lens, light source and CCD camera) are mounted on a motor, enabling bright-field imaging with a temporal resolution of 30 frames/sec during rotation. Almost all AMs started to move toward 10g and some reached the one side of endodermal cell within 30 sec. These results suggest that redistribution of AMs toward gravity resulting from the directional movement is involved in gravity sensing of Arabidopsis inflorescence stems. To further test this idea, we analyzed the well-known shoot gravitropic mutant, *sgr2*, which has non-sedimentable AMs and shows little gravitropism at 1g. Both redistribution of AMs toward hypergravity and an obvious gravitropism were induced by 30g for 5 min, strongly supporting the above idea.

2P-267 蛍光タンパク質の2量体界面への変異が FRET インジケータのダイナミックレンジに及ぼす影響

The effect on the dynamic range of FRET indicators by mutation in fluorescent protein dimerization interface

Ippei Kotera (1), Takeharu Nagai (1), Takuya Iwasaki (1) ((1) *Research Institute for Electronic Science, Hokkaido University*)

Genetically encoded fluorescent protein (FP)-based FRET indicators have become indispensable tools in molecular and cellular biology as well as other biology-related disciplines. Due to the *Aequorea victoria* FP's tendency to dimerize at high concentration, an A206K repulsive mutation has been introduced to monomerize the FP dimers by adding a charged residue at a dimerization interface. Because of the popularity and wide usage of the mutation among GFP-based FRET probes, we have analyzed the effect of the charge introduction toward the dynamic range of the indicators. A genetically encoded Ca^{2+} indicator Cameleon utilizes calmodulin and M13 peptide sandwiched by color variants of the fluorescent protein. Upon Ca^{2+} binding calmodulin and M13 undergo structural changes that alter both the distance between the chromophores and relative orientation of the dipole moments, which in turn affects the FRET signal of the indicator. We have constructed Cameleon indicators with the dimerization interface mutations and compared the dynamic range of the mutants with that of the non-mutant indicator. Indeed the dynamic range of the indicator was reduced as more charges were introduced to the dimerization interface. Spectroscopic analysis revealed that the Ca^{2+} -saturated samples, but not the Ca^{2+} -free sample, had the dramatic reduction in FRET signal. Moreover, the sticky mutations (S208F, V224L) which enhance the dimerization of FPs also reduced the dynamic range of the indicator. The reduction was due to the FRET signal increase in the Ca^{2+} -free sample. These results suggest that neither repulsive nor sticky mutations should be introduced to the dimerization interface as they both reduce the dynamic range of the indicator, by reduced FRET signal upon Ca^{2+} binding, and by increased basal FRET signal, respectively.

2P-268 FRET 指示薬における遷移双極子モーメント、FRET 効率、及びダイナミックレンジの相互関係

Correlation among dipole orientation, FRET efficiency and dynamic range of FRET-based indicators

Daisuke Nagai (1), Sanq-Yeob Kim (1), Tomomi Tani (1), Takeharu Nagai (1) ((1) *Research Institute for Electronic Science, Hokkaido University*)

Förster resonance energy transfer (FRET) is a physical phenomena in which an excited state energy of donor migrates into an acceptor non-radiatively. The FRET efficiency depends on both distance between donor and acceptor, and the relative orientation of the chromophore's transition dipoles. In the case of small chromophores, the orientation factor can be neglected due to their free rotation. Thus, FRET efficiency can be considered as a function of the distance between donor and acceptor. This is reason why many scientists have used FRET as a "spectroscopic ruler" to measure intermolecular distance. In contrast, the chromophore of fluorescent protein (FP) is fixed in the β -barrel structure with a slower rotation than the excited-state lifetime during which FRET occurs. Therefore, FP-based FRET should be strongly influenced by the orientation factor. Cameleon, YC3.60, is a FRET-based Ca^{2+} indicator with the biggest dynamic range (> 500 %) among the FP-based FRET indicators reported so far. The high dynamic range is achieved by high FRET efficiency in Ca^{2+} -saturated state, which is thought to be owing to the appropriate orientation of the transition dipoles, making YC3.60 a preferable model FRET indicator to investigate precise relationship among the orientation factors, the FRET efficiency and the dynamic range. Here, we report visualization of these three factors of regularly arrayed biotinylated-YC3.60 in the shape of two dimensions on the crystal of streptavidin by using a newly developed fluorescence polarization microscopy.

2P-269 植物細胞における長時間生理機能イメージングを目指した赤方化指示薬開発

Long time physiological imaging of plant cells with reduced autofluorescence by using a Ca^{2+} indicator composed of a red-shifted FRET pair.

