Direct observation of the rotation of F1 ATPase

Biochemistry 4000
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Noji et al., 1997
Introduction

- In most biological systems, the ATP synthase sits in the membrane and catalyses the synthesis of ATP from ADP and phosphate driven through proton gradient generated by electron transfer.

- The ATP synthase operates through a rotation mechanism in which the three active sites undergo a conformational change in binding affinity for the reactants, as originally predicted by Paul Boyer (1997).

- This rotation has been demonstrated experimentally by the work of Noji et al., 1997.
Structural elements...

- **ATP Synthase Structure...**
  - F1 (extrinsic) & F0 (intrinsic)

- **F1** 5 polypeptides
  - 3α, 3β, 1γ, 1δ & 1ε
  - α and β (513 and 460 aa) are homologous
  - 3 catalytic sites for ATP synthesis one on each β subunit

- **F0** (271 aa in E. Coli.) 3 polypeptides in ratio of 1a, 2b, and 12c (C-ring)
  - a-subunit formed half-channel that allows the passage of proton through the membrane
Crystal structures....

Bovine mitochondrial $F_1$

Yeast mitochondrial $F_o$
How ATPase works...

1. active site is on [β-subunits] & it changes conformation through 3 successive shapes (L-T-O)  
   O - open - site has low affinity to bind ATP - thus releases it  
   L - loose - ADP & P loosely bound to site  
   T - tight - ADP & P tightly bound favoring condensation without water

2. conformational changes result in rotation of subunits relative to central stalk (γ)
Objectives

- Principle objective was to observe the direct rotation of ATPase.
  
- Clockwise or anticlockwise rotation
  
- Dependency of rotation on heavy load and ATP
  
- Rotation speed and torque needed to make rotation
The subcomplex bound to the glass through 3 β subunit-

His-tag linked to N terminus of each β subunit.

The glass plate was coated with horseradish peroxidase conjugated with Ni-NTA.

Construct mutant (α-C193S, γ-S107C) of thermophilic bacillus, the α3β3γ subcomplex was purified,

- Cystein of γ subunit was biotinylated and a fluscerently labelled, biotinylated actin filament was attached to γ subunit through the streptavidin.
Rotating images…..

*1 out of 70 continuously
*15 out of 70 shows in and fro fluctuation
*rotary speed was not constant
  -tends to dwell in the bottom half

-axis length-2.6 \( \mu \text{m} \)
-axis length-2.4 \( \mu \text{m} \)

-rotary speed-0.5 r.p.s
-rotary speed-1.3 r.p.s

-time interval-133ms
-time interval-33ms

>45pN
>23pN
Unpredicted test…

-1800 filaments were under each test
  -Conditions were-
    1. +ATP
    2. -ATP
    3. +ATP+NaN3

-25 filaments made more than 5 turns in first 25s observations.
- No filaments made more than 2 turns in 25s.
- Pauses
- filament immobilized -39 /90 was torn off-7/90
- rotation continued >1 min-44/90
- some filament continued rotation >10min

1μm=0.5-1.4μm
2μm=1.5-2.4μm
3μm=2.5-3.4μm
Summary and Discussion

• Principle objective was to observe the direct rotation of ATPase. Rotation is seen under different length of actin filament.

• Clockwise or anticlockwise rotation - they found anticlockwise rotation as they considered ATP hydrolysis.

• Dependency of rotation on heavy load and ATP - slower movement under higher load and in absence of ATP no rotary motion.

• Rotation speed and torque needed to make rotation - high torque and slow rotation under larger actin filament.
Future direction......

Hirono-Hara et al., 2001 proposed that F1 ATPase has at least three phases during catalysis: continuous rotation, short and long pauses. They also proposed it rotates in discrete 120 degree steps and is further divided into 90 and 30 degree.

Lino et al., 2006 suggested that ε subunit works as ‘switch’ that change the F0F1 between ATP hydrolysis and synthesis mode.

Moser et al., 2001 established ATP metabolism play a role in angiostatin response.

- that binding of angiostatin or specific antibodies to the α/β-subunits inhibit the enzyme by blocking conformational changes required for ATP synthesis or hydrolysis.