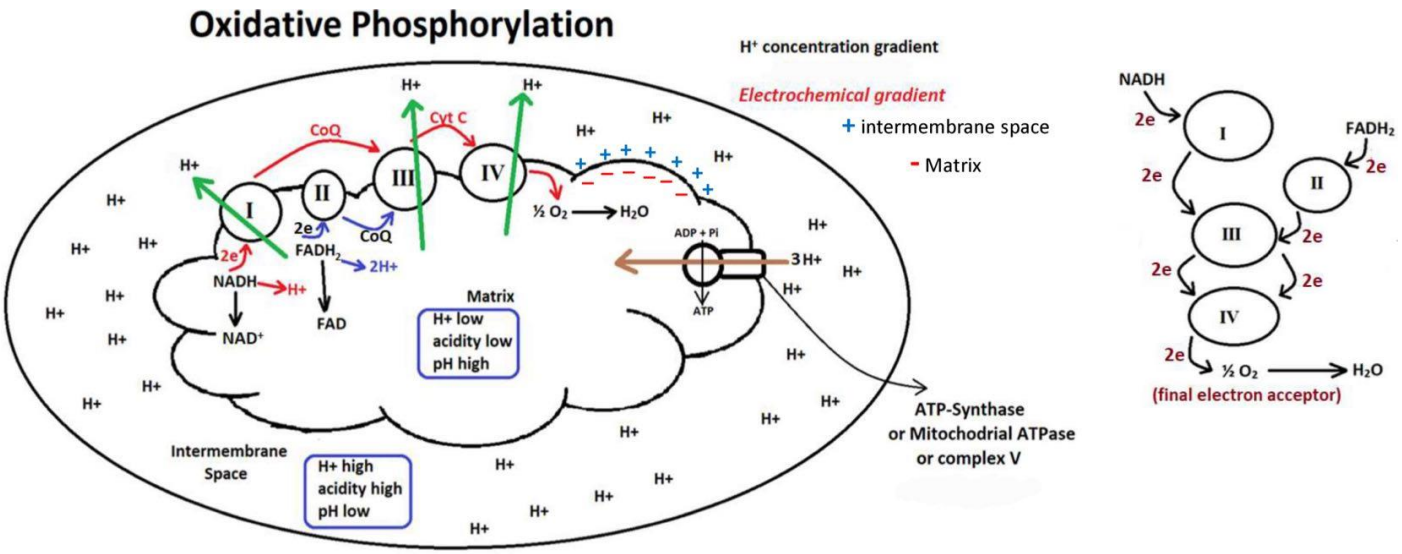
- In this reaction, reduction potential (E°) of A is higher than B
- If E° is **positive** → high tendency to accept electrons (stronger oxidizing agents)
- If E° is **negative** → low tendency to accept electrons so high tendency to lose electrons (stronger reducing agents)
- In **catabolism** of fuel (carbohydrate, fat and proteins), the fuel is **oxidized** (lose H/electrons), these electrons transferred initially to Coenzymes mainly NAD^+ and FAD
- NAD^+ (oxidized) accepts $2e^-$ and $1H^+$ forming NADH (reduced)
- FAD (oxidized) accepts $2e^-$ and $2H^+$ forming FADH2 (reduced)
- Now the reduced Coenzymes (NADH and FADH2) donate their electrons to **O2 (final ultimate electron acceptor)** through **electron transport chain** complexes in the inner mitochondrial membrane
- Movement of electrons from NADH and FADH2 toward O2 is **exergonic** and the energy released is **used to regenerate ATP** (ADP + Pi → ATP), this process is called **oxidative phosphorylation**



