Embryonic stem cell (ESC) populations become unhomogeneous for differentiation induction. One theory holds that individual cells fluctuate over time between undifferentiated and quasi-differentiated states. However, the colony formation of cultured ESCs means that monitoring individual cells is difficult. No traditional ESCs culture system with feeder cells and serum can be used to control the tight conditions needed for differentiation induction. Additionally, no differentiation of normal living cells can be identified.

To clarify the fluctuation of states within individual cells, mouse ESC expressed as green fluorescent protein in an undifferentiated state was cultured on an E-Cadherin/Fc Chimera (E-Cad-Fc) coating dish with differentiation induction and an undifferentiation maintenance medium. Two differentiation induction media were used. One is 'passive', i.e., without factors which maintain ESCs in an undifferentiated state, the other is 'active', i.e., with a differentiation-inducing factor. Using E-Cad-Fc makes cultured cells monolayer ones. After their medium was replaced by a differentiation induction medium, we observed the shifts of cell states at the single cell level.

Using a combination of our culture system and a medium perfusion system will make it possible to measure the states of the cells over a long time. Understanding differentiation from pluripotent to diverse cell states will encourage development in methods of high-precision differentiation induction.

3C1346 ベイズ推定を用いた PC12 細胞の自発的運命決定の定量解析 Quantitative analysis of spontaneous cell fate decisions in PC12 cells using Bayesian inference

Kazunari Mouri, Yasushi Sako (RIKEN ASI)

PC12, a cell line derived from rat pheochromocytoma is a model system of the differentiation of sympathetic neurons. In population, PC12 cells proliferate in the presence of EGF, and extend neuritis to differentiate into neuron-like phenotype in the presence of NGF. Individual single cells, however, determines its fate stochastically in the absence and even in the presence of external stimuli. Here we characterize such stochastic cell fate decisions after quantitative analysis.

We counted the number of cells divided into three fates, undifferentiated, differentiated, and dead cells in cultures under several serum conditions with or without EGF/NGF stimuli. Then, we developed a differential equation model of cell fate decision. To fit the model to the experimental data, we applied Bayesian inference using Markov chain Monte Carlo methods to determine the model parameters, i.e., the rates of proliferation, differentiation, and death. The cell fate was decided stochastically in every serum condition independent of external stimuli. Cellular responses to EGF/NGF changed depending on serum conditions, and the stimuli were only used to modulate the rates of cell fate decision. We defined an entropy to quantify the heterogeneity of a population. Cells maximized the entropy under the unstimulated ground state, and the entropy decreased as serum concentrations increased. Therefore, we conclude that the entropy or the heterogeneity of a population is basically determined by serum conditions, and EGF/NGF stimuli modify the cell response rates.

3C1358 動物の発生における細胞極性のスケーリング機構とその遺伝学的制御 P42 Genetic regulation of protein dynamics in scaled polarity-protein localization in *C. elegans* embryos

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Cell polarity is regulated by asymmetric localization of polarity proteins. In the embryo of *C. elegans*, aPKC kinase (PKC-3) and a ring finger protein PAR-2 asymmetrically localize in antagonistic pattern in the series of asymmetric cell division of 1-cell embryo and germline cells. In these divisions, cells progressively decrease their volume (The smallest cell is ~1/80-times smaller than 1-cell embryo in volume). How are the asymmetric localizations in different-sized cells maintained? By single-molecule detection and fluorescent correlation spectroscopy (FCS) in living embryos, we revealed that PAR-2 molecules move on the membrane in free diffusion ($D \sim 0.04 \mu\text{m}^2\text{s}^{-1}$), and the off-rate from cell membrane was twice faster in the anterior region than in the posterior region, while the on-rate was uniform. This result suggests that anteriorly-localized PKC-3 increases the off-rate in the anterior region. In mutants of *ooc-3* and *ooc-5*, encoding a membrane-integrated protein and torsinA like protein, respectively, the diffusion rate was faster than in the wild-type embryos ($D \sim 0.5 \mu\text{m}^2\text{s}^{-1}$). Using a reaction-diffusion model, we found that asymmetric PAR-2 localization in size-different cells is endured by slow

diffusion on the membrane. In faster diffusion rate ($D = 0.5 \mu\text{m}^2\text{s}^{-1}$), asymmetric localization maintained by asymmetric off-rate is more robust than that by asymmetric on-rate. In this study, we found that protein dynamics are adjusted through genetic regulations for asymmetric localization in different-sized cells.

3C1422 生物ネットワークにおける、アトラクターの幾つかの構造

Some properties of Attractors in Biological Network

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Biological network systems such as gene regulatory networks, metabolic networks and protein interaction networks depend on their network structures and dynamics. In this contribution, we report on attractors and their dynamical stability to rewiring in the core of the gene regulatory network of *Escherichia coli* and compare them with those of artificial networks with scale-free topology, using both threshold dynamics and Kauffman dynamics.

