Molecular tools for acute spatiotemporal manipulation of signal transduction

Brian Ross[1,2], Sohum Mehta[1] and Jin Zhang[1,3]

The biochemical activities involved in signal transduction in cells are under tight spatiotemporal regulation. To study the effects of the spatial patterning and temporal dynamics of biochemical activities on downstream signaling, researchers require methods to manipulate signaling pathways acutely and rapidly. In this review, we summarize recent developments in the design of three broad classes of molecular tools for perturbing signal transduction, classified by their type of input signal: chemically induced, optically induced, and magnetically induced.

Addresses
[1] Department of Pharmacology, University of California, San Diego, La Jolla, CA, USA
[2] Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA
[3] Department of Pharmacology and Molecular Sciences, Johns Hopkins University, Baltimore, MD, USA

Corresponding author: Zhang, Jin (jzhang32@ucsd.edu)

Introduction

To adapt to constantly changing environmental conditions, a cell must sense signals from its environment and elicit appropriate functional responses. The information it collects from its environment is transmitted and processed *via* a complex, interconnected, and highly coordinated network of signaling cascades. The biochemical activities involved in these pathways, including enzymatic activities, recruitment of second messengers, and protein–protein interactions, are under tight regulation both spatially and temporally within the cell. To study this regulation, researchers have developed a wide array of genetically targetable tools to activate or inhibit a particular biochemical activity at specific subcellular locations rapidly and at will. These tools connect an input signal (*i.e.* inducer) controllable by the researcher, such as the addition of a drug, exposure to light, or application of a magnetic field, with the modulation of a particular biochemical activity. The researcher can then use these tools to perturb the temporal dynamics and spatial patterning of particular signaling activities within a pathway and study downstream effects. Temporal control is achieved by manipulating the level of the input signal in time. Spatial control can be achieved by targeting these genetically targetable tools to specific subcellular locations or by applying the input signal only to certain regions of the cell.

The development of these tools is guided by certain design considerations, including speed, specificity, sensitivity, tunability, spatial control, reversibility, and modularity, whose relative importance depends on the particular experiment being performed. The perturbation should be induced rapidly upon addition of the inducer and should remain inactive in the absence of that signal. Likewise, the tool should have minimal off-target downstream effects. The tools should be sensitive enough so that there is no need for use of an excessive amount of inducer. In some experimental designs, the amount of modulation of the biochemical activity should scale with the level of input signal, while in other applications, an all-or-nothing response may be more appropriate. Moreover, for studying complex signaling dynamics, it is useful that the tool be reversible — that the associated biochemical activity turns off when the input signal is removed. Lastly, the design of these tools should be modular so that the same approach can be generalized for a wide range of biochemical activities.
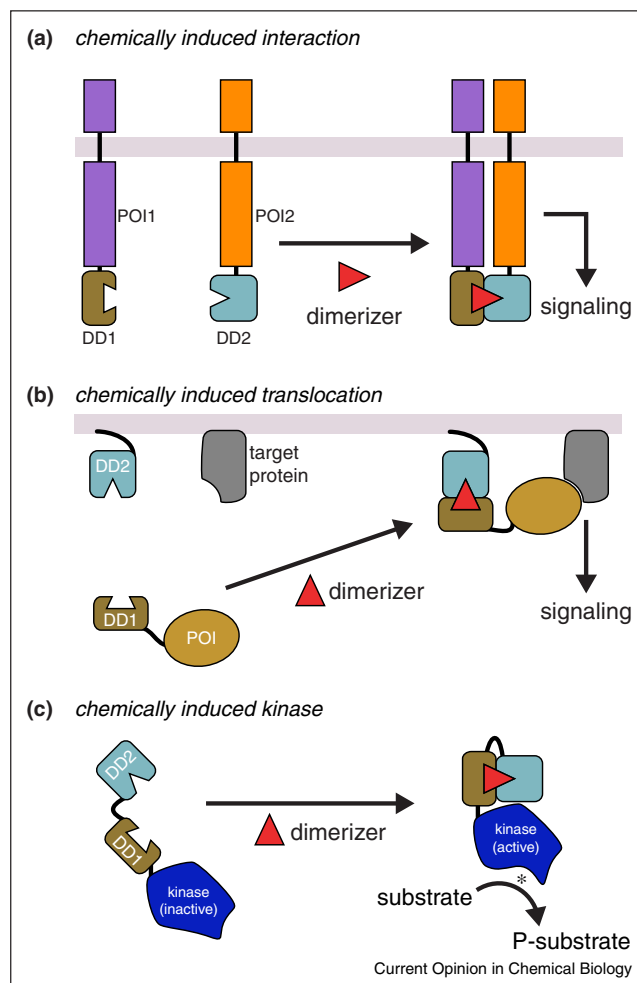
Chemically inducible tools

Small molecule drugs, such as receptor agonists and pharmacological enzyme inhibitors, have been used extensively to manipulate signal transduction. However, specific activators and inhibitors of certain activities are unavailable, and spatial control of pathway activation is difficult with this approach. On the other hand, genetically targetable molecular components that bind synthetic ligands can be used to design modular tools for manipulating cell signaling in ways impossible with standard pharmacological agents.

