2P152 微小管におけるキネシンとの相互作用部位のマッピング
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Mapping of kinesin-interaction sites on microtubule
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Kinesin moves along a microtubule by alternately switching between the strong- and weak-binding states depending on the species of the bound nucleotide. By mutational analysis of tubulin in yeast expression system, we previously identified E410, D417, and E421 in helix12 (H12) of β -tubulin as critical residues for the strong binding of kinesin (Uchimura et al., EMBO J., 2006). However, structural studies indicate that these residues might form only a part of the interface between tubulin and kinesin.

To conclusively identify the residues involved in the interaction, a comprehensive set of charged-to-alanine mutants was generated in the microtubule area spanning H11, H12, and the loop between these helices in both α - and β -tubulin. A total of 34 mutants were tested, and 6 acidic residues and 2 basic residues (including the 2 previously reported acidic residues in H12 of β -tubulin) were found to be haploid lethal in yeast cells, indicating these 8 amino acids might play vital roles in *in vivo* microtubule functions. When the motility of the two-headed kinesin on microtubules purified from these lethal mutants was analyzed, the kinesin could scarcely move along the microtubules that had charged-to-alanine mutations in the loop between H11 and H12 of α -tubulin (E416A and E418A). Measurement of the unbinding force is currently underway in order to determine the binding state modulated in these mutants. The mechanism of how each of these critical residues is involved in kinesin motility will be discussed by referring to structural data.

2P154 蛍光標識ATP アナログを用いたイネキネシン O12 の生化学的解析
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Biochemical analysis of the Rice Kinesin O12 using Fluorescent-ATP Analogue
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Kinesin is a motor protein that plays important physiological roles in intracellular transport, mitosis and meiosis, control of microtubule dynamics and signal transduction. Kinesin converts chemical energy from ATP into mechanical force. Kinesin family is classified into some subfamilies. Some species of kinesin derived from vertebrate have been well studied on their characterization. However, not so many studies for kinesins of plants have been done yet. Recently, the genome sequences of rice have been completed. Our preliminary bioinformatics analysis revealed at least more than 35 kinesins related protein encoded on the rice genome. In this study, we focused on rice specific kinesin O12. The cDNAs of the kinesin motor domain was cloned into expression vector pET21a and transformed into *E.coli* BL21 (DE3) LysE. There expressed O12 and purified by Ni-NTA column. The biochemical characterizations of the kinesin O12 motor were studied. ATPase activities, interaction with fluorescent ATP analogues, NBD-ATP were analyzed and compared with the results of other rice kinesin and mouse conventional kinesin. The binding and release of the ATP or ADP analogues were extremely slow compared with other kinesin. It is suggested that the rice kinesin O12 may has unique enzymatic properties and structure.

2P153 蛍光標識ATP アナログを用いた新規イネキネシンK16 のATPase kinetic pathway の観察
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Monitoring the ATPase kinetic pathway of the novel rice kinesin K16 using fluorescent ATP analogue
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Previously, we have succeeded to express the motor domain of the novel rice kinesin K16 by *E. coli*. The novel rice kinesin K16 has several unique enzymatic characteristics comparing with conventional kinesin. The most interesting property is that the ADP-free K16 motor domain is very stable, contrast to conventional kinesin that is very labile in ADP-free state. Our crystallographic study demonstrated that the neck-linker of the ADP bound K16 motor domain showed an ordered conformation in a position quite different from that observed in conventional kinesin. This may reflect the unique enzymatic characteristics of rice kinesin K16. Therefore, it is expected that the novel rice kinesin K16 has unique ATPase kinetic pathway coupling with motility. In this study, we investigated the ATPase kinetics of K16 with fluorescent ATP analogue, NBD-ATP using a stopped flow apparatus. When the NBD-ADP binds to the ATPase site of K16, the fluorescence intensity increased approximately threefold. The rate constants of binding of NBD-ATP and release of NBD-ADP were measured from the alteration of fluorescence intensity. The binding of K16 to microtubules was monitored by light scattering. The rate constants of these steps were almost similar to those of conventional kinesin. Currently, we are trying to measure the step of Pi release using phosphate-binding protein labeled by fluorescent probe.

2P155 運動中のキネシンのステップ方向の二次元観察
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Two-dimensional observation of stepping motion of single kinesin molecules along microtubule
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Kinesin is a motor protein that transports intracellular cargoes by moving processively along microtubules with 8nm-steps. Stepping motion of kinesin along the longitudinal axial of the microtubule has been studied extensively using optical trapping bead assays. However, the limited spatial resolution of the optical trap in the axis perpendicular to the displacement or the applied load made it difficult to observe stepping motion of kinesin in the lateral axis of microtubule ("sideway step") under low load, and it remains to be answered whether kinesin moves along single protofilament without sideway movement or not. Here, we analyzed two-dimensional movement of kinesin along microtubules under low applied load with improved spatial resolutions. We labeled kinesin with quantum dot (Qdot) and observed the movement of Qdot-kinesin using FIONA (Fluorescence Imaging with One Nanometer Accuracy) with spatial resolution of 3 nm and temporal resolution of 2 ms (under the best condition). We observed one-dimensional stepping motion of kinesin as has been reported previously but also observed sideway steps of 6 nm which may represent a transfer of kinesin to the adjacent protofilament. When we extended the size of the neck linker by inserting extra glycine residues, the frequency of sideway steps and backwards steps increased. These results suggest that the size of the neck linker is optimally designed to efficiently walk along a single protofilament.