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Living cells act as input-output (I/O) units, in which their environment and/or internal states are recognized on the cell surface and processed within the cell, leading to an adaptive response to any changes that occur (Figure 1). This cellular information processing is mainly controlled by intracellular signal transduction, which is comprised of a series of chemical reactions, most commonly protein phosphorylation. Dysregulation of this process 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 a perceived increase in the pathway's complexity. Computer-assisted simulation is one of the most promising approaches to understanding signal transduction pathway as a system. Indeed, chemical and physical reactions constituting the signal transduction can be described by a set of ordinary differential equations, and can be solved numerically by computers. In fact, a number of signaling pathway simulation models have been reported to date. However, most of the kinetic parameters utilized for these simulation models were not measured experimentally, but rather were assumed by fitting the experimental data with the simulation model or were simply determined arbitrarily. Consequently, there are substantial differences in the kinetic parameters between these studies, thereby making it difficult to quantitatively evaluate these simulation models.

To address these issues, we are currently focusing 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 become possible to visualize kinetic reactions at the single cell level. Förster (or fluorescence) resonance energy transfer (FRET) is a non-radiative process, in which the excitation energy

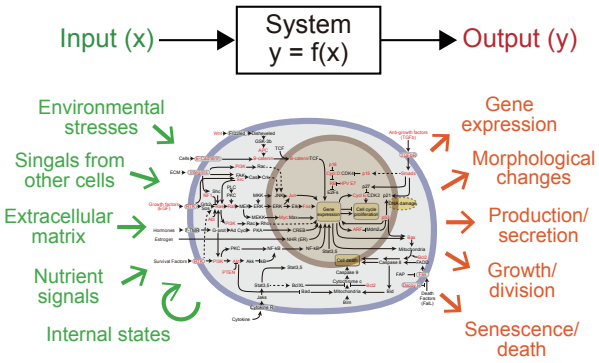

Figure 1. Information processing by intracellular signaling devices and networks.

of a donor fluorophore is transferred to a nearby acceptor fluorophore. Taking advantage of this principle, FRET-based biosensors allowed us to detect the kinase activity of PKA, ERK, Akt, JNK, PKC, and S6K in a living cell with high temporal and spatial resolution (Komatsu N, Mol. Biol. Cell, 2011). By using a FRET biosensor, we revealed the role of temporal and spatial dynamics of ERK activation in cell proliferation (Aoki K, Mol Cell, 2013) and collective cell migration (Aoki K, Dev Cell, 2017).

Although FRET biosensors enable us to monitor cell signaling events, FRET imaging is not suitable for multiplexed imaging, because these biosensors are composed of two different fluorophores in many cases. To circumvent this limitation, we are currently developing biosensors based on the

