DIVISION OF QUANTITATIVE BIOLOGY

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The Division of Quantitative Biology has been launched in April 2016. We are interested in the molecular mechanisms underlying cell-fate decision in mammalian cells.

Living cells act as input-output (I/O) units, in which environment and/or internal states are recognized on the cell surface and processed within a cell, leading to the adaptive response to these changes. This cellular information processing is mainly controlled by intracellular signal transduction, which is a series of chemical reactions, most commonly protein phosphorylation. Importantly, the dysregulation of signal transduction by gene mutation results in pathological diseases such as cancer.

The intracellular signaling pathway has been extensively studied over the last few decades, and most of its proteins and regulations have been identified, causing the perceived increase in the pathway's complexity. Computer-assisted simulation is one of the most promising approaches for the understanding of the signal transduction pathway as a system. Indeed, chemical and physical reactions comprising signal transduction can be described by ordinary differential equations, and solved numerically by computers. A number of simulation models of signaling pathways have, in fact, been reported to date. However, most of the kinetic parameters utilized for the simulation models were not measured experimentally, but rather were assumed by fitting the experimental data with the simulation model or simply determined arbitrarily. Consequently, there are substantial differences in the kinetic parameters between these studies, thereby making it difficult to evaluate these simulation models quantitatively.

To address these issues, we currently focus on the development of research tools enabling us to (1) visualize, (2) quantify, and (3) manipulate intracellular signaling pathways with fluorescence imaging techniques and computational approaches.

I. Visualization of cell signaling

With the advent of fluorescent proteins, it has been possible to visualize kinetic reactions at the single cell level. We are using the following two different approaches to unveil when and where the intracellular signaling event takes place:

1-1. Development of FRET-based biosensors

Förster (or fluorescence) resonance energy transfer (FRET) is a non-radiative process, in which excitation energy of donor fluorophore is transferred to a nearby acceptor fluorophore. By taking advantage of the principles of FRET, we have developed several biosensors that allowed us to detect kinase activity of PKA, ERK, Akt, JNK, PKC, S6K, and so on in a living cell with high temporal and spatial resolution (Figure 1).

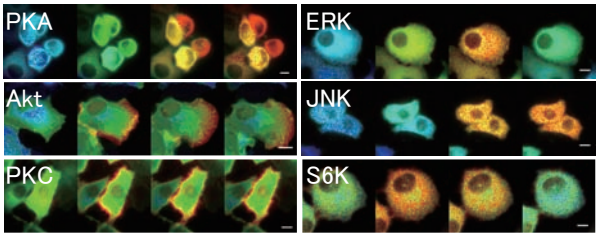

Figure 1. Visualization of PKA, ERK, Akt, JNK, PKC and S6K activities by FRET imaging. HeLa cells expressing FRET biosensors for the indicated kinases were stimulated with each ligand. Red and blue colors indicate activation and inactivation of the kinase, respectively.

1-2. Imaging of multiple kinase activities

Although FRET biosensors enable us to monitor cell signaling events, FRET imaging is not suited for multiplexed imaging, because these biosensors are authentically comprised of two different fluorophores in many cases. To circumvent this limitation, we are currently developing biosensors based on the principle of the kinase translocation reporter (KTR) system; the reporter is translocated from nucleus to cytoplasm when it is phosphorylated by its target kinase (Figure 2A). This approach yields several kinase reporters, e.g., ERK, Akt, p38, and JNK, and will provide clues as to how cells emerge all-or-none and irreversible cellular events such as cell cycle progression and apoptosis by using analog and continuous kinase signals as an input (Figure 2B).

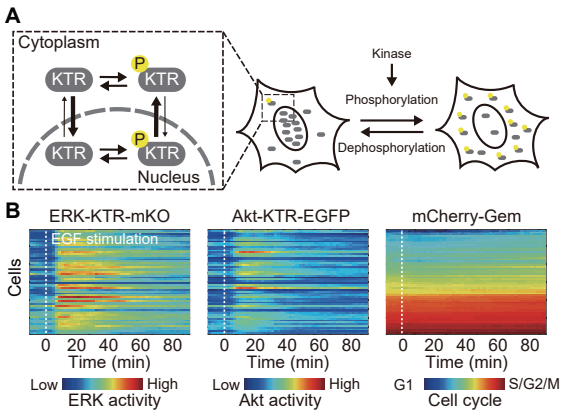

Figure 2. Multiplexed imaging of ERK and Akt activities and cell cycle state. A, Schematic representation of the principle of KTR system. B, Shown here are heat maps of ERK activity (left), Akt activity (middle), and cell cycle (right) obtained by KTR systems.

