Program

September 17(WED)-19(FRI),2025

International Symposium on Cellular Structural Biology

17th September, Wednesday

13:00~13:10

Opening remarks by Toshiya Endo (Director of the CREST program "Cell Dynamics: spatiotemporal dynamics of intracellular components")

Session I: Structure analysis of biomolecular complexes

13:10~14:50（25 min × 4）

Osamu Nureki (Univ. of Tokyo)

"Crosstalk between membrane and nucleic acids: DNA/RNA transport across cell membranes"

Mikako Shirouzu (RIKEN)

"Molecular conformational dynamics of the DOCK protein complexes in signal transduction"

Yoshitaka Matsuo (Univ. of Tokyo)

"Molecular basis for resolving ribosome collisions induced by aberrant translational stalling"

Roland Beckmann (Univ. of Munich)

"Molecular basis for RQC and CAT tailing in the cytosol and at the ER"

（coffee break 20 min）

Session II: Cross-scale analysis of structural dynamics in biological processes

15:10~16:50（25 min × 4）

Ryo Nitta (Kobe Univ.)

"Deciphering microtubule–orchestrated biological phenomena through cross-scale measurements"

Erina Kuranaga (Kyoto Univ.)

“Revealing the mechanism of dynamic mesoscale actin ring organization in collective cell migration”

Takeshi Fukuma (Kanazawa Univ.)

“Cross-scale biology studies by advanced atomic force microscopy techniques”

Kenichi Umeda (Kanazawa Univ.)

“Submolecular video-imaging of Smc5/6 complex by high-speed atomic force microscopy”

（coffee break 20 min）

Session III: Short talks from poster presentation

17:10~18:10 (10 min × 6)

Hirokazu Sakamoto (Univ. of Tokyo)

“Nanoscale phase separation generates highly ordered nanostructures of synaptic active zones”

Masafumi Minoshima (Univ. of Osaka)

“Development of protein labeling method using fluorescent dye cycling for imaging applications”

Masataka Yanagawa (Kyoto Univ.)

“TRPV4—GPCR crosstalk mediated by membrane remodeling”

Rina Nagata (Kyoto Univ.)

“Mechanism of non-cell autonomous autophagy that drives cell competition”

Shungo Adachi (National Cancer Center)

“Analysis of Intracellular Non-membrane Structures Using Mass Spectrometry” (working title)

Takuro Tojima (RIKEN)

“High-speed super-resolution imaging of the Golgi apparatus in living cells”

18th September, Thursday

Session IV: Liquid droplet formation of biomolecules for biological function

9:00~10:40（25 min × 4）

Tetsuro Hirose (Univ. of Osaka)

“Multifaceted roles of HSATIII architectural noncoding RNAs in the formation and function of nuclear stress bodies”

Nobuo Noda (Hokkaido Univ.)

“Molecular roles of phase separation in autophagy initiation”

Hayashi Yamamoto (Nippon Medical School)

“Autophagy and exosome secretion of liquid-like ferritin condensates”

Roland Knorr (Univ. of Colongne)

“Condensates organize morphology and topology of cellular membranes”

（coffee break 20 min）

Session V: Biomolecular assembly and organization for biological function

11:00~12:15（25 min × 3）

Tatsuo Fukagawa (Univ. of Osaka)

“Kinetochore assembly and organization in vertebrate cells”

Natsumi Ageta-Ishihara (Toho Univ.)

“Septin 3 (SEPT3) regulates memory and L-LTP-dependent extension of endoplasmic reticulum into spines”

Haruo Kasai (Univ. of Tokyo)

“Mechanical synaptic transmission at dendritic spines: molecular mechanisms and functional implications”

（Group photo & lunch break 80 min）

Session VI: Super-resolution imaging of biological events

13:35~15:15（25 min × 4）

Tetsuya Higashiyama (Univ. of Tokyo)

“Membrane trafficking underlying precise pollen tube chemotropism”

Hotaka Kobayashi (Tokushima Univ. & Univ. of Tokyo)

“Single-molecule imaging of RBP-mediated translational regulation in cells”

Satoru Fujiyoshi (Inst. of Sci. Tokyo)

“Correlative light and electron microscope for cross scale cell biology”

Francesca Bottanelli（Freie Universitat Berlin）

“Unravelling the inner secrets of cells with gene editing and live-cell super-resolution microscopy”

（coffee break 20 min）

Session VII: Organelle biology revealed by structural and microscopic analyses

15:35~17:15（25 min × 4）

Koichi Kato (National Inst Natural Sci.)

“Exploring the Golgi landscape to decipher the glycosylation program”

Kenji Inaba (Kyushu Univ.)

“Cross-scale analysis unveils the mechanism of calcium homeostasis in the early secretory pathway”

Akiko K. Satoh（Hiroshima Univ.）

“Reversible control of post-Golgi transport by Brefeldin A using GBF1 Brefeldin A resistant cells”

Yusuke Hirabayashi（Univ. of Tokyo）

“Membrane protein condensates drive ER–mitochondria contacts via phase separation”

17:20~18:40

Poster presentation（40 min × 2 groups）

19:00~

Social gathering

19th September, Friday

Session VIII: In-cell structure analysis of biomolecular complexes

9:00~10:40（25 min × 4）

Masahide Kikkawa (Univ. of Tokyo)

“Cross-scale structural studies of epithelial cells by cryo-electron tomography”

Genji Kurisu (Univ. of Osaka)

“Structural basis of the protein shell facilitating efficient CO_2 fixation in diatom pyrenoids”

Friedrich Förster (Utrecht Univ.)

“Visualization of protein biogenesis at the endoplasmic reticulum and stress response”

Noritaka Nishida (Chiba Univ.)

“Structural insights into the formation and maturation of FUS phase-separated condensates revealed by in-cell NMR”

（coffee break 15 min）

Session IX: Cutting-edge technologies for in-cell molecular dynamics study

10:55~12:10（25 min × 3）

Hitoshi Kurumizaka (Univ. of Tokyo)

“Decoding chromatin structure for functional insights into genome regulation”

Shin Mizukami (Tohoku Univ.)

“Small molecule–protein hybrid probes for investigating cross-scale dynamic structures in cells”

Yuji Sugita（RIKEN）

“Multi-scale molecular dynamics study of biomolecular condensate formations and regulations”

（lunch break 70 min）

Session X: Biomolecular condensation revealed by cross-scale analysis

13:20~15:00（25 min × 4）

Eiji Yamamoto (Keio Univ.)