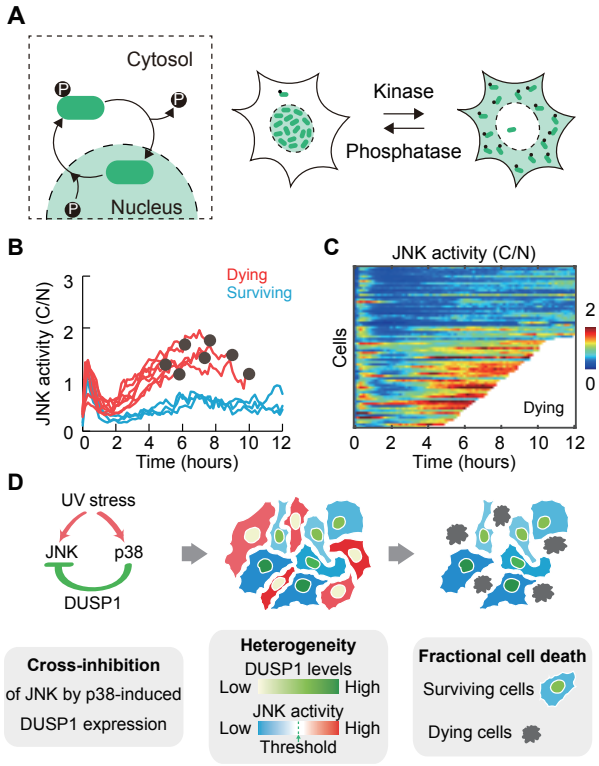

Figure 2. Multiplexed imaging of JNK and p38, stress-activating protein kinases (SAPKs) signaling. (A) Schematic representation of kinase translocation reporter (KTR). (B) Time-course of UV-C-induced JNK activation in surviving (blue) and dying (red) cells. (C) A heatmap shows time-courses of JNK activation and cell death. (D) The schematic shows p38-induced cross-inhibition through DUSP1 expression emerges JNK heterogeneity, leading to the fractional killing upon UV-C stress.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2018. The former title is indicated by an asterisk (*).

principle of the kinase translocation reporter (KTR) system. The reporter itself is translocated from nucleus to cytoplasm when it is phosphorylated by its target kinase (Figure 2A). This approach yields several ERK and Akt kinase reporters (Maryu G, Cell Strut, Funct, 2016). We further applied KTRs for p38 and JNK, stress-activating protein kinases (SAPKs) to investigate how stress stimuli induced p38 and JNK activation, and subsequently lead to cell death. Various stresses activated JNK and p38 within various dynamics. In all cases, p38 suppressed JNK activity in a cross-inhibitory manner. We demonstrated that p38 antagonizes JNK through both transcriptional and post-translational mechanisms. This cross-inhibition generates cellular heterogeneity in JNK activity after stress exposure (Figure 2B and 2C). Our data indicates that this heterogeneity in JNK activity plays a role in fractional killing in response to UV stress (Figure 2D) (Miura H, Cell Reports, 2018).

II. Quantification of cell signaling and physical parameters

Kinetic parameters such as protein concentration and dissociation constant, Kd, have traditionally 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 (Sadaie W, MCB, 2014). Therefore, it is critical to measure kinetic parameters in living cells. To this end, we launched a research project of quantitative cell cycle modeling in fission yeast *S. pombe* and mammalian cultured cells. We developed an efficient knock-in system with CRISPR/Cas9-mediated genome editing techniquescombined with fluorescence correlation spectroscopy (FCS) and fluorescence cross-correlation spectroscopy (FCCS) to quantitatively determine the protein concentration and the dissociation constant of endogenous protein (Komatsubara AT, Goto Y, bioRxiv, 2018).

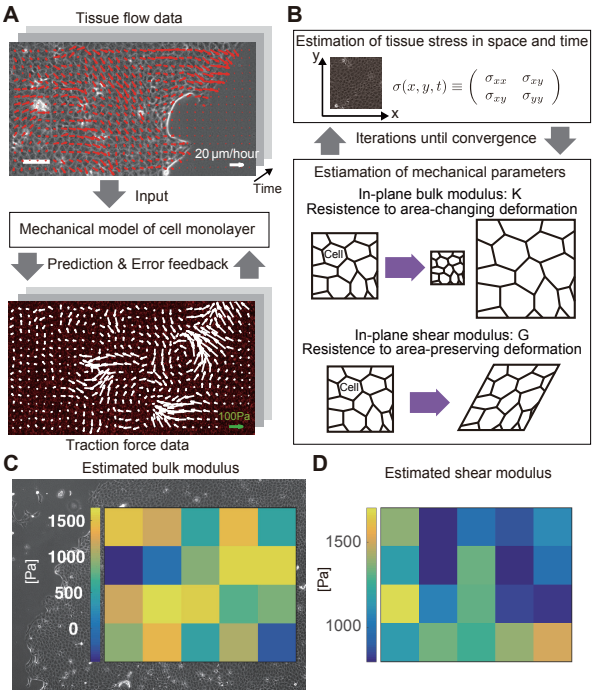

Figure 3. Overview of the inference of mechanical parameters. (A and B) Schematic representation of the inference scheme and algorithm of mechanical properties. (C and D) Spatial distribution of the estimated elastic moduli.

