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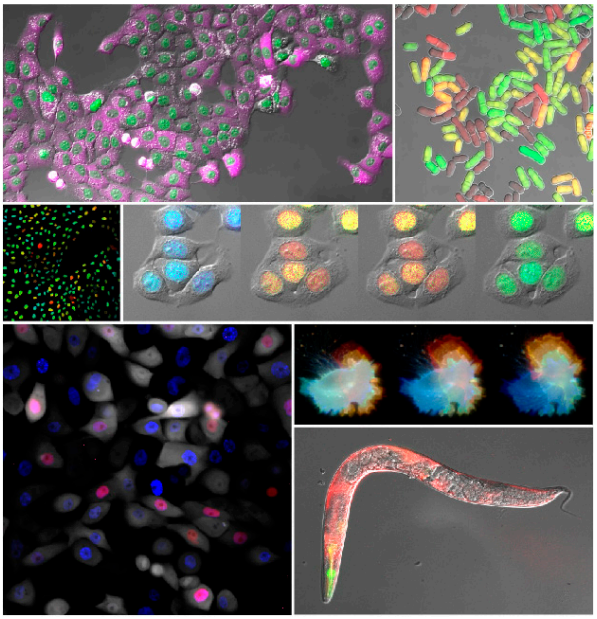

Visual overview of this lab's work, showing fluorescence images of mammalian cells, fission yeast, and *C.elegans*

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).

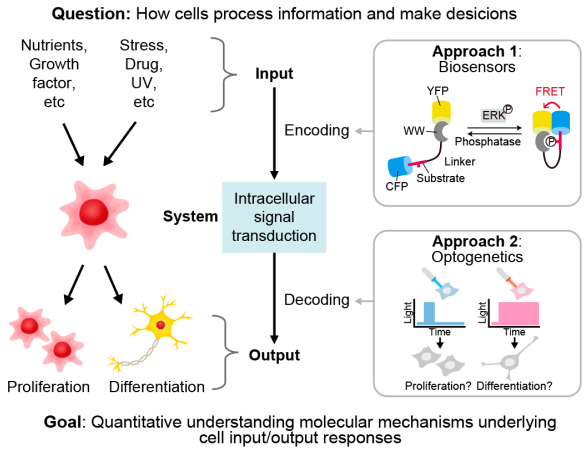

Figure 1. Information processing by intracellular signaling devices and networks.

I. Visualization of cell signaling

With the advent of fluorescent proteins, it has become possible to visualize kinetic reactions at the 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; Aoki K, Mol Cell, 2013; 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 (Figures 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, Mol Brain, 2021).

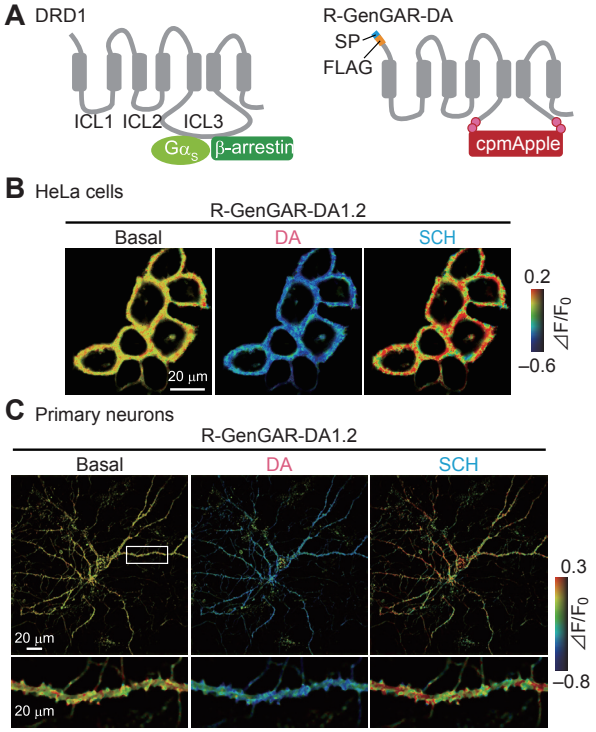

Figure 2. Development of a genetically encoded red fluorescent dopamine (DA) reporter. (A) Schematic representation of a dopamine receptor, DRD1 (left), and Red DA reporter, R-GenGAR-DA. (B and C) HeLa cells (B) or mouse primary hippocampal neurons (C) expressing R-GenGAR-DA were stimulated with DA,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

