as low as ~0.1 photons per an effective pixel as signal for the classification. Theoretically, wave-number of a pixel giving such a limiting photon-count defines the attainable resolution. To estimate attainable resolution even for structure-unknown molecule, we also developed a new concept of standard model protein. As a result, we can estimate attainable resolution by this new technology as a function of incident intensity and sample size.

2P012 ショウジョウバエ由来 GGA の溶液構造解析 Solution structural analysis of Drosophila GGA

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GGA is a multi-domain protein comprised of VHS, GAT, and GAE domains, which is an adaptor protein essential for the protein trafficking and targeting. GGA can interact with various proteins. How can GGA recognize its partners and how can GGA bind and release them during the protein trafficking? In order to understand the molecular mechanism of protein trafficking by GGA, it is essential to clarify the structure of GGA in the multi-domain state. The VHS domain recognizes the cytoplasmic acidic-cluster-dileucine (ACLL) sorting signal. Our previous study on mammalian GGA revealed that the domain rearrangement between VHS and GAT occurs when an ACLL peptide is bound to the VHS with the use of the fragment comprised of VHS and GAT. In this study, the effect of the binding of the ACLL peptide to VHS on the domain arrangement of the intact GGA was investigated using *drosophila* GGA (dGGA). We succeeded to purify the intact dGGA. Dynamic light scattering showed that the molecular size of dGGA is 150 Å in the absence of the ACLL peptide. Upon adding the peptide, the size was slightly increased to 155 Å, suggesting a structural change in the intact GGA. To reveal the structural change in detail, we are going to measure X-ray solution scattering profiles in the absence and the presence of the peptide. We will discuss the molecular mechanism of dGGA interaction with its cognate peptides based on the solution structures.

2P013 電子線位相差トモグラフィーによるべん毛基部位と蛋白質輸送装置 FliH/FliI との複合体の構造解析 Structural analysis of the flagellar basal body-FliH/FliI complex by phase contrast electron cryotomography

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The flagellar type III protein export apparatus is composed of six transmembrane proteins that form the export gate and three cytoplasmic proteins, FliH, FliI, FliJ, which promote the export process. The export apparatus translocates flagellar axial proteins into the central channel of the growing flagellum to export them to the distal end for self-assembly. It has been proposed that the export mechanism involves temporary binding of the FliH/FliI/FliJ/substrate complex to the flagellar basal body C ring before the complex is recruited to the export gate in order to facilitate the export process. However, the structure of the basal body with the export apparatus during the translocation process has not been observed yet due to the low stability of their binding and the use of detergents for the purification of the basal body. To visualize the structure of the export apparatus attached to the basal body by electron cryomicroscopy, we purified the FliH/FliI complex and the basal-body separately and cross-linked them by the method called Grafix, which uses a density gradient centrifugation step with weak chemical fixation. We succeeded in visualizing the structure of the basal body that is considered as the initial state of the export process. We are now analyzing the structure of the basal body with the FliH/FliI complex by phase contrast electron cryotomography, which can visualize the structure more clearly than conventional tomography.

2P014 細胞外マトリックス制御因子 RECK タンパク質の単粒子構造解析 Single particle analysis of the membrane-anchored protease inhibitor RECK

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The membrane-anchored protease regulator RECK (reversion-inducing cysteine-rich protein with Kazal motifs) plays important roles in mammalian development and tumor suppression, but its structure has been unclear. Electron microscopy-based single particle reconstruction, a computer-aided structure determination method, reconstructs a three-dimensional (3D) structure

from projections of mono dispersed protein. A large number of particle images are picked up from EM films, aligned and classified to generate two-dimensional (2D) averages, and, using the Euler angle of each 2D average, reconstructed into a 3D structure. From the negatively stained 2,325 particles, the 3D structure of RECK was reconstructed at 25.2 angstrom resolution assuming C2 symmetry. The resulting RECK-His model has a larger dimension than expected from its molecular size, most probably because of an internal large cavity. The RECK dimer seems to anchor to the membrane at its bell top and holds its opening with two protuberances outward. This configuration looks advantageous for docking and interactions with MMPs and other molecules.

2P015 大気圧走査型電子顕微鏡を用いた水中免疫電顕法によるタンパク質局在の高分解能観察 Atmospheric scanning electron microscope with immuno-labeling technique in solution determines protein localization at high resolution

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Determination of protein localization at high resolution is essential for the understanding of the physiological functions of protein. We have developed an immuno-labeling technique for the Atmospheric Scanning Electron Microscope (ASEM), which enables observation of labeled cells in a water solution. In the inverted ASEM system, an electron-permeable window made of pressure-resistant film allows the electron beam to be projected up from underneath the sample. The electrons backscattered from the sample are captured by a detector positioned below. In the immuno-labeling method, target proteins in cells are labeled with an antibody conjugated both with fluorescent dye and gold particles. Expression and rough localization of the proteins are visualized using a fluorescence microscope positioned above the sample, while higher-resolution images in the same area are obtained using the ASEM. In this technique, the gold particle is observed as a strong signal. The antigenicity of proteins and fine subcellular structures are well maintained because the cells are not dehydrated. Using this immuno-labeling method, we obtained high-magnification images of cytoskeletal structures and of ion channel localization in cells.

2P016 ヒトプリオンタンパク質における His96 と His111 の Cu²⁺ 結合能力 Cu²⁺ binding ability of His₉₆ and His₁₁₁ in human prion protein

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The N-terminal domain of prion protein (PrP) residues 23-125 includes four octarepeat regions, which has high affinity for binding Cu²⁺ leading to stabilize the structure. In this report, we tried to search the another site to bind Cu²⁺ in the N-terminal domain of PrP. HuPrP₉₅₋₁₁₄ containing His₉₆ and His₁₁₁, outside the octarepeats, was prepared and Cu²⁺ binding ability was assayed using Circular Dichroism (CD) with varying pH. By the addition of 1 mol eq. of Cu²⁺, the growth of the negative band at 200-250 nm and the blue shift of a negative peak at 200 nm were observed. However, further addition of Cu²⁺ did not achieve further change in the CD spectra. CD measurements in the UV region showed that huPrP₉₅₋₁₁₄ bound to Cu²⁺ at both physiological and acidic pH. In addition, to investigate the role of each site of His₉₆ and His₁₁₁ in Cu²⁺ binding, we also made similar experiments for huPrP₁₀₃₋₁₁₄ containing His₁₁₁. The negative peak at 200 nm did not show significant change, but slightly changes in negative band at 215 nm and 230-250 nm were observed by the addition of Cu²⁺ up to 2 mol eq.. At below pH 6.0, even Cu²⁺ was added, CD spectra for huPrP₁₀₃₋₁₁₄ never changed. These results suggest that His₁₁₁ binds to Cu²⁺ at only physiological pH, while His₉₆ even at acidic pH. Hence, we will plan to examine Cu²⁺ binding ability of His₉₆ by using huPrP₉₅₋₁₀₄ containing His₉₆. We will also examine more detailed conformation of Cu²⁺ in PrP with magnetic resonance spectroscopy.

2P017 分子動力学シミュレーションによる高圧における深海魚の α アクチンの構造変化の解析 Structure Change Analysis of Deep-sea Fish α-Actin at High Pressure by Molecular Dynamics Simulations

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α-actin plays important roles in numerous cellular functions in muscle and non-muscle cells. The amino acid sequence of α-actin is highly conserved in