RESEARCH ACTIVITIES X
Okazaki Institute for Integrative Bioscience

X-A Single-Molecule Physiology

A single molecule of protein (or RNA) enzyme acts as a machine which carries out a unique function in cellular activities. To elucidate the mechanisms of various molecular machines, we need to observe closely the behavior of individual molecules, because these machines, unlike man-made machines, operate stochastically and thus cannot be synchronized with each other. By attaching a tag that is huge compared to the size of a molecular machine, or a small tag such as a single fluorophore, we have been able to image the individual behaviors in real time under an optical microscope. Stepping rotation of the central subunit in a single molecule of F_1-ATPase has been videotaped, and now we can discuss its detailed mechanism. RNA polymerase has been shown to be a helical motor that rotates DNA during transcription. Myosin V and VI are also helical motors that move as a left- or right-handed spiral on the right-handed actin helix. Single-molecule physiology is an emerging field of science in which one closely watches individual, 'live' protein/RNA machines at work and examines their responses to external perturbations such as pulling and twisting. I personally believe that molecular machines operate by changing their conformations. Thus, detection of the conformational changes during function is our prime goal. Complementary use of huge and small tags is our major strategy towards this end.

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X-A-1 One Rotary Mechanism for F_1-ATPase over ATP Concentrations from Millimolar down to Nanomolar

SAKAKI, Naoyoshi; SHIMO-KON, Rieko; ADACHI, Kengo; ITOH, Hiroyasu[1,2]; FURUIKE, Shou; MUNEYUKI, Eiro[3]; YOSHIDA, Masasuke[3,4]; KINOSITA, Kazuhiko, Jr.
([1]Hamamatsu Photonics; [2]CREST; [3]Tokyo Inst. Tech.; [4]ERATO)

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F_1-ATPase is a rotary molecular motor in which the central γ-subunit rotates inside a cylinder made of $\alpha_3\beta_3$-subunits. The rotation is driven by ATP hydrolysis in three catalytic sites on the β-subunits. How many of the three catalytic sites are filled with a nucleotide during the course of rotation is an important yet unsettled question. Here we inquire whether F_1 rotates at extremely low ATP concentrations where the site occupancy is expected to be low. We observed under an optical microscope rotation of individual F_1 molecules that carried a bead duplex on the γ-subunit. Time-averaged rotation rate was proportional to the ATP concentration down to 200 pM, giving an apparent rate constant for ATP binding of 2×10^7 $M^{-1}s^{-1}$. A similar rate constant characterized bulk ATP hydrolysis in solution, which obeyed a simple Michaelis-Menten scheme between 6 mM and 60 nM ATP. F_1 produced the same torque of ~40 pN·nm at 2 mM, 60 nM, and 2 nM ATP. These results point to one rotary mechanism governing the entire range of nanomolar to millimolar ATP, although a switchover between two mechanisms cannot be dismissed. Below 1 nM ATP, we observed less regular rotations, indicative of the appearance of another reaction scheme.

X-A-2 ATP-Driven Stepwise Rotation of F_oF_1-ATP Synthase

UENO, Hiroshi[1]; SUZUKI, Toshiharu[1,2]; KINOSITA, Kazuhiko, Jr.; YOSHIDA, Masasuke[1,2]
([1]Tokyo Inst. Tech.; [2]ERATO)

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F_oF_1-ATP synthase (F_oF_1) is a motor enzyme that couples ATP synthesis/hydrolysis with a transmembrane proton translocation. F_1, a water-soluble ATPase portion of F_oF_1, rotates by repeating ATP-waiting dwell, 80° substep rotation, catalytic dwell, and 40°-substep rotation. Compared with F_1, rotation of F_oF_1 has yet been poorly understood, and, here, we analyzed ATP-driven rotations of F_oF_1. Rotation was probed with an 80-nm bead attached to the ring of c subunits in the immobilized F_oF_1 and recorded with a submillisecond fast camera. The rotation rates at various ATP concentrations obeyed the curve defined by a K_m of ≈30 μM and a V_{max} of ≈350 revolutions per second (at 37 °C). At low ATP, ATP-waiting dwell was seen and the $k_{on\text{-}ATP}$ was estimated to be 3.6×10^7 $M^{-1}\cdot s^{-1}$. At high ATP, fast, poorly defined stepwise motions were observed that probably reflect the catalytic dwells. When a slowly hydrolyzable substrate, adenosine 5'-[γ-thio]triphosphate, was used, the catalytic dwells consisting of two events were seen more clearly at the angular position of ≈80°. The rotational behavior of F_oF_1 resembles that of F_1. This finding indicates that "friction" in F_o motor is negligible during the ATP-driven rotation. Tributyltin chloride, a specific inhibitor of proton translocation, slowed the rotation rate by 96%. However, dwells at clearly defined angular positions were not observed under these conditions, indicating that inhibition by tributyltin chloride is complex.

X-A-3 Activation of Pausing F_1 Motor by External Force

ONO-HARA, Yoko[1]; ISHIZUKA, Koji[2]; KINOSITA, Kazuhiko, Jr.; YOSHIDA, Masasuke[2];

NOJI, Hiroyuki[1]
([1]Tokyo Inst. Tech.; [2]Univ. Tokyo)

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A rotary motor F_1, a catalytic part of ATP synthase, makes a 120° step rotation driven by hydrolysis of one ATP, which consists of 80° and 40° substeps initiated by ATP binding and probably by ADP and/or P_i dissociation, respectively. During active rotations, F_1 spontaneously fails in ADP release and pauses after a 80° substep, which is called the ADP-inhibited form. In the present work, we found that, when pushed >+40° with magnetic tweezers, the pausing F_1 resumes its active rotation after releasing inhibitory ADP. The rate constant of the mechanical activation exponentially increased with the pushed angle, implying that F_1 weakens the affinity of its catalytic site for ADP as the angle goes forward. This finding explains not only its unidirectional nature of rotation, but also its physiological function in ATP synthesis; it would readily bind ADP from solution when rotated backward by an F_o motor in the ATP synthase. Furthermore, the mechanical work for the forced rotation was efficiently converted into work for expelling ADP from the catalytic site, supporting the tight coupling between the rotation and catalytic event.