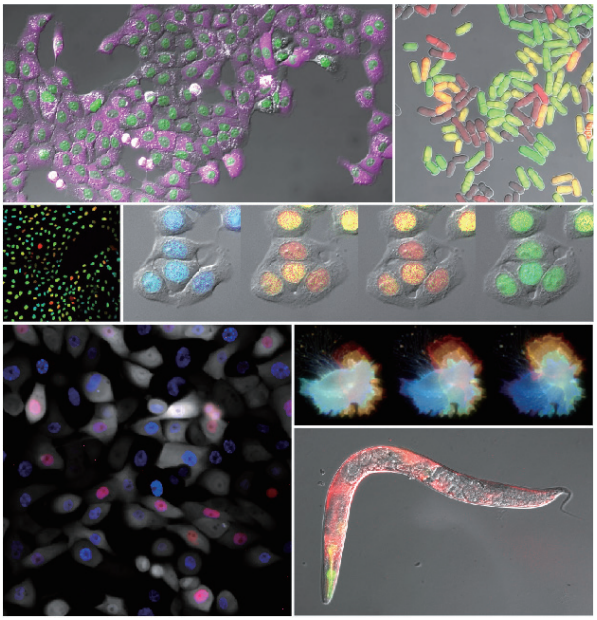
DIVISION OF QUANTITATIVE BIOLOGY

Professor
AOKI, Kazuhiro

Assistant Professor:	*KONDO, Yohei*
	GOTO, Yuhei
Technical Staff:	*BINO, Takahiro*
Postdoctoral Fellow:	*NAKAMURA, Akinobu*
SOKENDAI Graduate Student:	*TANII, Ryosuke*
	MUKAI, Masaya
	YAMAMOTO, Kei
	SAKAI, Keiichiro
	TSURUOKA, Tatsuki
Visiting Scientist:	*ITO, Reina*
Technical Assistant:	*EBINE, Emi*
	GOTO, Yoko
Admin Support Staff:	*ONODA, Kaori*



Visual overview of this lab's work, showing fluorescence images of mammalian cells, fission yeast, and *C.elegans*

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, thus leading to an adaptive response (Figure 1). This cellular information processing is mainly implemented by an intracellular signal transduction system, which is comprised of a series of chemical reactions such as protein-protein interactions and protein phosphorylation. Dysregulation of cell signaling by gene mutation is widely known to result in various pathologies, such as malignant tumors.

The intracellular signal transduction system has been extensively studied over the past few decades through approaches utilizing both biochemistry and cell biology approaches. As a result, many proteins and regulations have been identified, which has resulted in an increase in the pathway's complexity. The complicated signaling network makes it difficult to understand how cells process information and quantitatively make decisions.

To address these issues, we are currently focusing on the development of two types of research tools that enable us to (1) visualize and (2) manipulate intracellular signaling pathways (Figure 1).

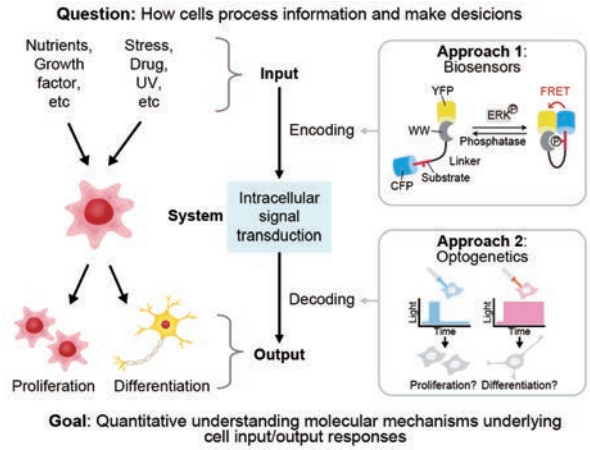


Figure 1. Information processing by intracellular signaling devices and networks.

I. Visualization of cell signaling

With the advent of fluorescent proteins, it has become possible to visualize kinetic reactions at a single-cell level. Förster (or fluorescence) resonance energy transfer (FRET) is a non-radiative process in which the excitation energy of a donor fluorophore is transferred to a nearby acceptor fluorophore. FRET-based biosensors allow us to detect PKA, ERK, Akt, JNK, PKC, and S6K's kinase activity in living cells at a high temporal and spatial resolution (Komatsu N, Mol. Biol. Cell, 2011). By using a FRET biosensor, we have been able to reveal the role played by ERK activation dynamics 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 the biosensors are composed of two different fluorophores in many cases. To circumvent this limitation, we are currently developing single-fluorophore-based biosensors. We recently developed a red-fluorescence dopamine (DA) reporter. DA, a neuromodulator, is involved in many neuronal functions, and to monitor it in living cells and animals, we have designed a red-fluorescent DA reporter. This reporter features a circular-permutated mApple (cpmApple), a red fluorescent protein, and a cpmApple was inserted into the third intracellular loop of a DA receptor, DRD1 (Figure 2A). The linker sequences between DRD and cpmApple were optimized by random mutagenesis and subsequent live-cell screening. Finally, we picked up the best performance reporter and named the red genetically encoded

