BIOGRAPHICAL MEMOIRS

Eugene P. Kennedy

September 4, 1919–September 22, 2011
Elected to the NAS, 1964

A Biographical Memoir by Howard Schulman

Eugene Patrick Kennedy, Hamilton Kuhn Professor of Biological Chemistry and Molecular Pharmacology Emeritus at the Harvard Medical School, was one of the giants of twentieth century biochemistry, renowned for his groundbreaking work on phospholipid biosynthesis and membrane function. His research helped shift biochemical leadership from pre-World War II Europe to the United States in the years after the war. His distinguished career spanned from the start of his Ph.D. research in 1947 at the University of Chicago to the closure of his lab in 1993.

Early Life and Education Amidst Turmoil

Eugene Kennedy was born on September 4, 1919, in Chicago, to Irish immigrant parents Catherine (née Frawley) Kennedy and Michael Kennedy. His father worked as a motorman on Chicago's streetcar service, earning just enough to keep the family of seven within the lower middle class. Like many immigrants, his parents strongly encouraged their children to pursue an education. Under the tutelage of Servite priests in the parochial high school he attended, young Kennedy excelled at clear expository writing and the humanities. This talent gave him the skill to undertake the task of editing his high school newspaper and, later, his college newspaper and, much later, his own scientific communications.

Kennedy enrolled in De Paul University in 1937 on a scholarship with a major in chemistry, perhaps a more practical choice than pursuing a humanities major. This period coincided with the grim unfolding of events in Europe and the onset of World War II. Kennedy closely followed international affairs and recalled decades later the "nightmare quality" of May 1940, as the seemingly inexorable triumph of Nazism cast a shadow over Europe.[1] It drove many European scientists to England and to the United States, where they would come to have an influence on Kennedy's education and research.[2]

Figure 1 Gene and Adelaide Kennedy, Cape Cod, Massachusetts. *Photo courtesy of the Kennedy family.*

After graduation, Kennedy entered the Ph.D. program in organic chemistry at the University of Chicago. Training grants were almost non-existent at the time, so he supported himself with a full-time job in the wartime research department of Armour and Company, which was engaged in plasma fractionation. He challenged himself with a grueling schedule, working the midnight shift in cold rooms at Armour

before attending morning classes at the university. Remarkably, one of the courses was taught by Frank Westheimer, with Aaron Novick as the teaching assistant and Daniel E. Koshland Jr. as a classmate, all of whom became significant leaders in science. When Armour opened a plasma facility in Fort Worth, Texas, Kennedy was sent to help design large-scale protein isolation. Joining him was Adelaide Majewski, a college classmate and former fellow newspaper editor, and they married on October 27, 1943.

Graduate Studies: The "Gold Rush" of Biochemistry

Kennedy's wartime experience ignited a fascination with biochemistry, and he transferred to the Department of Biochemistry, which was a vibrant intellectual hub populated by brilliant investigators like Konrad E. Bloch, Albert L. Lehninger, and Earl A. Evans. Kennedy vividly recalled the "heady sense of excitement and adventure" of entering the "intellectual gold rush" of biochemistry, where "nuggets" of discovery seemed to be "lying around everywhere."[3] Biochemical research was expanding, facilitated by new techniques, such as isotope tracers, and by a large seismic increase in the federal investment in biomedical research and training.

In 1947, on the recommendation of his fellow student and friend Morris E. Friedkin, Kennedy joined the laboratory of Albert Lehninger. At this time, neither the linked reactions for biosynthesis and metabolism of cell constituents nor their cellular organization were known. Lehninger and Kennedy observed that two apparently unrelated processes, fatty acid oxidation and oxidative phosphorylation, were similarly disrupted by hypotonic buffers and surmised that these processes occurred within a membrane-bound organelle sensitive to osmotic pressure. In a landmark discovery, Kennedy provided convincing evidence that fatty acid oxidation and oxidative phosphorylation, as well as the reactions of the Krebs tricarboxylic acid cycle, are all localized inside mitochondria.[4] It was the first demonstration that his keen observational skills and curiosity could be rewarded with discoveries. Their published findings solidified the mitochondrion's role as the "powerhouse" of the cell.

