The report of the 2014 ascb/ ifcb meeting (December 6-10/ philadelphia, pennsylvania)

by Kazumi Kawata

12/8 Poster Presentations

[Canonical Wnt signaling pathway regulates not only the odontobast differentiation through primary cilia but also formation of primary cilia. By K. Kawata]

受けた質問はすべて、①一次繊毛がWNTシグナルを調整している。
②WNTシグナルが一次繊毛形成を調整している。
では、WNTシグナル活性化と一次繊毛形成どちらが最初なのか？

自身の回答は、WNTシグナルの活性が抑制されることで、一次繊毛の形成が促進される。

12/6 Special Interest Subgroups

Q. Nonconventional Functions of Molecular Motors

[Unconventional functions of KIFs and diseases caused by their defects. By N. Hirokawa]

12/7 Minisymposium 4: Endocytic Trafficking

[KIF13B enhances the endocytosis of LRP1 by recruiting LRP1 to caveolae. By Y. Kanai]

Multifunctional low-density lipoprotein (LDL) receptor-related protein 1 (LRP1) recognizes and internalizes a large number of diverse ligands, including LDL and factor VIII. However, little is known about the regulation of LRP1 endocytosis. Here, we show that a microtubule-based motor protein, KIF13B, in an unexpected and unconventional function, enhances caveolin-dependent endocytosis of LRP1. KIF13B was highly expressed in the liver and was localized on the sinusoidal plasma membrane of hepatocytes. KIF13B KO mice showed elevated levels of serum cholesterol and factor VIII, and KO MEFs showed decreased uptake of LDL. Exogenous KIF13B, initially localized on the plasma membrane with caveolae, was translocated to the vesicles in the cytoplasm with LRP1 and caveolin-1. KIF13B bound to hDLG1 and utrophin, which, in turn, bound to LRP1 and caveolae, respectively. These linkages were required for the KIF13B-enhanced endocytosis of LRP1. Thus, we propose that KIF13B, working as a scaffold, recruits LRP1 to caveolae via LRP1-hDLG1-KIF13B-utrophin-caveolae linkage and enhances the endocytosis of LRP1.

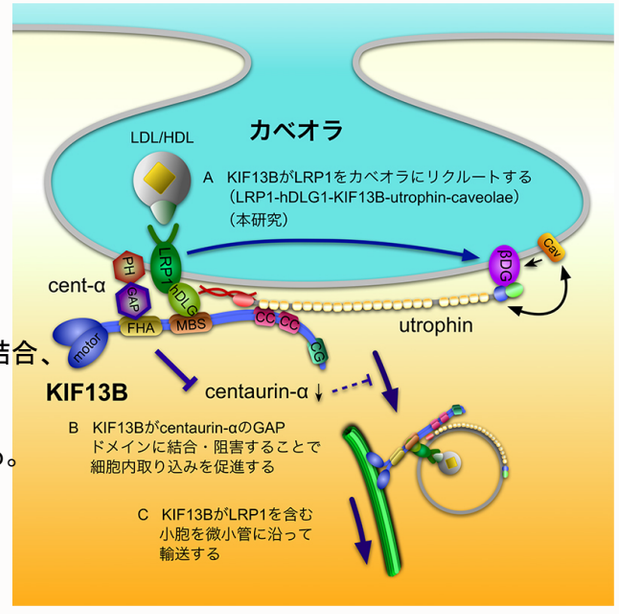
12/8 Symposium 4: Machinery of the Cell

[Kinesin Superfamily Molecular Motors, KIFs and Intracellular Transport : from Regulation of Learning/Memory and Development to Diseases. By N. Hirokawa]

KIF13B KO mouse

形態的に目立ったphenotypeなし。
しかし、血中コレステロールの上昇、KO MEFsでは低密度リポタンパク質 (LDL)の取り込みの減少が認められた。

・LDLのendocytosisには、LDL受容体(LDLR)や
LDLR関連タンパク質1（LRP1）が働く。
しかし、KIF13BはLRP1との み共局在する。
・また、clathrin依存性ではなく、
caveolin1依存性にLRP1のendocytosisが行われる。
・しかし、LRP1はKIF13Bと結合するが、
caveolinとは結合しない。
↓
KIF13Bがhuman Discs Large 1 (hDLG1)を介してLRP1と結合、
また、KIF13Bがutrophinを介してcaveolin1結合する。
これにより、KIF13BがLRP1とcaveolin1を結びつけ、
細胞内への血中コレステロールの取り込みを促進する。



http://www.u-tokyo.ac.jp/ja/utokyo-research/research-news/mechanism-of-caveolae-mediated-endocytosis-of-lrp1/より