GPCR activation reporter for DA, R-GenGAR-DA. The red fluorescence intensity decreased in response to DA and returned to the basal level upon a DRD1 antagonist treatment (Figure 2B and 2C). In addition, by using a previously reported green fluorescence norepinephrine (NE) reporter, we succeeded in simultaneously visualizing DA and NE in living neurons (Nakamoto C, Goto Y, *et al.*, bioRxiv, 2020).

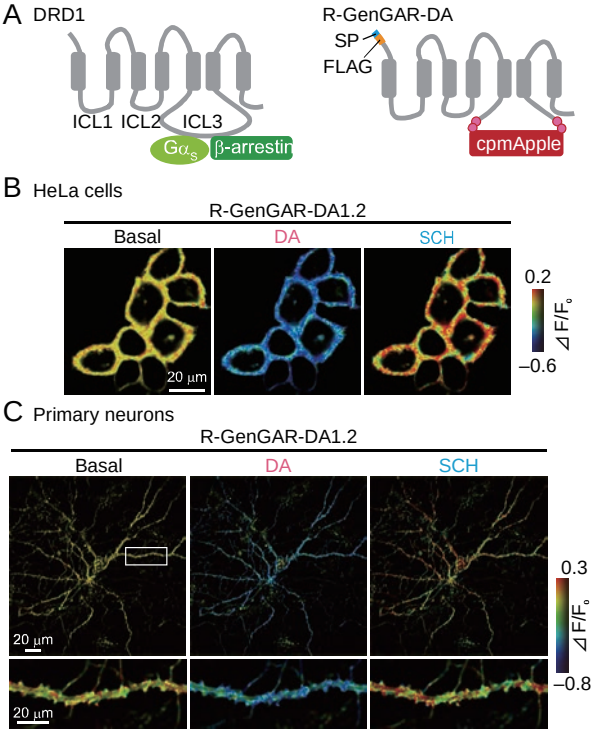


Figure 2. Development of a genetically encoded red fluorescent dopamine (DA) reporter. (A) Schematic representation of a dopamine receptor, DRD1 (left), and Red DA reporter, R-GenGAR-DA. (B and C) HeLa cells (B) or mouse primary hippocampal neurons (C) expressing R-GenGAR-DA were stimulated with DA, followed by a DRD1 antagonist, SCH.

II. Manipulation of cell signaling

Artificial manipulation of biochemical networks is well established 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 more beneficial in terms of temporal and spatial manipulation. The photo-responsive proteins derived from fungi, cyanobacteria, plants, and modified fluorescent proteins are used in this system.

We have reported a novel optogenetic tool to induce relaxation of actomyosin contractility with a blue light-responsive protein, such as cryptochrome 2 (CRY2) and iLID. Actomyosin contractility, which is generated cooperatively by nonmuscle myosin II (NMII) and actin filaments, plays essential roles in a wide range of biological processes such as cell motility and cytokinesis. Yet, it remains elusive as to how actomyosin contractility generates force and maintains cellular morphology. To elucidate this, we have developed a novel optogenetic tool, OptoMYPT, which combines a catalytic subunit of type I phosphatase-binding domain of MYPT1 with an optogenetic dimerizer, so that it allows light-dependent recruitment of endogenous PP1c to the plasma membrane (Figure 3A). We confirmed that blue-light illumination was sufficient to induce dephosphorylation of MLC, resulting in lamellipodial membrane protrusion (Figure 3B). The OptoMYPT was further employed to understand the mechanics of actomyosin-based cortical tension and contractile ring tension during cytokinesis (Figure 3C). We found that the relaxation of cortical tension at both poles by OptoMYPT accelerated furrow ingression rate, thus revealing that the cortical tension substantially antagonizes constriction of the cleavage furrow. These results provide new opportunities to freely design cell and tissue morphology through light (Yamamoto, *et al.*, bioRxiv, 2021).

