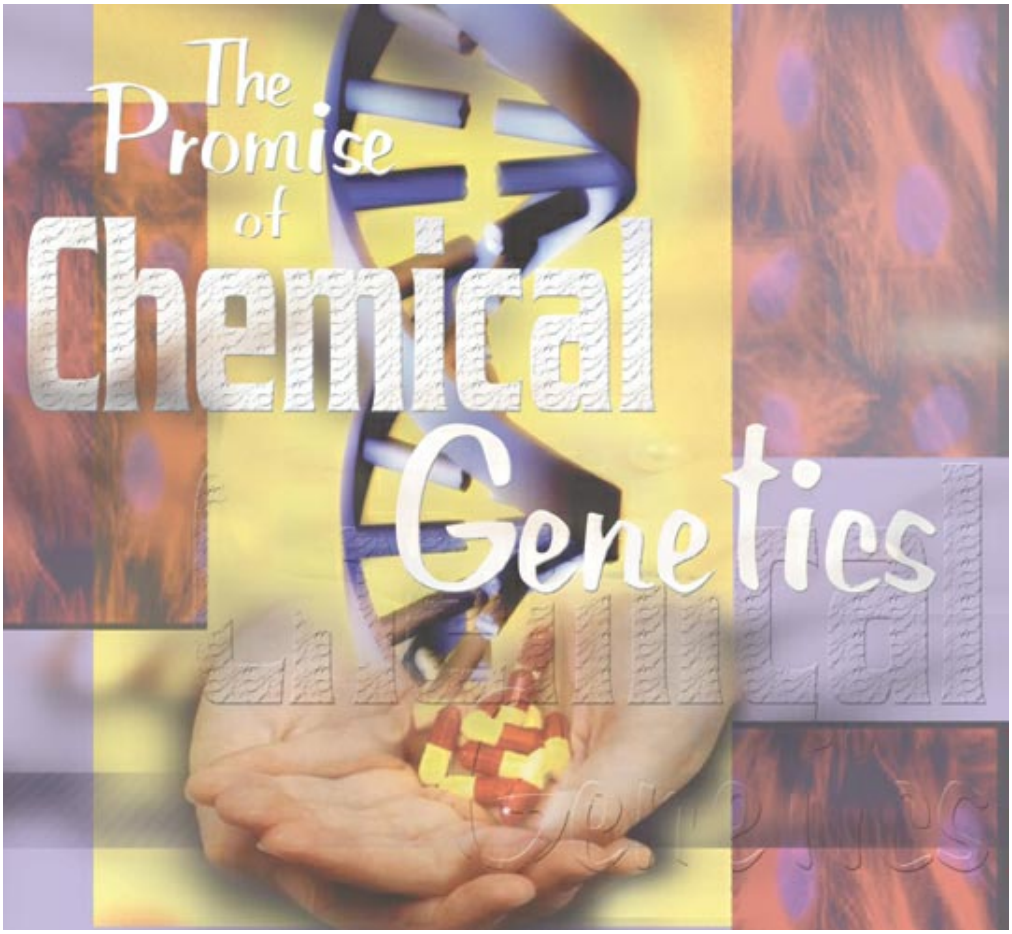

By Randy Wedin

Chemical genetics, or "chemical biology", is a rapidly emerging field in which researchers pursue small molecules that act as genetic switches, activating or inactivating gene products. Its promises include new drugs and new careers for chemists.

"To use a picture, I will say that enzyme and glucoside must join one another as lock and key to be able to exert a chemical effect."

—Emil Fischer, 1894

If the Royal Swedish Academy of Sciences ever presents a Nobel Prize in Metaphors, Emil Fischer will no doubt receive the first award. Fischer, the renowned German chemist who received the 1902 Nobel Prize in Chemistry for sugar and purine syntheses, coined one of science's most helpful and picturesque metaphors. Fischer's lock-and-key image illuminated how enzymes work at the molecular level and inspired many advances in chemistry and biochemistry throughout the 20th century.

As we move into the 21st century, chemists and biologists are poised to unlock even more secrets of biology and medicine using finely tuned molecular keys and the powerful principles of an emerging discipline known as chemical genetics.