12/6 Special Interest Subgroups

Q. Nonconventional Functions of Molecular Motors

[Kinesin-4 proteins: Regulating microtubule-based architectures required for cell division and hedgehog signaling in the primary cilium. By R. Subramanian]

・WT MEFsにShhの添加すると、primary ciliaの先端にKIF7が局在する。
・しかし、Kif7^{L130P} MEFsやKif7$^{-/-}$ MEFsにShhの添加しても、primary ciliaの先端にKIF7は局在しない。

・WT MEFsをSerum starvationにし、primary ciliaの形成を誘導すると、primary ciliaの先端にKIF7が局在する。
また、Gli2, Sufuもprimary ciliaの先端に局在する。
・しかし、Kif7^{L130P} MEFsやKif7$^{-/-}$ MEFsで同様の処理をしても、primary ciliaの先端以外にもKIF7は存在する。
Gli2, Sufuについても同様の結果を示す。
・さらにKif7^{L130P} MEFsやKif7$^{-/-}$ MEFsではprimary ciliaが長くなる。
↓
Kif7は延長中の微小管の先端に局在し、
微小管のdynamicsの調整を
行っている。

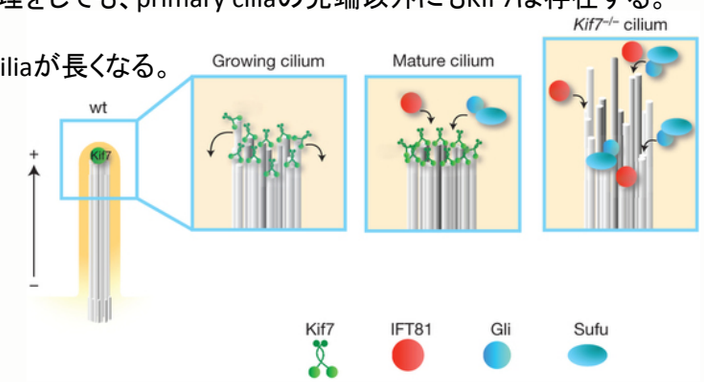


Figure 8: Model for Kif7-dependent regulation of plus-end dynamics in primary cilia. Model for Kif7 function at the tip of the primary cilium. In the wild type, Kif7 acts at the distal end of the growing cilium to prevent overgrowth of individual microtubules and to coordinate the growth of nine-doublet microtubules; only organized microtubule arrays are a suitable substrate for post-translational modifications. In the mature cilium, Kif7 creates a single distal tip compartment where IFT81 and Gli–Sufu complexes are enriched. In the absence of Kif7, growth of axonemal microtubules is not limited and synchronized, leading to longer and unstable cilia and ectopic tip-like compartments that contain IFT81 along the axoneme. Gli–Sufu complexes localized to the ectopic tip compartments, where they can be inappropriately activated in the absence of Shh ligand.

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12/7 Minisymposium 3: Cell Organization and Polarity

[Spontaneous and electric field-induced polarization and motility initiation utilize different mechanochemical pathways. By Y. Sun]

Stationary symmetric fish keratocytes break symmetry spontaneously and start moving within tens of minutes. It is known that the spontaneous symmetry break starts from the prospective rear when elevated contractility increases centripetal actin flow.

・The motile cells respond to an electric field (EF) and move to cathode. In the process of polarization, cells exhibit waves of protrusion-contraction. We observed that EF drastically accelerates keratocytes' polarization and motility initiation.

EF + → -

WT

~~cGMP~~ (ミオシンの脱リン酸化を誘導), ~~PI3K~~

・しかし、
Microscopy demonstrated that while actin and myosin in stationary and motile cells are distributed similarly without and in the presence of EF, adhesion (vinculin) distributions are different: in spontaneously motile cells, vinculin is swept to the rear, while in EF-induced cells it is biased to the front.