The Blue light-responsive optical dimerizers are useful, but its activation light often overlaps with the excitation light of fluorescent proteins, which in turn hampers the application of GFP or FRET biosensors together with their optogenetic tools. To circumvent this issue, we are focusing on the phytochrome B (PhyB)-PIF LID system. PhyB binds to PIF upon red-light illumination, and the PhyB-PIF complex dissociates from each other by far-red light exposure (Figure 4A). The

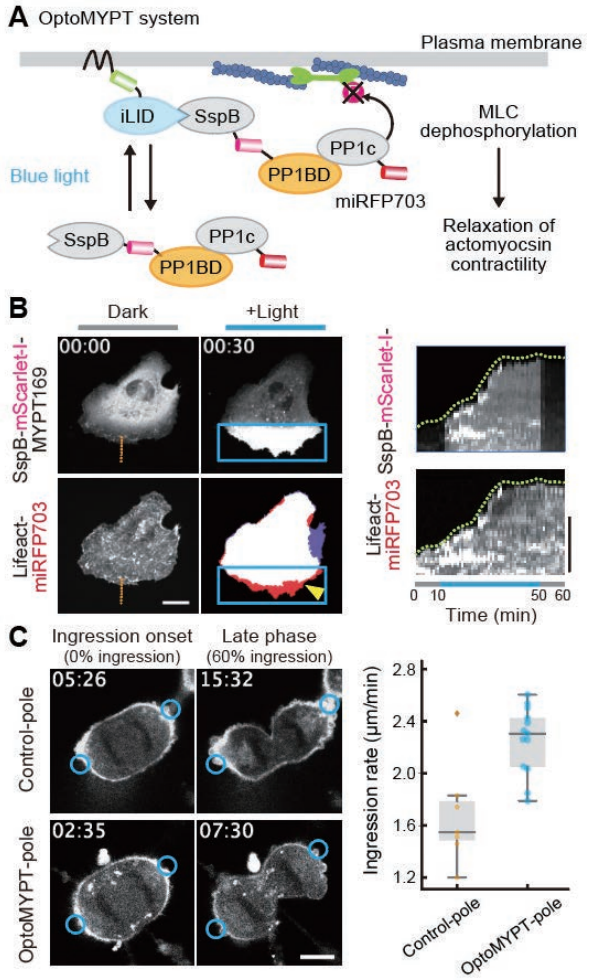


Figure 3. OptoMYPT: Optogenetic relaxation of actomyosin contractility. (A) Schematic representation of OptoMYPT. (B) Local recruitment of OptoMYPT induced myosin regulatory light chain dephosphorylation, leading to the induction of membrane protrusion. (C) Local relaxation of actomyosin cortical tension at the both poles in mitotic cells accelerated ingression of contractile ring.

reversible control of association and dissociation by light is a unique feature in the PhyB-PIF system because other LID systems can only control association or dissociation by light. One drawback is that covalent attachment of a chromophore, phycocyanobilin (PCB), is required for the light-responsive function of PhyB-PIF. To overcome this issue, we have developed a method for PCB biosynthesis in mammalian cells by introducing the gene products of *HO1*, *PcyA*, *Fd*, and *Fnr* into the mitochondria (Uda Y, *et al*., PNAS, 2017) (Figure 4A). We recently improved the PCB synthesis system, named SynPCB2.1, which allowed establishing stable cell lines that synthesize PCB and light-induced control of protein localization at the cell population level (Figure 4B and 4C) (Uda Y, *et al*., ACS Chem Biol, 2020).

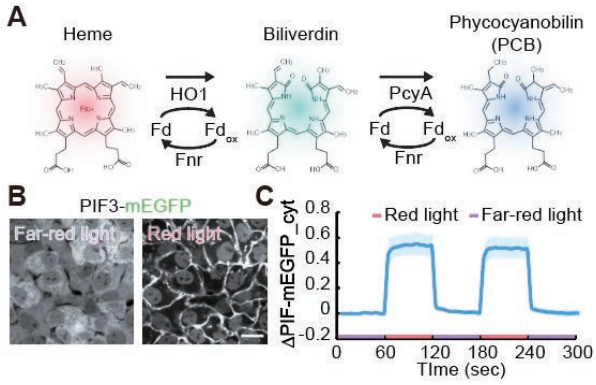


Figure 4. PhyB-PIF light-inducible dimerization (LID) system. (A) The metabolic pathway of phytochrome chromophores, PCB. (B and C) Light-induced translocation of PIF3-mEGFP to the plasma membrane, where PhyB is localized (B). Membrane translocation of PIF3-mEGFP is quantified (C).

