Rotation of some mutants of thermophilic F_1-ATPase

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A single molecule of F_1-ATPase works as a rotary protein motor. It is a water soluble portion of the ATP synthase that catalyzes the synthesis of adenosine tri-phosphate (ATP) in animals, plants and bacteria. The minimal stable sub-complex of F_1-ATPase for catalysis consists of $\alpha_3\beta_3\gamma$ subunits. A high resolution structure of bovine mitochondrial F_1-ATPase (MF_1) determined by Abrahams *et al.* (1994), has revealed that three α- and three β-subunits are alternately arranged to form a stator cylinder. Three β subunits bind and hydrolyze ATP whereas three α subunits bind ATP without hydrolysis. An α-helical coiled coil made of the amino- and carboxy-termini of the γ-subunit deeply penetrates the central cavity of the stator cylinder and is held by the cylinder wall at two positions, at the orifice and the bottom. The tip of the carboxy-terminus of the γ-subunit held by the bottom of the stator cylinder was suggested to play important roles in rotation of γ-subunit. Whether the tip of γ subunit plays any role in rotation, however, remains an intriguing question. In this study, an $\alpha_3\beta_3\gamma$ sub-complex of F_1-ATPase derived from thermophilic Bacillus PS3 (TF_1) having a structure similar to MF_1 was expressed in *Escherichia coli*. The γ-subunit was genetically truncated both at its amino- and carboxy- termini step by step until the remaining rotor portion would be outside the stator cavity and simply sit on the concave entrance of the stator orifice. Rotation of the wild type and mutants were observed adopting single molecule techniques. It was found that all truncation mutants rotated in the correct direction, though some mutants exhibited moments of irregular motion. The time-averaged rate of rotation as well as the rate of ATP hydrolysis was low in the mutants compared to the wild type, indicating that most of the interactions between the rotor and the stator cylinder are important for the rapid progress of catalysis. These interactions, however, are not essential for rotation.

Abrahams JP, Leslie AGW, Lutter R & Walker JE. (1994) *Nature*, **370:** 621-8.