**REVIEW**

**CELL BIOLOGY**

**Toward total synthesis of cell function: Reconstructing cell dynamics with synthetic biology**

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Biological phenomena, such as cellular differentiation and phagocytosis, are fundamental processes that enable cells to fulfill important physiological roles in multicellular organisms. In the field of synthetic biology, the study of these behaviors relies on the use of a broad range of molecular tools that enable the real-time manipulation and measurement of key components in the underlying signaling pathways. This Review will focus on a subset of synthetic biology tools known as bottom-up techniques, which use technologies such as optogenetics and chemically induced dimerization to reconstitute cellular behavior in cells. These techniques have been crucial not only in revealing causal relationships within signaling networks but also in identifying the minimal signaling components that are necessary for a given cellular function. We discuss studies that used these systems in a broad range of cellular and molecular phenomena, including the time-dependent modulation of protein activity in cellular proliferation and differentiation, the reconstitution of phagocytosis, the reconstitution of chemotaxis, and the regulation of actin reorganization. Finally, we discuss the potential contribution of synthetic biology to medicine.

**Introduction**

One of the prevailing aspirations of synthetic biology is the intelligent design of biological systems to perform a specific function, which is achieved through the assembly of modular components consisting of genes and proteins into coherent cellular systems (1, 2). By these means, cells can be endowed with new functionality, such as the ability to release and synthesize chemicals upon stimulation. Whereas these are applications of synthetic biology in engineering novel cellular behaviors, an alternative and increasingly important application of synthetic biology has been its emerging role in the study of biological phenomena. Chemotaxis, secretion, and phagocytosis are products of complex signaling pathways that have been subjects of synthetic biology studies. Attempts to study these pathways are often confounded by the presence of redundancies and feedback loops in the underlying mechanisms, which present a challenge for traditional knockout, overexpression, and pharmacological techniques. These issues are further exacerbated by either the longer time scale necessary to establish the perturbation or the off-target effects of pharmacological inhibitors. Over the past decade, new synthetic molecular tools have emerged as an appealing alternative that enable the rapid and specific modulation of key components in a given signaling pathway.

The approaches of synthetic biology range from tools that enable the measurement of specific protein activity to those that enable the manipulation of biomolecules with subcellular resolution. One of the ultimate goals of synthetic biology is the recapitulation of these behaviors in a cell-free system, in which all of the components are introduced in a controlled manner. Momentum toward this objective has been demonstrated by studies that identified the core components in a pathway necessary to elicit particular cellular behaviors. For example, these studies have proved useful in rigorously demonstrating that the Src homology 2 (SH2) domain, common in membrane proteins implicated in chemotaxis, is not required for phosphorylation and is simply for membrane binding (3). Space does not permit a detailed discussion here of the many results enabled by synthetic biology techniques; interested readers are referred to reviews that cover these topics in greater detail (3, 4).

Synthetic biology techniques in biological studies can rely on the use of external stimuli to induce intracellular changes. In this Review, we will cover a small subset of techniques that broadly fall under the categories of optogenetics, chemically induced dimerization (CID), and receptor activated solely by a synthetic ligand (RASSL). Optogenetics includes photodimerization systems and photoactivatable gene expression systems that use light as a form of external stimulation. The former systems were developed on the basis of the observation that certain complementary domains in proteins dimerize when illuminated by light. The individual dimerizable domains can be attached to proteins without perturbing native function, which enables their recruitment to different subcellular localizations. For example, CRY2 (cryptochrome 2) and CIB1 [cryptochrome-interacting basic helix-loop-helix (bHLH)] are proteins originally discovered in Arabidopsis that have the capability of dimerizing under the illumination of a blue light with subsecond responses (5). Alternative photodimerization systems include the protein domain pair Phy (phytochrome B) and PIF (phytochrome-interacting factor 6), which, in contrast to the CRY2-CIB1 pair, require two different wavelengths of light for operation (6). Exposure of light with a wavelength of 650 nm causes the dimerization of Phy and PIF, whereas exposure with a second wavelength of 750 nm induces dissociation. A third photodimerization system, known as TULIP (tunable light-inducible dimerization tags), uses the domain pair LOV2 (light-oxygen-voltage) and PDZ domains, which dimerize...
under blue light (7). This particular system has the advantage that known mutations exist in the LOV2 and PDZ domains that control the affinity of binding. These mutations provide both the abilities to adapt and to change the kinetics of given components in a signaling pathway. At the gene expression level, optical methods can be used to induce gene expression and the production of a specific protein. Photoactivatable gene expression systems that use DNA binding components from single guide RNA (8), synthetic zinc finger proteins (9), Gal4 (10), and TALEs (transcription activator-like effectors) (11) have been developed. These systems use photodimerization systems to recruit transcription factors to the gene of interest in response to light, and they faithfully generate time-dependent gene expression patterns from changes in light.