“Mesoscale simulations of protein dynamics in heterogeneous biomolecular condensates”

Shoji Hata (Univ. of Tokyo)

“Molecular properties for liquid crystallization drive assembly of centrosomal structures”

Makito Miyazaki（RIKEN）

“Optogenetic control of actin cytoskeletal dynamics in reconstituted systems”

Motomasa Tanaka (RIKEN)

“Cross-scale analysis of disease-associated amyloid fibrils”

15:00~15:05

Closing remarks by Masahide Kikkawa (Leader of transformative and innovative research group “Cross-scale Biology”)

Abstracts

INTERNATIONAL SYMPOSIUM ON CELLULAR STRUCTURAL BIOLOGY

Crosstalk between membrane and Nucleic acids: DNA/RNA transport across cell membranes

Osamu Nureki (Biol. Sci., Grad. Sci., The Univ. of Tokyo)

We have long progressed nucleic acid research in parallel to membrane protein research, which are now converging. Bacterial Type-II secretion apparatus takes up DNA from outside through the membrane to alter their genetic properties (transformation). Cryo-EM structure of ComEC responsible for DNA permeabilization revealed that one of the double-stranded DNA is degraded by the nuclease domain and the remaining single-stranded DNA permeates the membrane through a ratcheting mechanism by a single-stranded DNA binding protein localized inside the cell. In contrast, systemic RNA interference in Caenorhabditis elegans relies on the transmembrane protein SID1 for double-stranded RNA uptake. We determined the cryo-EM structures of SID1 in complex with dsRNA, which reveal that SID1 lacks a channel-like pore, instead forming self-organized multimeric complexes with dsRNA. Live-cell imaging demonstrates SID1-dependent dsRNA internalization apparently against concentration gradients, which is inhibited under endocytosis-suppressing conditions. Imaging at single-molecule levels shows SID1 dimers multimerize upon dsRNA binding, followed by clathrin accumulation and the subsequent dynamin recruitment. The dodecameric SID1-dsRNA complex structure suggests that SID1 multimer folds a large dsRNA molecule into a compact size to be packaged in a size-limited endosome.

[○Osamu Nureki[1] , Akira Takai[1,2], Kaoru Kumazaki1,Yasushi Okada[1,2]

([1]Biol. Sci., Grad. Sci., The Univ. of Tokyo, [2]Grad. Med., The Univ. of Tokyo)]

Session I

Molecular conformational dynamics of the DOCK protein complexes in signal transduction

Mikako Shirouzu (RIKEN)

The dedicator of cytokinesis (DOCK) family proteins function as guanine nucleotide exchange factors (GEFs) that activate Rho-family small GTPases, Rac and/or Cdc42, in several cellular processes including migration, phagocytosis, and cancer invasion. DOCK-A subfamily members (DOCK1, 2, and 5) bind to ELMO protein, and another small GTPase RhoG activates the ELMO-DOCK-Rac signaling pathway. Although we have previously reported the cryo-EM structure of the DOCK5–ELMO1–Rac1–RhoG complex (Kukimoto-Niino *et al., J. Biol. Chem.*, 2024), the structural mechanisms regulating DOCK activity on the plasma membrane have remained elusive. Here, we used cryo-electron microscopy with lipid membrane-coated grids to visualize a new conformation in which DOCK5, ELMO1, RhoG, and Rac1 are aligned on a plane and flattened. Biochemical and cellular experiments suggested that lipid-mediated conformational changes are important for the downstream signaling.

On the other hand, the structural basis for the regulation of DOCK-C family members (DOCK6, 7, and 8), which function independently of ELMO proteins, has remained unknown. Here, we determined the cryo-EM structures of full-length DOCK6 in three distinct states: apo, Rac1-bound, and Cdc42-bound forms. Surprisingly, we found that DOCK6 not only forms dimers but also assembles into tetramers and octamers. In these higher-order oligomers, DOCK6 adopts a closed conformation in which the catalytic and membrane-binding domains from different dimers interact with one another. This suggests a novel regulatory mechanism in which DOCK6 activity is modulated through oligomerization.

Session I

Molecular basis for resolving ribosome collisions induced by aberrant translational stalling

Yoshitaka Matsuo (Univ. of Tokyo)

Translation elongation speed is regulated by synonymous codon usage and is tightly coupled with processes such as nascent chain folding, protein targeting, and co-translational complex assembly. Aberrant translational stalling can lead to the production of malfunctioning proteins, and cells counteract this by employing quality control mechanisms that detect and terminate faulty translation.

A key challenge has been understanding how cells distinguish abnormal stalling from the diverse, codon-adapted elongation rates characteristic of each mRNA. Recent advances, including ribosome profiling, cryo-EM, and high-speed AFM, have revealed that ribosome collisions serve as a hallmark of aberrant translation. When elongation stalls, trailing ribosomes collide with the stalled one, forming a “ribosome traffic jam.” The sensor protein Hel2 detects these collisions and ubiquitinates the stalled ribosomes, thereby marking them for clearance by the Ribosome Quality control Trigger (RQT) complex.

This presentation will highlight recent insights into the molecular machinery that resolves ribosome collisions to maintain proteostasis and prevent excessive cellular stress responses.

Session I

Molecular basis for RQC and CAT tailing in the cytosol and at the ER

Roland Beckmann
Gene Center and Department of Biochemistry, Feodor-Lynen-Str. 25, University of Munich, 81377 Munich, Germany.

Abstract:
Problems in translation due to faulty mRNA or other modes of cellular stress lead to ribosomal collisions which are sensed by specific cellular factors for stress signaling and for clearance of problematic mRNA and incomplete nascent polypeptides. RQC, ribosome-associated quality control, is a conserved process degrading these potentially toxic truncated nascent peptides whose malfunction underlies neurodegeneration and proteostasis decline in aging. During RQC, dissociation of stalled ribosomes is followed by elongation of the nascent peptide with alanine and threonine residues, which in humans is driven by NEMF (Rqc2 in yeast). The resulting CAT tails (carboxy-terminal tails) and ubiquitination by the LTN1 listerin E3 ubiquitin protein ligase 1 (Ltn1 in yeast) together with the RQC factor TCF25 (Rqc1 in yeast) mark nascent peptides for proteasomal degradation. Here we present cryo-electron microscopy (cryo-EM) structures, revealing the mechanistic basis of individual steps of the CAT tailing cycle in humans. Recognition of the amino-acylated tRNA by NEMF in the ribosomal A-site appeared to be slightly different from the yeast system indicating potentially distinct recruitment specificity and CAT tail composition between yeast and humans. Moreover, we observed that TCF25 binds ubiquitin as well as the RING domain of listerin near the ribosomal tunnel exit, explaining the role of TCF25 in specifying K48 linkage of the poly-ubiquitin chains on the nascent peptide chain. We also discovered how on the 60S ribosomal subunit the RQC machinery can work together with the UFMylation machinery at the endoplasmic reticulum for coordinating ER-RQC of stalled secretory and membrane proteins.

