- 1P342 Br 修飾されたヒトテロメア DNA の G-quadruplex 立体構造の単一分子 FRET 計測
○岡本 憲二¹、三戸 祐太¹、真下 知子¹、杉山 弘¹、寺嶋 正秀¹
¹京大院・理化
Single molecule FRET measurement of three dimensional G-quadruplex structure of human telomere DNA with Br-modification
Kenji Okamoto(1), Yuta Sannohe(1), Tomoko Mashimo(1), Hiroshi Sugiyama(1) and Masahide Terazima(1). (1:Dept. Chemistry, Graduate School of Schience, Kyoto Univ)

Eucaryotic chromosomes have the 'telomere' region at their ends, which consist of tandem repeats of the short G-rich sequence. Telomere is sacrificed in inevitable shortening of DNA after each duplication and limits cell's lifetime. In cancer cells, the enzyme 'telomerase' actively elongates telomeres and allows the cell to limitlessly grow, whereas the normal somatic cells somehow inhibits telomerase's activity. The understanding of the inhibition mechanism is expected to be applicable to, e.g., anticancer drugs.

Human telomere DNA is known to date to form three dimensional 'G-quadruplex' structure, which consists of quadruple strand of G-tracts and linkers connecting them, and block telomerase's binding. However, the stable structure in physiological solution had been unmeasurable for years presumably because they are in equilibrium between multiple structures. In 2006, three groups independently identified the same structure, which is called "mixed-chair" structure, by NMR and CD measurements. However, the diversity of conformation and the dynamics between them are still under investigation.

Here the single molecule FRET measurements were carried out using human telomere DNA. FRET efficiency of single molecules are calculated from confocal fluorescence images of immobilized telomere DNAs. We employed Br-modification technique, which enables us to control G-quadruplex structure based on its dependence on the Br-modification positions. The FRET distributions are compared among unmodified and Br-modified DNAs.

- 1P344 蛍光偏光法を用いた DNA の一塩基ミスマッチの検出
○清水 雅史¹、南川 典子¹、荒井 宏美¹、鶴岡 誠¹
¹東京工科大
One Mismatch Detection of DNA Hybridization Using Fluorescence Polarization
Masafumi Shimizu(1), Noriko Minamikawa(1), Hiromi Arai(1), Makoto Tsuruoka(1)

It is known that single nucleotide polymorphism (SNP) is one of the factors that determine individual genetic features. For the detection of SNP, a simple and high-throughput analysis method using a small quantity of sample is desirable. In recent years, many SNP analysis methods have been reported, however, these methods are not very suitable for high-throughput analysis because they require extremely time-consuming processes.

Here, we focused on fluorescence polarization analysis (FPA) as a candidate for one mismatch detection method. In FPA, a fluorescently labeled molecule is excited with a polarized light, and the emitted fluorescence is depolarized due to the rotational Brownian motion of the labeled molecule. The degree of depolarization is strongly affected by the effective volume of the molecule. FPA is considered to be more suited to high-throughput SNP detection than electrophoresis or solid phase methods.

In this research, we investigated the potential of FPA as a method for detection of mismatch DNA hybridization. We monitored hybridization between DNAs using FPA with optimizing salt conditions for the hybridization. Furthermore, for the target DNAs, which contain one base replacement in the sequence where the probe hybridizes, time courses of the hybridization process were monitored with fluorescence polarization and compared with those of the non-mismatch target. We successfully distinguished the aspects of hybridization of non-mismatch target DNA from those of one mismatch target DNA using FPA.

- 1P343 蛍光相互相関分光法による誘導アポトーシス時の Caspase 3, Caspase 9 の In vivo の解析
○孫 凡¹、三國 新太郎¹、金城 政孝¹
¹北大院・生命科学・細胞機能
In situ simultaneous measurement fluorescence cross correlation spectroscopy for activity of caspase 3 and caspase 9 in apoptosis
Sun Fan, Shintaro Mikuni and Masataka Kinjo Laboratory of Molecular Cell Dynamics, Graduate school of life science, Hokkaido University

Caspases are enzymes known as proteases, which play essential roles in apoptosis (cell death) and inflammation. Caspase 9 is initiator caspase, the beginning signal is received from the upstream of the cell signal route of apoptosis, once activated, and it has the role to activate as effectors. Caspase 3 is an effectors caspase, it is activated depending on the initiator etc. In this study, we aimed to construct caspase substrate by using three kinds of fluorescence proteins, and to simultaneously pursue the activity of caspase 3 and caspase 9 in living cells through the fluorescence cross correlation spectroscopy (FCCS) measurement. At first, we constructed three kinds of fusion fluorescence proteins. Two of these contained only a recognition site. Another contained two recognition sites for each caspase 3 and caspase 9. Next, living cells fluorescence imaging was performed using an inverted confocal laser scanning microscope. Then the FCCS measurements were carried out. After transfected HeLa cells by the plasmids, we detected two colors fluorescence in single cell as well as cross correlation signals in each control. On the other hand, three colors were also detected in three colors substrate expressed cell, each two colors cross signals were detected. We are planning to detect the releasing the fluorescence protein in apoptosis by using the three colors laser system in next study. To prevent FRET between proteins, we construct other fusion fluorescence proteins.

- 1P345 発光ダイオードと CMOS を利用した小型蛍光イメージング
○三澤 宣雄¹、竹内 昌治¹
¹東大・生産研
1aK13 A COMPACT FLUORESCENCE MICROSCOPY USING A LIGHT-EMITTING DIODE AND A COMPLEMENTARY METAL OXIDE SEMICONDUCTOR
N. Misawa (1) and S. Takeuchi (1). (1:IIS, The University of Tokyo)

This study reports a compact and simple system for fluorescence observations using a light-emitting diode (LED) and a complementary metal-oxide-semiconductor (CMOS). For the first trial, we used fluorescent particles (excitation and emission peaks are 510 nm and 560 nm respectively) for observations and LED as an excitation light source (wavelength 510-520 nm). The fluorescence emission of the particles was detected through a band pass emission filter (575-625 nm) by a commercial CMOS device connected to a computer via universal serial bus (USB). We succeeded in getting fluorescence images of particles with diameters 15-19 μm using the CMOS device with a lens as focusing element. Preliminary experiments indicate that this system is applicable to the fluorescence imaging of small objects such as single cells. Furthermore, this observation system is highly compact, composing of only six simple elements: battery, LED, sample, filter, CMOS and computer. Compared to a conventional fluorescence microscopy system, our proposed integrated system is much more economical, light and compact, making it possible to develop a truly portable imaging system for fluorescence imaging analysis of chemical reactions and biomaterial's interactions during fieldwork operations.