In contrast to light-based methods, synthetic biology techniques can use small molecules as a form of stimulation, such as in the cases of CID and RASSL. Unlike photodimerization systems, CID relies on the use of small molecules to induce dimerization between complementary protein domains. A classic example of CID involves the use of rapamycin to cause FK506-binding protein (FKBP) to form dimers with the FKBP-rapamycin binding domain of mTOR (mechanistic target of rapamycin) (FRB). The dissociation constant observed between FKBP-rapamycin and FRB is 12 nM, which enables effective manipulations for most experimental and physiological purposes (12). Alternative CID systems that used different dimer-inducing agents and dimerizing domains exist (13–15). A second class of chemical methods discussed here are RASSLs, which are heterotrimetric guanine nucleotide–binding protein (G protein)–coupled receptors (GPCRs) that have been specifically mutated to be responsive to synthetic ligands but unresponsive to their native ligands. These receptors, which are genetically encoded, can be used to activate members of the Gα, Gβ, and Gγ families of G proteins (16). Although RASSLs activate downstream signaling cascades that are similar to those activated by native GPCRs, the use of a synthetic ligand to activate RASSLs enables researchers to activate a particular pathway without contributions from extracellular ligands.

Here, we outline how synthetic biology has been used to study and reconstitute various biological phenomena (Fig. 1). We begin by discussing the use of light in studying cellular proliferation and differentiation. We then describe the development of a system for the induced presentation of proteins to the extracellular space to reconstitute phagocytosis in normally non-phagocytic cell lines. This is followed by a discussion of studies of chemotaxis that reconstitute this process with RASSLs, CID, and light. Finally, we end with an overview of a study that examined the role of membrane phospholipids in actin regulation.

Optogenetic Approaches in Studies of Cellular Proliferation and Differentiation

Traditional experimental techniques provide limited control to experimen- ters over protein activity in both space and time. One of the advantages of synthetic biology is the spatiotemporal resolution that is often required to mimic biological processes that are dynamic and local. Optogenetics, a technique derived from light-sensitive proteins, provides the ability to rapidly activate and deactivate protein activity with light illumination at different spatial locations. In this section, we introduce some findings in the field that were enabled by optogenetic tools that were developed for controlling Raf–mitogen-activated and extracellular signal–regulated kinase (MEK)–extracellular signal–regulated kinase (ERK) and bHLH signaling pathways.