When Fischer proposed his lock-and-key metaphor, he was thinking about one lock (a specific enzyme) and one key (a specific small molecule). With today's technologies, however, scientists have the tools to work simultaneously with a large number of "locks" or proteins. The human proteome (the complete set of proteins produced by genes) numbers in the hundreds of thousands. The human genome (the complete set of genes in a person) consists of perhaps 30,000 genes. Remember, however, that one gene can produce several proteins, which are in turn further modified by cellular machinery. The complete set of small

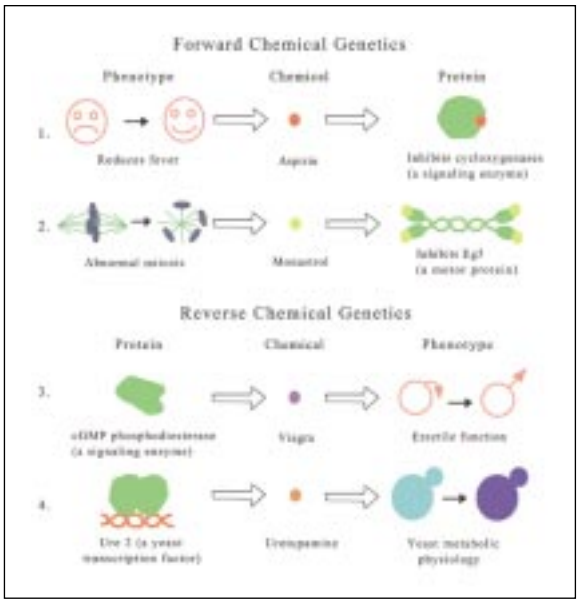

Figure 1. *This diagram shows two examples of discovery by forward chemical genetics, and two by reverse. In each case, the first is a medically important example from the literature, and the second a recent discovery at Harvard's Institute of Chemistry and Cell Biology (ICCB). Large arrows denote the discovery pathway, small arrows the effect of the chemical. The forward route starts with a biological effect (the phenotype) through a chemical that causes the effect to the target protein of the chemical. The reverse route starts with a protein of known importance and proceeds to a chemical by screening for binding to, or inhibition of, that protein. Then the phenotypic effect of the chemical is tested.*

(1) Aspirin, synthesized around 1850, was the first synthetic chemical to be used as a drug in humans, though it was used in the form of bark extracts much earlier. Aspirin was used to discover its target protein, the signaling enzyme cyclooxygenase, in the 1970s. Since then, many new drugs that target cyclooxygenase have been made.

(2) Monastrol was discovered at ICCB in 1999, using disruption of cell division as a phenotypic screen. It was found to target the motor protein Eg5. Monastrol has become a key tool in mitosis research, and it may become the lead for a new class of anti-cancer drug.

(3) Viagra (sildenafil) was discovered as an inhibitor of cGMP phosphodiesterase, a signaling enzyme known to be important in heart disease. Its phenotypic effect on erectile function was discovered by accident during a clinical trial. This is an example of an unexpected biological discovery from reverse chemical genetics.

(4) Uretupamine was discovered at ICCB in 2000 as an inhibitor of the yeast transcription factor Ure2p. This chemical has a specific effect on yeast metabolic physiology by targeting one aspect of the function of Ure2p, and it will be used as a tool to dissect the transcriptional regulation of metabolic physiology. This aspect of yeast physiology is related to human diabetes.

molecules—the "keys" to fit receptors on those proteins—may number in the millions or billions.

With the principles, strategies, and techniques of chemical genetics, scientists are beginning to sift systematically through all these locks and keys to find matched pairs. And, once they've found a matched pair, they are using that molecular key to open the lock, swing open the door, and explore a new room in the human body.

What biochemical discoveries await scientists inside these newly unlocked rooms? Some rooms will be dead ends. Others almost certainly will be passageways to whole new wings of the building—entire new frontiers for biological exploration. And some rooms might just contain the knowledge that underpins blockbuster drugs. With potential rewards like these, it's easy to understand why scientists in both academe and industry are eagerly embracing the tools of chemical genetics. Students interested in an exciting career, and established chemists considering a career change, should check the sidebar (page 21) for hints and advice from leaders in the field.