II. Quantification of cell signaling

Classically, kinetic parameters such as protein concentration and dissociation constant have been measured by *in vitro* biochemical analyses. However, some kinetic parameters might significantly differ between *in vitro* and *in vivo*. For instance, the Kd values measured *in vivo* were higher than

the *in vitro* Kd values by an order of 1 or 2. Therefore, it is critical to measure kinetic parameters in living cells. To this end, we combine CRISPR/Cas9-mediated genome editing with fluorescence correlation spectroscopy (FCS) and fluorescence cross-correlation spectroscopy (FCCS) to quantitatively determine the endogenous protein concentration and the dissociation constant (Figure 3). These parameters will be applied to the computer simulation to predict the dynamics of singling pathways.

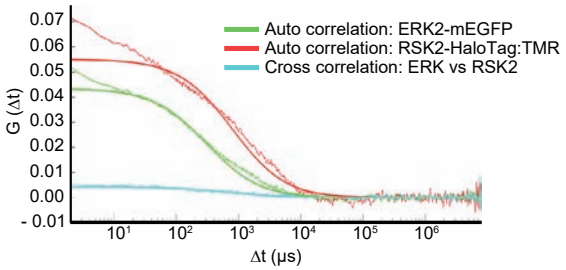

Figure 3. Auto- and cross-correlation functions of ERK2-mEGFP and RSK2-HaloTag. *mEGFP* and *HaloTag* genes were knocked-in at the site of 3' *ERK2* and *RSK2* genes, respectively. These auto- and cross-correlation functions were obtained by FCS and FCCS, providing endogenous concentration of ERK2 and RSK2 and the dissociation constant of ERK2-RSK2 binding.

III. Manipulation of cell signaling

Optogenetics is a powerful tool for the control of the intracellular signaling pathway with high spatial and temporal precision. Our laboratory focuses on the light-induced dimerization (LID) system with plant cryptochrome 2 (CRY2) and phytochrome B (PhyB), in which blue- and red-light induce heterodimerization to their binding partners, respectively. By using these systems, we attempt to reconstitute the spatial and temporal pattern of cell signaling in order to directly validate the physiological meaning of the dynamics of cell signaling that were observed by time-lapse fluorescence imaging.

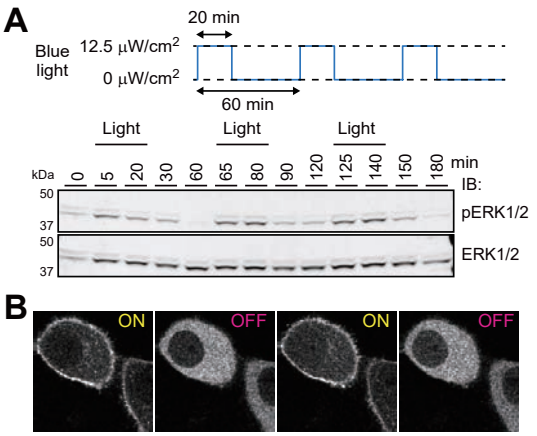

Figure 4. Optogenetic manipulation of intracellular signaling. A, Blue light-induced repetitive ERK activation with CRY2 system. B, Red and infrared light-induced shuttling of phytochrome interacting factor (PIF) -EGFP between cytoplasm and the plasma membrane.

Publication List:

〔Original papers〕

- Inaba, K., Oda, K., Aoki, K., Sone, K., Ikeda, Y., Miyasaka, A., Kashiyama, T., Fukuda, T., Makii, C., Arimoto, T., Wada-Hiraike, O., Kawana, K., Yano, T., Osuga, Y., and Fujii, T. (2016). Synergistic antitumor effects of combination of PI3K/mTOR and MEK inhibition (SAR245409 and pimasertib) in mucinous ovarian carcinoma cells by fluorescence resonance energy transfer imaging. Oncotarget *7*, 29577-29591.
- Kamezaki, A., Sato, F., Aoki, K., Asakawa, K., Kawakami, K., Matsuzaki, F., and Sehara-Fujiwara, A. (2016). Visualization of Neuregulin 1 ectodomain shedding reveals its local processing in vitro and in vivo. Sci. Rep. *6*, 28873.
- Maryu, G., Matsuda, M., and Aoki, K. (2016). Multiplexed fluorescence imaging of ERK and Akt activities and cell-cycle progression. Cell Struct. Funct. *41*, 81-92.
- Yamao, M., Aoki, K., Yukinawa, N., Ishii, S., Matsuda, M., and Naoki, H. (2016). Two new FRET imaging measures: linearly proportional to and highly contrasting the fraction of active molecules. PLoS One *11*, e0164254.