Cell proliferation control by the MEK-ERK pathway

The use of optogenetics enables the time-dependent activation and in-activation of the MEK-ERK pathway. Activation of this pathway leads to the translocation of ERK from the cytosol to the nucleus, which leads to further activation of numerous transcription factors and the stim-ulation of cellular proliferation. In two independent studies, the CRY2-CIB1 and Phy-PIF systems were used to recruit components of the MEK-ERK pathway from the cytosol to the plasma membrane, which led to the activation of this pathway. Aoki et al. fused cRaf to CRY2 and localized CIBN, a truncated form of CIB1, to the plasma membrane by fusing to a CAAX motif derived from small guanosine triphosphatase (GTPase) K-Ras (17). Dimerization of CRY2 and CIBN was induced with blue light illumination. Toetcher et al. similarly anchored the Phy domain to the plasma membrane and fused PIF to SOS-phos, a catalytically active fragment of SOS (Son of Sevenless) that activates Ras when localized to the membrane (18). In contrast to the CRY2-CIB1 system, the Phy-PIF system requires light of two different wavelengths: 650-nm light is required for dimerization, whereas 750-nm light is required for dissociation of the dimer. The recruitment of both these respective proteins to the plasma membrane caused the activation of ERK, which was observable by its translocation to the nucleus. These studies have demonstrated that light can be used to generate specific temporal profiles of ERK activation by modulating the patterns of light illumination in time. Experimental re-quirements ultimately determine which photodimerization system is used in a given study. In experiments requiring protein pairs to be dimerized for extended periods of time, the Phy-PIF system may be preferable, because the effects of phototoxicity that arise from sustained illumination can be limited. In contrast, experiments requiring more dynamic dimerization of protein pairs would benefit from the use of the CRY2-CIB1 system, because the system is less complex than the Phy-PIF system.

Control of neuronal differentiation by bHLH transcription factors

The studies discussed earlier used a photodimerization system to control the activation of the MEK-ERK pathway by recruiting an activating protein to
the plasma membrane. Alternatively, the activation of a particular pathway can be modulated at the level of transcription with a photoactivatable gene expression system. In their study, Imayoshi et al. synthetically reconstituted the differentiation of neuronal progenitor cells (NPCs) (19). In a developing murine nervous system, NPCs are capable of differentiating into three different cell types: neurons, oligodendrocytes, and astrocytes. The bHLH transcription factors Ascl1, Hes1, and Olig2 are required for the differentiation of NPCs to neurons, astrocytes, and oligodendrocytes, respectively. However, bHLH proteins also exert contradictory functions; for example, Hes1 promotes both the maintenance of NPCs and their differentiation into astrocytes. To clarify the role of bHLH factors in determining cell fate, Imayoshi et al. studied protein abundance at the single-cell level and found that (i) the relative abundances of Hes1, Ascl1, and Olig2 proteins oscillate in NPCs during the proliferation phase, and (ii) the cell type into which NPCs differentiated was determined through a process in which the abundance of a single bHLH factor was maintained whereas those of the other two factors were reduced. To confirm this observation by means of a synthetic approach, the authors first optimized a chimera consisting of the Gal4 protein fused to the LOV domain (Gal4-VVD). Gal4, which contains a DNA binding domain toward UASG (upstream activating sequence), is active when dimerized. Illumination with blue light causes the LOV domains to dimerize, which results in the activation of Gal4. With this system, the authors successfully generated oscillating and sustained abundances of the various bHLH factors in NPCs by modulating light exposure intervals. The synthetically generated oscillatory or light-dependent sustained abundance of a given bHLH factor showed that distinct expression patterns, demonstrated by relative abundances, are important for determining cell fate. This synthetic biology approach directly revealed the importance of certain transcription factors for maintaining NPC multipotency and establishing neural identity.

**Synthetic Biology Approaches in Reconstituting Phagocytosis**

Synthetic biology techniques have traditionally centered on the activation or sequestration of key proteins in a signal transduction pathway. In this regard, reconstituting the process of phagocytosis does not fall under the traditional synthetic biology paradigm, because it requires the establishment of cell-cell contact and the recognition of specific ligands before engulfment can take place. One of the challenges in studying phagocytosis has thus been the lack of a technique that enables the inducible presentation of proteins to the extracellular space. A previous report showed that modulation of Hes1, Ascl1, and Olig2 proteins oscillate in NPCs during the proliferation phase, and (ii) the cell type into which NPCs differentiated was determined through a process in which the abundance of a single bHLH factor was maintained whereas those of the other two factors were reduced. To confirm this observation by means of a synthetic approach, the authors first optimized a chimera consisting of the Gal4 protein fused to the LOV domain (Gal4-VVD). Gal4, which contains a DNA binding domain toward UASG (upstream activating sequence), is active when dimerized. Illumination with blue light causes the LOV domains to dimerize, which results in the activation of Gal4. With this system, the authors successfully generated oscillating and sustained abundances of the various bHLH factors in NPCs by modulating light exposure intervals. The synthetically generated oscillatory or light-dependent sustained abundance of a given bHLH factor showed that distinct expression patterns, demonstrated by relative abundances, are important for determining cell fate. This synthetic biology approach directly revealed the importance of certain transcription factors for maintaining NPC multipotency and establishing neural identity.

