ESSAY: AMERSHAM PHARMACIA BIOTECH & *SCIENCE* PRIZE

The Rotary Enzyme of the Cell: The Rotation of F1-ATPase

Hiroyuki Noji

Amersham Pharmacia Biotech & *Science* are pleased to announce the 1998 grand prize winner of the Amersham Pharmacia Biotech & *Science* Prize for Young Scientists. The grand prize has been awarded to a regional winner from Japan, Hiroyuki Noji.

AMERSHAM PHARMACIA BIOTECH & SCIENCE *prize* FOR YOUNG SCIENTISTS

Is there really a rotating enzyme within the cell? Every day, our body synthesizes approximately its own weight in ATP, which acts as an energy source for cells. ATP synthase (*1*), the enzyme responsible for synthesizing most of this ATP, is located on the inner membrane of the mitochondria. This common enzyme is also found in the plasma membrane of bacteria and the thylakoid membrane in chloroplasts. For ATP synthesis, it utilizes a proton electrochemical potential generated by biomembranes containing respiratory chains and photochemical systems. ATP hydrolysis in turn generates this potential when the ATP synthase pumps protons in the opposite direction. Its reverse reaction, proton transport, is accompanied by ATP hydrolysis. ATP synthesis and degradation are performed primarily by the portion of the enzyme exposed on the membrane (termed F1-ATPase); alone, F1-ATPase exhibits high ATP hydrolytic activity. Proton translocation is made possible by the F_0 portion of the enzyme, which crosses the membrane. Electron microscopy (*2*) has shown that the catalytic site of F1-ATPase is separated by approximately 80 Å from the F_0 proton-transport region, and the two are in contact with the F1-ATPase γ subunit. Energy transformation between ATP synthesis-hydrolysis and proton translocation is thus communicated by the γ subunit. Therefore, analysis of structural changes in the γ subunit can illuminate the mechanism of energy transmission for the enzyme.

On the basis of extensive kinetic analyses from a large number of researchers, Boyer proposed a model for the catalytic mechanism of F1-ATPase. Among other properties, this "binding-change mechanism" predicted that the energy was transmitted through rotation of the γ subunit in the center of the F1-ATPase molecule (*3*). Boyer's model was almost entirely substantiated by experimental results, with the exception of the rotation hypothesis. In 1994, Walker and colleagues reported the (at the time biggest) x-ray crystal structure of F1-ATPase (*4*), which strongly indicated that the enzyme acts as a "motor." The structure that they described was a ring formation consisting of three α subunits alternating with three β subunits having catalytic sites. The rod-shaped γ subunit spanned the center of the ring. This x-ray crystal structure provided a specific and easily understood image that permitted rotational movement of the γ subunit, a concept that had initially been considered unconventional. Does this enzyme really rotate? At the time, I remained unconvinced.

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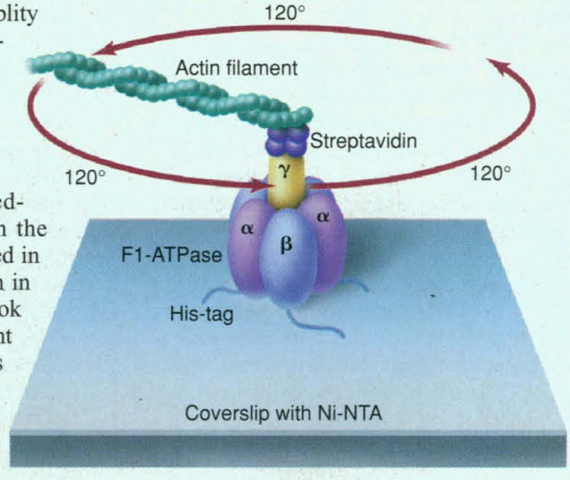
Unchanged Secondary Structure of γ

What might be another possiblity besides rotational motion? Because Jagendorf and colleagues reported the use of a tritium exchange reaction to study secondary structural changes within F1-ATPase (*5*), I first focused on the "coiled-coil" configuration by which the two longest helices are wrapped in the γ subunit, as could be seen in the x-ray crystal structure. I took this approach because in recent years there have been reports of major changes in the coiled-coil configuration of proteins. Perhaps this structure of the γ subunit changed greatly during the process of the catalytic reaction. To test this hypothesis, I determined whether the stability of the helixes within F1-ATPase could be assessed by an amide proton exchange reaction (*6*). After labeling isolated γ subunits with tritium, I added α and β subunits to reconstitute the molecule. Subsequently, I compared the amount of tritium remaining in the γ subunit before and after hydrolysis. However, no difference in tritium content before and after the catalytic reaction could be found. In other words, the γ subunit did not undergo major secondary structural changes during ATP hydrolysis. So was the rotation yet the correct model? I could think of no other that was consistent with the x-ray crystal structure image.

Observation of Rotational Motion

Most biochemical experiments are based on the observation of the behavior of many molecules. In case of the detection of the rotation, however, it is impossible to detect rotation from the average behavior of all molecules. Nevertheless, by using some very elaborate methods, several groups reported results supporting the rotation (*7, 8*). Simply showing that the γ subunit moved, however, did not prove that it actually rotated. It was a case of "seeing is believing." Only the direct observation of the single rotating F1-ATPase molecule could provide this evidence. Since the rotation of the γ subunit itself was far too small to be detected directly, I decided to attach a giant probe (fluorescently labeled actin filaments) to the portion of the γ subunit protruding on the F_0 side of the membrane. In addition, to eliminate rotational Brownian movement throughout the whole molecule, I introduced a histidine tag to the amino-terminal end of the three β subunits opposite to the



