3P152  Dynein moves in a short-pitch helical path around a microtubule
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Dynein is a microtubule (MT)-based motor protein. *Tetrahymena*
axonomal outer arm dynein (OAD) rotated sliding MTs around their axis in
an *in vitro* MT gliding assay, indicating that OAD generate torque.
However, the MT gliding assay may not accurately quantify the torque
generation of OAD, because a lot of OADs simultaneously act on a MT
and rotational marker attached to MTs may be a sterile hindrance to
rotation. To overcome them, we tracked a moving bead coated with OADs
along a MT anchored on an etched glass as a suspension bridge to
investigate movement of a small number of OADs without hindrance to
rotation. We find that the beads moved in a right-handed helical path, and
the pitch is 0.8 μm, which is shorter than MT superwrist pitches.

3P153  Investigating the torque-generating mechanism of kinesin-6
using unbinding force measurement
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Kinesin-6 (ZEN-4) is a microtubule (MT)-based motor protein which
localizes at central spindle in metaphase cells. We previously found that
ZEN-4 rotates sliding MTs in an *in vitro* 3-D gliding assay. This means
that ZEN-4 generates forces not only along the longitudinal axis of MT but
also along the rotational direction. To generate a unidirectional MT
rotation, ZEN-4 is considered to tend to unbind from MT after a step
against the direction of rotation and not to unbind after a step toward the
direction of rotation. In this study, we focused on the unbinding force of
ZEN-4-MT complexes and measured the unbinding force imposing
external load in every direction with optical tweezers in order to reveal the
mechanism of the generation of rotational force of ZEN-4.

3P154  Autoinhibition and synergistic activation of cytoplasmic dynein
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Yoko Toyoshima1 (1Dept. Life Sciences, Graduate School of Arts and
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Cytoplasmic dynein is involved in a wide range of cellular activities. To
perform diverse functions, dyneins are required to up- and down-regulated.
However, single-molecule motility of mammalian cytoplasmic dynein has
been controversial among research groups, which makes it unclear how
dynein’s activity is regulated and tuned for a specific function. Here, we
show that single dyneins are autoinhibited, in which two motor heads are
stacked together, whereas multiple dyneins can be activated when clustered
on a cargo. Optical trapping suggested that this activation is mediated by
dynein’s unconventional force response, which leads to mutual activation
among multiple dyneins. We propose that this synergistic property enables
self-regulation depending on cellular context.

3P155  Bicaudal-D2 による微小管系輸送の制御機構
Regulatory mechanism of microtubule-based molecular motors
by Bicaudal-D2
Takaya Kobayashi1,2, Akira Hanashima2, Yoko Y. Toyoshima1, Takashi
Murasawa2 (1Department of Life Sciences, Graduate School of Arts and
Sciences, The University of Tokyo, 2Department of Pharmacology, Juntendo
University School of Medicine.)
Bicaudal-D2 (BICD2) is a dynein adaptor which plays an important role in
intracellular transport. It has been suggested that BICD2 interacts with two
microtubule-based molecular motors: kinesin and dynein. However, it
remains unclear how BICD2 regulates these motors. To address the
question, we here investigated interaction between BICD2 and the
molecular motors in vivo and in vitro. In live-cell imaging, full-length
BICD2 was observed as foci moving along microtubules. A series of
deletion mutants of each domain showed that the mutants are localized
toward cell edges or cell center, suggesting that the corresponding domains
may be involved in interaction between kinesin or dynein motor. In vitro
interaction experiments are now in progress.

3P156  Molecular architecture of dynactin p150
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Dynactin is known to be a regulator of cytoplasmic dynein and play a part
in intracellular transport. Dynactin is a large complex composed of
multiple subunits including p150, p50 and Arp1, and has a characteristic
architecture. It has been shown that p150 forms a dimer and appears two-
headed structure and thin rod which extends from the Arp1 rod as a side
arm. Here, we investigated the molecular architecture of dynactin p150 by
electron microscopy using recombinant human proteins expressed in HEK
cells, and found that the dynein binding region of p150 forms a new
protrusion from the head of p150. Our results provide a new insight into
the dynein-dynactin interaction.

3P157  Subunit structure of *Tetrahymena* outer dynein arm complex
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Numata2, Yoko T. Toyoshima1 (1Department of Life Sciences, Graduate School
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Environmental Sciences, University of Tsukuba)
Outer dynein arm (ODA) complex is a major component in cilia and drives
its beating. *Tetrahymena* ODA complex is composed of three heavy chains
(α, β, γHCs), two intermediate chains (ICs) and several light chains (LCs),
but the exact subunit arrangement has not been determined. We engineered a codon-optimized hEGFP-tagged ODA component by homologous
recombination. Western blot and live cell imaging verified that hEGFP-
tagged ODA component was incorporated into cilia. PCR analysis
confirmed complete replacement of the target locus with the hEGFP-
tagged ODA complex. By Ni-NTA-gold labeling and electron microscopy of the
His-tagged ODA complex, we will elucidate the subunit architecture of
*Tetrahymena* ODA complex.