Kennedy had entered an elite scientific community that helped chart his training. He was deeply influenced by these figures, especially Lehninger, Fritz A. Lipmann, and Bloch, who shaped his view of cell physiology and how to approach problems experimentally. He benefited tremendously, of course, from entering biochemistry during this transformative time and, in turn, he helped transform biochemistry. Although Kennedy did not begin his Ph.D. training with the expectation of becoming a professor, he found his calling and contemplated an academic career.

At Lehninger's suggestion, Kennedy joined the lab of Horace A. Barker at the University of California, Berkeley, as a postdoctoral fellow. Barker and graduate student Earl R. Stadtman had recently made waves in enzymology by discovering the benefits of cell-free preparations from the anaerobic Gram-positive bacterium *Clostridium kluyveri* for unraveling metabolic pathways. Kennedy and Barker interpreted an effect of phosphate on cell free oxidation of butyrate to reveal a role of a high-energy intermediate, acetoacetate-X, and correctly conjectured that X could be Coenzyme A,[5] which Lipmann had discovered in 1945 and for which he received the Nobel Prize in Physiology or Medicine in 1953. The studies of Kennedy and Barker were crucial in bridging fatty acid metabolism with energy transfer reactions, helping to illuminate how cells couple catabolism to ATP generation.

Barker convinced Kennedy to further his postdoctoral training with Lipmann at the Massachusetts General Hospital in Boston. Lipmann was one of the towering figures of twentieth-century biochemistry and a profound thinker. He provided the framework for understanding how high-energy phosphate transfer drives metabolism, unifying disparate biochemical reactions under a single energetic principle. It was a formative year for Kennedy even though his time with Lipmann did not produce any published works. One important lesson for Kennedy was Lipmann's strategy of testing hypothesized intermediates by synthesizing the suspected molecules chemically and then examining their activity in biological systems, rather than first struggling to isolate these same compounds from tissue extracts—an approach Kennedy later applied in his own work.

Return to Chicago: The Kennedy Pathway

In 1951, Kennedy joined the faculty of the University of Chicago with a joint appointment in the Department of Biochemistry and the Ben May Laboratory for Cancer Research, headed by Charles B. Huggins. Kennedy began investigating the incorporation of radioactive inorganic phosphate [$^{32}PO_4^{3-}$] into phosphoproteins, inspired by leads in Friedkin's earlier work. With George Burnett, Kennedy discovered and partially purified an enzyme that catalyzed the transfer of phosphate from [γ-^{32}P]ATP to serine residues in casein.[6] This report was the first demonstration of a protein kinase, but the significance of protein phosphorylation was then unknown. Kennedy later reflected that he missed a golden opportunity by not pursuing protein phosphorylation, which later was found to control practically all cellular functions in response to extracellular signals.[7] Still, he did not choose badly by pursuing phospholipid biosynthesis. As fate would have it, phosphatidylinositol, whose biosynthesis Kennedy and his graduate student Henry Paulus later elaborated is, as we now know, a foundational molecule for phosphoinositides