Session II

Deciphering Microtubule-Orchestrated Biological Phenomena through Cross-Scale Measurements

Ryo Nitta (Kobe University Graduate School of Medicine)

Microtubules are tubular polymers composed of α- and β-tubulin dimers, playing a fundamental role in key cellular processes, including morphogenesis, cell division, and intracellular transport. In cells, microtubule polymerization is tightly regulated and typically requires a microtubule-organizing center (MTOC). The centrosome, which forms at both poles of the mitotic spindle, is a well-known example of an MTOC. However, recent studies have revealed that in highly polarized cells such as cardiomyocytes, neurons, and epithelial cells, microtubule networks are formed independently of the centrosome through non-centrosomal microtubules.

We have discovered that in non-centrosomal microtubules, the microtubule minus-end binding proteins CAMSAPs contribute to MTOC formation through liquid–liquid phase separation. In this presentation, we will introduce our findings on how CAMSAPs facilitate microtubule network formation, elucidated through cross-scale measurements, including cryo-electron microscopy, atomic force microscopy, total internal reflection fluorescence microscopy, and molecular dynamics simulations.

Session II

Revealing the mechanism of dynamic mesoscale actin ring organization in collective cell migration

Erina Kuranaga (Kyoto University/ Tohoku University)

Collective cell migration plays important roles in tissue morphogenesis, wound healing, and cancer metastasis. How the cell state transitions from static to migratory phase is largely unrevealed. Previously, we have found that epithelial cells around the *Drosophila* male external genitalia exhibit asymmetric myosin II distribution at cell boundaries. The asymmetric contractility drives cell intercalation that leads to unidirectional collective cell migration. In this study, a high-resolution analysis of actin distribution is performed. Interestingly, actin forms multiple unstable ring structures with a diameter of about 1 μm at the apical side of the migrating cells. Membrane vesicles are found inside the "actin rings" and the ultrastructural analysis reveals numerous vesicles with low electron density, resembling macropinosomes. The fluorescent Dextran internalization assay reveals that the epithelial cells indeed undergo pinocytosis. Downregulating macropinocytosis causes a decrease in actin rings. The polarized activation of micropinocytosis may be the key to understanding unidirectional collective cell migration.

Session II

Cross-Scale Biology Studies by Advanced Atomic Force Microscopy Techniques

Takeshi Fukuma (Kanazawa University)

Atomic force microscopy (AFM) is a powerful technique that enables direct, label-free imaging of biomolecular dynamics in liquids. To broaden its applicability in biological research, we have developed a suite of advanced AFM techniques. For instance, our in-liquid frequency modulation AFM (FM-AFM) has achieved subnanoscale imaging of surface structures of diverse biological molecules and their assemblies. We also developed three-dimensional AFM (3D-AFM), which allows visualization of subnanoscale 3D hydration structures and flexible molecular chains. More recently, we introduced in-cell AFM, enabling nanoscale measurements of dynamic and mechanical properties within living cells. Furthermore, as Director of the Nano Life Science Institute (NanoLSI) at Kanazawa University, one of the WPI centers, we have access to state-of-the-art AFM platforms, including high-speed AFM originally developed by Ando and colleagues.

Within the framework of the Transformative Research Area (A) project, “Cross-Scale New Biology,” we have conducted a range of collaborative studies utilizing these advanced AFM modalities. For example, in collaboration with the Tanaka group, we visualized the three-dimensional distribution of fuzzy coat structures on amyloid fibers and revealed their strong pH dependence. In partnership with the Fujiwara group, we employed in-cell AFM to capture the growth dynamics of focal adhesions and associated actin fibers. Additionally, in collaboration with the Nitta group, we investigated changes in nuclear elasticity associated with cardiomyopathy by in-cell AFM and also elucidated the molecular mechanisms of microtubule assembly and aster formation mediated by CAMSAP2 by high-speed AFM.

Session II

Submolecular video-imaging of Smc5/6 complex by high-speed atomic force microscopy

Kenichi Umeda (Kanazawa Univ.)

The human genome, totaling approximately 2 meters in length, is compactly folded within the micron-scale confines of the cell nucleus. This genome folding changes dynamically throughout the cell cycle and is intimately linked to essential chromosome functions, including cell division and gene expression. Structural Maintenance of Chromosomes (SMC) complexes, such as cohesin, condensin, and Smc5/6, play central roles in organizing the genome's higher-order 3D architecture. These ATPase motor proteins form ring-shaped structures that are thought to encircle DNA, functioning as molecular clamps. However, the precise molecular mechanisms underlying their interaction with DNA remain unresolved. Although recent advances using single-molecule fluorescence and cryo-EM have shed light on SMC functions, the dynamic behavior of SMC complexes topologically bound to DNA at the submolecular level remains largely unexplored.

In this study, we used high-speed atomic force microscopy to visualize the submolecular dynamics of Smc5/6, the least understood SMC complex. With time resolutions on the order of 100 milliseconds, we successfully captured Smc5/6 diffusing along DNA and undergoing structural transitions in real time. Further analysis using ATPase-deficient mutants revealed that Smc5/6 first accesses DNA via its ATPase domains and then transitions into a more stable DNA-bound state. These findings provide new insights into the molecular mechanism of DNA engagement by Smc5/6.

Session IV

Multifaceted Roles of HSATIII Architectural Noncoding RNAs in the Formation and Function of Nuclear Stress Bodies

Tetsuro Hirose (Graduate School of Frontier Biosciences, Osaka University)

Specific long noncoding RNAs (lncRNAs), known as architectural RNAs (arcRNAs), act as structural scaffolds for membraneless organelles and regulate gene expression through distinct mechanisms. We have been intensively studying nuclear stress bodies (nSBs), which are formed on HSATIII arcRNAs. These HSATIII arcRNAs are transcribed from Satellite III genomic regions in primate genomes under thermal stress and facilitate nSB assembly by recruiting multiple proteins, including splicing factors. Our study revealed that nSBs regulate temperature-dependent pre-mRNA splicing immediately after stress removal via two distinct mechanisms. First, nSBs serve as a "reaction crucible" where splicing factors are phosphorylated. Second, they function as a "molecular sponge," sequestering specific splicing factors. In the crucible mechanism, the protein kinase CLK1 is specifically phosphorylated and recruited to nSBs upon stress removal, where it encounters unphosphorylated serine/arginine-rich splicing factors (SRSFs), which serve as its substrates. The colocalization of CLK1 and SRSFs within nSBs enables efficient phosphorylation of SRSFs, thereby reactivating splicing regulation during the early phase of stress recovery. We also identified a kinase/phosphatase pair that functions as a temperature sensor, regulating the phosphorylation status of CLK1 and thus modulating its localization to nSBs. Surprisingly, during the later stages of stress recovery, we observed that HSATIII RNAs migrate from the nucleus to the cytoplasm. There, they either form distinct membraneless cytoplasmic bodies or are translated into polypeptides. In this symposium, we will provide an overview of the multifaceted roles of satellite RNAs and discuss the potential functions embedded within satellite genomic regions.