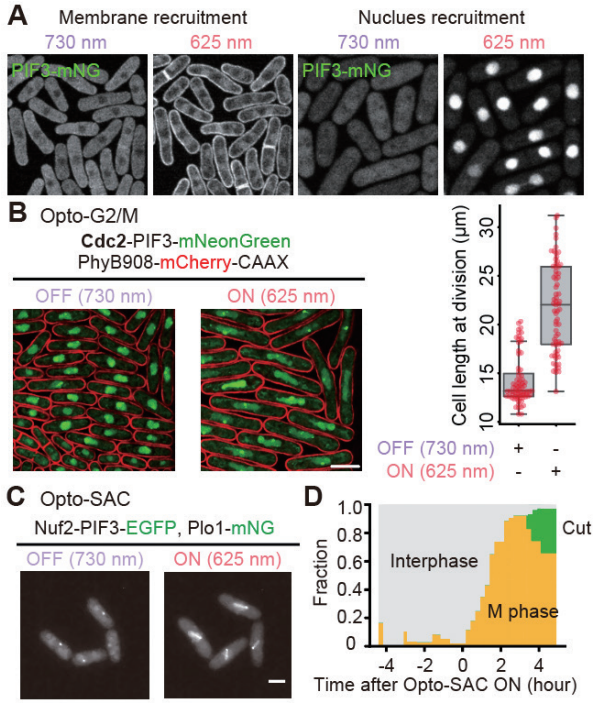


Figure 5. Optogenetic control of cell cycle in fission yeast. (A) Light-induced recruitment of PIF3-mNeonGreen (mNG) to the plasma membrane (left) and nucleus (right). (B) Opto-G2/M: Optical control of G2/M transition. (left) Opto-G2/M OFF cells showed normal morphology, while Opto-G2/M ON cells exhibited longer cell length, a typical phenotype of G2/M arrest fission yeast. (C) Opto-SAC: Optical control of spindle assembly checkpoint. Representative cells undergoing metaphase arrest (right) by Opto-SAC. (D) Long-term metaphase arrest by Opto-SAC leads to a mitotic failure and cut phenotype.

To take full advantage of this, we applied the genetically encoded PCB synthesis system to *S. pombe* and *C. elegans*, both of which are incapable of having purified PCB delivered to them. As we expected, the expression of *HO1*, *PcyA*, *Fd*, and *Fnr* genes induced PCB biosynthesis in *S. pombe* and *C. elegans*. Using this system, we developed optogenetic control of the cell cycle in two ways: the Opto-G2/M checkpoint triggered G2/M cell cycle arrest, and Opto-SAC induced a spindle assembly checkpoint (SAC) in response to red light (Figure 5) (Goto Y, and Aoki K., bioRxiv, 2020).

Publication List:

〔Original papers〕

- Asakura, Y., Kondo, Y., Aoki, K., and Naoki, H. (2021). Hierarchical modeling of mechano-chemical dynamics of epithelial sheets across cells and tissue. Sci. Rep. *11*. DOI: 10.1038/s41598-021-83396-6
- Hino, N., Rossetti, L., Marin-Llaurado, A., Aoki, K., Trepat, X., Matsuda, M., and Hirashima, T. (2020). ERK-mediated mechanochemical waves direct collective cell polarization. Dev. Cell *53*, 646+. DOI: 10.1016/j.devcel.2020.05.011
- Nakamura, A., Oki, C., Kato, K., Fujinuma S., Maryu, G., Kuwata, K., Yoshii, T., Matsuda, M., Aoki, K., and Tsukiji, S. (2020). Engineering orthogonal, plasma membrane-specific SLIPT systems for multiplexed chemical control of signaling pathways in living single cells. ACS Chem. Biol. *15*, 1004–1015. DOI: 10.1021/acschembio.0c00024
- Uda, Y., Miura, H., Goto, Y., Yamamoto, K., Mii, Y., Kondo, Y., Takada, S., and Aoki, K. (2020). Improvement of phycocyanobilin synthesis for genetically encoded phytochrome-based optogenetics. ACS Chem. Biol. *15*, 2896–2906. DOI: 10.1021/acschembio.0c00477
- Wen, C., Miura, T., Voleti, V., Yamaguchi, K., Tsutsumi, M., Yamamoto, K., Otomo, K., Fujie, Y., Teramoto, T., Ishihara, T., *et al*. (2021). 3DeeCellTracker, a deep learning-based pipeline for segmenting and tracking cells in 3D time lapse images. eLife*10*. DOI: 10.7554/eLife.59187
- Yano, T., Tsukita, K., Kanoh, H., Nakayama, S., Kashihara, H., Mizuno, T., Tanaka, H., Matsui, T., Goto, Y., Komatsubara, A., *et al*. (2021). A microtubule-LUZP1 association around tight junction promotes epithelial cell apical constriction. EMBO J. *40*. DOI: 10.15252/embj.2020104712

〔Review Articles〕

- Akiyama, S., Aoki, K., and Kubo, Y. (2020). Biophysical research in Okazaki, Japan. Biophys. Rev. *12*, 237–243. DOI: 10.1007/s12551-020-00633-4
- Goto Y., Kondo Y., Aoki K. (2021). Visualization and Manipulation of Intracellular Signaling. Adv. Exp. Med. Biol. *1293*, 225–234. DOI: 10.1007/978-981-15-8763-4_13