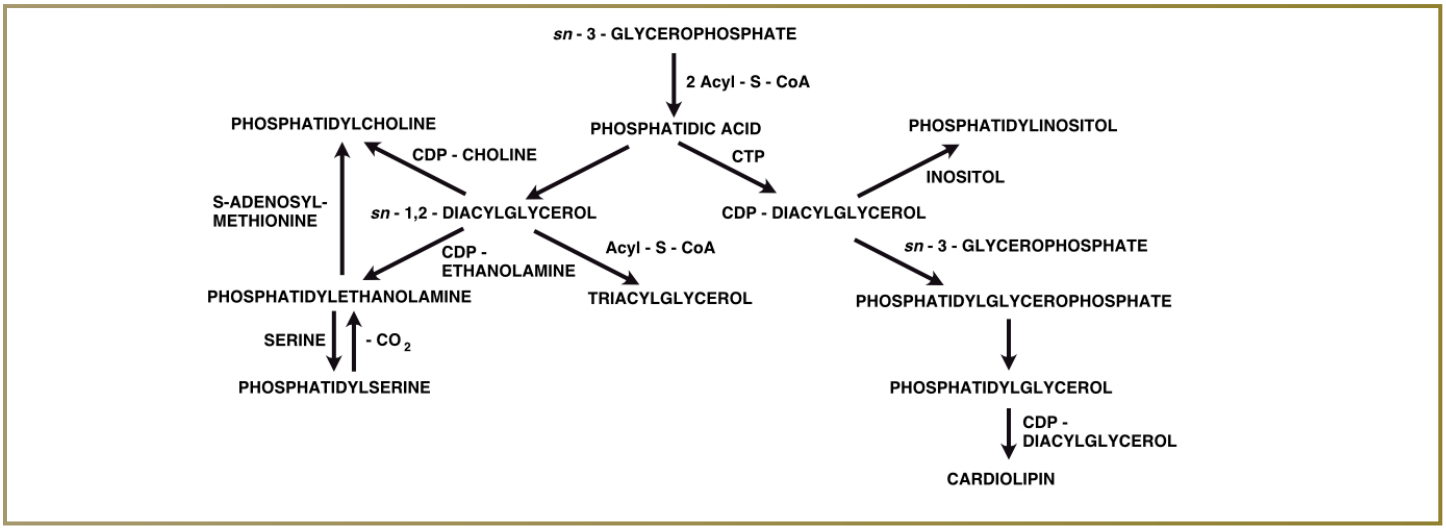

Figure 2 The Kennedy pathway of phospholipid biosynthesis in eukaryotic cells. *Redrawn from William Wickner.*[10]

generated by lipid kinases. In addition to their role in membrane organization, they are precursors for second messengers that can activate certain protein kinases.

Kennedy then began a long-term effort to delineate the biosynthesis of phospholipids, such as the abundant phosphatidylcholine. He was particularly interested in the mechanism of phosphodiester bond formation, as he hoped that it would shed light on biosynthesis of the backbone phosphodiester bonds in nucleic acids. Kennedy found that *sn*-3-glycerophosphate was an intermediate but thought that he was "scooped" when he saw an abstract in the *Federation Proceedings* by Arthur Kornberg and William E. Pricer Jr. in which they reported phosphocholine incorporation into phosphatidylcholine in the presence of ATP.

In 1954, puzzled by discrepancies between his findings and Kornberg's and Pricer's report, Kennedy and his first postdoctoral fellow, Samuel B. Weiss, re-examined phosphatidylcholine biosynthesis—a careful inquiry that produced an unforeseen result leading to a major discovery. They noticed that phosphocholine conversion to phospholipid in their cell extracts only occurred with certain commercial ATP preparations (such as amorphous ATP, Lot no. 116, from Pabst Laboratories), but not others. Suspicious that batches of ATP that "worked" contained an active impurity, they obtained a new lot of ATP (crystalline, Lot no. 122, also from Pabst Laboratories) and, as suspected, this purer ATP did not work as the cofactor. They then applied a lesson from Lipmann: rather than try to isolate the active contaminant, they surmised that it could be a nucleoside triphosphate other than ATP. Indeed, they discovered that only **c**ytidine triphosphate (CTP) was able to activate phosphocholine. Again, they synthesized (rather than isolated) a hypothesized intermediate, CDP-choline, which also supported phosphatidylcholine synthesis in their system. In a seminal discovery, they then revealed an enzyme activity that converts CTP and phosphocholine to CDP-choline, which then acts as the proximal choline donor to diacylglycerol and yields phosphatidylcholine.[8] In the process, they extended the repertoire of high-energy phosphate compounds. According to lab legend, these bottles of ATP and CTP could still be found years later stored in a freezer at the Harvard lab.

