system, we directly observed the proton-driven rotation of F_0F_1 in clockwise, which is the reverse direction of ATP-driven rotation, and also obtained various information related to the “dynamics”, such as the stepwise rotary motion coupled with the proton transport and ATP synthesis, which provided a clue for understanding the rotary catalysis mechanism of F_0F_1 in the physiological condition.

1PS034 分子動力学計算を用いて明らかにする γ 16° 回転前後の Yeast F1-ATPase の構造的特徴

Molecular Dynamics Simulation on Structural Characteristics of Yeast F1-ATPase before and after 16-degree Rotation of Gamma Subunit

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We performed molecular dynamics simulations for crystal structures of yeast F1-ATPase whose crystallographic unit contains two different states. One complex binds Pi in the β E subunit, and the other liberated it. In the latter structure, the position of central stalk is also rotated +16 degree in the hydrolysis direction. Because single molecule experiments have proven that Pi release occurs at the β E subunit, and the γ subunit is rotated after ATP hydrolysis and Pi release, these structures are supposed to represent snapshots before and after Pi releases for the 40 degree substep, respectively. From their motional trajectories, we describe how the F1-ATPase complex changes the structure after Pi release. Then, we propose a possible mechanism of the 40 degree rotation of the γ subunit.

1PS035 Single-molecule analyses of the rotation and regulation of human F1-ATPase

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F1-ATPase (F_1) is the smallest rotary motor in life. The rotation and regulation mechanisms have been deeply investigated by using bacterial enzymes, especially Bacillus PS3 F_1 (TF₁). One hydrolysis reaction of ATP makes 120°-rotation and during the 120°, two kinds of dwelling events, named ATP-binding dwell and catalytic dwell, are observable by single molecule microscopic analyses. Although researchers have desired to precisely compare the experimental results to structural information from X-ray crystal study to understand the rotary catalysis mechanism, available structural information is only from eukaryotic enzymes. Additionally, bacterial F_1 s have no sensitivity toward most of chemical and protein factors affecting eukaryotic F_1 , especially human F_1 . To overcome the difficulties, we have established systems for recombinant human F_1 .

Single molecule analyses showed counterclockwise rotation of the human F_1 by the energy of ATP as seen in bacterial F_1 , but its rotation was remarkably fast, 705 ± 75 rps at 26°C and over 1,366 ± 43 rps at human body temperature, which was roughly 3-folds faster than TF₁. Slow hydrolysable ATP-analogue, ATP- γ S, clarified the presence of catalytic dwell in it, although the angle was different from that of TF₁. Rotation analyses using several factors, such as inhibitory factor-I, revealed that another novel 3rd dwelling position is present in human F_1 . These results suggest that rotation in mammalian F_1 is not necessarily same to that of bacterial enzymes.

1PS036 一分子 FRET 法によるキネシン頭部のヌクレオチド依存的な構造変化の観察

Single molecule FRET observation of the nucleotide-dependent conformational changes of the kinesin motor domain

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Kinesin-1 is a motor protein that moves along microtubules by alternately exchanging two motor heads. Our lab recently showed that ATP hydrolysis cycles of trailing and leading heads are differently regulated depending on the direction of the neck linker extension, although it is still unknown how the tension posed to the neck linker affects the nucleotide-binding site distal from

the neck linker. Based on the recently solved crystal structure which revealed nucleotide-dependent rotational motion within the motor domain, we hypothesized that ATP binding/hydrolysis can be regulated by the neck linker tension through restricting the subdomain rotation. To test this hypothesis, we developed a single-molecule FRET probe to detect the rotational motion in the kinesin head. We labeled a residue in the rotating subdomain and a residue in the microtubule-interface in kinesin monomer with donor and acceptor dyes. The FRET efficiency distribution in nucleotide-free and AMPPNP states showed distinct peaks separated by about 10%, indicating that the conformational difference before and after rotation can be distinguishable. Then, we labeled with this probe the leading head of the heterodimeric kinesin in which trailing head is a immotile mutant head, and showed that the leading head mainly takes pre-rotating conformation even in the presence of ATP. These results are consistent with the idea that ATP binding in the leading head is prohibited because the backward tension on the neck linker suppresses the rotation in the head.

1PS037 キネシンのヘッド間張力がヌクレオチド結合部位に与える影響 (分子シミュレーションによる調査)

The effect of the internal tension between two heads on the nucleotide binding site of kinesin-1 studied by All-atom MD

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The dimeric motor protein kinesin-1 walks along microtubules (MT) in a hand-over-hand manner by alternately hydrolyzing ATP and moving two heads. The internal tension between two heads may be related to the regulation for the coordinated walking of kinesin, although the detail mechanism remains unknown. In this study, we focus on the mechanism how the internal tension imposed on the neck-linker region affects the nucleotide binding site in motor domain allosterically. To address this problem, we constructed the dimeric kinesin models bound on MT at on-path way "Tatp-Lapo" state (where the trailing head is ATP bounded state and the leading head is in nucleotide-free) and the monomeric model on MT at apo-state by using the new resolved x-ray structures. We performed all-atom MD and compared the structures and fluctuations at nucleotide binding sites between the dimeric (with internal-tension) and the monomeric model (without tension). Based on the comparison, we will discuss the validity of “front-head gating” mechanism which proposes ATP binding to the leading head is prohibited due to the internal-strain.

1PS038 スピンラベル ESR によるキネシンのネックリンカードッキング、リンカー間歪み、ヌクレオチド-リンカーサイト間伝達の動的構造解析

Structural dynamics of necklinker docking, interlinker strain and nucleotide-to-linker transmission in kinesin probed by spin-label ESR

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We have used site-directed spin label (SDSL) ESR to explore 3 structural events in kinesin monomer (KM) and dimer (KD) at atomic resolution; neck linker (NL) motion, the NL strain by 2 motor heads (MHs) and intersite transmission or communication. 1) We directly measured the distance between 2 SLs at motor domain (MD) (at C223) and NL (at C336), by availing spectral broadening. The NL-MD distance of KM on MTs was 1-2 nm (dock) in AMPPNP state whereas >2 nm (undock) in the no nucleotide (NN) or ADP state. 2) The distance between the C336s of NLs close to an NL-stalk junction of KD on MT was 1-2 nm (unstrained) in NN state whereas >2 nm (strained) in AMPPNP or ADP state. Using ESR, we for the first time detected the stress imposed by 2 docked NLs of front and rear MHs in AMPPNP state or by the dissociated and mobile MH in ADP state. 3) α -1 helix spans between nucleotide (nuc) and NL sites. We estimated steric hindrance, from SL mobility, around SL-cys residue 61, 62, 66, 67, 70, or 72 of α -1 in KM on MT. In NN state, the mobility of C66 and C67 was changed reversely; the mobility of C66 decreased, whereas that of C67 increased. Therefore, it is likely that nuc and NL sites communicate with each other through “rotation” of this helix that induces structural changes in both sites to regulate nuc affinity or NL docking to MD. We would discuss the structural transmission mechanism in KD by combining these data with the previous data on undock-dock transition and the length of NLs in the KD (Sugata et al. BBRC '04, JMB '09).