2P128 レバーアームを用いないミオシンVの運動機構
○齋藤 優太¹、名倉 直希¹、田中 祐介¹、安藤 敏夫^{1,2}
¹金沢大院・自然科学・数物科学、²JST/CREST
Myosin V S1 tethered at its head can propel actin filaments movement
Yuta Saito (1), Naoki Nagura (1), Yusuke Tanaka (1) and Toshio Ando (1,2).
(1:Dept. of Physics, Kanazawa Univ.; 2:JST/CREST)

Myosin V is a processive motor whose movement along actin tracks has already been demonstrated to take place in a hand-over-hand manner. The neck region swings to produce unidirectional force for this movement. However, we observed a phenomenon that does not seem to reconcile with the swinging lever arm model. Myosin V subfragment-1 (BMV-S1) having the first two IQ motifs was tethered to a glass surface at the head portion via PEG-streptavidin. We found that actin filaments could move over this surface. In this tethering arrangement, the lever arm makes a swing that misses. Therefore, our observation suggests two possibilities: (1) myosin V uses not only the swinging lever arm mechanism but also an additional mechanism or (2) not only the lever arm but also the head portion swings. In addition, we obtained data that the actin filaments movement observed above seemed to occur in the opposite direction. A recent report indicated that myosin VI S1 alters the directionality of actin filaments movement depending on the tethering positions in the neck [Zev Bryant *et al.* (2007) *PNAS* 104:772-777]. This was explained by the swinging lever arm model. Because our tethering position of BMV-S1 head is far from the converter domain, it is unlikely that the reverse of directionality with BMV-S1 can be explained in the same way as myosin VI S1. At the poster presentation, we will report more details of our observations.

2P130 Dynamic cooperative binding of myosin V on actin filament
○小塚 淳^{1,2}、石井 由晴²、柳田 敏雄^{1,2}
¹阪大院・生命機能、²JST
Dynamic cooperative binding of myosin V on actin filament
Jun Kozuka(1,2), Yoshiharu Ishii(2) and Toshio Yanagida(1,2)(1: Soft Biosystem Group, Laboratories for Nanobiology, Graduate School of Frontier Biosciences, University of Osaka; 2: Formation of Soft Nanomachines, Core Research for Evolution Science and Technology, Japan Science and Technology Agency)

Myosin V is an unconventional myosin that transports organelles along actin filaments in cells. Our previous study on the binding of myosin V heavy meromyosin (HMM) to actin filament in the presence of adenosine triphosphate (ATP) showed bunching of myosin molecules on the actin filaments. The observation of the binding suggested several modes of interaction between myosin V and actin filament depending on the concentration of myosin V (EABS & BSJ 2006, 1P285). These mode changes were accompanied by changes in the kinetic parameters of the movements of the myosin V bunches in low concentration, increase in the heterogeneity of actin filaments complex with myosin V in intermediate concentration, and sigmoidal increase in the total amount of bound myosin V in high concentration. The changes in the interaction mode may be related to the conformational changes of actin observed in the previous work (Kozuka et al, Nat. chem. bio., 2006). Actin conformation is in dynamic equilibrium between two states and the binding of myosin V shifts the equilibrium to the active state for myosin motility. To relate the cooperative interaction between myosin V and actin to the dynamic conformational change of actin, we observed the binding patterns of Myosin V fused green fluorescent protein along actin filament in real time using total internal reflection fluorescence microscopy (TIRFM) techniques and analyzed them. We will discuss the mechanism of the dynamic cooperative binding of myosin V on actin filament based on the conformational state of actin.

2P129 ミオシンVのカルシウム調節
○田中 慎一郎¹、小出 博史¹、表 冨子¹、安藤 敏夫^{1,2}
¹金沢大院・自然科学研究科、²CREST/JST
Calcium regulation of Myosin V
Shin'ichiro Tanaka(1), Hiroshi Koide(1), Saeko Omote(1) and Toshio Ando(1)(2)(1:Department of physics, Kanazawa University; 2:CREST/JST)

The motor activity of myosin V is regulated by Ca²⁺. Using chick brain myosin V, we previously identified the specific IQ motif from which CaM dissociates in high Ca²⁺ to be IQ2. In addition, we investigated the Ca²⁺ effect on the motility of myosin V subfragment-1 (S1) that possessed the first two IQ motifs adjacent to the motor domain. In low Ca²⁺, S1 has the motor activity even when CaM is absent at IQ2. On the other hand, in high Ca²⁺, S1 loses its motor activity. Namely, CaM dissociation from IQ2 is prerequisite but not sufficient for the motor activity loss. Here, we propose "CaM-bridging model" for the Ca²⁺ regulation; on binding to Ca²⁺, CaM on IQ2 dissociates from IQ2 and then CaM that was on IQ1 moves toward the empty IQ2, forming a bridge between IQ1 and IQ2. This CaM bridging weakens the head-neck connection, which leads to no mechanical transmission from the head to the neck, and thus, results in the motor activity loss. However, this bridging state seems to be in equilibrium with the non-bridging state (i.e., the CaM resides only on IQ1) because actin sliding velocity in high Ca²⁺ depended on the S1 density; at a high density, actin filaments moved even at pCa 4 whereas at a low density, no movement was observed at pCa 5. The proposed model can explain various observations made by us and others. More details will be presented at the poster.

2P131 吊られたアクチンフィラメント上の束縛されないミオシンVの運動の直接観察
○田島 勇治¹、城口 克之²、木下 一彦²
¹早大院・先進理工・物理応物、²早大・先進理工・物理応物
1aB04 Direct observation of unconstrained motion of myosin V on a suspended actin filament
Yuji Tajima (1), Katsuyuki Shiroguchi (1) and Kazuhiko Kinoshita Jr. (1). (1: Dept Physics, Graduate School of Science and Engineering, Waseda University)

Myosin V is a processive motor that takes 35-nm steps along an actin filament for each ATP hydrolyzed. It is a homodimer. Each monomer has a head domain that has the ATPase activity and an actin-binding site(s), and also a long neck containing six calmodulin-binding IQ motifs. The two heads of myosin V move alternately forward in a "hand-over-hand" fashion. In our previous report, myosin V moved as a left-handed spiral on the right-handed actin double helix when the myosin was allowed to rotate freely around an actin filament, indicating that the natural step size of myosin V is slightly shorter than the actin helical repeat of 36 nm. In this study, we attempt to alter the spiral pitch by modifying myosin V. The six IQ motifs in the neck are fully occupied by calmodulin (CaM) at low Ca²⁺ concentrations. At higher Ca²⁺ concentrations, CaMs tend to dissociate from the necks. After treatment at Ca²⁺ 3 or 5 μM, we found that some myosin molecules moved straight on a suspended actin. We are also testing several other modifications of myosin V motion to see how it spirals.