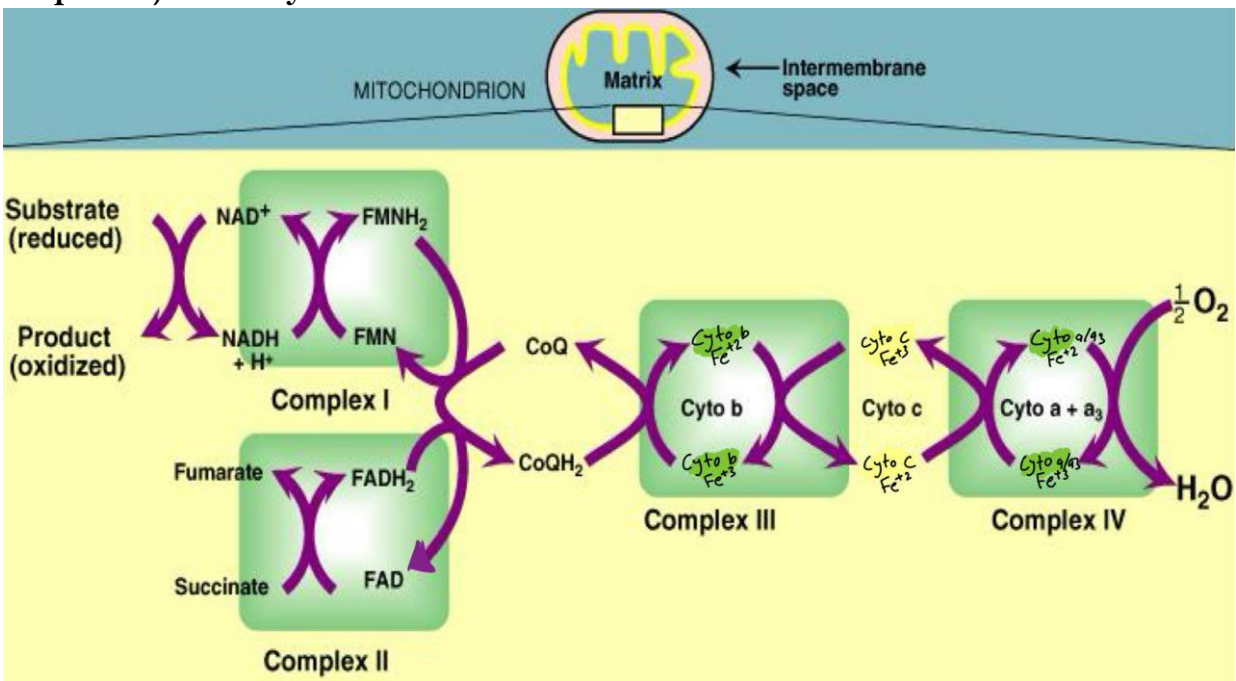
- In ETC electrons moves according to reduction potential from **low E° to high E°**
- So NADH and FADH2 have the **lowest E°** while O2 has the **highest E°**

Electron Transport Chain (ETC)/Respiratory chain

- Oxidative phosphorylation: is the oxidation of NADH and FADH2 using ETC and O2 that will lead to phosphorylation of ADP and production of ATP
- All NADH and FADH2 are produced from catabolism (oxidation) of carbohydrates, proteins and lipids. These NADH and FADH2 give their electrons to O2 through electron transport chain complex
- There are 5 complexes in ETC, complex 3 and 4 contains **cytochrome proteins**



- According to the picture, the process of ETC occurs in the **inner mitochondrial membrane**
- We have 3 related reactions in ETC
  1) Electron transport from one complex to another is an **exergonic process** (produce energy)
  2) The energy produced from electron transport is used to actively pump H^+ from the matrix to the intermembrane space **creating an electrochemical gradient**
  3) This gradient represents an energy that **drive H^+ back to the matrix through ATP-synthase (complex V) which synthesize ATP**
- The picture to the right is the ETC complexes in more details
- You have to know that before complex 3, when the substance is reduced it will have H. After complex 3,

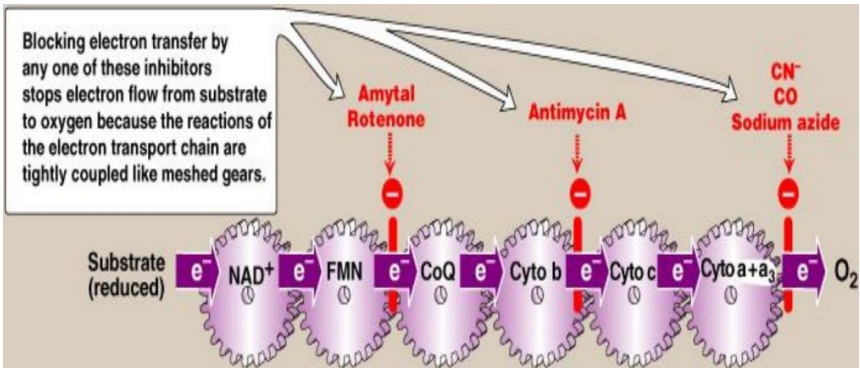


when the substance is reduced its Fe will be Fe^{+2} not Fe^{+3}.

