2P302 FRET による elongin B と elongin C の相互作用解析
FRET - based analysis of interactions between elongin B and elongin C

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Elongin B and elongin C are found together with elongin A in the elongin (SIII) complex that increases the efficiency of elongation by RNA polymerase II via suppressing transient transcriptional pausing. Elongin B and elongin C bind stably to each other in the complex. The interactions of two proteins were investigated using FRET-FLIM. However, the mechanism of suppression of pausing by the elongin complex is not known in details. We focused on conformational change of elongin C induced by binding of elongin B. We measured intramolecular FRET using a protein of elongin C linked to fluorescent proteins at N- and C-terminus, respectively. We will discuss suppression of pausing of Pol II by using this probe.

2P303 FRAP と 1 分子蛍光イメージングを用いた転写活性化時 Arp4β 動態の定量解析
Quantitative analysis of molecular dynamics of Arp4β upon transcriptional activation by single-molecule fluorescence imaging and FRAP

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Actin-related protein 4β (Arp4β) is a component of chromatin remodeling complex. It is thought to control transcription through binding to histone directly. However, the detailed mechanism remains elusive. Aiming to clarify the dynamics of Arp4β in transcriptional control, we performed quantitative imaging analysis of Arp4β in the nucleus. Both single molecule imaging and FRAP analysis revealed dynamic movements of Arp4β in the nucleus. After stimulation with PMA, the residence time of Arp4β became shorter. These results suggest dynamic interaction of Arp4β with either chromatin remodeling complex or chromatin. We will further discuss the dynamics of Arp4β.

2P304 炎症反応抑制タンパク質 PDLIM2 の局在制御機構の解明
The elucidation of the mechanism of PDLIM2 localization regulation

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NF-κB plays an important role in the immune responses through activation of inflammatory gene transcription. As excessive inflammatory responses cause massive damage to host cells, activation of NF-κB should be tightly regulated. Recently PDLIM2 was found to target NF-κB and terminates NF-κB activation in the nucleus. We observed translocation of GFP fusion proteins of PDLIM2 from the cytoplasm to the nucleus upon stimulation. In this study, aiming to elucidate localization regulation mechanism of PDLIM2, we analyzed the localization of phosphorylation mutants of PDLIM2. We found that 72Ser phosphorylation may have an important role of PDLIM2 localization regulation.

2P305 3 色同時 1 分子イメージングによる T 細胞マイクロクラスターとシグナル膜タンパク質の相互作用解析
Single molecule analysis of signaling membrane proteins in T cell microcluster by multicolor live cell imaging

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T cell receptor (TCR) microcluster is important to initiate and sustain T cell activation. Although various signaling molecules were found enriched in microcluster, the mechanism of microcluster formation remains unclear. To visualize the dynamics of membrane proteins related to microcluster formation, we performed single molecule imaging of TCR and phosphatase CD45 (excluded from microcluster) with GFP labeled microcluster simultaneously. On the activated T cell surface, TCR molecules showed slower diffusion and longer residence time in microclusters than CD45. It suggests the dynamic interaction of these molecules with microcluster. Based on the quantitative imaging analysis, we will discuss the mechanisms of microcluster formation in T cell activation.

2P306 カルシウムイオン刺激による微小管伸長の動態解析
Imaging analysis of effect of Ca²⁺ ion on microtubule polymerization

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EB1 (end-binding protein1) is one of the microtubule binding proteins and it dynamically tracks microtubule plus ends. EB1 is also known to associate with an ER resident Ca²⁺ sensor protein, STIM1 (stromal interaction molecule 1). In spite of the important role in microtubule dynamics, the molecular dynamics of EB1 especially in relation with STIM1 remains unclear. In this study we imaged movements of EB1 in HeLa cells and measured the polymerization speed of microtubules. We will discuss the effect of Ca²⁺ ion in microtubule dynamics.

2P307 T 細胞活性化における微小管形成中心の動態
Microtubules Organizing Center (MTOC) Dynamics and Migration upon T Cell Activation

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Cellular polarity and division is related to microtubule (MT) dynamics. As MT performs major roles in cellular activities notably cell division and morphological structuring, a comprehensive insight of MT and MTOC mechanism is crucial. The phenomena of MTOC migration during T cell activation has been well established, suggesting MT interaction with polymerized actin as the main influence. However, recent studies showed kinase protein exhibits a crucial role in manipulating MT dynamics and critical potential initiating MTOC migration. Our data on instability of MTOC structure enables further deduction of ER transfer along MTOC migration. We will further discuss the dynamics of MTOC using quantitative molecular imaging analysis.