Network structures and dynamics can model some biological and living functions. In general, distributions of in-degrees $P(k_{in})$ and out-degrees $P(k_{out})$ are different even in same network. Present data for many biological networks, including gene regulatory networks (GRNs), suggest an inhomogeneous out-degree distribution and a Poissonian or exponential in-degree distribution. In this study, we investigate the stability of attractors to the rewiring of links in biological networks. We obtain information of biological networks from bioinformatic databases containing many experimental results. As examples, we use the GRN of *Escherichia coli* (*E. coli*) and the metabolic network of *Bacillus subtilis* (*B. subtilis*) and adopt threshold dynamics and Kauffman dynamics to investigate the stability of the attractors to rewiring of the links as a perturbation.

3C1434 出芽酵母の核内染色体の分子動力学シミュレーション

Molecular Dynamics Simulation of Interphase Chromosomes in Budding Yeast

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Gene expression is not only controlled by regulatory DNA regions such as insulators and enhancers but is also controlled by nuclear organization such as nuclear envelope [1, 2]. Recently, conformation of all interphase chromosomes, in other words, conformation of the genome in nucleus, has been inferred from Chromosome Conformation Capture-on-Chip (4C) data [3]. However, this inferred 3D structure of the genome does not reflect the dynamical aspect of fluctuating chromosomes, so that we investigate dynamics of chromosomes by building a coarse-grained molecular dynamics simulation model based on the Kremer-Grest model of connected beads. In this model, the intra- and inter-chromosome Go-like interactions are introduced using the 4C data, and effects of nuclear envelope and nucleolus on the chromosome fluctuations are also taken into account. In this presentation, I will talk about the validity of the model and the results of molecular dynamics simulation regarding the role of nuclear envelope in transcriptional regulation.

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3C1446 X線によって誘導されるバイスタンダー細胞死による自然発生突然変異の抑制

Suppression of spontaneous mutagenesis by X-ray-induced bystander cell killing

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Radiation-induced bystander responses are biological effects observed in cells that receive signals from irradiated cells but are not directly exposed to ionizing radiations. Using a synchrotron X-ray microbeam irradiation system developed at the Photon Factory, KEK, we recently showed that nitric oxide (NO)-mediated bystander cell killing is parabolically enhanced in a dose-dependent manner. In this study, we irradiated 5 nuclei of V79 cells with $10 \times 10 \mu\text{m}^2$ 5.35 keV X-ray beams and then measured the mutation frequency in the bystander

cells by using the *HPRT* mutation assay. The mutation frequency with the null dose was 2.6×10^{-5} (background level) and decreased to 5.3×10^{-6} with a dose of approximately 1 Gy (absorbed-dose in the nucleus); at higher doses, however, the frequency returned to the background level. Same type of biphasic dose-response was observed during the bystander cell killing, as shown in our previous study. Similarity of these behaviors in dose-response may suggest a correlation between the enhancement of bystander cell killing and the suppression of spontaneous mutagenesis in the bystander cells. Increase in oxidative damage in unstable cells which have lost an antioxidative ability is responsible for spontaneous mutagenesis. The genetically unstable cells in the population of bystander cells might be selectively excluded by the bystander cell killing as NO caused mitochondrial degeneration, and hence the suppression of mutagenesis was observed in the bystander cells.

3C1458 軟X線照射によって生じるバystanダー細胞死における p53 の役割 Role of p53 in the soft X-ray Induced Bystander Cell Killing Effect

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Bystander response is defined as cellular responses of the cells, which have not been directly traversed by radiation, are induced in the neighborhood of cells which have been directly traversed by radiation. Due to its nature, the bystander response may have important biological consequences under low dose irradiation conditions where non-irradiated cells become major fraction in the population compared to the really irradiated. In this report, the role of p53 in the bystander response was studied using a focused aluminum K-shell X-ray microbeam. When 5 cell nuclei of normal human fibroblast WI-38 cells bearing wild-type *p53* were irradiated in the population, the mode of bystander cell killing showed a biphasic behavior, namely decrease to 90% cell survival rate at the dose of 0.47 Gy, partially suppressed between 2.3 and 7.0 Gy, followed by level-off around 90% with higher dose. In addition, the bystander surviving fraction of non-small cell lung carcinoma H1299 cells expressing transfected wild-type *p53* also decreased to about 90% when the 5 cell nuclei were irradiated with 0.45 Gy and then recovered to 98% at 2 Gy. On the other hand, the cell survival of bystander H1299 cells expressing transfected mutated *p53* decreased about 90% at 0.45 Gy and 85% at 2 Gy. These results suggest that the *p53* status is important in determining dose response of soft X-ray-induced bystander cell killing. Since nitric oxide (NO) is one of chief initiators/mediators of this response, importance of NO in the bystander response will be discussed.

