3P136 哺乳類細胞における細胞質ダイニン複合体の IC74 と LC8 の相互作用の役割
○小林 琢也¹、古田 健也²、豊島 陽子²、片山 栄作³、村山 尚¹
3aB01 ¹順天堂大・医・薬理、²東大院・総合・生命、³東大・医科研・分子構造解析
Potential role of interaction between IC74 and LC8 of cytoplasmic dynein complex in intracellular transport in mammalian cells
Takuya Kobayashi (1), Ken'ya Furuta (2), Yoko Y. Toyoshima (2), Eisaku Katayama (3) and Takashi Murayama (1). (1:Dept Pharmacology, Juntendo Univ School of Medicine; 2:Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo; 3:The Institute of Medical Science, The Univ of Tokyo)

Cytoplasmic dynein complex is a large multi-subunit microtubule-associated molecular motor involved in various cell functions including organelle positioning, vesicle transport and cell division. Dynein heavy chains contain motor domain together with intermediate and light chains are responsible for the cargo binding. Though the cargo binding is believed to be regulated by interactions of light chains and/or various other proteins with the intermediate chain, its operation mechanism is not yet clearly understood. In this study, we investigated the molecular interactions of the dynein complex by a series of in vivo and in vitro experiments. Dynein intermediate chain 74 (IC74) was labeled with a novel GFP-based fluorescent affinity tag (IC74-tag) and expressed in mammalian cells. IC74-tag distributed diffusely in the cytoplasm, in accord with the known distribution of endogenous IC74. A dynein complex purified from the expressed cells via IC74-fused tag showed reasonable motor activity and microtubule binding in *in vitro* motility assay. We also labeled dynein light chain LC8 by the tag (LC8-tag). Unlike IC74-tag, LC8-tag localized mainly at centrosome. This localization was abolished by S88E mutation in LC8 that promotes dissociation of LC8 from IC74. These findings suggest that IC74-LC8 interaction may have an important role in LC8 transport to the centrosome. We will also report the structure-based analysis of the interactions between IC74 and LC8 by electron microscopy.

3P138 zinc-induced tubulin sheet 上における細胞質ダイニンの1分子観察
○三浦 未知¹、古田 健也¹、枝松 正樹¹、豊島 陽子¹
3aB02 ¹東大院・総合文化・生命環境
Observation of single dynein molecules on zinc-induced tubulin sheets
Michi Miura, Ken'ya Furuta, Masaki Edamatsu, Yoko Y. Toyoshima
Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo

Cytoplasmic dynein is a double headed, minus-end directed motor of microtubules. Dynein motor domain is so large that it is not elucidated whether dynein uses only a single protofilament when it moves on microtubules as kinesin does. To answer this question, we exploited the property of zinc-induced tubulin sheets that the motor binding sites are only exposed at either edge of the two. We have previously shown that multiple dynein molecules on glass surface can glide zinc-sheets. However, it is possible that multiple dynein molecules glide zinc-sheets and the gliding does not necessarily mean that single dynein molecules can walk on single protofilament processively. In this study, we investigated the single molecule motility assay on zinc-sheets by TIRFM. We first tried to fix zinc-sheets with the dynein binding edge exposed in solution, using antibody or MAPs on glass surface. To observe single dynein molecules, we prepared GFP-tagged yeast cytoplasmic dynein and confirmed that the GFP-tagged dynein moves processively on microtubules. To verify that the experimental condition is appropriate, we used GFP-tagged kinesin as a positive control and observed that kinesin molecules were able to bind to zinc-sheets in the presence of AMP-PNP. After we establish the condition to observe the movement of single molecules of kinesin, we aim to analyze whether dynein moves processively on zinc-sheets or not.

