

Profile of Christian R. H. Raetz

DNA may carry all of the instructions, and proteins may do most of the work, but a cell would not be a cell without lipids. These molecules, which number in the thousands, include important natural products such as fatty acids, phospholipids, and sterols (1) and work at jobs as varied as forming the protective membranes that shelter cells from the outside world to acting as targets for receptors or second messengers in signal transduction cascades. Indeed, problems with lipid metabolism contribute to atherosclerosis, diabetes, and inflammation.

Perhaps no scientist has contributed as much to the important field of lipid biochemistry in recent years as Chris Raetz, the George Barth Geller Professor for Research in Molecular Biology at Duke University Medical Center (Durham, NC). Raetz has made fundamental contributions to understanding how these building blocks of biological membranes are assembled, particularly in bacteria. To date, he has uncovered and characterized over 30 different enzymes responsible for synthesizing or modifying lipid molecules, including the entire nine-enzyme pathway for the biosynthesis of lipid A, an essential part of bacterial outer membranes and a significant contributor to the virulence of some microbes. His work in this field has been pivotal in aiding current efforts to develop new antibiotics and vaccines.

In his Inaugural Article (2), which appeared in a previous issue of PNAS, Raetz provides the crystal structure of the first enzyme in the lipid A pathway, an enzyme known as UDP-GlcNAc acyltransferase or simply LpxA, along with its bound lipid product. "We've managed to take a snapshot of how LpxA recognizes the right lipid," he says. "And we find that it's quite picky about the length of the fatty acid chain it will let in." This paper adds new insights into the world of lipid biosynthesis at the molecular level and is just a small example of why another picky group, the National Academy of Sciences, elected Raetz in 2006.

Finding the Right Chemistry

Chris Raetz was born in East Berlin in 1946, the son of two industrial chemists. His family immigrated to Columbus, Ohio six years later, after Olin Mathieson Chemical Corporation (now Olin Chemical) recruited his father. Growing up in a chemically oriented family, Raetz did not have to stray far to acquire his initial scientific interests. "I



Chris Raetz with his research group in June 2007 outside the Department of Biochemistry at Duke University Medical Center. Front row, right to left: Chris Raetz, Teresa Garrett, Louis Metzger, Andrea Ryan, Bing Ma, Lori Robins, Hak Suk Chung, and Brian Coggins. Back row, right to left: Mike Reynolds, Allison Williams, David Six, Craig Bartling, Brian Ingram, Jinshi Zhao, Feng Song, Ziqiang Guan, and Adam Barb. Photo by Pei Zhou.

can still recall many of my first images, being surrounded by test tubes, distillation flasks, and chemistry books," he says. "Later, my father would bring home chemicals from the lab, and we would do little experiments in the basement."

Still, it was the life sciences, not chemistry, that drew in Raetz during high school and later during his first years at Yale University (New Haven, CT). Then, a fabulous Yale organic chemistry course, taught by Professor William von E. Doering, brought Raetz back to chemistry. "He was a truly inspiring teacher; he could make all those complex concepts sound crystal clear," he says.

In the wake of Doering's course, Raetz combined his interests in biology and chemistry to study enzymology, learning all of the intricate details about how enzymes bind to and act on their target molecules. After receiving his bachelor's degree in chemistry in 1967, Raetz enrolled in the combined medical/doctoral program at Harvard Medical School (Boston, MA). Although he primarily wanted to feed his passion for science, he admits that the atmosphere with the ongoing Vietnam War also played a role in his choice to further his education.

A Path to Lipid A

During his first year at Harvard, Raetz began searching for an advisor for his

doctoral studies, and he found one through his roommate, fellow predoctoral student Bill Wickner. "Bill had started working for Eugene Kennedy, an expert in lipid biochemistry, and he suggested that his lab might appeal to me." Wickner was right, so Raetz joined the team and began purifying and analyzing the enzymes involved in the production of membrane lipids in *Escherichia coli*.

"That was an interesting time in lipid research," recalls Raetz. "Scientists had been doing biochemical studies for a while, and they thought they knew most everything that there was to know about how lipids were made." As Raetz sat in on a genetics class taught by Jon Beckwith, however, he discovered that researchers had overlooked one big area. "Back in the 1970s, molecular genetics was just starting to come into its own," he says, "and Beckwith taught me to appreciate the power of using genetic reasoning in conjunction with biochemistry. Then I realized that researchers had not really applied a systematic genetic approach to lipids."