Toshiaki Uematsu (1), Ippei Kotera (1), Kenta Saito (1), Noriyuki Hatsugai (1), Kazuki Horikawa (1), Takeharu Nagai (1) ((1) *Research Institute for Electronic Science, Hokkaido University*)

Förster resonance energy transfer (FRET) is a physical phenomena in which an excited energy of donor molecule migrates non-radiatively into an acceptor molecule. So far, fluorescent protein-based FRET has been widely applied to develop functional indicators. In most studies, combination of cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) has been used as the FRET donor and acceptor, respectively. In spite of the many successful application, it remains a fundamental drawback in terms of the excitation wavelength since the FRET pair uses a relatively photo-toxic violet light (~430 nm) for specific excitation of CFP. To overcome this, we chose a green fluorescent protein (GFP) as the FRET donor, which can be excited by blue light such as a conventional 488 nm argon ion laser that is physiologically much gentle to live cells than violet light. Among various longer wavelength fluorescent proteins, mKO gave the highest FRET signal in combination with GFP. Thus, we replaced CFP and YFP in a Ca^{2+} indicator, cameleon YC3.60, with GFP and mKO, respectively, to obtain orange cameleon (OC3.60). When expressed in mammalian cells, OC3.60 reported Ca^{2+} transient and oscillation as shown by other Ca^{2+} indicator. Furthermore, we succeeded long term Ca^{2+} imaging with reduced autofluorescence signal in guard cells of plant, Arabidopsis thaliana that stably expresses OC3.60, demonstrating superiority of the FRET pair in bioimaging in plant.

2P-270 単一細胞エレクトロポレーションによって培養小脳神経細胞に導入された siRNA の効果の長期リアルタイムモニタリング

Long-term and real-time monitoring of effects of small interfering RNA introduced by single-cell electroporation in cultured cerebellar neurons

Masahiko Tanaka (1), Yuchio Yanagawa (2), Naohide Hirashima (1) ((1) *Graduate School of Pharmaceutical Sciences, Nagoya City University*; (2) *Graduate School of Medicine, Gunma University*)

RNA interference (RNAi) is a powerful means to elucidate gene function. Recently, we developed the method for introducing small interfering RNA (siRNA) by single-cell electroporation using micropipettes in neuronal cell cultures. In contrast to widely used transfection methods, our method allows the introduction of siRNA into single and identified cells. This feature is advantageous especially in investigations of the nervous system because the nervous system is composed of various types of cells. To explore the availability of this method in detail, we introduced siRNA against green fluorescent protein (GFP) into GFP-expressing Purkinje and Golgi cells in cerebellar cell cultures by single-cell electroporation. The temporal changes in the intensity of GFP fluorescence in the same electroporated cells were monitored in real-time over 10 days after electroporation by confocal microscopic imaging. Several parameters, including tip diameter and resistance of micropipettes, concentrations of siRNA and a fluorescent dye marker, voltage and time of pulses, were optimized to maximize both the efficacy of RNAi and the viability of the electroporated cells. Under the optimal conditions, transfer of GFP siRNA markedly reduced GFP fluorescence in the electroporated cells by 4 days after electroporation at the latest, whereas that of negative control siRNA had no effects. GFP siRNA was more efficient in Purkinje cells than in Golgi cells. Sufficient reduction in GFP fluorescence continued until at least 7 days after electroporation. After that, GFP fluorescence began to be recovered in some electroporated cells, although the recovery levels varied among cells. Thus, it is suggested that the most effective gene-silencing by this method is achieved at least between 4 and 7 days after electroporation.

2P-271 投影型X線顕微鏡によるケジラミのマイクロCT

Micro-CT of *Pthirus pubis* by projection X-ray microscopy

Junichi Moriya (1), Toshiaki Higashikawa (2), Hideyuki Yoshimura (1) ((1) *Meiji university*; (2) *Higashikawa Dermatology Office*)

The projection X-ray microscope utilized a very small X-ray source from a thin (0.1-3 μm) target metal film that is excited by the focused electron beam of a scanning electron microscope (SEM). If an object is placed just below the target metal film, the diverging X-rays enlarge the shadow of the object. Because no X-ray optics such as zone-plate is used, focal depth is infinite in principle. Taking this advantage, we have applied projection X-ray microscope for three-dimensional (3-D) structure analysis of a louse (*Pthirus pubis*, 1 mm in length) by means of cone-beam computed tomography (CT).

In the present study, a 3 μm thick copper film was used with an electron beam accelerating voltage of 18 keV. Thus, the characteristic X-ray of copper K α (0.15 nm) was the main X-ray. The projection images of a louse were recorded in every 3 degree for whole direction (360 degree) with a stepping motor controlled sample rotator. The 3-D image was reconstructed from cone-beam projections using filtered back-projection algorithm. Because an entire structure can be inspected using a single specimen without drying, coating, or making sections, the X-ray micro-CT technique method presented here is a powerful tool for the study of biological specimens. Three dimensional, inside and outside, structure of *Pthirus pubis* will be shown.