3P137 LIS1 と NDEL1 によるダイニン分子運動機能調節のメカニズム
○島澤 嵩征¹、中山 明子¹、古田 健也¹、枝松 正樹¹、広常 真治²、豊島 陽子¹
¹東大・総合文化・生命、²大阪市大・医・細胞機能
Molecular mechanism of the regulation of dynein motility by LIS1 and NDEL1
Takayuki Torisawa (1), Akiko Nakayama (1), Ken'ya Furuta (1), Masaki Edamatsu (1), Shinji Hirotsune (2), Yoko Yano Toyoshima (1). (1:Dept Life Sciences, Graduate School of Arts and Sciences, Univ. of Tokyo; 2: Dept Genetic Disease Research, Osaka City Univ. Graduate School of Medicine)

LIS1 and NDEL1 are known to be regulators of cytoplasmic dynein and essential for cargo transports in living cells. We have previously demonstrated that LIS1 and NDEL1 regulate the dynein motor activity in vitro; the binding of either LIS1 or NDEL1 to cytoplasmic dynein impaired its motor activity and the dual binding of these proteins restored the dynein motility. To investigate the molecular mechanism of the regulation by LIS1 and NDEL1, we analyzed interactions among these three proteins. As an anti-parallel dimer arrangement was suggested for NDEL1 (Tarricone et al. 2004), this possibility was tested using a series of cyst-substituted mutants of coiled-coil region of NDEL1. The electrophoresis of these mutants in an oxidative and a reductive conditions revealed that the coiled-coil region made a parallel dimer. Next, we dissected NDEL1 into two functionally different halves; N-terminal half which contain Lis1 binding region and C-terminal half which involve dynein binding domain. The N-terminal fragment of NDEL1 could not impair the dynein motor activity, but gave almost the same effects as full length molecule of NDEL1 in restoring the dynein motor activity from the inhibition by LIS1 binding. The order of the application of these proteins to dynein was critical. Applying LIS1 first and then N-terminal fragment of NDEL1 to dynein was effective, but the reverse order was not effective. These observations suggest that NDEL1 directly binds to LIS1 and the dynein motility is restored from the inhibition by LIS1.

3P139 in vitro 滑り運動系における微小管の三次元トラッキング
○水谷 佳奈¹、大岩 和弘²、小嶋 寛明²、榊原 斉²、政池 知子¹、西坂 崇之¹
3aB03 ¹学習院大・理・物理、²情報通信研・関西
Three-dimensional tracking of microtubule on surfaces coated by kinesin and dynein
Kana Mizutani(1), Kazuhiro Oiwa(2), Hiroaki Kojima(2), Hitoshi Sakakibara(2), Tomoko Masaake(1) and Takayuki Nishizaka(1). (1: Dept. Phys., Gakushuin Univ. 2: Kansai Adv. Res. Ctr.)

We have developed a microscope technique to track single particles or fluorophores in three dimensions by locating a wedge prism at an equivalent back focal plane. This technique was applied to a modified *in vitro* motility assay, in which fragments of Cy3-labeled microtubule (Cy3-MT) were copolymerized in a Cy5-MT and appeared as spots of Cy3. The copolymers slid on either kinesin and dynein immobilized on the glass surface. The Cy3-spots, which typically consist of ~ 40 fluorescent molecules, were tracked in Z-direction with ~ 8 nm accuracy at 100 ms resolution with a mercury light as an exciter, whereas each whole filament was simultaneously visualized as an image of the Cy5-MT. We focused on the aspects of the behavior of MT when two sliding MTs collided. On the surface coated with 72 µg/ml (~ 80 molecules/µm²) of dynein (Inner-arm dynein subspecies c), either one or both MTs changed their directions in XY-plane; MTs slid together and followed the same trajectory with no z-directional change (95 %, *n* = 60). On the contrary, this behavior was not observed in the case of kinesin (kinesin-1) surface. When one MT hit another MT, 'lifting' of the front end was always observed (with only one exception, *n* = 120). The top of arc of the MT typically reached 100 nm in height, and lifting occurred on the surface coated with kinesin at various concentrations, 8-800 µg/ml (~ 80-8000 molecules/µm²). The behavior is likely due to the difference in lateral binding force to a MT and/or flexibility of single kinesin and dynein molecules.