They quickly followed up with synthesis of CDP-ethanolamine, demonstrating its parallel role as an ethanolamine donor for the enzymic generation of phosphatidylethanolamine. But the evolutionary strategy of activating the headgroup diverges in the synthesis of phosphatidylinositol, as shown in Kennedy's 1960 paper with Henry Paulus.[9] It is the backbone phosphatidic acid that is activated to form CDP-diacylglycerol. Myo-inositol then displaces CMP to form phosphatidylinositol.

Harvard Years: Membranes, Permeases, and Oligosaccharides

In 1959, Kennedy was invited to head the Department of Biological Chemistry at Harvard Medical School as the Hamilton Kuhn Professor and continued studies on membrane lipids. The biosynthesis of phosphatidylglycerol was found to be like that of phosphatidylinositol, with the backbone phosphatidic acid activated as CDP-diacylglycerol and the headgroup added by enzymatic attack by the nucleophilic hydroxyl of *sn*-3-glycerolphosphate, followed by dephosphorylation. This is a minor phospholipid in eukaryotes, as it is largely converted to cardiolipin by displacement of CMP from CDP-diacyglycerol by phosphatidylglycerol. Thus, by 1961 the basic outline of the Kennedy pathway could be

accurately described (see Figure 2). The lab soon turned to bacteria and yeast for phospholipid biosynthesis and membrane studies. One difference in *Escherichia coli* (*E. coli*), as demonstrated by postdoc Carlos Hirschberg and Kennedy, is that synthesis of cardiolipin involves condensation of two phosphatidylglycerols and release of glycerol, with CDP-diacylglycerol only acting to stimulate the reaction.[11] Kennedy's work significantly advanced the pathway and initial understanding of the enzymology and genetic regulation of phospholipid biosynthesis in *E. coli.* His phospholipid team during the 1960s included Julian Kanfer, Ying-ying Chang, John Kiyasu, Ronald Pieringer, James Carter, and Dennis Voelker, and in the 1970s, William Wickner, William Dowhan, Ed Hawrot, Christian Raetz, Lawrence Rizzolo, Martin Snider, Gayle Schneider, Richard Tyhach, Michel Satre, Edward Dennis, and Carlos Hirschberg.

At Harvard, Kennedy's lab and department mirrored the expanding U.S. research enterprise, with graduate students now supported by NIH training grants and a dozen potential Ph.D. mentors for postdocs funded by fellowships. After a year of introductory courses, graduate students chose from advanced seminar courses, such as Kennedy's membrane course, in which presenting before distinguished Boston area colleagues that Kennedy invited (including even Nobel laureates) was both inspiring and daunting. On at least one occasion, Kennedy had to save a student who was unprepared or overwhelmed by the occasion. Biochemistry was a thriving field, reflected in the growth of the American Society for Biochemistry and Molecular Biology from 2000 members in the 1950s to 12,000 members today, with lively exchanges at annual meetings. The Gordon Research Conference on Lipid Metabolism at the spartan Kimball Union Academy in Meriden, New Hampshire, provided a more intimate venue.

Each faculty member in the department had a lab situated next to their office, which for full professors at Harvard in those days were quite large and wood-paneled; one even had a fireplace. One of Kennedy's dictums was to place a higher value on a clever experiment than on a new piece of equipment. He participated in the clever experiments, and his wise administrator in the adjoining office, Phyllis Elfman, kept a tight rein on new equipment. Students and postdoctoral fellows worked long hours, and glass pipettes were sometimes in short supply despite the quick work of Mrs. Berzins at her glass washing station. We arranged to get used pipettes from Charles C. Richardson's neighboring lab, which otherwise discarded any with even cosmetic scratches. This source became less attractive when they ordered pipettes monogrammed with CCR.

