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A mutational study of the energy transmission in *E. coli* ATP synthase

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Abstract

ATP synthase catalyzes ATP synthesis in oxidative phosphorylation. With the unique mechanism and structure of ATP synthase, the energy transmission between the rotor and stator complex plays a vital role to maintain its proper function. Our previous study has discussed that in γC87K mutation, a stronger interaction with βE381 could refrain the rotor complex from smooth spin, leading to insufficient energy coupling between ATP hydrolysis/synthesis and proton translocation. In this study, we engineered alanine mutations to mimic a weaker rotor/stator interaction. We found that multiple alanine mutations also impair the enzyme performance. This research will add more pieces to understand the energy flow in ATP synthase.

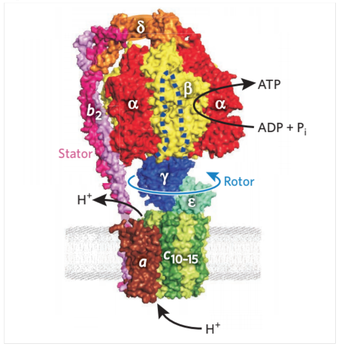


Figure 1: The structure of *E. coli* F_1F_0-ATP synthase. ab2$\alpha_3\beta_3\delta$ subunits form the stator complex; $c_{10}\gamma\varepsilon$ subunits form the rotor complex. Proton flowing across the membrane pushes the rotation of the rotor and generates a torque against the stator. The conformational alternation of the catalytic "crown" leads to ATP synthesis.

Methods and Techniques

Plasmid purification
- Obtain the pSN6 (parent) plasmid template.
- NEB plasmid purification kit.

Site-directed mutagenesis
- Make mutations in the original DNA strand.
- Single primer PCR strategy.

Transformation I
- Digest template DNA by *Dpn* I.
- Transform into NEB DH5α strain.

Colony verification I
- Colony PCR.
- Plasmid purification.

Colony verification II
- Restriction enzyme digestion.
- Agarose gel electrophoresis.
- DNA sequencing.

Transformation II
- Transform plasmids with desired mutations.
- DK8 strain (an *E. coli* strain lacking of *atp* operon).

Phenotype assay
- Measure growth yield of the WT and mutants.
- 8 mM succinate medium.
- Turbidity at Abs 590 nm.

ATPase activity assay
- Prepare membrane vesicles.
- ATP hydrolysis assay.

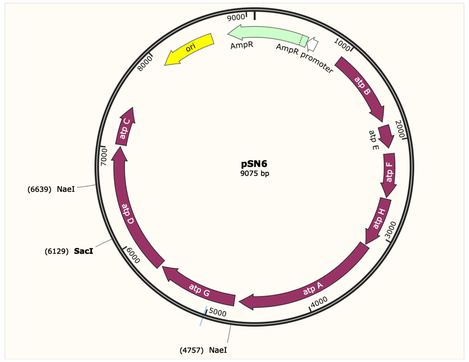
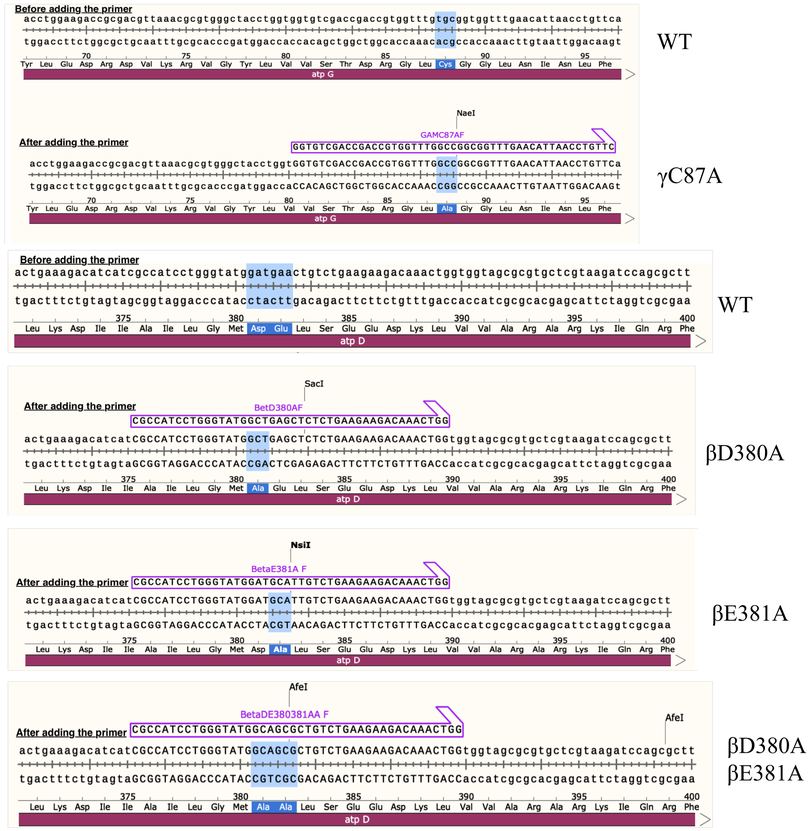
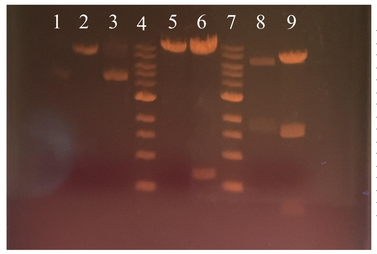


Figure 3:
Upper: Plasmid pSN6 carries the *atp* operon. It was used as the parent DNA in mutagenesis.