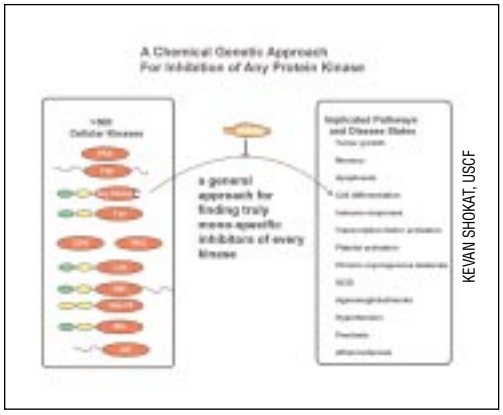

Figure 2. *Chemical genetics studies of proteins in the kinase family promise to improve understanding of biochemical pathways involved in a variety of human diseases.*

WHAT IS CHEMICAL GENETICS?

The term "chemical genetics" was first used in the inaugural issue of *Chemistry and Biology* in 1994–exactly 100 years after Fischer proposed the lock-and-key metaphor. The launching of this interdisciplinary journal, with Stuart L. Schreiber (ACS '77) of Harvard University and K. C. Nicolaou (ACS '73) of the Scripps Research Institute as founding editors, serves as a convenient milestone for the birth of chemical genetics. Other terms sometimes used interchangeably with chemical genetics include chemical genomics, chemogenomics, and chemical biology.

Chemical genetics, as most commonly defined, involves the use of small molecules to perturb, understand, and control the cellular and physiological function of proteins. Just as geneticists use mutations to perturb cellular function, researchers can use small molecules to activate or inactivate gene products. Small molecules are used, in effect, as switches.

Of course, scientists have been using small molecules to study proteins and biology for many years. In fact, the entire pharmaceutical industry is built on the interaction of small molecules and a group of about 450 target proteins. So what's unique about chemical genetics?

Schreiber provided the answer.

"The previous century of using small molecules to explore biological functions illustrated all the principles of chemical genetics, but they were used on a more-or-less ad hoc basis," he explained. "It was not systematic." Proponents of chemical genetics believe that it can become as systematic and general in the 21st century as genetics became in the 20th century. Eventually, they hope, scientists will identify a small-molecule partner for every gene product.

Why did it take scientists until the late 1990s to appreciate the possibility of a systematic approach called chemical genetics? "The answer to that is really simple," Schreiber said, "and no one can take a lot of credit for it. It's just simply that science—and engineering in particular—has evolved to the point where a whole lot of new techniques exist that can be adapted in a chemical genetics world." Included among those new technologies are robotics and high-throughput screening; bioinformatics and data-mining tools; combinatorial chemistry, including strategies like split-and-pool synthesis and diversity-oriented synthesis; microarrays for DNA, small molecules, and proteins; and increasingly sensitive and elegant biological assays.

To highlight the parallelism between the genetics approach and the chemical genetics approach to biological problems, scientists trained originally as chemists often use the language of geneticists to describe their experiments. They speak about epistasis analysis, modifier screens, gene knockouts, and forward genetics. "The name 'chemical genetics' pays homage to genetics," said Schreiber. "It's useful because it allows us to go back to the long history of genetics, to think about the well-established principles of genetics, as well as some of the more subtle principles, and to purposefully apply them with small molecules."

FORWARD CHEMICAL GENETICS

In a classic genetic screen, also called "forward genetics", mutagens or point mutations cause random mutations throughout the genome of a model organism. Mutants that show a change in a specific characteristic (a phenotype) are then used to discover the identity of genes responsible for that phenotype. In the chemical genetic counterpart to this method of discovery, a wide variety of small molecules are screened, and the ones that cause specific phenotypes in cells and organisms are then used to determine the protein target of the chemicals.

To use the lock-and-key metaphor, forward chemical genetics involves taking a few keys into a big house and randomly trying as many locks as possible. Once you discover something interesting behind one of the unlocked doors—such as a novel phenotype—you can go back to identify the lock that was involved. In doing so, maybe, just maybe, you've identified a new target for drug discovery. And to help you get started on studying this new lock, you can just use the key that unlocked the door, which can serve as a lead compound for new drug discovery.

REVERSE CHEMICAL GENETICS

Geneticists have recently developed a new strategy, often called "reverse genetics", involving gene knockouts. Mutations are used on a specific, previously identified gene to create and study a biological system in which that gene is no longer expressed.