3C1522 低レベルγ線による DNA 二重鎖切断の定量的評価：単一 DNA 分子観察手法の活用 Quantitative evaluation of DNA double-strand breaks by low gamma-ray radiation: Application of single DNA observation

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It is known that both, single-strand breaks (SSB) and double-strand breaks (DSB) of DNA are caused by various physically and chemically induced damages. Although the great majority of SSB are thought to be repairable using opposite complementary base pairs, a very small number of DSB in one cell can cause serious damage that may lead even to cancer cells or cell death. Until now individual DSB have been detected indirectly in various ways, however, to the best of our knowledge, there is no study to quantify DSB by direct detection of individual DSB at low dose (less than 100Gy) yet. Hence, we investigate in the processes of DSB by low dose of gamma-ray radiation (less than 100Gy) and evaluate DSB quantitatively by adapting single DNA observation measurements in solution. We measured the lengths of single long-DNA molecules (T4 phage) using fluorescence microscopy and analyzed the obtained data to understand underlying mechanism. Our results show that the number of DSB per base pair increases linearly as the dose of radiation increases. We discuss the kinetics of the reactions of DSB.

3C1534 266-nm フェムト秒パルスの2光子吸収によるDNA二本鎖切断の直接生成

Direct production of DNA double strand break induced by two-photon absorption of 266-nm femtosecond pulses

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We have investigated DNA strand breaks induced by 266-nm femtosecond (fs) laser pulses in the solid state. The amounts of open- circular (oc) and linear (L) forms produced from closed circular plasmid DNA (pUC18, 2686 base pairs) are quantified as a function of the laser intensity. The amount of L- form produced per pulse is proportional to the square of the intensity within $10^0 \sim 10^2$ GW/cm² region, while that of oc- form is almost proportional to the intensity. This indicates that the direct double strand break (DSB) formation occurs by the two-photon absorption corresponding to one-photon absorption in a vacuum ultraviolet (VUV) range (133 nm, 9.4 eV). The mechanism of the strand break is discussed on the basis of the ionization potential of DNA. The present results demonstrate that the two-photon absorption by 266-nm fs laser pulses is not only useful for investigating DNA damage induced by VUV - range energy absorption in air and in physiological condition but also potential in studying relationship between an individual biological response and a damaged biomolecule by in situ ionization.

3C1546 Inositol hexaphosphate and its apatite cements -induced ROS generation and their biological effects on human cells

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Inositol hexaphosphate (IP₆), phytic acid, is a potent metal chelator, and is known as an anti-cancer reagent. Recently, IP₆ has received a lot of attention as ingredient in Hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂; HAp) material of artificial bones and teeth. In this report, we examined the antioxidant effects of IP₆ and HAp-IP₆ cements, which are created by mixing the HAp powder modified with IP₆, on cell-free and cell culture systems. The enzymatic superoxide (O₂⁻) from Hypoxanthine-xanthine oxidase (XO) and hydroxyl radical (HO[•]) from a fenton reaction (Fe(II)-H₂O₂) were employed for antioxidant assay using electron spin resonance (ESR) combined with spin-trapping. In vitro systems, lower concentrations of IP₆ (3 mM or less) inhibited O₂⁻ and HO[•]; however, higher concentrations of IP₆ (10 mM or more) showed a significant increase in the quantity of HO[•] from a fenton reaction. Same results were given in HAp-IP₆. It have not yet been fully defined the mechanism of interaction between IP₆ and reactive oxygen species. We also report pH-dependent of IP₆ and HAp-IP₆ for HO[•]. For the biological assay, human leukemia PLB-985 cell culture was utilized. Cells were incubated with 1-10 mM of IP₆ (pH7.4) for 24 hours. Both the inhibition of cell proliferation and the induction of apoptosis were detected in the cells treated with a concentration higher than 4 mM of IP₆. Cell cycle remained approximately static up to 3 mM of IP₆. We are due to report the biological effects of HAp-IP₆.

3C1558 大気圧低温プラズマを用いたアミロイド線維の破壊

Destruction of Amyloid Fibrils by Low-Temperature Atmospheric Pressure Plasma

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The amyloid deposition of amyloid-β peptides (Aβ) is a critical pathological event in Alzheimer disease (AD). Preventing the amyloid deposits and removing preformed fibrils in tissues are important therapeutic strategies against AD. Previously, we reported inactivation of protein in aqueous solution due to the chemical modification by Low-Temperature Atmospheric Pressure Plasma (LTAPP). Here, we studied the effects of LTAPP on amyloid fibrils of Aβ, hen egg white lysozyme, and bovine serum albumin. In dry condition, reduction and degradation of amyloid fibrils were occurred by LTAPP. In aqueous solution, amyloid fibrils were conformational change and aggregate. Superoxide anion radical and hydroxyl radical produced by LTAPP in solutions had been detected by electron spin resonance. These data indicate that these free radicals are the primarily cause of the destruction of amyloid fibrils. This study is the first step to clarify the molecular mechanism of LTAPP as a new medical application.