

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 Catalysis and Rotation of F_1 Motor: Cleavage of ATP at the Catalytic Site Occurs in 1 ms before 40° Substep Rotation

SHIMABUKURO, Katsuya¹; YASUDA, Ryohei²; MUNEYUKI, Eiro¹; HARA, Kiyotaka Y.³; KINOSITA, Kazuhiko, Jr.; YOSHIDA, Masasuke^{1,3}
(¹Tokyo Inst. Tech.; ²Cold Spring Harbor Laboratory; ³ERATO)

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F_1 , a water-soluble portion of $F_0 F_1$ -ATP synthase, is an ATP hydrolysis-driven rotary motor. The central γ -subunit rotates in the $\alpha_3\beta_3$ cylinder by repeating the following four stages of rotation: ATP-binding dwell, rapid 80° substep rotation, interim dwell, and rapid 40° substep rotation. At least two 1-ms catalytic events occur in the interim dwell, but it is still unclear which steps in the ATPase cycle, except for ATP binding, correspond to these events. To discover which steps, we analyzed rotations of F_1 subcomplex ($\alpha_3\beta_3\gamma$) from thermophilic *Bacillus PS3* under conditions where cleavage of ATP at the catalytic site is decelerated: hydrolysis of ATP by the catalytic-site mutant F_1 and hydrolysis of a slowly hydrolyzable substrate ATP γ S (adenosine 5'-[γ -thio]triphosphate) by wild-type F_1 . In both cases, interim dwells were extended as expected from bulk phase kinetics, confirming that cleavage of ATP takes place during the interim dwell. Furthermore, the results of ATP γ S hydrolysis by the mutant F_1 ensure that cleavage of ATP most likely corresponds to one of the two 1-ms events and not some other faster undetected event. Thus, cleavage of ATP on F_1 occurs in 1 ms during the interim dwell, and we call this interim dwell catalytic dwell.

X-A-2 Mechanically Driven ATP Synthesis by F_1 -ATPase

ITOH, Hiroyasu^{1,2}; TAKAHASHI, Akira¹; ADACHI, Kengo; NOJI, Hiroyuki³; YASUDA, Ryohei⁴; YOSHIDA, Masasuke⁵; KINOSITA, Kazuhiko, Jr.
(¹Hamamatsu Photonics; ²CREST; ³Univ. Tokyo; ⁴Cold Spring Harbor Laboratory; ⁵ERATO)

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ATP, the main biological energy currency, is synthesized from ADP and inorganic phosphate by ATP synthase in an energy-requiring reaction. The F_1 portion of ATP synthase, also known as F_1 -ATPase, functions as a rotary molecular motor: *in vitro* its γ -subunit rotates against the surrounding $\alpha_3\beta_3$ subunits, hydrolysing ATP in three separate catalytic sites on the β -subunits. It is widely believed that reverse rotation of the γ -subunit, driven by proton flow through the associated F_0 portion of ATP synthase, leads to ATP synthesis in biological systems. Here we present direct evidence for the chemical synthesis of ATP driven by mechanical energy. We attached a magnetic bead to the γ -subunit of isolated F_1 on a glass surface, and rotated the bead using electrical magnets. Rotation in the appropriate direction resulted in the appearance of ATP in the medium as detected by the luciferase–luciferin reaction. This shows that a vectorial force (torque) working at one particular point on a protein machine can influence a chemical reaction occurring in physically remote catalytic sites, driving the reaction far from equilibrium.

X-A-3 Chemomechanical Coupling in F_1 -ATPase Revealed by Simultaneous Observation of Nucleotide Kinetics and Rotation

NISHIZAKA, Takayuki^{1,2,6}; OIWA, Kazuhiro¹; NOJI, Hiroyuki³; KIMURA, Shigeki¹; MUNEYUKI, Eiro⁴; YOSHIDA, Masasuke^{4,5}; KINOSITA, Kazuhiko, Jr.

(¹Kansai Adv. Res. Cent.; ²PRESTO; ³Univ. Tokyo;
⁴Tokyo Inst. Tech; ⁵ERATO; ⁶Gakushuin Univ.)

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F₁-ATPase is a rotary molecular motor in which unidirectional rotation of the central γ subunit is powered by ATP hydrolysis in three catalytic sites arranged 120° apart around γ . To study how hydrolysis reactions produce mechanical rotation, we observed rotation under an optical microscope to see which of the three sites bound and released a fluorescent ATP analog. Assuming that the analog mimics authentic ATP, the following scheme emerges: (i) in the ATP-waiting state, one site, dictated by the orientation of γ , is empty, whereas the other two bind a nucleotide; (ii) ATP binding to the empty site drives an ~80° rotation of γ ; (iii) this triggers a reaction(s), hydrolysis and/or phosphate release, but not ADP release in the site that bound ATP one step earlier; (iv) completion of this reaction induces further ~40° rotation.

X-A-4 Unconstrained Steps of Myosin VI Appear Longest among Known Molecular Motors

ALI, Md. Yusuf¹; HOMMA, Kazuaki²; IWANE, Atsuko H.³; ADACHI, Kengo; ITOH, Hiroyasu^{4,5}; KINOSITA, Kazuhiko, Jr.; YANAGIDA, Toshio³; IKEBE, Mitsuo²

(¹Shahjalal Univ., Bangladesh; ²Univ. Massachusetts Medical School, USA; ³Osaka Univ.; ⁴Hamamatsu Photonics; ⁵CREST)

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Myosin VI is a two-headed molecular motor that moves along an actin filament in the direction opposite to most other myosins. Previously, a single myosin VI molecule has been shown to proceed with steps that are large compared to its neck size: either it walks by somehow extending its neck or one head slides along actin for a long distance before the other head lands. To inquire into these and other possible mechanism of motility, we suspended an actin filament between two plastic beads, and let a single myosin VI molecule carrying a bead duplex move along the actin. This configuration, unlike previous studies, allows unconstrained rotation of myosin VI around the right-handed double helix of actin. Myosin VI moved almost straight or as a right-handed spiral with a pitch of several micrometers, indicating that the molecule walks with strides slightly longer than the actin helical repeat of 36 nm. The large steps without much rotation suggest kinesin-type walking with extended and flexible necks, but how to move forward with flexible necks, even under a backward load, is not clear. As an answer, we propose that a conformational change in the lifted head would facilitate landing on a forward, rather than backward, site. This mechanism may underlie stepping of all two-headed molecular motors including kinesin and myosin V.