

Describe the mitochondrial ATP synthesising enzyme. How is it driven by the respiratory chain and how does it work?

Introduction

- Oxidative phosphorylation is the coupling of TCA cycle to ATP production
- Enzyme involved is the F_0F_1 ATPase via discharge of the PMF

The enzyme

- Transmembrane spanning; inner mitochondrial membrane. Two parts
- a) F_1 (ATPase independently) consists of 3α and 3β subunits (orange) and 3 single copy peptides: γ , δ and ϵ . β units are catalytically active
- b) F_0 cylindrical complex of C subunits acting as a proton channel
- γ subunit couples the F_0 and F_1 parts. Inserts asymmetrically into $\alpha_3\beta_3$ core
- γ unit is able to rotate within the core due to torque development in F_0 unit.
- F_0F_1 ATPase is a rotary molecular motor
- Stator joins $\alpha_3\beta_3$ core with F_0 segment to prevent rotation of $\alpha_3\beta_3$ core

How is it driven by respiratory chain?

- In metabolic pathways reduced coenzymes NAD and FAD are generated
- Regeneration to allow continuation of pathways = electron transport chain
- Coenzymes pass reducing equivalents through a series of protein complexes in inner membrane that are increasingly oxidative. O is terminal acceptor – H_2O
- Some complexes pump protons against concentration gradient into intermembrane space using energy from endergonic oxidation of coenzymes
- Complex I, III pump 4 protons; complex II none and complex IV two protons
- $NADH_2 = 10$ protons; $FADH_2 = 6$ protons
- Properties of membrane lead to generation of potential difference across membrane – a.k.a. Proton Motive Force
- Peter Mitchells ‘chemiosmotic theory’; discharge of PMF through F_0F_1 ATPase results in the synthesis of ATP (F_0 acts as proton channel)

How does it work?

- Proton movement generates torque in F_0 segment of F_0F_1 ATPase
- γ subunit caused to rotate. Due to asymmetrical insertion into $\alpha_3\beta_3$ core rotation causes conformational change in β catalytic units = results in the catalytic activity: $ADP + Pi \rightarrow ATP$
- β unit can exist in three conformations: open, loose binding, tight binding
- Open state – very low affinity for substrate which enters active site
- Loose binding – substrates take up conformation ready for catalysis
- Tight binding – catalytic event occurs, ATP bound tightly
- Open state – very low affinity for ATP which dissociates
- Each step involves a rotation of the γ subunit within the $\alpha_3\beta_3$ core
- The process is known as the Binding Change Mechanism
- Suggested that 3 protons must be discharged through F_0F_1 ATPase for the synthesis of 1 ATP molecule (4 in total due to discharge of one to facilitate the transport of the substrates for the reaction across the mitochondrial membrane)