Session IV

Molecular roles of phase separation in autophagy initiation

Nobuo N. Noda (Hokkaido University)

Autophagy is an intracellular degradation pathway that requires the de novo formation of autophagosomes. In yeast, starvation triggers phase separation of the autophagy-initiating Atg1 complex, giving rise to the pre-autophagosomal structure (PAS), from which autophagosome biogenesis is thought to proceed. The physiological roles of the PAS droplet, however, remain poorly defined. Here, we introduced downstream Atg factors into in vitro-reconstituted early PAS droplets formed by phase-separated Atg1 complexes and found that each factor localized to the droplets with distinct efficiencies. Among the core Atg proteins, the Atg12–Atg5–Atg16 E3-ligase complex for Atg8 lipidation was enriched most efficiently. Further reconstitution experiments showed that early PAS droplets enriched with this E3 complex markedly promote Atg8 lipidation, and that Atg8 coating of vesicle membranes is both necessary and sufficient for their incorporation into the droplets. Together, these findings indicate that the PAS serves as an efficient production site for lipidated Atg8 and as a reservoir of membrane seeds that drive autophagosome formation.

Autophagy and exosome secretion of liquid-like ferritin condensates

Hayashi Yamamoto (Nippon Medical School)

Ferritin, an iron storage protein that forms a cage-like complex, plays a central role in intracellular iron homeostasis. To reuse stored iron ions, ferritin complexes are transported into lysosomes via two pathways: macroautophagy (autophagy) and microautophagy (the MVB-like pathway). In this study, we investigated the mechanisms by which ferritin complexes are transported through two distinct routes and found that ferritin complexes undergo further assembly via LLPS, forming liquid-like condensates. Time-lapse imaging and 3D-CLEM revealed that, during macroautophagy, the autophagosomal membrane closely associates with liquid-like ferritin condensates through 'membrane wetting', a characteristic feature of 'fluidophagy'. This interaction is mediated by TAX1BP1 on the ferritin condensates and LC3 on the autophagosomal membrane. In microautophagy, ferritin condensates are incorporated into endosomes in a TAX1BP1-dependent manner, indicating that TAX1BP1 functions as a key regulator in both macroautophagy and microautophagy. In addition to TAX1BP1, microautophagy also requires OPTN, which binds to K63- or M1-linked ubiquitin chains via its UBAN domain. Specifically, microautophagy is mediated by two parallel axes—TAX1BP1–LC3 and OPTN–ubiquitin—whereas macroautophagy is regulated by the TAX1BP1–LC3 axis. These mechanistic differences suggest that selective engagement of these pathways determines the route of ferritin sorting between macroautophagy and microautophagy.

In addition, we found that ferritin condensates incorporated into endosomes via microautophagy are also secreted extracellularly as exosomes. This pathway may represent a mechanism by which intracellular components are concentrated into liquid-like condensates, packaged into exosomes, and efficiently exported. We are currently investigating the molecular mechanisms underlying this secretion process.

Session IV

Condensates organize morphology and topology of cellular membranes

Roland L. Knorr
University of Cologne, Faculty of Medicine and University Hospital Cologne, Germany;
CECAD - Cluster of Excellence for Aging Research, Cologne, Germany;
Graduate School and Faculty of Medicine, The University of Tokyo, Japan

Membrane-bound organelles have been considered the fundamental organizational compartments of cells for decades. However, more recent research has revealed that condensed biomolecular phases (aka membrane-less organelles) play an additional intricate role in cells. Combining in vivo and in vitro experimentation with theoretical approaches allowed us to demonstrate that condensates and membrane interact in cells, which gives rise to novel capillarity-based force generation mechanisms that are implicated in fundamental physiological processes.
In my talk I will introduce capillarity and highlight how condensates drive morphogenesis of membrane-bound organelles by promoting membrane bending events and also, by cutting membrane neckswithout ATP-consuming protein machineries like ESCRTs. Examples include the formation of intraluminal vesicles in multivesicular bodies, of autophagosomes during autophagy and of protein storage vacuoles during plant embryogenesis. Our findings unveil previously unrecognized physiological roles of the condensate-membrane interplay, exemplifying how key compartments jointly contribute to intracellular organization.

Session V

Kinetochore assembly and organization in vertebrate cells

Tatsuo Fukagawa (FBS, The University of Osaka)

Chromosomes replicated during the S-phase must be divided into daughter cells during mitosis to transmit the genetic information to the progeny. The accurate chromosome segregation requires the kinetochore, which is formed at the centromere on each chromosome to attach chromosomes to spindle microtubules. If this attachment is incorrect, chromosome mis-segregation is caused, leading to many of genetic disorders including cancer formation. Therefore, a study on chromosome segregation is an important subject for both basic biology and medical research.

We focus on clarifying mechanisms of kinetochore assembly. The kinetochore is composed of more than 100 kinds of proteins, and it contains two major protein complexes: Constitutive Centromere Associated Network (CCAN) and Knl1-Mis1-Ndc80 complexes (KMN) network. CCAN, which consists of 16 components, constitutively localizes to the centromere throughout the cell cycle and forms a base of the kinetochore. In contrast KMN is recruited to the CCAN during mitosis to establish a functional kinetochore that binds to microtubules. While many biochemical and structural studies propose assembly models of the kinetochore, genetic studies are not always consistent with such models, based on the biochemical and structural studies. We took a combination study of biochemistry, structural biology and genetics and are proposing a model for kinetochore assembly. In this talk, we present our recent progresses and would like to discuss how the kinetochore assembles and functions.

Septin 3 (SEPT3) regulates memory and L-LTP-dependent extension of endoplasmic reticulum into spines

Natsumi Ageta-Ishihara (Toho Univ.)

Transient memories are converted to persistent memories at the synapse and circuit/systems levels. The synapse-level consolidation parallels electrophysiological transition from early- to late-phase long-term potentiation of synaptic transmission (E-/L-LTP). Excitatory synaptic activities trigger immediate mobilization of glutamate receptors and other neurotransmission machinery, followed by actin polymerization-dependent dendritic spine enlargement, which cooperatively increase synaptic conductance and structural stability. However, it remains unclear whether E- and L-LTP differ only in the magnitude and duration of the shared processes, or any other synaptic processes unique to L-LTP confer persistence by making qualitative differences from E-LTP.

The septin cytoskeleton is one of GTP-binding proteins that assemble into hetero-oligomeric complexes. In dividing cells, septins are associated with cytokinesis, cell motility, and ciliogenesis, as a scaffold that organizes other molecules and/or as a diffusion barrier that asymmetrically compartmentalizes the dividing membrane. In the nervous system, septins have emerged as pivotal players in neuronal migration, neurite outgrowth, spine morphogenesis, synaptic transmission, and neurodegenerative diseases.

