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IN MEMORIAM

Personal reflections on a mentor extraordinaire: Paul Greengard, Ph.D. (1925–2019)

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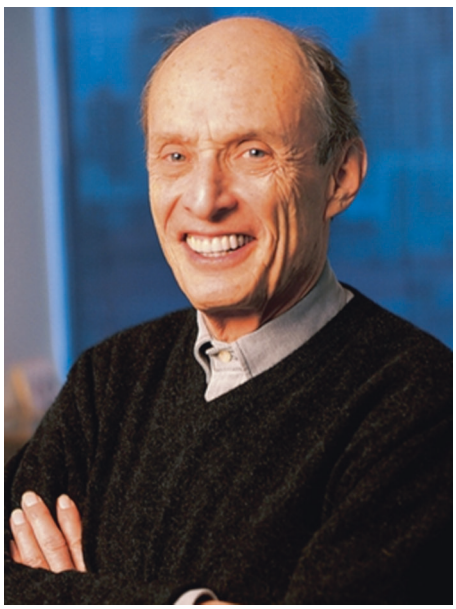

I first met Paul Greengard in September 1974. I had just returned to Yale College for my junior year after having spent the prior summer at Rockefeller University as a research volunteer where I studied regulation of cyclic GMP synthesis in the exocrine pancreas. As part of that project, I learned that Paul, then a professor of pharmacology at Yale, was a leader in cyclic nucleotide research. I thus made an appointment to meet with Paul when I returned to campus in the hope of doing a senior research thesis in his laboratory. I was told by his secretary to knock on his office door and enter and, when I did, I was faced by two large huskies—glaring at me head on from less than five feet away; Paul was nowhere to be seen. Suddenly, from behind the desk, I heard Paul say: "Come on in. They won't bother you." Paul was lying flat on his back on the floor of his office working on a manuscript—something he did often in those days to nurse a bad back. The rest, as they say, is history. I completed my senior thesis in the Greengard laboratory and stayed on at Yale for my M.D.-Ph.D. training most importantly so that I could do my graduate work with Paul. Paul became a second father to me and we remained very close—both scientifically and personally—until his recent death on April 13, 2019 at the age of 93.

From a scientific perspective, it is difficult to overstate the impact that Paul had on our understanding of cell signaling in general, and in the nervous system in particular. In the 1970s, the field of neuroscience was focused primarily on the control of the electrical activity of nerve cells and the ability of one nerve cell to affect the electrical activity of other nerve cells through synaptic transmission. Synaptic transmission itself was viewed as a primarily electrical phenomenon, whereby neurotransmitters controlled the opening and closing of postsynaptic proteins that served as ligand-gated or voltage-gated ion channels. In the background, there was an associated field of "neurochemistry," with investigators focused on the biochemical composition of brain tissue with limited regard for the underlying neural circuitry. Paul once told me that these two approaches to the nervous system operated almost completely separately with very limited crosstalk, and in fact that many of the leaders of each approach did not much like one another either! Paul saw the world differently. In 1968, Ed Krebs, Ed Fischer, and colleagues discovered cyclic AMP-dependent protein kinase (protein kinase A) in peripheral tissues, which they showed mediated the ability of circulating glucagon and epinephrine to stimulate glycogenolysis. Paul's son Leslie reports that when Paul saw that discovery he posited that the brain operates in the same way—that neurotransmitters also act in part like peripheral hormones—and that he was going to prove it. And that's exactly what he did over the ensuing five decades.

Paul spent most of his early career at the pharmaceutical company Geigy and, after brief sabbaticals at Vanderbilt University and at Albert Einstein College of Medicine in 1968, he joined the Yale pharmacology department in 1969 to establish his first academic laboratory at the age of 43. He quickly set out to test his hypothesis, first by showing that protein kinase A is expressed at high levels in brain, including synaptic fractions, and demonstrating in brain tissue the existence of substrates for protein kinase A that were not detectable in peripheral tissues. One of the first substrates discovered was named "Protein 1" (classic Paul!). Parenthetically, Protein 1 was the subject of my Ph.D. thesis in Paul's laboratory years later; my small part of the story was to demonstrate that Protein 1 phosphorylation is regulated by nerve impulses, thereby directly linking a nerve cell's electrical activity to a biochemical process in a defined circuit. The later demonstration that Protein 1 is enriched at synapses and associates with synaptic vesicles—for which it gained the new name, synapsin 1, lent further credence to Paul's organizing hypothesis that protein phosphorylation contributes to synaptic transmission. This discovery also stimulated ensuing decades of research by numerous

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laboratories on synaptic vesicle-associated proteins and their role in exocytosis.

