Understanding the Role of Periplasmic Loop Residues of *E. coli* ATP synthase

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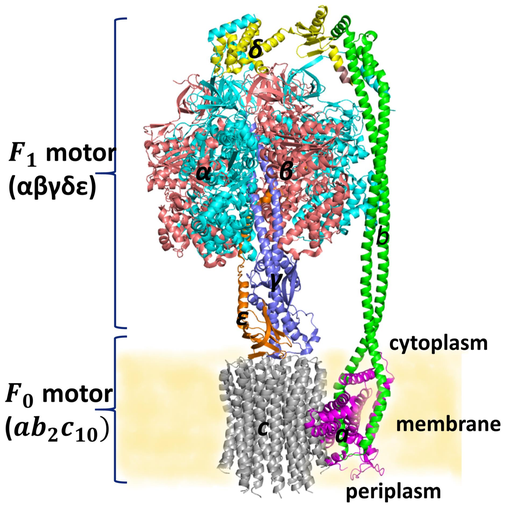
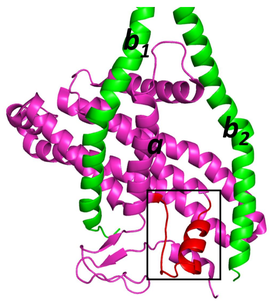
Background



- ATP synthase is a biological macromolecular complex that catalyzes the production of ATP.
- It consists of two unique nanomotors: a membrane bound F_0 motor and soluble F_1 motor.
- In presence of the electrochemical gradient, proton movement down the gradient rotates the F_0 motor which induces conformation change in the F_1 motor, synthesizing ATP from ADP and inorganic phosphate.
- Under certain circumstances, ATP synthase can hydrolyze ATP to transport protons against the electrochemical gradient



- Dysfunction of ATP synthase imbalances cellular energy, resulting in disease and even cell death.
- The increase in drug-resistant bacterial infections necessitate the identification of novel drug target sites on ATP synthase.
- Difference in sequence and composition of TM 2-3 loop of subunit *a* across species may help selectively target ATP synthase of different bacteria.

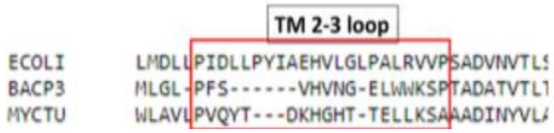
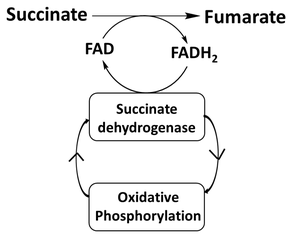


Figure 1: Subunit *a* of bacterial ATP Synthase: A) Residues T M 2-3 loop, shown in red, suggested to interact with subunit b_2. B) Sequence alignment of subunit a from *E.coli*, *Bacillus PS3* and *Mycobacterium tuberculosis*, highlighting the TM 2-3 loop.

Growth on Succinate

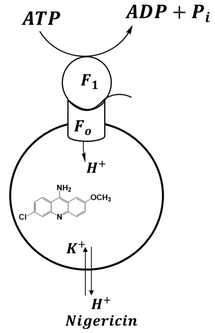
- Succinate is a nonfermentable carbon source that can be metabolized only in presence of functional ATP synthase
- Colony size is measured to determine defect in growth
- Slight defects in growth is seen for TM loop delete, G135C and L136C mutants

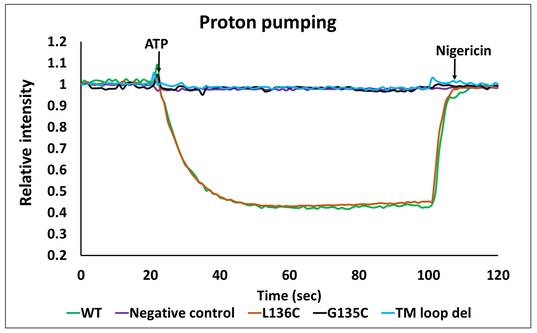


Cell Type	Growth	Colony Size
Wild Type	Positive	2 mm
Negative Control	Negative	N/A
TM loop del	Negative	1.5 mm
G135C	Positive	1.75mm
L136C	Positive	1.75 mm