NoEF EF

vinculin

・でも、やはり、

+Latrunculin (an actin polymerization inhibitor)

EF + → -

+Latrunculin

+Blebbistatin (a selective myosin II ATPase activity inhibitor)

EF + → -

→ EFによるdirectional response (motility)と極性は違う経路により制御されている。

12/8 Symposium 3: Machinery of the Cell

[Looking at tumor invasion and angiogenesis from a biomechanical, dynamical and correlative point of view. By J.G. Goetz]

Three mains reasons explain why most of the critical events driving normal and pathological scenarios had been less investigated: they occur rarely in space and time, they are highly dynamic, they differ when studied in situ in an entire living organism. Here, we have decided to develop imaging approaches with high temporal resolution, in living organisms, and with a correlative imaging framework (from light to electron microscopy) that would allow us to dissect these singular events with the highest resolution possible. Those techniques allowed us to highlight the importance of the mechanical remodeling of the stroma in tumor progression. We showed that caveolin-1 (Cav1) promotes Rho- and force-dependent contraction, matrix alignment, and microenvironment stiffening. Fibroblastic expression of Cav1 thereby remodels peri- and intratumoral microenvironments to facilitate both tumor invasion and metastatic potency. We are currently developping an intravital and correlative imaging approach in two model organisms (mouse and zebrafish) in order to capture cancer cells at crucial steps of metastasis formation. We also used whole-embryo live microscopy in the zebrafish embryo for testing the biomechanical input of hemodynamics to angiogenesis. Using a correlative combination of live-cell imaging at high temporal resolutions and electron microscopy coupled to electron tomography, we dissected the mechano-detection mechanisms at play at the cellular scale allowing the developing endothelium to sense flow-mediated forces. This allowed us to study, in its most details, the behavior, the nature and the architecture of endothelial primary cilia and how it is capable of relaying the biomechanical information carried by blood flow.

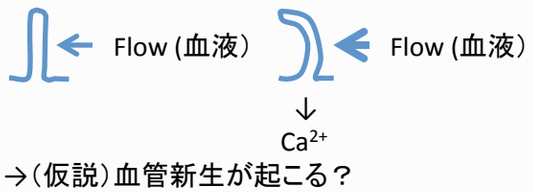
In conclusion, using two distinct events representative of normal and pathological situations and appropriated imaging techniques, our studies highlight how cells sense static extracellular forces mediated by the extracellular matrix and dynamic forces resulting from fluid flows.

Zebrafish

24hpt → 26hpt → 28hptと発生が進むにつれ、血液の流れが速くなる。

それにより、primary ciliaの折れ曲がりも起こるようになる。 → Ca^{2+}の流入が起こるようになる。

24hpt　　28hpt



→（仮説）血管新生が起こる？

腫瘍形成時の血管新生もこのメカニズム？

12/8 Poster Presentations　&　Minisymposium 9: Optical Microscopy and Superresolution Imaging

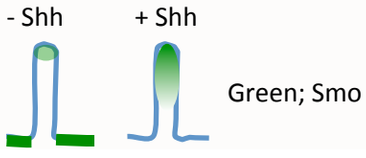
[Single-molecule tracking of Smoothened reveals binding in the primary cilium that is altered by pathway agonists. By L.E. Weiss]

Hedgehog (Hh) signaling plays an essential role in cell division and differentiation in embryonic and adult stem cells. Disruption of the pathway can often lead to fatal defects in embryos, as well as a wide variety of cancer types later in life. The pathway is initially switched on by the binding of the Hh ligand to the transmembrane protein Patched. Shortly after, anther transmembrane protein, Smoothened (Smo), is derepressed and relocalizes to a small microtubule-structured organelle that protrudes from the cell's surface called the primary cilium. Once there, it influences members of the Gli family of transcription factors that then upregulate target genes in the nucleus. Although the ciliary structure is known to be very complex, standard fluorescence microscopy measurements have been hindered by the small dimensions (~400 nanometers in diameter and 2-5 microns long) which are on the order of the diffraction limit. To overcome this barrier, we have used highly sensitive, single-molecule microscopy to obtain the trajectories of individual Smo proteins on the surface of cilia with high temporal and spatial resolution (10 millisecond and 30 nanometers, respectively). By analyzing their movements, we have observed three distinctive modes of motion: diffusion, directed motion, and binding, the last of which is altered in the presence of natural and synthetic small-molecule agonists.

Shh添加後、Smoの細胞膜上の局在が急激に減少し、primary ciliumへの局在が増える。

Primary ciliumへ移動したSmoはprimary cilium 内で自由に動くが、

primary ciliumの先端に留まる確立が高い。



12/9　Minisymposium 18: Small GTPases and Lipids in Membrane Dynamics

[The exchange factor DENND2B activates Rab13 at the leading edge of migrating cells driving cancer cell metastasis. By M. Ioannou]

The small GTPase Rab13 functions in exocytic vesicle trafficking in epithelial cells. Alterations in Rab13 activity have been observed in human cancers including carcinomas, yet the mechanism of Rab13 activation and its role in carcinoma progression have not been demonstrated.

