3P324 転写因子の免疫刺激による共局在の変化：マルチカラー分子イメージング
○新倉 和実¹、十川 久美子²、木村 宏³、徳永 万壽洋^{1,2,4}
¹遺伝研、²理研・免疫センター、³情通研・未来ICT、⁴総研大
Stimulation-induced changes in colocalization of transcription factors using multi-color molecular imaging
Kazumi Shinkura(1), Kumiko Sakata-Sogawa(2), Hiroshi Kimura(3), Makio Tokunaga(1,2,4). (1:Structural Biology Center, National Institute of Genetics; 2:Research Center for Allergy and Immunology, RIKEN; 3:Kansai Advanced Research Center, NICT; 4:Department of Genetics, The Graduate Univ for Advanced Studies)

To understanding how cellular activities are generated by molecular interactions, multi-color imaging with above 3 colors is very effective in visualizing and quantifying molecular interactions in cells. Specimen preparation of fluorescence labeling with above 3 kinds of dyes, however, is not facile. Recent advances in molecular imaging technologies, especially improvement of sensitivity, now allow the molecular imaging without fluorescence enhancement techniques, such as second antibody labeling, and thus made multi-color imaging with above 3 colors available.
Productive engagement of T cells by stimulation results in formation of microclusters of TCR and signaling molecules. Ultimately activation of the transcription factors NFAT, AP-1, and NF-kappaB, result in gene transcription. Why and how combinations of pathways are used for cellular functions? To answer this question, we examined multi-color molecular imaging by immunofluorescence. In non-stimulated Jurkat cells, NFAT, AP-1, and NFkB localized in the nuclei to a greater or less extent, but colocalization of all the three proteins rarely occurred. Upon stimulation, phosphorylated c-Jun increased in the almost whole area of the nucleus. NFAT and NF-kappaB were increased in a stimulation-dependent manner. Colocalization of two proteins were seen in a somewhat wide area. However, colocalization of the three proteins occurred in a restricted region. We will discuss colocalization with other molecules and three-dimensional distributions.

3P326 細胞内ATPを蛍光で可視化する
○今村 博臣¹、Kim Huynh Nhat Phuong¹、齋藤 健太²、飯野 亮太¹、山田 康之³、永井 健治²、野地 博行¹
1pB02 ¹阪大・産研、²北大・電子研、³立大・理
Fluorescent visualization of intracellular ATP
Hiromi Imamura (1), Huynh Nhat Phuong Kim (1), Kenta Saito (2), Ryota Iino (1), Yasuyuki Kato-Yamada (3), Takeharu Nagai (2), and Hiroyuki Noji (1). (1: ISIR, Osaka Univ; 2: RIES, Hokkaido Univ; 3: Dept Life Science)

Adenosine 5'-triphosphate (ATP) is fuel of all living organism. In spite of its important role, spatio-temporal dynamics and distribution of ATP within living cells are not well understood, because it has been difficult to quantitatively monitor intracellular ATP. We will report here the visualization of intracellular ATP using a newly developed fluorescent resonance energy transfer (FRET) probe. The probe consists of cyan (CFP) and yellow (YFP) fluorescent proteins connected by a regulatory ϵ subunit of F₁F₀-ATP synthase, which selectively binds but does not hydrolyze ATP. Probes with ϵ subunit from different bacteria showed different affinity to ATP, from the order of μ M to mM. Among them, the probe with *Bacillus subtilis* ϵ subunit had suitable affinity for detecting intracellular ATP (usually the order of mM). By introducing some modifications including circular permutation of YFP, the probe finally showed about 150% increase in YFP/CFP ratio at saturated ATP *in vitro*. Next, we recorded fluorescence from living HeLa cells expressing the probe. Although ratio of YFP/CFP emission from living cells is virtually constant without stimulations, it decreased significantly after addition of 2-deoxyglucose and oligomycin, inhibitors of ATP synthesis, indicating that it can detect changes in intracellular ATP concentrations. The probes will enable us to analyze ATP within single living cells during various cellular processes. Furthermore, these probes may be used to investigate many ATP-consuming or producing processes *in vitro*.

3P325 高度発達型培養筋細胞における GLUT4 分子動態イメージング
○藤田 英明¹、渡邊 朋信¹、根建 拓¹、樋口 秀男¹、神崎 展¹
¹東北大・先進医工
Live cell imaging of the movement of GLUT4 molecule in highly differentiated C2C12 myotubes.
Hideaki Fujita (1), Tomonobu Watanabe (1), Taku Nedachi (1), Hideo Higuchi (1) and Makoto Kanzaki (1). (1: TUBERO, Tohoku Univ)

Insulin stimulates glucose transport by inducing the translocation of GLUT4 molecule from intracellular storage compartment to the plasma membrane in muscle and adipose cells. Previously, we have demonstrated that real-time tracking of GLUT4 molecule in live cell are possible using Qdot under real-time confocal and TIRF microscopes, and reported the change in the movement of the molecule by insulin stimulation in adipocytes. In this report, we have tracked the movement of GLUT4 molecule in highly differentiated C2C12 myotubes displaying contractility and observed the change in its mobility by insulin. C2C12 myotubes expressing exofacial-myc-GLUT4 are differentiated and electric pulse stimulation was applied to enhance the formation of sarcomere structure as reported previously (Fujita et al., Exp. Cell Res., 2007). GLUT4 molecules are labeled by Qdot conjugated with Fab fragment of anti-myc-antibody in the presence of insulin. Observation by real-time confocal microscope revealed that in non-moving muscle cells, a large number of GLUT4 molecules was immobile even under insulin stimulation. On the other hand, most of GLUT4 molecules in muscle cells contracting periodically became mobile after insulin stimulation. These observations suggest that GLUT4 molecule in muscle translocates using different mechanism compared to adipocytes.

3P327 細胞内RNA解析を目指した新規蛍光オリゴヌクレオチド
○久保田 健¹、池田 修司¹、岡本 晃充¹
¹理研 フロンティア
Hybridization dependent fluorescent oligonucleotide for intracellular RNA analysis
Takeshi Kubota, Shuji Ikeda and Akimitsu Okamoto. (Frontier Research System, Riken)

We have designed and synthesized a novel modified oligonucleotide probe named NABiT (Nucleic Acids with Bis-Thiazole), which displayed green fluorescence only when hybridizing with a complementary nucleotide. Because the fluorescence ratio between hybridization/single strand states is large enough to detect the target sequence, there is no need to wash an excess amount of the probe out. To show usefulness, a polyA targeting NABiT probe was synthesized. The probe contained a fluorescence-modified thymidine in an oligoT DNA sequence. This probe had negligible fluorescence at single strand state. Addition of A13mer RNA into the solution of this probe induced strong fluorescence within several seconds. Mixtures with other irrelevant RNAs had little fluorescence. This showed that this probe has high sequence selectivity. Then, we applied this probe to cultured cells. Microinjection of the polyA targeting NABiT probe induced strong fluorescence at nucleus region, whereas other non-targeting NABiT probes showed little fluorescence. We conclude that this probe has high selectivity and sensitivity *in vitro* and *in vivo*. The present method would be applicable to an RNA live imaging for investigating RNA functions.