GATA1 (Gamsjaeger et al., 2011) and individual BD1 and BD2 of BRD4 association with the K310-acetylated RelA subunit of inflammatory transcription factor NF-κB (Zou et al., 2014).

The availability of BD1-selective chemical inhibitor (BrD1*i*) Olinone, distinct from the previously characterized broad BET inhibitors (BET*i*) MS417, JQ1, and I-BET that target both BD1 and BD2 and a BD2-selective inhibitor (BrD2*i*) MS765/RVX-208 (Figure 1B), allows the use of these pharmacological agents to address the target selectivity and functional significance of BD1 and BD2. Using mouse oligodendrocyte progenitor cells (OPCs) that can differentiate into myelin-producing oligodendrocytes, Gacias et al. (2014) found that treating OPCs with Olinone promotes oligodendrocyte differentiation, as reflected by enhanced myelin-specific *Mag*, *Mog*, and *Mbp* gene expression, accompanied by reduced progenitor *Hes1*, *Hes5*, and *Gmnn* marker expression; but, surprisingly, treating oligodendrocytes with broad BET inhibitors such as MS417 that target both BD1 and BD2 actually hinders differentiation (Figure 1C). This observation was further confirmed via the use of additional bromodomain-selective BET inhibitors, including MS611 BrD1*i* and RVX-208/MS765 BrD2*i* (Figure 1B). Enhanced myelin formation by BrD1*i*, but not BrD2*i* and BET*i*, highlights the need to develop more selective bromodomain inhibitors to enrich our molecular understanding of BD1- and BD2-specific function in gene targeting and disease treatment. It would be interesting to determine whether oligodendrocyte lineage gene expression is indeed regulated by BRD2 that is predominantly expressed in these cells and whether BRD4 and BRD3 could independently or collaboratively regulate progenitor and differentiated oligodendrocyte gene expression with BRD2. The existence of other evolutionarily conserved regions (e.g., ET, NPS, and BID) that regulate chromatin binding and partner association of the BET family proteins also predicts new drug development targeting other functionally important regions of the BET proteins. The recent finding that many protein kinase inhibitors targeting PLK1 (e.g., BI-2536), JAK2 (e.g., TG-101209 and TG-101348), PI3K (e.g., LY294002 and LY303511), and other kinases also exhibit strong binding affinity to both BD1 and BD2 or specifically to BD1 (Ciceri et al., 2014; Dittmann et al., 2014; Ember et al., 2014; see Figure 1B) raises not only interest in developing dual kinase/BET inhibitors for cancer therapeutics but also concerns of off-target effects that require further mechanistic studies of drug action in various biological systems.

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Lighting up FGFR Signaling

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In this issue of *Chemistry & Biology*, Kim and colleagues describe their work on optogenetic control of fibroblast growth factor receptor (FGFR) signaling. By engineering a chimeric receptor, the authors demonstrate that FGFR intracellular signaling can be controlled in space and time by blue light.

Intracellular signal transduction transmits external signals into the cell interior to ensure proper cellular decision making. Fibroblast growth factor receptor (FGFR) signaling belongs to a classical family of signal transduction pathways that regulates a wide spectrum of biological events such as development, wound healing, and angiogenesis. Dysregulation of FGFR signaling has been associated with developmental disorders and cancers.

Puzzlingly, many of the key cellular signaling modules initiated by membrane-bound receptor tyrosine kinases, like FGFR, activate overlapping sets of downstream pathways, but with distinct outcomes. Consequently, a central

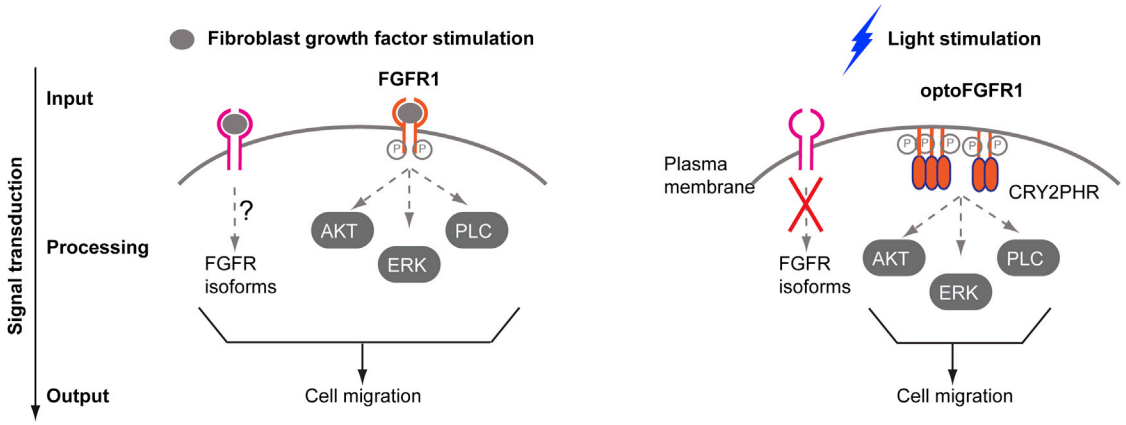

Figure 1. Comparison between FGFR Signaling Activated by FGF and Light Stimulation
FGF may activate multiple isoforms of FGFR through receptor dimerization, while light-controlled optoFGFR1 signaling only activates FGFR1 through CRY2PHR oligomerization. Light-controlled signaling activation enables superior spatial and temporal dissection of FGFR1 signaling networks.

question in growth-factor-mediated signal transduction is how a similar set of downstream signaling cascades can elicit diverse yet specific cellular outcomes. In this issue of *Chemistry & Biology*, Kim et al. (2014) introduce a new tool for addressing this question, showing that light-controlled activation of signal transduction enables superior spatial and temporal regulation, thus enabling dissection of the roles of specific receptor types.