As he completed his doctoral dissertation and began his own scientific career, first as a research associate with Herb Tabor at the National Institute of General Medical Sciences (Bethesda, MD)

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in 1974 and then as an assistant professor of biochemistry at the University of Wisconsin (Madison, WI) two years later, Raetz decided to incorporate genetic methodology into lipid research. He thought that screening for mutants that formed defective cell membrane lipids could unearth more players in lipid biosynthesis, especially the genes encoding the relevant enzymes.

He first developed a technique to transfer bacterial cells to filter paper, where they could be chemically tested for the activity of a specific enzyme (3); this approach enabled him to screen tens of thousands of bacterial mutant cells in a matter of days, greatly speeding up the mutant search process. Within a few years, he managed to identify many of the genes that encoded the enzymes in the pathway of *E. coli* membrane lipid production that his doctoral advisor Kennedy had discovered (4). “In a sense, I was just using genetics to confirm some of the biochemical results that Kennedy and others had produced,” Raetz says. However, one of his searches would soon take him down a different road.

While studying mutants with a defect in an enzyme called phosphatidylglycerophosphate synthetase (5), Raetz discovered that the novel lipids building up in these mutants looked strikingly similar to a precursor to a membrane component known as lipid A (6). This was an intriguing discovery because lipid A anchors a molecule known as lipopolysaccharide (LPS) to the outer membrane of *E. coli*. LPS, in turn, plays a big role in making many Gram-negative bacteria such as *E. coli* or *Salmonella* toxic.

At that time, the steps involved in synthesizing lipid A remained a mystery. Raetz now understood why. “Other researchers had identified lipid A as a major component of the outer membrane LPS several decades earlier,” he says. “But they had incorrectly predicted its structure; you cannot do the biochemistry if the chemistry is wrong.”

With the structure of a key precursor of lipid A in hand, in conjunction with emerging information from several laboratories on the correct structure of lipid A, Raetz began backtracking to fill in all of the enzymes in the lipid A pathway. He eventually found that the first step involved the LpxA enzyme (7).

The first step of the lipid A biosynthetic pathway proved to be exciting because it demonstrated that lipid biology did hold more mysteries than scientists appreciated in the 1970s. “The prevailing thought was that all membrane lipids derived from glycerol,” Raetz says. Lipid A, however, originated from a dif-

ferent molecule, glucosamine. Raetz had just uncovered an entirely new class of lipids.

Joining the Ranks of Industry

Beyond opening a new set of scientific doors, Raetz knew that his work could have additional clinical implications. “The enzymes that produce lipid A are essential to the survival of many of these toxic bacteria,” he says. “If we can interfere with the natural process, we can kill them.” Although his work had focused on *E. coli*, Raetz believed that the early steps of the pathway were common to multiple bacterial strains; if he targeted one of those enzymes, it might kill a wide range of bacteria.

An ideal opportunity to pursue this avenue of research soon presented itself when Roy Vagelos, recently appointed as the CEO of Merck, invited Raetz to join the pharmaceutical giant in 1987.

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“I knew Vagelos before he joined Merck. He was a terrific lipid biochemist, who together with Al Alberts had discovered acyl carrier protein, a key part of the fatty acid biosynthesis machinery,” says Raetz. “He knew the value that other lipid people like me could bring to the company. He also allowed me to bring my whole lab to Merck, which would greatly smooth the transition and definitely helped convince me.”

Raetz would spend the next several years at Merck, where he oversaw several drug-development projects, two notable ones being the cholesterol-lowering drug Zocor and the prostate-shrinking drug Proscar. In 1992, he became the vice president for biochemistry and microbiology research at Merck, where he oversaw a team of more than 400 people. He continued to conduct his own research as well and succeeded in finding some compounds that could inhibit the critical second step of lipid A biosynthesis, mediated by a deacetylase known as LpxC. These drugs were almost as effective at killing *E. coli* as ampicillin, the gold standard of *E. coli* antibiotics (8). Somewhat oddly, however, these drugs did not affect a related bacterial pathogen known as *Pseudomonas aeruginosa*, and Raetz could not figure out why.

