

THE MECHANISM OF ADP PHOSPHORYLATION BY ATP SYNTHASE

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F₁F₀-ATP synthase catalyzes the final step of oxidative phosphorylation, the synthesis of ATP from ADP and phosphate, driven by a transmembrane proton gradient. In bacteria, under certain physiological conditions, the enzyme can also hydrolyze ATP to generate a proton gradient. ATP synthesis/hydrolysis and proton translocation are coupled in a unique way, via subunit rotation. While our knowledge of the molecular basis of ATP hydrolysis and its integration with rotation is still far from complete, it is much better understood than ADP phosphorylation because it is easier experimentally accessible [1].

We have used a combination of fluorescence spectroscopy, site-directed mutagenesis, and biochemical analysis to investigate the mechanism of ATP synthase. We demonstrated that all three catalytic nucleotide binding sites have to be filled for catalysis to occur [2], and we developed models for the coordination of the essential Mg²⁺ ion in the enzyme-bound Mg²⁺-nucleotide complex [3] and for the catalytic transition state [4]. The experimental results allowed us to derive a hypothesis for the molecular mechanism of ATP synthesis. The model attempts to explain how ADP is bound despite the presence of an excess of ATP, how phosphate is bound, how rotation brings ADP and phosphate close enough to form first a transition state complex and then ATP, and how hydrolysis of the newly-formed ATP is prevented.

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