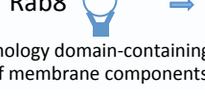
・DENN domain-containing protein 2B (DENND2B) / suppression of tumorigenicity 5 (ST5)の結合により、Rab13は活性化する。
・活性型Rab13は、leading edgeに局在する。
・Rab13 knockdownにより、癌細胞の遊走や浸潤が減少する。
・Rab13 knockdownにより、腫瘍の転移が減少する。

12/9 Poster Presentations

[Primary cilia bend and pivot in response to intracellular and extracellular forces. By C.M. Ott]

Primary cilia are ubiquitous, microtubule-based organelles which play diverse roles in sensory transduction in many eukaryotic cells. They interrogate the cellular environment through chemosensing, osmosensing and mechanosensing, using receptors and ion channels in the ciliary membrane. Little is known about the mechanical and structural properties of the cilium and how these properties contribute to ciliary perception. We probed the mechanical responses of primary cilia from kidney epithelial cells (MDCK-II), whose role it is to sense fluid flow in renal ducts. We found that upon manipulation with an optical trap, cilia deflect by bending along their length and by pivoting around an effective hinge located below the basal body. The calculated bending rigidity indicates weak microtubule doublet coupling. Primary cilia of MDCK cells lack inter-doublet dynein motors. Nevertheless we found that primary cilia display active motility. Three-dimensional tracking demonstrated correlated fluctuations of the cilium and basal body. These angular movements appeared random, but were dependent on ATP and cytoplasmic myosin-II in the cell cortex. We conclude that force generation by the actin cytoskeleton surrounding the basal body results in active ciliary movement. We speculate that actin-driven ciliary movement might tune and calibrate ciliary sensory functions.

Basal bodyが一次繊毛の傾きを調整する。



12/10 Minisymposium 24: Protein Sorting to Intracellular Compartments

[EHD proteins coordinate membrane reorganization and fusion to initiate early steps of ciliogesesis. By C. Insinna (Kettenhofen)]

The primary cilium is a membrane-bound, microtubule based sensory organelle that plays essential roles in development and disease pathways. Cilia biogenesis requires coordination of a series of processes including, mother centriole to basal body transformation, recruitment of intraflagellar transport (IFT) and transition zone (TZ) proteins, and axoneme formation and association with a developing ciliary membrane. Membrane association with the distal appendages of the mother centriole is a critical step in ciliogenesis initiation. The membrane trafficking Rab GTPase Rab11-Rab8 cascade plays key roles in early ciliary membrane assembly, but the molecular details remain unclear. Here, we show that the membrane shaping proteins EHD1 and EHD3, in association with the Rab11-Rab8 ciliogenesis cascade,function in cilia assembly in zebrafish and mammalian cells. We discovered that EHD proteins localize to Rab11 pre ciliary vesicles that dock to the distal appendages of the mother centriole, membranes we refer to as distal appendage vesicles (DAV). Using live and super-resolution imaging, as well as electron microscopy approaches, we established that EHD proteins are essential for the formation of the larger pre-axonemal ciliary vesicle (CV) from DAVs. Furthermore, we show that EHD1-dependent CV formation is critical for initiating mother centriole to basal body transformation and recruitment of IFT20 and transition zone proteins. Surprisingly, we found that Rab8 is recruited for ciliary membrane growth only after these steps, and in coordination with axonemal assembly. Investigations into the molecular mechanism of these early ciliogenesis initiation steps suggested that EHD proteins tubulate DAVs, bringing them in close proximity to allow fusion into the CV. This step is required for CP110 removal from the distal end of the mother centriole prior to recruitment of IFT20 and TZ proteins. Based on these findings we predicted that SNAREs, regulators of membrane fusion, would be important for CV assembly and ciliogenesis progression. We show that the SNARE SNAP29, an EHD1 interacting protein, is required for ciliogenesis and localizes with EHD1/3 on early ciliary membrane structures. Together, our studies provide new molecular mechanisms informing the classically described intracellular ciliogenesis　pathway and uncover a previously uncharacterized step in ciliary assembly.

The C-terminal Eps15 homology domain-containing protein 1 (EHD1) is ubiquitously expressed and regulates the endocytic trafficking and recycling of membrane components and several transmembrane receptors.