The inability to find multistrain applicability represented an aspect of his tenure at Merck that frustrated Raetz. “The nature of pharmaceutical research was just a bit too focused on what’s in front of you,” he says. “In academia, you can push forward with interesting or unexpected findings; you don’t have to drop a project because it’s only a 250 million dollar idea and not a 1 billion dollar idea.” He also began to miss teaching graduate students, so he eventually decided to leave the industrial realm.

“I certainly don’t want to suggest that I didn’t enjoy my time at Merck,” Raetz says. “I had great colleagues, learned a good deal about chemical biology—and this was even before Stu Schreiber coined the term—and made a lot of advances on the drug front. I just felt that I belong in academia.”

Adding Structure to the Mix

Raetz came to Duke in 1993. With the freedom to pursue more “pure” research, he decided to refine his understanding of the multistep lipid A biosynthetic pathway. He knew most of the players in the game, so he began focusing on structural biology to better understand the details of how the enzymes construct this important lipid from its varied component parts. In fact, this pursuit was one of the reasons he chose Duke—they offered a top-notch nuclear magnetic resonance and structural biology center.

He first tackled the enzyme that launched his lipid A research: LpxA. Together with his collaborator, crystallographer Steven Roderick at Albert Einstein College of Medicine (Bronx, New York), he determined the 3D structure of LpxA, revealing that it had an unusual architectural feature. “Part of the protein arranges itself in a series of flat sheets that fold on top of each other in a counterclockwise, or left-handed, fashion,” he says (9). The known rules of protein folding had suggested that such folds could only arrange in a clockwise manner.

Despite the presence of that unusual motif, the mechanism of LpxA was not clear based solely on its structure. The key would be developing a 3D structure of the enzyme with its lipid product, UDP-GlcNAc with an acyl chain attached to it. That is exactly what Raetz achieved in his Inaugural Article (2). With this visual image available, Raetz found which amino acids contribute to the enzymatic process and discovered that the positioning of one amino acid in particular, glycine 173, enables it to function like a “hydrocarbon ruler” to measure incoming acyl chains and en-

sure that they are the proper size. Raetz also managed to acquire a 3D structure of LpxA with a small peptide inhibitor bound to it (10), a structure that could serve as a template to design future LpxA antibiotics.

Raetz has also taken advantage of the recent advances in both genomics and structural biology to return to an old mystery: why the *E. coli* LpxC inhibitor that he developed at Merck was ineffective against other bacteria. With the ability to quickly compare the genes of many different bacteria, he discovered that slight differences in the enzyme shape meant that the inhibitor was not binding tightly enough in some cases (11). Teaming up with the drug company Chiron, Raetz and his collaborators used this comparative analysis to design new broad-spectrum deacetylase inhibitors, which in the preliminary stages show good promise (12, 13).

Future Horizons

In 2008, Raetz will shake things up with a move to the University of Colorado

(Boulder, CO), where he will be professor of chemistry and biochemistry. "I think that every 10–15 years, professors need to seek out some new horizons," he says. "They're setting up a new biotechnology center up there, and they have a lot of great young researchers as part of it, although currently no one specializing in lipids. So it provides for a wonderful environment for me to both teach and learn."

One area that Raetz would like to know more about is how lipid A and other outer membrane components reach their destination. "Many of these molecules are put together inside the cell," Raetz explains. "In Gram-negative bacteria, they have to pass through an inner membrane to reach the outer one. And we've only started to make mutants to see where these lipids might get stuck during the transport process (14)." In addition, he is beginning to delve further into the reasons why LPS and other bacterial surface components are potent

activators of our immune system and contribute to inflammation.

Raetz is not done with the search for novel lipids, however. In fact, he recently joined a National Institutes of Health-funded consortium known as Lipid MAPS, organized by Edward Dennis at the University of California (San Diego, CA). It is an ambitious project using a combination of genetics, biochemistry, and mass spectroscopy to create a comprehensive database detailing the structure and function of all of the lipids within a cell.

"Many people don't appreciate the large diversity of lipids out there, including an entire class of minor lipids that make up about 0.01% of a cell's lipid content and therefore are difficult to isolate," he says. "For all the ones we know about, there are probably just as many we haven't found yet." Unlike some of his predecessors, Raetz will tell you we still have much to learn about lipids.

Nick Zagorski, *Freelance Science Writer*

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