Kennedy's office opened onto the first room of his lab suite, in which his excellent research assistant, Marilyn Rumley, worked on experiments with him. He could be seen in his white lab coat sitting alongside Marilyn at a low bench going over an experiment. At times he sat at the bench quietly immersed in thought. Students and postdocs were spread out in the other adjoining lab spaces, each with their own project, bench, and juxtaposed desk. For projects at crucial points, particularly graduate student projects, he would step into the lab with the greeting *Wie gehts*? or "How's it going?" It was not a prompt to discuss how the student's new roommate was working out, although he could engage in small talk when the occasion arose. He wanted to see the outcome of the experiment discussed the previous week or day. Membrane protein purification and other projects were often difficult, and made even more challenging for postdocs by the fact that a typical postdoctoral stint in that era was just two years.

Membrane protein isolation was still in its infancy, and several papers were titled "Partial purification of …" Still, many postdocs and students from this period went on to successful academic careers, including some becoming department chairs, National Academy of Sciences members, and even a Nobel laureate. William Dowhan, William Wickner, and Kennedy did achieve a rare feat at the time, the first complete purification of an integral membrane protein, phosphatidylserine decarboxylase.[12] Beyond phospholipid biosynthesis, members of the lab explored important areas of membrane biogenesis, such as phospholipid "flip-flop" in the membrane, transport to the outer membrane, and purification of F_1F_0 ATPase (James Rothman, Keith Langley, Ronald Hanson, James Hare, and Robert Fillingame).

A long-standing focus of the Kennedy lab was the transport of cellular constituents across membranes *(*studied by Joan Lusk, David Nelson, Fred Fox, Alvin Tarlov, Lawrence Rothfield, and James Carter*),* including transport of magnesium and of lactose. The lactose permease or M protein was part of the iconic *lac* operon, a set of genes that became a cornerstone for insights on bacterial genetics, gene expression control, and allosteric regulation of proteins. The lab had established the M protein as a target for identification and purification. Kennedy and Fred Fox then devised an elegant double-labeling technique using M protein-induced and uninduced cells that allowed them to specifically tag the M protein on a sulfhydryl in its binding site.[13] The strategy involved broadly inactivating protein sulfhydryls with N-ethylmaleimide (NEM) in the presence of thiodigalactoside, an M protein substrate that protected a key sulfhydryl. After washout of the sugar, induced cells (high M protein level) were labeled with ^{14}C-NEM and uninduced cells (low M protein level) with ^{3}H-NEM. In a mixture of the two types of cells, the permease protein would be enriched for ^{14}C over ^{3}H. It was a crucial step in its identification, opening a new

era in its study as well as a paradigm for selectively tagging receptor proteins.

Kennedy was still troubled by gaps in phospholipid pathways that he needed to pursue. He and Kanfer had noted that ^{32}P-phosphatidylglycerol turned over rapidly during exponential growth, losing ^{32}P to the aqueous phase. His memory was long, and ten years later he returned to the observation with Lambert van Golde to discover that the phosphoglycerol headgroup was transferred from the lipid to soluble polymers of glucose, referred to as membrane-derived oligosaccharides or MDOs.[14] The biosynthesis and function of the MDO was the last long-term project in the lab and was personally driven by him with many contributors (Lambert van Golde, Howard Schulman, Marilyn Rumley, Daniel Goldberg, Barbara Jackson, Jeanette Schneider, Audrey Weissborn, Helene Therisod, Karen Miller, Vernon Reinhold, Jean-Pierre Bohin, and Otto Geiger). To the bemusement and perhaps the jealousy of some, the MDO project had Kennedy's persistent attention.