Here we show that L-LTP induced at the perforant path-hippocampal dentate gyrus (DG) synapses accompanies cytoskeletal remodeling that involves actin and the septin subunit SEPT3. L-LTP in DG neurons causes fast spine enlargement, followed by SEPT3-dependent smooth endoplasmic reticulum (sER) extension into enlarged spines. Spines containing sER show greater Ca^{2+} responses upon synaptic input and local synaptic activity. Consistently, *Sept3* knockout in mice (*Sept3*$^{-/-}$) impairs memory consolidation and causes a scarcity of sER-containing spines. These findings indicate a concept that sER extension into active spines serves as a synaptic basis of memory consolidation.

Session V

Mechanical Synaptic Transmission at Dendritic Spines: Molecular Mechanisms and Functional Implications

Haruo Kasai (The University of Tokyo)

The majority of excitatory synapses in the brain are formed on dendritic spines—tiny protrusions from neuronal dendrites—that enlarge rapidly in response to associative pre- and postsynaptic activity during learning processes. We have uncovered a novel, third form of synaptic transmission whereby dendritic spine enlargement mechanically enhances presynaptic functions. This mechanical synaptic transmission occurs significantly faster than the postsynaptic increases in glutamate sensitivity. Furthermore, we recently developed a chemogenetic tool, SYNCit, allowing precise manipulation of dendritic spine dynamics across extensive cortical regions. Our current findings indicate that such mechanical interactions at dendritic spines play critical roles in regulating cognitive functions and maintaining wakefulness. Ongoing investigations into the molecular mechanisms underlying the rapid induction of presynaptic enhancement are elucidating how mechanical transmission achieves both speed and efficiency.

Session VI

Membrane trafficking underlying precise pollen tube chemotropism

Tetsuya Higashiyama (The University of Tokyo)

Pollen tube cells emerging from pollen grains exhibit tip growth, driven by active secretion at the tube tip, at a rate that is among the fastest known in living organisms. Moreover, they are precisely guided by directional cues from female tissues, such as LURE attractant peptides secreted by two specific cells adjacent to the egg cell. Membrane trafficking in pollen tubes has been proposed as a key mechanism supporting both rapid elongation and precise chemotropism; however, its spatiotemporal dynamics remain poorly understood. In this talk, I will introduce the dynamics of membrane trafficking in pollen tubes as revealed by SCLIM super-resolution microscopy. Membrane trafficking in pollen tubes is more diverse than previously assumed, and distinct pathways regulate key plasma membrane receptors involved in tip growth. These receptors include PRK6, which acts as an accelerator of tip growth, and ANXUR, which functions as a brake, monitoring tip growth via an autocrine pathway. PRK6 also perceives LURE attractant peptides, though how PRK6 and LURE together control growth direction remains unclear. I will discuss the possibility that such diverse membrane trafficking pathways contribute to the robustness of chemotropism. I will also present ongoing efforts using single-molecule imaging to elucidate the potential role of attractant receptor endocytosis in regulating the chemotropic responses of the pollen tube.

Session VI

Single-molecule imaging of RBP-mediated translational regulation in cells

Hotaka Kobayashi (Tokushima Univ. & Univ. of Tokyo)

Translation, in which ribosomes decode mRNAs to synthesize nascent peptides, is a central mechanism in gene expression. A variety of RNA-binding proteins (RBPs) regulate this process by interacting with their target mRNAs. For decades, this type of regulation has been studied using ensemble biochemical methods that analyze bulk populations of mRNAs in test tubes. As a result, how individual mRNAs behave during translational regulation – and how this regulation occurs in time and space within cells – remains poorly understood. To bridge this gap, we established a method to image translational regulation by Argonaute (AGO) proteins, a class of RBPs, at single-mRNA resolution in cells. This method utilizes three-color fluorescence microscopy to simultaneously detect mRNAs, nascent peptides, and AGO proteins, and can be broadly applied to study translational regulation by other RBPs as well. In this symposium, I will introduce the principles of this method and share our latest findings obtained through its application.

Session VI

Correlative light and electron microscope for cross scale cell biology

Satoru Fujiyoshi (Institute of Science Tokyo)

Cryofixation has attracted attention as a method to preserve the spatial information of living cells at the angstrom scale within a few milliseconds. This represents a major advantage over chemical fixation. In cryo-electron microscopy (cryo-EM), cryofixed cells are physically sectioned into approximately 100-nm-thick lamellae. These thick cell lamellae also provide an ideal environment for single-molecule fluorescence microscopy because their thickness is comparable to the photo-excitation depth of total internal reflection fluorescence (TIRF) microscopy, and efficiently suppresses background emission. Imaging the same cell lamella with both cryo-electron microscopy and single-molecule fluorescence microscopy should enable molecular-level visualization of the cell interior. However, such attempts are often hindered by crystalline ice contamination that occurs during measurement or sample transfer. In this work, we developed a cryogenic single-molecule fluorescence microscope for cryogenic correlative light and electron microscopy (cryo-CLEM). The microscope consists of a cryostat equipped with crystalline ice contamination protection and a confocal fluorescence microscope unit stabilized to a single-nanometer precision over the course of a day. As a result, we successfully demonstrated cryo-CLEM imaging of subcellular structures.

Session VI

Unravelling the inner secrets of cells with gene editing and live-cell super-resolution microscopy

Francesca Bottanelli
Biochemistry department at Freie Universität in Berlin (Germany)

In our laboratory, we integrate endogenous tagging with live-cell super-resolution STED microscopy to illuminate dynamic processes occurring at the nanoscale and in the unperturbed physiological environment of the cell. We have generated a comprehensive library of proteins involved in intracellular communication. The ability to explore endogenous cellular dynamics is revealing novel and unexplored sorting mechanisms for trafficking in and out of the Golgi apparatus. We have provided functional characterization of a novel sorting compartment that we named ARF1 compartment. ARF1 compartments orchestrate clathrin-dependent post-Golgi trafficking via maturing into recycling endosomes. Additionally, we investigate how tubular-vesicular ER exit sites drive ER-to-Golgi transport via an analogous maturation mechanism. We also study the function of actin-rich protrusion as a signaling organelle and investigate how signalling molecules dynamically re-organize on membrane protrusions during chimeric antigenic receptor (CAR)–mediated activation.

Session VII

Exploring the Golgi Landscape to Decipher the Glycosylation Program

Koichi Kato[1,2] ([1]Exploratory Research Center on Life and Living Systems (ExCELLS), National Institutes of Natural Sciences, [2]Graduate School of Pharmaceutical Sciences, Nagoya City University)

Glycosylation, a critical post-translational modification, profoundly influences protein function and plays a central role in regulating cellular processes and intercellular communication. While artificial intelligence has revolutionized protein structure prediction, accurately modeling glycoproteins remains challenging, primarily because glycan modifications are not directly encoded in the genome. However, despite appearing stochastic, protein glycosylation largely follows deterministic rules governed by interactions with glycosylation-related enzymes, most of which are localized in the Golgi apparatus. Deciphering these mechanisms is key to enabling precise control over glycosylation, with significant implications for therapeutic engineering.

