2P007 PELDORによる時計タンパク質KaiBの構造変化の検出**PELDOR detection of structural changes of clock protein KaiB**

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The circadian clock is an endogenous biological mechanism that generates autonomous daily cycles of physiological activity. The cyanobacterial circadian clock, which consists of three clock proteins, KaiA, KaiB, and KaiC, works as a clock oscillator by forming the KaiABC complex.

We examined the structural changes of KaiB during incubation with both KaiA and KaiC by PELDOR method. The PELDOR is a well-established method to determine the distance between radicals with high accuracy. PELDOR results show that the inter-distance between the spin labels in KaiB was initially about 33.0 Å, and changed after incubation for several hours. These results indicate structural changes of KaiB in the formation of KaiABC complex.

2P008 2.5 kbar におけるユビキチン高エネルギー状態の立体構造解析**Solution structure of the "pure" high-energy state of ubiquitin: Q41N at 2.5 kbar**

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By using high-pressure NMR spectroscopy, solution structure of the alternatively folded state N2 of ubiquitin was determined. Comparing solution structures between N1 and N2, we designed ubiquitin mutants stabilizing N2 state of the protein. In the case of the Q41N mutant, in which hydrogen bond between I36 carboxyl group and Q41 side chain amide group is weakened, the N2 state is 71% populated even at 1 bar. Here, we demonstrate structural determination of Q41N at 2500 bar by high-pressure NMR spectroscopy, where the N2 state is about 98% populated. This allows us to characterize the structure of the "pure" N2 state. The combination of high-pressure NMR spectroscopy and amino acid substitution is powerful tool to characterize important intermediates of the protein.

2P009 X線小角散乱と電子顕微鏡像を用いたハイブリッド構造解析 Hybrid structure analysis with small-angle x-ray scattering and cryo-electron microscopic image

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Recently, small-angle x-ray scattering (SAXS) analysis on protein complexes by integrating other data such as cryo-electron microscopic (EM) images has been reported to be useful for improving the accuracy of the modeling. We attempted a hybrid structural analysis by use of two data of SAXS and EM image database (EMDB1313) on active oligomeric nitrilase complex in solution, the association of which is closely related to its activity. First, the EM data was used for homology modeling of the basic building block, a dimer. Second, the helical structure of active nitrilase complex was determined by SAXS. We report the result of the structure analysis and discuss about the effect of EM data in structure refinement process.

2P010 Structural analysis of the 26S proteasome by cryo-electron microscopy and Single-Particle Analysis

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The 26S proteasome (26S) undertakes the controlled degradation of proteins. The 2.5-MDa molecular machine comprises two types of subcomplexes: the barrel 20S core particle (CP) and the two 19S regulatory particles, which associate with the CP and prepare substrates for degradation. Since the weak assembly of the 26S makes structural analysis more difficult, we added 10% glycerol in the specimen for stabilization. But the low-contrast which made 'pick up' more difficult has been found in the 26S photograms. Thus we used the Gradient Fixation method to purify and stabilize the 26S. Then, CTF compensation using Wiener filter has been used to improve the contrast. As a result, particles could be visible and easy to be picked up. The refined 3D maps will be reported here.

2P011 Comparative survey of image processing packages for electron computed tomography

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Electron tomography (ET) is one of widely applicable methods to obtain the three-dimensional structure of organelles or cell components. Using tilt series of phantom data and axonemes by cryo-transmission electron microscopy, We compared the 3D reconstruction by three ET software packages (IMOD, Inspect3D and TEMography) and Eos, respectively. IMOD can automatically determine tilt axis by using fiducial marker, while Inspect3D needs to manually determine tilt axis but gave us more precise alignment than that of IMOD in case of no markers, judging from reconstructed 3D. We also compared them systematically to make our developed Eos more convenient for higher resolution analysis. More comparison between them will here be reported and discussed.

2P012 Possibility of metallothionein Labelling for CLEM method

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CLEM (Correlative light electron microscopy) is one of the powerful techniques to elucidate the localization and structure of the target proteins or their complexes in cell. Our previous study, metallothionein with cadmium ions functions as a label for electron microscopy [Nishino et al., 2007] whereas, in the presence of cadmium ions and selenium ions, it forms Qdot-like nano-particles, which generate fluorescence as reported by Park et al., (2010). We have also been challenging to use it as a fluorescent and heavy metal label for CLEM. We here report the possibility of metallothionein as a CLEM label and the trial to detect localizations and structures of two proteins, FAP20 and PACRG, in the axoneme of *Chlamydomonas reinhardtii*.