FGFR signaling initiates with ligand binding. Similar to the activation of other membrane receptor tyrosine kinases, ligand binding to the extracellular domain leads to the activation of dimeric FGFRs and their intracellular kinase domains, and then they trans-phosphorylate each other. This event leads to the activation of multiple downstream signaling cascades, including the mitogen-activated protein kinase (MAPK/ERK), phosphoinositide 3-kinase (PI3K), and phospholipase C (PLC). Intriguingly, these downstream pathways can also be activated by many other growth factors, including epidermal growth factors and nerve growth factors, which lead to completely distinct cellular functions such as proliferation, growth, differentiation, migration, survival, and apoptosis.

Previous research has suggested that differences in spatiotemporal regulation of intracellular signaling pathways can confer specificity to cellular responses (Marshall, 1995). Conventional approaches based on gain- or loss-of-function genetic manipulations or small-molecule inhibitors, however, lack the necessary resolution to modulate specific changes in space and time to test this hypothesis. A better understanding of signaling mechanisms therefore calls for new tools that can precisely control intracellular signaling in both space and time. Recently, several optogenetic tools have emerged, and they could potentially transform conventional ways of studying intracellular signaling (Kennedy et al., 2010; Levskaya et al., 2009; Wu et al., 2009; Yazawa et al., 2009).

Optogenetics relies on light-induced protein interactions to control the activation state of engineered signaling components in cells. Kim and colleagues use blue light-induced cryptochrome oligomerization to trigger the activation of an engineered FGFR (optoFGFR1) and subsequent signaling pathways (Kim et al., 2014). Light-controlled activation of this pathway opens the door for experiments that rely on spatial and temporal regulation aimed at dissecting the roles of specific receptor types (Figure 1).

To make an FGFR that can be activated by blue light (optoFGFR1), the authors engineered a chimeric receptor by inserting the cytoplasmic regions of FGFR1 between the N-terminal photolyase homology domain of cryptochrome (CRY2PHR) and a membrane-targeting myristoylation peptide. CRY2PHR has been shown to undergo blue light-mediated oligomerization (Bugaj et al., 2013; Wend et al., 2013). Therefore, when optoFGFR1 is exposed to blue light, CRY2PHR oligomerizes and brings the catalytic domains of FGFR into proximity, mimicking ligand-induced FGFR dimerization and subsequent activation. Using live cell imaging, a FRET based sensor, and other standard approaches for analyzing signaling pathways, the authors demonstrated that blue light can indeed induce phosphorylation of optoFGFR1 and activate downstream ERK, AKT, and PLCγ signaling cascades.

By controlling the temporal patterns of excitation light, the authors characterized ERK signaling in response to modulated light frequency and duration. They found that high-frequency light stimulation (10 min interval) leads to sustained ERK activation, whereas low-frequency light stimulation (30 min and 60 min) gives pulsatile patterns of ERK activation. This result is consistent with a previous study showing that the Ras/ERK signaling module functions as a low-pass filter in transmitting extracellular growth factor signals (Toettcher et al., 2013).

For spatial control, the authors first localized the illumination area to a small circle (5 μm radius) at the cell periphery and demonstrated that subcellular activation of FGFR signaling is sufficient to establish cell polarity and direct cell migration. Then, the authors established a "photo-taxis" model by expanding the illumination to a circular field (160 μm radius). Cells expressing optoFGFR1 were guided into the light-illuminated area, similar to directed cell migration in chemotaxis. Finally, the authors showed that PI3K and PLCγ signaling pathways are actively involved in the regulation of cell directionality, whereas inhibiting ERK activity has a negligible effect. These

results support previous findings that PI3K is an upstream regulator of the Rho GTPases. Taken together, these results provide an initial validation of optoFGFR1 for controlling FGFR signaling in space and time.

Looking forward, the current study opens up exciting opportunities for studying receptor-mediated intracellular signaling pathways. Because receptor activation is a general mechanism that cells use to regulate intracellular signal pathways, optogenetic chimeras can be conveniently generalized to control receptors of other ligands and subsequent signaling pathways. However, it should be noted that blue light induces receptor oligomerization, in contrast to ligand-bound receptor dimerization. Oligomerization and dimerization may orient the kinase domain differently and may lead to a different phosphorylation ratio of multiple amino acid residues in the kinase domain. Therefore, the system should be used with caution for quantifying relative outputs of downstream signaling pathways.

Several previous reports have used the PhyB-PIF or CRY2-CIB1 binding pairs to demonstrate optogenetic control of individual signaling cascades downstream of growth factor stimulation including the ERK (Toettcher et al., 2013; Zhang et al., 2014) and the AKT (Idevall-Hagren et al., 2012) pathways. This work adds another node of regulation at the level of membrane receptor. Overall, these light-based regulation studies promise greater insights into understanding the spatial and temporal dimensions of intracellular signal transduction.

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