By integrating cutting-edge bioimaging and omics technologies, our study reveals the remarkable complexity of the Golgi apparatus, with its dynamic morphology and finely tuned spatial distribution of enzymes. We systematically demonstrate that the compartmentalized localization of glycosylation-related enzymes within Golgi cisternae orchestrates the stepwise modification of glycoproteins. Furthermore, we identify key molecular codes and regulatory networks that govern glycoprotein trafficking and selective interactions with glycosyltransferases, providing new insight into the spatial regulation of glycosylation.

Our results underscore the critical role of Golgi architecture and its orchestrated molecular environment in shaping glycosylation outcomes. This multi-level investigation advances our understanding of glycobiology and provides a foundation for engineering glycosylation pathways with enhanced precision and therapeutic potential.

Cross-scale analysis unveils the mechanism of calcium homeostasis in the early secretory pathway

Kenji Inaba (Medical Institute of Bioregulation, Kyushu Univ.)

Calcium ion (Ca^{2+}) homeostasis is tightly regulated within cells. Based on the preceding studies using conventional Ca^{2+} probes, such as aequorin and FRET-based sensors, the cytosolic Ca^{2+} concentration is maintained below 100 nM, whereas the endoplasmic reticulum (ER) sustains a much higher Ca^{2+} concentration of nearly 1 mM, primarily through the action of the SERCA calcium pump, making it the main intracellular Ca^{2+} reservoir. The Golgi apparatus also contains a calcium pump, SPCA1, which maintains its luminal Ca^{2+} concentration at ~100 mM. However, quantifying Ca^{2+} with these conventional probes often lacks accuracy under physiological conditions, particularly in acidic organelles like the Golgi. To overcome this limitation, we have developed a novel Golgi-targeted fluorescent Ca^{2+} probe, named CEPIA-Golgi, which enables highly accurate measurement of Ca^{2+} concentrations within individual Golgi cisternae.

Consequently, we discovered a substantial gap in Ca^{2+} concentrations between the ER and cis-Golgi, and found that the Ca^{2+} concentration progressively increases from the cis- to trans-Golgi. This distribution pattern contrasts with the previously proposed model suggesting a gradual decline in Ca^{2+} concentration from the ER toward the Golgi. Furthermore, our systematic quantification of Ca^{2+} under SERCA2 and SPCA1 knockout conditions revealed that Ca^{2+} concentrations in the ER and Golgi are regulated specifically and separately by SERCA2 and SPCA1, respectively. Immunofluorescence and immunoelectron microscopy targeting these Ca^{2+} pumps demonstrated that SPCA1 is localized to the trans-Golgi and TGN, whereas SERCA2 is confined to the ER. These distinct localizations explain the authentic Ca^{2+} distribution map in the early secretory pathway, which we determined using CEPIA-Golgi.

Session VII

Reversible control of post-Golgi transport by Brefeldin A using GBF1 Brefeldin A resistant cells

Akiko K. Satoh (Graduate School of Integrated Sciences for Life, Hiroshima University)

Brefeldin A (BFA) is a well-known ARF-GEF inhibitor, which induces the Golgi absorption into the endoplasmic reticulum (ER) in human cell lines. This absorption depends on the inhibition of an *cis*-Golgi locating ARFGEF, GBF1, which activates ARF small GTPase on the *cis*-side of Golgi stacks and recruits COPI coats. Here we construct cell lines carrying a BFA-resistant GBF1 allele, $GBF1^{M832L}$. When applied on nocodazole-treated $GBF1^{M832L}$ cells, BFA does not induce Golgi absorption into the ER but rather inhibits the post-Golgi transport. Subsequent BFA washout immediately restarts post-Golgi transport, probably by recovery of TGN/RE-resident ARFGEFs, BIG1/2. Therefore, BFA combined with nocodazole-treated $GBF1^{M832L}$ cells can be used as the system to manipulate post-Golgi transport, enabling the detailed live imaging, electron microscopy and the biochemical analysis.

Using this system, we demonstrated that BFA arrests the transport of Glycosylphosphatidylinositol-anchored proteins (GPI-APs) at the *trans*-side of the Golgi stacks in $GBF1^{M832L}$ cells, and subsequent BFA washout quickly recruits ARF1, ARF3, AP1 and clathrin to the TGN, thereby gradually restore the translocation of the GPI-APs to the Golgi-associated recycling endosomes (GA-REs). GA-REs occasionally become larger during GPI-AP uptake and release from the Golgi stack, resulting in the formation of free REs. Electron microscopies show that, at this time, massive tubule- and beads-on-thread like structures positive for GPI-AP extend from the TGN. These results indicate that these tubule- and beads-like structures are intermediate compartments carrying GPI-APs from the TGN to the REs.

Session VII

Membrane protein condensates drive ER–mitochondria contacts via phase separation

Yusuke Hirabayashi (The University of Tokyo)

Mitochondria–ER membrane contact sites (MERCS) are essential ultrastructural features that support key biochemical and physiological processes. These sites serve as platforms for metabolite and lipid exchange between mitochondria and the endoplasmic reticulum (ER). The ER-resident protein PDZD8 has emerged as a critical tethering factor at MERCS, but the identity of its outer mitochondrial membrane (OMM) binding partner and the mechanism by which it establishes distinct contact sites remained unclear. Recently, we identified FKBP8 as the mitochondrial tethering partner of PDZD8 using unbiased proximity proteomics, CRISPR-Cas9-mediated endogenous tagging, cryo-electron tomography, and correlative light-electron microscopy. Single-molecule tracking reveals dynamic diffusion of PDZD8 along the ER membrane with frequent pausing at MERCS. Furthermore, PDZD8 promotes mitochondrial complexity in a FKBP8-dependent manner.

Here, we show that PDZD8 undergoes liquid–liquid phase separation via its intrinsically disordered region (IDR) under physiological conditions, forming membrane-bound condensates both in vitro and in vivo. Electron microscopy analyses reveal that full-length PDZD8—but not IDR-deleted PDZD8—rescues MERCS formation in PDZD8 knockout cells.

This study demonstrates that the membrane contact site proteins can form biomolecular condensates at the interface of lipid membranes stitching together the neighboring organelles.

Session VIII

Structural basis of IFT-motor-cargo interaction and flagellar asymmetry

Masahide Kikkawa (The Univ. of Tokyo)

Intraflagellar transport relies on motor proteins, such as kinesin-2 and IFT-dynein, to deliver essential cargoes along microtubules; however, the mechanisms of cargo recognition remain unclear. Here, we present high-resolution cryo-electron microscopy structures of the heterotrimeric kinesin-2 complex (KIF3A/KIF3B/KAP3) bound to the adenomatous polyposis coli (APC) cargo. We found that the KIF3 tail has a unique motif, which we named “Hitchdock domain”. This domain plays a pivotal role in mediating interactions with both the KAP3 adaptor and the APC cargo. The KIF3A helical regions facilitate specific cargo binding, while the β-hairpin region and KIF3B provide structural support. Interestingly, the Hitchdock/KAP3 structure suggests a conserved structural basis for cargo recognition across molecular motors, including kinesin-1 and dynein, which utilize similar hook-like architectures, highlighting the potential universality of this mechanism.