In a parallel manner, the strategy of reverse chemical genetics involves finding small molecules that will bind to and/or disrupt the function of pure proteins in vitro. The small molecules are then used to study the effects of deleting the function of that specific protein in a cell or organism.

To use the lock-and-key metaphor, reverse chemical genetics involves starting with a specific lock and door you've already identified (i.e., you already have your target protein). Then you try a very wide range of keys in order to find one that fits. Once you've found one that fits, you open the door and see what you can discover.

Figure 1 shows examples of discoveries made using the principles of forward and reverse chemical genetics.

USING CHEMICAL GENETICS TO INVESTIGATE KINASES

The tools of chemical genetics can provide some specific advantages over the standard techniques of genetics, as illustrated by recent studies of protein kinases. Kinases are enzymes with a key role in transmitting signals between cells and inside cells. They are involved in a variety of important cellular functions. Kinases work by phosphorylating other proteins, which then become activated and able to perform specific functions. Certain kinases control the activation of proteins that cause diseases and, thus, are prime drug targets for drug development. Kinases are involved in cardiovascular disease, cancer, autoimmune disorders, inflammation, metabolic disorders and neurological diseases.

Although kinases play a significant role in cellular signaling pathways, no kinase drug has yet reached the marketplace. This large family of homologous enzymes has been difficult to study by either chemical or genetic techniques. Because the ATP-binding site is highly conserved throughout the kinase family, it has been difficult to find small molecules of suitable specificity for chemical studies. And knockout genetic studies fail to account for "compensation", in which one kinase enzyme can compensate for the absence of another kinase over the development process of an organism.

Kevan Shokat (ACS '96), now at the University of California–San Francisco, and his colleagues elegantly combined the best features of genetics and chemistry to create a general approach that can be used to study any kinase. Using site-directed mutagenesis, they tinkered with one of the kinase genes, removing a hydrophobic residue and creating a pocket in its ATP-binding site. Although this didn't change the enzyme's functionality, it did change its ability to bind different ligands. Then they found a small molecule that would bind in this new site and inhibit the mutant protein, without binding and affecting any other kinase proteins.

Using the lock-and-key analogy, Shokat explained, "We had the idea of engineering the lock on one of the kinases. Then we created a new key with a notch on it that won't fit into any normal lock in nature but will fit into one of our engineered locks. If we're good locksmiths, we can make this same change in any lock, and then use this same key." By mutating different kinase genes, one at a time, they can now systematically study the function of each individual kinase protein.

The kinase example illustrates the power and precision of chemical genetics. These researchers have been able to construct a system that is highly specific, tunable, rapid, reversible, and conditional. And they've discovered that the physiological results of chemical disruption are not always the same as those found with genetic disruption. Shokat continued, "What we found, very surprisingly, is that the chemical disruption of kinases gives a different biological result (e.g., a different cell cycle disruption point) than the genetic disruption. Over the years, geneticists have constructed genetic maps that tell us how cells function. In our experiments,

Kevan Shokat

we've found, in kinase after kinase, that we get subtly different answers. So we think there's a pharmacological map of the cell, and it's different from the genetic map of the cell."

Peter Brown

CHEMICAL GENETICS IN ACADEME

Harvard University's Institute of Chemistry and Cell Biology (ICCB) is one of the leading academic centers for chemical genetics research. Founded in 1997 by Schreiber and Timothy Mitchison, who serve as co-directors, the ICCB (**http://iccb.med.Harvard.edu**) embodies the interdisciplinary nature of this field. Schreiber was trained as an organic chemist, Mitchison as a cell biologist. In addition to advancing the science of chemical genetics through a diverse set of research programs, one of ICCB's major goals is to develop research techniques that can be readily transferred to other institutions as students and postdocs leave to start research programs of their own. "Our mantra is accessibility, portability, and training," said Schreiber. "We want to make our discoveries widely available to the public."

Two other hotbeds of academic activity are the Scripps Research Institute and the Genomics Institute of the Novartis Research Foundation (GNF), both located in La Jolla, CA. Researchers in the biological chemistry group at GNF (**www.gnf.org**) are developing tools and investigating the function of large protein families such as kinases, proteases, GTP-dependent proteins, and methyltransferases. GNF director Peter Schultz (ACS '85) and his colleagues described one example of such a tool. It is a small-molecule switch for studying protein–protein interactions.