**Synthetic Biology Approaches in Reconstituting Chemotaxis**

Despite the widespread interest in the process of chemotaxis in cancer, immunology, and developmental biology, the chemotaxis field has presented many experimental challenges that have only recently been suitably addressed through synthetic biology techniques. The canonical chemotaxis pathways involve the binding of an external factor to a receptor tyrosine kinase or a GPCR (Fig. 3A). This binding event eventually stimulates multiple signaling pathways that branch downstream and result in changes that affect the actin cytoskeleton and cell adhesion. The redundancy arising from this branching confers the important biological advantage of being robust and being able to compensate for many perturbations that may be introduced into the system. This robustness raises an interesting, yet perplexing, question of all of the known pathways’ interconnectedness. One of the outstanding challenges is to identify the minimal signaling components necessary to achieve chemotaxis. Synthetic reconstitution is an alternative to traditional approaches, because it offers a well-controlled environment of a given pathway that can be manipulated at will by the experimenter. Here, we present a general survey of synthetic approaches for reconstituting chemotaxis.

**RASSLs in activating chemotaxis**

Findings from studies of in vivo models of GPCRs can often be confounded by the presence of other extracellular ligands. The development of RASSLs, GPCRs that have been mutated to be specifically responsive to synthetic ligands, was motivated by the need for receptors that can be controlled by the experimenter independently of the extracellular environment. In the study of chemotaxis, a subset of GPCRs known as chemokine receptors is of particular interest and has been important in reconstituting chemotaxis. Cells expressing RASSLs can be induced to migrate toward a synthetic ligand (Fig. 3B). Yagi et al. used RASSLs to identify the previously uncharacterized involvement of G proteins of the G12/13 family in the mechanism of chemotaxis of MDA-MB-231 and SUM-159 breast cells toward stromal-derived factor-1 (SDF-1; also known as CXCL12) (25). Pertussis toxin (PTX), which inhibits the activation of Goi proteins, failed to block SDF-1–stimulated chemotaxis in these cells, which suggested the involvement of other G proteins in mediating this response. This result was confirmed in experiments with MDA-MB-213 cells expressing a Goi-coupled RASSL, whereby in the presence of PTX, concentration gradients of the chemical CNO, the ligand for the RASSL, were insufficient to induce the directional migration of the same cells stably expressing the Goi-coupled RASSL. Subsequent knockdown experiments indicated that the Go12/13 G protein family contributes to SDF-1–dependent chemotaxis in MDA-MB-213 cells. This idea was further explored by the development of a chimeric form of the Go13 subunit that was responsive to the Goi-coupled RASSL. Coexpression of these constructs endowed breast cancer cells with the ability to directionally migrate toward CNO in various assays, thus confirming a role for Go12/13 in directed migration toward SDF-1.

In contrast, Park et al. expressed a RASSL in neutrophil-like HL-60 cells and other cell types to test their ability to migrate toward a synthetic ligand in vitro and in vivo (26). The authors also discovered that the expression of the Goi-coupled RASSL, Di, was sufficient to support the chemotaxis of HL-60 cells, T cells, and human umbilical vein endothelial cells toward CNO, which suggests that the approach can be generalized (Fig. 3B). T cells expressing Di trafficked toward CNO-loaded microspheres.
CID-based microfluidics methods in reconstituting chemotaxis