Chemically inducible dimerization (CID) systems are a widely used class of chemically induced, genetically targetable tools for acutely perturbing signal transduction in live cells. CID systems are composed of two protein components that only bind in the presence of an exogenous chemical agent. The most widely used CID systems are based on FK506 Binding Protein (FKBP), which

binds tightly to the immunosuppressant drugs FK506 and rapamycin. The first CID system was a homodimerization system developed in 1993 by creating a synthetic derivative of FK506 with two FKBP-binding moieties, called FK1012 [1], which could be used to induce the homodimerization of FKBP-tagged proteins. A heterodimerization system was developed a few years later by exploiting the ligand-mediated interaction between FKBP and mTOR [2]. An 89-amino acid fragment from mTOR called FRB was found to be sufficient for binding the FKBP-rapamycin complex but does not bind FKBP in the absence of rapamycin. Rapamycin addition induces the heterodimerization of FRB- and FKBP-fused proteins rapidly, on the order of seconds, yet irreversibly due to the high affinity of the FKBP-FRB interaction. Being able to interact with endogenous mTOR, moreover, the rapamycin input is not truly orthogonal to the cell's native signaling pathways. To address this concern, several rapamycin analogues such as iRap, AP21967, and AP23102 [3,4] have been developed to bind only to engineered FRB but not to endogenous mTOR. Furthermore, the FKBP-FRB design is highly modular, as it can be adapted to control a wide array of signaling activities.

These systems can be used to perturb cell signaling by bringing two proteins into proximity (Figure 1a), recruiting a protein to a particular subcellular location (Figure 1b), or mediating allosteric regulation of an enzyme (Figure 1c). Modulating molecular proximity using CID can be used to mediate the interaction between the two proteins or protein fragments, which can promote downstream signaling (Figure 1a), for example by facilitating the dimerization of receptor tyrosine kinases [5]. It can also be used to mediate the complementation and reconstitution of split protein fragments, as was demonstrated by fusing FKBP and FRB to fragments of the split tobacco etch virus (TEV) protease to modulate caspase activation [6]. In addition to mediating protein interactions, CID can control signal transduction by controlling the translocation of a protein of interest toward or away from its site of action (Figure 1b). One CID component is targeted to a particular subcellular location, while the other is fused to a protein of interest and is diffusible in the cytosol or is hidden at a relatively inert location [7]. Upon addition of the dimerizer, the diffusible component is rapidly recruited to the target site [8]. This approach has been extensively used to control the activity of enzymes involved in phosphoinositide metabolism [9,10] as well as small GTPases [3,11,12]. Another approach, called RapR, is to use the FKBP-FRB heterodimerization system to create allosterically regulated variants of kinases (Figure 1c) [13]. A modified FKBP, called iFKBP, is inserted into the kinase to make it catalytically inactive. When FRB heterodimerizes with the iFKBP domain upon the addition of rapamycin, a change in the conformational rigidity of iFKBP restores the catalytic activity of the kinase. Because the iFKBP is

Figure 1

(a) chemically induced interaction

(b) chemically induced translocation

(c) chemically induced kinase

Current Opinion in Chemical Biology

Strategies for manipulating signal transduction using chemically inducible dimerization (CID) systems. **(a)** CID can be used to activate signaling by mediating the interaction between two proteins of interest, for example by inducing the dimerization of receptor tyrosine kinases. **(b)** CID can also modulate signal transduction by inducing the translocation of a protein of interest to a particular subcellular location. One dimerization domain is fused to a protein of interest, while the other is fused to targeting sequence that anchors it to the site of action of the protein of interest. Addition of the dimerizer results in recruitment of the protein of interest to its site of action, resulting in activation of downstream signal transduction. **(c)** Chemically inducible kinases, such as in the UniRapR design [14], can be created by using CID to regulate their catalytic activity allosterically. DD: dimerization domain, POI: protein of interest.

inserted in a highly conserved region of the kinase, the design is modular and can be applied to a wide array of kinases. A unimolecular version of RapR, known as uniRapR, was also developed by fusing elements from FRB directly to a modified version of iFKBP and inserting the resulting fusion into the kinase (Figure 1c) [14].

To control multiple activities simultaneously in the same cell using CID, our toolbox has been expanded to include