Near-infrared fluorescent protein (iRFP) is a fluorescent protein with near-infrared excitation and emission maxima. Unlike the other conventional fluorescent proteins, iRFP requires biliverdin (BV) as a chromophore (Figure 3A). We accidentally found that phycocyanobilin (PCB) functions as a brighter chromophore for iRFP than BV and that biosynthesis of PCB allows live-cell imaging with iRFP in the fission yeast *Schizosaccharomyces pombe* (Figure 3B). We initially found that fission yeast cells did not produce BV and therefore did not show any iRFP fluorescence (Figure 3C). The brightness of iRFP–PCB was higher than that of iRFP–BV both *in vitro* and in fission yeast. We introduced SynPCB2.1, a PCB biosynthesis system (Uda, Miura, ACS Chem Biol, 2020), into fission yeast, resulting in the brightest iRFP fluorescence. These tools not only enable the easy use of multiplexed live-cell imaging in fission yeast with a broader color palette, but also open the door to new opportunities for near-infrared fluorescence imaging in a broader range of living organisms (Sakai, JCS, 2021).

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 control-

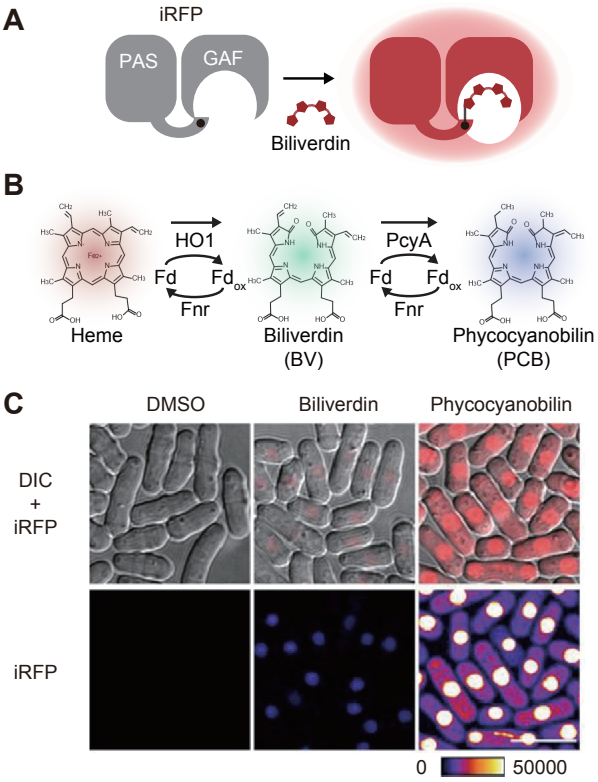

Figure 3. Phycocyanobilin (PCB) as a brighter chromophore for iRFP. (A) Schematic representation of iRFP with its chromphore Bliverdin. (B) The metabolic pathway of PCB. (C) DIC and iRFP fluorescence images are shown in fission yeats treated with DMSO, Biliverdin, or PCB.

ling 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 4A). We confirmed that blue-light illumination was sufficient to induce dephosphorylation of MLC, resulting in lamellipodial membrane protrusion (Figure 4B). The OptoMYPT was further employed to understand the mechanics of actomyosin-based cortical tension and contractile ring tension during cytokinesis (Figure 4C). We found that the relaxation of cortical tension at both poles by OptoMYPT accelerated the 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, Nat Comm, 2021).