Visualizing rotation. Experimental system for the observation of the rotation of the γ subunit in F1-ATPase ($\alpha_3\beta_3\gamma$ subcomplex). The His-tagged F1-ATPase was immobilized, and fluorescent actin filaments were attached to the subcomplex through streptavidin for observation with an epifluorescence microscope. When ATP was infused into the chamber, the actin filaments always rotated counterclockwise. In the presence of low concentrations of ATP, they rotated in discrete 120° steps.

probe, and fixed the entire molecule to a glass substrate coated with Ni-nitrilloacetic acid (NI-NTA). After preparing this experimental system, I observed the motion of the actin filaments using a fluorescent microscope. The actin filaments attached to the F1-ATPase only rotated in the presence of Mg-ATP (*9*). Moreover, the direction of rotation was without exception counterclockwise, and continued for at least 10 min. This was not Brownian movement, but rotation of the γ subunit. In addition, the direction of rotation corresponded to the direction that was predicted from the x-ray crystal structure. Specifically, the rotational movement I observed provided evidence that the three catalytic sites in the three β subunits catalyze synchronously and also that the x-ray crystal structure indeed corresponds to a reaction intermediate state.

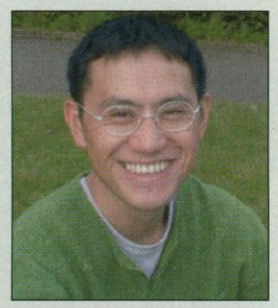
By observing this rotation at extremely low ATP concentrations, I found that the rotation occurs in increments of 120°, one step per molecule of ATP hydrolyzed (*10*). The orientation of the γ subunit changes with each ATP hydrolysis, occurring sequentially at β subunits positioned 120° apart on the molecular ring.

In these measurements, the actin filaments attached to the γ subunit rotated against the viscous resistance of water. The amount of rotational torque required for this movement could be estimated from the length of the actin filaments and the rate of rotation to provide a mean value of approximately 40 pN nm. For a single step of 120° and, hence, a single ATP hydrolysis, this amount to work of 80 pN nm. This value is in close agreement with those calculated for the release of free energy from the hydrolysis of a single ATP molecule under physiological conditions. The addition of ADP and P_i caused a shift in the free energy change for a single ATP molecule to 110 or 90 pN nm, but the work required for 120° rotation remained unchanged. These findings clearly indicate that the energy conversion efficiency for this enzyme is extremely high. This may be a necessary characteristic that allows the reverse reaction to occur.

The results of these experiments provided direct proof of Boyer's rotational model, which had once been considered unconventional. My findings show this enzyme to be a newly recognized motor protein with a conformation very similar to that of the portions of other proteins, such as myosin, which are also involved in structural changes accompanying ATP hydrolysis. It seems probable that these motor proteins share the same fundamental energy conversion mechanism (*11*). I hope to continue my research in comparing these motor proteins that convert chemical energy to mechanical energy and vice versa. In the long run, this might deepen our understanding of how specific properties could be acquired starting from a common principle, as it is seen so often throughout the biosphere.

1998 Grand Prize Winner

Hiroyuki Noji was born on 8 September 1969 in Sapporo, Hokkaido. He earned his masters thesis in the laboratory of Tairo Oshima in the Department of Bioscience of the Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, in Yokohama. His thesis topic was heat stabilization of 3-isopropylmalate dehydrogenase by random screening. He received his doctoral training at the Tokyo Institute of Technology in 1995 in the Department of Electronic Chemistry, Interdisciplinary Graduate School of Science and Engineering, in the laboratory of Masasuke Yoshida.



Hiroyuki Noji

Dr. Noji's Ph.D. thesis topic was on the detection of the rotation of the γ subunit of F1-ATPase. As part of his search for methods to detect the rotation, Dr. Noji participated in the 33rd Biophysical Society meeting of Japan in Sapporo. While there, he learned of techniques for the detection of single molecules and met with Ichiro Sase, a student in the laboratory of Kazuhiko Kinosita Jr., of Keio University, who reported the detection of the polarization of a single molecule of fluorescent dye. Soon afterward, he began collaborating with Ryohei Yasuda in Kinosita's laboratory on the detection of the rotation using the method of single molecule detection. They detected the rotation of the γ subunit in September 1996, and Dr. Noji completed his doctoral work in December 1997.

Since January, Dr. Noji has studied the mechanism for the energy conversion of ATP synthase as a postdoctoral researcher in the CREST genetic programming team 13, organized by Keio University's Kinosita.

Regional Winners

Europe: Stephane Marcand, for his essay, "A Protein-Counting Mechanism Regulates Telomere Length," which is based on his doctoral research in the laboratory of David Shore at Columbia University, College of Physicians and Surgeons, Department of Microbiology, and in the laboratory of Eric Gilson, Department of Molecular and Cellular Biology, Ecole Normale Superieure, Lyon, France.

North America: Jamie H. Cate, for his essay, "Structural Basis for an RNA World," reporting work done at Yale University, New Haven, CT; Arul M. Chinnaiyan, for his essay, "Destined to Die: Molecular Dissection of the Cell Death Machine," on work done at the University of Michigan, Ann Arbor, MI; and Camilla M. Kao, for her essay, "Modular Polyketide Synthases: Programming and Engineering Chemical Diversity," describing work performed at Stanford University, Stanford, CA.

Japan: Taro Nishinaka, for his essay, "A Novel Configuration of DNA Induced by Homologous Recombination," reporting work done at the University of Tokyo, Tokyo, Japan.

The other finalists were as follows: from Europe, James P. J. Chong and Francois Ichas; from North America, Thomas R. Chandinin and Wallace Marshall; and from all other countries, Ehud Gazit.

The full text of the essays written by the regional winners and information about applying for next year's award can be seen in *Science* Online at http://www.sciencemag.org/feature/data/pharmacia/1998.shl

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