ATP-driven Proton Pumping

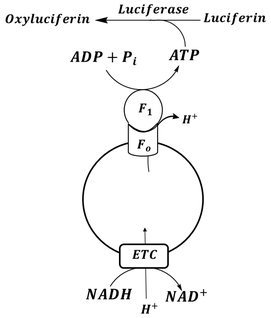
- Relies on the fluorescence of ACMA dye, which freely diffuses through the membrane
- Addition of ATP pumps proton into the vesicle and protonates ACMA, quenching the fluorescence
- Addition of nigericin destabilizes the gradient restoring fluorescence
- G135C and TM loop delete mutants inhibit proton pumping
- L136C is able to pump protons similarly to wild type

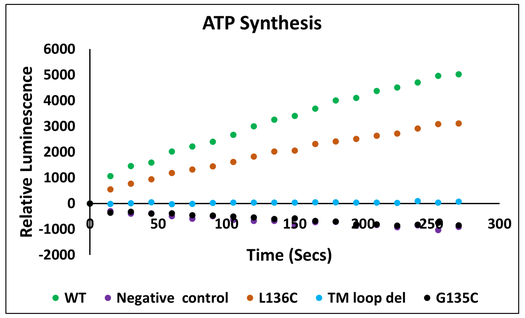




ATP Synthesis

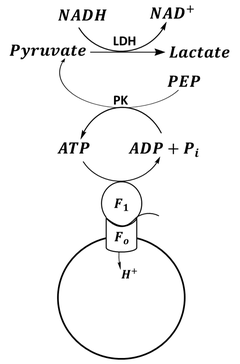
- Concentration of ATP over time is measured using ATP-dependent luciferase/luciferin system
- NADH activates electron transport chain (ETC) and pumps proton into the vesicle driving synthesis of ATP, which is required for oxidation of luciferin to oxyluciferin.
- G135C and TM loop delete mutants show no ATP synthesis activity
- L136C mutant shows decrease in luminescence indicating slight defect in ATP synthesis

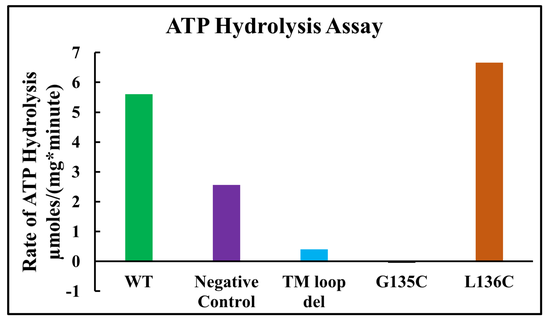




ATP Hydrolysis

- Rate of ATP hydrolysis is measured using NADH-linked ATP-regeneration system.
- ATP hydrolysis is coupled to NADH oxidation via lactate dehydrogenase and pyruvate kinase.
- G135C and TM loop delete mutants show defect in ATP hydrolysis consistent with the proton pumping assay
- L136C enhances the rate of hydrolysis.





Conclusion/ Future Directions

- TM 2-3 loop delete mutant affects all the activities of ATP synthase suggesting its importance for the function of the enzyme.
- G135C mutation suggests that glycine at position 135 seems to be an important residue. However, additional mutations will give more insight to the essential characteristics of this residue.
- L136C mutation reveals asymmetric effects on proton movement.
- The experiments with these residues will need to be replicated for validation as well as the expression levels of each mutant needs to be determined.
- We need to optimize the ATP hydrolysis assay to decrease the activity of negative control.

Acknowledgement & References

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