New tool illuminates cell signaling pathways key to disease

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In a major advance for fundamental biological research, UC San Francisco scientists have developed a tool capable of illuminating previously inscrutable cellular signaling networks that play a wide variety of roles in human biology and disease. In particular, the technique opens up exciting new avenues for understanding and treating psychiatric disease, the researchers say.

The new technology, described in a paper published April 6, 2016 in *Cell*, makes it vastly easier for scientists to study the complex workings of a large family of sensor proteins called G-protein-coupled receptors (GPCRs), which sit in cell membranes and enable cells to respond to chemical signals from other parts of the body or the outside world. In a first proof-of-principle study, the UCSF team used their new approach to identify new biochemical players involved in the development of tolerance to opioid painkillers—which target a particular type of GPCR—findings they anticipate will enable researchers to develop safer and more effective pain control.

"This technology will let us understand how these critical signaling molecules work in a way we've never been able to before," said Nevan Krogan, PhD, a professor of cellular and molecular pharmacology and director of the Quantitative Biosciences Institute (QBI) at UCSF and a senior investigator at the Gladstone Institutes, who was one of the new paper's senior authors.

Roughly 800 different types of GPCR play crucial roles throughout the

body, including regulating heart rate, blood pressure and digestion; mediating the senses of sight, smell, and taste; and enabling many forms of chemical communication between cells in the brain. Approximately 40 percent of medicines target one type of GPCR or another, including schizophrenia drugs that target dopamine receptors, painkillers that target opioid receptors, and allergy and heartburn drugs that target different types of histamine receptors, just to name a few.

These many types of GPCR have one feature in common that makes them particularly difficult to study: when they are activated (whether by a beam of light or a blood-borne hormone), they set off a rapid cascade of biochemical reactions, in which the GPCRs themselves physically move from one location to another within the cell and trigger signals that are passed among dozens or hundreds of different protein messengers. Together, these changes end up altering a cell's behavior—for example changing the excitability of neurons or reprogramming their genetic activity.

Technique Lets Scientist Sleuth Out Secretive Biochemical Networks

The last major breakthrough in understanding GPCR biology was the resolution of their chemical structure, research which garnered the 2012 Nobel Prize in Chemistry. But taking the next step towards understanding GPCR biology has been slow: without better tools for charting the chemical cascades triggered by GPCRs, it has been extremely challenging for researchers to get a clear picture of how these signals work, how they go awry in disease, or how to better control them with drugs. But Krogan and von Zastrow believe their new technique will change all that:

"The methodology that our collaborative team developed allows one to

precisely define the local protein environment of receptors as they dynamically change partners and move within the cell," said Mark von Zastrow, MD, PhD, a professor of psychiatry and cellular and molecular pharmacology at UCSF and the paper's other senior author. "We ourselves were surprised by the high degree of spatial and temporal resolution that this methodology can achieve."

Postdoctoral researchers Braden T. Lobingier, PhD, and Ruth Hüttenhain, PhD, who were co-first authors on the new study, led the development of the new tool, which lets researchers study GPCR signaling cascades by operating like police detectives mapping the criminal network of a secretive crime boss: Starting with a list of proteins that are known collaborators of a particular GPCR, researchers trigger GPCR activity and use a biochemical tracking device to identify these proteins' associates in other parts of the cell.

To build this network of associates, the researchers turned their receptor of interest into an "informant" by outfitting it with a tracking device in the form of an enzyme called APEX, which can be triggered to spray any nearby proteins with a chemical tag. Researchers can then use this tag to track down and identify suspected participants in the GPCR cascade using a technique called mass spectrometry. By triggering APEX tagging at different times after activating the GPCR, the researchers were able to begin building a detailed and unbiased map of the protein network underlying a cell's response to activation of a particular GPCR.

Study Reveals Potential Mechanisms of Opioid Painkiller Tolerance

In a proof-of-principle experiment, Krogan and von Zastrow's team used their technique to answer a long-standing mystery about the biological mechanisms of opioid tolerance—the phenomenon by which, over time,

patients tend to need higher and higher doses of opioid painkillers such as morphine to achieve the same level of pain management.

This is an important puzzle to solve, because increased opioid use in response to tolerance puts patients at risk of serious adverse side effects and also promotes addiction. Researchers know that tolerance occurs when cells respond to long-term opioid use by destroying or "down-regulating" the GPCR opioid receptors that these drugs target, but what triggers cells to do this is unknown.

Using their APEX-based tool, the UCSF researchers found that two cellular proteins not previously known to interact with opioid receptors in fact partner closely with delta-opioid receptors (a subtype of opioid receptor) at precisely the time and place at which the cell targets these receptors for destruction. They then confirmed, using genetic manipulations, that both proteins are essential for the down-regulation process.

Understanding the protein partners involved in opioid tolerance could enable researchers to develop improved pain control strategies, or adapt present strategies to be safer and more effective, the researchers say.

Krogan and von Zastrow emphasize that not all suspects revealed by their technique will prove to be important in a given GPCR cascade. But the ability to quickly and easily identify likely participants in a given cascade should dramatically quicken the pace toward understanding these complex signaling processes, and to develop more targeted treatments for diseases in which they go awry.

Researchers Set Their Sights on Common Mechanisms of Psychiatric Disease

Krogan and von Zastrow are particularly interested in the many classes of GPCR that mediate chemical signaling in the brain. The new approach is the centerpiece of a new large-scale collaborative project Krogan and colleagues are launching within QBI, called the Psychiatric Cell Mapping Initiative, the goal of which is to understand how abnormal biochemical network activity in different cell types in the brain might contribute to many different psychiatric disorders.

Most of the brain's chemical signals - neurotransmitters such as dopamine, serotonin, glutamate, and GABA - bind to their own class of GPCR to influence brain activity. These neurotransmitter receptors are deeply involved in many psychiatric diseases, including major depression, schizophrenia, and addiction, and are the targets of many psychiatric and psychoactive drugs.

"We still know so little about the biology of psychiatric disease, and even less about psychiatric drugs," Krogan said. "Our goal with this initiative is to use this and other new tools to gain a better understanding of the common cell biology behind major psychiatric diseases. This new tool will let us study how GPCRs work differently in psychiatric diseases, which could help us understand why these disorders arise, and will also let us test how psychiatric drugs actually alter the workings of their target cells in a way no one has ever been able to before."

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