CHEMICAL GENETICS IN INDUSTRY

The techniques of chemical genetics have quickly spread from academe to industry. Among the biotech companies that are especially active at the interface of chemistry and biology are ARIAD Pharmaceuticals (**www.ariad.com**), Cellular Genomics (**www.cellulargenomics.com**), Morphochem (**www.morphochem.com**), Myriad Genetics (**www.myriad.com**), Syrrx (**www.syrrx.com**), and Vertex Pharmaceuticals (**www.vpharm.com**).

But it's not just the small biotech companies that are taking advantage of the tools of chemical genetics. Big pharmaceutical companies are expanding their research programs and setting up partnerships with small companies.

At GlaxoSmithKline (GSK), according to Peter J. Brown, senior research investigator, chemical genetics/genomics represents "a marriage of the chemical technology of combinatorial chemistry with the genomic technologies of differential gene expression and proteomics." Brown and colleagues have been using reverse chemical genetics to uncover the physiological function of several orphan nuclear receptors. Many pharmaceutical companies are looking to orphan nuclear receptors, which are ligand-activated transcription factors whose ligands are not yet identified, as a rich source of tractable drug targets. "In each case," said Brown, "we developed a potent and selective ligand for the orphan receptor as a chemical tool." For example, GSK researchers recently used this technique to identify the role of peroxisome proliferator-activated receptor delta in the regulation of reverse cholesterol transport.

THE FUTURE OF CHEMICAL GENETICS

Given the flurry of research activity in both academic and industrial laboratories, we'll be hearing a great deal more about chemical genetics in the months and years ahead. Within a few years, we'll see clinical trials of new drugs that can be traced back to today's chemical genetics research. And if the proponents of chemical genetics achieve their vision, chemical genetics will take its place alongside biochemistry, genetics, and genomics as a systematic and general approach to investigate any biological question.

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Considering a Career in Chemical Genetics?

Because of chemical genetics' interdisciplinary nature, training for a career in the field can present students and teachers with a real dilemma. Stuart L. Schreiber of Harvard University described the quandary this way: "Multidisciplinary or interdisciplinary science is where the action is. Many modern research problems require integrating chemistry, biology, physics, computer science, engineering, and mathematics. Does that create an impossible situation?"

After acknowledging the complex challenge facing students, both Schreiber and Kevan Shokat, of the University of California–San Francisco, contend that it is possible to get the right kind of training. But it requires an approach that emphasizes both depth and breadth.

"The most important part of graduate training," said Shokat, "is critical thinking, experimental design, and learning to tease apart a problem. And sometimes it's best to learn that by studying one field in great detail." Schreiber said, "My advice is to specialize first in the physical sciences, because they are so enabling. Chemistry is perfectly situated—it's rigorous enough that you understand the rigors of science and yet it's not as abstract as particle physics."

But specialization in one field is not enough any more.

Both scientists cite the importance of understanding the language, the challenges, and the capabilities of neighboring disciplines. Schreiber advised, "Attend general lectures. Take general courses. Read general review articles. Interact with your classmates in neighboring disciplines." Shokat, who followed his Ph.D. training in organic chemistry with a postdoc in immunology, explained, "Chemists have to know the biology well enough so that they don't rediscover the wheel. You have to be savvy enough to know the newer problems in biology."

Professional success in today's workplace, however, will require more than just specialized knowledge and broad understanding of scientific principles. As Peter Brown from GlaxoSmithKline pointed out, interpersonal skills and personal qualities also play a role: "The ability to collaborate across a number of disciplines is essential, as is the openness to take risks. As with most scientific endeavors, there is no script for success, as each project offers its own challenges. However, good decision-making techniques and teamwork make the path to success a little smoother."

And then there's the bottom-line question: If you manage to put together this complete package of training, skills, and personal qualities, will there be anybody interested in hiring you?

When asked about research funding and employment in chemical genetics, Shokat is very upbeat. "I think all of that is going incredibly well," he noted. "A lot of new institutes are popping up, so there are many new positions. Chemistry departments are interested in hiring people who do more biologically relevant chemistry. And biology departments are hiring people who apply chemical approaches to biological problems."

—RW