The MDOs were a novel class of glucose-containing oligosaccharides comprising about 0.5 to 1 percent of the dry weight of growing *E. coli*. The oligosaccharides are acceptors of the acidic phospholipid headgroups—*sn*-glycero-1-phosphate (and phosphoethanolamine). Some species also contain succinic acid in O-ester linkage, likely transferred from succinyl-CoA. MDOs are highly branched, containing ten to twelve glucose residues in backbone β1-2 bonds catalyzed by a glucosyltransferase and branches via β1-6 bonds. They are present in the periplasmic space of all Gram-negative bacteria tested and absent from Gram-positive bacteria. This field expanded further following Kennedy's observation that the MDOs are important for osmoregulation in *E. coli* and other Gram-negative bacteria, reaching almost 7 percent of the dry weight of the cell when grown at low osmotic pressure, comparable to the mass of phospholipids. Osmoregulation prevents swelling of the inner cell membrane; hence, the MDOs are now also termed osmoregulated periplasmic glucans (OPGs). Thus, pursuing an observation on phospholipid turnover led to identification of a major class of periplasmic molecules critical for growth in enteric bacteria and related structures in soil bacteria.

A Philosophy of Science and an Enduring Legacy

Eugene Kennedy retired quietly from his laboratory in 1993. His later years were dedicated to recounting the rise of biochemistry in the first half of the twentieth century. Many of his colleagues and friends, including Fritz Lipmann, Rudolf Schoenheimer, and Konrad Bloch, were driven out of Germany by the brutal antisemitism of the Nazi regime, and he honored their contributions.[15] The center of biomedical research kept shifting toward the United States, as reflected

Figure 3 Gathering at the first Kennedy festschrift in Woods Hole, Massachusetts, in 1985. Identified by number are participants specifically mentioned in the text: 1. Albert Lehninger; 2. Fritz Lipmann; 3. Edward Dennis; 4. Marilyn Rumley; 5. Adelaide Kennedy; 6. Roberta Friedkin; 7. Phyllis Elfman; 8. Morris Friedkin; 9. Eugene Kennedy; 10. Samuel Weiss; 11. Howard Schulman; 12. Carlos Hirschberg; 13. Fred Fox; 14. William Dowhan; 15. Henry Paulus. Missing from the photo are James Rothman and William Wicker, who were absorbed in a discussion of membrane fusion. *Courtesy of Ed Dennis*.

by the many postdoctoral fellows who came to his lab from Europe, Asia, and Latin America.

Kennedy largely heeded Huggins's advice to "stay out of airports"—his less-than-subtle admonishment to avoid chasing after scientific fame—yet Kennedy's contributions were widely recognized nonetheless. Among his honors were the Pfizer Award in Enzyme Chemistry; election to the American Philosophical Society, the American Academy of Arts & Sciences, and the National Academy of Sciences; the Distinguished Service Award from the University of Chicago; the Gairdner Foundation International Award; the Passano Award; and the Heinrich Wieland Prize. He passed away peacefully at his Cambridge home on September 22, 2011, at the age of ninety-two, leaving behind three accomplished daughters (Sheila, Lisa, and Katherine) and their families, and a profound impact on generations of scientists. During his Harvard years, members of his lab expressed their affection and gratitude at not one, but three, festschrifts—on his sixty-fifth birthday (in Woods Hole, Massachusetts), his seventieth birthday (at Princeton University), and his ninetieth birthday (at Harvard Medical School).

Kennedy ended his excellent autobiographical essay, "Sailing to Byzantium," with the poem by that name by William Butler Yeats to contrast the legacy of artists and scientists.[16] For Yeats, Byzantium represented a timeless realm in which the creations of artists are indelibly stamped in their work. By contrast, Kennedy believed that scientists are somewhat interchangeable, with their contributions ultimately woven into the fabric of scientific knowledge. He agreed with

Lippman that fully exploring nature is one of the most important activities of humanity and is its own reward. After all, even contributions of Otto Warburg, who dominated biochemistry in the first half the twentieth century, could not be recalled by most students today. But Kennedy, like an artist, has had an imprint. His papers have a clear prose explaining the logic of each experiment. He demonstrated how attention to experimental anomalies leads to discoveries. Throughout, he made clever use of double labeling, pulse-chase experiments, and application of chemistry. His signature approaches have been carried out by his disciples in their study of lipid metabolism, membrane biology, and other fields.

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