Protein activity gradients are a commonly observed property of actively polarizing cells. Dimerization systems can be used to generate an intracellular protein activity gradient by generating a concentration gradient of the dimerizing chemical agent such as rapamycin. This is effectively achieved by establishing a scenario in which one dimerizing domain is anchored to the plasma membrane, whereas the complementary domain is fused to the soluble protein of interest. This system was implemented by Lin et al. in a microfluidic device with Tiam1, an activator of Rac1. A gradient of the dimerizer rapamycin led to a corresponding graded recruitment of Tiam1 to the plasma membrane between the leading and trailing edge of transfected HeLa cells (27). The resulting graded activity of Rac was confirmed with a fluorescence resonance energy transfer–based biosensor and was demonstrated to be sufficient to induce the chemotaxis of the HeLa cells. Changes in the time interval of rapamycin exposure revealed the existence of a threshold for Rac activity that needed to be overcome before the cells would polarize. This combination of microfluidics and CID is a promising platform for exploring the sufficiency of the graded activation of other proteins in inducing chemotaxis.

Light-based methods with potential applications in chemotaxis

An alternative method for generating a protein activity gradient in cells is to use photoactivatable proteins. Local illumination of light at the leading edge of a cell causes an enhancement in the signaling activity of proteins implicated in cell polarization. This particular technique was first made possible in the field of directed cell migration by the development of photoactivatable Rac by Wu et al. (28). The authors demonstrated repetitive Rac activation at a subcellular region resulting in directional guidance of the cell by light (Fig. 3D, top). In contrast to CID systems, the use of light has unique advantages in that it can be applied with spatiotemporal precision not only in cultured cells but also in model organisms such as Caenorhabditis elegans and Drosophila melanogaster. Here, we discuss findings from studies of photoactivatable Rac and the photoactivatable chemokine receptor CXCR4 (PA-CXCR4).

Wang et al. showed that Rac activation in a single cell directed the migration of border cells, a collection of 6 to 10 cells in the oocyte, during oogenesis (29). Wang et al. observed that local inhibition of Rac activity through a dominant-negative photoactivatable Rac was sufficient to abolish the extending protrusions from the illuminated cell, but also resulted in the formation of randomly oriented protrusions in the other border cells. This result illustrated that Rac-mediated protrusive forces can coordinate migration within a group of cells, and these findings have since been supported by a study that implicated E-cadherin in this process (30).

Yoo et al. found that local Rac activation in Danio rerio was sufficient to cause the direct migration of neutrophils in vivo (31) even in the presence
of endogenous guidance cues that were generated by wounding (Fig. 3D, bottom). Surprisingly, Rac activation stimulated the formation of protrusions by neutrophils that were treated with an inhibitor of PI3K (phosphatidylinositol 3-kinase), a kinase that is traditionally understood to be a key component of chemotaxis. In the context of PI3K inhibition, however, local Rac activation failed to stimulate migration, and cells had defects in the anteroposterior polarity of F-actin dynamics. The use of fluorescent reporters enabled the authors to distinguish between stable and dynamic forms of actin and its regulation by PI3K and Rac.

In an in vivo mouse melanoma model, Xu et al. used a light-activated chemokine receptor to guide the migration of T cells (32). The authors engineered PA-CXCR4, a chimera formed from the fusion of rhodopsin and the chemokine receptor, which transduces light into intracellular signaling. In response to 505-nm light, PA-CXCR4 stimulated the polarization and migration of T cells. In particular, PA-CXCR4-expressing cytotoxic T cells were recruited to tumors, where they substantially decreased tumor growth. This general strategy was also applied by Kim et al. to an optically controlled fibroblast growth factor receptor to induce phototaxis (33). These studies collectively demonstrate that photoactivatable proteins are powerful tools in model organisms to generate the steep protein activity gradients inside cells that are necessary for cell migration without the use of chemokines.

