Solving the Riddle of the Role of Sphingolipids in Cell Signaling

Sphingosine Inhibition of Protein Kinase C Activity and of Phorbol Dibutyrate Binding in Vitro and in Human Platelets

Inhibition of Phorbol Ester-dependent Differentiation of Human Promyelocytic Leukemic (HL-60) Cells by Sphinganine and Other Long-chain Bases

Inhibition of the Oxidative Burst in Human Neutrophils by Sphingoid Long-chain Bases. Role of Protein Kinase C in Activation of the Burst
(Wilson, E., Olcott, M. C., Bell, R. M., Merrill, A. H., Jr., and Lambeth, J. D. (1986) J. Biol. Chem. 261, 12616–12623)

When Johann L. W. Thudichum, a German-born physician, discovered a puzzling class of lipids in 1881, he dubbed them “sphingolipids,” a reference to the mysterious Sphinx of Greek legend who taunted travelers with her riddles. Although the structures of sphingolipids were worked out in the 1940s, their important roles in cellular metabolism were not apparent until the mid-1980s. “From the very beginning, these lipids were dubbed as enigmatic,” says Yusuf Hannun at Stony Brook University Cancer Center. “No one touched them for decades.”

Robert Bell, a lipid researcher, was at Duke University in the 1980s. At that time, “lipids were thought to be just components of membranes,” says Bell. “Medical students hated them. Graduate students ignored them.” Which is why, when the common lipid molecule diacylglycerol was found to activate the newly discovered enzyme known as protein kinase C, many in the field were astounded.

Protein kinase C phosphorylates many proteins in the cell; these proteins trigger a plethora of cellular responses, such as transcription, cell growth, and immune responses. “I couldn’t believe this was the case,” says Bell. “Diacylglycerol was an ordinary, everyday intermediate in lipid metabolism. How could it have this special second-messenger function ascribed to it?” Bell’s lab set out to refute the hypothesis that diacylglycerol regulated protein kinase C. When this proved unsuccessful, they wondered if other lipids might have an effect on the kinase.

Hannun was a postdoctoral fellow in Bell’s lab. Working alongside postdoctoral fellow Carson Loomis, he tested an array of common lipids, including sphingosine. Sphingosine can be either a precursor or breakdown product of complex sphingolipids. “No one knew why Bob’s lab had sphingosine, what it was doing on the shelf, or what sphingosine even was,” recalls Hannun. When the tests showed that sphingosine had the opposite effect and inhibited protein kinase C, the researchers were dumbfounded. “My mind started racing. What is this sphingolipid? What about other sphingolipids?” recalls Hannun. “I knew very little about sphingolipids. I would say most people who called themselves lipid biochemists knew very little about them.”

Ultimately, Bell and his collaborators submitted three papers as a set to the Journal of Biological Chemistry. The first paper described the primary observation that sphingosine inhibits protein kinase C and provided examples both in the test tube and human platelets. In the other two papers, other authors who collaborated with Bell’s group expanded on the physiological relevance of the primary observation. Alfred Merrill, a former postdoctoral fellow of Bell’s, who was then an assistant professor at Emory University, had been studying sphingolipids for several years. He describes the collaboration as “a coalescing of experiences that got everybody very excited.”

The second and third papers reported on the roles of sphingosine and other sphingoid bases. Sphingoid bases are the building blocks of the sphingolipid backbone. The two papers explored
the roles of sphingoid bases in oxidative burst, which is the release of chemicals from immune cells, and differentiation of bone marrow cells. The three papers together presented a more cohesive body of evidence for the important roles of sphingolipids in signaling than a one-off study would have provided. “At Duke, I worked very hard to make lipids interesting to medical students,” says Bell. “Suddenly, we started to understand that sphingolipids could play roles in cell signaling. It was very exciting.”

Still, their findings were controversial. Bell recalls presenting his research at a Gordon conference: “As soon as I sat down, everybody working in the sphingolipid field jumped up and started arguing,” he recalls. “The pushback was instant.”

Many lipid researchers had difficulty accepting that a lipid breakdown product could serve a regulatory function. “They all went back to their labs and started studying it,” says Bell. “Some of them are still studying it today.”

Ever enigmatic, sphingolipids proved difficult to handle for many researchers, some of whom initially had trouble verifying some of the cellular results. Once the community came up to speed, however, sphingolipid signaling proved to be fertile ground for scientific discovery. “Now there are thousands of papers on bioactive sphingolipids,” says Hannun. “They do so many things—regulate blood vessel formation, cell death, cell migration, immune responses.”

“You usually get the feeling that the best work you do is always resisted,” says Bell, who has been retired since 2010. “People aren’t going to accept it easily. I think this has now stood the test of time, which is great to know.”

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_JBC Associate Editor George M. Carman at Rutgers University nominated this set of papers as a Classic. Alexandra Taylor (alexandraataylor@gmail.com) is a master’s candidate in science and medical writing at Johns Hopkins University._