Lower: Mutagenetic nucleotide oligomers were used. An extra restriction site was added (or removed) in the primers for colony screen.



Results



Lane 1: WT + *Nsi* I
Lane 2: βE381A + *Nsi* I
Lane 3: βD380A/E381A + *Nsi* I
Lane 4 and 7: NEB 1-kb DNA ladder
Lane 5: WT + *Sac* I
Lane 6: βD380A + *Sac* I
Lane 8:WT + *Nae* I
Lane 9: γC87A + *Nae* I

Figure 4: Agarose gel electrophoresis shows the restriction patterns of the WT and mutant plasmid. A different pattern against it parent plasmid indicates that a mutation has been introduced. The presence of desired mutations was verified by DNA sequencing.

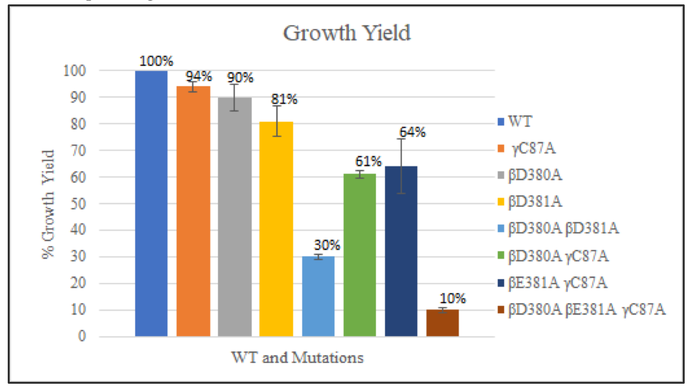


Figure 5: WT and mutants were inoculated into medium containing 8 mM succinate, allowing it to grow aerobically until saturation at 37 °C. Growth yield was evaluated from turbidity of the liquid culture by measuring its absorbance at 590 nm. The error bars represent the standard deviation of the percent growth yield mean of four samples tested.

	WT	γC87A	βD380A	βE381A	βD380A βE381A	βD380A γC87A	βE381A γC87A	βD380A βE381A γC87A
ATPase Activity (U/mg)	1.5 ± 0.2	1.3 ± 0.2	2.0 ± 0.4	1.0 ± 0.1	1.3 ± 0.3	2.9 ± 0.4	1.4 ± 0.2	1.0 ± 0.2

Table 1: ATPase activities were assayed in a buffer containing 50 mM Tris/H_2SO_4, 4 mM $MgSO_4$, 10 mM ATP and 1 μM carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), pH 8.0, at 25 °C. Inorganic phosphate released from the reaction was quantified using molybdate method. Each of the two biological samples were assayed in duplicate.

Mutations

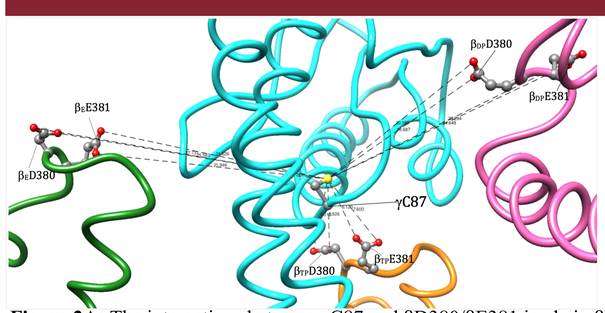


Figure 2A: The interactions between γC87 and βD380/βE381 in chain β_{DP} (pink), β_E (green) and β_{TP} (orange). (Protein Data Bank ID: 3OAA)

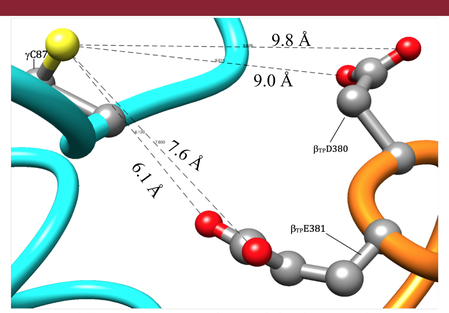


Figure 2B: A close-up view of the interaction between γC87 and β_{TP}D380/β_{TP}E381.

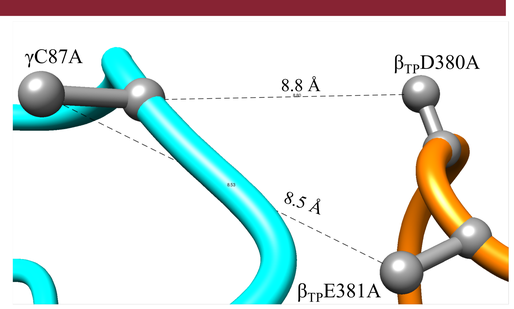


Figure 2C: Alanine replacements will lead to weaker interaction between γC87A and β_{TP}D380A/β_{TP}E381A.

Conclusion

When the native side chains of βD380, βE381, and γC87 in *E.coli* ATP synthase were replaced with alanine, the inter-residue interaction became weaker.

This weaker rotor/stator interaction would result in energy uncoupling between proton translocation and ATP synthesis/hydrolysis.

References

1. Weber, J. (2010). Structural biology: Toward the ATP synthase mechanism. *Nature Chemical Biology, 6*(11), 794-795.
2. Mnatsakanyan, N., Li, Y., Weber, J. (2019). Identification of two segments of the γ subunit of ATP synthase responsible for the different affinities of the catalytic nucleotide-binding sites. *Journal of Biological Chemistry,294*(4), 1152-1160.