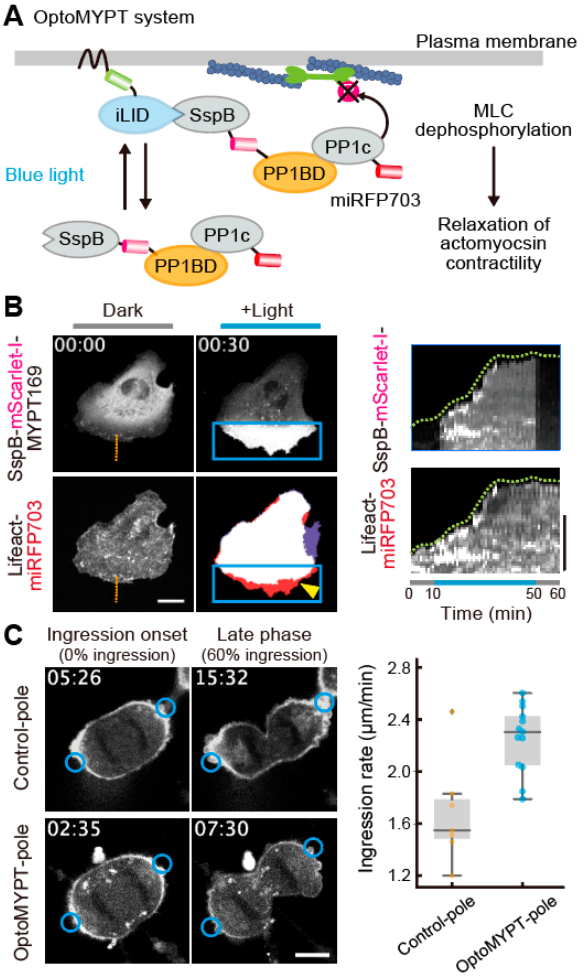

Figure 4. PhyB-PIF light-inducible dimerization (LID) system. (A) The metabolic pathway of phytochrome chromophores, PCB. (B and C) Light-induced translocation of PIF3-mEGFP to the plasma membrane, where PhyB is localized (B). Membrane translocation of PIF3-mEGFP is quantified (C).

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 focus on the phytochrome B (PhyB)-PIF LID system. Upon red-light illumination, PhyB binds to PIF, and the PhyB-PIF complex dissociate from each other by far-red light exposure. One drawback of PhyB-PIF LID is that a chromophore, *e.g.*, PCB, is required for the light sensing. To overcome this issue, we have developed a method for PCB biosynthesis, called SynPCB, in mammalian cells by introducing the gene products of *HO1*, *PcyA*, *Fd*, and *Fnr* into the mitochondria (Uda Y, PNAS, 2017; Uda Y, ACS Chem Biol, 2020).

To take full advantage of this system, we applied the SynPCB 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).

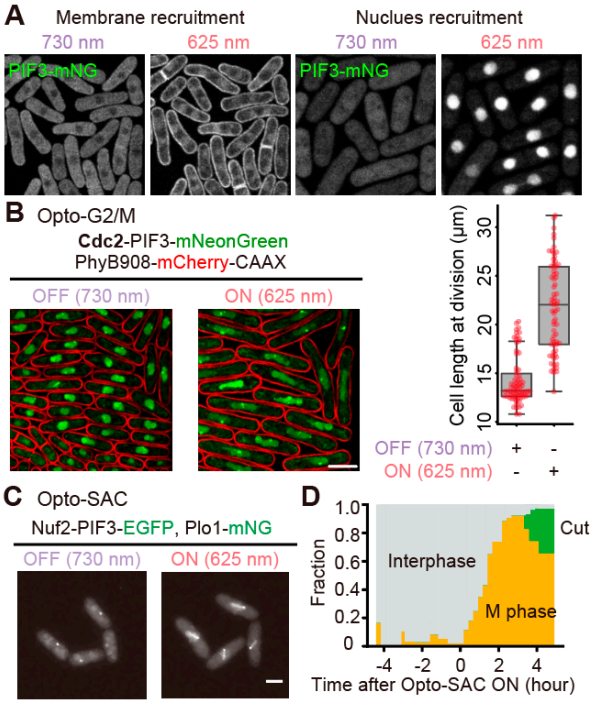

Figure 5. Optogenetic control of cell cycle in fission yeast. (A) Light-induced recruitment of PIF3-mNeonGreen (mNG) to the plasma membrane (left) and nucleus (right). (B) Opto-G2/M: Optical control of G2/M transition. (left) Opto-G2/M OFF cells showed normal morphology, while Opto-G2/M ON cells exhibited 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.

Publication List:

〔Original papers〕

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〔Review Articles〕

- Farahani, P.E., Reed, E.H., Underhill, E.J., Aoki, K., and Toettcher, J.E. (2021). Signaling, Deconstructed: Using Optogenetics to Dissect and Direct Information Flow in Biological Systems. Annu. Rev. Biomed. Eng. *23*, 61–87. DOI: 10.1146/annurev-bioeng-083120-111648
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