- The cytochromes in complex 3 and 4 contains of <u>polypeptide + **Heme**</u>
  - Heme contains **Iron Fe^{+3} (ferric, oxidized iron)** that becomes **Fe^{+2} (ferrous, reduced iron)**

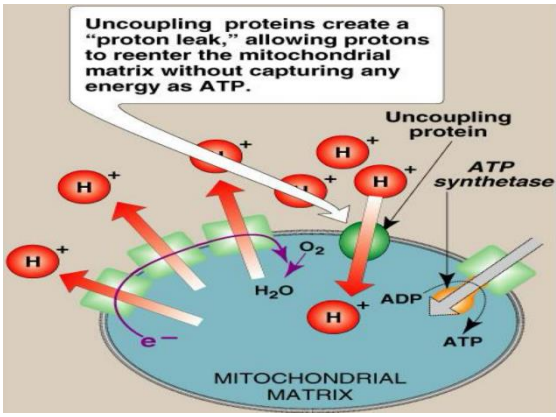
ETC/respiratory inhibitors

- When we use a respiratory inhibitor that block the transfer of electrons at specific point:
  - Carriers **before** the site of inhibition will accumulate in their **reduced form**
  - Carriers **after** the site of inhibition will accumulate in their **oxidized form**



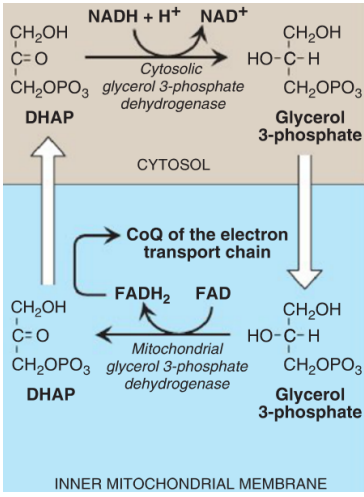
Uncoupling proteins (UCPs)

- UCPs are channel proteins found in the inner mitochondrial membrane that enable H^+ to return matrix **without passing through ATP synthase** → so the energy of returning H^+ will not be used to produce ATP, **it will be released as <u>heat</u>**
- An example of synthetic UCP is **2,4 Dinitophenol (DNP)** which is used to **decrease weight**



NADH shuttling

- A lot of NADH are synthesized outside the mitochondria and for them to participate in ETC they have to be translocated across the outer and inner mitochondrial membrane to the matrix
- It can simply diffuse through the outer membrane, but needs a **specific shuttle** to be transported through **inner membrane** to the matrix. We have 2 types of shuttle:



1) <u>Glycerol-3-P Dehydrogenase shuttle</u>
   - NADH in the cytosol gives its 2 electrons to Dihydroxyacetone-P (**DHAP**) producing **Glycerol-3-P** by enzyme called **<u>cytosolic</u> Glycerol-3-P Dehydrogenase**
   - Then Glycerol-3-P enters the mitochondria
   - In mitochondria Glycerol-3-P gives the 2 electrons to FAD by an enzyme called **<u>mitochondrial</u> Glycerol-3-P Dehydrogenase**
   - Now FAD becomes **FADH2 in the mitochondrial matrix**
2) <u>Malate – Aspartate shuttle</u>
   - NADH in the cytosol gives its 2 electrons to **Oxaloacetate** producing **Malate** by enzyme called **<u>cytoplasmic</u> Malate Dehydrogenase**
   - Then Malate enters the mitochondria
   - In mitochondria Malate gives the 2 electrons to NAD^+ by an enzyme called **<u>mitochondrial</u> Malate dehydrogenase**
   - Now NAD^+ becomes **NADH in the mitochondrial matrix** and Malate becomes Oxaloacetate. Then Oxaloacetate will be converted to Aspartate by Aminotransferase enzyme

- Aspartate gets out of mitochondria to cytosol where it is converted back to Oxaloacetate by Aminotransferase as well