In order to quantitatively understand the morphogenesis of living tissues, we need to elucidate the mechanical properties which describe how the tissues form in response to self-generated forces. To this end, we proposed a non-invasive approach for the statistical estimation of the mechanical properties, by combining tissue mechanics modeling and statistical machine learning (Figure 3A and 3B). This method was applied to the collective migration of Madin-Darby canine kidney cells, where the tissue flow and force were simultaneously observed by phase contrast imaging and traction force microscopy. As a result, the estimated elastic moduli were detected in the order of kPa μm (Figure 3C and 3D). We confirmed that our elastic solid tissue model outperformed null-hypothetical models in terms of forecast accuracy for the traction force fields, indicating that mechanical dynamics are dominated by elasticity. The results validate our framework, which paves the way to estimate *in vivo* mechanical properties of tissues during morphogenesis (Kondo Y, PLoS Comp, 2018).

III. Manipulation of cell signaling

Artificial manipulation of biochemical networks may be useful in the quantitative understanding of biological systems. Multiple methods have been suggested for controlling signal transduction, including light-induced dimerization (LID) and chemically induced dimerization (CID) systems. Of these two, the LID system is stronger in terms of temporal and spatial manipulations. The photo-responsive proteins derived from fungi, cyanobacteria and plants, and modified fluorescent proteins are used in this system.

We focus on the phytochrome B (PhyB)-PIF LID system: Upon red-light illumination, PhyB binds to PIF, and the two parts of the PhyB-PIF complex dissociate from each other by infra-red light exposure (Figure 4A). The reversible control of association and dissociation by light is a unique feature in the PhyB-PIF system, because association or dissociation only are regulated by light in other LID systems. One drawback is that covalent attachment of a chromophore, *e.g.* phycocyanobilin (PCB), is required for the light-responsive function of PhyB-PIF. To overcome this issue, we have developed a method for synthesis of PCB in mammalian cells by introduction of the gene products of *HO1*, *PcyA*, *Fd*, and *Fnr* into mitochondria (Uda Y, PNAS, 2017) (Figure 4B).

To take full advantage, we applied the genetically encoded PCB synthesis system to fission yeast *S. pombe* and *C. elegans*, of which both experienced difficulty in taking up externally-delivered PCB. As we expected, the expression of *HO1*, *PcyA*, *Fd*, and *Fnr* genes in *S. pombe* increased PCB synthesis, allowing us to manipulate subcellular distribution of proteins by light (Figure 4C). *C. elegans* demonstrated uptake of externally delivered PCB only in the gut, but not in the muscles or neurons (Figure 4D), while the introduction of *HO1*, *PcyA*, *Fd*, and *Fnr* genes enabled to reconstitute PCB synthesis in the muscles, guts and neurons (Figure 4D and 4E). In addition to this, we were able to demonstrate light-

induced PhyB-PIF hetero-dimerization in *C. elegans* for the first time (Figure 4F). The genetically encoded system of PCB synthesis will provide a potential advantage for establishing transgenic animals that stably synthesize PCB endogenously, thereby enabling the optogenetic manipulation of cell signaling in deeper tissues without injecting PCB.

