3P304 金属微粒子の生細胞内導入における表面増強ラマンスペクトル測定
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Surface enhanced Raman spectroscopy of living cells with gold nanoparticles
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Surface enhanced Raman scattering (SERS) is one of promising technique for analysis of molecules because the SERS spectra sensitively monitor vibrations of molecules due to strong enhancement caused by the resonant effect between collective oscillations of free electrons at a metal surface (i.e. plasmon) and incident light. In this research, we examined the SERS effect with gold nanoparticles in living cells. Since SERS is highly sensitive to molecular structure and its environment, SERS spectra may provide rich information about biological molecules in situ. We introduced gold nanoparticles into living macrophage and HeLa cells via endocytosis. Gold nanoparticles of which diameter was about 50nm were used. Uptake of the nanoparticles was observed by time-lapse observation with a dark-field microscope. We confirmed that several nanoparticles are taken by both cells in 2 hours after inserting the nanoparticles of which concentration was 4.5×10^9 particles/ml to the surrounding medium. The SERS from the gold nanoparticles was detected with a Raman microscope using a 785nm laser for a light source. We also observed the same sample with 532nm light. However, fluorescence background was strongly enhanced and prevented SERS from being detected. We measured temporal change of a SERS spectrum from a single spot in a cell. With the measurement of the temporal resolution of 0.63 sec with the exposure time of 0.5 sec, we have found that SERS spectra temporally changed by reflecting molecules, such as DNAs and proteins, around the particles.

3P306 緑色蛍光タンパクを用いたミトコンドリア形態観察
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Monitoring of mitochondrial morphology in a single living cell using green fluorescent protein
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Efficiency of oxidative phosphorylation is thought to be linked to mitochondrial morphology. Several authors reported alterations of mitochondrial shape and number in primary culture of fibroblasts from patients with mitochondrial dysfunction using fluorescent dye such as rhodamine 123, which was taken into the matrix depending on mitochondrial membrane potential. However, since the potential changes heterogeneously within a single cell, mitochondrial morphology alone is not always measured by rhodamine 123. In the present study, we used cells transfected with a plasmid containing green fluorescent protein gene which was fused to a mitochondrial targeting signal (mtGFP; Clontech). In contrast to a cytosol fraction obtained by centrifugation, both bands of cytochrome *c* and green fluorescent protein appeared in the western blot of a mitochondrial fraction. Uncoupler (CCCP) had no effect on fluorescence intensity of mtGFP. Fluorescence images of a cell expressing mtGFP were analyzed, and mitochondrial morphology was assessed by mitochondrial number within a cell for fragmentation and area per mitochondrion for swelling. Although the number was constant, the area tended to increase when cells were exposed to 0.4 mM H₂O₂, where mitochondrial DNA was subjected to be damaged and cell growth was suppressed. Next, we will use specific inhibitors of respiratory chain and determine the possibility of the diagnosis of mitochondrial dysfunction by the use of mtGFP.

3P305 群青色蛍光蛋白質の開発
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Development of a fluorescent protein with deep blue colour
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Genetically encoded fusions of cellular proteins with a green fluorescent protein (GFP) from the jellyfish *Aequorea Victoria* or its multicolour homologues from another organisms including *Anthozoa* have become indispensable tools in cell biology. So far, GFPs with altered fluorescence spectra have been created: blue and cyan variants were made by substitution of Tyr at 66 (Y66) in the *Aequorea* GFP chromophore for His and Trp, respectively. Here we describe a new color variant of fluorescent protein which was made by introduction of several amino acid substitutions into *Aequorea* GFP by combination of site-directed and semi-random mutagenesis. Spectroscopical analysis of the fluorescent protein revealed that the absorption and emission peaks were 360 nm and 420 nm, respectively. Both peaks are the shortest among the fluorescent proteins reported so far. Because of its deep blue color, this protein was named Ultra-Marine Fluorescent Protein, or UMFP in short. The critical feature of UMFP is its high fluorescence stability in a wide range of pH, enabling quantitative fluorescence imaging in acidic environment such as lysosome and the Golgi body. In addition to this feature, UMFP can be an ideal FRET donor with a CFP acceptor since the emission spectrum of UMFP is completely overlapping with the absorption spectrum of CFP. Indeed, UMFP-CFP fusion protein showed high FRET efficiency, which was observed in an experiment where UMFP signal was dequenched to 65%. The application of UMFP for cell biological analysis will be further discussed.

3P307 蛍光相関分光法とマイクロウェルを用いた遺伝子移送とタンパク質発現の単一細胞解析
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Single cell analysis of gene transfer and protein expression using fluorescence correlation spectroscopy and microwell
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Analysis of cell function is usually performed in the averaging procedure that loses information of individual cells and each cellular signal. For addressing single cell analysis, we combined Fluorescence Correlation Spectroscopy (FCS) and the microwells system. The microwells are picoliter sized cavities, were fabricated by poly-dimethylsiloxane (PDMS), which can be moved in a vertical direction by the micrometer scale on cover slip. This method has advantages for the trapping single cell and changing the environment of each cell. We can discriminate the signal from single cell and quantify the product from the cell using this system. In this report, we will demonstrate the determination of the relationship between introduced foreign DNAs and expressed EGFPs from these DNAs in single cell level. Cy5 labeled linear dsDNAs which had EGFP coding region were transfected into HeLa cells. After 24hours, we isolated the EGFP expressed cell from the other cells using the microwells and treated the lysis buffer. To quantify the number of introduced DNAs and expressed EGFPs in single cell, we carried out two-color FCS measurement in the microwell. We estimated the expression efficiency from the number of introduced DNA molecule and expressed EGFP in single cell. Our result suggests that the combination of FCS and the microwells system enables molecular quantification in single cell level.