**Synthetic Biology Approaches in Studies of Actin**

Actin remodeling near the cell surface is a fundamental aspect of cellular functions because numerous motile processes in biology are driven by actin polymerization (34). Actin dynamics are tightly regulated by various mechanisms, including nucleation, stabilization, bundling, and capping. Since Oosawa and co-workers pioneered the classic in vitro actin reconstitution assay of the cytoskeleton in the 1960s (35), dozens of synthetic biology approaches have been developed to study the properties of actin. These techniques include precise manipulation and measurement of the kinetics and parameters underlying actin assembly.

Synthetic biology applications in actin studies take advantage of the fact that the correct localization of proteins is a prerequisite for their activity. Through dimerization systems, proteins can be recruited toward or sequestered away from cellular regions where their substrates or interacting partners are present to modulate protein activity. This was effectively demonstrated by Ueno et al. in a study in which the recruitment of the Sec7 domain of cytohesin-1 to the plasma membrane led to the formation of actin comets, bundles of motile actin filaments that play a role in vesicular trafficking and bacterial locomotion inside cells (36). The translocation of Sec7 to the plasma membrane leads to activation of ADP (adenosine 5′-diphosphate) ribosylation factor 6 (Arf6), which bypasses the native pathway to increase the amount of P(4,5)P2 (phosphatidylinositol 4,5-bisphosphate) through a pathway involving P(4)P (phosphatidylinositol 4-phosphate) kinase. In the same study, a different system was developed that enabled the synthetic enrichment or depletion of P(4,5)P2 (Fig. 4). This second system was based on two known mechanisms of P(4,5)P2 generation. The first involved the phosphorylation of P(4)P by a kinase, whereas the second involved the dissociation of the polybasic domain of MARCKS (myristoylated alanine-rich C-kinase substrate) from a previously bound state with P(4,5)P2.
dance of PI(4,5)P2 through enhancing PI(4)P kinase activity caused the polymerization as well as eventual cellular behaviors. Increasing the abun-
dlative contributions of the two molecules [that is, PI(4)P and PI(4,5)P2] which this enrichment takes place. This finding implies that it is the re-
PI(4,5)P2 that determines cellular behavior but also the mechanism by
ruffling. These findings suggest that it is not simply the abundance of
delta isotype of phospholipase C [PLC-
have yet to be approached from the perspective of synthetic biology.

These reconstitution experiments revealed that the mechanism by which PI(4,5)P2 is generated critically determines the types of actin po-
limerization as well as eventual cellular behaviors. Increasing the abun-
dance of PI(4,5)P2 through enhancing PI(4)P kinase activity caused the formation of actin comets, whereas increasing the effective concentration of PI(4,5)P2 by sequestering the pleckstrin homology (PH) domain of the
dscription of phospholipase C (PLC) ISO-
resulted in membrane

Conclusions

Advances in synthetic biology have led to the development of various tools beyond those traditionally available to experimenters. In this Review, we described studies that have taken advantage of the unprecedented spatio-temporal precision provided by both optogenetics and CID systems in re-
vealing biological insights. In addition to the techniques discussed here, other studies have used different aspects of synthetic biology. For example, using a variation of the dimerization system described previously, Norris et al. used scaffolds that linked two proteins to reveal that motor proteins behave independently, not cooperatively (37). Despite the promises of these tech-
niques and the insights that they have revealed, many biological problems have yet to be approached from the perspective of synthetic biology.

Reconstitution of cellular processes and the manipulation of proteins in a pathway are only the first steps in the road ahead for synthetic biology. These studies have demonstrated the ability to induce specific cellular behaviors, such as phagocytosis and chemotaxis, in cells that inherently lack these abilities. A better understanding of these processes is a stepping stone to the development of engineered cells with broad applications, including the development of a new generation of therapeutics, a future that Fischbach et al. described in a review on cell therapy (38). This new generation of therapeutics includes the ability to engineer chemotactic and phagocytic capabilities into a patient’s cells, which in turn could be used to track and eliminate undesirable particles from the body. The ability to bypass native pathways and harness cell functions with minimal interven-
tions is a key development in ensuring that these mechanisms are eventually proven to be the bridge that enables the promise of cells as thera-
peutic vehicles.

REFERENCES AND NOTES


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