We are also trying to identify the structural basis of flagellar asymmetry by analyzing the high-resolution structures of Zebrafish sperm. The longitudinal asymmetry is regulated by calcium, which is sensed by calaxin. We are also trying to characterize the rotationally asymmetric structure in the 9+2 structures of flagella using cryo-electron tomography

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Session VIII

Structural basis of the protein shell facilitating efficient CO_2 fixation in diatom pyrenoids

Genji Kurisu (Institute for Protein Research, The University of Osaka)

Pyrenoids are subcompartments of algal chloroplasts that concentrate Rubisco enzymes and their CO_2 substrate, thereby increasing the efficiency of carbon fixation. Diatoms perform up to 20% of global CO_2 fixation, but their pyrenoids remain poorly characterized at a molecular level. *In vivo* photo-crosslinking to catalogue components of diatom pyrenoids identified pyrenoid shell (PyShell) proteins localized to the pyrenoid periphery of both the pennate diatom, *Pheaodactylum tricornutum*, and the centric diatom, *Thalassiosira pseudonana*. *In situ* cryo-electron tomography (cryo-ET) revealed that the pyrenoids of both diatom species are encased in a lattice-like protein sheath. Disruption of PyShell expression in *T. pseudonana* resulted in the absence of this protein sheath, altered pyrenoid morphology, and a high-CO_2 requiring phenotype, with reduced photosynthetic efficiency and impaired growth under standard atmospheric conditions. Pyrenoids in mutant cells were fragmented and lacked the thylakoid membranes that normally traverse the Rubisco matrix, demonstrating how the PyShell plays a guiding role in establishing pyrenoid architecture. Recombinant PyShell proteins self-assembled into helical tubes and sheets, enabling us to determine a 2.4 Å-resolution PyShell structure. This *in vitro* structure was fitted into an *in situ* subtomogram average of the pyrenoid's protein sheath, yielding a putative atomic model of the PyShell within diatom cells. The structure and function of the diatom PyShell provides a new molecular view of how CO_2 is assimilated in the ocean, a crucial biome that is on the front lines of climate change.

Session VIII

Visualization of protein biogenesis at the endoplasmic reticulum and stress response

Friedrich Förster
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A hallmark of eukaryotic cells is the presence of distinct organelles that create specialized biochemical environments. The endoplasmic reticulum (ER) is the entry point of the secretory pathway, which handles roughly one-fourth of all human proteins, including secreted proteins and integral membrane proteins. For most of these proteins, the dynamic ribosome–translocon complex acts as the gateway into the ER.

Using cryo-electron tomography, extensive classification, and molecular modeling, we capture molecular-resolution snapshots of mRNA translation and protein maturation at the ER membrane. Distinct polysomes bind to specialized ER translocons that synthesize secreted proteins or multipass transmembrane proteins. Our analyses provide atomic-level insights into ribosomal intermediates and their associated translocon complexes responsible for co-translational processing.

We further explore how the translation machinery reorganizes at the ER and in the cytosol under different types of cellular stress. Lastly, we use cryo-ET to study oxidative stress response in the extreme thermophilic archaeon *Pyrococcus furiosus*, revealing an unexpected compartmentalization mediated by giant oxidoreductase nanotubes.

In summary, we use cryo-electron tomography to uncover the structure and dynamics of context-dependent, transient molecular complexes in both human and archaeal cells.

Structural insights into the formation and maturation of FUS phase-separated condensates revealed by in-cell NMR

Noritaka Nishida（Chiba University）

Fused in sarcoma (FUS) is an RNA-binding protein that forms reversible biomolecular condensates through liquid–liquid phase separation (LLPS), contributing to physiological processes such as RNA metabolism. However, aberrant transitions from liquid-like droplets to fibrillar aggregates have been implicated in the pathogenesis of neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). LLPS of FUS is primarily driven by its N-terminal low-complexity (LC) domain, an intrinsically disordered region (IDR) that lacks stable tertiary structure and engages in multivalent interactions. NMR spectroscopy is well-suited to analyze such flexible proteins, as it provides atomic-resolution insights into their dynamic and heterogeneous conformations. However, to understand the mechanism of FUS droplet formation in living cells, it is necessary to consider intracellular factors—such as macromolecular crowding and endogenous interactions—that are not recapitulated in vitro. To address this, we employed in-cell NMR to investigate FUS droplet formation under physiological conditions.

We introduced ^{15}N-labeled FUS LC into HeLa S3 cells and monitored chemical shift changes and time-dependent signal attenuation to monitor intracellular droplet formation. To identify key interaction regions essential for droplet maturation, we performed in-cell NMR analysis of a series of FUS deletion mutants. Furthermore, to overcome spectral overlap caused by the low sequence complexity of FUS, we employed a segmental isotopic labeling strategy using split inteins, thereby improving spectral resolution. In this presentation, we will report the molecular mechanisms underlying FUS droplet formation and maturation, as well as how disease-associated mutations accelerate fibril formation.

Decoding chromatin structure for functional insights into genome regulation

Hitoshi Kurumizaka (Institute for Quantitative Biosciences, The University of Tokyo)

In eukaryotic cells, DNA is tightly packaged into chromatin to fit within the cell nucleus. The fundamental unit of chromatin is the nucleosome, composed of a histone octamer (H2A, H2B, H3, and H4) wrapped by ~145-147 base pairs of DNA. These nucleosomes are connected by linker DNA, forming a "beads-on-a-string" structure observed under certain conditions. While nucleosomes help condense and protect DNA, they also introduce regulatory complexity. Acting as both physical barriers and active modulators, nucleosomes influence essential processes such as transcription, replication, recombination, and repair. Through epigenetic mechanisms, they dynamically regulate DNA accessibility, enabling or restricting the binding of regulatory proteins. For instance, during transcription, RNA polymerase must navigate or displace nucleosomes to proceed along the DNA strand. To better understand the functional role of nucleosomes in genome regulation, we have developed and optimized various biochemical approaches to study their structure and dynamics. These methods have revealed critical insights into how nucleosomes undergo structural transitions in response to different genomic states. In this symposium, I will share our recent findings on nucleosome plasticity and its impact on the regulation of gene expression, highlighting the central and dynamic role of chromatin architecture in genome function.

Small molecule–protein hybrid probes for investigating cross-scale dynamic structures in cells

Shin Mizukami (Tohoku University)

Intracellular functions are governed by dynamic molecular interactions intricately associated with mesoscale structures. Therefore, technologies that enable the visualization and manipulation of these structures are crucial for understanding cellular processes. Fluorescence imaging, widely employed for its high sensitivity, continues to evolve through the development of both small-molecule (SM) and fluorescent protein probes. In parallel, optical manipulation techniques have progressed from caged compounds to optogenetic tools. These approaches typically rely on either SM- or protein-based probes, each with distinct strengths and limitations. Hybrid probes that integrate both components offer the potential to overcome these limitations and provide enhanced functionality.