In short order, Paul's laboratory discovered the existence in brain and peripheral tissues of a distinct protein kinase that was activated by cyclic GMP and not cyclic AMP, termed protein kinase G. The laboratory demonstrated that it too phosphorylates substrates specific to the brain and distinct from those phosphorylated by protein kinase A. By the late 1970s, phosphorylase kinase—a substrate for protein kinase A in peripheral tissues which activates phosphorylase and glycogenolysis—was shown to be a Ca^{2+}/calmodulin-activated protein kinase (CaMK). Paul's laboratory subsequently discovered two new forms of CaMK, including CaMKII, which is highly expressed in brain and particularly enriched at synapses where it phosphorylates specific synaptic proteins. Later discoveries likewise demonstrated the existence of several types of protein phosphatases in the brain—responsible for dephosphorylating phosphoproteins—along with numerous types of regulatory subunits of protein phosphatases which themselves are regulated by protein kinases and expressed in brain in a highly region-specific manner. DARPP32 (dopamine and cyclic AMP regulated phosphoprotein of 32 kDa) was discovered by virtue of its phosphorylation by protein kinase A (and later by several other kinases) and demonstrated subsequently to serve as a highly potent inhibitor of protein phosphatase 1. Incidentally, studies such as these—on synapsin 1 or DARPP32, for example—were among the first uses of an open-ended "proteomic" approach to discover novel mechanisms of brain regulation, although the word proteomic wasn't in use at the time.

These discoveries led Paul, in 1978, to propose his overarching hypothesis: that a wide range of neurotransmitters, through the activation of a wide range of protein kinases and protein phosphatases, control virtually every aspect of neuronal function through the phosphorylation of virtually every type of neuronal protein, including ion channels, neurotransmitter receptors, neurotransmitter synthetic and degradative enzymes, cytoskeletal proteins, and nuclear proteins, along with a host of intracellular regulatory proteins that create highly complex signaling networks. This view of protein phosphorylation as the major mode of cell regulation is now second nature to students and postdoctoral fellows entering the field of neuroscience today, but it was greeted initially with extreme skepticism by many leaders in the field who resisted the notion that neural phenomena could be controlled by biochemical processes. Despite this skepticism, the Greengard laboratory doggedly persevered and provided ever more comprehensive and compelling evidence for the validity of such "Greengard Cascades" over the next several decades, first at Yale and starting in 1983 at Rockefeller University, where Paul served as Head of the Laboratory of Molecular and Cellular Neuroscience—with 61 active researchers (the largest at Rockefeller) at last count—until his death. It was therefore most deserved when, in 2000, Paul received the Nobel Prize in Physiology or Medicine, shared with Arvid Carlsson and Eric Kandel, for his paradigm-shifting advances in our understanding of signal transduction mechanisms in the nervous system.

Paul's impact on our field, however, goes far beyond the papers he published. He trained hundreds of students and postdocs many of whom are now leaders in academic medicine, including deans, department chairs, institute and center directors, and innumerable leading research groups in neuroscience and other fields. Paul was unusually generative, not only in promoting the careers of members of his own laboratory, but also countless other faculty colleagues to whom he gave regular scientific and career advice.

Paul had a truly unique brain: he was able to cut incisively to the major question at hand, infer biological relationships where few others saw them, and focus like a laser beam for hours after colleagues several decades his junior grew fatigued. Paul made everyone who worked with him a better scientist by challenging us to do better, to be more precise with our own scientific writing and talks, and to think about our science in the most rational, linear, and exacting manner possible. Another essential ingredient was Paul's very special sense of humor. He had one of the quickest wits—I still laugh out loud when I think of things that Paul has said over the years. He was hysterical but also irreverent and mischievous and loved to challenge the status quo, just as he did with his science.

But Paul was far more than a scientific mentor to the hundreds of people who worked in or collaborated with his laboratory. He gave of himself selflessly to so many of us, helping us with our personal life challenges and not just our careers. Despite the fact that I left the Greengard laboratory in 1983, I relied on Paul for personal and professional advice for several decades thereafter. I would schedule trips to NYC just to have dinner with him to get that advice, and I left each and every visit reinfused with that distinctive sense of wellbeing and confidence that only Paul was able to offer me over the course of my own career. And I know dozens of other leading scientists nationally and internationally who feel exactly the same way about their time with Paul.

It is a special privilege to provide these reflections about Paul for this journal, which is sponsored by the American College of Neuropsychopharmacology (ACNP), in which Paul played a defining role. Paul was a founding member of the College and was elected Fellow in 1961. In 1996, I had the spectacular opportunity to interview Paul for the College's historical archives (https://acnp.org/videos/paul-greengard-by-eric-nestler/). In 2011, I served as president of the ACNP, another data point in Paul's lasting contributions through the many people whose lives he touched.

Meeting Paul at the age of 20, and working with him closely for almost 45 years thereafter, changed my life for the better in so many ways. To this day, I often will ask myself: what would Paul do (WWPD)? I am a better scientist, a better person, and a better mentor and leader because of what I learned from him. Paul Greengard was a mentor and role model for the ages. We will miss him terribly but his unparalleled legacy continues through the many generations of scientists who will always be part of the extended Greengard family.