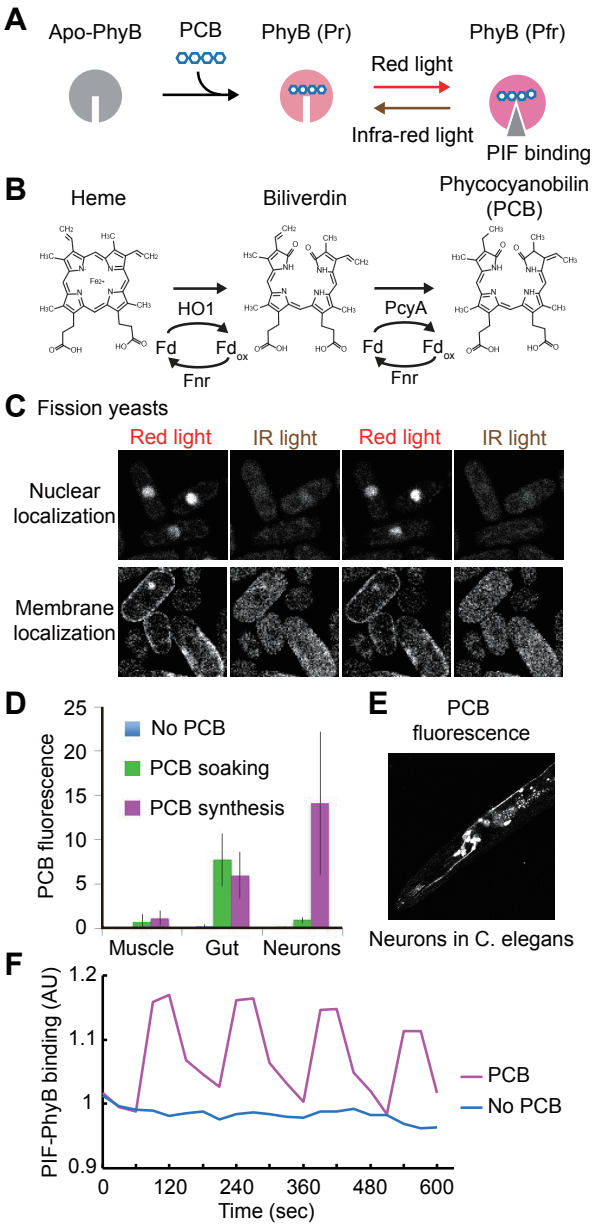

Figure 4. PhyB-PIF light-inducible dimerization (LID) system and its application to the manipulation of cell signaling. (A) Apo-PhyB covalently attaches its chromophore, which is phycocyanobilin (PCB) to produce holo-PhyB. There are two holo-PhyB forms, PhyB (Pr) and PhyB (Pfr), which change over in manner dependent on the chromophore status. Only PhyB (Pfr) associates with PIF. (B) The metabolic pathway of phytochrome chromophores, PCB. (C) Light-induced translocation to nucleus (upper) and plasma membrane (lower) of PIF3-EGFP protein in fission yeasts expressing PcyA, HO1, Fd, and Fnr. (D) *C. elegans* was soaked with DMSO (blue) or PCB (green), or introduced by PcyA, HO1, Fd, and Fnr genes to synthesize PCB (magenta). PCB fluorescence was quantified. (E) A representative image of PCB fluorescence in neurons of C. elegans. (F) Light-induced PhyB-PIF binding in gut of *C. elegans*.

Publication List:

〔Original papers〕

- Hori, S., Oda, S., Suehiro, Y., Iino, Y., and Mitani, S. (2018). OFF-responses of interneurons optimize avoidance behaviors depending on stimulus strength via electrical synapses. PLoS genetics *14*, e1007477.
- Kondo, Y., Aoki, K., and Ishii, S. (2018). Inverse tissue mechanics of cell monolayer expansion. PLoS Comput Biol. *14*, e1006029.
- Miura, H., Kondo, Y., Matsuda, M., and Aoki, K. (2018). Cell-to-cell heterogeneity in p38-mediated cross-inhibition of JNK causes stochastic cell death. Cell Reports *24*, 2658-2668.
- Muta, Y., Fujita, Y., Sumiyama, K., Sakurai, A., Taketo, M. M., Chiba, T., Seno, H., Aoki, K., Matsuda, M., and Imajo, M. (2018). Composite regulation of ERK activity dynamics underlying tumour-specific traits in the intestine. Nat Commun. *9*, 2174.

〔Review articles〕

- Maryu, G., Miura, H., Uda, Y., Komatsubara, A.T., Matsuda, M., and Aoki K. (2018). Live-cell imaging with genetically encoded protein kinase activity reporters. Cell Struct. Funct. *43*, 61-74.
- Oda, S., Uda, Y., Goto, Y., Miura, H., and Aoki, K. (2018). Optogenetic tools for quantitative biology: The genetically encoded PhyB–PIF light-inducible dimerization system and its application for controlling signal transduction. In Optogenetics: Light-driven Actuators and Light-emitting Sensors in Cell Biology, S. Vriz, and T. Ozawa, eds., pp. 137-148.