In this presentation, I will introduce our recent work on SM–protein hybrid probes that enable both visualization and optical control of intracellular mesoscale structures. For imaging, we improved the temporal resolution of single-molecule localization microscopy (SMLM), which is often limited by the slow blinking kinetics of fluorophores. By chemically tuning the blinking rate, we achieved rapid sequential SMLM imaging within seconds.

For optical control, we developed a photoswitchable ligand by incorporating a photochromic azobenzene moiety into methotrexate (MTX), a conventional ligand for eDHFR, enabling reversible binding in response to light of different wavelengths. By linking this photoswitchable ligand to another tag protein ligand (e.g., HaloTag), we created a modular platform for light-induced protein dimerization, allowing reversible control of protein translocation and mesoscale organization in living cells.

Session IX

Multi-scale molecular dynamics study of biomolecular condensate formations and regulations

Yuji Sugita[1,2,3] ([1]RIKEN PRI, [2]RIKEN R-CCS, [3]U. Tokyo)

Biomolecular condensates play key roles in many cellular functions. They contain highly disordered proteins or single-chain RNAs at high concentrations without reducing their fluidity. To study such dynamic systems in computational science, molecular dynamics simulations based on coarse-grained molecular models of biomolecules have often been employed because of the balance between computational efficiency and accuracy/reliability of the simulation results. In RIKEN, we introduce several coarse-grained models of proteins, disordered proteins, RNAs, DNAs, and lipid molecules in the MD software GENESIS. Recently, we have developed a highly parallel MD engine for implicit solvent CG MD simulations, which we call CGDYN [1]. Using CGDYN, we could perform large-scale simulations of biological systems, including biomolecular condensates. We have studied the formation and regulation of biomolecular condensates with other proteins, such as HERO proteins [2]. With a backmapping from coarse-grained to all-atom models, we could examine the role of water and ions in the formation and regulation at the atomic resolution. We also developed a new backmapping method based on machine learning called CGBack [3]. Based on the latest developments, multi-scale molecular dynamics can tackle many cellular functions at mesoscopic scales.

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Session X

Mesoscale simulations of protein dynamics in heterogeneous biomolecular condensates

Eiji Yamamoto (Keio Univ.)

In living cells, proteins and nucleic acids often form biomolecular condensates via phase separation. These membraneless organelles (MLOs) are involved in diverse processes such as stress response, signaling, and neurogenesis. A variety of intrinsically disordered proteins (IDPs) contribute to the formation of condensates through multivalent interactions and modulate their internal structure and material properties. Here, using multiscale molecular dynamics simulations, we investigate the dynamics and interactions of water, proteins, and nanoscale domains formed by specific proteins within condensates. Our results highlight how spatial heterogeneity within condensates governs molecular behavior, including conformational fluctuations, diffusivity, and the emergence of localized dynamic domains.

Molecular properties for liquid crystallization drive assembly of centrosomal structures

Shoji Hata (Univ. of Tokyo)

The centrosome controls diverse cellular functions by anchoring microtubules. During interphase, the centrosome anchors microtubules via structures called the subdistal appendages (SDAs). Upon mitotic entry, SDAs rapidly disassemble, and they quickly re-assemble upon mitotic exit. While the assembly dynamics of SDAs is considered important for the cell cycle-dependent microtubule network reorganization, their molecular mechanisms remain largely unknown. SDAs are huge and solid structures with numerous constituent proteins in regular orientation. Therefore, we aimed to reveal the molecular mechanisms enabling the rapid construction of this huge and ordered structure.

We focused on liquid-liquid phase separation (LLPS) as a potential mechanism for efficiently concentrating components for rapid SDA assembly. We found that CEP128, a major SDA component, undergoes LLPS to form condensates that concentrate other SDA components, suggesting that CEP128 condensates serve as a platform for SDA assembly. However, given the highly ordered molecular arrangement in SDAs, a mechanism is needed to explain how such order emerges from a liquid phase with random molecular orientations. Surprisingly, some CEP128 condensates exhibited liquid crystal-like properties, with molecules aligning in a layered fashion. A CEP128 mutant lacking this liquid crystal-forming ability failed to form proper molecular orientation within SDAs, leading to abnormal SDA structures. Collectively, our findings suggest that the huge and ordered SDAs are rapidly constructed through concentration of components via LLPS and their spontaneous alignment driven by liquid crystallization.

Optogenetic control of actin cytoskeletal dynamics in reconstituted systems

Makito Miyazaki (RIKEN)

The actin cytoskeleton forms a dynamic mesh-like network that drives cellular deformations. The structural and functional properties of actin network are defined by its density and the activity of actin-binding proteins. However, how network density impacts the penetration ability and activities of actin-binding proteins remains unclear. Here, we developed a method to spatiotemporally control actin network assembly on a supported lipid bilayer using photolithography and optogenetics. This approach enables precise manipulation of the density, thickness, and shape of Arp2/3-mediated actin network assembly. Using this reconstituted system, we investigated how network density affects the interaction of two representative actin-binding proteins: myosin and ADF/cofilin. We found that the penetration of myosin filaments into the network was strictly inhibited by only a several-fold increase in network density due to the steric hindrance. Furthermore, penetrated myosin filaments induced directional actin flow when the network has a density gradient. On the other hand, ADF/cofilin penetrated into the network regardless of network density. However, network disassembly was dramatically inhibited by only a several-fold increase in network density. These findings reveal the network-density-dependent functions of actin-binding proteins, shedding light on the mechanical regulation of cytoskeletal dynamics by the actin network density.

Cross-scale analysis of disease-associated amyloid fibrils

Motomasa Tanaka (RIKEN Center for Brain Science)

In the [*PSI*$^{+}$] prion system, the yeast prion protein Sup35 can form structurally distinct amyloid fibrils that lead to distinct transmissible prion states, or strains. However, our understanding of how different Sup35 fibril structures arise and translate to phenotypic variations is limited. Here we used cryo-EM and single-monomer force spectroscopy with optical tweezers to reveal the structural basis of yeast prion propagation in four wild-type and S17R mutant variants of Sup35 that underlie different [*PSI*$^{+}$] strains. Force spectroscopy shows distinct monomer conformational ensembles give rise to distinct fibril structures. Further, cryo-EM structures suggest prion strain strength is correlated with enhanced fibril propagation that is caused by a combination of low fibril stability and a large separation between the Sup35 fibril core and the Ssa1/Sis1 chaperone-binding region. These results provide a structure-based mechanism for the yeast prion strain phenomenon with implications for understanding amyloid propagation in human neurodegenerative diseases. In the presentation, the structure-phenotype relationships of distinct *in vitro*-generated tau fibrils will also be discussed.